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# Reviews

## Biofabrication with Chitosan

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The traditional motivation for integrating biological components into microfabricated devices has been to create biosensors that meld the molecular recognition capabilities of biology with the signal processing capabilities of electronic devices. However, a different motivation is emerging; biological components are being explored to radically change how fabrication is achieved at the micro- and nanoscales. Here we review biofabrication, the use of biological materials for fabrication, and focus on three specific biofabrication approaches: directed assembly, where localized external stimuli are employed to guide assembly; enzymatic assembly, where selective biocatalysts are enlisted to build macromolecular structure; and self-assembly, where information internal to the biological material guides its own assembly. Also reviewed are recent results with the aminopolysaccharide chitosan, a material that offers a combination of properties uniquely suited for biofabrication. In particular, chitosan can be directed to assemble in response to locally applied electrical signals, and the chitosan backbone provides sites that can be employed for the assembly of proteins, nucleic acids, and virus particles.

### Introduction

In the past, the primary reason for integrating biological components into microfabricated devices was to enlist the molecular recognition capabilities of nucleic acids, enzymes, and antibodies to perform biosensing functions.[1−3] The coupling of these biosensing components with the signal processing capabilities of microfabricated devices allowed the creation of rapid and sensitive biosensors for detection and quantification (e.g., to diagnose disease). More recently, different biological components are being examined to perform a different function: to facilitate fabrication. Interest in biofabrication (the use of biological materials for fabrication) is driven by the opportunity to access a wider range of fabrication options for construction at the micro- and nanoscale.[4] Potentially, biofabrication may facilitate the fabrication of devices with reduced "minimum feature sizes", the integration of labile biological components into high throughput testing instruments, and the generation of biocompatible systems for implantation.

Traditionally, there are three general approaches to fabricate micro- and nanoscale features into materials. Photo-

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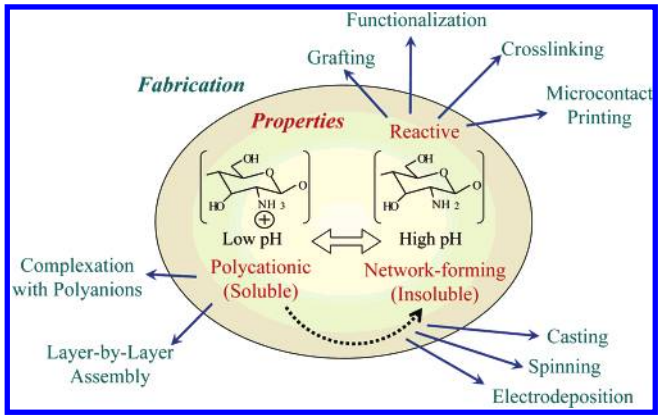


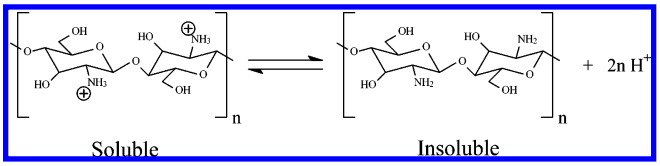

**Figure 1.** Schematic illustrating chitosan's versatility for fabrication. At low pH (less than about 6), chitosan's amines are protonated conferring polycationic behavior to chitosan. At higher pH (above about 6.5), chitosan's amines are deprontonated and reactive. Also at higher pH, chitosan can undergo interpolymer associations that can lead to fiber and network (i.e., film and gel) formation.

lithography is the primary approach used to fabricate highly organized surfaces in the microelectronics industry, and photolithographic patterning is based on applying localized optical stimuli to initiate selective photochemistries. An alternative patterning approach is soft lithography that includes a variety of techniques, one of which is microcontact printing.[5,6] In microcontact printing, a "molecular ink" is mechanically "stamped" onto a substrate, and a specific surface chemistry is often used to ensure the ink is retained at the stamped locations. Finally, dip-pen and related printing approaches emerged to mechanically deliver (i.e., "spot") biological probe molecules (particularly nucleic acids) onto slides for genome analysis. Once these biological probe molecules are spotted, coupling chemistries are often used to retain the probe at its specific address. A broad (admittedly oversimplified) generalization of these three traditional fabrication approaches is that they confer patterns and structures to materials by coupling *localized external stimuli* (optical or mechanical) with *specific chemistries*.

Interest in biofabrication is driven by the potential that a broader range of fabrication options can be accessed when biological materials are employed.[7−10] Well-recognized is the potential of biological materials to self-assemble. Information within their structure enables many biological materials to assemble over a hierarchy of length scales without the need for externally applied stimuli. However, biological materials offer additional opportunities for biofabrication. We review three fabrication options that are enabled by the use of biological materials: directed assembly, enzymatic assembly, and self-assembly. Also, we summarize recent results suggesting the aminopolysaccharide chitosan may be a key enabling material for biofabrication.

## Unique Properties of Chitosan for Biofabrication

In this section, we briefly summarize chitosan's unique properties for biofabrication (readers interested in chitosan's use in food, medical, and textile applications are referred to recent reviews[11−15]). Chitosan is a linear $\beta$-1,4-linked polysaccharide (similar to cellulose) that is obtained by the partial deacetylation of chitin. Because chitin deacetylation is incomplete, chitosan is formally a copolymer composed of glucosamine and *N*-acetylglucosamine. It is important to note that the term "chitosan" does not refer to a single well-defined structure, and chitosans can differ in molecular weight, degree of acetylation, and sequence (i.e., whether the acetylated residues are distributed along the backbone in a random or blocky manner). As a result of these structural differences, the properties of chitosan (e.g., the p$K_a$) can also vary somewhat. In the following, we consider the behavior of a typical chitosan with a degree of acetylation of 20% or less and a molecular weight on the order of 200 kDa. The unique structural feature of chitosan is the presence of the primary amine at the C-2 position of the glucosamine residues. Few biological polymers have such a high content of primary amines, and these amines confer important functional properties to chitosan that can be exploited for biofabrication.




The inner region of Figure 1 indicates that pH substantially alters the charged state and properties of chitosan. At low pH, these amines are protonated and positively charged, and chitosan is a water-soluble cationic polyelectrolyte. At high pH, chitosan's amines become deprotonated and the polymer loses its charge and becomes insoluble. Importantly, chitosan's p$K_a$ is near neutrality,[16−20] and the soluble−insoluble transition occurs at pHs between 6 and 6.5 which is a particularly convenient range for biological applications. In contrast, polylysine has a considerably higher p$K_a$ ($\approx$10) and exists only as a polycation at pHs where biological systems are stable. At high pH, chitosan's electrostatic repulsions are reduced allowing the formation of inter-polymer associations (e.g., liquid crystalline domains or network junctions) that can yield fibers, films, or hydrogels, depending on the conditions used to initiate the soluble−insoluble transition.[21] Finally, Figure 1 indicates that chitosan's amines are reactive allowing a range of chemistries to be employed to graft substituents to functionalize chitosan or to cross-link the chitosan backbone to confer elasticity.[22]

**Fabricating Chitosan Membranes, Films, and Three-Dimensional Structure.** The outer region in Figure 1 illustrates that chitosan's properties allow various methods to be used to fabricate membranes, thin films, and three-dimensional structures. Because chitosan can be dissolved under mildly acidic aqueous conditions, it can be readily cast into membranes and films that can be converted into insoluble networks by neutralization, as suggested by the dotted line in Figure 1. In addition to generating films and membranes, chitosan's pH-responsive solubility has also been used to fabricate three-dimensional scaffolds using rapid prototyping robotic dispensing.[23] Alternatively, cast chitosan membranes/films can be made insoluble over the entire pH-range by covalently cross-linking the chitosan chains, a procedure that is facilitated by the abundance of chitosan's reactive amines. In addition to casting films, microcontact printing methods have been used to stamp materials onto

**Table 1.** Recent Reports on Chitosan's Fabrication into Membranes, Films, and Three-Dimensional Structures

| fabrication approach and results | ref |
|---|---|
| solutions of chitosan and gold nanoparticles were cast into composite films for potential uses in trace analyses | 32 |
| colloidal solutions of carbon nanotubes and chitosan were cast onto an electrode, and then an enzyme was covalently tethered to the chitosan (using glutaraldehyde) for biosensing applications | 33 |
| solutions containing carbon nanotubes and a redox mediator (the mediator was covalently attached to the chitosan backbone using glutaraldehyde) were cast into films that permit NADH oxidation for biosensors, bioreactors or biological fuel cells | 34 |
| chitosan, an enzyme, and CdSe quantum dots were assembled layer-by-layer for biosensor construction | 35 |
| chitosan was spin-coated and covalently cross-linked into films for metal ion detection | 37 |
| chitosan and dextran sulfate were assembled layer-by-layer to create multilayer films with alternating pro-coagulant and anti-coagulant properties | 39 |
| chitosan and an anionic polysaccharide were assembled layer-by-layer to generate films that were able to undergo enzymatic hydrolysis (e.g. degradation) | 40, 41 |
| chitosan solutions were cast into films that were electrochemically patterned to alter optical, mechanical, and conducting properties | 42 |
| chitosan solutions were spin-coated and then stabilized lipid bilayers were deposited using the Langmuir−Blodgett trough method | 43 |
| chitosan and an azo dye were assembled layer-by-layer for light-induced storage | 177 |
| chitosan solutions were used for rapid prototyping robotic dispensing to construct a three-dimensional scaffold with a fully interconnected channel architecture | 23 |
| chitosan solutions were spin-coated and then plasticizer-assisted imprinting was used to create nanoscale topographical features | 178 |
| chitosan was microcontact printed onto glutaraldehyde-activated glass to provide patterned regions for cell binding | 26 |
| an anionic copolymer was microcontact printed onto a thin chitosan film to pattern regions that promote cell-adhesion (exposed chitosan) or resist cell-adhesion (exposed copolymer) | 24 |

chitosan films[24,25] or to stamp chitosan onto activated glass surfaces.[26] Finally, chitosan's polyelectrolyte behavior allows complexation with anionic polyelectrolytes (e.g., to create chitosan−DNA complexes), and layer-by-layer assembly (e.g., to create chitosan-based multilayer films).[27−31]

The ease of forming chitosan films, along with some of the unique properties conferred by chitosan has led to an explosive growth in the literature as highlighted in Table 1. In some instances, chitosan serves simply as a matrix to entrap components within the film's network. Examples include the entrapment of nanoparticles (e.g., quantum dots[32] and carbon nanotubes[33,34]) and biologically active components (e.g., enzymes).[35] In other instances, chitosan confers unique functional properties to the films, and the versatility

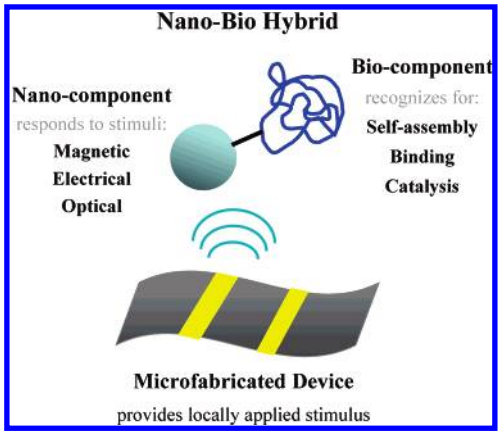



**Figure 2.** Hybrid materials can be directed to assemble if one component (e.g., the nano-component) can respond to an external stimulus. Microfabrication allows devices to be constructed that can apply a variety of external stimuli with spatial and temporal control.

of this aminopolysaccharide is illustrated by a few such examples. Chitosan's metal binding properties allow thin films to be exploited for metal detection.[36,37] Layer-by-layer (LbL) assembly of chitosan with dextran sulfate allows the construction of multilayer films with alternating procoagulation (based on chitosan) and anti-coagulation (based on dextran sulfate) properties.[38,39] Chitosan-based polyelectrolyte films can also be "dis-assembled" (i.e., degraded) by chitosan-hydrolyzing enzymes.[40,41] The ease with which chitosan's amines undergo reaction allows flexible films to be electrochemically micropatterned.[42] Langmuir−Blodgett methods have been utilized to assemble supported lipid bilayers onto chitosan films.[43] To summarize, chitosan's versatility is attracting growing attention in thin film applications for environmental, medical and consumer purposes.

## Directed Assembly

As mentioned in the Introduction, photolithography, soft lithography, and printing approaches rely on localized external stimuli to create spatial order. In these conventional fabrication approaches, the external stimulus is either optical (for photolithography) or mechanical (for soft lithography or printing). There is an emerging trend to use a broader range of external stimuli to direct components to assemble and to guide spatial ordering. As suggested in Figure 2, this trend is emerging because of the increasing capabilities to construct hybrid materials that consist of one component (typically an inorganic nanoparticle) that confers responsiveness to a specific stimulus (e.g., magnetic).

Currently, hybrid materials composed of nanoparticles and biological components are under intense study for various applications (e.g., medical imaging or therapeutic intervention).[10,44] We cite only two examples to illustrate the potential of hybrid materials to direct or detect assembly. First, Lvov and co-workers used layer-by-layer (LbL) assembly to create core−shell particles with a co-immobilized enzyme and magnetic nanoparticle. These hybrid particles retained both their biocatalytic activity and their responsiveness to locally applied magnetic fields.[45] Second, Willner and co-workers created a hybrid material from a biological sensing component and a magnetic particle. This hybrid allowed biomolecular recognition to be detected by the magnetome-

chanical deflection of a cantilever.[46] Although these examples illustrate the potential of directing the assembly (or delivery) of bio-nano hybrids, there are also exciting advances in the synthesis of such bio-nano hydrids. In particular, molecular biological methods are being applied to discover/evolve peptide sequences that allow biological components (e.g., proteins, virus particles, or cells) to selectively recognize and couple to nanoparticles.[47−50]

In the above paragraph, we cited two examples in which externally applied magnetic stimuli were used to guide assembly. Even more convenient are electrical stimuli that can also be applied with high spatial and temporal control. Further, biological components predictably respond to localized electrical stimuli. Nucleic acids are anionic polyelectrolytes and electric fields are routinely used to guide their migration for electrophoretic separations. Proteins are zwitterionic and electric fields can be used to "focus" them at their isoelectric point.

**Electrodeposition: Chitosan's Directed Assembly in Response to Electrical Stimuli.** One of chitosan's key assets for microfabrication is its unique response to applied electrical stimuli. Specifically, chitosan can be electrodeposited at a cathode surface by the mechanism illustrated in Figure 3a. When the applied voltage is sufficient for protons to be reduced at the cathode surface, then a localized pH gradient is generated.[51] If this localized gradient is created in the presence of chitosan (i.e., if the electrodes are immersed and biased in a slightly acidic chitosan solution), then chitosan chains that experience the high localized pH at the cathode surface can deposit as a thin film. To our knowledge, there are no reports of the nano- and microscale morphologies of the electrodeposited chitosan.

Figure 3b shows representative results indicating that the thickness of the electrodeposited chitosan film can be varied depending on deposition conditions.[52] Interestingly, when high current densities are imposed to generate a high localized pH that is expected to extend further from the cathode surface, chitosan deposits as a thick (several mm) hydrogel rather than a thin film.[53] In summary, chitosan's pH-responsive properties allow it to be directed to assemble (i.e., to electrodeposit) in response to locally applied electrical stimuli.

Whereas Figure 3b shows that chitosan can be controllably electrodeposited normal to the cathode surface (i.e., in the $z$ direction), it is also possible to control chitosan's deposition laterally (i.e., in the $x-y$ directions). In particular, chitosan can be deposited onto electrodes that have been microfabricated onto standard substrates (i.e., silicon wafers) and that have arbitrarily complex surface patterns. This microscale electrodeposition that is illustrated in Figure 3c, which shows the deposition of a fluorescently labeled chitosan onto 20 $\mu$m gold electrodes, is achieved with high lateral resolution.[54]

The potential utility of chitosan's electrodeposition can be illustrated by a couple of examples. First, Figure 4a illustrates that chitosan can mediate the spatially selective assembly of nanoparticles that are suspended in a chitosan solution. To demonstrate this capability, 100 nm fluorescent latex spheres were suspended in a 1% chitosan solution at pH 5. Figure 4b shows the silicon wafer fabricated with 20

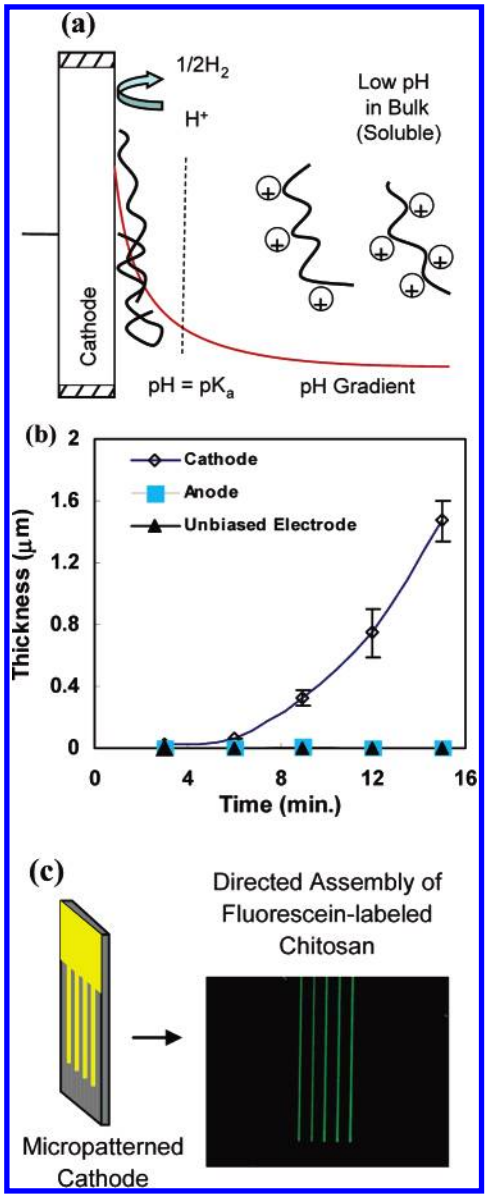



**Figure 3.** Directed assembly of chitosan in response to locally applied electrical stimuli. (a) Mechanism for chitosan's directed assembly (i.e., electrodeposition).[53] (b) Thickness of deposited chitosan can be controlled by deposition conditions (results for deposition at an applied voltage of 2.5 V from a 1% chitosan solution with a bulk pH of 5[52]). (c) Deposition is spatially selective as evidenced by deposition of fluorescently labeled chitosan onto micropatterned 20 $\mu$m gold electrodes (results for 2 min deposition at current density of 1 A/m$^2$ from a 0.8% labeled-chitosan solution with a bulk pH of 5.6[54]).

$\mu$m patterned gold lines that was immersed in the chitosan-containing suspension and biased to serve as the cathode (the anode in this experiment was an unpatterned gold-coated wafer). The fluorescence photomicrograph and image analysis in Figure 4c show that the 100 nm particles were assembled onto the cathode surface with high lateral resolution (i.e., in the $x-y$ directions). Control experiments demonstrate that chitosan is required for nanoparticle assembly, whereas further analysis indicated that the nanoparticles are entrapped throughout the chitosan matrix (i.e., in the $z$ direction). Potentially chitosan-mediated electrodeposition provides a means to assemble nanoscale particles into higher-order structures, a requirement that is necessary to exploit many of the unique properties of nanoparticles.[55]

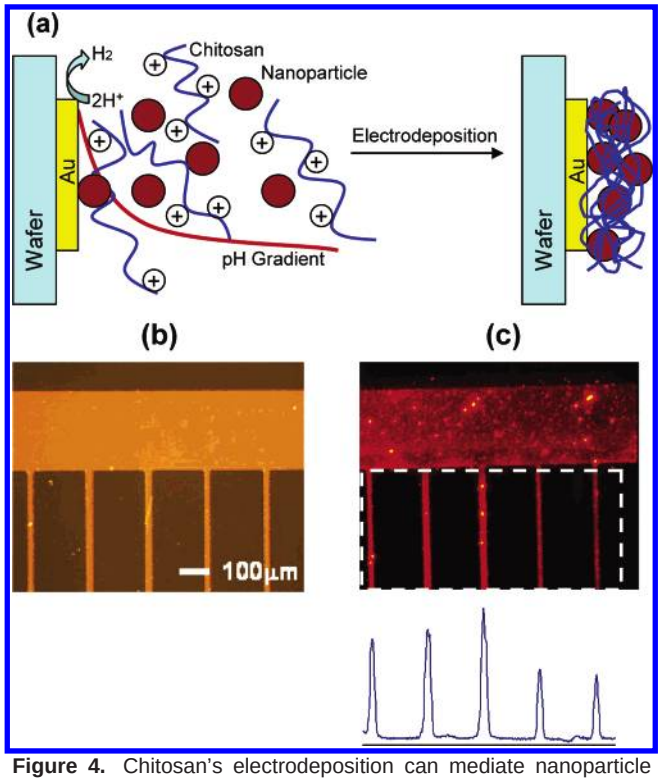



**Figure 4.** Chitosan's electrodeposition can mediate nanoparticle assembly. (a) Mechanism of chitosan-mediated nanoparticle deposition at the cathode. (b) Photomicrograph of silicon wafer patterned with 20 $\mu$m gold lines that were biased for deposition. (c) Fluorescence photomicrograph showing spatially localized deposition of 100 nm fluorescent latex spheres from a 1% chitosan solution (pH = 5).[55]

In a second example, Chen and co-workers used chitosan-mediated electrodeposition to provide a simple means to assemble components for a glucose biosensor. For one biosensor, they dispersed gold nanoparticles (17 nm) in a solution containing chitosan (0.5%) and the enzyme glucose oxidase (5 gm/L) and then electrodeposited to form a hydrogel-based nanoparticle-enzyme "biocomposite". The authors report that the gold nanoparticles enhanced the enzyme's stability and facilitated the electrochemical detection of the $H_2O_2$ reaction product.[56] The authors assembled a second glucose biosensor by dispersing carbon nanotubes (0.5 g/L) into a chitosan solution (1%; pH 5), adding glucose oxidase (5 gm/L) to the solution, and then electrodepositing (10 min at 3 V) to generate a chitosan film containing both carbon nanotubes and the enzyme. The authors report the carbon nanotubes were homogeneously distributed throughout the film and catalyzed $H_2O_2$ reactions.[57] In both cases, chitosan's electrodeposition provided a simple one-step method to assemble the components, and the method was sufficiently mild to ensure retention of enzyme activity.[56,57]

In summary, the application of localized signals to direct assembly is particularly promising because (i) microfabrication allows devices to be created that can impose a range of stimuli (e.g., electrical, optical, mechanical, and magnetic) with high spatial and temporal control and (ii) various materials (e.g., nanoparticle hybrids) can now be synthesized to respond to these stimuli. Chitosan is a particularly promising material because it responds to locally imposed electrical signals by depositing as a thin film (or thicker hydrogel), and this directed assembly can be controlled spatially and temporally. Further, the above examples illustrate that chitosan deposition can be used to mediate the assembly of additional components (e.g., nanoparticles and enzymes). Finally, it is important to note that chitosan's deposition is not confined to two-dimensional surfaces, as recent results show that chitosan's deposition can be extended to the base and sidewalls of microfluidic channels.[58,59]

## Enzymatic Assembly

As mentioned in the Introduction, traditional microfabrication methods create patterns and structure by employing external stimuli and specific chemistries. In principle, enzymes should be well-suited for microfabrication because they catalyze chemical reactions with exquisite selectivity and they impose less-burdensome requirements on fabrication (enzymes function in aqueous solution and do not require the extreme purities or extensive clean-room facilities common to traditional microfabrication). However, the selectivity of enzymes limits their use: many synthetic materials are not acted-upon by enzymes (that is why many synthetic polymers are not biodegradable). The most obvious way to enlist enzymes for biofabrication is to use biological materials that are substrates for enzymes.

As illustrated in Table 2, there have been some efforts to exploit biological materials and enzymes for fabrication, and many of these efforts have focused on hydrolytic enzymes.[60] For instance, peptides and proteins have been used as temporary fabrication aids (e.g., as scaffolds[61] or resists[62]) because proteases can chemoselectively remove these materials without damaging the desired, fabricated structure. There have also been efforts to combine the chemical selectivity of enzymes with the spatial resolution of the atomic force microscope (AFM) for nanolithographic patterning of lipid bilayers,[63] surface-bound peptides,[64] and a self-assembled monolayer (SAM) of oligonucleotides.[65]

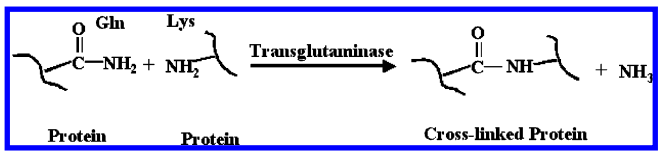
A second general approach for enlisting enzymes for fabrication is to access the extensive "toolbox" available for manipulating nucleic acids. In addition to the ability of nucleic acids to self-assemble (i.e., to undergo programmable hybridization), they can be manipulated by a variety of proteins. Table 2 lists some unique fabrication capabilities that are enabled by these enzymes and binding proteins. Probably the most thoroughly developed example enlisted homologous recombination with the RecA protein from *E. coli*.[66] In these studies, RecA polymerization on a single-stranded DNA provides a nucleoprotein filament that can recognize and bind to a homologous sequence (i.e., a specific address) on a double-stranded DNA (dsDNA) scaffold. The bound nucleoprotein filament acts as a sequence-specific resist that protects the underlying dsDNA scaffold from subsequent metallization steps that assemble gold onto the DNA scaffold. Homologous recombination thus provides a means to pattern insulating gaps into conducting nanowires.[67] In additional studies, the nucleoprotein filament was used to localize a single-walled carbon nanotube to fill the insulating gap and ultimately to yield a field-effect transistor.[68]

The final entry in Table 2 is an example in which a motor protein was integrated into a microfabricated device. Although this protein is not being used for fabrication (the topic

**Table 2.** Examples of the Use of Enzymes for Fabrication

| enzyme | use | ref |
|---|---|---|
| | **Hydrolytic Enzymes** | |
| protease | to digest gelatin resist and to selectively etch | 60 |
| protease | to digest a peptide scaffold after it had been used as a template for the construction of a silver nanowire | 61 |
| protease | to digest a gelatin resist without destruction of an assembled nucleic acid probe or polysaccharide sublayer | 62 |
| phospholipase $A_2$ | to pattern supported lipid membrane gel for "enzyme-assisted nanoscale lithography" | 63 |
| protease | to pattern surface-bound peptides with a protease tethered to an AFM tip for "enzymatic lithography" | 64 |
| DNase I | to pattern a self-assembled monolayer of oligonucleotide using an AFM tip inked with DNase and then activating the written DNase with $Mg^{2+}$ for "enzymatic nanolithography" | 65 |
| amylase | to destabilize and precipitate starch-stabilized Au nanoparticles | 179 |
| acetylcholinesterase | to convert acetylthiolcholine into thiolcholine that can self-assemble onto micropatterned gold surface | 180 |
| | **Enzymes/Proteins that Act on Nucleic Acids** | |
| RecA | to provide a sequence-specific resist for spatially selective DNA metallization ("sequence-specific molecular lithography") | 67 |
| RecA | to create a field-effect transistor using homologous recombination to provide a sequence-specific resist and a means to localize a single walled carbon nanotube | 68 |
| DNA methyltransferase | to introduce sequence-specific conformational perturbations to DNA | 181 |
| restriction endonuclease and DNA ligase | to program DNA self-assembly using a restriction endonuclease to de-protect and create cohesive ends for hybridization, and then DNA ligase to covalently connect the strands ("programmed enzymatic self-assembly of DNA") | 182 |
| Taq DNA polymerase | to tether oligo d(A−T)s to surfaces by polymerization of dATP and dTTP in the absence of added primer/template | 183 |
| | **Oxidation/Reduction Enzymes** | |
| horseradish peroxidase | to pattern conducting polymers by combining dip pen nanolithography (to write the monomer onto a surface) with enzyme-catalyzed polymerization | 184, 185 |
| horseradish peroxidase | to polymerize aniline onto a DNA template | 186 |
| alcohol dehydrogenase | to enzymatically generate NADH that mediates the shape-selective growth of nanoparticles | 187 |
| | **Motor Proteins** | |
| $F_1$-ATPase | to power a nano-device using biomolecular motor (the motor was engineered through rDNA to selectively interface with the device through His fusion tags) | 71 |

of this review), its use represents an exciting effort to exploit biological systems to transduce chemical energy (in the form of ATP) into mechanical function.[69] This example does however illustrate the potential of harnessing molecular biology to facilitate assembly. Specifically, the $F_1$-ATPase was engineered to have a histidine tag[70] that allowed the protein to be oriented onto the fabricated nickel posts of their device.[71,72] Further, an allosteric site based on zinc binding was incorporated to regulate motor function.[73]

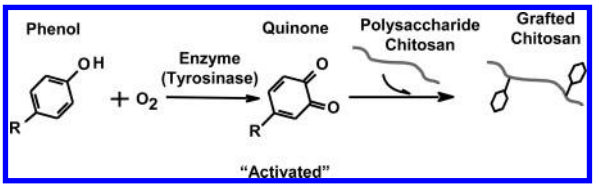



In most of the examples in Table 2, the enzymes either catalyze hydrolytic reactions that cleave structure (i.e., reduce molecular weight) or require complex cofactors (e.g., ATP) or activated substrates (*S*-adenosylmethionine) to build structure. It would be desirable if simple, noncofactor-requiring enzymes were available to build macromolecular structure; however, few candidate enzymes are known. One candidate is transglutaminase that catalyzes transamidation reactions between the glutamine and lysine residues of proteins.

This single enzymatic activity has been used to site-selectively modify proteins[74−78] and peptides,[79] and thus enables control of macromolecular architecture. Also, transglutaminases have been used to catalyze covalent cross-linking reactions[80−87] that confer mechanical properties (i.e., elasticity). Since these networks are constructed under mild conditions, labile biological components (viable cells[88] and biologically active peptides[89−94]) can be incorporated into these matrixes without loss of biological function. Interestingly, while transglutaminase is quite effective in cross-linking open chain proteins (when Lys and Gln residues are

accessible), it is less able (or unable) to react with globular proteins[95] unless their native structures are partially unfolded.[96−98] This apparent limitation allowed Nagamune and co-workers to engineer proteins to have N-terminal fusion tags with accessible residues for transglutaminase-catalyzed reactions. Proteins with these fusion tags were enzymatically conjugated to yield homo- and heterodimeric proteins[99,100] and functional heterodimers.[101,102] Although the above studies were not focused on microfabrication, they demonstrate that this single noncofactor-requiring enzymatic activity allows complex macromolecular architectures to be constructed, important mechanical properties to be conferred, and labile biological functions to be added. Further, molecular biological methods could be employed to construct specific sites for transglutaminase catalysis. Not surprisingly, transglutaminase's potential is beginning to attract attention for microfabrication applications.

A second, noncofactor-requiring enzymatic approach for building macromolecular structure is based on the enzyme tyrosinase. Tyrosinases (and related phenol oxidases) are oxidative enzymes that convert a broad range of phenols into *o*-quinones. Tyrosinases are simple to use because molecular oxygen is the oxidant and no complex cofactors are required. The *o*-quinones that are produced are quite reactive and after diffusing from the enzyme's active site can undergo a cascade of uncatalyzed reactions. In nature, this enzyme is responsible for the enzymatic browning of foods, the hardening of insect cuticles, and the setting of the mussel's water resistant adhesive protein.[103−106] Previous studies have also shown that tyrosinase-generated *o*-quinones can react with the reactive amines of chitosan to create grafted and cross-linked chitosan derivatives.[107−114]




**Conjugation: Enzymatic Assembly of Proteins onto Chitosan.** Figure 5 shows two important features of the tyrosinase-initiated grafting to chitosan. The scheme in Figure 5a illustrates that tyrosinase's substrate range is not limited to low molecular weight phenols, but rather this enzyme can oxidize accessible tyrosine residues of proteins. Thus, tyrosinase serves to "activate" proteins for their assembly (i.e., conjugation) onto chitosan.[115]

The second feature of tyrosinase-initiated conjugation is that it offers the potential of conferring chitosan's pH-responsive properties to proteins.[116] This potential is demonstrated by studies with a green fluorescent protein (GFP) that was constructed to have an N-terminal hexa-histidine tag (to facilitate purification from cell extracts) and a C-terminal penta-tyrosine tag (to provide additional tyrosine residues for tyrosinase-initiated conjugation). Figure 5b shows that, when this His-GFP-Tyr protein was studied as a control, it was observed to be soluble at varying pHs and its fluorescence was independent of pH. When the His-GFP-Tyr protein was conjugated to chitosan using tyrosinase, the

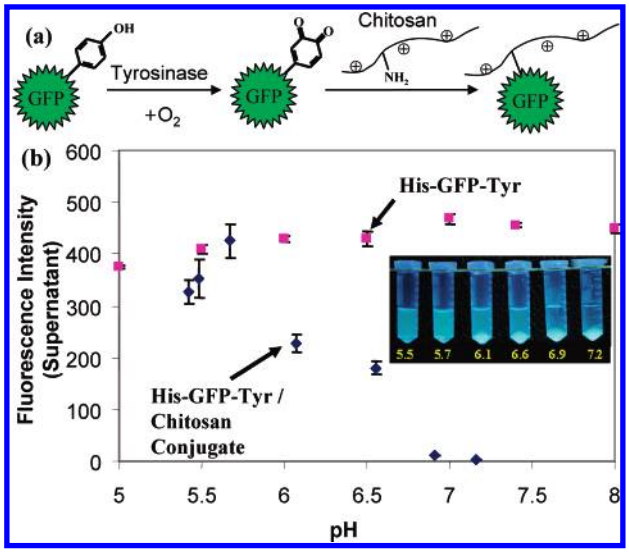



**Figure 5.** Enzymatic assembly of proteins onto the chitosan backbone. (a) Schematic of the tyrosinase-initiated enzymatic assembly of proteins onto chitosan (i.e., conjugation). (b) Conjugation can confer chitosan's pH-responsive solubility to the model green fluorescent protein (GFP). The unconjugated control (designated "His-GFP-Tyr") is soluble over the entire pH range, whereas the conjugated protein is soluble at low pH but becomes insoluble at higher pHs. Insert shows that increasing pH leads to the formation of a fluorescent precipitate for the conjugate of His-GFP-Tyr and chitosan.[117]

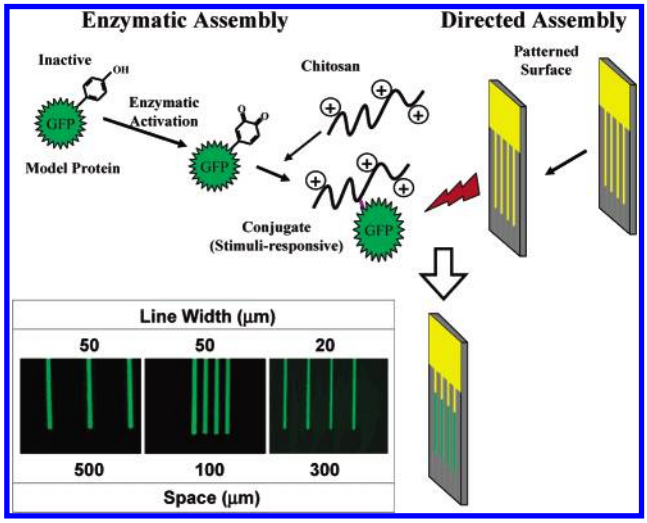



**Figure 6.** Coupling directed assembly with enzymatic assembly to selectively assemble a protein at a microfabricated address. Schematic showing that conjugation requires enzymatic activation, electrodeposition requires an imposed electrical signal, both provide added control to protein assembly. Fluorescence photomicrographs show the assembly of the GFP−chitosan conjugates onto micropatterned cathodes of varying sizes and spacing.

GFP-chitosan conjugate was observed to be soluble at low pH but to precipitate as the pH was raised near chitosan's p$K_a$. The conjugate's pH-responsive behavior is illustrated in Figure 5b by the series of fluorescence photographs and the plot of the supernatant's fluorescence versus pH.[117]

**Coupling Chitosan's Enzymatic and Directed Assemblies.** Tyrosinase provides the opportunity to assemble proteins onto the chitosan backbone, and the protein−chitosan conjugates can offer the pH-responsive properties that allow for their electrodeposition. This coupling of enzymatic and directed assembly is illustrated in Figure 6 which shows that chitosan is uniquely suited to "interface"

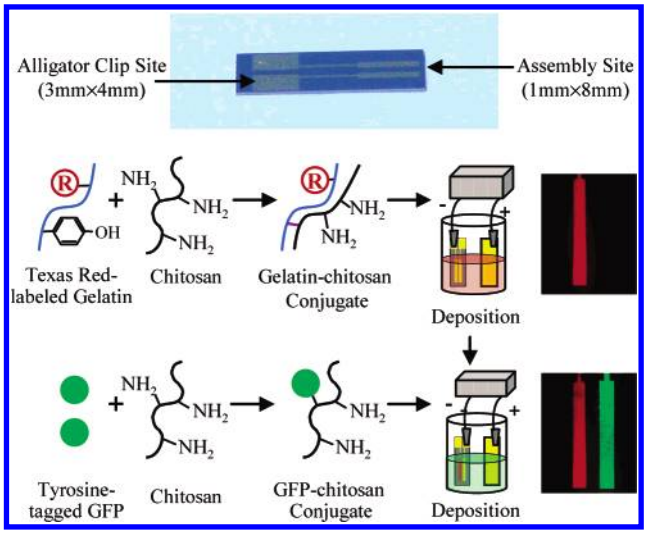



**Figure 7.** Sequential, signal-guided assembly of two proteins at different addresses. Upper photograph shows the "chip" was patterned to have two electrically independent addresses (the assembly site for each gold surface was 1 mm wide by 8 mm long). Upper reaction scheme shows initial step of conjugation and deposition of Texas-Red-labeled gelatin. Reaction scheme at the bottom shows second step of conjugation and deposition of GFP. The right-most images are fluorescence photomicrographs after each deposition.[118]

proteins to microfabricated devices. The left side of the schematic of Figure 6 shows that chitosan's reactivity allows conjugation through the protein's activated *o*-quinone residue. Activation is required for this "enzymatic assembly" as GFP is not conjugated to chitosan in the absence of tyrosinase. Chitosan confers stimuli-responsive properties to the conjugate, thus allowing localized electrical stimuli to direct the conjugate's assembly onto microfabricated surfaces. The fluorescence photomicrographs of Figure 6 show that the GFP−chitosan conjugate can be assembled onto gold-patterned cathodes of varying widths and spacing. Additionally, the fact that GFP retains its fluorescence provides evidence that this protein's tertiary structure is unaffected by the combination of tyrosinase-mediated conjugation and electrodeposition. This combination of enzymatic and directed assembly provides a simple and potentially generic means to guide the spatially selective assembly of proteins in response to locally applied external signals.[117]

Chitosan not only allows localized electrical stimuli to be enlisted for spatially controlled assembly but it also allows temporally controlled assembly. This spatial-temporal control is demonstrated by the results in Figure 7 where two proteins were sequentially assembled at separate addresses. The upper photograph in Figure 7 shows that the "chip" in this study is patterned to have two electrically independent gold surfaces on a silicon substrate. For deposition, the chip is partially immersed such that the assembly site is submerged in the deposition solution and the lead is connected to the power supply using an alligator clip. The first protein to be assembled was a gelatin that had been labeled with the fluorescent dye Texas Red. As illustrated in the upper-most reaction scheme to Figure 7, this fluorescently labeled gelatin was enzymatically conjugated to chitosan, and then the gelatin−chitosan conjugate was directed to assemble onto the left electrode. The fluorescence photomicrograph shows the Texas Red-labeled gelatin−chitosan conjugate only deposited on this left electrode. After rinsing, the chip was immersed in a solution containing the GFP−chitosan conjugate and this conjugate was assembled on the right electrode. The fluorescence photomicrograph shows that the gelatin−chitosan conjugate was not displaced from the left electrode, while the GFP−chitosan conjugate was selectively assembled onto the right electrode. This result demonstrates chitosan's potential for the sequential, signal-guided assembly of proteins from solution to individual addresses.[118]

**Broader Perspective of Enzymatic Assembly.** Results with transglutaminase and tyrosinase illustrate the considerable potential offered by noncofactor-requiring enzymes that can build macromolecular structures. Unfortunately, few such enzymes are known and they typically fall into two categories. First are enzymes that confine reactions to the active site (e.g., transglutaminase). These enzymes offer high selectivities that limit their substrate range (a disadvantage), but since they confine reactive intermediates to the active site they minimize uncatalyzed reactions that can generate undesired byproducts. A remarkable feature of transglutaminase is that its enzyme-bound intermediate (i.e., the acyl-enzyme intermediate) is efficiently attacked by an amine (i.e., the $\epsilon$-amine of lysine), as compared to attack by water that would lead to an unproductive hydrolytic reaction. The tendency for acyl-bound intermediates to be hydrolyzed in aqueous environments limits the ability of lipases and proteases to catalyze condensation reactions that could build macromolecular structure. In contrast to transglutaminase, the second category includes tyrosinases, peroxidases, and laccases that generate reactive intermediates that can diffuse away from the enzyme's active site and undergo further, uncatalyzed reactions. These enzymes offer the advantage that they can in situ activate a broad range of substrates for further reaction but have the disadvantage that controlling the subsequent nonenzymatic reactions can be problematic. The fact that these activated intermediates are electrophilic enables them to react with chitosan's reactive amines and provides the opportunity of creating pH-responsive conjugates.

In summary, the integration of enzymes into microfabrication offers exciting possibilities and a handful of reports have begun to appear. As more groups begin fabricating with enzymes, it seems likely that molecular biology will be used to improve the capabilities of known enzymes or to evolve enzymes with entirely new activities. Potentially, if simple cofactor regenerating systems become widely available, then it may be possible to employ ATP- and NADH-requiring enzymes for "routine" biofabrication.

## Self-Assembly

Self-assembly is probably the feature of biology that has most captured the imagination of those who fabricate at the micro- and nanoscale. The coupling of self-assembly with traditional organic chemistry led to the use of both covalent and noncovalent interactions to "synthesize" supramolecular structures over a broad range of length scales. Because supramolecular structures reversibly form, they can self-fabricate, correct errors, and heal.[119,120] More recent efforts to couple self-assembly with microfabrication could have

revolutionary technological effects. Conventional top-down microfabrication is having increasing difficulty keeping pace with the relentless reductions in feature sizes required to meet the insatiable demand for greater computational power.[121,122] Many biological structures self-assemble at attractive size scales, and these materials could serve as building blocks or templates for fabrication,[123,124] or as models to mimic.[125]

Lipid bilayers are probably the simplest self-assembled structures in biology, and they have sparked various technical applications. They have been used to template[126,127] or to localize components either within the membrane bilayer[128,129] or within an internal aqueous compartment(s).[130−133] There are even efforts to use lipids to construct soft microfluidic networks.[134] Lessons from lipid self-assembly are also being mimicked in the construction of block polymers that can phase separate to form domains of various thickness, that form stronger interfaces, and that confer long range order.[135]

Nucleic acids offer a range of capabilities useful for fabrication. As mentioned earlier, nucleic acids have been used as templates for metallization to create nanoscale wires and field effect transistors.[66,136,137] DNA self-assembly (i.e., base-pairing) is programmable and predictable allowing DNA-based materials to be engineered to assemble into complex architectures (e.g., dendrimers) over a range of length scales and to perform mechanical actions.[138−141] Finally, DNA has been conjugated to nanoparticles to allow DNA's programmable assembly to be employed for the thermally reversible formation of nanoparticle networks.[142−144]

Protein self-assembly also offers a remarkable array of opportunities even in the absence of a solution to the "protein-folding problem".[8] Despite an inability to predict the self-assembly of large polypeptides, there are growing efforts to exploit predictable secondary structural elements (e.g., α-helices and $\beta$-sheets) to generate self-assembling peptide-based materials.[145−158] Further, there are important examples of known protein systems that self-assemble into useful structures, eliminating the requirement for de novo polypeptide design for these cases. Examples include the filament-forming proteins actin and tubulin that have each been used as templates for nanowire synthesis.[159,160] Additionally, the self-assembling coat proteins of virus particles provide a rich source of nanostructures[123] that can be enlisted to compartmentalize[161,162] or to template.[163−168] A significant advantage of protein-based materials is that biotechnological methods can extend their capabilities in two important ways. First, known sequences can be genetically fused to proteins to confer added functionality (e.g., fusion tags of elastin-like peptides confer thermally reversible surface assembly capabilities[169]). Second, unknown sequences can be "discovered" to confer desired functionality (e.g., peptides that bind to inorganics[47,49,50,163]).

**Coupling Self-Assembly with Chitosan's Directed Assembly.** Although self-assembly offers the potential for bottom-up self-fabrication of nanoscale structures, it is not yet obvious how to interface these biological or biomimetic structures to specific addresses of microfabricated surfaces.[170] Potentially, chitosan can serve as the device−biology interface by integrating chitosan's ability to selectively electrodeposit at the device surface with its ability to be readily functionalized with biological components. Specifically, the reactivity of chitosan's amines allows previously deposited films to be functionalized using standard coupling chemistries based on homobifunctional (e.g., glutaraldehyde), heterobifunctional (e.g., $\gamma$-maleimido butyric acid succinimidyl ester), or biological (e.g., biotin) agents. In our studies, we used glutaraldehyde as a model to covalently tether an amine-terminated single stranded DNA oligonucleotide[171] onto electrodeposited chitosan films. We reasoned that the use of a covalent bond to tether the oligonucleotide to chitosan could potentially permit better control of the oligonucleotide's orientation and possibly lead to a more robust platform for repeated hybridization. Figure 8a shows a sequence of steps in which chitosan is first electrodeposited onto the patterned surface of our "chip", the deposited chitosan is next activated by glutaraldehyde, and then an amine-terminated single stranded DNA probe (20 bases) is conjugated onto the activated chitosan film. This chitosan-bound probe DNA can hybridize with a fluorescently labeled target nucleic acid that has a complementary sequence.[172]

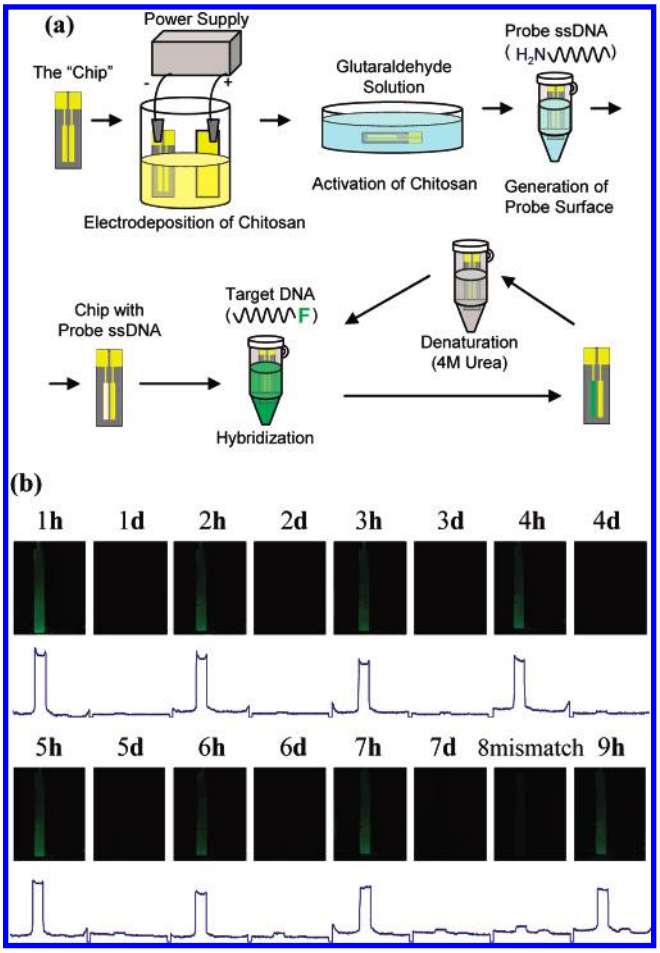



**Figure 8.** Coupling directed assembly with biofunctionalization to create a site for nucleic acid hybridization (i.e., self-assembly). (a) Schematic showing the sequence of steps; chitosan electrodeposition, glutaraldehyde activation, conjugation with amine-terminated single stranded DNA probe, hybridization with fluorescently labeled target DNA, and denaturation with 4M urea, at 65 °C for 30 min. (b) Fluorescence photomicrographs and associated image analysis that show fluorescently labeled target DNA can be repeatedly hybridized (h) and denatured (d), whereas no hybridization is observed in the 8th cycle when the probe DNA was challenged with a mismatched target DNA (i.e., DNA with a noncomplementary sequence). See text for further details.[172]

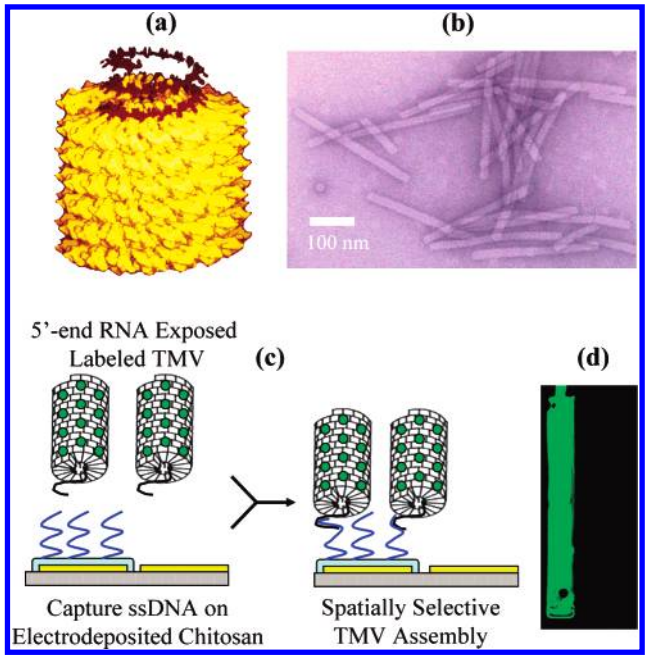



**Figure 9.** Coupling chitosan's directed assembly with TMV self-assembly. (a) TMV nanotubes are formed from thousands of coat protein subunits that self-assemble around the virus' single stranded RNA genome (Adapted from ref 188 with permission from Namba et al. *Science* **1985**, *227* (4688), 773−6. Copyright 1985 AAAS). (b) Transmission electron micrograph of TMV nanotubes. (c) Schematic showing that partial disassembly of the virus particle exposes the 5′ end of the viral RNA allowing hybridization to the surface-bound DNA probe. (d) Fluorescence photomicrograph showing capture of fluorescein-labeled TMV nanotubes on the left electrode containing TMV-specific probe DNA.[173]

In addition, this target can be reversibly "dis-assembled" by subjecting the chip to denaturing conditions (4 M urea and 65 °C).

Results from a sequence of hybridization (h) and denaturation (d) steps are shown in Figure 8b as a series of fluorescence photomicrographs and their associated image analysis. These results demonstrate that the deposited chitosan provides a robust platform for tethering probe nucleic acids that can serve as a nucleation site for reversible self-assembly (i.e., hybridization). Figure 8b also indicates that hybridization to the chitosan-bound probe relies on the molecular recognition capabilities inherent to DNA base-pairing. Specifically, during the 8th hybridization attempt in Figure 8b, the chitosan-bound probe was challenged with high concentrations of a fluorescently labeled target DNA that had a mismatched (i.e., noncomplementary) sequence. After observing that this mismatched target did not hybridize to the chitosan-bound probe (designated "8mismatch"), we confirmed that the probe could still hybridize to the fluorescently labeled target DNA of complementary sequence (designated "9h").[172]

To further illustrate the potential of chitosan to serve as a device−biology interface, we used surface-bound ssDNA to tether nanotubes of the tobacco mosaic virus (TMV). Each TMV particle consists of a single strand of genomic RNA encapsidated within a protein nanotube (inner cavity and outer diameter of 4 and 18 nm, respectively) that is composed of 2130 identical capsid protein subunits (subunit molecular weight of 17.5 kDa). Figure 9a illustrates TMV self-assembly, whereas Figure 9b is a transmission electron micrograph showing these nanotubes. In our experiments, we labeled the virus particles with fluorescein to facilitate visualization. To assemble the TMV nanotubes onto electrodeposited chitosan, we began by partially disassembling the fluorescein-labeled virus particles to expose the 5′ end of the viral RNA. As illustrated in Figure 9c, the exposed viral RNA can hybridize to a complementary ssDNA 25-mer that had been previously anchored to electrodeposited chitosan. Thus, the chitosan-bound DNA serves as a "connection" site to capture the TMV particles. Experimental evidence for this hybridization-based capture is shown in Figure 9d that shows the fluorescently labeled TMV is "targeted" to the left electrode that had the complementary DNA strand. No TMV assembly was observed on the untreated gold surface of the right electrode.[173] This result illustrates the coupling of electrodeposition and functionalization and further demonstrates the versatility of chitosan as a device−biology interface.

More broadly, the results in Figure 9 suggest chitosan may provide a key interconnect between conventional microfabrication and molecular-level self-assembly. Microfabrication allows devices to be created that can impose controlled electrical stimuli to direct chitosan's assembly. Biological components (e.g., ssDNA) can be readily conjugated to the electrodeposited chitosan using facile methods (e.g., glutaraldehyde) to create sites to either "connect" the self-assembled system or to "nucleate" self-assembly. Finally, molecular biological techniques can be integrated to confer desired functionality to the self-assembled structure. For the case of the self-assembled TMV, the coat protein can be engineered to enhance its role as a template for metal assembly.[168,174−176]

## Conclusions

Nature employs a small number of organic materials as building blocks to construct a diversity of structures that perform a range of functions. These capabilities have sparked interest in fabricating devices using these same biological building blocks or their mimics. Self-assembly is the feature that has attracted the most attention, and several studies have demonstrated the potential of biological components to self-fabricate nanoscale structures of controlled size and shape. However, the use of biological materials also allows access to enzymes for selective catalysis under mild conditions. Enzymatic assembly offers exciting opportunities that will be further broadened if the tools of modern biology can be enlisted to engineer/evolve/design enzymes to build macromolecular structure or catalyze surface assembly. Finally, a range of localized external stimuli are beginning to be used to guide the assembly of nano-bio hybrids. Directed assembly often employs the stimuli-responsive character of the nano component, whereas molecular biological methods are providing the means to link the bio to the nano. Directed assembly also allows the strengths of microfabrication to be accessed by creating devices that can impose stimuli with high spatial and temporal control.

Chitosan is an aminopolysaccharide that offers pH-responsive-solubility, forms films and hydrogels, and con-

tains readily modifiable primary amine substituents. Chitosan's pH-responsive solubility allows it to "recognize" localized electrical stimuli and respond by assembling (i.e., depositing) as a thin film. Chitosan's reactivity allows it to be readily functionalized: proteins can be enzymatically assembled onto the stimuli-responsive backbone, whereas nucleic acids can be tethered to electrodeposited films to serve as sites for self-assembly. These capabilities confer considerable versatility to chitosan and suggest this biopolymer has a particularly bright future for biofabrication of the device−biology interface.

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## References and Notes

(1) Cote, G. L.; Lec, R. M.; Pishko, M. V. Emerging biomedical sensing technologies and their applications. *IEEE Sens. J.* **2003**, *3* (3), 251−266.
(2) Grayson, A. C. R.; Shawgo, R. S.; Johnson, A. M.; Flynn, N. T.; Li, Y. W.; Cima, M. J.; Langer, R. A BioMEMS review: MEMS technology for physiologically integrated devices. *Proc. IEEE* **2004**, *92* (1), 6−21.
(3) Baeumner, A. J. Biosensors for environmental pollutants and food contaminants. *Anal. Bioanal. Chem.* **2003**, *377* (3), 434−445.
(4) Wu, L. Q.; Payne, G. F. Biofabrication: using biological materials and biocatalysts to construct nanostructured assemblies. *Trends Biotechnol.* **2004**, *22* (11), 593−9.
(5) Xia, Y.; Whitesides, G. M. Soft Lithography. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 550−575.
(6) Gates, B. D.; Xu, Q.; Love, C.; Wolfe, B. D.; Whitesides, G. M. Unconventional Nanofabrication. *Annu. Rev. Mater. Res.* **2004**, *34*, 339−372.
(7) Ball, P. Natural strategies for the molecular engineer. *Nanotechnology* **2002**, *13* (5), R15−R28.
(8) Goodsell, D. *Bionanotechnology*; Wiley-Liss: Hoboken, NJ, 2004.
(9) Jones, R. *Soft Machines. Nanotechnology and Life*; Oxford University Press: Oxford, 2004.
(10) Niemeyer, C. M. Nanoparticles, proteins, and nucleic acids: Biotechnology meets materials science. *Angew. Chem. Int. Ed.* **2001**, *40* (22), 4128−4158.
(11) Kumar, M. N.; Muzzarelli, R. A.; Muzzarelli, C.; Sashiwa, H.; Domb, A. J. Chitosan chemistry and pharmaceutical perspectives. *Chem. Rev.* **2004**, *104* (12), 6017−84.
(12) Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Peppas, N. A.; Gurny, R. Structure and interactions in covalently and ionically cross-linked chitosan hydrogels for biomedical applications. *Eur. J. Pharm. Biopharm.* **2004**, *57* (1), 19−34.
(13) Kumar, M. N. V. R. A review of chitin and chitosan applications. *React. Funct. Polym.* **2000**, *46* (1), 1−27.
(14) Shahidi, F.; Arachchi, J. K. V.; Jeon, Y. J. Food applications of chitin and chitosans. *Trends Food Sci. Technol.* **1999**, *10* (2), 37−51.
(15) Lim, S. H.; Hudson, S. M. Review of chitosan and its derivatives as antimicrobial agents and their uses as textile chemicals. *J. Macromol. Sci. Polym. Rev.* **2003**, *C43* (2), 223−269.
(16) Rinaudo, M.; Pavlov, G.; Desbrieres, J. Influence of acetic acid concentration on the solubilization of chitosan. *Polymer* **1999**, *40*, 7029−7032.
(17) Sorlier, P.; Denuziere, A.; Viton, C.; Domard, A. Relation between the Degree of Acetylation and the Electrostatic Properties of Chitin and Chitosan. *Biomacromolecules* **2001**, *2* (3), 765−772.
(18) Strand, S. P.; Tommeraas, K.; Varum, K. M.; Ostgaard, K. Electrophoretic light scattering studies of chitosans with different degrees of *N*-acetylation. *Biomacromolecules* **2001**, *2* (4), 1310−1314.
(19) Anthonsen, M. W.; Smidsrod, O. Hydrogen-Ion Titration of Chitosans with Varying Degrees of N−Acetylation by Monitoring Induced H-1-Nmr Chemical-Shifts. *Carbohydr. Polym.* **1995**, *26* (4), 303−305.
(20) Varum, K. M.; Ottoy, M. H.; Smidsrod, O. Water-solubility of partially *N*-acetylated chitosans as a function of pH: Effect of chemical composition and depolymerization. *Carbohydr. Polym.* **1994**, *25*, 65−70.
(21) Montembault, A.; Viton, C.; Domard, A. Rheometric study of the gelation of chitosan in aqueous solution without cross-linking agent. *Biomacromolecules* **2005**, *6* (2), 653−62.
(22) Kurita, A. Controlled functionalization of the polysaccharide chitin. *Prog. Polym. Sci.* **2001**, *26*, 1921−1971.
(23) Ang, T. H.; Sultana, F. S. A.; Hutmacher, D. W.; Wong, Y. S.; Fuh, J. Y. H.; Mo, X. M.; Loh, H. T.; Burdet, E.; Teoh, S. H. Fabrication of 3D chitosan-hydroxyapatite scaffolds using a robotic dispensing system. *Mat. Sci. Eng. C* **2002**, *20* (1−2), 35−42.
(24) Co, C. C.; Wang, Y. C.; Ho, C. C. Biocompatible micropatterning of two different cell types. *J. Am. Chem. Soc.* **2005**, *127* (6), 1598−9.
(25) Kumar, G.; Wang, Y. C.; Co, C.; Ho, C. C. Spatially controlled cell engineering on biomaterials using polyelectrolytes. *Langmuir* **2003**, *19* (25), 10550−10556.
(26) Feng, J.; Gao, C. Y.; Wang, B.; Shen, J. C. Co-patterning chitosan and bovine serum albumin on an aldehyde-enriched glass substrate by microcontact printing. *Thin Solid Films* **2004**, *460* (1−2), 286−290.
(27) Serizawa, T.; Goto, H.; Kishida, A.; Endo, T.; Akashi, M. Improved alternate deposition of biodegradable naturally occurring polymers onto a quartz crystal microbalance. *J. Polym. Sci. A Polym. Chem.* **1999**, *37* (6), 801−804.
(28) Huang, H. Z.; Yang, X. R. Chitosan mediated assembly of gold nanoparticles multilayer. *Colloids Surf., A* **2003**, *226* (1−3), 77−86.
(29) Lvov, Y.; Onda, M.; Ariga, K.; Kunitake, T. Ultrathin films of charged polysaccharides assembled alternately with linear polyions. *J. Biomater. Sci. Polym. Ed.* **1998**, *9* (4), 345−355.
(30) Richert, L.; Lavalle, P.; Payan, E.; Shu, X. Z.; Prestwich, G. D.; Stoltz, J. F.; Schaaf, P.; Voegel, J. C.; Picart, C. Layer by layer buildup of polysaccharide films: Physical chemistry and cellular adhesion aspects. *Langmuir* **2004**, *20* (2), 448−458.
(31) Thierry, B.; Winnik, F. M.; Merhi, Y.; Silver, J.; Tabrizian, M. Bioactive coatings of endovascular stents based on polyelectrolyte multilayers. *Biomacromolecules* **2003**, *4* (6), 1564−1571.
(32) dos Santos, D. S.; Goulet, P. J. G.; Pieczonka, N. P. W.; Oliveira, O. N.; Aroca, R. F. Gold nanoparticle embedded, self-sustained chitosan films as substrates for surface-enhanced Raman scattering. *Langmuir* **2004**, *20* (23), 10273−10277.
(33) Zhang, M. G.; Smith, A.; Gorski, W. Carbon nanotube-chitosan system for electrochemical sensing based on dehydrogenase enzymes. *Anal. Chem.* **2004**, *76* (17), 5045−5050.
(34) Zhang, M. G.; Gorski, W. Electrochemical sensing platform based on the carbon nanotubes/redox mediators-biopolymer system. *J. Am. Chem. Soc.* **2005**, *127* (7), 2058−2059.
(35) Constantine, C. A.; Gattas-Asfura, K. M.; Mello, S. V.; Crespo, G.; Rastogi, V.; Cheng, T. C.; DeFrank, J. J.; Leblanc, R. M. Layer-by-layer biosensor assembly incorporating functionalized quantum dots. *Langmuir* **2003**, *19* (23), 9863−9867.
(36) Schauer, C. L.; Chen, M. S.; Chatterley, M.; Eisemann, K.; Welsh, E. R.; Price, R. R.; Schoen, P. E.; Ligler, F. S. Color changes in chitosan and poly(allylamine) films upon metal binding. *Thin Solid Films* **2003**, *434* (1−2), 250−257.
(37) Schauer, C. L.; Chen, M. S.; Price, R. R.; Schoen, P. E.; Ligler, F. S. Colored thin films for specific metal ion detection. *Environ. Sci. Technol.* **2004**, *38* (16), 4409−13.
(38) Serizawa, T.; Yamaguchi, M.; Matsuyama, T.; Akashi, M. Alternating bioactivity of polymeric layer-by-layer assemblies: Anti- vs procoagulation of human blood on chitosan and dextran sulfate layers. *Biomacromolecules* **2000**, *1* (3), 306−309.
(39) Serizawa, T.; Yamaguchi, M.; Akashi, M. Alternating bioactivity of polymeric layer-by-layer assemblies: Anticoagulation vs procoagulation of human blood. *Biomacromolecules* **2002**, *3* (4), 724−731.
(40) Serizawa, T.; Yamaguchi, M.; Akashi, M. Enzymatic hydrolysis of a layer-by-layer assembly prepared from chitosan and dextran sulfate. *Macromolecules* **2002**, *35* (23), 8656−8658.
(41) Etienne, O.; Schneider, A.; Taddei, C.; Richert, L.; Schaaf, P.; Voegel, J. C.; Egles, C.; Picart, C. Degradability of polysaccharides multilayer films in the oral environment: an in vitro and in vivo study. *Biomacromolecules* **2005**, *6* (2), 726−733.
(42) Wu, L. Q.; Ghodssi, R.; Elabd, Y. A.; Payne, G. F. Biomimetic Pattern Transfer. *Adv. Funct. Mater.* **2005**, *15*, 189−195.
(43) Baumgart, T.; Offenhausser, A. Polysaccharide-supported planar bilayer lipid model membranes. *Langmuir* **2003**, *19* (5), 1730−1737.
(44) Katz, E.; Willner, I. Integrated nanoparticle-biomolecule hybrid systems: synthesis, properties, and applications. *Angew. Chem. Int. Ed. Engl.* **2004**, *43* (45), 6042−108.
(45) Fang, M.; Grant, P. S.; McShane, M. J.; Sukhorukov, G. B.; Golub, V. O.; Lvov, Y. M. Magnetic bio/nanoreactor with multilayer shells

of glucose oxidase and inorganic nanoparticles. *Langmuir* **2002**, *18* (16), 6338−6344.
(46) Weizmann, Y.; Patolsky, F.; Lioubashevski, O.; Willner, I. Magneto-mechanical detection of nucleic acids and telomerase activity in cancer cells. *J. Am. Chem. Soc.* **2004**, *126* (4), 1073−80.
(47) Sarikaya, M.; Tamerler, C.; Schwartz, D. T.; Baneyx, F. O. Materials assembly and formation using engineered polypeptides. *Annu. Rev. Mater. Res.* **2004**, *34*, 373−408.
(48) Sarikaya, M.; Tamerler, C.; Jen, A. K.; Schulten, K.; Baneyx, F. Molecular biomimetics: nanotechnology through biology. *Nat. Mater.* **2003**, *2* (9), 577−85.
(49) Naik, R. R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. Biomimetic synthesis and patterning of silver nanoparticles. *Nat. Mater.* **2002**, *1* (3), 169−172.
(50) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly. *Nature* **2000**, *405* (6787), 665−668.
(51) Shacham, R.; Avnir, D.; Mandler, D. Electrodeposition of methylated sol−gel films on conducting surfaces. *Adv. Mater.* **1999**, *11* (5), 384−388.
(52) Wu, L. Q.; Gadre, A. P.; Yi, H. M.; Kastantin, M. J.; Rubloff, G. W.; Bentley, W. E.; Payne, G. F.; Ghodssi, R. Voltage-dependent assembly of the polysaccharide chitosan onto an electrode surface. *Langmuir* **2002**, *18* (22), 8620−8625.
(53) Fernandes, R.; Wu, L. Q.; Chen, T. H.; Yi, H. M.; Rubloff, G. W.; Ghodssi, R.; Bentley, W. E.; Payne, G. F. Electrochemically induced deposition of a polysaccharide hydrogel onto a patterned surface. *Langmuir* **2003**, *19* (10), 4058−4062.
(54) Wu, L.-Q.; Yi, H.; Li, S.; Rubloff, G. W.; Bentley, W. E.; Ghodssi, R.; Payne, G. F. Spatially selective Deposition of a Reactive Polysaccharide Layer onto a Patterned Template. *Langmuir* **2003**, *19*, 519−524.
(55) Wu, L. Q.; Lee, K.; Wang, X.; English, D. S.; Losert, W.; Payne, G. F. Chitosan-Mediated and Spatially Selective Electrodeposition of Nanoscale Particles. *Langmuir* **2005**, *21* (8), 3641−3646.
(56) Luo, X. L.; Xu, J. J.; Du, Y.; Chen, H. Y. A glucose biosensor based on chitosan-glucose oxidase-gold nanoparticles biocomposite formed by one-step electrodeposition. *Anal. Biochem.* **2004**, *334* (2), 284−289.
(57) Luo, X. L.; Xu, J. J.; Wang, J. L.; Chen, H. Y. Electrochemically deposited nanocomposite of chitosan and carbon nanotubes for biosensor application. *Chem. Comm.* **2005** (16), 2169−2171.
(58) Kastantin, M. J.; Li, S.; Gadre, A. P.; Wu, L. Q.; Bentley, W. E.; Payne, G. F.; Rubloff, G. W.; Ghodssi, R. Integrated fabrication of polymeric devices for biological applications. *Sensors Mater.* **2003**, *15* (6), 295−311.
(59) Powers, M. A.; Koev, S. T.; Schleunitz, A.; Yi, H.; Hodzic, V.; Bentley, W. E.; Payne, G. F.; Rubloff, G. W.; Ghodssi, R. A fabrication platform for electrically mediated optically active bio-functionalized sites in BioMEMS. *Lab Chip* **2005**, *5*, 583−586.
(60) Yang, L. J.; Lin, W. Z.; J, Y. T.; Tai, Y. C. Photopatternable gelatin as protection layers in low-temperature surface micromachinings. *Sens. Actuators A* **2003**, *103*, 284−290.
(61) Reches, M.; Gazit, E. Casting metal nanowires within discrete self-assembled peptide nanotubes. *Science* **2003**, *300* (5619), 625−7.
(62) Fernandes, R.; Yi, H. M.; Wu, L. Q.; Rubloff, G. W.; Ghodssi, R.; Bentley, W. E.; Payne, G. F. Thermo-biolithography: A technique for patterning nucleic acids and proteins. *Langmuir* **2004**, *20* (3), 906−913.
(63) Clausen-Schaumann, H.; Grandbois, M.; Gaub, H. Enzyme-assisted Nanoscale Lithography in Lipid Membranes. *Adv. Mater.* **1998**, *10* (12), 949−952.
(64) Takeda, S.; Nakamura, C.; Miyamoto, C.; Nakamura, N.; Kageshima, M.; Tokumoto, H.; Miyake, J. Lithographing of Biomolecules on a Substrate Surface Using an Enzyme-Immobilized AFM Tip. *Nano Lett.* **2003**, *3* (11), 1471−1474.
(65) Hyun, J.; Kim, J.; Craig, S. L.; Chilkoti, A. Enzymatic Nanolithography of a Self-Assembled Oligonucleotide Monolayer on Gold. *J. Am. Chem. Soc.* **2004**, *126* (15), 4770−1.
(66) Braun, E.; Keren, K. From DNA to Transistors. *Adv. Phys.* **2004**, *53* (4), 441−496.
(67) Keren, K.; Krueger, M.; Gilad, R.; Ben-Yoseph, G.; Sivan, U.; Braun, E. Sequence-specific molecular lithography on single DNA molecules. *Science* **2002**, *297* (5578), 72−5.
(68) Keren, K.; Berman, R. S.; Buchstab, E.; Sivan, U.; Braun, E. DNA-templated carbon nanotube field-effect transistor. *Science* **2003**, *302* (5649), 1380−1382.
(69) Mavroidis, C.; Dubey, A.; Yarmush, M. L. Molecular machines. *Annu. Rev. Biomed. Eng.* **2004**, *6*, 363−95.
(70) Noji, H.; Yasuda, R.; Yoshida, M.; Kinosita, K., Jr. Direct observation of the rotation of F1-ATPase. *Nature* **1997**, *386* (6622), 299−302.
(71) Soong, R. K.; Bachand, G. D.; Neves, H. P.; Olkhovets, A. G.; Craighead, H. G.; Montemagno, C. D. Powering an inorganic nanodevice with a biomolecular motor. *Science* **2000**, *290* (5496), 1555−1558.
(72) Montemagno, C.; Bachand, G. Constructing nanomechanical devices powered by biomolecular motors. *Nanotechnology* **1999**, *10* (3), 225−231.
(73) Liu, H.; Schmidt, J. J.; Bachand, G. D.; Rizk, S. S.; Looger, L. L.; Hellinga, H. W.; Montemagno, C. D. Control of a biomolecular motor-powered nanodevice with an engineered chemical switch. *Nat. Mater.* **2002**, *1* (3), 173−7.
(74) Ohtsuka, T.; Sawa, A.; Kawabata, R.; Nio, N.; Motoki, M. Substrate specificities of microbial transglutaminase for primary amines. *J. Agric. Food Chem.* **2000**, *48* (12), 6230−6233.
(75) Sato, H.; Hayashi, E.; Yamada, N.; Yatagai, M.; Takahara, Y. Further studies on the site-specific protein modification by microbial transglutaminase. *Bioconj. Chem.* **2001**, *12* (5), 701−710.
(76) Villalonga, R.; Fernandez, M.; Fragoso, A.; Cao, R.; Di Pierro, P.; Mariniello, L.; Porta, R. Transglutaminase-catalyzed synthesis of trypsin-cyclodextrin conjugates: kinetics and stability properties. *Biotechnol. Bioeng.* **2003**, *81* (6), 732−7.
(77) Villalonga, R.; Fernandez, M.; Fragoso, A.; Cao, R.; Mariniello, L.; Porta, R. Thermal stabilization of trypsin by enzymic modification with beta-cyclodextrin derivatives. *Biotechnol. Appl. Biochem.* **2003**, *38*, 53−59.
(78) Josten, A.; Meusel, M.; Spener, F. Microbial transglutaminase-mediated synthesis of hapten-protein conjugates for immunoassays. *Anal. Biochem.* **1998**, *258* (2), 202−208.
(79) Collier, J. H.; Messersmith, P. B. Enzymatic modification of self-assembled peptide structures with tissue transglutaminase. *Bioconjugate Chem.* **2003**, *14* (4), 748−55.
(80) Babin, H.; Dickinson, E. Influence of transglutaminase treatment on the thermoreversible gelation of gelatin. *Food Hydrocolloids* **2001**, *15*, 271−276.
(81) Lim, L. T.; Mine, Y.; Tung, M. A. Barrier and tensile properties of transglutaminase cross-linked gelatin films as affected by relative humidity, temperature, and glycerol content. *J. Food Sci.* **1999**, *64* (4), 616−622.
(82) Crescenzi, V.; Francescangeli, A.; Taglienti, A. New Gelatin-Based Hydrogels via Enzymatic Networking. *Biomacromolecules* **2002**, *3* (6), 1384−1391.
(83) Fuchsbauer, H. L.; Gerber, U.; Engelmann, J.; Seeger, T.; Sinks, C.; Hecht, T. Influence of gelatin matrixes cross-linked with transglutaminase on the properties of an enclosed bioactive material using beta-galactosidase as model system. *Biomaterials* **1996**, *17* (15), 1481−1488.
(84) Chen, T. H.; Embree, H. D.; Brown, E. M.; Taylor, M. M.; Payne, G. F. Enzyme-catalyzed gel formation of gelatin and chitosan: potential for in situ applications. *Biomaterials* **2003**, *24* (17), 2831−2841.
(85) Sperinde, J.; Griffith, L. Synthesis and Characterization of Enzymatically-Cross-Linked Poly(ethylene glycol) Hydrogels. *Macromolecules* **1997**, *30*, 5255−5264.
(86) Sperinde, J.; Griffith, L. Control and Prediction of Gelation Kinetics in Enzymatically Cross-Linked Poly(ethylene glycol) Hydrogels. *Macromolecules* **2000**, *33*, 5476−5480.
(87) Hu, B. H.; Messersmith, P. B. Rational design of transglutaminase substrate peptides for rapid enzymatic formation of hydrogels. *J. Am. Chem. Soc.* **2003**, *125* (47), 14298−9.
(88) Chen, T.; Small, D. A.; McDermott, M. K.; Bentley, W. E.; Payne, G. F. Enzymatic Methods for In situ Cell Entrapment and Cell Release. *Biomacromolecules* **2003**, *4*, 1558−1563.
(89) Schense, J. C.; Hubbell, J. A. Cross-linking exogenous bifunctional peptides into fibrin gels with factor XIIIa. *Bioconjug. Chem.* **1999**, *10* (1), 75−81.
(90) Schense, J. C.; Bloch, J.; Aebischer, P.; Hubbell, J. A. Enzymatic incorporation of bioactive peptides into fibrin matrixes enhances neurite extension. *Nat. Biotechnol.* **2000**, *18* (4), 415−9.
(91) Schense, J. C.; Hubbell, J. A. Three-dimensional migration of neurites is mediated by adhesion site density and affinity. *J. Biol. Chem.* **2000**, *275* (10), 6813−8.
(92) Sakiyama, S. E.; Schense, J. C.; Hubbell, J. A. Incorporation of heparin-binding peptides into fibrin gels enhances neurite extension: an example of designer matrixes in tissue engineering. *FASEB J.* **1999**, *13* (15), 2214−24.

(93) Sakiyama-Elbert, S. E.; Hubbell, J. A. Controlled release of nerve growth factor from a heparin-containing fibrin-based cell ingrowth matrix. *J. Controlled Release* **2000**, *69* (1), 149−58.

(94) Sakiyama-Elbert, S. E.; Hubbell, J. A. Development of fibrin derivatives for controlled release of heparin-binding growth factors. *J. Controlled Release* **2000**, *65* (3), 389−402.

(95) Seitz, A.; Schneider, F.; Pasternack, R.; Fuchsbauer, H. L.; Hampp, N. Enzymatic cross-linking of purple membranes catalyzed by bacterial transglutaminase. *Biomacromolecules* **2001**, *2* (1), 233−8.

(96) Nieuwenhuizen, W. F.; Dekker, H. L.; De Koning, L. J.; Groneveld, T.; De Koster, C. G.; De Jong, G. A. H. Modification of glutamine and lysine residues in holo and apo alpha-lactalbumin with microbial transglutaminase. *J. Agric. Food Chem.* **2003**, *51* (24), 7132−7139.

(97) Nieuwenhuizen, W. F.; Dekker, H. L.; Groneveld, T.; de Koster, C. G.; de Jong, G. A. H. Transglutaminase-mediated modification of glutamine and lysine residues in native bovine beta-lactoglobulin. *Biotechnol. Bioeng.* **2004**, *85* (3), 248−258.

(98) Matsumura, Y.; Chanyongvorakul, Y.; Kumazawa, Y.; Ohtsuka, T.; Mori, T. Enhanced susceptibility to transglutaminase reaction of alpha-lactalbumin in the molten globule state. *Biochim. Biophys. Acta-Protein Struct. Mol. Enzymol.* **1996**, *1292* (1), 69−76.

(99) Kamiya, N.; Tanaka, T.; Suzuki, T.; Takazawa, T.; Takeda, S.; Watanabe, K.; Nagamune, T. S-peptide as a potent peptidyl linker for protein cross-linking by microbial transglutaminase from Streptomyces mobaraensis. *Bioconjugate Chem.* **2003**, *14* (2), 351−357.

(100) Tanaka, T.; Kamiya, N.; Nagamune, T. Peptidyl linkers for protein heterodimerization catalyzed by microbial transglutaminase. *Bioconjugate Chem.* **2004**, *15* (3), 491−497.

(101) Kamiya, N.; Takazawa, T.; Tanaka, T.; Ueda, H.; Nagamune, T. Site-specific cross-linking of functional proteins by transglutamination. *Enzyme Microb. Technol.* **2003**, *33* (4), 492−496.

(102) Takazawa, T.; Kamiya, N.; Ueda, H.; Nagamune, T. Enzymatic labeling of a single chain variable fragment of an antibody with alkaline phosphatase by mircobial transglutaminase. *Biotechnol. Bioeng.* **2004**, *86* (4), 399−404.

(103) Burzio, L. A.; Waite, J. H. Reactivity of peptidyl-tyrosine to hydroxylation and cross-linking. *Protein Sci.* **2001**, *10*, 735−740.

(104) Burzio, L. A.; Waite, J. H. Cross-linking in adhesive quinoproteins: studies with model decapeptides. *Biochemistry* **2000**, *39* (36), 11147−53.

(105) Sugumaran, M. Molecular mechanisms for cuticular sclerotization. *Adv. Insect Physiol.* **1988**, *21*, 179−231.

(106) Sugumaran, M. Oxidation chemistry of 1,2-dehydro-*N*-acetyldopamines: Direct evidence for the formation of 1,2-dehydro-*N*-acetyldopamine quinone. *Arch. Biochem. Biophys.* **2000**, *378* (2), 404−410.

(107) Kerwin, J. L.; Whitney, D. L.; Sheikh, A. Mass spectrometric profiling of glucosamine, glucosamine polymers and their catecholamine adducts − Model reactions and cuticular hydrolysates of Toxorhynchites amboinensis (Culicidae) pupae. *Insect Biochem. Mol. Biol.* **1999**, *29* (7), 599−607.

(108) Aberg, C. M.; Chen, T. H.; Olumide, A.; Raghavan, S. R.; Payne, G. F. Enzymatic Grafting of Peptides from Casein Hydrolysate to Chitosan. Potential for Value-Added Byproducts from Food-Processing Wastes. *J. Agric. Food Chem.* **2004**, *52* (4), 788−793.

(109) Wu, L. Q.; Embree, H. D.; Balgley, B. M.; Smith, P. J.; Payne, G. F. Utilizing renewable resources to create functional polymers: Chitosan-based associative thickener. *Environ. Sci. Technol.* **2002**, *36* (15), 3446−3454.

(110) Muzzarelli, C.; Muzzarelli, R. A. A. Reactivity of quinones toward chitosan. *Trends Glycosci. Glycotechnol.* **2002**, *14*, 223−229.

(111) Payne, G. F.; Chaubal, M. V.; Barbari, T. A. Enzyme-catalyzed polymer modification: Reaction of phenolic compounds with chitosan films. *Polymer* **1996**, *37* (20), 4643−4648.

(112) Yamada, K.; Chen, T.; Kumar, G.; Vesnovsky, O.; Topoleski, L. D.; Payne, G. F. Chitosan Based Water-Resistant Adhesive. Analogy to Mussel Glue. *Biomacromolecules* **2000**, *1* (2), 252−8.

(113) Sun, W. Q.; Payne, G. F.; Moas, M. S. G. L.; Chu, J. H.; Wallace, K. K. Tyrosinase Reaction Chitosan Adsorption for Removing Phenols from Wastewater. *Biotechnol. Prog.* **1992**, *8* (3), 179−186.

(114) Muzzarelli, R. A. A.; Ilari, P.; Xia, W. S.; Pinotti, M.; Tomasetti, M. Tyrosinase-Mediated Quinone Tanning of Chitinous Materials. *Carbohydr. Polym.* **1994**, *24* (4), 295−300.

(115) Chen, T. H.; Embree, H. D.; Wu, L. Q.; Payne, G. F. In vitro protein-polysaccharide conjugation: Tyrosinase-catalyzed conjugation of gelatin and chitosan. *Biopolymers* **2002**, *64* (6), 292−302.

(116) Vazquez-Duhalt, R.; Tinoco, R.; D'Antonio, P.; Topoleski, L. D. T.; Payne, G. F. Enzyme conjugation to the polysaccharide chitosan: Smart biocatalysts and biocatalytic hydrogels. *Bioconjugate Chem.* **2001**, *12* (2), 301−306.

(117) Chen, T.; Small, D. A.; Wu, L.-Q.; Rubloff, G. W.; Ghodssi, R.; Vazquez-Duhalt, R.; Bentley, W. E.; Payne, G. F. Nature-Inspired Creation of Protein-Polysaccharide Conjugate and its Subsequent Assembly onto a Patterned Surface. *Langmuir* **2003**, *19*, 9382−9386.

(118) Yi, H.; Wu, L. Q.; Ghodssi, R.; Rubloff, G. W.; Payne, G. F.; Bentley, W. E. Signal-Directed Sequential Assembly of Biomolecules on Patterned Surfaces. *Langmuir* **2005**, *21* (6), 2104−7.

(119) Lehn, J. M. Toward self-organization and complex matter. *Science* **2002**, *295* (5564), 2400−3.

(120) Reinhoudt, D. N.; Crego-Calama, M. Synthesis beyond the molecule. *Science* **2002**, *295* (5564), 2403−2407.

(121) Whitesides, G. M.; Grzybowski, B. Self-assembly at all scales. *Science* **2002**, *295* (5564), 2418−21.

(122) Whitesides, G. M. The 'right' size in nanobiotechnology. *Nat. Biotechnol.* **2003**, *21* (10), 1161−1165.

(123) Bittner, A. M. Biomolecular rods and tubes in nanotechnology. *Naturwissenschaften* **2005**, *92* (2), 51−64.

(124) Seeman, N. C.; Belcher, A. M. Emulating biology: building nanostructures from the bottom up. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* Suppl 2, 6451−5.

(125) Ball, P. Life's lessons in design. *Nature* **2001**, *409* (6818), 413−6.

(126) Shimizu, T.; Masuda, M.; Minamikawa, H. Supramolecular Nanotube Architectures based on Amphiphilic Molecules. *Chem. Rev.* **2005**, *105* (4), 1401−43.

(127) Hatano, T.; Bae, A. H.; Takeuchi, M.; Fujita, N.; Kaneko, K.; Ihara, H.; Takafuji, M.; Shinkai, S. Helical superstructure of conductive polymers as created by electrochemical polymerization by using synthetic lipid assemblies as a template. *Angew. Chem. Int. Ed. Engl.* **2004**, *43* (4), 465−9.

(128) Steinberg-Yfrach, G.; Rigaud, J. L.; Durantini, E. N.; Moore, A. L.; Gust, D.; Moore, T. A. Light-driven production of ATP catalyzed by F0F1-ATP synthase in an artificial photosynthetic membrane. *Nature* **1998**, *392* (6675), 479−82.

(129) Fang, Y.; Frutos, A. G.; Lahiri, J. Membrane protein microarrays. *J. Am. Chem. Soc.* **2002**, *124* (11), 2394−5.

(130) Karlsson, M.; Davidson, M.; Karlsson, R.; Karlsson, A.; Bergenholtz, J.; Konkoli, Z.; Jesorka, A.; Lobovkina, T.; Hurtig, J.; Voinova, M.; Orwar, O. Biomimetic nanoscale reactors and networks. *Annu. Rev. Phys. Chem.* **2004**, *55*, 613−649.

(131) Karlsson, A.; Sott, K.; Markstrom, M.; Davidson, M.; Konkoli, Z.; Orwar, O. Controlled Initiation of Enzymatic Reactions in Micrometer-Sized Biomimetic Compartments. *J. Phys. Chem. B* **2005**, *109* (4), 1609−1617.

(132) Graff, A.; Winterhalter, M.; Meier, W. Nanoreactors from Polymer-Stabilized Liposomes. *Langmuir* **2001**, *17* (3), 919−923.

(133) Andresen, T. L.; Jensen, S. S.; Jorgensen, K. Advanced strategies in liposomal cancer therapy: Problems and prospects of active and tumor specific drug release. *Prog. Lipid Res.* **2005**, *44* (1), 68−97.

(134) Karlsson, M.; Sott, K.; Davidson, M.; Cans, A. S.; Linderholm, P.; Chiu, D.; Orwar, O. Formation of geometrically complex lipid nanotube-vesicle networks of higher-order topologies. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99* (18), 11573−8.

(135) Discher, B. M.; Hammer, D. A.; Bates, F. S.; Discher, D. E. Polymer vesicles in various media. *Curr. Opin. Colloid Interface Sci.* **2000**, *5* (1−2), 125−131.

(136) Braun, E.; Eichen, Y.; Sivan, U.; Ben-Yoseph, G. DNA-templated assembly and electrode attachment of a conducting silver wire. *Nature* **1998**, *391* (6669), 775−778.

(137) Keren, K.; Berman, R. S.; Braun, E. Patterned DNA Metallization by Sequence-Specific Localization of a Reducing Agent. *Nano Lett.* **2004**, *4* (2), 323−326.

(138) Seeman, N. C. DNA engineering and its application to nanotechnology. *Trends Biotechnol.* **1999**, *17* (11), 437−43.

(139) Seeman, N. C. At the Crossroads of Chemistry, Biology, and Materials: Structural DNA Nanotechnology. *Chem. Biol.* **2003**, *10*, 1151−1159.

(140) Li, Y.; Tseng, Y. D.; Kwon, S. Y.; D'Espaux, L.; Bunch, J. S.; McEuen, P. L.; Luo, D. Controlled assembly of dendrimer-like DNA. *Nat. Mater.* **2004**, *3* (1), 38−42.

(141) Yan, H.; Zhang, X.; Shen, Z.; Seeman, N. C. A robust DNA mechanical device controlled by hybridization topology. *Nature* **2002**, *415* (6867), 62−5.

(142) Mirkin, C. A.; Letsinger, R. L.; Mucic, R. C.; Storhoff, J. J. A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature* **1996**, *382* (6592), 607−609.

(143) Mitchell, G. P.; Mirkin, C. A.; Letsinger, R. L. Programmed Assembly of DNA Functionalized Quantum Dots. *J. Am. Chem. Soc.* **1999**, *121* (35), 8122−8123.
(144) Storhoff, J. J.; Mirkin, C. A. Programmed materials synthesis with DNA. *Chem. Rev.* **1999**, *99* (7), 1849−1862.
(145) Schneider, J. P.; Pochan, D. J.; Ozbas, B.; Rajagopal, K.; Pakstis, L.; Kretsinger, J. Responsive Hydrogels from the Intramolecular Folding and Self-Assembly of a Designed Peptide. *J. Am. Chem. Soc.* **2002**, *124* (50), 15030−15037.
(146) Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. Rapidly recovering hydrogel scaffolds from self-assembling diblock copolypeptide amphiphiles. *Nature* **2002**, *417*, 424−428.
(147) Bellomo, E. G.; Wyrsta, M. D.; Pakstis, L.; Pochan, D. J.; Deming, T. J. Stimuli-responsive polypeptide vesicles by conformation-specific assembly. *Nat. Mater.* **2004**, *3* (4), 244−8.
(148) Zhao, X.; Zhang, S. Fabrication of molecular materials using peptide construction motifs. *Trends. Biotechnol.* **2004**, *22* (9), 470−6.
(149) Rajagopal, K.; Schneider, J. P. Self-assembling peptides and proteins for nanotechnological applications. *Curr. Opin. Struct. Biol.* **2004**, *14* (4), 480−6.
(150) Urry, D. W. Five axioms for the functional design of peptide-based polymers as molecular machines and materials: Principle for macromolecular assemblies. *Biopolymers* **1998**, *47*, 167−178.
(151) Soldatov, D. V.; Moudrakovski, I. L.; Ripmeester, J. A. Dipeptides as microporous materials. *Angew. Chem. Int. Ed. Engl.* **2004**, *43* (46), 6308−11.
(152) Zhang, S. G. Fabrication of novel biomaterials through molecular self-assembly. *Nat. Biotechnol.* **2003**, *21* (10), 1171−1178.
(153) Collier, J. H.; Messersmith, P. B. Self-assembling polymer-peptide conjugates: Nanostructural tailoring. *Adv. Mat.* **2004**, *16* (11), 907−910.
(154) Yemini, M.; Reches, M.; Rishpon, J.; Gazit, E. Novel electrochemical biosensing platform using self-assembled peptide nanotubes. *Nano Lett.* **2005**, *5* (1), 183−186.
(155) Farmer, R. S.; Kiick, K. L. Conformational Behavior of Chemically Reactive Alanine-Rich Repetitive Protein Polymers. *Biomacromolecules* **2005**, *6* (3), 1531−1539.
(156) Stevens, M. M.; Allen, S.; Davies, M. C.; Roberts, C. J.; Sakata, J. K.; Tendler, S. J. B.; Tirrell, D. A.; Williams, P. M. Molecular Level Investigations of the Inter- and Intramolecular Interactions of pH-Responsive Artificial Triblock Proteins. *Biomacromolecules* **2005**, *6* (3), 1266−1271.
(157) Ramachandran, S.; Tseng, Y.; Yu, Y. B. Repeated Rapid Shear-Responsiveness of Peptide Hydrogels with Tunable Shear Modulus. *Biomacromolecules* **2005**, *6* (3), 1316−1321.
(158) Checot, F.; Brulet, A.; Oberdisse, J.; Gnanou, Y.; Mondain-Monval, O.; Lecommandoux, S. Structure of Polypeptide-Based Diblock Copolymers in Solution: Stimuli-Responsive Vesicles and Micelles. *Langmuir* **2005**, *21* (10), 4308−4315.
(159) Patolsky, F.; Weizmann, Y.; Willner, I. Actin-based metallic nanowires as bio-nanotransporters. *Nat. Mater.* **2004**, *3* (10), 692−695.
(160) Fritzsche, W.; Bohm, K.; Unger, E.; Kohler, J. Metallic nanowires created by biopolymer masking. *Appl. Phys. Lett.* **1999**, *75* (18), 2854−2856.
(161) Douglas, T.; Young, M. Host−guest encapsulation of materials by assembled virus protein cages. *Nature* **1998**, *393*, 152−155.
(162) Hooker, J. M.; Kovacs, E. W.; Francis, M. B. Interior Surface Modification of Bacteriophage MS2. *J. Am. Chem. Soc.* **2004**, *126* (12), 3718−3719.
(163) Mao, C.; Flynn, C. E.; Hayhurst, A.; Sweeney, R.; Qi, J.; Georgiou, G.; Iverson, B.; Belcher, A. M. Viral assembly of oriented quantum dot nanowires. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100* (12), 6946−51.
(164) Mao, C.; Solis, D. J.; Reiss, B. D.; Kottmann, S. T.; Sweeney, R. Y.; Hayhurst, A.; Georgiou, G.; Iverson, B.; Belcher, A. M. Virus-based toolkit for the directed synthesis of magnetic and semiconducting nanowires. *Science* **2004**, *303* (5655), 213−7.
(165) Lee, S. W.; Mao, C.; Flynn, C. E.; Belcher, A. M. Ordering of quantum dots using genetically engineered viruses. *Science* **2002**, *296* (5569), 892−5.
(166) Schlick, T. L.; Ding, Z. B.; Kovacs, E. W.; Francis, M. B. Dual-Surface Modification of the Tobacco Mosaic Virus. *J. Am. Chem. Soc.* **2005**, *127* (11), 3718−3723.
(167) Chatterji, A.; Ochoa, W. F.; Ueno, T.; Lin, T. W.; Johnson, J. E. A Virus-Based Nanoblock with Tunable Electrostatic Properties. *Nano Lett.* **2005**, *5* (4), 597−602.
(168) Nam, K. T.; Peelle, B. R.; Lee, S. W.; Belcher, A. M. Genetically Driven Assembly of Nanorings based on the M13 Virus. *Nano Lett.* **2004**, *4* (1), 23−27.
(169) Hyun, J.; Lee, W. K.; Nath, N.; Chilkoti, A.; Zauscher, S. Capture and Release of Proteins on the Nanoscale by Stimuli-Responsive Elastin-Like Polypeptide "Switches". *J. Am. Chem. Soc.* **2004**, *126* (23), 7330−5.
(170) Ball, P. Synthetic biology for nanotechnology. *Nanotechnology* **2005**, *16* (1), R1−R8.
(171) Yi, H. M.; Wu, L. Q.; Sumner, J. J.; Gillespie, J. B.; Payne, G. F.; Bentley, W. E. Chitosan scaffolds for biomolecular assembly: Coupling nucleic acid probes for detecting hybridization. *Biotechnol. Bioeng.* **2003**, *83* (6), 646−652.
(172) Yi, H. M.; Wu, L. Q.; Ghodssi, R.; Rubloff, G. W.; Payne, G. F.; Bentley, W. E. Robust Technique for Assembly of Nucleic Acid Hybridization Chips based on Electrochemically Templated Chitosan. *Anal. Chem.* **2004**, *76* (2), 365−372.
(173) Yi, H.; Nisar, S.; Lee, S.-Y.; Powers, M. A.; Bentley, W. E.; Payne, G. F.; Ghodssi, R.; Rubloff, G. W.; Harris, M. T.; Culver, J. N. Patterned Assembly of Genetically Modified Viral Nanotemplates via Nucleic Acid Hybridization. *Nano Lett.* **2005**, in press.
(174) Lee, S.-Y.; Royston, E.; Culver, J. N.; Harris, M. T. Improved metal cluster deposition on a genetically engineered tobacco mosaic virus template. *Nanotechnology* **2005**, *16*, S435−S441.
(175) Knez, M.; Sumser, M.; Bittner, A. M.; Wege, C.; Jeske, H.; Martin, T. P.; Kern, K. Spatially selective nucleation of metal clusters on the tobacco mosaic virus. *Adv. Funct. Mater.* **2004**, *14* (2), 116−124.
(176) Knez, M.; Bittner, A. M.; Boes, F.; Wege, C.; Jeske, H.; Maiss, E.; Kern, K. Biotemplate Synthesis of 3-nm Nickel and Cobalt Nanowires. *Nano Lett.* **2003**, *3* (8), 1079−1082.
(177) dos Santos, D. S.; Bassi, A.; Rodrigues, J. J.; Misoguti, L.; Oliveira, O. N.; Mendonca, C. R. Light-Induced Storage in Layer-by-Layer Films of Chitosan and an Azo Dye. *Biomacromolecules* **2003**, *4* (6), 1502−1505.
(178) Tan, L.; Kong, Y. P.; Pang, S. W.; Yee, A. F. Imprinting of polymer at low temperature and pressure. *J. Vac. Sci. Technol. B* **2004**, *22* (5), 2486-2492.
(179) Sarma, T. K.; Chattopadhyay, A. Starch-Mediated Shape-Selective Synthesis of Au Nanoparticles with Tunable Longitudinal Plasmon Resonance. *Langmuir* **2004**, *20* (9), 3520−3524.
(180) Jang, C. H.; Stevens, B. D.; Carlier, P. R.; Calter, M. A.; Ducker, W. A. Immobilized Enzymes as Catalytically Active Tools for Nanofabrication. *J. Am. Chem. Soc.* **2002**, *124* (41), 12114−12115.
(181) Yun, C. S.; Khitrov, G. A.; Vergona, D. E.; Reich, N. O.; Strouse, G. F. Enzymatic Manipulation of DNA−Nanomaterial Constructs. *J. Am. Chem. Soc.* **2002**, *124* (26), 7644−7645.
(182) Kanaras, A. G.; Wang, Z. X.; Bates, A. D.; Cosstick, R.; Brust, M. Toward multistep nanostructure synthesis: Programmed enzymatic self-assembly of DNA/gold systems. *Angew. Chem. Int. Ed.* **2003**, *42* (2), 191-+.
(183) Chi, Y. S.; Jung, Y. H.; Choi, I. S.; Kim, Y. G. Surface-Initiated Growth of poly d(A−T) by Taq DNA Polymerase. *Langmuir* **2005**, *21* (10), 4669−4673.
(184) Xu, P.; Kaplan, D. L. Horseradish peroxidase catalyzed polymerization of tyrosine derivatives for nanoscale surface patterning. *J. Macromol. Sci. Pure Appl. Chem.* **2004**, *A41* (12), 1437−1445.
(185) Xu, P.; Kaplan, D. L. Nanoscale surface patterning of enzyme-catalyzed polymeric conducting wires. *Adv. Mat.* **2004**, *16* (7), 628-+.
(186) Nagarajan, R.; Liu, W.; Kumar, J.; Tripathy, S. K.; Bruno, F. F.; Samuelson, L. A. Manipulating DNA Conformation using Intertwined Conducting Polymer Chains. *Macromolecules* **2001**, *34* (12), 3921−3927.
(187) Xiao, Y.; Shlyahovsky, B.; Popov, I.; Pavlov, V.; Willner, I. Shape and Color of Au Nanoparticles Follow Biocatalytic Processes. *Langmuir* **2005**, *21* (13), 5659−5662.
(188) Namba, K.; Caspar, D. L.; Stubbs, G. J. Computer graphics representation of levels of organization in tobacco mosaic virus structure. *Science* **1985**, *227* (4688), 773−6.

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