

Structural characterization of FZD7, the importance of water network and cholesterol for class F GPCRS

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Background

For more than 30 years, since the discovery of WNTs, WNT/ β -catenin and planar cell polarity signaling has formed the basis for what we understand to be the primary output of the interaction between the protein ligands of the WNT family and their receptors known as FZDs (ten isoforms: FZD1-10). FZD7 is one of the best characterized receptors within the family and plays a critical role in many biological processes including migration of mesendoderm cells during development and renewal of intestinal stem cells in adults. Moreover, FZD7 has been highlighted for its involvement in tumor development predominantly in the gastrointestinal tract. This research aims to provide a better understanding of FZDs in general with a highlight on FZD7 by combining structural, computational, and pharmacological tools.

Methods

In this study, we apply a combination of conventional cryo-electron microscopy (cryo-EM) single particle analysis, MD simulations, and phylogenetic analysis to draw FZD family-wide conclusions on structural aspects and mechanisms of FZD activation. These data are complemented with pharmacological experiments employing genetically encoded biosensors to functionally validate our structural findings. This comprehensive approach provides us with insights into the function of FZD7 specifically, as well as FZDs in a broader context.

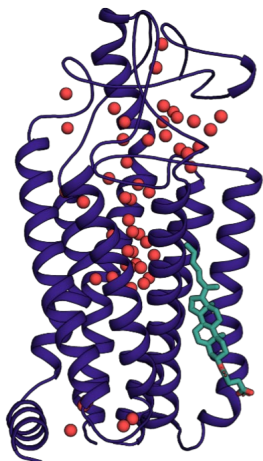
Results

We report the structure of inactive FZD7, without any stabilizing mutations, determined by cryo-EM. This allowed us to provide a direct comparison with the G protein bound FZD7 and to confirm previously identified residues involved in G protein binding mechanism. We characterized a fluctuating water pocket in the core of the receptor important for FZD7 dynamics and used a phylogenetic analysis to define conserved residue defining the water pocket base that remains hermetic upon G protein binding unlike what is observed in Class A GPCRS. Molecular dynamics simulations were then used to investigate the temporal distribution of those water molecules and their importance for potential conformational changes in FZD7. Additionally, we discovered lipids that interact with the receptor core and a conserved cholesterol binding site. This site plays a pivotal role in the association of FZD7 with a transducer protein, Dishevelled (DVL), and in the initiation of downstream signaling and the formation of signalosomes.

Conclusion

We provided a high-resolution structure of FZD7 and defined functionally relevant features of FZDs dynamic and signaling.

Graphic:



Keywords:

GPCRs, FZDs, FZD7, Cryo-EM

Reference:

Turku, A., Schihada, H., Kozielowicz, P., Bowin, C. F., & Schulte, G. (2021). Residue 6.43 defines receptor function in class F GPCRs. *Nature Communications*, 12(1), 1–14.

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