2236-Plat
Analysis of the Cellulose-Cellulase Interaction
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Cellulose holds great potential as a source of biofuel energy. It is a complex carbohydrate that forms the cell walls of plants and gives them rigidity. The sugar subunits can be unlocked and fermented to produce ethanol. Plants have developed over time defense mechanism which locks up sugars and makes the fermentation process difficult and not cost-competitive [1]. The cellulase enzyme CBH1 [2] is capable to break up this sugar chains, it consists of two parts, the binding module and the catalytic domain which are hold together by a linker peptide. One of the major research questions is to analyze the mechanical process how the cellulase enzyme accesses the cellulose fiber. There is evidence that the linker peptide shows a high flexibility and could be essential for the understanding of the cellulase dynamic.
To guarantee statistical unbiased results a set of Brownian dynamics simulations are performed. The huge configuration space is discretized by applying different clustering algorithms. A Markov state model (MSM) [3,4] was used to analyze the dynamics of the system. The MSM description gives a mathematically rigorous approach to combine the statistical information of several independent simulations. There is no clear experimental evidence in which order the different enzyme parts access the fiber. In the context of a MSM the probability for different docking conformations of the fiber are calculated to answer this question. Depending on the bundling schema of the cellulose fibers, different phases are visible. It is further analyzed if the enzyme favors one of the fiber phases to split up the sugar chains.
[1] Himmel et al, Science, 2007
[2] Brady et al, Cellulose, 2008
[3] Bremaud, Springer, 1999
[4] Singhal et al, JPC, 2004

2237-Plat
Single Molecule Kv2.1 Channel Dynamics in Live Mammalian Cells
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Neuronal Kv2.1 potassium channels localize into micron-sized clusters which are regulated by extracellular glutamate and intracellular Ca2+ levels. The physical mechanisms underlying the formation and maintenance of these unique structures are largely unknown. We are investigating the dynamics of clustered Kv2.1 channels using high resolution total internal reflection fluorescence microscopy (TIRFM) to track single molecules with 8 nm accuracy.
Transfected human embryonic kidney (HEK) cells expressing biotinylated and GFP-tagged Kv2.1 channels are detected with streptavidin-conjugated red quantum dots (QD). While the red QDs enable tracking of individual channels, GFP fluorescence provides characteristics of clusters as an ensemble. The channel dynamics inside Kv2.1 clusters are analyzed in the membrane of live cells in terms of their mean square displacement (MSD) and cumulative distribution function (CDF).
In our current model, the actin cytoskeleton plays a dominant role in Kv2.1 cluster formation and maintenance. To test this model we are studying the effects of depolymerization agents such as Cytochalasin and Swinholide A on the individual channel dynamics.
Clustered channels remain confined within the cluster perimeter throughout the entire imaging time, up to 25 minutes. MSD analysis indicates similar diffusion constants for clustered and non-clustered channels, $D = 0.013 \pm 0.017\ \mu m^2/s$ and $0.014 \pm 0.011\ \mu m^2/s$ respectively. The CDF of all analyzed trajectories (n=900) deviates from a monoexponential, indicating a discrepancy with Brownian diffusion. Instead, the data can be accurately fit to a double exponential. Our results show a bimodal distribution of channels (clustered and non-clustered) and indicate that both populations experience anomalous subdiffusion. The double exponential term of the CDF suggests two stochastic processes which have slow and fast mobility respectively. Single molecule tracking with simultaneous channel cluster imaging is shown to be an effective way to study the mechanisms underlying clustering phenomena.

2238-Plat
Illuminating Dynamic Regions Associated with the Heterotropic Allosteric Communication In Bacillus Stearothermophilus Phosphofructokinase
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Van't Hoff analysis has revealed that the positive sign of the coupling free energy between the allosteric inhibitor, phosphoenolpyruvate (PEP), and the substrate, fructose 6-phosphate (F6P), of Bacillus stearothermophilus phosphofructokinase (BsPFK) is determined by the large entropy component of the system. BsPFK can exist in four different ligation states: BsPFK-F6P, PEP-BsPFK, PEP-BsPFK-F6P, and apo-BsPFK. The coupling free energy derives from the standard free energy associated with the equilibrium between the first 2 and the last 2 species, respectively. However the entropic properties of these species are poorly understood. The dynamic character of these four species can be expected to contribute to the coupling entropy which we have investigated using intrinsic tryptophan fluorescence anisotropy to report on the local restrictions to side-chain motion. Hybrid forms of BsPFK containing a single tryptophan that report on a single heterotropic interaction have been constructed. Tryptophan was positioned by constructing conservative tryptophan-shift modifications in which one of the sixteen native phenylalanine or tyrosine residues in one subunit was substituted with tryptophan, and the native tryptophan was changed to a tyrosine. Six positions dispersed throughout the monomer were initially targeted for this modification. Steady-state anisotropy measurements combined with time-resolved experiments were used to detect significant changes in the rotational correlation times of each ligated species of the BsPFK hybrid that isolates the strongest single heterotropic interaction. The dynamic changes observed in this manner suggest that specific regions are involved in the heterotropic allosteric coupling. Of the dynamics measured between the enzyme forms relevant to the coupling, F139 and F240 exhibit the largest difference in dynamics suggesting these residues identify regions particularly important for the allosteric communication of the dominant heterotropic interaction in BsPFK. Supported by NIH Grant GM33216 and Welch Foundation Grant A1548.

2239-Plat
Proteins of Functioning Flagellar Rotor Turnover but only in the Presence of Signalling Proteins
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For many bacteria, motility is achieved by means of one or more filaments, each being driven by a rotary motor embedded in the cell membrane. The bacterial flagellar rotary motor is one of nature's most intricate molecular machines and is composed of two main parts: the rotor and the stator. Whilst much is known about its static structure, there are little data on the dynamics and interactions of its constituents under natural conditions in living cells. Recent results revealed the rapid exchange undergone by the membrane spanning protein complex MotAB which localizes around the rotor and forms the torque generating units. The C-ring, also called ''switch complex'', is part of the rotor and is localized to the cytoplasmic region of the motor. The response regulator CheY-P binds one of the C-ring components, FliM, causing the rotor to switch rotational direction thus making FliM the interface with the chemosensory pathway.
Here, we use single-molecule fluorescence imaging in *Escherichia coli* cells expressing genomically-encoded YPet derivatives of FliM at physiological levels. Analysis of functional flagellar motors revealed that each contains ~34 FliM molecules. We found that two FliM populations coexist within the same motor, one undergoing constant turnover and one remaining ''fixed''. Surprisingly, exchange within the dynamic population relies on the presence of the response regulator protein linking the complex to the rest of the sensory pathway and may therefore play an active part of signal processing within the cell.
These results show the strength of combining molecular genetics with *in vivo* imaging and in this case illustrate the highly dynamic and adaptive nature of the bacterial flagellar motor. Further data will be presented on the different FliM complexes observed in the cells and their possible interaction with CheY-P.

Platform AP: Membrane Pumps & Transporters

2240-Plat
Single Molecule Rotation of F1-ATPase from *S. cerevisiae*
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Single molecule studies of the thermophilic *Bacillus* PS3 F_1-ATPase have revealed kinetic and structural information that cannot be discerned using other methods, including the presence of 40 and 80 degree physical substeps (*Yasuda et al. 2001*) and the order and kinetics of chemical substeps. We use single molecule techniques to observe the effects of Mitochondrial Genome Integrity