and a laser trap based position sensing scheme. This 'Thermal Noise Imaging' can provide real-time tracking of 3D structural transitions. We present details of the technique and a comparison of thermally excited structure fluctuations with functional transitions.

1942-Plat
The Flexibility of Unbound Importin-beta studied by Molecular Dynamics
Christian Kappel, Helmut Grubmuller.
Max-Planck Institute for Biophysical Chemistry, Göttingen, Germany.
The transport of macromolecules between the nucleus and the cytoplasm takes place through nuclear pore complexes (NPC). NPCs act as an barrier against the diffusion of larger molecules. Karyopherins mediate the selective transport of proteins and RNA across this barrier.
A particularly well studied and crucial karyopherin is importin-beta. This protein binds in the cytoplasm to a cargo and transports it into the nucleus. Here, the complex is dissociated by RanGTP, which itself binds to importin-beta and is transported back into the cytoplasm, where it dissociates after hydrolysis.
All these processes are mediated by different conformations of importin-beta [1]. A number of these conformations have been resolved, revealing an inherent flexibility of importin-beta. Furthermore, recent molecular dynamics studies [2] as well as small angle x-ray scattering data [3] suggested an extended conformation of the free, unbound state of importin-beta. According to the ''loaded spring'' hypothesis, the elasticity of importin-beta plays a crucial role in this context which, however, is not accessible experimentally so far.
In this work, the energetics and the mechanical properties of importin-beta are studied by both force probe and free molecular dynamics simulations. Based on the outcome of the simulations, mechanical models are developed to further gain insight into the large scale motions of importin-beta.
[1] Conti, Muller, Stewart, Current Opinion in Structural Biology 16, 237-244 (2006)
[2] Zachariae, Grubmuller, Structure 16, 906-915 (2008).
[3] Fukuhara et. al., Journal of Biological Chemistry 279, 2176-2181 (2004).

1943-Plat
Cooperative long range protein-protein dynamics in Purple Membrane
Maikel C. Rheinstadter[1], Karin Schmalzl[2], Kathleen Wood[3], Dieter Strauch[4].
[1]University of Missouri-Columbia, Columbia, MO, USA, [2]Institut Laue-Langevin, Grenoble, France, [3]Institut de Biologie Structurale, Grenoble, France, [4]Universitat Regensburg, Regensburg, Germany.
The understanding of dynamics and functioning of biological membranes and in particular of membrane embedded proteins is one of the most fundamental problems and challenges in modern biology and biophysics. In particular the impact of membrane composition and properties and of structure and dynamics of the surrounding hydration water on protein function is an upcoming hot topic, which can be addressed by modern experimental and computational techniques. Very recently, interprotein motions in a carboxymyoglobin protein crystal were reported from a molecular dynamics simulation [Phys. Rev. Lett. 100, 138102 (2008)]. We present experimental evidence for a cooperative long range protein-protein interaction in purple membrane (PM). The dynamics was quantified by measuring the spectrum of the acoustic phonons in the 2d Bacteriorhodopsin (BR) protein lattice using inelastic neutron scattering. The data were compared to an analytical model and the effective spring constant for the interaction between protein trimers was determined to be k=53.49 N/m. The experimental results are in very good agreement to the computer simulations, which reported interaction energy of 1 meV.
[1] Maikel C. Rheinstädter, Karin Schmalzl, Kathleen Wood, Dieter Strauch, http://arxiv.org/abs/0803.0959.

1944-Plat
The Bcd Morphogenetic Concentration Gradient is Formed by Diffusion
Asmahan Abu-Arish, Cecile Fradin.
McMaster University, Hamilton, ON, Canada.
The morphogenic protein Bicoid is an essential activator of cellular differentiation and pattern formation in the fruit fly *Drosophila melanogaster*. It forms an exponential concentration gradient along the anterior-posterior axis of fly embryo and acts as a transcription factor that activates a cascade of target genes. The currently accepted model, known as the Synthesis, Diffusion & Degradation (SDD) model, assumes that the protein spreads across the embryo by simple diffusion, as was initially proposed by Francis Crick in 1970. This Model, however, has been called into question by several recent studies. To test the validity of the SDD model, we studied the localization and dynamics of a Bcd-EGFP fusion protein in live embryos using complementary fluorescence techniques: Fluorescence Recovery after Photobleaching (FRAP) and Fluorescence Correlation spectroscopy (FCS). We observed that Bcd-EGFP concentration decayed exponentially along the anterior-posterior axis of the embryo with a characteristic length of ~100 μm, as previously reported by other groups, and we estimated the absolute nuclear and cytoplasmic Bcd-EGFP concentrations at the anterior pole to be 120 nM and 15 nM, respectively. In the cytoplasm, we found that the overwhelming majority of Bcd molecules were undergoing diffusive motion, with an average diffusion coefficient D~5 $\mu m^2/s$. This is an important result, because it provides the first experimental evidence that the mobility of cytoplasmic Bcd is high enough to support the establishment of a concentration gradient across the embryo before the beginning of cellularization, as envisioned in the SDD model. We also observed that 35% of the nuclear Bcd population was engaged in transient binding to immobile structures, with an average binding time $\tau_B=1/k_{off}=120$ ms, a result consistent with the fact that Bcd functions as a transcription factor.

Platform AJ: Interfacial Protein-Lipid Interactions II

1945-Plat
Curvature and Specific Lipid-Protein Interactions Modulate Activity of Rhodopsin
Olivier Soubias, Shui-Lin Niu, Drake C. Mitchell, Klaus Gawrisch.
NIH, Bethesda, MD, USA.
Membrane composition strongly modulates the ability of photo-activated rhodopsin to achieve the G-protein binding competent metarhodopsin II conformation (MII). In particular, MII concentration increases linearly with the bilayer concentration of non-bilayer forming PE lipids. This observation has prompted the membrane curvature hypothesis, which states that the continuum elastic properties of the lipid matrix play a dominant role on MII formation. Here, we aimed to separate the effect of membrane curvature elasticity from specific interactions between rhodopsin and PE headgroups. In a series of rhodopsin-containing proteoliposomes of different intrinsic curvature the level of rhodopsin activation was determined by steady-state and time-resolved UV/vis spectroscopy, membrane order and dynamics parameters were probed by ^2H NMR and ^{13}C MAS nuclear relaxation, and the structural response of rhodopsin to changes in membrane composition was followed by circular dichroism (CD). MII formation was increased by 18:0-22:6 PC and 18:0-22:6 PE, agents promoting negative curvature and decreased by lysophosphatidylcholine which promotes positive curvature, in agreement with the membrane curvature hypothesis. However, MII formation was also augmented by curvature-neutral lysophosphatidylethanolamine. In parallel, significant changes in helical content were observed by CD. Our results suggest that the structure and function of rhodopsin are modulated not only by membrane curvature elasticity, but also by specific interactions between rhodopsin and PE headgroups. The role of headgroup hydration, cation-pi interactions or salt bridge formation between rhodopsin and the annular lipids will be discussed on the basis of NMR experiments.

1946-Plat
Synaptotagmin Perturbs The Acyl Chain Order Of Lipid Bilayer Membranes
Alex Liqi Lai, David S. Cafiso.
University of virginia, Charlottesville, VA, USA.
The perturbation of lipid acyl chain order by fusion proteins is widely reported in the membrane of viral entry and fertilization process. Synaptotagmin is the Ca2+ trigger for membrane fusion in neuronal exocytosis, and it may act by modulating lipid packing or membrane curvature strain. The effects of soluble synaptotagmin (C2AB) and separate C2 domains (C2A and C2B) on the lipid order of POPC:POPS (3:1) membrane bilayer were examined with attenuated total reflection Fourier transformed infrared spectroscopy (ATR-FTIR). Our results show that C2AB and more noticeably C2B decrease the lipid order and C2A increases the lipid order in low concentrations. However, in concentrations higher than certain threshold values, the effects reduce or even reverse. The presence of 1% PIP2 in the lipid bilayer lowers these threshold concentrations. The role of Ca++ is ambiguous: Ca enhances the perturbation effect in presence of PIP2, and reduces the effect in absence of PIP2. Experiments with membrane bilayers composed of deuterated POPC and normal POPS indicate that the change in lipid order are largely due to POPS. These data suggest that lipid demixing and membrane curvature strain may play a role in the mechanisms of Ca2 ± mediated fusion in the central nervous system.

1947-Plat
Membrane structure and the activity of phospholipase and sphingomyelinase D
Kerstin Wagner[1], Gerald Brezesinski[2], Roberto Pablo Stock[3], Luis Alberto Bagatolli[1].
[1]University of Southern Denmark, Odense, Denmark, [2]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, [3]National Autonomous University of Mexico, Cuernavaca, Mexico.