Structure of the PTEN Tumor Suppressor Associated with the Fluid Lipid Membranes

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PTEN (3,4,5)P3, which is a phosphatase involved in the regulation of PI(3,4,5)P3. It consists of a phosphatase and a C2 domain that interact synergistically with anionic lipids in membranes. A single-point mutation of the wild type (wt) protein, H93R, has the same secondary structure but significantly reduced enzyme activity. As for many membrane proteins, the crystal structure of a truncated PTEN has been determined, but the association of the protein with lipid membranes has only been indirectly inferred. We study the association of wt PTEN and H93R with membranes using neutron reflectometry (NR) of tethered bilayer lipid membranes (tBLMs) which are long-term stable and retain their fluidity with in-plane dynamics similar to that in vesicles. Surface Plasmon Resonance (SPR) spectroscopy has also been used to investigate the binding of these two variants of PTEN.

Data analysis uses a composition-space model that resolves the thermally disordered membrane with Ångstrom resolution, enabling us to characterize PTEN association with the bilayer in its physiologically relevant, disordered state. The crystal structure serves as a starting point for model refinement. Computational techniques are used to explore the conformational flexibility of the peptide stretches deleted for crystallization. We observed slight differences in the peripheral association of H93R and wt PTEN with the bilayer’s headgroup region and speculate how these may be related to their functional distinctions.

(2) J. Lee et al., 1999, Cell 99:323.
(3) D. J. McGillivray et al., 2007, Biotheriophases 2:21.
(5) S. Shenoy et al., 2010, Soft Matter 6, 1263.

An Improved Open Microfluidic Flow Cell for Measuring Solute Adsorption to Monolayers

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We earlier described an apparatus and optical method that permit quantitative measurement of the adsorption of a solute to a gas-liquid interface [K.C. Hoang, D. Malakhov, W.E. Momsen and H.L. Brockman (2006) Anal. Chem. 78, 1657-1664]. The interface may or may not support a protein, lipid or other monolayer. A potential limitation to such a flow cell, being probed optically through the monolayer, is concomitant solute adsorption to the solid trough bottom, which is parallel to but displaced from the monolayer being studied. To circumvent this unwanted adsorption we introduced a flowing sucrose solution under the flowing solute solution that supports the monolayer. In this way solute is prevented from reaching the trough bottom and any solute that might adsorb to or diffuse through the sucrose-solute solution interface is rapidly moved from the optical path by flow to the trough drain. Eliminating the need to chemically modify the trough bottom by introducing a sucrose layer allows trough dimensions to be reduced, allows robust and fully automated cleaning between experiments and improves reproducibility of solute adsorption measurements. An additional feature of the new configuration is control of the thickness of the solute solution layer, which conserves reagents and enhances the sensitivity of solute adsorption measurements. The apparatus was used to characterize the interaction of a fluorescent protein antigen with an antibody monolayer and to the initial rate of adsorption of bovine serum albumin to phospholipid-dicyclohexylmonolayers of differing lipid compositions but approximately constant surface pressure.

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Achieving Ultralow Fouling Performance for Poly(Hydroxy-Functional Methacrylates) Grafted Surfaces via Atom Transfer Radical Polymerization

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The development of nonfouling biomaterials to prevent nonspecific protein adsorption and cell/bacterial adhesion is critical for many biomedical applications, such as antithrombogenic implants and biosensors. In this work, we synthesize and characterize two polymer brushes on the gold substrate by using surface-initiated atom transfer radical polymerization (SI-ATRP) of Hydroxy-Functional Methacrylates monomers: HEMA and HPMA. We investigate the effect of film thickness on protein adsorption from single protein solution to complex human blood media, as well as bacterial adhesion. Surface plasmon resonance (SPR) results show a correlation between antifouling properties and film thickness, that is, optimal film thickness of 25-45 nm for polyHPMA and 20-45 nm for polyHEMA is obtained to achieve almost zero protein adsorption (<0.3 ng/cm²) from single protein solution, 10% human blood plasma, and 10% human blood serum. Furthermore, polyHEMA brushes remain their excellent resistance to 100% human serum and plasma with <5 ng/cm² adsorption amount, while polyHPMA brushes adsorb more proteins of 13.5 ng/cm² and 50.0 ng/cm² from 100% human serum and plasma, respectively. More strikingly, static bacteria adhesion assay shows that there are almost no bacteria adhered to polyHEMA and polyHPMA surfaces as compared to full coverage of bacteria on the bare gold surface. In addition, stability tests show a very stable polyHPMA and polyHEMA surfaces without polymer degradation within 20 days. PolyHEMA and PolyHPMA provide effective nonfouling biomaterials, alternative to poly(ethylene glycol), to highly resist nonspecific protein adsorption and cell/bacteria adhesion.

Association-Dissociation Behavior of the Apolipoprotein E Proteins: Implications for Lipid Binding

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Apolipoprotein-E4 (ApoE4) is a risk factor for Alzheimer’s diseases but the structural or functional differences between the isoforms viz, ApoE2, ApoE3 and ApoE4 are unknown. Lipidation of ApoE is important for its functions. However, the molecular mechanism and the isoform specificity of ApoE-lipid interactions are unclear. In vitro lipid free ApoE undergoes self association but which oligomeric form of ApoE interacts with lipids is unknown. Using Fluorescence Correlation Spectroscopy, intermolecular FRET and sedimentation methods we find that association-dissociation reaction of ApoE can be modeled by a monomer-dimer-tetramer process. Dissociation kinetics as measured by changes in FRET show two phases reflecting dissociation of tetramer to dimer and of dimer to monomer. The rate constants are found to be different for the ApoE isoforms. The kinetics of lipidation of ApoE in presence of unilamellar vesicles of DMPC show striking similarity with the kinetics of dissociation of ApoE multimers to monomers. Furthermore, lipidation kinetics are slower at higher ApoE concentrations and kinetic data are consistent with only monomers binding to lipids. The results imply that differences in lipidation properties between apoE isoforms arise due to their differences in association-dissociation behavior.

Ligand Induced Conformational Redistribution in Synaptotagmin I C2A

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Thermodynamic parameters capture the overall contribution to a system’s energetics. In the case of binding proteins, such as Synaptotagmin I, ascertaining the overall magnitude of the interactions within the protein is the first step toward addressing how energy is distributed throughout the body of the protein. Our aim is to understand how the signal of ligand binding is disseminated through the protein during the role it plays in regulated exocytosis. While several detailed molecular approaches have identified putative regions where interactions occur, it is the energetics that are key to understanding Synaptotagmin I’s functional response. Here, denaturation studies of the C2A domain of Synaptotagmin I were carried out in conditions that are physiologically relevant to regulated exocytosis where calcium ions and phospholipids were either present or absent. Denaturation was carried out using two techniques: differential scanning calorimetry (DSC) and fluorescence lifetime (FLT). A global analysis approach combining these data sets was used where the data was simultaneously fit to models derived from thermodynamic principles. The enthalpy associated to the denaturation of the C2A domain of Synaptotagmin I in the absence of all ligands was found to be quite low when compared to other proteins of the similar molecular weight, which suggests that the protein exhibits conformational flexibility. In addition, the denaturation behavior is shown to change...