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Cyanobacterial Control of Carbonate Mineralization under Variable Geochemical Conditions Revealed by High-throughput Correlative Imaging and Raman Spectromicroscopy

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Minerals formed by microorganisms frequently exhibit properties that distinguish them from abiotic counterparts, including complex morphologies, metastable structures, and close organic association. These characteristics determine the reactivity, stability, and ecological functions of microbial biominerals in the environment, thereby regulating elemental cycling and biogeochemical processes. Microbial biominerals may also serve as potential biosignatures, preserving evidence of biological activity in ancient terrestrial and planetary environments. Despite their importance, the biomolecular and physicochemical controls of microbial biomineralization remain mechanistically understood in only a limited number of systems.

Because biomineral formation emerges from the complex interplay between microbial metabolism, extracellular polymeric substances (EPS), and surrounding geochemical conditions, disentangling biological and environmental controls requires systematic exploration across multidimensional geochemical space. Conventional low-throughput approaches typically examine only a restricted subset of variables and often rely on sample preparation steps that may induce mineralogical or morphological artefacts. Resolving mineral–organic–microbe interactions therefore requires in situ, non-destructive techniques capable of probing mineral growth directly in aqueous, physiologically relevant environments and at sufficiently large experimental scale. To enable this, we developed a high-throughput, in situ correlative imaging–Raman workflow to map carbonate phase and growth dynamics across varied geochemical conditions.

Using this approach, we investigated carbonate mineralization in the presence of two cyanobacterial strains (*Synechococcus* sp. PCC 11901 and *Cyanobacterium aponinum* UTEX 3222) under varied temperature, CO_2 concentrations, Ca^{2+} availability, Mg/Ca ratios, and bicarbonate concentrations, alongside abiotic controls. Experiments were conducted in multi-well format and analysed using automated bright-field microscopy, correlative fluorescence imaging leveraging natural cyanobacterial autofluorescence, and targeted Raman spectromicroscopy.

Preliminary results show that under conditions of enhanced mineralization, calcite is the dominant precipitated phase and preferentially nucleates in spatial association with dense cyanobacterial clusters, indicating microbial modification of local precipitation microenvironments. Compared to abiotic controls, cyanobacteria-mediated precipitates display pronounced anisotropic growth and complex aggregation textures. Time-lapse correlative bright-field and fluorescence imaging further reveals a directional spatial coupling between increasing autofluorescence intensity and particle growth fronts, consistent with localized microbial control of calcite mineralization.