Biologically-Templated Metal Oxide and Metal Nanostructures for Photovoltaic Applications

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ABSTRACT

In several electronic, electrochemical and photonic systems, the organization of materials at the nanoscale is critical. Specifically, in nanostructured heterojunction solar cells, active materials with high surface area and continuous shapes tend to improve charge transport and collection, and to minimize recombination. Organizing nanoparticles, quantum dots or organic molecules intro three-dimensional structures can thus improve device efficiency. To do so, biotemplates with a wide variety of shapes and length scales can be used to nucleate nanoparticles and to organize them into complex structures.

In this work, we have used microorganisms as templates to assemble metal oxide and metal nano- and microstructures that can enhance the performance of photovoltaic devices.

First, we used M13 bacteriophages for their high aspect ratio and ability to bind noble metal nanoparticles, to create plasmonic nanowire arrays. We developed a novel process to assemble bacteriophages into nanoporous thin films via layer-by-layer assembly, and we mineralized the structure with titania. The resulting porous titania network was infiltrated with lead sulfide quantum dots to construct functional solar cells. We then used this system as a platform to study the effects of morphology and plasmonics on device performance, and observed significant improvements in photocurrent for devices containing bacteriophages.

Next, we developed a process to magnesiothermally reduce biotemplated and solution-processed metal oxide structures into useful metallic materials that cannot be otherwise synthesized in solution. We applied the process to the synthesis of silicon nanostructures for use as semiconductors or photoactive materials. As starting materials, we obtained diatomaceous earth, a natural source of biotemplated silica, and we also mineralized M13 bacteriophages with silica to produce porous nanonetworks, and Spirulina major, a spiral-shaped algae, to produce micro-coils. We successfully reduced all silica structures to nanocrystalline silicon while preserving their shape.

Overall, this work provides insights into incorporating biological materials in energy-related devices, doping materials to create semiconductors, characterizing their morphology and composition, and measuring their performance. The versatility and simplicity of the bottom-up assembly processes described here could contribute to the production of more accessible and inexpensive nanostructured energy conversion devices.

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À mes parents,
Hélène et André,
sans qui tout ceci
n’aurait pas été
possible.
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Chapter 1:
Introduction and Motivation
Chapter 1. Introduction and Motivation

1.1 Nanotechnology for Solar Energy

Solar energy is the most abundant renewable energy source. Every year, $5.5 \times 10^{17}$ kWh of solar energy strikes the Earth’s surface, which represents 720 times the world annual human energy consumption.\(^1\) The access to this energy is unlimited and therefore the interest in developing efficient photovoltaic devices is growing within the science and engineering communities. However, there currently exists an obvious discrepancy between the abundance of solar energy and the use of this energy as a supply in the United States and worldwide, as illustrated in Figure 1-1. While solar energy represents over 99% of the total available renewable energy sources, it only consisted in 3% of the energy supplied in the United States in 2013. In addition, the total energy supplies that derive from renewable sources sum up to only 9.5% of the total, and the remaining 90.5% still originate from non-renewable sources.\(^2\)

![Figure 1-1](image_url)

**Figure 1-1.** A discrepancy exists between the large abundance of the solar resource and its poor use as an energy supply in the United States. A. Percentage of renewable energy amongst all energy supplies in the U.S. in 2013 and corresponding distribution of renewable energy supplies. B. Availability of renewable energy and distribution of renewable energy supplies. These charts were created using data from the Institute for Energy Research and the U.S. Energy Information Administration.\(^2\)
The poor utilization of solar energy originates in part from the high materials and manufacturing costs to produce photovoltaic devices. In order for solar energy to become competitive with natural gas, its price should decrease below 20 cents per kWh, and to compete with coal, its price should approach 5 cents per kWh. Currently, only crystalline silicon solar cells and multijunction devices have the potential to reach parities with non-renewable resources because of their high power conversion efficiencies, ranging between 20 to 45%. Still, the economic and environmental costs of these technologies is high. For instance, crystalline silicon solar cells require refining highly pure electronic grade silicon, texturing the wafers via chemical etching, and slicing the wafers. These complex manufacturing steps result in a costly, high temperature and energy-intensive process.

Nanomaterials can be used to simplify the processing steps, using technologies like roll-to-roll manufacturing, and various solution-based thin film deposition methods like spincoating, dipping, dropcasting, etc. The cost of nanomaterials and solution-processable materials like quantum dots, nanoparticles and conjugated polymers, is also generally lower than the cost of crystalline silicon, and they may be synthesized using solution chemistries. In addition, thinner films of nanomaterials that can strongly absorb the sunlight can be used to harvest the same quantity of light than silicon wafers that are orders of magnitude thicker. The bandgap, and therefore the wavelengths at which light is absorbed, can also be tuned for quantum dots, as a function of their size which can be controlled by the synthesis conditions. Photoactive nanomaterials therefore represent a versatile class of materials that can be used to develop novel photovoltaic devices at low cost.

Thin film photovoltaic technologies constructed with less expensive polycrystalline materials, including nano and microcrystalline silicon, and with organic and inorganic nanomaterials, have attracted more interest in the past decade and have increased in efficiency (see Figure 1-2). Organic polymer solar cells, quantum dot solar cells, and nano- or microcrystalline silicon solar cells reached efficiencies around 10 %, and amorphous silicon devices increased above 13 % efficiency.
Figure 1-2. Emerging photovoltaic devices constructed with nanomaterials have increased their efficiency significantly during the past decade. Efficiency of various solar technologies (thin-films and emerging PV) compiled by the National Center for Photovoltaics at the National Renewable Energy Laboratory (NREL). This figure was adapted from the latest NREL research solar cell efficiency chart.

Nanomaterials are often organized in heterojunction structures to construct devices. A heterojunction solar cell is composed of two materials, a p-type (or electron donor) material that transports holes to the cathode, and an n-type (or electron acceptor) material that transports electrons and is connected to the anode. Scheme 1-1 illustrates the general mechanism of operation of a heterojunction solar cell. When light is absorbed, electron-hole pairs, called excitons, form. Because of the difference in energy levels of the p-type and n-type materials, electrons generated in the p-type layer can dissociate from the holes at the interface of the two materials.

Scheme 1-1. Nanomaterials can be organized into heterojunctions to create thin film photovoltaic devices. Schematic representation of the energy levels of the p-type (donor) and n-type materials and the electrons (filled circle) and holes (empty circle) flow through the materials. LUMOd and HOMOd are the energy levels of the donor, and LUMOa and HOMOa are the energy levels of the acceptor.
The driving force for exciton dissociation is proportional to the difference in lower unoccupied molecular orbital (LUMO) level between the p-type and the n-type materials, and the rate of charge transport within each phase depends on the hole and electron mobilities in a given material. The efficiencies of charge dissociation, transport and collection are also strongly affected by the morphology of the heterojunction.

1.2 The Importance of Nanoscale Morphology in Energy Conversion, Catalytic and Electronic Devices

1.2.1 Nanostructures Photovoltaic Devices

Morphology is of primary importance when designing thin film solar cells. A continuous interpenetrated nanostructured morphology is desirable because of several reasons illustrated in Scheme 1-2: 1) High aspect ratio continuous structures allow for charges to be transported efficiently along direct pathways towards the electrodes. 2) Nano-sized domains minimize recombination of electrons and holes by decreasing the distance that excitons must travel to reach the interface between the p-type and n-type materials. This advantage applies in systems that are diffusion-limited, in other words, for materials with short exciton diffusion lengths, such as conjugated polymers. 3) In depleted nanostructured heterojunctions, thicker active layers can be constructed to harvest more light, without compromising charge collection. Improvements in device performance, especially increases in photo-generated current, have been observed for several type of solar cells, from all organic to hybrid, and all inorganic devices, when the materials were organized in a bulk heterojunction structure as opposed to a planar heterojunction.
Scheme 1-2. Continuous nanostructured morphologies can improve the efficiency of heterojunction devices through three mechanisms: 1) Improved charge transport, 2) Decreased recombination, and 3) Increased charge collection. Schematic diagram of the active layer of heterojunction solar cells (n-type material in yellow, and p-type in brown).

In polymer solar cells, various methods have been employed to create nanostructured morphologies, including the use of self-assembling block copolymers, the formation of polymer nanowires, template methods, nanoimprint lithography, etc. The morphology of the two polymer phase has been widely characterized, and researchers determined that nano-sized domains lead to an increase in photogenerated current by reducing bimolecular recombination and increasing charge mobility in conjugated polymers.

While the mechanism of charge generation and collection slightly differs between organic bulk heterojunctions and inorganic depleted heterojunctions, morphology also impacts the performance of quantum dot devices. Continuous percolated networks allow for more efficient charge transport and for the increase of the depletion region, which in consequence, allows for constructing thicker devices that can absorb more light and thus have higher power conversion efficiencies. For instance, it was demonstrated that a heterojunction composed of lead sulfide and bismuth sulfide quantum dots performed three times better when a blend layer was created compared to a planar morphology, due to an increased carrier lifetime in the bulk heterojunction structure.

Similar principles apply to thin film hybrid metal oxide-organic and all inorganic metal oxide-quantum dot devices. Metal oxides such as titania or zinc oxide are widely used as
n-type materials in heterojunction devices, and they can easily be used to create various nanostructures that impact device performance. For instance, they can be deposited as porous nanoparticle pastes, nucleated along templates with defined shapes, or chemically grown as nanopillars or nanowires. In particular, it has been reported that the performance of zinc oxide-lead sulfide quantum dot devices can be improved by constructing high aspect ratio zinc oxide arrays and infiltrating quantum dots within the porous oxide structures. Analogous observations were made for titania nanopillar arrays infiltrated with quantum dots. In these metal oxide-quantum dot devices, charge collection efficiency was enhanced when high aspect ratio metal oxide structures were introduced. Simply blending metal oxide particles with conjugated polymers or quantum dots also has the potential improving charge transport in the device. Challenges with devices involving metal oxides remain the contact between the two material phases, and problems with poor infiltration of polymers of quantum dots within porous metal oxide structures. Materials interface engineering and novel synthesis methods are currently studied to decrease resistances between oxides and organic or inorganic molecules.

Nanostructured morphologies also benefit the performance of novel micro or nano-crystalline silicon solar cells and hybrid silicon-organic devices. In these devices, charge transport can be improved using continuous nanostructures, and the reflectance of the device can simultaneously be decreased because of surface texturing acting as anti-reflection coatings. Various morphologies have led to enhanced currents and power conversion efficiencies, such as silicon nanocones, nanowires, nanotubes, and pyramid-textured silicon contacted with polymers of small organic molecules.

### 1.2.2 Other Devices with Nanostructured Morphologies

In addition to benefiting photovoltaic devices, high aspect ratio, continuous and porous morphologies at the nano- and microscale are desirable for other energy conversion, catalytic and electronic devices. For instance, silicon is a promising material for anodes for lithium-ion batteries. Continuous silicon structures can improve directional charge transport in these batteries, and highly porous materials are desirable in order to sustain the strain produced during lithiation-delithiation cycles. Porous metals or oxides are also often used as catalysts. Catalysts with morphologies that can maximize active surface area or active material loading are desired.
Apart from porous and high aspect ratio structures, being able to control even more complex morphologies could be useful for other applications. For instance, shape-memory materials can change shape in response to a stimulus and reform to their original shape. Currently, they are studied as nanoparticles or thin films, but producing other shapes could be useful for a variety of applications. Designing coil-shaped shape-memory alloys at the micro- or nanoscale could lead to the formation of heat-responsible coils that could expand and retract on demand. Spiral-shaped metal could also be used in light bulbs, or could be integrated as novel elements of electronic circuits. Integrated circuit may also benefit from novel synthesis methods for microstructures with turns, edges, angles and curves.

1.2.3 Current Methods for Producing Nanostructured Oxides or Metals

Two main strategies can be employed to create nanostructures: bottom-up and top-down approaches. Top-down approaches use patterning techniques, such as lithography, to introduce micro- and nanostructure into bulk materials, wafers, substrates, etc. On the other hand, bottom-up approaches being with nano-sized building blocks and involve molecular synthesis, colloidal chemistry, and macromolecular assemblies to create organized structures.

Current industrial microfabrication methods generally employ top-down approaches to produce metallic and oxide nanostructures. These methods include photolithography and harsh chemical etching, electron-beam or X-ray lithography, ion beam machining, anodization, and even mechanical deformations like ball milling. Although they can be applied to large scale material syntheses, top-down approaches require expensive equipment, and are often thermally and energy-intensive. They may also involve several serial protection, etching and deprotection steps, which slow down the process.

In contrast, bottom-up methods allow for less expensive, faster and more versatile assemblies. Bottom-up approaches allow for the formation of complex shapes using building blocks at the nanoscale and solution-based assembly methods, under mild conditions.
1.3 Self-Assembly and Biomineralization for Creating Nanostructures

1.3.1 Biological Templates and Biomineralization

Biotemplates are biological materials, including microorganisms, macromolecules, proteins and even DNA, that can serve as scaffolds for the creation of micro or nano-scale architectures. They provide a mean for creating nanostructures at low cost, via solution-based processes, and using bottom-up versatile assembly methods.

Biotemplates can be used to nucleate inorganic nanoparticles from precursors in solution. As illustrated in Scheme 1-3, precursor molecules are first attracted onto the surface of the biotemplate, often via electrostatic or other molecular interactions, and nanoparticles begin to nucleate on the surface of the template. The nanoparticles are then aligned on the biotemplated and cover its full surface. Finally, the biotemplated can be degraded and removed through heat treatment and combustion, chemical treatments, or enzymatic degradation for example. The final product is an inorganic material with the morphology of the biotemplate. By the shapes obtained, or by the mechanisms used to nucleate synthetic materials, biomineralization strategies often mimic nature.

![Scheme 1-3. Biological materials can serve as templates for the growth of inorganic nanowires by 1) nucleating precursors, 2) aligning nanoparticles, and finally 3) annealing the inorganic nanoparticles and burning off the biotemplate. This image of the M13 bacteriophage was adapted from Mao, C., et al., Science, 2004.](image)

Examples of commonly used biotemplates include viruses, such as the high aspect ratio M13 bacteriophage, the rod-like tobacco mosaic virus, and many other viruses with interesting and precise three-dimensional capsid structures. The M13 bacteriophage is of
particular interest because of its nanowire-like structure, and has been used so far to template a variety of metal nanoparticles, semiconductors and oxides.\textsuperscript{42,47-49} In addition, its tunable surface chemistry, mediated through the amino acid sequence of its 2700 copies of the pVIII coat protein, allows for engineering or selecting bacteriophage variants with binding affinities for various materials.\textsuperscript{50} For example, it can be used to bind and disperse noble metal nanoparticles and create plasmonic arrays.\textsuperscript{51}

Larger microorganisms, like bacteria and algae can also be used as templates for the creation of microstructures. Bacteria can be used to create hollow structures by nucleating inorganic particles onto their surface,\textsuperscript{52,53} and the structure of their cell walls can even be used to generate materials with precise symmetries.\textsuperscript{54} Most recently, proteins structures derived from bacteria, such as flagella\textsuperscript{55} and curli fibers\textsuperscript{56} have also served as biotemplates for nanofibers. Algae have also been used for the biomineralization of materials with a wide range of morphologies. For instance, \textit{Spirulina} algae have been used to produce coil-shaped metallic structures with different helical geometries.\textsuperscript{57} Some algae can also act as biotemplates during their natural life cycle. For instance, diatoms, through their long chain polyamines and silaffin peptides, nucleate biosilica inside of their silica deposition vesicles.\textsuperscript{58} The remains of these algae consist in fossilized skeletal silica structures that exhibit nanoscale features and porosities, and that can be used for biotechnological applications.

1.3.2 Nanoscale Assembly Methods

Various bottom-up approaches can be used to assemble biotemplates with themselves, with polymers, or with nanoparticles and other nano-objects. Nanomaterials can be assembled through several types of interactions: electrostatic, hydrogen bonding, covalent bonds, or other molecular affinities. A technique that can take advantage of all of these interactions to create thin composite films is layer-by-layer (LbL) assembly. Layer-by-layer assembly involves the sequential adsorption of nanomaterials through complementary interactions, traditionally electrostatic.\textsuperscript{59} It can be used to create homogeneous blends of materials at the nanoscale, since the layers of materials blend together as opposed to remaining segregated. Advantages of this technique include the precise control of thickness and composition of the film, as well as the low cost, water-based, ambient pressure and temperature process conditions.\textsuperscript{60} Layer-by-layer assembly can be modified to accommodate a wider variety of molecular interactions. For
instance, as shown in Scheme 1-4, non-covalent and non-electrostatic interactions such as hydrogen bonding can be exploited, and the process can also be turned into a reactive layer-by-layer assembly during which covalent bonds are formed between the layers of materials.

Scheme 1-4. Layer-by-layer assembly can be accomplished using two components with complementary functionalities. Complementary functionalities can include A. opposite charge (electrostatic LbL), B. non-electrostatic and non-covalent interaction such as hydrogen bonding or protein-protein interactions, or C. reactive groups that can participate in a covalent coupling chemistry.

Examples of covalent chemistries that can be applied to biotemplates generally involve the participation of chemical moieties ubiquitously present in amino acids, like carboxylic acid and amines. Carbodiimide chemistry can be used to couple an amine and a carboxylic acid to form a stable amide bond, and glutaraldehyde chemistry can be used to crosslink amines present at the surface of proteins. Sulphydryl groups can also be found in biotemplates (in cysteines) and they can participate in the formation of disulfide bonds, and in maleimide-thiol chemistry. Using such bioconjugate chemistries, biotemplates can be crosslinked together to form randomly-organized three-dimensional networks if a “one-pot” reaction is accomplished, or thin films of materials can be assembled when the reactions are integrated in a layer-by-layer assembly process.
It has been shown that M13 bacteriophages can be incorporated into layer-by-layer assembled films along with polymers, creating macroporous films. M13 bacteriophage have also been crosslinked using gluteraldehyde in a “one-pot” reaction to form hydrogels with pores in the order of hundreds of nanometers and high aspect ratio fibers made of bundles of several bacteriophages crosslinked together. Both of these three-dimensional structures were stable and could be coated with a layer of metal or metal oxide.

Complexes of biotemplates and nanoparticles or quantum dots can also be integrated into thin films or gels. Nanoparticles may initially be attracted to the biotemplate through engineered affinity, and the complexes can further be coated with another layer of materials, creating core-shell or complex hierarchical structures.

1.4 Thesis Overview and Objectives

This thesis focuses on the assembly and biomineralization of biotemplates to create three-dimensional structures at the biological, metal oxide and metal levels. This work is motivated by the need for fine nano-scale morphologies in thin films solar cells and other energy-related devices. Beginning with the assembly of a highly porous bacteriophage nanotemplate, metal oxides are nucleated onto the bacteriophage scaffold for thin film heterojunction solar cell applications. The work is then expanded to other biotemplates with various shapes and dimensions, and a process is developed to convert biotemplated metal oxides into metals that could be used for photovoltaic, catalytic or other energy-storage and conversion applications. The metal oxide and metal nanostructures presented here would be difficult to achieve using conventional fabrication methods, giving a unique essence to this thesis.

Scheme 1-5 presents a visual overview of the different aspects of this thesis.
Scheme 1-5. Starting with biotemplates, three-dimensional materials are formed and can serve as scaffolds for the synthesis of metal oxide and metal networks with photovoltaic applications. M13 bacteriophage, Spirulina major and diatomaceous earth are used as biotemplates to construct three-dimensional networks. Oxide structures are reduced to metals via magnesiothermal reduction (MGTR). Both oxide and metal biotemplated structures can be used for photovoltaic applications.

In Chapter 2, strategies to electrostatically assemble M13 bacteriophages using layer-by-layer assembly are explored. Methods for increasing the isoelectric point of M13 bacteriophages and producing a positively charged bacteriophage are described.

In Chapter 3, a novel covalent layer-by-layer assembly of M13 bacteriophage is developed. Using this process, highly porous thin bacteriophage films are assembled, with features at the nanometer scale and pores in the order of ten nanometers. The bacteriophage scaffold is mineralized with titania, producing a continuous and porous titania mesh. In addition, bacteriophage-nanoparticle complexes are incorporated into the thin films. We show that the distribution of nanoparticles with the films can be controlled, and that the nanoparticles remain dispersed even after mineralizing the structure with titania. Detailed characterization of the bacteriophage and titania film morphologies are performed and Appendix B presents methods for imaging the cross-section of thin films.
Appendices A and C present, respectively, an alternate method for creating bacteriophage-templated titania films, and the biomineralization of other semiconducting materials onto layer-by-layer assembled scaffolds.

In Chapter 4, thin film solar cells are then constructed using the bacteriophage-templated titania nanoporous structure. Lead sulfide quantum dots are infiltrated into the porous titania film to create a model nanostructured heterojunction. To improve device performance, two features of the M13 bacteriophage are taken advantage of: 1) its high aspect ratio to create continuous structures, and 2) its ability to specifically bind noble metal nanoparticles and create plasmonic arrays. The effects of film morphology and localized surface plasmon resonance are studied, and we confirm that M13 bacteriophages can contribute to increasing the photocurrent.

Appendices D through F present alternative methods for creating photoactive composites with high aspect ratio continuous structures, including the formation of titania-conjugated polymer composite thin films, and the synthesis of bacteriophage-quantum dot nanowires.

In Chapter 5, two new biotemplates are introduced, *Spirulina major*, and diatomaceous earth. Along with the M13 bacteriophages, these templates are used to form silica structures. The biotemplated silica is then magnesiothermally reduced to metalloid silicon, while preserving the initial shape of the microorganisms used as templates. We demonstrate that high surface area nanocrystalline silicon materials are obtained with various length scale and morphologies. The composition of the biotemplated silicon materials can be tuned, and the process could be extended to the formation of other metals, carbides, and nitrides. Appendix G specifically gives examples of titania mineralization and magnesiothermal reduction.

Finally, Chapter 6 extends the magnesiothermal reduction concept to nanoparticle coatings on various substrates, planar and curved surfaces, with the objective being the facile solution-processed coating of metals. Characterization of the electrochemical, electrical and thermal properties of silicon coatings, including biotemplated and nanoparticle-based, are performed to determine their most promising applications.

The primary conclusions of all chapters are presented in Chapter 7, along with recommendations for future work based on this thesis.
1.5 References


30. Liu, N.; Huo, K.; McDowell, M. T.; Zhao, J.; Cui, Y., Rice husks as a sustainable source of nanostructured silicon for high performance Li-ion battery anodes. *Scientific Reports* **2013**, *3*.


Chapter 2:
Genetic, Covalent and Electrostatic Modifications for Increasing the Isoelectric Point of M13 Bacteriophages
Chapter 2. Genetic, Covalent and Electrostatic Modifications for Increasing the Isoelectric Point of M13 Bacteriophages

This chapter summarizes the strategies employed to change and increase the isoelectric point of M13 bacteriophages. A bacteriophage with a higher isoelectric point could be layer-by-layer assembled with existing negatively charged M13 bacteriophages. Attempts at genetically modifying the pVIII protein sequence, covalently attaching positively-charged molecules to the pVIII proteins, or electrostatically coating M13 bacteriophages with positively charged polymers are described. The surface charge and morphology of the modified bacteriophage particles were characterized, and their potential for use as polycationic nano-objects in layer-by-layer assembly was evaluated.

2.1 Introduction

M13 bacteriophages are filamentous bacteriophages containing a single-strand circular DNA that encodes for 2700 copies of a major coat protein, pVIII, 5 copies of pIII and pVI proteins on one end of the virus particle, and 5 copies of pVII and pIX on the other end.1 Because of their high aspect ratio structure (~ 880 nm in length by 6 to 7 nm in diameter) and their programmable protein functionalities, M13 bacteriophages are widely used in biotechnology. By genetically engineering the pVIII coat protein, the surface properties of the phages can be modified to specifically bind nanomaterials, and they can be used as building-blocks in bottom-up material syntheses.2

The structure of the M13 bacteriophage is highly determined by its assembly and by its bacterial infection mechanism that requires an efficient extrusion through bacterial cell membranes. Bacteriophage proteins assemble onto the circular DNA, and use it as a substrate for bacteriophage particle assembly. Several phage proteins form an initiation site for the assembly and form complexes with the DNA. They are then replaced with pVIII proteins, as the DNA is extruded through the bacterial membrane.3 Basic residues of the pVIII proteins interact with the DNA, while acidic residues located in the N-terminus of the pVIII are exposed to the outside of the bacteriophage particle.3,4 Scheme 2-1 illustrates the helical structure of the pVIII proteins that results from these interactions, and the intrinsic polarity generated by the positioning of positively and negatively charged amino acids on the pVIII protein.
Primary structure of the engineered M13 phage major coat protein:

\[ \text{AEEEEDPAKAAFNSLQASATEYGYAWAMVVVIVGATIGIKLFKKFTSKAS} \]

**Scheme 2-1.** pVIII proteins are organized in a helical structure along the M13 bacteriophage, with negatively charged extremities exposed to the outside, and positively charged ends oriented towards the inside and the DNA. This figure was reproduced from Lee B.Y., et al., Nature Nanotechnology, 2012.4

A. Schematic representation of the M13 bacteriophage. B. Side view of the electrostatic potential of an E4 bacteriophage variant. The dipole moments generated by ten α-helical pVIII proteins are directed from the N-terminus (blue) to the C-terminus (red). C. Vertical cross-section view of the electrostatic potential of M13 bacteriophages. The pVIII proteins assemble with five-fold rotational and two-fold screw symmetry. D. Side-view representation of the electrostatic potential of a single pVIII protein, tilted 20° relative to the phage axis. E. Amino acid sequence of the pVIII protein for an E4 variant. The pVIII variable region is underlined in blue. The negatively charged amino acids are shown in blue and the positive ones in red.

In addition to the basic residues of the pVIII proteins that govern DNA interactions, two hydrophobic regions on the pVIII proteins have also been identified and are thought to mediate intermolecular interactions.5 Electrostatic and hydrophobic interactions therefore guide M13 bacteriophage assembly, and allow for the elongation of the bacteriophage particle. Once these requirements are satisfied, the bacteriophages can assemble, but some flexibility in the pVIII sequence is allowed in the N-terminus region, which can be extended by up to six amino acids, or modified.3 This variable region has the sequence “EGD” in the wild-type bacteriophage, and has been modified to “EEEE” for instance in the E4 bacteriophage variant shown in Scheme 2-1. However, experiments have shown that the polarity of the pVIII protein must be conserved and that the N-terminus of the pVIII should remain basic, or in other words negatively charged, in order for the bacteriophage particles to properly assemble and to avoid potential undesired interactions of the N-terminus with the DNA.
Since the N-terminus of the pVIII protein is exposed to the outside of the bacteriophage particle, it determines the surface charge of the bacteriophages. M13 bacteriophages are thus negatively charged at neutral pH, and in general at pHs above 5. However, synthesizing a positively charged bacteriophage would open up new uses for M13 bacteriophages. For instance, a positively charged bacteriophage could be electrostatically assembled with a wild-type M13 bacteriophage to create nanostructures. Similarly, layer-by-layer (LbL) assembly could be used to layer positively and negatively charged bacteriophages and create nanostructured thin films. Positively charged M13 bacteriophages could also allow to engineer bacteriophages with affinities for new materials, inorganic anions, and molecules that carry a negative charge.

Here, we describe how genetic engineering, post-assembly covalent chemistry and electrostatic modifications can be used to modify the charge of M13 bacteriophages. The engineered bacteriophage particle should have dimensions similar to wild-type bacteriophages, but with a higher isoelectric point (pI), allowing the particles to remain positively charged at higher pHs. Using zeta potential measurements to characterize the surface charge of the bacteriophages and various imaging tools to observe their morphology, we evaluated the potential of the modified phages for incorporation in LbL thin films.

2.2 Results and Discussion

2.2.1 Genetic Modifications

First, the zeta potential of existing bacteriophage variants was measured as a function of pH to determine their isoelectric point. Figure 2-1 shows zeta potential profiles for four bacteriophage variants: E3, E4, EFE and DSPH. It can be observed that the surface charge of all variants is negative at neutral pH, but that the isoelectric point differs significant from a variant to another. These differences can be explained from the amino acid composition of the N-terminus region of the pVIII proteins of each bacteriophage (see Table 2-1 for amino acid sequence and surface charge information for each bacteriophage variant). When amino acids with a low pKa are present in the solvent-exposed N-terminus region, the pI of the bacteriophage tends to be lower, and vice-versa. In addition, an increased number of negatively charged amino acids tends to also increase the magnitude of the negative surface charge, as observed between the E3 and E4 variants.
Figure 2-1. Different M13 bacteriophage variants exhibit different surface charge profiles and isoelectric points ranging between pH 3.7 and 5.2. Zeta potential titration measured with a 10 mM NaCl background electrolyte for four different bacteriophage variants.

As an attempt at further increasing the isoelectric point of M13 bacteriophages, several bacteriophages with pVIII proteins containing positively charged amino acids were transformed into bacteria. The DNA sequence of these bacteriophages was obtained from existing bacteriophage libraries or were custom-designed for this purpose. Among several sequences transformed, a few were successfully expressed and resulted in fully assembled bacteriophages. The zeta potential of two of these bacteriophages, EGHVHATM and EDHLHKSM, are presented in Figure 2-2, and their surface charge was analyzed in Table 2-1. Starting at around pH 8, the phages were titered and their surface charge was measured. The initial decrease in degree of ionization indicated that, by extrapolation, the isoelectric point of these bacteriophage could be located around pH 5.5 to 6 for EDHLHKSM, and around pH 4.5 to 5 for EGHVHATM. However, when the pH continued to decrease, severe aggregation of the bacteriophages occurred when the pI was reached, and the surface charge of the aggregates fluctuated significantly between pH 3 and 6 for EDHLHKSM, and at below pH 4.5 for EGHVHATM. These observations indicated that, although an increase in the apparent isoelectric point was observed for these variants, they are not stable around or below their isoelectric point.
The incorporation of positively charged amino acids in the pVIII sequence increases the theoretical isoelectric point, but also increases aggregation of phage particles. Zeta potential titration measured with a 10 mM NaCl background electrolyte for two phage variants with two or three positively charged amino acids, with E3 as a reference.

Table 2-1. The net surface charge of bacteriophage particles can be predicted from the pVIII sequence. The variable pVIII sequence is comprised between “SFAA…DPAK”, at the n-terminus of the pVIII protein. The number of positive and negative charges are reported only for the variable region. In the non-variable N-terminus region, there are additional negative (D) and positive (K) charges.

<table>
<thead>
<tr>
<th>Bacteriophage name</th>
<th>Variable pVIII sequence</th>
<th>Number of positive charges</th>
<th>Number of negative charges</th>
<th>Net charge</th>
</tr>
</thead>
<tbody>
<tr>
<td>E3</td>
<td>EEE</td>
<td>0</td>
<td>3</td>
<td>-3</td>
</tr>
<tr>
<td>E4</td>
<td>EEEE or EEAEE</td>
<td>0</td>
<td>4</td>
<td>-4</td>
</tr>
<tr>
<td>DSPH</td>
<td>DSPHTELP</td>
<td>1</td>
<td>2</td>
<td>-1</td>
</tr>
<tr>
<td>EFE</td>
<td>DVYESALP</td>
<td>0</td>
<td>2</td>
<td>-2</td>
</tr>
<tr>
<td>EGHVHATM</td>
<td>EGHVHATM</td>
<td>2</td>
<td>1</td>
<td>+1</td>
</tr>
<tr>
<td>EDHLHKSM</td>
<td>EDHLHKSM</td>
<td>3</td>
<td>2</td>
<td>+1</td>
</tr>
</tbody>
</table>

Among the variants that demonstrated no significant aggregation, the DSPH and EFE bacteriophages exhibited the lowest and highest isoelectric points, respectively. After analyzing their zeta potential curves, it was determined that they might be layered together at pHs ranging roughly between 4 and 5. Figure 2-3 presents this analysis and the results from a LbL assembly experiment. pH 4.3 was chosen for the layering experiment because it maximized the absolute value of the charge for both bacteriophages. At this pH, their zeta potential reached
approximately 15 mV, as shown in Figure 2-3A. After 60 bilayers, high aspect ratio features could be observed on the surface of the films via atomic force microscopy (AFM) (Figure 2-3B), confirming the sequential adsorption of bacteriophages onto the substrate.

![Figure 2-3. DSPH and EFE bacteriophages can be layer-by-layer assembled at pH 4.3. A. Zeta potential curves for DSPH and EFE showing the pH range at which the bacteriophages could be layered. B. Surface morphology imaged by AFM (amplitude images) for 60 bilayer films, at two different magnifications. C. 60 bilayer film scratched and imaged with AFM to determine its thickness. D. Growth curve showing the thickness and roughness of thin films containing 60 and 120 DSPH-EFE bilayers.]

Since the LbL films obtained were very thin, AFM was used to determine both their thickness and roughness. After scratching the films, they were imaged (as shown in Figure 2-3C), and image analysis and 3D projections with the Nanoscope Analysis software allowed for the construction of the growth curve shown in Figure 2-3D. Although a film growth is observed, the growth rate is very slow (less than 1 nm/bilayer), and the roughness of the film was calculated to be very large, especially for the thinner 60 BL film. Considering a 10 min dipping time in each bacteriophage bath and the observed growth rate, constructing a 200 nm thick film
would take between 6 and 7 days. This is likely due to the low ionization of both bacteriophages at the assembly pH, which reduced the driving force for assembly. Because of the slow growth and large roughness, the experiment was not further continued, but one could expect that the roughness would become less significant as the film thickness would increase.

2.2.2 Covalent Modifications

2.2.2.1 EDC Chemistry

Carbodiimide chemistry can easily be applied to M13 bacteriophage because they contain an abundance of exposed carboxylic acid and amine groups that can react with carbodiimides like 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). To increase the isoelectric point of bacteriophages, the exposed negatively charged carboxylic acid groups on the pVIII proteins can be covalently linked to small polyamine molecules via EDC chemistry, as shown in Scheme 2-2. Two polyamine molecules of interest are spermidine and spermine. They contain respectively three and four amines that can confer positive charges to the bacteriophages when protonated. In addition, these polyamines have been shown to catalyze the formation of titania, which could be of interest for the synthesis of a bacteriophage-templated titania network.

A. The structure of two candidate polyamine molecules, spermine and spermidine. B. The reaction mechanism for 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) chemistry applied to M13 bacteriophages. N-hydroxysulfosuccinimide (sulfo-NHS) was added to increase the solubility and stability of the reaction intermediate.

Scheme 2-2. Positive charges can be introduced on the surface of bacteriophages by conjugating available carboxylic acid groups on pVIII with polyamine molecules via carbodiimide chemistry. A. The structure of two candidate polyamine molecules, spermine and spermidine. B. The reaction mechanism for 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) chemistry applied to M13 bacteriophages. N-hydroxysulfosuccinimide (sulfo-NHS) was added to increase the solubility and stability of the reaction intermediate.
Two different bacteriophage variants were selected for reaction with polyamines, E3 and DSPH. E3 was selected because of the four carboxylic acid groups present on the N-terminus of each pVIII. Each of these groups can react with a polyamine molecule. On the other hand, DSPH was selected because it was the stable bacteriophage variant with the highest isoelectric point. DSPH exhibits three carboxylic acids on the N-terminus of its pVIII, but also contains a histidine that contributes to increasing its isoelectric point.

The covalent linkage between small polyamine molecules and the pVIII of bacteriophages was confirmed via mass spectrometry, which showed that variable fractions of the pVIII proteins carried at least one spermine or spermidine molecule after reaction. The number of pVIII proteins functionalized depended on excess of polyamine used during the reaction. Covalently linking spermine had a greater effect on the charge of the bacteriophages compared with spermidine. The zeta potential profiles of E3 and DSPH covalently modified with spermine are shown in Figure 2-4.

As observed in Figure 2-4A, the isoelectric point of E3 is increased by approximately half a pH unit when modified with spermine. Increasing the excess of spermine during the reaction did not further increase the pI, but reduced the absolute value of the bacteriophage charge at pHs above the pI. In the case of DSPH, it appears like the isoelectric was not significantly modified by the addition of spermine, but that, again, the magnitude of its negative surface charge was reduce at high pHs.
Figure 2-4. The isoelectric point of bacteriophages conjugated with spermine molecules is slightly increased. Zeta potential titrations measured with a 10 mM NaCl background electrolyte for A. E3 and E3 conjugated with two different ratios of spermine, and B. DSPH and DSPH conjugated with spermine.

2.2.2.2 Maleimide-Thiol Chemistry

As second type of covalent chemistry that could be applied to M13 bacteriophages is the maleimide-thiol reaction. It is known that maleimides can react with sulfhydryl groups to block them or attach a molecule or peptide. Therefore, a bacteriophage expressing two cysteines on each of its pVIII, the cysteine phage, was selected for this reaction. The variable pVIII amino acid sequence of the cysteine phage is “SCPDCGAE”, and natively, the two cysteines tend to form disulfide bonds that need to be broken before reacting them with a maleimide. To do so, reducing agents, such as tris(2-carboxyethyl)phosphine (TCEP) can be employed, and a maleimide-functionalized positively charged peptide can be introduced into the reaction mixture.
Scheme 2-3 illustrates how such as peptide can be coupled to a pVIII protein with an available sulfhydryl group.

Scheme 2-3. Maleimide-functionalized positively charged peptides can be attached to pVIII proteins via maleimide-thiol chemistry. A single pVIII protein with a reduced cysteine is shown in yellow.

We have chosen to use a biologically-inspired peptide that could confer a positive charge to the bacteriophages, but could also mimic peptides found in nature that nucleate metal oxide ions. We therefore chose the peptide silaffin, which is involved in the biogenesis of silica in diatoms. Silaffin has also been shown to promote titania formation \textit{in vitro}. The structure of this peptide is shown in Scheme 2-4, where it can be observed that several of its amino acids are phosphorylated or modified with polyamine chains, giving an amphiphilic character to the peptide. It has to be noted that the amino acid sequence itself is also amphiphilic, as it contains several lysines (positively charged) and serines (containing hydroxyl groups). For simplicity, a synthetic silaffin peptide without phosphorylation or polyamine modifications was synthesized for covalent attachment to the cysteine phage.

Scheme 2-4. Silaffin peptide is an amphiphilic molecule containing several positively charged amino acids. This image was reproduced from Cole K.E. & Valentine A.M., Biomacromolecules, 2007.
After reacting the cysteine phage with a maleimide-functionalized silaffin peptide under reducing conditions, the products were analyzed using electrophoresis and mass spectroscopy (MALDI-TOF). The results are shown in Figure 2-5. The amino acid sequence of each protein involved in the reaction was used to analyze the results. These sequences are shown in Table 2-2.

Figure 2-5A shows the reaction products for different concentrations of reducing agent, TCEP. It appears that, as the concentration of TCEP increases, the band corresponding to the pVIII-silaffin product increases in intensity, indicating the need for reducing the disulfide bonds of the cysteine phage before proceeding to the maleimide-thiol coupling. In all of these reactions, unreacted silaffin is also observed. Figure 2-5B shows that the product can be purified via polyethylene glycol/sodium chloride precipitation and centrifugation, and that all unreacted silaffin can be removed from the product. Finally, mass spectroscopy was difficult to analyze because of the low purity of the cysteine phage used. Because cysteine phages tend to amplify slowly and attract other bacterial proteins as contaminants, the spectrum for the final phage-silaffin complexes after purification was cleaner and easier to analyze. A peak located at the expected position for pVIII was located, along with additional peaks that could correspond to reaction products.

Although a cysteine phage-silaffin complex was formed, it was not further analyzed or used because of the low amplification yields of cysteine phages. In order to use bacteriophages for LbL assembly, tens of milliliters of bacteriophage at $10^{13}$ phage/mL are required, and this quantity could not be obtained efficiently for the cysteine phage, and thus for cysteine phage-silaffin complexes.
Figure 2-5. Gel electrophoresis and mass spectroscopy confirm the covalent crosslink between the pVIII protein of cysteine phage with silaffin. Protein SDS-PAGE gels stained with coomassie blue showing A. reactions carried out with TCEP concentrations increasing from 0 to 250 equivalents per pVIII compared with a reaction without silaffin, the cysteine phage only and silaffin only. B. a reaction purified or not via polyethylene glycol/sodium chloride precipitation. The bands for the pVIII of cysteine phage, silaffin, and the product are shown in black, yellow and red respectively. C. MALDI-TOF spectra for cysteine phage, silaffin and the reaction product.
Table 2-2. The amino acid sequences of the peptides involved in the maleimide-thiol coupling of phage and silaffin are predicted. The his-tag added to silaffin is underlined.

<table>
<thead>
<tr>
<th>Protein or peptide</th>
<th>Amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maleimide-functionalized,</td>
<td>Maleimide – SSKSGSYSGSKGSKHHHHHHH - COOH</td>
</tr>
<tr>
<td>his-tagged silaffin</td>
<td>SCPDGAEDPAKAAPKNSLQASATEYIGYAWAMVVMVIVG</td>
</tr>
<tr>
<td>pVIII of cysteine phage</td>
<td>ATIGIKLFKFTSKAS</td>
</tr>
<tr>
<td>Reaction product</td>
<td>HHHHHHKSGKSGYSGSKKSS - maleimide-thiol bond - SCPDGAEDPAKAAPKNSLQASATEYIGYAWAMVVMVIVG</td>
</tr>
</tbody>
</table>

2.2.2.3 Sortase-Mediated Modifications

Sortagging was first developed in 2007, and it was demonstrated to be a versatile method to label or conjugate proteins, using bacterial enzymes that that recognize specific amino acid motifs. Sortagging was applied to M13 bacteriophages a few years later in the Belcher group. Scheme 2-5 shows how sortase A (SrtA) from *Streptococcus aureus* can be used to attached positively charged peptides to pVIII proteins. SrtA recognizes the amino acid motif LPXTA, and cleaves between the threonine and the alanine to produce a sortase-acyl peptide complex. This is followed by a nucleophilic attack of the N-terminal of the pVIII containing an oligo-alanine.

![Scheme 2-5](image)

**Scheme 2-5.** Positively charged peptides with the motif LPXTA can be covalently attached to modified pVIII proteins with a N-terminus oligo-alanine via sortagging. SrtA designates the enzyme sortase A from *Streptococcus pyogenes.*
To attach a silaffin peptide to the pVIII proteins of a bacteriophage, a synthetic silaffin peptide containing a LPETAA fragment was obtained, and a modified DSPH bacteriophage with two exposed alanines was amplified. The peptide sequences of each of the proteins involved in the sortagging reaction are shown in Table 2-3.

**Table 2-3.** The amino acid sequences of the peptides involved in the sortase-mediated coupling of phage and silaffin are predicted. His-tags are underlined, and sequences required for sortase reaction are in bold. The chemistry of the C-terminal amino acid of silaffin is indicated in italic.

<table>
<thead>
<tr>
<th>Protein or peptide</th>
<th>Amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hig-tagged silaffin with LPETAA sequence</td>
<td>SSKKSGSYSGSKGSKHHHHHHHLPETAA – CONH₂</td>
</tr>
<tr>
<td>pVIII of AADSPH phage</td>
<td>AADSPHTELPDPAAAFNSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS</td>
</tr>
<tr>
<td>Sortase pyogenes</td>
<td>MRSSHHHHHH SSGLVPRGSV LQAQMAAQQL VIGGIAPE LGINLPIFKG LGNTELIYGA GTMKEQVMG ENNYSLASH HIFGITGSSQ MLFSPLERAQ NGMSIYLTDK EKIYEYIIKD VFTVAPERVER VIDDATAGLKE VTLMRSSHHH HHHSSGLVPR GSVLQAQMAA QQLPVIGGIA IPELGINLP1 FKGLGNTELI YGAGTMKEQ VMGGENNYSL SHHIFGITG SSQMLFSPLE RAQNGMSIYL TDKEKIYEYI IKDVFTVAPE RVDVDDTAG LKEVTLVTCT DIERATIIV KGEKTEYDF DKAPADVILA FNHSYNQVST</td>
</tr>
<tr>
<td>Reaction product</td>
<td>SSKKSGSYSGSKGSKHHHHHHHLPETAADSPHTELPDPAAK1 AFNSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS</td>
</tr>
</tbody>
</table>

To confirm that a covalent linkage formed between the pVIII of AADSPH and silaffin, gel electrophoresis was used in combination with mass spectroscopy. First, gel electrophoresis visually demonstrated the formation of a sortase-silaffin complex for a control reaction carried out without bacteriophage, and then the formation of a pVIII-silaffin product for a full reaction, as shown by the shift of the pVIII band in the last row of Figure 2-6A. MALDI-TOF also showed the presence of additional peaks after sortagging, and the decrease in intensity of the AADSPH peak, which could be indicative of the formation of the AADSPH-silaffin complexes.
Figure 2-6. Gel electrophoresis and mass spectroscopy confirm the presence of crosslinks between silaffin and the pVIII proteins of AADSPH. A. Protein SDS-PAGE gel stained with coomassie blue showing the bands for AADSPH alone, silaffin alone, sortase alone, a reaction carried out without phage (sortase + silaffin only), and a full reaction. The pVIII of AADSPH is boxed in black, the sortase-silaffin intermediate in a dashed-black line, and the product in red. Silaffin and sortase alone are boxed in yellow and green respectively. B. MALDI-TOF spectra for AADSPH alone, and the product after sortase reaction.

The surface charge of the AADSPH before and after sortase reaction was measured as a function of pH. Figure 2-7A shows that silaffin shifted the isoelectric point of AADSPH by approximately half a pH unit, and also caused the surface charge of the phage to be slightly more positive at all pHs.

Since silaffin was a large peptide that should increase the overall size of the bacteriophage once covalently attached to the pVIII proteins, the size of the product was also monitored as a function of pH, and compared with the size of free AADSPH bacteriophages. As shown in Figure 2-7B, the size of the bacteriophage particles with silaffin attached is larger than for naked bacteriophages at all pHs. It has to be noted that this size corresponds to a hydrodynamic radius for particles in solution, and therefore, it does not give a precise measurement for the size of a bacteriophage due to its high aspect ratio. However, it provides an estimation of relative hydrodynamic radii before and after reaction with silaffin. The right panel of Figure 2-7B shows the differences in size of the bacteriophages at high pH, before any
aggregation occurred. For fully dispersed AADSPH, the minimum size of a bacteriophage was measured to be 239 nm (at pH 7.1), while fully dispersed AADSPH with silaffin are no smaller than 329 nm (at pH 7.9). This difference in size is indicative of the presence of the silaffin peptide on the surface of the bacteriophages, causing their size to increase. In addition, the aggregation of bacteriophages (or sudden increase in size) occurs at a higher pH for the AADSPH-silaffin complexes, which corroborates the increase in isoelectric point of the phage after silaffin attachment.

**Figure 2-7.** Attaching silaffin to AADSPH bacteriophages increases their isoelectric point and their size. Zeta potential (A) and size, or hydrodynamic radius, (B) of AADSPH bacteriophages, before and after sortagging silaffin, in a 10 mM NaCl background electrolyte.
2.2.3 Electrostatic Assemblies

2.2.3.1 Polymer-Coated Bacteriophages

Electrostatic interactions were also explored as a mean to modify the isoelectric point of M13 bacteriophages. Since the E3 bacteriophages carry a negative surface charge at pHs above 4.2, they can be coated with common polycations that are positively charged at these pHs. As illustrated in Scheme 2-6, the goal is to conformally layer a polymer around the bacteriophages to form individual phage-polymer complexes with a positive surface charge. Ideally, the polymer should not significantly change the size and aspect ratio of the bacteriophage particles so that they could be used LbL assembly to create bacteriophage thin films with high aspect ratio features. Previous reports indicate that it is possible to coat bacteriophages with polymers, but detailed studies of the charge and morphology of the products have not been carried out.\(^{13,14}\) The coating or layering process is analogous to the layer-by-layer coating of nanoparticles.

\[+\]

Scheme 2-6. Polycations can be layered onto the surface of negatively charged M13 bacteriophages. A polymer chain is shown in red.

Three polycations were selected for this study, linear polyethyleneimine (LPEI), poly(allylamine hydrochloride) (PAH), and poly-L-lysine (PLL). The molecular weight of the polymers was selected to be as low as possible in order to create small bacteriophage-polymer complexes. The chosen polymers are listed in Table 2-4. The pH used for layering onto bacteriophages was determined based on the isoelectric point of the polymers and of E3 bacteriophages.
Table 2-4. Polycationic polymers were chosen to layer the M13 bacteriophages. The molecular weight of the available polymers are presented as well as their isoelectric point and the chosen pH for layering the M13 bacteriophages.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>MW (kDa)</th>
<th>pI</th>
<th>Chosen layering pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethyleneimine</td>
<td>40</td>
<td>4.8\textsuperscript{15} to 5.5\textsuperscript{17}</td>
<td>3 and 5</td>
</tr>
<tr>
<td>Poly(allylamine hydrochloride)</td>
<td>15</td>
<td>8.8\textsuperscript{18}</td>
<td>7</td>
</tr>
<tr>
<td>Poly-L-lysine</td>
<td>15-30</td>
<td>10.5\textsuperscript{19}</td>
<td>7</td>
</tr>
</tbody>
</table>

First, LPEI was layered on bacteriophages at pH 3 or pH 5. Since the isoelectric point of LPEI is relatively low, layering pHs were close to the pI of E3 bacteriophages. Nonetheless bacteriophage-complexes were formed in both cases. At pH 5, E3 bacteriophages have a negative charge of approximately 25 mV, which is large enough for an electrostatic interaction to occur. However, at pH 3, the net charge of the bacteriophages is positive. The formation of complexes at this pH could therefore be explained by the availability of several carboxylic acid groups on the surface of the bacteriophages that might still be able to interact with the positively charged amine groups of the polymer.

The zeta potential curves of the bacteriophage-LPEI complexes were measured for different polymer to bacteriophage ratios at pH 3 and pH 5. The ratios were varied from 10:1 to 1000:1 LPEI:phage, and as the ratio increases, the zeta potential curves shift to more positive values, as shown in Figure 2-8. At ratios of 100:1 and 1000:1, the isoelectric point of the complexes is significantly shifted compared free bacteriophages.
Figure 2-8. The isoelectric point of bacteriophage-LPEI complexes shifts towards higher pH values as the polymer to phage ratio increases. Zeta potential titrations measured with a 10 mM NaCl background electrolyte for bacteriophage-LPEI complexes synthesized at pH 3 or 5, and various LPEI:phage ratios.

A 1000:1 LPEI:phage ratio produced well-defined fibers, shown in Figure 2-9. Lower ratios tend to produce complexes of free floating bacteriophages with aggregated polymer particles, while higher ratios produce large aggregates without defined structure. An interesting observation was made when the polymer/bacteriophage mixture was heated up to 50 or 80 °C during the layering process. It is known that, at high temperature, carboxylic acids can react with amines to form amide crosslinks. This process likely occurred in the bacteriophage-LPEI complexes, since the shape of the fibers became better defined and thicker after heating up the samples. Figure 2-9B shows the morphology of the fibers obtained under these conditions.
Figure 2-9. Large fibers are formed after layering LPEI into the surface of M13 bacteriophage. AFM amplitude images showing fibers formed for A. layering at pH 3, with a 1000:1 LPEI:phage ratio, and B. layering under the same conditions but at an elevated temperature of 50 °C.

However, as shown in Figure 2-9A (left panel), aggregates were observed even for the optimal 1000:1 ratio. In addition, the diameter of the fibers ranges between 100 to 500 nm, for the thinnest fibers observed, which is considerably larger than the diameter of a single bacteriophage. The length of the complexes also exceed the length of M13 bacteriophages by orders of magnitudes. Therefore no bacteriophage-LPEI complexes are not suitable for LbL assembly of thin films with single bacteriophages.

Next, polymers with a higher isoelectric point, which would allow for layering with fully ionized M13 bacteriophages, were selected. A higher degree of ionization of the bacteriophages might allow for the formation of less aggregates. In addition, polymers with a lower molecular weight were used, in order to reduce the size of the bacteriophage-polymer complexes.
A 15 kDa PAH was layered onto E3 bacteriophages at pH 7, and the zeta potential of the products was characterized. The curves are reported in Figure 2-10. As observed with LPEI, the zeta potential of the bacteriophage-PAH complexes shifts towards more positive values as the polymer to phage ratio increases, producing exclusively positively charged fibers for a 100 and 1000X excess polymer relative to bacteriophages.

![Zeta potential titrations](image)

**Figure 2-10.** The isoelectric point of bacteriophage-PAH complexes shifts towards higher pH values as the polymer to phage ratio increases. Zeta potential titrations measured with a 10 mM NaCl background electrolyte for bacteriophage-PAH complexes synthesized at pH 7, and various PAH concentrations relatively to the number of bacteriophage.

While complexes of bacteriophage and PAH were formed, the morphology of these complexes is not desirable. As shown in Figure 2-11, for low polymer ratios, individual bacteriophage and aggregated polymer particles can be observed. Although these aggregates colocalize, the polymer does not seem to conformally coat the bacteriophages. As the polymer concentration increases, larger aggregates are observed, but no high aspect ratio structures were obtained.
Figure 2-11. Bacteriophage-PAH complexes form aggregates. AFM amplitude images showing the morphology of bacteriophage-PAH complexes for different concentrations of PAH (10X, 100X, 1000X and 10000X relative to the number of bacteriophages).

The bacteriophage-polymer complexes with the optimal morphology were obtained by coating E3 bacteriophages with PLL, at pH 7, using a 1000X excess of PLL. The zeta potential of the bacteriophage-PLL complexes was positive up to the isoelectric point at pH 7 (see Figure 2-12), corresponding in a large shift compared to the zeta potential of free E3 bacteriophages.

Using PLL as polycation allowed for the formation of individually-coated bacteriophages. Figure 2-13A and B show the difference between free uncoated bacteriophages, and bacteriophage-PLL complexes, and Figure 2-13C shows higher magnification images of the complexes. The particles obtained have a length that roughly corresponds to the length of a single bacteriophage (880 nm), and their diameter ranges between tens of nanometers to approximately 200 nm.
Figure 2-12. Bacteriophage-PLL complexes have an isoelectric point around pH 7. Zeta potential titration measured with a 10 mM NaCl background electrolyte for bacteriophage-PLL complexes synthesized with a 1000:1 PLL:phage ratio, at pH 7.

A. 

B. 

C. 

Figure 2-13. PLL can conformally coated bacteriophage particles, forming individual complexes. AFM amplitude images comparing A. free uncoated E3 bacteriophages, with B. bacteriophage-PLL complexes, and C. showing high magnification images of the complexes. PLL was layered onto the bacteriophages at pH 7 using a 1000:1 polymer:phage ratio, and the product was dialyzed to remove excess polymer.
Judging that the bacteriophage-PLL complexes could be suitable for LbL assembly with free E3 bacteriophages, we then proceeded to a LbL assembly on silicon substrates. The substrate was plasma treated and the film was assembled by dipping 15 minutes in the bacteriophage-PLL complexes followed by rinses and dipping in E3 bacteriophages. The pH of the bacteriophages and bacteriophage-PLL complex solution was maintained at 4.9 using NaOAc buffer. The surface morphology of the films obtained is shown in Figure 2-14. At low or high magnification, it is difficult to distinguish bacteriophage-like features in these images. This could be due to the PLL layer detaching from the bacteriophage-PLL complexes during the process, filling the pores between free and complexed bacteriophages, and smoothing the film morphology. The PLL coating approach therefore provides a mean for incorporating bacteriophages with other negatively charged nano-objects in LbL thin films, but not for forming porous thin films that exhibit continuous high aspect ratio features characteristic of the M13 bacteriophages.

![Figure 2-14](image)

**Figure 2-14.** The morphology of LbL films constructed with E3 and E3 coated with PLL does not clearly show high aspect ratio structures. AFM images showing the surface morphology of 30 bilayer films. A. Low magnification amplitude image, and B. corresponding height image. C. Higher magnification amplitude image.

### 2.2.3.2 Bacteriophage-Dendrimer Fibers

Poly(amido amine) (PAMAM) dendrimers were selected as another promising macromolecule for electrostatic modifications of bacteriophages. Because of their small diameter, and their high number of available amine groups, several PAMAM dendrimers could interact with the surface of a single bacteriophage, as illustrated in Scheme 2-7. The dendrimers...
can interact with bacteriophages via electrostatic interactions, and also be crosslinked to the bacteriophages using EDC chemistry.

Scheme 2-7. Amine-containing dendrimers can interact with the surface of M13 bacteriophages to create positively charged complexes.

The effect of the size of the dendrimers (or their generation) on the morphology of the bacteriophage-dendrimer complexes was studied. Generation two (G2) and generation five (G5) dendrimers were chosen. The properties of these dendrimers are shown in Table 2-5, and the morphology of the complexes obtained is shown in Figure 2-15.

Table 2-5. Generation 2 and 5 PAMAM dendrimers were selected for complexation with M13 bacteriophages. The dendrimers were purchased from Dendritech Inc., and had a cystamine core and an amine-terminated surface.

<table>
<thead>
<tr>
<th>PAMAM dendrimer</th>
<th>Generation</th>
<th>MW (Da)</th>
<th>Diameter (Å)</th>
<th>Number of surface amines</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNT-295</td>
<td>2</td>
<td>3,256</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>DNT-298</td>
<td>5</td>
<td>28,836</td>
<td>54</td>
<td>128</td>
</tr>
</tbody>
</table>

The first observation drawn from these pictures is that the dendrimers did not form complexes with a single bacteriophage, but formed large fibers, with a high aspect ratio. This is likely due to the large number of surface amine groups of the dendrimers, which can interact simultaneously with multiple bacteriophages to create large bundles. Second, the morphology of the fibers obtained with the two different dendrimers reflect clearly the size of the dendrimer particles. For G2 dendrimers, fibers are linear and it is difficult to distinguish individual dendrimers, while for G5 dendrimers, spherical polymer aggregates can be observed all along the
fibers. Larger dendrimers therefore alter the morphology of the fibers and create more aggregation.

**Figure 2-15.** Complexing bacteriophages with dendrimers of different generations produces fibers with different morphologies. AFM amplitude images showing bacteriophage-PAMAM complexes for generation 2 (left) and 5 (right) dendrimers. 100:1 dendrimer:phage ratio was used.

The bacteriophage-PAMAM complexes with G2 dendrimers were further studied. Their zeta potential was measured as function of pH and the results are shown in Figure 2-16. The isoelectric of the complexes is significantly higher compared to that of free E3 bacteriophages. In fact, the bacteriophage-dendrimer complexes have a positive surface charge up to pH 8.7, which is close to the theoretical isoelectric point of amine-terminated PAMAM dendrimers of ~9.2.21

**Figure 2-16.** E3 bacteriophages complexed with PAMAM have an isoelectric point shifted by almost 5 pH units compared to free bacteriophages. Zeta potential titration measured with a 10 mM NaCl background electrolyte for bacteriophage-PAMAM (G2.0) complexes.
The morphology of the complexes was then observed with TEM, as shown in Figure 2-17. The left panel shows large fibers, comparable to those observed with AFM, but further imaging revealed the presence of thinner fibers as well. A multiscale morphology is therefore present within the bacteriophage-PAMAM complexes.

![Figure 2-17. The morphology of bacteriophage-PAMAM dendrimer complexes encompasses larger and thinner fiber structures. TEM images showing fibers of different sizes formed using G2 PAMAM dendrimers.](image)

Next steps consisted in reducing the overall size of the bacteriophage-dendrimer fibers. The best results were obtained when the ratio of phage to dendrimer was maintained constant, but their concentration was reduced by a factor of 10 to 100 during the complexation. Diluting the reaction mixture contributed to reducing the formation of aggregates, as shown in Figure 2-18. The diameter of the fibers synthesized in dilute mixtures did not exceed 200 to 500 nm, while some aggregates observed previously could have diameters of 2 to 3 μm.

Although bacteriophage-PAMAM fibers exhibited a desired high isoelectric point and could be tuned to have diameters in the order of one hundred nanometers, their length remained considerably larger than the length a single bacteriophage. These complexes were therefore not used for LbL assembly with free bacteriophages, but could have other applications where high aspect ratio structures with several exposed amine groups are desirable.
Figure 2-18. Thinner bacteriophage-PAMAM dendrimer fibers are obtained when the reaction mixture is diluted. AFM amplitude images for bacteriophage-PAMAM complexes formed with G2 dendrimers when the reaction mixture was diluted A. 100 times, or B. 10 times compared to the images presented in Figure 2-15.

2.3 Conclusions

In conclusion, several bacteriophage-based materials with an increased isoelectric point were produced by modifying different M13 bacteriophage variants genetically, covalently, or electrostatically. First, the variability in the pVIII protein sequences results in significant changes in isoelectric points, but bacteriophages tend to aggregate as the pH is decreased and approaches their isoelectric point. Dramatic aggregation was observed for bacteriophage variants containing more positively charged amino acids. Covalent modifications then allow for slight modifications of the zeta potential profiles. Third, electrostatic modifications have shown to be very efficient in modifying the surface charge of bacteriophages, but polymer coatings or dendrimer complexation also drastically change the morphology of the bacteriophages and generally yield larger bacteriophage-polymer fibers. Only poly-L-lysine was conformally coated onto the surface of bacteriophages and produced individual bacteriophage-polymer particles.

Several difficulties were encountered in producing bacteriophages with an isoelectric point high enough for layer-by-layer assembly with existing negatively charged bacteriophages. When using bacteriophages with a low degree of ionization, LbL films grow at a very slow rate, and when using polymer-coated bacteriophages, the morphology of the film is altered and no high aspect ratio nanowire-like feature can be observed.

While the modified bacteriophages presented here are not suitable for the formation of LbL films with nanowire-like features, they might be appropriate for other applications, such as
drug or gene delivery. Bacteriophage-polymer complexes could also be used to incorporate bacteriophages or bacteriophages complexed with nanoparticles, quantum dots or drug molecules, into LbL films if a nanostructured morphology is not desired or critical.

Finally, this study also presents an exhaustive list of methods for functionalizing M13 bacteriophage particles. The genetic, covalent and electrostatic approaches described here could be used for modifying other surface properties of the bacteriophages by replacing different amino acids, modifying the molecules to be covalently attached, and changing the polymers used to coat the bacteriophages. One could imagine not only altering the charge of the bacteriophages, but also their hydrophilicity or hydrophobicity, their affinity for specific materials or substrates, and their shape or size.

2.4 Materials and Methods

**Materials:** Poly(allylamine hydrochloride) (15kDa) and polyethylenimine (40 kDa) were purchased from Polysciences Inc. (Warrington, PA). Poly-L-lysine (15-30kDa), spermine, spermidine and tris(2-carboxyethyl)phosphine (TCEP) were purchased from Sigma (St-Louis, MO). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysulfosuccimide (sulfo-NHS) were purchased from Pierce Biotechnology Inc. (Rockford, IL). PAMAM dendrimers (DNT-295 and DNT-298) were purchased from Dendritic Nanotechnologies Inc. (Midland, MI). Modified silaffin peptides were synthesized in the Biopolymers and Proteomics Laboratory (Koch Institute, MIT).

**Engineering pVIII proteins:** Plasmidic DNA was obtained from bacteriophage libraries containing pVIII sequences with positively charged amino acids, or custom DNA sequences were amplified with PCR and ligated into a vector. XL-1 Blue Supercompetent Cells (Agilent Technologies) were transformed with the plasmidic DNA using electroporation, and the cells were grown in Super Optimal broth with Catabolite repression (SOC) media for 1h. The cells were plated on tetracycline (Tet), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) and Isopropyl β-D-1-thiogalactopyranoside (IPTG)-containing agar plates, and colonies were counted and selected for amplification and DNA sequencing.

Bacteriophages were amplified by infecting exponentially growing *Escherichia coli* ER 2739 bacteria, and purified via polyethylene glycol/sodium chloride (PEG/NaCl) precipitation and
centrifugation cycles. The DNA sequence coding for the pVIII protein of each variant was confirmed via DNA sequencing.

**LbL of DSPH and EFE:** Bacteriophage solutions were prepared at a final concentration of $10^{13}$ phage/mL in sodium acetate buffer (NaOAc) at pH 4.3. The film was built by successively dipping an oxygen plasma treated substrate in the DSPH and EFE bacteriophage solutions, and rinsing in milli-Q water after the dip in each solution. Films of different thicknesses were assembled by varying the number of dipping repetitions. The thickness and roughness of the films was characterized via atomic force microscopy using a MultiMode Atomic Force Microscope (Veeco Metrology) in the tapping mode.

**EDC chemistry with polyamines:** Bacteriophage solutions were prepared in milli-Q water, at a final concentration of $2 \times 10^{12}$ phage/mL. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS) were added in a 10 to 1000X excess with respect to the number of carboxylic acid groups available on the surface of the bacteriophages (2700 pVIII proteins/phage, with a given number of COOH groups on each pVIII protein depending on the phage variant). For example, with four COOH groups per pVIII for E3, there are a total of 10 800 COOH per phage. After incubating the bacteriophages with EDC and sulfo-NHS for ~10 min to activate the carboxylic acid groups on the surface of the bacteriophages, the polyamine molecule was added in a 10 to 1000X excess. Simultaneously, 10X phosphate buffer saline (PBS) was added to a final concentration of 1X, and the mixture was agitated at room temperature for at least 1 h. The product was then precipitated down using polyethylene glycol/sodium chloride precipitation and centrifugation. To remove any excess polyamine molecule, the product was also dialyzed in milli-Q, in a 12-14 kDa MWCO (Spectrum Laboratories Inc., CA).

**Maleimide chemistry:** An his-tagged and maleimide-terminated silaffin peptide was synthesized in the Biopolymers & Proteomics Laboratory. Cysteine phage was mixed with tris(2-carboxyethyl)phosphine (TCEP) in a 1:2.5 molar ratio of pVIII:TCEP, and maleimide-functionalized silaffin was added in a 1:5 pVIII:peptide ratio. Phosphate buffer saline (PBS) or tris-buffered saline (TBS) were used to regulate the pH. The mixture was left at room temperature for 1 to 2 h. The product was then collected via PEG/NaCl precipitation and centrifugation, and resuspended in milli-Q water.
Sortase-mediated modifications: An his-tagged silaffin peptide with a LPETAA C-terminus fragment was synthesized in the Biopolymers & Proteomics Laboratory. Sortase A from Streptococcus pyogenes was expressed and purified as previously described. In TBS buffer, sortase was mixed with silaffin and AADSPH phage at concentrations of 50 μM, 20 μM and 8 nM respectively. The mixture was incubated at 37 °C for 3 h, and the product was purified via PEG/NaCl precipitation and centrifugation.

Polymer coatings: Polymers selected for coating were dissolved in milli-Q water, and the pH of the solution was adjusted to the chosen pH for layering. M13 bacteriophages dissolved in water were added to the polymer solution under agitation. The polymer to bacteriophage ratio was varied between 10 and 10000. After adding the bacteriophages, the mixture was sonicated 5 min, and then left without agitation at room temperature. The mixture was again sonicated 5 min, and the product was dialyzed in water, in a 100 kDa MWCO membrane (Spectrum Laboratories Inc., CA). The bacteriophage-polymer complexes were observed by AFM using a MultiMode Atomic Force Microscope (Veeco Metrology).

Dendrimer-bacteriophage fiber formation: Dendrimers were diluted in milli-Q water from stock solution in methanol. E3 bacteriophages were added to the dendrimer solution dropwise under agitation, to a final concentration of 10^{12} phage/mL. The ratio of dendrimer:phage was varied from 10:1 to 270:1 (corresponding to 10 dendrimers per pVIII). EDC was something added to the mixture in order to crosslink the dendrimers to the bacteriophages, but fibers were obtained with or without EDC. The bacteriophage- dendrimer complexes were purified from excess reactants via dialysis or centrifugation. The products were observed by AFM (MultiMode Atomic Force Microscope (Veeco Metrology)) and TEM (JEOL 2100 FEG TEM).

Zeta potential and particle size characterization: Surface zeta potential and particle hydrodynamic radius were measured using a Malvern ZetaSizer Nano-ZS90 and disposable capillary cells. All zeta potential measurements were carried out in a 10 mM NaCl background electrolyte solution, and the pH was adjusted dropwise under agitation with 0.1 to 0.001 M NaOH and HCl solutions. The concentration of bacteriophages was maintained above 10^{12} phage/mL for all measurements and three data points were averaged. Errors bars report the standard deviation between these measurements in all zeta potential titration figures.
2.5 References


Chapter 3:
Layer-by-layer Assembled
M13 Bacteriophage Nanoporous Thin Films as Scaffolds for Semiconducting Networks and Nanocomposites
Chapter 3. Layer-by-layer Assembled M13 Bacteriophage Nanoporous Thin Films as Scaffolds for Semiconducting Networks and Nanocomposites

This chapter describes the assembly and biomineralization of thin M13 bacteriophage films that are highly porous and exhibit features at the nanometer level. The resulting films are suitable to serve as scaffolds in thin film solar cells and other energy devices that require porous and continuous structures. The film growth, morphology and porosity were characterized, and it was demonstrated that M13 bacteriophages can serve as vehicles to carry and disperse nano-objects in the layer-by-layer assembled thin films. After biomineralization with titania or other semiconductors, crystalline nanostructured networks and composites can be obtained.


3.1 Introduction

For several electrochemical and photovoltaic applications, nanoporous structures confer advantages over bulk, macro-, or micro-porous materials. As the pore size decreases, the available surface area increases for a constant volume of material. Such an active surface can be inherently catalytic or photoactive, modified to display functional groups, or loaded with active materials. Porous nanostructures have been employed to improve the device performance for batteries,\textsuperscript{1} catalytic electrodes,\textsuperscript{2, 3} biosensors,\textsuperscript{4} and photovoltaics.\textsuperscript{5, 6}

For instance, in bulk heterojunction solar cells, especially those relying on conjugated polymers for light absorption, a nanoporous bi-continuous morphology is essential to achieving high performance because of the limited lifetime of photogenerated excitons in conjugated polymers.\textsuperscript{7, 8} In addition, systems such as tandem solar cells require the stacking of different layers of materials for selective light absorption, which calls for a high degree of control over the spatial organization of nanomaterials inside the thin film.\textsuperscript{9-11} For electroluminescent, photovoltaic, and photocatalytic devices, electron donor and electron acceptor materials must be in close contact and share a large interfacial area to improve charge transport and dissociation. Finally, for any form of electro- or photocatalysis, the pathways for charge transport to the
electrodes must be as continuous and direct as possible. Therefore, a nanoporous network of nanowires can serve as a useful template for the assembly of many classes of active materials.

Typically, nanopore arrays are created with energy intensive specialized processes such as electron beam etching, nanoimprinting lithography, or atomic layer deposition. The simple template approach is also commonly used, and generally consists of assembling biological scaffolds or block co-polymers into organized networks followed by nucleating materials, specifically or not, onto the resulting structure. However, most of these templating techniques generate mesopores or micropores, as opposed to nanopores.

It has previously been demonstrated that the M13 bacteriophage, which is ~ 880 nm in length and 6.6 nm in diameter, can be utilized as a template to generate singular nanowires through a sequential nucleation, growth, and annealing process. The 2,700 copies of its pVIII coat protein can be engineered to display affinity for specific materials, making these phages excellent templates for nanoscale manipulation of metals, metal oxides, conjugation of organic compounds, and even binding of carbon nanostructures such as nanotubes and graphene. We have found that it is possible to construct nanoporous networks via layer-by-layer (LbL) assembly of the high aspect ratio M13 bacteriophage and that these networks can be assembled with nanoscale control of their composition and maintenance of their binding and templating capabilities. With such functional networks, the performance of photovoltaic, optical and electrochemical devices relying on high aspect ratio structures for charge transport and nanoscale features could be greatly improved.

Layer-by-layer assembly provides a means to assemble nano-objects in a tightly controlled manner in terms of thickness, morphology, and composition at the nanoscale. Additionally, it has the key advantage of being a low-cost, aqueous solution-based technique. LbL assembly leverages electrostatic interactions, hydrogen bonding, or covalent crosslinks to build films. The latter allows for the assembly of a singular nano-object with itself, such as the M13 bacteriophage, onto a substrate. Conversely, techniques that randomly crosslink nanomaterials such as phage in the bulk state using conventional ‘one-pot’ chemistry often result in gels with high porosity but contain aggregated or bundled nanostructures and larger pores, which decreases the functional surface area available for electrochemical or photocatalytic reactions.
In previous work, M13 bacteriophages have been adsorbed onto the surface of layer-by-layer assembled polymer films\textsuperscript{44} and have been used as polyanions in combination with polymers to tune the morphology of LbL films.\textsuperscript{20} However, because of their intrinsic negative charge at neutral pH, and the great challenges encountered in trying to increase their isoelectric points genetically or chemically, M13 bacteriophages have only been layer-by-layered with other positively charged nanomaterials or polymers. As described in Chapter 2, electrostatic LbL assembly of bacteriophages with complementary charge has not been achieved yet. Herein, we describe a novel method to assemble nanoporous LbL films composed solely of M13 bacteriophages, based on covalent carbodiimide chemistry as opposed to electrostatic assembly. As illustrated in Scheme 3-1, these bacteriophages can be functionalized with nanoparticles or quantum dots, and then serve as vehicles for controllably incorporating them into the thin film. The resulting bacteriophage scaffolds can be further used to template a variety of semiconducting or metallic materials via sol-gel syntheses, thereby generating nanoporous networks of inorganic wires.

![Scheme 3-1](image_url)

**Scheme 3-1.** M13 bacteriophage complexation with nanoparticles, layer-by-layer assembly onto a substrate, and biomineralization.

This work focuses on presenting the properties of the bacteriophage nanotemplate, and demonstrating its ability to assemble porous nanocomposites by controllably introducing gold nanoparticles in the structure, and synthesizing crystalline titania nanoporous networks via biomineralization. The unique nanoporous and continuous titania structures reported here cannot be achieved via other assembly methods, such as the random mixing of titania nanoparticles with
bacteriophages, or the LbL assembly of pre-synthesized titania nanoparticles with bacteriophages (See Appendix A “Layer-by-Layer Assembly of Titania Nanoparticles with M13 Bacteriophages”).

3.2 Results and Discussion

3.2.1 Layer-by-Layer Assembly and Bacteriophage Film Growth

The bacteriophages are assembled in an LbL fashion using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a crosslinking agent in a novel and versatile covalent LbL process. **Figure 3-1A** shows a schematic of EDC chemistry applied to M13 bacteriophages, and Figure 3-1C a representation of the layer-by-layer assembly process. This process relies on the crosslinking of carboxylic acid and amine groups, which are ubiquitously present on the surface of any M13 bacteriophage variant. Thus, the process is very versatile and allows for films to be built using bacteriophages with peptides that display many different functionalities. Covalent LbL assembly based on EDC chemistry has been employed by other groups to assemble films of proteins or polymers; the growth of these films was reported to be linear with the number of repeated cycles. A single protein with a negative surface charge at neutral pH, human serum albumin, could be layered with itself in this fashion, which indicates that repulsive electrostatic interactions do not override the reaction-diffusion driving force that transports the charged nanomaterials from the bulk solution to the surface, enabling the formation of covalent amide bonds.

The films can also be grown on a variety of substrates provided that the surfaces can be either silanized or rendered negatively charged via plasma treatment. As an example, Figure 3-1B shows a silicon substrate functionalized using an amino-silane subsequently linked covalently to M13 bacteriophages with EDC. We have successfully constructed bacteriophage films on glass, silicon wafers, plastics, and metal foils, and have shown that different surface functionalization methods result in the growth of films with the same morphology using covalent LbL assembly (see Figure 3-1D).

The bacteriophage films grow in a linear manner, characteristic of LbL assembly, as shown in Figure 3-1E and F. Figure 3-1E shows that the thickness of the film can be predicted based on the number of EDC-phage bilayers (bL), regardless of the solution concentrations and dipping parameters. However, as shown in Figure 3-1F, the time-rate of growth of a film is
dependent on the concentrations of EDC and the bacteriophage, as well as the dwell time during
dips.

Figure 3-1. M13 bacteriophage thin films grow linearly via a covalent layer-by-layer assembly process.
A. Schematic representation of EDC chemistry applied to M13 bacteriophages. B. Atomic force
microscopy of a silicon substrate functionalized with a single layer of bacteriophages. C. Schematic
representation of the covalent LbL process. D. Growth curve from zero to 100 bilayers for substrates
functionalized with three different methods: 1) using aminopropyltrimethoxysilane (APTMES) and
then functionalizing with a layer of bacteriophages; 2) using carboxyethylsilane (CES); 3) by
constructing 4.5 (LPEI/PAA) base layers. The films were constructed with a concentration of 75 mM EDC, and $10^{13}$ bacteriophages mL$^{-1}$, and a total time of 14 min bl$^{-1}$. E. Grow curve as a function of the number of EDC-phage bilayers, for three different assembly conditions: Diamond (1 bl = 36 min; 75 mM EDC, $5 \times 10^{12}$ phage mL$^{-1}$), Circle (1 bl = 14 min; 75 mM EDC, $10^{13}$ phage mL$^{-1}$), Triangle (1 bl = 14 min; 150 mM EDC, $10^{13}$ phage mL$^{-1}$); F. Growth curve as a function of the total assembly time for the same conditions.

To monitor the incorporation of M13 bacteriophages within LbL films, and to show a control over their distribution within the film, M13 bacteriophages were fluorescently-labeled with a succinimidyl ester-activated Oregon green dye and then incorporated within LbL films with different architectures. Figure 3-2A shows the chemical structure of the dye conjugated to the bacteriophages by covalently binding to the terminal exposed amine group of the pVII proteins, and Figure 3-2B shows a solution of fluorescently-labeled bacteriophages. Via mass spectroscopy, the covalent attachment of the dye to the pVIII proteins was confirmed, and it was determined that approximately 315 of the total 2700 pVIII proteins were labeled on each bacteriophage (Figure 3-2C). After LbL assembly of the films illustrated in Figure 3-2D, their relative fluorescence was measured and confirmed that fluorescently-labeled bacteriophages can be incorporated within the films. The fluorescence was only observed for films containing fluorescently-labeled bacteriophages, and the fluorescence intensity was diluted if only portions of the films were assembled using the labeled bacteriophages. It can be noted that the fluorescence of Film D (in which labeled bacteriophages were assembled at the bottom of the film) is slightly higher than that of Film C (containing bacteriophages in the top layers on the film), and this could be caused by the slow depletion of EDC during assembly, since the EDC solution was not refreshed for this experiment. Therefore, the film grew more efficiently at the beginning of the assembly and allowed for the incorporation of more fluorescent bacteriophages in Film D.
A.

Oregon green

![Chemical Structure of Oregon Green](image)

**MW = 509.38 g/mol**

B.

Fluorescently-labeled M13 bacteriophage solution.

C.

Mass spectroscopy showing the presence of pVIII proteins conjugated with Oregon green after coupling, with a yield of approximately 315 Oregon green molecules per bacteriophage.

D.

**Film A**

100bL

**Film B**

Fluorescent E3

**Film C**

Fluorescent E3

50bL

50bL

**Film D**

E3

Fluorescent E3

Figure 3-2. Fluorescently labeled bacteriophages can be incorporated into layers of the LbL films. A. Structure of Oregon green dye with a reactive succinimidyl ester. B. Fluorescently-labeled M13 bacteriophage solution. C. Mass spectroscopy showing the presence of pVIII proteins conjugated with Oregon green after coupling, with a yield of approximately 315 Oregon green molecules per bacteriophage. D. LbL film architectures to demonstrate the incorporation of fluorescently-labeled bacteriophages, and corresponding relative fluorescence intensity as determined by ImageJ. The fluorescence intensity of Film A and Film B were set to 0% and 100% respectively. The slight fluorescence detected for Film A is due to contamination in the common rinse baths used for all films during LbL.

Noble metal nanoparticles embedded in semiconducting networks produce plasmons, which can enhance the performance of light-absorbing and light-emitting devices. Therefore, they represent an attractive material to incorporate into LbL-assembled bacteriophage thin films that could afterwards be mineralized with semiconducting materials. In the bacteriophage films, the distribution of nanoparticles varies significantly with the method of incorporation. Gold nanoparticles (AuNPs) can be added to the structure post-assembly, either by immersing the film into a nanoparticle solution, or by dropcasting this solution onto the film and allowing it to dry. When immersed in a NP solution, the majority of the particles remains on the surface of the film; whereas, capillary forces tend to pull the NPs deeper into the film when they are dropcast on the
surface of the film (see XPS depth profiling results in Figure 3-3C, left panel). The dropcast method results in a more uniform NP distribution, although the concentration of NPs is still higher on the surface. For different M13 variants, different kinds of complexing behavior can be observed within the networks. For instance, the p8#9 variant expresses pVIII proteins with several serine amino acids, which through their hydroxyl groups, can coordinate, stabilize, and organize gold or silver nanoparticles.17-19 This variant was chosen to perform all LbL assemblies with bacteriophage-metal nanoparticle complexes.

A significant advantage of the LbL assembly method is that nanoparticles can also be incorporated in a more controlled fashion. In the covalently-assembled bacteriophage films, we show that layers of viruses can be precisely assembled on top of each other and remain segregated. These covalent crosslinks provide a means to tightly control the spatial organization of different templates and their resulting nanomaterials within the films.

As reported previously in our group, M13 bacteriophages can be used as a vehicle to carry nanoparticles.27 To demonstrate control of this process during LbL assembly, two different film architectures were constructed with bacteriophages that were complexed with AuNPs in solution pre-assembly and incorporated in LbL films (Figure 3-3A): (1) the AuNP-phage complexes were assembled in the layers at the bottom half of a multilayer film, while naked bacteriophages were assembled in the layers at top half, or (2) vice-versa. Through XPS depth profiling analysis, as shown in the right panel of Figure 3-3C, we show that the NPs can be localized near the bottom or top regions of the film, with control within a resolution of 50 nm when using 8 to 20 nm diameter AuNPs. When AuNPs are incorporated during the film assembly, their presence can be visualized macroscopically, as they give a uniform red color to the film (see optical images of films constructed on glass slides in Figure 3-3B). When only bacteriophages are assembled, the resulting film is colorless.
Figure 3-3. Precise spatial distribution control of the bacteriophage-mediated incorporation of gold nanoparticles. A. Schematic of gold nanoparticle incorporation during LbL assembly. B. Optical image of bacteriophage films constructed on glass. Left, without nanoparticles; Right, with gold nanoparticles incorporated using bacteriophages as carriers during LbL assembly. C. XPS depth profiling analysis of the gold distribution as a function of the film depth, converted to nanoparticle to phage ratio for different film architectures. The left panel shows the distribution for nanoparticles infiltrated post-assembly, while the right panel shows films that were assembled with phage-nanoparticle complexes.

The precise control over localization of nanoparticles inside bacteriophage films would allow for the flexible design of functional and porous thin films. One could imagine tuning the distribution and concentration of NPs as a function of film thickness, and as a result, create on-demand localized plasmon resonances that affect only certain regions of the film. If, instead, the type of metallic NP is varied in different layers of the film, then the plasmon resonance
wavelength could be defined in different sections of the film. Both of these scenarios find applications in designing photovoltaic devices that enhance the electric field and optimize light absorption in specific photoactive materials. Of course, all nanomaterials that M13 bacteriophages are programmed to bind could be incorporated in different layers of the film. For instance, the viruses could carry different nanoparticles into the LbL films in a hierarchical order, for instance quantum dots with different bandgaps for tandem solar cells or optical displays, particles with different dielectric constants for various optical nanostructures (e.g. antireflective coatings, gradient index materials, etc.), and metal nanoparticles for plasmonic-based biosensing or photocatalytic applications.

3.2.2 Bacteriophage Film Morphology and Porosity

As the thin bacteriophage film grows, it evolves from a flat bacteriophage-functionalized surface to a porous and rough film, as illustrated in Figure 3-4A. In the AFM images shown in this figure, the high aspect ratio features observed in the 16 bL image correspond to deposited bacteriophages. These individual features become less detectable as the thickness and roughness of the films increase. The surface roughness is generally proportional to 40% of the overall film thickness. As shown in Figure 3-4B, a film built on a silicon wafer appears uniform and translucent, yet a TEM cross-section demonstrates its nanoscale features. The diameter of the wire-like features in this TEM image is approximately equivalent to the diameter of a bacteriophage (6 to 7 nm). A detailed protocol for preparing cross-sectional TEM samples can be found in Appendix B “Preparation of Cross-Sectional SEM and TEM Samples for High Resolution Imaging”.

Cross-sectional images were also used to determine the pore size distribution within the bacteriophage-film cross-section. By enhancing the contrast of the image, the ImageJ software can be used to identify the pores (see Figure 3-4C). The pore size distribution shown in Figure 3-4D reveals that equivalent pore diameters remain below 30 nm, with the majority of the pores ranging between 0 and 10 nm.
Figure 3-4. M13 bacteriophage films grow into nanoporous networks. A. Surface morphology of the bacteriophage film after 16, 32, 48, 64 and 80 (EDC-phage) bilayers, characterized by AFM. B. Optical image of a 300 bL bacteriophage film constructed on silicon and cross-sectional TEM image of the film, illustrating the nanoporous morphology of the film. C and D. ImageJ analysis of the pore size distribution of the bacteriophage thin films. C. Cross-sectional images with enhanced contrast (left), and with identified pores in ref (right). D. Pore size distribution and cumulative distribution for calculated equivalent pore diameters based on pore area measured with ImageJ.

The porosity of a bacteriophage film was estimated to be 59% based on quartz crystal microbalance measurements, and on an approximation of the density of M13 bacteriophages.
For estimating the density of a single bacteriophage, its geometry was approximated as a cylinder, as illustrated in Scheme 3-2.

Scheme 3-2. Approximation of the M13 bacteriophage as a cylinder with a molecular weight corresponding to the sum of its 2700 pVIII proteins.

The bacteriophage film mass can then be extracted by using the following equation:

\[
\frac{\Delta f}{f_0} = \frac{\Delta l}{l_0} = \frac{-2f_0\Delta m}{A \sqrt{\rho_{\text{quartz}}\mu}}
\]

Equation 3-1

where \(f_0\) is the resonant frequency, \(\Delta m\) the mass deposited on an area \(A\), \(l_0\) the thickness of the quartz plate, \(A\) the area (here 4.91 cm\(^2\)), \(\rho_{\text{quartz}}\) the density of the quartz, and \(\mu\) the shear modulus of the quartz.

Thus, the total mass of bacteriophages added during layer-by-layer assembly (\(\delta m\)) can be calculated from the resulting simplified equation:

\[
\delta f \text{ (Hz)} = -56.6 \frac{\delta m \text{ (\(\mu g\))}}{A \text{ (cm}^2\text{)}}
\]

Equation 3-2

The film density and porosities can then be derived with Equation 3-3 and Equation 3-4, since the film thickness is known from profilometry measurements at various numbers of bilayers. The results for films of different thicknesses are compiled in Table 3-1.

\[
\rho_{\text{film}} = \frac{\delta m}{V} = \frac{\delta m}{A \times L}
\]

Equation 3-3
\[ \varphi = \left[ 1 - \frac{\rho_{\text{film}}}{\rho_{\text{phage}}} \right] \times 100\% \]  

Equation 3-4

where \( \rho_{\text{film}} \) is the density of the bacteriophage film, \( V \) the total volume of the bacteriophage film, \( L \) the thickness of the bacteriophage film measured by profilometry, \( \varphi \) the film porosity, and \( \rho_{\text{phage}} \) the density of a single bacteriophage calculated from the approximations presented in Scheme 3-2.

Table 3-1. Quartz crystal microbalance frequency measurements and porosity results for bacteriophage films of 40, 60 and 80 bilayers. The porosity of the phage film was calculated from an approximation of a single bacteriophage density (9.3x10\(^{-7}\) µg µm\(^{-3}\)), and from the frequencies measured for a phage film constructed on a quartz crystal for different numbers of bilayers. Zero bilayer refers to the bare substrate.

<table>
<thead>
<tr>
<th>Number of bilayers</th>
<th>Frequency [Hz]</th>
<th>Total mass added [µg]</th>
<th>Film density [10(^{-7}) µg µm(^{-3})]</th>
<th>Porosity [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4990158</td>
<td>0</td>
<td>0</td>
<td>-----</td>
</tr>
<tr>
<td>40</td>
<td>4989721</td>
<td>14.0</td>
<td>3.84</td>
<td>58.8</td>
</tr>
<tr>
<td>60</td>
<td>4989572</td>
<td>18.7</td>
<td>3.95</td>
<td>57.7</td>
</tr>
<tr>
<td>80</td>
<td>4989467</td>
<td>22.1</td>
<td>3.79</td>
<td>59.4</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>3.86 ± 0.08</td>
<td>58.6 ± 0.9</td>
</tr>
</tbody>
</table>

2.2.3 Biomineralization of Bacteriophage Nanoporous Scaffolds with Titania

Sol-gel syntheses are employed to nucleate nanoparticles onto the phage scaffold and these nanoparticles are then sintered together to form a continuous mesh. Titania, a solution-processable photoactive wide-bandgap semiconductor, is an example of a useful material that can be nucleated on the bacteriophage template. To do so, the films are dipped vertically into an aqueous titanium tetrachloride (TiCl\(_4\)) solution and hydrolysis is allowed to proceed at 80 °C for an hour. Because covalent bonds are linking the bacteriophages layers, the film does not disassemble upon changes in pH or salt concentration, as evidenced by its resistance to the highly acidic TiCl\(_4\) solution used for titania nucleation. The films are then rinsed with deionized water, dried, and annealed at 450 °C in air. This annealing step has a dual role: 1) it removes all organic materials from the film via combustion (see Figure 3-5), and 2) it converts the amorphous titania to crystalline anatase nanoparticles (See Figure 3-6G and H for XRD and
TEM characterization of the crystalline nanoparticles) and forms continuous structures along the virus template by interconnecting the titania nanoparticles.

Figure 3-5. Only traces of carbon are observed after annealing at 450°C, as shown by XPS depth profiling of the TiO$_2$ coated phage film. A. Overview of the atomic concentrations of Ti, O, C and Si, by monitoring Ti2p, O1s, C1s and Si2p respectively. B. A closer look at the carbon concentration inside of the film.

The structure of a titania film templated from the bacteriophage network is depicted in Figure 3-6. The surface morphology reveals a nanowire-like interconnected mesh, with a wire diameter of 10 nm. Both on the surface and through the cross-section of the film, the vast majority of the pores have a diameter between 4 and 15 nm (see pore size distribution in Figure 3-6C), which is a critical size scale for many energy applications.

Precise control over the reaction time for TiO$_2$ formation is critical for obtaining a nanoporous film that maintains the structure of the bacteriophage template. The dilute TiCl$_4$ solution is initially transparent, but its color changes as nanoparticles are formed. The solution turns light blue and then white as the nanometer-sized particles grow and scatter light. If the hydrolysis reaction is stopped prematurely (Figure 3-6A), not enough titania has nucleated on the virus to produce well-defined interconnected wires, as compared with films made with a longer reaction time (Figure 3-6B). However, if the reaction proceeds for too long, the film grows thicker on the template surfaces and the nanoporosity is lost, as nanoparticles merge and occlude the pores.

Figure 3-6D and E show cross-sectional TEM images of a titania-coated bacteriophage film. The dark field image shown in Figure 3-6D uses contrast to reveal that the titania film is
highly porous throughout its depth. In Figure 3-6E, the morphology of the cross-section can be visualized and a fine interconnected mesh structure is observed uniformly throughout the section.

TEM also allows for a clearer visualization of the bacteriophage-mediated organization of the titania nanoparticles. Figure 3-6F shows a STEM image of a thin film (10 EDC-phage bilayers) constructed, templated with titania, and annealed on a silicon nitride support film. The pores can clearly be visualized, as well as the sintered nanoparticles arranged into wire-like structures. An elemental mapping of titanium is also shown in Figure 3-6I, and presents the co-localization of titanium atoms with the visible nanowire-like structures.

In addition, it was found that the rate at which the temperature is elevated to 450 °C during annealing of the titania is also a key parameter for the successful preparation of the nanoporous titania. If the temperature is elevated slowly (over more than 30 min), then the bacteriophage scaffolds burn off before the titania nanoparticles are sintered together, resulting in a loss of structure, and the collapse of the film onto the substrate. A fast increase in temperature preserves the three-dimensional structure, but can also cause cracking of the film if the rate is too high (if increased to 450 °C over 10 min or less), as shown in Figure 3-7.
Figure 3-6. Titanium dioxide nanoporous structures. A and B. Top surface SEM images of the phage film coated with titania and annealed at 450°C. In A, the reaction was stopped early (after 40 minutes) and in B the coating was allowed to proceed fully for an optimal reaction time of an hour. The color of the titania solution at each time point is show in the respective inset. Images of films coated with TiO₂ for an hour are shown at two different magnifications. C. Pore size distribution based on 5 different top surface SEM images. The mean pore diameter is 8.1 nm, with a standard deviation of 6.5 nm for an hour reaction in TiCl₄. D and E. TEM images of the cross-section of a titania network formed after reacting in TiCl₄ for one hour reaction. The film was constructed on silicon and cross-sectioned with a focused ion beam, (D – STEM-Bright Field (BF) image, E – TEM image). F. STEM-HAADF (high angle annular dark field) image contrasting the titania nanowire mesh (brighter), from the silicon nitride support film that the phage film was constructed on (pale grey), and empty spaces (black). G and H. Crystallinity of titania-coated phage films after annealing at 450°C (G – TEM image showing lattice fringes of crystalline titania nanoparticles, H – X-Ray diffraction spectrum of resulting anatase titania). I. STEM-Bright Field image and elemental mapping of titanium for a titania nanowire mesh constructed on a silicon nitride support film.
Figure 3-7. The rate of temperature increase during annealing plays an important role for maintaining the integrity of the film. SEM images of the top surface of titania coated bacteriophage films for different rates of temperature increase during the annealing step. The temperature was increased from 20 to 450°C in 10, 20 or 30 minutes from left to right.

Bacteriophage thin films containing gold nanoparticles were also mineralized with titania. On the right panel of Figure 3-8, a titania-coated p8#9 phage film into which AuNPs were incorporated before titania deposition is shown. Visually, the gold nanoparticles seem to be dispersed within the titania film. The functional groups on the p8#9 pVIII coat protein complex the NPs and uniformly distribute them on the surface, but this phenomenon is not observed for all M13 bacteriophage variants. For example, more aggregation is observed when NPs are dropcast onto E3 bacteriophage films, which have a greater tendency to bind metals with less specificity, as clearly observed on the left of Figure 3-8. The E3 bacteriophage expresses three glutamic acids on each of its pVIII proteins, and thus has a negative surface charge at neutral pH\(^{20}\) due to its available carboxylic acid groups, but it does not exhibit functional groups that specifically bind noble metal nanoparticles. In contrast, the p8#9 bacteriophages contain a total of four serines on each pVII protein, exposing several hydroxyl groups that are able to bind and disperse metal nanoparticles.
3.2.4 Optical Properties of Bacteriophage Scaffolds and Bacteriophage-Based Titania Films Containing Metal Nanoparticles

3.2.4.1 Optical Analysis of Gold Nanoparticles in Bacteriophage Films

In order to further investigate the ability of bacteriophages to organize nanoparticles within the film, bacteriophage-AuNP complexes were assembled via LbL and the absorption spectrum of the film was experimentally collected for comparison against controls. Due to the phenomenon of localized surface plasmon resonance (LSPR), the location and width of the absorption peak for spherical AuNPs is very sensitive to two factors: (1) the dielectric function of the medium surrounding the nanoparticle and (2) the distance between neighboring nanoparticles in the system. By comparing the absorption measurements of AuNPs in different environments to theoretical predictions, inferences can be made about the degree of particle aggregation present in virus-based composites. Mie theory provides the extinction cross-section, \( C_{\text{ext}} \), for a nanoparticle much smaller than the wavelength of incident light, which can be employed to predict the absorption spectra of isolated particles immersed in different media as follow.\(^{28}\)

\[
C_{\text{ext}} = \frac{24\pi^2a^3\varepsilon_m^2}{\lambda} \left( \frac{\varepsilon_2}{(\varepsilon_1 + 2\varepsilon_m)^2 + \varepsilon_2^2} \right)
\]

Equation 3-5

Figure 3-8. p8#9 bacteriophages disperse gold nanoparticles within the LbL films, with dispersity remaining so even after titania mineralization, while films containing E3 bacteriophages show nanoparticle aggregation. SEM images of the top surface of an E3 (left) and p8#9 bacteriophage film infiltrated with gold nanoparticles under the same conditions, and subsequently coated with TiO\(_2\) and annealed at 450°C.
where, $a$ is the nanoparticle radius, $\epsilon = \epsilon_1 + i\epsilon_2$ is the complex dielectric function of the nanoparticle, and $\epsilon_m$ is the dielectric function of the medium surrounding it.

The dielectric function of a bacteriophage film with a porosity of 59% for wavelengths between 500 and 1700 nm was experimentally determined using ellipsometry. By fitting the ellipsometry results to the Bruggeman equation for an effective medium, estimates of the complex dielectric function for pure phage were obtained, which enabled the estimation of the effective dielectric constant observed by AuNPs when complexed onto the surfaces of bacteriophages suspended in aqueous solution.

Because the pores of the bacteriophage are significantly smaller than the wavelength of incident light, light cannot distinguish between the individual domains of bacteriophage and air in the film, rather it perceives an effective dielectric medium that is a blending of the material properties of the two components. The Bruggeman model for an effective dielectric function was chosen because it is valid for a two-component inhomogeneous medium and does not assume the presence of particulate inclusions like the Maxwell Garnett model does. The expression for the complex effective dielectric function $\epsilon_{\text{eff}}$, according to the Bruggeman model is as follow:

$$ f \frac{\epsilon_{pp} - \epsilon_{\text{eff}}}{\epsilon_{pp} + 2\epsilon_{\text{eff}}} + (1 - f) \frac{\epsilon_m - \epsilon_{\text{eff}}}{\epsilon_m + 2\epsilon_{\text{eff}}} = 0 \quad \text{Equation 3-6} $$

where $\epsilon_{pp}$ is the complex dielectric function of pure bacteriophage, $\epsilon_m$ is the dielectric function of the other medium (either air, $\epsilon_{\text{air}} = 1.000$, or water, $\epsilon_{\text{water}} = 1.777$), and $f$ is the volumetric fill fraction of the bacteriophage in the two-component material. The effective dielectric function, $\epsilon_{\text{eff}}$, must be solved for numerically using Equation 3-6, however, since the effective dielectric constant of a 59% porous bacteriophage film (i.e. 41% volumetric bacteriophage fill fraction) is known from ellipsometry, this data allows the dielectric function for pure phage, $\epsilon_{pp}$, (i.e. 0% porous phage film) to be estimated. Rearranging Equation 3-6 and solving for $\epsilon_{pp}$ provides:
\[ \epsilon_{pp} = \epsilon_{film} \left( \frac{2(f_{film} - 1)}{f_{film}} \right) \left( \frac{\epsilon_{air} - \epsilon_{film}}{\epsilon_{air} + 2\epsilon_{film}} \right) + 1 \]  

Equation 3-7

where \( f_{film} \) is the bacteriophage fill fraction (41% for measured sample) and \( \epsilon_{film} \) is the dielectric function for the bacteriophage film obtained from ellipsometry measurements. Also, note that the complex index of refraction for a non-magnetic material is simply:

\[ n + i\kappa = \sqrt{\epsilon_1 + i\epsilon_2} \]  

Equation 3-8

where \( \epsilon = \epsilon_1 + i\epsilon_2 \) is the form of the complex dielectric constant, \( n \) is the real part of the refractive index, and \( \kappa \) is the imaginary part. Figure 3-9A shows both \( n \) and \( \kappa \) for the 59% porous phage film as determined by ellipsometry measurements and the estimated values for a pure bacteriophage film from Equation 3-7 and Equation 3-8.

Then, in order to predict the refraction index for bacteriophage-AuNP complexes in solution, the geometry of the complex had to be approximated. By approximating the filamentous bacteriophage as a cylinder with a radius, \( r_{virus} = 3.5 \) nm and a length of 880 nm, the volume fraction that the virus-cylinder occupies in a sphere with radius \( 2a = 8 \) nm is (see Figure 3-9B, inset):

\[ f_{complex} = \frac{V_{virus}}{V_{sphere}} \approx \frac{\pi r_{virus}^2 (4a)}{\frac{4}{3} \pi (2a)^3} = \frac{\pi (3.5\text{nm})^2 (4 \cdot 4\text{nm})}{\frac{4}{3} \pi (2 \cdot 4\text{nm})^3} = 0.287 \]  

Equation 3-9

By substituting \( f_{complex} \) for \( f \), the estimated dielectric function for pure phage obtained from Equation 3-7 for \( \epsilon_{pp} \), and the dielectric constant of water, \( \epsilon_{water} = 1.777 \), for \( \epsilon_m \) into the Bruggeman effective medium equation provided in Equation 3-6, the effective dielectric function for the bacteriophage-water complexes can be found by numerically solving for \( \epsilon_{eff} \). The resulting complex effective index of a bacteriophage complex in water as perceived by a gold nanoparticle is presented in Figure 3-9B.
Figure 3-9. The refractive index of pure bacteriophage films and of bacteriophage-AuNP complexes can be determined from the measured refractive index of porous layer-by-layer assembled bacteriophage films. A. Real (blue) and imaginary (red) components of the index of refraction for the 59% porous bacteriophage films (dotted lines) as determined through ellipsometry measurements and the estimated function for pure bacteriophage films (i.e. 0% porous films, solid lines) as predicted by Bruggeman effective medium theory. B. Real (blue) and imaginary (red) components of the effective index of refraction calculated for bacteriophage-water complexes with a bacteriophage fill-fraction of \( f_{\text{complex}} = 0.287 \), as predicted by Bruggeman effective medium theory. Inset: Schematic of geometry for gold nanoparticle complexed onto a bacteriophage used to calculate the fill-fraction occupied by the bacteriophage.
By inserting these dielectric functions into $\varepsilon_m$ of Equation 3-5 and using the experimental dielectric function for gold as measured by Johnson and Christy for $\varepsilon$, the absorption peaks were predicted for 8 nm diameter AuNPs suspended in aqueous solution, complexed onto the bacteriophage in aqueous solution, or incorporated into a dried bacteriophage film. The measured and predicted absorption peaks for AuNPs in these different media are shown in Figure 3-10. The exact positions of the peaks are also reported in Table 3-2.

![Figure 3-10. Comparison of optical properties of AuNPs under different conditions; freely suspended in water, complexed to bacteriophages in solution, and incorporated into 59% porous dried bacteriophage films during LbL, or dropcast onto a film post-assembly. The theoretical prediction for each peak is shown with a dotted vertical line corresponding to the color of the experimental data.](image)

**Table 3-2.** Predicted and measured absorption peak positions for 8 nm gold nanoparticles in different media.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measured Absorption Peak Wavelength [nm]</th>
<th>Predicted ExtinctionCross-Section Peak Wavelength [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs in Water</td>
<td>521.5</td>
<td>521.5</td>
</tr>
<tr>
<td>AuNP-Phage Complexes in Water</td>
<td>533.5</td>
<td>533.5</td>
</tr>
<tr>
<td>AuNPs in 59% Porous Phage Film</td>
<td>525.0</td>
<td>523.5</td>
</tr>
</tbody>
</table>
It is well known that the formation of AuNP aggregates drastically changes the observed absorption spectra because higher order modes arise with resonance at lower energies, which collectively act to generate a broadened and red-shifted absorption peak. In the simplest case of a AuNP dimer, the electromagnetic fields will couple and a additional orientation-dependent resonance peaks will arise at lower energies due to the longitudinal plasmon mode. As shown in Table 3-2, the predicted peak positions are in excellent agreement with experimental observations and additional peaks or shoulders are not observed in any of the absorption measurements. Together these two observations indicate that aggregates do not occur in significant enough abundance to be detected in the samples.

In contrast, when AuNPs are dropcast onto the bacteriophage film after it has been formed, they tend to fill the pores and form larger aggregates because the citrate capping agent loses its negative charge once the solvent is evaporated and an attractive van der Waals force causes neighboring nanoparticles to cluster. As shown in Figure 3-10, the measured absorption peak of such films is significantly broadened and red-shifted from the LbL film peak, because, as opposed to isolated sub-wavelength AuNPs that only support the dipole resonance mode, AuNP clusters are able to support multiple higher-order resonance modes. The effect is the same as that observed when citrate-capped gold nanoparticles lose stability in the presence of salt and ethanol or are aggregated via ligand exchange.

By exploring the optical properties of the AuNP-bacteriophage films, two important observations have arisen: first, the effective index of bacteriophage-air films for wavelengths between 500 and 700 nm is close to that of water ($n_{film}(\lambda = 500 \text{ nm}) = 1.33+i0.10$ to $n_{film}(\lambda = 700 \text{ nm}) = 1.45+i0.07$ and $n_{H2O} = 1.33$, see Figure 3-9). Therefore, if a plasmonic nanoparticle has a LSPR peak in this range when dispersed in water, the peak will be only slightly shifted when incorporated into the nanoporous bacteriophage film via LbL. Secondly, AuNPs must be incorporated into the bacteriophage films during the LbL process in order to remain well-dispersed and preserve the resonance peak location. AuNPs incorporated after the film has been built by LbL will result in aggregation with significantly red-shifted and broadened spectra.
3.2.4.2 Optical Analysis of Gold Nanoparticles in Titania Films

When the AuNP-bacteriophage films created by LbL are templated with amorphous titania and then converted to anatase by annealing, we observe a red-shift in the measured absorbance spectra (Figure 3-11). This shift is not a result of aggregation, but is due rather to the increase in the effective index of the porous medium upon the introduction of TiO$_2$ into the system. Although the exact peak shift is dependent upon the degree of titania crystallinity and shell thickness, the finding is consistent with previously reported silver-titania\textsuperscript{35} and gold-titania\textsuperscript{20} nanoparticle composites.

![Normalized absorption vs Wavelength](image)

**Figure 3-11.** Due to the change in local environment, a slight shift in AuNPs absorption peak observed upon coating of the bacteriophage film with titania, before and after crystallization. Measured absorption spectra for AuNPs assembled in a porous bacteriophage film, a bacteriophage film mineralized with amorphous titania, and a bacteriophage-templated anatase titania film after annealing at 450 °C.

Quartz crystal microbalance (QCM) was not used to measure the porosity of the anatase titania films because the QCM substrate could not resist the required annealing temperatures. However, by using Bruggeman effective medium theory, the film porosity can be estimated by the position of the absorption peak. Based on the observed absorption peak at 567 nm, we estimated the final phage-based anatase titania film to be 53 % porous for an hour reaction in titanium tetrachloride.
To do so, the complex dielectric function of pure, solid anatase titania was first calculated from the values reported by Kim et al.\textsuperscript{36} for a 16 % porous anatase titania thin film using the same method discussed above to estimate the complex index of pure, non-porous bacteriophage films. In short, the reported dielectric function for the 16 % porous film was substituted for $\varepsilon_{\text{film}}$ in Equation 3-7 and a titania volumetric fill fraction of 84 % ($i.e.$ 100-16 = 84) was used for $f_{\text{film}}$ to extrapolate the dielectric function of pure, solid anatase titania (i.e. $f_{\text{film}} = 100\%$), $\varepsilon_{pp}$. The complex index of refraction was then calculated using Equation 3-8 and the result presented in Figure 3-12A.

Once the complex dielectric function of pure anatase was calculated, Bruggeman effective medium theory was again used to calculate the effective complex dielectric function, $\varepsilon_{eff}$, of a nanoporous anatase film with an arbitrary volumetric fill fraction of titania, $f$, by numerically solving Equation 3-6. However this time, the calculated complex function of pure titania was substituted into $\varepsilon_{pp}$. Furthermore, by calculating the extinction peak location for AuNPs in nanoporous anatase films as a function of film porosity, the porosity of any nanoporous bacteriophage or titania films can be estimated by simply measuring the absorption peak of AuNPs dispersed in the film and comparing it to Figure 3-12B. Absorption measurements performed on the bacteriophage-templated anatase titania film specimen showed a peak at 567nm, which is predicted using this method to be 53% porous. This is a reasonable value as it is expected that the biomineralization process would increase the diameters of the nanowire mesh. The estimated complex effective index of this sample is reported in Figure 3-12A.
Figure 3-12. From the absorption spectra of thin films, the refractive index of porous titania, and the porosity of any porous bacteriophage or titania film can be predicted. A. Real (blue) and imaginary (red) components of the index of refraction for pure (i.e. 0% porous films, dotted lines) and 53% porous anatase titania films. B. Extinction peak location predicted by Mie Theory for AuNPs embedded in nanoporous phage (solid blue line) or nanoporous anatase titania films (solid red line) as a function of film porosity ($i p = 1 - f$). Dotted lines represent experimentally measured absorption peak locations for nanoporous phage (dotted blue line) and nanoporous anatase titania (dotted red line) films.
3.3 Conclusions

In conclusion, this chapter demonstrates that a covalent LbL assembled film composed only of bacteriophages generates a nanoporous structure that can serve as a scaffold for the nucleation of nanoparticles and nanowire meshes synthesized by a sol-gel method. Although this work focused on nucleating titania, the bacteriophage film can also serve as a scaffold for other materials, including other oxides (silicon oxide, zinc oxide, etc.), or sulfides (zinc sulfide), and metals that can be reduced from precursors in solution (See Appendix C “Biomineralization of Various Semiconductors and Metals onto M13 Bacteriophage Thin Films” for details). Because the bacteriophages have an affinity for gold and are assembled in an LbL fashion, AuNPs can be controllably loaded into specific regions of the film. This method is not limited to AuNPs but could be extended to other nanomaterials that can be directly bound to other M13 bacteriophage variants. For example, certain variants can bind photoactive quantum dots or silver nanoparticles, which could facilitate the assembly of tandem or plasmon-enhanced solar devices. Furthermore, the nanoporosity of titania films described here and the nanoscale compositional control demonstrated herein are difficult to generate by other methods, making the bacteriophage nanotemplate promising for a variety of thin film devices where both nanoscale pores and high porosity are essential. Finally, the method described here to predict the optical properties of gold nanoparticles in nanoporous organic and inorganic films could be used to rationally design plasmonic-bacteriophages systems for a variety of optical applications.

3.4 Materials and Methods

Materials: Titanium tetrachloride, aminopropyltrimethoxysilane triol (APTMES), gold(III) chloride (>= 99.99% trace metal basis), and sodium citrate dihydrate were purchased from Sigma (St-Louis, MO). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysulfosuccinimide (sulfo-NHS) were purchased from Pierce Biotechnology Inc. (Rockford, IL). The substrates, silicon wafers and microscope glass slides were obtained from University Wafer Inc. (Boston, MA) and VWR Scientific Products (Edison, NJ) respectively. The polymers, linear polyethyleneimine (LPEI, 25000 g mol⁻¹) and polyacrylic acid (PAA, > 250000 g mol⁻¹) were purchased from Polysciences Inc. (Warrington, PA). Carboxyethylsilanetriol was purchased from Gelest Inc. (Morrisville, PA). Oregon Green 488 carboxylic acid, succinimidyl ester was purchased from Invitrogen, Thermo Scientific Fisher Inc. (Waltham, MA).
**M13 bacteriophages:** Different variants of the M13 bacteriophage were amplified by infecting exponentially growing *Escherichia coli* ER2739 bacteria, and then purifying the synthesized bacteriophages from the bacterial debris via a series of polyethylene glycol-sodium chloride precipitation and centrifugation cycles. The identity of each variant was confirmed via DNA sequencing.

The concentration of the bacteriophage solutions was quantified by measuring the absorbance of the bacteriophage DNA at 269 nm, using a NanoDrop ND-1000 Spectrophotometer.

**Conjugation of Oregon green with M13 bacteriophages:** E3 bacteriophages were diluted in PBS buffer pH 7.4 to a final concentration of 6x10^{12} phage mL^{-1} and 1000 equivalents of Oregon green 488 succinimidyl ester dissolved in PBS buffer were added. The mixture was stirred at room temperature for 4 hours. The fluorescently-labeled bacteriophages were separated from unreacted dye via polyethylene glycol-sodium chloride precipitation and centrifugation. The product was then dialyzed in water to remove excess salt. The labeling was confirmed via mass spectroscopy with a Bruker MicroFlex MALDI-TOF using sinapinic acid as matrix.

**Gold nanoparticle synthesis:** Gold nanoparticles with ~8 nm diameters were synthesized following the Turkevich method.\(^{37}\) In summary, 46.8 mL of deionized water and 3.2 mL of 20 mM gold(III) chloride were combined in a 100 mL round-bottom flask and brought to a boil while stirring in a silicone oil bath. While boiling, 5 mL of 1% wt sodium citrate dihydratre was quickly added while vigorously stirring. The color of the solution quickly turned black and then ripened into a deep ruby red. The solution was kept boiling and stirring for an additional 20 minutes. The solutions was transferred to a glass vial and stored at 4°C without further purification.

**Complexation of gold nanoparticles to the bacteriophage:** The as-synthesized AuNP solution was diluted in deionized water. Gold nanoparticles were complexed onto the virus by combining them in ratios varying between 0.1 to 5 AuNP per phage.

**Surface functionalization:** Cleaned and plasma treated silicon or glass substrates were functionalized with aminopropyltrimethoxysilaneetriol (APTMES) (2 % v/v) using 90 % v/v acetone in water as solvent, for 2 h at room temperature. The silanized substrates are rinsed with acetone to remove excess silane, and cured at 80 °C for 1 h or in a dessicator overnight.
Crosslinking of a first layer of bacteriophages was formed by contacting a solution of EDC and sulfo-NHS, with the silanized substrate. EDC and sulfo-NHS are first added to a bacteriophage solution in water and agitated for 10 min to activate the carboxylic acid groups on the phage. The pH is then increased by diluting the solution to a phage concentration of 10^{11} phage mL^{-1} with PBS at pH 7.4, and the solution is deposited immediately on the silanized substrate. After two hours, the substrates were rinsed with water. Alternatively, the substrates can be silanized with carboxyethylsilanetriol under the same conditions as described for the APTMES, but using ethanol as a solvent. The resulting exposed carboxylic acid groups can directly be used for LbL assembly. A third method involves the construct of polymer based layers onto a plasma treated substrate. The substrate can be successively dipped into 20 mM LPEI, water rinses, and 20 mM PAA solutions followed by water rinses. A layer of bacteriophages can then be adsorbed electrostatically onto a 4.5 (LPEI-PAA) bilayer film, by dipping the film in a dilute aqueous bacteriophage solution at pH 4.9.

**Film assembly:** The film was built by successively dipping the functionalized substrates in an aqueous EDC solution at pH 5 to activate the carboxylic acid groups, and then in a dilute bacteriophage solution in PBS (pH 6 to 8) to form amide bonds between bacteriophage layers. The bacteriophage could be complexed or not to nanoparticles before assembly. Two rinses in water are done after the dip in each solution. The dipping time in the EDC and phage solutions was varied from 5 to 30 min. The EDC concentration was adjusted between 75 and 150 mM, and the phage concentration between 10^{12} and 10^{13} phage mL^{-1}. Films of different thickness are generated by selecting different numbers of dipping repetitions and by varying the concentration and pH of the solutions.

**Bacteriophage film characterization:** The thickness of the film was determined using a Veeco Dektak 150 profilometer. The porosity of the bacteriophage film was estimated from density data obtained from QCM analysis (Masscal Model G1), from the thickness of the film, and the volume of a bacteriophage. The surface morphology of the bacteriophage film was characterized by atomic force microscopy (AFM) using a MultiMode Atomic Force Microscope (Veeco Metrology) in the tapping mode. XPS with depth profiling was used to determine the distribution of nanoparticles in the bacteriophage film using a PHI Versa-Probe II X-ray photoelectron spectrometer with a scanning monochromated Al source (1,486.6 eV; 50 W; spot size, 200 μm), and the sputtering was done using an argon source. The data was collected every minute, and the
sample was exposed to zalar rotation at a rate of 1 rpm. The absorption spectra of the films (constructed on glass slides) were characterized using a Beckman Coulter DU 800 Spectrophotometer and performing a wavelength scan at a rate of 600 nm min$^{-1}$. The complex refractive index of the film was determined using a spectroscopic rotating compensator ellipsometer (M-2000, J. A. Woollam Co., Inc.). The ellipsometric parameters \( \Psi \) and \( \Delta \) were collected at three incident angles of 50°, 60°, and 70° and the transmission intensity at a normal incidence. They were regressed simultaneously to break the correlation between the thickness and the optical constants of the film.$^{[8]}$ The film optical constants were modeled using a Kramers-Kronig consistent B-spline function$^{[9]}$ in the spectral range from 500 nm to 1700 nm and extrapolated to 450 nm.

**Titania coating:** TiO$_2$ was synthesized on the bacteriophage template via incubation in a 20 mM aqueous TiCl$_4$ precursor solution for 1 h at 80 °C. The film was positioned vertically in the solution to avoid sedimentation of titania nanoparticles at the surface of the film. The film was rinsed with dI water, and the TiO$_2$ was then annealed and the bacteriophages burnt off by heating at 450 °C for 1 h.

**Titania film characterization:** The surface morphology was observed by scanning electron microscopy (SEM) using a Helios Nanolab 600 Dual Beam Focused Ion Beam Milling System, and the cross-sections were observed after fracturing the sample constructed on a silicon wafer using a diamond knife. The pore size distribution was obtained from ImageJ, using five different SEM images, and analyzing the difference in contrast between the pores and the nanowires. The area of the pores, identified with a color threshold, was converted into an equivalent pore diameter assuming circular pores. The crystallinity of the titania was assessed by X-ray diffraction using a PANalytical Multipurpose Diffractometer. TEM characterizations of the films were performed with a JEOL 2100 FEG TEM (EDX, X-MAX 80mm$^2$). Thin films coated with titania, for TEM, were constructed on a silicon nitride support film (Ted Pella Inc., Redding, CA). Cross-sectional TEM samples were prepared using a JEOL 9320-FIB.
3.5 References


Chapter 4:
Constructing Bacteriophage-Based Thin Film Solar Cells: The Contributions of Morphology and Optics on Device Performance
Chapter 4. Constructing Bacteriophage-Based Thin Film Solar Cells: The Contributions of Morphology and Optics on Device Performance

The goal of this chapter is to present a versatile bacteriophage-based platform for constructing nanostructured plasmon-enhanced thin film solar cells. Applying the bacteriophage nanotemplate to titania – lead sulfide quantum dot heterojunction solar cells, the effects of the bacteriophage continuous nanowire-like morphology on the device performance were studied. The ability of M13 bacteriophages to specifically bind metal nanoparticles was also exploited to create localized surface plasmon resonances that increased the generated photocurrent of the devices. Apart from optimizing the assembly process, characterizing the morphology of the active layer and measuring the performance parameters, the system was also modeled to predict the photocurrent enhancement upon addition of plasmonic nanoparticles.


4.1 Introduction

Strategies for improving solar cells generally fall into one of two categories; the first focuses on synthesizing better materials, while the other focuses on generating architectures that optimize performance. New materials with improved photovoltaic properties are constantly emerging, however, device architectures that can capitalize on a material’s strengths, while compensating for its weaknesses are necessary for maximizing the performance of any given solar cell. For instance, the careful selection of materials in heterojunction solar cells can maximize photovoltage and reduce recombination, whereas nanostructured architectures can enhance photocurrent by improving light absorption and carrier collection efficiencies. Such nanostructured morphologies have been shown to improve the performance of several heterojunction types, including organic, inorganic, hybrid, and colloidal quantum dot devices. Likewise, optical structures such as gratings, plasmonic nanoparticles, and
photonic crystals \(^{17}\) have been used to improve the ability of photovoltaics to harvest light, and thus offer a second pathway for increasing photocurrent. Therefore, a well-designed architecture that complements excellent active materials is required to achieve champion photovoltaic performance.

Biotemplating is a bottom-up approach to constructing nanostructures that uses biological material as a scaffold to nucleate inorganic material. By programming the biotemplate at the genetic level to express proteins with specific functionality, multifunctional composites can be assembled and further mineralized for use in non-biological applications. One of the most well-characterized and versatile biotemplates is the filamentous M13 bacteriophage, which has been used previously to (1) bind a variety of inorganic nanomaterials including carbon nanotubes,\(^{18}\) graphene,\(^{3}\) and metal nanoparticles,\(^{19-21}\) (2) assist in the nucleation and templating of inorganic materials such as metals, semiconductors,\(^{21,22}\) polymers,\(^{23}\) and complex metal-oxides,\(^{24}\) and (3) act as building blocks for the assembly of hierarchical structures.\(^{21-23}\) All of these virus-templated materials exhibit nanowire-like morphologies consistent with the filamentous structure of the individual virions. The availability of surface functional groups allows M13 bacteriophages to be easily cross-linked to generate porous scaffolds, films, and gels suitable for energy applications. As previously demonstrated in dye-sensitized solar cells (DSSCs), virus hydrogels and virus-polymer composites are able to create percolation pathways that improve electron diffusion lengths by acting as a scaffold for the nucleation of titania.\(^{22,25}\) Furthermore, genetically encoding the virus to bind with either carbon nanotubes\(^{18}\) or gold nanoparticles\(^{22}\) has enhanced device performance by respectively improving either the charge collection efficiency or light harvesting ability of the photoanode.

The aim of this work is to use the M13 bacteriophage to construct solution-processable thin film solar cells that are light weight, solid-state alternatives to the thicker, liquid-based dye-sensitized counterparts. This class of devices uses heterojunctions comprised of either organic or inorganic materials to generate photovoltage and extract charges to the proper electrodes. However, these photoactive materials generally exhibit shorter carrier extraction and absorption lengths than dye-sensitized solar cells, and thus require thinner films with feature sizes that match these length scales. Therefore, building a virus-templated morphology suitable for use in solution-processed solar devices that are only a few hundred nanometers thick requires synthesis
techniques that offer more precise control over device thickness and porosity than the methods previously employed for building dye-sensitized solar cell (DSSC) photoanodes. The procedures used to synthesize the tens of microns thick photoanodes necessary for DSSCs resulted in rough films with macroscopic pores that varied in size throughout the film thickness, which are unsuitable for thin film devices. In contrast, we have previously reported a method to assemble nanoporous bacteriophage-based anatase titania films of controllable thickness and uniform porosity by combining a covalent cross-linking process with layer-by-layer (LbL) assembly. It was also shown that by binding metal nanoparticles to the virus prior to film assembly, the particles could be evenly distributed throughout the final nanoporous anatase titania film without aggregation. Using this framework, we aim to construct a multifunctional device architecture that is capable of enhancing photocurrent generation in a variety of solution-processed thin film solar devices. As illustrated by Scheme 4-1, our two-fold approach consists of (1) controllably constructing a M13 bacteriophage-based nanoporous titania nanowire network with nanoscale features to efficiently collect and transport charges from the bulk material, and (2) loading it with plasmonic metal nanoparticles capable of increasing light harvesting via localized surface plasmon resonance (LSPR).

Note that we investigated other approaches for constructing nanostructured photoactive layers using layer-by-layer assembly, including the direct LbL of n-type and p-type materials (See Appendix D “Layer-by-Layer Assembly of Titania Nanomaterials with Charged Conjugated Polymers to Create Flexible Photoactive Composites”), and the assembly of high aspect ratio structures using the M13 bacteriophage to organize quantum dots (See Appendix E “Assembly of High Aspect Ratio M13 Bacteriophage-Quantum Dot Complexes”). However, while these strategies could generate nanostructured morphologies, the poor contact between individual nanomaterials would likely have led to reduced photocurrent and poor device performance. In contrast, the strategy based on a bacteriophage nanoporous scaffold described here results in a highly connected and continuous network of titania.
Scheme 4-1. Two fold-strategy employed for constructing a bacteriophage-based nanoscale device architecture, as applied to PbS QD solar cells, to enhance the photocurrent generation over a randomly-organized geometry. A. The control device has a disorganized geometry that consists of a layer of PbS QDs infiltrated into a porous layer of titania nanoparticles. The percolation pathways for electrons can be circuitous depending on how the TiO$_2$ NPs sinter together. B. Virus-templated titania network provides a blended heterojunction morphology that allows photogenerated charges to be collected from QDs that have infiltrated into the network and provides more direct pathways to conduct electrons. C. Metal nanoparticles act as nano-antennas to concentrate light in their near-field region, thereby producing a higher photon flux on nearby QDs and an increased rate of carrier generation.

This strategy is applicable to many different solution-processed solar cell types because (1) anatase titania is popularly employed as the n-type component in hybrid organic-inorganic, colloidal quantum dot, and perovskite systems, (2) nanostructured titania is commonly used to assist in charge extraction in bulk-heterojunction devices and in perovskite solar cells, and (3) the resonance position of the plasmonic nanoparticles can be tuned by geometry and material choice to capture light that is poorly absorbed for any given active material. To evaluate the feasibility of our approach and the suitability of the multifunctional virus-based films for solid-
state thin film solar cells, we have chosen to assemble colloidal quantum dot bulk heterojunction solar cells. This is a class of solid-state photovoltaic devices that are low-cost, compatible with solution-processing methods, and have recently demonstrated efficiencies as high as 8.6%.\textsuperscript{6,27,28} Specifically, lead sulfide quantum dots (PbS QDs) are excellent active materials for solar cells because they are efficient charge transporters, their band gaps can be tuned throughout the infrared spectrum, their band edges can be modified with various ligand molecules,\textsuperscript{29} and they are often paired with anatase titania to form a heterojunction. We used PbS QDs with an average diameter of 2.9 nm and a first excitonic absorption peak at 905 nm. This corresponds to a bandgap of 1.37 eV, which is close to the optimal bandgap for an ideal single junction photoconverter under the AM 1.5G solar spectrum.

Traditionally, PbS QDs are stacked on top of a nonporous titania film to form a depleted heterojunction. In order to extract carriers efficiently in this planar device, the QD film thickness must be thinner than the carrier collection length (~200 to 250 nm), which is the sum of the depletion width and the minority carrier diffusion length.\textsuperscript{30} On the other hand, complete light absorption requires that the QD film thickness be greater than the absorption length (>1 \textmu m). Instead, a bulk heterojunction structure can be constructed by infiltrating QDs into nanostructured titania, which can extend the width of the depletion region and allow for more light to be absorbed with thicker QD films. However, these structures are more prone to trap-assisted recombination at defects if the conduction pathways are tortuous and if the interfacial surface area is high. For this reason, nanowire-based titania morphologies that provide continuous and direct pathways for charge transport are advantageous over disorganized mesoporous nanoparticle films commonly used in solution-processed solar cells.\textsuperscript{9,27} It has been shown that using an array of titania\textsuperscript{7,8,31} or zinc oxide\textsuperscript{6} nanopillars or nanowires infiltrated with PbS quantum dots enhances the device performance compared to planar oxide layers because the photocurrent increases enabled by extended depletion zones overcome any undesirable increases in non-radiative recombination that result from a larger interfacial area between the active material and the titania nanostructures.

Our plasmonic virus-templated porous composite is rationally designed to generally compensate for the weaknesses of photoactive materials with relatively short lifetimes. Specifically for titania-PbS QD depleted bulk heterojunctions, our system offers the following
advantages: first, the highly porous film allows for substantial infiltration of QDs into the titania networks, thereby extending the depletion region of the device. Second, compared to a porous film made using a titania nanoparticle paste, the interconnected network of titania nanowires formed by the high aspect ratio viruses provides direct pathways for transporting charges from the bulk of the device to the electrodes. Likewise, forming titania along a high aspect ratio template like the M13 bacteriophage produces a smaller overall surface area compared to a porous film comprised of spherical particles, and is therefore expected to have less surface defects to promote non-radiative recombination. Third, PbS QDs suffer from an absorption spectrum that preferentially absorbs high energy photons. Therefore, it is susceptible to the classic tradeoff in solar cells between light harvesting and carrier collection, where thick active layers are required to absorb light throughout the visible and infrared ranges, but carrier generated in the quasi-neutral region are less likely to diffuse to the depletion region before recombining. By using the M13 bacteriophage to carry and assemble metal nanoparticles with plasmon resonances in the visible range within nanoporous films, photons at poorly absorbed wavelengths can be concentrated in the near-field surrounding the NPs and light harvesting is improved without further increasing the QD film thickness. Lastly, the uniformity and sub-wavelength pore size combined with the ability of the virus to evenly disperse metal nanoparticles throughout the film offers an ideal environment where effective medium theory can be paired with finite-difference time-domain (FDTD) simulations to theoretically evaluate how several metal nanoparticle types can enhance light absorption in the devices.

This work presents the overall process for constructing virus-based depleted bulk heterojunction PbS QD solar cells, with a focusing on the optimization of various critical steps to maximize device performance, such as (1) PbS QD infiltration into the titania pore network (2) surface preparation prior to assembly, (3) biominalerization time and control of the morphology, and (4) nanoparticle-virus complexation procedures with different nanoparticles including gold nanoparticles (Au NPs), silver nanoparticles (Ag NPs) or silver nanoplates (Ag NPLs). Through this optimization, we were able to diminish parasitic resistances and improve light harvesting in the devices, yielding enhanced photocurrents and power conversion efficiencies. By comparing a nanoparticle paste control device against our virus-based system, we demonstrate significant photocurrent enhancement and separate the contributions due to morphology and plasmon enhancement.
4.2 Results and Discussion

4.2.1 Device Design, Assembly and Patterning

A process for patterning bottom and top electrodes, assembling thin film solar cells, and testing them was developed. Scheme 4-2A illustrates the general device geometry. First, a fluorine-doped tin oxide (FTO) substrate is patterned using combination of etching with zinc powder in concentrated hydrochloric acid (HCl), and a subsequent etching with hydrofluoric acid (HF). In order to protect an area of the FTO substrate that will define the solar cell area, photolithography was used to deposit a photoresist on the black areas shown in Scheme 4-2B, preventing removal of FTO in these areas. After cleaning the resulting substrates with soap, water, acetone and isopropanol, the active layer can be deposited, but a section of the substrate must remain clean in order to make contact with the counter electrode during device testing. A shadow mask (Scheme 4-2C) was designed to evaporate six-pad top metal electrodes onto the active layer. Each of the six pads can be tested independently and has an area of 0.0547 cm$^2$ corresponding to the overlap between the FTO electrode and the top metal electrode.
Scheme 4-2. Fluorine-doped tin oxide (FTO) substrates are patterned via photolithography before assembling the active layer of the devices and finally evaporating a patterning of six tops metal electrodes. A. Schematic of the process for 1) patterning FTO, 2) constructing a photoactive film and dissolving or scratching off a thin layer to allow for contact with the FTO electrode, 3) evaporating metal electrodes in a six-pad patterning, and 4) connecting each electrode to a conductive pin linked to the testing setup. B. Mask for patterning a photoresist onto a 10 cm sheet of FTO-coated glass. The black region is covered with the photoresist and is protected during the etching. C. Shadow mask for evaporating six-pad electrodes on 16 devices at a time. D. Drawing of the testing holder. The lid is open to allow for a top illumination, and device are placed face-down in the holder to allow for each electrode to contact conductive pins.

For bacteriophage-templated titania-PbS QD thin film solar cells specifically, the device assembly process is schematized in Scheme 4-3. On a patterned FTO substrate, a dense and compact layer of titania paste is first spin-coated, and acts as a hole blocking layer to prevent shorting of the device. After annealing this base layer at 500 °C, the viruses or virus-metal nanoparticle complexes are assembled via LbL, as previously described. Various methods to functionalize the titania surface and prepare it for virus attachment can be employed, and will be discussed later. Once the virus film is constructed, it is coated with titania using titanium tetrachloride as the precursor, and annealed again to burn off the viruses and generate an anatase crystalline titania nanoporous film. PbS QDs are then infiltrated into the pores of the film via dropecasting or spin-coating to create the titania-lead sulfide composite. Finally, a 25 nm thick hole transporting layer of MoO₃ is thermally evaporated, followed by a 100 nm thick gold electrode.
1. Pattern FTO
2. Spincoat Compact TiO₂ Layer
3. Build Nanoporous Virus Film via LbL
4. Biomineralize Virus Film with TiO₂
5. Infiltrate and Spincoat PbS QDs
6. Evaporate Top Metal Electrodes

Scheme 4-3. Bacteriophage-based titania-PbS QD composites are assembled via sequential solution-processing to result in the final solar cells. A compact titania layer is spincoated onto patterned FTO substrates. This layer is then functionalized with bacteriophages and a layer-by-layer assembled bacteriophage film is constructed and mineralized with titania. Lead sulfide quantum dots are infiltrated within the pores of the titania network to create the photoactive composite before a final evaporation of metal electrodes.

4.2.2 Infiltration of Quantum Dots within Bacteriophage-Templated Titania

The bacteriophage-templated titania film was designed to have a high porosity and to be infiltrated with p-type materials, including conjugated polymers (See Appendix F “Infiltration of Conjugated Polymers within Porous Bacteriophage-Templated Titania Networks”) and quantum dots. A cross-section of the final titania film shows the difference in morphology between the compact titania nanoparticle base layer and the porous virus-templated titania film (Figure 4-1). The high porosity of the bacteriophage-templated portion can be observed as well as nanowire-like features, creating direct pathways for electrons to travel in the titania phase.
Figure 4-1. Porous bacteriophage-templated titania can be constructed on top of a compact titania based layer. SEM image of the cross-section of a titania-coated virus film on a compact titania paste base layer. The dash line indicates the interface between the base layer and the virus-templated titania film.

For titania-PbS QD solar cells, we have demonstrated that PbS QDs can be infiltrated throughout ~ 90 nm of a bacteriophage-templated titania film, with a concentration gradient decreasing from the top to the bottom of the film. Figure 4-2 shows TEM characterization of titania films infiltrated with QDs. First, the titania-PbS composite was scratched off and deposited on a TEM grid as shown in Figure 4-2A. While two materials can be distinguished in the composite (~ 2.9 nm diameter quantum dots within larger ~ 10 nm titania particles), cross-sectional TEM images allowed for a clearer visualization of the infiltration of PbS QDs within the titania films. Figure 4-2B shows a TEM cross-section image where a titania-PbS blend layer can be distinguished from a top PbS QD-only layer, and the TEM image with elemental mapping in Figure 4-2C demonstrates that a titania-lead sulfide composite is achieved after QD infiltration. The QDs not only infiltrate completely through the nanoporous virus-template titania network but also form a packed layer above it. Others have previously infiltrated PbS quantum dots into nanowire arrays, but the inter-spacing of the nanowires (on the order of 50 to 100 nm) was considerably larger than the pores of the virus-templated titania film presented here, which are 8.1 nm diameter in average. Elemental mapping shows that a concentration gradient occurs within the infiltrated virus-templated film, and that the fraction of QDs gradually decreases with the film depth.
Figure 4-2. Lead sulfide quantum dots can infiltrate porous bacteriophage-templated titania networks to form nanocomposites. A. STEM-Bright Field image showing a piece of titania-PbS QD composite. The small particles within the larger aggregate are PbS QDs. B. Cross-sectional TEM image showing the titania-PbS QD blend layer stacked with a top PbS-only layer and protective layers used to prepare the TEM sample. C. Elemental mapping of titanium (red) and lead (green) on a TEM image showing the PbS QD-infiltrated titania film.

The gradient in PbS QD concentration within the titania film was quantitatively measured for various infiltration methods, including dip-coating, spin-coating and dropcasting, and performing ligand exchanges during the process. X-ray photoelectron spectroscopy (XPS) with depth profiling was used to monitor the atomic concentration of lead and titanium as a function of film depth and these atomic concentrations were converted to lead sulfide/titania molar ratios and subsequently to volume fractions using the molecular weight and crystalline density of anatase titania and lead sulfide. First, the mole fractions of lead (Pb) and titanium (Ti) are equal respectively to the mole fractions of lead sulfide (PbS) and titania (TiO₂) and can be taken as the atomic concentrations measured by XPS.

\[
{n_{\text{PbS}}} = {n_{\text{Pb}}} \quad \text{and} \quad {n_{\text{TiO₂}}} = {n_{\text{Ti}}}
\]

\[
{x_{\text{PbS}}} = \frac{{n_{\text{PbS}}}}{{n_{\text{PbS}} + n_{\text{TiO₂}}}} \times 100\%
\]

\[
{x_{\text{TiO₂}}} = \frac{{n_{\text{TiO₂}}}}{{n_{\text{PbS}} + n_{\text{TiO₂}}}} \times 100\%
\]

where \(x_{\text{PbS}}\) and \(x_{\text{TiO₂}}\) are the mole fractions of lead sulfide and titania respectively, \(n_{\text{PbS}}, n_{\text{Pb}}, n_{\text{TiO₂}}\) and \(n_{\text{Ti}}\) are the number of moles of PbS, Pb, TiO₂ and Ti respectively.
Then, from the known crystalline volumes of anatase titania and lead sulfide, relative volumes of titania and lead sulfide can be calculated on a 100 mol basis.

\[ V_{TiO_2} = V_{TiO_2\text{molecule}} \times x_{TiO_2} \]  \hspace{1cm} \text{Equation 4-4}

\[ V_{PbS} = V_{PbSmolecule} \times x_{PbS} \]  \hspace{1cm} \text{Equation 4-5}

where \( V_{TiO_2} \) and \( V_{PbS} \) are the relative volumes of bulk titania and lead sulfide within the film, and \( V_{TiO_2\text{molecule}} \) and \( V_{PbSmolecule} \) the known volumes of a molecule of TiO\(_2\) and PbS in their crystalline phases, respectively.

From this data, the pore filling fraction, or the percentage of the pores in the titania film that are filled with PbS QDs, was calculated as a function of depth in the titania film. This calculation was done using a measured porosity of 42% for the titania film (see Figure 4-8B), and assuming that the maximum possible QD packing density inside the pores is a loose random-packing configuration\(^{33}\) or 56%. A total value for the volume of the blend layer film (including the titania, the loosely-packed PbS and air) can be determined, as well as the volume of the pores within the titania film before infiltration:

\[ V_{\text{total}} = \frac{V_{TiO_2}}{1 - \rho_{TiO_2}} \]  \hspace{1cm} \text{Equation 4-6}

\[ V_{\text{pores}} = \rho_{TiO_2} x V_{\text{total}} \]  \hspace{1cm} \text{Equation 4-7}

where \( V_{TiO_2} \) is the volume of TiO\(_2\) derived from XPS measurements; \( \rho_{TiO_2} \) the known porosity of the titania film; \( V_{\text{total}} \) the total film volume including TiO\(_2\), loosely-packed PbS and air; and \( V_{\text{pores}} \) the volume of the pores within the TiO\(_2\) film before infiltration.

From the packing density of PbS, the volume of loosely-packed quantum dots at each film depth can be calculated, as well as the pore filling fraction, which is simply the ratio of the loose-packed QD volume over the volume of the pores in the titania film.

\[ V_{l-p\text{PbS}} = \frac{V_{PbS}}{f_{PbS}} \]  \hspace{1cm} \text{Equation 4-8}

\[ \eta_{\text{filling}} = \frac{V_{l-p\text{PbS}}}{V_{\text{pores}}} \]  \hspace{1cm} \text{Equation 4-9}

where \( f_{PbS} \) is the packing fraction of the quantum dots; \( V_{PbS} \) the volume of PbS particles derived from XPS measurements; \( V_{l-p\text{PbS}} \) the volume of loosely-packed quantum dots; and \( \eta_{\text{filling}} \) the
pore filling fraction or, in other words, the fraction of the initial volume of the pores in the TiO$_2$ film that is now filled with PbS QDs taking into account their packing density.

The resulting profiles of PbD QD infiltration in bacteriophage-templated titania are shown in Figure 4-3. A first infiltration study was carried out via dip-coating, with or without ligand exchange (Figure 4-3A). When the QDs were coated ten times onto a titania film without ligand exchange, very little infiltration occurred, and the QDs did not penetrate further than 30 nm in depth. When an exchange of the oleic acid ligands to benzene-1,3-dithiol (BDT) was performed, the infiltration slightly increased, but a thick PbS QD layer formed on top of the titania film. The adherence of the QDs was thus improved by the exchange with BDT, but the infiltration was marginally affected. A third sample was prepared by dip-coating a titania film that was previously incubated overnight in a 1\% mercaptopropionic acid (MPA) solution. This treatment allowed for a deeper infiltration than the control, but since only one dip was done, the pore filling fraction was not improved. Next, an infiltration study was carried out by dropcasting the PbS QDs onto titania films and air-drying the samples (Figure 4-3B). With and without ligand exchange with MPA, the PbS content within the titania film increased compared to the dip-coat method. With MPA exchanges after each dropcast, the PbS QDs were, again, able to infiltrate deeper into the film. Based on these observations, spincoating and dropcasting methods were compared for a more concentrated PbS QD solution, and with MPA ligand exchange, which yielded the most efficient infiltrations (Figure 4-3C).

Dropcasting a dilute PbS solution, followed by slowly drying at room temperature allows for more PbS QDs to infiltrate the film via capillary action, and maintains a high pore filling efficiency that is above 40\% throughout the 100 nm thick virus-templated titania film. However, drying rings are observed on the surface of the film and poor film quality is produced when the dropcasting method is employed. In contrast, spin-coating produces a very smooth and uniform film. The concentration profile of PbS decreases more sharply with film depth, but the QDs still penetrate deeper than 80 nm into the virus-templated titania film. Therefore, a combination of these two methods is used to prepare the photovoltaic devices: PbS QDs are first dropcast onto the surface of the titania film, and then allowed to penetrate the film for 1 min; the solution is then spun off of the substrate at 2500 rpm in order to produce a smooth film, followed by a mercaptopropionic acid (MPA) ligand exchange and rinse. Subsequent layer of PbS quantum dot
are then spin-coated and ligand exchanged in a layer-by-layer fashion. Figure 4-4 shows the cross-sectional and top surface morphology of smooth PbS QD films prepared with this method.

Figure 4-3. PbS QDs infiltrate porous bacteriophage-templated titania films to different extents depending on the infiltration method. Pore filling fractions as a function of film depth calculated from XPS depth profiling results for A. Thin films dip-coated in a 5 mg/mL PbS solution with or without ligand exchange. A sample was first incubated in MPA overnight and then dipped in the PbS QD solution for 1h. The other two samples were dipped successively 10 times in the PbS QD solution with or without exchange with BDT between each dip. B. PbS QDs dropcast onto titania films at 5 mg/mL 10 times with or without exchanging the ligands with MPA between each dropcast. C. PbS QDs spincoated or dropcast at 50 mg/mL, with MPA ligand exchange. A depth of 0 nm corresponds to the interface between the top PbS QD layer and the TiO$_2$-PbS blend layer.
4.2.3 Optimization of Bacteriophage-Based Solar Cell Assembly

To assess the impact of the virus nanocomposite system on device performance, we fixed the thickness of the titania layers and kept the PbS deposition procedure (concentration, ligand, number of deposited layers) consistent between devices. To determine these parameters, simple planar devices were fabricated using conventional procedures and commercially available titania paste (Solaronix Ti-Nanoxide HT/SC). By varying the thicknesses of both layers, it was established that a total titania thickness of approximately 200 nm is optimal, and that beyond five layers of spin-coated PbS QDs, no improvement is observed (See Figure 4-5). In order to ensure full infiltration of PbS QDs within the virus-templated titania layer, we fixed the electron transporting compact titania base layer to 100 nm and the virus-templated titania layer to 100 nm, for a total of 200 nm of titania. Then, using these fixed thicknesses, the process for constructing the bacteriophage film and mineralizing it was optimized.

Figure 4-4. Quantum dots spincoated in a layer-by-layer fashion result uniform films. A. Cross-sectional SEM image showing five layers of spincoated PbS QDs staking onto a substrate. B. Top surface morphology visualized by SEM showing minimal porosity and the absence of pinholes within the PbS QD film.
Figure 4-5. Device performance is a function of the thickness of the titania and PbS layers for a planar device. Maximum and average power conversion efficiency as a function of A. thickness of titania, and B. number of layers of PbS QDs spin-coated.

A critical design step in assembling photoactive materials using biomolecules is controlling the surface chemistry of inorganic components. The chosen surface functionalization method must provide uniform surface coverage with a high loading density of the biotemplate, but it must also involve reagents that can be completely degraded and removed from the device afterwards to avoid interfering with charge transport. In this work, M13 bacteriophages must be adsorbed or covalently attached to the titania base layer as a first step in the virus film assembly. After the film assembly and biomineralization with titania, the bacteriophages are burnt off at a temperature of 500 °C. Therefore, the reagents used for surface functionalization must also burn off at this temperature.

Three surface modifications were considered for functionalizing the titania base layer with a monolayer of viruses: silanization with (3-aminopropyl)trimethoxysilane (APTMES), construction of polymer base layers via electrostatic LbL assembly, and spin-coating a thin layer of a charged short chain polymer. These modifications are illustrated in Figure 4-6A, and the corresponding performance of each virus-based titania-PbS solar cell is shown in Figure 4-6B. The silanization method has the advantage of forming covalent amide bonds between the amine groups of the silane molecules and the carboxylic acid groups of the virus through a reaction catalyzed by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). However, APTMES can leave silica, silicon, polysiloxane and other silicon derivative residues after combustion at 500°C. In consequence, high series and low shunt resistances are observed for devices made using
silanization, producing low fill factors. The high series resistance is likely due to the presence of insulating residues, such as silica, at the titania-substrate interface while the shunt resistance could be created by the presence of conducting residue, such as silicon, providing alternate pathways for electrons to travel to the compact titania layer.

In contrast, polymers such as poly(allylamine hydrochloride) (PAH), poly(acrylic acid) (PAA), and polyethyleneimine (PEI) are excellent candidates to replace silanes because they burn off at temperatures below 500 °C, with the major combustion product being carbon dioxide in the gas phase. Using LbL assembly, cationic PAH and anionic PAA can be layered electrostatically on an oxygen plasma-treated titania surface. We thus assembled 4.5 bilayers of PAH and PAA, ending with a top positively charged PAH layered onto which negatively charged M13 bacteriophages can be electrostatically adsorbed. The virus adsorption was carried out, as described previously, in sodium acetate buffer at pH 4.9 to obtain a dense assembly of viruses. This thin polymer base layer film is expected to have a thickness of approximately 10 nm according to literature for a comparable system. To further decrease the thickness of the polymer base layer formed, a short branched PEI polymer (1.3kDa) was spin-coated onto a plasma-treated titania base layer surface. The resulting thickness of this positively charged layer is only ~2 nm, and similarly, M13 bacteriophage can be electrostatically adsorbed to this surface. Figure 4-6B shows the improvement in device performance, as (1) the amount of inorganic residues decreases, and (2) the thickness of the base layer decreases. In fact, the short-circuit current is significantly larger for the devices constructed with polymers compared to silanes, and is higher when the 2 nm thin PEI layer is used as opposed to a thicker PAH/PAA film.

These results are also consistent with XPS measurements confirming the presence of the polymer layers after deposition, and their subsequent disappearance after annealing at 500 °C. Figure 4-7 and Table 4-1 respectively show the XPS surveys scans measured before and after annealing, and the resulting atomic composition of the titania surface. When the polymers are deposited, peaks for nitrogen can be observed due to the presence of amine groups in PEI and in PAH. For the samples with PEI, the nitrogen signal is weak and titanium atoms can still be detected, indicating that that PEI layer is, as expected, thinner than 10 nm (maximum detection depth by XPS). For the samples with PAH/PAA base layers, the nitrogen signal is stronger, as
well as the carbon signal, but no titania is detected on the surface. This observation confirms that the PAH/PAA base layers are 10 nm thick or greater. Then, after burning off the polymers at 500 °C, the nitrogen peak disappears from all samples, revealing a clear titania surface. It has to be noted that oxygen and carbon are present in all samples (including the titania surface before any polymer is deposited) because of natural oxidation and contamination with carbon.

Although all polymers burn off at high temperatures, the thicker PAH/PAA layer may lead to higher series resistance because it could create a gap in between the compact titania base layer and the bacteriophage-templated titania film, preventing a good contact between the titania layers and potentially promoting film delamination.

Based on the $JV$ curves in Figure 4-6B, series and shunt resistances were calculated from the inverse of the slope of the $JV$ curve at $V = 0$ V and $J = 0$ mA/cm$^2$ respectively. The resistances were normalized to the characteristic resistance of the cell, $R_{CH}$, in Ω/cm$^2$, which is calculated as follow.

$$R_{CH} = \frac{V_{oc}}{J_{sc}}$$  \hspace{1cm} \text{Equation 4-10}

where $V_{oc}$ is the open circuit voltage in V, and $J_{sc}$ is the short-circuit current density in A/cm$^2$.

Dividing the series and shunt resistances by the cell resistance gives the normalized resistances, as shown in Equation 4-11 and Equation 4-12.

$$r_{SH} = \frac{R_{SH}}{R_{CH}}$$  \hspace{1cm} \text{Equation 4-11}

$$r_{SR} = \frac{R_{SR}}{R_{CH}}$$  \hspace{1cm} \text{Equation 4-12}

where $r_{SH}$ and $r_{SR}$ are the normalized shunt and series resistances, and $R_{SH}$ and $R_{SR}$ the shunt and series resistances, respectively, in Ω/cm$^2$.

Accordingly, the calculated series and shunt resistances are improved when thin polymer films are used, as shown in Figure 4-6C. The series resistance decreases when the silane residues disappear, and the shunt resistance increases, indicating that charges are travelling more efficiently through the titania pathways. All device results presented hereafter employ the optimal PEI spin-coating surface functionalization method.
Figure 4-6. Series and shunt resistances can be improved by changing the surface functionalization method prior to LbL assembly. A. Three methods for functionalization the surface and allow for bacteriophage attachment, either via covalent bonding or electrostatic adsorption. From left to right, silanization using APTMES, LbL-assembled PAH/PAA base layers, and thin PEI layer. B. Comparison of the device JV curves with the three different surface preparations. C. Normalized series and shunt resistance calculated from the JV curves. The series and shunt resistances are calculated from the slope at zero current and zero voltage respectively, and are normalized by the characteristic resistance of the device.
Figure 4-7. Polymer base layers completely burn off after annealing at 500 °C. XPS surface analysis for a thin PEI layer and PAH/PAA base layers deposited onto compact titania paste. A. Survey scans. B. N1s region of the survey scan showing the disappearance of nitrogen after annealing.
Table 4-1. Atomic composition of the surface of compact titania or titania with polymer layers gives insights about the presence and thickness of polymer layers. Atomic concentrations of titanium, oxygen, carbon and nitrogen determined by XPS before and after annealing at 500 °C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ti (%)</th>
<th>O (%)</th>
<th>C (%)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titania base layer</td>
<td>23.2</td>
<td>59.9</td>
<td>16.8</td>
<td>0</td>
</tr>
<tr>
<td>Titania with PEI</td>
<td>21.2</td>
<td>54.0</td>
<td>20.7</td>
<td>4.1</td>
</tr>
<tr>
<td>Titania with PEI burnt off</td>
<td>21.8</td>
<td>60.2</td>
<td>18.0</td>
<td>0</td>
</tr>
<tr>
<td>Titania with PAH/ PAA</td>
<td>0</td>
<td>31.0</td>
<td>62.5</td>
<td>6.5</td>
</tr>
<tr>
<td>Titania with PAH/PAA burnt off</td>
<td>20.1</td>
<td>54.4</td>
<td>25.6</td>
<td>0</td>
</tr>
</tbody>
</table>

4.2.4 The Effect of Morphology in Bacteriophage-Based Titania-PbS QD Solar Cells

Biomineralization time strongly affects the morphology of the virus-templated titania film, and consequently the device performance. M13 bacteriophages are 6 nm in diameter by 880 nm in length, and can therefore create very fine titania nanostructures, as thin as 6 to 7 nm in diameter, after burning them off and sintering the crystallites. The reaction time in a 0.2 M TiCl₄ solution was varied between 1.5 and 2.5 hours, and the morphology, porosity and pore size distribution of the resulting titania network were compared. Figure 4-8A shows the surface morphology of the virus-templated titania films after 1.5, 2 and 2.5 hours of titania nucleation and subsequent annealing at 500 °C. It is readily observed that the porosity tends to decrease with increasing reaction time and that the diameter of nanowire-like features increase. The structure is highly porous after 1.5 h, and the pores begin to occlude after 2.5h in the reaction mixture. Therefore over time, the nanostructured and porous titania film become less porous and more planar. Figure 4-8B summarizes results obtained from analysis of SEM images with ImageJ, which reveal that the porosity decreased from 44.3 % to 41.9 % and 36.1 % after 1.5, 2 and 2.5 hour reactions respectively (see Figure 4-9 for high contrast images used for the analysis and mapping of the pores on the top surface of the films). However, the pore size distribution is only slightly affected, with somewhat smaller pores being formed on average for shorter reaction times. A striking change in morphology between 1.5 and 2 hour reaction time is the diameter of nanowire-like features, which increase from an average of 6.2 nm to 11.8 nm.
Figure 4-8. The biomineralization reaction time allows for tuning the feature size and porosity in bacteriophage-based titania. A. Surface morphology of the virus-templated titania film captured by SEM, as a function of reaction time in TiCl$_4$ (1.5, 2, and 2.5 h). B. Pore size distribution, porosity and average nanowire (nw) size for the different reaction times. Values were determined using image analysis.
Figure 4-9. Image analysis for the estimation of porosity, pore size distribution and size of nanowire-like features. The analysis was performed using the ImageJ software. The contrast between the pores and the titania was enhanced (top tow), and the area corresponding to the pores was calculated (area in red in the bottom row). To calculate the equivalent diameter of a pore, the area was converted into diameter assuming a circular pore. The red arrows show nanowire features in each image. To determine the average nanowire size, several nanowires were measured in each enhanced-contrast image and the measurements were averaged.

The highest performing virus-templated device was obtained with a reaction time of 2 h (JV curve shown alone in Figure 4-10C). Therefore, the increase in nanowire diameter appears to have strongly affected the efficiency of the device and significantly increased the short-circuit current density, as observed in Figure 4-10A (see also Table 4-2). The average short-circuit current density and power conversion efficiency increased by about 2-fold in the devices biomineralized for 2 h compared to those biomineralized for only 1.5 h. The fill factor also increased from 1.5 to 2 h, indicating that thicker nanowires decrease the series resistances by improving the electron conductivity through the titania mesh. In addition, it is possible that incomplete coverage of the bacteriophage surface for the 1.5 h samples is causing local breakpoints in the titania networks, which decreases photocurrent and fill factor, and also increases the variability between devices. The virus films biomineralized for 2.5 h hours also perform better than the ones reacted for 1.5 h, but the short-circuit current decreases compared to the 2 h biomineralization, likely due to the decrease in titania film porosity, which not only provides less pore volume for QDs to fill but also diminishes their ability to infiltrate into them. Figure 4-10B presents the JV curves for the devices with the best performance for each biomineralization time,
and also shows the increase in photogenerated current from 1.5 to 2 h and the subsequent decrease for 2.5 h mineralization. In summary, we found that the biomineralization time has a strong effect on device performance and that increasing time to an optimum of 2 h increases both the short-circuit current and the fill factor. For longer times, the efficiency might continue to plateau or decrease due to pore occlusion and flattening of the titania surface. These findings demonstrate that fine tuning the morphology of the titania-PbS layer is critical.

**Figure 4-10.** Biomineralization time affects short-circuit current, fill factor and device performance. A. Average and maximum device performance parameters as a function of the reaction time. Error bars represent standard deviation. Paired t-tests were performed and the statistically significant differences between pairs of results are shown by * for a p-value < 0.05 and ** for a p-value < 0.005. Exact values are reported in Table 4-2. B. JV curves for the device with the best performance for each biomineralization time. C. JV curve for device with best performance with a 2 h reaction time.

In order to demonstrate the advantages of using M13 bacteriophages as a scaffold to construct a continuous and porous titania matrix, our optimized virus-templated device was compared to an analogous device built using a titania nanoparticle paste. A titania paste was prepared using the same TiCl₄ precursor at 0.2 M in water and collecting the particles formed after a 2 h hydrolysis reaction. The particles were resuspended in a mixture of terpineol.
containing ethylcellulose, which acts as a sacrificial polymer, and the final titania paste was spin-coated onto a silicon substrate to observe its morphology (Figure 4-11A). The resulting film porosity was estimated to 40% using ImageJ (Figure 4-11B), which is comparable to that of the phage film biomineralized for 2 h. However, as seen in Figure 4-11A, the nanoparticles are randomly oriented and particle size is polydisperse. When the phage film is immersed into a TiCl₄ solution, only the crystallites nucleated on the surface of the viruses remain in the film and create the uniform and organized networks of nanowires observed in Figure 4-1 and Figure 4-8. In contrast, the paste is formed with all nanoparticles synthesized in solution during TiCl₄ hydrolysis, and cannot be easily purified to exclude larger aggregates or assembled in an organized manner. Although the PbS infiltration profiles measured by XPS depth profiling follow a trend similar to the one observed for the virus films (Figure 4-11C), the short-circuit current density in the paste device was found to be almost 1.5 times lower than the virus-templated device, as shown in Figure 4-11D, and on average the performance of the optimized virus-template was two times higher than the paste control (see Table 4-2). This suggests that organizing titania nanoparticles into an interconnected network of nanowires allows for charges to be transported more efficiently than in a randomly distributed nanoparticle paste film.
Figure 4-11. Randomly organized TiO₂ nanoparticles prepared from the same TiCl₄ precursor result in a devices with a lower efficiency. A. Cross-sectional morphology of a TiCl₄-based TiO₂ paste after annealing. The paste was spun at 2000 rpm onto a silicon substrate. B. Image analysis of the TiCl₄-derived TiO₂ paste film. The analysis was performed with the ImageJ software to estimate the film porosity. Mapping of the pores is in red. C. PbS QD infiltration profile by dropcasting or spin-coating into the TiO₂ paste film, resembling the infiltration profile in a virus-templated TiO₂ film. A depth of 0 nm corresponds to the interface between the top PbS layer and the TiO₂-PbS composite film. D. Comparison between the JV curve of a virus-templated TiO₂ film and a randomly organized nanoparticle film.

Table 4-2. Solar cell performance parameters for TiO₂ NP paste control device and virus-based devices at different biomineralization times. Average values and standard deviations are presented for each parameter, as well as the maximum power conversion efficiency (PCE) for each type of device.

<table>
<thead>
<tr>
<th>Device</th>
<th>Jsc (mA/cm²)</th>
<th>Voc (V)</th>
<th>FF</th>
<th>PCE (%)</th>
<th>Max PCE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paste control</td>
<td>-7.3 ± 2.5</td>
<td>0.53 ± 0.03</td>
<td>0.24 ± 0.03</td>
<td>0.99 ± 0.47</td>
<td>1.99</td>
</tr>
<tr>
<td>1.5 h, virus only</td>
<td>-7.4 ± 4.5</td>
<td>0.50 ± 0.04</td>
<td>0.27 ± 0.05</td>
<td>1.06 ± 0.82</td>
<td>2.45</td>
</tr>
<tr>
<td>2 h, virus only</td>
<td>-13.2 ± 2.0</td>
<td>0.51 ± 0.04</td>
<td>0.32 ± 0.03</td>
<td>2.18 ± 0.40</td>
<td>2.93</td>
</tr>
<tr>
<td>2.5 h, virus only</td>
<td>-11.8 ± 2.2</td>
<td>0.54 ± 0.04</td>
<td>0.31 ± 0.03</td>
<td>1.94 ± 0.52</td>
<td>2.89</td>
</tr>
</tbody>
</table>
4.2.5 Plasmon-Enhanced Bacteriophage-Based Thin Film Solar Cells

In addition to improving charge collection via the morphology of the nanoporous titania film, the performance of the solar cells was further improved by enhancing their ability to harvest light with metal nanoparticles (NPs), which concentrate light in the near-field via localized surface plasmon resonance (LSPR). By measuring the absorption spectrum of bacteriophage-templated titania films constructed in glass and infiltrated with PbS QDs, we observed that light is poorly absorbed at wavelengths above ~600 nm (Figure 4-12) and thus metal nanoparticles that could compensate for this absorption would be beneficial to the device performance.

![Absorption Spectrum](image)

**Figure 4-12.** The absorption spectrum of bacteriophage-templated titania infiltrated with PbS QDs shows poor absorption at wavelengths above ~600 nm. Absorption spectrum for a titania film infiltrated with PbS QDs, and ideal region of metal nanoparticle absorption highlighted in yellow.

This LSPR approach to device improvement leverages the ability of the M13 bacteriophage to be genetically programmed to exhibit affinity for noble metals, which allows silver and gold nanoparticles to be complexed onto the viruses while in aqueous solution. The complexation process spaces the nanoparticles fairly evenly along the bacteriophages, which avoids aggregation and allows the characteristic absorption peaks of plasmonic particles to be preserved. TEM images of viruses complexed with spherical gold nanoparticles (Au NPs), spherical silver nanoparticles (Ag NPs), and silver triangular nanoplates (Ag NPLs) are shown in Figure 4-13A. As discussed in our previous report\(^2\), the act of binding to the virus causes the characteristic plasmon peak positions of noble metal NPs to be slightly red-shifted compared to aqueous colloids due to the presence of the virus proteins in the medium directly surrounding the
Figure 4-13B presents the absorption spectra of all three NP-virus complexes in solution and clearly shows the characteristic dipole peak positions of Au NP at 520 nm, Ag NP at 400 nm, and both the dipole and quadrupole peaks of Ag NPLs at 620 and 400 nm, respectively.

![Image of TEM images and absorption spectra](image)

**Figure 4-13.** Noble metal nanoparticles with different shapes and absorption peaks can be complexed with M13 bacteriophages. TEM images (A) and corresponding absorption spectra (B) for virus-nanoparticle complexes that were incorporated into the films.

Using our previously reported LbL-assembly process\textsuperscript{21}, Au NPs, Ag NPs, and Ag NPLs were evenly distributed throughout the nanoporous virus films without aggregation with loadings 1 NP per virus or less, and subsequently coated with a thin film of titania during the biomineralization process. These plasmonic nanoporous titania films were then used as the n-type component of the heterojunction QD solar cells. We first studied the effects of adding Ag NPLs at different concentrations (Figure 4-14), and then constructed devices with all three types of nanoparticles at a concentration of 0.1 NPs per virus.
Figure 4-14. The power conversion efficiency varies with the concentration of silver nanoplates in bacteriophage-templated devices. Optimal surface functionalization and biomineralization conditions were used for this study. The highest efficiency is observed for a 0.1 NPL/phage ratio.

All types of nanoparticles improved the overall power conversion efficiency of the titania-PbS devices with the Ag NPLs producing the largest photocurrent enhancement, as shown in Figure 4-15A. The short-circuit current was enhanced in the presence of each nanoparticle type, with statistically significant improvement occurring with Ag NPs and Ag NPLs. In addition, the open circuit voltage increased by approximately 50 mV when adding any type of metal nanoparticle to the devices, but the fill factor was not significantly affected by the presence of nanoparticles. The improvement in power conversion efficiency (PCE) caused by the metal nanoparticles in the virus-templated devices was calculated to be 22.2% for Au NPs, 25.1% for Ag NPs, and 36.5% for Ag NPLs on average (Figure 4-15B and Table 4-3). The top performing Ag NPL device reached a PCE close to 4%, and its JV curve is compared to a virus-templated device without plasmonic nanoparticles in Figure 4-15C.
Figure 4-15. The incorporation of noble metal nanoparticles improves the efficiency of the virus-templated titania-PbS QD depleted bulk heterojunction devices. A. Comparison of power conversion efficiency, short-circuit current density and open circuit voltage for devices with naked virus, or for viruses complexed with Au NPs, Ag NPs or Ag NPLs. Paired t-tests were performed and the statistically significant differences between pairs of results are shown by ** for a p-value < 0.005 and *** for a p-value < 0.0005. B. Average percentage improvement in efficiency with addition of metal nanoparticles. C. Comparison of the JV curves for the best device with and without metal nanoparticles. Overall, the nanoparticle device with the highest efficiency contained Ag NPLs. Exact values are reported in Table 4-3.
Table 4-3. Solar cell performance parameters for the optimized virus-based device, and plasmonic virus-based devices. Average values and standard deviations are presented for each parameter, as well as the maximum power conversion efficiency (PCE) for each type of device.

<table>
<thead>
<tr>
<th>Device</th>
<th>$J_{sc}$ (mA/cm$^2$)</th>
<th>$V_{oc}$ (V)</th>
<th>FF</th>
<th>PCE (%)</th>
<th>Max PCE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus only</td>
<td>-13.2 ± 2.0</td>
<td>0.51 ± 0.04</td>
<td>0.32 ± 0.03</td>
<td>2.18 ± 0.40</td>
<td>2.93</td>
</tr>
<tr>
<td>With Au NPs</td>
<td>-14.1 ± 1.6</td>
<td>0.55 ± 0.02</td>
<td>0.35 ± 0.03</td>
<td>2.67 ± 0.36</td>
<td>3.15</td>
</tr>
<tr>
<td>With Ag NPs</td>
<td>-15.5 ± 1.0</td>
<td>0.56 ± 0.01</td>
<td>0.32 ± 0.02</td>
<td>2.73 ± 0.16</td>
<td>3.04</td>
</tr>
<tr>
<td>With Ag NPLs</td>
<td>-15.5 ± 1.2</td>
<td>0.55 ± 0.04</td>
<td>0.35 ± 0.04</td>
<td>2.98 ± 0.58</td>
<td>3.96</td>
</tr>
</tbody>
</table>

It has to be noted that higher device performances can be obtained for devices with smaller areas. Figure 4-16 compares the performance of virus-based devices containing Au NPs with a 0.0547 cm$^2$ area (as reported throughout this work) and for areas approximately 5 times smaller of 0.0121 cm$^2$. The higher average performance observed for smaller area devices is likely due to the reduced probability of defects in the film, which significantly increases the short-circuit current and the fill factor for a constant open circuit voltage. We however believe that reporting values for larger areas are more valuable since the ultimate goal is to produce large area photovoltaic devices and panels.
Figure 4-16. Smaller area devices can reach higher efficiencies. A. Best JV curves and B. Average power conversion efficiency, short-circuit current, open circuit voltage and fill factor, for virus and Au NPs device with areas of 0.0547 cm$^2$ or 0.0121 cm$^2$ (small area).

The effect of each nanoparticle type on the ability of the devices to harvest light was computationally predicted prior to device fabrication by combining effective medium theory with finite-difference time-domain (FDTD) calculations. As illustrated in Figure 4-17A, the simulation geometry follows the approximate dimensions of an actual device cross-section, which consists of stacked slabs of homogeneous materials starting with an FTO base layer, a 100 nm thick compact anatase titania layer, a 100 nm thick blended titania-PbS QD layer that contains a single plasmonic NP, a 100 nm thick layer of random-packed PbS QDs, and a 25 nm thick MoO$_3$ layer on top. Periodic boundary conditions were placed on both the $x$ and $y$ lateral directions in order to simulate a unit cell of the solar cell slab, which allowed any light scattered out of the sides of the simulated domain to be reabsorbed elsewhere in the unit cell. The extent of the unit cell in the $x$ and $y$ lateral directions was set to 60 nm, which is the estimated approximate distance between nanoparticles in the virus-templated titania film. Finally, the unit cell was illuminated through the FTO layer by a plane wave with wavelengths ranging from 200 to 1200 nm and a three-dimensional power monitor was placed throughout the unit cell to sample the power density of light absorbed at each node in the mesh for each simulated wavelength.
Because a nanoparticle can be located at any vertical position within the blend layer, five separate FDTD simulations were performed for each nanoparticle type, each with the nanoparticle center positioned at a different elevation (10, 30, 50, 70, and 90 nm into the blend layer). A sixth simulation was performed with the nanoparticle positioned 10 nm into the QD film layer to account for roughness of the virus film, which would cause a NP located near the top of the blend layer to be surrounded by a higher fraction of QDs. The ensemble average over all six configurations was taken during the analysis to approximate the effect of inherent randomness of homogeneously distributed NPs throughout the thickness of the virus-templated film has upon light absorption.

Aside from the geometry of the model, developing a realistic description of the complex dielectric function of the photoactive materials in the optical model is critical to capturing how light is absorbed as it propagates through the device architecture and how far the near-field generated by the plasmonic NP extends into the active regions. The complex dielectric function of the compact titania, titania-PbS blend layer, and packed PbS QD layers were calculated using effective medium theory in conjunction with values adjusted from previous reports for anatase thin films and PbS quantum dots (Figure 4-17B, C and D). When a monodisperse solution of PbS QDs is deposited onto a planar film, the nanospheres will pack into a porous layer with voids existing between them. Because the size of a quantum dot is substantially smaller than the wavelength of light, light cannot distinguish between an individual particle and the air in the voids, rather it perceives an effective dielectric medium that is a blending of the material properties of the two components. Therefore, the dielectric function of a randomly-packed PbS QD layer deposited on top of the nanoporous titania film was calculated using the Maxwell-Garnett effective medium model. Similarly, when the pores of the virus-templated titania film are infiltrated with the PbS QDs, the resulting film is a blend of anatase titania, PbS, and air void-space, which was modelled using the Bruggeman model as an effective homogeneous medium with a dielectric function that depends on the porosity of the virus-templated film. For simplicity, it was assumed that the PbS QDs are homogeneously distributed throughout both the blend and QD film layers with a volume fraction of 56% in the spin-coated QD layer and 15% in the blend layer. A value of 56% in the QD film corresponds to a loose random-packing, which is considered more appropriate than the 74% value associated with regular close-packed beds because spin-coated films of QDs have been shown to be less-regularly packed with packing
densities around 50%. Likewise, a 15% PbS volume fraction in the blend layer was calculated by averaging the infiltration data of spin-coated PbS QDs into the virus film reported in Figure 4-3C over the film thickness.

By illuminating the simulated device with a flat irradiance across all wavelengths and integrating the absorbed power density over the spatial extent of the photoactive regions of the device, i.e. the titania-PbS QD blend layer and the random-packed QD layer, we predicted how introducing the plasmonic nanoparticles into the solar cell architecture would influence its overall absorption efficiency, $\eta_a$. However, the performance of solar cells depends not only on its ability to absorb photons, but also on how well those photons can be converted into charges and how efficiently those charges can be collected at the electrodes. Therefore, some basic assumptions were applied to build a simple model for charge collection efficiency, $\eta_c$, mainly: (1) it is assumed that all of the absorbed power in the blend layer can be converted to charges and collected because it is within the depletion region. Secondly, (2) it is assumed that carriers generated deeper in the close-packed QD layer are less likely to migrate to the titania in the blend layer before recombination occurs than those generated closer to the blend layer interface. Therefore, an exponential decay envelop with a decay length of 30 nm was applied to the absorbed power density values in the quasi-neutral region in order to capture the fact that carriers generated closer to the blend layer are more likely to diffuse to the depletion region for collection and contribute to the device photocurrent:

$$\eta_c = \begin{cases} 1, & 0 \leq z \leq L_b \\ e^{-(z-L_b)/L_d}, & L_b < z \leq L_b + L_{QD} \end{cases}$$  

**Equation 4-13**

where $L_b$ is the thickness of the depletion region, $L_{QD}$ is the thickness of the quasi-neutral region, and $L_d$ is the electron decay length in PbS QD films.
Figure 4-17. Geometry of FDTD simulations. A. Schematic of the titania, titania-PbS blend, and PbS layers. B. Real (blue) and imaginary (red) components of the complex dielectric
function for the pure PbS QDs modified from Moreels et al.\textsuperscript{38} for 904 nm QDs (solid lines) and the estimated effective function for a spin-coated film of loosely random-packed PbS QDs (56% volume fraction of PbS QD and 44% air) as predicted by Maxwell Garnett effective medium theory. C. Real (Left) and imaginary (Right) parts of the complex refractive index computed for pure anatase titania (blue lines), the 35% PbS QD 75% air mixture in the pores of the blend layer (red lines), and the final blend layer (black lines) consisting of a 58% volume fraction of anatase titania, 15% PbS QDs, and 27% air. D. Real (blue) and imaginary (red) components of the index of refraction for the 16% porous anatase TiO\textsubscript{2} film reported by Kim et al.\textsuperscript{37} (dotted lines) and the estimated function for pure anatase films (i.e. 0% porous films, solid lines) as predicted by Bruggeman effective medium theory.

The product of the absorption and collection efficiencies provides an estimate of the external quantum efficiency (EQE) spectra for each device, which quantifies how efficiently incident photons of a given wavelength are converted into collected charges. Figure 4-18A and B, respectively, show the computed and measured EQE spectra for devices loaded with each type of silver plasmonic particle. The overall shape of the simulated and measured EQE spectra for the devices without nanoparticles are remarkably similar, with a main peak arising around 375 nm followed by a slowly decaying profile at higher wavelengths. The simulations predict a clear plasmon-induced performance enhancement starting around 400 nm for Ag NPs and 525 nm for Au NPs, which is in good agreement with the observed regions of power enhancement in the actual devices.

![Figure 4-18](image_url)

**Figure 4-18.** FDTD simulations can be used to predict the external quantum efficiency of the devices. A. Predicted and B. Measured EQE spectra for devices containing Au NPs, Ag NPs or Ag NPLs, compared to virus-only devices. All EQE curves were adjusted based on measured average short-circuit current values for each type of device.
In contrast, the predictions for Ag NPLs dramatically underestimate the observed performance increase in devices. This is likely due to the Ag NPLs changing shape and size during the device fabrication process. First, monitoring their shape and absorption spectrum during LbL assembly indicated an evolution from triangular nanoplates to circular nanodiscs and nanoparticles (Figure 4-19). Second, it is expected that the nanoplates originally incorporated into the films transformed into a polydisperse population of nanospheres during the device annealing process performed to crystallize the titania network. It has been observed that colloidal nanoplate solutions tend to stack upon one another when dried on TEM grids (Figure 4-20). It is possible that during the LbL process used to build the virus films, free nanoplates could stack upon those that have been already bound to the virus, which later convert into various sizes of silver nanospheres during annealing. A polydisperse nanosphere population would result in a broader enhancement wavelength range consistent with the wide region of enhancement demonstrated by the EQE measurement shown in Figure 4-18B. This hypothesis was also investigated computationally by running simulations using Ag nanospheres whose volume is conserved from stacks of 1, 2, or 3 Ag NPLs. Averaging the results produces a corrected simulated EQE spectrum that is much closer to the measurements made for devices originally made using Ag NPLs.
Figure 4.19. The optical properties and morphology of silver nanoplates evolve during the LbL assembly process. Three different ratios of Ag NPLs/phage were used (0.1, 0.2 and 0.4), and the absorption spectra and morphology of the Ag NPL-bacteriophage complexes were monitored initially, and after 82, 188, 284 and 325 bilayers. A. Normalized absorption spectra of Ag NPL-bacteriophage complexes through the process. The spectra are blue shifted as the number of bilayer increases as indicated by the arrows. B. Change in peak positions as a function of the number of bilayers. The peak positioned around 625 nm corresponds to the dipole resonance of the NPLs. As the corners of the NPLs become more rounded, this peak shifts towards shorter wavelengths. The 390 nm peak position corresponds to the dipole peak of silver nanospheres. This indicates that during the LbL process the morphology of the NPLs change into spheres or discs as the particles interact with the virus proteins and likely lose the protective citrate ions that preserve the triangular shape. Higher NPL loadings of the virus results in more spheres as indicated by an increase in the 390 nm peak, whereas lower loadings result in the NPLs becoming cylindrical discs. C. TEM images showing the morphology of the NPLs as a function of the number of bilayers for 0.4 NPL/phage.
Figure 4-20. Evidence of Ag NPL stacking, as shown by a TEM image of Ag NPLs dropcast from solution.

Although a metal nanoparticle absorbs light, the power contained within its interior does not contribute to the photogeneration of carriers and is generally lost as heat. Only light concentrated in the NP’s near-field that extends into the surrounding active layer will contribute to photocurrent enhancement. Placing a NP inside the blend layer occupies volume that would have otherwise been filled with a blend of titania and QDs in its absence, therefore the near-field generated by the NP must overcome this loss. Thus, enhancement is only expected to occur at wavelengths where LSPR occurs and performance is expected to be slightly reduced at off-resonant wavelengths because less QDs are physically present in the model to absorb photons at these energies. This is the reason why the simulated EQE spectra of the plasmonic devices are slightly reduced from the devices without NPs at short wavelengths. Discrepancies between the predicted and measured EQE spectra are likely due to local spatial variations in the effective complex dielectric function of the media in the device arising from (1) PbS QDs not being homogeneously distributed throughout the actual blend layer thickness, and (2) roughness at the interfaces between layers. In actuality, a QD concentration gradient exists as is shown in Figure 4-3C, which is not explicitly considered by the model. Similarly, roughness at the interface between the blend layer and the random-packed QD layer would cause a nearby NP to sense an effective medium comprised of a higher fraction of PbS than a NP buried within the blend layer. The result of both cases is to broaden the wavelength range of plasmon enhancement as metal nanoparticles located at different positions in the actual active layers would experience different effective local dielectric functions of the surrounding medium, thereby producing LSPR peaks at
different wavelength positions and broadening the spectra of the ensemble average. Since the idealized model presented herein is sufficient to capture the observed trends in the measurements, any further modeling of local variations in geometry and material properties is considered to be beyond the scope of this report.

The presented model clearly demonstrates for the Ag nanosphere case that the wavelengths between 400 and 600 nm where significant EQE enhancement occurs can be explained by LSPR acting to concentrate light in the near-field region surrounding the noble metal nanoparticles. This increases the photon flux incident on QDs located within the near-field, thereby increasing their rates of charge carrier generation. The ability of a plasmonic NP to enhance photocurrent generation in a solar cell can be quantified by defining an enhancement factor, $EF$, which compares the predicted short circuit current density ($J_{sc}$) resulting in the unit cell geometry that contains a NP to the prediction of the $J_{sc}$ in the same unit cell without the NP:

$$EF = \frac{J_{sc}}{J_{sc}}$$

$$\frac{\int_{\text{volume in active region}} p_{abs}(\vec{x}, \lambda)\eta_c(z) d^3 x}{\int_{\text{region outside NP}} p_{abs}(\vec{x}, \lambda)\eta_c(z) d^3 x} \frac{\lambda}{h c} M_{AM1.5}(\lambda) d\lambda$$

where $p_{abs}$ is the absorption efficiency density as a function of position and wavelength for a unit cell containing a plasmonic NP, $p_{abs}^0$ is the absorption efficiency density as a function of position and wavelength for the same unit cell without a NP, $M_{AM1.5}$ is the solar irradiance for the AM1.5 global solar spectrum, $h$ is Planck’s constant, $c$ is the speed of light, and $\eta_c$ is the carrier collection probability. The enhancement factor can be directly compared to the ratio of the short circuit current density, $J_{sc}$, for a unit cell with NPs to that without, $J_{sc}^{NP} / J_{sc}^{NO NP}$, which is presented in Figure 4-21A. The measured devices demonstrate higher photocurrent enhancement than the simulations predicted with the measured enhancement being 6.2%, 16.3%, and 16.5% for solar cells with Au NPs, Ag NPs, and Ag NPLs respectively, whereas simulations predicted 3.8, 10.9, and 4.2%. When the photocurrent is calculated for a polydisperse population of Ag NPs that have been transformed from Ag NPLs, the predicted enhancement rises to 11.3%. Although the predictions were lower than observed photocurrent enhancement, the trend is clearly captured. It is worth noting that devices with Ag NPs in both the simulated and measured
devices produce a photocurrent enhancement roughly three times larger than devices with Au NPs.

The model presented can only explicitly account for enhancement of the device photocurrent and cannot account for any differences in parasitic resistances and the fill factor between devices, which fortunately do not change significantly between virus-based solar cells with and without plasmonics NPs. However, the increase in open-circuit voltage ($V_{oc}$) that is observed in plasmonic devices likely arises directly from their corresponding increase in photocurrent due to enhanced light harvesting. The standard diode equation model for solar cells couples the current and voltage together; therefore, the open circuit voltage of a device will change if it can output a higher photocurrent. By fitting each of the measured $JV$ curves for virus-based devices with and without plasmonic NPs to the standard diode equation for solar cells, it was revealed that the $V_{oc}$ increase observed for the device with Ag NPLs (Figure 4-15C) corresponds to the $V_{oc}$ predicted by the standard solar cell model if the virus-based device without plasmonic NPs happened to experience an increase in photocurrent equal to that observed in the virus-based device with Ag NPLs. Therefore, the effect of LSPR conveniently causes both the $J_{sc}$ and $V_{oc}$ to rise in the plasmonic devices, causing the observed overall enhancement in PCE to be even higher.

Lastly, as demonstrated in Figure 4-21B, blending the PbS QDs with the titania nanoporous network provides an effective medium that allows the near-field surrounding the metal NPs to propagate further into the blend layer than they would if incorporated into the random-packed QD layer. This is a direct result of the effective complex index of the blend layer being lower than the random-packed QD film by virtue of its lower volume fraction of PbS. This is useful from a practical standpoint because the larger the extent of the near-field, the greater the chance a PbS QD can experience a higher photon-flux and exist in a defect-free region that can efficiently generate and transport charge carriers.
**Figure 4-21.** The trends of FDTD simulated photocurrent enhancements and plasmon decay lengths upon addition of plasmonic nanoparticles follow the experimentally measured trends. A. Predicted and measured photocurrent enhancement compared to virus-templated devices without nanoparticles. B. Predicted plasmon decay length for different nanoparticles embedded in the blend and random-packed QD layers.

### 4.3 Conclusions

In conclusion, we demonstrated that the high aspect ratio M13 bacteriophage can serve as a template to form nanocomposites that are suitable for thin film solar devices. However, in order to make an effective device, many processing parameters had to be optimized to maximize device efficiency. First, we found that a short chain PEI polymer was the optimal surface functionalization molecule to adsorb the bacteriophages to the titania base layer because it could form a very thin layer that was minimally insulating. Second, we showed that the biomineralization time could be optimized to allow for the thickening of the nanowire-features while maintaining the film porosity. Third, the best method for infiltrating PbS QDs into the pores was found to be a combination of dropcasting and spincoating as determined by XPS depth profiling measurements. Although the pores are on average 10 nm in size, the 2.9 nm diameter QDs could substantially infiltrate throughout the virus-templated titania film. Last, we determined the optimal amount of plasmonic nanoparticles that would maximize the enhancement in photocurrent.

Compared to a randomly organized nanoparticle paste control, we confirmed that the virus-based titania morphology had a significant impact on the photocurrent of the device. A two-fold improvement in device efficiency suggests that the high aspect ratio of the virus improves charge transport within the active layer. Devices with Ag NPs and Ag NPLs incorporated into the active layers of solar cells showed an improved light harvesting ability in
the EQE spectra near the expected LSPR peak positions and subsequent enhancement in the $J_{sc}$, $V_{oc}$, and PCE, with PCE enhancements reaching 36.5% over virus-based control devices with Ag NPLs. How the virus-templated nanoporous morphology affected the propagation of light through the solar cells was numerically computed by modelling the nanostructured device layers with effective medium theory and using the FDTD method to calculate how power is absorbed throughout the devices. The main features of the predicted EQE spectra and photocurrent enhancement for devices with Ag NPs and Ag NPLs were in good agreement with the trends observed in measurements, indicating that LSPR is primarily responsible for the observed improvements in light harvesting and photocurrent in plasmon-enabled solar cells. Furthermore, the simulations indicate that generating a blended titania-PbS active layer produces a larger near-field compared with the random-packed QD layer, thereby increasing the chance that light concentration occurs at a point without structural or electronic defects that impair charge generation and transport. Therefore, the virus-templated geometry not only improves the ability of the device to collect and transport charges from the bulk of the device, but also the practical ability of plasmons to enhance light harvesting.

4.4 Materials and Methods

**Materials:** 1-octadecene (technical grade, 90 %) (ODE), oleic acid (technical grade, 90 %) (OA), lead (II) oxide (technical grade, 99.999 %), cadmium chloride (99.99 %), hexamethyldisilathiane (synthesis grade) (TMS-S), titanium tetrachloride, (3-aminopropyl)trimethoxysilane (APTMES), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), gold(III) chloride (>= 99.99% trace metal basis), silver nitrate, coumalic acid, hydrogen peroxide (Perdrogen, 30% solution), sodium borohydride, glycerol, branched PEI (1.3kDa), 3-mercaptopropionic acid (MPA), and 1,3-benzenedithiol (BDT) were purchased from Sigma (St-Louis, MO). 1-tetradecylphosphonic acid (98%) (TDPA) and sodium citrate dihydrate were purchased from Alfa Aesar (Ward Hill, MA). Oleylamine (80-90 %) was purchased from Acros (Fair Lawn, NJ). N-hydroxysulfosuccinimide (sulfo-NHS) was purchased from Pierce Biotechnology Inc. (Rockford, IL). TEC 15 FTO-coated glass substrates were purchased from Pilkington Glass (Northwood, OH). Polyallylamine hydrochloride (PAH, 120000 to 200000 g mol$^{-1}$) and polyacrylic acid (PAA, >250000 g mol$^{-1}$) were purchased from Polysciences Inc. (Warrington, PA). Titania paste (HT/SC, 8-10 nm particles) was purchased from Solaronix SA (Switzerland).
**M13 bacteriophage:** The E3 M13 bacteriophage variant was amplified by infecting exponentially growing *Escherichia coli* bacteria. The bacteriophage was separated from cell debris via centrifugation and further purified with two cycles of polyethylene glycol-sodium chloride precipitation and centrifugation. The final product was diluted in a PBS buffer, and the EEE peptide displayed on each pVIII protein was confirmed with DNA sequencing.

For metal nanoparticle binding, the p8#9 variant was used, and amplified as described above. The VSGSSPDS peptide displayed on the pVIII proteins was confirmed with DNA sequencing.

**Gold nanoparticle (Au NP) synthesis:** Gold nanoparticles with ~20 nm diameters were synthesized following the Turkevich method.\(^{41}\) In summary, 95 mL of deionized water and 2.6 mL of 50 mM gold(III) chloride were combined in a 250 mL round-bottom flask and brought to a boil while stirring in a silicone oil bath. While boiling, 10 mL of 1% wt sodium citrate dihydrate was quickly added while vigorously stirring. The color of the solution quickly turned black and then ripened into a deep ruby red. The solution was kept boiling and stirring for an additional 20 minutes. The solution was cooled to room temperature and stored without further purification.

**Silver nanoparticle (Ag NP) synthesis:** Silver nanoparticles were synthesized following a modified Turkevich method.\(^{41}\) In summary, 95 mL of deionized water and 4 mL of 30 mM silver nitrate were combined in a 250 mL round-bottom flask and brought to a boil while stirring in a silicone oil bath. While boiling, 10 mL of 5% wt sodium citrate dihydrate was quickly added while vigorously stirring. After stirring for a minute, 6 mL of 100 mM coumalic acid was quickly added. The color of the solution slowly ripened into a deep yellow-brown color. The solution was kept boiling and stirring for an additional 20 minutes. The solution was cooled to room temperature and stored without further purification.

**Silver triangular nanoplate (Ag NPL) synthesis:** Silver nanoparticles were synthesized by modifying the method previously reported by Zhang *et al.*\(^{42}\) In a 1 L Erlenmeyer flask, 240 mL of deionized water was combined with 5 mL 50 mM silver nitrate and 5 mL 750 mM sodium citrate dihydrate while stirring at room-temperature, followed by 6 mL of 30% hydrogen peroxide stock solution. While stirring at room-temperature, 10 mL of freshly prepared 100 mM sodium borohydride was added dropwise to the solution. The color of the solution should change from yellow to brown to amber-red within about fifteen minutes. Once the color has stabilized, add 3 mL of 30% hydrogen peroxide solution. Repeat addition of hydorgen peroxide, if necessary, until...
the solution has changed color and stabilized to a deep blue. Add 1 mL of glycerol to stabilize the solution for long-term storage.

**PbS quantum dot synthesis:** PbS quantum (QD) synthesis followed previously published methods. A metal halide precursor was first prepared by dissolving 0.3 g CdCl₂ and 0.033 g of TDPA in 5 mL of oleylamine in a three-neck flask and degassed at 100 °C under vacuum. The metal halide solution was then kept at 80 °C under nitrogen.

In a separate three-neck flask, 0.45 g of PbO, 1.5mL of oleic acid, and 18 mL of ODE were degassed under vacuum at 125 °C. A sulfur precursor was prepared by mixing 0.18g of TMS-S (weighed out in an argon atmosphere glovebox) with 10mL of degassed ODE. The sulfur precursor solution was then rapidly injected into the reaction flask at 125 °C. Immediately after injection, the reaction mixture was removed from heat and allowed to cool down to room temperature. When the mixture cooled down to 60-70 °C, 1 mL of the metal halide precursor solution was rapidly injected. Once the entire mixture reached room temperature, the QDs were purified and isolated through several centrifugation wash cycles. The QDs were first precipitated with acetone and isolated by centrifugation. The QDs were subsequently re-dispersed in toluene and precipitated again with excess acetone and methanol, followed by centrifugation. Finally, the QDs were washed several more times with methanol and stored as a dry powder.

**Device assembly:** Patterned FTO substrates were cleaned sequentially in soap, water, acetone and isopropanol with sonication. After drying with N₂ gas, the substrates were plasma treated for 2 min, and a base layer of titania paste (Solaronix, HT/SC) was spun at 2000 rpm and annealed at 500 °C for 1 h.

The titania paste layer was functionalized either with aminopropylsilanetriol and by attaching a first layer of viruses with EDC as previously described, or by functionalizing the surface either with 4.5 bilayers of PAH/PAA or with a branched PEI (1.3kDa) spincoated at 0.1 w% in water at 5000 rpm, and electrostatically adsorbing the first layer of viruses in a NaOAc buffer (pH 4.9) at 50 °C for 45 min. After each functionalization, the films were rinsed with milli-Q water and dried with N₂ gas. Virus films were assembled via covalent layer-by-layer, as described previously. To remove any residual salts or organics, the films were rinse in milli-Q water for at least 30 min after LbL.
Titania was nucleated onto the virus films using a 0.2 M TiCl₄ solution in water. The hydrolysis occurred at 80 °C for 1.5, 2 or 2.5 h. The films were then sonicated for a few seconds in milli-Q water, and rinsed for at least 30 min. The titania-coated virus films were annealed at 500 °C for 1 h.

As a control, porous titania paste was synthesized using 0.2 M TiCl₄ as a precursor and reacting it for 2 h at 80 °C. The resulting nanoparticles were collected via centrifugation, rinsed and resuspended with ethanol. 5-15 mPa ethylcellulose and 30-50 mPa ethylcellulose were added as sacrificial polymers (each at an equivalent of ~10 w% of the titania nanoparticles), along with terpineol as solvent and the ethanol was evaporated. The mixture in terpineol was spincoated onto the compact titania base layer to prepare control devices, and the titania was annealed at 500 °C, simultaneously burning off the sacrificial polymers.

Oleic acid-capped PbS QDs at 50 mg/mL in octane were dropped onto the titania films for 1 min, and then spun at 2500 rpm for 10 s. Ligand exchange was performed with MPA (1 % in methanol), which was spun for 10s, then rinsed with methanol and spun for 10s, and rinsed with octane and spun for 10s. The cycle was repeated a total of five times.

Patterned electrodes of MoOₓ (25 nm) and Au (100 nm) were thermally evaporated onto the PbS film. The resulting area of each device was 0.0547 cm².

**Thin film characterization:** The thickness of the films was determined using a Veeco Dektak 150 profilometer. The cross-section and surface morphology of the films were observed by scanning electron microscopy (SEM) using a Helios Nanolab 600 Dual Beam Focused Ion Beam Milling System. The porosity, pore size distribution and nanowire size were estimated from the SEM images using the Image J software. The cross-sectional TEM sample was prepared using a JEOL 9320-FIB, and the mapping of Ti and Pb was performed with a JEOL 2100 FEG TEM (EDX, X-MAX 80 mm²). XPS with depth profiling was used to determine the atomic concentration of PbS and TiO₂ through the film depth and was converted to volumetric fractions using the molecular weight and the density of the materials. The XPS used was a PHI Versa-Probe II X-ray photoelectron spectrometer with a scanning monochromated Al source (1,486.6eV; 50 W; spot size, 200 µm), and an argon source was used for sputtering, with zalar rotation at 1 rpm.
Device testing: A solar simulator (150 W Newport 96000 xenon arc-lamp, with AM1.5G filter and diffuser lens) was calibrated to 100 mA cm\(^{-2}\) using a silicon reference cell. J-V curves were obtained by applying an external bias to the devices and measuring the resulting photocurrent, with a Keithley 6487 picoammeter. External quantum efficiency (EQE) spectra were obtained from 300 nm to 1100 nm without bias illumination, and the useful range of data from ~ 300 nm to 900 nm was presented.

4.5 References


Chapter 5:
Synthesis of Biologically-Templated Silica and Silicon Materials as Building Blocks for Micro- to Nanostructures
Chapter 5. Synthesis of Biologically-Templated Silica and Silicon Materials as Building Blocks for Micro- to Nanostructures

This chapter presents the biomineralization of silica onto various biotemplates, and the magnesiothermal reduction of biotemplated silica products to form metalloid silicon, while preserving the shape of the biotemplates. High aspect ratio M13 bacteriophages and coil-shaped Spirulina major algae were used as templates to create novel nano- and microstructures, and natural sources of silica, like diatomaceous earth, were also magnesiothermally reduced to optimize the reduction process and post-treatment steps. The morphology, crystallinity, composition and surface area of the biotemplated silica and silicon materials were characterized to demonstrate successful conversion of oxide materials to metals, and the reliability of the process to conserve key features of the biotemplate morphologies throughout the synthesis. The method presented here can be applied to the reduction of a wide range of oxides to metals (See Appendix G “Synthesis and Magnesiothermal Reduction of Titania on M13 Bacteriophage and Spirulina Biotemplates” for an example of magnesiothermal reduction of titania).


5.1 Introduction

Metalloid silicon is of primordial importance for several industrial applications, including the fabrication of semiconductors, solar panels and high strength alloys. In addition to using bulk silicon, there has been a growing interest in creating micro- and nanostructured silicon materials for applications in hybrid silicon-organic photovoltaics,\(^1\) lithium-ion batteries,\(^2,3\) micro-electronics,\(^4\) and even sensing devices.\(^5,6\) Organizing silicon into continuous structures can be used to improve charge transport, and creating high surface area networks could contribute to its reactivity and efficiency as an anode material. Silicon has the highest known theoretical charge capacity as an anodic materials in lithium ion batteries,\(^7,9\) and therefore developing new methods for the synthesis of high surface area silicon materials with on-demand nanostructures is of great interest. In addition, silicon is widely used for photovoltaic applications, and nanostructured silicon thin films are currently being explored for use as
antireflection coatings, and in hybrid silicon solar cells which are inexpensive, low-temperature alternatives to conventional silicon solar cells.1,10

Currently, patterning silicon is carried out via top-down approaches, which include lithography combined with harsh chemical etching techniques,11-14 electron-beam lithography,15-17 or anodization18,19 of silicon wafers. While these methods have been widely employed to create structures with simple geometries like silicon posts, pillars or nanowires, complex shapes cannot be achieved with these top-down approaches. The ability to template metalloid silicon onto pre-assembled scaffolds with a desired shape would open up new possibilities for this material in the field of nanotechnology.

While silicon, due to its highly negative reduction potential, cannot be reduced from precursors in aqueous solution,20 silicon dioxide or silica, is a material that can easily be synthesized via sol-gel reactions. Silica nanoparticle gels can be formed in solution, and subsequently nucleated onto scaffolds and templates that take on a variety of shapes. Now, with magnesiothermal reduction (MGTR), a process that has been recently developed to reduce metal oxides into their metal counterparts while preserving their shape, we can envisage converting silica structures into metalloid silicon. MGTR was first investigated in the 1970’s and 80’s to prepare metals like boron21 and titanium.22,23 It was applied to silica in 2007,24 and since then, interest in creating various silicon nanostructures via MGTR has grown.

MGTR is the vaporization of magnesium at relatively low temperature (650 to 700 °C), and the reduction of a metal oxide, such as silica, upon contact with its vapor. While silica is reduced to silicon, the vaporized magnesium is oxidized to magnesium oxide. This process can be carried out at temperatures considerably lower than the melting point of silicon (1414 °C), which allow for preserving the shape of the silica structure that is being reduced, in contrast with carbothermal processes that require temperatures above 1700 °C and cannot be used to create nanostructures.25,26 It therefore allows for the formation of a variety of silicon structures with a precise control over the porosity, three-dimensional structure and grain size by exploiting and controlling well-known silica chemistries. During the past decade, MGTR has been employed to produce nanoporous silicon using diatoms27, mesoporous silica granules,28, silica particles grown on inorganic templates,29 and rice husks30 as precursors.
In order to produce a broader variety of silica shapes, with more complex or finer structures, we turned our attention to biological templates. Viruses, bacteria, algae and other microorganisms have a plethora of shapes that could be of interest for technological applications. They often exhibit complex morphologies containing turns, coils, angles, and pores, and their size varies from tens of microns for algae to nanometers for viruses. In addition, they can be organized into three-dimensional hierarchical structures via bioconjugation techniques to create porous films or arrays.\textsuperscript{31,32} Silica can be mineralized onto biotemplates with different shapes and length scales, and the silica structures can be reduced while preserving the shape of the template. \textbf{Scheme 5-1} shows an overview of the biomineralization and magnesiothermal reduction process.

The magnesiothermal reduction of silica proceeds as shown in Equation 5-1. Solid silicon powder is contacted with magnesium vapor, and is reduced to silicon.

\begin{equation}
\text{SiO}_2(s) + 2 \text{Mg}(v) \rightarrow \text{Si}(s) + 2\text{MgO}(s) (+\text{Mg}_2\text{Si}(s)) \tag{Equation 5-1}
\end{equation}

Magnesium oxide and magnesium silicide are formed as solid by-products, but can be easily rinsed off using an acid. For instance, with hydrochloric acid, magnesium oxide reacts to form magnesium chloride which is soluble in water (Equation 5-2), and magnesium silicide converts to micro-quantities of silane gas which bubbles out of solution (Equation 5-3). All purifications of the silicon products should be carried out in a fumehood to contain the toxic silane vapors and prevent exposure.

\begin{equation}
\text{MgO}(s) + 2 \text{HCl}(aq) \rightarrow \text{MgCl}_2(aq) + \text{H}_2\text{O} \tag{Equation 5-2}
\end{equation}

\begin{equation}
\text{Mg}_2\text{Si}(s) + 4 \text{HCl}(aq) \rightarrow \text{SiH}_4(g) + 2 \text{MgCl}_2(aq) \tag{Equation 5-3}
\end{equation}

After rinsing off the by-products, the product is a pure silicon structure with the same shape as the biotemplate. The silicon product can be porous or continuous depending on the shape of the template and the silica mineralization conditions.
Of particular interest for the formation of silicon is diatomaceous earth (DE), a natural and abundant source of silica formed by the skeletal remains of diatoms. We used these natural fossilized silica structures as model low aspect ratio structures. In addition, many other microorganisms including viruses and algae can be used to nucleate silica nanoparticles. In this work, the M13 bacteriophage, a filamentous bacteriophage, is used as a template to create silica nanowires. With its high aspect ratio – the phage has a length of 880 nm and diameter of 6.5 nm - the M13 bacteriophage is well known to generate fine nanowire-like structures upon biomineralization.\(^{31,33}\) *Spirulina* major, a coil-shaped blue-green algae also serves as a template for silicon micro-coils. *Spirulina major* was recently used to mineralize titania\(^ {34}\) and copper\(^ {35,36}\) while preserving its spiral-like structure. We are therefore interested in demonstrating the use of this complex shape for the formation of silicon.

In this work we demonstrate our ability to bio-mineralize silica using the M13 bacteriophage and *Spirulina major* as templates, and to magnesiothermally reduce the products to
silicon to form high surface area materials with defined nanostructures. Although the magnesiothermal reduction of DE has been previously demonstrated and applied to battery anodes and photoelectrodes, we also use DE as a model organism to optimize the magnesiothermal reduction process. We then apply the optimal conditions to our biotemplated silica to produce novel silicon morphologies. We provide characterization of the morphology, elemental composition, and crystallinity of all biotemplated silica and silicon samples, and show that upon reduction, the as-synthesized amorphous silica is converted to nanocrystalline silicon and that the shape of the biotemplate is preserved throughout the process. In addition, we show that it is possible to first assemble the biotemplates into porous thin films, and then nucleate silica and reduce the thin films via magnesiothermal reduction to create porous silicon films with nanowire-like features. Interestingly, our results indicate that the novel coil and nanowire-like silicon morphologies that we present here could also act as semiconductors. We show that microorganisms have the potential for serving both as templates and sources of dopants for the production of semiconducting silicon. Our ability to produce high surface area and porous silicon materials could be extended to other metals and open up opportunities for using these materials to create aerogels, light weight structures and other useful porous metallic structures.

5.2 Results and Discussion

5.2.1 Magnesiothermal Reduction of Diatomaceous Earth and Process Optimization

Diatomaceous earth was used as a model microorganism to demonstrate and optimize our magnesiothermal reduction process. Using this abundant source of biotemplated silica, the reduction time and temperature were varied, as well as the mass of magnesium and the nature of the acid used for the post-reduction rinse. The conversion of DE from amorphous silica to nanocrystalline silicon was monitored via XRD, and the results are shown in Figure 5-1. We established that the reduction must be carried out for 3 h in order to fully convert amorphous silica into crystalline silicon, and that the temperature should be kept at 650 °C or higher in order for the reaction to proceed. For a 10 mg silica sample, an equivalent mass of magnesium was found to be optimal for the reduction, which corresponds to a 2.5:1 Mg:SiO₂ molar ratio. A slight excess of magnesium is therefore desirable for the reaction to proceed. The by-products were easily removed by a rinse with a strong acid like HCl, while traces of MgO could still be
detected by XRD after rinsing with a combination of two weak acids, acetic acid and formic acid.

**Figure 5-1.** Magnesiothermal reduction is optimized with diatomaceous earth as a model organism, varying A. the acid used to rinse the product, B. the reduction temperature, C. reduction time at 650 °C, and D. the mass of magnesium for a 10 mg silica sample at 650 °C. Conversion of amorphous silica to nanocrystalline silicon indicates a successful reaction, and complete removal of MgO yields a clean product.

**Figure 5-2** shows the morphology of DE before reduction, and of reduced DE under optimized conditions. Before and after MGTR, it consists of a mixture of diatom shapes. As indicated on the images, the size of the DE features decreased by almost two times. The diameter of cylindrical diatomaceous earth changed from 10-15 μm to 6-7 μm. In addition, a drastic change in color from beige-white to dark brown-black occurred, indicating the presence of nano-sized silicon after reduction.
Figure 5-2. Upon magnesiothermal reduction, diatomaceous earth features shrink, but their shape is preserved. SEM and optical images of DE before (top) and after (bottom) reduction.

For a 3 h magnesiothermal reduction at 725 °C, the surface composition and crystallinity of DE were characterized by XPS and XRD, as shown in Figure 5-3. While only silica can be observed on the surface of the sample before reduction, silicon can be detected in the final product (XPS Si2p peak around 98 to 99 eV). XRD also indicates the presence of amorphous silica in DE before reduction, and shows peaks characteristic of nanocrystalline silicon after reduction. The additional peaks observed before reduction are attributed to salts and trace clay minerals that are also present in DE.\textsuperscript{39}
5.2.2 Synthesis of M13 Bacteriophage-Templated Silica and Silicon Nanoporous Networks

The magnesiothermal reduction process was then applied to biomineralized silica using M13 bacteriophage as a template. Silica nanoparticles were nucleated onto M13 bacteriophages in solution using TMOS as a precursor, and the reaction proceeded as shown in Equation 5-4. Dimethylsulfoxide (DMSO) was selected as a solvent in order to prevent denaturation of the bacteriophage template.

\[
\text{Si(OCH}_3)_4 + 2 \text{H}_2\text{O} \rightarrow \text{SiO}_2 + 4 \text{CH}_3\text{OH}
\]

Equation 5-4

As shown in Figure 5-4, the reaction time was optimized. After only a few minutes of incubation in TMOS, almost no silica structures could be observed, but after a 3 h reaction, interconnected bacteriophage-templated silica could be clearly observed. The high aspect ratio structures formed after 3 h exhibit features resembling entangled nanowires, with a nanowire diameter in the order of 10 to 15 nm. However, after 5 h in TMOS, the thickness of these features increased significantly to form wires with a diameter close to 100 nm. A 3 h reaction time was therefore selected.
Figure 5-4. The morphology of M13 bacteriophage-templated silica nanowires changes drastically with TMOS hydrolysis time. TEM images of the reaction product after 30 min, 3 h and 5 h.

As a control, the same reaction was executed, but without the presence of any biotemplate. As shown in Figure 5-5, the result without biotemplate is simply a silica gel without an interconnected nanowire morphology.

Figure 5-5. Silica nanoparticles synthesized without biotemplate do not exhibit an interconnected morphology. TEM image showing a silica nanoparticle gel synthesized with TMOS for 3 h.

Figure 5-6 shows the change in morphology and color of the bacteriophage-based nanoporous networks upon magnesiothermal reduction. After drying the silica product, annealing the structure and burning off the bacteriophage template at 500 °C in air, a white powder was obtained. The right panel of Figure 5-6 shows the product after magnesiothermal reduction and acid rinse. This product exhibits a high porosity derived from the silica nanowire network, but its macroscopic color changed from white to dark brown/black, indicative of the conversion of silica to silicon.
Figure 5-6. Silica and silicon interconnected nanoporous networks are produced with the M13 bacteriophage as a template in solution. TEM and optical images of M13 bacteriophage-templated silica (left) and reduced silicon (right) nanowires synthesized in solution.

Silica can also be nucleated onto a pre-assembled biotemplated network. For instance, M13 bacteriophages assembled into a porous thin film using a covalent layer-by-layer assembly process\textsuperscript{31} can be fully infiltrated with silicon tetrachloride as a precursor for the synthesis of silica. By immersing the bacteriophage thin film in an aqueous silicon tetrachloride solution, the precursor hydrolyzes and silica nucleates specifically onto the thin film (Equation 5-5).

\[ \text{SiCl}_4 + 2 \text{H}_2\text{O} \rightarrow \text{SiO}_2 + 4 \text{HCl} \quad \text{Equation 5-5} \]

The morphology of bacteriophage-templated silica thin films was optimized until a porous structure could be achieved. As shown in Figure 5-7, at low concentration, the bacteriophage thin film is not fully coated with silica, and only a thin non-uniform layer of silica is obtained after burning off the bacteriophages. As the concentration increases, the silica structure becomes more porous, and nanowire-like structures can be identified. The optimal concentration for the biomineralization of silica using silicon tetrachloride was found to be 200 mM.
The morphology of bacteriophage-templated silica films varies as a function of the concentration of precursor. SEM images for silicon tetrachloride concentrations ranging from 10 to 200 mM for a 1 h biomineralization, after annealing at 450 °C.

Figure 5-7. The morphology of bacteriophage-templated silica films varies as a function of the concentration of precursor. SEM images for silicon tetrachloride concentrations ranging from 10 to 200 mM for a 1 h biomineralization, after annealing at 450 °C.

Figure 5-8 (left) shows the structure of the resulting bacteriophage-templated silica film after burning off the bacteriophage template. Similarly, this thin film was magnesiothermally reduced, and its final surface morphology is shown at the right of Figure 5-8. A well-defined network of silicon nanowires is then created uniformly onto the substrate, which exhibits a dark color after reduction. This method provides a means to uniformly coat large surfaces with porous silicon.
Figure 5-8. Silica and silicon nanoporous thin films can be formed using layer-by-layer assembled M13 bacteriophage scaffolds as templates. SEM and optical images of silica (left) and reduced silicon (right) nanoporous networks templated by an LbL-assembled M13 bacteriophage thin film.

Bacteriophage-templated silica and silicon powders were characterized via XPS and XRD (Figure 5-9). XPS shows that the surface content of silica decreases upon reduction and that silicon is also present (Si2p peak showing the presence of silicon around 99.5 eV). From XRD measurements, it is determined that bacteriophage-templated silica was amorphous, and that it is converted to nanocrystalline silicon after magnesiothermal reduction. Figure 5-9B clearly shows a broad amorphous shoulder indicative of amorphous silica only before reduction, and the appearance of sharp peaks characteristic of nanocrystalline silicon after MGTR.

Figure 5-9. M13 bacteriophage-templated amorphous silica nanowires are converted to nanocrystalline porous networks of silicon. A. XPS spectra showing the Si2p region and B. XRD spectra, for M13 bacteriophage-based silica and silicon nanowires synthesized in solution before and after reduction.
5.2.3 Synthesis of *Spirulina*-Templated Silica and Silicon Micro-Coils

*Spirulina major* was used as a third biotemplate to produce silica and silicon structures. After at least 10 days of growth, the algae could be harvested as biofilms, and observed under optical microscope to reveal loose coil structures (see **Figure 5-10A**). The diameter of the *Spirulina* coils ranges between 2.5 and 3 µm, and a turn spans ~ 4.5 µm. Conveniently, *Spirulina* biofilms exhibit a mechanical resistance that allows them to stay intact when removed from the growth medium, and to maintain their shape even after silica nucleation. To nucleate silica on *Spirulina* biofilms, a silane with an isocyanate functional group was used to first nucleate silane molecules onto the polysaccharide layer that is known to surround each *Spirulina* algae (see Figure 5-10B, showing a TEM image showing *Spirulina* with its native polysaccharide layer). This takes place because the hydroxyl groups that are present on the surface of *Spirulina* can react with the isocyanate functionalized silane molecules. Then, TMOS was used to synthesize a thicker layer of silica along the algae. **Figure 5-11** shows a *Spirulina* biofilm lyophilized after silica biomineralization. Spiral-like entangled structures can be observed by SEM.

**Figure 5-10.** *Spirulina major* grows in biofilms, creating entangled networks of spirals with outer polysaccharide layers. A. *Spirulina major* biofilm, as grown. B. TEM image of *Spirulina major* with its polysaccharide outer layer.
Figure 5-11. Once mineralized with silica, the *Spirulina* biofilms maintain their integrity. Lyophilized silica-coated *Spirulina* biofilm. Optical image (left), and SEM (right).

To remove the *Spirulina* template, the silica-coated biofilm was oxygen plasma treated for 1 h (Figure 5-12 shows that plasma treatment removes a significant fraction of the carbon prior to annealing), and then annealed in air at 500 °C for 12 h.

Figure 5-12. The concentration of carbon within Spirulina-templated silica decreases after plasma treating the silica-coated biofilm for 1 h. XPS Cls spectrum before and after 1 h plasma treatment.

Figure 5-13 shows that the micro-coil shapes are preserved after burning off the template, and that individual spiral structures can be observed. After magnesiothermal reduction, similar silicon structures can be observed. Both for the *Spirulina*-templated silica and silicon, large entanglements of micro-coils are found and result directly from the structure of the *Spirulina* biofilm. Individual coils correspond to free algae that were mineralized with silica, or algae at the edge of the biofilms. SEM with elemental mapping confirmed the presence of silicon co-localizing with the coils observed, as shown in Figure 5-14.
Figure 5-13. Silica and silicon micro-coils are formed using *Spirulina major* as a template. A. *Spirulina major* biofilm, as grown. B. Lyophilized silica-coated Spirulina biofilm. Optical image (left) and SEM image (right). C. Magnesiothermal reduction of *Spirulina*-templated silica. SEM and optical images of *Spirulina*-templated silica after annealing (top), and resulting *Spirulina*-templated silicon (bottom).
Figure 5-14. Elemental mapping shows co-localization of silicon with Spirulina-templated coils. SEM image with elemental mapping of silicon (in red) for Spirulina-templated silicon deposited onto a titanium foil substrate.

In addition to the change in color of the Spirulina-templated biofilms observed before and after reduction (see Figure 5-15), XPS confirmed the presence of additional silicon chemistries after reduction, including a peak corresponding to metalloid silicon (around 99.5 eV). A single silica peak was measured before reduction, while fitting indicates that silicon is present in addition to surface oxides in the sample after reduction (Figure 5-15A). As shown in Figure 5-15B, XRD also confirmed the transformation of amorphous silica into bulk nanocrystalline silicon after reduction.

Figure 5-15. Spirulina-templated amorphous silica is converted to nanocrystalline silicon upon magnesiothermal reduction. A. XPS spectra showing the Si2p region, and B. XRD spectra for Spirulina-templated silica and silicon micro-coils.
5.2.4 Effect of Acid Rinse on Composition of Biotemplated Silicon Materials

The acid rinse step used to purify the final silicon products was studied in details. First, the disappearance of any remaining magnesium oxide was confirmed via XPS, and corroborates the XRD spectrum after HCl rinse presented in Figure 5-1. XPS also confirmed that the silicon product is covered with MgO before acid etch, and that after etching, the metalloid silicon chemistry is still visible in addition to surface oxides. Figure 5-16A shows the complete disappearance of MgO from biotemplated silicon samples and the detection of silicon before and after magnesiothermal reduction of silicon templated onto layer-by-layer assembled bacteriophage thin films. The peak observed around 50.2 eV, indicative of the presence of MgO after magnesiothermal reduction, disappears upon rinsing with HCl. In addition, Figure 5-16B shows that, before acid etching, the silicon signal was weak, but it becomes apparent that the sample contains bulk metalloid silicon after rinsing with acid and uncovering the silicon surface.

![Figure 5-16A](image)

**Figure 5-16.** After rinsing with hydrochloric acid, magnesium oxide is completely cleaned off of the silicon product, uncovering the surface of reduced biotemplated silicon. XPS spectra of the A. Mg2p and B. Si2p regions for silicon templated onto LbL assembled M13 bacteriophage thin films, before and after rinsing with acid. Solid lines indicate the raw data, and dotted lines show fitted data, using Casa XPS.

5.2.5 Crystallite Size Calculation

The crystallite size in silicon materials can be of importance in terms of concentration of surface defects and interfaces within the material, and also for determining the properties of nanoscale silicon materials. The particle size of silicon in each of the samples was therefore calculated from the XRD spectra presented above, using the Scherrer equation, as follow.
\[ L = \frac{k\lambda}{\beta \cos \theta} \]

Equation 5-6

where \( k \) is a dimensionless shape factor corresponding to 0.94 for cubic crystals, \( \lambda \) is the X-ray wavelength (0.154056 nm for Cu k-alpha radiation), \( \beta \) is the line broadening at the half maximum intensity, in radians, and \( \theta \) is the Bragg angle.

The results are shown in Table 5-1. Overall, the particle size ranges between \( \sim 10 \) and 25 nm. Silicon templated using DE and the M13 bacteriophage have similar crystallite size, while silicon templated with *Spirulina major* has significantly larger particle size. This can be explained by the longer silicon nucleation performed on the *Spirulina* templates. Because the features of *Spirulina major* are much larger than that of the M13 bacteriophage, a longer TMOS hydrolysis and condensation is necessary to fully cover the surface of the algae. Consequently, the silica and silicon particle size also increases since the reaction proceeds for longer.

### Table 5-1. The crystallite size for biotemplated silicon was calculated from XRD spectra using the Scherrer equation.

<table>
<thead>
<tr>
<th>Biotemplate</th>
<th>Crystallite size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatomaceous earth</td>
<td>11.9 ± 2.9</td>
</tr>
<tr>
<td>M13 bacteriophage</td>
<td>13.3 ± 1.3</td>
</tr>
<tr>
<td><em>Spirulina major</em></td>
<td>23.9 ± 2.9</td>
</tr>
</tbody>
</table>

#### 5.2.6 Determination of Surface Area of Biotemplated Silica and Silicon Materials

Quantifying the surface area is of great importance for using biotemplated silicon in photovoltaic, electronic or electrochemical devices. Large surface area materials are often desired to create large contact areas between two materials that may lead to increased reaction rates, or more efficient transport phenomena. On the other hand, a lower surface area might be desirable to reduce recombination rates in photoactive devices. We therefore assessed the surface area of our materials before and after magnesiothermal reduction. To do so, our syntheses were scaled-up to produce sufficient amounts of materials for BET analysis. For diatomaceous earth before and after reduction, and for silica nanoparticles as control, BET with nitrogen adsorption could be performed because sufficient surface area was available for analysis. However, for *Spirulina*-templated and bacteriophage-templated silica and silicon
powders, only 10 to 20 milligrams of materials could be obtained from scaled-up syntheses, providing only enough surface area for BET analysis with krypton adsorption.

First, compared with a silica nanoparticle control synthesized without biotemplate, the surface area of all biotemplated silica samples is lower (See Figure 5-17 and Table 5-2). This difference results from the higher order organization of the nanoparticles along the biotemplates, to create high aspect ratio structures with reduced exposed surface area. In addition, BET measurements on control silica nanoparticles revealed that the 500 °C annealing treatment causes a slight decrease in exposed surface area due to the sintering of silica nanoparticles.

**Figure 5-17.** Nanoparticle control demonstrates that the surface area of silica before reduction decreases after annealing the structure. BET adsorption and desorption isotherms for silica nanoparticles as synthesized, and after a 500 °C annealing step.

Second, the surface area of all biotemplated samples was compared before and after MGTR. Isotherms for the measurements are shown in Figure 5-18. The BET surface area data presented in Table 5-2 shows that, for all biotemplates, the surface area significantly increases upon reduction. This increase is due to the loss of oxygen atoms from the materials, as well as the change in crystallinity. M13 bacteriophage-templated nanoporous silicon exhibits the largest surface area of approximately 271 m²/g. Conversely, *Spirulina*-templated silica and silicon have a smaller surface area compared to their diatom or bacteriophage-templated counterparts, which is consistent with the larger length scales of the *Spirulina* templates.
Figure 5-18. Biotemplated silica and silicon materials exhibit a higher surface area after magnesiothermal reduction. BET adsorption and desorption isotherms for the data presented in Table 5-2 are shown. For a small amount of material, krypton was used as the adsorbing gas to replace nitrogen.

Table 5-2. BET surface area (m²/g) of biotemplated materials increases when silica is reduced to silicon. The data was calculated from the isotherms presented in Figure 5-18.

<table>
<thead>
<tr>
<th>Biotemplate</th>
<th>Silica</th>
<th>Silicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatomaceous earth</td>
<td>64</td>
<td>188</td>
</tr>
<tr>
<td>M13 bacteriophage</td>
<td>51</td>
<td>271</td>
</tr>
<tr>
<td><em>Spirulina major</em></td>
<td>19</td>
<td>43</td>
</tr>
</tbody>
</table>
5.2.5 Composition and Doping in Biotemplated Silicon Materials

Although the conversion of silica to silicon is complete upon magnesiothermal reduction, other trace elements are found in the final biotemplated silicon materials, and their role needs to be understood in order to apply the materials to energy-relevant devices. In DE-based silicon, the main trace element found is aluminum, at an atomic concentration ranging between 0.2 and 1 % (Figure 5-19 left panel). The presence of Al is not surprising since DE is composed primarily of silica, but can also contain some clay minerals such as alumina. Upon magnesiothermal reduction, the alumina present in DE is reduced to Al, which could act as a p-type dopant in the final product. On the contrary, phosphorous is present in the final bacteriophage-templated nanoporous silicon, at an atomic concentration of 0.4 to 2 % (Figure 5-19, middle panel). This P content likely originates from the DNA of the bacteriophage, and from the buffer solution chosen to suspend the bacteriophages. Nonetheless, the presence of P in silicon nanowires is promising for applications as n-type semiconductors. In *Spirulina*-templated silicon, magnesium is observed at an atomic concentration of ~ 0.3 % (Figure 5-19 right panel). The presence of Mg is due to the chlorophyll pigment found in this algae, which contains an Mg center. Even after acid rinsing the magnesiothermally reduced *Spirulina*-templated silicon materials, Mg is still present, indicating that it must be trapped within the silicon structure. Such Mg-doped silicon spirals could be used as n-type semiconductors.41

![Figure 5-19](image)

**Figure 5-19.** Biotemplated silicon products contain various trace elements that could act as p-type or n-type dopants. XPS survey scans for silicon materials templated with diatomaceous earth, the M13 bacteriophage or *Spirulina major* algae.

In addition to phosphorous, a high sodium content was initially found in the bacteriophage-based nanowires if no water rinse was performed after synthesis. High sodium concentrations are due to the highly concentrated polyethylene glycol/sodium chloride solutions.
used to precipitate the bacteriophages during purification steps prior to biomineralization. Figure 5-20A shows the differences in sodium to silicon atomic ratio when rinse steps are added after drying the silica-mineralized bacteriophages, or after annealing the product to burn off the template. As more rinse steps are performed the content of Na decreases and can be reduced to practically zero. Therefore, although large amounts of Na are initially present in the phage-templated structures, rinses allow us to control final Na concentration.

Rinsing silicon templated onto LbL-assembled bacteriophage films also allows for tuning the concentration of phosphorous in the nanoporous silicon thin films from approximately 1 to 2.5 %, as shown in Figure 5-20B. If the bacteriophage films are taken directly after LbL assembly, they can contain as much as ~ 3 % phosphorous, due to the phosphate buffer used during LbL assembly. However, when the films are thoroughly rinsed with water after assembly, the phosphorous content is decreased to ~ 1%. Adding dopants, such as phosphoric acid during the silica mineralization, also allows for modifying the phosphorous content in the final silicon film.

The 1 % P content observed after rinsing the LbL films is consistent with the theoretical maximum amount of phosphorous that could dope silicon directly from the bacteriophage DNA. Considering the crystalline density of cubic crystals of silicon, one can determine that 8 Si atoms compose one unit cell, with a known cell volume of (0.543 nm)$^3$. This corresponds to a silicon atom density of 5e$^{22}$ Si atoms/cm$^3$. Then, the volume of a bacteriophage-based silicon nanowire can be approximated as a cylinder of 880 nm in length, and the total number of silicon found in such a wire can be easily calculated. The phosphorous concentration deriving from the bacteriophage DNA is thus simply the ratio of the 7222 P atoms found in the 7222 bases that constitutes the single-stranded DNA genome of a bacteriophage, over the number of silicon atoms in a phage-based silicon wire. Depending on the assumptions made regarding the geometry of the nanowires (if the nanowires are hollow or filled with silicon, and if their diameter corresponds to the diameter of bacteriophage or to a thicker silica-coated bacteriophage), the phosphorous content due to DNA in a bacteriophage-templated silicon nanowire reaches 0.2 to 1 %. This percentage thus refers to the minimum phosphorous content in bacteriophage-templated silicon, and is equivalent to a dopant concentration of 1e$^{21}$ to 5e$^{21}$ P atoms/cm$^3$, resulting in heavily n-type doped materials.
Other trace elements could be introduced on-demand in the various biotemplated silicon materials by changing the microorganism growth and purification conditions, and the buffer solutions used at different stages of the process. Further experiments could lead to the production of useful biotemplated semiconductors and the precise quantification and tuning of the resulting n-type or p-type carrier concentrations.

5.2.6 Synthesis of Biotemplated Silicon Nitride and Silicon Carbide

In addition to tuning the composition of the final biotemplated silicon products with dopants, the nature of the products can be modified by the process conditions. More specifically, carbides and nitrides can be synthesized instead of metals if biotemplated oxides are magnesiothermally reduced without burning off the organic template, or if the MGTR is carried
out under a nitrogen environment, respectively. **Scheme 5-2** summarizes the materials that have been synthesize so far by varying process conditions. For all the data presented in the above section, the first process flow was followed; (1) the biotemplate was burnt off at high temperatures or using a combination of plasma treatment and high temperature annealing, (2) inert argon gas was flowed through the MGTR reactor, and (3) a metal or metalloid was obtained. If this process flow is modified by leaving the biotemplate in, the large amount of carbon present during the MGTR will cause the appearance of carbides. In the case of silica, the expected product will be silicon carbide. Alternatively, if the biotemplate is burnt off, but nitrogen is flowed through the reactor instead of argon, then nitrides can be formed. After reducing silica, silicon nitride is the expected product.

**Scheme 5-2.** Three different process routes lead to different products after magnesiothermal reduction: metals, carbides or nitrides.

**Figure 5-21** shows a XPS analysis for LbL-assembled M13 bacteriophage film coated with silica, and subsequently magnesiothermally reduced under nitrogen, without burning off the bacteriophage scaffold. As expected, both silicon nitride (Si2p peak around 101.8 eV) and silicon carbide (Si2p peak around 100.2 eV) are found in the final product. Significant quantities of carbon and nitrogen were detected in the final product, with a ratio of Si:C of approximately 1:2, and Si:N of 14:1.
Figure 5-21. Magnesiothermal reduction of biotemplate silica under nitrogen and without burning off the biotemplate results in the synthesis of silicon carbide and silicon nitride. XPS spectra of A. the Si2p region before reduction, after MGTR and after acid rinse, and B. the N1s, and C1s regions for the final product after MGTR and acid rinse. The biotemplate used here was a LbL assembled M13 bacteriophage thin film.

Silicon carbide and silicon nitride materials could also be applied to useful devices. For instance, porous silicon carbide could be of interest for the fabrication of temperature-resistant membranes due to the high thermal shock resistance of this material combined with its remarkable mechanical properties, or for use as a thermoelectric material due to its high thermal conductivity.\textsuperscript{45,46} Silicon nitride also has attractive properties for a variety of applications. For example, it can act as antireflective coatings in photovoltaic devices,\textsuperscript{47} and has also been studied for incorporation in waveguides.\textsuperscript{48} Combining silicon nitride and silicon carbide materials has also attracted interest for taking advantage of the properties of both materials and improving mechanical and dielectric properties of the product.\textsuperscript{49}
5.3 Conclusions

In conclusion, we have demonstrated that combining a biotemplated approach to nucleate silica nanoparticles along with magnesiothermal reduction represents a versatile process to create silicon nano- and microstructures. Complex shapes such as coils and entangled nanowires can be produced with this method. Individual microorganisms can serve as templates for discrete shapes, or they can be assembled into thin films to create three-dimensional net-works. As-grown biofilms can even be used directly as templates, like the *Spirulina major* biofilm, or cross-linked microorganisms that can be synthetically drawn into higher order structures, like M13 bacteriophage porous thin films.

We have shown that amorphous silica is first nucleated onto the biotemplates via hydrolysis reactions, and that the template can be removed with heat treatments. After magnesiothermal reduction, the remaining silica is converted into nanocrystalline silicon, while preserving the original shape of the template. During the reduction process, the size of the features decreases, and as a consequence the specific surface area of the material increases. The process can therefore be used to synthesize high-surface-area bio-templated silicon building blocks.

In addition, our investigations of the elemental composition of the final silicon products reveal that biotemplates can serve as a sources of dopants to produce potentially semiconducting materials. Changing the reaction conditions would also allow for modifying the composition of the biotemplated silicon products.

While we have demonstrated this process with diatomaceous earth, the M13 bacteriophage, and *Spirulina major*, the concept could be extended to a broader range of microorganisms. Different oxide nanoparticles could also be synthesized on the biotemplates, and subsequently reduced to produce metallic structures with different compositions, including pure metals, doped semiconductors, and alloys.

5.4 Materials and Methods

**Materials:** Tetramethyloxysilicate (98%) (TMOS), silicon tetrachloride, ammonium hydroxide solution, dimethyl sulfoxide (DMSO), and magnesium powder were purchased from Sigma (St-
Louis, MO). 3-isocyanatopropyltrimethoxysilane was purchased from Gelest (Morrisville, PA). Diatomaceous earth powder was purchased from DiatomaceousEarth.com (Lewisville, ID).

The E3 M13 bacteriophage variant was amplified by infecting exponentially growing Escherichia coli bacteria, and was separated and purified via centrifugation. DNA sequencing confirmed that the SFAAEEEDPAK peptide was displayed on each pVIII protein.

A starter culture of Spirulina major Alga-Gro® Seawater Medium were purchased from Carolina Biological Supply Company (Burlington, NC). 8 mL of Spirulina culture was added to 200 mL of growth medium in a sterile flask, and grown for at least 10 days without agitation, alternating between 12 hours in light and 12 hours in the dark.

**Bacteriophage-templated silica nanoporous network synthesis:** 100 μL of NH₄OH solution was added to 5.6 mL of DMSO and thoroughly mixed. On ice, 8e14 pfu of bacteriophage dissolved in 1.5 mL of water was added to the mixture. Ice was used to cool down the heat of mixing that was produced. The DMSO/NH₄OH/phage mixture was then added to 50 μL of TMOS in a separate vial, which was left at room temperature for 3 hours. The resulting entangled silica nanowires were precipitated by adding ethanol and toluene, and centrifuged down at 3000 rpm for 10 min. After further rinsing with ethanol, the silica nanowire pellet was dried in a vacuum oven at 80 °C over-night. To remove residual salts, the dried phage-templated silica was rinsed with milli-Q water, collected by centrifugation and dried again, twice. The bacteriophages were burnt off and the silica annealed in a tube furnace in air at 500 °C for 12 hours. Two more rinses with water, centrifugation and drying steps were performed to clean the nanoporous silica.

**Porous bacteriophage-based silica thin film synthesis:** Nanoporous bacteriophage thin films were assembled via covalent layer-by-layer, as described previously. The films were rinsed with milli-Q water. A 0.2 M SiCl₄ solution was prepared by slowly pouring concentrated SiCl₄ on iced water, with agitation and allowing the ice to melt to form a solution. The bacteriophage films were immersed in this solution and incubated for 1 h at 80 °C. The silica-coated bacteriophage films were then rinsed with milli-Q water, blow-dried with nitrogen and annealed in air at 500 °C for 12 hours.
**Spirulina-templated micro-coil synthesis:** A section of *Spirulina* biofilm was taken directly from the growth flask and transferred into milli-Q water to rinse off minerals and salts. The rinsed film was deposited on a non-stick Teflon-lined surface and rinsed several times with acetonitrile. The film was then incubated on this surface for an hour in a 20 vol% 3-isocyanatopropyltrimethoxysilane solution in acetonitrile, and then rinsed with acetonitrile. An overnight incubation in a solution containing TMOS and ammonium hydroxide (NH₄OH) in DMSO followed, and the silica-coated biofilm was finally rinsed several times with DMSO and dried via lyophilization. To remove carbon and burn off the biotemplate, the silica-coated *Spirulina* film was exposed to oxygen plasma for 1 hour, and annealed at 500 °C for 12 hours in air in a tube furnace. Final rinses with milli-Q water and drying cycles were performed to clean the micro-coils.

**Magnesiothermal reduction:** A known mass of silica powder was placed in a stainless steel boat, along with magnesium powder in an adjacent boat (in general 10 mg of silica and 10 mg of magnesium), and pushed to the middle of a (1 inch in diameter by 10 inches in length) tubular reactor. Alternatively, a ~1 cm² thin film of silicon was placed in the reactor along with 2 mg magnesium. Argon was flowed through the reactor at a rate of 100 sccm, and the temperature was elevated to 725 °C for 3 hours. After cooling down, the silicon product was removed from the reactor and residual MgO and Mg₂Si by-products were dissolved by immersing the sample in 1 M hydrochloric acid for 12 hours. The purified silicon product was thoroughly rinsed in milli-Q water, and dried in air.

**Nanomaterial characterization:** The morphology of the nanomaterials before and after magnesiothermal reduction was characterized using transmission electron microscopy (TEM) with a JEOL 2010 FEG Analytical Electron Microscope and scanning electron microscopy (SEM) with a Helios Nanolab 600 Dual Beam Focused Ion Beam Milling System. *Spirulina*-based materials were also observed by optical microscopy using a Leica LEITZ DMRX light microscope with a Nikon digital camera DXM1200F. The surface composition and chemistry of the samples was analyzed by X-ray photoelectron spectroscopy (XPS) with a PHI Versa-Probe II X-ray photoelectron spectrometer with a scanning monochromated Al source (1,486.6 eV; 50 W; spot size, 200 μm). The XPS data was fitted using the Casa XPS software. X-ray diffraction (XRD) was used to evaluate the crystallinity of the samples, using a PANalytical Multipurpose
Diffractometer and zero background holders. The surface area of the materials was measured by the Brunauer, Emmett and Teller (BET) technique, using an ASAP 2020 BET and monitoring nitrogen or krypton gas adsorption and desorption.

5.5 References


Chapter 6:
Towards Biologically-Templated and Solution-Processed Silicon Semiconducting Coatings and Devices
Chapter 6. Towards Biologically-Templated and Solution-Processed Silicon Semiconducting Coatings and Devices

The aim of this chapter is to demonstrate that silicon, and by extension other metals, can be coated onto various substrates from solution processes, and that these coatings may be used as semiconductors, isothermal conductors, photoactive materials, etc. Materials characterization is performed on biologically-templated or solution-processed silicon coatings and powders to determine their electrochemical, electrical and thermal properties. Preliminary device results are also reported, where the silicon materials synthesized via magnesiothermal reduction are used as a photoactive layer.

Photoluminescence measurements were performed with Mark Weidman, in Professor William Tisdale’s laboratory. Ultraviolet photoelectron spectroscopy measurements were carried out by Patrick Brown in the Organic and Nanostructured Electronics Laboratory. Thermal conductivity measurements were carried out by Lingping Zheng, in Professor Gang Chen’s laboratory.

6.1 Introduction

Several metals are used in nano-devices and are molded, etched or mechanically deformed to produce nano or micro-structures because they cannot be synthesized as nanoparticles in solution. In fact, due to their negative reduction potential, metals like silicon, titanium, tungsten, aluminum and niobium tend to readily oxidize in solution. However, oxides of these metals can be synthesized from precursors in solution, in the form of nanoparticle sol-gels or particles nucleated on templates. Magnesiothermal reduction (MGTR), which can be used to reduce metal oxides to metals while simultaneously oxidizing magnesium metal to magnesium oxide, could therefore be applied to a variety of solution-processed metal oxide coatings to produce metallic coatings on planar, curved or rough surfaces.

Interest in producing solution-processed metallic and semiconducting coatings is growing. For instance, a liquid silane precursor has been recently used to produce amorphous silicon coatings with high mobilities for applications as transistors\(^1\), or as active layers in solar cells.\(^2\) Nanocrystalline silicon crystal inks have also been produced using plasma-based syntheses,\(^3\) and were then assembled into thin films.\(^4\) However, these processes require inert gas
environment during the synthesis, highly pure silane precursors, as well as the use of vacuum systems and radiofrequencies. Magnesiothermal reduction could eliminate the use of vacuum systems, and dangerous or pure precursors such as silane gases. All metals synthesized with MGTR can be first processed under ambient conditions, in air, to form oxides, and then reduced to metals in a one-step MGTR reaction. Sintered nanoparticles or three-dimensional silica structures can also be reduced while preserving their morphology.

Magnesiothermally-prepared silicon semiconductors should exhibit a bandgap similar to traditional crystalline silicon wafers, and should allow for the incorporation of dopants that could change its properties from an n-type to a p-type semiconductor. These properties may vary between silicon nanoparticles synthesized from sol-gel mixtures, and biologically-templated silicon structures that use microorganisms as templates to create three-dimensional structures, as described in Chapter 5.

Here, we demonstrate that uniform silicon coatings can be prepared via solution-processing of silica and subsequent magnesiothermal reduction. We also show that n-type and p-type dopants can be introduced into the coatings, leading to the formation of semiconducting coatings that could be applied to planar or curved surfaces. Next, we characterize the optical, electrochemical, electrical and thermal properties of doped biotemplated silicon building blocks and thin films synthesized in Chapter 5. We also propose photovoltaic device architectures based on solution-processed nanoparticle and biotemplated coatings and demonstrate that biotemplated silicon materials have properties that make them attractive for photovoltaic applications, in addition to potential uses in electronic circuits and as thermally insulating layers.

6.2 Results and Discussion

6.2.1 Coating Planar and Curved Surfaces with Sol-Gel Derived Silicon Thin Films

In order to demonstrate the versatility and simplicity of metal coating production via magnesiothermal reduction, sol-gel derived coatings were synthesized and substrates were coated using dip-coating, spincoating or dropcasting. Silica sol-gels were prepared using tetramethylorthosilicate (TMOS) or tetraethylorthosilicate (TEOS) as precursors with a hydrochloric acid as catalyst. Titanium foil or titanium wires were then coated in one-step, dried in air, and sintered at 500 °C, prior to MGTR.
Both planar and curved surfaces can be conformally coated with a thin layer of silicon using this process, and any metal oxide that can be prepared using sol-gel chemistries could be coated in a substrate and reduced to metal. **Figure 6-1** shows that visible silica and silicon layers can be observed titanium substrates or wires.

![Figure 6-1](image)

**Figure 6-1.** Flat and curved surfaces can be dip-coated with silica and silicon. Optical images showing titanium foil and titanium wires coated with silica via dip-coating and vertical drying in air, and with silicon after magnesiothermal reduction.

Coatings of hundreds of nanometers were obtained and the thickness was varied with the number of dipping repetitions. As an example, the morphology of silica and reduced silicon coatings on a titanium wire is shown in **Figure 6-2**. The bare titanium nanowire has a very rough surface, as illustrated in Figure 6-2A. Apart from some cracking observed after the 500 °C annealing, likely due to differences in thermal expansion coefficients of titanium and silica, uniform coatings were obtained. The surface of the coatings is very smooth prior to MGTR (Figure 6-2C and D), and nanoparticles can be observed more clearly after MGTR (Figure 6-2E and F).
In addition to preparing pure silica sol-gels, dopants were introduced in the reaction mixtures to form n-type and p-type doped silicon coatings. Using phosphoric acid and boric as sources of phosphorous and boron respectively, silicon coatings coating traces of phosphorous and boron were obtained, as shown in Figure 6-3. The precise quantity of phosphorous and boron atoms that were incorporated in the silicon crystals has not been determined yet because
the concentration was too low to be detected by X-ray photoelectron spectroscopy with accuracy. However, different silicon to dopant ratios can be prepared by varying the concentration of boric acid or phosphoric acid in the sol-gel mixture.

![Figure 6-3](image)

**Figure 6-3.** Dopants can be introduced in the sol-gel derived silicon coatings using phosphoric or boric acids. EDX mapping of coatings after magnesiothermal reduction showing the presence of silicon and trace phosphorous or boron in red.

While no complex morphologies can be achieved using sol-gel processing without biotemplates, nanoparticle thin films can be obtained via a processing that is simple and versatile.

### 6.2.2 Electrochemical Characterization of Biotemplated Silicon Thin Films

In order to determine if the magnesiothermally reduced silicon is electrochemically active and to estimate its energy levels, cyclic voltammetry was performed on bacteriophage-templated silicon thin films. These biotemplated films are particularly interesting for energy-related applications because of their highly porous and continuous silicon morphology. **Figure 6-4** compares the cyclic voltammograms of a control n-doped single-crystal silicon wafer with a 100 nm thick bacteriophage-templated silicon film. For both samples, oxidation and reduction peaks can be observed, but the current density recorded is approximately an order of magnitude greater...
for the bacteriophage-templated silicon film, which might be due to its high surface area compared to a planar silicon wafer.

![Cyclic voltammogram](image)

**Figure 6-4.** Oxidation and reduction peaks are observed for bacteriophage-templated silicon thin films via cyclic voltammetry. Cyclic voltammogram measured at a rate of 5 mV/s for a bacteriophage-templated silicon thin film and a bare n-doped silicon wafer. Bacteriophage-templated films were prepared from layer-by-layer assembled phage films on a 0.5” by 0.5” silicon substrate, mineralized with silica for 1 h and reduced via MGTR to produce a 100 nm thick porous silicon film.

The position of the oxidation and reduction peaks, measured here relative to an Ag/AgCl reference electrode, were converted to potentials relative to the vacuum level. The oxidation peak can therefore provide an estimate for the valence band of the silicon, and the reduction peak an estimation of the conduction band,\\(^6\) as follow:

\[
E_V = E^{\text{ox}} + E^{\text{ref}} \tag{6-1}
\]

\[
E_C = E^{\text{red}} + E^{\text{ref}} \tag{6-2}
\]

where \(E_V\) and \(E_C\) are the positions of the valence and conduction bands, respectively, in eV, \(E^{\text{ref}}\) the potential of the reference electrode relative to the vacuum level (-4.4 eV for Ag/AgCl), and \(E^{\text{ox}}\) and \(E^{\text{red}}\) the oxidation and reduction potentials, respectively, measured via cyclic voltammetry.

The measured oxidation and reduction potentials and their corresponding calculated conduction and valence bands are reported in **Table 6-1**. Due to some variation in the position
of the reduction peaks between cycles, a range of values is reported for the conduction band of bacteriophage-templated silicon. In addition, error could be introduced in these measurements due to the fact that the surface of the silicon is likely oxidized, which may modify the electrochemical properties of the material.

Table 6-1. Estimations of the valence band ($E_v$), conduction band ($E_c$) and bandgap ($E_g$) of biotemplated silicon were calculated based on cyclic voltammetry results.

<table>
<thead>
<tr>
<th>Material</th>
<th>$E_{ox}$ (V)</th>
<th>$E_{red}$ (V)</th>
<th>$E_v$ (eV)</th>
<th>$E_c$ (eV)</th>
<th>$E_g$ (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteriophage-templated Si</td>
<td>0.65</td>
<td>0.40 to 0.47</td>
<td>-5.05</td>
<td>-4.00 to -3.93</td>
<td>1.05 to 1.12</td>
</tr>
<tr>
<td>Si wafer, single-crystal</td>
<td>0.61</td>
<td>0.51</td>
<td>-5.01</td>
<td>-3.89</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Based on the cyclic voltammetry measurements, the band gap (difference between the conduction and valence bands) of bacteriophage-templated silicon is approximately 1.1 eV, just like the single crystal silicon wafer. These values correspond very closely to the 1.12 eV bandgap reported in literature for silicon.

6.2.3 Spectroscopic Measurements and Optical Properties

The optical properties of biotemplated silicon powders were investigated via absorption and photoluminescence measurements. The absorption spectra of magnesiothermal reduced diatomaceous earth (DE) and phage-templated silicon nanoporous networks, suspended in water, are shown in Figure 6-5A. A large increase in absorption is observed in the 900-1100 nm range for the phage-templated silicon powder, and a slight peak can also be distinguished for reduced DE at the same wavelengths. These peaks suggest that the biotemplated silicon materials could be semiconducting and would have a bandgap ranging between 1.1 and 1.4 eV, as calculated from the equation below.

$$E_g = \frac{hc}{\lambda}$$  \hspace{1cm} \text{Equation 6-3}

where $E_g$ is the bandgap energy, $h$ is the Planck constant, $c$ is the speed of light, and $\lambda$ is the wavelength.
Figure 6-5. Biotemplated silicon materials exhibit broad absorption and photoluminescence spectra at wavelengths larger than 900 nm. A. Absorption spectra of reduced diatomaceous earth, and bacteriophage-templated silicon. B. Photoluminescence for diatomaceous earth before and after MGTR, and bacteriophage- and *Spirulina*-templated silicon. A 405 nm laser was used to excite the samples.

To further examine the optical properties of biotemplated silicon materials, photoluminescence of magnesiothermally reduced DE, phage-templated silicon, *Spirulina*-templated silicon, DE before MGTR were recorded, using the excitation of a 405 nm laser. For DE before MGTR, no signal was recorded at wavelengths larger than 900 nm, but signals were measured for all magnesiothermally reduced samples. Major peaks were observed in the 950-1100 nm range and seem to have a maximum at shorter wavelength values, and minor peaks were also detected at longer wavelengths. Unfortunately, the major peaks are not well defined because the sensitivity of the detector used decreases dramatically below 1000 nm and large correction factors are applied to the data.

Photoluminescence signals are not expected to be detected for large silicon crystals, but are often reported for quantum confined silicon nanoparticles. In fact, broad emissions with peaks ranging between 600 and 900 nm have been observed, and red shift with increasing silicon nanocrystal sizes. In addition, the photoluminescence of silicon is highly dependent on its surface chemistry. Therefore, although the particle size found in the bulk of biotemplated silicon materials was determined to be around 10 to 20 nm by X-ray diffraction, it is possible that silicon crystals on the order of 5 nm diameter are also present and responsible for the photoluminescence signal observed around 900 nm. The surface of these particles is likely oxidized with silica. Minor peaks at larger wavelengths could be due to defects and trap states.
Measuring photoluminescence of the biotemplated silicon materials in the visible range might help understanding their behavior.

Ultraviolet photoelectron spectroscopy (UPS) provided more insight in the energy levels of the biotemplated silicon thin films. The UPS spectra of two 100nm thick bacteriophage-templated silicon thin films was compared to an n-type single-crystal silicon wafer, and the results are shown in Figure 6-6 and Table 6-2. By performing a linear extrapolation of the secondary electron cutoff region with the binding energy axis (left panel of Figure 6-6, at high binding energies), the Fermi level can be determined, and similarly a linear interpolation of the primary electron cutoff region (right panel of Figure 6-6, at low binding energies) allows for determining the valence band. The Fermi levels were successfully estimated for all samples, but only the silicon wafer spectra exhibited a clear linear shape in the low-binding energy region. Therefore, valence band estimates could not be calculated for the phage-templated silicon thin films. The absence of the linear region in the phage-templated samples could be due to sample roughness, porosity, variable thickness or inhomogeneity. UPS is a technique that is highly sensitive to surface roughness because it depends on the takeoff angle of the emitted electrons. In consequence, surface roughness can lead to the measurement of a range of takeoff angles and to the broadening of the primary electron cutoff region.

![Figure 6-6](image)

Figure 6-6. UPS spectra allow for determining the Fermi level and valence bands based on linear interpolations at the intercept of the secondary and primary electron energy cutoffs respectively. The complete spectra are shown in the middle panel, and the detailed shapes of the regions used to determine the Fermi level and valence band are shown in the left and right panels, respectively. $E_{\text{vac}}$ represents the energy of the vacuum, at -21.22 eV, $E_F$ the Fermi level, and $E_V$ the difference in energy between the Fermi level and the valence band.
Table 6-2. Fermi level and valence band can be estimated using ultraviolet photoelectron spectroscopy.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fermi level (± 0.1 eV)</th>
<th>Valence band (± 0.2 eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-doped single-crystal Si wafer</td>
<td>3.91</td>
<td>5.15</td>
</tr>
<tr>
<td>Phage-templated Si film 1</td>
<td>3.90</td>
<td>-</td>
</tr>
<tr>
<td>Phage-templated Si film 2</td>
<td>4.01</td>
<td>-</td>
</tr>
</tbody>
</table>

The Fermi levels of all samples vary between 3.9 and 4.01 eV, which correspond to the Fermi levels of highly doped n-type silicon. This is consistent with the high phosphorous content measured via X-ray photoelectron spectroscopy in bacteriophage-templated silicon. As the n-dopant content increases, the Fermi level moves closer to the expect position of the conduction band. Here, for a bandgap similar to single-crystal silicon, the conduction band is expected to be around 3.9 eV.

Overall, based on electrochemical and spectroscopic measurements, biotemplated silicon thin films and powders seem to consistently show semiconductor properties and have measured bandgaps similar to single-crystal silicon wafers or slightly larger (as determined by the broad absorption peaks). Bandgaps in the 1.1 to 1.4 eV range would be consistent with nanocrystalline silicon since the bandgap of single-crystal silicon is expected to be 1.1 eV and the bandgap of amorphous silicon has been reported to be around 1.7 eV. Intermediate values could be due to nano-size effects or defect states.

6.2.4 Electrical properties

The electrical properties of porous bacteriophage-templated silicon thin films was studied by performing four-point probe measurements. The thin films were constructed in a highly resistive silicon carbide substrate with a resistivity between $10^5$ to $10^7 \Omega \cdot \text{cm}$ (confirmed experimentally by four-point probe measurements). The sheet resistance, as measured in kΩ/□, is reported in Table 6-3 for samples containing different phosphorous dopant concentrations that we quantified by XPS in Chapter 5. From known film thicknesses, sheet resistance can be converted to resistivity, using Equation 6-4.

$$\rho = R_s \times t$$  

Equation 6-4
where $\rho$ is the resistivity in $\Omega \cdot \text{cm}$, $R_s$ is the sheet resistance in $\Omega/$ $\square$ and $t$ the thickness of the film in cm.

The conductivity can also be calculated as follow.

$$\sigma = \frac{1}{{\rho}} \quad \text{Equation 6-5}$$

where $\sigma$ is the electrical conductivity in S/m, and for $\rho$ the resistivity in $\Omega \cdot \text{m}$.

Table 6-3. Electrical conductivity of porous silicon thin films is in the order of 20 to 30 S/m. Sheet resistance measured using a 4-point probe and corresponding resistivity and conductivity for 100 nm and 115 nm thick films (1 h coating and 1.5 h coating respectively). After LbL, the films were rinsed or not in water. For sample 3, H$_3$PO$_4$ was added to a 1:5000 P:Si ratio.

<table>
<thead>
<tr>
<th>Sample</th>
<th>P content (%)</th>
<th>Sheet resistance (k$\Omega/$ $\square$)</th>
<th>Resistivity ($\Omega$/$\text{cm}$)</th>
<th>Conductivity (S/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Water rinsed, 1 h coating</td>
<td>1.2 ± 0.4</td>
<td>320 ± 70</td>
<td>3.2 ± 0.7</td>
<td>32.6 ± 7.1</td>
</tr>
<tr>
<td>2. Water rinsed, 1.5 h coating</td>
<td>1.2 ± 0.4</td>
<td>386 ± 200</td>
<td>4.5 ± 2.4</td>
<td>29.6 ± 17.6</td>
</tr>
<tr>
<td>3. Water rinsed, H$_3$PO$_4$ added, 1 h coating</td>
<td>1.6 ± 0.2</td>
<td>751 ± 310</td>
<td>7.5 ± 3.2</td>
<td>18.0 ± 12.6</td>
</tr>
<tr>
<td>4. No rinse, 1 h coating</td>
<td>2.4 ± 0.4</td>
<td>633 ± 220</td>
<td>6.3 ± 2.2</td>
<td>18.0 ± 6.9</td>
</tr>
</tbody>
</table>

Slightly lower sheet resistances, and higher conductivities, were measured for silicon thin films that we prepared from bacteriophage films thoroughly rinsed with milli-Q water after LbL assembly, prior to silica mineralization. However, a decrease in resistivity is generally expected for silicon as the dopant concentration increases.$^{11,12}$ Large variations were observed between individual measurements, and it is possible that the differences between the various dopant concentrations tested here are not large enough to observe differences in conductivity. These results could also be explained by the fact that sodium salts and other impurities are removed during the rinses in addition to decreasing the phosphorous content in the films. It is also possible that higher phosphorous concentrations do not fully get incorporated in the silicon crystal lattice since these bacteriophage-templated silicon thin films are heavily doped with phosphorous.
The phosphorous contents calculated here correspond to dopant concentrations varying from $6.2 \times 10^0$ to $1.2 \times 10^{21}$ cm$^{-3}$, based on the crystalline density of silicon of $5 \times 10^{22}$ atoms/cm$^3$. For such phosphorous concentrations, the resistivity of silicon would be expected to have values in the order of $10^{-3}$ Ω·cm. The measured resistivity values for bacteriophage-based silicon thin films are three orders of magnitude greater. This result is not surprising because the bacteriophage-based silicon thin films are highly porous and rough, and electrical resistivity is known to increase with increasing porosity and roughness. Our biotemplated silicon also has a high surface area compared to bulk silicon, which increases the surface that is exposed to air and that can be oxidized to form insulating silica. Nonetheless, our measurements indicate that biotemplated silicon thin films do behave like semiconductors.

The electrical properties of silicon nanoparticle and biotemplate silicon powders were also evaluated for powders compressed into compact pellets, but their calculated resistivities were orders of magnitude greater than the resistivities of the thin films reported above. These results could be due to the fact that pellets are not truly interconnected and contain many oxidized surfaces that could increase resistances. In addition, while pressing may be sufficient to form pellets that maintain structural integrity during the measurements, it may not generate a good contact between the particles. Still, these measurements allowed to observe significant differences between doped and undoped silicon nanoparticles, and indicated that doped magnesiothermally reduced silicon is considerably more conductive than undoped silicon. Performing more accurate measurements on biotemplated silicon powder may give insights in how each biotemplate may affect the conductivity of the silicon products.

6.2.5 Thermal Properties

To determine if the biotemplated silicon materials could be used as thermal insulators, the thermal conductivity of bacteriophage-templated silicon thin films was measured. Since the thermal conductivity of silicon is expected to decrease as its porosity increases, bacteriophage-templated silicon with different porosities was prepared by varying the silica mineralization time prior to MGTR. Smooth thin films were required for these measurements, and therefore, the thermal conductivity of silicon powders was not measured.

Figure 6-7 shows the morphology of the silicon films prepared with different mineralization times, and the ImageJ analysis of SEM images used to determine the porosity of
the film cross-sections. As the mineralization time increased from 1 h to 1.5 h and 2 h, the porosity decreased from 29 to 16 and then 13 %. The thickness of the films was also determined based on the SEM images, and the density and heat capacity of the silicon thin films were estimated using volumetric weighted averages based on their porosities. These values, reported in Table 6-4, were used to fit the data obtained from time-domain thermoreflectance (TDTR) measurements and obtain estimates of the thermal conductivity for each sample.

As observed in Figure 6-7E, the thermal conductivities measured for all three samples are low, and vary between 0.7 and 1.1 W/mK. The use of biotemplated silicon thin films as insulators is therefore promising. However, it is difficult to distinguish clear trends in the thermal conductivity data as a function of porosity. As the porosity decreases from 1.5 to 2 h, a slight increase in thermal conductivity is observed, but the highest average thermal conductivity value was calculated for the 1 h sample. This could be due to several factors. First, while the porosity of the sample mineralized for 1 h is the largest, its morphology is also the most continuous and pathways are more direct for heat to travel. It has been observed in literature that high aspect ratio structures tend to have higher thermal conductivities. On the other hand, several sources of error may also explain the highest thermal conductivity of the film mineralized for 1 h and its largest standard deviation between measurements. Because this thin film is highly porous and an aluminum electrode must be evaporated on top of the samples prior to measuring the thermal conductivity, the aluminum might infiltrate some of the pores of the film causing errors in the measurements. In fact, fitting the data for the 1 h sample was most difficult and imprecise. In addition, the samples had a significant roughness and were not perfectly flat, which can also contribute to the variations.

Although no trend can be detect with certainty between the samples, it is clear that porous silicon thin films could be used as insulators. This insulating property combined with their semiconducting properties could lead to their use as isothermal electric materials.
Figure 6-7. Low thermal conductivity values were estimated for porous bacteriophage-templated porous silicon thin films. A. Images of thin film mineralized with silica for various durations, on titanium foil. No film was formed after 0.5 h. B. Cross-sectional morphology of the thin films, observed by SEM. C. Porosity analysis using the samples cross-section and the ImageJ software to identify the pores (in red). D. Porosity estimated with ImageJ as a function of mineralization time. E. Thermal conductivity averaged from four modulation frequencies and three locations on each sample, as a function of mineralization time.
**Table 6-4.** Film thickness, porosity, density and heat capacity were estimated and served for the fitting of thermal conductivity data. Thickness and porosity were determined from SEM images. Density and heat capacity were estimated as a weighted average of the properties of bulk silicon (2.329 g/cm$^3$, 710 J/kg*K) and air (0.001225 g/cm$^3$, 1005 J/kg*K), based on the estimated film porosities.

<table>
<thead>
<tr>
<th>Mineralization time (h)</th>
<th>Thickness (nm)</th>
<th>Porosity (%)</th>
<th>Density (g/cm$^3$)</th>
<th>Heat capacity (J/kg*K)</th>
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<td>1</td>
<td>95</td>
<td>29</td>
<td>1.65</td>
<td>919</td>
</tr>
<tr>
<td>1.5</td>
<td>115</td>
<td>16</td>
<td>1.96</td>
<td>958</td>
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<tr>
<td>2</td>
<td>230</td>
<td>13</td>
<td>2.03</td>
<td>967</td>
</tr>
</tbody>
</table>

### 6.2.6 Design and Fabrication of Hybrid Silicon Photovoltaic Devices

Photovoltaic devices were designed using magnesiothermally reduced silicon layers. Preliminary results were obtained for control silicon-organic hybrid devices, and biotemplated silicon-organic devices, but **Scheme 6-1** presents more device architectures that could be constructed with solution-processed and biotemplated silicon. On the left, a planar control devices; and a nanostructured device containing a bacteriophage-templated silicon layer are shown. These devices are constructed using n-type silicon, and p-type organic layers (spiro-OMeTAD, and PEDOT:PSS can be employed here). Then, on the right, all-silicon device architectures are presented. The organic layer could be replaced with p-type silicon, in the form of a nanoparticle coating, or biotemplated films or foams. Ultimately, self-standing biotemplated silicon films could fully replace silicon wafer substrates.

In all cases, an aluminum electrode is used as the anode, and a silver electrode that is either shaded or transparent can be used as top-electrode. Shaded electrodes have been used before to construct hybrid silicon-organic photovoltaic devices,$^{23}$ and can have a finger conformation, as shown in **Figure 6-8A** and B. In the metal evaporation mask shown in Figure 6-8A, the fingers result in a 20% shading, and the remaining area of each of the four pads constructed on a substrate is 0.1219 cm$^2$.  

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Scheme 6-1. Design of hybrid biotemplated and solution-processed silicon photovoltaic devices. From left to right, a planar hybrid silicon-polymer device, a modified device with a biotemplated coating, an all-silicon device constructed with a sol-gel derived silicon coating, and an all-biotemplated silicon device without substrate or silicon wafer.

Device results are shown in Figure 6-8C and D. Results indicate that etching away the silica layer that forms on silicon surface with hydrofluoric acid (HF) is crucial to prepare functional hybrid silicon solar cells. In Figure 6-8C, a biotemplated device is compared to a planar control, and a large increase in short-circuit current can be observed for the biotemplated device, likely due to the surface texturing. These devices were treated with hydrofluoric acid a few hours prior to spincoating the spiro-OMeTAD layer. However, when comparing two planar control devices that were either not treated with HF, or treated with HF immediately prior to spincoating the organic layer, the shape of the curve changes and the short-circuit current greatly increases when the silicon is treated with HF. The s-shaped curved observed for devices without HF treatment indicate high resistance through the silicon wafer due the resistive surface silica layer. A non-optimized planar control exhibited a power conversion efficiency approaching 1 %, indicating that upon optimization and addition of nanostructures, the performance could be even higher. Decreasing the thickness of the organic layer or modifying its composition might further increase device performance.
Figure 6-8. Planar hybrid silicon devices and biotemplated devices were constructed.
A. Shadow mask for evaporating 4-pad finger electrodes, B. Pictures of a planar control and a biotemplated device with silver finger electrodes. C. JV curves comparing planar and biotemplated devices, D. JV curves comparing planar devices constructed with or without etching the silicon surface with HF prior to spincoating the spiro-OMeTAD layer.

A challenge encountered with the construction of these devices is the reactivity of silicon wafers with acids after magnesiothermal reduction. Magnesium silicide can be a by-product formed during magnesiothermal reduction of silica to silicon, or upon exposure of silicon with magnesium at high temperatures. When silicon wafers are used as substrates, as opposed to titanium foil, the samples react violently with hydrochloric acid after MGTR, and release more silane gas. This is likely because the silicon substrate reacts with magnesium to form significant quantities of magnesium silicide.

The morphology of bacteriophage-templated silicon thin films constructed in silicon wafers was observed after MGTR and acid rinse. The results, shown in Figure 6-9, indicate that the formation of silane gas could be causing cavitation at the surface of the wafer, and disrupting the phage-templated silicon nanostructure while also creating large cavities on the surface of the wafer. Figure 6-9A and B shows that some nanostructure remains, but that larger features are created. In addition, device cross-sections shown that, when using a wafer that has not been
Magnesiothermally reduced, a flat device can be assembled, but that significant roughness is introduced in devices exposed to MGTR.

**Figure 6-9.** Magnesiothermally reduced silicon wafers react with acids forming silane gas and causing cavitation and deformation of the thin film morphology. A and B. Surface morphology of bacteriophage-templated silicon thin films constructed on silicon wafers after magnesiothermal reduction, hydrochloric acid rinse and hydrofluoric acid rinse. C. Cross-sectional images of control devices constructed with bare silicon wafers no exposed to magnesium. D. Device cross-section for a biotemplated devices.

While the structures formed after acid etching have features orders of magnitude larger than the phage-templated silicon films, they might still contribute to increase the directionality of charge transport through the device. In addition, coating polished silicon wafers with biotemplated silicon or sol-gel derived silicon reduce their reflectance to almost zero (see **Figure 6-10**).
Figure 6-10. Biotemplated and sol-gel derived silicon thin films significantly reduce the reflectance of polished silicon wafers. Reflectance measured as a function of wavelength for a bare polished silicon wafer and polished silicon wafers with a phage-templated or sol-gel derived silicon coating. The right panel has an adjusted scale to show the curves of the coated wafers. Discontinuities in the curves correspond to changes of detectors.

If high quality silicon can be produced via magnesiothermal reduction, then the silicon wafer substrate may not be necessary, and could be replaced with a titanium substrate that would also act as the anode, or a free-standing thick sol-gel derived or biotemplated silicon film could be used without substrate. Figure 6-2 shows schematic of devices that could be constructed on titanium wires or titanium foil substrates (the anode).

Scheme 6-2. Solution-processed silicon devices could be constructed directly on flat or wire metal anodes. Device architectures for silicon photovoltaic devices to be assembled on titanium wires, analogous to planar device structures on titanium foil.


6.3 Conclusions

In conclusion, we have demonstrated that solution-processed silicon coatings can be synthesized on planar and curved substrates. In Chapter 5, biotemplated silicon building blocks and thin films were produced, and here nanoparticle coatings were prepared. These results therefore demonstrate that it is possible to prepare uniform metallic coatings via solution processes.

Using biotemplated silicon materials as models, our results suggest that magnesiothermally reduced silicon materials act as semiconductors, based on electrochemical, spectroscopic and electrical measurements. Specifically, UPS measurements indicated that bacteriophage-templated silicon thin films are heavily n-doped semiconductors. While some measurements could only be carried out on silicon powders, and others on silicon thin films due to technical constraints, it would be interesting to complete the measurements sets to obtain a comprehensive data set for the bandgap, Fermi level, electrical and electrochemical properties of these materials, as a function of their dopant concentrations and processing conditions.

In addition, biotemplated silicon thin films have attractive properties for the construction of photovoltaic devices. For instance, they considerably decrease the surface reflectance of polished silicon wafers. Functional hybrid silicon-organic devices were also constructed using magnesiothermally reduced silicon, indicating its potential for use as a photoactive material. Other potential applications for biotemplated and solution-processed silicon thin films include isothermal electric materials, or semiconducting components of electronic circuits.

6.4 Materials and Methods

Materials: N-doped single-crystal silicon wafers purchased from University Wafers (Boston, MA). Tetramethylorthosilicate, tetraethylorthosilicate, 4-tert-butylpyridine, and lithium bis(trifluoromethylsulfonyl) imide were purchased from Sigma (St-Louis, MO). 2,2’,7,7’-tetrakis(N,N-di-p-methoxyphenylamine)-9,9’-spirobifluorene (spiro-OMeTAD) was purchased from Luminescence Technology Corp. (Taiwan). Highly resistivity silicon carbide wafers were purchased from University Wafer (Boston, MA).

Sol-gel silica coatings: Titanium substrates were coated with silica gels by after being plasma treated for 2 minutes at medium intensity. The sol-gels were prepared by first mixing TMOS or
TEOS in ethanol for 15 minutes. The concentration of TMOS or TEOS was adjusted to a ratio of ethanol:precursor of 100:1. A higher ratio (10:1) resulted in fast gelation and the formation of aggregates. A mixture of water and ethanol (adjusted to a ratio of water:precursor of 2:1) was then added dropwise under agitation. A few drops of 37% hydrochloric acid were then added under agitation to adjust the pH of the mixture to 2. The reaction mixtures were aged at room temperature from minutes to a day before coating the substrates.

**Magnesiothermal reduction:** Magnesiothermal reduction was carried out as described in Chapter 5.

**Materials characterization:** The morphology of silicon coatings was observed by scanning electron microscopy (SEM) using a Helios Nanolab 600 Dual Beam Focused Ion Beam Milling System, and elemental composition was performed using the same instrument with EDX.

Electrochemical characterization of the bacteriophage-templated silicon thin film was carried out by cyclic voltammetry with a three-electrode configuration (silicon thin film as working electrode, Ag/AgCl reference electrode, and platinum counter electrode) using 10 mM tetrabutylammonium perchlorate in acetonitrile as electrolyte. Current-voltage curves were recorded from -0.4 to 1.0 V using a potentiostat/galvanostat (Princeton Applied Research Model 263A).

Absorption spectra were measured for silicon powders suspended in water using a Beckman Coulter DU 800 Spectrophotometer. Photoluminescence was measured for an excitation with a 405 nm laser, with a SuperGamut NIR Spectrometer detector (BaySpec Inc.). Ultraviolet photoelectron spectroscopy (UPS) measurements were carried out with an Omicron ultrahigh vacuum system with a base pressure of $10^{-10}$ mbar.

Sheet resistance of the thin films was measured using a RM3-AR four point probe station from Jandel Engineering LTD.

Thermal conductivities were measured using time-domain thermoreflectance (TDTR) at four modulation frequencies (3, 6, 9 and 12 MHz), and the values were averaged for three locations on the silicon films. For this measurement, phage-templated silicon samples with different porosities were prepared using different silica mineralization times, and 100 nm aluminum was evaporated on top of the films.
Photovoltaic device construction and testing: Solar cells were constructed using n-doped single-crystal silicon wafers as substrates. Bacteriophage thin films were layer-by-layer assembled, mineralized with silica and magnesiothermally reduced, as described in Chapter 5. The silicon wafers or silicon wafer with a biotemplated silicon layer were treated with 5% hydrofluoric acid for 2 minutes to remove the intrinsic surface silica. A spiro-OMeTAD solution was prepared in in chlorobenzene. For 1 mL chlorobenzene, 72.3 mg spiro-OMeTAD was dissolved, and 28.8 μL of 4-tert-butylpyridine, and 17.5 μL of a stock solution of 520 mg/mL lithium bis(trifluoromethylsulfonyl) imide in acetonitrile were added. Spiro-OMeTAD was spincoated at 3000 rpm, and a layer of PEDOT:PSS was then spincoated at 6000 rpm. The devices were kept in container with dessicant overnight prior to electrode evaporation, in order to oxidize the spiro-OMeTAD layer, but prevent exposure to water. 100 nm of aluminum was evaporated on the back side of the silicon wafer, and 100 nm silver finger electrodes were evaporated on top of the PEDOT:PSS layer. A solar simulator (150 W Newport 96000 xenon arc-lamp, with AM1.5G filter and diffuser lens) was calibrated to 100 mA/cm² using a silicon reference cell, and J-V curves were obtained by applying an external bias to the devices and measuring the photocurrent, with a Keithley 6487 picoammeter. The devices were illuminated from the front side through the silver fingers.

6.5 References

6. Petrova, P.; Ivanov, P.; Marcheva, Y.; Tomova, R., Estimation of energy levels of new Iridium cyclometalated complexes via cyclic voltammetry. *Bulgarian Chemical Communications* 2013, 45 (SPEC. ISSUE B), 159-164.


Chapter 7: Conclusions and Recommendations
Chapter 7. Conclusions and Recommendations

7.1 Thesis Summary

Overall, this thesis presented the use of M13 bacteriophages, *Spirulina major* algae, and diatomaceous earth as biotemplates to assemble three-dimensional biological scaffolds. These scaffolds served for the nucleation and spatial organization of metal oxide and metal nanoparticles, creating porous materials that can be used in photovoltaic and other energy-related devices. Bacteriophage-templated titania thin films were used to create functional heterojunction solar cells, and biologically-templated doped silicon materials with various shapes were synthesized for use as semiconductors for a variety of applications. Throughout this work, focus was placed on controlling the morphology of materials and synthesizing materials with novel nanostructures. Experiments were carried out to demonstrate that nano- and micro-scale morphology affects materials properties and device performance.

In Chapter 2, several methods for modifying the surface charge of M13 bacteriophages were presented, with the goal of using the modified bacteriophage particles in electrostatic layer-by-layer assembly. We demonstrated that the variable pVIII amino acid sequence directly affects the isoelectric point of the bacteriophages, but as expected from the bacteriophage assembly mechanisms, the bacteriophages are generally negatively charged around neutral pHs. We then showed that small polyamine molecules or synthetic peptides could be covalently attached onto the pVIII proteins to modifying their isoelectric point, and next that larger polymers or dendrimers could be used as a positively charged coating to produce bacteriophages with a positive surface charge over a wide pH range. However, our results indicated that changes in the morphology of the bacteriophage particles occurred when their charge was reversed. For instance, aggregation was often observed, as well as the formation of thicker bacteriophages or large bacteriophage fibers.

In Chapter 3, a covalent layer-by-layer assembly process was developed to form thin porous M13 bacteriophage films. The porosity of the bacteriophage films was measured to be as high as 59 %, and the films exhibited nanowire-like features with the diameter of a single phage. The films were then used as a scaffold to mineralize titania via titanium tetrachloride hydrolysis, and the resulting titania films remained highly porous. To demonstrate that layer-by-layer assembled bacteriophage films could be used to spatially organize nanomaterials, bacteriophages
were also complexed with gold nanoparticles and the complexes were introduced in the films during LbL assembly. We showed that the distribution of nanoparticles within the film could be controlled at the nanoscale, and that the nanoparticles remained dispersed before and after mineralization with titania. Given the high porosity and versatility of the titania-nanoparticle composites that can be formed with this process, they could be used for a wide range of applications including optical sensing, photovoltaics, catalysis, etc.

In Chapter 4, bacteriophage-templated nanostructured heterojunction solar cells were constructed. Using titania-mineralized bacteriophage films infiltrated with lead sulfide quantum dots as a model materials system, we demonstrated that a continuous morphology and dispersed noble metal nanoparticles can increase the photocurrent in heterojunction devices. To create films with different morphologies, the titania mineralization time was varied and it was found to significantly affect the photocurrent. Compared to a randomly organized nanoparticle control, our optimized bacteriophage-based device on average doubled the photocurrent. In addition, the incorporation of various types of nanoparticles within the bacteriophage film further increased the photocurrent by creating localized surface plasmon resonance. Silver nanoplate-bacteriophage complexes were found to increase the performance of the devices the most, resulting in devices with up to 4% power conversion efficiencies, and an overall 36% improvement over devices without plasmonic nanoparticles. The layer-by-layer assembled bacteriophage thin film provided an ideal platform for the creation of nanostructured morphologies that improve charge collection, and for the dispersion of metal nanoparticles that enhance light absorption.

In Chapter 5, M13 bacteriophages, *Spirulina major* algae, and diatomaceous earth were used as templated for the synthesizing of nanostructures silica and silicon materials. Via magnesiothermal reduction, biotemplated silica structures were reduced to metalloid silicon. Diatomaceous earth was first used to optimize the process conditions, and silica nanoparticles were then mineralized on the surface of free floating bacteriophages, layer-by-layer assembled bacteriophage films, and *Spirulina* algae. After reducing the structures to silicon, the shapes of the biotemplates were preserved, forming high aspect ratio, porous or coil-shaped nanocrystalline silicon building blocks. Interestingly, the composition of the biotemplate was affecting the final composition and doping of the silicon materials, potentially allowing for the
direct synthesis of n-doped or p-doped semiconductors. Using the biotemplating and magnesiothermal reduction approaches, it is possible to synthesize high surface area metallic structures with complex and diverse shapes.

Lastly, in Chapter 6, the work on solution-processed metallic materials and coatings was continued and important properties of these materials were characterized. We showed that doped and undoped silicon coatings could be prepared via sol-gel chemistry and magnesiothermal reduction, and could conformally coat planar, curved or rough surfaces. We then investigated the optical, electrochemical, electrical and thermal properties of silicon nanoparticle coatings, biotemplated silicon coatings and biotemplated silicon powders. The results indicated the biotemplated silicon materials exhibit a bandgap similar to single-crystal silicon wafers, and that they could be used as semiconductors in electronic circuits, photovoltaic devices, etc. In addition, with their high porosity and low measured thermal conductivity, bacteriophage-templated silicon thin films can act as thermal insulators. Finally, with further optimization and adequate substrate selection, efficient solution-processed silicon photovoltaic devices could be constructed.

7.2 Recommendations for Future Directions

This thesis work addresses important questions regarding the interaction of morphology and device performance, and presents several novel assembly and biomineralization strategies. Nonetheless, it could still be further deepened by improving processes and device performance, and by continuing the work and developing more nanostructured metal oxides and metals. Some of these aspects were discussed in chapters and appendices, and main recommendations for future work are listed below.

7.2.1 Layer-by-layer assembly of M13 bacteriophages

Electrostatic assembly of M13 bacteriophages was explored in Chapter 2, but it was determined that synthesizing a bacteriophage with a high isoelectric point is challenging without simultaneously modifying the shape of the phage particles. In Chapter 3, covalent layer-by-layer assembly successfully replaced electrostatic layer-by-layer assembly, making use of a carbodiimide cross-linker (EDC). However, the cost of covalent layer-by-layer assembly is high due to the solutions of EDC that must be prepared and refreshed frequently because of the molecule’s degradation over time. Alternative layer-by-layer assembly processes, that would be
based on other types of interactions might be feasible and could reduce the cost of the process. For instance, known protein-protein affinities such as biotin-streptavidin or antigen-antibody could be used. Two different M13 bacteriophages could be engineered to display protein domains that would interact together during layer-by-layer assembly. After completing the initial genetic engineering work, the bacteriophage variants could be used directly, simply by amplifying in *Escherichia coli* and purifying them prior to a layer-by-layer experiment.

### 7.2.2 Bacteriophage film mineralization and use in heterojunction devices

Titania and silica were mineralized successfully onto M13 bacteriophage nanoporous thin films, but other metal oxides or sulfides that could be used as photoactive materials were more challenging to mineralize. In some cases described in Appendix C, the particles were only nucleated on the surface of the films, and pore infiltration was an issue. However, materials like zinc oxide and zinc sulfide can uniformly coat free floating M13 bacteriophage in solution. Therefore, it may be possible to extend the range of materials used in bacteriophage-templated heterojunction solar cells, by using n-type nanostructures formed in solution and creating blends with p-type quantum dots or conjugated polymers to create an interpenetrated active layer.

Apart from diversifying the materials used in solar cells, the performance of the titania-lead sulfide devices presented in Chapter 3 could further be improved. While the optimized system reported here allowed us to study the effects of morphology and plasmonics on device performance, the efficiency of the solar cells could be increased by employing novel processing methods reported in the past few years. For instance, several new ligands for quantum dots have been explored recently and some were found to have a dramatic effect on device performance. Varying quantum dot sizes, ligands, and surface passivation could all play a role in improving the efficiency of titania-lead sulfide heterojunctions, as well as their long term stability.

### 7.2.3 Magnesiothermal reduction of biotemplated metal oxides to metals

Magnesiothermally reduced silicon materials have been characterized in terms on their electrochemical, electrical, optical and thermal properties. However, some measurements that were challenging with powders were not performed, and the same is true with thin films. Fully characterizing the materials as a function of their shape, porosity, and doping concentration would provide a better understanding of the materials properties and how they could be used in devices.
Preliminary results were obtained for hybrid silicon photovoltaic devices, but challenges were encountered with the silicon substrate reacting with magnesium during magnesiothermal reduction. Therefore, selecting other substrates, or preparing hundreds of microns thick biotemplated silicon foams could allow for the construction of devices with nanostructured morphologies.

The magnesiothermal reduction of biotemplated metal oxides is only beginning to be explored and many more experiments could be carried out to develop novel materials based on this process. The reduction of a variety of biotemplated or porous metal oxide foams is being explored for the production or nanostructured aluminum, tungsten, titanium, and niobium. Alloys could also be produced with this method, including stimuli-responsive nickel-titanium structures. In addition, further exploring the synthesis of nitrides and carbides via magnesiothermal reduction could lead to the development of a wider variety of catalysts or semiconductors. Finally, extending our process to different microorganisms would lead to the production of different shapes at the nanoscale. For instance, *Spirulina* algae cultivated under different light and temperature conditions can take on coil shapes with slightly different geometries. Specific strains of diatoms could also be grown and their silica shells could be harvested to produce silica structures with defined shapes as opposed to the mixture of silica morphologies found in diatomaceous earth.

### 7.3 Concluding Remarks

Throughout this thesis, we observed the great versatility of biotemplates, making them valuable tools for assembling nanostructured composites. Biotemplates can specifically bind materials and organize them into complex architectures. On the other hand, using biotemplates in photonic or electronic devices can also present challenges, such as introduction of insulating organic materials, salts or other contaminants. It is therefore important to have a deep knowledge of the biotemplate composition, and to understand the processing steps required to assemble it and use it as a scaffold in devices. By anticipating the effects of the chosen biotemplate on the final material, we can use it to our advantage to controllably introduce dopants or tune surface chemistries.

Focus was also placed on evaluating how morphology affects device performance. Our investigations revealed that continuous interpenetrated morphologies can in general improve
charge transport in energy conversion and storage devices. However, morphology is not the only parameter to optimize when designing such devices. Other important aspects include purity of materials, surface chemistry, passivation, ligands, crystallinity, particle size, etc. Champion devices can only be achieved through an optimization of these equally important parameters combined.

In conclusion, this thesis work has contributed to developing novel organic and inorganic nanostructured materials using low-cost solution-processed bottom-up approaches, and to applying biologically-derived materials to photovoltaic devices. The novel materials processing strategies employed here could contribute to synthesizing oxide or metallic thin films and powders with on-demand complex nanostructures. Impacts were made in the fields of nanostructured and porous materials, layer-by-layer assembly and surface coatings, as well as in the characterization of biotemplated materials and the construction of thin film nanostructured solar cells. In the future, biotemplates could be used with highly efficient photovoltaic materials systems to further improve the device performance through engineering of the pathways for charge transport. Biological scaffolds and their programmable DNA, various surface chemistries, functional groups and morphologies represent a rich matrix for binding and organizing nanomaterials. Ultimately, microorganisms and other biological materials could be incorporated as scaffolds in our daily-life energy conversion and storage devices, allowing for the assembly of devices under mild conditions and for the production of clean energy at low cost.
Appendices
Appendix A. Layer-by-Layer Assembly of Titania Nanoparticles with M13 Bacteriophages

This appendix presents a method to electrostatically assemble titania nanoparticles with M13 bacteriophages to create a composite thin film. While negatively charged M13 bacteriophages can be layered with a variety of positively charged materials, assembling them with titania nanoparticles is of importance for the creation of nanostructured titania films for photovoltaic, catalysis, or other applications. As opposed to the strategy described in Chapter 3, consisting in pre-assembling a film composed solely of bacteriophages and then mineralizing it with titania, the strategy described here could be advantageous if the infiltration of precursors to synthesize titania onto a bacteriophage template was not possible. The nanoparticle-bacteriophage LbL method is not limited to titania and could also be applicable to other inorganic nanoparticles.

This work was done in collaboration with Dr. Md Nasim Hyder, who synthesized the titania nanoparticles.

A.1 Assembly and Growth

Assembling negatively charged M13 bacteriophages with positively charged titania nanoparticles or nanotubes could allow for creating a nanostructured titania film directly from layer-by-layer assembly, as opposed to using a pre-assembled scaffold to nucleate titania. Here, we explored the assembly of poly(allylamine hydrochloride) (PAH)-stabilized titania nanoparticles with bacteriophages. A similar approach was previously used to assemble high aspect ratio structures of titania and carbon nanotubes via LbL assembly. 1

Because of their thin PAH coating, the titania nanoparticles exhibit a positive charge up to approximately pH 5.5, which leaves a pH range between ~ 4 and 5 for LbL assembly with bacteriophages, as shown by the zeta potential curves in Figure A-1. However, as the charge of the titania nanoparticles decreases and as the pH approaches the isoelectric point, the particles become unstable. Therefore, we selected pH 3.5 and pH 4.5 for the titania solution, and the bacteriophage solution was maintained at pH 4.9 to maximize their charge.
Figure A-1. E3 M13 bacteriophages can be layer-by-layer assembled with titania nanoparticles. A. Schematic of the materials used for LbL assembly and the resulting nanostructured film. B. Zeta potential titration in a 10 mM NaCl background electrolyte for E3 bacteriophages and titania nanoparticles coated with PAH.

At pH 3.5, the charge of the M13 bacteriophages is close to neutral, and we therefore expect less bacteriophages to be incorporated into the films when the titania nanoparticles are at pH 3.5 compared to pH 4.5. Consistently, the growth of the films is significantly faster at pH 4.5 for equal or similar titania concentrations. In fact, at pH 3.5, the film barely grows when the titania concentration is 0.01 mg/mL, as shown in Figure A-2. pH 4.5 was therefore selected as the optimal pH for this LbL assembly. It was also observed that the growth rate increases as the concentration of titania increases at both pHs, for a fixed bacteriophage concentration.

Figure A-2. The growth rate varies as a function of concentration for layer-by-layer assembled M13 bacteriophages and titania nanoparticles. A. Thickness, and B. Roughness, both measured via profilometry, for titania-bacteriophage LbL films assembled using titania solution at different pHs and concentrations.
A.2 Film Morphology

While the film growth was strongly affected by the pH of the solution, the film morphology was mainly affected by the titania concentration, and therefore by titania nanoparticle to bacteriophage ratio. Figure A-3 shows the changes in surface morphology as a function of titania concentration for a fixed bacteriophage concentration of $5 \times 10^{12}$ phage/mL. It can be observed that at low titania concentrations, finer features appear on the surface of the film, and these features are difficult to observe at higher concentrations. At high titania concentrations, the abundance of titania nanoparticles in the film masks the high aspect ratio features of the M13 bacteriophages, and they become more visible as thinner layers of titania nanoparticles adsorb onto the surface of bacteriophages.

![Figure A-3](image)

**Figure A-3.** Bacteriophage-like features are more visible at low titania concentrations in LbL assembled titania-bacteriophage films. AFM amplitude images showing the surface morphology of the LbL films for various titania concentrations. The titania solution were maintained at pH 4.5.

The morphology of LbL films was also observed by scanning electron microscopy (SEM), before and after annealing at 450 °C to sinter the titania nanoparticles and burn off the bacteriophages. Figure A-4 presents a low and a high magnification views of the films, as well as an image of regions showing nanowire-like features before and after annealing. Prior to annealing the film, porous structures can be observed, as well as some high aspect ratio features. After annealing, several aggregates are observed in the films, as well as region that are still porous. Large and small titania aggregates of particles are found, resulting in a non-uniform film. However, underneath the film surface, it is possible to find nanowire-like structures made of titania, which may result from the bacteriophage-titania nanoparticle complexes formed during LbL assembly.
B. After annealing

Figure A-4. Porous and high aspect ratio structures are observed in the titania-bacteriophage films before and after annealing, along with larger aggregates. SEM images showing the film surface morphology before and after annealing for films assembled at pH 4.5 with 0.01 mg/mL titania.

A.3 Conclusions

In conclusion, we have demonstrated that it is possible to layer-by-layer assemble M13 bacteriophages directly nanoparticles, and to control the thickness of the resulting films. The morphology of the thin films is dependent on the titania to bacteriophage ratio, and fine tuning this ratio might allow for a better control over the titania film morphology.

Under the conditions explored here, porous titania films are obtained, but the annealing causes the formation of titania aggregates. It might be possible to prevent this aggregation by using lower titania to phage ratios, and by modifying the parameters of the annealing step like the temperature increase rate. Using other titania nanomaterials, like nanotubes or nanowires, and assembling them with M13 bacteriophages could also generate different morphologies.

A.4 Materials and Methods

Titania nanoparticle synthesis: Hydrothermal synthesis was used to prepare sub-8 nm titania nanoparticles, stabilized with poly(allylamine hydrochloride) (PAH) as previously described.¹
**LbL assembly:** The films were constructed by successively dipping plasma treated substrated in an aqueous solution of titania nanoparticles coated with PAH, and then in a dilute bacteriophage solution. Two rinses in milli-Q water were done after the dip in each solution. The titania solution was prepared at pH 3.5 or 4.5 (adjusted with hydrochloric acid), and the bacteriophages were dispersed in sodium acetate buffer (NaOAc) at pH 4.9. The bacteriophage concentration was set to $5 \times 10^{12}$ phage/mL, and the titania concentration was varied from 0.001 to 0.125 mg/mL. Films of different thicknesses were produced by varying the number of dipping repetitions. To minimize titania nanoparticle aggregation, the films were assembled using Nanostrata robotic dippers (Nanostrata Inc., FL) that allow for mixing of the solution while dipping.

**Film characterization:** The thickness and roughness of the films were measured using a Veeco Dektak 150 profilometer. The surface morphology was characterized via atomic force microscopy (AFM) using a MultiMode Atomic Force Microscope (Veeco Metrology) in the tapping mode, and higher resolution images were obtained using scanning electron microscopy (SEM) using a JEOL 6700F Scanning electron microscope (FEG-SEM).

**A.5 References**

Appendix B. Preparation of Cross-Sectional SEM and TEM Samples for High Resolution Imaging

This appendix details the procedures employed to prepare scanning electron microscopy (SEM) and transmission electron microscopy (TEM) samples for imaging the cross-section of thin films assembled on a substrate.

B.1 Cross-Sectional SEM Samples

To prepare cross-sectional SEM samples, the proper holder is required, a 90° cross-sectional holder onto which the sample can be positioned vertically, as shown in Figure B-1. This holder is specific for the Helios Nanolab 600 Dual Beam focused Ion Beam Milling System (at the Electron Microscopy Facilities of the Center for Material Science and Engineering (CMSE)), a SEM that allows for high resolution imaging of thin films and cross-sections.

For samples constructed on rigid substrates, such as silicon wafers, the samples can be simply sectioned using a diamond knife and applying pressure on the edge of the sample to fracture it. If the substrate is softer or bendable, then liquid nitrogen may be used to fracture the sample. In any case, the chosen substrate should be conductive.

After fracturing the sample to expose the cross-section, the sample can be stuck onto the cross-sectional holder using carbon tape or conductive paste. For better quality images, it is recommended to a graphite paste, the PELCO© Colloidal Graphite (Isopropanol Base) (Ted Pella Inc., CA). This paste allows for a better adhesion of the sample to the holder and minimizes motions when imaging. The paste should be applied on the surface of the holder, and then on top of the base of the sample to create an electrical connection between the top surface of the sample and the holder.
Cross-section of interest

Conductive graphite paste

**Figure B-1.** Fractured thin film on silicon on a cross-sectional holder.

Examples of SEM images obtained using this method are shown in **Figure B-2,** and fine features in the order of 10 nm can be observed.

**Figure B-2.** Cross-sectional images of nanostructured titania thin films on silicon wafers. A. 75 000x magnification, B. 750 000x magnification.

### B.2 Cross-Sectional TEM Samples

The preparation of cross-sectional TEM samples requires more complicated sample preparation techniques because the samples are required to be thinner than ~100 nm in order to obtain a clear image with TEM.

#### B.2.1 Thinning by Polishing and Ion Milling

Ion milling and polishing can be used to prepare cross-sectional TEM samples from thin films that are resistant to heat and to ion beams. Organic and soft samples, or samples with a complex or fine nanostructure might be damaged through the procedure.
The overall protocol for preparing TEM samples using polishing and ion milling is illustrated in **Scheme B-1**. The first step consists in embedding the sample in an epoxy resin, with protective layers on each sides of the sample. Then, the sample has to be cut into disks and then polished with sand paper using a polisher. When the thickness of the sample is approximately 70 μm, it can be installed on a Single Slot Grids GA1500-Cu 1.5mm aperture (Electron Microscopy Sciences, PA) using M-Bond 610 glue (Electron Microscopy Sciences, PA). After solidifying the glue overnight, the sample is then ion milled using a Fischione 1010 Ion Mill until a small hole forms in the middle of the sample.

Alternatively, a thin film deposited on a substrate can be directly polished to decrease the thickness of the substrate, and glued to a TEM grid to pierce a hole in the direction perpendicular to the substrate. This allows for imaging a section of the film parallel to the substrate.

**Scheme B-1.** Procedure for preparing cross-sectional TEM samples using polishing and ion milling. This protocol was provided by Dr. Dong Soo Yun.
After the small hole forms in the sample, the thickness of the sample increases with the distance from the hole, and TEM imaging should be done as close as possible to the hole. Examples of TEM images obtained using this technique, using a JEOL 2010 Advanced High Performance TEM (at the Electron Microscopy Facilities of CMSE), are shown in Figure B-3. In Figure B-3A, the hole can be seen in the top right corner of the image. Higher resolution images are shown at a distance from the hole (Figure B-3B), and very close to the hole (Figure B-3C). The greatest color contrast can be obtained immediately next to the hole.

![Figure B-3. TEM images of titania thin films obtained with the polishing and ion milling method.](image)

**B.2.1 Sectioning with a Microtome**

Cutting sample sections using a microtome is applicable for soft samples and substrates that are resistant to mechanical stress. Thin films can be constructed on polymeric substrates or, if required, thin metal foils. Difficulties with cutting arise when using rigid substrates such as silicon wafers.

The first step is to embed the sample into a resin, such as the LR White Resin, Hard grade (Ladd Research Industries, VT). After solidifying overnight, the sample is then secured in a microtome (RMC MT-X cryo-ultramicrotome) and preliminary cuts into the resin are performed.
using a glass knife. Once the sample is reached, the glass knife is replaced with a sharp diamond knife and the velocity of the cut is decreased. Sections of the sample fall down into a water boat on the knife. The size of the sections can be set on the microtome, and should be around 100 nm or less for high resolution imaging. The sections are then picked up directly onto a TEM grid using tweezers, and immersing the grid in the water boat of the knife. Holey carbon grids are often used for thin film cross-sections since they provide support, but also empty grid sections without background.

Figure B-4 shows TEM images obtained using this method. First, it has to be noted that it is often difficult to distinguish sliced pieces of resin and a sliced sample, especially if the sample is organic. For instance, Figure B-4A shows how the resin is also a nanostructured material, and Figure B-4D illustrates pieces of samples that can be identified within a large piece of resin. In B and C, images illustrate the mechanical damages that can be caused to the cross-sections. Those images should show interconnected titania structures, but upon sectioning the slices get damages and the film can break, decompose into pieces or deform. Figure B-4E also shows only a fragment on a section, but the structure of the titania can be observed. In F, a high magnification image of this structure was obtained, demonstrating that it is possible to gather high resolution images with this method. However, this requires (1) locating the sample within the resin material, (2) preventing damages to the sample by slicing slowly, using a sharp knife, and only soft substrates. In order to simplify the sample search within the resin, a layer of high conducting material, gold for instance, could be deposited onto the thin film prior to embedding in epoxy. The high contrast between the gold and the sample or the resin, should allow for determining where the sample is located on the grid.
B.2.3 Cutting with a Focused Ion Beam

The last method employed in this work to prepare cross-sectional TEM sample has been the most successful. It consists in cutting thin slices of the thin film with a focused ion beam, and then imaging the sample with TEM. Ideally, the sample should be resistant to the ion beam, but protective layers are deposited on top of thin film, as shown in Scheme B-2. These layers prevent the focused ion beam from damaging and melting the thin film. Using this technique, inorganic thin films, but also organic and biological (made of M13 bacteriophages) thin films were cut and imaged successfully.
Focused ion beam

Scheme B-2. Protective layers are added on top of the thin film to protect from damages caused by the focused ion beam. A gold layer is sputtered onto the thin film, and then a layer of Sharpie marker is deposited.

A JEOL 9320-FIB is used to cut the samples (by Dr. Steve Kooi at the Institute for Soldiers Nanotechnologies). They are then deposited on holey TEM carbon grids. Examples of images obtained with this method are shown in Figure B-5. In all of the cases, the interface between the substrate and the protective layers was easy to identify and the structure of the thin film remained intact.
B.3 Conclusions

In conclusion, a universal method for cross-sectional SEM sample preparation was described, along with three methods for the preparation of TEM samples that may be applicable to different types of samples. The focused ion beam method is recommended for any sample that can resist gold and sharpie deposition along with ion beam exposure, since the shape of the thin film remains completely intact, and allows for imaging of the nanostructure of the sample without damages.

The instruments used for preparing the samples are located in the Center for Materials Science and Engineering (CMSE) and at the Institute for Soldiers Nanotechnologies (ISN). Dr. Dong Soo Yun, Dr. Steve Kooi, Dr. Yong Zhang, Dr. Shaoyan Chu, and Patrick Boisvert assisted with different steps of the methods described above for TEM sample preparation or with the use of equipment related to these samples. Dr. Shiahn Chen assisted with the use of cross-sectional holders for SEM sample imaging.
Appendix C. Biomineralization of Various Semiconductors and Metals onto M13 Bacteriophage Thin Films

The aim of this appendix is to present the biomineralization of other oxides and metals, apart from titania, silica and silicon, onto layer-by-layer assembled M13 bacteriophage scaffolds. Semiconductors relevant for photovoltaic applications were studied, including zinc oxide, zinc sulfide and silver sulfide. In addition, metals like gold and silver can also be mineralized onto bacteriophage scaffolds from precursors in solution. The coating of bacteriophages with gold was studied to compare the plasmonic properties of high aspect ratio gold structures with that of discrete gold nanoparticles, and for potential catalytic applications with highly porous gold structures.

This work was done in collaboration with Matthew T. Klug, and with the help of Victor J. Cantú.

C.1 Biomineralization of Zinc Oxide and Zinc Sulfide

Zinc oxide and zinc sulfide both are n-type materials that have energy levels similar to titania (see Scheme C-1) and could be paired with several organic or inorganic p-type materials to form heterojunction solar cells. Therefore, organizing these materials along a M13 bacteriophage scaffold could allow for the formation of a nanostructured heterojunction similar to those discussed with titania in Chapters 3 and 4.

\[
\begin{array}{ccc}
\text{ZnS} & \text{ZnO} & \text{TiO}_2 \\
\hline
-3.9\text{eV} & -4.4\text{eV} & -4.2\text{eV} \\
-7.5\text{eV} & -7.7\text{eV} & -7.4\text{eV}
\end{array}
\]

Scheme C-1. Zinc oxide and zinc sulfide are n-type materials with energy levels comparable to titania. ZnO could be paired with lead sulfide quantum dots or with poly-3-hexylthiophene (P3HT) to form a heterojunction solar cell, and ZnS could be paired with P3HT.

Positively charged zinc ions can be nucleated onto the surface of negatively charged M13 bacteriophages at pH 4.9 or higher. Therefore, the biomineralization of zinc sulfide of zinc oxide
begins by incubating the bacteriophage thin films into zinc salt solutions, such as zinc nitrate or zinc chloride.

\[
Zn(NO_3)_2 \rightarrow Zn^{2+} + 2 NO_3^- \quad \text{Equation C-1}
\]

\[
ZnCl_2 \rightarrow Zn^{2+} + 2 Cl^- \quad \text{Equation C-2}
\]

Then, to form zinc oxide where the zinc ions are nucleated, hydroxyl ions are added to form zinc hydroxide, and the product is then heated up to convert zinc hydroxide into zinc oxide, as follow.

\[
Zn^{2+} + OH^- \rightarrow Zn(OH)_2 \quad \text{Equation C-3}
\]

\[
Zn(OH)_2 \rightarrow ZnO + H_2O \quad \text{Equation C-4}
\]

This method is very common for synthesizing zinc oxide, and has been applied before to the growth of zinc oxide nanostructures on templates such as chitosan\(^1\) and carbon nanotubes\(^2\), or using nanoparticles as nucleation sites.\(^3\) However, the features of the materials synthesized in previous reports were considerable larger than the features of nanoporous bacteriophage films.

**Figure C-1** shows a typical zinc oxide coating obtained on bacteriophage thin films. The structure is clearly porous and shows features at the nanoscale, comparable to the porous structures observed in bacteriophage thin films mineralized with titania.

![Figure C-1](image-url)

**Figure C-1.** Zinc oxide biomineralization yields a nanoporous morphology, but only partial coverage is obtained. SEM images showing the porous ZnO structure partially covering the bacteriophage film.

Instead, to form zinc sulfide, the bacteriophage film is incubate into a solution containing a source of sulfur, such as sodium sulfide. Sodium sulfide dissociates in aqueous solution, and the negatively charged sulfur ions can react with the zinc ions to form zinc sulfide.
\[ \text{Equation C-5} \]

\[ Na_2S \rightarrow 2 Na^+ + S^{2-} \]

\[ \text{Equation C-6} \]

\[ Zn^{2+} + S^{2-} \rightarrow ZnS \]

This method has been reported before for the synthesis of multilayered ZnS films, by successively dipping a substrate into the zinc salt and sodium sulfide solutions.\(^4\) When applying this strategy to a bacteriophage thin film, a coating was observed (Figure C-2), but only on the surface of the film. In addition, the coating was not uniformly covering the surface but only forming islands of ZnS on the film. Repeating the mineralization process several times to allow for the coating of multiple layers of ZnS onto the bacteriophage scaffold did not help increase the uniformity of the ZnS coating.

![Figure C-2](image)

**Figure C-2.** Zinc sulfide biomineralization yields partial coverage of the bacteriophage scaffold. SEM images showing the surface morphology of films mineralized with ZnS.

In order to confirm the presence of zinc oxide and zinc sulfide on the surface of the bacteriophage films, elemental analysis with EDX was performed. **Figure C-3** shows that Zn, and O or S, are detected on the bacteriophage films that were mineralized with ZnO or ZnS. Therefore, although the coating is not complete, the target compounds have been synthesized.
Figure C-3. Elemental analysis confirmed the presence of ZnO or ZnS on the bacteriophage thin films. SEM with EDX showing the elemental mapping for Zn, and O or S.

C.2 Biomineralization of Gold

To mineralize gold onto thin bacteriophage films, chloroauric acid was used as a precursor and it was reduced to gold by introducing a reducing agent, sodium borohydride, into the mixture. The reaction proceeds as follow:

$$HAuCl_4 + NaBH_4 \rightarrow Au^0 + NaCl + BH_3 + H_2(g) \quad \text{Equation C-7}$$

The structures obtained after gold mineralization with a 2 mM chloroauric acid solution are shown in Figure C-4A and B. Although conformal coverage of the bacteriophages was achieved over tens of microns, the overall coverage of the surface is only partial, and gold only coats bacteriophages located on the surface of the films, as illustrated in Figure C-4C and D. At least 200 nm of a ~600 nm thick bacteriophage film is not coated with gold close to the substrate.

Although the gold structures are located on the surface of the film, the porosity and pore size distribution achieved are analogous to those obtained with titania mineralization, with the majority of the pore diameters ranging between 5 and 20 nm. The pore size distribution of bacteriophage-templated gold thin films is presented in Figure C-5. Such highly porous gold thin films with a high surface area may have applications in catalysis.
Figure C-4. Gold biominalization onto bacteriophage thin film creates nanowire-like structures on the surface of the bacteriophage film. A and B. Top surface morphology of bacteriophage-templated gold films, imaged with SEM. The mineralization was carried out in a 2 mM chloroauric acid solution. C. SEM image showing the partial coverage of gold onto the bacteriophage film. D. Cross-sectional SEM image showing the gold nucleation on the top surface of the bacteriophage film, and an uncoated portion of the bacteriophage scaffold near the substrate.

Figure C-5. The pore size distribution of bacteriophage films mineralized with gold is similar to the pore size distribution observed for bacteriophage-templated titania film. Pore size distribution as determined with ImageJ.

In order to evaluate if this bacteriophage-templated gold structure could have applications as plasmonic material, the absorption spectrum of a gold-coated bacteriophage film constructed
on glass was measured. The results were compared to free gold nanoparticles, and are shown in Figure C-6. It can be seen that, as opposed to spherical gold nanoparticles exhibiting a clear plasmon resonance peak at around 520 nm, the higher aspect ratio porous gold structures constructed onto the bacteriophage scaffold do not show any plasmon resonance peak. This result is not surprising as gold nanorods typically shows more than one resonance peaks and have a broader absorption as their aspect ratio increases. Therefore as the aspect ratio of the gold structure increases and then forms a porous continuous film, the absorption spectrum flattens down.

![Figure C-6. Gold-coated bacteriophage film exhibits no plasmon resonance peak compared to individual 10 nm gold nanoparticles in solution. Absorption spectra for bacteriophage films coated with gold using chloroauric acid as a precursor, and the same gold-coated films mineralized with an additional layer of titania. These films were assembled on glass slides, and compared to 10 nm gold nanoparticles in aqueous solution.](image)

C.3 Mineralization of Silver and Silver Sulfide

Silver sulfide is another material of interest for photovoltaic devices. Combined with poly-3-hexylthiophene (P3HT), it has yielded high current densities reaching 20 mA/cm². Therefore, organizing this material into a tridimensional structure along M13 bacteriophage scaffolds could lead to the development of highly efficient solar cells.

Silver sulfide can be synthesized starting from metallic silver. Thus, the first step of the synthesis consisted in mineralizing silver onto bacteriophage thin films. This was accomplished by nucleating silver ions onto the bacteriophages. In order to fully infiltrate the film with Ag⁺ ions, silver nitrate was added to the rinse baths during the layer-by-layer assembly of
bacteriophages. Then, the silver ions were reduced to metallic silver using a reducing agent such as sodium borohydride.

\[ Ag(NO_3)_2 \rightarrow Ag^{2+} + 2 NO_3^- \quad \text{Equation C-8} \]

\[ Ag^{2+} + NaBH_4 \rightarrow Ag^0 + + NaCl + BH_3 + H_2(g) \quad \text{Equation C-9} \]

While the structure obtained was porous, the features were considerably larger than the pore size or nanowire diameter formed by M13 bacteriophages, as shown in Figure C-7A. Then, to synthesize silver sulfide, the silver films were incubated into an ammonium sulfide solution, and Ag$_2$S was formed as follow.

\[ Ag^0 + Na_2S \rightarrow Ag_2S + 2 Na^+_{(aq)} \quad \text{Equation C-10} \]

As expected, the size of the silver sulfide crystals was comparable to that of the silver structures previously obtained. The Ag$_2$S film is shown in Figure C-7B. Although the morphology of the silver sulfide films did not correspond to the desired morphology for thin film heterojunction solar cells, the nature of the silver sulfide product was confirmed via XPS. Figure C-8 shows that silver is converted to silver sulfide after reaction with ammonium sulfide. A shift in the Ag3d3/2 peak is observed from metallic silver (368 to 368.2 eV) to silver sulfide (366.9 eV). Therefore, assembling bacteriophage-based Ag$_2$S nanowires from free-floating bacteriophages, or on bacteriophage thin films would be promising if the thickness of the silver coatings can be reliably controlled prior to the conversion to silver sulfide.
Figure C-7. Silver ions can be reduced silver onto bacteriophage thin films, and subsequently converted to silver sulfide. SEM images of LbL film assembled with rinses in 10 mM silver nitrate, reduced to silver and reacted with ammonium sulfide to form silver sulfide.

Figure C-8. Silver is converted to silver sulfide after reaction with ammonium sulfide. XPS spectra showing the Ag3d region before and after reaction with ammonium sulfide. The Ag3d3/2 peak (in purple) shows a characteristic shift from metallic silver to silver sulfide.
C.4 Conclusions

In conclusion, several metal oxides and metals can be synthesized from precursors in solution, but the mineralization of bacteriophage thin films was only optimized for titania (See Chapter 3) and silica (See Chapter 5). While oxides like titania and silica can form a conformal coating on M13 bacteriophage nanoporous thin films, other oxides and metals appear to only form on the surface of the thin films. When synthesized on free floating bacteriophage, uniform bacteriophage-based zinc oxide or gold nanowires can be formed, indicating that the precursors used for the synthesis may not be able to fully infiltrate thin films with pores in the order of 10 nm, due possibly to unfavorable ionic interactions or steric hindrance. For other materials, the crystal size plays a critical role in impeding the formation of fine structures onto the bacteriophage thin films. It is the case of silver sulfide crystals that are too large to preserve the features of nanoporous thin films.

Tetrachloride precursors used for the biomineralization of titania and silica are efficient at infiltrating nanoporous bacteriophage scaffolds and uniformly mineralizing them. Therefore, it is recommended to use tetrachloride precursors, which are commercially available for the synthesis of various metal oxides, for the mineralization of bacteriophage thin films. Other metal oxide or metal precursors can be employed in solution, or potentially on very thin bacteriophage layers assembled onto a substrate.

C.5 Materials and Methods

Zinc oxide biomineralization: Bacteriophage thin films were incubated at least 15 min in a 10 mM Zn(NO₃)₂ or ZnCl₂ aqueous solution to allow for nucleation of zinc ions onto the surface of bacteriophages. For 1.6 mL of zinc nitrate or zinc chloride solution, 8 µl of 1M NaOH was added and the bacteriophage film was left into this mixture overnight at room temperature. The thin films were then rinsed with milli-Q water and dried with nitrogen gas. The films were heated up to 80 °C in an oven for 2 h to convert zinc hydroxide to zinc oxide. The bacteriophages were then burn off at 450 °C for 1 h.

Zinc sulfide biomineralization: Bacteriophage thin films were incubated at least 15 min in 1.5 mL of a 10 mM Zn(NO₃)₂ or ZnCl₂ or Zn(OAc)₂ aqueous solution to allow for nucleation of zinc ions onto the surface of bacteriophages. 1.5 mL of 20 mM Na₂S in water was added to the zinc salt solution, and the vial with the bacteriophage film and the solution was placed at 4 °C for 24
h. The vial was taken out of the refrigerator and left at room temperature for a few hours, and the bacteriophage film was rinsed with milli-Q water and dried with nitrogen gas.

**Gold biomineralization:** Bacteriophage thin films were incubated in an aqueous chloroauric acid solution (concentrations were varied from 2 mM to 10 mM) with pH adjusted to ~7.5 using NaOH. After 30 min, sodium borohydride was added to the mixture at a final concentration of 10 mM to allow for reduction of gold onto the viruses. After 1 h with the reducing agent, the bacteriophage film was removed from the mixture, rinses with milli-Q water, and dried with nitrogen.

**Silver and silver sulfide biomineralization:** To nucleate silver ions onto bacteriophage films, the films were constructed by rinsing in a silver nitrate solution at 10 mM during LbL assembly. To reduce the silver ions to silver metal, the films were incubated in a 0.01 to 0.1 M sodium borohydride solution at room temperature for 20 min. The films were then rinsed with milli-Q water. To convert the silver into silver sulfide, the films were incubated in a 1 w% solution of (NH₄)₂S for 1 h, and then rinsed with milli-Q water and dried with nitrogen gas.

**Characterization:** The morphology of mineralized thin films was characterized by scanning electron microscopy (SEM) using a Helios Nanolab 600 Dual Beam Focused Ion Beam Milling System. SEM with EDX was used to perform elemental mapping on the samples. The absorption spectrum of the gold-coated bacteriophage film (constructed on glass slides) was carried out using a Beckman Coulter DU 800 Spectrophotometer and performing a wavelength scan at a rate of 600 nm min⁻¹. PHI Versa-Probe II X-ray photoelectron spectrometer with a scanning monochromated Al source was used to analyze the chemical composition of the products.

**C.6 References**


Appendix D. Layer-by-Layer Assembly of Titania Nanomaterials with Charged Conjugated Polymers to Create Flexible Photoactive Composites

The goal of this appendix is to present a method to layer-by-layer assemble conjugated polymer and titania thin films, and to study properties of these films that are relevant to photovoltaic applications. Metal oxide-polymer composites can be formed using different methods, including the infiltration of polymer chains without porous oxide structures. Here, we report a LbL assembly method, which allows for creating a uniform blend of titania and conjugated polymer, with tunable thickness and composition.

This work was done in collaboration with Dr. Md Nasim Hyder, who synthesized the titania nanoparticles and nanotubes, and with the help of Summer 2012 CMSE MPC REU intern Margaret Stevens.

D.1 Assembly, Growth and Composition

Conjugated polymers can act as p-type materials when paired with n-type metal oxides like titania. Creating a nanoscale blend of these materials could allow for the construction of a nanostructured bulk heterojunction solar cell with small domains in the order of the exciton diffusion length in conjugated polymers. 1

Charged conjugated polymers and metal oxide nano-objects can be incorporated into layer-by-layer (LbL) assembled thin films if they carry a complementary surface charge. For instance, a modified version of poly(3-hexylthiophene) (P3HT) containing a carboxylic acid group attached to each carbon ring is commercially available and carries a negative charge at neutral pH. The structure of this polymer is shown in Figure D-1, along with its surface charge as a function of pH. This polymer can be LbL assembled with positively charged particles, such as titania nanoparticles, nanotubes or nanowires with a positively charged polymer absorbed on their surface.

Figure D-1 shows the structure of anatase titania nanoparticles and nanotubes that can be coated with a thin poly(allylamine hydrochloride) (PAH) layer to change their surface charge and create a positive charge around neutral pH.
Figure D-1. Carboxylic acid-modified polyhexylthiophenes can be layer-by-layer assembled with polymer-stabilized titania nanoparticles or nanotubes. A. The structure of poly [3-(3-carboxypropyl)thiophene-2,5-diyl] (P3CProT), and TEM images of titania nanoparticles and nanotubes stabilized with a thin poly(allylamine hydrochloride) (PAH) positively charged coating. B. Zeta potential titration curve with a 10 mM NaCl background electrolyte for P3CProT, titania nanotubes without PAH coating, and titania nanotubes coated with PAH.

This appendix discusses the assembly of titania nanoparticles and nanotubes, but the assembly method could be extended to a broader range of titania nanomaterials. For instance, higher aspect ratio nanowires could also be layered with polymers, as well as bacteriophage-based titania structures, and bacteriophage-metal nanoparticle complexes mineralized with titania (see Scheme D-1). These materials should generate different nanostructures that could affect the performance of photovoltaic devices. In addition, if noble metal nanoparticles are incorporated in the structure, localized surface plasmon resonances could be produced.
Scheme D-1. Various titania nano-objects could be layer-by-layer assembled with charged conjugated polymers to create hybrid nanocomposites. A. The strategies for LbL assembly of titania nanoparticles or nanotubes with a conjugated polymer, described in this appendix. B. By extension, nanowires or bacteriophage-templated titania could be assembled with conjugated polymers. The TEM images shows bacteriophage-gold nanoparticle-titania complexes that could be LbL assembled with polymers (TEM image by Matthew T. Klug).

Films were assembled both with titania nanoparticles and with titania nanotubes. The charged conjugated polymer was solubilized in dimethyl sulfoxide (DMSO), and then diluted in a DMSO-PBS (phosphate buffer saline) solution mixture, and the titania nano-objects were dissolved in PBS. More experiments were carried out with the titania nanotubes since they could form high aspect ratio structures within the films and result in architectures that might favor charge transport in solar cells. The growth curves for both systems are shown in Figure D-2 and Figure D-3 for the titania nanoparticles, and nanotubes respectively. The grow rate was considerably faster for the nanotubes compared with the nanoparticles because of the larger dimensions of the nanotubes. All films grew linearly, and the dipping times were varied for the nanotube-polymer films. The highest growth rate was observed for films dipped for 5 minutes in each solution, and decreasing the dipping time significantly decreased the growth rate, likely indicating that polymer requires a few minutes to fully adsorb onto the surface of the film and
create a layer that is thick enough to allow for the adsorption of the next titania layer. In contrast, when decreasing the dipping time in the titania nanotube solution, the growth rate did not appear to be significantly affected.

Figure D-2. Layer-by-layer assembled films of conjugated polymer and titania nanoparticles grow linearly. Growth curve for thin films assembled using spherical titania nanoparticles of ~ 8 nm diameter.

Figure D-3. Film growth depends on dipping time in conjugated polymer and titania nanotube solutions. Growth curves for A. A constant 5 min dip in titania, with varied dipping times in P3CProT, and B. A constant 5 min dip in P3CProT with varied dipping times in titania. The ratios indicated correspond to times in minutes.

The composition of three films constructed with different dipping times in the polymer solution was then studied using thermogravimetric analysis (TGA). After elevating the temperature to 900 °C, only titania is still present, while PAH and P3CProT burn off at temperatures around 400 to 600 °C. Therefore, when analyzing the thin films, the weight fraction of material remaining after burning off the film at 900 °C corresponds to the weight
fraction of titania within the thin film, as shown in Figure D-4. Table D-1 summarizes the titania content (weight and volume fractions) in thin films with varied dipping time ratios.

**Table D-1.** The weight and volume fractions of titania within titania nanotube-P3CProT thin films varies as a function of dipping time. Weight fraction of titania within the thin films, and corresponding volume fraction calculated from the crystalline density of titania.

<table>
<thead>
<tr>
<th>Dipping time in P3CProT:TiO₂ nanotubes (min)</th>
<th>Weight fraction of titania (%)</th>
<th>Volume fraction of titania (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:5</td>
<td>27.7</td>
<td>10.0</td>
</tr>
<tr>
<td>2.5:5</td>
<td>15.6</td>
<td>5.1</td>
</tr>
<tr>
<td>5:2.5</td>
<td>4.7</td>
<td>1.4</td>
</tr>
</tbody>
</table>
D.2 Film Morphology

The surface morphology of the films was first observed via optical microscopy for different dipping times in the conjugated polymer solution (see Figure D-5). It can be clearly observed that the films are rough, and that the roughness and non-uniform features are more pronounced for shorter dipping times in the polymer solution.

![Figure D-5](image)

Figure D-5. Optical microscopy reveals rough surfaces for films dipped 1.25 and 2.5 minutes in P3CPRT and a smoother surface for a 5 min dip. Optical microscopy images of LbL film containing titania nanotubes and P3CPRT.

The morphology of films prepared using titania nanoparticles and nanotubes was also compared. Figure D-6 shows surface and cross-sectional images for both types of films. The films constructed with spherical nanoparticles seem highly porous, while, apart from the surface roughness observed in the nanotube films, they seem denser from the cross-sectional images. A layer-like structure can also be observed in the film cross-sections, but no individual nanotube could be distinguished within the film. Since the titania content in the film only reached approximately 10 vol %, the nanotubes are expected to be well embedded within polymer layers and it is not surprising that they cannot be detected visually.
Figure D-6. The morphology of the films varies with the shape of the titania nanomaterial. SEM images showing the surface and cross-section of LbL films assembled with A. titania nanoparticles, or B. titania nanotubes.

D.3 Relevant Properties for Photovoltaic Applications

D.3.1 Light Absorption

The absorption spectrum of P3CProt was compared to the spectrum of titania-P3CProT thin films. As shown in Figure D-7, the position of the absorption peak is the same for P3CProT in solution, and for the dried P3CProT-titania thin film, but the peak is slightly broader for the thin film. This could be due to the presence of titania, which creates a background absorption.

The clear absorption peak observed indicates that the LbL films constructed might be suitable for photovoltaic applications. Visually, the thin films appear very dark, as shown in Figure D-7B, and they can be constructed on polymeric transparent flexible substrates since they do not require any post-assembly heat treatment.
Figure D-7. The absorption peak of P3CProT is maintained after incorporation into thin films with titania nanotubes. A. Absorption spectra of P3CProT in a DMSO solution, and in a dried LbL thin film with titania nanotubes. The data was normalized to the peak height of P3CProT in solution. B. Optical image of a thin film constructed in an ITO-coated polymeric flexible substrate.

D.3.2 Percolation Threshold and Conductivity

Finally, the percolation threshold of electrons in a thin film containing titania nanotubes was estimated. The titania nanotubes have an aspect ratio of approximately 7, and using the fitting presented by Zheng et al.\textsuperscript{2} for the percolation threshold as a function of the aspect ratio of randomly oriented nanorods, it corresponds to a theoretical percolation threshold of 9.7\%. Therefore, if the thin films contain a volume fraction equal or greater than 9.7\%, the nanotubes should form a percolated networks in which the electrons can migrate from the top to the bottom.

The conductivity of titania nanotubes-P3CProT thin films containing different volume fractions of titania was studied in order to examine the impact of a percolated titania network on conductivity. The film conductivity was low in general and standard deviation was large between the various measurements, but estimate values are presented in Table D-2, where it can be see that the conductivity increases with the titania content, and that it remains approximately equal once it reaches a volume fraction of titania of 5.1 vol\% or higher. This could indicate that the experimental percolation threshold is lower than expected and that a 5.1 vol\% of titania is sufficient to create a percolated network. However, the low conductivity measured could also result from a poor contact between the individual titania nanotubes, and it may be difficult to further increase it by increasing the titania content in the film.
Table D-2. The conductivity increases near and above the theoretical percolation threshold of titania nanotubes. Conductivity approximated via 4-point probe measurements for films containing different volume fractions of titania.

<table>
<thead>
<tr>
<th>Dipping time in P3CProT:TiO&lt;sub&gt;2&lt;/sub&gt; nanotubes (min)</th>
<th>Volume fraction of titania (%)</th>
<th>Conductivity (S/cm) (± 0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:5</td>
<td>10.0</td>
<td>0.02</td>
</tr>
<tr>
<td>2.5:5</td>
<td>5.1</td>
<td>0.02</td>
</tr>
<tr>
<td>5:2.5</td>
<td>1.4</td>
<td>0.01</td>
</tr>
</tbody>
</table>

It has previously been reported that layer-by-layer assembled hybrid photovoltaic devices, composed of polymer and quantum dots<sup>3</sup> or titania nanoparticles,<sup>4</sup> are limited by low short-circuit current due to poor charge transfer. High temperature annealing of titania nanoparticles results in higher photocurrents due to better contacts between individual titania nanoparticles, and between titania nanoparticles and the substrate.<sup>4</sup> Annealing the P3CProT-titania nanotube devices would damage the polymer, which begins to decompose below 400 °C, as observed with TGA in Figure D-4. Therefore, producing highly conductive layer-by-layer assembled P3CProT-titania devices would be challenging.

D.4 Conclusions

In conclusion, we have demonstrated that titania nanoparticles and nanotubes can be assembled with a modified charged version of poly(3-hexylthiophene) (P3HT), namely poly [3-(3-carboxypropyl)thiophene-2,5-diyl] (P3CProT). The LbL films grow linearly to form titania-conjugated polymer composites that could be used for constructing flexible photovoltaic devices. Because the titania nanoparticles or nanotubes synthesized are in the anatase crystalline phase, no post-assembly heat treatment is required, but this also implies that no sintering of the titania nanomaterials occurs which may result in a poor conductivity and less efficient electron transport. To overcome this potential issue, different titania nanostructures could be used. For instance, higher aspect ratio nanowires, or pre-synthesized interconnected bacteriophage-based titania networks could be used to improve the charge percolation.
D.5 Materials and Methods

Materials: P3CProT was purchased from Rieke Metals, Inc. (Lincoln, NE).

Titania nanoparticle and nanotube synthesis: Hydrothermal synthesis was used to prepare sub-8 nm titania nanoparticles, stabilized with poly(allylamine hydrochloride) (PAH) as previously described. For nanotube synthesis, 0.5 g of TiO₂ particles were mixed with 50 ml of a 10 M NaOH aqueous solution, followed by hydrothermal treatment at 150 °C in an autoclave for 10 h. After the hydrothermal reaction, the treated powders were washed thoroughly with distilled water and 0.1 M HCl and subsequently filtered and dried at 60 °C for 2 days.

Film assembly: Films were assembled by successively dipping a plasma treated substrate in titania nanoparticle or nanotube solution, and in a P3CProT solution, under agitation using Nanostrata robotic dippers (Nanostrata Inc., FL). Two rinses were performed in milli-Q water after the dip in each solution. The P3CProT solution was prepared at 10 mM in a 50:50 mixture of PBS buffer and dimethyl sulfoxide (DMSO), and the titania solution at 0.1 mg/mL in PBS.

Film characterization: The thickness of the thin films was measured using a Veeco Dektak 150 profilometer. The surface morphology was observed by optical microscopy using a Leica LEITZ DMRX light microscope with a Nikon digital camera DXM1200F, and by scanning electron microscopy (SEM) using a JEOL 6700F Scanning electron microscope (FEG-SEM) or a Helios Nanolab 600 Dual Beam Focused Ion Beam Milling System. The film composition was determined using thermogravimetric analysis (TGA) with a TA Instruments Thermogravimetric Analyzer Discovery. The conductivity of the composite was estimated using a Keithley SCS-4200 4-Point Probe. The absorption spectra of the films (constructed on glass slides) were characterized using a Beckman Coulter DU 800 Spectrophotometer.

D.6 References


Appendix E. Assembly of High Aspect Ratio M13 Bacteriophage-Quantum Dot Complexes

This appendix presents the assembly of M13 bacteriophages with lead sulfide quantum dots. First, we present a method to transfer PbS QDs from an inorganic phase to an aqueous phase, and to give them a positive charge. The high aspect ratio structures formed from the bacteriophage-quantum dot complexes were characterized via atomic force microscopy (AFM) and transmission electron microscopy (TEM).

This work was done in collaboration with Kevin J. Huang, who synthesized the quantum dots, Matthew T. Klug who contributed to the assembly strategies, and with the help of Summer 2014 CMSE MPC REU intern John Je Lee.

E.1 Ligand Exchange and Assembly

The strategy used to assemble lead sulfide (PbS) quantum dots (QDs) onto the surface of M13 bacteriophages is detailed in Scheme E-1. If the surface charge of PbS QDs can be modified to be positively charged, then they could spontaneously electrostatically assemble with bacteriophages. To do so, PbS QDs must first be transferred from an organic phase to an organic phase. As synthesized PbS QDs are oleic acid-capped and soluble in organic solvents like octane.

The phase transfer of PbS QDs can be performed with a variety of ligand that interact with the quantum dots and have a water-soluble moiety. For instance, reports present the use of several bifunctional thiols like cysteine, dihydrolipoic acid, mercaptopropionic acid (MPA), glutathione etc. to transfer quantum dots to an aqueous phase. While these ligands all confer quantum dots a negative charge after the transfer to aqueous phase, a second layer can be added around the quantum dot to revert its charge and make it positive. For instance, the strategy employed here involved the use of the MPA ligand to perform a phase transfer from chloroform to water. After collecting the aqueous phase, an amine-containing polymer, poly(allylamine hydrochloride) (PAH) was used to form a thin coating around the water-soluble quantum dots and change the surface charge.

Another method would consist in directly using a thiol molecule that contains an amine group, or even better, an ammonium group. Such a molecule exists and is called 11-Amino-1-
undecanethiol. We have used this molecule to perform quantum dot phase transfers very efficiently, but it costs $430 for 50 mg and does not represent a cost-efficient option for producing large quantities of water-soluble quantum dots.

Scheme E-1. The strategy for assembling bacteriophage-PbS QD complexes involves wrapping water-soluble QDs with a positively charged polymer and electrostatically assembling them with negatively charged bacteriophages. A. Electrostatic assembly of positively charged PbS QDs with negatively charged bacteriophages. B. The layers functionalizing the PbS QDs. First, the MPA ligand gives a negative charge to the QDs, which is reverted by adding a layer of poly(allylamine hydrochloride) (PAH).

Figure E-1 shows that the phase transfer can produce well-dispersed water-soluble QDs. Figure E-1A shows a vial containing the organic and aqueous phases after phase transferring PbS QDs to the top aqueous phase. In Figure E-1B, it can be observed that the main absorption peak of the PbS QDS only slightly shifted after the phase transfer, and this shift corresponds to an increase in bandgap of approximately 0.024 eV. The optical properties of the QDs are thus minimally affected by the phase transfer.
Figure E-1. PbS QDs can be transferred from octane to water while preserving their light absorption properties. A. Image of PbS QDs transferred from a chloroform phase (bottom) to an aqueous phase (top). B. Absorption spectra of PbS QDs transferred from octane (and then chloroform) to water. The absorption was normalized to the concentration of QDs.

The surface charge of the quantum dots transferred in water was measured in phosphate buffered saline (PBS) solution at pH 7.4, and it was measured again after introducing PAH into the solution. As shown in Figure E-2, the surface charge of the QDs changes from highly negative to positive after adding the positively charged PAH layer. The charge of the product was also verified at pH 4.9 in sodium acetate buffer (NaOAc), and it remained positive in the presence of PAH. Since M13 bacteriophages are negatively charged over a wide pH range, any pH above approximately 4.9 is suitable for electrostatic assembly of the two components.

Figure E-2. Once coated with PAH, the PbS QDs exhibit a positive surface charges and could be electrostatically assembled with negatively charged E3 bacteriophages. Zeta potential titration of E3 bacteriophage in 10 mM NaCl background electrolyte, and zeta potential values at pH 7.4 (PBS buffer) or pH 4.9 (NaOAc buffer) for water-soluble PbS QDs with MPA ligand and coated with PAH.
E.2 Morphology of Bacteriophage-QD complexes

M13 bacteriophages were assembled simply by mixing with positively charged PbS QDs in PBS at pH 7.4. After a few minutes, a precipitate begins to form, as the PbS QDs nucleate onto the bacteriophages and form large complexes (see Figure E-3A). The morphology of these complexes was observed using atomic force microscopy (AFM) and transmission electron microscopy (TEM), as shown in Figure E-3.

These images show that high aspect ratio structures are formed, but that the diameter of these structures is larger than the diameter of a single M13 bacteriophage. The presence of PAH might create a polymeric layer around the bacteriophages that increases their thickness.

The PbS QDs seem to co-localize with the high aspect ratio structures and therefore with the bacteriophages. At some locations, they form continuous QD chains along the bacteriophages, but they also seem absent from some sections of the nanowire-like structures. It
is possible that by increasing the ratio of PbS QD to phage, a full coating of quantum dots along the bacteriophages could be obtained.

E.3 Conclusions

To conclude, we have demonstrate that quantum dots can be electrostatically assembled along the length of M13 bacteriophages to form high aspect ratio structures. The quantum dots used for this assembly were transferred from organic solvents to aqueous solutions, while maintaining their light absorption properties. Therefore, the bacteriophage-quantum dot complexes could be used in photovoltaic devices, in combination with metal oxide nanoparticles or with small n-type molecules like fullerenes. However, if the PAH content is too high within the bacteriophage-PbS QD complexes, the polymer might create an insulating layer that would decrease the device performance. In order to construct devices that would be efficient, a positively charged conductive polymer could be used. Such a structure could also serve for the assembly of supercapacitors, or photo-activated flexible semiconductors.

E.4 Materials and Methods

Materials: Mercaptopropionic acid (MPA) was purchased from and poly(allylamine hydrochloride) (PAH) were purchased from Sigma (St-Louis, MO).

Lead sulfide quantum dot synthesis: Oleic acid-capped PbS QD synthesis followed previously published methods,\textsuperscript{5,6} also detailed in Chapter 4.

Lead sulfide quantum dot phase transfer and charge reversal: PbS QDs were either taken as a dry powder or were dried from a solution octane using a rotary evaporator. After resuspending in a few milliliters (~5 mL) of chloroform, the oleic acid-capped PbS QDs were vortexed for 15 minutes, and sonicated for 15 minutes until fully dispersed in chloroform. To transfer the PbS QDs from a chloroform phase to an aqueous phase, a solution of MPA in methanol and water at high pH was prepared (1 mL methanol, 0.3 mL water, 40 µL NaOH 40 w%, 18.2 µL MPA). The MPA solution was mixed with the PbS QDs in chloroform and the mixture was vigorously agitated for 2 hours at room temperature. The agitation was then stopped and the mixture was left standing at least 5 min for phases to separate. 7 mL of water was added gently to the mixture without disturbing the phases. The top phase is the aqueous phase now containing the PbS QDs and can be collected carefully using a Pasteur pipette.
**Bacteriophage-quantum dot assembly:** For the formation of bacteriophage-PbS QD complexes, the water soluble PbS QDs were diluted 10 times in a PBS buffer solution at pH 7.4 (1mL of PbS QDs, 8 mL of water, 1 mL of 10X PBS). To this mixture, 10 µL of a PAH solution at 20 w% was added and mixed. A total of $6 \times 10^{12}$ phages were added to the PbS QDs and the solution was gently mixed. The formation of a precipitate was observed.

**Materials characterization:** The absorption spectra of PbS QDs in octane and in water was measured using a Beckman Coulter DU 800 Spectrophotometer. The surface charge of the PbS QD particles was measured with a Malvern ZetaSizer Nano-ZS90 using disposable capillary cells. The morphology of the bacteriophage-PbS complexes was characterized by AFM using a MultiMode Atomic Force Microscope (Veeco Metrology) and by TEM using a JEOL 2010 Advanced High Performance TEM.

**E.5 References**


Appendix F. Infiltration of Conjugated Polymers within Porous Bacteriophage-Templated Titania Networks

The work presented here summarizes the methods used to infiltrate conjugated polymers into porous titania networks. Infiltrated titania films could then serve as the active layer in hybrid heterojunction solar cells. Polymers with different molecular weights were infiltrated, and the infiltration temperature was varied. The degree of infiltration was quantified using X-ray photoelectron spectroscopy (XPS) with depth profiling. The crystallinity and absorption properties of the polymers were also characterized in order to determine if degradation occurred upon infiltration.

F.1 Polymer Infiltration and Analysis via XPS Depth Profiling

Two conjugated polymers were infiltrated into porous bacteriophage-templated titania networks, poly(3-hexylthiophene) (P3HT) and poly[3-(3-carboxypropyl)thiophene-2,5-diyl] (P3CProT). The molecular structure of these polymers only differ by the number of carbon atom presents on the side chain attached to the conjugated backbone of the polymer, and by the presence of a carboxylic acid group at the end of this side chain for P3CProT. These structures, and the corresponding absorption spectra of the polymers are shown in Figure F-1. While the absorption peak of P3CProT is slightly shifted to longer wavelengths compared to P3HT, they are both suitable for photovoltaic applications, and could be combined with titania to form a heterojunction device.

Figure F-1. P3HT and P3CProT have different molecular structures, which result in different absorption profiles. A. Absorption spectra for P3HT and P3CProT solutions in dichlorobenzene (DCB) and dimethylsulfoxide (DMSO) respectively, at the same concentration. B. The structure of a P3HT and a P3CProT monomer.
The differences in molecular structures of the polymers also result in a difference in hydrophilicity. While P3HT can be dissolved in solvents like dichlorobenzene (DCB), P3CProT has to be dissolved in more polar solvents like dimethylsulfoxide (DMSO).

Conjugated polymers have been reported to be efficiently infiltrated into vertically-oriented porous networks by spincoating a layer of polymer onto the porous titania and heating up the sample at temperatures between 100 and 200 °C.\textsuperscript{1,2} Based on absorption measurements, the authors estimated that 33 % of the total film volume was composed of the infiltrated polymer, and from XPS depth profiling data, they concluded that the polymer infiltrated the titania film completely down to the bottom.\textsuperscript{1} A similar method was applied here to infiltrate polymers into the randomly-oriented pores of bacteriophage-templated titania thin films.

XPS with depth profiling was used to convert raw data measured in atomic concentration (example of raw data shown in Figure F-2) to volumetric fractions of polymer within the film. To do so, based on the carbon and titanium atomic fractions, a molar ratio of monomer to titania was calculated considering that each P3HT monomer contains 10 carbon atoms, and each P3CProT monomer has 7 carbon atoms. Then, knowing the polymer chain length, the monomer molar ratio was converted to a polymer molar ratio. Finally, assuming that the crystalline density of the polymers is 1.1 g/cm\textsuperscript{3}, and using the crystalline density of anatase titania, the molar ratios were converted to volumetric fractions.

![Figure F-2](image.png)

\textbf{Figure F-2.} XPS with depth profiling is used to measure the atomic concentration of Ti, O, C, S and Si (substrate) as a function of etching time. XPS raw data for P3HT 97kDa infiltrated at 200 °C for 4 h. Initially, only carbon is observed, which corresponds to a polymer over layer deposited on top of the titania film. Then, titanium and oxygen are detected, corresponding to the titania-polymer composite layer, and finally, the substrate signal (Si) increases indicating that the bottom of the film is reached.
First, a long chain P3HT (97 kDa) was spincoated onto a porous titania film and its infiltration profile was studied via XPS depth profiling as a function of the infiltration temperature (from no heat treatment to 200 °C) applied for 4 hours. The results are shown in Figure F-3A. A first observation can be made by looking at the thickness of the top polymer-only layer (the longer the etching time is for a 100 % volumetric fraction of polymer, the thicker the polymer over layer is). For the same amount of polymer spincoated onto the film, as the temperature increases, the thickness of the top polymer layer decreases indicating that more polymer has infiltrated the film and reduced the thickness of the top layer. Consistently, the total volumetric percentage of polymer within the film is the highest for an infiltration at 200 °C, and seems to follow a trend by decreasing slightly from 150 to 125 and to room temperature.

Next, considering that the polymer might be damaged at high temperatures, we tried infiltrating the polymer at a lower temperature (100 °C), but for a longer time (16 hours), and compared to an infiltration at 200 °C for 4 hours. Figure F-3B shows that, compared to a room temperature infiltration, a significant increase in the polymer content within the film is observed for the long infiltration at 100 °C, but this content is still lower than for an infiltration at 200 °C. Figure F-3B also shows a curve where two consecutive polymer infiltrations were performed at 200 °C. This curve presents the highest polymer volumetric content.

Finally, the effect of changing the polymer nature or the molecular weight of the polymer was studied. As shown in Figure F-3C, infiltrating P3CProT from a solution in DMSO produced the highest volumetric filling fraction compared to P3HT infiltrated at the same temperature and for the same amount of time. This could be due to the hydrophilicity of the polymer and the carboxylic acid groups that may interact with the titania surface. In fact, it is less likely that the difference in infiltration is due to the difference in molecular weights of the two polymers (16 kDa P3CProT compared with 97 kDa or 33 kDa P3HT) because the shorter P3HT polymer consistently produced films with a lower polymer content compared to the longer P3HT polymer.
Figure F-3. Polymer infiltration varies as a function of temperature, infiltration time, polymer molecular weight and hydrophilicity. XPS depth profiling results converted into total volumetric percentage of polymer within the film as a function of depth (the remaining percentage is titania) for A. 97 kDa P3HT infiltrated at different temperatures, for a fixed time of 4 h, B. 97 kDa and 33 kDa P3HT infiltrated at 100 °C for 16 h or at 200 °C for 4 h, and C. Different polymers (P3HT 33 kDa or 97 kDa, or P3CProT) all infiltrated at 200 °C for 4 h. The interface between the top polymer layer and the polymer-titania composite corresponds to the point where the polymer volumetric fraction begins to decrease from 100 %.
It has to be noted that the titania films used for this study were estimated to be approximately 51% porous (for 1 h mineralization in titanium tetrachloride), and that therefore, when the total volumetric polymer content reaches this percentage, the polymer is fully filling the pores of the titania film. The results obtained here therefore show a higher degree of infiltration compared to previously published reports. Our more efficient infiltration could be due to the longer infiltration times used, in the order of hours here as opposed to minutes in the previous reports.

F.2 Morphology of Titania-Polymer Composites

The film thickness was measured for several infiltration conditions studied above. For films constructed in silicon wafers, a macroscopic color change could also be observed after infiltration for different infiltration temperatures. Figure F-4 shows that, consistently with the longer etching times required to etch through thick polymer over layers formed at low infiltration temperatures, the final film thickness is dependent on the infiltration temperature. At higher temperatures, the thickness of the film decreases indicating that more polymer has infiltrated the titania network, and for longer infiltration times (16 h), the thickness of the film further decreases.

Figure F-4. The final film thickness and macroscopic morphology varies as a function of the polymer infiltration conditions in titania networks. Optical images of titania films infiltrated with 97 kDa or 33 kDa P3HT, on silicon wafers, and corresponding total film thickness (nm). The initial titania film thickness was approximately 100 nm.
Microscopy was also used to visualize the polymer infiltration. Figure F-5 shows SEM images, with a 53° angle, of the cross-section of porous titania films infiltrated with P3HT at room temperature or at 200 °C for 4 hours. For the infiltration at room temperature, the titania film still appears porous, especially near the bottom. When the temperature is elevated, the titania film appears glassy and completely filled with polymer.

![Figure F-5](image)

**Figure F-5.** Polymer infiltration within porous titania films can be observed with SEM. SEM cross-section images (samples at a 53° angle) of titania films infiltrated with 97 kDa P3HT at room temperature and at 200 °C for 4 h. The films were scratched using a razor blade and intact film cross-section were observed by tilting the sample.

**F.3 Polymer Crystallinity and Absorption as a Function of Infiltration Temperature**

To investigate if the polymers can resist the infiltration steps without decomposing or losing some of their light absorption properties, their stability as a function of temperature was studied. We also examined their crystallinity after the various infiltration procedures.

First, thermogravimetric analysis (TGA) was used to demonstrate that the polymers do not combust at the temperatures used for infiltration (100 to 200 °C). As shown in Figure F-6, the weight of P3CProT begins to decrease significantly at around 365 °C, and another sudden decrease if observed above 440 °C. The weight of P3HT only begins decrease around 465 °C.
Figure F-6. P3HT and P3CProT become to decompose at around 465 and 365 °C respectively, according to thermogravimetric analysis.

Although the polymers do not decompose below 365 °C, some damage might still occur at 100 or 200 °C. Therefore, for several of the conditions used for infiltration, the absorption spectrum of the infiltrated polymer was measured, as well as its crystallinity, via XRD. Figure F-7 presents the changes in absorption for two different heat treatments (100 °C for 16 hours, or 200 °C for 4 hours) and two different P3HT molecular weight. Compared to samples that were not heated, the light absorption at around 504 nm only slightly decreases when the polymer is heated to 100 °C, even when heated for several hours. However, when the temperature is elevated to 200 °C, a significant decrease in absorption occurs, and is accompanied with a shift in the absorption peak. The crystallinity of the polymers, regardless of the molecular weight, also changes significantly when temperature increases. The major XRD peak for P3HT, found around a 2 theta value of 5.25 degrees, increases with temperature to reach a maximum 150 °C, and then decreases drastically when the temperature reaches 200 °C.
Figure F-7. The crystallinity and light absorption properties of poly(3-hexylthiophene) (P3HT) changes as a function of temperature exposure. For two molecular weights of P3HT (33 kDa and 97 kDa), A. Absorption spectra, B. XRD crystalline peak, and C. Results summary as a function of temperature. The absorption and crystallinity of the polymers were measured for polymers infiltrated in titania films on glass or silicon substrates, under different conditions (16 or 4 hours heat treatment at 100 or 200 °C respectively, compared with no heat treatment).

Therefore, there might be an optimal temperature, in between 100 and 150 °C, where the polymer absorption is still high, but its crystallinity is also high. An optimal infiltration temperature of 125 °C was previously reported, and yielded a high power conversion efficiency compared to devices prepared at 165 or 210 °C.3

A highly crystalline polymer could increase the rate of charge transport within the film if the polymer chains are properly oriented. Some reports claim that polymer chains aligned parallel to the side walls of the pores, with their side chains perpendicular to the pores, could improve charge transport because charges travel from monomer to monomer. This orientation would then promote a more direct charge transport to the electrodes, and would be induced by the nano-confinement of the polymer.4 Other reports assert that no preferred polymer oriented is
observed within nanometer-sized pores, but that nano-confinement still enhances charge transport mechanisms in conjugated polymers. Nonetheless, it appears like the size and geometry of the pores plays a significant role in the alignment of the polymer chains. Further experiments would need to be carried out to determine which infiltration conditions yields the optimal device performance in bacteriophage-templated hybrid titania-polymer devices with randomly-distributed pores.

F.4 Conclusions

In conclusion, we have demonstrated here that conjugated polymers can be efficiently infiltrated within porous bacteriophage-templated titania networks with pore diameters in the order of 10 nm. The total volumetric percentages of polymer measured here are, for several infiltration conditions, higher than those previously reported in literature for vertically-aligned pores of similar sizes. While higher temperatures promote polymer chain mobility and infiltration within small pores, the optical properties of the polymers may be affected by heat treatments above 100 °C. The crystallinity of the polymers also varies considerably with the infiltration conditions. Device performance should be measured for the various infiltration conditions to determine if crystallinity is important for device performance, or if significant polymer degradation occurs during the infiltration step.

F.5 Materials and Methods

Materials: Poly(3-hexylthiophene) (P3HT) 97kDa and 33kDa were purchased from Sigma (St-Louis, MO). P3CProT was purchased from Rieke Metals Inc. (Lincoln, NE).

Polymer infiltration: Polymer were infiltrated in the porous bacteriophage-templated titania films by dropcasting or spincoating a polymer solution onto the titania film, and allowing it to dry in a glovebox. Heat was applied using a hot plate inside of a glovebox.

XPS with depth profiling: XPS with depth profiling was used to quantify the polymer infiltration with titania films using a PHI Versa-Probe II X-ray photoelectron spectrometer with a scanning monochromated Al source (1,486.6 eV; 50 W; spot size, 200 μm), and the sputtering was done using an argon source. The data was collected every minute or two, and the sample was exposed to zalar rotation at a rate of 1 rpm. Atomic concentrations were converted into volumetric fractions using the crystalline density of titania and conjugated polymer.
F.6 References


Appendix G. Synthesis and Magnesiothermal Reduction of Titania on M13 Bacteriophage and *Spirulina* Biotemplates

The goal of this appendix is to show that *Spirulina major* can be mineralized with titania, and that biotemplated titania structures can be magnesiothermally reduced to produce metals, carbides and nitrides, while preserving the morphology of the template.

This work was done in collaboration with Dr. Stephen A. Steiner III, and Victor J. Cantú.

G.1 *Spirulina*-Templated Titania and Titania-Nickel Oxide Composites

Mineralizing *Spirulina* algae with various metal oxides and subsequently magnesiothermally reducing the structures could lead to the product of various spiral or coil-shaped metallic materials. We have demonstrated, in Chapter 5, that *Spirulina major* could be used to produce spiral-like silicon microstructures. In addition, *Spirulina* has been used recently to synthesize copper micro-coils with various helical geometries. (Kamata, 2014 #1) Growth conditions, including light intensity and temperature, affect strongly the final shape of the spiral-like algae and allows for controlling parameters like the pitch of the helix, which can be tighter for growth under higher temperatures and light intensities. (Kamata, 2014 #1)

Using this concept to prepare more coil-shaped metallic structures could allow for the formation of micro-coils that could be stimuli-responsive and contract or retract upon, for instance, a change in temperature. Nickel-titanium alloys (NiTi) are such stimuli-responsive and shape-memory materials. The goal here is to take a step towards the production of useful materials on *Spirulina* templates, like a NiTi coating. A first step in this direction is to mineralize titania onto the algae to produce titania micro-coils.

Titania can be mineralized onto *Spirulina* algae using titanium tetrachloride as a precursor, but the concentration of precursor and reaction time has to be precisely controlled in order to obtained spiral-like structures. If the precursor concentration is too high (around 0.2 M), then large titania aggregates are formed and no spirals can be observed. The same is applicable to reactions that occur for too long (~ 1 h). Reducing the TiCl₄ concentration 10 times, and the reaction time to approximately 35 minutes, or until the solution begins to change color to a light blue, allows for the synthesis of some spiral-like structures, as shown in Figure G-1. These reaction conditions allow for maintaining the titania nanoparticle size small enough for an
efficient separation of the large algae-titania structures from the nanometer-sized free titania nanoparticles via centrifugation.

Figure G-1. Titania can be mineralized onto *Spirulina major* algae, forming titania spiral bundles. SEM images showing the titania structures after burning off the algae template at 500 °C.

However, it remains difficult to obtain perfectly individual spiral-shaped titania structures, which might be due to the low specificity of the reaction. Just like in Chapter 5, where a first silica layer was nucleated onto the polysaccharide surface of *Spirulina major*, using an isocyanate-functionalized silane, targeting the surface of *Spirulina* to specifically nucleate titania might promote the formation of individual spirals.

In an attempt at preparing a titania-nickel oxide composite coating onto *Spirulina*, a solution was prepared containing a total of 0.02 M precursors (0.01 M TiCl$_4$, and 0.01 M nickel chloride), and the algae were incubated in this mixture as described above. However, several aggregates were observed after reaction, and the no three-dimensional spiral structures could be observed. Only spiral-like traces confirmed that *Spirulina* was present in the reaction mixture and produced void spaces after annealing the sample at 500 °C (see Figure G-2). The drastically different morphology observed here could be due to differences in reaction mechanisms between the titania and nickel oxide precursors, and to an acceleration of the reaction. Further optimization would be required to determine the reaction time and molar ratio of precursors required to produce titania-nickel oxide composites that could be magnesiothermally reduced to temperature-responsive NiTi alloys.
Figure G-2. Spiral traces can be observed through a matrix of titania and nickel oxide particles. SEM image showing the result of a reaction containing 0.01 M titanium tetrachloride, and 0.01 M nickel chloride. The algae were burnt off at 500 °C.

G.2 M13 Bacteriophage-Templated Titania and Titanium Nitride

One of the first magnesiothermal reductions performed in the Belcher lab was carried out on bacteriophage-templated titania films. The bacteriophages were burnt off at 500 °C, but the reaction was carried in a closed stainless steel vessel sealed under nitrogen as opposed to a reactor containing argon gas. The morphology of the bacteriophage-templated titania film before and after magnesiothermal reduction (MGTR) is shown in Figure G-3.

Figure G-3. Bacteriophage thin films mineralized with titania can be reduced via magnesiothermal reduction and preserve their initial morphology. SEM images before and after magnesiothermal reduction for bacteriophage films mineralized with titania.

As a result of the nitrogen environment, titanium nitride was observed in the final product. In addition, XPS spectra revealed the presence of titanium monoxide (see Figure G-4). Titanium metal could not be clearly identified, indicating that the reaction did not fully proceed. In literature, titania has been reported to be reduced to titanium via MGTR, but at higher

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temperatures and using addition de-oxidation steps. This could indicate that the temperature at which the reaction is carried out here (650 to 750 °C) is not sufficient for the reduction to occur. In addition, since the reaction was carried out in the presence of nitrogen, the titanium nitride formation might be favored over the formation of titanium metal.

![Figure G-4](image)

Figure G-4. Bacteriophage-templated titania is converted to titanium oxide, nitride and carbide after magnesiothermal reduction in nitrogen gas. XPS spectra showing the Ti2p and N1s regions before and after MGTR.

Although titanium metal could not be obtained, titania nitride, if porous or nanostructured, has applications in catalysis, and magnesiothermal reduction could become an efficient method for producing catalysts and catalyst supports with defined shapes.

G.3 Conclusions

In summary, titania can be mineralized onto bacteriophage thin films, but also on *Spirulina major* algae to create coil-shaped structures. With further optimization, individual titania spirals may be synthesized, and alloys that may act as stimuli-responsive materials could also be produced. Upon magnesiothermal reduction of bacteriophage-templated titania films under nitrogen gas, titanium nitride is formed and could be used for catalytic applications. Similarly, if the biotemplate was left into the structure, titanium carbide could be formed. Finally, in order to produce titanium metal, the reaction should be carried out under argon atmosphere, and higher reaction temperatures might be required.
**G.4 Materials and Methods**

**Titania biomineralization on *Spirulina major***: *Spirulina major* biofilms were thoroughly agitated, vortex and pipetted in order to break them into individual algae or small pieces of biofilm. The free algae were incubated with agitation in a TiCl$_4$ solution at 0.2 or 0.02 M for 35 min to 1 h, at 80 °C. The algae coated with titania were separated from free titania nanoparticles or excess reactants via centrifugation and rinse cycles with milli-Q water. The mineralized algae were deposited onto silicon substrates and annealed at 500 °C.

**Titania biomineralization on bacteriophage films**: Layer-by-layer assembled bacteriophage thin films were mineralized with titania as described in Chapters 3 and 4, by immersing the thin film in a 0.2 M TiCl$_4$ solution at 80 °C for 1.5 h. After mineralization, the films were rinsed with milli-Q water and annealed at 500 °C for 1 h.

**G.5 References**
