All iGEM Teams Ever (2008-2016)

This is a list of all iGEM Teams where we could find abstracts to. 2016 and 2014 are missing unfortunately. Please use this as a reference and if you want to see if you idea has been done before, simply press CTRL+F or Apple+F and search for some key words of your idea.

You can also click the team names to get to their wiki page.

One sentence summaries:
http://diyhpl.us/wiki/dna/projects/#igem-2013

diybio mailing list thread:
https://groups.google.com/d/msg/diybio/Cu8PfDh2lHY/wIkaH9iMEQAJ

2015

Team AHUT China: Shining Sanctifier

Water, the origin of life, is the necessary and elementary component of our daily life. Various kinds of means have been developed to dispose nitrite and ammonium which are the main contaminants of this type of effluent. One of them is anaerobic ammonium oxidation bacteria (anammox) which can convert the fomite in the water into nitrogen. Our goal is to design a wastewater treatment system which can absorb the pollutant efficiently while transform it into luminous energy. We plan to use E.coli to design a bacterium that can digest the nitrite and ammonium in its interior using the disposal system from the anammox. Through the introduction of luciferase, the energy can be transformed into bioluminescence. Therefore, we named it Shining Sanctifier. This new star in synthetic biology will be applied to the sewage treatment system on a large scale while it can also be made into illuminating system.

Team AITM-Nepal: siRNA MEDIATED IMMUNE MODULATION FOR INNATE AND ADAPTIVE RESPONSE USING GENETICALLY ENGINEERED Escherichia coli

Canonical small interfering RNA (siRNA) duplexes are potent activators of the mammalian innate immune system. The induction of innate immunity by siRNA is dependent on siRNA structure and sequence, method of delivery, and cell type. The delivery of siRNA in a packaged outer membrane vesicle of gram negative bacteria is the theme of our work. The toll like receptor-7/8 activation by siRNA in order to boost the production of Interferon type -1 molecules to inhibit the viral and outer membrane LPS structure to activate Toll like receptor -4 to inhibit bacterial pathogens is the objective of this work. The delivery is made dependent on the peptide fragment which mediated the fusogenic mechanism so as to escape the endosomal compartment once endocytosed inside host(mamalian) cell. Thus freeing the siRNA to silence the myD88 transcriptin host cytoplasm making RISC complex and hence, activating TLR-7/8 in endosomal membrane formerly.

Team BIT: A New Strategy to Detect Antibiotics in Milk: Based on Sensors with Controllable Bio-enhanced Blocks

Bio-amplification, especially controllable bio-amplification is significant for biological detection. In a synthetic biological way, 2013 BIT iGEM assembled the T7 RNA polymerase gene and T7 promoter as an

amplification block (amplifier), which is based on the high activity of T7 promoter to amplify the signal. To make the magnification controllable, a lacO operator regulated by lacI was assembled in downstream as a control block (controller), by adjusting the concentration of IPTG. With this block, several sensors of materials including but not limited to antibiotics are able to be enhanced controllable. This year, a sensor of beta-lactam newly designed and one of tetracycline are applied to detect the residual of antibiotics in milk which endangers human health. To make the detection faster and more convenient, milk samples and engineered E.coli are mixed in a tailor-made bio-chip and the green fluorescence will be detected and shown on a tailor-made electronic equipment.

Team BIT-China: Intelligent Microbial Heat Regulating Engine

To keep the cells in a good condition, cooling system is used to control the temperature in fermentation process. However, the cooling system can result in a great consumption of energy, which increases the cost of production and causes resources wasting, global warming indirectly. To settle this problem, we constructed an Intelligent Microbial Heat Regulating Engine (I'MHeRE), which includes the customized thermo-tolerance system and the intelligent quorum regulating system, to help cells resist heat by regulating the expression of heat shock proteins and controlling the density of cells. The chassis host with I'MHeRE may make the fermentation less depend on the cooling system and shrink cost. Besides, cells could live well in higher temperature, because we extend their optimum living temperature and make them live in optimizing density. Owing to this, the activity of the enzymes in cells could be increased and the efficiency of microbial metabolism could be improved.

Team Biwako Nagahama: AgRePaper&E.coli-ink

Cellulose is used as raw material for paper, so our team experimented various ways to increase the amount of cellulose produced by agrobacterium and using it to make papers. For this we developed the different parts to insert into the system of agrobacterium. Among them are the genes used for expression of the curdlan. Similarly, genetic parts in order to increase the expression of the cellulose, along with the agrobacterium type binary vector were also developed . We are also working on recycling the produced paper by degrading the cellulose to D-Glucose using various enzymes. We worked for the preparation of the biological ink using the sperm whale's cells by genetically modification to increase amount of myoglobin. Then, we observed the change on the color of the product by altering the formation of myoglobin and the production amount of myoglobin with the insertion of T7 promoter to the cell system.

Team CAU China: Alcohol-detoxic Beverage

Alcoholism is prevalent in China. Here we decide to invent an alcohol-detoxic beverage that can considerably prevent alcoholism by adding one healthy bacterium-lactobacillus. In principle, this engineered bacteria can survive in the extremely acidic stomach environment and reduce the toxicity by converting alcohol to corresponding carboxylic acid through a two-step reaction. The two-step reaction is catalyzed by intracellular alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH),respectively.We try to engineer both enzymes, ADH and ALDH, to be acid resistant for higher performance in human stomach.

Team Chiba: Magnetic E. coli

In nature, there exist a variety of magnetotactic bacteria. Recently, it was reported that non-magnetotactic cells such as yeast can be magnetized to some extent. We set the goal to transform E. coli into those that are attracted by magnets. By magnetizing E. coli, the cell harvesting process will be much simpler and

more economical than the conventional processes such as centrifugation and filtration. To this end, we are conducting three itemized projects. (1) modification of iron transportation network to import as much Fe ions as possible in E. coli, (2) sequestering/ storing iron into human ferritin, and (3) converting cytosolic space from reducing to oxidizing in order to elevate Fe(II)/ Fe(III) ratio within. Because all such manipulations significantly impact the physiology of the host cell, we are establishing the BioBrick platform that enables the temporal knockdown of multiple genes using recently control technology such as CRISPRi.

Team Fudan: ALeader: leading the advance of RNA synthetic biology

RNA regulation patterns, which have not been fully understood so far, is a research hotspot still deserving exploiting. A recently-discovered riboswitch ALeader updated our ideas by its delicate, 75nt-structure consisting of an aptamer, a recombination site, and even a bicistron motif. Inspired by this natural design, we proposed a series of novel strategies this summer, with dynamic rather than static perspectives. Guided by the theoretical study on functional multistable states and semi-static states of a riboswitch, and the kinetics involving impacts from other systems such as CRISPR, RNA polymerases, ribosomes, and degradation complex, the ALeader-based functional multi-phase and tricistron switches are designed. We also tried to regulate aptamer's function by manipulating its working environment instead of itself, with SpinachALeader-based real-time monitors to avoid the signal distortion. Furthermore, to demonstrate the advantages of RNA biobricks, we constructed an antibiotic-detector with ALeader, optimized by a network with a RNA-OUT/IN translational regulatory system.

Team HIT-Harbin: B-POM: Biological proportional operational Mu-circuit

The composition of B-POM is that hrpR's promoter depends on the input, but hrpS'promoter is always Ptet and tet owns PhrpL, while the output gene follows tet and shares PhrpL. Once the input is sensed, the input promoter triggers hrpR's transcription. The activity of Ptet is constitutive, which means HrpS protein is ample. As HrpR accumulates, HrpS binds to HrpR and form HrpRS which then triggers PhrpL, and tetR and output begin to accumulate. tetR can inhibit Ptet. As a feedback, HrpS and HrpRS will decrease. PhrpL will be of lower activity, so the amount of tetR and the level of output will decline. The decrease of tetR will enhance the hrpS' expression. All these construct a feedback cycle. Finally, the output will stabilize and be in a certain proportion with the input. By manipulating the RBS of hrpR, hrpS, tetR and output gene, we can control input-output proportion.

Team HokkaidoU Japan: "Maestro E. coli" ~optimization kit for expression~

Thousands of genes are expressed in living cells. Their expression is cleverly controlled by promoters and RBSs. Precise regulation of recombinant genes is hard to achieve. Imbalance in regulation results in little production. However, it is hard to objectively select promoters and RBSs. We thought that E. coli could do the selection for us. We created a kit for E. coli to find the best suited promoters and RBSs. It enables our lab E. coli to be like "Maestro" who creates excellent harmonies with lots of instruments. For the kit we created an original promoter and RBS families with different strengths. We checked and made these parts to be reliable. And it only takes a single golden-gate assembly to get your construct! We made the promoters and RBSs by selecting from randomized libraries. Using the kit, E. coli can choose optimal promoters and RBSs by her/it/him-self, just like the maestro.

Team Hong Kong CUHK: Switch off PAHs

To rapidly regulate biological process, we designed a novel transmembrane protein called Voltage Switch

(VS), which is a fusion protein utilizing the voltage sensing domain from potassium ion channels. Triggered by change in potential across the cell membrane, VS can separate or bring targeting enzymes into proximity, thus allowing an instant control of enzymatic reaction. We also utilized VS to accelerate the polycyclic aromatic hydrocarbons (PAHs) degradation system – another highlight of our project. The metabolites of certain PAHs are mutagenic and carcinogenic. We codon-optimized laccase from Bacillus sp. HR03 and catechol 1,2-dioxygenase from Pseudomonas putida KT2440 for Escherichia coli, which when forming a cascade, PAH degradation into less toxic simple carboxylic acid would occur. Since quinones are intermediates in the degradation of PAHs, we also added quinone sensing and response repressor (QsrR) to control the degradation process.

Team Hong Kong HKU: E. capsi: Reducing phosphate pollution using engineered E. coli that harvests polyphosphate

Phosphate pollution in waterways and water treatment plants is a major problem. Removal of phosphate from wastewater is required to treat phosphate-containing discharge to reduce eutrophication, algal blooms and “dead zones” in lakes, rivers and coastal marine ecosystems. The aim of this project was to remove or reduce the levels of inorganic phosphate from a system or environment by employing engineered bacteria E. capsi, capable of accumulating phosphate in the form of polyphosphate. Our strategy is to express polyphosphate kinase together with the ethanolamine utilization (eut) bacterial microcompartment from Salmonella enterica to provide an environment for polyphosphate synthesis. Furthermore, the project provides a novel way to recover accumulated polyphosphate, an energy rich macromolecule with many industrial uses. This paves a way towards living system-based phosphate pollution treatment to tackle critical environmental challenges.

Team Hong Kong HKUST: FATBUSTER - The Artificial Futile Cycle

While low-fat diet and regular exercise are popular approaches to fight with obesity, one easy alternative is simply to increase energy metabolism. In a synthetic biology approach, we are working to create an artificial futile cycle in mammalian cell by introducing glyoxylate enzymes native to bacteria. Past research has shown that mice expressing enzymes constituting an active glyoxylate shunt are shown to be resistant to diet-induced obesity. Our team plans to introduce an inducible system that allows us to couple the sensing of circulating fatty acid concentrations with an inducible circuit of glyoxylate shunt. Our inducible system is intended to prevent the risk of fatty acid deficiency, while facilitating greater fatty acid uptake at higher fatty acid circulating concentrations. Such a system should increase the feasibility of a glyoxylate cycle engineered to function in vivo.

Team HUST-China: Antihypertensive Ecoli

Hypertension causes grave concern worldwide for its notoriety, there’re not many therapeutic methods to hypertension besides various antihypertensive drugs. However, this comes along with heavy financial burden to developing or underdeveloped countries. In addition, almost all these drugs have side effects to liver and renal. Here is a novel method to treat Hypertension by constructing human-friendly engineering bacteria that can produce short-chain fatty acids (SCFA) periodically and naturally to help maintain the blood pressure in safe level. SCFA, especially acetate and propionate, has been proved to induce vasodilatation and ensuing hypotensive response via receptors in smooth muscle cells of vessels. This year we have found a metabolic pathway in Escherichia coli that converts succinate to propionate through Wood-Werkman reaction. An operon consisting four genes encodes enzymes in this pathway. By combining bio-oscillator and key gene together, we want to make E. Coli release propionate periodically in

patients' intestine periodically.

Team HZAU-China: Safe moving vaccine factory

For HZAU-2013iGEM project, we are creating a safe moving vaccine factory by synthetic biology which can spread Rabies vaccine in dogs rapidly and actively. Our aim is to help in the achievement of the WHO goal of being free of human rabies by 2020 through the improvement of the vaccination coverage in dogs. The idea comes from Yersinia pestis and fleas. We make use of fleas as our moving injector. When flea feed blood from dogs, our vaccine vector Bacillus subtilis will be regurgitated into blood and successfully transferred to mammalian host. Bacillus subtilis can express antigens which can stimulate the immunity of dogs. Meanwhile, endogenous or exogenous expression of 'Antimicrobial Peptides' by B. subtilis can kill Yersinia pestis in fleas. In this way we achieved a safe moving vaccine factory.

Team IIT Delhi: pHColi

pH induced response elicited by certain promoters in bacteria may have major practical applications. The response can be targeted for specific pH ranges, for example in tracking the anomalies associated with the gut micro-biota or detecting pH inside a bioreactor. There are only limited studies reported in the area. In the present project, a genetic circuit has been created, using the promoters of the acid shock response gene from E.coli and the F0F1 ATPase operon from C. glutamicum that produces a pH dependent colour gradient, much like a universal pH indicator. A mathematical model has been developed to simulate the experimental findings. The present study will form the basis for further research in the field of synthetic biology.

Team IIT Madras: COMBATING SHIGA TOXIN : A SYNTHETIC BIOLOGY APPROACH

Shiga toxin, a worldwide menace, has killed over 1 million people to date and continues to afflict almost 150 million people each year. Currently, there is no treatment for Shiga toxicosis and it leads to complications in the human system like hemolytic uremic syndrome (HUS) and renal failure. Here, we propose a two-fold, novel synthetic biology approach to combat the lethal effect of the toxin. We aim to neutralize the already produced toxin through a nine amino acid Gb3 mimic peptide. We have engineered the Gb3 mimic along with a cellular export signal (ompF) downstream of AHL(quorum sensing molecule) inducible promoter (pLuxR). We also plan to prevent further toxin production by inhibiting the biofilm formation of shigatoxigenic E.coli using indole-3-acetaldehyde (I3A). We expect to validate our approach through functional assays and in silico modelling. Our findings can potentially initiate a new perspective of tackling Shiga toxicosis using synthetic biology tools.

Team ITB Indonesia: Aflatoxin Biosensor

Aflatoxins are naturally occuring mycotoxins that are mutagenic and carcinogenic. Aflatoxin contamination of foods that are found in many developing countries may cause a serious problem for human health. ITB_Indonesia team for iGEM 2013 focuses on designing a whole cell biosensor for aflatoxin B1 detection in foods. The biosensor uses Escherichia coli as the chassis to build a genetic circuit using SOS response system to detect DNA damage caused by aflatoxin B1-oxide attack. The SOS response promoter is followed by a reporter gene coding a chromoprotein, therefore the concentration of aflatoxin B1 in food samples could be easily detected by the color change of the bacteria. For the ease of usage, we will design a syringe shaped device with our whole cell biosensor in it. This device would allow aflatoxin B1 to enter the device, but would not permit the cells to leave the device.

Team KAIT Japan: Hay fever curE.coli

Japanese on of six people is troubled now by hay fever. These people take a medicine for the hay fever. But, If they take it , they become sleepy. If become sleepy, they cannot work and study. So, we are working on a project to relive hay fever by Escherichia coli to improve these. Mechanism of hay fever When an allergen invades it in the living body, naïve T cell differentiates in Th2. There is more Th2 than Th1, and the mast cell and others that is humoral immunity become active, and inflammation is in this way caused. We perform following four this time. ①Expression of IL-10 receptor to E.coli. ②Phosphorylation of STAT3. ③Preparation of gene array with HlyA and L-12 promoter and receiving the STAT3. ④Preparation of gene array with TolC and HlyB and HlyD promoter and to receive the STAT3.

Team KIT-Kyoto: Fregrance coli

We are trying to construct a novel E.coli that has fruity flavor like Japanese rice wine (Japanese sake). In order to accomplish the purpose, yeast genes related with production of the Japanese sake fragrance were introduced into E. coli cells. We also tried to develop a way to eliminate bad smells of E. coli in parallel. Although we previously won a gold prize by the development of a novel pen (E. coli Pen) in 2010, its bad smells were weak points and must be improved. We will overcome this problem through the progress of our new project in 2013. So far, “smell” is not a popular keyword and not a major field in iGEM. However, we believe that our project will provide a new point of view to iGEM friends

Team Korea U Seoul: Pearl-coli: E. coli converting CO2 into a pearl powder (nacre)

The Korea_U_Seoul team aims to design Pearl-coli that is E. coli able to convert atmospheric CO2 into pearl powder materials. The design is based on cell surface display of nacrein in E.coli. Nacrein is a major protein component in nacre(an organic-inorganic composite layer found in outer coating of pearls). We divided nacrein into functional regions - carbonic anhydrase(CA), calcium binding and scaffold repeats. CA domain fixes CO2 into carbonic acid changing to bicarbonate ion in aqueous solution. We will examine if displayed nacrein in E. coli can make a pearl powder in a solution or fabricate a nacre-like structure while atmospheric CO2 is fixed into bicarbonate. Once a nacre material can be prepared from Pearl-coli, we will grow E. coli in a confined container to make synthetic pearl. The Pearl-coli has dual-function such as (1) mitigate the global warming by CO2 reduction, (2) prepare valuable pearl-like raw materials.

Team Kyoto: Oscille.coli

Every organism has its own cycle such as the periodicity of cell division, ordered patterns of its body. Some kinds of the cycles are regulated just by two factors. Using E. coli, we applied this kind of periodicity formation.</p> Firstly, we focused on oscillation regulated by RNA. We suspected if RNA world hypothesis is correct, there could be protein-depended oscillatory system. To show the possibility of cycle formation by RNA, we constructed an oscillator by utilizing two different types of functional RNA, which are transcriptional activator and repressor.</p> Secondly, we also targeted on planar oscillation forming mechanism. A. Turing (1952) suggested a simple principle containing just two variables explains many organisms’ epidermal pattern formation. However, it is not confirmed the pattern formation is only based on Turing’s discourses. To check this, we used two types of E. coli, which secrete different factors, and regulated their population.

Team LZU-China: Twinkle Cancer Hunter

To construct a regulating vector of NF-κB signaling pathway by gene recombination technology, introducing into tumor cells with NF-κB to form a signal feedback control system. Using NF-κB binding elements as promoter,and IκB-GFP fusion protein as reporter.Then inverted into HEK-293T cells and DU-145

cells.Through the observation of the GFP to probe the expression of IκB. The expressed protein was identified by Western blot, etc.The constructing of a regulating vector of NF-κB signaling pathwayprovides a new method and thought for tumor gene therapy, and propel forward the research of NF-κB signaling pathway.

Team Macquarie Australia: Green is the new black - Expression of Chlorophyll within Escherichia coli

Photosynthesis is a key biological pathway that uses sunlight energy to convert water and carbon dioxide into ATP, glucose and oxygen. Chlorophyll is a green pigment that facilitates this energy production in photosynthetic organisms. Although the biosynthesis pathway for chlorophyll has been thoroughly investigated, the reproduction of this pathway in a non-photosynthetic organism has, to date, not been achieved. Successful production of chlorophyll in a bacterial host is the first step towards the synthetic construction of photosystem II, and the eventual creation of a renewable energy source. Our research involves expression of twelve genes (from Chlamydomonas reinhardtii) necessary for the chlorophyll biosynthesis pathway in a bacterial host (E. coli). Gene sequences have been synthetically designed to allow for prokaryotic expression. By utilising Gibson assembly, we plan on being able to successfully produce chlorophyll in prokaryotic cells. This will be evident from the growth of green E. colicolonies.

Team Nanjing-China: Atrazine Elf

Atrazine, a widely used herbicide, persists for a long period in the environment onced used. It causes metabolic disorders in both animals and humankind. Our team utilized the ribosome switch induced by atrazine, a QS system of Plux and a degrading enzyme to control E.coil's motility through regulating it's CheZ gene. Therefore, E.coli can recognize atrazine, recruit team workers, and degrade atrazine. Our team found a transporter of atrazine, which we call TRM. We also mutated the degrading enzyme, TrzN, making it better at degradation. We combined TRM and the TrzN to improve atrazine absorbance and degradation. Moreover, our team are trying to analyze and compare several systems with computer, hoping to find the best one which is equipped with faster moving and quicker degrading. Overall,we believe our system will boost the industrialization, universalization as well as standardization in the field of treatment for atrazine and other versatile small molecules.

Team NCTU Formosa: E.colightuner

We have proven a sRNA-regulated system of our own to be an effective and competent way for regulating gene expressions. Recent studies have shown that sRNA-mediated regulation is an important factor to bacterial growth. sRNAs work by base pairing with limited or extended complementary target mRNAs, regulating proteinproductions. Using sRNA mechanism, we can control gene expression in RNA level, in contrast to common promoters that functions on DNA level. Since the existing sRNAs in Escherishia Coli have important functions in other metabolic processes, we designed an artificial sRNA with high specificity to avoid undesired base binding in vitro. By using the sRNA-regulated system, red light induced operator, and thirty seven degree Celsius ribosome binding site (RBS), we constructed a manipulatable system that is capable of expressing four different genes under different conditions. In other words, it is a multitask machine.</p>

Team NJU China: Biomissile: a novel drug delivery system with microvesicle

Recently, small interfering RNA (siRNA) has emerged as a promising therapeutic drug against a wide array of diseases. However, site-specific delivery has always been a challenge in gene therapy. Microvesicles

(MVs) are lipid-bilayer vesicles which are naturally secreted by almost all cell types, playing crucial roles in intercellular transport of bioactive molecules. Given the intrinsic ability to naturally transport functional RNAs between cells, MVs potentially represent a novel and exciting drug carrier. In our project we are trying to express both anti-virus siRNA within the cell and target protein on the surface of the MVs by engineering the HEK 293T cell, which is capable of producing large amounts of MVs. Thus, the MVs produced by our engineered HEK 293T cells will contain the siRNA and be able to specifically deliver the siRNA to the sites we want, acting as biomissile for the targeted destruction of the disease.

Team NJU NJUT China: The Application of Cas9 as a Gene 'Missiles'

Most bacteria and archaea can resist invading DNA and/or RNA elements via the clusters of regularly interspaced short palindromic repeats (CRISPRs).It is believed that the integrated CRISPR sequences have the ability to form a genetic memory which prevents the host from being infected.The memory exist as a DNA library in genome, artificially modified to set its target. The CRISPRs and Cas (CRISPR-associated) interact and form this prokaryotic adaptive immune system. Cas9, as a core of CRISPRsystem, can play a role of targeted-attacking gene 'missiles'. Therefore, we build a sort of plasmids, loading CRISPR system, to realize the 'killing' of harmful genes and/or organisms.

Team NTU-Taida: QS array

Bacterial infection is the invasion of the body by pathogenic bacteria, which causes pneumonia, urethral infection, bacteremia and other symptoms in hospital and community. The efficiency of traditional detection and diagnostic approaches is impeded by the time-consuming laboratory procedures, yet many of which grow poorly in bacterial cultures. All these limitations call for a new rapid and direct bacterial identification method to improve patient management and antimicrobial therapy. Quorum sensing is a type of bacterial cell-cell communication correlates with the population size. Many bacteria have one or several species-specific quorum sensing molecules released in different growth state and environment. Quorum sensing signals are shown to be involved in many physiological functions, including virulence, biofilm formation and drug-resistance. We aim to establish a novel bacterial identification method in clinical samples based on the quorum sensing profiles.

Team NTU Taiwan: YeasTherm - against the cold

During winter season, due to low temperatures, fish farming is one of the most heavily affected economic venues. Due to this, year after year, several farmers are faced with many problems as a result of a loss of fish product. </p>Using our background in bioengineering we suggest an innovative alternative: Our idea is based on heterologues expression of SrUCP in *Saccharomyces cerevisiae* and *Rhodotorula glutinis*. Through the expression plasmid, yeasts are transformed from the wild-typephenotype into a thermogenic phenotype. </p>To implement this idea and make it simple and efficient, we plan to drive the expression of SrUCP under the control of cold shock promoter Tir1. In this way, yeasts will generate heat only when the temperatures drop. Moreover, the temperature-responsive range of Tir1 may be regulated by applying genetic circuits, providing the means to manipulate the biological device to suit different temperature conditions and needs in application.

Team NU Kazakhstan: Detection of Carcinoembryonic antigen with sandwich-biosensor

Carcinoembryonic antigen (CEA) is the cancer biomarker at early stages of several cancers including colorectal carcinoma, lung carcinoma and others. The aim of the study is to develop a biosensor that can

be used in the detection of CEA. In the first part of the study ssDNA aptamers, with strong affinity for CEA, were selected by 12 cycles of Systematic Evolution of Ligands by Exponential Enrichment procedure, and characterized with dot-blot analysis and Surface Plasmon Resonance methods. In the last part, it is planned to clone the genes that will assist in expression of streptavidin on the surface of E. coli and S. cerevisiae membrane. E. coli will deliver streptavidin on the surface via Lpp-Omp expression system, while S. cerevisiae via Aga1 – Aga2 system. Modified model organisms, aptamers and CEA will be used to constructsandwich-biosensor.

Team NYMU-Taipei: Bee. coli: to bee, or not to bee

To save bees from Nosema ceranae, the culprit of colony collapse disorder, we created Bee. coli. from E. coli K-12 MG1655, a bacterium residing natively in bees. Bee. coli is strategically designed to work as follows. First, it continuously secretes mannosidase to inhibit the sprouting of N. ceranae spores. Second, if the bee is infected, the fungus-killing-circuit with a positive feedback design will be turned on to wipe out N. ceranae. Third, if these designer weapons should fail to conquer N. ceranae, our designed bee-suicide-operon will be activated to kill the infected bee and save its companions. Fourth, a light-inducible lysis system is included to ensure our Bee. coli only lives inside of the bee. Fifth, we apply encapsulation as the way to send Bee. coli into the bee. Since the capsule will only dissolve in a bee's gut, our Bee. coli will not spread to the environment.

Team Osaka: Beat the discrimination against E.coli !

Since the middle of the 20th century, Escherichia coli(E.coli) have made great contributions to various field of our society. Although they have played essential roles in the society, it seems that they are not properly appreciated by general public. People's common images to E.coli are very negative (dirty, stinky, dangerous etc). So in our project, to wipe away the negative images to E.coli, we have created a circle that enable them to communicate with each other via nutrient production.And we made "empathetic E.coli" that lives cooperatively with each other. Then, by conducting experiments and using computer simulation, we have examined how they live and grow in liquid medium culture and what kind of pattern they form on solid medium culture.

Team OUC-China: Reconstructing the Magnetosome Membrane in E. coli

Membranous organelles are unique structures of eukaryotic cells, rare bacteria and paleontology. Magnetospirillum magneticum is an important biological model system of prokaryotic organelle study because the structure of magnetosome in Magnetospirillum magneticum has similar traits to eukaryotic organelles with membranes. Our task is to reconstruct the magnetosome membrane in Escherichia coli. Magnetospirillum magneticum requires a micro-aerobic and oligotrophic environment in order to produce magnetosome, so the significance of our project lies in simplifying the magnetosome produce method, opening up the path for futher functional gene research. We use homologous recombination to transfer the mamAB gene into E.coli to build an IMS part. Also, as the mamK gene is crucial to the IMS construction. We want to improve the mamK gene's expression by stabilizing its mRNA with a new method, hoping it can be used to promote the IMS construction. So we design a DNA segment to slow down mRNA degradation.

Team Peking: Aromatics Busted

Aromatic pollution is becoming a worldwide concern, and monitoring aromatics remains challenging. Noting the abundant genomic data of prokaryotes from aromatics-rich environment, Peking iGEM applied part mining to the genetic repertoire to develop a comprehensive set of biosensors for aromatics. The

transcriptional regulators for each typical class of aromatic compounds were bioinformatically determined and promoter engineering and protein engineering were performed to tune their function. To expand the detection range, enzymes in upper pathways, working as plug-ins, were coupled with biosensors to degrade aromatics to detectable compounds. For environmental detection, we construct the band pass filter to detect a certain range of concentration. Responses of biosensors equipped with band-pass filter can robustly reflect the concentration of environmental samples. Peking iGEM has remarkably enriched the library of biosensors for aromatics and enabled quantitative detection for environmental monitoring. These biosensors will be also potent for metabolic engineering and well-characterized synthetic biological tools.

Team SCAU-China: Detection and degradation of organophosphorus compounds

Synthetic organophosphorus (OP) compounds, which are highly toxic contaminants in agro-environment and food security, have been widely applied to pesticides. Parathion is a typical representative of organophosphorus pesticides. This year, our goal is to construct a p-Nitrophenol sensor in E.coli, which is the degradation product of parathion, in order to reflect the existence of parathion. Besides, we try constructing a degradation system to solve the pollution problem. Considering the biosafety problem, we also design a suicide system in which the lethal genes are only triggered by declining p-Nitrophenol concentration. This will enable the bacteria to commit suicide when p-Nitrophnol is sufficiently degraded.

Team SCUT: E.cerevisiae

E.cerevisiae is a sophisticated signal transport system between E.coli and S.cerevisiae. Producer, the E.coli, is assigned to distribute a special volatile—butanedione periodically with a stable oscillation circuit, which defines the meaning of the signal. On the other side, Sniffer□the yeast, transplanted with a nose from nematode, can respond to the signal immediately. We hope this can realize the communication between prokaryotes and eukaryotes for the further research on symbiosis.

Team SCU China: Imitations of Gametogenesis & Sexual Reproduction using E.coli

We intend to construct two groups of differentiated E.coli,one imitates the male multicellular organism ,the other for the female. When cultured separately, the male/female multicellular system gets bigger and matures, and cells will differentiate into gametes,which cannot divide any more but are capable of gene transfer.</p> After that, you mix this two liquid cultures,the male gametes will recognize the female cells and begin to transfer modified F plasmids into female gametes through sex pili. The conjugationmakes female gametes return to the state of un-differentiation(called G cells),which means they can divide again but are not sexually determined.Then,after several cell divisions,one G cell will differentiate into a G+ or G-,which, like zygote, can grow into next generation of the multicellular system maybe containing genes from both male and female gametes.

Team Shenzhen BGIC 0101: Genovo

Genovo is a Computer-Aided Design (CAD) tool used for denovo design of genome. The current version consists of 4 parts. The first, Chromosome Construction will grap genes in a common pathway and chromosome features to build a new genome and let user to define the order and orientation in drap-drop way. The second, NucleotideModification will optimize and soften the sequence of CDSs. It also help design the CRIPSR sites so that we can silence the wild type genes. The third, ChromosomeSegmentation will cut chromosome into pieces and add 3A & Gibson & Goldengate & Homologous Recombination adaptors to the pieces automatically for assembly. The last one, OLS Design will guide users to gain the chromosome by microarray. Genovo will enable user to design their innovative chromosome as their

wishes and further the research on genome on pathway level.

Team Shenzhen BGIC ATCG: Cell Magic

Cell Magic plays a gorgeous movie show in the both E.coli and S.cerevisiae.Various colors are blooming in different branchs & buds: plasma membrane, nucleus matrix, mitochondria membrane & matrix, vacuolar membrane, peroxisomal membrane, centrosome, and also actin. But the scene is far from static, colors will show up in order under the sophisticated cell cycle system at G1, S, G2 or M phase. Accelerator—degradation system is applied to run this movie faster, and freezer—sic1 system will put off the cell cycle during G1 phase. Beside, the editor—intron will expands a random dimension, leading to produce more combining form.

Team SJTU-BioX-Shanghai: Metabolic Gear Box

Few researches have been done to regulate gene expression levels in genomic scale so far. This year we aim to combine two systems together in order to provide a universal and convenient tool which can be used to regulate different genomic genes simultaneously and independently in a quantitative way.

Our project involves the newly developed gene regulating tool CRISPRi and three light-controlled expression systems induced by red, green, and blue light respectively. Simply by changing the regulating parts in CRISPRi system towards mRFP, luciferase, and three enzymes, we hope to prove our system can be used qualitatively, quantitatively and practically step by step.

We have also designed a box and written a software as our experiment measurements. Simply by typing in several parameters, different gene expression levels can be controlled. This system can also be improved to predict the maximized producing efficiency after some simple tests in future.

Team Sumbawagen: E. coli which able to measure the level of sugar in honey by emitting light

Glucose and fructose are major sugar component of honey. Sumbawa honey is protected as geographical indication by Indonesian patent office. Sode Lab at Tokyo University of Agriculture and Technology has created a fusion of mutant glucose binding protein and firefly luciferase, which able to measure glucose level by emitting light - intended initially for blood glucose sensor application (Taneoka et al, 2009). In this project, we plan to create this construct in Biobrick format, and evaluate the ability of transgenic E. coli for the measurement of glucose in honey. Our final goal is to create a device which can be used for quality control of Sumbawa honey, which we call 'ECONEY'.

Team SUSTC-Shenzhen-A: Game Theory--Stategy for the Classic Prisoners' Dilemma

There are many applications of the game theory in some aspects of our life. Each individual has two kinds of choices--to betray or stay silent, and the choice you make would determine your fate. To betray the other side, you may risk being revenged. While staying silent, companion's betrayal may hurt you deeply. As for our project, we work out a new way to imitate the game theory by constructing a community of two E. Coli bacteria. Here we use the growth rate of each species to represent its fate. The effect of one's silent or betrayal on the other species' fate is acted through intercellular signal molecules of two quorum sensing systems. Each signal molecule regulates the expression of toxic genes in the other species and reduces its

growth rate. We characterize the consequence of each strategy by quantitatively measure the growth rates of each species in the community.

Team SUSTC-Shenzhen-B: Circuit+

To standardize genetic circuits and bring the idea that relations exist in circuit between parts back to synthetic biology, we proposed our Technical Standard RFC 101 and RFC 102 to define genetic circuits and logical gene gates. To solve the problem that synthetic biology lacks such a database to systematically record genetic circuits and to make the standards work, we built Circuit+, an online registry of standard genetic circuit which records information of circuits based on the two standards. Users can retrieve circuits, browse information, share by exporting SBOL and upload new circuits. We also have developed Clotho version Circuit+, Circuit List and transplanted TTEC to Clotho. And we have developed an online platform for synthetic biology lab management. We also did human practice to promote synthetic biology and iGEM.

Team SydneyUni Australia: Keeping DCA at Bay - Assembly of synthetic constructs and cassettes for degradation of dichloroethane.

The picturesque city of Sydney is marred by industrial efflux of chlorinated hydrocarbons into the aquifers around Botany Bay. 1,2-dichloroethane (DCA) is toxic and a suspected carcinogenic agent, and one of the more soluble and mobile contaminants. Conventional DCA treatment is both costly and time-consuming, involving pumping and heat-stripping groundwater. We propose a biological alternative which may be cheaper and more effective. There are strains of bacteria able to degrade low levels of organochlorine compounds in selective conditions. Polaromonas JS666 and Xanthobacter autotrophicus GJ10 contain two pathways of particular interest. Our goal is to construct our own versions of two metabolic pathways of DCA biodegradation for comparison in a BioBrick-compatible vector, and characterise their effectiveness in utilising DCA as a sole carbon source for growth. We hope to create friendly strains of bacteria capable of removing DCA at greatly reduced cost and effort, and reduce the environmental impact of industry.

Team SYSU-China: iPSC safeguarding Device

Since Shinya Yamanaka published the epoch-making paper in 2006, the induced pluripotent stem cells(iPSCs) has become one of the most promising techniques in regenerative medicine. Like embryonic stem cells(ESC), iPS Cells can be differentiated into any tissues. Compared with ESC, iPSC is easier to attain, immune rejection-free, and ethical issue-free. However, Rurther application of human induced pluripotent stem cells(hiPSCs) in tranlational medicine requires the concerns of two problems: the specificity of directional differentiation and the safety of the transplant. Here we design a new device which can spontaneously select hepatocytes from iPS differentiated cell mass and prevent potential carcinogenesis. To achieve accurate spatiotemporal control,we build a miRNA-122 sensor and make use of the tetracycline induction system. Our work may also be extended to the field of gene therapy, and provide a new direction to our train of thought about how to solve the safety problem in genetic manipulation of human cells.

Team SYSU-Software: CAST (Computer Aided Synbio Tool)- An Integrated Tool for Synthetic Biology

Accurate simulation and gene circuit design are essential but difficult parts in synthetic biology.Here, we designed CAST to cover the workflow from beginning to end, users can focus on function design and the gene circuit would be automatically designed. Furthermore, we developed a new simulation model that work with standard dynamic characteristic and verified by wetlab experiments. Moreover, we build an

expandable database that users can contribute their own dynamic information which would lead to more accurate and sufficient dynamic information of all the Biobricks. Finally, our software is designed as an easy deployed server so that it can be used on personal purpose or shared by a whole lab or institution.

Team Tianjin: Alk-Sensor, a Novel Detector Applied for the Selection of Alkane Producers

Biosynthesized alkanes are promising candidates for drop-in replacement of petroleum. We constructed and characterized a device named Alk-Sensor, which can sensitively detect a wide range of alkanes and generate certain response. Alk-Sensor is composed of ALKR protein——a transcriptional regulatory protein, and promoteralkM. ALKR recognizes alkanes and their interaction triggered a conformation change of ALKR dimers which isomerizes the promoter-RNAP complex and led to activate the downstream genes of PalkM.

Based on Alk-Sensor, we built a relationship between productivity of alkanes with strain's growth rate under certain environmental stress. Starting from this relationship we further designed a novel selection method to select out the engineered strains with highest productivity of alkanes. We demonstrated that this novel selection method could enable us to select out the optimized strains effectively and efficiently.

Team TMU-Tokyo: Genomic 'Pythagorean Devices'

In this year, TMU-Tokyo created Genomic 'Pythagorean Devices'. Pythagorean Device appears Japanese famous educational TV program 'Pythagorean Switch' Pythagorean Devices are known in the US as 'Rube Goldberg machines'. Pythagorean Devices are deliberately over-engineered or overdone machines that performs a very simple task in a very complex fashion, usually including a chain reaction. We constructed a Pythagorean device in Escherichia coli genome, using lambda phagerecombination system 'RED'.

Team Tokyo-NoKoGen: Twinkle.coli -Fast cycle! Fast response!-

We created Twinkle.coli, which "blinks" fast like a firefly. An oscillator is a system that responds in periodic changes. This response is usually regulated by positive or negative feedbacks by using inducer or repressor proteins. However, the use of proteins might delay the response because transcription and translation must happen before the next output. To design an artificial fast responding oscillating circuit, we designed an RNA-based oscillator. We used RNA-responsive self-cleavage ribozymes whose cleavage is regulated by an RNA molecule. The ribozyme cleavage cuts-off an "RNA scaffold" that harbors RNA aptamers. This aptamer binds to its specific target proteins, which are directly fused to reporter protein. This binding recruits the already translated split reporter protein complementation resulting in the output (twinkles). Our system enabled fast response and short oscillation cycle.

Team Tokyo Tech: 'Mutant Ninja. coli'

In our project, we propose to create E. coli that mimic some of the qualities of Japan's ancient 'ninja' warrior-spies. A ninja must receive and pass on correct information at all times. A mistake will be fatal. We have created a circuit that avoids crosstalk between two signals in cell-to-cell communication, and we are also looking into applications for it. Ninjas are also known for their star-shaped 'shuriken' throwing knives. Our E. coli ninja has a similar weapon, an M13 phage which it releases to infect other E. coli, injecting plasmid DNA into them. Finally, ninja must harmonize with the natural environment, so their relationship to it is very important. Plant hormones help plants to grow efficiently, and we are attempting to construct a

circuit that synthesizes two plant hormones depending on the soil environment.

Team Tsinghua: Mobile Health---Pathogen detector

In a long term, the testing of pathogenic diseases is via comparably complex procedures. This year, we aim to design a sensing yeast powder based portable test paper, that is, the 'mobile' testing system, take advantage of querom sensing system in bacteria, to achieve the testing of specific microorganism caused disease. In the same time, we built a frame of testing any pathogen that will cause diseases, using different the input and output combination. Furthermore, in order to achieve the simultaneous testing of different pathogens, we design a “fast-shifting box” to accomplish the combination of input and output signaling. This will in theory

Team Tsinghua-A: Synthetic gene switch shows adaptation to DNA copy number variation

In some natural and synthetic biological networks, DNA copy number which transfection into cells is fluctuant-influencing gene expression. We hope target gene expressionlevel has a strong adaptability and ability to DNA copy number by using the method of engineering and bringing in incoherent feed-forward circuit. The robust circuits we designed may apply to cancer detection and gene therapy in the future. Generally speaking, we modeled three and four nodes motifs to find some appropriate circuits, which function reliably in the face of fluctuating stoichiometry of their molecular components. Two designed circuits have been tested and we found that the motifs has certain robustness to DNA copy number.

Team Tsinghua-E: Darwinian evolution for microbial cell factory:in vivo evolution engineering towards tryptophan-overproduction superbug

Darwinian evolution shows great power in creating incredible biological function in amazing speed. Inspired by this, our team aimed at creating novel fast and irrational microbial cell factory by simulating natural Darwinian evolution process. With tryptophan as target product, a novel tryptophan biosensor utilizing translating ribosomemechanism was firstly developed as the foundation for tryptophan productivity and selection pressure switch module. We further constructed this tryptophan overproduction selection gene circuit coupling with in vivo mutation machine (mutator gene of mutD). By fine-tuning the selection conditions, our selection circuit showed good tryptophan dependent growth property, which provides the foundation for further evolution. As a preliminary result of this project, we successfully evolved an ancestor with zero productivity to a high-tryptophan producer only after several rounds of evolution.

Team TzuChiU Formosa: Hypnoseq.

The new pattern of antibiotic resistance is a spreading global issue that may soon leave us defenseless against bacterial infections. Taking a closer look, the lack of comprehensive pharmaceutical management system in Taiwan has come to our concern as it results in easy access to antibiotics. Large amount of antibiotics are added in the forage of animal husbandry and aquaculture,hence, leading to the increase of antibiotic resistance in Taiwan. In order to ameliorate this growing threat, we attempt to carry out “Hypnoseq.” to make this world a better place. Our aim in this project is to combine the sense and antisense mRNA of the antibiotic resistance gene to inhibit the expression of the antibiotic resistance gene. Knowing that they have the ability to conjugate and deliver our designed plasmid to other bacteria, we are able to predict that they can decrease the percentage of antibiotic resistance in the environment.

Team UESTC: Nebula

Nebula is a biological circuit design tool composed of Interactive Part & Automatic Part. We classified the

parts released in 2013 and constructed a database for users to choose what they want. In the first part, you are free to link any parts that we have already classified together to meet your requirement. In the second part, once you determine the inducer and the product, our software will offer you the optimized circuit with the input and output that you designated. We use Analytic Hierarchy Process to score every part and edges (passage linking two parts) according to attributions including availability, usefulness, sample status, part status and sequencing. According to weight of edges, we regard the shortest passage between input and output as the optimum presented to users. You can also save the circuits made in Nebula in case you want to check or change it later.

Team UESTC Life: Multistage Degradation of Environment Haloalkanes Contaminant by Co-expression Enzymes

1,2,3-Trichloropropane (TCP) and an organic pesticide-Hexachlorocyclohexane (Lindane-HCH) have been shown to be serious pollutants as they are toxic and quite persistent in the environment, and need to be removed to low levels from polluted sites. Microbial degradation of these compounds represents an important and efficient way to fulfill the target. In order to improve biodegradation efficiency, several powerful genetically engineered E. coli strains have been constructed by the co-expression of key enzymes involving in the biodegradation pathways of the two compounds. For this, foot and mouth disease virus 2A peptide and polycistronic co-expression strategies were adpoted. The results showed that all enzymes could co-expressed as a soluble protein with 2A peptide acting as a linker. Moreover, the resulting engineered E. coliexhibited an excellent capability for the degradation of TCP.

Team UI-Indonesia: Project Blue Ivy - scFv with Blue Indicator as a Biosensor for TB

Tuberculosis (TB) is a worldwide major health problem which infects one third of the world's population. The absence of reliable diagnostic tool in suburban area, where TB cases are most likely found, is still a great obstacle in TB eradication effort. Seeing Indonesia as one of the high burden countries for TB, UI-Indonesia iGEM team are trying to create a reliable, portable, and easy to use diagnostic tool for detecting TB. We are constructing a biosensor consist of highly specific antibody bound to a fragment of β-Galactosidase as a reporter to detect the presence of protein Ag85, a novel TB biomarker. Our goal is to make a biosensor that will detect the presence of antigen 85 in blood serum of TB suspect. Positive result will be indicated with easy to detect blue color, and when it's negative, no response will be observed.

Team USTC-Software: Gene Network Analyze and Predict (gNAP)

Synthetic biology creates and uses standardized parts such as Biobricks to build engineered bacteria for various function. To realize those purposes, importing exogenous genes to target bacteria is universal and essential. In this approach, improve or reduce the expression of target genes through interaction is inevitable. Experiments in wet lab could find the effect and choose the best of imported exogenous genes but take a long period of time. gNap utilizes Internet databases to construct a gene regulatory network (GRN) and analyze the effect of exogenous gene by Michaelis-Menten equation and sequence alignment algorithm. Meanwhile, to guide wet lab experimenters to find the best imported gene in the whole network, we use PSO method to figure out the best regulation patterns of new imported genes meeting experimenters' goal. To realize those ideas, we build gNAP that provides researchers with gene network analysis and prediction.

Team USTC CHINA: T-VACCINE

T-VACCINE is a vaccine initiating immune response by penetrating the skin with the aid of transdermal

peptide. From now on, injections are simply history.Based on the theory of user-friendly, a special group of engineering bacteria which produce T-VACCINE is used to create a brand-new 'band-aid' serving as a guardian of our health .We have found a kind of transdermal peptide TD-1,a magical molecule that enhances the permeability of the skin as well as draw filamentous bacteriophages into the skin.By combining the gene fragments of antigen,immune adjuvant LTB and Luman-recruiting factor TNLFα with that of the TD-1, our team got the permeable fusion protein. In order to obtain large amount of extracelluar protein, we chose bacillus subtilis WB800N as our expression chassis. Further more, the universality of our experimental method is verified by the adoption of various antigen of existing vaccine, such as HBsAg, PA and AG85B.

Team UT-Tokyo: Multicellular Analog Clock

We designed a 'multicellular' E.coli clock with a clock hand. Your naked eyes see the red clock hand moving along a circle of E. coli population on an agar plate. The clock hand, expression of mCherry gene, is driven by an “engine” which is constructed under the inspiration of the mechanism of action potential conduction in nerve cells. The engine consists of a positive feedback loop of AHL and negative feedback loops of TetR, AiiA and 2 types of artificial sRNA. We also designed UV reset devices using UV sensor construct. In addition, small RNAs were designed for metabolic engineering of E. coli, which is the first trial in iGEM competition. We show you the new and easy approach in genetic engineering with the BioBrick parts, which will lead to future application of sRNAs in synthetic biology.

Team WHU-China: Master of Regulation: dcas9-based Multi-stage Gene Expression Regulator

Cas9 is an RNA-guided dsDNA nuclease utilized by bacteria immune system. The genetically engineered Cas9 has recently been shown to have the ability to repress or activate desired gene expression. </p>In practical research and industrial application, we usually face the problem to express a gene at different levels, not only “on” or “off ”, so a more flexible regulation method is needed. To achieve multi-stage regulation of target genes, we further develop several dCas9 devices in which dCas9 alone or fused with omega subunit of RNAP is directed by various guide RNAs to different regions of designed double promoters. Therefore, promoters with disparate strength can be either activated or repressed respectively and multi-stage gene expression can be achieved. Also, based on such novel technology platform, we are developing diverse applications such as a guide RNA-mediated oscillator.

Team XMU-China: A SynBio Oscillation Signal Converter

Oscillations permeate every corner of the world, from the alternative current AC in power lines to our tiny microorganism friends. To use oscillations in bacteria as a strong and steady signal transmission method like AC, we need to tackle with the noise of transcription and translation in the cellular environment by coupling millions of cells through the synchronizing genetic oscillations in E.coli. At the colony level cells could be synchronized via quorum sensing, which is limited to tens of micrometers by the AHL, and between colonies a gas-phase redox (mainly H2O2) will serve as a signal that can give positive feedback to the whole circuit over millimeter scales simultaneously. On a liquid crystal display (LCD)-like microfluidic array bacteria grow in separate colonies, so that synchronization in both levels could be verified visually. Now a robust synthetic biology signal converter is accomplished and ready to show the growth environment of cells.

Team XMU Software: Biobrick evaluation and optimization software suit and lab

assistant tool

The biobrick evaluation and optimization software tool suit (Brick Worker) provide analysis of biobrick sequences, namely, promoter, RBS, protein coding sequence and terminator. We use PWM algorithm to evaluate the relative strength of promoters and RBS and precisely locate the key region of the sequence that affect its performance. Through codon optimization and GA algorithm our program can analyze and then optimize the protein coding sequence so as to enhance the protein expression level. Terminator efficiency prediction is also included in this suit. As for the lab assistant tool (E'Note), it is a powerful experimental recording platform with exhilarating functions such as multi-line operating, software tool integration and template customization, providing a all-round as well as customized tool to significantly enhance the efficiency of experimental work.

Team ZJU-China: A Tale of Aptamers: Ghost and Elf

This year we aim to utilize aptamer to specifically detect and clear molecules of different sizes. In order to detect and clear certain protein, we make tunneled E.coli called bacterial ghost that allow protein to diffuse in. We then build two types of inner-membrane protein scaffold, which will dimerize when pulled together by two aptamers attached to two sites of the protein. The dimerized proteins have enzymatic activity that can be detected via commercial test strips. The device will also sequester the proteins and allow us to clear them. In order to efficiently detect and clear a small molecule called atrazine, which is an herbicide causing tremendous environmental problems, we split our aptamer-based detection module and clear module into two strains. The first strain is chemotaxic to atrazine and will release quorum sensingmolecules to attract the second strain, which contains atrazine hydrolase to clear it.

EUROPE

Team AMU-Poznan: sh-miR designer - tool for construction of RNA interference reagents: sh-miRs

sh-miR Designer will be a software aimed at fast and efficient design of effective RNA interference (RNAi) reagents - sh-miRs, also known as artificial miRNAs. sh-miRs are RNA particles whose structure is based on miRNA precursor pri-miRNA, but sequence interacting with transcript is changed depending on research purpose. Maintenance of structure of pri-miRNA is very important to enable cellular processing and therefore ensure functionality of artificial particles. sh-miRs delivered to cells on genetic vectors- plasmids or viral vectors - enter natural RNAi pathway and silence target mRNA. They can be used in genetic therapies and basic biomedical research.

Team ATOMS-Turkiye: Project Oncoli

According to the World Cancer Research Fund, the estimated number of cancer cases around the world every year is 12.7 million and is expected to increase up to 21 million by the year 2030. Taking this widely popular and alarming obstacle into attention, we have devised a system which is aiming to tackle cancer from a very different perspective to before. Our choice of bacteria Nissle 1917, a probiotic strain of Escherichia Coli, once inside the body will secrete a cancer tracing protein which recognizes and builds up around the cancer cells. Using the quorum sensing system, E.coli Nissle 1917 detects the bacteria inducing substance AI-2 produced by the tracing proteins. Nissle 1917 bacteria motion towards the region of AI-2 and once in the region, produce our cancer killing protein called apoptin. Apoptin enters the cancer cells

and induces apoptosis thereby eliminating their existence.

Team Baskent Meds: Killing Legionella pneumophila Softly

Legionella pneumophila is the cause of the Legionnaires' disease which is a type of pneumonia. The bacterium is found in warm water environments, particularly in artificial water supply systems such as air conditioning systems and cooling towers. The enfection occures by inhalation by small droplets of contaminated water. Our aim, as the team “Baskent_Meds”, is developing bacteria which can recognize Legionella pneumophila specifically at species level by legionella quorum sensing, and respond by producing anti-Legionella peptide which is produced by some Staphylococcus strains. Quarum means “minimum”. Legionella pneumophila should sense the minimum amount of cells around to colonize in the environment and express its virulence. So our modified E. coli may sense the presence of Legionella pneumophila in any contaminated surface and kill it.

Team BGU Israel: P.A.S.E. - Programmable Autonomous Self Elimination

Bioremediation and biosensors often require the release of genetically modified organisms (GMOs) to the environment. After being released, these GMOs are no longer under direct control. As their effect on the environment is unknown, they pose a potential threat. In order to eliminate this threat, we are developing a genetic circuit, using e. coli as a model GMO, that limits the lifetime of a bacterial population after it is released to the environment. Our goal is to allow the end user to program a GMO population to survive in the environment until it has completed its task, after which the entire population will disappear without any further external intervention. We employ two approaches to achieve this goal: One relies on the dilution of a synthetic control element through cell division, and the second is based on the lifetime of an essential proteincontaining an unnatural amino acid.

Team Bielefeld-Germany: Ecolectricity – currently available

There is a growing interest in the use of ecologically friendly alternative energy sources because of the depletion of fossil fuels and an increasing environmental pollution. Therefore, we are developing a Microbial Fuel Cell (MFC). The goal of this project is to generate electricity with a modified Escherichia coli in a self-constructed fuel cell. Besides the technical optimization of the fuel cell, we investigate different genetic approaches like integrating porines and cytochromes as well as endogenous mediators. Using heterologous expression of pore-forming transmembrane proteins, we are able to enhance the extracellular electron transfer, leading to higher membrane permeability. Direct electron transfer can be achieved by integrating cytochromes into the cellular membrane, whereas a production of endogenous mediators enhances the electron transport to the electrode. With different aspects for technical and genetic optimization we enable Ecolectricity, the use of E. coli for direct energy production.

Team Bonn: LOV Wars - May the light be with you

A reliable, yet easily adaptable mechanism for controlling protein activity is key to most areas of life and medical science research. Still, the most common approaches suffer from various flaws. iGEM Bonn 2013 aims to overcome these drawbacks by engineering a novel tool based on blue light-inducible degradation of targeted proteins.</br> The use of a modified ClpXP protease system allows a significant increase in rate and scale of activity change while keeping the modification of the target protein to a minimum. Combining this system with a tool for photo-activatable heterodimerisation based on a LOV domain results in a superior tempero-spatial control.</br> To demonstrate the capabilities of our device, we designed a photosensitive kill-switch. This contributes to the security of synthetic biology in such a way that

bacteriaaccidentally brought out of a safe work environment, for example a red-light-hood, would be killed by sunlight within a short period of time.

Team Bordeaux: The Dairy Planet

The economical stakes of food-processing industry have always been a concern in society. Technological innovations have improved the yield and production costs of daily use products. Advances in health sector and biotechnology made it possible to offer food products rich in substances that are nutritious and possess medicinal properties. Our project aims at producing a new range of lactic cultures able to produce natural flavours and colouring substances in a yogurt; including ones producing resveratrol, a molecule responsible for the red wine beneficial effects, implicated in the 'French paradox". Necessary routes of biosynthesis will be introduced in Lactobacillus bulgaricus and Streptococcus thermophilus, agents of lactic fermentation. Thus, a work of optimization on the genetical modifications of lactic bacteria has been done. This project will allow an easier production of custom yogurts with beneficial and healing properties, avoiding the use of substances derived from expensive chemical synthesis harmful to the environment.

Team Braunschweig: Engineering synthetic microbial consortia

Bacterial consortia offer a great benefit for synthetic biology due to the ability to perform complex tasks by splitting the whole reaction into smaller reactions and share the task among different specialized strains. Also, a self-regulating bacterial culture with intra consortial dependencies offers great advances in biosafety. To shut down the whole bacterial consortium, only on strain has to be eliminated. We engineer three different E. coli strains to grow in a consortium exploiting different Quorum Sensingsystems. Each strain maintains a constitutive expression of an inactive transcription activator (LuxR, LasR or RhlR). Inducers are synthesized by different synthases (LuxI, LasI or RhlI) that are each expressed in one strain and subsequently secreted into the medium. Once taken up by a cell, the inducers bind to the corresponding, inactive transcription factors to render them functional. As a result, an antibiotic resistance under the control of an inducible promoter is expressed.

Team DTU-Denmark: Requiem for a Stream: From Ammonia Pollution to Energy Production via Denitrification

Global demand for fixed nitrogen has increased to the point that half the human population now relies on chemical fertilizer to grow their food. While fertilizer is a requirement for modern life, runoff from over-fertilized farmland can cause eutrophication. In the presence of abundant ammonia, algae overgrow and consume much of the available oxygen in the water. This results in decreased biodiversity throughout the watershed. Within Europe, 53% of lakes are eutrophic. Using two E. coli mutants built with genes from Nitrosomonas europaea and Pseudomonas aeruginosa, we provide a system to reverse nitrogen fixation. Our mutants consume ammonia and produce nitrous oxide, and release a sustainable source of energy when decomposed into nitrogen and oxygen. We also provide a prototype of a bioreactor that could be scaled up and deployed in the field to simultaneously clean the water and produce energy.

Team Dundee: ToxiMop

The ToxiMop project attempts to tackle the problem of freshwater algal blooms by detecting, reducing, and reporting the levels of the algal toxin microcystin. This toxin causes liver damage and is also speculated to be a carcinogen. Microcystin's toxic action lies in its ability to bind to the human Protein Phosphatase 1 (PP1), which is a major regulator of cell division, protein synthesis and other essential processes. Using synthetic biology techniques, we engineered bacterial chassis (E. coli and B. subtilis) to express PP1,

which covalently binds to microcystin. The engineered bacteria can then be used as a molecular mop, the ToxiMop, to remove microcystin from contaminated water. Applying mathematical modelling to our experiments, we optimised our prototype ToxiMop. Additionally, we attempted to develop a biological detector for microcystin, which was combined with our electronic device, the Moptopus. This device has the potential for real-time monitoring and analysis of water bodies.

Team Edinburgh: WastED

The Edinburgh iGEM 2013 team, WastED, is focusing on remediation and valorization of industrial waste streams, with a particular focus on Scottish leather and whisky industry waste waters, containing toxic heavy metal ions as well as fermentable organic components. Using Bacillus subtilis as chassis, we are engineering organisms to capture ions using chelators and metal binding proteins, and to ferment organic components to produce biofuels. We are also testing a new assembly procedure, GenBrick, based on the Genabler assembly system. GenBrick allows assembly of multiple RFC10-compatible BioBricks in a single reaction, and is also well suited to the preparation of fusion proteins and addition of terminal tags. Enzyme fusions may enhance metabolic pathways through substrate channeling. We are testing the effect of protein fusions on fermentation efficiency for biofuel production. In addition, we are examining the implications of possible Scottish independence, following the 2014 referendum, for synthetic biology in Scotland.

Team EPF Lausanne: Taxi.Coli: smart drug delivery

EPF_Lausanne's team is proud to participate to iGEM 2013 and excited to present their project: Taxi.Coli: smart drug delivery. The team's vision is to build a biosynthetic drug delivery concept. The key word of this project is "adaptability". Our goal is to explore a way of using E.Coli as a highly modular carrier, opening the gate to several applications and alternatives in disease treatments. Using the principles of synthetic biology, we engineered a gelatinase secreting E. Coli able to bind gelatin nanoparticles using a biotin-streptavidin interaction and release them in a corresponding location. The drug delivery system is built in three parts: 1) the nanoparticle binding and 2) the environment sensing that 3) triggers the gelatinase release of the engineered E. Coli, liberating the content of the nanoparticle. The nanoparticles made of gelatin are able to carry any type of organic compound leading to a wide range of applications.

Team ETH Zurich: Colisweeper: The world's first biological Minesweeper game

Colisweeper is an interactive, biological version of the Minesweeper computer game, based on luxI/luxR quorum sensing and chromogenic enzymatic reactions. The goal is to clear an agar "minefield" without detonating mines. Genetically engineered Escherichia coli colonies are used as sender-cells (mines) and receiver-cells (non-mines). Mines secrete the signaling molecule N-(3-oxohexanoyl)-l-homoserine lactone (OHHL) whereas non-mines process the signal. To distinguish between OHHL-levels, a library of PLuxR promoters with various sensitivities was created through site-saturation mutagenesis. High-pass filters were constructed to control the expression of different orthogonal hydrolases in non-mines, depending on the number of surrounding mines. Additionally, the mines express their own hydrolase. A spatiotemporal reaction-diffusion model was established to evaluate and improve the system. To play Colisweeper, a colorless substrate solution is pipetted onto a colony of choice. The result is a defined color change within minutes, allowing identification of the played colony and the number of mines surrounding it.

Team Evry: Iron coli Project

This year, our project focuses on diseases that are subsequent to an iron overload such as hemochromatosis and thalassemia. </p> Nowadays, iron overload is mainly treated by bloodlettings for

hemochromatosic patients but this treatment cannot be extended to thalassemic patients who suffer from anaemia. The aim of our project is to prevent the intestinal absorption of iron by engineering Escherichia coli to produce siderophores, chelators of iron. This strategy acts directly at the source. </p> We engineer Escherichia coli using the Ferric Uptake Regulation (FUR) couple to an inverter system, in order to produce these siderophores in presence of iron. To reduce the patient's iron absorption, our bacteria is encapsulated in a pill. Once it arrives in the duodenum, our bacteria will produce the siderophore at their full potential and chelate the iron. </p>

Team Exeter: Paint by coli: Creating a Colour Bio-camera Using Escherichia coli via complete optical control

Synthetic biology has lead to microorganisms being pushed into an unprecedented range of novel functions. Many bacterial systems currently rely on external stimuli to induce transcription. One dimensional protocols often require constant monitoring of applied chemical concentrations, leading to them becoming inept for more complex systems. A triplet of NOT gated photoreceptors in Escherichia coli, will be used to create a system which is finely controlled using only light. This will be showcased using magenta, cyan and yellow pigments as outputs. Varying the intensity and wavelength of light projected onto E. coli will control the shade and colour produced, respectively. Hence, this will show the versatility of the optical control by creating a full colour bio-camera. Additionally, using bacteria to produce an image vastly increases the resolution when compared to conventional cameras, due to the micrometre scale of bacteria.

Team Frankfurt: Steviomyces - sweeter than sugar

The Stevia plant produces several sweeteners known as Steviolglycosides which have only recently been admitted as a foodadditive in the European Union. The iGEM-Team Frankfurt 2013 searches for ways to transfer the pathway of the plant into Saccharomyces cerevisiae in order to make stevia production possible with both lower effort and lower costs. Several of known problems with carbohydratesweeteners like diabetes or caries could be overcome by the Steviolglycosides which are produced by Stevia rebaudiana. We're building upon results gained from last year's competition which gave us the possibility to transfer a mevalonat plasmid into yeast to increase the production of a steviol-precursor Geranylgeranyl-diphosphate. This year we're searching for a further reconstruction of the pathway and transfering the 2nd plasmid for synthesis of Steviol from Geranylgeranyl-diphosphate into yeast. Thus the whole pathway can take place in a microbial organism and easify the production by lowering the costs.

Team Freiburg: uniCAS - The Toolkit for Gene Regulation

Our Team developed a universal toolkit, termed uniCAS, that enables customizable gene regulation in mammalian cells. Therefore, we engineered the recently discovered and highly promising CRISPR/CAS9 system. The regulation is based on the RNA-guided CAS9 protein, which allows targeting of specific DNA sequences. Our toolkit comprises not only a standardized CAS9 protein, but also different effector domains for efficient gene activation or repression. We further engineered a modular RNAplasmid for easy implementation of RNA guide sequences. As an additional feature, we established an innovative screening method for assessing the functionality of our uniCAS fusion proteins. Single genes and even whole genetic networks can be modified using our uniCAS toolkit. We think that our toolbox of standardized parts of the CRISPR/CAS9 system offers broad application in research fields such as tissue engineering, stem cell reprogramming and fundamental research.

Team Gdansk-UG: MetOli

The aim of our project was to construct a biological system that would be able to detect methanol in ethanol solutions. Our idea was to create a test that could be performed not only in the laboratory, but also at home. We believe that such test would reduce the rate of intoxications by methanol during ethanol consumption. To achieve it, we used a methanol-dependent promoter from Methylobacterium organophilum which would control the production of a dye, for instance GFP, or an enzyme that would produce visible product, such as catechol oxidase. Our eventual goal is to find a bacterium that would not only react to methanol, but also survive in high concentrations of ethanol.

Team Goettingen: The beast and its Achilles heel: A novel target to fight multi-resistant bacteria

Since the discovery of penicillin by Alexander Fleming in 1928, antibiotics have marked a major victory of mankind in the battle against infectious diseases. However, after 90 years, the antibiotics are now losing their old time glory: Bacteria acquire resistance against antibiotics and become unbridled. We must control the use of antibiotics, meanwhile, we need new antibiotics, which can suffiently eliminate the invaders without hurting the 'good' bacteria. Therefore, c-di-AMP, an important, recently discovered signaling molecule in gram-positive bacteria, has come to our sight. Our project is to build a screening system targeting c-di-AMP, which could be applied in novel-drug screening. With this system, the level of c-di-AMP in the cell can be visualised and measured.

Team Grenoble-EMSE-LSU: Light Automated Cell Control by Talk'E. coli

Maintaining cell growth state during culturing is generally difficult due to metabolic adaptation and changing cell division rates. Using light-induced promoters and a phototoxic fluorescent protein, we've designed Talk'E. coli. It uses light signals to communicate with bacteria allowing the researcher to remotely control the cultures using a computer. Cell density is monitored through fluorescence recordings and, thanks to a predictive model, Talk'E. coli responds by illuminating the culture with one or more wavelengths to obtain different effects: killing off cells beyond a threshold density, or producing or degrading protein. The tool is portable and mountable in an incubator making it a handy device for research.

Team Groningen: Engineering Bacillus subtilis to self-assemble into a biofilm that coats medical implants with spider silk.

Approximately half of all implanted medical devices result in one or more medical complication, which have been found to increase mortality rates by 25%, and to cost the amerian society an additional 30 billion dollars every year. A possible solution for these complications is to form a protective biocompatible layer between the implant and the body by means of a spider silk coating. This is achieved through mathematical modelling, techniques from the synthetic biology, and the Gram-positive bacteria Bacillus subtilis, which is redesigned to secrete silk and to self-assemble into a biofilm surrounding the implant. It uses a modified chemotaxis system coupled to the DesK heat sensing system to do so. B. subtilis is furthermore often used in the industry for the commercial production of extracellular proteins, and is generally regarded as safe.

Team Heidelberg: THE PHILOSOPHER'S STONE

Several secondary metabolites, such as commonly used antibiotics, pigments and detoxifying enzymes, are synthesized by non-ribosomal peptide synthetases (NRPSs). These enzymes beautifully reflect one of the fundamental principles of synthetic biology, as they are remarkably modular. We will assemble new NRPSs by combining individual domains and modules of different origin, thus setting the basis for novel and customized synthesis of non-ribosomal peptides. To make the use of NRPSs amenable to a wider

community, we will devise a new software-tool, called “NRPS Designer”, which predicts the optimal modular composition of synthetic NRPSs for production of any desired peptide and outputs a cloning strategy based on Gibson assembly. As an application relevant to society, we will engineer Escherichia coli to recycle gold from electronic waste in a cost- and energy-efficient way through the heterologous expression of the NRPS pathway of Delftia acidovorans that naturally enables precipitation of gold ions from solution.

Team Imperial College: Plasticity: Engineering microbes to make environmentally friendly plastics from non-recyclable waste

Accumulation of waste represents a considerable problem to humanity. Over the next 50 years, the global community will produce approximately 2 trillion tonnes of waste, or 2.5 times the weight of Mount Everest. Traditionally, mixed non-recyclable waste is sent to landfill or for incineration, both of which result in environmental damage. The detrimental effects are perpetrated by the plastic degradation into toxic byproducts and the production of greenhouse gases by these processes. As an alternative we propose to upcycle this mixed waste into the bioplastic poly-3-hydroxybutyrate (P3HB) to create a closed loop recycling system. Our engineered E. coli will operate within sealed bioreactors. In the future we picture the use of our system in a variety of contexts as part of our M.A.P.L.E. (Modular And Plastic Looping E.coli) system.

Team INSA Toulouse: E. calculus Project

The E. calculus project consists constructing a full n-bits adder capable of transmitting a carry to the next step. The designed strain contains specific devices that should ensure a relatively precise calculation and will be decomposed as follows:

- Various logic gates using specially designed recombinases and recombination sites to avoid reversibility of the gates states.

- A strict control of the expression of recombinases via a tight riboregulation control of the translation of recombinases genes

- A general inducer, switching the strain from inactive to active counting.

- A carry system based on the diffusion of a messenger molecule to the second bit.

- An artificial input system based on photoreceptors sensible to blue and red lights.

The envisioned system should approach as much possible the reliability of an electronic two digit device and may help the Synthetic Biology community designing strong and robust Genetic Boolean Operators.

Team ITU MOBGAM Turkey: Intrinsic Factor-y

Pernicious anemia is described first by James S. Combe in 1822. Pernicious anemia is a type of anemia occurs due to malabsorption of vitamin B12 in the small intestine due to problems with the production of Intrinsic Factor, which is responsible for the absorption of vitamine B12. Pernicious anemia shows its stiking effects on blood, gastro-intestinal tract and nervous system and pernicious anemia usually develops together with an autoimmune disease. Our aim as ITU MOBGAM IGEM Team, is to design a bacterium that is capable of surviving in small intestine and secreting Intrinsic Factor dependent on pH. Also, we design a genetic circuit for controlling the overgrowth and containment of bacteria.

Team Kent: No to NO: A novel approach to reduce greenhouse gas

In today’s rapidly changing environment greenhouse gases such as NO are an issue that need to be

addressed. NO has been proven to have a detrimental impact on the environment and iGem Team Kent 2013 will provide a solution that focuses on reducing the amount of NO formed in waste water. Our system will utilise an engineered strain of E. coli which will be capable of converting this excess NO into ammonia. Our Biobricks have been designed to enable the detection of NO using the norV promoter. The NO can then be converted into ammonia via the nitrite reductase enzyme encoded by the E. coli gene NrfA. Our solution will have many advantages over the current approaches to waste water treatment such as reduced cost and risk of contamination. Our system will provide a source of recycled ammonia and could be a greener alternative to the Haber Bosch process.

Team KU Leuven: E. coligy: Plants with BanAphids

Aphids, the little green plant-sucking bugs, can pose serious threats to a farmer's proceeds. Not only physical damage to the crops caused by the sucking is a problem, but aphids also transmit harmful viruses to the plants. The magnitude of loss is difficult to quantify as it changes with aphid species, crop species, location, year and other factors. The use of insecticides to control aphid population is contested, as it has a negative effect on the natural predators and aphids grow resistant. That's why we, the KU Leuven iGEM 2013 team, decided to do something about it in a sustainable way, using an insecticide-free controlling mechanism. With E.coligy: Plants with BanAphids we will teach E.coli cells to hack into insects signaling systems to drive off the aphids and attract the natural predators, such as the ladybug.

Team Leeds: The Micro-beagle - A living biosensor

Micro-Beagle is a novel reporter system for E-coli that, as an iGEM first, has been designed to dynamically detect arbitrary target solids (including other cells) through a mechanism activated by cell surface binding. Micro-Beagle is a modular system, utilising Ice Nucleation Protein to express and position target-binding peptides on the cell surface. Target binding induces membrane stress that activates the Cpx signalling pathway, and Micro-Beagle thus utilises a promoter from this pathway (pCpxR) to initiate expression of a reporter protein, such as GFP. As a proof of concept, we have used silica beads as a model diagnostic target (a pathogen surrogate) and the silica-binding “Si4” sequence as the target-binding peptide. We foresee Micro-Beagle being adapted for both the detection of waterborne pathogens and a variety of other diagnostic applications, and we envision future multisensor Micro-Beagles in which diverse pathogens can be simultaneously and quantitatively measured from a single water sample.

Team Leicester: Biological routes to recycling, re-using and re-purposing polystyrene

Polystyrene is a useful material, but also a visible pollutant that locks up oil-derived hydrocarbons. For 2013 we are diversifying, to reduce polystyrene’s various environmental impacts: Recycling - Building on 2012’s project, we are adapting the toluene degradation pathway from Pseudomonas species to work on polystyrene, in E. coli.</p> Re-using - Consumer 3D printers use a variety of thermoplastics but virgin plastic is usually required. Recycled polystyrene can be a support for making complex 3D shapes, and removed later. Polystyrene is soluble in limonene (an environmentally friendly solvent) so we are adapting limonene biosynthesis biobricks, to enable biological 'finishing' of 3D printed objects.</p> Re-purposing - Polystyrene is a great building insulator, but needs to be flame retardant. Currently this involves adding halogenated hydrocarbons, proven environmental pollutants. Recently DNA was shown to be an effective flame retardant, so we are using synthetic biology to generate cheap DNA, for flame retardant polystyrene.</p>

Team Linkoping Sweden: A novel immunochemical detection system for food

allergens.

Antibodies are usefull for recognition of antigens in food. Antibodies have, however, a very complex structure that is not suitable for expression in E. Coli. The Camelid antibody IgG (cIgG), however, has lower complexity than the Human IgG. We present a new approach for recognition of food allergens with a synthetizised cIgG for expression in E. Coli. The epitope of cIgG is designed for Hen Egg White Lysosyme (HEWL). The cIgG is designed with a linker that connects to the bioluminescent enzyme Luciferase. We also synthetizised an HEWL antigen carrying the protein RFP, A-HRFP, that reacts to the luminescence of luciferase as the A-HRFP attaches to the cIgG. The recognition of HEWL in a sample leads to the release of luminescent green-light as a result of HEWL binding to the cIgG. If, however, no HEWL antigen is present in the sample, A-HRFP binds to cIgG resulting in a luminescent red-shift.

Team Manchester: E. c(oil)i; The Lean, Green, Fat-Producing SynBio Machine

From food products, to cosmetics and biodiesel, palm oil is the world's most widely used vegetable oil. Its demand is ever increasing; however the current method of extracting palm oil is severely unsustainable. Massive deforestation is required to build oil palm plantations, ruining the land of locals in Malaysia and Indonesia. Manchester iGEM aims to combat this by providing a more eco-friendly source of the four main components of palm oil. We reengineered the fatty acid biosynthesis pathway of E. coli to overproduce palmitic and stearic acid and introduced two new genes, desaturase and desaturase, to yield oleic and linoleic acid. To explore the scale-up potential of synthetic palm oil production in E. coli, we developed a fully parameterised kinetic model of the engineered fatty acid biosynthesis pathway.

Team Marburg: Phaectory

The diatom Phaeodactylum tricornutum is a widely spread organism in marine waters. It belongs to the group of diatoms. As a group of great ecological relevance diatoms are responsible for up to 20% of the global CO2 fixation and generate about 40 % of the marine biomass of primary producers. In addition, diatoms represent an important source of lipids and silicate making them interesting for various biotechnological applications e.g. in biofuel industry, food industry and nanofabrication. Furthermore, a relatively easy biolistic method for transfection is established. A simple cultivation eases a putative industrial use of the diatom. Former researches not only proved a possible expression of antibodies, bioplastic and other recombinant proteins, but also demonstrated a direct secretion of the expressed proteins in the outer medium, making it easier to filter the wanted proteins. These characteristics make P. tricornutum an interesting organism for putative industrial use.

Team METU Turkey: Bee subtilis

Taking a major role in pollination, bees are one of the most important organisms within an ecosystem. However their populations are in serious decline. Colony Collapse Disorder has been found as the most common cause of the disappearance of bees in large numbers. In this study, we aimed decrease the number of hives affected by chemical compounds such as imidacloprid.Our plan is to turn the mutualistic bacteria living in bees' guts into a shield mechanism to protect the bees against these factors. A protein CYP6G1 found in Drosophilia melanogaster has the ability to degrade imidacloprid into harmless substances. Moreover, coumaric acid increases the general immunity of bees against harmful components and we aim to increase the level of coumaric acid in bees' guts. The main objective of this study is the transformation of the genes coding for these two proteins to Bacillus subtilis, which mutualistically live in bees' guts.

Team Newcastle: L-forms: Bacteria without a cell wall - a novel chassis for synthetic biology

L-forms are bacterial without cell walls that are still able to divide without the normally essential cell division machinery. The lack of a cell wall imparts a range of interesting properties and we show that L-forms can be used as a novel chassis for a range of fundamental applications in synthetic biology. We produced a BioBrick for Bacillus subtilis, that allows cell morphology to be toggled from normal to L-form. We have explored some of the interesting opportunities that L-forms provide including cell fusion, genome shuffling and the generation of differently shaped cells using microfluidics. L-forms are thought to exist naturally within plant tissues and we also studied their use as agents for delivering novel functionality into plants. For project outreach, we created a game as an Android application and considered the implications raised by our project and also look at the exciting relationship between synthetic biology and architecture.

Team NRP-UEA-Norwich: Developing Biosensors to Identify Antimycin-Producing Actinomycetes

Antimycins, anti-fungal compounds primarily produced by Streptomyces (a sub-set of actinomycetes), function by inhibiting the final stage of the electron transport chain. Our aim is to develop Biosensors to aid identification of novel antimycin-producing actinomycetes. Homologues of the AntA sigma factor, the key regulatory protein in antimycin biosynthesis, are present in all 14 known biosynthetic gene clusters. Due to this property, Biosensors have been designed with the AntA-regulated promoter(antGp) controlling the expression of three reporters: neomycin resistance gene, RFP (red fluorescent protein) and GUS (providing β-glucoronidase activity). The Biosensors will be produced, trialled and optimised where possible after sub-cloning into two actinomycete-specific integrative plasmids, pMS82 (BT1 integrase) and pAU3-45 (C31 integrase). Worldwide soil and sediment samples have been collected to produce a library of actinomycete strains, which will be screened using our Biosensors, the ultimate goal being to screen bacterial strains for antimycin production.

Team NTNU-Trondheim: VesiColi

Gram negative bacteria produce outer membrane vesicles (OMV) in the size range of 20-200nm. Whereas their function and contents has been studied for decades, their potential as drug carriers has not been investigated before. We want to introduce protein G from Streptococcus dysgalactiae subsp. equisimilis into Escherichia coli OMV's. Protein G is known to bind to human serum albumin (HSA) which helps S. dysgalactiae subsp. equisimilis hide from the immune system.

The second part of our project is to introduce fluorescent proteins (FP's) linked together into the vesicles. Introducing protein G and linked FP's into the vesicles will demonstrate that it is indeed possible to manipulate the content, and therefore the properties, of OMV's.

Team Paris Bettencourt: Fight Tuberculosis with Modern Weapons!

We are testing new weapons for the global war against Mycobacterium tuberculosis (MTb), a pathogen that infects nearly 2 billion people. Our 4 synergistic projects aim to help in the prevention, diagnosis, and treatment of tuberculosis. 1) We are reproducing an essential MTb metabolic pathway in E. coli, where it can be easily and safely targeted in a drug screen. 2) We are building a phage-based biosensor to allow the rapid diagnosis specifically drug-resistant MTb strains. 3) We are constructing a mycobacteriophage to detect and counterselect drug-resistant Mtb in the environment. 4) We are programming E. coli to follow

MTb into human macrophages and saturate it with bacteriolytic enzymes. We want to vanquish tuberculosis and build a TB-free world.

Team Paris Saclay: PCBbusters

PCBs (Polychlorobiphenyls) are synthetic chemicals widely used during the late 20th century. These compounds are extraordinarily stable, not readily biodegradable and have accumulated in the environment. PCBs also accumulate in animal fatty tissues including human tissues. As PCBs are probably carcinogenic and some are endocrine disruptors, they constitute an important health issue. Although PCBs have no natural equivalents, some bacterial communities have developed the capacity to degrade PCBs. Highly chlorinated PCBs undergo anaerobic reductive dechlorination, lowering the chlorine atom number. Lightly chlorinated PCBs are then degraded via the aerobic biphenyl degradation pathway. Our project is to construct an Escherichia coli strain capable of degrading PCBs by introducing in the strain genes involved in PCB degradation in various bacteria. Because some steps are anaerobic and others aerobic, we want to use an oxygen-based regulation of gene expression. We also want to develop a sensor system to detect PCBs in the environment.

Team Poznan-BioInf: SR-MUX: a biological multiplexer with 3-bit editable transcriptional memory.

Our goal is to engineer a device allowing to save up to three binary input signals in living E. coli cells, resulting in expression of red, blue and green fluorescent proteins as reporters. Converting inducer signals into expression of serine recombinases, enzymes capable of specific DNA editing, we are able to create three transcriptional analogues of transistors - transcriptors - and to use them as elemental memory units called SR-latches under control of a fourth, strobe signal, providing a mean to reset the system to its original state. This complex biological memory unit opens the way to cheap, reversible gene induction, useful both to the industry and researchers, not only lowering inducing cost but also being less stressful for the studied organisms, e.g. plants. It is also another step towards Von Neumann-inspired biocomputers.

Team SDU-Denmark: Bacteriorganic Rubber

The growing demand for natural rubber causes deforestation of the rainforest or occupation of arable lands, all due to the founding of new plantations. If producing rubber by bacteria succeeds, production of natural rubber will not be limited to the regions where the rubber tree can grow. Our project aims to make an E. coli strain able to produce natural rubber while grown under controlled conditions. Natural rubber is composed of polymerized IPP (isopentenyl pyrophoshate) units. E. coli already possesses the ability to produce IPP, but it lacks the polymerization enzyme, prenyltransferase, from the rubber tree. In this project we introduce prenyltransferase into E. coli and simultaneously manipulate the bacteria to produce more of the IPP links, consequently leading to the production of natural rubber in the bacterial setting.

Team TU-Delft: Peptidor: Detection and killing of resistant S. aureus using antimicrobial peptides

Methicillin-Resistant Staphylococcus aureus causes major problems, especially in hospitals, leading to over half a million infections annually in the US alone. Of the alternative treatments currently under investigation one of the more promising is through antimicrobial peptides (AMPs). These small, highly-specific peptides attack the membrane of target organisms. Thousands of AMPs are known to exist and little resistance against them has been developed. The Peptidor project consists of an E. colithat can detect S. aureus, using S. aureus' native quorum sensing system, in order to locally produce and deliver AMPs. Upon

detection, peptides inactivated by a SUMO-tag fusion, are overexpressed. After a delay period, introduced through a negative transcriptional cascade, a SUMO protease is expressed cleaving off the inactivating tag. Using this mechanism, high concentrations of peptide are delivered at the infection to efficiently kill S. aureus. As a safety mechanism, the timer also activates an E. coli kill-switch.

Team TU-Eindhoven: MRiGEM: Creating a production and delivery system for a CEST MRI contrast agent

Our project presents an alternative solution to the use of heavy metals MRI contrast agents by focusing on CEST MRI. Within CEST imaging, proteins enclosing hydrogen atoms generate high quality images. We use Escherichia coli to create CEST proteins when the bacteria sense a hypoxic environment due to a promoter designed for this purpose, thus working as a production and delivery system for the CEST MRI contrast agent. Hypoxic regions are related to tumors, therefore our eventual goal is to use this device to target and image tumors in humans by injecting the bacteria into the bloodstream. A second application is tracking bacteria in bacterial infections studies. For the iGEM competition however, the proteins are only expressed ex-vivo: in aerobic and anaerobic conditions. We aim to achieve an efficient testing of the CEST properties of the proteins and confirm the promoter's ability to express each protein.

Team TU-Munich: PhyscoFilter – Clean different

The contamination of aquatic ecosystems with multiple anthropogenic pollutants has become a problem since the industrial revolution. Antibiotics, hormones and various noxious substances threaten environmental health and are not effectively removed by conventional waste water treatment. We propose to employ transgenic plants which produce effectors for enzymatic degradation (BioDegradation) or specific binding (BioAccumulation) of pollutants. The autotrophic, sedentary, aquatic nature of the moss Physcomitrella patens makes it an ideal chassis for a self-renewing, low-maintenance and cheap water filter. A light-triggered kill switch prevents unintended environmental spreading by limiting viability to places where the spectrum of sun light is appropriately filtered. Furthermore, we have developed a device to implement this biological filter in an aquatic environment, investigated the application of this new technology and examined its economic feasibility. Based on our results, PhyscoFilter may become a game-changing approach to improve global water quality in an affordable and sustainable fashion.

Team Tuebingen: Tuebingen Yeast Based Progestin Measurement System

Detrimental alterations caused to water bodies by endocrine disruptive chemicals are an increasing problem in our environment. Especially steroid hormones influence the development and generative behavior of fish. The binding of those hormones to progestogen receptors can mistime the reproductive behavior of aquatic organisms and thereby endanger population balance. Our aim is to construct a yeast-based measurement system for progestin concentration in water samples. Many currently used methods are either very expensive or significantly slower than our method will be. We take advantage of membrane bound receptors in order to achieve high specificity and to speed up measurement. The binding of the ligand to the receptor stops inhibition of the reporter and thereby initiates its expression through a sensitive signaling-chain. This transcriptional switch allows measurement of very small amounts of substrate. To improve our system we use different interchangeable parts for assembly to get a high variety of possible applications.

Team TU Darmstadt: Hunting Fungi

The danger of fungal contamination of grains and cereals but also other food sources has severe

consequences. Undetected contaminations can render large quantities of food stocks useless – with detrimental effects on the economy and the food supply. We want to develop a handy device which allows an easy, fast and reliable detection of mycotoxins. For that our team uses various methods from the fields of synthetic biology, electrical engineering and information processing. Our system relies on E. coli with modified TAR receptor interacting with specific mycotoxins. If these are present in the sample they induce a conformational change of TAR and thereby generates a measurable FRET-beacon by bringing two fluorophores in close distance to each other. The modified E. coli will be embedded in exchangeable capsules. Together with a handheld-device and a controlling Smartphone App they will guarantee that measurements can be done quickly, easy to operate and secure.

Team UCL: Spotless Mind

This year, the UCL iGEM team is taking a radical new step with synthetic biology. We intend to explore the potential application genetic engineering techniques on the brain, by tackling Alzheimer's disease, which is linked to the presence of amyloid plaques in the brain. Targets for the project include: establishing microglia cells as a new Synthetic Biology chassis and constructing new BioBricks to enable engineered Microglia to detect and destroy disease-associated amyloid plaques.

Team UCL PG: Spectra

Spectra aim to use a novel configuration of synthetic gene networks (SGNs) to drive evolution of a fluorescent protein with dramatically improved spectroscopic properties. In future we intend to use the capabilities this enhanced fluorescent protein will provide to enable better dissection of differentiation pathways in stem cells.

Team UGent: A new model for chromosomal evolution: Eliminating antibiotic resistance

The main goal of industrial biotechnology is to increase the yield of biochemical products using microorganisms as production hosts. This includes engineering large synthetic pathways and improving their expression. Overexpression of genes has hitherto mainly been achieved by using high or medium copy plasmids. However, studies have demonstrated that plasmid-bearing cells lose their productivity fairly quickly as a result of genetic instability. Therefore a new method was developed for the overexpression of a gene of interest in the bacterial chromosome: Chemically Inducible Chromosomal evolution (CIChE). In this technique the chromosome is evolved to contain a higher number of gene copies by adding a chemical inducer. The original model for CIChE, however, results in bacterial strains containing a large number of antibiotic resistance genes. To make this valuable technique more widely applicable in the industry, we developed a model for chromosomal evolution based on a toxin-antitoxin system instead of antibiotic resistance.

Team UNIK Copenhagen: Project Magneto

Project Magneto is a biological system that allows us to find better ways to treat cancer, acts as a sustainable energy source or just enables us to visualize our environment in a new way. We created it using magnetosomes. Thanks to these specialized organelles magnetotactic bacteria are able to navigate in the earth’s magnetic field. The magnetosome is a nanomagnet which consists of a magnetic crystal housed inside a lipid membrane. Magnetosomes arrange together in chains and act as a compass needle thereby orienting the cell. They show various properties that give them an advantage over industrially synthesized nanomagnets. We demonstrate their usability by fusing fluorescent proteins to their

membrane. Through this we open the way for using magnetosomes in various different applications where the fluorescent protein could be simply replaced by a drug for targeted cancer therapy, an ATP-synthase to create a biological dynamo or dye for magnetic paint.

Team UniSalento Lecce: NICKBUSTERS: developing a nickel detection and remediation platform

Nickel is one of the most widespread heavy metals in the ecosystem and, though essential, its excess could be toxic, leading to various noxious effects; nowadays bacteria-mediated bioremediation from inorganic substances seems to be a considerably relevant frontier in microbic biotechnologies. Our project aims to develop a living system in two easy monitorable bacterial platforms who would work as a Nickel detector and a Nickel remediation system. The devices are based on genetic parts from Helicobacter pylori: from the nickel sensing device, H.pylori NikR protein, to the Nickel storage system, Hpn protein, whose role is to store the Nickel ions inside the cell. The two devices are split in two separate populations, which intercommunicate through Quorum Sensing. The system allows to remove the Nickel ions from polluted environmental substrates through bioaccumulation and could be easily implemented in purification plants.

Team UNITN-Trento: B. fruity

B. fruity envisions an environmentally friendly way to control fruit ripening by exploiting an engineered, light regulated strain of B. subtilis. The system works by synthesising ethylene or methyl salicylate (MeSA) upon photoinduction. Everything is housed in a vending machine-like enclosure that regulates fruit ripening in response to consumer demand. Ethylene is a natural plant hormone that is widely used to ripen fruit, such as bananas and kiwi. However, the synthesis, handling, and storage of ethylene is expensive and dangerous. In contrast, B. fruity produces ethylene from inexpensive material by exploiting a TCA cycle intermediate, 2-oxoglutarate, and the activity of P. syringae 2-oxoglutarate decarboxylase. The inhibition of fruit ripening results from the synthesis of MeSA via a pathway built with wintergreen parts. As a proof of concept, we engineered E. coli with the above systems plus the YF1/FixJ blue light receptor device.

Team Uppsala: LactoNutritious

Malnutrition is today a major global problem that affects people both in affluent and developing countries. Even if you get the right amount of calories, if these do not contain sufficient amounts of micronutrients, like vitamins and minerals, serious illness and even death can be the result. The goal of our project is to alleviate this problem by applying synthetic biology to probiotic bacteria. With our project, we will make the lactobacillus genus the new probiotic platform for metabolic engineering of nutritional compounds. We will engineer probiotics to produce for example beta-carotene, resveratrol, p-coumaric acid, miraculin and saffron. To exemplify what this combination of probiotics and metabolic engineering can accomplish we used our modified bacteria to create nutritionally enriched yoghurt. We have also put great effort into addressing the ethical and safety issues that naturally follow when creating GM food.

Team Valencia-CIPF: Project - Freshellent Yeast

Our team will try to develop a project based on the production of aromas and repellents. The aim is to create a biological platform within a model organism, such as common yeast, to develop an alternative method for production of several aromatic monoterpenoids. The advantage of this organism as producer lies in its capabilities of genetic modification, robustness and culture simplicity. We can also control the production of these compounds using different promoters, so we can choose our favourite aroma while there is repellent activity. The microorganism is completely harmless as it is responsible for fermenting

bread and beer. The project aims to establish the basis for future production of repellents in a sustainable and organical manner in developing homes that are under the risk of pandemics caused by mosquitoes and other insects.

Team Valencia Biocampus: Wormboys

Bacteria are essential in biotechnology, but they can hardly move. Nematodes, such a C. elegans, are fast crawling organisms, but they have limited biotechnological applications. By combining the best from both organisms, we present the first artificial synthetic symbiosis with bacteria engineered to ride on worms, which concentrate in hotspots where bacteria perform a desired biotechnological process, such as bioplastic (PHA) production. We have engineered Pseudomas putida with a whole operon that allows the formation of a biofilm on the worm. Biofilm formation is swhitched on and off depending on the media, and thus bacteria get on and off the worm like travellers on a bus. We have also engineered a third partner, E. coli, to express an interference RNA that promotes clumping. Taken together, our artificial symbiosis allows biotechnologically interesting bacteria to travel on nematodes, reach nutrient-rich biomass spots and maximize the efficiency of biotechnological fermentations in heterogenous substrates.

Team Wageningen UR: Aspergillus niGEM: A lov story

The fact that secondary metabolites are often synthesized as polymer backbones that are subsequently diversified greatly via the actions of tailoring enzymes sets the stage for combinatorial biochemistry because their biosynthesis is modular. One of the goals is to establish a modular system of domain shuffling to generate a plethora of novel enzymes with new and improved functionalities. The production of lovastatin, a drug used in lowering LDL cholesterol for patients suffering from cardiovascular disease, has been chosen as a proof of principle. The aim is to transfer the entire lovastatin metabolic pathway from A. terreus into a GRAS organism like Aspergillus niger. To expand our scope we will also be working on host engineering, trying to create a single cell phenotype of Aspergillus niger. To increase the accessibility of our host we also deliver a set of tools, which include ATP and pH biosensors, cytoskeletal gfp-fusions and chromoproteins.

Team Warsaw: FluoSafe

We are presenting to you FluoSafe- a biosensor for acrylamide, known for its carcinogenic and neurotoxic effect! This compound is present not only in biological laboratories but also in starch-based food products (fries, chips etc.). We aim to construct a bacterial strain that would serve as a detector of acrylic amide. This will be attempted in two ways: through the use of roGFP (redox sensitive GFP) fused with glutaredoxin 1 (the presence of acrylamide is known to affect the cellular gluthatione pool) and by expressing hemoglobin α- and β- subunits fused with split fluorophore (adducts formed by acrylamide on the N-terminal valine are known to affect interactions between subunits). We also constructed a BiFC toolbox in BioBrick standard. We sought to find out what was the effect of acrylamide on a variety of human cell lines and asses the toxicity of different concentrations of this compound.

Team Westminster: Hungry for chitin

This year the Westminster iGEM team are tackling the growing bed bug problem. Serratia marcescens has been identified as an efficient chitin degrader, however as it is a pathogenic organism it can not be used as a biocontrol agent. Our idea is to use the chitin genes from this bacterium and create a chitin degrading E.coli. We will test the efficiency of the activity of chitinase which is expressed by our engineered E.coli compared to that of S. marcescens by using a chitin azure assay.

Team York UK: Electricus Aureus: Our greatest source of power comes from the smallest organisms on Earth

We envisage a world where your mobile phone my one day be powered by synthetically engineered microorganisms, when non-renewable energy is a thing of the past. Our project comes at a time when all sources of energy are fighting to be the lesser of many evils; we would therefore like to propose a cheaper, greener and more effective source of energy. Currently, fuel cells do not produce sufficient power to be used for household appliances. Our genetically engineered organism will help us change this and be the first step in the Renewable Revolution. Bacteria are the most abundant form of life on Earth, they survive in harsh environments and they divide rapidly. Thus, they can be a renewable, sustainable source of energy. Our organism will deposit gold nanoparticles on the battery to increase its conductivity. These gold ions come from toxic pharmaceutical waste which is extremely harmful to the environment.

LATIN AMERICA

Team BIOSINT Mexico: Smartpro

This year Biosint Mexico team will be developed a smart probiotic. Along the competition have been present several projects about probiotics, nevertheless the main disadvantage was that most of them were not being created in a lactobacillus strain. Because of this we constructed a Lactobacillus platform for others iGEM teams. Continuing with the idea of the smart probiotic system we include a sensor for xenobiotic substances that could detect and decrease intoxications by pesticides. Also the team implemented a kill switch for safety issues. This project contributes to resolve one of the Mexican food and health problems.

Team Buenos Aires: To drink or not to drink

Our project is focused on developing a biosensor specific for certain water pollutants, with a modular and scalable approach. This approach would make it easy to adapt the response for the detection of different substances. In contrast to other iGEM biosensors, it does not rely on expensive equipment or qualified people to interpret the results. Being aware that most of the populations affected by consumption of contaminated groundwater don't have scientific or technical training, we intend the device to be cheap and easily distributed. We have designed it in a way that any user could easily determine the presence and level of the contaminant on drinking water, using image-based instructions. The project will focus on measuring a primary pollutant: arsenic. However, its modular and scalable design provides an easy way to measure various contaminants such as nitrate/nitrite among others.

Team Ciencias-UNAM: Skully coli

The human peptide LL-37 is an antimicrobial peptide shown to protect against H.pylori and other pathogenic bacteria. Synthetic expression of active LL-37 in vivo is challenging due to the cytotoxic effects it has in the host. To make a resistant host that can export LL-37 to the media we intend to overexpress the E.coli acrAB and tolC operons, which activate the AcrAB-TolC efflux pump, a mechanism related with resistance to this and similar peptides by expulsion. To create a system in which E.coliexpels LL-37 only in the presence of specific pathogenic bacteria, we use the LsrA promoter, which allows transcription in the presence of AI-2, a molecule produced by these bacteria to communicate via quorum-sensing. To avoid self-induction we designed an antisense RNA with specific secondary structure to inhibit the translation of

LuxS, the enzyme responsible of the production of AI-2 in E.coli.

Team Colombia Uniandes: Nicko & Chimi: The magneto and the chimera

This year we are developing two projects: The first one, that we call Nicko, is an alternative solution for water pollution caused mainly by mining, it is a system capable to detect and absorb nickel, to later be removed magnetically, using parts of the homeostatic system of E. coli and Ralstonia metallidurans and the magnetotactic property from Magnetospirillum magneticum AMB-1 which will be used as our final chassis.

The second one, Chimi, is a stress-tester for animals (or even humans). It is based in a glucocorticoid sensor that is able to discern between basal levels and stress levels of glucocorticoid hormones in a sample with an easily recognizable signal, such as color, to allow the sensor to be used in the field, household or the laboratory.

Team Costa Rica Cibus: Genetic transformation of Bacillus subtilis for lactose consumption

Cibus 3.0 takes biodiesel production to a new level using dairy industry wastes. Annually, about 675 thousand tons of whey are thrown into rivers. This because at the present time there isn't a program for reusing this waste, and producers find it difficult to treat them properly because of its chemical composition. Our idea consists in the modification of the bacteria Rhodococcus opacus to enhance the absorption of lactose and the overexpression of the natural triglycerides (TGA) producing ability of R. opacus, achieved by inserting an optimized sequence of a DGA acyltransferase gene and lactose absorption genes, constitutively expressed, also with an optimized sequence of a lipase from B. cepacia which is the responsible to break down the TGAs and an inducible "suicide device" in order to extract them with ease. Now all what it takes to finish the job is adding some ethanol to obtain our biodiesel!

Team Manaus Amazonas-Brazil: Electrobacter: from used frying oil to electricity

Used-frying-oil is produced in deep-fried food preparations and is one of the most serious environment hazards.In our project we are using the Shewanella which is a genus of proteobacteria widely found in Amazon region(called also as Shewie).They can reduce long-chain fatty acids, being a versatile new chassis to study and work in the iGEM competition. The fat acid degradation via Beta-oxidation is done by enzymes which expression is regulated by the genes FadR, FadL, FadD, FadE producing acetyl –CoA. All these features are remarkable for bioremediation of fat and oil spills.Besides that,is also known for its ability in "delivering" electrons to external media.we modified Shewie β-oxidation pathway silencing regulators and enhancing expression of some genes for fat degradation.In this years' project we aim to make a micro power plant using a bacteria hungry for used-frying-oil.

Team TecMonterrey: Modular, synthetic biology approach for the development of a bacterial cancer therapy in Escherichia coli.

By harnessing the inherent ability of facultative anaerobic bacteria to colonize and grow in tumoral environments, this project aims to prove the functionality of four different modules that would work together as a bacterial cancer therapy using Escherichia coli as chasis: Toxicity module, Secretion module, Localized induction module, and Internalization module. The expression of tumor specific therapeutic proteins, Apoptin and TRAIL, conforms the toxicity module. For these proteins to have their effect they need to be located in the extracellular matrix, therefore we are developing a module with a secretion function using hemolysin secretory mechanism. The hypoxic microenvironment present in tumors can be used for

the localized induction module of tumor specific proteins, using the promoters HIP and nirB. Finally, Apoptin needs mechanisms to enter tumor cells' cytoplasm. Proteins with this requirement could reach the cytoplasm when coupled with the internalization module, resulting in a fusion with the TAT peptide.

Team UANL Mty-Mexico: Integrating transcriptional and post-transcriptional regulation through the use of two synthetic RNAthermometers

Temperature sensing RNA sequences, known as RNA thermometers, regulate translation by preventing the ribosome from binding the transcript until higher temperatures shift it to an open structure. Several naturally occurring RNA thermometers have been described, and synthetic sequences that emulate them have been designed and proved to regulate genetic expression at different temperature ranges. Here, we intend to build a genetic circuit that results in three discrete states whose transition can be regulated by temperature changes only. Most notably, our circuit integrates transcriptional and post-transcriptional regulation, widening the spectrum of potential genetic circuit topologies for synthetic biology, with applications that range from basic research to the replacement of chemical inducers for industrial-scale processes.

Team UC Chile: Whateversisome: create your own bacterial functional organelle

Compartmentalization is a characteristic of complex biological systems. Carboxysomes are proteinaceous bacterial microcompartments that evolved to optimize bacterial metabolic reactions. We sought to take advantage of this biological principle to design a platform for in vitro metabolic engineering. Whateversisome it's based on two hypotheses: the targeting signal to the microcompartment is present in a subunit of RuBisCO and that after isolation Carboxysomes can maintain their metabolic capacity in vitro. To address these hypotheses, we designed a system to target proteins of interest to the Carboxysome using RuBisCO subunits as targeting signals. We showed colocalization of GFP fusion proteins and Carboxysome shell-proteins fused to RFP. Second, we designed a simple system for Carboxysome purification based on biotinylation that should enable easy isolation of recombinant Whateversisomes. Our approach would enable combinatorial in vitro metabolic engineering by producing and combining arbitrary Whateversisomes. This project takes advantage of subcellular organizational principles for metabolic engineering.

Team UFMG Brazil: CardBio (Cardiovascular disease biomarkers sensor)

Death by heart diseases is very common worldwide, being Acute Coronary Syndrome (ACS) its main cause. This fact is deeply related to late diagnosis, which is usually made after the cardiac event had already occurred. We, from UFMG team, decided to explore this problem building a system capable of providing a precocious diagnosis for ACS based in 3 biomarkers: Brain Natriuretic Peptide (BNP), Trimethylamine-N-Oxide (TMAO) and Ischemia Modified Albumin (IMA). The main goal is to detect each of these biomarkers using our engineered E. coli by integrating the signals CFP, YFP and RFP produced when BNP, IMA and TMAO, respectively, are present in a sample of patient serum. This diagnosis is based on color intensity of the fluorescent proteins. So, we can establish the presence or absence and severity of ACS disease and predict earlier a myocardial event, thus providing information for fast treatment.

Team USP-Brazil: Detecthol: Methanol detection system

Our product is a bioengineered sensor, which will be able to detect levels of methanol above 2% in common alcoholic drinks. This will allow government to make high-throughput screening of ethanol drinks tainted will methanol. The device will be used as an initial low cost and portable test. The construction is based on the pAOX promoter, which is activated by methanol and repressed by ethanol. Several parts of

the device must be tuned for proper function in Pichia pastoris; pAOX promoter, red fluorescent protein (RFP), Mxr1p transcriptional factor and FLD promoter. Since we aim to develop a product ready for the consumer to use, we plan to develop a plastic container for the lyophilized yeast, printed by 3D printer, that will help perform the test and will also contain the yeast. After use, the container will be able to apply bleach to eliminate the yeast.

NORTH AMERICA

Team Alberta: The Littlest Mapmaker

Inspired by a 2007 iGEM joint project by Davidson College and Missouri Western State University, 'The Littlest Mapmaker' is the University of Alberta's effort to create a biological computer capable of solving the Travelling Salesman Problem, a logistical challenge in which a hypothetical salesman must find the shortest route through a series of destinations. Our travelling salesman computer is built from a combination of raw DNA chemistry and bacterial colonies: first it assembles the routes by stringing genes together, treating them like roads on the salesman's map, then the bacteria sort the good routes from the bad, identifying the answer through the quantity of bacterial colonies making use of each route. The most commonly used route is the winner!

Team Arizona State: BactoVax: A Modular, Bacterial-based Cancer Vaccine Platform

Cancer kills eight million people each year, a number unchanged over the past five decades. The current paradigm for cancer treatment involves non-specific therapies such as chemotherapy and radiation that cannot differentiate between cancerous and healthy tissue. We propose a novel vaccine delivery system of tumor associated antigens and immunomodular agents encapsulated within probiotic bacteria to harness the patient's own immune system to fight cancer. The bacterial vaccine should activate macrophages and dendritic cells in order to teach the immune system to recognize cell surface antigens that distinguish tumors from healthy tissue. We aim to engineer lab-strain Escherichia coli and E. coli Nissle 1917, a commercial probiotic, to train the immune system to target and destroy tumor cells. This provides a distinct advantage over current bacterial vaccinology platforms, which rely on pathogenic bacterial chassis such as Salmonella and Listeria.

Team Berkeley: Genes to Jeans: a green solution to blue denim

The world consumes over 40 million kilograms of indigo annually, primarily for dyeing denim. Indigo is currently derived from petroleum using a high energy process, and commercial dyeing involves the use of reducing agents to solubilize the dye. The development of biosynthetic and bioprocessing methodologies for indigo dyeing could have environmental and economic advantages. By combining the biosynthesis of indigo and the use of the natural indigo precursor indican, we propose a more sustainable dyeing method as an alternative to chemically-reduced indigo in the large scale production of indigo textiles. We achieved in vivo indigo production in high titers, and efficient cleavage of indican using a non-native glucosidase. Inspired by natural systems, we isolated and characterized several plant and bacterial glucosyl transferases hypothesized to produce indican. Lastly, we compare the cost and environmental impact of our alternative with the present chemical process.

Team BostonU: Fuse, or Die: The Case for the MoClo Revolution

In order for synthetic biologists to be able to use automation technologies, we need a well-characterized

library of basic biological components that can then be used to design more complex systems. MoClo is a one-pot digestion-ligation assembly technique developed by Weber in 2011, which enables faster and more efficient construction of genetic circuits when compared to BioBricks, the current iGEM standard. With our project, we are proposing that iGEM teams replace the inefficient BioBricks format with MoClo. We have expanded our library of basic MoClo DNA Parts and characterized devices using various promoter-RBS combinations via flow cytometry. We also designed and implemented a standardized data sheet with a JavaScript software program in order to easily share our library and data with the community. Our MoClo library, characterization data, and data sheet tool fill an essential role in the implementation of automated synthetic biology protocols.

Team British Columbia: CRISPR MADE BY U – CRISPR Mediated Automated Design Employed to Bring You Ultrabiotics

The past decade has seen the emergence of robust bioprocessing strains engineered to synthesize discrete molecular products. The next-generation of strains could be “programmable,” with on demand generation of molecules within a bioreactor e.g. a yogurt fermentation capable of making any combination of flavouring, nutrients or pharmaceuticals. While merging all this potential into single hosts seems efficient, it would also bring added risk in the case of a process failure due to bacteriophageinfection. Here, we not only rationally design widespread immunity to phage infection, but also hack this immunity system to yield programmable biosynthesis at the community level. We demonstrate this by building both broadly and specifically neutralizing CRISPR systems that were paired with biosynthetic capabilities for vanillin, caffeine and cinnamaldehyde production. Eventually, a fermentative process could exist that is vaccinated to phage infection but susceptible to targeted phage addition that results in a programmable probiotic – or ultrabiotic.

Team BYU Provo: Phage Pharming: Two Approaches to Expanding the Use of Bacteriophage in Synthetic Biology

Bacteriophages are the most abundant organism on the planet, yet most are still uncharacterized. Current research is focused on finding new ways to use bacteriophageeither in their wild-type state or after they have been modified for use in synthetic biology. We studied two ways to modify existing well-characterized bacteriophages. First, we employed random mutagenesis, CsCl purification, and plaque-size selection to isolate T4 and T7 bacteriophage with altered capsid sizes. A library of capsid sizes will allow researchers to select the appropriate bacteriophage for use in biotechnology or nanotechnology applications. Second, we designed a cholera sensing and destruction circuit using bacteriophage lambda. In this circuit, lambda contains biofilm-degrading enzymes controlled by a cholera quorum-sensing system transferred to E. coli. Upon sensing cholera, this E. coli will activate lambda, leading to cell lysis and biofilm degradation. This research demonstrates the versatility and utility of bacteriophages in the field of synthetic biology.

Team Calgary: The FerriTALE

Outbreaks of foodborne illnesses are a growing problem for food safety and public health. Whether in your water, salad or steak, pathogenic E. coli causes upwards of 250,000 illnesses every year. To solve this problem, iGEM Calgary is developing the FerriTALE to detect harmful E. coli. It uses engineered proteins that detect and report the presence of dangerous E. coli in a sample. The detector, TALE, binds to genomic markers specific to dangerous E. coli. Next, our TALEs are attached to the scaffold and reporter, Ferritin, that rapidly alerts the user to the presence of E. coli through a visible color change. We have integrated these proteins into a handheld device, similar to a home pregnancy test, that tells the user if dangerous E. coli is present. Moving forward, the FerriTALE can be tailored to detect other pathogens as the basis of a

powerful new detection platform.

Team Carnegie Mellon: Light-Activated Antimicrobial Phage

Due to the widespread misuse and overuse of antibiotics, drug resistant bacteria now pose significant risks to health, agriculture and the environment. An alternative to conventional antibiotics is phage therapy. However, many temperate phage also form prophage. Our approach to antibiotic resistance is to engineer a temperate phage, Lambda, with light-activated production of superoxide. The fluorescent protein KillerRed was cloned into a plasmid vector and lambda gt11 with the IPTG inducible lac promoter. Lysogens were isolated and these strains were characterized and compared to E. coli with KillerRed from high-copy plasmids. Light activation of KillerRed resulted in decreased cell numbers. In addition, we modeled our system at multiple scales, including populations of phage and bacteria, KillerRed gene expression, ROS production, and effects of light. Having two methods of killing, lysis and superoxide, decreases the probability of developing resistance and our system overcomes the prior limitations of using wild-type temperate phages.

Team Clemson: Development of a Universal Self-Amplified (USA) Biosensor for Repaid Detection of Viable Pathogens

Many regulatory agencies such as the Department of Agriculture and the Environmental Protection Agency have specific standards for pathogen concentrations in sample materials, including “zero-tolerance” for some foodborne pathogens. However, current detection methods for these disease-causing bacteria suffer from one or more of the following limitations: 1) requiring sample enrichment, 2) inability of low-level detection, 3) indiscrimination between viable and non-viable cells, 4) small sample volume capacity, 5) tedious procedures, and 6) high assay cost. Our Universal Self-Amplified (USA) Biosensor uses a genetically modified detection bacteria to solve many of the aforementioned issues. The engineered USA bacteria will recognize a target chemical produced by the pathogen of study, which will trigger a cascade of genes to both amplify the chemical signal and produce a visible alert to the pathogen’s presence. The USA pathogen detection mechanism strives for rapidity, economy, and simplicity.

Team Concordia: Comput-E.coli

Our aim is to achieve universally computational cells through the exploitation of cell-cell communication to generate biological cellular automata. To achieve this, we are using an array of E. coli colonies, which implement the same logical functionality while using different input/output interfaces. The strains process their inputs (two inputs from neighbors plus their own current state) to decide what their next state will be, after the application of a global clock.

At the heart of our project is the construction of the clock, to synchronize information processing. We are making a fusion protein of ETR1 from A. thaliana and EnvZ from E. coli to allow cells to respond to ethylene gas concentrations. All cells will produce this gas at cyclic intervals, thus creating a truly autonomous clock. We have also devised an RNA-based system that can process information reliably and expediently with the use of ribozyme-based XOR and AND gates.

Team Cornell: Organofoam: Genetically Engineering Fungal Mycelium for Biomaterials Development

The goal of Organofoam is to develop a fundamental toolkit of genetic parts for engineering complex fungi, particularly plant-pathogenic basidiomycetes. We were inspired to do so by a local company, Ecovative Design, that uses lignin-degrading fungi and plant matter to produce a biodegradable Styrofoam substitute.

The existing product that we are seeking to improve, known as “mushroom packaging,” is a sustainable and necessary alternative to Styrofoam. Polystyrene can take hundreds of years to degrade in landfills, produces dozens of identified chemical toxins upon combustion, and is tremendously inefficient to recycle, thus posing difficulties for disposal and polluting the environment. However, the production efficiency of Ecovative’s substitute suffers due to contamination from pathogenic molds, a problem that we seek to solve using synthetic biology. Using the complex, plant-pathogenic basidiomycete, Ganoderma lucidum, as a chassis, we are expanding the accessibility of fungal genetic engineering and demonstrating its utility for commercial purposes.

Team CU-Boulder: Cheap protein and DNA purification methods for DIY Bio

The focus iGEM at CU-Boulder has been to make synthetic biology more accessible and affordable. We spent the summer developing parts, procedures, and documentation to help make this vision a reality. The original goal was to create the constructs and purification methods necessary to produce and isolate restriction enzymes. Along the way we explored some novel approaches to DNA and protein purification and developed experimentally tested protocols for these and other procedures essential to Biobrick assembly. Our purification methods exemplify the ideal of using common lab materials to make performing everyday lab techniques as accessible and inexpensive as possible. A related aspect of our project was exploring methods of recycling consumables associated with lab work in order to reduce waste and material expenses. We hope that our findings using this 'do-it-yourself' approach of synthetic biology help make this type of research more accessible for those where funding is limited.

Team Duke: Designing Synthetic Gene Networks Using Artificial Transcription Factors in Yeast

Synthetic gene circuits have the potential to revolutionize gene therapies and bio-industrial methods by allowing predictable, customized control of gene expression. Bistable switches and oscillators, key building blocks of more complex gene networks, have been constructed using naturally occurring and well-characterized regulatory elements. In order to expand the versatility and variety of these circuits, we designed and constructed gene networks using artificial transcription factors (ATFs). The ATFs are of two classes: inhibitory TAL proteins and a catalytically inactive dCas9 protein with small guide RNA elements, each orthogonal to the yeast genome. Using mathematical modeling, we determined the parameters expected to create bistability and oscillation, using tandem binding site kinetics to achieve cooperativity. Based on these results, we assembled a library of plasmids containing ATFs, binding sites, regulatory elements, and fluorescent reporters. We then integrated these genes into the genome of Saccharomyces cerevisiae and are currently characterizing them using flow cytometry.

Team Gaston Day School: Fluorescent Detection of Cadmium in Water Supplies

Heavy metal contaminants pose a serious health threat around the world, especially in locations with poor irrigation. Cadmium, in particular, is a known carcinogen that affects the cardiovascular, gastrointestinal, and respiratory systems. The Agency for Toxic Substances and Disease Registry compiled a Priority List of toxic substances, on which Cadmium was seventh. We combined the Green Fluorescent Protein coding region with a Cadmium Sensitive Promoter to create our detector, which provides a simple and inexpensive test for the presence of Cadmium in water supplies. We will incorporate sensitivity tuners to decrease the detection threshold, and we will use mutagenic PCR on both the promoter and the entire detector to increase its sensitivity. Proper use of this BioBrick could result in early detection of cadmium polluted water and potentially prevent deaths worldwide.

Team Georgia State: Mamba Juice: Expression of Exogenous Mambalgin Peptide Using the pGAPzα Vector System

Pharmaceutical companies have invested considerable financial resources in developing analgesics. Often, the compounds used in these medicines are naturally occurring, such as aspirin and opoids. In 2012, Diochot et al from the Institut de Pharmacologie Moléculaire et Cellulaire in France, was able to extract and isolate an analgesic peptide found in the African Black Mamba. The peptide, Mambalgin-1, has been shown to have comparable analgesic effects to morphine but does not induce the debilitating withdrawal affects. However, extracting this peptide is expensive and dangerous work. Using a cassette of standardized systems (the pGAPzα expression vectors) in P. pastoris, we sought to produce high quantities of pure, functional proteins in a safe and cost effective manner.

Team GeorgiaTech: Bacterial BioBots: Integrin-Based BioSensors

Our team goal is to develop novel bacterial BioBots that respond to the extracellular tissue environment. Mammalian cells communicate with the extracellular matrix (ECM) using heterodimeric cell surface receptors, called integrins, which can signal in a bidirectional manner between the cell interior and ECM. We aimed to express the integrin αIIbβ3 in E. coli cells. To promote dimerization of the integrin subunits, we attempted to optimize bimolecular fluorescence complementation of split GFP using surface display technologies. We cloned split GFP parts, assembling the T7-promoter, LacI-operator, and ribosomal binding site (RBS) upstream of the protein-coding region. To verify αIIbβ3 function, we developed an integrin activity sensor consisting of the ligand derived from fibrinogen (KQAGDV) coupled to GFP. Finally, we successfully created a new standard for RBS addition that inputs the strong RBS (BBa_B0034) in front of any standard BioBrick part, which is efficient and more successful than the usual 3A/standard assembly.

Team Greensboro-Austin: Bioadhesive Production Using an Expanded Genetic Code

Mussel adhesive proteins (MAPs) are water-resistant bioadhesives that have a variety of biomedical and construction applications. Replicating the natural properties of MAPs through in vivo production using microbes is difficult as it depends on post-translational modifications, primarily the hydroxylation of tyrosine residues to L-DOPA. Since adhesiveness is correlated with L-DOPA content, our project aims to improve the adhesive properties of MAPs by utilizing a strain which inserts the non-canonical amino acid L-DOPA at UAG stop codons during translation. When combined with the in vivo production of free L-DOPA, this system is a significant step towards rapid, cost-effective MAPs production. Our team also worked on degrading odorous compounds, computationally modeled the detection of potentially dangerous oligo orders, and created BactoArt using inducible fluorescent proteins. Additionally, we created the Open Sequence Initiative, which is focused on updating the standards for submitting BioBricks to the iGEM Registry.

Team Lethbridge: Frame-Changer: Shifting Translation for Multiple Protein Expression

The current growth in synthetic biology research promises more complex and useful engineered systems. However, increased complexity often requires more genetic material that can be difficult to introduce into organisms. We propose the development of a new library of regulatory gene expression elements that allow for compression of multiple coding sequences into a smaller amount of genetic space. Using a pseudoknot RNA structural motif, commonly used by viruses to minimize their genome size, we will show the utility of dual-coding gene sequences to give useful protein products whose expression can be regulated by the pseudoknot's ability to induce ribosomal frameshifting. A software tool will also be used to overlap multiple coding sequences into different reading frames. Ultimately, this library of standardized

parts will be available for use in a variety of engineered systems requiring minimal coding space and multiple protein expression.

Team Michigan: A Completely Unidirectional Biological Transistor Utilizing an Engineered Fim Switch

Recent studies have just started to explore the possibility of utilizing existing recombination systems, to store information and perform computations. However, the only systems studied so far are not completely unidirectonal in their ability to flip a segment of DNA. Instead, previous systems have relied on “recombination directionalityfactors”, which when complexed with the unidirectional recombinase, reverse the direction in which it flips the DNA segment. The fim system from E. coli, has been shown to contain 2 unidirectional recombinases, hbif and fime, which flip a promoter containing segment of DNA. Our project seeks to engineer the fim switch by replacing the native promoter with another promoter. We demonstrate that it can function as a reliable and efficient biological transistor, or “transcriptor”. Beyond storing information and performing basic computations, the system would serve as a very useful, tightly controlled switch.

Team Minnesota: The pMNBB vector system: A toolkit approach for engineering Pichia pastoris

Currently, there is a significant gap in available resources for transformation and expression of foreign proteins in the industrially-relevant yeast model, Pichia pastoris. To address this, we have constructed pMNBB, a versatile shuttle vector system. pMNBB vectors provide researchers a modular BioBrick platform to introduce synthetic pathways into E. coli and also the option to rapidly transfer these constructs into P. pastoris. Not only is this vector system designed for integration of expression cassettes into the P. pastoris genome, but we have also added elements which allow for the episomal maintenance of these plasmids. Moreover, these vectors are amenable to trans-kingdom conjugation (TKC). Rather than following a yeast transformation protocol that can take days to weeks, our constructs may be introduced to a yeast culture in roughly one hour. Finally, as a proof-of-concept, we use the pMNBB vector system to produce and secrete active human insulin from P. pastoris.

Team MIT: Engineered mammalian cell-cell communication mediated by synthetic exosomal cargoes.

Coordinating behavior across cell populations to form synthetic tissues requires spacial communication between individual cells. While there has been some success engineering single signals, sending multiple signaling elements spanning spatial scales for multicellular coordination remains a significant hurdle. Here, we describe a method for mammalian cell-cell communication utilizing engineered exosomes containing miRNA or protein signals. First, we demonstrate selectively packaging signalingmiRNAs (miR-451 and miR-503) and synthetic fusion proteins (GFP, Cas9, and Cre recombinase each individually fused to the oligomerizing membrane targeting domain Acyl-TyA) into exosomes within cells engineered with sender genetic circuits. Next, we demonstrate that these miRNA and protein signals can modulate gene expressionwithin cells engineered with receiver genetic circuits. Finally, we present preliminary cell-cell signaling results on populations of cocultured sender and receiver cells. Our method may enable multiplexed communication among populations of various cell types and the creation of sophisticated synthetic tissues.

Team MSOE Milwaukee: Synthesizing Eucalyptol from Spent Grain Waste using a Three E. coli System

Eucalyptol, the main component of Eucalyptus oil, is a valuable product with multiple potential industrial uses. Current methods are costly, which is a major deterrent for research on these applications. We have designed a system consisting of three strains of Escherichia coli (E. coli) which will convert spent grain waste from breweries into Eucalyptol. The design utilizes two strains of E. coli overexpressing and secreting enzymes to degrade hemicellulose into xylose. The third strain of E. coli will utilize the xylose and convert the molecule into Eucalyptol using the Mevalonate pathway. A scale-up model was developed to assess industrial feasibility. The cost of production would drop two-thirds, which would make industrial uses plausible. Future studies will be transforming our E. coli with the genes of interest and testing for production of Eucalyptol.

Team Nevada: Lysesavers: A novel endolysin-based bactericide for the treatment of gram-negative pathogens

Endolysins are the bacteriophage proteins responsible for lysing target bacteria by degrading the peptidoglycan layer. The use of endolysins to fight bacterial plant disease is an emerging field, but it has largely been limited to gram-positive bacteria, as the outer membrane of gram-negative bacteria prevents endolysins from accessing the peptidoglycan. Recombinant E. coli have been created to express endolysins which target various plant pathogens. These proteins can be used as a novel gram-negative bactericide when coupled with a treatment to disrupt the outer membrane. A system to easily detect outer membrane disruption will be critical to our project, as well as any future studies on gram-negative endolysins. To address this, a registered iGEM part used for cell surface detection was improved and incorporated into a fluorescence-based assay. Erwinia amylovora, a gram-negative bacterium responsible for fire blight in fruit trees, was chosen as a model target for proof of concept.

Team Northwestern: NU Balance

The purpose of this iGEM project is to combat oral diseases by engineering a bacteria to neutralize the lactic acid produced by Streptococcus mutans. Due to the time constraint, the scope of the project is limited to a dual-state promoter where an acid-responsive promoter is placed upstream of a constitutive promoter. This novel transcription regulation element will have both constitutive and inducible activity. The construct will be tested by linking green fluorescent protein to the downstreampromoter. Based on preliminary results, the extra promoter should allow for a significant increase in gene expression. These pieces have wide implications in any fields that require a quick upregulation of any gene in response to acidic environments.

Team OU-Norman OK: A Shuttle Vector for Clostridial Chassis Organisms

Concerns about energy security, sustainability, and the environmental impact of fossil fuels have renewed the interest of both the public and the scientific community in the development of renewable energy sources, including biofuels. Recent research in synthetic biology has resulted in the production of alcohols not known to be produced naturally, and the extension of carbon chain length. These systems have been expressed in E. coli. Historically, the alcohol titer obtained with native alcohol producers, such as Clostridium acetobutylicum, an acetone/butanol/ethanol producer, and Clostridium beijerinckii, an isopropanol/butanol/ethanol producer, has been greater than that obtained wtih non-native producers. One major problem with these systems is the lack of tools available for genetically manipulating them. The goal of our project has been to develop a shuttle vector that will allow the heterologous expression of these and similar biosynthetic pathways in these organisms.

Team Penn: Engineering the Epigenome

The code of life is more than a sequence of A's, C's, T's and G's; epigenetic modifications, such as DNA methylation, are powerful and heritable regulators of gene expression. Targeted methyltransferases are enzymes that catalyze sequence-specific methylation – the most useful tool for engineering the epigenome. With a synthetic biology approach, we developed an assay to test targeted methyltransferases without expensive, time-consuming traditional methods. Our modular single-plasmid system allows methyltransferases to be easily cloned and tested via inexpensive digestion assays, quickly measuring the existence and extent of targeted methylation. Additionally, our plasmid contains standardized primer-binding sites for methylation-sensitive sequencing, and our E. coli chassis effectively eliminated noise associated with methylation studies. We are using this assay to characterize our novel targeted methyltransferases, which could be used to study epigenetic modifications. In the future, synthetic biologists could embrace these tools to explore the next frontier in engineering biological systems: the epigenome.

Team Penn State: Plants as Plants: Natural Factories Producing Fuel, Plastic, Flavoring, and More

Plants as Plants: Natural Factories provides a green approach to the manufacturing of valuable chemicals and materials. Through synthetic biology, we are able to control the expression of genes that regulate the production of desired secondary metabolites. Via the manipulation of established metabolic pathways, we hope to produce vanillin and butanol. The prospect of being able to synthetically produce a biofuel provides vast possibilities for the scope of synthetic biology and green energy. Additionally through the manipulation of the cellulose synthase genes, we hope to increase the biomass of plants by a hybrid plant cell wall. As shown through these projects, the use of plants provides various green energy possibilities. However, due to the limited use of plants within synthetic biology there are various regulation issues. Thus we have additionally worked on characterizing a range of plant promoters as well as introducing the Cas9 crisper system into plants.

Team Purdue: Back to the Basics

Synthetic biology has striven to prove that classical engineering principles are applicable in the field of biology. Several challenges have yet to be overcome including design of robust genetic circuits, reliable gene expression, and a standard way to characterize parts. To assess circuit robustness, we utilized the power of the Taguchi Method, a statistical analysis which optimizes a set of parameters to form a robust system against outside noise while minimizing experimental time and cost. Making bicistronic expression operating units, which reduce the variability of gene expression, available in the Registry of Standard Biology Parts will enable efficient engineering of large networks. Finally, collaboration among teams allowed for a new standardized form of submitting characterization of parts to the Parts Registry. These three approaches to improve part standardization and robustness will help move the field of synthetic biology one step closer to proving that biology can, in fact, be engineered.

Team Queens Canada: Biosynthesis and breakdown of human odour compounds for the behavioural manipulation of malarial mosquitos

Malarial mosquitoes are developing resistance to key insecticides and drugs, and are becoming diurnal to avoid treated mosquito nets. Recent studies have shown that the African mosquito uses human foot odour to locate its host, a trait that is enhanced when the insect is carrying malaria. We plan to combine a carboxylic acid reductase and an acetyl transferase, in order to create E. coli capable of converting a major component of foot odour (isovaleric acid) into banana smell (isoamyl ester). This could have both

commercial and humanitarian applications. Our second goal is to deliberately synthesize mosquito attractants inside traps. Recent research has shown that a mixture of CO2 and foot odour volatiles can be more attractive than a human. We have chosen indole as our first attractant, a compound naturally found in human sweat. We hope our project will show that bioremediation and biosynthesis techniques have applications in mosquito control.

Team RHIT: Constructing a Unique Platform for Interspecies-Dependence (CUPID): The evolution of multicellular machines.

CUPID aims for stable obligate symbiosis for the model prokaryote Escherichia coli (bacteria) and the model eukaryote Saccharomyces cerevisiae (yeast). We address this goal by constructing a unique platform for interspecies-dependence based on inducible expression of a required histidine biosynthetic gene (HIS) in each species. Expression of the bacterial HIS gene is induced by lactic acid produced by constitutive expression of a lactate dehydrogenase gene in the eukaryote. Expression of the yeast HIS gene is induced by the binding of its mating factor receptor to mating factor expressed constitutively on the surface of the prokaryote. Histidine deprivation necessitates physical contact and symbiosis for survival. Such pressure may facilitate evolution of a stable exosymbiotic form of the two species. Study of isolates and further manipulation could provide insight into the use of obligate symbiosis in synthetic biology and yield a chassis for the synthesis of novel multicellular machines.

Team Stanford-Brown: Synthetic Bio-Communication

Communication is a dynamic requirement for life as we know it. We are using cellular and molecular messaging of different magnitudes to improve the broadcasting and reception of information. Starting on the atomic level, our BioWires project has created silver-incorporating DNA to act as nanowires, which could improve the cost and effectiveness of electronic products. Our CRISPR project is creating a system for DNA messages and resistances to be passed from cell to cell, in effect, creating transmissible probiotics and changing the way that cells communicate. We are also building a chromogenic biosensor to detect sucrose secretion that will be launched on a satellite (EuCROPIS) into low-Earth orbit. Finally, our De-Extinction project involves decoding messages from the past to better understand early life on Earth.

We are the Stanford-Brown iGEM team, and we're connecting life on Earth, to help us prepare for life beyond it.

Team Toronto: Biofilm: System engineering in E. coli

Microorganisms frequently adopt a lifestyle in which they excrete extracellular biopolymers to aggregate and form biofilms. We are researching the pathways that induce biofilm formation and maturation in E. coli, for modulating surface-specific adhesion of E. coli biofilms. We are constructing and characterizing E. coli strains with targeted deletions or recombinant protein expressions that are crucial in biofilm formation pathways. In response to environmental stimuli such as temperature, blue light, or sodium, the phenotype of each mutant E. coli strain will be characterized using a specially developed high-throughput protocol. Biofilm formation control has applications for bioremediation, in which we are pursuing in a related project on heavy metal precipitation. Another potential application is in medical treatment of pathogenic infections, since the generally slow diffusion rate in biofilms gives pathogenic bacteria/fungi an additional mechanism for antibiotic resistance phenotypes.

Team UC Davis: RiboTALe: A Tunable and Modular System for Control of Gene Expression

Despite the fact that the Registry of Standard Biological Parts contains a large number of inducible promoters, the actual usage of these parts is dominated by a very select few. In order to increase the versatility of expression control systems, we propose a new system coupling transcription activator-like effectors (TALEs) with inducibleriboswitches. TALEs are proteins secreted by the bacterial pathogen Xanthomonas that contain engineerable, sequence-specific DNA binding domains and can act as transcriptional repressors or activators. We plan to manipulate TALE activity by subjecting them to inducible expression through riboswitches and promoters. By pairing TALEs with riboswitches, we can expand the existing library of inducible repression systems. In addition, we hope to modify the parameters of our system to show the tunability and modularity of our overall construct. Through proper characterization, we believe that iGEM teams may also use these modular repression systems for the development of future devices.

Team Rutgers: Sensteria: Quorum Sensing E. Coli

Our project aims at developing a self-regulatory system for the degradation of virulent factors. We plan to integrate quorum sensing and pon1 to create this system of self-regulation. Quorum sensing will be used to detect the presence of high cell densities and to activate pon1 upon detecting high cell density. Pon1, acting as a repressor, will degrade the signal molecules needed for quorum sensing and thus turn off quorum sensing as well as itself. It will reactivate upon the reactivation of quorum sensing and this cycle of self-regulation will persist. This prototype system is meant to test the application of this system but the main goal is to introduce this system to pseudomonas aeruginosa as a means to prevent the harm that it causes.

Team UChicago: Keratinase Expression System in E. coli and B. subtilis

Each year, the poultry industry produces over two billion pounds of feather waste that is mostly processed into nutrient-poor animal feed but recent research has shown that feather keratin can be used to produce biodegradable plastics, fertilizers, detergents, and pharmaceuticals. However, current chemical methods for keratin degradation are energetically costly and previous efforts at keratinase production in heterologous hosts have been stymied by poor protease expression. To address both these problems, our team constructed BioBricks based on kerA, a serine keratinase gene native to B. licheniformis active on whole poultry feathers. We designed two biobricks for expression in E. coli and constructed a high copy number BioBrick plasmid with an origin of replication compatible with B. subtilis. By designing a new keratinase expression system in B. subtilis, we hope to provide a faster, cheaper alternative to current methods of industrial keratinase production.

Team UCLA: DiversiPhage: Library Generation for Protein Selection

Both the mammalian immune system's complex defenses and a bacteriophage's targeting mechanism depend upon protein diversification. These models have inspired innovations ranging from targeted drug delivery to protein display. Using the major tropism determining (MTD) protein expressed on the Bordatella bacteriophage BPP-1, we aim to develop an in vitro system for generating antibody-like proteins that bind specified targets. The MTD protein expressed at the phage's tail fiber is naturally modified at its variable region to produce nearly 10e13 possible binding variants while preserving its structure. Mutating the MTD's variable region by PCR can match the massive diversity of MTD in vitro. A library of MTD protein-DNA fusions generated by mRNA display can then be screened for binding against specified protein targets. This in vitro analog to phage display and immune clonal selection can be a powerful tool for constructing

target-binding MTD variants with equally many varied applications.

Team UCSF: Operation CRISPR: Deploying precision guided tools to target unique species in a complex microbiome

In microbial communities, bacterial populations are commonly controlled using indiscriminate, broad range antibiotics. There are few ways to target specific strains effectively without disrupting the entire microbiome and local environment. The goal of our project is to take advantage of a natural horizontal gene transfer mechanism in bacteria to precisely affect gene expression in selected strains. We combine bacterial conjugation with CRISPRi, an RNAi-like repression system developed from bacteria, to regulate gene expression in targeted strains within a complex microbial community. One possible application is to selectively repress pathogenic genes in a microbiome, leaving the community makeup unaffected. In addition, we use CRISPRi to lay the groundwork for transferring large circuits that enable complex functionality and decision-making in cells.

Team UGA-Georgia: Geraniol production via novel protein expression tools in Methanococcus maripaludis

Geraniol is an intriguing 10 carbon compound with diverse applications including use as an agent for cancer prevention, fragrance, insect repellent, proposed biofuel etc. We explored and engineered a novel gene expression tool (BBa_K890000) for Methanococcus with the capability of expressing geraniol synthase from Ocimum basilicum (BBa_K1138000). We report the biosynthesis of geraniol at over 5% of DCW by transforming the vector into Methanococcus thereby expanding its native isoprenoid pathway. Furthermore we engineered new vectors (BBa_K1138001 & BBa_K1138002) with the potential capability of regulating and quantifying the expression of desired proteins via red fluorescence. This work demonstrates the use of Methanococcus as a cell factory for chemical production and highlights synthetic biology advancement by engineering new systems over traditional biological systems such as Escherichia coli.

Team UIUC Illinois: Cardiobiotics - A Genetically Engineered Approach to Cardiovascular Health

Cardiovascular disease (CVD) has been the leading cause of death in the United States for over twenty years and is a rising global health issue. Recent studies demonstrate a correlation between CVD and atherosclerosis, the buildup of plaque in the arteries. One associated risk for atherosclerosis is the production of Trimethylamine N-oxide (TMAO) by natural gut flora when metabolizing L-carnitine, a chemical found primarily in red meat and energy drinks. We created a probiotic to attack the root of this problem by outcompeting the gut bacteria for L-carnitine in order to suppress the production of TMAO. L-carnitine transporters (caiX and cbcWV) and L-carnitine dehydrogenase (CDH) derived from Pseudomonas aeruginosa were expressed in a safe strain of E. coli (Nissle 1917). This engineered E. coli can uptake and metabolize L-carnitine along an alternative, safe pathway into 3-dehydrocarnitine. Together, this system offers a novel solution in preventing TMAO-related cardiovascular health conditions.

Team uOttawa: Fold-change molecule detection using the Type-I Incoherent Feedforward Loop

Many synthetic gene networks are susceptible to cellular noise, as they rely upon the absolute levels of gene regulators which can vary greatly between individual cells. To address this, uOttawa has engineered a network in S. cerevisiae that is responsive to fold-changes as opposed to absolute changes in stimulus. This allows the network to maintain sensitivity despite noise, and also permits response to stimuli in a much larger dynamic range. By modifying the promoter driving the stimulus, the network can be engineered to

detect fold-changes of any molecule with a responsive promoter, thereby serving as a structural chassis for the next generation of molecule detectors. In addition, we have also authored a children's book aimed at disseminating the concepts of synthetic biology to the public, and have designed an online interface that will facilitate the rapid construction of devices built from the Registry of Standard Biological Parts.

Team UT Dallas: A Multifaceted Approach Against Tooth Decay

Cavities are a problem faced by people and animals worldwide and are primarily caused by the bacteria Streptococcus mutans. S. mutans digests certain sugars, consequently forming lactic acid that can lead to tooth decay. Our system starts with the detection of saccharides essential for S. mutans' survival. Using dextranase, the E. coli are able to attach onto the dextran layer that S. mutans normally live on. This allows the E. coli to deliver a targeted dose of norspermidine, helping with the breakdown of S. mutans biofilms. We also took another approach that utilizes a natural quorum signaling molecule, competence stimulating peptide (CSP). This peptide is responsible for population control in S. mutans. By using these approaches, we hope to aid in the treatment of cavities in both humans and animals.

Team Utah State: AMPed up E. coli

Antimicrobial peptides (AMPs) are peptides that have activity against a wide range of microorganisms. AMPs can vary greatly in their size, structure, and mode of antimicrobial activity. Due to the immense interest in both testing and characterizing AMPs (from various forms of life) a sustainable method to produce and purify them is necessary. Using Synthetic Biology as a platform, the production of AMPs in E.coli could potentially offer a cost-effective approach for large-scale peptide manufacture, as opposed to isolation from natural sources. After successfully manufacturing several AMPs using E.coli, these peptides will be tested on a wide variety of organisms to screen for antimicrobial activity.

Team UTK-Knoxville: Modular Design of Chimeric Biosensors

The major limitation in synthetic biology today is the lack of numerous, well characterized sensors. Our project aims to provide a reliable scaffold to test potential sensing domains with unknown substrates. We have created a standard platform to test a range of intracellular and transmembrane domains. Positive results are reported with red fluorescent protein for easy identification which can be done with high throughput methods such as 96 well plates and flow cytometry. We test our platform on sensors with interesting known responses. The chimera proteins are also useful in creating signals orthogonal to the cell.

Team Virginia: Minicells: Multi-Purpose Nano Chassis

Overexpression of the tubulin-homolog FtsZ leads to asymmetric cell division in E. coli that yields achromosomal 'minicells.' The lack of a chromosome renders minicells unable to replicate and cause infection, yet they still retain and express plasmid genes. Furthermore, minicells inherit the stable, non-leaky membranes and cytosolic composition from their parent cell. Our project design is centered on the creation of an IPTG-inducible FtsZ Biobrick that permits tunable overexpression for optimal minicell production. With the development of a multi-purpose, innocuous bacterial chassis as our ultimate goal, we incorporated three additional safety elements: the Ail protein, a polysialic acid capsule and de-acylated lipopolysaccharide. Both Ail and the PSA capsule serve to prevent complement deposition on the surface of the minicells, with PSA also protecting against antibody opsonization. Finally, LPS toxicity is reduced by inducing minicell formation in an lpxM mutant strain that lacks a critical myristoyl transferase for late-stage acyl modifications.

Team Washington: Red Light! Green Light!

Refinement of functional systems is a key aspect of engineering. Biological systems are not immune to this fact and must be continuously improved to function consistently, and reliably. Creating easy methods for tuning biological systems using light was the goal of this year's effort. Our team sought to improve on a previous light-regulated gene expression method, integrate the use of tablet device for testing, and create multiple functional biobrick constructs. In 2012, we developed an app that affords any researcher with access to an android device with a LED screen the ability to illuminate cells in a controlled manner for synthetic biology applications. We improved the functionality of the app by adding a mini petri dish setting and demonstrated the advantages of paralization by performing experiments in a 96 well plate format. This tablet application represents the first real life example of using a mobile device as synthetic biology instrument.

Team WashU StLouis: Converting E. Coli into a Nitrogen Bio-Fertilizer Using a Cyanobacterial nif Cluster: an iGEM project

The nif cluster of Cyanothece 51142 consists of 29 genes that construct and regulate a nitrogenase protein complex. This year, our iGEM team worked on harnessing the power of nif to produce ammonia in Escherichia coli. After synthesizing a nif-containing plasmid (28 kbp) using the DNA assembler method (Shao et al 2009) and transforming that plasmid into E. coli, our team tested for nitrogenase activity using the acetylene reduction assay. The transformed E. coli were then compared to wild-typeunder limited nitrogen conditions to check for a competitive advantage. Tests were used to evaluate the expression of various nitrogenase subunits. Our team also aimed to further characterize the promoter sequences of the Cyanothece 51142 nif cluster. Between the cysE and nifB genes, there is a 958bp uncharacterized, bidirectional promoter region of particular interest. We used fluorescent reporters to identify key regions within this promoter sequence under various environmental conditions.

Team Waterloo: Controlled Modification and Intercellular Transmission of a DNA Message

In nature, intercellular communication allows coordinated cellular behavior on a population level. Engineers seeking complex programmed population-level behavior require tools enabling controlled, information-rich intercellular messaging. Given its versatility and universality as an information-encoding molecule, DNA suggests itself as a message-carrying molecule to enable information-rich messaging. The Endy group at Stanford recently published a proof-of-principle demonstration of such DNAmessaging wherein a DNA message was transmitted from one bacterial population to another carried by M13 bacteriophage particles. Here we propose an intercellular communication program that extends DNA messaging by controlling modification and transmission of a DNA message. Modification is controlled through flipping a DNAswitch on the message DNA – a promoter sequence that is invertible using serine integrases and recombination directionality factors (RDFs). Transmission is controlled by placing expression of the M13 major coat protein, which is required for viral packaging of message DNA, under control of such a switch.

Team Wellesley Desyne: Enhancing Bio-Design with Next-Generation Human-Computer Interaction

Systems that integrate the wide array of technological tools available to synthetic biologists are needed more than ever. As the field of synthetic biology continues to advance, it is critical to communicate the applications, goals, and limitations of synbio research to the public. Our team is creating a software suite, which addresses technical synbio challenges while improving end-user experience and harnessing human-

computer interaction (HCI) to engage the public in synbio concepts. Eugenie is a visual language and integrated development environment for Eugene that allows biologists to specify biological parts, properties, and device composition rules. zTree is a tool for visually representing the Registry of Standard Biological Parts to support sense-making of complex, hierarchical data sets. Bac to the Future is a web-based, interactive application that utilizes Twitter to illustrate synbio ideas to the public. The application of HCI techniques to synbio fosters more effective, collaborative, and intuitive software tools.

Team WLC-Milwaukee: The One Ring to Secrete Them All

Our construct utilizes removable purification and secretion tags separated by a single enzyme cut site NheI that is the insertion point for any coding sequence of interest. The induced gene can be secreted from the cytoplasm to the extracellular space via a tripartite secI pump. A cymR-regulated T5cumate promoter within the plasmidcontrols a Tse2 toxin to avoid horizontal gene transfer, while the plasmid also encodes a chimeric ompA-monoclonal antibody gene, which when expressed, inserts into the outer membrane and the antibody selectively binds norovirus within the gut to hinder infection. This plasmid construct was used to over-express and secrete plant-based, polymer-degrading enzymes bglS, yesZ, and xynA isolated from the cellulosome of Bacillus subtilis subtilis 168 to digest plant material. We utilized Escherichia coli Nissle 1917 containing a chromosome-integrated cymR gene to secrete these enzymes in the hope to accentuate dietary uptake for humans and livestock in impoverished countries.

Team Yale: Converting E. coli into a foundry for bioplastics

Poly(lactic acid) (PLA) is a biodegradable, biocompatible, bioresorbable, thermoplastic bioplastic that offers many advantages over other biomaterials in both commercial and medical applications. The current chemical method of synthesizing PLA is expensive, and the required processing and purifying steps use many environmentally unfriendly chemicals. Recently, E. coli has been engineered to produce PLA, but low yields and short chain lengths prevent the approach’s commercial use. Here, we evaluate the potential of using multiplex automated genome engineering to raise both yields and chain lengths of biosynthesized PLA by directing the E. coli metabolism to funnel resources toward PLA production without sacrificing the organism’s viability. Efficient biosynthesis of PLA, which may be thus achieved, would be a significant step in reducing the impact of plastic waste, and would benefit those receiving bone implants. It would also open up a new possibility in rapid manufacturing of personalized bone implants using three-dimensional printers.

2013

Team AHUT China: Shining Sanctifier

Water, the origin of life, is the necessary and elementary component of our daily life. Various kinds of means have been developed to dispose nitrite and ammonium which are the main contaminants of this type of effluent. One of them is anaerobic ammonium oxidation bacteria (anammox) which can convert the fomite in the water into nitrogen. Our goal is to design a wastewater treatment system which can absorb the pollutant efficiently while transform it into luminous energy. We plan to use E.coli to design a bacterium that can digest the nitrite and ammonium in its interior using the disposal system from the anammox. Through the introduction of luciferase, the energy can be transformed into bioluminescence. Therefore, we named it Shining Sanctifier. This new star in synthetic biology will be applied to the sewage treatment

system on a large scale while it can also be made into illuminating system.

Team AITM-Nepal: siRNA MEDIATED IMMUNE MODULATION FOR INNATE AND ADAPTIVE RESPONSE USING GENETICALLY ENGINEERED Escherichia coli

Canonical small interfering RNA (siRNA) duplexes are potent activators of the mammalian innate immune system. The induction of innate immunity by siRNA is dependent on siRNA structure and sequence, method of delivery, and cell type. The delivery of siRNA in a packaged outer membrane vesicle of gram negative bacteria is the theme of our work. The toll like receptor-7/8 activation by siRNA in order to boost the production of Interferon type -1 molecules to inhibit the viral and outer membrane LPS structure to activate Toll like receptor -4 to inhibit bacterial pathogens is the objective of this work. The delivery is made dependent on the peptide fragment which mediated the fusogenic mechanism so as to escape the endosomal compartment once endocytosed inside host(mamalian) cell. Thus freeing the siRNA to silence the myD88 transcriptin host cytoplasm making RISC complex and hence, activating TLR-7/8 in endosomal membrane formerly.

Team BIT: A New Strategy to Detect Antibiotics in Milk: Based on Sensors with Controllable Bio-enhanced Blocks

Bio-amplification, especially controllable bio-amplification is significant for biological detection. In a synthetic biological way, 2013 BIT iGEM assembled the T7 RNA polymerase gene and T7 promoter as an amplification block (amplifier), which is based on the high activity of T7 promoter to amplify the signal. To make the magnification controllable, a lacO operator regulated by lacI was assembled in downstream as a control block (controller), by adjusting the concentration of IPTG. With this block, several sensors of materials including but not limited to antibiotics are able to be enhanced controllable. This year, a sensor of beta-lactam newly designed and one of tetracycline are applied to detect the residual of antibiotics in milk which endangers human health. To make the detection faster and more convenient, milk samples and engineered E.coli are mixed in a tailor-made bio-chip and the green fluorescence will be detected and shown on a tailor-made electronic equipment.

Team BIT-China: Intelligent Microbial Heat Regulating Engine

To keep the cells in a good condition, cooling system is used to control the temperature in fermentation process. However, the cooling system can result in a great consumption of energy, which increases the cost of production and causes resources wasting, global warming indirectly. To settle this problem, we constructed an Intelligent Microbial Heat Regulating Engine (I'MHeRE), which includes the customized thermo-tolerance system and the intelligent quorum regulating system, to help cells resist heat by regulating the expression of heat shock proteins and controlling the density of cells. The chassis host with I'MHeRE may make the fermentation less depend on the cooling system and shrink cost. Besides, cells could live well in higher temperature, because we extend their optimum living temperature and make them live in optimizing density. Owing to this, the activity of the enzymes in cells could be increased and the efficiency of microbial metabolism could be improved.

Team Biwako Nagahama: AgRePaper&E.coli-ink

Cellulose is used as raw material for paper, so our team experimented various ways to increase the amount of cellulose produced by agrobacterium and using it to make papers. For this we developed the different parts to insert into the system of agrobacterium. Among them are the genes used for expression of the curdlan. Similarly, genetic parts in order to increase the expression of the cellulose, along with the

agrobacterium type binary vector were also developed . We are also working on recycling the produced paper by degrading the cellulose to D-Glucose using various enzymes. We worked for the preparation of the biological ink using the sperm whale's cells by genetically modification to increase amount of myoglobin. Then, we observed the change on the color of the product by altering the formation of myoglobin and the production amount of myoglobin with the insertion of T7 promoter to the cell system.

Team CAU China: Alcohol-detoxic Beverage

Alcoholism is prevalent in China. Here we decide to invent an alcohol-detoxic beverage that can considerably prevent alcoholism by adding one healthy bacterium-lactobacillus. In principle, this engineered bacteria can survive in the extremely acidic stomach environment and reduce the toxicity by converting alcohol to corresponding carboxylic acid through a two-step reaction. The two-step reaction is catalyzed by intracellular alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH),respectively.We try to engineer both enzymes, ADH and ALDH, to be acid resistant for higher performance in human stomach.

Team Chiba: Magnetic E. coli

In nature, there exist a variety of magnetotactic bacteria. Recently, it was reported that non-magnetotactic cells such as yeast can be magnetized to some extent. We set the goal to transform E. coli into those that are attracted by magnets. By magnetizing E. coli, the cell harvesting process will be much simpler and more economical than the conventional processes such as centrifugation and filtration. To this end, we are conducting three itemized projects. (1) modification of iron transportation network to import as much Fe ions as possible in E. coli, (2) sequestering/ storing iron into human ferritin, and (3) converting cytosolic space from reducing to oxidizing in order to elevate Fe(II)/ Fe(III) ratio within. Because all such manipulations significantly impact the physiology of the host cell, we are establishing the BioBrick platform that enables the temporal knockdown of multiple genes using recently control technology such as CRISPRi.

Team Fudan: ALeader: leading the advance of RNA synthetic biology

RNA regulation patterns, which have not been fully understood so far, is a research hotspot still deserving exploiting. A recently-discovered riboswitch ALeader updated our ideas by its delicate, 75nt-structure consisting of an aptamer, a recombination site, and even a bicistron motif. Inspired by this natural design, we proposed a series of novel strategies this summer, with dynamic rather than static perspectives. Guided by the theoretical study on functional multistable states and semi-static states of a riboswitch, and the kinetics involving impacts from other systems such as CRISPR, RNA polymerases, ribosomes, and degradation complex, the ALeader-based functional multi-phase and tricistron switches are designed. We also tried to regulate aptamer’s function by manipulating its working environment instead of itself, with SpinachALeader-based real-time monitors to avoid the signal distortion. Furthermore, to demonstrate the advantages of RNA biobricks, we constructed an antibiotic-detector with ALeader, optimized by a network with a RNA-OUT/IN translational regulatory system.

Team HIT-Harbin: B-POM: Biological proportional operational Mu-circuit

The composition of B-POM is that hrpR's promoter depends on the input, but hrpS’promoter is always Ptet and tet owns PhrpL, while the output gene follows tet and shares PhrpL. Once the input is sensed, the input promoter triggers hrpR's transcription. The activity of Ptet is constitutive, which means HrpS protein is ample. As HrpR accumulates, HrpS binds to HrpR and form HrpRS which then triggers PhrpL, and tetR

and output begin to accumulate. tetR can inhibit Ptet. As a feedback, HrpS and HrpRS will decrease. PhrpL will be of lower activity, so the amount of tetR and the level of output will decline. The decrease of tetR will enhance the hrpS' expression. All these construct a feedback cycle. Finally, the output will stabilize and be in a certain proportion with the input. By manipulating the RBS of hrpR, hrpS, tetR and output gene, we can control input-output proportion.

Team HokkaidoU Japan: “Maestro E. coli” ~optimization kit for expression~

Thousands of genes are expressed in living cells. Their expression is cleverly controlled by promoters and RBSs. Precise regulation of recombinant genes is hard to achieve. Imbalance in regulation results in little production. However, it is hard to objectively select promoters and RBSs. We thought that E. coli could do the selection for us. We created a kit for E. coli to find the best suited promoters and RBSs. It enables our lab E. coli to be like “Maestro” who creates excellent harmonies with lots of instruments. For the kit we created an original promoter and RBS families with different strengths. We checked and made these parts to be reliable. And it only takes a single golden-gate assembly to get your construct! We made the promoters and RBSs by selecting from randomized libraries. Using the kit, E. coli can choose optimal promoters and RBSs by her/it/him-self, just like the maestro.

Team Hong Kong CUHK: Switch off PAHs

To rapidly regulate biological process, we designed a novel transmembrane protein called Voltage Switch (VS), which is a fusion protein utilizing the voltage sensing domain from potassium ion channels. Triggered by change in potential across the cell membrane, VS can separate or bring targeting enzymes into proximity, thus allowing an instant control of enzymatic reaction. We also utilized VS to accelerate the polycyclic aromatic hydrocarbons (PAHs) degradation system – another highlight of our project. The metabolites of certain PAHs are mutagenic and carcinogenic. We codon-optimized laccase from Bacillus sp. HR03 and catechol 1,2-dioxygenase from Pseudomonas putida KT2440 for Escherichia coli, which when forming a cascade, PAH degradation into less toxic simple carboxylic acid would occur. Since quinones are intermediates in the degradation of PAHs, we also added quinone sensing and response repressor (QsrR) to control the degradation process.

Team Hong Kong HKU: E. capsi: Reducing phosphate pollution using engineered E. coli that harvests polyphosphate

Phosphate pollution in waterways and water treatment plants is a major problem. Removal of phosphate from wastewater is required to treat phosphate-containing discharge to reduce eutrophication, algal blooms and “dead zones” in lakes, rivers and coastal marine ecosystems. The aim of this project was to remove or reduce the levels of inorganic phosphate from a system or environment by employing engineered bacteria E. capsi, capable of accumulating phosphate in the form of polyphosphate. Our strategy is to express polyphosphate kinase together with the ethanolamine utilization (eut) bacterial microcompartment from Salmonella enterica to provide an environment for polyphosphate synthesis. Furthermore, the project provides a novel way to recover accumulated polyphosphate, an energy rich macromolecule with many industrial uses. This paves a way towards living system-based phosphate pollution treatment to tackle critical environmental challenges.

Team Hong Kong HKUST: FATBUSTER - The Artificial Futile Cycle

While low-fat diet and regular exercise are popular approaches to fight with obesity, one easy alternative is simply to increase energy metabolism. In a synthetic biology approach, we are working to create an

artificial futile cycle in mammalian cell by introducing glyoxylate enzymes native to bacteria. Past research has shown that mice expressing enzymes constituting an active glyoxylate shunt are shown to be resistant to diet-induced obesity. Our team plans to introduce an inducible system that allows us to couple the sensing of circulating fatty acid concentrations with an inducible circuit of glyoxylate shunt. Our inducible system is intended to prevent the risk of fatty acid deficiency, while facilitating greater fatty acid uptake at higher fatty acid circulating concentrations. Such a system should increase the feasibility of a glyoxylate cycle engineered to function in vivo.

Team HUST-China: Antihypertensive Ecoli

Hypertension causes grave concern worldwide for its notoriety, there're not many therapeutic methods to hypertension besides various antihypertensive drugs. However, this comes along with heavy financial burden to developing or underdeveloped countries. In addition, almost all these drugs have side effects to liver and renal. Here is a novel method to treat Hypertension by constructing human-friendly engineering bacteria that can produce short-chain fatty acids (SCFA) periodically and naturally to help maintain the blood pressure in safe level. SCFA, especially acetate and propionate, has been proved to induce vasodilatation and ensuing hypotensive response via receptors in smooth muscle cells of vessels. This year we have found a metabolic pathway in Escherichia coli that converts succinate to propionate through Wood-Werkman reaction. An operon consisting four genes encodes enzymes in this pathway. By combining bio-oscillator and key gene together, we want to make E. Coli release propionate periodically in patients' intestine periodically.

Team HZAU-China: Safe moving vaccine factory

For HZAU-2013iGEM project, we are creating a safe moving vaccine factory by synthetic biology which can spread Rabies vaccine in dogs rapidly and actively. Our aim is to help in the achievement of the WHO goal of being free of human rabies by 2020 through the improvement of the vaccination coverage in dogs. The idea comes from Yersinia pestis and fleas. We make use of fleas as our moving injector. When flea feed blood from dogs, our vaccine vector Bacillus subtilis will be regurgitated into blood and successfully transferred to mammalian host. Bacillus subtilis can express antigens which can stimulate the immunity of dogs. Meanwhile, endogenous or exogenous expression of 'Antimicrobial Peptides' by B. subtilis can kill Yersinia pestis in fleas. In this way we achieved a safe moving vaccine factory.

Team IIT Delhi: pHColi

pH induced response elicited by certain promoters in bacteria may have major practical applications. The response can be targeted for specific pH ranges, for example in tracking the anomalies associated with the gut micro-biota or detecting pH inside a bioreactor. There are only limited studies reported in the area. In the present project, a genetic circuit has been created, using the promoters of the acid shock response gene from E.coli and the F0F1 ATPase operon from C. glutamicum that produces a pH dependent colour gradient, much like a universal pH indicator. A mathematical model has been developed to simulate the experimental findings. The present study will form the basis for further research in the field of synthetic biology.

Team IIT Madras: COMBATING SHIGA TOXIN : A SYNTHETIC BIOLOGY APPROACH

Shiga toxin, a worldwide menace, has killed over 1 million people to date and continues to afflict almost 150 million people each year. Currently, there is no treatment for Shiga toxicosis and it leads to complications in the human system like hemolytic uremic syndrome (HUS) and renal failure. Here, we

propose a two-fold, novel synthetic biology approach to combat the lethal effect of the toxin. We aim to neutralize the already produced toxin through a nine amino acid Gb3 mimic peptide. We have engineered the Gb3 mimic along with a cellular export signal (ompF) downstream of AHL(quorum sensing molecule) inducible promoter (pLuxR). We also plan to prevent further toxin production by inhibiting the biofilm formation of shigatoxigenic E.coli using indole-3-acetaldehyde (I3A). We expect to validate our approach through functional assays and in silico modelling. Our findings can potentially initiate a new perspective of tackling Shiga toxicosis using synthetic biology tools.

Team ITB Indonesia: Aflatoxin Biosensor

Aflatoxins are naturally occuring mycotoxins that are mutagenic and carcinogenic. Aflatoxin contamination of foods that are found in many developing countries may cause a serious problem for human health. ITB_Indonesia team for iGEM 2013 focuses on designing a whole cell biosensor for aflatoxin B1 detection in foods. The biosensor uses Escherichia coli as the chassis to build a genetic circuit using SOS response system to detect DNA damage caused by aflatoxin B1-oxide attack. The SOS response promoter is followed by a reporter gene coding a chromoprotein, therefore the concentration of aflatoxin B1 in food samples could be easily detected by the color change of the bacteria. For the ease of usage, we will design a syringe shaped device with our whole cell biosensor in it. This device would allow aflatoxin B1 to enter the device, but would not permit the cells to leave the device.

Team KAIT Japan: Hay fever curE.coli

Japanese on of six people is troubled now by hay fever. These people take a medicine for the hay fever. But, If they take it , they become sleepy. If become sleepy, they cannot work and study. So, we are working on a project to relive hay fever by Escherichia coli to improve these. Mechanism of hay fever When an allergen invades it in the living body, naïve T cell differentiates in Th2. There is more Th2 than Th1, and the mast cell and others that is humoral immunity become active, and inflammation is in this way caused. We perform following four this time. ①Expression of IL-10 receptor to E.coli. ②Phosphorylation of STAT3. ③Preparation of gene array with HlyA and L-12 promoter and receiving the STAT3. ④Preparation of gene array with TolC and HlyB and HlyD promoter and to receive the STAT3.

Team KIT-Kyoto: Fregrance coli

We are trying to construct a novel E.coli that has fruity flavor like Japanese rice wine (Japanese sake). In order to accomplish the purpose, yeast genes related with production of the Japanese sake fragrance were introduced into E. coli cells. We also tried to develop a way to eliminate bad smells of E. coli in parallel. Although we previously won a gold prize by the development of a novel pen (E. coli Pen) in 2010, its bad smells were weak points and must be improved. We will overcome this problem through the progress of our new project in 2013. So far, “smell” is not a popular keyword and not a major field in iGEM. However, we believe that our project will provide a new point of view to iGEM friends

Team Korea U Seoul: Pearl-coli: E. coli converting CO2 into a pearl powder (nacre)

The Korea_U_Seoul team aims to design Pearl-coli that is E. coli able to convert atmospheric CO2 into pearl powder materials. The design is based on cell surface display of nacrein in E.coli. Nacrein is a major protein component in nacre(an organic-inorganic composite layer found in outer coating of pearls). We divided nacrein into functional regions - carbonic anhydrase(CA), calcium binding and scaffold repeats. CA domain fixes CO2 into carbonic acid changing to bicarbonate ion in aqueous solution. We will examine if displayed nacrein in E. coli can make a pearl powder in a solution or fabricate a nacre-like structure while

atmospheric CO2 is fixed into bicarbonate. Once a nacre material can be prepared from Pearl-coli, we will grow E. coli in a confined container to make synthetic pearl. The Pearl-coli has dual-function such as (1) mitigate the global warming by CO2 reduction, (2) prepare valuable pearl-like raw materials.

Team Kyoto: Oscille.coli

Every organism has its own cycle such as the periodicity of cell division, ordered patterns of its body. Some kinds of the cycles are regulated just by two factors. Using E. coli, we applied this kind of periodicity formation.</p> Firstly, we focused on oscillation regulated by RNA. We suspected if RNA world hypothesis is correct, there could be protein-depended oscillatory system. To show the possibility of cycle formation by RNA, we constructed an oscillator by utilizing two different types of functional RNA, which are transcriptional activator and repressor.</p> Secondly, we also targeted on planar oscillation forming mechanism. A. Turing (1952) suggested a simple principle containing just two variables explains many organisms' epidermal pattern formation. However, it is not confirmed the pattern formation is only based on Turing's discourses. To check this, we used two types of E. coli, which secrete different factors, and regulated their population.

Team LZU-China: Twinkle Cancer Hunter

To construct a regulating vector of NF-κB signaling pathway by gene recombination technology, introducing into tumor cells with NF-κB to form a signal feedback control system. Using NF-κB binding elements as promoter,and IκB-GFP fusion protein as reporter.Then inverted into HEK-293T cells and DU-145 cells.Through the observation of the GFP to probe the expression of IκB. The expressed protein was identified by Western blot, etc.The constructing of a regulating vector of NF-κB signaling pathwayprovides a new method and thought for tumor gene therapy, and propel forward the research of NF-κB signaling pathway.

Team Macquarie Australia: Green is the new black - Expression of Chlorophyll within Escherichia coli

Photosynthesis is a key biological pathway that uses sunlight energy to convert water and carbon dioxide into ATP, glucose and oxygen. Chlorophyll is a green pigment that facilitates this energy production in photosynthetic organisms. Although the biosynthesis pathway for chlorophyll has been thoroughly investigated, the reproduction of this pathway in a non-photosynthetic organism has, to date, not been achieved. Successful production of chlorophyll in a bacterial host is the first step towards the synthetic construction of photosystem II, and the eventual creation of a renewable energy source. Our research involves expression of twelve genes (from Chlamydomonas reinhardtii) necessary for the chlorophyll biosynthesis pathway in a bacterial host (E. coli). Gene sequences have been synthetically designed to allow for prokaryotic expression. By utilising Gibson assembly, we plan on being able to successfully produce chlorophyll in prokaryotic cells. This will be evident from the growth of green E. colicolonies.

Team Nanjing-China: Atrazine Elf

Atrazine, a widely used herbicide, persists for a long period in the environment onced used. It causes metabolic disorders in both animals and humankind. Our team utilized the ribosome switch induced by atrazine, a QS system of Plux and a degrading enzyme to control E.coil's motility through regulating it's CheZ gene. Therefore, E.coli can recognize atrazine, recruit team workers, and degrade atrazine. Our team found a transporter of atrazine, which we call TRM. We also mutated the degrading enzyme, TrzN, making it better at degradation. We combined TRM and the TrzN to improve atrazine absorbance and

degradation. Moreover, our team are trying to analyze and compare several systems with computer, hoping to find the best one which is equipped with faster moving and quicker degrading. Overall,we believe our system will boost the industrialization, universalization as well as standardization in the field of treatment for atrazine and other versatile small molecules.

Team NCTU Formosa: E.colightuner

We have proven a sRNA-regulated system of our own to be an effective and competent way for regulating gene expressions. Recent studies have shown that sRNA-mediated regulation is an important factor to bacterial growth. sRNAs work by base pairing with limited or extended complementary target mRNAs, regulating proteinproductions. Using sRNA mechanism, we can control gene expression in RNA level, in contrast to common promoters that functions on DNA level. Since the existing sRNAs in Escherishia Coli have important functions in other metabolic processes, we designed an artificial sRNA with high specificity to avoid undesired base binding in vitro. By using the sRNA-regulated system, red light induced operator, and thirty seven degree Celsius ribosome binding site (RBS), we constructed a manipulatable system that is capable of expressing four different genes under different conditions. In other words, it is a multitask machine.</p>

Team NJU China: Biomissile: a novel drug delivery system with microvesicle

Recently, small interfering RNA (siRNA) has emerged as a promising therapeutic drug against a wide array of diseases. However, site-specific delivery has always been a challenge in gene therapy. Microvesicles (MVs) are lipid-bilayer vesicles which are naturally secreted by almost all cell types, playing crucial roles in intercellular transport of bioactive molecules. Given the intrinsic ability to naturally transport functional RNAs between cells, MVs potentially represent a novel and exciting drug carrier. In our project we are trying to express both anti-virus siRNA within the cell and target protein on the surface of the MVs by engineering the HEK 293T cell, which is capable of producing large amounts of MVs. Thus, the MVs produced by our engineered HEK 293T cells will contain the siRNA and be able to specifically deliver the siRNA to the sites we want, acting as biomissile for the targeted destruction of the disease.

Team NJU NJUT China: The Application of Cas9 as a Gene 'Missiles'

Most bacteria and archaea can resist invading DNA and/or RNA elements via the clusters of regularly interspaced short palindromic repeats (CRISPRs).It is believed that the integrated CRISPR sequences have the ability to form a genetic memory which prevents the host from being infected.The memory exist as a DNA library in genome, artificially modified to set its target. The CRISPRs and Cas (CRISPR-associated) interact and form this prokaryotic adaptive immune system. Cas9, as a core of CRISPRsystem, can play a role of targeted-attacking gene 'missiles'. Therefore, we build a sort of plasmids, loading CRISPR system, to realize the 'killing' of harmful genes and/or organisms.

Team NTU-Taida: QS array

Bacterial infection is the invasion of the body by pathogenic bacteria, which causes pneumonia, urethral infection, bacteremia and other symptoms in hospital and community. The efficiency of traditional detection and diagnostic approaches is impeded by the time-consuming laboratory procedures, yet many of which grow poorly in bacterial cultures. All these limitations call for a new rapid and direct bacterial identification method to improve patient management and antimicrobial therapy. Quorum sensing is a type of bacterial cell-cell communication correlates with the population size. Many bacteria have one or several species-specific quorum sensing molecules released in different growth state and environment. Quorum sensing

signals are shown to be involved in many physiological functions, including virulence, biofilm formation and drug-resistance. We aim to establish a novel bacterial identification method in clinical samples based on the quorum sensing profiles.

Team NTU Taiwan: YeasTherm - against the cold

During winter season, due to low temperatures, fish farming is one of the most heavily affected economic venues. Due to this, year after year, several farmers are faced with many problems as a result of a loss of fish product. </p>Using our background in bioengineering we suggest an innovative alternative: Our idea is based on heterologues expression of SrUCP in *Saccharomyces cerevisiae* and *Rhodotorula glutinis*. Through the expression plasmid, yeasts are transformed from the wild-typephenotype into a thermogenic phenotype. </p>To implement this idea and make it simple and efficient, we plan to drive the expression of SrUCP under the control of cold shock promoter Tir1. In this way, yeasts will generate heat only when the temperatures drop. Moreover, the temperature-responsive range of Tir1 may be regulated by applying genetic circuits, providing the means to manipulate the biological device to suit different temperature conditions and needs in application.

Team NU Kazakhstan: Detection of Carcinoembryonic antigen with sandwich-biosensor

Carcinoembryonic antigen (CEA) is the cancer biomarker at early stages of several cancers including colorectal carcinoma, lung carcinoma and others. The aim of the study is to develop a biosensor that can be used in the detection of CEA. In the first part of the study ssDNA aptamers, with strong affinity for CEA, were selected by 12 cycles of Systematic Evolution of Ligands by Exponential Enrichment procedure, and characterized with dot-blot analysis and Surface Plasmon Resonance methods. In the last part, it is planned to clone the genes that will assist in expression of streptavidin on the surface of E. coli and S. cerevisiae membrane. E. coli will deliver streptavidin on the surface via Lpp-Omp expression system, while S. cerevisiae via Aga1 – Aga2 system. Modified model organisms, aptamers and CEA will be used to constructsandwich-biosensor.

Team NYMU-Taipei: Bee. coli: to bee, or not to bee

To save bees from Nosema ceranae, the culprit of colony collapse disorder, we created Bee. coli. from E. coli K-12 MG1655, a bacterium residing natively in bees. Bee. coli is strategically designed to work as follows. First, it continuously secretes mannosidase to inhibit the sprouting of N. ceranae spores. Second, if the bee is infected, the fungus-killing-circuit with a positive feedback design will be turned on to wipe out N. ceranae. Third, if these designer weapons should fail to conquer N. ceranae, our designed bee-suicide-operon will be activated to kill the infected bee and save its companions. Fourth, a light-inducible lysis system is included to ensure our Bee. coli only lives inside of the bee. Fifth, we apply encapsulation as the way to send Bee. coli into the bee. Since the capsule will only dissolve in a bee’s gut, our Bee. coli will not spread to the environment.

Team Osaka: Beat the discrimination against E.coli !

Since the middle of the 20th century, Escherichia coli(E.coli) have made great contributions to various field of our society. Although they have played essential roles in the society, it seems that they are not properly appreciated by general public. People's common images to E.coli are very negative (dirty, stinky, dangerous etc). So in our project, to wipe away the negative images to E.coli, we have created a circle that enable them to communicate with each other via nutrient production.And we made “empathetic E.coli” that

lives cooperatively with each other. Then, by conducting experiments and using computer simulation, we have examined how they live and grow in liquid medium culture and what kind of pattern they form on solid medium culture.

Team OUC-China: Reconstructing the Magnetosome Membrane in E. coli

Membranous organelles are unique structures of eukaryotic cells, rare bacteria and paleontology. Magnetospirillum magneticum is an important biological model system of prokaryotic organelle study because the structure of magnetosome in Magnetospirillum magneticum has similar traits to eukaryotic organelles with membranes. Our task is to reconstruct the magnetosome membrane in Escherichia coli. Magnetospirillum magneticum requires a micro-aerobic and oligotrophic environment in order to produce magnetosome, so the significance of our project lies in simplifying the magnetosome produce method, opening up the path for futher functional gene research. We use homologous recombination to transfer the mamAB gene into E.coli to build an IMS part. Also, as the mamK gene is crucial to the IMS construction. We want to improve the mamK gene's expression by stabilizing its mRNA with a new method, hoping it can be used to promote the IMS construction. So we design a DNA segment to slow down mRNA degradation.

Team Peking: Aromatics Busted

Aromatic pollution is becoming a worldwide concern, and monitoring aromatics remains challenging. Noting the abundant genomic data of prokaryotes from aromatics-rich environment, Peking iGEM applied part mining to the genetic repertoire to develop a comprehensive set of biosensors for aromatics. The transcriptional regulators for each typical class of aromatic compounds were bioinformatically determined and promoter engineering and protein engineering were performed to tune their function. To expand the detection range, enzymes in upper pathways, working as plug-ins, were coupled with biosensors to degrade aromatics to detectable compounds. For environmental detection, we construct the band pass filter to detect a certain range of concentration. Responses of biosensors equipped with band-pass filter can robustly reflect the concentration of environmental samples. Peking iGEM has remarkably enriched the library of biosensors for aromatics and enabled quantitative detection for environmental monitoring. These biosensors will be also potent for metabolic engineering and well-characterized synthetic biological tools.

Team SCAU-China: Detection and degradation of organophosphorus compounds

Synthetic organophosphorus (OP) compounds, which are highly toxic contaminants in agro-environment and food security, have been widely applied to pesticides. Parathion is a typical representative of organophosphorus pesticides. This year, our goal is to construct a p-Nitrophenol sensor in E.coli, which is the degradation product of parathion, in order to reflect the existence of parathion. Besides, we try constructing a degradation system to solve the pollution problem. Considering the biosafety problem, we also design a suicide system in which the lethal genes are only triggered by declining p-Nitrophenol concentration. This will enable the bacteria to commit suicide when p-Nitrophnol is sufficiently degraded.

Team SCUT: E.cerevisiae

E.cerevisiae is a sophisticated signal transport system between E.coli and S.cerevisiae. Producer, the E.coli, is assigned to distribute a special volatile—butanedione periodically with a stable oscillation circuit, which defines the meaning of the signal. On the other side, Sniffer□the yeast, transplanted with a nose from nematode, can respond to the signal immediately. We hope this can realize the communication between prokaryotes and eukaryotes for the further research on symbiosis.

Team SCU China: Imitations of Gametogenesis & Sexual Reproduction using E.coli

We intend to construct two groups of differentiated E.coli,one imitates the male multicellular organism ,the other for the female. When cultured separately, the male/female multicellular system gets bigger and matures, and cells will differentiate into gametes,which cannot divide any more but are capable of gene transfer.</p> After that, you mix this two liquid cultures,the male gametes will recognize the female cells and begin to transfer modified F plasmids into female gametes through sex pili. The conjugationmakes female gametes return to the state of un-differentiation(called G cells),which means they can divide again but are not sexually determined.Then,after several cell divisions,one G cell will differentiate into a G+ or G-,which, like zygote, can grow into next generation of the multicellular system maybe containing genes from both male and female gametes.

Team Shenzhen BGIC 0101: Genovo

Genovo is a Computer-Aided Design (CAD) tool used for denovo design of genome. The current version consists of 4 parts. The first, Chromosome Construction will grap genes in a common pathway and chromosome features to build a new genome and let user to define the order and orientation in drap-drop way. The second, NucleotideModification will optimize and soften the sequence of CDSs. It also help design the CRIPSR sites so that we can silence the wild type genes. The third, ChromosomeSegmentation will cut chromosome into pieces and add 3A & Gibson & Goldengate & Homologous Recombination adaptors to the pieces automatically for assembly. The last one, OLS Design will guide users to gain the chromosome by microarray. Genovo will enable user to design their innovative chromosome as their wishes and further the research on genome on pathway level.

Team Shenzhen BGIC ATCG: Cell Magic

Cell Magic plays a gorgeous movie show in the both E.coli and S.cerevisiae.Various colors are blooming in different branchs & buds: plasma membrane, nucleus matrix, mitochondria membrane & matrix, vacuolar membrane, peroxisomal membrane, centrosome, and also actin. But the scene is far from static, colors will show up in order under the sophisticated cell cycle system at G1, S, G2 or M phase. Accelerator—degradation system is applied to run this movie faster, and freezer—sic1 system will put off the cell cycle during G1 phase. Beside, the editor—intron will expands a random dimension, leading to produce more combining form.

Team SJTU-BioX-Shanghai: Metabolic Gear Box

Few researches have been done to regulate gene expression levels in genomic scale so far. This year we aim to combine two systems together in order to provide a universal and convenient tool which can be used to regulate different genomic genes simultaneously and independently in a quantitative way.

Our project involves the newly developed gene regulating tool CRISPRi and three light-controlled expression systems induced by red, green, and blue light respectively. Simply by changing the regulating parts in CRISPRi system towards mRFP, luciferase, and three enzymes, we hope to prove our system can be used qualitatively, quantitatively and practically step by step.

We have also designed a box and written a software as our experiment measurements. Simply by typing in several parameters, different gene expression levels can be controlled. This system can also be improved to predict the maximized producing efficiency after some simple tests in future.

Team Sumbawagen: E. coli which able to measure the level of sugar in honey by emitting light

Glucose and fructose are major sugar component of honey. Sumbawa honey is protected as geographical indication by Indonesian patent office. Sode Lab at Tokyo University of Agriculture and Technology has created a fusion of mutant glucose binding protein and firefly luciferase, which able to measure glucose level by emitting light - intended initially for blood glucose sensor application (Taneoka et al, 2009). In this project, we plan to create this construct in Biobrick format, and evaluate the ability of transgenic E. coli for the measurement of glucose in honey. Our final goal is to create a device which can be used for quality control of Sumbawa honey, which we call 'ECONEY'.

Team SUSTC-Shenzhen-A: Game Theory--Stategy for the Classic Prisoners' Dilemma

There are many applications of the game theory in some aspects of our life. Each individual has two kinds of choices--to betray or stay silent, and the choice you make would determine your fate. To betray the other side, you may risk being revenged. While staying silent, companion's betrayal may hurt you deeply. As for our project, we work out a new way to imitate the game theory by constructing a community of two E. Coli bacteria. Here we use the growth rate of each species to represent its fate. The effect of one's silent or betrayal on the other species' fate is acted through intercellular signal molecules of two quorum sensing systems. Each signal molecule regulates the expression of toxic genes in the other species and reduces its growth rate. We characterize the consequence of each strategy by quantitatively measure the growth rates of each species in the community.

Team SUSTC-Shenzhen-B: Circuit+

To standardize genetic circuits and bring the idea that relations exist in circuit between parts back to synthetic biology, we proposed our Technical Standard RFC 101 and RFC 102 to define genetic circuits and logical gene gates. To solve the problem that synthetic biology lacks such a database to systematically record genetic circuits and to make the standards work, we built Circuit+, an online registry of standard genetic circuit which records information of circuits based on the two standards. Users can retrieve circuits, browse information, share by exporting SBOL and upload new circuits. We also have developed Clotho version Circuit+, Circuit List and transplanted TTEC to Clotho. And we have developed an online platform for synthetic biology lab management. We also did human practice to promote synthetic biology and iGEM.

Team SydneyUni Australia: Keeping DCA at Bay - Assembly of synthetic constructs and cassettes for degradation of dichloroethane.

The picturesque city of Sydney is marred by industrial efflux of chlorinated hydrocarbons into the aquifers around Botany Bay. 1,2-dichloroethane (DCA) is toxic and a suspected carcinogenic agent, and one of the more soluble and mobile contaminants. Conventional DCA treatment is both costly and time-consuming, involving pumping and heat-stripping groundwater. We propose a biological alternative which may be cheaper and more effective. There are strains of bacteria able to degrade low levels of organochlorine compounds in selective conditions. Polaromonas JS666 and Xanthobacter autotrophicus GJ10 contain two pathways of particular interest. Our goal is to construct our own versions of two metabolic pathways of DCA biodegradation for comparison in a BioBrick-compatible vector, and characterise their effectiveness in utilising DCA as a sole carbon source for growth. We hope to create friendly strains of bacteria capable of removing DCA at greatly reduced cost and effort, and reduce the environmental impact of industry.

Team SYSU-China: iPSC safeguarding Device

Since Shinya Yamanaka published the epoch-making paper in 2006, the induced pluripotent stem cells(iPSCs) has become one of the most promising techniques in regenerative medicine. Like embryonic stem cells(ESC), iPS Cells can be differentiated into any tissues. Compared with ESC, iPSC is easier to attain, immune rejection-free, and ethical issue-free. However, Rurther application of human induced pluripotent stem cells(hiPSCs) in tranlational medicine requires the concerns of two problems: the specificity of directional differentiation and the safety of the transplant. Here we design a new device which can spontaneously select hepatocytes from iPS differentiated cell mass and prevent potential carcinogenesis. To achieve accurate spatiotemporal control,we build a miRNA-122 sensor and make use of the tetracycline induction system. Our work may also be extended to the field of gene therapy, and provide a new direction to our train of thought about how to solve the safety problem in genetic manipulation of human cells.

Team SYSU-Software: CAST (Computer Aided Synbio Tool)- An Integrated Tool for Synthetic Biology

Accurate simulation and gene circuit design are essential but difficult parts in synthetic biology.Here, we designed CAST to cover the workflow from beginning to end, users can focus on function design and the gene circuit would be automatically designed. Furthermore, we developed a new simulation model that work with standard dynamic characteristic and verified by wetlab experiments. Moreover, we build an expandable database that users can contribute their own dynamic information which would lead to more accurate and sufficient dynamic information of all the Biobricks. Finally, our software is designed as an easy deployed server so that it can be used on personal purpose or shared by a whole lab or institution.

Team Tianjin: Alk-Sensor, a Novel Detector Applied for the Selection of Alkane Producers

Biosynthesized alkanes are promising candidates for drop-in replacement of petroleum. We constructed and characterized a device named Alk-Sensor, which can sensitively detect a wide range of alkanes and generate certain response. Alk-Sensor is composed of ALKR protein——a transcriptional regulatory protein, and promoteralkM. ALKR recognizes alkanes and their interaction triggered a conformation change of ALKR dimers which isomerizes the promoter-RNAP complex and led to activate the downstream genes of PalkM.

Based on Alk-Sensor, we built a relationship between productivity of alkanes with strain’s growth rate under certain environmental stress. Starting from this relationship we further designed a novel selection method to select out the engineered strains with highest productivity of alkanes. We demonstrated that this novel selection method could enable us to select out the optimized strains effectively and efficiently.

Team TMU-Tokyo: Genomic 'Pythagorean Devices'

In this year, TMU-Tokyo created Genomic 'Pythagorean Devices'. Pythagorean Device appears Japanese famous educational TV program 'Pythagorean Switch' Pythagorean Devices are known in the US as 'Rube Goldberg machines'. Pythagorean Devices are deliberately over-engineered or overdone machines that performs a very simple task in a very complex fashion, usually including a chain reaction. We constructed a Pythagorean device in Escherichia coli genome, using lambda phagerecombination system 'RED'.

Team Tokyo-NoKoGen: Twinkle.coli -Fast cycle! Fast response!-

We created Twinkle.coli, which “blinks” fast like a firefly. An oscillator is a system that responds in periodic

changes. This response is usually regulated by positive or negative feedbacks by using inducer or repressor proteins. However, the use of proteins might delay the response because transcription and translation must happen before the next output. To design an artificial fast responding oscillating circuit, we designed an RNA-based oscillator. We used RNA-responsive self-cleavage ribozymes whose cleavage is regulated by an RNA molecule. The ribozyme cleavage cuts-off an “RNA scaffold” that harbors RNA aptamers. This aptamer binds to its specific target proteins, which are directly fused to reporter protein. This binding recruits the already translated split reporter protein complementation resulting in the output (twinkles). Our system enabled fast response and short oscillation cycle.

Team Tokyo Tech: ‘Mutant Ninja. coli’

In our project, we propose to create E. coli that mimic some of the qualities of Japan’s ancient ‘ninja’ warrior-spies. A ninja must receive and pass on correct information at all times. A mistake will be fatal. We have created a circuit that avoids crosstalk between two signals in cell-to-cell communication, and we are also looking into applications for it. Ninjas are also known for their star-shaped ‘shuriken’ throwing knives. Our E. coli ninja has a similar weapon, an M13 phage which it releases to infect other E. coli, injecting plasmid DNA into them. Finally, ninja must harmonize with the natural environment, so their relationship to it is very important. Plant hormones help plants to grow efficiently, and we are attempting to construct a circuit that synthesizes two plant hormones depending on the soil environment.

Team Tsinghua: Mobile Health---Pathogen detector

In a long term, the testing of pathogenic diseases is via comparably complex procedures. This year, we aim to design a sensing yeast powder based portable test paper, that is, the 'mobile' testing system, take advantage of querom sensing system in bacteria, to achieve the testing of specific microorganism caused disease. In the same time, we built a frame of testing any pathogen that will cause diseases, using different the input and output combination. Furthermore, in order to achieve the simultaneous testing of different pathogens, we design a “fast-shifting box” to accomplish the combination of input and output signaling. This will in theory

Team Tsinghua-A: Synthetic gene switch shows adaptation to DNA copy number variation

In some natural and synthetic biological networks, DNA copy number which transfection into cells is fluctuant-influencing gene expression. We hope target gene expressionlevel has a strong adaptability and ability to DNA copy number by using the method of engineering and bringing in incoherent feed-forward circuit. The robust circuits we designed may apply to cancer detection and gene therapy in the future. Generally speaking, we modeled three and four nodes motifs to find some appropriate circuits, which function reliably in the face of fluctuating stoichiometry of their molecular components. Two designed circuits have been tested and we found that the motifs has certain robustness to DNA copy number.

Team Tsinghua-E: Darwinian evolution for microbial cell factory:in vivo evolution engineering towards tryptophan-overproduction superbug

Darwinian evolution shows great power in creating incredible biological function in amazing speed. Inspired by this, our team aimed at creating novel fast and irrational microbial cell factory by simulating natural Darwinian evolution process. With tryptophan as target product, a novel tryptophan biosensor utilizing translating ribosomemechanism was firstly developed as the foundation for tryptophan productivity and selection pressure switch module. We further constructed this tryptophan overproduction selection gene

circuit coupling with in vivo mutation machine (mutator gene of mutD). By fine-tuning the selection conditions, our selection circuit showed good tryptophan dependent growth property, which provides the foundation for further evolution. As a preliminary result of this project, we successfully evolved an ancestor with zero productivity to a high-tryptophan producer only after several rounds of evolution.

Team TzuChiU Formosa: Hypnoseq.

The new pattern of antibiotic resistance is a spreading global issue that may soon leave us defenseless against bacterial infections. Taking a closer look, the lack of comprehensive pharmaceutical management system in Taiwan has come to our concern as it results in easy access to antibiotics. Large amount of antibiotics are added in the forage of animal husbandry and aquaculture,hence, leading to the increase of antibiotic resistance in Taiwan. In order to ameliorate this growing threat, we attempt to carry out “Hypnoseq.” to make this world a better place. Our aim in this project is to combine the sense and antisense mRNA of the antibiotic resistance gene to inhibit the expression of the antibiotic resistance gene. Knowing that they have the ability to conjugate and deliver our designed plasmid to other bacteria, we are able to predict that they can decrease the percentage of antibiotic resistance in the environment.

Team UESTC: Nebula

Nebula is a biological circuit design tool composed of Interactive Part & Automatic Part. We classified the parts released in 2013 and constructed a database for users to choose what they want. In the first part, you are free to link any parts that we have already classified together to meet your requirement. In the second part, once you determine the inducer and the product, our software will offer you the optimized circuit with the input and output that you designated. We use Analytic Hierarchy Process to score every part and edges (passage linking two parts) according to attributions including availability, usefulness, sample status, part status and sequencing. According to weight of edges, we regard the shortest passage between input and output as the optimum presented to users. You can also save the circuits made in Nebula in case you want to check or change it later.

Team UESTC Life: Multistage Degradation of Environment Haloalkanes Contaminant by Co-expression Enzymes

1,2,3-Trichloropropane (TCP) and an organic pesticide-Hexachlorocyclohexane (Lindane-HCH) have been shown to be serious pollutants as they are toxic and quite persistent in the environment, and need to be removed to low levels from polluted sites. Microbial degradation of these compounds represents an important and efficient way to fulfill the target. In order to improve biodegradation efficiency, several powerful genetically engineered E. coli strains have been constructed by the co-expression of key enzymes involving in the biodegradation pathways of the two compounds. For this, foot and mouth disease virus 2A peptide and polycistronic co-expression strategies were adpoted. The results showed that all enzymes could co-expressed as a soluble protein with 2A peptide acting as a linker. Moreover, the resulting engineered E. coliexhibited an excellent capability for the degradation of TCP.

Team UI-Indonesia: Project Blue Ivy - scFv with Blue Indicator as a Biosensor for TB

Tuberculosis (TB) is a worldwide major health problem which infects one third of the world’s population. The absence of reliable diagnostic tool in suburban area, where TB cases are most likely found, is still a great obstacle in TB eradication effort. Seeing Indonesia as one of the high burden countries for TB, UI-Indonesia iGEM team are trying to create a reliable, portable, and easy to use diagnostic tool for detecting TB. We are constructing a biosensor consist of highly specific antibody bound to a fragment of β-

Galactosidase as a reporter to detect the presence of protein Ag85, a novel TB biomarker. Our goal is to make a biosensor that will detect the presence of antigen 85 in blood serum of TB suspect. Positive result will be indicated with easy to detect blue color, and when it's negative, no response will be observed.

Team USTC-Software: Gene Network Analyze and Predict (gNAP)

Synthetic biology creates and uses standardized parts such as Biobricks to build engineered bacteria for various function. To realize those purposes, importing exogenous genes to target bacteria is universal and essential. In this approach, improve or reduce the expression of target genes through interaction is inevitable. Experiments in wet lab could find the effect and choose the best of imported exogenous genes but take a long period of time. gNap utilizes Internet databases to construct a gene regulatory network (GRN) and analyze the effect of exogenous gene by Michaelis-Menten equation and sequence alignment algorithm. Meanwhile, to guide wet lab experimenters to find the best imported gene in the whole network, we use PSO method to figure out the best regulation patterns of new imported genes meeting experimenters' goal. To realize those ideas, we build gNAP that provides researchers with gene network analysis and prediction.

Team USTC CHINA: T-VACCINE

T-VACCINE is a vaccine initiating immune response by penetrating the skin with the aid of transdermal peptide. From now on, injections are simply history.Based on the theory of user-friendly, a special group of engineering bacteria which produce T-VACCINE is used to create a brand-new 'band-aid' serving as a guardian of our health .We have found a kind of transdermal peptide TD-1,a magical molecule that enhances the permeability of the skin as well as draw filamentous bacteriophages into the skin.By combining the gene fragments of antigen,immune adjuvant LTB and Luman-recruiting factor TNLFα with that of the TD-1, our team got the permeable fusion protein. In order to obtain large amount of extracelluar protein, we chose bacillus subtilis WB800N as our expression chassis. Further more, the universality of our experimental method is verified by the adoption of various antigen of existing vaccine, such as HBsAg, PA and AG85B.

Team UT-Tokyo: Multicellular Analog Clock

We designed a 'multicellular' E.coli clock with a clock hand. Your naked eyes see the red clock hand moving along a circle of E. coli population on an agar plate. The clock hand, expression of mCherry gene, is driven by an "engine" which is constructed under the inspiration of the mechanism of action potential conduction in nerve cells. The engine consists of a positive feedback loop of AHL and negative feedback loops of TetR, AiiA and 2 types of artificial sRNA. We also designed UV reset devices using UV sensor construct. In addition, small RNAs were designed for metabolic engineering of E. coli, which is the first trial in iGEM competition. We show you the new and easy approach in genetic engineering with the BioBrick parts, which will lead to future application of sRNAs in synthetic biology.

Team WHU-China: Master of Regulation: dcas9-based Multi-stage Gene Expression Regulator

Cas9 is an RNA-guided dsDNA nuclease utilized by bacteria immune system. The genetically engineered Cas9 has recently been shown to have the ability to repress or activate desired gene expression. </p>In practical research and industrial application, we usually face the problem to express a gene at different levels, not only "on" or "off ", so a more flexible regulation method is needed. To achieve multi-stage regulation of target genes, we further develop several dCas9 devices in which dCas9 alone or fused with

omega subunit of RNAP is directed by various guide RNAs to different regions of designed double promoters. Therefore, promoters with disparate strength can be either activated or repressed respectively and multi-stage gene expression can be achieved. Also, based on such novel technology platform, we are developing diverse applications such as a guide RNA-mediated oscillator.

Team XMU-China: A SynBio Oscillation Signal Converter

Oscillations permeate every corner of the world, from the alternative current AC in power lines to our tiny microorganism friends. To use oscillations in bacteria as a strong and steady signal transmission method like AC, we need to tackle with the noise of transcription and translation in the cellular environment by coupling millions of cells through the synchronizing genetic oscillations in E.coli. At the colony level cells could be synchronized via quorum sensing, which is limited to tens of micrometers by the AHL, and between colonies a gas-phase redox (mainly H2O2) will serve as a signal that can give positive feedback to the whole circuit over millimeter scales simultaneously. On a liquid crystal display (LCD)-like microfluidic array bacteria grow in separate colonies, so that synchronization in both levels could be verified visually. Now a robust synthetic biology signal converter is accomplished and ready to show the growth environment of cells.

Team XMU Software: Biobrick evaluation and optimization software suit and lab assistant tool

The biobrick evaluation and optimization software tool suit (Brick Worker) provide analysis of biobrick sequences, namely, promoter, RBS, protein coding sequence and terminator. We use PWM algorithm to evaluate the relative strength of promoters and RBS and precisely locate the key region of the sequence that affect its performance. Through codon optimization and GA algorithm our program can analyze and then optimize the protein coding sequence so as to enhance the protein expression level. Terminator efficiency prediction is also included in this suit. As for the lab assistant tool (E'Note), it is a powerful experimental recording platform with exhilarating functions such as multi-line operating, software tool integration and template customization, providing a all-round as well as customized tool to significantly enhance the efficiency of experimental work.

Team ZJU-China: A Tale of Aptamers: Ghost and Elf

This year we aim to utilize aptamer to specifically detect and clear molecules of different sizes. In order to detect and clear certain protein, we make tunneled E.coli called bacterial ghost that allow protein to diffuse in. We then build two types of inner-membrane protein scaffold, which will dimerize when pulled together by two aptamers attached to two sites of the protein. The dimerized proteins have enzymatic activity that can be detected via commercial test strips. The device will also sequester the proteins and allow us to clear them. In order to efficiently detect and clear a small molecule called atrazine, which is an herbicide causing tremendous environmental problems, we split our aptamer-based detection module and clear module into two strains. The first strain is chemotaxic to atrazine and will release quorum sensingmolecules to attract the second strain, which contains atrazine hydrolase to clear it.

EUROPE

Team AMU-Poznan: sh-miR designer - tool for construction of RNA interference reagents: sh-miRs

sh-miR Designer will be a software aimed at fast and efficient design of effective RNA interference (RNAi) reagents - sh-miRs, also known as artificial miRNAs. sh-miRs are RNA particles whose structure is based on miRNA precursor pri-miRNA, but sequence interacting with transcript is changed depending on research purpose. Maintenance of structure of pri-miRNA is very important to enable cellular processing and therefore ensure functionality of artificial particles. sh-miRs delivered to cells on genetic vectors- plasmids or viral vectors - enter natural RNAi pathway and silence target mRNA. They can be used in genetic therapies and basic biomedical research.

Team ATOMS-Turkiye: Project Oncoli

According to the World Cancer Research Fund, the estimated number of cancer cases around the world every year is 12.7 million and is expected to increase up to 21 million by the year 2030. Taking this widely popular and alarming obstacle into attention, we have devised a system which is aiming to tackle cancer from a very different perspective to before. Our choice of bacteria Nissle 1917, a probiotic strain of Escherichia Coli, once inside the body will secrete a cancer tracing protein which recognizes and builds up around the cancer cells. Using the quorum sensing system, E.coli Nissle 1917 detects the bacteria inducing substance AI-2 produced by the tracing proteins. Nissle 1917 bacteria motion towards the region of AI-2 and once in the region, produce our cancer killing protein called apoptin. Apoptin enters the cancer cells and induces apoptosis thereby eliminating their existence.

Team Baskent Meds: Killing Legionella pneumophila Softly

Legionella pneumophila is the cause of the Legionnaires' disease which is a type of pneumonia. The bacterium is found in warm water environments, particularly in artificial water supply systems such as air conditioning systems and cooling towers. The enfection occures by inhalation by small droplets of contaminated water. Our aim, as the team “Baskent_Meds”, is developing bacteria which can recognize Legionella pneumophila specifically at species level by legionella quorum sensing, and respond by producing anti-Legionella peptide which is produced by some Staphylococcus strains. Quarum means “minimum”. Legionella pneumophila should sense the minimum amount of cells around to colonize in the environment and express its virulence. So our modified E. coli may sense the presence of Legionella pneumophila in any contaminated surface and kill it.

Team BGU Israel: P.A.S.E. - Programmable Autonomous Self Elimination

Bioremediation and biosensors often require the release of genetically modified organisms (GMOs) to the environment. After being released, these GMOs are no longer under direct control. As their effect on the environment is unknown, they pose a potential threat. In order to eliminate this threat, we are developing a genetic circuit, using e. coli as a model GMO, that limits the lifetime of a bacterial population after it is released to the environment. Our goal is to allow the end user to program a GMO population to survive in the environment until it has completed its task, after which the entire population will disappear without any further external intervention. We employ two approaches to achieve this goal: One relies on the dilution of a synthetic control element through cell division, and the second is based on the lifetime of an essential proteincontaining an unnatural amino acid.

Team Bielefeld-Germany: Ecolectricity – currently available

There is a growing interest in the use of ecologically friendly alternative energy sources because of the depletion of fossil fuels and an increasing environmental pollution. Therefore, we are developing a Microbial Fuel Cell (MFC). The goal of this project is to generate electricity with a modified Escherichia coli

in a self-constructed fuel cell. Besides the technical optimization of the fuel cell, we investigate different genetic approaches like integrating porines and cytochromes as well as endogenous mediators. Using heterologous expression of pore-forming transmembrane proteins, we are able to enhance the extracellular electron transfer, leading to higher membrane permeability. Direct electron transfer can be achieved by integrating cytochromes into the cellular membrane, whereas a production of endogenous mediators enhances the electron transport to the electrode. With different aspects for technical and genetic optimization we enable Ecolectricity, the use of E. coli for direct energy production.

Team Bonn: LOV Wars - May the light be with you

A reliable, yet easily adaptable mechanism for controlling protein activity is key to most areas of life and medical science research. Still, the most common approaches suffer from various flaws. iGEM Bonn 2013 aims to overcome these drawbacks by engineering a novel tool based on blue light-inducible degradation of targeted proteins.</br> The use of a modified ClpXP protease system allows a significant increase in rate and scale of activity change while keeping the modification of the target protein to a minimum. Combining this system with a tool for photo-activatable heterodimerisation based on a LOV domain results in a superior tempero-spatial control.</br> To demonstrate the capabilities of our device, we designed a photosensitive kill-switch. This contributes to the security of synthetic biology in such a way that bacteriaaccidentally brought out of a safe work environment, for example a red-light-hood, would be killed by sunlight within a short period of time.

Team Bordeaux: The Dairy Planet

The economical stakes of food-processing industry have always been a concern in society. Technological innovations have improved the yield and production costs of daily use products. Advances in health sector and biotechnology made it possible to offer food products rich in substances that are nutritious and possess medicinal properties. Our project aims at producing a new range of lactic cultures able to produce natural flavours and colouring substances in a yogurt; including ones producing resveratrol, a molecule responsible for the red wine beneficial effects, implicated in the 'French paradox”. Necessary routes of biosynthesis will be introduced in Lactobacillus bulgaricus and Streptococcus thermophilus, agents of lactic fermentation. Thus, a work of optimization on the genetical modifications of lactic bacteria has been done. This project will allow an easier production of custom yogurts with beneficial and healing properties, avoiding the use of substances derived from expensive chemical synthesis harmful to the environment.

Team Braunschweig: Engineering synthetic microbial consortia

Bacterial consortia offer a great benefit for synthetic biology due to the ability to perform complex tasks by splitting the whole reaction into smaller reactions and share the task among different specialized strains. Also, a self-regulating bacterial culture with intra consortial dependencies offers great advances in biosafety. To shut down the whole bacterial consortium, only on strain has to be eliminated. We engineer three different E. coli strains to grow in a consortium exploiting different Quorum Sensingsystems. Each strain maintains a constitutive expression of an inactive transcription activator (LuxR, LasR or RhlR). Inducers are synthesized by different synthases (LuxI, LasI or RhlI) that are each expressed in one strain and subsequently secreted into the medium. Once taken up by a cell, the inducers bind to the corresponding, inactive transcription factors to render them functional. As a result, an antibiotic resistance under the control of an inducible promoter is expressed.

Team DTU-Denmark: Requiem for a Stream: From Ammonia Pollution to Energy

Production via Denitrification

Global demand for fixed nitrogen has increased to the point that half the human population now relies on chemical fertilizer to grow their food. While fertilizer is a requirement for modern life, runoff from over-fertilized farmland can cause eutrophication. In the presence of abundant ammonia, algae overgrow and consume much of the available oxygen in the water. This results in decreased biodiversity throughout the watershed. Within Europe, 53% of lakes are eutrophic. Using two E. coli mutants built with genes from Nitrosomonas europaea and Pseudomonas aeruginosa, we provide a system to reverse nitrogen fixation. Our mutants consume ammonia and produce nitrous oxide, and release a sustainable source of energy when decomposed into nitrogen and oxygen. We also provide a prototype of a bioreactor that could be scaled up and deployed in the field to simultaneously clean the water and produce energy.

Team Dundee: ToxiMop

The ToxiMop project attempts to tackle the problem of freshwater algal blooms by detecting, reducing, and reporting the levels of the algal toxin microcystin. This toxin causes liver damage and is also speculated to be a carcinogen. Microcystin's toxic action lies in its ability to bind to the human Protein Phosphatase 1 (PP1), which is a major regulator of cell division, protein synthesis and other essential processes. Using synthetic biology techniques, we engineered bacterial chassis (E. coli and B. subtilis) to express PP1, which covalently binds to microcystin. The engineered bacteria can then be used as a molecular mop, the ToxiMop, to remove microcystin from contaminated water. Applying mathematical modelling to our experiments, we optimised our prototype ToxiMop. Additionally, we attempted to develop a biological detector for microcystin, which was combined with our electronic device, the Moptopus. This device has the potential for real-time monitoring and analysis of water bodies.

Team Edinburgh: WastED

The Edinburgh iGEM 2013 team, WastED, is focusing on remediation and valorization of industrial waste streams, with a particular focus on Scottish leather and whisky industry waste waters, containing toxic heavy metal ions as well as fermentable organic components. Using Bacillus subtilis as chassis, we are engineering organisms to capture ions using chelators and metal binding proteins, and to ferment organic components to produce biofuels. We are also testing a new assembly procedure, GenBrick, based on the Genabler assembly system. GenBrick allows assembly of multiple RFC10-compatible BioBricks in a single reaction, and is also well suited to the preparation of fusion proteins and addition of terminal tags. Enzyme fusions may enhance metabolic pathways through substrate channeling. We are testing the effect of protein fusions on fermentation efficiency for biofuel production. In addition, we are examining the implications of possible Scottish independence, following the 2014 referendum, for synthetic biology in Scotland.

Team EPF Lausanne: Taxi.Coli: smart drug delivery

EPF_Lausanne's team is proud to participate to iGEM 2013 and excited to present their project: Taxi.Coli: smart drug delivery. The team's vision is to build a biosynthetic drug delivery concept. The key word of this project is "adaptability". Our goal is to explore a way of using E.Coli as a highly modular carrier, opening the gate to several applications and alternatives in disease treatments. Using the principles of synthetic biology, we engineered a gelatinase secreting E. Coli able to bind gelatin nanoparticles using a biotin-streptavidin interaction and release them in a corresponding location. The drug delivery system is built in three parts: 1) the nanoparticle binding and 2) the environment sensing that 3) triggers the gelatinase release of the engineered E. Coli, liberating the content of the nanoparticle. The nanoparticles made of

gelatin are able to carry any type of organic compound leading to a wide range of applications.

Team ETH Zurich: Colisweeper: The world's first biological Minesweeper game

Colisweeper is an interactive, biological version of the Minesweeper computer game, based on luxI/luxR quorum sensing and chromogenic enzymatic reactions. The goal is to clear an agar “minefield” without detonating mines. Genetically engineered Escherichia coli colonies are used as sender-cells (mines) and receiver-cells (non-mines). Mines secrete the signaling molecule N-(3-oxohexanoyl)-l-homoserine lactone (OHHL) whereas non-mines process the signal. To distinguish between OHHL-levels, a library of PLuxR promoters with various sensitivities was created through site-saturation mutagenesis. High-pass filters were constructed to control the expression of different orthogonal hydrolases in non-mines, depending on the number of surrounding mines. Additionally, the mines express their own hydrolase. A spatiotemporal reaction-diffusion model was established to evaluate and improve the system. To play Colisweeper, a colorless substrate solution is pipetted onto a colony of choice. The result is a defined color change within minutes, allowing identification of the played colony and the number of mines surrounding it.

Team Evry: Iron coli Project

This year, our project focuses on diseases that are subsequent to an iron overload such as hemochromatosis and thalassemia. </p> Nowadays, iron overload is mainly treated by bloodlettings for hemochromatosic patients but this treatment cannot be extended to thalassemic patients who suffer from anaemia. The aim of our project is to prevent the intestinal absorption of iron by engineering Escherichia coli to produce siderophores, chelators of iron. This strategy acts directly at the source. </p> We engineer Escherichia coli using the Ferric Uptake Regulation (FUR) couple to an inverter system, in order to produce these siderophores in presence of iron. To reduce the patient's iron absorption, our bacteria is encapsulated in a pill. Once it arrives in the duodenum, our bacteria will produce the siderophore at their full potential and chelate the iron. </p>

Team Exeter: Paint by coli: Creating a Colour Bio-camera Using Escherichia coli via complete optical control

Synthetic biology has lead to microorganisms being pushed into an unprecedented range of novel functions. Many bacterial systems currently rely on external stimuli to induce transcription. One dimensional protocols often require constant monitoring of applied chemical concentrations, leading to them becoming inept for more complex systems. A triplet of NOT gated photoreceptors in Escherichia coli, will be used to create a system which is finely controlled using only light. This will be showcased using magenta, cyan and yellow pigments as outputs. Varying the intensity and wavelength of light projected onto E. coli will control the shade and colour produced, respectively. Hence, this will show the versatility of the optical control by creating a full colour bio-camera. Additionally, using bacteria to produce an image vastly increases the resolution when compared to conventional cameras, due to the micrometre scale of bacteria.

Team Frankfurt: Steviomyces - sweeter than sugar

The Stevia plant produces several sweeteners known as Steviolglycosides which have only recently been admitted as a foodadditive in the European Union. The iGEM-Team Frankfurt 2013 searches for ways to transfer the pathway of the plant into Saccharomyces cerevisiae in order to make stevia production possible with both lower effort and lower costs. Several of known problems with carbohydratesweeteners like diabetes or caries could be overcome by the Steviolglycosides which are produced by Stevia rebaudiana. We're building upon results gained from last year's competition which gave us the possibility to

transfer a mevalonat plasmid into yeast to increase the production of a steviol-precursor Geranylgeranyl-diphosphate. This year we're searching for a further reconstruction of the pathway and transfering the 2nd plasmid for synthesis of Steviol from Geranylgeranyl-diphosphate into yeast. Thus the whole pathway can take place in a microbial organism and easify the production by lowering the costs.

Team Freiburg: uniCAS - The Toolkit for Gene Regulation

Our Team developed a universal toolkit, termed uniCAS, that enables customizable gene regulation in mammalian cells. Therefore, we engineered the recently discovered and highly promising CRISPR/CAS9 system. The regulation is based on the RNA-guided CAS9 protein, which allows targeting of specific DNA sequences. Our toolkit comprises not only a standardized CAS9 protein, but also different effector domains for efficient gene activation or repression. We further engineered a modular RNAplasmid for easy implementation of RNA guide sequences. As an additional feature, we established an innovative screening method for assessing the functionality of our uniCAS fusion proteins. Single genes and even whole genetic networks can be modified using our uniCAS toolkit. We think that our toolbox of standardized parts of the CRISPR/CAS9 system offers broad application in research fields such as tissue engineering, stem cell reprogramming and fundamental research.

Team Gdansk-UG: MetOli

The aim of our project was to construct a biological system that would be able to detect methanol in ethanol solutions. Our idea was to create a test that could be performed not only in the laboratory, but also at home. We believe that such test would reduce the rate of intoxications by methanol during ethanol consumption. To achieve it, we used a methanol-dependent promoter from Methylobacterium organophilum which would control the production of a dye, for instance GFP, or an enzyme that would produce visible product, such as catechol oxidase. Our eventual goal is to find a bacterium that would not only react to methanol, but also survive in high concentrations of ethanol.

Team Goettingen: The beast and its Achilles heel: A novel target to fight multi-resistant bacteria

Since the discovery of penicillin by Alexander Fleming in 1928, antibiotics have marked a major victory of mankind in the battle against infectious diseases. However, after 90 years, the antibiotics are now losing their old time glory: Bacteria acquire resistance against antibiotics and become unbridled. We must control the use of antibiotics, meanwhile, we need new antibiotics, which can suffiently eliminate the invaders without hurting the 'good' bacteria. Therefore, c-di-AMP, an important, recently discovered signaling molecule in gram-positive bacteria, has come to our sight. Our project is to build a screening system targeting c-di-AMP, which could be applied in novel-drug screening. With this system, the level of c-di-AMP in the cell can be visualised and measured.

Team Grenoble-EMSE-LSU: Light Automated Cell Control by Talk’E. coli

Maintaining cell growth state during culturing is generally difficult due to metabolic adaptation and changing cell division rates. Using light-induced promoters and a phototoxic fluorescent protein, we've designed Talk’E. coli. It uses light signals to communicate with bacteria allowing the researcher to remotely control the cultures using a computer. Cell density is monitored through fluorescence recordings and, thanks to a predictive model, Talk’E. coli responds by illuminating the culture with one or more wavelengths to obtain different effects: killing off cells beyond a threshold density, or producing or degrading protein. The tool is portable and mountable in an incubator making it a handy device for research.

Team Groningen: Engineering Bacillus subtilis to self-assemble into a biofilm that coats medical implants with spider silk.

Approximately half of all implanted medical devices result in one or more medical complication, which have been found to increase mortality rates by 25%, and to cost the amerian society an additional 30 billion dollars every year. A possible solution for these complications is to form a protective biocompatible layer between the implant and the body by means of a spider silk coating. This is achieved through mathematical modelling, techniques from the synthetic biology, and the Gram-positive bacteria Bacillus subtilis, which is redesigned to secrete silk and to self-assemble into a biofilm surrounding the implant. It uses a modified chemotaxis system coupled to the DesK heat sensing system to do so. B. subtilis is furthermore often used in the industry for the commercial production of extracellular proteins, and is generally regarded as safe.

Team Heidelberg: THE PHILOSOPHER'S STONE

Several secondary metabolites, such as commonly used antibiotics, pigments and detoxifying enzymes, are synthesized by non-ribosomal peptide synthetases (NRPSs). These enzymes beautifully reflect one of the fundamental principles of synthetic biology, as they are remarkably modular. We will assemble new NRPSs by combining individual domains and modules of different origin, thus setting the basis for novel and customized synthesis of non-ribosomal peptides. To make the use of NRPSs amenable to a wider community, we will devise a new software-tool, called "NRPS Designer", which predicts the optimal modular composition of synthetic NRPSs for production of any desired peptide and outputs a cloning strategy based on Gibson assembly. As an application relevant to society, we will engineer Escherichia coli to recycle gold from electronic waste in a cost- and energy-efficient way through the heterologous expression of the NRPS pathway of Delftia acidovorans that naturally enables precipitation of gold ions from solution.

Team Imperial College: Plasticity: Engineering microbes to make environmentally friendly plastics from non-recyclable waste

Accumulation of waste represents a considerable problem to humanity. Over the next 50 years, the global community will produce approximately 2 trillion tonnes of waste, or 2.5 times the weight of Mount Everest. Traditionally, mixed non-recyclable waste is sent to landfill or for incineration, both of which result in environmental damage. The detrimental effects are perpetrated by the plastic degradation into toxic byproducts and the production of greenhouse gases by these processes. As an alternative we propose to upcycle this mixed waste into the bioplastic poly-3-hydroxybutyrate (P3HB) to create a closed loop recycling system. Our engineered E. coli will operate within sealed bioreactors. In the future we picture the use of our system in a variety of contexts as part of our M.A.P.L.E. (Modular And Plastic Looping E.coli) system.

Team INSA Toulouse: E. calculus Project

The E. calculus project consists constructing a full n-bits adder capable of transmitting a carry to the next step. The designed strain contains specific devices that should ensure a relatively precise calculation and will be decomposed as follows:

- Various logic gates using specially designed recombinases and recombination sites to avoid reversibility of the gates states.

- A strict control of the expression of recombinases via a tight riboregulation control of the translation of recombinases genes

- A general inducer, switching the strain from inactive to active counting.

- A carry system based on the diffusion of a messenger molecule to the second bit.

- An artificial input system based on photoreceptors sensible to blue and red lights.

The envisioned system should approach as much possible the reliability of an electronic two digit device and may help the Synthetic Biology community designing strong and robust Genetic Boolean Operators.

Team ITU MOBGAM Turkey: Intrinsic Factor-y

Pernicious anemia is described first by James S. Combe in 1822. Pernicious anemia is a type of anemia occurs due to malabsorption of vitamin B12 in the small intestine due to problems with the production of Intrinsic Factor, which is responsible for the absorption of vitamine B12. Pernicious anemia shows its stiking effects on blood, gastro-intestinal tract and nervous system and pernicious anemia usually develops together with an autoimmune disease. Our aim as ITU MOBGAM IGEM Team, is to design a bacterium that is capable of surviving in small intestine and secreting Intrinsic Factor dependent on pH. Also, we design a genetic circuit for controlling the overgrowth and containment of bacteria.

Team Kent: No to NO: A novel approach to reduce greenhouse gas

In today's rapidly changing environment greenhouse gases such as NO are an issue that need to be addressed. NO has been proven to have a detrimental impact on the environment and iGem Team Kent 2013 will provide a solution that focuses on reducing the amount of NO formed in waste water. Our system will utilise an engineered strain of E. coli which will be capable of converting this excess NO into ammonia. Our Biobricks have been designed to enable the detection of NO using the norV promoter. The NO can then be converted into ammonia via the nitrite reductase enzyme encoded by the E. coli gene NrfA. Our solution will have many advantages over the current approaches to waste water treatment such as reduced cost and risk of contamination. Our system will provide a source of recycled ammonia and could be a greener alternative to the Haber Bosch process.

Team KU Leuven: E. coligy: Plants with BanAphids

Aphids, the little green plant-sucking bugs, can pose serious threats to a farmer's proceeds. Not only physical damage to the crops caused by the sucking is a problem, but aphids also transmit harmful viruses to the plants. The magnitude of loss is difficult to quantify as it changes with aphid species, crop species, location, year and other factors. The use of insecticides to control aphid population is contested, as it has a negative effect on the natural predators and aphids grow resistant. That's why we, the KU Leuven iGEM 2013 team, decided to do something about it in a sustainable way, using an insecticide-free controlling mechanism. With E.coligy: Plants with BanAphids we will teach E.coli cells to hack into insects signaling systems to drive off the aphids and attract the natural predators, such as the ladybug.

Team Leeds: The Micro-beagle - A living biosensor

Micro-Beagle is a novel reporter system for E-coli that, as an iGEM first, has been designed to dynamically detect arbitrary target solids (including other cells) through a mechanism activated by cell surface binding. Micro-Beagle is a modular system, utilising Ice Nucleation Protein to express and position target-binding peptides on the cell surface. Target binding induces membrane stress that activates the Cpx signalling pathway, and Micro-Beagle thus utilises a promoter from this pathway (pCpxR) to initiate expression of a reporter protein, such as GFP. As a proof of concept, we have used silica beads as a model diagnostic target (a pathogen surrogate) and the silica-binding "Si4" sequence as the target-binding peptide. We foresee Micro-Beagle being adapted for both the detection of waterborne pathogens and a variety of other

diagnostic applications, and we envision future multisensor Micro-Beagles in which diverse pathogens can be simultaneously and quantitatively measured from a single water sample.

Team Leicester: Biological routes to recycling, re-using and re-purposing polystyrene

Polystyrene is a useful material, but also a visible pollutant that locks up oil-derived hydrocarbons. For 2013 we are diversifying, to reduce polystyrene's various environmental impacts: Recycling - Building on 2012's project, we are adapting the toluene degradation pathway from Pseudomonas species to work on polystyrene, in E. coli.</p> Re-using - Consumer 3D printers use a variety of thermoplastics but virgin plastic is usually required. Recycled polystyrene can be a support for making complex 3D shapes, and removed later. Polystyrene is soluble in limonene (an environmentally friendly solvent) so we are adapting limonene biosynthesis biobricks, to enable biological 'finishing' of 3D printed objects.</p> Re-purposing - Polystyrene is a great building insulator, but needs to be flame retardant. Currently this involves adding halogenated hydrocarbons, proven environmental pollutants. Recently DNA was shown to be an effective flame retardant, so we are using synthetic biology to generate cheap DNA, for flame retardant polystyrene.</p>

Team Linkoping Sweden: A novel immunochemical detection system for food allergens.

Antibodies are usefull for recognition of antigens in food. Antibodies have, however, a very complex structure that is not suitable for expression in E. Coli. The Camelid antibody IgG (cIgG), however, has lower complexity than the Human IgG. We present a new approach for recognition of food allergens with a synthetizised cIgG for expression in E. Coli. The epitope of cIgG is designed for Hen Egg White Lysosyme (HEWL). The cIgG is designed with a linker that connects to the bioluminescent enzyme Luciferase. We also synthetizised an HEWL antigen carrying the protein RFP, A-HRFP, that reacts to the luminescence of luciferase as the A-HRFP attaches to the cIgG. The recognition of HEWL in a sample leads to the release of luminescent green-light as a result of HEWL binding to the cIgG. If, however, no HEWL antigen is present in the sample, A-HRFP binds to cIgG resulting in a luminescent red-shift.

Team Manchester: E. c(oil)i; The Lean, Green, Fat-Producing SynBio Machine

From food products, to cosmetics and biodiesel, palm oil is the world's most widely used vegetable oil. Its demand is ever increasing; however the current method of extracting palm oil is severely unsustainable. Massive deforestation is required to build oil palm plantations, ruining the land of locals in Malaysia and Indonesia. Manchester iGEM aims to combat this by providing a more eco-friendly source of the four main components of palm oil. We reengineered the fatty acid biosynthesis pathway of E. coli to overproduce palmitic and stearic acid and introduced two new genes, desaturase and desaturase, to yield oleic and linoleic acid. To explore the scale-up potential of synthetic palm oil production in E. coli, we developed a fully parameterised kinetic model of the engineered fatty acid biosynthesis pathway.

Team Marburg: Phaectory

The diatom Phaeodactylum tricornutum is a widely spread organism in marine waters. It belongs to the group of diatoms. As a group of great ecological relevance diatoms are responsible for up to 20% of the global CO2 fixation and generate about 40 % of the marine biomass of primary producers. In addition, diatoms represent an important source of lipids and silicate making them interesting for various biotechnological applications e.g. in biofuel industry, food industry and nanofabrication. Furthermore, a relatively easy biolistic method for transfection is established. A simple cultivation eases a putative

industrial use of the diatom. Former researches not only proved a possible expression of antibodies, bioplastic and other recombinant proteins, but also demonstrated a direct secretion of the expressed proteins in the outer medium, making it easier to filter the wanted proteins. These characteristics make P. tricornutum an interesting organism for putative industrial use.

Team METU Turkey: Bee subtilis

Taking a major role in pollination, bees are one of the most important organisms within an ecosystem. However their populations are in serious decline. Colony Collapse Disorder has been found as the most common cause of the disappearance of bees in large numbers. In this study, we aimed decrease the number of hives affected by chemical compounds such as imidacloprid.Our plan is to turn the mutualistic bacteria living in bees' guts into a shield mechanism to protect the bees against these factors. A protein CYP6G1 found in Drosophilia melanogaster has the ability to degrade imidacloprid into harmless substances. Moreover, coumaric acid increases the general immunity of bees against harmful components and we aim to increase the level of coumaric acid in bees' guts. The main objective of this study is the transformation of the genes coding for these two proteins to Bacillus subtilis, which mutualistically live in bees' guts.

Team Newcastle: L-forms: Bacteria without a cell wall - a novel chassis for synthetic biology

L-forms are bacterial without cell walls that are still able to divide without the normally essential cell division machinery. The lack of a cell wall imparts a range of interesting properties and we show that L-forms can be used as a novel chassis for a range of fundamental applications in synthetic biology. We produced a BioBrick for Bacillus subtilis, that allows cell morphology to be toggled from normal to L-form. We have explored some of the interesting opportunities that L-forms provide including cell fusion, genome shuffling and the generation of differently shaped cells using microfluidics. L-forms are thought to exist naturally within plant tissues and we also studied their use as agents for delivering novel functionality into plants. For project outreach, we created a game as an Android application and considered the implications raised by our project and also look at the exciting relationship between synthetic biology and architecture.

Team NRP-UEA-Norwich: Developing Biosensors to Identify Antimycin-Producing Actinomycetes

Antimycins, anti-fungal compounds primarily produced by Streptomyces (a sub-set of actinomycetes), function by inhibiting the final stage of the electron transport chain. Our aim is to develop Biosensors to aid identification of novel antimycin-producing actinomycetes. Homologues of the AntA sigma factor, the key regulatory protein in antimycin biosynthesis, are present in all 14 known biosynthetic gene clusters. Due to this property, Biosensors have been designed with the AntA-regulated promoter(antGp) controlling the expression of three reporters: neomycin resistance gene, RFP (red fluorescent protein) and GUS (providing β-glucoronidase activity). The Biosensors will be produced, trialled and optimised where possible after sub-cloning into two actinomycete-specific integrative plasmids, pMS82 (BT1 integrase) and pAU3-45 (C31 integrase). Worldwide soil and sediment samples have been collected to produce a library of actinomycete strains, which will be screened using our Biosensors, the ultimate goal being to screen bacterial strains for antimycin production.

Team NTNU-Trondheim: VesiColi

Gram negative bacteria produce outer membrane vesicles (OMV) in the size range of 20-200nm. Whereas

their function and contents has been studied for decades, their potential as drug carriers has not been investigated before. We want to introduce protein G from Streptococcus dysgalactiae subsp. equisimilis into Escherichia coli OMV's. Protein G is known to bind to human serum albumin (HSA) which helps S. dysgalactiae subsp. equisimilis hide from the immune system.

The second part of our project is to introduce fluorescent proteins (FP's) linked together into the vesicles. Introducing protein G and linked FP's into the vesicles will demonstrate that it is indeed possible to manipulate the content, and therefore the properties, of OMV's.

Team Paris Bettencourt: Fight Tuberculosis with Modern Weapons!

We are testing new weapons for the global war against Mycobacterium tuberculosis (MTb), a pathogen that infects nearly 2 billion people. Our 4 synergistic projects aim to help in the prevention, diagnosis, and treatment of tuberculosis. 1) We are reproducing an essential MTb metabolic pathway in E. coli, where it can be easily and safely targeted in a drug screen. 2) We are building a phage-based biosensor to allow the rapid diagnosis specifically drug-resistant MTb strains. 3) We are constructing a mycobacteriophage to detect and counterselect drug-resistant Mtb in the environment. 4) We are programming E. coli to follow MTb into human macrophages and saturate it with bacteriolytic enzymes. We want to vanquish tuberculosis and build a TB-free world.

Team Paris Saclay: PCBbusters

PCBs (Polychlorobiphenyls) are synthetic chemicals widely used during the late 20th century. These compounds are extraordinarily stable, not readily biodegradable and have accumulated in the environment. PCBs also accumulate in animal fatty tissues including human tissues. As PCBs are probably carcinogenic and some are endocrine disruptors, they constitute an important health issue. Although PCBs have no natural equivalents, some bacterial communities have developed the capacity to degrade PCBs. Highly chlorinated PCBs undergo anaerobic reductive dechlorination, lowering the chlorine atom number. Lightly chlorinated PCBs are then degraded via the aerobic biphenyl degradation pathway. Our project is to construct an Escherichia coli strain capable of degrading PCBs by introducing in the strain genes involved in PCB degradation in various bacteria. Because some steps are anaerobic and others aerobic, we want to use an oxygen-based regulation of gene expression. We also want to develop a sensor system to detect PCBs in the environment.

Team Poznan-BioInf: SR-MUX: a biological multiplexer with 3-bit editable transcriptional memory.

Our goal is to engineer a device allowing to save up to three binary input signals in living E. coli cells, resulting in expression of red, blue and green fluorescent proteins as reporters. Converting inducer signals into expression of serine recombinases, enzymes capable of specific DNA editing, we are able to create three transcriptional analogues of transistors - transcriptors - and to use them as elemental memory units called SR-latches under control of a fourth, strobe signal, providing a mean to reset the system to its original state. This complex biological memory unit opens the way to cheap, reversible gene induction, useful both to the industry and researchers, not only lowering inducing cost but also being less stressful for the studied organisms, e.g. plants. It is also another step towards Von Neumann-inspired biocomputers.

Team SDU-Denmark: Bacteriorganic Rubber

The growing demand for natural rubber causes deforestation of the rainforest or occupation of arable lands, all due to the founding of new plantations. If producing rubber by bacteria succeeds, production of natural rubber will not be limited to the regions where the rubber tree can grow. Our project aims to make an E. coli strain able to produce natural rubber while grown under controlled conditions. Natural rubber is composed of polymerized IPP (isopentenyl pyrophoshate) units. E. coli already possesses the ability to produce IPP, but it lacks the polymerization enzyme, prenyltransferase, from the rubber tree. In this project we introduce prenyltransferase into E. coli and simultaneously manipulate the bacteria to produce more of the IPP links, consequently leading to the production of natural rubber in the bacterial setting.

Team TU-Delft: Peptidor: Detection and killing of resistant S. aureus using antimicrobial peptides

Methicillin-Resistant Staphylococcus aureus causes major problems, especially in hospitals, leading to over half a million infections annually in the US alone. Of the alternative treatments currently under investigation one of the more promising is through antimicrobial peptides (AMPs). These small, highly-specific peptides attack the membrane of target organisms. Thousands of AMPs are known to exist and little resistance against them has been developed. The Peptidor project consists of an E. colithat can detect S. aureus, using S. aureus' native quorum sensing system, in order to locally produce and deliver AMPs. Upon detection, peptides inactivated by a SUMO-tag fusion, are overexpressed. After a delay period, introduced through a negative transcriptional cascade, a SUMO protease is expressed cleaving off the inactivating tag. Using this mechanism, high concentrations of peptide are delivered at the infection to efficiently kill S. aureus. As a safety mechanism, the timer also activates an E. coli kill-switch.

Team TU-Eindhoven: MRiGEM: Creating a production and delivery system for a CEST MRI contrast agent

Our project presents an alternative solution to the use of heavy metals MRI contrast agents by focusing on CEST MRI. Within CEST imaging, proteins enclosing hydrogen atoms generate high quality images. We use Escherichia coli to create CEST proteins when the bacteria sense a hypoxic environment due to a promoter designed for this purpose, thus working as a production and delivery system for the CEST MRI contrast agent. Hypoxic regions are related to tumors, therefore our eventual goal is to use this device to target and image tumors in humans by injecting the bacteria into the bloodstream. A second application is tracking bacteria in bacterial infections studies. For the iGEM competition however, the proteins are only expressed ex-vivo: in aerobic and anaerobic conditions. We aim to achieve an efficient testing of the CEST properties of the proteins and confirm the promoter's ability to express each protein.

Team TU-Munich: PhyscoFilter – Clean different

The contamination of aquatic ecosystems with multiple anthropogenic pollutants has become a problem since the industrial revolution. Antibiotics, hormones and various noxious substances threaten environmental health and are not effectively removed by conventional waste water treatment. We propose to employ transgenic plants which produce effectors for enzymatic degradation (BioDegradation) or specific binding (BioAccumulation) of pollutants. The autotrophic, sedentary, aquatic nature of the moss Physcomitrella patens makes it an ideal chassis for a self-renewing, low-maintenance and cheap water filter. A light-triggered kill switch prevents unintended environmental spreading by limiting viability to places where the spectrum of sun light is appropriately filtered. Furthermore, we have developed a device to implement this biological filter in an aquatic environment, investigated the application of this new technology and examined its economic feasibility. Based on our results, PhyscoFilter may become a game-

changing approach to improve global water quality in an affordable and sustainable fashion.

Team Tuebingen: Tuebingen Yeast Based Progestin Measurement System

Detrimental alterations caused to water bodies by endocrine disruptive chemicals are an increasing problem in our environment. Especially steroid hormones influence the development and generative behavior of fish. The binding of those hormones to progestogen receptors can mistime the reproductive behavior of aquatic organisms and thereby endanger population balance. Our aim is to construct a yeast-based measurement system for progestin concentration in water samples. Many currently used methods are either very expensive or significantly slower than our method will be. We take advantage of membrane bound receptors in order to achieve high specificity and to speed up measurement. The binding of the ligand to the receptor stops inhibition of the reporter and thereby initiates its expression through a sensitive signaling-chain. This transcriptional switch allows measurement of very small amounts of substrate. To improve our system we use different interchangeable parts for assembly to get a high variety of possible applications.

Team TU Darmstadt: Hunting Fungi

The danger of fungal contamination of grains and cereals but also other food sources has severe consequences. Undetected contaminations can render large quantities of food stocks useless – with detrimental effects on the economy and the food supply. We want to develop a handy device which allows an easy, fast and reliable detection of mycotoxins. For that our team uses various methods from the fields of synthetic biology, electrical engineering and information processing. Our system relies on E. coli with modified TAR receptor interacting with specific mycotoxins. If these are present in the sample they induce a conformational change of TAR and thereby generates a measurable FRET-beacon by bringing two fluorophores in close distance to each other. The modified E. coli will be embedded in exchangeable capsules. Together with a handheld-device and a controlling Smartphone App they will guarantee that measurements can be done quickly, easy to operate and secure.

Team UCL: Spotless Mind

This year, the UCL iGEM team is taking a radical new step with synthetic biology. We intend to explore the potential application genetic engineering techniques on the brain, by tackling Alzheimer's disease, which is linked to the presence of amyloid plaques in the brain. Targets for the project include: establishing microglia cells as a new Synthetic Biology chassis and constructing new BioBricks to enable engineered Microglia to detect and destroy disease-associated amyloid plaques.

Team UCL PG: Spectra

Spectra aim to use a novel configuration of synthetic gene networks (SGNs) to drive evolution of a fluorescent protein with dramatically improved spectroscopic properties. In future we intend to use the capabilities this enhanced fluorescent protein will provide to enable better dissection of differentiation pathways in stem cells.

Team UGent: A new model for chromosomal evolution: Eliminating antibiotic resistance

The main goal of industrial biotechnology is to increase the yield of biochemical products using microorganisms as production hosts. This includes engineering large synthetic pathways and improving their expression. Overexpression of genes has hitherto mainly been achieved by using high or medium copy plasmids. However, studies have demonstrated that plasmid-bearing cells lose their productivity fairly

quickly as a result of genetic instability. Therefore a new method was developed for the overexpression of a gene of interest in the bacterial chromosome: Chemically Inducible Chromosomal evolution (CIChE). In this technique the chromosome is evolved to contain a higher number of gene copies by adding a chemical inducer. The original model for CIChE, however, results in bacterial strains containing a large number of antibiotic resistance genes. To make this valuable technique more widely applicable in the industry, we developed a model for chromosomal evolution based on a toxin-antitoxin system instead of antibiotic resistance.

Team UNIK Copenhagen: Project Magneto

Project Magneto is a biological system that allows us to find better ways to treat cancer, acts as a sustainable energy source or just enables us to visualize our environment in a new way. We created it using magnetosomes. Thanks to these specialized organelles magnetotactic bacteria are able to navigate in the earth's magnetic field. The magnetosome is a nanomagnet which consists of a magnetic crystal housed inside a lipid membrane. Magnetosomes arrange together in chains and act as a compass needle thereby orienting the cell. They show various properties that give them an advantage over industrially synthesized nanomagnets. We demonstrate their usability by fusing fluorescent proteins to their membrane. Through this we open the way for using magnetosomes in various different applications where the fluorescent protein could be simply replaced by a drug for targeted cancer therapy, an ATP-synthase to create a biological dynamo or dye for magnetic paint.

Team UniSalento Lecce: NICKBUSTERS: developing a nickel detection and remediation platform

Nickel is one of the most widespread heavy metals in the ecosystem and, though essential, its excess could be toxic, leading to various noxious effects; nowadays bacteria-mediated bioremediation from inorganic substances seems to be a considerably relevant frontier in microbic biotechnologies. Our project aims to develop a living system in two easy monitorable bacterial platforms who would work as a Nickel detector and a Nickel remediation system. The devices are based on genetic parts from Helicobacter pylori: from the nickel sensing device, H.pylori NikR protein, to the Nickel storage system, Hpn protein, whose role is to store the Nickel ions inside the cell. The two devices are split in two separate populations, which intercommunicate through Quorum Sensing. The system allows to remove the Nickel ions from polluted environmental substrates through bioaccumulation and could be easily implemented in purification plants.

Team UNITN-Trento: B. fruity

B. fruity envisions an environmentally friendly way to control fruit ripening by exploiting an engineered, light regulated strain of B. subtilis. The system works by synthesising ethylene or methyl salicylate (MeSA) upon photoinduction. Everything is housed in a vending machine-like enclosure that regulates fruit ripening in response to consumer demand. Ethylene is a natural plant hormone that is widely used to ripen fruit, such as bananas and kiwi. However, the synthesis, handling, and storage of ethylene is expensive and dangerous. In contrast, B. fruity produces ethylene from inexpensive material by exploiting a TCA cycle intermediate, 2-oxoglutarate, and the activity of P. syringae 2-oxoglutarate decarboxylase. The inhibition of fruit ripening results from the synthesis of MeSA via a pathway built with wintergreen parts. As a proof of concept, we engineered E. coli with the above systems plus the YF1/FixJ blue light receptor device.

Team Uppsala: LactoNutritious

Malnutrition is today a major global problem that affects people both in affluent and developing countries.

Even if you get the right amount of calories, if these do not contain sufficient amounts of micronutrients, like vitamins and minerals, serious illness and even death can be the result. The goal of our project is to alleviate this problem by applying synthetic biology to probiotic bacteria. With our project, we will make the lactobacillus genus the new probiotic platform for metabolic engineering of nutritional compounds. We will engineer probiotics to produce for example beta-carotene, resveratrol, p-coumaric acid, miraculin and saffron. To exemplify what this combination of probiotics and metabolic engineering can accomplish we used our modified bacteria to create nutritionally enriched yoghurt. We have also put great effort into addressing the ethical and safety issues that naturally follow when creating GM food.

Team Valencia-CIPF: Project - Freshellent Yeast

Our team will try to develop a project based on the production of aromas and repellents. The aim is to create a biological platform within a model organism, such as common yeast, to develop an alternative method for production of several aromatic monoterpenoids. The advantage of this organism as producer lies in its capabilities of genetic modification, robustness and culture simplicity. We can also control the production of these compounds using different promoters, so we can choose our favourite aroma while there is repellent activity. The microorganism is completely harmless as it is responsible for fermenting bread and beer. The project aims to establish the basis for future production of repellents in a sustainable and organical manner in developing homes that are under the risk of pandemics caused by mosquitoes and other insects.

Team Valencia Biocampus: Wormboys

Bacteria are essential in biotechnology, but they can hardly move. Nematodes, such a C. elegans, are fast crawling organisms, but they have limited biotechnological applications. By combining the best from both organisms, we present the first artificial synthetic symbiosis with bacteria engineered to ride on worms, which concentrate in hotspots where bacteria perform a desired biotechnological process, such as bioplastic (PHA) production. We have engineered Pseudomas putida with a whole operon that allows the formation of a biofilm on the worm. Biofilm formation is swhitched on and off depending on the media, and thus bacteria get on and off the worm like travellers on a bus. We have also engineered a third partner, E. coli, to express an interference RNA that promotes clumping. Taken together, our artificial symbiosis allows biotechnologically interesting bacteria to travel on nematodes, reach nutrient-rich biomass spots and maximize the efficiency of biotechnological fermentations in heterogenous substrates.

Team Wageningen UR: Aspergillus niGEM: A lov story

The fact that secondary metabolites are often synthesized as polymer backbones that are subsequently diversified greatly via the actions of tailoring enzymes sets the stage for combinatorial biochemistry because their biosynthesis is modular. One of the goals is to establish a modular system of domain shuffling to generate a plethora of novel enzymes with new and improved functionalities. The production of lovastatin, a drug used in lowering LDL cholesterol for patients suffering from cardiovascular disease, has been chosen as a proof of principle. The aim is to transfer the entire lovastatin metabolic pathway from A. terreus into a GRAS organism like Aspergillus niger. To expand our scope we will also be working on host engineering, trying to create a single cell phenotype of Aspergillus niger. To increase the accessibility of our host we also deliver a set of tools, which include ATP and pH biosensors, cytoskeletal gfp-fusions and chromoproteins.

Team Warsaw: FluoSafe

We are presenting to you FluoSafe- a biosensor for acrylamide, known for its carcinogenic and neurotoxic effect! This compound is present not only in biological laboratories but also in starch-based food products (fries, chips etc.). We aim to construct a bacterial strain that would serve as a detector of acrylic amide. This will be attempted in two ways: through the use of roGFP (redox sensitive GFP) fused with glutaredoxin 1 (the presence of acrylamide is known to affect the cellular gluthatione pool) and by expressing hemoglobin α- and β- subunits fused with split fluorophore (adducts formed by acrylamide on the N-terminal valine are known to affect interactions between subunits). We also constructed a BiFC toolbox in BioBrick standard. We sought to find out what was the effect of acrylamide on a variety of human cell lines and asses the toxicity of different concentrations of this compound.

Team Westminster: Hungry for chitin

This year the Westminster iGEM team are tackling the growing bed bug problem. Serratia marcescens has been identified as an efficient chitin degrader, however as it is a pathogenic organism it can not be used as a biocontrol agent. Our idea is to use the chitin genes from this bacterium and create a chitin degrading E.coli. We will test the efficiency of the activity of chitinase which is expressed by our engineered E.coli compared to that of S. marcescens by using a chitin azure assay.

Team York UK: Electricus Aureus: Our greatest source of power comes from the smallest organisms on Earth

We envisage a world where your mobile phone my one day be powered by synthetically engineered microorganisms, when non-renewable energy is a thing of the past. Our project comes at a time when all sources of energy are fighting to be the lesser of many evils; we would therefore like to propose a cheaper, greener and more effective source of energy. Currently, fuel cells do not produce sufficient power to be used for household appliances. Our genetically engineered organism will help us change this and be the first step in the Renewable Revolution. Bacteria are the most abundant form of life on Earth, they survive in harsh environments and they divide rapidly. Thus, they can be a renewable, sustainable source of energy. Our organism will deposit gold nanoparticles on the battery to increase its conductivity. These gold ions come from toxic pharmaceutical waste which is extremely harmful to the environment.

LATIN AMERICA

Team BIOSINT Mexico: Smartpro

This year Biosint Mexico team will be developed a smart probiotic. Along the competition have been present several projects about probiotics, nevertheless the main disadvantage was that most of them were not being created in a lactobacillus strain. Because of this we constructed a Lactobacillus platform for others iGEM teams. Continuing with the idea of the smart probiotic system we include a sensor for xenobiotic substances that could detect and decrease intoxications by pesticides. Also the team implemented a kill switch for safety issues. This project contributes to resolve one of the Mexican food and health problems.

Team Buenos Aires: To drink or not to drink

Our project is focused on developing a biosensor specific for certain water pollutants, with a modular and scalable approach. This approach would make it easy to adapt the response for the detection of different substances. In contrast to other iGEM biosensors, it does not rely on expensive equipment or qualified

people to interpret the results. Being aware that most of the populations affected by consumption of contaminated groundwater don't have scientific or technical training, we intend the device to be cheap and easily distributed. We have designed it in a way that any user could easily determine the presence and level of the contaminant on drinking water, using image-based instructions. The project will focus on measuring a primary pollutant: arsenic. However, its modular and scalable design provides an easy way to measure various contaminants such as nitrate/nitrite among others.

Team Ciencias-UNAM: Skully coli

The human peptide LL-37 is an antimicrobial peptide shown to protect against H.pylori and other pathogenic bacteria. Synthetic expression of active LL-37 in vivo is challenging due to the cytotoxic effects it has in the host. To make a resistant host that can export LL-37 to the media we intend to overexpress the E.coli acrAB and tolC operons, which activate the AcrAB-TolC efflux pump, a mechanism related with resistance to this and similar peptides by expulsion. To create a system in which E.coliexpels LL-37 only in the presence of specific pathogenic bacteria, we use the LsrA promoter, which allows transcription in the presence of AI-2, a molecule produced by these bacteria to communicate via quorum-sensing. To avoid self-induction we designed an antisense RNA with specific secondary structure to inhibit the translation of LuxS, the enzyme responsible of the production of AI-2 in E.coli.

Team Colombia Uniandes: Nicko & Chimi: The magneto and the chimera

This year we are developing two projects: The first one, that we call Nicko, is an alternative solution for water pollution caused mainly by mining, it is a system capable to detect and absorb nickel, to later be removed magnetically, using parts of the homeostatic system of E. coli and Ralstonia metallidurans and the magnetotactic property from Magnetospirillum magneticum AMB-1 which will be used as our final chassis.

The second one, Chimi, is a stress-tester for animals (or even humans). It is based in a glucocorticoid sensor that is able to discern between basal levels and stress levels of glucocorticoid hormones in a sample with an easily recognizable signal, such as color, to allow the sensor to be used in the field, household or the laboratory.

Team Costa Rica Cibus: Genetic transformation of Bacillus subtilis for lactose consumption

Cibus 3.0 takes biodiesel production to a new level using dairy industry wastes. Annually, about 675 thousand tons of whey are thrown into rivers. This because at the present time there isn't a program for reusing this waste, and producers find it difficult to treat them properly because of its chemical composition. Our idea consists in the modification of the bacteria Rhodococcus opacus to enhance the absorption of lactose and the overexpression of the natural triglycerides (TGA) producing ability of R. opacus, achieved by inserting an optimized sequence of a DGA acyltransferase gene and lactose absorption genes, constitutively expressed, also with an optimized sequence of a lipase from B. cepacia which is the responsible to break down the TGAs and an inducible "suicide device" in order to extract them with ease. Now all what it takes to finish the job is adding some ethanol to obtain our biodiesel!

Team Manaus Amazonas-Brazil: Electrobacter: from used frying oil to electricity

Used-frying-oil is produced in deep-fried food preparations and is one of the most serious environment hazards.In our project we are using the Shewanella which is a genus of proteobacteria widely found in

Amazon region(called also as Shewie).They can reduce long-chain fatty acids, being a versatile new chassis to study and work in the iGEM competition. The fat acid degradation via Beta-oxidation is done by enzymes which expression is regulated by the genes FadR, FadL, FadD, FadE producing acetyl –CoA. All these features are remarkable for bioremediation of fat and oil spills.Besides that,is also known for its ability in “delivering” electrons to external media.we modified Shewie β-oxidation pathway silencing regulators and enhancing expression of some genes for fat degradation.In this years' project we aim to make a micro power plant using a bacteria hungry for used-frying-oil.

Team TecMonterrey: Modular, synthetic biology approach for the development of a bacterial cancer therapy in Escherichia coli.

By harnessing the inherent ability of facultative anaerobic bacteria to colonize and grow in tumoral environments, this project aims to prove the functionality of four different modules that would work together as a bacterial cancer therapy using Escherichia coli as chasis: Toxicity module, Secretion module, Localized induction module, and Internalization module. The expression of tumor specific therapeutic proteins, Apoptin and TRAIL, conforms the toxicity module. For these proteins to have their effect they need to be located in the extracellular matrix, therefore we are developing a module with a secretion function using hemolysin secretory mechanism. The hypoxic microenvironment present in tumors can be used for the localized induction module of tumor specific proteins, using the promoters HIP and nirB. Finally, Apoptin needs mechanisms to enter tumor cells’ cytoplasm. Proteins with this requirement could reach the cytoplasm when coupled with the internalization module, resulting in a fusion with the TAT peptide.

Team UANL Mty-Mexico: Integrating transcriptional and post-transcriptional regulation through the use of two synthetic RNAthermometers

Temperature sensing RNA sequences, known as RNA thermometers, regulate translation by preventing the ribosome from binding the transcript until higher temperatures shift it to an open structure. Several naturally occurring RNA thermometers have been described, and synthetic sequences that emulate them have been designed and proved to regulate genetic expression at different temperature ranges. Here, we intend to build a genetic circuit that results in three discrete states whose transition can be regulated by temperature changes only. Most notably, our circuit integrates transcriptional and post-transcriptional regulation, widening the spectrum of potential genetic circuit topologies for synthetic biology, with applications that range from basic research to the replacement of chemical inducers for industrial-scale processes.

Team UC Chile: Whateversisome: create your own bacterial functional organelle

Compartmentalization is a characteristic of complex biological systems. Carboxysomes are proteinaceous bacterial microcompartments that evolved to optimize bacterial metabolic reactions. We sought to take advantage of this biological principle to design a platform for in vitro metabolic engineering. Whateversisome it’s based on two hypotheses: the targeting signal to the microcompartment is present in a subunit of RuBisCO and that after isolation Carboxysomes can maintain their metabolic capacity in vitro. To address these hypotheses, we designed a system to target proteins of interest to the Carboxysome using RuBisCO subunits as targeting signals. We showed colocalization of GFP fusion proteins and Carboxysome shell-proteins fused to RFP. Second, we designed a simple system for Carboxysome purification based on biotinylation that should enable easy isolation of recombinant Whateversisomes. Our approach would enable combinatorial in vitro metabolic engineering by producing and combining arbitrary Whateversisomes. This project takes advantage of subcellular organizational principles for metabolic engineering.

Team UFMG Brazil: CardBio (Cardiovascular disease biomarkers sensor)

Death by heart diseases is very common worldwide, being Acute Coronary Syndrome (ACS) its main cause. This fact is deeply related to late diagnosis, which is usually made after the cardiac event had already occurred. We, from UFMG team, decided to explore this problem building a system capable of providing a precocious diagnosis for ACS based in 3 biomarkers: Brain Natriuretic Peptide (BNP), Trimethylamine-N-Oxide (TMAO) and Ischemia Modified Albumin (IMA). The main goal is to detect each of these biomarkers using our engineered E. coli by integrating the signals CFP, YFP and RFP produced when BNP, IMA and TMAO, respectively, are present in a sample of patient serum. This diagnosis is based on color intensity of the fluorescent proteins. So, we can establish the presence or absence and severity of ACS disease and predict earlier a myocardial event, thus providing information for fast treatment.

Team USP-Brazil: Detecthol: Methanol detection system

Our product is a bioengineered sensor, which will be able to detect levels of methanol above 2% in common alcoholic drinks. This will allow government to make high-throughput screening of ethanol drinks tainted will methanol. The device will be used as an initial low cost and portable test. The construction is based on the pAOX promoter, which is activated by methanol and repressed by ethanol. Several parts of the device must be tuned for proper function in Pichia pastoris; pAOX promoter, red fluorescent protein (RFP), Mxr1p transcriptional factor and FLD promoter. Since we aim to develop a product ready for the consumer to use, we plan to develop a plastic container for the lyophilized yeast, printed by 3D printer, that will help perform the test and will also contain the yeast. After use, the container will be able to apply bleach to eliminate the yeast.

NORTH AMERICA

Team Alberta: The Littlest Mapmaker

Inspired by a 2007 iGEM joint project by Davidson College and Missouri Western State University, 'The Littlest Mapmaker' is the University of Alberta's effort to create a biological computer capable of solving the Travelling Salesman Problem, a logistical challenge in which a hypothetical salesman must find the shortest route through a series of destinations. Our travelling salesman computer is built from a combination of raw DNA chemistry and bacterial colonies: first it assembles the routes by stringing genes together, treating them like roads on the salesman's map, then the bacteria sort the good routes from the bad, identifying the answer through the quantity of bacterial colonies making use of each route. The most commonly used route is the winner!

Team Arizona State: BactoVax: A Modular, Bacterial-based Cancer Vaccine Platform

Cancer kills eight million people each year, a number unchanged over the past five decades. The current paradigm for cancer treatment involves non-specific therapies such as chemotherapy and radiation that cannot differentiate between cancerous and healthy tissue. We propose a novel vaccine delivery system of tumor associated antigens and immunomodular agents encapsulated within probiotic bacteria to harness the patient's own immune system to fight cancer. The bacterial vaccine should activate macrophages and dendritic cells in order to teach the immune system to recognize cell surface antigens that distinguish tumors from healthy tissue. We aim to engineer lab-strain Escherichia coli and E. coli Nissle 1917, a commercial probiotic, to train the immune system to target and destroy tumor cells. This provides a distinct

advantage over current bacterial vaccinology platforms, which rely on pathogenic bacterial chassis such as Salmonella and Listeria.

Team Berkeley: Genes to Jeans: a green solution to blue denim

The world consumes over 40 million kilograms of indigo annually, primarily for dyeing denim. Indigo is currently derived from petroleum using a high energy process, and commercial dyeing involves the use of reducing agents to solubilize the dye. The development of biosynthetic and bioprocessing methodologies for indigo dyeing could have environmental and economic advantages. By combining the biosynthesis of indigo and the use of the natural indigo precursor indican, we propose a more sustainable dyeing method as an alternative to chemically-reduced indigo in the large scale production of indigo textiles. We achieved in vivo indigo production in high titers, and efficient cleavage of indican using a non-native glucosidase. Inspired by natural systems, we isolated and characterized several plant and bacterial glucosyl transferases hypothesized to produce indican. Lastly, we compare the cost and environmental impact of our alternative with the present chemical process.

Team BostonU: Fuse, or Die: The Case for the MoClo Revolution

In order for synthetic biologists to be able to use automation technologies, we need a well-characterized library of basic biological components that can then be used to design more complex systems. MoClo is a one-pot digestion-ligation assembly technique developed by Weber in 2011, which enables faster and more efficient construction of genetic circuits when compared to BioBricks, the current iGEM standard. With our project, we are proposing that iGEM teams replace the inefficient BioBricks format with MoClo. We have expanded our library of basic MoClo DNA Parts and characterized devices using various promoter-RBS combinations via flow cytometry. We also designed and implemented a standardized data sheet with a JavaScript software program in order to easily share our library and data with the community. Our MoClo library, characterization data, and data sheet tool fill an essential role in the implementation of automated synthetic biology protocols.

Team British Columbia: CRISPR MADE BY U – CRISPR Mediated Automated Design Employed to Bring You Ultrabiotics

The past decade has seen the emergence of robust bioprocessing strains engineered to synthesize discrete molecular products. The next-generation of strains could be “programmable,” with on demand generation of molecules within a bioreactor e.g. a yogurt fermentation capable of making any combination of flavouring, nutrients or pharmaceuticals. While merging all this potential into single hosts seems efficient, it would also bring added risk in the case of a process failure due to bacteriophageinfection. Here, we not only rationally design widespread immunity to phage infection, but also hack this immunity system to yield programmable biosynthesis at the community level. We demonstrate this by building both broadly and specifically neutralizing CRISPR systems that were paired with biosynthetic capabilities for vanillin, caffeine and cinnamaldehyde production. Eventually, a fermentative process could exist that is vaccinated to phage infection but susceptible to targeted phage addition that results in a programmable probiotic – or ultrabiotic.

Team BYU Provo: Phage Pharming: Two Approaches to Expanding the Use of Bacteriophage in Synthetic Biology

Bacteriophages are the most abundant organism on the planet, yet most are still uncharacterized. Current research is focused on finding new ways to use bacteriophageeither in their wild-type state or after they have been modified for use in synthetic biology. We studied two ways to modify existing well-characterized

bacteriophages. First, we employed random mutagenesis, CsCl purification, and plaque-size selection to isolate T4 and T7 bacteriophage with altered capsid sizes. A library of capsid sizes will allow researchers to select the appropriate bacteriophage for use in biotechnology or nanotechnology applications. Second, we designed a cholera sensing and destruction circuit using bacteriophage lambda. In this circuit, lambda contains biofilm-degrading enzymes controlled by a cholera quorum-sensing system transferred to E. coli. Upon sensing cholera, this E. coli will activate lambda, leading to cell lysis and biofilm degradation. This research demonstrates the versatility and utility of bacteriophages in the field of synthetic biology.

Team Calgary: The FerriTALE

Outbreaks of foodborne illnesses are a growing problem for food safety and public health. Whether in your water, salad or steak, pathogenic E. coli causes upwards of 250,000 illnesses every year. To solve this problem, iGEM Calgary is developing the FerriTALE to detect harmful E. coli. It uses engineered proteins that detect and report the presence of dangerous E. coli in a sample. The detector, TALE, binds to genomic markers specific to dangerous E. coli. Next, our TALEs are attached to the scaffold and reporter, Ferritin, that rapidly alerts the user to the presence of E. coli through a visible color change. We have integrated these proteins into a handheld device, similar to a home pregnancy test, that tells the user if dangerous E. coli is present. Moving forward, the FerriTALE can be tailored to detect other pathogens as the basis of a powerful new detection platform.

Team Carnegie Mellon: Light-Activated Antimicrobial Phage

Due to the widespread misuse and overuse of antibiotics, drug resistant bacteria now pose significant risks to health, agriculture and the environment. An alternative to conventional antibiotics is phage therapy. However, many temperate phage also form prophage. Our approach to antibiotic resistance is to engineer a temperate phage, Lambda, with light-activated production of superoxide. The fluorescent protein KillerRed was cloned into a plasmid vector and lambda gt11 with the IPTG inducible lac promoter. Lysogens were isolated and these strains were characterized and compared to E. coli with KillerRed from high-copy plasmids. Light activation of KillerRed resulted in decreased cell numbers. In addition, we modeled our system at multiple scales, including populations of phage and bacteria, KillerRed gene expression, ROS production, and effects of light. Having two methods of killing, lysis and superoxide, decreases the probability of developing resistance and our system overcomes the prior limitations of using wild-type temperate phages.

Team Clemson: Development of a Universal Self-Amplified (USA) Biosensor for Repaid Detection of Viable Pathogens

Many regulatory agencies such as the Department of Agriculture and the Environmental Protection Agency have specific standards for pathogen concentrations in sample materials, including “zero-tolerance” for some foodborne pathogens. However, current detection methods for these disease-causing bacteria suffer from one or more of the following limitations: 1) requiring sample enrichment, 2) inability of low-level detection, 3) indiscrimination between viable and non-viable cells, 4) small sample volume capacity, 5) tedious procedures, and 6) high assay cost. Our Universal Self-Amplified (USA) Biosensor uses a genetically modified detection bacteria to solve many of the aforementioned issues. The engineered USA bacteria will recognize a target chemical produced by the pathogen of study, which will trigger a cascade of genes to both amplify the chemical signal and produce a visible alert to the pathogen’s presence. The USA pathogen detection mechanism strives for rapidity, economy, and simplicity.

Team Concordia: Comput-E.coli

Our aim is to achieve universally computational cells through the exploitation of cell-cell communication to generate biological cellular automata. To achieve this, we are using an array of E. coli colonies, which implement the same logical functionality while using different input/output interfaces. The strains process their inputs (two inputs from neighbors plus their own current state) to decide what their next state will be, after the application of a global clock.

At the heart of our project is the construction of the clock, to synchronize information processing. We are making a fusion protein of ETR1 from A. thaliana and EnvZ from E. coli to allow cells to respond to ethylene gas concentrations. All cells will produce this gas at cyclic intervals, thus creating a truly autonomous clock. We have also devised an RNA-based system that can process information reliably and expediently with the use of ribozyme-based XOR and AND gates.

Team Cornell: Organofoam: Genetically Engineering Fungal Mycelium for Biomaterials Development

The goal of Organofoam is to develop a fundamental toolkit of genetic parts for engineering complex fungi, particularly plant-pathogenic basidiomycetes. We were inspired to do so by a local company, Ecovative Design, that uses lignin-degrading fungi and plant matter to produce a biodegradable Styrofoam substitute. The existing product that we are seeking to improve, known as “mushroom packaging,” is a sustainable and necessary alternative to Styrofoam. Polystyrene can take hundreds of years to degrade in landfills, produces dozens of identified chemical toxins upon combustion, and is tremendously inefficient to recycle, thus posing difficulties for disposal and polluting the environment. However, the production efficiency of Ecovative’s substitute suffers due to contamination from pathogenic molds, a problem that we seek to solve using synthetic biology. Using the complex, plant-pathogenic basidiomycete, Ganoderma lucidum, as a chassis, we are expanding the accessibility of fungal genetic engineering and demonstrating its utility for commercial purposes.

Team CU-Boulder: Cheap protein and DNA purification methods for DIY Bio

The focus iGEM at CU-Boulder has been to make synthetic biology more accessible and affordable. We spent the summer developing parts, procedures, and documentation to help make this vision a reality. The original goal was to create the constructs and purification methods necessary to produce and isolate restriction enzymes. Along the way we explored some novel approaches to DNA and protein purification and developed experimentally tested protocols for these and other procedures essential to Biobrick assembly. Our purification methods exemplify the ideal of using common lab materials to make performing everyday lab techniques as accessible and inexpensive as possible. A related aspect of our project was exploring methods of recycling consumables associated with lab work in order to reduce waste and material expenses. We hope that our findings using this 'do-it-yourself' approach of synthetic biology help make this type of research more accessible for those where funding is limited.

Team Duke: Designing Synthetic Gene Networks Using Artificial Transcription Factors in Yeast

Synthetic gene circuits have the potential to revolutionize gene therapies and bio-industrial methods by allowing predictable, customized control of gene expression. Bistable switches and oscillators, key building blocks of more complex gene networks, have been constructed using naturally occurring and well-characterized regulatory elements. In order to expand the versatility and variety of these circuits, we

designed and constructed gene networks using artificial transcription factors (ATFs). The ATFs are of two classes: inhibitory TAL proteins and a catalytically inactive dCas9 protein with small guide RNA elements, each orthogonal to the yeast genome. Using mathematical modeling, we determined the parameters expected to create bistability and oscillation, using tandem binding site kinetics to achieve cooperativity. Based on these results, we assembled a library of plasmids containing ATFs, binding sites, regulatory elements, and fluorescent reporters. We then integrated these genes into the genome of Saccharomyces cerevisiae and are currently characterizing them using flow cytometry.

Team Gaston Day School: Fluorescent Detection of Cadmium in Water Supplies

Heavy metal contaminants pose a serious health threat around the world, especially in locations with poor irrigation. Cadmium, in particular, is a known carcinogen that affects the cardiovascular, gastrointestinal, and respiratory systems. The Agency for Toxic Substances and Disease Registry compiled a Priority List of toxic substances, on which Cadmium was seventh. We combined the Green Fluorescent Protein coding region with a Cadmium Sensitive Promoter to create our detector, which provides a simple and inexpensive test for the presence of Cadmium in water supplies. We will incorporate sensitivity tuners to decrease the detection threshold, and we will use mutagenic PCR on both the promoter and the entire detector to increase its sensitivity. Proper use of this BioBrick could result in early detection of cadmium polluted water and potentially prevent deaths worldwide.

Team Georgia State: Mamba Juice: Expression of Exogenous Mambalgin Peptide Using the pGAPzα Vector System

Pharmaceutical companies have invested considerable financial resources in developing analgesics. Often, the compounds used in these medicines are naturally occurring, such as aspirin and opoids. In 2012, Diochot et al from the Institut de Pharmacologie Moléculaire et Cellulaire in France, was able to extract and isolate an analgesic peptide found in the African Black Mamba. The peptide, Mambalgin-1, has been shown to have comparable analgesic effects to morphine but does not induce the debilitating withdrawal affects. However, extracting this peptide is expensive and dangerous work. Using a cassette of standardized systems (the pGAPzα expression vectors) in P. pastoris, we sought to produce high quantities of pure, functional proteins in a safe and cost effective manner.

Team GeorgiaTech: Bacterial BioBots: Integrin-Based BioSensors

Our team goal is to develop novel bacterial BioBots that respond to the extracellular tissue environment. Mammalian cells communicate with the extracellular matrix (ECM) using heterodimeric cell surface receptors, called integrins, which can signal in a bidirectional manner between the cell interior and ECM. We aimed to express the integrin αIIbβ3 in E. coli cells. To promote dimerization of the integrin subunits, we attempted to optimize bimolecular fluorescence complementation of split GFP using surface display technologies. We cloned split GFP parts, assembling the T7-promoter, LacI-operator, and ribosomal binding site (RBS) upstream of the protein-coding region. To verify αIIbβ3 function, we developed an integrin activity sensor consisting of the ligand derived from fibrinogen (KQAGDV) coupled to GFP. Finally, we successfully created a new standard for RBS addition that inputs the strong RBS (BBa_B0034) in front of any standard BioBrick part, which is efficient and more successful than the usual 3A/standard assembly.

Team Greensboro-Austin: Bioadhesive Production Using an Expanded Genetic Code

Mussel adhesive proteins (MAPs) are water-resistant bioadhesives that have a variety of biomedical and construction applications. Replicating the natural properties of MAPs through in vivo production using

microbes is difficult as it depends on post-translational modifications, primarily the hydroxylation of tyrosine residues to L-DOPA. Since adhesiveness is correlated with L-DOPA content, our project aims to improve the adhesive properties of MAPs by utilizing a strain which inserts the non-canonical amino acid L-DOPA at UAG stop codons during translation. When combined with the in vivo production of free L-DOPA, this system is a significant step towards rapid, cost-effective MAPs production. Our team also worked on degrading odorous compounds, computationally modeled the detection of potentially dangerous oligo orders, and created BactoArt using inducible fluorescent proteins. Additionally, we created the Open Sequence Initiative, which is focused on updating the standards for submitting BioBricks to the iGEM Registry.

Team Lethbridge: Frame-Changer: Shifting Translation for Multiple Protein Expression

The current growth in synthetic biology research promises more complex and useful engineered systems. However, increased complexity often requires more genetic material that can be difficult to introduce into organisms. We propose the development of a new library of regulatory gene expression elements that allow for compression of multiple coding sequences into a smaller amount of genetic space. Using a pseudoknot RNA structural motif, commonly used by viruses to minimize their genome size, we will show the utility of dual-coding gene sequences to give useful protein products whose expression can be regulated by the pseudoknot's ability to induce ribosomal frameshifting. A software tool will also be used to overlap multiple coding sequences into different reading frames. Ultimately, this library of standardized parts will be available for use in a variety of engineered systems requiring minimal coding space and multiple protein expression.

Team Michigan: A Completely Unidirectional Biological Transistor Utilizing an Engineered Fim Switch

Recent studies have just started to explore the possibility of utilizing existing recombination systems, to store information and perform computations. However, the only systems studied so far are not completely unidirectonal in their ability to flip a segment of DNA. Instead, previous systems have relied on "recombination directionalityfactors", which when complexed with the unidirectional recombinase, reverse the direction in which it flips the DNA segment. The fim system from E. coli, has been shown to contain 2 unidirectional recombinases, hbif and fime, which flip a promoter containing segment of DNA. Our project seeks to engineer the fim switch by replacing the native promoter with another promoter. We demonstrate that it can function as a reliable and efficient biological transistor, or "transcriptor". Beyond storing information and performing basic computations, the system would serve as a very useful, tightly controlled switch.

Team Minnesota: The pMNBB vector system: A toolkit approach for engineering Pichia pastoris

Currently, there is a significant gap in available resources for transformation and expression of foreign proteins in the industrially-relevant yeast model, Pichia pastoris. To address this, we have constructed pMNBB, a versatile shuttle vector system. pMNBB vectors provide researchers a modular BioBrick platform to introduce synthetic pathways into E. coli and also the option to rapidly transfer these constructs into P. pastoris. Not only is this vector system designed for integration of expression cassettes into the P. pastoris genome, but we have also added elements which allow for the episomal maintenance of these plasmids. Moreover, these vectors are amenable to trans-kingdom conjugation (TKC). Rather than following a yeast transformation protocol that can take days to weeks, our constructs may be introduced to a yeast culture in

roughly one hour. Finally, as a proof-of-concept, we use the pMNBB vector system to produce and secrete active human insulin from P. pastoris.

Team MIT: Engineered mammalian cell-cell communication mediated by synthetic exosomal cargoes.

Coordinating behavior across cell populations to form synthetic tissues requires spacial communication between individual cells. While there has been some success engineering single signals, sending multiple signaling elements spanning spatial scales for multicellular coordination remains a significant hurdle. Here, we describe a method for mammalian cell-cell communication utilizing engineered exosomes containing miRNA or protein signals. First, we demonstrate selectively packaging signalingmiRNAs (miR-451 and miR-503) and synthetic fusion proteins (GFP, Cas9, and Cre recombinase each individually fused to the oligomerizing membrane targeting domain Acyl-TyA) into exosomes within cells engineered with sender genetic circuits. Next, we demonstrate that these miRNA and protein signals can modulate gene expressionwithin cells engineered with receiver genetic circuits. Finally, we present preliminary cell-cell signaling results on populations of cocultured sender and receiver cells. Our method may enable multiplexed communication among populations of various cell types and the creation of sophisticated synthetic tissues.

Team MSOE Milwaukee: Synthesizing Eucalyptol from Spent Grain Waste using a Three E. coli System

Eucalyptol, the main component of Eucalyptus oil, is a valuable product with multiple potential industrial uses. Current methods are costly, which is a major deterrent for research on these applications. We have designed a system consisting of three strains of Escherichia coli (E. coli) which will convert spent grain waste from breweries into Eucalyptol. The design utilizes two strains of E. coli overexpressing and secreting enzymes to degrade hemicellulose into xylose. The third strain of E. coli will utilize the xylose and convert the molecule into Eucalyptol using the Mevalonate pathway. A scale-up model was developed to assess industrial feasibility. The cost of production would drop two-thirds, which would make industrial uses plausible. Future studies will be transforming our E. coli with the genes of interest and testing for production of Eucalyptol.

Team Nevada: Lysesavers: A novel endolysin-based bactericide for the treatment of gram-negative pathogens

Endolysins are the bacteriophage proteins responsible for lysing target bacteria by degrading the peptidoglycan layer. The use of endolysins to fight bacterial plant disease is an emerging field, but it has largely been limited to gram-positive bacteria, as the outer membrane of gram-negative bacteria prevents endolysins from accessing the peptidoglycan. Recombinant E. coli have been created to express endolysins which target various plant pathogens. These proteins can be used as a novel gram-negative bactericide when coupled with a treatment to disrupt the outer membrane. A system to easily detect outer membrane disruption will be critical to our project, as well as any future studies on gram-negative endolysins. To address this, a registered iGEM part used for cell surface detection was improved and incorporated into a fluorescence-based assay. Erwinia amylovora, a gram-negative bacterium responsible for fire blight in fruit trees, was chosen as a model target for proof of concept.

Team Northwestern: NU Balance

The purpose of this iGEM project is to combat oral diseases by engineering a bacteria to neutralize the lactic acid produced by Streptococcus mutans. Due to the time constraint, the scope of the project is limited

to a dual-state promoter where an acid-responsive promoter is placed upstream of a constitutive promoter. This novel transcription regulation element will have both constitutive and inducible activity. The construct will be tested by linking green fluorescent protein to the downstreampromoter. Based on preliminary results, the extra promoter should allow for a significant increase in gene expression. These pieces have wide implications in any fields that require a quick upregulation of any gene in response to acidic environments.

Team OU-Norman OK: A Shuttle Vector for Clostridial Chassis Organisms

Concerns about energy security, sustainability, and the environmental impact of fossil fuels have renewed the interest of both the public and the scientific community in the development of renewable energy sources, including biofuels. Recent research in synthetic biology has resulted in the production of alcohols not known to be produced naturally, and the extension of carbon chain length. These systems have been expressed in E. coli. Historically, the alcohol titer obtained with native alcohol producers, such as Clostridium acetobutylicum, an acetone/butanol/ethanol producer, and Clostridium beijerinckii, an isopropanol/butanol/ethanol producer, has been greater than that obtained wtih non-native producers. One major problem with these systems is the lack of tools available for genetically manipulating them. The goal of our project has been to develop a shuttle vector that will allow the heterologous expression of these and similar biosynthetic pathways in these organisms.

Team Penn: Engineering the Epigenome

The code of life is more than a sequence of A's, C's, T's and G's; epigenetic modifications, such as DNA methylation, are powerful and heritable regulators of gene expression. Targeted methyltransferases are enzymes that catalyze sequence-specific methylation – the most useful tool for engineering the epigenome. With a synthetic biology approach, we developed an assay to test targeted methyltransferases without expensive, time-consuming traditional methods. Our modular single-plasmid system allows methyltransferases to be easily cloned and tested via inexpensive digestion assays, quickly measuring the existence and extent of targeted methylation. Additionally, our plasmid contains standardized primer-binding sites for methylation-sensitive sequencing, and our E. coli chassis effectively eliminated noise associated with methylation studies. We are using this assay to characterize our novel targeted methyltransferases, which could be used to study epigenetic modifications. In the future, synthetic biologists could embrace these tools to explore the next frontier in engineering biological systems: the epigenome.

Team Penn State: Plants as Plants: Natural Factories Producing Fuel, Plastic, Flavoring, and More

Plants as Plants: Natural Factories provides a green approach to the manufacturing of valuable chemicals and materials. Through synthetic biology, we are able to control the expression of genes that regulate the production of desired secondary metabolites. Via the manipulation of established metabolic pathways, we hope to produce vanillin and butanol. The prospect of being able to synthetically produce a biofuel provides vast possibilities for the scope of synthetic biology and green energy. Additionally through the manipulation of the cellulose synthase genes, we hope to increase the biomass of plants by a hybrid plant cell wall. As shown through these projects, the use of plants provides various green energy possibilities. However, due to the limited use of plants within synthetic biology there are various regulation issues. Thus we have additionally worked on characterizing a range of plant promoters as well as introducing the Cas9 crisper system into plants.

Team Purdue: Back to the Basics

Synthetic biology has striven to prove that classical engineering principles are applicable in the field of biology. Several challenges have yet to be overcome including design of robust genetic circuits, reliable gene expression, and a standard way to characterize parts. To assess circuit robustness, we utilized the power of the Taguchi Method, a statistical analysis which optimizes a set of parameters to form a robust system against outside noise while minimizing experimental time and cost. Making bicistronic expression operating units, which reduce the variability of gene expression, available in the Registry of Standard Biology Parts will enable efficient engineering of large networks. Finally, collaboration among teams allowed for a new standardized form of submitting characterization of parts to the Parts Registry. These three approaches to improve part standardization and robustness will help move the field of synthetic biology one step closer to proving that biology can, in fact, be engineered.

Team Queens Canada: Biosynthesis and breakdown of human odour compounds for the behavioural manipulation of malarial mosquitos

Malarial mosquitoes are developing resistance to key insecticides and drugs, and are becoming diurnal to avoid treated mosquito nets. Recent studies have shown that the African mosquito uses human foot odour to locate its host, a trait that is enhanced when the insect is carrying malaria. We plan to combine a carboxylic acid reductase and an acetyl transferase, in order to create E. coli capable of converting a major component of foot odour (isovaleric acid) into banana smell (isoamyl ester). This could have both commercial and humanitarian applications. Our second goal is to deliberately synthesize mosquito attractants inside traps. Recent research has shown that a mixture of CO2 and foot odour volatiles can be more attractive than a human. We have chosen indole as our first attractant, a compound naturally found in human sweat. We hope our project will show that bioremediation and biosynthesis techniques have applications in mosquito control.

Team RHIT: Constructing a Unique Platform for Interspecies-Dependence (CUPID): The evolution of multicellular machines.

CUPID aims for stable obligate symbiosis for the model prokaryote Escherichia coli (bacteria) and the model eukaryote Saccharomyces cerevisiae (yeast). We address this goal by constructing a unique platform for interspecies-dependence based on inducible expression of a required histidine biosynthetic gene (HIS) in each species. Expression of the bacterial HIS gene is induced by lactic acid produced by constitutive expression of a lactate dehydrogenase gene in the eukaryote. Expression of the yeast HIS gene is induced by the binding of its mating factor receptor to mating factor expressed constitutively on the surface of the prokaryote. Histidine deprivation necessitates physical contact and symbiosis for survival. Such pressure may facilitate evolution of a stable exosymbiotic form of the two species. Study of isolates and further manipulation could provide insight into the use of obligate symbiosis in synthetic biology and yield a chassis for the synthesis of novel multicellular machines.

Team Rutgers: Sensteria: Quorum Sensing E. Coli

Our project aims at developing a self-regulatory system for the degradation of virulent factors. We plan to integrate quorum sensing and pon1 to create this system of self-regulation. Quorum sensing will be used to detect the presence of high cell densities and to activate pon1 upon detecting high cell density. Pon1, acting as a repressor, will degrade the signal molecules needed for quorum sensing and thus turn off quorum sensing as well as itself. It will reactivate upon the reactivation of quorum sensing and this cycle of self-regulation will persist. This prototype system is meant to test the application of this system but the main

goal is to introduce this system to pseudomonas aeruginosa as a means to prevent the harm that it causes.

Team Stanford-Brown: Synthetic Bio-Communication

Communication is a dynamic requirement for life as we know it. We are using cellular and molecular messaging of different magnitudes to improve the broadcasting and reception of information. Starting on the atomic level, our BioWires project has created silver-incorporating DNA to act as nanowires, which could improve the cost and effectiveness of electronic products. Our CRISPR project is creating a system for DNA messages and resistances to be passed from cell to cell, in effect, creating transmissible probiotics and changing the way that cells communicate. We are also building a chromogenic biosensor to detect sucrose secretion that will be launched on a satellite (EuCROPIS) into low-Earth orbit. Finally, our De-Extinction project involves decoding messages from the past to better understand early life on Earth.

We are the Stanford-Brown iGEM team, and we're connecting life on Earth, to help us prepare for life beyond it.

Team Toronto: Biofilm: System engineering in E. coli

Microorganisms frequently adopt a lifestyle in which they excrete extracellular biopolymers to aggregate and form biofilms. We are researching the pathways that induce biofilm formation and maturation in E. coli, for modulating surface-specific adhesion of E. coli biofilms. We are constructing and characterizing E. coli strains with targeted deletions or recombinant protein expressions that are crucial in biofilm formation pathways. In response to environmental stimuli such as temperature, blue light, or sodium, the phenotype of each mutant E. coli strain will be characterized using a specially developed high-throughput protocol. Biofilm formation control has applications for bioremediation, in which we are pursuing in a related project on heavy metal precipitation. Another potential application is in medical treatment of pathogenic infections, since the generally slow diffusion rate in biofilms gives pathogenic bacteria/fungi an additional mechanism for antibiotic resistance phenotypes.

Team UC Davis: RiboTALe: A Tunable and Modular System for Control of Gene Expression

Despite the fact that the Registry of Standard Biological Parts contains a large number of inducible promoters, the actual usage of these parts is dominated by a very select few. In order to increase the versatility of expression control systems, we propose a new system coupling transcription activator-like effectors (TALEs) with inducibleriboswitches. TALEs are proteins secreted by the bacterial pathogen Xanthomonas that contain engineerable, sequence-specific DNA binding domains and can act as transcriptional repressors or activators. We plan to manipulate TALE activity by subjecting them to inducible expression through riboswitches and promoters. By pairing TALEs with riboswitches, we can expand the existing library of inducible repression systems. In addition, we hope to modify the parameters of our system to show the tunability and modularity of our overall construct. Through proper characterization, we believe that iGEM teams may also use these modular repression systems for the development of future devices.

Team UChicago: Keratinase Expression System in E. coli and B. subtilis

Each year, the poultry industry produces over two billion pounds of feather waste that is mostly processed into nutrient-poor animal feed but recent research has shown that feather keratin can be used to produce

biodegradable plastics, fertilizers, detergents, and pharmaceuticals. However, current chemical methods for keratin degradation are energetically costly and previous efforts at keratinase production in heterologous hosts have been stymied by poor protease expression. To address both these problems, our team constructed BioBricks based on kerA, a serine keratinase gene native to B. licheniformis active on whole poultry feathers. We designed two biobricks for expression in E. coli and constructed a high copy number BioBrick plasmid with an origin of replication compatible with B. subtilis. By designing a new keratinase expression system in B. subtilis, we hope to provide a faster, cheaper alternative to current methods of industrial keratinase production.

Team UCLA: DiversiPhage: Library Generation for Protein Selection

Both the mammalian immune system's complex defenses and a bacteriophage's targeting mechanism depend upon protein diversification. These models have inspired innovations ranging from targeted drug delivery to protein display. Using the major tropism determining (MTD) protein expressed on the Bordatella bacteriophage BPP-1, we aim to develop an in vitro system for generating antibody-like proteins that bind specified targets. The MTD protein expressed at the phage's tail fiber is naturally modified at its variable region to produce nearly 10e13 possible binding variants while preserving its structure. Mutating the MTD's variable region by PCR can match the massive diversity of MTD in vitro. A library of MTD protein-DNA fusions generated by mRNA display can then be screened for binding against specified protein targets. This in vitro analog to phage display and immune clonal selection can be a powerful tool for constructing target-binding MTD variants with equally many varied applications.

Team UCSF: Operation CRISPR: Deploying precision guided tools to target unique species in a complex microbiome

In microbial communities, bacterial populations are commonly controlled using indiscriminate, broad range antibiotics. There are few ways to target specific strains effectively without disrupting the entire microbiome and local environment. The goal of our project is to take advantage of a natural horizontal gene transfer mechanism in bacteria to precisely affect gene expression in selected strains. We combine bacterial conjugation with CRISPRi, an RNAi-like repression system developed from bacteria, to regulate gene expression in targeted strains within a complex microbial community. One possible application is to selectively repress pathogenic genes in a microbiome, leaving the community makeup unaffected. In addition, we use CRISPRi to lay the groundwork for transferring large circuits that enable complex functionality and decision-making in cells.

Team UGA-Georgia: Geraniol production via novel protein expression tools in Methanococcus maripaludis

Geraniol is an intriguing 10 carbon compound with diverse applications including use as an agent for cancer prevention, fragrance, insect repellent, proposed biofuel etc. We explored and engineered a novel gene expression tool (BBa_K890000) for Methanococcus with the capability of expressing geraniol synthase from Ocimum basilicum (BBa_K1138000). We report the biosynthesis of geraniol at over 5% of DCW by transforming the vector into Methanococcus thereby expanding its native isoprenoid pathway. Furthermore we engineered new vectors (BBa_K1138001 & BBa_K1138002) with the potential capability of regulating and quantifying the expression of desired proteins via red fluorescence. This work demonstrates the use of Methanococcus as a cell factory for chemical production and highlights synthetic biology advancement by engineering new systems over traditional biological systems such as Escherichia coli.

Team UIUC Illinois: Cardiobiotics - A Genetically Engineered Approach to Cardiovascular Health

Cardiovascular disease (CVD) has been the leading cause of death in the United States for over twenty years and is a rising global health issue. Recent studies demonstrate a correlation between CVD and atherosclerosis, the buildup of plaque in the arteries. One associated risk for atherosclerosis is the production of Trimethylamine N-oxide (TMAO) by natural gut flora when metabolizing L-carnitine, a chemical found primarily in red meat and energy drinks. We created a probiotic to attack the root of this problem by outcompeting the gut bacteria for L-carnitine in order to suppress the production of TMAO. L-carnitine transporters (caiX and cbcWV) and L-carnitine dehydrogenase (CDH) derived from Pseudomonas aeruginosa were expressed in a safe strain of E. coli (Nissle 1917). This engineered E. coli can uptake and metabolize L-carnitine along an alternative, safe pathway into 3-dehydrocarnitine. Together, this system offers a novel solution in preventing TMAO-related cardiovascular health conditions.

Team uOttawa: Fold-change molecule detection using the Type-I Incoherent Feedforward Loop

Many synthetic gene networks are susceptible to cellular noise, as they rely upon the absolute levels of gene regulators which can vary greatly between individual cells. To address this, uOttawa has engineered a network in S. cerevisiae that is responsive to fold-changes as opposed to absolute changes in stimulus. This allows the network to maintain sensitivity despite noise, and also permits response to stimuli in a much larger dynamic range. By modifying the promoter driving the stimulus, the network can be engineered to detect fold-changes of any molecule with a responsive promoter, thereby serving as a structural chassis for the next generation of molecule detectors. In addition, we have also authored a children's book aimed at disseminating the concepts of synthetic biology to the public, and have designed an online interface that will facilitate the rapid construction of devices built from the Registry of Standard Biological Parts.

Team UT Dallas: A Multifaceted Approach Against Tooth Decay

Cavities are a problem faced by people and animals worldwide and are primarily caused by the bacteria Streptococcus mutans. S. mutans digests certain sugars, consequently forming lactic acid that can lead to tooth decay. Our system starts with the detection of saccharides essential for S. mutans' survival. Using dextranase, the E. coli are able to attach onto the dextran layer that S. mutans normally live on. This allows the E. coli to deliver a targeted dose of norspermidine, helping with the breakdown of S. mutans biofilms. We also took another approach that utilizes a natural quorum signaling molecule, competence stimulating peptide (CSP). This peptide is responsible for population control in S. mutans. By using these approaches, we hope to aid in the treatment of cavities in both humans and animals.

Team Utah State: AMPed up E. coli

Antimicrobial peptides (AMPs) are peptides that have activity against a wide range of microorganisms. AMPs can vary greatly in their size, structure, and mode of antimicrobial activity. Due to the immense interest in both testing and characterizing AMPs (from various forms of life) a sustainable method to produce and purify them is necessary. Using Synthetic Biology as a platform, the production of AMPs in E.coli could potentially offer a cost-effective approach for large-scale peptide manufacture, as opposed to isolation from natural sources. After successfully manufacturing several AMPs using E.coli, these peptides will be tested on a wide variety of organisms to screen for antimicrobial activity.

Team UTK-Knoxville: Modular Design of Chimeric Biosensors

The major limitation in synthetic biology today is the lack of numerous, well characterized sensors. Our project aims to provide a reliable scaffold to test potential sensing domains with unknown substrates. We have created a standard platform to test a range of intracellular and transmembrane domains. Positive results are reported with red fluorescent protein for easy identification which can be done with high throughput methods such as 96 well plates and flow cytometry. We test our platform on sensors with interesting known responses. The chimera proteins are also useful in creating signals orthogonal to the cell.

Team Virginia: Minicells: Multi-Purpose Nano Chassis

Overexpression of the tubulin-homolog FtsZ leads to asymmetric cell division in E. coli that yields achromosomal 'minicells.' The lack of a chromosome renders minicells unable to replicate and cause infection, yet they still retain and express plasmid genes. Furthermore, minicells inherit the stable, non-leaky membranes and cytosolic composition from their parent cell. Our project design is centered on the creation of an IPTG-inducible FtsZ Biobrick that permits tunable overexpression for optimal minicell production. With the development of a multi-purpose, innocuous bacterial chassis as our ultimate goal, we incorporated three additional safety elements: the Ail protein, a polysialic acid capsule and de-acylated lipopolysaccharide. Both Ail and the PSA capsule serve to prevent complement deposition on the surface of the minicells, with PSA also protecting against antibody opsonization. Finally, LPS toxicity is reduced by inducing minicell formation in an lpxM mutant strain that lacks a critical myristoyl transferase for late-stage acyl modifications.

Team Washington: Red Light! Green Light!

Refinement of functional systems is a key aspect of engineering. Biological systems are not immune to this fact and must be continuously improved to function consistently, and reliably. Creating easy methods for tuning biological systems using light was the goal of this year's effort. Our team sought to improve on a previous light-regulated gene expression method, integrate the use of tablet device for testing, and create multiple functional biobrick constructs. In 2012, we developed an app that affords any researcher with access to an android device with a LED screen the ability to illuminate cells in a controlled manner for synthetic biology applications. We improved the functionality of the app by adding a mini petri dish setting and demonstrated the advantages of paralization by performing experiments in a 96 well plate format. This tablet application represents the first real life example of using a mobile device as synthetic biology instrument.

Team WashU StLouis: Converting E. Coli into a Nitrogen Bio-Fertilizer Using a Cyanobacterial nif Cluster: an iGEM project

The nif cluster of Cyanothece 51142 consists of 29 genes that construct and regulate a nitrogenase protein complex. This year, our iGEM team worked on harnessing the power of nif to produce ammonia in Escherichia coli. After synthesizing a nif-containing plasmid (28 kbp) using the DNA assembler method (Shao et al 2009) and transforming that plasmid into E. coli, our team tested for nitrogenase activity using the acetylene reduction assay. The transformed E. coli were then compared to wild-typeunder limited nitrogen conditions to check for a competitive advantage. Tests were used to evaluate the expression of various nitrogenase subunits. Our team also aimed to further characterize the promoter sequences of the Cyanothece 51142 nif cluster. Between the cysE and nifB genes, there is a 958bp uncharacterized, bidirectional promoter region of particular interest. We used fluorescent reporters to identify key regions within this promoter sequence under various environmental conditions.

Team Waterloo: Controlled Modification and Intercellular Transmission of a DNA Message

In nature, intercellular communication allows coordinated cellular behavior on a population level. Engineers seeking complex programmed population-level behavior require tools enabling controlled, information-rich intercellular messaging. Given its versatility and universality as an information-encoding molecule, DNA suggests itself as a message-carrying molecule to enable information-rich messaging. The Endy group at Stanford recently published a proof-of-principle demonstration of such DNAmessaging wherein a DNA message was transmitted from one bacterial population to another carried by M13 bacteriophage particles. Here we propose an intercellular communication program that extends DNA messaging by controlling modification and transmission of a DNA message. Modification is controlled through flipping a DNAswitch on the message DNA – a promoter sequence that is invertible using serine integrases and recombination directionality factors (RDFs). Transmission is controlled by placing expression of the M13 major coat protein, which is required for viral packaging of message DNA, under control of such a switch.

Team Wellesley Desyne: Enhancing Bio-Design with Next-Generation Human-Computer Interaction

Systems that integrate the wide array of technological tools available to synthetic biologists are needed more than ever. As the field of synthetic biology continues to advance, it is critical to communicate the applications, goals, and limitations of synbio research to the public. Our team is creating a software suite, which addresses technical synbio challenges while improving end-user experience and harnessing human-computer interaction (HCI) to engage the public in synbio concepts. Eugenie is a visual language and integrated development environment for Eugene that allows biologists to specify biological parts, properties, and device composition rules. zTree is a tool for visually representing the Registry of Standard Biological Parts to support sense-making of complex, hierarchical data sets. Bac to the Future is a web-based, interactive application that utilizes Twitter to illustrate synbio ideas to the public. The application of HCI techniques to synbio fosters more effective, collaborative, and intuitive software tools.

Team WLC-Milwaukee: The One Ring to Secrete Them All

Our construct utilizes removable purification and secretion tags separated by a single enzyme cut site NheI that is the insertion point for any coding sequence of interest. The induced gene can be secreted from the cytoplasm to the extracellular space via a tripartite secI pump. A cymR-regulated T5cumate promoter within the plasmidcontrols a Tse2 toxin to avoid horizontal gene transfer, while the plasmid also encodes a chimeric ompA-monoclonal antibody gene, which when expressed, inserts into the outer membrane and the antibody selectively binds norovirus within the gut to hinder infection. This plasmid construct was used to over-express and secrete plant-based, polymer-degrading enzymes bglS, yesZ, and xynA isolated from the cellulosome of Bacillus subtilis subtilis 168 to digest plant material. We utilized Escherichia coli Nissle 1917 containing a chromosome-integrated cymR gene to secrete these enzymes in the hope to accentuate dietary uptake for humans and livestock in impoverished countries.

Team Yale: Converting E. coli into a foundry for bioplastics

Poly(lactic acid) (PLA) is a biodegradable, biocompatible, bioresorbable, thermoplastic bioplastic that offers many advantages over other biomaterials in both commercial and medical applications. The current chemical method of synthesizing PLA is expensive, and the required processing and purifying steps use many environmentally unfriendly chemicals. Recently, E. coli has been engineered to produce PLA, but low yields and short chain lengths prevent the approach's commercial use. Here, we evaluate the potential of

using multiplex automated genome engineering to raise both yields and chain lengths of biosynthesized PLA by directing the E. coli metabolism to funnel resources toward PLA production without sacrificing the organism's viability. Efficient biosynthesis of PLA, which may be thus achieved, would be a significant step in reducing the impact of plastic waste, and would benefit those receiving bone implants. It would also open up a new possibility in rapid manufacturing of personalized bone implants using three-dimensional printers.

2011

Team Alberta: Genetically engineering a common fungus to produce biodiesel from cellulosic waste.

Converting food into biofuel is an unsustainable proposition. Our project focuses on the creation of cellulosic biodiesel using waste products. We are engineering Neurospora crassa, a highly efficient cellulose metabolizer, to produce an excess of fatty acids by both inhibiting beta oxidation and up-regulating fatty acid synthesis by the one-step replacement of the FadD gene with a thioesterase gene. We are testing the growth of Neurospora on a variety of waste substances and are developing an efficient chemical esterification method to convert the fatty acids into fatty acid methyl esters, a common biodiesel requiring no changes to current fuel delivery infrastructure.Neurospora crassa's broad substrate preferences give it unique advantages for bioproduction from cellulose. We have therefore developed an efficient and reliable system for modular bioengineering of Neurospora including a starter kit of basic reusable parts with the intent of creating a novel chassis for metabolic engineering and synthetic biology.

Team Arizona_State: CRISPR Assisted Genetic Engineering

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are a genomic feature of many prokaryotic and archeal species. CRISPR functions as an adaptive immune system, targeting exogenous sequences that match spacers integrated into the genome. Our project focuses on developing a set of tools for synthetic control over the CRISPR pathway. This includes a method for creating polymers of repeat-spacer-repeat units, the development of CRISPR biobricks (CAS genes, leader sequences) for several CRISPR subtypes (E. coli, B. halodurans, and L. innocua), testing these components on plasmids containing GFP, and a software tool to collect and display CRISPR information, as well as select spacers from a particular sequence. Given the relatively recent progress in the scientific understanding of this system, we see the potential for a wide range of biotechnological applications of CRISPR in the future.

Team Baltimore: Making Synthetic Biology More Accessible: Making Wetware and Dryware for the Synthetic Biology Lab

The overall mission of our team is to attempt to overcome some practical barriers to entry of groups and laboratories that may not be well-funded or may not have the capital requirements to realize their synthetic biology dreams. We approach this problem from two angles- "wet-ware" and "dry-ware." Our wet-ware goal this year is to provide a way for anyone to be able to make their own Taq polymerase instead of having to purchase it. Our dry-ware goal this year is to create a prototype for a PCR machine that can be assembled from an inexpensive kit.

Team Bard-Annandale: "Zener Diode" Quorum Sensing: Communication in One Direction at a Time

A directional quorum sensing system was made using E. coli immobilized in a microfluidic chip. One strain

of E. coli carrying the LuxI and LuxAB genes was trapped in hydrogel at one end of microfluidic channel. Another strain of E. coli carrying a LuxR operon attached with a GFP reporter gene and LuxCDE gene was similarly trapped at the other end of the channel. Depending on the direction of flow, one group of E. coli will be downstream of the other and therefore capable of receiving a chemical signal. Acting as an analog of zener diode, this device is capable of controlling the direction of communication between the E. coli. The orthogonal visual responses, one fluorescent and one luminescent, should be clear indicators of which way communication took place. This device is a novel member of the growing toolkit available to perform logic operations with living systems.

Team Berkeley: DETECTR

Biosensors have widespread applications ranging from diagnostics to environmental monitoring. Vibrio cholerae's ToxR system can be used as a component in biological devices capable of detecting a wide variety of molecules. A periplasmic domain causes ToxR homodimerization, activating transcription of the ctx promoter. By replacing the periplasmic domain of ToxR with existing or engineered ligand-dependent homodimers, we hope to link ToxR dimerization (and gene expression) to the presence of specific ligands. Initially, ToxR constructs proved to be toxic to E. coli. To address ToxR toxicity, we screened microarray data for promoters that exhibited stress-based down regulation. We constructed a negative feedback system with the rffGH promoter, which permits the use of potentially toxic proteins like our various ToxR chimeras. By fusing existing or engineered ligand dependent homodimers to ToxR, this modular system can be applied to develop new biosensors.

Team British_Columbia: iSynthase: Mass production of terpenes in yeast

In nature, terpenes are mostly synthesized and secreted by plants as a defense against pathogenic attacks by insects and fungi, such as the case of the mountain pine beetle infestation. These compounds are also utilized in pharmaceuticals, fragrances, food, and energy industries, which drives interest for high-scale production. Hence, we aim to optimize production of terpenes in Saccharomyces cerevisiae yeast by constructing the biosynthetic pathways necessary to synthesize and retain these compounds. To simulate the system, we are also developing a model of terpene production in yeast using SimBiology Toolkit in MATLAB. In parallel, we are constructing a mathematical model to predict the dynamics of the mountain pine beetle populations in British Columbia, Canada under the influence of our synthetic yeast.

Team Brown-Stanford: Mars BioTools: Synthetic Biology for Space Exploration

One of the major challenges of space exploration is the enormous cost of launching materials, limiting the size and affordability of long-term missions. Synthetic Biology can revolutionize space exploration and settlement by providing a microbial platform for catalyzing critical reactions and manufacturing essential products. Biological devices have a major advantage over classical machines: the ability to self-replicate and regenerate.

Project RegoBrick uses bacteria to cement Martian and Lunar regolith simulant into a concrete-like compound. Extraterrestrial settlements will be able to use such a process to build structures using resources readily available in the environment, instead of having to transport materials from Earth.

Project PowerCell develops a universal energy source from engineered cyanobacteria, which generate carbon and nitrogenous nutrients from sunlight and air and secrete them to sustain other microbes. This system will allow future settlers to transform resources on other planets into fuel, food, drugs, and other useful products.

Team BU_Wellesley_Software: Trumpet, Puppeteer, E-Notebook, Optimus Primer, and Gnome-Surfer: A Workflow for Collaborative Research, Design, and Assembly

We present tools which facilitate the research, design, and fabrication of biological constructs. Our workflow comprises Gnome Surfer for research, Trumpet and Optimus Primer for design, and Puppeteer and E-Notebook for the construction of those designs. Gnome surfer promotes collaborative research by allowing users to browse genes, Parts, and other DNA along with their associated literature on a table-top surface. Using Optimus Primer, primers can be designed for the selected Parts. Trumpet generates permutable constructs from these Parts by interleaving invertase sites among them. To assemble these permutable constructs, we present a Protocol Automation Stack comprising a high-level programming language called Puppeteer, executable on a robot. For improving manual protocol execution, we are developing an Electronic Lab Notebook that helps capture data, and schedule resources and lab activities. Our unique tools offer an end-to-end workflow that is collaborative, includes wetlab automation and organization, and provides algorithms for designing configurable constructs.

Team BYU_Provo: E. colinoscopy

We constructed a novel molecular AND gate in E. coli. An AND gate requires two positive inputs to generate a single output. Either positive input without the other does not generate an output. Our AND gate expresses a reporter in the presence of both ROS and high temperature. We selected an OxyR-responsive promoter (HemH) and a thermo-sensitive riboswitch (derived from Listeria) as detectors for ROS and temperature, respectively.The OxyR-responsive promoter is used to drive transcription of the Listera thermo-sensitive riboswitch coupled to a Cre/Lox system which, when activated, removes a double-terminator sequence and allows constitutive transcription of the reporter molecule. This system may be further modified and adapted to various applications, including early detection of colon cancer.

Team Calgary: *Senseomonas NAstytoxins*

The University of Calgary's iGEM team is working on developing an electrochemical biosensor for Naphthenic Acids (NAs). NAs are toxic surfactants released into tailings ponds as a by-product of the bitumen extraction process of oil sands. Microorganisms indigenous to tailings ponds that are uniquely capable of degrading NAs suggest that bioremediation may be a viable solution. To be successful, however, levels of NAs need to be monitored and existing methods for detection are costly and offsite. Using two NA-degrading organisms relatively new to iGEM: microalgae and pseudomonads, we used bioinformatics and a novel NA affinity-based screen in an attempt to identify a sensory element. In the process, we have characterized an electrochemical reporter system and built a working measurement device. We have also submitted new parts for future work in microalgae, as well as novel parts to move constructs between Pseudomonas and E. coli.

Team Caltech: Bioremediation of Endocrine Disruptors Using Genetically Modified Escherichia Coli

Endocrine disruptors, or substances that mimic estrogen in the body, have detrimental biological effects on the reproduction of several species of fish and birds; the Caltech team focuses on bioremediation of these toxins. Our goal is to create a system housed in E. coli that can be used to process water and remove endocrine disruptors on a large scale. We focus on isolating degradation systems for the common endocrine disruptors bisphenol A (BPA), DDT, nonylphenol and estradiol. We synthesized known degradation enzymes DDT dehydrochlorinase, BisdA and BisdB, and characterized the behavior of these enzymes when acting on our target endocrine disruptors. In addition, we explored the potential of certain

cytochrome p450s to initiate degradation of these chemicals, focusing on WT-F87A degradation of BPA. Finally, we characterized the functionality of E. coli protein processing when E. coli is deployed as an easily containable biofilm on various substances in aqueous environments.

Team Colombia: Bacterial prevention of rust headaches

Our objective is to create a bacterial “detect and alert” system as an aid for defending coffee plantations against fungi (rust). Our bacteria will detect chitin, an organic compound found in fungal cell walls, and alert the plant by stimulating an early hypersensitive response.

Two strains of bacteria will be created: one that detects chitin using a two component system from V. fischerii and produces chitinase to attack the fungus as well as a signaling molecule to activate the second strain. This second strain will process and amplify the signal, and if it decides that there is a threat it will produce a second signal that mimics the internal alarm system of the plant to activate the hypersensitive response. The design is modular to allow other combinations of fungus-host plant systems to be created easily.

We expect this biocontrol method to prove very useful for farmers and reduce fungicide use.

Team Columbia-Cooper: DOT DOT DOT... Environmentally-Friendly Manufacture of Quantum Dots in E. coli

Quantum dots (QDs) are semiconducting nanoscale crystals with unique optical properties. They have many applications, including medical imaging, enhanced LEDs, solar cells, and solid state quantum computation. Our project pioneers a greener manufacturing process for QDs. We are BioBricking several metal binding peptides, expressing them in E. coli, and characterizing their ability to nucleate QDs from cadmium salts. In addition, since cadmium is dangerous to the environment, we will create QDs using less toxic metals such as zinc. We are also building a manufacturing “tuning” device that will sense specific light wavelengths emitted by nucleated QDS and activate antibiotic resistance.

Team Cornell: BioFactory

Cornell’s 2011 iGEM team has designed a new, scalable, and cell-free method to produce complex biomolecules. Current methods for purification from cellular lysate are expensive and time consuming. Biofactory utilizes modified enzymes, capable of being attached to surfaces, in the creation of a modular microfluidic chip for each enzyme. The surface bonding is performed by the well characterized biotin-avidin mechanism. When combined in series, these chips operate as a linear biochemical pathway for continous flow reactions. Additionally, we engineered E. Coli with the mechanism for light-induced apoptosis to easily lyse cultures producing the necessary enzymes. The resulting lysate is flowed through the microfluidic channels, coating them with the desired enzyme. We believe these methods will reduce unwanted side reactions, and lower the costs of producing bio-pharmaceuticals in the future.

Team Duke: Engineering Bacterial Genetic Toggle Switch Controllers Using Synthetic Zinc Finger Transcription Factors

Additions to the synthetic biologists' toolkit are expected to be interoperable and modular in order to facilitate standardization of these tools. The Genetic Toggle Switch originally presented by Collins et al (2000) was a major breakthrough in synthetic biology and utilized constitutive promoters. Here, we use Zinc Fingers as transcriptional repressors in a newly designed interfacing network, the controller, for a modified version of the original toggle switch. The controller interface is made with emphasis on interoperability and applications in mind. Specifically, we use zinc finger repressors and degradation tags to reduce cross-talk

and make the network more robust. Nine ZF transcription factors are computationally characterized and submitted to the registry. The interfacing controller and toggle switch are stochastically modeled to predict gene expression levels and are subjected to experimental testing.

Team Gaston_Day_School: Red Fluorescent Nitrate Detector

Increasing levels of fertilizer required for mechanized farming can result in elevated nitrate levels in soil and groundwater. Due to contaminated food and water, humans are at risk for methemoglobinemia caused by enterohepatic metabolism of nitrates into ammonia. This process also oxidizes the iron in hemoglobin, rendering it unable to carry oxygen. Infants in particular are susceptible to methemoglobinemia, also known as “blue baby syndrome”, when formula is reconstituted using water contaminated with nitrates. By combining the red fluorescent protein coding region with a nitrate sensitive promoter, we are developing an inexpensive, simple, visual test for nitrate contaminated water. Use of this detector in agricultural areas could alert families to the presence of nitrates in groundwater and prevent blue baby syndrome.

Team GeorgiaState: Isolation & Characterization of P. pastoris promoters in E. coli

Pichia pastoris is a methylotrophic yeast used as an alternative host for protein production in addition to Escherichia coli and Saccharomyces cerevisiae. There are several reasons why P. Pastoris is an ideal host organism. Its ability to perform eukaryotic post-translational modifications, high yields of recombinant protein, and its genetic similarity to S. cerevisiae are very attractive traits (Cereghino and Cregg, 2000). The Georgia State University iGEM team has isolated three promoters from P. pastoris. These promoters have been inserted into standard biobrick vectors. The team plans to transform these parts into E. coli and characterize their productivity using fluorescence.

Team GeorgiaTech: De Novo Adaptation of Streptococcus thermophilus CRISPR1 Defense in Bacillus Subtilis

A diverse range of Bacteria and Archaea acquire resistance to foreign DNA by integrating short fragments of the invading nucleic acid into clusters of regularly interspaced short palindromic repeats (CRISPRs) on their genomic DNA. For our project we have PCR amplified the CRISPR1 locus from the chromosome of Streptococcus thermophilus [DGCC7710] and ligated it into an integration vector to place it on the chromosome of Bacillus subtilis through allelic recombination on the chromosome. B. subtilis served as our model organism because it does not naturally posses a CRISPR mechanism. This should demostrate that the S. thermophilus CRISPR1/Cas system can be transferred into Bacillus subtilis and provide heterologous protection against plasmid transformation and phage infection.

Team Grinnell: Exploiting the Secretion System of Environmental Caulobacter crescentus to Deliver Biofilm-inhibiting Proteins

Caulobacter crescentus is a non-pathogenic aquatic bacterium that can grow to high densities in low-nutrient environments. It has a robust Type I secretion system that secretes a single protein, RsaA. This is a surface layer protein that totals 10-12 percent of all of the protein in the cell. We created a toolbox of biobrick parts that enable this system to secrete any protein of interest when fused to the C-terminal secretion signal of RsaA. Because Caulobacter is cheap and easy to grow but cannot survive in a human host, it has the potential to be an efficient chassis for enzyme-based drug delivery. As a proof-of-concept, we engineered non-biofilm forming strains of Caulobacter to secrete two enzymes, Esp and DspB, which have been shown to degrade biofilms. We tested our strains and demonstrated that our recombinant enzymes are secreted and that they do inhibit Staphylococcus aureus biofilm formation.

Team Harvard: Engineering customized zinc finger protein arrays by massively multiplexed protein design and selection

Gene therapy is a powerful approach for the treatment of disease; however, current therapies using viral vectors carry significant risk of tumorigenesis due to their use of non-specific gene insertion. To meet this challenge, we engineered zinc finger proteins (ZFPs), which are tailored to bind to DNA with high specificity, enabling precise genome editing. Our group has developed a foundational technology for synthesizing nearly 50,000 unique ZFPs using chip-based DNA synthesis based on bioinformatic analysis and for identifying the best binder using a novel genomically encoded 1-hybrid genetic selection scheme in a massively multiplexed fashion. Further, we employed multiplex automated genome engineering (MAGE) for facile editing of the E. coli genome enabling rapid modification of ZFP target sites, gene knockouts and silent codonsubstitutions. These tools allow for low-cost creation of ZFPs targeting any endogenous human gene, which will increase the accessibility of customized genome editing for gene therapy.

Team ITESM_Mexico: SensE.coli: Dual light controlled arabinose biosensor

Integrating the work of many other previous iGEM teams (Tokyo NoKoGen 2010, Chiba 2009, 2010, British Columbia 2009, Cambridge 2010, UNAM-Genomics México 2010, ITESM Monterrey 2010), the aim of this project is to develop a way of giving a cell the command to perform a function at user's will, improving current lock-and-key designs. A novel mechanism based on an E.coli chassis, was designed with two main objectives: to sense arabinose reporting its concentration and to use light receptors to trigger the expression of the required pathways. The first receptor enables E.coli to activate (express), the arabinose sensing mechanism; whereas the second receptoractivates a quick deactivation(degradation), of the sensing mechanism depriving the cell of that capability.

Team Johns_Hopkins: VitaYeast: addressing malnutrition with synthetic vitamin production in baker's yeast

While hunger is a major issue in developing nations, it is often a lack of specific nutrients rather than total calories that underlies specific health issues including infant mortality, birth defects, and blindness. To ameliorate worldwide malnutrition, we engineered baker's yeast to produce vitamins A and C. "VitaYeast" can be used to bake bread without special equipment, training, orreagents. We designed our genetic constructs using state-of-the-art optimization techniques, simulations, and data from our parts characterization experiments. In order to realize this design, we developed an extensive yeast toolkit including promoters, UTRs, and vectors. We synthesized the violacein pathway, which, combined with our vitamins and intermediate metabolites, represent a new set of yeast reporters. Finally, to assess the potential of VitaYeast to combat hunger in the real world, we have sought IRB approval to distribute an extensive survey on attitudes toward genetic engineering of food world-wide.

Team Lethbridge: Tailings pond clean up kit, a synthetic biology approach to bioremediation

Mining extraction and refining processes produce toxic by-products that are often stored in tailings ponds. Tailings ponds are artificial reservoirs where the by-products such as toxic organic compounds, heavy metals or fine clay particles are stored until they are remediated by industrial treatment or natural degradation, which can require decades. We are developing the components necessary to create a tailings pond clean up kit for removal of harmful by-products. The first component uses the xylene degradation pathway of Pseudomonas putida optimized by the use of a protein microcompartment produced from the engineered Aquifex aeolicus protein lumazine synthase. The second component removes heavy metals by

producing nanoparticles with the Magnetospirillum magneticum protein Mms6. The third component causes sedimentation of fine clay particles using natural properties of Eschericia coli and cell aggregation with Antigen 43. The final component removes the genetically modified organism's DNA by using restriction endonucleases.

Team McGill: All You Need is LOV: Photoswitchable dimerization in mammalian cells

Optogenetics involves the use of light to remotely control cellular function via light responsive proteins. It is a promising tool for engineering optical regulation of cellular behavior. Unlike most stimuli, light signals have the advantage of being highly precise with regards to temporal and spatial action as well as having readily tunable intensity. Within cells, a variety of effectors can be controlled using light, including DNA binding proteins, enzymes and mediators in signal transduction. Specifically, our projects focus on building light-responsive biobricks for control of mammalian cells by fusing photo-switchable domains found in plant, algal and fungal light sensing proteins to such effectors. Mammalian biochemistry is complex and well regulated, necessitating synthetic effectors that can be easily and dynamically adjusted to meet the needs of the application. Photo-switchable systems provide this versatility.

Team Michigan: DNA directed cell immobilization using outer membrane protein containing zinc finger domain

The ability of zinc finger domains to selectively bind specific double stranded DNA sequences have largely been applied intracellularly, such as in engineered zinc finger nucleases for genomic manipulations. Proteins containing zinc finger domains can also be used extracellularly to precisely adhere objects to surfaces containing bound oligonucleotides. This project aims to utilize the specificity of zinc finger protein to direct binding of Escherichia coli to oligonucleotides bound on surfaces. The fusion protein engineered to contain a fragment of the OmpA membrane domain and a zinc finger domain allows the protein to be expressed on the outside of the cell while remaining bound to the host cell. Possible applications of this project include creating patterns with fluorescently labeled cells or studying cell-cell interactions.

Team Minnesota: E. coli Based Biotemplating

The objective of our project is the construction of a light-inducible system in which E. coli is engineered to express silacatein in order to create a biotemplating system. This system has many potential uses, such as the creation of precise nano-structures or biomemetic bone. The coliroid light-inducible system was assembled by cloning a fusion protein of Cph1 and EnvZ, called Cph8, from a previous iGEM group, and isolating and cloning the two other genes (Heme Oxigenase and PcyA) required for functionality of the system and putting all the parts into a vector. Then the silicatein alpha gene isolated from Subterities domuncula was fused with E. coli outer membrane protein A (OmpA) and ice nucleation protein (INP) and inserted in the vector under control of the coliroid system.

Team Missouri_Miners: Glucose Sensor

In the bodies of people with diabetes, the ability to recognize and respond to glucose concentrations in the blood has been compromised. As a result, glucose accumulates to dangerous levels. High blood glucose concentrations can cause irreversible damage to critical organs, impairing their functionality. With parts from the iGEM registry, our team created a glucose-controlled promoter linked to a yellow fluorescence production gene in E. coli. The concentrations of glucose to which the promoter responds can be determined. Once the concentration is known, the promoter can be mutated so that it will be activated by varying concentrations of glucose and be used as a glucose sensor for people with diabetes. In the future,

an insulin gene could be added to this system for use in insulin pumps, where specific glucose levels trigger insulin production in E. coli.

Team MIT: Towards Tissue Self-Assembly via Juxtacrine Signaling

Current medical practices are only able to scratch the surface of tissue engineering or organ development. Fortunately, nature has provided us with robust, cellular systems capable of governing the autonomous formation of complex structures. To pursue control of multicellular systems, we engineered a number of ligand-receptor signalingmechanisms: the Notch-Delta juxtacrine signaling pathway and an assortment of G-Protein Coupled Receptors. Both signaling systems were modified to activate orthogonal genetic circuits, allowing for processing and integration of numerous signals. In order to facilitate and maintain pattern formation, we introduced cadherin, a natural intercellular glue. To understand and predict multicellular behavior, we developed a simulation framework based on the Synthetic Biology Open Language and CompuCell 3D modeling environment. Our models motivated several circuit designs we subsequently tested in the laboratory. Altogether, our developments establish a paradigm for manipulation of intercellular communication systems to drive self-organization of tissues.

Team Nevada: So Happy Together: A Cooperative Relationship between Cyanobacteria and E. Coli for production of biofuels

Traditional methods for obtaining biofuels have relied on the fermentation of agricultural crops. The problems are reduction in land available for food production, relatively low levels of CO2 biofixation, and large biomass requirements. Our project aims to overcome these problems by utilizing E. coli for the production of biodiesel (C-12 fatty acids) and bioethanol. There have been a number of examples of biofuel production in E. coli; however 30-40% of production cost is based on media costs. Our project will surmount these high production costs by engineering the cyanobacteria, Synechocystis PCC6803, to secrete large quantities of glucose that will feed our biofuel-producing E .coli. Cyanobacteria and E.coli will be co-cultivated to allow the mutual transfer of carbon to produce biofuels. This project provides an efficient means for producing biofuels without a carbon source. It will also create a novel cooperative system between bacterial species that may have further industrial implications.

Team Northwestern: My NU P.A.L.

Pseudomonas aeruginosa is an opportunistic pathogen commonly found in immunocompromised patients. In addition to being the primary cause of lung infections in cystic fibrosis patients, many severe nosocomial infections can be attributed to P. aeruginosa. Currently, the standard detection method requires a potential sample to be grown overnight and then screened for the pathogen of interest. Our goal is to create a faster detection method without sacrificing reliability or experimental resolution. To realize our objective, we harnessed the native cell signaling and quorum sensing machinery of P. aeruginosa. Quorum sensing in P. aeruginosa is a complex hierarchy that governs the expression of numerous virulence genes. Quorum sensing elements from P. aeruginosa were transplanted into E. coli and used to express detectable reporters. We are thus creating a novel biosensor capable of detecting the presence of P. Aeruginosa both quickly and effectively.

Team NYC_Software: Deinococcus Genome Sequencing & Registry/ Biobrick Tools

Our goal is to sequence the genomes of several Deinococcus bacteria, construct phylogenetic trees and identify links between radiation resistant species using a combination of publicly available genome analysis tools. By sequencing the genomes of species of the radiation-resistant Deinococcus genus, we hope to

identify genesthat may be responsible for increased protection against superoxide radicals and ionizing radiation. We are also performing RNA-seq experiments to ascertain what genesare upregulated by radiation exposure. We also realize that there are significant gains to be made on the software side of synthetic biology, so we are working with others to code tools to integrate into the Registry and into iGEM teams' biobricking pipelines.

Team NYC_Wetware: Radiation-resistance? There's a Gene for that!

Extremophile organisms have adapted to extreme chemical and physical environments here on Earth. Deinococcus radiodurans, also know as Conan the Bacterium, is famous for being resistant to 1000 times the amount of radiation that would be lethal to humans. By identifying genes that could be responsible for such extreme radiation resistance in D.rad as well as other organisms, we hope to enable future engineering of radiation-resistant organisms to use in toxic conditions for bioremediation here on Earth or in the high radiation background during future space travel to Mars.

Team Panama: Alternative E. coli Oil Spill Bioremediation Kit

Since late April 2010, the world has experienced a devastating oil spill throughout the Gulf of Mexico that has become one of the worst environmental disasters in worldwide history. Oil spills can pollute air and water and alter ecosystems for years. For this reason, last year's Panama team designed a BioBrick that can biosynthesize rhamnolipids under the presence of rhamnose and β-hydroxyalkanoic acids. Rhamnolipids have emulsifying properties that reduces the surface tension of water, making the hydrocarbons easier to recover and biodegrade. This year we want to create a BioBrick that provides rhamnose for rhamnolipid biosynthesis and for other purposes as well. We also want to create a bioremediation system using existing biobricks that will produce the rhamnolipid while biodegrading the hydrocarbons in vivo. The system will be designed to operate with a lysis gene that will kill the cells once bioremediation of the contamination site is done.

Team Penn: "Cellular phones" - an optogenetic cellular signaling system

Paracrine cell signaling typically occurs through chemical messengers. Such signaling is limited by diffusion coefficients and unique cell properties, and cannot easily be controlled in therapeutic applications. The goal of this project was to demonstrate light-based cell signaling in mammalian cells. Using luminescent proteins and optogenetic tools, we constructed "Sender" and "Receiver" HEK 293T cells which send and receive blue light, respectively. The Sender Cell expresses a 480nm light emitting protein. The Receiver Cell expresses Channelrhodopsin-2, an ion channel gated by 480nm light. When the Receiver Cell is illuminated, Channelrhodopsin-2 opens, triggering a second luminescent protein via calcium influx. Since the luciferases in this system require an externally added luciferin substrate, we have also been developing Pre-coelenterazine, a genetically encoded luciferin which could allow our system to function autonomously. Our light signaling system has eventual applications in interkingdom signaling and optical controlling of synthetic networks.

Team Penn_State: Bacterial Dosimeter--Detecting Levels of Harmful Radiation

Ionizing radiation and radiation pollution is an important environmental problem that not only affects those working around radiation facilities, but those dealing with the aftermath of widespread nuclear disasters such as those at the Fukushima Daiichi nuclear reactor. Penn State's team project designed and constructed a genetic circuit introduced into E. coli bacterial cells, in order to rapidly detect and report the presence of harmful ionizing radiation. We are working to create a robust and reliable biosensor that

utilizes the lambda phage lytic-lysogenic switch as the radiation sensor. When the sensor detects radiation, it triggers one of three fast acting reporters we developed based on the concept developed by Imperial College's 2010 iGEM team. Each of the reporters features a different enzyme/substrate reaction (β-galactosodise/β-D-galactose, β-glucurodinase/β-D-glucuronide or C23O/catechol). We believe that the final construct may have the potential to rival current radiation detection methods, such as digital dosimeters.

Team Queens_Canada: Nemoremediation: Engineering C. Elegans into a Toolkit for Soil Bioremediation

Naphthalene is a pollutant produced by oil sands operations. The Queen's team has engineered the nematode worm C. elegans into a toolkit for dealing with this compound in the soil. We have produced constructs with GPCRs from M. musculus, R. norvegicus, and H. sapiens intended to enhance the worm's ability to chemotax toward naphthalene. We are working on a field bioassay based on fluorescent proteins that will indicate the presence of naphthalene in a soil sample. The goal is to have a population of green fluorescent worms chemotaxing toward and a population of red fluorescent worms chemotaxing away from the napthalene in the soil sample. Finally, we have biobricked the P. putida gene nahD, which encodes a degradative enzyme as part of a naphthalene catabolic pathway. The nahD gene encodes the enzyme 2-hydroxychromene-2-carboxylic acid isomerase, which catalyzes the fourth step in the catabolic pathway.

Team Rutgers: Complex Circuits in Synthetic Biology

The Rutgers iGEM Team designed two complex genetic circuits, Etch-a-Sketch and Full Adder, and created a software tool, MYS!S. The Etch-a-Sketch circuit enables a lawn of bacteria to be drawn on with a laser. This seemingly inconsequential task presents many engineering challenges: the bacteria need to be sensitive in order to respond to a laser pulse, yet selective to use in ambient lighting. The second circuit allows bacteria to emulate a digital full adder. The circuit makes use of individually non-functional split reporters that can reform functional reporters with the help of fused "zipper" domains. In addition to the circuit, we have made easily fuse-able BioBricks of these domains in order to facilitate the engineering of more split proteins, which should assist in the creation of logic circuits. MYS!S aims to improve the parts registry by checking and giving directions to modify Biobricks to conform to assembly standards.

Team Tec-Monterrey: E. Coli's Sweet Deal

Production of sugarcane used to be a high profit activity in the Mexican industry. Nonetheless, the increasing demand of high fructose syrup has become a rising threat to most sugar companies. Our project expects to apply synthetic biology to use sugar, obtained from sugarcane, in an industrial sugar-fructose process intending to make it easier and more profitable. The new genetic construct will be able to immobilize invertase by fusing it to bacterial natural membrane protein fragments using a technique for cell surface display. This system will catalyze the transformation of sucrose into fructose directly, without the need of any chemical or mechanical purification process to obtain the enzyme, reducing the amount of unit operations, and cutting production costs. Also, we will use the same principle to immobilize cellulase, converting cellulose from bagasse into something useful to produce biofuels.

Team Toronto: Magnetasense

Magnetotactic bacteria use the Earth's magnetic field to guide them to favourable environments. This is known as magnetotaxis, and is achieved in these bacteria by creating uniformly shaped cubo-octahedral magnetite nanoparticles. Mms6 is a critical catalytic protein which binds to magnetite in order to facilitate the formation of an uniform crystal structure. Using the Mms6 gene from Magnetospirillium Magneticum

AMB-1 we intend to create a magnetite synthesis pathway in E.Coli. Finally as a proof of concept, we will also show how we can use the formation of magnetite as a novel gene expression system. We intend to achieve this through the use a ToxR - Mms6 fusion protein and the ctx promoter from Vibrio cholerae. This novel gene expression system can be used to help bridge the gap between biological and digital systems.

Team UANL_Mty-Mexico: Simple Light Code Interpretation Enabling Circuit in Escherichia coli

Information processing through living things remains a challenge to science. Genetic logic-gates and switches have been used to this purpose[1]. Moreover, light induction systems have been recently constructed[2]. Our project aims to enable a bacterial community, constituted by three E. coli strains, to interpret a simple light based code. Chromosome insertion of light induction's genes will be performed in order to create light responsive chassis. Furthermore, each strain will contain plasmids carrying the genetic constructions needed for the interpretation of the code, which relies on logic-gates and switches. Phage lambda's based biphasic switch[3], which theoretically allows controlling the independent expression of two different proteins through a single input, is introduced to iGEM.

Team UC_Davis: The Generation and Characterization of Mutant Libraries for BioBrick Circuit Synthesis

The Registry of Standard Biological Parts offers inducible BioBrick promoters and their corresponding repressors in a limited range of strengths and activities. To broaden the application of this key part type, we have produced and characterized libraries for the LacI, TetR repressible and lambda c1 regulated promoters, as well as the LacI, TetR and cI repressors, some of the most commonly used repressor and promoter parts. These new libraries can be used to engineer genetic circuits requiring inducibleparts with varying activity levels and chemical sensitivities. To demonstrate their utility, we used our new parts to construct diverse circuits that capitalize on differences in their activity levels.

Team UCSF: Building a Synthetic Community: Yeast We Can!

Many microbial cells form biofilms as a means of survival. Biofilms are formed when a large number of microbial cells aggregate together. This year, the UCSF iGEM team has engineered artificial biofilms via yeast cell surface display. We synthetically engineered S. cerevisiae to form tunable biofilm-like structures by inducing the display of adhesive proteins on their surface. By combining the natural yeast mating receptors – Aga1 and Aga2 – with adhesive proteins from a variety of organisms, we created several adhesive interactions among yeast cells. Our synthetic cell adhesions can serve as a model for biofilm formation and primitive multicellular structures.

Team UIUC-Illinois: E. chiver

Our project, E. chiver, drew inspiration from the commonly used CRIM system, a series of plasmids that allows the user to integrate constructs into lambdoid phage sites common to many bacterial chromosomes. Our E. chiver system adds several elements yielding new applications. Our team designed two E. chiver constructs utilizing Lambda and P21 machinery. Each can in theory be used to shuttle a plasmid construct between two forms: a single chromosomal insert and a high copy number plasmid. In their current designs the systems must function separately, but possible routes have been identified by our team to make the co-functioning of these systems possible. We can see elements of our project being used in drug delivery systems as a method to keep a gene of interest dormant unless in the correct condition/location, and with further exploration into the co-functioning routes it may be used to create a 'bacterial filing cabinet'.

Team UNAM-Genomics_Mexico: Hydrobium etli

Among the biological systems that produce hydrogen, the most efficient ones achieve it through reactions catalyzed by enzymes with iron-sulfur clusters which require hypoxic microenvironments to work. The bacterium Rhizobium etli, during its symbiotic relationship with the common bean Phaseolus vulgaris, can transform nitrogen gas into ammonia in a process called nitrogen fixation. In exchange the plant provides the bacteria with carbon sources and a protected niche inside its root, where Rhizobium etli reaches a hypoxic state. We will exploit this microenvironment to produce hydrogen in Rhizobium etli introducing a pathway assembled with elements from Clostridium acetobutylicum, Desulfovibrio africanus and Chlamydomonas reinhardtii, while maintaining nitrogen fixation.

The two goals of our project are to make Rhizobium etli a powerful agent in environmental protection by nitrifying soils and producing hydrogen from solar energy, and to standardize the work in Rhizobials.

Team UNICAMP-EMSE_Brazil: STRESS WARS – Jedi bacteria designed to fight against stress derived immune imbalance

Stress and autoimmune diseases cause imbalances in immune system, observed in the biased naive T-CD4+ Lymphocytes differentiation towards T-lymphocyte-"helper"-1 in autoimmune diseases, or Th2 in stressful condition, favoring cellular or humoral adaptive responses. This can lead to bacterial evasion of host defense systems and susceptibility to some pathogens. Since nowadays we are exposed to continuously stress, perhaps we can avoid the negative effects caused by this condition. Thus, a mechanism that counteracts this imbalance is highly desirable. The ability of some bacteria to sense stress hormones such as Catecholamines will be used to produce Interleukin-12 and inducing Th1 lineage. On the other hand, the ability to sense Nitric-Oxide released in inflammatory conditions will be used to trigger Interleukin-10 production, counteracting excessive immunity. A switch control system will sustain the balance. The “Jedi Bacteria” containing these devices would be a useful probiotic to fight against the battle imposed by stress.

Team uOttawa: A platform for robust quantification of transcriptional regulators and promoters and a new assembly protocol

Currently, there are few tools for quantitatively characterizing BioBricks™ in the model organism S. cerevisiae. Furthermore, current standard BioBrick™ assembly protocols continue to be laborious, and have the tendency to deliver inconsistent results. To address this issue, team uOttawa has engineered two strains of S. cerevisiae that allow for the rapid integration of both transcriptional regulators and their trans-regulated promoters. Transcriptional factors (TF) and promoters transformed into these strains yield fluorescently tagged TFs or fluorescently reported promoters respectively. This dual-colour system enables the quantification of the relationship between TF expression and promoter responsiveness. To expand the registry with well-characterized parts, all of these tagged transcriptional regulators and promoters will be submitted. Finally, we also describe a fast, efficient and cost-effective assembly protocol developed by our lab that was used to assemble all constructs built by our team.

Team USC: Bioengineering a mechanism to override plasmid-based antibiotic resistance

Bacteria protect their genome and remove foreign DNA through a primitive immune-like system called clustered-regularly-interspaced-short-palindromic-repeats (CRISPR). Plasmids commonly used in molecular biology are mobile genetic elements that facilitate horizontal gene transfer in the wild and enable the spreading of antibioticresistance genes between microorganisms. Plasmids contain unique DNA

sequences that can be targeted by the CRISPR system. We exploited this system by engineering E.coli with an inducible mechanism of self-curing plasmid based antibiotic resistance. We synthesized a version of CRISPR encoding a spacer that matches the GFP DNA sequence. We tested the synthetic CRISPR array against E.coli harboring a tetO::GFP plasmid that confers ampicillin resistance. Activation ofCRISPER-gfp destroys the gfp-containing plasmid restoring the bacterial host's sensitivity to ampicillin. We will use the synthetic CRISPR system as a biological tool, combining it with other BioBricks for use in applications that will impact health and medicine, biotechnology, molecular biology, and genetics.

Team UT_Dallas: "Immunobots: a step towards intelligent probiotics"

The human bowel hosts a rich diversity of symbiotic microflora that provides a powerful engineering platform for intelligent probiotics. These "immunobots" will ideally work in-sync and enhance natural self-repair mechanisms for a range of intestinal diseases associated with tissue damage. Towards this end, we engineered a bacterial chemotaxis pathway that utilizes a chimeric receptor to successfully interface with the immune system. In addition, we introduced an inducible secondary bacterial population that can trigger system-wide self-destruction, conferring an additional level of user control. Each module of our system was characterized using fluorescent reporters and the integrated parts were evaluated by controlled experiments involving wound signal gradients. We envision a probiotic solution that can facilitate localized tissue repair for damage resulting from inflammatory bowel diseases, including ulcerative colitis and Crohn's disease.

Team Utah_State: CyanoBricks: Expression Testing and Bioproduct Development

Building upon the CyanoBrick toolkit developed by the 2010 Utah State iGEM team, our project focuses on producing valuable bioproducts using Synechocystis sp. PCC 6803. Our project will attempt to produce three different bioproducts: fatty alcohols, wax esters, and alkanes/alkenes. In order to optimize expression levels of various geneproducts, we constructed a dual luciferase expression measurement device. We used this device to provide more detailed characterization of promoters and ribosomebinding sites from E. coli and Synechocystis. We also produced a variety of useful intermediate parts for the dual luciferase device, which are currently not available through the registry, allowing this measurement system to be easily adapted to new organisms and new reference standards.

Team UTP-Panama: THERMOGENIC RESPONSE NUTRIENT BIOSENSOR (THE RENBO)

To develop flexible and better sensors for environmental, agricultural and engineering applications are the aims of the UTP-Panama Team "SynBio Engineering Tool kit". In this way we work with Nitrate Biosensor (PyeaR - GFP composite) developed by Team BCCS-Bristol 2010, which expresses fluorescent signals upon nutrient detection, producing a high-resolution map of arable land. To achieve this goal we use the collateral effect of the AOX enzyme (Alternative oxidase) mainly designed to generate heat in response to a cold-shock, using the hybB promoter which increases the bacteria growth at temperatures below 20°C.

Finally we design a prototype device with a better cold shock promoter (CspA) developed by UNAM-CINVESTAV Team in 2010, in order to give our E. coli an "Intelligent Coat"", which means that not only survives a cold-shock but is also able to keep up with its duties, due of improving their expression mechanisms at low temperature."

Team VCU: Production of Isoprenoids in Synechococcus: A model for sustainable manufacturing

Cyanobacteria, such as Synechococcus elongatus, are prokaryotic photoautotrophic model organisms, which are responsible for a large proportion of global photosynthesis. Our group endeavored to develop S. elongatus as an emergent platform for synthetic biology and metabolic engineering.

While Synechococcus elongatus has been used in previous synthetic biology projects, our group sought not only to characterize functional promoters, but also promoterscapable of tailoring expression to circadian rhythms or transcriptional factors. Concurrently, our team experimented with fluorescent proteins to find a quick-folding, robust, and transient reporter that may be used to characterize dynamic parts (such as those involved in circadian rhythms).

As proof of concept, the VCU iGEM team also aspired to integrate isoprenoid pathways into Synechococcus - isoprenoids being precursors of many commercially and pharmaceutically relevant molecules. By demonstrating production of these industrially important metabolites we hope to show the practicality of utilizing cyanobacteria as a superior sustainable production platform.

Team Virginia: A Synthetic Biology Approach to Promoting Angiogenesis at Traumatic Wound Sites

We use a synthetic biology approach to promote tissue regeneration at traumatic wound sites. Tissue regeneration is composed of three primary processes: the regrowth of functional parenchymal tissue, the regrowth of support tissues, and the regrowth of vasculature to sustain the nascent tissue formation (angiogenesis). Although tissue engineering has offered several effective solutions to address the first two processes, our project attempts to build upon these ideas and develop a more cost-effective and robust method to promote angiogenesis at traumatic wound sites. We have devised a circuit to be incorporated in a yeast chassis that efficiently expresses two vital angiogenic proteins--vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF-B)--in a sequential and time-dependent manner that approximates the natural cascade of growth factor release in the human body. We also intend to submit a Biobrick-compatible yeast plasmid backbone for future use.

Team Virginia_Tech: Fluorescent Protein Characterization

Fluorescent proteins have become ubiquitous tools for studying cellular processes. To be particularly effective for these applications, fluorescent proteins must feature fast maturation and degradation rates, and these rates must be well-characterized and documented. The 2011 VT iGEM team has worked to find and characterize fluorescent proteins and degradation tags that more quickly degrade them. Here, we present chemical and mathematical models based on two parameters, maturation and degradation rates, and in doing so, we explored difficulties in the process of characterizing parts. In conducting our experimentation, we tested fluorescent proteins with different degradation tags in Escherichia coli and Saccharomyces cerevisiae using automated fluorescent microscopy techniques, and then worked to determine a comprehensive, accurate mathematical basis for fluorescent protein characterization.

Team Washington: Make it or Break it: Diesel Production and Gluten Destruction, the Synthetic Biology Way

Synthetic biology holds great promise regarding the production of important compounds, and the degradation of harmful ones. This summer, we harnessed the power of synthetic biology to meet the world's needs for fuel and medicine. Make It: We constructed a strain of Escherichia coli that produces a variety of alkanes, the main constituents of diesel fuel, by introducing a pair of genes recently shown convert fatty acid synthesis intermediates into alkanes. Break It: We identified a protease with gluten-

degradation potential, and then reengineered it to have greatly increased gluten-degrading activity, allowing for the breakdown of gluten in the digestive track when taken in pill form. Finally, to enable next-generation cloning of standard biological parts, BioBrick vectors optimized for Gibson assembly were constructed and used to construct the Magnetosome Toolkit: genes for biofabrication of magnetic particles.

Team WashU: Engineering Carotenoid Biosynthesis in Saccharomyces cerevisiae

Vitamin A deficiency causes blindness in over 250,000 children annually. The WashU iGEM team hopes to address this issue by creating a transgenic strain of Saccharomyces cerevisiae (baker's yeast) that produces β-carotene, the precursor to vitamin A. The WashU team created four DNA constructs for homologous recombination into the S. cerevisiae genome that will catalyze the production of β-carotene. Each construct consisted of a gene encoding an enzyme from Xanthophyllomyces dendrorhous, a bacterium that produces β-carotene. Three of the constructs encode for enzymes in the metabolic pathway required for β-carotene production, while a fourth enzyme cleaves β-carotene to form β-ionone, a rose-scented compound used in the fragrance industry. Additionally, the WashU team has established spectraphotometric assays to detect β-carotene and β-ionone in yeast extract. Although we have yet to successfully incorporate these four genes into S. cerevisiae, we have prepared all four constructs and biobricked these genes for future use.

Team Waterloo: In vivo Fusion Protein Assembly Using Self Excising Ribozyme.

Introns, self-excising ribozymes, can become a useful tool to create in vivo protein fusions of BioBrick parts. To make this possible, intron sequences are used to flank non-protein parts embedded in coding sequences. An intron sequence with an embedded recombination site is capable of in vivo insertion of a compatible protein fusion part. As an example, a GFP-fusion was created with an intervening lox site that is removed from the final protein using the intron to form a fully functional GFP protein. In vivoprotein fusions can be applied to a larger number of modular systems to make complicated expression systems, such as synthetic antibodies or plants capable of Cry-toxin domain shuffling.

Team West_Point: Visual Detection of Cholera via Modified E. Coli

Cholera (Vibrio cholerae) is a bacterium that causes intestinal infections in humans. While it is rare in industrialized nations, cholera remains a major threat in developing nations; there are an estimated 3-5 million cases resulting in over 100,000 deaths annually. West Point's 2011 iGEM team is developing a simple test kit to determine the presence of cholera contamination in drinking water. Inserting a plasmid coding for Beta-Galactosidase (β-Gal) enzyme induced by an arabinose promoter (araBAD) into E. coli creates a biological specimen that will produce the β-Gal in the presence of arabinose. When the modified specimen is mixed with cholera the β-Gal is released into solution where it reacts with X-gal, also in the solution, producing a blue-violet color. If no cholera is present, the solution remains clear. Our technique should produce visible results in the order of minutes, rather than the typical day(s) it currently takes for similar techniques.

Team Wisconsin-Madison: Optimizing biosensors through a two-phase directed evolution

The University of Wisconsin - Madison 2011 iGEM team sought to create fluorescent E. coli biosensors for ethanol and alkanes, two molecules with significance for sustainable fuel production. Through expression of heterologous genes, E. coli strains which appear to have a response to a specific biofuel were generated. However, these biosensors did not produce the low uninduced and high induced levels of

expression desired. This situation appears to be common among biosensor-based iGEMprojects. In order to improve the biofuel sensors, as well as provide a framework for other teams to do the same, a universal biosensor directed evolution construct was assembled. The device can be used to both select for high expression in the presence of an analyte, as well as select for no expression in its absence. In conjunction with mutagenesis of key genes, this device could be used to significantly improve existing and novel biosensors.

Team Yale: Nature's Antifreeze: Microbial Expression and Characterization of a Novel Insect Antifreeze Protein for De-icing Solutions

Antifreeze proteins have applications in cryopreservation of food, cells, and organs, as well as in cryosurgery and agriculture. The purpose of this study was to express, purify, and characterize a novel, hyperactive antifreeze protein recently isolated from the Siberian beetle, Rhagium inquisitor (RiAFP). Large scale (150mg/L), stable production of RiAFP and a RiAFP-GFP fusion protein was achieved in E. coli. Proteins were purified using Ni-NTA affinity chromatography. E. coli expressing RiAFP exhibited increased survival post-freezing. RiAFP inhibited ice recrystallization in both splat and capillary assay. To optimize the activity of the hypothesized RiAFP ice binding site, we are using directed evolution through multiplex automated genome engineering (MAGE). Finally, we are further optimizinge our crystallization conditions for RiAFP to better understand the structure-function relationship, as well as conducting post-freezing survival assays in C. elegans.

ASIA

Team ArtScienceBangalore: Everything is Everywhere but our PCR selects (Ubiquitous Genetically engineered Machines)

The Biobrick has been used as an abstraction or template for creating standardized functional parts. This year's ArtScienceBangalore project proposes alternate re-appropriations of the BioBrick by using existing BioBrick primers as random-PCR primers in investigating soil samples. This random PCR will provide a succinct signature of the biological diversity present in these samples. These investigations of soil lead us to ask questions about citizen's science "performed" by non-institutional actors using accessible tools as well as gives us a glimpse into the "post-natural world" where BioBricks may end up in our environment and may very well show up as bands in a gel. By imagining a world in which the Biobrick has become the accepted standard for synthetic biology, and where these engineered products are ubiquitous in our lives and environments, the samples we archive will serve as the baseline from which the subsequent extent of human influence can be measured.

Team CBNU-Korea: GOD, Genome Organization & Design for synthetic minimal genome.

Synthetic minimal genome is the smallest possible group of genes that would be sufficient to sustain cellular life form. However, synthesizing genome is kind of tough way. So, we decide to synthesize minimal chromosome which consists of essential genes and has normal nature of normal chromosome such as self-replication, partition and control. For this project, we employed chromosome II of v.cholerae and used some genes about replication system(rctA, rctB and origin of chromosome II) and partition system(parA, parB and several parS sites) of chromosome II.

Also, we are developing our own essential gene database and genome organization design software

named "GOD" that can solve design problem until unsolved. Both are based on pattern, arrangement and direction data of genes from DEG and NCBI with statistical analyzing.

Team Fudan-Shanghai: E.tree, neon light and the dinner service

What is the first thing that comes to your mind when you see this: tree, neon lights and dinner? Christmas! Well, exactly, and that is what our project all about.

Part I: E.tree The “leaves” change color according to the nutrients in the “soil”: if the soil is rich in nitrates, the “leaves” are healthy and green; otherwise, the leaves turn yellow.

Part II: neon lights Each engineered E.coli emits one color of fluorescence; after a while, the light fades and another color of light is emitted. Different combination of such E.coli could therefore achieve the effect of neon lights.

Part III: dinner service The genetically modified bacteria involve a certain self-feedback system. When the “customer” is starving, it orders dinner from the “chef”; and the chef serves meals. While the “customer” is full, it tells the “chef” that no more food is wanted, so the “chef” stops cooking.

Team HIT-Harbin: The Reform of Two Strains in Yogurt

Since lots of people in China are lactose intolerance, they have no access to drinking milk in the past. With the development of the dairy industry in China, yogurt has become highly accepted by consumers, including those lactose intolerance people. And postacidification has always been the most vital factor which affects the shelf life and flavor of yogurt. So our team takes it as our track. Through the literature, we have discovered a gene, called lacR, which could combine with the lactose operon to inhibit the production of lactic acid in Bulgaria Lactobacillus. If lacR could be highly transcripted in Bulgaria Lactobacillus when the pH value of yogurt declines to a certain level, the acidification of yogurt would be postponed. Meanwhile, we also want to transfer part of human collagen genes to Streptococcus Thermophilus in order to enhance the nutrition of yogurt.

Team HKU-Hong_Kong: Development of a Novel Inducible Transcriptional Repressor mediates the formation of heterochromatin-like complex in E.coli

In eukaryotes, heterochromatin plays an important role in gene regulation. Here we use a synthetic biology approach to imitate heterochromatin in E.coli to achieve genesilencing. Specifically, fusion proteins comprising tetR and different parts of HNS (histone-like nucleoid structuring protein) were synthesized, they are expected to bind DNAspecifically and carry out polymerization among the fusion HNS and the native HNS to create a densely packed DNA form, which may block the transcription. We produced constructs with tetO sites upstream or downstream of lac promoter and EGFP gene. Then we used standard constitutive promoters with different activity to drive our fusion proteins to find the optimum expression level. Moreover, tetR, HNS and fusion proteins were purified and gel shift assay was utilized to detect the interaction between those proteins with DNA. This study presents a novel approach to introduce a mimic heterochromatin-like structure into prokaryotes to achieve inducible gene silencing.

Team HKUST-Hong_Kong: E. trojan – Boosting the Effectiveness of Antibiotics through Quorum-sensing Disruption

Adding to the already massive arsenal of bacteria, highly-resistant (HR) /E. coli/ are found to be capable of supporting less-resistant (LR) individuals experiencing antibiotic stress through indole signalling - allowing LR individuals to survive in antibiotic concentrations that would otherwise be lethal. Our team has

engineered disruptor /E. coli/ expressing mutated toluene-4-monooxygenase, which facilitates indole degradation. Introducing this disruptor strain into an /E. coli/ culture of HR and LR individuals is thus hypothesized to result in massive LR cell death at a lower-than-expected antibiotic concentration. If successful, indole degradation may become a possible strategy in boosting antibiotics effectiveness in medical practices against bacteria relying on similar signalling methods. We are also constructing a novel strain of /E. coli/ that utilizes an essential gene (/nadE/) for antibiotic-free transformation and plasmid maintenance. This strain can help future iGEM teams reduce their antibiotics consumption without deviating significantly from widely used transformation protocols.

Team HokkaidoU_Japan: Advancement of Dr. *E. coli*: The world's smallest potein injector

Bacteria living around us evolved ways to effect their surrounding environment. Some bacteria can change its surrounding environment by injecting whole protein molecules into targeted eukaryotic cells through Type 3 secretion system (T3SS). During iGEM 2010 we showed that *E. coli* containing a part of *Salmonella* genome expresses T3SS. We thought this system can be applied to direct reprogramming of somatic cells. This year we tried to make the system more convenient. To accomplish this, we designed Bsa I cloning site and developed plasmid backbone which can instantly produce ready-to-inject fusion proteins from biobrick parts to be injected.

Team Hong_Kong-CUHK: ChloriColight

Control of gene switch by phototuning Conventional induction systems usually involve addition of a chemical agent that functions either by turning on or off an output event, which is equivalent to flipping an on/off switch. In our project, we aim to replace such analog system with a phototunable cascade, which can trigger a measurable output (e.g. gene expression) according to the wavelengths, intensities, and durations of a light source in a quantitative manner. In the long run, this system could be further modified to convert light into other types of output, such as osmolality and electric current.

Team HUST-China: Super E.coli Architect

Super E.coli Architect is a design software of engineering bacteria. It offers a convenient and efficient design platform,which contributes to making simple ideas into a complete solutions. The existing synthetic biology softwares involve some specific functions such as finding genes and metabolism pathways, modeling and biobrickdesign.And these softwares are often oriented to scientific researchers. But we think that there is a hope in synthetic biology to turn the science of artificial biosystem into a engineering program having a standard process. Therefore, we constructed a software, Super E.coli Architect, oriented to designers more than scientists.Providing all the necessary technical support,Super E.coli Architect can release desighners from technical details,helping them work as the architects,allowing them to take more attention into constructing kinds engineering bacterias for solving practical problems.

Team IIT_Madras: P.rex: Photonivorous bacteria for Resolution and Efficient eXpression

“Make ATP when the sun shines”. Proteorhodopsin, the inspiration for our motto is a proton pump, native to marine bacteria, that uses light to generate proton gradient across the cell membrane. This leads to Photophosphorylation, as the proton gradient drives ATP-synthase to produce ATP. We have designed a cloning vector containing a Proteorhodopsin generator which confers a survival advantage to transformants in nutrient limiting conditions. And thus, we propose a novel screening technique that uses metabolic stress

to screen for transformants in the presence of light. In addition, using the stress alleviation due to proteorhodopsin activity, we intend to enhance the yield of recombinant proteins and substrate consumption efficiency.

Team KAIST-Korea: E. Casso: Artistic E. Coli inspired by random color generation

E. Casso is an E. coli system engineered to perform art. The system consists of two types of genetically modified E. coli: the random signal generating E. coli ("Brush E. coli") and the color generating E. coli ("Paint E. coli"). Brush E. coli possesses genes (lasI, luxI, rhlI, and cinI) in its gDNA that produce quorum molecules. On the other hand, by modified Cre-loxP mechanism, gDNA is randomly set to secrete only one of the four types of quorum molecules. Paint E.coli possesses plasmids that express one of four colors (red, cyan, yellow, and green) by producing fluorescent proteins in response to the type of quorum it receives from Brush E.coli. As Paint E.coli expresses corresponding fluorescence in response, it also produces quorum molecule that is identical to the inducer to start propagation. Ultimately, using this system, the "random" contribution of cells will create art on an LB plate.

Team KAIT_Japan: Colony on Colony -Colony formation like layer cake-

We tried to overlap colony on colony. Generally, colony and colony don't overlap and approach each other, because of the cell-cell communications. Bacterias also have a system, so called quorum sensing, to control of production of signal chemicals depend on the bacteria's density.

We pay attention quorum sensing system. Bacterias produce and secret autoinducer against other bacterias by quorum sensing system, as a result of the system, bacteriasenses other bacterias. Most of Gram-negative bacterias make use of autoinducer named AHL. We applied E. coli to our experiment and we use aiia which is catabolic enzyme to inhibit AHL. We tried to make a recombinant bacteria to cancelled sensing of other bacterias and expected to form the colony of bacterias on the other bacterias.

Team KIT-Kyoto: Mr.D -who will cure leukemia-

Our team, KIT-Kyoto challenges making a leukemia disease model in Drosophila melanogaster. Drosophila is being used as many hereditary disease model because over 70% of known human disease genes have similarities to Drosophila genes. This year, we focus on a leukemia in which the etiology and the therapy have not been established. We therefore decided to make a leukemia disease model in Drosophila. We insert human leukemia genes into Drosophila genome and also fuse a green fluorescent protein(GFP) with a leukemic protein to monitor its expression in E. coli or Drosophila. We expect that establishing a leukemia disease model in Drosophila will be a first step to determine the etiology and to establish the method of therapy in future.

Team Korea_U_Seoul: Synthesis of Synthetic Micro-Alkanes ("Synfuels") in Engineered Escherichia coli

Our team concentrated on finding the solution to the world's diminishing natural oil and gas resources and greenhouse gas emissions. The aim of our project is the production of biofuels, alkanes, using bacterial cells as factories. Alkanes, so called "Green" hydrocarbon fuels, are chemically energetically the same as petroleum-based fuels, thus no penalty for use of conventional engines is encountered from their use. For alkane biosynthesis, we designed a synthetic circuit using bacterial bioluminescence system and aldehyde decarbonylase from Vibrio harveyi and cyanobacteria, respectively. Free fatty acids in the cells firstly are reduced and converted to fatty aldehydes by LuxC, LuxD and LuxE and then fatty aldehydes finally are decarbonylated and turned into alkanes.

Team Kyoto: Creation of carnivorous bacterium which can catch and digest bugs

Many unique bacteria have been created in iGEM. However, they are not so ‘creatures’, but new tools or machines. We try to make ‘new’ creature, carnivorous E.coli which can prey on insects when they are hungry. In poor availability of combined nitrogen, carnivorous E.coli emit light and attract bugs by using bioluciferase, then catch and digest them by mucilage and catabolic enzymes. To create carnivorous E.coli, we set up four sub-goals: hunger, luminescence, predation and digestion. In “hunger”, We measure activity of σ54 promoter with nitrogen regulatory protein, the activator. These promoter and protein enable E.coli to responce concentration of amino acids. We also check the response of fruit fry to light emitted by bioluciferase, and weather E.coli can catch and digest bugs with mucilage and protease in the other three sub-goals.

Team Macquarie_Australia: Switch-a-roo: engineering a photoresponsive 'E.colight switch'

Photoreceptors are ubiquitous proteins that allow an organism to sense light. These proteins have evolved in unique environments to sense light intensity in different colour ranges. This experiment focuses on constructing a biological switch that uses two photoreceptors from separate organisms – Deinococcus radiodurans and Agrobacterium tumafaciens. The coupling of heme oxygenase supplies our photoreceptor proteins with biliverdin, allowing for the self-assembly of the switch within host systems. The switch is the first stage of a two component light sensor and when expressed at high level, there is a noticeable colour change of the cell when it is activated by light.

Team NCTU_Formosa: Pathway Commander

Controlling the flux through a synthetic metabolic pathway lies in selecting well-matched genetic components that when coupled, can reliably produce the desired behavior. Each generating different protein expression levels in order to test performance in reliability and consistency, but this process is both tedious and time consuming. To overcome this problem, our team provided a novel circuit design method_ Pathway Commander. We construct a single version of a synthetic metabolic pathway circuit that can use culture temperature shifts to control the expression levels of a series of metabolic proteins at the precise times. We have implemented the Pathway_Commander design in (1) Carotenoid synthesis Pathway, (2) Violacein biosynthesis pathways and (3) Butanol synthesis pathway in E. coli. This circuit design utilizes a temperature controlled system that gives precision control over metabolic protein expression which amounts to optimal synthesis that can maximize synthesis of a given compound or drug.

Team NYMU-Taipei: Tailoring Your Avatar

Optogenetics, the latest neuroscientific method, has improved specificity for stimulating certain cell types of neurons, reversible bi-directional stimulation, and elevated spatiotemporal precision. However, to achieve neuronal network stimulation, light cables are still needed, leaving long-standing annoying issues regarding immune responses unresolved. This year NYMU-Taipei iGEM team creates wireless neuro-stimulator, focusing on achieving remote neuro-stimulation to minimize the invasion and damage to the neuron. To achieve this goal, we use a species of magnetic bacteria, Magnetospirillum magneticum AMB-1. We have chosen mms13, a transmembrane protein as our target for protein design, as it serves as a linker between reception of wireless magnetic field and optogenetic neuro-stimulation output. Regarding the neuroimmune response, we choose three genes to achieve symbiosis within glial cell: MinC, a division inhibitor, INV, a gene for invasion and LLO, a gene for facilitated escape from phagosomes. Overall, our project will make optogenetic neuro-stimulation wireless and safe.

Team Osaka: Bio-dosimeter

On March 11, 2011, the Great East Japan Earthquake struck off the coast of Eastern Japan and triggered a series of events that led to a nationwide nuclear crisis. The need for low-cost, portable and easy-to-use dosimeters became apparent as radiation measurements could only be conducted at sparsely-distributed installations and the values reported infrequently. We have decided to tackle the issue by building a biological dosimeter. Focus is placed on modularly transferring radiation response and DNArepair genes from the extremophilic bacterium Deinococcus radiodurans to the well-characterized, easily cultivable chassis Escherichia coli. The native DNA damage detection and repair systems of E. coli will be also modified to efficiently sense ionizing radiation through detection of the resultant DNA damage. Finally, detection will be connected to visible outputs such as color pigment production, resulting in a biological device capable of detecting ionizing radiation and alerting users to it through color change.

Team OUC-China: Theory of Five Elements—Bacteria are performing philosophy in theatre of science

Our work aims to illuminate the ancient Chinese philosophical theory called “Wu Xing”, also Theory of FIVE Elements, by artificially assembling a communication system between bacteria. This theory explains the composition and phenomena of the physical universe. In the system there exists close relationships classified as mutual promoting and restraining under certain conditions, by which functions of the various systems are coordinated and homeostasis of universe maintained. In our project, five modified nutritionally deficient bacteria represent the five elements in the theory. They talk to each other emitting and receiving specific signals called AHLs. Five pairs of signal receivers and producers, like luxR and luxI, are involved in achieving the process. Moreover, one will help another producing the essential nutrition for living while limit the production of a third member. Through such mechanism, the whole group survive and live in moderate coexistence, just like the effect of the philosophical theory.

Team Peking_R: Soft-coding of genetic program for synthetic biology

During genetic program tuning, mutagenesis and following screening of each mutant, which can be named as hardcoding approach, is usually the only choice, although laborious and time-consuming. But actually there can be an alternative - softcoding approach, which refers to designs that allow for the customization of performance of genetic programs via external input without having to edit the DNA sequence case by case.

This year our team aims to establish a genetic fine-tuning platform based on the softcoding of genetic program in bacteria, composed of a toolbox and a methodology -- The toolbox consists of interoperable and modular ligand-responsive riboswitches/ribozymes, while the methodology is automated design of synthetic RBS with customized translation rate. When combining them together, the configuration of RBS strength in genetic program can be fast and easily determined by variable concentration of external ligand and an RBS that meets this configuration will be automatically generated.

Team Peking_S: A ‘Chemical Wire’ Toolbox for Synthetic Microbial Consortia

Cell-cell communication-based multicellular networks provide an extended vista for synthetic biology. However, ‘chemical wires’ that allow versatile concurrent communications are far from sufficient.

Accordingly, our project intends to develop a versatile ‘chemical wire’ toolbox for both multicellular Boolean computing and non-Boolean dynamics by two approaches. Firstly, a set of recently reported novel quorum sensing systems have been characterized. Secondly, quorum sensing (QS) based transcriptional

repression system have been built from the ground up by conversing LuxR family of transcription activators into repressors. We next sought to develop design rules of microbial consortia as supplements to this toolbox. To validate this toolbox together with the design rules, several robust combinational and sequential logic circuits that are difficult to be implemented in single cell have been constructed as a proof of concept for Boolean logic. As for non-Boolean dynamics, a balancer of microbial population density have been created with supporting microfluid device.

Team SJTU-BioX-Shanghai: Codon Switch Controlling Protein Biosynthesis

SJTU-BioX-Shanghai iGEM team is designing a modulating device that achieves fine tuning of target protein biosynthesis (translation). The translation of the protein can be finely turned up/down with the control of the number of rare codons and the different strength of tRNA induction. Besides, our device can be made into a real switch that can be turned on/off without background protein expression in two ways. One is to use any codon but initial codons to initiate translation, the other is to use stop codon as the controlling element. Moreover, our design would be a brand-new way to selectively express part of a gene or introduce point mutations into target residues in proteins, thus favoring the study of the important domains or residues of a target protein.

Team SYSU-China: Nuclear-Leakage Rescuer

As the nuclear-leakage issue in Japan has caught world-wide attention this year, we consider that engineered E. coli nuclear cleaner will be effective to this issue. Our project consists of two parts. On one hand, the radiation-sensitive promoter PrecA and gene CheZ will build chemotaxis to Ionising radiation and lead engineered E.coli to move toward seriously affected places. On the other hand, when moved in a proper radiation level, the less radiation-sensitive promoter PrecN and gene trkD will activate a kind of ion channel which can absorb the Cs+ ions. Our nuclear cleaner will become capable in dealing with the nuclear-leakage issue. We also tried to get the idea of synthetic biology known by designing an iPhone app and a card game, carrying a survey and holding forums.

Team Tianjin: "Expecto Patronum"——Reconstruction of TOR pathway to increase the tolerance of yeast to composite inhibitors

Although “Harry Potter and the Deathly Hallows” marks the termination of J. K. Rowling’s popular novels, we recreate a fantastic world where patronus are produced by Saccharomyces cerevisiae to fight against dementors inside the cell. Ethanol fermented by yeast from lignocellulosic materials can be an environmentally friendly fuel. However, rapid and efficient fermentation of lignocellulosic hydrolysates is limited because of inhibitors generated during pretreatment in addition to monomeric sugars. Inhibitors strongly affect the normal physiology of yeast as well as its ethanol productivity, just like the dementors taking away people’s hope and happiness. Nevertheless, we reconstruct TOR protein, a central component of major signaling transduction network controlling cell growth, to increase the tolerance of yeast. A new TOR after directional mutation will play the role of patronus to defend the influence of inhibitors, keep the overall signaling networks in good order, and finally provide a prosperous world for ethanol production.

Team Tokyo_Metropolitan: BeE.coli

We propose the project "BeE.coli" as a micro injection system. BeE.coli has the abilities to move fast and target pathogens concentrated area. Getting close to pathogens, it sends the killer gene to them with conjugation. Then pathogens will be killed. To accomplish this project, we planned to add three functions to E.coli. First, expression of the mutant H-NS proteins enables E.coli to move faster. This contributes to

increasing the frequency of conjugation. Second function is new taxis for AHL. We make the target bacteria as a model of pathogens, and they produce AHL. BeE.coli goes for the area where high concentrations of AHL that means concentration of target bacteria. Last, BeE.coli kills pathogens by sending the killer gene with the conjugative plasmid. BeE.coli has the anti killer gene repressing the killer gene. In the future, BeE.coli will be useful for medical scenes.

Team Tokyo_Tech: Cool down in summer with our rock-paper-scissors game

In Japan, summer is terribly hot. To have more pleasant summers, we created a rock-paper-scissors game in which the winner gets a refreshing shower of rain.

We make it rain by producing isoprene, which is the base for the creation of condensation nuclei that are used to make rain. As it rains and water evaporates things will cool down by giving away the heat of evaporation to water.

To make it even fresher, we also created urea coolers! They are pocket size and contain E. coli-synthetized urea that has been dried. By adding water to them they will cool down as the heat of solution is used to dissolve the urea.

The RPS game works based on a genetic randomizer, and we have confirmed this by creating mathematical models of it. We have also optimized the urea and isoprene production by flux analysis. Let's play rock-paper-scissors and make it rain!

Team Tokyo-NoKoGen: EcoLion - an E. coli collecting heavy metal ions in BMC

We developed the EcoLion, which collects heavy metals from the environment, aimed at cleaning up heavy-metal pollution as well as for the mining of valuable metals for industrial applications. A bacterial micro-compartment (BMC) was engineered into our E. coli micromachine to act as a tank to accumulate heavy metals. Heavy metal ions, such as Zn2+, Cd2+, and As2+, that are taken up by the cell will be trapped inside the tank by a metallothionein fused to a BMC-localizing tag. Using BMC in such a way will be very advantageous as it may achieve the accumulation of high concentrations of heavy metals in one place. The EcoLion that has stored heavy metals will be conveniently collected by light using phototaxis or self-aggregation. This EcoLion may ultimately be applied for collecting a number of different toxic or valuable molecules by using specific target-binding peptides or proteins.

Team Tsinghua: E. colimousine

We aim at constructing an E. Coli strain that can transport target protein along the concentration gradient of a cue molecule back and forth, acting as a bus in the information processing network.

We first conjugated domain of outer membrane protein A (OmpA) with SH3 domain which has high affinity for proline-rich motif. These two together would function as a binding vehicle directly sensing substrate in the media.

Considering efficient and specific release from strong binding, HIV-protease site was incorporated into the binding cassette. Another bacteria strain expressing the proteasewould wait on the finish line to cut off target protein.

Besides loading and unloading, we took advantage of “tar” and “tar*” receptor to manipulate E. Coli chemotaxis along aspartate and PAA concentration gradients and control the direction of the movement and binding and releasing with transcription factors.

Team Tsinghua-A: ECHO: an E.Coli Homochronous Oscillator.

Tsinghua-A iGEMers dedicate in pursuing the goal of the construction of a biological oscillator with two Escherichia coli populations expressing gene one after another, giving red and green fluorescent light alternatively. E.coli populations communicate bi-directionally by a class of signaling molecules involves in bacteria quorum sensing, that is, N-Acyl homoserine lactones (AHL), to regulate the gene expression of each other. By engineering their gene circuits, two groups (we call them CELL-A and CELL-B) will form a network, with B inducing A and A restricting B, thus cycle in a homochronous way. We have established a mechanism to change the rate of the AHL expression, allowing us to control the period and the phase of the oscillatory cycle. Moreover, a mathematical model has been made to analyze the dynamics of the system, and computer simulation software is introduced into the process.

Team TzuChiU_Formosa: Photo-Paper

For centuries, paper-making has been a traditional but indispensable industry. Wood pulp is the major raw material for paper-making, moreover, the complicated processes toward paper-making may contribute to deforestation and environmental pollution. Gluconacetobacter hansenii is a bacterium which produces bacterial cellulose. It has an acs operon, consisting of genes that called acsAB, acsC, and acsD. Together these genes used UDP-glucose as substrate and synthesize. We aim to clone acs operonand transform it into environmental friendly and economical microbes in order to produce large amount of cellulose for paper industry. In doing so we minimized global deforestation as well as CO2 emission. We also aim to introduce acs operon into cyanobacteria which would used only light, CO2 and water to produce glucose and the acs operon to produce cellulose. With manufacturing processes, we believe this project can develop into a new, eco-friendly thus revolutionized the paper industrial.

Team UNIST_Korea: Engineering Synthetic Self-Killing Device for Microbial Cell Factories (CHOp-Coli-LATE)

Recently, microbe-driven fermentation products are gaining increased importance. However, release of these microbes to the open environment would pose increasing threat to the society due to the possibility of changes expected in the indigenous microbial population. Hence, we have engineered a synthetic self-killing system for the famous industrial workhorse, Escherichia coli. High temperature (370C), native quorum sensing molecule (AI-2) and the darkness present in the fermentor will keep the self-killing system turned off. Environmental signals such as low temperature (25 0C), foreign quorum sensing molecules and light encountered by the E. coli outside of the fermentor would trigger the self-killing device. Unlike other lysis device, we have introduced a novel self-killing device that chops up the DNA. Thus, this system would not only favor cell death but also ensure that all the genetic materials are destroyed and guarantee that there would be no horizontal gene transfer.

Team UQ-Australia: Timely E. coli: Engineering a novel cellular oscillator

The human circadian rhythm drives many important processes in the body in accordance with the sleep/wake cycle. A characteristic of this biological clock is the periodic oscillation of gene expression. Current parts in the Registry designed to regulate periodic oscillations of gene expression have shown limited success. Here we demonstrate a biological clock being standardised as a set of BioBrick parts. Our network is controlled by an engineered promoter, Plac/ara, which features both an activator and a repressor domain. This controls the production of downstream genes to activate other inducible promoters, pBAD and GlnAp2, eventually leading to the production of a repressor protein, lacI, which inhibits Plac/ara, resulting in oscillatory expression. This project shows the feasibility of standardising the biological clock in

E. coli and grounds further development for applications in regulated drug/hormone delivery and ion channel control.

Team UST-Beijing: Gene H-transfer: bile acid receptor in E.coli & proteorhodpsin in mitochindrial inner membrane

In order to celebrate the power of gene H(orizontal)-transfer between pro- and eukaryotes, we constructed two fusion proteins and tested their function: (1). a synthetic bile acid receptor in E.coli using a mammalian nuclear receptor LXR. As proof-of-principle, the regulatory circuit in symbiotic bacteria could be harmoniously linked to metabolic pathway of their host. Potential application includes in situ synthesis of pharmaceuticals on-demand in the digestive tract. (2) a synthetic light-driven proton pump in human mitocondrial inner membrane using a bacterial proteorhodopsin. Preliminary testing demonstrated cellular sensitivity to light radiation. Application and utility relies on result of in-depth characterization of such system design.

Team USTC-China: Bacterial 'Amitosis'

So far we have successfully constructed a novel system in which bacterial colonies will automatically divide into two parts after certain time. Over the summer we have been working on assembling riboswitches finely tuned by small molecules, which will act as the main power to drive two parts away from each other, and toggle switches pushed on and off while memorizing current state, which will play the role of giving birth to two 'different' kinds of bacteria in one colony. Furthermore, we have been modulating the toggle switch to produce a more balanced ratio and creatively integrating quorum sensing into our system to optimize our results.

As to modeling, we have not only been building models of the movement in this 'amitosis', but also been collecting and analyzing data for a aptamers database for small molecules and corresponding genomic sequences and structures in guiding bacteria.

Team USTC-Software: Lachesis

USTC dry team as a one has worked diligently on designing and implementing a user friendly and interacting-prone software which will get nearer to biology reality and free synthetic biologist from considering unnecessary minutia as well as help both layman and expert draw deep understanding of the mechanism on how the gene circuit run. We offer a visualizing tool which give insight into the dynamics of a biology network. User dominated parameter adjustment process is also provided to assist in getting the required behavior. In order to assess the network's immunology to parameter perturbation, a PCA analysis approach is exploited to depict the structure of a 'good' behaved region.

Team UT-Tokyo: SMART E.coli: Self Mustering with Aspartate-Responsive Taxis

Bio-systems as a means for environmental remediation have been extensively investigated. However, these devices have often been limited by the requirement of a high cell density at the target site in order to achieve higher efficiencies. To overcome this, we devise an inducible self- assembling system in Escherichia coli utilizing L-aspartate (L-Asp) chemotaxis. Our system consists of two cell types: "guiders" and "workers". When exposed to a signal, the former discharge and generate a signal-centered spatial L-Asp gradient, and the latter lose motility by repression of a flagellum-regulating gene (cheZ). These cells remain at the source of the signal and cannot escape. Using E. coli-derived stress-sensitive promoters cloned de novo, we provide evidence that our system enables auto-assembly and localization after exposure to ultraviolet radiation. Since the input can be varied to other inducible promoters, we anticipate

that our system to greatly enhance the potential of engineered cellular machineries.

Team VIT_Vellore: In vivo Drug Factory

In our project we propose a novel approach to the problem of sustained drug release, controlled using the concentrations of the target molecule and using synthetic biology.

Our 'in vivo drug factory' involves using E. coli. strains which are located in the human gut to manufacture and deliver drugs in the required concentration – controlled by a promoter. We have incorporated two safety features.

· hok/sok post-segregational killing mechanism. In case of plasmid loss during replication -minimizing the growth of unwanted bacteria

· 'kill-switch' – using bacteriophage holins ensures that in the case of adverse reactions, the production can be shut off.

We have chosen lactose intolerance for our pilot study as it is high prevalent and it involves a pre-characterized lacZ system. Our system is controlled by intestinal glucose concentration.

Our system could be potentially used for treating other inborn errors of metabolism, when coupled with the appropriate promoter.

Team WHU-China: Colorful E.coli Weave Time and Space.

Our project focuses on constructing colorful E.coli, which includes two parts. We plan to construct two systems consisting of several strains of E.coli: one produces different pigments due to the change of time, and the other produces different pigments with the change of position. In the first part, the strain of E.coli works as an oscillator which can yield different kinds of pigment periodically with the help of a signal transformation system. In the second part, we came up with the idea of ¡°colorful E.film¡±. In hope to create a colorful film, we will construct three E.coli strains which can produce and secrete three primary colors respectively in the presence of the three primary lights. Therefore, if we use a color picture as an input signal, the output will be the copy of it on the plate.

Team XMU-China: i-ccdB: Intelligent Control of Cell Density in Bacteria

We have developed a series of devices which program a bacteria population to maintain at different cell densities. A genetic circuit has been designed and characterized to establish a bacterial 'population-control' device in E. coli based on the well-known quorum-sensing system from Vibrio fischeri, which autonomously regulates the density of an E. coli population. The cell density is influenced by the expression levels of a killer gene (ccdB) in our device. We have successfully controlled the expression levels of ccdB by site-directed mutagenesis of a luxR promoter (lux pR) and error-prone PCR of gene luxR, and we are building a database for a series of mutation sites corresponding to different cell densities and fluorescent intensities. An artificial neural network will be built to model and predict the cell density of an E. coli population. This work can serve as a foundation for future advances involving fermentation industry and information processing.

Team ZJU-China: Rainbofilm

Rainbofilm is a stratified expression system in biofilm, a self-organized module extensible for various needs. Researchers found a vertical oxygen gradient establishes in the biofilm. Such property allows us to use oxygen sensitive promoters to artificially induce differentiated functions through the spatial distribution

of cells. Thus, the multi-step reaction can be processed within the different layers of the biofilm.

The biofilm and its layered structure form spontaneously. Also biofilm has the natural resistance to high levels of toxin. These two properties render the Rainbofilm a convenient stable system for bio-production and bio-sensor. The system can cater to different needs simply by changing downstream genes. One possible application is ethanol production. The cellulose is degraded to monose from the bottom to the middle layer, and the ethanol is produced and secreted in the surface to minimize the toxicity to the inner cells.

EUROPE

Team Amsterdam: icE. coli: enhancing E. coli's growth rate at low temperatures by psychrophile chaperones

Escherichia coli's optimal growth temperature is 37°C. Its growth rate decreases drastically at lower temperatures and growth completely halts below 7°C.

The aim of our project is to increase the cold tolerance of E. coli by expressing and combining several chaperone proteins that are normally found in psychrophillic bacteria. Chaperone proteins are essential in maintaining correct protein folding following changes in temperature. Therefore, expressing these proteins will likely enable enhanced growth rates at temperatures below 37°C and shift the minimal growth temperature down from 7°C, possibly even allowing growth near 0°C.

Our project is fundamental in its nature, but any application outside the labs, from biofuel production to bio-sensors, will benefit from the ability of icE.coli to grow at low temperatures, since this will relieve the burden and costs of maintaining the temperature constant at 37°C. Moreover, we envision a temperature-based selection as an alternative to antibiotics-based selection.

Team Bielefeld-Germany: The Bisphenol A-Team: A Cell-free Approach for Biosensors

The development of sensitive and selective biosensors is an important topic in synthetic biology. We want to provide nanobiotechnological building blocks as a basis for cell-free biosensors. Therefore we worked with S-layers (crystalline bacterial surface layers) which build up well-defined nanosurfaces and can be attached to the surface of beads. As an example we are developing a cell-free bisphenol A (BPA) biosensor based on a coupled enzyme reaction fused to S-layer proteins for everyday use. BPA is a supposedly harmful substance which is e.g. used in the production of polycarbonate. To detect BPA it is degraded by a fusion protein under formation of NAD+ which is detected by a NAD+-dependent enzymatic reaction with a molecular beacon.

Team Bilkent_UNAM_Turkey: Biodegradation of TNT and TNT derivatives by nfsI-transfected Chlamydomonas reinhardtii

We aim for the genetic modification of the unicellular microalga Chlamydomonas reinhardtii by introducing the nfsI gene belonging to the bacterium Enterobacter cloacae in order to investigate how nitroreductase expressing-microalgae respond to trinitrotoluene (TNT) exposure. Our experimental design is as follows: firstly, obtain a synthetic gene of nfsI with flanking prefix and suffix of standard Biobricks, and ligate this insert to pRbcBRL, a vector with appropriate expression and selection system for Chlamydomonas reinhardtii and obtain pRbcnfsI. Then, Chlamydomonas reinhardtii will be transfected with the with the designed plasmid. The transfected algae will then be grown in presence of TNT and/or TNT derivatives and

the effectiveness of nitroreductase activity on biological degradation of TNT will be investigated.

Team Cambridge: Bactiridescence

Nature's colours don't just come from pigments, but from structure too. Cephalopods camouflage themselves using intracellular, iridescent structures made of proteinscalled reflectins. These are the only known proteinaceous materials that use thin film interference to generate colour. They are inspiring a new class of responsive optical materials.

We hope to demonstrate the potential of reflectins as photonic materials by producing optical devices which exhibit instantaneous colour change. In addition we intend to characterise export pathways in E. coli and optimise protein production for commercial viability. We will submit constructs for the expression of reflectins in a variety of organisms.

Our team is producing a report examining the impact of iGEM, focusing on innovation in the biotechnology industry. Alongside this we are contributing to Gibthon, a collection of software tools aimed at fragment library management and construct design, building on standards developed by previous Cambridge teams.

Team Copenhagen: Expressing and standardizing cytochrome P450 in E.coli to create the oxime producing CyperMan

A cytochrome P450 (CYP) is an enzyme able to perfom complex hydroxylations. The regio- and stereospecfic hydroxylations performed by CYPs are often difficult to do using conventional chemical methods and it can therefore be greatly beneficial to utilize our CyperMan as a small synthetic biology factory.

CYPs from the plant CYP79 family produce small molecules called oximes. Oximes are toxic to fungus, as they inhibit the ability of the mitochondrial peroxidases to breakdown potentially harmful hydrogen peroxides. The oximes are produced from a variety of amino acids. As a proof of concept we aim at generating bacteria or CyperMen able to combat fungus on their own turf. The main goal is however to standardize different plant CYP79s and deliver them as BioBricks to iGEM.

Team Debrecen_Hungary: Oilrig or nuclear hormone receptors: How to find lipids in the environment?

Nuclear hormone receptors (NHRs) are ligand activated transcription factors. They are able to regulate the expression of their target genes by direct DNA-binding, in a ligand-dependent manner. NHRs bear high homology to each other and are modular into distinct regions: N-terminal regulatory region, DNA-binding domain, a Hinge region, Ligand binding domain (LBD) and a C-terminal region. Some Nematode NHRs can be activated by extracted oil contamination of the soil so they can use as possible oil sensors in the future. Zinc finger motifs are the tools of NHRs to bind DNA and regulate gene expression directly. These tiny elements can be tested as direct gene regulators. Controlled gene induction can also lead to programmed cell death which is a less harmful tool in order to quit non-functionable cells.

Team DTU-Denmark: Tuning regulation with a non-coding RNA trap

Small regulatory RNA is an active area of research with untapped possibilities for application in biotechnology. One such application could be the optimization and fine-tuning of synthetic biological circuits, which is currently a cumbersome process of trial and error. We have investigated a novel type of RNA regulation, where the inhibitioncaused by a small regulatory RNA is relieved by another RNA called trap-RNA. The system displays a large dynamic range and can uniquely target and repress any geneof

interest providing unprecedented flexibility. We suspect that any level of repression is achievable by simply altering the sequences of the involved RNAs. Multiple such systems can coexist without interfering and are thus compatible with more complex designs. Furthermore the trap-RNA can be fused to any transcript in effect allowing any gene to act as an activator.

Team DTU-Denmark-2: Plug'n'Play with DNA: a novel assembly system

The DTU-Denmark 2 team is designing a novel standardized assembly system, called "Plug'n'Play with DNA", where any biological parts can be gathered without use of restriction enzymes and ligases. Our goal is to create a new assembly standard of biological parts in the form of pre-produced PCR-products, which can be directly mixed with a vector. This will make synthetic biology faster and assembly of an expression vector possible within a few hours. We have created a library of standardized biological parts for mammalian cells and fungi ready to plug'n'play. The simple and easy use of this new assembly system have been demonstrated by developing a reporter targeting system a mammalian cell line as well as for fungi. This novel assembly system represents an improvement of the conventional BioBrick assembly, which have its limits when creating synthetic biology with eukaryotic parts.

Team Dundee: The Sphereactor - a synthetic bacterial microcompartment

Bacterial microcompartments are proteinaceous reaction chambers designed to 'cage in' metabolic pathways and increase efficiency. Potentially, these could be engineered to house any chemical reaction imaginable; to sequester toxic material; or to confer new physical properties to a host. Here, a synthetic microcompartment ("The Sphereactor") was designed and built. This was assisted by the creation of new mobile apps and web-based tools for DNA analysis. A synthetic operon was constructed, based on the pduABJKNTU genes from Salmonella, that assembled into the empty Sphereactor, which was also affinity-tagged to allow its isolation for downstreamapplications. A new targeting sequence comprising 20 residues of PduD was shown to target GFP into The Sphereactor. Attempts were made to pack the Sphereactor with many other proteins. Together, the Sphereactor and its new targeting sequence is a foundational advance that could influence the design of new metabolic pathways or inspire new bioremediation or biomedical projects.

Team Edinburgh: Improved biorefineries using synergy: a feasibility study

A biorefinery is a special type of refinery in which biomass, such as plant cellulose, is converted by microorganisms into useful products. Edinburgh's 2011 iGEM project is a feasibility study into the creation of biorefineries using E. coli, the workhorse of synthetic biology; and whether biorefineries can be improved by arranging for the different enzymes involved to be in close proximity to each other, so as to create synergy between them. We investigate two methods of bringing the enzymes close together: cell surface display via Ice Nucleation Protein, and phage display via M13's major coat protein. We attempt a new DNA assembly protocol, provisionally named "BioSandwich". We construct computer models of synergy. Finally, we consider the broader economic and social questions surrounding the construction of a biorefinery: can it be done, and should it be done?

Team ENSPS-Strasbourg: Biobricks model generator for electronic simulators

Despite the research made in the field of automation of the design of synthetic biosystems, there is no existing generic tool for the moment. However, in the field of microelectronics, automated system design has been proven over 40 years experience. By analogy with the behavior of biosystems and the working of some electronic circuits, it seems possible to rely on microelectronics tools to design biosystems. This

project is aimed to create a graphical user interface for designers of synthetic biosystems. This would help them in their design process by simulating the system. The midterm objective is to base the software on the database of biological material, fed by other iGEM teams. It will model the studied system in an electronic modeling language and provide simulation results to the user.

Team EPF-Lausanne: Teenage Mutant Ninja TetRs: A Transcription Factor Development Pipeline

To create novel devices, and to fine-tune systems for a specific response to a stimulus, synthetic biology relies on a library of parts with specific functionalities and characteristics. Here, we present our efforts to expand the choice of regulatory parameters in a system by engineering TetR-based transcription factors with a large range of DNA-binding specificities and affinities. Specifically, we have generated several TetR and pTet promoter variants using site-directed mutagenesis, and characterized their respective interactions using fluorescent reporter assays and MITOMI-based microfluidic devices. In addition, we are developing a proof-of-concept high-throughput measurement system based on bacterial cell lysis to in vivo select desirable variant protein-DNA interactions . This system is based on a repression cascade, in which TetR binding to the pTet promoter element triggers cell lysis in an affinity-dependent manner, enabling us to collect the plasmids of suitable TetR variants for sequence analysis and downstream processing.

Team ETH_Zurich: SmoColi

SmoColi is a bacterio-quantifier of acetaldehyde concentration that can be used as a passive smoke detector. Acetaldehyde is a toxic and carcinogenic component of cigarette smoke. It has a boiling point of 20.2 °C and is very volatile, thus can be used as an information carrier through air. The SmoColi cells are immobilized in an agarose-filled microfluidic device. The test solution is fed on one end of a microfluidic channel, in which an acetaldehyde gradient is established by synthetic cellular degradation. The cells are engineered to sense acetaldehyde by a synthetically re-designed fungal acetaldehyde-responsive transactivator. The sensor is linked to a band-pass filter that drives GFP expression. This allows establishment of an input-concentration-dependent, spatially located fluorescent band displaying quantitative information about acetaldehyde. Finally, if the acetaldehyde concentration exceeds the threshold of malignance, a quorum-sensing-based mCherry alarm system springs into action, turning the whole device red.

Team Fatih_Turkey: The Rainbow Graveyard

E. Coli is one of the gram-negative bacteria and most of their types are harmless. However some of E.Coli types such as enterohaemorrhagic E.Coli (EHEC) can cause serious disease.

In our project we designed an innovative model to prevent gram-negative growth and infection. In our model, to prevent E.Coli growth we modified non-infectious gram-positive bacteria B.Subtilis by transforming it with a construct, which was designed to produce limulus anti-LPS factor (LALF) together with a signal peptide. LALF is expected to bind and neutralize Lipopolysaccharide (LPS) found in gram-negative bacteria cell wall. On the other hand, to show E.Coli growth inhibition, we prepared another construct, which carries reflectin sequence with a signal peptide sequence and transferred into E.Coli. Reflectin protein produces color by reflecting light in different wavelenghts. This ability helps us to detect whether E.Coli is dead or alive.

Team Freiburg: Lab in a cell - protein purification

In the future more therapeutic proteins will be produced by the pharmaceutical industry to cure various

diseases. The key to allow less developed countries to improve their own research in this field lies in making large scale protein purification fast and affordable but also ecofriendly to save precious resources. By eliminating routine use of expensive materials, our novel tool will utilize sustainable laboratory equipment and widespread His-Tag technology to guarantee reliable protein purification for all.

We propose an expression system induced by blue, green and red light, combined with subsequent temperature controlled autolysis of E. coli. Purification of the his-tagged protein of interest will be accomplished by an adaptor protein of our own design which binds the His-Tag on one side and the surface of serological pipettes on the other. Two subsequent pipetting steps for washing and purification of the cell lysate will quickly elute the product.

Team Glasgow: DISColi: Bio-photolithography in Device Engineering Using Different Wavelengths of Light

The DISColi project aims to design and construct a novel bio-photolithographic system for the engineering of biofilms into functional 2D and 3D structures and devices in response to different patterns and wavelengths of light. In this project we worked with light responsive promoters, a novel biofilm-forming synthetic biology chassis, E. coliNissle 1917, and novel biobricks including several designed for biofilm dispersal and fluorescent reporters with wider utility than GFP. The main aims of our project can be separated into three light-controlled components: the designed sculpting of biofilms; 3D printing for encapsulation of cells; and the controlled modular synthesis of a variety of products. We expect this technology to have applications for material synthesis and compound manufacture in remote locations, for example outer space.

Team Grenoble: MercuroColi: A new way to quantify heavy metals.

Our project aims at constructing an easy to use, transportable sensor capable of quantifying the concentration of mercury, in an aqueous sample. Our system is based on a comparison between an unknown mercury concentration and a known IPTG concentration. A linear IPTG gradient is present on a test-strip containing the engineered bacteria. When the mercury solution is added, the regulatory network will switch to one of two states depending on the IPTG/mercury ratio. Bacteria become either“sender” or “receiver”. The bacteria sensing a predominance of mercury over IPTG, the “senders”, will release a quorum sensing molecule which is detected by the nearby “receivers”. The reception of quorum sensing molecules will induce the expression of a red dye in the “receivers”. In this way, a red line emerges at a position in the IPTG gradient from which the unknown mercury concentration can be deduced.

Team Groningen: Count Coli – A synthetic genetic counter

Our project aims to design a genetic device able to count and memorize the occurrences of an input signal. We achieved this by utilization of auto-inducing loops, that act as memory units, and an engineered riboregulator, acting as an AND gate. The design of the device is modular, allowing free change of both input and output signals. Each increase of the counter results in a different output signal. The design allows implementation of any number of memory units, as the AND gate design enables to extend the system in a hassle-free way. In order to tweak bistable autoinducing loops we need a very fast and robust method for characterizing parts. For this we have created a genetic algorithm that will enable us to find parameters of the parts used in the design. It also allows the combination of data from multiple experiments across models with overlapping components.

Team Imperial_College_London: When AuxIn met Root

In an effort to combat soil erosion, we have developed the AuxIn system. This system is comprised of three modules combined in an E. coli chassis. The first involves secretion of the plant growth factor indole 3-acetic acid (auxin). This plant hormone will promote root growth which is essential for anchoring soil.

The second module rewires the chemotactic mobility of the cell by introducing a novel receptor protein which is sensitive to root exudates. The bacteria can then be naturally taken up by root cells for targeted auxin delivery. The final module uses a toxin-antitoxin system to prevent horizontal gene transfer. While the plasmid containing the AuxIn system can be maintained inside our chassis, it will induce lysis in any other bacteria.

By improving root growth, the AuxIn system provides a synthetic biology approach to tackling worldwide problems such as soil erosion and desertification.

Team KULeuven: E.D. FROSTI: CONTROLLING ICE FORMATION

Our team will engineer a bacterium that can perform 2 different functions; depending on the stimulus used, it will either induce ice crystallization or inhibit ice formation.

To stimulate ice formation, we will let our bacterium produce Ice Nucleating Proteins (INP), which stimulate the formation of ice crystals. These bacteria could be used in lakes to make the ice stronger, for the creation of fluorescent ice on ski-slopes or for trendy cocktails with glowing ice cubes in it. Also, it would decrease ice-melting of glaciers, thereby effectively slowing down global warming.

The second function of E.D. Frosti is the anti-freeze function by the production of Anti Freeze Proteins (AFP). These bacteria could then be used as anti-freeze biofilms on roads, which would help the roads become ice and snow free in winters. Also other applications which require ice melting could benefit from our bacterium.

Team LMU-Munich: WOO-HOO! - The Bacterial Heavy Metal Detector Kit

Heavy metal ions of human origin contaminate waters worldwide and represent a major threat to human health, especially in lesser developed countries. The compliance with strict drinking water quality standards as a prerequisite to a healthy living requires qualitative and quantitative methods for monitoring metal ion concentration. Applying standard chemical methods is costly, complicated and sometimes requires high-tech machinery, which is often not available.

We therefore aim at creating a set of bacterial biosensors for some of the most toxic heavy metal ions found in drinking water, by fusing metal-responsive promoters under the control of transcriptional regulators with reporter genes such as GFP. The biosensors will be evaluated to qualitatively determine the metal ion specificity and subsequently quantitatively describing the concentration-dependent output of the reporters. Such a tool kit can be applied to quantify the metal ion content in water samples in an easier and cheaper way.

Team Lyon-INSA-ENS: "Cobalt Buster" : decontamination of trace cobalt from radioactive effluents.

The activity of nuclear power plants releases many radioactive elements in water. Cobalt is one of them, and is a major concern for these industries because of its high level of radiation.

Currently, nonspecific ion exchange resins are used. Our E. coli “Cobalt buster” strain aims at capturing cobalt specifically, even at extremely low concentrations, to increase resin lifetime and decrease the

volume of nuclear waste. In a previous study, an E. coli strain has been modified by adding a transporter for cobalt and inactivating cobalt efflux pump.

Then, our work focused on making this strain adherent in response to cobalt, via cobalt inducible production of curlis. These properties will enable to create an efficient biofilter, which will be easily separated from the effluent, allowing efficient and cheap bioremediation of trace cobalt in nuclear wastewater.

Team METU-Ankara: MethanE.COLIc : Decreasing the Greenhouse effect and Saving the workers' life in one system

Firedamp explosions are frequently seen cases at all mines over the world. In Turkey, every year, around 50 miners lose their lives because of firedamp explosions. Firedamp is a flammable gas found in coal mines and it mainly contains methane. Beside its explosive property, methane is also the main contributor to global warming. However, recent mechanisms in mines release the methane that is collected into air. By offers of Synthetic biology, we aimed to design a device which will work on E.colithat provides solutions for side effects of methane. Device that we are planning to construct involves the genes of bacteria (Methylococcus capsulatus) and insect (Drosophilia melanogaster) Our compact system in E.coli is fabricated as sensation of methane, the conversion of methane to methanol and then entrapment of methanol to handle for biofuel and death of bacteria at 42 C by kill switch mechanism.

Team METU-BIN_Ankara: M4B: Mining for Biobricks

Main goal of METU-BIN 2011 project is to provide a web based tool that helps synthetic biologists at the pre-experimental step to design their genetic constructs using the biobricks according to their input and output parameters. 1- A network of all bioparts in 2011 distribution will be generated, which describes the functional relations between the subatomic bioparts. 2- A search algorithm will be developed to reveal all possible device combinations for the user defined input and output within bioparts of 2011 distribution. 3- Visualization tools will be applied for graphical representaiton of the results. 4- A web-based user interface will be provided for the developed software. The functional relations of subatomic bioparts will be examined and each combination will be registered as one incidence. The most frequently used combinations will be given a priority while providing the possible device combinations as a list which satisfies the user defined requirements.

Team NTNU_Trondheim: Fluorescent Stress Sensor

ppGpp (Guanosine pyrophosfate) is a global regulator of gene expression in bacteria. Production of ppGpp up-regulates central genes for starvation survival, and down-regulates genes involved in growth and proliferation, e.g. rRNA operons and the rrnB P1 promoter. ppGpp production is induced by amino acid starvation and other general stress and starvation factors, making this signal molecule suitable for monitoring stress in bacteria.

The sensor consists of two parts. mCherry (a red fluorescent protein) controlled by the Lac promoter, and the Lac Inhibitor controlled by the rrnB P1 promoter.

We observed a statistically significant reduction in fluorescence during growth compared to cultures with IPTG-inhibited LacI, demonstrating lacI production by rrnB P1. Results suggest that the rrnB P1 promoter produces less lacI when the cells are grown in minimal medium, possibly due to amino-acid starvation and ppGpp shutting it down. Computer modeling supports these results.

Team Paris_Bettencourt: TuBe or not TuBe? Toward a new bacterial cell-to-cell communication

Bacterial communication and resource sharing, hitherto thought to be mediated through the medium has been challenged by a recent paper (G.P.Dubey et al.) suggesting an extraordinary new form of communication between B.subtilis cells and even exchanges with E.coli through nanotubes. We set to provide new evidence for this cell-to-cell communication and to allow the synthetic biology community to harness its potential for amorphous computing and metabolic engineering. We developed and characterized new B.subtilis BioBricks designed to validate this finding by testing a wide range of molecules that could potentially travel through the nanotubes and be detected via signal amplification. We worked on molecules of different size and nature to best characterize the transfer. Modeling suggests that we will be able to follow the diffusion through the nanotube network by fluorescence microscopy. TuBe or not TuBe? Our ongoing experiments will shed light on this elusive question.

Team Potsdam_Bioware: Modification, Selection and Production of Cyclic Peptides for Therapy

One key task of biopharmaceuticals is the binding and blocking of deregulated proteins. Towards this goal, we mutate and select microviridins, which are tricyclic depsipeptides from cyanobacteria. They are small but stable due to their post-translational side-chain crosslinking. Microviridins have a high potential for therapy as they can block disease-relevant proteases. Yet, the possibilities of cyclic peptides are largely untapped since genetic systems for optimization are not well established. Thus, we developed synthetic systems for the mutation, selection and production of such peptides. We use the 6.5 kb microviridin (mdn) gene cluster cloned in E. coli plasmids, established random mutagenesis and generated focused libraries of microviridins. For selection against a panel of proteases, we are applying and testing phage display, and we are constructing a novel in-vivo selection device, which links protease blocking to antibiotic resistance. Our systems, including the 6.5 kb cluster, adhere to the BioBrick standards.

Team Sevilla: Arcanum Project

We propose a new standard in order to do synthetic biology at a higher level of abstraction, above the biobrick. Our intention is to define a universal substance (Ubbit) for communication between modules of bacteria. Each module is composed of different transformed bacterial strains that work co-ordinately so that the whole module performs a logic operation based on a binary code. Having a unique communication substance means there's no need to understand how the modules work or what's inside, the only important thing is knowing which operation it performs and the inputs and outputs it has. This would allow us to build bigger circuits with different modules that could carry out more complex operations.

Team St_Andrews: Kill Switch Engage: Intracellular Protegrin-1 Production and its Potential Applications

For the 2011 St Andrews iGEM Team project, we are creating an intracellular Escherichia coli "kill switch" that functions differently from any found in nature. Our kill switch is designed by inserting an antimicrobial peptide (AMP) gene into E. coli. The AMP in question is protegrin-1, an 18 amino acid residue. Protegrin-1's secondary structure is a beta -sheet conformation including a b-hairpin turn, which allows it to imbed itself into the phospholipid bilayer and disrupt bacterial cell walls by creating pores within the membrane.

The antimicrobial activity of protegrin-1, including the action of pore formation, provides us with a wealth of potential applications for this kill switch, including drug delivery, conjugation both in vivo and in vitro, and its use as a basic biosafety tool."

Team TU_Munich: E.XPRESS3D - Three-Dimensional Printer Based on Optogenetics

This year, we aim to develop a light-controlled 3D-printer by innovative utilization of optogenetics. As a first step, we want to develop a genetic logical AND-gate sensitive to light of two different wavelengths (e.g. blue and red light). The bacteria are then immobilized in a transparent gel matrix, where they can be precisely actuated when hit by both blue and red light at the same time. If both of these inputs are positive, gene expression is induced. Various different gene products can be expressed using this system. For example, a simple colored pigment will allow us to create colored three-dimensional objects. Expressing collagen and consecutive biomineralization and generation of hydroxylapatite could be used to create bone.

Team TU-Delft: StickE. Coli : Single Protein Attachment of Escherichia coli

Natural attachment of micro-organisms relies on a complex network of varying compounds known as biofilms. This complexity hinders an easy control and regulation of attachment and detachment. We will give Escherichia coli a simple, effective and controllable mechanism for biofilm formation based on the strong glue from mussel feet. E. coli, expressing the strongest-binding mussel foot protein Mfp5 on the outer cell surface, can robustly attach to a wide variety of surfaces, including glass, plastic and itself.

Using highly sensitive TIRF microscopy and atomic force measurements we visualize and characterize the localization and attachment of cells. Combining these results with our mathematical models allows us to predict the attachment speed and stability as well as cell clustering and settling. The controllable, strong attachment opens up new possibilities for the use of bacterial machines in environmental applications, medicine and industry.

Team UCL_London: E.coili - Making supercoiled pDNA technology viable

We are developing a toolkit for the industrial manufacturing of supercoiled plasmid DNA. This will enable a widespread adoption of pDNA-based technologies, most relevantly DNA vaccines - the future of immunization. Therefore we are a manufacturing project with a strong impact on the global health sector. Our toolkit consists of five modules tackling different bottlenecks in the manufacturing process from production to lysis and filtration as well as providing tools for monitoring and improving the overall quantity and quality of the product.

Recognizing that the adoption of vaccines and other biological technologies also depends strongly on their social acceptance, we're investing heavily into public engagement for synthetic biology and are exploring the process by which new scientific disciplines arrive in the public sphere.

Team UEA-JIC_Norwich: The evolution of synthetic biology; The introduction of new photosynthetic eukaryotes as model organisms.

There are challenges using plants in iGEM, namely growth time and the complexity with adapting synthetic biology approaches for plants. However, plants are a major focus of synthetic biology due to their potential use in an array of applications from food security to synthesis of biofuels. The short time scale of the iGEM competition has often meant that plant based projects are challenging and there have been very few in previous years. As the first iGEM team at UEA and in co-operation with the JIC, we felt that we could make a significant contribution to plant based synthetic biology. The overall aim of our project is to help develop and where possible pioneer some of the fundamental technologies and methodologies needed to make plant based synthetic biology projects possible. To achieve this we hope to adapt existing synthetic biology approaches which are successful in Escherichia coli for use in plants.

Team ULB-Brussels: Pindel: One step insertion / deletion system

One of the most common actions of all engineers is the assembly or deletion of parts. Bearing in mind that one of the purposes of iGEM is to link biology and engineering sciences, we'd like to implement an easy way to manage those simple steps in biological systems.

Unfortunately, in E. coli, it's still difficult to assemble or delete in one step, because there is a lack of genetic tools to execute homologous recombination with linear DNA. By the assembly of a unique plasmid containing different phages' genes, and the design and construction of helper plasmids, we aim to provide the iGEM community with a system that would confer to E. coli the useful properties of controlled homologous recombination.

We called this plasmid for insertion and deletion of genes : Pindel.

Team UNIPV-Pavia: Ctrl+E. - Signalling is nothing without control

Our work aims at implementing the engineering concept of closed-loop control in E. coli, exploiting quorum sensing. As a proof of concept, we designed a simple genetic controller that regulates the concentration of 3OC6-HSL signalling molecule around a user-defined set-point. The controlled variable (3OC6-HSL) increases as a function of the exogenous anhydroTetracycline input, that triggers LuxI expression. The controller senses the 3OC6-HSL concentration and activates the production of AiiA, that degrades it. To observe the desired behaviour, a fine tuning of the system was necessary. The transcriptional/translational strength of the regulatory elements (promoter+RBS in several combinations) and the enzyme activities were measured and exploited to identify a mathematical model able to predict the behaviour of the controlled system. These predictions made possible an in silico rational fine tuning of the circuit: the most promising modules were selected and assembled into the final circuit, avoiding a cost and time expensive combinatorial approach.

Team UNITS_Trieste: Synbiome: a three-cell type interkingdom consortium

The synbiome project exploits synthetic biology to obtain a synthetic stable community of eukaryotic and prokaryotic cells. Two different bacterial strains 'A', 'B' and one eukaryotic cell type 'C' will be engineered to establish mutualism: 'A' produces a N-acyl homoserine lactone (AHL) sensed by 'B', which in turn produces a different AHL sensed by 'A'. In addition, both bacterial cells activate, through AHL, an enzyme necessary to convert cellobiose to glucose, which represents the only energy source for the whole consortium. The eukaryotic cell 'C' responds to AHL through a hybrid protein, thereby producing a secreted beta-lactamase, which allows the bacterial cells to grow in the presence of ampicillin. The creation of a consortium of inter-dependent cells from different kingdoms is expected to pave the way to multiple applications, since different cells might cooperate and, for instance, better produce complex molecules.

Team UPO-Sevilla: FLASHBACTER: Improving robustness in bistable systems

The core of our project is the development of molecular systems that reproduce the behavior of an electronic flip-flop, allowing the construction of living memory devices closer to the robustness of informatics. Several iGEM teams based their work on the construction or use of biological flip-flop systems, with more problems than expected. The UPO-Sevilla team considers the design, construction and improvement of this type of systems, encompassing different regulation levels (transcription, translation, protein proteolysis and epigenetic regulation) in different model organisms, from bacteria to yeast. We leverage our informatics knowledge to improve biological systems, so we can control precisely the cellular machinery.

In addition, we intend to develop a Foundational Advance for Synthetic Biology: the miniTn7 BioBrick toolkit, a transposon-based system to integrate BioBricks into microbial genomes. And a Software tool: the BioBrick Creator, to facilitate the design of BioBrick sequences and their inclusion in the Parts' Registry.

Team Uppsala-Sweden: Expand the colidroids into colorful light sensing

Regulation of gene expression by light-sensing is a milestone in synthetic biology. In this project we strive to upgrade the existing coliroid system by making it triple-chromatic.

Our triple-chromatic coliroid system employs sensors for red light, green light and blue light in one and same platform. These sensors are to be activated both independently and together. This functions as a triple-switch of genetic regulation. In combination of switching the individual sensors on-and-off, one can achieve higher dimensions of transcriptional control. The proof of concept is shown by coupling each type of sensor with chromoproteins, proteins that naturally show color when expressed.

Instead of visualization by e.g. UV light, our colidroid system can be visualized with naked eye. Other than a bacterial piece of art, our system will be very useful in the future concerning e.g. biomaterial.

Team Valencia: "Water Colicin Cleaner: disinfected water by E. coli"

Water Colicin Cleaner is a biological alternative for disinfecting contaminated water using synthetic biology. We kill targeted pathogens by means of antimicrobial peptides in Escherichia coli. As a proof of concept we have chosen to aim for enterobacteria, focusing on E. coli contamined water, as a proxy of fecal water. The cleaning of E. coli will be possible thanks to the colicin protein. The activity of these proteins will be controlled by a biological pH-stat, using light-driven cyanobacteria to control pH of the media. We will deliver a DIY kit as a prototype platform for killing pathogens responsible of diseases such as cholera, diarrhea and typhoid fever, ending with more than 2 million deaths in the last year according to the 2011 WHO Guidelines for Drinking-Water Quality.

W.C. Cleaner is an effective, low-cost device, that will help people that struggle to find pathogen-free drinking water avoid important diseases.

Team Wageningen_UR: The Synchroscillator: Controlling and Visualizing Synchronized Oscillations in Real Time

One aim of Synthetic biology is to re-engineer naturally occurring gene circuits to produce artificial systems that behave predictably. Our project involved streamlining and providing additional functionality to a recently published synchronized oscillatory circuit, in an attempt to reproduce and further characterize its dynamics. Our genetic circuit consists of modified (and BioBricked) elements of the Vibrio fischeri lux quorum sensing system composed to form interconnected positive and negative feedback loops, which dynamically regulate the expression of GFP. In order to provide our E. coli host with the right environment required for population-wide oscillations, we designed and manufactured a custom flow-chamber capable of maintaining a defined cell population while independently varying the growth conditions. The chamber was specifically designed for time-lapse studies with a fluorescence microscope. We detected synchronized oscillatory gene expression under zero-flow conditions, suggesting an unexpected level of robustness. This should facilitate its integration with more advanced genetic circuits.

Team Warsaw: Synthetic Cloning and Expression Control

Our goal is to set up an easy and quick protocol for cell free cloning. It skips plasmid propagation in bacteria. This speeds up the cloning procedure at least three times and allows cloning of toxic genes. We

make sure that no bacteria get harmed during our project. Moreover we have measured the RBS parts with various fluorescent proteinsand they are not as standard as we would like them to be. The strength of a RBS part depends on the protein used. Why? Because the beginning of the protein influences the mRNA fold. We came up with the idea of RBS parts fused with short 'protein beginnings' - expression adapters. Using genetic algorithm we designed expression adapters that would provide standardized protein expression or increase expression of your favorite protein. We are testing our design in the wet lab.

Team WITS-CSIR_SA: Biotweet: A riboswitch controlled location-based networking framework

Bacterial chemotaxis is controlled via a signalling cascade, where CheZ is a protein integral in the directed movement of bacteria towards a stimulus. The aim was to control chemotaxis such that bacteria will be attracted to a defined substance followed by the ability to travel back to another stimulus at the start location, upon activation of an IPTG inducible toggle switch. Two riboswitches were used to control the translation of a CheZ fluorescent protein fusion, the first sensitive to theophylline and the second to atrazine. Fluorometry was used to prove the activation of the riboswitches. A theophylline concentration of 1.5mM resulted in the highest expression of the fusion protein. Motility experiments indicated that CheZ mutants regained motility in the presence of theophylline. Since riboswitches can be engineered for many substances, this system has possible applications as a networking template in multiple situations, be they industrial or medical.

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Team BostonU: Abandon All Hope, Ye Who PCR: MoClo and the Quest for Genetic Circuit Characterization

Our project aims to introduce a standardized protocol for the characterization of genetic circuits using flow cytometry. We built a vast number of both simple and complex genetic circuits that were characterized using flow cytometry. These genetic circuits were built using an assembly technique called MoClo (developed by Weber et al., 2011), which involves a multi-way, one-pot digestion-ligation reaction, enabling faster and more efficient construction of genetic circuits. We converted a large subset of BioBrick™ Parts from the Registry (http://partsregistry.org/) into MoClo Parts using PCR and cloning strategies. We built and characterized various genetic circuits using MoClo Parts and compared them against their pre-existing BioBrick™ counterparts in order to compare the characterization results from the two assembly techniques. We also created a standardized data sheet to be included in the Registry of Standard Biological Parts for each Part we characterized to easily share our data with the synthetic biology community.

Team Carnegie Mellon: Real-time quantitative measurement of RNA and protein levels using fluorogen-activated biosensors

The design and implementation of synthetic biological systems often require quantitative information on both transcription and translation rates. However, quantitative information about the expression strength of a synthetic promoter has been difficult to obtain due to the lack of noninvasive and real-time approaches to measure the levels of both RNA and protein in cells. Here, we engineer a fluorogen-activated bio-sensor that can provide information on both transcription strength and translation efficiency. This biosensor is noninvasive, easily applied to a variety of promoters, and more efficient than existing technologies. To demonstrate the utility of our biosensor, we constructed and characterized several designed T7Lac hybrid promoters. Furthermore, we developed a mathematical model of our synthetic system to guide experiments and an open-source electronic kit that mimics experimental setup and well suited for education purposes. Our results could have a broad impact on the measurement and standardization of synthetic biological parts.

Team Clemson: Biphenyl degradation by pollutant targeting, biosurfactant production, and overexpression of catabolic enzymes

Polychlorinated biphenyls (PCBs) are widespread, cancer-causing pollutants left-over mainly from manufacture of capacitors and electric motors. There are over 200 possible PCBs, derivatives of biphenyl, which share the same biodegradation pathways in bacteria. Our team is using a genetic engineering approach to produce a small consortium of E. coli that should efficiently degrade biphenyl, and it is hoped that this same system can be adapted for the bioremediation of PCBs. Natural bioremediation by native bacterial communities is exceedingly slow due to the recalcitrant nature of PCBs and their hydrophobic properties which reduce the bioavailability to potential catabolizers. We are taking a three-pronged approach in an attempt to increase the efficiency of biphenyl bioremediation—attraction of biphenyl-degrading E. coli by other guiding bacteria, overexpression of the biphenyl catabolic enzymes, and production of a biosurfactant to increase the solubility of biphenyl. Together, this system should significantly increase the rate of biphenyl degradation.

Team Columbia-Cooper-NYC: Light Sensitive Spatially Controlled Micromachining of Copper Wafers Using Acidithiobacillus Ferrooxidans

The Columbia-Cooper iGEM team is working with Acidithiobacillus ferrooxidans to create a light-controlled printed circuit board manufacturing process. This bacteria's metabolism relies on its ability to oxidize iron; the iron can then be used to oxidize, and in turn solubilize, copper. By genetically altering the bacteria, we intend to install a light sensitive mechanism which will enable us to etch copper in a desired pattern, leaving a finished circuit board. Once a blank printed circuit board is placed in a thin layer of solid media, the bacteria will be applied onto the surface of the media and light will be focused on it in a desired pattern. The light sensitive mechanism in ferrooxidans would activate and self-destruct in the pathway of the light. In the end, the circuit board will be 'etched' by the bacteria everywhere but the illuminated spots, leaving the desired pattern behind on the circuit board.

Team Cornell: SAFE BET: The Shewanella Assay for Extended Biomonitoring of Environmental Toxins

Cell-based biosensors have potential uses in environmental monitoring for toxins, medical diagnostics, and drug discovery. However, current methods for information output from whole cells (fluorescence, luminescence, pH) are very cumbersome to measure. To overcome this obstacle, the Cornell iGEM team has developed a new generation of biosensors capable of a direct current output which can be recorded easily with high precision. By upregulating the metal-reduction pathway of Shewanella oneidensis in the presence of a target compound, these sensors can act as a continuos monitoring system. While our system is adaptable to sensing a wide range of analytes, we have focused on the detection of arsenic-containing compounds and naphthalene, which are common contaminants in oil sands tailings. Furthermore, we have integrated these organisms within a field-deployable device capable of wireless data transmission – a fully autonomous electrochemical biosensor.

Team Duke: A High-Throughput Optogenetic Toolkit for Rapid Screening of Genetic Therapeutic Targets

Alzheimer's and other hereditary conditions are caused by small mutations in key genes. Medical genetics focuses on screening and treatment of hereditary genetic disorders. However, current high throughput screening methods are time consuming and extremely limited by cell growth rate and rate of gene activation. In order to address these issues we've created an optogenetic tool kit in Saccharomyces cerevisiae using the CIB1/CRY2 optogenetic system. In this system, the CIB1-VP16AD and CRY2-Gal4BD fusion proteins dimerize in the presence of blue light leading to gene expression at the Gal1 promoter. Using flow cytometry we characterized four different fluorescent proteins for use in our tool kit using a galactose activation assay. After characterizing our network, also using flow cytometry, we developed several proteinexpression assays for medical genetics. Finally, in order to confirm network success in silico, we utilized the modeling software TinkerCell to generate a stochastic model of our optogenetic network.

Team Gaston Day School: Detection of Heavy Metal Contaminants in Water

Heavy metal contaminants in water pose serious health problems; the lungs, liver, kidneys, blood, digestive system, and the nervous system are all affected by contamination. The Agency for Toxic Substances and Disease registry released a Priority List of Hazardous Substances (ASTDR). Heavy metals accounted for almost half of the top 10 substances; therefore, we have constructed a set of sensors that detects heavy metal contaminants in water. Our sensors provide an inexpensive, simple, and visual test for Arsenic (the number one substance from ASTDR's list), Lead (number two), and Cadmium (number seven). One sensor

paired a promoter responsive to both Cadmium and Arsenic with GFP as a reporter. Another was created for the detection of Lead and a third sensor was specific to cadmium. Use of the detector could potentially save lives around the world through early detection of the contamination.

Team GeorgiaState: Modification of Shuttle Vectors for the Expression of Recombinant Proteins Within Pichia and Plants

The GSU iGEM Team strives to modify shuttle vectors for the expression of proteins in Pichia pastoris and within recombinant plants. Adapting the glyceraldehyde-3-phosphate dehydrogenase (pGAP) shuttle vector to iGEM standards enables the expression of complex proteins in Pichia pastoris, due to post-translational modifications and a secretion system. We also plan to standardize the pPic9 shuttle vector for the same purposes. Our second project involves the use of Agrobacterium tumefaciens, the causative agent of Crown-Gall disease in plants. The ability of this organism's Ti plasmid to insert foreign DNA into a plant's chromosome is used to manipulate plants to express desired traits. These small plasmids house a cloning site and a selectable marker between the left and right border of the TDNA. The aim of this project is to modify a binary vector system to be compatible with the iGEM standard for the expression of foreign proteins within plants.

Team Georgia Tech: An intragenic complementation approach to engineer a faster fluorescence biosensor

Our goal is to engineer a novel biosensor with a faster readout than is currently available. Many bacteria produce, secrete, and respond to chemicals called autoinducers to monitor population density and to synchronize gene expression, a process called quorum sensing. In quorum sensing based biosensors, detection of autoinducer activates transcription of a reporter gene, which must then be translated and accumulate to detectable levels, which can take two to four hours. In our system, we will use TraR, a protein used in the quorum sensing response of Agrobacterium tumefaciens, which dimerizes only in the presence of its autoinducer. We have successfully fused traR to sequence for two separate complementary fragments of GFP. Upon addition of autoinducer, we predict that already accumulated TraR-GFP fragment monomers will dimerize, allowing the GFP fragments to interact and fluoresce. This new approach may drastically reduce the time necessary for future biosensors to produce detectable output.

Team IvyTech-South Bend: Optimization of a Bacteria-based Biosensor for Arsenic in Drinking Water

Millions of people worldwide are exposed to toxic levels of arsenic in drinking water. Bacteria have an efflux operon regulated by an arsenic sensitive inducible promoter. It is possible through recombinant DNA technology to isolate this promoter and combine it to a reporter system and transform bacteria to create a biosensor for arsenic. Induction of the arsenic-sensitive promoter occurs by the binding of arsenite to an inhibitory protein, de-repressing transcription. We have observed the arsenic responsive promoter from E. coli to have a consistent, low level of background induction. We have tested the hypothesis that by increasing the quantity of the inhibitory protein in the cell, we can quantifiably raise the threshold of the response. Our intention is to create a tunable biosensor to form the basis of a low-tech device that can reliably detect dangerous levels of arsenic in water for use in the developing world.

Team Johns Hopkins-Software: AutoGene

Autogene is an innovative CAD tool used to automate the design process of synthetic DNA sequences. The first module, AutoPlasmid, leverages the power of cloud computing, sophisticated bioinformatics algorithms, and an expert curated feature database containing over 40,000 features to automatically

annotate natural/synthetic DNA sequences, finding both perfect and imperfect matches. It also provides an effective solution to the Registry of Parts for annotation automation and pathogen sequence detection. The second module, AutoDesign, provides users with a drag-and-drop design environment to construct new sequences using user-imported features as well as those from our database. The third module AutoFab, which is still being developed, will provide users with guidelines of fabricating and optimizing their synthetic DNA. Compatible with other common bioinformatics tools such as ApE and capable of documenting in SBOL, genbank, and fasta formats, we hope that Autogene will allow synthetic biologists to take their research to the next level.

Team Johns Hopkins-Wetware: OptiYeast: optimizing production in yeast by ethanol regulation and optogenetic gain- and loss-of-function

Our global community deserves access to healthcare and nutrition currently available to only the most fortunate among us. Thanks to synthetic biology applications exploiting the yeast chassis, valuable compounds such as anti-malarial drugs and specialty chemicals can now be produced inexpensively. Using Golden Gate assembly designed for BioBrick compatibility, we have developed two tools to improve yeast expression of non-native pathways. First, we engineered an ethanol control system that reduces yeast's endogenous stress response and diverts more cellular resources towards product synthesis. Second, we constructed a light-induced system for instantaneous gain- and loss-of-function at the protein level. These tools will allow engineers to optimize heterologous pathways by monitoring toxic intermediates or regulating flux in a controllable, time-dependent manner. We hope our ideas will shape the future of industrial cell-based manufacturing.

Team McMaster-Ontario: Mutans Murder Machine: A Targeted Treatment for Dental Cavities

The oral microbiome comprises a variety of both commensal and detrimental microbes. Oral health requires a fine balance of these organisms, which can be upset by broad-spectrum antibiotics. Our experiments involved the use of the peptide based antibiotic actagardine, known to have activity against Streptococci. Homologs of actagardine were also incorporated into the designed gene cluster, in an effort to develop novel antimicrobial compounds. We sought to use synthetic biology tools to create a targeting system for an antibiotic to kill only Streptococcus mutans, the primary causative agent of dental cavities. A combinatorial approach applying phage display and heterologous expression of modified lantibiotics was applied to develop this targeted S. mutans killing machine.

Team Michigan: Utilizing FimE and HBif Recombinases to Tightly Control a Bi-directional and Inheritable Switch

Recombinases can be used to create responsive, low background, boolean genetic circuits in biological systems. Further, it is theoretically possible to create complex control circuits using combinations of invertible DNA sequences. We utilized the recombinase HbiF to augment an existing system in Escherichia coli that relied on the recombinase FimE. A burst of induced, low level expression of one recombinase will invert the promoter flanked by the recombinase binding sites, triggering a switch from strong expression of one set of proteins to another set. Induced expression of the second recombinase will revert the promoter to its original orientation, triggering the original set of protein expression. The inversion will be sustained across cell divisions with little leaky protein expression and negligible performance degradation after repeated inversions. This is a heritable, binary memory system and can be used as a component in more complex systems.

Team Minnesota: Construction of organism-extrinsic synthetic pathways for the biosynthesis of beneficial natural compounds.

Team Minnesota aims to change the paradigm regarding the synthesis of natural products. Rather than depending upon slow and expensive chemical synthesis, our team has developed two cohesive platforms utilizing the BioBrick strategy and synthetic biology to produce compounds for public health and nutrition using industrially-relevant microorganisms. First, we constructed and optimized designer pathways, using the BioBrick platform, for the production of a suite of sunscreen-like compounds that inhibit the effects of ultraviolet radiation, which we hope to incorporate into bacteria found on the skin microbiome for prolong ultraviolet protection. We also developed a novel and modular BioBrick backbone for expression in Saccharomyces cerevisiae. For demonstration, we constructed a caffeine production pathway in this backbone, generating a yeast strain which produces caffeine. Both of these projects gain impact from their synergistic application of synthetic biology and bioengineering for products that apply to real-world situations for researchers and the general public.

Team Missouri Miners: Adjustable Multi-Enzyme to Cell Surface Anchoring Protein

There are a plethora of enzymes that occur in the natural world which perform reactions that could be immensely useful to humans. Unfortunately, the efficiency of some of these reactions may render their applications logistically unrealistic. The cellulosome scaffolding protein produced by Clostridium thermocellum has been shown to significantly increase the efficiency of cellulose degradation. The scaffolding protein can be reduced in size and adapted for the cell surface of Escherichia coli. Different cohesion sites on the new cell surface display protein can also be introduced to allow for attachment of desired enzymes. Future applications would include producing a collection of distinct versions of the scaffolding protein for unique arrangements and concentrations of enzymes, enabling construction of an extra-cellular assembly line for a variety of multi-enzymatic reactions. This would lay the foundation for making previously infeasible applications of reactions possible through increased efficiency.

Team MIT: RNA Strand Displacement for Sensing, Information Processing, and Actuation in Mammalian Cells

The complexity of engineered genetic circuits in eukaryotic systems is limited by the availability of regulatory components and further hampered by the inability to assemble and deliver large DNA constructs. In contrast, in vitro synthetic DNA circuits utilizing strand displacement have demonstrated complex digital logic with reliable and scalable behaviors in a small base-pair footprint. The possible adaption of such circuits into cellular environments can amplify the scale and complexity of biological circuits, broadening synthetic biology's application space. Our project leverages strand displacement to create a process technology that supports multi-input sensing, sophisticated information processing, and precisely-regulated actuation in mammalian cells. We construct RNA strand displacement circuits that detect endogenous mRNA, perform digital logic computation, and output desired proteins through programmable RNA interference pathways. We envision in-vivo RNA strand displacement as a new foundation for scaling up complexity in engineered biological systems, with applications in biosynthesis, biomedical diagnostics and therapeutics.

Team Northwestern: The Phytastic Probiotic: Increasing the Bioavailability of Nutrients in the Digestive System

Iron deficiency affects 2 billion people - or over 30% of the world's population – and can lead to anemia, ill health, and even death. Surprisingly, this deficiency is typically not due to a lack of dietary iron, but rather

due to low bio-availability, and thus poor absorption of iron. Phytic acid is a prevalent chelator of iron and other nutrients in food. Our mission is to build a system that breaks down phytic acid in the digestive system, releasing bound iron for the body to absorb. Our solution comprises two engineered components: a module that constitutively produces phytase to break down phytic acid and a pH-sensitive module that causes cells to lyse and release the accumulated phytase in the stomach. If successful, our strain would be a low-cost sustainable solution to preventing iron deficiency without the need for constant supplies of iron supplements.

Team NYC Hunter: Developing a bacterial XOR gate and hash function.

Our team set out to construct a functional XOR gate in e.coli by building on and improving previous designs based on signalling systems. Our ultimate goal was to engineer a system of bacterial logic gates that could be used in combination for higher order computations like hash functions. We approached issues in promoter design and using bioinformatics and devised a plan for site mutagensis to modify promoter activity. We considered and worked with several promoters and considered different approaches to integrating together series of gate components.

Team NYC Hunter Software: Modeling and buliding computational circuits from biological logic gates

Our first goal was to make an abstract software model and kinetic reaction model of the bacterial XOR gate we designed on our wet lab team. We used python/pygame and BioNetGen software to approach these problems. We also wanted to demonstrate how biological logic gates could be assembled through hypothetical manufacturing processes into more complex computational circuits including hash function based algorithms. We wanted to model and help design various spatially arranged computational elements. Bacteria use signalling pathways ways that are highly dependent on spatial considerations and computational flexibility is likewise highly spatially dependent.

Team NYU Gallatin: Aseatobacter (not your mother's chair)

Aseatobacter (A-Seat-Obacter) began as a vision; a vision of fully formed seats and chairs emerging from giant vats of colorful bioengineered bacteria. Acetobacter xylinum naturally produces mats of cellulose that can be used for a variety of purposes. We wanted to create a broader spectrum of materials, so we altered the properties of the cellulose mats by engineering Acetobacter to express enzymes that synthesize N-acetyl glucosamine, a subunit of chitin. The result is a chitin-cellulose copolymer with unique properties. We have also engineered colors into the mats, and demonstrated their use in modern architectural design.

Team Penn: pDAWN Of A New Era: Engineering Bacterial Therapeutics

We are engineering E. coli bacteria which may enable highly targeted eradication of human epidermal growth factor receptor 2 (HER2) overexpressing cancer cells. Upon binding to HER2 overexpressing cells, bacterial cytotoxicity can be triggered with spatial and temporal precision by illumination with blue light, which activates overexpression and secretion of Cytolysin A (ClyA) under the control of the pDawn transcriptional module. Furthermore, we are also investigating the feasibility of engineering bacterial biofilms that can act as antimicrobial surfaces. We are engineering E. coli bacteria to form non-pathogenic biofilms that express bacteriolytic proteinscapable of inhibiting the formation of pathogenic biofilms that are potential sources of hospital acquired infections. These cells carry the a gene encoding lysostaphin (lss), which selectively destroys the cell walls of Streptococcus bacteria, a common pathogen in many hospital settings.

Team Penn State: Questioning the Central Dogma of Molecular Biology

The central dogma of molecular biology does not always accurately predict results acquired in the lab. A construct containing two adjacent start codons in different reading frames measures the E. coli DH10B ribosome's proclivity for either one start codon or the other through a fluorescent protein reporter in each respective reading frame. Variations in RBS translation initiation rates and length between start codons provide additional data. Repeating sequences of non-degenerative threonine and alanine codons measure codon bias and determine E. coli DH10B's ability to translate varying lengths of identical codons through the use of mCherry and GFP reporters. Promoters are tested for bidirectionality in protein translation by measuring the rate of forward expression through downstream GFP or reverse expression through upstream RFP. A ratio of fluorescence characterizes each tested promoter.

Team Purdue: Synthetic Biology in the Community: Accessible Biotechnology for Water Treatment

Polluted water is the world's largest health risk, killing over three million people a year. Our project focused on enhancing biofilms used in water treatment. We designed a system to accelerate the adhesion of bacteria to surfaces. On biofilm aggregation, expression of silica-binding peptides works to build silica matrices on the surface of cells. These matrices act as a mechanical filter for large particles and a barrier between the biofilm and fluid shear, decreasing dislodgment of organisms that could otherwise lead to fouling. We envision these improved biofilms being used in municipal water treatment to help recycle and filter home waste water streams, a concept we implemented in lab-scale membrane bioreactors. Bringing awareness of synthetic biology closer to our community, we initiated a community bio-lab and a Girl Scout biotechnology badge. Ultimately, we hope to take synthetic biology from benchtop to park bench.

Team Queens Canada: ChimeriQ x SynthetiQ: Chimeric flagella scaffold enhancing bioremediation and manufacturing, presented with dance!

This year, Queen's iGEM team is using flagella to host heterologous proteins that will result in thousands of useful enzymes organized in an extensive scaffold, with the benefits of extracellular synthesis, degradation and arrangement. The fliC (flagellin) protein is known to spontaneously polymerizes to form the length of flagella in E.coli. By replacing the variable D3 domain of the fliC protein with proteins for binding, degradation, adhesion, and synthesis, we can increase the efficiency of bioremediation and biosynthesis, and facilitate the collection of products in situ or ex situ. This year we will also introduce dance as a presentation form and part of our human practices project. Known as SynthetiQ, we will be the first group ever to use dance to replace powerpoint slides at a research conference.

Team RHIT: Checkmate: A Rapid Yeast Mating Type Detector

Easily manipulated genetics make the yeast Saccharomyces cerevisiae a versatile and widely used model eukaryote. To progress, researchers must often determine the mating type of haploid strains, which typically takes days. The goal of our project is to reduce that time to hours. So we designed a novel promoter harboring Ste12 and LexA binding sequences and placed it upstream of an ORF encoding a red fluorescent reporter fused to LexA binding and VP64 activator domains. Others have shown that this fusion protein induces its own expression from a LexA promoter. We propose that Ste12, activated in the pheromone response pathway, will bind the hybrid promoterand induce expression of the fusion protein, which will amplify and maintain its own expression. Therefore, when mating pheromone receptors on a haploid harboring this latch-type circuit are bound and activated, the cell will fluoresce and function as a rapid mating type detector.

Team Rutgers: Biofuels in Bacteria and Genetic Circuits

The current fossil fuel-dependent economy drives a demand for sustainable energy resources. Although much effort has gone into the production of ethanol, other biofuels, such as butanol, are superior. Butanol has a higher energy content, is less volatile, and is safer to use than ethanol. To develop strains of bacteria that produce high levels of 1-butanol we have introduced the genes coding for a biochemical pathway from Clostridium acetobutylicum into a mutant E. coli strain that produces a high level of NADH. The combination of these chemical pathways is predicted to increase the level of butanol production. Our second project, the Bacterial Etch-a-Sketch, features a complex network of gene expression and repression that enables a lawn of bacteria to respond to 470nm light. This task presents many engineering challenges: the bacteria need to be sensitive enough to respond to a laser pulse, yet selective enough to use in ambient lighting.

Team Toronto: Extracellular secretion of Aspergillus phytase and constitutive expression of Rhagium antifreeze: Genetically Engineering Super-Plants

This year's project is two-fold, and it involves engineering Arabidopsis thaliana with two constructs that would be important proof of concepts for further studies of feasibility in crops. The first construct would allow for extracellular secretion of Aspergillus phytase from Arabidopsis roots allowing the plants to utilize the accumulated forms of soil organic phosphorus (primarily, phytate), which otherwise would not be available to the plant. The second construct, building on Yale's 2011 project, aims to increase the range of tolerance to low temperature stress in A.thaliana by incorporating a Rhagium inquisitor antifreeze protein and ensuring it is constitutively expressed in the plant.

Team UConn: VitaYeast - Transformation of S.cerevisiae for the production of Vitamin D3

S. cerevisiae (yeast) is a commonly used organism in food preparation processes around the world. This fungi naturally produces Vitamin D2 - which is less potent in terms of biological benefit for humans than D3. In the past, Yeast has been modified to increase production of D2 but no attempt on converting it for D3 production has been made. Our team aims to insert the necessary genes to allow for simultaneous D2 and D3 synthesis when the modified Yeast is exposed to Ultraviolet light. If successful, this new strain of Yeast could provide an extremely cheap and efficient alternative to current Vitamin D supplementation.

Team UGA-Georgia: Genetically Modifying Methanococcus maripaludis into an Air Freshener Producer

The methane-producing archaeon Methanococcus maripaludis was synthetically modified via expression of a gene to produce geraniol synthase (GS) from Ocimum basilicum. The GS gene was cloned on a methanococcal shuttle vector downstream of a strong promoter, and transformation of methanococci was confirmed via PCR. GS catalyzes the conversion of geranyl diphosphate, an intermediate in biosynthesis of the isoprenoid lipids of these archaea, to geraniol, the major aromatic compound in roses and a potential biofuel. Small amounts of geraniol biosynthesis were detected in cultures of the transformants by GC/MS. Methanogens are archaea that live in the guts of humans and animals and are responsible for the methane content of flatulence. Thus, this project could convert personal polluters into an air freshener. Because of the prevalence of flatulence among the aging population of the United States, the potential impact of this research is very high.

Team UIUC-Illinois: PUF, The Magic RNA Binding Protein: Programmable RNA Binding Protein with Custom Functions

RNA has characteristics that are important in human gene expression (i.e. alternative splicing of mRNA, noncoding RNA). Therefore, a modular RNA binding protein is an invaluable tool for gene regulation. The PUF domain of human PUM1 gene contains eight tandem repeats, each recognizing one of the four nucleotide bases. In theory, a PUF protein can be programmed to recognize any 8-nt ssRNA sequence. Here we demonstrate that PUF can be tethered with other functional domains for applications in E. Coli. Specifically, we show that a PUF/endonuclease fusion protein acts as RNA scissors, silencing gene expression through site specific mRNA cleavage. PUF was also tethered to split GFP to test its ability to co-localize proteins using a RNA scaffold. PUF biobricks offer a wide range of possible functions including gene expressionmodulation and scaffolding of metabolic pathways.

Team uOttawa CA: A Comprehensive Approach to Universal Network Design

In order for synthetic biology to advance as a field it must be made simpler for large gene networks to be designed and built. The goal of the uOttawa team this year was to tackle this problem by characterizing parts and improving gene assembly by taking advantage of S. Cerevisiae's ability to exist in both the haploid and diploid form. Inducible systems were used to externally manipulate gene expression and allowed for a fine-tuning of the designed networks. A shuttle vector was designed that will take advantage of the assembly abilities of yeast and the replicative abilities of bacteria. To expand the Biobrick registry we will be submitting new inducible activators, promotersand our shuttle vector.

Team Virginia: Genetically engineered bacteriophage for diagnosis of whooping cough

Whooping cough, the infectious respiratory disease caused by Bordetella pertussis, is diagnosed in tens of millions of people and results in almost 300,000 deaths globally each year. Low-income and unvaccinated individuals as well as infants are especially susceptible. Current diagnostic procedures are complicated, costly, and can take up to a week, by which time the disease may have progressed or spread. The enormous impact of this disease urgently motivates the development of a faster, cheaper, and more reliable diagnostic test. Our epidemiology models suggest that earlier diagnosis could drastically reduce the incidence and impact of the disease. We propose an engineered bacteriophage diagnostic system for rapid clinical detection of pertussis. We first engineered T7 bacteriophage to demonstrate this approach in E. coli. Our modular diagnostic approach can be applied to the high-sensitivity detection of other bacteria.

Team Waterloo: In Vivo Protein Fusion Assembly Using Self Excising Ribozyme

Continuing from last year, the Waterloo iGEM team has repeated the project in the hopes to finalize the project. Introns, self-excising ribozymes, can become a useful tool to create in vivo protein fusions of BioBrick parts. To make this possible, intron sequences are used to flank non-protein parts embedded in coding sequences. An intronsequence with an embedded recombination site is capable of in vivo insertion of a compatible protein fusion part. As an example, a GFP-fusion was created with an intervening lox site that is removed from the final protein using the intron to form a fully functional GFP protein.

Team Wellesley HCI: Enhancing Bio-Design with Touch-Based Human-Computer Interaction

Synthetic biology will require a multidisciplinary, collaborative design environment in order to engineer the complex biological systems of the future. Our team created a collection of software tools, which address specific technical synthetic biology challenges while advancing the way in which users interact with computing environments. We also utilize advances in human-computer interaction (HCI) to communicate

core concepts of synthetic biology to the public. Synbio Search is an online tool that generates data sheets for biological parts by aggregating data from various publicly available resources. MoClo Planner visualizes the Golden Gate Modular Cloning process and facilitates hierarchical design and production of multi-gene constructs. SynFlo is an interactive installation that utilizes tangible and tabletop HCI techniques to illustrate core concepts of synthetic biology in outreach programs. The application of novel HCI techniques to synthetic biology fosters the development of more effective, collaborative, and intuitive software tools, which enhance the design-build-test methodology.

Team Wisconsin-Madison: A tool to evaluate the translation of heterologous genes in Eschericia coli

In synthetic biology, a powerful method for the production of novel metabolites is the expression of heterologous genes in Escherichia coli. A common challenge when using non-native genes in metabolic engineering is determining if they are being properly expressed. To address this issue, we have constructed a BioFusion compatible system for testing the translation of a gene of interest. This system couples the translation of the target gene to a fluorescent reporter gene. Fluorescence will only be detected when the target gene is entirely translated. This construct enables synthetic biologists to quickly determine if a gene is being expressed without the need for costly antibodies or analytical instruments (e.g. mass spectrometry). Currently, we are utilizing this cassette to troubleshoot the expression of limonene synthase, an enzyme that catalyzes the production of limonene, a monoterpene with potential as a renewable jet fuel.</p>

Team WLC-Milwaukee: iDifferentiate: The SAVE (Selection for Atrial and Ventricular cardiomyocytes through Engineering) Assay

A clear understanding of stem cell differentiation pathways is important to advance regenerative medicine therapies using stem cells. An incomplete knowledge base of developmental mechanisms impedes stem cell research and innovation. The iDifferentiate system is a genetic engineering platform that may be used to elucidate differentiation pathways of any cell type for which there is a known lineage-specific cis-regulatory element. To demonstrate this system we developed the SAVE Assay, which uses visual cues to indicate the overall quantity and relative percentage of atrial and ventricular cardiomyocytes amongst differentiated stem cells. The assay uses a dual plasmid system that selects for successfully transformed stem cells via neomycin and puromycin resistance along with fluorescent reporter genes regulated by atrial and ventricular promoters. Altering the basic protocol by using different reagents and induction factors will allow scientists to quickly and accurately determine differentiation pathways of two or more related cell types.

Team Yale: Multiplex Automated Genome Engineering (MAGE) in Naturally Competent Bacteria: An Alternative to Cloning

Traditional plasmid-based cloning methods are limited by tedious protocols that make targeted genetic changes within the cell. Multiplex Automated Genome Engineering (MAGE), an alternative technique for rapidly generating genomic diversity using the recombination ability of the λ-phage ssDNA-binding protein β, has to date only been introduced in E. coli. These cells must be transformed via electroporation for each MAGE cycle to facilitate efficient uptake of mutagenic oligonucleotides, but this process kills a significant portion of otherwise viable cells. For our project, we designed and created a universal test cassette system to introduce MAGE to diverse bacteria as well as a library of β homologs for testing. Finally, we optimized the technique for the naturally competent organisms B. subtilis and A. baylyi to eliminate the costly

electroporation step and developed computational algorithms to aid in the design and prediction of MAGE experiments.

AMERICAS WEST

Team Alberta: Towards a microbial color wheel: spatial control of gene expression

As a young team composed of high schoolers and junior undergraduates, we selected a project aimed at giving ourselves a firm understanding in the fundamentals of genetic engineering and control. The end goal of our project was to create spatial color patterns using bacteria, such as a color wheel and a rainbow, that required control over several color outputs in response to spatial gradients of chemical inducers. Colour gradients were achieved using a high-copy plasmid that contained both an inducible colour gene and its corresponding repressor. Colour banding was achieved by a novel means of adjusting gene expression through plasmid copy number control that varied from 0 to ~1000 copies/cell as a function of inducer concentration. Note that the rapid loss of plasmid that occurs in the absence of inducer also constitutes a novel and extremely effective safety switch for genetically engineered organisms which might enter the environment.

Team Arizona State: Chimerasensors

Diarrheic pathogens including E.coli O157:H7 serotype, campylobacter, shigella, and salmonella often contaminate drinking water supplies in developing nations and are responsible for approximately 1.5 million worldwide annual deaths. Current technologies for detection of bacteria include DNA hybridization FRET signaling, electrical detection via immobilized antimicrobial peptides, and PCR amplification followed by gel visualization. Our method of bacterial detection fills a niche in biosensor technology. Our design implies lower costs, higher portability, and a more rapid signal output than most bacterial biosensors. Additionally, our interchangeable DNA probe confers modularity, allowing for a range of bacterial detection. Using a split beta-galactosidase complementation assay, we have designed three unique chimeric proteins that recognize and bind to specific pathogenic markers and create a functioning beta-galactosidase enzyme. This functioning enzyme unit then cleaves x-gal and produces a colorimetric output signal. Our research demonstrates success in initial stages of chimeric protein assembly.

Team Austin Texas: Caffeinated coli: An addicted E. coli for biosensing and bioremediation of methylxanthines

The widespread use of caffeine (1,3,7–trimethylxanthine) and other methylxanthines in beverages and pharmaceuticals has led to significant environmental pollution. We have developed a novel detection and bioremediation strategy for caffeine contamination by refactoring the methylxanthine degradation operon native to Pseudomonas putida CBB5. Escherichia coli cells with this synthetic operon degrade caffeine by N-demethylation to the guanine precursor, xanthine. Cells deficient in guanine biosynthesis and containing our refactored operon were addicted to caffeine; their growth density was limited by the availability of caffeine. Remarkably, they were able to sense the caffeine content of several common beverages. Characterization of nearby genes in the P. putida operon revealed a potential methylxanthine regulatory system for use in biological circuit design. The synthetic N-demethylation operon could be useful for cheaply producing pharmaceuticals or precursor molecules and for detoxifying waste so that it can be recycled into animal feed and biofuels.

Team Berkeley: MiCodes - enabling library screens with microscopy by connecting genotypes to observable phenotypes

Many applications in synthetic biology demand precise control over subcellular localization, cell morphology, motility, and other such phenotypes that are only observable via microscopy. At present, engineering these properties is challenging due in large part to the inherent throughput limitation imposed by microscopy. We have developed a strategy that enables high-throughput library screening with microscopy by coupling a unique fluorescence signature with each genotype present in a library. These MiCodes (microscopy barcodes) are generated by targeting combinations of fluorophores to several organelles within yeast, and they eliminate the need to isolate and observe clonal populations separately. MiCodes can potentially scale to library sizes of 10^6 or more, and their analysis can be largely automated using existing image processing software. As a proof of principle, we applied MiCodes to the problem of finding unique pairs of protein-protein interaction parts.

Team British Columbia: Synthetic Syntrophy

The field of synthetic biology has seen the development of many biological monocultures capable of performing a wide range of novel functions. In contrast to this current paradigm, microbes have naturally evolved to survive as members of dynamic communities with distributed metabolism. This “divide and conquer” strategy allows the community to perform more complicated metabolic processing than would be possible in single microorganisms while being resilient to environmental changes. Despite very recent proof of concepts in developing model microbial consortia, or synthetic ecology, questions remain as to whether complex metabolic pathways can be engineered in context of microbial populations. The 2012 University of British Columbia iGEM team sets a precedent by engineering a tunable consortium with a distributed 4S desulfurization pathway for increased efficiency in the removal of organosulfurs in heavy oils and bitumen resources.

Team BYUProvo: E. colin: A Two-Circuit System for Early Colon Cancer Detection

In the initial stages of colon cancer, malignant cells give off excess heat, reactive oxygen species (ROS), and lactate. Last year, the BYU iGEM team genetically engineered E. coli to detect heat or ROS. This year we developed E. coli capable of simultaneously sensing lactate, heat and ROS, implemented a novel Cre-Lox system, and constructed a library of thermosensors. Our project uses two circuits, each with a unique reporter. The first circuit contains a RNA thermosensor driven by a ROS-inducible promoter, allowing expression of Cre recombinase when both heat and ROS are present. Although heat is transient, Cre ensures continued expression of the first reporter gene. The second circuit contains a periplasmic lactate sensor coupled to a second reporter. Finally, we have evolved a library of thermosensors that work in a narrow physiological range. Together, this two-circuit system may allow accurate and specific detection of early colon cancer cells.

Team Calgary: Detect and Destroy: Engineering FRED and OSCAR

Tailings ponds are concentrated pools of toxic and corrosive compounds resulting from oil and mining extraction. The Calgary iGEM team aims to alleviate this potential environmental and economic threat by developing a detection and bioremediation system for these toxins: FRED (Functional, Robust Electrochemical Detector) and OSCAR (Optimized System for Carboxylic Acid Remediation). FRED detects multiple compounds within one sample using an electrochemical output. We created an open-source hardware and software platform to be used as a biosensor prototype. For OSCAR, we designed and modeled a bioreactor to remove impurities (sulfur, nitrogen, and carboxylic acids) from tailings ponds.

Known degradative microbial pathways were combined with unique engineering solutions in a bioreactor model. Furthermore, we developed ‘Ribo-kill-switches’ to prevent antibiotic resistance and disturbing natural flora. Overall, this system aims to detect and convert toxins into clean hydrocarbons in an economical, safe, and self-contained process.

Team Caltech: Biofuels and BioFilms: Optimizing Biofuel Production and Animating Bacteria

We aimed to develop a system capable of converting recalcitrant biopolymers into substrates for biofuel synthesis. From pond water, we isolated bacteria capable of metabolizing lignin and polystyrene. We attempted to identify the degradation genes and express them in Escherichia coli. In parallel, we worked to optimize ethanol production in E. coli by diverting electron flow from normal cell metabolism to alcohol fermentation. We also explored using Zymomonas mobilis, a more efficient ethanol producer, as an expression host for our biodegradation enzymes. </p> We also aimed to improve the spatial and temporal control of bacterial behavior. We modified the coliroid system to produce a degradable output, allowing a bacterial image to change over time. With this animated coliroid, we worked to create an interface between digital animation and biology using a simple light projector.

Team Colorado State: More Than a Great Beer, a Gluten-Free Beer

Fort Collins is a major brewing hub, so it was natural for our team to gravitate toward a beer-related project. Knowing full well the problems caused by Celiac disease, and the affinity many others have for reducing gluten in their diets, we decided to design and create a yeast strain capable of both fermenting quality beer, and breaking down gluten. Our search for an enzyme capable of breaking down gluten and neutralizing its toxicity led us to the enzyme mutated by the 2011 UW IGEM team. The modified Kumamolisin-As has a maximal activity at a pH of 4 and would work well in the pH range of 5.2-5.5 found in beer. For expression in yeast we had to account for codon bias, and optimized the sequence so it could more easily be moved from a prokaryotic system to a eukaryotic one.

Team CU-Boulder: Inhibition of Quorum Sensing and Biofilm Degradation

The CU-Boulder team aims to manage quorum-sensing and the resulting biofilm that contributes to food rot and general bacterial contamination. We improved the characterization of the pre-existing AHLase, Aiia. Aiia inhibits quorum sensing which is proven to reduce bacterial viability. Additionally, we developed a construct for treatment and submitted a new part to the registry, NucB, which is a nuclease that targets extracellular DNA necessary for biofilm. Future applications of this project include incorporation into plants to naturally prevent food rot as well as the possible development of a probiotic for human consumption to prevent the pathogenesis of bacteriawhose toxicity is dependent on quorum sensing. In addition to this project, we isolated and submitted the 6 essential genes in the Lux brick (LuxA,B,C,D,E, and G) from its original source, Vibrio Fisheri, because many teams have been unsuccessful with the previously submitted Lux parts.

Team Harvey Mudd: Scalable, Orthogonal Buffer Amplifier

In digital electronics, a buffer amplifier is used to filter noise, isolate parts of a circuit, and make low signals lower/high signals higher. The ability to make many orthogonal buffer amps is critical to scaling up digital information processing in vivo. For our project, we first provide two mathematical proofs: (1) the buffer amp is 'equivalent' to a bistable circuit, in that the ability to create one implies the ability to create the other, and (2) inhibitors with first-order binding cannot allow bistability (without other elements). This means that

methods such as TALORs and many sRNA strategies cannot build a buffer amp. Thus, we test a new method: sequence-specific formation of a DNA-DNA-RNA triple helix to block a promoter. By using a dimer for the RNA, we can achieve second-order binding, allowing us to build sequence-specific buffer amps.

Team Lethbridge: CAB Extraction: A Synthetic Biology Approach to Microbial Enhanced Oil Recovery Lethbridge 2012 iGEM

Increasing global oil demands require innovative technologies for the extraction of unconventional oil sources such as those found in Alberta's Carbonate Triangle. Microbial enhanced oil recovery (MEOR) has been utilized across the world to increase the productivity of these difficult resources. Using a synthetic biology approach, we have designed the CAB (CO_2, acetic acid, biosurfactant) extraction method for a modified MEOR to extract carbonate oil deposits. CAB extraction will utilize natural carbon fixation machinery in the cyanobacteria Synechococcus elongatus to convert CO_2 into sugars to fuel acetic acid and biosurfactant production in Escherichia coli. Acetic acid and biosurfactant applied to carbonate rock will facilitate and enhance extraction. The use of carbon fixation to feed downstream systems can be tailored for many applications requiring inexpensive methods for fueling biological systems, while simultaneously reducing greenhouse gas emissions. CAB extraction provides an alternative, inexpensive, and environmentally sustainable MEOR method for carbonate oil deposits.

Team Nevada: iRICE: A Novel, Non-GM Approach to Biofortification of Rice

Even though white rice is a major source of calories for over half the world's population, it is a poor source of nutrients. While rice can be fortified using vitamin powders, such approaches have had limited success because many vitamins are leeched away during the washing process prior to cooking. To address this problem, we have engineered proteins that will adhere nutrients to rice grains and prevent losses. These proteins contain a starch-binding domain that is fused to specific nutrient-binding domains. Because rice is composed mainly of starch, the starch-binding domain prevents nutrient leeching during washing. Upon cooking, the nutrient-binding domain denatures and releases the nutrients into the cooked rice. Supplementing rice with these fusion proteins will provide a novel, non-GMO approach to fortifying rice. Proteinswith a starch-binding domain connected to a vitamin B12-binding domain, a thiamine-binding domain, a lysine-rich protein, and a RFP have been created.

Team Stanford-Brown: The Transit of Synthetic Astrobiology

Astrobiology revolves around three central questions: "Where do we come from?", "Where are we going?", and "Are we alone?" The Stanford-Brown iGEM team explored synthetic biology's untapped potential to address these questions. To approach the second question, the Hell Cell subgroup developed BioBricks that allow a cell to survive harsh extraterrestrial conditions. Such a toolset could create a space-ready synthetic organism to perform useful functions off-world. For example, the Biomining branch attempted to engineer bacteria to recycle used electronics by degenerating silica and extracting metal ions in situ. The Venus Life subproject grappled with the third key astrobiological question by exploring Carl Sagan's theory that life could exist in Venusian clouds. To this end, Venus Life designed a cell-cycle reporter to test for growth in aerosol within an adapted Millikan apparatus. Through this triad of projects, Stanford-Brown iGEM aimed to illuminate synthetic biology's value as a tool for astrobiology.

Team UC Davis: Engineering Pathways for Polyethylene Terephthalate Degradation in E. coli

Current plastic recycling practices successfully reduce the accumulation of non-degradable waste in the

environment and landfills. However, they remain surprisingly expensive. Synthetic biology holds the potential to transform the recycling industry by altering the economics of waste processing. To this end, we are engineering a model organism, E. coli, to degrade polyethylene terephthalate (PET), a common plastic found in soda bottles, carpets, clothing, food packaging, and even space blankets. We engineer and express a gene originally found in leaf-branch compost encoding a cutinase enzyme whose product degrades PET into two products: ethylene glycol and terephthalic acid. Through rational and directed evolution of the E. coli chassis, we also create strains that utilize the breakdown product ethylene glycol as their sole carbon source.

Team UC-Merced: E. hydro Express: Streamlining Bacterial Production of Hydrogen Gas

To exploit the fermentative capabilities of Escherichia coli to produce hydrogen gas, we performed P1 transduction on strain FMJ39 from JW1228-1 to produce the desired triple mutant with the necessary metabolic flux to hydrogen production. In the fermentation process E. coli converts glucose into various intermediate states to generate energy. The transduction of the adhE knockout found in JW1228-2 to FMJ39 will produce a triple mutant with the following genes deleted: ldhA, pflB, and adhE. From these deletions insertions of mhpF, pyruvate decarboxylase, and ferredoxin oxidoreductase will result in a more direct metabolic line towards hydrogen production.

Team UCSF: Cell Mates: Engineering Metabolic Cooperation and Cellular Codependence

One major goal of synthetic biology is to use common chassis (E. coli, yeast) for the production of drugs and useful natural products. This practice often requires placing large enzymatic pathways into one cell. Production of the desired product is usually affected by increased metabolic burden or negative feedback on the cell. In nature, however, many organisms work symbiotically to accomplish a task and/or provide mutual benefits to one another. For the first part of our project, we have studied two systems to create cellular codependence in E. coli - using either pairs of auxotrophs or toxin/antitoxins. In the second part of our project, we split a model metabolic pathway (violacein production) between two separate strains. Our goal is to create a tunable system to control population ratio of strains in co-culture in order to maximize the yield of a product.

Team USC: E. musici: Facilitating communication between bacteria and researchers through song

We have created a method of communication with Escherichia coli by engineering a system that causes a predictable response to a controlled environmental stimulus. Many strains of E.coli possess flagella which are controlled by a key group of genetic factors for assembly and chemotactic control. Regulation of these genes can be harnessed by creating combinations of promoters and individual components of the flagella apparatus. By promoting the synthesis of E.coli flagella genes and flagella activity under various conditions, such as salt concentration, nitrate concentration, pH and temperature, we can measure changes in flagella rotation and frequency. This frequency can be translated into an audible range, which indicates the bacteria's distress and providing the researcher with a bacterial response to controlled growth conditions. Our system provides a new mechanism of bacterial communication with the researcher, through a spectrum of musical outputs. As such, we have named our system E. musici.

Team UT Dallas: Distributed cellular processing units: a synergistic approach to biological computing

The goal of the 2012 University of Texas at Dallas IGEM team is to redefine biological information processing using quorum signaling-based biological circuitry in bacteria. Quorum signaling allows bacteria to communicate with each other through the use of chemical signals. Bacteria use this form of signaling in nature to coordinate their behavior. Using three quorum signaling molecules we create unique connections between different populations of engineered bacteria and perform coordinated computing functions. We design and characterize standard and novel modules such as toggle switches, oscillators, signal propagators, and logic gates. As compared to engineering molecular circuitry in single populations, we aim to show that the synergistic approach to information processing leads to improved, scalable, and tunable operation.

Team Utah State: ArachniColi

Spider silk is the strongest known biomaterial, with a large variety of applications. These applications include artificial tendons and ligaments, biomedical sutures, athletic gear, parachute cords, air bags, and other yet discovered products which require a high tensile strength with amazing extendibility. Spiders however cannot be farmed because they are territorial and cannibalistic. Thus, an alternative to producing spider silk must be found. We aim to engineer spider silk genes into E. coli to produce this highly valuable product. Spider silk production in bacteria has been limited due to the highly repetitive nature of the spider silk amino acids in the protein. To overcome this obstacle we are using various synthetic biology techniques to boost spider silk protein production and increase cellular fitness. After successful production, spider silk protein is artificially spun into usable fibers and tested for physical properties.

Team Washington: Apptogenetics: Purpose-Built Computational Applications for Biological Research

Biological systems must often be painstakingly tuned before they will efficiently produce drugs or biofuels, degrade chemicals, or perform other useful tasks. Our team implemented broadly applicable methods to optimize biological systems through directed evolution, light-regulated gene expression, and computer aided protein design. We characterized light-inducible protein expression systems for multichromatic tuning of biological pathways. To provide an inexpensive method for tuning gene expression with light, we developed a tablet application that is freely available. We also used computer-aided design to develop proteins that more effectively bind isotypes of the flu proteinHemagglutinin. Finally, we implemented a continuous culture device (turbidostat) in order to apply directed evolution to the metabolism of ethylene glycol in E. coli. We have termed the research conducted this year “Apptogenetics” as all projects utilize purpose-built computational applications for biological research.

ASIA

Team BAU-Indonesia: Plastic Terminator

Indonesia is known as the 4th highest population densities around the world. Nowadays, 1.5 million tons/year from human activity which is used in the world is PET. PET is a thermoplastic polymer resin, not easily degraded naturally. Based on this background, the BAU-Indonesia team designs a plasmid which is contains of encoding cutinase degrading enzymes of producing PET. The early stage of this project was done by the preservation of plastic waste bacteria from landfills at Galuga. The bacteria were cultured in liquid media which is contained yeast extract powder and PET enrichment. The result of this preservation

will be followed by the isolation of DNA and PCR with Cutinase F primer'ACGCGCCGGGCGTCACCGAGCA'3 and R 5'ACGCGTCGTGCCGTCAGGGCCA'3. Cutinase gen that were amplified will be inserted to plasmidpSB1C3. The recombinant plasmid which contained the cutinase gene will be introduced into E. coli. Finally it will be used as PET biodegradator product.

Team CBNU-Korea: BUGS(Brick and Unique minimal Genome Software)

We have developed two distinct software tools. The first tool, MG-designer, is functionally divided into designer and viewer. The viewer shows the information of genomes in both linear and circular form. So it is easier for users to understand the characteristic of genomes. By the designer, user can design minimal genomes by essential geneswhich are analogized by our team in this year. The minimal genome can be designed depending on characteristics of species by inserting the function of genes into particular locations. With the second tool, brick-designer, user can design new bio-bricks. It is also able to synthesize bricks by using the bricks registered in partsregistry. User can also utilize bricks he just designed. We tried to enhance software potability by enabling the bricks to save as Genbank and SBOL types. Brick also can be saved as picture file so that it is helpful in the Wiki implementation.

Team CD-SCU-CHINA: The construction of engineering E.coli for eliminate hydrocarbone pollution

The proccess of our work consists of three parts including the sensing system, emulsification system and the degradation system.the emulsification system invovles one gene ,oprf/omp ,which is found the prominent constituent for emulsification of oil.and the the sensing system is about alkane-sensitive. we use the Alks ,a kind of transcription factor which can bind to the alkane.when the binding complex is generated, the E.coli will turn on a subset of gene for degradating alkane.The last system is about degradation of alkane,which involves of two kinds of enzymes ,the P450 for degradation of medium chain alkane and alkb2 for short chain alkane.The purpose of this work is to achieve the function of degradating oil leaking in the sea and eliminate the pollution to the environment.

Team Ehime-Japan: E.colingual!

We are trying to realize three projects below.

E.co-mail: We created a mailing system with E.coli and an optical fiber.

E.co-Domino: E.co-Domino is domino toppling. We tried to make a timer and a firework by using it.

E.colingual: 'E.colingual' is a tool to know feelings of E.coli.

Our goal is to make E.colingual. E.co-mail functions as a connection part and E.co-Domino is the screen of E.colingual. In addition, we also use quorum sensing system in order to construct E.colingual. Light sensor genes from cyanobacteria are used for E.co-mail and E.co-Domino.

Team Fudan D: Telomere related construction of programmable time switch in yeast

For this year's iGEM competition we create a programmable time switch in yeast(Saccharomyces cerevisiae) employing the counting mechanism of telemere. The time switch we made counts the replication number of a single yeast cell, and triggers the activation of a certain gene (death gene, for example) when the replication number of the cell reaches the pre-set level. The work is an attempt to use the special quality of telemere and the end of chromosomes in the construction of synthetic biology device.

Our system utilizes a whole new mechanism that is seldom used in cell counting device, and it enables us to delay the expression of a certain gene for generations. And by control the life span of a certain cell, it sheds new light on the biosafety concerns and can be used for fields such as the protection of intellectual property rights.

Team Fudan Lux: Biowave | Nano-tubular Highways | Labcloud

Using light as messager is rarely seen in the biological system. In project BIOWAVE, we want to create such a light driven feedback system including artificial light sensor and bioiluminescence. With the properties of feedback system and time-lapse of gene expression,colony could form a detectable wave like pattern in a macroscopic level. Project Nano-tubular Highways is about constructing a brand-new biological model using a recently discovered cellular structure termed Tunneling Nanotubes(TNT) and bacteria with green fluorescence protein. Studying the distribution of the bacteria which could transport through the TNT and its pattern format is helpful for the optimal model problem. Project LabCloud aims to provide a mobile app for iGEMers share their experiments, ideas, files and others in and between teams. It will also provide group's shared calendar, instruments management and other powerful functions to help iGEMers' cooperation. At last, it has the Push Notification to ensure communication in time.

Team HIT-Harbin: Staphylococcus aureus Monitor

Staphylococcus aureus infections are major causes of morbidity and mortality in community and hospital settings. Since bacterial sensors are attracting more and more biologists' attention owing to its' specific, fast and accurate detecting, we plan to construct a E.coli biofilm consisting of two different engineered populations, which are designed to detect and eradicate S.aureus, respectively. The two engineered populations communicate with each other by AHL signal transduction. We hope that compartmentalization of functions can lessen metabolism load and cross-reactions interfere, and achieve the assembly of different functions in bacterial level. The whole system comprises sensing, killing and biofilm formation devices. </br>Detecting device: to detect the existence of S.aureus through sensing the AIPs secreted only from S.aureus.</br> Killing device: to eradicate S.aureus through the production and release of lysostaphin.</br> Biofilm formation device: to enhance biofilm formation by over-expression of yddV, a di-guanylate cyclase, which catalyzes GTP into c-di-GMP.

Team HKU HongKong: Inhibition of biofilm formation with engineered Escherichia coli

HKU's iGEM team aims to introduce an acyl homoerine lactone (AHL)-degrading genetic system into the non-biolfilm-forming and non-virulent BL21 Escherichia coli strain. PvdQ, an enzyme naturally produced by Pseudomonas aeruginosa, is an acylase that functions to degrade long chain AHLs that bacteria like Pseudomonas putida or aeruginosa itself utilize for biofilm formation. Biofilms are population density-dependent structures formed by quorum sensing bacteria that produce and secrete auto-inducers, which signal selective gene transcription. These signaling molecules, namely the AHLs, are responsible for most bacterial pathogenicity including the opportunistic respiratory infections caused by P.aeuroginosa in immunocompromised patients.

Team HKUST-Hong Kong: B. hercules---The Terminator of Colon Cancer

The dispersal of toxic anti-tumor chemicals in the circulatory system during conventional cancer treatment prompts us to consider the need of alternative cancer therapies. In an effort to combat with colorectal carcinoma, we aim to use genetically modified Bacillus subtilis to execute targeted drug delivery to cancer cells in the digestive tract, offering an advantage of generating minimal adverse effect on normal colon

epithelial cells. Targeting is achieved by expressing RPMrel, a colon tumor specific binding peptide, on the cell wall using a LytC cell wall binding system. The anti-tumor cytokine, bone morphogenetic protein 2 (BMP-2), is synthesized and secreted out from the bacteria with the help of a signaling peptide fused to the protein. To control the timing and amount of BMP2 release, two regulatory systems, xylose-inducible system and ydcE/ydcD toxin-antitoxin system are introduced to minimize the harmful effect from BMP2 overdose.

Team HokkaidoU Japan: Bio-capsule E. coli - E. coli bio-capsules that can accumulate bio-plastic in it-

We designed two modules to make “Bio-capsule E. coli' that accumulates bio-plastic. First module is to form bio-capsule by aggregation, using cell-cell interactive protein“Ag43” located on the surface of E. coli. Aggregate of E. coli enables collecting them by simple filtration, so production of valuable materials will be more efficient. Second module is to produce bio-plastic (poly-3-hydroxybutyrate, P3HB). Development of cost-efficient method to manufacture biodegradable plastic is one of the most important issues for making sustainable future society. We optimized culture conditions for more efficient production of “Bio-capsule E. coli” and P3HB. This is the first successful production of bio-plastic as an iGEM team. We will try to extend the applicability of this system for producing other high-value macromolecules in the capsule. Also, we created new Bio-Communication tool, named 'Biobrick Reviews and Issues' to share iGEMer's opinions about each biobrick.

Team Hong Kong-CUHK: Light of No Return

Although the sensory technology has been deeply explored and implemented in various means, most of the developed sensors are chemically-dependent promoters which regulate downstream gene expression. We exploited the use of halobacterial sensors, the sensory rhodopsins which are sensitive to a wide spectrum of readily available light source and build a series of sensing systems to control cellular movement and gene regulation. This system can be executed as a fundamental part for further applications, such as cell targeting and refining. Furthermore, to counter the safety issues caused by the leakage of bioengineered cells, this sensing method altogether with the CRISPR/Cas sytem can targart and achieve the cleavage of the transformed plasmid under the stimulation of natural light sources.

Team HUST-China: Synthetic Biofactory: Lignocellulose Decomposer and Microbial Fuel Cell

The fossil fuels on earth are so limited today and will disappear in less than 50 years. In order to mitigate energy crisis, the HUST-China team has designed two systems to produce power sources.

Lignocellulose Decomposer: We constructed three strains of Pichia pastoris that pretreat lignocellulose, an important biomass resource, degrade cellulose, hemicellulose and lignin, the three polymers of lignocellulose, and finally generate ethanol. We introduced several genes and used external secretion and cell surface co-display techniques to express the corresponding enzymes.

Microbial Fuel Cell: Microbial Fuel Cell (MFC) can generate electricity using glucose, acetate or lactate, especially when the substrate is simple organic. Our project would firstly construct a signal regulated network to control the formation or depolymerization of biofilm. And then we will build a metabolic pathway to decomposition pyruvate into CO2, so that the NADH can be consumed and regenerated for electric energy export.

Team IIT Madras: Novel Applications of a Chimeric Estrogen Receptor

We aim to express codon-optimized ligand binding domain of Estrogen Receptor. in conjugation with the ToxR DNA binding domain from Vibrio cholerae in E. coli to separate stereoisomers that have profoundly different impacts on biological systems. Isolation and concentration of specific isomers is of immense biological importance to pharmaceutical industries. We plan to try and simplify this process by separating commercially important compounds using a biological system instead of traditional chemical methods which can be very resource intensive and time consuming. We also plan to develop a high-throughput system for screening of drugs which act on the Estrogen receptor using E. coli. Finally, we wish to provide an efficient means for the bioremediation of endocrine disruptors prevalent in the Indian subcontinent which have an adverse impact on the country's population.

Team JUIT-India: Captain Green - Reducing the Greenhouse gas to increase the soil fertility in paddy fields.

'Global warming is too serious for the world any longer to ignore its danger or split into opposing factions on it", quoted Tony Blair. Rice, which is the staple diet of India and many other countries around the world, is believed to engender many potential greenhouse gases or global warming gases per se like carbon dioxide, methane and nitrous oxide. Nitrous oxide, is released due to the inevitable use of nitrogen fertilizers which are added in the paddy fields. We are dealing with the conversion of nitrous oxide into nitrate form using synthetic biology tools to insert two genes into a bacterial cassette along with its detection systems. This nitrate, can be utilized by the plant itself, solving our purpose and adding a new dimension to this diversion and in turn being beneficial for the farmers reducing the compromise factor that would, otherwise, have been done.

Team KAIST Korea: CO2 Fixation Pathway and Pathway Switching Module

1. CO2 Fixation Pathway </br> Reductive acetyl-CoA pathway is a pathway for carbon dioxide (CO2) fixation in many anaerobes. Acetogenic use this pathway to synthesize acetic acid from carbon dioxide. Because the pathway is non-regenerative, reductive acetyl CoA pathway is a appropriate target pathway to consume atmospheric carbon dioxide (CO2). Nowadays, full genome sequences of bunch of acetogens are available. Also, the enzymes consisting the pathway are elucidated allowing us to reconstruct the pathway in Escherichia coli. </br></br> 2. Pathway Switching Module</br> Throughout past iGEM competitions, many kinds of bio-modules were proposed and tested. In our project, we are suggesting dual-phase switching module using DNA recombination system that is new to iGEM part registry. With this module we will be able to control metabolic pathway we are targeting. Coupling of suggested module with cell growth, we expect to enable our cells to control their metabolisms according to cell growth.

Team KAIT Japan: E.coli which has ability to kill the cancer cell.

We try to make E.coli which has ability to kill the cancer cell. Regulatory T cells move toward to cancer cells by which produced CCL22. Killer T cells and helper T cells are inactivated by reguratory T cells, so, the cancer cells elude from the immune system. We think to make use of this mecanism underhand. We will give E.coli three functions. First,we give E.coli the function to have the chemotaxis to cancer cells by recognision of CCL22 which is a chemokine produced from cancer cells. Second, we give E.colithe function that they combine with cancer cells. Cancer cells express the MICA on their cell membranes. NKG2D recepter from NK cells combine MICA. Third,We give E.coli function that they release the azurin to cancer cells. The azurin induced apoptosis in cancer cells by binding with p53. We would like to be help the treatment of cancer .

Team KIT-Kyoto: Drosophila Melanogaster Workshop

Drosophila melanogaster has been used for a genetic study as model organism for a long time and brought us much discovery. And we are sure that the benefit continues from now on. Therefore we KIT-Kyoto team aim at the production of the disease model Drosophila which expresses the responsible gene of MALT lymphoma that is one of leukemia. It is thought that we can contribute to elucidation of the mechanism of this disease and the development of the therapeutic drug by promoting this project. In addition, we think about what we can do in order to continue researches using Drosophila melanogaster in the world. So, this year we aim at the design of the parts with which a study that we use the Drosophila can expand in iGEM in future. If these projects are realized, the study using D. melanogaster will step forward to the new one step again.

Team Korea U Seoul: Project 1 : Rice Guardian

Bacterial leaf blight disease (BLB) is one of the preeminent vascular diseases inirrigated rice. Bacterial leaf blight in rice is caused by infection of bacteria known as X. oryzae pv. oryzae. Based on previous researches, it was proven that bacterial rax gene complex (rax A, B, C, P, Q, R, H) and their protein products(Ax21) are responsible for BLB.

Since Ax 21 is a major pathogen that causes BLB and ever present molecule that signifies presence of X. oryzae pv. oryzae, we decided to make synthetic bacteria that detect Ax21 and furthermore, kill them. We will use rax R and H gene promoters to detect Ax21. As a result of transcription activation, gene will synthesize bacteriocin to kill X. oryzae species.

Team Kyoto: Flower Fairy E.coli

A flower fairy had been merely a creature of imagination until October 5 2012, but not more. Our Flower Fairy E.coli are capable of blooming flowers on demand by producing FLOWERING LOCUS T (FT) protein, called Florigen, a kind of plant hormone composed of 175 amino acids. To make it possible for FT protein to access to plant cells directly from E.coli, we established a new protein translocation system, R-TAT. Our R-TAT system can carry proteins from the cytoplasm to plant cells while maintaining appropriate folding of target proteins. We will show that FT protein induces expression of genes involved in anthesis and functions effectively at low doses by confirming that FT protein activates some key blooming-related genes such as AP1. We will also provide iGEMers incredible promoters constructed through Golden Gate Assembly. Our Giant Controllable-Promoter, for example, is composed of 5x promoter regions following a Lac repressor element.

Team Macquarie Australia: Flick of the Switch: Employing Light-Sensitive Bacteriophytochromes to Control Gene Expression

Phytochromes, or photoreceptors with the ability to control the expression of genes, exist in bacteria as bacteriophytochromes. This project creates a light-dependent biological switch using the bacteriophytochromes from Deinococcus radiodurans and Agrobacterium tumefaciens. When coupled with heme oxygenase, these bacteriophytochromes are supplied with biliverdin, a pigment which allows for the self-assembly of a switch within the host system. In the presence of red light, the conformation of the bacteriophytochrome is modified. This reaction produces a visible colour change in the presence of red light, and can be used to control expression of a targeted gene when coupled with the appropriate response regulator. Exposure to far-red light will cause the bacteriophytochrome to revert to its original conformation, thus repressing the gene and reversing the colour change.

Team Nanjing China Bio: Bacterial Cancer Therapy: Tumor-targeted Salmonella

Bacteria targeting cancer therapy: Salmonella typhimurium-VNP20009 with its unique characteristic of accumulating in nutrient-rich or hypoxic tissues can be adapted to tumor targeted therapy. However, recent researches revealed that S. typhimurium can survive in other normal tissues, resulting in damage or inflammatory response. Our project aims to improve the therapeutic property of S. typhimurium by modifying its amino acids-synthesizing genes and screen highly tumor-targeted strains. Then we standardize them to construct a general element used specifically in hypoxic tissues. The hypoxic feature in the core areas of tumors made it possible for S. typhimurium to target tumors specifically. Our goal is to screen the anaerobic promoter that can express efficiently in S. typhimurium and ligate it with genes of anti-cancer drugs. The double properties of the strains and promoters enable anti-cancer drugs to express specifically in tumors, fulfilling our goal of decreasing toxic and side effects of the drugs.

Team Nanjing-China: si-Veg

Exogenous RNAs are flowing and working in our body, and food carries them besides traditional nutrients. Evidence shows that natural plant miRNAs can be ingested into mammal bodies and target specific genes. Such discoveries show us a promising approach to perform cross-kingdom information transpotation and gene regulation. We propose a method of controlling animal gene expression and helping cure disease by creating vegetable that produces artificial siRNAs targeting critical genes for some disease. This time, we chosed PGC-1 alpha gene, which is over-expressed in fatty liver and contributes to insulin resistance, as the target. This concept can provide us a better perception of our daily diet and a new way of curing disease. On the other hand, we expand the boarder of iGEM by working on green plant. Firstly standardized binary vector is constructed. Also, we designed a brand divece for transgenic plant to help solving potential safety problems.

Team NCTU Formosa: EcoFuel E.coLine

Greenhouse Effect and the limitation of the fossil fuels have been a huge concern to people on Earth. Research shows that higher alcohols possess qualities making them more suitable as a biofuel than ethanol, including lower vapor pressure, lower hygroscopicity, and higher energy density. So our team (NCTU_Formosa) managed to produce isobutanol by E. coli. With the temperature control system, we can reduce the toxic intermediates of synthetic pathway to enhance isobutanol yield. Furthermore, we add 4 zinc fingers to the synthetic enzymes trying to increase the chance of protein interaction, making E. coli much as a production line to produce isobutanol more efficiently. This Ecofuel E.coLine gives an insight of yielding biomass energy, providing better biofuel and, in the long run lowering the burden of our Earth.

Team NTU-Taida: PepdEx: Smart Peptide-based Therapies

In our project, we aim to utilize a microbe that responds to conditions in human body as an approach to administer smart peptide-based therapies. GLP-1, a human innate neuro-peptide for energy balance, is chosen to combat for obesity and metabolic syndrome. We engineer the non-pathogenic E. coli which senses fatty acids in intestines and secretes synthetic GLP-1. Appropriate signal peptides and penetratin are used to facilitate peptide secretion and intestinal uptake. Furthermore, we design a circuit with quorum sensing and double repressors, which aims to generate quick but sustainable responses and serves as an anti-noise filter. Plasmid stabilization modules including partition system and multimer resolution system are also incorporated to circumvent the undesirable loss or segregational instability of our artificial device. With this general concept of delivery of short peptide into human body, we can also target other human diseases

with alternative circuit designs.

Team NYMU-Taipei: Venus Marvel

Nowadays, pollution spreads through the world and our environment is deteriorating day by day. Our project is mainly about the removal of several pollutants, including nitrogen oxides, sulfur oxides and carbon oxides, from exhaust air and waste water. We planned to cultivate a special strain of genetically engineered cyanobacteria. With reductases metabolizing nitrogen, sulfur and carbon oxides, our organisms reduce three major pollutants in the modern day. Furthermore, we also focus on the removal of cadmium ions from soil. We tried to engineer E.coli to gain better capability of collecting cadmium ions. In fact, our engineered E.coli could stay inside of Dictyostelium discoideum, which allows us to build a biosafety system to make sure our GMOs won't become another threat to the environment. Combining our engineered cyanobacteria and the concept of endosymbiosis, we grant eukaryotes, ultimately human being, the ability to colonize Venus and expand our territory.

Team Osaka: Bio-dosimeter

It is still sharp in our memory that, on March 11, 2011, the Great East Japan Earthquake struck off the coast of Eastern Japan and triggered a series of events that led to the nationwide nuclear crisis. Moved by that accident in iGEM 2011, we have built a synthetic biological dosimeter to detect the radiation. In this year we further develop that 'Bio-dosimeter'. Our 'Bio-dosimeter' consists of two points: damage tolerance and radiation detection. To introduce the tolerance to E. coli, we are trying to put in some radiation resistance genes from Deinococcus radiodurans. For the detection of the radiation, we are trying to connect the native DNA damage response system of E. coli to production of pigment lycopene as a reporter. Now, we are attempting to assess its tolerance to various types of DNA damage and to evaluate DNA damage detection more clearly

Team OUC-China: oceanfilm and oceanfeel(a portable ratio sensor that can float)

Our projects focus on warning and countermeasure against red tide. A precise sensor and an effective processor is coming to solve it. N/P is recognized as the key indicator and floatable E.coli is needed for survival. The second one was successfully solved by means of engineering our E.coli with a brand-new gvp gene clusters that possess far shorter length and better property for bacteria to float. Characterization and analysis of the gene cluster is underway. Phosphate and nitrate sensor have been finished respectively, together with three test devices which facilitate our quantitative analysis. More detailed measurements are underway. Once those sensors work as expected, N/P as input would better match our model. Fine-tuned comparators and ratio sensors with sRNA-mRNA interactions serve as the processor for decision-making. This model-driven part would take advantage of our fine-tuned N/P sensors and synthetical RNA interactions together to accurately alarm red tide.

Team Peking: Luminesensor: Programming Cells through Light

Optogenetic tools have made significant impact on life sciences and beyond. However, several serious issues remain: cytotoxicy, narrow dynamic range, and dependency on laser and exogenous chromophore. To circumvent these, Peking iGEM has rationally constructed a hypersensitive sensor of luminance-Luminesensor. Primarily, the sensor was designed by fusing blue-light-sensing protein domain from Neurospora with DNA binding domain of LexA from E.coli, following which protein structure inspection and kinetic simulation were conducted to rationally perform optimization. Amazingly, Luminesensor was proved to be as sensitive as to sense natural light and even bioluminescence. With this sensor, spatiotemporal

control of cellular behavior, such as phototaxis, high-resolution 2-D and 3-D bio-printing using dim light and even luminescence of iPad were shown to be very easy. What's more, we successfully implemented cell-cell signaling using light, which is the very first time in synthetic biology and of great importance for biotechnological use.

Team SEU A: don't hide from me, bacteria

Since people gradually rely on various antibiotics, people come across a big dilemma in drug resistance. Hence, we come up with two innovative ways which have great advantages over the traditional one. The first one, we try to take advantage of the bacteria's nature selection system. We construct a new plasmid which contains two types of genes, sweet and fatal for bacteria. Regularly, the sweet genes will be expressed, promoting the spread of bacteria transfer plasmid through conjugation. Once the amount of bacteria reaches a certain threshold, the dead gene will turn on, resulting in the death of the host bacteria. The second method derive from the idea of dog-eat-dog, we attempt to cultivate a Bdellovibrio bacteriovorus strain, which live in cracking other bacteria. We consider to improve the sterilization efficiency to a certain bacteria. All the two methods may avoid the probability of bacteria resistance at the same time.

Team SEU O China: Breaking the symmetry

Our team,SEU_Omega aims to execute a synthetic biology project based on colony of bacteria. An initial idea concerns the control of the pattern of colony, which would be in the shape of a pentagram.Light sensing would be used as a switch to manipulate the differentiation of cells and the qurum sensing system of AHL would govern the holistic pattern with antisense RNA effecting the division rate. Further cellur differentiation would automatically lead aggregating cells with separated division rates and similar phenotype into percific patterns. Available applications may include bacterial quantitive biosensor,logical gates,cellur automata and so on.

Team Shenzhen: YAO #1.0 (Yeast Artifical Organell)

Project: Yeast Artifical Organell

Synthetic biologists have been engineering genes and pathways in the cell to let it perform functions they desire. However, these man-made pathways incorporated in cytoplasm may suffer inferences from the original genes and pathways within the cell. Eukaryotic cells have organells that separate important pathways from that in the cytoplasm. Thus we want to make our own organells that perform the designed function. There has been some works on man-made organells, however this year the team of Shenzhen plan to create our man-made organell and apply it to organic synthesis by engineering yeast mitochondria, which we call Yeast Artificial Organell, and YAO for short.

Team SJTU-BioX-Shanghai: Membrane Magic

In this year's project, we aim at constructing a set of protein systems on the E.coli cell membrane as carriers of enzymes of assorted reactions. Distinct from linear DNA or RNA scaffolds in the traditional sense, the membrane protein system expands the dimension of reaction space, making possible the framework of numerous complex reactions on the two-dimensional plane, for example, switchable or circular reactions. In such a device, the membrane replaces DNA or RNA scaffolds as an extensive surface for proteins to anchor without limitation of expression amount. More importantly, by gathering the downstream enzymes through signal regulation, the reaction can be accelerated sharply. Besides, products can be transported much more efficiently from the inside to the outside of the cell in that the enzymes are tied to the membrane proteins. Hence the membrane is where the magic happens.

Team SUSTC-Shenzhen-A: BioSearch-An iPhone App for Partsregistry

The era of Partsregistry on mobile phone has arrived! With BioSearch on your iPhone, you can now check biobricks and partsregistry in the seminar room; You can design your genetic circuits when you are waiting for a bus! BioSearch is fully interacting with Partsregistry(http://partsregistry.org) and has all parts information of Partsregistry database with enhanced user-friendly interface. BioSearch has a powerful search engine. Users can search parts and devices by type, by category, by keywords, etc. Our online survey shows that BioSearch has major improvement in search result ranking. In addition, our iPhone App has new functions including sharing, rating, adding bookmarks and downloading to local system. These new functions shall promote the commuting and sharing between synthetic biologists. The BioSearch is going to be available on Apple Store and is free to use.

Team SUSTC-Shenzhen-B: Theoretical modeling and experimental measurement of transcription terminator efficiency

Transcription terminator is an essential part of biobrick circuits, but is not well characterized. We studied the rho-independent transcription terminators using both theoretical modeling and experiment method. We first developed a theoretical model. This model calculate the free energy of RNA folding and can predict the secondary structure of terminators. From the secondary structure, we proposed an algorithm that can calculate terminator efficiency. In the aspect of experiment, we construct 100 terminators. We measure the the terminator efficiency by measuring the GFP and RFP which are placed before and after terminators. The efficiency calculated from theoretical model fit quite well with experimental results. We also created a software and a web surver for people to calculate their terminators and also built a database of terminator efficiency which we believe to be the largest database of such kind. Our work is by far the most comprehensive study on terminator efficiency.

Team SYSU-China: An Asymmetric Cell Differentiation for maintaining a Stable and Efficient System

In a specific system composed of two kinds of homologous cells, the cells with higher growth rate could gain the upper hand in numbers and then replace the slower ones. This process will cause the system unstable. But a stable and proper ratio of numbers of the two cells is essential for an efficient system like stem cells and the mature ones. So we are constructing a model of an asymmetric cell differentiation to maintain a stable system. We want to construct a system where the cells with higher growth rate could transform into the slower ones which has a different function. And with this process the system would become efficient and stable. We are constructing the part of regulation with toggle switch and Gene A, which is supposed to slow down the cycle of divisions when transfected successfully, to accomplish an automatic asymmetric cell differentiation.

Team SYSU-Software: BiArkit, A Versatile Toolkit For Synthetic Biology

BiArkit is a versatile toolkit that integrates different modules together and helps researchers approach information on synthetic biology. The first function is GenomeBrowser, which visualizes the genomes of some model microorganisms, locates the genes on the genome and make it easy to study the genome. Secondly, Regulator Designer helps the design of regulatory elements, mainly non-coding RNA,in which we firstly develop Riboswitch Designer. Thirdly, we optimize the methods of scanning and output of the existing database of pathways. Fourthly, to analyze the dynamic change in various metabolic networks, we present a simulator that help the researchers analyze the network in silico, with the application of flux balance analysis (FBA). Further, to make it more convenient, the software is localized; that is to say, all functions

mentioned above can be achieved without linkage to Internet.

Team Tianjin: AegiSafe O-Key

The Shine-Dalgarno (SD) sequence is a ribosome binding site several basepair ahead of start codon AUG. It interacts with the anti-Shine-Dalgarno (ASD) sequence in the 16S rRNA in the ribosome to initiate protein translation. By mutate the basepair in the SD and ASD sequence, we produced an orthogonal translation system where the canonical ribosome cannot translate the orthogonal mRNA, and vice versa. We call this system O-Key. Using the O-Key, we are able to strictly control the synthesis of desired product and prevent potential contamination to the publics and environment. We can even build a completely new orthogonal phage that can only infect our engineered E. coli. With these successful examples, we demonstrated a bright and secure future that guarantees the safety of the human and environment with our O-Key.

Team TMU-Tokyo: Chef Ant E.coli

Formaldehyde is a common harmful chemical, and it has a bad effect in relatively low concentration. (For example, in agricultural chemicals, in disinfectant at hospitals and in paint of building materials) Also, since formaldehyde is mass-produced in factories, it is highly possible to exceed over the permissible amount in the environment. This year, in Japan, the detection of formaldehyde in Tone river became an issue. (1) We planned to create E.coli with an ability to detect and detoxify formaldehyde named Chef Ant E.coli. About detection, we try to visualize formaldehyde by ligating regulated promoter, frmR and GFP. Moreover we plan to overexpress two enzymes in Chef Ant E.coli. First, formaldehyde dehydrogenase decomposes formaldehyde to formic acid. The gene of formaldehyde dehydrogenase is from Pseudomonas Putida. Second, formic acid dehydrogenase converts formic acid to CO2 and H2O. The gene of formic acid dehydrogenase is from Methylbacterium extorquens.

Team Tokyo Tech: 'Romeo and Juliet' by E.coli cell-cell communication

A love story contains several processes. Two people fall in love and their love burning wildly. However, no forever exists in the world, in most occasions, love will eventually burn to only a pile of ashes of the last remaining wind drift away. In our project, we have recreated the story of 'Romeo & Juliet' by Shakespeare vividly by two kinds of Escherichia coli. We aim to generate a circuit involving regulatory mechanism of positive feedback rather than commonly-used negative feedback to control the fate of E.coliby signaling between two types of E.coli. Besides, Rose represents love. We will challenge to be the first iGEM group ever to synthesize PHA (a kind of bio-plastics) from glucose using the whole PHA gene sequence to represent rose.

Team Tokyo-NoKoGen: Coli express for long distance communication

We created an E. coli for long distance communication. This “Communicheria coli” was inspired by the Pony Express, a rapid mail delivery service in the American Wild West, where mail was relayed by horseback riders. Communicheria coli sends a message, in the form of light, to distant cells, which then relay the message to other distant cells. Communicheria coli has light sensors constructed using the light receptor domain of bacterial sensory rhodopsin or the cyanobacterial green light sensor CcaS. In response to light signals, cells will induce their own lux operon to send the message to other distant cells, for example in a separate flask, which will in turn relay the message to other distant cells. To improve the effectiveness of our new signal delivery system, we set out to enhance the light intensity, change the light color, and shorten the response time.

Team Tsinghua: Domino E.coli community

The domino effect, a chain reaction that occurs when a small change causes similar changes nearby and leading to a set of changes in linear sequence, can be viewed as a form of information processing and signal amplification. In prokaryotes, information transmission through slow diffusion of chemical compounds is limited either in width or rate. In our project, we constructed a bio-film like Domino E.coli community, aimed at achieving an expansive and rapid biological signal processing system. Domino E.colicommunity, as its name suggests, is capable of amplifying a weak starting signal via geometrical progression, taking advantage of quorum sensing effect. Meanwhile, our system undergoes multi-signal integration, logical computation and transformation of chemical inputs to visual outputs, suggesting the approach of constructing multi-cellular biocomputing.

Team Tsinghua-A: CPLD: a Cell-based Programmable Logic Device

The ambitious Tsinghua-A iGEMers are still dedicated to a beautiful combination of biology and engineering, and this year, the realization of a programmable logic device (PLD) on the gene sequence has become the focus of our attention. A series of symmetric logic-toggle modules, or briefly speaking, AND-OR switching gates, are designed to act as the basic parts of this Cell PLD. The idea comes from PLD which is widely used in electronics engineering. Hopefully, the construction of these modules in the cell will be achieved, with the help of the site-specific recombination systems. Feedforward control theory is introduced into the module and mechanism on the behavior has long been under our analysis, all aiming at a better performance of the logic gate. Modeling as well as computer simulation will help to evaluate and thus improve the robustness in this process.

Team Tsinghua-D: Designable Thermoswitch

By creating the term ‘Designable thermoswitch”, we are trying to deliver an idea that metabolic controllers responded to given temperatures can be designed. Besides explanation and prediction, the ultimate goal of science is creation. Here, we create several regulatory RNAs as thermal metabolic controller. Pre-set a ‘switch-on’ temperature and a ‘switch-off’ temperature, in silico simulation will give the sequence of the regulatory RNA that meet the requirement. A step further, we apply our ‘Designable Thermoswitches’ to the field of fermentation industry. For a long time, engineers are trying to find a more economic and more automatic way to extract fermentation product inside the engineered microorganism. We align our ‘Designable Thermoswitches’ and gene of lysozyme together and put them into E.Coli to solve this problem. The reconstructed E.Coli will switch on the procedure of self-lysate at the given temperature. Thus, the fermentation product inside the engineered microorganismcan be released.

Team USTC-China: Anti-phage E.coli

Bacteriophage is one of the most severe threats the fermentation factories have to face. To help solve the problem, we design a gene circuit which can both detect and defend against the phages. We use the modified promoter pRM to sense the phage’s infection and initiate the defence. The lysis gene which can make bacteria lyse is installed in our circuit. When it works, the phage won’t be able to take advantage of its host to replicate any longer. To win more time for lysis to function well, we design antisense RNA to prevent the phage from turning into lytic life cycle. Thus, when the lysis protein kills the host, the phage is still at lysogenic life cycle or the newly assembled phages are still immature. By using the quorum sensing system, the bacteria around the host will prepare to defend in advance. Attribute to these parts, our bacteria survive.

Team USTC-Software: Reverse Engineering for Biological Regulatory Networks

Traditional synthetic biological design creates or uses standardized parts such as BioBricks to build the genetic circuits, and uses mathematics to model the behavior. In this approach, biological design guides both experiments and mathematical modeling, but is it possible to use experimental data to reversely engineer the mathematical model and guide backwards the biological design? This project answers the question. We use reverse engineering techniques to get mathematical models such as ordinary differential equations(ODEs), directly from the experimental data and build the feasible designs according to the models. In this sense, we not only fully connect biology, experiments and mathematics, but also get feasible designs that have certain behaviors. To realize this idea, we build a suite of applications that provide researchers with efficient workflows.

Team UT-Tokyo: Sweetaholic Energy Generator: Hydrogen Production from Sugar-rich Waste by E.coli

Today, large quantities of food and drinks, together with the enormous amounts of energy they contain, are dumped without being reused. However, the moisture in this waste prevents it from being used as a energy source by burning. Our project aims to reuse such nutritious garbage by Escherichia coli digesting glucose to synthesize hydrogen, which is expected to be used in various useful ways, such as in fuel cells. E. coli cells have intrinsic metabolic systems related to synthesis of hydrogen from glucose via formic acid. We are trying to improve the latter part of this metabolic system, the formic acid-hydrogen pathway, by overexpressing a gene which controls a step in this pathway. If we are successful, what we have to do for getting energy is to share our sweets and juices with E. coli.

Team UT-Tokyo-Software: Software tools for iGEMers: BioBrick/Project Search & Tutorials

We developed new search and educational tools to assist iGEM teams. For many teams, the majority of team members are new-comers. Our primary goal therefore is to aid these beginners get used to iGEM earlier to make project initiation swift. All of our tools are web-based and have user-friendly interfaces enabling users to gain quick access to needed information. Our project consists of the following four tools.

“BioBrick Search” improves convenience in searching BioBrick parts by a sophisticated interface and an ordering algorithm taking into acount the parts' frequency of use. With “Past Project Search”, you can run a keyword search for all past projects and access past teams' presentation material easily. “BioBrick Puzzle” and “Gene Network Game” are educational games intended for beginners to acquire knowledge about BioBricks and gene networks, which aides them to plan their projects and conduct experiments.

Team WHU-China: E. coslim: Synthetic Probiotics Help Defy Obesity

Utilizing human microbiota to tackle diseases has long been the keen desire of scientists. This year, we WHU-China team engineered a probiotics “E. coslim” from Escherichia coli, hoping to provide a new approach for treating obesity. Specifically, three genetic devices were designed. The first two devices were assembled to sense and response to fatty acids and glucose. To achieve these goals, promoters repressed by FadR and CRP were devised and synthesized respectively. When functional genes are placed downstream of these promoters appropriately, the two devices are supposed to degrade fatty acids and convert glucose into cellulose rapidly, thus preventing excessive calorie intake as well as producing prebiotics. Meanwhile, the third device was designed to control the densities of “E. coslim” and forestall horizontal gene transfer in future applications. As a whole, by simulating, we are developing “E. coslim” to

regulate the microbiome composition in intestine to reduce risks of obesity.

Team XMU-China: E.Lumoli: a shining synthetic device for digit or time-course display

We haved constructed a fluorescent digital display device with synthetic logic gates, which is able to respond to signals by displaying and switching numbers. We put GFP equipped with degradation tags in downstream to illuminate our numbers and change them quickly as well. Considering our engineering background, we accordingly employ cell immobilization to build our device. Engineering bacteria have been embedded in intra-hallow calcium alginate microcapsules and in PDMDAAC-NaCS microcapsules, respectively. In addition, 3D CAD design is performed for a perfect device. Our genetic circuits vary in length and RBS strength, leading to different durations of time delay for GFP expression. This inspired us to extend our work last year. By altering the strength of RBS at five grades, another five circuits have been built. After the induction by arabinose, the duration of response time for GFP expression increases as the strength of RBS declines, bringing about a time-course display.

Team ZJU-China: Riboscaffold

ZJU-China aims to design and realize a tunable RNA scaffold to accelerate biological pathways and turn their on and off. RNA scaffold is designed to colocalize enzymesthrough interactions between binding domains on the scaffold and target peptides fused to each enzyme in engineered biological pathways in vivo, which may suffered from low efficiency of production caused by relative lack of spatial organization of non-homologous enzymes. The scaffold allows efficient channeling of substrates to products over several enzymatic steps by limiting the diffusion of intermediates thus providing a bright future for solving the problem. Meanwhile, we plan to add an aptamer structure on RNA scaffold as a switch to regulate biological pathways by micromolecular ligands. Then we can control the all-or-none binding relationship between the enzymes and scaffold by whether the special ligands are presented or not.

EUROPE

Team Amsterdam: Cellular Logbook - A methylation-based reporter system

Multi-sensing genetic devices offer great future perspectives for biotechnology, environmental monitoring and medical diagnostics. In light of this we have created an innovative DNA-methylation based reporter system in E. coli, named Cellular Logbook, that has the potential of simultaneously reporting on significantly more signals than current fluorescence-based systems (eg. GFP). The Cellular Logbook can be used to detect and store the presence of any compound linked to a transcriptional regulator. This system allows for offline monitoring by functioning as a memory module. Assessment of the memory status is performed by digesting with restriction endonucleasesfollowed by gel electrophoresis. Furthermore, the Cellular Logbook is able to infer the time of signal-onset or signal-intensity using the natural dilution of the registered signal’s due to cell division. In short our exciting new memory module could potentially be utilized as a platform for many groundbreaking technologies.

Team Bielefeld-Germany: TOXIC COMPOUNDS IN NATURAL WATER - A CASE FOR LACCASE

The accumulation of endocrine disruptors and toxic substances in wastewater has serious consequences for aquatic life and may lead to severe damages in humans. Especially the use of synthetic estrogen in birth control pills results in increasing the concentrations of this disruptor in wastewater. Therefore, 'iGEM

Team Bielefeld' is developing a biological filter using immobilized laccases, enzymes able to radicalize and break down a broad range of aromatic substances. For the production of laccases from different bacteria, fungi and plants, two expression systems are used: 'Escherichia coli' and the yeast 'Pichia pastoris'. Immobilization is carried out either by using cpc-silica beads or by fusing the enzymes to cellulose binding domains. The concept could be extended to other toxic pollutants in drinking and wastewater, as well as to industrial applications in paper and textile industries or even for bioremediation of contaminated soil.

Team Bonn: All You Need is LOV!

Fusion protein design has always been time- and design-intensive, to say the least. We are developing and characterizing a fusion construct containing a light sensitive domain, providing easy coupling and light activation of proteins of interest to investigators, thus developing a protein-level light-induced knockout. Using the LOV (Light, Oxygen, Voltage) domain commonly found in plants, where it enables light-directed growth, we are establishing guidelines for coupling proteins of interest to the LOV domain, which allows control of protein activity through blue wavelength light. Designing our reversible knockout at the protein level allows quick response times (2.2 microseconds activation time, 85 seconds deactivation time). A device of that kind could be of great importance as a tool for disinfection on a laboratory scale or mutant selection via blue light. Further potential applications of our LOV fusion system include bioreactor regulation or site-specific drug activation.

Team Bordeaux: A bacterial eyespot

This project aims at creating a regulatory system in the bacteria Escherichia coli. Our main goal is to engineer a single strain of bacteria able produce concentric patterns on the dishes. The challenge is to model a regulatory mechanism which mimics both cell differentiation and cell-to-cell communication observed in eukaryotes. We chose to create four operons (a total of 21 assemblies): three to allow the communication and expression of a visible phenotype, the fourth containing the genes needed for signal transduction. Each of the three first operons will respond to a specific quorum-sensing system (QSS) and trigger another QSS resulting in a chain reaction communicating a unique signal to all bacteria nearby. We also developed our model in silico to run simulation and test parameters that influence pattern propagation on a petri dish.

Team Cambridge: Parts for a reliable and field ready biosensing platform

Implementation of biosensors in real world situations has been made difficult by the unpredictable and non-quantified outputs of existing solutions, as well as a lack of appropriate storage, distribution and utilization systems. This leaves a large gap between a simple, functional sensing mechanism and a fully realised product that can be used in the field. We aim to bridge this gap at all points by developing a standardised ratiometric luciferase output in a Bacillus chassis. This output can be linked up with prototyped instrumentation and software for obtaining reliable quantified results. Additionally, we have reduced the specialized requirements for the storage and distribution of our bacteria by using Bacillus' sporulation system. To improve the performance of our biosensing platform we have genetically modified Bacillus' germination speed. Lastly, we demonstrated the robustness of our system by testing it with a new fluoride riboswitch, providing the opportunity to tackle real life problems.

Team Chalmers-Gothenburg: Biodetection of hCG hormone - Development of a biodegradable pregnancy test kit

The goal of this project was to construct a biosensor for the hCG hormone consisting of S. cerevisiae. The

human luteinizing hormone receptor (LH/CG), a GPCR with high affinity for hCG, was therefore expressed in yeast. The yeast strain used contains a yeast/human chimeric G-subunit, enabling coupling of the LH/CG-receptor with the pheromone pathway in yeast. Binding of hCG should consequently result in activation of the pathway. The genes tnaA and fmo, encoding tryptophanase and flavin-containing monooxygenase respectively, were introduced into the yeast strain. These enzymes catalyze the conversion of tryptophan to indigo. tnaA was set under the control of the pheromone induced FIG1 promoter and fmo was expressed constitutively. Hence, detection of hCG should result in the production of bio-indigo, the output signal of the biosensor. In order to ensure hCG to pass the cell wall, the gene CWP2, encoding a cell wall mannoprotein, was deleted.

Team Copenhagen: CyanoDelux

Our overall objective is to create cyanobacteria that glow exclusively in darkness. To accomplish this, we will use a native promoter (lrtA) that normally functions as a light-regulated promoter in cyanobacteria. We will insert it into a plasmid together with the luxCDABE cassette. The cassette contains the luciferase enzyme and enzymesnecessary for regeneration of its substrates. The final goal is to make cyanobacteria (Synechococcus elongatus PCC 7002) glow because cyanobacteria perform photosynthesis and therefore do not need supplied nutrients. First, the experiment is carried out in E. coli and afterwards the plasmid is transferred to the cyanobacteria. Both of the systems will subsequently be thoroughly analyzed to determine important characteristics of the system including kinetics and efficiency of the expression levels. To achieve this quantification we will collaborate with a fellow Physics student at University of Copenhagen.

Team Dundee: Six, Lyse and Obliterate: a synthetic silver bullet against healthcare acquired infection.

Hospital acquired infections are a global problem. One example is Clostridium difficile, a bacterial pathogen that infects patients undergoing prolonged antibiotic treatment and results in pseudomembranous colitis, a potentially fatal gut infection. This project aimed to design a synthetic bacterium that would respond to C. difficile infection and kill the pathogen in situ. Escherichia coli was engineered to secrete an endolysin from a bacteriophage that would specifically attack the C. difficile cell wall. The endolysin was fused to the extracellular components of an engineered Type VI Secretion System from Salmonella, which itself comprised 13 different proteins. In addition, a synthetic ‘inflammation biosensor’ was developed, based on a two-component system from Salmonella, with the aim of restricting endolysin secretion to the diseased colon only. Mathematical modelling was used to assist in the development of the laboratory work and to investigate potential therapeutic strategies beyond the scope of the experimental programme.

Team Edinburgh: Tools that make synthetic biology easier and safer - questioning legacy and friendliness

Edinburgh’s 2012 iGEM project focuses on developing tools that expand the range of synthetic biology applications. We are characterizing Citrobacter freundii as a chassisin order to investigate the potential of a new host organism as an alternative to Escherichia coli in synthetic biology. The team is also looking at novel selectable and counter-selectable markers as a substitute for antibiotic based systems which facilitate the spread of antibiotic resistance in the environment. We seek to implement the MtrCAB electron transfer system from Shewanella oneidensis into E. coli, and test the resulting electron output from the organisms using microbial fuel cells. We are constructing computer models of the electron transfer chain and of cell survival with non-antibiotic markers. This tools-based project responds directly to legislation and safety. We considered how iGEM gives us the freedom to pursue blue-sky research and whether our work

is driven by preconceptions of public opinion.

Team EPF-Lausanne: SWITCH: Direct, Light-induced Gene Expression for Optimal Drug Production in Mammalian Cells

The fusion protein our team aims to characterize is a version of the LovTAP construct (submitted as a BioBrick by the 2009 EPFL iGEM team) adapted to mammalian generegulation. It allows for tight regulation of conditional gene expression (started upon illumination with a blue light) through a photo-sensitive domain coupled to DNA-binding and activating domains. We are also developing and building a custom bioreactor setup to create the appropriate conditions for the LovTAP switch to work, and modeling the behavior of our system. Development of optogenetics has mainly been focused on bacteria but we are also comparing our project to another mammalian system, developed by Fussenegger et al. (Science Vol. 23, 2011), that uses a melanopsin switch to trigger endogenous calcium-driven promoters. Light-induced gene expressioneliminates the need for activating molecules in sensitive applications such as the production of therapeutic proteins in the pharmaceutical industry.

Team ETH Zurich: E.colipse – Who's your pABA: intelligent sun protection

E.colipse is an intelligent and adaptive sun radiation protection system which responds to UV exposure with the production of the protective agent pABA. To detect hazardous levels of sun radiation our system is based on UVR-8, a UV sensing protein from plants. In its dark state, this protein forms a homodimer that dissociates upon UV radiation. We fused UVR-8 with the DNA binding domain from TetR, which is unable to dimerize and to bind DNA in monomeric form. UV-exposure might force the TetR-UVR8 fusion dimer to split, release the DNA and enable transcription. Thus, TetR-UVR8 might act as a light-activated on-switch in bacteria. We plan to use this novel switch to start the production of para-aminobenzoic acid (pABA), a common ingredient of sunscreen, and - dependent on the intensity and duration of exposure as determined by our detailed in silico model - a colored pigment as a visible warning signal.

Team Evry: A synthetic hormonal system for the vertebrate chassis Xenopus tropicalis

Building on a long-standing French fascination for frogs, we wanted to spread this enthusiasm to the world of synthetic biology by introducing a new, vertebrate chassis to the community: Xenopus tropicalis. This leap towards multicellular biological engineering required new tools, so we first developed a new set of frog compatible vectors, biobricked tissue specific promoters and a new technique to assemble them in a single shot. To benefit from tissue compartmentalisation, we created a synthetic, orthogonal hormonal system using the plant molecule auxin. We also investigated E. coli/Xenopus interfacing, effectively creating a synthetic ecosystem. We modelled our system at the organism scale, using a multi-level and multi-technique approach. Finally, working with whole animals during iGEM brought a load of difficult ethical questions regarding animal biotechnologies and experimentation. This led us to wonder: Are we a chassis?

Team Exeter: e-candi: Engineering the Fourth Polymer of Life

Polysaccharides have a spectacular range of properties and uses, from the structural and medicinal, to foods and glues. These properties stem from the relationships between the chemical nature of the sugars, their arrangement within the polymer and the arrangement of the polymer itself. Scientists rely on chemical modification of polysaccharides or expensive and time-consuming production via synthetic chemistry to understand these relationships. This project, e-candi, asks if synthetic biology could generate designer polysaccharides. We created biobricks for the biosynthesis of useful polysaccharides in Escherichia coli

and asked whether we could synthesise a novel polymer sequence in E. coli by targeting endogenous polysaccharide biosynthesis. We developed this work further through the generation of a GTase database with a user-friendly interface to aid polymer construction, and by investigating a GTase donor/acceptor characterization assay alongside mathematical modeling of our biosynthetic system in order to improve system understanding and performance.

Team Fatih-Medical: Cancel the Cancer

Our project is mainly based on the early diagnosis of cancer. EpCAM (Epithelial cell adhesion molecule) is a pan-epithelial differentiation antigen overexpressed on the basolateral surface of most carcinomas and Circulating Tumor Cells(CTC); the cells which are released into blood in early phases of cancer. Our objective is to fix appropriate antibodies for EpCAM antigens to the E.coli cell wall so that we will be able to detect CTCs before the cancer precipitates its way to metastasis. For the next step, we plan to enhance the detection signal in our bacteria by the means of quorum sensing mechanism. Finally, to prevent the production of possible undesirable and detrimental genetically modified organisms (GMOs), we aim to induce self-destruction device in our E.coli via emission of light.

Team Frankfurt: Steviomyces - It´s gonna be sweet

The Stevia plant produces several sweeteners known as Steviolglycosides which have only recently been admitted as a food additive in the European Union. However it has been used as a traditional food ingredient by Paraguayan natives, for example to sweeten mate tea. The iGEM Team Frankfurt wants to transfer the pathway of the plant into baker yeast (Saccharomyces cerevisiae) to make stevia production much cheaper. Furthermore microbial production of these sweetening compounds could also lower the environmental costs of Sweetener production. In addition to these advantages, it would be possible to selectively produce only the most flavorful compounds. Several of known problems with carbohydrate sweeteners like diabetes or caries could be overcome by the Steviolglycosides which are produced by Stevia rebaudiana. Another interesting perspective is the capability of Steviolglycosides to reduce the blood sugar value.

Team Freiburg: Let us tell you a fabulous TALE ...

Transactivator-Like Effectors (TALEs) are a brand-new technology that currently revolutionizes the way researchers manipulate DNA with exceptional site specificity. Originally derived from Xanthomonas spp., this type of protein comprizes an effector domain and a modular DNA binding domain that can be rationally designed to bind to virtually any target sequence of DNA. Over the past two years, universal endonucleases (TALENs) and transcription factors have been tested in various organisms ranging from bacteria to humans. According to existing protocols, TALE assembly requires several weeks of work and substantial lab skills. In order to bring this technology within reach for iGEM students, we invented an extremely fast and easy TALE assembly strategy and developed a TALE platform with expression plasmids and new classes of TALEs. With our so called GATE assembly kit, future iGEM students will be able to precisely manipulate genomic loci easier and faster than anyone else in the world.

Team Goettingen: Homing coli: Engineering E. coli to become “tracking dogs”

The model organism Escherichia coli is naturally capable of sensing substances in its environment and consequently moves directionally towards these, a phenomenon known as chemotaxis. Here, we apply directed evolution to chemoreceptors by targeting five amino acid residues in the ligand binding site to enable E. coli to perceive novel substances. In order to investigate mobility and directed movement

towards a substance, an effective mobility selection method using special “swimming plates” is designed. Additionally, we attempt to improve E. coli’s swimming velocity by creating new parts derived from its own motility apparatus. Based on our selection system, we identify variants of chemoreceptors with new binding specificities in the mutant library. By these means, we aim to train the bacterium to detect new molecules such as tumor cell markers. Once having established E. coli as our “tracking dogs”, the possible applications in medicine but also to environmental issues are virtually countless.

Team Grenoble: sEnsiColi: A tunable and reliable ultra-sensitive detector

Multi-resistant bacteria are a worldwide issue which in a very near future will have huge impacts on our societies and ways diagnosis and prevention will be performed. In this optic, the Grenoble iGEM team has built an ultra-sensitive pathogen detector. It consists of three interconnected modules: 1- Detection, 2- Amplification/ Communication and 3- Output. The detection module consists of a recombinant membrane receptor that, once activated, actuates an amplification loop. The amplification system contains a genetic feed forward loop, which filters out false positive outputs. Once amplified and filtered, the signal is transmitted to neighboring bacteria via a diffusible molecule. In turn, the amplification loop is triggered which leads to the production of a measurable fluorescence output. The design of our network is easily adaptable to different input signals by using other receptor domains.

Team Groningen: The Food Warden. It’s rotten and you know it!

Every year, one third of global food production -1.3 billion tons of food- is thrown away, partially due to the “best before” dating system. iGEM Groningen 2012 seeks to provide an alternative method of assessing edibility: The Food Warden. It uses an engineered strain of Bacillus subtilis to detect and report volatiles in spoiling meat. The introduced genetic construct uses a promoter to trigger a pigment coding gene. This promoter, identified by microarray analysis, is significantly up-regulated in the presence of volatiles from spoiled meat. The activity of the promoter regulates the expression of the pigment reporter and will be visible to the naked eye. For safe usage of the system, spores of our engineered strain are placed into one half of a semi-permeable capsule, the second containing a calibrated amount of nutrients. Breaking the barrier between the two compartments allows germination and growth, thereby activating the spoiling meat sensor.

Team Leicester: A Synthetic Biology Solution To Polystyrene Degradation.

Objective - Naturally occurring organisms using polystyrene as their sole source of carbon have been recently identified, by analysing the occurrence of polystyrene breakdown products. However these metabolites accumulate very slowly, explaining why polystyrene is so persistent in the environment. Polystyrene can currently be recycled, but due to the low density of the majority of polystyrene products it is economically unfavourable, due to the high energy demands. If inexpensive biological degradation can be achieved this would assist recycling, but we also hope to use products of this reaction to make useful organic chemicals. Aim - To construct BioBricksfrom the genes encoding enzymes involved in this pathway and manipulate their expression and properties to maximise the rate of polystyrene degradation. Hypothesis - genes encoding the enzymes of the polystyrene breakdown pathway can be isolated and expressed in a host microorganism and the rate of the process increased by genetic manipulation.

Team LMU-Munich: Beadzillus: Fundamental BioBricks for Bacillus subtilis and spores as a platform for protein display

We chose to work with Bacillus subtilis to set new horizons and offer tools for this model organism to the

Escherichia coli-dominated world of iGEM. Therefore, we created a BacillusBioBrickBox (BBBB) composed of reporter genes, defined promoters, as well as reporter, expression, and empty vectors in BioBrick standard. B. subtilis naturally produces stress resistant endospores which can germinate in response to suitable environmental conditions. To highlight this unique feature using the BBBB, we developed Sporobeads. These are spores displaying fusion proteins on their surface. As a proof of principle, we fused GFP to the outermost layer. Expanding this idea, we designed a Sporovector to easily create any Sporobead imaginable. Because the Sporobeads must be biologically safe and stable vehicles, we prevented germination by knocking out involved genes and developed a Suicideswitch turned on in case of germination. With the project Beadzillus, our team demonstrates the powerful nature of B. subtilis.

Team Lyon-INSA: Biofilm Killer: long-term destruction of biofilms in an industrial context.

Biofilms are responsible for billions of dollars in production losses and treatment costs in the industry every year. Biofilm-related problems are major concerns in the food industry where it can cause food spoilage or poisoning, in health industry because of pathogens' persistence and dispersal, or in the oil and water industry where it causes corrosion. Assuming that the environment is already over-saturated with harmful chemicals such as biocides, whose long term health effects remains to be elucidated, there is a great need for innovating solutions to reduce detrimental biofilm effects. To reduce the use of biocides, the INSA-Lyon iGEM team aims to engineer a bacterial 'torpedo' capable to infiltrate and destroy biofilms formed on industrial equipments, pipes or reservoirs. Industrial surfaces will then be protected from further deleterious contamination by either a surfactant coating, or the establishment of a protective biofilm produced by the torpedo bacteria.

Team Marburg SYNMIKRO: “The Recombinator”: an intelligent Genetically Engineered Slot Machine (iGESM)

The vertebrate immune system produces billions of different antibodies. This diversity is generated by random VDJ-recombination of a limited number of antibody subfragments. This inspired us to construct an automatic recombination system in E. coli that generates large numbers of novel proteins by combinatorial fusion of functional domains. The site-specific DNA recombinase Gin of bacteriophage Mu depends on the presence of a DNA enhancer element for efficient recombination. This allowed us to construct a system, called “The Recombinator”, which automatically shuts down after successful recombination. We visualized the randomizing function of our genetically engineered slot machine by combining colors with cellular localization domains. By scaling up the number of recombination modules and functional domains our system will be able to generate a multitude of new proteins. We envision that “The Recombinator” will serve as a tool to create novel enzymatic activities for innovative drug design, environmental detoxification and metabolic engineering.

Team METU: eCO Filter

Carbon monoxide (CO) poisoning is one of the most harmful types of air poisoning around the world.CO gas is mostly released from the internal combustion of engines as well as the use of fuels such as wood and coal.Since CO is highly produced in urban areas,it presents a big danger for any living organism.The aim of our project is to convert CO into CO2 biologically,which then can be converted into oxygen with photosynthesis by photoautotrophic organisms.In order to achieve this,we plan to constructa biofilm containing the enzyme Carbon Monoxide Dehydrogenase (CODH).With the production of this biofilm,it may be possible to obtain a biological filter that can fix the ratio of CO and CO2 present in the environment.We

also try to integrate a kill switch,previously developed by Berkeley,to our system for safer use of our biofilm as well as a cell limiter for better characterization of the biofilm activity.

Team NRP-UEA-Norwich: A future using quantitative computing and its applications using a dual promoter.

Imagine a world in which all sectors of industry use synthetic biology to meet specific needs. The NRP-UEA team have developed novel biobricks, which provide a foundation for a system with this level of complexity. The project began with a simple idea with widespread applications: the detection of exogenous nitric oxide (NO). However it soon became clear the detection of highly reactive NO was challenging, and this was addressed in two main ways. A bacterial promoter, PyeaR, was fused to its mammalian counterpart, CArG. The functionality of this flexible dual promoter was determined in both mammalian and bacterial chassis. Yet it was determined that further specificity was still needed, leading to the comparator circuit, that subtracts the expression of one promoter from that of another, allowing for signal integration and quantitative computing. This system thus allows for the detection of any chemical, providing the promoters have overlapping specificity.

Team NTNU Trondheim: Bacterial Anti Cancer Kamikaze

One of the biggest problems with the cancer treatment used today is that normal chemotherapy is harming healthy cells in addition to cancer cells. Our approach for solving this problem has been to develop a genetic circuit that makes E.coli cells able to release toxic molecules only when in presence of cancer cells. As cancer cells grow faster than healthy cells, they also consume more oxygen and release more lactate than a healthy cell would do, so to make the E.coli cells recognize cancer cells we have made a system where the input signals are high lactate concentration and low oxygen concentration. When these criteria are met, the E.coli cells will undergo lysis, and release the toxin colicin, which our cells are producing constitutively. With our project, we want to show that one of the biggest challenges in medicine can be solved by synthetic biology.

Team Paris Bettencourt: bWARE

Many synthetic biology projects propose the application of Genetically Engineered Organisms (GEOs) in natural environments. However, issues of biosafety and ethics constrain the use of GEOs outside the lab. A primary concern is the Horizontal Gene Transfer (HGT) of synthetic genes to natural populations. Strategies developed to address this problem provide varying levels of containment, however, the substantial elimination of HGT remains difficult or perhaps impossible. We have developed a new containment system to expand the range of environments where GEOs can be used safely. To do so, we rely on three levels of containment: physical containment with alginate capsules, semantic containment using an amber suppressor system, and an improved killswitch featuring delayed population-level suicide through complete genome degradation. We aim to raise the issue of biosafety by engaging the general public and scientific community through debate, and to advocate the discerning use of biosafety circuits in future iGEM projects.

Team Paris-Saclay: GEMOTE: a new tool to control gene expression by temperature

We designed a system that allows controlling the expression of a gene or an operon over a specific temperature interval (between 32 and 42 degrees Celsius). This system consists of an RNA thermometer controlling the translation of a thermosensitive transcriptional repressor, which itself controls the expression of the targeted gene or operon. In our current construction, the crtEBI operon directing lycopene

biosynthesis is used as a reporter, allowing us to check our system's performance. However, the possible applications of this system are extremely numerous. For example, controlling the expression of a toxin would allow creating a “suicidal bacterium” that would bring on its own death outside the specified temperature range. This will help preventing its spread in the environment. And this is just one example... The only limit is our imagination !

Team Potsdam Bioware: Antibody Generation System - Maturation, Selection and Production in CHO Cells

Antibodies are of utmost importance for research and therapy but their generation is laborious and time consuming. We established a novel streamlined workflow for obtaining antibodies by incorporating all natural steps such as antibody maturation, selection and production in one genetic system implemented into a eukaryotic cell line. We stably transfect an antibody construct into CHO cells and mimic maturation by using the enzyme AID (activation-induced deaminase), which is known to induce somatic hypermutation. For selection, we are testing and deploying a versatile and continuous viral system as well as magnetic beads and cell sorting. Finally, a genetic switch enables the transition from surface expression to production of soluble antibodies. In addition, we pursue phage display with an antibody fragment to study mutation rate and evolution by AID in prokaryotes. Our system supersedes animal immunization, and the smooth process will increase the ready availability of antibodies in various formats.

Team SDU-Denmark: Novel approach in the fight against obesity: modulating gut microbiota by probiotic inulin producing bacteria

Obesity is associated with a low-grade inflammatory response, which among other things, is triggered by bacterial plasma lipopolysaccharide (LPS). A high-energy diet, increases the amount of LPS-producing gut microbiota, and increased LPS levels has been observed in obese individuals. By inducing changes in the gut microbiota by prebiotics, like inulin, it is possible to decrease the plasma LPS level. This is associated with the stimulation of bifidobacterial growth. We have designed a novel approach to address this issue of plasma LPS, by probioticly induce changes in the gut flora by genetically modifying a bacteria to produce plant originated inulin. We cloned the two genes encoding sucrose:sucrose fructosyltransferase (SST) and fructose:fructose fructosyltransferase (FFT) from the Jerusalem artichoke into a E. coli, where it will produce inulin by using sucrose as an acceptor molecules. In the future this construct should be introduced by a probiotic lactobacillus, into the gut.

Team Slovenia: Switch-IT (Inducible therapeutics)

Currently, biological drug-based therapies require periodic invasive application. Often, due to their systemic administration, adverse effects are observed. Furthermore, large quantities of these substances are needed because of their distribution throughout the body. This, coupled with expensive production and especially purification, imposes a great burden on health systems. We aim to develop a safe and cost-effective biological delivery system for biopharmaceuticals, which would increase the quality of patients' lives, because it would minimize the number of required procedures. This type of delivery system would increase patient compliance to the therapy while the local administration will reduce the side-effects associated with current treatments. We plan to design the mammalian cells-based delivery system to be regulated by the digital logic from the outside.

Team St Andrews: Mind-full of Resources: Alternative Omega-3 Production and Novel Metal Recovery Methods

Omega-3 – known to prevent heart disease – is now causing governments to keep their finger on the

pulse... of the fishing industry. Fish stocks are fast depleting and alternative sources of these essential fatty acids are urgently required. Our re-sourcing idea: the creation of an Omega-3 biosynthetic pathway in E. coli, using genes from a Cyanobacterium. Mass spectrometry analysis detected polyunsaturated fatty acids in cells expressing our desaturase enzymes; normal cells have none.</br> Additionally, in seeking modern resource management solutions, specifically designed short peptide chains on the C-terminus of a GST fusion protein were expressed allowing the binding of precious and toxic metals. Such metals are often deposited in the environment. Ultraviolet-visible spectroscopy was used to demonstrate binding to our novel proteins.</br> Finally, we modelled the impact our 'Fatty Acid Factory' could have on total fish biomass before investigating the effect the iGEM Competition has in Science and elsewhere.

Team Technion: Trojan Phage

Viruses can be described as complex 3D structures capable of efficient infection of their target organism. Because of their highly specific infection ability, they can be used as vessels for 'smart' therapeutic strategies which rely on an agent that can effectively analyze the cellular environment and compute an appropriate response. To demonstrate the potential of a 'smart' strategy, we are developing a 'Trojan Horse' type of approach based on bactriophage-lambda.

Our project uses phage lambda and its target organism, E.coli, as a proof of concept for creating a system with predefined actions that demonstrates the described strategy. The design is based on a high specificity system which combines several different cell elements that will function as a type of logic AND gate. The phage will not harm the bacteria unless three independent conditions are met, activating the phage's lytic cycle and resulting in the bacteria's death; imitating a 'Trojan Horse'.

Team Trieste: The JOLLY JoCARE

Recent studies have evidenced that having a beneficial and healthy intestinal microflora is very important for human health. Our aim is to modify a bacteria normally found in human gut and create a safe, controllable and versatile molecular platform which can be used to produce a wide range of molecules leading to a beneficial probiotic. For this purpose we have chosen the E. coli strain Nissle 1917 which has been used for many years as a probiotic. We designed a robust gene guard system regulated by a novel and easy to control inducible cumate switch that activates the production of a human antimicrobial peptide LL-37 that can kill the bacteria and also avoid horizontal transfer. The safe probiotic constructed here can be used to produce nutritious, preventive or therapeutic molecules. For example, we have used it to produce an antibody against the emerging virus, Norovirus.

Team TU Darmstadt: From trash to cash: The PET.erminators are breaking new grounds in biological recycling

Polyethylene terephthalate (PET) has become the most widely manufactured synthetic polymer. With annual production exceeding 100 million tons (2010), it creates an issue of PET waste. In Western countries less than 70% of PET production is recovered by recycling. Biological processes play no role so far, only expensive chemical processes are applicable yet. PET waste left to erosion in the environment creates nanoparticles which tend to accumulate toxic substances. This poses a growing environmental threat and a serious health risk. Thus, developing new methods for PET degradation has become an urgent issue. Team TUD designed a bacterial recycling system that uses PET waste as a resource for synthesis of new chemical compounds. The proposed solution pursues PET decomposition into its monomers, transportation into E. coli and leading via terephthalic acid (TPA) to a high-value end product. The latter's

specification is determined by the inserted enzymes to build new metabolic pathways.

Team TU Munich: TUM-Brew: iGEM's first and finest SynBio Beer

The TU Munich iGEM Team engineers *Saccharomyces cerevisiae*, also known as baker's yeast, in order to lay the foundations for a new generation of functional foods with nutritionally valuable ingredients.

As an example, for iGEM's first "SynBio Beer" the compounds Xanthohumol (anticancerogenic), Limonene (limeflavor), Caffeine (stimulant) as well as the Thaumatin (protein sweetener) were chosen to demonstrate the spectrum of possibilities to complement traditional foods or beverages.

The metabolic pathways for these substances were converted to genetic BioBricks. Using the shuttle vector pYES2, which was adapted to the iGEM standard, transient transfection and expression in yeast were achieved. The gene products were subsequently characterized and their biosynthetic activities investigated.

Constitutive, alcohol-inducible and light-switchable promoter systems were developed, to individually regulate the expression of these gene cassettes. By combining these BioBricks our team has been able to brew iGEM's first and finest SynBio Beer.

Team TU-Delft: Snifferomyces

The aim of this year's iGEM project will be the synthesis of an olfactory device for the purpose of characterization of volatile compound. Here, the aim is to introduce olfactory receptor gene fusions into Saccharomyces cerevisiae and linking these receptors to a transcription response. Aims:

1. The diagnostics of the presence of tuberculosis bacteria in the lungs by sensing chemical compound methyl nicotinate by S. cerevisiae. For diagnostics, the response to these molecules is light, generated by the Lux proteins (visible blue light) or GFP (fluorescent green).
2. Introducing receptors for sensing the presence of banana-smell (iso-amyl acetate). This is done to see whether communication between S. cerevisiae and E. coli is possible by this volatile intermediate.
3. Supplying a toolkit which allows scientists to introduce olfactory receptors in yeast with minimal effort. Further we want to characterize the receptor parts submitted by the 2009 Hong Kong University.

Team TU-Eindhoven: SOMY – LCD, the Super Optimized Modified Yeast – Light-emitting Cell Display

Eindhoven, the city famous for its light bulbs, is the place where the roots of Dutch television lie. The iGEM team of the Eindhoven University of Technology developed an innovative electro-biological equipment which will be the replacement of your old television screen in the future! They proudly present to you the SOMY – LCD, the Super Optimized Modified Yeast – Light-emitting Cell Display. It's a multicolor display, in which genetically engineered yeast cells are electrically stimulated to induce a fluorescent light response and consequently function as pixels. Since calcium takes the leading part in this process, the yeast cells are engineered with fluorescent calcium sensors and extra voltage-gated calcium channels.

Team Tuebingen: Yeast based measurement system for endocrine disruptors in aquatic environments

Lacking a genetically strict sex determination system, fish are very sensitive to hormonally active agents in water. The extensive use of fertilizers and the inability of sewage treatment plants to break down drug waste lead to increasingly high concentrations of so-called endocrine disruptors in rivers. As a result, male fish have been found to be less fertile and even develop female sex tissue, so called ovotesties. Since fish spawn is constantly exposed to river waters, fish development is easily disturbed, and while the ratio of female fish increases, population numbers decrease. For sensing we use a membrane-bound receptor of Danio rerio. Activation will lead to bioluminescence which can be read out by photometric measurement.

Team ULB-Brussels: InteGreator

In synthetic biology, one of the main issues scientists and engineers must tackle is biochemical pathways optimization. In this project, we are going to develop an exceptional natural tool that could be used to optimize bio-production pathways: the integron. Integrons are genetic platforms which contain (re)movable gene cassettes. These integrons are mostly known to carry resistances to antibiotics. They are flanked with recombination sites which allow gene shuffling inside the integron thanks to a specific enzyme: the integrase. With appropriate selective pressure, this shuffling should result in optimized production. As a proof of concept, we are going to produce two antibiotics: Microcin C7 and Microcin B17. Two bacteria possessing the integron containing the antibiotics production gene cassettes, the integrase and a low resistance to the opposite antibiotic will be put in competition. With the integrase, we could change the natural order of the genes in order to optimize production.

Team UNITN-Trento: Crust Away

Statues and monuments all over the world are often covered in a disfiguring black crust caused by weather and pollution. Current methods to clean black crust are either too destructive or non-effective. The aim of our project is to develop a system to more gently restore statues and monuments. To achieve this goal, we engineered E. coli to eat the black crust. More specifically, we introduced an aerobic sulfate reducing pathway and a hydrogen sulfide producing pathway into E. coli. In this way, the sulfate component of the black crust is transformed into a gas, thereby degrading the offending substance without degrading the original material of the statue. In addition to our black crust project, we developed a ratiometric fluorescence platform to test transcriptional terminators and subsequently used the platform to compare the efficiencies of T7 and E. coli transcriptional terminators with T7 and E. coli RNA polymerases.

Team University College London: Plastic Republic - Bioremediation of Marine Microplastic Waste

It is in the Great Pacific Garbage Patch that we are confronted with the real consequences of human plastic dependency: an immense mass of accumulating microplastic particles floating just beneath the surface of the North Pacific Ocean. Where attempts at physical removal and biodegradable plastics have failed to solve this pollution disaster, synthetic biology steps in. UCL's project proposes the bioremediation of microplastic waste by two systems: degradation using a laccase enzyme or aggregation by controlled expression of curli. Ultimately we envisage the construction of habitable islands - turning waste into a resource. We used novel chassis: two marine bacteria, Oceanibulbus indoliflex and Roseobacter denitrificans. In line with considering the viability of our project, we questioned the access ordinary citizens should have to these tools. Initiating a new partnership, UCL teamed up with a group of 'biohackers' (citizen scientists in molecular biology) to create the world's first 'Public BioBrick'.

Team Uppsala University: Combating antibiotic resistance - Resistance is futile!

Serious infections caused by antibiotic resistant bacteria are a global healthcare problem. As the discovery of new antibiotics lags behind, we are developing new methods for targeting the resistance itself - making resistant bacteria sensitive to old antibiotics once again. Working with real-world resistance genes from multi-resistant bacteriaisolated at hospitals, we are developing anti-resistance systems to strike at three different levels: DNA, transcriptional and translational level. At DNA level, we develop a method for increasing resistance plasmid loss rate. At transcriptional level, we use super-repressors to repress transcription of resistance genes and native defense mechanisms. At translational level, we develop a modular system for high-throughput screening of sRNAs to silence resistance genes. We also provide tools useful for the whole synbio community, such as new standard backbones and methods for scarless gene deletion. With this team on this project, there is no question about it: Resistance is futile!

Team UTBC-RDCongo: E. coli as biodegradeur of organic waste (E. coli comme Biodegradeur des dechets organiques)

In our work, we used the Streptomyces coelicolor, which is known for degrading organic waste, and eschirichia coli as biological materials. We searched the gene of s. coelicolor responsible for the degradation of organic waste and have inserted it in the e. coli so that it can express this activity. We have genetically transformed bacteria in biodégradeur organic waste. We have cloned the expression on biodegradation for the s. coelicolor to the e. coli.

Team Valencia: Project Synechosunshine: photosynthetically powered biolamp

We present an artificial consortium between 2 specialized bacteria by the means of genetic engineering, in order to obtain a photosynthetically fed biolamp. It is a novel proposal of synthetic ecology, based on the use of an efficient photosynthesizer (the cyanobacterium Synechococcus elongatus) modified to become an exporter of sucrose and diel switch of the activity of Aliivibrio fischeri, a marine bacterium widely known for its bioluminescent properties in response to quorum sensing signals. Our modified cyanobacteria feed the population of A. fischeri through a transporter protein and produce AHL to induce bioluminescence in response to the activity of a photosensitive operator, which would activate only at night. We also have tried to transform different microalgae with bioluminescence genes to test their effectiveness. We look forward to develop an efficient and autosufficient environmentally friendly biolamp, with potential application to cover the illumination needs of many infrastructure sectors.

Team Valencia Biocampus: Talking Life

Do you speak to your bacteria? We do. We have designed, constructed and characterized an inter-specific translator based on light pulses that allows to literally dialogue with microorganisms. We have built seven biobricks with fluorescent proteins under the control of environmentally-sensitive promoters. The process is as follows: human voice messages are electronically- and then light-encoded in excitation wavelengths, and microbial proteins' emission wavelengths are electronically- and voice-encoded back. We have used this system to find out the fermentative status of budding yeast and to dialogue with E. coli allowing it to answer questions such as "are you hungry?" The three pillars of our project (human practices, modeling and wetlab) yielded continuous feedback with each other, illustrating an integrated interdisciplinary approach. For example, in human practices, we qualitatively analysed the risk of cheater mutants ("liers"), which was quantitatively supported by our results in both our modeling simulations and in the wetlab.

Team Wageningen UR: A standardized tool for site specific drug delivery using Virus-

Like Particles

Medicines are generally active in a non-site-specific fashion, affecting the whole patient, including healthy tissue. Therefore, we attempt to specifically target diseased areas by packaging medicines inside Virus-Like Particles (VLPs). VLPs are not infectious, as they are built solely from viral coat proteins. We designed a modular Plug and Apply system that enables modifications to these coat proteins. The system facilitates the linkage of numerous ligands to the coat protein, thereby creating site-specific carriers. After expression of coat protein genes in Escherichia coli the VLPs were assembled in vitro, yielding modified Virus-Like Particles. Medicines can be packed using the Plug and Apply system or simply by addition during VLP assembly. Concluding, VLPs can be used as universal carriers for site-specific drug delivery, allowing customization to a variety of diseases while decreasing side effects for patients during treatment.

Team Warsaw: B. subtilis: supporting actor of the iGEM stage

The iGEM community is far focused on Escherichia coli as the model organism, and a vast majority of available BioBricks is designed to work in this chassis. We would like to encourage working with another important bacteria, Gram-positive Bacillus subtilis, thus our project aims at obtaining new parts dedicated to this micro-organism. We also design a mammalian BioBrick that opens a new pathway into 'bricking' eucaryotic cells. Our idea is to construct a system enabling us to achieve expression of gene of choice inside a mammalian cell. This system consists of two parts: a shuttle vector, working both in B. subtilis and in eucaryotic cells, and an invasive strain of B. subtilis. Invasiveness would be achieved by expression of listeriolysin.

Team Westminster: iSTEM (Intelligent Synthetic Tumor Eliminating Machine)

We have created a genetically engineered machine to identify, isolate and eliminate Cancer Stem Cells (CSCs). According to the latest Cancer Stem Cell Theory, not all the cancer cells have the same ability to generate new tumors. Tumor growth is mostly driven by a small proportion of cells, the CSCs. In addition to having high proliferation rates, CSCs are more resistant to chemotherapy. This indicates that while regular cancer cells are killed, CSCs may remain unaffected and give rise to new tumors once the treatment stops. CSCs produce increased levels of a particular enzyme, Aldehyde Dehydrogenase. We have identified its 3 most frequent isoforms (ALDH1A1, ALDH1A3 and ALDH3A1)in aggressive types of cancer, and used their gene promoters to build our CSC-targeting constructs: the iSTEM -Intelligent Synthetic Tumor Eliminating Machine.

LATIN AMERICA

Team Buenos Aires: Synthetic ecology

We aimed to create a stable community of microorganisms that could be used as a standard tool. Our system would allow the co-culture of several genetically engineered machines in tunable proportions. Hence the engineered organism would be a standard part! This defines a new level of modularity allowing the increase of the complexity of the system by moving to the community level. We´ve come up with several plausible circuits designs and in silico predictions and decided to build a “crossfeeding” system in which each strain produces and secretes an aminoacid the other strains need to grow. We characterized two auxotrophic yeast strains (for tryptophan and histidine) and designed novel biobricks that regulate the export of Trp and His rich peptides. In the future this would allow for other modules to control the

proportions of each strain, thus allowing dynamic and stimulus dependent changes in the abundances of each strain.

Team Ciencias-UNAM: Synthetic CO2 biosensor

Our goal is to generate a bio-brick which indirectly detects atmospheric CO2 by noticing the compounds formed by the CO2 dissociation in water as result of the enzymecarbonic anhydrase. This reaction is done almost instantly and it is used as a signal by distinct organisms like E.coli, C.albicans associated in the regulation of emergency signaling. Also, an association of the H+/HCO3- ratio and the signaling pathway of adenilate cyclase has been seen. The employment of these genes with reporter proteinscan be used to indirectly calculate the concentration of environmental CO2. The CO2 concentration depends on the partial pressure and the speed with it is assimilated by the liquid. The fastest it gets to the saturation point 5%, the higher is the CO2 concentration.

Team CINVESTAV-IPN-UNAM MX: Rhodofactory, controlling genetic expression: an oxygen and light response

The metabolic versatility of purple non-sulfur photosynthetic bacteria allows them to grow in light, darkness and with or without oxygen; all it is due to their genetic regulation mechanisms. Taking advantage of this, our project aims to build two genetic control systems based on R. sphaeroides photosynthesis cluster regulation. The first one is a light dependent system controlled by two proteins AppA/PpsR that works like an antirepresor/repressor mechanism, and the second one is an oxygen dependent system of two-component called PrrA/PrrB. This two devices were tested on R. palustris chassis, using a cassete in which a reporter (GFP) is regulated by external conditions that activate or repress its expresion. Once we have characterized the functionality of these networks, our perspective is to develop a Rhodofactory, it means to control the produccion of differents metabolites, such as biodiesel and butanol, using simple signals.

Team Colombia: Pest-busters

We are developing a modular synthetic system that is able to recognize pathogen-associated molecules from either fungi or bacteria, aiming to speed up the activation of the plant immune system in an infection process. Three major parts comprise the system: A chitin-sensor system that is activated in the presence of Hemileia vastatrix (coffee rust) or, alternatively, a device that senses 3OH-PAME, a diffusible signal of Ralstonia solanacearum, cause of bacterial wilt. A second construct, the communication device, receives the input from the sensor device and starts the production of salicylic acid, a plant hormone that stimulates an hypersensitive response in the plant. The third part, toxin-antitoxin systems, will be either placed in the final plasmids or in the chromosome in different combinations, causing the cells to stay dormant most of the time without pathogen presence, and also ensuring no transfer of plasmids to other cells.

Team Costa Rica-TEC-UNA: Cibus 3.0: A novel bacterial system for biodiesel production using whey as feedstock

Cibus 3.0 takes biodiesel production to a new level. Our idea consists in the modification of two bacteria: Rhodococcus opacus and Escherichia coli, both maintained in whey based medium (which in our country is produced by hundreds of thousands tons per year). Overexpression of the natural TGA producing ability of R. opacus is achieved by inserting an optimized sequence of a DGA acyltransferase gene, constitutively expressed, and an inducible “suicide device” in order to extract them with ease. </p> On the other hand, E. coli is transformed with an optimized sequence of a B. cepacia lipase which is secreted to the medium where we extract it continuously and encapsulate it. Now all what it takes to finish the job is adding our

encapsulated enzymes to the extracted TGAs and mixing them with some ethanol to obtain our biodiesel! </p>

Team Panama INDICASAT: Genetically Modified E. coli as an Alternative Biosensor of Cyanide and Cyanide Compounds

Cyanide is considered an extremely harmful toxic for the environment and living organism's compound, since it inhibits the cellular respiration at the level of electron transport chain. In the industrial sector, cyanide is used to produce paper, paints, textiles, plastics and in the mining industry as a way to recover metals. In this project, we will incorporate genes that will allow the bacteria to become a biosensor with the capacity to detect the presence of cyanide and cyanide compounds by adding the expression of cyanide resistant genes (cioAB) and a reporter gene under the control of an inducible promoter. This new technique will also become a platform so that in the future we could incorporate a gene that allows the bacteria, not only detect, but also to degrade these compounds using a method that is accessible and environmentally friendly through bioremediation.

Team Tec-Monterrey: Development of a freeze resistant E.coli strain and an allergy detection kit produced by P.pastoris.

The development of synthetic biology has eased the production of innovative materials, two scenarios have come to our attention: the medical/clinical field and the improvement of laboratory protocols. Our team, Tec-Monterrey, decided to develop two projects including a freeze resistant cloning Escherichia coli strain and the harnessing of bioproducts from Pichia pastoris followed by their assembly into a novel standardized allergy detection kit. The aim of our first project is to develop a new strain of E.coli capable of expressing an antifreeze protein from the Rhagium inquisitor (RiAFP), resulting in a cloning strain capable of sustaining its viability over the cryopreservation cycle. The goal of our second project is to develop an affordable, standardized allergy detection kit, integrating components that are both easily manufactured and purified. Moreover, the kit's components can be produced either in P. pastoris or E. coli thanks to the design of our engineered shuttle-expression sequence.

Team Tec-Monterrey EKAM: Implementation of a Fine-Tuned Modular Expression System in the P. pastoris Yeast

Pichia pastoris, an alternative expression system for gene products requiring post-translational modification, is hereby utilized to construct an optimized system for producing terpenoids (aromatic hydrocarbons with advantageous biochemical activity: antimicrobial, antineoplastic, and other pharmaceutical properties). Truncating an enzyme previously shown to limit counterproductive metabolic regulation in terpenoid synthesis, together with a genetic construct design enabling the setup of a modular biofactory, potentially poses a foundation for applied interchangeable systems in synthetic biology. The need for reliable promoters for P. pastoris is addressed by characterizing four inducible promoters as standardized parts useful in controlling gene expression, with GFP as a reporter. The functionality of peroxisome-targeting signal PTS1 for use on the C-terminus of gene products is also analyzed. Difficulties in designing laboratory protocols and engineering genetic constructs are approached by developing a multi-purpose software tool to solve methodological obstacles, as well as facilitating implementation of a modular design on future projects.

Team UANL Mty-Mexico: E. cologic: Arsenic biosensor and chelator with scalable silica-binding recovery system

One of the major environmental problems in northeastern Mexico is arsenic contamination of groundwater.

Several projects have previously aimed to biorremediate heavy metals and metalloids using bacteria, but without scalable potential due to the lack of an efficient cell recovery system. We aim to develop an easy-to-recover arsenic biosensor and chelator. Recovery strategy will consist of a new adhesion mechanism that enables bacteria to bind to silica surfaces through the expression of the L2 ribosomal protein, attached to the outer membrane protein AIDA-I. A quantifiable, highly-sensitive luciferase-based reporter system coupled to an oligomeric metallothionein is expected to increase our system's capability of arsenic sensing and chelation.

Team UC Chile: Luxilla: a light rechargeable and programmable biolamp

Synchronization of biological processes in populations is essential to achieve strong measurable and functional traits. Circadian rhythms are one of nature's most exquisite mechanisms to regulate and synchronize biological processes over time. Our team has taken advantage of the fine time control offered by the circadian clock machinery to construct a genetic circuit that allows robust oscillatory behavior in a synchronized and predictable manner. We have coupled the expression of genes of a bioluminescent pathway to the endogenous circadian clock of Synechocystis PCC 6803. The benefits of using Synechocystis as our chassis for practical applications include minimal production costs due to its autotrophic growth capacity and precise synchronization to time-dependent events using environmental cues such as light. As a direct application we designed the first self-rechargeable programmable bio-lamp. Secondary projects include a novel secretion system, the first spider-silk biobrick and an optimization of the Gibson assembly reaction for small parts.

Team UNAM Genomics Mexico: Bacillus booleanus

Bacillus booleanus is a project that wants to create a "molecular computer". How it works? We are working on the creation of different strains of Bacillus subtilis, each one will be able to perform a single Boolean operation just like a transistor. A single transistor is not a computer, they need to communicate with others to perform new logic operations, but how our bacterial transistors can communicate? In 2011, Ben-Yehuda et. al. identified a type of bacterial communication mediated by nanotubes that bridge neighboring cells, providing a network for exchange of cellular molecules within and between species. By using these nanotubes our bacterium will be capable to communicate with others so that create complex networks of logic gates. Using this it could be possible to develop a complex network of 'transistors' to create, for example, a synthetic metabolic pathway.

Team USP-UNESP-Brazil: Hackology - Hacking BioSystems

Our group purpose is to discover and develop new ways of hacking and modifying biological systems. We developed two projects, which aims are to introduce new properties in a system and to gain control over the information processing. The first one hacks the way of transforming cells. It inserts and transcribes any protein inside E. coli, using only two steps: PCR and transformation. Using the Cre recombinase action and sequences flanked by loxP modified sites any open reading frame could be inserted and expressed in a plasmid ready to receive it inside the bacteria, called Plug&Play Machine. The second one is a way to build a bacteria network with memory capacity, which works as a Hopfield Network. This network could, by means of quorum sensing, recognize a given pattern (input), process the pattern and reach an output state. The output depends on two possibilities already imprinted in the memory.

Team UTP-Software: Biofuel Tool Kit

Today, there is a growing need for new energy sources that are accessible and inexpensive. The most

popular and greens sources are the biofuels. One of the main problem is that the processes to produce biofuels are not cheap neither efficient. With this in mind, the team UTP-Software 2012 seeks to develop a tool that help other teams and researchers to work and study the production of biofuels through synthetic biology. Our target biofuels are: • Biohydrogen • Bioethanol • Biodiesel • Methane Our tool aims to facilitate the study and development of these bio fuels by analyzing routes from the substrates for the reactions to identify the genes responsible for each enzymatic reactions that could produce these biofuels.

2010

Team Aberdeen_Scotland: The AyeSwitch: a translationally regulated genetic toggle switch in yeast

A novel genetic toggle switch regulated at the translational level was engineered in yeast that allowed the mutually exclusive expression of either green or cyan fluorescent protein. Using cell cytometry (FACS) and fluorimetry, we demonstrated in yeast the successful expression and translational regulation of a fusion of mRNA binding proteinand fluorescent protein. These results, along with published parameter values, were used to predict via deterministic and stochastic models that the probability of successful bistability for our switch was 0.96%, but this could be improved theoretically to a maximum of 51.27% by limiting the range of variation of the most sensitive parameters. The models also predicted that co-operative binding of the mRNA binding protein to its mRNA stem loop was essential for generating switch-like behaviour. These results suggest that a translationally regulated genetic toggle switch is a viable and novel engineering concept applicable to medicinal, environmental and technological problems.

Team Alberta: GENOMIKON: An Educational Tool Kit for Rapid Genetic Construction

Building DNA is too hard. Democratizing Synthetic Biology will demand fundamental advances to make DNA construction easier and cheaper, thereby enabling broader access to biotechnology by the public. Our team has tackled this challenge with the design of an inexpensive self-contained kit called GENOMIKON, currently targeted for the high school and DIY communities but with clear potential for professional use. The kit contains prefabricated parts that are sequentially assembled on a solid support using cycle times of 5 min./part with a coupling efficiency of ~95%. The parts exist with sufficient diversity and quantity for hundreds of unique experiments. The kit is accompanied by an online resource that serves as lab manual, notebook, information management system and social network for the exchange of ideas. While similar in concept to our last year’s project, GENOMIKON differs in most technical aspects and is far superior in performance.

Team ArtScienceBangalore: Synthetic and Post-Natural Ecologies

In our second year as artists and designers at IGEM, we have decided to investigate the consequences of creating a Synthetic Ecology: an ecosystem in which organisms designed for a techno-scientific environment interact with organisms in the wild. C.elegans live on a diet of a variety of bacteria, E.coli being such strain. Genetically-modified E.coli can be fed to C.elegans which can then express any double stranded RNA of interest. The dsRNA can knock off specific genes in C.elegans. In our experiments, we are using C.Elegans as a marker to expres a range of external factors in two sets, temperature and IPTG. On a utilitarian level, our project investigates the use of C.elegans as a visual marker for changes in environmental conditions. On a more critical level, C.elegans is used to study the consequences of interactions between engineered organisms and the 'natural' world.

Team Baltimore_US: DIY-GEM: a path towards low cost high throughput

gene synthesis

Synthetic biology research requires more cost effective approaches toward reagents and hardware accessibility. We are developing low-cost alternatives to existing hardware and enzymes in an attempt to expand participation in biological research and development. Our project expands the accessibility of Taq Polymerase by engineering it in a form compatible with BioBrick assembly. This allows use of the over-expressed enzyme from a crude bacterial extract in a PCR reaction at a fraction of the cost of highly purified commercial enzyme. In addition, we have developed inexpensive and easily assembled lab equipment such as a gel electrophoresis apparatus and a PCR thermal cycler. Enabling researchers to synthesize their own enzymes and having access to inexpensive tools will allow for increased participation among the DIY-bio community, stretch increasingly scarce educational funds, and allow rapid scale up of large scale gene synthesis projects.

Team BCCS-Bristol: agrEcoli: Smarter farming through bacterial soil fertility sensors

Fertiliser production is a major contributor to global carbon emissions, and excess fertiliser can cause immense damage to local ecosystems. Our lab has developed and characterised a cheap, versatile soil fertility sensor based on an E.coli chassis. It expresses fluorescent signals upon nutrient detection, producing a high-resolution nutrient distribution map of arable land. The ratio of two fluorescent signals allows farmers to quantify soil nutrient content. agrEcoli bacteria, encapsulated within a gel container to improve visibility and prevent escape, have been shown to work on soil in lab conditions. We have explored the marketing of our device, considering public perceptions of synthetic biology. BSim, our prize-winning modelling framework, has been extended to analyse our new biobricks' behaviour within gel capsules. In addition, a new interface for BSim has improved its accessibility to the wider synthetic biology community, facilitating collaboration. agrEcoli optimises fertiliser use, saving farmers' money and reducing environmental damage.

Team Berkeley: Choachoa's Delivery Service

Single-celled phagocytic eukaryotes like Choanoflagellates are of great interest to developmental biologists because they may be the last living immediate precursor on the evolutionary tree to animals. These easy to culture and robust organisms are also a desirable eukaryotic chassis for synthetic biology, but there are few tools for delivering biomolecules into these organisms. So, we engineered E. coli to deliver proteins and/or DNA payloads into these bacteria-devouring eukaryotes. Once ingested, our E. coliare programmed to self-lyse and porate the phagosome, releasing their payloads into the cytosol. This delivery mechanism has the potential to deliver payload to any phagocytic organism with a cholesterol-based membrane. As part of our parallel software effort to rework the Clotho plugin environment and API, we made automatic biosafety handling an intrinsic feature of the core. Together, these tools provide a foundation for metazoan synthetic biology and a framework for improving safety in our field.

Team Bielefeld-Germany: MARSS - Modulated Acetosyringone Receptor Sensor System Defining Spiciness since 2010

The iGEM-Team Bielefeld is going to modulate an Agrobacteria receptor in Escherichia coli in order to detect capsaicin which is responsible for the hot taste of chilies. The intention is to make the spiciness in fare visible using a gradient light signal. The original receptor is the acetosyringone detection system of Agrobacterium tumefaciens. By using directed evolution, we aim to modulate the receptor binding domain

to enable the interaction with similar phenolic substances like capsaicin. Brought into E. coli, this modulated system will induce light effects of different intensities - depending on the concentration of capsaicin respectively the spiciness of the sample. The capsaicin detection is a proof of principle concept. We aim to establish a system, which is characterized by a high sensitivity and specifity and is capable to replace slow and high priced diagnostics or analytic methods. The targets of the system could be allergy-triggers, explosives and toxins.

Team BIOTEC_Dresden: SensorBricks

SensorBricks is a reliable and modular system for antigen recognition, signal amplification and quantification. Initial steps of SensorBricks will focus on the detection of CD33 and other leukemic markers to increase diagnostic stringency. There are three major components in SensorBricks: (i) monoclonal antibodies that bind to an antigen of interest, (ii) a LuxI-Protein A fusion construct which non-specifically binds antibodies and produces the autoinducer N- Acyl homoserine lactone (AHL), and (iii) a Escherichia coli based biosensor which strongly amplifies the production of a fluorescence protein in the presence of AHL. By coupling signal detection to a genetic circuit, we would be able to amplify the signal in a quantifiable manner, allowing the identification of cancer markers expressed in minute quantities.

Team British_Columbia: A Multi-pronged Approach to Eliminating Staphylococcus aureus Biofilms Using RecombinantBacteriophage and Biofilm-Degrading Enzymes

Biofilms are ubiquitous microbial communities that often display greater resistance and pathogenicity compared to individual microbes. Biofilms commonly cause complications in both industrial and medical settings and represent a significant source of morbidity and mortality. A synthetic biology approach to tackling biofilms has only recently been applied to Escherichia coli biofilms. To eliminate the more clinically relevant Staphylococcus aureus biofilms, our team aims to break new ground at iGEM by using S. aureus as a model host and developing a standard for genetically engineering bacteriophages. Our design incorporates DspB, a biofilm matrix-degrading enzymeinto the Φ13 phage genome, which is altered to operate under the regulation of the S. aureus agr quorum sensing pathway and thus upon contact with biofilms. As a complement, we have also developed a mathematical model that simulates the dynamics of our system under different conditions.

Team Brown: Light Pattern Control of Cell Circuits

Biological manufacturing of complex compounds often requires the synthesis of many intermediate products. Production of these intermediates is currently triggered by inefficient methods, such as chemical inputs (tetracycline, estrogen-analogs, arabinose, etc) or drastic changes to the cellular environment (pH, oxygen levels, temperature, etc). On an industrial scale, this chemical induction requires large quantities of reagents and extensive purification, while environmental induction requires conditions that can adversely affect cell vitality and yield. To this end, we have designed an E. coli genetic circuit that can pass through four stable states of protein production triggered solely by ON/OFF patterns of light. To efficiently test the components of our circuit, we have also created a system for the transient delivery of transcription factors through the cell and nuclear membranes. With this production method, we can link multiple synthesis steps to a single, clean and rapidly scalable input.

Team Calgary: Translating Stress Into Success

The majority of projects in synthetic biology involve the over expression of recombinant proteins in microorganisms. A major stumbling block however, is often an inability to express functional protein. This situation is difficult to manage and troubleshoot as it is often unclear why expression is failing. We have designed a system that can accurately and visually report whether a gene is being transcribed and/or translated. The system also differentiates whether expression is failing due to misfolding in the periplasm or cytoplasm. In the case of misfolding, our system can also fine tune expression levels of a given protein to optimize production, increasing the likelihood of obtaining functional protein. To further understand protein misfolding we have built an equation-based, multivariant model of inclusion body formation. Finally, we used a series of podcasts to explore the social implications of our project in the context of the growing synthetic biology industry.

Team Caltech: Towards the Production of a Bioplastic Bioprinter and Design for a General Printing Framework

Our goal for the was to create and print a bioplastic, polyhydroxybutyrate (PHB), from soybean oil using E. coli. Our proposed design uses a radical crosslinking reagent to crosslink PHB monomers in cell lysate, released upon a light-induced lysis gene network. We hope to apply this printing ability to three-dimensional printing, offering a cheap alternative to current rapid-prototyping technologies. Our work involves characterizing an infrared promoter for light-lysis, experimenting with PHB production in cells, and the design of a dual-wavelength printing system. We discuss how this system could be generalized to create a framework for actuating groups of cells in any 3D volume to theoretically modulate behavior more complex than lysis. We also plan to apply special consideration to the ramifications of possible commercial enterprises developed in iGEM competitions with open source biological materials, such as BioBricks™.

Team Cambridge: E.glowli: a bioluminescent future

Bioluminescence is one of the most striking spectacles in the natural world. Taking genes from fireflies and Vibrio fischeri, the Cambridge team have constructed BioBrickswhich allow light output at a wide range of wavelengths. Firefly luciferase is already used as a reporter, but requires continual addition of the expensive substrate luciferin. We have created codon-optimised operons combining luciferase with a luciferin regenerating enzyme (LRE). This allows recycling of luciferin for sustained light output. In addition, we have submitted the first lux operon to the registry, taking genes from bacteria which form symbiotic relationships with squid. This is the first BioBrick to emit light without addition of substrate and can be used as a reporter with any promoter. These two approaches will allow cheaper assays with brighter signals. We also hope they will lay the foundations for natural light sources that help to address the energy crisis facing our planet.

Team CBNU-Korea: Design and Construction of Synthetic Minimal Chromosomes

Most of all bacteria have single circular chromosome. But some bacteria have two or more circular chromosomes. In Vibrio cholerae, there are two circular chromosomes, chromosome I and chromosome II, and each perfectly works as a chromosome. We've been motivated by V.cholerae's two chromosome system. So we employed some essential genes, parA, parB, parS, dif, and origin of chromosome II and contstructed a tiny miniature of V.cholerae's two chromosome system in E.coli, using BioBrickassembly method. Also, we built software and database of essential genes for designing of minimal synthetic chromosome and genome. Essential gene informations were gathered from some databases, DEG, EGGS,

NCBI and java language was used. Our final goal is making useful, safe and stable synthetic minimal genome for Synthetic Minimal Cell. Although our project is feeble, we extremely believe that our project in this year will be worth first step for that.

Team Chiba: Eliminating the False-Input ~Genetic Double-Click System~

We daily double-click the icons to open the files or to exert the program: this is clearly distinguished from the single click, which is often for selecting or highlighting the program. This year, iGEM CHIBA is constructing genetic double-click system whose output is released only when the input (inducing agent) is given twice within a limited time. To discriminate double-click from two separated single-clicks, the 1st input is to be memorized temporarily. If the 2nd input is added before the memory gets lost, output will be produced. If the 2nd input is not added within the given time, the system will be reset to the original state. This mechanism could work as a sort of safety device; by requiring the 2nd 'confirmation' input, one can drastically reduce, or even eliminate, the frequency of false-inputs. This system could be useful in operating the potent or potentially-hazardous biochemical processes.

Team Cornell: OMG OMVs!

Outer membrane vesicles (OMVs) are natural secretions by gram-negative bacteria that can transport various proteins, lipids, and nucleic acids in interactions with mammalian host cells. OMV technology presents an affordable, non-toxic, and direct method of drug delivery and antigen tracking. We have designed a method for visualizing the interactions of mammalian cells with outer membrane vesicles by utilizing the ClyA surface protein as an attachment site for fluorescent proteins. The current goal of this project is to characterize the distribution of varying ClyA-fluorescent protein complexes on OMVs. Future work will be to develop a tracking system employing a ClyA-fluorescent protein construct for in vitro microscope imaging. An antibody fragment will also be attached to another ClyA complex, allowing the OMV tracking system to target specific regions of an organism. This method allows in vitro characterization of OMVs and provides integral data for developing a future OMV delivery platform in vivo.

Team Davidson-MissouriW: Foundational Advances in Biology and the Knapsack Problem

We focused on the Knapsack Problem which asks, "Given a set of weighted items and a knapsack of fixed capacity, is there some subset of these items that fills the knapsack?" Weighted items are represented by TetA alleles that confer measurably distinct levels of tetracycline resistance in E. coli. Excess TetA kills the cells; insufficient TetA can be screened by plating on tetracycline plates. Each TetA allele is coupled with a distinctive fluorescent gene, and both are flanked by variant lox sites. Cre proteincan invert or excise floxed DNA, yielding different combinations of expressed TetA alleles. We constructed different TetA alleles by altering codon optimization and characterized the consequence of changing the order of two genes (TetA and RFP). Furthermore, we designed and tested a total of 11 new lox sites for site specific recombination. We developed several open access software tools for the wider synthetic biology community.

Team Debrecen-Hungary: The lipid sensor eukariotic toolkit

Eukaryotic synthetic biology has huge potential, yet it is still in need of more diverse molecular tools for defined gene regulation. Nuclear receptors are a conserved family of proteins responsible for sensing lipids; they may be viewed as lipid activated transcription factors. We have successfully developed a kit with a variety of lipid responsive domains (from H.sapines, D.melanogaster and C.elegans) for the rational construction of synthetic transcription factors. The domains respond only to predefined lipids and

selectively activate predetermined gene expression. To characterize theses domains, we used standardized protocols for comparable measurements. In vivo gene expression was measured as a function of ligand concentration using luciferase activity. The potential for these tools is immense; e.g. from the ultra sensitive detection of lipid contaminants in the environment to the opportunity of titration specific gene expression canges in patients undergoing gene therapy.

Team DTU-Denmark: Bi[o]stable – Engineering a bistable switch

The aim of this project is to engineer a genetic bi-stable switch that produces two different, mutually exclusive outputs when given two different inputs. The switch is based on the repressor-anti-repressor system of the salmonella phages Gifsy1 and Gifsy2 and the λ-phage anti-termination system. The latest induced output will remain stable through generations, even once the input ceases, due to the phage regulatory systems. We present the framework for this development and characterize the regulatory mechanisms by using fluorescent proteins as the reporter (outputs). The dynamics of the system have been modeled and we have also attempted to characterize and submit the promoters, repressors and anti-repressors from the salmonella phages, as well as the two anti-terminator proteins from the lambda phage, as BioBricks. We have hereby demonstrated the engineering of a multipurpose bi-stable switch sensor/reporter tool that can have numerous applications.

Team Duke: Engineering a Robust Genetic Switch

Our project aims to produce a genetic transistor which, unlike most bistable switch mechanisms available to synthetic biologists, does not exhibit basal regulatory noise. The transistor will be based on a protein sequestration pathway that uses leucine zippers (bZIPs) Fos and Jun alongside synthetically designed dominant negatives thereof, eliciting a response dynamic similar to a signal titration. Furthermore, we intend to apply such transistors to function as signal amplifiers due to the ultrasensitive responses that can be generated in this mechanism. For the application of this project and others, we are also developing a high throughput gene expression screen for synthetic genelibraries and codon variants, allowing for the possibility of tunable gene expression levels.

Team ECUST-Shanghai: _

Team Edinburgh: Communicating Through Bridges: Bridging with Biology, Bridging with Light, Bridging with People

The engineering equivalent of Genetic Engineering is to get a bunch of concrete and steel, throw it into a river, and if you can walk across it, call it a bridge. Synthetic biology and iGEM have long?attempted to refine this process of 'bridge-building'. The 2010 University of Edinburgh team has applied this idea comprehensively throughout their project. The BRIDGE protocol (BioBrick Recombineering In Direct Genome Editing) is a protocol for markerless insertion of BioBricks onto the bacterial chromosome, which will bridge ideas and reality in synthetic biology. Bacterial BRIDGEs aim to foster non-chemical means of communication between bacteria by pairing light-producing and light-sensing BioBricks; future teams may make use of them in a variety of novel applications. Finally, human BRIDGEs examine synthetic biology as ways of thinking and the permeation of human aspects, bridging the so-called 'divides' between disciplines and individuals. The question is... how do you think?

Team EPF_Lausanne: Asaia, the pink force against malaria

Malaria is a tropical disease that kills more than 1 million people each year and no effective cure or vaccine exists yet. The EPFL iGEM project aims to stop malaria propagation by acting on the vector: the mosquito.

We are engineering Asaia, a bacterium that naturally lives in the mosquito's gut, to express an immunotoxin that can prevent the malaria agent Plasmodium falciparum from infecting the mosquito, thereby eliminating the transmission of this parasite to humans. Asaia is an organism that is easy to grow and genetically manipulate. We are establishing Asaia as a new chassis so that future iGEM teams can quickly and efficiently engineer new and more potent Asaia strains. This will provide the synthetic biology community with a useful tool in the fight against malaria and other mosquito-borne diseases.

Team ESBS-Strasbourg: A light-controllable specific protein degradation system as new standard for synthetic biology

The aim of our project is to engineer a new fundamental component that could be universally used to build more complex or more controllable biological circuits inside chassis organisms. This new component consists of the E. coli protease ClpXP to which the phytochrome B (PhyB) of Arabidopsis thaliana is fused. Any given protein can be degraded as long as it is fused with the Phytochrome Interacting Factor (PIF)-degradation tag biobrick. The activity of this system is tightly controlled and switchable by light inducement.

Team ETHZ_Basel: E. lemming – a remote controlled living robot

We control the movement of a single E. coli cell by light. In wild type E. coli flagella movement is controlled by proteins of the chemotaxis pathway, so called Che proteins. In our engineered cells one of these Che proteins is fused to a synthetic light-sensitive localization system. Two external inputs – red light and far red light - induce the relocation of the fused proteins, thus reversibly changing flagella movement direction. Cells, imaged by bright field microscopy, are automatically detected and tracked while a closed loop controller guides the cell into a user defined direction by autonomously sending light inputs. This makes our engineered cell the smallest remote controllable living robot on earth.

Team Freiburg_Bioware: A Modular Virus Construction Kit for Therapeutic Applications

Gene delivery using viral vectors holds great promise for the treatment of acquired and inherited diseases. The human Adeno-Associated Virus (AAV) is a small, non-pathogenic, single-stranded DNA virus gaining increasing attention being both versatile and effective. Taking current knowledge into account, we generated a recombinant, modularized, BioBrick-compatible AAV ‘Virus Construction Kit’. We provide parts for modified capsid proteins, targeting modules, tumor-specific promoters, and prodrug-activating enzymes as well as readily assembled vectors for gene delivery and production of non-replicative virus particles. The viral tropism is altered by N-terminal fusion or by loop replacement of the capsid proteins. Functionality of viruses constructed from our kit was demonstrated by fluorescent protein expression in infected cells and by prodrug-induced killing of tumor cells upon viral delivery of a thymidine kinase. Incorporating multiple layers of safety, we provide a general tool to the growing field of personalized medicine and demonstrate its use in tumor therapy.

Team Freiburg_Software: SynBioWave 2.0 – A Collaborative Toolkit for Synthetic Biology

SynBioWave is an open-source, Synthetic Biology software suite based on Google’s open-source communication tool Wave. SynBioWave enables research collaboration by real-time sharing of parts, design and documentation. Moreover, biologists can record and share the process of creating research data. Last year our team developed the basic SynBioWave robot. This year we ported the main program (Robot) to Wave API 2.0 and improved user friendliness, separated the input and output from the sequence

database operations by creating a linked wave for data storage. We also provide the “blueprint-robot”, a framework easing new robot development. Furthermore, we are adding new functionality by creating add-on robots that perform tasks such as BLAST-searches, ORF-finding, translation, sequence alignments and restriction site mapping. The main robot is available at SynBioWave@appspot.com, the source code at http://synbiowave.sourceforge.net and the homepage of the project is http://www.synbiowave.org.

Team Fudan-Shanghai: _

Team Gaston_Day_School: Construction of a Biological Iron Detector in a Secondary School Environment

Our team’s project was to create a biological iron detector using techniques and procedures available to an ordinary high school laboratory that replicate methods used in university research laboratories. We constructed our reporter by combining an iron-sensitive promoter with a red fluorescent protein (RFP) coding sequence. We chose RFP because of its high visibility and easy detection. Although the assembly was successful, the resulting detector is leaky with measurable RFP even in conditions with no iron present. In our lab environment, we found that it was necessary to work with relatively high concentrations of bacteria and DNA. We developed simplified procedures for transformations, digests, and ligations, but we continue to face problems with DNA visualization and measuring the pigments from the bacteria.

Team Georgia_State: Pichia pastoris: A Novel Chassis for iGEM

The methylotrophic yeast, Pichia pastoris, is increasingly used as an alternative host for heterologous protein production. P. Pastoris is advantageous because it is able to perform eukaryotic post-translational modifications, produce high yields of recombinant protein, and it is genetically similar to Saccharomyces cerevisiae. (Cereghino and Cregg, 2000). The 2010 Georgia State team believes P. pastoris would be an excellent chassis for the iGEM competition. The purpose of this project is to provide a tool box of parts necessary for the genetic manipulation of this organism. These parts include a variety of promoter systems, multiple selectivity options, and a plasmid backbone. In addition, the tool box will be used to produce a flu virus antigen in P. pastoris as a representation of the applicability of this system. These contributions will enable future users to maximize the use and further explore the incredible potential P. pastoris has to offer.

Team GeorgiaTech: Inducing a Thermogenic Response to Cold-shock in Bacteria

Alternative Oxidase (AOX) is a terminal oxidase protein found in the respiratory chain of various organisms ranging from aquatic prokaryotes to plants and animals. In the AOX pathway, electrons are transferred from ubiquonone to AOX, and then directly used to reduce oxygen. The drop in the electric potential energy of the electrons transferred from AOX to oxygen is dissipated as heat. Our project has focused on 1) cloning the AOX gene from a thermogenic plant (Sacred Lotus) into E. coli to induce a thermogenic response to a cold-shock, and 2) calculating a theoretical rate of heat production per bacterial colony to select for an appropriate calorimetric technique. Further, numerical methods in MATLAB will be employed to model the steady-state temperature profile of the synthetic bacterial colony, and to potentially corroborate later experimental findings. Engineering a controlled thermogenic response in bacteria could lead to improved bacterial functioning in cold shock environments.

Team Groningen: Hydrophobofilm --- a self assembling hydrophobic biofilm

Surface hydrophobicity is a useful property and has many applications. Hydrophobicity keeps a surface clean and dry preventing micro-organisms from attaching to a surface. Most chemical coatings used

presently are costly or harmful to the environment. Our idea is to engineer Bacillus subtilis which when applied to a surface, automatically forms a hydrophobic biofilm coating. Succesful biofilm formation will serve as a trigger for the expression of hydrophobic proteins called Chaplins. The result of this process will be a rigid biofilm with embedded hydrophobic proteins, leaving a coated surface which is extremely hydrophobic. Producing a self-assembling hydrophobic biofilm is cheap, there is no high-tech treatment involved and there are no hazardous chemicals necessary to attain a hydrophobic coating. Applications of this hydrophobic biofilm range from anti-fouling coatings on ships to anti-corrosion coatings used to protect sensory equipment.

Team Harvard: iGarden: an Open Source Toolkit for Plant Engineering

The Harvard iGarden is a venture into plant engineering. We aim to create a toolkit for the cultivation of a personalized garden containing features introduced through synthetic biology. In addition to a "genetic fence" designed to prevent the spread of introduced genetic material, we have developed three independent features to be included in this toolkit - inclusion of novel flavors, knockdown of plant allergens, and modification of petal color. All parts are BioBrick compatible and introduced into plants through agrobacterium-mediated transformation, using existing plant vectors modified with the BioBrick multiple cloning site. The Harvard iGarden is an effort to raise public awareness of synthetic biology, production of food, and how the two can intertwine. We envision the iGarden as a medium through which the non-scientist can see the power and potential of synthetic biology, and apply it to everyday life.

Team Heidelberg: miBricks: DNA is not enough

The key to successful gene therapy is integration of tissue specificity and fine-tuned target gene expression. The iGEM Team Heidelberg 2010 unlocks the world of synthetic microRNAs, since focusing solely on DNA has often been inconvenient for medical purposes. We engineered a toolkit for standardized measurements of interactions between artificial miRNAs and their binding sites. From this data we were able to compute an in silico model integrating binding site properties and knockdown percentages. Thus, the expression level of any gene of choice could be arbitrarily adjusted by employing the corresponding binding site design. To produce tissue specific miRNA gene shuttles, we developed an evolution-based method for synthesis of new adeno associated viruses. This enabled us to overcome the natural limitations of virusselectivity. In the future, miBricks could be applied for treatment of diseases like Diabetes and Hemophilia, opening the doors to new Synthetic Biology based medical approaches.

Team HKU-Hong_Kong: The bio-safety net

Our team’s project is a “bio-safety net” that limits the survival of bacteria according to tailored conditions. Bacteria could be designed to perform promising tasks, such as the biodegradation of oil to clean up oil spills. Yet, there are risks associated with the possibility of living bacteria performing undesired activities. Our goal is to introduce a “bio-safety net” that will be applicable to virtually all genetically engineered bacteria as a vital termination step after their tasks have finished. We have made this possible by introducing a "suicide" mechanism, that will be triggered under specific conditions. By combining different promoters, the system can respond to changes in environmental factors and control expression specific to chosen factors. Such mechanism can be easily assembled and incorporated to bacteria through the use of biobricks.

Team HKUST: Engineered Lactobacillus against S. aureus Infection

Our project aims at establishing an interspecies quorum quenching system in which engineered

Lactobacillus can sense and reduce the virulence of potentially pathogenic Staphylococcus aureus. To accomplish this, we are constructing chimeric quorum sensing receptors that can localize on Lactobacillus membrane and detect autoinducing peptides (AIPs) released by S. aureus. The ligand binding to the chimeric receptor will trigger downstream plnABCD pathway and initiate the synthesis and secretion of RNAIII inhibiting peptide (RIP), a heptapeptide with proven effectiveness in attenuating S. aureus virulence. The possibility of achieving this lies in the structural homology of the catalytic domain of the quorum sensing receptors in Lactobacillus and S. aureus. Both receptors belong to the HPK10 subfamily of a two-component histidine kinasefamily. Attenuation of S. aureus virulence by quorum-sensing inhibitors should not yield a strong selective pressure for development of resistance, and would therefore be an attractive concept for preventive medicine.

Team HokkaidoU_Japan: Dr. E. coli: World Smallest Protein Injector

Our project is on Type III Secretion Apparatus which is one of the most amazing biological devices. It can pass a whole protein molecule from a bacterial cell to a target eukaryotic cell. This apparatus which looks like a syringe is an organelle of pathogenic gram-negative bacterium such as Salmonella and Yersinia. We are aiming at making this device available for E. coli. Because it will not involve usage of pathogenic strains, it will be safer to use. Also we will show how to construct proteins for secretion and how to measure if it is really secreted using GFP.

Team Hong_Kong-CUHK: Bio-cryptography: information en/decryption and storage in E. cryptor

Data encryption and storage has always been an important branch of research in computer engineering. In our project, we explored the possibility of harnessing a biological system as an alternative solution for data en/decryption and storage. By using E. coli, we engineered and devised a prototype, dubbed E. cryptor, for 1) bio-encryption and -decryption with error checking; and 2) data storage in a bacterial system. In the age of synthetic biology, designed microorganisms may carry a specific DNA barcode to be distinguished from their natural counterparts. Our system could turn such barcode into more than simply a tag. In the future, can we also store text, pictures, and even videos into these tiny bacteria and protect the contents?

Team IBB-Pune: _

Team IIT_Delhi_1: Dr.coli

The use of bacteria for sensing applications has been around for a while now, and they have been used to produce recombinant proteins as needed for even longer time. The current project focuses on integrating these two components to create a device capable of responding to external stimuli in the form of quantitative protein production. For this device to function, it needed to be capable of producing and secreting the protein extracellularly. Further the dynamics of elicitor interaction with the bacteria in a flow stream and concomitant product release have also been a part of the study. We believe that such a system can play a major role in drug delivery systems that treat as needed and further in creating artificial glands for diseases such as insulin.

Team IIT_Madras: Pro-biotic Sweetener, under the control of a pseudo AND gate

We aim to use synthetic biology to engineer pro-biotic lactic acid bacteria to produce Monellin, a heat and pH stable sweetening protein. If we are successful in engineering Lactobacillus lactis, a Gram positive bacteria to express and secrete Monellin, we will be able to produce dairy products low in poly-saccharide-

based sweeteners, radically reducing the calorific content of these products. In order to be able to control the level of expression in this system, we plan to develop a regulatory system(s) that simulates a logical AND gate in response to two bioloical inputs. We plan to use the decreasing pH during curdling and the addition of nicin as the two inputs. To achieve the AND gate we will be using the CRE gene in combination with loxP sites. By placing the loxP sites appropriately, we will create an expression system that will produce the Monellin in a window of conditions.

Team Imperial_College_London: Parasight – Parasite detection with a rapid response

More than two billion people around the world live with unrelenting illness due to parasites" - WHO Director General Lee Jong-wook. Synthetic biology offers great opportunity for biosensors, however current designs require hours before useful output. To tackle this issue in the field, it's crucial that our project can respond in minutes, hence we have engineered a fast, modular sensor framework. This allows detection of a range of different parasites, and may also be used as an environmental tool for mapping their spread. We have developed two new technologies that enable our modular input/output - a novel cell surface biosensor, customisable for specific parasitic proteases, linked through quorum-sensing to a new 'fast-response' module capable of producing a detectable output in minutes. To demonstrate the concept, we've designed and fabricated B. subtilis to give a striking colour readout upon detecting the waterborne Schistosoma parasite which affects 200 million people worldwide.

Team Indiana: Plant Time Machines

Because of their 3D structure, multiple organelles, dynamic environmental responses, and other unique properties plants make interesting synthetic biology platforms. To demonstrate one application of power of plants in synthetic biology and introduce plants to iGEM, we aim to create plants that initiate different gene programs dependent on the time of day. To build off of current Biobricks, we chose to design plants that smell like wintergreen during the day and banana at night. We believe this can be accomplished by tapping into the promoter sequence of circadian regulated genes. In addition, we will also create an iGEM BBA 10 standardized plant transformationvector which can accept Biobricks and help deliver them to the genome of Arabidopsis.

Team INSA-Lyon: Droppy Coli : factory of PHB, application and improvement

Polyhydroxyalcanoates granules (PHAs) are universal prokaryotic storage compounds of carbon and energy. We aim to control their production in E. coli thanks to a new part: a strong promoter sensitive to the shaking speed and the temperature of the water bath. By controlling this production, our team focuses on two final purposes : (1) the granule as a storage system for overproduced lipids with medical applications, such as DHA or EPA and (2) the granule as self-cleaving micro-beads in order to purify a recombinant protein of interest. In bacteria, three separate monofunctional enzymes are required for PHA synthesis. In order to improve this pathway, we intend to model a single multifunctional enzyme based on the study of natural evolution of fatty acid synthesis in animals.

Team IvyTech-South_Bend: To Swim or Not to Swim?

Anyone who wants to enjoy bathing in natural bodies of water in or near areas populated by humans or livestock may encounter unsafe levels of enteric bacteria. Contemporary methods of assessing water quality have a slow turn-around time so we have taken steps to perfect a biosensor for rapidly indirectly

quantifying the presence of enteric bacteria in natural water samples through the detection of quorum sensing factors. Previous IGEMS have exploited the LuxR/pLux system for the detection of a variety of N-acylhomoserine lactone autoinducers. We have taken steps to further perfect a biosensor based on this device by transforming a gram-positive bacteria host to eliminate any background autoinducer signal and to build-in an enzymatic “read-out” to obtain an analog output. We envision the development of a handheld monitor that uses this IGEM biosensor, immobilized on input paper strips, to rapidly detect unsafe levels of enteric bacteria in water samples.

Team Johns_Hopkins: Synthetic Voltage Sensitivity at The Transcriptional Level in Saccharomyces cerevisiae

If the goal of iGEM and the Parts Registry is to take the messy world of genetic engineering and transform it into something like the standardized world of electrical engineering, it may be useful if electronic systems could directly interface with biological systems. Past iGEM projects have used chemical or optical stimuli to actuate transcriptional responses. Our project, however, seeks to add voltage sensitivity to Saccharomyces cerevisiae. Baker’s yeast was chosen because in some sense yeasthave a system that responds to voltage input. With a voltage stimulus one can open the voltage-gated calcium channels of yeast, causing calcium ions to rush into the cytoplasm. This causes calcineurin to dephosphorylate Crz1, which enters the nucleus and binds various promoters. Our group presents a library of characterized Crz1-sensitive promoters of both naturally-occurring and synthetic varieties. Genes downstream of these promoters are thus voltage-regulated in media containing calcium.

Team KAIST-Korea: DiscoverY: universal diagnostic yeast

Large portion of the world is still suffering from diseases despite of the availability of treatment -tuberculosis in Africa for instance. Such trouble originates from unavailability of cheap and effective diagnostic method. Team KAIST will present DiscoverY that is capable of diagnosing multiple diseases. S.Pombe chassis holds FGFR1-STAT1 pathway with modification in FGFR1, which becomes fusion antibody receptor in our system. When fusion antibody receptors on the surface come in contact with antigens, the pathway is initiated. The pathway ends with GFP expression as diagnostic display. The system will be tested with tuberculosis antibody, and simple replacement of antibody will make DiscoverY the universal diagnostic yeast.

Team KIT-Kyoto: E.coli Pen": Draw with your own color

Our team, KIT-Kyoto suggests an “E.coli Pen” as a new Art Tool. This brand-new pen uses no ink but medium in which genetically modified E.coli has been cultured. The Pen is able to express more than four colors in various intensities with single bacterial culture. This will be achieved by constructing plasmids carrying genes coding for four different fluorescent proteins under the control of seven promoters having different sensitivity to oxidative stress. The E. coli carrying these plasmids will produce different colors with various intensities by differentially responding to the gradient of hydrogen peroxide treatment. Different from previous passive BioArt in iGEM, the genetically engineered “E. coli Pen” provides an active and wonderful tool for us to purely enjoy the Art having a feeling for biotechnology.

Team Korea_U_Seoul: Heavy Metal Gang Captured By Capsule Cop

Toxic heavy metals such as arsenic, zinc, and cadmium in water are very harmful. Detecting these heavy metals is an important task. So we designed a heavy-metal-detecting E. coli. In order to design the system, we employed two fluorescence proteins (GFP, RFP) and aryl acylamidase as signal reporters. The aryl

acylamidase converts a colorless acetaminophen(Tylenol TM) to a brown color substrate. Since the detecting E. coli has three heavy metal promoters, if more than two heavy metals coexist in a solution, the E. coli emit mixed fluorescence, so we simultaneously detect metals. Our goal is to synthetic modules put these three genes for different heavy metals in a row in E. coli and then utilized in the form of a lyophilized powder, which can be stored in a drug capsule to make it portable so that analysis of water is easily processed. We call it a "Capsule Cop".

Team Kyoto: The Fantastic Lysisbox

Genetic engineered cell death is imperative for biotechnological usage, such as bioremediation area. For controlling cell death, we designed “Lysisbox” consists of a pair of modules: “Killer gene” and “Anti-killer gene.” As the Killer gene for E.coli, we noted the lysis cassette [SRRz/Rz1gene] of λ phage coding for a holin and an endolysin. The holin forms pores in the inner membrane, and the endolysin access to and degrade the peptidoglycan by passing through the pores, leading the E.coli to death. As the Anti-killer gene, we chose SΔTMD1 coding for a dominant-negative holin that inhibits the formation of the fatal pores. The balance of these two genes expression level has a key of the E.Coli’s life or death. In addition, such controllable membrane pores must show critical functions for all living organisms with lipid membranes. “Lysisbox” will contribute a lot to future projects, thus you must say “FANTASTIC!!!”

Team Lethbridge: A synthetic biology based approach for bioremediation of the tailings ponds

The industrial methods, used to harvest the oil sands, produce contaminated water in the form of tailings ponds with many harmful chemicals such as naphthalic acids, catechol and heavy metals. We are targeting catechol for degradation into common metabolic intermediates of the Krebs Cycle by using xylE from Pseudomonas putida that codes for the protein catechol-2,3-dioxygenase. Catechol-2,3-dioxygenase is being targeted into microcompartments, formed by engineered Aquifex aeolicus protein, lumazine synthase, to reduce cross-talk and increase concentration. The complex will then be purified and applied to the tailings for catechol degradation. By funnelling other pathways through catechol we can develop efficient methods for the decontamination of the tailings ponds. Mms6 from Magnetospirillum magneticum removes heavy metals from solution by forming nanoparticles. The Mms6 protein will be secreted from the cell into the tailings for the removal of metals such as iron and cobalt for creating an efficient bioremediation process.

Team LMU-Munich: Production of azobenzene derivates in E.coli and selection of successful transformants by apoptosis

We are engaged in two projects: Project “Pathway” involves the creation of an artificial metabolic pathway for the synthesis of azobenzene derivates in E. coli. This would be accomplished by expressing the required enzymes, encased in a proteinaceous bacterial microcompartment. This construct is necessary in order to shield the cell from toxic intermediates which would otherwise make this biosynthesis impossible. Azobenzene derivates are interesting in the field of biochemistry because of their properties as synthetic molecular switches. Project “ApoControl” is divided into three subprojects on controllable cell-death. The goal is to develop a system to improve the efficiency and specificity of gene expression in eukaryotic cell-lines and more specifically, to select cells expressing the target gene against cells that do not. Here, proapoptotic genesinstead of antibiotic resistance are used as a selection marker to induce clean cell-death at different stimuli.

Team Macquarie_Australia: Engineering a Bacteriophytochrome switch – creating a controllable E. coli chameleon

Photoreceptors are utilized by almost every organism to adapt to their ambient light environment. Our aim is to engineer a novel, reversible molecular 'light switch' within E. coli by introducing a photoreceptor from non-photosynthetic bacteria (Deinococcus radiodurans and Agrobacterium tumafaciens). By cloning the bacteriophytochrome coupled with heme- oxygenase, an enzyme producing biliverdin, the created colonies are able to respond to red and far-red light environments. This novel approach will result in the colour of E. coli to 'switch' from blue to green reversibly. Our E. coli chameleon will serve as a fundamental 'bio-brick' for future applications by providing a simple and photo-reversible switch.

Team METU_Turkey: E-CO Sensor

Cells can sense and respond to the presence of various gas molecules such as oxygen, nitrogen and carbon monoxide using gas sensor proteins. CooA is a carbon monoxide (CO) sensing transcription factor. It is a member of the cAMP receptor protein (CRP)/fumavate nitrate reduction (FNR) family of transcriptional regulators. CooA switches on oxidation enzymes in Rhodospirillum rubrum (a purple, nonsulfur, phototrophic bacterium) which enables the bacterium to use CO as a carbon source. CO is an odorless and colorless gas which can be extremely lethal. Our aim is to develop a cell sensor which can detect a wide range of CO concentration in the environment. We are building CooA and CooA-responsive promoter biobricks which will be transformed into E.coli. Fluorescent proteins (GFP and RFP) will be utilized as dose-responsive signals of ambient CO.

Team METU_Turkey_Software: BIO-GUIDE

As Synthetic Biology is on the rise, iGEM also grows up and part numbers in partsregistry increase with submission of more constructs each year. Our first milestone is to perform more useful standardization on parts-entry due to facing some difficulty while running our algorithms. We also used Software Requirements Specification, Software Design Description and Quality Plan approaches to define requirements for each part and building blocks, risks and design art elements of the designed software program. Next, we have used graph theoretic modeling to visualize relations between parts and to standardize representation of the parts as much as possible. It will help us while trying to find input-output relations between either biobrick parts or constructs. By this way, our program as a Biobrick Guide will provide alternative pathway choices to users for construction of the most reliable devices with respect to given inputs and expected outputs.

Team Mexico-UNAM-CINVESTAV: A very cool E. coli

We begin by proposing a biosynthetic construction that enables Escherichia coli to produce an antifreeze protein, AFP at less than 15 degrees Celsius. This proteinprevents ice crystal formation in the cell, which in turn allows survival at very low temperatures. We develop a switch by adapting the cold-shock E. coli operon with AFP from a fish (Macrozoarces americanus) using a positive feedback circuit. A very important potential application we are interested in is the use of AFP in designing systems helping crops to avoid potential damage from frosts. There are other possible important applications in tissue and organ preservation.

Team Michigan: Algae Bioflocculation for Biofuel Production and Bioremediation of Oil Sands Tailings Water

Our team worked on two projects this year. Our first project aims to improve the economics of algal biofuel production by creating a cost efficient microalgae bioflocculant out of E. coli. To achieve this, we over-

express Type I pili to increase the cell's adhesiveness, and also express a chlorovirus protein on the cell surface which specifically binds Chlorella species, a promising algal feedstock for the biofuel industry. We are also participating in the Oil Sands Initiative and seeking to improve the biodegradation rate of naphthenic acids (NAs), a toxic by-product of the oil extraction process which can linger in the environment for decades. Two Pseudomonas strains have been found to synergistically degrade 95% of NAs. Our project focuses on engineering these Pseudomonas strains to form biofilms in the harsh tailings water environment, which can potentially increase degradation rates by two orders of magnitude, by expressing a self-associating E. coli protein.

Team Minnesota: Metabolic Engineering: In vivo Nanobioreactors

Modern microbial engineering methods allow the introduction of useful exogenous metabolic pathways into cells. Metabolism of certain organic compounds is sometimes limited by the production of toxic intermediates. Several bacteria have evolved protein based microcompartments capable of sequestering such reactions, thus protecting cytosolic machinery and processes from interference by these intermediates. For our project, we will identify and transform the genes encoding proteins responsible for the production and assembly of bacterial microcompartment. Additionally, we will confirm the signal sequences that target enzymes to the protein compartments by fusing this sequence to reporter genes. To demonstrate the microcompartment's potential to serve as nanobioreactors, we will target genes encoding a short catabolic pathway into recombinant microcompartments assembled in E. coli.

Team Missouri_Miners: The Electric Microbe: Making A Fuel Cell With E. coli

The growing need for alternative fuel sources has sparked interest and research across many scientific and engineering disciplines. The fledgling field of microbial fuel cell development has previously relied on anaerobic metal reducing organisms such as Geobacter sulfurreduccens. This project sought to isolate genes from the electron shuttling pathway in Geobacter and transform them into the more manageable aerobic Escherichia coli. The Missouri University of Science and Technology iGEM team isolated four outer membrane cytochrome (omc) genes from Geobacter, vital to the extracellular transportation of electrons. The four genes; omcB, omcE, omcS and omcT, were cloned into individual plasmids. The eventual goal is to combine all four genes into one plasmid to transform into E. coli to create an aerobic, electron transporting microbial system.

Team MIT: Programmable, Self-Constructing Biomaterials

Our goal is to produce adaptive, living biomaterials that can be reliably controlled in two different systems: mammalian cells and bacteria. Our mammalian system uses newly isolated mechano-sensing promoters and a bi-stable toggle to stimulate osteogenesis via transient mechanical signals. Our bacterial system uses a toggle that takes advantage of quorum sensing and cell response to UV light and triggers the production of fluorescent proteins, and a polymer composed of a matrix of cross-linked phage. Our systems are remarkable because they translate a macroscale input into a pattern that emerges from the growth and re-modeling of cells. This technology not only has applications in the field of self-repairing nanotechnology and medicine, but it is also shedding light on artificial differentiation and the use of phage display technology in a new and innovative way.

Team Monash_Australia: Design and construction of a biological ethylene generation device

The Monash University iGEM team has identified that ethylene, a common organic compound, is under increasing production demands by the plastics and food industries. Current methods of production are energy intensive, and rely on processing of non-renewable fossil fuels. However many plants produce ethylene from L-methionine by use of the Yang cycle, which has lower energy requirements. We aim to introduce the genes that are required for ethylene production into Escherichia coli under the control of an inducible promoter, in an attempt to develop a cleaner and non-energy intensive method of production. At lower yields, this device may also provide a useful module for signal transduction between the E. coli and plants.

Team NCTU_Formosa: Mosquito Intelligent Terminator, a genetically engineered, temperature controlled E. coli for killing wrigglers

The Mosquito Intelligent Terminator (MIT) is designed and optimized to be an ecological and environmental friendly mosquito pesticide. MIT is an engineered E. colisecreting crystal proteins isolated from Bacillus thuringiensis to kill mosquito larvae, or known as wrigglers. These crystal proteins are toxic to certain types of mosquitoes and are not pathogenic to mammals. We designed a temperature-dependent genetic circuit expressing high levels of crystal proteins at room temperature only, thus production does not occur at incubation temperature 37°C. In order to make an environmentally safe insecticide, our design also incorporates a genetic circuit controlling the population size of E. coli. This intelligent terminator is not limited to mosquitos, as it can be custom fitted with different cry genes to other insect species. Currently, with more than one hundred crystal proteins targeting various insect species, our design may potentially serve as a promising pest control solution in the future.

Team Nevada: Development of Plant Biosensors for Environmental Monitoring Using Nicotiana tabacum Protoplasts as Transgenic Plant Models

The 2010 Nevada iGEM team has three objectives for this year's competition. One, we want our highlight to be the first team to provide the iGEM registry with stress-inducible promoters to be used in plants. These promoters can be valuable tools in monitoring the environment for salt, heavy metals, temperature, and more. Second, we want to develop a real-time monitoring model of these stress-inducible promoters by having fluorescent reporters linked to their expression. Current research typically uses microarray, a technique that takes a 'snapshot' of a system, where as we want to hold a 'video camera' up to specific genes. Third, we will show the advantages of using Nicotiana tabacum protoplasts (NT cells). Our NT cell system provides a faster, cheaper, and safer method of obtaining a transgenic plant model than transforming an actual plant, benefits future iGEM teams may want to take into consideration.

Team Newcastle: BacillaFilla: Filling Microcracks in Concrete

BacillaFilla, an engineered Bacillus subtilis, aims to repair microcracks in concrete, which can cause catastrophic structural failure. BacillaFilla would be applied to structures by spraying onto their surface. The Bacillus swims deep into the microcracks. Repair is effected by production of $CaCO_3$, filamentous cells and Levansucrose. $CaCO_3$ expands at the same rate as concrete, making it the ideal filler. A filamentous cell mesh provides reinforcement. Levansucrose glues $CaCO_3$ and filamentous cells in place. B. subtilis 168 sporulates, making it ideal for storage and transportation. The cells are naturally tolerant to concrete's high pH. We repaired 168's defective swrA and sfp, regaining motility. At the end of the crack the quorum communication peptide subtilin triggers a co-ordinated population response from a subtilin-inducible

promoter. Upregulating SR1 and rocF promotes arginine and urea production, increasing exogenous $CaCO_3$ deposition. Over-producing yneA induces the filamentous cell phenotype, while SacB converts extracellular sucrose to levansucrose glue.

Team Northwestern: SCIN - Self-regenerating Chitin INduction

Chitin, found in the exoskeletons of insects and crustaceans, is one of the most abudant substances in nature. Like keratin in skin, it comprises the protective outer layer of these animals. Our chitin expression platform involves generating a layer of chitin from a lawn of bacteria in response to an external molecular cue. This cue induces chitin synthesis (fast) and cell lysis (slow). This system allows for a build-up of chitin followed by cell lysis and subsequent release into the top layer of the lawn. Abrasions expose cells to the external cue for self-repair. In this way, we create a regenerative chitin biolayer with potential medical and industrial applications.

Team NYMU-Taipei: SpeedyBac

For iGEM2010, our NYMU-Taipei team is interested in resolving the fundamental need of rapid reporting response from bacterial gene expression. Our goal is to speed up the reporting response of bacterial gene expression through shortening the time needed for routine iGEM experiments. We intend to construct systems aimed at reducing experimental time and increasing efficiency of construction production. To achieve the goal, our design process is split up into three parts: Riboswitch, mRNA Binding, and SsrA. (1) Riboswitch - faster production of proteins by inducing the translation of pre-transcribed RNA molecules. (2) mRNA Binding - using mRNA aptamers and split GFP/RFP-eIF4A reporter systems to show faster promoter activity or the expression of mRNA transcripts. (3) SsrA - fast, specific, and constitutive proteolysis achieved by engineering fluorescent proteins with LVA tags.

Team NYU: ImmunoYeast : antibody discovery and production in one simple system

The goal of our project is to increase the speed and efficiency of the antibody discovery process. We constructed a yeast strain that is capable of screening a library of antibody fragments against an antigen of interest, processing the antibody genes through recombination and secreting an easily-purified form of antibody protein for research use. Our hope is to demonstrate the feasibility of using the yeast cell to not only discover antibodies but to provide a streamlined processing unit that can quickly and easily transition from antibody discovery to protein production.

Team Osaka: Continuous Greening Cycle

Desertification all over the world causes famine, drought and suffering. We aim to develop micro-machines that can stop and even reverse desertification by recovering vegetation in these areas. We envison a ‘Continuous Greening Cycle” in which engineered microorganisms decompose plant fibers into nutrients through the action of cellulolytic enzymes. They then produce water-absorbant polymers such as poly(gamma-glutamic) acid that retain water in the soil to help plants grow. When the plants die they will be decomposed to start the cycle anew. In addition to aiming for the continuous and self-expanding greening of desert areas, we hope to contribute to iGEM by developing useful BioBricks!

Team Panama: Standardization of Rhamnosiltransferase 1 gene (rhlAB) into a Biobrick for rhamnolipid production in E. coli

There is considerable interest among bio-industries in bioremediation products such as Rhamnolipids.

Rhamnolipids as biosurfactants are important in the remediation of oil spill areas. The cleanup of the Exxon Valdez oil spill using rhamnolipids as biosurfactants was too expensive and complicated, therefore impractical for large-scale bioremediation. However, with advances genetic engineering and synthetic biology offer a viable solution to oil spill pollution clean up. In this project we use genetic engineering as a tool to integrate genetic parts through the BioBrick assembly standard protocol of iGEM to develop a BioBrick for rhamnosiltransferase 1 complex (rhlAB) gene expression in Escherichia coli for standardized rhamnolipid production. Our BioBrick integrates a promoter, a RBS (ribosomal binding site), our part rh1AB genesequence isolated from Pseudomonas aeruginosa, a GFP reporter and a terminator. All the parts fit into a plasmid backbone that can be transformed into E. coli strains, which can then produce rhamnolipids.

Team Paris_Liliane_Bettencourt: Every bacteria counts!

Counting is the action of finding the number of elements in a set. Past attempts at developing counters in cells have mostly attempted to mimic the binary methods that computers use to count.Our first counter takes a new approach to counting in cells, essentially a mechanical rotary counter implemented on a micro scale. Each time the counter detects an input, it performs an excision and integration directly down-stream of the active site, turning on a reporter and rotating over one "notch" on the counter.Our second counter operates on the wholly different principle that the statistical occurrence of a rare event in a large population can be modeled. Each cell in our population harbors a construct that when stimulated has a small chance of excising a terminator and expressing a resistance gene. The number of resistant cells is thus an accurate count of the number of input stimuli.

Team Peking: Heavy Metal Decontamination Kit

During this summer, our group has developed a method to engineer bacteria into heavy metal decontamination kits. First of all, we analyzed the function, structure and operation of the transcription factor MerR, a mercury-responsive regulator in detail via bioware experiments and modeling. Then appropriate topology candidates for proper bioreporters were carefully searched. We selected a candidate and re-designed genetic components to accomplish certain bioreporter function in need, which was verified by following bioware experiments. For bioabsorbents, we engineered MerR into a metal binding peptide. This was followed by inductive expression of engineered peptide on surface, periplasm and cytosol of E.coli. This reverse engineering method was then expanded to lead-responsive regulator, PbrR, to confirm the validness of this method. Results demonstrated that the procedure mentioned above is streamlined enough to construct valid whole-cell bioreporters and bioabsorbents of various heavy metals for field application in the near future.

Team Penn_State: _

Team Purdue: Development and Characterization of Hypoxic Stress Response Systems in Mammalian and Plant Models

From water-logged soils to overpopulated regions of tumors, low-oxygen environments distress plant and mammalian systems. Plants with inadequate levels of oxygen move from aerobic respiration to alcohol fermentation to sustain their metabolism. This switch causes the accumulation of byproducts that are detrimental to the plant. A synthetic biological circuit, centering on the alcohol dehydrogenase (Adh) promoter, has been developed indicating when low oxygen levels (< 5% O2) are present in plants. Similarly, low oxygen zones can develop in solid tumors in numerous mammalian cancer models. Substantial evidence indicates that hypoxia in tumors initiates angiogenesis, a process that aids in tumor

proliferation. Accordingly, an additional hypoxia-sensitive circuit that up-regulates the activity of a reporter protein in low oxygen (<1% O2) environments has been created for mammalian systems. The development and characterization of these circuits will provide tools to explore the consequences and identity of hypoxic environments in mammalian and plant systems.

Team Queens-Canada: WormWorks: Introducing the nematode C. elegans as a multicellular chassis

Historically, the iGEM competition has tended away from working with eukaryotic and multicellular organisms, limiting prospects for higher levels of project complexity in favor of simpler and easier-to-understand bacteria. The nematode worm Caenorhabditis elegans was examined as a prospective chassis for use in the competition. Once it was decided that the opportunities presented by the organism appeared to outweigh the challenges involved in working with it, a foundational library of parts was built and tested within the organism. This collection includes useful promoters, reporters, effectors, and a terminator. An educational resource specifically targeted at iGEMparticipants was written and incorporated into the team wiki in order to assist future teams in learning about and exploring the possibilities offered by C. elegans.

Team RMIT_Australia: A Recombinant Peptide Expression System That Exploits Taq Polymerase as a carrier Molecule

The RMIT 2010 iGEM team has attempted to create a biological system that will produce peptides at a low economic cost. This biological machine includes the use of a T7 promoter regulated by the Lac elements to express a soluble thermostable protein carrier molecule with an attached peptide of interest. Taq polymerase will be attached to this peptide via a thermolabile bond allowing the peptide to be cleaved using just temperature. The polymerase has furthermore been mutated in such a way to avoid interactions with nucleic acids, thus diminishing the effects it may have in the bacterial host cell. This system can be adopted and enhanced to produce libraries or large scales of peptides/drugs without the high price tag attached to then be distributed to large communities that otherwise cannot afford the cost of research nor treatment.

Team SDU-Denmark: Flow-E, a bacterial flow inducer

When generating flow through a microcapillary tube, engineers are met with problems in generating force and keeping fluids mixed or separated. Inspired by an article by Kim et al on bacterial carpets that could generate flow, we have designed a biological system in E. coli that can induce flow and increase mixing in a fluid, essentially working as a microscopic flow actuator. The actuator uses E. coli's flagellas and can be switched on and off using light. Our system is composed of several novel parts: a photosensor, that controls the tumbling frequency of E. coli's flagella via chemical pathways; a hyperflagellation part, to increase the number of flagella and the power of the system; a retinal producing part, that works with the 2009 Cambridge's iGEM team's β-Carotene brick, which we have helped further characterize. Our human practices focused on watermarking synthetic genetic material and improve security in synthetic biology.

Team Sheffield: iCOLI: A water-borne pathogen detection system and an exploration of identity in synthetic biology

The Sheffield iGEM 2010 team organised its activities around a general theme: 'identification'. This notion, for instance, is at the heart of the scientific and modeling projects, which have made steps towards the design and production of a multi-target, water-borne pathogen detection system. Identification is also central to the human practices projects, which explicated and analysed the concept of identity in relation to the disciplinary backgrounds brought together in the project and the field of synthetic biology itself. Vibrio

cholera was chosen as a test-case, giving us two potential routes to engineer E.coli to recognise the pathogen: 1. Fusion of cholera's receptor (CqsS) for its quorum sensing molecule (CAI-1) with the internal apparatus of one of E.coli's general stress detecting systems (BarA). 2. Engineer the whole cholera quorum sensing system into E.coli.Each of these would then be connected to a representation system (GFP or E.chromi) to visualize the pathogen.

Team SJTU-BioX-Shanghai: Synthetic-biological Approaches to Osteoarthritis

Osteoarthritis (OA) is a chronic disease in which joint matrix is degraded and chondrocytes undergo disordered and hypertrophic differentiation, symptoms including joint pain, tenderness and stiffness. We proposed two synthetic-biological approaches to OA, one with a eukaryotic genetic circuit and another prokaryotic. Both circuits are composed of three systems: "Detector", "Actuator" and "Supervisor". As for Detector, we built tissue-specific promoters in the eukaryotic circuit, while inflammation factors are employed as signals of OA in the prokaryotic circuit. The same Actuator shared by two circuits generates proteins col2a1, which replenishes the degraded matrix, and oct4, which reverses the disordered differentiation. The eukaryotic Supervisor part has an original design in which a photo-sensitive cation channel crosstalks with certain cellular signaling pathways, resulting in the light-controlled expression of col2a1 and oct4; in the counterpart of prokaryotic circuit, both injected inducers and over-population lead the engineered bacteria to suicide, thus attenuating possible side effects.

Team SJTU-Oncology-Shanghai: _

Team Slovenia: DNA coding beyond triplets

Slovenian iGEM team is designing a cell based system in which we will be able to control the sequence of steps in a multi-step biosynthetic pathway. Our novel approach is designed in such a way that it will enable control of the order and sequence of reactions in a particular biosynthetic pathway. Our goal is to use synthetic biology approach to significantly increase the speed , efficiency or direction of a particular biosynthetic reaction. We will also attempt to use our approach to create a novel chemical product of the existing biosynthetic reactions. This novel cell based device represents a universally applicable principle and offers a significant improvement of current industrially important biotechnological processes. Similar approach will be applied to demonstrate the advantages for cell based oscillators and information within the cell.

Team St_Andrews: Tackling Vibrio cholerae by introducing genetically re-engineered Escherichia coli to the human alimentary tract

Every year five million people are infected by cholera. An effective vaccine has yet to be found. Our aim is to confer resistance to cholera by taking advantage of quorum-sensing regulated pathogenicity. CAI-1, the cholera autoinducer, is produced by the enzyme, CqsA. E. coli engineered to express CqsA could grow along with regular gut flora and render V. cholerae avirulent by maintaining high CAI-1 concentrations. To lessen its metabolic burden, the system should respond to low concentrations of autoinducer produced by V. cholerae and co-ordinate this response using the Lux quorum-sensing system. We have explored rearranging the network architecture of the Lux system to give bistable expression. A model to describe bistability was formed using ODEs and implemented in C++. To assess the perception of synthetic biology, software was written to gather data from social networking and news sites and quantify the change in opinions with time.

Team Stanford: EscheRatio coli: Novel Sensors to Detect a Ratio of Environmental Inputs

The majority of sensors currently used in synthetic biology respond to the absolute concentration of a chemical. However, many important biological processes are governed by the ratio between the concentrations of multiple chemicals. To create systems capable of responding to more complex input data, the Stanford team designed two types of ratio-measuring sensors. The first sensor utilizes two unique small RNAs to inhibit the transcription of two operons, each responding to an environmental factor and having a specific flourescent protein (or output gene). By manipulating the number of small RNAs produced, a tipping point between outputs can be established for different input ratios. The second sensor uses a phosphorylatable protein to control the transcription rate of an output promoter. By linearly associating two chemical inputs to the production of a kinase and a phosphatase, the ratio of the two environmental factors indirectly dictates the activity of the output promoter.

Team Stockholm: Spot on Treatment

For our first participation in iGEM, Team Stockholm have explored the possibilities to fight skin disorders using novel cell-penetrating peptides. We have focused on vitiligo in where pigment cells are destroyed, resulting in white patches on the skin. Studies have shown that vitiligo patients have decreased levels of antioxidants and elevated levels of antibodies targeting the pigment cells in the skin. We have used synthetic biology to fuse cell-penetrating peptides to proteins that are deficient in skin of vitiligo patients. The cell-penetrating peptides will aid in passing the proteins through the skin and target the pigment cells to reach repigmentation. Currently there are no treatments like ours for vitiligo patients. However, we strongly believe that synthetic biology can help bacteriotherapy getting a major role in fighting several skin disorders in the future, and that our first explorations in this field inspires others to continue exploring.

Team Tec-Monterrey: Development of a genetic frame for the creation of a concentration-sensitive bacterial sensor

Bacterial reporters or whole-cell bacterial sensors have always been an area of application for genetic manipulation and synthetic biology. The first bacterial reportersappeared 20 years ago, although these early tests didn't use genetically modified microorganisms. Further research and development in the area of genetic engineering has resulted in newer and more sophisticated bacterial sensors, capable of detecting the presence of contaminants, sugars and amino acids in different media such as soil and water. However, most bacterial sensors can only detect the presence of a compound at a certain concentration and currently there are few documented bacterial sensors that can detect and report different concentrations of the compound of interest. Our objective was to develop a genetic frame, compatible with the BioBrickstandard, for the creation of a concentration-sensitive bacterial sensor. In the process we also developed and characterized BioBricks for two new families of phageactivators.

Team The_Citadel-Charleston: Appetuners: A System for the Expression and Control of Appetite Regulation Peptides in E.coli

Appetite can shape a person's life, from body composition to mood (to budget!), yet the means for controlling appetite are very limited. Imagine if a complex neurological state such as the desire to eat could be influenced by an engineered strain of intestinal microflora. In this regard, Peptide Tyrosine Tyrosine (PYY) and its associated molecules offer a unique opportunity for synthetic biologists. These peptides permit microorganisms native to the gastrointestinal tract to interact with the central nervous system and to

influence the perception of hunger. The Citadel-Charleston Team is working to express the PYY family of peptides in E.coli, to implement a secretion pathwayfor those peptides, and to ensure that their expression is strictly controlled by means of a cellular population limit.

Team Tianjin: Lignin degreadation yeast

We make some Saccharomyces cerevisiae which could synthesize lignin degrading enzymes and put them on the out surface of the yeast through the yeast surface display technology. Those cells, which propagate themselves rapidly, could affix to the wood cellulose material and begin to degrade the lignin quickly with the enzymes of their surface. This yeast will make the lignin degradation pretreatment more secure to our environment and lower the cost. The products of this process are celluase and hemicellulase, which could be used to produce alcohol fuels and papermaking in the next steps. Yeast pharmacist: What we what to design is a model which can facilitate the discovery of various drugs in the traditional Chinese Medicine, the active components of which are usually unknown. Since nicotine receptor is the target of many medicines, especially medicines for smoking cession, our system aims at helping to find medicines that could act as partial agonist.

Team Tokyo_Metropolitan: Life Design: Fine Clothing, Color Housing and Delicious Food by using E. coli

Our team theme is “Life Design.” We make our life comfortable by following three projects. [E. coli Fiber Project – Fine Clothing] Bacteria cellulose is very useful material. People try to get bacteria cellulose. But it is high cost and low efficiency. Aim of our project is to make low cost and high efficiency cellulose factories where E.coli works! [E. coli Pattern Formation Project – Color Housing] Give an experimental proof of Turing pattern. We use two kinds of E. coli and make various patterns! The patterns are dynamic and stereoscopic!? [E. coli Rice Master Project – Delicious Food] Japanese like rice, our staple food. We are particular about the quality of rice. Rice Master of E. coli judges the quality of rice. In future, other kinds of food “Master” provide us some information about quality of foods.

Team Tokyo_Tech: The “Wolfman” Coli

Have you heard the legend of “The Wolfman”? They’re ordinary man at daytime, but suddenly transform into a ferocious wolf in the full-moon night. Our project aim to imitate the character of Wolfman, more specifically, designing two types of E.coli that help each other to survive at daytime, whereas competing at nighttime when cocultured. Firstly, two cells have different types of antibiotic-resistance, however this expression is repressed in absence of outer stimulation. Two factors of stimulation, cell density and light, are required at once to activate the antibiotic resistance. We took advantage of photoregulation system (Cph1-EnvZ) to make sure downstreampromoter is activated with light. This promoter is followed by circuit designed to sense the cell density of its counterpart and activate the counterpart cell’s antibiotic-resistant gene. With this entire system, we could observe the “Wolfman” Coli in our laboratory working by AND gate of two stimulation.

Team Tokyo-NoKoGen: An EcoTanker for the easy collection and delivery of target compounds

We propose an entirely new Escherichia coli micro machine that behaves like a tanker, the EcoTanker. The EcoTanker takes up a target compound from the environment and delivers it to a desired location for easy harvesting. The target compound is taken up and stored inside the cell in an EcoTank, a bacterial microcompartment (pduABJKNU). Blue light then directs the delivery of the “cargo” to a desired destination

by phototaxis, using the N. pharaonis blue light responsive domain (NpSRII-NpHtrII-Tar) linked to an E. coli two component system. The EcoTankers are then signaled by green light to self-aggregate and autolyse in response to quorum sensing. We can then easily collect the EcoTanks filled with the target compound, which could be an environmentally toxic substance or an industrially desirable chemical. We expect that EcoTanker to be a powerful machine in various bioremediation and industrial applications.

Team Toronto: Enhanced Catechol Degradation via Metabolic Channeling in E. coli

Oil sands, consisting of a mixture of clay, sand and bitumen represent a significant fraction of remaining petroleum reserves. However, extracting the hydrocarbons requires large volumes of water which are then contaminated with naphthenic acids, heavy metals and residual hydrocarbons. These 'tailings' are acutely toxic to higher organisms, though some bacteria and algae can survive in these environs. Our knowledge of metabolic pathways capable of degrading such contaminants (bioremediation) is limited. However, the known pathways tend to be slow and inefficient. The breakdown of many polycyclic aromatic hydrocarbons generates a common intermediate, catechol that is further degraded to Acetyl-CoA. Metabolic channeling is an effect whereby intermediates are shuttled between enzymes in a pathway circumventing free diffusion into the cytosol. Here, we present a design, modeling and baseline experiments aimed at demonstrating optimization of catechol degradation using the principle of metabolic channeling in an E. coli proof of concept system.

Team Tsinghua: E.mmunology: Bacteria-based Antibody Production System

Tsinghua iGEM 2010 team focus on simulating antibody generation and selection technology with Bacteria, thus develops a new Antibody Production Method. Traditional antibody production method is expensive and time consuming. Thanks to the simple and easy-to-industrialize nature of prokaryotic systems, our antibody production system, once established, will facilitate the cheap and efficient production of antibodies. Production of antibodies in the mammalian immune system involves two steps: Random production of a large numbers of antibodies; Selection of a specific antibody matching the antigen. Therefore, our Antibody Production System would be composed of two devices: Module I: Generation of antibody library; Module II: Selection of specific antibodies.

Team TU_Delft: Alkanivore: Enabling hydrocarbon degradation in aqueous environments

Pollution of soil and water environments by crude oil has been, and is still today, an important environmental issue. This was once more confirmed with the oil-spill in the Gulf of Mexico, but is also an issue that has to be faced continuously during the process of oil extraction from oil sands. Cleaning has proven to be challenging, but synthetic biology may hold the key to sustainable bio-remedial solutions for the future. What if we could design a small, autonomous, self-replicating, inexpensive method to remove oil from aqueous environments? The TU Delft iGEM 2010 team spent their summer designing a system that can tolerate, sense, dissolve & degrade hydrocarbons in aqueous environments, which could open new doors for the oil-industry.

Team TU_Munich: bioLOGICS: Logical RNA-Devices Enabling BioBrick-Network Formation

Among the goals of iGEM is the creation of synthetic biological parts and their utilization to achieve novel

features and behavior in biological systems. The emphasis of our project is put on this latter, "systems" aspect of iGEM. More precisely, we aim at the development and experimental demonstration of a scalable approach for the realization of logical functions in vivo. By developing a computational biological network based on RNA logical devices we will offer everyone the opportunity to 'program' their own cells with individual AND/OR/NOT connections between BioBricks of their choice. Thereby, BioBricks can finally fulfill their original assignment as biological parts that can be connected in many different ways. We will achieve this by engineering simple and easy-to-handle switches based on predictable RNA/RNA-interactions regulating transcriptional termination. These switches represent a complete set of logical functions and are capable of forming arbitrarily complex networks.

Team TzuChiU_Formosa: Nutrient synthesizer

Global nutrient deficiency issue has never been solved for centuries, 5.6 millions children died from malnutrition and hunger yearly(UN statistics). We aim to create a synthetic biological device to produce nutrient for people who needed. The device contains sensing, synthesis, and lysis system which is operated in E.coli. When the device senses appropriate signal, it will activate synthesis system to produce nutrient. When products reach a critical mass, the lysis system will take over to release nutrient. We takeβ-carotene as one of our source of nutrient, as it is the precursor of vitamin A. 40 millions people worldwide suffer night blindness each year because vitamin A deficiency. In our device, we use hydrophobic promoter as our sensing system (β-carotene is lipid soluble), and lysogenic enzyme as our lysis system. Our next step is to transform our synthesizer to lactobacillus which is harmless to human so we could solve the global food crisis problem.

Team UC_Davis: Engineering a Spatial Oscillatory Network for Spontaneous Two-Dimensional Pattern Generation in E. Coli

Genetic circuits regulating spatial pattern formation play vital roles in organismal development throughout the eukaryotic domain. We believe that we can use related design principles to synthesize spatial patterning in bacterial populations. Previous genetic devices have allowed bacterial lawns to faithfully reproduce spatial patterns and, with the inclusion of of cell-cell communication, to construct of a bacterial edge detection device. Our device builds on these ideas but differs significantly by aspiring to produce complex spatial patterns in response to simple stimuli rather than by copying the input stimulus. This genetic circuit could drive the creation of biological systems capable of generating spatially varying gene expression profiles in response to simple chemical stimuli. Such devices should have application in fields such as nanofabrication, environmental engineering and tissue engineering.

Team UCL_London: Hypoxon: Improved manufacturing of biopharmaceuticals by process-triggered positive-feedback loop in E. coli.

Biopharmaceuticals are commonly synthesized using E. coli as production chassis. Typically, the production of biopharmaceuticals is triggered by the addition of an induction agent, often IPTG, during the bioprocess. The UCL iGEM team aims to create “independent” cells in which the production phase is triggered by an external stimulus, removing the need for IPTG. The stationary phase growth in bioreactors is preceded by a dip in dissolved oxygen tension (DOT) followed by a DOT spike. We have developed a genetic circuit in which a series of promoters will cause the production to be triggered by the hypoxic condition. This auto-induction promises an economically and manufacturing improved production of biopharmaceuticals. There is the potential of applying this "Pavlovian" circuit principle in near future to

yeast, mammalian or other expression systems for the production of complex biomolecules for the treatment of major diseases.

Team UCSF: Synthetic Killers - engineering immune cells for cancer therapy

Cytotoxic cells of the immune system (Natural killers and cytotoxic T cells) identify cancer and virally-infected cells and kill them. These potent killers travel throughout the body, recognizing proteins and other molecules on the surface of target cells. If the target cell is deemed potentially dangerous, the cytotoxic cell grips the target cell tightly and creates an immunological synapse at the site of adhesion. Within this immunological synapse, the killer cell releases cytotoxic granules to kill the target cell without harming any nearby cells allowing for a direct, apoptotic death. Our team will focus on improving the specificity and killing efficiency of cytotoxic cells towards certain cancertypes. By using synthetic biology tools and logic gates' design, we hope to create powerful killing biomachines for the fight against cancer. Our newly engineered synthetic devices would have the potential to enhance current adoptive cell-based immunotherapy for cancer patients.

Team UIUC-Illinois: sRNAs in Artifical Gene Circuits and Bioremediation Applications

Previously, the majority of engineered, genetic regulation within bacteria has been achieved through the use of transcriptional regulators. However, the recent explosion of interest from the emerging field of RNA regulation provides new insights into the dynamic nature of genetic regulation. Small non-coding RNAs (sRNAs) comprise the chief regulatory mechanism for eliciting primary responses to environmental stresses. Acting in conjunction with proteins such as hfq (RNA chaperone), sRNAs provide a cost-effective, specific and rapid response that is essential in targeting gene transcripts for regulation. The Illinois iGEM team has worked to create a set of endogenous and artificial sRNA regulator Biobricks to be used in cellular stress responses and which contribute to bistability in artificial gene circuits. A bacterial metal detection system of arsenic and gold demonstrates the capacity of sRNA regulation in artificial gene circuits.

Team UIUC-Illinois-Software: BioMORTAR

In order to facilitate the design process for novel bacterial metabolism, our team has created a tool suite known as BioMortar which will automate plasmid design for metabolic processes as well as model cell growth. BioMortar begins with a much improved version of IMPtools, which uses an algorithm over a network generated by the KEGG database, to determine the optimal metabolic pathway according to specified conditions. At this point, it accesses the DNA sequences for each recommended enzyme for each reaction and searches the BioBrick database for related gene sequences. Then, BioMORTAR designs and displays the advised, usable plasmid(s) in BioBrick format for the user. Finally, the program models the growth of the organism, with the addition of the new metabolic pathway(s). By automating the design process, BioMORTAR streamlines the process of designing bacteria with new metabolic processes.

Team ULB-Brussels: Hydrocoli : How to make wastewater our new green energy source

Current hydrogen production processes are not sufficiently energy-efficient to provide a viable source of green energy. Our project is to design a genetically engineered Escherichia coli which could be used for hydrogen production. To offset the low yields of hydrogen production through dark fermentation, the

substrate would be the organic compounds found in wastewater. The mixed acid fermentation pathway, leading to hydrogen production, can be improved by both the overexpression and the deletion of several genes involved. In addition, we would like to implement various features to enable the strain to perform other tasks related to wastewater treatment, such as biosensor, eliminating nitrogen compounds, or hindering hydrogen consumption by methanogenic bacteria, as well as a planned death system in order to prevent its proliferation in the environnement. We also plan to adapt to iGEM standards the homologous recombination tool used for the deletions.

Team UNAM-Genomics_Mexico: WiFi Coli, a Communicolight System

Synthetic Biology has been enabling changes in all bio-domains, one such being communication. Traditional cellular communication has relied since time immemorial on chemical messengers to exchange information. Regardless of their scope, these messengers are constrained to a system; eg: even far reaching couriers such as hormones are bound within the chemical structure that is the human body. But this is about to change. In this project, our goal is to render the chemical barrier deprecated by using a non-chemical herald: photons. These will transport information between our engineered cells, creating a photon-based inter-cellular communication system. These messengers are produced through bio-luminescent reactions, and are quite capable of traversing multiple environments. This enables the transmission of information beyond the chemical, biological, and even spatial limitations. As the courier is effectively decoupled from the chemical layer, it is an innovative step in establishing communications between organic-based and silicon-based systems, such as computers.

Team UNIPV-Pavia: ProteInProgress: a cellular assembly line for protein manufacturing

Large-scale production and purification of recombinant proteins by cell cultures represent a key-area in manufacturing field. The production process still has several drawbacks affecting cost/efficiency. In this work, three modular systems were designed to overcome some of these bottlenecks. A library of self-inducible promoters was built and characterized to start the peptide production at a desired culture density, without expensive inducer molecules. Two standard integrative vectors were realized to insert BioBrick parts in user-defined positions of E. coli or S. cerevisiae genome, to ensure genetic stability without using selection markers. Finally, two promising techniques were combined for an "in-cell" protein purification: PolyHydroxyAlkanoate (PHA) granules were used as a substrate for PHA-binding peptides (Phasins) fused to the target protein, thus replacing affinity resins/columns and tags, while a pH-based self-cleaving peptide (Intein) was used instead of a protease cleavage site. These solutions are modular and provide useful BioBrick parts for other applications.

Team uOttawa: Characterization of toggle switch components for use in engineered, tunable networks

The generation rate of complex genetic networks has slowed in the past decade due to a lack of characterized components. We aim to solve this issue by contributing a library of well-characterized yeast toggle switch components with varying dynamics. In order to characterize these switches, each of the two repressors, driven by mutually repressible promoters, is tagged with a distinct fluorescent protein that can be detected and quantified using flow cytometry. To expand the BioBrick database we will submit functionally-tested yeast-enhanced fluorescent proteins, repressors, and a library of repressible promoters. Furthermore, to demonstrate the applicability of these components in fine tuning genetic networks, we hope

to use these toggle switches to build a DNA damage sensor with varying levels of sensitivity. We also describe novel methods for BioBrick construction based on natural homologous recombination mechanisms in S. cerevisiae.

Team UPO-Sevilla: Bacterial Crowding

The possibility of specifically targeting bacteria to biological or abiotic surfaces is a promising technology of potential use in therapy, pest control and bioremediation, among others. However, since in most cases bacteria are not drawn towards their targets, the possibility of specific interaction is limited to those bacterial cells that randomly collide with the surface, thus requiring a high bacterial population to achieve efficient targeting. On the other hand, most bacteria are specifically attracted by gradients of a variety of chemicals, thus achieving high cell densities in the areas where the chemoattractants are present at higher concentration. The aim of “Bacterial Crowding” is to exploit chemotaxis for directing a relatively small population of bacteria to interact efficiently with a non-diffusible target exposed on a biotic or abiotic surface.

Team Uppsala-Sweden: Levande Klocka (Living Clock)

Our project aims for building a biological concentration band detection sensor and demonstrating its usefulness in building a bio-clock. Many previous iGEM teams have come up with sensors for various applications ranging from toxic metals to life saving enzymes. Our attempt is to create a concentration band detect component that can quantify the signal. This concentration band detection sensor thus serves as a quantitative sensor, which can work in combination with any of these chemical sensors. Apart from this utility, we believe the concentration band detect sensor in different configurations can allow the creation of complex circuits ranging from simple oscillators to complex data processing machines which can exist on the same system without affecting other components of the system owing to its concentration band specificity. With the bio-clock we test the robustness of the concentration band detection mechanism and if it could be used to build more complex devices.

Team UQ_Australia: _

Team USTC: An Integrated Platform Based on Bacterial Microcompartment for de novo Proteinaceous Artificial Organelles

In synthetic biology, we are in great need of an independent compartment and an integrated line to assemble disparate elements in cells. Thus we design, model and construct a platform for artificial organelles. The shell genes of Citrobacter freundii pdu bacterial microcompartment (BMC) were first constructed into BioBrick parts, assembled and expressed to form an empty porous multi-protein shell of ~100nm. Then target protein can be located either inside or outside the empty shell by fusing the protein with signal sequences, using a novel and convenient assembly standard compatible with the RFC 10. Onto this platform, artificial organelles with different functions can be constructed. With enzymes or binding proteins inside the shell, different nanoreactors or nanoreservoirs can be produced. Modifications outside the shell can be applied to build multi-protein super-complexes or facilitate downstream purification with affinity tags. Such a platform will make E. coil an integrated factory.

Team USTC_Software: iGaME: Synthetic Biology for Gamers

To promote public awareness of synthetic biology and introduce its basic ideas to the laymen, our team devoted to the development of an experimental video game which aims at instructing non-biologists to design and improve biological systems. Following the games-with-a-purpose paradigm in which players

help solve scientific problems, we attempt to apply the human brain's puzzle-solving abilities to the complex designs of biological systems. While most of developed simulation tools are designed for experts to model the reaction networks from scratch, our game integrates a modeling environment in which users only need to submit their assembling of parts for our program to discover and generate the biological model automatically. With a mass of data for the use of modeling, we propose the Standard Biological Parts Modeling Database Language, which enables descriptions of complicated biological processes. Furthermore, previous iGEM project models will be featured to demonstrate the availability of our idea.

Team UT-Tokyo: E.coli solves SUDOKU

Information-processing by means of cellular machinery has flourished recently, as exemplified by the creation of bacteria capable of counting or performing AND/OR boolean logic. However, the successful assembly of organisms that integrate multiple (>3) pieces of information into an elongated AND gate has yet to be seen. Our “4C3 leak-switch” realizes such a tool with the use of homologous recombination and leaky transcriptional terminators. This switch turns on only when three of four types of information are transmitted, regardless of the order of transmission, and outputs differently depending on the combinations of input. Our current project aims at creating bacteria that collectively solve the popular puzzle game Sudoku. This aim is achieved by combining this switch with our second subproject, the “signal-virus,” which relays information only to the relevant targets, based on an antisense-RNA key system. This system realizes parallel-computing and we believe will greatly advance the field of information-processing.

Team Utah_State: CyanoBricks: Genomic Engineering Tools for the Photosynthetic Cyanobacterium Synechocystis

The future of synthetic biology lies in expanding our ability to engineer genes in new organisms. Our project develops a system to engineer the genome of the photosynthetic cyanobacterium Synechocystis sp. PCC6803, establishes expression standards for this species, and adds a set of characterized Synechocystis promotersand ribosome binding sites to the BioBrick toolbox. We developed a BioBrick vector that can be used to assemble parts and devices in E. coli. Upon transformation into Synechocystis, it integrates the device directly into the genome through homologous recombination. We utilized genes that were activated under a variety of conditions, from those responding to heat stress to ones oscillating under a circadian rhythm. The promoters and ribosome binding sites were converted into BioBrick-compatible parts, and subsequently characterized. Our success will enable the use of existing parts in new species, and will expand the range of devices that can be built.

Team UTDallas: Enlisting E. Scherichia Holmes: A modular whole-cell biosensor for the detection of environmental pollutants

Recalcitrant pollutants such as petroleum constituents and nitrates are regularly introduced to the environment through oil spills, natural geological seepage and eutrophication. The UN’s flagship water protection initiative enumerates a host of health risks associated with these chemicals. UT Dallas iGEM addresses the eminent need to mitigate their circulation by developing novel whole-cell biosensors that can detect alkanes, aromatics and nitrates and execute combinatorial logic, feedback and noise-reduction functions inspired by synthetic biology. This work has wide ranging applications requiring a cheap chemical sensor that can dynamically process heterogeneous inputs and express a user-friendly output.

Team Valencia: Mad yeast on Mars

We present an intermediate scenario in the pathway towards Mars Terraformation. The project is focused in

two essential conditions on this process: Increase the planet temperature, resistance of microorganism to thermal changes. The proposal is that dark yeast cells retain the arriving radiation and heat the surface. But, once the temperature reaches its optimum on the planetary surface the color production should be switched off. In order to achieve that and taking advantage of Synthetic Biology principles, a switch based on prion proteins (on mad yeast!) will be used. The work will be complemented with the implementation of the expression of LEA (late embryogenesis abundant) “antifreeze” protein. Summarizing, we are going to build a engineered yeast resistant to temperature changes and able to produce a dark pigment which will be the responsible of a global temperature increase on Mars.

Team VictoriaBC: Exploring AHL inducible fluorescence

AHL mediated quorum sensing is utilized by many gram negative bacteria for both intra and interspecies communication. Cells containing synthetic DNA constructs that produce fluorescent proteins in the presence of AHLs could be used as in situ reporters of the AHL milleu in dynamic natural systems like biofilms and plant root nodulation. Additionally, cells that can produce AHLs could be used to alter the signalling in these systems and examine the results. Finally, different combinations and spacial arrangements of AHL inducible reporters, AHL producing cells and AHL degrading cells all regulated by the AHL milleu of the media could allow for information processing through the interactions between such nodes. Out team designed a number of AHL regulated nodes, devices that each respond to AHL levels in different ways, and attempted to build some of these nodes to characterize their behavior.

Team Virginia_United: Quorum Sensing Amplifiers and a Codesign Approach for Information Processing

Synthetic biology endeavors to create information processing systems modeled on digital electronics. The use of quorum sensing can help transform an inherently analog molecular signal into a binary response and simultaneously allow the tuning of input response thresholds and signal amplification. This project demonstrates these capabilities through experimentation and modeling. Another candidate for reapplying an electronic engineering technique is the codesign of hardware and software to implement a function. In synthetic biology, codesign might mean implementing a design spec in different expression control regimes and comparing their relative merits. Our work examines the codesign concept by constructing an AND gate in three different design domains. We explore the application of these ideas with an environmental sensor. A unique aspect of our project is the collaborative nature involving five institutions at three locations.

Team VT-ENSIMAG_Biosecurity: Design and development of the GenoTHREAT gene sequence screening software

In order to mitigate the biosecurity risks associated with the potential dual use of gene synthesis, the U.S. Government published a draft version of a “Screening Framework Guidance for Synthetic Double-Stranded DNA Providers.” This document outlines a minimal DNA sequence screening protocol that gene synthesis companies are encouraged to use prior to fulfilling an order. The protocol relies on the “Best Match” method developed in response to the limitations of other screening protocols previously proposed by trade organizations. The objective of the “Best Match” method is to identify sequences which are uniquely related to Select Agents or Toxins. The GenoTHREAT software is being developed in accordance with the Government guidance and, to our knowledge, is the first implementation of the sequence screening procedure outlined in the guidance. Although software characterization has elucidated both strengths and limitations, GenoTHREAT appears to be a viable tool for sequence screening.

Team Warsaw: An universal platform for protein delivery to the mammalian cells

Our project consists of two parts: BactoDHL and RBS Measurement. BactoDHL is a universal platform for protein and DNA delivery to the mammalian cells. It's based on E. coli strain expressing invasion determinants: invasin from Yersinia pestis and listeriolysin (LLO) from Listeria monocytogenes. Invasin causes uptake of the bacterium into the mammalian cell by induction of endocytosis. Bacterial cells are lysed in the endosome and then LLO is released. LLO is a pore-forming toxin which causes endosomal membrane disruption and release of the payload (either protein or DNA) into cytoplasm of the mammalian cell. In order to fine-tune expression of genes used in our project we conducted measurement of various RBS parts included in 2010 spring distribution both from Community and Anderson's collections. We used standard measurement kit composed of promoter BBa_J23100 and GFP+terminator part BBa_I130401. We performed both relative and absolute RBS strength measurement.

Team Washington: Antibiotics for the 21st Century

While vital to our quality of life, traditional antibiotics face the serious problems of widespread bacterial resistance and destruction of natural gut flora - problems which call for improved twenty-first century antibiotics. Using synthetic biology tools, we designed, built, and tested two new systems to fight infections by both broad types of bacteria- Gram-positive and Gram-negative. Our first project targets Bacillus anthracis, the Gram-positive pathogen that causes anthrax. We re-engineered an enzyme to remove the pathogen's protective coating, rendering it defenseless against the immune system. In our second project, we re-engineered and transplanted a protein secretion system capable of combating Gram-negative bacteria into E. coli. This system was designed to target Gram-negative pathogens in a modular and controllable fashion. These two systems are the vanguard of a new era of antibiotics using the power of nature harnessed with the tools of synthetic biology.

Team WashU: A new set of synthetic biology tools for Saccharomyces cerevisiae

Saccharomyces cerevisiae is a model unicellular eukaryotic chassis; however when compared with Escherichia coli the available synthetic biology tools are lacking. To remedy this problem the 2010 Washington University iGEM team has introduced a synthetic alternative splicing tool, as well as designed and produced new BioBricks parts to ease transformation of synthetic constructs into S. cerevisiae. A mutually exclusive exon splicing system was formulated in which Sex-lethal interacts with the native splicing machinery to affect splice site choice. Two vectors have been designed to facilitate simple bacterial BioBrick manipulation and subsequent chromosomal integration into the yeast genome. A yeast positive selection marker BioBrick has been produced for the first time. Chromosomal integration with positive selection will stabilize and streamline BioBrick transformations into S. cerevisiae. A synthetic splicing assembly will allow for new synthetic biology techniques such as isoform engineering of proteinsor combinatorial logic.

Team Waterloo: Staphiscope: diagnosis of S. aureus through bacterial wiretapping

Superbugs, or antibiotic resistant microorganisms, are microbes that have become resistant to traditional treatments. These types of infections are difficult to diagnose, treat, and eradicate, making the healing process time consuming and resource intensive. The native quorum-sensing unit from S.aureus (the Agr

system), will be introduced into a non-pathogenic strain of E.coli. The E.coli will then effectively have the ability to eavesdrop on the activity of the pathogenic organism and emit an indication of the magnitude of the infection in the form of RFP. Using sensitivity tuners the system can be designed such that the response will occur at an exact level, when the size of the population poses a threat to the host. Upon a positive result from a diagnosis, further tests could be done to specify whether MRSA (methicillin-resistant S. aureus) or MSSA (Methicillin-sensitive S. aureus) are present.

Team Weimar-Heidelberg_Arts: Super Cell - the synthetic biology supermarket

Super Cell is a fictional supermarket offering speculative products which are all based on Synthetic Biology. The Super Cell website already gives us a glimpse of the products that will be available in local stores in the near future. By putting already existing and far-out in the future applications next to each other in a familiar environment such as a webshop, the project aims to improve public awareness about Synthetic Biology and at the same time foster a debate on how we want to see it manifested in our everyday lives. This kind of discussion, we feel, is urgent to have if we as a society want to influence how this powerful science will affect our future lives.

Team Wisconsin-Madison: Universal Platform for Polypeptide Delivery: Intelligent Delivery of Ingestible EnzymeTreatment (iDIET)

We have designed a universal platform for polypeptide release within the small intestine of the human gut. Our model system release beta-galactosidase, a functional homologue of human lactase, once it reaches the duodenum to help a lactose intolerant patient metabolize lactose. The chassis for this system is the common probiotic in yoghurt, Lactobacillus acidophilus. Once the Lactobacillus acidophilus has reached the duodenum, they will lyse by either by a timed inducible/repressible system, a bile-inducible system, or an encryption system. Using DNA we can mimic the functionality of a combination lock, and produce a "locked" gene, which can be effectively "unlocked" only after a specific sequence of inputs. Since DNA functions as a logical medium, the "locked" and "unlocked" states are heritable, which makes this system useful as the computational basis for many higher-order genetic devices from bacterial calculators to engineering of new metabolic pathways to bacterial drug delivery systems.

Team WITS-South_Africa: Lactoguard: A commensal whole-cell bionsensor for the diagnosis of sexually transmitted infections

Whole-cell bacterial biosensors have been developed for a range of applications. In this project, the concept of a whole-cell biosensor has been adapted for medical use as a diagnostic for viral infection through engineering a commensal bacterium. Lactobacilli are the predominant commensal organisms in the human vaginal mucosa and are ideally placed to detect the presence of a sexually transmitted infection, such as Human Papillomavirus. A strain of this bacterium has been modified to produce a chromogenic reporter, which is visible to the naked eye, when ‘switched on’ through exposure to the input signal (viral infection). However, in order for this to be clearly visible in vivo, this signal needs to be transmitted throughout the entire bacterial population. Thus, this project focused on a) modifying a Gram-positive bacterium to produce the chromogenic reporter; b) using a heterologous quorum-sensing mechanism to propagate an auto-regulated feedback loop amongst a bacterial population.

Team Yale: Manufacturing electrical circuits using localized microbial metal deposition

What does it take to make bacteria produce an electrical circuit? One of the most exciting uses for synthetic biology is in the design of biological systems that can replace and improve industrial processes. By achieving industrial goals using biological processes, we predict dramatic reductions in economic and environmental manufacturing costs. Our project is a first step towards biologically synthesized electronic circuits. Based on precedence of naturally redox-capable bacteria, we generated a system in E. coli that reduces metal in solution. Depending on the application, this system has the ability to form a conductive copper sulfide that can be localized with high precision. In the future our bacteria could catalyze metal deposition to form electrical circuits of any desired dimension and complexity.

Team ZJU-China: Bach: gene composer

While former iGEM software teams were mainly focusing on the assembly of biobricks into a predictable system, our software Bach targets at the coding sequence of biobrick itself. We first built a mathematical model to quantitatively predict RIPS (ribosome initiation per sec) of any given coding sequence in any organism. We then constructed three modules (synonymous substitution, optimization and RIPS design) to process input coding sequences. Bach could recompose the input sequence into an output one that better suits the host organism and performs the specific translational rate of desire. In this way, the discordant tunes of codon bias in biobricks from different genetic backgrounds could be synchronized, and the realization of a more predictable and robust system would be possible. Our work not only benefits current works involving exogenous protein expression, but also makes a big step towards standardization and characterization of biobricks.

2009

Team Aberdeen_Scotland: A Synthetic Biology Approach to Pipe Repair: The Pico-Plumber

Damage to inaccessible pipe systems, such as computer cooling circuits, is difficult to rectify. An Escherichia coli synthetic biology circuit for pipe repair was designed. Pipe breach detection and the restoration of pipe integrity were implemented through exploitation of chemotaxis, and cell lysis that releases a two-component protein-based glue (lysyl oxidase and tropoelastin). Control was achieved using an AND gate with quorum sensing and the lac inducer IPTG (released from the breach) as inputs. Deterministic and stochastic models of the genetic circuit, integrated with an agent-based model of E.coli cells, were used to define the effective radii of cell migration and timing of lysis. Constructed AND gate, quorum sensing and lysis timing modules were experimentally tested. The two-component glue concept was successfully validated using in vitroalpha-omega complementation of beta-galatosidase activity. Finally, a proposal for an igem.org-based parameter database was developed to aid the rapid identifation of BioBricks parameter values.

Team Alberta: A Synthetic Biology Tool Kit for Artificial Genome Design and Construction

The creation of simplified artificial cells with specialized functions, along design principles that are compatible with the goals of synthetic biology, requires advances in two key areas. In Silico modelling tools are needed to assess the performance of artificial networks prior to assembly. Genome biofabrication must achieve rates well beyond existing methods using a modular design so that the extent to which natural systems can be made artificial can be tested. We have taken our first steps towards these goals by directing our efforts to the rational refactoring of the E. coli genome. Using flux balance analysis we have identified 117 new genes that may be essential for survival. We have developed and validated a rapid, modular biofabrication method (BioBytes) and have produced BioBytes for 150 of our 447 essential gene list. We have also built a Lego Mindstorm-based DIY biofab robot and extended the concept to a BioFab-on-a-chip prototype.

Team ArtScienceBangalore:

We consider ourselves amateurs/novices within the context of the IGEM competition. Our endeavor as “outsiders” is to bring our training in the arts and design to synthetic biology. Over this summer, we learnt the tools and techniques of synthetic biology and developed a piece of life which reflects our concerns, namely, the cultural, ethical and aesthetic implications of Synthetic Biology. Using a DIY approach and getting our hands “wet” was a critical element in the learning process. Our construct synthesizes Geosmin, an enzyme normally produced by cyanobacteria and actinobacteria. The biosynthesis of geosmin from farnesyl diphosphate is catalyzed by a single enzymegermacradienol/germacrene D synthase.E. coli, does not bear a gene that codes for this enzyme. We have expressed this gene in different strains of E. coli. Geosmin is responsible for producing the earthy smell when rain falls after a dry spell of weather.

Team Bay_Area_RSI: Breast cancer cell targeting phage

Breast cancer is the second most common type of cancer diagnosed in women. RNAi has proven to be an effective mechanism in the silencing of oncogenes. Therefore, we have attempted to build a viable system for the delivery of RNAi into breast cancer cells. First, we inserted a shRNA sequence coding for the Raf-1 protein into an AAV cassette containing two ITR's, allowing it to reproduce itself in mammalian cells. This cassette was inserted into our chosen vector, the filamentous bacteriophage FUSE-55. An antibody sequence was then added to the phage plasmid near the coat protein sequence in order to target HER2. As an additional feature, we have fused Silicatein and Silintaphin to mStrepavidin, which will bind to a protein tag in the coat, forming silicate structures on the coat of the phage, thereby reducing the immunotoxicity of the bacteriophage in vivo.

Team BCCS-Bristol: VESECURE

Directed delivery of specific proteins into cells would have dramatic consequences for drug delivery and expand the horizons of synthetic biology into the multicellular domain via discrete, targetted communication. Gram-negative bacteria naturally produce outer member vesicles (OMVs): spherical, bilayered proteolipids from 20-200nm in diameter. OMVs carry outer membrane, periplasmic and cytoplasmic proteins, DNA, RNA and other biological molecules. They protect their cargo from the extracellular environment and deliver it to a multitude of target cells via membrane fusion. We investigate the possibility of allowing the secretion of any protein in OMVs via fusion with novel, non-toxic partners enhanced in OMVs, using a novel Bioscaffold compatible with the current assembly standard. A new version of the award winning BSim software has been developed to study applications at the population level such as communication. The ultimate goal is to create a safe and standardised system for directed delivery of proteins into cells.

Team Berkeley_Software: Eugene, Spectacles, and Kepler: Managing Synthetic Biology Device Development

Three crucial activities in synthetic biology are the creation of standardized parts, the construction and specification of devices from these parts, and the automatic assembly of these devices. Each of these activities requires software tools. Tools give users access to data as well as provide algorithmic support and abstraction to design large scale systems. We have created three software tools for these tasks. The first is a domain specific language called Eugene for the specification of biological constructs and rules for their creation. The second contribution is a visual design environment for device creation called Spectacles. Finally, we have created workflows for the Kepler design environment. This work is integrated within the Clotho design framework. We show that together they offer a powerful solution to the problems of today while also providing a path to the more exotic design activities of the future.

Team Berkeley_Wetlab: Automated assembly of cell surface display devices

The University of California Berkeley iGEM team has developed an automated approach to large-scale parts assembly that is accurate, high-throughput, reduces labor, and decreases cost. As a test bed for our system we have chosen to explore novel applications of cell surface display within Escherichia coli, the gold standard organism for bacterial engineering. Displaying peptides and proteins on a cell's surface is difficult, and many attempts may have to be made to generate a given functional protein. By automatically generating and testing a large set of diverse proteins paired with various display methods, we can search a large design space and develop guidelines for rational design of projects involving surface display.

Team BIOTEC_Dresden: Temporal and spatial control of protein synthesis by in vitro recombination inside picoliter reactors

Manufacturing functionalized proteins in vitro poses a challenge, as it requires coordinated molecular assemblies and multi-step reactions. In this project we aim to control, over time and space, the production of proteins tagged with a silver-binding peptide for in situ silver nanoparticle nucleation inside microdroplets generated by microfluidic devices. Combining a transcription-translation system with protein coding genes and a recombination logic inside microdroplets provides spatial control. Moreover, in the microfluidic chamber we can pinpoint the beginning of synthesis, and easily track and isolate the droplets. Site-specific recombination generates a molecular timer for temporal control of protein synthesis. Unlike transcriptional regulation, this method gives true all-or-none induction due to covalent modification of DNA by Flp recombinase. Determining the transfer curve of inter-FRT site distance versus average recombination time allows the onset of gene expression to be predicted. We then apply this Flp reporter system as a powerful PoPS measurement device.

Team Bologna: T-REX: Trans-Repression of Expression. A BioBrick gene-independent control of translation

The project aims to realize a device with standard biological parts for the post-transcriptional control of gene expression, regardless of the gene sequence to be silenced. We designed the T-REX device, composed of two non-coding DNA sequences: the TRANS-repressor and the CIS-repressing parts. TRANS-repressor acts as a silencer of CIS-repressing RNA target. This target includes a region complementary to the TRANS-repressor sequence antisense, ends with RBS, and is assembled upstream of the coding sequence to be silenced. Upon binding of TRANS-repressor and CIS-repressing RNAs, the access to RBS by ribosomes is hampered, silencing translation. Accordingly, the amount of TRANS-repressor controls the translation rate of the regulated gene. The TRANS-repressor sequence was determined by a computational analysis performed to minimize the interference with the genomic mRNAs and to maximize the base-pairing interaction to the CIS-repressing RNA. The T-REX device is proposed as a universal and fast switch in synthetic gene circuits.

Team British_Columbia: Development of a modular, analog E. coli biosensor

To date, efforts to design a whole-cell biosensor capable of detecting levels of one or more biological inputs and responding in an analog mode has been elusive. We have designed a system of synthetic constructs implemented in an E. coli chassis that will allow detection of continuously varying levels of a single metabolic input and report on the concentration with qualitative output depending on threshold levels of the input. Our system design utilizes RNA-level hairpin hybridization and antisense technologies linked to various reporters. Because our approach is modular and does not depend on either endogenous protein processing or exogenous RNA, we envision that such a system could find applications in many different fields, including environmental sensing, detection of diagnostic of therapeutic biomarkers, and systems biology.

Team Brown: Engineering Staphylococcus Epidermidis to Secrete Recombinant Histamine Binding Protein in Response to Changing Histamine Concentration

The 2009 Brown iGEM Team aims to treat allergic rhinitis (hay fever) by engineering Staphylococcus epidermidis to secrete a histamine-binding protein, rEV131, in response to elevated histamine

concentrations during an allergic attack. rEV131 was cloned from a species of tick, Rhipicephalus appendiculatus. We are putting the rEV131 gene into an endogenous element of human nasal flora, Staphylococcus epidermidis. rEV131 will have a secretion tag specific for S. epidermidis. To synchronize rEV131 production with elevation of histamine concentration, we are computationally designing a novel histamine receptor. This histamine-responsive receptor will induce expression of rEV131. Although S. epidermidis is a non-pathogenic species, when it reaches a certain population threshold it produces potentially hazardous biofilms. To mitigate this concern, we have engineered safety measures that prevent excessive growth by repurposing S. epidmidis' natural population sensor to cue each cell's "suicide" when a population has reached a dangerous size.

Team Calgary: Reprogramming a Language and a Community

iGEM Calgary contributed a second quorum sensing (QS) system to the Registry. The Vibrio harveyi AI-2 QS signalling system has been engineered in Escherichia coli . Coupled with quorum quenching, our system allows us to target biofilm maintenance. The robustness of AI-2 signalling in E. coli was characterized in the lab and compared to data from mathematical models of the system built using the Matlab Simbiology toolbox and the emerging Membrane Computing framework in Mathematica. We also undertook community outreach projects in order to enhance the synthetic biology community. Specifically, the Second Life platform was used to create an educational tool to train future synthetic biologists in an accessible, user-friendly, virtual environment. Moreover, we examined the implications of our project in light of the recently proposed proactionary and precautionary frameworks with special focus on ethical, environmental, economic, legal and social (E3LS) impact.

Team Cambridge: E. Chromi: Triggering Pigment Production in E. Coli

Previous iGEM teams have focused on genetically engineering bacterial biosensors by enabling bacteria to respond to novel inputs, especially biologically significant compounds. There is an unmistakable need to also develop devices that can 1) manipulate the input by changing the behaviour of the response of the input-sensitive promoter, and that can 2) report a response using clear, user-friendly outputs. The most popular output is the expression of a fluorescent protein, detectable using fluorescence microscopy. But, what if we could simply see the output with our own eyes? The Cambridge 2009 iGEM team is engineering E. coli to produce different pigments in response to different concentrations of an inducer.

Team CBNU-Korea: Essarker: An Essential Remarker for a Minimal, Synthetic Genome

It is challengeable to create a synthetic genome for fulfilling the needs of energy and food. Without the assistance of computing tools, moreover, it would be much more difficult to make the synthetic genome. We here propose a key tool to help the creation of a genome as the essential step. The goal of Essarker is to help users design a minimal genome synthesized through the fundamental frame comprising the essential genes of replication. Essarker is a standalone software to manage and retrieve required sequences of genomes, and explore the essential gene order and direction and the related orthologous genes. It also identifies and visualizes the positions and orientations of genes. In addition, it shows optimal ordering of essential genes and orthologs by statistical analysis.

Team Chiba: E. coli Time Manager Since 2008

Since 2008, we have been constructing the bacteria timer that "work together". The mechanism is very simple; (1) the "Transmitter cells" generates the signal molecules, whose concentration gradually

increases, (2) when it reaches a certain level, the "Receiver cells" switch on the expression of any given genes. Precise control of the time of delay of this entire process, one can pre-set the time of expression of genetic functions in a predicable manner. By using Asyl-Homoserine Lactones(AHLs) that can freely pass through the cell membrane as signal molecules, the time can be shared, in real time, by all cells within the pot. This way, receiver (timer) cells would take the action all at once in right timing, minimizing the distribution in each cell's response time. This year, we are trying to make a platform for generating an animated pictures using series of new timer cells we have constructed.

Team CityColSanFrancisco:

We at CCSF have begun constructing a bacterial powered battery. The design has been generated with sustainability in mind, and aims to create an alternative to traditional fossil fuel technologies. The battery owes its capabilities to two strains of bacteria: the heterotroph Rhodoferax ferrireducens, and the photoautotroph Rhodopseudomonas palustris. Each strain will occupy its own concentration cell and after being cultured anaerobically, will either oxidize (in the case of R. palustris) ferris iron or reduce (in the case of R. ferrireducens) ferric iron. The resulting current will be collected and used to demonstrate the functionality of the battery. The reduction and oxidation reaction will be self-substaining. This process is further aided by the genetic modification of R. palustris. As a photosynthetic prokaryote, R. palustris generates glucose readily. We intend to share this glucose with R. ferrireducens by inserting a passive glucose transporter into the cells of R. palustris.

Team Cornell: Engineering the Bacillus Subtilis Metal Ion Homeostasis System to Serve as a Cadmium Responsive Biosensor

The goal of our project is to create a whole cell cadmium biosensor by attaching cadmium responsive promoters in Bacillus subtilis to fluorescent reporter proteins. Cadmium is a toxic heavy metal which has no known biological function. Ingestion of cadmium contaminated water can induce bone fractures and severe renal damage. Major sources of cadmium contamination include fertilizers, sewage sludge, manure and atmospheric deposition. Cadmium contaminated sewage is often used for irrigation purposes in many parts of the world, especially in developing nations. Crops grown in these contaminated soils are then sold in markets without any detoxification treatment. Current analytical methods such as atomic absorption spectroscopy, though highly sensitive, are significantly more expensive than bacterial biosensors and are unable to measure the amount of bioavailable cadmium.

Team DTU_Denmark: The redoxilator, and the USER fusion assembly standard

The Redoxilator: By in silico design and computer modelling followed by gene synthesis, we have constructed a molecular NAD/NADH ratio sensing system in Saccharomyces cerevisiae. The sensor works as an inducible transcription factor being active only at certain levels of the NAD/NADH ratios. By the coupling of a yeastoptimized fast degradable GFP, the system can be used for in vivo monitoring of NAD/NADH redox poise. A future novel application of the system is heterologous redox coupled protein production in yeast. The USER fusion standard: Another part of our project is the proposal of a new parts-assembly standard for Biobricks based on USER(TradeMark) cloning. With this technique, not based on restriction enzymes, all parts independent of function can be assembled without leaving any ‘scars’ from the restriction enzyme digestions.

Team Duke: One-Step Construction of a Bioplastic Production Pathway in

E. coli

A convenient ligation-free, sequence-independent one-step plasmid assembly and cloning method is developed [Quan J, Tian J (2009) Circular Polymerase Extension Cloning of Complex Gene Libraries and Pathways. PLoS ONE 4(7): e6441]. The strategy, called Circular Polymerase Assembly Cloning (CPEC), relies on polymerase extension to assemble and clone multiple fragments into any vector. Using this method, we are able to quickly assemble a metabolic pathway consisting of multiple enzymes and regulatory elements for the production of a biocompatible as well as biodegradable plastic polymer in E. coli.

Team Edinburgh: Defusing a dangerous world: a biological method for detection of landmines

Landmines left over from past conflicts are a major hazard in the world, killing and maiming many people every year. We have sought to engineer a bacterium able to detect TNT and its degradation products, nitrites, in the environment. Our system is based around a previously published computationally designed TNT-sensing protein derived from the periplasmic ribose binding protein, which interacts with an EnvZ-Trg transmembrane hybrid fusion protein and a nitrite-responsive repressor to trigger a pathway of TNT degradation and visualization using combined output from a bacterial luciferase and Yellow Fluorescent Protein. We envisage that the detection system could be applied by spraying the organism on soil where the presence of landmines is suspected, and detecting luminescence using low-light sensing. Once located, the mines could be safely removed. This system could be extended to detect other analytes in the environment.

Team EPF-Lausanne: E. Colight

Recent discoveries of photoreceptors in many organisms have given us insights into the interest of using light-responsive genetic tools in synthetic biology. The final goal of our project is to induce a change in gene expression, more specifically to turn a gene on or off, in a living organism, in response to a light stimulus. For this we use light-sensitive DNA binding proteins (or light-sensitive proteins that activate DNA binding proteins) to convert a light input into a chosen output, for example fluorescence, through a reporter gene such as RFP. Demonstrating that the light-induced gene switch tool works in vivo would show that easier and faster tools can potentially be made available in several fields of biology, as such tools can induce more localized, more precise (time resolution and reversibility) and drastically faster genetic changes than currently used ones, thus allowing research to evolve even better.

Team Freiburg_bioware: Universal endonuclease – cutting edge technology

Gene technology is driven by the use of restriction endonucleases. Yet, constraints of limited sequence length and variation recognized by available restriction enzymespose a major roadblock for synthetic biology. We developed the basis for universal restriction enzymes, primarily for routine cloning but also with potential for in-vivo applications. We use a nucleotide cleavage domain fused to a binding domain, which recognizes a programmable adapter that mediates DNA binding and thus cleavage. As adapter we use readily available modified oligonucleotides, as binding domain anticalins, and as cleavage domain FokI moieties engineered for heterodimerization and activity. For application, this universal enzyme has merely to be mixed with the sequence-specific oligonucleotide and the target DNA. Binding and release are addressed by thermocycling. We provide concepts for in-vivo applications by external adapter delivery and activity regulation by photo switching. Additionally, an argonaute protein is engineered towards a DNA

endonuclease.

Team Freiburg_software: SynBioWave – A Collaborative Synthetic Biology Software Suite

Synthetic Biology, which aims at constructing whole new genomes, is pushed forward by many users and relies on the assembly of genetic elements to devices and later systems. The construction process needs to be transparent and even at final stages control at the basepair level is required. We are building a software environment enabling multiple distributed users to analyze and construct genetic parts and ultimately genomes with real-time communication. Our current version demonstrates the principle use as well as the power of the underlying Google Wave protocol for collaborative synthetic biology efforts. Many wave-robots with a manageable set of capabilities will divide and conquer the complex task of creating a genome in silico. The initial developments of 'SynBioWave' lay the ground for basic layout, calling and data exchange of wave-robots in a clear and open process, so that future robots can be added and shared easily

Team Gaston_Day_School: Development of a Red Fluorescent Nitrate Detector

Increasing levels of fertilizer required for mechanized farming can result in elevated nitrate levels in soil and groundwater. Due to contaminated food and water, humans are at risk for Methemoglobinemia caused by enterohepatic metabolism of nitrates into ammonia. This process also oxidizes the iron in hemoglobin, rendering it unable to carry oxygen. Infants in particular are susceptible to Methemoglobinemia, also known as “Blue Baby Syndrome”, when formula is reconstituted using contaminated water. In order to prevent Methemoglobinemia, it is essential to detect high concentrations of nitrates. Fnr-NarG is an aerobic mutation of the nitrogen-sensitive promoter NarG that was provided by Dr. Lindow at UC Berkeley. By combining Red Fluorescent Protein with an aerobic mutant strain of NarG, the creation of Red Fluorescent Nitrate Detector (RFND) is possible. RFND is economically efficient because of its ability to self-replicate.

Team Groningen: Heavy metal scavengers with a vertical gas drive

Heavy metal pollution of water and sediment endangers human health and the environment. To battle this problem, a purification strategy was developed in which arsenic, zinc or copper are removed from metal-polluted water and sediment. In this approach Escherichia coli bacteria accumulate metal ions from solutions, after which they produce gas vesicles and start floating. This biological device encompasses two integrated systems: one for metal accumulation, the other for metal-induced buoyancy. The uptake and storage system consists of a metal transporter and metallothioneins (metal binding proteins). The buoyancy system is made up of a metal-induced promoterupstream of a gas vesicle gene cluster. This device can be changed to scavenge for any compound by altering the accumulation and the induction modules. The combination of both systems enables the efficient decontamination of polluted water and sediment in a biological manner.

Team Harvard: Interspecies Optical Communication Between Bacteria and Yeast

Optical communication is central to interactions between many multicellular organisms. However, it is virtually unknown between unicellular organisms, much less between unicellular organisms of different kingdoms of life. Our team has constructed a system that allows for interspecies, bacteria-to-yeast optical communication. To permit bacteria to send an optical signal, we expressed in E. coli a red firefly luciferase

under IPTG induction. In yeast, we used a yeast-two-hybrid-system based on the interaction between the red-light-sensitive Arabidopsis thaliana phytochrome PhyB and its interacting factor PIF3. Interaction between PhyB and PIF3 is induced by the red light from the bacteria, resulting in transcription of the lacZ gene. This is an excellent demonstration of the principles and potential of synthetic biology: this system not only allows for interspecies optical communication, but enables us to optically bridge a physically separated canonical lac operon using light as a trans-acting factor.

Team Heidelberg: Spybricks - a starter kit for synthetic biology in mammalian cells

Mammalian synthetic biology has a huge potential, but it is in need of new standards and a systematic construction of comprehensive part libraries. Promoters are the fundamental elements of every synthetic biological system. We have developed and successfully applied two novel, in silico guided methods for the rational construction of synthetic promoters which respond only to predefined transcription factors. Thus, we have been able to create a library of promoters of different strength and inducibility. To characterize the promoters, we have developed standardized protocols for comparable measurements of promoter strength by either transient or stable transfection. These synthetic promoters can be used as “spybricks” which enable the construction of assays for simultaneously monitoring several pathways in a cell. However, the potential of synthetic promoters goes far beyond this application: e.g. in virotherapy, these promoters could be used for selective gene expression in target cells.

Team HKU-HKBU: Biomotor

Much hope has been laid on nanorobots in their application in therapeutics in this era of catheters and minimally invasive surgery, but the problem remains that purely mechanical nanorobots lack sufficient locomotive power to perform their intended tasks. Our 'bio-motor' aims to breach this gap to bring a foundational advancement. In our model, Escherichia coli cells are engineered to specifically express streptavidin at pole(s), which allows cells to adhere in the same orientation to a microrotary motor through biotin-streptavidin interaction. Thus, with the propulsion generated by bacterial flagella, this synthetic device is capable to convert biological energy into mechanical work. Furthermore, the propulsion energy was programmed to be adjustable by controlling E.coli swimming speed, i.e. putting E.coli cheZ gene under the control of ptet. This technology has tremendous potential to be applied in various fields including biomedicine, bio-energy, and bioengineering.

Team HKUST: SynBiological Bug Buster

We aim to engineer a novel yeast strain that can detect, attract and eliminate pests. This strain would serve as an environmental-friendly substitute for pesticides. The idea is demonstrated by constructing an odorant sensing module, coupled production of chemical attractant and production of pest-killing binary toxin in yeast to kill pests lured to the yeast culture. A chimera G-protein coupled receptor (GPCR) responsive to an odorant chemical is coupled to the yeast mating pathway that can be activated upon ligand binding. It leads to over-expression of an endogenous yeast transaminase that catalyzes a reaction to yield 2-phenylethanol. Constitutively expressed binary toxin in the yeasts would poison the attracted pests after their consumption, as tested by feeding drosophilae. In addition to being a pesticide substitute, this cheaply-maintained engineered yeast strain also serves as a research reagent to screen for GPCRs that bind to certain ligands.

Team IBB_Pune: Constructing multi-strain computational modules using

Nucleotide and Protein mediated cell-cell signaling.

Building complex genetic circuits in a single cell becomes difficult due to the formidable task of co-transforming large nucleotide sequences in addition to the imposed metabolic burden on the cell. Can a complex system be divided into independent modules that reside in different cells and interact with each other using nucleotide and protein mediated cell-cell signalling to act as a single unit? We seek to address this problem using a three pronged approach. Firstly, we are trying to introduce natural competance genes into the biobrick framework which will act as nucleotide importers. We are also building a protein export system using the TAT dependent export pathway. Finally, we are attempting to construct a multi-state turing machine which is a compound, modular computational system that has independent, interacting states which applies the above principle. We hope that this approach overcomes the obstacles in building more complex and composite circuits.

Team IIT_Bombay_India: Analysis of multiple feedback loops using Synthetic Biology

One of the major objectives of synthetic biology is to unveil the inherent design principles prevailing in biological circuits. Multiple feedback loops (having both positive and negative regulation) are highly prevalent in biological systems. The relevance of such a design in biological systems is unclear. Our team will use synthetic biology approaches to answer these questions. Our team comprises of nine undergraduates, 3 graduate students as student mentor and two faculty mentors, one each from biology and engineering background. The project specifically deals with the analysis of effect of single and multiple feedback loops on gene expression. This project will involve theoretical and experimental studies. We have designed synthetic constructs to mimic multiple feedbacks. The focus of our experimental work will be to visualize the effect of multiple feedback loops on the synthetic construct using single cell analysis. The project will provide insights into the roles of multiple feedback loops in biological systems.

Team IIT_Madras: PLASMID: Plasmid Locking Assembly for Sustaining Multiple Inserted DNA

Any episome introduced into the cell shows segregational asymmetry accompanied with differential growth rates in the absence and presence of episome leading to an overall loss of the episomal unit in the absence of any selective pressure. We have designed a versatile system which maintains any given plasmid DNA in E.coli by using user-defined selection pressures, limited only by the presence of a response element to said pressure, like most antibiotics, certain chemicals and physical conditions. Depending on this selection pressure, a custom plasmid retaining system can be designed and co-transformed with the plasmid of interest to maintain it. A similar system can be used to “lock” the function of a gene of interest, like a combination lock, which is unlocked only when the cultures are grown in a pre-determined order of selection pressures. In principle, using this locking system, multiple plasmids can be maintained using a single selection pressure.

Team Illinois: Bacterial Decoder

The Illinois iGEM team has been working to engineer a decoder function within E. coli. Decoders are logic devices used frequently in low-level computer architecture. We are creating a 2 to 4 decoder, which takes two binary inputs to activate one of four outputs. Each output corresponds to a specific combination of the inputs. With the presence of lactose and arabinose, our Bacterial Decoder will express Green Fluorescent Protein. If only lactose is present, a different fluorescent protein will be expressed. This goes for the other

two combinations as well (only arabinose, or neither sugars). To implement logic we use combinations of small non-coding RNAs and transcriptionfactors. The system allows the next engineer to swap standard parts in and out to change the inputs and outputs. Our Bacterial Decoder can help sense for multiple environmental cues, having implications for medical diagnostics and environmental and water contaminant detection.

Team Illinois-Tools: Interactive Metabolic Pathway Tools

Interactive Metabolic Pathway Tools (IMP Tools) is an open source, web based program that involves model-guided cellular engineering where new metabolic functions can be added to existing microorganisms. This program will assist in the design stage of synthetic biology research. IMP tools is written primarily in python using the Django web framework. It takes a user-defined input compound, output compound, and weighting scheme and determines the ideal pathway from the starting to the ending compound. Our program presents an exciting capability to help transform important processes in the world for applications ranging from bioremediation to biofuels.

Team Imperial College London: The E.ncapsulator

For iGEM 2009 the Imperial College London team present you with The E.ncapsulator; a versatile manufacture and delivery platform by which therapeutics can be reliably targeted to the intestine. Our E.coli chassis progresses through a series of defined stages culminating in the production of a safe, inanimate pill. This sequential process involves drug production, self-encapsulation in a protective coating and genome deletion. The temporal transition through each of these stages has been individually optimised by both media and temperature. The E.ncapsulator provides an innovative method to deliver any biologically synthesisable compound and bypasses the need for expensive storage, packaging and purification processes. The E.ncapsulator is an attractive candidate for commercial pill development and demonstrates the massive manufacturing potential in Synthetic Biology.

Team IPN-UNAM-Mexico: Turing meets synthetic biology: self-emerging patterns in an activator-inhibitor network.

We present a synthetic network that emulates an activator-inhibitor system. Our goal is to show that spatio-temporal structures can be generated by the behavior of a genetic regulatory network. We implement the model by means of several biobricks. We construct a self activating module and correspondingly an inhibitory one. Self-activation dynamics is given by the las operon, while the inhibitory part is provided by the lux operon. Quorum sensing and diffusion of AHL provide the reaction-diffusion mechanism responsible for the formation of Turing patterns. The importance of our work relies on the fact that we show that the action of the morphogenes as originally proposed by Turing is equivalent to the effect of diffusion of chemicals interacting with the synthetic network, which accounts for the reactive part, a possibility implicit in Turing's original work in the context of morphogenesis of biological patterns.

Team IPOC1-Colombia: Molecular Device to Detect Sea Salinity

Different gene parts are being assembled in order to construct a device that is able to detect different salinity levels in the sea. The device is tested against different concentrations of sodium chloride, fluoride, calcium, and magnesium. Different parameters, such as reporter fluorescence, DNA concentration, growth of bacterial device will be used to measure the efficacy of the device. Computational modeling will be used in the project to complement the laboratory work. Importance of project: Colombia borders two oceans: the Atlantic and the Pacific.

Team IPOC2-Colombia: Molecular Device that Biodegrades Pesticides

Different gene parts are being assembled, in order to construct a device that is able to mineralize and biodegrade recalcitrant pesticides. The device will be tested against different concentrations of different recalcitrant pesticides. Specific chassis will be assembled with gene parts from different metabolic pathways in order to finally reach mineralization of the pesticide. Different parameters, such as DNA concentration, ATP concetration, fluorescence of reporters, growth of bacterial device, and reduction of pesticide concentration, will be used to assess the efficacy of the device. Computational modeling will be used in order to complement the laboratory work.

Team Johns_Hopkins-BAG: Synthetic yeast genome Sc2.0 and Build-A-Genome

The JHU team will present the work of the Build-A-Genome course, powering the fabrication of synthetic yeast genome Sc2.0. Build-A-Genome provides students tools to meld seamless arrays of DNA into predesigned synthetic chromosomes. Our team develops new technologies for synthetic genomic fabrication. We developed a new standard, the Building Block, allowing production of much longer DNA sequences that can encode for more complicated cellular operations than allowed by current iGEMbiobrick standards, as well as more standard iGEM-y devices. Through multiple rounds of homologous recombination we can create chromosome segments and eventually full chromosomes. We will present our improved methodology for building block synthesis, the software we have created to aid in our synthesis, applications of the yeastgenome redesign and the new standard we have created. We will emphasize the Build-A-Genome course and its implications on future genomic technologies that both rely on and teach students.

Team KU_Seoul: Integrated Heavy Metal Detection System

Our team project is designing synthetic modules for simultaneous detection of multiple heavy metals such as arsenic, zinc, and cadmium in E. coli. The ultimate goal is to build a micromachine sensing and determining of the concentration of heavy metals in a sample solution (e.g. the waste water). In order to design the system, we will employ two fluorescence proteins (GFP and RFP) and aryl acylamidase as signal reporters. Since each heavy metal promoter produces unique fluorescence or color by those reporters, if more than two heavy metals coexist in a solution, the results would be interpreted from the convoluted fluorescence and/or color rather than a single signal detection. The successful construction of the synthetic modules in E. coli can be utilized in the form of a lyophilized powder, which can be stored in a drug capsule to make it portable.

Team KULeuven: Essencia coli, the fragrance factory

'Essencia coli' is a vanillin producing bacterium equipped with a control system that keeps the concentration of vanillin at a constant level. The showpiece of the project is the feedback mechanism. Vanillin synthesis is initiated by irradiation with blue light. The preferred concentration can be modulated using the intensity of that light. At the same time the bacterium measures the amount of vanillin outside the cell and controls its production to maintain the set point. The designed system is universal in nature and has therefore potential benefits in different areas. The concept can easily be applied to other flavours and odours. In fact, any application that requires a constant concentration of a molecular substance is possible.

Team Kyoto: Time Bomb & Cells in cells

We have two projects. The first is “GSDD”, the project to make a "time bomb"---a unique system to control

the time of cell death. We create timer mechanism by taking advantage of the end-replication problem and the protecting effect of lacI (bound to each end of DNA) against exonuclease digestion. As the cell divides, due to the end-replication problem, the "timer" DNA gets shorter, and eventually, the repressor expression level falls. Then the downstream killer gene becomes expressed. The other one, “Cells in Cells” is the project to make a cell. We defined making cells as making liposomes that can divide like mitochondria do. To approach our goal, we set two subgoals. One is to enable cells to take in liposomes. The other is to enable the liposomes to import proteins needed for mitochondrial division. We suppose this could be the first step to create artificial cells.

Team LCG-UNAM-Mexico: Fight fire with fire: phage mediated bacterial bite back

Bacteriophage infection represents a common matter in science and industry. We propose to contend with such infections at a population level by triggering a defense system delivered by an engineered P4 phage. P4 is a satellite of P2 phage, so it cannot assembly unless some P2 genes are present. Those indispensable genes will be expressed by an E.Coli strain, hence creating a production line of a P4 which will be able to deliver (transduce) standardized synthetic systems to E. Coli and possibly similar species. The defense system will consist of toxins for DNA and rRNA degradation, transcribed by T3 or T7 RNA-Polymerases, fast enough to stop phage's assembly and scattering. The system includes the spread of an AHL, hence "warning" the population to prepare against further T3 or T7 infection. We will implement a stochastic population model to simulate the infection processes and quantify the efficiency of our system.

Team Lethbridge: A Synthetic Future: Microcompartments, Nanoparticles and the BioBattery

The issues surrounding energy production are becoming more prominent with increasing environmental concerns and the rising cost of energy. Microbial fuel cells (MFCs) use biological systems to produce an electrical current. Cyanobacteria are organisms which have been studied in MFCs and have been found to create a current, although not highly efficient (Tsujimura et al., 2001). We wish to increase the efficiency of the cyanobacteria MFC by introducing microcompartments to create a BioBattery. The microcompartments are created by the production of the protein lumazine synthase forms icosahedral capsids. As a proof of principle we will create this system within Escherichia coli and target two different fluorescent proteins within the microcompartment to observe fluorescence resonance energy transfer. Furthermore, we will be exploring a novel method for the mass production of uniform nanoparticles, which is more efficient and cost effective than current methods.

Team McGill: Activation-inactivation signaling in one-and two-dimensions

Intercellular signaling constitutes the foundation of may disparate research fields such as neurophysiology, embryology, cancer research, and several others. We investigated a simple representational intercellular signaling network where a population of cells synthesizes and secretes an activator molecule capable of activation a second population of cells into synthesizing and secreting an inhibitor molecular which feeds back and inhibits the production of the activating molecule. This is known as an activation-inhibition system. We began by using a partial differential equation model of the system to explore the effect of varying the separation distance of the two populations of cells. We found that three types of dynamics were present: steady states, periodic oscillations, and quasi-periodic oscillations. We further designed two strains of E. coli capable of interacting with each other as an activation-inhibition system and endeavored to validate our

modeling results in a biological system.

Team METU-Gene: A Fast Healing Mechanism; Wound Dressing

In case of bulk loss of tissue or non-healing wounds such as burns, trauma, diabetic, decubitus and venous stasis ulcers, a proper wound dressing is needed to cover the wound area, protect the damaged tissue, and if possible to activate the cell proliferation and stimulate the healing process. By this purpose, designing a wound dressing which is natural, non-toxic, and biodegradable and imitating the actual wound healing mechanism which is forming on open wounds in mammalian tissues is our main purpose.By this wound covering, we will fasten the healing process, and protect the wounded area from infectious agents. In this wound dressing, there will be 4 layers including polyurethane layers and our bacteria colonies. Our bacteria colonies will be capable of synthesizing human epidermal growth factor and keratinocyte growth factor. The communication between these bacteria colonies will be dependent on quorum sensing molecules.

Team Michigan: The Toluene Terminator

Toluene is a toxic substance used in petrol, paint, paint-thinners and adhesives. Through spills and improper disposal, toluene can contaminate soil and ground water environments. Using microorganisms to clean up toluene-contaminated sites can be an effective and economical way of degrading the pollution before it can spread throughout the environment. There is concern, however, that these non-native microorganisms may upset the balance of the ecosystem through unnatural competition or horizontal synthetic gene transfer. We are engineering the Toluene Terminator as a way to neutralize toluene pollution while addressing these concerns. It will have the capabilities of sensing and mineralizing the toluene into carbon dioxide and water, but this terminator will not be back. The Toluene Terminator will have a suicide mechanism which kills the bacteria in the absence of toluene.

Team Minnesota: Computational synthetic biology: How the Synthetic Biology Software Suite can guide wet-lab experiments

Synthetic biology has all the characteristic features of an engineering discipline: applying technical and scientific knowledge to design and implement devices, systems, and processes that safely realize a desired objective. Mathematical modeling has always been an important component of engineering disciplines: models and computer simulations can quickly provide a clear picture of how different components influence the behavior of the whole, reaching objectives quickly. Our presentation focuses on sophisticated mathematical models of synthetic biological systems that connect the targeted biological phenotype to the DNA sequence. The activities for iGEM 2009 included the development and testing of simulation tools that connect multiple levels of organization from molecules and their interactions, to gene regulatory relations, to emerging logical architectures in bacteria. We connected out tools to the Registry and validated the simulations with a significant experimental component, constructing and testing these synthetic biological systems in Escherichia coli.

Team Missouri_Miners: A Synthetic Biology Apporach to Microbial Fuel Cell Development Utilizing E. Coli

Optimization of electron shuffle to external surfaces such as anodes was a primary goal. Geobacter sulfurreducens happened to be our model bacteria due to its ability in nature to efficiently export electrons extracelluarly. E. coli was the chassis for this experiment due to its well documentation and the fact that its genome already containing some key proteins in our preferred pathway. The proteins, such as extracellular

pilin, MacA, and many other cytochromes, which E. coli does not have were isolated from Geobacter sulfurreducens and introduced into E. coli to formulate the most optimal pathway for generating electromotive force in a microbial fuel cell apparatus. Some problems were faced concerning plasmid engineering and the simple fact that Geobacter is anaerobic and E. coli is aerobic. The current work includes production and optimization of a microbial fuel cell into which our modified bacteria will be placed.

Team MIT: Photolocalizer

There has been growing interest in designing fast and reversible switchable controls over all steps of gene expression, from transcription to post-translational modification. Our project involves engineering S. cerevisiae to localize proteins to various points in the cell in response to light exposure. Under red light, a tagged protein of interest localizes to a specific target, while exposure to far-red light causes the protein to rapidly delocalize and diffuse throughout the cell. This is accomplished using the PhyB-PIF3 system, a light-based transcriptional regulation system found in Arabidopsis. This project has two components. 1) Metabolically engineering yeast to endogenously produce PCB, a tetrapyrrole necessary for system activation, and 2) adapting the PhyB-PIF3 system to localize proteins of interest to different targets in the cell. The versatility and applications for this system are vast, ranging from cellular diffusion studies to easily synchronizing cell division for entire populations.

Team MoWestern_Davidson: Rolling Clones: Can't get no SATisfaction

Our team goal was to advance the developing field of bacterial computing. The Satisfiability (SAT) problem was the first mathematical problem proven to be NP-complete. A SAT problem is formed by connecting true-false variables with OR to form clauses and connecting clauses with AND. The goal is to determine if true-false values can be assigned to each variable to make the overall logical expression true. Our designed system uses frameshift suppressor tRNAs as inputs and frameshift suppressor leaders (FSLs) that process the inputs to enable the translation of fluorescent proteins exactly when an appropriate combination of inputs is present. The results illustrate the potential of engineered living cells to evaluate challenging mathematical problems. Our project also explored two aspects of synthetic biology education: a survey and analysis of public opinion and teachers' knowledge of synthetic biology and the design and construction of physical models of a frameshift suppressor.

Team NCTU_Formosa: Bacterial referee with the adjustable timer and counter functions

Our team constructed a controllable synthetic genetic circuit in Escherichia coli which has timer and counter functions. The circuit works as an OR gate to integrate temporal and environmental signals. The output (red fluorescent protein: RFP) of the OR gate is ON when one of the input signals is ON. The timer function is controlled by Lac promoter, and the concentration of lactose determines timer's working length. After added lactose is consumed by E. coli, the RFP will be translated to remind us that time's up. The counter function can detect the bacteria population with LuxI/LuxR device; moreover, the counter sensitivity is controlled by the strength of TetR repressible promoter. If external bacteria invade the system, the extra AHL produced by them will induce the RFP translation to warn us the contamination. Our project can be applied to storage warning signs of fresh food, contact lens, and wound dressing.

Team Nevada: Cinnamicide: Producing a Natural Insecticide against Mosquito Larvae in E. coli and Duckweed

Cinnamaldehyde is a natural insecticide against mosquito larvae that shows low toxicity towards other

organisms. The objective of this project is to engineer the cinnamaldehyde biosynthetic pathway into E. coli to develop an inexpensive and readily available source of this compound. By introducing the genes encoding phenylalanine ammonia lyase, cinnamate-CoA ligase, and cinnamoyl-CoA reductase, it should be possible to produce cinnamaldehyde from available phenylalanine in E. coli. Once we have proven that we can produce cinnamaldehyde in E. coli, we will engineer cinnamaldehyde production in duckweed, a small aquatic plant. Because mosquito larvae feed on duckweed detritus, the engineered plant will serve as an excellent vehicle to deliver cinnamaldehyde for mosquito control.

Team Newcastle: Bac-man: sequestering cadmium into Bacillus spores

Cadmium contamination can be a serious problem in countries where polluting industries are located close to agricultural sites. Our team developed a design to address this problem using the resiliant spore-forming bacterium Bacillus subtilis. We engineered B. subtilis to sense and sequester cadmium from the environment into metallothionein containing spores, rendering it bio-unavailable. Germination of the spores can be disabled, making retrieval of the cadmium unnecessary since they can persist intact for thousands of years. We computationally simulated the life cycles of individual cells and entire cell populations, to estimate the parameter values necessary to maintain sustainable populations of sporulating, germinating and vegetative cells. Our design required us to engineer stochastic differentiation processes at a single cell level. A sporulation rate tuner was developed and we also engineered a tuneable stochastic invertase switch to stochastically control cell differention and fate.

Team NTU-Singapore: Plaque Out!

The NTU iGEM ’09 team is proud to be working on a proposed alternative treatment for atherosclerosis. Our system, pLaqUe Out!, ideally based in a macrophage chassis, when introduced into the bloodstream, will be activated by a symptom typical of plaque buildup. Upon activation, our system will release a cholesterol degrading enzyme, a novel reporter protein and a vasodilator. The cholesterol degrading enzyme will break down the plaque. The reporter protein was chosen for expression because of its unique fluorescent property. This allows the plaque site to be identified in a manner similar to X-ray visualization. Finally the vasodilator will simultaneously dilate the blood vessels for better flow, as well as switch off the extended activity of our system. In the interests of time, this system is first modeled using E.Coli.

Team NYMU-Taipei: ViroCatcher

1. The objective: Binding viruses to designer ViroCatcher cells that cannot support viral replication to diagnose, attenuate, and prevent infection. 2. What we intend to do: (1) Make our designer cell safe, (2) Express specific cell surface receptors and antibodies to catch the virus, (3) Transduce the signal after viruses attached for feedback control, and (4) Remove the viruses along with ViroCatcher itself. 3. Anticipated results: the ViroCatcher is made safe for the bloodstream. When it is injected into the bloodstream, our ViroCatcher passively lies around letting viruses attach to it by using its 4 receptors: CD4 (for HIV), Integrin (for various viruses), Sialic Acid (for Influenza), and Antibodies (for Influenza). After enough viruses attach to it, or after a certain amount of time elapses, it removes itself from the bloodstream by calling macrophages to eat it up.

Team Osaka: ColorColi: Painting tools toward bio-art

Bio-art has appeared as a crossover of life science and art in 21st century. Such artworks can give rise to a number of issues and metaphors accompanying the advance of science and technology. Astonishingly, there are still few collaborations between bio-art and synthetic biology. In this context, we engineered

Salmonella enterica cells to function as new painting tools in bio-art. Specifically, we try to program cells to automatically form various pattern and gradation of colors by sensing cell identity and density by means of quorum sensing. Moreover, we will extend these tools for actual paintings and artworks to consider the ethical implications such as 'view of life'. This collaborative project can show the social situation or question of life science.

Team Paris: Message in a Bubble: a robust inter-cellular communication system based on outer membrane vesicles.

Sending a message across the ocean… Outer membrane vesicles (OMV), naturally produced by gram negative bacteria as E. Coli, are strong candidates for long-distance messaging. Our engineered communication platform consists of controlling OMV production by destabilizing membrane integrity through over-expression of specific periplasmic proteins (e.g., TolR). In order to control and modulate message content, we used fusions with OmpA signal sequence and the ClyA hemolysin as delivery tags. A targeting system was developed, based on the outer-membrane expression of Jun/Fos leucine zippers to control the vesicle flux between donor and recipient cells. Once received, the signal from incoming vesicles is transduced through a modified Fec pathway, whereby the receptor is provided by the OMV. Computational models provided insight to all of the above steps. Such reliable communications systems have wide biotechnological implications, ranging from targeted drugs delivery and detoxification to advanced division of labor or even cell-based computing.

Team PKU_Beijing: Conditioned Reflex Mimicking in E.coli

We are engineering our E. coli cells to process the correlation information of two enviornmental signal, similar to the process of conditioning in higher orgamisms. We have constructed and tested a series of AND gates which can sense the two signals: the conditioned and unconditioned stimuli. With the presence of both signals, the AND gate outputs a repressor protein and then changes the state of the bistable switch, which acts as a memory module. In this way, our E. coli cells can convert the information about the concurrence of the two signals into its memory. After the memory module is switched and given the "conditioned stimulus", the E. coli cells will pass the information to the reporter module and thus exhibit the "conditioned response."

Team Purdue: Engineered Microglia to Locate CD133+ Tumor-Initiating Cells

Glioblastoma multiforme (GBM) is one of the most common forms of primary brain cancer, which usually results in fatality. To date, it has been difficult to overcome primary brain cancer resulting from GBM, primarily because the cancer-initiating cells are suspected to be highly resistant to current cancer therapies. Specifically, CD133+ cells have shown resistance to hypoxia, irradiation, and some forms of chemotherapy. CD133+ hunting machines will be created by genetically engineering microglial cells (BV-2) with mammalian expression vectors. The project will also take advantage of inherent qualities of the microglia such as constant environmental sensing and quick motility. The engineered BV-2s will be equipped to locate the specific GBMs and label the targeted cells with a tat-GFP fusion protein. It is the goal of this study to show an alternative approach to cancer treatment, and to emphasize the power of biologically available options to fight the disease.

Team Queens: Plaque Busters: A Synthetic Biology Approach to Targeted Drug Delivery Treatment of Atherosclerosis

Atherosclerosis is associated with the buildup of plaques in the vascular walls. Currently, treatment for atherosclerosis involves preventative measures and surgical removal of plaque, angioplasty, and stent placement. We sought to develop an E. coli chassis delivering anti-atherosclerotic substances to the site of plaque in vasculature. Inflamed endothelial cells express VCAM-1, a receptor normally binds to the leukocyte antigen VLA-4. We attempted to express a VLA-4 fragment in E. coli, in order to selectively attach the cells to plaques. In vitro binding test uses inflamed murine endothelial cells which express VCAM-1. Results are pending. Our bacterial chassis also carries several inducible “effector” systems which, upon binding, release substances that facilitate plaque stabilization and regression. Effector systems include heme oxygenase-1, serum amyloid A and atrial natriuretic peptide. Expression of HO-1 in E. coli has been confirmed using spectroscopy. Testing for SAA secretion and ANP-induced gene expression in endothelial cells is ongoing.

Team SDU-Denmark: Bacto Bandage - Quorum-quenching S. Aureus Biofilm Formation, One Peptide at a Time

Our goal is to create an E. coli strain, which inhibits Staphylococcus aureus biofilm formation in wounds by producing RNA III-inhibiting-peptide (RIP). S. aureus is one of the largest causes of hospital infections, each year infecting millions of people around the globe. S. aureus is normally commensal, but can create biofilms on implanted medical devices and in post-operational wounds. Biofilm is increasingly hard to treat, as a result of growing resistance to many types of antibiotics. By manipulating E. colito express a synthetic RIP peptide tagged with an export signal, we hope to reach this goal. RIP has been shown to hinder the quorum-sensing processes essential for biofilm development in S. aureus, thereby making it harder for the bacteria to cause infections. We propose making a bandage that contains our engineered bacteria behind a semipermeable membrane, allowing only small peptides such as RIP to pass through, into the wound.

Team Sheffield: E. Coli Switch

By modifying E.coli so that it can use a phytochrome- with a light receptor- from cyanobacteria as a trigger of protein generation. This pathway is controlled by a certain wavelength of red light, acting as a system switch for lacZ production. LacZ can react with substrate X-gal and form a blue precipitate as a reporter. However, other reportergenes can be attached to the lacZ gene, so different reporters can be expressed. From the fact that this mechanism is sensitive to a certain wavelength of light, we hope to create a system that can be sensitive to various wavelengths and hence triggering different protein generation. Through this the E.coli can become a wavelength sensor; a different wavelength can trigger a different production of protein, for example various types of fluorescent protein, giving a different a colour-scaled indication of the wavelength of the environment around the E.coli.

Team SJTU-BioX-Shanghai: Hypnos' Curse: E.coli the napper

Inspired by the natural regulator of circadian bioclock exhibited in most eukaryotic organisms, our team has designed an E.coli-based genetic network derived from the toxin-antitoxin system (TA system). The relE protein□toxin□, is an RNase that preferentially cleaves mRNA stop codons, severely inhibiting translation and preventing colony formation. Whereas expression of relB protein□antitoxin□ and tmRNA forms a rescue system to reverse inhibitory effects. Based on these mechanisms our network functions as a bacterial bioclock oscillating between the two states of dormancy and activity. Potential applications of our project include lifespan prolongation of prokaryotes and eukaryotes, since the metabolic process of microbes is vastly decelerated during the dormancy state, just like that of bears and hedgehogs in their

hibernation.

Team Slovenia: nanoBRICKsPRO – synthetic smart nanomaterials from nano to macro

Nanotechnology designs materials with advanced properties based on the control of structure at the nanoscale. Biological systems provide an attractive opportunity to design and easily manufacture material with programmable properties. DNA origami demonstrated the power of this technology by creating a variety of assemblies that can be easily encoded in the nucleotide sequence. However, for biological nanodevices nature favors polypeptides over nucleic acids due to stability and versatility of amino-acid side chains. With few exceptions protein and peptide assemblies have been considered too difficult for the bottom-up design due to complex interactions and manufacturing problems specific for each case. We present technology for manufacturing nanomaterials based on combinations of modular peptide elements and proteindomains, which allow self-assembly into complex tertiary structures with designed macroscopic properties. We will demonstrate the feasibility and potentials of proteinnanotechnology by design, streamlining the production and technological application of nanomaterials based on nanoBRICKsPRO.

Team Southampton: E.colYMPIC GAMES

The project exploits quorum sensing in E. coli to engineer interactions between 'species' such that complex spatiotemporal patterns are generated. We have two systems that correspond the game ³Rock, Paper, Scissors² (RPS) and to Conway¹s ³Game of Life² (GoL). In GoL expression/diffusion of lactones is exploited to create local rules that modulate expression of a fluorescent protein. Fluorescence patterns for different combinations of conditions are modelled using a new simulation tool designed to be generically applicable to inter-bacterial communication. In the RPS system three 'species' each produce a different fluorescent protein whose expression is downregulated by a lactone from one of the other 'species' and hence the interaction network is intransitive. Simulations indicate that when this game is played out on culture plates, a range of complex patterns evolve with time. Also, selective patterning of the different 'species' allows for new 'racetrack' or 'playing field' type of interactivity.

Team Stanford: Immuni-T. coli: A Probiotic Approach to Diagnosing and Treating Inflammatory Bowel Disease (IBD)

Homeostasis relies on the balance between immune cell types, disruption of which leads to autoimmunity. The Stanford team has applied synthetic biology to a longstanding objective of immunotherapy: restoration and maintenance of homeostasis. Stanford's Escherichia coli-based probiotic will polarize T cell differentiation along antagonistic fates - immunosuppressive Treg and inflammatory Th17 phenotypes - in response to local conditions. Our device design consists of two parts: one that modulates deleterious Treg-driven immunosuppression and another that engages Th17-mediated inflammation. Through specific sensors and effectors, therapy will oscillate between dampening pathologic inflammation and immunosuppression until a balance in the local T cell population is achieved. Securing such homeostasis between these populations has therapeutic implications for autoimmune disorders like IBD, HIV and cancer. We envision our novel and directed probiotic therapy for IBD as acting at the interface between commensal bacteria and human lymphocytes, integrating cutting-edge immunology with synthetic biology.

Team SupBiotech-Paris: Double vectorisation system (DVS)

As a part of the iGEM competition, we decided to develop a new process, allowing the protection of the active biological principle. This type of process exists already, and it is called a vector. It may be biological

as viruses, or chemicals as polymeric nanoparticles. Whatsoever its nature, the vector encounters many problems : Stability, targeting, membrane passage, and the immune response. Therefore, we tried to achieve the ideal vector, being the most stable as possible, can easily penetrate its targets, and outwit the immune system. We have created a double vectorization system, by using jointly a bacteria and a phage. The first vector which is bacterial, will target the tissue, and resist to the immune system. The second vector is a phage, will be used for cell targeting and membrane penetration. The combination of the two systems improves the intrinsic abilities of vectors, and offers new possibilities for applications.

Team Sweden: The Linguistic Cell: Sentence Parsing Bacteria

Language is an essential part of our civilization. But making sense out of a series of words can only be achieved by certain rules that underlie the language. This set of rules is called a grammar. A grammar tells us how to order words in a meaningful way. These rules can be implemented as a Finite State Automaton (FSA), which for every new word input moves from the current state to the next until it reaches the end of input. We propose in our project a biological model which is based on this con- cept of language parsing from computational linguistics.

Team Tianjin: Cyanobacteria convertor & Microcystins detector

Project 1: Inspired by several features of cyanobacteria, which is low-grade, fast-growing, photosynthetic and easy to operate. We aim to construct an pathway in cyanobacteria so that when it is carrying photosynthesis□carbon dioxide can be transferred into target production, ethanol. Project 2: This project is to design a Yeast Two-hybrid system aimed at Microcystins(MCs) detection in waters. The MCs detection device we design takes the advantage of Gal4 promoter, which consists of two domains, one is AD, the other is BD. When AD and BD are close enough to each other, the report gene transcription LacZ will be trigged. We selected and modified two peptides that have specific interactions with MCs and engineered them into two vectors to construct the Yeast Two-hybrid system. In the presence of MCs at different concentrations, blue dots in different shades of colors can be seen directly.

Team Todai-Tokyo: Prevention of Lifestyle Diseases Using Synthetic Organisms

Lifestyle diseases, diseases caused by unhealthy living habits, comprise one of the major problems in modern society, especially as they may lead to fatal heart problems or even cancer. However, preventing or curing these diseases is presently of extreme difficulty. Our team, Todai-Tokyo, has been tackling treatment of lifestyle diseases such as hypercholesterolemia, diabetes, circadian rhythm dysfunction, and bad smoking habits by using synthetic living systems, utilizing their ability to incorporate complex logic functions and dispensability of external control once in operation. To do this, we aim to create the following: cells that ingest cholesterol to decrease blood cholesterol levels, healthy low calorie breads, a system in which periodic gene expression is controlled, and bacteria that encourage smokers to quit smoking, respectively. By applying similar synthetic biology methodologies to these, prevention of numerous lifestyle-related diseases may become reality, serving as a first step towards their eradication.

Team Tokyo_Tech: 2009 Space Odyssey: Terraforming of Mars with genetically engineered bacteria

Have any life forms existed on Mars? If so, what kind of features could they have possessed? Today, the Martian environment is severe for any life to inhabit because of some constrained conditions. For instances, the surface temperature having a range from -80°C to 15°C, CO2 occupying 95% of the

atmosphere and the absence of organic substances on the surface don't allow aerobic organisms or heterotrophic bacteria to grow. Our project objective is to create a genetically engineered iron-oxidizing bacteria surviving on Mars and to establish a new model organism playing an important role to terraform Mars. We engineered Acidithiobacillus ferrooxidans by introducing a synthetic pathway of both Melanin and Anti Freeze Protein with temperature-regulated systems. Anti Freeze Protein contributes to enhance tolerance of cryogenic condition and Melanin to blacken the Martian surface eventually resulting in melt of ice cap and generation of atmosphere and sea.

Team Tokyo-Nokogen: Escape tedious work with Escherichia coli Auto Protein Synthesizer (ESCAPES).

Tokyo-NokoGen has developed the Escherichia coli Auto Protein Synthesizer (ESCAPES), an E. coli machine that greatly simplifies the production of your favorite protein. We created a green light-activated actuator to respond to external light signals, as well as a riboregulator-based signal counter to count the number of flashes. In ESCAPES, the first green light flash induces the E. coli to self-aggregate, while the second flash causes them to auto-lyse, thus greatly simplifying the protein preparation process. The light-activated actuator was constructed by fusing the light responsive domain of the Synechocystis photoreceptor CcaS with the EnvZ histidine kinasedomain. Self-aggregation is achieved by the induction of the Antigen43 gene, which we isolated from E. coli, while autolysis took advantage of the available BioBrick parts endolysin and holin. ESCAPES helps you “escape” from tedious protein preparation steps, such as centrifugation and cell disruption.

Team TorontoMaRSDiscovery: Engineering bacterial micro-compartments to investigate metabolic channeling and its potential uses in biotechnological applications

A key challenge in metabolic engineering is to improve productivity and yield. Potential applications range from the production of valuable compounds such as therapeutic molecules and biofuels to the degradation of toxic wastes. There is increasing recognition that spatial organization can play an important role in optimizing pathwayefficiency. Specifically, the spatial co-localization of consecutive enzymes in a pathway can result in efficient translocation of substrates between enzymes, an effect known as enzyme "channeling". Here we report the design, modeling and construction of a bacterial micro-organelle based system for the targeted co-localization of selected enzymes. Our "Encapsulator" represents a fundamentally new class of parts which, in nature consist of metabolic enzymes encased within a multi-protein shell reminiscent of a viral capsid. Micro-compartments based on encapsulin (and similar proteins) represent an experimentally amenable system to investigate the effects of channeling in potential downstream applications.

Team Tsinghua: Syn-genome Based Gensniper

Our aim is to construct a targeted gene therapy vector with high cellular specificity, considerable capacity and the potential for mass production and universal modification. Analogizing the characteristics of bacteriophage lambda and adenovirus, we genomically engineered the fiber protein of adenovirus with the pC of bacteriophage lambda, with the knob region modified by cell-specific peptides generated by phage display (called targeted biobrick). After inducing the vector genome (generated by bottom-up or top-down approach) into BL21 DE3 E.coli strain, we applied a co-transformed therapeutic DNA (namely a cosmid with a capacity of 40-50 kb) for mass production of our targeted gene therapy vectors containing the

desired genes to be delivered. With the targeted biobrick immediating the attachment and RGD domain immediating the internalization of the targeted vector, we are able to accomplish the targeted gene therapy.

Team TUDelft: Bacterial Relay Race

In our project, we aim at creating a cell-to-cell communication system that allows the propagation of a set of instructions coded on a plasmid, and not just binary information as in quorum sensing. To achieve this goal, we have designed a communication system based on three different modules: a conjugation system, a time-delay genetic circuit, and a self- destructive plasmid. Cell-to-cell communication systems are important because, in most synthetic biology applications, the desired tasks are generally accomplished by a population of cells, rather than by a single cell. The proposed communication system could be used for creating a distributed sensors network, or it could help to better understand and possibly reduce antibiotic resistance in bacteria. Furthermore, we have conducted a survey to study the perception on synthetic biology and related ethical issues, among iGEM participants, students and supervisors. We have focused on the top-down and bottom-up approaches as applied to biology.

Team TzuChiU_Formosa: Midnight Apollo

In Taiwan there are 9 power stations generating energy by coal, and produce 269.1 million tons of CO2 every year. Power stations are major causes of global warming. Therefore, we would like to create a ” biolight” system that can reduce CO2 production and attenuate degree of global warming. We plan to create a new organism that doesn’t need electricity and cause no pollution. We named it “Midnight Apollo”. The “Midnight Apollo” will be turned on when surrounding area turns dark and will be turned off automatically when the environment becomes bright. The idea is based on two systems, Cph8 and aeoquorin/GFP. The Cph8 is regulated by visible light that can activate protein translation of an illuminating system. Subsequently, this illuminating system would use aeoquorin/GFP to light up the environment. We hope The Midnight Apollo could be applied in producing energy-saved Bio-streetlamp, emergency Biolighting, or Biosearchlight.

Team UAB-Barcelona: A toxics biosensor. Could bacteria detect instantaneous and simultaneously several types of pollutants?

We would like to construct a recombinant /Escherichia coli /strain that/ /could detect different aggressive pollutants, like toxics compounds. The first approach was the halogenated compounds (and more specifically chloroform) detector. Tap water usually contains it due to the chlorination process in drinking water production or other activities like swimming pools, etc. and it can become harmful to public health at high concentrations. /Nitrosomonas/ /europaea/’s promoters (/mbla/ and /clpb/) are specifically sensitive to chloroform, so coupling them with an output (a fluorescence protein) should allow quantifying its concentration. This was our first approximation towards our final aim of making a complete circuit that would allow to assign simultaneously to every pollutants family a certain color (fluorophore) thanks to the selective activation of different promoters.

Team UC_Davis: A Bacterial Secretion System Motivated the Goal of Managing Celiac

The current estimate of the number of Americans with Celiac Disease/gluten intolerance is one out of 133. Not being able to digest gluten properly inside the small intestine leads to an immune system response that leads to a variety of symptoms. We have designed a bacterial secretion system that could be used in a probiotic organism to secrete an enzyme to degrade the allergen gliadin before it reaches the intestine. A

putative advantage a probiotic treatment over direct enzyme therapy approaches is the potential for administering fewer doses, thus making it less troublesome, less costly, and more convenient. Our secretion system consists of an inducible promoter, ribosome binding site, an extracellular anchor (ompA/INPNC), cleavage signal sequence, 6 HIS Tag and a terminator. We have tested its behavior in E. coli on two proteinsof varying sizes (GFP/Luciferase).

Team UChicago: An enhanced yeast-based system for detection and decontamination of organophosphate neurotoxins.

Organophophates (OPs) are highly toxic compounds used as pesticides and chemical ware-fare agents around the world, including sarin, soman, and VX gas. To combat these toxins, which act as acetylcholinesterase inhibitors, we designed a highly efficient whole-cell S. cerevisiae sensor and biocatalyst system for the detection and remediation of the model organophosphate compound paraoxon and its degradation products. For our biosensor device, reporter constructs were incorporated into the genome downstream of paraoxon and paraoxon-hydrolyis sensitive promoters. Our degradation device was designed in three parts, targeting paraoxon through expression of organophosphate hydrolase from Flavobacterium, sp and two of its degradation byproducts p-nitrophenol and diethyl phosphate. Each device was genomically integrated in order to bypass selective the need for selective condition, while the degradation devices were constitutively expressed for maximum efficiency. Combined, this two-part system allows for both the detection and remediation of a broad range of common and deadly neurotoxins.

Team UCL_London: Stress Light

Our project “Stress Light” will produce a series of synthetic biosensor devises, which can improve on the traditional sensors in bio-processing; by using green fluorescent protein (GFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP) and red fluorescent protein (RFP) expression as indicators of different stresses for e.colibacteria during cultivation. Our product and system is called “the traffic-light stress sensor”. It is constructed to express fluorescent proteins of different colors in response to different stimuli which are inhibiting growth or harming production. We aim to build a sensor that can detect shear stress and low oxygen levels. We believe these two stresses are critical in a bioprocessing environment. We are applying e.coli’s native or modified promoters for DegP, Spy and NarK in order to induce transcription. Ideally the cells responses to stressed conditions should be sufficiently accurate, reliable and rapid for the stresses to be detected and mitigated.

Team UCSF: Engineering the Movement of Cellular Robots

Some eukaryotic cells, such as white blood cells, have the amazing ability to sense specific external chemical signals, and move toward those signals. This behavior, known as chemotaxis, is a fundamental biological process crucial to such diverse functions as development, wound healing and immune response. Our project focuses on using a synthetic biology approach to manipulate signaling pathways that mediate chemotaxis. We are attempting to reprogram the movements that the cells undergo by altering the guidance and movement machinery of these cells in a modular way. For example, can we steer them to migrate toward new signals? Can we make cells move faster? Slower? We hope to better understand how chemotaxis works, and eventually build cells that can perform useful tasks. Imagine, for example, therapeutic cell-bots that could home to a directed site in the body and execute complex, user-defined functions (e.g., kill tumors, deliver drugs, guide stem cell migration).

Team ULB-Brussels: GluColi, a new generation of glue

Whether you want to stop a leaking ship's hull, or repair a fractured bone, you will need a top quality, strong adhesive. Our project aims to create a new generation of glue. In contrast to most glues, ours is natural, biodegradable, efficient on wet surfaces and has exceptional resistance (up to 3 times better than super glue). It is composed of a polysaccharide released by a Gram-negative bacterium, Caulobacter crescentus. Our aim is to use BioBrickTM standard biological parts in order to create a new strain of Escherichia coli which will synthesise this adhesive material. Moreover we are going to use a new plasmid stabilisation technique, the StabyTM system developed by Delphi Genetics. This system allows us to avoid the use of antibiotics and has been shown to be more efficient as far as protein secretion is concerned.

Team UNICAMP-Brazil: The Microguards

Industrial production processes based on microorganisms, such as biofuels, fine chemicals and enzymes, are threatened by contaminants that cause losses of up to 10% of the gross production. To solve this problem, the aim of our project is to engineer strains of the industrial workhorses E. coli and S. cerevisiae to recognize and destroy contaminants. The engineered yeast will recognize lactic acid bacteria contaminants, which greatly affect Brazilian´s ethanol production. The presence of lactic acid will activate a lysozyme-based killing mechanism, effective against gram-positive contamination. The engineered E. coli will recognize contaminants based on a non-self recognition mechanism and a percentage of the population will differentiate into a killing lineage releasing colicins and endonucleases. The killing mechanisms will be regulated by promoter Py, probably activated by conjugation. The characterization of Py and a lactate-inducible promoter that is not subjected to glucose catabolic repression are the main challenges of our project.

Team UNIPV-Pavia: Ethanol? Whey not!

Cheese whey is classified as a special waste for its high biochemical and chemical oxygen demand. Even if whey can be valorized by extracting high value substances, like whey-proteins, at the end of the treatment the residual liquid is still a special waste for its high lactose content (4%). E. coli was engineered to convert efficiently lactose into ethanol, a precious biofuel. Three main enzymes are involved in this transformation: beta-galactosidase, pyruvate-decarboxylase and alcohol-dehydrogenaseII. Beta-galactosidase (lacZ gene) was over-expressed to obtain higher lactose-glucose conversion yield. Coding sequences of pyruvate-decarboxylase (pdc) and alcohol-dehydrogenaseII (adhB), essential in alcoholic fermentation pathway, were designed by DNA chemical synthesis and codon-optimized for E. coli. The final circuit includes the device to metabolize lactose and the ethanol-producing operon, containing pdc and adhB. It has a theoretical yield of 20kilos/tons of whey. Finally, 3OC6HSL, aTc and lactose/IPTG inducible systems were characterized to be used in this circuit.

Team uOttawa: A probiotic Lactobacillus strain which produces cellulose

This year's project focuses on genetically engineering the bacterium Lactobacillus plantarum to produce cellulose, as a food additive. L. plantarum was selected as it is already commonly found in yogurt. The aim of generating this novel probiotic is to reduce human morbidity via the subsequent increase in dietary fibre in the gut. The sequestering of glucose for fibre production by L. plantarum provides the additional benefit of effectively reducing dietary sugars. We have successfully extracted the four genes that code for cellulose synthase from the Acetobacter xylinum. These genes were then placed under the control of a strong constitutive promoter, and transformed into Lactobacillus plantarum. Plasmid and genome based expression of the synthase genes are being evaluated and characterized. In the future, cellulose production

assays, evaluation of biofilm formation, and in vivo testing will be performed to determine viability as potential health benefits.

Team Uppsala-Sweden: Booze Bugs : Sun To Alcohol

In the long run our crude oil resources will be on the decline but most importantly the effects of the climate change demand a quick shift to a sustainable fuel economy. Approaching biofuel production by direct synthesis from sunlight has the potential to solve the problems that arise with the conventional fermentation of starches and sugars such as the direct competition of fuel feedstock with food crops. Thus the Uppsala iGEM Team 2009 investigated the production of ethanol and butanol with the use of the cyanobacteria Synechocystis sp PCC6803. Also known as blue-green algae, cyanobacteria possess the ability to directly convert sunlight into biofuels. We engineered constructs for ethanol and butanol production as well as strategies to increase the yields of photosynthetic ethanol production.

Team UQ-Australia: Mercury sequestration using a multicomponent operon, and increasing the temperature tolerance range of P. syringae.

Microbes such as Escherichia coli and Cuprivadis metallidurans have an endogenous multicomponent mercury (Hg2+) uptake and reduction operon, under the control of a metal responsive transcription factor, MerR. By utilising elements of this pathway, with a novel recovery mechanism, mercury can be accumulated intracellularly and efficiently removed from the environment. The presence of mercury activates MerR, driving the expression of Antigen 43 (Ag43), a self-adhering surface protein. Coupling a mercury sensitive promoter to the expression of Ag43 enables cells to accumulate mercury then aggregate in solution. P. syringae is a ubiquitous airborne bacterium which expresses a unique protein, InaZ. This protein acts as a scaffold for ice nucleation, inducing precipitation. Optimal growth of P. syringae occurs at 22oC. By introducing five heat-shock genes, the tolerance range will be increased to better suit the Australian climate. This modification has the potential to increase the availability of Australia's most precious resource; water.

Team USTC: E. coli Automatic Directed Evolution Machine: a Universal Framework for Evolutionary Approaches in Synthetic Biology

Evolution is powerful enough to create everything, from biomolecules to ecosystems. The ultimate goal of E. coli Automatic Directed Evolution Machine (E.ADEM) project is to manage the power of evolution, by engineering a robust system framework that can automatically create anything we want in synthetic biology, from various types of parts to complex systems. Each demand can be converted into designing a scoring function to give the evolution process a direction. E.ADEM is designed by implementing evolutionary algorithm back into biology. The core of E.ADEM is a self-adaptive controller that can adjust variation rate and selection pressure, based on fitness score, population size and average fitness score calculated by a quorum sensing device. After comprehensive measurement using constitutive promoter family stimulus signals and modeling of the components, a prototype machine is built. Modular design and PoPS device boundary standard will ensure the extensibility and universality of the machine.

Team USTC_Software: Automatic Biological Circuit Design

The ultimate goal of synthetic biology is to program complex biological networks that could achieve desired phenotype and produce significant metabolites in purpose of real world application, by fabricating standard components from an engineering-driven perspective. This project explores the application of theoretical approaches to automatically design synthetic complex biological networks with desired functions defined as

dynamical behavior and input-output property. We propose a novel design scheme highlighted in the notion of trade-off that synthetic networks could be obtained by a compromise between performance and robustness. Moreover, series of eligible strategies, which consist of various topologies and possible standard components such as BioBricks, provide multiple choices to facilitate the wet experiment procedure. Description of all feasible solutions takes advantage of SBML and SBGN standard to guarantee extensibility and compatibility.

Team Utah_State: BioBricks without Borders: Investigating a multi-host BioBrick vector and secretion of cellular products

The aim of the Utah State University iGEM project is to develop improved upstream and downstream processing strategies for manufacturing cellular products using the standardized BioBrick system. First, we altered the broad-host range vector pRL1383a to comply with BioBrick standards and enable use of BioBrick constructs in organisms like Pseudomonas putida, Rhodobacter sphaeroides, and Synechocystis PCC6803. This vector will facilitate exploitation of advantageous characteristics of these organisms, such as photosynthetic carbon assimilation. Following expression, product recovery poses a difficult and expensive challenge. Downstream processing of cellular compounds, like polyhydroxyalkanoates (PHAs), commonly represents more than half of the total production expense. To counter this problem, secretion-promoting BioBrick devices were constructed through genetic fusion of signal peptides with protein-coding regions. To demonstrate this, the secretion of PHA granule-associated proteins and their affinity to PHA was investigated. Project success will facilitate expression and recovery of BioBrick-coded products in multiple organisms.

Team Valencia: iLCD: iGEM Lighting Cell Display

The Valencia Team project consists of developing a “bio-screen” of voltage-activated cells, where every “cellular pixel” produces light. It is known that for instance neurons, cardiomyocites or muscle cells are able to sense and respond to electrical signals. These cells use a common second messenger system, calcium ion, which promotes a defined response when an electrical pulse is supplied to them. Nevertheless, these cultures present several technical disadvantages in order to make a handily use of them. Valencia team uses this property on yeast to produce luminescence as a response to electrical stimulus. This project constitutes the first time in which the electrical response of Saccharomyces and its potential applications are going to be tested. The obtained results will be used to build the first iLCD in history. We will reflect the perception that different groups of people have about Synthetic Biology in the survey http://igemvalencia.questionpro.com.

Team Victoria_Australia: An environmentally sustainable biological lighting system

Our aim is to build a biological lighting system via cell free transcription and translation. We will be focusing on developing a prototype using two cell free systems: E. coliand wheat germ in which the proteins will fluoresce. We are using the fluorescent proteins BFP, GFP, Vic green, blueberry, yellow and cherry in the cell free systems. Our main aim was to develop an alternative light source, which could possibly be powered by a waste material as simple as grass clippings (cell lysate). We are also attempting to develop a new registry part that is a blueberry fluorescent protein using the yellow protein (part # BBa_E0030) through mutagenesis.

Team VictoriaBC: Signal Integration: Applications of RNA Riboregulator

Capabilities

This project explores some of the ways that the secondary structure of messenger RNA can be used to control the rate of protein expression. The 32°C ribothermometer made by the 2008 TUDelft team will be coupled to fluorescent proteins to visually confirm temperature-dependent translation. The "ribolock" made by the 2006 Berkeley team will be tested at various temperatures to determine if it could double for use as a ribothermometer. Finally, a proof-of-concept NAND logic gate will be constructed: a ribolock will be used to interpret two concurrent environmental signals into an on/off control for mCherry output.

Team Virginia: Arsenic Sequestration for Groundwater Decontamination

As many as 137 million people in 70 countries may be affected by groundwater contaminated with arsenic. Existing treatment options are too expensive for the majority of affected areas. Therefore we are developing a bioremediation tool using Escherichia coli to absorb and bind arsenic and remove it from its surrounding environment. Natural and synthetic peptides are employed to sequester the toxic ions and a pump knockout ensures that arsenic stays in the cell. Measurement of growth capacity of the engineered strain in arsenic containing media and quantitative analysis of arsenic sequestration will be performed. Characterization and integration of an arsenic-responsive promoter will allow the sequestration system to dynamically adjust to current conditions. A simple, well-implemented system for biosequestration of arsenic may become part of a solution to a problem denying access to clean drinking water for many.

Team Virginia_Commonwealth: Promoter design, characterization and consequences

The generation of well-characterized genetic parts is a prerequisite for the rational design and construction of reliable genetically-encoded devices and systems. However, most publicly available parts (including those in the Registry) remain largely uncharacterized. Therefore, we propose a minimal measurement standard for the quantitative characterization of one of the most frequently used parts, promoters. This approach uses both mRNA and protein measurements to provide a tractable and universal analysis of relevant promoter characteristics. In an effort to elucidate promoter design principles, we have also designed and characterized new promoter and enhancer sequences. Our goal is to contribute to the advancement of fundamental synthetic biology by evaluating the performance of new and existing promoters and enhancers, which may serve as a model for describing other basic parts such as ribosome binding sites and transcriptional terminators.

Team Warsaw: BacInVader – a new system for cancer genetic therapy

The main aim of our project is to design a model system based on genetically modified Escherichia coli, capable of entering into eukaryotic cells. We have developed a regulatory system composed of three distinct functional modules. The whole system is activated by thermal degradation of the repressor protein, which leads to internalisation of E. coli by mammalian cells. When in endosome, pH-dependent two-component regulatory system activity enables the bacterium to escape to cytoplasm. Once the bacterium is in the cytoplasm some proteins are secreted due to expression of specific genes. In our case, secretion of p53 or bax proteins to mitochondria leads to apoptosis without cell cycle arrest thus enabling complementation of traditional chemotherapeutical agents, which affect only proliferating cells.

Team Wash_U: Improved Photosynthetic Productivity for Rhodobacter sphaeroides via Synthetic Regulation of the Light Harvesting Antenna LH2

Photosynthetic light harvesting antennas function to collect light and transfer energy to a reaction center for

photochemistry. Phototrophs evolved large antennas to compete for photons in natural environments where light is scarce. Consequently, cells at the surface of photobioreactors over-absorb light, leading to attenuated photobioreactor light penetration and starving cells on the interior of photons. This reduction of photosynthetic productivity has been identified as the primary impediment to improving photobioreactor efficiency. While reduction of antenna size improves photosynthetic productivity, current approaches to this end uniformly truncate antennas and are difficult to manipulate from the perspective of bioengineering. We aim to create a modifiable system to optimize antenna size throughout the bioreactor by utilizing a synthetic regulatory mechanism that correlates expression of the pucB/A LH2 antenna genes with incident light intensity. This new application of synthetic biology serves to transform the science of antenna reduction into the engineering of antenna optimization.

Team Washington: The Ideal Protein Purification System

The use of recombinant protein production using E. coli-based expression systems has revolutionized the fields of biotechnology and medicine. However, the ability to utilize such proteins hinges upon their capacity to be isolated from their expression systems. Our project aims to create an all-in-one protein expression and purification system using BioBrick standards to greatly simplify protein production for synthetic biologists, reducing the time and cost involved in standard protein purification methods. Our method uses a novel combination of two systems: secretion and display. By fusing two tags to the protein it can be secreted into the expression media, and subsequently directed to bind to the outside of the cell. To collect the pure proteins, cells only need to be spun down and then resuspended in an elution buffer, releasing the protein of interest. Our research exhibits the utility of synthetic biology for developing new techniques that improve upon established practices.

Team Washington-Software: LegoRoboBricks for Automated BioBrick Assembly

Commercial Liquid Handling Systems are extremely expensive, and are typically beyond the reach of the average molecular biologist interested in performing high throughput methods. To address this problem, we design and implement a liquid handling system built from commonly accessible Legos. Our goal is the automation of BioBrick assembly on a platform that can itself be easily replicated and we demonstrate a proof-of-principle for this system by transferring colored dye solutions on a 96-well plate. We introduce a new concept called LegoRoboBrick. The liquid handling system is build from 3 new LegoRoboBrick modular components: ALPHA (Automated Lego Pipette Head Assembly), BETA (BioBrick Environmental Testing Apparatus), and PHI (Pneumatic Handling Interface). We will demonstrate that the same BioBrick assembly software can run on multiple plug-and-play LegoRoboBrick instances with different physical dimensions and geometric configurations. The modular design of LegoRoboBricks allows easy extension of new laboratory functionalities in the future.

Team Waterloo: Chromobricks: A Platform for Chromosome Engineering with BioBricks

The aim of our project is to develop a fully-featured platform for chromosome engineering, allowing the in vivo assembly of a synthetic chromosome from interchangeable parts, followed by selective degradation of the native chromosome. We have designed a proof-of-concept for chromosome-building that will use the site-specific integrase of phage ΦC31 to integrate a BioBrick into a defined locus of the E. coli genome. Six pairs of integrase-targeted att sites have been designed to be non-cross-reactive in order to support

repeatable cassette-exchange reactions for chromosome building. We have also written software to model the integrase-mediated rearrangement of DNAmolecules containing att sites, to aid the design of more elaborate chromosome-building systems. To selectively degrade the native chromosome we designed a nuclease-based, inducible genome-degradation system. In its simplest form, our system can be used to integrate biological devices into a chromosome in situations requiring stable copy number and selection-free maintenance.

Team Wisconsin-Madison: Ocean Fuel: increased salt tolerance through glycine betaine production

Biofuels represent a potential solution to current world energy demands. Total crude oil replacement based on a 20% fuel titer and current fuel demands would require 5.6 trillion gallons of fresh water per year. Current fresh water supplies may not support this added demand. Alternatively, a sustainable approach may use a portion of the Earth's 3.5x1020 gallons of ocean water. However, current fuel-producing organisms are unable to thrive in ocean-level osmolarities. Glycine betaine, a powerful osmoprotectant, shields organisms from salt-induced stress. Wild-type Escherichia coli can acquire glycine betaine from their surroundings or synthesize it from environmental choline. Two enzymes, glycine/sarcosine methyltransferase, and sarcosine/dimethyglycine methyltransferase, catalyze three successive methylations of glycine for de novo synthesis of glycine betaine. Here, we demonstrate an engineered E. coli with an increased growth rate under salt induced stress. We highlight utility by demonstrating the improved growth of fuel producing bacteria in ocean water.

Team Yeshiva_NYC: Spatially encoding temporal information: using diffusional escape of periplasmic reporter proteinsas a clock.

We were inspired by iGEM projects that utilize colored or fluorescent reporter molecules. Specifically, we thought "wouldn't it be nice to be able to leave a plate out in the field, collect it a day or two later, and be able to tell at what time the synthesis of the reporter molecules was triggered?" Ideally, protein reporters would leave the cell and diffuse through the agar, leaving a clear history of their expression. To this end, we are 1) creating a library of Sec and Tat leader sequence biobricks for directing expressed proteins to the periplasm, 2) making an E. coli expression strain with a weakened outer cell wall that leaks most of the periplasmic proteins to the environment, and 3) measuring and modeling the diffusion of small molecules and proteins in agar to ascertain the ability to derive the times and conditions under which they originated.

2008

Alberta NINT

Project Logi-col[i]: Terminator/Attenuator anti-sense Logic (T/AasL)

Two major hurdles facing the development of complex genetic logic circuits are device connectibility and device extensibility. Connectibility refers to the ability to connect the output of one device to the input of another device, while extensibility refers to the dual abilities to rationally design new devices and combine multiple devices in one organism. Our project uses terminator/attenuator (T/A) hairpin sequences (gates) to control transcription and anti-sense RNA as input/output signals to/from the devices. We call this approach Terminator/Attenuator anti-sense Logic (T/AasL – pronounced “taw-ssel”). It solves the connectibility problems of common protein-based approaches because the anti-sense output of one device is used to disrupt formation of T/A hairpin structures of downstream devices, thus activating them. In addition, because RNAsecondary structures can be rationally designed (using our m-fold derived analysis program) we can readily construct a large family of devices with minimal cross-talk for inclusion in a single cell.

Bay Area RSI

Differentiation and Targeting of Stem Cells to Infarcted Cardiac Tissue

Every year over 1.2 million people suffer myocardial infarction. The resulting heart damage requires new approaches for effective repair. Stem cell therapies provide hope. However none of the stem cell therapies currently in clinical trials addresses the need for efficient stem cell targeting to cardiac tissue or the need to replace efficiently dead tissue with new cardiomyocytes. To address these problems, we have built several genetic circuits that work sequentially to repair the heart. First, we have built an inducibledifferentiation circuit that closely resembles the endogenous differentiation pathway, to program cells to become cardiomyocytes. Second, we have built circuits that use the extracellular domains of chimeric proteins to target cells to damaged cardiac tissue. Upon binding, novel receptor-coupled intein-mediated signaling domains activate effector genes that then aid in integration, inhibition of cell death, and the alteration of the tissue microenvironment.

BCCS-Bristol

Bacto-Builders

Assembling particles at microscopic scales into desired patterns or structures is usually difficult or impossible. All construction projects require the manipulation of varying size components, many much larger than any individual. To make this possible, teams of individuals work together towards a common goal. To find out how to transfer this behaviour to our ``Bacto-Builders*, we investigate the possibility of using large numbers of E. coli to perform a task too great for any individual cell. Specifically, this involves the physical movement of particles through direct contact with a swarm of bacteria working together in a co-ordinated manner. The ultimate goal is to engineer the bacteriato follow a set of simple rules, so that collective behaviour emerges, and particles are assembled into a desired pattern. Furthermore, patterns or*

structures could be evolved in real time with bacteria adapting to new dynamic requirements or autonomously forming new structures.

Beijing Normal

Intelligent PCBs detector and degrader

Polychlorinated biphenyls (PCBs) are a group of organic pollutants that are persistent when released into the environment. Our task is to design an effective as well as intelligent PCBs degrader. According to the recent research, ortho-chlorinated PCB metabolites (DHBs) are potent and physiologically significant inhibitors of DHBD, so we design a feedback activation pathway to increase the BphC transcription and expression under a 2, 3-DHBP and 4-CB inducible promoter Ppcb. As dihydrodiols and dihydroxybiphenyls are very toxic to bacterial even after short incubation time, we design a feedback repression pathway use sRNA components— sodB and rhyB. As to the sensor part, dihydroxylated PCBs are substrate of the clcA-encoded chlorocatechol dioxygenase and thus induce the clcR and related promoter, so we use this as the sensing system. T7 amplification system is added to the downstream to amplify the signal.

Bologna

Ecoli.PROM: an Erasable and Programmable Genetic Memory with E. coli

The project aims to design a bacterial reprogrammable memory with genetically engineered E.coli colonies in solid medium working as an array of binary memory cells. To engineer bacteria we designed a genetic flip-flop composed of a binary memory (toggle switch) and an UV sensitive trigger. We chose UV to have a fine spatial selectivity in programming the cells and IPTG to reset the memory. We designed a circuit with high UV sensitivity by computer-model analysis. Core elements of the genetic memory are two mutually regulated promoters, designed as independent operator sites flanking a constitutive promoter. Thus, promoter transcriptional strength and repressor binding affinity can be independently fixed. Operator libraries for LacI, TetR, Lambda and LexA repressors were cloned as BioBricks to allow the rational design of regulated promoters that is still lacking in the Registry We expect this approach to be a benefit in many Synthetic Biology applications.

Brown

Toxipop: Conductance Measurement of Cell Lysis as a Reporter of Toxin Presence

Around the world, primarily in third world countries, contamination of drinking water is an immense problem that is difficult and expensive to detect with current technology. As such, there is a need for an economically feasible, transportable, and user-friendly detection system for water contamination that can reliably be used in the field. Our goal was to design and implement a novel biosensor with the ability to detect the presence of certain water contaminants and report that information back via a change in the conductance of a bacterial solution. An inducer specific promoter transcribes and leads to the translation of a "Lysis Gene Cassette." The subsequent lysis of the bacteria results in an increase in the solution's conductivity, indicating the presence of the inducer.

BrownTwo

A Genetic Limiter Circuit in S. cerevisiae

Numerous disease states in multicellular organisms involve anomalous expression patterns of endogenous genes. Tumor growth, associated with the overexpression of oncogenes, is one vexing example in which this occurs. While extremes of gene expression can damage living systems, normal expression is necessary for healthy function. We have designed a modular genetic circuit to limit the expression level of a gene of interest to a user-defined, tunable threshold. The limiter network reacts to the transcription of an endogenous gene within each cell, entering a regulatory state only where and when the rate of transcription lies beyond an acceptable range of activity. Along with its potential therapeutic utility, we offer our device as a foundational tool for researching gene expression in a eukaryotic model.

Calgary Ethics

An exploration of ethical, environmental, economic, legal and social (E3LS) issues of synthetic biology

Synthetic biology is a rapidly advancing field of scientific and technological inquiry. To reach its full potential its (E3LS) issues have to be investigated in a proactive and foresight manner. We are the first iGEM team focusing exclusively on investigating synthetic biology (E3LS) issues. We pursued various projects: a) development, distribution and interpretation of two online surveys, one for high school- one for non-high school students; b) development of an online course on synthetic biology (E3LS) issues; c) dialogue with the University of Calgary wetware iGEM team and the University of Guelph iGEM team about (E3LS) issues attached to their respective projects; e) involvement in the Synthetic Biology 4.0 Poster “Forward-Engineering a Regulatory Framework for Synthetic Biology: How Existing Regulatory Architecture Could Lend to the Creation of Our Own” by Laura Dress from the University of Maryland.

Calgary Wetware

Quorum-coupled Bacteriocin Release: Engineering a Champion

Microorganisms use pheromones to interact amongst themselves and with other microbial species in a process known as Quorum Sensing. In a similar sense, we have exploited the natural communication systems involving Autoinducer-1 (AI-1) from Vibrio fischeri and Autoinducer-2 (AI-2) from Vibrio harveyi, to create a model biosensor system in Escherichia coli. We have engineered the genetic circuits necessary for the production of these pheromones into two populations of E. coli (termed Bad guy #1 and Bad guy #2, as per their respective Autoinducer). In addition, our third population of E. coli (termed Champion cell) acts as a biosensor by receiving these signal inputs and subsequently initiating transcription of specific E. coli-targeted bacteriocins (i.e. colicins) in tandem with specific fluorescent proteins. The presence of AI-1 induces the Champion to produce a colicin to which Bad guy #1 is susceptible, but to which Bad guy #2 is resistant, and vice-versa for AI-2.

Caltech

Engineering multi-functional probiotic bacteria

The human gut houses a diverse collection of microorganisms, with important implications for the health and welfare of the host. We aim to engineer a member of this microbial community to provide innovative medical treatments. Our work focuses on four main areas: (1) pathogen defense, either by expression of pathogen-specific bacteriophage or by targeted bursts of reactive oxygen species; (2) prevention of birth defects by folate over-expression and delivery; (3) treatment of lactose intolerance, by cleaving lactose to allow absorption in the large intestine; and (4) regulation of these three treatment functions to produce renewable subpopulations specialized for each function. Our research demonstrates that synthetic biology techniques can be used to modify naturally occurring microbial communities for applications in biomedicine and biotechnology.

Cambridge

Cambridge iBrain: Foundations for an Artificial Nervous System using Self-Organizing Electrical Patterning

We have developed a system which creates spatially organised electrical features in a genetically identical bacterial population, allowing for simulation of action potentials and other complex phenomena. This system generates electrical potentials in bacterial cells using artificially formed potassium gradients, released upon chemical stimulation. We have designed the genetic circuitry to establish a two-component Reaction-Diffusion system involving the well-characterised Lux and Agr signalling pathways, and we have modelled the intercellular interactions between these pathways to produce complex self-organising designs known as Turing patterns. To support this system we have developed the gram-positive bacteria Bacillus subtilis as a BioBrick chassis, including direct chromosomal single-copy insertion, peptide signalling, and BioBrick-compatible vectors for expression in both gram-negative and -positive bacteria. We have also tested a new assembly method for rapidly generating constructs by joining multiple PCR fragments. This work can serve as a foundation for future advances involving cellular patterning, signalling, and self-organisation.

Chiba

E.coli time manager

We control the timing of gene expression by using multiple signaling devices. To this end,we utilize molecules associated with Quorum sensing, a phenomenon that allows bacteria to communicate with each other. Our project uses two classes of bacteria: senders and receivers. Senders produce signaling molecules, and receivers are activated only after a particular concentration of this molecule is reached. Although different quorum sensing species have slightly different signaling molecules, these molecules are not completely specific to their hosts and cross-species reactivity is observed. Communication using non endogenous molecules is less sensitive, and requires a higher signal concentration to take effect. This results in slower activation of receivers.

CPU-NanJing

Adding new notes to the song of life / Customizing a biomacromolecule

In our project, we designed a novel device by which we could insert different unnatural amino acids into a certain site in target protein expressed in E coli. Of course these unnatural amino acids would bring some new characteristics of the target protein. #2: In our project, we intend to design a device which composed of a bio-timer and alternatively expressed two glycosyltransferases. The timer could be controlled by the concentration of the inducer, and the glycosyltransferase are in charge of synthesizing the polysaccharide. As a result, the molecular weight of polysaccharide could be controlled by concentration of the inducer. By exchanging glycosyltransferase, this device would provide a useful tool to obtain different polysaccharide with certain molecular weight.

Davidson-Missouri Western

E. nigma: XOR Gates, a Bacterial Hash Function, and Viz-A-Brick

The team designed, modeled, and constructed a bacterial computer that uses XOR logic to compute a cryptographic hash function. Hash functions are used to authenticate the integrity of a document by computing its digital “fingerprint,” an integer value that can be compared to the publicized value. Our bacterial computers recognize the presence or absence of two chemical signals, converting biological information into binary numbers. Given a starting “key” and a binary message of arbitrary length, various configurations of the designed system produce the hash function output. Mathematical modeling of these computers has shown that our hash functions are difficult to corrupt. We also produced a graphical interface for exploring the Registry of Standard Biological Parts called Viz-A-Brick (http://gcat.davidson.edu/VizABrick/), and other web-based tools to improve the construction of new parts with BioBrick ends (http://gcat.davidson.edu/iGEM08/tools.html).

Duke

Attacking the plastic waste problem: a two-pronged approach

Faced with the issues of plastic waste accumulation and environmental pollution, a two-pronged approach with the potential to solve these problems has been developed. Firstly, biologically produced plastics such as polyhydroxyalkanoates (PHAs) are superior to petroleum-based plastics because they are both biodegradable and biocompatible. By focusing on modulating the ratio of two PHA monomers, 3-hydroxybutyrate and 4-hydroxybutyrate, the copolymer poly(3HB-co-4HB) can be created featuring increased elasticity and utility over any particular PHA monomer. Secondly, a novel polyethylene-degradation pathway is being engineered based on the oxidation of long-chain alkanes by alkane monooxygenase LadA. The region inhibiting the binding and catalysis of polyethylene has been computationally identified and site-directed mutagenesis is being conducted at this region to yield a mutant of LadA that oxidizes polyethylene and thereby increases its biodegradability. The combination of the production of an eco-friendly bioplastic with the degradation of petroleum-based plastics is a promising method of waste reduction.

Edinburgh

A weapon of mass nutrition: The conversion of waste cellulosic biomass into starch and beta-carotene

Cellulose, in the form of biomass, is the ultimate renewable resource. Its conversion to starch would provide a hugely abundant source of material which could be used for the manufacture of biofuels or other biological products, as an animal feed supplement to release grain for human food use, or even as the basis of a food for human use. Given the present food and energy shortages, the advantages of such a process are clear. With this in mind, Edinburgh iGEM 2008 have devised systems for E. coli to degrade cellulose into glucose, to upregulate glycogen and terpenoid production, and to convert glycogen into starch. We have also designed software capable of generating a model in SBML format from a list of genes and promoters entered by the user. This is supported by a background database allowing users to build models based on published data.

EPF-Lausanne

Genetic network generating spatial patterns through cell-cell communication and controlled information processing

Biological systems are unique in their ability to combine information and energy to generate complex entities. Genetically encoded networks drive many of these patterning processes. Furthermore, developmental studies have highlighted the importance of gradient formation and cell-cell communication for the generation of cellular patterns in the early stages of life. It has been shown that simple networks can form both static and dynamic patterns. Nonetheless, a system whose pattern formation is dependent on combinations of multiple signals has yet to be demonstrated. Here we address this question by designing a network, involving two different quorum-sensing based signalingmechanisms. Upon introduction in E.coli, the system can sense the relative amounts of two input molecules. Using a pre-define set of rules which was selected on its ability to generate spatial patterns, the cell can then express its final state by emitting red or green fluorescence and transmit its state to its neighbors.

ESBS-Strasbourg

Cell Cycle Dependent Toggle Switch in Eucaryotic Cells: Approach to a Binary Cell Division Counter

Our team aims to establish a regulatory network over several cell generations in budding yeast. This model organism as chassis offers ideal conditions as it has been the primary source for studies on the cell cycle. More specifically, we want to construct a toggle switch that is triggered by cell cycle dependent factors. The construction consists of two subassemblies of identical composition, each with a positive feedback loop for the own expression pattern and a repression of the competing module. Switching is achieved by directed degradation of transcription factors at a specific time frame within the cell cycle. This should result in a binary expression pattern like for example GFP expression in every other cell cycle (0-1-0-1). The system shall be extendable by adding further "bits" of similar construction (e.g. for the second bit the pattern 0-0-1-1). The device would thus be an approach to a binary cell division counter.

ETH Zurich

Make yourself simpler, stupid! Or how engineering a self-minimizing cell leads to the Minimal Genome

This year's ETH Zurich project tackles a fundamental problem of synthetic biology: the minimal genome. Exploring the minimal set of genes that is able to support life is a question of significant biological interest.

Additionally, one of the main complications when implementing genetic circuits is possible cross-talk with endogenous pathways. Thus, an organism carrying a minimal genome would provide a simple chassis for biological engineering. Our approach is based on an iterative cycle of genome reduction and strain selection. We propose a novel method to randomly delete chromosomal fragments by controlled expression of restriction enzymes and ligases in vivo. Furthermore we develop a chemostat-based selective condition for cells having a smaller genome by constraining nucleotide availability. Computationally, we analyze the genome for optimal cutting sites, and perform flux balance analysis on a genome scale model to predict growth of reduced genome strains. Finally, we simulate the restriction enzyme control circuit and the selection mechanism.

Freiburg

Modular Synthetic Transmembrane Receptor Systems Interfaced with Nano Breadboards

Signaling through membranes is a characteristic of life. Transmembrane proteins control proliferation, differentiation, and cellular response and are key for the formation of multicellular organisms. Controlling such proteins enables one to modify cellular behavior and ultimately program cells at will. The complex rules for transmembrane signaling often require engagement of several proteins in a fine-tuned spatial and temporal manner. To tap into the possibilities of transmembrane programming, the Freiburg 2008 iGEM team provides an extensible system comprising an external framework with spatial resolution, a concept for modifying natural receptors, and a modular set of fusion-Biobricks for the construction of synthetic receptors. Spatial resolution in nanometer scale is provided by DNA-Origami modified with distinct patterns and combinations of ligands. Receptors are decoupled from their natural ligands by fusion with artificial binding domains. The Biobrick collection contains signal sequences, binding domains, transmembrane domains, and effector domains featuring split enzymes and split fluorescent proteins for immediate readout.

Groningen

Conway's Game of Life in Real Life

Conway's Game of Life is a simple cellular automaton famous for generating complex "life-like" patterns. The goal of this project is to explore the possibility of implementing cellular automata, particularly the Game of Life, as a regular spatial arrangement of bacteria. Communicating the number of neighbors is implemented using the well-known HSL quorum sensing system. A novel component is the circuit implementing the automaton's ruleset, to determine the state to switch to upon detecting "too few" , "enough" or "too many" neighbors. This "interval switch" was designed and implemented by altering the binding site affinity of the signal molecule complexes to correspond to the levels of HSL coming from the neighbors. Finally, the "ON" state of the cells is indicated by GFP production and production of new HSL signals, and the "OFF" state by their absence. The system was implemented partially in vivo and we have developed in silico models.

Guelph

Reprogramming microbes to cater to or silence their hosts: beta carotene production and RNAi delivery

In humans microbes help digest our food and produce vitamins to supplement our diet, while plants such

as corn harbour microbes within their tissues, which can extend the metabolic capacity of their host. In order to exploit these patterns of microbial habitation, we attempted to modify the broad host range plasmid pDSK-GFPuv to contain either a synthetic operon of metabolic genes belonging to the soil microbe /Erwinia uredovora/, or Biobrick compatible RNAi constructs targetting expression of either GFP or corn TB1 genes. These plasmids were to be electroporated into either probiotic /Escherichia coli/ /Nissle //1917 /or endophytic/ Klebsiella pneumonii /342. Assays will then show whether a genetically modified enteric microbe could be made to produce vitamin A in a modelled human intestine, or whether a common corn endophyte could stably express and deliver RNAi signals against expression of GFP and corn TB1 genes while living inside a growing corn plant.

Harvard

BACTRICITY: Bacterial Biosensors with Electrical Output*

The metabolically versatile bacterium Shewanella oneidensis adapts to anaerobic environments by transporting electrons to its exterior, reducing a variety of environmental substrates. When grown anaerobically and provided with lactate as a carbon source, S. oneidensis transfers electrons to an electrode of a microbial fuel cell. We sought to engineer S. oneidensis to report variations in environmental conditions through changes in current production. A previous study has shown that S. oneidensis mutants deficient in the mtrB gene produce less current than the wildtype strain, and that current production in these mutants can be restored by the addition of exogenous mtrB. We attempted to control current production in mtrB knockouts by introducing mtrB on lactose, tetracycline, and heat inducible systems. These novel biosensors integrate directly with electrical circuits, paving the way for the development of automated, biological measurement and reporter systems. *Bacteria As Current Transmitters Report Induced Changes Important To You

Hawaii

A BioBrick toolkit for cyanobacteria

We aim to extend the current BioBrick registry to a greater range of organisms, including cyanobacteria. Cyanobacteria are studied for their ability to produce useful compounds, including biofuels and biopolymers. These "little green factories" require only salts, light, water, and carbon dioxide for photoautotrophic growth. A cyanobacterial "toolkit" would enhance our ability to utilize this system. We designed: 1) mobilizable broad-host range BioBrick vectors derived from RSF1010, 2) a cassette for protein secretion from Synechocystis sp. PCC 6803, and 3) a nitrate-inducible cyanobacterial promoter BioBrick. Our toolkit was designed for conjugative gene transfer from Escherichia coli to Synechocystis to achieve the controlled production and recovery or bioproducts, demonstrable by induced secretion of green fluorescent protein. Though our parts were targeted for work in cyanobacteria, they may be compatible with other Gram-negative systems including Agrobacterium, which is capable of plant transformation.

Heidelberg

Ecolicence to kill: Engineering E.coli for targeting pathogenic microorganisms

Microbial communities known as biofilms are particularly resistant to conventional therapies. Biofilm formation depends on signalling molecules called autoinducers. Our aim is to exploit this communication mechanism by engineering synthetic bacteria that are able to target harmful autoinducer-secreting species and to kill them. We engineered “killer” E. coli cells with two complementary modules. The “sensing module” comprises the modification of E. coli’s chemotaxis system to make killer cells move towards a prey-secreted autoinducer stimulus. The “killing module” ensures that once in the vicinity of the prey, at high levels of the stimulus, a bacteriocidal mechanism is activated. In our model system, autoinducer-secreting “prey” cells are represented by a second E. coli strain. We further developed computer models that show the dynamics of both modules and probe the efficiency of the system in defined spatial environments. Future directions include adjusting the system to target real pathogens or even cancer cells.

HKUSTers

Does God play dice with the cell?

Stochastic fluctuation in a cellular context and the lambda-phage bifurcation have been extensively studied. However, from a bottom-up synthetic aspect, we aim to exploit the cellular "noise" to build an E. coli version of a computational device, the "Random Number Generator". One random binary digit can be generated by capturing an initial Polymerase binding event with a pair of mutually exclusive promoters. Reciprocal inhibition using two repressors shall achieve unilateral expression of the "switch", with fluorescence reporters indicating the probability of each alternative occurrence. Balancing the two sets of affinity and kinetic parameters and maintaining a single copy of this synthetic device integrated into the bacterial chromosome shall improve performance. If successful, coupled with other reporters we envision multiple extensions of this "Randomizer", including a Memorizer that utilizes a hierarchy of XOR-calculations to "store" a multi-digit random number, and intriguing pattern generation involving chemical gradients and random "population behavior".

iHKU

Formation of new patterns by programming cell motility

The ability of living organisms to form patterns is an untapped resource for synthetic biology. The HKU iGEM2008 team aims to generate unique patterns by rewiring the genetic circuitry controlling cell motility. Specifically, E. coli cells are programmed to autonomously regulate their movement by sensing local cell density. Interesting patterns are formed by two types of newly engineered cells. The high cell-density motility-off cells spread outwards and spontaneously form a distinctive ring of low cell densitysurrounded by rings of high cell density whilst the high cell-density motility-on cells form a Fuji-mountain-like structure. Moreover, we build a theoretical model that satisfactorily fits our current experimental data, and also predicts some parameters which may significantly affect the ring formation. The study of this self-organized spatial distribution of cells helps us to understand principles underlying the formation of natural biological patterns, and synthetic non-natural patterns have various potential applied uses.

IIT_Madras

StressKit: A BioBrick library of Lac-repressed σ24, σ28, σ32 and σ38 promoters for Escherichia coli

Regulated gene expression is an essential part of the synthetic biologist's toolkit. Bacteria have evolved 'generalized stress responses' which generate genome-wide changes as responses to globally-integrated information. Specific types of stress upregulate specific 'alternative σ factors', which activate transcription by binding to nucleotide signatures at the -10 and -35 boxes of their cognate promoters. We set out to design, construct, and validate a library of σ dependent promoters for E.coli, with the following specifications: the promoters must conform to the BioBrick standard; they must be modular so they can be used multiply in devices; and they must be LacI repressed but σ dependent, off by default but behaving like native σ dependent promoters in the presence of IPTG. We're currently characterizing the library of promoters(σ24, σ28, σ32 and σ38) against the unmodified Lutz-Bujard promoter, using spectrophotometry and fluorescence microscopy.

Illinois

Cell-based and in vitro antigenic sensors for medical diagnostics

The unifying motivation behind our research this year is the creation of novel diagnostic tools for medicine: we are conducting three parallel research projects to create cell-based and in vitro biosensors. We are engineering a bimolecular fluorescence system in which two halves of a fluorescent protein, each fused to an antigenic epitope, will bind to the two sites on an antibody in human serum to cause a detectable fluorescent signal when antibodies against this specific antigen are present. These proteins can be produced in bulk through a bacterial expression system. We are also pursuing similar diagnostic objectives using a eukaryotic system; we are designing strains of yeastable to respond specifically to immunogenic epitopes or antibodies, and activate a fluorometric or enzymatic response accordingly. We are fusing antibodies against immunological targets to cell surface receptors of transcriptional signaling pathways, which would become activated only in the presence of these pathogens.

Imperial College

Designer Genes – Biofabricator subtilis

The Imperial College iGEM Team has constructed a genetically engineered Biofabricator, using the Gram-positive bacterium Bacillus subtilis, with application from BioCouture to tissue engineering. Our Biofabricator subtilis is designed to produce self-assembling biomaterials using light as a trigger, and it achieves this in three stages: (i) based on the principles of holography and an endogenous light-sensing mechanism, our engineered bacteria is captured at desired locations; (ii) next, bacterial locomotion is suspended by using a recently-discovered clutch mechanism that disengages the flagellum from the motor protein; (iii) finally, once bacteria are stationary, biomaterial production is triggered leading to self-assembly and the formation of bio-scaffolds at specific locations.

Johns Hopkins

The Yeast Sex Detector: Visual Mating Type Determination System for S. cerevisiae

A haploid S. cerevisiae yeast cell is either mating type ‘a’ (MATa) or mating type ‘α’ (MATα). In the elucidation of biochemical and genetic processes in yeast, it is often necessary to initiate sporulation of

diploid yeast cells. The meiotic products of sporulation are four haploid cells; two MATa and two MAT α. To continue analysis, differentiating between the haploid cells is often crucial, and the necessary assay can take 2 to 3 days. Our detector, consisting of fluorescent proteins that are preferentially expressed depending on the mating type, will cut this time to seconds. Simply shining a UV lamp over the cells will reveal the mating type, allowing for the cells to be easily separated. This device could assist most yeast geneticists on a daily basis, as well as aid in the study of HO strains of yeast that switch mating-type at every mitotic division.

KULeuven

Dr. Coli, the bacterial drug delivery system

Imagine a bacterium that produces a drug when and where it is needed in the human body. It would have several advantages over classical drugs and could have many medical applications. In this framework we proudly present our team's project: Dr. Coli, the bacterial drug delivery system. Dr. Coli senses the disease signal and produces the appropriate amount of drugs to meet the individual patient's needs. And when the patient is cured, Dr. Coli self-destructs. To do this, a molecular timer registers the time since the last disease signal sensed. But when the disease flares up again, this timer is reset and drug production is resumed. Within the time frame of the iGEMcompetition, we developed a proof of concept of Dr. Coli. The most important assets are massive reuse of standard biobricks, different control mechanisms and extensive modeling.

Kyoto

Cells as physical power suppliers: Raise the Titanic!

In many biotechnological contexts, bacterial cells are considered as "chemical facilities." A number of studies have genetically engineered cells to produce various desired compounds. They further aim at accurate and precise regulation of material production. Cells are also power suppliers in terms of their motility. This aspect, however, has been much less featured. Our project started with the gigantic goals of lifting up the Titanic from the deep-sea with bacterial power. We worked towards engineering cells to carry larger objects and have been designing and constructing cells so that these micro-order entities can move a centimeter or larger objects. We have equipped E. coliwith the ability to attach to an object surface, cell density dependent buoyancy production and regulated flagella, and examined by quantitating the parameters to what extent our goal is achieved. Our study presents the possibility of bacterial physical power.

LCG-UNAM-Mexico

Singing bacteria: Controlling Escherichia coli's nickel efflux pump

Our project is to make bacteria sing. This will be achieved through the control of E. coli's nickel efflux pump, RcnA. The main idea is that a change in the concentration of extracellular nickel will translate into a change in the medium's conductivity, which we will measure. This will be read by a computer and, depending on the value, emit a sound. This way, bacteria are "singing"! The RcnA gene is placed under the control of phage lambda's CI repressor, which is itself produced in the presence of AHL and LuxR. LuxR is produced

constitutively in the cell, so the addition of AHL will be the input signal and limiting step. The final objective is to express the extent of RcnA's repression (and so the extracellular nickel concentration) as a function of AHL present in the cell.

Lethbridge CCS

Ligase-Independent Cloning as a Standard for BioBrick Preparation

While there is an established BioBrick format, there is not yet a standard method for turning a gene of interest into a BioBrick. Ideally, such a standard method would be easily adopted, even by amateurs, and would lend itself to automation. A significant drawback of several existing techniques is their dependence on ligase treatment, which is often problematic. We propose a ligase-independent cloning (LIC) method, based on the technique of Aslanidis & de Jong (1990), as a possible standard for novel BioBrick preparation. Instead of short overhangs and ligase treatment, LIC uses long overhangs to circularize plasmid vectors for transformation without the use of ligase. The LIC method reduces the number of enzyme steps required for cloning, thus lending itself to easy adoption, automation, and real biological 'engineering.'

Melbourne

Building a temporal controller in E. coli using red-light sensor and riboswitches

This year Melbourne iGEM competition team seeks to build a temporal controller in E. coli. The idea is to build a system, which is modular, has all components in the form of biobricks, and expresses gene(s) at a specific time in a sequential manner. In this study, we show the design, modeling and some experimental results towards a proof of principle of the system. The design uses the leverage of existing biobricks of red light bacterial photography system, positive feedback loops and riboswitches. We propose that the architecture presented should scale well with increasing number of genes to be temporally regulated. It is anticipated that such system will be useful in metabolic engineering because enzymes can be turn on and off in a sequential manner.

METU Turkey

Light Controlled Metal Carrying E. coli

Heavy metal contamination of drinking water is a major problem in many developing countries. It requires expensive techniques to get rid of these contaminants. In this project we aimed to develop metal cleaning techniques which (1) should be cost effective (2) and should not let further contamination in the course of cleaning. By using available systems from the nature we tried to develop a bacterial machine which can bind/release heavy metals and whose movement can be controlled by providing specific light wavelengths. To accomplish our aims we introduced metal binding proteins and bacteriorhodopsin to control pH which are located on the extracellular surface of membrane and phototactic capability to control movement by light.

Mexico UNAM-IPN

Design of an experimental device to detect events of horizontal gene transfer in Escherichia coli

Horizontal gene transfer is an evolutionary mechanism that contributes to the acquisition of new genetic material among organisms; as such it helps bacteria to acquire antibiotic resistance and other genetic devices. The main goal is to design a devise that would detect events of horizontal gene transfer among bacteria. Genetically modified E. coli were monitored until a detectable sign appears in the media, indicating an event of horizontal transfer. In order to detect such events, we will use plasmidsas the genetic material that could be transferred in a bacterial culture.

Michigan

Circadian Clocking... in E. Coli

The human body's "clock" regulates the daily cycles of many physiological and metabolic processes, such as the sleep-wake cycle and feeding rhythms. It is controlled by the interplay of numerous molecular factors that orchestrate complex feedback loops and processes that are fundamentally mediated by gene expression and the events that follow it. We are working on constructing a synthetic clock, affectionately deemed "The Sequestilator," that is analogous to the mammalian clock. Our clock consists of two parts: an activator with constitutive expression and a promoter that drives the production of a repressor that binds and "sequesters" the activator away from the promoter. While intuitively it seems that this system may reach a steady state rather than oscillate, simulations have shown that under certain rapid equilibrium and tight binding conditions, this circuit does exhibit oscillations. We are currently involved the in building and testing of this device.

Minnesota

Minnesota, Hats Off To Thee: Bacterial suicide, comparator and computer-aided synthetic biology

The University of Minnesota is sending their first team to the iGEM competition this year. Our group is composed of two subgroups: Team Comparator and Team Timebomb, each of which is working on an individual project. Team Timebomb is working to engineer a bacterial clock, based on which bacterial cells will 'commit suicide' after a predetermined number of divisions has been reached. Team Comparator is engineering a bacterial comparator, which is one element of a feedback controller. Team Comparator is also developing two computational tools: the SynBioSS Designer and the SynBioSS Wiki. SynBioSS stands for the Synthetic Biology Suite, which is freely available at synbioss.sourceforge.net . It is a suite of algorithms for automatically generating, storing and retrieving networks of reactions, which can model and simulate BioBricks gene networks. Computer-aided synthetic biology at its best!

Mississippi State

Genetically Engineered System for Lignin Biodegradation using Lignin Peroxidase A

Lignin is a ubiquitous, extremely complex biopolymer found in plant cells. It is the most recalcitrant part of the cell wall, and only a few organisms can degrade it. As a result, a huge proportion of the earth's biomass resources are trapped in a highly degradation resistant lignin matrix. To make these resources viable for energy and chemical needs, lignin must be broken down to separate the chemical components of biomass. We have isolated a single gene from the Lignin Peroxidase gene family. It produces the enzyme responsible for initiating the breakdown of lignin. Upon this fundamental research can be built a

characterized and controllable system for the breakdown of biomass. Our project is vital to developing a biological process for degrading biomass. We want to make our resources a reality, and this project is the first step.

Missouri Miners

Constructing an Ethanol Sensor

In Pichia pastoris, alcohol oxidase (AOX) is the first enzyme in the methanol utilization pathway. This enzyme is encoded by the AOXI gene. If exposed to an environment containing both methanol and ethanol, P. pastoris preferentially metabolizes ethanol. The production of the AOX enzyme is subject to the concentration of ethanol. This diauxic metabolism may be utilized as an ethanol sensor. When the AOXI promoter is fused with a gene encoding a fluorescent protein, the activation of the AOXI promotermay be detected by direct observation of fluorescence. Our project is the development of a device containing the AOXI promoter fused with a fluorescent protein gene to create an inexpensive ethanol sensor for a variety of applications. The concentration of ethanol in the environment may be deduced from the time period between exposure of bacteria carrying the device to ethanol and methanol, until the detection of fluorescence.

MIT

Streptococcus mutans is the main cause of dental caries. A clinical study (Kelly CG et al.; Nature Biotechnol. 1999) isolated the 20aa functional segment (p1025) that S.mutans uses to attach to teeth. p1025 competitively inhibits binding of S.mutans, preventing the recolonization of S.mutans for 90 days.

We are engineering Lactobacillus bulgaricus, a bacteria common in yogurt, to produce and secrete p1025. Since a new batch of yogurt is made using some of an old batch, a continuous supply of teeth- cleaning yogurt will be available since all descendants of the original bacteria will also express p1025. This expression system can be used to produce other peptides by replacing the p1025 gene with another. Yogurt with modified bacteria is a cheap and efficient way to distribute vitamins, vaccines and more in underdeveloped rural communities.

Newcastle University

A Computational Intelligence Approach to Developing a Diagnostic Biosensor: The Newcastle BugBusters Project

Computational tools for the design and simulation of circuits are widely used within the engineering community, but have been under-utilized in synthetic biology. Of particular promise is a computational intelligence focus, using algorithms such as artificial neural networks (ANNs) and evolutionary computation, which are designed specifically for the generation of "good-enough" solutions to problems in complex, poorly understood systems. We use an Evolutionary Algorithm to computationally design a genetic regulatory circuit that behaves like an ANN. The circuits are composed bottom-up from modular parts and are modelled in CellML. The aim of the project is to engineer an extensible signalling network to allow Bacillus subtilis 168 to detect, classify and indicate the presence of selected pathogens in its environment.

We use bacterial two-component systems as the input layer of the in vivo ANN that responds to specific profiles of quorum sensing signalling peptides by expressing genes for selected fluorescent proteins.

NTU-Singapore

Engineering Colicin E7 production system to inhibit Enterohemorrhagic Escherichia Coli O157:H7

The focus of the NTU Team's iGEM 2008 project is the use of bacteriocins (i.e. colicin E7) for the inhibition of the Escherichia Coli O157:H7 enterohemorrhagic strain (EHEC), which causes colitis and bloody diarrhea by producing a toxin (i.e. Shiga toxin) that damages the intestines. This is a prevalent medical problem that has effected a wide population. The team intends to achieve its objective by engineering a biological system that i) produces Colicin E7 through the regulation of LacI gene, and ii) releases E7 through lysis upon detection of symptoms and presence of pathogenic E. Coli. The production of the lysis protein functions under the control of an AND gate, and the inputs are Fe2+ ions (attributed to presence of blood) and Ai2 (attributed to presence of O157:H7). The team intends to characterize the parts and devices used and developed, and to understand the system via computational modeling.

Paris

Bacterio'clock : First-In-First-Out temporal gene expression control

Modulating the temporal expression of genes is at the heart of many biological processes. The aim of our project is to introduce a logical order of expression of genes within the context of an oscillating system. In our system the period of oscillation would allow the sequential switching of three genes in a "FIFO : First In, First Out" manner. This FIFO behavior is implemented as a network of Feed-Forward Loop motives. For this purpose we chose to base our synthetic FIFO system on the naturally existing E. coliflagella system where FIFO of the flagellar machinery genes expression was demonstrated. The FIFO system is then coupled at the population level to an oscillator based on the las quorum sensing system. In parallel we established quantitative computational models with experimentally measured parameters to explore the dynamics of this system.

Peking University

A genetic circuit to direct evolution of proteins in vivo

Directed evolution method could be a powerful tool for answering scientific questions or for constructing novel biological systems. Here we present a simple genetic circuit for in vivo evolution, which is comprised of functional elements for random mutation and artificial selection. We engineered yeast to generate the mutator AID, an essential protein in adaptive immunity, and target it specifically to a gene of interest. The target gene will be mutated at a high rate and consequently evolves at rapid pace. The mutation rate inversely correlates with the functionality of the desired gene by self-regulated expression of AID. This circuit may be adopted for in vivo evolution in eukaryotic system on virtually any genetically encoded target. It has a variety of potential applications in academic and industrial contexts, including almost any inter-molecular interaction that involves proteins and RNAs.

PennState

Diauxie Elimination by Xylose Inducible Promoters

Microorganisms typically preferentially utilize glucose over other sugar carbon sources such as xylose. This is largely regulated through control of gene expression based on the response of regulatory elements to sugars available to the cell. In E. coli, the xylose metabolism operon is controlled by both the xylose-inducible XylR activatorprotein and the cAMP receptor protein (CRP). In this project we attempt to eliminate glucose control over xylose-inducible gene expression in E. coli by altering the natural transcriptional control region of the xylose operon. Designs constructed and tested include scrambling the CRP binding site, increasing the strength of the xyl promoter, and over expressing XylR. Xylose-inducible gene expression that functions independently of glucose regulation provides a useful approach to improving microbial utilization of biomass feedstocks containing mixtures of glucose and xylose.

Prairie View

Modeling Molecular Biosensor: Use of eNOSE and Neural Network System

Biosensors are functional molecules and/or cells including microbial cells that allow detection of the presence of different molecules and/or metal ions such as iron , vanadium, nickel, and other elements, even at detection levels beyond limits of conventional methods. Therefore, the aim of the denoted project was to disign a divice for detection of different levels of Fe (II), Ni (II), and V (II). The response of the biosensor was measured by DNA and protein fluorescence, bacterial growth (CFU), and ATPproduction. The device was tested at different concentrations of the metal ions.A computational modeling, neural network system coupled to an eNose system was developed to accurately assemble and predict the efficacy of the final biosensor device.

Princeton

Genetically engineered neuronal circuits: the fast and the furious

The electrical and chemical excitability of biological neurons make them excellent components for synthetic biology systems. We designed and partially constructed genetic programs that drive the formation of several specific neuronal cell types from embryonic stem cells. A two-phase genetic program is used to first drive stem celldifferentiation into neuronal precursors followed by differentiation into mature neurons that synthesize and respond to specific neurotransmitters. We arrange populations of three types of genetically engineered neurons in a topology that implements a (very fast) bi-stable toggle switch. Pacemaker cells serve as the ‘power source’ and constantly transmit excitatory dopamine-based action potentials to the other two cells types. These two cell types cross-repress each other using inhibitory neurotransmitters (e.g. GABA and glycine) such that only of these cell types is active. The system is switched between the two stable states through external induction with the inhibitory neurotransmitters.

Purdue

Engineering a Real-Time Living Biosensor: DNA Damage cause by Ultra-violet Irradiation

Early detection of ultra-violet (UV) exposure is critical to minimizing the risk of developing skin cancer due to DNA damage. Chemically based sensors, such as UV sensitive beads that change color with progressive exposure to sunlight and color changing sunscreen, are both available on the market. These

products allow the consumer to visibly check their level of UV protection and provide an early warning when the sunscreen becomes less effective. This project involves utilizing *Escherichia coli* and standard genetic manipulation to mimic these effects from a biological standpoint. Utilizing two pathways common to genetic engineers, SOS and β-gal blue/white markers; we strive to transform an *E. coli* that will visibly change color when exposed to a large amount of ultra-violet radiation. This construct allows us to measure direct DNA damage due to UV irradiation.

Rice University

BioBeer

Resveratrol, a phytochemical used for defense in plants, has been implicated as a natural product that increases life span and prevents cancer. Unfortunately, significant levels of resveratrol are present in only a small number of foods, such as red wine, peanuts, and blueberries. To create an alternative source for resveratrol consumption, we are introducing a biosynthetic pathway for this compound into a brewing strain of *Saccharomyces cerevisiae* and examining whether this strain can be engineered to produce resveratrol during beer fermentation. Given the high worldwide consumption of beer and the low cost of production, unfiltered beer brewed using our genetically modified *S. cerevisiae* should provide a cost-effective source of pharmacologically-active resveratrol.

Slovenia

Immunobricks

Almost half of the world population is infected with bacteria *Helicobacter pylori* which is also recognized as a type I carcinogen by WHO. Effective vaccine against *H. pylori* is not available, although it would be a durable solution, particularly in a formulation affordable to the third world population. *H. pylori* evades the immune surveillance by modifying several of its components to avoid detection by several Toll-like receptors. Recent discoveries demonstrate that synergy between innate and adaptive immune response is essential for an effective vaccine. We used principles of synthetic biology to assemble well defined synthetic vaccine, composed of the functional “immunobricks”, which combine the activation of innate immune receptors, appropriate cellular localization for processing of antigens and antigenic segments to stimulate formation of antibodies and cellular adaptive response. Our engineered vaccine was implemented in three different types of vaccines based on recombinant protein, engineered bacteria and DNA vaccine.

Tianjin

A synthetic convertible ecosystem & A foolproof genetic self-assembly system

Tianjin’s program is composed of two projects: in project #1 a Prisoner’s Dilemma will be imposed to two cocultured strains of E coli, while in project #2, an effort has been made to improve the methodology of gene cloning experiments. #1. A bistable ecosystem comprised of two strains that could switch between mutualism and competition has been built. The relationship between the two could be regulated by changing culture conditions. By doing this, we explored the possibilities of improving the coexistent ecosystems that function in industries. #2. A genetic self-assembly system was built to reduce the labor and cost involved in gene cloning experiments. Via the mechanism of site-specific recombination and

incompatibility of plasmids, our device could make it possible that the recombination of the genes of interest as well as the dilution of the undesired recombinant genes will be automatically performed by the cells, upon introducing the foreign genes.

Tokyo Tech

Coli.Touch – implementation of a pressure-responsive genetic circuit in E. Coli

Our project is to construct a bacterial `touch panel' which is colored by the pressure. We name it E. coli touch or Coli. touch. In iGEM, genetic circuits that respond to various inputs -- heat, small molecule, and light -- have been constructed. However, a pressure-responsive genetic circuit has not been constructed yet. Therefore, we constructed a pressure responsive circuit using a pressure-inducible promoter. Under high pressure, the affinity of LacI for the lac operator in lac promoter is known to decrease due to a tetramer to dimer transition of LacI. However, we need 30 MPa pressure for induction of the lac promoter. Therefore, we created a withstand high-pressure display, and we tried to create a promoter induced by the lower pressure. In order to implement rewritable function in Coli. touch, we are planning to construct a toggle switch circuit using the lac promoter.

Tsinghua

Modeling and reconstruction of the Escherichia coli chemotaxis system/Construction of a Polyhydroxyalkanoates(PHA) production induced-lysis cell

1□Inspired by the chemotaxis system of bacteria, we isolated and reconstructed a set of genetic modules in order to reconstitute an independent and interchangeable chemotactic device used as pollutant detector. Novel cybernetics terms and methods are introduced, while in silico modeling together with related softwares are also established to simulate the effects.

2□In this project we are going to establish a novel bacteria strain which will sense the production of PHA, a degradable material used in environmentally friendly plastics. The key of this construction is to find a link between the amount of PHA particles and gene expression. A wildtype circuit and an artificial device are combined together to achieve this purpose. Lysis genes from the phage are introduced to break the cell and release the particles.

TUDelft

Engineering Bio-thermometers at Delft University of Technology

The goal of our project is to construct temperature-sensing bacteria Escherichia coli that changes color at different temperatures. Such a thermometer can be applied as a temperature reporter system in large-scale fermentations, or as a temperature-inducible protein production system. The functionality of this thermometer relies on the post-transcriptional regulation of a temperature-sensitive RNA structure. It opens and enables the ribosome to bind, only when the temperature exceeds a certain threshold. We designed new artificial temperature sensitive RNA sequences, and developed protocols, using luciferase as a reporter, to test their functionality. For the colour output, we built upon the existing carotene biosynthesis pathway and converted all new elements to the BioBrick standard. Furthermore, we developed mathematical models describing both the temperature sensitive parts and the colour mevalonate pathway, and estimated parameters using the experimental data. The ethical issues in design and possible implementation of a commercial product are also addressed.

UC Berkeley

Clonebots

In an effort to optimize the manufacture of parts, we have designed Clonebots - a collection of devices and strains that aid in the synthesis and analysis of new parts. Our team has programmed Clonebots to perform processes critical for efficient manufacture of biological products. We created systems capable of in vivo genetic manipulations and constructed an inducible self-lysis device designed to reclaim a variety of products without the need for conventional methods of lysis. By replacing traditional mechanical operations with biologically encoded alternatives, Clonebots are capable of accomplishing many operations with a single automated liquid handling unit - a cost-effective, BioCAD-friendly approach to large-scale projects.

UC Berkeley Tools

Clotho: A Platform-Based Design Tool for the Development of Synthetic Biological Systems

Clotho is an open source BioCAD software platform with a unified set of tools for the management (via flexible databases), development (via algorithms and analysis), and deployment (via part packaging standards) of biological parts and systems. It follows the principle of Platform based design (PBD), a methodology that enforces a strict separation between what a system does and how it is implemented. In this methodology, a platform should continually provide more accessible, variegated workspaces while widening its array of possible applications. Clotho achieves this by providing a core data structure that connects to external databases as well as plugin tools, including tools for sequence editing and annotation, design tools for constructing BioBrick composite parts, and an algorithm development manager. Data objects can be passed between design and analysis tools within Clotho and shared between users. Additionally, we have developed the infrastructure for community developers to write custom plugins for Clotho.

UCSF

Chromatin Memories: A New Tool for Synthetic Biology

The cells of higher eukaryotes utilize chromatin state to encode "permanent" epigenetic changes in gene expression. For example, signals received by a cell during the course of development can induce the partitioning of the genome into accessible (euchromatin) and inaccessible (heterochromatin) regions that specify the fate of that cell. This epigenetic profile, in which blocks of gene are "silenced" by heterochromatin, is stably maintained and inherited by daughter cells. Thus, chromatin state provides a higher level of gene expression control that is regional (many genes at once), dominant over transcription factors, ultra-cooperative (all or none), and highly stable (memory). We have constructed and characterized a synthetic silencing system in S. cerevisiae that inducibly silences specific loci in the genome. This foundational technology will facilitate the construction of complex genetic circuits with memory, and has potential application in the engineering of cell differentiation in higher eukaryotes.

UNIPV-Pavia

Engineering E. coli to multiplex and demultiplex signals

The goal of this project is to provide multiplexing and demultiplexing capabilities in E. coli. Multiplexing is a process where one of multiple input signals is conveyed into a single output channel, whereas in demultiplexing a single input signal is conveyed into one of multiple output channels. The choice of input channel in multiplexing and output channel in demultiplexing is controlled by a selector. The devices implementing these functions are called Multiplexer (Mux) and Demultiplexer (Demux) respectively. In a digital framework, signals can only assume 0/1 values and then the two components can be represented as logic networks. To reach our goal, we chose a biological implementation for all the logic gates involved in the networks and we connected these biological gates to build up two genetic circuits that behave respectively as a 2:1 Mux and a 1:2 Demux, that can be used in several contexts.

University of Alberta

The Detection and Degradation of Bisphenol A in the Environment: An Antidote For Poisonous Plastic.

Bisphenol A (BPA) is a chemical building block used to make polycarbonate plastic and epoxy resins, which are used throughout society. It is also an endocrine disruptor that has been implicated in cancer, obesity and developmental problems. These health dangers arise, in part, because BPA is an estrogen-like compound capable of activating the human estrogen receptor, and thereby, affecting gene expression. Using our novel NOT-gate design, we have constructed a system that detects, and ultimately degrades BPA by utilizing BPA's chemical similarities to estrogen. This prototype system tests the validity of a novel ERα/TetR NOT-gate. The effectiveness of BPA degradation via gene products of the newly characterized BisdA and BisdB genes is also examined.

University of Chicago

Efficient production of mussel adhesive proteins in E. coli and Caulobacter crescentus

Our research group is interested in engineering E. coli and caulobacter to express recombinant mefp-5, fp-151 and which are found naturally in mussels (Mytilus edulis and Mytilus galloprovincialis). These mussel foot proteins are strong bioadhesives and powerful anti-biofouling agents, with applications for biomaterials and biomedical research . We aim to produce results that will achieve the initial goals of genetic engineering, as well as further the conceptual goals of synthetic biology. The final goal of this research is to produce 5 biological Systems that meet the specifications of the Standard Registry of Biological Parts: 1) Expression of recombinant MAPs 2) secretion of MAPs 3) Surface display of MAPs 4) expression of tyrosinase and 5) concomitant production of tyrosinase with MAPs.

University of Lethbridge

The "Bacuum" Cleaner - an intelligent self-propelling keener cleaner

Tailing ponds used to store discarded waste from oil refineries pose a major environmental dilemma. Our goal is to create modified E. coli capable of seeking out and degrading toxic aromatic pollutants created during the oil refinery and mining processes. Our "Bacuum" cleaner will respond to a destructive compound through interaction with a programmable riboswitch. The riboswitches will switch at varying concentrations of target ligand, thus altering the induced signal. At low concentrations, we intend to have our riboswitch

express the motility protein cheZ in E. coli, directing the bacterium towards higher concentrations of our target molecule. Once it reaches a threshold concentration, a catabolic pathway capable of degrading our target pollutant will be activated. To create these riboswitches we plan to use SELEX to reprogram the theophylline riboswitch. We chose 2-chlorobenzoate, a compound related to polychlorinated biphenyls (PCBs), as our target molecule.

University of Ottawa

A Pulse Generator in Yeast for Sustained Expression of Recombinant Proteins

Large-scale production of recombinant proteins typically involves growing genetically modified microorganisms in bioreactors in which selective pressure tends to diminish culture productivity over time. To alleviate the detrimental effects of continual high-rate synthesis, we have designed a yeast strain capable of producing bursts of gene expression in a controlled and inducible manner. These "pulses" are generated by the action of an inducer molecule that triggers the synthesis of a protein of interest and simultaneously induces a repressor protein to terminate expression as well as an enzyme to degrade the inducer signal, thereby returning the system to its initial state. By co-culturing populations of inducer-synthesizing cells and pulse-generating receiver cells, we hope to achieve self-sustaining oscillatory gene expression dynamics that could render long term culture-based recombinant protein synthesis more sustainable. This would open the door for the production of considerably toxic proteins for numerous applications including anti-cancer therapeutics and antiseptics.

University of Sheffield

Fusion receptors – an approach to confer new features on bacteria

Expression of non-native receptor proteins in bacteria often involves extensive genetic modifications that can be difficult to execute. One way of addressing this problem is making a fusion receptor protein consisting of the sensing part derived from a foreign species, and a signal transmitting part that is native to the organism in which the receptor is to be expressed. The fusion receptor we have designed consists of Vibrio cholerae's sensing module fused to E.coli's signal conveyer. The receiver part of a receptor should bind to signaling molecules excreted by V.cholera and pass it downstream through signal transmitters to DNA. Expression of reporter molecules will indicate water contamination. As a result, the fusion receptor could be applied in real life as a basis for a cheap device sensing water contamination.

University of Washington

The VectorJector: Engineering a Microbial Gene Delivery System

Transferring novel abilities into eukaryotes has many potential applications. Our project attempts to control transfer of genetic material across phylogenetic domains. We attempt to direct the prokaryote Escherichia coli (domain Bacteria) to transfer DNA encoding potentially useful traits from to the yeast Saccharomyces cervisiae (domain Fungi). The design utilizes standard engineering and synthetic biology techniques to modularize this process, in order to enable usage across varying organisms and conditions. To achieve control over our system, bacteria transfer DNA via conjugation only if certain conditions are met. In our design, E. coli transfers the genes to metabolize lactose in S. cerevisiae, but only where lactose is

prevalent, glucose is minimal, and yeast proximity is sensed via a yeast-produced signaling molecule. It therefore provides a means for conditional, not constitutive, gene transfer between diverse organisms. Applications might include the production of transgenic plants and animals, clinical gene delivery, and interacting multiple-organism systems.

USTC

Self-organized multiple-cell system

It is an amazing process in nature that the evolution from Protozoa to Metazoa. Even in the development of each Metazoa, it is still unknown how the genome regulates the stem cells to develop into different kinds of cells, which can compose different organism, according to where they are in the body. There should be a self-organized procedure. Here we are trying to build a self-organized multiple-cell system based on the quorum sensing system to understand the mechanism of this process. We employed the small molecules in AHL family as the messenger to transmit the orders of differentiation and response and Cre recombinase as the executor of differentiation. Through an artificial designed network, we are trying to construct a new kind of cells, which a cycle composed by GFP will be seen on the plate if the colony is big enough.

Utah State

Efficient systems for monitoring polyhydroxyalkanoates production in microorganisms

The increasing cost and negative environmental effect of fossil hydrocarbon-derived conventional plastics has escalated the need for economically realistic alternatives. Polyhydroxyalkanoates (PHAs)are a class of microbially synthesized, biodegradable thermoplastics that exhibit material properties comparable to those of conventional plastics. The Utah State University iGEM team project is focused on creating an efficient system for production and monitoring PHA production in microorganisms. One goal of our research is to develop and optimize a method, using fluorescent proteins, for the detection of maximum product yield of polyhydroxybuterate (PHB, a bioplastic) in recombinant E. coli and in Cupriavidus nectror. In order to develop an optimal PHB detection system, we focused on the identification of the most efficient reporter genes, and the best promoter sequences that would allow our reporter to indicate when PHB production was maximized.

Valencia

The Hot Yeast Project: Heat production in UCP-1-expressing yeasts

The present project aims to demonstrate that the temperature of a microbial culture might be modulated through the expression of the mammalian uncoupling protein UCP-1. Saccharomyces cerevisiae strains genetically modified to express wild type UCP-1, mutant sequences with increased uncoupling activity, as well as a control strain were cultured in an Liquid Culture Calorimeter (LCC) we developed. The system consisted of a modified thermo flask with an inserted thermocouple allowing real-time accurate temperature measurements. Different conditions, such as initial densities, amounts of inductor, or shaking speeds were tested. We succeeded to obtain significant temperature increases in the mutant strains compared with the other strains. We also developed an effective model of our system. Although the system is not always stable and might be sensitive to external perturbations, this is the first time a significant increase in

temperature associated to UCP-1 expression in yeast is reported.

Virginia

Transcription attenuation for metabolic control by engineering intrinsic terminators

A main challenge in constructing synthetic biological systems is the inability to precisely regulate gene expression using artificial means. Tightly-regulated control of any given set of related transcriptional, translational and posttranslational events will likely require a combination of powerful strategies. Therefore, the 2008 Virginia iGEM team is developing a library of transcriptional terminators intentionally redesigned to be functionally inefficient. Well-characterized, standardized terminators of various efficiencies should allow finely-tuned transcription attenuation and represents yet another step toward global biological control. This work complements other gene expression control methods that focus on initiation of transcription. The desired result is quantitative control of transcript levels, which is often necessary to balance flux through a synthetic metabolic pathway. To demonstrate its potential for real-world application, the team is planning to employ this approach to control the expression of a heterologous pathwayin E. coli for the biosynthesis of polyhydroxybutyrate (PHB), a biodegradable polyester plastic.

Warsaw

Bacterial device for creating and production of interactors for any given bait protein

We have developed a system allowing to search for antibodies with new specificities or screen protein libraries in order to generate interactors for a given bait. Our system changes a protein sequence to maximize its interaction with a given partner. Proteins with modified sequences are then directed to the bacterial outer membrane, where the best interactors are selected. Protein presented on the cell surface is fused with part of β-lactamase protein, while its bait is fused with the complementing part of the enzyme and added to medium. The stronger the interaction between proteins of interest, the more efficient the binding of the two halves of β-lactamase, leading to resistance to ampicillin and survival. Cells expressing less interacting variants die as they don't achieve sufficient complementation of the reporter enzyme. This allows us to select strains producing interactors for any given bait.

Waterloo

A plasmid-safe, inducible genome-degradation strain for post-kill gene expression

The aim of our project is to engineer a genome-free, cell-based expression system capable of producing a desired protein or activating a pathway in response to an environmental signal. Genome degradation is achieved using the combined activity of a restriction endonuclease to fragment the genome and an exonuclease to hasten degradation. The gene for the protein of interest will be located in a plasmid lacking recognition sites for the endonuclease, allowing it to remain intact after genomedegradation. The plasmid genes will be expressed using the remaining cell resources until they expire. The primary application of this design would be an in situ compound production and delivery system for agricultural, industrial or therapeutic use.

Wisconsin

Examining Biofuel Precursors Via Increased Sorbitol Flux and Lignin Peroxidase Expression in Escherichia

coli

The global fuel crisis impacts our economy, national security, and environment. The need for alternative fuels is of utmost importance. Team Wisconsin used Escherichia coli to produce biofuel precursors in an effort to find these alternative fuel sources. One project focused on using E. coli to efficiently produce sorbitol, a biofuel precursor. Using computer modeling, we determined a way to funnel glycolysis' intermediates towards the production of sorbitol via sorbitol-6-phosphate dehydrogenase. Wisconsin's second aim was to isolate high-energy precursors from the plant cell wall through E. coli mediated breakdown of cell wall lignin. This was achieved by inserting the geneencoding lignin peroxidase , found in the white rot fungus Phanerochaete chrysosporium, into genetically modified E. coli, capable of producing and exporting the enzyme. Both projects improve current methods in the production of alternative fuels via two different, unique routes, and have the potential to move sustainable biofuel research forward.