of spectroscopic and calorimetric techniques was used to investigate the unfolding of both DNA molecules and polycation-DNA complexes, and to determine their thermodynamic binding profiles. The resulting polycation-DNA complexes were stable in aqueous solution at room temperature. The binding of each copolymer to DNA stabilized the helix-coil transition of all DNA molecules, yielding binding affinities of ~104 M-1, which were lowered by the increase in salt concentration. However, binding affinities of 105 were obtained with the ethhidium bromide displacement essay. Isothermal titration calorimetric experiments yielded negligible heats of interaction. Therefore, the favorable formation of the copolymer-DNA complexes is entropy driven which was rationalized in terms of the release of both counterions and water molecules upon complex formation. In summary, polycation binding to DNA was found to be electrostatic in nature, i.e., the positively charged lysine groups formed ion pairs with the negatively charged phosphate groups of DNA. Supported by Grant MCB-1122029 from the National Science Foundation.

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Correlating Drug Binding Affinities with Base Pair Opening Rates in DNA

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The cyclic AMP responsive element (Cre) is a highly conserved stretch of DNA that is involved in the activation of gene transcription. Our group has studied the drug binding of a fluorescent derivative of a DNA intercalating anti-cancer drug, 7-amino actinomycin D (7-AMD), to the Cre sequence in different sequence contexts. We initially analyzed the DNA backbone conformation of Cre samples with varying flanking sequences and correlated these values to the binding affinities of 7-AMD. These studies revealed several anomalies that suggest that the conformation described by BI/BII content of the DNA backbone is, at most, only partially responsible for the 7-AMD binding affinity to the Cre sequence. This result is not surprising as 7-AMD has a conjugated ring structure in addition to its peptidyl side chains which interact with the backbone. As a result, we began studying DNA base pair opening rates and correlating these with 7-AMD binding affinities to account for the intercalation of 7-AMD into the Cre sequence. DNA base pair opening rates were determined by tracking imino proton exchange via NMR spectroscopy in the presence of a varying concentration of base catalyst. In this study, both two-dimensional NOESY and one-dimensional H^1 NMR spectroscopy are used to track the change in line widths of the imino protons of the central Cre binding site for five sequences with varying flanking sequences. Trends in opening rates reveal that sequences with strong 7-AMD binding feature slower base pair opening dynamics than sequences with weaker 7-AMD binding. These results suggest that local base stacking is important for 7-AMD binding, a hypothesis we are currently investigating with UV spectroscopy.

Platform: Exocytosis, Endocytosis, and Membrane Fusion

1772-Plat

Microtubule Motors Drive Plasma Membrane Tubulation in Clathrin-Independent Endocytosis

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How the plasma membrane is bent to accommodate clathrin-independent endocytosis is poorly understood. Recent studies suggest the exogenous clathrin independent cargo molecules Shiga toxin and cholera toxin induce the negative membrane curvature required for endocytic uptake by binding and crosslinking multiple copies of their glycosphingolipid receptors on the plasma membrane. But it remains unclear if toxin-induced sphingolipid crosslinking provides sufficient mechanical force for deforming the plasma membrane, or if host cell factors also contribute to this process. To test this, we imaged the uptake of cholera toxin B-subunit into surface-attached tubular invaginations in live cells. We found that a cholera toxin mutant that binds to only one glycosphingolipid receptor accumulates in tubules, and that toxin binding is entirely dispensable for membrane tubulations to form. Unexpectedly, the driving force for tubule extension was found to be supplied by the combination of microtubules, dynein, and dynactin, thus defining a novel mechanism for generation or extension of membrane curvature during endocytic uptake at the plasma membrane.

1773-Plat

High-Speed Atomic Force Microscopy of ESCRT Protein Assembly

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The endosomal sorting complex required for transport (ESCRT) mediates membrane remodelling in cells. When ESCRT oligomerize, it is able to bud the membrane forming constriction necks that will break resulting in vesicular bodies or the viral envelope, to name a few of its implications. So far, relatively little is known about the molecular fine structure and less about the dynamics of ESCRT assembly, essential for our understanding how it deforms and cleaves the membrane.

In this work, we used high-speed atomic force microscopy (HS-AFM) to study the ESCRT machinery, in particular the ESCRT-III complex, Snf7. HS-AFM allows simultaneous observation of structure, dynamics and function of biological assemblies, with nanometer spatial and sub-second temporal resolution. We show HS-AFM movies of the Snf7 complex formation and its dynamics from filament to the maturated circular assembly around the membrane constriction site. We observe interfilament dynamics that provide a basis for a mechanistic explanation how the machinery creates tension for membrane fission by a buckling mechanism.

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Mechanisms of Membrane Shaping by Peripheral Proteins

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Membrane curvature has developed into a forefront of membrane biophysics. Numerous proteins involved in membrane curvature sensing and membrane curvature generation have recently been discovered, and the structure of these proteins and their multimeric complexes is increasingly well-understood. Substantially less understood, however, are thermodynamic and kinetic aspects and the detailed mechanisms of how these proteins interact with membranes in a curvature-dependent manner. New experimental approaches need to be combined with established techniques to be able to fill in these missing details. Here we use model membrane systems in combination with a variety of biophysical techniques to characterize mechanistic aspects of the function of peripheral proteins such as BAR domains, ENTH domains, and synucleins. This includes a characterization of membrane curvature sensing and curvature generation. We also establish kinetic and thermodynamic aspects of BAR protein dimerization in solution, and investigate kinetic aspects of membrane binding. We present two new approaches to investigate membrane shape instabilities leading to stable membrane curvature. We demonstrate that membrane shape instabilities can be controlled by factors such as protein binding, lateral membrane tension, lipid shape and asymmetric bilayer distribution, and macromolecular crowding on the membrane.

Our findings are relevant to the mechanistic understanding of membrane trafficking phenomena, including endocytosis.

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Role of Hemagglutinin Palmitoylation in Assembly and Fusion of Influenza Virus-Like Particles

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Influenza A virus is a major human pathogen causing annual epidemics and occasional pandemics. Hemagglutinin (HA), the influenza virus fusion protein, contains on its cytoplasmic tail three conserved cysteins which are palmitoylated. Contradictory data have been reported regarding the role of HA palmitoylation in either membrane fusion or virion assembly. Here we analyzed the role of HA palmitoylation on assembly and fusion of influenza virus-like particles (VLPs) by using fusion assay, cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET). VLP assembly, release, morphology, as well as glycoprotein spacing on the surface of the VLP, were not affected by mutation of all three cysteins. However, using both cell-cell and VLP-cell fusion assays we found that palmitoylation plays a role in fusion pore enlargement. We tested HA from three different influenza strains (H2 (A/Japan/305/57), H3 (A/Aichi/2/68), H3 (A/Udorn/72)). In all cases HA depalmitoylation impaired pore enlargement, suggesting that the role of palmitoylation in