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Biomimetic synthesis of inorganic materials and their applications*

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Abstract: Mimicking the evolution processes of Nature, the combinatorial approach to biomolecular recognition properties attracts much attention due to the potential as a generic scheme to achieving complex material structures and hierarchical assemblies with molecular precision from the bottom up. In this paper, some recent efforts in the biomimetic synthesis of inorganic materials are reviewed, with emphasis placed on in vitro material formation with the use of protein/peptide molecules found in natural organisms as well as those with specific affinities to inorganic materials selected through the molecular evolution process. The applications of material-specific peptides and proteins in sensing and guiding hierarchical material assembly are also briefly discussed at the end.

Keywords: biomimetics; biorecognition; bottom–up assembly; nanomaterials; proteins and peptides.

INTRODUCTION

Materials with dimensions in nanoscale have attracted a lot of attention over the past two decades owing to the novel properties that appear with the finite dimension [1–5]. One of the main challenges to bringing nanoscale materials to practical applications is the controlled synthesis and assembly of these tiny building blocks [6–10]. The lithography-based "top–down" approach will soon reach its limit in creating patterns in the deep nanometer regime [11,12]. Alternatively, the bottom–up approach, with which material structures are hierarchically assembled from individual nanoscale building blocks, can circumvent the limitations of lithography and lead to the formation of functional nanosystems with molecular precision [13–15]. The main hurdle now faced by the bottom–up approach is the lack of a general scheme for the creation and assembly of nanomaterials [16,17]. On the other hand, through millions of years of evolution, Nature has selected organisms that can create diversified inorganic material structures in nano- or microscale, which is known as biomineralization. Examples include calcification and silification found in diatoms, and the formation of magnetite crystals in magnetotactic bacteria, as shown in Fig. 1 [18–21]. These organisms are capable of organizing nanoscale building blocks into large-scale hierarchical structures to form hard tissues serving different functions, such as mechanical support, filtration, light harvesting, gravity sensing, and locomotion [22–24]. Scientists have long been exploring the approaches and mechanisms adopted by Nature to create functional structures for technical applications, which is usually referred to as biomimetics [25,26]. With the recent advances in molecular biology, biomimetics has now advanced to the molecular level—adopting the bottom–up scheme

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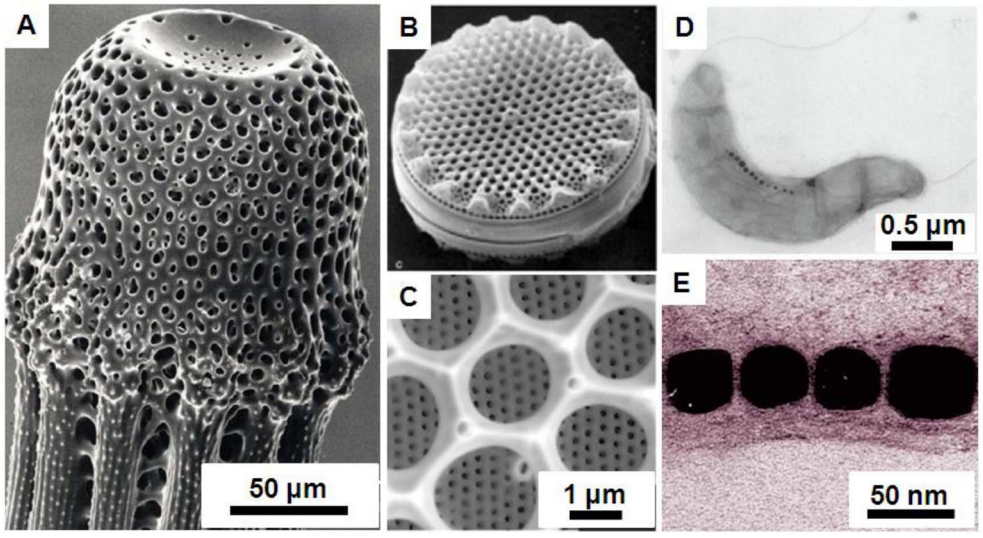

Fig. 1 (A) Scanning electron micrograph (SEM) of a mature sea urchin spine, which is a composite of organic macromolecules and oriented $CaCO_3$ (calcite) [18]. (B) SEM image of cell walls from diatom. (C) High-magnification SEM image of diatom silica cell walls [20]. (D) A magnetospirillum gryphiswaldense cell. (E) Magnetite crystals observed at the ends of the chain in magnetotactic bacteria [21].

used by biosystems in building hierarchical structures. For example, in vitro biomimetic studies have revealed that the proteins play an important role in controlling the size and morphology of hard-tissue minerals, as well as in directing the biomaterial assembly through their specific interactions with inorganic crystal surfaces evolved through millions of years, which has inspired considerable efforts in biomimetic material synthesis and assembly using the biomolecular recognition properties [27,28].

In this article, we will review recent efforts in biomimetic synthesis of inorganic materials. Emphasis will be placed on in vitro material formation with the use of protein/peptide molecules that are extracted from the organisms or identified through the molecular evolution process, showing specific affinity to an arbitrary inorganic material. We note that although there are great biomimetic achievements in producing material structures using whole biological entities/organisms such as the whole virus and algae [29–34], or using DNA/RNAs [35,36], this review article will limit the scope of discussion to protein- and peptide-based biomimetic material synthesis.

PROTEIN-DIRECTED BIOMINERAL SYNTHESIS IN VITRO

Through millions of years of evolution, Nature has created proteins that are capable of directing the formation and assembly of minerals into hard tissues, and doing so with minimal energetic requirements [37]. There are many efforts at trying to decipher the roles of the proteins in directing the formation of the biominerals by carrying out in vitro mineralization studies with the presence of the proteins that are extracted from the hard tissues. For example, the cell wall of the unicellular diatom is formed by amorphous silica, the morphologies of which vary among different species [38–40]. Through in vitro studies, researchers found that two protein groups are responsible for the morphologies of the silica cell wall. Long-chain polyamines and silaffins are the two major groups of molecules that are extracted from the silica cell wall by rigorous treatment using hydrofluoric acid, and have been found to be involved in the silification process [41–45]. In vivo studies have suggested that these proteins can induce the silica polymerization in a special vesicle called silica deposition vesicle (SDV) [46]. Later studies have also successfully demonstrated that these protein groups can induce the silification in vitro, as shown

in Fig. 2A. At the molecular level, it is suggested that the polyamines containing N-methylated oligopropyleneimine condense the monomer silicic acid to silica by serving as an acid–base catalyst, in which one of two appropriately arranged amino groups along the polyamine backbone is deprotonated (as a base) while the other is protonated (as an acid) to stabilize the transition state. By deprotonation with a silicic acid molecule, the base would help the formation of the silanolate group, whereas the acid facilitates the water release through protonation from the attached silicic acid, leading to the condensation reaction between two silicic acid molecules [47,48]. Silaffins are polycationic molecules containing a large amount of hydroxyamino acids (mainly serine), which may form hydrogen bonds or have ionic interactions with the surface of silica particles [43,49]. At the same time, silaffins are covalently modified with oligo-*N*-methyl-propylamine, which resembles the chemical structure of polyamine and possesses the catalytic activity for silicic acid polymerization, and has been demonstrated to catalyze the formation of irregular silica particles and porous silica blocks [42,43].

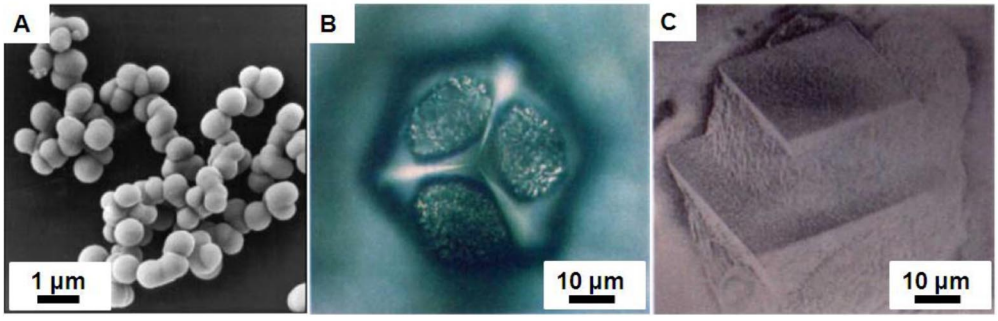

Fig. 2 (A) SEM micrographs of silica precipitated by silaffin-1A [43]. (B) Addition of polyanionic protein causes aragonite overgrowth as needles on the rhombohedral faces. (C) SEM image of sequential phase switching induced by sequential addition and removal of aragonitic polyanionic protein [60].

It has also been discovered that polyamines of different chain lengths can lead to the silica formation of different sizes and morphologies. For example, longer-chain polyamines (with mass range of 1000–1250 Da) produce spherical silica of size 800–1000 nm, while shorter polyamines (between 600 and 700 Da) produce much smaller silica spheres from 100 to 200 nm [42]. Further studies revealed that a synergistic action of long-chain polyamines and silaffins helps produce different silica morphologies. For example, when polyamines and silaffins are both added into the silicic acid solution, a hybrid structure of silica blocks composed of spherical silica particles is formed, which is reminiscent of both the precipitate that forms with polyamine only and the precipitate that forms with silaffin only [42]. So far, the mechanism of the polyamine- and silaffin-induced silica precipitation is not completely understood, but it is believed that the ability of the two protein groups in catalyzing the siloxane-bond formation plays a key role.

In addition to the diatoms, silica is found in sponges in the form of needle-like spicules that support the organisms and protect them from the predators. All spicules produced in membrane-enclosed vesicles in the cells known as sclerocytes contain an axial filament of protein [50,51]. These protein filaments serve as the templates to direct the deposition of silica [52]. Three similar protein subunits were found in the protein filament extracts from a marine sponge *Tethya aurantia*, named silicateins α, β, and γ [53]. It has also been demonstrated that the isolated protein filaments can direct the silica condensation from silicon alkoxide precursors in vitro, indicating the role of silicateins in the silification process [54]. Detailed genetic studies have revealed the similarity of the silicateins α and β to the well-known proteolytic enzyme cathepsin L points, thus postulating the catalytic role of the histidine–serine–asparagine triad in the silicateins [54]. In addition, similar to diatom extracts, long-

chain polyamines, with lengths between 5–15 units, were also found to precipitate silica in vitro but only with the existence of multivalent anions which can induce the microscopic phase separation due to their electrostatic interaction with the polyamines [55]. It has also been reported that the ratio between polyamines and sulfate anions is critical in fabricating silica with different morphologies, probably as a result of the formation of different long-chain polyamine-derived macromolecules, which affects the microscopic phase-separation process [56].

The molecular mechanisms by which the proteins and peptides control the material formation are still largely unknown [37]. However, some key insights have been offered through the ex situ and in situ crystallization studies on calcium minerals with the influence of proteins. Calcium mineral is another group of abundant biominerals found in Nature, such as the calcium carbonate and calcium oxalate. Calcium carbonate is the main component of mollusk shell, usually existing in two forms—aragonite in the nacre layer and calcite in the prismatic layer [57]. The formation of the same compound in different polymorphs is attributed to the proteins involved in the mineralization process. Two proteins, aragonitic and calcitic polyanionic proteins, were extracted from the abalone shell and were found to generate totally different polymorphs (aragonite and calcite) as well as morphologies [27,58,59]. By switching the dissolved proteins during the growth, the calcium carbonate can be switched between two polymorphs, generating different morphologies: needle-like structure under the influence of aragonitic polyanionic protein and planar structure under the influence of calcitic protein, as shown in Figs. 2B,C. The in vitro study showed, for the first time, the phase transition between calcite and aragonite catalyzed by material/phase-specific proteins [60]. In situ atomic force microscopy (AFM) study further revealed that the selective binding of proteins to different crystallographic step edges affects the crystal formation in vitro. For instance, some protein binds to **[48 $\bar{1}$]** acute edge while some binds to **[4$\bar{4}$ $\bar{1}$]** acute edge on calcite (104) surface, generating distinctive symmetries from the crystals grown without proteins. This study indicated that proteins may affect the crystal morphology by preferred binding to specific facets or steps and thus helps to understand the distinctive shell formation mechanism in oysters [61]. In addition, in situ AFM study on calcium oxalate monohydrate (COM) crystallization under the influence of osteopontin (OPN) also reveals some key roles of protein–crystal interaction in controlling the crystallization processes. COM is a mineral commonly found in kidney stones, therefore there is considerable interest in inhibiting COM mineralization through understanding its crystallization process [62]. OPN is a protein found in bone and eggshell that has been found to inhibit the mineralization of COM [63–66]. In situ AFM studies reveal that the whole OPN protein inhibits the COM growth by stopping the growth along (010) facets, as the greater step height of quadruple steps on (010) facilitates the binding of carboxylic acid and phosphate groups to the step riser and the basal plane, leading to a strong OPN–step interaction that pins the steps [64]. Since the OPN protein is rich with aspartic acid and glutamic acid, poly(aspartic acid) and poly(glutamic acid) were synthesized as mimics of OPN and were found to inhibit the growth of COM [67–69]. Later COM inhibition studies with truncated rat OPN of several highly phosphorylated, aspartic acid-, and glutamic acid-rich sequences showed similar phosphorylation-dependent inhibition manner, indicating the importance of phosphate groups in inhibiting the {100} facets of COM [70].

The crystallization studies with extracted proteins demonstrated the possibility of understanding and using material specific biomolecules to direct mineral formation as found in natural organisms in vitro. However, the extraction of protein is time-consuming and labor-intensive, and the quantity produced is usually low. Furthermore, naturally occurring proteins are usually limited in recognizing material elements that are technically important, such as semiconductors and various metals. To this end, scientists have been seeking a general biomimetic approach that can enable the discovery of biomolecules that recognize a wide range of materials and to do so in a more efficient process than that of natural evolution.

BIOMIMETIC EVOLUTION USING COMBINATORIAL DISPLAY

The biomolecules responsible for controlling the mineralization of materials vary in their composition, conformation, and origin [71–74]. The chemical and physical properties of biomolecules contribute to their unique specificity in binding on materials or mediating materials assembly [75–77]. To obtain the specific biomolecules, the most straightforward method is to extract them from the organisms, during which complicated procedures including growing organisms and purification are involved [78–80]. The extracted biomolecules usually can only be used in reproducing the materials they are originally associated with and are difficult to modify or engineer, limiting their practical uses. Recently, the in vitro evolutionary selection technique emerged as a general route for identifying biomolecules that show specific affinities to various arbitrary materials of interest, presenting an important step forward in biomimetic research [81]. The combinatorial library technique brings the natural evolution to the lab, condensing the millions of years of selection process to a few weeks to identify material-specific biomolecules. The commonly used tool box is the library of random peptides sequences displayed on phages or cell-surfaces to screen and select specific sequences showing binding affinities to the chosen material surfaces [82]. The so-called phage display (PD, schematic is shown in Fig. 3A) and cell-surface display (CSD) techniques have been well developed and used for identifying peptide sequences exhibiting affinity for various inorganics [81,83]. The libraries are generated by inserting random oligonucleotides into phage genome or bacterial plasmids. The resultant surface of organisms such as the coat proteins of phage or the outer membrane of a cell is incorporated and displayed with a random polypeptide sequence [84]. During the biomimetic evolution process, libraries are exposed to inorganic substrates and several washings can exclude nonbinders. In the case of a PD library, bound phages are eluted and amplified by reinfecting their host. The amplified phages are then used as the sublibrary in the next round of selection. This complete process is called "biopanning". Usually, three to five rounds of biopanning are repeated to select strong binders. Eventually, clones are sequenced to identify the inorganic substrate-binding peptide sequences. The identified peptide sequences show specific and selective binding affinity to the surface of a particular material. As illustrated in Figs. 3B,C, the fluorescently labeled G12-3 clone, with PIII protein fused with peptide selected against GaAs surface, specifically recognized the GaAs (the red line) but not the SiO_2 surface (the dark space area). So far,

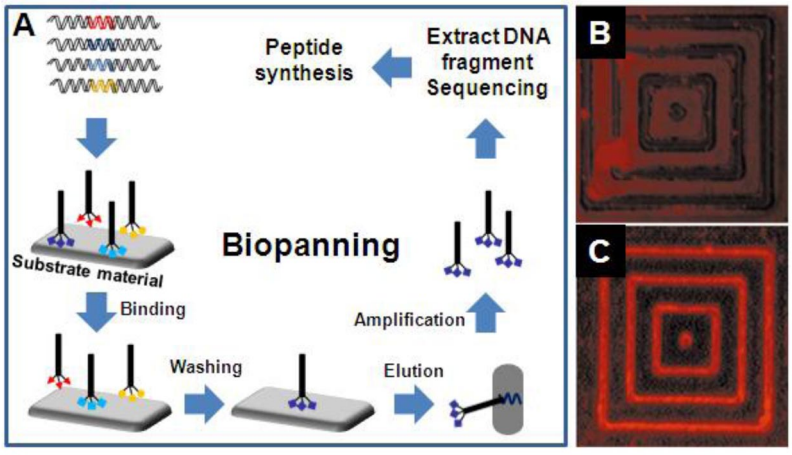

Fig. 3 (A) Schematic of phage display technique. (B) and (C) are fluorescence images related to GaAs recognition by phage. (B) Control experiment: no phage is present, but primary antibody and streptavidin-(TMR) are present. (C) The GaAs clone G12-3 was interacted with a substrate patterned with 1-mm GaAs lines and 4-mm SiO_2 spaces. The phages were then fluorescently labeled with TMR. The G12-3 clone specifically recognized the GaAs and not the SiO_2 surface [83].

specific biomolecules have been selected for various materials, such as metals (Au, Ag, Cu, Co, Ni, Pt, Pd, etc.) [85–91]; magnetic materials (CoPt, FePt, etc.) [89,92]; oxides (TiO_2, GeO_2, ZnO, etc.) [93–95]; and semiconductors (CdS, ZnS, PbS, etc.) [96].

Amino acids are the building blocks of polypeptides. The binding nature of a sequence for a surface is contributed by chemical and structural recognition mechanisms [85]. Although the molecular-level details of the peptide binding to material surfaces is still not known, generally, in terms of chemical specificity, noble metal binding peptide sequences are found to be rich in hydrophobic- and hydroxyl-group-containing amino acids such as serine and threonine [81,86,97]. Metal oxide and ionic crystal-peptide binding sequence are characteristic of basic and positively charged amino acids [81,98,99]. Binding peptide sequences to the surface of particular materials may share similar chemical functional motifs but are different in spatial distribution, which may involve placing a periodic structure of the binding domain into a lattice where their binding strength is accordingly changed [100,101]. The combinatorial display technique prevails as it provides a fast approach to discover material-recognizing biomolecules efficiently even without detailed knowledge of the binding nature.

BIOMIMETIC SYNTHESIS USING SPECIFIC PEPTIDES

Biomimetic synthesis of nanostructures of technically important materials has been studied extensively during the past decade due to their possible applications in sensing, catalysis, imaging, cancer therapy, and environmentally friendly synthetic routes [102,103]. The synthesis of nanostructures, especially nanocrystals (NCs) of different sizes and morphologies of various materials such as semiconductors, metals, and minerals, using the conventional chemical approaches has achieved great success after tens of years of development [8,9]. However, elaborate control over the NCs was mostly achieved at rigorous synthetic conditions such as high temperatures, organic solvents, or high pressures, etc. In addition, the synthetic control by the chemical approaches is usually achieved through the trial-and-error process by testing different conditions and chemicals used therein. For example, the colloidal NC growth is believed to be a kinetically controlled process, in which the low-energy facets persist while the high-energy facets vanish, leading to a particular resultant shape enclosed by the low-energy surfaces [8,9]. Therefore, it is important to identify a surfactant that can specifically bind to a particular crystal facet, lowering its surface energy, and lead to NCs with well-controlled shapes. In conventional chemical synthetic routes, shape control is achieved by employing various surfactants identified through the trial-and-error process. On the other hand, biomolecules can be specifically selected to recognize a chosen surface through a biomimetic molecular evolution process and therefore have the potential to be explored for generalizing the nanomaterial synthesis process with predictable control. It has therefore triggered many efforts in using surface-specific biomolecules in directing the formation of NCs [85–95].

Earlier work on using selected biomolecules to mediate the formation of various technically important inorganic NCs have demonstrated some exciting results (Figs. 4A–D). ZnO (Fig. 4A) and TiO_2 (Fig. 4B) nanoparticles (NPs) have been synthesized in solution with ZnO-1 and Ti-1 peptides, which were selected against the ZnO particle surface and the rutile TiO_2 single-crystal surface, respectively [93,95]. In addition, when ZnO-1 was immobilized on gold plate via an added cysteine onto the C terminus, it could induce the nucleation and formation of flower-like ZnO NCs on Au plates (Fig. 4A) [95]. Au NCs in Fig. 4C was synthesized with A3-Flg peptide where the A3 is selected with PD technique and found to bind to both Ag and Au. Flg is a commonly used biomolecular recognition domain for tagging proteins and, in this case, fused to the A3 to stabilize the Au NCs on the substrate through the anti-Flg linker [87]. Another work by Naik et al. demonstrated that well-shaped Ag NPs, as shown in Fig. 4D, can be synthesized with AG4 peptide, selected against the Ag crystal surface, without reducing agent. It was proposed that the free peptide AG4 binds to the {111} facets of Ag nuclei and provides a chemically reducing environment around the cluster, thereby allowing further accelerated reduction of Ag ions at the interface between peptide and metal [86].

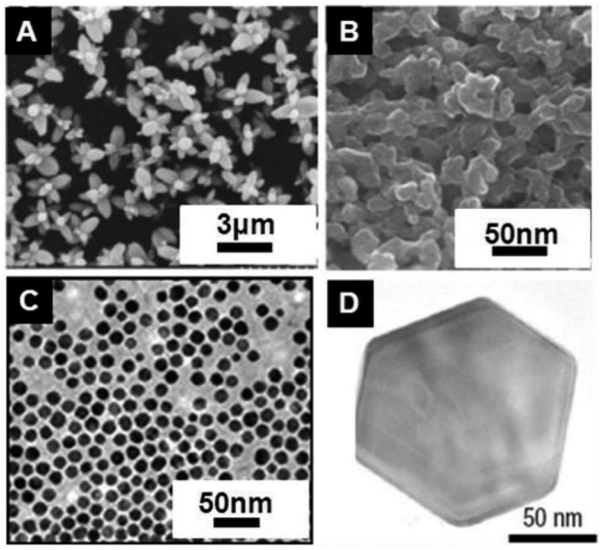

Fig. 4 (A) TEM image of the solid particles precipitated by the GGGSC-conjugated ZnO-1 peptide [95]. (B) Secondary electron image of titania particles generated in the presence of Ti-1 peptide [93]. (C) TEM image of Au NPs synthesized by the reduction of $HAuCl_4$ by A3-Flg peptide [87]. (D) TEM image of Ag NPs obtained using AG4 peptide [86].

The above-mentioned success indicated the feasibility and great potential in using specific peptides to direct the formation of various materials, although the degree of synthetic control has yet to be improved. Our group has recently demonstrated morphology- and size-controlled Pt and Pd NCs using specific peptides identified using M13 PD library [90,91,97]. Notably, we have demonstrated the synthesis of ultra-small Pt NCs with atomic layer control over the size and Pt NC multipod structures with crystallographic control in growth directions, demonstrating the potential to tailor material structures with molecular-level precision using biomolecules.

Our studies on Pt NCs have demonstrated that the synthesized free peptide molecule Thr-Leu-His-Val-Ser-Ser-Tyr (TLHVSSY), although identified as part of M13 protein through the biopanning process, is able to bind to the Pt NC surface and thus can perform as stabilizer to regulate crystal nucleation and growth, and therefore control both the morphology and size of the resulting Pt NCs. Our studies were carried out in aqueous solution at room temperature, using potassium tetrachloroplatinate (K_2PtCl_4) as the precursor and sodium borohydride ($NaBH_4$) as the reducing agent. Liquid-chromatogram and mass spectrometry (LC-MS) studies on peptide molecules before and after reaction confirmed the integrity of peptides under the synthetic conditions. The tightly bound peptides on the NC surface greatly slows down the growth rate of the NCs and prevents NCs from coalescence, which has allowed us to achieve excellent control over the size of ultra-small Pt NCs (Fig. 5) in a burst nucleation growth. Figures 5A–C show the TEM images of NCs taken out from reaction at 10 s, 60 s, and 5 h. High-resolution TEM (HRTEM) images in Figs. 5D–F show that the atomic layers of Pt NCs in [111] direction, as marked by the arrows, grow from 7 to 12 layers, and finally to 16 layers. With this approach, ultra-small Pt NCs of variable sizes below 5 nm can be synthesized in a highly controllable fashion. The schematic in Fig. 5G shows that the peptide binds to all facets on the surface of NCs, leading to the formation of nanospheres [97]. Similar work on Pd NCs has demonstrated the tunable NC sizes from 2.2 to 6.6 nm using Pd-specific peptides as capping agents [91].

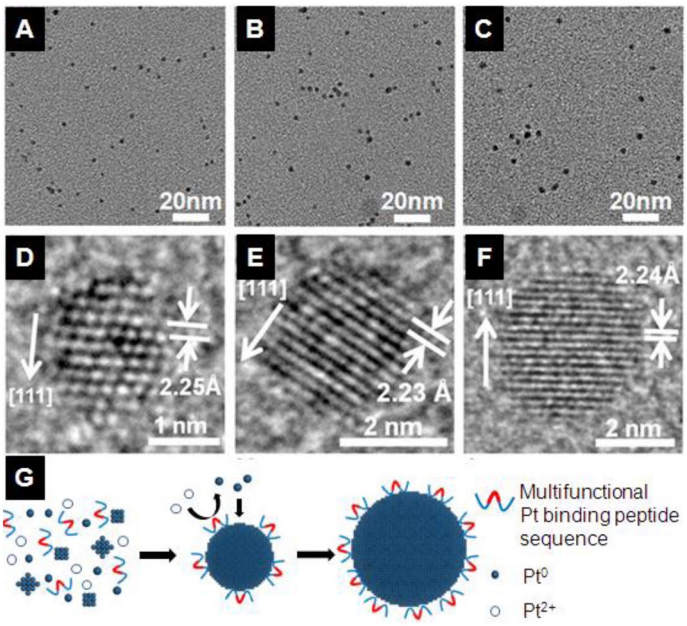

Fig. 5 (A–C) TEM images of reaction samples taken at 10 s, 60 s, and 5 h. (D–F) High-resolution images of the samples in (A–C), respectively. (G) is the schematic of the peptide-directed Pt NC growth [97].

Furthermore, we have also achieved uniform multipod structures of Pt NCs through slow injection of $NaBH_4$, which enhances the growth stage of Pt NCs and allows for the control of the peptide to further reveal its effects on NC crystal growth (Fig. 6). It is shown that lower TLHVSSY peptide concentration led to the formation of long pods (Fig. 6A), with pod length gradually decreasing with increasing peptide concentrations (Figs. 6B,C). At sufficiently high peptide concentrations, all Pt NCs appear spherical (Fig. 6D). Lattice analysis with HRTEM further revealed the single-crystal nature of the multipod Pt NCs, and that most of the pods grew along the [111] direction with the d spacing of 2.26 Å correspondingly. Some pods were found to grow along [100] directions. No pods growing along the [110] direction were observed. Our studies indicate that the Pt-binding peptide preferentially bind to {110} facets. At low peptide concentrations, the peptide molecules selectively bound to {110} facets and lowered their surface energy, and therefore promoted the growth along the [111] and [100] directions. At higher peptide concentrations, as there were more than enough peptide molecules to cover the {110} facets, excessive peptides also bound to {111} and {100} facets, leading to convergence of surface energies. This can explain the resulting less-distinctive multipod structures (Figs. 6F,G). At sufficiently high peptide concentrations, when there were enough peptide molecules to bind to all facets, growth along all directions was inhibited, leading to the spherical morphology (Figs. 6H,I). These studies suggest that peptides can discern the fine structural differences among different crystal facets and paved the way for predictable morphology control of NCs and other nanostructures using specifically engineered peptides.

In addition to synthetic efforts, scientists are searching for a possible mechanism by which peptides regulate the crystal formation process. It is believed that the properties of amino acids, the building blocks of peptides, play important roles in the NC synthesis. For example, a systematic study on the effects that 20 individual natural α-amino acids may have on producing Au NCs suggested that histidine (H) exhibits the strongest binding ability to Au while tryptophan (W) shows the strongest reduction ability to reduce Au precursor [104]. It has been suggested that not only the types of amino acids, but also the sequence and interference between neighboring amino acids might have a profound effect

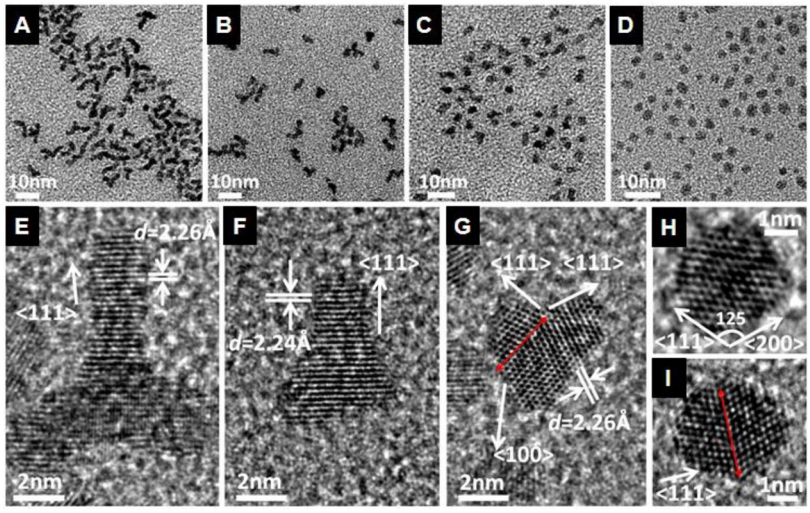

Fig. 6 (A–D) TEM images of Pt NCs from reactions with 22.5, 50, 100, and 250 μg/ml peptide concentrations, respectively. (E–G) HRTEM images of Pt NCs from reactions with 22.5, 50, and 100 μg/ml BP7A, and (H,I) crystals obtained from the reaction with 250 μg/ml BP7A. The double-headed arrows indicate the twin planes [90].

on the nucleation of NCs, due to the modified complexation behavior between metal ions and different amino acids [104]. These studies helped to understand how peptide molecules may have facilitated the nucleation and growth of NCs. But there is still a long way to go to entirely unveil the underlying mechanism of biomolecule-mediated materials formation. A significant amount of both experimental and theoretical efforts are expected to eventually solve the mystery and to eventually allow for the rational design and engineering of biomolecules that can lead to the formation of complex hierarchical material structures with tailored functionalities from the "bottom–up" scheme.

APPLICATIONS OF PEPTIDE/PROTEIN MATERIAL-SPECIFIC BINDING BEHAVIOR

The specific interaction between the biomolecule and material surface has inspired applications including sensing, assembly, catalysis, etc. (Fig. 7) [105]. The molecular sensing application based on the peptide affinities has been explored with single-wall carbon nanotubes (SWNTs) field-effect transistor (FET) sensors. FET is a commonly used device for sensing applications due to the real-time electronic response [106,107]. SWNT-FET with peptide-modified polymers was fabricated for the detection of heavy-metal ions such as Ni^{2+} and Cu^{2+}. It was found that the sensitivity of the FET is dependent on the binding specificity between ions and peptides [108]. A later work used a bifunctional peptide which contains a SWNT-binding region and a small-molecule-binding region and fabricated the SWNT-FET for the trinitrotoluene (TNT) detection [109]. Along the similar line, a colorimetric sensor was developed to detect a collection of metal ions (Pb^{2+}, Hg^{2+}, Co^{2+}, and Pd^{4+}) using bifunctional peptide synthesized Au NCs [110]. The addition of different metal ions will change the surface-plasmon-resonance behavior of the Au NCs due to the complexation between metal ions and peptide, demonstrating different colors [110]. Multifunctional peptides have also been explored to create functional hybrid structures by facilitating the assembly of multicomponent material structures, such as assembly of NCs [111]. The appropriate design of peptide molecules has been demonstrated to simplify the synthesis of multicomponent NCs while enhancing the performance. For instance, by combining two functional peptide sequences into one peptide molecule, A3 domain for Au binding and Flg domain for Pd/Pt binding ability, Au NCs with attached Pd clusters were synthesized in a two-step procedure [112]. It represents a general approach to achieve hybrid structures by programming the amino acid sequence. With

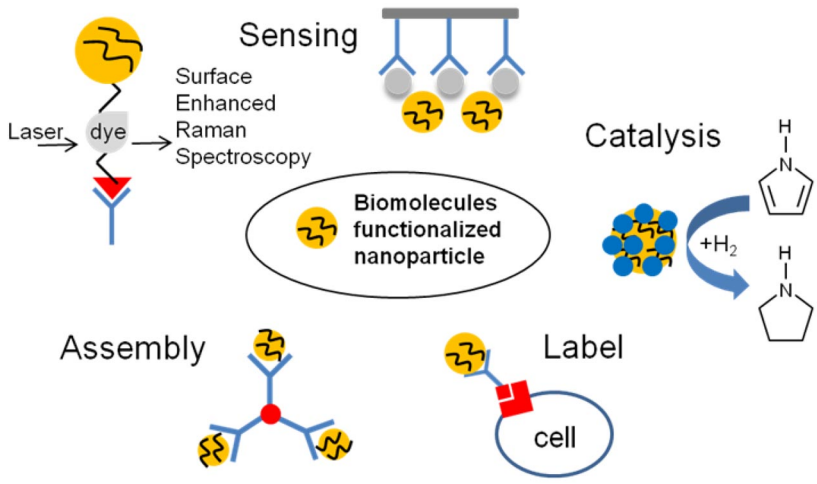

Fig. 7 Schematic of possible applications of biomolecule-functionalized nanomaterials.

the same method, CdS NCs coated with Pt clusters were also synthesized as a mimic of the enzyme to enhance the photoinduced nitrate reduction activity, which benefits from the fact that the peptide-templated NC configuration eliminates the need for electron mediators and exogenous sacrificial electron donors, both of which must be considered if one uses enzyme for the nitrate reduction [113].

To take a step further, by incorporating the binding peptides into various protein structures, one has been able to create material assembly structures in larger scale in one, two, and three dimensions by employing the self-assembly nature of the biomolecules [32,114–123]. Stable protein 1 (SP1) is a protein that can resist high temperature and assemble into a lateral organization of ultra-dense docking arrays. It has an inner core diameter of 2–3 nm and a total of 11 nm in diameter. A six-histidine tag was incorporated into the N termini of the SP1 (6His-SP1) to enable binding to the ligand Ni-NTA on Au NPs. By forming a docking array, the SP1 can direct the formation of Au NP arrays with controllable distance between particles, as shown in Figs. 8A,B [117]. Wild-type SP1s can assemble into hexagonal patterns so they can also be potentially used for the two-dimensional (2D) assembly of Au NPs, as shown in Figs. 8C,D [116]. Other proteins including ferritin-like protein cages, CCMV and CPMV, as well as chaperonin proteins have been used for the 2D assembly [118–120]. Another potential candidate for the 2D assembly is crystalline bacteria cell-surface layer (S-layer), which is the monomolecular array composed of a single protein or glycoprotein species and represents the simplest biological membrane. It assembles into a 2D array to enclose the whole cell, which inspired the biomimetic use in assembling NPs such as CdSe, Au, and CdSe/ZnS core/shell quantum dots (QDs) [121,122]. Peptide-specificity-assisted material assembly in 3D has been demonstrated with liquid-crystal formation of M13 phages. M13 phage with PIII minor coat protein inserted with ZnS binding peptide sequence A7 was engineered, amplified, and used to precipitate QD NCs which were attached at the end of the phages. By employing the liquid-crystalline behavior of the phage, the phage/QD hybrid structure assembled into a 3D highly oriented and self-supporting film with thickness up to 15 μm, with the attached ZnS QDs assembled in multilength scale, as shown in Figs. 8E,F [32]. In addition, electrospinning was also found to be useful in aligning the long axis of the rod-shaped phages parallel to the spun fiber axis, thus potentially helping to align NCs in 3D [123].

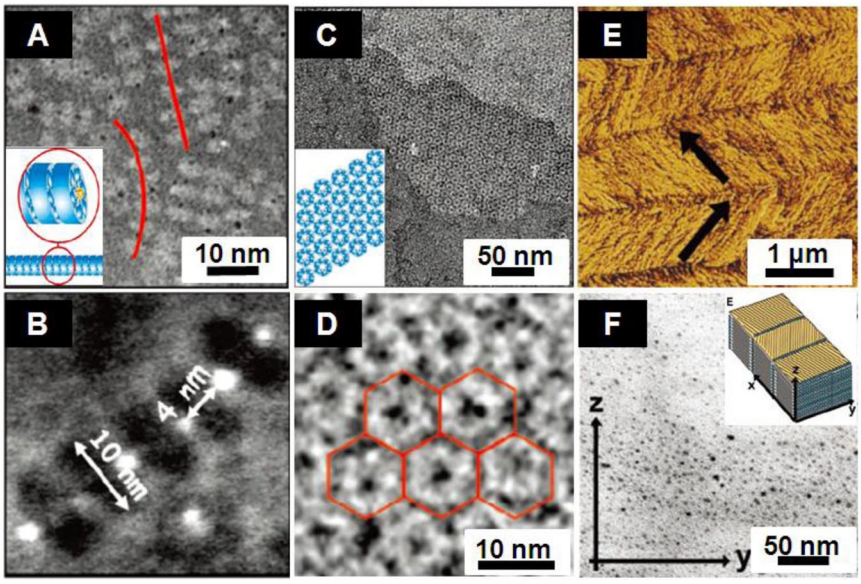

Fig. 8 (A) TEM image (phosphotungstic acid staining) of 6His-SP1-Au NP (GNP) chains (marked by red lines). Inset: Schematic of the 6His-SP1 chain. (B) High-angle annular dark field scanning transmission electron microscopy (HAADF-STEM) image of 6His-SP1-GNP chains with 4 nm GNP separation (vanadium staining) [117]. (C) TEM image of ordered wild-type (WT) SP1 arrays (one and two monolayers). Inset: Schematic representation of a WT SP1 array. (D) Higher magnification of highly ordered and continuous array of WT SP1 [117]. (E) AFM image of the film surface. The phage forms parallel aligned herringbone patterns that have almost right angles between the adjacent director (arrows). (F) Low-resolution TEM image of film viewed in the *y*–*z* direction, showing ZnS NCs. Inset: schematic structural diagram of the A7-ZnS composite film [32].

SUMMARY

Nature serves as a good example for producing functional structures from the bottom up through genetic programming, using biomolecules like proteins. The biomimetic approach offers the potential to create material structures with complexity and precision of unprecedented level, benefitting from the inherent recognition properties and the self-assembly nature of the biomolecules. Specifically, the selective interactions between proteins/peptides and materials have inspired various ideas of producing and assembling nanoscale materials into complex and hierarchical functional systems, and offer the possibilities of realizing the "bottom–up" approach for future applications. Over the past decade, biomimetic research has rendered tremendous knowledge and encouraging results on understanding and employing the specific interaction between proteins and materials. Biomineralization studies with proteins/peptides extracted from the organisms have confirmed the ability of material-specific proteins to control the precipitation and phase transformation of inorganic materials, both in vivo and in vitro. In situ imaging studies of the mineralization suggested that proteins interact with specific crystallographic steps or planes to control the crystallization process. Furthermore, with the advances in molecular biology, we now can mimic the natural evolution process in lab to evolve biomolecular recognitions to arbitrary material surfaces using the combinatorial display techniques. This has allowed scientists to expand the use of biomolecules beyond natural minerals to technically important materials such as semiconductors and catalytic materials. Together with the molecular biology techniques, these peptides may be expressed in various protein structures to guide the hierarchical assembly of materials from nano- to micro-, and even to the macroscale. Despite all these achievements, however, we are still far from totally unveiling the mysteries of the biomolecular specificity. This has limited further advances of bio-

mimetics. For example, so far the biomimetic material synthesis is still lack of predictable and programmable control over material structures. To eventually achieve the structure complexity and superior function of material systems as observed in Nature, significant collective efforts in biology, chemistry, materials, biophysics, as well as simulations are necessary to understand the fundamental mechanisms by which the biomolecules control the formation and assembly of materials.

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