



Cell-Free Systems For Studying and Engineering Protein Assemblies

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Abstract: Protein assemblies play an important role in many biological processes and constitute a unique class of building blocks for materials of various types, owing to our ability to precisely tune their sequence. Our ability to harness these assemblies in and beyond biological contexts is contingent on understanding the driving forces for their formation. However, production and study of these assembling proteins typically depends on intracellular protein production followed by lysis and soluble protein purification—steps that are not necessarily compatible with maintaining the integrity of the protein assemblies of interest. Cell-free protein synthesis (CFPS) offers the opportunity to shift this paradigm for protein assemblies, providing on-demand synthesis of protein assemblies in an unconstrained open reaction environment. In this talk, I will describe how we are using CFPS in the study of two different types of protein assemblies.

First, I will demonstrate how we used CFPS to study and prototype assemblies formed by hexameric bacterial microcompartment (BMC-H) proteins that drive the formation of bacterial microcompartments. This work focuses on two homologous BMC-H proteins that come from the same bacterial microcompartment system, PduA and PduJ. Using immunostaining and confocal fluorescence microscopy, we find that both PduA and PduJ form structures hundreds of microns large in CFPS, highlighting the role that the cell boundary plays in checking protein self-assembly *in vivo*. Further, we find that while PduA and PduJ are similar in both biological function and sequence, these two proteins form significantly different supramolecular structures in CFPS, a testament to the significant impact seemingly small differences can have on self-assembly—a finding that is further supported by characterization of single PduA and PduJ point mutants.

The second portion of my talk will focus on applying CFPS to the study of proteins that form bacterial biomolecular condensates. Specifically, using a panel of model condensate forming proteins, we are studying how condensate behavior varies across *in vitro* buffer systems, *in vivo* bacterial systems, and *in vitro* CFPS systems, with the ultimate goal of addressing the limitations of current *in vitro* and *in vivo* approaches through the use of CFPS.

Bio: Dr. Carolyn Mills (she/they) is an Assistant Professor in the Department of Bioengineering at the University of California, Santa Barbara (UCSB). Carolyn's research at UCSB focuses on molecular engineering of proteins that control spatial organization in biological systems, with applications in plastic remediation and oral vaccine delivery. Carolyn completed her B.S. in Chemical Engineering in 2013 at UCSB, and her PhD in Chemical Engineering in 2019 at Massachusetts Institute of Technology, working in Professor Bradley Olsen's lab on self-assembly and high-throughput processing of fusion protein materials. During her PhD, Carolyn was supported by an NSF Graduate Research Fellowship. Carolyn completed her postdoctoral work with Professor Danielle

Tullman-Ercek at Northwestern University, researching self-assembling protein nanoreactors. During her time at Northwestern, Carolyn was recognized by the Chemical and Biological Engineering Department's Distinguished Postdoctoral Service Award. At UCSB, Carolyn is actively involved in collaborations at both the NSF ExFAB Biofoundry and BioPACIFIC Materials Innovation Platform.

Hosted by: Prof. Ryan Hayes