Membrane Protein Structure & Function III

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Nanosecond Time-Resolved Infrared Basis for a Bulge of the Transmembrane Helix Between Hemes *A* and *A*3 to Facilitate Highly Efficient Proton Pumping by Bovine Heart Cytochrome C Oxidase

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Recent X-ray structural analyses show that net positive charges on a heme iron site (heme a) of bovine cytochrome c oxidase, created upon electron donation to the O_2-reduction site, electrostatically drives proton-pump through a hydrogen-bond network to the positive side of mitochondrial inner membrane [1, 2]. The four electron equivalents for complete reduction of O_2 at the fully reduced O_2-reduction site are transferred one at a time, each, coupled with pumping of one proton equivalent, giving four intermediate species, F, O, E and R (from P). X-ray structures of P, F, O and R suggested that the water channel which connects the negative side space with the hydrogen bond network is kept closed by Ser382 bulge of the trans-membrane-helix during the transfer of four electron equivalents for complete reduction of the bound O_2. The closure blocks effectively proton back-leakage from the hydrogen-bond network.

The structural basis for complete protonation of the hydrogen-bond network before the O_2-binding prerequisite for the efficient energy transduction was explored using a newly developed nanosecond time-resolved infrared apparatus for aqueous protein system. A transient CO-binding to Cu_B, after Fe_{a3}-CO photolysis, was discovered to open the water-channel by eliminating the Ser382 bulge. The infrared and X-ray structural results suggest that, sensing protonation state of the hydrogen-bond network, a relay system including Cu_B, O_2, Fe_{a3} and two a-helix turns extending to Ser382 facilitates effective proton collection and timely water-channel closure by conformational changes in the Ser382-containing segment, thereby ensuring efficient energy transduction.

[1] Muramoto K., et al (2010) Proc. Natl. Acad. Sci. USA 107: 7740-7745.
[2] Yoshikawa S., et al (2011) Ann. Rev. Biophys. 40: 205-223.

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Oligomerization and its Effect on Function in 7-Transmembrane Proteins

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Proteorhodopsin (PR) is a solar-powered membrane protein–a proton pump from marine bacteria that has significant structural and dynamical commonalities to seven-transmembrane (7TM) mammalian proteins, including the G-protein Coupled Receptors (GPCRs). Furthermore, PR associates with itself in the membrane to form oligomers similarly to GPCRs, and these different structural forms are thought to play a large role in function. Unfortunately, studying the structure and function of membrane proteins in oligomeric assemblies is very challenging at the molecular level due to the oligomers' large size, disordered nature, and ability to resist crystallization–making PR a facile model system for capturing elusive details of dynamics and function. Past work has revealed PR's hexameric interface in dodecylmaltoside (DDM) micelles, and here we focus on understanding the effect of oligomerization on function. We seek to probe oligomeric interfaces as we vary surfactant environment and oligomeric state. using fast protein liquid chromatography (FPLC) and optical absorption experiments we show that the hexameric state of PR in DDM surfactant has a lower pKa value for the proton accepting residue than both the monomeric and dimeric protein, suggesting that the hexamer is more optimized for proton transport. Time-resolved electron paramagnetic resonance (EPR) and optical absorption experiments show that the hexameric state of PR has much slower photocycle dynamics than the monomeric state. Our work shows that varying surfactant environment appears to have less of an effect on kinetics and function than does oligomerization. Protein-protein interactions therefore have a central role in tuning protein function, a result that lends insight into the functional mechanisms of more complex mammalian membrane proteins.

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Computational and Experimental Analyses of the Transmembrane Domain Dimerization of IRE1α Protein

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The Inositol-requiring 1α protein (IRE1α) is a type I transmembrane protein essential for the endoplasmic reticulum (ER) unfolded protein response that promotes cell survival by reducing the level of misfolded/unfolded proteins. IRE1α consists of an N-terminal luminal domain, a single-pass transmembrane (TM) spanning segment and a cytosolic region, which contains a kinase domain and an endoribonuclease (RNase) domain. Upon ER stress, the IRE1α protein dimerizes to activate its cytoplasmic kinase and RNase domains. Dimerization by either the luminal or the cytosolic domains can enhance IRE1α enzymatic activity. Current information on the TM domain of the IRE1α protein does not exist. Therefore, we designed a model TM segment which contains the potential TM domain sequence predicted by several TM prediction servers. using circular dichroism spectroscopy, we confirmed that the model TM peptide has α-helical conformation in POPC liposome. SDS-PAGE and FRET analyses further showed that the TM peptide forms dimers and higher-order oligomers. Molecular dynamics (MD) simulations were performed to determine potential mechanism(s) wherein the TM domain of IRE1α could dimerize and regulate IRE1α activity. We arbitrarily assigned three different conformations (face-to-face, face-to-back, and back-to-back) and embedded two TM molecules in POPC bilayers for each conformation in the MD simulations. The simulations revealed that the back-to-back is the most favorable conformation for inducing dimerization of the TM peptide. In addition, the computational results predicted that the TM dimerization is mediated by the SxxLxxx sequence motif. Further mutation analyses in cells showed that the TM domain plays a key role in regulating the dimerization as well as the protein activities. The combination of computational and experimental studies provided potential insight into how the TM domain could impact the dimerization of IRE1α.

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Structure-Function Relationship in Human Phospholipid Scramblase 1: Role of C-Terminal α-Helix

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Phospholipid scramblases (PLSCRs) constitute a group of homologous bidirectional lipid translocators that are conserved in all eukaryotic organisms. In humans, four related PLSCR genes have been identified, named hPLSCR1-hPLSCR4. The first described member and prototype of this family is hPLSCR1, a 37 kDa type II endofacial membrane protein, that is multipalmitoylated and widely expressed in most human tissues. hPLSCR1 is involved in the rapid calcium dependent translocation of plasma membrane phospholipids, although neither the detailed calcium-induced conformational change nor the mechanism of phospholipid scrambling are known yet. In addition to this role hPLSCR1 may also function in regulating processes including signaling, cell differentiation, apoptosis, injury, cell proliferation and transcription. In the present contribution we have studied the role of the C-terminal α-helix (30 aa residues) in the structure-function relationship of hPLSCR1. With that aim a truncated mutant was constructed lacking the C-terminal α-helix (hPLSCR1αC290). A combination of structural and functional studies (fluorescence and infrared spectroscopies, partial trypsin digestion and functional characterization using liposomes) reveal that the α-helix is crucial for the scramblase activity. Furthermore in the presence of calcium the truncated mutant displays a much lower affinity for this ion and, although it still undergoes conformational changes, it requires higher Ca^{2+} concentrations than the wild type. Calcium binding increases the truncated mutant stability, inducing protection against trypsin digestion and thermal denaturation.

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Regulation of Connexin26 by Ca^{2+} and Calmodulin

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Gap junction channels are key pathways for direct cell-cell communication that enables the intercellular diffusion of ions, second messengers, small metabolites and electrical coupling. To date, there are over 20 members of the gap junction channel family identified in human and they serve distinct roles in different cells and tissues. Among them, Cx26 is one of the most prominently expressed connexins (including Cx26, 29, 30 and 43) in the cochlea, which is primarily responsible of sound transduction in the inner ear. Over 100 mutations of Cx26 genes account for the 14% of the hearing loss. Here, we report a novel calcium binding pocket conserved in various family of Cxs using our established computer algorithms. In addition, we have also identified