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 M0796 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.

**991-Plat**

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 μL 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.

**992-Plat**

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 providing a stabilizing agent. Subsequent rehydration in physiological buffer led to the fast and reproducible formation of GUVs (10-20 μm diameter) harboring functional GltPh in their membrane.

In vitro studies on the transport properties of GltPh revealed that it is a...