Design of phase-separated protein assemblies in living cells

In recent years, the discovery of phase-separated protein assemblies has added to our understanding of numerous cellular processes, including metabolism, signal transduction, and cellular memory. The molecular mechanisms underlying the formation of such assemblies are, however, largely uncharacterized. Here, we use synthetic biology to dissect how properties of phase-separated protein assemblies can emerge from molecular properties of their building blocks. We designed genetically encoded protein building blocks that work in pairs, similarly to epoxy resins. These building blocks interact non-covalently and exclusively intermolecularly, while affinity and valence are controlled precisely by design. The system undergoes self-assembly in vivo to form round-shaped phase-separated protein granules observable by fluorescence microscopy. Electron microscopy shows the granules to be independent of membranes, and their formation is reversible upon treatment of cells with hexanediol. Remarkably, the material state of the granules is tunable. While high-affinity interactions yield gel-like structures, weaker affinities yield liquid granules where building blocks rearrange rapidly. This synthetic system provides a well-controlled molecular toolkit for viewing and modeling micron-scale self-assembly in vivo, and for designing more advanced structures and scaffolds.