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Aragonite formation in confinements: a step towards understanding polymorph control

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Calcium carbonate ($CaCO_3$) is one of the most common minerals on the earth, which not only forms rocks like limestone or marble, but is also a main component of biominerals such as pearls, the nacre of sea shells and sea urchin skeletons.(1) Despite many years of research, the polymorphism of $CaCO_3$ is still far from being understood. $CaCO_3$ has three anhydrous crystalline forms: calcite, aragonite and vaterite, with a decreasing thermodynamic stability under aqueous ambient conditions (calcite>aragonite>vaterite).(2) While vaterite is rare in Nature, calcite and aragonite are both frequently found in rocks or biominerals.(1) A well-known example is the aragonite structure of nacre,(3) where the organization of the crystals leads to extraordinary mechanical performances. However, in synthetic systems, crystallization experiments only generate a small fraction of aragonite compared to calcite at ambient conditions and in the absence of additives.(4) So how is the formation of aragonite facilitated in Nature, especially in biominerals? In their recent PNAS paper, Zeng et al. shed light on this matter by showing that aragonite formation is dramatically promoted within confinements.(5)

In recent decades, great efforts have been spent towards understanding the strategies exploited by organisms to regulate aragonite formation, and several key factors have been identified. Up to now, the effect of Mg^{2+} additive is most well-established. Mg^{2+} is abundant in seawater and is expected to be present during the formation of many marine biominerals.(6, 7) At high Mg^{2+}: Ca^{2+} ratios, aragonite forms as the major crystalline form instead of calcite at room temperature. This was recently explained by Sun et al. in PNAS,(8) who show that Mg^{2+} can significantly increase the surface energy of calcite and raise its nucleation barrier, while aragonite is much less affected. Meanwhile, insoluble organic matrices and soluble acidic macromolecules extracted from aragonite-forming tissues also favor aragonite formation to different extents.(9, 10) Detailed mechanisms of the effects remain unclear, but were generally attributed to the interaction between the acidic functional groups of the biomacromolecules and the mineral components. Additionally it was reported that a macromolecular hydrogel-like 3D network is formed prior to the mineralization of nacre,(11) which may play a role in the crystallization process by confining the crystallization to defined small volumes. Nonetheless, confinement has never been directly correlated with aragonite formation in biominerals, although its capability on controlling crystal orientation and polymorphism has been shown in many recent reports.(12-15)

Now, Zeng et al. explored for the first time the impact of confinement on aragonite formation.(5) By precipitating $CaCO_3$ within the cylindrical pores of track-etched membranes (Fig. 1), they investigated the relationship between pore size and the polymorphism of $CaCO_3$. Strikingly, a high level of aragonite formation was detected within these nanosized confinements. Using the same concentrations of Mg^{2+}

and SO_4^{2-} as additives, the aragonite proportion in bulk solution was only 7%, while this value increased to 69% in 200 nm sized nanopores, and reached 100% in 50 nm sized pores. When even smaller sized pores were used (25 nm), pure aragonite crystals were obtained even in the absence of any additives. More than that, the aragonite crystallized within these nanopores were mainly single crystal rods, highly oriented along the c-axis.

The authors subsequently examined several possible origins for the preferred formation of aragonite within these confinements. Confinement is known to increase the incubation time for crystallization nucleation, inhibiting the formation of thermodynamically stable phases (e.g., calcite) and in favor of metastable phases.(13) This is, however, unlikely the reason for aragonite formation here since aragonite is seldom seen as a precursor to calcite. Computations demonstrated that the reaction was also not affected by a variation of diffusion rates. Hence, the only reasonable cause for the promotion of aragonite formation seems to be the influence of the pore surface on crystal nucleation. Indeed, aragonite formation was further promoted when smaller sized pores were used and larger pore surfaces were generated. Zeng et al. suggest that the pore surface may modulate ion activity and thus facilitate aragonite formation. Unfortunately, experimental confirmation was hindered by the difficulty to directly measure the ionic profiles within the nanopores.

By showing that pure aragonite single crystals can be synthesized at ambient condition by only using nanosized confinements, the work of Zeng et al. provides a route for $CaCO_3$ polymorph control that may possibly also be applied by biomineralizing organisms. In particular, it points to the importance of surface effects in controlling aragonite formation. This also aligns with the computational work of Sun et al.,(8) showing that the presence of Mg^{2+} favors aragonite formation by modulating the surface energy of the crystals. Nevertheless, the details of the mechanism by which surface effects facilitate aragonite growth for now remain unknown. The surface interactions may modulate the $CaCO_3$ polymorph through tuning the distribution of ions, as proposed by the authors; alternatively, the effect may be due to a lower interfacial energy between the pore surface and aragonite, as compared to calcite. Another intriguing question is how the confinement promotes the formation of oriented single crystals, as was also reported for other mineral systems.(14-16) Clearly, future investigations of crystallization within porous membranes are likely to give us more important insights on the control of crystal orientation and polymorphism with possible relevance for both synthetic and biological systems.

Meanwhile, to what extent organisms indeed deploy nano-sized confinements to promote aragonite formation is a question that needs further discussion. One interesting system is nacre where lamellar

organic matrices were found between adjacent ~500 nm thick aragonite layers.(17) As this dimension is still a bit above the largest effective pore size (200 nm) reported by Zeng et al., it is likely that in addition to confinement also the interaction between the mineral and biomacromolecular matrix(9-11) plays a role in the preferred aragonite formation in nacre.

A similar fundamental question remains on how confinement is correlated to the selection of the aragonite polymorph. Zeng et al. show that the two main volume effects of confinement, i.e. inhibiting nucleation and limiting diffusion rate, are both irrelevant to the preferred aragonite formation, and the main role of confinement appears to be enhancing the surface effect.(5) Indeed, when track-etch membranes from different manufacturers were used, different $CaCO_3$ polymorphs form within the same sized pores.(15) This was attributed to possible differences in the density or conformation of the chemical species lining the membrane pores, as only minor differences of surface roughness and no differences in composition were detected for these membranes. Hence, maybe not only aragonite, but also other polymorphs of $CaCO_3$ can be selected by nano-sized confinements with the appropriate surface chemistry. This could certainly be a strategy employed in biomineralization, but could also potentially provide a new window for controlling polymorphism in the synthesis of crystalline materials. In conclusion, the results of Zeng et al. are of great significance for the understanding of polymorph control, which has seen some recent interesting advances,(18, 19) but is still in its infancy despite its importance, in particular in the preparation of pharmaceutics.

Author Contributions

Y. X and N. A. J. M. S co-wrote the paper.

Conflict of Interest

The author declares no conflict of interest.

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Figure Captions

Fig. 1. Scheme of $CaCO_3$ crystallization within the cylindrical nanopores of track-etched membranes. While crystals formed in the bulk solution is mainly calcite, aragonite single crystals oriented in c-axis are formed within the nanopores.