Crystal structure of the human Frizzled-4 receptor

10 Frizzled receptors (FZDs) belong to a class of unconventional G-protein-coupled receptors (GPCRs) that function in WNT signal transductions and are gatekeeping proteins in regulating the fundamental signaling axis in embryonic development and tumorigenesis [1] (Fig. 1). Thus, the FZDs have been recognized as promising cancer drug targets for decades yet no drugs have been yielded from efforts toward discovering anti-FZD therapeutics. Uncovering the structures of extracellular soluble region in FZDs, the cysteine-rich domain (CRD) and its complex with WNT led to the discovery of small-molecule ligands and biologics targeting on this region [2]. However, until now, there has been no reported ligand targeting on the transmembrane domain of FZDs, the traditional pocket on which numerous GPCR drugs act. In order to understand why no one has been able to develop good tool ligands or drug molecules for FZDs and to tackle the mystery of FZD signaling, we solved the intact transmembrane domain structure of Frizzled4 receptor (FZD4) at 2.4 Å resolution [3].

As a representative of the Frizzled family, FZD4 mediates the canonical WNT signaling. It harbors a multi-domain architecture including the family-conserved extracellular CRD, a 7-helical transmembrane bundle (7TM or TMD), and a hinge domain that we previously defined for Smoothened (SMO) [4], a close homology of Frizzled. Uniquely for FZD4, it also recognizes Norrin protein to mediate the canonical β-catenin signaling. Structural investigation of FZD4 will provide the first insight to understand the structure-function relationship of other FZDs which are emerging cancer drug targets.

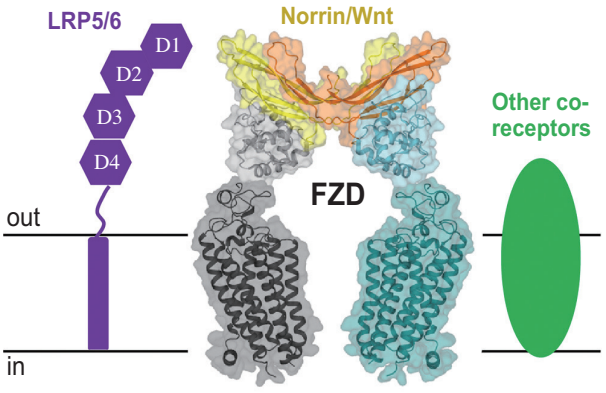

Fig. 1. Simplified illustration of Wnt (Norrin)-Frizzled signaling pathway. Wnt (or Norrin) induced Frizzled signaling requires clustering of transmembrane components such as Frizzled, LRP5/6 and other co-receptors. The intact signalosome also requires intracellular components such as Axin, DVL, GSK and other factors.

To generate a stable human FZD4 protein amenable for structure determination in the absence of a ligand (apo state), we screened hundreds of constructs, optimized purification procedures, and tried thousands of possible crystallization methods. It turns out to be extremely challenging to obtain diffraction-quality crystals, likely due to the lack of a tool ligand to lock the flexible region in the protein. During the course of crystal optimization, the efficient feedback from SPring-8 data collection results provided us the right direction. Finally, with introduction of stabilizing mutations and further optimization of crystallization conditions we were able to collect a complete data set at SPring-8 **BL41XU**. We solved the structure of FZD4 in the apo state, which is the first structure of the Frizzled family GPCRs and the first apo structure of a ligand-regulated GPCR as far as we know.

It is surprisingly observed that the traditional orthosteric ligand binding site is very narrow making it hard for small molecules to enter or bind (Fig. 2). More importantly, such a narrow and hydrophilic pocket is highly conserved among 10 FZDs, but not in SMO. These findings improved our understanding of the FZD ligand and signaling, and showed that ligand design for this pocket may require special considerations that could be inspired by this crystal structure.

Although both FZD4 and SMO belong to Class-F GPCRs, and they share similar overall architecture, the Frizzled and SMO are distinguishable in several ways: (1) from the structure perspective, the helix VI extension and ECL3 in FZD4 are much shorter than that in SMO (Fig. 3(a)). In SMO, the long helix VI extension and ECL3 create a large interface for CRD interaction and contribute to the stable “straight-up” conformation of CRD relatively to TMD. In contrast, such a short ECL3 in FZD4 may result in flexible CRD connection leading to swinging dynamics in the extracellular region as seen in MD simulations of the full-length model [3]. This comparison suggests different ways of CRD connection and extracellular ligand recognition. Additionally, the intracellular end of helix V in FZD4 moves outward from the helical bundle by about 13°. These differences between FZD4 and SMO are consistent with the varied upstream and downstream signaling of these two receptors. (2) Family-conserved residues such as $Y250^{2.39f}$ (transplanting the B&W numbering system

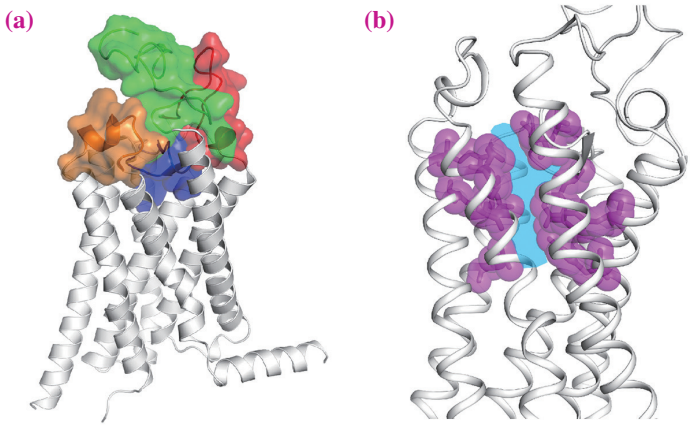

Fig. 2. **(a)** Overall structure of ΔCRD-Frizzled4. The hinge domain, ECL1, ECL2, ECL3 are colored in green, red, blue, and orange, respectively. **(b)** Highlight of the narrow pocket at the transmembrane domain. Residues constituting the transmembrane pocket are represented in stick-ball in magenta.

from Class A GPCRs), F440$^{6.36f}$ and W494$^{7.55f}$ adopt different conformations in the two structures (Figs. 3(b,c)). It is conceivable that these residues play important roles in downstream signaling, and evolution has endowed these key residues distinct conformations for respective function. (3) Molecular dynamics simulations at microsecond-time scale and mutational analysis uncovered two coupled, dynamic kinks located at helix VII that are involved in FZD4 activation. However, residues constituting these two kinks are conserved in 10 FZDs but not in SMO, suggesting that FZDs and SMO may undergo different conformational changes while being activated.

In addition to the discovery of a vacant and narrow pocket, the structure also reveals an unusual packing in transmembrane helices, providing new insight into a potential activation mechanism of this family of receptors. Such a remarkable structure provides a more accurate template to redirect the efforts on Frizzled drug discovery.

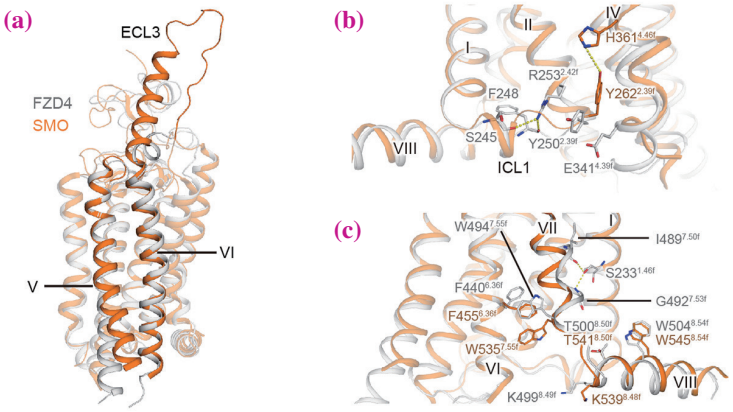

Fig. 3. **(a)** Comparison of ΔCRD-FZD4 (gray) with ΔCRD-SMO (orange, truncated from PDB: 5L7D). **(b)** and **(c)**, Conformational rearrangement of Y2.39f, F6.36f and W7.55f were observed between FZD4 and SMO.

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References

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