MS8-04 The structure of a sodium pumping pyrophosphatase: clues to catalytic mechanism

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Membrane-bound pyrophosphatases (M-PPases) couple pyrophosphate hydrolysis or synthesis to Na^+ or H^+ pumping. They are found in plants, bacteria and protozoans and are crucial for survival in various stress condition (such as low light intensity, anoxia, cold and mineral deficiency) and are also important for plant maturation. We have solved the metal bound resting state (TmPPase:CaMg) and product bound (TmPPase:MgPi) structures of *Thermotoga maritima*sodium pumping M-PPase (TmPPase) at 2.6 Å and 4 Å resolution, respectively . The resting state structure (Figure 1) shows an open active site cavity with the hydrolytic center at the top (20 Å above the membrane), followed by a "coupling funnel" formed by conserved, charged residues lining six a-helices. The "coupling funnel" ends at the gate formed by the conserved Asp243, Glu246 and Lys707 and below this is an exit channel leading to the periplasmic space. Comparison of our two TmPPase structures with the recently solved crystal structure of *Vigna radiata* H^+-pumping pyrophosphatase in the state with product analogue bound (VrPPase:PNP) shows movement of the helix 12 in VrPPase:PNP and TmPPase:MgPi (Figure 2)**.** We presume that upon substrate binding a transient state is formed in which sliding of the helix 12 towards the periplasm/vacuolar lumen opens the gate and the exit channel and leads to ion pumping. Superimposing helices 3-6, 9-12 and 13-16 of TmPPase suggests that M-PPases arose through gene triplication.

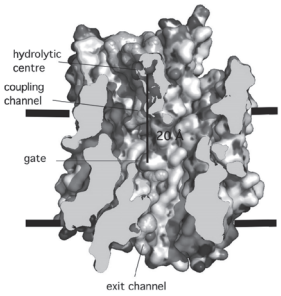

Figure 1. General overview of TmPPase structure (blue = active site cavity, green = exit channel, image from).

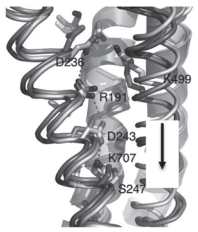

Figure 2. Movement ofhelix 12 (arrow) duringthe catalytic cycle of an M-PPase (blue = resting state TmPPase, green = product bound TmPPase, brown = substrate analogue bound VrPPase, image from).

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MS8-05 Serial Femtosecond Crystallography Using Crystals Grown in Lipidic-Sponge Phases.

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Membrane proteins are difficult to crystallize and rarely diffract to high resolution in an X-ray beam. The main obstacles are either insufficient X-ray intensity or the size of the crystals. Both these issues can be overcome by the availability of a hard X-ray Free-Electron Laser (XFEL) such as LCLS in Menlo Park, CA, USA. The high intensity beam enables data collection from protein crystals consisting only of a few unit cells, thus bypassing the time-consuming optimization steps for growing large membrane protein crystals. The protein of choice in the experiment was Reaction center (RC) from *Bl. viridis*, which, in lipidic-sponge phases, give large crystals suitable for room temperature time-resolved Laue experiments [1]. These crystal conditions were used as a starting point for optimization of submicron crystals grown in lipidic-sponge phase batch setups. Successive rounds of optimization and characterization were necessary, including polarization techniques and SEM studies, to provide crystals of the appropriate size and amounts for a femtosecond nano-crystallography experiment.The RC crystals were delivered to the XFEL beam at the AMO beamline at LCLS, via a liquid micro-jet (3 μm in diameter) at a similar rate and pressure to that of water. The XFEL pulse duration was 70 fs and contained 2 keV X-rays (wavelength = 6.2 Å) focused into a 3 μm diameter spot. The diffraction data was collected on two pnCCDs, which were read out after each X-ray exposure at a repetition rate of 60 Hz. The RC crystals were found to diffract beyond the edge of the detector (maximum resolution at this experiment was 6.2 Å), and in total 360,000 diffraction patterns were collected, of which approximately 0.5% were characterized as "hits". The structure was solved by molecular replacement and refined using REFMAC5 [2] to 8.2 Å.

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