Beyond DNA origami: A look on the bright future of nucleic acid nanotechnology

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Abstract

Nucleic acid nanotechnology exploits the programmable molecular recognition properties of natural and synthetic nucleic acids to assemble structures with nanometer-scale precision. In 2006, DNA origami transformed the field by providing a versatile platform for self-assembly of arbitrary shapes from one long DNA strand held in place by hundreds of short, site-specific (spatially addressable) DNA "staples". This revolutionary approach has led to the creation of a multitude of 2D and 3D scaffolds that form the basis for functional nanodevices. Not limited to nucleic acids, these nanodevices can incorporate other structural and functional materials, such as proteins and nanoparticles, making them broadly useful for current and future applications in emerging fields such as nanomedicine, nanoelectronics, and alternative energy.

Introduction

Nucleic acid nanotechnology has been utilized by nature for billions of years\(^1,2\). DNA in particular is chemically inert enough to reliably store genetic information over even millions of years\(^3\). Packaged in genomes, DNA is expressed in a regulated fashion with assistance from its more rapidly hydrolyzed structural analog, RNA, as well as protein factors\(^2,4,5\). Complementarily, the enhanced functionality of RNA is exploited in many biological nanomachines, such as the ribosome\(^6\) and the spliceosome\(^7,8\), for the “data processing” that accompanies gene expression. Nature also uses the specificity of base pairing by nucleic

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acids for database maintenance and readout via the modulation of gene expression by non-coding RNAs. Inspired by nature, researchers over the past four decades have explored nucleic acids as convenient building blocks to synthesize novel nanodevices. Because they are composed of only four different chemical building blocks and follow relatively simple, yet highly specific and thus predictable organizational base pairing rules at the molecular scale, nucleic acids are the preferred biological material for the design of structures with nanometer precision when compared with other candidates such as proteins. Short (typically < 50 nucleotides (nt) for RNA and < 100 nt for DNA) specific sequences of the building blocks can be chemically synthesized at low cost, whereas longer DNA strands of predefined sequence are provided by nature in the form of genomic DNA. A combination of these two sources led to the advent of the DNA origami method in 2006, which dramatically accelerated progress in nucleic acid nanotechnology by further increasing the simplicity, precision, and fidelity of the design principles available for generating spatially addressable nanoscale structures (see “DNA Origami” insert). Numerous DNA-origami-based nucleic acid nanodevices and nanomaterials have since been constructed with great potential for a multitude of useful applications, and with abundant prospects for innovation.

THE BUILDING MATERIALS: NATURAL NUCLEIC ACIDS

Nucleic acids are linear biopolymers found in all organisms, as well as many viruses, as they are the means by which genetic information is stored, transferred, and regulated. Their monomeric building blocks (nucleotides) each consist of three moieties (Figure 1a–c): a nucleobase, a five-carbon sugar, and a phosphate group. The phosphate group enables the formation of a phosphodiester bond between the sugars of adjacent nucleotides, creating a polymer known as a single-stranded nucleic acid. Complementary nucleobases can form hydrogen bonds which, along with stacking interactions between adjacent base pairs, results in two fully complementary polymers hybridizing to form a relatively rigid antiparallel double-stranded helix (Figure 1d). This famous Watson-Crick duplex has an average dimension (rise) of 0.34 nm (for canonical B-form DNA) between the base pairs along the helical axis and a diameter of 2 nm, ideally suited to engineer nanotechnological structures.

Nature has optimized two different types of nucleic acids through evolution: DNA and RNA, which both consist of four nucleotide building blocks that tend to pair in a predictable Watson-Crick fashion (Figure 1d). The guanine-cytosine (G:C) base pair forms three hydrogen bonds, stronger than the two hydrogen bonds of the adenosine-thymine (A:T) pair, used in DNA, or the adenosine-uracil (A:U) pair, used in RNA. The primary chemical difference between DNA and RNA is their sugars (Figure 1a); ribose, the sugar found in RNA, contains an extra 2'-hydroxyl group not present in deoxyribose, the sugar found in DNA, causing the backbone of RNA to hydrolyze ~10^8- to 10^10-fold more quickly than that of DNA (Figure 1a). The A-form RNA duplex, however, is more thermally stable than the B-form DNA duplex, even though RNA is predominantly found single-stranded in nature and forms mostly intramolecular helices. These structural differences between DNA and RNA are functionally exploited by nature.

DNA

While the most notable purpose of DNA is storing and maintaining the genetic blueprint of an organism, DNA performs other structural roles in the cell as well. For instance, the guanine-rich single-stranded telomeres at the ends of chromosomes can form topologically more complex quadruplex structures built around a square arrangement of four stacked guanine-bases stabilized by a chelated metal ion (preferably K⁺) at their center. G-quadruplexes thus play important roles in the maintenance of linear eukaryotic genomes and...
possibly in gene expression regulation. Similarly, so-called Holliday four-way junctions of two entwined DNA duplexes play critical roles in DNA recombination. Forming and resolving such higher-order structures often requires the aid of external factors such as proteins. For example, complexes of histone proteins facilitate the compact folding of ~1 m of DNA into chromosomes that fit inside a ~6-µm-wide nucleus while still remaining accessible for gene expression. In cases where decreased stability would be most beneficial, a lower melting temperature may be achieved by increasing the ratio of A:T to G:C base pairs, as is desirable for promoter regions in DNA (regions that need to melt for an RNA polymerase to initiate gene expression).

**RNA**

In the central dogma of molecular biology, the portrayed function of RNA is as the messenger between DNA and protein, carrying the blueprints for protein formation from the DNA genome so that they may be translated by the ribosome into proteins. However, over the past few decades the pervasiveness and importance of RNA that does not code for proteins has been increasingly appreciated. Typically, these non-coding RNAs (ncRNAs) directly or indirectly regulate or mediate gene expression at the transcriptional or translational level, making them candidates for therapeutic applications. Some ncRNAs simply make use of molecular recognition through base pairing, such as in the RNA interference pathway. Others exploit conformational dynamics of RNA. For example, riboswitches regulate gene expression via the conformational change induced by the RNA binding a small-molecule metabolite or second messenger. Still other ncRNAs promote catalysis, such as in peptide bond formation within the ribosome or cleavage of RNA via hydrolysis of the sugar-phosphate backbone; the activity of each of these RNA enzymes (ribozymes) is dictated by its tertiary structure. While these activities are currently difficult to engineer from scratch due to the large number (hundreds to thousands) of nucleotides required and our limited understanding of the underlying mechanisms, these functional, structurally complex ncRNAs have already been optimized by nature as active components in cellular machines.

**THE BUILDING MATERIALS: MODIFIED NUCLEIC ACIDS**

Nucleic acids undergo chemical base modifications in vivo, impacting their cellular functions. For example, cytosine methylation prevents DNA from being digested by methylation-sensitive restriction enzymes in bacteria, and in many eukaryotic organisms is involved in the epigenetic regulation of gene expression. In an analogous fashion, researchers have synthetically modified nucleotides to increase thermodynamic and chemical stability as well as specificity of interactions. For instance, locked nucleic acids (LNA) are engineered with an extra methylene bridge between the 2'-oxygen and 4'-carbon of ribose, “locking” the ribose in the 3'′-endo conformation (Figure 1c), which leads to a particularly stable A-form helix with enhanced base stacking and backbone pre-organization and significantly increased melting temperature, specificity of base pairing, and nuclease resistance. Other chemical modifications of the nucleic acid backbone commonly used for enhancing intracellular stability while still often supporting biological function of an ncRNA include 2'-O-methyl, 2'-amino, 2'-fluoro, and phosphorothioate substitutions (Figure 1a). As a more drastic modification, peptide nucleic acids (PNA) have an uncharged backbone of N-(2-aminoethyl)-glycine units joined by a peptide bond, resulting in higher thermal stability between PNA/DNA strands than corresponding DNA/DNA strands (Figure 1b). The uncharged nature and nuclease resistance of PNA make it an appealing option for nanodevices and 2D nucleic acid arrays.

Not only can the nucleic acid backbone be modified, but also the nucleobases themselves. A particularly far-reaching example is the artificial expansion of the genetic alphabet by an

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additional, differently hydrogen bonded base pair such as Z or P (or 6-amino-5-nitro-3-(1'-β-D-2'-deoxyribofuranosyl)-2(1H)-pyridone and 2-amino-8-(1'β-D-2' deoxyribofuranosyl)-imidazo[1,2-a]-1,3,5-triazin-4(8H)-one, respectively, see Figure 1d), which increases hybridization specificity while still allowing for specific recognition by DNA-binding enzymes 39.

Whereas the improved thermal and chemical stability often found in modified nucleic acids will prove useful for nanostructures, incorporation is nontrivial; nanostructure design is heavily dependent on helical twist, which is typically different between modified and unmodified nucleic acids 10. This hurdle will, however, be overcome with improved and expanded design rules. In addition, these materials can already be used to site-specifically decorate DNA scaffolds since they form canonical Watson-Crick base pairs.

THE PRESENT STATE OF NUCLEIC ACID NANOTECHNOLOGY

By employing the knowledge acquired from observing nature and building upon it, nanostructures not found in nature have been engineered that encompass a broad range of design objectives. The following are examples of successful nanostructures grouped according to their purpose.

Scaffolding—Nucleic acid scaffolds are nanostructures that may be functionalized for ordering and arraying materials with nanometer precision. Scaffolds were originally formed by combining different short double-stranded DNA (dsDNA) domains joined in a programmable fashion using single-stranded DNA (ssDNA) overhangs known as “sticky ends” 40. While the stiffness of dsDNA makes it a suitable raw material for the edges of stable 2- and 3-dimensional structures, the vertices of such structures remain flexible, resulting in a range of angles between domains 40. Inspired by naturally occurring Holliday junctions 21, 41, more rigid structures were accomplished using reciprocal exchange to generate double 42 and triple crossover motifs 43 (Figure 2). As an alternative to combining multiple domains, which results in variable yields of assembled structures without defined boundaries, increased yield and stability are accomplished using Rothemund’s DNA origami method (see “Origami” insert and Figure 3), leading to more sophisticated 2D and 3D structures ranging from smiley faces 11 to octahedra 44 and nanoflasks 45 that can be concatenated into larger structures 11, 46.

Dynamic Devices Constructed on Nucleic Acid Scaffolds—To add functionality to the scaffolds, DNA, RNA and protein have further been used to construct dynamic devices serving as proofs-of-principle for directed transport and nanoscale assembly factories. Molecular walkers analogous to the motor proteins myosin, kinesin, and dynein have been constructed completely or primarily from DNA, and have attained increasing levels of autonomy as well as responsiveness to external instruction (Figure 4a) 52, 53. Some such transporters are capable of dozens to potentially thousands of directed steps and have been shown to walk processively along tracks on DNA origami 47, 52. Directed walkers have also aided in the combinatorial assembly of different-sized gold nanoparticles 48 or the multistep synthesis of a small organic compound 49, in the former case aided by two-state DNA conformational switches (Figure 4b). Coupling such devices with DNA computing 54, either on the scaffold or in solution, may further improve the complexity and range of responses to environmental stimuli or instructions.

In addition to the above devices made primarily of nucleic acids, several groups have sought to augment the functional repertoire of DNA and RNA nanotechnology through conjugation to other nanomaterials, using self-assembled DNA structures as a scaffold on which to position non-nucleic-acid components that impart some new catalytic, electronic, photonic,
or structural functionality. For instance, placing the metabolically linked enzymes luciferase and NAD(P)H-dependent FMN oxidoreductase (NFOR) in close proximity on a DNA template led to more efficient recycling of their common FMN/FMNH\textsubscript{2} cofactor and an overall enhancement of the catalyzed reaction rates, mimicking the strategy of substrate channeling often employed by nature\textsuperscript{55}. DNA origami has been used to create junctions of carbon nanotubes with precisely defined geometry\textsuperscript{50, 51}, in one case behaving like a nanoscale field-effect transistor (Figure 4c)\textsuperscript{50}. Furthermore, DNA templates have directed the assembly of chromophores such as organic dyes and quantum dots for the directed transfer of photonic energy through FRET\textsuperscript{56–58}, with the prospect of developing photonic circuits or artificial photosynthetic antenna complexes (Figure 4d). Conjugating DNA to small organic molecules or polymers has yielded materials with interesting thermal properties, such as solid DNA that only melts at 95°C\textsuperscript{59}. Hybrids of nucleic acids and synthetic polymers show promise for drug delivery and diagnostics\textsuperscript{60}. Finally, proteins may also be employed as integral structural components of nucleic acid nanossemblies. For instance, assemblies shaped like equilateral triangles were formed from an RNA-binding protein and an RNA strand bearing the appropriate binding motif\textsuperscript{61}.

**Combining Bottom-Up with Top-Down Techniques**—So far we have described nanoassemblies built using bottom-up techniques (i.e., the creation of more complicated structures self-assembled from fundamental units). While these techniques yield highly resolved structures that may be generated in parallel, they are so far still limited in their dimensions, making them unappealing for applications where truly large-scale patterning is required. By contrast, most current technology outside of nanotechnology uses top-down techniques, namely the ordered assembly of components by externally-controlled means. For example, many lithographic top-down fabrication methods are able to serially generate structures with features typically on the order of microns (e.g., for silicon-based computer chips), but at great capital expense for equipment. By combining top-down and bottom-up techniques, a more affordable large-scale (> 1 mm) patterned array with nanometer addressability may be accomplished\textsuperscript{62}. Electron beam\textsuperscript{63} and soft\textsuperscript{64} lithography techniques have been used to pattern DNA nanostructures across a surface with spacings of ~300 nm and ~5 µm, respectively (Figure 4e,f).

**ENVISIONING FUTURE APPLICATIONS**

We envision that the basic materials and concepts of nucleic acid nanotechnology outlined so far will further be recombined to create devices capable of accomplishing an ever wider variety of tasks, particularly in the following fields (Figure 5).

**Molecular Computing**—Modern day computers are capable of performing incredibly complex calculations with impressive speeds. At the computer’s core, these complex calculations are made possible by large series of binary digital circuits assembled in logic gates. Analogously, nucleic acids can behave as logic gates. For instance, the output “true” for an AND logic gate can be represented by a deoxyribozyme cleaving its substrate if and only if both DNA strand A and DNA strand B are present in solution\textsuperscript{65} (Figure 6). Using variations on this basic concept, nucleic acids have completed tasks with a range of complexities from competitively playing tic-tac-toe\textsuperscript{66} to mimicking field programmable gate arrays\textsuperscript{67} and neural networks\textsuperscript{68}. Nucleic-acid-based logic gates inherently function based on thermodynamic mass action and chemical kinetics laws (i.e., probabilistic behavior), rather than the deterministic, digital on-off switches of silicon-based computers. Computing by nucleic acid nanotechnology therefore will more closely mimic the “fuzzy logic” and feedback loops of ant colonies and the human brain, with corresponding advantages in adaptability and complex pattern analysis\textsuperscript{69}. As such, it will likely find ample application in both probing and interfacing with biology.
**Plasmonics**—When metallic nanoparticles interact with visible electromagnetic waves, their conduction band electrons are excited, resulting in a phenomenon known as plasmon resonance\(^{70, 71}\). These nanoparticles placed in close proximity (<2.5 times the diameter of the nanoparticle) undergo strong near-field coupling with field enhancements\(^{72}\). However, the optimal field enhancement is dependent on the geometry of the nanoparticle array, requiring precise nanoparticle placement and spacing\(^{70, 72}\). To this end, gold nanoparticles have been assembled on DNA origami triangles\(^{73, 74}\). While DNA origami tiles are advantageous in their rigidity and addressability, they are limited in length (~100 nm) unless concatenated, in turn limiting the propagation of the plasmon resonance signal. In the future, the longer scaffolds, such as the nanopeapod\(^{75}\) or DNA origami nanotube\(^{76}\) with lengths on the order of tens of microns, will be paired with top-down microfabrication techniques to construct larger nanoparticle arrays with precision and spacing on the order of tens of nanometers. Applications will likely include communication systems that merge electronics and photonics (optics) at the nanoscale\(^{70}\).

**Biosensors**—Biosensors are small, self-contained devices that detect and report the concentration of an analyte through physical interaction\(^{77}\). Several types of biosensors have been constructed from nucleic acids (e.g., aptazyme-based\(^{78}\) or pH-sensitive\(^{79}\)), the most common being optical biosensors in which the hybridization between the analyte and its complementary capture strand immobilized on the surface results in a change in fluorescent signal\(^{77}\). To be effective, such a technique requires a highly specific interaction between the capture strand and the analyte, and low yield of false positive results. Increased specificity has already been accomplished by incorporating synthetic nucleic acids\(^{80, 81}\), although there is still room for improvement, for example, with the further development of modified nucleotides. One of the causes of false positive signals is crosstalk between neighboring capture strands\(^{82}\). The spacing between neighboring biosensor units may be determined with nanometer precision by using a DNA origami scaffold, enabling a high density of capture strands on the surface while limiting their interactions. Such scaffold techniques have recently been utilized to detect analyte concentrations as low as 200 pM, and it was found that the hybridization sensitivity was dependent on probe position\(^{83}\). In addition, precisely arranged biosensor arrays with enhanced and possibly synergistic detection properties for complex samples are now within grasp.

**Organic Synthesis**—Unlike conventional organic synthesis, which is performed one step at a time with pure reagents, the cell is capable of catalyzing thousands of reactions simultaneously in the same vessel with extremely high specificity and efficiency. DNA scaffolds and devices may provide a way to emulate this approach by virtue of their programmable nanoscale structure and ability to spatially organize catalysts and reagents. DNA origami, in combination with single-stranded overhangs, conformational DNA switches, and/or DNA walkers, have been used for multi-step organic synthesis\(^{49}\), programmable assembly of dendrimeric oligomers\(^{84}\), and dynamically controlled combinatorial assembly of gold nanoparticles\(^{48}\). Since DNA origami are currently expensive to produce in large quantities, practical applications may be restricted in the short term to small-scale syntheses, those that must be performed under mild conditions, or those that are not feasible using conventional synthetic chemistry – for example, when high-resolution control of reactant positioning (different than that afforded by surface or metal ion coordination chemistry)\(^{84}\) is required. Such syntheses are abundant in nature and exemplified by the assembly lines of multi-domain polyketide synthases that generate a large number of bioactive compounds\(^{85}\). Another exciting prospect enabled by coupling DNA origami to other dynamic devices is the ability to make chemical reactions or noncovalent molecular assembly responsive to environmental cues\(^{48}\). For instance, signals from a host organism might provide cues for a smart molecular “assembly line” to
synthesize one of many possible products depending intracellular conditions at a particular time.

**Synthetic Biology**—Synthetic biology is concerned with engineering natural or artificial biological systems to better understand biological phenomena or for practical uses such as food, drug, or fuel production, or for novel therapeutic approaches. While most synthetic biology has made use of pre-existing pathways by altering them for a particular purpose or inserting them into a different organism (top-down approach), it will be desirable to construct entirely novel artificial organisms and pathways to allow for more predictable and tractable performance (bottom-up approach). DNA origami and similar scaffolds could contribute to such systems by allowing for spatial and chemical coupling of related enzymes in a manner analogous to natural multi-enzyme complexes, a concept that has recently been demonstrated on simpler DNA templates in vitro and in highly concatenated RNA scaffolds in bacteria. (The latter demonstrates one clear advantage of RNA for many synthetic-biology applications: it is readily transcribed from a plasmid inserted into the host organism.) Three-dimensional nucleic-acid scaffolds could also serve to physically compartmentalize artificial biochemical reaction networks like natural organelles, since they can be engineered to assemble with high efficiency into vessels of arbitrary shape with well-defined dimensions. Furthermore, due to the relative ease of engineering nucleic-acid-based reaction networks and the existence of numerous elementary components such as aptamers, ribozymes, and riboswitches, some of the first artificial biological systems or organisms may have nucleic acids as integral components of their information networks. Although true bottom-up synthetic biology remains an elusive prospect for now, nucleic acid scaffolds and devices are beginning to serve as a useful means to perturb biochemical networks. Since DNA origami are stable in cells and cell lysate, ever more complex structures may soon find similar uses in interfacing with and programming of natural biological pathways.

**EMERGENT INTERDISCIPLINARY FIELDS**

Beyond the horizon of advantageous applications in more or less well-established fields, it is illuminating to explore the current state of some emerging interdisciplinary fields (Figure 5) to better understand how they are expected to benefit from future advancements in nucleic acid nanotechnology.

**Nanoelectronics**—There exists a strong effort to make more highly sophisticated, conveniently sized electronic devices, requiring the further minimization of electronic components. Currently, top-down-produced assemblies are limited by the size of nanowires and use of lithographic techniques to tens of nanometers in spatial resolution. The wires are also limited in the flux of digital information they are able to transport when compared to optical digitization techniques such as optical fibers. Exploiting plasmonic properties between precisely-placed nanoparticles is a plausible alternative. Positioned using nucleic acid scaffolds, such an architecture would not only allow for signal enhancement of the propagated information, but theoretically, the nanoparticles could behave as various, reconfigurable circuit components based on their materials and geometries (Figure 6). Integrating this approach with established lithographic techniques will lead to synergy. Scaffolded components could also serve as templates for metal deposition to generate solid-state devices with nanometer precision, although it will be necessary to develop methods for sustaining the template in the adverse conditions required for deposition. Arraying various bottom-up circuit elements using top-down assembling techniques may generate larger, more complex circuits.
**Alternative Energy**—Rising global demand for energy and concerns about the environmental impact of fossil fuel use motivate the search for viable alternative energy sources. While progress has been made toward improving alternative energy production and storage using nanomaterials, emphasis has been placed on inorganic materials rather than biomolecules. Owing to their complexity, stand-alone nucleic acid nanodevices are not likely to meet large-scale energy needs anytime soon, but become more formidable when combined with living systems or inorganic materials. For example, genetically encoded nucleic acid scaffolds can be used to increase the flux through engineered biochemical pathways by colocalizing functionally related enzymes, including in the production of fuels such as hydrogen. Nanoelectronic and photonic devices constructed using nucleic acid scaffolds and suitable chromophores could, with sufficient improvements in efficiency, mimic photosynthetic antenna complexes for nanoscale solar energy harvesting.

**Nanomedicine**—Nanomedicine has been defined as the utilization of nanotechnology for medicinal purposes including diagnostic assays, therapeutic agents, and monitoring devices. One of the current main objectives in the field is targeted drug delivery in which a drug carrier is able to selectively deliver a drug only to diseased cells, resulting in lower drug dosages and reduced harm to healthy cells. Three-dimensional DNA smart materials have several features that make them appealing candidates for nanocarriers: they are biocompatible; they can selectively contain particles, fully encapsulate them, and release them when presented with a trigger; the exteriors may be decorated with targeting signals; and differing functional components may be incorporated into their design with defined spatial positioning. Current designs initiate cargo release by introducing a nucleic acid strand to the carrier’s environment. By alternatively incorporating functional strands that are sensitive to natural changes in cellular environments, such as potassium-sensitive deoxyribozymes or DNA strands that undergo conformational changes in response to pH or an intrinsic cellular component, cargo release may be controlled intracellularly by natural means (much like intracellular genome release from a viral capsid). Computational nucleic acid devices that mimic ncRNAs are being created to implement this goal by using self-cleaving deoxyribozyme hairpins that contain within their sequence a short ssDNA strand that is complementary to the portion of mRNA needing to be suppressed, but is not active unless triggered to be cleaved by disease-indicators in the cellular environment. These devices could be integrated with the nanocarrier design to be transported only to the diseased cells. Beyond that, complex molecular “communities” could integrate biosensors, molecular computation, and chemical synthesis to diagnose and treat illnesses at the level of individual cells. For instance, one such system might enter a cell through a carrier-mediated interaction, detect abnormal levels of a certain subset of messenger RNAs, compute the probability that a given cell is cancerous, and then decide whether to initiate apoptosis through an intrinsic cellular pathway (Figure 6). While such an application remains technically very challenging and expensive, most of the required device classes have been constructed.

DNA does have the disadvantage that it is susceptible to nucleases, potentially leading to release of cargo before triggered to do so. To overcome this challenge, modified nucleic acids may be incorporated, although introducing foreign materials will need to be rigorously tested for cytotoxicity and unexpected side effects. Before these devices can be used as therapeutic agents, failsafe features (that lead, for example, to instability of the device outside of its target area) will need to be included in the design to prevent any undesired side effects.

Another primary objective of nanomedicine is the creation of point-of-care devices, which provide a convenient way of testing and diagnosing a patient immediately. Biosensors
constructed with top-down techniques hold much promise for this goal. However, the currently limited sensitivity of biosensors generally requires the amplification of the analyte, typically accomplished using PCR, which limits the detection efficiency. Plasmonics may help amplify fluorescent signals, enabling a binding event to be detected even with low concentrations of the analyte. Integration of biosensors with nanoelectronic devices could potentially accomplish the types of analysis required to accurately diagnose a patient.

Conclusion

Nucleic acid nanotechnology has come a long way since its inception a quarter century ago. It has advanced from purely static scaffolds to dynamic functional devices that include other natural and synthetic materials. The currently existing nanodevices show great promise for useful future applications in a broad variety of fields as sampled here. It is even more exciting to contemplate that the precise structures and functional versatility of devices such as these will spawn new applications and perhaps entire new fields that lie beyond the current bounds of our insight. Buttressing these advances will be the further development and exploitation of single molecule tools that can monitor the dynamic (bio)chemical reactions, actuation, movement (diffusion), and integrity of individual nanodevices, for example, super-resolution single molecule fluorescence microscopy.

References


DNA Origami

The invention of scaffolded DNA origami was a milestone in the advancement of nucleic acid nanotechnology. Developed by Paul Rothemund, DNA origami is the assisted folding of one long single-stranded DNA “scaffold” strand from a bacterial phage genome consisting of 7,249 nucleotides into a predetermined shape by ~200 typically 32-nt-long single-stranded DNA “staple” strands containing sequences that are complementary to specific regions of the scaffold strand. Two or more nonadjacent segments of the scaffold strand are brought together and held in place by hybridization to different portions of the same staple oligonucleotide in aggregate (Figure 3a), enabling the creation of arbitrary shapes based solely on the staple sequences (Figure 3b,c). The staples may be extended at their 5’ ends to make them addressable for patterning with a resolution of 6 nm on the assembled origami, imposed by the inter-staple distance on the assembled DNA duplexes of the origami11 (Figure 3d).

While building on previous approaches to self-assembly of DNA nanostructures, the DNA origami technique has multiple advantages: (i) It accomplishes highly specific topologies with a spatially addressable resolution comparable to that accomplished by AFM or STM surface manipulation. (ii) The well-formed, redundant self-assembly is relatively insensitive to varying stoichiometric ratios of the staples, eliminating the need for intermittent purification steps and resulting in higher yields. (iii) Multiple nanostructures may be obtained simultaneously and possibly further assembled into larger structures with high fidelity. (iv) Creating arbitrary shapes that are largely unrestricted by symmetry considerations is relatively straightforward11.

The DNA origami method has so far provided a scaffold for many applications including forming the track for molecular nanorobots47, constructing ordered molecular assembly lines48, 49, and assembling components of putative nanoelectronic circuits50, 51. The scope and limitations of these applications are enumerated throughout.
Figure 1.
Chemical structures of nucleic acids. Nucleotide backbone structures for (a) DNA (left), RNA (right), (b) PNA, and (c) LNA. The right side of panel a also indicates the backbone hydrolysis reaction of RNA, where “AH⁺⁺” and “B” are an acid and base catalyst, respectively. (d) Structure of a double-stranded nucleic acid incorporating the four principal natural nucleobases A, T/U, G, and C, as well as artificial P:Z base pair.
Figure 2.
DNA crossover motifs. The (a) double and (b) triple crossover motifs are demonstrated by the red strand.
**Figure 3.**
The DNA origami method. (a) The single-stranded DNA “scaffold” strand (purple) is folded and held in place by specifically hybridizing “staple” strands (red and green). (b) Resultant rectangular origami tile once all the staples have bound to the scaffold. (c) Triple crossover motifs demonstrated by the staples interacting with the scaffold. (d) 5’ ends of the staples are extended to create overhangs, which then can be decorated with partially complementary oligonucleotides (purple).
Figure 4.
Current state-of-the-art of nucleic-acid-based nanotechnology. (a) Molecular nanorobot walking along a track generated by decorating a rectangular origami with leg footholds (substrates); below, tile (boxed) imaged using AFM over time, indicating spider movement. (b) Multistep synthesis of an organic compound mediated by a deoxyribozyme. (c) Junctions of carbon nanotubes and origami to create a field-effect transistor. (d) Transfer of photonic energy along a DNA template using FRET. (e) Triangular DNA origami arranged using electron beam lithography. (f) DNA nanotubes arranged using soft lithography. Each panel was reproduced with permission from the respective publisher.
Figure 5.
Hierarchy of areas impacted by DNA origami technology. The foundational DNA scaffolds (green) are used to create devices (blue) for a broad variety of applications (orange) that can be combined to enhance numerous emerging interdisciplinary fields (purple). Disclaimer: the nucleic acid nanotechnology field will not be limited by our current vision.
Figure 6. Progression in DNA and RNA nanotechnology. In nature, DNA forms structures such as the Holliday junction, which has inspired scientists to create more complex structures such as the rectangular DNA-origami tile. In the future, such tiles may be used in fields including nanoelectronics as a scaffold for plasmonic circuit components to generate circuits that mimic neuron behavior. As the simple example in the figure depicts, a new circuit connection (purple) may be strengthened by repeated cooperative stimuli from excitatory pathways (green and blue) and hindered by the stimuli from an inhibitory pathway (orange). In nature, RNA plays a catalytic role in peptide bond formation by the ribosome, arguably the most important enzyme on earth. The catalytic and exquisite molecular recognition activity of RNA is exploited in (deoxy)ribozyme computing, which may be used in the future for complex therapeutic nanomedicine applications. For instance, a drug carrier, specifically delivered to a diseased cell through endocytosis triggered by binding to a protein on the cell surface, opens after entering the cell to release the drug. The contents of the drug carrier include a miRNA mimic that causes repression of a specific protein that otherwise
would inhibit the surface marker, resulting in a cell that becomes more receptive to the drug carriers.