

# Virus-enabled Synthesis of Titanium Oxide Nanowires

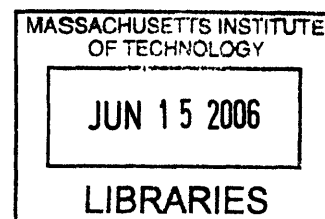
by

Forrest Liou

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## **Abstract**

Bio-assisted materials fabrication methods allow for the production of high technology materials and devices at lower costs and with less environmental impact. To expand the biological toolkit for synthesizing materials, we demonstrated titanium oxide nanowire synthesis with use of engineered M13 virus at room temperature. In this virus-enabled synthesis process, negatively-charged titanium fluoro complexes nucleate at positive amine sites on the virus, and a subsequent anion-scavenging reaction drives the synthesis of titanium oxide on the virus. TEM imagery provided visual validation of the nanowire formation, and XRD analysis identified the crystalline structure as anatase.

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# 1. Motivation

## 1.1. *Bio-enabled Materials Synthesis and Assembly*

Nature demonstrates the astonishing possibilities of biological self-assembly for creating intricate functional systems at room temperature in aqueous solution. As nanoscale materials become increasingly important for emerging technological applications, there is much incentive for us to look to nature for inspiration [1-5].

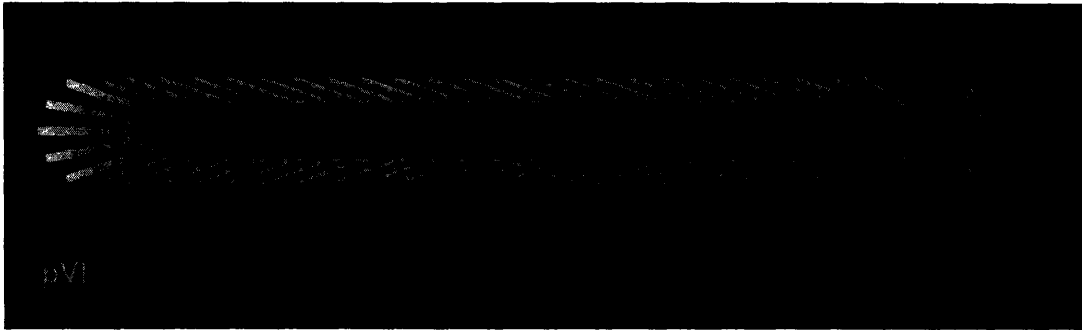
Already, biomolecules have been used to nucleate technologically important inorganic materials, such as semiconductor materials, magnetic materials, and metals [6-9]. More complex biological entities, including genetically engineered viruses and yeasts, have also been employed to nucleate and arrange quantum dots [10], nucleate nanowires [11,12], and form multidimensional liquid crystals and films [13-15]. Expanding this biological toolkit will facilitate further technological innovation in nanobiotechnology for a wide range of applications, such as in computing, medicine, and energy.

## 1.2. *The M13 Bacteriophage as Model Organism*

### 1.2.1. Background

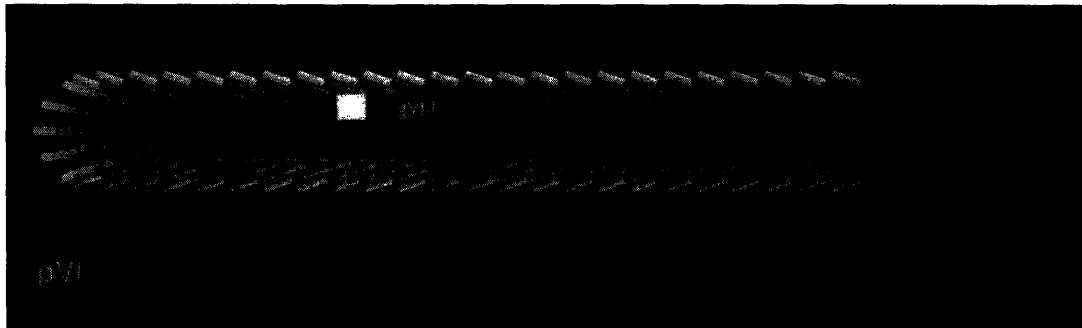
The M13 filamentous bacteriophage (genus *Inovirus*) contains a circular single-stranded DNA genome encased in a long, cylindrical, and flexible protein capsid [16]. This virus specifically infects *Escherichia coli* bacteria by attaching to the tip of the F conjugative pilus. After infection, the viral DNA is replicated by a mixture of both virus-encoded and bacterial components, which results in a newly synthesized, single-stranded DNA bound to copies of virus-encoded DNA-binding protein. Concurrently, viral capsid proteins are synthesized and integrated into the bacteria membrane. During virus assembly, the new viral DNA is extruded through assembly sites of the bacteria membrane, at which the DNA-binding proteins are removed and the capsid proteins in the membrane are packaged in a helical, five-fold symmetry around the viral DNA. The resulting structure is approximately 7 nm wide and 1  $\mu\text{m}$  long in wild-type form.

This virus is highly useful for its genetically modifiable major (gP8) and minor (gP3, gP6, gP7, gP9) coat proteins (Fig. 1). The capsid comprises approximately 2,700 copies of gP8 protein. The functionality of these proteins can be modified through additions in the viral genome. As such, genetic modification has been used to produce virus-based templates for synthesizing and arranging nanomaterials [17, 18], including nanowires [19, 20] that can self-assemble into a liquid crystal film [21, 22].



**Figure 1. Wild-type virus.** Genetically modifiable coat proteins surround the virus DNA to form a structure approximately 7 nm wide and 1  $\mu\text{m}$  long.

Our study utilized the E4 clone of the M13 virus. This clone, produced through genetic modification and expression using a phagemid system, contains gP8 coat proteins with tetra-glutamate (EEEE-) peptides fused to the N termini (Fig. 2).



**Figure 2. E4 virus clone.** Tetra-glutamate (EEEE-) is fused to the N termini of the gP8 coat proteins.

### **1.2.2. Technological Significance**

The M13 virus can serve as a genetically programmable universal template for the synthesis and assembly of materials. By fusing peptide inserts into appropriate regions of the virus, it can be engineered to nucleate materials and self-organize into useful structures. For example, nanorings have been made by genetically modifying the ends of a virus to bind to a linker molecule [23]. Furthermore, nanostructured cobalt oxide lithium ion battery electrodes have been synthesized and assembled with the E4 clone through a polyelectrolyte multilayer dipping process [24], thereby demonstrating the practicality of the biological approach.

This kind of bio-enabled materials production may lead to higher performance products in smaller, more flexible packaging. Additionally, the products would be manufactured without the use of highly expensive equipment (e.g., vacuum furnaces) or toxic organic solvents. Furthermore, the knowledge derived from working with the M13 virus could be applied to other types of biological entities in the future to create an even greater variety of products.

### **1.3. *Titanium Oxide as Material of Study***



Titanium oxide is a technologically important material because of its unique electronic and photoelectrochemical properties. It is a promising material for self-cleaning, photocatalytic surfaces [25, 26], high lifetime batteries [27], rugged gas sensors [28], and dye-sensitized solar cells [29]. Virus-enabled synthesis and assembly methods have the potential to improve titanium oxide based devices by producing novel titanium oxide microstructures and enabling advantageous product form factors (e.g., thin films or fibers).

## 2. Specific Project Goals

Liquid phase deposition of titanium oxide can involve reacting titanium as part of a negative ion complex with fluorine ( $[\text{TiF}_6]^{-2}$ ). As current virus-enabled methods employ either the molecular recognition of specific crystallographic faces of materials or the negative charge attraction to positive metal ions, a new method for interacting with negative species was necessary. Accordingly, the project objectives were to find a method to attach negative ions and ion complexes to engineered viruses for greater flexibility in virus-enabled materials synthesis. In particular, we investigated the nucleation of an inorganic material (titanium oxide) through the use of the amine functionality of an engineered virus. Results from this study would provide a possible future means for producing hybrid nanomaterials, such as  $\text{Li}_4\text{Ti}_5\text{O}_{12}$  for batteries and solar cells.

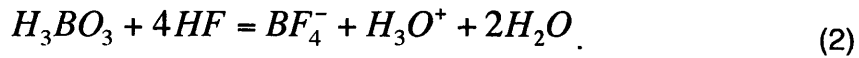
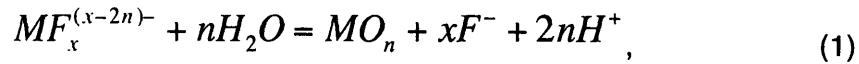
### **3. Experimental**

#### **3.1. *Virus Preparation***

Tetraglutamate (EEEE-) was fused to the N terminus of each copy of the major coat p8 protein with 100% expression. The E4 clone was chosen because it had been successfully used with charged polymers in electrostatic multilayer thin film assembly [30, 31].

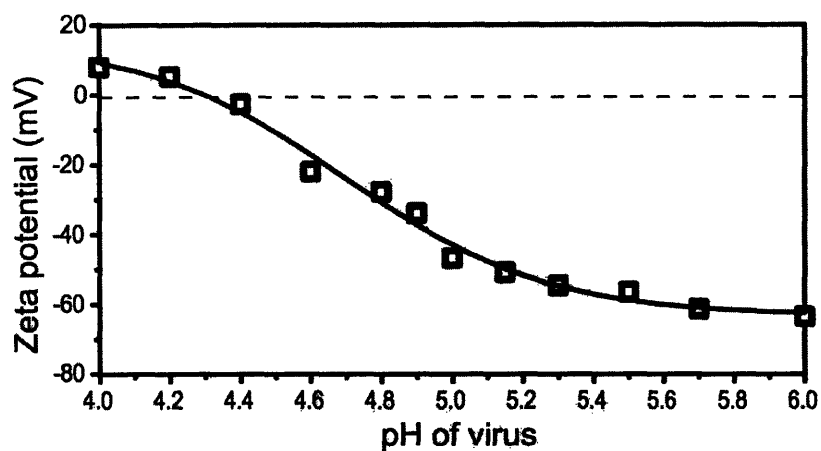
#### **3.2. *Titanium Oxide Formation***

Liquid Phase Deposition (LPD) is a wet process for depositing metal oxide or hydroxide thin films homogeneously on various types of surfaces [32]. The relatively simple process involves immersing a substrate in an aqueous solution of the reactant, which allows a metal oxide thin film to form through a ligand-exchange (hydrolysis) equilibrium reaction. In the reaction, a metal-fluoro complex species reacts with water to produce metal oxide, and an F<sup>-</sup> consumption reaction with boric acid or aluminum metal acting as the F<sup>-</sup> scavenger accelerates the ligand-exchange reaction by shifting the equilibrium reaction to the right-hand side. For example:

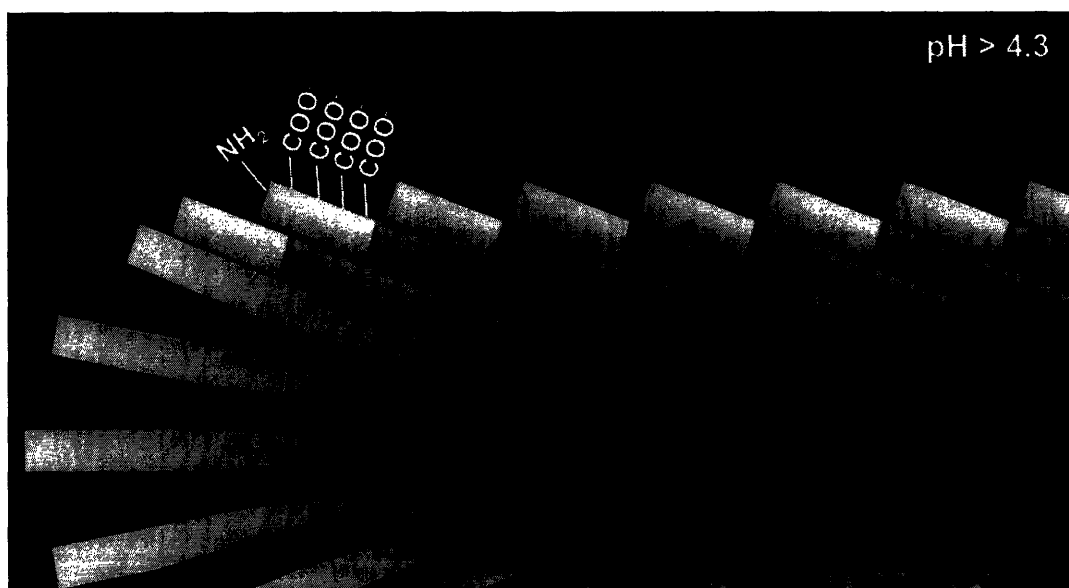


This technique has been used to fabricate various titanium oxide conformal structures, including macroporous films using a colloidal template [33], and gold-dispersed thin films [34].

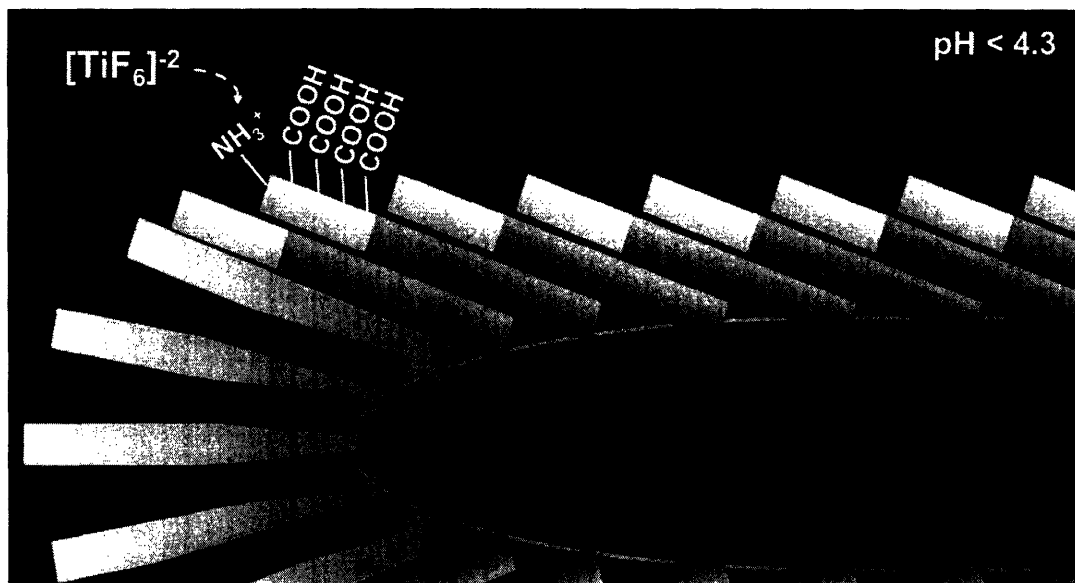
To utilize this titanium oxide synthesis technique on an E4 virus template, we needed to activate the virus' amine functionality. As shown in Fig. 3, the zeta potential (overall surface charge) of the virus changes from negative to positive as the pH of the surrounding solution is lowered to less than approximately 4.3 [35]. When near neutral pH, the virus can serve as a template for nucleating positive ions or ion complexes (Fig. 4). At a low pH, the positive amine functionality would serve as sites for preferential nucleation of the  $[TiF_6]^{2-}$  negative ion complex for targeted liquid phase deposition (Fig. 5).



**Figure 3. Zeta potential of E4 virus as a function of solution pH.** The virus changes from an overall negative surface charge to an overall positive surface charge as the pH is below approximately 4.3.



**Figure 4. Tetraglutamate (EEEE-) fused to the N-terminus of a viral p8 major coat protein, shown near neutral pH.** The representative graphic shows only one copy of the fused peptide. The carboxyl negative functionalities have been used to nucleate positive metal ions such as cobalt for synthesizing cobalt oxide nanowires that can be electrostatically assembled with charged polymers into a multilayer thin film structure.



**Figure 5. Tetraglutamate (EEEE-) fused to the N-terminus of a viral p8 major coat protein, shown at low pH.** The representative graphic shows only one copy of the fused peptide. The positive amine functionality can be used to nucleate the negatively charged titanium fluoro complex for synthesizing titanium oxide.

Virus-templated titanium oxide formation was achieved by first incubating  $10^{10}$  mL<sup>-1</sup> E4 virus in 200 mL of 5 mM ammonium hexafluorotitanate (99.99% metals basis, Alfa Aesar) for 30 minutes to allow the [TiF<sub>6</sub>]<sup>-2</sup> ion complexes to attach to the virus' positive amine sites. Subsequently, 200 mL of 350 mM boric acid (Puratronic® 99.9995% metals basis, Alfa Aesar) was added to drive the ligand-exchange reaction overnight.

### **3.3. *Transmission Electron Microscopy***

In transmission electron microscopy (TEM), an electron beam is focused onto a specimen. And as the electrons pass through the specimen, interactions with the specimen create beam distortions that are visible in a magnified projection on a phosphor screen [36]. TEM images were captured on a JOEL JEM-2001X1A1.

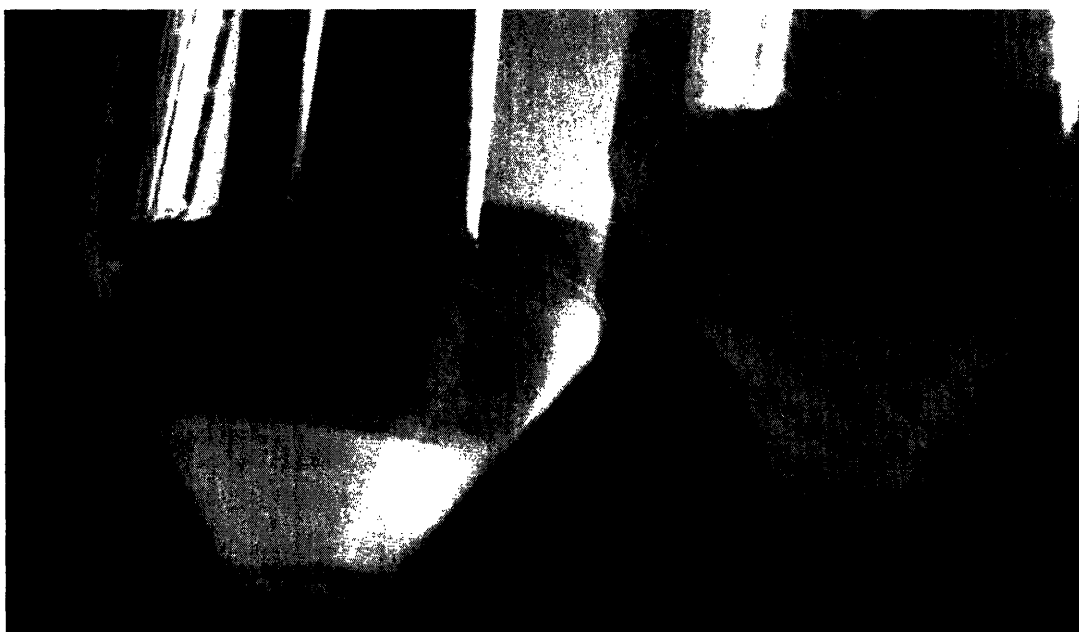
### **3.4. *X-Ray Diffraction***

In x-ray diffraction (XRD), x-rays travel through a specimen and are scattered by the regularly spaced atoms in the specimen material. The crystal structure(s) of the powdered specimen are then determined by matching the characteristic diffraction peaks with those in a database. XRD data was collected on a Rigaku Rotaflex RU 300.

## **4. Results**

### **4.1. *Visual Inspection***

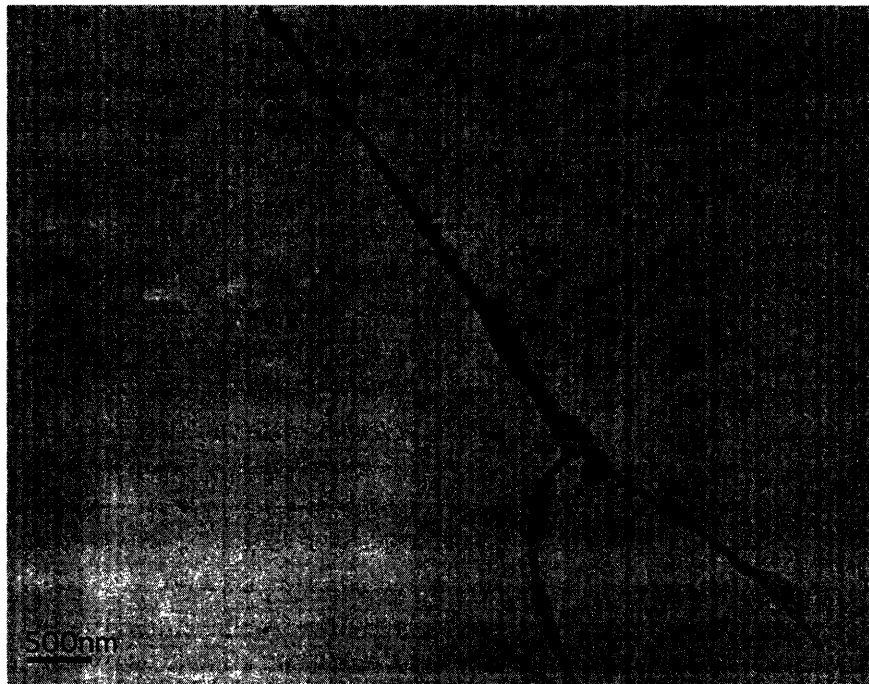
The acid-driven reaction produced a semi-opaque, thin, white film near the bottom of its plastic vessel and a noticeable amount of white, suspended particulates (Fig. 6). Droplets from this suspension were viewed via TEM. Spinning out the reaction products using a centrifuge yielded an aggregate of white material at the button of the vessels. After pouring out the fluid and letting the material air-dry to powder form, the material was inspected via XRD.



**Figure 6. Titanium oxide formation.** The virus-templated titanium oxide product is a result of the room temperature ligand-exchange equilibrium reaction of ammonium hexafluorotitanate with water, accelerated through the addition of boric acid. Best results were obtained by incubating  $10^{10}$  mL<sup>-1</sup> virus in 200 mL of 5 mM ammonium hexafluorotitanate for 30 minutes, then adding 200 mL of 350 mM boric acid and leaving overnight.

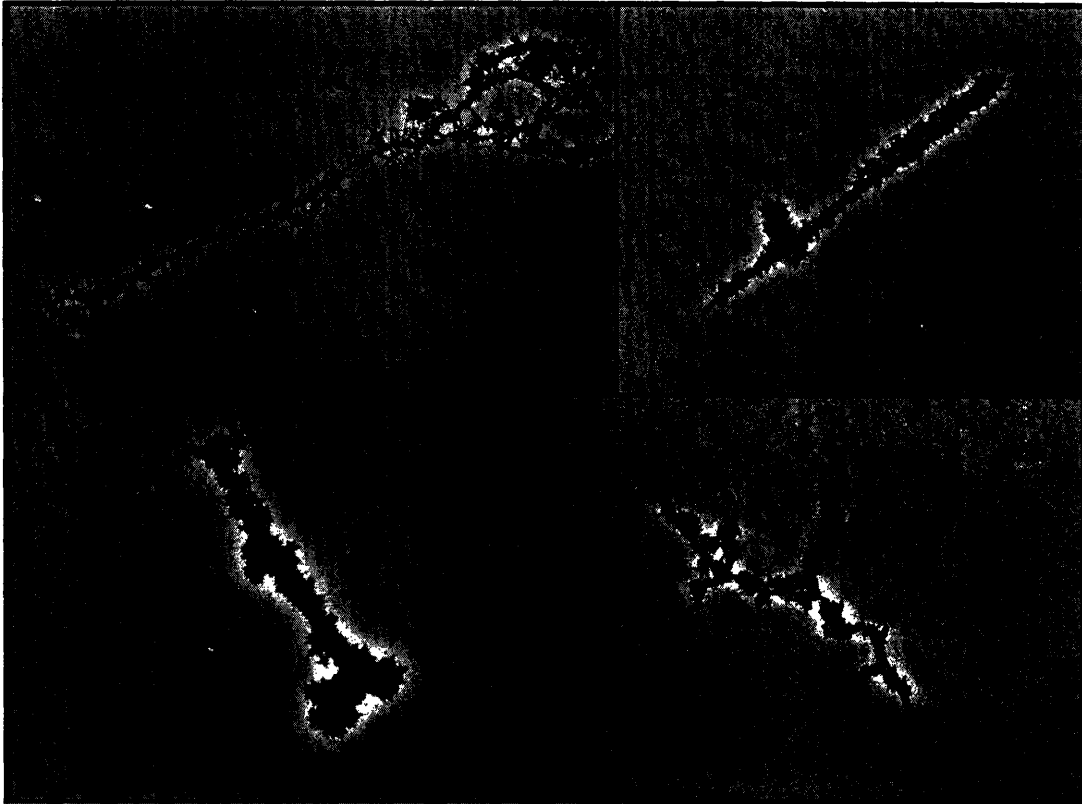
## 4.2. *Transmission Electron Microscope Analysis*

A virus aggregate without deposited titanium oxide is shown in Fig. 7 as a visual control. Exemplary virus aggregates with titanium oxide are shown in Fig. 8. We see that the nanowires are a result of titanium oxide nucleating and growing on virus aggregates, since individual viruses are only approximately 1  $\mu\text{m}$  long. The rough texture may be due to an excessively fast reaction.



**Figure 7. Transmission electron microscope image of a virus aggregate.** Individual viruses are typically approximately 1  $\mu\text{m}$  long.

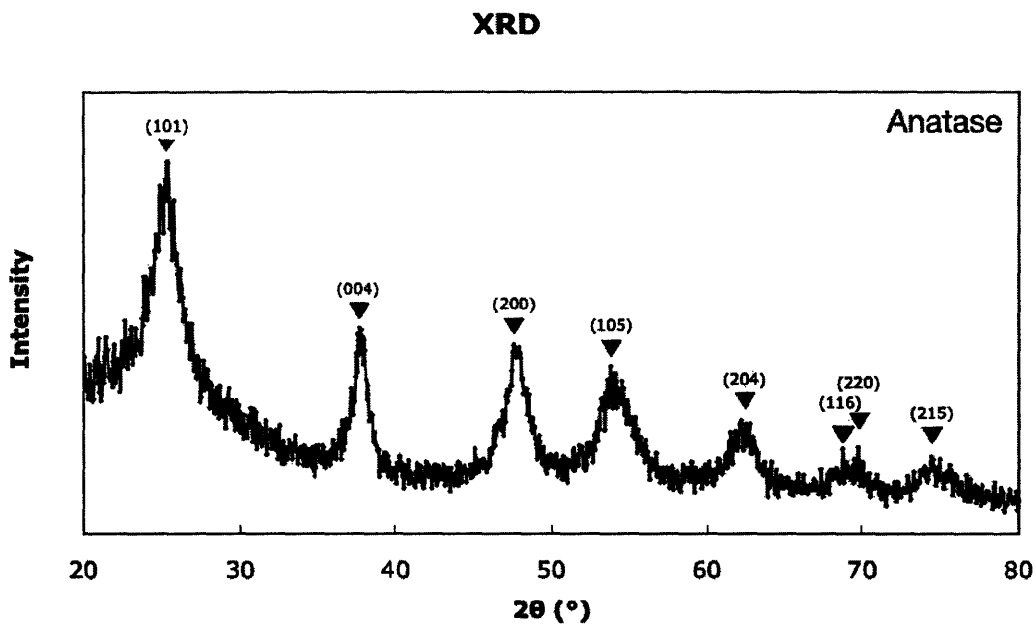




**Figure 8. Transmission electron microscope image of virus aggregates with titanium oxide synthesized at room temperature.** Individual viruses are typically approximately 1  $\mu\text{m}$  long. All four images are scaled proportionally.

### ***4.3. X-Ray Diffraction Analysis***

The x-ray diffraction peaks match those for the anatase form of titanium oxide (Fig. 9). The two other common crystal forms for titanium oxide are rutile and brookite. Of the three forms, anatase is the most preferred, as it exhibits unique electronic and electrophotocatalytic properties that are useful for the aforementioned titanium oxide applications.



**Figure 9. X-ray diffraction spectrum of the virus-templated titanium oxide product.** The diffraction peaks match those of the anatase crystallographic form of titanium oxide.

## 5. Discussion

The TEM and XRD analyses show that anisotropic anatase titanium oxide nanostructures formed on the E4 virus templates at room temperature. Tuning the reaction conditions and parameters could control the synthesis efficiency and product morphology. We found that when the ammonium hexafluorotitanate concentration greatly exceeded 5 mM, messy film growth occurred. On the other hand, when the concentration was smaller than 5 mM, little reaction occurred overnight. For the boric acid, when the concentration exceeded 350 mM, small particles nucleated throughout the solution. And when the concentration was

much smaller than 350 mM, slow film growth occurred. With further experimentation with the reaction parameters and conditions, it may be possible to create nanowires consisting of a uniform cylindrical shell of discrete nanoparticles.

## 6. Future Work

As titanium oxide is a promising material for battery anodes, an appropriate next step for this project is to measure the electrochemical properties of the synthesized nanowires, and to improve those properties by optimizing the synthesis process to achieve favorable texture and morphology.

Another important area for further investigation includes examining the titanium oxide synthesis efficacy of the E4 virus clone versus that of the wild-type virus. Nanowire synthesis using other fusion peptide sequences, including those with more than one amine groups, should be attempted and the results compared.

Once sufficient control of the titanium oxide synthesis is achieved, the creation of hybrid materials such as  $\text{Li}_4\text{Ti}_5\text{O}_{12}$  can be attempted. The advantage of using an engineered virus such as the E4 is that the bifunctionality (from having both amine and carboxyl functionalities for nucleating different materials) can be leveraged to synthesize multiple materials on the same virus template, and allow

the synthesized hybrid nanowires to be arranged by means of electrostatic assembly. This work and future extended efforts will serve to expand the virus-based biological toolkit for producing increasingly sophisticated materials and devices.

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