DISTINGUISHED LECTURE in BIOLOGICAL ENGINEERING

“Analysis and Design of Proton Transporters”

Tuesday – March 29, 2016 – 12:15 p.m.
EPFL – room SV1717.1

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host: Prof. Matteo Dal Peraro

Abstract:
The mechanisms by which membrane proteins conduct protons and use proton gradients to drive the transport of other molecules and ions up their concentrations are important problems in structural biology. This talk will focus on a designed transporter, Rocker, and a natural proton transporter, M2. The M2 proton channel from influenza A virus is required for the acidification of the interior of endosomally entrapped virus during the life cycle of the virus. To investigate the mechanism by which protons are transported through the channel we previously determined crystal structures of the channel using crystals that diffract to 1.05 Å resolution using conventional synchrotron radiation at low and high pH, and at cryogenic as well as room temperature. At cryogenic temperatures we observe strings of water molecules that appear well oriented to transmit protons to a cluster of histidine residues deep within the pore. However, the water was far less ordered in crystal structures determined at room temperature, leaving two possibilities: 1) the lack of order might be associated with radiation damage; or 2) the water wires seen at low temperature might be an artifact of low temperatures. Most recently, we are using the X-ray free electron laser (XFELs) to investigate the room temperature structures. Although work is still in progress, our preliminary results indicate that ordered water wires are present in the r.t. structures solved using XFELs crystallography, although the geometry differs from that seen at room temperature. Thus, the lack of order seen using conventional synchrotron radiation was due to radiation damage. We also are using these structures to assess the relative merits of different water models used in molecular mechanics force fields. M2 is also the target of the amantadine class of drugs, but the emergence of drug-resistant viruses has become a major problem that curtailed the use of this class of therapeutics. We have solved the structure of the drug-resistant mutants, and used this information to design new drugs that inhibit the most problematic mutant forms of the channel.

The second portion of the talk will focus on the de novo design of a transporter, which uses a proton gradient to drive the transport of Zn(II) and vice versa. The protein was designed to test concepts previously suggested by other investigators to be important for the evolution of this class of proteins. These mechanisms feature gene duplication of a primordial unit and “frustrated symmetry” of the otherwise symmetrical protein to cause it to rock between states in which the substrates can alternately access the pore from either side of the membrane (but not both sides simultaneously). Using computational design algorithms to stabilize the desired asymmetric states relative to the fully symmetrical state, we designed a membrane-spanning four-helical bundle that transports transition metal ions Zn(II) and Co(II) – but not Ca(II) – across a membrane. The conduction path was designed to contain two di-metal binding sites, which display negative cooperativity in their binding characteristics. X-ray crystallography, solids NMR, solution NMR and molecular dynamics calculations indicate that the overall helical bundle is formed from two tightly interacting pairs of helices, which form individual domains that interact weakly along a more dynamic interface to allow conduction of ions between the binding sites. Vesicle flux experiments show that as Zn(II) ions diffuse down their concentration gradients into the vesicles, protons diffuse outward, even in the presence of an unfavorable pH gradient. Current work is focused on improving overall antiport efficiency.

Sandwiches will be provided

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