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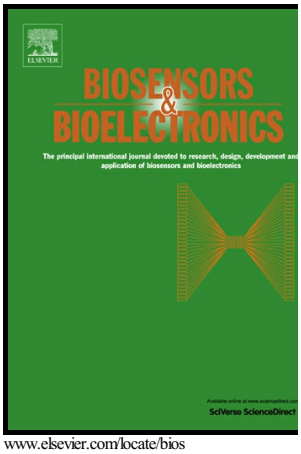

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Switchable bioelectronics

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Abstract

We review the rapidly emerging field of switchable interfaces and its implications for bioelectronics. We seek to piece together early breakthroughs and key developments, and highlight and discuss the future of switchable bioelectronics by focusing on bio-electrochemical processes based on mimicking and controlling biological environments with external stimuli. All these studies strive to answer a fundamental question: "how do living systems probe and respond to their surroundings? And, following on from that: "how one can transform these concepts to serve the practical world of bioelectronics?" The central obstacle to this vision is the absence of versatile interfaces that are able to control and regulate the means of communication between biological and electronic systems. Here, we review the overall progress made to date in building such interfaces at the level of individual biomolecules and focus on the latest efforts to generate device platforms that integrate bio-interfaces with electronics.

Keywords: Switchable bioelectronics, stimuli-responsive bio-interface, controllable catalysis.

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1. Introduction

The interface between the biological and electronic worlds has generated a new branch of science to respond to long-standing fundamental questions about living systems (Turner *et al*. 1987; Willner and Katz 2006). Milestone discoveries in last century in both biotechnology and electronics have facilitated the engineering of bio-interfaces and shown that electronics

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can be integrated with biotechnology to build new sensors, drive synthetic reactions and generate power (Aston and Turner 1984; Cass *et al.* 1984; Clark and Lyons 1962; Clark *et al.* 1953; Turner *et al.* 1982). These advances have opened up a new era of "bioelectronics" which helps us to understand the basis of various biochemical and/or biophysical events in living systems and to build functional bio-devices (Berggren and Richter-Dahlfors 2007; Noy 2011; Willner and Willner 2001). Moreover, the tremendous contribution made by advances in electronic components to biochemical and biotechnological progress cannot be underestimated. This has been achieved by the design and development of new methods or engineering new biomaterials with various functionalities to advance the level of bioelectronics (Joachim *et al*. 2000). In particular the top-down approaches in electronics, developed recently with the help of advances in materials science, provide for miniaturisation of each and every element and make possible the pocket-size and wearable devices that now predominate in our future thinking (Maloberti 2011; Mayergoyz and Lawson 2012; Turner 2013). Ultimately, the harnessing of these materials yields a broad platform of functional units for integration as bioelectronic interfaces (Benson *et al.* 2001; Du *et al.* 2014; Heiduschka and Thanos 1998; Hill *et al.* 2005; Hook *et al.* 2009). The last few decades have witnessed the transformation of these lab-scale bioelectronic interfaces into real commercial bioelectronic devices (Katz 2014; Zhirnov and Cavin 2015). To date, several types of bio-device have contributed towards practical realisation of this vision, such as the amperometric blood glucose biosensor (Cass *et al.* 1984; Newman and Turner 2005), real-time affinity sensors (Daniels and Pourmand 2007; Hunt and Armani 2010; Luo and Davis 2013), sensors based on surface plasmon resonance (Lee *et al.* 2015; Liedberg *et al.* 1983; Wang and Tang 2015), and field-effect devices (Lundstrom and Winquist 1987; Shen *et al.* 2014). There have, of course, been many important challenges and technical hurdles in the research and development of bioelectronic devices, whether at the lab-scale or in commercial applications. For example, barriers to charge transport in biological matrices and electron transfer between redox proteins

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hinder the construction of efficient communication between biological materials and electronic components, and scaling up systems mimicking the complexity of biological systems for mass production, still await improved solutions. Many elegant discussions, reviews and valuable hypotheses have been published (Berlin *et al.* 2001; Bocharova and Katz 2012; Davis and Higson 2005; Gray and Winkler 2003; Katz 2010; Katz *et al.* 2012; Katz *et al.* 2013; Katz and Pita 2009; Scott *et al.* 2009; Shleev *et al.* 2005) and researchers around the world have continuously strived to overcome these problems, but many of these ideas deserve separate discussion and fall out of the scope of the current review.

Here, we focus on the recently emerging field of switchable interfaces and their implications for bioelectronics. We seek to piece together early breakthroughs and key developments, highlight and discuss the future of switchable bioelectronics by concentrating on recent studies which have focused on understanding the chemical or physical mechanisms in living systems, and find plausible explanations for bio-electrochemical processes based on mimicking and controlling biological environments with external stimuli. But first, we ponder the question: "why do we need switchable bioelectronics?"

Why switchable bioelectronics?

All living systems, from prokaryotes to the more complex life forms of animals, have the innate ability to adapt themselves reversibly to their surroundings, for example, heat-shock in bacteria (Requena 2012), change of wettability and/or adhesion skills (gecko, mussels) (Autumn *et al.* 2000; Lee *et* al. 2007), and control and regulation of the transport of ions or molecules (cells) (Nilius and Droogmans 2001). In contrast, man-made materials/devices are essentially static; their function is essentially invariable and predetermined by the immobilised bio-molecule in both form and function. Only very recently have efforts begun to change the *status quo* from static to dynamic (so called "switchable") enabled by advances in

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material science and synthetic chemistry (Alarcon *et al.* 2005; Roy *et al.* 2010; Stuart *et al.* 2010). The ultimate goal of these studies on switchable bio-interfaces is to develop a range of novel technologies that merge the functions and properties of biological systems, such as bio-catalysis, control of ion transfer and molecular recognition, with the pervasive, programmable world of electronics. Aside from being important for fundamental studies, switchable materials have several advantages over their static counterparts, such as facilitating selective regulatory control to allow biomolecules to be switched on and off in response to specific stimuli and providing the opportunity for mechanistic studies of the pathways by which biomaterials can sense, integrate and respond to changes in their environments. Emerging applications include "smart" control of adhesion of biomolecules such as cells or proteins for tissue engineering, controllable interaction of biomolecules and ions with responsive surfaces for bio-separation and switchable bio-catalysis for the construction of energy efficient biofuel cells, to name just a few (Hou *et al.* 2011; Lowik *et al.* 2010; Lutz 2011; Mart *et al.* 2006; Mendes 2008; Roy and Gupta 2003; Xia and Jiang 2008).

Switchable bioelectronics comprising stimuli-responsive surfaces are essential for the construction and realisation of highly engineered bio-interfaces, which have the capability to alter their macroscopic properties on demand (Sun and Qing 2011; Tanaka and Chujo 2012; Yoshida 2005). Surfaces equipped with molecular cues that mimic certain aspects of the structure or function of natural environments, offer new opportunities to change their physical and chemical properties or convert chemical and biochemical messages into electrical signals, and *vice versa* (Buenger *et al.* 2012; Chen *et al.* 2013; Domachuk *et al.* 2010; Shanmuganathan *et al.* 2010). Over the past decade, a variety of approaches have been pursued to create various switchable bioelectronic interfaces capable of changing their properties in response to several different stimuli (Marsden and Kros 2009). For instance, various signal-responsive materials can positively respond to light irradiation, changes in

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temperature or pH, and electric or magnetic fields. Polymers (Gil and Hudson 2004; Qiu and Park 2001), nanoparticles, (supra) molecules or self-assembled monolayers (SAM) (Rybtchinski 2011; Xin and Hao 2010) can be employed or modified on various nanostructured materials or electrode surfaces to produce switchable interfaces to control and modulate enzyme catalysis, sensing of analyte or binding and release of cells and proteins in response to external stimuli.

Switchable systems covered in this review are categorised into two groups stimulated by either: external physical or external chemical stimuli. The former stimuli include light, temperature, applied potential and magnetic fields, and the latter involves addition of some specific chemicals, enzymatic interactions, changes in pH or ionic strength fluctuations. Each section concludes with a discussion of practical applications such as biosensing, biocatalysis, biofuel cells and other possible applications in bioelectronics.

2. Physically stimulated bioelectronic systems

2.1. Light-switchable bio-interfaces

Switchable interfaces that can positively respond to different external stimuli have numerous advantages in diverse areas such as biosensing, organic electronics and microfluidics (Beebe *et al.* 2000; Dong *et al.* 2006; Olsen and Segalman 2008; Orgiu and Samori 2014). Despite the fact that light-switchable interfaces face some problems, such as difficulties in achieving high photo-stationary states, lack of full reversibility and low resilience, the high spatial and temporal precision offered by this approach, together with the absence of side-reactions, favours light as the preferred stimulus. Light switchable interfaces are particularly well suited to information processing in optoelectronic devices such as flip-flop memory units (Chatterjee *et al.* 2006; de Ruiter and van der Boom 2011) or controllable biocatalysis in enzyme cascade reactions (Hu *et al*. 2012; Roy and Ulijn 2010).

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Many different light responsive molecular systems have been employed as light-harvesting elements to construct light-switchable interfaces, including azobenzenes (Schumers *et al.* 2010), diarylethenes (Browne and Feringa 2009), spiropyrans (Florea *et al.* 2012), fulgides (Yokoyama 2000), azulenes (Amir *et al.* 2011), overcrowded alkenes (Feringa *et al.* 2000) and others (Feringa 2001) (**Figure 1**). These switchable elements have been used in different forms such as individual molecules or monolayers (SAM), covalently bound to polymer backbones or conjugated with nanoparticles to construct different forms of interface (Bunzli and Piguet 2005; Jeong and Gutowska 2002; Qiu and Park 2001). In addition to synthetic molecules, some natural light-sensitive proteins (photoreceptor proteins) such as rhodopsin, phytochromes (in plants) or bacteriorhodopsin in some bacteria, have also been employed to construct light-driven bioelectronic devices, especially in the form of proton pumps, whereby light energy is captured to move protons across a membrane (Balashov *et al.* 2005; Blaustein and Lederer 1999; Nagel *et al.* 2002).

Different mechanisms are involved in the switching process depending on the molecules in use. The interface constructed by employing azobenzene moities, for example, works by a reversible transition between two isomeric conformations “trans-to-cis” upon ultraviolet (UV) light irradiation and allows controllable accessibility of the bio-interface for the diffusion of analyte and/or electroactive species, depending on a change of volume and permeability of the interface. However, the yield of the isomerisation is usually far from quantitative, which hinders the use of azobenzenes as a bioelectronic interface (Schumers *et al.* 2010). The mechanism in diarylethenes, on the other hand, depends on a change in electrochemical properties upon photoisomerisation, being redox inactive in one state and exhibiting reversible redox activity in the alternative state, thus permitting mediated reactions or amplification as a result of the interfacial redox transformation. The change in electrochemical properties of diarylethenes is a consequence of the different rates of electron

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transfer reactions in the two different forms resulting from disproportionation (Browne and Feringa 2009). Even though they undergo only a small change in molecular conformation, they usually possess high resistance to photo-degradation, which makes them superior in terms of stability to other light-switchable counterparts. Spiropyrans, both in the form of assembled-monolayers or conjugated with a polymer backbone, show reversible isomerisation comprising protonation-deprotonation of the isomeric states, which results from heterocyclic C-O bond cleavage, or cyclic ring opening leading to a charged interface. The closed and open ring isomeric states are called as spiropyran (SP) and merocyanine (MC), respectively. The charge separation in the MC form creates a large dipole moment relative to the SP state and also the significant structural difference occupies more volume than the SP form in addition to a lower packing factor (Klajn 2014).

Figure 2 illustrates the use of light-responsive spiropyran molecules embedded in a mechano-sensitive channel protein of large conductance (MscL from *Escherichia coli*) as a molecular valve to provide external control over transport through the channel using the electrophysiological patch-clamp technique, which allows the study of single multiple-ion channels in cells (Kocer *et al.* 2005). MscL plays an important role as a safety valve for many cells (Levina *et al.* 1999). In certain cases, such as osmotic downshifts in bacterial cells or instant influx of water, which results high tension the membrane, these mechanosensitive channels open to around 3 nm to allow the flux of ions and proteins to save the cells from lysis (Cruickshank *et al.* 1997). The functionality of the resulting nanovalve can be examined by measuring the ionic current level of single molecules flowing through the modified channel in a patch-clamp experiment. Single-channel recordings are performed at +20 mV without any pressure gradient, and channel openings are shown as upward currents. The patch is sequentially illuminated with UV and visible light by alternating the filter in the light source. This technique offers considerable promise as a means to understand ion transport

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phenomena in cells and also utilisation of the nanovalve as an externally controlled ion pump for various applications.

In another exciting study, Noy *et al.* showed the use of a biological regulation mechanism in a light-driven bioelectronic device based on a one-dimensional lipid bilayer on a microfabricated Si nanowire field-effect transistor (SiNW FET) (**Figure 3**a) (Tunuguntla *et al.* 2015). In this device, a lipid bilayer containing bacteriorhodopsin protein, bR, covered the nanowire surface. Upon exposure to green light (λ_{max}= 560 nm), the protein covered SiNW device delivered a large response (red curve, Figure 3b) due to the strong absorbance of light by the protein resulting in a multistate photo-cycle that moves the proton across the membrane. After the system was switched off, the current gradually returned to its original level without any significant loss. In the absence of the protein, the SiNW device did not produce the photocurrent (black curve, Figure 3b). Based on the same principle, researchers also developed a light-powered biocapacitor based on another photoreceptor protein, proteorhodopsin (pR) with a modified alumina nanochannel to mimic membrane ion-pump proteins and ion-channel proteins (Rao *et al.* 2014). In the biocapacitor system, while pR generates potential by light irradiation, nanochannels provide a regulated resistance resulting in charging-discharging actions as in typical electrical double-layer capacitors. The capacitor-like behaviour of the system was studied by measuring photocurrent duration time and nanochannel resistance. These studies and techniques offer interesting opportunities to construct bioelectronic devices, such as biosensors or neural interfaces, powered by proton gradients and with tunable performance.

In addition to the aforementioned bioelectronic devices, switchable interfaces have been employed to control and modulate the communication between biomolecules and/or a redox probe at an electrode surface. A variety of modulated electrode surfaces based on light-responsive materials have been developed and these are discussed in the following section

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(Doron *et al.* 1996; Mandler and Willner 1984; Willner and Maidan 1988; Willner and Rubin 1996; Willner and Willner 1991).

Willner *et al.* have demonstrated the reversible binding of anti-dinitrophenol (anti-DNP) antibody (AB) to an electrode surface via a switchable activation-deactivation mechanism (Willner *et al.* 1994). In this study, the amperometric response of a fero/ferri cyanide redox probe, $[Fe(CN)_6]^{3-}$ / $[Fe(CN)_6]^{4-}$, was reversibly controlled depending on the state of the light-responsive molecular unit on a gold electrode. When the state of the responsive molecule is merocyanine (i.e. carries both negative and positive charge) anti-DNP cannot approach the electrode surface due to the lack of affinity and the redox probe can easily diffuse through to the electrode surface, which results in the generation of high electrochemical signals. However, when the system is exposed to visible light, isomerisation occurs on the modified surface with the merocyanine state switching to the spiropyran, neutral state, and anti-DNP can easily interact and insulate the electrode surface. The ease of interaction is explained by the presence of two NO_2 groups in the spiropyran state, which act as an antigen for the anti-DNP antibody. The interaction is similar to an antigen-antibody reaction and hinders the diffusion of redox probe through to the electrode surface and the electrochemical signal gradually decreases upon exposure to visible light (**Figure 4**a). Besides electrochemical analysis, the light switchable activation-deactivation of the electrode surface was extensively studied using a quartz crystal micro balance (QCMD) and by surface plasmon resonance (SPR) techniques. A similar trend was observed when pyrroloquinoline quinone (PQQ) was employed as a redox probe on the same type of electrode surface (Katz and Willner 1995). The strong electro-static interaction between negatively charged PQQ and a positively charged MC-modified electrode resulted in efficient interfacial electron transfer. However, electrochemical reduction was suppressed when the electrode surface was exposed to visible light due to the loss of the electrostatic interaction between the redox probe and the surface. The ability to switch surface charge upon exposure to different wavelengths of light,

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provides the ideal platform to study electrochemical reaction kinetics (Frumkin effect) (Batrakov and Damaskin 1975) of different switchable interfaces including electron-transfer mediators, enzymes and catalytic nanoparticles. A more detailed description of the photo-control of different types of surfaces and their interaction with various biomolecules can be found elsewhere (Cole *et al.* 2009; Xia and Jiang 2008; Yoshida *et al.* 2006; Zhang *et al.* 2012).

2.2. Electrically-switchable bio-interfaces

Electrical control and regulation of biomolecular interactions at engineered interfaces presents considerable opportunities for understanding biological processes in living systems and also for developing highly efficient substrates and devices for a wide range of biomedical applications (Mendes 2008; Skorb and Andreeva 2013). The main advantage of electrically-switchable bio-interfaces is that they provide direct control over the bio-system (non-invasive) and are quantitative. A number of different electroactive groups have been successfully employed to obtain switchable interfaces to control interactions of proteins, peptides, DNA and cells, with the electrode surface modified by various molecules having switchable characters in the form of SAMs and polymeric films (Lahann *et al.* 2003; Mendes 2013; Pranzetti *et al.* 2014; Pranzetti *et al.* 2012; Sun and Lahann 2009).

One of the main research targets in this field is improved understanding of the adhesive behaviour of biological systems such as cells and bacteria to man-made surfaces, which is of crucial importance to fabricate functional biomaterials for marine biofouling, tissue engineering and *in vivo* diagnostic tools. To elucidate these challenges, dynamic interfaces are required with a particular focus on the possibility of creating switchable interfaces to control surface properties on-demand. Recently, Pranzetti *et al.* demonstrated an electrically-switchable interface consisting of a SAM to control the early stage of bacterial

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adhesion (Pranzetti *et al.* 2013). The switching mechanism is based on controlling the charge of the end group in the layer via an applied potential (**Figure 5**a). The adhesive properties of the bacterium was tested under open-circuit conditions by sequentially applying positive (+0.25 V) and negative (-0.25 V) potentials for a certain period of time. Confocal images clearly showed that the adhesion of bacteria (*Marinobacter hydrocarbonoclasticus*) was successfully controlled at three different applied electrical potentials (Figure 5b). However, it was observed that the attachment of bacteria became irreversible when the switching time increased (20 min.). Even though the method had some drawbacks with respect to repeatability, this study clearly shows that real-time switching of non-specific cell attachment is possible using an applied potential as an external stimulus, which is highly desirable for several biomedical applications, such as genetic expression systems and the control of fouling by biomaterials.

A similar potential-induced electrochemical switching process has been investigated to measure *in-situ* and real-time conformation change of end-tethered dsDNA on a gold electrode surface (Spuhler *et al.* 2010). Here researchers sought to combine the electrical orientation of surface-bound DNA with an optical technique to detect an oligonucleotide target based on the switchable response of surface confined DNA molecules upon an applied potential. It has long been known that DNA molecules are negatively charged due to the phosphate groups in the sugar-phosphate backbone, which makes DNA molecules sensitive to electrode potential. A DNA-modified electrode surface can easily be manipulated by changing the polarity of an applied potential from positive to negative; in this way the molecules can readily be driven away from or pulled towards the electrode surface. At a negative applied potential, the DNA molecules tend to stand straight on the electrode surface, whereas when a positive potential is applied to the electrode, the DNA molecules tend to lay flat (**Figure 6**a). In this study, the detection mechanism was based on fluorescence intensity

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changes relative to the orientation of the DNA molecules to the surface. When a positive potential was applied to the electrode, the DNA molecules began to bend, which quenched the fluorescent signal of Cy3. However, when the potential moved to the negative, the DNA molecules tended to stand straight and produced higher fluorescent signals. The same trend is observed when hybridisation of a target sequence with its complimentary ss-DNA occurs on the surface, which amplifies the fluorescent signal (Figure 6b). This conformational switching of DNA offers a convenient and sensitive way to understand binding kinetics and the affinity constant of DNA-based sensor systems.

Sustained efforts to provide electrical contact between redox enzymes and electrodes has underpinned developments in the areas of biosensors, bioelectrochemically catalysed chemical transformations and the advancement of biofuel cells. The so-called "wiring" of an electroactive interface to redox proteins or the incorporation of redox proteins in an electrochemically active polymer medium are common ways to provide electrical contact and use redox enzymes efficiently. These approaches have facilitated the design of electrochemically-controlled redox functionalities in biomolecular systems for information storage, processing systems and new bioelectronic devices. Katz *et al*. reported a hybrid copper-poly(acrylic acid) (PAA) polymer-modified electrode which has switchable redox activity depending on the position of Cu ions or particles in the polymeric medium (Katz 2010; Katz and Willner 2003). When copper aggregates near to the electrode in the polymer matrix (Cu^0 nanoparticles), the system provides metallic conductivity across the polymer film, whereas the system turns to a nonconductive state when the polymer contains electrochemically oxidised Cu^{2+} ions. Based on this principle, an electro-switchable and tunable biofuel cell was developed based on the oxidation of glucose. The biofuel cell was tuned and switched on and off by applying reductive (-0.5 V) and oxidative potentials (+0.5 V) to the electrode surface, which reversibly yielded the conductive Cu^0-PAA (on state) or the

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non-conductive Cu^{2+}-PAA (off state) forms of the polymeric film. Another interesting approach to wiring enzyme (glucose oxidase, GOx) and/or enzyme cofactor (FAD) to the electrode surface using an electrochemically-stimulated molecule, was demonstrated using electron-accepting rotaxane with cyclobis(paraquat-*p*-phenylene) macrocycle on a diaminobenzene donor-site containing "molecular wire" (**Figure 7**) (Katz *et al.* 2004b). The tetracationic macrocycle of rotaxane is used as redox relay and a mediator, and is able to control bioelectrocatalysis by shutting the electrical transport on and off upon application of a potential. This offers an important way to obtain tunable operation of the output signal, which facilitates the design of power on-demand approaches for biofuel cells and enables tailoring of enzyme electrodes for various bioelectronic applications.

2.3. Temperature-switchable bio-interfaces

Temperature switching is an alternative technique which is commonly employed to control and regulate the function of interfaces for various bio-applications (Ge and Liu 2013; Kulkarni and Biswanath 2007; Tirelli 2006; Weber *et al.* 2012). While SAMs on electrode surfaces have been used in some specific applications, the use polymers having temperature sensitivity dominates most of the reports of thermo-responsive interfaces. Various forms and morphology of polymers, including polymer brushes, hydrogels or layer-by-layer films, are widely preferred due to their ease of preparation and modification on the electrode surface (Hatakeyama *et al.* 2006; Kharlampieva *et al.* 2009). It is worth mentioning that the morphology of polymeric structures is primarily responsible for the behaviour of the interface. The rate of switching, diffusibility of ions/analyte/redox species, resistance to ionic strength and fatigue failure as a result of the number of times the polymer is switched varies from one form to another, even if the same polymer is used.

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A significant body of work has recently been established using different temperature-responsive polymeric bio-interfaces which reversibly control molecular recognition events, cell attachment or biocatalysis (Gil and Hudson 2004; Hou et al. 2011; Kudina et al. 2014; Li *et al.* 2011a; Li *et al.* 2011b; Li *et al.* 2010; Li and Cao 2014; Meyer and Chilkoti 1999; Xiao *et al.* 2014; Zhang *et al.* 2010). The most commonly used polymeric system is poly(*N*-isopropylacrylamide- PNIPAAM), a thermo-responsive polymer that possess a lower critical solution temperature of 32 °C (LCST) and undergoes a phase transition owing to changes in temperature and solvent quality, which is attributed to an alteration in the hydrogen bonding interactions of the amide group (Toomey *et al.* 2004; Wei *et al.* 2006). In contrast to other temperature-responsive polymers, the phase transition range lies conveniently between body temperature and room temperature, which make PNIPAAM the superior choice, especially for biological applications. Below the LCST, the polymer chains are in an extended and solvent-swelled conformation, whereas when the system is heated up the polymer chains undergo a phase transition which yields a collapsed morphology and a hydrophobic interface (Ju *et al.* 2001; Rzaev *et al.* 2007).

The sharp change in the solution properties of PNIPAAM near to physiological temperature provides a useful platform to control reversible adsorption and release properties of biomolecules on demand. As with cell adhesion, changes in surface properties governed by the phase transition of the polymer can easily mediate reversible bioadhesion. The programmable adsorption and release of proteins in microfluidic devices has recently been developed. One recent study described a 4 nm thick PNIPAAM layer in a microfluidic device with a network of gold heater wires on a Si_3N_4 membrane (**Figure 8**a). Each gold wire could be selectively heated or cooled, which provided reversible control to capture or release biomolecules, such as myoglobin, cyctochrome C, bovin-serum albumin (BSA) or haemoglobin, on demand in less than 1 second (Figure 8c). This study shows that the rapid

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response characteristics of such devices can be manipulated for proteomic functions, including pre-concentration or could be used as a protein trap for programmable protein separations in integrated fluidic chips (Huber *et al.* 2003).

Temperature-responsive polymers have also been widely used as a switchable interface for biocatalytic applications. There are three main approaches that have been employed to generate thermo-responsive interfaces containing PNIPAAM. In the first, thermo-responsive polymers are conjugated with redox groups and used as an electron mediator for enzyme-based catalytic reactions (**Figure 9**) (Nagel *et al.* 2007). In this method, ferrocene-modified PNIPAAM co-polymer bearing oxirane groups were covalently bonded to an amine terminated gold electrode via "grafting". Here, the co-polymer acts as a mediator for controllable electron transfer between the cofactor, PQQ, of glucose dehydrogenase (sGGH) on the electrode surface. At temperatures below the LSCT, the co-polymer is in the swollen state and provides optimal conditions for mediated electron transfer, whereas when the co-polymer undergoes phase transition and the polymer chains show a tendency to shrink, communication between the enzyme and the cofactor is hindered and efficient electron transfer is supressed. One of the possible disadvantages of extension of this work might be a change in the LCST after modification of the PNIPAAM polymer with another molecule intended to add more functions, such as redox activity or another switching character (dual switchability). A small change in the composition of PNIPAAM on addition of a foreign molecule may increase or decrease the LCST of the polymer and render it useless for biological applications (Gil and Hudson 2004). In order to overcome such challenges, other methods have recently been developed. In an improved approach, amine-terminated PNIPAAM polymer (without any coupling to different molecules) was assembled electrostatically on a sulfonated electrode surface containing graphene, to develop an auto-switchable bio-interface that is capable of positively responding to thermal excitation with the help of a nanostructured "zipper-like" interface (**Figure 10**) (Parlak *et al.* 2014). The zipper-

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like design contains sulfonated graphene as donor and PNIPAAM as an acceptor branch, which are assembled together based on stoichiometric donor-acceptor interactions. The novelty in this work is that the system responds positively to temperature change rather than negatively, as in previous studies. Here, the modified electrode generates a higher signal at 40 °C relative to 20 °C, due to the ease of access of the redox probe to the electrode surface or of the bio-substrate to the enzyme (co-immobilised to electrode surface with sulfonated graphene) to facilitate bioelectrocatalysis. In addition to subversion of the donor-acceptor interaction between the polymer and graphene, the polymer morphology changes to a shrunken conformation, which increases the available surface area for efficient electrocatalysis. In a third approach, researchers employed layered double hydroxide (LDH) nanoparticles and PNIPAAM to modify an electrode surface using a layer-by-layer (LbL) technique. The designed system also had positive responding ability towards the oxidation of glucose in a similar way to the second approach described above. In another interesting study, on/off switchable catalysis by a smart enzyme-like imprinted polymer has been developed using positively responding PNIPAAM containing a p-nitrophenyl phosphate-imprinted network (Li *et al.* 2011c). Here, the network system shows temperature dependent hydrophobicity/hydrophilicity. The polymeric network exhibited vigorous catalytic activity for the hydrolysis f p-nitrophenyl acetate due to its hydrophilic networks, which enabled access to the imprinted framework. In contrast, at relatively higher temperatures (such as 40 °C) the same polymer network exhibited poor catalysis resulting from increased hydrophobicity, which inhibited access to the imprinted sites. This work clearly shows that this novel imprinted polymer exploiting thermosensitive poly (N-isopropylacrylamide) networks could be used to control and regulate switchable catalysis, unlike any previously reported imprinted polymers. The examples reported herein demonstrate the versatility and broad potential of polymers having phase transition under physiologically convenient conditions of temperature.

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2.4. Magneto-switchable bio-interfaces

Magnetic nanoparticles with different size and shape have been precisely synthesised and used for various applications, including magnetic resonance imagining (MRI), targeted drug delivery, fuel-driven artificial nanomotors/carriers and magnetic separation of DNA, proteins and cells. They find wide utility due to their low toxicity and ease of processing (Gupta and Gupta 2005; Wang and Manesh 2010; Willner and Katz 2003). The use of magnetic fields to switch on/off electrochemical reactions, upon translocation of external magnet in order to generate an "on-demand bio-interface", is also another interesting and recently growing research area (Dosev *et al.* 2007; Gangopadhyay and De 2000; Jimenez *et al.* 2008; Lutz 2011). The combination of redox molecules with magnetite particles is the general way to form magnetically-controllable electrochemical systems. To date, many different types of electroactive relay, including quinones, ferrocyanide and ferrocene derivatives, have been employed by modifying the surface of magnetic nanoparticles, mainly iron oxide (Fe_3O_4), in order to control electrochemical processes (Willner and Katz 2003).

The general mechanism of magneto-switchable electrocatalytic interfaces is described in **Figure 11**a-b (Willner and Katz 2003). Here, the attraction of redox-modified nanoparticles to the electrode surface by an external magnet, which is positioned under the electro-chemical cell, activates the communication between the electrical contact of the redox unit and the electrode, which results in switching the system on. However, when the position of magnet is moved in the opposite direction (above the electrochemical cell), the magnetic particles are pulled away from the electrode surface and suspended in the solution, which switches off the electrochemical activity of the system. This simple switching mechanism has been used to control various biocatalytic reactions mediated by redox-species. The bio-electrocatalytic oxidation of glucose in the presence of glucose oxidase coupled with primary electrochemical reaction of the diffusional redox species has also been shown by controlling the electrode

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interfacial properties (Jimenez *et al*. 2008). Here, magnetic $CoFe_2O_4$ gold core-shell particles were used to generate conducting nanowires in the presence of an external magnetic field. The applied magnetic field directs all core-shell particles in the solution and assembles them in a wire structure that generates an array of nanoelectrodes, extending the electrode support and increasing the effective surface area (Figure 11c). This assembling process enhances the electrochemical response for a diffusional redox probe around five fold (Figure 11d). The process is switched off when the external magnet is removed, resulting in disassembling of the nanowires on the surface and all core-shell particles return to a free-state and suspend into the solution. This approach is interesting for both control of bioelectrocatalytic reactions and directed self-assembling of nanoparticles in a confined space.

Another way of controlling electrode interfacial properties is to generate switchable and selective interfacial electrochemical reactions employing magnetic nanoparticles coated with hydrophobic groups (Katz *et al.* 2004a). The interfacial properties of a functionalised surface with redox molecules can be controlled by using a two-phase systems consisting of aqueous buffer and organic solutions (toluene). Here, hydrophobic nanoparticles are suspended in the organic phase on top of an aqueous phase, which contains glucose and glucose oxidase as diffusional components (**Figure 12**). The attraction of magnetic nanoparticles to form a hydrophobic layer on the electrode surface results in inhibition of the bioelectrochemical process, due to their insertion between the biocatalyst and the surface-bound redox molecules. In other words, the bioelectrocatalytic oxidation of glucose is blocked due to the directed aggregation of magnetic nanoparticles on the electrode surface, preventing electrical contact between the solubilised GOx and the functionalised electrode. However, when the magnetic field is moved to the opposite position (top of the cell) the hydrophobic nanoparticles are retracted from surface, returned to the organic phase and make the surface available for mediated electron transfer for the oxidation of glucose.

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Even though development of magnetically-switchable interactions was one of the first examples of stimuli-responsive applications, it has not yet lived up to expectation. Nevertheless, the use of functional magnetic particles may still provide a comprehensive solution in the development of bioelectrocatalytic systems. Assembling of particles on a surface is a powerful way to concentrate and separate the components involved in an analytical processes and localisation of redox-functionalised magnetic particles can enable the electronic transduction of the biosensing event.

3. Chemically stimulated bioelectronic systems

3.1. Chemically switchable bio-interfaces

The design and development of chemically and biochemically-responsive interfaces has recently evolved and started to be used to generate novel reagentless and re-usable biosensors, smart adhesive surfaces and delivery systems with controllable functionalities (Bocharova and Katz 2012; Katz *et al.* 2013; Privman *et al.* 2009; Tam *et al.* 2008b). The addition of different chemical/biochemical species, such as enzyme and DNA molecules, or changing the reaction environment with respect to pH or ionic strength, have similar impact on the control and regulation of many bioelectrocatalytic reactions as external physical stimuli.

One promising development is in the area of electrochemical DNA-based sensor technology, or so called "e-DNA". There are a couple of different approaches that have recently been developed in this area. These reversible sensor systems work in a similar way to optical molecular beacons, where oligonucleotide probes become fluorescent upon hybridisation with target DNA molecules. In e-DNA sensors, the sensing mechanism is based on the variation of electron transfer dynamics as a result of structural rearrangement initiated by hybridisation on electrode surface. The first e-DNA sensor approach comprised a surface confined DNA labelled with a ferrocene-based redox probe as a hybridisation sensing element

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(**Figure 13**a) (Fan *et al.* 2003). In the absence of complementary DNA, the redox probe locates close to the electrode surface, resulting in the generation of a strong faradaic current. However, introducing a complementary nucleic acid sequence induces a large conformational change to the thermodynamically more stable, rigid rod-like form of this surface-confined DNA structure, which in turn significantly alters the electron-transfer tunnelling distance between the electrode and the redox label. The resulting conformational change in electron transfer efficiency is readily measured by electrochemical techniques at target DNA concentrations as low as 10 pM. One of the drawbacks of this signal-off sensor is the limitation of signal suppression, which technically cannot be more than 100%. To overcome this difficulty and increase the sensitivity, signal-on devices have been developed using ferrocene-labelled triblock macromolecules consisting of oligonucleotide-poly(ethylene) glycol-oligonucleotide (Immoos *et al.* 2004). The electrochemical signal is generated due to the large conformational change induced by the hybridisation of target molecule with both the bottom and top sequence of the surface-confined macromolecules (Figure 13b). In a similar fashion, Xiao *et al.* developed another interesting way to increase the sensitivity using methylene-blue (MB) modified duplex DNA, which is able to change its conformation on introduction of target DNA resulting in significant strand displacement (Figure 13c) (Xiao *et al.* 2006). In the absence of target, the duplex DNA is able to keep the redox-probe away from the surface and hinder electrochemical communication between MB and the electrode, thus limiting the observed redox current. When complementary DNA is introduced, enhancement of the redox signal is observed due to the formation of the single-stranded element in the signalling probe, which relocates the MB close to the surface, and efficient electron-transfer occurs. These two signal-on approaches offer many advantages over signal-off device in terms of analytical performance, such as high sensitivity and a low detection limit of down to 0.4 pM. Using similar approaches, researchers have recently started to extend the applicability of these techniques to produce sensors using aptamers against diverse target such as dyes,

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proteins, peptides, small aromatic molecules and antibiotics (Lu *et al.* 2008; Peng *et al.* 2009; Xiao *et al.* 2005).

An intriguing method to transduce the cellular activities of mammalian cells into measurable electronic signals has recently been developed by Collier and Mrksich (2006). In this study, the authors describe an important approach by integrating cellular activities and electrical processes in an underlying substrate. The concept of molecular communication between cells and a material surface based on an enzyme-responsive, self-assembled monolayer is introduced. They showed that a self-assembled monolayer of 4-hydroxyphenyl valerate can be switched enzymatically by cutinase from a redox-inactive to a redox-active state (**Figure 14**). The cells were engineered with a cell surface chimeric receptor that presents the non-mammalian enzyme, cutinase. The action of this cell-surface cutinase on the enzyme substrate self-assembled monolayers switches a non-electroactive hydroxyphenyl ester (off state) to an electroactive hydroquinone (on state), providing electrical activity that can be identified with cyclic voltammetry. In this way, cell-surface enzymatic activity is transduced into electronic signals. The development of strategies to directly interface the activities of cells with materials will be important to enabling a broad class of hybrid microsystems that combine living and non-living components.

Advances in stimuli-responsive polymer synthesis also facilitate new approaches to fabricate switchable electrodes to control chemical or biochemical process producing pH changes. In this method, pH-sensitive polymer films, hydrogels or polymeric brushes are covalently linked with metal-based redox complexes to generate pH sensitive interfaces for efficient electrocatalysis. When the polyelectrolyte is close to neutral pH ($pH \geq 6$), the interface becomes impermeable to ionic redox species and electrode activity is turned off, whereas when polymer is charged ($pH \leq 5$), the polymeric interface becomes permeable to ionic species and allows their access to the conducting support, thus switching the electrode to

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on state (**Figure 15**a). In addition to changing the permeability and ionisation ability of the polymer, morphology change also play an important role in generating controllable interfaces. In some cases, polymer brushes at the interface show a tendency to shrink or extend depending on the pH. There are different variations of same approach by employing more than one polyelectrolyte having different pH responsive behaviour over various pH ranges, such as mixed polymer brushes, which can expand the area of interest and control multi-step reactions (Tam *et al.* 2008a).

The pH-controlled, reversible transition of Os-complex (Osmium-complex) functionalised electrodes has been developed to control the electrocatalytic oxidation of glucose via accompanying enzymatic reactions which change the pH value *in situ* (Figure 15b) (Contin *et al.* 2015; Tam *et al.* 2008b). Here, the coupling of a redox-silent biocatalytic process for analyte detection, with an enzyme-catalysed redox reaction, is demonstrated by the stimulation of electrostatic interaction between a pH-responsive polymer and a redox enzyme, to control the on-off transition for electrochemical signal generation. The electrode surface is modified with a poly(vinyl)imidazole polymer complex, with an Os(bipyridine)$_2$Cl redox hydrogel film entrapping urease and PQQ-dependant glucose dehydrogenase, while glucose is present in the solution. The on/off transition mechanism depends the presence of urea as an analyte, which is hydrolised to ammonia by the urease enzyme in the polymeric matrix. This reaction increases the local pH, resulting in the deprotonation of imidazole groups in the polymer chain. The decrease of positive charge at the polymer backbone decreases electrostatic repulsion between the polymer and the positively charged PQQ-dependant glucose dehydrogenase. Overall, the electron transfer rates between the polymer-bound Osmium complexes and PQQ inside the enzyme are enhanced, activating the electrocatalytic oxidation of glucose. This process generates the electrochemical signal for switchable urea detection.

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4. Concluding remarks and perspectives

In this review, we have sought to reflect the emerging field of switchable interfaces and their implications for bioelectronics by highlighting early breakthroughs and key developments in order to show the possible advantages and discuss the future of switchable bioelectronics. Recently, a significant number of switchable bio-interfaces based on self-assembled monolayers, polymers including polymer brushes, hydrogels and polymer-particle composites have been described, which modulate interactions with biomolecules including proteins, DNA and cells that interact with the surface of electrodes. A variety of physical and chemical stimuli have been used to achieve this target. Most of the studies concern reversible signal responses in which interfacial properties are switched on and off. However, since the current area of interest is "bioelectronics" one should ask "how all these switchable bio-interfaces will be used in real devices" or "which features of switchable bio-interfaces will be beneficial for the general realisation of bioelectronics"? Answers to these questions still remain unclear. In many papers, authors have attempted to respond to these conundrums, but all too often the discussions descend into elaborate rhetoric and persuasive phraseology that lacks strong underpinning arguments. We believe that some fundamental challenges remain to be solved for the evolution of switchable bio-interfaces through switchable bioelectronics. First, biological organisms use signal transduction at a single molecule level, so optimisation of electronic platforms is required in order to scale up/down the system, depending on the application or device, to achieve comparable spatial resolution. Secondly, improvement in the reversibility of these systems is crucial. Most of the publications mentioned in this review did not discuss the stability or selectivity of their designs, and yet this should be one of the first concerns of an author seeking to generate a practical switchable system.

Switchable bioelectronics, therefore, is still in its infancy and there is long way to go before we can clearly see the route to manufacture robust hybrid devices that can deliver the promise

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offered by harnessing biological interactions in programmable electronic devices. Nevertheless we believe there is considerable potential for future progress in this field, and we await further creative experimentation and perspicacity to reveal the true efficacy of this interdisciplinary interface. There is no doubt that future research will progress the precise manipulation of the response of biomolecules with expanding sets of complex materials to generate a variety of new "instrumented bio-interfaces".

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Scheme 1. Schematic illustration of how biotechnology, electronics and materials science merge together to form the concept of bioelectronics.

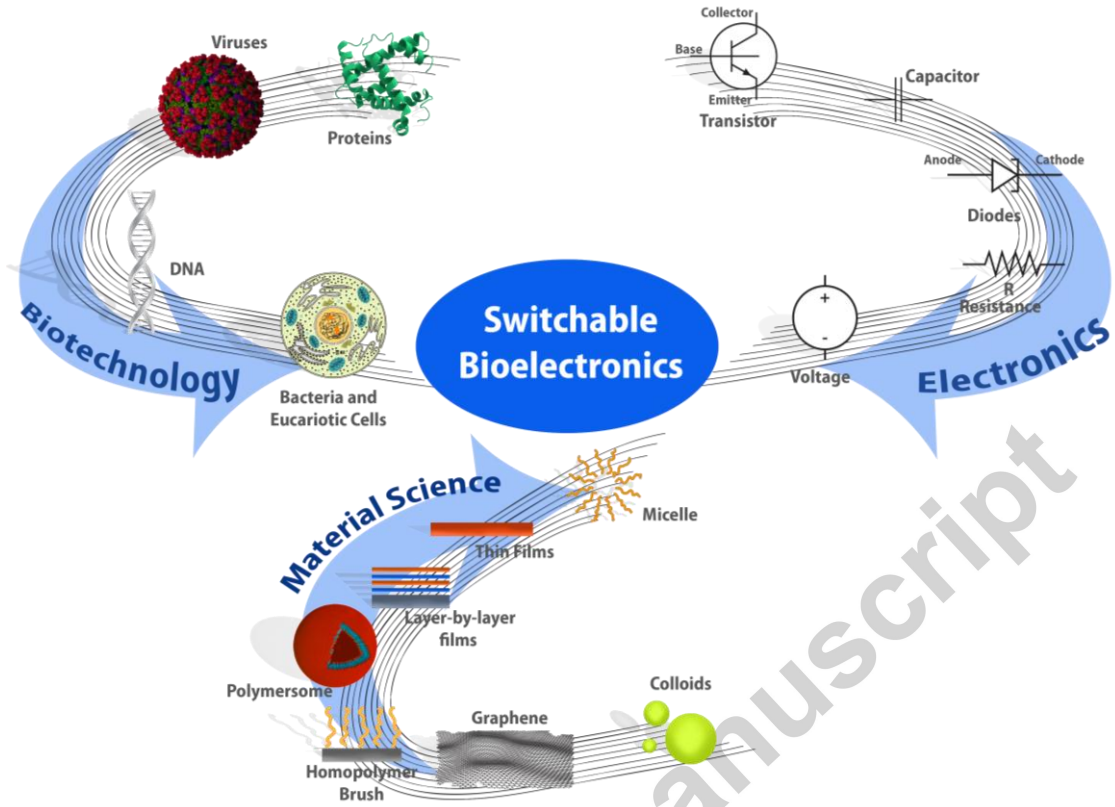

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Figure 1. Examples of the more commonly used photo-switchable molecular systems.

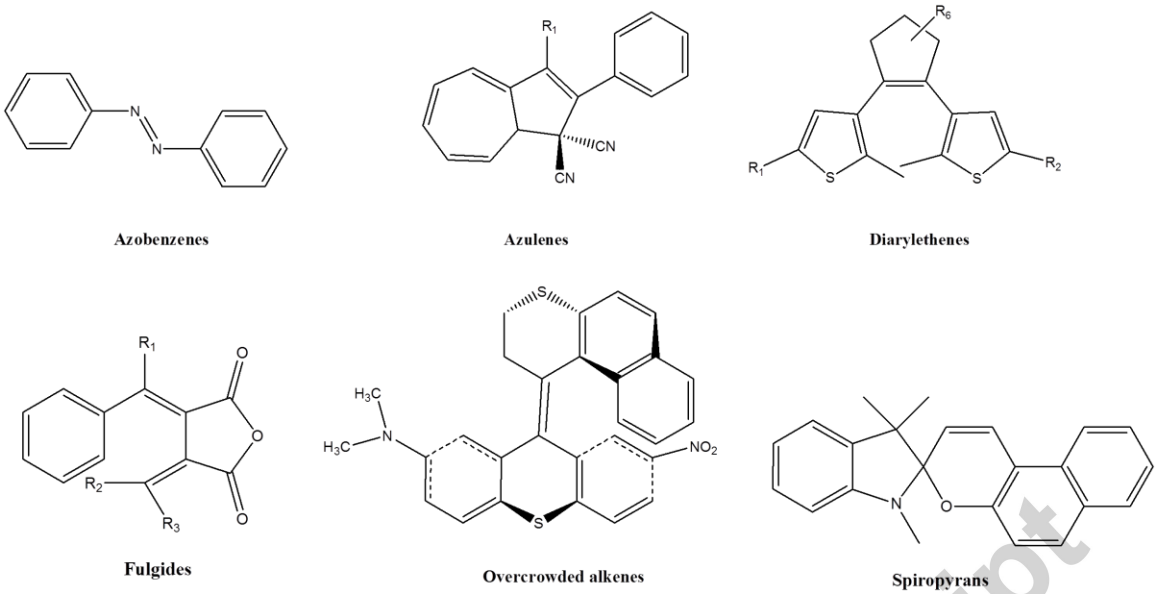

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Figure 2. Electrophysiological analysis of the reversible functioning of modified MscL by the patch-clamp technique (a) and illustration of a switchable bio-hybrid nanovalve based on the mechanosensitive channel protein of large conductance together with spiropyran molecular units (b). Reprinted with permission from ref (Browne and Feringa 2009) Copyright 2015 Annual Reviews.

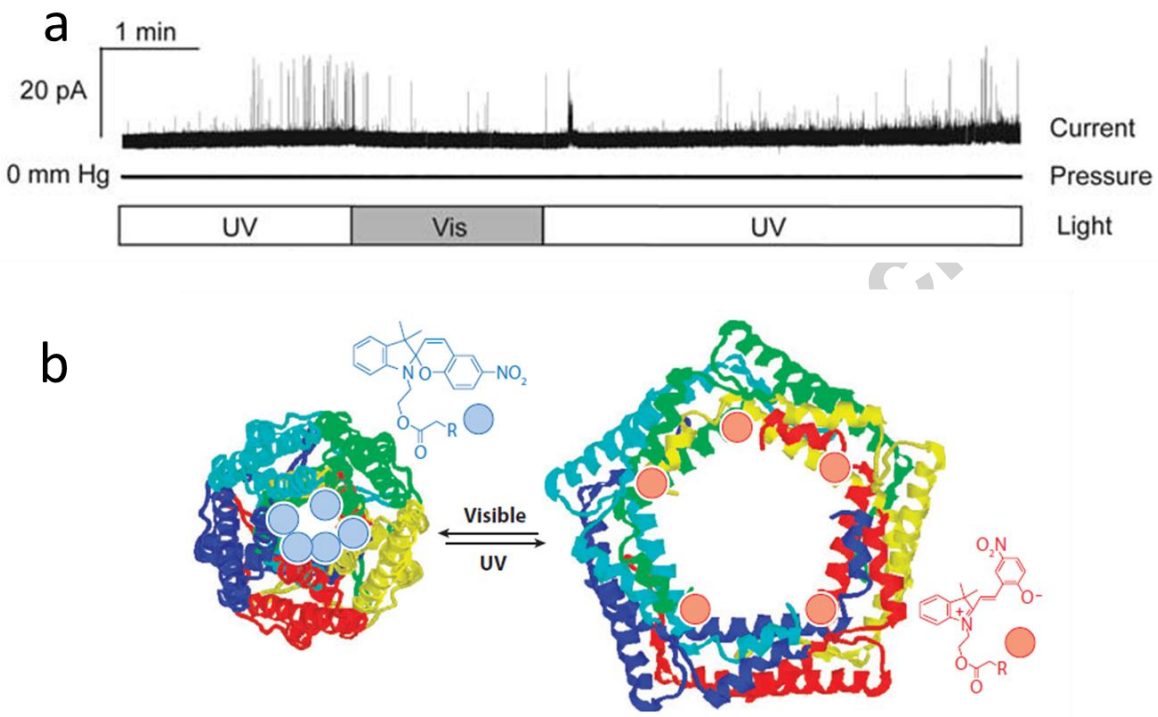

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Figure 3. Schematic illustration of: (a) a light-activated SiNW-based FET device containing a nanowire covered with a lipid bilayer and bacteriorhodopsin protein and (b) the on-off switchable source-drain current of a SiNW-based FET device. Reprinted with permission from ref (Tunuguntla 2015). Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

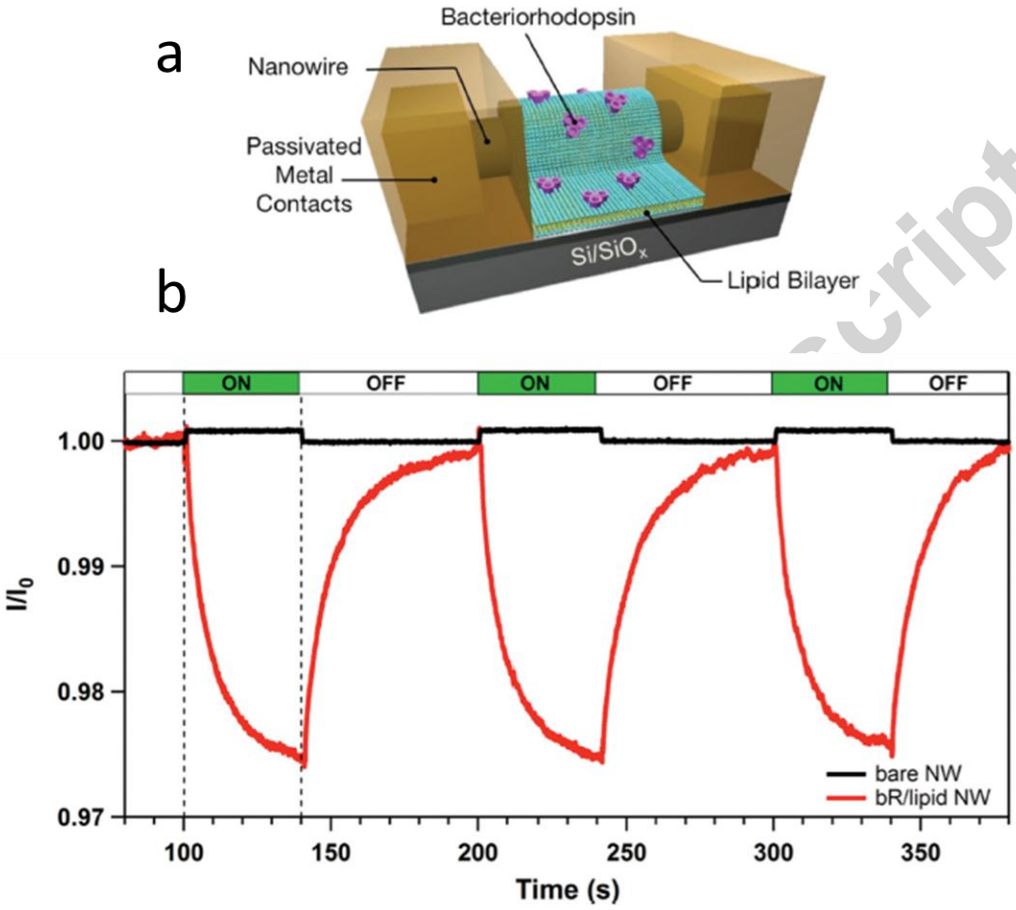

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Figure 4. Schematics of: (a) light modulated electrochemical behaviours of modified electrodes. Reversible binding-releasing of anti-dinitrophenol (anti-DNP) antibody on a light-responsive spiropyran-modified gold surface which shows reversible binding ability and the interactions are followed by the diffusion of redox prbobe by cyclic voltammetry; (b) light-switchable electro-static interaction of pyrroloquinoline quinone (PQQ^{3-}) with two different isomers of light-responsive spiropyran molecular units modified gold surface and corresponding response by cyclic voltammetry. Reprinted with permission from ref (Wan and Chen 2014) Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

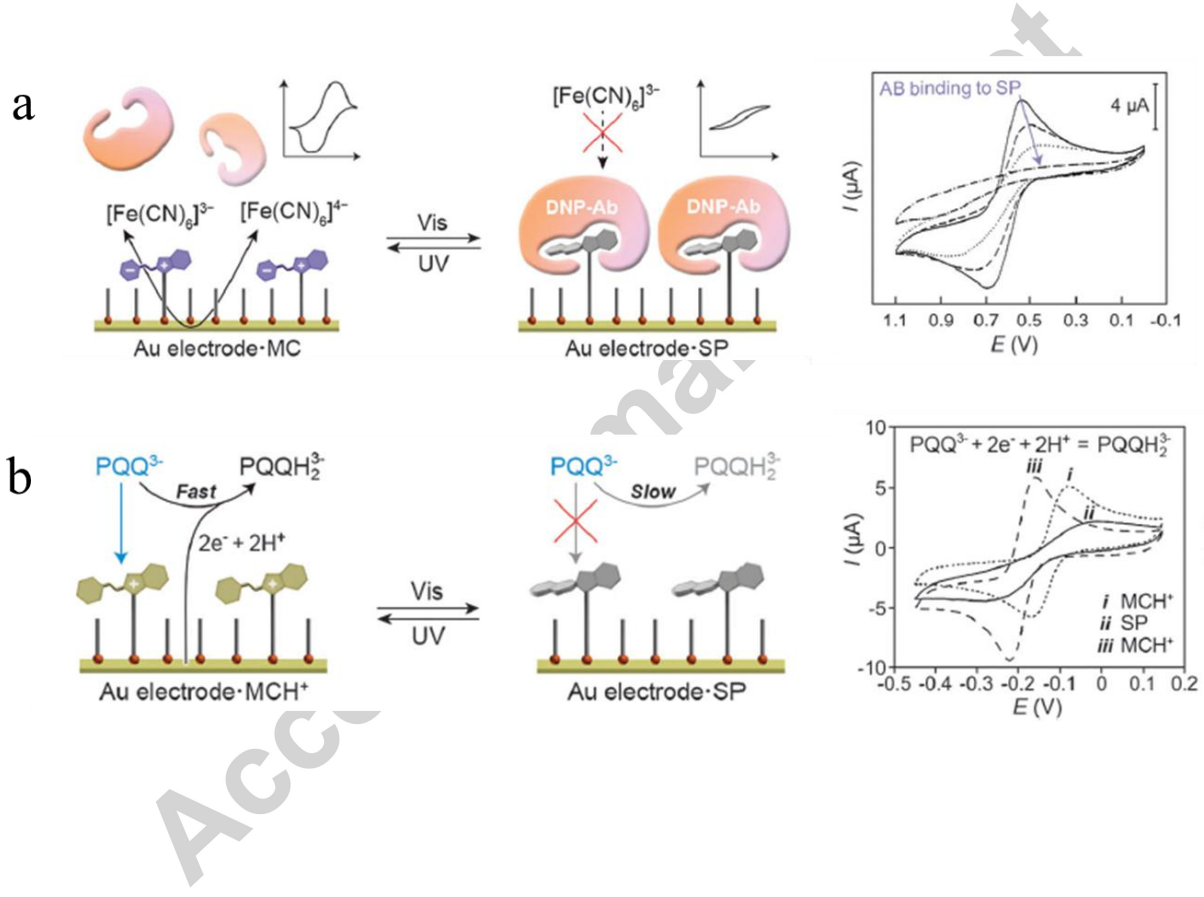

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Figure 5. Schematic representation of: (a) switchable adhesion of bacterial cells based on charge and conformational change of surface-modified groups upon applied potential. The conformation of surface molecule is changed upon applying potential and adhesion of bacterial cells is controlled; (b) confocal microscopy images and cell counting graph in three different applied potential conditions such as -0.25 V, open-circuit (OC) and +0.25 V, respectively. Reprinted with permission from ref (Pranzetti *et al.* 2013) Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

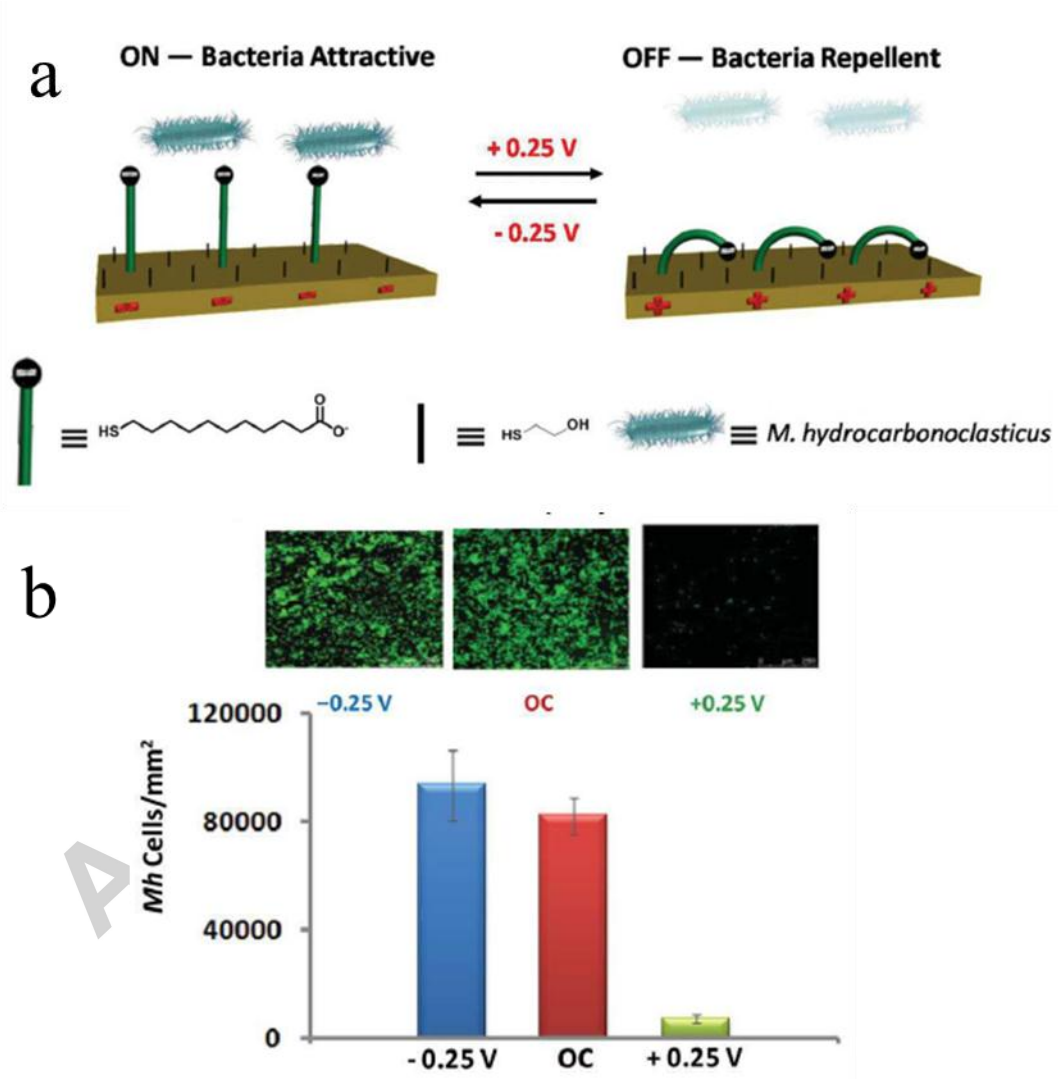

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Figure 6. Schematic illustration of: (a) electrical-switching and conformational change of single-stranded oligonucleotide immobilised (left) and hybridisation of target sequence to the surface confined single-stranded oligonucleotide on gold electrode (right). When the target DNA is introduced to the surface, conformation of surface-tethered DNA molecule changes upon hydridisation; (b) potential triggered fluorescent intensity change upon hybridisation in microfluidic channel. The hybridisation on electrode surface amplifies the fluorescent signals. Reprinted with permission from ref (Mendes 2008). Copyright 2015 Royal Society of Chemistry (a), reprinted with permission from ref (Spuhler *et al.* 2010). Copyright 2015 Proceedings of the National Academy of Sciences of the United States of America (b).

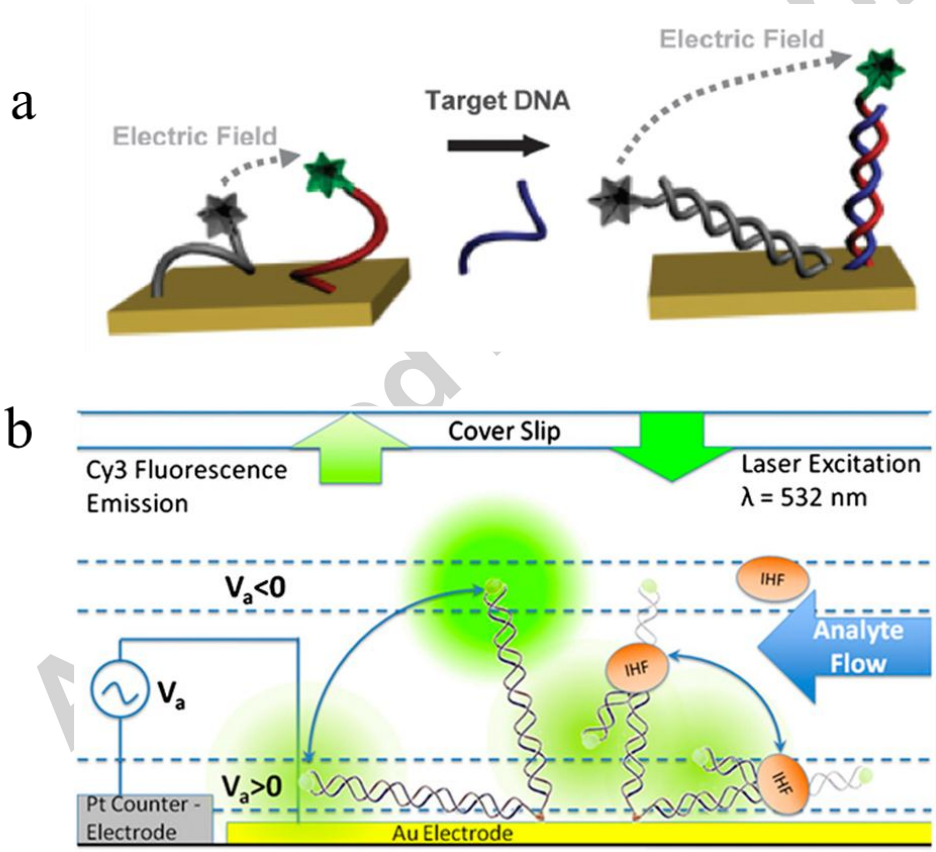

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Figure 7. Schematic illustrations of electrochemically stimulated rotaxane on gold electrode as an electron-transfer mediator to control bioelectrocatalytic oxidation of glucose. Reprinted with permission from ref (Wan and Chen 2014) Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

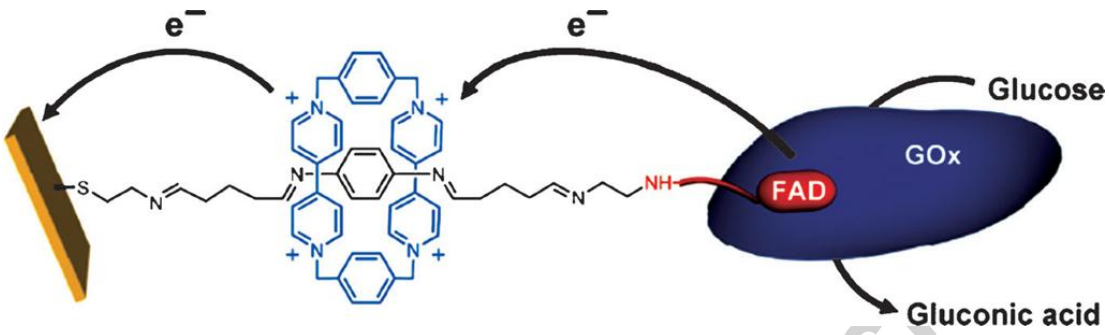

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Figure 8. A photomicrograph of a microfluidic chip showing: (a) an array of gold heater tracks on top of a 200 µm-wide Si_3N_4 membrane (white) array; (b) water-contact angle measurements of PNIPAAM films as function of temperature; (c) fluorescence microscopy images of fluorescein labelled myoglobin (green) interacting with a single heated track. The image obtained on heating a track above the PNIPAM transition temperature after exposure to a 0.5 mg/ml myoglobin solution followed by rinsing in myoglobin-free buffer. The images were obtained 0.8 and 1.2 s after the hot track was turned off, releasing a plume of protein into a stagnant solution. Reprinted with permission from ref (Huber *et al.* 2003) Copyright 2015 AAS.

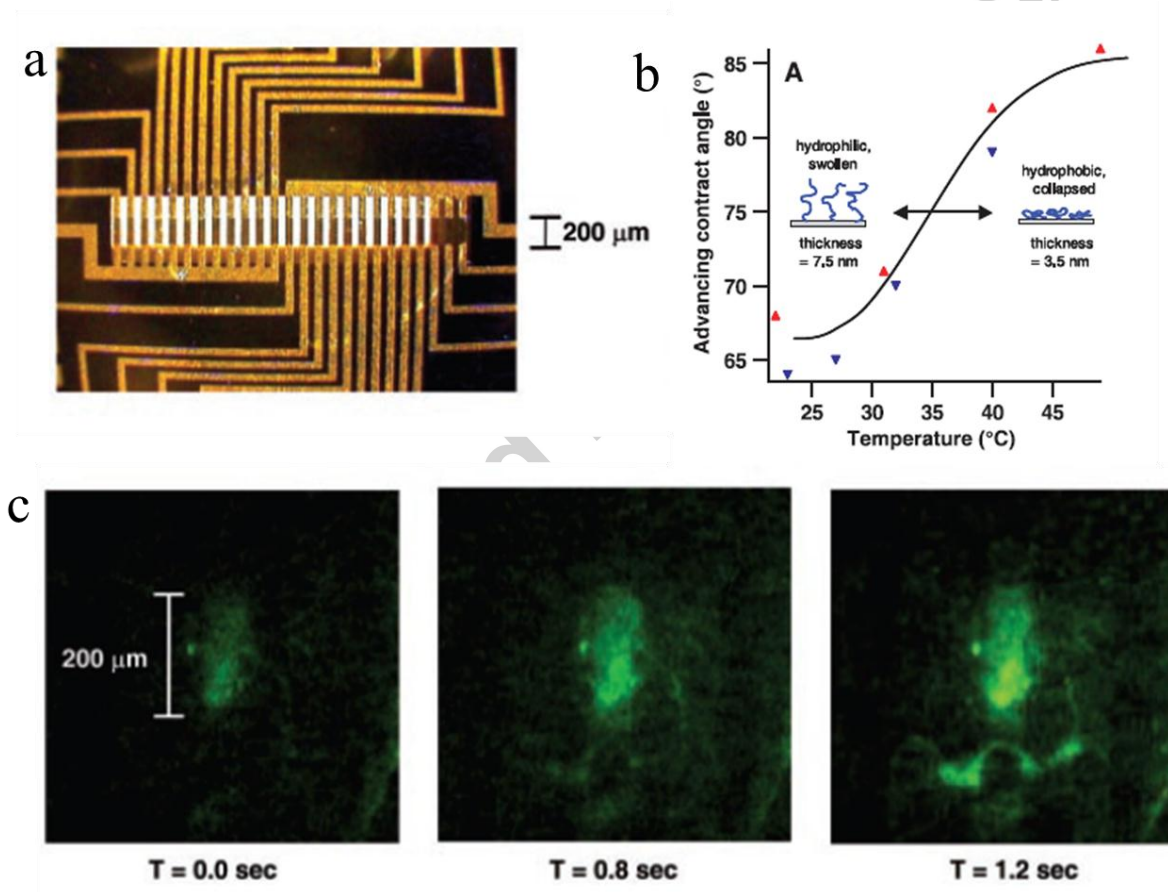

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Figure 9. Schematic illustrations of amine-terminated gold surface modification with redox-active PNIPAAM-ferrocene polymer and temperature-responsive bioelectrocatalysis. Reprinted with permission from ref (Wan and Chen 2014) Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

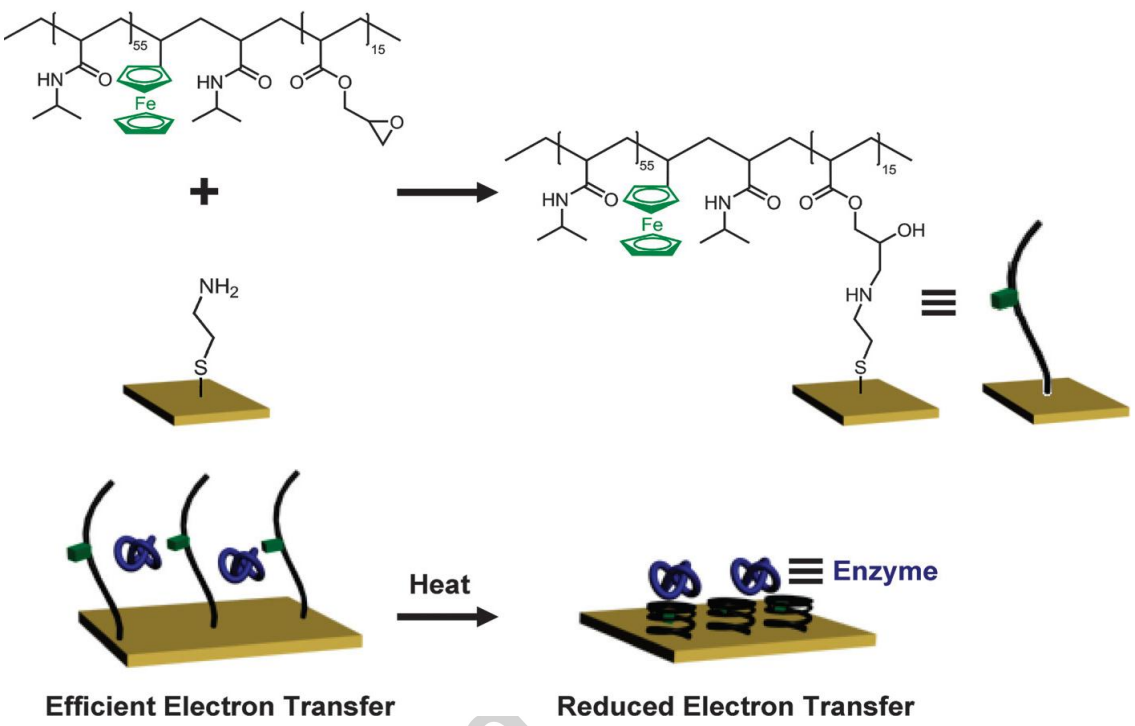

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Figure 10. Schematic representations of an on/off switchable bioelectrocatalytic graphene interfaces in two different states. Reprinted with permission from ref (Parlak *et al.* 2014). Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

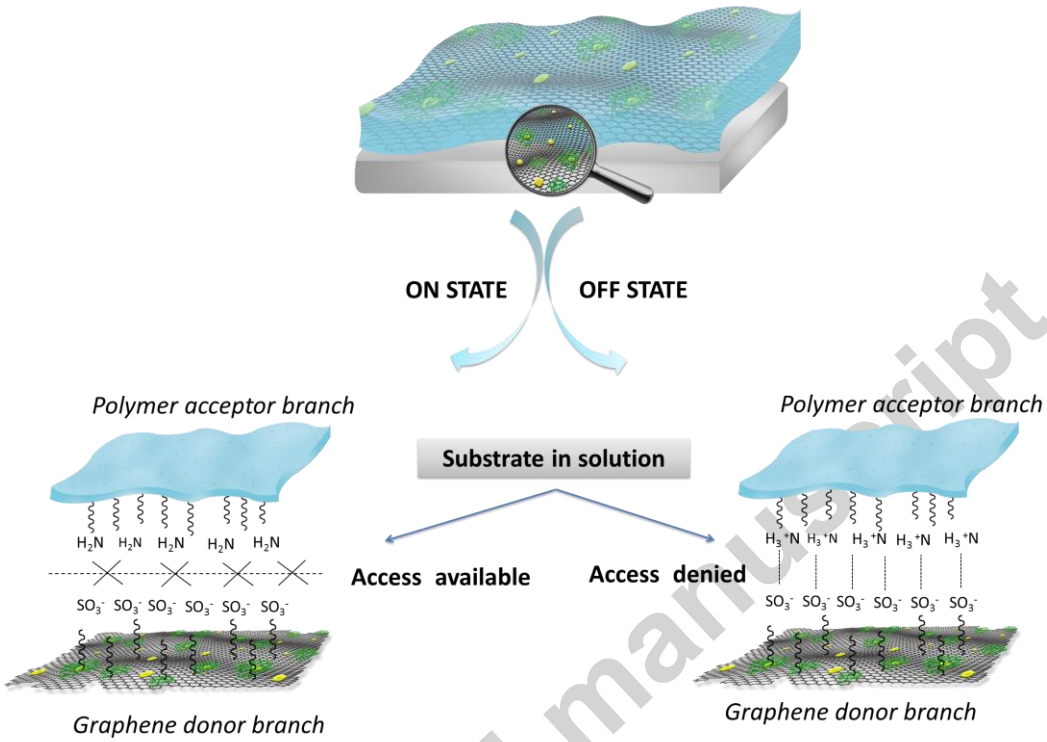

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Figure 11. Schematic illustrations of: (a-b) a reversible electro-catalytic reaction with functional magnetic nanoparticles (Fe_3O_4-R) controlled by an external magnetic field; (c) schematics of magnetically-controlled reversible assembling of gold-coated magnetic nanoparticles on electrode surface; and (d) response of mediated electrochemical oxidation glucose in the presence (black curve) and absence (red curve) of the magnetic field. Reprinted with permission from ref (Bocharova and Katz 2012). Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

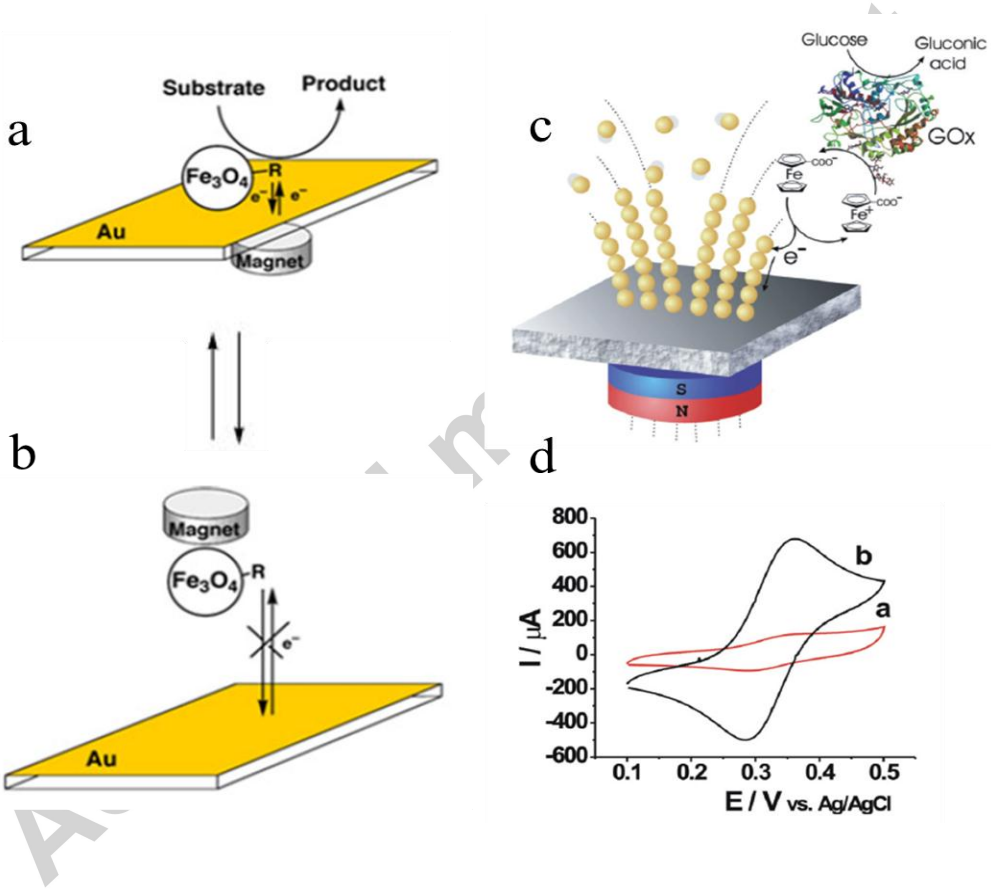

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Figure 12. The reversible permeations of electrode surface with magnetic nanoparticles to control the bioelectrocatalytic oxidation of glucose. Reprinted with permission from ref (Katz *et al.* 2005) Copyright 2015 American Chemical Society.

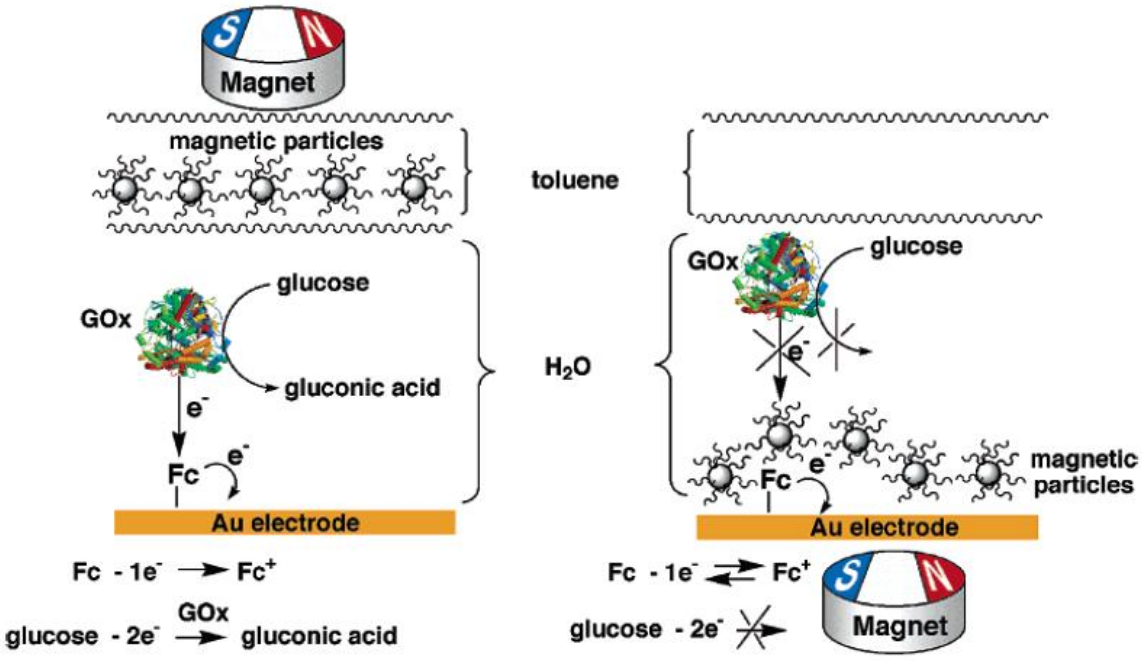

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Figure 13. Schematic representations of: (a) "signal-off" and (b-c) "signal-on" electrochemical DNA-based sensors. The electrochemical signals are controlled based on conformation change of each stem-loop oligonucleotide and translocation of their conjugated electroactive ferrocene (a-b) methylene blue (c) groups. Reprinted with permission from ref (Wan and Chen 2014) Copyright © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

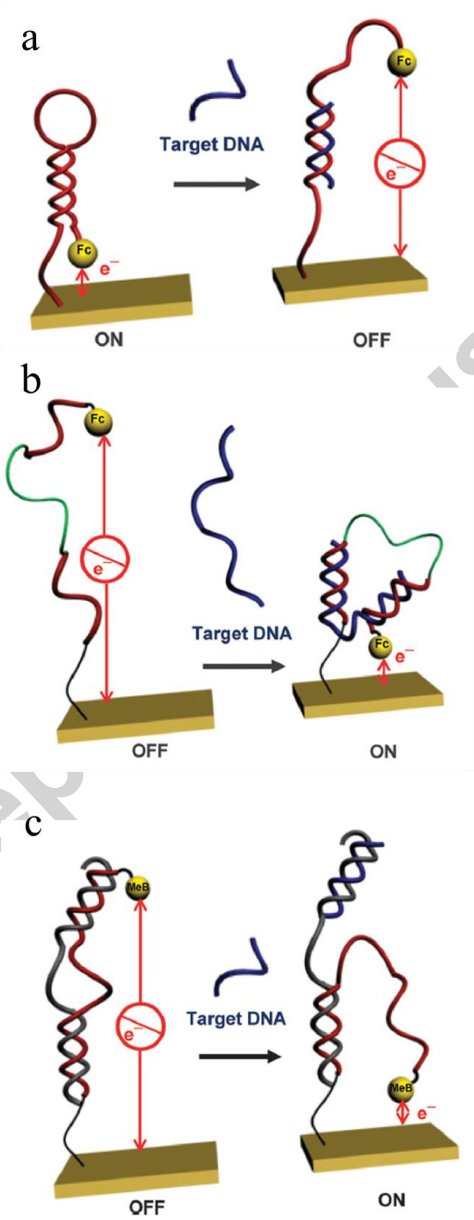

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Figure 14. Diagrams showing transducing of cellular activities into electrical signals. The non-mammalian enzyme cutinase reversibly switches non-electroactive hydroxypheyl ester-terminated monolayer to an electroactive hydroquinone terminated monolayer, which can be reversibly oxidised to give the corresponding benzoquinone. Reprinted with permission from ref (Collier and Mrksich 2006) Copyright 2015 Proceedings of the National Academy of Sciences of the United States of America

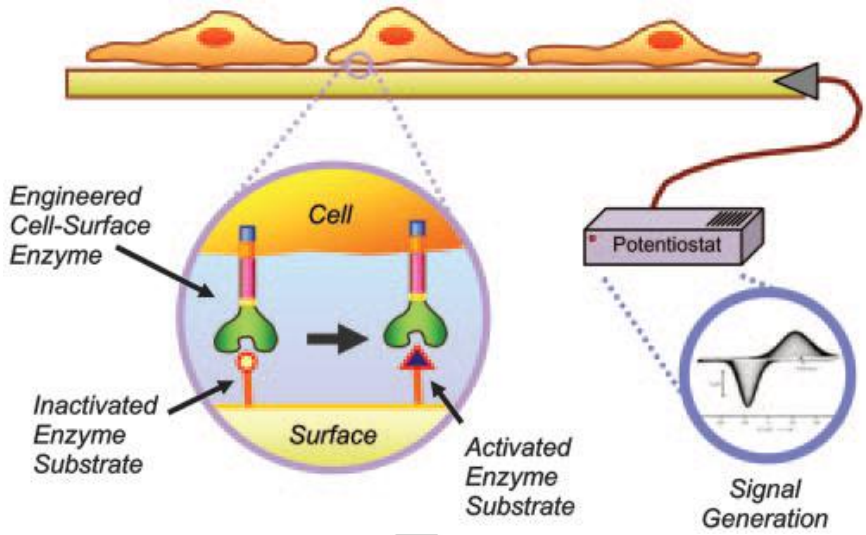

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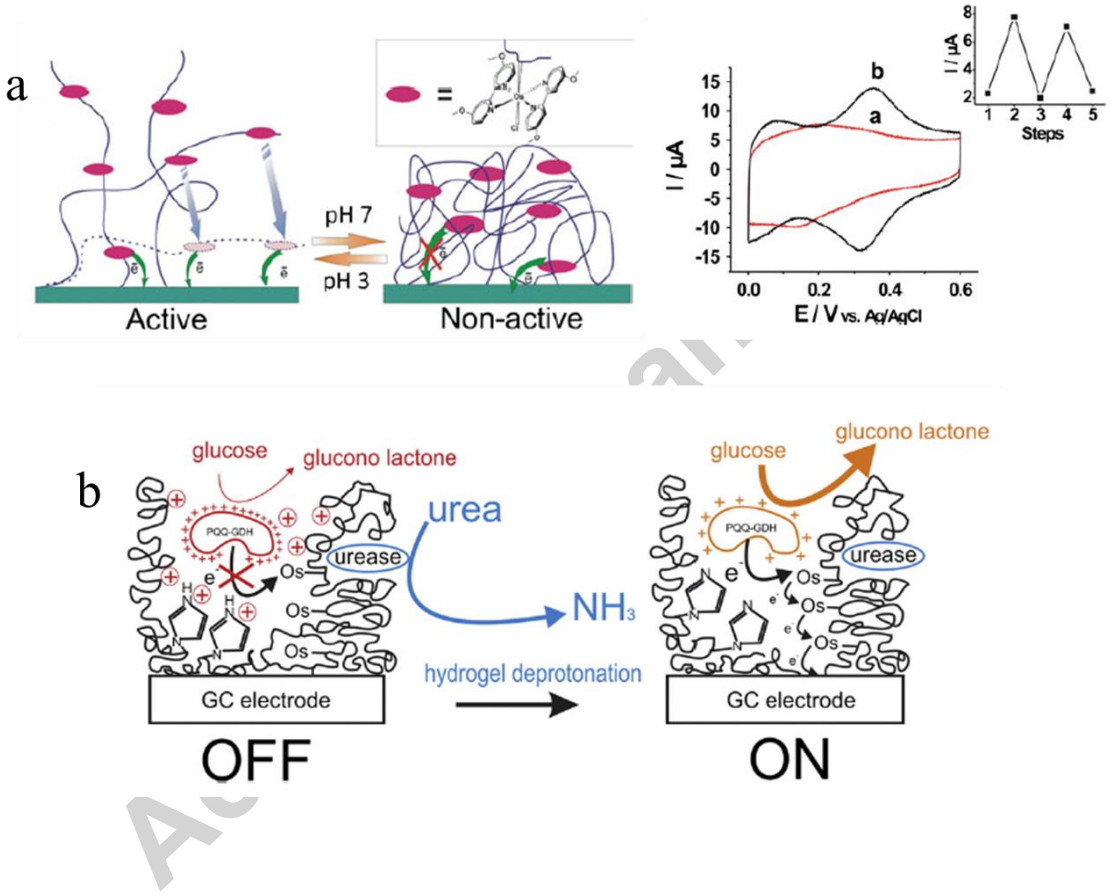
Figure 15. Illustrations of (a); pH switchable Os-poly(vinylpyrridine) polymeric interfaces in active and passive states and cyclic voltammograms in two different pH conditions. (The red and black curves represent conditions at pH 7 and pH 3, respectively); (b) the representation of reversibly regulated electrostatic interaction between the polymer backbone and PQQ-GDH. Reprinted with permission from ref (Bocharova and Katz 2012). Copyright 2014 Wiley-VCH Verlag GmbH (a), reprinted with permission from ref (Contin *et al.* 2015) Copyright 2015 Elsevier (b).



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Highlights

- We review the rapidly emerging field of switchable interfaces and its implications for bioelectronics.
- In this review we try to answer a fundamental question: “how do living systems probe and respond to their surroundings?
- Why do we need switchable bioelectronics?
- Switchable bioelectronics comprising stimuli-responsive surfaces are essential for the construction and realisation of highly engineered bio-interfaces, which have the capability to alter their macroscopic properties on demand.
- Surfaces equipped with molecular cues that mimic certain aspects of the structure or function of natural environments.

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