that under physiological conditions, the function(s) of BACE is influenced by Ca^{2+}.

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Investigation Of The Multidrug ABC-transporter LmrA By Multinuclear MAS-NMR And EPR

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The multidrug ABC-transporter LmrA from *Lactococcus lactis* is a structural and functional homologue of human P-glycoprotein. Just like its famous relative, LmrA extrudes hydrophobic drugs from the membrane and can thus confer resistance to its host cell. The energy for this process is provided by ATP hydrolysis in the two nucleotide binding domains (NBDs). During a hydrolysis-transport cycle, the NBDs communicate a structural change to the transmembrane domains (TMDs), where the substrate is recognized and extruded. However, it is not understood for any ABC-transporter at which point during those cycles the two domains interact and how ATP hydrolysis and substrate recognition and extrusion are coupled. In order to some shed light on these phenomena, we have trapped the transporter during the hydrolysis cycle with fluorinated phosphor analogues (BeFx, AlFx) and investigated the electronic environment within the NBD with a complementary $^{19}F/^{31}P$ MAS ssNMR approach. Additionally, we have labelled the TMDs with ^{19}F- and EPR spinlabels for ssNMR and EPR in order to probe the relationship between drug recognition, transport and coupling to the hydrolysis cycle. Activity of the transporter under MAS conditions has been verified by an ATPase Assay with 31P NMR that can also be employed to probe substrate phosphorylation within membranes.

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A Voltage Sensitive Phosphatase from Xenopus Laevis Testis

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Voltage sensitive phosphatases (VSPs) are transmembrane proteins comprised of a voltage sensor domain characteristic of an ion channel, coupled to a phosphatidylinositol phosphatase; their phosphatase activity is activated by membrane depolarization (Murata and Okamura, 2007, *J. Physiol.* 583:875-889). Because VSPs are expressed predominantly in sperm, it has been proposed that they might function in the voltage-dependent regulation of sperm-egg membrane fusion, which in many species provides a fast block to polyspermy. To characterize the properties of a VSP from a species amenable to transgenesis and fertilization studies, we identified a VSP homolog from *Xenopus laevis* testis and expressed it in *Xenopus* oocytes, together with the fluorescent PIP_2 sensor PLCδPH-GFP. Using a photodiode to measure PLCδPH-GFP fluorescence from the pigmented surface of voltage clamped oocytes, we showed that XlVSP enzymatic activity is regulated over a range of membrane potentials (50% activation at ~ +10 mV) similar to those that regulate sperm-egg fusion. In agreement with previous studies of ascidian and zebrafish VSPs (Hossain et al., 2008, *J. Biol. Chem*. 283: 18248-59), an R152Q point mutation shifted the XlVSP activation curve towards more hyperpolarized membrane potentials (50% activation at ~ −12mV). Future studies of the voltage dependence of fertilization using sperm from transgenic frogs expressing $XlVSP^{R152Q}$ should allow investigation of the functional significance of voltage sensitive phosphatases in sperm-egg fusion.

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The Substrate Translocation Pathway and Transport Mechanism in the Dopamine Transporter

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The crystal structure of a prokaryotic homolog of the dopamine transporter (DAT), LeuT provided essential structural insights about the family of sodium symport transporters, but only limited clues regarding the molecular mechanism of substrate transport. Utilizing this structure as a template we constructed an experimentally-validated homology model for DAT, and used SMD simulations to explore the substrate translocation pathways. The substrate was first pulled from its primary binding site (S1) toward the extracellular side to evaluate the presence of a secondary binding site (S2) in DAT analogous to the one identified in LeuT (Shi et al, Mol Cell 2008). With such a site identified, a doubly occupied (substrate in S1 and S2) model was constructed and equilibrated. During this MD equilibration, the S1,S2-DAT was found to open towards the intracellular side allowing water to enter. This is accompanied by specific changes in local conformations, e.g., the rotamer of residue F1.42, and a downwards move of the dopamine in S1, which primes it for translocation. Further pulling this substrate towards the cytoplasmic side causes further inward opening, and conformational changes of specific residues that correlate with SCAM data. Thus, residues lining the transport pathway and key interaction-networks stabilizing either inward-facing or outward-facing conformations (e.g., involving Y3.35-E8.66) are revealed. Large-scale helix rearrangements involved in transition between different states are identified, such as (TM1-TM4-TM5 and TM8-TM9) moving as one group, and (TM2-TM6-TM7 and TM10-TM11) moving as another. Hinge regions involved in these movements contain conserved residues such as G/P/S/T/C that could disrupt helix rigid-body motions and are known to be necessary for maintaining dopamine uptake or efflux. Together, our findings delineate both the mechanism and the pathway for substrate translocation at a level of detail that is directly amenable to experimental validation.

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Purification and Characterization of an Activated Rhodopsin/Transducin Complex

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Light-induced conformational changes of the dim-light photoreceptor rhodopsin promote efficient binding and activation of the intracellular guanine nucleotide-binding protein, transducin. This activation event initiates GDP/GTP exchange and subsequent dissociation of transducin, from the activated photoreceptor. In this work we have developed a method to assemble and purify an activated rhodopsin/transducin complex in both detergent micelles and lipid bilayers. Activated rhodopsin was immobilized on an affinity resin, allowed to bind to transducin, and extensively washed to remove nonbinding material prior to elution. Evaluation of the eluate by SDS-PAGE and UV-visible absorption spectoscopy confirm the presence of rhodopsin and transducin. The incubation of inactive rhodopsin with transducin in a control experiment resulted in the elution of rhodopsin but no transducin. Activity of the rhodopsin/transducin complex was measured by rhodopsin-dependent GDP release, the uptake of GTPγS, and the nucleotide-dependent release of transducin.