Halobacterium Salinarum, has been studied by multidisciplinary approaches to reveal the molecular machinery of proton transfer and photocycle. For example, to understand the functional role of structural change in M-state intermediate, different media with a high pH value have been used to extend the lifetime of M state. However, not much attention has been paid to the conformational changes that occur in the ground state, especially around the retinal binding pocket, due to local environment.

Here, the conformation changes to the B r ground state, especially around the retinal binding pocket, have been studied by solid-state NMR through chemical shifts and torsion angle measurements, combined with the light-induced kinetic and UV spectroscopies. All the experimental results have been discussed in the context of X-ray crystal structures, and possible mechanisms have been proposed.

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Stripping the CLC-ec1 Dimerization Interface: An Investigation into the Role of Van Der Waals Interactions in Membrane Protein Assembly
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What drives membrane proteins to fold and oligomerize in lipid bilayers? One possibility is that protein-protein interactions stabilize the assembled state. In membrane proteins, interfaces are typically lined by non-polar residues offering weak van der Waals interactions (VDW), yet a large network of these might confer strong stability. To investigate the role of side-chain VDW interactions in the free energy of membrane protein complex formation, we have made a library of mutations on the dimerization interface of the CLC-ec1 Cl−/H+ antiporter - a model system that we have developed to measure equilibrium dimerization in lipid bilayers. The interface is comprised of four alpha-helices lined by non-polar residues: I: -F219, I220, I223, I227; H: -L194, I197, I199, I1201; P: -L406, I409, I140, I1413; Q: -I422A, L421A, I426A, I430A, I434A. We systematically mutated each helix surface to alanine (all-ALA), stripping the interface of its VDW interactions. In all cases the protein expresses, is stable and upon reconstitution in membranes, shows Cl− transport activity comparable to wild-type CLC-ec1. We ran gel-filtration chromatography over the course of one week to screen the stoichiometry of the protein in detergent. All-ALA helix I, P & Q are stable dimers, however all-ALA helix H showed shifts to monomer directly after purification. Furthermore, single-mutant L194A was sufficient to shift the protein to a monomer-dimer mixture. Because helix H interacts with helix P, and all-ALA helix P is still dimeric, these results suggest that dimer stability cannot be explained by VDW interactions alone, at least in detergent. We are currently measuring the change in free energy of CLC-ec1 dimerization with these subtractive mutations to quantify the role of VDW interactions in membrane protein assembly.

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Sarcolin-Mediated Regulation of SERCA by Computer Simulations
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The sarco-endoplasmic reticulum Ca2+-ATPase (SERCA) is a transmembrane pump that, upon ATP hydrolysis, pumps Ca2+ against a concentration gradient from the cytosol to the sarco- or endoplasmic reticulum, thus terminating muscle contraction and priming the cell for the next excitation-contraction stimulus1. SERCA function in skeletal muscle cells is regulated by sarcoplasmic (SLN), a 31 amino acid transmembrane peptide that inhibits SERCA by lowering its apparent Ca2+ affinity. By combining solid-state NMR and cross-linking experiments, we characterized the structure of the SERCA/SLN complex in its natural membrane environment in both Ca2+-E1:ATP and H+/E2:ATP states of the catalytic cycle. In this work, we employ molecular dynamics computer simulations to investigate SERCA’s mechanism of regulation by mapping the effects of SLN binding on SERCA’s free energy landscape, sub-microsecond structural dynamics, and allosteric coupling between the transmembrane and cytoplasmic domains. Our calculations indicate that upon binding of SLN, SERCA SLN increases its average tilt angle with respect the unbound state. The interaction between the two proteins results in a shift of the free energy basin of SERCA and in an altered coupling between its transmembrane and cytoplasmic domains, which may be responsible for the reduced activity of SERCA in its SLN-bound state.

References: