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What we have learned about membrane-bound pyrophosphatases (mPPases) from X-ray crystallography, MD simulations, FRET and PELDOR

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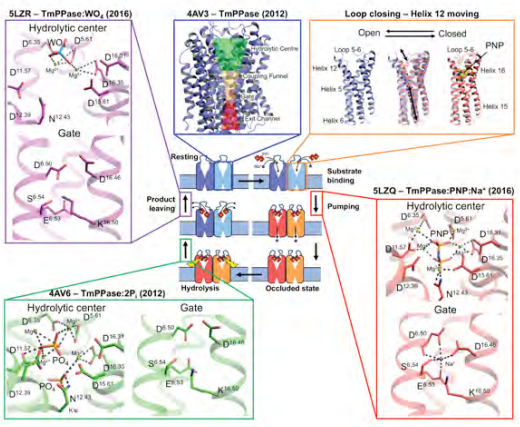
Membrane-bound pyrophosphatases (mPPases) couple the hydrolysis of inorganic pyrophosphate to the pumping of ions (sodium or protons) across a membrane in order to generate an electrochemical gradient. This class of membrane protein is widely conserved across plants, fungi, archaea and bacteria, but absent in multicellular animals. The absence of mPPases from higher eukaryotes makes them an attractive drug target against *protozoan* parasites such as *Plasmodium falciparum* and pathogens such as *Bactoroides vulgatus* [1]. During one catalytic turnover, an mPPase must bind its substrate (PP_i), pump an ion(s) across the membrane, hydrolyze PP_i and release $2P_i$ as product. The mPPases of different species differ in respect to the ion they pump, either sodium, protons or both; and they display varying dependence on potassium ions. We have used X-ray crystallography to solve numerous structures of the *Thermotoga maritima* mPPase, providing us with snapshots of the protein throughout the catalytic cycle and an excellent understanding between three-dimensional structure and enzyme function [2]. Some questions still remain, including the molecular details of potassium dependence for catalysis, and residues involved in defining single-pumping *versus* dual-pumping of sodium ions and protons. We also wanted to explore the dynamics and kinetics of the catalytic cycle between the static snapshots afforded to us by crystallography. We employed molecular dynamics (MD) simulation [3], pulsed electron-electron double resonance (PELDOR/DEER) and single-molecule Förster resonance energy transfer (FRET) measurements to determine the dynamic range and frequency of conformations available to the enzyme in a lipid bilayer during the catalytic cycle, leading to further insights into the mechanism of this enzyme.



References:

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