A Novel Method of Quantitative Analysis for Lipid Membrane Interleaflet Asymmetry

R. Wang; T. Yang and P.S. Cremer

Abstract: The asymmetrical arrangement of phospholipids within the dual leaflets of a eukaryotic plasma membrane is an integral part of cell function. Despite the wide use of the supported lipid bilayer (SLB) as a mimetic model for cellular membranes over the past several decades, comprehending the inter-leaflet distribution of lipids within these ultra-thin lipid membranes has been somewhat elusive. Inspired by the graphene exfoliation technique, we have created the lipid bilayer unzipping assay, a novel method enabling the separation of a lipid bilayer membrane into two monolayers. Our investigation has found that oxidized polydimethylsiloxane (PDMS) is a highly promising material for lipid bilayer unzipping. This preference arises from its exceptional chemical stability, mechanical flexibility, and optical transparency, which together promote maximal conformal contact when forming the substrate-lipid-substrate sandwich structure. Following the successful separation of the two leaflets, we employed a combination of fluorescence microscopy and High-Performance Liquid Chromatography - Mass Spectrometry (HPLC-MS) to quantify the lipids on the two unzipped monolayer individually. Surprisingly, we found that the distribution of phosphatidylserine (PS) and phosphatidylethanolamine (PE) lipids within SLBs is highly asymmetric, with a greater abundance observed in the lower leaflet. This asymmetry has been attributed to the interactions between the lipid head group and the glass surface, including hydrogen bonding and electrostatic interactions. Furthermore, we have demonstrated our capacity to manipulate lipid asymmetry within SLBs by altering parameters such as salt concentration, pH levels, and buffer conditions, in order to better mimic the cellular membranes.