PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL PHYSICAL & ENGINEERING SCIENCES

Architecture in the microcosm: biocolloids, self-assembly and pattern formation

Alan R. Hemsley and Peter C. Griffiths

Phil. Trans. R. Soc. Lond. A 2000 **358**, 547-564
doi: 10.1098/rsta.2000.0545

THE ROYAL SOCIETY

Architecture in the microcosm: biocolloids, self-assembly and pattern formation

By Alan R. Hemsley[1] and Peter C. Griffiths[2]

[1]*Department of Earth Sciences, University of Cardiff, PO Box 914, Cardiff CF1 3YE, UK*

[2]*Department of Chemistry, University of Cardiff, PO Box 912, Cardiff CF1 3TB, UK*

Complex microscopic structure is a common feature in biology; the mineral shells of single-celled aquatic plants and animals such as diatoms, coccolithophores, radiolaria, the organic coatings of pollen grains and the surfaces of many seeds are all familiar examples. To the human eye, viewing this exquisite complexity, the method of construction is often far from obvious. Operating on the microscopic scale, at the size range called the colloidal dimension by synthetic chemists, is a gamut of interactions between the various components, which in many cases can lead to the formation of complex structure as an entropically favourable process. The importance of these 'colloidal interactions' is becoming increasingly apparent to biologists seeking the link between the genetic basis of structure and its ultimate expression. It is an emerging theme that through the evolutionary history of life, self-assembly of structure from colloidal building blocks has become integral to the process of organismal development.

Colloidal interactions, however, are themselves complex. Chemists therefore tend to restrict the number and diversity of components within any system being studied in order to minimize this complexity. The interactions of spherical polystyrene particles in an aqueous or organic fluid, for example, have been well documented. The introduction of a third component into such a system clearly increases the diversity of interaction (and concomitantly, the difficulty of interpretation). Yet such a system is unrealistically simple to the biologist! The investigation of the behaviour of mixed colloidal systems is essential in the formulation of concepts regarding microscopic structural development in order to further both our understanding of biological construction, and to give rise to new developments in microscopic materials technology.

Here we assess the developments in the understanding of colloidal systems in microscopic biological construction and demonstrate how these have given rise to new concepts regarding the relationships and evolution of the gene and organismal structure. We show how development of these new concepts may give rise to new materials with properties that have been tried and tested by organisms over millions of years of evolution and which, by their very nature, are more compatible with humans and their environment. We suggest how self-assembling microstructure might be used in the development of new surface coatings and drug delivery mechanisms.

Keywords: biocolloids; self assembly; microarchitecture; colloids; biological constructions; pattern formation

1. The biology of microarchitecture and self-assembly

There is little doubt that the information encoded in the genes of living things has a great impact on their ultimate form. Dogs, daisies and diatoms (figure 1*a*) are what they are, largely because they have a set of genes that, in combination, code for the production of such things as fur, flowers or frustules. However, working in tandem with the genetic code is a diversity of mechanisms which cannot be mapped to any gene, but which contribute much to the production of structure, architecture and pattern (Ingber 1998). The existence of such mechanisms is in part obvious. As explained by Cohen (1995), the imaginary changeling-like introduction of fly DNA into the egg of a chicken would produce neither fly nor chicken since fly information and chicken constructional mechanisms would be largely incompatible. A fly needs fly construction mechanisms while the constructional apparatus in a chicken's egg cannot use fly information to make a chicken.

'Man-made' structures and architecture operate under similar constraints. Three factors come together to produce the final object. There is a design in the form of a blueprint, the workforce to manipulate the components, and the components themselves whose physical properties also play a role in determining the ultimate form. One cannot build a car engine from rubber or Wellington boots from steel. Classical Greek architecture embodies these principles and has provided structures which were functional and have stood the test of time. Furthermore, the Greeks were aware of some fundamental patterns in nature. Their architects recognized the intrinsic aesthetic value of the 'golden ratio' (1:1.618), which is derived from adjacent numbers in the Fibonacci series. The same mathematical series governs many space-filling operations in nature, seen most obviously in the arrangement of scales in a pine cone or of seeds on a sunflower head.

The DNA (our blueprint) gives rise to proteins (commonly our components) by converting the genetic code into a sequence of linked amino acid units. The proteins roll up in a specific (self-assembling) way governed by the interactions of the side chains. Some, by a long history of chance and evolutionary selection, behave as efficient catalysts (enzymes) to bring about the formation of other types of molecule from the same simple components. Others break apart molecules releasing energy to power these processes. The self-assembly of biological molecules and synthetic analogues has received some attention (Whitesides *et al*. 1991; Raymo & Stoddart 1996) and is usually the consideration of the biochemist. But how does an organism progress from such a molecular cocktail to something with a spinal column, a stem or a complex silica shell? What is the 'workforce' that operates to achieve construction from our genetic blueprint?

In his inspiring work, 'On growth and form', D'Arcy Thompson (1961) saw that the production of many relatively small-scale biological structures such as radiolarian skeletons and the spiral shells of many marine organisms resulted from packing phenomena (as in pine cones or sunflowers) upon surfaces or in three dimensions. Today his work is perhaps seen as being overly directed to the description of nature by 'natural' mathematical rules, very much in the Greek tradition. However, the nub of his argument still has great merit; rules do apply in development and they are those of biophysics and chemistry acting at the interfaces of components derived from the molecular soup within cells (Kauffman 1993). Further, it is the interaction between cells so constructed and constrained that gives rise to the varied shapes of

multicellular organisms, including ourselves. Nonetheless, it is at the scale of single-celled organisms that the mechanisms of self-assembly are most apparent and close observation of the often spectacular architecture displayed at this level, should give clues to the nature of these mechanisms. These interactions, as noted by Thompson, occur at the colloidal dimension. Given this connection, it is surprising that there are few studies attempting to correlate architecture and organic colloid chemistry.

Proteins are not the only structures within cells to adopt a particular form dependent upon the intrinsic characteristics of their components. Self-assembly has been demonstrated in microtubules; cell components built from proteins that act like tug boats and guide large components to the interaction sites. Their various conformations are a result of concentration-specific self-assembly processes (Nédélec *et al.* 1997). Similarly, the form taken by membranes is governed by the concentration of the components, the nature of the surrounding fluids, and physical parameters such as temperature (Lipowsky 1991). The formation of periodic minimal surfaces and other bicontinuous structures (Thomas *et al.* 1990) may be an inherent consequence, as seen in the prolamellar bodies of chloroplasts in plants (Gunning & Steer 1996). In both cases, the genetic code need not define all possible conformations, merely the required concentration of the components in order to initiate the 'desired' structure. It is perhaps noteworthy that the formation of complex membrane systems, and indeed the positioning of the structural units, is often aided by microtubules presenting clear evidence of a hierarchy of developmental self-organization and assembly.

Micro-organisms may produce complex microscopic architecture involving inorganic components (Wilcock *et al.* 1988, 1989). Common among these additions are calcium and silica. Small, golden-brown algae produce surface discs of calcium carbonate (coccoliths), which can resemble miniature car hub caps. These structures, although small, are the principal component of the White Cliffs of Dover, having accumulated for millennia upon a Cretaceous sea bed. The siliceous frustules (shells) of diatoms (figure 1*a*) enclose the single-celled alga in much the same way as a Petri dish; one larger half, overlapping the edges of the smaller. Like the coccoliths, and many other microstructures, these shells are composed of networks of bars, ridges, pores and spines (Li & Volcani 1984; Leadbeater 1984; Mann & Ozin 1996). Siliceous architecture also occurs on the surface of some higher plant spores (figure 2*a*) and has been shown to have a colloidal origin (Tryon & Lugardon 1978).

The production of artificial microscopic structures with similar architecture to that produced by micro-organisms has been pioneered by Stephen Mann (Mann & Ozin 1996). As in our experiments (below), the production of microstructure relies on the behaviour of the component which will form the structure (in this case calcium bicarbonate) in a bicontinuous mixture of oil–water–surfactant (Walsh & Mann 1995). We concur with the views of Mann & Ozin (1996) that complex three-dimensional surfaces (such as that of the prolamellar body) provide a potential template for the accumulation of more robust structural units, be they inorganic or organic.

In the case of diatom frustules, foam-like aggregations adjacent to the surface membrane of the organism restrict the deposition of the mineral phase (Crawford & Schmid 1986). This is the self-assembling aspect of pattern formation. What is less clear (and probably more directly under genetic influence) is how consistency of form is maintained within a species and how different forms are produced by different species. This is not a problem restricted to mineral microarchitecture. The

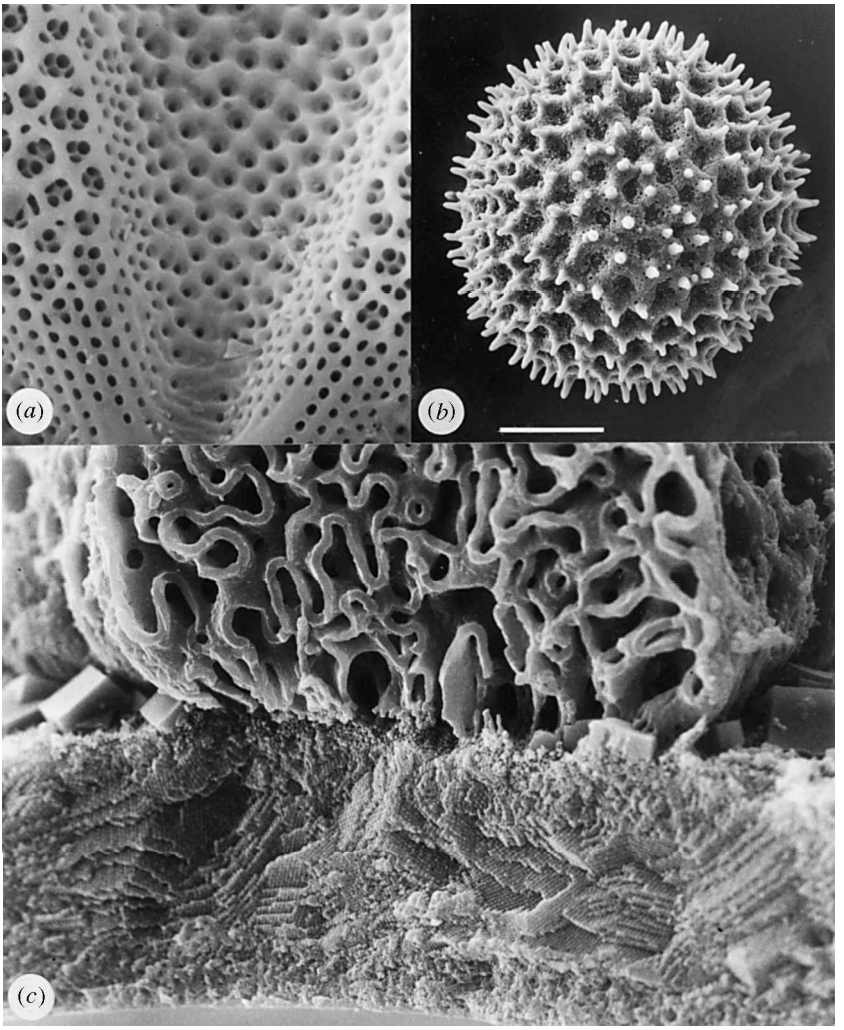

Figure 1. All scales refer to bar in (*b*). (*a*) The siliceous frustule of the colonial diatom *Actinoptychus*. Scale = 10 μm. (*b*) Pollen grain of *Ipomoea indica*. Scale = 40 μm. (*c*) A broken section of spore wall from *Selaginella myosurus* showing complex internal structure, including a colloidal crystal region. Scale = 5 μm.

organic (sporopollenin) surfaces of spores and pollen (figure 1*b*) all seem to have species-unique patterning, of great use to taxonomists working with both living and extinct plants. These very different microarchitectures can only arise through slight modifications in the building process; the question that needs addressing is how?

Flexibility of pattern formation may well be the consequence of self-assembly mechanisms acting upon 'digital' information such as that contained within DNA. The nature of proteins is such that a single base change in the genetic sequence can code for a different amino acid, which in turn can give rise to a different molecular configuration. A different protein within a construction sequence will have no effect (possibly by coding for the same amino acid), cause it to fail, or occasionally cause it to produce something different that does something useful within the

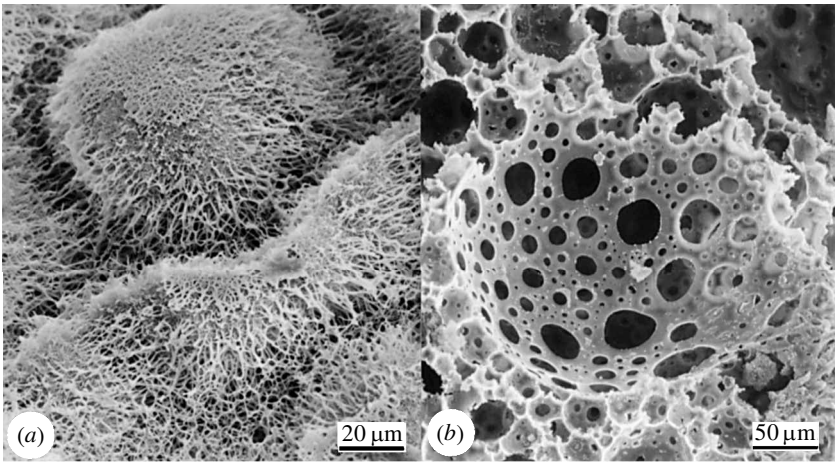

Figure 2. (*a*) Colloidal silica network on the surface of spores from *Isoetes pantii*. Scale = 20 μm. (*b*) Polystyrene networks and foams produced as a byproduct of latex formation (Hemsley *et al*. 1998). Scale = 50 μm.

organism. The nature of such mechanisms is essentially chaotic in that they exhibit both robustness and fragility (Goodwin *et al*. 1993). The substitution of many amino acids within a protein need not significantly change its folding pattern if these are chosen with care with respect to the influence they have on folding (robustness). However, the substitution of any one critical amino acid will cause the adoption of a different configuration (fragility). Alongside such potential generators of robust difference are so-called 'antichaotic' factors (Kauffman 1991). In antichaotic systems, components within the cellular soup are seen to be fully interactive with each other at a complex level. These systems can be perturbed, but are in a sense self-generating and in a state of balance (Bak & Chen 1991). Such systems act to maintain this equilibrium but, if distorted to excess, will 'snap' to an alternative stable state.

It is against this background that we have been investigating the structure and development of spores from the clubmoss *Selaginella*. These show complex microscopic architecture within their relatively thick walls (figure 1*c*). The presence of an apparently colloidal crystal region within the wall, which consists of more or less spherical particles of sporopollenin, has been determined. This has focused attention on constructional mechanisms involving processes of colloidal interaction (Collinson *et al*. 1993; Hemsley *et al*. 1994) in order to account for the crystalline region and the other structures encountered within the complex walls. It has become apparent that a full understanding of this mode of microarchitectural construction lies as much with an appreciation of synthetic colloid and surfactant interactions (Adams *et al*. 1998) as it does with 'biological' control mechanisms.

2. Consideration of colloidal interactions and self-assembly

While our understanding of the relevant factors important to colloid science in terms of synthetic applications and materials is quite advanced, as we have seen the same cannot be said for the 'colloid science' operating in natural environments.

This is surprising since many of the same types of materials are present in the natural environment, e.g. spherical particles comprising silica or a monomer, free polymer, salt and other additives such as fatty acids. Furthermore, the synthetic colloid scientist can manipulate the components within a system in ways that are not accessible to nature, i.e. there is unlikely to be a genetic mechanism that can suddenly add 0.2 g of polymer or increase the ionic strength to 0.1 M! Genetic input is simply not responsive enough in relation to the speed of reactions. However, nature is a far better chemist than humanity, although she has had many more millennia to get it right and discovering the finesse and natural controlling factors would certainly enhance the ability of the relatively crude synthetic chemist. Nature may prepare systems at the boundary of stability and through subtle changes in one parameter, tip the system over the edge resulting in significant architectural changes. The approach taken in our work has been to try to manipulate the behaviour of synthetic organic colloids with a view to reproducing patterns and architecture present in the natural materials; this will *inter alia* uncover the controlling factors used by nature. Use of organic components in synthetic biological self-assembly is new and presents complexity of interpretation. However, it is essential if we are to progress beyond qualitative description to quantitative and defined understanding.

First though, we must outline albeit very briefly, the basic factors important to colloidal stability and self-assembly. It is these areas that clearly hold the insights we require. Throughout the section, we highlight possible control mechanisms available to the natural system.

The Greeks also believed that only two forces, love and hate, could account for all fundamental phenomena. There are in reality four distinct forces; the strong nuclear interactions that bind nuclei together, weak interactions associated with electron clouds and the electrostatic and gravitational forces (Israelachvili 1991). Actually, the Greeks did observe these latter two interactions, but could not explain them. In the 17th century, Newton showed that the interaction between molecules within an ensemble affected their bulk physical properties. Phenomena such as capillary rise led to the suggestion that different glass–liquid and liquid–liquid interactions which operate over very small distances must exist. It was the Dutch scientist van der Waals who made the breakthrough; in order to explain why gases do not obey the ideal gas law, van der Waals introduced a force (which now bears his name) to account for an attractive interaction between molecules. However, it was not until the advent of quantum theory in the 1920s and the ability to elucidate the electronic structure of molecules, that it become clear that all intermolecular interactions are in fact, electrostatic in origin. Today, intermolecular forces can be calculated from a knowledge of the distribution of electron clouds associated with the molecules.

The characteristics of colloidal particles are somewhat different to those of a molecule, yet the same basic forces operate (Vincent 1980). Consider a generalized interaction between identical spherical colloid particles dispersed in a solvent. The interaction energy can be written as the product of some property of the particle, X_{P}, and the solvent, X_{S}. The binding energies of the particles and solvent molecules can be expressed as $W_{\mathrm{SS}} = -X_{\mathrm{S}}^2$ and $W_{\mathrm{PP}} = -X_{\mathrm{P}}^2$ for like-binary combinations, while $W_{\mathrm{SP}} = -X_{\mathrm{P}}X_{\mathrm{S}}$ for unlike-binary combinations. In the aggregated state, there is a specific molecular organization with n_{PP} and n_{SS} 'contacts' between particles

and solvent molecules, respectively. However, when the particles are dispersed, the organization becomes random, i.e. the n_{PP} and n_{SS} contacts are broken and $2n_{\mathrm{PS}}$ contacts are formed. The difference in the binding energies of the aggregated W_{agg} and dispersed W_{dis} states is fundamental to colloid science, namely

$$\Delta W = W_{\mathrm{agg}} - W_{\mathrm{dis}} = -n(X_{\mathrm{P}} - X_{\mathrm{S}})^2$$

and since both n and $(X_{\mathrm{P}} - X_{\mathrm{S}})^2$ can only be positive, ΔW must be negative, i.e. $W_{\mathrm{agg}} < W_{\mathrm{dis}}$. There is therefore always an attractive interaction between like particles in a solution, tending to induce aggregation. Colloidal dispersions are therefore inherently thermodynamically unstable. So, if an organism can synthesize a colloidal dispersion, either through aggregation of dissolved minerals or polymerization of self-assembled molecules, the formation of the colloidal crystals such as that present in some spore walls (figure 1*c*) should come as no surprise! It is this very potential, i.e. to form aggregates rather than dispersions, that organisms have used to great effect.

Our simple thermodynamic discussion can be generalized to two particle types, X_{P1} and X_{P2} in a solvent. Since we have no idea of the relative numbers of P_1/S and P_2/S contacts, we can write

$$\Delta W = W_{\mathrm{agg}} - W_{\mathrm{dis}} \propto -(X_{\mathrm{P1}} - X_{\mathrm{S}})(X_{\mathrm{P2}} - X_{\mathrm{S}}).$$

Interestingly, the differences in binding energies now depend on the relative magnitudes of X_{P1}, X_{P2} and X_{S} and can be either positive (favouring dispersal) or negative (favouring aggregation). This again could be the basis for a natural control mechanism; as the number and composition of the colloidal building blocks evolve, subtle changes in X_{P1} and X_{P2} or more likely X_{S}, could switch a dispersion from stable to unstable.

This interaction between particles is the van der Waals interaction, but is somewhat different to that operating between discrete molecules as it comprises a pairwise summation of the intermolecular forces present between the molecules themselves which comprise the particles. The interparticle interaction, for spherical particles of radius a, can be described by a free energy ΔG^{att}:

$$\Delta G^{\mathrm{att}} = -A_{\mathrm{H}}a/12H \qquad (\text{for } H \ll a),$$

where A_{H} is the Hamaker constant and H is the surface separation. Note that the interaction energy falls off much more slowly for particles than for the constituent molecules and hence, the van der Waals interaction is termed long range. The drawback of this approach is that it is difficult to estimate the Hamaker constant with sufficient accuracy; various approaches are possible, the simple being essentially incorrect and the complex requiring bulk optical/dielectric constants over a wide frequency range. The Hamaker constant depends on the nature of the particle and the solvent; A_{H} must be replaced by an effective Hamaker constant,

$$A_{\mathrm{H}} = (A_1^{1/2} - A_2^{1/2})^2,$$

where A_1 and A_2 are the Hamaker constants for the particles and solvent molecules, respectively. Values for the Hamaker constant between common colloidal particles are typically 1–100kT, where kT is the thermal energy of the particles whose random motion favours dispersal.

The overall interaction between colloidal particles in solution sometimes includes a further electrostatic term arising through the presence of charged groups on the surface of the particle (Shaw 1980). Several mechanisms lead to surface charge: dissociation of ionic groups, adsorption/desorption of potential determining ions and other ionic materials such as surfactants. The presence of surface charges induces a redistribution of nearby ions; like-charges are repelled and unlike-charges attracted. Combined with their thermal motion, this leads to an 'electric double layer' consisting essentially of two distinct regions; an inner region of adsorbed ions called the Stern layer and a more diffuse region. When two such diffuse layers overlap, a repulsive interaction is introduced. For typical ionic strengths, e.g. 10^{-3} mol dm^{-3}, the thickness of the double layer is *ca.* 10 nm. If the ionic strength is substantially higher, the double-layer interaction is sufficiently reduced and it can no longer provide stabilization against the van der Waals driven aggregation. This double layer can be quantified using the potential at the Stern layer $_{\mathrm{d}}$, the permittivity ε and screening length κ via the Gouy–Chapman model. The repulsive electrostatic potential at a separation H for large particles with low surface charges can be approximated by

$$V_{\mathrm{r}}(H) = 2\pi\varepsilon\varepsilon_0 a \ {}^{2}_{\mathrm{d}} \ln[1 + \exp(-\kappa H)],$$

where ε is the permittivity of free space, ε_0 is the permittivity of the medium. In contrast to the van der Waals interaction, which falls off reciprocally with distance, the electrostatic repulsion falls off exponentially with distance. Consequently, the van der Waals interaction dominates at small and large distances, while the double-layer interaction dominates at intermediate distances. At very small distances, i.e. particle contact, there is a very sharp rise in repulsive interaction as the electron clouds of adjacent particles overlap.

Overall, interparticle interactions have the form shown in figure 3. The maximum in the potential corresponds to the barrier to aggregation: the inherent stability of the dispersion. If this barrier is larger than the thermal energy kT, the dispersion will be stable. The height of the barrier depends on $_0$ and κ. Simply modifying the ionic strength can significantly alter the phase behaviour, another potential control mechanism.

The stability of colloids can be dramatically altered by inclusion of polymeric materials (Vincent 1987; Fleer *et al.* 1993). For example, if the polymer interacts favourably with the particle surfaces, i.e. it 'adsorbs', then both an increase and a reduction in stability are possible. If the polymeric material is charged, the adsorbed polymer layer modifies the electrostatic interaction. When the polymer and particle carry the same charge, the electrostatic repulsion in increased since the surface charge is increased. Concomitantly, the absorbed polymer layer may reduce the van der Waals attraction also promoting enhanced stability.

More importantly perhaps, are the new contributions to the overall interaction that the polymer layers introduce. For instance, two particles approaching one another may lead to desorption of the polymer, compression of the polymer layer; both of which are unfavourable. Furthermore, increases in the local polymer concentration as the polymer layers overlap can be favourable or unfavourable depending on the relative affinities of the solvent for the polymer and particle.

The criteria for 'steric stabilization' are rather straightforward; essentially (i) the polymer needs to be of sufficient coverage to coat all the particle surface with a polymer layer of thickness δ, such that when $H = 2\delta$, the van der Waals interaction

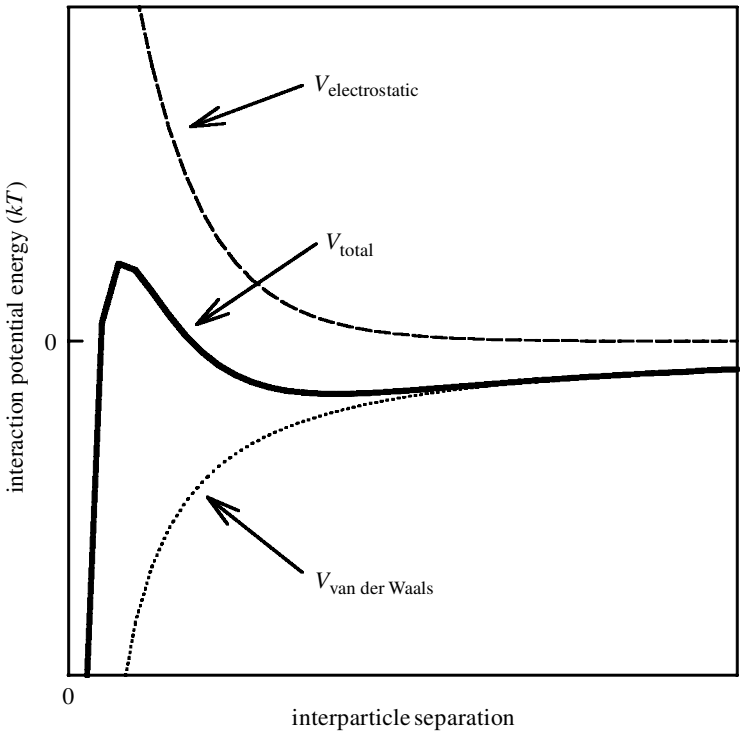

Figure 3. Schematic potential energy curves describing the interactions between colloidal particles.

energy is small compared with kT, and (ii) the polymer layer needs to be firmly attached to the surface. How this is engineered is beyond the scope of this article, but the consequences of not satisfying these criteria are informative in understanding the effect that polymers have on the overall interparticle potential. Since complete or incomplete coverage of the particles will result in different properties, this is clearly one way in which minimal change in initial conditions can lead to major differences in stability.

As the polymer-coated particles approach one another, the steric interaction V_{s} is modified in two distinct manners, one due to the elastic nature of the polymer chains and the other due to the mixing of the layers, namely

$$V_{\text{s}}(H) = \frac{2\pi akTV_2^2\Gamma_2^2}{V_1}(\tfrac{1}{2} - \chi)S_{\text{mix}} + 2\pi akT\Gamma_2 S_{\text{elastic}},$$

where V_{i} corresponds to the molecular volume of the solvent (subscript 1) and polymer (subscript 2), Γ_2 is the amount of polymer adsorbed on the particle surfaces and χ is the Flory interaction parameter. S_{mix} and S_{elastic} are geometric terms dependant on the concentration profile of the polymer normal to the surface. The first term, the mixing contribution, can be both positive and negative depending on the value of the Flory interaction parameter χ. If $\chi > 0.5$, by, for example, adding a non-solvent for the polymer or altering the ionic strength for certain polymers, the mixing term becomes negative and an attractive component to the steric interaction is present.

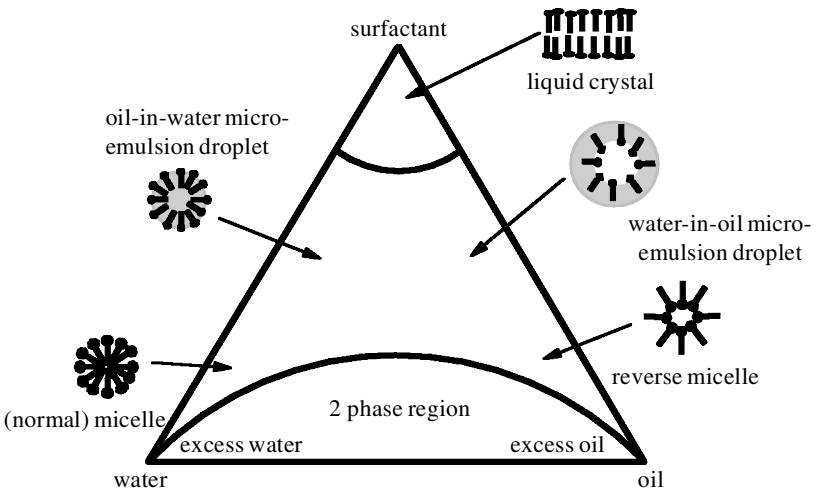

Figure 4. Schematic phase diagram for a three-component (oil, water, surfactant) system showing some of the self-assembled structures which form in the various regions.

This will reduce the overall stability; again another possible natural control mechanism if the molecular weight or concentration of the polymer could be varied easily. The elastic term is, however, always positive, i.e. repulsive.

If insufficient polymer layer is present, when the particles attain a separation $\delta \approx H$, the polymer layers on adjacent particles may bridge between the particles leading to a favourable interaction for enthalpic reasons. This is termed bridging flocculation. One way to destabilize a sterically stabilized dispersion for instance, is to add a second polymer which displaces the first but does not itself form an adsorbed polymer layer that would satisfy the two main criteria for steric stabilization. Similarly, a non-adsorbing polymer in solution can also destabilize a dispersion through a mechanism called depletion flocculation. When polymer molecules do not interact favourably with the particle surfaces from an enthalpic perspective, they are repelled from the surface regions due to entropic reasons. A 'depletion zone' around the particles is created which has a lower average polymer concentration than the bulk solution. The osmotic pressure difference results in solvent being pulled from the depletion zone—in essence, pulling the particles closer together. This is equivalent to an attractive interparticle interaction. Interactions involving surface bound polymers are of great interest in explaining biological microarchitectures as in many cases, the likely components will be separated from the supporting fluids by mixed polymeric membranes involving lipids, proteins and polysaccharides.

Another important interaction that needs to be considered is the hydrophobic interaction (Tanford 1980). This can be most easily thought of in terms of two immiscible liquids such as oil and water being induced to mix by adding surfactants, to form (micro) emulsions. The exact structure of the phase formed depends heavily on the relative compositions of the various phases and the structure of the surfactant.

Below some critical surfactant concentration, the system is two phase with excess oil or water depending on the oil/water concentration ratio (Fennell Evans & Wennerström 1994). On adding more surfactant, the system moves into a one-phase region with normal micelles forming in water-rich systems. The water constitutes

the continuous phase, solvating the headgroups of the surfactant whose hydrophobic tails solubilize oil in the core of the micelle. In oil-rich systems, reverse-micelles form. With further increases in surfactant composition, oil-in-water or water-in-oil (micro) emulsion droplets form. Ultimately, at high surfactant compositions, liquid crystalline (lamellar) structures form.

The region in the middle of this phase diagram (figure 4), where bicontinuous structures are often located, consists of 'oil-in-water-in-oil-in-water'-type systems, shown below. In the organism, if these types of system were present and were to contain monomers or minerals dissolved in either phase, after polymerization or aggregation a rigid three-dimensional 'gel' structure would be formed.

The sites of spore wall formation, i.e. the sporangial loculus, act as mini-reactor vessels in which the above interactions can occur. If a polymerization occurs within one such structure, the resulting (polymer) architectures will probably closely resemble the self-assembled ones formed in our artificial sporangia.

3. Synthetic self-assembled architecture and evolutionary implications

Following identification of the colloidal crystal layer within our spore walls, an attempt was made to use a simple colloid consisting of polystyrene particles in water (a latex) to mimic the natural structure. To cause flocculation of the particles, carboxymethylcellulose (CMC) was introduced with the intention of initiating a depletion interaction as described above (Hemsley *et al.* 1996). Although different from sporopollenin, polystyrene shares some properties and is at least reasonably well understood with regard to its colloidal behaviour. CMC was chosen as a relatively 'natural' polysaccharide. These initial experiments proved successful and resulted in the formation of colloidal crystals like those within the spore walls, but more significantly, they were built by processes and components which we believe behave in a similar manner to those in the natural system. Similar particle flocculations, but of an amorphous nature and formed from particles of inconsistent size, could be produced by either depletion or bridging flocculation. Subsequent experiments (Hemsley *et al.* 1998, 2000) have used hydrocarbons and lipids (known from the natural system of wall production) to synthesize mimics resembling other types of spore wall with some success.

It is disconcerting how 'life-like' some structures built from synthetic colloidal particles can be (figures 2*b* and 5*a–d*)). Hollow spheres of aggregated particles and particle aggregates ('raspberries') are self-assembling from polystyrene latex in a water–cyclohexane emulsion. These are comparable with 'raspberries' and aggregated particles of sporopollenin formed during the development of *Selaginella* spores (figure 5*g*). Similar structures occurring in water–rape-seed-oil emulsions (figure 5*d*) closely resemble some *Selaginella* spores in surface architecture and internal organization (figure 5*e*,*f*).

The following hypothetical situation might arise reflecting that found in synthetic systems. An oil-in-water emulsion forms, comprising a monomer such as a hydroxycinnamic acid (figure 6) stabilized by fatty acids. The polymerization of 'sporopollenin' can occur through a free radical mechanism involving the vinyl group, although the concentration of free radicals is likely to be low in natural systems, or through an alcohol + acid condensation to form an ester. The latter polymerization, cer-

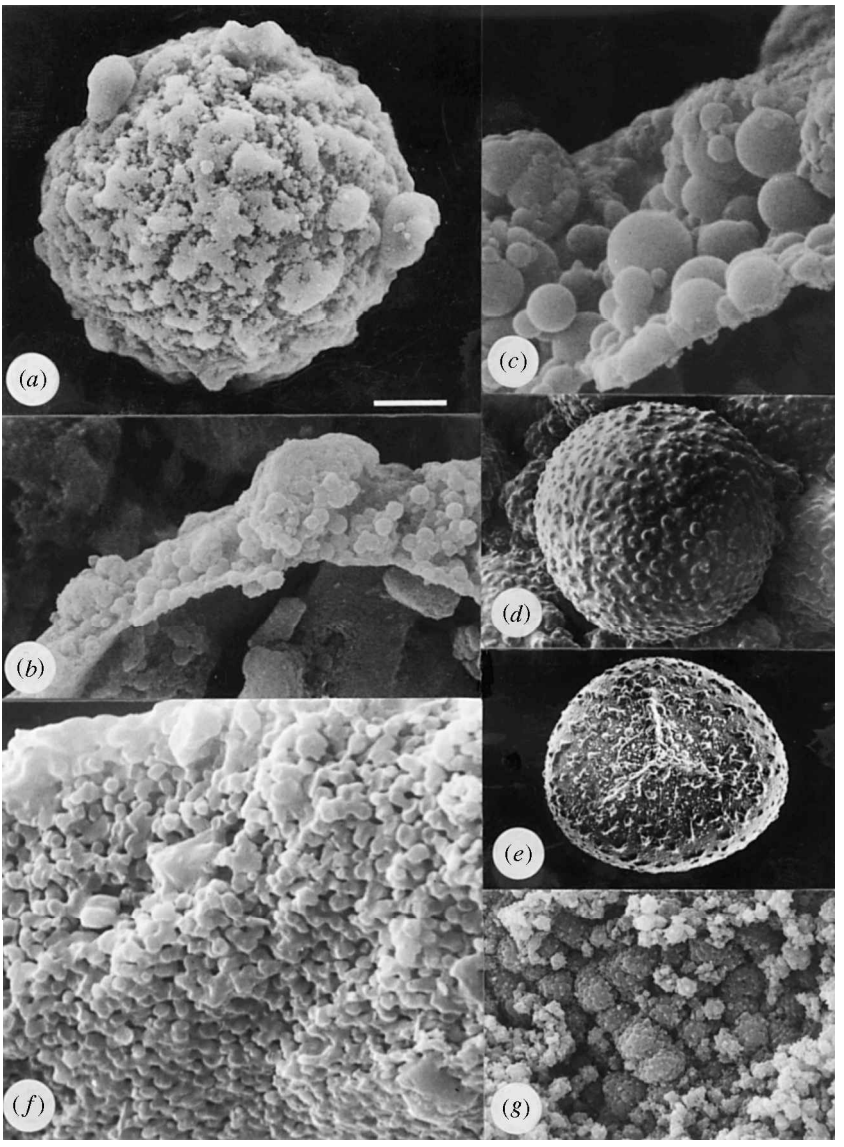

Figure 5. All scales refer to the bar in (*a*). (*a*) Spore-like structure of sporopollenin particles and particle aggregates formed around a droplet of hydrocarbon. Scale = 10 μm. (*b*) A broken structure like that shown in (*a*). Scale = 5 μm. (*c*) Detail of the composition of the wall of the mimic spore shown in (*b*). Scale = 2 μm. (*d*) Large-scale particle aggregates formed in the presence of lipids, again around a hydrocarbon droplet. Scale = 500 μm. (*e*) Spore of *Selaginella selaginoides*. Scale = 400 μm. (*f*) The wall structure of a broken spore of *Selaginella selaginoides*. Scale = 3 μm. (*g*) Sporopollenin particle aggregates and colloidal sporopollenin occurring during wall development in *Selaginella laevigata*. Scale = 10 μm.

tainly in a synthetic application, is very slow in the absence of any added (acid) catalyst although a second molecule of acid could self-catalyse the reaction (Cowie 1991). Nevertheless, the kinetics of this reaction are very sensitive to concentration.

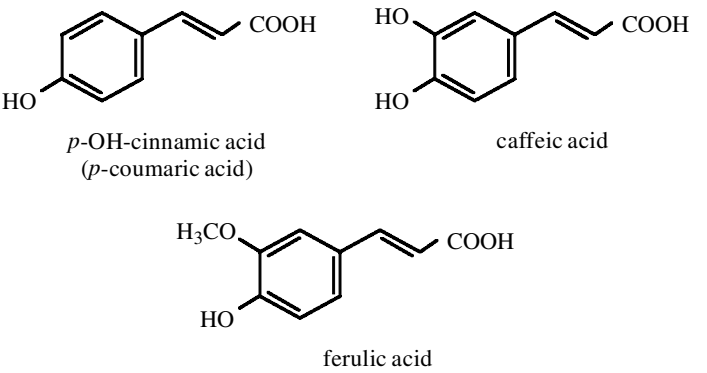

Figure 6. Three hydroxycinnamic acids common in plants and of interest as potential sporopollenin components (Wehling *et al*. 1989).

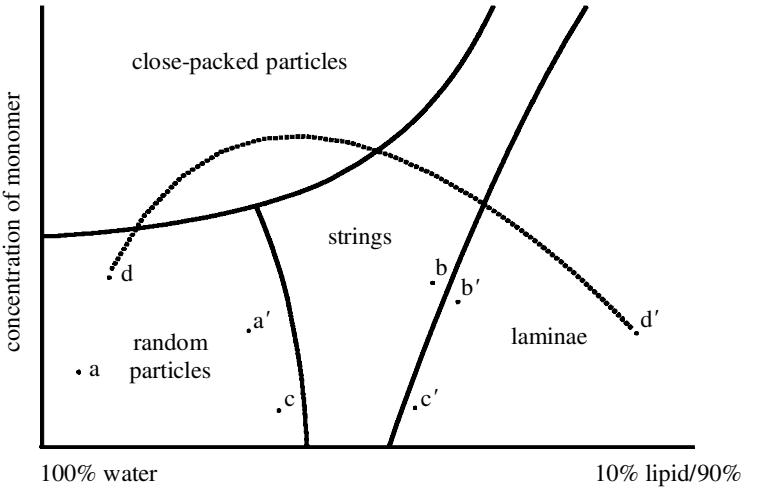

Figure 7. Hypothetical representation of a set of architectural domains defined by monomer concentration and proportion of lipid. Each defines structure regardless of the exact composition, providing this lies within its boundary. Letters a to d and a′ to d′ represent specific concentrations of components. The dotted line d to d′ shows a pathway of changing concentration by which a spore wall such as that shown in figure 1*c* might be constructed.

Furthermore, should free radicals be present, the vinyl groups would much more rapidly polymerize depleting the emulsion droplets of monomer, providing the control required for a particular particle size. The composition of the solution thus determines not only the phase behaviour, but the rate of polymerization and the particle size. If, the organism has in its genetic code, the ability to synthesize the monomer, it presumably has the information to degrade any excess. This natural equilibrium could also create the initiator species as a by-product of the reaction which breaks down the excess monomer.

Differences in microarchitecture in relation to component concentration would appear to occur in our simulations of *Selaginella* megaspore wall construction. Imagine an example in which our synthetic wall structure is determined by concentration

of styrene and cyclohexane (in the plant, these would be sporopollenin monomer and a fatty acid) all in water. Different arrangements (and sizes) of polystyrene particles occur depending upon the conditions at the initiation of polymerization. In the hypothetical example shown in figure 7, compositions and conditions represented by a and a′ are different. They result from slightly different genetic codings but despite this, they both give rise to the same ultimate structure (they are within the same domain of the diagram). Examples b and b′ may have much more similar genetic codings (they may differ only in a single base pair and thus produce similar concentrations of components), but because this gives rise to initiation points either side of a domain boundary, the resulting structure is different, possibly very different (strings or laminae). Points c and c′, although probably closer to each other (genetically) than a and a′, may be considered to exhibit the greatest difference in microarchitectural expression since these are separated by two domain boundaries. Significantly, it may not matter for any subsequent stage of development from where within each domain the original composition was positioned since what matters is how the new components interact to initiate the next stage of development. It is abundantly clear from this illustration that assessment of relationships of organisms based on comparison of the genetic code would differ somewhat from any assessment based on patterning and structure. Consider the likely outcome of such an analysis on a, a′, c and c′. There are further complications in that composition will usually change as wall development occurs (consider arrow from d to d′ and compare with figure 1*c*, development is from bottom to top). In addition, any *in vivo* self-assembly system such as this is reliant upon second-hand manipulation by proteins/enzymes which have already been through a similar selection process.

The incorporation of self-assembly mechanisms in development is clearly advantageous to an organism if the processes involved are sufficiently robust and the results consistent. Such systems represent a saving in terms of both the required genetic code and its decryption (via ribosomal RNA) into enzymic regulatory proteins. The genetic code need only describe the initial conditions and not the complexity of the ultimate structure (e.g. Regier & Hatzopoulos 1988). Over the great expanse of time involved in the evolution of life (particularly simple, single-celled organisms) many self-assembly mechanisms have been included by chance, much as proteins with a specific function have been retained and elaborated. Among organisms, many self-assembly mechanisms are shared (although they may result in different patterns and architecture due to different initial conditions), while others may be unique. However, the identification of such mechanisms and an assessment of their distribution amongst organisms will surely assist in both an understanding of organismal relationships and the meaning of structural, architectural and pattern diversity between ‘species’. The observation that self-assembly systems can switch from the production of one pattern to another with only minor modification of the initial conditions (supported by our colloidal work) adds weight to the view that evolutionary change (in the form of speciation) could be relatively rapid (Hemsley 1998).

The evidence we offer above for the microarchitectural development mechanisms occurring within spore walls serves to underline the significance of colloids in biological construction and pattern formation. As we have demonstrated, an understanding of colloidal mechanisms has the potential to explain certain aspects of biological complexity. As a first approximation to reality, our organic mimics have already revealed

much about the way in which spore walls form. Furthermore, they have begun to indicate just how much of our ultimate structure is governed by the ways in which our microscopic components interact.

4. Future applications of biocolloid self-assembly

Clearly, the improved understanding of colloidal behaviour within living systems that we are developing offers the eventual prospect of our being able to manipulate such systems. The control of microarchitecture in both living and synthetic systems has many potential applications. The most important aspect is the ability to define the particular conditions under which a certain pattern or structure will be formed such that the products will be uniform. This clearly happens in nature, but natural systems have been subject to trial and error for considerably longer than any experiment involving synthetic systems.

Natural materials, particularly compounds such as sporopollenin with almost total resistance to digestion, could be used in the manufacture of cosmetic and drug delivery capsules (Caruso *et al*. 1998; Loxley & Vincent 1998), and would be both safe and efficient. Our studies of the colloidal construction of spore walls reveals how we might design such capsules with a high degree of control over size, wall thickness, solubility, and porosity leading to complete regulation of dosage. Such capsules could be self-assembled in vast numbers, possibly even around micelles containing the intended active content. As our understanding of the interaction of plant genetics and colloidal construction mechanisms improves, we may eventually be able to manipulate plants into producing both the required capsule and content.

Regulation of microarchitecture has applications in the production of surface coatings. Again, control of the consistency of pattern offers the prospect of the self-assembly of periodic surface features on a scale that would interact with incident light. Paints could be designed to produce iridescent effects or to produce specific finishes upon drying. The use of natural water-based colloidal systems could eliminate the need for potentially harmful or irritating volatile components. Were consistent surface patterns to be of a highly repetitive nature over relatively large scales, they may potentially be of use in the production of computer chip technology, providing a template for microcircuitry. Again, it might be feasible to extract the required component chemicals from genetically engineered plants, much as we can extract clove oil, ephedrine or opium now.

The use of colloidal chemistry in the production of synthetic organic microarchitecture based on that produced by living systems is in its infancy. Its development will naturally run parallel to the greater use of genetic manipulation of organisms both as a whole and as organismal components in test tubes. We perceive a time, within the new millennium, in which we are able to control nature, not just through genes, but by making use of the inherent properties of biological construction materials and processes. These substances and mechanisms will be, by their very nature, 'friendly' to both humans and the environment as a whole.

We thank B. Vincent for many useful discussions, J. Pike for the *Actinoptychus*, L. Axe, P. Fisher, A. Oldroyd, and V. Williams for technical assistance and the Royal Society for a URF award to A.R.H.

References

Adams, M., Dogic, Z., Keller, S. L. & Fraden, S. 1998 Entropically driven microphase transitions in mixtures of colloidal rods and spheres. *Nature* **393**, 349–352.

Bak, P. & Chen, K. 1991 Self-organised criticality. *Scient. Am.* **264**, 26–33.

Caruso, F., Caruso, R. A. & Möhwald, H. 1998 Nanoengineering of inorganic and hybrid hollow spheres by colloidal templating. *Science* **282**, 1111–1114.

Cohen, J. 1995 Who do we blame for what we are. In *How things are* (ed. J. Brockman & K. Matson), pp. 51–60. London: Weidenfeld & Nicholson.

Collinson, M. E., Hemsley, A. R. & Taylor, W. A. 1993 Sporopollenin exhibiting colloidal organization in spore walls. *Grana Supplement* **1**, 31–39.

Cowie, J. M. G. 1991 *Polymers: chemistry and physics of modern materials*, 2nd edn. London: Chapman & Hall.

Crawford, R. M. & Schmid, A. M. 1986 Ultrastructure of silica deposition in diatoms. In *Biomineralisation in lower plants and animals* (ed. B. S. C. Leadbeater & R. Riding), pp. 291–314. Systematics Association Special Vol. 30. Oxford: Clarendon Press.

Fennel Evans, D. & Wennerström, H. 1994 *The colloidal domain where physics, chemistry and biology meet.* New York: VCH.

Fleer, G. J., Cohen Stuart, M. A., Scheutjens, J. M. H. M., Cosgrove, T. & Vincent, B. 1993 *Polymers at interfaces*, 1st edn. London: Chapman & Hall.

Goodwin, B. C., Kauffman, S. & Murray, J. D. 1993 Is morphogenesis an intrinsically robust process. *J. Theor. Biol.* **163**, 135–144.

Gunning, B. E. S. & Steer, M. W. 1996 *Plant cell biology, structure and function.* Boston: Jones and Bartlett Publishers.

Hemsley, A. R. 1998 Non-linear variation in simulated complex pattern development. *J. Theor. Biol.* **192**, 73–79.

Hemsley, A. R., Collinson, M. E., Kovach, W. L., Vincent, B. & Williams, T. 1994 The role of self-assembly in biological systems: evidence from iridescent colloidal sporopollenin in *Selaginella* megaspore walls. *Phil. Trans. R. Soc. Lond.* B **345**, 163–173.

Hemsley, A. R., Jenkins, P. D., Collinson, M. E. & Vincent, B. 1996 Experimental modelling of exine self-assembly. *Bot. J. Linnean Soc.* **121**, 177–187.

Hemsley, A. R., Vincent, B., Collinson, M. E. & Griffiths, P. C. 1998 Simulated self-assembly of spore exines. *Ann. Bot.* **82**, 105–109.

Hemsley, A. R., Collinson, M. E., Vincent, B., Griffiths, P. C. & Jenkins, P. D. 2000 Self-assembly of colloidal units in exine development. In *Pollen and spores, morphology and biology* (ed. M. M. Harley, C. M. Morton & S. Blackmore). Kew: Royal Botanic Gardens.

Ingber, D. E. 1998 The architecture of life. *Scient. Am.* **278**, 30–39.

Israelachvili, J. 1991 *Intermolecular and surface forces*, 2nd edn. Academic.

Kauffman, S. A. 1991 Antichaos and adaptation. *Scient. Am.* **265**, 64–70.

Kauffman, S. A. 1993 *The origins of order.* Oxford University Press.

Leadbeater, B. S. C. 1984 Silicification of 'cell walls' of certain protistan flagellates. *Phil. Trans. R. Soc. Lond.* B **304**, 529–536.

Li, C.-W. & Volcani, B. E. 1984 Aspects of silicification in wall morphogenesis of diatoms. *Phil. Trans. R. Soc. Lond.* B **304**, 519–528.

Lipowsky, R. 1991 The conformation of membranes. *Nature* **349**, 475–481.

Loxley, A. & Vincent, B. 1998 Preparation of poly(methylmethacrylate) microcapsules with liquid cores. *J. Coll. Interface Sci.* **208**, 49–62.

Mann, S. & Ozin, G. A. 1996 Synthesis of inorganic materials with complex form. *Nature* **382**, 313–318.

Nédélec, F. J., Surrey, T., Maggs, A. C. & Leibler, S. 1997 Self-organization of microtubules and motors. *Nature* **389**, 305–308.

Raymo, F. M. & Stoddart, J. F. 1996 Self-assembling wholly synthetic systems. *J. Coll. Interface Sci.* **1**, 116–126.

Regier, J. C. & Hatzopoulos, A. K. 1988 Evolution in steps: the role of regulatory alterations in the diversification of the moth chorion morphogenic pathway. In *Self-assembling architecture* (ed. J. E. Varner), pp. 179–202. New York: Alan R. Liss.

Shaw, D. J. 1980 *Introduction to colloid and surface chemistry*, 3rd edn. London: Butterworths.

Tanford, C. 1980 *The hydrophobic effect. Formation of micelles and biological membranes*, 2nd edn. Wiley.

Thomas, E. L., Reffner, J. R. & Bellare, J. 1990 A menagerie of interface structures in copolymer systems. *Colloq. Physique* **51**, 363–374.

Thompson, D. W. 1961 *On growth and form*, abridged edition (ed. J. T. Bonner). Cambridge University Press.

Tryon, A. F. & Lugardon, B. 1978 Wall structure and mineral content in *Selaginella* spores. *Pollen Spores* **20**, 315–340.

Vincent, B. 1980 The stability of particulate suspensions. *Chem. Industry* **15**, 218–224.

Vincent, B. 1987 The stability of solid–liquid dispersions in the presence of polymers. In *Solid/liquid dispersions*. Academic Press.

Walsh, D. & Mann, S. 1995 Fabrication of hollow porous shells of calcium carbonate from self-organising media. *Nature* **377**, 320–323.

Wehling, K., Niester, Ch., Boon, J. J., Willemse, M. T. M. & Wiermann, R. 1989 *p*-Coumaric acid—a monomer in the sporopollenin skeleton. *Planta* **179**, 376–380.

Whitesides, G. M., Mathias, J. P. & Seto, C. T. 1991 Molecular self-assembly and nanochemistry: a chemical strategy for the synthesis of nanostructures. *Science* **254**, 1312–1319.

Wilcock, J. R., Perry, C. C., Williams, R. J. P. & Mantoura, R. F. C. 1988 Crystallographic and morphological studies of the celestite skeleton of the acantharian species *Phyllostaurus siculus*. *Proc. R. Soc. Lond.* B **233**, 393–405.

Wilcock, J. R., Perry, C. C., Williams, R. J. P. & Brook, A. J. 1989 Biological minerals formed from strontium and barium sulphates. II. Crystallography and control of mineral morphology in desmids. *Proc. R. Soc. Lond.* B **238**, 223–233.

AUTHOR PROFILES

A. R. Hemsley

Alan Hemsley (below, standing) studied botany at Bedford College, London (1982–85), and Reading (1986–87) before returning to Royal Holloway, University of London, to study palaeopalynology for his PhD (1990). Research into heterospory in fossil plants took him to Montpellier, France, and eventually to Cardiff, where in 1995 he was awarded a Royal Society University Research Fellowship. Aged 36, Alan is an editor for *Palaeontology*, and has recently co-authored a revision of a popular botany textbook. His interests still lie principally in the study of fossil spores, particularly their evolution, wall development, structure and chemistry.

P. C. Griffiths

Originating from Cornwall, Peter Griffiths (below left) studied initially at University College, North Wales (1985–88), and subsequently the University of Bristol (PhD 1991). After postdoctoral positions in Bristol and Stockholm, he moved to a lectureship at Cardiff in 1995. Aged 32, his research interests centre around colloidal systems, in particular polymer–surfactant interactions.