have high affinity for specific materials (carbon nanotubes, glass, polystyrene) were studied for the first time at the single molecule level with optical tweezers. One-micron DNA tethers are used to connect the peptide to the trapped bead. Single-molecule force spectroscopy studies revealed similar rupture forces between the aptamers of ~20pN at loading rates in the range of 1-10pN/s. Optical tweezers were found to be a powerful tool to probe this type of non-covalent biomolecular interactions.

The single molecule rupture force probabilities are fitted to force spectroscopy models [1] in order to extract information such as the lifetime of the aptamer-material bond as a function of force, the distance along the pulling direction between the free-energy minimum and the transition state, and the free energy of activation.

In order to demonstrate the generality of our single molecule assay for several types of biomolecular interactions, antibody-antigen rupture forces were also measured following the same procedure and kinetic information was obtained from the fits. Extracting off-rates from single-molecule techniques can aid ligand optimization, receptor design, and screening processes. We include a comparison study of the interaction between the antigen fluorescein and its murine monoclonal antibody, clone 4-4-20 using this tethered bead assay.

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3210-Plat

Strength of Non-Covalent Biomolecular Interactions Probed at the Microsecond Timescale

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We have measured strength of non-covalent interactions at the microsecond timescale by using a recently developed high-speed force spectroscopy technique. The resulting loading rates narrow the gap between time scales of experimental methods and molecular dynamics simulations substantially. Measurements on biotin-streptavidin complexes provide direct experimental verification of forces predicted by steered molecular dynamics simulations [1]. This technique uses a T-shaped atomic force microscope cantilever with its tip placed offset from the longitudinal axis [2,3]. When this cantilever vibrates at its vertical resonance, instantaneous forces acting on the tip are detected by the twisting motion due to its large mechanical bandwidth. As a result, force-distance curves are generated at every cycle of the vertical oscillations. Approximately ten thousand force curves are generated every second and analyzed in real time. The dramatic enhancement in measurement speed also enables a chemically specific imaging technique based on single molecule force spectroscopy.

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Workshop 4: Membrane Zoology: Model Membranes of Increasing Complexity

3211-Wkshp

Measurements of Reductionist Membranes that Beautifully Fit Physics Theories

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Micron-scale liquid domains appear in lipid membranes containing three lipid types (lipids with high melting temperature, lipids with low melting temperature, and cholesterol or a similar sterol) when the membrane is below a miscibility transition. When this transition occurs at a critical point, large fluctuations appear within the membranes. The fluctuations are described by beautiful physics: the critical exponents for correlation length and for the difference in composition between the two phases are consistent with the universality class of the 2-dimensional Ising model (Honerkamp-Smith et al., BJ, 2008). Complex mixtures of lipids and proteins derived from cell membranes in GPMVs (giant plasma membrane vesicles) exhibit the same critical behavior (Veatch et al., ACS Chem. Biol., 2008). Recently, we measured the effective dynamic critical exponent relating the decay time of membrane composition fluctuations to the wavenumber (an inverse length). We find that at temperatures far from the critical point, the exponent is 2, as expected from diffusion. As the temperature approaches the critical point, the exponent increases. We find that submicron membrane fluctuations corresponding to a wavenumber of 1/(50nm) persist for at least 0.8 ± 0.3ms, on the order of times required for changes in protein configuration (e.g. 1ms). Therefore, similar and longer-lived fluctuations in cell membranes can potentially alter protein function.

3212-Wkshp

Membrane Interactions Mediated by DNA Hybridization

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Our lab has been involved for some time in the development of strategies for assembling and partitioning model membranes on solid supports. While ideally suited for analysis by surface-sensitive methods, the close proximity of the lower leaflet of the supported bilayer to the solid support limits its application, especially for transmembrane proteins. In order circumvent this limitation, we have developed three model membrane architectures in which the bilayer is separated from the support: tethered vesicles using DNA-lipid conjugates which can be used to study vesicle-vesicle interactions and fusion; structures that position a black lipid membrane in close proximity to a highly reflective mirror for interferometry in combination with electrical measurements; and membrane patches tethered to solid supports or to fluid supported bilayers using DNA-lipid conjugates. Each architecture offers specific advantages and opportunities, and recent results will be described.

3213-Wkshp

The Language of Shape: Biological Reactions are Dramatically Affected by the Shape of Lipid Membranes

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To date the fields of biophysics, biochemistry, molecular and cellular biology and have established exhaustive correlations between the lipid composition of membranes and its impact on membrane properties and protein function. However, in addition to composition the shape of cellular membranes appears to be a well-conserved phenotype in evolution. The characteristic membrane topology of organelles e.g. the folded structure of the endoplasmatic reticulum, is strictly retained in most types of cells. Nevertheless we largely ignore what are the consequences of membrane shape/curvature to biological functions that make it so critical for sustaining life. The lack of information on the significance of membrane shape has predominantly been due to the absence of reliable assays that allow us to perform systematic experiments as a function of membrane shape/curvature. We have recently demonstrated the possibility to construct a high throughput array of unique nanoscale membrane curvatures. The assay is based on unilamellar liposomes of different diameters (30 nm to 700 nm), and therefore curvature, that are immobilized on a surface at dilute densities allowing for imaging of single liposomes with fluorescence microscopy.

Here I will discuss published and unpublished data on two important classes of biomolecular interactions that exhibited dramatic curvature dependence: i) SNARE-mediated docking of single lipid vesicles and ii) membrane anchoring of lipidated proteins, and reveal previously unsuspected consequences of membrane curvature to biological function.

References: Biophys. J. 2008. 95 (3): p. 1176; PNAS. 2009, 106 (30): p. 12341; Angew. Chem. Int. Ed. 2003, 42, p. 5580; Nat. Chem. Biol., doi:10.1038/nchembio.213; EMBO J., in press; Methods in Enzymol., in press.

3214-Wkshp

Sorting of Proteins and Lipids in Membrane Curvature and Composition Gradients

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The sorting of lipids and proteins in cellular membrane sorting centers such as the trans-Golgi network, the plasma membrane, and the endocytic recycling compartment, lies at the heart of fundamental biological phenomena such as organelle homeostasis, membrane signaling, and trafficking. Our research is directed at understanding biophysical contributions to the sorting of membrane components, using experimental lipid model membranes, and analytical thermodynamic and membrane elasticity theory.

We will present measurements of thermodynamically reversible membrane curvature sensing for several peripherally binding membrane proteins, including toxins, endocytic accessory proteins, as well as naturally unfolded proteins. For example, whereas the cholera toxin subunit B is observed to partition away from regions of high positive membrane curvature, we show that the Epsin N-terminal homology domain enriches in such regions.

Our recent research has furthermore shown that ideally diluted lipids are not significantly sorted in curvature gradients presented by a cylindrical membrane