information, we have succeeded to build an atomistic model of the VS ribozyme from SAXS data [2]. A particular strength of SAXS as a solution technique is that conformations under different solution conditions can be explored. We have dissected the conformations of the glycine riboswitch [3] and of a TPT riboswitch [4] in the absence and presence of Mg2+ required for folding and in the absence of presence of the functional ligands.
Secondly, the highly negatively charged backbone of nucleic acids presents an important barrier to folding and packing and makes these processes strongly dependent on counterions. We show that low and intermediate resolution models derived from SAXS in combination with computational modeling can provide a rigorous framework to understand RNA-ion interactions and to distinguish the effects of electrostatic relaxation and specific ion binding [5].
Finally, I will describe how single-molecule experiments can present an complementary window to study the (salt-dependent) mechanical properties of double stranded DNA and RNA [6].
[1] Lipfert, et al., JAC (2007)
[2] Lipfert, Ouellet, et al., Structure (2008)
[3] Lipfert, et al. JMB (2007)
[4] Ali, et al. JMB (2010)
[5] Lipfert, Sim, et al. RNA (2010)
[6] Lipfert, et al. Nature Methods, in press

7-Subg
Studying Protein DNA Binding with PELDOR
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Pulsed Electron-Electron Double Resonance (PELDOR or DEER) is an Electron Paramagnetic Resonance (EPR) method by which distance can be measured very precisely in the range from ~1-8nm1. As Fluorescence methods, PELDOR usually requires labels but which are considerable smaller and rigid2 making a translation of distance into structure easier.
To overcome challenging label synthesis, we introduce the next generation of labels for DNA/RNA which make use of non-covalent binding. We can show that the specificity and strength of binding is large enough to yield high-quality PELDOR data yielding not only distances but also information on orientations and dynamics. This non-covalent labelling concept proves also successful for the study of protein binding and concomitant oligonucleotide bending.
Labeling the helicases Hel308 and PcrA, we have been able to resolve the ATP, ADP, and DNA induced conformational switching of these molecular motors. This clearly shows that PELDOR does not only provide mere distances but access to orientations, dynamics and changes between conformational states of large oligonucleotide/protein complexes difficult to access otherwise.
1) O. Schiemann, T.F. Prisner Quart. Rev. Biophys. 2007, 40, 1.
2) O. Schiemann, P. Cekan, D. Margraf, T.F. Prisner, S.T. Sigurdsson Angew. Chem. 2009, 121, 3342.

SUBGROUP: Bioenergetics

8-Subg
Quantitative Analysis of Integrated Energy Metabolism of Muscle Cells: Experimental and Theoretical Studies
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Quantitative analysis, in combination with detailed experimental studies, is the basis of Molecular System Bioenergetics (MSB) which takes into account specific interactions between cellular components, formation of metabolic dissipative structures and resulting system level properties such as metabolic compartmentation. There are several experimental and theoretical approaches used: studies in vivo kinetics of mitochondrial respiration regulation; Metabolic Control Analysis of integrated energy metabolism; mathematical modeling. Cardiac cells are characterized by high level of structural organization with very regular arrangement of mitochondria and their interaction with cytoskeleton. Heterodimeric tubulin containing beta II isoform is connected to mitochondrial outer membrane, specifically controls VDAC permeability and is coexpressed with mitochondrial creatine kinase. These proteins form with ATP Synthasome a supercomplex Mitochondrial Interactosome (MI) in which the kinetics of regulation of respiration by MtCK is significantly altered in comparison with mitochondria in vitro affinity for extramitochondrial ATP is strongly decreased due to limited permeability of VDAC but for creatine increased. Metabolic Control Analysis of reactions in MI showed that flux control coefficients are significantly increased for reactions of ADP/ATP recycling coupled with phosphocreatine production by MtCK. These experimental data are the basis of the mathematical model of compartmentalized energy transfer and show that about 90 % of energy is carried out of mitochondria by phosphocreatine due to specific structure of MI. This model explains well the metabolic aspects of Frank -Starling law of the heart. In cancerous HL −1 cells of cardiac phenotype MtCK and beta II tubulin are not expressed. Mathematical models based on the theory of homogenous intracellular medium and CK equilibrium do not explain adequately the experimental observations. In future development, application of single-molecule enzyme kinetics and theory of nonlinear biochemical reaction networks is necessary.

SUBGROUP: Motility

9-Subg
Tethered Diffusion and Strain-Dependent Gating in Kinesin-2 Motors
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Kinesin-2 motors carry out a number of transport roles in cells, including intraflagellar transport, melanosome dispersion, and axonal transport. In almost all cases, cargo transported by Kinesin-2 motors also have dynein attached, and the cargo display bidirectional movement. To understand the properties of Kinesin-2 motors that underlie its diverse transport roles, we are investigating wild-type and mutant mouse KIF3A/B motors. The flexible neck linker domain of Kinesin-2 is 17 residues long, compared to only 14 residues for Kinesin-1. Shortening the Kinesin-2 neck linker enhances processivity and lengthening the Kinesin-1 neck linker reduces processivity in a single-molecule fluorescence assay. From stochastic simulations of the kinesin chemomechanical cycle, these processivity differences are consistent with the hypothesis that enhancing the compliance of the neck linker domain reduces the force-dependent head-head coordination that underlies processive kinesin stepping. Interestingly, because kinesin stepping involves tethered diffusion of the free head to the next binding site, lengthening the neck linker is expected to enhance this diffusional search and thus increase processivity; however this appears not to be the case. The force-dependencies of motor velocity and processivity were also measured using an optical tweezer in force-clamped mode. Interestingly, while the speed of Kinesin-2 stepping is affected less by force than Kinesin-1, under resisting loads Kinesin-2 processivity drops steeply. This suggests that in a tug-of-war with dynein, Kinesin-2 leads to enhanced dynamics of bidirectional switching.

10-Subg
Dynamics of Myosin-X in Filopodia
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Myosin-X (Myo10) is an unconventional myosin that localizes to the tips of filopodia, has potent filopodia inducing activity, and is also required for proper spindle orientation. The Myo10 heavy chain consists of a myosin motor domain, 3 IQ motifs, and a complex tail that includes an alpha helical region, 3 PH domains, a MyTH4 domain, and a FERM domain. One of the PH domains binds to the important signaling lipid PIP3, the MyTH4 domain can bind to microtubules, and the FERM domain can bind to the cytoplasmic domain of B-integrins. We have previously used conventional fluorescence imaging to show that bright puncta of GFP-Myo10 undergo forward movements in filopodia at ~80 nm/s as well as rearward movement at the retrograde flow rate of ~20 nm/s. Using Total Internal Reflection Fluorescence (TIRF) microscope, we can now detect extremely faint particles of GFP-Myo10 that move in a rapid (578 +/−174 nm/s) and directed fashion to the tip of a filopodium. These particles appear to be at or near the single molecule level and their movements suggest that Myo10 functions as a motor for an intrafilopodial transport system. Similar movements are observed for two other proteins that localize to the tips of filopodia, GFP-VASP, and GFP-mDia2. Interestingly, faint particles of GFP-Myo5a exhibit similar movements, although at a slower velocity (~250 nm/s). The rapid movements of GFP-Myo10 in filopodia indicate the existence of a novel form of directed transport at or near the single molecule level.

11-Subg
Actin Filament Patterning by Myosin Motors
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We have studied the bulk alignment of actin filament sliding movement, driven by randomly oriented myosin molecules. We performed conventional, actin filament gliding assays, but using micromolar concentrations of actin which are roughly 100,000,000 times greater than normally used in such assays, but close to the concentration of actin found in living cells. Under these conditions, actin filament movement takes up a preferred orientation. The oriented patterns of movement extend over a length scale of ~100 micrometres,