Molecular biomimetics: nanotechnology through biology

Proteins, through their unique and specific interactions with other macromolecules and inorganics, control structures and functions of all biological hard and soft tissues in organisms. Molecular biomimetics is an emerging field in which hybrid technologies are developed by using the tools of molecular biology and nanotechnology. Taking lessons from biology, polypeptides can now be genetically engineered to specifically bind to selected inorganic compounds for applications in nano- and biotechnology. This review discusses combinatorial biological protocols, that is, bacterial cell surface and phage-display technologies, in the selection of short sequences that have affinity to (noble) metals, semiconducting oxides and other technological compounds. These genetically engineered proteins for inorganics (GEPIs) can be used in the assembly of functional nanostructures. Based on the three fundamental principles of molecular recognition, self-assembly and DNA manipulation, we highlight successful uses of GEPI in nanotechnology.

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There is a rich and long history of gaining inspiration from nature for the design of practical materials and systems[1–4]. Traditionally, biomimeticists, inspired by biological structures and their functions, focused on emulating or duplicating biosystems using mostly synthetic components and following traditional approaches[5–7]. With the recent developments of molecular[8] and nanoscale[9] engineering in physical sciences, and advances in molecular biology[10], biomimetics is now entering the molecular scale[11,12]. By combining nature's molecular tools with synthetic nanoscale constructs, molecular biomimetics is emerging as a hybrid methodology[13].

Materials have uniquely functional properties at nanometre-scale dimensions that, if harnessed effectively, could lead to novel engineering systems with highly useful characteristics[14,15]. Mechanical properties of nanostructured composites, electronic properties of low-dimensional semiconductors, magnetic properties of single-domained particles, and solution properties of colloidal suspensions, are all attractive and interesting, and correlate directly to the nanometre-scale structures that characterize these systems[16,17]. 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[14,18].

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[19] and hard tissues[20] (Fig. 1)[21–23]. 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[6,7,20]. 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[11–13].

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[24]. 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[24,25] (Fig. 2). By contrast, in biological

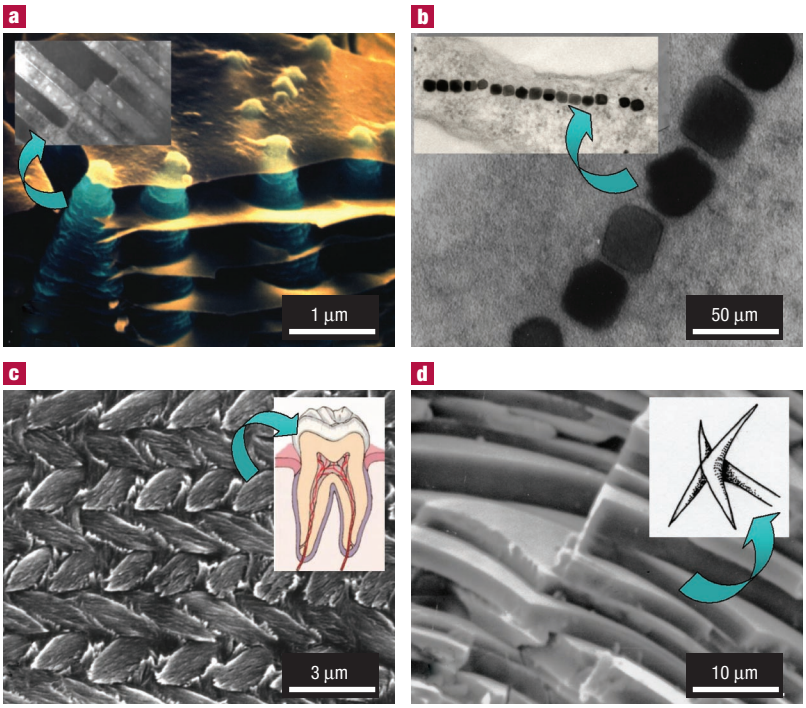

Figure 1 Examples of biologically synthesized complex materials. **a**, Scanning electron microscope (SEM) image of a growth edge of abalone (*Haliotis rufescens*) displaying aragonite platelets (blue) separated by organic film (orange) that eventually becomes nacre (mother-of-pearl). This is a layered, tough, and high-strength biocomposite (inset: transmission electron microscope (TEM) image[21]. **b**, Magnetite nanoparticles formed by magnetotactic bacterium (*Aquaspirillum magnetotacticum*, inset: TEM image) are single-crystalline, single-domained and crystallographically aligned[6]. **c**, Mouse enamel (SEM image) is hard, wear-resistant material with highly ordered micro/nano architecture consisting of hydroxyapatite crystallites that assemble into woven rod structure (inset: schematic cross-section of a human tooth)[22]. **d**, Sponge spicule (with a cross-shaped apex shown in inset) of Rosella has layered silica with excellent optical and mechanical properties, a biological optical fibre (SEM image)[23].

systems, evolutionary selection processes result in specific molecular recognition[26,27].

In molecular biomimetics[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[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[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[11].

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[30]. This is currently impractical because it is time consuming and expensive. Another possibility would be to extract biomineralizing proteins from hard tissues followed by their isolation, purification and cloning[31–35]. Several such proteins have been used as nucleators, growth modifiers, or enzymes in the synthesis of certain inorganics[33–40]. 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 biomineralization and each distributed spatially and temporally in complex ways[33–35]. 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[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[29,42–45].

Since their inception, well-established *in vivo* combinatorial biology protocols (for example, phage display (PD)[46,47] and cell-surface display (CSD)[48]) 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[49–52]. The power of display technologies relies on the fact that an *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. *In vitro* methods[53], such as ribosomal and messenger RNA display technologies, have been developed for increased library size (10^{15}) compared to those of *in vivo* systems ($10^{7–10}$).

Combinatorial biology 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[29,43–45]. Libraries are generated by inserting randomized oligonucleotides within certain genes encoded on phage genomes[44,45] or on bacterial plasmids[29,42,43] (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

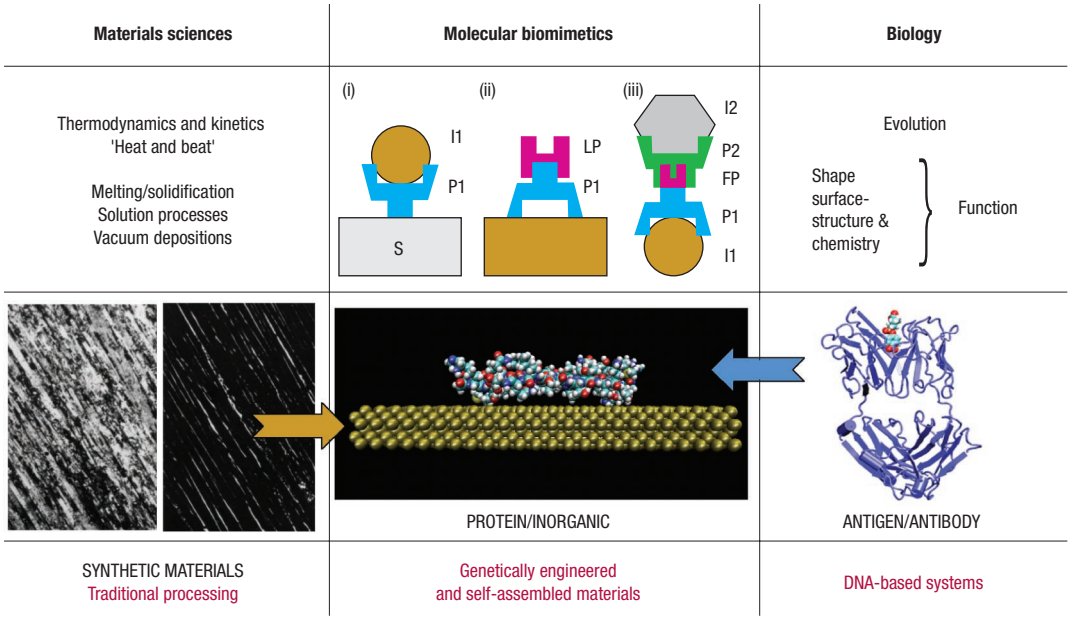

Figure 2 Molecular biomimetics. This is the marriage of materials science engineering and molecular biology for development of functional hybrid systems, composed of inorganics and inorganic-binding proteins. The new approach takes advantage of DNA-based design, recognition, and self-assembly characteristics of biomolecules[27]. Traditional materials science engineering produces materials (for example, medium-carbon steels depicted in the bright- and dark-field TEM images)[25], that have been successfully used over the last century. Molecular biology focuses on structure–function relations in biomacromolecules, for example, proteins. In molecular biomimetics, inorganic-binding proteins could potentially be used as (i) linkers for nanoparticle immobilization; (ii) functional molecules assembled on specific substrates; and (iii) heterobifunctional linkers involving two (or more) binding proteins linking several nanoinorganic units. (I1: inorganic-1, I2: Inorganic-2, P1 and P2: inorganic specific proteins, LP: linker protein, FP: fusion protein).

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 λ, 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[46–52]. 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[41,53].

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[42–45,54,55], including the seminal work of Brown[29]. 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 (Cu_2O 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[29,43] selected by CSD and/or the 12-aa

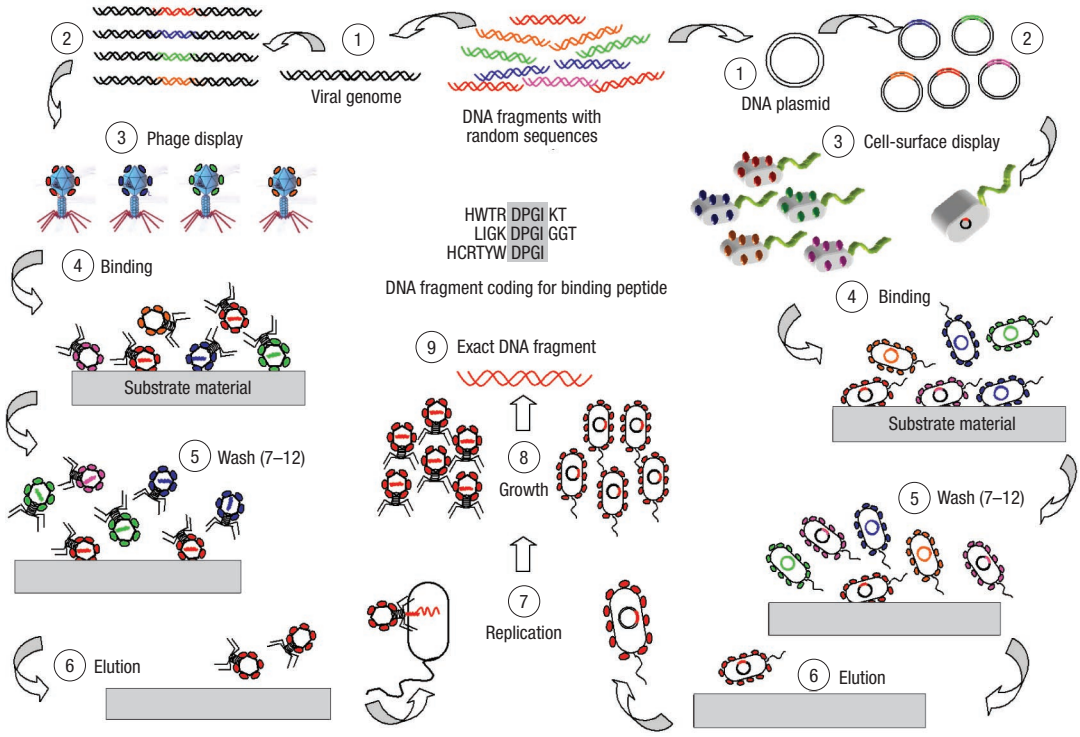

Figure 3 Phage display and cell-surface display. Principles of the protocols used for selecting polypeptide sequences that have binding affinity to given inorganic substrates.

Ag-binding sequences[54] 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 (SiO_2, ZnO, and zeolites, refs 45, 56 and 57 respectively), and binders to ionic crystals ($CaCO_3$, Cr_20_3, and Fe_20_3/Fe_30_4, refs 58, 59 and 60 respectively).

The specificity of a protein for a surface may originate from both chemical[61] (for example, hydrogen bonding, polarity and charge effects) and structural[62] (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[29]. 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 *Escherchia coli* alkaline phosphotase even in the presence of a detergent. Our initial assembly studies[63] 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[29,43]. Similar tetrapeptide repeats (SEKL and GASL) were observed in the sequences identified that control the morphology of gold crystals[43] (Table 1, also see Fig. 5). The Ag-, Pt-, and Pd-binders were all selected using M13 PD libraries. For Ag binders[54], 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[64]. 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[59] and, to a lesser extent, in SiO_2 (ref. 45). Of the ionic crystals, Cr_2O_3 (ref. 59) and Fe_2O_3 (ref. 60) binders have similar characteristics, such as being basic and charged. Unlike biological $CaCO_3$-binding proteins where asparagines and glutamine dominante[36–39,62], PD-selected $CaCO_3$-binders contained mostly uncharged or basic amino acids[58], 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

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

Materials	Sequences	Size	pI[a]	MW[b]	Charge[c]	Display[Ref]
	MHGKTQATSGTIQS	14	8.52	1446.60	+1	
Au	SKTSLGQSGASLQGSEKLTNG	21	8.31	2050.21	+1	CSD[29,43]
	QATSEKLVRGMEGASLHPAKT	21	8.60	2211.52	+1	
	DRTSTWR	7	9.60	920.98	+1	
Pt	QSVTSTK	7	8.75	749.82	+1	PD[d]
	SSSHLNK	7	8.49	771.83	+1	
	SVTQNKY	7	8.31	838.92	+1	
Pd	SPHPGPY	7	6.46	753.81	0	PD[d]
	HAPTPML	7	6.74	765.93	0	
	AYSSGAPPMPPF[e]	12	5.57	1221.39	0	
Ag	NPSSLFRYLPSD[e]	12	6.09	1395.53	0	PD[54]
	SLATQPPRTPPV[e]	12	9.47	1263.46	+1	
	MSPHPHPRHHHT[e]	12	9.59	1470.63	+1	
SiO_2	RGRRRRLSCRLL[e]	12	12.30	1541.89	+6	PD[45]
	KPSHHHHHTGAN	12	8.78	1359.43	+1	
	VKTQATSREEPPRLPSKHRPG	21	10.90	2371.68	+3	
Zeolites	MDHGKYRQKQATPG	14	9.70	1616.82	+2	CSD[59]
	NTRMTARQHRSANHKSTQRA[e]	20	12.48	2351.59	+4	
ZnO	YDSRSMRPH	9	8.75	1148.26	+1	CSD[56]
$CaCO_3$	HTQNMRMYEPWF	12	6.75	1639.87	0	
	DVFSSFNLKHMR	12	8.75	1480.70	+1	PD[58]
Cr_2O_3	VVRPKAATN	9	11.00	955.13	+2	
	RIRHRLVGQ	9	12.30	1134.35	+3	CSD[59]
Fe_2O_3	RRTVKHHVN[e]	9	12.01	1146.32	+3	CSD[60]
GaAs	AQNPSDNNTHTH	12	5.97	1335.31	0	PD[44]
	RLELAIPLQGSG	12	6.00	1253.46	0	
	TPPRPIQYNHTS	12	8.44	1410.55	+1	
ZnS	NNPMHQN[e]	7	6.74	853.91	0	PD[55]

[a] Isoelectric points and [b]Molecular masses of peptides are calculated using Compute pI/Mw tool (http://us.expasy.org/tools/pi_tool.html).

[c] Calculated by subtracting the number of basic residues (R and K) from the number acidic residues (D and E).

[d] Unpublished results by the authors.

[e] Most frequently observed sequences.

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).

PHYSICAL SPECIFICITY OF PEPTIDE BINDING

Ideally, selection of sequences should be performed using an inorganic material of specific morphology, size, crystallography or surface stereochemistry[29–45]. 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

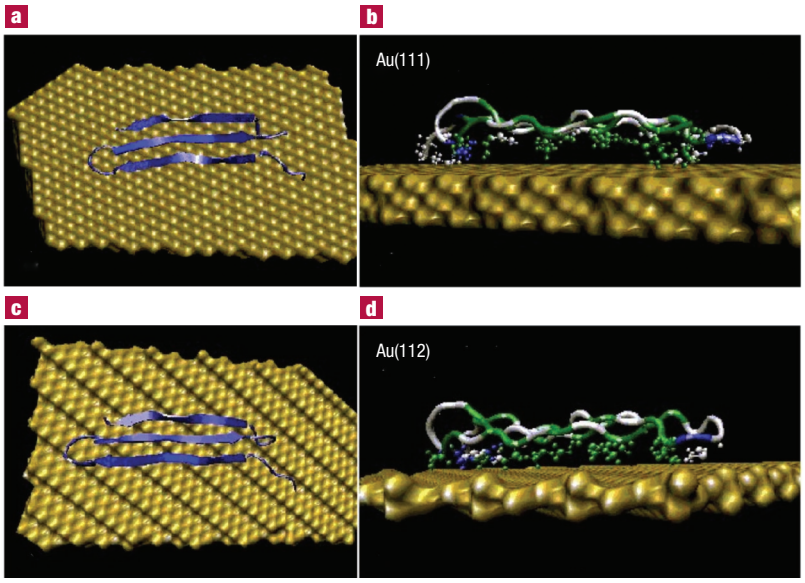

Figure 4 A gold-binding protein (3-repeat GBP1) on Au(111) and Au(112) surfaces. Viewed from above (**a** and **c**) and edge-on (**b** and **d**), respectively. The colouring corresponds to residue type: polar residues are highlighted in green, charged in blue, and hydrophobic in white.

GEPI binding to a material of a certain size may also bind to a smaller particle of the same material, but less strongly. Similarly, a GEPI binding strongly to a specific crystallographic surface may bind with an altered affinity to another surface of the same material. Finally, a GEPI strongly binding to a material of composition A may bind less strongly to a material B with a different composition but having similar structure (for example, perovskites). Therefore, if one seeks highly specific binders, the physical and chemical characteristics of the material must be known. An alternative approach is that, once a relatively large number of binders have been identified by panning on powders, a subset specific for morphology, size or surface could be identified on well-defined materials.

We performed structure-modelling studies to predict the shape of the 3-repeat Au-binding peptide GBP1 in solution[65]. This work was carried out in the hope that a match between amino acids of GBP1 and the spacing of the inorganic atomic lattice would shed light on the mechanism of binding. Figure 4 shows the polypeptide placed on Au atomic lattices. Simulation results indicate that the repeats form an antiparallel β-sheet, and that their close contacts between peptide and Au(111) surfaces mainly involve the polar side chains of serine and threonine, which places a periodic structure of OH^- groups into a regular lattice. In the same study, we also showed that GBP1 does not bind to Au(112) as tightly, because of the migration of water molecule through the atomic grooves of the crystallographic surface, which decouples the polypeptide from the surface[65].

INORGANIC-BINDING POLYPEPTIDES AND NANOASSEMBLY

Nanometre-sized particles and nanostructured inorganics could be fundamental building blocks for future technological materials and devices[8,14,18]. Numerous challenges must be addressed before they are successfully implemented into working systems. 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[32], dental structures[33], mollusc shells[40], and particles formed by single-celled organisms[34,66–68]. 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[43], we characterized the effects of GBP1 on the morphology of gold particles. Using the well-known Faraday technique[69], monodispersed nanogold particles 12 nm in diameter can be formed by reducing $AuCl_3$ with sodium citrate under ambient conditions. Reducing the gold concentration and temperature allows particle formation at a slower rate, giving the protein time to interact 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)[43]. The polypeptides, in spite of being slightly basic, may have caused the

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[54] 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[67].

PEPTIDE-MEDIATED NANOPARTICLE ASSEMBLY

Organization and immobilization of inorganic nanoparticles in two- or three-dimensional geometries are fundamental in the use of nanoscale effects[14,70]. For example, quantum dots can be produced using vacuum techniques[14,71], 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[71]. 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[8]. Using the recognition properties of the coupling agents, novel materials have been generated[8] and controlled growth has been achieved[72,73]. These molecules, however, do not exhibit specificity for a given material. For example, thiols couple gold as well as silver nanoparticles in similar ways[74,75]. Likewise, citrate ions cap noble metals indiscriminately[8].

A desirable next step would be to use GEPIs that specifically recognize inorganics[63,76] for nanoparticle assembly. An advantage of this approach is that GEPI 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[63], 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[54] using, for example, dip-pen lithography[77].

FUTURE PROSPECTS

Controlled binding and assembly of proteins onto inorganics is at the core of biological materials science and engineering with wide-ranging applications[78–81].

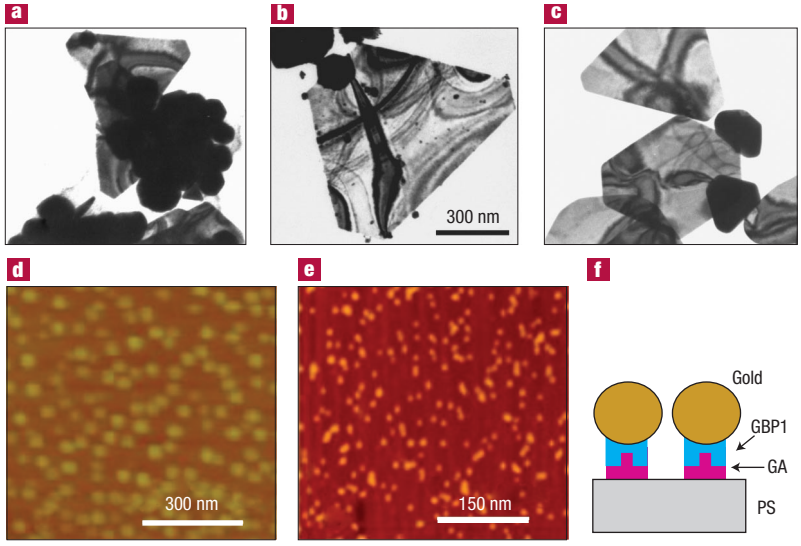

Figure 5 Effect of GEPI on nanocrystal morphology. **a–c**, One of the two mutants (RP1) from a library of gold-binding GEPIs were tested in the formation of flat gold particles, shown in **a**, similar to those formed under acidic (**b**) or boiling (**c**) conditions. Particles formed in the presence of vector-encoded alkaline phosphatase and neutral conditions do not result in morphological change of gold particles (not shown)[43]. **d**,**e**, The atomic force microscope images show quantum (GaInAs) dots assembled on GaAs substrate; **d**, through high-vacuum (molecular beam epitaxy) strain-induced self-assembly (courtesy of T. Pearsall, University of Washington), and **e**, through 7-repeat GBP1. **f**, Schematic illustration of **e**. PS: polystyrene substrate, GA: glutaraldehyde, GBP: 7-repeat GBP1, and gold: 12-nm-diameter colloidal gold particles.

Protein adsorption and macromolecular interactions at solid surfaces play key roles in the performance of implants[78] and hard-tissue engineering[33,40,82]. DNA and proteins adsorbed specifically onto probe substrates have been used to build microarrays suitable for modern genomics[83], pharmogenetics[84] and proteomics[85].

The preliminary results of self-assembly and molecular-recognition properties of combinatorially selected polypeptides provide insights into potential future applications of GEPI. Thiol and silane linkages, two major molecular linkers for noble metal and oxide surfaces, respectively, have so far dominated the field of self-assembled molecules on solid substrates[74,75]. Self-assembled GEPI monolayers could open up new avenues for designing and engineering novel surfaces for a wide variety of nano- and biotechnology applications (Fig. 6). For example, a GEPI recognizing and assembling on the surface of a therapeutic device could be fused to a human protein to enhance biocompatibility[78], or used for drug delivery through colloidal inorganic particles[86]. A GEPI may also be conjugated to a peptide–amphiphilic system leading to a molecular biomineralization substrate[87–89]. Taking advantage of recognition and self-assembly properties, DNA[90] or proteins[91,92] could be fused to GEPIs to create functional molecular substrates. Coupled with a molecular motor[93,94], a GEPI may provide a critical step towards creating dynamic nanostructures. Ultimately, using nanopatterned multimetallic or multisemiconducting particles, and localized surface plasmon effects (for example, surface-enhanced Raman[95] or surface plasmon resonance[96] spectroscopies), several different GEPI molecules could serve as specific linkers in creating nanoscale platforms for rapid development of nanoarrays for proteomics[97].

Although significant advances have been made in developing protocols for the selection of surface-binding polypeptides through display technologies, many questions remain before their robust genetic design and practical applications as building blocks are realized. These include, for example, the physical and chemical basis for GEPI recognition of inorganic

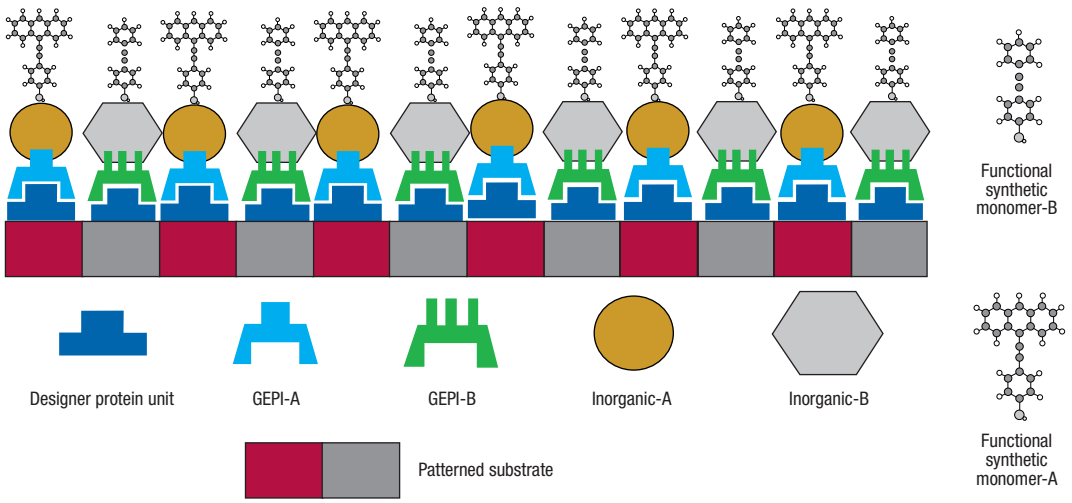

Figure 6 The potential of using GEPI as 'molecular erector' sets. Two different GEPI proteins (GEPI-A and GEPI-B) are assembled on ordered molecular or nanoscale substrates. One could use either a designer protein, followed by genetic fusion of the respective GEPIs, or directly assemble GEPIs on the patterned substrate. The inorganic particles A and B are immobilized selectively on GEPI-A and GEPI-B, respectively. Synthetic molecules (that is, conducting[17] or photonic[18]) are assembled using functionalized side-groups on the nanoparticles. Size, shape, separation, and distribution of nanostructural units, as well as self-assembly, are parameters unique to this approach.

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.

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Competing financial interests

The authors declare that they have no competing financial interests.