and transmembrane domains M1-M3, and the other the fourth transmembrane domain M4) results in the assembly of functional pLGICs indistinguishable in their electrophysiological properties from wt pLGICs assembled from contiguous subunits. Alanine scanning of M1, M3 and M4 of the GlyR α1 subunit identified a total of 12 aromatic residues as important or crucial for pentameric assembly. The assembly-relevant aromatic residues cluster in one face of each helix. Homology modelling based on crystal structures (Hilf & Dutzler 2008; Bocquet et al 2008) predicted π- π interactions between the aromatic face of the M4 helix and three or two aromatic residues located in the M1 helix (Tyr228, Trp239, and Phe242) and the M3 helix (Trp286, Phe293), respectively . The loss of homopentamer formation and function seen upon alanine replacement of any of these contact residues strongly supports the existence of a membrane-embedded network of pairwisely interacting aromatic side chains that compacts and stabilizes the membrane core region of the GlyR. We infer from these results that a precise geometric arrangement of transmembrane helices defined by the tri-helical aromatic network is a prerequisite to allow the circular arrangement of the subunits stabilized essentially by earlier occurring random subunit interactions between the ectodomains.

2157-Plat

Conformational Changes in GPCR Surface and Core Probed by [^{13}C]-Methyl NMR Spectroscopy

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Recent crystal structures reveal the inactive states of non-rhodopsin G-protein coupled receptors (GPCRs) in beautiful detail. Solution NMR spectroscopy is ideally suited to contribute dynamic information regarding GPCR activation. However, these eukaryotically-expressed membrane proteins remain challenging NMR targets. We apply selective labeling with [^{13}C]methyl probes and two-dimensional NMR to analyze ligand-induced conformational changes in beta2-adrenergic receptor (b2AR).

Lysine side chains were labeled with [^{13}C]dimethyl probes to explore conformational changes in the b2AR extracellular surface. Lys305 forms a salt bridge connecting the extracellular end of transmembrane (TM) helix 7 with extracellular loop 2. The Lys305 NMR resonances are sensitive to conformational changes in the receptor extracellular surface. Using NMR, we observe disruption of the Lys305 salt bridge upon receptor activation by agonist. Computational modeling suggests that a lateral displacement of TM7 occurs in concert with an inward motion at the extracellular end of TM6 (thus extending the "global toggle switch" model of Schwartz (2006) *Annu. Rev. Pharmacol. Toxicol.*) Different conformational changes occur upon inverse agonist binding. Molecular dynamics simulations suggest that a conserved phenylalanine (Phe193) in the orthosteric ligand binding site is key for inverse agonism. Taken as a whole, these results demonstrate conformational coupling between the GPCR extracellular surface and orthosteric ligand binding site within the transmembrane domains (Ahuja (2009) *Nat. Struct. Mol. Biol.*) This provides rationale for developing allosteric pharmaceuticals targeting the GPCR extracellular surface.

Conformational changes within the b2AR transmembrane core are also observed by NMR using selective epsilon-[$^{13}CH_3$] labeling of methionines. While assignments are pending, clear conformational changes are seen with activation or inverse agonist binding. [^{13}C]methyl NMR spectroscopy, in combination with crystal structures and molecular dynamics simulation, provides a dynamic view of the conformational changes intrinsic to GPCR function.

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Observation of Structural Changes on Activation of the NTS1 G-Protein-Coupled Receptor on DNA-Templated Protein Arrays by cryo-EM

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We report the use of self-assembled DNA templates to create dense, orientationally disordered protein arrays that are optimized for single-particle cryo-EM. The nanostructure templates dramatically simplify data collection and have allowed us to obtain the first structures of a wild-type, neuropeptide-binding GPCR, the rat neurotensin receptor type 1 (NTS1), in both its ligand-free and liganded forms. Resolution better than 7Å allows clear identification of the 7 trans-membrane (TM) alpha-helices. Comparison of the structures provides the first direct observations and measurements of helix excursions during the conformational changes associated with activation of a ligand-binding GPCR. Conformational changes in the TM helices are observed upon ligand binding, namely shifts in TM1 and TM2 at the extracellular side of the membrane and pronounced shifts in TM5 and TM6 on the intracellular, G-protein interacting side that are the hallmark of the GPCR-activated state. Our results suggest a mechanism for ligand activation of a class A GPCR.

This is the highest resolution achieved to date by single-particle cryo-EM of a membrane protein, and NTS1 (43kDa) is an order of magnitude smaller than the nearest similar existing reconstruction of an asymmetric protein at comparable resolution. Our results suggest that the use of DNA-templated protein arrays has the potential to make high-resolution structure determination for small, asymmetric and hard-to-crystallize proteins routinely achievable.

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The Structure and Transport Mechanism of AdiC - an Arginine/agmatine Antiporter

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AdiC transports arginine and agmatine (the decarboxylation product of arginine) across the membrane of certain enteric bacteria including *E. coli*, as well as pathognic organisms such as *Salmonella*. It belongs to the APC (amino acids, polyamines and organic cations) superfamily. Its major role is to maintain the internal pH of the cell in the acidic environment (such as stomach) by functioning as a virtual proton pump - transporting arginine (+1 charge) into and agmatine (decarboxylated arginine, +2 charge) out of the cell, resulting in export of 1 proton out during each turnover.

We recently solved the crystal structure of AdiC with a Fab fragment at 3.2 Å. The protein is captured in an outward-open, substrate-free conformation. Both structure and functional data have shown that some aromatic residues (Y93, W293 and Y365) are important for the substrate binding and transport, yet we don't have the direct picture of the structure in the presence of either arginine or agmatine. Therefore, our current work is focused on obtaining the conformation with substrate-bound. One approach is to design pair wise cysteines that can crosslink and mimic the conformation with substrate. Comparing the structure of AdiC and several other proteins with similar fold, we are targeting TM2 and TM8 for the cysteine crosslinking now. A second approach, based on our experience with Fab production, is to find another Fab that can stabilize the substrate-bound conformation. Currently we have twelve more monoclonal antibodies that bind AdiC and need to be tested.

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Crystal Structure of the Membrane Fusion Protein CusB from Escherichia Coli

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Gram-negative bacteria, such as Escherichia coli, frequently utilize tripartite efflux complexes belonging to the resistance-nodulation-division family to expel diverse toxic compounds from the cell. These systems contain a periplasmic membrane fusion protein that is critical for substrate transport. We here present the x-ray structures of the CusB membrane fusion protein from the copper/silver efflux system of E. coli. This is the first structure of any membrane fusion proteins associated with heavy-metal efflux transporters. CusB bridges the inner membrane efflux pump CusA and outer membrane channel CusC to mediate resistance to Cu+ and Ag+ ions. Two distinct structures of the elongated molecules of CusB were found in the asymmetric unit of a single crystal, which suggests the flexible nature of this protein. Each protomer of CusB can be divided into four different domains, whereby the first three domains are mostly β-strands and the last domain adopts an entirely helical architecture. Unlike other known structures of membrane fusion proteins, the α-helical domain of CusB is folded into a three-helix bundle. This three-helix bundle presumably interacts with the periplasmic domain of CusC. The N and C-termini of CusB form the first β-strand domain, which is found to interact with the periplasmic domain of the CusA efflux pump. Atomic details of how this efflux protein binds Cu+ and Ag+ were revealed by the crystals of the CusB-Cu(I) and CusB-Ag(I) complexes. The structures indicate that CusB consists of multiple binding sites for these metal ions. These findings reveal novel structural features of a membrane fusion protein in the resistance-nodulation-division efflux system, and provide direct evidence that this protein specifically interacts with transported substrates.