Physical and molecular sensing of single carboxysomes in an anti-Brownian electrokinetic trap

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In response to the slow catalytic rate and low specificity of the carbon fixation enzyme rubisco, autotrophic bacteria evolved the carboxysome, a bacterial microcompartment designed to locally concentrate CO_2 and enhance carbon fixation. The carboxysome encloses rubisco and other proteins with a proteinaceous shell permeable to small, metabolically important anions such as bicarbonate and ribulose-1,5-biphosphate. Direct observation of small-molecule transport into the carboxysome has proven challenging, and heterogeneity in carboxysome size and shape is expected to impact how small molecule species enter and exit the carboxysome. To address these challenges, we have used the ISABEL trap to observe molecular kinetics from single carboxysomes. The ISABEL trap applies electrokinetic feedback on a carboxysome's position using interferometric scattering signal in the near infrared. For molecular monitoring, we use fluorescent biosensors to study, for instance, the permeation of small redox molecules into single carboxysomes. We have also used a combination of scattering and fluorescence sensing to estimate the mass and cargo loading of single carboxysomes, as well as exploring the incorporation of shell protein hexamers on the carboxysome surface. These experiments benefit from multiplexed, correlated measurements utilizing both scattering and fluorescence, with many exciting future capabilities within reach.

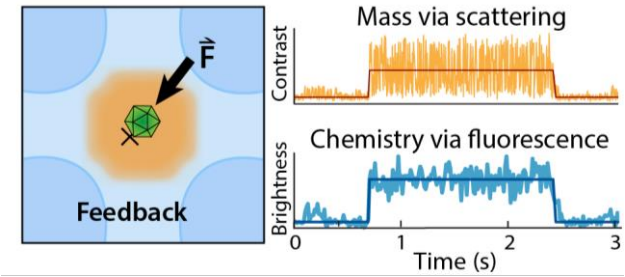

Figure 1. The left panel illustrates that a single carboxysome is held in solution for extended times by applying electrokinetic feedback forces. The right panel illustrates simultaneous long-term measurements on a single carboxysome, highlighting the capability to extract physical parameters and chemical properties from single microcompartments.