C98S) in a cardiac TnI core structure (McTnI-ND_{29}-Cys) did not affect the COOH-terminal conformation of TnI and preserved binding to TnT and TnC. McTnI-ND_{29}-Cys purified from bacterial culture was fluorescently labeled with the Alexa Fluor 532 dye and used to reconstitute troponin complex. After verifying the ratio of fluorophore to protein conjugation by spectrophotometer and SDS-PAGE, Ca^{2+}-titrations were performed for fluorescence intensity and polarization changes. The results demonstrated Ca^{2+} regulated conformational/environmental changes as well as flexibility change in the COOH terminus of TnI. Further experiments are performed to measure the Ca^{2+}-induced structural changes in reconstituted myofilaments to understand the function of TnI COOH terminal domain in calcium-regulation of muscle contraction.

1936-Plat

Cardiomyopathy Causing Mutations Stabilize an Intermediate State of Thin Filaments

Mohit C. Mathur[1], Tomoyoshi Kobayashi[2], Joseph M. Chalovich[1].
[1]Brody School of Medicine, East Carolina University, Greenville, NC, USA, [2]University of Illinois at Chicago, Chicago, IL, USA.

Congenital cardiomyopathies are initiated by changes in ATP hydrolysis and result in hypertrophy, fibrosis, and myofibrillar disarray. We studied the mechanism by which mutations in troponin and tropomyosin change ATPase rates and have linked several mutants to inappropriate switching between the inactive and active states of the actin thin filament. We have shown that troponin I mutants mimicking protein kinase C phosphorylation stabilize the inactive state of actin filaments whereas the Δ14 TnT mutant stabilizes the active state. We have now shown that two mutations on troponin I, R146G and R146W, which cause cardiomyopathy produce complex effects on ATPase activity. These TnI mutations produced increased ATPase rates in the absence of calcium and decreased rates in the presence of calcium compared to wild type. These differences were maintained at high actin concentrations. Saturating concentrations of the activator NEM-S1 equalized the rates of both the mutants and wild type. The NEM-S1 data rule out alterations in rate constants of transitions (i.e. product release) along the active pathway. The results from the R146G and R146W mutants have implications for the function of the 3 structural states of regulated actin that have been observed. That is, the results can be explained most readily if the mutants stabilize an intermediate state in both calcium and EGTA with an activity between that of the inactive and active states. In the past we have assumed that the intermediate state had properties identical to the inactive state. Our current data show that while the intermediate more closely resembles the inactive state it has unique properties. Our present results, as well as previous results, indicate that inappropriate stabilization of any state of regulated actin can result in cardiac dysfunction.

Platform AI: Protein Dynamics I

1937-Plat

Probing Conformational Motion of Serpin by Time-Resolved and Single Molecule Fluorescence

Chin Wing Ko[1], Wenhua Zhou[1], Richard J. Marsh[2], Daven A. Armoogum[2], Nick Nicolaou[2], Angus J. Bain[2], Zhenquan Wei[3], Aiwu Zhou[3], **Liming Ying**[1].
[1]Imperial College London, London, United Kingdom, [2]University College London, London, United Kingdom, [3]University of Cambridge and Cambridge Institute for Medical Research, Cambridge, United Kingdom.

Serpin (serine protease inhibitor) is a structural prototype for the study of the molecular mechanism of many diseases due to the conformational instability which leads to protein aggregation. The inhibitory function of serpin relys on a flexible loop undertaking a striking conformational transition, but this property also leaves serpin at risk of polymerization. We have investigated the conformational dynamics of the reaction center loop (RCL) of the plasminogen activator inhibitor-1 (PAI-1) by time resolved fluorescence spectroscopy. The RCL becomes more solvent exposed and exhibits faster rotation when PAI-1 interacts with an octapeptide which blocks the loop insertion pathway, indicating that the RCL is well displaced from the protein surface. A heterogeneous population model with three rotational correlation times has been developed to account for the ''dip and rise'' observed in fluorescence anisotropy decays. We have also employed single molecule FRET to probe the conformational change of serpin under different environment and the early stage of its ploymerization process. Preliminary results will be presented.

1938-Plat

Evolution of enzyme fold: Linking protein dynamics and catalysis

Pratul K. Agarwal.
Oak Ridge National Laboratory, Oak RIdge, TN, USA.

Enzymes are dynamic molecules. In the past, enzymes have been viewed as static entities and their high catalytic power has been explained on the basis of direct structural interactions between the enzyme and the substrate. Recent evidence has linked protein dynamics to catalytic efficiency of enzymes. Further, motions in hydration-shell/bulk solvent have been shown to impact protein motions, therefore, function.

Theoretical and computational studies of protein dynamics linked to enzyme catalysis will be discussed. Investigations of cyclophilin A and dihydrofolate reductase have lead to the discovery of networks of protein vibrations promoting catalysis. Results indicate that the reaction promoting dynamics in these enzymes is conserved across several species. Moreover, we have characterized the protein dynamics of a diverse super-family of dinucleotide binding enzymes. These enzymes share very low sequence similarity and have different structural features. The results show that the reaction promoting dynamics is remarkably similar in this enzyme super-family.

1939-Plat

Function And Activity Of Von Willebrand Factor Is Regulated By A Hierarchy Of Mechanical Forces

Carsten Baldauf[1], Tobias Obser[2], Antje Pieconka[3], Sonja Schneppenheim[3], Ulrich Budde[3], Reinhard Schneppenheim[2], Frauke Graeter[1].
[1]CAS-MPG PICB, Shanghai, China, [2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [3]AescuLabor Hamburg, Coagulation Lab, Hamburg, Germany.

The von Willebrand factor (VWF) is a shear-flow sensitive multimeric protein. Under normal flow conditions VWF is in a globular state, it unfolds at high shear rates and is activated for adhesion at the blood vessel wall [Schneider et al. 2007 PNAS p7899]. The elongation of multimeric VWF results in a force pulling along the VWF length axis. Based on a model of the VWF A domain organization, we performed force probe molecular dynamics simulations. We reveal the basis of two force-sensing VWF functions, and test the results by experiments. Our results indicate a competition between VWF A2 domain and glycoprotein Ib (GPIb) for the same binding site of the VWF A1 domain. When the stretching force along VWF reaches a critical point, the A1 A2 interaction is lost. The domains remain connected by a linker that gives space for GPIb to bind to the A1 domain. We thus suggest a force-dependent platelet binding to VWF as mediated by GPIb, which is experimentally testable and represents an alternative mechanism to recently published studies [Chen et al. 2008 Biophys J p1303; Lou et al. 2008 PNAS p13847].

We show how proteolysis of the VWF is activated under shear conditions. The specific proteolytic site is buried in the VWF A2 domain [Sutherland et al. 2004 J Mol Model p259]. At extreme forces as present in high molecular weight VWF multimers, the A2 domain C terminus unfolds until the ADAMTS13 cleavage site is uncovered. Introducing a disulfide bond by mutagensis prevents VWF cleavage. This explains the size regulation of VWF by ADAMTS13: larger multimers involve higher pulling forces and therefore higher unfolding rates under shear flow. Larger VWF is cleaved faster, preventing blood clots and thrombosis [in preparation].

1940-Plat

Experimental Confirmation of an NtrC Transition Pathway Predicted by Targeted MD

Janice Velos, Ming Lei, Ce Feng Liu, Phillip Steindel, Dorothee Kern.
Brandeis University, Waltham, MA, USA.

The infinitely short lifetime of transition states makes characterization extremely difficult. We have used a combination of molecular dynamics and experimental approaches to determine two important rate-limiting interactions involved in the allosteric transition of a signaling protein. Targeted MD simulations of the receiver domain of NtrC (Nitrogen Regulatory Protein C) were used to predict interactions that are important in stabilizing the transition state between the known inactive and active structures of the protein. Mutations were made to test these predictions and the rate of exchange between the two substates were measured by 15N-CPMG relaxation dispersion experiments. The results verify the importance of these key interactions in the transition pathway of NtrC. This work shows that targeted molecular dynamics together with experimental validation can be an invaluable tool at elucidating the structure and rate-limiting interactions of conformational transitions.

1941-Plat

Real-time 3D Tracking of Structural Transitions in Adenylate Kinase by Thermal Noise Imaging

Yunhsiang Hsu, Vijay Rana, Arnd Pralle.
SUNY Buffalo, Amherst, NY, USA.

Proteins have to be flexible enough to support turn-over rates up to hundreds per second, yet stable enough to maintain their three-dimensional structure over hours and days. As result of thermal excitation they fluctuate between structural conformations. We measured thermally excited structural fluctuations in the Adenylate Kinase using a site-specifically attached nanoparticle

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.