ELSEVIER

Phage as templates for hybrid materials and mediators for nanomaterial synthesis

Anna Merzlyak and Seung-Wuk Lee

Genetic engineering of phage provides unprecedented opportunities to build novel nanomaterials by integrating biology, chemistry and materials science principles. By mimicking the evolution process in nature, the phage is used as an information-mining tool to identify specific peptide information that can recognize desired materials at the molecular level. The unique recognition peptides of the phage can direct peptide-mediated mineralization processes to grow many useful nanometer-scale electronic and medical materials. The monodispersity and long rod shape of the phage, bearing specific recognition motifs, enable the organization of various nanomaterials into periodically ordered hierarchical structures that could be useful for electronic, optical and biotechnological applications.

Addresses
Department of Bioengineering, University of California, Berkeley, Physical Bioscience Division, Lawrence Berkeley National Lab, Berkeley Nanoscience and Nanotechnology Institute, UCSF and UCB Joint Graduate Group in Bioengineering, Berkeley CA 94720, USA

Corresponding author: Lee, Seung-Wuk (leesw@berkeley.edu)

Current Opinion in Chemical Biology 2006, **10**:246–252

This review comes from a themed issue on
Combinatorial chemistry and molecular diversity
Edited by Scott K Silverman and Paul J Hergenrother

Available online 4th May 2006

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2006.04.008

Introduction

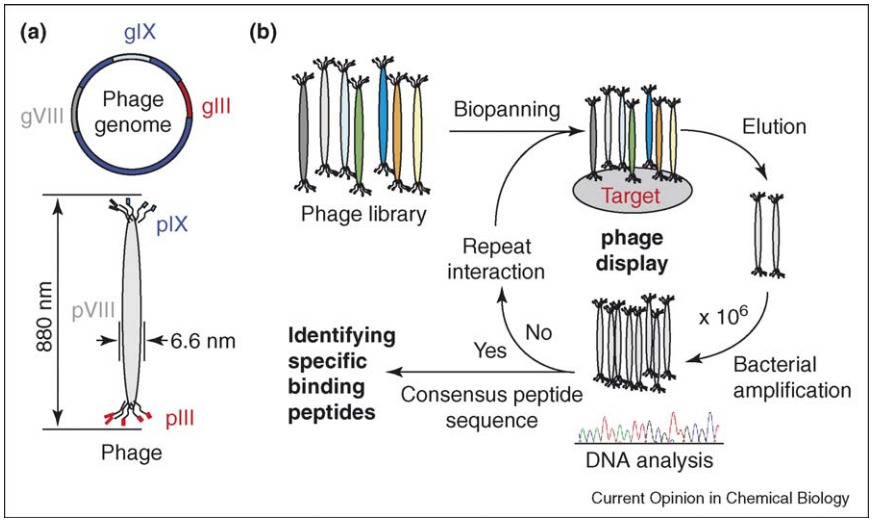
A fundamental challenge in nanoscience is to accomplish two 'complementary' goals simultaneously: to synthesize high-performance functional materials on the nanometer scale, and assemble them into well-defined structures that can surpass current lithographic capabilities [1,2]. Although many synthesized inorganic and organic nanomaterials have shown unique and interesting properties (e.g. light emission, data storage, and switching) [1,3–6] attributed to the exquisite design of their structures, compositions and dimensions, few methods have been able to integrate them into large-scale devices in cost-effective ways. On the other hand, in nature, numerous nanomaterials have been created and then perfected by evolution over millions of years [7–11]. Proteins and genes mutually orchestrate spatial and temporal control over the synthesis of organic and inorganic nanomaterials, which often results in hierarchically organized, functional structures. Some examples include glass sponges (optical fibers) [7], brittle stars (optical lens array) [8], diatoms (sophisticated periodic structures) [9], abalone shells (fracture-resistance materials) [10], and bones (support structure for vertebrates) [11]. Although protein-based 'bottom-up' synthesis of nanoscale functional materials and devices is one of the most promising areas in bio-inspired nanotechnology [5,6,12,13••,14], identifying active basic buildings blocks from biological examples is still challenging because of their complex biological nature (long encrypted peptides and genes). Many research groups have recently been successful in addressing the challenge of identifying specific functional motifs for a number of technologically useful materials (e.g. semiconductors, metals and ferromagnetic materials) by using a variety of combinatorial libraries displayed on bacteria and phage [2,15–17,18•,19–22]. Several recent review articles have summarized the use of such combinatorial methods and their application to synthesize nanomaterials through the biomineralization processes [15,23,24•,25•]. In this review, we focus on recent accomplishments in phage engineering for synthesis of functional nanomaterials as well as its potential future application and directions.

Phage display

Phage display is a combinatorial process to identify specific binding peptides using phage and bacterial biology through a fast, directed-evolution process. M13 bacteriophage is a bacterial virus composed of a single-stranded DNA encapsulated by various major and minor coat proteins. It has a long-rod filament shape that is approximately 880 nm long and 6.6 nm wide (Figure 1a) [26]. The viral capsid is composed of 2700 copies of helically arranged major coat protein, pVIII, and 5–7 copies of pIII, pVI, pIX and pVII, located at either of its ends [26,27]. All these proteins can be genetically modified to display short (<8–12 amino acid) peptide sequences at various locations on the phage. By inserting randomized DNA sequences into specific locations of phage genome, a highly diverse library of peptides (up to 10^{11} random sequences) can be displayed on the viral particles [26,27].

To select the best peptide binding sequence for a given target material, the engineered phage library pool goes through several rounds of selection processes (Figure 1b). Initially, the phage are allowed to bind to the target. The non-bound phage are then washed away, and the bound ones are eluted, and amplified through *E. coli* bacterial host infection. This evolutionary approach to select the

Figure 1



Phage structure and phage display selection process. **(a)** Schematic diagram of phage and its genome and **(b)** phage-display process to identify specific binding peptide motifs against desired targets.

fittest binding peptide sequence is repeated several times to enrich the phage with the best affinity peptide for the target. Finally the dominant binding peptides are identified by DNA analysis of the phage genome. Traditionally this technique has been used to identify protein epitopes, small antibodies, and study protein interaction [28,29]. Recently phage display has also been used to identify affinity peptide sequences for a variety of inorganic substances, such as semiconductor, magnetic, metallic and optically interesting materials [2,15–17,18•,19–22,30–33]. Specific binding peptide sequences identified through such directed evolution screening methods have been summarized in recent reviews [24•,25•].

Molecular complementarity at the peptide–material interface

Phage display performed with inorganic substrates can uncover novel information about the specific binding interaction between inorganic crystals and organic peptides. Belcher and co-workers have demonstrated that a genetically engineered phage can specifically recognize various semiconductor substrates. Whaley *et al.* demonstrated that the phage selected for the GaAs (III–V semiconductor) was able to distinguish between different crystallographic orientations, such as (1 0 0) and (1 1 1), and structurally similar materials, such as the (1 1 1)A (gallium terminated) or (1 1 1)B (arsenic terminated) face of GaAs, with a high selectivity [30]. The phage identified as binding the GaAs (1 0 0) surfaces contained amino acid residues rich in hydroxyl groups (serine, threonine) and amine Lewis base groups (asparagine, glutamine). These specific amino acid patterns were revealed through the directed evolution phage screening process. Other peptides that bind specifically to II–VI semiconductor (ZnS, CdS, PbS, etc.) have been identified, and predominantly display Lewis base groups and positively charged amino acids (arginine, histidine, lysine) [15]. A phage display screening for carbon nanotubes and graphite surfaces showed selective binding sequences with multiple tryptophan residues that could be attributed to aromatic ring interaction between the peptides and nanotubes [31,32]. Through comparative panning using a series of alanine substituted phage and deletion mutant phage, amphoteric titanium oxide binding peptides have shown that both negatively and positively charged, as well as proline residues play an important role in specific binding of isolated peptides [21].

A variety of methods have been used to show the chemical and crystallographic specificity of binding peptides. To analyze a specific binding phage (or peptides) with X-ray photoelectron spectroscopy and fluorescence microscopy, the phage can be coupled with traceable nanoparticles (Au, CdS, fluorescent tags, etc.) and labeled with secondary antibodies [15]. Surface probe microscopy can be used to quantify the adhesion of synthetic peptides [33]. Finally, an increasing affinity between target substrates and binding peptides has been observed by comparing the electronegativity of the substrate and the acidity of the amino acid side chains. In spite of the various efforts described above, understanding of the molecular level of binding specificity between identified peptides target substrates remains elusive.

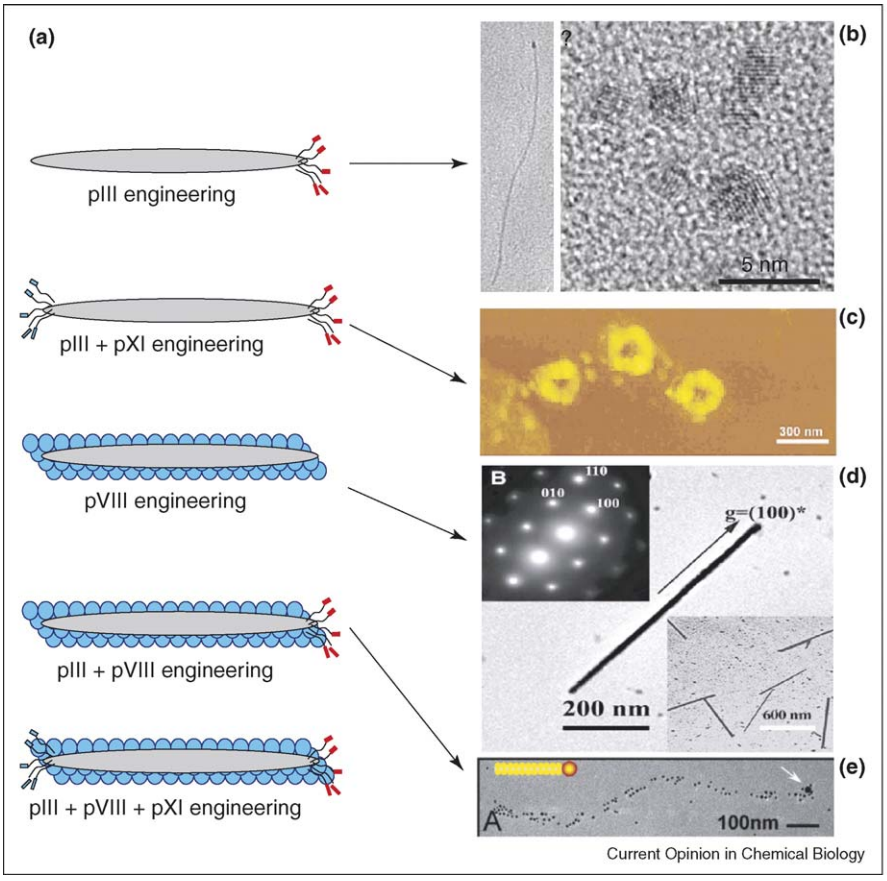
Inorganic nanocrystal and nanomaterial synthesis

Specific recognition peptides identified through phage library display for the desired crystal surfaces have been

exploited to template the growth of various inorganic crystals and nanomaterials. Flynn *et al.* identified specific binding peptide motifs with high affinity for various II-VI semiconductor materials (ZnS, CdS, PbS, etc.), and used them to direct mineralization of different phases of the inorganic crystal in controlled shape and morphology (Figure 2b) [15]. Such linear and constrained ZnS binding peptides specifically nucleated cubic (zinc blend) and hexagonal phases (wurzite) of ZnS nanocrystals, respectively.

Fusion of such crystal-nucleating peptides onto multiple phage coat proteins, enabled construction of a variety of interesting nanomaterials and nanostructures. Five demonstrated schemes of phage functionalization through genetic modification are depicted in Figure 2a. Single modification of pIII minor coat protein was previously described to synthesize ZnS and CdS nanoparticles on the tip of the phage (Figure 2b) [2]. Nam *et al.* modified both of the end minor coat proteins (pIII and pIX) with six histidine and HPQ (His–Pro–Gln) streptavidin binding peptide motifs. These phage were then used to construct nanoscale viral ring structures through specific binding of chemically conjugated streptavidin and Ni-NTA linkers (Figure 2c) [34]. By expressing the semiconductor and metal binding peptides on the major coat proteins using phagemid vectors, one-dimensional polycrystalline semiconductor, magnetic and metallic nanowires were synthesized on phagemid templates (Figure 2d) [16,18•]. Through an annealing process, high-quality single crystal monodisperse ZnS nanowires were fabricated with promising electro-optical and magnetic properties [18•]. A variety of architectures of phage-templated nanostructures were successfully demonstrated with concurrent modification of pIII and pVIII coat proteins (Figure 2e) [35••]. Both ends and major coat protein engineered phage might conduct multiple programmed operations. Such engineered phage are expected to be able to serve as electronic components as well as provide interconnections within electronic

Figure 2



Genetic modification of the phage for programmed material synthesis. **(a)** Possible genetic modifications of phage and their multiple combinations to synthesize nanomaterials. Example of nanoparticle synthesis on phage coat proteins: **(b)** ZnS nanoparticle synthesis on pIII minor coat proteins [2]; **(c)** pIII and pIX modification to construct viral ring structures [34]; **(d)** pVIII protein coat engineering to synthesize ZnS nanowires [16]; **(e)** pIII and pVIII engineering to construct hetero-nanoparticle arrayed structures [35••]. (b) and (d) were reproduced with permission from Science. Copyright 2002 and 2004 American Association for the Advancement of Science. (c) and (e) were reproduced with permission from American Chemical Society. Copyright 2004, 2005 American Chemical Society.

circuits, potentially resulting in self-interconnected electronic devices.

Other complex nanostructures were fabricated by incorporating specific binding peptide information with solid substrates or organisms. Using microfluidic channels, Naik *et al.* deposited phage-display identified silver binding peptides into a desired pattern, which was then reproduced by the biomineralization of silver nanoparticles [22]. Sweeney *et al.* modified bacterial outer membrane to nucleate CdS nanocrystals [36]. Klem *et al.* nucleated magnetic nanoparticles in protein cages (*Methanococcus jannaschii*) by incorporating CoPt specific peptides [37•]. Even the assembly of carbon nanotubes could potentially be facilitated by using peptide binding sequences isolated through phage display [31,32]. The specific functional peptide motifs are expected to be incorporated into many other organic/inorganic, polymeric and biological templates to build novel functional hybrid materials.

Nanomaterial synthesis through phage self-assembly

Besides the ability of viruses to specifically recognize and template specific crystal growth, they can also self-assemble into periodically ordered structures. Because of their monodispersity and long-rod shape, filamentous viruses have been extensively studied as liquid crystalline model systems [38–41]. By changing variables such as concentration of virus suspension, ionic strength of solution, and the externally applied force fields, the viruses are able to form into ordered liquid crystalline structures.

In an isotropic concentration range (<5 mg/ml), the molecular long axes of the viruses are randomly oriented. With increased concentration (10–20 mg/ml), virus particles begin to align into orientationally ordered structures, called nematic phase liquid crystals. The helical arrangement of pVIII subunits on the virus surfaces causes twisting of the nematic layers (cholesteric phase; 20–80 mg/ml). Finally, with concentrations above 100 mg/ml, the viruses obtain both positional and orientational order (smectic phase).

Similarly, the M13 viruses have been studied in much higher concentration ranges as solid-phase films [40] and one-dimensional fibers [42]. Viral liquid crystalline films were grown on air–liquid–solid interfaces and were shown to have a helically rotating smectic C-layered structure in the longitudinal direction (chiral smectic C phase). The observed order was maintained even in the sub-centimeter scale long-range structures [40]. One-dimensional microscale and nanoscale viral fibers were fabricated by using the genetically engineered viruses in the conventional wet-spinning and electrospinning processes [42]. Microscale virus-based fibers were spun by extruding the virus liquid crystalline suspensions through microsyringes into a cross-linking solution such as glutaraldehyde. In addition, the nanoscale virus-based fibers were fabricated with a high electric field electrospinning process.

The self-assembling nature of viruses combined with peptide display via genetic modification has been exploited to organize a variety of nanocomponents into periodically ordered films and fibers [2,41,42]. After the identification of ZnS-specific binding peptide through phage display, ZnS nanoparticles were self-assembled into periodically ordered film structures. Similarly anti-streptavidin viruses were used to align various nanoparticles, including gold, organic dyes and biological molecules [41]. Magnetic nanoparticle-virus composite films (FePt, CoPt etc.) were made through both the phagemid and pVIII engineering of viruses [17,43]. Finally, spontaneously ordered virus-based thin films with various electronic nanoparticles (GaN, Au) have been fabricated on multilayered electrolyte polymer films with layer-by-layer deposition methods [44].

Besides electronic and magnetic particles, viruses may be used for the assembly of biomedical hard tissues. A hydroxyapatite crystal, an inorganic component of bone, has been used as a target for phage display to isolate a specific binding peptide from a pVIII-engineered library. The identified sequence was shown to contain a collagen-like short peptide motif, and with 2700 copies of pVIII, fully covered the viral coat. Using the cloned phage, liquid crystalline collagen-like scaffolds were fabricated and templated with calcium and phosphate ions to create artificial bone structures (Lee SW *et al.*, unpublished data). Even though the results are promising, some of the challenges such as cytotoxicity and cellular viability, as well as the mechanical properties of the formed structures need to be addressed before further application of bone tissue regeneration to *in vivo* models.

The virus can also be endowed with multiple functionalities through displaying different peptide groups on pIII and pVIII. Using such multi-functional viruses a variety of architecturally complex structures have been rationally designed and shown to self-assemble [34,35••]. A technologically challenging example is the use of the phage to organize magnetic nanoparticles in a smooth thin film structure that could have potential applications in high-density memory devices [18•,43].

Future potential applications

One of the potential future arenas for genetically engineered phage might be as a template material for tissue engineering and regenerative medicine. Such templates would need to imitate the native extracellular matrix (ECM) environment, which is composed of a fibrous protein mesh and provides cells with a physical support, and guidance by chemical signaling and directional

alignment. Spatial control of dense chemical signaling arrays, proven desirable for controlling cellular activity [13••], can be obtained on the virus particles by genetic modification of desired virus coat proteins [26,27]. The replication of the phage through *E. coli* bacteria provides an easy and cost-effective method to obtain a large monodisperse population of the basic building units for the template. Finally, the ability of the phage to self-assemble into directionally organized networks can provide the cells with the physical and directional guidance present in many biological tissues (Figure 3) [45].

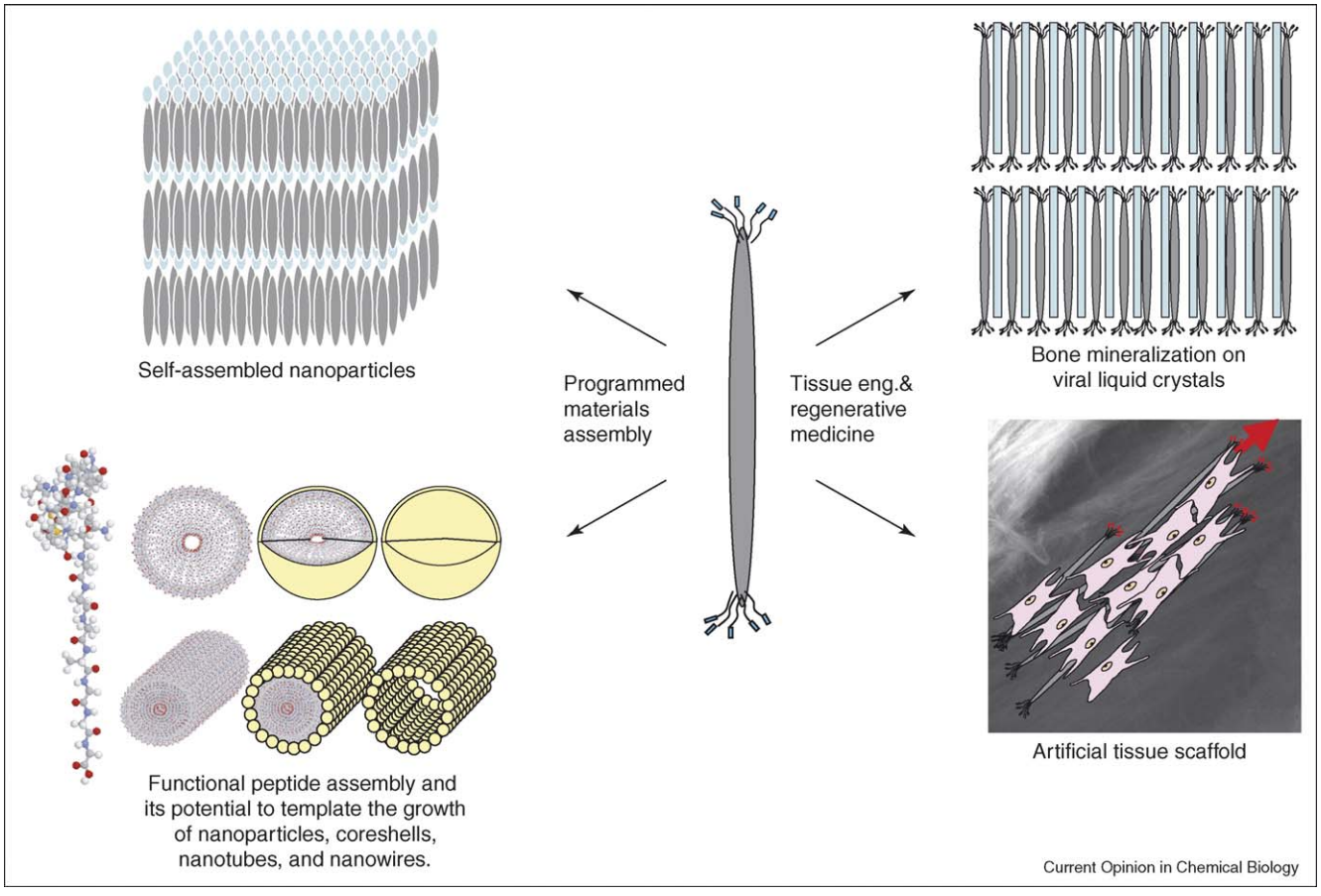
Another direction where phage display can be exploited is to fully harness the potential of proteins as the binding linkers between organic and inorganic substrates by identifying peptides with specific affinity for a variety of novel materials. Peptides selective for a material of desired functionality can be conjugated with other structural self-assembling peptides [12,13••,14] to provide alternative routes to self-assembly and nanometer-scale organization of high-density electronic components. In turn, such structures might be useful for future applications such as optical computing, data storage, sensors and photovoltaic cells (Figure 3).

Conclusion

Phage engineering and its phage-display products provide novel opportunities to integrate various disciplines including molecular biology, organic/inorganic chemistry, materials science and electric engineering. Through genetic modification, library information is constructed in the phage template. The directed evolution selection process enables an unprecedented level of novel peptide information to be mined at the organic and inorganic interface. Once identified, the specific recognition abilities encoded on phage can direct the growth of useful materials including semiconductors, magnets and metals as well as hard biological tissues such as bones and teeth. Finally, identical clones of such functionalized phage can self-assemble to form highly organized hierarchical structures.

We would like to define an emerging multidisciplinary field that uses genetically engineered viruses to build various electronic and medical materials with precise molecular level control, as '*virotronics*'. The discipline would incorporate the unique biological advantages that can be exploited from phage, such as evolution, specific recognition and self-replication with the engineering

Figure 3



Potential applications of phage in electronics, tissue engineering and peptide engineering.

aspects including information mining, storage and translation, as well as structural self-assembly, self-templating and organization of various materials into functional devices. In the near future, we hope that virotronic products will aid a variety of emerging fields, from self-assembled nanoscale computers and machines to drug delivery vehicles and self-healing regenerative tissues.

Acknowledgements

SL thanks Dr Carolyn Bertozzi for valuable discussion and support for the bone research at Lawrence Berkeley National Lab. SL also thanks Dr Angela Belcher for providing a pVIII-engineered phage library to work in artificial bone related work described here. This paper is supported by start-up funds from University of California, Berkeley and Lawrence Berkeley National Lab. AM thanks the UCSF/UCB Joint Graduate Group in Bioengineering for support.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Shevchenko EV, Talapin DV, Kotov NA, O'Brien S, Murray CB: **Structural diversity in binary nanoparticle superlattices**. *Nature* 2006, **489**:55-59.

2. Lee SW, Mao C, Flynn CE, Belcher AM: **Ordering of quantum dots using genetically engineered viruses**. *Science* 2002, **296**:892-895.

3. Wu Y, Fan R, Yang P: **Block-by-block growth of single-crystalline Si/SiGe superlattice nanowires**. *Nano Lett* 2002, **2**:83-86.

4. Milliron DJ, Hughes SM, Cui Y, Manna L, Li J, Wang L, Alivisatos AP: **Colloidal nanocrystal heterostructures with linear and branched topology**. *Nature* 2004, **430**:190-195.

5. Huang Y, Duan X, Cui Y, Lauhon LJ, Kim K, Lieber CM: **Logic gates and computation from assembled nanowire building blocks**. *Science* 2001, **294**:1313-1317.

6. Nam JM, Thaxton CS, Mirkin CA: **Nanoparticle-based bio–bar codes for the ultrasensitive detection of proteins**. *Science* 2003, **301**:1884-1886.

7. Sundar VC, Yablon AD, Grazul JL, Ilan M, Aizenberg J: **Fiber-optical features of a glass sponge**. *Nature* 2003, **424**:899-900.

8. Aizenberg J, Tkachenko A, Weiner S, Addadi L, Hendler G: **Calcitic microlenses as part of the photoreceptor system in brittlestars**. *Nature* 2001, **412**:819-822.

9. Morse DE: **Silicon biotechnology: harnessing biological silica production to construct new materials**. *Trends Biotechnol* 1999, **17**:230-232.

10. Smith BL, Shaffer TE, Viani M, Thompson JB, Frederick NA, Kindt J, Belcher A, Stucky GD, Morse DE, Hansma PK: **Molecular mechanistic origin of the toughness of natural adhesives, fibres and composites**. *Nature* 1999, **399**:761-763.

11. Weiner S, Wagner HD: **The material bone: structure-mechanical function relations**. *Annu Rev Mater Sci* 1998, **28**:271-298.

12. Stupp SI: **Biomaterials for regenerative medicine**. *MRS Bull* 2005, **30**:546-553.

13. •• Silva GA, Czeisler C, Niece KL, Beniash E, Harrington DA, Kessler JA, Stupp SI: **Selective differentiation of neural progenitor cells by high-epitope density nanofibers**. *Science* 2004, **303**:1352-1355.
An elegant demonstration of how peptide amphiphile systems can self-assemble to form nanotube networks, which are able to promote selective differentiation of neural progenitor cells into neurons, due to high density of the displayed IKVAV epitopes.

14. Zhang S: **Fabrication of novel materials through molecular self-assembly**. *Nat Biotechnol* 2003, **21**:1171-1178.

15. Flynn CE, Lee SW, Peele BR, Belcher AM: **Viruses as vehicles for growth, organization and assembly of materials**. *Acta Materialia* 2003, **51**:5867-5880.

16. Mao C, Flynn CE, Hayhurst A, Sweeney R, Qi J, Georgiou G, Iverson B, Belcher AM: **Viral assembly of oriented quantum dot nanowires**. *Proc Natl Acad Sci USA* 2003, **100**:6946-6951.

17. Lee SK, Yun DS, Belcher AM: **Cobalt ion mediated self-assembly of genetically engineered bacteriophage for biomimetic Co-Pt hybrid material**. *Biomacromolecules* 2006, **7**:14-17.

18. • Mao C, Solis DJ, Reiss BD, Kottmann ST, Sweeney RY, Hayhurst A, Georgiou G, Iverson B, Belcher AM: **Virus-based toolkit for the directed synthesis of magnetic and semiconducting nanowires**. *Science* 2004, **303**:213-217.
An elegant method of creating crystalline semiconducting and magnetic nanowires is described by varying the binding peptides fused to the major coat protein pVIII through phagemid engineering, nucleating the crystals, and annealing the structure to remove the original viral template.

19. Dai H, Choe WS, Thai CK, Sarikaya M, Traxler BA, Baneyx F, Schwartz DT: **Nonequilibrium synthesis and assembly of hybrid inorganic-protein nanostructures using an engineered DNA binding protein**. *J Am Chem Soc* 2005, **127**:15637-15643.

20. Brown S: **Metal-recognition by repeating polypeptides**. *Nat Biotechnol* 1997, **15**:269-272.

21. Sano K, Shiba K: **A hexapeptide motif that electrostatically binds to the surface of titanium**. *J Am Chem Soc* 2003, **125**:14234-14235.

22. Naik RR, Stringer SJ, Agarwal G, Jones SE, Stone MO: **Biomimetic synthesis and patterning of silver nanoparticles**. *Nat Mater* 2002, **1**:169-172.

23. Seeman NC, Belcher AM: **Emulating biology: building nanostructure from the bottom up**. *Proc Natl Acad Sci USA* 2002, **99(Suppl 2)**:6451-6455.

24. • Sarikaya M, Tamerler C, Jen AKY, Schulten K, Baneyx F: **Molecular biomimetics: nanotechnology through biology**. *Nat Mater* 2003, **2**:577-585.
A thorough review of how genetically engineered proteins can be selected for inorganic materials through phage-display method, and how these proteins can be used to mimic nature in combining both organic and inorganic materials to form nano-structures of controlled organization and function.

25. • Kriplani U, Kay BK: **Selecting peptides for use in nanoscale materials using phage-displayed combinatorial peptide libraries**. *Curr Opin Biotechnol* 2005, **16**:470-475.
A comprehensive review about the use of a phage-display library as a tool for production of novel nanoscale materials, as well as the variety of target materials, including semiconductors, metals and carbon nanotubes, against which highly selective peptide sequences have been identified.

26. Smith GP, Petrenko VA: **Phage display**. *Chem Rev* 1997, **97**:391-410.

27. Petrenko VA, Smith GP, Gong X, Quinn T: **A library of organic landscapes on filamentous phage**. *Protein Eng* 1996, **9**:797-801.

28. Presta LG: **Selection, design, and engineering of therapeutic antibodies**. *J Allergy Clin Immunol* 2005, **116**:731-736.

29. Mullaney BP, Pallavicini MG: **Protein-protein interactions in hematology and phage display**. *Exp Hematol* 2001, **29**:1136-1146.

30. Whaley SR, English DS, Hu EL, Barbara PF, Belcher AM: **Selection of peptides with semiconductor binding specificity for directed nanocrystal assembly**. *Nature* 2000, **405**:665-668.

31. Wang S, Humphreys ES, Chung S, Delduco DF, Lustig SR, Wang H, Parker KN, Rizzo NW, Subramoney S, Chiang Y, Jagota A: **Peptides with selective affinity for carbon nanotubes**. *Nat Mater* 2003, **2**:196-200.

32. Kulp JL III, Shiba K, Evans JS: **Probing the conformational features of a phage display polypeptide sequence directed against single-walled carbon nanohorn surfaces**. *Langmuir* 2005, **21**:11907-11914.

33. Goede K, Busch P, Grundmann M: **Binding specificity of a peptide on semiconductor surfaces**. *Nano Lett* 2004, **4**:2115-2120.

34. Nam K, Beau RP, Lee SW, Belcher AM: **Genetically driven assembly of nanorings based on the M13 virus**. *Nano Lett* 2004, **4**:23-27.

35. •• Huang Y, Chiang CY, Lee SK, Gao Y, Hu EL, Yoreo JD, Belcher AM: **Programmable assembly of nanoarchitectures using genetically engineered viruses**. *Nano Lett* 2005, **5**:1429-1434.
Viruses were made multifunctional by genetically engineering two coat proteins, to express a gold-binding motif on pVIII and streptavidin-binding motifs on pIII. Viral nanowire covered with gold nanoparticles was deposited on silicon substrates and their electronic properties measured.

36. Sweeney RY, Mao C, Gao X, Burt JL, Belcher AM, Georgiou G, Iverson BL: **Bacterial biosynthesis of cadmium sulfide nanocrystals**. *Chem Biol* 2004, **11**:1553-1559.

37. • Klem MT, Willits D, Solis DJ, Belcher AM, Young M, Douglas T: **Bio-inspired synthesis of protein-encapsulated CoPt nanoparticles**. *Adv Func Mater* 2005, **15**:1489-1494.
Magnetic CoPt nanoparticles were synthesized by protein cage formation of *Methanococcus jannaschii* heat shock proteins, previously modified with a phage-display selected dodecapeptide with a high binding affinity for $L1_0$ phase of CoPt.

38. Dogic Z, Fraden S: **Smectic phase is a colloidal suspension of semiflexible virus particles**. *Phys Rev Lett* 1997, **78**:2417-2420.

39. Dogic Z, Fraden S: **Cholesteric phase in virus suspensions**. *Langmuir* 2000, **16**:7820-7824.

40. Lee SW, Wood BM, Belcher AM: **Chiral smectic C structures of virus-based films**. *Langmuir* 2003, **19**:1592-1598.

41. Lee SW, Lee SK, Belcher AM: **Virus-based alignment of inorganic, organic, and biological nanosized materials**. *Adv Mater* 2003, **15**:689-692.

42. Lee SW, Belcher AM: **Virus-based fabrication of micro and nanofibers using electrospinning**. *Nano Lett* 2004, **4**:387-390.

43. Reiss BD, Mao C, Solis DJ, Ryan KS, Thomson T, Belcher AM: **Biological routes to metal alloys ferromagnetic nanostructures**. *Nano Lett* 2004, **4**:1127-1132.

44. Yoo PJ, Nam KT, Qi J, Lee SK, Park J, Belcher AM, Hammond PT: **Spontaneous assembly of viruses on multilayered polymer surfaces**. *Nat Mater* 2006, **5**:234-240.

45. Schoen FJ: **Tissues in biomaterials science: an introduction to materials in medicine**. Edited by Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, Academic Press, 1996:147–167.