Neutron Spin Echo Detects Effects of the pH-Low Insertion Peptide on Membrane Thickness Fluctuations

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Lipid membranes are highly dynamic assemblies, exhibiting a hierarchy of motions ranging from rotation of an individual lipid molecule to collective membrane fluctuations. The latter, and in particular thickness fluctuations, have recently become experimentally accessible with the development of neutron spin echo (NSE) techniques. Biological membranes contain surface-associated and transmembrane proteins, both of which are key to a cell's well-being. However, how these proteins affect the membrane's dynamics, including thickness fluctuations, remains an open question. Here, we use pH-low insertion peptide (pHLIP) to address this question. pHLIP is highly sensitive to changes in pH, transitioning from a surface-associated to a transmembrane configuration below a critical pH value, and thereby allowing both classes of membrane proteins to be studied in a single system. We used NSE to measure membrane thickness fluctuations, and small-angle neutron and X-ray scattering (SANS and SAXS, respectively) to measure the average membrane thickness. Both SANS and SAXS showed that pHLIP conformation does not affect the average bilayer thickness of a phosphatidylcholine/phosphatidylserine/cholesterol (PC/PS/Chol) membrane. In contrast, NSE detected differences in membrane dynamics between the two protein states. Specifically, pHLIP in the surface-associated state decreased the amplitude of the membrane's fluctuations while increasing their frequency. Strikingly, as a transmembrane helix, pHLIP dampened the membrane's fluctuations. We hypothesize that the suppression of thickness fluctuations may result from hydrophobic matching between the peptide and membrane, effectively pinning the membrane in place.