Biotemplated nanostructures: directed assembly of electronic and optical materials using nanoscale complementarity

Nan Ma,[a] Edward H. Sargent*[b] and Shana O. Kelley*[ac]

Received 1st August 2007, Accepted 25th October 2007
First published as an Advance Article on the web 22nd November 2007
DOI: 10.1039/b711764g

The worlds of biology and materials engineering have traditionally been quite distinct. The spontaneous assembly of biological materials presents a stark contrast to the rational fabrication conventionally required for high-performance materials. The merger of these diverse fields represents a tremendous opportunity, given that biomolecules can organize into intricate, functionally sophisticated structures—exactly the sort of precise control urgently needed to make the next generation of materials for medicine, computing, communications, energy, and the environment. In the last several years, tremendous advances have been made towards using the structures of biomolecules as scaffolds and templates for nanomaterials. This overview discusses how the sequence and structural information encoded within proteins and nucleic acids can be used to program the synthesis of nanomaterials.

Introduction

Biomolecules are sophisticated nanostructures that are programmed by sequence information and tailored by evolution. The structure of a biomolecule dictates how biological functions are performed by influencing specific interactions between partners: single-stranded DNA molecules hybridize with complementary sequences through hydrogen bonding to form double-stranded helices; proteins and RNA molecules can fold into complicated three-dimensional structures to form binding domains or pockets that can accommodate other molecules. The structural diversity and combinatorial assembly of nucleotides and amino acids permits the generation of libraries of bionanostructures, with each member possessing unique properties.

Thus, contemplating the integration of these bionanostructures with inorganic nanomaterials presents a tantalizing possibility in the world of materials chemistry, where access to such structural versatility would be of extraordinary value. Already, solution-synthesized inorganic crystals such as colloidal

[a]*Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Canada. E-mail: shana.kelley@utoronto.ca*
[b]*Department of Electrical and Computer Engineering, Faculty of Applied Science and Engineering, University of Toronto, Canada. E-mail: ted.sargent@utoronto.ca*
[c]*Department of Biochemistry, Faculty of Medicine, University of Toronto, Canada*

Edward H. Sargent

Ted Sargent is the Canada Research Chair in Nanotechnology and Professor, Department of Electrical and Computer Engineering (Applied Science and Engineering) at the University of Toronto. He has published over 100 papers in refereed journals, and his most recent work has been in the application of colloidal quantum dots in optoelectronic devices and systems. In 2004–2005 he was Visiting Professor in Nanotechnology and Photonics at MIT. In 2003 he was named "one of the world's top young innovators" by MIT's Technology Review. In 2002 he was honoured by the Canadian Institute for Advanced Research as one of Canada's top twenty researchers under age forty. He received a B.Sc.Eng. (Engineering Physics) from Queen's University and a Ph.D. in Electrical and Computer Engineering (Photonics) from the University of Toronto in 1998. His book The Dance of Molecules: How Nanotechnology is Changing Our Lives *was released in 2006.*

Shana O. Kelley

Shana Kelley is a Professor in the Departments of Biochemistry (Medicine) and Pharmaceutical Sciences (Pharmacy) at the University of Toronto. She has co-authored 50 scientific publications, and has developed new biosensing methods, nanomaterials for ultrasensitive biodetection, and novel bioconjugates for cellular studies. Since 2000, she has received a Research Corporation Innovation Award, a Dreyfus New Faculty Award, a National Science Foundation CAREER Award, an Alfred P. Sloan Fellowship, a Camille Dreyfus Teacher-Scholar Award, the Pittsburgh Conference Achievement Award, and was named to Technology Review's list of Top 100 innovators. She holds a Ph.D. from California Institute of Technology, and was an NIH Postdoctoral Fellow at the Scripps Research Institute.

quantum dots have been shown to compete with, or in cases even outperform, traditional semiconductor devices in applications as diverse as optical sensing,[1] photovoltaics,[2] and electronics.[3] There are suggestions that imparting a more complex structure at the nanoscale—early examples include branched tetrapods[4] and core–shell nanoparticles[5]—would offer significant improvements in the performance of such devices.

Enhanced nanostructuring capabilities may be just the tip of the iceberg. Just as biological self-assembly acts over many length scales—from protein to ribosome to organelle to cell to organ to animal—could a unified bionanochemistry do the same? For example, could self-assembled semiconductor quantum dots organize directly into microstructured inverse opals, possessing optical resonances engineered to harvest specific wavelengths of light?[6] Could a highly efficient multi-junction solar cell be made not by sequential small-molecule evaporation,[7] but by spraying a single layer that subsequently segregates into suitably ordered phases? While the field of biotemplated nanostructures is in its infancy—with most of the work done to date demonstrating proof-of-principle for architectural control—applications like these should be realized in the near future.

This Feature Article will focus on recent studies that exploit biomolecules as templates and scaffolds for the synthesis and manipulation of inorganic nanomaterials including metals, semiconductors and carbon nanotubes (CNTs). A survey of recent achievements in this area makes it apparent that the sequences and structures of biomolecules can be used to exert bottom-up control over the synthesis and properties of nanomaterials. In addition, the use of combinatorial methods in molecular biology, which have been invaluable as a tool for materials synthesis, will be discussed in this Feature Article.

Metallic nanostructures templated by nucleic acids and peptides

Deposition of metallic nanostructures on DNA templates

Moore's Law—which describes the empirical observation of a doubling of computer chip speed and complexity every 18 months since the 1960s—is subject to physical limits. One of these constraints is the finite capacity of top-down lithography to define smaller and smaller electronic structures, such as the gate length of a field-effect transistor. Bottom-up self-assembly is already known to be highly effective at the shortest length scales—1–10 nm—defining structures such as metallic nanowires with refined precision. However, integrated semiconductor circuit architecture requires a union of top-down control with bottom-up self-assembly. Nanoscale circuits must be linked together, ideally without recourse, with nanofabrication techniques possessing the highest resolution. Self-assembly could provide a solution to this problem, if a sufficiently directed, versatile, and robust system could be put in place with defined rules for the formation of electrical linkages.

The nucleic acids (DNA and RNA) are perfectly suited for this task, as complementary sequences bind to each other in pairs with extremely high specificity. The Watson–Crick base pairing rules, dictating that cytidine (C) nucleotides pair with guanine (G) nucleotides and that adenine (A) nucleotides pair with thymine (T) nucleotides, provide a means for the generation of sophisticated patterns. To turn these biomolecular assemblies into circuits, the critical element needed is a method to turn DNA sequences into wires after assembly. By using electroless deposition of metals templated by complexes of metal ions bound to nucleic acids helices, several groups have demonstrated that they can convert DNA molecules into wires, and that the wires possess excellent electrical characteristics.

DNA helices, possessing diameters of 2 nm and lengths that can range from 2 nm to over 2 μm, have been used as quasi-1D deposition sites to grow conductive nanowires and then exploited to fabricate nanoelectronics (Fig. 1A).[8,9] This type of deposition relies on the fact that metal ions such as Ag(I), Pd(II),[10] Pt(II)[11,12] and Cu(II)[13] bind to the anionic DNA phosphate backbone, producing a scaffold for wire synthesis. A metallization step relying on a reduction agent or UV light[14] facilitates the heterogeneous nucleation and growth of nanoclusters on DNA that then leads to the formation of continuous nanowires.[15] Importantly, DNA-templated nanowires made in this fashion are highly conductive.[9,16]

An elegant advance on this approach was developed by Braun and co-workers; this group developed a strategy for molecular-level lithography by selectively metallizing DNA molecules.[17] As a masking agent, the RecA protein was used (Fig. 1B); this protein catalyzes the pairing of single-stranded DNA with complementary regions of double-stranded DNA during homologous DNA recombination and DNA repair.[18] To achieve lithography-like patterning, RecA was polymerized along a ssDNA probe to facilitate assembly with a dsDNA substrate and protect the selected DNA region from metallization (Fig. 1B). RecA polymerization is not DNA sequence-specific and thus the probe–substrate assembly is dictated by the sequences' homology. Once the nucleic acids complex is formed, Ag(I) ions bound to the unprotected DNA region can be reduced to form tiny Ag aggregates along the exposed DNA strands. The Ag aggregates subsequently catalyzed gold deposition, which then formed two continuous gold nanowires separated by the RecA gap. This approach enables nanowire formation at the desired region and represents a very precise means to program more complicated metallic nanostructure patterns dictated by designed DNA sequences.

In addition to forming linear structures, DNA molecules can be engineered to assemble two-dimensional arrays,[19] and consequently can serve as a template for the generation of multi-dimensional nanostructures. Woolley and co-workers designed a three-branched DNA motif to fabricate a three-terminal nanoelectronic device.[20] Three different DNA sequences simultaneously assembled into a three-branched DNA template with each branch exhibiting a mean length of 26 nm (Fig. 1C). Either silver or copper was deposited on the DNA template and highly specific metallization patterns were observed in the product obtained—three branched metallic nanostructures as well as higher-order structures were observed by TEM in comparison with irregular shaped products formed in the absence of the DNA. Thus, complex, rationally designed DNA motifs can be used to assemble building blocks into multi-dimensional nanoelectronic devices.

Peptide- and protein-based templating of metallic nanostructures

The discovery of biomineralization proteins in living organisms has inspired the use of peptides and proteins as templates to

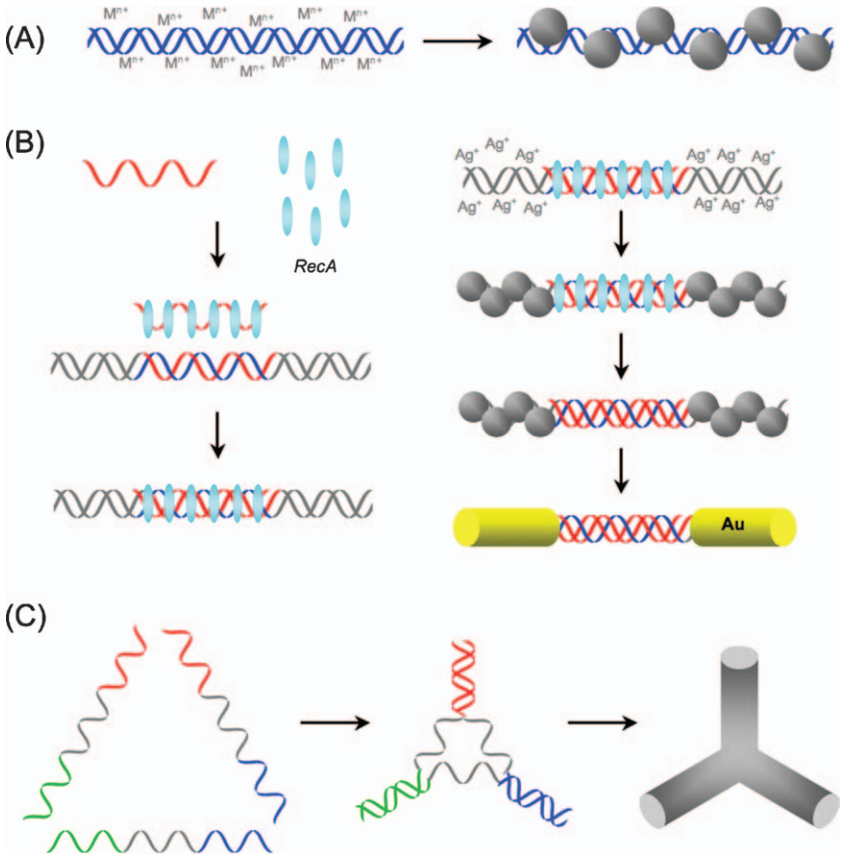

Fig. 1 DNA as a metallization template. (A) Metal particles formed along DNA strands upon reduction of metal ions bound to DNA phosphate backbone.[8] (B) Selective DNA metallization assisted by RecA protein.[17] (C) Branched metallic nanostructures formed along a self-assembled three-arm DNA motif.[20]

synthesize inorganic nanomaterials.[21,22] Small histidine-rich peptides such as AHHAHHAAD (HRE) have been used to synthesize various types of metal nanostructures including those made of Au and Ag.[23] The nanoparticles generated are recognized by antibodies, indicating that native peptide structure is retained in the complex. This finding indicates that nanoparticle–antibody recognition could be used to prepare higher-order structures with greater complexity. In addition, Matsui and co-workers have rationally tuned metallic nanoparticle sizes by altering peptide conformations and assembling the nanoparticles on nanotube templates.[24–26] For example, the conformation of a designed histidine-rich peptide (HG12) with coordinated Cu ions was altered by changing its pH value, which resulted in the formation of Cu nanocrystals with different sizes and dispersities. This approach provides a feasible way to achieve bottom-up control over the properties of nanoelectronic devices using biomolecule structures.

Proteins—possessing more variable, and more globular structures than peptide fragments—have been used to achieve more stringent control over particle growth. Protein cages,[27,28] peptide assembled nanotubes[29] or nanorings[30,31] have been used as reaction vessels (Fig. 2). For example, ferritin is a robust iron-storage protein self-assembled from 24 subunits that has a total internal diameter of 8 nm. Apoferritin, lacking the iron oxide core, therefore serves as a nanoreactor for the mineralization of metal particles such as cobalt, nickel and palladium. By infusing metal ions into the protein cavity through hydrophilic channels and then adding a reducing agent, metal nanoparticles could be prepared with dimensions close to that of the protein interior.[32] A recent adaptation of this approach was reported by Naik and co-workers, who fused a peptide selected to synthesize silver nanoparticles to the inner C-terminal end of the human L chain ferritin, and obtained a chimeric protein that constrained silver nanoparticle formation inside the cavity and produced monodisperse product.[33]

Semiconductor nanocrystals synthesized with biological ligands

The sequence information encoded within biomolecules provides a means to use biostructures as templating units for non-specific plating procedures once self-assembly has occurred. However, biomolecular building blocks can play an additional—and potentially more important—role in nanomaterials synthesis by providing a set of organized and diverse chemical functional groups that can exert control over a synthetic reaction. In this way, the sequence composition of a biomolecule could control the outcome of a reaction and dictate the properties (*e.g.* size, morphology) of a nanomaterial. One very exciting area where this principle could be applied is to the synthesis of colloidal

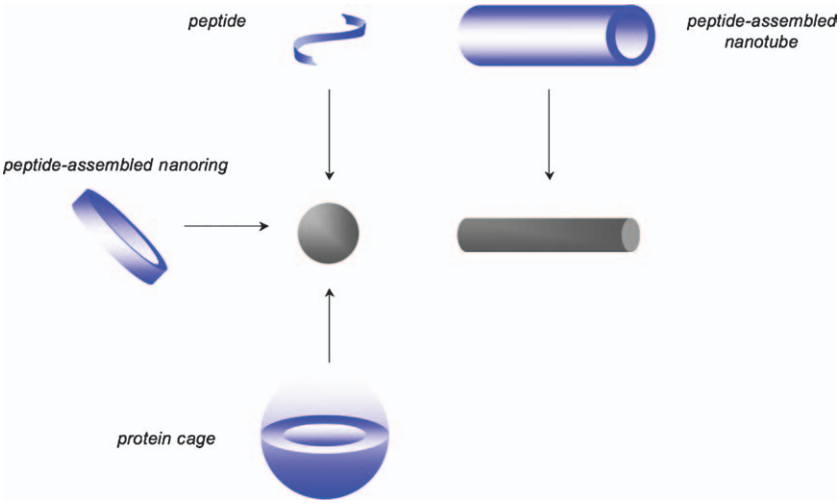

Fig. 2 Schematic illustration of metallic nanoparticles and nanowires synthesized with peptides[23] and peptide-assembled nanorings,[30,31] nanotubes[29] as well as protein cages.[27,28]

semiconductor nanocrystals (quantum dots). These materials are important synthetic targets because they exhibit tunable electronic and optical properties that can be exploited for biological imaging and the fabrication of optoelectronic devices.[34–36] Synthetic control in the preparation of these materials is typically achieved through brute-force optimization of reaction conditions, and general rules governing the production of quantum dots with defined sizes or properties have not yet been formulated. By using biomolecules as templating agents, the potential exists for programmable reactions where biological sequences could be used to control the outcome of quantum dot synthesis.

In order to evaluate peptides as agents that could bind to and passivate semiconductor surfaces, Belcher and co-workers used an *in vitro* selection method and combinatorial phage display libraries to interrogate a large region of sequence space.[37,38] Several peptide sequences were identified that exhibited specific binding affinities towards different semiconductor crystal compositions and crystal faces. This work was of tremendous significance because it was the first to illustrate that relationships based on structural complementarity could be established for biomolecules and inorganic materials. Peptide sequences composed of a high number of uncharged polar and Lewis-base containing functional groups (*e.g.* serine (S), threonine (T), asparagine (N), glutamine (Q)) were among those isolated, indicating that the donation of electrons to the semiconductor surface was important for binding (Fig. 3).[39] It was demonstrated that high specificity could be achieved in the binding complexes, with a GaAs binding peptide not displaying affinity for AlGaAs, suggesting that the peptide can discriminate very subtle changes in composition and crystal structure.[37]

Interactions between peptides and semiconductors have also been harnessed to build nanostructures in solution.[40,41] Peptides that specifically bind to ZnS and CdS semiconductor nanocrystals can be genetically fused to the virus coat protein pVIII to direct nanoparticles' nucleation and growth. ZnS nanocrystals formed along the virus are not only systematically arranged spatially by the viral topography, but the crystal faces display good alignment towards the viral surface. This degree of control over

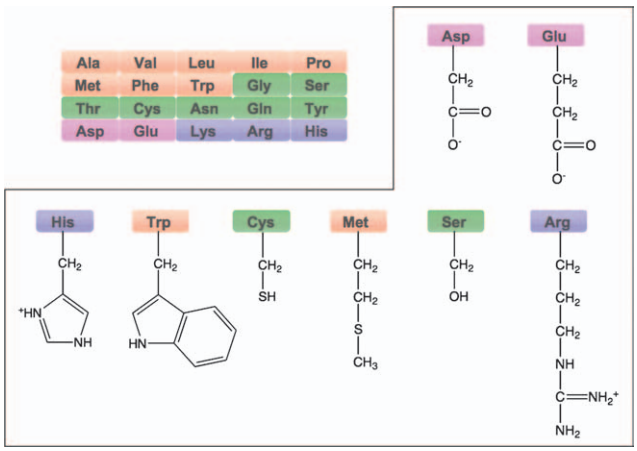

Fig. 3 Structures of amino acid residues that bind to semiconductor or metal surfaces.[37–40]

nanostructure positioning illustrates the power of a biomolecule-based approach to nanomaterials synthesis.

Nucleic acids including DNA and RNA have also been used to template semiconductor nanocrystals synthesis.[42–47] The negatively charged phosphate backbone and base functionalities present sites that can interact with and stabilize quantum dots (Fig. 4A). The first step in the assembly of a quantum dot involves seeding a ligand or templating moiety with metal ions. For nucleic acids, the phosphate backbone presents an ideal substrate to fulfill this role, as this structure can preorganize metal ions for subsequent reaction with an appropriate sulfur source that will produce the semiconductor. In the final stage of the reaction, ligand moieties must cap the quantum dot to stop the growth process and protect the surface of the structure so that aggregation does not occur (Fig. 4B). Functionalities on the nucleic acid bases, primarily the endocyclic and exocyclic amines, are able to serve as ligands and allow stable materials to be formed.

The utility of nucleic acid-templated quantum dot synthesis has been demonstrated in several studies where the sequences

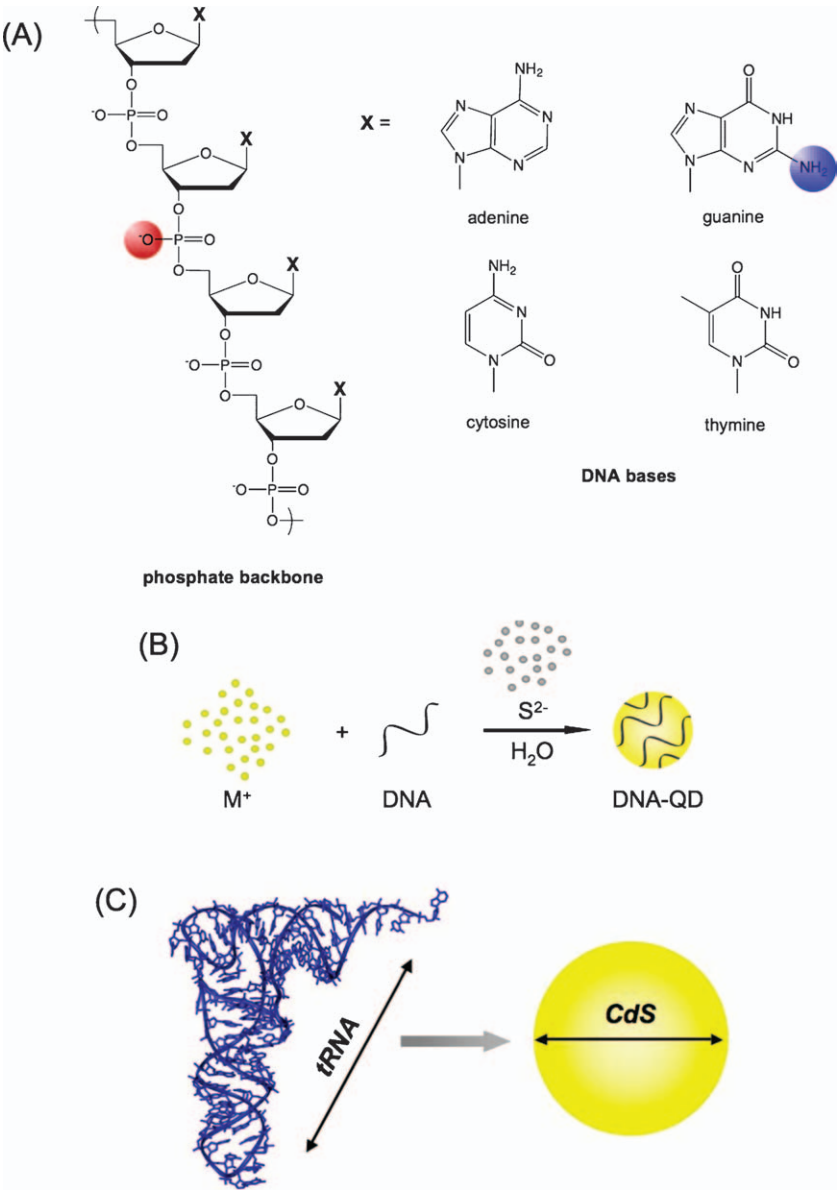

Fig. 4 Nucleic acid-templated semiconductor nanocrystal synthesis. (A) Structure of DNA phosphate backbone and base moieties. (B) Quantum dot synthesis templated by DNA oligonucleotides. (C) Transfer RNA as a template for CdS quantum dot formation.[44]

and structures of nucleic acids were used to control the size and properties of semiconductor products. Monomeric nucleotides have been used as ligands for PbS[46] and CdS,[47] and in each case it was discovered that individual DNA functionalities can control the synthesis and modulate the emission properties of the products obtained. These studies also revealed that the phosphate moiety displayed on a nucleotide is critical for the production of soluble, stable materials, and it was proposed that this functional group plays important roles both in the seeding the reaction by binding metal ions, and by promoting water solubility once the nucleotide is bound to the nanocrystal as a ligand. This approach—with atomic-level analyses of the relationships between nucleic acid structures and semiconductor quantum dot properties indicating that different DNA residues can modulate the synthesis of a nanomaterial and give rise to discrete outcomes—demonstrated that nucleic acids can be used to program the properties of nanomaterials with rational control.

Bioactive nucleic acids have also been used as templates for quantum dot synthesis, and can be used to gain structure-based control over nanocrystal size. Transfer RNA, with a well-defined three-dimensional structure, was used as a scaffold and ligand system in the aqueous synthesis of CdS, and it was found that nanocrystal structure can be modulated by the structure of the templating tRNA (Fig. 4C).[44] When possessing a folded three-dimensional structure, tRNA-templated synthesis yields a single product: spherical CdS particles with hydrodynamic diameters of ~6 nm and nanocrystal diameters of ~5 nm. When the same tRNA was rendered unstructured through the introduction of destabilizing mutations, a range of products are observed with hydrodynamic diameters of 7–12 nm and nanocrystal diameters that range between 5 and 10 nm. These findings indicate that

biomolecules can be used to systematically engineer the structures and properties of semiconductor-based materials, and that the synergy between the dimensions of nanostructures and biomolecules provides a means to tune the properties of materials with nanoscale precision.

Toxicity resulting from decomposition and release of heavy metal ions and/or ligand moieties has hampered efforts to use quantum dots as lumiphores in biological systems.[48–51] Often, materials used for biological applications are synthesized in organic solution and then transferred to aqueous solution by a ligand exchange step that increases the polarity of the nanocrystal coating.[48] Many materials prepared in this manner do not exhibit good stability, likely because the ligand exchange does not provide complete protection from water and decomposition of the particle then occurs when the new solvent interacts with the crystal surface. In contrast, nucleic acid templated quantum dots are made in water, and therefore the isolation of stable products provides an excellent starting point for obtaining materials with good stability in biological fluids. Indeed, a recent study of CdS nanocrystals synthesized using oligomeric DNA oligonucleotides demonstrated that very low levels of toxicity resulted from the exposure of cells to these biotemplated materials.[52] Thus, in addition to producing a means to achieve structural control over quantum dot structure, the use of nucleic acid based ligands also provides a way to obtain materials with improved toxicity profiles.

Semiconductor quantum dots made using peptides as templates have also been produced, and highly emissive materials can be obtained using this approach.[53] For example, a small tripeptide glutathione can be used to synthesize CdTe quantum dots with >60% quantum yield and narrow spectral features, *e.g.* a full-width at half-maximum of 33 nm. The tripeptide-templated products exhibited size-tunable optical properties with the emission wavelength ranging from 480 nm to 650 nm. The synthesis conditions are mild and less time-intensive compared to conventional organometallic routes, thus this biotemplated approach constitutes a useful advance over existing synthetic methods.[54]

Complex heteronanostructures assembled by biological coding

Many of the applications envisioned for nanomaterials—including assembling new electronic devices and energy conversion and storage—involve bringing together materials with diverse properties. In this capacity, biological molecules serve as ideal vehicles for programmable and self-assembling heterostructure fabrication. Viruses can serve as useful tools to assemble complex structures, as rational design can be used to include different substrate-specific peptide motifs. For example, complex arrays of gold nanoparticles and CdSe quantum dots have been prepared through the incorporation of gold-binding motifs into the capsid and streptavidin-binding motifs of viruses.[55] A particularly elegant example of the biotemplated assembly of a complex heteronanostructure produced a virus-based lithium ion battery with improved capacity (Fig. 5A).[56] A bifunctional virus template was designed to simultaneously express a tetraglutamate Co_3O_4 nucleation motif and a gold-binding motif. Au–Co_3O_4 hybrid nanowires, synthesized for the first time using this method, were formed along the virus and subsequently assembled into macroscopic liquid crystalline films as electrodes. The incorporation of Au nanoparticles into Co_3O_4 nanowires remarkably improved the electrochemical performance and imparted 30% greater capacity. The generation of a novel material using biotemplating, and the demonstration of enhanced performance attained with this material, illustrates the power of merging biomolecular and materials chemistry.

DNA can also serve as a scaffold to assemble different materials into complex heteronanostructures.[57–59] For example, sequence-specific DNA lithography can enable the integration of various types of materials into metal nanowire gaps to form heteronanostructures.[57] RecA nucleoprotein filaments, which are resistant to DNA metallization, can bind to RecA antibodies and biotin-conjugated antibodies which then bind with streptavidin coated single-wall carbon nanotubes (SWNT). The resulting DNA–SWNT complex can then be metallized: the exposed area is coated with metallic wire, and a robust contact is established with the SWNT. More complicated circuits can be generated this way by integrating distinct nanoelectronics at different addresses using a DNA network as a scaffold.

Another example of a DNA-directed approach to heteronanostructure assembly produced CNT–ZnO hybrid structures (Fig. 5C).[58,59] Gold nanoparticle catalysts were delivered site-specifically onto the tips of carbon nanotube arrays, and ZnO nanorods were grown with one end contacting a gold nanoparticle and the other end affixed to a carbon nanotube. The heterojunctions produced were electronically functional, demonstrating that a multimaterial, optically active nanowire platform could be generated using DNA-directed assembly.

Nanomaterial separation with biomolecular carriers

The purification of nanostructures is a challenging and important step required for commercial implementation of these materials. In order to separate mixtures of nanomaterials that may be similar in size and structure, it is necessary to enlist a carrier or separation matrix that can detect and harness molecular-scale features. The complementary sizes of nanostructures and biomolecules can be leveraged to purify nanomaterials, as the interactions of biological molecules will be specific for certain types of nanostructures—just as they are specific to their biological partners. Achieving nanomaterial separation with biomolecular carriers has been realized for both metal nanoparticles and carbon nanotubes.[60–63] Both DNA molecules and synthetic peptides are known to make specific complexes with nanostructures that then enable efficient purification. Moreover, the effectiveness of the bio-assisted purification is modulated by biomolecular sequence and controlled by coded properties.

The polydispersity and poor aqueous solubility of carbon nanotubes restrict their further application, and thus efficient purification methods are critical for the commercial use of CNTs. Exploiting the similar size scales of biomolecules and CNTs, biomolecule-assisted separation for carbon nanotube purification has been used with great success.[60,61] DNA is able to disperse and stabilize carbon nanotubes, presumably because the hydrophobic bases can interact with carbon nanotube surfaces through π-stacking (Fig. 6A). Carbon nanotube dispersion efficiency is DNA-sequence dependent, and poly(T) is known

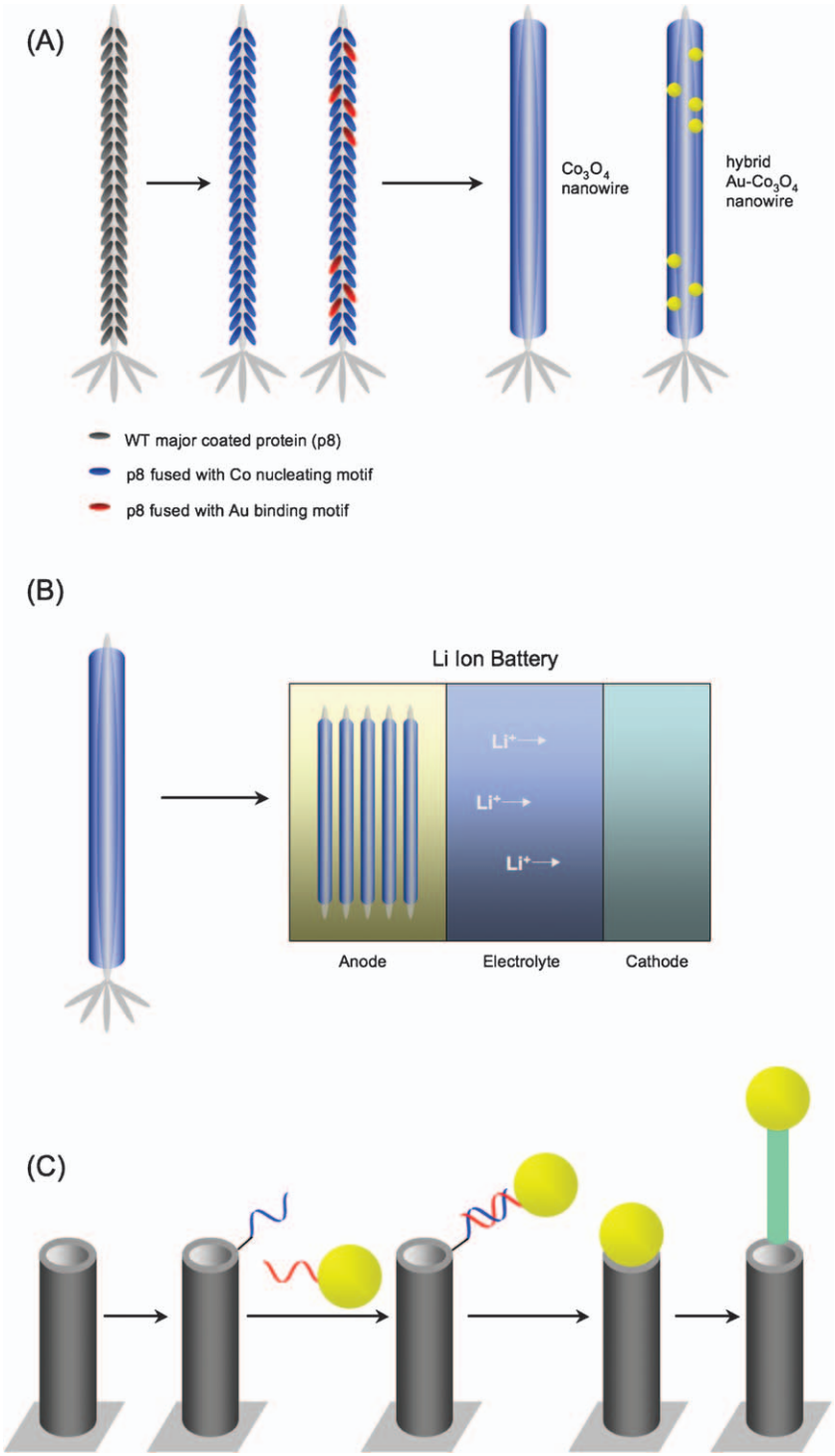

Fig. 5 Biotemplated complex heteronanostructures. (A) Hybrid Au–Co_3O_4 nanowires formed along a bifunctional virus template featuring Co_3O_4 nucleation and Au-binding motifs and (B) the subsequent assembling of nanowires for Li ion battery fabrication.[56] (C) CNT–ZnO–Au heteronanostructure formation controlled by DNA hybridization.[58,59]

to be the homopolymer providing the highest dispersion efficiency.[60] Once complexed with DNA, CNTs can then be separated based on anion-exchange column chromatography, with the net negative charge density of DNA–carbon nanotube hybrids discriminating different types of carbon nanotubes. Metallic tubes were separated from semiconducting nanotubes using this approach. In addition, the charge density also depends on the diameter of the tubes, thus carbon nanotubes can be separated by diameter rather than length. The separation efficiency achieved with this approach was DNA-sequence dependent, with a d(GT) sequence performing best: this sequence is believed to adopt a unique double-helical structure that minimizes the

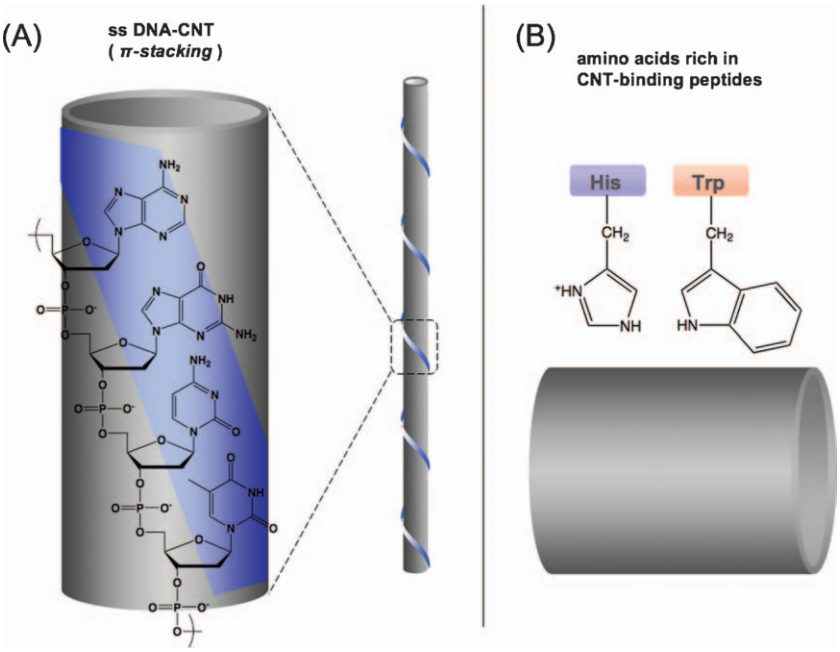

Fig. 6 Interactions between biomolecules and carbon nanotubes exploited for CNT purification. (A) π-Stacking of aromatic DNA bases with CNT and helical wrapping of ssDNA on CNT.[60,61] (B) Amino acids showing affinity towards CNTs.[62]

allowed conformations on the tubes and thus provided the best separation efficiency.[61] Peptides can also be used for the same application, and sequences rich in histidine and tryptophan are known to be effective complexation and separation agents (Fig. 6B).[62] If in the future, different diameters or chiralities of carbon nanotubes can be separated with specific peptide sequences, this strategy will provide a complete toolbox for purification of these important materials.

Tools for improved biotemplated nanomaterials

The techniques that revolutionized molecular biology several decades ago are of great utility in manipulating nanomaterials. Indeed, many of the most spectacular accomplishments in the area of biotemplated materials have harnessed the highly combinatorial methods that can be applied to select peptide, DNA and RNA sequences producing specific materials. The use of *in vitro* selection of nucleic acids and phage display of peptide sequences has yielded biomolecules that can direct the synthesis of metallic and semiconductor nanostructures of defined shapes and sizes, with the next goal being the generation of materials with specific electronic or optical properties through combinatorial sequence searches.

Phage display allows the insertion of foreign DNA fragments into a minor coat protein gene of filamentous phage so that a library of fusion proteins can be expressed on the surface of virions.[64,65] This method enables the high-throughput screening of billions of random peptide sequences towards a target using affinity selection. Initially, phage display was used to map and discover epitopes of antibodies. As described above, this technique has been adapted for inorganic materials, enabling the discovery of metal-[66] and semiconductor-recognizing[37] peptides. A typical phage display selection strategy is illustrated in Fig. 7A. The selection starts with a mixture of phages containing 10^9 randomized peptide inserts that are incubated with a bulk substrate. Peptides exhibiting binding affinity for the substrate are captured while the non-specific peptides are rinsed off and removed, and finally, retained phages are eluted with reduced pH buffer and amplified using a bacterial host. After each selection round the binding peptides will be enriched among the total population and finally the peptide-encoding DNA will be sequenced to identify the peptide sequence. Several semiconductor and metal recognition peptides have been selected including those binding to GaAs, ZnS, CdS, PbS, Au, Ag, and Co.[37,38,40,67–69] Peptides that selectively bind to carbon nanotubes have also been discovered.[62]

Compared to peptides, nucleic acids have more limited functionalities with only four different natural nucleotides that are combined to exert function. However, the discovery of RNA catalysis showed that these residues can be used to promote chemical reactions,[70] and eventually prompted the use of this biopolymer in nanomaterials synthesis. Recently, RNA was shown to be able to mediate metal–metal bond formation *via in vitro* selection.[71] The evolved RNA sequences were shown to catalyze palladium particle growth to a few micrometers with uniform morphology present.

The selection cycle used to isolate RNA sequences capable of mediating Pd nanoparticle growth is shown in Fig. 7B. A DNA library containing 40 randomized positions (10^{14} unique sequences) flanked by two primer binding sites were synthesized and used as templates to synthesize RNA transcripts containing modified UTP nucleotides. The single stranded RNA product was mixed with metal precursors to mediate particle growth. The active RNA sequences that became complexed with particles were separated with inactive sequences by size cutoff membrane or native polyacrylamide gel electrophoresis and then amplified by polymerase chain reaction (PCR). After several selection rounds, the conserved sequence regions were identified. Interestingly, different RNA sequence families were shown to generate

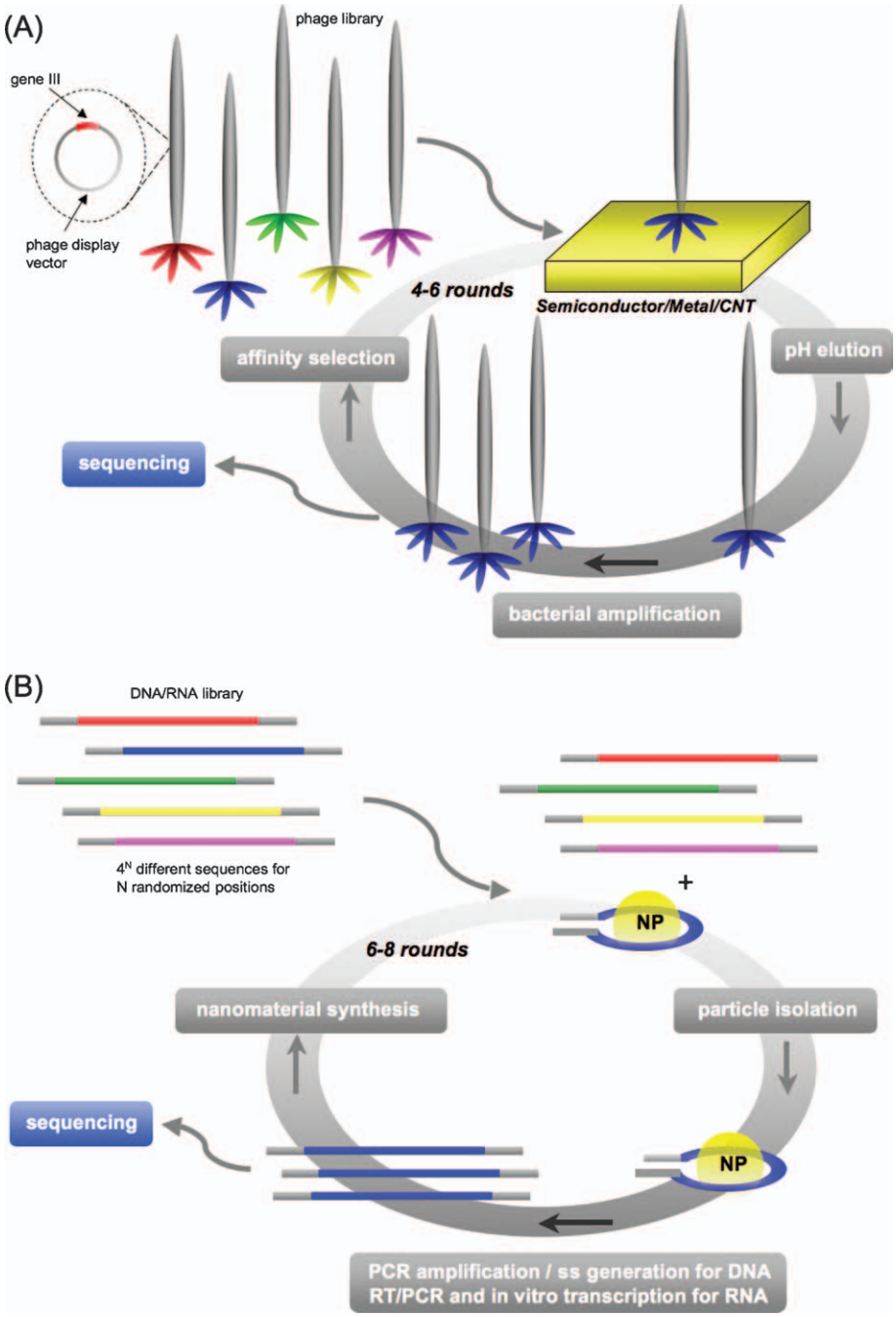

Fig. 7 Combinatorial approaches for nanomaterials fabrication. (A) Phage display for the selection of peptide sequences possessing high affinities for specific inorganic material surfaces.[37,38] (B) *In vitro* selection of DNA or RNA sequences catalyzing inorganic nanoparticle formation.[71]

palladium particles with distinct shapes.[72] Several reports of the use of *in vitro* selection for nanomaterials development have now appeared, indicating that the *in vitro* selection is a promising method to manipulate and optimize nanostructures.

Frontiers and applications of biotemplated nanomaterials

The first wave of nanobiotechnology demonstrated that biomolecules can influence—and, with rational understanding of their mechanisms of action, direct—the creation of nanostructured materials.

The second wave of discoveries demonstrated that such manipulations can lead to control not only over morphology, but also over properties—including conductivity, light emission, dielectric constant, polarization, *etc*. In some cases, the materials obtained are superior to those generated without the intervention of biomolecule-based self-assembly.

The third wave—on whose cusp we lie today—is the demonstration that enhanced materials properties can be exploited to

build improved devices. Energy storage has been amongst the first frontiers in this regard; accelerated progress in photovoltaics, environmental sensing, and electronics lies close at hand. As these practical applications are realized, the sensitivities of biotemplated nanomaterials to temperature and other environmental factors will have to be addressed in order to ensure that robust devices can be generated.

The fourth wave—which lies in sight—is to understand and then exploit biology's capacity to engineer along many length scales. The nanometer scale offers quantum confinement; the tens of nanometers scale is ideal for devices detecting single molecules; the hundreds of nanometers to multiple microns scale offers resonances with light ranging from the ultraviolet through the visible and into the infrared; and from thenceforth into the terahertz and gigahertz regimes that merge with the realm of human experience. Nature provides proof by example that—even when we begin at the bottom, at the nanometer length-scale—the realization of robust, versatile, human-scale devices is within reach.

References

1 G. Konstantatos, I. Howard, A. Fischer, S. Hoogland, J. Clifford, E. Klem, L. Levina and E. H. Sargent, *Nature*, 2006, **442**, 180.
2 I. Gur, N. A. Fromer, M. L. Geier and A. P. Alivisatos, *Science*, 2005, **310**, 462.
3 D. V. Talapin and C. B. Murray, *Science*, 2005, **310**, 86.
4 L. Manna, E. C. Scher and A. P. Alivisatos, *J. Am. Chem. Soc.*, 2000, **122**, 12700.
5 B. O. Dabbousi, J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen and M. G. Bawendi, *J. Phys. Chem. B*, 1997, **101**, 9463.
6 Y. A. Vlasov, N. Yao and D. J. Norris, *Adv. Mater.*, 1999, **11**, 165.
7 J. Xue, S. Uchida, B. P. Rand and S. R. Forrest, *Appl. Phys. Lett.*, 2004, **85**, 5757.
8 E. Braun, Y. Eichen, U. Sivan and G. Ben-Yoseph, *Nature*, 1998, **391**, 775.
9 J. Richter, M. Mertig and W. Pompe, *Appl. Phys. Lett.*, 2001, **78**, 536.
10 J. Richter, R. Seidel, R. Kirsch, M. Mertig, W. Pompe, J. Plaschke and H. K. Schackert, *Adv. Mater.*, 2000, **12**, 507.
11 M. Mertig, L. C. Ciacchi, R. Seidel and W. Pompe, *Nano Lett.*, 2002, **2**, 841.
12 R. Seidel, L. C. Ciacchi, M. Weigel, W. Pompe and M. Mertig, *J. Phys. Chem. B*, 2004, **108**, 10801.
13 A. T. Woolley and C. F. Monson, *Nano Lett.*, 2003, **3**, 359.
14 L. Berti, A. Alessandrini and P. Facci, *J. Am. Chem. Soc.*, 2005, **127**, 11216.
15 J. T. Petty, J. Zheng, N. V. Hud and R. M. Dickson, *J. Am. Chem. Soc.*, 2004, **126**, 5207.
16 H. Yan, S. H. Park, G. Finkelstein, J. H. Reif and T. H. LaBean, *Science*, 2003, **301**, 1882.
17 K. Keren, M. Krueger, R. Gilad, G. Ben-Yoseph, U. Sivan and E. Braun, *Science*, 2002, **297**, 72.
18 R. M. Story, I. T. Weber and T. A. Steitz, *Nature*, 1992, **355**, 318.
19 N. C. Seeman, *Nature*, 2003, **421**, 427.
20 H. A. Becerril, R. M. Stoltenberg, D. R. Wheeler, R. C. Davis, J. N. Harb and A. T. Woolley, *J. Am. Chem. Soc.*, 2005, **127**, 2828.
21 J. Ziegler, R. T. Chang and D. W. Wright, *J. Am. Chem. Soc.*, 1999, **121**, 2395.
22 R. Djalali, Y. Chen and H. Matsui, *J. Am. Chem. Soc.*, 2002, **124**, 13660.
23 J. M. Slocik, J. T. Moore and D. W. Wright, *Nano Lett.*, 2002, **2**, 169.
24 I. A. Banerjee, L. Yu and H. Matsui, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 14678.
25 R. Djalali, Y. Chen and H. Matsui, *J. Am. Chem. Soc.*, 2003, **125**, 5873.
26 L. Yu, I. A. Banerjee, M. Shima, K. Rajan and H. Matsui, *Adv. Mater.*, 2004, **16**, 709.
27 F. C. Meldrum, V. J. Wade, D. L. Nimmo, B. R. Heywood and S. Mann, *Nature*, 1991, **349**, 684.
28 M. Allen, D. Willits, J. Mosolf, M. Young and T. Douglas, *Adv. Mater.*, 2002, **14**, 1562.
29 M. Reches and E. Gazit, *Science*, 2003, **300**, 625.
30 N. Nuraje, K. Su, A. Haboosheh, J. Samson, E. P. Manning, N. Yang and H. Matsui, *Adv. Mater.*, 2006, **18**, 807.
31 S.-Y. Lee, X. Gao and H. Matsui, *J. Am. Chem. Soc.*, 2007, **129**, 2954.
32 T. Ueno, M. Suzuki, T. Goto, T. Matsumoto, K. Nagayama and Y. Watanabe, *Angew. Chem., Int. Ed.*, 2004, **43**, 2527.
33 R. M. Kramer, C. Li, D. C. Carter, M. O. Stone and R. R. Naik, *J. Am. Chem. Soc.*, 2004, **126**, 13282.
34 A. P. Alivisatos, *Science*, 1996, **271**, 933.
35 X. Michalet, F. F. Pinaud, L. A. Bentolila, J. M. Tsay, S. Doose, J. J. Li, G. Sundaresan, A. M. Wu, S. S. Gambhir and S. Weiss, *Science*, 2004, **307**, 538.
36 I. L. Medintz, H. T. Uyeda, E. R. Goldman and H. Mattoussi, *Nat. Mater.*, 2005, **4**, 435.
37 S. R. Whaley, D. S. English, E. L. Hu, P. F. Barbara and A. M. Belcher, *Nature*, 2000, **405**, 665.
38 S.-W. Lee, C. Mao, C. E. Flynn and A. M. Belcher, *Science*, 2002, **296**, 892.
39 B. R. Peelle, E. M. Krauland, K. D. Wittrup and A. M. Belcher, *Langmuir*, 2005, **21**, 6929.
40 C. Mao, C. E. Flynn, A. Hayhurst, R. Sweeney, J. Qi, G. Georgiou, B. Iverson and A. M. Belcher, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 6946.
41 C. Mao, D. J. Solis, B. D. Reiss, S. T. Kottmann, R. Y. Sweeney, A. Hayhurst, G. Georgiou, B. Iverson and A. M. Belcher, *Science*, 2004, **303**, 213.
42 L. Levina, V. Sukhovatkin, S. Musikhin, S. Cauchi, R. Nisman, D. P. Bazett-Jones and E. H. Sargent, *Adv. Mater.*, 2005, **17**, 1854.
43 J. H. Choi, K. H. Chen and M. S. Strano, *J. Am. Chem. Soc.*, 2006, **128**, 15584.
44 N. Ma, C. J. Dooley and S. O. Kelley, *J. Am. Chem. Soc.*, 2006, **128**, 12598.
45 A. Kumar and A. Jakhmola, *Langmuir*, 2007, **23**, 2915.
46 S. Hinds, B. J. Taft, L. Levina, V. Sukhovatkin, C. J. Dooley, M. D. Roy, D. D. MacNeil, E. H. Sargent and S. O. Kelley, *J. Am. Chem. Soc.*, 2006, **128**, 64.
47 C. J. Dooley, J. Rouge, N. Ma, M. Invernale and S. O. Kelley, *J. Mater. Chem.*, 2007, **17**, 1687.
48 A. M. Derfus, W. C. W. Chan and S. N. Bhatia, *Nano Lett.*, 2004, **4**, 11.
49 G. Oberdorster, V. Stone and K. Donaldson, *Nanotoxicology*, 2007, **1**, 2.
50 E. Chang, N. Thekkek, W. W. Yu, V. L. Colvin and R. Drezek, *Small*, 2006, **2**, 1412.
51 C. Kirchner, T. Liedl, S. Kudera, T. Pellegrino, A. M. Javier, H. E. Gaub, S. Stölzle, N. Fertig and W. J. Parak, *Nano Lett.*, 2005, **5**, 331.
52 N. Ma, J. Yang, K. M. Stewart and S. O. Kelley, *Langmuir*, 2007, in press.
53 H. Qian, C. Dong, J. Weng and J. Ren, *Small*, 2006, **2**, 747.
54 C. B. Murray, D. J. Norris and M. G. Bawendi, *J. Am. Chem. Soc.*, 1993, **115**, 8706.
55 Y. Huang, C.-Y. Chiang, S. K. Lee, Y. Gao, E. L. Hu, J. D. Yoreo and A. M. Belcher, *Nano Lett.*, 2005, **5**, 1429.
56 K. T. Nam, D.-W. Kim, P. J. Yoo, C.-Y. Chiang, N. Meethong, P. T. Hammond, Y.-M. Chiang and A. M. Belcher, *Science*, 2006, **312**, 885.
57 K. Keren, R. S. Berman, E. Buchstab, U. Sivan and E. Braun, *Science*, 2003, **302**, 1380.
58 A. D. Lazareck, S. G. Cloutier, T.-F. Kuo, B. J. Taft, S. O. Kelley and J. M. Xu, *Nanotechnology*, 2006, **17**, 2661.
59 A. D. Lazareck, S. G. Cloutier, T.-F. Kuo, B. J. Taft, S. O. Kelley and J. M. Xu, *Appl. Phys. Lett.*, 2006, **89**, 103109.
60 M. Zheng, A. Jagota, E. D. Semke, B. A. Diner, R. S. Mclean, S. R. Lustig, R. E. Richardson and N. G. Tassi, *Nat. Mater.*, 2003, **2**, 338.
61 M. Zheng, A. Jagota, M. S. Strano, A. P. Santos, P. Barone, S. G. Chou, B. A. Diner, M. S. Dresselhaus, R. S. Mclean, G. B. Onoa, G. G. Samsonidze, E. D. Semke, M. Usrey and D. J. Walls, *Science*, 2003, **302**, 1545.

62 S. Wang, E. S. Humphreys, S.-Y. Chung, D. F. Delduco, S. R. Lustig, H. Wang, K. N. Parker, N. W. Rizzo, S. Subramoney, Y.-M. Chiang and A. Jagota, *Nat. Mater.*, 2003, **2**, 196.
63 J. S. Lee, S. I. Stoeva and C. A. Mirkin, *J. Am. Chem. Soc.*, 2006, **128**, 8899.
64 G. P. Smith, *Science*, 1985, **228**, 1315.
65 S. F. Parmley and G. P. Smith, *Gene*, 1988, **73**, 305.
66 S. Brown, *Nat. Biotechnol.*, 1997, **15**, 269.
67 R. R. Naik, S. J. Stringer, G. Agarwal, S. E. Jones and M. O. Stone, *Nat. Mater.*, 2002, **1**, 169.
68 C. E. Flynn, C. Mao, A. Hayhurst, J. L. Williams, G. Georgiou, B. Iverson and A. M. Belcher, *J. Mater. Chem.*, 2003, **13**, 2414.
69 R. R. Naik, S. E. Jones, C. J. Murray, J. C. McAuliffe, R. A. Vaia and M. O. Stone, *Adv. Funct. Mater.*, 2004, **14**, 25.
70 T. M. Tarasow, S. L. Tarasow and B. E. Eaton, *Nature*, 1997, **389**, 54.
71 L. A. Gugliotti, D. L. Feldheim and B. E. Eaton, *Science*, 2004, **304**, 850.
72 L. A. Gugliotti, D. L. Feldheim and B. E. Eaton, *J. Am. Chem. Soc.*, 2005, **127**, 17814.

