Biotemplated synthesis of inorganic materials: An emerging paradigm for nanomaterial synthesis inspired by nature

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Abstract

Biomineralization, the process by which biological systems direct the synthesis of inorganic structures from organic templates, is an exquisite example of nanomaterial self-assembly in nature. Its products include the shells of mollusks and the bones and teeth of vertebrates. By comparison, conventional inorganic synthesis techniques provide limited control over inorganic nanomaterial architecture. Inspired by biomineralization in nature, over the last two decades, the field of biotemplating has emerged as a new paradigm for inorganic nanomaterial assembly, wherein researchers seek to design novel nanostructures in which inorganic nanomaterial synthesis is directed from an underlying biomolecular template. Here, we review the motivation, mechanistic understanding, progress, and challenges for the field of biotemplating. We highlight the interdisciplinary nature of this field, and survey a broad range of examples of bio-templated engineering: ranging from strategies that exploit the inherent capabilities of proteins in nature, to genetically-engineered systems that unlock new capabilities for self-assembly with biomolecules. We illustrate that the use of biological materials as templates for inorganic self-assembly holds tremendous potential for nanomaterial engineering, with applications that range from electronics and energy to medicine.

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1. Introduction

Biological systems are exquisite examples of self-assembly in nature. Examples of biological self-assembly can be found throughout a vast hierarchy of length-scales, from the assembly of proteins and nucleic acids that form nanoscale viruses, to the development of complex anatomies in multi-cellular animals. By comparison, the ability of materials scientists to design self-assembling architectures with multi-scale precision remains quite limited. However, the possibility of engineering novel functional materials with structures and resultant functions that are precisely tailored at the nanoscale excites tremendous interest that continues to grow. The field of biotemplating emerged from the union of these two pursuits: the study of biological self-assembly in nature, and the development of nanostructured inorganic materials with properties exceeding their bulk counterparts. In nature, through the process of biomineralization, a wide variety of biological systems direct the growth of complex hierarchical inorganic mineral structures whose crystal phases and multi-scale architectures are precisely dictated by an underlying template composed of biomolecules. Drawing inspiration from biomineralization in nature, in the past two decades, biotemplated synthesis of inorganic nanostructures has burgeoned as a field, wherein researchers seek to engineer new inorganic materials whose structures are directed by the unique recognition and self-assembly properties of biomolecules.

In this review, we provide a broad overview of the motivation, mechanistic understanding, progress, and challenges for biotemplated synthesis of inorganic materials. To this end, we begin in Section 2 with a brief overview of the nanomaterials field, together with a survey of its potential applications, and current challenges in nanomaterials synthesis. The remainder of the review focuses on biotemplating of inorganic materials, organized from least-engineered to most-engineered strategies (illustrated in Fig. 1). Thus, in Section 3, we consider biomineralization as it occurs in nature, assess current models for its underlying mechanism, and preview the potential for mechanistic understanding of biomineralization to be applied toward nanoscale control of inorganic material synthesis. In Section 4, we review recent progress in using proteins as templates for exercising control over nanoparticle structure and organization. In Section 5, we review advances in peptide systems as molecular recognition motifs for directing nucleation of nanoparticles and controlling their growth and assembly at various length-scales. In Section 6, we provide examples in the literature in which peptide molecular recognition and protein self-assembly are combined into protein-peptide fusion systems that direct inorganic material synthesis. Finally, in Section 7, we offer a perspective on the current challenges facing the field of biotemplating, including practical synthetic challenges, as well as environmental and human health concerns.
As we intend to illustrate in this review, the global market for nanomaterials is broad and growing as nanomaterials become more prevalent in the biomedical, semiconductor, and energy fields. The precise structure of nanomaterials dictates their properties, and nanoscale materials have shown promise in applications where bulk material properties are insufficient for the task. The development of scaffolds capable of reproducibly templating inorganic nanomaterials will be essential to the development of next generation materials, because the unique properties of nanomaterials are highly dependent on their size and precise structures. Drawing inspiration from biomineralization in nature, researchers are increasingly investigating the use of proteins and peptides in inorganic nanotemplating. Protein and peptide based systems enable architectural control over nanomaterial structure that is difficult to obtain using conventional synthetic techniques. The multi-faceted potential for biotemplating includes shape and size control over nanoparticles, chemo-selection of specific inorganic species, spatially directed localization of nanomaterials, and exquisite 3D self-assembly capabilities (Fig. 2). In addition, the mild
conditions associated with protein and peptide based templating is highly advantageous, especially in light of the current uncertainty about the environmental impacts of nanomaterials. While peptide-based templating of inorganic nanomaterials is still an immature field, interest has risen tremendously in recent years and should continue to grow in tandem with the rapidly developing market for novel nanomaterials.

2. Nanomaterials: prospects, applications, and challenges

2.1. Nanomaterials market forecast

As a result of their size, nanomaterials frequently possess unusual physical (structural, electronic, magnetic, and optical) and chemical (catalytic) properties. The global market for nanomaterials, valued in the billions of dollars, is massive and far-reaching with applications ranging from medicine to battery production [1,2]. The pharmaceutical, cosmetic, food, textile, automotive, and construction industries all sell a number of consumer products with incorporated nanomaterials, and academic and industrial research in the area of nanomaterials has exploded over the past two decades [2–4]. Nanomaterials design and synthesis represents an inherently interdisciplinary field of research, bringing together theoreticians and experimental researchers from diverse communities including organometallics, colloids, catalysis, soft matter, biology, polymer chemistry, and electrical engineering. The intersection between diverse scientific fields has led to some surprising hybrid technologies, such as lithium batteries templated by viruses [5], and many manufacturing challenges in electrical engineering may ultimately be solved using protein-based systems.
2.2. Applications of nanomaterials

2.2.1. Biomedical applications

Nanomaterials have enjoyed widespread popularity in the biomedical industry. Nanomaterials can be used in a variety of biomedical contexts, including drug delivery, biosensing, and imaging [6–12]. Cancer diagnostics and targeted drug delivery to tumors are among the most well studied applications of nanomaterials in healthcare [7,12]. Networks of gold nanoparticles and bacteriophage have shown promise as biological sensors and cell-targeting agents [9], and composite organic-inorganic nanoparticles have shown utility as Raman labels for tissue analysis [10]. Due to their small size, versatile surface functionalization, and useful imaging properties, gold and silver nanoparticles have been widely studied for targeted treatment of tumors, including pancreatic tumors [12]. However, the biocompatibility of inorganic nanoparticles is shape, size, surface chemistry, and cell-type dependent [13,14]. Thus, the physical and chemical properties of nanoparticles must be carefully considered and controlled for biomedical applications.

2.2.2. Semiconductor applications

Since the precise arrangement of metals and metal oxides into nanostructures influences the resulting optical and electronic properties, the role of nano-architecture has been widely studied in the semiconductor industry [15]. For example, the electronic properties of nanoparticles for field emission applications has been a topic of extensive research [16,17]. Peptides with semiconductor binding specificity for directed nanocrystal assembly have been identified to facilitate the production and study of complex inorganic nanostructures [18].

2.2.3. Catalysis and electrochemistry applications

Nanomaterials have also been rapidly adopted in the electrocatalysis field. Catalysts can be highly clustered on the surface of nanomaterials, leading to an electrocatalytically active interface that can be exploited in fuel cells and other electrocatalysis-based devices. Moreover, the size- and geometry-dependent electronic properties of nanoparticles present the opportunity for developing catalysts with superior properties. Nanodevices currently used in electrocatalysis include carbon nanotubes [19–21], platinum nanoparticles [19], palladium nanoparticle-cored dendrimers [22], and colloidal gold nanoparticles [23]. Given the importance of materials development to the success of next generation energy technologies,

![Fig. 3. Biomineralization in nature produces diverse hierarchical structures of inorganic materials. (a) SEM images of cell walls from diatoms. Diatoms exhibit a wide-range of multi-scale structures containing biomineralized silica, such as the cell walls from *Thalassiosira pseudonana* (left) and *Stephanopyxis turris* (right). (b) SEM images of the microstructure of silica cell walls in *Gyrosigma balticum* (left) and *Ditylum brightwellii* (right). (c) SEM image of the nacreous layer in the mollusk shell of *Atrina rigida*. The nacreous layer forms the inner structure of the mollusk shell and consists of layered tablets of mineralized aragonite. (d) SEM image of the prismatic layer of the mollusk shell from *Atrina rigida* after partially dissolving inorganic minerals. The prismatic layer contains a fibrous matrix of proteins that direct mineralization of calcite. Images from (a and b) adapted with permission from Ref. [163]. Copyright 2008 American Chemical Society. Images (c and d) were reproduced with permission from Ref. [39] with permission of The Royal Society of Chemistry.](image-url)
the range of nanostructures used for electrocatalysis will likely expand as more complicated architectures are produced and the relationship between nanostructure and activity becomes better understood.

2.2.4. Synthetic challenges in nanomaterial chemistry

Although nanomaterials excite broad interest in the scientific community, their synthesis presents both practical challenges and environmental concerns. Standard inorganic syntheses used to produce inorganic nanomaterials generally involve the use of harsh conditions and solvents. In many cases, organic solvents and ionic liquids are used to control the composition, crystal structure, and assembly properties of nanomaterials, often under elevated temperature and pressure conditions [24–26]. The disposal of synthesis byproducts and used solvent, in addition to the energy required to maintain elevated temperature for large-scale reactions, represents an environmental burden that should be addressed as commercial scale production of inorganic nanoparticles becomes increasingly common [27,28]. Moreover, the ability to design nanomaterials with complex architectures using standard inorganic synthesis techniques is currently limited, so alternative methods are needed to meet the demand for new nanomaterials with novel, structure-dependent properties [29].

2.2.5. Biotemplating for “greener” nanomaterial synthesis

While material synthesis routes that utilize milder conditions are increasingly being pursued and utilized [28,30,31], many researchers have taken inspiration from nature for greener inorganic materials synthesis [32–34]. Natural biomineralization produces complicated, tunable nanostructures under environmentally friendly conditions. By utilizing naturally occurring or naturally inspired templates, nanomaterials may be produced without the use of harsh solvents or high temperatures. In addition, natural inorganic nanocomposites possess a wide variety of complex 3D architectures not currently accessible through conventional chemical techniques, making their use appealing from both an environmental and a design perspective.

3. Biomineralization

3.1. Biomineralization in nature

Biomineralization is a widely observed process whereby biological organisms direct the assembly of inorganic mineral structures [35]. In contrast to the growth of inorganic materials typically achieved in laboratories, this directed assembly in biological systems often gives rise to elaborate architectures with hierarchical levels of complex spatial organization, spanning nanoscale control of crystal patterning and growth up to meso- and macroscale morphological patterns [36,37]. Products of biomineralization can be observed, for instance, in the intracellular micro-skeletons of unicellular radiolarian,
the morphologically diverse frustules that enclose diatoms, the shells of mollusks, and the bones and teeth of vertebrates [37–39] (Fig. 3). Although widely varying in form and biological origin, biomineralization products are unified by their emergence from organic templates, wherein macromolecular scaffolds and binding motifs are hypothesized to orchestrate the patterned nucleation and controlled subsequent growth of inorganic minerals into higher-order assemblies [40,41]. Beyond its obvious biological significance, the elegant multi-scale control of inorganic structures achieved in biomineralization, which is unrivaled by conventional synthetic materials chemistry, provides inspiration for novel pathways to materials design and synthesis through biomimetic templates.

3.2. Mechanisms of biomineralization

Although biomineralization in nature proceeds through a complex interplay of physiochemical and biological processes at a hierarchy of length-scales, at the most basic level, biomineralization is initiated by organic scaffolds capable of exerting precise control over spatial localization of mineral nucleation and growth [40]. Thus, a natural starting point for the development of a rational framework for design of inorganic nanostructures via biomimetic templates is understanding the fundamental principles of crystal nucleation and growth. A deep understanding of the fundamental physio-chemical processes that govern biomineralization may provide key insights into the design of nanostructured materials that are assembled from the bottom-up.

3.2.1. Classical nucleation theory

The most commonly consulted theoretical framework for understanding growth of crystals from supersaturated solutions is provided by classical nucleation theory [42,43] (Fig. 4). Within classical nucleation theory, crystal growth is viewed as a thermodynamically-driven process governed by the balance of free energies between the ionic or molecular precursors in supersaturated solution, the species in the bulk of the crystal, and the excess free energy at the crystal/solution interface (the interfacial energy). Crystallization of atoms or molecules from the supersaturated solution is driven by the lower free energy in the bulk crystal, but growth of small crystals is kinetically hindered by the excess interfacial energy relative to the free energy in solution. Since the surface area grows with the crystal radius \( r \) as \( r^2 \), whereas the crystal volume grows as \( r^3 \), the interfacial energy dominates for small particles, and the bulk free energy prevails for sufficiently large particles. This gives rise to a dependence of the crystal free energy on the radius that exhibits a local maximum at a corresponding critical nucleation radius. Spontaneous density fluctuations in the supersaturated solution due to thermal fluctuations yield crystal aggregates with varying sizes. Those with sizes below the critical radius are thermodynamically unstable with respect to growth, and those exceeding the critical radius overcome the nucleation barrier and proceed to grow bulk crystals through addition of atoms or molecules. During the course of subsequent crystal growth through the creation of atomic steps, the formation of new crystal step edges also presents a free energy barrier, which gives rise to a critical step size for crystal growth propagation from the existing crystal surface [36].

3.2.2. Controversy over classical mechanisms

Although appealing in their simplicity, the principles of classical nucleation theory have been vigorously challenged in the last two decades by experimental observations of mineral crystallization, general theoretical considerations, and computer simulations [42]. The phenomenology predicted by the classical theory relies on the assumptions that aggregates of ions or molecules form nuclei with internal energies comparable to the bulk crystal, and that the notion of a phase interface in such aggregates is well-defined in terms of classical thermodynamics. Theoretically, such assumptions may be regarded as questionable at nanoscopic length-scales, and recent experimental observations suggest a more complex reality. For instance, multiple studies of crystallization mechanisms in calcium carbonate reported the existence of (meta)stable aggregates, “pre-nucleation clusters,” that serve as precursors to crystal nucleation, rather than nucleation and growth by direct addition of ions [44–46]. Pre-nucleation clusters also have been observed in calcium phosphate systems [47]. Moreover, it has been proposed that in calcium carbonate systems, nucleation of an amorphous precursor phase represents an alternative pathway to crystallization in addition to direct nucleation of the crystal phase [48,49]. Such observations contrast with the classical theory, wherein crystal nucleation proceeds through the accretion of individual ions or molecules, and the crystal structure resembling the bulk is nucleated directly, rather than through maturation of metastable intermediates.

However, mounting evidence suggests that crystallization cannot be described by a single universal pathway. Other studies of organic template directed crystallization of calcium carbonate [43] and glucose isomerase [50] have demonstrated crystallization in accordance with classical nucleation theory, challenging the notion that pre-nucleation clusters and metastable intermediates represent a universal pathway to mineralization. Indeed, in a recent study on crystallization of magnetite, it was proposed that direct nucleation from ionic precursors, pre-nucleation clustering, and nucleation of metastable intermediates together form a system of possible crystallization mechanisms that are available to supersaturated mineral solutions (middle panel of Fig. 4). The selection of these is dictated by the mixture phase diagram and the energetic landscapes of aggregates, metastable phases, and the bulk crystal [51,52]. Moreover, nucleation can be bypassed during mineralization without invoking pre-nucleation clusters or metastable intermediates under solutions conditions near the critical point for liquid-liquid spinodal decomposition. In the vicinity of the critical point, the liquid-cluster interfacial energy vanishes, leading to unstable density fluctuations at all length scales, and broad distributions of cluster sizes that form without an apparent nucleation energy barrier [53]. Similarly, a recent molecular dynamics study predicted that amorphous pre-
cursors will arise in calcium carbonate mixtures when the degree of supersaturation coincides with the liquid-liquid coexistence regime [53]. These studies indicate that the crystallization pathway can be modified by the regime of the thermodynamic phase diagram within which the mixture resides. Together, these findings paint a picture of crystal nucleation and growth that is richer and more complex than that provided by classical theory, with a network of possible pathways replacing a single universal nucleation mechanism.

Despite controversy over crystallization mechanisms, it is becoming increasingly accepted that the formation of precursor aggregates and intermediate metastable states represent important possible mechanisms for biomineralization [42,54]. However, many mechanistic studies have emphasized mineralization from homogeneous saturated solutions, where the role of organic interfaces in heterogeneous nucleation, such as that which occurs in biological systems, cannot be probed. From a classical perspective, organic interfaces may facilitate and direct mineralization by reduction of interfacial energy of nucleated crystals, thereby reducing the critical nucleus size. Such a model for mineralization at organic interfaces has been validated in studies involving calcium carbonate crystallization on alkyl thiol self-assembled monolayers, where direct manipulation of interfacial energies by modulation of the self-assembled monolayer leads to corresponding control over calcium carbonate crystallization consistent with classical nucleation theory [43]. Recently, experiments have also revealed the capacity of biomimetic organic matrices with negatively charged acid groups to localize the nucleation of metastable amorphous calcium carbonate through enhanced concentration of calcium ions in the vicinity of acid-bearing sites [55]. This work demonstrates that biomimetic matrices are capable of organizing the spatial localization of crystal precursors in a supersaturated solution, suggesting that organic interfaces are capable of mediating controlled mineralization through non-classical mechanisms. These studies provide emerging evidence that organic interfaces can direct both spatial organization and mechanistic pathways of inorganic mineralization.

3.2.3. Biomineralization mechanisms: insight for nanoscale engineering

A detailed mechanistic understanding of nucleation and growth processes in biomineralization is not only of fundamental interest, but also may provide a predictive framework for the rational design of novel synthetic nanomaterials. Recent work provides indications of the potential for a mechanistically-informed biomimetic approach toward engineering nanostructures with structural control that surpasses conventional materials chemistry. For instance, in a simple model system for organic templating involving self-assembled organic thiol monolayers, cooperative reorganization of the underlying organic template was strongly coupled to the ability of the template to direct the growth of crystals with preferred crystal orientation [56]. Thus, the flexibility of organic templates to reorganize may provide an important design parameter for controlling the crystal orientation of nanostructures. Observations of biomineralization mechanisms in vivo also may provide direct insights into design of templating systems in vitro. For example, inspired by the observation of ferrihydrite precursors in bacterial magnetite mineralization, an in vitro peptide-based scheme was developed for crystallizing magnetite with superior control over particle shape and size compared to traditional coprecipitation of iron(II) and iron(III) [57]. Similarly, following identification of a matrix protein implicated in the directed mineralization of calcium oxalate needles in Musa spp. (banana), a highly conserved peptide sequence in the matrix protein was utilized to direct the growth of similarly elongated crystal fibers in vitro [58]. Although our understanding of underlying mechanisms for organic template directed mineralization remains in a nascent stage, these findings reflect a growing potential to harness mechanistic understanding of biomineralization in the development of nanostructures with unprecedented bottom-up architectural control.

4. Natural and recombinant protein inorganic templating

Proteins in nature offer a multitude of compelling examples of their ability to direct the synthesis of complex, hierarchical structures through biomineralization. Moreover, outside of biomineralization, proteins, themselves, are well-known for their ability to self-assemble into a variety of organizations not easily achieved by synthetic organic materials. However, with the development and increasing sophistication of recombinant techniques, proteins are no longer limited purely to those found in nature, and a vast design space exists for developing protein technologies with self-assembly capabilities not accessible synthetically or in nature. Consequently, inspiration from natural biomineralization scaffolds combined with opportunities through recombinant protein technology inspires researchers to pursue a variety of novel protein systems as templates in nanoparticle engineering.

4.1. Organic scaffolds in abalone shells: inspiration from nature

Some of the earliest evidence of the capacity of proteins to self-assemble nanostructures with properties exceeding those of synthetic systems arose from biomineralization studies on abalone shells. Abalone shells comprise a multi-lamellar hierarchy of highly-oriented, interdigitating calcium carbonate crystals that are separated by thin organic lamellae [59]. Their nano- and micro-structure confers a tensile fracture toughness that is ~3000 times that of synthetically produced calcium carbonate crystals [60]. The unique properties and multi-scale structure are intimately connected to the proteins that constitute the organic phase. Even in solution, polyatomic proteins isolated from either the calcite or aragonite phases of abalone shells were shown to direct mineralization of calcium carbonate crystals with distinct phases (calcite or aragonite) and morphologies, depending on the chemical identity of the protein templates, suggesting that protein expression provides a
switchable mechanism for controlling phase and orientation of crystal growth \textit{in vivo} \cite{61}. At a larger scale, atomic force microscopy (AFM) and scanning electron microscopy (SEM) studies of shell formation in mollusks suggest that the 2D geometry and pore structure of the protein-based organic lamellae guides the growth of more complex 3D mineral structures with mineral bridges that interconnect hierarchically stacked pearls \cite{59}. These studies highlight two promising features of protein-based inorganic templating: precise molecular recognition for control of crystal location, phase, and orientation, and self-assembling higher-order structures that orchestrate the controlled formation of elaborate 3D geometries.

4.2. Fibrous proteins

Due to inspiration from biomineralization in nature, fibrous proteins and biopolymers have attracted interest in the materials science community as scaffolds for templated synthesis of inorganic materials \cite{62–68}. Collagen and chitin are two prominent examples of fiber-forming biopolymers that serve as organic templates for mineralization \textit{in vivo}. Their tendency to form fibers at multiple length-scales (nano-fibers, micro-fibers, and macro-fibers) results in hierarchically organized scaffolds that function both to guide the morphology of deposited minerals as well as to localize mineral forming precursors within the matrix, leading to unique morphologies and properties \cite{64}. Fibrous proteins have been demonstrated to provide similar functionality \textit{in vitro}. Collagen scaffolds were shown to direct mineralization of alumina mesoporous materials \cite{66} (Fig. 5), as well as titanium dioxide nanofibers with unusual catalytic properties for degradation of organic toxins \cite{68}. Similarly, native fish scales, which comprise a chitin scaffold that recruits a number of small proteins, proved to be a template for producing porous carbon materials with promising performance as electrochemical capacitors \cite{67}. On a smaller length-scale, fibers formed from lysozyme were found to electrostatically direct the assembly of gold nanoparticles along the fibers.
into arrays with tunable particle spacing [65] (Fig. 6). These works, among others, suggest that fibrous proteins represent a promising route toward templated synthesis of complex 3D inorganic architectures.

4.3. Cage proteins

4.3.1. Viral capsids

It is often desirable to control size and organization of inorganic nanoparticles on scales much smaller than the size scales of organization in fibrous proteins. Viral capsids are a simple and elegant example of the ability of proteins to self-assemble into precise, responsive nano-structures (see Fig. 7 for examples). In naturally occurring viruses, the viral capsid self-assembles from protein subunits encoded by the viral genome to form a protective coat that serves to package and protect nucleic acids and functions as a responsive delivery system for infecting a target host with the enclosed genetic cargo [69–72]. The biological challenges encountered by viruses impose stringent demands on the ability of the capsid to robustly and stably self-assemble in its target host, and to remodel in response to environmental cues. In addition, viral capsids typically possess a high degree of structural regularity and symmetry, and are found to exist in a variety of self-assembled architectures spanning a range of length-scales, including helical, icosahedral, and more complex geometries [69,71].

In vitro studies reveal that even a particular protein coat composition can give way to a rich self-assembly phase diagram with diverse morphologies governed by ionic strength and pH [73]. These appealing properties of naturally occurring capsids, and the immense potential for engineering new protein capsids through recombinant approaches, excites considerable interest in the nanoscience and nanotechnology communities to develop virus-like particles with new functional properties, either as delivery systems or templates for self-assembly.

A number of groups have investigated the potential use of viral capsids as templates for engineering inorganic nanoparticle assemblies with constrained sizes and/or spatial organization. For example, multiple variants of cowpea chlorotic mottle virus (CCMV) capsids are capable of directing reduction of gold(III) precursors to form gold nanoparticle-decorated capsids through tyrosine residues, with high selectively for reduction of gold(III) over several other metal precursors [74]. Alternatively, the endogenous histidine residues on the CCMV capsid can serve as highly specific nucleation sites for gold in the presence of a non-reducible precursor, yielding gold nanoparticle-patterned capsids with spatial organization dictated by the highly symmetric, repeating organization of native histidine residues on the icosahedral capsid [74]. Capitalizing on the cylindrical geometry of tobacco mosaic viral capsids, cylindrical nano-arrays of gold, platinum, and palladium nanoparticles were formed through reduction of cationic precursors by acidic residues [75]. It was further demonstrated that introducing mutations in the viral capsid to alter the density of negative charges produced distinct changes in the spatial

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**Fig. 6.** Lysozyme amyloid nanofibers direct the deposition of gold nanoparticles into 1D arrays. (a) Atomic force microscopy images of amyloid nanofibers on glass during the time-course of deposition with gold. Total elapsed deposition time is indicated in the upper right of each panel. (b) Atomic force microscopy images of amyloid nanofibers decorated with gold nanoparticles with indicated diameters (upper right of each panel). The spacing between nanoparticles deposited on the nanofibers can be tuned based on the nanoparticle diameter. Lower-right panel shows the scale-bar for the z-height in panels (a and b). Images were adapted with permission from Ref. [65]. Copyright (2014) American Chemical Society.
organization of nucleated nanoparticles. In addition, the well-defined sizes of viral cages and their porous structures, which allow diffusion of reaction precursors, enable them to behave as size-constrained nano-reactors that direct the synthesis of inorganic nanoparticles with controlled size distributions within the interior of the capsid [76]. These studies demonstrate the potential of viral capsids to produce nano-arrays of metal nanoparticles with geometry and dimensionality dictated by the underlying geometry of the self-assembled capsid, and spatial distributions that can be tuned by engineering the presentation of nucleation-directing amino acid residues. Viral capsids thus provide a versatile platform for nano-particle mineralization and assembly.

Alternatively, inspired by the ability of many wild-type viral capsids to self-assemble around their electrostatically charged genetic cargo, other researchers explored inorganic-organic hybrid materials in which the viral protein cage encapsulates functionalized nanoparticle templates. The potential to enclose nanoparticles in protein coats similar to native viruses offers the possibility of producing nanoparticles equipped with proteins that are engineered for specific responsiveness or other functionality. For example, gold nanoparticles functionalized with carboxylate-terminated thiolalkylated tetraethylene glycol chains can serve as templates for self-assembling protein cages from brome mosaic virus proteins [77]. Under suitable conditions, a protein coat efficiently assembled around the gold nanoparticles to form caged structures with protein stoichiometry, symmetry, and low polydispersity reminiscent of the icosahedral capsids that form around the RNA template in the native brome mosaic virus. In order to develop a deeper understanding of the mechanisms that underlie the protein cage assembly around charged nanoparticles, a combinatorial study was designed to decouple geometric and electrostatic effects. Below a critical charge density essentially no protein cages are formed, irrespective of the total nanoparticle

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**Fig. 7.** Diverse strategies for organic-inorganic hybrid material self-assembly using protein cages. Nanoparticles (represented schematically as silver, yellow, and copper colored spheres) can be patterned using self-assembling protein cages for spatially directed synthesis and nanoparticle shape and size control. (a) Nanoparticles can be synthesized or selectively bound on the surface of viral capsids with spatial organization dictated by the underlying symmetry of the capsid. In the schematic illustration provided, the viral capsid presents inorganic binding motifs on the purple colored domains of the self-assembled capsid, resulting in selective patterning of the nanoparticles (silver spheres) onto the surface that mirrors the underlying symmetry of the template. (b) Nanoparticles can be assembled in arrays whose geometries are directed by the protein cage, such as the cylindrical Tobacco Mosaic Virus cage. In this schematic, gold nanoparticles are synthesized within the interior of a cylindrically shaped viral capsid due to presentation of gold binding residues on the surface. (c) The well-defined size and porous structure of protein cages can be exploited for the size-constrained synthesis of nanoparticles within the interior. In this schematic, a porous protein cage permits diffusion of precursors into the interior of the self-assembled cage and promotes the synthesis of a nanoparticle (copper colored sphere) within the interior whose size is dictated by the size of the enclosing capsid. (d) Inorganic nanoparticles can be functionalized to template the self-assembly of virus-like-particles from protein cage subunits. In this schematic, a nanoparticle (yellow sphere) is decorated with amphiphilic molecules possessing an inorganic binding domain (red tails) and a charged domain (green spheres) that interact with protein monomers of a self-assembling capsid. The protein monomers self-assemble around the functionalized inorganic nanoparticle, forming a virus-like particle with an inorganic nanoparticle in the interior. Viral capsid illustrations were generated using the UCSF Chimera package from the Computer Graphics Laboratory [85] using files contributed to the Protein Data Bank from Refs. [164–167].
charge [78]. This furnishes evidence of the possibility to rationally design protein cage assembly based on physical principles and independently tunable parameters.

4.3.2. Ferritin cages

Protein cage structures for biotemplated inorganic nanoparticle synthesis are not limited to viral capsids. One of the most common non-viral proteins used for synthesizing inorganic nanomaterials is ferritin. Ferritin is a class of spherical protein cages made up of 24 identical self-assembling subunits. It performs a crucial role in maintaining iron homeostasis by sequestering iron as a nanoparticle of iron oxide (ferrihydrite) in the interior cavity of the cage [79]. This demonstration of naturally occurring nanoparticle synthesis has made ferritin a popular target for developing alternative syntheses. In addition to iron oxide [80], ferritin has been used to generate particles of silver [81], cobalt oxide [82], nickel [83], chromium [83], and calcium carbonate [84], among others.

4.3.3. Heat shock proteins

Heat shock proteins represent yet another example of naturally occurring proteins that can self-assemble into symmetric cage structures, which can serve as nano-reactors that are amenable to chemical or genetic engineering. The self-assembled structures possess pores that enable exchange of reactants and products between the interior and the environment, and present amino acid residues that function as spatially defined reactive sites. For example, the heat shock protein cage from Methanococcus jannaschii can direct the size-constrained synthesis of ferrihydrite nanoparticles from iron precursors via acidic amino acid residues in the interior of the protein cage [85]. Moreover, both endogenous lysine groups and genetically engineered thiols served as reactive sites for conjugation to fluorophores with well-defined spatial locations. Capitalizing on the ability of the same heat shock protein to nucleate controlled synthesis of platinum nanoparticles, a biomimetic hydrogenase was developed to efficiently catalyze hydrogen gas production [86] by platinum catalyzed reduction of protons. Due to their ability to crystallize into well-defined arrays, protein cages formed from heat shock proteins also can direct the self-assembly of micron-sized nanoparticle arrays, such as quantum dot arrays for electronic or photonic applications [87]. Such arrays are amenable to genetic modification to design non-native pore sizes for quantum dot incorporation [87].

5. Peptide only systems

A powerful feature of proteins that direct biomineralization in living organisms is the existence of specific peptide binding motifs that enable spatial localization of inorganic precursors. The vast diversity of peptide sequences available for exploration, together with the existence of high throughput combinatorial techniques for their selection [88], renders the design of novel self-assembling peptides with high affinities for crystal precursors a promising route toward synthesis of nanostructures with refined control over composition, morphology, and spatial distribution. The ability of peptides to provide molecular recognition functionalities has been demonstrated not only to promote nanoparticle nucleation in solution conditions

Fig. 8. Phage display enables high-throughput evolution of molecular-recognition peptides. (a) A large combinatorial library of peptide-encoding DNA sequences (green, yellow, and red colored segments) is generated and integrated into the genome of the desired phage (black circles). (b) Phages are amplified in a bacterial host, generating phages that present peptides (green, yellow, and red colored ellipsoids) from the combinatorial library on the surface of their protein coat. (c) Phage capsids that present peptides with high affinities for a desired material (e.g., a nanocrystal, represented here by a red sphere) are purified by affinity selection. (d) Sequences of the affinity-selected, molecular-recognition peptide sequence are directly determined by sequencing the genomes of the purified phage capsids. Phage capsid illustrations were generated using the UCSF Chimera package from the Computer Graphics Laboratory [85] using files contributed to the Electron Microscopy Databank from Refs. [168,169].
under which it would otherwise not occur, but also to provide morphological control of crystal growth, such as by high specificity of binding to specific crystal faces [89–91].

5.1. Peptide evolution by phage display

Naturally occurring peptides are highly evolved to serve specific functionality in living systems, but they are capable of nucleating only a limited range of crystal morphologies and compositions. A broad range of potential synthetic nanomaterials with important technological applications, such as semiconductors, are not naturally synthesized in biological systems. Thus, the development of peptide-based organic templating systems for nanoparticle synthesis hinges crucially on the ability to design novel peptide sequences with functionalities not currently found in nature. To this end, evolution of peptides by phage display is a powerful combinatorial technique for screening vast arrays of peptide sequences to identify molecular recognition peptides with high binding affinities for nanocrystals or crystal precursors [88] (Fig. 8).

Phage display is an in vitro technique in which a large library (~10^12) of peptides are fused into the coat protein genes of phage virions, thereby producing an array of phages that display distinct peptide sequences on their capsids. These virions can subsequently be separated by binding affinity purification with a desired binding ligand, thereby isolating peptides with high binding specificity. The physical linkage between the phage phenotype and the corresponding genome encoding for the peptide allows the specific peptide sequences to be directly identified. Although this technique is predicated on evolution based on affinity to the final crystal, and not nucleation kinetics, it was recently demonstrated that binding affinity to model self-assembled monolayers is inversely related to the free energy barrier to nucleation, consistent with classical nucleation theory [92]. Thus, binding affinity to the desired crystal product may serve as a proxy for nucleation efficiency for some systems. Phage display has been successfully utilized to evolve novel peptide sequences with high affinities for a variety of substrates, including Al [93], carbon nanotubes [94], graphene [95], and semiconductors [18], and also for discovery of peptides with catalytic activity for inorganic nanocrystal synthesis [96]. Moreover, the incorporation of non-natural amino acids further expands the available peptide library, and was implemented to develop peptides with high metal ion binding affinities [97]. Thus, the high-throughput nature of phage display and the possibility of incorporating a variety of non-natural amino acids enables the development of peptide sequences for assembly of nano-architectures that are not only biomimetic, but that yield products that extend beyond those found in the biological realm. The ability to directly evolve peptide sequences based on their desired functional properties also circumvents challenges associated with predicting peptide properties in de novo peptide design.

5.2. Mechanisms of biomineralization by peptides

Phage display technology enables high throughput selection of peptide sequences with desired binding properties without a priori knowledge, but it does not provide direct understanding of the underlying molecular mechanisms that confer molecular recognition properties of peptides [98]. Moreover, peptide design based purely on combinatorial techniques may overlook peptides with desirable functional properties due to biased and incomplete libraries, [98] or desired functionality that does not directly translate from binding affinity alone. For example, a peptide sequence selected by affinity-based phage display may direct growth of widely varying morphologies under different reaction conditions [99]. Thus, knowledge of the fundamental principles that mediate peptide molecular recognition may offer powerful input for further refinement and discovery of peptide structures.

Although recent studies on calcite nucleation by model self-assembled monolayers demonstrate crystallization behavior adherent to classical nucleation theory [92], where crystal binding energy directly correlates with nucleation efficiency, a number of studies in peptide-mediated nucleation point to the addition of more subtle and complex factors at work. Non-classical effects aside, peptide binding itself depends on a multitude of factors, including electrostatic and hydrophobic properties of the substrate [100], as well as flexibility and conformational entropy of the peptide adsorbed to the target surface [101–103]. Even within a particular amino acid primary sequence, constraints that impose changes in secondary structure can have profound changes on binding energies, as demonstrated in a series of gold adsorption studies involving linear and cyclized forms of peptides with identical primary sequences [104]. The situation is further complicated by non-classical nucleation mechanisms. For instance, a recent study combining experiments and molecular dynamics simulations on peptide-mediated binding and nucleation of silver revealed pronounced differences in growth of silver nanoparticles between peptides with comparable binding energies [105], but different simulated 3D conformations. Overall, the current body of work reveals a complex structure-function landscape for peptide recognition systems involving classical and non-classical mechanisms, where a variety of approaches, including combinatorial screening, post-screening site-specific mutations [99,102,106], molecular simulations [101–105], and computational bioinformatics [107], are required to elucidate the molecular underpinnings of technologically-relevant functionality.

5.3. Examples of peptide systems

5.3.1. Soluble peptides

A number of specific peptide template systems for biomineralization have been developed, which exhibit varying levels of structural complexity, ranging from single peptides to hierarchical assemblies. The simplest technologically relevant peptide
systems are soluble peptides, which have been successfully implemented to direct growth of metal nanoparticles with a variety of sizes, morphologies, and consequent optical and or catalytic properties. Such peptides often contain α-helical domains that direct spatial localization of metal ions, with subsequent growth that is morphologically tunable through a range of factors. Even a single peptide sequence can orchestrate the synthesis of distinct morphological properties under different environmental conditions. This has been demonstrated, for instance, in work where a single peptide sequence can form an α-helical secondary structure at mildly alkaline conditions, but random coil structure under strongly alkaline conditions. Consequently, this peptide directs the assembly of fluorescent silver nanoparticles with distinctly different sizes and emission spectra at different pH [108]. The ability of metal-binding peptides to strongly localize metal ions also leads to a delicate dependence of particle morphology and functionality on peptide to metal ratios, as shown in a study of peptide-mediated synthesis of catalytic palladium nanoparticles [109] (Fig. 9). Tertiary structure also provides an important tuning parameter for nanoparticle synthesis. For example, α-helical peptides that spontaneously assemble into supercoiled trimers, tetramers, and hexamers promote nucleation of fluorescent silver nanoparticles with varying optical properties that are directly related to the expected number of metal ions sequestered by the coiled-coil multimers [110]. Similarly, it was found that the number of tandem α-helical repeats in homologous repeat proteins could control the morphology and optical properties of gold nanoparticles in template-directed synthesis [111]. Therefore, even comparatively simple soluble peptide systems yield opportunities for template-directed synthesis of nanoparticles with a variety of technologically-relevant properties, which may be tuned at the level of primary, secondary, and tertiary structures.

5.3.2. Peptide arrays

Soluble peptide systems enable growth of nanoparticles with some degree of tunability, but they provide limited control over hierarchical spatial organization of nanoparticle assemblies. A promising strategy for exerting more elaborate self-assembly of superstructures is the use of 2D peptide arrays as programmable templates that direct nucleation of nanopar-
articles into pre-determined spatial distributions. Inspiration for such peptide arrays can be found in nature, such as in the S-shells that enclose many prokaryotic organisms, which are composed of proteins that self-assemble into 2D lattices of a variety of forms [112]. S-shells containing regularly ordered functional groups can be exploited as templates to guide the growth of nanoparticle superlattices with a variety of pre-programmed symmetries and compositions, including metallic and semiconducting arrays [113–117]. Furthermore, S-shells isolated from living organisms can be further chemically modified to incorporate novel functionality and specificity not found in naturally occurring protein lattices, opening broader possibilities for bottom-up assembly of supramolecular architectures [113]. Potentially even greater flexibility may be afforded by the

Fig. 10. Self-assembling collagen-like peptides serve as templates for metallic nanowire synthesis. (a) Schematic of the collagen-like peptide nanofiber template system. In step 1, collagen-like peptides equipped with lysine residues can be functionalized with amine-reactive gold nanoparticles, which serve as nucleation sites for electroless plating by reduction of gold or silver. In step 2, the gold-decorated collagen-like peptides self-assemble into nanofibers. Finally, in step 3, electroless plating by reduction of silver produces metallic nanowires whose morphology is dictated by the underlying peptide nanofiber template. (b) TEM images of nanofibers following 1, 2, and 3 electroless silver deposition cycles (left to right). Scale bars are 100 nm. Images were reproduced from Ref. [124] with permission of the Royal Society of Chemistry.
design of bio-inspired synthetic peptide array systems, such as the peptide-functionalized graphene templates developed to pattern nanoparticle arrays with electrocatalytic functionality [118]. In direct contrast with conventional lithographic patterning techniques, which involve expensive, top-down sequential processing [115], these peptide array approaches enable bottom-up, in situ self-assembly of superstructures with architectures that are dictated by the spatial distribution of physiochemical properties of the underlying template.

5.3.3. **Self-assembling peptides**

In addition, peptides can be designed to spontaneously self-assemble into other 2D or 3D architectures through intermolecular associations, such as nanofibers and nanotubes. Self-assembled scaffolds comprise individual components that spontaneously self-assemble into stable supramolecular structures under specific conditions. Spontaneous self-assembly often occurs in response to shifts in pH, ionic strength, and concentration, [119] and the non-covalent bonds that stabilize the resulting structures typically include hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals interactions [120]. Self-assembly of peptide-amphiphiles is often driven by the tendency of the aliphatic tails to aggregate in aqueous solution at high concentrations. Several peptide-based self-assembling systems have been developed in recent years [121], including some materials applicable to inorganic nanotemplating. For example, single tail peptide-amphiphile molecules that self-assemble into nanofibers of varying morphology, surface chemistry [119,122–129], and bioactivity have been designed to template minerals such as hydroxyapatite [125].

Through judicious amino acid selection and alkyl tail modification, peptide-amphiphiles have been developed to undergo pH-induced self-assembly into nanofibrous structures that mimic the architecture of the native extracellular matrix [125]. Drawing inspiration from the natural tendency of collagen fibrils to promote oriented hydroxyapatite crystal growth, it was discovered that incorporation of acidic phosphoserine residues into the peptide sequence of a known self-assembling peptide could promote the formation of calcium phosphate minerals along the fibrillar surface post-assembly. Acidic phosphorylated moieties are known to play an essential role in biomineralization processes, and the designed peptide sequence resulted in a surface display of phosphate similar to the repetitive organization of phosphate groups found in natural phosphophoryn proteins.

Moreover, peptide sequences may be modified to produce scaffolds with less common natural functions, such as conducting nanowires. For example, metal-binding peptide sequences may be introduced into self-assembling peptide sequences to promote the association of inorganic precursors with the peptide surface. The diversity of peptide sequences available and their inherent responsiveness to solution conditions offers the possibility for bottom-up self-assembly of functionalized nanowires with morphologies tuned by peptide composition or solution conditions. In recent years, peptide nanofiber-directed synthesis has been used to robustly produce a variety of inorganic nanomaterials with controllable morphologies, including 1D silver nanowires [124] (Fig. 10), 1D platinum nanostructures [129], lipophilic inorganic nanoparticles [126], silica nanotubes [127], and coaxial metal tubes [122].

Peptide-directed inorganic templating may be used to produce more complex inorganic nano-assemblies. While 1D and 2D peptide scaffolds have served as the primary biologically inspired templates to date, 3D hierarchical nanomaterial architectures are becoming increasingly prevalent. For example, doughnut-shaped peptide nano-assemblies have been utilized to create nanoreactors [130,131]. In another exemplary case, alternative 3D architectures were used to produce palladium nanoparticle networks and ferroelectric barium titanate nanoparticles under mild conditions [132]. As peptide self-assembly becomes better understood and more predictable, peptide templating will likely become an increasingly facile and effective method of synthesizing complex inorganic nano-assemblies not accessible via more conventional assembly techniques.

6. **Protein-peptide hybrid systems**

While a wide range of inorganic structures have been produced using exclusively protein or exclusively peptide templates, a number of innovative templating systems were recently developed that utilize both peptides and proteins to create novel and broadly applicable scaffolds. Such systems combine the high specificity of molecular recognition peptides and the 3D architecture conferred by protein self-assembly, opening the potential for engineering precisely spatially organized binding motifs for nucleation of inorganic materials along with other functionalities.

6.1. **Phage capsid-peptide systems**

Viral capsids are a natural platform for functionalization with peptides. For example, a highly versatile protein-peptide platform was developed using genetically engineered M13 bacteriophage that recognizes specific inorganic molecules and selectively directs their growth into higher-ordered structures. The engineered M13 system has been used produce nanomaterials with a diverse range of functions, including nanocrystals applicable in the semiconductor industry [18,133], cathodes for lithium-oxygen batteries [134], and ordered arrays of quantum dots. Other applications for the M13 bacteriophage system include the production of dye-sensitized solar cells [135,136], templating of magnetic nanoparticles for targeted in vivo imaging of prostate cancer [137], and stabilization of single-walled carbon nanotubes for use in noninvasive imaging and surgical guidance of submillimeter tumors [138]. Like many other protein-peptide fusion systems, the M13 phage system
combines the ability of the capsid proteins to self-assemble into a well-defined architecture with the precise recognition features of engineered peptides.

6.2. Ferritin-peptide systems

Similar to phage capsids, the self-assembled ferritin protein cage offers a variety of opportunities for molecular engineering through genetic or post-translational incorporation of molecular recognition peptides. For example, ferritin protein cages were genetically engineered to incorporate either titanium or carbon nanotube binding peptide aptamers with selective affinity to their target substrates while retaining the ability of endogenous ferritin subunits to self-assemble into cage structures and mineralize iron [139]. A similar strategy was pursued to achieve the selective growth of silver nanoparticles in the interior of a ferritin cage genetically modified to display a peptide known to reduce silver(I) ions to silver [140]. Even broader opportunities exist for producing inorganic-ferritin hybrid materials functionalized with molecular recognition peptides for a variety of applications. For instance, ferritin cages were engineered to present surface peptides that block protein receptors implicated in asthma, and the symmetric organization of peptide “bunches” mediated significantly improved affinity of the protein-peptide chimera to the target receptor compared to the soluble peptide alone [141].

Fig. 11. Template engineering through epitope recognition (TETHER): Clathrin-peptide hybrid systems provide tunable inorganic nanoparticle synthesis through modular design. (a) Schematic of a clathrin cage. The clathrin cage (left) is formed from repeat units of triskelion monomers (highlighted in light blue and shown on the right) that self-assemble into a protein cage. (b) Design of TETHER peptides. Heterobifunctional peptides are synthesized that consist of a clathrin binding domain and an inorganic binding domain separated by a glycine spacer. The inorganic binding domain can be designed in a modular fashion to produce peptides with affinity for the inorganic material of interest. Here, TP1, TP2, and TP3 represent peptides whose inorganic binding domains exhibit affinity for titanium dioxide, cobalt oxide, and gold, respectively. (c) Schematic of the TETHER templating process. Clathrin triskelion monomers self-assemble into clathrin cages in solution (left). The desired TETHER peptide can be bound to the clathrin cage through epitope recognition at the clathrin binding domain, yielding a decorated clathrin cage that presents inorganic binding domains on its surface (middle). Incorporation of the appropriate inorganic precursors yields directed synthesis of inorganic nanoparticles within or on the surface of the clathrin cage (right). In the schematic provided, the clathrin template is functionalized with either titanium oxide (red), cobalt oxide (green), or gold (blue) nanoparticles, depending on the choice of TETHER peptide and inorganic precursors. Images from a) were reproduced from Ref. [170] under a CC-BY-4.0 license agreement. Images from b) and c) were adapted with permission from Ref. [142]. Copyright (2011) American Chemical Society.
6.3. Clathrin-peptide systems

Clathrin protein cages are similarly amenable to augmentation with molecular recognition peptides. To move away from the need for chemical and genetic modifications which may disrupt protein integrity or assembly properties, a strategy was developed to mimic the naturally occurring phenomenon of adaptor proteins that bind to a central protein complex at specific sites to modify the overall function of the complex (Fig. 11). Peptides were designed to functionalize clathrin cage scaffolds through site-specific molecular recognition using short binding sequences from clathrin-binding proteins. These peptides also contained inorganic-nucleating peptide sequences similar to those discussed above, rendering them bifunctional. The use of non-covalent, site-specific functionalization allowed the components to be treated in a mix-and-match manner, making the self-assembled clathrin platform versatile for a range of modifications. Clathrin cages, complexed with a family of engineered peptides, was used to separately synthesize nanoparticles of titania, cobalt oxide, and gold [142]. This strategy, termed TETHER (Template Engineering Through Epitope Recognition), was extended to the use of multiple peptides aimed at distinct binding sites on the clathrin inner and outer surfaces to generate multi-material nanoparticles containing silver and gold in different arrangements. Particles were synthesized with mixed domains or core-shell arrangements of silver and gold [143]. Furthermore, careful control of the ratio of inorganic precursor, bi-functional peptide, and clathrin cage scaffold was shown to lead to tunable arrangements of synthesized gold particles into solutions of dispersed or clustered particles, illustrating the potential of this non-covalent functionalization strategy for predictable and controlled hierarchical assembly [144].

7. Challenges in nanomaterials

7.1. Reproducibility in nanomaterial synthesis

While biomaterial templates are a promising and innovative platform for the production of nanomaterials with complex architecture, lower costs, high-throughput production methods, and increased reproducibility will be essential to the development of next-generation commercial optical, electronic, and magnetic materials and devices. Many complicated new nanomaterials will rely on robust connections between multiple components, and defects in any of the 2D and 3D nanostuctures used in these systems could impair function and reduce or eliminate their industrial utility [145]. Theoretical guidance has been successfully used to guide experimental design and reduce defect formation in some conventional templating systems, including one scaffold for the growth of zinc oxide nanowire arrays [146]. However, similar approaches are not commonly used in peptide-based templating. Moreover, techniques to quickly and cost-effectively characterize nanomaterials to screen for defects and enhanced properties are still in the early developmental stage. Benchtop production methods, which are commonly used to produce nanomaterials in academic settings, rarely include advanced control systems. While nanomaterials created using benchtop production methods are useful in many industrial applications in spite of the relatively low level of automation and reaction control involved in their synthesis, cost effective reaction scale-up presents a barrier to industrial adoption. More fundamentally, the use of nanomaterials is also limited by the current level of understanding of what nanoarchitectures are most useful in a given application [147]. Future research into nanostructure-property relationships will be necessary to fully exploit the complex inorganic nanoarchitectures that may be produced from engineered biological templates.

7.2. Mechanical properties of organic-inorganic hybrid materials

Although protein-templated nanomaterials are highly versatile and could be used in diverse industrial applications, the low mechanical strength [148,149] of most protein-templated nanomaterials has limited their adoption outside the biomedical industry. Even in nonstructural applications, such as optical and electronic devices, certain mechanical properties are required to ensure functionality. However, natural inorganic-protein nanocomposites such as bone, teeth, and nacre exhibit mechanical toughness and strength that notably exceed their individual components [150]. Increased understanding of the viscoelastic properties of strong naturally occurring nanocomposites will likely inform future design strategies, leading to tougher bioinorganic nanocomposites that can be exploited in a wider range of commercial products [151,152].

7.3. Environmental concerns

The influence of nanomaterials, including protein-templated inorganic nanocomposites, on the environment and human safety is still poorly understood. While the market for nanomaterials is rapidly expanding, research into their environmental impact is in its infancy. Reports have discussed environmental hazards; however, the cause and extent of the environmental burden is currently unknown [153–155]. This is due in part to the vast range of nanomaterials that have been developed, and their rapid ongoing discovery. The small size of nanomaterials increases the risk of their unintentional release into the environment through atmospheric emissions and waste streams at production facilities. Unintentional release of nanomaterials into the atmosphere could ultimately result in soil and water contamination as the airborne nanomaterials deposit on available surfaces. While low-level contamination may not be harmful to the environment, significant levels of environmental
accumulation due to spillage or other production or disposal methods could prove to be toxic to surrounding wild life and, indirectly, to humans.

7.4. Health concerns

In addition to environmental considerations, human safety must be considered when designing nanomaterials, especially nanomaterials intended for clinical use [14,156–159]. Off target cytotoxicity is one of the primary safety issues encountered during clinical nanomaterial administration. Nanomaterials often acquire protein coronas and interact with secondary sites in vivo [158,160]. Nanomaterial shape and exposure route often dictate material toxicity by affecting site-specific accumulation of nanoparticles and subsequent local toxicity. Shape in particular plays a large role in determining biodistribution, as rod shaped particles are more easily internalized by cells and more frequently exocytosed than spherical nanoparticles [14]. Moreover, shape and administration route can affect the interaction between nanomaterials and phagocytic cells. Ingestion of nanomaterials by phagocytic cells can lead to undesired removal and accumulation of particles in the organs such as the liver, spleen, and lungs, which may reduce the potency of nanomaterial-based treatments while increasing the risk of adverse immune response following recognition of the nanomaterials by phagocytic cells. Intelligent materials design could potentially mitigate many off-target effects, facilitating the use of nanomaterials in the treatment of diseases other than cancer, where cytotoxic side effects are the norm.

Other safety issues to consider include unintentional exposure to nanomaterials, including those not intended for clinical use, through inhalation and skin exposure [159]. Inhaled nanoparticles, especially very small nanoparticles (20 nm particles), have very high deposition efficiencies in the lungs, where their accumulation may lead to severe immune responses. The effects of nanoparticles on the skin are unclear since it is not yet known how many nanomaterials can penetrate the skin and how nanomaterial properties change under different exposure conditions. However, introduction of nanomaterials into the bloodstream via initial skin contact could have detrimental health consequences, especially in the case of toxic metal nanoparticles. Inhalation and skin exposure are especially important concerns in the context of manufacturing, since worker safety may be compromised if nanomaterial toxicology is not well understood.

8. Conclusion and outlook

In spite of the potential challenges associated with their design, manufacture, and use, nanomaterials will become more prevalent in the biomedical, semiconductor, and energy fields. The unique properties derived from the nanoscale structure of these materials will enable new and useful products to be produced on increasingly large scales as the field develops. The ability to precisely template inorganic nanomaterials will be key to developing next-generation nanomaterials, since complex nanostructures are likely to produce different effects than the simple 1D and 2D architectures used at present. While conventional synthetic techniques are increasingly being adapted to meet nanomaterial design challenges, proteins and peptides enable facile control over nanomaterial structure under mild conditions. Biotemplates, inspired from biomineralization in nature, promise to provide multifaceted control over inorganic nanomaterial synthesis. As we describe in this review, evidence already is emerging for their potential to control the shape and size of nanoparticles, their molecular recognition capabilities for selective binding to specific inorganic species, their ability to spatially localize inorganic materials to specific underlying patterns, and their 3D self-assembly capabilities. The integration of these control strategies in next-generation biotemplates may yield unprecedented control in synthesizing hierarchical nanostructures. The reduced environmental and health burden associated with the production of nanomaterials at ambient temperature in aqueous solvent using peptide or protein-based templates makes them an attractive platform for next generation materials design. However, the challenges described above associated with bio-templated nanomaterials will need to be addressed before the platform’s promise can be fulfilled. While a great deal of progress has been made in the past decade to elucidate design principles and explore potential applications, bioengineered templating of inorganic nanomaterials is still an immature field with considerable untapped potential.

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