correlated with alterations in cell homeostasis and physiological output. These changes often result in disease. Therefore controlling these signaling pathways and ion fluxes are of major importance for developing strategies, which can re-adjust and reset the altered signals. It is also important to control precisely the intracellular signaling pathways for understanding signaling events and signaling crosstalk of G protein coupled receptor pathways. Rhodopsins and green algae channelrhodopsin are light activated proteins which are currently used to control ion fluxes and GPCR pathways in cells. Here, we will present out data related to the use of vertebrate rhodopsin and channelrhodopsin for ion channel modulation in heterologous systems, control of neuronal excitability in single neurons and in spinal cord.

869-Symp Interplay between Single-Cell and Multi-Cellular Signaling during Glucose-Stimulated Insulin Secretion

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The islet of Langerhans is the functional unit responsible for glucose-stimulated insulin secretion (GSIS), and thus plays a key role in blood glucose homeostasis. The importance of the islet is demonstrated by the proven ability of islet transplants to reverse Type I diabetes pathologies in human patients. Over the last 10 years, we have been interested in understanding the multicellular mechanisms of islet function, and their role in the regulation of blood glucose under normal and pathological conditions. In many ways, the islet appears to function as a syncytium, which exhibits synchronous behavior of membrane action potentials, Ca^{2+} oscillations, and pulsatile insulin secretion across all β-cells in the islet. In other ways, the islet works as individual cells, especially in the regulation of gene transcription. Using our unique quantitative optical imaging methods and novel microfluidic devices, the *dynamics* of these molecular mechanisms can be followed quantitatively in living cells within intact islets. These investigations utilize transgenic and tissue-specific knock-out mouse models with demonstrated phenotypes, as well as traditional biochemical and molecular biological approaches.

870-Symp High-Speed Imaging of Cellular Dynamics in Freely Moving Mice Using Portable Fluorescence Microscopy

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A longstanding research goal has been to develop flexible fiber-optic fluorescence microscopes that provide micron-scale resolution, for the purpose of studying animal behavior and underlying cellular properties concurrently. To date, in vivo fluorescence imaging studies in mammalian subjects have typically been limited to anesthetized animals. To overcome this limitation, we have created high-speed, portable fluorescence microscopes based on micro-optics, fiber-optics, and miniaturized focusing actuators. We concentrated our instrumentation design efforts on the use of mice as our subjects, because of the wide availability of transgenic mouse lines with genetically targeted alterations to cellular properties and resulting behavioral deficits. Our portable microscopes are sufficiently small - about 1 cm in lateral extent and 2.5 g in mass - to be borne on the head of an adult mouse that is freely behaving but tethered via flexible fiber optics and electronic control lines. Using these microscopes we have performed high-speed imaging (up to 100 Hz frame rates) of cerebral microcirculatory dynamics, cerebellar neuronal dynamics, and Bergmann glial calcium transients in freely behaving animals. We expect that fluorescence imaging in freely moving mice will become increasingly prevalent, allowing detailed comparisons of animal behavior, physiological dynamics, and cellular properties between normal and transgenic mouse subjects.

Platform P: Protein Dynamics

871-Plat The Physics of Protein Fluctuations and Conformational Changes

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The different aspects of protein dynamics are investigated by X-ray structure analysis, Mössbauer effect with synchrotron radiation, incoherent neutron scattering and Mössbauer absorption spectroscopy. Experiments were performed in a wide temperature range. Although several different proteins have been investigated yielding similar results, only experiments on myoglobin are discussed. For this protein the most complete data set is available. X-ray structure analysis reveals structural distributions even at 0K. The slightly different structures are called Conformational Substates (CS). With the help of synchrotron radiation the density of phonons coupling to the heme iron of myoglobin was determined in a time regime between ps and fs. The corresponding mean square displacements, msd, increase linearly with temperature indicating a harmonic behavior, which was also seen in the msd obtained from X-ray structure analysis. Incoherent neutron scattering as well as Mössbauer absorption spectroscopy show a non harmonic behavior of the msd above a characteristic temperature T_c (∼180K). An analysis of the spectral shape of the Mössbauer absorption proves that molecules fluctuate between CSs if they can reach the so-called flexible state where slow Brownian oscillations of molecule segments occur. Molecules which do not reach the flexible state can not perform conformational changes. This was proved by CO and H_2O flash photolysis. The kinetics of conformational changes was investigated by the relaxation of a metastable state of myoglobin, created by reducing at low temperatures the Fe^{3+} of met-myoglobin by X-rays. The obtained Fe^{2+} low spin state with H_2O as ligand relaxes with time and temperature to deoxy-myoglobin. Molecules in the flexible state can change the conformation by surmounting the barrier between the states. This process is analyzed by Kramers theory.

872-Plat The Role Of Collective, Ms Timescale Dynamics In The Regulation Of Multi-domain Proteins. A Phosphorescence Approach

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In complex protein systems a question of general interest is, how information is transmitted through the multi-domain structure. We have used phosphorescence lifetime measurements in the temperature range of 10–270°K to assess the role of collective, ms - seconds timscale dynamics. In both cases we have avoided the use of cryosolvents, which enabled us the separation of intrinsic and slaved dynamics.

In the case of hemoglobin, we have shown [1] that upon the binding of allosteric effectors tertial structural changes take place, which affect the interdimeric interfaces. To monitor possible changes in the overall dynamics, we have used a fluorescent T-state analog, and calculated the enthalpy and entropy changes characterising the onset of this dynamics. We found a correlation between effector strength and these parameters, and suggest that collective dynamics may significantly contribute to Hb regulation.

In the case of phosphoglycerate kinase it was shown that interdomain interactions may play an important role in the folding process[2]. We have used single-tryptophan mutants to monitor possible changes in the dynamics of the subunits by the absence of the other subunit. The intrinsic dynamics was found to be sensitive to the presence of the other domain, and we suggest that coupling of the dynamics of the domains may carry an important part of the information flow between the subunits.

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873-Plat Coarse Master Equations for Peptide Folding Dynamics

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We construct coarse master equations for peptide folding dynamics from atomistic molecular dynamics simulations. A maximum-likelihood propagator-based method allows us to extract accurate rates for the transitions between the different conformational states of small helix-forming peptides. Different procedures for the construction of master equations are compared, and we find that the resulting kinetic models properly capture the character and relaxation times of the entire spectrum of conformational relaxation processes. We find that the master equation models not only give access to the slow conformational dynamics, but also shed light on the molecular mechanisms involved in peptide folding dynamics.

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2Osváth S, Köhler G, Závodszky P, Fidy J Asymmetric Effect of Domain Interactionson the Kinetics of Folding in Yeast Phosphoglycerate Kinase-ProtSci *14*, 1609–1616

874-Plat Modeling Side-chain Entropy And Correlation In Proteins' Native States

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Fluctuations within protein structures are essential to their biological function. At the same time, folded protein interiors are dense enough to be considered almost crystalline in nature. However, a vast number of side-chain rearrangements have been shown previously to be consistent with excluded-volume constraints. We use Monte Carlo sampling to explore how, in addition to these steric constraints, implicit solvent, salt bridges, and hydrogen bonds influence the exploration of side-chain conformational space in proteins with natively folded backbones. By examining the equilibrium ensemble of side-chain conformations, we have calculated an overall entropy for side-chain fluctuations in several proteins, as well as the distribution of entropy amongst the side-chains. In addition, we have quantified correlations between various side-chain motions. Results indicate that these restraints can propagate changes in structure and mobility over unexpectedly large distances.

875-Plat NMR Identification Of Transient Complexes Critical To Adenylate Kinase Catalysis

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A fundamental question in protein chemistry is how the native energy landscape of enzymes can enable efficient catalysis of chemical reactions. Adenylate kinase is a small monomeric enzyme that catalyzes the reversible interconversion of AMP and ATP into two ADP molecules. Previous structural studies have revealed that substrate binding is accompanied by large rate-limiting spatial displacements of both the ATP and AMP binding motifs. Here, a solution state NMR approach was used to probe the native energy landscape of adenylate kinase in its free form, and in complex with its natural substrates and in presence of a tight binding inhibitor. Binding of ATP induces a dynamic equilibrium in which the ATP binding motif populates both open and closed conformations with almost equal weights. A similar scenario is observed for AMP binding that induces an equilibrium between open and closed conformations for the AMP binding motif. These ATP and AMP bound structural ensembles represent complexes that are populated transiently during the enzymatic reaction cycle. The dynamic mode of protein-ligand interaction observed for adenylate kinase stands in contrast to the traditional view of bound complexes as rigid, low entropy states. Simultaneous binding of AMP and ATP is required to force both substrate binding motifs to close cooperatively enabling

the chemical step to occur. In addition, a previously unknown unidirectional energetic coupling between the ATP and AMP binding sites was discovered. Based on these and previous results we propose that adenylate kinase belongs to a group of enzymes whose substrates acts to shift preexisting equilibria towards catalytically active states.

876-Plat Protein Allosteric Dynamics in Src Kinase: From Coarse-grained and Atomistic Models

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The Src-family kinases are large allosteric enzymes playing a key role in the regulation of cell growth and proliferation. They can undergo large conformational changes, thereby "switching" between different inactive and active "states" in response to cellular signals. Computational models can be used to simulate the thermodynamic and dynamical behaviors of conformational changes in Src kinases. First, "coarse-grained" models that capture the energetics using a substantially simplified representation of the system are used to address the long timescale of transition dynamics. We construct a coarse-grained structure-based model of the catalytic domain incorporating experimental structures for the two stable states, and simulate the dynamics of conformational transitions in kinase activation. We then explore the transition energy landscapes by constructing a structural network among clusters of conformations from the simulations. Two major ensembles of pathways for the activation are identified. In one pathway, we find a coordinated switching mechanism of interactions among aC, A-loop, and beta5. In the other pathway, the conformational change is coupled to partial unfolding of the N-lobe. Second, all-atom molecular dynamics simulations with explicit solvent are used to explore the switching mechanism involved in the first pathway as revealed from the coarse-grained model. A complete transition pathway from the inactive to the active state is mapped out, for the first time, using the aggregate information from independent simulations. The activation mechanism of Src kinase is analyzed in atomic details. Taken together, these results provide a broad framework for understanding the main features of the conformational transition taking place upon Src activation.

877-Plat Real-time Sub-molecular Imaging of Single-molecule Photodynamics of Bacteriorhodopsin in Liquid by High-speed AFM

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Membrane proteins comprise about a third of the proteome of most organisms. However, determining their structure-function relationship remains a major challenge, mostly because of the absence of techniques able to directly monitor the structural dynamics of label-free, native proteins in their natural environment.

Bacteriorhodopsin (bR), the best understood membrane protein, functions as a light-driven proton pump. It exhibits structural analogies with G-protein coupled receptors: bR is composed of a 7 transmembrane alpha-helices (labeled A-G) enclosing the retinal chromophore. bR naturally occurs as trimers arranged in a hexagonal lattice with a variety of specific lipids. Spectroscopic data have demonstrated the existence of a 10–15 ms photocycle during which bR isomerizes and pumps one proton across the membrane.

Using high-speed atomic force microscopy (1), we directly image for the first time the photo-dynamics of individual bR in a saline solution at room temperature, with a spatial resolution of $\sim$5 Å at scanning rate of up to 50 frames/s ($<$ 5 ms/bR monomer). Upon illumination, we observe a large conformational change with the EF-loop moving $\sim$1 nm away from the rest of the protein. This structural change, $\sim$2 times larger than predicted by crystallography, mechanically propagates within the trimer and could be important for photo-cooperative effects. Importantly, our movies capture the thermal fluctuations essential for the protein activity, providing a new platform to complement sequential dynamics derived from crystallographic studies of bR mutants cryo-trapped in the different intermediate states of the photocycle.

Our results open unprecedented possibilities to investigate the dynamics of native membrane proteins (e.g. receptor-ligand binding) in biologically relevant conditions, in real-time and with sub-molecular resolution.

References

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878-Plat Water Dynamics In and Around Proteins

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Multinuclear magnetic relaxation dispersion measurements over wide frequency ranges combined with the use of paramagnetic surface probes to shift the effective frequency driving spin relaxation provides a comprehensive dynamical description of water translational and rotational motions at the protein surface and the rare long-lived interior water binding sites. Translational correlation times for water at the surface deduced from intermolecular proton-proton dipole relaxation and proton-electron spin relaxation are found to be in the range 6–30 ps depending on how the distance from the interface is weighted by a particular experiment. These values are within a factor of 3 of bulk water translational correlation times. Rotation at the protein surface is too rapid to measure accurately; however, the rotational correlation function has a long time tail with a correlation time of order 15 ps which may be averaged by translational displacement along the surface from a region of one surface electrostatic charge bias to a region of opposite charge bias. Quenching the global rotational motion of the protein by cross-linking reactions permits characterization of the internal dynamics of rare bound water sites. The local restricted reorientational motion of these internal water molecules in bovine serum albumin has an average correlation time of 25 ns and these sites exchange with the

bulk pool in a time of a few microseconds. Combined with the chain dynamics of the protein, these collective dynamics account quantitatively for the observable proton and deuteron spin-lattice relaxation in protein systems including whole tissues. Of particular note is the importance of the internal water molecule dynamics rather then the water at the protein surface in making a significant and sometimes dominant contribution to the spin-lattice relaxation rate constants in the range of most magnetic imaging experiments.

Platform Q: Ligand-gated Channels

879-Plat The Free Energy Landscapes Governing Conformational Changes in a Glutamate Receptor Ligand-Binding Domain

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Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels activated by the agonist glutamate. The extracellular ligand-binding domain of these receptors responds to agonist binding by undergoing a conformational change that opens a cation-permeable channel in the postsynaptic neuron. iGluRs have been the subject of intense study, and a wealth of insight has been gained into the structure and dynamics of the ligand-binding domain. The energetic basis controlling the conformational response of the ligand-binding domain underlying iGluR function, however, has yet to be revealed. We computed the free energy landscapes governing the opening/closing of the GluR2 ligand-binding domain in the apo, DNQX-, and glutamate-bound forms using all-atom molecular dynamics simulations with explicit solvent, in conjunction with an umbrella sampling strategy. The apo S1S2 is found to easily access low-energy conformations that are more open than observed in X-ray crystal structures. A conformational free energy of 9 to 12 kcal/mol becomes available upon glutamate binding for driving the conformational changes in S1S2 associated with receptor activation. Features in the glutamate-bound S1S2 free energy landscape suggest a sequence of interactions in the cleft that correspond to different states of cleft closure. Small-angle X-ray scattering profiles calculated from computed ensemble averages were found to agree better with experimental results than profiles calculated from static X-ray crystal structures. A cluster of water molecules in the cleft may contribute to stabilizing the apo S1S2 in open conformations. Free energy landscapes were also computed for the glutamate-bound T686A and T686S S1S2 mutants, and the results elaborate on findings from experimental functional studies.

880-Plat *T*esting Mechanisms Of AChR Gating By Φ-value Analysis

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After binding agonists, diliganded neuromuscular acetylcholine receptors (AChRs) gate between C(losed) and O(pen) conformations. A number of specific αsubunit structural transitions have been proposed as being important in C ↔ O gating of cys-loop receptors, including the rotation of the extracellular domain (ECD) β-core, a *cis-trans* isomerization of an M2-M3 linker proline and the perturbation of a salt bridge between R209 (in pre-M1) and E45 (in loop L2). To test these proposals, we measured rate constants and Φ values from ∼**200** different α subunit mutant constructs of the salient and nearby residues (mouse $\alpha_2\beta\delta\varepsilon$, cell-attached, HEK cells, PBS, 22°C, + 70 mV pipette, 500mM ACh/20mM choline/5mM carbachol). The ECD residues K145 and Y127 have Φ-values of 0.96 and 0.77 respectively, which suggests that these residues move early in the gating reaction but asynchronously, so a rotation of the β-core ECD, if present, is not as a rigid body. In the M2-M3 linker, mutations of P272 and G275 produced functional AChRs, so the full isomerization of these backbone bonds is not essential for gating. Overall, the M2-M3 linker had an average Φ-value of 0.64. The putative salt bridge partners E45 and R209 have similar Φ-values (0.80 and 0.74, respectively), with other pre-M1 residues either showing no sign of movement (M207, Q208 and P211) or moving late (L210; Φ=0.36). Several results argue against the salt bridge hypothesis:

(i) E45R is a gain-of function mutation,
(ii) a number of uncharged R209 mutants produce functional AChRs, and positions 45 and 209 show little energetic interaction.

We think that there is no single 'on-off' switch for the channel gating, but that the conformational trajectory through the protein is complex, with energy spread across many residues and along broad boundaries.

881-Plat Using Molecular Dynamics Simulations To Study The Channel Gating Mechanism Of The Nicotinic Receptor

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We previously reported that in target molecular dynamics (MD) simulations with the nicotinic acetylcholine receptor, structural changes at the ligand binding-sites contributed to the channel pore wideness. We also showed in the normal MD simulation that hyperpolarized electric potentials prompted cations translocation through the channel and widened the pore also. Now, we performed target MD simulations including electric-field bias as the hyperpolarized potential, to illustrate how ligand-binding triggers the cations translocation and initiates the electrical current passing through the channel.

882-Plat Crystal Structure Of A Cyclic Nucleotide Regulated Channel

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