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Continuous Microfluidic Fabrication of Synthetic Asymmetric Vesicles for Membrane Biology Studies LI LU, JEFFREY SCHERTZER, PAUL CHIAROT, State University of New York at Binghamton — Membrane vesicles are spherical structures comprised of a single lipid bilayer enclosing an aqueous lumen. In nature, vesicles carry out many important functions in both eukaryotic and prokaryotic organisms. When preparing vesicles artificially, it is difficult to simultaneously control vesicle membrane asymmetry, size, unilamellarity, throughput, and monodispersity. Membrane asymmetry, where each leaflet of the lipid bilayer consists of a different lipid distribution, is of particular importance as it is a feature of nearly all natural membranes. In this study, we report on a novel microfluidic strategy to build monodisperse asymmetric vesicles with customized membrane composition, size, and luminal content at high-throughput. The microfluidic device consists of a triangular post region and two flow-focusing regions. The major steps of the vesicle fabrication process include: (1) assembly of the inner-leaflet, (2) continuous flow separation - replacing the inner-leaflet-lipid with the outer-leaflet-lipid, (3) assembly of the outer-leaflet, and (4) extraction of the intermediate oil layer. Membrane asymmetry and unilamellarity are confirmed using a fluorescence quenching assay and a membrane protein insertion assay, respectively. Our vesicle fabrication method can yield membrane asymmetries as high as 95%, which is maintained at a high-degree for over 30 hours. In addition, over 80% of the vesicles remain stable for at least 6 weeks. The effect of bilayer composition on the mechanical properties of the membrane and the role of small molecules on membrane architecture will be investigated.

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