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Determining the structure of the seminal biomineral/protein interface by cryo-EM

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Biomineralization is orchestrated by the coordinated action of a large number of proteins. How these proteins regulate the formation of minerals is one of the fundamental questions in biomineralization research. High-resolution structures of protein/mineral complexes at various growth stages of sediment growth are crucial for understanding proteins' ability to sense, respond, and regulate mineral formation.

The interaction between biomineralization proteins and minerals is often non-uniform [1]. As a result, the straightforward application of high-resolution structure determination methods such as X-ray crystallography and cryo-EM is challenging. To circumvent these challenges, we devised an experimental strategy for placing a biomineralization protein near nano-sediments of various sizes and watching how they interact. To this end, we adopted ferritin as a nano-reactor and a scaffold. As a nano-reactor, ferritin mediated the in-situ formation of iron-oxide nanoparticles. As a scaffold, ferritin was used for placing the in-situ formed iron-oxide nanoparticles within interaction distance from a biomineralization protein.

Using this experimental strategy, we determined the cryo-EM structures of the iron-binding segment (M6A) of the magnetite biomineralization protein Mms6 [2-3] from a magnetotactic bacteria while interacting with iron-oxide sediments of three different sizes (Figure 1a-b) [4].

We found that M6A, which is intrinsically disordered in the absence of a sediment, becomes structured in response to the formation and growth of the sediments. Unexpectedly, the stabilization of the M6A's structure starts away from the sediment and propagates towards the sediment as it grows. These findings demonstrate that transient interactions on one side of M6A are sensed on its other side, resulting in the structural stabilization of the latter. This phenomenon can be the structural basis for coordination between the various components of the biomineralization machinery in response to the formation of the sediment. M6A forms stable interactions with larger sediments (Figure 1c). These interactions can directly affect the sediment's growth by altering its surface energy and surface-diffusion rates.

Our results support and extend the long-suspected mechanism for the interaction and control of minerals' morphology by biomineralization proteins [5]. This mechanism suggests that unstructured regions in the proteins increase their order in response to the formation of sediments. According to our findings, the interactions between the intrinsically disordered domains and the sediments can function as a sensor, leading to structural changes away from the sediment; and as a direct effector on sediment's growth by changing its surface properties [4].

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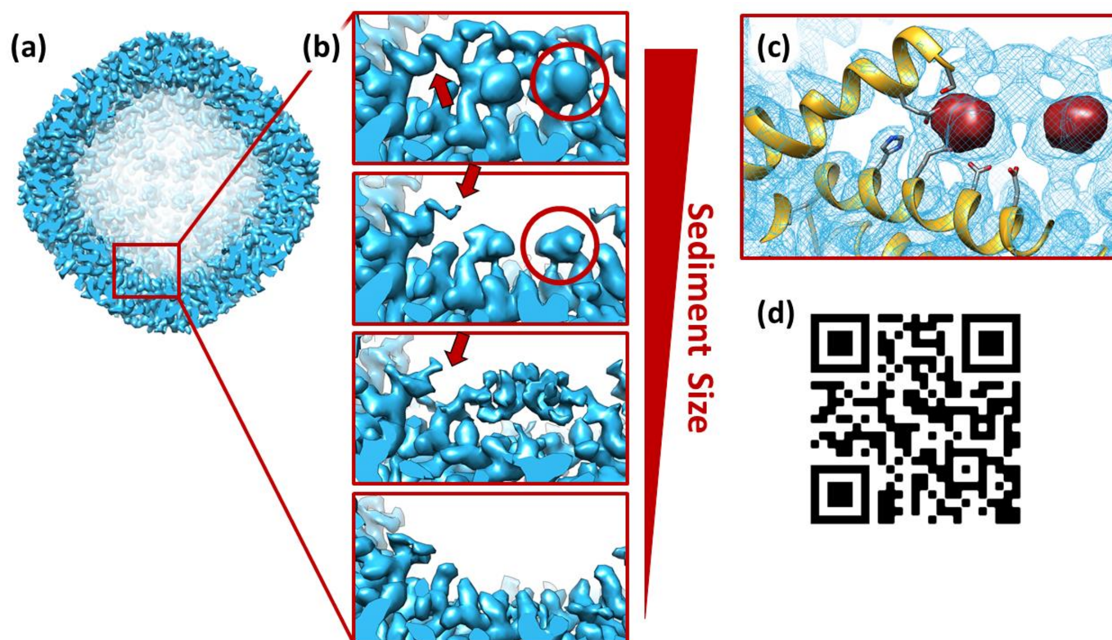

Figure 1. M6A becomes gradually more structured in conjunction with the growth of the sediment. (a) A slice through the electron density map of L-ferritin without a sediment, providing an overview of ferritin's structure. (b) Enlarged views of the framed region in (a) showing four different stages of the experiment, each of which with different sediment size, starting with no sediment (bottom) and ending with the largest sediment (top). The gradually stabilized structure of M6A and one of the two growing sediments are designated by arrows and circles, respectively. (c) A molecular model of ferritin and M6A showing the charged residues which mediate interactions between the protein and the sediment. (d) a movie (https://www.youtube.com/watch?v=YucYMYtmRzQ&feature=youtu.be) showing the structural organization of M6A and the sediments inside ferritin (adapted from [4]).

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