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.

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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

MT ends that contact the barrier start to shrink with a speed slightly slower that the speed of dynein, which is much slower than the shrinkage speed of free MTs, occasionally switching back to a growing state. These results suggest that, on its own, dynein attached to a surface can capture and stabilize dynamic MT plus ends.

162-Wkshp Single-Molecule Studies of the Eukaryotic RNA Polymerase II

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Single-molecule methods allow for the real-time analysis of movement and conformational changes of proteins, nucleic acids as well as protein-nucleic acid complexes. Therefore, in contrast to other structural methods such as x-ray crystallography these methods allow for the direct visualisation of flexible domains within large multiprotein/nucleic acid complexes.

I will present a novel hybrid method of high-resolution crystallographic data and single-pair FRET measurements which enables us to find a previously unknown position of a flexible domain within a large complex whose overall structure is well known. This method is applied to elongation complexes of yeast RNA polymerase II, helping us to determine the exit pathway of the nascent RNA.

163-Wkshp Biomolecular Confinement, Mixing, and Interactions in Hydrosomes

Lori S. Goldner

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To observe the dynamics or kinetics of individual molecules or molecular complexes on timescales longer than 1 ms, it is often necessary to confine the molecule to an observation region. Techniques previously used to confine or immobilize molecules or complexes include surface attachment, gel encapsulation, and more recently liposome encapsulation [1]. Water-in-oil emulsions have been used to sequester biomolecules into discrete microdroplets; the first measurement of single enzyme activity was demonstrated in this way [2]. We introduce the use of optically trappable aqueous nanodroplets (hydrosomes) [3] for isolation, confinement and study of individual biomolecules and biomolecular complexes. Hydrosomes fuse on contact, which facilitates assembly of separate moities to form a molecular complex. In this talk I will describe techniques for generating hydrosomes and the fluorescence detection of single molecules confined in them. A comparison between single molecule-pair fluorescence resonance energy transfer (spFRET) data taken on a surface and in hydrosomes will be shown. The results of fluorescence polarization anisotropy lifetime measurements of water soluble proteins and nucleic acids in hydrosomes will be discussed; we find these molecules can be freely rotating in this confining environment. We use hydrosomes to confine and study transiently interacting molecular complexes on an individual basis. For example, building upon previous work [4], we use spFRET to study the reaction between the serpin alpha-1 protease inhibitor and the protease rat trypsin.

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Workshop 3: Structural Genomics: A Discussion

164-Wkshp Structure Genomics: An Integral Partner with Functional and Chemical Genomics for Biology and Medicine

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Just as "traditional" structural biology seeks to understand protein biochemical function via integration of structural knowledge with biochemical, biophysical and other functional data, structural genomics offers the opportunity to integrate these types of data in the context of entire genomes or protein families.

I will present examples in which structural coverage of entire protein families combined with enzymatic and/or binding specificity screens has provided functional and mechanistic information that could not have been obtained from a traditional one-protein-at-a-time approach. By using the hundreds of purified proteins generated in structural genomics we are able to

1. identify compounds that promote protein purification or crystallization,
2. identify potential inhibitors or other modulatory molecules, and
3. identify potential substrates for orphan members of gene families,
4. compare substrate or ligand specificity across a family of related proteins, and
5. map out mechanistic details of enzymatic activity by integrating structure and activity profiles for an enzyme family.

Data will be presented from The Structural Genomics Consortium (www.theSGConline.org), an international charity which focuses on human and malarial proteins, and the Northeast Structural Genomics Consortium (www.NESG.org), part of the PSI-2 which focuses on eukaryotic proteins.

165-Wkshp Structural genomics - genome inspired and enabled structural biology

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