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nucleotide-binding sites (NBSs) in the dimer is formed by residues from the two NBDs. It is still unresolved whether hydrolysis leads to dissociation of the ATP-induced dimers or opening of the dimers (with the NBDs remaining in contact during the hydrolysis cycle), and also whether the presence of two NBSs is required for ATP hydrolysis or formation of the NBD dimer. Here we performed steady-state and kinetic studies of mutants of the prototypical NBD MJ0796 from *M. jannaschii* using luminescence resonance energy transfer (LRET) to assess association/dissociation of the NBDs. We show that dissociation is complete and follows hydrolysis at only one of the two NBSs. We also show that binding of two ATP molecules is necessary for NBD dimerization. We conclude that ATP hydrolysis at one nucleotide-binding site drives NBD dissociation, but two binding sites are required to form the ATP-sandwich NBD dimer necessary for hydrolysis. This work was supported by CPRIT grant RP101073.

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Reconstitution of Human ABC Transporter Mrp3 into Giant Unilamellar Vesicles for Single Molecule Transport Recordings on Micro- Structured Biochips

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The functional reconstitution of large and complex membrane proteins such as eukaryotic ABC transporters into giant unilamellar liposomes (GUVs) represents a major challenge as GUV formation usually involves the presence of organic solvents and/or dehydration in high vacuum making it incompatible with delicate protein samples. To overcome this limitation, we developed a solvent-free method for the transformation of proteoliposomes into GUVs. MRP3-containing proteoliposomes were partly dehydrated on an agarose-based hydrogel under controlled humidity and in the presence of trehalose as a stabilizing agent. Subsequent rehydration in physiological buffer led to the fast and reproducible formation of GUVs (10-20 μm diameter) harboring functional MRP3 in their membrane.

To observe the transport of substrates by single MRP3 molecules, MRP3-GUVs were fused onto the surface of a silicon-based biochip featuring a rectangular grid of thousands of cylindrical cavities (0.8 μm diameter, 6 fL volume) with open tops and optically transparent closed bottoms allowing highly parallel three-channel fluorescent readout on an inverted microscope set-up. Fluorescently labeled lipids in the bilayer and a fluorescent dye that is not transported by MRP3 served as in situ controls to continuously monitor the integrity of the pore-spanning lipid bilayer.

ATP-dependent transport of autofluorescent substrates into the cavities by MRP3 could be monitored in real-time and revealed a distribution of rate constants in good agreement with previous bulk measurements. Furthermore, the competitive inhibition of MRP3-mediated transport by non-fluorescent co-substrates or inhibitors could also be observed.

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Molecular Dynamics Simulation Study of a Mutant Construct of the Archaeal Glutamate Transporter GltPh with Transport Rates as Fast as its Human Counterpart

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The glutamate transporter GltPh is a homolog of mammalian excitatory amino acid transporters (EAATs) that mediate glutamate re-uptake after discharge at the neuronal synaptic cleft, thereby enabling repeated signaling cycles and preventing excitotoxicity. While the structure of EAATs is not yet known, several stages of the transport cycle have been captured in crystallographic studies of the homotrimeric GltPh, suggesting a mechanism of separate elevator-like motions of three transport domains relative to a scaffold composed of the trimerization domains from each monomer. Dynamic aspects of the transport cycle monitored in single-molecule FRET experiments, revealed quiescent phases in which GltPh appears to be ''locked'' in the inward- or outward-facing states. Significantly higher transport rates, typical for human EAATs, were recently reported for a GltPh construct in which key residues were mutated to mimic the sequence of mammalian EAAT1. The mutant adopted a novel ''unlocked'' conformation, in which the transport domains were separated from the trimerization domains. Molecular dynamics simulations of this GltPh mutant in lipid bilayers found the unlocked conformation to be unstable if the transport/trimerization domain interface is solvated by water only, but stabilized by insertion of one or several hydrophobic moieties such as lipid tails. Analysis of the effect of the mutations on the local dynamics in several known stages of the transport cycle showed that a charged side chain introduced at the protein interface with the membrane produces membrane deformation and a destabilizing energy cost due to residual hydrophobic mismatch. Free energy perturbation calculations were used to estimate the impact of the mutation in the explored stages of the transport cycle in order to gain insights on the key structural determinants of EAAT transport efficiency.

Symposium: Bacterial Subcellular Dynamics at Super-Resolution: This Brings Super-Resolution to a Dynamic Sense

993-Symp

Beyond Model Systems: Super-Resolving the Subcellular Dynamics of Starch Digestion in the Human Gut Microbiome

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By beating the diffraction limit that restricts traditional microscopy, single-molecule fluorescence imaging provides a flexible, noninvasive way to super-resolve position and dynamics. Single-molecule methods are ideally suited to the small size of bacterial cells, and are being applied to central processes that remain contested by microbiologists. Pushing the scope of single-molecule microscopy further, we are keenly interested in the realm of non-model systems, including pathogens and beneficial bacteria, which play a key role in human health and nutrition. In a synergistic collaboration with microbiologists, we have used super-resolution microscopy, single-molecule tracking, and gene knockouts to elucidate for the first time in live cells the mechanism of starch-utilization proteins in the human symbiont *Bacteroides thetaiotaomicron*, which colonizes our guts to unlock calories from otherwise indigestible sugars.

Our live-cell super-resolution imaging reveals the transient interactions, assembly and collaboration of the *Bacteroides thetaiotaomicron* Starch Utilization System (*Bt*-Sus) outer membrane proteins. Overall, we demonstrate that the polymeric starch substrate dynamically recruits Sus proteins, serving as an external scaffold for bacterial membrane assembly of the Sus complex, which may promote efficient capturing and degradation of starch. Furthermore, by simultaneously localizing multiple Sus outer membrane proteins on the *Bt* cell surface, we have characterized the dynamics and stoichiometry of starch-induced Sus complex assembly on the molecular scale. Finally, based on Sus protein knockout strains, we have discerned the mechanism of starch-induced Sus complex assembly in live anaerobic cells with nanometer-scale resolution.

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Bacterial Chromosome Segregation at the Single-Molecule Level

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How does a bacterial cell without a nucleus, without mitosis, and without extensive sister cohesion, organize, replicate, repair and segregate its chromosomes? We use live-cell super-resolution PALM and 3-D SIM imaging, alongside classical biochemistry and molecular genetics, and *in vitro* single-molecule biophysical techniques to address these questions. The presentation will focus on the sequential and coordinated action of Topoisomerase IV and the SMC complex, MukBEF, in *E. coli* chromosome segregation.

References

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995-Symp

Bacterial Cell Wall Peptidoglycan Architecture and Dynamics

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Bacterial cell wall peptidoglycan is essential for the life of most bacteria. It determines cell shape, and its biosynthesis is the target for many important antibiotics. The fundamental chemical building blocks of peptidoglycan are conserved: repeating disaccharides cross-linked by peptides. However, despite this relatively simple chemistry, how this is manifested into the myriad bacterial shapes and how this single macromolecule remains dynamic permitting cell growth and division has largely remained elusive. The advent of new microscopy approaches is beginning to revolutionize our understanding of