from the Protein Kinase Cα (PKCα), that are covalently linked to one another in the primary structure of the parent protein. Using NMR relaxation dispersion techniques, paramagnetic relaxation enhancement (PRE) experiments, and NMR-detected ligand binding studies, we demonstrate the role of conformational plasticity and initial membrane pre-association in modulating the affinity of the C1 domain to its natural cofactor, diacylglycerol. We show that the C2 domain employs a drastically different mechanism of membrane insertion that involves modulation of its electrostatic potential by divalent metal ions. The dynamics of loop and N- and C-terminal regions of C2 changes as a function of metal ligation state, suggesting a possible mechanism for propagating the information about the metal-binding event to other PKCα domains. In aggregate, our studies provide a view of conditional membrane domains as highly dynamic entities, in which conformational plasticity and synergistic action of cytosolic and membrane-embedded ligands define their nuanced signaling response. The functional and structural interplay between C1 and C2 domains will also be discussed in the context of the only two existing multi-domain structures of Protein Kinase C, one of which came from our laboratory.

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**1256-Plat**

**The Role of Protein and Membrane Context in the Interaction of Polyglutamine Peptides with Lipid Membranes**

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Huntington's disease is a dominant genetic neurodegenerative disorder associated with motor and cognitive decline, caused by a mutation in the polyglutamine (polyQ) region near the N-terminus of the huntingtin (htt) protein. Expansion of the polyQ region above 35-40 repeats results in the disease that is characterized by inclusion body aggregates of mutated protein. The polyQ expansion in htt flanked by a 17 amino acid N-terminal sequence (Nt17) and a proline-rich (polyP) region. To investigate the interaction between htt exon1 and lipid membranes, a combination of Langmuir trough techniques and vesicle permeability assays measuring calcein leakage were used to directly monitor the interaction of a variety of synthetic polyQ peptides with different combinations of flanking sequences (KK-$Q_{35}$-KK, KK-$Q_{35}$-$P_{10}$-KK, Nt17-$Q_{35}$-KK, and $Nt_{17}$-$Q_{35}$-$P_{10}$-KK) on total brain lipid extract (TBLE) model membranes. PolyQ peptides that lacked the Nt17 domain did not appreciably aggregate on or insert into lipid membranes. Nt17 facilitated the interaction of peptides with lipid surfaces while the polyP region enhanced this interaction. Our data suggests that the Nt17 domain plays a critical role in htt binding and aggregation on lipid membranes, and this lipid/htt interaction can be further modulated by the presence of the polyP domain. The addition of cholesterol to TBLE model membranes, to determine its role in htt-membrane interactions, resulted in reduced peptide insertion into lipid monolayers and decreased levels of induced vesicle permeability, though the effect does not scale linearly with cholesterol concentration. Results from parallel studies performed with ganglioside $GM_1$ and sphingomyelin to determine their role will also be presented.

**1257-Plat**

**Association of α-Synuclein with Lipid Vesicles. Stopped-Flow Kinetics of Concerted Binding and Conformational Change**

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Alpha-synuclein (AS), a 140aa intrinsically disordered protein, self-associates into oligomeric forms and aggregates into amyloid fibrils in Parkinson's disease. Certain mutations affect these processes and accelerate disease pathogenesis. The physiological roles of AS are a matter of speculation. Membrane binding is undoubtedly involved and the protein acquires α-helical structure in the process (1). We have studied the thermodynamics and kinetics of AS-membrane association utilizing vesicles (SUVs) of differing composition. Functionally neutral single cysteine mutants of AS were labeled with a polarity sensitive excited-state intramolecular proton transfer (ESIPT) probe (MFE). Double cysteine mutants were labeled with a FRET pair (Alexa Fluor488, Alexa Fluor568) at a series of selected positions in the primary sequence. Kinetic studies were conducted by stopped-flow, using 5-20 nM protein concentrations and increasing levels of SUVs (generally 20-200 μM) Signal changes indicative of membrane association were observed: increased intensity and shape change of dual band ESIPT emission, and altered FRET with the Alexa dyes. The analysis revealed a two-step reaction sequence in the time range $<10$ s. We attribute the first step to binding, and from the dependence on lipid concentration determined the second order rate constants and corresponding spectroscopic parameters. The second concentration independent step (1-10 s range) presumably arises from conformational changes in the protein (α-helix formation) and its accommodation to or perturbation of the lipid microenvironment (ESIPT dye).
Accompanying thermodynamic measurements led to estimates of dissociation constants as a function of membrane composition, charge, and shape (SUVs, LUVs). A new experimental protocol (*slopes*), implemented in a microplate reader, circumvented technical problems usually manifested in titrations of protein with lipid.

[1] Pfefferkorn C M, Jiang, Lee J C (2012). Biophysics of α-synuclein membrane interactions. *Biochim Biophys Acta***1818**:162-171.

**1258-Plat**

**Fluorinated Aromatic Amino Acids Distinguish Cation-ϕ Interactions from Membrane Insertion**

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Peripheral membrane proteins can be targeted to membranes via specific lipid interactions. Molecular dynamics (MD) simulations suggest that phosphatidylcholine (PC) cation / amino acid π complexes are important for the membrane binding of Bacillus thuringenisis phosphatidylinositol-specific phospholipase C (PI-PLC). This peripheral membrane protein specifically binds to PC-rich vesicles, and both mutagenesis and MD simulations suggest that choline cation / tyrosine π complexes provide most of the binding energy. The related Staphylococcus aureus PI-PLC has little affinity for PC, but introduction of two tyrosines at Asn-254 and His-258, mimicking the Bacillus enzyme, leads to PC specificity presumably due to cation-π interactions. However, there are not facile experimental methods for differentiating cation-π interactions from membrane insertion of aromatic side chains. By site-specific incorporation of pentafluorophenylalanine (F5-F) and difluorotyrosine (F2-Y) using the pEVOL system, we can distinguish these two types of interactions. Fluorinated amino acids are more hydrophobic, and are therefore likely to enhance binding by insertion. However, the altered electrostatics of the fluorinated aromatic ring should destabilize cation-π interactions. S. aureus F249(F5-F) has higher binding affinities than wildtype for small unilamellar vesicles (SUV) at all mole fractions of PC (XPC), and a ΔΔG relative to wildtype of $-3.4$ kJ/mol for binding to PC-rich SUVs(XPC=0.8) suggesting that the Phe inserts the membrane. In contrast, N254Y/H258(F2-Y) loses significant binding affinity for PC-rich vesicles compared to the parent N254Y/H258Y, with a ΔΔG relative to N254Y/H258Y of $+7.5$ kJ/mol at XPC=0.8, consistent with role of Tyr residues in cation-π complexes with choline headgroups. Thus fluorinated amino acids allow us to directly test how aromatic residues interact with membranes elucidating protein membrane interactions at molecular level, and provide both a direct test of MD simulations results and data that can be used to design membrane binding interfaces.

**1259-Plat**

**In Vitro Reconstitution of Transcellular Tunnels Closure**

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Several bacteria, such as Staphylococcus Aureus, are able to cross the endothelial barrier by inducing transcellular tunnels, called Transendothelial Macroapertures (TEM), in endothelial cells. The closure of these TEMs is critical to prevent endothelial permeability and cell death. Several proteins have been identified to play a role in this process. In particular, the I-BAR domain proteins MIM and ABBA have been shown to accumulate at the edge of the aperture shortly after the opening event. They subsequently recruit actin, followed by actin-rich membrane wave extension over the aperture. Interestingly, the related protein IRSp53, that unlike MIM and ABBA proteins does not have amphipathic alpha-helices on its I-BAR domain, has not been found at the edge of the TEM.
The details of this mechanism remain unknown. Our objective is to characterize the physics underlying the first step in TEM closure. Our hypothesis is that MIM and ABBA have the ability to recognize the newly negatively-curved membrane at the edge of the TEM through their I-BAR domain. We use a minimal system where the protein is encapsulated in a giant unilamellar vesicle and can interact with the negatively-curved inner surface of a membrane tube that has been pulled out of the vesicle. We study protein-membrane interactions through fluorescence (confocal microscopy) and force (optical tweezers) measurements. By combining the two types of measurement, we quantify the affinity of the proteins for curved interfaces, ranging in radii of curvature from 10 to 100 nm, as well as their potential mechanical effect on the membrane. Our results show an original behavior where ABBA and IRSp53 are maximally enriched in membrane tubes of specific radii of curvature.