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Hiv-1 Tat Membrane Translocation Probed by Low- and Wide-Angle X-Ray Scattering, Neutron Scattering, CD Spectroscopy and MD Simulations

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In an effort to understand membrane translocation of a cell-penetrating peptide, interactions of HIV-1 Tat peptide (GRKKRRQRRRPPQ) with DOPC, DOPC/DOPE, DOPC/DOPS, and nuclear membrane mimics were investigated using low- and wide-angle x-ray scattering (LAXS and WAXS), neutron scattering, and circular dichroism (CD) spectroscopy. The diffuse scattering analysis applied to LAXS collected at CHESS revealed that Tat-membrane interactions reduce the membrane thickness by ~1 Å. In DOPC and DOPC/DOPE membranes, the position of Tat was found to transition from the vicinity of the glycerol-carbonyl headgroup to the phosphate headgroup as Tat mole fraction was increased from 0.009 to 0.06. The area per lipid for DOPC and DOPC/DOPE membranes increased by ~2 $Å^2$ at the highest Tat mole fraction. The membrane bending modulus was found to decrease by roughly a factor of 2 at the highest Tat mole fraction except for the nuclear mimic. The chain-orientational order parameter, S_{xray}, calculated from WAXS and corrected for mosaic spread, showed Tat slightly disordered chains. Neutron scattering collected at NIST from fully hydrated samples consisting of DOPC:DOPE (3:1) membranes and Tat at 0.06 mole fraction showed a prominent, broad peak corresponding to a Tat-membrane correlation of ~100 Å. The secondary structure of Tat calculated from CD spectra using DichroWEB was found to be the same in pure water as in lipid thin films and primarily consisted of β-sheet and random coil with small helical content. Our findings are consistent with the results from MD simulations by Herce and Garcia, which suggested that Tat interacts with phosphate headgroups across the bilayer, facilitating the formation of pores. The ensemble of configurations obtained from a new MD simulation allows visualization of Tat/membrane interactions. Funded by GM44976, GM86801, DMR-0936384(CHESS), and DOE(NIST).

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A Systematic Study of Phase Changes Induced by Trans-Membrane Peptide Gramicidin-A in Multi-Component Lipid Membranes

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What are the effects of proteins on lipid membrane domains? In order to answer this question, we systematically investigated the phase changes induced by trans-membrane peptide gramicidin-A in 16:0-18:2PC(PLPC)/di18:0PC(DSPC)/cholesterol and 16:0-18:2PC/di16:0PC(DPPC)/cholesterol lipid bilayers. Quaternary giant unilamellar vesicles (GUV) were prepared using our recently developed Damp-Film method. The phase boundaries of liquid-ordered and liquid-disordered (Lo+Ld) coexisting region as well as the critical points were determined using video fluorescence microscopy. Within the phase coexisting regions, thermodynamic tie-lines were determined using a fluorescence assay. Our results show that adding 1 mol% of gramicidin produces significant and complex phase changes to the lipid bilayers: at some lipid compositions, gramicidin can induce lipid domains; at others, gramicidin completely abolish the phase separation; even if the phase separation is preserved, gramicidin significantly alters the lipid compositions of membrane domains and tie-lines. In the biological relevant critical region, these changes could be quite dramatic. We also measured gramicidin-A partition coefficients between coexisting Lo+Ld lipid phases. Away from the critical point, the coefficient is close to 2, indicating that gramicidin slightly prefers the disordered Ld lipid domains with smaller bilayer thickness. However, the partition coefficient continuously changes with lipid composition. Near the critical point, the partition coefficient approaches to the theoretical value of 1.

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Physical Properties of Model Membranes Containing Pope and Phytosterol

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We have studied the effect of phytosterol on the physical properties of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) multilamellar vesicles using deuterium nuclear magnetic resonance (^{2}H NMR). The *sn-1* chain of POPE is deuterium labeled. The NMR spectra were taken as a function of temperature and phytosterol concentration. The order of POPE-d31 membranes, measured through the spectral first moment, is almost not affected by the addition of phytosterol in the gel phase, while it increases with phytosterol concentration in the liquid-crystalline phase. A significant difference in the ability of phytosterol to disorder the gel-phase and to order the liquid-phase POPE membranes is observed. This finding differs from those observed in POPE/chol and other lipid/sterol systems. Furthermore, the temperature-composition phase diagram will be discussed.

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Measuring the Dimerization Propensities of Mucin1 Transmembrane and Juxtamembrane Domains

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Overexpression of the membrane protein mucin 1 (MUC1) has been linked to 75% of all human solid tumor cancers, including 90% of breast carcinomas. In cancer cells, MUC1-MUC1 homodimerization has been associated with cell migration and adhesion. Furthermore, this interaction is necessary for forming complexes with growth factor receptors and targeting to the nucleus, where MUC1 can interact with effector proteins regulating gene expression. Thus, understanding how MUC1 forms homodimers is essential for developing novel therapeutic strategies to block its oncogenic effects. A recent study has shown that the membrane proximal CQC motif promotes dimerization under oxidizing conditions, suggesting that the motif may act as a redox switch in response to changes of cytosolic oxidant levels. Aside from these few studies focusing on the CQC motif, very little is known regarding the mechanism of MUC1 homodimerization. Currently, we are using the ToxR and AraTM assays to investigate if the transmembrane domain, without the cytosolic CQC motif, is able to dimerize by itself. We are also measuring if the dimerization propensity of the TMD changes with the membrane proximal CQC motif. The two assays allow us to compare the dimerization propensity when the CQC motif is in reducing and oxidizing environments.

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Characterizing the Curve: A Mechanistic Study of CPLA2-Mediated Membrane Bending

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Lipid membranes play a critical role in cellular signaling through selective protein-lipid interactions. The membrane composition of organelles often drives specific proteins to localize in cells. Lipid binding proteins, including those harboring BAR and ENTH domains, have been shown to shape biological membranes into vesicles necessary to transport cargo across the membrane. Recently, we observed that the calcium-dependent enzyme cytosolic phospholipase A_2 ($cPLA_2$), bends model membranes through its N-terminal C2 domain, which is dependent upon its membrane penetration (Ward et al. JLR, 2012). Thus, in addition to its role in generating arachidonic acid from membrane phospholipids, this enzyme may have a role in regulating membrane curvature changes. This hypothesis is supported by roles for $cPLA_2$ described in the literature including intra-Golgi trafficking, Golgi tubulation, Golgi vesiculation and Fc-receptor-mediated phagocytosis. We found that membrane bending by $cPLA_2$ translated into A549 and HeLa cells, supporting the physiological relevance of our earlier findings. Thus, we sought to characterize the molecular forces driving $cPLA_2$-dependent membrane bending *in vitro* and in cells. Using a variety of mCherry and mEGFP protein chimeras, we investigated the hypothesis that $cPLA_2$ oligomerizes on membranes with a series of correlation spectroscopy experiments. These results show that $cPLA_2$ forms large protein oligomers on cytoplasmic vesicles using number and brightness analysis and with an *in vitro* crosslinking assay. Taken together, using a variety of biophysical methods, we have consistently found $cPLA_2$ to oligomerize through its C2 domain *in vitro* and in cells.

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Cubic - Inverted Hexagonal Phase Transition Kinetics in Monoolein-Sucrose Mixtures

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Sugars play key roles in the biology, yet much remains unknown about their interactions with lipids. In particular, we examine the effect of different concentrations of sucrose-water solutions on the cubic - inverted hexagonal transition in monoolein. Using DSC (differential scanning calorimetry), we ramp the temperature up and down through the transition and measure the ramp-rate