Calcium carbonate crystallization driven by engineered protein

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The formation of biogenic minerals is a highly complex process in which proteins play a key role. Protein control over crystallization results in exceptional biomineral structures with unique mechanical properties. Proteins are able to exert control over crystal polymorphism, to control the location and orientation of biogenic mineral phases as well as acting as templates providing specific sites for nucleation[1]. For instance, the transformation of amorphous calcium carbonate precursors (ACC) into the crystalline polymorph (calcite) in *S. purpuratus* spicules seems to be regulated *in vivo* by the so-called soluble organic matrix, composed by more than 45 proteins[6]. However, the mechanism by which proteins influenced crystallization is still far to be understood, especially with regard to its interpretation within classical or alternative nucleation mechanisms.

Additives containing functional motifs of typical biomineralization proteins (e. g. Asp-rich proteins) have been shown to direct *in vitro* crystallization producing structures that are reminiscent of biogenic minerals. However, the engineering of proteins with bio-inspired functional groups and motifs for controlling crystallization has not been widely exploited. Our aim is to engineer and design proteins that develop strong intermolecular interactions with mineral precursors and, therefore, are able to direct $CaCO_3$ crystallization. In this study, the effect of three types of genetically engineered Ubiquitin (Ub) protein (Ub3met, Ub3Aha and Ub3Aha with linked phosphate groups) were tested in $CaCO_3$ precipitation using gas diffusion experiments and analyzed using electron microscopy and infrared spectroscopy. Ubiquitin (Ub) is a globular, very stable (T_m near 100°C at neutral pH) protein, compatible with unnatural amino acids (UAA) incorporation. The UAA azidohomoalanine (Aha) was incorporate as methionine (met) surrogate with the aim of later introduction of functionalities via click reaction. We observed the temporal stabilization of a liquid phase and a strong control on $CaCO_3$ polymorph selection when the engineered proteins were present in the media.

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