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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[3] M. D. Dong, S. Husale, and O. Sahin, ''Determination of protein structural flexibility by microsecond force spectroscopy'' *Nature Nanotechnology* **4** 514-517 (2009).

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.

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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

in contact with a large thermodynamic reservoir (a giant unilamellar vesicle). This finding is consistent with analytical theories as well as molecular dynamics simulations and indicates that lipid sorting needs to be amplified by cooperative interactions, as is indeed observed in vesicles composed of ternary lipid mixtures. Two regimes of cooperatively amplified curvature demixing are distinguished: a) the sorting in the weak segregation limit in compositions near a demixing phase boundary and b) the sorting in the strong segregation limit, deep in the coexistence region. We will describe both regimes by means of thermodynamic models and also discuss dynamic aspects of curvature sorting.

3215-Wkshp
Spatial Organization and the Mechanics of Signal Transduction in Cell Membranes
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Signal transduction in living cells is carried out through cascades of chemical reactions, which generally begin on the cell membrane surface. In recent years, there has been growing realization that the large-scale spatial arrangement of cell surface receptors can regulate the outcome of ensuing signal transduction process. Signaling through the T cell receptor (TCR) in the context of the immunological synapse provides a case in point. Spatial reorganization of TCRs occurs on multiple length-scales, and apparently with multiple purposes, during antigen recognition by T cells. The cell membrane and cytoskeleton, working as an inseparable unit in this case, create the mechanical framework within which TCR signaling processes occur. To better study these phenomena, a new experimental strategy, in which the spatial positions of cell membrane receptors are directly manipulated through mechanical means, has emerged. By physically inducing a 'spatial mutation' of the signaling apparatus, the role of spatial organization in signal transduction as well as the mechanisms by which it arises can be illuminated. Specific applications of this strategy to TCR signaling and other cell-cell signaling systems will be discussed.

Workshop 5: Superresolution: Imaging and Probes

3216-Wkshp
PALM-Based Super-Resolution Imaging and its Applications
Jennifer Lippincott-Schwartz, Suliana Manley, Dylan Burnette, Jennifer Gillette, George Patterson.
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Superresolution techniques such as photoactivated localization microscopy (PALM) enable the imaging of fluorescent protein chimeras to reveal the organization of genetically-expressed proteins on the nanoscale with a density of molecules high enough to provide structural context. Here, various new applications of this recent technology will be discussed. One application involves dual-color PALM imaging of PA-GFP and PAmCherry fused to different proteins-of-interest. Imaging is performed using low level 405 nm laser for simultaneous activation of the two chimeras followed by sequential collection of 488-nm excited PA-GFP and 561-nm excited PA-mCherry single molecule fluorescence. Using dual-color PALM imaging, we show the fine architecture and molecular specification of the ER-Golgi interface and the midbody during cytokinesis. Another approach combines the techniques of PALM and single particle tracking to resolve the dynamics of individual molecules by tracking them in live cells. Called single particle tracking PALM (sptPALM), the technique involves activating, localizing and bleaching many subsets of photoactivatated fluorescent protein chimeras in live cells. By obtaining spatially-resolved maps of single molecule motions through sptPALM, we explore the behavior of proteins embedded in the plasma membrane and characterize the directed motions of actin molecules at the cell cortex. Examples such as these will be presented to illustrate the value of PALM-based super-resolution imaging in providing quantitative insights into protein organization and dynamics at the nanoscale.

3217-Wkshp
iPALM: 3D Optical Imaging of Protein Locations at the Nanometer Level
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Accurate determination of protein locations in 3D gives insight to cellular organization on the molecular scale. Here we describe a single photon, simultaneous multi-phase interferometric technique, providing 10-nm vertical localization. When combined with photoactivated localization microscopy PALM which can provide molecular coordinate based 2D resolution, a new technique termed iPALM resolves 3D molecular coordinates of individual fluorescent protein-tagged proteins with sub-20 nm resolution. The excellent photon sensitivity enables it to maintain these high resolution standards with the less bright but biologically preferable endogeously labeled fluorescent proteins. This technique is applied to plasma membranes, microtubules, endoplasmic reticulum, and focal adhesions. In the focal adhesions several protein specific layers can be characterized with < 4 nm reproducibiliy using iPALM.

3218-Wkshp
Structured Illumination and Image Inversion Interferometry
Kai Wicker, Marie Walde, Enno R. Oldewurtel, Liisa Hirvonen, Ondrej Mandula, Simon Sindbert, **Rainer Heintzmann**.
Kings College London, London, United Kingdom.
An overview of recent advances in high resolution fluorescence microscopy will be given.
In structured illumination the sample is illuminated with a number of different patterns of light. In our case this is a series of sinusoidal grids at different grid positions and orientations generated by a programmable spatial light modulator or a physical phase grating. Experimental datasets acquired under these conditions and reconstructed results from these data, demonstrating a resolution improvement of up to a factor of two over standard widefield microscopy are presented.
The non-linear approach of saturating optical transitions (for structured illumination as well as beam-scanning approaches) has a great potential especially in combination with photo-switchable dyes such as the recently described IrisFP protein from Ulrich Nienhaus'group or the Cy3-Alexa647 system used in Xiaowei Zhuang's group. An interesting approach is to push molecules into dark states in a patterned way shortly before imaging and exploiting the saturation of this transition.
Finally a method will be presented in which the emitted fluorescence of a confocal microscope passes through two separate paths. These paths are interferometrically recombined in such a way that the images undergo a mutual rotation of 180 degrees. The self-interference of the fluorescent light is only constructive, if it originated from the optical axis of the scanning laser beam, thus leading to an efficient detection of a high resolution fluorescence images.
K. Wicker, S. Sindbert, R. Heintzmann, Characterisation of resolution enhancing image inversion interferometers, Optics Express 17, 15491-15501, 2009
L. Hirvonen, K. Wicker., O. Mandula and R. Heintzmann, Structured illumination microscopy of a living cell, Europ. Biophys. J. 38, 807-812, 2009

3219-Wkshp
Exploring Membrane Dynamics by Fluorescence Nanoscopy
Christian Eggeling[1], Veronika Mueller[1], Christian Ringemann[1], Steffen J. Sahl[1], Marcel Leutenegger[1], Günter Schwarzmann[2], Vladimir Belov[1], Andreas Schönle[1], Stefan W. Hell[1].
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Cholesterol-assisted lipid interactions such as the integration into lipid nanodomains ('rafts') are considered to play a functional part in a whole range of membrane-associated processes, but their direct and non-invasive observation in living cells is impeded by the resolution limit of >200nm of a conventional far-field optical microscope. We report the detection of single diffusing lipid molecules in nanosized areas in the plasma membrane of living cells using the superior spatial resolution of stimulated emission depletion (STED) far-field nanoscopy. Combining a (tunable) resolution of down to 30 nm with tools such as fluorescence correlation spectroscopy (FCS) or other single-molecule techniques, we obtain new details of molecular membrane dynamics. For example, unlike phosphoglycerolipids, sphingolipids or 'raft'-associated proteins are transiently (~ 10 ms) trapped on the nanoscale in cholesterol-mediated molecular complexes.

3220-Wkshp
High-Contrast Fluorescence Imaging Using new Optical Switches and Optical Lock-in Detection Imaging Microscopy
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We have developed a new microscope imaging technique to isolate specific fluorescence signals from background signals by using lock-in detection of the modulated fluorescence of a unique class of optical probe. This optical lock-in detection (OLID) approach involves modulating the fluorescence emission of an optical switch probe through defined optical manipulation of its fluorescent and non-fluorescent states - a digital lock-in detection method is employed to isolate the modulated signal of interest from non-modulated signals in the sample, such as conventional fluorescent probes and natural fluorophores. I will discuss the spectroscopic and photochemical properties of several new synthetic and genetically-encoded optical switches and illustrate their applications in high contrast (OLID) imaging studies of specific structures and proteins in cultured cells and in living organisms.