Allosteric modulation of ligand-gated ion channels

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Pentameric ligand-gated ion channels (pLGICs) or Cys-loop receptors are a class of ion channels involved in fast synaptic transmission in the central and peripheral nervous systems. Members of this family include the nicotinic acetylcholine receptors, serotonin type-3 receptors, $GABA_{A/C}$ (gamma-aminobutyric acid) receptors and glycine receptors. These receptors are the target for a wide variety of therapeutics, including benzodiazepines such as diazepam (Valium), which act as positive allosteric modulators (PAMs) of the $GABA_A$ receptor and are prescribed as a sedative, anxiolytic or anti-epileptic. In this study, we used a structural approach to better understand the structural mechanisms of allosteric modulation in this class of ion channels. In one line of research we employed nanobodies, which are antibody fragments derived from camelids and have the potential to trap conformationally transient states, and used them as crystallization chaperones for the prokaryote ligand-gated ion channel ELIC [1]. Functional characterization of ELIC nanobodies using electrophysiological techniques demonstrated that these nanobodies are active as channel modulators. We demonstrate that nanobodies can either inhibit or potentiate agonist-evoked channel responses, suggesting that nanobodies can act as negative (NAMs) or positive allosteric modulators (PAMs), respectively. The X-ray crystal structure of ELIC in complex with a nanobody reveals that a nanobody binds at an allosteric binding site and near to a binding site previously known to mediate potentiation of ELIC by the benzodiazepine flurazepam. In a parallel line of research, we employed fragment-based screening via the iNEXT platform to target novel allosteric binding sites in the α7-Acetylcholine Binding Protein (AChBP), which is a homologue of the extracellular ligand binding domain of the human α7 nicotinic acetylcholine receptor [2]. Strikingly, we found that a number of fragment hits converge on the same allosteric binding site that was identified using nanobodies directed against ELIC. Given the structural conservation of allosteric binding sites between ELIC and human receptors our study reveals common mechanisms for allosteric modulation in this family of channels. Our expanded knowledge of the structural determinants of allosteric modulation opens opportunities for the development of new therapeutics targeting pLGICs in ion channel-related disorders.

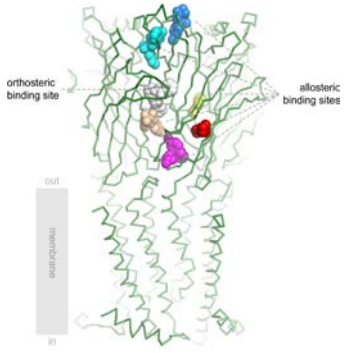

Figure 1: Overview of different allosteric binding sites in the extracellular ligand binding domain of a pentameric ligand-gated ion channel ELIC.

References

[1] - X-ray structure of a prokaryotic pentameric ligand-gated ion channel. R.J. Hilf, R. Dutzler, Nature. 2008;452(7185):375-9.

[2] - Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. K. Brejc, W.J. van Dijk, R.V. Klaassen, M. Schuurmans, J. van Der Oost, A.B. Smit, T.K. Sixma Nature. 2001;411(6835):269-76.