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TOPICAL REVIEW

# Biofabrication to build the biology–device interface

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**Abstract**

The last century witnessed spectacular advances in both microelectronics and biotechnology yet there was little synergy between the two. A challenge to their integration is that biological and electronic systems are constructed using divergent fabrication paradigms. Biology fabricates bottom-up with labile components, while microelectronic devices are fabricated top-down using methods that are 'bio-incompatible'. Biofabrication—the use of biological materials and mechanisms for construction—offers the opportunity to span these fabrication paradigms by providing convergent approaches for building the bio–device interface. Integral to biofabrication are stimuli-responsive materials (e.g. film-forming polysaccharides) that allow *directed assembly* under near physiological conditions in response to device-imposed signals. *Biomolecular engineering*, through recombinant technology, allows biological components to be endowed with information for assembly (e.g. encoded in a protein's amino acid sequence). Finally, *self-assembly* and *enzymatic assembly* provide the mechanisms for construction over a hierarchy of length scales. Here, we review recent advances in the use of biofabrication to build the bio–device interface. We anticipate that the biofabrication toolbox will expand over the next decade as more researchers enlist the unique construction capabilities of biology. Further, we look forward to observing the application of this toolbox to create devices that can better diagnose disease, detect pathogens and discover drugs. Finally, we expect that biofabrication will enable the effective interfacing of biology with electronics to create implantable devices for personalized and regenerative medicine.

(Some figures in this article are in colour only in the electronic version)

## 1. Introduction

### *1.1. The opportunity for integrating biology and electronics*

Two of the most transformative technologies that emerged in the 20th century were microelectronics and biotechnology. Microelectronics enabled unprecedented capabilities for data collection, processing and storage as well as convenient wireless communication. It is difficult to over-state the impact that microelectronics had in changing our way of life. Biotechnology provided broad access to the capabilities of biology for selective molecular recognition and specific

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**Table 1.** Examples of opportunities enabled by bio–device integration.

| | |
|---|---|
| Analysis | Biosensors—multiplexed analysis in hand-held devices<br>Lab-on-a-chip—high-throughput screening<br>Smart fabrics—remote monitoring of first responders |
| Energy | Biofuel cells—efficient conversion of chemical and solar energy<br>Nanostructured batteries—compact storage of energy |
| Implantable devices | Devices to personalize medicine—smart delivery of therapeutics<br>Neural prosthetics—effective repair or restoration of function |

catalysis as well for probing the detailed genetic and molecular mechanisms of life. While the impact of biotechnology is most apparent in the area of medical diagnosis and therapy, biotechnology transformed the study of biology and accelerated progress in understanding how genes and their expression allow cells and tissue to develop and interact with their environment.

In the 21st century, we anticipate that transformative capabilities will result from the integration of biology with microelectronics. As illustrated in table 1, the emerging field of bioelectronics [1] offers incredible potential. In the area of analysis, the effective integration of biology with microelectronics will enable hand-held devices to perform multiplexed analysis at the point-of-care (for medical diagnosis) or on-site (for environmental sampling). Further, lab-on-a-chip devices (or micro total analytical systems; $\mu$TAS) will enable miniaturized analysis in a highly parallel fashion for high-throughput screening (e.g. of drug candidates). Finally, the coupling of biological recognition elements (enzymes, antibodies and receptors) with smart fabrics [2, 3] will enable chemical information to be added to physical measures of temperature, pressure and motion to provide greater capabilities for remote sensing (e.g. to monitor the status of a firefighter).

The coupling of biology with electronics also promises to enhance energy harvesting, storage and conversion. Recent studies aim to develop biofuel cells to convert energy directly into electricity. Cell-based biofuel cells may enable the generation of electricity from organic wastes (e.g. cellulosics) [4–6] or from sunlight [7], while enzyme-based fuel cells aim to access the body's circulating fuel (e.g. glucose) to power implantable devices [8, 9]. Also, nature's nanoscale particles are being enlisted as templates for high surface area batteries [10].

In the longer term, the coupling of biology with microelectronics should enable implantable devices that monitor and assess *in vivo* information, evaluate therapeutic options and implement decisions—all in real time while in constant communication with outside observers (e.g. physicians) [11]. The potential for feedback control of therapeutic interventions allows medicine to be 'personalized' by rapidly delivering the correct agent at the right time in the right place and in the right dose. Today, a common goal is an implant that can monitor glucose and deliver insulin, while more ambitious goals are devices that communicate with cells, tissue and organs to repair damage or restore function. A long-standing dream is an artificial eye that can provide sight (even partial sight) to the blind.

**Table 2.** Divergent fabrication paradigms for electronics and biology.

| | |
|---|---|
| Electronics fabrication | Top-down fabrication<br>Permanent features<br>Error-free methods (to prevent defects)<br>Control chemistry by excluding contaminants |
| Biological fabrication | Bottom-up fabrication<br>Labile components and structures<br>Error-correction and healing<br>Control chemistry by molecular recognition |

### *1.2. The fabrication challenge*

There are many challenges on the path to successfully integrating biology with electronics, and one such challenge is bridging the fabrication gap. Table 2 highlights the divergent fabrication paradigms between microelectronics and biology [12–14]. Microelectronic devices are fabricated using top-down, monolithic methods to pattern surfaces (e.g. silicon wafers). In contrast, biology is assembled bottom-up using genetic information to code for an amino acid sequence that contains the information for protein folding ($\approx$1 nm) and further hierarchical assembly. In addition to differences in fabrication approaches, the lifetimes of the assemblies are markedly different. Electronics are built to last with the devices generally becoming obsolete before they cease to function. Biology is built from labile components and it even has mechanisms to guide its own destruction (e.g. programmed cell death) to ensure that biological systems can undergo appropriate developmental programs and can adapt to its environment. Biology also has mechanisms for repair so the function of damaged components can be restored.

Fabrication, whether for microelectronics or biology, must employ strategies to control chemistry to ensure that patterns/structures are generated at the desired location and within an appropriate time-scale, and to ensure that undesired side-reactions are prevented. The strategy for controlling chemistry in microelectronics fabrication is to exclude contaminants and limit side-reactions using clean rooms, ultra-pure reagents and high vacuum. In contrast, biology performs its fabrication in a complex milieu and controls chemistry through molecular recognition both for the enzymatic generation of covalent bonds and the non-enzymatic self-assembly of components through non-covalent bonds.

## 2. Biofabrication: construction with biological materials and mechanisms

The benefits of integrating biology with microelectronics will only be realized when the divergent fabrication paradigms

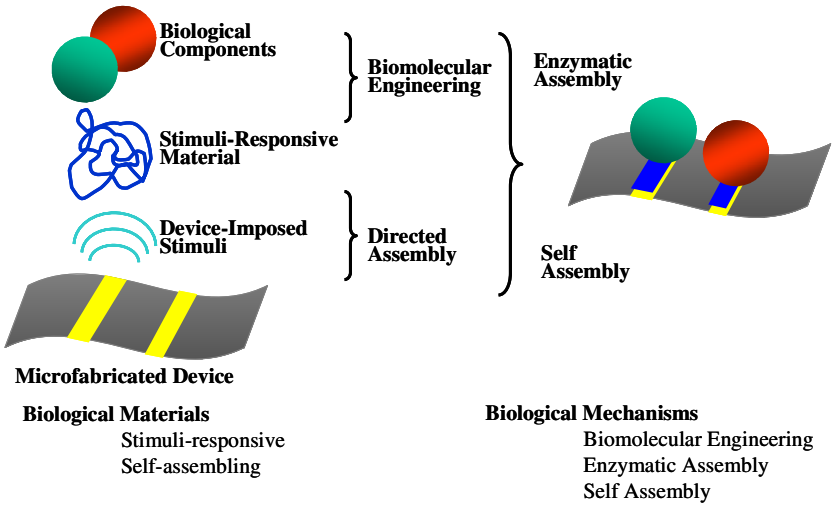



**Figure 1.** Convergent biofabrication methods to construct the bio–device interface.

can be bridged. Our groups are exploring a convergent approach to bridge this fabrication gap and we define our approach as biofabrication [15, 16]—the use of biological materials and mechanisms for construction. It is worth noting that the word 'biofabrication' has been used by several groups with slightly different definitions. An early use of the term emerged from biomineralization studies aimed at understanding and controlling the synthetic mechanisms for calcium and silica-based materials (e.g. pearls) [17–20]. The US Defense Advanced Research Projects Agency (DARPA) employed a somewhat expanded definition of biofabrication to include the mimicking of biology's methods for creating three-dimensional structures with angstrom-level control. Mironov and co-workers define biofabrication as 'the production of complex living and non-living biological products from raw materials such as living cells, molecules, extracellular matrices and biomaterials' [21]. The major differences in definition appear to involve the products and applications—DARPA's products would be abiotic (e.g. high efficiency solar cells), while many aim to use biofabrication for tissue engineering applications [22, 23].

Our definition for biofabrication focuses on the fabrication steps (irrelevant to the applications) and emphasizes that biological materials and mechanisms offer unique capabilities for controlling fabrication over a hierarchy of length scales. The recent review by Mironov *et al* [21] summarizes biofabrication efforts for tissue engineering, while here we review how biofabrication enables convergent fabrication approaches for building the bio–device interface.

Figure 1 illustrates our vision for biofabricating the bio–device interface. Device-imposed stimuli provide the signals to initiate integration and to spatially localize the biological components. Stimuli-responsive materials that recognize and respond to these device-imposed signals are essential to this 'directed assembly'. The biological components to be interfaced to the device could be engineered (e.g. genetically engineered) to facilitate assembly. Finally, the hierarchical assembly of these components is achieved by self-assembly or enzymatic assembly.

**Table 3.** The biological materials for biofabrication (excluding inorganics).

| | |
|---|---|
| Small molecules | Lipids—for compartmentalization and organization |
| | Amino acids and peptides—for assembly functions |
| Macromolecules | Proteins—for assembly, structural and biological functions |
| | Polysaccharides—for assembly and structural functions |
| | Nucleic acids—for programmable assembly |

### *2.1. The biological materials*

Table 3 indicates that we focus on fabricating the bio–device interface using organic biological materials (versus inorganic biomaterials [20, 24]). Lipids are common self-assembling small molecules used by biology to compartmentalize (e.g. in organelles and vesicles) and organize (e.g. the electron transport proteins in the respiratory chain). Relatively recent studies have demonstrated that self-assembly can occur with amino acids and short peptides [25–29] and these may become important materials for biofabrication. Traditionally, the biological macromolecules of interest are proteins, polysaccharides and nucleic acids. Proteins and polysaccharides are obvious candidates for biofabrication because they are commonly used as structural materials in biology (e.g. collagen and cellulose). Nucleic acids typically serve information storage and transfer functions in biology; however, nucleic acids have attracted considerable attention because base-pairing provides a programmable means for assembly [30, 31].

Figure 1 indicates two major materials properties that are convenient for biofabrication—stimuli-responsiveness and self-assembling. These properties are not mutually exclusive, and, as will be discussed, many biological materials self-assemble in response to externally imposed stimuli. With respect to building the bio–device interface, the most appropriate stimuli are those that can be conveniently applied with high spatiotemporal control. While optical,

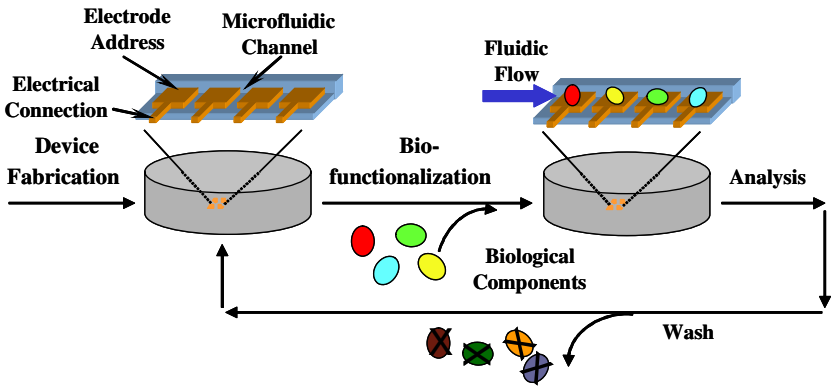



**Figure 2.** Biofabrication allows the biological components to be interfaced after the chip/device has been fully microfabricated and also allows for reuse of the device. De-linking device fabrication from biofunctionalization should enable fuller access to the power of microelectronics.

mechanical and magnetic stimuli are potential candidates, we focus on enlisting electrical stimuli since devices can be readily fabricated to impose electrical stimuli with exquisite spatiotemporal control.

### *2.2. Biological mechanisms*

Biology employs templated polymerization processes to guide the high-fidelity amplification of information (i.e. the replication of genetic information) and its conversion into functional components (i.e. the translation into proteins). Recombinant technology enables these templated biological processes to be accessed for the *biomolecular engineering* of proteins with precisely controlled amino acid sequences. While recombinant technology is well known for generating biologically active proteins, this technology is also capable of precision manufacturing at the nanoscale. In particular, this technology allows monodisperse particles (i.e. proteins) to be created with defined sizes and shapes, and with chemical functionalities that can be localized with sub-nanoscale precision [32]. In contrast, common synthetic methods to form nanoparticles achieve statistical control of size, shape and surface chemistry. In addition, recombinant technology allows short sequences of amino acids (i.e. fusion tags) or longer protein domains to be genetically fused to proteins to facilitate further assembly (e.g. to a surface).

In addition to employing templated polymerization to generate functional nanoscale components (i.e. proteins), biology also assembles these nanocomponents over a hierarchy of length scales. For example, viral coat proteins (≈1 nm) self-assemble to form functional virus particles (10–1000 nm) that are held together by non-covalent interactions. Also, collagen forms a hierarchy of structures from individual polymer chains to triple helices, fibrils, fibers and fiber bundles—in some cases these structures are stabilized by the enzyme-catalyzed introduction of covalent crosslinks. Importantly, hierarchical assembly in biology generally involves molecular recognition either at the interface between the self-assembling particles or in the active site of the enzyme. *Self-assembly* and *enzymatic assembly* provide convergent mechanisms for the hierarchical assembly of pre-formed nanoparticles into functional structures. Such hierarchical assembly poses significant challenges for conventional microfabrication.

## 3. De-link device fabrication from biofunctionalization

Our collaborative efforts in biofabrication were sparked nearly a decade ago by the recognition that there had been stunning advances in both microelectronics and biotechnology, but little synergy between the two. Specifically, few of the advances in biotechnology were accessing the power of microelectronics. For instance, the slides used for DNA microarrays were passive in the sense that they could neither guide spotting of the probe nucleotides nor report hybridization of the target—large and expensive printing and imaging instruments were required to perform these tasks. Also, the trend for inexpensive diagnostic testing in remote regions was focused on single-use and disposable test strips (e.g. dip sticks) that offered few capabilities for automated data collection, storage and wireless transmission (e.g. for real-time epidemiological analysis). Our goal was to develop convergent fabrication approaches that enabled the more effective bridging of biology and electronics.

A key challenge suggested in table 2 is that conventional microfabrication methods are not 'biocompatible' and have limited capabilities for accommodating labile biological components. We reasoned that modifying conventional microfabrication methods to be biocompatible would result in unnecessary compromises in performance. Our vision was to develop methods that allowed the biological components to be integrated into the chip or device after it had been fully fabricated as illustrated in figure 2. This approach would allow the entire suite of microfabrication methods to be enlisted to generate devices with the most sophisticated electronics. Further, our vision of biofabrication aims to generate a bio–device interface that is not permanent so the chip/device can be washed, re-functionalized and reused. Thus, we envision devices that can perform functions (e.g. analysis) while fully accessing the power of biology (for molecular recognition) and electronics (for data collection, analysis and transmission), while device reusability will allow these functions to be performed economically.

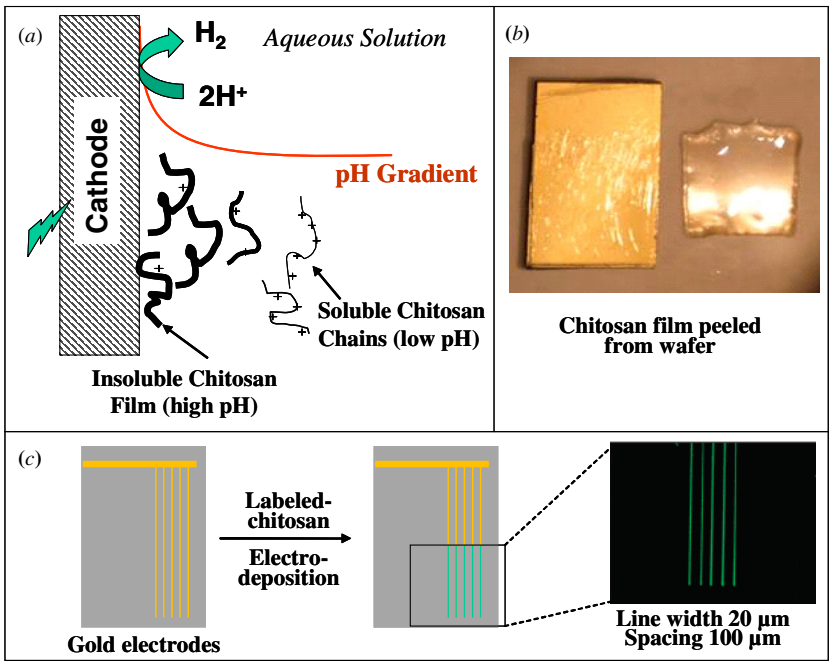


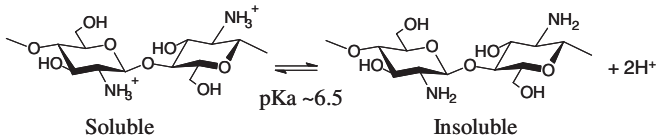

**Figure 3.** Electrodeposition allows chitosan to be directed to assemble in response to electrode-imposed signals. (*a*) Mechanism for chitosan electrodeposition at the cathode. (*b*) The deposited chitosan film is stable in the absence of an applied potential (although it can be re-dissolved under acidic conditions). (*c*) Chitosan can be electrodeposited with spatial resolution as evidenced by the deposition of fluorescein-labeled chitosan onto gold electrodes patterned onto a silicon wafer. Adapted with permission from [44]. (Copyright 2003 American Chemical Society.)

## 4. Biofabrication methods to build the bio–device interface

### *4.1. Directed assembly by enlisting electrical stimuli*

Directed assembly aims to enlist device-imposed signals to 'address' the biological components with high spatial selectivity. From a device perspective, electrical signals are particularly convenient and several potential mechanisms are being explored for electroaddressing [33–37]. A key enabling discovery was that stimuli-responsive, film-forming biopolymers can respond to device-imposed electrical signals by depositing as a thin film.

*4.1.1. Electrodeposition of stimuli-responsive film-forming polysaccharides.* To our knowledge, the pH-responsive aminopolysaccharide chitosan was the first biopolymer to be electrodeposited [38, 39]. Chitosan is pH-responsive because its primary amines can be protonated at low pH making chitosan a soluble cationic polyelectrolyte. At high pH, the amines are deprotonated, chitosan loses its charge and becomes insoluble. Importantly, chitosan undergoes its soluble-to-insoluble transition in the pH range of 6–7 which is particularly convenient for biological applications. It is also important that when chitosan becomes insoluble, it does not necessarily precipitate as insoluble particles. Rather, chitosan can form a three-dimensional hydrogel film [40]. These pH responsive and film-forming properties are integral to chitosan's ability to be electrodeposited.




The chitosan's electrodeposition mechanism is illustrated in figure 3(*a*) which shows that cathodic reactions in an aqueous solution result in the net consumption of protons (or net production of $OH^-$ ions) and the generation of a pH gradient. If this electrolysis is performed in the presence of chitosan, then polymer chains far from the cathode that experience the low bulk pH (less than about 6) will be protonated and soluble, while chains closer to the cathode will experience higher pHs and become less charged and less soluble. Ultimately, chitosan chains very close to the cathode surface will experience a sufficiently high pH that they become insoluble and deposit as a hydrogel film [41, 42]. The deposited chitosan film is stable in the absence of an applied voltage provided it is retained at a pH greater than about 6.5. Figure 3(*b*) shows a thick chitosan film that has been deposited and peeled from the electrode surface to illustrate the stability of the film. Early studies demonstrated that chitosan's electrodeposition is readily controlled by deposition conditions (chitosan concentration, voltage and time) [38]. In particular, the thickness of the electrodeposited film [38, 43] and the spatial resolution in the lateral dimensions can be controlled at the micron level [44–46]. The lateral resolution of chitosan electrodeposition is illustrated in figure 3(*c*) which

shows fluorescently labeled chitosan electrodeposited onto gold electrodes that had been patterned onto a chip.

Initial applications from the Chen group at Nanjing University employed chitosan electrodeposition for biosensor assembly [47, 48]. Specifically, they observed that enzymes could be mixed with the chitosan solution and co-deposited within the chitosan films. These films were observed to retain enzymatic activity (e.g. glucose oxidase [47] and peroxidase activities [49]). In addition to co-depositing enzymes, this group also reported that various nanoparticles (e.g. gold nanoparticles [50], $Fe_3O_4$ [51], $MnO_2$ [52–54], and carbon nanotubes [55]) could be co-deposited with chitosan and retained within the deposited films.

Emerging research has confirmed that chitosan electrodeposition provides a simple, rapid and reagentless means to assemble components for biosensing applications and several groups have extended the capabilities in important ways. Zhou *et al* reported that the addition of benzoquinone as an oxidant could enhance cathodic proton consumption to facilitate chitosan electrodeposition [56]. The use of chitosan electrodeposition for biofunctionalization has been expanded from studies with glucose oxidase [57] and horseradish peroxidase [58, 59] to additional enzymes (e.g. acetylcholinesterase [60–63] and lysozyme [64]), multiple enzymes, (glucose oxidase plus peroxidase [65]), antibodies for immunosensing [66] and oligonucleotides for detecting hybridization of complementary nucleic acid [67]. In addition to incorporating biological components (proteins and nucleic acid), chitosan films have been electrodeposited to include components that facilitate signal transduction. For instance, the chitosan chains have been modified with the electron transfer mediator ferrocene [68, 69] while soluble mediators [59, 70], and insoluble nanoparticles (e.g. $Fe_2O_3$ [71], gold [72] and carbon [73, 74]) have also been included in the deposited chitosan films to facilitate electrochemical reactions. Finally, chitosan electrodeposition has been extended to the co-deposition of intact vesicles [75].

In addition to employing chitosan electrodeposition for biosensor development, there is complementary research aimed at the use of chitosan electrodeposition for creating composite coating [76]. Zhitomirsky and co-workers have devoted considerable effort in co-depositing hydroxyapatite with chitosan to generate composite coating on hard materials with potential applications in bone replacements [43, 77–80]. Results from this group demonstrate how chitosan electrodeposition can be extended in several important ways. First, chitosan can be electrodeposited onto complex surfaces [81] which suggests the potential for extending biosensing from flat electrodes to wires [82]. Second, chitosan electrodeposition can be coupled with the electrosynthesis by employing the same cathodic conditions to convert soluble salts (e.g. $CoCl_2$ [83], $FeCl_2$ [84]) into inorganic nanoparticles (e.g. $Co_3O_4$, $Fe_2O_3$) [85–87]. Third, the Zhitomirsky group has extended electrodeposition to other polysaccharides either by co-deposition with chitosan (e.g. heparin) [88, 89] or through new electrodeposition mechanisms [90]. For instance, figure 4(*a*) shows that the acidic polysaccharide alginic acid is believed to electrodeposit at the anode by a protonation

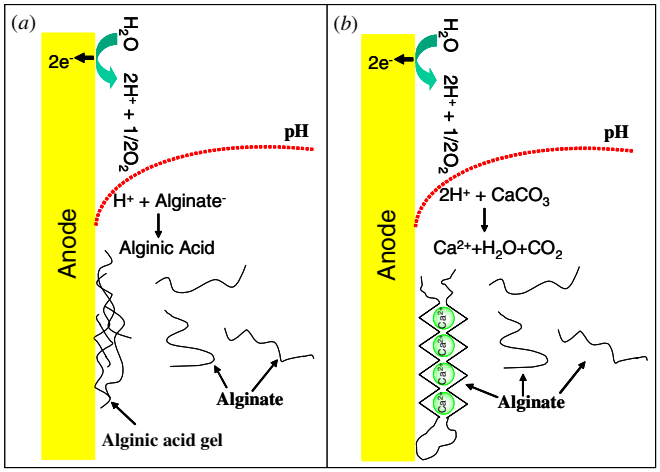



**Figure 4.** Separate mechanisms for alginate electrodeposition. (*a*) Neutralization mechanism in which the low pH at the anode induces a sol–gel transition to form alginic acid [91]. (*b*) The localized solubilization of $Ca^{2+}$ (from $CaCO_3$) at the anode induces the sol–gel transition of calcium alginate [103].

reaction that neutralizes the polymer [91, 92]. A similar mechanism may be responsible for the anodic deposition of the acidic polysaccharide hyaluronic acid [93].

The ability to electrodeposit alginate is especially interesting because calcium alginate gels are commonly used for cultivating prokaryotic [94] and eukaryotic cells [95–101]. Figure 4(*b*) shows a mechanism for the electrodeposition of the calcium-responsive alginate polysaccharide. Soluble sodium alginate is blended with insoluble particles of calcium carbonate ($CaCO_3$). The low pH generated at the anode triggers the localized 'solubilization' reaction:

$$CaCO_3 + 2H^+ \longrightarrow Ca^{2+} + H_2O + CO_2$$

The local release of $Ca^{2+}$ near the anode allows localized associations between $Ca^{2+}$ and alginate chains to form the physically crosslinked hydrogel network [102]. Recent studies have shown that bacterial cells can be codeposited with $Ca^{2+}$ alginate and these cells remain viable, can grow and respond to their environment (i.e. they can be induced to express a recombinant protein or report environmental stimuli) [103]. Because $Ca^{2+}$ alginate gels are physically (i.e. reversibly) crosslinked, these gels can be re-dissolved using agents that chelate $Ca^{2+}$ (e.g. sodium citrate).

*4.1.2. Electroaddressing of biological components.* While stimuli-responsive film-forming properties are integral to electrodeposition, polysaccharides can offer additional capabilities that facilitate addressing [104, 105]. For instance, polysaccharides can be partially oxidized to convert their hydroxyl groups into aldehydes and such partial oxidations are commonly used for the grafting of amino-containing components (e.g. proteins). It appears possible to induce this partial oxidation electrochemically as illustrated in figure 5(*a*) which suggests that anodic oxidation in the presence of NaCl results in the generation of a reactive mediator (possibly hypochlorite $OCl^-$) that can react with

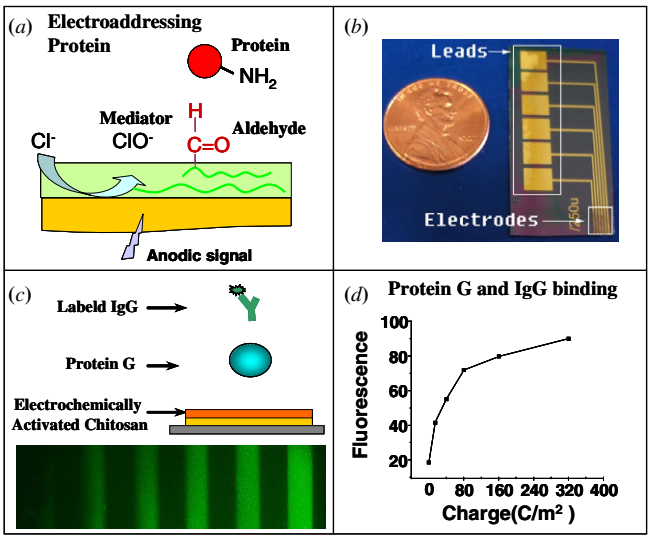



**Figure 5.** Protein electroaddressing with chitosan. (*a*) Proposed mechanism for the anodic activation of the electrodeposited chitosan film. (*b*) Chip with six electrically independent electrode addresses (250 $\mu$m wide gold lines spaced 250 $\mu$m apart). (*c*) Schematic and fluorescence image of the electroaddressing of Protein G and the subsequent binding of labeled human IgG. (*d*) Correlation between protein assembly (as determined from image analysis of fluorescence intensity) and the extent of chitosan-film activation (as measured by the charge transfer, $Q$). Adapted from [106]. (Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

and partially oxidize the chitosan film [106]. The sequential application of cathodic signals (for chitosan electrodeposition) with anodic signals (for oxidation) allows a rapid, simple and reagentless means to electroaddress proteins.

In an initial demonstration study, we used the chip as shown in figure 5(*b*) which possesses six electrically independent gold electrodes (250 $\mu$m wide gold lines). Chitosan was first electrodeposited onto all six electrode addresses. Next, the individual chitosan films were activated by sequentially biasing the underlying electrode (0.9 V versus Ag/AgCl) in the presence of NaCl for varying times (total time for activation $\approx$2 min). Finally, the bacterial IgG-binding protein, Protein G, was assembled by immersing the activated chip in a solution containing Protein G. Protein assembly was visualized by incubation with fluorescently labeled human IgG. Figure 5(*c*) shows a progressive increase in fluorescence intensity with an increase in film activation (left to right in the images), while figure 5(*d*) shows that protein assembly can be quantitatively controlled by controlling the charge transferred ($Q = \int i\,dt$ where $i$ is current) during anodic oxidation [106].

In a second demonstration study, we assembled the common biosensing enzyme glucose oxidase (GOx) and used this for the electrochemical detection of glucose [82]. To illustrate the versatility of this protein assembly approach, we employed a gold wire rather than a patterned electrode. Figure 6(*a*) shows a schematic of the electroaddressing approach, while figure 6(*b*) shows a schematic of the experiment to detect glucose. The reactions in figure 6(*a*) illustrate the basis for this biosensing; GOx catalyzes the oxidation of glucose with the formation of $H_2O_2$ which is anodically oxidized to generate an electrical signal (i.e. an anodic current). The insert in figure 6(*c*) shows that as each aliquot of glucose is added to the test solution, there is a step increase in the steady state current. The standard curve given in figure 6(*c*) shows the correlation between the electrical signal and the glucose concentration. Potentially, wires could be functionalized with varying biosensing components and 'woven' into fabrics to generate clothing capable of multiplexed analysis.

While chitosan allows the electroaddressing of proteins, alginate offers the potential for electroaddressing cells. This potential is illustrated by a study in which different bacterial populations were electrodeposited onto different electrode addresses using the mechanism outlined in figure 4(*b*). Transparent indium tin oxide (ITO) electrodes were used for our demonstration experiment. Figure 7(*a*) illustrates that an *E. coli* population engineered for the inducible expression of red fluorescent protein (RFP) was co-deposited with calcium alginate onto the left-most ITO electrode, while a second population engineered for the inducible expression of GFP was co-deposited onto the middle electrode. The right-most electrode was a control in which calcium alginate was electrodeposited without cells. This patterned slide was incubated in growth medium for 2 h and then cultivated in induction medium for 16 h after which it was observed using fluorescence microscopy. Figure 7(*b*) indicates that the RFP-expressing cells were confined to the left electrode address, while the GFP-expressing cells were confined to the middle address while no fluorescence was observed on the right, control electrode. This result indicates that different cell populations can be addressed and cultivated at specific electrodes [103].

*4.1.3. Studies with microfabricated devices.* As indicated above, the discovery of chitosan's ability to electrodeposit in response to device-imposed electrical signals enabled a variety of components (both biological and non-biological) to be co-deposited and assembled at electrode addresses. Device studies demonstrated that electrodeposition can be performed in microfluidic channels [107] even when the channel is covered [108, 109]. For instance, figure 8 shows a device with six microfluidic channels (left to right) passing over six electrodes (top to bottom) that are patterned onto the base of the channel. The fluorescence photomicrograph on the right in figure 8 shows a deposit of fluorescently labeled chitosan on one of these electrode addresses. Later studies demonstrated that enzymes could be assembled with the electrodeposited chitosan to permit their reactions to be studied in a lab-on-a-chip device [110]. These results demonstrate that a fully fabricated device can be biofunctionalized with proteins simply, rapidly and without the need for reactive reagents (required for many chemical functionalization methods), direct contact (required for printing methods) or a 'line-of-sight' (required for photolithographic methods). Further, these results demonstrate that biofabrication allows the de-linking of device fabrication from biofunctionalization and enables devices to be washed and reused.

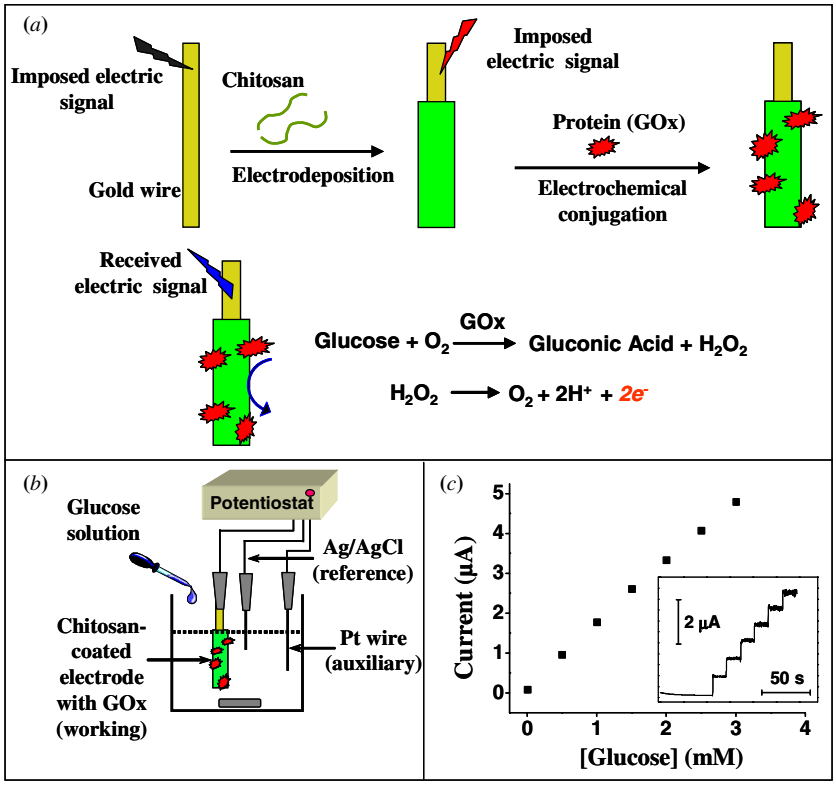



**Figure 6.** Assembly of a standard biosensing enzyme on a gold wire. (*a*) Schematic of the electrodeposition and electrochemical conjugation of glucose oxidase (GOx) onto a conducting wire and subsequent glucose sensing. (*b*) Schematic of glucose sensing experiment. (*c*) Standard curve for electrochemical detection of glucose. Adapted with permission from [82]. (Copyright 2009 American Chemical Society.)

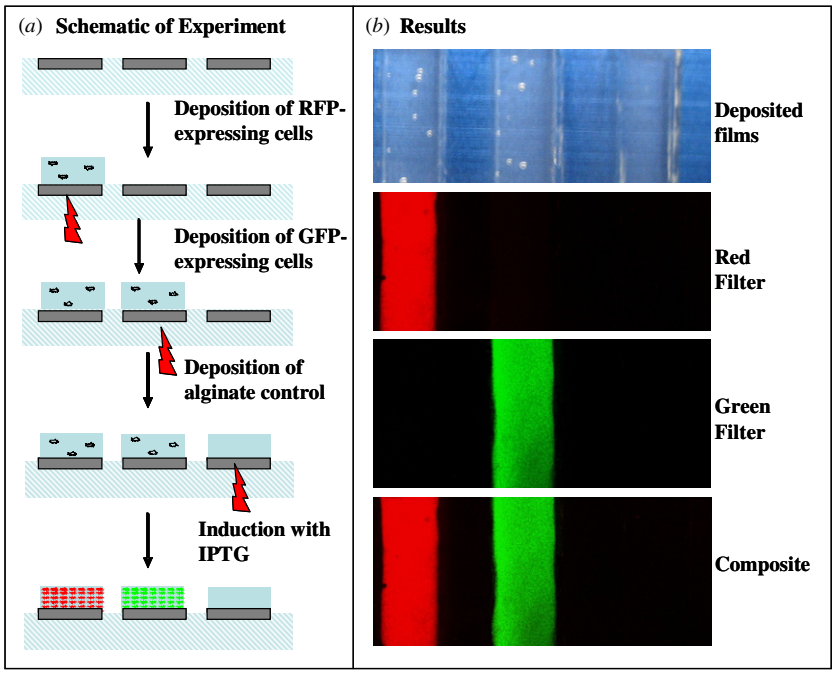



**Figure 7.** Electroaddressing two different *E. coli* populations onto patterned ITO electrodes (2 mm wide). (*a*) Schematic of electroaddressing experiment with sequential co-deposition of cells that express RFP (left electrode) and GFP (middle electrode) and a control alginate gel (right electrode). (*b*) Images of patterned slide after growth (2 h) and induction (16 h). Adapted from Shi *et al* [103]. (Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

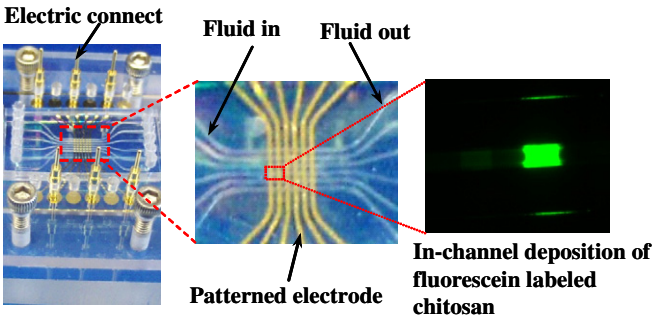



**Figure 8.** Electrodeposition in a closed microfluidic device. The left-most photographs show a microfluidic device with the fluidic channels shown left to right and the gold electrodes patterned onto the base shown top to bottom. The fluorescence image shows fluorescein-labeled chitosan deposited at a single electrode address within a channel.

Another interesting feature of chitosan is that its nucleophilic properties allow the deposited film to serve as an 'active film'. For instance, chitosan films are being examined for the multi-modal detection of phenols that are abundant in natural and technological systems (e.g. antioxidant food phenols, catecholic neurotransmitters or environmental contaminants). As illustrated in figure 9(*a*), low-molecular-weight phenols can readily diffuse through electrodeposited chitosan films and be anodically oxidized at the underlying electrode. The oxidation products are reactive and in many cases rapidly graft to chitosan's amine groups [111, 112]. This phenol grafting reaction imparts UV–visible absorbance to the otherwise transparent chitosan films and may provide a means for phenol analysis [113]. Figure 9(*b*) illustrates a device that was designed and fabricated for on-chip phenol detection using an integrated optical waveguide that can monitor changes in the film's UV–visible absorbance [114]. In addition to imparting optical properties to chitosan, the phenol grafting reaction alters the mass and modulus of the chitosan film which allows detection by mechanosensors such as the gold-coated microcantilever shown in figure 9(*c*) [115]. Thus, the electrodeposited chitosan film is 'active' potentially enabling the multi-modal detection of phenols; oxidation generates an electrical signal while the post-electrode grafting to the film can be detected optically and mechanically.

One final example of how stimuli-responsive biopolymers can be enlisted for construction exploits microfluidic (and not electrical) 'signaling'. Microfluidics allow precise control of the contacting of laminar flow streams as suggested in figure 10(*a*) and this contacting provides an alternative approach for precisely imposing localized stimuli. In this example, figure 10(*b*) illustrates that a slightly acidic, chitosan-containing solution (pH $\approx$ 5; shown as the upper stream) is contacted with a high pH buffer (pH = 10; shown as the lower stream). This contacting allows the generation of a pH gradient that induces chitosan's sol–gel transition at the interface between the two streams. Figure 10(*c*) shows a thin chitosan membrane formed at the interface between these two fluids. This chitosan membrane is semi-permeable in that it allows water to pass through but can restrain larger macromolecules (e.g. antibodies) and particles. Further the

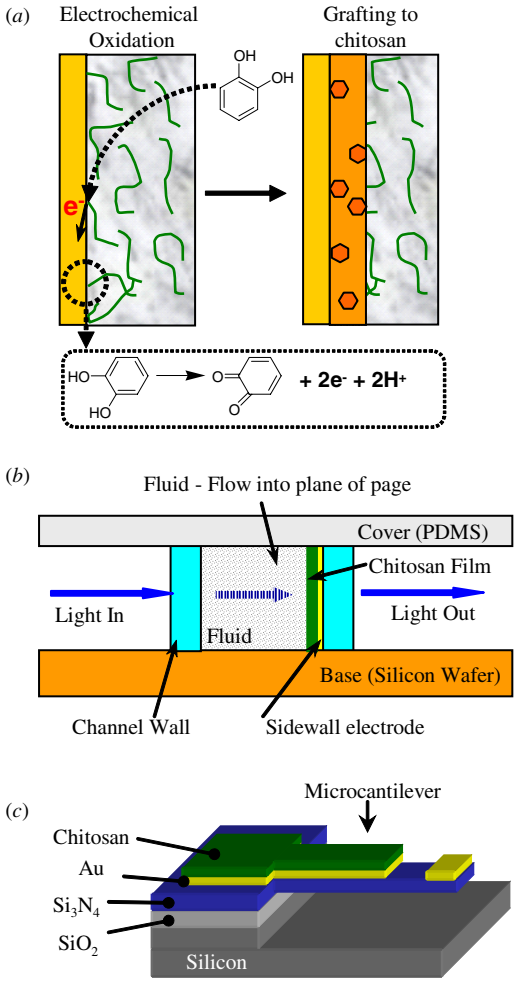



**Figure 9.** Multi-modal chemical sensing of phenol with chitosan films. (*a*) Schematic illustrates that anodically oxidized phenols graft to chitosan film. (*b*) Grafting alters the UV-visible absorbance properties of the films allowing on-chip optical detection through an integrated optical waveguide. Adapted from [114]. (Copyright (2009), with permission from Elsevier.) (*c*) Grafting alters the mechanical properties of the films allowing on-chip detection by a microcantilever mechanosensor. Adapted from [115]. (Reproduced by permission of The Royal Society of Chemistry (http://dx.doi.org/10.1039/b609149k).)

membrane is not permanent but can be dissolved under slightly acidic conditions to provide a means to 'erect' a temporary barrier to compartmentalize microfluidic flows [116]. Free-standing membranes have also been generated with collagen [117, 118], and analogous microfluidic contacting of laminar flow streams has been used to generate alginate hydrogels with controlled sizes and shapes (e.g. microbeads and bars) [95–99, 119–122].

### *4.2. Enzymatic assembly*

As indicated in table 2, methods to fabricate microelectronic devices and biological systems must control chemical reactions. Because enzymes offer exquisite catalytic efficiencies and selectivities under mild conditions, there is considerable appeal to employing these biological catalysts for

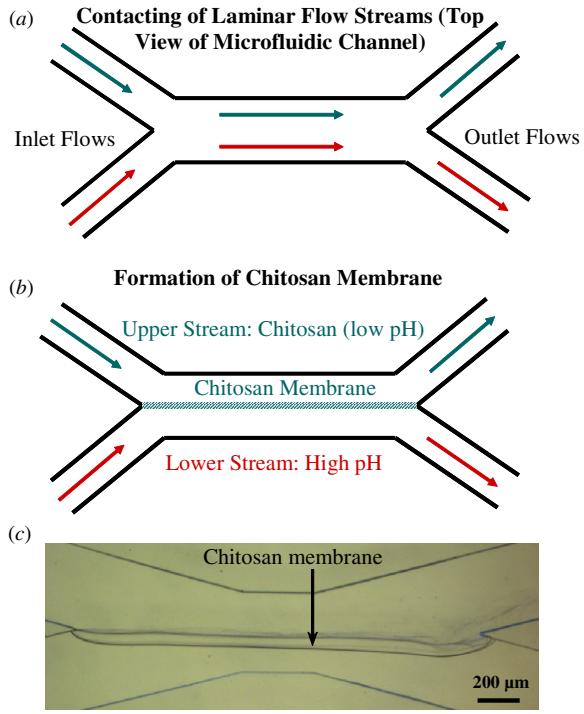
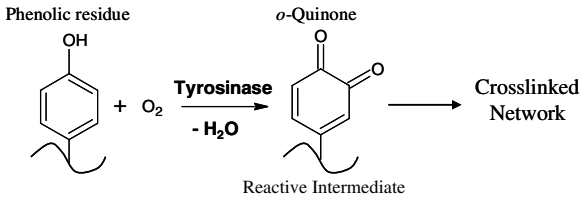


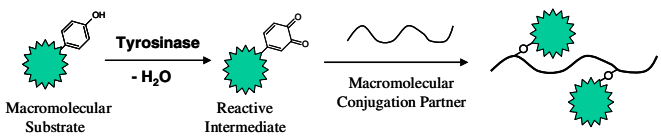

**Figure 10.** Directing assembly by imposing stimuli through the contacting of laminar flows in microfluidic channels. (*a*) Schematic of microfluidic contacting. (*b*) Schematic showing the contacting of a slightly acidic chitosan solution (upper stream) with a basic buffer (lower stream) to impose a pH gradient, induce a localized sol–gel transition, and form a chitosan membrane at the interface between the two streams. (*c*) Micrograph showing chitosan membrane formed within the microfluidic channels. Adapted from [116]. (Reproduced by permission of The Royal Society of Chemistry (http://dx.doi.org/10.1039/b916548g).)

construction at the nanoscale [123–126]. Despite the appeal, there are significant constraints to the broad utilization of enzymes for fabrication. First, an enzyme must be available to catalyze a desired reaction, and few enzymes are available that offer useful activities with inorganic or polymeric substrates. The common industrial enzymes that do act on polymers (e.g. proteases and cellulases) typically act on biological (versus synthetic) polymers and typically catalyze hydrolytic reactions that reduce molecular weight rather than build structure. A second constraint for *in vitro* enzymatic assembly is that the co-substrate requirements must be simple. This latter constraint precludes the use of the physiological enzymes used for protein, nucleic acid and polysaccharide biosyntheses because they require ATP or monomer activation. These constraints limit the options for *in vitro* enzymatic –assembly, and the commonly studied enzymes can be divided into two broad groups based on whether the intermediate is free or enzyme bound.

*4.2.1. Enzymes that generate a diffusible reactive intermediate.* A lock-and-key analogy is commonly invoked to explain the exquisite selectivities of enzymes—the substrate (key) fits specifically into the enzyme's catalytic site (lock). In contrast, there are some enzymes (often oxidative enzymes) that react with a broad range of substrates and generate diffusible intermediates. These enzymes serve functions such as initiation, degradation (e.g. peroxidases that degrade lignin), detoxification (e.g. cytochrome P450 enzymes in the liver) or adhesion (e.g. tyrosinases that initiate 'curing' of the mussel glue). In the latter example, tyrosinase uses molecular oxygen to oxidize accessible phenolic moieties (i.e. tyrosine or dihydroxyphenylalanine residues) of the mussel's adhesive protein. This adhesive protein has an open-chain structure which facilitates access to the tyrosinase enzyme [127, 128]. Oxidation converts the phenolic moieties into reactive *o*-quinones that can leave the enzyme's active site to undergo subsequent uncatalyzed crosslinking reactions.




As suggested by the above reaction, tyrosinase has simple co-substrate requirements ($O_2$), reacts with specific substituents (phenolic moieties) and activates these residues for subsequent reactions. Thus, tyrosinase offers the potential to initiate the covalent conjugation of macromolecules (e.g. proteins) onto other macromolecules or onto surfaces. Many of the reports of tyrosinase for *in vitro* conjugation examined substrates such as peptides [129–131], structural proteins (e.g. the silk fibroin [132–134]) or their derivatives (e.g. gelatin [135, 136]), non-protein polymers with phenolic substituents [137–140], or surfaces with phenolic moieties [141]. These examples indicate that tyrosinase-mediated coupling can be somewhat generic provided phenolic residues are present and accessible for enzymatic activation.




Many important functions of proteins (e.g. selective binding and catalysis) are performed by globular proteins, and the tyrosine residues of globular proteins are often not accessible for enzymatic activation. Thus, native globular proteins may not be amenable to tyrosinase-mediated coupling. If protein engineering methods can be applied, then short tyrosine-containing sequences can be 'genetically fused' to the protein (e.g. to the amino- or carboxy terminus) to permit tyrosinase-mediated conjugation [142]. Importantly, if the native protein lacks accessible tyrosine residues then the tyr-tag may provide a means to orient the protein by limiting conjugation through the fusion tag. To date, tyrosine fusion tags have been reported for the tyrosinase-mediated conjugation of the model green fluorescence protein (GFP) [142, 143], an IgG-binding protein (Protein G) [144] and enzymes responsible for bacterial quorum sensing [145, 146].

The 'conjugation partners' for reaction with the tyrosinase-activated intermediates typically possess nucleophilic moieties (e.g. sulfhydryl or amino groups) that can react with the electrophilic *o*-quinone residues. Many of the

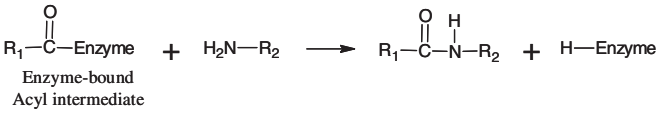
studies cited above employed chitosan since the primary amino groups of this polysaccharide readily react with *o*-quinones [147, 148]. In addition, tyrosinase-mediated grafting has been performed to anchor proteins to other amine-containing polymers such as polyethyleneimine [149] and polyallylamine [150].

While tyrosinase has been the most commonly studied enzyme for macromolecular assembly, other enzymes (e.g. laccases and peroxidases) that generate reactive intermediates could also be considered. For instance, phenolic moieties have been grafted onto gelatin [151], carboxymethylcellulose [152] and hyaluronic acid [153, 154] to enable peroxidase to oxidize these polymers to initiate crosslinking and gel formation.

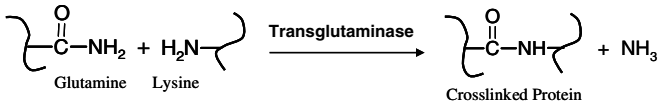
The advantages of enzymes that generate diffusible and reactive intermediates are that conjugation can be simple, rapid and reagentless. Further, these enzymes have broad substrate ranges that make this enzymatic strategy somewhat generic while still offering some selectivity. For instance, tyrosinase is residue specific (it activates only 1 of the 20 normal amino acids) but is not protein specific (and presumably not sequence specific).

The disadvantage of this enzymatic strategy is that the nonenzymatic reaction of the reactive intermediate cannot be precisely controlled. For instance, chitosan is a useful conjugation partner because the low pKa of chitosan's amines make them a preferential nucleophile for reaction with tyrosinase-generated *o*-quinones. However, the grafting location on the chitosan backbone or the number of grafted proteins per chitosan chain cannot be precisely controlled.

*4.2.2. Enzymes that perform catalysis with enzyme-bound intermediates.* Many enzymatic reactions involve enzyme-bound acyl intermediates. These enzymes include lipases and proteases that employ water at the active site to hydrolyze ester and amide linkages. In some cases, these enzymes can catalyze condensation reactions (e.g. in nonaqueous environments), and the potential of 'reversing' hydrolytic enzymes for polymer synthesis has attracted considerable attention [155, 156]. Two enzymes, transglutaminase and sortase, naturally employ acyl-bound intermediates for macromolecular synthesis and there is growing efforts to enlist these enzymes for biofabrication.




Transglutaminases catalyze transamidation reactions between lysine and glutamine residues of proteins to generate *N*-$\varepsilon$-($\gamma$-glutamyl)lysine crosslinks. The most commonly known transglutaminase (Factor XIIIa in blood) catalyzes the crosslinking of fibrin in the last stage of blood coagulation. More recently, a microbial transglutaminase (mTG) became available that is not derived from blood and is simpler to use [157–159]. This mTG can catalyze reactions with a broad range of proteins and does not appear to have strong sequence specificity. With respect to residue specificity, mTG appears to be specific for glutamine but not lysine as it can react with a broad range of primary amines provided these amines have a flexible alkyl linker [160]. Because mTG catalysis requires the residues to be accessible, many studies report reactions with open chain or structural proteins (e.g. gelatin [136, 161–164] and collagen [165–169]). Because the residues of globular proteins are often inaccessible [170], mTG catalysis has been achieved by either partially unfolding a globular protein [171, 172] or by engineering proteins with fusion tags [173–177].




Much of the research with mTG has focused on generating crosslinked networks for applications in food processing [178], biotechnology [179] and medicine [136, 165, 166, 180–186]. In addition to generating crosslinked networks, mTG is being used to site-selectively graft substituents to proteins using either small molecules [187, 188] or macromolecules [189–194], and to immobilize proteins to surfaces [176, 177].

Sortase is a bacterial transpeptidase enzyme that recognizes a specific peptide sequence of proteins and catalyzes their covalent attachment to cell wall peptidoglycan. Like mTG, sortase employs an acyl-bound enzyme intermediate that undergoes nucleophilic attack at the active site by an amine (typically the amine of an N-terminal glycine residue) [195, 196]. Also, like mTG, sortase has a relaxed specificity for the amine nucleophile which allows sortase to be used to generate various conjugates [197–205]. There is an emerging interest in enlisting sortases to assemble proteins to bead or flat surfaces for technological applications [200, 206].

*4.2.3. Enzymatic assembly on patterned surfaces and microfluidic devices.* Tyrosinase provides a simple means to conjugate proteins to chitosan. If the protein–chitosan conjugate retains chitosan's pH-responsive film-forming properties, then it can be electrodeposited [142]. The potential for sequentially electroaddressing the protein–chitosan conjugates was demonstrated using the chip shown in figure 11(*a*). In this study, tyrosinase was first used to conjugate a fluorescently labeled gelatin to chitosan and then the gelatin–chitosan conjugate was electrodeposited on the left electrode of a chip (figure 11(*b*)). Next, a GFP with a tyrosine tag was enzymatically conjugated to chitosan and the GFP–chitosan conjugate was electrodeposited on the right electrode as illustrated in figure 11(*c*). Thus, the combination of tyrosinase conjugation and electrodeposition allows the spatially selective sequential deposition of these two protein-chitosan conjugates [207].

In the above experiments, tyrosinase was used to conjugate proteins to soluble chitosan chains (at pH values near or below 6) and then the conjugates were electrodeposited. An alternative approach would be to electrodeposit the chitosan first and then conjugate the protein to the chitosan films [146]. This deposit-then-conjugate approach was used to assemble IgG antibodies through a bacterial IgG-binding protein, Protein G. Figure 12(*a*) illustrates that chitosan was first deposited onto one of the electrode addresses, then the Protein G was enzymatically conjugated through a tyrosine

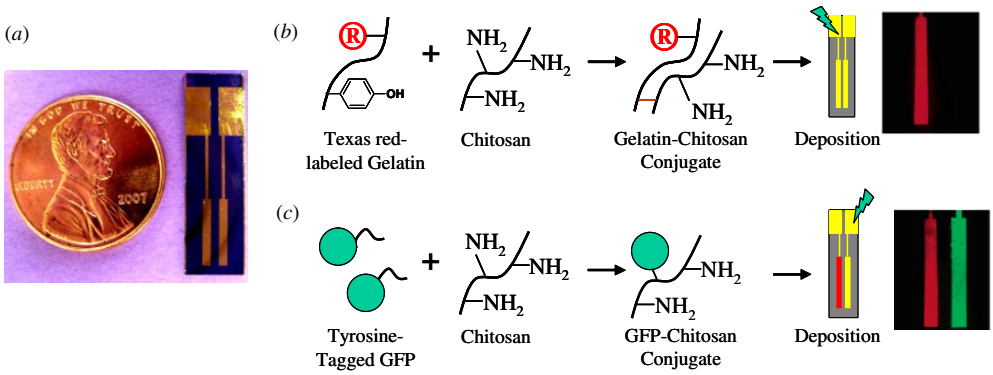



**Figure 11.** The sequential assembly of two proteins using tyrosinase-mediated conjugation and electrodeposition. (*a*) Chip with two electrically independent electrodes. (*b*) A fluorescently labeled gelatin is conjugated and electrodeposited on the left electrode address. (*c*) A GFP with a tyrosine tag is conjugated and electrodeposited onto the right electrode address. Adapted with permission from [207]. (Copyright 2005 American Chemical Society.)

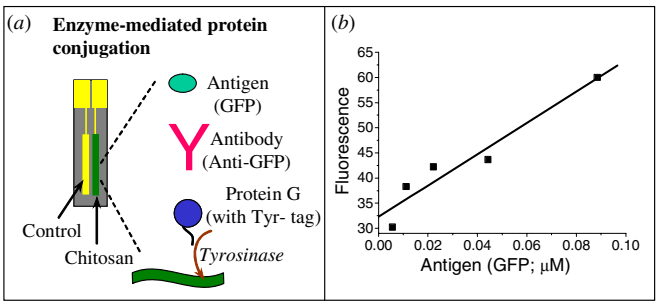



**Figure 12.** Antibody assembly on-chip address using chitosan electrodeposition followed by tyrosinase-mediated conjugation of Protein G. (*a*) Schematic illustrating assembly of anti-GFP antibody and subsequent binding of the GFP antigen. (*b*) Standard curve showing fluorescence intensity associated with antigen (GFP) binding versus antigen concentration in a solution. Adapted with permission from [144]. (Copyright 2009 John Wiley & Sons Inc.)

fusion tag. Antibodies were then bound to the assembled Protein G and for purposes of demonstration we used an antibody that recognizes GFP (anti-GFP). To demonstrate antigen binding, the chips with anti-GFP antibodies were contacted with varying levels of the GFP antigen. Figure 12(*b*) shows a reasonably quantitative relationship between fluorescence intensity of the chip and the antigen (i.e. GFP) concentration in the solution. These results suggest the potential of this simple assembly approach for functionalizing chips/devices for immunoanalysis [144].

A tyrosine tag has also been engineered to the carboxy terminus of an enzyme in the biosynthetic pathway for a low-molecular-weight bacterial signaling molecule (autoinducer II) [146]. This tyr-tag allows the biosynthetic enzyme to be conjugated to chitosan using tyrosinase and the enzyme–chitosan conjugate was electrodeposited onto an electrode address within a microfluidic device. This biofunctionalized device was then used to study the enzyme kinetics [110]. Ultimately the goal is to re-constitute metabolic pathways on-chip to allow the screening of inhibitors that could provide novel therapeutic agents (e.g. antimicrobials). This example illustrates that biofabrication may assist in realizing the potential of lab-on-a-chip devices for high throughput screening in drug discovery.

Tyrosinase and microbial transglutaminase (mTG) provide orthogonal methods for protein assembly as illustrated in figure 13(*a*). In this example, tyrosinase is used to conjugate gelatin to electrodeposited chitosan. Gelatin has an open-chain structure with a small number of tyrosine residues that are located at the terminus (in the telopeptide region) and thus gelatin serves as a tether for subsequent protein assembly as illustrated in figure 13(*a*). Gelatin also possesses multiple lysine and glutamine residues allowing the mTG-catalyzed assembly of either a glutamine-tagged GFP or a lysine-tagged RFP. The plot in figure 13(*b*) indicates that this orthogonal assembly approach yields high levels of protein assembly that exceed monolayer coverage [208] (monolayer protein coverage would be less than 10 pmole $cm^{-2}$).

The above example illustrates the use of mTG for assembling proteins at device addresses. An alternative motivation for using mTG is to confer structural or mechanical properties. For instance, mTG-catalyzed crosslinking has been coupled with molding to generate gelatin-based microfluidic channels that can be used for the localized cultivation of cells in microenvironments that may better mimic *in vivo* environments [209, 210]. Also, mTG has been used to generate a thermally stable gelatin-based optical waveguide that is biodegradable, biocompatible and disposable for biosensing and implantable devices [211].

### *4.3. Self-assembly*

The self-assembling capabilities of biology are well known and there have been extensive efforts to enlist or mimic biological materials for self-assembly [212, 213]. We briefly discuss self-assembly and especially focus on opportunities and issues for biofabrication.

*4.3.1. Some examples of self-assembly for biofabrication.* The self-assembling capabilities of polysaccharides to form films and hydrogels in response to various stimuli have been discussed above. While these three-dimensional networks are well known, the underlying molecular details of these

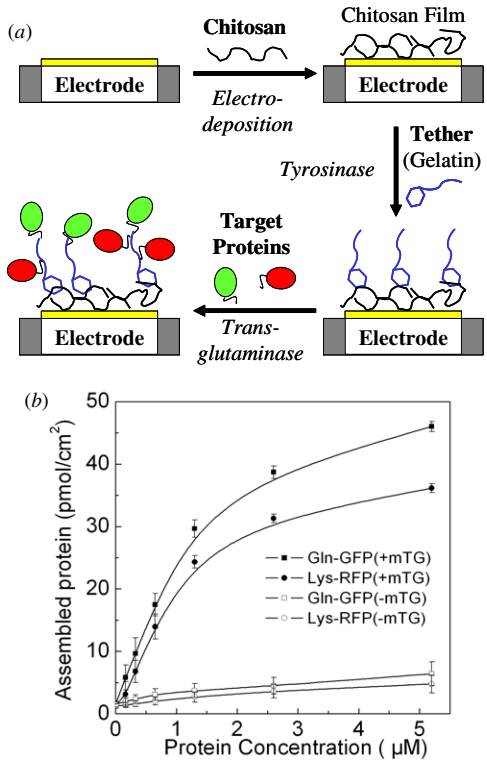



**Figure 13.** Orthogonal enzymatic assembly using tyrosinase to anchor the gelatin tether to chitosan and microbial transglutaminase (mTG) to conjugate target proteins to the tether. (*a*) Schematic of the procedure. (*b*) Quantitative analysis demonstrating significant protein assembly. Adapted with permission from [208]. (Copyright 2009 American Chemical Society.)

gels are often unclear. Lipids self-assemble into bilayers that are used in biology to compartmentalize and to organize and lipid-based structures (e.g. vesicles) may provide unique opportunities with fabricated chips and devices [75, 214, 215]. As mentioned earlier, the programmable self-assembly of nucleic acids (by base pairing) has also attracted interest for selectively assembling components to device addresses and for creating materials with precise three-dimensional structures [30, 31].

Amino acids, peptides and proteins offer many exciting opportunities for enlisting molecular recognition and self-assembly for biofabrication. Two classic examples are (i) the binding of the (strept)avidin protein to biotin that is widely used in biotechnology and (ii) the adhesion of cells through cell surface receptor binding to surfaces that present an arginine–glycine–aspartic acid (RGD) sequence. Especially important for integrating biology into devices is the opportunity to utilize genetic engineering approaches to facilitate assembly. In particular, amino acid residues, polypeptide sequences or entire protein domains can be genetically 'fused' to exploit specific recognition or stimuli responsiveness to guide assembly. For instance, the addition of short fusion tags (containing histidine or cysteine residues) allows the assembly of proteins onto a variety of surfaces [216–220]. Longer peptide sequences have been discovered or designed to extend the capabilities for selective binding [10, 221–224]. A common sequence is derived from the elastin protein and elastin-like polypeptides (ELPs) are commonly used to confer stimuli-responsive properties [32, 225–227]. For instance, ELPs have been conjugated or fused to proteins to allow proteins to be bound to, or released from, specific surface addresses in response to precise changes in temperature [228–231].

*4.3.2. Self-assembly on chips and devices.* There is a large and growing literature on the use of self-assembly to integrate biology with electronic devices. Here, we highlight two examples in which biological components are used for self-assembly while the ultimate products perform abiotic electrical functions. In these examples the self-assembling of biological components creates the desired nanoscale structures that serve as templates for subsequent metallization reactions that confer electronic conductivity.

The first example is the work from Technion-Israel Institute of Technology to use DNA templating for the self-assembly of molecular electronics [232, 233]. The upper scheme in figure 14 shows that in initial studies, double-stranded DNA (dsDNA) was assembled between two electrodes using sticky ends (single stranded segments) that allowed connection to separate electrodes by hybridization. This dsDNA served as a template for metallization by allowing the binding of silver ions and the subsequent reduction of these ions to metallic silver that conferred electronic conductivity [234]. In a subsequent study illustrated by the middle scheme in figure 14, the templated wire was patterned. Patterning was achieved using the self-assembly of RecA 'monomers' and a ssDNA probe to form a nucleoprotein filament composed of polymerized RecA and the ssDNA. This nucleoprotein is able to bind to the dsDNA with sequence specificity, and binding prevents this site from being metalized (i.e. the nucleoprotein serves as a sequence-specific resist). After metallization, the nanowire has an insulating gap between the conducting regions [235]. In a final study illustrated by the lower scheme in figure 14, a field effect transistor (FET) was created using an anti-RecA antibody to bind to the nucleoprotein and streptavidin-mediated binding to assemble a single-walled carbon nanotube (details not illustrated in figure 14) [236]. These studies demonstrate the potential of self-assembly for the fabrication of functional nanoscale components.

Viruses are a second example of self-assembling biological systems that are useful as templates for device fabrication [237, 238]. Specifically, *Tobacco mosaic virus* (TMV) has been used as a nanotemplate for the assembly of high surface area electrodes [239–241]. Figure 15(*a*) shows that TMV is a rigid rod consisting of about 2130 copies of a coat protein subunit (subunit molecular weight 17.5 kDa) stacked in a helix around its single strand of the plus-sense RNA, leaving a 4 nm diameter channel through the 300 nm long virion axis. Using an infectious cDNA clone of the virus genome, precise genetic modifications can be introduced to the virus coat protein [237]. One such modification, the introduction of a cysteine residue at the N-terminus of the coat protein (designated TMV1cys), imparts metal binding properties [238, 239]. Self-assembly of the TMV1cys coat proteins produces a rod-shaped virus

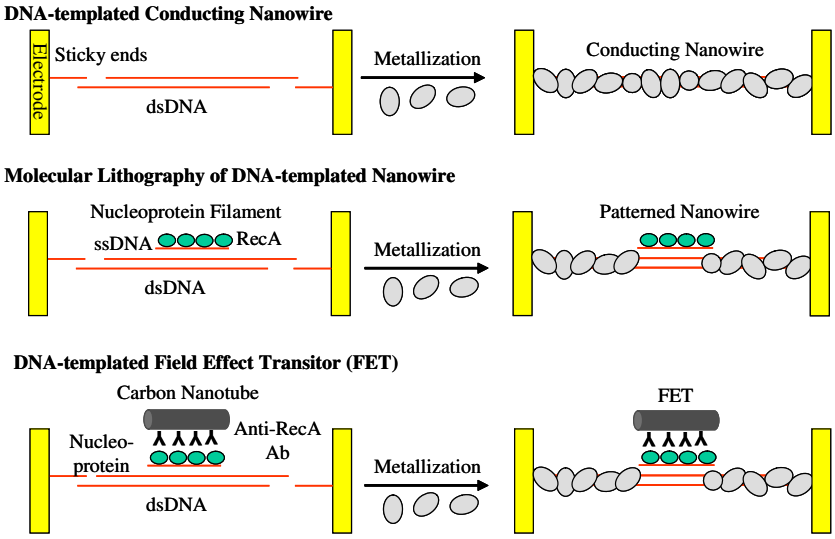



**Figure 14.** DNA-based self-assembly for molecular electronics. Upper scheme illustrates the use of DNA hybridization to establish a nanoscale connection between separate electrodes [234]. The middle scheme illustrates the use of biological components (the Rec A protein and a single stranded DNA probe) to pattern the wire through 'molecular lithography' [235]. The lower scheme illustrates how antibody binding allows the assembly of a single-walled carbon nanotube to generate a field effect transistor [236].

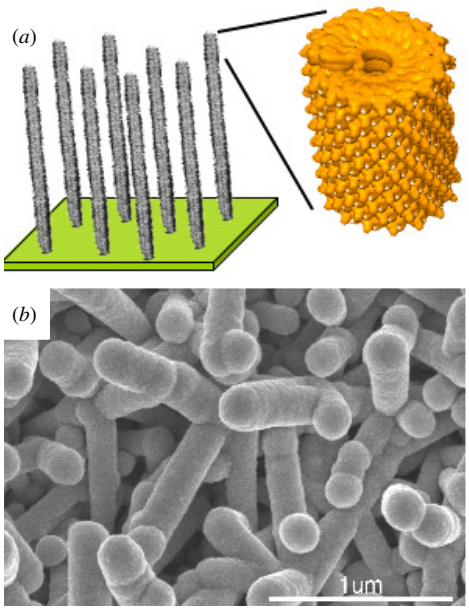



**Figure 15.** Self-assembly of virus particles for a nanostructured battery. (*a*) Schematic of the assembly of the tobacco mosaic virus (TMV) from thousands of copies of a coat protein subunit. The addition of a single cysteine residue to the coat protein subunit introduces recessed sites for metal ion binding along the length of the virus rod, while the end of the rod can bind to a metal surface. (*b*) SEM image showing a nickel-coated TMV1cys attached perpendicular to a gold-coated mica surface.

with a repeating pattern of cysteine residues that serve as metal binding sites. Along the length of the virus rod, the cysteine residues are solvent exposed and can bind metal ions but because these residues are recessed they cannot bind metal surfaces. In contrast, the cysteine residues at the 3′ end of the rod are accessible for surface binding which can orient the virus rod into a near vertical configuration as illustrated in figure 15(*a*). These unique metal-binding properties allow TMV1cys to be assembled perpendicular to a metal surface and then subsequently metalized along the entire virus template as shown by the scanning electron micrograph (SEM) in figure 15(*b*) [239]. These processes of surface assembly and metallization of virus rods yield a high surface area electrode. Since surface area is a key limiting factor in electrode capacity, TMV1cys-structured battery electrodes offer a significant enhancement in capacity [239, 240].

## 5. Conclusions

The effective integration of biology with microelectronics will have transformative impacts but will require a bridging of their divergent fabrication paradigms. Biological materials and mechanisms enable convergent approaches capable of spanning these construction paradigms. Integral to this biofabrication approach are stimuli-responsive materials that can recognize and respond to device-compatible signals for the *directed assembly* of biological components. *Biomolecular engineering* (e.g. through recombinant technology) can be enlisted to endow biological components with the information needed to guide assembly at the bio–device interface. Construction over a hierarchy of length scales can be achieved by *self-assembly* and *enzymatic assembly*. Over the last decade, a suite of biofabrication techniques emerged to couple biological components to electronic devices. More importantly, a growing number of research labs across the world began enlisting the unique capabilities of biological materials and mechanisms to build the bio–device interface. Over the next decade we anticipate further additions to the biofabrication toolbox and hope to see initial applications of devices that can effectively couple the capabilities of biology with the power of microelectronics.

## Acknowledgments

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