DOI: 10.1002/anie.201106715

Materials Synthesis

Biomineralization as an Inspiration for Materials Chemistry

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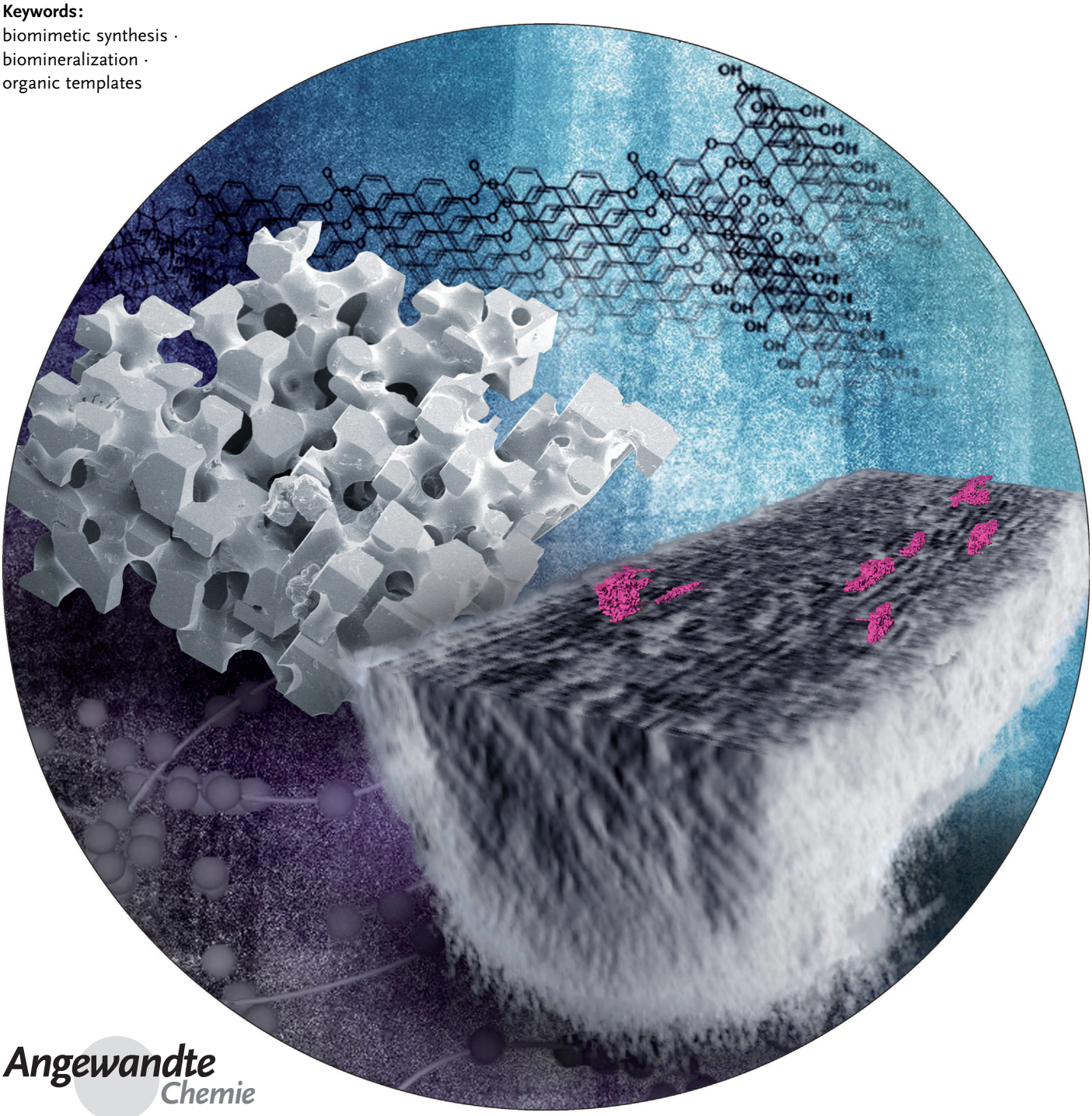
Keywords:
biomimetic synthesis · biomineralization · organic templates



Angewandte International Edition Chemie

Living organisms are well known for building a wide range of specially designed organic–inorganic hybrid materials such as bone, teeth, and shells, which are highly sophisticated in terms of their adaptation to function. This has inspired physicists, chemists, and materials scientists to mimic such structures and their properties. In this Review we describe how strategies used by nature to build and tune the properties of biominerals have been applied to the synthesis of materials for biomedical, industrial, and technological purposes. Bio-inspired approaches such as molecular templating, supramolecular templating, organized surfaces, and phage display as well as methods to replicate the structure and function of biominerals are discussed. We also show that the application of in situ techniques to study and visualize the bio-inspired materials is of paramount importance to understand, control, and optimize their preparation. Biominerals are synthesized in aqueous media under ambient conditions, and these approaches can lead to materials with a reduced ecological footprint than can traditional methods.

From the Contents

1. Introduction

Living organisms are well known to exploit the material properties of amorphous and crystalline minerals when building a wide range of organic–inorganic hybrid materials for a variety of purposes, such as navigation, mechanical support, photonics, and protection of the soft parts of the body. The high level of control over the composition, structure, size, and morphology of biominerals results in materials of amazing complexity and fascinating properties that strongly contrast with those of geological minerals and often surpass those of synthetic analogues.[1] It is no surprise, then, that biominerals have intrigued scientists for many decades and served as a source of inspiration in the development of materials with highly controllable and specialized properties. In this Review we aim to provide an overview of the different nature-drawn strategies that have been applied to produce materials for biomedical, industrial, and technological applications. We will first illustrate the diversity of biogenic minerals and their overall properties, and describe the most general approaches used by organisms to produce such materials. We will then discuss several approaches inspired by the mechanisms of biomineralization in nature, and how they can be applied to the synthesis of functional and advanced materials such as bone implants, nanowires, semiconductors, and nanostructured silica. In the final section, we will discuss methods that are necessary to study and visualize the formation of synthetic materials in situ so as to better understand, control, and optimize their synthesis and properties. Although a large part of the literature focuses on $CaCO_3$, we will take examples from all four main biominerals (silica, magnetite, $CaCO_3$, and calcium phosphate) to give an overview of the different materials that are found in nature, their functions, and their biomimetic strategies, which have been learned from their mechanisms of formation.

1.1. Biomineralization: An Overview

The diversity of mineralized structures found in nature, in terms of mineral composition, morphologies, properties, and functions, is quite astounding. More than 60 different types of minerals are known to be used by organisms from all 5 kingdoms, where each organism has evolved its own strategies for building materials that are tailor-made for their function. In this section we will discuss a few well-known examples of biominerals, their properties, and how their design strategies can be of interest for the synthesis of synthetic materials.

Many microorganisms and animals as well as lower and higher plants form remarkable structures from amorphous silica. The best know representatives of biosilicifying organisms are the diatoms and radiolarians, which are unicellular algae that are abundant in fresh water and in the ocean. In fact, most of the biosilica formation in the oceans is governed by these species.[1] These organisms use the silica to build their cell walls and microskeletons, which are nano- and micropatterned, thus highlighting the remarkable level of control that they exert over the formation of these structures.[2] Silica formation occurs inside a specialized organelle, called the silica deposition vesicle.[3,4] Two families of phosphate- and amine-rich proteins,[5,6] the silaffins[7–9] and long-chain polyamines (LPCAs),[10] function in conjunction with another family of proteins, the silacidins,[11] to control silica polymerization. The latter are proteins rich in phosphorylated serine, aspartic acid, and glutamic acid,[11] and serve as cross-linking agents for the assembly of long-chain polyamines, which help to mediate silica precipitation and the formation of higher

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order silica morphologies. Ultimately, the morphologies of the silica structures, from the nano- to the microscopic level, are genetically regulated to the extent that the silica structures, morphologies, and patterns are species-specific. To date, the synthesis of diatom-like silica structures has not yet been reproduced in the laboratory.

Another fascinating example of biosilica is the skeleton of the glass sponge *Euplectella* (Figure 1),[12,13] which resides in the deep sea at depths ranging from 35 to 5000 m. The

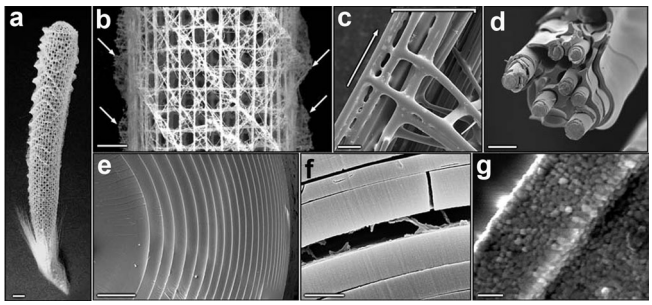

Figure 1. Hierarchical levels in the structure of the skeletal system of Euplectella sp. a) Entire skeleton (scale bar: 1 cm). b) Higher magnification of the cage (scale bar: 5 mm). c) Scanning-electron microscopy (SEM) image of a section of a strut, composed of bundled spicules (scale bar: 100 μm). d) SEM image of a fractured and partially HF-etched single beam (scale bar: 20 μm). e) SEM image of a cross-section through a spicule in a strut, showing its characteristic laminated architecture (scale bar: 5 μm). f) SEM image of a fractured spicule, revealing an organic interlayer (scale bar: 1 μm). g) Nanoparticulate nature of the biosilica (scale bar: 500 nm). Adapted from Ref. [12] with permission, Copyright 2005 AAAS.

mineralized skeleton of *E. aspergillum* has a convoluted cylindrical structure similar to a cage,[12] with a hierarchical structure that overcomes the low strength of glass and provides an exceptional mechanical stability.

Another class of minerals commonly produced by organisms are iron oxides, which are used for a broad range of functions such as iron storage, navigation, sensing of magnetic fields, stretching of tissues, and hardening of teeth.[1] The magnetotactic bacteria (Figure 2), which produce nanoparticles of magnetite (Fe_3O_4) or greigite (Fe_3S_4) to navigate through the Earth's magnetic field along chemical gradients in aquatic habitats, are well studied.[14] These bacteria produce nanocrystals that are aligned into an intracellular chain, with each crystal located inside specialized compartments, called

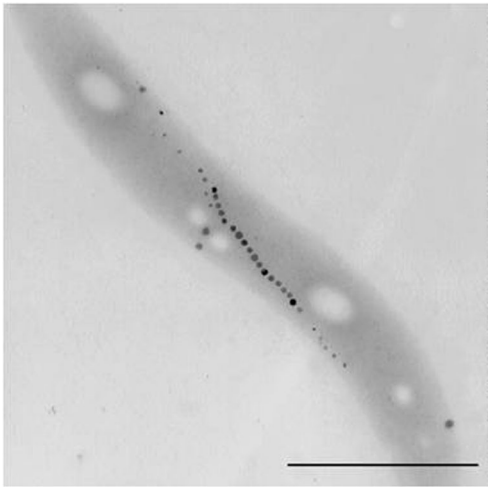

Figure 2. Transmission electron microscopy (TEM) image of a spirilium with a single chain of cubooctahedral magnetosomes (scale bar, 1 μm). Adapted from from Ref. [14] with permission, Copyright 2008 American Chemical Society.

magnetosomes. This compartment is where the magnetite crystals form and they are aligned in well-ordered chains. A single magnetite crystal is a highly efficient permanent magnetic carrier, and its size of 30–140 nm lies in the single magnet domain.[14] Moreover, magnetotactic bacteria can precisely control the oxidation state of the iron atoms, preventing the transformation of magnetite to maghemite, which is still an issue in synthetic samples.[15]

Calcium carbonate based biominerals are the most abundant crystalline biogenic minerals found in nature. $CaCO_3$ is present, in particular, in fresh water and marine organisms, such as sea urchins, mollusks, sponges, corals, and crustaceans, where it exerts a variety of functions: for structural purposes as in coral skeletons and sea urchin tests; protection as in sea urchin spines, mollusk shells, crustacean carapaces; and mechanical as in sea urchin teeth.[1] Furthermore, calcium carbonate also forms gravity sensors in marine and land animals,[1] and is part of the photosensory organ in brittlestars.[16] Calcium carbonate can occur in the form of three anhydrous crystalline polymorphs, all of which are found in the biominerals vaterite, aragonite, and calcite, with calcite being the most thermodynamically stable form under ambient conditions.[17] Furthermore, calcium carbonate can also occur in three hydrated forms: amorphous calcium

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carbonate (ACC), calcium carbonate monohydrate, and calcium carbonate hexahydrate. Of these, ACC is particularly important, since it is often used by organisms either as an integral constituent of a biomineral or as a precursor phase to calcite or aragonite.[18–21]

Calcite is found in many cases as large single crystals, ranging from hundreds of micrometers to several millimeters in size, and forming a skeleton to protect the soft parts of the animal. Typical examples are mollusk shells,[22,23] sea urchin spines, sponges, and corals. Interestingly, inorganically formed calcite is very brittle, since it cleaves easily along the {10.4} plane, and therefore is not a very suitable material for such a purpose.[24] Organisms overcome this limitation by having proteins occluded inside the crystal, preferentially in the planes parallel to the *c*-axis, which cause dislocations on the planes that are oblique to the cleavage planes.[24–26] This strategy forms an efficient crack-deviation mechanism, such that the spines cleave conchoidally, as if they were composed of glassy materials.[24,27] This design strategy essentially introduces anisotropic fracture behavior into a material that is still highly anisotropic at the atomic level. The result is the enhancement of the mechanical properties of the calcite crystals and a decrease in their brittleness. This approach, in fact, may have wide implications in the fabrication of synthetic materials.

Another strategy employed to produce biominerals capable of withstanding stresses is the organization of crystals into large superstructures. This strategy is particularly evident on the aragonitic nacreous layer of mollusk shells. Much of the mechanical strength of nacre derives from its superstructure, where plate-shaped aragonite crystals approximately 500 nm thick are arranged into parallel layers that are separated by a sheet of organic matrix (Figure 3).[28–31] This arrangement, and the combination of organic–inorganic materials, makes the nacre 3000 times tougher than pure inorganic aragonite.

Calcium phosphate minerals are known to exist in various compositions, and the most well-known examples are found in vertebrate bones and teeth. Bone is a nanocomposite with unique mechanical properties that are defined by its chemical composition and structural organization.[32] The nanostructure of bone is composed of fibrils of type I collagen, in which hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) crystals are embedded.[33,34] Although collagen is the major organic component of bone, a small fraction of highly acidic noncollagenous proteins are also present, and are involved in controlling the formation of apatite inside the collagen fibrils.[35] One of the most interesting characteristics of bone is its hierarchical structure, going from the nanometer to the macroscopic scale.[32,36] As such, while all types of bone are composed of mineralized collagen fibrils, arrays of fibrils can be organized in different patterns, thereby generating a structural diversity that is optimized to the functional need. Typical examples are woven bone, where fibrils are loosely packed and poorly oriented; the rotated plywood structure that is common to lamellar bone; arrays of parallel fibers, which are found in mineralized tendons; and radial fibril arrays, as found in dentin (Figure 4).[32] All these different arrangements will lead to structures with different mechanical properties and hence are suited to withstanding different types of stresses.

The above examples are but a small fraction of the diversity of biominerals, in terms of their morphologies, structures, compositions, and functions, that exist in nature. Nevertheless, they illustrate the level of sophistication of these materials, particularly in terms of their adaptation to function. Therefore, many chemists, physicists, and materials scientists have turned to these biogenic minerals as a source of inspiration for the synthesis of novel materials that could have wide applications in medicine as well as industrial and technological processes. However, controlling the structure and the morphology of these organic–inorganic composite materials is still a challenge and requires a profound fundamental knowledge of the mechanisms involved in the biogenic processes. In the next section we will address the different approaches that have been employed to learn nature's strategies for building functional, tailor-made minerals, with the aim of applying these principles to the synthesis of materials with controllable properties.

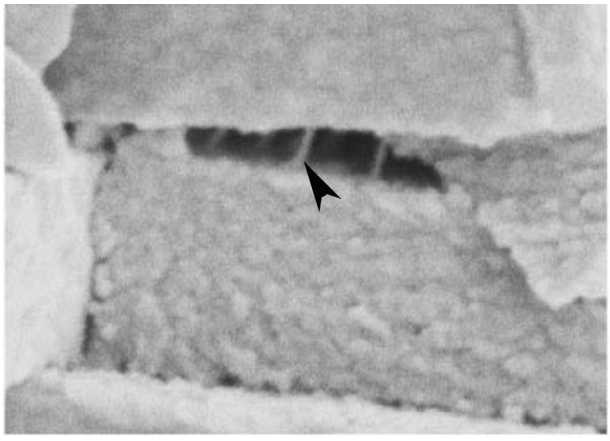

Figure 3. SEM image of fractured nacre, showing the adhesive organic matrix between two aragonite crystals (black arrowhead). Adapted from Ref. [31] with permission, Copyright 1998 Macmillan Publishers Ltd.

2. Understanding Nature's Strategies To Form Functional Materials

Biogenic minerals have morphologies, crystal habits, and material properties that are quite different from those of their geological counterparts. These differences are due to the tight control over all the processes in the formation of the biominerals such as crystal nucleation, growth, morphology, polymorph type, and composition. Although it is impossible to understand the different specific mechanisms that lead to the formation of every particular biogenic mineral, there are some basic strategies for controlling mineralization that seem to be common to many organisms. These strategies, as discussed by Mann,[37] involve: a) chemical control, b) spatial control, c) structural control, d) morphological control, and e) constructional control. In chemical control, physicochemical factors such as solubility, supersaturation, nucleation, and crystal growth are regulated by controlling the ionic compo-

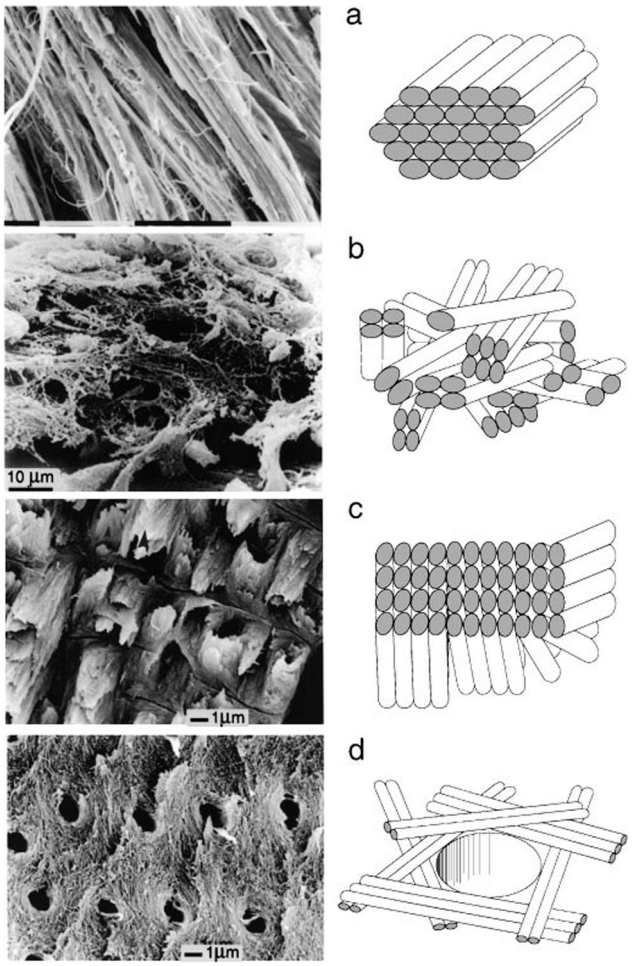

Figure 4. Four of the most common fibril array patterns of organization. SEM images (left) of fractured surfaces and respective schematic representations (right, not drawn to scale) of the basic organizational motifs. a) Array of parallel fibrils from a mineralized turkey tendon (scale bar: 0.1 mm). b) Woven fiber structure from the outer layer of a 19-week old human fetus femur. c) Plywood-like structure present in lamellar bone, from the fracture surface of a baboon tibia. d) Radial fibril arrays from human dentin, fractured roughly parallel to the pulp cavity surface. The tubules (holes) are surrounded by collagen fibrils that are all more or less in one plane. Adapted from Ref. [32] with permission, Copyright 1998 Annual Reviews.

sition of the medium as well as through specialized macromolecules such as polysaccharides and (glyco)proteins that act as promoters or inhibitors of nucleation, growth, and phase transformation.[24,38–40] Spatial control means that the mineral is formed in an enclosed space into which ions and molecules can be selectively transported, thereby resulting in control over the chemistry and kinetics of the growth of the crystal. Structural control is where an organic scaffold functionalized with active chemical groups templates the mineral formation,[41] inducing the nucleation of the crystal along a preferential face or axis. Morphological control relates to the shaping of biominerals into the complex morphologies that are often found. Control over the morphology is exerted by precipitating the mineral within an enclosed space. The confinement provides boundaries that direct crystal growth along specific directions determined not by the intrinsic crystallographic axes of the unit cell, but by the biological program. Here, the use of amorphous phases as precursors to the crystalline materials is essential, since amorphous materials are isotropic and easily moldable, thus allowing the directional restrictions of crystals to be overcome when building mineralized structures.[18–20,42,43] Constructional control is where hierarchical architectures are produced from the assembly of mineral-based building blocks. This process occurs across several length scales, from the nanometer to the macroscopic level. These strategies have formed the basis of many studies over the years that have aimed to elucidate the function of the specialized macromolecules and to reproduce the morphology and/or properties of the biominerals in the laboratory. These studies can be divided into two main groups: those aiming to understand controlled crystal nucleation and those aiming to elucidate how to control crystal morphology.

Much research on biogenic crystal nucleation has been performed using surfaces with acidic functionalities, where the interactions between the functional groups and the growing crystals have been investigated. These studies were done on Langmuir monolayers of fatty acids[44] and other surfactants[45] on aqueous subphases, as well as on self-assembled monolayers (SAMs) on solid substrates.[46–49] These studies have shown that the nature of the functional groups (i.e. COO^-, OH, SO_3^-, and PO_3^{2-}), their organization and orientation, and their ability to adapt to the demands of the developing mineral are important for effective nucleation and to achieve a high degree of orientational specificity.[50] These results suggest that the orientation of the crystal is dictated by the stereochemical and geometrical match between the functional groups in the organic template and the ions in the organic phase. However, there have also been reports showing that monolayers that were structurally highly dissimilar could induce the formation of calcite crystals with the same preferential (01.2) orientation, thus ruling out the effect of geometrical or stereochemical matching on crystal nucleation and growth.[51–54] It was then suggested that it is the average charge density or the mean dipole moment of the surface that actually determines the orientation and polymorph of the growing crystals. However, these studies did not rule out the possibility that the functional head groups in the monolayer could orient themselves to match the orientation of the carbonate groups in the (01.2) face of the calcite. Indeed, it was later demonstrated that the ability of the surfactants of the monolayer to adapt to the demands of the nucleating mineral phase is an important factor in the templating process and determines their activity in controlling nucleation.[55–57]

Control over crystal morphology is modulated in three ways in biogenic minerals: the first is by the interaction of the acidic proteins with specific crystal faces, thus changing the crystal habit.[39,58,59] The second is by growing the mineral in an enclosed space with a predefined shape that acts as a mold for the incipient crystal.[1,60,61] The third form of control is by providing a regulating, hydrogel-like environment inside the mineralization compartment in which the minerals grow.[62,63]

A combination of methods has been employed to understand how the acidic proteins are capable of modulating

crystal morphology. The proteins were first identified and sequenced, and then their function mimicked in in vitro crystallization assays. Many such proteins have been purified and sequenced, most notably from mollusk shells,[64,65] bone apatite,[35] diatoms,[6,66] and magnetite in magnetotactic bacteria, by using biochemical and molecular biology tools.[14] Several of these proteins were used as additives during crystal growth, and provided valuable mechanistic insights into the control of mineral formation. One example is the Mms6 protein from the magnetotactic bacteria, which was shown in vitro to be capable of forming superparamagnetic cuboidal crystals 20–30 nm in size.[67–69] The next step would be to translate the function of Mms6 to synthetic polymers that could modulate magnetite formation in a similar way.[70] Synthetic polymers such as polyaspartic acid, polyacrylic acid, and polystyrene sulfonate, among others, have been used successfully to tune the type of polymorph and morphology of calcium carbonate crystals.[50,71,72] Most interesting is the formation of chiral morphologies in calcium carbonate by interaction with chiral molecules,[73] and the formation of hierarchical structures by using low-molecular-weight and polymeric additives.[74,75]

The effect of confinement was demonstrated in the molding of crystal shapes, as for example by growing calcium carbonate in a polymer membrane that had the same morphology as the sea urchin skeletal plate.[76] The result was that single crystals of calcite formed with a morphology that perfectly replicated the membrane structure (Figure 5).

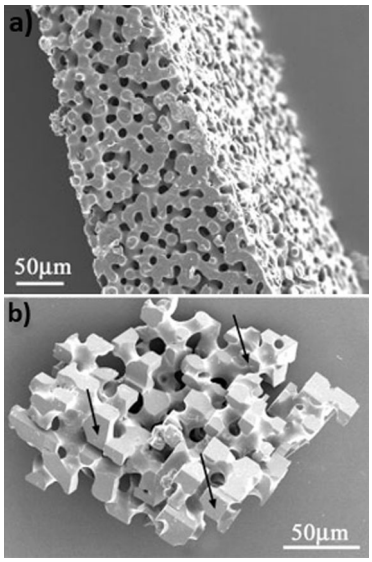

Figure 5. a) SEM image of a polymer replica of the sea urchin skeletal plate. b) SEM image of the singe crystal of calcite templated by the polymer replica in (a). Adapted from Ref. [76] with permission, Copyright 2002 Wiley-VCH.

Furthermore, rod-shaped pores of polycarbonate track-etch membranes have been used successfully as templates for the formation of single crystals of calcite nanowires with high aspect ratio.[77] The stabilization of ACC by small molecules such as phosphoenolpyruvate and 3-phosphoglycerate[78] or synthetic polymers such as polyaspartic acid, polyacrylic acid,[77,79] and polyalylamine hydrochloride,[80] which form a "polymer-induced liquid-precursor phase" (PILP),[71] is often instrumental in molding the morphology of calcium carbonate crystals. Confinement was also shown to affect the crystallization kinetics, and lead to the stabilization of ACC.[81,82]

Growing crystals in a gel-like environment was shown to change the crystal morphology by altering the kinetics of growth and the interface energy of the crystals.[83,84] Furthermore, it can also to lead to the inclusion of the gel inside the particle. Such organic inclusions, as are present in biominerals,[85] lead to structural alterations of the crystal lattice and significantly affect the mechanical properties of the resulting mineral.[86]

There is one important point that always needs to be kept in mind when trying to investigate the function of the organic matrix components with the aim of mimicking their effects in vitro, or to produce materials with similar properties as biominerals: the organic matrix components do not function in isolation. Rather, the three-dimensional assembly of the biomolecules into a framework is crucial for proper control over mineralization and over the properties of the material. Therefore, an understanding of the structure–function relationship of the organic matrix/mineral composite is very important for the development of bio-inspired materials. Furthermore, the interaction between the organic and inorganic phases also plays a critical role in determining the material properties of the biominerals. Hierarchical materials such as bone, teeth, and the skeleton of the glass sponge *Euplectela* illustrate this principle well.[12] Their mechanical properties, and hence their function, are highly dependent on the assembly of the basic building blocks, from the nanometer to the macroscopic level.

3. Applying Lessons from Nature: Synthesis of Biomimetic and Bio-Inspired Materials

So far we have discussed the major principles behind the formation of biogenic minerals in nature, and how it is possible to learn from the biological systems. In this section we will discuss how one can apply what we have learned from nature to the synthesis of bio-inspired materials with tunable morphologies and properties.

3.1. Molecular Templates

The ability of organisms to use macromolecules to control the nucleation and growth of biominerals has served as the basis of several methods for materials chemistry, especially for the synthesis of semiconductors, nanowires, and nanoparticles. The main idea behind this approach is to use functional molecules that can selectively induce the nucleation of specific inorganic materials and can control the crystal structure and size of the incipient particles by adsorbing to specific crystal faces. Thus, by tuning the chemistry and specificity of the molecule according to the desired inorganic compound, a very highly controlled formation of (nano)-

crystals can be obtained. This approach can be further expanded by using bifunctional molecules, such as peptides with two different domains, each being specific for a different compound. Each domain of the peptide will then control the nucleation of a different mineral phase, thereby resulting in the synthesis of hybrid particles that are composed of two different materials.

This method was exploited in the synthesis of core–shell CdSe–ZnS nanocrystals, where a peptide containing a CdSe-specific domain (Cys-Thr-Tyr-Ser-Arg-Lys-His-Lys-Cys, with the two Cys residues forming a disulfide bridge) and a ZnS-binding domain (Lys-Arg-Arg-Ser-Ser-Glu-Ala-His-Asn-Ser-Ile-Val) was employed (Figure 6).[87] In the first step, a CdSe core was synthesized. The CdSe domain of the peptide induced the nucleation of the CdSe nanocrystals with precise control over their size, thereby resulting in particles 4–5 nm in diameter. In the second step, these nanocrystals were allowed to interact with $ZnCl_2$ and Na_2S, where the second domain of the peptide induced the formation of the ZnS shell around the CdSe core. The thickness of the shell can be further modulated by using other functional peptides that can control the growth of the ZnS shell. The strength of this approach is that the peptide sequences can be fine-tuned to control the size and composition of the particles and to direct the formation of other inorganic materials and structures.

Molecules are also able to direct the morphology of minerals, thereby leading to the formation of synthetic materials with well-defined structures, sizes, and shapes. This level of control is particularly evident in the case of silica-based materials, since silica is amorphous and in the absence of any control it precipitates as formless gels or spherical colloidal particles. When polycationic peptides or polyamino acids derived from proteins involved in silica biomineralization are present, silica particles with morphologies such as nanospheres, hexagonal plates, organized fibrillar structures, and three-dimensional structures with periodic voids are obtained, depending on the shear forces applied during the reaction.[88,89]

All the above examples illustrate how molecular templates can be used to direct different steps in the synthesis of materials, namely the nucleation phase, crystal growth, and morphology. The first step in this process is to design the organic template (synthetic polymers, peptides) such that it can specifically interact and control the formation of the desired material. The second step is to fine-tune the size, selectivity, and chemistry of the template and the reaction conditions to precisely control the size, morphology, and structure of the product.

3.2. Supramolecular Templates

The supramolecular structure and the self-assembly properties of large molecules are exploited by organisms to template the formation of their mineralized structures, and this principle can also be employed in synthetic chemistry. The aggregates generated by using molecules that self-assemble into long or large structures will control the self-organization of nanoparticles by serving as a substrate for their deposition. Thus, the morphology of the resulting material will follow that of the organic template.

The use of DNA strands as templates for the organization of nanoparticles takes advantage of two characteristics of the molecule: the negatively charged phosphate groups in the DNA backbone can control the adsorption and binding of positively charged metal ions, while the DNA strands themselves can be synthesized with tailor-made structures and patterns.[90–92] The DNA has two functions in this process, the first is to template the morphology of the nanowires, and the second is to control their formation through its interaction with the metal ions during the deposition process. Thus, an array of nanoparticles that follows the architecture of the DNA strands can be produced, thus generating, for example, a nanowire connecting two electrodes[93] or one-dimensional and two-dimensional crossed-metallic nanowires.[94]

A further step in the application of supramolecular complexes is to use aggregates of molecules that are capable of translating their structure and morphology directly to the inorganic phase. This effect is observed when molecules with a triblock architecture termed dendron rodcoils are used to

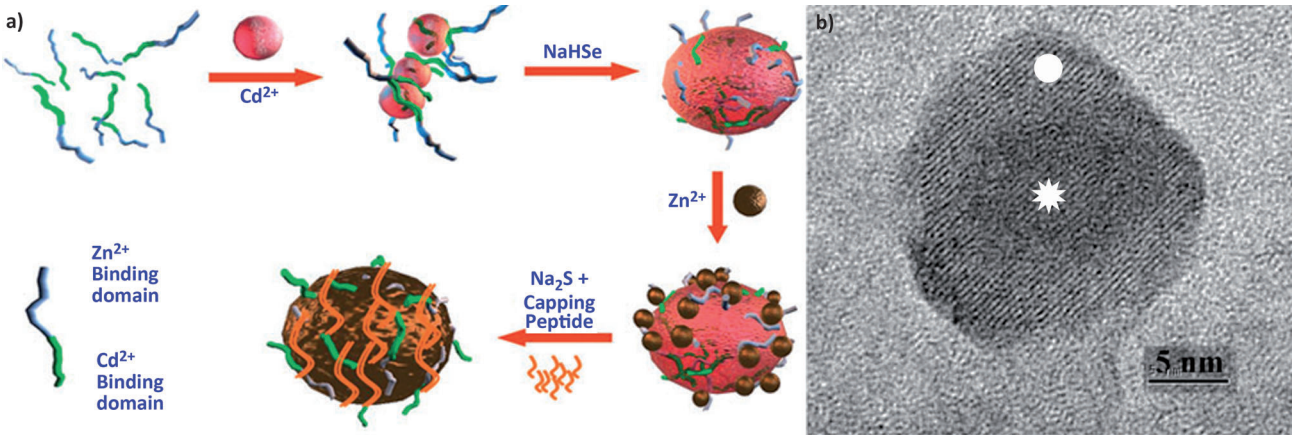

Figure 6. a) Schematic representation of the process of synthesis of core–shell nanoparticles by using a bifunctional peptide. b) High-resolution TEM image of a CdSe–ZnS core–shell nanoparticle, where the ZnS (white circle) has grown around the CdSe core (white star). Adapted from Ref. [87] with permission, Copyright 2010 Royal Society of Chemistry.

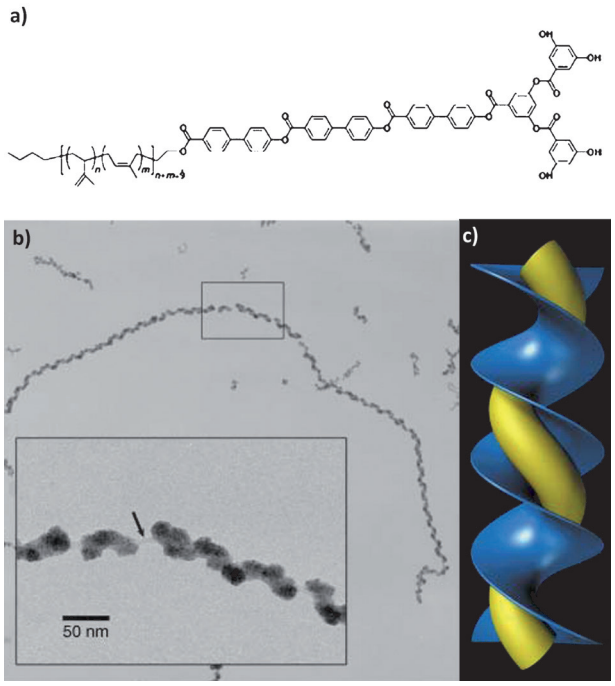

Figure 7. a) Chemical structure of the dendron rodcoil. b) TEM image of CdS helices precipitated in a suspension of dendron rodcoil nanoribbons in ethyl methacrylate. c) Schematic representation of a possible templating mechanism, in which a coiled CdS helix (in yellow) is produced from a twisted helical template through growth along one face of the template (blue). Adapted from Ref. [96] with permission, Copyright 2002 Wiley-VCH.

control the synthesis of CdS.[95,96] These molecules self-assemble into nanoribbons which act as templates for the production of CdS helices composed of polycrystalline domains of 4–8 nm (Figure 7).[96] Similarly, amine-containing organogels that self-assemble into fiberlike structures have been used to produce hollow silica fibers with linear, helical, and multilayered morphologies,[97,98] silica fibrils with a double-stranded helical structure,[99] silica nanotubes with adjustable meso- or macroscale inner diameters,[100] and chiral silica nanotubes.[101]

Polymers that assemble into vesicles and form emulsions in water also provide interesting supramolecular templates. Emulsions based on the EO_{76}-PO_{29}-EO_{76} triblock copolymer were used to produce silica spheres with well-defined multilamellar structures and high monodispersity,[102,103] while reverse emulsions based on EO_{39}-BO_{47}-EO_{39} generated hollow spheres with ultralarge mesoporous structures. Furthermore, block copolypeptides with specific recognition domains for silica and gold nanoparticles were employed to template the formation of hollow spheres with amorphous walls composed of two distinct layers of silica–gold nanoparticles.[104] This method is quite interesting since it represents an approach to produce silica by a hierarchical organization of nanoparticles into multidimensional composite arrays.

A last approach to be discussed here is where the self-assembly properties of macromolecules is exploited to form an enclosed compartment that will serve as a template for the formation of inorganic materials. One example is the use of amyloid-based polypeptides, which form hollow tubes that are a few micrometers in length and act as a casting mold for the formation of silver nanowires.[105] In this case, the function of the polypeptide is not to control the nucleation and growth of the nanoparticles as in the examples discussed above, but to serve as a template for the morphology of the silver deposits. Therefore, controlling the self-assembly of the polypeptide chain into higher structures is the crucial step in templating the deposition of the metal into desired morphologies.

3.3. Organized Surfaces as Templates

The use of surfaces to control the formation of minerals and inorganic phases is a very interesting approach that can lead to the formation of films and organized arrays of crystals. This method is based on using surfaces with active functional groups that will directly interact with the forming mineral phase, directing its nucleation, crystal orientation, polymorph type, and morphology.

Monolayers of surfactants can be effective in templating crystal formation, since they can provide an epitaxial match between the functional groups of the surfactant and the inorganic phase, thereby leading to controlled and oriented nucleation.[44] Organic surfactants were thus used to produce organized arrays of II-VI semiconductor nanocrystals with a homogeneous size and morphology,[106–112] while Langmuir monolayers were used as templates for the growth of semiconductors, and resulted in nanocrystals with different morphologies, such as rods, triangles, or as a continuous network.[113–115]

A three-dimensional template also provides an interesting approach to control the formation of organized arrays of nanoparticles. The power of this approach can be demonstrated by the use of non-ionic organic amphiphiles that form hexagonally packed cylindrical aggregates. Oleyl moieties form the hydrophobic core of the segments and the hydrophilic blocks composed of oligoethylene oxide are exposed to the solution and thus form a functionalized surface (Figure 8). Thus, the semiconductor material, in this case CdS, precipitates in the hydrophilic regions of the self-assembled structure, while the hydrophobic core remains empty of inorganic material. The symmetry and structure of the aggregates are preserved, thus resulting in nanostructured particles that consist of a polycrystalline semiconducting continuum with periodic nanometer-sized cavities.[116,117] This type of material is particularly interesting because the cavities can generate a periodic array of antidots that could modify the electronic properties of the material.[118–120] These cavities can, in turn, selectively adsorb, transport, or transform diffusing molecules according to the electronic and photonic properties of the semiconductor.[111] This approach, therefore, seems promising for organizing semiconductors into functional macroscopic structures.

Soluble additives can be employed in combination with a templating surface to control the formation of well-ordered inorganic structures. Such an effect could be observed in the case of aragonite films formed on a chitosan substrate in the

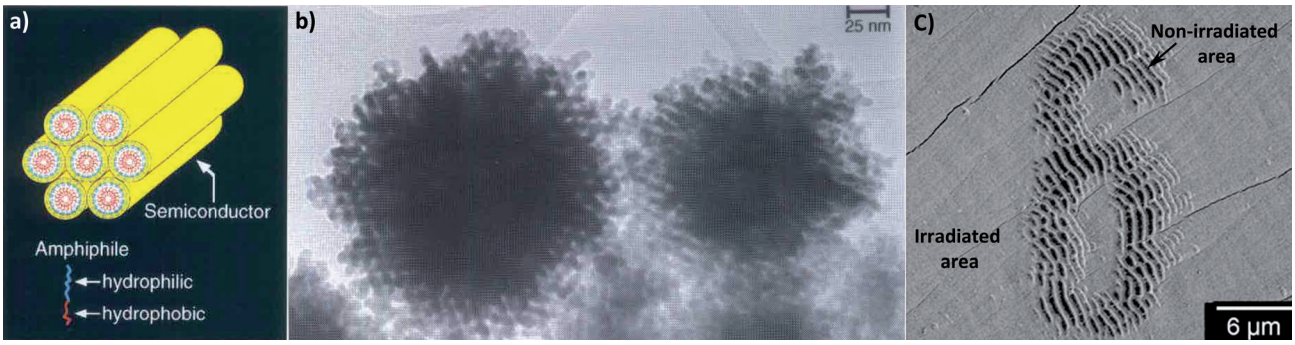

Figure 8. a) Schematic representation of an ordered organic–inorganic composite, in which the organic phase consists of hexagonally close-packed tubules of self-assembled amphiphiles, and the inorganic phase consists of CdS (yellow regions) that precipitated on the hydrophilic chains of the amphiphile. b) TEM images of the nanostructured CdS–amphiphile aggregate. Adapted from Ref. [116] with permission, Copyright 1996 Macmillan Publishers Ltd. c) SEM image of the $CaCO_3$ crystals formed on the photoimaged PVA–SbQ matrix, where the "6"-shaped area was protected from irradiation by a photomask. Adapted from Ref. [121] with permission, Copyright 2011 Wiley-VCH.

presence of polyaspartic acid and Mg^{2+} ions.[122] Periodic relief structures of calcite were formed when the chitosan matrix was replaced by cholesterol-bearing pullulans, which is a soft gel matrix, and polyacrylic acid (PAA) was used as an additive.[123] Here, the gel matrix prevents the transport of calcium and carbonate ions, so that when the calcium carbonate precipitates, the ions are rapidly depleted and precipitation stops. When the ion concentration recovers, the mineral can precipitate again, in a cyclic manner, thus forming the relief patterns. A similar effect was observed when the substrate is composed of poly(vinyl alcohol) (PVA).[124] The cooperative effects of the PVA matrix and the additive polyacrylic acid results in the formation of a flat, thin film of calcite in the first stage, which later develops into three-dimensional relief structures composed of needlelike calcite crystals. The templating effect of surfaces on crystal growth can be further exploited to create patterned surfaces through the use of lithography methods, where for example photoreactive polymers are used as substrates for crystal growth. This approach was demonstrated with the use of a photosensitive PVA derivative with styrylpyridinium groups (SbQ) as the matrix, which acts as the substrate on which to grow $CaCO_3$ films (Figure 7b).[121] The SbQ groups are UV-sensitive and dimerize upon irradiation. By using a photomask with a checkered or a "6" pattern, the authors have shown that flat, smooth $CaCO_3$ films grew on the UV-irradiated areas, while self-organized regular surface relief structures formed on the non-irradiated areas. The mechanism of formation of these relief structures is similar to the one described above for the pullulan matrix, and occurs only on the non-irradiated part of the substrate.

3.4. Phage-Display Libraries

Phage-display libraries have proven very powerful in selecting peptides that bind to a variety of semiconductor particles with high specificity. This technique is a high-throughput method to screen protein–substrate interactions by expressing different proteins or peptides on the capsid of the bacteriophage virus. The substrate of interest is then immobilized on the surface of a well and exposed to the virus. The one that displays the protein capable of binding to the substrate will remain, while the others will be washed away. The viruses can then be eluted and further multiplied by bacterial infection to produce more of the relevant phage. The DNA of the virus can also be extracted and sequenced to identify and characterize the protein or peptide of interest.

This technique has allowed the identification of peptide sequences capable of binding to semiconductor substrates.[125] In addition, it has opened up the possibility to exploit viruses as substrates for the synthesis of semiconductors by expressing peptides that control the size, composition, and phase during the nucleation of nanoparticles on the capsid. Thus, while these peptides will control the nucleation and growth of the nanocrystals, the capsid will serve as a scaffold for the formation of the semiconductor (Figure 9a,b).[126] CdS and ZnS nanocrystals 3–5 nm in size were produced by this approach as polycrystalline aggregates, preferentially aligned on the capsid of the virus. The organic template was removed by annealing, and the nanocrystals formed single-crystal nanowires with high aspect ratio, being several hundreds of nanometers in length and only about 20 nm in width (Figure 9c,d). This approach is also versatile, since the peptide sequence can be fine-tuned according to the material, crystal size, morphology, etc. For example, nanowires of ferromagnetic FePt (Figure 9e,f) and CoPt were also produced by changing the substrate-specific peptide.

3.5. Replicating the Structure and Function of Biominerals In Vitro

So far we have discussed how bio-inspired approaches have been applied to the synthesis of synthetic materials with technological and industrial applications. In addition, much research has been devoted to learning how to produce synthetic materials that replicate the structural and functional properties of biominerals. Research in this area is particularly evident in regenerative medicine and biomedical engineering, where the aim is to either induce the regeneration of a tissue or to completely replace it.

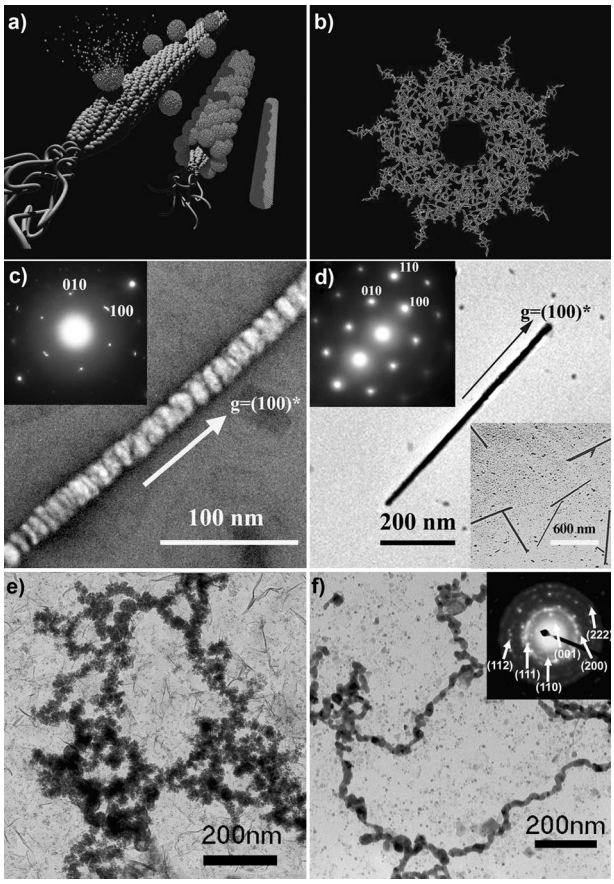

Figure 9. a) Schematic representation of the nanowire synthesis, showing the different steps for the nucleation, ordering, and annealing of virus–particle aggregates. b) The symmetry of the virus allows the nucleated particles to be ordered along the *x*, *y*, and *z* directions, thus fulfilling the requirements for aggregation-based annealing. c) Dark-field diffraction-contrast imaging of the preannealed ZnS system using the (100) reflection, showing the crystallographic ordering of the nucleated nanocrystals. Inset: electron diffraction pattern of the polycrystalline preannealed wire, showing the wurtzite crystal structure and the single-crystal-type [001] zone axis pattern. d) Bright-field TEM image of an individual ZnS single-crystal nanowire formed after annealing. Inset, upper left: electron diffraction pattern along the [001] zone axis showing a single-crystal wurtzite structure of the annealed ZnS nanowire. Inset, lower right: low-magnification TEM image showing the monodisperse, isolated single-crystal nanowires. e) TEM image of the unannealed FePt wires. f) TEM image of the annealed FePt wires. Inset: ED pattern showing the crystalline nature of the material. Adapted from Ref. [126] with permission, Copyright 2004 AAAS.

Thus, biologically inspired materials have great potential in the fields of regenerative medicine and biomedical engineering.[127] One biological tissue that has been the subject of research for biomimetic replacement materials is bone. Bone has unique mechanical properties that arise from its hierarchical structure and may vary according to the function that a particular bone performs at a particular location in the body.[32] In the case of severe traumas, the tissue needs to be replaced using artificial materials to restore its function. Therefore, there is great interest in developing bio-inspired materials that possess osteoinductive properties, namely being capable of inducing bone regeneration and eventually being resorbed by the organism and replaced by bone, or that can be directly used as a replacement material. Several approaches have been under development. The osteoconductive properties of biogenic calcium carbonate have inspired the use of synthetic calcium carbonate as coatings for three-dimensional polymers or ceramic scaffolds to improve their biocompatibility. These induce the attachment and differentiation of bone cells, thus inducing bone repair.[128,129] A significant advance was made a few years ago in the development of implant materials that better mimic bone structure and composition when it was demonstrated that collagen mineralization could be mimicked in vitro by substituting the noncollagenous proteins by the synthetic polymer polyaspartic acid.[130] For the first time, intrafibrillar mineralization of collagen was achieved under in vitro conditions, where the apatite crystals formed inside the collagen fibrils had the same morphology and orientation as in bone.[130,131] These findings open up new possibilities in developing bone-replacement scaffolds composed entirely of mineralized collagen and that have optimal osteoconductive properties. In attempting to mimic the hierarchical structure and mechanical properties of bone, an approach has been under development where wood templates are chemically converted to obtain a hydroxyapatite scaffold.[132] During the transformation process, the hierarchical structure and three-dimensional morphology of wood is preserved and translated to the mineral, thereby generating a scaffold that could potentially support cell growth, vascularization, and at the same time satisfy biomechanical requirements, thus providing the mechanical properties that are required for the tissue.

Ultimately, an ideal biomimetic scaffold should be able to reproduce the composition, three-dimensional structure, and overall properties of a biological tissue and thus be able to restore its function. However, producing such a scaffold is still a challenge since biological tissues, and bone in particular, have quite complex architectures that are directly correlated to their function and overall properties. Nevertheless, biomimetic approaches to try to understand and mimic the way that mineralized biological tissues are designed and formed has provided significant advances in the design of bio-inspired materials that have great potential for tissue engineering (reviewed in Ref. [133,134]).

4. In Situ Methods To Investigate the Synthesis of Bio-Inspired Materials

The often astonishing material properties of crystalline biominerals are generally related to the hierarchical assembly of specifically interacting organic and inorganic components. When trying to synthesize new materials with similar advanced properties by applying nature's biomimeralization strategies, it is not possible to employ approaches based solely on empirical routes (trial and error). Given the complexity of these systems, such approaches would be highly inefficient in producing materials with desired properties. Without a proper understanding of the mechanisms of the biomimetically controlled formation of minerals one cannot predict or

control the morphology, structure, and overall properties of the material being synthesized. The complex dynamics involved in organic-matrix-controlled mineralization means that in situ, time-resolved techniques are essential to understand the mechanisms of formation. Such techniques allow the investigation and monitoring of the changes in shape, morphology, and crystallinity, as well as the structural dynamics of both the organic and inorganic phases in (real) time.[135]

Several techniques including dynamic light scattering, X-ray scattering, infrared, and Raman spectroscopy are very useful to obtain in situ information about biological models as well as biomimetic systems. Light scattering, for example, can give information on the dynamics of particle formation, revealing the evolution of size and mass over time.[136,137] X-ray scattering, on the other hand, can give morphological and structural information, depending on the scattering vector used. Small-angle X-ray scattering (SAXS) probes the overall size, aggregation behavior, mass density, morphology, and number density of evolving particles, while wide-angle X-ray scattering probes the crystallographic structure of the particles.[135] Infrared and Raman spectroscopy both probe atomic interactions in compounds, being useful to detect changes in the chemistry or structure of materials. For example, both techniques can be used to study the transformation of amorphous calcium carbonate into the more stable polymorphs vaterite, aragonite, and calcite.[138]

Microscopy comprises another powerful set of tools for the in situ study of the synthesis of bio-inspired materials. Optical microscopy is highly suitable for visualizing materials in the micrometer range, and the possibility of using crossed polarizers enables the development of crystallinity in mineral compounds to be monitored.[129,139] Atomic force microscopy is generally used to study surface morphology and topography at the nanoscale. It can be used, for example, for time-resolved in situ studies of the thermodynamics and kinetics of mineral formation,[135] the effect of additives in solution on the development of growth steps on mineral crystals,[73] and for the study of two-dimensional arrays such as nanowires or nanoparticles.[94]

Electron microscopy is the most powerful tool to investigate the formation, structure, and chemical composition of biogenic and synthetic biomimetic minerals, as it combines imaging with the possibility of obtaining structural (through electron diffraction) and chemical (through electron-dispersive X-ray spectroscopy, EDX, and electron-energy loss spectroscopy, EELS) information on the same part of the sample.[140,141] However the use of conventional electron microscopy is limited, since the samples have to be dehydrated, which may not only cause morphological changes, but also hampers the in situ study of biomimetic mineral formation. This limitation was circumvented with the advent of cryogenic electron microscopy (cryoEM), in which samples are rapidly frozen to preserve their native hydrated state, and observed either by SEM or TEM under cryogenic conditions. In particular, cryoTEM provides unique possibilities for quasi in situ and time-resolved analysis of biomimetic mineralization.[142,143] The high-resolution provided by TEM means that features with dimensions of less than a nanometer and up to a few hundred nanometers can be imaged. Furthermore, cryoTEM can be used in combination with cryo-electron tomography (cryoET)[144] to obtain three-dimensional morphological and spatial information on the biomimetic mineralization system.[131,145–147] CryoTEM can be supported by electron-diffraction, EDX, and scanning-transmission electron microscopy (STEM), which give compositional and structural information on the specimen.

CryoTEM has been successfully applied to show the presence of the recently discovered prenucleation clusters[148] in biomimetic calcium carbonate mineralization and their aggregation into aggregates 2–4 nm in size, which form the onset of the nucleation of amorphous nanoparticles in solution.[147] In a following stage, it was shown how a template constituted of a Langmuir monolayer of stearic acid could direct the first assembly of the nanoparticles into amorphous calcium carbonate and subsequently their transformation into oriented calcite through a vaterite intermediate (Figure 10).

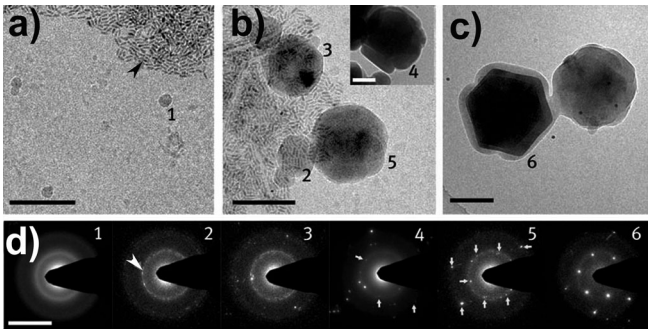

Figure 10. a–c) CryoTEM images of the early, intermediate, and mature stages of development of calcium carbonate particles, templated by a monolayer of stearic acid (arrow in a). Scale bars: 200 nm. d) Low-dose selected-area electron diffraction patterns of the particles marked in (a–c), showing the development of crystallinity during mineralization. Adapted from Ref. [147] with permission, Copyright 2009 AAAS.

A more detailed study was performed on calcium phosphate, and also showed the role of the template surface, in this case a monolayer of arachidic acid, in directing the aggregation of prenucleation clusters, followed by the formation of amorphous calcium phosphate and the development of oriented apatite crystals.[145] Unraveling the mechanisms of the surface-controlled formation of apatite is particularly important, since calcium phosphate is the major constituent of bone and teeth, and its deposition is also involved in certain cases of pathological mineralization, such as atherosclerosis.

In regard to biomimetic bone materials, we discussed previously that inhibitors of apatite nucleation such as polyaspartic acid can be used to induce the intrafibrillar mineralization of collagen, thereby resulting in mineralized collagen fibrils that resemble bone collagen both in composition and in structure.[130] CryoTEM and cryoET have been instrumental in revealing the mechanisms of mineral formation under these synthetic conditions, and has shown the twofold role of collagen in controlling the mineralization process: the charge interactions between its surface and the

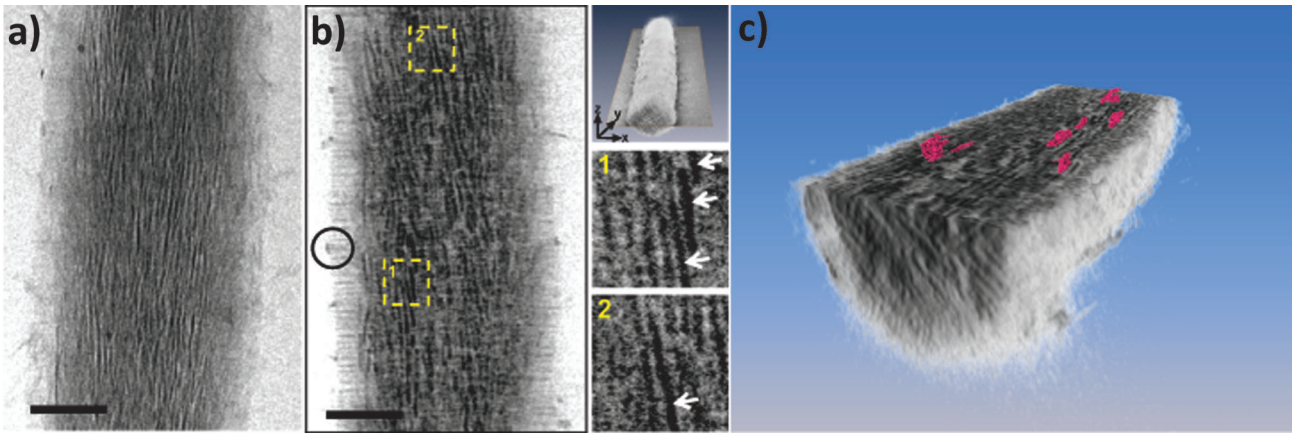

Figure 11. Cryo-electron tomography images of a collagen fibril mineralized in vitro in the presence of polyaspartic acid and stained with uranyl acetate. a) Two-dimensional cryoTEM image. b) Slice from a section of the three-dimensional volume along the *xy*-plane (top inset), where crystals are visible edge-on (insets 1 and 2, white arrows). Black circle: amorphous calcium phosphate infiltrating the fibril. The crystals nucleate on the uranyl acetate staining bands, which mark the location of clusters of charged amino acids of collagen that mediate the transformation of amorphous calcium phosphate into oriented hydroxyapatite. c) Computer-generated three-dimensional visualization of mineralized collagen. The fibril is sectioned through the *xy*-plane, revealing plate-shaped apatite crystals (pink) embedded in the collagen matrix. Scale bars: 100 nm. Adapted from Ref. [131] with permission, Copyright 2010 Macmillan Publishers Ltd.

calcium phosphate–polymer complex was found to be essential to mediate the infiltration of the mineral into the fibril, and the charged amino acids in collagen form nucleation sites that control the transformation of amorphous calcium phosphate into apatite (Figure 11).[131] These findings, therefore, are important for the further development of biomimetic materials and scaffolds for tissue engineering.

In the field of nanoparticles, several studies have made use of cryoTEM to investigate the synthesis of liposome–nanoparticle hybrids with potential use in medical imaging, drug delivery, and nanotoxicology.[149–154] This technique provided sufficient resolution to visualize the individual nanoparticles, such as Au, CdSe, Fe_2O_3, and their location within the vesicles and their native environment, thus providing new insights into the interactions between the particles and the liposomes. These studies are fundamental for the synthesis of such liposome–nanoparticle hybrids, since they help to determine how the nanoparticle size, hydrophobic layer ligand, layer thickness, and chemistry as well as lipid composition influence the structure of these hybrids.

The application of cryoTEM and cryoET to materials chemistry can help to provide the required combined morphological, structural, and chemical information necessary for the development of biomimetic synthesis routes to materials with predefined structures and properties. Nevertheless, one must realize that every technique has its own limitations and can only generate part of the information required. For a fundamental understanding of the processes involved in the formation and properties of biomimetic materials, a combination of different techniques is required to collectively provide detailed information on the structure, morphology, chemistry, and other properties of the material. Different techniques should be used to cover different size ranges and time scales as well as achieve different spatial and temporal resolutions.

5. Summary and Outlook

Biomimetic approaches are very promising for the design of advanced materials. The application of self-organization, such as supramolecular template synthesis, template-directed crystal growth, phase separation, and self-assembly, has great potential for tailoring the structure, size, function, and properties of materials from the nanometer to the macrometer level. The feasibility of these approaches has been successfully demonstrated in the fields of nanoelectronics, semiconductors, nanowires, silicification, and biomedical engineering. Organic scaffolds and templates have been used to produce materials with controllable sizes, composition, morphology, crystallinity, and hierarchical organization. However, so far such functional materials have only been produced on a laboratory scale, and their synthesis on an industrial scale with direct technological applications and in a cost-effective way is still very limited. Expanding our capacity to produce advanced materials—particularly on a larger scale—goes hand in hand with increasing our understanding on the mechanisms of formation and on the structure–function relationship of biomaterials.

Moreover the formation of biominerals in nature demonstrates that such advanced hybrid materials can be obtained from renewable feedstocks by synthetic routes that use aqueous reaction media and ambient conditions exclusively. At present, our modern day society uses natural resources approximately 1.4 times as fast as the Earth's ecological systems can replace them. This puts a strong demand on the development of new materials with a reduced ecological footprint, that is, materials, the production of which puts a lower demand on the Earth's natural resources. It is our strong belief that the investigation of biomimetic synthesis routes may lead to more sustainable alternatives to many of the materials and the production routes used today. As we

learn more, new possibilities will open in the fields of material science, chemistry, and biomedical engineering to sustainably produce materials with tunable functions, morphologies, and properties.

We thank the Netherlands Science Foundation, NWO, for financial support.

Received: September 21, 2011
Revised: January 19, 2012
Published online: May 25, 2012

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