Supporting Information

Fabrication of Ordered Nanostructures of Sulfide Nanocrystal Assemblies over Self-Assembled Genetically Engineered P22 Coat Protein

Liming Shen,[†] Ningzhong Bao,[†] Peter E. Prevelige,*[,‡] and Arunava Gupta*[,†]

[†]Center for Materials for Information Technology, University of Alabama, Tuscaloosa AL 35487, and [‡]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294, USA.

Content

Experimental Details

Materials: The synthesis of ZnS and CdS was carried out using commercially available reagents. Sodium sulfide (Na_2S) and cadmium acetate ($Cd(CH_3COO)_2$) were purchased from Acros Organics and zinc acetate ($Zn(CH_3COO)_2{\cdot}2H_2O$) was obtained from Fisher Scientific. All the chemicals were used as-received without any further purification.

Preparation of Genetically Engineered Bacteriophage P22 Coat Protein Assemblies: Two peptide sequences, CNNPMHQNC and SLTPLTTSHLRS, isolated by Belcher's group through screening of M13 phage display library and confirmed to having binding specificity to ZnS and CdS, were used in this work.[1] The nucleotide sequence encoding the specific peptide was introduced into a pET-based plasmid encoding the bacteriophage P22 scaffolding and coat protein genes at coat protein residue 182 by PCR based mutagenesis as described previously.[2] The coat and scaffolding proteins were expressed in *E. coli* BL21 DE3 at 37 ^{o}C. Cells were harvested by centrifugation 3-4 hours after induction with 1 mM IPTG and lysed by repeated freeze-thaw cycles in 50 mM Tris, 100 mM NaCl, 20 mM $MgSO_4$, pH 7.6. The lysate was clarified by centrifugation at 12,000 x g for 45 min and the supernatant containing the procapsid-like particles (PLPs) was centrifuged through a 20% sucrose cushion at 185,000 x g for 2 h to pellet the procapsids. The procapsids were re-suspended in 0.5 M Guanidinium-Cl buffer to remove the scaffolding proteins and the procapsid shells were pelleted at 40,000 x g for 1 h. The coat protein assemblies were re-suspended in 50 mM NaCl, 25 mM Tris, 2 mM EDTA, pH 7.6 and band purified by sucrose gradient centrifugation on a 5 mL 5-20 % sucrose gradient, followed by dialysis against 50 mM NaCl, 25 mM Tris, 2 mM EDTA, pH 7.6 to remove the sucrose.

Inorganic Synthesis over Genetically Engineered Bacteriophage P22 Coat Protein Assemblies: Genetically engineered bacteriophage P22 coat protein assemblies stored in EDTA buffer were 4,000 x dialyzed against deionized water before being utilized as biosynthetic templates. Water based protein assembly solution (10 μL) was dispersed in 1 mL of aqueous $Zn(CH_3COO)_2$ (1 mM) for interaction at 4^{o}C for 12 h, allowing the protein-Zn(II) interaction. 1 mL of aqueous Na_2S solution (1 mM) was then added into the above solution and the mixture was placed at room temperature for 2 h. Aqueous $Cd(CH_3COO)_2$ (1 mM) was used as the Cd(II) source in the synthesis of CdS. All the reported experimental procedures were carried out in a fume hood with appropriate handling of the chemicals and biological samples for safety.

Materials Characterization: The morphology and structure of the products was observed using transmission electron microscopy (TEM) coupled with high resolution (HR) (Tecnai F-20) and selected area electron diffraction (SAED). Only the original P22 protein assemblies were stained by 2 % uranyl acetate for the TEM characterization. All the P22 protein-templated sulfide samples were imaged without staining, and the observed contrast is from the electron-dense inorganic material grown on the protein template.

Reference:

(1) Flynn, C. E.; Mao, C.; Hayhurst, A.; Williams, J. L.; Georgiou, G.; Iversona, B.; Belcher, A. M. *J. Mater. Chem.* **2003**, *13*, 2414-2421.

(2) Kang, S.; Lander, G. C.; Johnson, J. E.; Prevelige, P. E. *Chembiochem.* **2008**, *9*, 514-518.

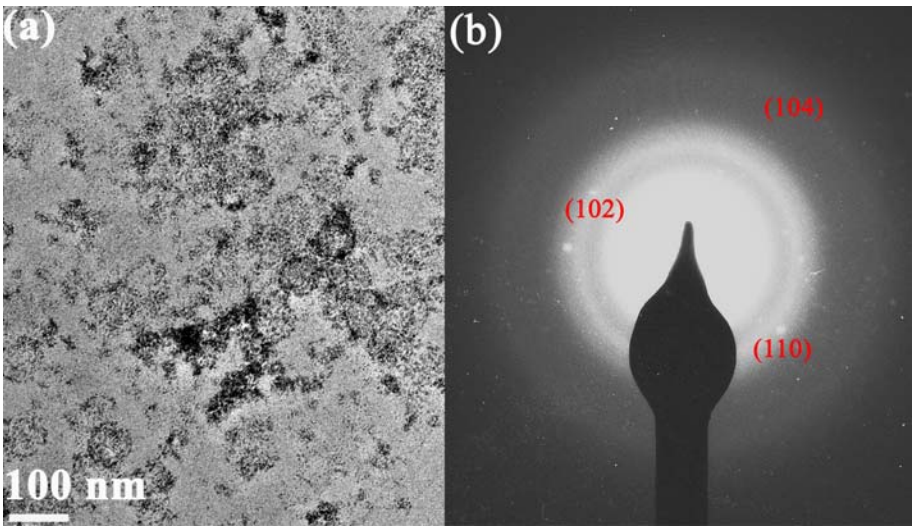

Figure S1. (a) TEM image of ZnS nanostructures and (b) corresponding SAED of the hexagonal wurtzite structure.

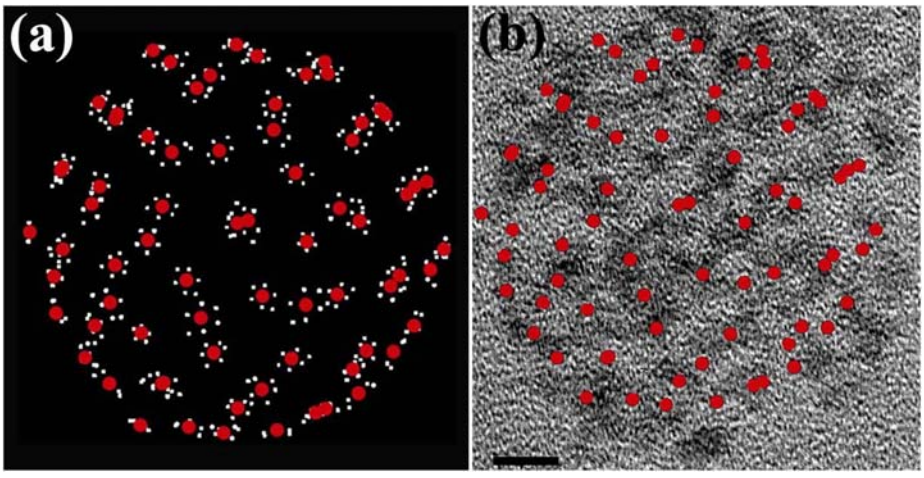

Figure S2. (a) Two-dimensional projection of a simulated P22 coat protein assembly generated by PyMOL molecular visualization system, only showing the individual protein residue 182 as a white dot. The red dots indicate the center of the hexamers and pentamers. (b) Matching (a) with a ZnS nanostructure. The scale bar represents 10 nm.

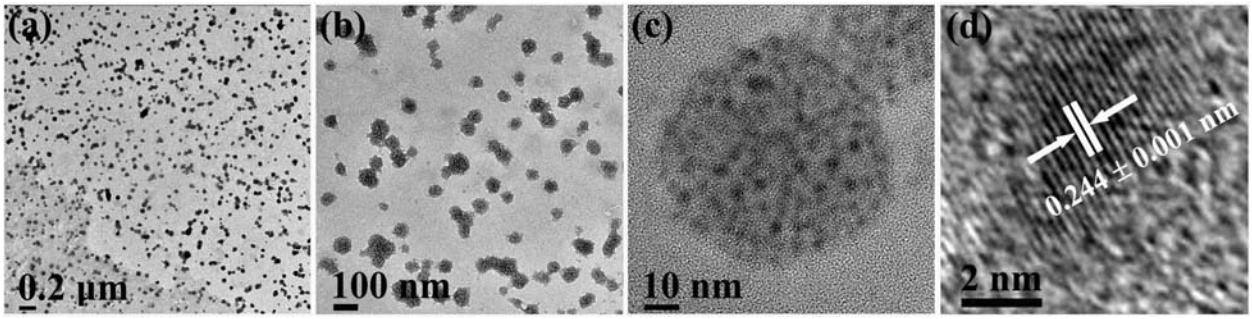

Figure S3. (a-c) TEM and (d) HRTEM images of nearly monodisperse CdS nanocrystal assemblies formed over genetically engineered P22 coat protein assemblies after a short reaction time of 2 hours at a low reactant concentration (1 mM).

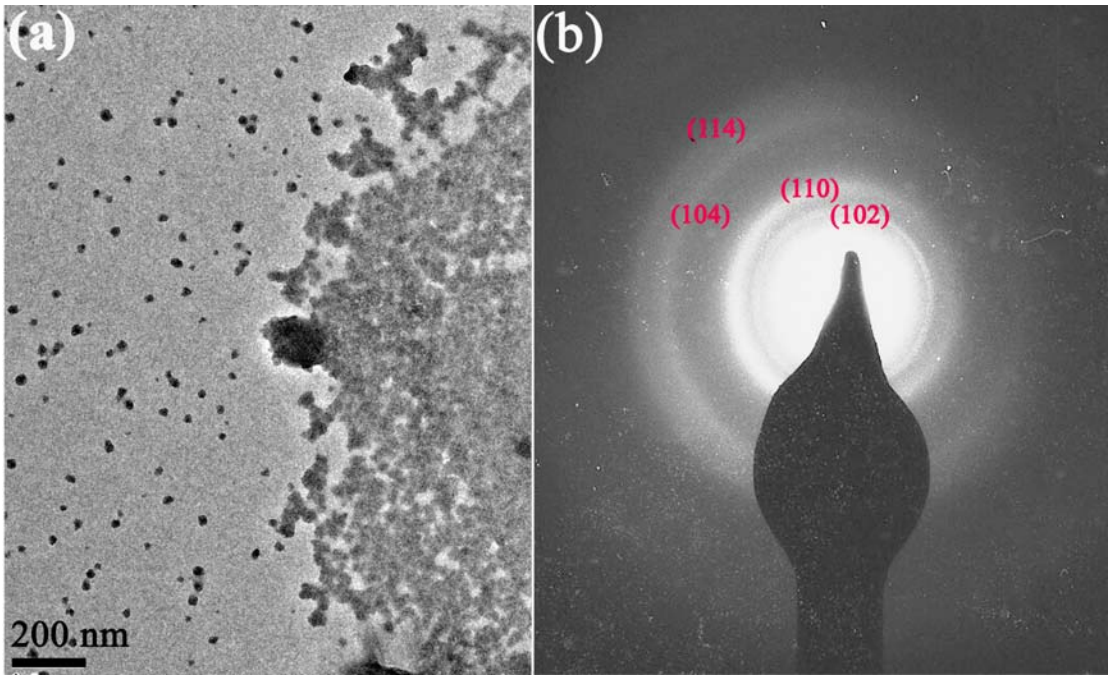

Figure S4. (a) TEM image of CdS nanostructures and (b) corresponding SAED of the hexagonal wurtzite structure.

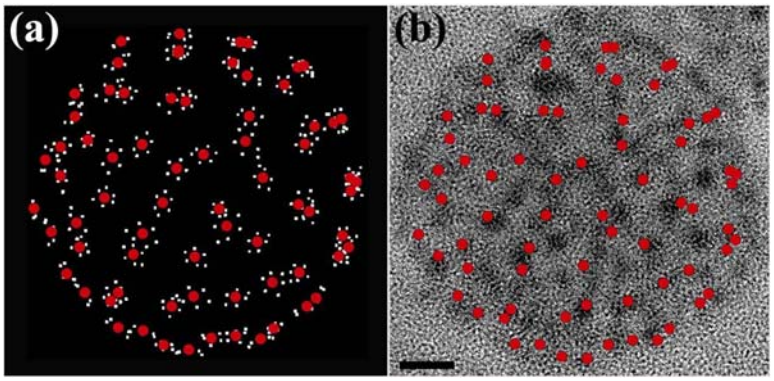

Figure S5. (a) Two-dimensional projection of a simulated P22 coat protein assembly generated by PyMOL molecular visualization system, only showing the individual protein residue 182 as a white dot. The red dots indicate the center of the hexamers and pentamers. (b) Matching (a) with one CdS nanostructure. The scale bar represents 10 nm.

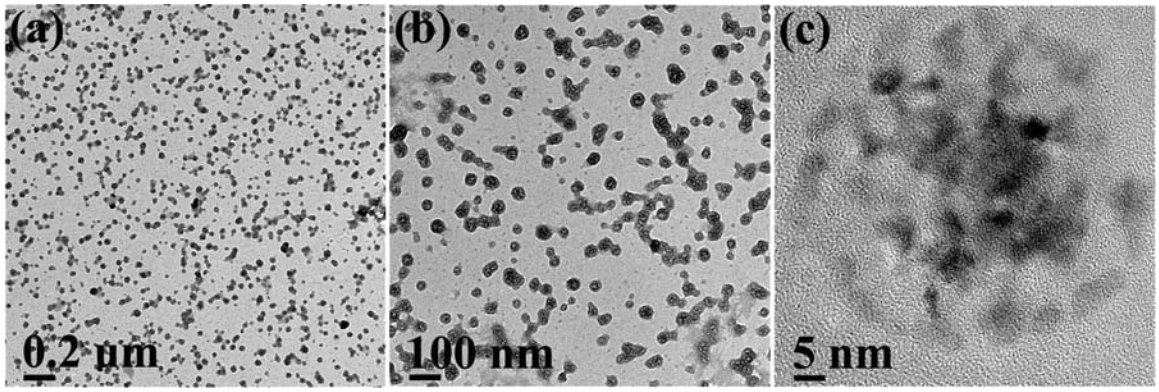

Figure S6. TEM images of nearly monodisperse CdS nanocrystal assemblies formed over genetically engineered P22 coat protein assemblies after a longer reaction time of 5 hours at the low reactant concentration.

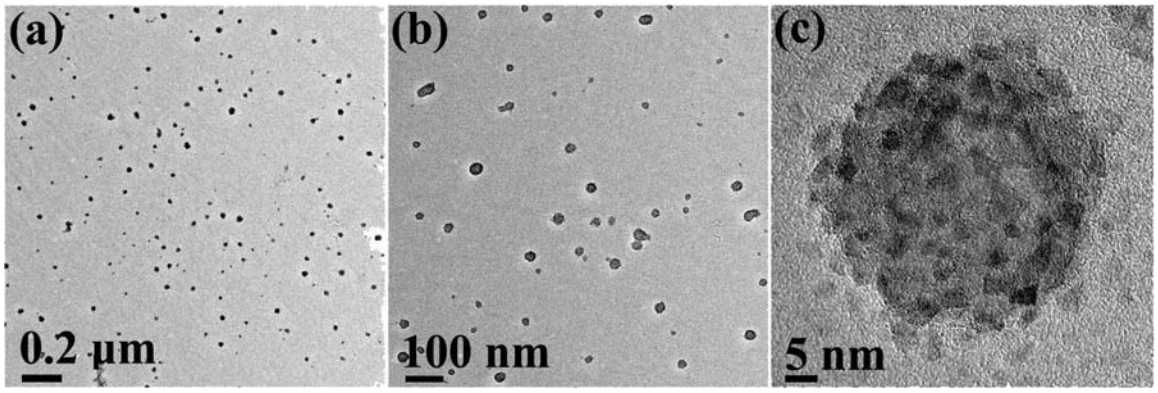

Figure S7. TEM images of nearly monodisperse spherical hollow nanostructures of CdS nanocrystals assembled over genetically engineered P22 coat protein assemblies after an even longer reaction time of 12 hours and at a higher reactant concentration (2 mM).

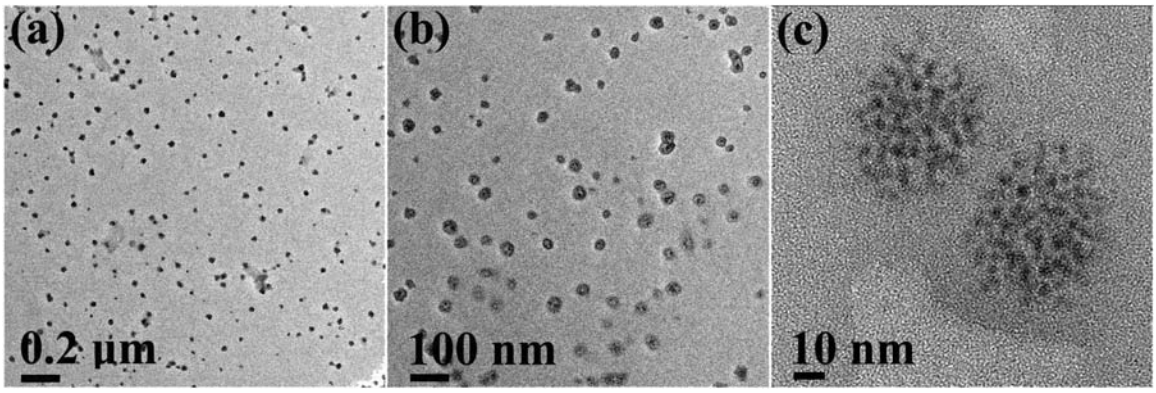

Figure S8. TEM images of nearly monodisperse ZnS nanocrystal assemblies formed over genetically engineered P22 coat protein assemblies after a short reaction time of 2 hours.

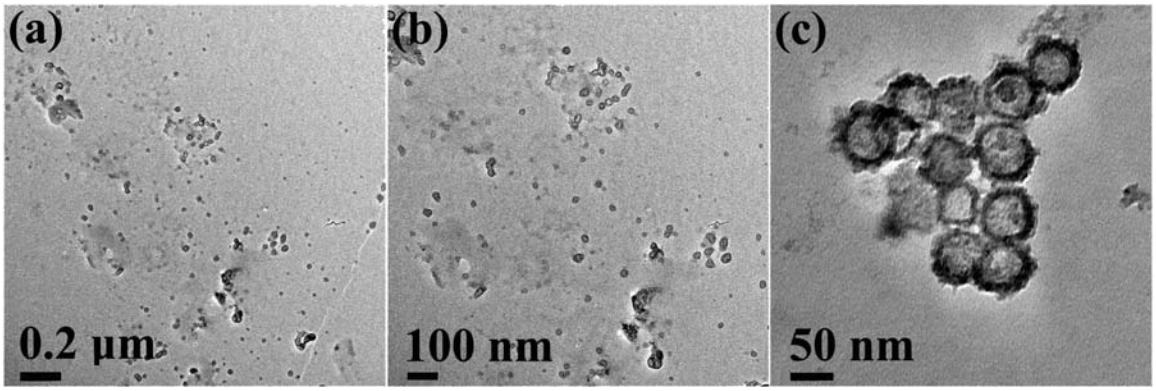

Figure S9. TEM images of nearly monodisperse spherical hollow nanostructures of ZnS nanocrystals assembled over genetically engineered P22 coat protein assemblies after a longer reaction time of 12 hours.

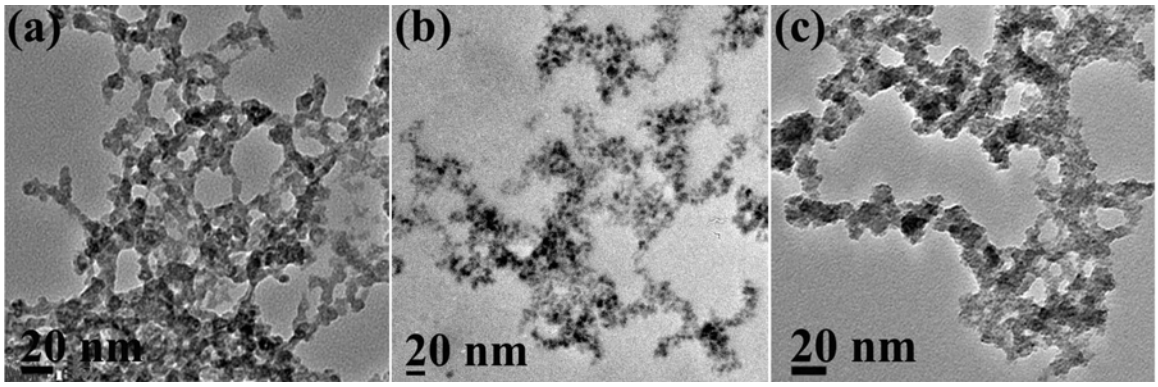

Figure S10. TEM image of (a) ZnS product prepared in the presence of wild P22 procapsids; (b) CdS product prepared in the presence of self-assembled P22 coat proteins engineered with ZnS-specific peptide; and (c) ZnS product prepared in the absence of any type of P22 coat protein.