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FINAL REPORT

Multicomponent Protein Cage Architectures for Photocatalysis

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1. PROGRAM SCOPE

The primary goal of the project was to develop protein-templated approaches for the synthesis and directed assembly of semiconductor nanomaterials that are efficient for visible light absorption and hydrogen production. In general, visible-light-driven photocatalysis reactions exhibit low quantum efficiency for solar energy conversion primarily because of materials-related issues and limitations, such as the control of the band gap, band structure, photochemical stability, and available reactive surface area of the photocatalyst. Synthesis of multicomponent hierarchical nano-architectures, consisting of semiconductor nanoparticles (NPs) with desired optical properties fabricated to maximize spatial proximity for optimum electron and energy transfer represents an attractive route for addressing the problem. Virus capsids are highly symmetrical, self-assembling protein cage nanoparticles that exist in a range of sizes and symmetries. Selective deposition of inorganic, by design, at specific locations on virus capsids affords precise control over the size, spacing, and assembly of nanomaterials, resulting in uniform and reproducible nano-architectures. We utilized the self-assembling capabilities of the 420 subunit, 60 nm icosahedral, P22 virus capsid to direct the nucleation, growth, and proximity of a range of component materials. Controlled fabrication on the exterior of the temperature stable shell was achieved by genetically encoding specific binding peptides into an externally exposed loop which is displayed on each of the 420 coat protein subunits. Localization of complimentary materials to the interior of the particle was achieved through the use "scaffolding-fusion proteins. The scaffolding domain drives coat protein polymerization resulting in a coat protein shell surrounding a core of approximately 300 scaffolding/fusion molecules. The fusion domain comprises a peptide which specifically binds the semiconductor material of interest.

2. ACCOMPLISHMENTS

The principal investigators of this proposal have considerable expertise in the areas of genetic engineering and assembly of virus templates, light harvesting nanomaterial synthesis, material properties, and photocatalysis. Based on their complimentary expertise they established a highly collaborative and productive research program during the course of this funding. This project has resulted in critical findings in areas related to virus-based self-assembly nano-architectures and semiconducting inorganic nanomaterials synthesis for energy applications. Specifically, a summary of the seventeen major publications from the past project period in pioneer journals such as *Journal of the American Chemical Society*, *Chemical Commun*ications, *Nature Chemistry*, *Scientific Reports*, *Langmuir*, *Biomacromolecules, Journal of Materials Chemistry*, *Chemistry of Materials*, and *Nanotechnology* are provided below.

- Shen, L.; Bao, N.; Prevelige, P. E.; Gupta, A., Fabrication of ordered nanostructures of sulfide nanocrystal assemblies over self-assembled P22 coat protein. *Journal of the American Chemical Society* **2010**, *132*, 17354-17357.

A general strategy was developed for the controlled synthesis of light harvesting NPs (e.g., CdS and ZnS) on the exterior of P22 VLPs, inspired by the biotemplated nanocrystal formation in magnetotactic bacteria. First, a genetically engineered P22 was designed with a foreign peptide sequence known to facilitate CdS and ZnS NP nucleation between the P22 coat proteins. Using this VLP template, protein-directed growth of ordered ZnS and CdS nanocrystals was obtained. 70 and 65 ZnS and CdS nanocrystals decorated the P22 surfaces, respectively (Figure 1). The size and packing density of the NPs could be tailored well with reactant concentration and

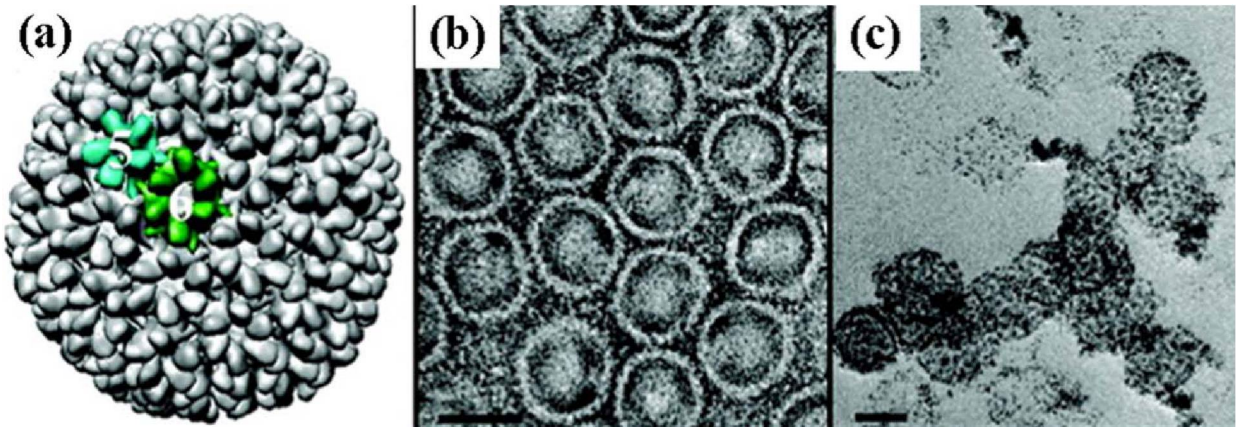

Figure 1. Synthesis of CdS NPs on the coat protein of genetically engineered P22 VLPs. (a) Three-dimensional surface representation of P22 procapsids (along 3-fold axis), (b) TEM image of P22 VLPs with engineered coat protein, and (c) TEM image of CdS NPs grown on sample (b). All scale bars represent 50 nm.

reaction time. By carefully denaturing the inner protein shell without affecting the outer sulfide nanocrystal shell, a hollow nanostructure assembly could also be designed. The reported strategy was a basic discovery towards obtaining dynamic control of nanocrystal nucleation and growth on the P22 VLP template.

- Kale, A.; Bao, Y.; Zhou, Z.; Prevelige, P. E.; Gupta, A., Directed self-assembly of CdS quantum dots on bacteriophage P22 coat protein template. *Nanotechnology* **2013**, *24*, 045603 (1-6).

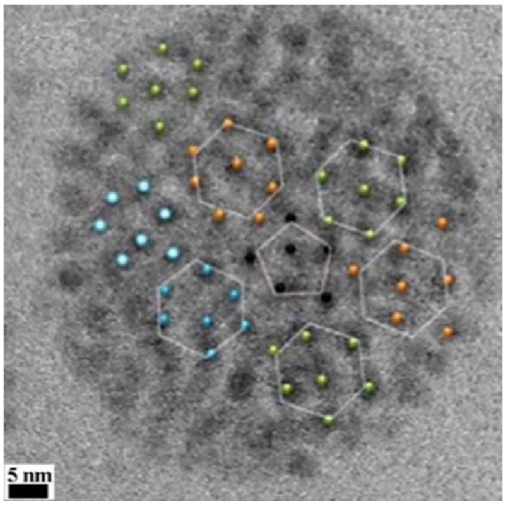

Figure 2. Selective binding of negatively-charged CdS quantum dots to the hexamers and pentamers of P22 procapsid.

In this research, the unique charge distribution on P22 procapsids was exploited to build ordered self-assembly of preformed CdS quantum dots. CdS NPs with negative ligand coating showed excellent binding affinity for the positively charged pockets of P22, irrespective of the overall negative charge on P22 procapsids. These light harvesting NPs packed on the surface of each P22 into hexameric and pentameric units via selective electrostatic attraction (Figure 2).

- Shen, L.; Bao, N.; Zhou, Z.; Prevelige, P. E.; Gupta, A., Materials design using genetically engineered proteins. *Journal of Material Chemistry* **2011**, *21*, 18868-18876.

This feature review article summarized the recent advances in biomimetic nano-architectures using genetically engineered protein cages. The synthesis strategies for one-dimensional and hierarchical self-assembly structures consisting of single NP components were described in detail.

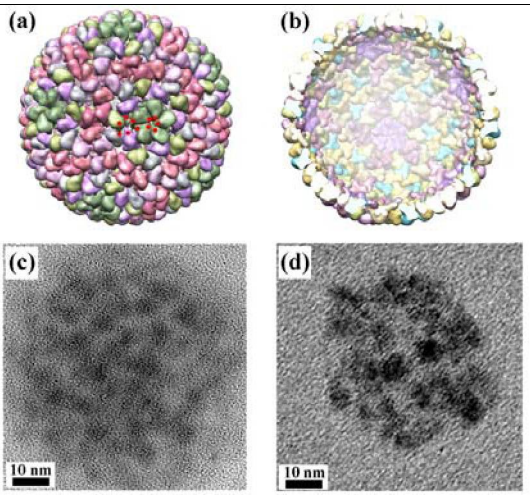

Figure 3. Synthesis of CdS NPs on the exterior and interior surfaces of P22. (a) Surface representation of genetically engineered P22 for exterior NP growth, (b) cutaway representation of engineered P22 for interior NP growth, (c) TEM image of NPs on the exterior P22 surface, and (d) TEM image of NPs grown on the interior P22.

- Zhou, Z.; Bedwell, G. J.; Li, R.; Prevelige, P. E.; Gupta, A., Formation mechanism of chalcogenide nanocrystals confined inside genetically engineered virus-like particles. *Scientific Reports* **2014**, *4*, 3832 (1-6).

Following the external binding of NPs over P22 procapsids, the next level of self-assembly was investigated. In this study, controlled confinement of the light harvesting NPs (CdS) within P22 procapsids was obtained through genetically engineered peptide sequences on P22 scaffold proteins (Figure 3b). The study provided a detailed understanding of the site selective nucleation and growth of NPs within P22 using CdS as a prototypical example.

- Zhou, Z.; Bedwell, G. J.; Li, R.; Bao, N.; Prevelige, P. E.; Gupta, A., Virus constructed Au/CdS plasmonic photocatalytic nanostructures for enhanced photoactivity. *Chemical Communications* **2015**, *51*, 1062-1065.

Plasmonic nanostructures are highly attractive in absorbing visible light and preventing recombination of electron-holes for efficient photocatalysis. A novel synthesis strategy was designed to form core-shell like structure with CdS NPs and Au NPs using genetically engineered P22 templates. In this approach, CdS NPs were confined within the VLP via selective peptide sequences on scaffold proteins, prior to nucleation and growth of plasmonic NPs (Au) on the outside coat proteins. The biotemplated Au/CdS nanostructures exhibited significantly enhanced activity for photodegradation of methylene blue as compared to VLPs with only CdS or Au NPs.

- Reichhardt, C.; Uchida, M.; O'Neil, A.; Li, R.; Prevelige, P. E.; Douglas, T., Templated assembly of organic-inorganic materials using the core shell structure of the P22 bacteriophage. *Chemical Communications* **2011**, *47*, 6326-6328.

A fusion protein in which a polyanionic peptide was fused to P22 scaffolding protein was successfully employed to nucleate the formation of Fe_2O_3 in a size-constrained manner inside P22 particles in aqueous solution.

- Uchida, M.; Morris, D. S.; Kang, S.; Jolley, C. C.; Lucon, J.; Liepold, L. O.; LaFrance, B.; Prevelige, P. E.; Douglas, T., Site-directed coordination chemistry with P22 virus-like particles. *Langmuir* **2012**, *28*, 1998-2006.

The interior surface of P22 particles was modified to allow site addressable chemical modification. The conjugation of a Ni/phenanthroline complex served as an anchor point for the directed synthesis of a Ni/phenanthroline co-ordination polymer.

- Lucon, J.; Qazi, S.; Uchida, M.; Bedwell, G. J.; LaFrance, B.; Prevelige, P. E.; Douglas, T., Use of the interior cavity of the P22 capsid for site-specific initiation of atom-transfer radical polymerization with high-density cargo loading. *Nature Chemistry* **2012**, *4*, 781-788.

An entirely internally contained polymer of 2-aminoethyl methacrylate (AEMA) was formed in P22 by atom-transfer radical polymerization initiated at a modified amino acid on the internal surface of P22. The pendant amino groups on the polymer were selectively modified by the addition of a fluorescent dye or a gadolinium chelate.

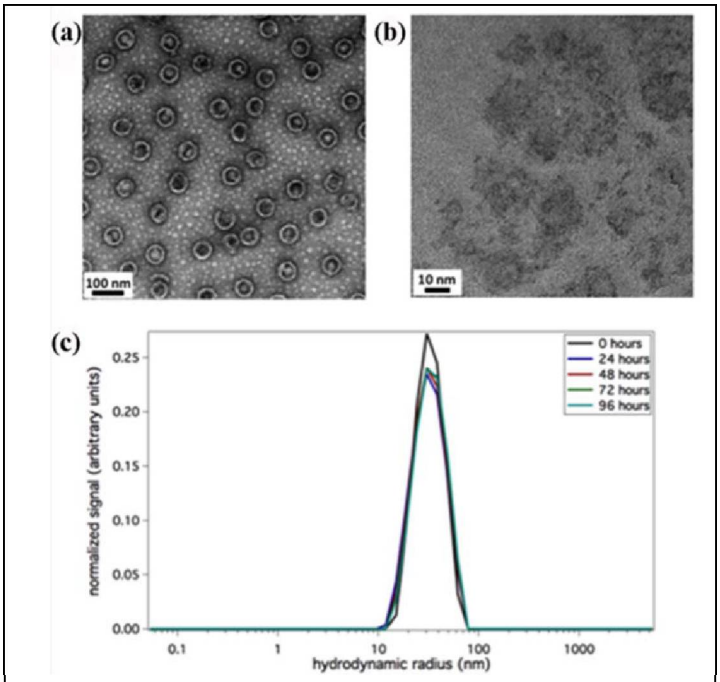

Figure 4. (a) TEM image of 2% uranyl acetate stained P22 VLPs from 24 hour mineralization reaction showing TiO_2 NPs on their interior surface, (b) unstained HRTEM image of sample (a) suggesting the presence of TiO_2 NPs inside the P22 cores, and (c) DLS particle size distributions for the starting material (unmineralized shells) and shells incubated up to 96 hours with TiBALDH. No change in size indicates that the mineralization reaction occurs on the interior of P22 VLPs.

- Bedwell, G. J.; Zhou, Z.; Uchida, M.; Douglas, T.; Prevelige, P. E.; Gupta, A., Formation mechanism of chalcogenide nanocrystals confined inside genetically engineered virus-like particles. *Biomacromolecules* **2015**, *16*, 214-218.

Short peptides reported to nucleate the crystallization of TiO_2 in both anatase and rutile forms were genetically fused to the C-terminal 162 residues (141-303) of P22 scaffolding protein. Analytical ultracentrifugation was employed to demonstrate that the fusion proteins re-entered the protein cage. Incubation of these particles with titanium (IV)-bis-ammonium-lactato-dihydroxide (TiBALDH) precursor resulted in the formation of multiple electron-dense NPs contained entirely within the shells. Dissociation of the shells at various times during mineralization allowed us to determine the kinetics of crystal growth and the average size of the NP (2.5 nm). HRTEM demonstrated that the TiO_2 was crystalline and that the phase (anatase or rutile) corresponded to that expected based on the fused peptide (Figure 4). Under illumination, the particles were capable of generating reducing equivalents as measured by breakdown of methylene blue.

- O'Neil, A.; Prevelige, P. E.; Basu, G.; Douglas, T., Coconfinement of fluorescent proteins: spatially enforced communication of GFP and mCherry encapsulated within the P22 capsid. *Biomacromolecules* **2012**, *13*, 3902-3907.

GFP and m-Cherry were co-encapulsated in P22 shells by fusion to scaffolding proteins. The proteins were properly folded and capable of FRET. Analysis of FRET efficiency was used to probe the effect of macromolecular crowding.

- Servid, A.; Jordan, P.; O'Neil, A.; Prevelige, P. E.; Douglas, T., Location of the bacteriophage P22 coat protein C-terminus provides opportunities for the design of capsid-based materials. Biomacromolecules 2013, 14, 2989-2995.

The C-terminus of P22 capsid protein was developed as an additional addressable display site. Introduction of peptide based tags (6XHis), chemically reactive amino acids (Cys), and short coiled-coil peptides demonstrated external exposure and provided a path to hierarchical assembly.

- Wang, Y-H. A.; Zhang, X.; Bao, N. Z.; Lin, B.; Gupta, A., Synthesis of shape-controlled monodisperse wurtzite $CuIn_xGa_{1-x}S_2$ semiconductor nanocrystals with tunable band gap. *Journal of the American Chemical Society* **2011**, *133*, 11072-11075 (Acknowledging partial DOE BES support).

Using a solution-based approach, syntheses of new wurtzite crystal phase NPs of $CuIn_xGa_{1-x}S_2$ over the whole composition range ($0 \leq x \leq 1$) were reported. The shape of the monodisperse NPs could be accurately controlled with changes in the chemical composition and synthesis conditions. The band gap of the NPs varied linearly from 1.5 - 2.4 eV with increasing Ga concentration.

- Zhang, X.; Bao, N.; Ramasamy, K.; Wang, Y-H. A.; Lin, B.; Gupta, A., Crystal phase-controlled synthesis of Cu_2FeSnS_4 nanocrystals with a band gap of around 1.5 eV. *Chemical Communications* **2012**, *48*, 4956-4958 (Acknowledging partial DOE BES support); Zhang, X.; Bao, N.; Lin, B.; Gupta, A., Synthesis of wurtzite Cu_2CoSnS_4 nanocrystals and the photoresponse of spray-deposited thin films. *Nanotechnology* **2013**, *24*, 105706 (Acknowledging partial DOE BES support).

In these two manuscripts, the synthesis of novel quaternary chalcogenide nanocrystals of Cu_2FeSnS_4 and Cu_2CoSnS_4 were reported as an attractive low-cost alternative for thin film solar cells. It was discovered that a tunable band gap around 1.5 eV could be obtained with changes in morphology of the NPs. The crystal structures of these photoactive NPs were investigated in detail. These optimal band gap NPs are attractive for photocatalytic applications because they are potentially composed of low-cost and earth abundant elements of low toxicity.

- Ramasamy, K.; Sims, H.; Butler, W. H.; Gupta, A., Mono-, few- and multiple layers of copper antimony sulfide ($CuSbS_2$): a ternary layered sulfide. *Journal of the American Chemical Society* **2014**, *136*, 1587-1598; Ramasamy, K.; Sims, H.; Butler, W. H.; Gupta, A., Selective nanocrystal synthesis and calculated electronic structure of all four phases of copper-antimony-sulfide. *Chemistry of Materials* **2014**, *26*, 2891-2899**;** Ramasamy, K.; Tien, B.; Archana, P. S.; Gupta, A., Copper antimony sulfide ($CuSbS_2$) mesocrystals: a potential counter electrode material for dye-sensitized solar cells. *Materials Letters* **2014**, *124*, 227-230.

These three recent publications reported for the first time the selective synthesis of four distinct phases of Cu-Sb-based semiconducting sulfides in the form of NPs with different morphologies and their optical properties. Bulk band structure calculation results for the different phases were also reported. Two of the phases ($CuSbS_2$ and Cu_3SbS_3) exhibit optimal band gap and high absorption coefficient at visible wavelengths for potential use in thin-film solar cells. In addition to desirable optical properties, the $CuSbS_2$ phase is interesting because of its 2D layered structure. Solution-based approaches were developed for the synthesis of mono-, few-, and multiple layers of $CuSbS_2$ and their tunable optical properties investigated. The performance of solar cells using

$CuSbS_2$ as a counter electrode in dye-sensitized solar cells was also explored and found to be comparable with that of platinum.

3. PUBLICATIONS

- Shen, L.; Bao, N.; Prevelige, P. E.; Gupta, A., Escherichia coli Bacteria-Templated Synthesis of Nanoporous Cadmium Sulfide Hollow Microrods for Efficient Photocatalytic Hydrogen Production. *J. Phys. Chem. C* **2010**, *114*, 2551-2559.
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- Shen, L.; Bao, N.; Zhou, Z.; Prevelige, P. E.; Gupta, A., Materials design using genetically engineered proteins. *Journal of Material Chemistry* **2011**, *21*, 18868-18876.
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- Zhou, Z.; Bedwell, G. J.; Li, R.; Prevelige, P. E.; Gupta, A., Formation mechanism of chalcogenide nanocrystals confined inside genetically engineered virus-like particles. *Scientific Reports* **2014**, *4*, 3832 (1-6).
- Ziyou Zhou, Gregory J. Bedwell, Rui Li, Peter E. Prevelige, Jr. and Arunava Gupta, Formation mechanism of inorganic nanocrystals confined inside genetically engineered virus-like particles. *Chemical Communications* **2015**, *51*, 1062-1065.
- Gregory J. Bedwell, Ziyou Zhou, Masaki Uchida, Trevor Douglas, Arunava Gupta, and Peter E. Prevelige, Jr., "Biotemplated synthesis and characterization of photoactive TiO_2

nanoparticles inside a P22-derived Protein Cage nanoarchitecture. *Biomacromolecules* **2015**, *16*, 214-218.

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- Ramasamy, K.; Sims, H.; Butler, W. H.; Gupta, A., Selective nanocrystal synthesis and calculated electronic structure of all four phases of copper-antimony-sulfide. *Chemistry of Materials* **2014**, *26*, 2891-2899.
- Ramasamy, K.; Tien, B.; Archana, P. S.; Gupta, A., Copper antimony sulfide ($CuSbS_2$) mesocrystals: a potential counter electrode material for dye-sensitized solar cells. *Materials Letters* **2014**, *124*, 227-230.
- Ramasamy, K.; Gupta, R. K.; Sims, H.; Palchoudhury, S.; Ivanov, S.; Gupta, A. Layered ternary sulfide $CuSbS_2$ nanoplates for flexible solid-state supercapacitors. *J. Mater. Chem. A* **2015**, *3*, 13263-13274.
- Ramasamy, K.; Gupta, R. K.; Palchoudhury, S.; Ivanov, S.; A. Gupta, A. Layer-Structured Copper Antimony Chalcogenides ($CuSbSe_xS_{2-x}$): Stable Electrode Materials for Supercapacitors. *Chem. Mater.* **2015**, *3*, 379-386.
- Ghosh, A.; Palchoudhury, S.; Thangavel, R.; Zhou, Z.; Naghibolashrafi, N.; Ramasamy, K.; Gupta, A., A new family of wurtzite-phase Cu_2ZnAS_{4-x} and $CuZn_2AS_4$ (A = Al, Ga, In) nanocrystals for solar energy conversion applications. *Chemical Communications* **2016**, *52*, 264-267 (Cover art).

3. SUPPORT

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The University of Alabama, Tuscaloosa

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