abilistically appear in a GUV. In such cases, the GUV first expanded its surface area at constant volume then suddenly changed to expanding its volume at constant area. Pore formation in individual GUVs exhibits three effects that have been deduced from the material properties of peptide-lipid mixtures:

1. binding of amphipathic peptides to the membrane interface stretches the membrane area,
2. when the membrane is stretched beyond a threshold value of fractional area expansion, pores are formed, and
3. peptide-induced pores are stable and have a well-defined size.

The statistical manner by which pores appeared in peptide-bound GUVs is consistent with pores occurred in stretched vesicles of pure lipids; both are dictated by nucleation of precursor defect. Knowing these principles will allow us to design molecules that can open pores in membranes in a controllable fashion for gene transfer and drug delivery.

Workshop 1: Modeling the Membrane

156-Wkshp Thermodynamics of Lipid Bilayer Perturbations

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Computer simulations have reached a point where thermodynamic properties of processes such as pore formation, lipid flipflop, and partitioning of small molecules can be accurately calculated. Simulation of pore formation by mechanical force or electric fields has revealed the molecular architecture of pores and the determinants of pore formation and stability. Free energy calculations on lipid flipflop show that the energy barrier in the process corresponds to the formation of small water-filled headgroup-lined pores. From this barrier, accurate flipflop rates and passive diffusion rates for ions can be calculated. Defects also play a key role in the energetics of partitioning of polar and charged amino acid side chains. Detailed simulations are required to calculate the energies involved in these processes; continuum models break down because they cannot take into account the flexibility of a lipid bilayer at the molecular scale. The results of systematic detailed simulations are useful in developing next-generation coarse grained models to simulate membranes at much larger length scales and much longer time scales.

157-Wkshp Concerted Simulation and Experimental Studies of Membrane Structure and Dynamics

Doug Tobias

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Over the past decade and a half, molecular dynamics (MD) simulations have emerged as a valuable tool for filling in details of the molecular structure, interactions, and dynamics of membranes that are not available from experiments. The images produced by MD simulations not only underscore the inherent disorder in membranes and the breadth and chemical heterogeneity of the membrane-water and membrane-protein interfaces, but they also offer the possibility of identifying and characterizing the myriad of interactions taking place, and dynamical processes playing out, at the atomic level. The exquisite detail afforded by MD simulations has also proven useful in the validation or refinement of models used in the interpretation of data from a variety experiments probing membrane structure and dynamics. In this talk I will demonstrate the power of a concerted simulation and experimental approach to studying membranes using four examples. With the first example I will show how neutron diffraction experiments inspired by MD simulations have revealed a greater degree of disorder in lipid acyl chains than was previously imagined. Next, based on MD simulations restrained using electron spin resonance and fluorescence quenching data, I will suggest that peripheral membrane proteins sculpt lipids to construct self-induced docking sites in membranes. In the third example, which is relevant to voltage sensing in voltage-gated ion channels, I will use MD simulations to rationalize the remarkably small energetic penalty, measured by translocon-mediated insertion experiments, for placing helical peptides containing arginine residues in transmembrane helical configurations. In the final example, I will show how we are using a combination of neutron spectroscopy and MD simulations to unravel the complex web of dynamical couplings between a membrane protein and its lipid and aqueous surroundings.

158-Wkshp Synthetic Peptides As Models For Intrinsic Membrane Proteins

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The aim of our research is to determine how the lipid environment can influence the structure and organization of membrane proteins by affecting their transmembrane segments. For this purpose we employ designed model peptides that mimick transmembrane parts of proteins. These peptides are incorporated into well-defined synthetic lipid bilayers of varying composition, and the systems are studied by a range of biophysical approaches, including solid state NMR methods, fluorescence spectroscopy, and mass spectrometry. This allows us to analyze in detail the influence of lipids on structural properties of transmembrane protein segments, such as structure and dynamics of the peptide backbone, helix tilt, and direction of tilt. In particular we focus on how these properties are affected by the extent of hydrophobic matching and what the role is of interfacial anchoring interactions of amino acids that flank the hydrophobic transmembrane segments. Results of these studies and

their implications for the behavior of natural membrane proteins will be discussed.

159-Wkshp Dynamics and Lateral Pressure Profiles of Lipid Rafts

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The understanding of biomembrane structure and dynamics has made considerable progress recently. In part, this is due to atomistic and coarse grained simulations of membrane models, which complement experimental studies significantly. Here we discuss two cases where simulations are particularly useful. First, we consider lipid rafts, highly ordered membrane domains rich in cholesterol and sphingolipids. Recent studies by atomistic simulations [1] have turned out to provide a great deal of insight into the structural as well as dynamical properties of rafts. Not only atomic and molecular details but also large-scale properties such as elasticity can be sorted out by simulations. Of particular interest is the lateral pressure profile of a membrane exerted on a protein embedded in the membrane, since the pressure profile bridges the atomistic and continuum (elastic) limits. We show how the pressure profile depends on membrane composition, providing examples for one-, two-, as well as three-component lipid bilayers, and discuss how changes in pressure profile can be related to membrane protein activation. As a second topic, we discuss the dynamics of membranes in terms of the mechanisms of basic dynamic processes such as lateral diffusion and flip-flop. These processes take place over molecular scales, for which reason detailed simulations can provide valuable insight that is usually not within reach by experimental approaches.

References

[1]. P. Niemela, S. Ollila, M. T. Hyvonen, M. Karttunen, and I. Vattulainen. PLoS Comput. Biol. *3*, 304–312 (2007).

Workshop 2: Single Molecule Biophysics

160-Wkshp Single Molecule Beyond the Membrane(s)

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The ability to observe individual biomolecules provides a powerful tool for investigating the molecular dynamics of each cell constituent. Nevertheless, within this purely reductionist approach, the experiments are performed out of the biological context: the cell. A priori, there is no reason to exclude that cytoplasmic and nuclear cofactors regulate the properties of a specific protein and modify considerably its behavior. Single-molecule imaging in living cells has become possible recently. In comparison to more established methods using GFP, it allows for a direct determination of molecular motions without averaging over a large number of molecules and therefore permits the identification of distinct molecular subpopulations and the analysis of their kinetic parameters. In this context, new fluorescent inorganic probes such as semiconductor quantum dots (QD) provide great prospects for ultrasensitive imaging in an optically noisy environment such as live cells.

Here, we characterize the motion of individual QD-tagged Myosin V motors in living HeLa cells. The Myosins V are conjugated to the quantum dots (QDs), via a biotinylated calmodulin, and are introduced into the cells either by pinocytosis or microinjection. Single-molecule measurements provide important parameters of the myosin V, such as its velocity, processivity, step size, as well as an estimate of the force necessary to carry a QD. Our measurements bridge the gap between in vitro and in vivo experiments on individual molecular transporters and demonstrate the importance of single molecule experiments in the investigation of intracellular transport as well as the potential of single quantum dot imaging for the study of important processes such as cellular trafficking, cell polarization, and division.

161-Wkshp Force Generation at Dynamic Microtubule Ends

Marileen Dogterom

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Forces generated at the ends of dynamic microtubules (MTs) play a role in cellular processes such as chromosome motions and positioning of the mitotic spindle. These forces are either generated by polymerization or depolymerization of the MT ends themselves or by motor proteins that specifically interact with MT ends at for example cortical attachment sites. We use microfabrication techniques and optical tweezers in vitro, and automated image analysis in vivo, to understand force generation by MT polymerization both in- and outside the cell. In addition, we are interested in the mechanism of MT capture and force generation by cortical motor proteins. For example pulling forces generated on MT ends by the minus-end directed motor protein dynein seem to play an important role in the positioning of the mitotic spindle both in budding yeast and C elegans embryos.

We study the capture of dynamic MT ends by dynein motor proteins in an in vitro model system. We grow MTs against a microfabricated gold barrier, to which we specifically attach purified dynein molecules (a generous gift from the lab of Ron Vale). The MTs are nucleated from nucleation sites immobilized on the surface. MT ends that contact the barrier stop growing and surprisingly do not rapidly switch to a shrinking state, but remain in an apparently ‘captured’ state up to 30 minutes. When we repeat the experiment with MTs that are not attached to a nucleation site, the