Cba. tepidum-S^0 interactions: Some attachment required

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The *Chlorobi* are anoxygenic phototrophic bacteria that produce solid, extracellular elemental sulfur (S^0) globules as an intermediate step in the oxidation of sulfide to sulfate. Because globules are deposited extracellularly, cells must prevent encrustation during globule formation, and access sulfur through direct contact or at-a-distance mechanisms while degrading globules. To understand how *Chlorobaculum tepidum*, a model organism of the *Chlorobi*, addresses these challenges, we characterized the spatial relationships of cells and globules by light, electron (SEM), and atomic force (AFM) microscopy.

Cba. tepidum oxidizes sulfide to form extracellular S^0 globules. Globules nucleate early on, then continue to grow during this stage, with cells generally outnumbering globules by a factor of 2-3. Using time-lapse light microscopy, globules were observed to form and grow at a distance from cells, with ~15% of globules never coming into contact with cells. This suggests that *Cba. tepidum* cells produce a pool of soluble sulfur compounds (e.g. polysulfides) that can nucleate into globules and accrete away from the cell surface.

Upon the exhaustion of sulfide, cells begin to oxidize and degrade S^0 globules. During this stage, most cell-sulfur contact is transitory. Only ~15% of cells are attached during globule degradation. Cells grow while not attached to globules, even in the absence of other electron donors. Likewise, globules can be degraded without any contact from cells. However, cells separated from globules by a dialysis membrane are unable to grow. Taken together, these results suggest that attached *Cba. tepidum* cells may produce soluble intermediates that can feed unattached cells, and potentially activate S_8 rings in unattached globules.

Overall, these observations suggest mechanisms for *Cba. tepidum* globule formation and degradation that are dependent on cell-sulfur contact in a system where contact is transient and dynamic. Additionally, we are using AFM and SEM to investigate how the biomineral structure and rigidity evolve over the formation and degradation stages.