

## Porphyrim Amphiphiles as Templates for the Nucleation of Calcium Carbonate

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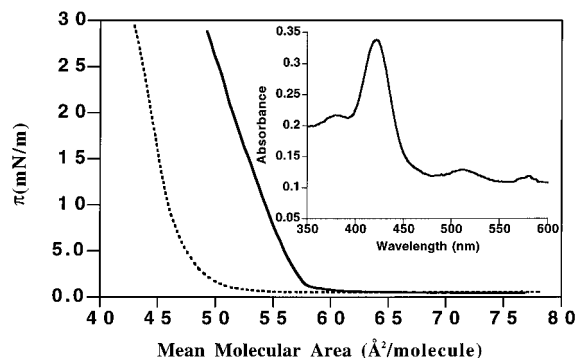
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The processes of biogenic crystal growth are of interest both to derive an understanding of the biochemistry for the development of mesoscopic structures and as an inspiration to devise new strategies for materials fabrication.<sup>1</sup> Template-mediated nucleation of calcite, a predominant polymorph in these structures, has been investigated using either proteins<sup>2,3</sup> or simple monofunctional amphiphiles.<sup>4–6</sup> Model studies with small amphiphilic molecules at compressed Langmuir interfaces, pioneered by Mann *et al.*,<sup>4</sup> have yielded important insights on the molecular recognition affecting the nucleation process of the inorganic phase. However, the morphological control mechanisms and numerous subtleties associated with protein templates remain unknown at the molecular level.

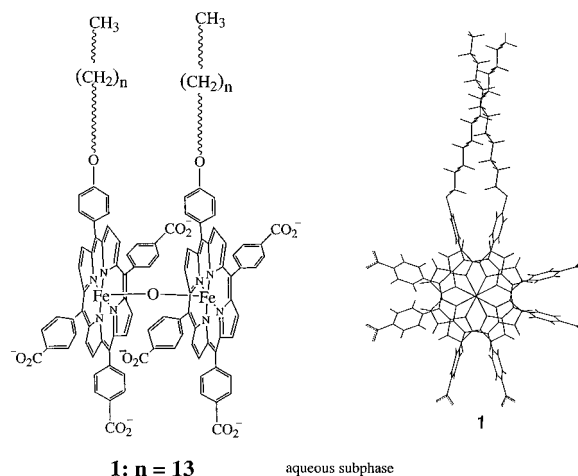
We have synthesized amphiphilic tricarboxyphenylporphyrin iron(III)  $\mu$ -oxo dimers, surface films of which mimic several aspects of nucleation and growth of calcium carbonate observed with native biological templates: (1) highly oriented crystals have been obtained; (2) self-assembly of the template occurs with no external mechanical forces to modulate crystal growth or morphology; (3) highly patterned excavations resulting in “chiral” crystals are observed. The porphyrin presents a semirigid surface array of carboxylate groups<sup>7</sup> intermediate in complexity between protein matrices and simple molecules. This approach could delineate the effects of cooperativity<sup>2</sup> and spatial–stereochemical effects,<sup>8</sup> and, especially, determine what minimal structure is required to mimic the controlled nucleation observed in biological systems.

Monolayers of the  $\mu$ -oxo iron(III) porphyrin dimer **1**<sup>7b</sup> were formed at the air/water interface using a solution of **1** in 3:1 chloroform/methanol (1 mg/mL) to spread the film. Isotherms obtained over a supersaturated calcium bicarbonate subphase by compression produced a limiting area of 56–58 Å<sup>2</sup>/porphyrin (Figure 1), corresponding to an approximately vertical orientation of the porphyrin plane at the interface.<sup>7,9</sup> A limiting area



**Figure 1.** LB isotherms for **1** over water (···) and calcium bicarbonate subphases (—),  $[Ca^{2+}] \approx 9$  mM. Inset: UV spectrum of **1** (in chloroform) extracted from calcium carbonate crystals grown from nucleation under **1**.<sup>10,15</sup>

of only 46–48 Å<sup>2</sup>/molecule (Figure 1) was obtained over distilled, deionized water. The higher limiting area in the presence of calcium ions is indicative of strong cation binding to the anionic template.<sup>5</sup> The total Ca concentration was estimated by EDTA titrimetric analysis to be  $\sim 9$  mmol dm<sup>-3</sup>.



Calcium carbonate crystals were obtained by the slow, spontaneous loss of CO<sub>2</sub> from the unstirred bicarbonate solutions beneath monolayers of **1** that were either compressed to 20–25 mN/m<sup>2</sup> or relaxed to the fluid region (0–5 mN/m).

Crystals were studied by *in situ* optical microscopy and scanning electron microscopy (SEM). Transfer of oriented CaCO<sub>3</sub> crystals was accomplished either by dipping and withdrawing glass cover slips through the surface films or by skipping the surface, following established procedures.<sup>4</sup> The dipping technique enables crystals to be viewed from above while the latter method affords a perspective of the crystals from the underside. Calcite was obtained as the predominant polymorph for nucleation (as verified by X-ray diffraction) by the porphyrin dimer **1**, although 5–10% (by number) of the crystals were vaterite.

Remarkably, the calcite crystals obtained were observed to be suspended from the amphiphilic surface by a corner of the calcite rhombohedron, very similar to the result reported by Addadi *et al.* for mollusk glycoproteins.<sup>2</sup> *In situ* optical microscopy viewed from above the porphyrin (**1**) monolayer revealed calcite crystals that were hexagonal in projection, indicative of their attachment to the amphiphilic surface at a corner, as shown in Figure 2a (inset). Approximately 80% of the crystals had this structural feature. Figure 2a shows an SEM image of one such calcite crystal, obtained using the “dipping” method. The crystals have smooth rhombohedral {10.4} side faces and a

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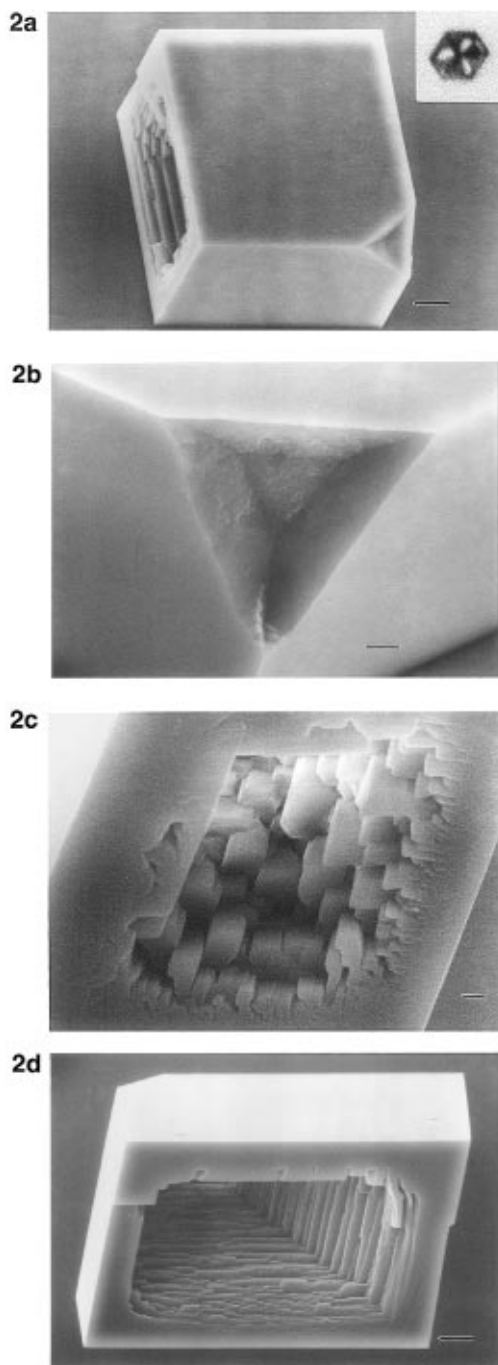
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**Figure 2.** SEM images of calcite crystals obtained from nucleation under **1** (2a–d). (a) Image of a single calcite crystal showing the truncated corner and cavity on a distal {10.4} plane, obtained by the “dipping” method. Inset: an *in situ* optical micrograph of a calcite crystal nucleated under **1**, viewed from above. This latter experiment was carried out in a circular glass trough. (b) Close-up of the truncated corner showing the three {01.2} planes radiating from a central flat (00.1) plane. (c, d) Views of the rectangular projections and a terraced gallery, respectively, on adjoining {10.4} planes distal to the truncated corner. Scale bars: (a) 5  $\mu\text{m}$ , (b) 2  $\mu\text{m}$ , (c) 1  $\mu\text{m}$ , (d) 5  $\mu\text{m}$ .

symmetrical, concave depression at the surface attachment point. Superficially, these truncations appeared to be flat. However, SEM images revealed that these corners consisted of three-symmetry related faces at  $120^\circ$  to each other radiating in some of the crystallites from a flat (00.1) face at the center of the truncation (Figure 2b). SEM stage-tilting experiments revealed an angle of  $\sim 17^\circ$  between each plane comprising the truncated corner and the adjacent {10.4} face. These correspond to the {01.2} planes on the basis of the known crystal structure of

calcite. All of the crystals surveyed with the truncated corners had this angular relationship. However, viewed from the underside, none of the crystals had corner truncations.

Interestingly,  $\sim 20\%$  of the three symmetry-related distal {10.4} faces had pronounced rectangular cavities, and some had two or three cavities on adjoining distal {10.4} faces. Distinct varieties of {10.4} cavities could be discerned. Some had layered and terraced galleries such as depicted in Figure 2a,d, and others had bundles of rectangular projections as shown in Figure 2c. Both sets of features were approximately 1  $\mu\text{m}$  in size. Significantly, the crystals with a layered excavation on one {10.4} face had rectangular projections on the other, suggesting different views of the same internal structure. Consequently, *these excavations produce an intrinsically chiral morphology* (even though calcite has a non-enantiomorphic crystal lattice). Both “enantiomorphs” of the crystal were observed. This chirality could only have originated from the porphyrin dimer template **1** which, according to molecular modeling, must have staggered porphyrin planes, and hence, the unit template is asymmetric. By desorption of porphyrin from accessible surfaces, we find that **1** is also incorporated inside the crystal.<sup>10</sup> Thus, the anisotropic adsorption of the template **1** on specific planes could result in the highly textured laminations.<sup>2c</sup>

An epitaxial match is possible with the (00.1) plane of calcite since there is a Ca–Ca distance of 4.99  $\text{\AA}$  which closely matches the 5  $\text{\AA}$  intercarboxylate distance in the dimer (**1**) as obtained from the molecular modeling.<sup>11</sup> We suggest that nucleation of these crystals occurred at or near the surface on the (00.1) face. The concave depression and the exposed {01.2} faces could be due either to inhibition of that face by the preferential adsorption of the porphyrin carboxylate cluster or limited diffusion of calcium ions to the truncated face due to the surface attachment.<sup>2,12</sup> While it is unlikely that all six carboxylates of a molecule of **1** could interact with a planar calcite face, it is clear that the carboxylate array afforded by the porphyrin template has induced the unusual morphologies observed. Since *the type of crystals obtained is independent of the compression state of the monolayer*, the template of porphyrin dimers required for oriented nucleation is spontaneously formed, without the need for compression to a “crystalline” state,<sup>5,13</sup> as is observed for the mollusk and chicken-egg shell proteins.<sup>14</sup> This is attributed to the intermolecular interactions between the porphyrinic amphiphiles, even at submonolayer coverage.<sup>15</sup>

The study demonstrates that a polyanionic porphyrin can assemble into a biomimetic nucleation template. Similar templates (with systematic variation) could delineate the requirements for the synergistic cooperative effects<sup>2</sup> and temporal phase changes<sup>3</sup> that are observed in biomineralization.

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(10) Calcite crystals washed extensively with Clorox showed 1.5% retention of porphyrin **1**.

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