Unraveling the $CaCO_3$/PSS Mesocrystal Formation Mechanism by *in situ* TEM and *in situ* AFM

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The interplay between mineral and organic species is central to the control of microbial systems over biomineral formation. Deciphering the underlying mechanisms requires *in vitro* studies in which the physical and chemical parameters can be well controlled.

Using negatively charged polyelectrolyte polystyrene sulfonate (PSS) as aproxy for a bio-organic mediator, we investigate particle-mediated crystallization of $CaCO_3$, one of the most abundant materials for biomineralization. To induce growth, we use a calcium source in combination with the ammonium carbonate diffusion method. Utilizing similar conditions, Wang *et al*. showed that this approach yielded a family of well-defined calcite-PSS mesocrystals – i.e. 3D regular but porous scaffolds composed of easily distinguished and almost perfectly aligned nanocrystals [1]. Although mesocrystal formation was suggested to proceed via assembly of an amorphous precursor, direct proof of this mechanism is lacking. Here we use *in situ* TEM and *in situ* AFM to follow the formation process. For *in situ* TEM a custom designed fluid cell was utilized, containing two Si/Si_3N_4 wafers with electron transparent Si_3N_4 membranes.

We find that the observed mesocrystal morphology can be obtained merely through $CaCO_3$ overgrowth in PSS solution on a single crystal seed, and *in situ* AFM demonstrates that the development of this morphology is due to polymer poisoning of atomic steps during classical layer-by-layer calcite growth. However, both *in situ* TEM and AFM reveal a complex pathway during the initial stages of formation. Ca- PSS particles first adsorb onto the substrate. After about 2h of reaction with ammonium carbonate in the TEM fluid cell, these then appear to transform into larger amorphous aggregates. We are now attempting to detail the structural evolution of these initial particles and draw inferences about their formation mechanism and role in crystallization.

[1] Wang *et al*. (2005), *J. Am. Chem. Soc*. **127**, 3246-3247

The Discovery and Role of Non-Stoichometric Complexes of Calcium Carbonate in the Solution Precipitation of Vaterite

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Calcium carbonate (CaC) is the most abundant biogenic mineral, found in marine organisms and their fossilized deposits. Its crystallization mechanism is heavily debated since recent studies proposed a non-classical crystallization pathway via pre-nucleation clusters [1]. In the present study, we used cryo-TEM in combination with a titration set-up and demonstrated the existence of subnanometer-sized complexes (~0.4-0.6 nm) as prevalent pre-nucleation species in CaC mineralization, even in under-saturated regimes, which persisted after nucleation.

We show that these pre-nucleation species contain only one calcium in the equilibrium structure, which makes them complexes rather than clusters, similar as what was recently shown for calcium phosphate [2]. From a detailed analysis of the titration data we calculated that the average CaC-pre-nucleation complex chemistry represents a Ca/C ratio <<1, contains at least 50 mol% of bicarbonate and has an overall negative charge. This observation is in contrast to the previously proposed neutral $CaCO_3$ clusters that have been reported to exist under similar conditions [1]. We used *ab-initio* calculations to identify the structure of these complexes.

Furthermore, a dense liquid phase [3] of sub-micron sized assemblies of complexes exist already before the nucleation event. We propose a nucleation mechanism in which a calcium carbonate nucleus forms within these assemblies through a transformation of the complexes, after which the bulk phase (vaterite) propagates by ion growth.

[1] Gebauer *et al*. (2008), *Science* **322**, 1819-1822. [2] Habraken *et al*. (2013), *Nature Communnications* **4**, 1507. [3] Bewernitz *et al*. (2012), *Faraday Discussions* **159**, 291-312

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