Molecular biomimetics: nanotechnology through biology

There is a rich and long history of gaining inspiration from nature for the design of practical materials and systems. Traditionally, biomimetics, inspired by biological structures and their functions, focused on emulating or duplicating biosystems using mostly synthetic components and following traditional approaches. With the recent developments of molecular and nanoscale engineering in physical sciences, and advances in molecular biology, biomimetics is now entering the molecular scale. By combining nature’s molecular tools with synthetic nanoscale constructs, molecular biomimetics is emerging as a hybrid methodology.

Materials have uniquely functional properties at nanometre-scale dimensions that, if harnessed effectively, could lead to novel engineering systems with highly useful characteristics. Mechanical properties of nanostructured composites, electronic properties of low-dimensional semiconductors, magnetic properties of single-domain particles, and solution properties of colloidal suspensions, are all attractive and interesting, and correlate directly to the nanometre-scale structures that characterize these systems. The realization of the full potential of nanotechnological systems, however, has so far been limited due to the difficulties in their synthesis and subsequent assembly into useful functional structures and devices. Despite all the promise of science and technology at the nanoscale, the control of nanostructures and ordered assemblies of materials in two- and three-dimensions still remains largely elusive.

Biomaterials, on the other hand, are highly organized from the molecular to the nano- and macroscales, often in a hierarchical manner, with intricate nano-architectures that ultimately make up a myriad of different functional soft and hard tissues. Under genetic control, biological tissues are synthesized in aqueous environments under mild physiological conditions using biomacromolecules, primarily proteins but also carbohydrates and lipids. Proteins both collect and transport raw materials, and consistently and uniformly self- and co-assemble subunits into short- and long-range-ordered nuclei and substrates. Whether in controlling tissue formation, biological functions or physical performance, proteins are an indispensable part of biological structures and systems. A simple conclusion is that next-generation biomimetic systems should include protein(s) in synthesis, assembly or function.

Biomaterials, on the other hand, are highly organized from the molecular to the nano- and macroscales, often in a hierarchical manner, with intricate nano-architectures that ultimately make up a myriad of different functional soft and hard tissues. Under genetic control, biological tissues are synthesized in aqueous environments under mild physiological conditions using biomacromolecules, primarily proteins but also carbohydrates and lipids. Proteins both collect and transport raw materials, and consistently and uniformly self- and co-assemble subunits into short- and long-range-ordered nuclei and substrates. Whether in controlling tissue formation, biological functions or physical performance, proteins are an indispensable part of biological structures and systems. A simple conclusion is that next-generation biomimetic systems should include protein(s) in synthesis, assembly or function.

Engineering materials are synthesized using a combination of approaches, for example, melting and solidification processes, followed by thermomechanical treatments, or solution/vacuum deposition and growth processes. In many cases, however, the final product is dictated by the kinetics and thermodynamics of the system and often achieved through ‘heat-and-beat’ approaches. By contrast, in biological...
systems, evolutionary selection processes result in specific molecular recognition\textsuperscript{26–27}.

In molecular biomimetics\textsuperscript{11–13}, a marriage of the physical and biological fields, hybrid materials could potentially be assembled from the molecular level using the recognition properties of proteins (Fig. 2) under the premise that inorganic surface-specific polypeptides could be used as binding agents to control the organization and specific functions of materials. Molecular biomimetics simultaneously offers three solutions to the development of hetero-functional nanostructures. The first is that protein templates are designed at the molecular level through genetics. This ensures complete control over the molecular structure of the protein template (that is, DNA-based technology). The second is that surface-specific proteins can be used as linkers to bind synthetic entities, including nanoparticles, functional polymers, or other nanostructures onto molecular templates (molecular and nanoscale recognition). The third solution harnesses the ability of biological molecules to self- and co-assemble into ordered nanostructures. This ensures a robust assembly process for achieving complex nano-, and possibly hierarchical structures, similar to those found in nature (self-assembly).

The current knowledge of protein-folding predictions and surface-binding chemistries does not provide sufficiently detailed information to perform rational design of proteins\textsuperscript{28}. To circumvent this problem, massive libraries of randomly generated peptides can be screened for binding activity to inorganic surfaces using phage and cell-surface display techniques\textsuperscript{29}. It may ultimately be possible to construct a ‘molecular erector’ set, in which different types of proteins, each designed to bind to a specific inorganic surface, could assemble into intricate, hybrid structures composed of inorganics and proteins. This would be a significant leap towards realizing molecularly designed, genetically engineered technological materials\textsuperscript{31}.

**Selection of Inorganic-Binding Proteins Through Display Technologies**

There are several possible ways of obtaining polypeptide sequences with specific affinity to inorganics. A number of proteins may fortuitously bind to inorganics, although they are rarely tested for this purpose. Inorganic-binding peptides may be designed using a theoretical molecular approach similar to that used for pharmaceutical drugs\textsuperscript{39}. This is currently impractical because it is time consuming and expensive. Another possibility would be to extract biominingating proteins from hard tissues followed by their isolation, purification and cloning\textsuperscript{40–45}.

Several such proteins have been used as catalystors, growth modifiers, or enzymes in the synthesis of certain inorganics\textsuperscript{43–45}. One of the major limitations of this approach is that a given hard tissue usually contains many proteins, not just one, all differently active in biomimeralization and each distributed spatially and temporally in complex ways\textsuperscript{43–45}.

Furthermore, tissue-extracted proteins may only be used for the regeneration of the inorganics that they are originally associated with, and would be of limited practical use. The preferred route, therefore, is to use combinatorial biology techniques\textsuperscript{29,41}. Here, a large random library of peptides with the same number of amino acids, but of different sequences, is used to mine specific sequences that strongly bind to a chosen inorganic surface\textsuperscript{42–45}.

Since their inception, well-established \textit{in vivo} combinatorial biology protocols (for example, phage display (PD)\textsuperscript{29,41} and cell-surface display (CSD)\textsuperscript{42,43}) have been used to identify biological ligands and to map the epitope (molecular recognition site) of antibodies. Libraries have also been screened for various biological activities, such as catalytic properties or altered affinity and specificity to target molecules in many applications including the design of new drugs, enzymes, antibodies, DNA-binding proteins and diagnostic agents\textsuperscript{29–42,45}.

The power of display technologies relies on the fact that an \textit{a priori} knowledge of the desired amino acid sequence is not necessary, as it can simply be selected and enriched if a large enough population of random sequences is available. \textit{In vitro} methods\textsuperscript{43, such as ribosomal and messenger RNA display technologies, have been developed for increased library size (10\textsuperscript{15}) compared to those of \textit{in vivo} systems (10\textsuperscript{7–10}).

Combinatorial biomolecular protocols can be followed in molecular biomimetics to select polypeptide sequences that preferentially bind to the surfaces of inorganic compounds chosen for their unique physical properties in nano- and biotechnology\textsuperscript{49–52}. Libraries are generated by inserting randomized oligonucleotides within certain genes encoded on phage genomes\textsuperscript{40,41} or on bacterial plasmids\textsuperscript{42,43} (step 1 in Fig. 3). This leads to the incorporation of a random polypeptide sequence within a protein residing on the surface of the organism (for example, the coat protein of a phage or an outer membrane or flagellar protein of a cell; step 2). The eventual result is that each phage or cell produces...
and displays a different, but random peptide (step 3). At this stage, a heterogeneous mixture of recombinant cells or phages are contacted with the inorganic substrate (step 4). Several washing cycles of the phages or the cells eliminate non-binders by disrupting weak interactions with the substrate (step 5). Bound phages or cells are next eluted from the surfaces (step 6). In PD, the eluted phages are amplified by reinfecting the host (step 7). Similarly in CSD, cells are allowed to grow (steps 7,8). This step completes a round of biopanning. Generally, three to five cycles of biopanning are repeated to enrich for tight binders. Finally, individual clones are sequenced (step 9) to obtain the amino acid sequence of the polypeptides binding to the target substrate material.

Outer membrane proteins, lipoproteins, fimbria and flagellar proteins have all been used for heterologous surface display on bacteria. In PD, most of the research has been performed using filamentous phages such as M13 or the closely related fd and f1. Random peptide libraries have been displayed on bacteriophages T7, T4 and A, but these systems are not yet used on a routine basis. PD and CSD need to be investigated and optimized in the selection of inorganic-surface-binding polypeptides. In general, cell surfaces are more complex than the coat of bacteriophages, but a single host is required. By contrast, PD requires bacteriophage and its host bacterium, and reinfection is necessary for the amplification of selected variants. Eluting bound phages from an inorganic surface might be problematic leading to a loss of good binders. This may be overcome by reamplifying unreleased phages from the substrate despite the low yields. Material instability in the screening buffers and non-specific binding of phages or bacteria to inorganic surfaces could also be a problem. These issues can be remedied by a careful choice of buffer combinations. It should be noted that even if a material appears to be unaffected in a particular screening buffer, its surface might be chemically modified, resulting in the recovery of peptides that bind to a surface or morphology different from the one that was originally intended. Growth competition or poor expression of a peptide due to rare codons, misfolding, degradation or inefficient export, may also bias the screen. Thus, parallel use of multiple screening techniques may allow greater flexibility in working with unstable materials and maximizing the number of useful screens. In the quest for inorganic-binding polypeptides, new methodologies are likely to be needed. For example, the use of in vitro systems and a combination of rational and random approaches could prove useful for the identification of very rare sequences, and to increase the diversity and affinity of sequences isolated in previous selections.

CHEMICAL SPECIFICITY OF INORGANIC-BINDING POLYPEPTIDES

A genetically engineered polypeptide for inorganics (GEPI) defines a sequence of amino acids that specifically and selectively binds to an inorganic surface. The surface could be well defined, such as a single crystal or a nanostructure. It might also be rough, or totally non-descriptive, such as a powder. Many early studies used powder samples, including the seminal work of Brown. Our recent research has focused on using materials that can be synthesized in aqueous environments under physiological conditions (biocompatible) and that exhibit fairly stable surface structures and compositions. These include noble metals (Pt and Pd) as well as oxide semiconductors (CuO and ZnO) that were biopanned using either PD or flagellar display (both studies unpublished). Some of the identified binders as well as sequences selected by other researchers are listed in Table 1.

In our PD panning, a disulphide-constrained M13 peptide library, seven amino acids long, was used. The purpose was to determine whether there were any similarities in metal-binding domains between the short Pt- and Pd-binding sequences and the 14-aa Au-binding sequences selected by CSD and/or the 12-aa...
Ag-binding sequences selected by PD. Others focused on PD-based sequences with interesting size-selective binding capabilities to semiconductors (GaAs and ZnS refs 44 and 55 respectively), metal-oxide-binding-motifs (SiO₂, ZnO, and zeolites, refs 45, 56 and 57 respectively), and binders to ionic crystals (CaCO₃, Cr₂O₃, and Fe₂O₃/Fe₃O₄, refs 58, 59 and 60 respectively).

The specificity of a protein for a surface may originate from both chemical (for example, hydrogen bonding, polarity and charge effects) and structural (size and morphology) recognition mechanisms. Some conclusions may be drawn from the available set of inorganic-binding sequences. For example, gold-binding sequences isolated by CSD could be moved from an extracellular loop of maltoporin to the N-terminus of alkaline phosphotase with retention of gold-binding activity, suggesting portability and independence of the surrounding protein framework.

Although many proteins bind to Au at low salt concentrations, the gold-binding sequences were selected for binding at high salt concentrations. As a result, they exhibit substantially improved binding compared with native *Escherichia coli* alkaline phosphatase even in the presence of a detergent. Our initial assembly studies focused on the 14-amino-acid-long Au-binding peptide, GBP1 (MHGKTQATSGTIQS). To increase binding activity, tandem repeats of the sequence were generated by genetic engineering, and it was found that at least three repeats were required for high-affinity binding. Similar tetrapeptide repeats (SEKL and GASL) were observed in the sequences identified that control the morphology of gold crystals (Table 1, also see Fig. 5). The Ag-, Pt-, and Pd-binders were all selected using M13 PD libraries. For Ag-binders, a 12-aa library was used whereas we used a constrained heptamer library to identify the smallest sequences capable of binding Pt and Pd. Inspection of the sequences of the noble-metal binders (Table 1) suggests that serine and threonine are important for binding. These amino acids have similar structures and contain aliphatic hydroxyl groups in their side chains. Participation in hydrogen bonding by nitrogen atoms was also a common feature of noble-metal binders. Interestingly, most of the sequences isolated to date have not contained cysteine, and only a few of them contained histidines, two residues known to bind to transition metal ions. Aromaticity was rarely observed, and only when it was coupled with a hydroxyl or amine functional group. Overall, noble-metal-binding sequences appear to predominantly consist of hydrophobic and hydroxyl-containing polar amino acids.

A similar analysis can be performed on the non-metal binding sequences listed in Table 1. For both metal oxides and zeolites, basic amino acids (arginine and lysine) and hydroxyl-containing residues were common, especially in ZnO (ref. 56), zeolite and, to a lesser extent, in SiO₂ (ref. 45). Of the ionic crystals, Cr₂O₃ (ref. 59) and Fe₂O₃ (ref. 60) binders have similar characteristics, such as being basic and charged. Unlike biological CaCO₃-binding proteins where asparagines and glutamine dominate (ref. 36–39, 62), PD-selected CaCO₃-binders contained mostly uncharged or basic amino acids with small differences possibly originating from different mineral forms, aragonite and calcite. Finally, sequences binding to semiconductors GaAs (ref. 44) and ZnS (ref. 55) contain polar amino acids composed of mostly uncharged ones. Overall, both...
metal oxide and ionic crystal binders exhibit strong basic characteristics and high positive charges, whereas non-oxide semiconductor binders are close to the neutral range, and metal binders are slightly basic (Table 1).

Table I Examples of polypeptide sequences exhibiting affinity for various inorganics.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Sequences</th>
<th>Size</th>
<th>pIa</th>
<th>MWb</th>
<th>Chargec</th>
<th>Displayed</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au</td>
<td>MHGKTQATSGTQG 14</td>
<td>8.52</td>
<td>1446.60</td>
<td>+1</td>
<td>CSD53,54</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SKTSLQGGAMLQGSEKLTNG 21</td>
<td>8.31</td>
<td>2050.21</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>QATSEKLVRGMEGASLHPAKT 21</td>
<td>8.60</td>
<td>2211.52</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt</td>
<td>DRTSTWR 7</td>
<td>9.60</td>
<td>920.98</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>QSTSTK 7</td>
<td>8.75</td>
<td>749.82</td>
<td>+1</td>
<td>PD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SSSHLNK 7</td>
<td>8.49</td>
<td>771.83</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pd</td>
<td>SVTOKNY 7</td>
<td>8.31</td>
<td>838.92</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SHPGPQY 7</td>
<td>6.46</td>
<td>753.81</td>
<td>0</td>
<td>PD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAPTPML 7</td>
<td>8.74</td>
<td>765.93</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>AYSSGAPPMPFF 12</td>
<td>5.57</td>
<td>1221.39</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NPSLFLRYLDSD 12</td>
<td>6.09</td>
<td>1395.53</td>
<td>0</td>
<td>PD34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SLAFOPRTPPP 12</td>
<td>9.47</td>
<td>1283.46</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SiO₂</td>
<td>MSPHPRHHHT 12</td>
<td>9.59</td>
<td>1470.63</td>
<td>+1</td>
<td>PD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RGRRRRLSCRL 12</td>
<td>12.30</td>
<td>1541.89</td>
<td>+6</td>
<td>PD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>KPHHHWHTGAN 12</td>
<td>8.78</td>
<td>1359.43</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zeolites</td>
<td>VKQTSPREPPRLPSKHPRG 21</td>
<td>10.90</td>
<td>2371.68</td>
<td>+3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MDHHGYOKQATPS 14</td>
<td>9.70</td>
<td>1616.82</td>
<td>+2</td>
<td>CSD39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NTRMTARHHsAWHKSTOR 20</td>
<td>12.48</td>
<td>2351.59</td>
<td>+4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZnO</td>
<td>YDSRSMRPH 9</td>
<td>8.75</td>
<td>1146.26</td>
<td>+1</td>
<td>CSD39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCO₃</td>
<td>HTGNRRMRVPWF 12</td>
<td>6.75</td>
<td>1639.87</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DVSSFNLHKMR 12</td>
<td>8.75</td>
<td>1480.70</td>
<td>+1</td>
<td>PD34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr₂O₃</td>
<td>WRPKAATN 9</td>
<td>11.00</td>
<td>955.13</td>
<td>+2</td>
<td>CSD39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RHRHLQGO 9</td>
<td>12.30</td>
<td>1134.35</td>
<td>+3</td>
<td>CSD39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>RRTVWHVHN 9</td>
<td>12.01</td>
<td>1146.32</td>
<td>+3</td>
<td>CSD39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GaAs</td>
<td>AQGSPDNTHHT 12</td>
<td>5.97</td>
<td>1335.31</td>
<td>0</td>
<td>PD44</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RLEAQPQGGSG 12</td>
<td>6.00</td>
<td>1253.46</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TPPRPIQYNHTS 12</td>
<td>8.44</td>
<td>1410.55</td>
<td>+1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZnS</td>
<td>NNPMHQN 7</td>
<td>6.74</td>
<td>853.91</td>
<td>0</td>
<td>PD43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

a Isoelectric points and bMolecular masses of peptides are calculated using Compute pI/MW tool (http://us.expasy.org/tools/pi_tool.html).
b Calculated by subtracting the number of basic residues (R and K) from the number acidic residues (D and E).
c Unpublished results by the authors.
d Most frequently observed sequences.

In practice, however, powders of various sizes and morphologies have been used for selection. The sequence space should be largest for powders, as peptides can attach to surfaces with various morphological features. On the other hand, because powders are non-descriptive, the selected polypeptides may exhibit little or no homology and 'solve' the binding problem through different strategies. Binders selected for a given size, morphology, crystallography or stereochemistry may share a higher degree of homology. For example, a
These include synthesizing nanostructures (for example, particles, rods and tubes) with uniform size and shape, controlling their mineralogy, surface structures and chemistry, and predicting their spatial distribution. The molecular biomimetics approach holds great promise in overcoming some of these difficulties. Because inorganic surface-binding polypeptides are selected to specifically bind to a material based on its physical or chemical characteristics, they may find applications in controlling materials morphology and uniformity, and could be used to generate heterostructures combining different inorganics and molecules that would normally phase-separate.

The availability of amino acid sequences binding to inorganics offers a fresh perspective towards future studies in self-assembling nanostructured components with distinct functionalities. One could select a GEPI from the available sequences (Table 1) and insert it within the framework of another protein exhibiting properties (for example, DNA-binding) and use the resulting designer protein or the DNA as a molecular substrate. Alternatively, a GEPI could be chemically linked to a synthetic polymer with functional properties (for example, conductive or light-sensitive) to create multifunctional hybrid polymeric units. Whether fused to biological or synthetic macromolecules, the role of the GEPI would be to endow them with another functionality: specific inorganic-binding. Examples attained using these three fundamental aspects of GEPI (genetic selection, molecular recognition and self-assembly) in the applications of material synthesis, formation and assembly are discussed below.

**CONTROL OF CRYSTAL GROWTH**

In biomineralization, a significant aspect of biological control over materials formation is through protein/inorganic interaction, such as in the biosynthesis of bone, dental structures, mollusc shells, and particles formed by single-celled organisms. With the emergence of combinatorially selected inorganic-binding peptides, a natural step was to examine how they would affect inorganic formation. In the first such study, we characterized the effects of GBP1 on the morphology of gold particles. Using the well-known Faraday technique, monodispersed nanogold particles 12 nm in diameter can be formed by reducing AuCl₃ with sodium citrate under ambient conditions. Reducing the gold concentration and temperature allows particle interaction with surfaces during growth, and provides conditions to examine the effect of gold-binding during colloidal gold formation. We conducted a search for mutants that modulated the morphology of gold crystallites by taking advantage of the change of colour in the gold colloid (from pale yellow to red), which is related to altered rates of crystallization. Out of fifty mutants, two accelerated crystal growth and changed the particle morphology from cubo-octahedral (the usual shape of the gold particles under equilibrium growth conditions) to flat, triangular or pseudo-hexagonal particles (Fig. 5a). The polypeptides, in spite of being slightly basic, may have caused the

**INORGANIC-BINDING POLYPEPTIDES AND NANOASSEMBLY**

Nanometre-sized particles and nanostructured inorganics could be fundamental building blocks for future technological materials and devices. Numerous challenges must be addressed before they are successfully implemented into working systems.
formation of gold crystals similar to those formed under boiling and acidic conditions (Figs 5b and c respectively) possibly by acting as an acid and modulating crystal growth rather than serving as a template. A similar study was also carried out using metallic Ag-binding polypeptides that were selected using PD. In this case, although the exact mechanism is not yet known, it was observed that metal ions were reduced to Ag precipitates in mostly flat morphology, an indication that 12-aa-long peptide may have directed the preferred mineral shape, similar to flat Ag-particles formed in bacteria.

PEPTIDE-MEDIATED NANOPARTICLE ASSEMBLY

Organization and immobilization of inorganic nanoparticles in two- or three-dimensional geometries are fundamental in the use of nanoscale effects. For example, quantum dots can be produced using vacuum techniques such as molecular beam epitaxy, shown in Fig. 5d for the GaInAs/GaAs system. However, this can only be accomplished under stringent conditions of high temperature, very low pressures and a toxic environment. A desirable alternative would be not only to synthesize inorganic nanodots under mild conditions, but also to immobilize/self-assemble them. Inorganic particles have been functionalized with synthetic molecules, including thiols and citrates, and with biological molecules, such as lipids, amino acids, polypeptides and ligand-functionalized DNA. Using the recognition properties of the coupling agents, novel materials have been generated and controlled growth has been achieved. These molecules, however, do not exhibit specificity for a given material. For example, thiols couple gold as well as silver nanoparticles in similar ways. Likewise, citrate ions cap noble metals indiscriminately.

A desirable next step would be to use GEPIs that specifically recognize inorganics for nanoparticle assembly. An advantage of this approach is that GEPIs can be genetically or synthetically fused to other functional biomolecular units or ligands to produce heterobifunctional (or multifunctional) molecular entities. Figure 5e,f shows the assembly of nanogold particles on GBP1-coated flat polystyrene surfaces, which resembles the distribution of quantum dots obtained by high-vacuum deposition techniques (Fig. 5d). The homogenous decoration of the surface with nanogold suggests that proteins may be useful in the production of tailored nanostructures under ambient conditions and aqueous solutions. Furthermore, the recognition activity of the protein could provide an ability to control the particle distribution, and particle preparation conditions could allow size control. This approach makes it possible to pattern inorganic-binding polypeptides into desirable arrays to produce inorganic particles through templating using, for example, dip-pen lithography.

FUTURE PROSPECTS

Controlled binding and assembly of proteins onto inorganics is at the core of biological materials science and engineering with wide-ranging applications. Protein adsorption and macromolecular interactions at solid surfaces play key roles in the performance of implants and hard-tissue engineering. DNA and proteins adsorbed specifically onto probe substrates with biological molecules, such as lipids, amino acids, polypeptides and ligand-functionalized DNA. Using the recognition properties of the coupling agents, novel materials have been generated and controlled growth has been achieved. These molecules, however, do not exhibit specificity for a given material. For example, thiols couple gold as well as silver nanoparticles in similar ways. Likewise, citrate ions cap noble metals indiscriminately.

Protein adsorption and macromolecular interactions at solid surfaces play key roles in the performance of implants and hard-tissue engineering. DNA and proteins adsorbed specifically onto probe substrates with biological molecules, such as lipids, amino acids, polypeptides and ligand-functionalized DNA. Using the recognition properties of the coupling agents, novel materials have been generated and controlled growth has been achieved. These molecules, however, do not exhibit specificity for a given material. For example, thiols couple gold as well as silver nanoparticles in similar ways. Likewise, citrate ions cap noble metals indiscriminately.

A desirable next step would be to use GEPIs that specifically recognize inorganics for nanoparticle assembly. An advantage of this approach is that GEPIs can be genetically or synthetically fused to other functional biomolecular units or ligands to produce heterobifunctional (or multifunctional) molecular entities. Figure 5e,f shows the assembly of nanogold particles on GBP1-coated flat polystyrene surfaces, which resembles the distribution of quantum dots obtained by high-vacuum deposition techniques (Fig. 5d). The homogenous decoration of the surface with nanogold suggests that proteins may be useful in the production of tailored nanostructures under ambient conditions and aqueous solutions. Furthermore, the recognition activity of the protein could provide an ability to control the particle distribution, and particle preparation conditions could allow size control. This approach makes it possible to pattern inorganic-binding polypeptides into desirable arrays to produce inorganic particles through templating using, for example, dip-pen lithography.
surfaces and quantification of their cross-specificity for diverse materials. Based on the insights achieved through these studies in the coming decade, and following the lead of molecular biology, a roadmap could be developed in which GEPI could be used as a versatile molecular linker and open new avenues in the self-assembly of molecular systems in nano- and nanobiotechnology.

doi:10.1038/nmat964

References


© 2003 Nature Publishing Group

Acknowledgements
We thank our colleagues S. Brown (University of Copenhagen, Denmark), D. Schwartz, F. Ouchi, and R. Tieler for invaluable discussions, and S. Dancer, D. Heidel, B. Braun (Beckman Institute, University of Illinois), M. H. Zarate, V. Bulmus and H. Fong (all at University of Washington) for technical help. This research was supported by the US Army Research Office (Program Manager: Robert Campbell) through a DURINT Program (Defense University Research Initiative on Nanotechnology).
Correspondence and requests for materials should be addressed to M.S.

Competing financial interests
The authors declare that they have no competing financial interests.