Self-Assembly Process of Soft Ferritin-PNIPAAm Conjugate Bionanoparticles at Polar-Apolar Interfaces

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Published in:
Langmuir

DOI:
10.1021/la3042988

Journal of Materials Chemistry

Cite this: *J. Mater. Chem.*, 2011, **21**, 18903

www.rsc.org/materials

FEATURE ARTICLE

Synthetic inorganic materials by mimicking biomineralization processes using native and non-native protein functions

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Received 1st June 2011, Accepted 15th September 2011
DOI: 10.1039/c1jm12490k

Nature is able to produce various inorganic structures with very specific fine structures in the micro- and nano-regime, which are facilitated and controlled by protein-based systems. Enzymes like silicateines catalyse biomineralization and provide organisms with exoskeletons with specific material properties. While these structures are interesting materials in biology, they also offer ample opportunities for material scientists to create man-made materials with the same biological species in a non-natural setting. While natural organisms rely on specific proteins for certain processes, other more accessible proteins show similar capabilities even though it is not their native function. Mimicking biomineralization provides a route for the formation of new materials of various shapes and compositions. In this article, synthetic processes and the resulting materials will be discussed, describing the tools and bio-inspired systems used and comparing the original biological function of the protein to its role in the non-natural process.

1 Introduction

Novel materials are essential for technological progress, and the use of biological processes for the development of these materials receives increasing attention. While in the past organic and inorganic materials attracted interest in separate disciplines, today the combination of those materials leads to hybrid materials displaying interesting properties. Although these materials are termed novel, nature has already realized their advantages a long time ago. The more naturally occurring materials are investigated, the more important properties of those and their potential use in various technologies are discovered. Potential applications are, amongst others, nano-catalytic reactors, nano-containers, high performance materials and all materials with the requirement of biocompatibility.

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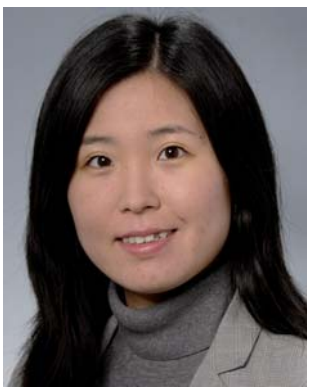

Huihui Wang

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Natural composites do not only consist of two or more components that are randomly combined, but they influence each other, and often the self-assembly of one part directs the formation of the other.[1] With this approach, nature is capable of building complex, hierarchically organized materials under ambient conditions. Even though the first investigations on biological hybrid materials were initiated in the first decades of the last century,[2,3] it seems that only the tip of the iceberg in terms of understanding the synthesis and the properties of these materials has been unveiled. However, what has been learned so far is already sufficient to design and produce artificial hybrid materials with extensive potential by the use of nature's concepts, thereby fully exploiting the advantages of natural materials. Many precursors for these materials are available in large amounts, the materials are often biocompatible, the reaction conditions are mild and the mechanical properties are tuned to fit the exact requirement of the desired materials application.

Here we present most recent insights into materials formed in ways similar to that of various organisms. Furthermore, we show novel approaches of using proteins for the production of composite materials as well as successful modifications of proteins for the production of biomimetically designed materials.

2 Matrix proteins

Most of the species forming biominerals use a matrix of proteins as a scaffold and to enhance the mechanical performance of the material. The diversity of proteins used by nature is extensive, and here we present a selection of those frequently used as a matrix for the process of biomineralization. Despite their diversity, there are general features which explain why the use of matrix proteins is advantageous for the formation of protein–inorganic composite materials.

Composite materials can offer new characteristics which are more than just a superposition of the properties of the individual components. A common feature of biominerals is a plywood-like structure with alternating layers of protein and mineral, having a large difference in the Young-moduli of the hard and soft layer. First, the mineral provides stability against tensile loads and the protein protects the material against shear loads.[4] Second, alternating layers prevent crack propagation and make the material tough. This effect works best if the ratio of the Young-moduli is larger than five, and it works even for very thin layers of protein.[5] This explains the mechanical properties of many materials, but it is not sufficient to explain the properties of bone.

In addition to improving the material, matrix proteins serve as a framework during the mineralization process. Many possible functions of the matrix have been formulated: It could provide cavities that enable the crystal growth and limit its size, create domains where local supersaturation enables mineralization due to the presence of charged groups, or control the mineral phase by periodic arrangement of charged groups located on the proteins.[6] The exact function of the matrix as found in the living organism is often difficult to determine as there is always a mixture of proteins. For the *in vitro* biomineralization process, the standard procedure is extraction and purification of the active protein, followed by a mineralization *in vitro*. The functions of proteins in the biomineralization process vary from protein to protein and an overview on important and promising proteins will be given later in this section.

The aforementioned cavities recently received attention as they could control the size and shape of the crystals by blocking further crystal growth. Most biomaterials are composed of nanometre-sized crystals which frequently contain protein impurities from co-precipitation, which behave similar to micro-cracks. It was shown that these crystals have the same mechanical properties as perfectly pure crystals if they are smaller than $\sim$ 30 nm, which explains the small size of crystallites in many materials.[4] The common anisotropy within the materials can be explained by means of optimization, ensuring that mineral and protein fail at the same time under critical mechanical loads. This optimization reduces the amount of mineral and the weight.[4]

Patrick van Rijn

Patrick van Rijn is an Alexander von Humboldt-fellow for Post-doctoral research in the group of Alexander Böker at the RWTH Aachen, which he joined 2010. He received a MSc in Organic Chemistry at the University of Groningen in 2005 after which he started his PhD research in the group of Prof. Jan H. Van Esch at the same University in the field of Supramolecular Chemistry. Early 2007, he joined the Self Assembling Systems' group in the move to Delft University of Technology and successfully defended his thesis in 2010 on the topic of "curved conjugated amphipathic amphiphiles".

Alexander Böker

Alexander Böker holds the Chair of Macromolecular Materials and Surfaces at RWTH Aachen and is Co-Director of the DWI an der RWTH Aachen e.V. He studied chemistry at the Johannes Gutenberg University Mainz and received his PhD from Bayreuth University in 2002. From 2002–2004 he was a post-doctoral fellow with Thomas P. Russell at the University of Massachusetts, Amherst. In 2006 he received a Lichtenberg-Professorship funded by the VolkswagenStiftung. The main research interests of his group include guided self-assembly of block copolymer systems, hierarchical (bio)nanoparticle assemblies and the control of self-assembly processes via external fields.

2.1 Proteins used for biomineralization by nature

Biominerals are organized hierarchically, and proteins are involved in every hierarchical level. If we want to build new materials, the understanding of the interactions between mineral and protein and their self-organization is crucial. Here, the most recent and interesting findings on biominerals will be discussed.

A general approach of natural biomineralization systems for the formation of materials is the use of an organized protein matrix as an overall framework. In bone, the collagen assembles itself into fibrils and fibers, being mechanically stable due to crosslinking. Collagen tends to build fibers composed of triple-helices due to its characteristic repetitive motifs in its sequence.[7,8] The mineralization with hydroxyapatite, a mineral phase of calcium phosphate, takes place inside the cavities in between the fibrils (interfibrillar) as well as inside the fibrils themselves (intrafibrillar).[9] The exact arrangement of collagen and hydroxyapatite was explained in detail by studying the mechanical properties with different models. The most suitable structure is composed of parallel mineralized collagen fibrils. The region between the end of one collagen fibril and the beginning of the next, known as the overlapping region, is less mineralized. The mineral platelets have a specific size that corresponds to the distance between one gap and the next, across one overlapping region (see also Fig. 3).[10]

An important factor of the resulting structure is its anisotropy. The hydroxyapatite starts to grow as a plate, with the elongated c-axis of the crystal aligned parallel to the fiber-axis. This in-plane growth is quick, followed by a much slower growth in thickness until the whole cavity is filled.[8] The total fraction of mineral to protein is 0.43–0.56, resulting in an increase in Young-modulus by a factor of 200, an increase in the elastic stress by a factor of 5, and a decrease of the elastic strain by a factor of 50.[10] The ratio of the mechanical properties along the fibre axis and its orthogonal axis reflects the anisotropy of the material. The ratio of the elastic moduli is 20 and the ratio of the tensile strength is 15, meaning that bones can take strong loads only along their main axis. The mechanical properties orthogonal to the main axis are dominated by the interfaces between hydroxyapatite and collagen.[11] The mechanical properties also show a strong dependence on humidity. Bone samples were kept wet over the whole process of sample preparation and were measured at a controlled humidity. The results show that both the strength and the elastic modulus increase with decreasing water content.[8,11]

The mineral component of teeth is also hydroxyapatite, but the protein components are different. The main component of the dental matrix is amelogenin. This protein is largely hydrophobic, adsorbs readily on different surfaces, including octacalcium-phosphate and creates nanospheres with a diameter of 20–60 nm in solution *via* self-assembly.[12] Depending on its concentration, the protein influences the morphology of growing crystals.[13] This influence on the growing crystallites was also shown in knockout mice (where the gene for amelogenin formation was disabled), where not only the structure of the teeth changed, but also the interaction of the osteoblasts with the teeth was disturbed.[14]

Another interesting and even more studied system is the basins of molluscs, the nacreous part in particular received a lot of attention. Nacre is often only a part of the basin of which the oyster is a good example. The outer layer, which is also called the prismatic layer, is composed of different proteins and calcite, the thermodynamically most stable calcium carbonate species. The inner layer, which is also called the nacreous layer, is composed of a mixture of proteins (which is still not completely understood) and the mineral aragonite.[15,16] The main non-mineral component of nacre is β-chitin, an abundant polysaccharide with big crystalline parts composed of parallel strands.[17] The assembly of β-chitin occurs, like in bone, without the mineral and is not directed by cells. Chitin crystals can form nematic phases, but this process is not dominant in nature as the system has not enough time to reach equilibrium. The matrix contains several proteins with a high affinity to chitin which act as a glue.[16] Furuhashi *et al.* claim that chitin might not be as important as generally accepted, because chitin is possibly not present in all molluscs and the tests for chitin might sometimes be incorrect. It should also be emphasized that the acidic proteins bound to the chitin-matrix could also have an important influence on the mineralization, as they can bind to the {001} side of an aragonite crystal. This crystal face is important, as the crystallites are oriented along this face. It has been suggested to focus on silk fibroins, which have a repetitive sequence with big β-sheet domains, as some molluscs have proteins which appear to be similar to silk fibroin.[18]

The organized matrix of chitin is filled with amorphous calcium carbonate as a precursor, which mineralizes to aragonite. While the chitin-matrix controls size and shape, solvated proteins change the mineral phase to the thermodynamically unstable aragonite as well as the shape of the crystal.[16] The interactions of extracted proteins with crystals have been studied in much more detail, because this is easier understood than the whole process with multiple components involved in the mineralization process. Various authors extracted, purified and sometimes also produced proteins synthetically or in a recombinant way. Their influence on the shape and phase of the minerals was examined. Many proteins from the nacreous layer lead to the growth of aragonite instead of calcite, and some of these proteins displayed interesting and promising properties. The protein N16N, extracted from nacre, was shown to influence the growth of aragonite in such a way that it results in a sandwich-like structure of protein and mineral. The protein itself does not orient the mineral; the mineral orients itself by mineral bridges through the protein layers. N16N occurs together with chitin in nature and might play an important role for biomineralization.[19] Metzler *et al.* studied the interaction of the protein with the crystal in more detail and found that the protein forms an ordered array due to the interaction of its negatively charged groups with the crystal surface.[20] The influence of proteins and peptides on the growing crystal and its defect structure was investigated directly *via in situ* AFM. Elhadj *et al.* compared various proteins and showed that the acceleration or deceleration of crystal growth is concentration-dependent. The effects of different proteins also depend on their charge, with the acidic groups being most important.[21] The combination of proteins is typical for every organism, and it is therefore improbable that the crystal growth proceeds in exactly the same way in every organism.[22]

Chitin is also the matrix component of the lobster's armor, but here the modification is crystalline α-chitin. The larger fraction of the partly crystalline chitin fibers has the ordering of its c-axis

(which is the fiber axis) parallel with respect to the surface, building a plywood-like structure. A small fraction of the fibers is aligned orthogonally with respect to the surface and interpenetrates the chitin-layers. The cavities of the material are filled with calcite localized in the fibers orthogonal to the surface and preferentially in the outer layers.[23] The reason for this is most probably the hardening of the outer layer against mechanical attacks. Amorphous calcium carbonate is the dominant phase in the layers far below the surface.[24] The chitin is not the only protein component that takes part in the biomineralization. An acidic protein from the crayfish directs the growth of calcite from square particles to round particles, and this protein also changes the structure of chitin. Yamamoto *et al.* showed *via* genetic modifications that the acidic part of the protein is crucial for its function.[25]

Calcium carbonate formation also occurs in the shell of eggs. Many extracted proteins show strong interactions with calcium carbonate and influence the crystal morphology in precipitation experiments.[26,27] Wang *et al.* examined the interactions between ovalbumin and calcium carbonate crystals *via in situ* AFM. They showed that calcite is the main phase, exhibiting many homocharged surfaces which are stabilized by the protein ovalbumin.[27] Another way to measure the interactions of proteins with calcium carbonate is cyclic voltammetry.[26] Pipich *et al.* investigated the growth of calcium carbonate crystals in detail using MD-simulations. Without protein, an amorphous calcium carbonate converts into calcite *via* metastable vaterite. The simulation shows that vaterite is not a metastable species anymore when ovocleidin-17 adsorbs on the crystals, so that the amorphous phase converts directly into calcite. They also showed that the protein binds tightly to small crystallites (192 formula units), while it desorbs from bigger crystals. This optimizes the phase transition from amorphous to crystalline for as many particles as possible, as the transition is accelerated by the adsorbed protein. When the transformed calcite crystal begins to grow, the protein desorbs again and is ready to transform another particle into the crystalline phase.[28] The transfer of the crystals from amorphous to crystalline was also investigated with SANS in the presence of ovalbumin. The complexation of the protein with calcium unfolds the protein, making its affinity to calcium higher, resulting in an self-amplifying process. These unfolded proteins build larger complexes that facilitate the growth of amorphous calcium carbonate particles, which convert to vaterite and finally to aragonite. The resulting crystals are still interconnected by the protein, which might be a potential mechanism of alignment to mesocrystals.[29]

Diatoms typically build complex and beautiful structures of silica, some examples are shown in Fig. 1. The deposition of silica is guided by silaffins, cingulines, long-chain polyamines and silicateins, whereas the latter ones form materials by catalytic transformations which will be discussed later on. Silaffins have catalytic activity in some cases, but they are also part of the structural matrix and because of that, we discuss them in this section as well as together with the enzymes. The mechanism of biomineralization for silaffins and cingulines differs from the ones found for bones and nacre. Diatoms do not possess a structural matrix that organizes itself before the biomineralization process of the shell, but rather a matrix that reorganizes itself frequently during the process of mineralization.

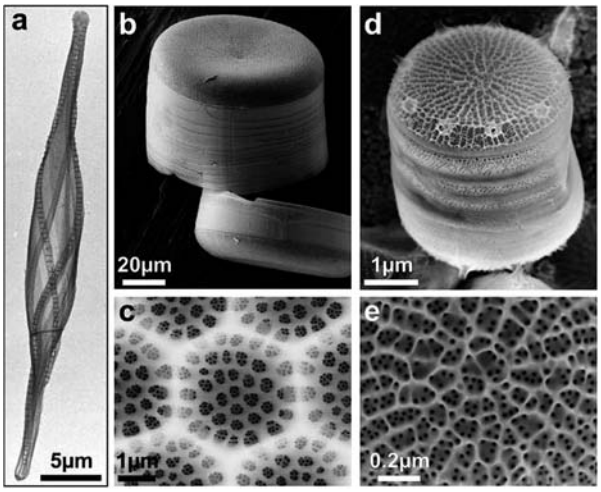

Fig. 1 Structures of diatom cell walls. Electron microscopy images of isolated cell walls from different diatom species: a) *Cylindrotheca fusiformis*; b,c) *Coscinodiscus asteromphalus*; d,e) *Thalassiosira pseudonana*. Figure and description are taken from ref. 30 and reprinted with permission of RSC, Copyright 2004.

The most important component in the matrix of diatoms are the silaffins, which are composed of a highly phosphorylated (and therefore negatively charged) peptidic backbone and polyamine (positively charged) side chains. The whole process of self-organization is illustrated in Fig. 2 which displays the silaffins stabilizing a hexagonally arranged emulsion in the silica deposition vesicles of the diatom. The silaffins at the interface mediate the precipitation of silica from silicic acid in the diatoms, and parts of the silaffins co-precipitate with the silica. After the formation of a silica shell around the droplet, smaller droplets are formed in the cavity of the old droplet.[30,31] The decreasing size of the droplets can be explained by a lower concentration of

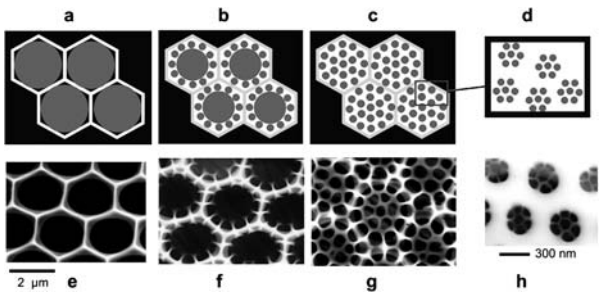

Fig. 2 Schematic drawing of the templating mechanism by the phase separation model (a–d) and comparison with different stages of the cell wall biogenesis of *C. wailesii* (e–h). a) The monolayer of polyamine-containing droplets in close-packed arrangement within the silica deposition vesicle guides silica deposition. b,c) Consecutive segregations of smaller (about 300nm) droplets open new routes for silica precipitation. d) Dispersion of 300nm droplets into 50nm droplets guides the final stage of silica deposition. Silica precipitation only occurs within the water phase (white areas). The repeated phase separations produce a hierarchy of self-similar patterns. e-h) Scanning electron micrographs of valves in *statu nascendi* at the corresponding stages of development. Figure and description are taken from ref. 31 and reprinted with permission of WILEY-VCH, Copyright 2006.

phosphate or by a drop of the pH value, which occurs during silica precipitation.[31,32] *Via* this route, the diatoms can produce silica with subsequent hierarchies of hexagonal patterns.[31]

The proteins that take part in the formation of silica are often extracted from diatomic silica by the use of HF. Even if the structure of some proteins is altered by this treatment,[33,34] the extraction of active species is often possible. Different silaffins were extracted from various diatoms,[30,33,35,36] and they induced the formation of silica in solutions that would not occur without protein. They can also influence the morphology of the silica. Especially interesting is the interaction of silaffins and polyamides, which often occur coupled in nature,[30,31] showing synergistic effects, yielding different morphologies.[35] Silaffins can build large aggregates which might be important for the construction of precursors or hierarchies. The phosphorylation is important for this assembly, as it changes the charge of the proteins. Unfortunately, the phosphorylation is often changed by the treatment with HF.[34] In addition to silaffins, cingulines also play an important role in the formation of the diatom shells. These proteins are the insoluble component that builds the girdle bands, which fuse the two main parts of the shell of diatoms. The cingulines do not influence the morphology of the mineral, but they accelerate the precipitation of silica and produce nonporous structures.[37]

The last example for biomineralization is that of the spiked shell of the sea urchin. Although each spike of the sea urchin appears to be a monocrystal in X-ray diffraction (XRD), this is not the case. The individual crystals of which one spike is composed of are perfectly aligned in one direction, and they are embedded in a matrix of proteins.[1] The proteins from the matrix of spicules from sea urchin embryos were extracted and identified. A complex mixture of proteins was found, containing not only various matrix proteins, but also membrane proteins which could confine the space for the growing crystal and take part in signalling events. The effect of the separated proteins on the mineralization has not been investigated so far.[38]

Other investigations on extracted proteins and their influence on precipitation were also performed for the sclerites of soft corals,[39,40] fish otoliths,[41,42] radulas[43] and sponges.[44]

2.2 Adaption of naturally occurring proteins for the synthesis of new materials

With increasing knowledge about biomineralization, conditions are being mimicked in order to achieve synthetic structures. Using the natural process of biomineralization provides new materials made from the same components, only templating and using the protein matrix differently. Here, some recently developed materials and systems are being discussed, not only copying nature, but also using various artificial additives.

Collagen is one of the frequently used proteins for the production of bioinspired materials. The production of synthetic bonelike material is very promising due to the potential applications in the field of medicine. Here, recombinant collagen is especially interesting, as it eliminates the risk of infections or immune reactions. It is already possible to mineralize the surface of self-assembled collagen fibrils. The c-axis of the resulting hydroxyapatite crystals is aligned along the fiber axis.[45,46] It is also possible to use only the helical part of collagen, which assembles into fibers like natural collagen. In contrast, helical collagen reduces precipitation of hydroxyapatite from a simulated body fluid solution. The collagen was used as a gel or in films, which slowed the mineralization even more.[47] Recently, there was a successful attempt to reproduce the main structural feature of bone, which is also depicted in Fig. 3. The collagen network with hydroxyapatite crystals in its cavities, but not on its surface. Olszta *et al.* used polyaspartate to produce amorphous calcium phosphate as a precursor. The amorphous precursor infiltrates the collagen network and converts into crystalline hydroxyapatite afterwards. The results suggest that only the cavities of the collagen network were mineralized, while the rest of the fibers remained uncovered.[9] Amorphous and metastable precursors are also used in organisms,[48,49] and these colloidal calciprotein–particles could also serve as precursors for similar mineralization processes.[50,51]

Other experiments are concerned with the synthetic reproduction of dentine, one of the mineral components of teeth. A straightforward attempt is the solvation of the mineral with acid; the demineralized protein matrix can be used for re-mineralization experiments afterwards. It was shown that re-mineralization of this matrix is possible, and that the newly formed crystals are similar to the natural material regarding their shape and crystal phase. The natural dental matrix protein was exchanged with

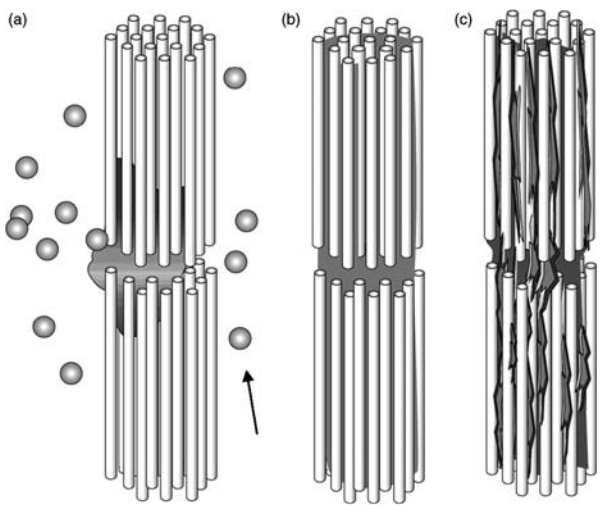

Fig. 3 Schematic depicting a proposed mechanism of intralar mineralization of collagen. Each of these pictures represents the whole zone region of a collagen fibril within the aqueous mineralizing solution containing the polymeric process-directing agent (*i.e.*, polyaspartate). (a) The negatively charged polymer sequesters ions and at some critical ion concentration generates liquid–liquid-phase separation within the crystallizing solution, forming nanoscopic droplets of a highly hydrated, amorphous calcium phosphate phase. The nanoscopic droplets of this polymer-induced liquid precursor (PILP) phase adsorb to the collagen fibril, and due to their fluidic character, become pulled up and into the whole zones and interstices of the collagen fibril by capillary action. (b) The collagen fibril becomes fully imbibed with the amorphous mineral precursor, which then solidifies as the hydration waters are excluded. (c) The amorphous precursor phase crystallizes, leaving the collagen fibril embedded with nanoscopic crystals of hydroxyapatite. The picture and the description were taken from ref. 9 and reprinted with permission of Elsevier, Copyright 2007.

polyacrylic acid and poly(vinyl phosphonic) acid, showing that mimicry of some protein functions is possible.[52] Gajjeraman and coworkers used the naturally occurring proteins to build materials. While collagen I causes the precipitation of brushite, the dental matrix protein changes the mineral phase to hydroxyapatite, irrespective of whether it is used alone or in combination with collagen I.[53] In all cases, the amorphous precursor phases are important, suggesting that this is a general route in natural systems for the formation of the crystalline phase. Gajjeraman *et al.* also measured the mechanical properties of the material after milling it. As anisotropy and the hierarchical structure of the material are important for biomaterials and their characteristic properties,[8,10,11] the measured values do not resemble the true properties of the produced material.[53]

As mentioned before, nature uses the polysaccharide chitin for composite materials, and there are approaches to produce biomimetic materials with chitin or its chemically modified form, chitosan. The most obvious way to produce mimetic materials is co-precipitation, which was recently done with chitosan and hydroxyapatite. Rusu *et al.* could show that the first mineral phase is brushite, which converts into hydroxyapatite, growing and increasing in crystallinity over time. The final size and crystallinity decrease with an increasing amount of chitosan. A bimodal fit of the diffraction peaks was also performed to show two size distributions, but it is not possible to identify the described linear increase of the fraction of small crystallites with increasing chitosan content. Furthermore, the fluctuations of the size of the crystallites in both size domains are strong, which might indicate that a bimodal fit of the given data is not appropriate.[54] Synergistic effects of chitin and N16N, whose natural occurrence was already discussed earlier, were also used for the production of materials. N16N changed the mineral phase from calcite to aragonite. Keene *et al.* investigated the influence of the sequence of N16N for this effect, and they could show that a change of the protein charge lowers the effect, while a scrambling of the sequence destroys it completely. Furthermore, N16N only interacts with β-chitin, the modification used in natural systems for biomaterials, but not with α-chitin. N16N adsorbs readily on β-chitin and still changes the mineral phase of the precipitate from calcite to aragonite.[55]

Silk fibroin is frequently used for biomineralization experiments, as it is similar in structure to matrix proteins occurring together with chitin.[18] Silk fibroin can be used to produce calcium carbonate particles from solution. The shape of the particles can be tuned by the molecular weight of the silk fibroin and the pH. The explanation for the appearance of rice-like particles at high molecular weights of silk fibroin is the alignment of β-sheets of the silk fibroin alongside each other, forming elongated structures, resulting in rice-like particles with a small cavity.[56] It was shown that silk fibroin can induce the precipitation of aragonite instead of calcite depending on the concentrations of protein and calcium as well as on the temperature. Spectroscopic methods showed that a surface with NH_2 functions induces a transition from silk I to silk II, which has more β-sheets and results in the formation of aragonite instead of a mixture of aragonite and calcite.[57] This could be related to the hierarchy in nature, where chitin is coated with other proteins whose structure may be changed by chitin. Composites of silk fibroin and hydroxyapatite are biocompatible and promote the cell differentiation and proliferation.[58] Films of silk fibroin can also be used to grow hydroxyapatite crystals. Li *et al.* also used silk II for this procedure, which they produced by the addition of ethanol to silk I, pointing out that the β-sheet structure should indeed be important for the mineralization processes.[59]

The mineralization of fibronectin, elastin and their mixture also yields interesting insights. Elastin shows no mineralization, while fibronectin itself and also its mixture with elastin are mineralized with hydroxyapatite. The mineral is at first amorphous and converts into needle-like hydroxyapatite crystals, showing clear diffraction patterns for fibronectin and also, a little less clear, for the mixture of fibronectin and elastin.[60] The structure of fibronectin and elastin on surfaces, where the proteins can unfold, was also examined. These proteins build fibrils which can be mineralized, accompanied by a growing volume and an increasing stiffness.[61]

Another protein associated with biomineralization is bovine serum albumin. *Via* assembly in a Langmuir trough, pure calcite can be deposited at the interface. The Langmuir trough can control the structure of the protein layer, offering new possibilities for controlling the material independent of the ion concentration in the mineralization solution.[62]

Others used the membranes from the nacreous layer of molluscs to produce various crystal shapes. Hou *et al.* claim that the membrane might have a periodicity that fits calcite crystals. The shape of the crystals is sensitive to the addition of glycine, which adsorbs on specific faces of the crystal, and asparagine, which directs the agglomeration of the crystals. It remains unclear why the used mineral phase is calcite instead of aragonite, which is the mineral phase of the nacreous layer. Nevertheless, the findings may be interesting for further work on biomimetic materials.[63] It is also possible to synthesize the protein AP7 from the nacreous layer. This protein disrupts the growth of crystals and makes their edges rounder. The important part for the mineralization is located in the structural labile region of the protein, showing that the unstructured regions are frequently important for mineralization.[64] Similar works were carried out for other membranes from molluscs.[65] It is also possible to synthesize proteins of the prismatic layer, like asprich. This protein changes the morphology of calcite particles during their growth from rhombohedral to round-shaped. Their crystallinity is also lowered, as parts of the round particles consist of amorphous calcium carbonate. Ndao *et al.* analyzed the structure of asprich and showed that the main parts of the protein are unstructured in solution, like many other proteins that influence the mineralization.[66]

In addition to proteins, bionanoparticles like viruses or bacteriophages can also be used as a template for mineralization, with many options for further modifications.[67,68] Bacteriophages can be aligned electrostatically with calcium and mineralized afterwards, resulting in an ordered structure of hydroxyapatite.[69] Moreover, the cowpea mosaic virus has recently been employed to produce silica nanoparticles.[70] Another possibility is the use of ferritin, which can be assembled at an oil/water interface where it induces the precipitation of hydroxyapatite from a saturated solution, resulting in hollow capsules.[71]

It is a common approach to use proteins for the synthesis of biominerals which are also used by organisms for this purpose. In contrast to that, it is also an option to use natural proteins that

are far from any mineralization processes in nature but offer specific processing advantages—like hydrophobins, a class of very surface-active, amphiphilic proteins.[73,74] This protein can be absorbed on a flat substrate to precipitate a smooth and crack-free polycrystalline film of TiO_2 under mild conditions.[75] In recent work from our group, a member of this class of proteins was assembled at an interface between water and different oils. Afterwards, the coated surface is mineralized from a saturated solution, resulting in well-mineralized capsules (as shown in Fig. 4) composed of protein and hydroxyapatite. The capsules can be treated with temperatures up to 900 °C to change their morphology.[72]

2.3 Design of new proteins for biomineralization

As described in the previous sections, nature provides a huge amount of different proteins that can be extracted and used for the process of biomineralization. Nevertheless, natural proteins might not have the exact properties that are desired. The previous sections showed that scientists are beginning to understand the functions of proteins and the influence of specific motifs on the mineralization process. Consequentially, there are attempts to produce active species to control the mineralization in a predetermined fashion—by mimicking nature with other substances, by using short motifs from the active center of proteins, or by modifying and creating new proteins.

A good example for partial mimicry is the use of collagen I for the mineralization of hydroxyapatite with the addition of different soluble additives to modify the growing crystals. With pure collagen I, no mineralization occurs. However, when polyacrylic acid and poly(vinyl phosphonic) acid are present, the mineralization of the collagen fibers starts with amorphous precursors, converting into small hydroxyapatite crystals that are aligned along the fiber axis. Polyacrylic acid is used to stabilize the amorphous precursor particles, while poly(vinyl phosphonic) acid binds to collagen and introduces negative charges on its surface. The process of mineralization is similar to the one in nature. The resulting aligned crystals are not only present inside the fibers, but also on their surface.[76]

In the process of creating new protein structures, the easiest route is to understand at first the role of single amino acids. For example, the solubility of calcite is increased by the addition of aspartic acid, and the kinetics of this process can be important for mineralization.[77] While many investigations are concerned with the effects on materials occurring in nature, amino acids can also be used to control the morphology and the mineral phase of non-natural substances like TiO_2.[78]

A short sequence of peptides can already have a great influence on the mineralization if it is similar to the active center of a protein. There are tests to reproduce the active part of the bone morphogenetic protein. It is possible to produce hydroxyapatite with the used peptide sequence, but the resulting material has to be characterized more carefully to evaluate its quality.[80] A study on the precipitation of calcium carbonate in the presence of three short peptide sequences was performed in great detail. One of the first results is that the frequently used technique of gas diffusion, which increases the concentration of carbonate in the solution by diffusion of carbon dioxide, is not reliable in all cases and should be used only for qualitative examinations. It is more reliable to measure and control the pH as well as the concentration of calcium over the whole reaction time. The investigated peptides influence the crystal growth, as shown in Fig. 5. The given data show that the peptides neither bind calcium nor influence the formation of clusters. Also, the net charge and slight changes in the secondary structures are not important for this process. Some of the peptides prevent the nucleation of amorphous phases. While the mineral phase can be switched between calcite and vaterite by a change of pH when no peptide is present, the mineral phase in the presence of all peptides is vaterite (with one unidentified peak in its diffractogram) at different pH values. The collected data shows that the mineral phase is pre-formed in the short-range order of the clusters. Therefore, the final mineral phase results from the least growth-inhibited clusters.[79]

The design of new proteins is challenging. There are initial successes to identify typical peptide motifs from the dentine matrix protein and use them in an artificially created protein. The new proteins were screened to investigate their influence on the transition of amorphous calcium phosphate to hydroxyapatite, which is also important in teeth. Some of the proteins influence the amorphous precursors by reorganizing them without changing their size, which points to a preorganization for the following crystallization which was already described above.[81] There are many discussions about the importance of secondary structures for mineralization proteins. The active motif of nacrein could not suppress the precipitation of calcium carbonate in its pure form, but it did suppress precipitation if it was integrated repetitively into an artificial protein without secondary structure.[82] The most challenging approach is the theoretical prediction of an active sequence. This is done by

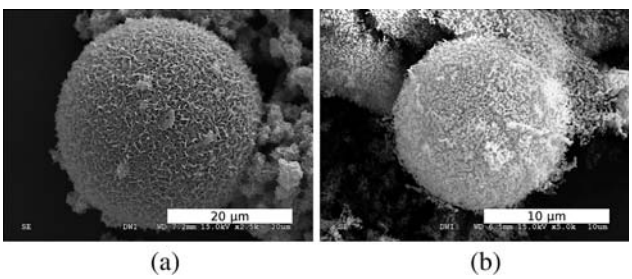

Fig. 4 Capsules of hydroxyapatite prepared from a hydrophobin-stabilized oil-in-water emulsion; (a) at room temperature, (b) after sintering at 900 °C. The picture was taken from ref. 72 and reprinted with permission of RSC, Copyright 2011.

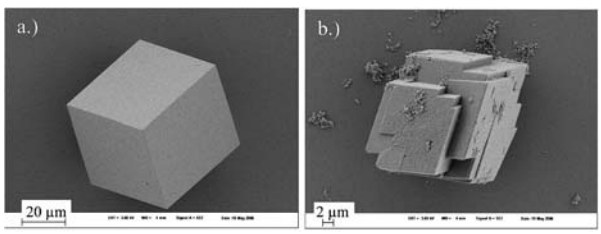

Fig. 5 (a) A typical calcite crystal grown in solution; (b) A calcite crystal grown in the presence of a synthetic oligopeptide (KRSKFPHKHDVI). Pictures are taken from ref. 79 and reprinted with permission of the American Chemical Society, Copyright 2009.

a simulation which optimizes the sequence of a small protein, so that folding and sequence are perfect for the interaction with the high-energy 001 site of the crystal. The simulations showed that positively charged amino acids preferably interact with the crystal. The most promising candidates were synthesized and analyzed experimentally. The proteins influenced the growth of calcite by specific binding to one crystal face, resulting in morphological changes like macro-stepping, twinning and kinking. The proteins were also tested after scrambling their sequence. While the negative proteins still had strong effects on the crystals, some of the positive proteins lost their function, meaning that obviously the structural sequence is important for the function of these proteins.[83]

3 Enzymes for biomineralization

3.1 Proteins used for enzymatic biomineralization by nature

Nature is able to produce minerals like silica with controlled shape at ambient temperatures and pressures and at near neutral pH while synthetic organosilicon chemistry requires high temperatures and pressures or extreme pH.[84] Organisms, including diatoms, radiolaria, choanoflagellates, sponges and higher plants, build up their skeleton with silica with the help of specialized proteins. It is possible to isolate specific proteins from these organisms that are involved in the biosilicification process, producing the hard exoskeleton.[85] A well-known example is silicatein (silica proteins) which is isolated from sponges.[84]

3.1.1 Silicatein. Silicatein was at first discovered by Morse and coworkers from the silica spicules, which constitute 75% of the dry weight of marine sponge *Tethya auranita*. Each spicule contains a central axial filament composed of silicatein (Fig. 6). Silicateins can be divided into three different types: silicatein α, β and γ. About 70% of the mass of the proteinaceous filament is composed of silicatein α.[84]

The silicateins enzymatically control the biosilicification process in siliceous sponges and at the same time serve as the organic matrix for the silica product.[87] In the report of Morse and coworkers it was shown that the native silicatein filaments and the recombinant silicatein α expressed from a recombinant DNA template in *Escherichia coli* could catalyze the polycondensation of tetraethoxysilane (TEOS) and also trialkoxysilanes to form silica *in vitro* at neutral pH and 20 °C. The absence and the use of denatured silicateins under the same conditions yielded little silica product, indicating the enzymatic activity of the silicateins in this process and the possible dependence on the native three-dimensional conformation of the proteins.[84]

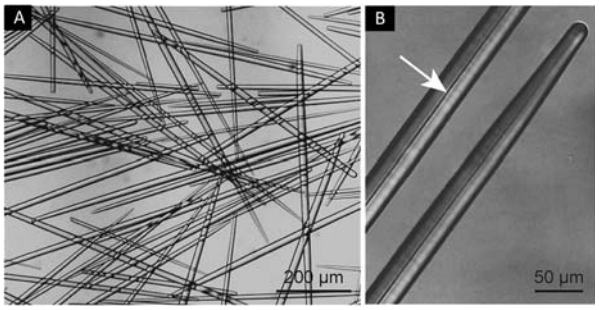

Fig. 6 Optical micrographs of isolated spicules from the marine sponge *Tethya auranita*. The higher magnification image reveals the axial protein filaments (indicated by white arrow). Reprinted with permission from Wiley-Liss, Copyright 2003.[86]

The key step in the silica synthesis is the Si–O–Si bond formation from the condensation of two Si–OH groups either in silica-forming organisms or *in vitro* silicification.[88] The hydrolysis of alkoxysilanes, which typically uses acids or bases as catalysts, is the rate-limiting step in the silica condensation, therefore it was proposed that silicateins catalyze this hydrolytic reaction at neutral pH. Silicateins were found to exhibit high similarities with respect to the hydrolytic enzyme cathepsin L, which belongs to the group of papain-like proteasse with the characteristic active triad histidine (His), asparagine (Asn) and cysteine (Cys). The similarities found include amino acid sequences and three-dimensional structures. In the silicateins, cysteine was replaced by serine (Ser) and the proposed hydrolytic activity of silicateins resulting from the Ser/His active site was supported by site-directed mutagenesis. Morse and coworkers have also suggested a possible mechanism of TEOS hydrolysis catalyzed by the Ser/His active site of silicatein α (Fig. 7a).[89]

Müller, Tremel and coworkers have determined the enzymatic activity of recombinant silicatein α in the condensation of dimethylsilane (DMS) to form oligomeric PDMS at ambient conditions which was analyzed by mass spectrometry and ^{29}Si NMR. The mass spectrum of the silicon product formed in the presence of silicatein showed the signal of the chain length up to twelve repeating monomer units, while without silicatein only

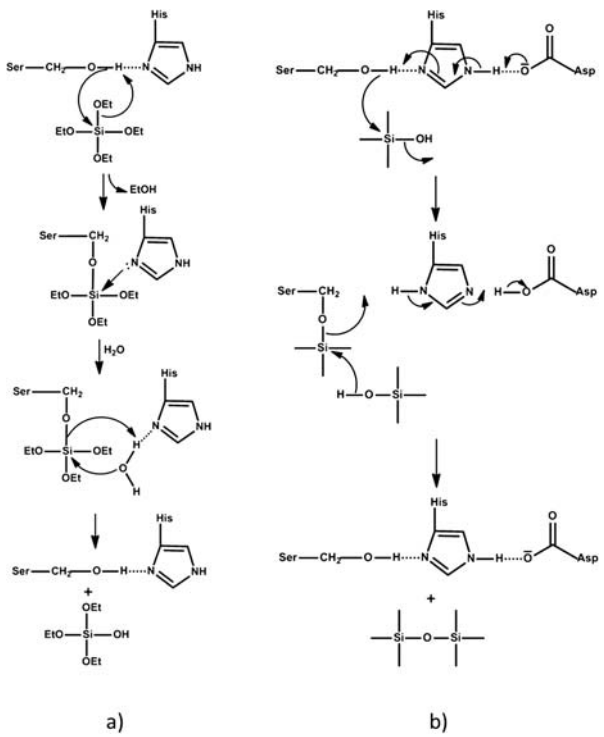

Fig. 7 Proposed mechanism of a) TEOS hydrolysis catalyzed by the Ser/His active site in silicatein α;[89] b) condensation of Me_3SiOEt catalyzed by the Ser/His/Asp triad.[88]

a weak signal of up to seven repeat units was present. In the ^{29}Si NMR analysis, intensive signals of hydrolysis intermediates and dimers of DMS were detected after 5 h of incubation with silicatein, suggesting catalytic activity in the hydrolysis of DMS. Because of the concentration limit of the NMR technique, no signals for higher oligomers could be detected. UV/Vis absorption spectroscopy was applied to analyze the reaction *in situ*. The methoxy group was substituted with *p*-aminophenoxy group and the concentration of the by-product *p*-aminophenol could be measured with UV/Vis spectroscopy during the condensation. The increasing absorption at 290nm of the *p*-aminophenol in the presence of silicatein revealed also that silicatein could catalyze the silica condensation (Fig. 7b).[90,91]

3.1.2 Native hydrolases with the capability to catalyze silicification. As mentioned in the section above, the silicateins are able to catalyze the polycondensation of alkoxysilanes such as TEOS and DMS, a series of commercially available hydrolases, including proteases, lipases and phosphatases, which are not naturally associated with silica production, were also studied by Bassindale *et al.* for the catalytic ability of *in vitro* siloxane bond formation from ethoxytrimethylsilane (Me_3SiOEt) at neutral pH and room temperature.[88,92] Several enzymes were found to catalyze the condensation of Me_3SiOEt forming hexamethyldisiloxane (HMDS) in aqueous solution. The found enzymes include: Bovin pancreatic trypsin, Bovin pancreatic trypsin (tosyl phenylalanyl chloromethyl ketone (TPCK) treated), α-chymotrypsin, *Aspergillus ficuum* phytase, *Aspergillus niger* phytase, chicken egg white lysozyme, *porcine gastric mucosa* pepsin and *phizopus oryzae* lipase. Trypsin was found to be the most effective one for the Si–O–Si bond formation and the Me_3SiOEt condensation in the presence of Trypsin was nearly complete after 3 h.[92] Trypsin and α-chymotrypsin could also act as catalysts for the polycondensation of TEOS.[93] Furthermore, it was also found that papain was able to mediate the formation of silica from tetramethoxysilane (TMOS).[94] All of these hydrolytic enzymes exhibit one or more serine, aspartate or histidine residues or a combination of them, which resembles the catalytic triad of the silicatein, and therefore are able to perform similar reactions like the silification, though with reduced activity. The proposed mechanism for a Ser/His/Asp triad involved silanol condensation extending the comprehension of the interaction between enzymes and silica precursors (Fig. 7b).[88]

3.2 Adaption of naturally occurring proteins for the synthesis of new materials

The enzymatic activities of these naturally occurring proteins in mineralization processes provide significant potential for the development of an environmentally friendly approach to create new materials, especially for hybrids of organic/inorganic substances.

3.2.1 Synthesis of inorganic materials or organic/inorganic hybrids with native and recombinant silicatein. The biocompatible biosilica, mediated by silicatein under near physiological conditions, could be advantageous for biomedical applications. Schröder *et al.* demonstrated that collagen-coated surfaces modified by enzymatically catalyzed silicification from TEOS with recombinant silicatein could increase calcium phosphate deposition *in vitro*.[95] A new implant material for bone reconstitution based on the osteoinduction of biosilica and the enzymatic activity of recombinant silicatein for silicification was reported by Wiens *et al.* In this work the silicatein and its substrate sodium metasilicate were encapsulated into biodegradable and biocompatible poly(D,L-lactide)/poly(vinyl pyrrolidone) microspheres. Furthermore, these silicatein/silica containing microspheres were integrated in a plastic-like matrix to form a functional implant material.[96] This hybrid material together with the collagen-coated surfaces modified by biosilicification could be potentially applied in the field of bone regeneration and tissue engineering. It was also possible to generate silica layers on teeth by biosilicification with silicatein to protect teeth from bacterial attack.[87] The research of Müller *et al.* indicated that biosilica nanoparticles isolated from marine sponge *S. domuncula* strongly increase the expression levels of enamilin and amelogenin *in vitro*, which contribute to hydroxyapatite crystallite formation. Therefore the enzymatic synthesis of biosilica by silicatein might be a novel approach for tooth reconstruction.[97]

Silicatein is not only able to produce silica enzymatically with structural control but is also capable of catalyzing and templating the formation of other metal oxides such as titanium dioxide and gallium oxide.[98] Sumerel *et al.* have reported the biocatalytically templated synthesis of titanium dioxide (TiO_2) from titanium bis-(ammonium-lactato)-dihydroxide (Ti[BALDH]) at neutral pH and mild temperature with native silicatein filaments isolated from Sponge *Tethya aurantia*. Further thermal annealing led to different crystallite diameters and phases of TiO_2, compared to the materials obtained from the same precursor *via* traditional alkali or thermal catalysis.[99]

Similarly, these protein filaments could, under mild conditions, enzymatically synthesize either gallium oxo-hydroxide (GaOOH) or spinel gallium oxide (γ-Ga_2O_3) from gallium(III) nitrate (GNO), depending on the concentration of the precursor solution. The diffraction results of the γ-Ga_2O_3 nanocrystallite also suggested the structure-directing capability of silicatein filaments.[100]

O'Leary *et al.* have tested the use of silicatein filaments to silicify trialkoxysilanes bearing an organometallic species, which could be useful for the immobilization of organometallic catalysts. In this work siloxy functionalized platinum (Pt) and palladium (Pd) complexes and TEOS were mixed together with native silicatein filaments and the complexes were successfully condensed and incorporated into the silica matrix *via* the enzymatic silicification. This benign method provides a new possibility for the future immobilization of the catalysts which are sensitive to specific environmental conditions.[101]

A recent report by Natalio *et al.* showed the first *in vivo* formation of silica/titania nanospicule by incubating primary sponge cell cultures from the marine sponge *Suberites somuncula* with Ti[BALDH]. In this procedure, silicatein assembled in a rod shape and enzymatically catalyzed the deposition of silica and titania simultaneously onto the silicatein-coated rod to form a nano-composite material with spicule-form (Fig. 8).[102]

Curnow *et al.* have applied bacterial *E. Coli* cells, whose cell-surface displayed recombinant silicatein after the transformation with a silicatein gene, as a whole-cell biocatalyst to fabricate ordered titanium phosphate on the cell surfaces at low

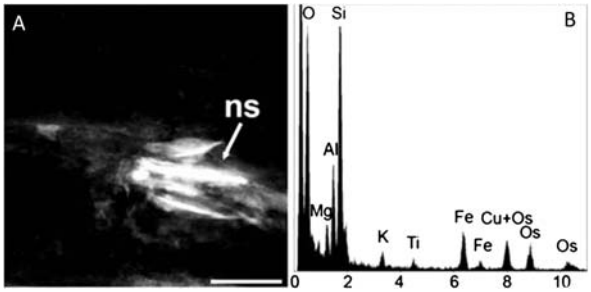

Fig. 8 Dark-field STEM image and EDX spectroscopy of nanospicule (ns) found in sponge cell culture incubated with 25μM Ti[BALDH] (scale bar: 200nm). EDX analysis reveals the presence of Si and Ti in the nanospicule with a ratio up to 16. Reprinted with permission from Springer, Copyright 2010.[102]

temperature and neutral pH from Ti[BALDH] in phosphate-buffered saline.[103] Müller *et al.* have also demonstrated that the silicatein was deposited on the surface of *E. coli* after the transformation and that the bacterial cells could be encapsulated with silica after further incubation with silicic acid under physiological conditions (Fig. 9). However, the growth kinetics of the bacteria remained unaffected, compared to bacteria without silica shell, suggesting that the bacteria retained their activity after the encapsulation. Thus, this mild method could improve the technique of immobilization of bacteria or yeast cells without affecting their activities.[104]

Silicatein can also be employed to coat materials which are sensitive to heat, alkaline or acidic conditions with silica or other metal oxides under mild conditions. The preparation of biosilica coatings initially requires the immobilization of silicatein on the surface.[85] Tahir and coworkers have successfully immobilized His-tagged recombinant silicatein onto gold surfaces to catalyze the formation of not only nanospheres of silica with a diameter between 70–300nm from TEOS[105] but also nanoparticles of titania from Ti[BALDH] and zirconia from anionic hexafluorozirconate $(ZrF_6)^{2-}$ with a diameter of 50–60nm on gold surfaces with a layer-like appearance.[106] Rai and Perry even achieved to fabricate uniform silica films with a thickness of about 20–100nm on the gold surfaces bound with silicatein. By varying the amount of absorbed silicatein and the reaction times with the precursor TMOS, the physical properties of the silica films such as thickness, roughness and hydrophilicity could be tuned.[107] In addition to gold, iron(III) oxide (Fe_2O_3) could be functionalized with silicatein and consecutively was coated with a silica layer. Shukoor *et al.* applied this approach to form

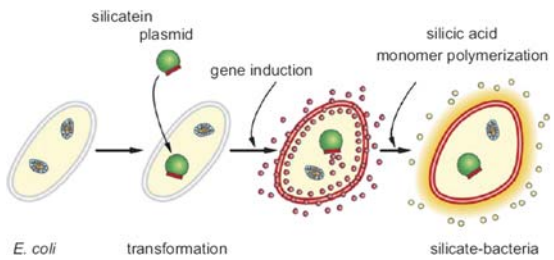

Fig. 9 Scheme of bioencapsulation of *E. Coli* with silica using silicatein. Reprinted with permission from Elsevier, Copyright 2008.[104]

magnetic γ-Fe_2O_3 nanoparticles with silica coating, which could offer protection to the magnetic nanoparticles from the environment and increase their biocompatibility for biomedical applications.[108] The His-tagged recombinant silicatein could also be bound on the surface of tungsten disulfide (WS_2) nanotubes and further incubation of the functionalized WS_2 nanotubes with Ti[BALDH] created a biotitania shell on the nanotube.[109]

In addition to the hydrolytic activity of silicatein, Tahir *et al.* have also found that silicatein could mediate the reduction of chloroauric acid ($HAuCl_4$) into gold nanoparticles at room temperature. Based on this effect they have produced TiO_2/Au nanowire-nanoparticle composites from TiO_2 nanowires, whose surface was modified with deposited His-tagged silicatein to allow the Au nanocrystal growth (Fig. 10a). This procedure offers the possibility to synthesize simple composites of nanowire/tube and nanoparticle, which opens a mild pathway towards combination of components and their properties.[110] In addition, the functionalization of the surface of polymeric core-shell colloids with His-tagged silicatein to grow gold nanoparticles from auric acid onto the colloids was performed by Lange *et al.* (Fig. 10b and c).[111] This extends the approach towards polymeric-inorganic composites, which would potentially be useful in selective layer deposition in organic-inorganic electronic devices.

3.2.2 Material synthesis with native hydrolases. Hydrolases, particularly lysozyme, which are capable of catalyzing the silicification process, are also of great interest for the design of new materials based on silica. Schiomi *et al.* reported the

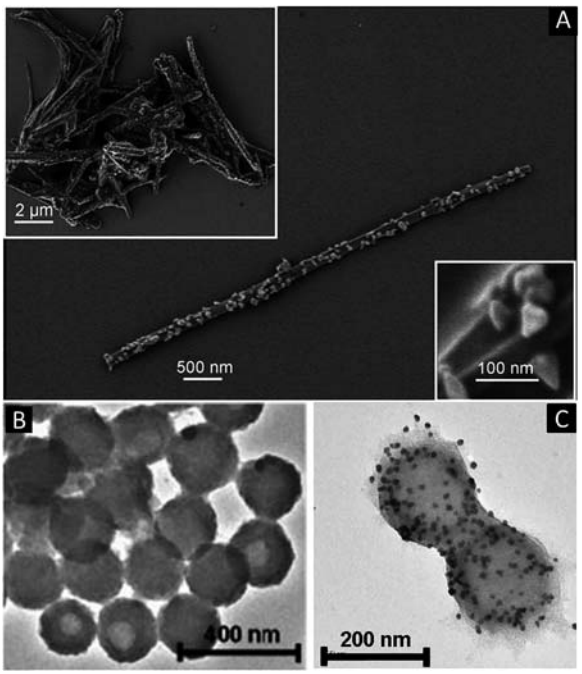

Fig. 10 a) SEM images of TiO_2 nanowires decorated with Au nanoparticles. Top left: overview, right bottom: magnified view. Reprinted with permission from Wiley-VCH, Copyright 2006[110] b) TEM images of surface functionalized core-shell colloids with His-tagged silicatein and c) the functionalized colloids with gold nanoparticles, which were grown from immobilized silicatein. Reprinted with permission from Wiley-VCH, Copyright 2007.[111]

synthesis of lysozyme-silica hybrid hollow spherical particles at ambient conditions, using the catalytic activity of lysozyme for silica formation from TEOS and the foaming effect of lysozyme. It was possible to control the morphologies of particles by varying the lysozyme concentration.[112] This method could avoid the use of other synthetic surfactants as a template for silica capsule formation[113,114] and furthermore, it was not necessary to remove the template, compared to other templated syntheses of hollow silica spheres.[115] The formation mechanism of hybrid particles with different morphologies by altering reaction conditions and varying lysozyme concentration was also demonstrated.[116] In the hybrid shell, lysozyme structures disperse uniformly within the silica matrix and by the removal of the lysozyme under calcination, cage-like spherical silica capsules were formed, with a silica shell thickness of 100 nm containing pores of 50–250 nm in diameter (Fig. 11), which has potential applications for controlled release systems for biomacromolecules like proteins and DNAs.[117]

In addition, cage-like hollow aluminosilicate spheres which have wormlike elongated holes (short axis: several tens of nanometres, long axis: about 100 nm or more) in the shell have also been prepared *via* hydrothermal treatment using an Al-containing alkaline (NaOH/NaAlO2/tetraethylammonium hydroxide (TEAOH)) solution of lysozyme-silica hybrid particles. Within these cage-like hollow aluminosilicate spheres, a red fluorescent protein from Discosoma coral was encapsulated and fixated *via* a cross-linking process.[118]

In addition to the catalytic activity of lysozyme with respect to the precipitation of silica from TEOS the formation of titania from potassium hexafluorotitanate (PHF-Ti) or Ti[BALDH] has also been found. Luckarift and coworkers reported a mild one-pot method to generate lysozyme-silica or lysozyme-titania bionanocomposites with the antibacterial properties of lysozyme. It was also possible to add an additional enzyme during the lysozyme-mediated mineralization so that the enzyme could be encapsulated in the silica or titania matrix. The benefit of this approach is that the silica or titania prevents the enzyme from physical denaturation and protects the lysozyme from microbial degradation.[119]

A simple preparation of another silica–enzyme composite with trypsin instead of lysozyme was reported by Bassindale *et al.* and in this composite at least 40% of the activity of trypsin could be detected after one week at ambient temperature.[120] These findings suggest that the silica matrix allows for the encapsulated enzyme to retain its activity.

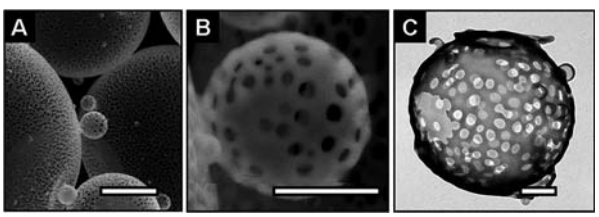

Fig. 11 SEM (A, B) and TEM (C) images of cage-like hollow silica particles obtained after calcination at 700 °C for 2h. Scale bars: (A) 3μm, (B) and (C) 500nm. Reprinted with permission from The Royal Society of Chemistry, Copyright 2007.[117]

Another example for the entrapment of an enzyme in a biologically synthesized silica matrix by lysozyme was the co-immobilization of carbon nanotubes and glucose oxidase for direct electron transfer. The glucose oxidase is a stable redox enzyme with high catalytic activity and the direct bio-electrocatalysis of glucose oxidation could be of great interest for the development of bioelectrodes for biosensors and biofuel cells. As the redox center was not accessible after the traditional immobilization of glucose oxidase, the electron transfer between the enzyme and the electrode surface was limited. The lysozyme catalyzed the silica formation from TMOS on conductive carbon paper with the addition of carbon nanotubes and glucose oxidase. During silicification the carbon nanotubes and the glucose oxidase were incorporated into the silica matrix. With the help of this co-immobilization technique, the carbon nanotubes could provide an electrical connection between the enzyme and the carbon paper surface and the electron-transfer rate was increased compared to that of unmodified electrode. Subsequently, the immobilized enzyme retained its catalytic activity for a period of one month.[121]

3.3 Design of new catalysts for biomineralization

Inspired by the catalytic capabilities of the silicatein, scientists have tried to design other analogues of silicatein for the catalysis of biomineralization. Morse and coworkers have applied synthetic cysteine–lysine diblock copolypeptides to catalyze the silicification of TEOS with controlled dimensions. In this block-copolypeptide the poly-lysine was the cationic polyelectrolyte with amine groups, which is water soluble at pH 7, and the poly-cysteine was the water insoluble domain with hydrolytic activity. The amphiphilic block-copolypeptide was able to self-assemble into aggregates in water and the reduced (under N_2) or oxidized (under air) forms of the block-copolymer initiated different aggregate morphologies due to the formation of disulfide bonds in oxidative environment, leading to different silica shapes.[98,122] They have also synthesized a non-peptide diblock copolymer (consisting of hydroxylated polybutadiene and poly(2-vinyl pyridine)) which contains both nucleophilic side chains with –OH groups and nitrogen containing side chains with pyridine groups (Fig. 12a) to mimic the enzymatic active site of silicatein

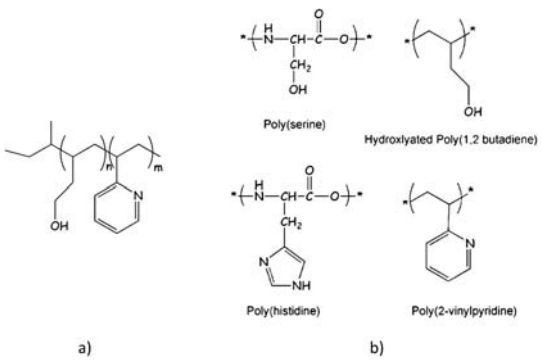

Fig. 12 a) Structure of synthetic non-peptide diblock copolymer consisting of hydroxylated polybutadiene and poly(2-vinyl pyridine); b) Comparison of amino acid chemical structure with the synthetic mimic. Reprinted with permission from American Chemical of Society, Copyright 2004.[123]

(the hydroxyl functionality acting as the serine-26 and the pyridine functionality acting as the histine-165) (Fig. 12b). This non-peptide diblock copolymer showed good catalytic activity for the condensation of TEOS at neutral pH.[123,124]

The catalytic capability for silica formation of an array of small molecules with bifunctionalities has been also tested by Morse and coworkers. These small molecules exhibit a nucleophilic group as well as hydrogen-bonding acceptor groups, such as –SH, –OH, $–SC_2H_5$, $–NH_2$, –NHR, $–NR_2$. The distance between the two functional groups is 2.9 Å and the distance between sulfur and nitrogen in the active site in cathepsin L and other hydrolases with serine, histidine and asparagine as catalytic triad is about 3.2 Å. Among those candidates cysteamine was the most successful biomimetic catalyst and the reaction with TEOS led to amorphous silica spheres with a diameter 40–100 nm. Furthermore silicification of TMOS catalyzed by cysteamine was successfully used for encapsulation of biomaterials such as Luciferase, green fluorescent protein (GFP) and *E. Coli* cells, and the biomaterials retained their activities after the encapsulation.[125]

Pogula *et al.* have fabricated continuous thick silica coatings with TMOS under benign conditions on glass fibers, which were treated with poly-L-lysine, poly(L-lysine-tyrosine(1 : 1)) and poly (allylamine hydrochloride) respectively.[126] Poly-L-lysine also showed the activity to catalyze silicic acid deposition. By varying the mixing sequence of a poly-L-lysine solution, phosphate buffer and silicic acid precursor, different silica structures were formed.[127] Goldberg *et al.* have applied poly-L-lysine with pre-hydrolyzed TMOS to form a silica coating on dentin surfaces, suggesting that also the biosilicification with synthetic polypeptide catalysts is a potential treatment strategy.[128]

Lee and coworkers have chosen dipeptide His–Ser to mimic the catalytic triad of silicatein for the synthesis of GaOOH, a solid precursor of the semiconductor Ga_2O_3 with controlled shape. The dipeptide catalyzed the hydrolysis and condensation of gallium species and templated the growth of the product. As a result, uniform nanospindles from GaOOH were produced (Fig. 13). In addition, the concentration of the dipeptide and the pH value were shown to affect the rate of the reaction and the shape of the product. A pH value between 3 and 5 was optimal for the catalytic activity of the dipeptide and GaOOH with a spindle shape was obtained at pH 3.2. At a pH value between 5 and 7 GaOOH with a blunt structure was observed. Under basic condition only amorphous aggregates were formed. At a pH value of 3.2, smaller spindles were obtained at higher dipeptide concentration.[129]

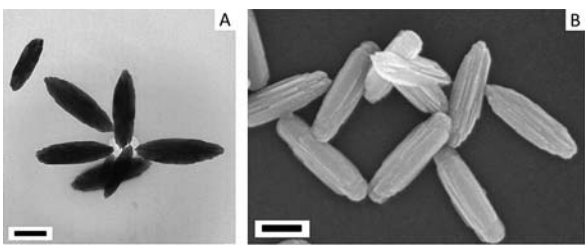

Fig. 13 TEM (left) and SEM (right) images of GaOOH nanospindles formed *via* a His–Ser dipeptide (scale bar: 100nm). Reprinted with permission from Elsevier, Copyright 2010.[129]

4 Conclusion

Hierarchy plays an important role in natural materials. In many cases, the structure develops *via* self-organisation of a protein matrix and subsequent organisation of other proteins into this matrix. The interactions of various proteins with the amorphous precursors control the mineralization process wherein crystallites form subsequently. The resulting materials often exhibit strong anisotropies, reflected in their mechanical properties. While biomineralization in nature is a complex process due to the large variety of substances involved, there are many successful attempts to adopt the basic mechanisms of biomineralization to the production of new materials. Especially the basic hierarchy of a pre-organized matrix, subsequently mineralized in a controlled manner, is reproduced and used *in vitro*.

Some studies also attempted biomineralization with matrix proteins not associated with mineralization processes in nature, for example hydrophobins or ferritin. It is even possible to use the understanding of biomineralization to create new proteins and peptides to influence the crystal structures. These successes reveal that our understanding already goes beyond the pure mimicry of nature, meaning that there are numerous possibilities to create new materials by adapting the principles of nature.

Nature produces materials under ambient conditions. While it often uses simple supersaturation, catalytic activity is needed in other cases. Especially the formation of silica with silicateins and silaffins, which are also important matrix components, has been studied in detail. The insights allow us to produce synthetic silicateins or its analogues, to mimic the active part of the protein, or to use other more abundant enzymes to produce materials from precursors under ambient conditions. This is not just important in terms of effectiveness, but also enables the co-precipitation or encapsulation of catalysts, drugs and even cells. The latest studies show that we can not only produce silica *via* this route, but also non-natural compounds like titanium dioxide and gallium oxide under ambient conditions.

The growing number of investigations on proteins, enzymes and materials shows that biomineralization is a valuable tool for the development and production of new material classes at increased efficiency compared to conventional processes.

References

1 An-Wu Xu, Yurong Ma and Helmut Cölfen, Biomimetic mineralization, *J. Mater. Chem.*, 2007, **17**, 415–449.
2 C. V. Raman, On iridescent shells, *Proceedings of the Indian Academy of Science*, 1935, **1**, 567–589.
3 W. V. Mayneord, An X-ray study of the crystal structure of some biological objects, *British Jour. Radiol.*, 1927, **23**, 21–30.
4 Huajian Gao, Baohua Ji, Ingomar L. Jager, Eduard Arzt and Peter Fratzl, Materials become insensitive to flaws at nanoscale: Lessons from nature, *Applied Physical Sciences*, 2003, **100**, 5597–5600.
5 Peter Fratzl, Himadri S. Gupta, Franz Dieter Fischer and Otmar Kolednik, Hindered crack propagation in materials with periodically varying young's modulus–lessons from biological materials, *Adv. Mater.*, 2007, **19**, 2657–2661.
6 Graeme K. Hunter, Jason O'oung, Bernd Grohe, Mikko Karttunen and Harvey A. Goldberg, The flexible polyelectrolyte hypothesis of protein-biomineral interaction, *Langmuir*, 2010, **26**, 18639–18646.
7 Kazuhiko Kawasaki, Anne V. Buchanan and Kenneth M. Weiss, Biomineralization in humans: Making the hard choices in life, *Annu. Rev. Genet.*, 2009, **43**, 119–142.

8 P. Fratzl, H. S. Gupta, E. P. Paschalisb and P. Roschger, Structure and mechanical quality of the collagen–mineral nano-composite in bone, *J. Mater. Chem.*, 2004, **14**, 2115–2123.

9 Matthew J. Olszta, Xingguo Cheng, Sang Soo Jee, Rajendra Kumar, Yi-Yeoun Kim, Michael J. Kaufman, Elliot P. Douglas and Laurie B. Gower, Bone structure and formation: A new perspective, *Mater. Sci. Eng., R*, 2007, **58**, 77–116.

10 Ingomar Jäger and Peter Fratzl, Mineralized collagen fibrils: A mechanical model with a staggered arrangement of mineral particles, *Biophys. J.*, 2000, **79**, 1737–1746.

11 Jong Seto, Himadri S. Gupta, Paul Zaslansky, H. Daniel Wagner and Peter Fratzl, Tough lessons from bone: Extreme mechanical anisotropy at the mesoscale, *Adv. Funct. Mater.*, 2008, **18**, 1905–1911.

12 Barbara J. Tarasevich, Scott Lea, William Bernt, Mark Engelhard and Wendy J. Shaw, Adsorption of amelogenin onto self-assembled and fluoroapatite surfaces, *J. Phys. Chem. B*, 2009, **113**, 1833–1842.

13 Lijun Wang, Xiangying Guan, Chang Du, Janet Moradian-Oldak and George H. Nancollas, Amelogenin promotes the formation of elongated apatite microstructures in a controlled crystallization system, *J. Phys. Chem.*, 2007, **111**, 6398–6404.

14 DanHong Zhu, Michael L. Paine, Wen Luo, Pablo Bringas Jr. and Malcolm L. Snead, Altering biomineralization by protein design, *J. Biol. Chem.*, 2006, **281**, 21173–21182.

15 Ryousuke Takagi and Tomoyuki Miyashita, Prismin: A new matrix protein family in the japanese pearl oyster (pinctada fucata) involved in prismatic layer formation, *Zool. Sci.*, 2010, **27**, 416–426.

16 Julyan H. E. Cartwright and Antonio G. Checa, The dynamics of nacre self-assembly, *J. R. Soc. Interface*, 2007, **4**, 491–504.

17 Michio Suzuki, Shohei Sakuda and Hiromichi Nagasawa, Identification of chitin in the prismatic layer of the shell and a chitin synthase gene from the japanese pearl oyster, pinctada fucata, *Biosci., Biotechnol., Biochem.*, 2007, **71**, 1735–1744.

18 Takeshi Furuhashi, Clemes Schwarzinger, Ivan Miksik, Miloslav Smrz and Anton Beran, Molluscan shell evolution with review of shell calcification hypothesis, *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.*, 2009, **154**, 351–357.

19 Rebecca A. Metzler, John Spencer Evans, Christopher E. Killian, Dong Zhou, Tyler H. Churchill, Narayana P. Appathurai, Susan N. Coppersmith and P. U. P. A. Gilbert, Nacre protein fragment templates lamellar aragonite growth, *J. Am. Chem. Soc.*, 2009, **132**, 6329–6334.

20 Rebecca A. Metzler, Won Kim, Katya Delak, John Spencer Evans, Dong Zhou, Elia Beniash, Fred Wilt, Mike Abrecht, Jau-Wern Chiou, Jinghua Guo, Susan N. Coppersmith and P. U. P. A. Gilbert, Probing the organic-mineral interface at the molecular level in model biominerals, *Langmuir*, 2008, **24**, 2680–2687.

21 S. Elhadj, J. J. De Yoreo, J. R. Hoyer and P. M. Dove, Role of molecular charge and hydrophilicity in regulating the kinetics of crystal growth, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 19237–19242.

22 Takeshi Furuhashi, Ivan Miksik, Miloslav Smrz, Bettina Germann, Dashnor Nebija, Bodo Lachmann and Christian Noe, Comparison of aragonitic molluscan shell proteins, *Comp. Biochem. Physiol., Part B: Biochem. Mol. Biol.*, 2010, **155**, 195–200.

23 D. Raabe, P. Romano, C. Sachs, H. Fabritius, A. Al-Sawalmih, S.-B. Yi, G. Servos and H. G. Hartwig, Microstructure and crystallographic texture of the chitin–protein network in the biological composite material of the exoskeleton of the lobster homarus americanus, *Mater. Sci. Eng., A*, 2006, **421**, 143–153.

24 Ali Al-Sawalmih, Chenghao Li, Stefan Siegel, Helge Fabritius, Sangbong Yi, Dierk Raabe, Peter Fratzl and Oskar Paris, Microtexture and chitin/calcite orientation relationship in the mineralized exoskeleton of the american lobster, *Adv. Funct. Mater.*, 2008, **18**, 3307–3314.

25 Yuya Yamamoto, Tatsuya Nishimura, Ayae Sugawara, Hirotaka Inoue, Hiromichi Nagasawa and Takashi Kato, Effects of peptides on caco3 crystallization: Mineralization properties of an acidic peptide isolated from exoskeleton of crayfish and its derivatives, *Cryst. Growth Des.*, 2008, **8**, 4062–4065.

26 Liliana Marín-García, Bernardo A. Frontana-Uribe, Juan Pablo Reyes-Grajeda, Vivian Stojanoff, Hugo Javier Serrano-Posada and Abel Moreno, Chemical recognition of carbonate anions by proteins involved in biomineralization processes and their influence on calcite crystal growth, *Cryst. Growth Des.*, 2008, **8**, 1340–1345.

27 Xiaoqiang Wang, Congmeng Wu, Kai Tao, Kang Zhao, Jiqian Wang, Hai Xu, Daohong Xia, Honghong Shan and Jian R. Lu, Influence of ovalbumin on caco3 precipitation during *in vitro* biomineralization, *J. Phys. Chem. B*, 2010, **114**, 5301–5308.

28 Colin L. Freeman, John H. Harding, David Quigley and P. Mark Rodger, Structural control of crystal nuclei by an eggshell protein, *Angew. Chem., Int. Ed.*, 2010, **49**, 5135–5137.

29 Vitaliy Pipich, Mathias Balz, Stephan E. Wolf, Wolfgang Tremel and Dietmar Schwahn, Nucleation and growth of $caco_3$ mediated by the egg-white protein ovalbumin: A time-resolved in situ study using small-angle neutron scattering, *J. Am. Chem. Soc.*, 2008, **130**, 6879–6892.

30 Manfred Sumper and Nils Kröger, Silica formation in diatoms: the function of long-chain polyamines and silaffins, *J. Mater. Chem.*, 2004, **14**, 2059–2065.

31 Manfred Sumper and Eike Brunner, Learning from diatoms: Nature's tools for the production of nanostructured silica, *Adv. Funct. Mater.*, 2006, **16**, 17–26.

32 Katharina Lutz, Christian Gröger, Manfred Sumper and Eike Brunner, Biomimetic silica formation: Analysis of the phosphate-induced self-assembly of polyamines, *Phys. Chem. Chem. Phys.*, 2005, **7**, 2812–2815.

33 Nicole Poulsen, Manfred Sumper and Nils Kröger, Biosilica formation in diatoms: Characterization of native silaffin-2 and its role in silica morphogenesis, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 12075–12080.

34 Nils Kröger, Sonja Lorenz, Eike Brunner and Manfred Sumper, Self-assembly of highly phosphorylated silaffins and their function in biosilica morphogenesis, *Science*, 2002, **298**, 584–586.

35 Nils Kröger, Rainer Deutzmann, Christian Bergsdorf and Manfred Sumper, Species-specific polyamines from diatoms control silica morphology, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 14133–14138.

36 Nils Kröger, Rainer Deutzmann and Manfred Sumper, Silica-precipitating peptides from diatoms, *J. Biol. Chem.*, 2001, **276**, 26066–26070.

37 André Scheffel, Nicole Poulsen, Samuel Shian and Nils Kröger, Nanopatterned protein microrings from a diatom that direct silica morphogenesis, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 3175–3180.

38 Karlheinz Mann, Fred H. Wilt and Albert J. Poustka, Proteomic analysis of sea urchin (strongylocentrotus purpuratus) spicule matrix, *Proteome Sci.*, 2010, **8**, 1–12.

39 M. Azizur Rahman and Tamotsu Oomori, Structure, crystallization and mineral composition of sclerites in the alcyonarian coral, *J. Cryst. Growth*, 2008, **310**, 3528–3534.

40 M. Azizur Rahman and Tamotsu Oomori, *In vitro* regulation of caco3 crystal growth by the highly acidic proteins of calcitic sclerites in soft coral, sinularia polydactyla, *Connective Tissue Research*, 2009, **50**, 285–293.

41 Dongni Ren, Zhuo Li, Yonghua Gao and Qingling Feng, Effects of functional groups and soluble matrices in fish otolith on calcium carbonate mineralization, *Biomed. Mater.*, 2010, **5**, 1–11.

42 Hidekazu Tohse, Kazuko Saruwatari, Toshihiro Kogure, Hiromichi Nagasawa and Yasuaki Takagi, Control of polymorphism and morphology of calcium carbonate crystals by a matrix protein aggregate in fish otoliths, *Cryst. Growth Des.*, 2009, **9**, 4897–4901.

43 Tzu-En Hua and Chia-Wei Li, Silica biomineralization in the radula of a limpet notoacmea schrenckii (gastropoda: Acmaeidae), *Zoological Studies*, 2007, **46**, 379–388.

44 Hermann Ehrlich, Paul Simon, Wilder Carrillo-Cabrera, Vasily V. Bazhenov, Joseph P. Botting, Micha Ilan, Alexander V. Ereskovsky, Guilherme Muricy, Hartmut Worch, Axel Mensch, René Born, Armin Springer and Kurt Kummer, Insights into chemistry of biological materials: Newly discovered silica-aragonite-chitin biocomposites in demosponges, *Chem. Mater.*, 2010, **22**, 1462–1471.

45 Y. Zhai and F. Z. Cui, Recombinant human-like collagen directed growth of hydroxyapatite nanocrystals, *J. Cryst. Growth*, 2006, **291**, 202–206.

46 Nadine Nassif, Frédéric Gobeaux, Jong Seto, Emmanuel Belamie, Patrick Davidson, Pierre Panine, Gervaise Mosser, Peter Fratzl and Marie-Madeleine Giraud Guille, Self-assembled collagen-apatite matrix with bone-like hierarchy, *Chem. Mater.*, 2010, **22**, 3307–3309.

47 Giuseppe Falini, Simona Fermani, Barbara Palazzo and Norberto Roveri, Helical domain collagen substrates mineralization in simulated body fluid, *Journal of Biomedical Materials Research*, 2008, **87**, 470–476.

48 Julia Mahamid, Amnon Sharir, Lia Addadi and Steve Weiner, Amorphous calcium phosphate is a major component of the forming fin bones of zebrafish: Indications for an amorphous precursor phase, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 12748–12753.

49 Laurie B. Gower, Biomimetic model systems for investigating the amorphous precursor pathway and its role in biomineralization, *Chem. Rev.*, 2008, **108**, 4551–4627.

50 Johanna Wald, Susanne Wiese, Thomas Eckert, Willi Jahnen-Dechent, Walter Richtering and Alexander Heiss, Formation and stability kinetics of calcium phosphate–fetuin-a colloidal particles probed by time-resolved dynamic light scattering, *Soft Matter*, 2011, **7**, 2869–2874.

51 Alexander Heiss, Thomas Eckert, Anke Aretz, Walter Richtering, Wim van Dorp, Cora Schäfer and Willi Jahnen-Dechen, Hierarchical role of fetuin-a and acidic serum proteins in the formation and stabilization of calcium phosphate particles, *J. Biol. Chem.*, 2008, **283**, 14815–14825.

52 Franklin R. Tay and David H. Pashley, Guided tissue remineralisation of partially demineralised human dentine, *Biomaterials*, 2008, **29**, 1127–1137.

53 Sivakumar Gajjeraman, Karthikeyan Narayanan, Jianjun Hao, Chunlin Qin and Anne George, Matrix macromolecules in hard tissues control the nucleation and hierarchical assembly of hydroxyapatite, *J. Biol. Chem.*, 2007, **282**, 1193–1204.

54 Viorel Marin Rusu, Chuen-How Ng, Max Wilke, Brigitte Tierscha, Peter Fratzl, and Martin G. Petera. Size-controlled hydroxyapatite nanoparticles as self-organized organic–inorganic composite materials, *Biomaterials*, 2005, **26**, 5414–5426.

55 Ellen C. Keene, John S. Evans and Lara A. Estroff, Matrix interactions in biomineralization: Aragonite nucleation by an intrinsically disordered nacre polypeptide, n16n, associated with a β-chitin substrate, *Cryst. Growth Des.*, 2010, **10**, 1383–1389.

56 Cheng Cheng, Zhengzhong Shao and Fritz Vollrath, Silk fibroin-regulated crystallization of calcium carbonate, *Adv. Funct. Mater.*, 2008, **18**, 2127–2179.

57 Xiaoqiang An and Chuanbao Cao, Coeffect of silk fibroin and self-assembly monolayers on the biomineralization of calcium carbonate, *J. Phys. Chem. B*, 2008, **112**, 15844–15849.

58 Yurong Cai, Jun Jin, Danping Mei, Nianxin Xia and Juming Yao, Effect of silk sericin on assembly of hydroxyapatite nanocrystals into enamel prism-like structure, *J. Mater. Chem.*, 2009, **19**, 5751–5758.

59 Yucheng Li, Yurong Cai, Xiangdong Kong and Juming Yao, Anisotropic growth of hydroxyapatite on the silk fibroin films, *Appl. Surf. Sci.*, 2008, **255**, 1681–1685.

60 Xiaolan Ba, Miriam Rafailovich, Yizhi Meng, Nadine Pernodet, Sue Wirick, Helga Füredi-Milhofer, Yi-Xian Qin and Elaine DiMasi, Complementary effects of multi-protein components on biomineralization *in vitro*, *J. Struct. Biol.*, 2010, **170**, 83–92.

61 K. Subburaman, N. Pernodet, S. Y. Kwak, E. DiMasi, S. Ge, V. Zaitsev, X. Ba, N. L. Yang and M. Rafailovich, Templated biomineralization on self-assembled protein fibers, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 14672–14677.

62 Zhong-Hui Xue, Shu-Xi Dai, Bin-Bin Hu and Zu-Liang Du, Effect of langmuir monolayer of bovine serum albumin protein on the morphology of calcium carbonate, *Mater. Sci. Eng., C*, 2009, **29**, 1998–2002.

63 Wen-Tao Hou and Qing-Ling Feng, Morphologies and growth model of biomimetic fabricated calcite crystals using amino acids and insoluble matrix membranes of mytilus edulis, *Cryst. Growth Des.*, 2006, **6**, 1086–1090.

64 Il Won Kim, Sebastiano Collino, Daniel E. Morse and John Spencer Evans, A crystal modulating protein from molluscan nacre that limits the growth of calcite in vitro, *Cryst. Growth Des.*, 2006, **6**, 1078–1082.

65 Michio Suzuki, Hiromichi Nagasawa and Toshihiro Kogure, Synthesis and structure of hollow calcite particles, *Cryst. Growth Des.*, 2006, **6**, 2004–2006.

66 Moise Ndao, Ellen Keene, Fairland F. Amos, Gita Rewari, Christopher B. Ponce, Lara Estroff and John Spencer Evans, Intrinsically disordered mollusk shell prismatic protein that modulates calcium carbonate crystal growth, *Biomacromolecules*, 2010, **11**, 2539–2544.

67 Günther Jutz and Alexander Böker, Bionanoparticles as functional macromolecular building blocks – a new class of nanomaterials, *Polymer*, 2011, **52**, 211–232.

68 Mark Young, Debbie Willits, Masaki Uchida and Trevor Douglas, Plant viruses as biotemplates for materials and their use in nanotechnology, *Annu. Rev. Phytopathol.*, 2008, **46**, 361–384.

69 Fuke Wang, Binrui Cao and Chuanbin Mao, Bacteriophage bundles with prealigned ca^{2+} initiate the oriented nucleation and growth of hydroxylapatite, *Chem. Mater.*, 2010, **22**, 3630–3636.

70 Nicole F. Steinmetz, Sachin N. Shah, J. Elaine Barclay, Ghanasyam Rallapalli, George P. Lomonossoff and J. David, Evans. Virus-templated silica nanoparticles, *Small*, 2009, **5**, 813–816.

71 Günther Jutz and Alexander Böker, Bio-inorganic microcapsules from templating protein- and bionanoparticle-stabilized pickering emulsions, *J. Mater. Chem.*, 2010, **20**, 4299–4304.

72 Alexander Schulz, Bernd Michael Liebeck, Daniela John, Alexander Heiss, Thomas Subkowski, and Alexander Böker. Protein-mineral hybrid capsules from emulsions stabilized with an amphiphilic protein. *J. Mater. Chem.*, DOI: 10.1039/C1JM10662G.

73 Markus B. Linder, Hydrophobins: Proteins that self assemble at interfaces, *Curr. Opin. Colloid Interface Sci.*, 2009, **14**, 356–363.

74 Wendel Wohlleben, Thomas Subkowski, Claus Bollschweiler, Bernhard von Vacano, Yaquian Liu, Wolfgang Schrepp and Ulf Baus, Recombinantly produced hydrophobins from fungal analogues as highly surface-active performance proteins, *Eur. Biophys. J.*, 2010, **39**, 457–468.

75 D. Santhiya, Z. Burghard, C. Greiner, Lars P. H. Jeurgens, T. Subkowski and J. Bill, Bioinspired deposition of tio_2 thin films induced by hydrophobins, *Langmuir*, 2010, **26**, 6494–6502.

76 Young Kyung Kim, Li sha Gu, Thomas E. Bryan, Jong R. Kim, Liang Chen, Yan Liu, James C. Yoon, Lorenzo Breschi, David H. Pashley and Franklin R. Tay, Mineralisation of reconstituted collagen using polyvinylphosphonic acid/polyacrylic acid templating matrix protein analogues in the presence of calcium, phosphate and hydroxyl ions, *Biomaterials*, 2010, **31**, 6618–6627.

77 Toru Yoshino and Hiroyuki Kagi, Effects of aspartic acid on calcite dissolution rate and solubility, *Chem. Lett.*, 2008, **37**, 508–509.

78 Olivier Durupthy, Joachim Bill and Fritz Aldinger, Bioinspired synthesis of crystalline tio2: Effect of amino acids on nanoparticles structure and shape, *Cryst. Growth Des.*, 2007, **7**, 2696–2704.

79 Denis Gebauer, Andreas Verch, Hans G. Borner and Helmut Cölfen, Influence of selected artificial peptides on calcium carbonate precipitation - a quantitative study, *Cryst. Growth Des.*, 2009, **9**, 2398–2403.

80 Yuan Quan, Guo Xiaodong, Zheng Qixin, Zhao Ming, Pan Zheng, Chen Shunguang and Quan Daping, Bioinspired growth of hydroxyapatite nanocrystals on plga- (peg-asp)n scaffolds modified with oligopeptide derived from bmp-2, *Key Engineering Materials*, 2007, **334–335**, 1261–1264.

81 Toru Tsuji, Kazuo Onuma, Akira Yamamoto, Mayumi Iijima and Kiyotaka Shiba, Direct transformation from amorphous to crystalline calcium phosphate facilitated by motif-programmed artificial proteins, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 16866–16870.

82 Kiyotaka Shiba and Tamiko Minamisawa, A synthesis approach to understanding repeated peptides conserved in mineralization proteins, *Biomacromolecules*, 2007, **8**, 2659–2664.

83 David L. Masica, Sarah B. Schrier, Elizabeth A. Specht and Jeffrey J. Gray, De novo design of peptide-calcite biomineralization systems, *J. Am. Chem. Soc.*, 2010, **132**, 12252–12262.

84 Jennifer N. Cha, Katsuhiko Schimizu, Yan Zhou, Sean C. Christiansen, Bradley F. Chmelka, Galen D. Stucky and Daniel E. Morse, Silicatein filaments and subunits from a marine sponge direct the polymerization of silica and silicones *in vitro*, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 361–365.

85 Heinz C. Schröder, Ute Schloßmacher, Alexandra Boreiko, Filipe Natalio, Malgorzata Baranowska, David Brandt, Xiaohong Wang, Wolfgang Tremel, Matthias Wiens and Werner E. G. Müller, Biosilica in Evolution, Morphogenesis, and Nanobiotechnology, *SpringerLink*, 2009, **47**, 295–314.

86 James C. Weaver and Daniel E. Morse, Molecular biology of demosponge axial filaments and their roles in biosilicification, *Microsc. Res. Tech.*, 2003, **62**, 356–367.

87 Xiaohong Wang, Matthias Wiens, Heinz C. Schröder, Shixue Hu, Enrico Mugnaioli, Ute Kolb, Wolfgang Tremel, Dario Pisignano and Werner E. G. Müller, Morphology of sponge spicules: Silicatein a structural protein for bio-silica formation, *Adv. Eng. Mater.*, 2010, **12**, B422–B437.

88 Vincenzo Abbate, Alan R. Bassindale, Kurt F. Brandstadt and Peter G. Taylor, A large scale enzyme screen in the search for new methods of silicon-oxygen bond formation, *J. Inorg. Biochem.*, 2011, **105**, 268–275.

89 Yan Zhou, Katsuhiko Shimizu, Jennifer N. Cha, Galen D. Stucky and Daniel E. Morse, Efficient catalysis of polysiloxane synthesis by silicatein α requires specific hydroxy and imidazole functionalities, *Angew. Chem., Int. Ed.*, 1999, **38**, 779–782.

90 Werner E. G. Müller, Ute Schloßmacher, Xiaohong Wang, Alexandra Boreiko, David Brandt, Stephan E. Wolf, Wolfgang Tremel and Heinz C. Schröder, Poly(silicate)-metabolizing silicatein in siliceous spicules and silicasomes of demosponges comprises dual enzymatic activities (silica polymerase and silica esterase), *FEBS J.*, 2008, **275**, 362–370.

91 Stephan E. Wolf, Ute Schlossmacher, Anna Pietuch, Bernd Mathiasch, Heinz-C. Schröder, Werner E. G. Müller and Wolfgang Tremel, Formation of silicones mediated by the sponge enzyme silicatein α, *Dalton Trans.*, 2010, **39**, 9245–9249.

92 Alan R. Bassindalea, Kurt F. Brandstadta, Thomas H. Laneb and Peter G. Taylora, Enzyme-catalysed siloxane bond formation, *J. Inorg. Biochem.*, 2003, **96**, 401–406.

93 Mark Frampton, Ateeya Vawda, Jackie Fletchera and Paul M. Zelisko, Enzyme-mediated sol–gel processing of alkoxysilanes, *Chem. Commun.*, 2008, **44**, 5544–5546.

94 Gregory P. Smith, Kelly J. Baustian, Christopher J. Ackerson and Daniel L. Feldheim, Metal oxide formation by serine and cysteine proteases, *J. Mater. Chem.*, 2009, **19**, 8299–8306.

95 Heinz C. Schröder, Oleksandra Boreiko, Anatoli Krasko, Andreas Reiber, Heiko Schwertner and Werner E. G. Müller, Mineralization of saos-2 cells on enzymatically (silicatein) modified bioactive osteoblast-stimulating surfaces, *J. Biomed. Mater. Res., Part B*, 2005, **75B**, 387–382.

96 Matthias Wiens, Xiaohong Wang, Filipe Natalio, Heinz C. Schröder, Ute Schlobmacher, Shunfeng Wang, Michael Korzhev, Werner Geurtsen and Werner E. G. Müller, Bioinspired fabrication of bio-silica-based bone-substitution materials, *Adv. Eng. Mater.*, 2010, **12**, B438–B450.

97 Werner E. G. Müller, Alexandra Boreiko, Xiaohong Wang, Anatoli Krasko, Werner Geurtsen, Márcio Reis Custódio, Thomas Winkler, Lada Lukić-Bilela, Thorben Link and Heinz C. Schröder, Morphogenetic activity of silica and bio-silica on the expression of genes controlling biomineralization using saos-2 cells, *Calcif. Tissue Int.*, 2007, **81**, 382–393.

98 Richard L. Brutchey and Daniel E. Morse, Silicatein and the translation of its molecular mechanism of biosilicification into low temperature nanomaterial synthesis, *Chem. Rev.*, 2008, **108**, 4915–4934.

99 Jan L. Sumerel, Wenjun Yang, David Kisailus, James C. Weaver, Joon Hwan Choi and Daniel E. Morse, Biocatalytically templated synthesis of titanium dioxide, *Chem. Mater.*, 2003, **15**, 4804–4809.

100 David Kisailus, Joon Hwan Choi, James C. Weaver, Wenjun Yang and Daniel E. Morse, Enzymatic synthesis and nanostructural control of gallium oxide at low temperature, *Adv. Mater.*, 2005, **17**, 314–318.

101 Patrick O'Leary, Cornelis A. van Walree, Nilesh C. Mehendale, Jan Sumerel, Daniel E. Morse, William C. Kaska, Gerard van Koten and Robertus J. M. Klein Gebbink, Enzymatic immobilization of organometallic species: biosilification of ncn- and pcp-pincer metal species using demosponge axial filaments, *Dalton Trans.*, 2009, **38**, 4289–4291.

102 Filipe Natalio, Enrico Mugnaioli, Matthias Wiens, Xiaohong Wang, Heinz C. Schröder, Muhammad Nawaz Tahir, Wolfgang Tremel, Ute Kolb and Werner E. G. Müller, Silicatein-mediated incorporation of titanium into spicules from the demosponge suberites domuncula, *Cell Tissue Res.*, 2010, **339**, 429–436.

103 Paul Curnow, Paul H. Bessette, David Kisailus, Meredith M. Murr, Patrick S. Daugherty and Daniel E. Morse, Enzymatic synthesis of layered titanium phosphates at low temperature and neutral ph by cell-surface display of silicatein, *J. Am. Chem. Soc.*, 2005, **127**, 15749–15755.

104 Werner E. G. Müller, Sylvia Engela, Xiaohong Wang, Stephan E. Wolf, Wolfgang Tremel, Narsinh L. Thakurd, Anatoli Kraskoa, Mugdha Divekara and Heinz C. Schröder, Bioencapsulation of living bacteria (escherichia coli) with poly (silicate) after transformation with silicatein-a gene, *Biomaterials*, 2008, **29**, 771–779.

105 Muhammad Nawaz Tahir, Patrick Théato, Werner E. G. Müller, Heinz C. Schröder, Andreas Janshoff, Jian Zhang, Joachim Huthe and Wolfgang Tremel, Monitoring the formation of biosilica catalysed by histidine-tagged silicatein, *Chem. Commun.*, 2004, 2848–2849.

106 Muhammad Nawaz Tahir, Patrick Theźato, Werner E. G. Müller, Heinz C. Schröder, Alexandra Borejko, Simon Faiß, Andreas Janshoff, Joachim Huthe and Wolfgang Tremel, Formation of layered titania and zirconia catalysed by surface-bound silicatein, *Chem. Commun.*, 2005, 5533–5535.

107 Akhilesh Rai and Carole C. Perry, Facile fabrication of uniform silica films with tunable physical properties using silicatein protein from sponges, *Langmuir*, 2010, **26**, 4152–4159.

108 Mohammed Ibrahim Shukoor, Filipe Natalio, Helen Annal Therese, Muhammad Nawaz Tahir, Vadim Ksenofontov, Martin Panthöfer, Marc Eberhardt, Patrick Theato, Heinz Christoph Schröder, Werner E. G. Müller and Wolfgang Tremel, Fabrication of a silica coating on magnetic γ-Fe2O3 nanoparticles by an immobilized enzyme, *Chem. Mater.*, 2008, **20**, 3567–3573.

109 Muhammad Nawaz Tahir, Filipe Natalio, Annal Therese, Aswani Yella, Nadine Metz, Muhammad Raza Shah, Enrico Mugnaioli, Rüdiger Berger, Patrick Theato, Heinz-Christoph Schroöder, Werner E. G. Müller and Wolfgang Tremel, Enzyme-mediate deposition of a TiO2 coating onto biofunctionalized WS2 chalcogenide nanotubes, *Adv. Funct. Mater.*, 2009, **19**, 285–291.

110 Muhammad Nawaz Tahir, Marc Eberhardt, Helen Annal Therese, Ute Kolb, Patrick Theato, Werner E. G. Müller, Heinz-Christoph Schröder and Wolfgang Tremel, From single molecules to nanoscopically structured functional materials: Au nanocrystal growth on TiO2 nanowires controlled by surface-bound silicatein, *Angew. Chem., Int. Ed.*, 2006, **45**, 4803–4809.

111 Birger Lange, Nadine Metz, Muhammad Nawaz Tahir, Friederike Fleischhaker, Patrick Theato, Heinz-Christoph Schröder, Werner E. G. Müller, Wolfgang Tremel and Rudolf Zentel, Functional polymer-opals from core-shell colloids, *Macromol. Rapid Commun.*, 2007, **28**, 1987–1994.

112 Toru Shiomi, Tatsuo Tsunoda, Akiko Kawai, Hiroyuki Chiku, Fujio Mizukamib and Kengo Sakaguchi, Synthesis of protein-silica hybrid hollow particles through the combination of protein catalysts and sonochemical treatment, *Chem. Commun.*, 2005, **41**, 5325–5327.

113 Hans-Peter Hentze, Srinivasa R. Raghavan, Craig A. McKelvey and Eric W. Kaler, Silica hollow spheres by templating of catanionic vesicles, *Langmuir*, 2003, **19**, 1069–1074.

114 Guilia Forsasieri, Stephane Badaire, Renal Backov, Oliver Mondain-Monval, Cecile Zakri and Philippe Poulin, Mesoporous and homothetic silica capsules in reverse-emulsion microreactors, *Adv. Mater.*, 2004, **16**, 1094–1097.

115 Jeroen J. L. M. Cornelissen, Eric. F. Connor, Ho-Cheol Kim, Victor Y. Lee, Teddie Magibitang, Philip M. Rice, Willi Volksen, Linda K. Sundberg and Robert D. Miller, Versatile synthesis of nanometer sized hollow silica spheres, *Chem. Commun.*, 2003, **39**, 1010–1011.

116 Toru Shiomi, Tatsuo Tsunoda, Akiko Kawai, Fujio Mizukami and Kengo Sakaguchi, Biomimetic synthesis of lysozyme-silica hybrid hollow particles using sonochemical treatment: Influence of ph and lysozyme concentration on morphology, *Chem. Mater.*, 2007, **19**, 4486–4493.

117 Toru Shiomi, Tatsuo Tsunoda, Akiko Kawai, Fujio Mizukamib and Kengo Sakaguchi, Formation of cage-like hollow spherical silica *via* a mesoporous structure by calcination of lysozyme-silica hybrid particles, *Chem. Commun.*, 2007, **43**, 4404–4406.

118 Toru Shiomi, Tatsuo Tsunoda, Akiko Kawai, Shun ichi Matsuura, Fujio Mizukami and Kengo Sakaguchi, Synthesis of a cagelike hollow aluminosilicate with vermiculate micro-through-holes and its application to ship-in-bottle encapsulation of protein, *Small*, 2009, **5**, 67–71.

119 Heather R. Luckarift, Matthew B. Dickerson, Kenneth H. Sandhage and Jim C. Spain, Rapid, room-temperature synthesis of antibacterial bionanocomposites of lysozyme with amorphous silica or titania, *Small*, 2006, **2**, 640–643.

120 Alan R. Bassindale, Peter G. Taylor, Vincenzo Abbatea and Kurt F. Brandstadt, Simple and mild preparation of silica-enzyme composites from silicic acid solution, *J. Mater. Chem.*, 2009, **19**, 7606–7609.

121 Dmitri Ivnitski, Kateryna Artyushkova, Rosalba A. Rincon, Plamen Atanassov, Heather R. Luckarift and Glenn R. Johnson, Entrapment of enzymes and carbon nanotubes in biologically synthesized silica: Glucose oxidase-catalyzed direct electron transfer, *Small*, 2008, **4**, 357–364.

122 Jennifer N. Cha, Galen D. Stucky, Daniel E. Morse and Timothy J. Deming, Biomimetic synthesis of ordered silica structures mediated by block copolypeptides, *Nature*, 2000, **403**, 289–292.

123 Douglas H. Adamson, Daniel M. Dabbs, Daniel E. Morse and Ilhan A. Aksay, Non-peptide, silicatein inspired silica condensation catalyst, *Polymeric Materials: Science & Engineering*, 2004, **90**, 239–240.

124 Douglas H. Adamson, Daniel M. Dabbs, Carlos R. Pacheco, Marcus V. Giotto, Daniel E. Morse and Ilhan A. Aksay, Non-peptide polymeric silicatein r mimic for neutral ph catalysis in the formation of silica, *Macromolecules*, 2007, **40**, 5710–5717.

125 Kristian M. Roth, Yan Zhou, Wenjun Yang and Daniel E. Morse, Bifunctional small molecules are biomimetic catalysts for silica synthesis at neutral ph, *J. Am. Chem. Soc.*, 2005, **127**, 325–330.

126 Suvarchala Devi Pogula, Siddharth V. Patwardhan, Carole C. Perry, John W. Gillespie, Shridhar Yarlagadda and Kristi L. Kiick, Continuous silica coatings on glass fibers *via* bioinspired approaches, *Langmuir*, 2007, **23**, 6677–6683.

127 Lin Xia and Zhibo Li, Poly(L-lysine)-mediated biomimetic silica synthesis: Effects of mixing sequences and counterion concentrations, *Langmuir*, 2011, **27**, 1116–1122.

128 A. J. Goldberg, M. C. Advincula, T. Komabayashi, P. A. Patel, P. T. Mather, D. G. Goberman and R. B. Kazemi, Polypeptide-catalyzed biosilicification of dentin surfaces, *J. Dent. Res.*, 2009, **88**, 377–381.

129 Inho Lee, Jinyoung Kwak, Seungjoo Haam and Sang-Yup Lee, Dipeptide-assisted growth of uniform gallium oxohydroxide spindles, *J. Cryst. Growth*, 2010, **312**, 2107–2112.

