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# Formation of Amorphous Calcium Carbonate and the Effect of pH, $Mg^{2+}$ and Amino Acids Revealed by *in situ* SAXS

**LUCAS KUHRTS**

Max Planck Institute of Colloids and Interfaces

The assembly of amorphous calcium carbonate (ACC) nanoparticles (NPs) is a widespread mineralization pathway in biomineral formation, utilized by numerous organisms such as corals, sea urchins, and mollusks. A narrow and well-controlled nanoparticle size distribution is crucial for the formation of the intermediate colloidal crystal and, ultimately, for producing defect-free biominerals.

Using in situ small-angle X-ray scattering combined with a stopped-flow device, we monitor the formation of synthetic ACC NPs with 10 ms time resolution while systematically varying pH, $Mg^{2+}$ concentration, and amino acid content. We find that exceptionally narrow size distributions emerge when ACC forms near the spinodal boundary of liquid–liquid phase separation (LLPS). Within the binodal regime, even a slight increase in pH accelerates nucleation kinetics, promoting the formation of highly monodisperse ACC NPs and reducing the size distribution width by a factor of two for a pH change of only 0.1 units. Vice versa, even a slight increase in pH can dramatically widen the ACC size distribution, hampering their use in the successful formation of biominerals and foreshadowing possible effects of ocean acidification.

Although $Mg^{2+}$ is widely recognized for stabilizing ACC, its influence on nanoparticle size and dispersity through shifts in LLPS boundaries has not previously been addressed. [1] In addition, close to the LLPS binodal line—where ACC formation proceeds more slowly—amino acids significantly narrow the particle size distribution. Temperature-dependent kinetic analysis shows that this effect is kinetic rather than thermodynamic in origin: adsorption of amino acids at the precursor–water interface selectively retards the growth of older particles, thereby enhancing size uniformity. [2]

Together, these results establish a combined kinetic and thermodynamic framework for ACC NP formation and provide insight into how ocean acidification may affect, and how organisms may control, ACC-based biomineralization.

[1] Kuhrts, L. PNAS, 122 (19), 2025, 1–9.

[2] Kuhrts, L. Adv. Funct. Mater., 2025, 20547.