be) to the measured shear spectrum equalled 1/3. This threshold was selected heuristically, and the resulting $r$ and $K_r$ are insensitive to the exact choice of value. $(V_{cm}^2)$ was computed by integrating the GM normalized shear spectrum over wavelengths as for $(V_{cm}^2)$. To estimate $R_{cm}$, a bulk shear-to-strain ratio as for $(V_{cm}^2)$ was diagnosed as $R_{cm} = \langle (V_{cm}^2) \rangle (\langle \xi \rangle)^2$. Here, $\langle \xi \rangle$ is the variance of strain, the vertical gradient in the vertical displacement of isopycnals induced by internal waves. We estimated strain from CTD density profiles using a scale separation assumption (20) and calculated $\langle \xi \rangle^2$ and $R_{cm}$ in 320-m segments identical to $(V_{cm}^2)$ bins. Despite being somewhat heuristic, the resulting $R_{cm}$ distribution shows a consistent mean value of 8 to 12 between different regions and cruises. We thus used a constant $F_{Re}$ of 1/3 throughout the analysis. That value is a conservative choice based on an internal wave dynamical model (29) validated with microstructure observations (37).

17. Our in situ estimates of widespread large $K_r$ values show some resemblance to diapycnal diffusivities diagnosed by an inverse calculation using ACC hydographic observations (40).

18. The vertically integrated dissipation rate $E$ is calculated as $\int \rho u^2 dz$, where $\rho$ is the in situ density, and the integral is over the water column.

19. This factor is assumed to include temporal variability, as suggested by the analysis of three repeats of the eastern Drake Passage transect (Fig. 1, station C) yielding very similar diffusivities.


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**R E P O R T S**

**Virus-Based Toolkit for the Directed Synthesis of Magnetic and Semiconducting Nanowires**

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We report a virus-based scaffold for the synthesis of single-crystal ZnS, CdS, and freestanding chemically ordered CoPt and FePt nanowires, with the means of modifying substrate specificity through standard biological methods. Peptides (selected through an evolutionary screening process) that exhibit control of composition, size, and phase during nanoparticle nucleation have been expressed on the highly ordered filamentous capsid of the M13 bacteriophage. The incorporation of specific, nucleating peptides into the generic scaffold of the M13 coat structure provides a viable template for the directed synthesis of semiconducting and magnetic materials. Removal of the viral template by means of annealing promoted oriented aggregation-based crystal growth, forming individual crystalline nanowires. The unique ability to interchange substrate-specific peptides into the linear self-assembled filamentous construct of the M13 virus introduces a material tunability that has not been seen in previous synthetic routes. Therefore, this system provides a genetic toolkit for growing and organizing nanowires from semiconducting and magnetic materials.

The reliance of future technologies on developing scalable and economic methods for the fabrication of one-dimensional (1D) systems has spurred intense and rapid progress in the area of materials synthesis. In particular, 1D materials have been enthusiastically pursued for their applications in the study of electrical transport (1), optical phenomena (2), and as functional units in nanocircuitry (3). Pursuit of “bottom-up” methods for the synthesis of semiconducting, metallic, and magnetic nanowires has yielded strategies including, but not limited to, vapor liquid solid (VLS) (4), chemical (5), solvothermal, vapor phase, and template-directed fabrication (6). Although each method developed for the production of nanowires has had success in achieving high-quality materials, no distinct strategy to date has yielded monodisperse, crystalline nanowires of radically different compositions. The realization of such a system
would require the combination of substrate-specific ligands with the predictability of self-assembly that is commonly found in nature. Recently, biological factors have been exploited as synthesis directors for nanofibers (7, 8), virus-based particle cages (9), virus-particle assemblies (10, 11), and nonspecific peptide templates (12). This is due to the high degree of organization, ease of chemical modification, and naturally occurring self-assembly motifs inherent in these systems.

The ability to store information about a material, including composition, phase, and crystallographic detail, within the genetic code of the M13 bacteriophage virus DNA has proven to be a viable means of synthesizing and organizing materials on the nanometer scale. The use of phage display techniques (using peptide libraries consisting of ~10^9 random sequences) has led to the discovery of materials-specific peptides that have preferential binding (13), control over nanoparticle nucleation (14), and the ability to order on the basis of the inherent shape anisotropy of the filamentous M13 virus (11). Because the protein sequences responsible for these attributes are gene linked and contained within the capsid of the virus, exact genetic copies of the virus scaffold are easily reproduced by infection into its bacterial host.

We report the general synthesis of 1D nanostructures based on a genetically modified virus scaffold for the directed growth and assembly of crystalline nanoparticles into 1D arrays, followed by annealing of the virus-particle assemblies into high aspect ratio crystalline nanowires through oriented aggregation-based crystal growth (15, 16) (Fig. 1A). The synthesis of analogous nanowire structures from fundamentally different materials, the II-VI semiconductors ZnS and CdS and the crystal structure L1_2 ferromagnetic alloys CoPt and FePt, demonstrates both the generality of the virus scaffold and the ability to precisely control material characteristics through genetic modification. In contrast to other synthetic methods (6), this approach allows for the genetic control of crystalline semiconducting, metallic, oxide, and magnetic materials with a universal template.

Evolution of substrate-specific peptides through phage display technologies for the directed nucleation of materials on the nanometer scale has been previously reported and serves as the basis for the material specificity in the virus template (13). Screening of the ZnS, CdS (14, 17), FePt, and CoPt systems (18) with commercially available bacteriophage libraries (New England Biolabs) expressing either a disulfide constrained (Cys-Cys) heptapeptide or a linear dodecapeptide as a fusion to the gene product (gP) 3 protein located at the proximal tip of the virus has yielded nucleating peptides with the following sequences: CNNPMHQNC (termed A7; ZnS), SLLPTLTTHRLS (termed J140; CdS), HNKHLPSTDPLA (termed FP12; FePt), and CNAHDHCAN (termed CP7; CoPt) (19). The incorporation of these peptides into the highly ordered, self-assembled capsid of the M13 bacteriophage virus provides a linear template that can simultaneously control particle phase and composition, while maintaining an ease of material adaptability through genetic tuning of the basic protein building blocks.

The M13 bacteriophage is a high-production rate virus (200 mg/liter) comprising five genetically modifiable proteins (20–22): gP3, gP6, gP8, and gP9. About 2700 copies of the gP8 protein form the capsid of the wild-type virus. The gP8 protein was genetically modified and expressed using a phagemid system, resulting in the fusion of the substrate-specific peptides to the N terminus of the gP8 protein (14). During assembly, stacking of the gP8 unit cell results in a fivefold symmetry down the length (c axis) of the virus; this stacking is also the origin of the ordering of fusion peptides in a 3D structure (Fig. 1B). Computational analysis of peptide expression on the capsid of the virus revealed that the nearest neighbor peptide separation stabilized around 3 nm at and above 20% incorporation (Fig. 1C). Consequently, high incorporation of the substrate-specific fusion peptides is not required for complete mineralization of the virus to occur. Trifunctional templates can be realized through further genetic modification of the proximal and remote tips of the virus [specifically, the gP3 and gP9 proteins (23)], which can be used to push the current system to higher aspect ratios and introduce materials that we screened, including noble metals, semiconductors, and oxides, to assemble functional heterostructured materials (Fig. 1D).

Mineralization of the ZnS and CdS systems has been described previously (11, 14, 17); the process involves incubating the viral template with metal salt precursors at reduced temperatures to promote uniform orientation of the peptide molecules during nucleation (24), which leads to the preferred crystallographic orientation of nucleated nanocrystals. Before annealing, wurtzite ZnS and CdS nanocrystals (3 to 5 nm) that were grown on the virus surface were in close contact and preferentially oriented with the [001] direc-

**Fig. 1.** Visualization of the M13 bacteriophage and the subsequent nanowire synthesis. The gP8 coat assembly was reconstructed from the x-ray fiber crystallographic data (PDB number 1ifj). The gP3 and gP9 proteins located at the proximal and remote ends of the virus are not to scale and serve as representations of the proteins. (A) The nanowire synthesis scheme is visualized for the nucleation, ordering, and annealing of virus-particle assemblies. (B) The symmetry of the virus allows for ordering of the nucleated particles along the x, y, and z directions, fulfilling the requirements for aggregation-based annealing. (C) The highly ordered nature of the self-assembled M13 bacteriophage promotes the preferred orientation seen in nucleated particles through the rigidity and packing of the expressed peptides, which is visualized at 20% incorporation. (D) The construct of the M13 bacteriophage virus showing the genetically modifiable capsid and ends, specifically the gP3, gP8, and gP9, which are coded for in the phagemid DNA enclosed within the virus capsid.
tion and the (100) (ZnS) and (001) (CdS) planes, which were perpendicular to the wire-length direction. These data are supported by electron diffraction (ED), high-resolution transmission electron microscopy (HRTEM), high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM), and dark-field diffraction-contrast imaging (Fig. 2) (25). Particles attached to the virus were prohibited from fusing under initial synthesis conditions because of the blocking effects of the nucleating peptides, and they therefore required removal of the template to form single-crystal nanowires. Thermal gravimetric analysis of the virus-particle system was used to obtain a critical temperature for the synthesis of crystalline nanowires, and it showed removal of the organic materials by 350°C (26). This agreed well with the minimum temperature observed for the fusion of adjacent particles by TEM with annealing, which we performed in situ using a thermal stage (27).

Annealing of the mineralized viruses at temperatures below the ZnS and CdS particle melting point (400°C to 500°C) allowed the polycrystalline assemblies to form single-crystal nanowires through the removal of the organic template and the minimization of the interfacial energy (28) (Fig. 2, B and E, wires measure 600 to 650 nm in length for ZnS and 475 to 500 nm for CdS; both have diameters of ~20 nm). ED and HRTEM revealed the single-crystal nature of individual nanowires that inherited the preferred orientation seen in the precursor polycrystalline wires when the grain boundaries were removed (29, 30) (Fig. 2, C and D). The [100] direction and (001) plane orientations of the observed ZnS nanowires were consistent with common elongation directions for II-VI nanowires, even though these are thermodynamically high-energy planes (Fig. 2, B and C) (13, 16, 31). HRTEM of the single-crystal CdS nanowires revealed a lattice spacing of 2.4 Å, which was consistent with the unique 2.451 Å separation between two (102) planes in bulk wurtzite CdS crystals [Joint Committee on Powder Diffraction Standards (JCPDS) card number 41-1049]. The 43.1° orientation of (102) lattice planes with respect to the nanowire axis indicated that the nanowire was elongated along the [001] direction and again confirmed the wurtzite structure (Fig. 2F).

Extending the virus-directed synthesis approach to the ferromagnetic L1 0 CoPt and FePt systems was a natural direction for both demonstrating the diversity of applicable materials and addressing current technological issues regarding the development of low-dimensional magnetic materials. Platinum-alloyed magnetic materials of the chemically ordered L1 0 phase have been of recent interest because of their high coercivity, resistance to oxidation, and inherent magnetic anisotropy, which are necessary for ultrahigh-density recording media (32). Although synthetic routes such as VLS yield exquisite 1D semiconducting structures, and nonspecific template schemes are applicable to a range of mate-

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Fig. 2. Electron microscopy of both the pre- and postannealed ZnS and CdS viral nanowires. (A) Dark-field diffraction-contrast imaging of the pre-annealed ZnS system using the (100) reflection reveals the crystallographic ordering of the nucleated nanocrystals, in which contrast stems from satisfying the (100) Bragg diffraction condition. (Inset) ED pattern of the polycrystalline pre-annealed wire showing the wurtzite crystal structure and the single-crystal type [001] zone axis pattern, suggesting a strong [001] zone axis preferred orientation of the nanocrystals on the viral template. g = (100)* denotes the reciprocal vector of (100) crystal planes, which is perpendicular to the (100) planes and has a length inversely proportional to the interplanar spacing of the (100) planes. (B) Bright-field TEM image of an individual ZnS single-crystal nanowire formed after annealing. (Inset, upper left) ED pattern along the [001] zone axis shows a single-crystal wurtzite structure of the annealed ZnS nanowire. (Inset, lower right) Low-magnification TEM image showing the monodisperse, isolated single-crystal nanowires. (C) A typical HRTEM of a ZnS single-crystal nanowire showing a lattice image that continually extends the length of the wire, confirming the single-crystal nature of the annealed nanowire. The measured lattice spacing of 0.33 nm corresponds to the (010) planes in wurtzite ZnS crystals. A 30° orientation of (010) lattice planes with respect to the nanowire axis is consistent with the (100) growth direction determined by ED. (D) HAADF-STEM image of single-crystal ZnS nanowires, which were annealed on a silicon wafer. (E) HAADF-STEM images of CdS single-crystal nanowires. (F) A HRTEM lattice image of an individual CdS nanowire. The experimental lattice fringe spacing, 0.24 nm, is consistent with the unique 0.24519-nm separation between two (102) planes in bulk wurtzite CdS crystals.
nucleation of the CoPt and FePt particles was achieved by means of the chemical reduction of metal precursor salts in the presence of gP8-modified viruses (18, 34). Annealing of the assemblies at 350°C was necessary for the removal of the virus template, and it promoted the growth of crystalline CoPt and FePt nanowires that retained the L1_0 phase of the as-prepared particles and that were uniform in diameter (10 nm ± 5%). The crystalline nature of the wires can be seen in the selected area ED pattern, which also shows the characteristic (001) and (110) L1_0 peaks, and in HRTEM lattice imaging (Fig. 3, C and D). The (111) plane that was perpendicular to the long axis of the CoPt wires and that had a lattice spacing of 2.177 Å was in agreement with the reported value of 2.176 Å, which again confirmed the highly crystalline nature of the material (Fig. 3D, JCPDS number 43-1358). The persistence of the L1_0 phase, which has traditionally been accessible only above 550°C (35), was attributed to the propensity of particles to maintain their original orientation during aggregation-based annealing.

Monte Carlo simulations of the A7 constrained sequence resulted in a 21% decrease in the standard deviation of backbone dihedral angles upon transfer of the peptide from isolation into the capsid environment, demonstrating the rigidity imposed on the fusion peptide (36). We believe that the ordering of the nucleated particles with regard to preferred crystallographic orientation along the length of the virus was a result of the stability of the peptide fusion and the symmetry of the virus coat. This nanocrystal ordering promoted the single-crystal nature of the annealed nanowires by satisfying the orientation requirements of the aggregation-based crystal growth mechanism (15). Although particles exhibiting orientations that were not coherent with that of the majority were to be expected, these minority nanocrystals should rotate to adopt the preferred crystallographic orientation and merge with the majority during annealing to minimize both the interfacial and grain-boundary energies (31, 37, 38).

The exploitation of the self-assembly motifs employed by the M13 bacteriophage to produce a biological scaffold provides a means of generating a complex, highly ordered, and economical template for the general synthesis of single-crystal nanowires. By introducing programmable genetic control over the composition, phase, and assembly of nanoparticles, a generic template for the universal synthesis of a variety of materials can be realized. Further advances in the fabrication of nanoscale materials and devices can be achieved through the modification of the remaining four proteins in the virus to incorporate device-assembly directors. Overall, modification of biological systems by the introduction of substrate-specific peptides presents a means of achieving well-ordered nanomaterials in a cost-effective and scalable manner.

References and Notes

Fig. 3. (A) CoPt wires, synthesized by the modified virus template, were soluble in water (right). The reduction of Co and Pt salts without the presence of the virus yielded large precipitates which immediately fell out of solution (left). (B) TEM image of the unannealed CoPt nanoparticle-virus system. (Inset) STEM image of the unannealed CoPt wires. Scale bar, 100 nm. (C) Low-resolution TEM image of crystalline L1_0 CoPt wires (650 × 20 nm). The tendency of the CoPt and FePt wires not to be straight stems from magnetic interactions between wires that are not present in the II-VI systems. (Inset) STEM image of the unannealed CoPt wires. Scale bar, 100 nm. (D) HRTEM of the CoPt wires with the (111) plane perpendicular to the c axis of the wire. (Inset) ED of an annealed CoPt wire reveals the superlattice structure unique to the L1_0 phase. (E) TEM imaging of the unannealed FePt wires. (F) TEM of the annealed FePt wires. (Inset) ED pattern confirming the L1_0 nature of the FePt wires and showing the crystalline nature of the material.
Boron Nitride Nanomesh

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A highly regular mesh of hexagonal boron nitride with a 3-nanometer periodicity and a 2-nanometer hole size was formed by self-assembly on a Rh(111) single crystalline surface. Two layers of mesh cover the surface uniformly after high-temperature exposure of the clean rhodium surface to argon, and forming gas (5% H2). Samples were prepared by centrifugation of the virus-particle suspension into 1-mg pellets and allowed to dry. TEM samples were analyzed with the JEOL 2010–Field Emission Gun (FEG) microscopes. HAADF analysis of the ZnS and CdS samples was performed on the JEOI 2010–FEG. Gantan Digital Micrograph is used to subtract the background of the HRTMEM lattice images. Thermal analysis of the CoPt system was performed in situ on the JEOL 200CX microscope operated at 200 kV, with a Gatan heat stage.  

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Pairs of boron and nitrogen atoms are iso-electronic to pairs of carbon atoms. Thus, boron nitrides show a structural variety similar to that of carbon solids, including graphitic hexagonal boron nitride (h-BN) and diamond-like cubic boron nitride (c-BN) (1), onion-like fullerenes (2), and multi- and single-wall nanotubes (3, 4). Some carbon allotropes, such as C60, are thermally very stable and can serve as a template to organize molecules, as is exemplified by the decoration of the mesh by C60 molecules.

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In the case of FePt, 1 ml of phase (1017 phase/ml) was mixed with 0.01 mM FeCl3 and 0.01 mM H2PtCl6. These mixtures were vortexed for 10 min to ensure mixing, and 0.1 M NaBH4 was added to reduce the metals forming the desired nanoparticles. The CoPt and FePt systems were applied directly to Si TEM grids and annealed under forming gas (5% H2) to prevent the onset of oxidation for 3 hours at 350°C.

The virus assembly was reconstructed from the gp8 protein structure obtained from the Protein Data Bank (PDB) [number 1f]{n} by application of the appropriate translation vectors. A random number generator was used to realistically incorporate peptide inserts in the gp8 assembly at a given incorporation percentage. The peptide inserts were modeled in the capsid environment with Monte Carlo software MCPRO (39) with solvent effects accounted for by the Poisson-Boltzmann tool-kit ZAP (40).


MCPRO: Version 1.68, W. L. Jorgensen, Yale Univer-
sity.

OpenEye Scientific Software, Cambridge, MA.

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