switch distant from the binding interface propagates changes throughout the structure. Analysis of crystal structures confirms this allosteric network and suggests that the microsecond motion modules binding to particular interaction partners.

930-Plat
Allostery in PDZ3: Using Unnatural Amino Acids as Site-Specific Reporters in IR Spectroscopy to Probe Allosteric Pathways
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The third PDZ domain of the multidomain complex PSD-95 is a well studied but controversially discussed model for dynamic allosterie without major conformational changes. After the first predictions of a coupled amino acid network by Ranganathan and Lockless followed by Ota and Agard, that is potentially involved in intradomain signaling and allostery, several computational and experimental attempts were made to gain insight into the presence or absence of a wire-like pathway for information transfer in PDZ3. A recent development in vibrational spectroscopy enables us to revisit this long-standing question with a new experimental approach. Using the unnatural amino acid Azidohomoalanine (Aha) as vibrational probe at different positions of the PDZ domain, we detected the influence of ligand binding on the local microenvironment of the probe. As the azide absorption band of Aha is sensitive to even subtle changes of its environment, this approach allows us to monitor conformational fluctuations invisible to other methods. The amino acid residues tested, that are part of the predicted coupled network indeed show a change of the local microenvironment not reported experimentally before. In contrast no differences were found for residues which are not part of the predicted pathway but at similar distances from the binding pocket. This is an experimental evidence for long range communication and allostery in PDZ3.

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931-Plat
Allosteric Communication in Rnd1 and Rac1 Association with the Plexin-B1 RhoGTPase Binding Domain Revealed by Hydrogen Exchange Mass Spectroscopy and by Solution NMR
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Rho family GTPases play important roles in the regulation of the cell’s actin cytoskeleton and mediate the repressive and attractive effects of guidance molecules. Their interaction with single transmembrane receptors Plexins are of particular importance, since Plexin receptors are important for axon guidance, angiogenesis and also cancer. Our previous study mapped the interactions of small Rho GTPases, Rnd1 and active Rac1, with the plexin-B1 through a common region, the Rho GTPase Binding Domain (RBD)[1]. And NMR relaxation experiments on the RBD: Rac1 complex revealed that the Rac1 is less dynamic in the Plexin bound state[2]. To further address the mechanism under the specificity and different functions of different Rho GTPase involving Plexin-B1, we present here the dynamical behaviour of Rac1 and Rnd1, and the changes upon their association with plexin-B1 RBD. Solution NMR as well as mass spectrometry experiments are conducted to monitor the amide hydrogen exchange process. Both Rnd1 and Rac1 become rigid in general, especially in the switch I and switch II regions. In addition, both GTPases show complimentary dynamics changes distal from the binding sites, indicating an allosteric signaling mechanism. This also suggests a more direct role of Rho GTPase upon binding to Plexin-B1, rather than simply being sequestered by Plexin-B1. More importantly, the dynamical study revealed the binding with Plexin-B1 RBD induces different changes in the dynamics and in different regions of the two GTPases. These are in accord with previous thermodynamic measurements and implies a difference in the mechanism of action of the two Rho GTPases-Rac1 and Rnd1, upon interaction with Plexin-B1[3]. These studies together suggest the origin of the specificity of the GTPase-protein interaction and signaling in the plexin-B1 system.

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932-Plat
Conformational Transitions in Switch Regions of the Ras-Like GTPase Rab1B Studied by Free Energy Simulations
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The family of small Ras-like GTPases plays a pivotal role in the coordination of cell signaling by acting as binary protein switches. The switching mechanism is triggered by the hydrolysis of co-factor GTP to GDP which induces a transition in conformation and dynamics of two functional regions designated as switch I and switch II located in the proximity of the co-factor binding site. The switch I and switch II regions typically adopt an ordered and well defined structure only in the presence of bound GTP and unfold in the presence of GDP. The conformational equilibrium of switch I and switch II regions can be further influenced by post-translational modifications. We employed umbrella sampling free energy simulations to systematically investigate the switch I and switch II conformational transitions in the Ras-like GTPase Rab1b in the presence of bound GTP or GDP and different posttranslational modifications. The free energy change was recorded along a collective coordinate composed of a network of predefined inter-atomic distances (dRMSD) coupled with Hamiltonian replica exchange simulations. In agreement with experiment the free energy simulations predicted a lower free energy barrier of unfolding switch I and switch II regions in the presence of GDP compared to GTP and are also compatible with the experimentally observed effects of posttranslational modifications. An energetic analysis indicates that electrostatic interactions play a decisive role for the conformational equilibrium. It was also possible to determine the order of structural transitions in the switch I and II regions upon induced unfolding. The results can have important implications for understanding the mechanism of signal transduction by GTPases.

Platform: Micro- and Nanotechnology

933-Plat
Probing the Motion of the Intrinsically Disordered Neuronal Protein Alpha-Synuclein through the VDAC Pore using a Single-Molecule Approach
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Recent studies demonstrate the direct interaction of α-synuclein with mitochondrial membrane proteins and point to the role of the voltage-dependent anion channel (VDAC) of the mitochondrial outer membrane (MOM) in facilitating translocation of α-synuclein through the MOM. Single-channel studies of the ionic current through VDAC reconstituted into a planar lipid membrane in the presence of α-synuclein reveal a complex interaction of α-synuclein with the VDAC pore that is characterized by the presence of two blocked states. The duration of both blocked states is biphasic with voltage: at low voltage, the blockage time increases with voltage, while at sufficiently large voltages, it decreases with voltage thus implying translocation. Detailed kinetic analysis of the transitions between the blocked states suggests that the second blocked state arises from simultaneous capture of two α-synuclein molecules. Selectivity studies at asymmetric electrolyte concentrations demonstrate a distinct splitting of the conductance of each blocked state into higher- and lower-selectivity sub-states. Transitions between these sub-states report on the stochastic dynamics of a single α-synuclein molecule within the pore. Finally, the shape of the blockage time distributions over the entire voltage range can be understood using a first-passage approach that reveals the potential experienced by α-synuclein molecules in the VDAC channel. These results highlight the suitability of the α-synuclein/VDAC system as a model for the investigation of the detailed molecular motion of disordered proteins in large β-barrel channels.

934-Plat
Using Nanoparticles to Control Cellular Membrane Potential
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All cells generate an electrical potential (V_m) across their plasma membrane driven by a concentration gradient of charged ions. A typical resting membrane potential ranges from ~40 to ~70 mV, with a net negative charge on the surface of the cell. Nanoparticles, such as gold nanoparticles, can be used to perturb the membrane potential by directly interacting with the membrane. These interactions can be used to control cellular processes, such as gene expression and drug delivery. In this study, we investigated the effect of gold nanoparticle treatment on the membrane potential of various cell types, including HeLa and mouse embryonic fibroblasts (MEFs). We found that the membrane potential decreased in a dose-dependent manner upon nanoparticle treatment, with the magnitude of the effect depending on the nanoparticle concentration and cell type. These results suggest that gold nanoparticles can be used as a tool to control cellular membrane potential, which may have implications for a variety of biological and medical applications. Future work will focus on understanding the mechanisms underlying these effects and exploring potential applications in cell therapy and drug delivery.
cytosolic side of the membrane. Disruption of this electrochemical equilibrium causes $V_m$ to become more positive or more negative relative to its resting state, referred to as "depolarization" or "hyperpolarization," respectively. Changes in membrane potential have proven to be pivotal not only in normal cell cycle progression, but also in malignant transformation. Using polystyrene nanoparticles as a model system, we use a combination of fluorescence microscopy and flow cytometry to measure changes in membrane potential in response to nanoparticle binding to the plasma membrane. We find that cationic nanoparticles depolarize both CHO-K1 and HeLa cells. The cellular binding of anionic nanoparticles does not lead to a discernible trend in altered membrane potential. Maintenance of the resting membrane potential depends on the presence of two-pore-domain potassium "leak" channels, which allow for outward diffusion of potassium ions along their concentration gradient. Using an assay that tests the diffusion of ions through these potassium channels, we observe reduced permeability of the channels when cells are treated with nanoparticles. Based on a dynamical system model of the cell, we conclude that this loss of permeability likely results from physical blockage of the channel itself by the particle. Prevention of potassium ion efflux due to blocked channels causes accumulation of positive charge inside the cell, resulting in a depolarized membrane. By understanding the ways in which nanoparticles can be utilized to selectively generate cellular responses, we can begin to consider them as active species that may alter the very systems they are currently designed to probe.

935-Plat
Electro-Wetting of a Hydrophobic Gate in a Biomimetic Nanopore
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Nanopores in membranes have a range of potential applications. Biomimetic design of nanopores aims to mimic key functions of biological pores within a stable template structure. Molecular dynamics simulations have been used to test whether a simple β-barrel protein nanopore can be modified to incorporate a hydrophobic barrier to permeation. Simulations have been used to evaluate functional properties of such nanopores, using water flux as a proxy for ionic conductance. Potential of mean force calculations have been used to calculate free energy landscapes for water and for ion permeation in pore models. These studies demonstrate that a hydrophobic barrier can indeed be designed into a β-barrel protein nanopore, and that the height of the barrier can be adjusted by modifying the number of consecutive rings of hydrophobic sidechains. A hydrophobic barrier prevents both water and ion permeation even though the pore is sterically unoccluded [1].

A clear prediction of the hydrophobic gating model is that of electro-wetting of the gate should occur, i.e. the pore can be functionally opened by applying a high transmembrane voltage. This has been seen experimentally in studies of hydrophobically-gated solid state nanopores [2]. We have explored electro-wetting of our model of a hydrophobic gate in a simple β-barrel protein nanopore using atomistic molecular dynamics simulations with either a constant pore using the G6pDH enzyme.

936-Plat
Deformation of MCF-7 Cells in Micropores with Undulating Diameter
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Pores used in current resistive-pulse experiments were shown to measure size and surface charge of translocating objects. With the addition of the undulation of the pore opening diameter it is possible to simultaneously characterize the size and mechanical properties of the object that has been passed through the pore. Here we will discuss in detail the characterization of a cell’s size and mechanical properties by examining the resistive-pulse of each individual cell. The cells translocated the pore electrokinetically and no external pressure difference was applied. Previous experiments with polystyrene and hydrogels particles as well as numerical modeling of electroosmotic fluid flow in our pores revealed existence of pressure drops along the pore axis. The local pressure gradients were predicted to deform biological cells even if the pore opening was larger than the cell at any axial position. Polystyrene particles suspended in a solution of HBSS and Twente 80 were first passed through single undulating micropores to measure their topography. MCF-7 cells were suspended in a solution of HBSS and pluronic, and passed through the same pores. Deformation of the cells was observed as a change of the relative amplitude of the pulse sub-peaks compared to the signature obtained with the hard polystyrene particles. Viability of the cells after their passage through the micropore was also established.

937-Plat
Fingerprinting Single Living Cells with Molecular Precision
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The secretome of a single living cell contains the totality of its secreted proteins, and therefore can act as a fingerprint by which to identify cell type. Although between 10 % and 20 % of the human genome encodes proteins that are secreted, measuring the secretome from an individual living cell is challenging as the secreted proteins are present in vanishingly small concentrations due to the very large dilutions involved.

However, a nanopore is able to detect single proteins through the distinctive blockage profile that develops in the ion conductance current when a protein passes through the nanopore [2]. Using a synthetic nanopore in a silicon membrane we investigate the distinctive blockage patterns, in essence the fingerprint, that arise from a living cell. The cell is placed in a micelle living cell culture cavity to the nanopore using optical tweezers and held stationary. The ion conductance current is measured across the nanopore, and translocation events (distinct blockage currents) are observed and measured. When the events are plotted in scatter plots (as dwell time versus average blockage current) the distinct fingerprint of individual cells can be observed. For instance, lymphoma cells (U937) and breast cancer cells (MCF7) produce distinct event patterns that enable them to be distinguished. This shows for the first time cell identification based entirely on the secretome, measured using a simple, non-invasive, non-destructive nanopore.


938-Plat
Mechanical Modulation of Enzyme Activity by Rationally Designed DNA Tweezers: From the Ensemble to the Single-Molecule Level
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Switchable nanomachines provide a platform to control dynamic functional states by altering distances at the nanoscale on demand. Recently, a tweezers-like DNA device was used to control the activity of an enzyme/cofactor pair juxtaposed on the two arms of the tweezers. Initial studies focused on bulk properties of the tweezers-mediated reactions, and hence lacked insight into the mechanism of enzymatic activation. Here, we used site-specific fluorescent labeling of the tweezers to monitor the arm-to-arm distance through single-molecule fluorescence resonance energy transfer (smFRET). Consistent with AFM measurements, smFRET showed that the tweezers only partially close in the proposed "closed" state and exhibit conformational sub-states. MD simulations showed bending and twisting of the tweezers arms, rationalizing the sub-states. Additionally, smFRET experiments on the isolated Holliday junction hinge suggested that the isomer resulting in the closest closing of the tweezers (isomer-1) is less favored, further explaining the only partial closing. We rationally improved the closing by increasing the stem length of the DNA hairpin bridging and actuating the tweezers from 3 to 4 base pairs, and by redesigning Holliday junction(s) of the tweezers to favor the optimal closing. Here, we used site-specific fluorescent labeling of the tweezers to monitor the arm-to-arm distance through a coupled enzymatic cascade. Using our optimized tweezers, we were able to enhance the bulk activity of G6PDH upon tweezers closure to up to ~12-fold. Currently, we are exploring the tweezers-manipulated enzymatic reaction...