P-1.145

Preparation and Investigation of Asymmetric Pore-spanning Membranes

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Biological membranes are dynamic structures with complex asymmetries, characterized by distinct lipid compositions in each leaflet. The maintenance of this asymmetry requires significant free energy, expended through the precise interplay of various integral membrane proteins. These include flippases, floppases and scramblases, which transport lipids between the inner and outer leaflets in a controlled manner. Loss of this asymmetry can have severe consequences, including apoptosis or Scott syndrome, a rare genetic blood coagulation disorder. Although lipid asymmetry is highly regulated in living cells, mimicking it in artificial membrane systems remains a major challenge, as most in vitro approaches yield symmetric bilayers.

Our quantitative understanding of membrane asymmetry remains limited due to the lack of effective in vitro methods for generating asymmetric model membranes. Therefore, this study focuses on the preparation and investigation of asymmetric pore-spanning membranes (PSMs) as a model system to study lipid asymmetry. To mimic this asymmetry in vitro, asymmetric giant unilamellar vesicles (GUVs) are generated using the inverted emulsion method by selectively varying the lipid composition in the monolayer and emulsion phase. These asymmetric GUVs are then spread onto porous substrates to form PSMs, which are subsequently analyzed using fluorescence microscopy.