Simon R Hall

BIOTEMPLATING

Complex Structures from Natural Materials

Imperial College Press

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Complex Structures from Natural Materials

This book is dedicated to Caroline, Corey, Emily
and all from 4W 2.25, W504 and S401.

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Contents

1. Introduction
   1.1 History of biotemplating 2
   1.2 Mechanisms and models 4
   1.3 Crystallization in nature/skeletons in the beaker 5
   1.4 References 12

2. Simple Mono- and Oligosaccharides
   2.1 Structure 14
   2.2 Use as a source of carbon 16
   2.3 Use as a chelating agent 20
   2.4 Other uses 23
   2.5 References 26

3. Complex Polysaccharides
   3.1 Structure and properties 28
   3.2 Cellulose 30
   3.3 Dextran 38
   3.4 Starch 52
   3.5 References 59

4. Hydrocolloids
   4.1 Structure and properties 63
   4.2 Carrageenan 65
   4.3 Alginate 74
   4.4 Gelatin 81
   4.5 Agar, curdlan, gellan, pectin and the gums 86
   4.6 References 89

5. Chitin/Chitosan
   5.1 Structure and properties 92
   5.2 Chitin/chitosan 93
   5.3 References 114

6. Proteins and Lipids
6.1 Proteins – structure and properties 117
6.2 Lipids 141
6.3 Structure and properties 141
6.4 References 149

7. Viruses and Bacteria
7.1 Viruses – structure and properties 154
7.2 Spherical/polyhedral viruses 156
7.3 Helical/filamentous viruses 157
7.4 Bacteria – structure and properties 162
7.5 DNA 170
7.6 References 172

8. Complex Biostructures as Templates
8.1 Complex biostructures 174
8.2 References 194

9. Into the Future – Genetic Engineering and Beyond
9.1 Genetic engineering 196
9.2 References 202

Index 205

Chapter 1

Introduction

In terms of structural complexity, the natural world produces examples of stunning beauty and high functionality, usually with the minimum of material and energy expenditure. Scientists can harness these amazing structures as ready-made scaffolds on which to grow inorganic phases which replicate them, thereby producing materials with greatly enhanced physical properties. With the recent explosion of research into nanotechnology, biomaterials provide ideal templates as complexity in biopolymers is invariably on the nanoscale. This book highlights the wide range of natural materials that have been used in this way and the inorganic phases which result from them. Covering simple molecules such as cellulose and chitin, to large biological constructs such as bacterial proteins, viruses and pollen, practically every inorganic material has been synthesized using biotemplating methods, from simple oxides and carbonates such as silica and calcite, to complex semi- and superconducting materials. The book also discusses the formation of these materials from a mechanistic point of view, thereby enabling the reader to better understand the processes involved in biotemplated mineralization.

Many of these materials can be classified in a number of different ways, for example alginate can be considered as a polysaccharide, a hydrocolloid and potentially owing to its behaviour, as a complex biostructure. Inclusion of a biopolymer in one section does not therefore preclude its consideration as one of the others, although to avoid repetition this multiple inclusion is largely avoided. The classifications in this work are based primarily on the properties of the biotemplate being utilized for a particular product.

1.1 History of biotemplating

Through the four billion years since the first prokaryotic cells appeared, evolution has worked and re-worked life on earth, continually adapting, amending and improving the survivability of organisms in response to a plethora of stimuli. At around 550 million years ago, organisms began to utilize their simple organic molecules in order to grow mineralic phases. These 'hard' materials conferred a significant evolutionary advantage, allowing the organisms to survive harsher environments, grow larger, evade predation and so on. As a result, these simple organic molecules eventually became part of structurally complex organic matrices, specifically tailored to biomineralize inorganic phases which precisely fit form to function. This means that today we have at our fingertips, an entire world full of organic matter, often with built-in nano-scale complexity, ready to be pressed into use as templates for the creation of complex functional materials. The processes involved in creating architectural elegance using the minimal amount of material has long fascinated Man, who has endeavoured to understand how such intricate construction can be accomplished through the simple flow of inorganic ions and strategically placed macromolecules. "When the demands of the environment are the blueprints of the construction, structures are produced with the utmost efficiency". This quotation from D'Arcy Thompson, in his seminal work '*On Growth and Form*' (1917) represents the kernel of what first drove Man to attempt synthesis based on naturally occurring materials and methods[1]. Even as far back as the 16th century, scientist and astronomer Johannes Kepler noted that 'Nature uses as little as possible of anything.' Both these and many other luminaries throughout history have held nature in the highest esteem as an engineer par excellence. This is perhaps best exemplified by the extraordinary feats of engineering undertaken throughout the Victorian-era, when engineers turned to nature for inspiration, when stable, complex constructions were required.

One of the earliest 'bioinspired' architectural projects was the construction of the Crystal Palace for the Great Exhibition of 1851. The architect Joseph Paxton conceived the Crystal Palace largely as a result of his work as Head Gardener to the Duke of Devonshire at Chatsworth House, Derbyshire. Whilst at Chatsworth, Paxton built the largest conservatory in the World at that time, utilizing glass and iron for strength and durability. In 1837, the arrival of a lily from Guyana required a custom-built heated pool which Paxton designed. He was intrigued by the huge leaves of the plant which he dubbed 'a natural feat of engineering' and

tested their strength by floating his daughter on one of them. The secret of their mechanical stability was clear to Paxton; an array of radiating ribs connected with flexible cross-ribs. Experimentation over the following years enabled Paxton to improve on his glass and iron structures, culminating in the incorporation of the waterlily's structural features in his design for the Crystal Palace. Another striking example of engineering inspired by nature can be seen in Isambard Kingdom Brunel's Royal Albert Bridge near Plymouth. The bridge is a clever combination of arch and suspension bridge. An arch bridge produces a net outward thrust at the abutments, whereas a suspension bridge pulls the abutments inwards. By combining the two concepts in one bridge, the overall force at the abutments is almost zero. In Brunel's bridge, the arches consist of iron tubes with an oval cross-section, which produce the outward thrust to balance the inward pull of the draped chains. The minimal force carried by the abutments allows for a lighter and more importantly, cheaper construction. Inspiration for this may have come from the observation that this method of force balancing is one which every four-legged animal adopts. For example, in an elephant, the legs are the abutments, the belly the chains and the spine the arch of the bridge. Although it is not known whether Brunel (in the manner of Paxton) first considered nature before embarking on his design, it is likely that such a natural analogue would not have been far from his mind.

In the 20th century, scientists began to take a more active interest in the architectural constructs of the biological world, particularly keen to understand the procedures used by flora and fauna in the production of inorganic structural elements. The father of this approach was R.J.P. (Bob) Williams of Oxford University, who instigated a study of the detailed functional use of inorganic elements in biological systems[2]. By applying principles from inorganic chemistry such as the complex-ion formation and redox potential, to biological systems, he was able to deduce many hitherto unknown biomineralization mechanisms. Among the discoveries from this time were the elucidation of the special inorganic chemistry of unusual metal binding sites in nature, and the role and mode of action of calcium in the formation of calcified structures[3]. One of the students of the Williams's 'Oxford School' was Stephen Mann. Mann realised that by understanding the processes of biomineralization in terms of the movement and precipitation of inorganic elements within a 'biological environment' it should be possible to replicate or mimic them under laboratory conditions[4]. Mann supposed that as mineralization usually takes place due to constraint within an organism, then by replicating those constraining factors

synthetically, either physically in the form of for example, vesicles, or chemically by control of localized supersaturation, bio-analogues could be created. These experiments in 'biomimetics' yielded complex and often strikingly 'lifelike' inorganic materials by following closely (but not exactly) the protocols used in the natural analogue[5]. Currently, research World-wide into biomimetic control of mineralization is strong, producing many diverse and often industrially valuable materials[6]. All syntheses however still rely either directly or indirectly on the 'boundary organized biomineralization' concepts introduced by Williams, Mann *et al.* The complexity of biological structures and the complex systems which give rise to them are not easily replicated in the laboratory. Even the most advanced and succinct synthetic protocols can only ever offer a poor imitation of the natural analogue. This has convinced many scientists to 'cut out the middle man' and directly utilize naturally occurring materials as part of their synthetic procedures. The advantage of this approach is clear; by using a pre-formed, often hierarchically complex material, the scientist aims to transfer the physical properties of the original, to that of the synthetic analogue. It is this simple and elegant synthetic protocol that is the *raison d'être* of this book. After two decades of concerted research, the time is right for an overview of the research that has been done and that can still be done in the field of biotemplating. This book gathers together for the first time, research on virtually every biomaterial that has been used as a template for mineralization; from simple monosaccharides and peptides, to macromolecular complex bioconstructs such as pollen, diatoms and cuttlebone. In doing so, it is hoped that the reader will get a feel for the breadth and depth of research in biotemplating and perhaps stimulate further research in this most fascinating of fields.

1.2 Mechanisms and models

The fact that biomineralization and therefore biotemplating succeeds so spectacularly is largely due to the complimentary interaction between oppositely charged entities (ions, molecules, etc.). There are however, other effects which play a part and other ways of conceptualizing the process of biomineralization/biotemplating which sometimes better describe the effect being observed. The rest of this first chapter discusses the interaction between organic and inorganic phases and explores what happens when soft meets hard. The mechanisms and models described in this chapter apply to all of the examples of biotemplating which follow in this book. If the mechanism is not explicitly stated

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in the discussion of these materials, the reader is invited to revisit this chapter and deduce the mode of interaction at play in that particular example.

1.3 Crystallization in nature/skeletons in the beaker

The process of crystallization is well understood, although in practice, there are many factors which can influence the growth of a crystalline solid from a solution. This means that even after careful determination of the potential for successfully growing a particular crystalline phase, it can be difficult to achieve in practice. Broadly speaking, the entire process can be broken down into a nucleation event followed by subsequent crystal growth. The inducement for nucleation is the formation of a stable cluster of ions in solution. This process is dynamic and many nucleation events occur in solution only for the cluster to dissipate as soon as it has been formed. It is only once a critical size has been reached that the stability conferred on the cluster by aggregation allows it to persist without reverting back to individual ions. Spontaneous nucleation and growth in the absence of any seed is considered to be a rare event. Even supersaturated solutions of some compounds will remain uncrystallized providing there is no contamination or disturbance to the system. Once a suitable seed has provided the impetus for a nuclei to persist, crystal growth can occur. Providing the system is in a state of supersaturation, this process will be repeated continuously, with the number of nucleation sites increasing with time until the concentration of ions in solution dips below supersaturation and the system reaches equilibrium. Crystal growth can then occur as ions in solution add to the nucleated clusters. It is obvious then, that the more seed sites that are present, the more likely (all other things being equal) that crystallization will occur. In these cases of 'classical' crystallization, both thermodynamic and kinetic factors play an important role in determining the rate of crystal growth and several models were proposed in order to account for the observed formation of crystals within any given system[7-10].

The kinetic control of crystallization is a key concept in biomineralization and biotemplating. Crystallization under kinetic control can be thought of conceptually as a progressive modification of the activation-energy barriers of nucleation and growth. This step-wise progression can lead to the appearance of several intermediate species in biomineralizing systems, often with the starting point an amorphous precursor phase (Figure 1.1). The progression along the sequence to the final form of the biomineral is entirely dependent on the

solubilities of the inorganic phases present and on the free energies of their interconversions[11].

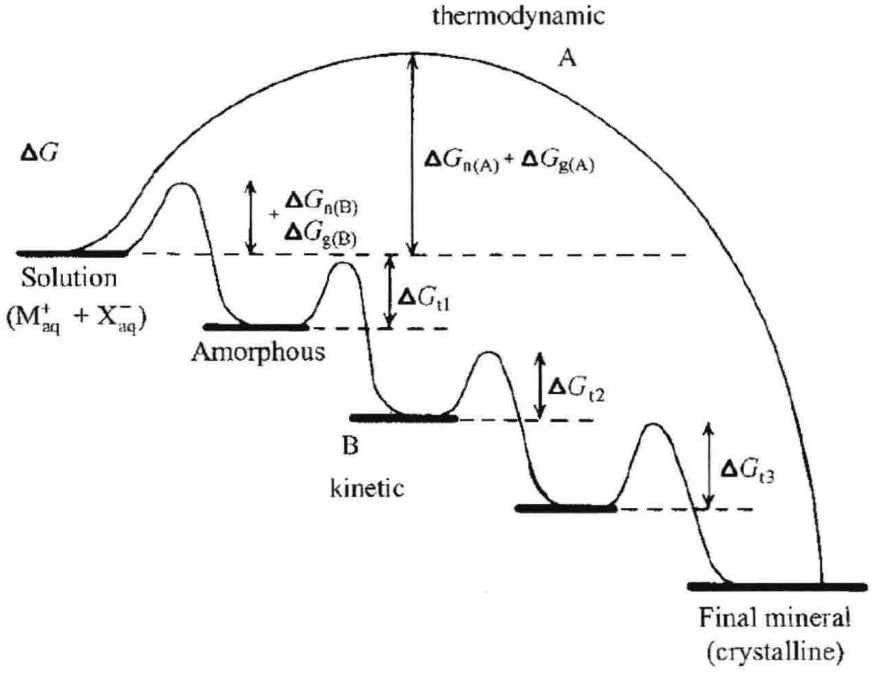

Figure 1.1 – Crystallization pathways under thermodynamic and kinetic control. Whether a system follows a one-step route to the final mineral phase (pathway A) or proceeds by sequential precipitation (pathway B), depends on the free energy of activation (ΔG) associated with nucleation (n), growth (g), and phase transformation (t). Amorphous phases are common under kinetic conditions. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

As these step-wise changes of structure in the inorganic phase proceed by way of dissolution-reprecipitation, this process is further complicated by the hydrodynamic properties of the ions in solution. In nature however, the mechanism of crystallization cannot simply be accounted for by these phenomena, as the presence of organic matter serves as a ready-made substrate for the formation of nuclei, thereby allowing crystallization to occur more favourably. One of the earliest studies on the effect of molecular additives to crystal growth was that of Buckley who proposed an essentially epitaxial mechanism to account for the observed morphology of certain crystals when

grown under these conditions[7]. By interacting with specific crystal faces, the molecular additive 'poisons' a particular face or faces to further growth by adhering preferentially to that surface. Crystals grown under the influence of molecular additives therefore have the potential to develop into non-classical morphologies, directed by the specificity of the additive to different crystal faces in different degrees. By extension, this concept can be applied to biomineralizing entities, with the organic elements not only inducing crystal growth and directing morphology, but also providing a macroscopic scaffold on which crystal growth occurs.

In an early work on how this concept can be applied to biotemplating, Mann and co-workers described that it is possible to draw analogies between the formation of inorganic nuclei on the surface of an organic matrix and the interaction between an enzyme and substrate[12]. In each case, nuclei can be considered to be kinetically stabilized by specific molecular interactions with the surface layer of the organic material. The overall effect that the organic substrate has is to lower the activation energy of nucleation ($\Delta G^{\#}$). Furthermore, it is entirely likely that as different sets of symmetry-related crystal faces show different levels of complimentarity for organic substrates, $\Delta G^{\#}$ may be dependent on the absolute 3D structure of the organic matrix, leading to further complexity of mineralization.

From experiments done since these early studies on complimentarity, it appears that the prime factor in organic-inorganic recognition is the charge matching between the inorganic ions and appropriate unlike charges on functional groups of the organic substrate. A good example of this in nature is in the biomineralization of calcitic structures. The majority of organisms which biomineralize calcium carbonate have organic fragments which are rich in carboxylate (COOH) groups. Charge matching is therefore possible between the COO^- anions and Ca^{2+} cations, leading to preferred sites of nucleation for the subsequent growth of calcium carbonate. This complimentarity immediately suggests a model for the long range directed growth of the inorganic phase on the substrate. As the organic matrix in a biomineralizing organism will be (usually) a protein or polysaccharide, the organic fragments which carry the appropriate charge for inducing inorganic ion binding will be disposed in a regular manner across the surface of the substrate. We will see in later chapters how the construction of macro-molecular assemblies with complex morphologies can be used effectively to replicate this complex form as a result of the regularity of reactive organic fragment disposition. This leads to preferred nucleation and

growth of inorganic material at specific locations on the organic fragment. This direct 'epitaxial matching' of organic to inorganic entity has been postulated and indeed observed to occur in certain cases. In work by Mann *et al.*, it was found that short-chain α-ω-dicarboxylic acids $[(CH_2)_n(CO_2H)_2]$ are particularly effective at stabilizing faces parallel to the $\{1\bar{1}.0\}$ surface of calcite provided that both carboxylate groups are in their ionized form[13]. The group postulate that this is due to the fact that these faces contain both Ca^{2+} and CO_3^{2-} ions with the latter oriented such that the plane of the triangular anion is perpendicular to the surface. This leads to a direct matching of the carbonate anions into the $\{1\bar{1}.0\}$ face during growth, through bidentate binding of two of the three oxygen atoms to Ca^{2+} ions in the surface. By simulating a calcite crystal surface, the group discovered that both carboxylate groups in the additive molecule would bind simultaneously to two different calcium ions if the spacing between the CO_2^- groups is close to 0.4 nm (Figure 1.2). This leads to the conclusion that by altering the chain length of the additive molecule, specificity can be controlled in a very precise manner. For example, both malonate (n=1) and the unsaturated diacid, maleate (cis-$^-O_2CCH{=}CHCO_2^-$) will adopt an epitaxially matched conformation on the calcite surface, but the more rigid conformation adopted by the maleate ion will reduce the binding affinity. The sensitivity of this epitaxial matching model to absolute conformation was confirmed by the group in experiments on the diacid *trans*-isomer, fumarate, which they found had no effect on the control of calcite morphology owing to the molecule being the "wrong" shape to take part in the co-operative binding. These epitaxial effects are pronounced at lower additive concentrations, as higher concentrations will lead to non-specific binding of the additive over all crystal faces[14]. Similar work by Mann and Heywood identified the formation of oriented barium sulfate phases by the interaction between long-chained sulfated molecules and barium ions[15]. Stabilization of the {011} set of faces in $BaSO_4$ were found to be a result not only of the ions possessing the correct stoichiometry to be structurally complimentary to the organic phase, but also to be highly polar, leading to a stronger interaction between the organic and inorganic phases. A study by Weiner *et al.* found that this model has a natural analogue[16]. Acidic macromolecules extracted from adult sea urchins were found to interact specifically with calcite prismatic faces lying almost parallel to the $\{1\bar{1}.0\}$ surface. These acidic molecules have a large number of glutamic and aspartic acid residues, which mimic the coordination environment of ions in the $\{1\bar{1}.0\}$ face by binding to the growing surface of the calcite. Low concentrations of

naturally-occurring longer biopolymers such as carrageenans and alginates have also been observed binding specifically to crystals, adopting preferred configurations along edge sites[17]. In this way, nucleation and growth of the edges of crystals are inhibited and non-classical morphologies begin to dominate.

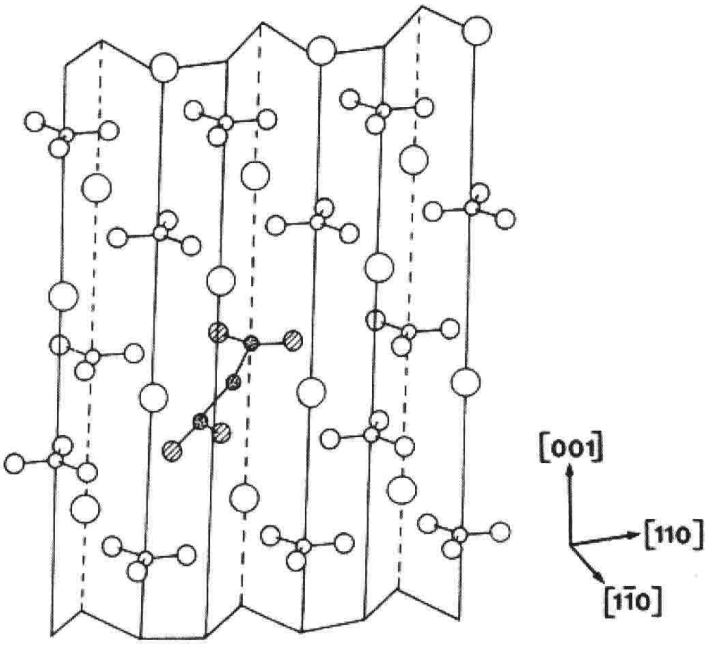

Figure 1.2 – Perspective drawing of the calcite {1-1.0} face showing a possible binding site for malonate anion. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

This epitaxial model of biotemplated growth is one which enables the researcher to reconcile the formation of inorganic phases with certain crystallographic features, but it only partially explains the growth of crystal phases in the presence of organic macromolecules. Another important consideration is the electrostatic environment which surrounds the nucleation centre. The localization of specific inorganic binding entities will concentrate areas of electrostatic charge, which will further improve the specificity of that part of the organic fragment for the inorganic phase. For example, the presence of glutamic acid residues in the inner cavity of the hollow spherical iron transport protein ferritin increase the electrostatic field in the inner surface of the protein relative to the outer surface. This increases the likelihood that iron sequestration

will take place inside the capsid rather than on the outside[18]. Work by Yamashita *et al.* revealed that the rate of growth of hydroxyapatite could be controlled by altering the electric polarization conditions of the synthesis[19]. Hydroxyapatite is polarizable owing to the ease of reorientation of dipole moments between O^{2-} and H^{+} of the OH^{-} ions in the crystal lattice. By performing the crystallization in the presence of an electric field of 1,000 V, larger crystals of hydroxyapatite could be formed than when the electric field was absent. In addition, the group found that there was a linear relationship between the field strength (and hence polarity of the hydroxyapatite) and speed of crystal growth. At an optimum polarization, crystals of hydroxyapatite were found to grow at six times the rate of non-polarized samples. When the same experiments were undertaken with dehydrated hydroxyapatite (an absence of hydroxyl groups), no enhancement of the crystal growth rate was observed. The presence of polarizable hydroxyl groups in this case clearly leads to improved crystallization. The mechanism of enhanced growth is clearly an electrostatically driven one as it is not difficult to imagine that cations (in this case Ca^{2+}) are preferentially adsorbed to the more polarized surface of the nucleation centre. The growth of these nuclei are therefore accelerated by the presence of stronger dipole moments.

Once a nucleation/growth event is underway and the inorganic phase is a viable (i.e. stable) entity, the possibility exists for the further growth of the newly formed crystallites following the topology of the organic material. In the aforementioned work by D'Arcy Thompson 'On Growth and Form', the scene was set for the conceptualization of inorganic crystal growth that was not of the 'classical' morphology of straight edges and fixed angles. At longer length scales, it is the influence of mechanical stress and gravity which determine the macroscopic shape of a scaffold. In order to better understand this process, Mann *et al.* proposed that conceptually, the production of macroscopic, three dimensional structures by the mineralization of an organic scaffold could be considered as either dependent on the chemical and spatial modification of crystal growth (contingent) or as a consequence of the spatial conformation adopted by the organic structures (prescribed)[20]. As examples, he cites the formation of calcitic spicules in some corals as contingent growth and the formation of delicate siliceous skeletons of diatoms as being an example of prescribed growth. Coralline spicules are polycrystalline calcitic structures which form in discrete vesicles within the body of the coral. The shape that the spicules adopt is determined by the local environment in which the deposition body finds itself; disturbances of the reaction volume and concentration fluctuations across

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