allosteric modulators bind at multiple binding sites, we find ligands at different sites in the channel pore, transmembrane domain and ligand-binding domain. In combination with mutagenesis in eukaryote receptors, we probe the importance of homologous residues involved in ligand contacts. Together, our results provide a framework for structure-based design of new allosteric modulators targeting different sites in pentameric ligand-gated ion channels.

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Structural Principles of Serotonin and Granisetron Recognition in a 5-HT3 / Binding Protein Chimera

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The 5-HT3 serotonin receptor belongs to the family of pentameric ligand-gated ion channels (LGIC). A wealth of structural information on molecular recognition of nicotinic ligands has emerged from high-resolution co-crystal structures of acetylcholine binding proteins (AChBPs), which are homologous to the extracellular domain of the nicotinic acetylcholine receptor. Such information is currently missing for other members of the LGIC family, including the 5-HT3 receptor. To overcome this limitation, we used a rational approach to engineer a binding protein with ligand recognition properties similar to the 5-HT3 receptor. Amino acids contributing to binding site loops A-F in AChBP were substituted to their corresponding residues in the 5-HT3 receptor and the effect of these substitutions were characterized with a competitive binding assay using 3H-granisetron. We present crystal structures of a binding protein chimera in complex with agonists and antagonists of the 5-HT3 receptor. Together, this information provides a structural framework for understanding ligand recognition in the 5-HT3 receptor.

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Differential Functional Requirements at the Binding Site Between Serotonin and the Partial Agonist M-Chlorophenylbiguanide in Serotonin 3A Receptors

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The Serotonin 3a Receptor ($5HT3_aR$) is a pentameric ligand gated ion channel found in the central and peripheral nervous systems and implicated in numerous diseases. In previous studies with the endogenous agonist serotonin, we have identified two interactions critical for receptor function: a cation-π interaction at W183 in loop B and a hydrogen bond at E129 in loop A. Here we employ mutant cycle analyses utilizing conventional and unnatural amino acid mutagenesis to investigate how a third residue, D124 of loop A, orients the aforementioned residues for proper receptor function. We also identify differences in receptor binding and the initiation of channel gating between serotonin and the competitive partial agonist *m*-chlorophenylbiguanide (mCPBG) at these and adjacent residues.

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The Molecular Mechanism for the Dual Alcohol Modulation of Cys-Loop Receptors

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Despite high sequence identity, pentameric ligand-gated ion channels exhibit remarkable diversity in function with anionic/cationic channels that are either potentiated or inhibited by allosteric ligands. The recently available structures of bacterial homologs of Cys-loop receptors provide an excellent framework for understanding this allosteric modulation and function, but the modeling can be complex; our earlier simulations of the prokaryotic anionic glycine receptor (GlyR) suggest inter-subunit binding for ethanol (Murail, Biophys J **100**, 1642, 2011), which at first sight appears to be incompatible with the experimental *Gloeobacter violaceus* (GLIC) ligand-gated ion channel structure showing binding intra-subunit.

Here, we present new simulations of GLIC that confirm the occurrence of multiple binding sites by showing intra-subunit binding for ethanol. By experimentally introducing the single-site F238A mutation in GLIC we can turn it into a highly ethanol-sensitive channel (Howard, PNAS **108**, 12149, 2011), similar to GlyR, and simulations of the mutated species confirm the occurrence of multiple binding sites. To critically test the results, we performed extensive docking and free energy calculations to identify alcohol-binding sites and determine their affinity. In the wild-type GLIC, short alcohols preferentially bind intra-subunit, with a very weak binding site inter-subunit. However, with the F238A mutation the inter-subunit site achieves a significantly lower free energy, and even becomes the highest-affinity site in the channel for some alcohols.

These results suggest a new model for pentameric ligand-gated channel potentiation and inhibition, where the intra-subunit cavity would control inhibition, and the inter-subunit cavity potentiation. The possibility of allosteric ligands interacting with both cavities - or even a single molecule stretching from one site to the other - offers an attractive explanation for the complex functional dynamics of ligand-gated ion channels as well as an potential explain for the alcohol cutoff effect.

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Assay Development for Ligand Gated Ion Channel Pharmacology

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Modulators of ligand gated ion channel (LGIC) activity are being actively developed by a number of leading pharmaceutical companies. Electrophysiology assays remain the gold standard for determining functional compound effects on these targets, and pose unique challenges due to the need for accurate temporal control of agonist and compound application.

In this study we present results from complex assays enabled by a novel microfluidic automated patch clamp platform. The assay development process include evaluation of a number of protocols including pre-incubation, co-application, and open channel modulation modalities. The data includes case studies from GABAA, nicotinic and NMDA receptors and trade-offs between the different available measurement modalities developed.

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Agonist Interactions and Selectivity in Inhibitory Cys-Loop Ligand-Gated Ion Channels

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Cys-loop ligand-gated ion channels constitute one of two major superfamilies of receptors mediating rapid chemical synaptic transmission in the central nervous system. Mammalian members of the superfamily include cation selective channels that are receptors for excitatory neurotransmitters, acetylcholine and serotonin, and anion selective channels that are receptors for inhibitory neurotransmitters, GABA and glycine. Structural information from snail acetylcholine binding proteins (AChBP), torpedo acetylcholine receptors provided a clear picture of the acetylcholine-binding site, including the conserved aromatic box which forms cation-pi bonds with bound agonists. From AChBP-based homology models, we proposed (Cromer 2002) that the 3-dimensional position of one of the aromatic box residues is replaced with an acidic residue in inhibitory receptors for GABA (GABAA/CR) and glycine (GlyR). Further that this residue forms a salt-bridge interaction with the cation group of bound agonists and is important for selectivity for primary amine agonists, such as GABA and glycine, over bulkier quaternary amines, such as acetylcholine.

We now present evidence in support of this hypothesis, particularly using the homopentameric Rho1 GABA receptor (GABAC) as a model for the broader family of inhibitory Cys-loop LGICs. We also present evidence for a series of other interactions in the agonist-binding site of inhibitory Cys-loop LGICs that are determinants of selectivity between agonists of different size, such as glycine versus GABA. These results are consistent with our initial hypothesis and provide a more detailed understanding of agonist-receptor interactions in inhibitory Cys-loop ligand-gated ion channels.

Cromer, B. A., Morton, C. J. & Parker, M. W. (2002). Anxiety over GABA(A) receptor structure relieved by AChBP. Trends Biochem Sci 27, 280-7.

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Structure and Function of ELIC Bound with the Antagonist Acetylcholine

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The bacterium Erwinia chrysanthemi ligand gated ion channel (ELIC) is a prototype for Cys-loop receptors, including nicotinic acetylcholine receptors (nAChRs). We co-crystallized ELIC with acetylcholine, an endogenous agonist of nAChRs, and solved the crystal structure to a resolution of 2.9 Å. Acetylcholine binds to the orthosteric agonist site for Cys-loop receptors. Upon acetylcholine binding, loop C exhibits a substantial contraction with a profound reduction in flexibility. Conformational changes in nearby regions of the ligand-binding domain are also observed. However, no substantial reorganization in the pore