



“Sequential Bottom-up Assembly of Synthetic Cells”

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Abstract

The evolution of cellular compartments for spatially and temporally controlled assembly of biological processes became an essential step in developing life. Synthetic approaches towards cellular-like compartments are still lacking well-controlled functionalities as would be needed for more complex synthetic cells. In part, this is due to the mechanical and chemical instabilities of the lipid-based protocells and a lack of technical means for their well-controlled manipulation. We developed droplet supported lipid bilayer vesicles by microfluidics to generate mechanically and chemically stable and, therefore, manipulable cell-like compartments with a well-defined chemical and biophysical microenvironment. The enhanced stability enabled the sequential loading of such compartments with biomolecules by pico-injection microfluidics without compromising their functionality as synthetic cells. We demonstrate a successful sequential bottom-up assembly of a compartment with lipids, transmembrane proteins (integrin, FoF1-ATP synthase) and cytoskeleton proteins which would not assemble in a fully functional way by mixing and including them in one pot at once.