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Reconstitution of Mineral Morphogenesis in Membrane-enclosed Compartments

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In nature biomineralization often takes place in membrane-enclosed compartments, where mineral precursors and accessory molecules are combined in a defined volume. Specialized vesicles transport the mineral precursors to these membrane-enclosed compartments, where the vesicles fuse with the compartment membrane. However, the transport and release of these precursors remain poorly understood. To get more insight into these processes we plan to establish an in vitro system that enables us to 1. reconstitute membrane-enclosed compartments, 2. add mineral-forming compounds via fusion of vesicles, and 3. quantitively investigate mineralization as a function of the chemical and physical conditions of the membrane and inside the compartment. Here, we focus on the mineralization of CaCO3.

Giant unilamellar vesicles are spread on porous substrates forming a pore-spanning membrane to create membrane-enclosed compartments. These pore-spanning membranes are accessible by fluorescence microscopy. A Ca^{2+}-sensitive dye inside the compartments enables detection of Ca^{2+} influx upon fusion of Ca^{2+}-filled large unilamellar vesicles. Moreover, we also established a bulk assay detecting the fusion of vesicles and their content release using a Ca2+-sensitive dye by fluorescence spectroscopy. Fusion is achieved through electrostatic interactions between oppositely charged membranes.