








# Learning lessons from nature – the future of biomimetics: general discussion

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DOI: 10.1039/d2fd90024f

**Fiona Meldrum** opened a general discussion of the paper by Fabio Nudelman: Do the crystals containing encapsulated cells form in bulk solution or only on the walls of the vessel?

**Fabio Nudelman** responded: It is hard to tell, we didn't check this. The cells will eventually sediment to the bottom of the crystallization vessel, so I would say that both are possible.

**Peter Vekilov** commented: I think I have an explanation of how one of the crystals overpowers all the other crystals that initially nucleate on the substrate. Owing to their displacements and misorientations with respect to the substrate, the nucleated crystals are strained, but to distinct degrees. The one which is the least strained has the lowest free energy and is exposed to highest degree of supersaturation. It grows the fastest and engulfs all the other crystals.

**Fabio Nudelman** replied: Yes, I agree.

**Ruel Cedeno** said: Does the surface charge of the cells play a role in the occlusion? Since the zeta potential is dependent on the pH, is the occlusion process sensitive to changes in pH as well?

**Fabio Nudelman** replied: The pH of the crystallization solution is basic, as we are mixing  $\text{CaCl}_2$  with sodium bicarbonate. We didn't try to vary the pH, so it is hard to tell how it will affect the occlusion. Regarding the cells, they normally have a negative surface charge because the coccoliths are coated by acidic, negatively charged polysaccharides. We did zeta-potential measurements that show that as polyallylamine hydrochloride is added, the surface charge of the cells increases, becoming positive. I assume that this is quite important for the occlusion.

**Ran Zhao** enquired: Are the 'Ehux' algae cells still alive and can they function well, *e.g.* to further produce coccoliths, once they are entrapped? If no, what do you think is the advantage to occluded cells compared to synthetic calcite spheres? Do you think it is possible to obtain other polymorphs or crystal structures if you regulate the growth medium or occlude vaterite/aragonite capsules?

**Fabio Nudelman** answered: The cells were initially transferred from their growth medium, which is sea water, to a solution containing 10 mM calcium chloride and sodium bicarbonate. They will not survive for long under these conditions. Even if they are still alive when entrapped into calcite, they will eventually die as they cannot exchange gas and nutrients with the environment. Using the cells was a proof-of-principle for the encapsulation of such micron-sized structures into calcite, and using them as carriers. Once we achieved that, we translated the system to a synthetic ones where the cells were replaced by calcitic hollow spheres. In terms of applications, it is better to use the hollow spheres than the cells to avoid, for example, the presence of the biological material, and the need to grow and maintain them.

What we observed regarding other polymorphs was that in the absence of cells, vaterite was obtained, but this was not occluded into calcite.

**Christian Kuttner** asked: Could you please briefly elaborate on the mechanism of void formation for the hollow calcite spheres? Is there anything special about the final surface chemistry inside or outside of the spheres that is essential for a successful occlusion?

**Fabio Nudelman** responded: We didn't look in detail at voids that appear in the calcite crystals containing the hollow spheres so I cannot comment on that. The only way we could get the spheres occluded into calcite was by using the positively charged polymer PAH as an additive in the crystallization medium, just like we did for the occlusion of the coccolithophores. There is a sweet spot there in terms of the optimal polymer concentration – too little and nothing happens, too much and it starts interfering with the calcite precipitation by producing those polymer-induced liquid precursor phases. The polymer does change the surface charge of the guest particles (whether the cells or the hollow spheres), making them positively charged. I believe that this is important for the occlusion. The surface chemistry inside the hollow spheres, on the other hand, shouldn't play any role in the occlusion as their inner surface does not interact with the growing calcite crystals.

**Lucia Maini** queried: In Fig. 2 of the paper (<https://doi.org/10.1039/D1FD00095K>), the crystal of calcite seems to cover the coccospheres, while in Fig. 8 of the paper, when the crystallization happens on the hollow calcite spheres, it looks like that there is the imprint of the pristine hollow sphere. Does it mean that the synthetic hollow calcite sphere was recrystallized into the main big crystal? Why doesn't this happen with the coccospheres?

**Kevin Roberts** said: Maybe the crystal is re-nucleating around the edges of the obstruction and then encompassing the "obstacle". If the secondary nucleation

process associated with this was epitaxial, then the crystallinity would, in principle, be maintained. This effect was demonstrated by the X-ray tomographic characterisation of agglomeration in hexamine, a high symmetry organic material with a cubic crystal structure.<sup>1</sup>

1 P. Gajjar, T. T. H. Nguyen, J. Sun, I. D. Styliari, H. Bale, S. McDonald, T. L. Burnett, B. Tordoff, E. Lauridson, R. B. Hammond, D. Murnane, P. J. Withers and K. Roberts, Crystallographic tomography and molecular modelling of structured organic polycrystalline powders, *CrystEngComm*, 2021, 23, 2520–2531.

**Fabio Nudelman** responded: It is difficult to tell what is really going on. From the images in the paper (<https://doi.org/10.1039/d1fd00095k>), it is clear that there is Ostwald ripening involved, but we cannot tell the mechanism. Most crystals are clearly dissolving, as we start with the nucleation of several calcite crystals ions on the surface of the coccoliths, and after several hours there is only one left that is growing and encompassing the cells. It is possible that there is secondary nucleation, as you are suggesting, but this needs to be checked.

**Ran Zhao** asked: If we think of the calcium carbonate protection layers in nature like egg shells or, as shown in the coccospheres, they usually work as intact units but also have porosities at the surfaces which allow for nutrition exchange, can you give an outlook on how we can proceed to achieve this in the field of fabricating biomineral-based delivery vehicles?

**Fabio Nudelman** replied: Reproducing calcium carbonate structures with morphologies that are similar to those found in coccoliths, or in other biominerals, is still very challenging. To do that, one needs to look at the templating mechanisms that control mineral formation in biological systems, and there are many factors in place. In terms of biomineral-based delivery vesicles, we show that this is possible, not just with coccoliths but also by producing hollow spheres of polycrystalline calcite. The challenge with those hollow spheres is that on their own, they will eventually leak the encapsulated materials. Hence, occluding them inside single crystals of calcite was necessary.

**Peter Vekilov** opened the discussion of the paper by Takeshi Fukuma: You interpret your results as evidence that dissolution rate is distinct from the growth rate. Asymmetry between growth and dissolution may happen on a macroscopic scale, but not on the molecular scale, where the growth–dissolution symmetry represents a manifestation of the principle of microscopic reversibility, a fundamental tenet of chemical kinetics.

**Takeshi Fukuma** answered: In the present paper (<https://doi.org/10.1039/d1fd00084e>), I compared the dissolution and growth from several aspects. As for the step dissolution and growth rates, we reported that they differ and depend on the step types (such as acute and obtuse steps). However, this is not new and has been previously reported.<sup>1,2</sup> I think such a difference in the step flow rates does not contradict the reversibility of the chemical reaction as the solution conditions are significantly different between these two situations. Namely, dissolution experiments are mostly performed in nearly pure water,

while the growth experiments are performed in a supersaturated calcium carbonate solution containing various ionic contents.

We also reported that non-linear steps are observed only in the growth process. This difference probably comes from the different step generation mechanisms. In the growth process, steps are generated from screw dislocations, around which steps are not necessarily linear and propagate in various directions. Meanwhile, the dissolution process is triggered by the formation of a rectangular pit, and the surrounding four linear steps linearly propagate in the four specific directions. Again, this does not contradict the reversibility of the chemical reaction at the step edges.

Lastly, we also discussed a few different chemical reaction pathways in the growth process, while we assumed only one reaction pathway for the dissolution. However, this is not due to the asymmetry of the chemical reaction but reflects the variations in the solution conditions. As for the dissolution, we assumed only one solution condition – pure water. For the growth process, we considered different concentrations of  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_2$  in the solution. Therefore, the multiple pathways reflect the multiple solution conditions and do not imply the asymmetry of the chemical reaction processes.

1 R. Shiraki *et al.*, *Aquat. Geochem.*, 2000, **6**, 87.

2 H. H. Teng *et al.*, *Geochim. Cosmochim. Acta*, 2000, **64**, 2255.

**Wenhao Sun** said: Dear Professor Fukuma, thank you for the wonderful videos and observations. I have a question on your last slide; since you have developed a microkinetic model regarding the transition region in calcite dissolution and growth, it appears that your equations have a dependence on the bicarbonate concentrations. I wonder if you have thought about how pH would change the kinetics of step-edge growth, based on how it influences the width and growth speed of the transition region? I also want to add that if you do an experiment that investigates this pH dependence, it would be very interesting to decouple the thermodynamics aspects (since pH also affects the supersaturation of  $\text{CaCO}_3$ ) from the kinetics of the step-edge growth velocity.

**Takeshi Fukuma** answered: We agree that pH is one of the major factors that may influence the step-growth process. Although we have not performed a systematic study with variable pH or temperature yet, that is one of the studies we would like to perform in the near future.

As for the TR width, we may decouple the contributions from the thermodynamics and kinetics aspects. Our previous MD simulation results showed that an extended hydration layer is formed above the upper terrace and TR. Thus, our current hypothesis is that the TR is formed due to this energetic merit to form the extended hydrogen-bonding network. However, the influence of the upper terrace on the hydration structure should decay with the distance from the upper terrace edge. Therefore, at a certain distance, the energetic merit to form the hydration layer should balance the demerit to constrain the thermal motion of the  $\text{Ca}^{2+}$  and  $\text{OH}^-$  ions under the hydration layer. This balance should determine the TR width. If the TR width does not show any dependence on pH, the TR width mostly reflects the thermodynamic balance. In contrast, if it depends on pH, it may also

reflect the competition between the reaction speeds at the TR edge and upper terrace edge. These reaction rates also determine the overall step flow rate.

In addition to pH, we should also consider the contributions from the other ionic species, such as  $\text{CO}_3^{2-}$ ,  $\text{HCO}_3^-$ , and  $\text{CO}_2$ . As discussed in the paper (<https://doi.org/10.1039/d1fd00084e>), the ratio of these species could alter the reaction pathway. Therefore, these ionic content ratios could heavily affect both the kinetics and thermodynamics. Due to the large parameter space, it would be reasonable to focus on pH as a first step.

**Matthew Bennett** asked: Have you tried varying the flow rate of  $\text{CO}_2$ ? Did you see any changes in reaction rates?

**Takeshi Fukuma** responded: No, we have not. In this experiment, we performed all the experiments with fixed conditions. Although we did not control the  $\text{CO}_2$  concentration in the solution or air, we estimated it to be 60  $\mu\text{M}$  from the measured pH value. As the next step, we would like to investigate the dependence of the step edge behavior on the pH or  $\text{CO}_3^{2-}/\text{Ca}^{2+}$  ratio. This is because the strong dependence of the reaction rate on these parameters was previously reported.

**Christian Kuttner** enquired: What is the difference in the hydration structure between the acute and obtuse steps and how does this mechanistically translate into differences in the transition region (TR) width?

**Takeshi Fukuma** replied: Strictly speaking, the hydration structures of the acute and obtuse steps are different. According to the previous MD simulation study, the work needed to remove a dissolution unit from the acute step is larger than that from the obtuse step.<sup>1</sup> Such difference should be partially influenced by the difference in the hydration structure. One clear difference between the acute and obtuse steps that we observed in our experiment was in the TR formation probability in the dissolution process. Our previous MD simulation suggests that the energetic merit to form a TR comes from the formation of an extended hydration layer over the upper terrace and TR. Thus, the difference in the hydration structures between the acute and obtuse steps may lead to the difference in the energetic barrier to form an extended hydration layer and, eventually, in the TR formation probability. However, as the TR width does not show a significant dependence on the step type, the stability of the extended hydration layer should not be very different. Of course, these arguments should be verified by MD simulation with a model taking into account the formation of a TR.

1 D. Spagnoli *et al.*, *J. Cryst. Growth*, 2006, **294**, 103.

**Michael Anderson** commented: The transition region is always the same separation from the main terrace. This means that a  $\text{Ca}(\text{OH})_2$  layer is growing at precisely the same rate as the carbonate terrace, which seems strange unless they are somehow coordinated. Can you think of a mechanism that would keep these two growth fronts in registry?

**Takeshi Fukuma** answered: Strictly speaking, the width of the transition region (TR) is not necessarily constant. If we closely look at the images, there are some variations even for the linear steps. For the non-linear steps observed in the growth process, this variation is very evident. These results suggest that the observed structures are not caused by the double tip artifact in the AFM observation.

However, as you pointed out, the TR width is surprisingly uniform for the linear steps observed in the growth and dissolution processes. According to our MD simulation results, an extended hydration layer is formed above the upper terrace and TR. Thus, our current hypothesis is that the TR is formed due to this energetic merit to form the extended hydrogen bonding network. However, the influence of the upper terrace on the hydration structure should decay with the distance from the upper terrace edge. Therefore, at a certain distance, the energetic merit to form the hydration layer should balance with the demerit to constrain the thermal motion of the  $\text{Ca}^{2+}$  and  $\text{OH}^-$  ions under the hydration layer. This balance should depend on the local solution condition, temperature, and interaction with the underlying crystal lattice.

The observed constant TR width suggests that these conditions are uniform around the observed step edges. In contrast, at the edge of the non-linear step edges observed in the growth process, the TR width was not uniform and exhibited dynamic changes. This suggests that the aforementioned conditions were not uniform around these step edges. This sounds reasonable, as the growth direction of the non-linear steps was not constant but dynamically changing. This should at least lead to the variation of the interaction with the underlying crystal lattice.

**Ivo B. Rietveld** commented: The results obtained by AFM are very impressive and the molecular layers of the crystal can clearly be seen. Is it also possible to see the hydration layer on the surface with the method? Moreover, is it possible to see density fluctuations as a result of an increase or decrease in the solute concentration near the surface?

**Takeshi Fukuma** responded: We recently expanded the capability of our HS-FM-AFM from 2D to 3D and succeeded in observing the 3D hydration structure around a dissolving calcite step edge. The result clearly demonstrates that a hydration layer on the upper terrace is indeed almost seamlessly extended over the TR. Unfortunately, our AFM is not sensitive enough to detect the density fluctuations caused by the inhomogeneity of the solute concentration. However, we sometimes see the contrast fluctuations in the 2D-AFM images, which appear to be related to the ion diffusion on the surface.

**Sten O. Nilsson Lill** remarked: Would it be possible to use isotopically substituted  $\text{HCO}_3^-$  to get an understanding of the competing pathways *via* isotope effects on the kinetics and rate-determining steps? Some other technique than AFM could be useful for this.

**Takeshi Fukuma** replied: Unfortunately, AFM itself is insensitive to isotopic difference. Thus, we plan to vary the ionic contents of  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$ , and  $\text{Ca}^{2+}$  to investigate the competitive pathways. However, if the isotopic difference alters the

kinetics, we may see such effects through the altered dynamic behavior around the dissolving or growing step edges. Combination with other surface analysis techniques sensitive to the isotopic difference may be useful. However, the precise control of the isotopic labeling may be a great challenge as all the carbon compounds are subject to equilibrium reactions.

**Jan Sefcik** asked: Would it be useful to think of the transition layer observed at the surface of a growing crystal as non-classical surface nucleation and growth mechanism?

**Takeshi Fukuma** answered: Yes, I think so. The growth mechanism should involve at least two steps: reactions at the edges of the transition region (TR) and the upper terrace. In addition, the nearly constant width of the TR suggests the interaction between the two steps through the hydration layer above the TR. Such interaction should also be taken into account. Therefore, I think that such a mathematical modeling to describe the calcite growth involving the TR formation is strongly demanded.

**Ian Ford** asked: What might be the expected scale of fluctuations in the edge position under these conditions? Is it possible that the observations might be consistent with a smeared image of an edge that is fluctuating back and forth?

**Takeshi Fukuma** replied: Under our experimental conditions, we rarely see switching between the growth and dissolution processes. In the dissolution experiments in pure water, we only see the retreating steps, while in the growth experiments in a supersaturated solution, we only see the growing steps. Only in very limited cases was such switching observed. Even in these cases, the TR edges moving back and forth were clearly visible in the AFM images. Thus, it is very unlikely that the observed TR corresponds to the time-averaged image of the fluctuating step edges. Strictly speaking, at the TR edges, there must be atomic-scale fluctuations corresponding to the adsorption or desorption of the growth or dissolution units, respectively. Although our present AFM is not fast enough to capture such events, the clear atomic-scale step edge structures visualized by our AFM suggest that the scale of such fluctuations should be on the order of the atomic scale.

## Conflicts of interest

There are no conflicts to declare.