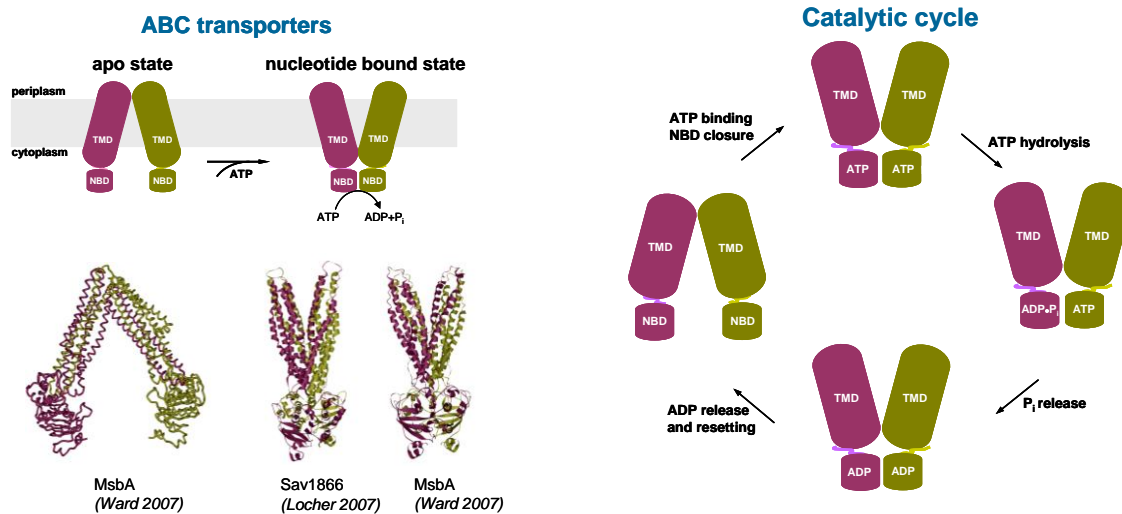
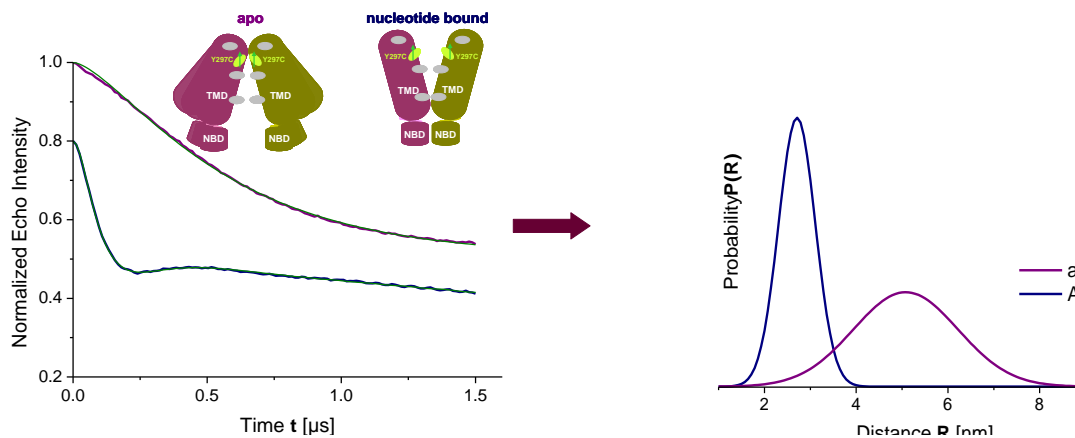


Pulsed electron-electron double resonance (PELDOR) in combination with site-directed spin labeling is a well established electron paramagnetic resonance (EPR) technique for distance determination.^{1,2} It has been proven to be a powerful tool in mapping the conformational changes associated with different intermediate states of the catalytic cycle of membrane transporters and their implication to the functional mechanism.³⁻⁶

ATP-binding cassette (ABC) transporters are a large family of integral membrane proteins that translocate a diverse range of substrates (ions, peptides, lipids, metabolites, chemotherapeutic drugs and antibiotics) across cellular membranes. The energy required for this biological work is gained through ATP hydrolysis. ABC transporters are conserved from bacteria to humans and share common structural features: two nucleotide binding domains (NBDs), which are the site of ATP hydrolysis and two transmembrane domains (TMDs) providing a translocation pathway for the substrate.⁷⁻⁹



The aim of the work will be to study structural rearrangements during the transport cycle of ABC transporters by means of PELDOR spectroscopy, using nitroxide spin labels as spin probes. To achieve that goal experiments will be performed on ABC transporters trapped in different intermediate states of the nucleotide cycle: resting state (without nucleotide), pre-hydrolysis (ATP bound) and post-hydrolysis ADP (bound) states. Furthermore a series of spin labeled mutants along the TMD and NBD domains will be measured in order to obtain more distance constraints and gain structural insights into the conformational dynamics during the catalytic cycle of ABC transporters.



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