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High-throughput Characterization of $CaCO_3$ Mineralization in Genetically Engineered Organisms

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Biologically sourced composite materials such as bone and nacre are a class of hybrid biomaterials that have unique materials properties due to a hierarchical structure of organic-inorganic constituents. One of the most remarkable traits of the organisms that produce these biomaterials is their ability to synthesize complex hierarchical structures with a high degree of control over the crystal morphology and composition over multiple length scales [1]. Recently, there has been more focus on characterizing the structures of biominerals and organic-inorganic composites found in various organisms [2–5]. Although the structures and compositions of these natural biomaterials are well characterized, there are significant gaps of knowledge in their synthesis processes, especially genetically controlled transport processes and the chemical and structural identities of intermediate phases. The ability to characterize inorganic structures in biomaterials fabricated by genetically engineered organisms is critical for the understanding of biomineralization pathways, elucidating cellular functions, and designing composite bioinspired materials with new functions.

The synthesis of biomaterials using engineered bacteria such as *E. coli* has attracted considerable interest, as these bacteria can precipitate calcium carbonate ($CaCO_3$) and other biogenic crystals at near room temperature and pressure. Recently, *E. coli* strains engineered with the urease gene cluster from the soil bacteria *S. pasteurii* have been shown to precipitate large quantities of $CaCO_3$ and produce brick-like products when combined with sand [6]. By introducing biomolecules such as peptides and proteins during crystal synthesis, further control of the polymorphic nature and morphology of the biomaterials can be achieved.

Here we present transmission electron microscopy (TEM), high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM), and electron diffraction of $CaCO_3$ crystals grown by a *E. coli* strain engineered with a urease gene cluster. We identified that the engineered *E. coli* strain produced vaterite, which is a metastable polymorph of $CaCO_3$ that is a precursor to calcite, and the addition of bovine serum albumin (BSA) protein to the same bacteria strain produced hollow vaterite microspheres (Figure 1). In order to better understand the 3D inorganic structures in the microspheres and how they evolve, we have performed HAADF-STEM tomography to visualize the outer and inner surfaces of the microspheres. The 3D reconstruction of selected hollow vaterite microspheres reveals that these large porous spheres mostly consist of small nanoparticles with a calcite crystal plate nucleating at the outer surface.

Furthermore, we also show that automated high-throughput HAADF imaging and data analysis can efficiently acquire substantial data on the 2D morphology of these $CaCO_3$ microspheres. By acquiring many images of these spheres, we determined that the majority of them have a radius between 700 nm to 1.2 μm and the eccentricity typically range from 0.4 to 0.6 (Figure 2). Additionally, large scale imaging reveals that the hollow centers have varying sizes and remarkably, some spheres do not have hollow centers. With an AI/ML approach, we can acquire large volumes of imaging data and quantitatively assess the sizes and shapes of the crystals synthesized using many different growth conditions such as time, temperature, peptides, and bacteria strains. By rapidly investigating the $CaCO_3$ mineralization in genetically engineered bacteria grown in varying conditions, we can infer the crystal growth dynamics and understand how to precisely control crystal morphology using synthetic biology.

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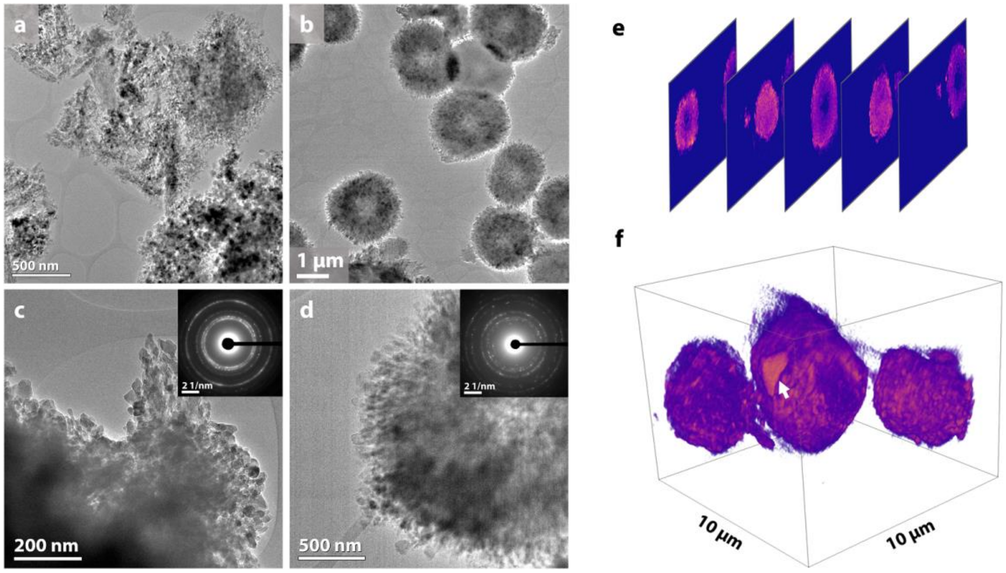

Figure 1. Figure 1. TEM images of CaCO3 crystals produced by (a) engineered E. coli and (b) engineered E. coli with BSA. (c) As confirmed by electron diffraction patterns (inset), crystals produced by the engineered E. coli strains typically form clusters of small vaterite crystallites, while (d) the same strain with BSA produced vaterite crystallites arranged in the structure of a hollow sphere. (e) Image slices from the 3D tomography reconstruction show the hollow centers of the microspheres. (f) The 3D reconstruction of the microspheres reveals that they mostly consist of small nanoparticles with a calcite crystal plate (white arrow) at the outer surface.

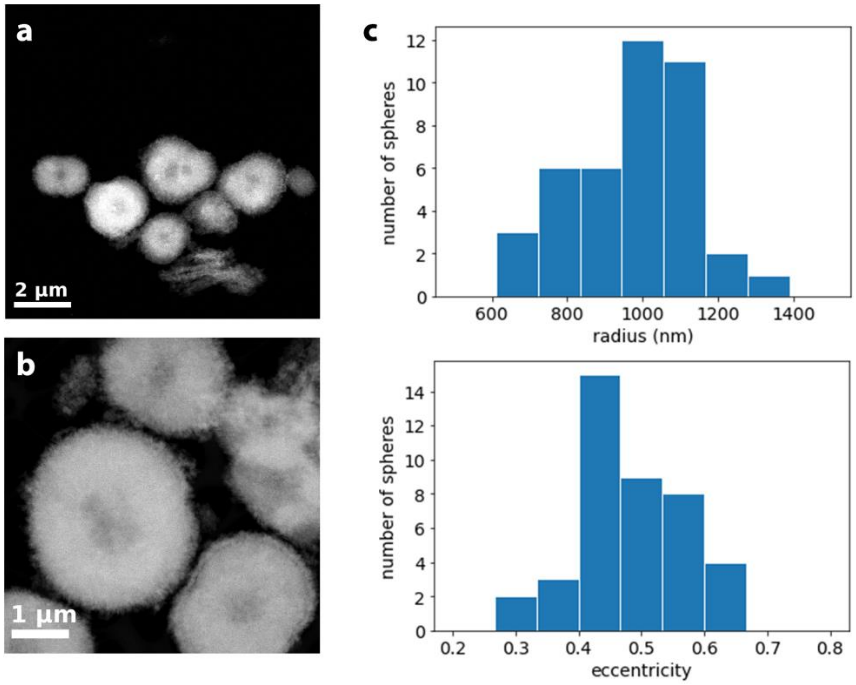

Figure 2. Figure 2. High-throughput characterization of CaCO3 microspheres. (a-b) HAADF imaging shows that the CaCO3 microspheres have varying shapes and sizes, and some of them do not have hollow centers. (c) Using automated image segmentation, the microspheres can be detected and their morphology can then be quantitatively classified.