SFB 35 Colloquia in Membrane Transport

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"Investigation of the consequences of nucleotide binding to a multidrug resistance ABC transporter with magnetic resonance techniques"

The multidrug ATP Binding Cassette (ABC) transporter LmrA is an integral membrane protein and a functional homologue of human P-glycoprotein involved in resistance against anti-cancer drugs in chemotherapy. LmrA forms a homodimer comprising the typical ABC transporter architecture of two nucleotide binding domains (NBDs) and two transmembrane do-mains (TMDs). It utilizes ATP binding or hydrolysis at its NBDs to drive the extrusion of toxic hydrophobic compounds via its TMDs. Combined, NMR and EPR present excellent tools to investigate all aspects associated with membrane transporter functionality, such as dynamics (e.g. side-chain or domain mobility) or structural (e.g. domain movements, substrate binding) effects. With a tri-fold magnetic resonance approach including solid-state NMR on the recon-stituted full-length protein, solution NMR on the isolated NBD as well as pulsed EPR on cysteine mutants of the full-length protein, a comprehensive picture on the dynamics of LmrA throughout its catalytic cycle could be developed. In order to investigate the ATPase activity of reconstituted LmrA, a 31P solid-state NMR based assay was developed that allows the simultaneous observation of all phosphor resonances in the ATP hydrolysis reaction. Because solid-state NMR is not limited by phase separation effects, this assay can be utilized to investigate simultaneous reactions in the aqueous as well as the lipid phase.