Progress Update for DE-FG02-02ER45975

Virus Assemblies as Templates for Nanocircuits

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Progress Report:

In the six months since receiving funding we have made significant advances in our stated project goals. Specifically, we have brought on board the personnel needed for the project (See personnel below) and are making rapid progress in developing specific viral-based biotemplates and methodologies for the production of novel nanoparticles and wires. A manuscript has been prepared on the stability of TMV particles in water-alcohol mixtures for planned submission to *Langmuir*.

Objective 1. Determine the methodology needed to nucleate different inorganic solids such as gold and copper onto and within the Tobacco mosaic virus (TMV) virion. Preliminary experiments have shown that the TMV virion can serve as a template for the nucleation of inorganics. Efforts in this objective have focused on optimizing these processes as well as modeling the interactions that lead to nucleation/mineralization.

Experiments have shown that TMV bioparticles can be coated with materials such as silica, and silver. To coat the TMV particles with silver, the TMV particles were first coated with a thin layer of silica and subsequently coated with the silver using a coupling agent. In each of the coating steps, the TMV particles tended to aggregate; therefore, it was decided that we needed to establish the stability of the TMV particles in alcohol/water mixtures when an electrolyte was present. This mixture was used because organometallic compounds are employed to coat the TMV particles. These compounds are immiscible in water; however, they are miscible in water/alcohol mixtures. Additionally, the organometallic compounds can produce ionic species that increase the ionic strength of the solution and, therefore, enhance the aggregation of the TMV particles.

To produce good nanowires and nanotubes, it is imperative that the TMV particles remain colloidally stable (unagglomerated). The TMV particles are stabilized by electrostatic repulsive forces. Electrolyte concentrations over a few mmol/L can result in the aggregation of the TMV particles due to a reduction in the electrostatic repulsive forces. Our studies have established the concentration of 1:1 electrolyte over which the TMV particles are stable in water and mixtures of water and several low molecular weight alcohols (methanol, ethanol, and 1-propanol). It was also found that both electrolyte and alcohol content affected the stability of the TMV particles. Thus, regions where solvophobic and electrostatic instabilities occur have been mapped. Dynamic laser light scattering (particle size measurements) and Laser Doppler Electrophoresis (electrophoretic mobility measurements) were used to establish the stability of the TMV particles.

Future research efforts are focusing on producing coated TMV particles at solution conditions where the TMV particles are stable. Both batch type experiments and continuous flow experiments are planned. In the batch type experiments, the TMV particles will be suspended in the reacting solutions or in a suspension of metal oxide, silica or metal nanoparticles. The suspension will be slowly agitated as the TMV particles are being coated. The flow through experiments will involve the use of a flow chamber where an electric field will be applied across the channel to trap the TMV particles and to concentrate any charged particles in the region to enhance the coating process.

Objective 2. Molecular alterations to improve the mineralization of Tobacco mosaic virus. The goal of this objective is to utilize existing molecular biology and protein engineering techniques to alter the surface characteristics of the TMV virion to enhance its ability to act as a biotemplate for the production of nanoparticles.

Repulsive carboxy-carboxylate interactions occur between individual coat protein subunits of the TMV virion and are known to control virus particle assembly and disassembly. We hypothesized that by altering these interactions we could create virus particles with novel assembly profiles and structures that would be useful in the production of nanoparticles. Specifically, we utilized two approaches to disrupt/alter carboxy-carboxylate interactions. In the first approach, we took advantage of several existing mutants in which the specific coat protein amino acids involved in the carboxy-carboxylate interactions were removed. The structural affect of these mutations is a virus coat protein that can self-assemble into rod-like particles of indefinite length. These mutant viruses should be useful in creating long wire-like biotemplates. At present we have purified sufficient quantities of these viruses to begin mineralization studies. A second approach is directed at altering the spatial location of carboxy-carboxylate interactions within the three dimensional structure of the virus

particle. By moving the location of these interactions we hope to change the ability of individual coat protein subunits to self-associate, such that the overall structure of the rod-like virus particles becomes more flexible and therefore more suitable as a wire template. Currently, we have created a set of seven specific coat protein mutants directed at changing the location of the carboxylate interactions. The biophysical properties of these mutants are currently under investigation.

Another goal of this objective is directed at adding a number of cysteine residues to the surface of the TMV virus particle. When oxidized the negatively charged cysteine sulfur groups are capable of bonding with positively charged metals such as gold or copper, thus, creating an enhanced surface for the deposition of these metals. To date we have engineered coat proteins with either one or two surface exposed cysteine residues. These mutant coat proteins are currently being cloned into the virus genome for expression and characterization.

Objective 3. Controlled assembly of mineralized TMV structures to produce novel composites. The known assembly process of TMV will be utilized and or modified to allow assembly of differentially mineralized TMV virion subunits with the goal of producing novel TMV based superstructures.

It is by controlling the formation of the initial nanostructures that the coating of the TMV virion subunits with silica, metal oxides and metals will be optimized. Thus, these initial structures must be monitored by in-situ and ex-situ techniques. Transmission electron microscopy and ultra-small angle X-ray scattering were used to determine the size and structure of the nanophases that are produced during the early stage formation of silica and silicalite-1 (a type of zeolite). One experiment was also done at the USAXS – UNICAT facility at the Argonne National Laboratory on TMV particles that were coated with silica. Additional experiments are required at SAXS and USAXS facilities to better characterize the structure of the final TMV coated particles and to investigate the dynamics of the coating process. Other experimental techniques such as Atomic Force Microscopy with a wet stage or a dry stage are being investigated as tools for characterizing the coated TMV particles, especially those particles that have been formed from the differentially mineralized TMV virion subunits.

Future Research Summary/Significance:

We will continue to address the research goals as stated in the above research objectives (See progress report). It is our belief that nanoscale technologies hold the potential to improve a broad range of energy, manufacturing, and environmental processes/issues. Furthermore, biological systems have evolved particularly sophisticated mechanisms to promote the ordering of molecules at

the nanoscale level. The aim of this research combines expertise in materials chemistry/colloids (Dr. Harris, Purdue University) with protein engineering/virology (Dr. Culver, University of Maryland Biotechnology Institute) to address the application of biologically derived molecules for the production of nanoparticles. The parameters and processes developed in this research program should have broad application for the use and engineering of biologically derived templates for the production of nanotubes, nanowires and nanoshells of varying geometries.

Project Personnel:

Purdue Laboratory:

Dr. Mike Harris	PI
Elizabeth Royston*	Graduate Student
Sang-Yup Lee	Graduate Student

Maryland Laboratory

Dr. James Culver	PI
Haimi Shiferaw	Research Associate
Elizabeth Royston*	Graduate Student

*Elizabeth Royston is a graduate student in the Dept. of Chemical Engineering, Purdue University. Elizabeth spent the summer of 2002 working in Dr. Culver's laboratory at the University of Maryland, learning techniques for the purification and manipulation of the virus coat protein. She is currently working in Dr. Harris's laboratory at Purdue.

Current and Pending Support (9/30/02)

Current:

NSF (0113536) \$330,000, 9/01 to 8/04. Genetic Analysis of Tobamovirus-Host Interactions.

DOE (DE-FG02-02ER45975; This Proposal) \$223,559, 5/02 to 4/05. Virus Assemblies as Templates for Nanocircuits.

Pending:

USDA, \$292,137, 7/03 to 6/06. Functional Dissection of the Tobamovirus Replicase.

Published Results:

Abstract to be presented at the American Institute of Chemical Engineers 2002 Annual Meeting, November 3 – 8, Indianapolis IN.

COLLOIDAL STABILITY OF TOBACCO MOSAIC VIRUS IN WATER AND ALCOHOL MIXTURE Sang-Yup Lee¹, Elizabeth Royston¹, James N. Culver², and Michael T. Harris¹

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Abstract

The dispersion of nano-sized biomaterials provides a new way for nano-scaled material synthesis; however fundamental study is necessary for the further applications. Tobacco Mosaic Virus (TMV) is a widely used virus as a template for its rod-like shape with high aspect ratio. In this research, the colloidal stability of TMV dispersion in water and alcohol mixtures is studied by measuring the hydrodynamic radius and the electrophoretic mobility. Dynamic light scattering (DLS) is used to measure the hydrodynamic radius that represents the flocculated particle size, and the DELSA, an electrophoresis system, is used for the electrophoretic mobility measurement.

With an increase of the salt contents in the mixture, the hydrodynamic radius gradually increases that is due to the decrease of electrostatic repulsion between the particles. On the other hand, the particle-agglomerated size drastically rises with an increase of the alcohol weight fraction, which originates from the biological instability of TMV at low water contents. These results are important for the initial step of bio-particle coating by wet chemistry, especially sol-gel coating or metal particle deposition on the bio-particles.