Crystal structure of the first orphan GPCR

G-protein-coupled receptors (GPCRs) are membrane proteins with seven-transmembrane (7TM) structural features. Of the over 800 members in the human genome, there are more than 100 are orphan receptors whose endogenous ligands are yet to be identified. GPR52 is a Class-A orphan GPCR highly expressed in the brain, particularly in the striatum. The psychiatric diseases in which it plays a role include hyperactivity, schizophrenia [1], psychiatric disorders, brain malformation [2], and cognitive symptoms. Therefore, GPR52 is a promising target for a variety of neurological disorders. Moreover, it was recently reported as a potential therapeutic target for Huntington's disease [3]. However, tool ligand and drug discovery have been largely hampered by a lack of structural understanding due largely to the low homology (< 20%) of GPR52 to any known GPCR structure. Orphan GPCR structures are highly demanded and we decided to solve the first one by focusing efforts on GPR52.

To obtain a stable GPR52 protein for structural determination, we generated and screened over 600 constructs, tried different purification procedures, and did crystallization trials for more than 100 constructs. Finally, we obtained multiple types of crystals for GPR52 and collected diffraction data at SPring-8 **BL41XU** and **BL45XU** with kind help of beamline scientists. Guided by the feedback of data collection at SPring-8 from the initial crystal hits, we immediately optimized the crystals following the right direction which allowed us to obtain the high-quality diffraction data for structural determination within a few months. Finally, we were able to collect three complete data sets at SPring-8 BL41XU and BL45XU beamlines and solved high-resolution structures for GPR52: one in complex with agonist c17 (at 2.2 Å) and two in ligand-free (apo) forms (at 2.8 Å, 2.9 Å) [4].

The two apo structures in the ligand-free state (GPR52-Rub-apo and GPR52-Fla-apo) were engineered with different ICL3 fusion partners and were crystallized in different space groups. Comparison of the two structures reveal that the overall conformations at the transmembrane region were essentially identical (root-mean-square deviation (RMSD) of helix bundle Cα is 1.1 Å), confirming that receptor conformation was not altered by crystal packing (Fig. 1(a)). we will not distinguish the two structures and named them GPR2-apo unless otherwise noted.

With close examination of the GPR52-apo structure, a 22-residue ECL2 caught our attention as it folds into a special configuration and occupies the orthosteric binding pocket of the receptor. To maintain this unique configuration, the side chain of $Y185^{ECL2}$ packs tightly into a local aromatic environment formed by the residues $Y281^{6.51}$, $Y284^{6.54}$ and $F285^{6.55}$ of TM6. In addition, $K182^{ECL2}$ forms a salt bridge with $D188^{ECL2}$, $C193^{ECL2}$ forms a disulfide bond with $C114^{3.25}$ in TM3, both interactions strongly hold the ECL2 in its registry (Fig. 1(b)). Alignment of the ECL2 with canonical ligand binding pocket in other GPCRs suggests that this motif may behave as an agonist intrinsically contributing to the high basal activity of GPR52. To test this hypothesis, mutagenesis

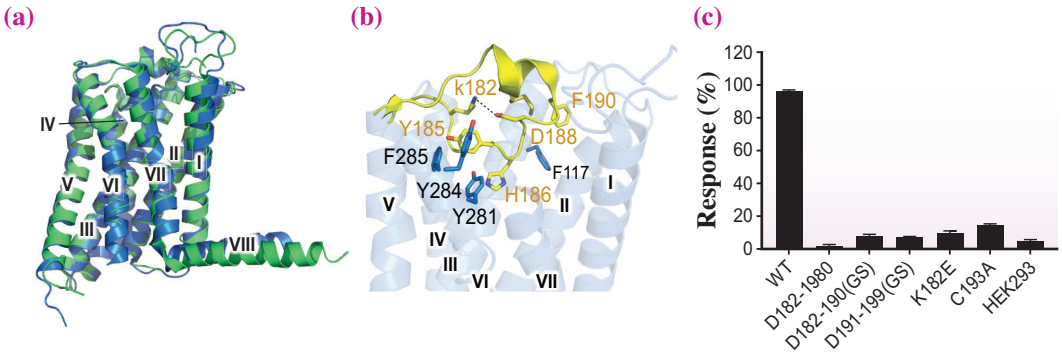

Fig. 1. **(a)** Overall structure of GPR52-rub-apo (green) and GPR52-Fla-apo (blue). **(b)** The close view of the ECL2 in the orthosteric binding pocket. Key residues are shown as sticks. **(c)** Mutations that interfere with the conformation of the ECL2 reduced downstream signaling in the cellular cAMP assay.

and cellular functional assays showed that deleting residues 182–198, replacing residues 182–190 or 191–199 with a 6-residue linker (GGSGGS), breaking the disulfide bond between $C193^{ECL2}$ and $C114^{3.25}$ or mutating the single key residue $K182^{ECL2}$ all markedly reduced the basal activity of GPR52 (Fig. 1(c)).

Next, we were curious where the tool ligand binds if the orthosteric pocket is already occupied by the ECL2. We therefore set out to investigate the binding mode of a GPR52 agonist by co-crystallizing GPR52 with the surrogate ligand c17. The overall conformation of GPR52-c17 is highly consistent with GPR52-apo and the C_α RMSD of the two structures at the helix bundle is 1.7 Å. The most remarkable difference occurs at the N-terminal loop. In the GPR52-c17 structure, it is well-folded and engaged in the coordination with the c17 ligand. In particular, the conformation of the ECL2 region is highly conserved in GPR52-apo and GPR52-c17 structures, suggesting that c17 may play a positive allosteric modulating role to further enhance the receptor activity without disturbing the intrinsic conformation of ECL2. In the GPR52–c17 complex, the N-terminal loop and ECL2 push the ligand towards one side and contribute to the formation of a new ligand pocket—side pocket (Fig. 2(a)).

We compared the side pocket of GPR52 to that of Class-A representative peptide receptors, non-lipid small-molecule receptors and lipid-activated receptors. We found that c17 in GPR52 is located closer to TM1, TM2 and TM7 while other ligands are closer to TM4–TM6—a ligand-binding mode that is commonly seen in other Class-A receptors (Fig. 2(b)). The GPR52 unique ligand-binding side pocket we have revealed can be targeted by rational structure-based ligand design and holds promise for selective drug screening owing to its allosteric-like features.

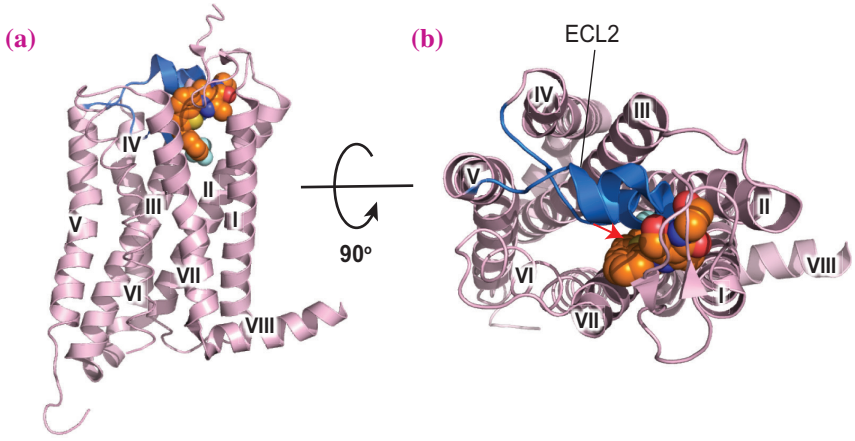

Fig. 2. Side view **(a)** and top view **(b)** of GPR52-c17 (pink-orange) complex, ECL2 is colored in blue.

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