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FEATURE ARTICLE

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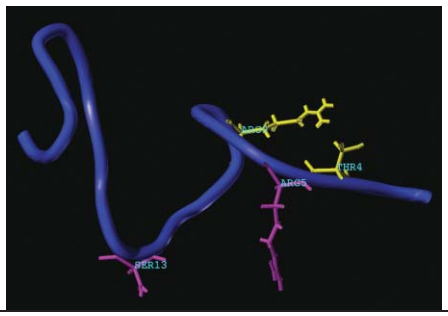
Interactions of biomolecules with inorganic materials: principles, applications and future prospects

Siddharth V. Patwardhan,* Geetanjali Patwardhan and Carole C. Perry*

The goal of this article is to overview the current understanding of biomolecule–inorganic materials interactions; to identify the 'rules' that govern interaction; to highlight the drawbacks of the present approaches and outline future challenges and opportunities.



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Interactions of biomolecules with inorganic materials: principles, applications and future prospects

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Interactions between inorganic materials and biomolecules at the molecular level, although complex, are commonplace. Examples include biominerals, which are, in most cases, facilitated by and in contact with biomolecules; implantable biomaterials; and food and drug handling. The effectiveness of these functional materials is dependant on the interfacial properties *i.e.* the extent of molecular level 'association' with biomolecules. The goal of this overview is four-fold: to present biomolecule–inorganic materials interactions and our current understanding using selected examples; to elaborate on approaches that have been used to expose the mechanisms underpinning such interactions; to identify the 'rules' or 'guiding principles' that govern interactions that could be used to explain and hence predict behaviour; and finally to highlight the drawbacks of the present approaches and outline future challenges and opportunities.

1. Introduction

In the search for new materials with useful properties, either as materials for use in the human body or as conjugates with, for example, novel electrical, catalytic or optical properties, a range of experimental approaches have been developed including the use of organic or 'soft' templates for the generation of inorganic materials and the semi-programmed or building block approach to materials assembly (in its widest sense).[1] Organic chemistry and more recently supramolecular chemistry has been very successful in creating structures with spectacular morphologies[1–9] but the synthesis of materials with shape and form using most elements of the periodic table (non carbon based chemistry) has lagged behind.

In biological organisms, organic molecules appear to exert a remarkable level of control over the nucleation, composition (principal and trace ions) and crystallographic phase of minerals including oxides and simple salts. What is even more remarkable is that these composite materials are produced under ambient conditions of temperature and pressure (4–70 °C), from aqueous solution usually at circumneutral pH in the presence of metal ions and anions that do not form part of the final material. Formation also occurs in the presence of a very large mixture of other organic molecules

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Siddharth V. Patwardhan

Siddharth obtained his first degree in Engineering from University of Pune, India (2000) and worked on bio-inspired silica synthesis for his Master of Science (2002) and PhD (2003) degrees at the University of Cincinnati, USA. In 2003 he was appointed as a Visiting Scholar and Postdoctoral Fellow at the University of Delaware where he worked on developing strategies for self-healing glass fibres using biomineralization techniques. Currently, he is a Postdoctoral Research Fellow at Nottingham Trent University. Siddharth's research interests include biominerals; bioinspired materials chemistry; biomolecule–mineral interactions and continuous flow systems. Dr Patwardhan has published several book chapters, reviews, articles and inventions.

Geetanjali Patwardhan

After receiving her first degree, Bachelor of Pharmacy from University of Pune, India, Geetanjali Patwardhan (formerly Jog) received her PhD in Medicinal Chemistry from University at Buffalo, USA in July 2005, where her research focused on using molecular modelling techniques in the design and synthesis of analogs for inhibiting bacterial quorum sensing. Before starting her current position as Research Fellow at the University of Nottingham, she worked with Prof. Perry at Nottingham Trent University, applying her molecular modelling skills to Materials Chemistry where she performed molecular dynamics studies on zinc oxide binding peptides. Her current work focuses on structure based drug design for anti-cancer therapeutics.

that are intentionally excluded from the mineral formed.[10–12] The ability to control structure applies both to the generation of apparently single crystals and to the organisation of crystallites and other nanoscale building blocks into complex hierarchically organised structures that have specific biological functions.[10–13] For the majority of biominerals explored to date, proteins are present in intimate association with the mineral phase and they are implicated in the directed assembly of nanosized particles (crystalline and amorphous) into sophisticated and functional structures. This ability to control nucleation and then to direct the assembly of nanosized objects into controlled and structurally sophisticated structures has motivated many researchers to develop bottom-up assembly methods that mimic or exploit the recognition capabilities identified in biological organisms. These assembly methods derive from an understanding of the interactions that biomolecules have with inorganic materials. Using such an approach a wide variety of 'small' and 'simple' biomolecules such as amino acids through to macromolecules such as proteins and DNA and more complex assembled structures thereof have been explored for fabricating novel materials.[1,14–27] In order to develop this approach further, it is essential to understand in detail how biomolecules interact with inorganic materials, and to be able to identify the 'rules' or 'guiding principles' that govern interaction which could then be used to explain and then predict behaviour. An understanding of biomolecule–inorganic materials interactions would be highly fruitful not only to understand biological mineralization processes but also to design novel materials and processing technologies for applications in fields as diverse as biological imaging and biosensors, implant integration,[28–32] food and drug handling, and electronic materials (Fig. 1).[30,32–36]

Carole C. Perry

Professor Carole C. Perry obtained her BA (1982) and DPhil (1985, supervisor Professor R. J. P. Williams, FRS) at Somerville College, Oxford, and was an E. P. A. Brereton-Sherman Junior Research Fellow at St Hilda's College, Oxford (1985–1987) before taking up a permanent position at Brunel University as a lecturer in Inorganic Chemistry. Professor Perry spent six months working with Professor S. Weiner at Weizmann Institute, Israel (1992) before moving to Nottingham Trent University in 1993. She was promoted to Reader in 2000 and Professor in 2003. She held a Visiting Professorship at Universite Pierre et Marie Curie, Paris VI in the laboratory of Professor J. Livage in 2002. She is currently Head of Chemistry. Carole C. Perry has extensively published articles and reviews on a range of biological materials related topics. Her current research interests include in vivo and in vitro silicon–biomolecule interactions, biomaterials, sol–gel derived materials and their interactions with peptides and proteins, super-hydrophobic surfaces, and correlation analysis methods in the study of material structures.

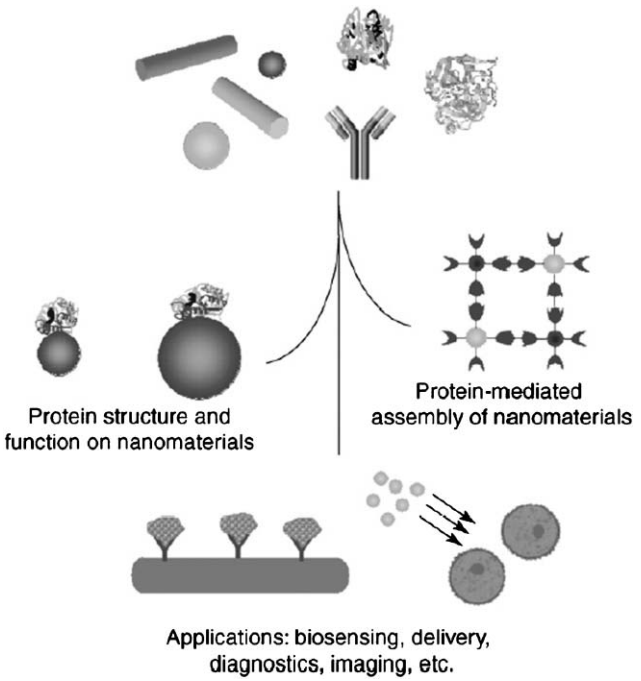


Fig. 1 Schematic presentation of the possible interactions between nanomaterials and biomolecules and their applications. Image reprinted from ref. 33 (Asuri *et al.*) with permission from Elsevier.

In this review we briefly present a few examples of our current level of understanding of inorganic materials–biomolecule interactions from the biomineralization perspective before discussing the role that combinatorial approaches such as phage and cell display methods can play in identifying peptides that interact with a wide range of natural and non-natural mineral surfaces. Here, the emphasis will be on the extent of understanding that can be obtained from such studies. Although the field of biomolecule–nanoparticle bioconjugates could be included in the present discussion, the topic has been omitted for simplicity and also because there is a considerable amount of literature already available including comprehensive reviews.[1,4,37] The article will conclude with an indication of future prospects for the field.

2. Biomineralization

Biomineralization is a process by which biological organisms produce inorganic minerals *in vivo*. Note: these materials are actually composites of biopolymers and inorganic salts or oxides and the materials have physical and structural properties that may be somewhat different to those of their individual components. The process of biomineral formation in many cases is known to be genetically regulated and it is this control that produces species-specific, often ornate biomineral structures with physical and mechanical properties fit for function.[10–12,38] It is known that organic biomolecules such as peptides, proteins and proteoglycans, lipids and polysaccharides are involved in most, if not all, stages of biomineral formation, from transport, to nucleation and growth through to structure stabilisation. In the generation of such well defined composite biomineralized structures, molecular recognition

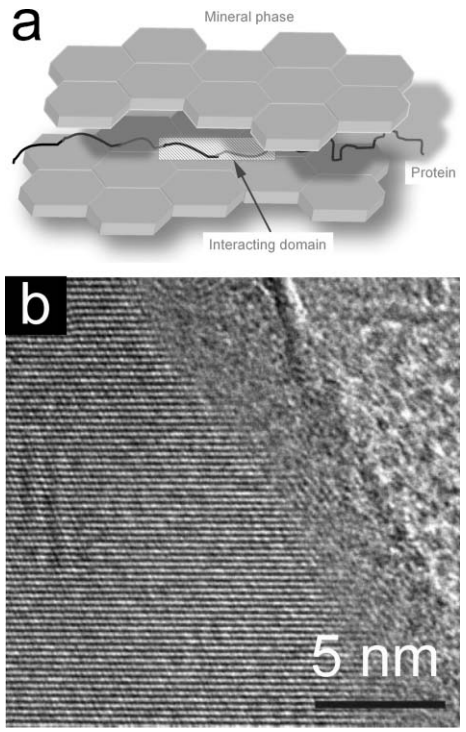


Fig. 2 (a) Schematic representation of the proposed interactions of proteins (Lustrin-A) and biominerals (aragonite).[43–45] (b) TEM images showing the presence of an amorphous calcium carbonate layer between the aragonite and organic phases [image reprinted from ref. 46 (Nassif *et al.*) with permission from National Academy of Sciences, U. S. A.].

between the organic and inorganic species has been proposed to be essential.[39] Recognition can arise from individual or combinations of interfacial or non-bonding interactions such as electrostatic interactions, hydrogen bonding, the hydrophobic effect *etc.* and may also include stereochemical effects.[11,36,39–41]

The chemical and physical interactions that exist between the inorganic and organic phases in the products of biomineralization are still greatly debated throughout the scientific community. A well known family of biomineralized structures that have been intensively studied are red abalone shells which although made from calcium carbonate are extremely tough.[10,11,42] The generally accepted view is that crystalline plates of aragonitic calcium carbonate interact with intercalated proteins in an epitaxial fashion giving rise to a layered material that is incredibly tough. In attempts to understand what is happening in such a material at the molecular level, nacreous layer matrix Lustrin proteins have been studied in the presence of relevant metal ions.[43–45] A 24 amino acid polyelectrolyte active domain from the protein Lustrin-A, termed D4, has been found to exhibit metal ion (calcium) binding ability. Particular chemical features of the domain were that it was rich in aspartic acid residues and also contained hydrogen donor/acceptor amino acids including asparagine, glutamine, arginine, threonine, serine and tyrosine. Spectroscopic studies of D4 have shown that it adopts an open chain conformation in solution enabling the side chain charged residues to access the inorganic surface [Fig. 2(a)]. Further detailed examination of this protein has revealed the presence of a nine amino acid sequence capable of binding Ca(II) ions in a 2 : 1 (Ca : peptide) stoichiometry *in vitro*. For $CaCO_3$ grown *in vitro*, the presence of a model peptide containing the D4 sequence in the reaction medium was found to affect the morphology of the crystals formed with the polypeptide being bound to the surfaces of the crystals.

In principal these experimental observations should be very important but their level of significance is called into question by a recent report on the structure of nacre from a different genus of red abalone.[46] In this study, the aragonite crystals examined were found to have a continuous coating of amorphous $CaCO_3$ which therefore would not readily show an epitaxial interaction with the organic matrix [Fig. 2(b)]. The chemical and/or physical nature of the interaction between the inorganic and organic phases in this genus is currently unknown but does bring into question the role of interactions of the organic matrix with biominerals in determining the structure and form of these materials.

Another example is of magnetotactic bacteria that contain single domain particles of magnetite (Fe_3O_4) that is formed within a magnetosome, the membrane of which contains proteins.[47,48] Analysis of magnetite crystal associated proteins have identified several low molecular mass proteins tightly bound to bacterial magnetite (but not within the crystals) which show common features in their amino acid sequences, namely hydrophobic N-terminal and hydrophilic C-terminal regions.[49,50] The N-terminal domain is suggested to be a transmembrane domain. Within the C-terminal region are found basic amino acids (function unknown) and clusters of carboxyl and hydroxyl containing amino acids that bind iron ions. The use of the recombinant version of one of these proteins in the chemical synthesis of magnetite yields particles with a morphology similar to that observed in the bacterium from which the biomineralizing protein had been isolated. The proposed role of this protein is to provide nucleation sites for precipitation of iron oxide within bacterial magnetosomes. The impact of the protein on morphology control is not yet clear.

A further example from the field of biomineralization is the generation of hierarchically ordered silica structures which occur in the presence of proteins, carbohydrates and simpler molecules according to the species investigated.[51–54] In the case of silicified diatoms, the current state of knowledge is that complex patterned macroscopic structures are built up from nanometre sized amorphous silica particles in the presence of proteins and/or polyamines. For the specific species investigated, silaffins containing heavily posttranslationally modified lysine (some modified with polyamines) and serine (all modified with phosphate) and/or long chain polyamines are thought to assist in silica formation on the atomic length scale as well as in the development of reaction domains.[53] However, the nature of the molecular level interactions is not clear. Laboratory based model studies using the non-posttranslationally modified peptide sequence found in silaffins also shows promotion of silica formation at physiological pH. This fact has led to the development of silica based composite materials for a variety of applications ranging from optical devices through to biomaterials.[27,55,56] However, nothing

much is really understood at the molecular level regarding the interactions involved.

The study of the biological, biophysical and chemical characteristics of biomolecules involved in biomineralization will continue to be explored so as to probe the interactions naturally occurring between biomolecules and inorganic materials that enable composite materials with interesting physical and mechanical properties to be generated. However, understanding of the structure of biomolecules and indeed the biomolecule matrix involved in biomineral formation is in its infancy, with very few biomolecules being fully characterised, let alone their activity understood. In addition, this approach is limited to a few materials only and excludes many commercially relevant materials such as CdS, oxides of Ti, Sn, *etc*. and metals. An alternative approach that can be used for materials that are synthesised by biological organisms is to use knowledge of the associated biomolecules and to investigate simpler moieties that contain the structural characteristics that are believed to be essential for activity. This field of biomimetics has been used to good effect for progress in understanding how to control the structure (on different length scales) of simple oxides and salts such as calcium carbonate.[11,23,57–59] However, this approach still does not address the issue that biological organisms do not process many different metals or cation–anion combinations in their generation of inorganic materials. In principal we believe that the approach used by biomineralizing organisms (the use of biomolecules to direct mineralization) could be applicable to a huge number of simple and complex inorganic materials.

3. Going forward—combinatorial methods

The approach that has been championed is the combinatorial (*i.e.* screening) approach, typically used in the pharmaceutical field for drug discovery. The screening technique is used to explore the importance of a large set of parameters controlling a given process, examples being chemical synthesis, molecular binding or processing.[60] In the present context of the biomolecule–materials interface, the combinatorial method involves the selection of peptides capable of interacting with inorganic materials from a random library (of over a billion unique sequences) and then taking this information and attempting to use it to generate materials *de novo in vitro*.[18,61–63] The peptides can be displayed on particles such as bacteriophages or cells, where one particle contains several identical copies of one amino acid sequence (of a desired number of residues) which constitutes part of the library (Fig. 3). Selection of the desired peptide(s) is achieved through multiple rounds of target binding, elution and amplification by a process known as 'biopanning'. The amino acid sequence of the displayed peptide is then obtained by sequencing the encoding DNA of the phage or cell displaying the peptide. This approach is thought to be a more practical approach to the identification of surface specific proteins than the more traditional approaches to gene identification through the isolation of proteins and amino acid sequencing followed by gene identification. For detailed information on this topic, readers are directed to recent reviews.[33,63–65]

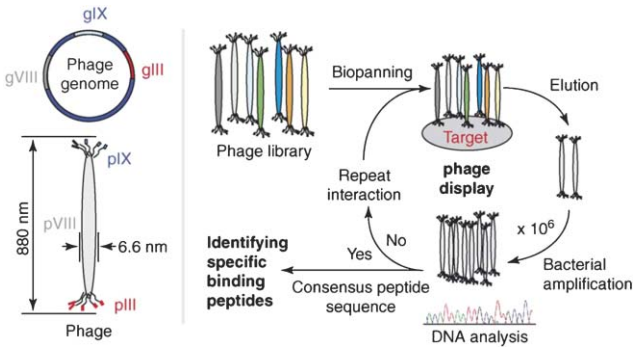


Fig. 3 Schematic of a phage (left) and the biopanning process (right). Image reprinted from ref. 64 (Merzlyak and Lee) with permission from Elsevier.

The goal of 'biopanning' is often to isolate biological 'catalysts' and 'templates' that can be used for the 'bottom-up' microfabrication of materials. In all instances a 'material of choice' is required for the panning procedure and the protocol used to synthesise this material is thought to ultimately determine which peptide compositions selectively bind to the particular surfaces expressed. The range of materials that has been selected for by this approach is large and includes metals and alloys *e.g.* Ag, Au, Pd, Gd, Ti, Co/Pt and Fe/Pt, oxides of Si, Fe, Ti, Zn, Sn, Ge, Mn, Cr, Co, Pb, sulfides of Zn, Cd, Pb, selenides of Cd, Zn, arsenides of Ga (pure and doped), zeolites, simple salts including $CaCO_3$, and other materials such as fullerenes, carbon nanotubes and polymers.[62–64,66–85] Recent reviews have summarised the sequences of various peptides binding inorganic materials.[18,63] The use of combinatorial methods such as this could also be used to deduce useful information such as predictive rules governing the interfacial interactions between minerals and biomolecules that could be applied to a variety of other materials systems. Selected examples to illustrate the diversity of experimental systems explored to date are presented below together with a brief discussion on the principles gleaned from such studies.

In one particular in depth study, a 12 amino acid random peptide library was screened for binding against metallic titanium (as used in artificial implants) with the discovery of a high occurrence of the peptide *–Ti*-12-3-1 (N-*RKLPDAPGMHTW*-C).[86] Further experimentation to investigate whether the whole 12 amino acid peptide was necessary for binding or whether smaller components were active found that only the N-terminal hexapeptide was important to retain metallic Ti binding activity. Mutants of the hexapeptide, with one amino acid being sequentially replaced with an alanine residue, were studied in order to find out which amino acids (and in which position) were required for binding to the material in question. The substitution of the first AA [arginine (R)], the fourth AA [proline (P)] and the fifth AA [glutamic acid (E)] residues resulted in a significant loss of binding of the peptide to the metal. Detailed evaluation of the titanium metal surface revealed the presence of an amphoteric oxide layer in which $Ti–O^-$ species are proposed to interact with the positively charged arginine residue, $Ti–OH_2^+$ species are proposed to bind with negatively charged aspartic acid residues, and the presence of proline provides a suitable

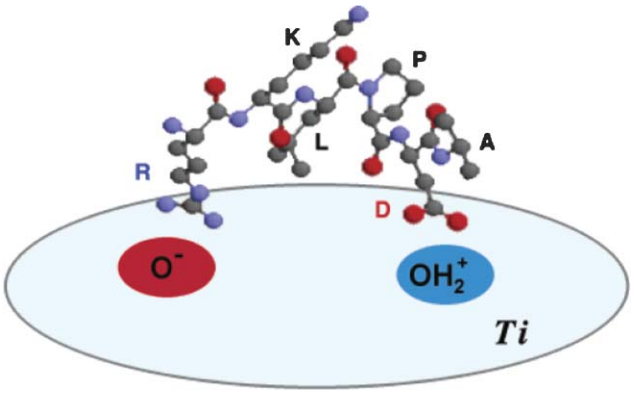


Fig. 4 Schematic of a model hexapeptide binding to a metallic titanium surface exhibiting an amphoteric oxide surface layer. Reprinted from ref. 86 (Sano *et al.*) with permission from the American Chemical Society.

constrained peptide conformation for binding as depicted in Fig. 4. This series of experiments highlights two key features of peptide–inorganic surface interactions. Firstly, it shows that interfacial interactions are dependent on both the chemical (amino acid sequence, pI, *etc.*) and physical (secondary structure, folding, stability, *etc.*) nature of the peptide. Secondly, we learn that the inorganic surface itself plays an important role in determining how biomolecules interact with the surface.

In another study of peptide binding to group II–VI semiconductors (CdS, CdSe, ZnS and ZnSe) and gold substrates, the effects of homohexapeptides displayed on yeast were explored.[72] The approach used in this study aimed to identify "(1) which amino acid functional groups are sufficient for binding short peptides to their chosen inorganic surfaces? (2) How do neighbouring amino acid functional groups and their spatial arrangement in a peptide sequence modulate binding strength? And (3) can the results be used to design peptides specific for the different materials surfaces?" The histidine hexapeptide (HHHHHH) was found to bind all the materials under investigation and tryptophan (WWWWWW), cysteine (CCCCCC) and methionine (MMMMMM) hexapeptides demonstrated selective binding which was dependant on the material's properties. In order to establish rules by which binding motifs for a whole range of mineral phases could be predicted, several heterohexapeptides containing three histidine residues and three residues of another amino acid were designed and their binding activities investigated. The results obtained revealed that the presence of some amino acids increased peptide binding to the materials under investigation, while others reduced peptide binding. For example, the presence of lysine (K) residues (KHKHKHK) showed 50% more coverage on a ZnS surface, while the presence of aspartic acid (D) residues (DHDHDHD) reduced the binding of the peptide by nearly half [both activities were reported in comparison with an alanine (A) histidine mix, AHAHAHA]. These 'rules' were further used to design substrate specific binding peptides by incorporation of residues that would either specifically enhance or diminish peptide binding. The experimental data generated from the binding of designer peptides on CdS, ZnS and Au surfaces revealed that the designed peptides obeyed the predicted binding rules to a large extent.

In another recent report, the binding of homopeptides (8–10 residues) from peptide solutions in three different solvents to metallic, semiconductor and insulator substrates has been explored.[87] Quantitative binding data was obtained by fluorescence measurement using markers on the N-terminus of the peptides. Charged residues were observed to exhibit higher binding affinities through electrostatic interactions, *e.g.* binding of peptides containing basic residues [histidine (H), lysine (K)] on the silicon-derived insulating materials tested. The hypothesis was further supported by the results obtained from pH dependent binding studies. However, the unexpected binding of a negatively charged aspartic acid homopeptide was observed suggesting that complex binding mechanisms must exist. Predictive rules were identified from the data and used to design peptides that specifically bound to a given substrate. As an example, surfaces containing alternate GaAs and AlGaAs patterns were constructed with varying separations. Triblock peptides were designed where the end blocks were non-binders to both GaAs and AlGaAs, while the central block was selected to bind only to AlGaAs. It was shown that the peptide adhesion could be controlled simply by varying the separation distances between GaAs and AlGaAs.[87]

4 Molecular modelling

Although in the latter two examples described above (and other investigations presented herein) the binding of peptides to a given surface has been correlated to the chemical characteristics of the peptides, there seems to be avoidance or a lack of understanding concerning the molecular features of the inorganic materials' surfaces and the biophysical properties of the peptides (*e.g.* folding/conformation and water accessible surface). Conformational details of peptides binding to surfaces, when compared with those from non-bound sequences could generate a fundamental understanding of the process of peptide binding. Additionally, one could identify the effects of subtle features such as the positions of important amino acids in the peptide sequence and the effect of their immediate environments within the peptide itself.

The principle of this approach is described below. In the example study, a screening of peptides displayed on cell surfaces against Cu_2O and ZnO was conducted [Fig. 5(a)] and detailed statistical analysis performed to identify statistically significant binding sequences.[70] The experimental results obtained showed that peptides that bound to the metal oxides were enriched in arginine (R), tryptophan (W) and glycine (G) residues with a specific R–X–X–R tetrapeptide motif (X = any other amino acid) being identified (Table 1). This motif was suggested to be a metal oxide binder, while the presence of an additional R–R and R–K motif was thought to distinguish between the two oxides studied. The results of conformational analysis performed on selected peptides by molecular dynamics simulations suggest that there are specific orientational requirements for binding to inorganic surfaces. The peptides exhibited corrugated surface features and characteristic angles between the arginine (R) and/or lysine (K) residues involved in the proposed binding motif [Fig. 5(b)] thus

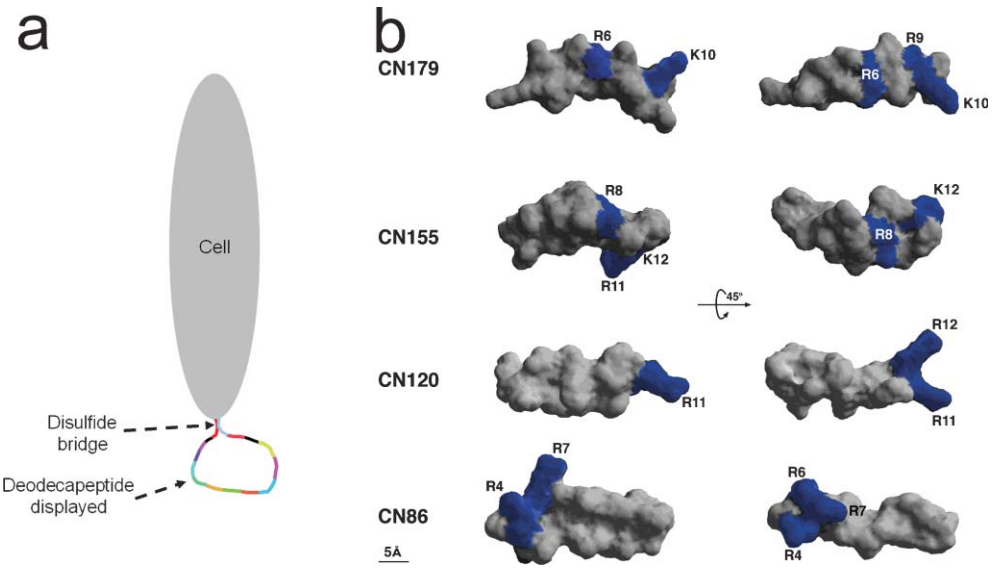


Fig. 5 (a) Schematic representation of the cell display used by Thai *et al.*[70] showing the disulfide constrained dodecapeptide displayed on cell surface. Only one peptide is shown for simplicity (scheme not to scale). (b) Molecular dynamic simulation of peptides screened for ZnO or Cu_2O binding. The peptides were constrained by adding CGP and CPG tripeptides on N- and C-termini respectively in order to mimic the cell display system used. Fig. 5(b) kindly provided by Dr. François Baneyx,[70] reproduced with permission from Wiley.

Table 1 List of peptides found to tightly bind ZnO. Reproduced from ref. 70 with permission from Wiley.

Binder	Sequence	Adhering cells	Charge	pI	Hydrophilicity
ZnO Class I					
CN179	RIGHGRQI***RK***PL	100 ± 17	+4	12.3	+0.53
CN155	VRTRDDARTH***RK***	69 ± 5	+3	11.6	+1.48
CN120	PASRVEKNGV***RR***	13 ± 6	+3	11.7	+1.00
ZnO Class II					
CN146	MRHSSSGEPRLL	84 ± 12	+1	9.4	+0.38
CN111	PAGLQVGFAVEV	78 ± 13	−1	4.0	−0.55
CN185	RTDDGVAGRTWL	61 ± 7	0	6.0	+0.33

supporting the hypothesis presented herein that the biophysical characteristics of peptides control the binding events. Similarities between R–R distances from the peptide sequences and the oxide crystal lattices were also suggested to play an important role in peptide binding.

Is there really a correlation between the surface properties of materials and peptide binding? Scientists have tried to address this question by performing a molecular dynamics simulation study of the binding abilities of genetically engineered gold binding proteins on two gold surfaces with different crystallographic faces—Au [111] and Au [211].[88] Protein modelling revealed that the preferred secondary conformation of the gold binding proteins was β-sheet, periodically displaying hydroxyl groups which were suggested to bind to the gold surface (Fig. 6). There was a noticeable difference in the binding energies on the two distinct gold surfaces. This difference in protein adsorption was suggested to be due to the tighter association of water molecules on the [211] surface in comparison with gold expressing [111] surfaces. Thus we know that the chemical composition of the surface and the materials' properties such as crystal faces expressed are important in determining biomolecule binding.

We have performed a study to test the generality of principles derived from molecular modelling approaches. As mentioned above, the RXXR/K motif is proposed to be involved in the binding of peptides to the zinc oxide surface and that the characteristic angles between R and/or K residues

Table 2 List of peptides found to tightly bind ZnO. Reproduced from ref. 85 with permission from the American Society of Microbiology.

Plasmid	Frequency	Enriched amino acid sequences
pJKS9	8/25	R S N T R M T A R Q H R S A N H K S T Q R A R S
pJKS10	2/25	R S V F L P S I L G W R S R L D D Q G V A A R S
pJKS12	3/25	R S T R N K H T T A R R S V A P G I G E P S R S
pJKS14	1/25	R S I M H V R L R A R R S A R H M K D A D P R S
pJKS17	1/25	R S P I I I R S R I N R S H G R T K A T P A R S
pJKS18	2/25	R S R G L R N I L M L R S Y D S R S M R P H R S
pJKS11	4/25	R S T R R G T H N K D R S
pJKS16	1/25	R S T V P K R H P K D R S
pJKS45	1/25	R S I A K K T H N K Q R S
pJKS15	1/25	R S Y D S R S M R P H R S
pJKS46	1/25	R S T A S R H T E P H R S

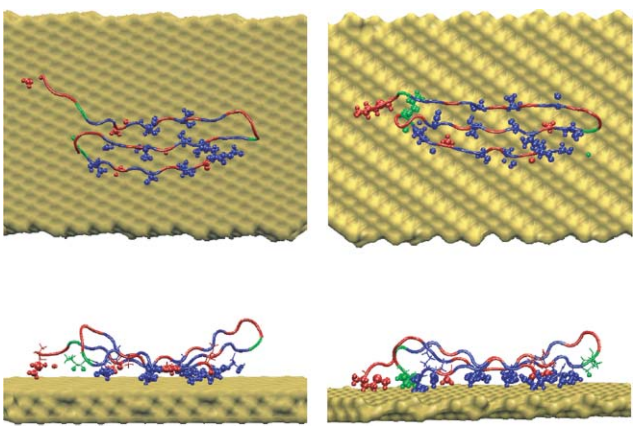


Fig. 6 Molecular dynamic simulation of genetically engineered proteins on two gold surfaces: left [111] and [211] right. Colour scheme: blue—polar residues, green—charged residues and red—hydrophobic residues. Reproduced with permission from BRILL.[88]

give surface-specific selectivity.[70] In another related study, peptide affinity and selectivity towards zinc oxide has been explored using an *E. coli.* based peptide display system with peptides being identified that contain the same RXXR/K motifs (Table 2).[85] In order to find out if the hypothesis proposed by Thai *et al.*[70] would apply to the set of ZnO binders identified by Kjærgaard *et al.*[85] we performed molecular modelling of the peptides obtained in the latter studies.

We selected two peptides for molecular dynamics studies—peptide pJKS9 (*P1*) which displayed higher ZnO binding activity and selectivity (with respect to CdO) and peptide pJKS17 (*P3*), with almost similar ZnO binding activity but reduced selectivity (Table 2). The molecular dynamics could explain the basis for selectivity and specificity of peptide binding between ZnO and CdO. Comparing peptides pJKS9 (*P1*) and pJKS17 (*P3*), modelling suggested that in the case of *P1* two binding sites are likely to be present, one consisting of Thr4 and Arg9 [highlighted in yellow in Fig. 7(a,b)] and the second one comprising Arg5 and Ser13 [highlighted in magenta in Fig. 7(a,b)]. These two binding sites are almost perpendicular to each other, as seen in Fig. 7(b). *P3* on the other hand was found to fold into a V-shaped cleft with Arg1, Arg7, Arg9 and Arg12 all lying on one side [highlighted in grey, Fig. 7(a)]. The only likely interaction that occurs is between Arg1 and Ser24 which lie at the ends of the V-shaped cleft. The lack of specificity of *P3* can be explained on the basis that display of basic residues on one side of the surface could interact with the surface oxide, regardless of the metal oxide in question.

It should be noted that these peptides bearing 24-AA residues have higher propensity to display secondary structures than those studied by Thai *et al.*[70] bearing 12-AA residues and also the fact that these peptides are not constrained into loops by disulfide bonds. Our results presented above suggest that the residues likely to interact are not necessarily the ones in the motif but that what is required is a basic amino acid residue (usually arginine) which may or may not be part of the motif and a hydrophilic residue (serine/threonine), typically at distances of 0.6–0.8 nm from each other.

From our study, it was realized that physical characteristics of the peptides and their display pattern could explain the observed specificity and activity. The inferences were drawn solely based on the properties of the peptides, with the contribution from the solid state binding partner not being taken into consideration. Thus no details were obtained about the manner in which these peptides interact with the surface. It could be likely that binding is solely dependant on electrostatic interaction between partial positive charges on the metal centres with an anionic site in the peptide and a partial negative charge on the oxide with a positively charged site on the peptide. The partial charge distribution would in turn vary between metal oxides and may allow selective binding of specific peptide sequences or motifs. What is needed to systematically investigate selectivity and specificity of binders is a study where a single peptide is screened for activity on various metal oxide surfaces. This would answer questions such as how well does the peptide differentiate between various metal oxides, shed light on the pattern of interaction and obtain information on whether it is generic for all metal oxides. Another factor to be considered is that most of the peptides

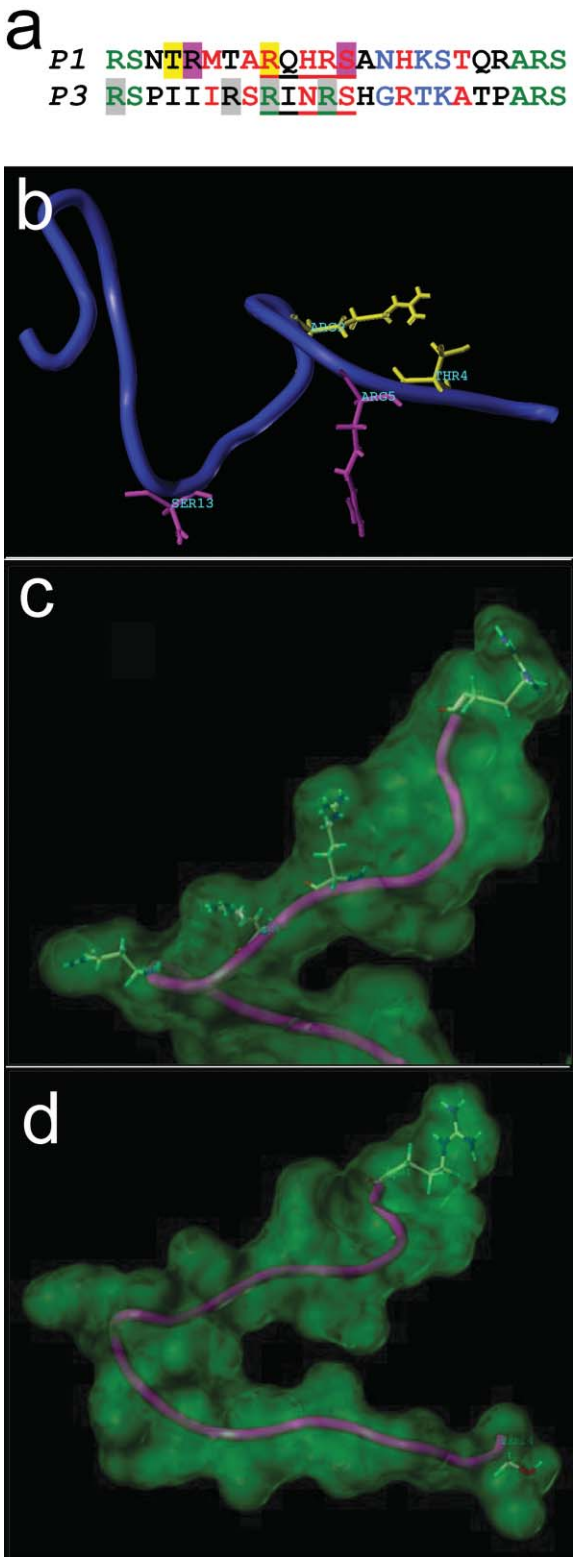


Fig. 7 (a) Sequence alignment of *P1* and *P3*. The proposed binding motif RXXRX is underlined. (b) *P1*, Relative orientation of the two binding sites is almost perpendicular to each other. (c),(d) *P3*, Top right V-shaped cleft, top right Arg1, Arg7, Arg9 and Arg12 on one side of the cleft; bottom left Arg1 and Ser24 at the end of the cleft.

binding experiments have been carried out in aqueous reaction conditions at near neutral pH which clearly affect selection for polar amino acids such as arginine, histidine, serine and threonine in the peptides that bind. In addition there is a strong possibility of water mediated interactions. Although our modelling investigations are of a preliminary nature, the results caution on the generality of the principles in question. The caveats involved with modelling should be considered before applying a given set of rules from one system to another.

5. Summary and future prospects

From the preceding discussion of recent literature it is clear how important and yet complex are the interactions of biological molecules with inorganic materials. Nonetheless, a close inspection of these findings would provide us with possible modes of and parameters affecting such interactions. These are classified as chemical and physical factors (listed below).

Chemical effects

○ Kinetic effect on material growth and dissolution in the presence of biomolecules

○ Molecular affinity/chemical specificity between inorganic species and biomolecules—complex formation

○ Biomolecule mediated stabilisation of intermediates and/or particular phases of inorganic materials

○ Weak forces between biomolecules and inorganic materials—electrostatic interactions, hydrogen bonding, van der Waal's forces, hydrophobic effect, *etc.*

○ Biomolecule chemistry (amino acid sequence for a peptide, pI, *etc.*).

Physical effects

○ Epitaxial recognition/matching between biomolecules and inorganic surfaces/materials

○ Structure direction—scaffolding or aggregation promotion by biomolecules in inorganic materials' synthesis

○ Mineral phase stabilisation mediated by biomolecules

○ Geometric complementarity

○ Adsorption of biomolecules inhibiting growth of inorganic material

○ Biomolecule (peptide and protein) confirmation and stability.

Although we have highlighted advantages of the biopanning techniques, there are some limitations to the approach. The phage display method, for example, is dependent on whether fusions are to coat proteins III or VIII as these will have different numbers of copies of the fusion product. pIII is present at 5 copies per virion and all 5 can be fused to short peptides. The major coat protein pVIII is present at *ca*. 2700 copies per virion and approximately 10% of these can be replaced giving *ca*. 200 copies per virion. The fusion products are some way from the surface of the virion and as such can adopt 'free' structures in solution. Furthermore, there is no information on how much peptide is displayed on the surface and what its exact location is in relation to the surface. There seems to be a real lack of understanding of how display particles and peptides associated with them 'see' surfaces and changes that occur with time. For example, the peptides displayed are bound on the host particle at one end, rendering that end incapable of binding to another material. In the case of biopanning against inorganic substrates, some researchers have used phage display systems while others employed cell displays, some have even used constrained peptide libraries. The differences between these display systems and their effects on identifying peptide sequences need to be taken into consideration. For example, for a given target material, would different random display systems yield the same information pertaining to peptide binding?

It is also important to note that in some cases commercially available metals, oxides and other salts have been used and in others freshly synthesised materials have been used. In almost all cases, however, there is usually little, if any, information given on the form of the materials including sizes and morphology of particles, crystal phase and crystal planes available for interaction or for amorphous materials, information on the surface characteristics including extent of hydration and hydroxide functionality as well as intrinsic particle sizes and porosity. As we have seen for some examples presented above, materials' characteristics have an important role to play in peptide binding and cannot be ignored.

If we wish to improve our understanding of peptide–mineral interactions in order to ultimately use that knowledge predictively then detailed molecular studies, both in solution and of peptides attached to surfaces, are required. Experiments will need to investigate the material composition as well as crystal faces expressed and/or porosity and/or surface functionality. It will also be important to undertake extensive simulation studies and compare the results with experimental data on the same systems. Questions which future studies should seek to answer include:

(1) What are the molecular scale interactions between mineral surfaces and biomolecules?

(2) Do these interactions arise from fundamental features of the chemical species *e.g.* Au or Cu and O involved and/or their disposition with respect to one another in the solid state phases and faces expressed, and/or is it the fundamental chemistry of the peptides (chemical functionality and spatial disposition) that is important?

(3) How can these interactions be used to direct materials synthesis, assembly and detection, *e.g.* phase, expression of particular crystal faces, morphologies, stabilisation and/or dissolution of particles *etc*?

(4) Can these interactions be used to provide predictive 'rules' for peptide binding to a range of other materials?

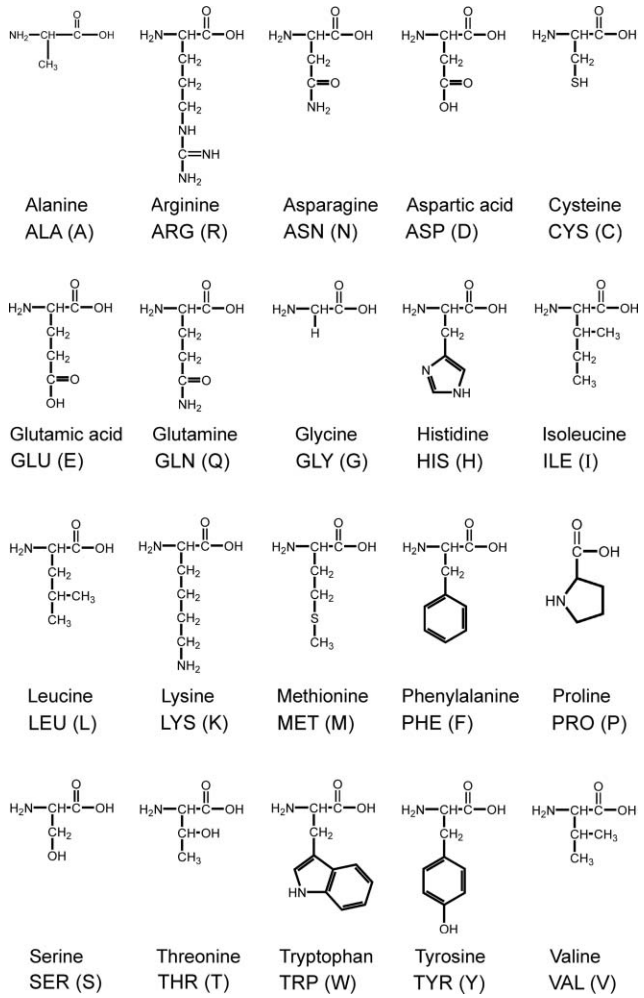
(5) Can these interactions be used to produce ordered assemblies of nanoscale materials?

(6) What does the observed behaviour tell us about the way that biological organisms control mineral production during biomineralization processes?

This area is ripe for investigation but genuine progress will only be made if great care is taken in the preparation of target materials and application of a combinatorial display system in biopanning.

Abbreviations

AA Amino acids/residues. Shown below are structures and two types of abbreviation for each AA.



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