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Affimer® Proteins-Directed Control over Calcium Carbonate Polymorphs

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A key factor in biomineralization is the use of organic molecules to direct the formation of inorganic materials. However, the identification of organic molecules that can selectively produce calcite or aragonite has proven extremely challenging. Here, we use a phage-display approach to identify proteins that can direct calcium carbonate formation. A $1.3 \cdot 10^{10}$ library of modified M13 phage displaying Affimer® proteins[1] was employed, where these comprise a small protein scaffold of 81 amino acid with two nine-amino acids variable regions.[2][3] The library was screened against calcite and aragonite polymorphs at different pH conditions. From the initial phage library, 14 different proteins were selected and purified. The ones that bound strongly to calcite are particularly rich in basic and nonpolar amino acids in both variable regions. For aragonite, we found a significant number of nonpolar amino acids, with the appearance of aspartate (D) and glutamate (E) residues in one of the two loops. The ability of some of these proteins to direct the precipitation of calcium carbonate was then investigated. Our experiments show that aragonite proteins select for aragonite polymorph in presence of a molar ratio Ca:Mg 1:1 while the calcite proteins select for calcite under the same solution conditions. Control experiments conducted in the absence of proteins also yield calcite. In the absence of Mg^{2+} ions, calcite alone forms in all samples. We believe that this is the first example where phage-display has been used to identify organic molecules that can support aragonite formation, where our results highlight the potential importance of Mg^{2+} ions – which are abundant in seawater – to reduce the barriers to aragonite formation.

References:

[1] “Affimer® is a registered trademark from Avacta® Life Sciences Ltd.” .

[2] C. Tiede *et al.*, “Adhiron : a stable and versatile peptide display scaffold for molecular recognition applications,” *Protein Eng Des Sel.*, vol. 27, no. 5, pp. 145–155, 2014.

[3] C. Tiede *et al.*, “Affimer proteins are versatile and renewable affinity reagents,” *Elife*, no. 6:e24903, pp. 1–35, 2017.