

## FOCUS REVIEW

# Macromolecular templates for the development of organic/inorganic hybrid materials

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**Biominerals and the formation mechanisms of these materials have been studied intensively for decades. Biominerals have attracted much attention from material scientists because these compounds have significant mechanical and optical properties because of their elaborate structures. We believe that novel functional hybrid materials with hierarchical structures are best prepared using abundant elements and mild conditions, similar to the formation of natural biominerals. This review focuses on using an organic (bio)polymer for the formation of these hybrid materials based on CaCO<sub>3</sub> and on understanding the formation of new hybrid materials via bio-inspired approaches. The structure–function relationships of biomineralization-related proteins are discussed. Molecular designs to control the properties of the hybrid materials are also described. The combination of experimentation and molecular simulation is also introduced. These studies provide useful ideas for the development of hybrid materials through biomimetic approaches.**

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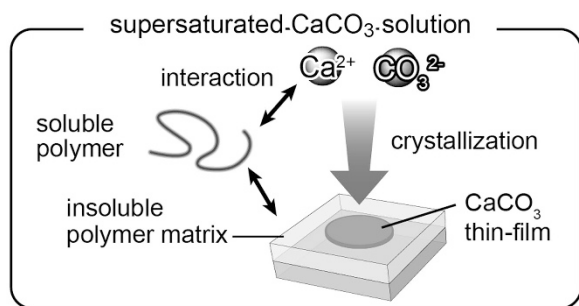
## INTRODUCTION

The hard tissues of living organisms such as teeth, bone, seashells and the exoskeletons of crayfish are called biominerals.<sup>1–4</sup> These materials are polymer/inorganic hybrid composites formed under mild conditions by molecularly controlled processes.<sup>1–4</sup> Owing to the hierarchical structures of biominerals at the molecular level, these materials exhibit significant mechanical,<sup>5,6</sup> optical<sup>7,8</sup> and magnetic properties,<sup>9</sup> which are exploited by the biomineral-producing organisms for a variety of purposes. It is known that biomacromolecules are essential for the formation of biominerals. In the process of biomineralization, well-ordered macromolecular templates are first formed via self-organization and are then subsequently used as substrates for inorganic crystallization.<sup>10</sup> The chemical and physical properties of biominerals, such as polymorphs, morphology, crystallographic orientation and crystal size, are strongly influenced by these templates.<sup>11</sup> Biomineralization has inspired material scientists in the design of novel functional hybrid materials for several decades.<sup>12–19</sup> Understanding the process of biomineral formation, and especially the roles of biomacromolecules in this process, is crucial to the development of new approaches to practical engineering processes. Although many studies on biomineralization-inspired crystallization and biomimetic hybrids have been reported, precisely controlled molecular processes for synthesizing hybrid materials under mild conditions have not yet been successfully achieved.

Kato and co-workers<sup>20–33</sup> have conducted studies on polymer/CaCO<sub>3</sub> hybrid materials, especially thin-film hybrids, using biomineralization-inspired methods (Figure 1). The use of cooperative interactions between soluble and insoluble polymer matrices is essential for the formation of polymer/CaCO<sub>3</sub> hybrids. The

production of thin-film hybrids containing both polymer and CaCO<sub>3</sub> with a wide variety of morphologies, including flat,<sup>20–23,29</sup> patterned,<sup>24,25,27,30</sup> unidirectionally oriented<sup>31–33</sup> and three-dimensional complex<sup>26,28</sup> structures, was achieved by changing the combination of polymer templates used in the hybrid formation. For example, photoimaged patterns on CaCO<sub>3</sub> thin films have been obtained by incorporating a combination of photo-crosslinkable polymer gel matrices and poly(acrylic acid) (PAA) additive into the hybrid material.<sup>27</sup> Thin films of hydroxyapatite crystals have also been prepared on chitosan matrices in the presence of PAA.<sup>34</sup> These results indicate that the use of polymer templates is crucial for controlling the formation of hybrid materials. Although intensive work regarding functional hybrids has been conducted over the decades, the study of functional hybrid production via the use of a macromolecular template has only just begun. Detailed formation mechanisms and appropriate molecular designs are still unclear.

This paper provides a review of the recent advances in understanding biomineralization processes and the development of new organic/CaCO<sub>3</sub> hybrids through biomineralization-inspired approaches. Three topics are reviewed in this paper, as described below: (1) The isolation of biomineralization-related peptides from living organisms and the roles of these peptides in the CaCO<sub>3</sub> mineralization process are examined. The structure–function relationships of the peptides are discussed. (2) The spontaneous formation of macroscopically oriented crystals on oriented chitin matrices that were prepared via liquid crystalline assembly is presented. (3) CaCO<sub>3</sub> hybrids have also been developed using amorphous mineral precursors, resulting in the formation of two- or three-dimensional composites. In the context of the above topics, the roles of the



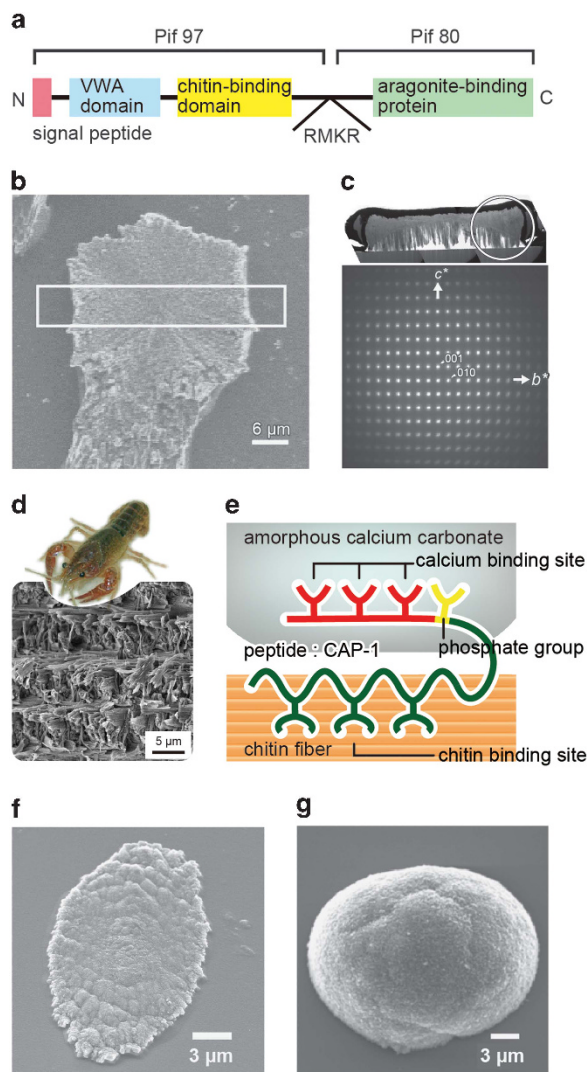
**Figure 1** Schematic illustration of the formation of thin-film  $\text{CaCO}_3$ .

macromolecular templates that affect the formation of novel polymer/ $\text{CaCO}_3$  hybrid materials prepared via approaches inspired by biomineralization are reviewed in this paper.

### STUDIES OF THE STRUCTURE–FUNCTION RELATIONSHIPS OF BIOMINERALIZATION-RELATED PEPTIDES

To date, the organic matrices of the nacre of seashells have attracted much attention because these biomacromolecules have a significant influence on the quality, including the color and luster, of pearls.<sup>35</sup> In 1960, Watabe and Wilbur<sup>36</sup> reported for the first time the effects of organic matrices on  $\text{CaCO}_3$  crystallization. The organic matrices used were extracted from the nacre of a seashell. These authors demonstrated the formation of aragonite in the presence of organic matrices *in vitro*; aragonite is one of the polymorphs of  $\text{CaCO}_3$  found in the nacre layer. Generally, it is difficult to obtain aragonite in test tubes without the use of additives. The results indicated that polymorph control was achieved by the molecular control provided by the organic matrices. However, the researchers were not able to isolate the peptides critical for this process from the crude organic matrices because of limitations in peptide analysis at that time. More recently, owing to advances in biotechnology, several principal peptides have been isolated from the nacre of seashells with reverse-phase high-pressure liquid chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the chemical structures of these peptides have been identified. Nacrein was the first protein isolated from a nacre layer by Miyamoto *et al.*,<sup>37</sup> and this protein functions as a carbonic anhydrase to produce bicarbonate ions. Nacrein also interacts with  $\text{CaCO}_3$  through its acidic amino-acid residues. Thereafter, many other proteins, including N14,<sup>38</sup> N16,<sup>39</sup> MSI60,<sup>40</sup> MSI7,<sup>41</sup> pearlins,<sup>42</sup> prismaticin-14,<sup>43</sup> perlucin<sup>44</sup> and lustrin A,<sup>45</sup> were successfully isolated from various mollusk shells. Some of the effects of these proteins on *in vitro* crystallization were also examined. It was found that these proteins interact with  $\text{CaCO}_3$  and have effects on the crystallization of  $\text{CaCO}_3$ . However, no aragonite nucleation was observed in the presence of these proteins *in vitro*. It was believed that major proteins might be essential for the formation of the nacre layer. Although these major proteins have been isolated, the formation mechanism is still unclear.

Recently, Suzuki *et al.*<sup>46</sup> isolated the matrix proteins Pif80 and Pif97 from the pearl oyster *Pinctada fucata*, as shown in Figure 2. They searched for aragonite-specific binding proteins using an aragonite-binding assay to identify the aragonite-inducing proteins. These investigators found that the novel protein Pif80, an 80 kDa protein, specifically interacts with geological aragonite crystal surfaces. Pif80 is an Asp-rich acidic protein that forms an *in vivo* complex through disulfide bonds with Pif97, an additional peptide that is posttranslationally cleaved from a precursor protein (Figure 2a). It was also found



**Figure 2** (a) Schematic illustration of the protein structure of the Pif complex. Boxes represent the signal peptide, the VWA domain and the peritrophin A-type chitin-binding domain. RMKR is a Kex2-like proteinase cleavage site. The right box is the aragonite-binding protein (Pif80). (b)  $\text{CaCO}_3$  crystal observed with scanning electron microscopy (SEM). (c) Electron diffraction pattern in a cross-section of the white circled area of the white box in (b). (d) SEM image of a crayfish exoskeleton. (e) Schematic illustration of CAP-1 in the exoskeleton structure. SEM images of  $\text{CaCO}_3$  formed on (f) chitin and (g) a glass substrate in the presence of CAP-1 ( $2.4 \times 10^{-3}$  wt%). Reproduced with permission from the reference.

that Pif80 has an aragonite-binding domain and Pif97 has chitin-binding domains, both of which are acidic, with pI (isoelectric point) values of 4.99 and 4.65, respectively. Suzuki *et al.*<sup>46</sup> also found that the Pif complex acts at the interface between the organic layer and the aragonite tablets via the complex's binding domains and regulates the formation of the nacre layers. The *in vitro* conditions of the crystallization of  $\text{CaCO}_3$  in the presence of the Pif complex were examined by using  $\text{CaCO}_3$  supersaturated solution<sup>21</sup> to understand how the Pif complex assists in this process. Notably, the Pif complex induces aragonite thin-film growth with the c axis aligned perpendicular to the surface of a chitin matrix, as observed for natural nacre (Figures 2b and c). These results confirmed that the Pif complex is essential for nacre formation. This study also revealed that the biomineralization-related proteins are able to control the process of biomineral

formation even when present in tiny amounts. Moreover, each step in the functional sequence, such as the  $\text{CaCO}_3$ - and chitin-binding steps, might be connected to one another to maximize the efficiency of the formation process.

The exoskeletons of crustaceans are one of the most extensively studied types of biominerals that are composed of  $\text{CaCO}_3$  and biomacromolecules.<sup>47–49</sup> Figure 2d shows the fractured surface of the exoskeleton of a crayfish. The hierarchical composite structure of the chitin fibrils arranged in a helical array along with amorphous  $\text{CaCO}_3$  (ACC) can be observed. Owing to their elaborate structures, exoskeletons have excellent mechanical properties, such as ductility and toughness, while also being lightweight. This structure is similar to that of fiber-reinforced plastics, and material scientists have focused on the formation process and mechanical properties of crustacean exoskeletons to inform the development of novel hybrid materials.<sup>50</sup> A significant feature of these exoskeletons is the interface between the chitin fibrils and  $\text{CaCO}_3$ . Although chitin fiber and  $\text{CaCO}_3$  are immiscible, they are closely associated with each other in the exoskeleton. Recently, it was found that several functional peptides

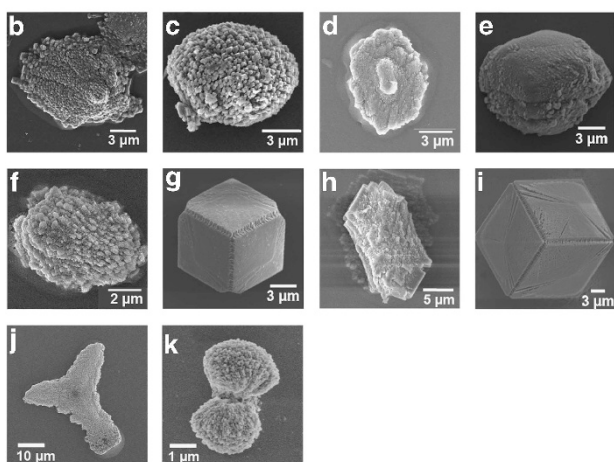
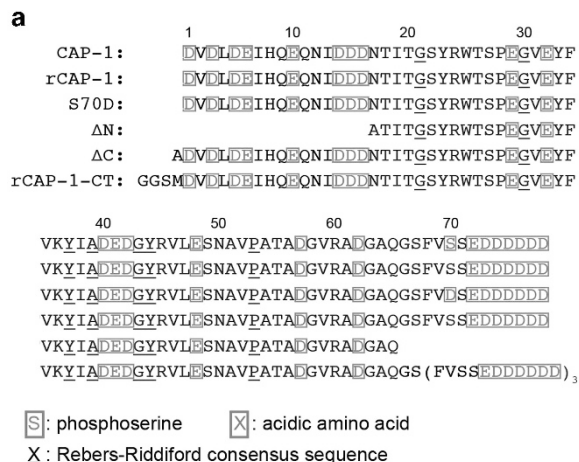
act at the interface of these two components, resulting in the fusion of the chitin framework with  $\text{CaCO}_3$  at the molecular level (Figure 2e). In 2000, the mineralization-related acidic peptide called CAP-1 was isolated from the crayfish exoskeleton by Inoue *et al.*<sup>51</sup> According to an assay of crystal growth inhibitory activity, CAP-1 is the most effective peptide for inducing  $\text{CaCO}_3$  crystallization within insoluble organic matrices in the cuticle. CAP-1 also has several functional domains similar to the Pif complex (Figure 2e). CAP-1 is composed of acidic peptides bearing an Asp-repeating fragment at the C terminus and a phosphoserine residue at position 70 that may interact with calcium ions and stabilize the amorphous precursors of  $\text{CaCO}_3$ . CAP-1 has a chitin-binding sequence in the central region of the peptide. These functional domains collaborate to concentrate  $\text{Ca}^{2+}$  ions at the chitin surface and to stabilize the amorphous precursors, resulting in the deposition of the amorphous precursors of  $\text{CaCO}_3$  onto the chitin fibrils. CAP-1 was also examined in a  $\text{CaCO}_3$  crystallization experiment by using  $\text{CaCO}_3$  supersaturated solution<sup>21</sup> to understand the structure–function relationships of the peptide. CAP-1 induces the formation of  $\text{CaCO}_3$  thin films on the chitin matrix with the *c* axis of the thin-film crystals unidirectionally aligned parallel to the surface (Figure 2f).<sup>31</sup> It is noteworthy that no oriented crystals are observed when a glass substrate is used (Figure 2g). The crystallographic orientation of the thin-film crystal depends on the specific interaction between the chitin-binding site of CAP-1 and the chitin fibrils in the matrix.

These biomineral-related proteins, such as Pif and CAP-1, exist at the biomacromolecule and mineral interface to bind these two components during the formation of an organism's hard tissues. These studies on the structure–function relationships of biomineralization-related proteins might illuminate useful information for the bio-inspired design of functional molecules that may be used for the development of new hybrid materials.

## MOLECULAR DESIGNS FOR THE FORMATION OF BIOMIMETIC HYBRID MATERIALS (I)

Understanding the roles of the biomacromolecules involved in biomineralization is a shortcut to developing novel functional hybrid materials through biomimetic approaches. To clarify the effects of each functional component of the crayfish peptides on the formation of biominerals, relevant peptides have been designed and synthesized (Figure 3a).<sup>31–33,49</sup> The types of  $\text{CaCO}_3$  obtained on chitin or glass substrates in the presence of the recombinant peptides as soluble additives are depicted in Figures 3b–k. All of the peptides influence the crystallization of  $\text{CaCO}_3$  because they are acidic peptides. We observed a significant difference in the morphology and orientation of the crystals, which suggests that these parameters may be dependent on the chemical structures of the recombinant peptides.

To investigate the control over morphology, the phosphorous group at the 70th position was substituted with OH and carboxylic acid in the recombinant peptides.<sup>31</sup> Significant changes were observed in the surface morphology of the  $\text{CaCO}_3$  formed on the glass substrates (Figure 3c). It was revealed that the functional group at position 70 greatly influences the crystallization of  $\text{CaCO}_3$ . Notably, the surface morphology of the crystals changed gradually according to the strength of the acidic groups present on the 70th residues (Figures 2f, g and 3b–e).<sup>32</sup>  $\Delta\text{N}$  and  $\Delta\text{C}$  are recombinant peptides that lack the 17 residues at the N or C terminus. A comparison between the crystals obtained with  $\Delta\text{N}$  and  $\Delta\text{C}$  shows that the Asp repeat at the C terminus also has significant effects on the morphology of  $\text{CaCO}_3$  (Figures 3e–i).<sup>32</sup> The deleted peptides were designed to possess the same number of acidic amino-acid residues. In the



**Figure 3** (a) Comparison of the sequences of CAP-1 and its related peptides. (b–k) Scanning electron microscopy (SEM) images of  $\text{CaCO}_3$  formed in the presence of various types of recombinant peptides. (b and c) rCAP-1, (d and e) rCAP-1-S70D, (f and g)  $\Delta\text{N}$ , (h and i)  $\Delta\text{C}$  and (j and k) rCAP-1-CT. Each image on the left side is  $\text{CaCO}_3$  formed on a chitin matrix, and the right image is  $\text{CaCO}_3$  formed on a glass substrate. Reproduced with permission from the reference. A full color version of this figure is available at *Polymer Journal* online.

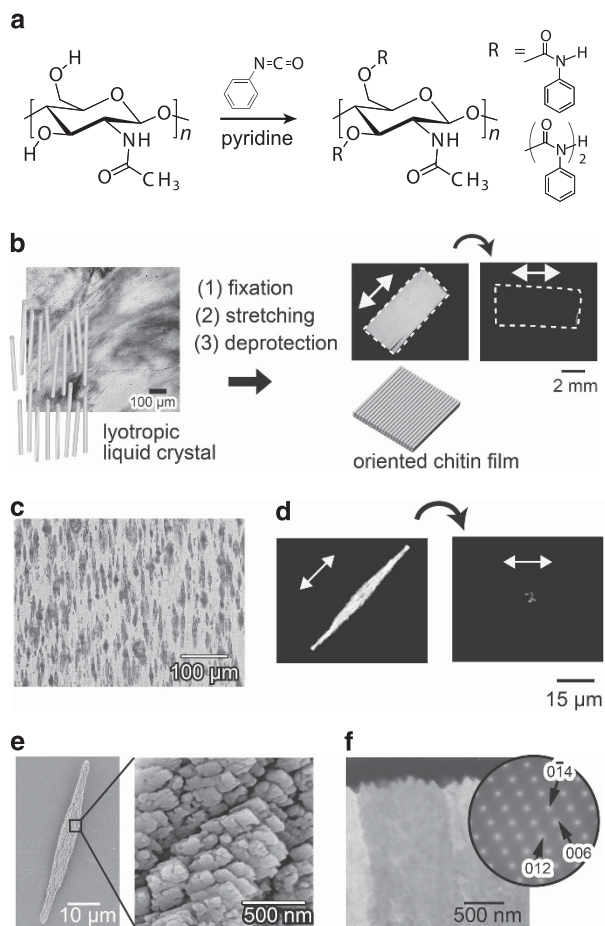
presence of  $\Delta C$ , the lack of a C terminus induced the formation of grains of  $\text{CaCO}_3$  on the chitin matrix, due to a decrease in the interaction with calcium ions. However, the effects of  $\Delta N$  on  $\text{CaCO}_3$  crystallization were similar to those of CAP-1, even though  $\Delta N$  lacked 17 residues. These results indicate that the C-terminal acidic region has a greater influence on  $\text{CaCO}_3$  crystallization than the N-terminal acidic region. When the acidic region at the C terminus was elongated, triangular  $\text{CaCO}_3$  thin films were obtained on the chitin matrix (Figures 3j and k).<sup>33</sup> The expanded acidic portion strongly stabilizes the amorphous precursors through polymer effects. The stabilization of the precursors subsequently reduces the crystal growth rate. The selective adsorption of the peptides onto specific faces of the  $\text{CaCO}_3$  through stereochemical recognition might lead to the formation of these plate-like tripodal crystals.

However, the crystallographic orientation of the thin films also strongly depends on the specific interaction between the chitin-binding sequence and the chitin fibrils in the matrix. All of the recombinant peptides, which have the chitin-binding sequence, induce the formation of unidirectionally oriented  $\text{CaCO}_3$  thin films on the chitin matrix identical to those obtained in the presence of natural

CAP-1. The stabilization of the amorphous precursors in the chitin matrix before nucleation might be a key process in the formation of macroscopically oriented crystals. The effects of the chitin-binding site on crystallization were investigated using a chitosan matrix.<sup>33</sup> Although chitosan is the deacetylated derivative of the chitin polymer, the binding strength of the recombinant peptides to chitosan is lower than that to chitin. When crystallization experiments were conducted by using a chitosan matrix in the presence of these recombinant peptides, round thin films were obtained instead of oriented thin films. The weak interactions between the peptides and the matrix suppressed the formation of oriented crystals. The lack of strong specific interactions changed these peptides from functional polymers to simple acidic polymers, such as PAA. Synthetic oligopeptides without the chitin-binding sequence also induce the formation of non-oriented small grains on a chitin matrix.<sup>31</sup> These results provide us with information regarding the molecular design of functional molecules for the development of organic/inorganic hybrids. The structures of the individual functional domains of the peptides are an important design feature for the formation of hybrid materials.

## MOLECULAR DESIGNS FOR THE FORMATION OF BIOMIMETIC HYBRID MATERIALS (II)

As mentioned earlier, the exoskeletons of crustaceans are good examples to explore to improve the design strategies of structural materials. The mechanical properties of the exoskeleton, such as ductility, toughness and a low weight, are directly related to its hierarchical structures. These elaborate structures and the mild conditions under which the structures form have been recognized as attractive strategies for the construction of biomimetic hybrid materials with hierarchical structures. It is worth studying the ordered structures of liquid crystalline materials as potential templates for inducing hierarchical structures in our target hybrid materials. Liquid crystals are a state of matter that combines the anisotropy of crystals and the fluidity of liquid.<sup>52,53</sup> Liquid crystals can easily form large domains of orientation on a macroscopic scale. Capitalizing on this characteristic, unidirectionally oriented chitin templates have been prepared with liquid crystalline precursors.<sup>54</sup> Although chitin is difficult to dissolve in any solvent because the chitin fibrils form aggregates through intermolecular hydrogen bonding, chitin phenylcarbamate, which has a protective group at the OH position, can be dissolved in polar solvents (Figure 4).<sup>55,56</sup> Moreover, chitin phenylcarbamate exhibits lyotropic nematic liquid crystallinity in concentrated solutions (somewhat more than 15 wt%) because of the rigidity and intermolecular interactions of this compound. These liquid crystals can be converted to the gel state when they are immersed in solvents in which they have poor solubility, such as methanol. After stretching and deprotection, a unidirectionally oriented chitin matrix was successfully obtained (Figure 4b). The oriented chitin film is composed of stretched chitin polymer chains that are oriented at a macroscopic scale. Calcium chloride aqueous solution containing PAA was used to carry out  $\text{CaCO}_3$  crystallization. The solution containing the matrices was placed in a closed desiccator together with ammonium carbonate. When the resultant gel matrices are used as template for the crystallization of  $\text{CaCO}_3$ , unidirectionally aligned rod-shaped crystals  $\sim 80 \mu\text{m}$  in size are formed within the oriented chitin matrix (Figures 4c and d). Scanning electron microscopy and tunneling electron microscopy observations of the rod-shaped crystals formed in the oriented chitin matrix (Figures 4e and f) reveal that the crystals are assemblies of submicron-size rhombohedral calcite crystals with a single crystalline orientation, which are known as mesocrystals.<sup>57</sup>



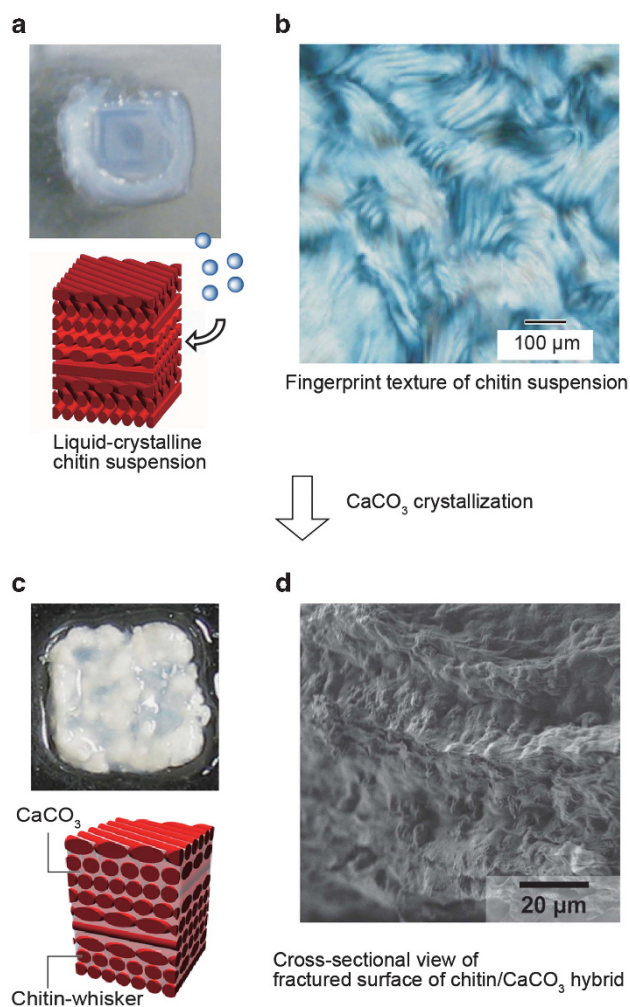
**Figure 4** Schematic illustration of the process for preparing macroscopically ordered  $\text{CaCO}_3$ /organic hybrids using oriented chitin templates. (a) Synthetic scheme of chitin phenylcarbamate. (b) Illustration of the preparation of oriented chitin templates. (c) Photograph of the obtained hybrids, (d) polarizing optical microscopic images of isolated crystals, (e) scanning electron microscopy (SEM) images and (f) tunneling electron microscopy (TEM) image of an oriented crystal. Reproduced with permission from the reference.

Another way of developing hybrid materials via the use of liquid crystalline templates has also been reported.<sup>58</sup> It is well known that polysaccharide nanowhiskers have lyotropic nematic and chiral nematic liquid crystallinity.<sup>59–61</sup> Because of the similarity between the hierarchical helical structure of crustacean exoskeletons and that of cholesteric liquid crystals, the liquid crystalline suspensions of polysaccharides were used as templates for the development of three-dimensional structural hybrid materials similar to the crustacean-derived biominerals. The lyotropic liquid crystalline chitin suspension was obtained by acid hydrolysis of  $\alpha$ -chitin, as reported by Revol and Marchessault.<sup>61</sup> After ultrasonication, the resulting chitin whiskers were dispersed in water with a desired pH value because of their electronic repulsion. At the appropriate concentration and pH, the whisker suspension displays a fingerprint texture, suggesting that the suspension forms a chiral nematic helical structure (cholesteric phase). The helical structure can be preserved in gel form and then serve as the template for  $\text{CaCO}_3$  crystallization (Figure 5). After the crystallization of  $\text{CaCO}_3$  in the presence of PAA, by using sublimation method, the  $\text{CaCO}_3$ /chitin hybrids are formed with a composition of 20 wt% adsorbed water molecules, 60 wt% organic polymer and

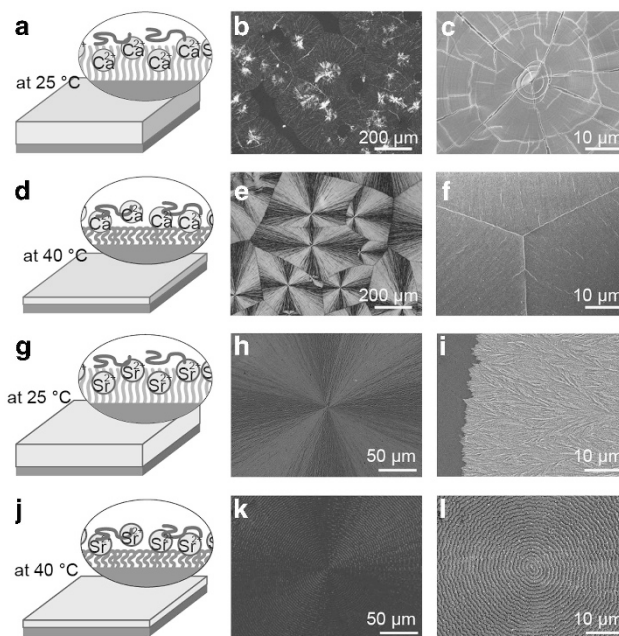
20 wt% inorganic crystal, as determined by thermogravimetric analysis.<sup>58</sup> The obtained hybrid materials have hierarchical ordered structures similar to that of the exoskeleton of crustaceans. These structures potentially enhance the mechanical as well as the optical properties of the resultant materials.<sup>62</sup>

The self-organized macroscopic orientation of liquid crystalline polymers is one of the advantages of using these polymers in the development of hybrid materials. Liquid crystal polymers that exhibit cholesteric, smectic and columnar phases could be applied as templates for new hybrid structures. This liquid crystalline methodology might be useful for the preparation of novel oriented hybrid materials based on a wide range of functional inorganic crystals.

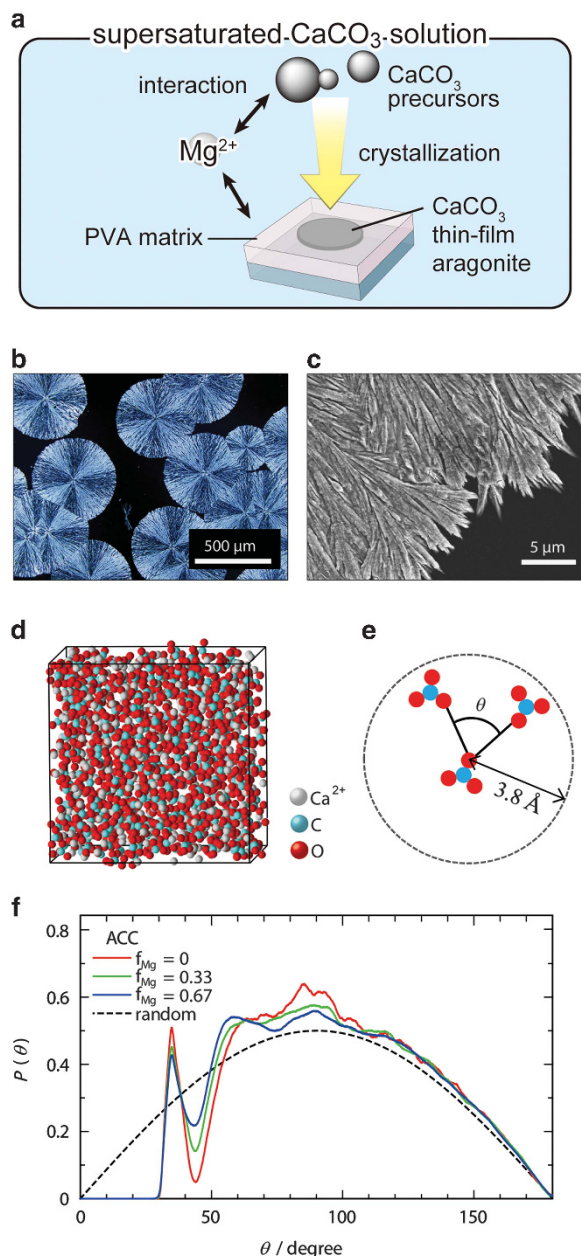
A polymer brush matrix is one of the organic template candidates that may be used in the development of novel hybrid materials with dynamic and stimuli-responsive properties. A polymer brush is a thin-film polymer that covalently attaches by one end of the polymer chain to the surface of a substrate.<sup>63</sup> Such polymer layers possess excellent stability over long time periods in various solvents at a wide range of temperatures. Because of recent advances in surface-initiated, controlled living radical polymerization techniques,<sup>64</sup> a more precise control of the thickness, composition, and density has been accomplished for polymer brushes compared with chemically grafted or physisorbed polymers. Moreover, polymer brushes are a particularly attractive approach in creating stimuli-responsive matrices. The formation of a responsive polymer involves the application of a water-soluble polymer coating that can be subsequently used as a platform for the nucleation of inorganic crystals.<sup>65</sup> When thermo-responsive PNIPAm (poly(*N*-isopropylacrylamide)) is used as a polymer brush template, for example, the formation of vaterite thin films is induced in the presence of PAA.<sup>65–67</sup> Furthermore, conformational changes in the PNIPAm brush matrix significantly influences the morphologies and preferential orientations of the resultant



**Figure 5** Schematic illustration of chitin/ $\text{CaCO}_3$  hybrids formed using a chitin suspension. (a and b) Photographs of lyotropic liquid crystalline chitin suspensions. (c) Photograph and (d) scanning electron microscopy (SEM) image of an obtained  $\text{CaCO}_3$ /chitin hybrid. Reproduced with permission from the reference.



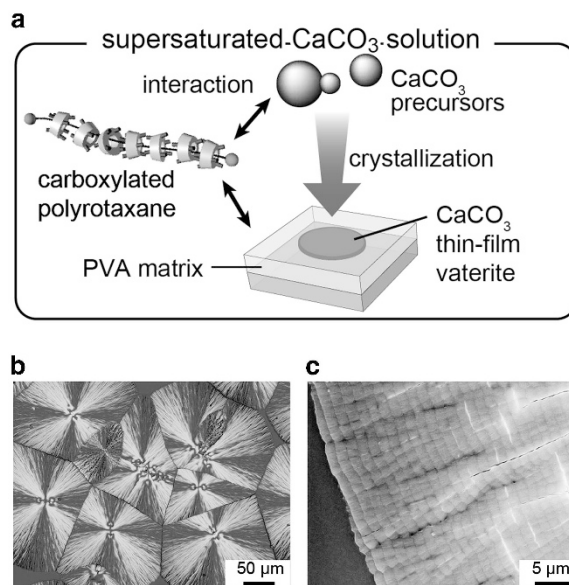
**Figure 6** (a–f) Illustration of  $\text{CaCO}_3$  thin films formed on a thermoresponsive polymer template. (b) Polarizing micrograph and (c) Scanning electron microscopy (SEM) image of  $\text{CaCO}_3$  thin films developed at 25 °C and (d–f) at 40 °C. (g–l) Illustration of  $\text{SrCO}_3$  thin films formed on a thermoresponsive polymer template. (g–i) at 25 °C and (j–l) at 40 °C. Reproduced with permission from the reference.



**Figure 7** (a) Formation of thin-film  $\text{CaCO}_3$  in the presence of  $\text{Mg}^{2+}$  ion. (b) Polarizing optical micrograph and (c) scanning electron microscopy (SEM) image of the  $\text{CaCO}_3$  thin films grown on PVA matrices in the presence of  $\text{Mg}^{2+}$  (5 mm). (d) Illustration of the  $\text{CaCO}_3$  simulation system. (e) The definition of  $\theta$  formed by the three nearest-neighbor O atoms of the  $\text{CO}_3^{2-}$  ions. (f)  $P(\theta)$  for ACC at each  $f_{\text{Mg}}$ .  $P(\theta) = 1/2 \sin \theta$  are also shown for the random distribution. Reproduced with permission from the reference.

vaterite/PNIPAm hybrid thin films (Figures 6a–f).  $\text{CaCO}_3$  thin films with the c axis preferentially perpendicular to the brush template are obtained below the lower critical solution temperature, whereas crystallization above the lower critical solution temperature induces the formation of vaterite thin films with the c axis parallel to the brush template. In addition, the surface morphology of  $\text{SrCO}_3$  thin films has also been tuned with a PNIPAm brush template (Figures 6g–l).<sup>67</sup>

The use of polymer brushes also contributes to the design of organic/inorganic functional hybrid-coating materials. These studies



**Figure 8** (a) Formation of thin-film  $\text{CaCO}_3$  via ACC precursors stabilized by supramolecules. (b) Polarizing optical microscopic image and (c) scanning electron microscopy (SEM) image of the obtained thin-film crystals formed on the PVA matrix in the presence of carboxylated polyrotaxane. Reproduced with permission from the reference.

demonstrate that polymer brush matrices are effective crystallization matrices for inorganic minerals. The use of a stimuli-responsive polymer brush matrix template is a promising method for realizing control over the morphology and orientation of hybrid materials.

#### CRYSTALLIZATION FROM ACC STATES AND THE FORMATION MECHANISM INVOLVED

It has been well recognized for a decade that ACC, the precursor form of crystalline  $\text{CaCO}_3$ , has an important role in the formation mechanisms of biominerals, sea urchin spicules,<sup>69</sup> crustacean exoskeletons<sup>70,71</sup> and the nacre of seashells.<sup>72</sup> Recently, material scientists have also demonstrated that the biomimetic synthesis of crystalline  $\text{CaCO}_3$  particles and thin films via stabilized ACC precursors is possible.<sup>28,62,73</sup> For example, Sommerdijk and co-workers<sup>74</sup> directly observed ACC particles before they crystallized on a Langmuir–Blodgett film template. In this section, the ACC precursor is described with a focus on its use in the development of hybrid materials. The formation mechanisms of the hybrid materials obtained with ACC precursors are also covered.

ACC is thermodynamically unstable and crystallizes rapidly in the presence of water. It is well known that acidic polymers containing carboxylic acids and phosphoric acids can stabilize ACC and suppress its crystallization. Recently, it was found that not only acidic polymers but also small molecules such as  $\text{Mg}^{2+}$  act as stabilizers for ACC.<sup>75</sup> Because  $\text{Mg}^{2+}$  is abundant in seawater<sup>76</sup> and is incorporated into the biominerals of marine organisms,<sup>77</sup> intensive research has already been reported concerning the role of  $\text{Mg}^{2+}$  in the biomineralization of  $\text{CaCO}_3$ . For example, in crustacean exoskeletons, the amorphous region was found to contain more  $\text{Mg}^{2+}$  than the crystalline region,<sup>78</sup> and the distribution of  $\text{Mg}^{2+}$  in the teeth of sea urchins is also regulated. Inspired by the role of  $\text{Mg}^{2+}$  in biomineralization,  $\text{Mg}^{2+}$  has been used as an additive in synthetic systems to control  $\text{CaCO}_3$  crystallization by decreasing the crystallization rate, stabilizing the

amorphous precursors and providing control over both morphology and polymorph formation.<sup>79</sup>

The role of  $Mg^{2+}$  in the crystallization of  $CaCO_3$  on organic templates was recently investigated to obtain useful information for the development of functional thin-film hybrid materials through sublimation method by using calcium chloride solution together with ammonium carbonate (Figure 7).<sup>29</sup> In the presence of  $Mg^{2+}$ , ACC precursors were stabilized in supersaturated  $CaCO_3$  solutions, and thin films of  $CaCO_3$  were formed on the annealed poly(vinyl alcohol) (PVA) matrices. The polymorph of the thin films was aragonite, as confirmed by infrared spectroscopy and X-ray diffraction measurements. It is of interest that the ionic additive alone (without additional polymer additives) resulted in thin films of  $CaCO_3$  on the PVA matrices. Both the growth rate and surface morphology of the aragonite thin films were dependent on the concentration of  $Mg^{2+}$  in the crystallization solution; in the absence of PVA matrices, no thin films were formed despite the presence of  $Mg^{2+}$ . To evaluate the effects of  $Mg^{2+}$  on the stabilization of ACC, a molecular dynamics simulation was conducted (Figure 7d).<sup>29,80</sup> When the  $Mg^{2+}$  content of the ACC solution increases, the peaks in the distribution functions, which indicate the local structure of the ACC (Figure 7e), become broad, and the complete distribution function approaches a random distribution function (Figure 7f). These results from the molecular dynamics simulation of the  $CaCO_3$  precursor suggest that the transition of ACC to a crystalline form is suppressed in the presence of  $Mg^{2+}$ . The possible mechanism of the formation of thin-film hybrid materials by  $Mg^{2+}$  can be explained as follows: In the crystallization solution, Mg ions stabilize the ACC precursor and suppress  $CaCO_3$  crystal nucleation. In contrast, the PVA matrix may promote  $CaCO_3$  crystallization by assisting aragonite nucleation via the interaction of the functional groups of the two species. When the amorphous precursor is attached to the surface of the PVA matrix, nucleation and crystal growth occur, and as a result, hybrid thin films are formed.<sup>29</sup>

Supramacromolecules<sup>81–83</sup> are also useful for developing hybrid materials.<sup>30</sup> To this end, acidic supramacromolecules have been designed and synthesized as additives for the stabilization of ACC precursors. Polyrotaxane structures composed of acidic cyclodextrin and polyethylene oxide were synthesized (Figure 8).<sup>84,85</sup> In the presence of carboxylated polyrotaxane, vaterite thin films were formed on PVA matrices (Figure 8).<sup>30</sup> The results suggest that the monomeric cyclodextrin units entrapped by polyrotaxane have a polymer-like effect on  $CaCO_3$  crystallization, despite the highly mobile characteristics of these cyclodextrin units in supramolecular structures. The ACC precursors, stabilized by the acidic supramacromolecules, are essential for the formation of thin-film hybrid materials. Thin films are not obtained when the individual carboxylated  $\alpha$ -cyclodextrin units are used as additives because of the lack of ACC stabilization. Previously, covalent polymers were used to develop the thin-film growth of  $CaCO_3$ . The use of supramacromolecules extends our understanding of the formation mechanisms of thin-film  $CaCO_3$ -based hybrid materials.<sup>86</sup>

## CONCLUSIONS

In this review, the roles of organic (macro)molecules as templates or additives have been discussed in the context of preparing new hybrid materials. Because of technological advancements in organic, polymer and materials sciences, we are able to control the structures and functions of organic materials. As a result, a variety of complex hybrid material structures have been achieved using designed macromolecular templates. However, the construction of hybrid materials using biomimetic methods inspired by natural materials still remains a

challenge. We should attempt to synthesize new materials that can overcome the limitations of biological materials and exhibit versatile characteristics such as semiconductivity<sup>87</sup> and special optical<sup>88</sup> and magnetic properties.<sup>89</sup> This might be accomplished through a collaboration among a wide variety of scientific fields, including organic chemistry, polymer chemistry, inorganic chemistry, physics, biology and engineering. Further understanding the process of biomineralization and the roles of biomineralization-relevant proteins will enable us to design macromolecular templates for functional inorganic materials, resulting in new functional hybrid materials.

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