Regulating Lipid Organization and Investigating Membrane Protein Properties in Physisorbed Polymer-tethered Membranes

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Abstract:

Cell membranes have remarkable properties both at the microscopic level and the molecular level. The current research describes the use of physisorbed polymer-grafted lipids in model membranes to investigate some of these properties on both of these length scales. On the microscopic scale, plasma membranes can be thought of as heterogenous thin films. Cell membranes adhered to elastic substrates are capable of sensing substrate/film mismatches and modulating their membrane stiffness to more closely match the substrate. Membrane/substrate mismatch can be modeled by constructing lipopolymer-enriched lipid monolayers with different bending stiffnesses and physisorbing them to rigid substrates which causes buckling. This report describes the use of

atomic force microscopy and epimicroscopy to characterize these buckled structures and to illustrate the use of the buckled structures as diffusion barriers in lipid bilayers. In addition, a series of monolayers with varying bending stiffnesses and thicknesses are constructed on rigid substrates to analyze changes in buckling patterns and relate the experimental results to thin film buckling theory. On the molecular scale, plasma membranes can also be thought of as heterogeneous mixtures of lipids where the specific lipid environment is a crucial factor affecting membrane protein function. Unfortunately, heterogeneities involving cholesterol, labeled lipid rafts, are small and transient in live cells. To address this difficulty, the present work describes a model platform based on polymer-supported lipid bilayers containing stable raft-mimicking domains into which transmembrane proteins are incorporated (αvβ3, and α5β1integrins). This flexible platform enables the use of confocal fluorescence fluctuation spectroscopy to quantitatively probe the effect of cholesterol concentrations and the binding of native ligands (vitronectin and fibronectin for αvβ3, and α5β1) on protein oligomerization state and on domain-specific protein sequestration. In particular, the report shows significant ligand-induced integrin sequestration with a low level of dimerization. Cholesterol concentration increases rate of dimerization, but only moderately. Ligand addition does not affect rate of dimerization in either system. The combined results strongly suggest that ligands induce changes to integrin conformation and/or dynamics without inducing changes in integrin oligomerization state, and in fact these ligand-induce conformational changes impact protein-lipid interactions.

Description:

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