Dalton Perspectives

Biomineralization: the Hard Part of Bioinorganic Chemistry!

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In a world of shifting scientific focus, there can be now no doubt that Inorganic Chemistry within the UK is in imminent danger of becoming seriously marginalized. The advent of strategic initiatives in Advanced Materials and Biomolecular Science has already hived off major sections of the UK Inorganic Chemistry Community. Combine this with the recent exhortation by the current President of the Dalton Division[1] for a fundamental change in style (not so much a paradigm lost but a paradigm regained), and UK inorganic chemists are clearly becoming an endangered species.

I write these remarks in an introduction to an article on *Biomineralization* because, as a scientist identified at the periphery of inorganic chemistry, I wish to place on record that the development of my research area has always been fostered by the activities and insights of more conventionally placed inorganic chemists. Indeed, much of the vitality of Inorganic Chemistry stems from this traditional reliance on a broad church of scientific enquiry unified in the principles of the Periodic Table. Our strength is based on an integrated but eclectic community and any internal or external narrowing of our scope of activity serves only to erode the quality of our teaching and inbue a growing parochialism in the research base. Instead, we must strive to enlarge our interests and responsibilities (the Dalton Division has a key role to play), in the belief that inorganic chemistry *in toto* will be strengthened by its engagement in new areas of scientific enquiry.

We have strong precedents for this attitude. For example, the field of bioinorganic chemistry highlights the fundamental impact that inorganic chemists continue to have on the understanding of the structure and function of molecules typically far removed from the systems studied in inorganic chemistry laboratories. And this impact has been made primarily by applying established principles of co-ordination chemistry (in its widest sense) to novel systems. In return, new fundamental knowledge of the structure, bonding, redox and electronic behaviour, and reactivity of molecules has been obtained. This synergy, which links tradition and innovation, is the life-blood of science. A similar perspective should be nurtured with regard to the recent side-stepping of synthetic inorganic chemists into Materials Science. If we fail to cultivate a fundamental interest in chemical (molecular) processes, then it follows that, in the not too distant future, our own resource base will be further depleted as funds are dissipated into peripheral disciplines.

With these comments in mind, I wish to present in this *Dalton Perspective* a somewhat personal view of a new field of inorganic chemistry, *viz. Bioinorganic solid state chemistry*. This field (which is usually called Biomineralization) concerns the structure and synthesis of inorganic minerals in biological environments. It is another example of how inorganic chemistry can drive the development of associated disciplines. My objective is not to provide a detailed overview (see refs. 2–7 for further reading) but to illustrate the major concepts and areas of interest, the difficulties that need to be overcome, and the potential for future research. Throughout, I wish to emphasize that the involvement of inorganic chemists is paramount to the development of this field.

Biominerals

Of the 20–25 essential elements required by living organisms, H, C, O, Mg, Si, P, S, Ca, Mn and Fe are common constituents of biological minerals (Table 1). Other essential elements such as N, F, Na, K, Cu and Zn are less widespread. Non-essential elements such as Ag, Au, Pb and U are found in association with the external cell walls of bacteria, whilst, surprisingly, Sr and Ba are accumulated and deposited as intracellular minerals. The major mineralized tissues such as bone and teeth, and shells are composed of calcium phosphate or carbonate minerals, respectively, in combination with a complex organic macromolecular matrix of proteins, polysaccharides and lipids. Simple considerations of ionic charge, size and packing, and their effect on hydration and lattice energies, are sufficient to explain the thermodynamic stability of these minerals (and other Group 2 oxyanion salts) in biological environments. Indeed, this strong thermodynamic driving force provides a very effective control of ionic concentrations in biological fluids (homeostasis and detoxification), but has the disadvantage that adventitious precipitation, resulting in kidney and urinary stones, dental calculus, *etc.*, can readily take place. Biological systems have evolved strategies to offset these thermodynamic limitations by establishing kinetic control of nucleation and crystal growth so that materials of specific size, structure, shape, orientation and organization are synthesised for precise functional use.

The predominance of calcium biominerals over other Group 2 metals can be explained by the low solubility products of the carbonates, phosphates, pyrophosphates, sulfates and oxalates and the relatively high levels of Ca in extracellular fluids (10^{-3} mol dm^{-3}). Significantly, the acid–base equilibria of many of these oxyanions (*e.g.* $HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$) provide a stringent means of regulating the activity products of supersaturated biological solutions. In some instances, this is so critical that enzymes such as carbonic anhydrase and alkaline phosphatase are employed. Magnesium salts are generally more soluble and no simple magnesium biominerals are known. However, Mg has an important role in influencing the structure of both carbonate and phosphate biominerals through lattice and surface substitution reactions. Other ions, such as Na^+, NH_4^+, K^+ and F^- in particular, also have a pronounced affect on the solubility of hydroxyapatite.

Although most biominerals are ionic salts, the stability of Si–O–Si units in water gives rise to hydrated inorganic polymers that are moulded into elaborate shapes by many unicellular organisms. Why some organisms utilize silica rather than calcium carbonate as a structural material is unknown. Moreover, silicon is considered to be an essential element although its biochemistry has not been elucidated in any detail.[8] The widespread occurrence of siliceous biominerals as structural elements in lower plants and animals suggests a role for Si

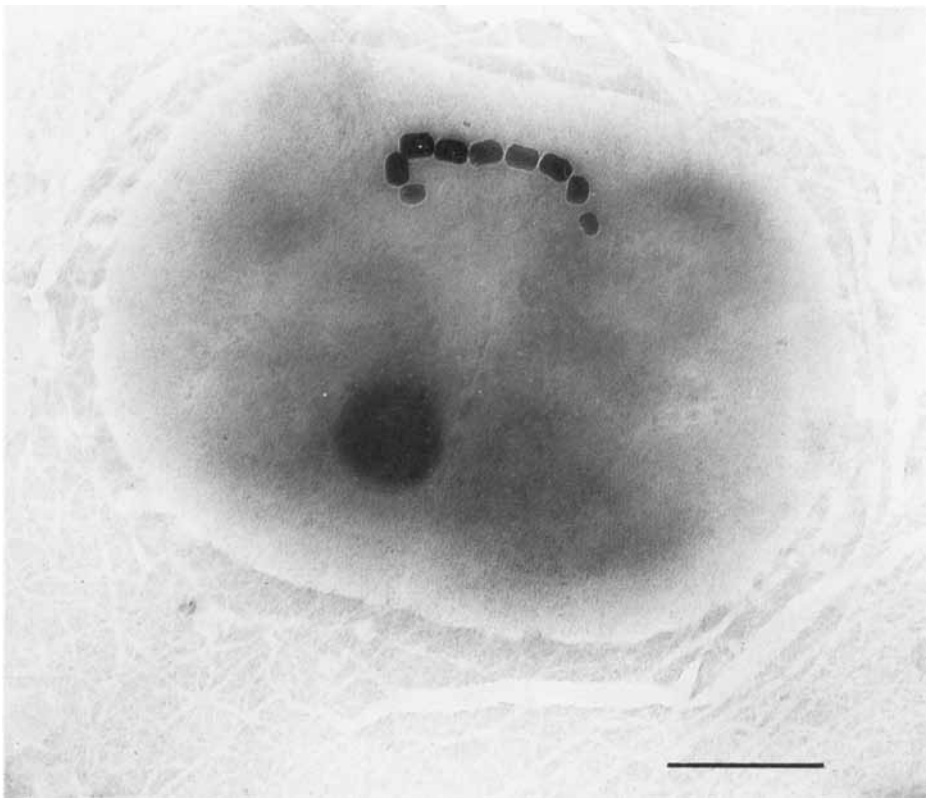
Table 1 The types and functions of the main biominerals

Mineral	Formula	Organism	Location	Function
Calcium carbonate				
Calcite	$CaCO_3$	Coccolithophorids	Cell wall scales	Exoskeleton
		Foraminifera	Test	Exoskeleton
		Trilobites	Eye lens	Optical imaging
		Molluscs	Shell	Exoskeleton
		Crustacea	Crab cuticle	Mechanical strength
		Aves	Eggshells	Protection
		Mammalia	Inner ear	Gravity receptor
Magnesium calcite	$(Mg,Ca)CO_3$	Octocorallia	Spicules	Mechanical support
		Echinoderms	Test/spines	Strength and protection
Aragonite	$CaCO_3$	Scleractinian corals	Cell wall	Exoskeleton
		Molluscs	Shell	Exoskeleton
		Gastropoda	Love dart	Reproduction
		Cephalopoda	Shell	Buoyancy device
		Fish	Head	Gravity receptor
Vaterite	$CaCO_3$	Gastropoda	Shell	Exoskeleton
		Ascidians	Spicules	Protection
Amorphous	$CaCO_3 \cdot nH_2O$	Crustacea	Crab cuticle	Mechanical strength
		Plants	Leaves	Calcium store
Calcium phosphate				
Hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$	Vertebrates	Bone	Endoskeleton/ion store
		Mammals	Teeth	Cutting/grinding
		Fish	Scales	Protection
Octacalcium phosphate	$Ca_8H_2(PO_4)_6$	Vertebrates	Bone/teeth	Precursor phase
Amorphous	?	Chitons	Teeth	Precursor phase
		Gastropoda	Gizzard plates	Crushing
		Bivalves	Gills	Ion store
		Mammalia	Mitochondria	Ion store
		Cow	Milk	Ion store
Group 2 sulfates				
Gypsum	$CaSO_4 \cdot 2H_2O$	Jellyfish	Statoconia	Gravity receptor
Celestite	$SrSO_4$	Acantharia	Cellular	Skeleton
Baryte	$BaSO_4$	Loxodes	Intracellular	Gravity device
		Xenophyophora	Intracellular	Unknown
		Chara	Statoliths	Gravity receptor
Silicon dioxide				
Amorphous silica	$SiO_2 \cdot nH_2O$	Diatoms	Cell wall	Exoskeleton
		Choanoflagellates	Lorica	Protection
		Radiolaria	Cellular	Skeleton
		Chrysophyta	Scales	Protection
		Limpets	Teeth	Mechanical strength
		Plants	Leaves	Protection
Iron oxides				
Magnetite	Fe_3O_4	Bacteria	Intracellular	Magnetotaxis
		Chitons	Teeth	Mechanical strength
		Tuna/salmon	Head	Magnetic navigation
Goethite	α-FeO(OH)	Limpets	Teeth	Mechanical strength
Lepidocrocite	γ-FeO(OH)	Sponges	Filaments	Unknown
		Chitons	Teeth	Mechanical strength
Ferrihydrite	$Fe_2O_3 \cdot nH_2O$	Plants/(in)vertebrates	Ferritin	Storage protein
		Chitons	Teeth	Precursor phase
		Beever/rat/fish	Tooth surface	Mechanical strength
	+ phosphate	Bacteria	Ferritin	Storage protein
		Sea cucumber	Dermis	Mechanical strength
Other minerals				
Greigite	Fe_3S_4	Bacteria	Intracellular	Magnetotaxis
Ice	H_2O	Bacteria	Cell wall	Unknown
Weddellite/whewellite	$CaC_2O_4 \cdot nH_2O$	Plants	Cellular	Ion store
Calcium pyrophosphate	$Ca_2P_2O_7$	Gastropoda	Hepatopancreas	Detoxification

in the production and maintenance of connective tissue in higher organisms, although this function has not been clearly established.

Of the many transition metals which display a rich biocoordination chemistry, only Fe, and to a lesser extent, Mn, have extensive roles in biomineralization. The bioinorganic solid-state chemistry of these elements is dominated by the redox behaviour of the II/III oxidation states, an affinity for O, S and OH ligands, and the ease of hydrolysis in aqueous solution. Like the calcium biominerals, biological iron oxides are used to strengthen soft tissues and as storage depots (Fe, OH^-, HPO_4^{2-}). Furthermore, the magnetic properties of mixed-valence phases are utilized by several types of bacteria as a means of navigation in the ambient geomagnetic field. Most magnetotactic bacteria synthesise intracellular magnetite (Fe_3O_4) [Plate 1(*a*)],[9] although recent studies have shown that species inhabiting sulfide-rich environments deposit the isomorphic mineral greigite (Fe_3S_4).[10] In both systems the crystals

(*a*)



(*b*)

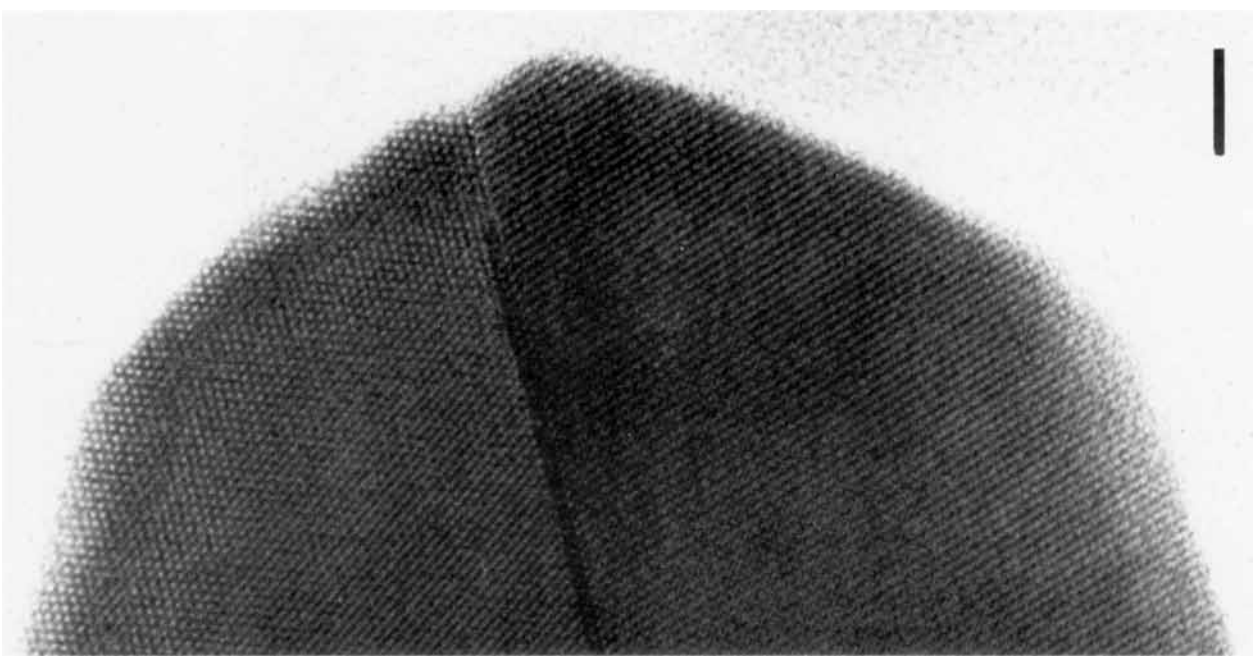


Plate 1 (*a*) Electron micrograph showing a coccus type bacterium containing a chain of nine intracellular magnetite crystals. Scale bar = 1 μm. (*b*) High-resolution lattice image of part of a bacterial magnetite crystal aligned along the [110] axis. Note the presence of a {111} twin boundary running from top to bottom in the centre of the micrograph. Scale bar = 5 nm

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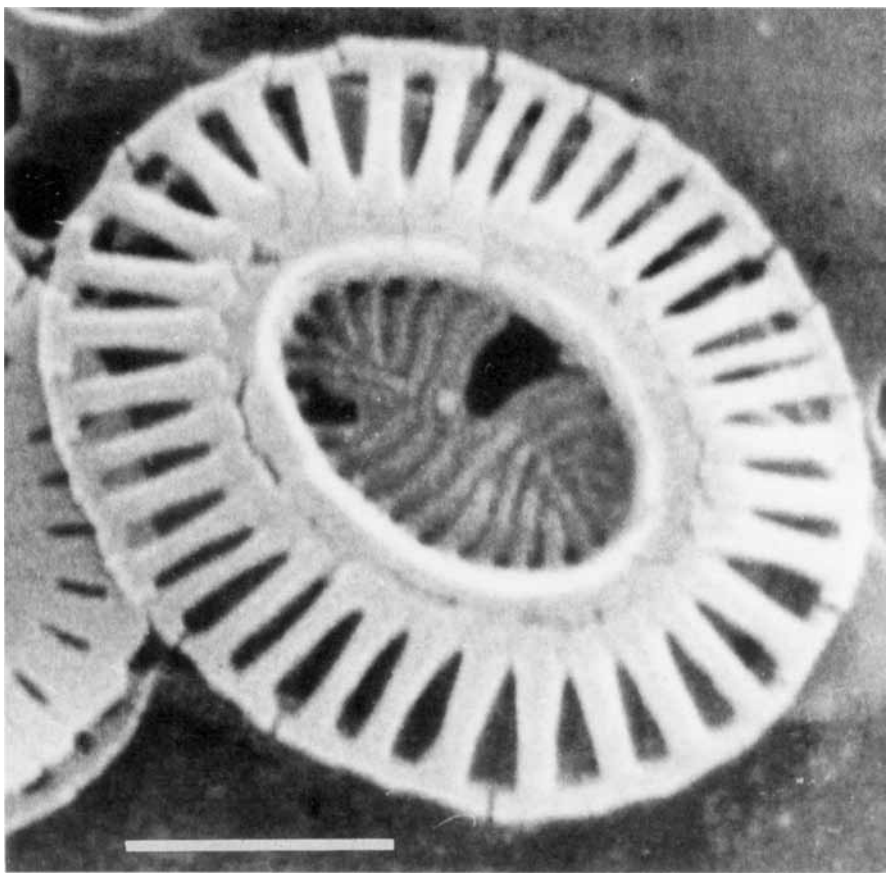
(*c*)



(*d*)

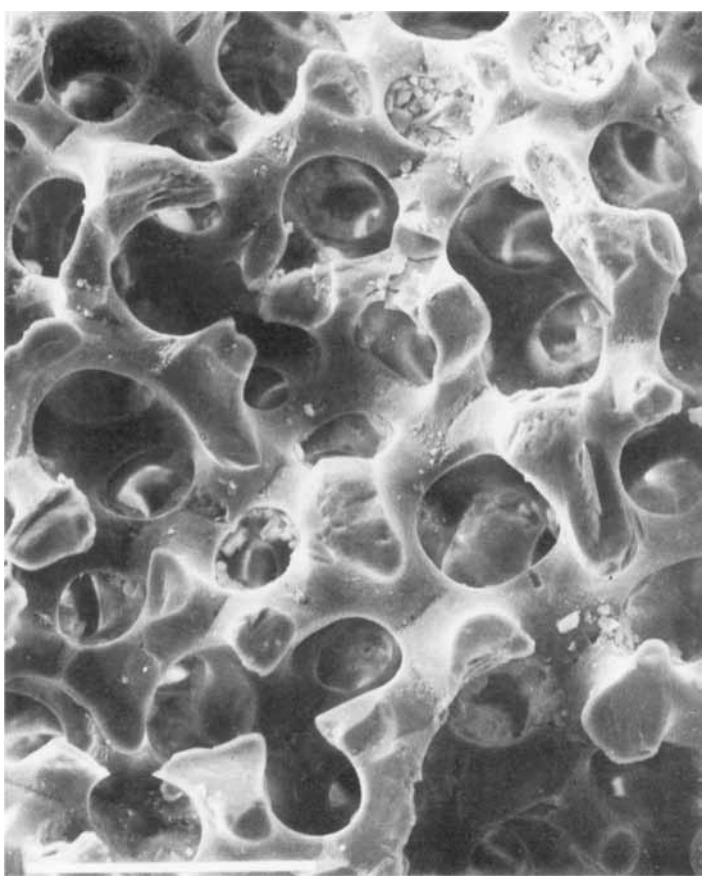


Plate 1 (*c*) Electron micrograph of a coccolith scale from the unicellular alga *Emiliania huxleyi*. The scale consists of a radial array of hammer-shaped single crystals of calcite which are crystallographically oriented. The shape of the crystals is species-specific and the crystallographic orientation is conserved throughout the fossil record, indicating that nucleation is controlled at the molecular level.[20] Scale bar = 1 μm. (*d*) Fractured mineralized plate of the sand dollar *Dendraster excentricus*, showing conchoidal fracture of the mineralized exoskeleton. Note that the labyrinthic ultrastructure represents a single crystal of magnesium calcite. Scale bar = 50 μm

Table 2 Bacterial biomineralization processes

Site	Mechanism	Mineral	Examples
Epicellular	Soluble biopolymers	Mn/FeO(OH)	*Leptothrix*
			Pedomicrobium
	Spore coats	MnO(OH)	*Bacillus*
	Gas/ion exchange		
	H_2S	Fe/CuS	*Desulfovibrio*
	CO_2/pH	$CaCO_3$	*Calothrix*
	pH	$MgNH_4PO_4$	*Proteus miribilis*
	Proteolipid/transport	$Ca_5(OH)(PO_4)_3$	*Streptococcus*
	Phosphatase activity	$(UO_2)_3(PO_4)_2$	*Citrobacter*
	Electron transfer	Fe_3O_4	GS-15
		UO_2	GS-15
		Au	*Pedomicrobium*
	Nucleation proteins	H_2O(ice)	*Pseudomonas syringae*
	S-layer templates?	FeO(OH)	*Leptothrix*
Intracellular	Vesicles	Fe_3O_4	*Aquaspirillum magnetotacticum*
		Fe_3S_4/FeS_2	Wild types
	Ferritin micelles	$FeO(OH)/PO_4$	*Pseudomonas aeruginosa*

must be aligned in chains and have dimensions compatible with that of a single magnetic domain, if they are to function efficiently as biomagnetic compasses.

Many elements, such as Cu, Zn and Pb, are deposited on the external surfaces of bacteria in the form of metal sulfides. Interestingly, some yeasts (fungi) mineralize nanometre-size intracellular CdS particles within short chelating peptides of general structure, $(\gamma\text{-Glu-Cys})_n\text{Gly}$.[11] As the number of CdS units per particle is small ($\approx$85), we can consider these peptide/mineral complexes as large-nuclearity clusters capped by cysteinyl thiolate ligands, analogous in a sense with proteins such as metallothionein which sequester three- and four-metal ion clusters. Finally, the only biometallization process currently documented involves the formation of gold crystallites on the surface of bacteria,[12,13] a process which may be responsible for the localized deposition of Alaskan gold nuggets!

The formation of biominerals takes place in well defined spatially delineated sites. In bacteria (Monera) and unicellular organisms (Protoctista) these sites are either epicellular (*i.e.* on or within the cell wall) or intracellular. Bacterial mineralization is generally associated with cell wall processes in which extruded metabolic products (ions, gases, polypeptides, electrons) coprecipitate with extraneous metal ions in the surrounding environment (Table 2). The resulting biominerals are often structurally ill defined, physically heterogeneous and spatially disorganized. Intracellular mineralization, on the other hand, is characterized by prodigious fine-tuning of the crystal chemistry. This process is uncommon in bacteria, but prevalent in algae and protozoa because intracellular compartments can be readily synthesised. In multicellular organisms, specialized cells have evolved to regulate mineralization in extracellular spaces. Secretion of biopolymers, such as collagen and chitin, into this space enables complex large-scale composite materials (*e.g.* bone, shell) to be fabricated.

Structural Challenges

At first sight, the minerals listed in Table 1 do not appear to hold out any immediate structural challenges. The crystallography is well documented and the mineral compositions appear straightforward. However, biominerals have several unusual properties. For example, the structural chemistry of bone mineral is exceedingly complex. Lattice-site substitutions of PO_4^{3-} with CO_3^{2-} have been investigated over a number of years, primarily by infrared spectroscopy, but the full details of the defect chemistry of calcium-deficient carbonated apatite remain to be determined. What is needed is a structural analysis on par with that often exerted in solid-state chemistry (*e.g.* $Fe_{1-x}O$, UO_{2+x}). Also, since structural and compositional properties influence the ability of bone to undergo remodelling (healing), inorganic chemists can make an important contribution to our understanding of bone diseases. Similarly, the major decrease in dental caries observed in the western world over the last two decades can be attributed to the simple chemical observation that substitution of OH^- with F^- in enamel crystals results in lattice contraction along the *c* axis and a consequent reduction in mineral solubility.[14]

Many organisms deposit biogenic iron oxides that are variable in composition and structure. Such systems are best characterized by a combination of methods usually involving high-resolution electron microscopy, electron diffraction and temperature-dependent ^{57}Fe Mössbauer spectroscopy. In the case of the iron storage protein, ferritin, the oxide particles are of nanometre dimension which gives rise to interesting superparamagnetic properties.[15] Other iron biominerals, such as Fe_3S_4, are poorly characterized at the present time. Besides these crystalline phases, there is a wide range of amorphous biominerals. The most common is hydrated SiO_2 which is deposited in a variety of forms (gel, particulate, fibrous).[16] The variability of the Si–O–Si bond angle and extent of hydroxylation {*i.e.* $[SiO_{n/2}(OH)_{4-n}]_m$} confounds structural analysis but some progress has been made by a combination of lattice imaging, infrared spectroscopy and solid-state ^{29}Si NMR spectroscopy.[17] Less effort has been addressed to the amorphous calcium carbonates, phosphates and pyrophosphates, which are often used as storage depots or as inert hosts for immobilizing toxic metals (bioglassification?). Extended X-ray absorption fine structure (EXAFS) appears to be a promising method for studying amorphous calcium biominerals.[18]

One of the limiting factors in these structural studies is the general lack of access within chemistry departments to electron microscopy facilities. At the current time there are few UK groups involved in biomineralization which have sufficient in-house facilities. Conventional methods such as X-ray diffraction are often not applicable because of the limited amount of material available or the requirement to study systems as near as possible to the native state. For example, the structural determination of magnetic crystals in magnetotactic bacteria has been achieved primarily by lattice imaging of crystals in *individual* cells viewed directly in the electron microscope [Plate 1(*b*)].

Synthetic Challenges

Even the most casual observer is surely impressed by the remarkable synthetic capability of organisms to produce materials such as seashells, pearls, bone, coral, sea-urchin tests, *etc.* In all cases there is a specific choice of mineral structure.

Sometimes, as in various shells, two polymorphs (calcite and aragonite) are deposited within close proximity. In chitons (molluscs) at least three different iron oxides [$Fe_2O_3 \cdot nH_2O$, γ-FeO(OH) and Fe_3O_4] are synthesised in distinct regions of the teeth.[19] How organisms regulate mineral structure in this way is not fully understood: a combination of chemical and structural (interfacial) controls over the mechanistic steps of nucleation and growth appears most probable. For example, chemical regulation of redox and pH in localized biological environments may dictate the course of magnetite biomineralization [equation (1)], whilst control over the concentration of

$$Fe_2O_3 \cdot nH_2O(s) + Fe(OH)^+(aq) \longrightarrow Fe_3O_4(s) + H^+ + nH_2O \quad (1)$$

extraneous ions such as Mg^{2+} can be responsible for polymorph selectivity in carbonate and phosphate mineralization [equations (2) and (3)].

$$CaCO_3 + Mg^{2+} \longrightarrow (Mg,Ca)CO_3(\text{calcite, } < 35\%\ Mg) + CaCO_3\ (\text{aragonite}) \quad (2)$$

$$Ca_n(HPO_4,PO_4,OH) + Mg^{2+} \longrightarrow Ca_{18}Mg_2(HPO_4)_2(PO_4)_{12}(\text{whitlockite}) \quad (3)$$

The aqueous precipitation of many of the minerals listed in Table 1 is a relatively straightforward laboratory procedure compared with the synthesis of complex solid phases often encountered in inorganic solid-state chemistry. (No biogenic 1,2,3 high-temperature superconducting phases, unfortunately!) However, whilst chemists tend to view synthesis as a form of molecular engineering, materials scientists are generally more aware of the importance of ultrastructural details. Thus it is one thing to be able to grow crystals of a new compound, but to control the size, shape, orientation and assembly of these crystals, as is typical of many biominerals, is an order of magnitude more complex. Yet chemists have a key role to play in the fabrication of ultrastructures because properties characteristic of this level of organization are ultimately governed by the nature of molecular interactions occurring at solid–liquid and solid–solid interfaces.

This is very apparent from studies of biominerals where the dimension, morphology, crystallographic alignment and assembly of mineral particles are highly controlled and replicated over millions of years [Plate 1(*c*)]. In many cases the control of particle size is achieved by confining the synthesis to discrete localized volumes through the use of supramolecular assemblies of organic molecules. These are often spherical or elongated phospholipid vesicles of variable size (20–1000 nm). However, the best characterized system is the protein micelle of ferritin.[21] This iron storage protein consists of a spherical polypeptide shell surrounding an inorganic core of the iron oxide mineral, ferrihydrite. The micelle is constructed from the self-assembly of 24 polypeptide subunits arranged in cubic symmetry such that molecular channels penetrate the shell. The internal cavity is *ca.* 8 nm in diameter which sets an upper limit on the number of Fe atoms that can be accommodated in the mineral core. Confinement of the mineralization reaction to this nanospace is achieved by specific molecular processes (oxidation and site-directed nucleation, see below) which compete successfully against non-specific reactions occurring in the external medium. Functionally, this is very important because there is no bulk precipitation of iron oxide (rust is a very toxic biomaterial!) and iron storage and transport can be closely regulated by the levels of ferritin biomineralization within the organism.

This intimate association of inorganic and organic phases is a hallmark of biomineralization. In many cases the integration is at the superstructural level where mineral particles and bipolymers, such as collagen or chitin, are organized to give composites of unusual strength and toughness. Sea-shells are a typical example; the calcite or aragonite crystals are organized within sheets or tubes of specific macromolecules. Similarly, bone is a complex composite of carbonated apatite crystals synthesised within and between the collagen fibrils. Much is known about the ultrastructures of these bioinorganic materials and how they vary in different organisms, but hardly anything has been revealed about the molecular interactions governing their construction. Several important questions are being addressed. How are the inorganic particles crystallographically oriented with respect to the organic matrix? Are there specific molecular interactions at the inorganic–organic interface which give rise to a precise templating of the inorganic lattice? What is the nature of the bonding between inorganic and organic components? The answers to these questions will not only explain much of the bioinorganic chemistry of these processes but open a new vista in the chemistry of heterogeneous interfaces.

There are other examples where the association of inorganic and organic components is more subtle. Surprisingly, a spine of a sea-urchin, with its elaborate (non-crystallographic) texture and porosity, generates a highly ordered single-crystal X-ray diffraction pattern, yet it contains 0.02% (w/w) of protein.[22] Where are the organic molecules? Similarly, sectioning through what appears to be (morphologically) a single crystal of calcite formed in the mammalian inner ear reveals an iso-oriented assembly of small primary crystallites.[23] It may turn out that many biominerals are of this form, *i.e.* a *nanocomposite* of inorganic and organic constituents, with the organic macromolecules (proteins, polysaccharides, lipids) residing at the interfaces between essentially coherent crystal domain boundaries. If this is the case then we have much to learn from biomineralization in terms of nanofabrication. Moreover, the biocomposites have modified physical properties; for example, echinoderm tests and spines fracture conchoidally (like a glass) and not along the low-energy {104} cleavage planes [Plate 1(*d*)]. Could a similar influence in mechanical properties be fashioned in more advanced crystalline inorganic materials?

Molecular Recognition in Biomineralization[24]

The specific interaction of molecules in solution as exemplified by host–guest complexes of macrocyclic ligands, enzyme–substrate reactions and antibody–antigen coupling is determined by complementarity in size, charge, molecular shape and dynamics. Could analogous interactions be responsible for the oriented nucleation of inorganic crystals on biological substrates? The last ten years has seen much activity in attempting to resolve this question. The major difficulties have been in the isolation and identification of the active biological components, the dearth of knowledge of their native molecular configurations, and the potential degradation of matrix constituents on isolation. Thus, most of the progress has been made using model systems where systematic experiments can be undertaken even if their relevance to the biological process remains tenuous.

We can consider the role of an organic matrix, such as collagen or ferritin, analogous to that of an enzyme in solution, with the incipient inorganic nucleus as the corresponding substrate. However, the long-range electrostatic forces of ionic surfaces and the requirement of space symmetry indicate that factors such as lattice geometry, spatial charge distribution, hydration, defect states and surface relaxation need to be considered along with the stereochemical requirements of ion binding at the interface. Although some progress has recently been made using computer modelling of the surface interaction and morphological influence of extraneous cations with inorganic crystals,[25] a description of the molecular forces operating at interfaces involving inorganic clusters and macromolecular frameworks is not currently available. In light of these comments it can be

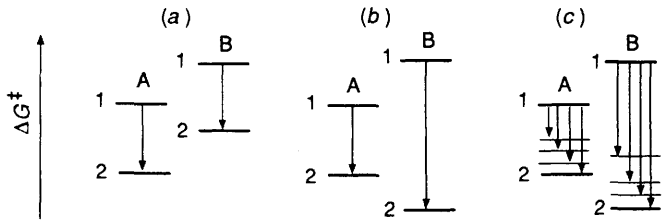


Fig. 1 Diagrammatic representation of the activation energies of nucleation, $\Delta G^{\ddagger}$, of inorganic minerals in the absence (state 1) and presence (state 2) of an organic surface involved in biomineralization. Three possibilities exist for a mineral of two polymorphic structures (or two nucleation orientations), A and B, where A is the more kinetically favoured in the absence of the organic matrix. (*a*) Non-specific nucleation catalysis in which both polymorphs (or crystal faces) have reduced activation energies due to the presence of the matrix surface but there is no change in the outcome of mineralization. (*b*) Structure-specific nucleation of polymorph (or crystal face) B due to molecular recognition and high-fidelity synthesis/replication of the matrix surface. (*c*) Combination of (*a*) and (*b*) depending on the levels of recognition of nuclei A and B and the fidelity of matrix production, factors which may be influenced by genetic, metabolic and environmental processes

stated that the role of an organic surface involved in inorganic crystallization is primarily to lower the activation energy of nucleation ($\Delta G^{\ddagger}$), where, for the most simple case, equation (4) is applicable [B = constant ($16\pi/3$ for a spherical

$$\Delta G^{\ddagger} = B\sigma^3 v^2/(kT \ln S)^2 \qquad (4)$$

nucleus), σ = interfacial energy, v = molecular volume, k = Boltzmann constant, T = temperature and S = supersaturation]. This equation is derived by assuming that nuclei will only develop into stable entities if the energy released through the formation of bonds in the solid state is greater than that required to maintain the newly created solid–liquid interface. No account is taken of the dependence of $\Delta G^{\ddagger}$ on the two-dimensional structure of different crystal faces or of the effect of extraneous surfaces in the medium. Clearly, both these factors influence the interfacial energy such that there may be an ensemble of nucleation profiles that are crystallographically specific and dependent on the nature of the substrate. Although unconventional, it is useful to consider the nucleation of biominerals in terms of the general ideas of transition-state theory, with incipient nuclei of different structure or orientation represented as a series of *activated clusters* of different $\Delta G^{\ddagger}$. Consequently, their steady-state concentration and frequency with which they transform into thermodynamically stable entities will be dependent on their corresponding reaction trajectories which, in turn, are determined by the specificity of molecular recognition processes. In this way, metastable polymorphs (*e.g.* vaterite, ferrihydrite) and specific crystal faces can be preferentially nucleated by the stabilization of particular transition states at the matrix surface (Fig. 1).

The specific lowering of the activation energy of nucleation reflects a requirement for structural and stereochemical complementarity between the inorganic and organic surfaces. Co-ordination environments in the mineral phase can be simulated by metal-ion binding to appropriate ligands exposed at the organic surface. Carbonate and phosphate biominerals tend to be associated with carboxylate-rich (aspartate, glutamate) and phosphorylated (phosphoserine) proteins, respectively;[26] in both cases, the organic residues can mimic the oxyanion stereochemistry of particular crystal faces, and this may be sufficient to induce oriented nucleation. Similarly, biological deposition of silica[27] and ice[28] is associated with hydroxy-rich macromolecules such as polysaccharides and serine/threonine-rich proteins. Again, the organic ligands complement the mineral chemistry.

Stereochemical recognition at the nearest-neighbour level is insufficient in generating the long-range translational symmetry of inorganic lattices, but this can be achieved by regulating the spatial disposition of functional groups across the matrix surface. In this respect, the secondary, tertiary and quaternary structures of macromolecules are key features of the pre-organization required for controlled nucleation. Molecular periodicity can be attained by the use of β-pleated sheets (shells), α-helices (fish antifreeze proteins), and phospholipid membranes, whilst curved surfaces with regions of high surface charge and reactivity can be generated from supramolecular assembly (ferritin), aggregation (ice-nucleating bacterial proteins) and controlled polymerization (collagen). It is notable that the construction of large-scale structures, such as shell and bone, relies on regiospecific nucleation so that active sites must be generated over relatively long distances (microns). This is accomplished by cellular regulation of synthesis, transport and deposition of the matrix components and is under metabolic (hormone) control.

It seems feasible that the earliest biological approach to nucleation control involved the clustering of redox-active charge centres. The localization of manganese-oxidizing proteins in bacterial cell walls[29] and iron-oxidation centres in ferritin[30] are typical examples. The primary role of these centres is to stabilize the transition state (nucleus) by reducing the time between ionic encounters, an effect that can be enhanced if the constituents of the nuclei are chemically generated *in situ*. This is what happens in ferritin, which will now be discussed.

As described above, the ferritin molecule is an aggregate of polypeptide subunits assembled into a micelle-like quaternary structure. The system is complicated by the fact that there are two different subunits, designated H (heavy) and L (light) according to their relative molecular masses. Some ferritins, such as that isolated from horse spleen, are enriched (90%) in the L-chain subunit whilst others, for example from human heart, are H-chain dominant. The presence of heteropolymeric ferritins has made it difficult to assess the role of the protein in mineralization, but has now been overcome by the ability to produce recombinant homopolymer ferritins (*100%* H or L subunits)[31] which can be crystallized and studied by X-ray diffraction.[32] A striking observation is that there is a specific metal-binding oxidation site in the H-chain but not in the L-chain polypeptide subunit.[32] This site is close to the inner surface of the protein shell such that iron(III) species formed at the ferroxidase centre readily migrate into the cavity (Fig. 2). The nucleation site consists of a cluster of three carboxylate ligands on the cavity surface (conserved in both H and L chains) which probably stabilize incipient iron(III) nuclei by charge and polar interactions. Experiments involving site-directed mutagenesis to produce ferritins depleted of the oxidation and/or the nucleation site indicate that both sites act co-operatively in achieving controlled nucleation of ferrihydrite.[33] In particular, residue Glu 61 is crucial because it can adopt two positions, either pendant from the inner surface or as an additional ligand in the co-ordination sphere of the metal bound at the oxidation centre.

Unfortunately, compared with ferritin, much less is known about other biomineralization matrices. The exception is collagen which has been studied in detail.[34] However, although it is generally accepted that bone crystals are nucleated in the interstices of a crystalline assembly of collagen fibrils[35] (a host–guest composite?), it remains unresolved whether nucleation is activated directly by the matrix or indirectly through non-collagenous proteins adsorbed in the hole zones. In invertebrates, electron diffraction studies of partially demineralized mollusc shells have shown that in some species both the *a* and *b* axes of an antiparallel β-pleated sheet protein are aligned with the *a* and *b* crystallographic directions of aragonite ($CaCO_3$).[36] Partial amino acid sequencing of these acidic proteins[37] has indicated that there are repeated domains of polyaspartate which could be the nucleation centres. In general, the evidence is mounting that surface-binding motifs involving blocks

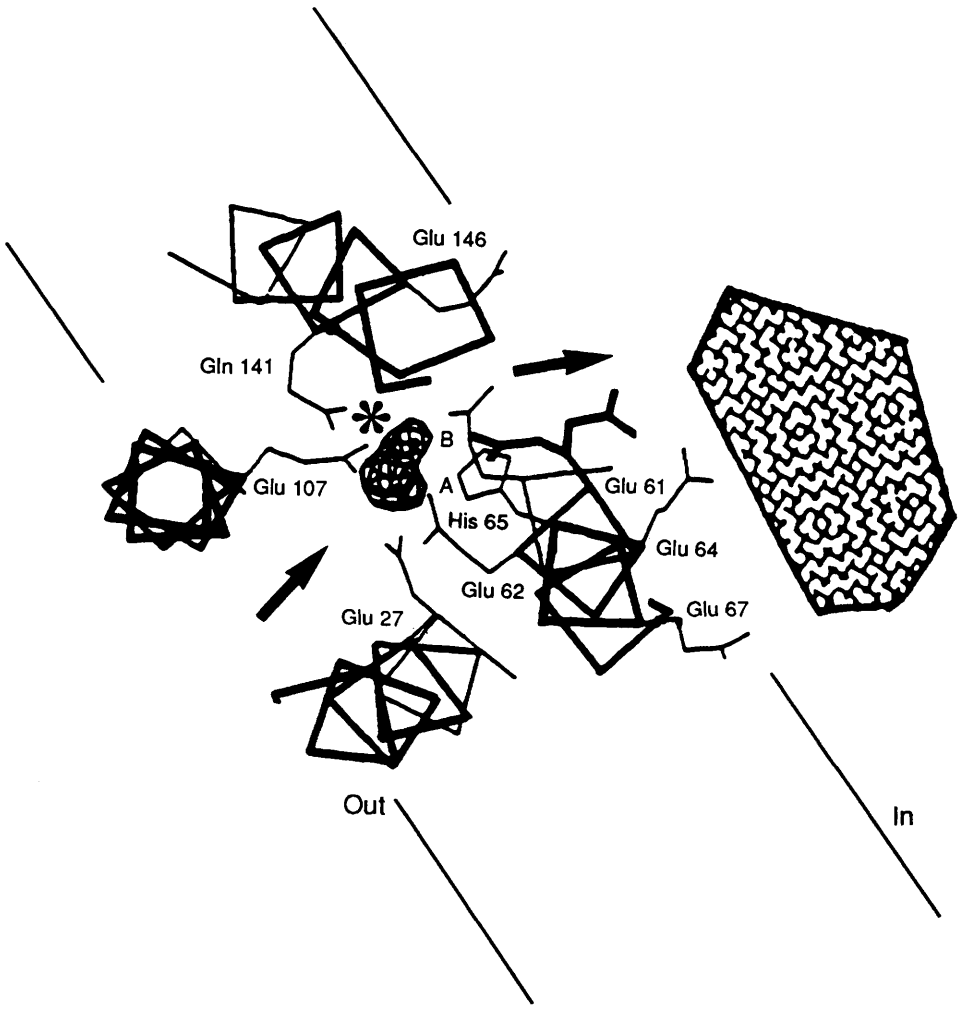


Fig. 2 Diagram showing possible mechanism of iron oxide nucleation in ferritin. One polypeptide subunit is shown spanning the protein shell. Iron(II) species, present in the external environment, bind at the ferroxidase centre (*) where they undergo rapid oxidation. Migration of Fe^{III} into the cavity results in mineral nucleation at a site comprising three glutamate residues (adapted from ref. 32)

Table 3 Amino acid motifs associated with biomineralization

Function	System	Macromolecule	Motif
Structural framework	Bone/dentine[4]	Collagen (type I)	$(GXZ)_{338}$
	Crab cuticle[4]	α-Chitin	β-(1,4)GlcNAc
	Mollusc shell[4]	α-Chitin	β-(1,4)GlcNAc
	Enamel[38]	Amelogenin	MPLPPHPGHPGYINFSYEVLTPLKWYQ (1–27) TDKTKREEVD (170–180)
	Plants[17]	Cellulose	β-(1,4)Glc
Binding/nucleation/inhibition	Bone (rat)[39]	Sialoprotein	EEEGEEEE (77–84) DEEEEEEEEEE (155–164)
		Osteopontin	DDDDDDDDDG (70–79)
		BAG-75	EEEEDDED/E (10–17)
	Dentine[40]	Phosphophoryn	DDDDDDYSDSDSSDSDD *SSSSSSSS*
	Oyster shell[37]	Glycoproteins	$(E)_{15-20}$, $(EX)_n$, $(S)_m$
	Sea-urchin Larval spicule[41]	Glycoproteins	$(WVGDNQAWVENPE)_{15}$
	Bovine milk[42]	β-Casein	E*SLSSS*EES (14–22)
	Cells/plasma[32]	Ferritin	EEE (61,64,67)
	Yeast[11]	CdS Peptides	$(\gamma\text{-EC})_nG$
	Bacteria[28]	Ice proteins	$(AGYGSTLT)_{122}$
	Polar fish[43]	α-Helical peptides	$(AAT)_n$
	Coccoliths[44]	Polysaccharides	$(Man)_n$ + GalA

Residue positions given in parentheses. A = Alanine, C = cysteine, D = aspartic acid, E = glutamic acid, F = phenylalanine, G = glycine, H = histidine, I = isoleucine, K = lysine, L = leucine, M = methionine, N = asparagine, P = proline, Q = glutamine, R = arginine, S = serine, T = threonine, V = valine, W = tryptophan, Y = tyrosine, X,Z = spacer residues, *T* = threonine *O*-linked to disaccharide, *S* = serine *O*-linked to phosphate, *Man* = sulfated mannose, GalA = D-galacturonic acid, Glc = glucose, GlcNAc = *N*-acetylglucosamine.

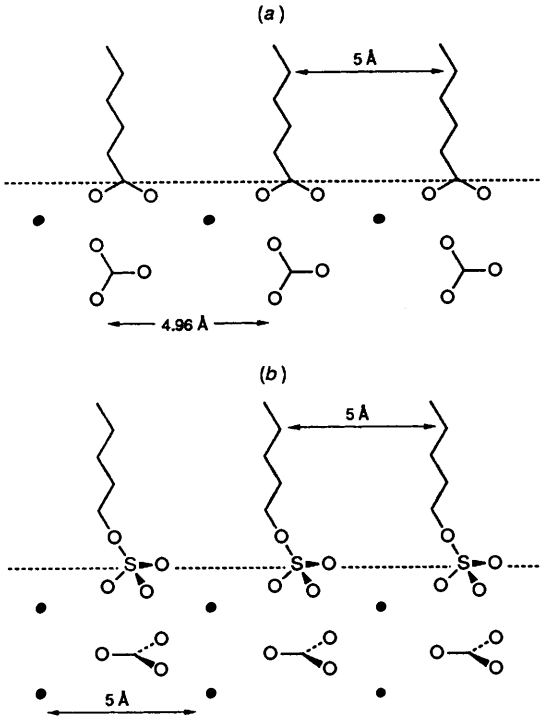


Fig. 3 Geometric and stereochemical complementarity at monolayer-crystal interfaces. (*a*) Nucleation of the calcite {1$\bar{1}$0} face under carboxylate monolayers. (*b*) Nucleation of the calcite (001) face under sulfate monolayers

of sequences such as $(Asp)_n$ and $(PhosphoSer)_n$ are common throughout biomineralization (Table 3).

How do these surfaces regulate oriented nucleation? One possibility is that there is geometric matching (epitaxy) between the lattice spacings of ions in crystal faces and functional groups arranged across the organic surface. For example, the distances between aspartic acid residues deployed along a β-pleated sheet are similar to the Ca–Ca distances in the nucleated aragonite (001) face observed in the mollusc shell.[36] As the binding constants for Ca at carboxylate sites are not high, other ligands such as sulfate and phosphate esters are required to increase the general binding capacity of the nucleation sites. Mollusc shells, for example, contain high levels of sulfated polysaccharide at their nucleation sites. Addadi and Weiner[7] have suggested that nucleation is a co-operative process involving structurally disordered sulfate groups of flexible oligosaccharide side chains and organized motifs of carboxylate ligands within β-pleated sheet surface domains of the matrix. The former provides a flux of Ca to the nucleation site whilst the latter induces oriented nucleation. These suggestions are borne out by model systems in which calcite crystals were grown on sulfonated polystyrene films with or without adsorbed polyaspartic acid.[45] Rigid, highly sulfonated films induce the preferential nucleation of the calcite (001) face and this is increased ten-fold in the presence of adsorbed polyaspartate in the β-sheet conformation. Adsorption of polyglutamate, which mainly adopts a random conformation, does not show this effect.

Another approach,[46–48] involving the spreading of Langmuir monolayers on the surface of supersaturated solutions, has shown that ion binding, lattice matching and stereochemical recognition are important factors responsible for oriented nucleation. For example, calcium binding to negatively charged stearate ($C_{17}CO_2^-$) monolayers results in the nucleation of the {1$\bar{1}$0} face of calcite.[49] The pseudo-hexagonal net of stearate molecules has an interheadgroup spacing of *ca.* 0.5 nm, which matches the distance between coplanar calcium ions on the {1$\bar{1}$0} face. Similar geometric correspondences are present for the (100) face of $BaSO_4$ crystals nucleated under long-chain alkyl sulfate or phosphonate monolayers.[50,51] In addition to these geometric relationships, the stereochemical arrangement of the surfactant headgroups are of fundamental importance. Nucleation of the calcite {1$\bar{1}$0} face, for example, is favoured by carboxylate headgroups because the bidentate motif mimics the carbonate stereochemistry exposed on this crystal surface; nucleation of the (001) face, on the other hand, is induced by sulfate headgroups because the tridentate arrangement simulates the oxygen positions of carbonate anions lying parallel to this crystal surface (Fig. 3).[52]

To conclude, it has been discussed how biomineralization can be controlled by molecular processes at inorganic–organic interfaces, details of which are currently being elucidated. There is much scope for inorganic chemists, particularly in modelling these interactions. Moreover, the application of this knowledge could be of fundamental importance in designing new approaches to materials synthesis. I now discuss this 'biomimetic' aspect of biomineralization research.

Biomimetics and Crystal Engineering

The last few years have seen a surge of research activity in the general area of 'intelligent materials'. Biomineralization is of central importance to this new field because the synthesis of bioinorganic materials is highly regulated and responsive to the surrounding environment; just the level of sophistication you need if the wing of a fighter plane is to 'heal' itself in battle! The adaptation of ideas and concepts derived from biomineralization research to the synthesis of inorganic materials with controlled properties appears to be a promising area of investigation.[53] Materials exhibiting uniform particle size (often nanoscale), polymorph selectivity, tailored morphology, oriented nucleation, organized assembly and composite inorganic–organic structures (organoceramics?) are realistic areas for investigation.

Several biomimetic approaches, based on biomineralization, are being currently explored:[54] (*i*) the use of supramolecular assemblies in nanoscale synthesis; (*ii*) organic surfaces as molecular templates for oriented nucleation; (*iii*) synthetic polymeric matrices as frameworks for composite structures; and (*iv*) organic additives in the control of crystal morphology.

The possibility of using a *'host–guest'* approach to confine the size of the reaction volume for materials synthesis is very attractive. Clearly, we require cavities that are an order of magnitude greater than those provided by conventional hosts such as crown ethers and cyclodextrins. Zeolites offer some exciting prospects in synthesising and immobilizing discrete inorganic clusters (*e.g.* CdS)[55] but the channel dimensions are usually well below 1.5 nm and the reaction products cannot be extracted from the host lattice. More versatility can be provided by organic supramolecular hosts such as reverse micelles and phospholipid vesicles since the range of reaction environments (1–100 nm) is more extensive and there is the potential for molecular engineering of the surface functional groups. A range of nanometre-dimension inorganic materials (*e.g.* Ag_2O,[56] Fe_3O_4,[57] calcium phosphates,[58] Al_2O_3[59] and CdS[60]) have been prepared within phospholipid vesicles. Since each particle is surrounded by a 4.5 nm thick bilayer membrane, particle–particle interactions are negligible and reaction rates can be diffusion controlled. For example, slow membrane diffusion of OH^- into iron(II)-loaded phosphatidylcholine vesicles results in the intravesicular crystallization of Fe_3O_4 compared with γ-FeO(OH) deposition under analogous conditions in bulk solution.[57]

One of the difficulties with the use of phospholipid or surfactant assemblies is their sensitivity to changes in phase behaviour. In addition, the dynamic behaviour of reverse micelles restricts their use in controlling the synthesis of inorganic particles less than 2 nm in diameter because aggregation of the primary particles readily takes place. To alleviate some of these difficulties, more robust systems such as the

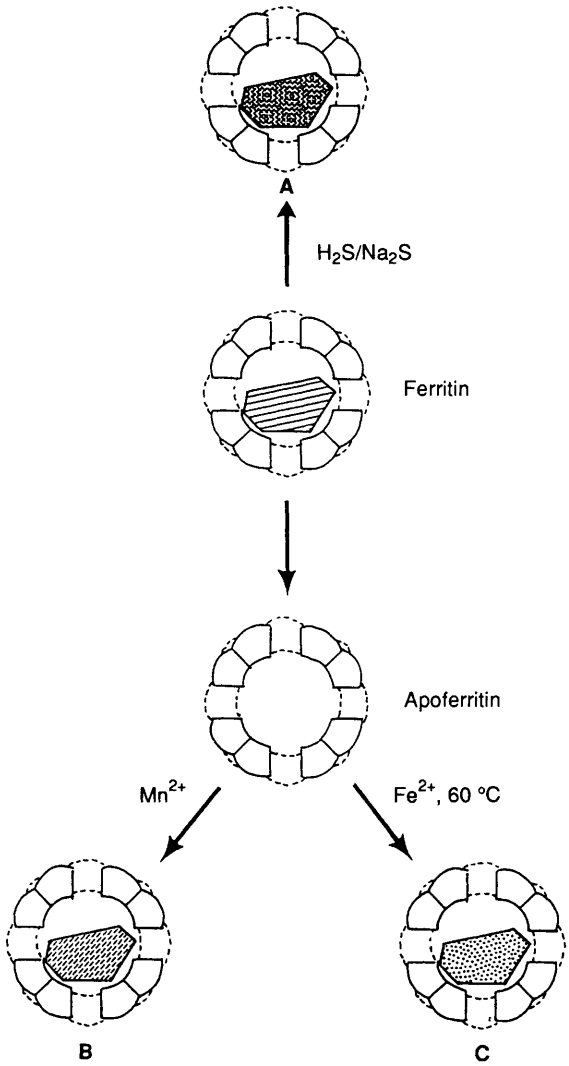


Fig. 4 The use of ferritin in the synthesis of nanophase materials; **A**, iron sulfide; **B**, manganese oxide; and **C**, Fe_3O_4

mineral-free protein shell of ferritin (Fig. 4) have been used. The similar redox and aqueous chemistries of Mn^{II}–Mn^{III} and Fe^{II}–Fe^{III} have been utilized in the room-temperature synthesis of nanophase manganese(III) oxides within the 8 nm internal cavity of the protein shell.[61] Furthermore, as the protein can tolerate pH values up to 9.5 and temperatures of 60–80 °C for limited periods, there is the possibility of extending the scope of this approach. For instance, we have recently reported a synthetic route to the *in situ* deposition of Fe_3O_4 within the protein cavity.[62] The result is a ferrimagnetic protein! This is exciting because not only could there be an important clinical use for such a protein in magnetic imaging, but it also indicates that the range and complexity of materials synthesised within ferritin may be significantly greater than originally conceived. Could we prepare finely divided monodisperse $BaTiO_3$, high T_c precursors and zeolites by this method?

I have already described how organized monolayers of surfactant molecules can induce the oriented nucleation of inorganic crystals by recognition processes at the inorganic–organic interface. We know that changes in headgroup charge, packing and stereochemistry have profound effects on the crystal chemistry. As yet, only a handful of inorganic materials have been studied ($CaCO_3$,[47] $BaSO_4$,[48] $SrSO_4$,[63] ice,[64] NaCl,[65] CdS,[66] PbS[67]), and further work is required to determine the general applicability of this experimental system. A related approach involves the use of organic polymers containing functional surface groups as active substrates for crystal nucleation.[68] Epoxidation of styrene–butadiene copolymers appears to be a useful method since a range of functionalized polymers can be prepared by ring opening on addition of various acids. The introduction of $-PO_3H_2$, $-COCH_3$, $-CO_2H$ or $-SO_3H$ residues converts the inert copolymer into substrates capable of nucleating salts such as hydroxyapatite, calcite or CdS.[69] In general, the advantage of using polymeric systems is that a range of chemical modifications is readily available through relatively straightforward organic chemistry. However, the disadvantage of these systems is that the structure of the functionalized surfaces is difficult to characterize.

Finally, there is the possibility of modifying crystal shape by the interaction of soluble molecules with growing crystal faces. The idea is based on molecular recognition at inorganic surfaces[70,71] and of immediate significance because the ability to engineer crystal habits is an important aspect in designing the optical, magnetic, catalytic, and rheological properties of inorganic materials. Soluble macromolecules, isolated from biominerals such as mollusc shell and sea-urchin tests, are very effective at inhibiting calcite crystallization by adsorption onto discrete and multiple growth sites; however, whilst the shell acidic glycoproteins are non-specific, the sea-urchin proteins interact specifically with the calcite $\{1\bar{1}0\}$ faces.[72] Polyanionic peptide analogues of the matrix proteins have been synthesised and assessed as crystal inhibitors. Peptides with poly(Asp) regions [*e.g.* $(Asp)_{19}$] were more effective than ternary repeats such as $(GlySerAsp)_{10}$,[73] although serine phosphorylation significantly enhanced the activity of the latter.

Conclusion

This has been a partisan article, flying the banner of Bioinorganic Chemistry through its interdependence with its mother subject, Inorganic Chemistry. Inorganic chemists should remain united in their commitment to studying the chemistry of the elements wherever they may be found. If there is an urgent need to explore this chemistry within the context of the natural environment then we should accept that challenge. However, we must do so without seriously compromising the tenets of our discipline; mainstream Inorganic Chemistry must also flourish if our contribution at the expanding periphery is to be profound, a point I have tried to make throughout this article. Perhaps we have, to some extent, eroded our allegience to an eclectic community, instilled a narrowness of vision and undermined our ability to respond creatively to new outlooks: if so, let us hope that the current hardships placed on the UK Inorganic Chemistry community galvanize a wider perspective amongst its members. If not, then the slide towards disintegration is irrevocable.

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