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G PROTEIN-COUPLED RECEPTORS

Illuminating orphan GPCRs

G protein-coupled receptors (GPCRs) have a central role in physiology and disease and represent the most productive drug targets. However, more than 100 non-olfactory GPCRs are 'orphan' receptors whose endogenous ligands, and often physiological roles, remain unknown. Here, Roth, Shoichet and colleagues describe an integrated experimental and computational approach to identify ligands for orphan GPCRs, identifying a potent modulator of GPR68 and a potential role for this receptor in learning and memory.

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GPR68 belongs to a family of proton-sensing GPCRs and is expressed in many tissues in mice, particularly in the cerebellum and hippocampus. Although GPR68 has been implicated in several processes, potential roles have not been well characterized in knockout mouse models. Moreover, this GPCR is considered 'pharmacologically dark', as it lacks small-molecule modulators that can probe its activity.

To identify ligands that modulate GPR68, Roth and colleagues first used yeast-based screens of a library of approved small-molecule drugs, finding the benzodiazepine lorazepam to activate GPR68. Further analysis revealed lorazepam activity to be pH dependent, indicating the compound to be functioning as a positive allosteric modulator (PAM) of GPR68.

Next, the authors set out to model the GPR68–lorazepam complex. To do this, they generated 407 three-dimensional homology models for GPR68 templated onto the structure of CXCR4 (which is the nearest template of GPR68, sharing 29% sequence identity). Using elastic network modelling, which samples backbone and loop conformations, the number of models was expanded to 3,307. Lorazepam, inactive compounds from the National Clinical Collection, and property-matched decoy molecules were then computationally docked in five candidate allosteric sites in each of the homology models, and the ranking of lorazepam versus decoy molecules was evaluated. Iterative cycles of modelling and optimization converged to a stable lorazepam docking pose, which they confirmed by mutating specific residues lining the site.

To identify optimized PAMs of GPR68, over 3 million available lead-like molecules were computationally docked against the putative lorazepam site in GPR68. Further testing and optimization of some of the top 0.1% of the docking-ranked molecules ultimately led to the identification of ogerin as a markedly more potent PAM than lorazepam. Screening ogerin for off-targets using the Similarity Ensemble Approach program demonstrated the specificity of ogerin for GPR68.

To explore possible functions of GPR68, the authors evaluated *Gpr68*-knockout and wild-type mice in contextual fear-conditioning, a hippocampus-dependent learning and memory test. Ogerin attenuated context-based fear memory in wild-type mice, but had no effect on memory retrieval in *Gpr68*-knockout mice, revealing a potential novel role for GPR68 in hippocampal-associated learning and memory.

Demonstrating the general applicability of this approach, the authors identified ligands for the related proton-sensing GPCR family member GPR65, which shares 37% sequence homology with GPR68. Using the recently identified GPR65 agonist BTB09089 to anchor modelling of GPR65, they generated homology models templated on GPR68. The final docked GPR65–BTB09089 model resembled that of GPR68–ogerin and computational docking of the same 3.1 million compounds identified a novel allosteric agonist and a negative allosteric modulator of GPR65.

In summary, this study demonstrates that combining physical and structure-based screening may be a broadly useful approach for deorphanizing GPCRs.

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