G PROTEIN-COUPLED RECEPTORS

Finding common threads

Despite substantial recent advances in the understanding of the structure and function of G protein-coupled receptors (GPCRs), many of these receptors remain poorly characterized. Now, two papers seek to help address such gaps by organizing GPCRs based on either ligand or structural similarities.

As some GPCR ligands can activate unrelated receptors (for example, serotonin (5-HT) can activate both GPCRs and ion channels), Shoichet and colleagues conducted a pioneering ligand-based classification of the class A (rhodopsin-like) family of GPCRs to see whether it would yield unexpected relationships in comparison with the more traditional sequence- and/or structure-based classification of such receptors.

They used the ChEMBL database and the chemoinformatic [similarity ensemble approach](#) to calculate dendrograms and an 'expectation value' (*E*-value) that scores how closely the receptors relate to each other based on the chemical characteristics of known ligands for each receptor.

In contrast to the sequence-based dendrogram, which clustered similar receptors together as expected, the ligand-based dendrogram revealed new relationships. For example, the muscarinic receptors were separated from other biogenic amine GPCRs and moved towards the chemokine receptors, even though they have a lower level of similarity based on the sequence of their orthosteric ligand-binding sites.

Interestingly, many of the GPCRs that became neighbours according to their ligand-based similarities did not share any known ligands, and

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so the authors next performed a virtual screen to prospectively identify candidates. For one such pair of neighbouring receptors — κ -opioid receptor (OPRK) and 5-HT_{2B} — an *in vitro* experiment showed that a compound identified by this approach had similar inhibition constant values for both of these receptors. Two other new GPCR pairs were similarly related by finding, for the first time, a ligand that bound to both.

Notably, 485 non-GPCRs were found to be closely related to at least one GPCR by ligand similarities. Three pairs of targets were also tested and shown to share a ligand, including the chemokine receptor CXCR2 and casein kinase 1. So, although there are important limitations to the approach, including a 50% false-positive rate regarding the *E*-values and the ligands not being distinguished by their mode of action (that is, binding to orthosteric or allosteric sites), it could be valuable in identifying molecules with desirable polypharmacological properties.

In the paper by Babu and colleagues, a systematic analysis of the published data of solved GPCR structures (more than 75, all belonging to the class A family) enabled them to identify key similarities among the three main GPCR domains: the extracellular, transmembrane (TM) and intracellular regions. Among the detailed observations noted by the authors, they identified a network of 24 non-covalent contacts

between the seven α -helices that are conserved across the structures (for both active and inactive states). They suggest that these contact points may be an evolutionarily conserved scaffold and could help in future GPCR modelling or engineering experiments.

Regarding the ligand-binding pocket and changes during receptor activation, most of the ligand-contacting residues reside on the TM3, TM6 and TM7 helices (except for the GPCRs CXCR4 and the neurotensin receptor NTS_{1R}), and receptor activation leads to common rearrangements in the TM bundle. Furthermore, given the involvement of almost every residue on the TM3 helix in maintaining conserved interhelical contacts upon binding to a ligand or G protein, the authors suggest that TM3 plays the part of a “structural and functional hub” in the GPCR fold.

Together, these approaches may help in characterizing other members of the GPCR family for which no ligand has been found (orphan receptors) or high-resolution structure obtained, as well as in identifying novel ligands such as those that have desirable activity against multiple GPCRs and other unrelated protein families.

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ORIGINAL RESEARCH PAPERS Lin, H. et al. A pharmacological organization of G protein-coupled receptors. *Nature Methods* **10**, 140–146 (2013) | Venkatakrishnan, A. J. et al. Molecular signatures of G protein-coupled receptors. *Nature* **494**, 185–194 (2013)