find that even small spontaneous curvatures have a very strong effect on the behavior of nanoparticles in contact with membranes. For a single nanoparticle interacting with a membrane, we predict four different stability regimes, leading to free, partially engulfed, or completely engulfed particles, as well as particles displaying bistability between the free and completely engulfed states. For the case of many nanoparticles in contact with a vesicle, we predict several distinct engulfment patterns, which should be observable in the optical microscope using fluorescently labeled particles. These patterns can be explored, e.g., by varying the particle size or the area-to-volume ratio of the vesicles.

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Membrane Fluidity in Cancer Cell Membranes as a Therapeutic Target: Validation using BPM 31510
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Membranes in cancer cells are relatively more fluid compared to healthy cells. Higher membrane fluidity in cancer cells closely relates to their invasive potential, proliferation, and metastatic ability. Normalization of membrane fluidity in cancer cells represents a novel therapeutic modality, however, there are no strategies currently focused on targeting this modality as cancer therapeutics. . This study describes the introduction BPM 31510, a proprietary CoQ10 based liposomal formulation that specifically targets cell membrane fluidity as one of the modalities influenced in cancer cell to effectuate a therapeutic end-point, i.e decrease in cell proliferation. First, CoQ10 concentrations was systematically varied in the liposomal formulation and membrane rigidity (Fluorescence Anisotropy) measured as function of temperature. Increasing concentrations of CoQ10 was associated with progressive and significant increase in rigidity of liposomal membranes followed by decrease at higher concentration. Interestingly, the concentration at which the local maxima in rigidity occurred matched with the composition of BPM 31510. Later, we demonstrate that BPM 31510 treatment temporarily increases cell membrane rigidity that orchestrates adaption in lipidome, proteome, and cell bioenergetics. To better understand differential response to BPM 31510, a spectrum of cancer and healthy cells were stratified based on intrinsic membrane rigidity, cell bioenergetics, and proliferation rates, and relative changes following treatment with BPM 31510 were compared. Collectively, the data provides novel insight into CoQ10 effect on cell membrane dynamics, suggesting an integration of biophysical, biochemical and molecular effects attributable to BPM 31510 mechanism of action in the treatment of cancer. Overall, the study provides compelling data in support of targeting of membrane fluidity, a biophysical characteristic of cell, as a novel target amenable to pharmacological manipulation in the treatment of cancer.

Biophysical Techniques for the Study of Protein-Lipid Interactions

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Using CW-EPR to Explore Substrate Binding and the Mechanism of TonB-Dependent Transport in BtuB
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Outer-membrane TonB-dependent transporters function in the uptake of essential nutrients, and are important for the success of many pathogenic bacteria These proteins consist of a 22 stranded β-barrel where the N-terminal 130 to 150 residues form a core domain that fills the barrel. During transport, these proteins undergo a cycle of binding and unbinding to the inner membrane protein TonB, through an interaction that is mediated by the Ton box, an energy-coupling segment near the transporter N-terminus. Over 50 high-resolution crystal structures have been obtained for 12 different TonB-dependent transporters, however the mechanisms of substrate transport remain unclear. During the coupling of the Ton box to TonB, transport is thought to involve a transient unfolding or rearrangement of the N-terminal core promoting the release of the substrate to the periplasm. Utilizing a combination of site-directed spin labeling (SDSL) and chemical denaturation we have examined the thermal stability of the core domain in the Escherichia coli vitamin B12 transporter, BtuB, as well as the effects of substrate binding on the stability of the core. The data indicate that core unfolds in a series of steps and that substrate, which alters the stability of the Ton box, also alters the thermal stability of the core. Pulse EPR methods are being used to determine the steps that occur during transport and to determine the position and binding sites for the substrate within the transporter.
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Cytoplasmic Domain of Dengue Virus Protein NS4A Preferentially Binds Highly Curved Membranes
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Dengue virus (DENV) is a mosquito-transmitted virus that causes dengue fever, dengue hemorrhagic fever and dengue shock syndrome. There is no vaccine available against DENV and no specific treatment for dengue fever. DENV is believed to replicate its RNA genome in association with modified intracellular membranes. DENV non-structural protein 4A (NS4A) has been implicated in the formation of the viral RNA replication complex (RC). However, the details of RC assembly are incompletely understood. We have previously identified a conserved region in the N-terminal 48 amino acids of NS4A containing putative amphipathic helices (AH). Mutations (L6E; M10E) designed to reduce the amphipathic character of the predicted AH, abolished viral replication and reduced NS4A oligomerization [1]. Solution state NMR spectroscopy was used to study the structure of recombinant wild type NS4A a.a. 1-48 peptide and a double mutant NS4A(1-48, L6E;M10E) peptide in the presence of membrane mimicking SDS micelles. The peptides are basically unstructured in aqueous buffer. However, two α-helical segments separated by a non-helical linker are observed for both peptides in presence of SDS micelles. Addition of liposomes induced formation of α-helical secondary structure in the wild type NS4A(1-48) but not in the mutant peptide. We used surface plasmon resonance, floatation assays, and circular dichroism spectroscopy to analyze the binding of recombinant NS4A(1-48) peptides to liposomes. We found that NS4A(1-48) binds to liposomes in a membrane curvature-dependent manner. The AH mutations reduced the affinity of NS4A(1-48) for lipid membranes. These results suggest that the two AHs in the N-terminus of NS4A may be crucial for membrane binding, curvature sensing and stabilization. Better understanding of the molecular details of the DENV RC formation might lead to novel anti-DENV strategies.
[1] O. Stern et al. (2013) *J. Virol.* 87:4080-85

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pH Titration and Acid Activation of the Full-Length Influenza a M2 Proton Channel in Lipid Bilayers
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The Influenza A M2 protein forms an acid-activated proton-selective tetrameric channel, in which His37 acts as both the pH sensor and selectivity filter. Here we report on the exquisite pH-dependent changes in conformation and dynamics of the His37 tetrad, for the first time, in the full-length (FL) protein, as probed by solid-state NMR spectroscopy (2D 13C-13C, 15N-13C, and 1D 15N) in lipid bilayers. From pH 8.8 to 7.3, the NMR signals show three different tau His37 sidechain conformations and one pai tautomer, with one of the tau Ne2 peaks significantly down-field shifted implicating a strong interaction (e.g., hydrogen bonding) at this site. At pH 6.6 the His37 tetrad starts to be protonated with the arising of new signals from the charged His37 and at below pH 5.8 the tetrad starts to enter the triply protonated state by quantification calculation. The narrow pH range over which the first two protons are added suggests cooperativity. Linewidths are significantly broadened at pH 5.8; this and additional data indicate considerable dynamics and conformational exchange. In addition, a cross peak between a tau Ce1 and a charged Cd2 appears at pH 5.8, indicating a short distance between them. The more complex spectra of M2FL than that of the M2 truncated versions reveal a functional mechanism in which the channel is blocked by His37-His37 hydrogen bonding at neutral pHs, whereas at low pHs sidechain dynamics allows the permeating proton to transfer from the extracellular side of the His37 tetrad to the intracellular side.