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Allosteric ligand subpocket of $S1P_5$ as a determinant of inverse agonism and ligand specificity

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Motivation and Aim: S1P is a bioactive lysophospholipid targeting GPCRs. It acts via five different subtypes of S1P receptors (S1PR)—$S1P_{1\text{-}5}$. Despite sequence similarity, these receptors differ in expression profiles: $S1P_{1\text{-}3}$ receptors are abundant, $S1P_4$ is limited to the immune system, and $S1P_5$ is mainly present in nervous and immune systems. The last one regulates cell migration for NK cells and oligodendrocyte progenitors, playing a role in autoimmune, neurodegenerative and oncogenic disorders. Although FDA approved multiple non-selective (fingolimod) or dual $S1P_1$/$S1P_5$ agonists, few selective modulators were available until the last year. Lack of these modulators leads to constraints in $S1P_5$ signaling research. To speed up the design of $S1P_5$ ligands, an inactive structure of this receptor can contribute.

Methods: Optimized for insect cell expression, the human *S1PR5* gene was fused with a partner protein in the 3rd intracellular loop, expression tags on N-term, and a C-term cleavable decahistidine tag. This construct was expressed in Sf9 cells using the baculovirus expression system, purified with metal-affinity chromatography and co-crystallized in the lipid cubic phase with an inverse agonist. The crystals undergone room temperature serial femtosecond crystallography (SFX) data collection at Pohang Accelerator Laboratory X-Ray Free-Electron Laser (PAL-XFEL) in South Korea.

Results: The crystal structure was solved at a 2.2 Å resolution in the $P2_12_12_1$ space group with identical unit cell constants as the single-crystal data. The receptor crystallized with two monomers per asymmetric unit, forming an antiparallel dimer through the TM4-TM4 interface. The structure shares the classical Edg-receptor architecture with seven transmembrane helices bundle, N-terminal and C-terminal helices. Receptor's microswitches conformations correspond to inactive position, indirectly locked by the ligand.

Conclusions: This inactive-state structure of $S1P_5$ in a complex with an inverse agonist. It reveals an uncommon allosteric binding mode of ligand targeting the variable sub-pocket of an S1PR and displays the way to design inverse agonists.

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