[1] A.R. Rogers, J.W. Driver, P.E. Constantinou, D.K. Jamison and M.R. Diehl, *Physical Chemistry Chemical Physics*, **11**, 4882 (2009).

2231-MiniSymp
Cargo Transport by Two Teams of Molecular Motors
Melanie J.I. Muller[1,2], Florian Berger[1], Stefan Klumpp[1], Reinhard Lipowsky[1].
[1]Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, [2]FAS Center for Systems Biology, Harvard University, Cambridge, MA, USA.
Intracellular transport is accomplished by molecular motors which pull cargos along cytoskeletal filaments. Many intracellular cargos are transported by two teams of molecular motors, for example by a team of kinesins and a team of dyneins. Such a cargo is observed to move bidirectionally along a microtubule, switching direction every few seconds.
We have developed a theoretical model for cargo transport by two motor teams, which describes the interaction of the motor teams as a stochastic tug-of-war, and which incorporates the results of single-molecule experiments [1]. This model can explain experimental in vivo data previously thought incompatible with a tug-of-war, in particular fast bidirectional motion and the response to perturbations. In addition, the model shows that the finite processivity, the low detachment force, and the low backward velocity of biological motors are favorable for bidirectional cargo transport, leading to fast motion, enhanced diffusion, and enhanced processivity of the cargo.
A similar model for cargo transport by a team of actin- and a team of microtubule based motors also results in enhanced processivity of the cargo on each filament type, in agreement with recent experimental results [2].
[1] M.J.I. Muller, S. Klumpp, R. Lipowsky, Proc. Natl. Acad. Sci. USA 105: 4609 (2008)
[2] F. Berger, M.J.I. Muller, R. Lipowsky, EPL 87: 28002 (2009)

Platform AO: Protein Dynamics II

2232-Plat
Functional Dynamics in the Enzyme Adenylate Kinase
Magnus Wolf-Watz.
Universitry of Umeå, Umeå, Sweden.
An important question in biology is how the energy landscape of enzymes can enable efficient catalysis of chemical reactions. We have undertaken an in-depth analysis of conformational exchange dynamics in the enzyme adenylate kinase (adk). Adk is an essential enzyme in higher organisms that catalyzes the reversible interconversion of AMP and ATP into two ADP molecules. The enzyme is modular and is composed of distinct ATP and AMP binding subdomains and in addition a core subdomain (where core is responsible for global thermodynamic and thermal stability). We have previously shown that substrate binding is accompanied by rate-limiting spatial displacements of both the ATP and AMP binding motifs. Here, we present a solution state chemical shift based NMR approach to probe the native energy landscape of adenylate kinase with and without its natural substrates present. Binding of ATP induces a dynamic equilibrium in which the ATP binding subdomain populates both open and closed conformations with equal weights. A similar scenario is observed upon AMP binding in the AMP binding subdomain. These structural ensembles represent complexes that are populated transiently during the enzymatic reaction cycle. Our proposed dynamic mode of protein-ligand interaction in adk stands in contrast to the traditional view of substrate/enzyme complexes as rigid, low entropy states. Finally, by using a combination of protein engineering and hydrogen to deuterium exchange experiments we have shown that the individual subdomains in adk can fold independently of each other. Independent folding of subdomains can, in principle, be utilized to accommodate the structural change during the functional open to closed transition.

2233-Plat
Molecular Details of the Apolipoprotein E and the Amyloid Beta Peptide Interaction: Analysis of a Potential Binding Site Responsible for ApoE4 Misfolding
Alex Perálvarez-Marín[1,2], Jinghui Luo[3], Jean-Didier Maréchal[2], Sebastian Wärmländer[3], Astrid Gräslund[3].
[1]Brigham and Women's Hospital, Boston, MA, USA, [2]Universitat Autònoma de Barcelona, Cerdanyola del Vallés, Spain, [3]Stockholm University, Stockholm, Sweden.
The relationship between Apolipoprotein E (ApoE) and the aggregation processes of the amyloid β (Aβ) peptide has been shown to be crucial for Alzheimer's disease (AD). ApoE4 is considered as a contributing risk factor for AD. Although various mechanisms have been proposed to explain the physiological and pathological role of this relationship, the detailed molecular properties of ApoE4 interacting with Aβ peptide are unknown. In our studies, a peptide-protein docking approach has been used to investigate the process of Aβ interaction with the N-terminal domain of the human ApoE4 isoform. The use of molecular dynamics simulations (10 ns in water) has allowed studying the interaction mechanism between the protein and the peptide. Our results show that ApoE4 forms a partially unfolded intermediate (molten globule) stabilized by the interaction with Aβ. The initial SDS-induced α-helix used as Aβ peptide model, becomes unstructured due to the interaction with ApoE4. Peptide interaction with the different ApoE isoforms changed the pattern of the salt bridges network in ApoE4 compared to ApoE4 alone. By analysis and statistics of these electrostatic interaction patterns, we present a model for the salt bridge network in the ApoE4- Aβ complex, crucial for the understanding of the interaction mechanism and relevant for potential drug design and therapeutics.

2234-Plat
Structural Dynamics by Time-Reolved EPR and Transient Time-Resolved FRET: Application to Myosin
Roman V. Agafonov[1], Igor V. Negrashov[1], Sarah E. Blakely[1], Margaret A. Titus[1], Yuri E. Nesmelov[2], David D. Thomas[1].
[1]University of Minnesota, Minneapolis, MN, USA, [2]University of North Carolina, Charlotte, NC, USA.
We have used three complementary spectroscopic techniques, pulsed EPR (DEER), time-resolved fluorescence resonance energy transfer (TR-FRET) and transient time-resolved FRET [$(TR)^2$FRET], to elucidate the mechanisms of energy transduction in myosin - an important motility protein involved in a variety of processes including muscle contraction. We engineered two double-Cys myosin mutants that were labeled with optical probes or spin labels, and determined structural changes in a single structural element of the myosin motor domain, the relay helix, as affected by nucleotide binding and hydrolysis. In all cases, time-domain detection permitted the reliable determination of the interprobe distance distribution, quantitating both order and disorder, and resolving coexisting structural states. While DEER offered superior distance resolution, $(TR)^2$FRET permitted detection of transient structural changes after rapid mixing (stopped-flow) with ATP. All methods demonstrated two structural states of myosin during the recovery stroke, corresponding to straight and bent conformations of the relay helix, in parallel with straight and bent conformations of the entire myosin head. A narrower interprobe distance distribution in the post-recovery state shows ordering of the relay helix structure during the recovery stroke. These experiments reveal changes in both structure and dynamics of the relay helix and identify it as a key player in the interdomain coupling mechanism. This methodology is applicable to any enzyme in which double Cys mutants can be engineered in a key region of energy transduction, permitting resolution of structural states and dynamics in real time during the transient phase of a biochemical reaction.

2235-Plat
Protein-Exchange Dynamics at GPCR Micro-Domains: a Case Study with the Parathyroid Hormone Receptor
Juan A. Ardura, Jean-Pierre Vilardaga, Peter A. Friedman.
University of Pittsburgh, Pittsburgh, PA, USA.
Na/H exchanger regulatory factor-1 (NHERF1) is a cytoplasmic protein that contains two PDZ domains and assembles macromolecular complexes and regulates localization, trafficking and mobility of a number of membrane transporters and receptors. Interactions among NHERF1 and its target proteins are stabilized by the bimolecular interaction of PDZ and PDZ-binding domains. The PTH receptor (PTHR) contains a PDZ-binding domain that enables direct association with NHERF1 and tethers the PTHR to the actin cytoskeleton. After activation, the PTHR is trafficked to clathrin. The fate of NHERF1 is unknown. We used PTHR as a model to identify the fate of NHERF and to determine the dynamic interactions with the PTHR. Fluorescence Recovery after Photobleaching (FRAP) and confocal fluorescence microscopy were used to measure mobility of PTHR and NHERF1 and the behavior of these proteins at the cell membrane. Fluorescent mCherryNHERF and Green Fluorescent Protein (GFP)-tagged PTHR were coexpressed in rat osteosarcoma cells. We observed a significant reduction in the mobility of PTHR in the presence of NHERF1. Upon stimulation of the receptor with PTH, PTHR internalization was triggered, while NHERF1 remained near the cell membrane. Furthermore, when the PTHR was immunologically immobilized at the cell membrane, mCherryNHERF mobility was high and equivalent to that of NHERF with non-immobilized PTHR. However, PTH interaction with PTHR caused a rapid and greater increase of NHERF1 mobility, even when the receptor was completely immobilized at the membrane. These results support the view that NHERF1 exhibits dynamic interactions with the PTHR, with receptor occupancy followed by rapid dissociation of NHERF1.