Unsaturated fatty acyl recognition by Frizzled receptors mediates dimerization upon Wnt ligand binding

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Edited by Roeland Nusse, Stanford University School of Medicine, Stanford, CA, and approved March 10, 2017 (received for review November 3, 2016)

Frizzled (FZD) receptors mediate Wnt signaling in diverse processes ranging from bone growth to stem cell activity. Moreover, high FZD receptor expression at the cell surface contributes to overactive Wnt signaling in subsets of pancreatic, ovarian, gastric, and colorectal tumors. Despite the progress in biochemical understanding of Wnt–FZD receptor interactions, the molecular basis for recognition of Wnt cis-unsaturated fatty acyl groups by the cysteine-rich domain (CRD) of FZD receptors remains elusive. Here, we determined a crystal structure of human FZD7 CRD unexpectedly bound to a 24-carbon fatty acid. We also report a crystal structure of human FZD5 CRD bound to C16:1 cis-Δ9 unsaturated fatty acid. Both structures reveal a dimeric arrangement of the CRD. The lipid-binding groove exhibits flexibility and spans both monomers, adopting a U-shaped geometry that accommodates the fatty acid. Re-evaluation of the published mouse FZD8 CRD structure reveals that it also shares the same architecture as FZD5 and FZD7 CRDs. Our results define a common molecular mechanism for recognition of the cis-unsaturated fatty acyl group, a necessary posttranslational modification of Wnts, by multiple FZD receptors. The fatty acid bridges two CRD monomers, implying that Wnt binding mediates FZD receptor dimerization. Our data uncover possibilities for the arrangement of Wnt–FZD CRD complexes and shed structural insights that could aide in the identification of pharmacological strategies to modulate FZD receptor function.

Wnt signaling is evolutionarily conserved from metazoa to humans (1). It is critical for tissue homeostasis and during development (2, 3). On the other hand, derangements in Wnt signaling are associated with severe pathologies in mammals, including cancer (4–6). Signaling is initiated at the cell surface where the secreted, lipid-modified Wnt glycoprotein interacts with the extracellular N-terminal cysteine-rich domain (CRD) of the frizzled (FZD) receptor (7). This high-affinity interaction occurs at two distinct contact sites, one comprising a protein–fatty acyl interface and another a protein–protein interaction interface (8–10). Sequence analysis revealed that there are 10 human frizzled genes grouped into four clusters (SI Appendix, Fig. S1A).

Among their multiple roles in physiology, FZD receptors have emerged as critical regulators of Wnt-dependent stem cell processes. For example, FZD7 is a critical component of the stem cell niche and was shown by genetic knockdown experiments to be essential for maintaining human embryonic and epithelial limbal stem cells in an undifferentiated state (11, 12). Of the 10 mammalian frizzled, FZDs 1, 2, and 7 are enriched at the base of mammalian adult intestinal crypts where the stem cells reside (13, 14), and FZD7 is required for stem cell-mediated regeneration of the intestinal epithelium (15). Moreover, high FZD receptor expression at the cell surface contributes to overactive Wnt signaling in subsets of pancreatic, ovarian, gastric, and colorectal tumors (16–19). Recently, antibody-mediated inhibition of FZD5 demonstrated a role for this receptor in pancreatic ductal adenocarcinoma and colorectal carcinoma models (20). Altogether, these findings highlight the importance of FZD receptors in Wnt-dependent stem cell and cancer biology.

Critical for Wnt secretion to the extracellular environment and activity is its fatty acylation. This covalent modification occurs at a conserved serine residue and is catalyzed by the O-acyltransferase porcupine, which efficiently transfers a 16-carbon cis-Δ9 unsaturated fatty acyl (C16:1n-7; palmitoleic acid) onto Wnt (21–23). Multiple lines of evidence support the notion that Wnt proteins are modified preferentially with cis-unsaturated fatty acids. First, pharmacological inhibition of stearoyl-CoA desaturase, an enzyme that generates cis-Δ9 unsaturated fatty acyl CoA substrates, abolishes fatty acylation of Wnt proteins in mammalian cells, unless exogenous cis-monounsaturated fatty acid is supplied to the cellular medium (22). Second, addition of exogenous palmitoleic acid abolishes metabolic incorporation of a nonradioactive alkyne palmitic acid probe into Wnt proteins, indicating that the palmitoleoyl is the preferred fatty acyl group for incorporation into Wnt (21, 24). Finally, mass spectrometry experiments uncovered C16:1n-7 as the major fatty acyl species on mWnt3a, with trace levels of unsaturated C14:1n-5 (myristoleic acid) detected (23).

Prior structural studies of apo mouse (m) FZD8 CRD (25) and mFZD8 CRD in complex with Xenopus (X) Wnt8a (9) provided a molecular view of the configuration of FZD8 CRD and its interaction with Wnt proteins, but stopped short of elucidating the molecular basis for CRD recognition of cis-unsaturated fatty acyl groups. Here, we present FZD CRD crystal structures in complex with free fatty acids. We show that the fatty acid bridges two CRD monomers, implying that Wnt binding mediates FZD receptor dimerization. Our data uncover possibilities for the arrangement of Wnt–FZD CRD complexes and shed structural insights that could aide in the identification of pharmacological strategies to modulate FZD receptor function.

Significance

Wnt proteins signal through frizzled (FZD) receptors to regulate physiological processes; however, the structural basis for recognition of the Wnt unsaturated fatty acyl group by FZD remains elusive. Here, we report the first structures of the extracellular cysteine-rich domain (CRD) of two members of the FZD family in complex with free fatty acids. We show that the fatty acid bridges two CRD molecules and occupies the lipid-binding groove, which adopts a U-shaped geometry and exhibits flexibility. Our findings suggest a common mechanism for fatty acyl recognition by multiple FZD receptors and imply that Wnt binding to FZD mediates its dimerization. Overall, this study provides structural insights into how cell-surface FZD receptors recognize cis-unsaturated fatty acyl groups on Wnt ligands.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes 5URV, 5URZ, 5URY, and 5T4E).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1618293114/-/DCSupplemental.
long-sought-after structural rationale for how FZD receptors bind to cis-unsaturated fatty acyl groups on Wnt ligands.

Results and Discussion

Structure of hFZD7 CRD in Complex with a C24 Fatty Acid Reveals a U-Shaped Lipid-Binding Groove. We initially set forth to determine the crystal structure of apo hFZD7 CRD. We expressed and purified hFZD7 CRD (residues Gln33–Gly168) as a soluble secreted protein in insect cells. The X-ray crystal structure of hFZD7 CRD was solved by molecular replacement and refined to a resolution of 2.20 Å (SI Appendix, Table S1). hFZD7 CRD adopted a homo-dimer arrangement, with an α-helical dimer interface between two protomers (chains A and B) that composed the crystallographic asymmetric unit. Unexpectedly, we observed an extra electron density in the lipid-binding cavity, showing an elongated shape that could be readily modeled as a free fatty acid molecule (C24) (Fig. 1; SI Appendix, Fig. S1B). Such a fatty acid presumably originated in the insect cell expression host. The observed electron density indicated that the carboxylate group of the fatty acid was present in one monomer (chain A), whereas the methyl end of the hydrocarbon chain was present in the other monomer (chain B) (Fig. 1). We could not unambiguously determine the degree of unsaturation within the fatty acyl chain from the electron density. Each monomer in the hFZD7 CRD dimer contained a lipophilic groove. The two lipid-binding grooves met at the dimer interface, forming a contiguous and bent (U-shaped) cavity (Fig. 1). We could not unambiguously determine the degree of unsaturation within the fatty acyl chain from the electron density. In particular, residues lining the hydrophobic cavity such as Tyr87 and Phe138 appeared to partially occupy the lipid-binding cavity in the apo structure (Fig. 1D) but adopted alternative rotamer conformations in the lipid-bound structure, suggesting flexibility in the lipid-binding cavity that may play a role in regulating lipid specificity.

Structure of apo hFZD7 CRD Reveals Flexibility in the Lipid-Binding Groove. Under different crystallization conditions, we also obtained the crystal structure of apo hFZD7 CRD, which was solved by molecular replacement and refined to a resolution of 2.00 Å (SI Appendix, Table S1). Intriguingly, hFZD7 CRD crystalized this time in the absence of any endogenous fatty acid originating from the expression host. The lack of the fatty acid could be attributed, in part, to differences in the crystallization conditions compared with C24-bound hFZD7 CRD. The apo hFZD7 CRD structure exhibited a similar dimer configuration and lipid-binding groove geometry as that observed in the structure of C24-bound hFZD7 CRD. The asymmetric unit consisted of two protomers (chain A and chain B) joined through an α-helical dimer interface (SI Appendix, Fig. S2A). Even though both apo- and lipid-bound structures superimposed well (SI Appendix, Fig. S2B), there were some key conformational changes observed. In particular, residues lining the hydrophobic cavity such as Tyr87 and Phe138 appeared to partially occupy the lipid-binding cavity in the apo structure (Fig. 1D) but adopted alternative rotamer conformations in the lipid-bound structure, suggesting flexibility in the lipid-binding cavity that may play a role in regulating lipid specificity.

Structural Basis for Recognition of cis- Unsaturated Fatty Acids by hFzd5 CRD. The above unexpected observations in the hFZD7 CRD:C24 complex structure, in particular the U-shaped geometry of the lipid-binding groove around the dimer interface and its asymmetric recognition of the fatty acid, prompted us to examine how other closely related FZD CRDs would bind to a physiologically relevant lipid present on Wnt proteins. There are no reported structures of FZD5 CRD or of any other FZD CRD in complex with unsaturated fatty acids. FZD5 CRD exhibits evolutionary proximity to FZD7 and 8 CRDs (SI Appendix, Fig. S1A). Therefore, we co-crystallized hFzd5 CRD (residues Ala27–Ala155) in complex with C16:1n-7 fatty acid, and the obtained X-ray crystal structure of the complex was solved by molecular replacement and refined to a resolution of 2.10 Å (SI Appendix, Table S1). The crystallographic asymmetric unit consisted of two protomers of hFzd5 CRD (chain A and chain B), and the dimer displayed a markedly different configuration from that observed in the asymmetric unit of the hFZD7 CRD structure. Each protomer was bound to one C16:1n-7 molecule (SI Appendix, Fig. S3 A and B), placing the fatty acids in an unfavorable solvent-exposed environment.
Further inspection of hFZD5 CRD structure and crystallographic symmetry mates revealed an additional protein–protein interface (chain A homodimer) that is similar to that observed in hFZD7 CRD (Fig. 2; SI Appendix, Figs. S3 C and D). There were two lipid-binding grooves in the chain A homodimer, each originating from one monomer, which formed a contiguous U-shaped cavity (Fig. 2 B and C), similar to what was observed in the hFZD7 CRD dimer structure (asymmetric unit). The bound C16:1n-7 fatty acid was refined with a 50% occupancy in the structure due to its special location on the twofold axis, which is equivalent to 100% occupancy within the hydrophobic cavity (Fig. 2A; SI Appendix, Figs. S3 C–E). The fatty acid could bind in either direction within the lipid-binding cavity, with the carboxylic acid head group positioned proximal to Trp71 and Met120 residues (Fig. 2C). Remarkably, the kinked cis-Δ9 unsaturation site (C9–C10) within the hydrocarbon chain was located at the base of the U-shaped lipid-binding cavity near residues Ile76 and Try123 (Val92 and Phe138 in hFZD7 CRD, respectively) (Fig. 2C). Moreover, the carboxylate head group of C16:1n-7 was positioned lower relative to that of C24 bound to hFZD7 CRD and did not engage in H-bond interactions (SI Appendix, Fig. S3F). However, the carboxylate head group of C16:1n-7 appeared to be within proximity of the ester group at the Ser187 palmitoylation site on Wnt when bound to mFZD8 CRD (9). Thus, our crystal structure of hFZD5 CRD in complex with C16:1n-7 reveals an unprecedented atomic resolution view of the geometry of the lipid-binding cavity and how it accommodates a free unsaturated fatty acid. Importantly, our findings indicate that a single Wnt ligand could bind to two CRD molecules and mediate dimerization of FZD receptors (see proposed model below).

We also obtained the crystal structure of hFZD5 CRD bound to detergent [n-octyl-β-D-glucoside (BOG)]; it displayed a CRD dimer architecture and U-shaped lipid-binding cavity similar to C16:1-bound hFZD5 CRD and C24-bound hFZD7 CRD (SI Appendix, Figs. S4 and S5 and Table S1).

mFZD8 CRD Shares a Conserved α-Helical Dimer Interface with FZD5 and FZD7 CRDs. Based on the structural similarity between apo hFZD7, hFZD7 CRD in complex with C24, and hFZD5 CRD in complex with C16:1n-7 or BOG, we reexamined the published crystallographic data of mFZD8 CRD (25), which belongs to the same evolutionary cluster as FZD5 CRD (SI Appendix, Fig. S1A). Although the asymmetric unit of mFZD8 CRD (chain A and chain B) forms a loop–loop dimer interface as reported earlier (25), symmetry mate analysis revealed a dimer configuration with an α-helical dimer interface, which is identical to both hFZD7 and hFZD5 CRDs (Fig. 3 A–C, and SI Appendix, Figs. S6 and S7). Dann et al. assigned the asymmetric unit based on its similarity to the secreted frizzled-related protein 3 and its favorable complementarity scores between the loop–loop regions that mediated the dimer interface in the asymmetric unit (chain A and chain B) (25). At the time of publication, neither the cis-unsaturated fatty acylation status of Wnts nor Wnt’s fatty acid-mediated binding to FZD CRD was known (9, 23), perhaps providing no clues about the U-shaped lipid-binding cavity. Our in silico analysis, based on energy and complementarity scores (28), supports a helix–helix (FZD7-like) dimer interface as the potential biological interface within the mFZD8 CRD dimer (SI Appendix, Fig. S8). Computationally, both loop–loop and helix–helix dimer interfaces are similar in their complementarity score (SI Appendix, Fig. S84); however, the helix–helix dimer interface is energetically more favorable compared with the loop–loop interface for hFZD5, hFZD7, and mFZD8 CRDs (SI Appendix, Fig. S8B).

Interestingly, the lipid-binding groove in the reported apo mFZD8 CRD structure seemed to be in a more open configuration compared with that of apo hFZD7 CRD, with the side-chain orientations resembling those of the ligand-bound FZD CRD structures (Fig. 3D).
These observations prompted us to re-examine the electron density within the homodimer interfaces. We found multiple electron densities, of which the most striking had a tubular U-shape and was located within the lipid-binding groove of mFZD8 CRD at the homodimer interfaces (Fig. 3E). Although these densities were not assigned or described in the earlier report (25), their presence within the hydrophobic cavity may explain the apparent lack of flexibility of the lipid-binding groove reported earlier when comparing the apo mFZD8 CRD structure (25) to mFZD8 CRD in complex with XWnt8a (9). Upon structural comparison of apo hFZD7 CRD with C24-bound hFZD7 CRD or mFZD8 CRD (containing the tubular electron density), we observed a conformational change in the GFG motif lining the hydrophobic cavity (Fig. 3D). The side chains of Phe138 and Phe140 residues displayed inward or outward orientations relative to the hydrophobic cavity in the apo or ligand-bound structures, respectively, highlighting flexibility in the lipid-binding groove.

Although mFZD8 CRD shares a conserved dimer configuration with other FZD family members (hFZD5/7 CRDs), it appears that the dimer configuration of hFZD4 CRD is different, lacking an α-helical dimer interface and a U-shaped lipid-binding cavity (29, 30). Moreover, we observed unassigned electron densities within the hydrophobic cavities of all three reported apo FZD4 CRD structures (PDB IDs 5CM4, 5BPB, and 5BPO). These data indicate that the lipid-binding grooves of FZD CRDs may often be occupied by hydrophobic ligands, the identity of which warrants further elucidations.

Unsaturated Fatty Acids Regulate the Oligomeric State of hFZD7 CRD in Solution. Our finding that a cis-unsaturated fatty acid bridged the FZD CRD α-helical dimer interface suggests that fatty acids may mediate CRD dimerization (Fig. 2). To test this hypothesis, we assessed the effect of saturated or cis-unsaturated fatty acids of variable hydrocarbon chain lengths on the oligomeric state of hFZD7 CRD-His. Earlier reports demonstrated that purified soluble FZD4,-5, and -8 CRDs are monomeric (30, 31). However, FZD receptors could also undergo homo- and hetero-oligomerization at the cell surface (32, 33). In our studies, we often detected multimeric species of hFZD7 CRD in solution at micromolar concentrations (~60–600 μM) (Fig. 4A), in agreement with the structural data demonstrating that hFZD7 CRD could form dimers. Due to the poor solubility of free fatty acids (34), we used a highly sensitive fluorescence size-exclusion chromatography (FSEC) method (35) to monitor the formation of low-abundance multimeric species of FZD CRD by using a multivalent nitriotrifluoroacetic acid fluorescence probe that binds to the polystyrene tag present at the C terminus of FZD7 CRD. The formation, albeit minor, of dimer species of hFZD7 CRD-His was detected in the presence of the long cis-unsaturated fatty acids C14:1n-5, C16:1n-7, and C20:4 (all cis-Δ5,8,11,14) with substantial dimer formation observed upon incubation with C18:2 (cis,cis-Δ9,12), C18:3 (all cis-Δ9,12,15), or C20:5 (all cis-Δ5,8,11,14,17) (Fig. 4B–H). There was no CRD dimer formation detected in the presence of long saturated fatty acids C14:0 and C16:0 or cis-mono-unsaturated fatty acids C18:1n-9 and C24:1n-9 (Fig. 4C–H), which is likely due to their poor solubility. Also, C18:0 fatty acid could not be tested in the assay buffer conditions due to severe precipitation. It is noteworthy that the solubility of fatty acids is dramatically affected by the degree and type of unsaturation of the hydrocarbon chain. Finally, no CRD dimer peak was observed in the presence of short fatty acids C8:0, C10:0, and C12:0 (Fig. 4B and C) despite their relatively high solubility, consistent with the notion that the fatty acyl chain length is not long enough to bridge two CRD molecules.

Wnt Regulators Recognize cis- Unsaturated Fatty Acids with Curved Hydrophobic Cavities. Given the conservation of the U-shaped hydrophobic cavity across FZD5/7/8 CRDs (SI Appendix, Fig. S9A and B), we compared the shape of this cavity with that present in other proteins that bind to cis-unsaturated fatty acids and regulate Wnt signaling. Notum, a Wnt deacylase, inhibits Wnt signaling by cleaving the Wnt fatty acyl moiety (36, 37). Examination of the Notum structure bound to peptide-conjugated C16:1 revealed an alternative geometry of the hydrophobic cavity with a narrow base where the cis-Δ9 desaturation site was positioned, allowing the hydrocarbon chain to fold onto itself (SI Appendix, Fig. S9C) (36). Moreover, recent structural studies of stearoyl-CoA desaturase 1 (38, 39), which acts upstream of porcupine-mediated O-fatty acylation of Wnts (21, 22), revealed a bent long hydrophobic tunnel. The C9–C10 carbons of C18:0-CoA are positioned at the apex of the curved hydrophobic cavity, enabling regio- and stereoselective dehydrogenation to occur (SI Appendix, Fig. S9D). Altogether, these findings indicate that multiple Wnt-regulating proteins use curved hydrophobic cavities with different geometries as a selection mechanism for recognition of fatty acyl moieties (8).

Proposed Model for Assembly of Wnt–FZD CRD Complexes with 1:2 Stoichiometry and Wnt-Mediated FZD CRD Dimerization. Several lines of evidence support the notion that the CRD helix–helix dimer interface configuration with a U-shaped lipid-binding cavity is likely the biological unit, based on the following observations: (i) structural and sequence conservation of the helix–helix dimer interface across FZD5, -7, and -8 family members (SI Appendix, Figs. S6 and S7); (ii) in silico simulations demonstrating that the helix–helix interface is energetically favorable compared with the loop-loop interface (SI Appendix, Fig. S8); (iii) structural similarity and conservation of the kinked hydrophobic cavity across multiple FZD family members (Fig. 3D); and (iv) the similar fatty acid-binding mode in distinct FZD CRD structures (FZD5 and -7) obtained independently under different crystallization conditions. These structural studies provide compelling support for a contiguous hydrophobic lipid-binding cavity that is occupied by a single fatty acid with the carboxylic acid end of the hydrocarbon chain located on one CRD monomer and the methyl end on the other CRD monomer (Figs. 1 and 2). Clearly, additional studies are required to explore the functional relevance of the observed dimer geometry and its regulation of the lipid-binding cavity.

Based on the structural data presented in this study, we propose a molecular model for Wnt interaction with FZD CRDs. The Wnt fatty acyl group occupies the U-shaped lipid-binding cavity of FZD CRD with the kinked cis-Δ9 unsaturation site positioned at the base of the cavity (Fig. 5A–C; SI Appendix, Fig. S10). This model implies that a single Wnt molecule could bind to and promote dimerization of two CRD molecules. Consistent with this
Proposed Mechanisms for Clustering of Wnt–FZD Complexes. FZD receptor clustering is an attractive model implied by our structural studies. Consistent with this notion, high-order oligomers of mFZD8 CRD–XWnt8 complexes were observed in solution (9), raising a question about how receptor clustering may occur at the cell surface. Our studies suggest that fatty acid-mediated FZD dimerization at the cell surface may enhance FZD receptor clustering provided there are additional (direct or indirect) interaction interfaces between FZD molecules. In addition to the two distinct contact sites mediating the Wnt–FZD CRD high-affinity interaction (8–10), there is a third pseudo-site 3, which appeared to mediate formation of asymmetric dimers in the crystal lattice. Pseudo-site 3 could potentially mediate clustering between different XWnt8–mFZD8 CRD pairs, thereby completely burying the solvent-exposed fatty acid that is protruding from site 1 (Fig. 6A) (9). However, oligomerization through pseudo-site 3 would be sterically incompatible with the α-helical dimer configuration of the FZD CRD reported in this study, due to the following reasons: (i) the N terminus of XWnt8 would block the CRD dimer interface, and (ii) the CRD dimer configuration would prevent access of XWnt8 to the ω-carbons of the fatty acyl chain (Fig. 6A). Additionally, in a 1:1 model for Wnt-FZD CRD binding, oligomerization through the CRD loop–loop dimer interface would also not be feasible due to steric clashing with site 2 (Fig. 6B). In our proposed 1:2 model for Wnt–FZD CRD binding, it is conceivable that FZD CRD dimers form clusters without posing proposal, superimposition of hFZD5 CRD reported in this study with the published mFZD8 CRD structure bound to XWnt8 (9) shows that a cis-unsaturated fatty acyl chain originating from a single XWnt8 could occupy both lipid-binding cavities simultaneously, thereby bridging the hFZD5 CRD dimer interface (SI Appendix, Fig. S10). The same is true for FZD7 and FZD8 CRD dimers. The 1:2 stoichiometry for Wnt–FZD CRD interaction proposed in this study (Fig. 5A–C) is distinct from the 1:1 stoichiometry depicted in the published structure of mFZD8 CRD bound to XWnt8 (Fig. 5D–F) (9). In the latter case, the lipid-binding groove is in an unfavorable solvent-exposed orientation and does not fully bury the 16-carbon fatty acyl chain within the hydrophobic cavity (9) (Fig. 5D and E). It is conceivable that the protein complex isolation methods used in the study and the nature of the mFZD8 CRD construct, including the Fc fragment and linker length, may not have favored formation of the FZD CRD dimer, thereby providing an explanation for the observed discrepancy. Alternative purification protocols and construct design may enable the generation of a Wnt–FZD complex with a 1:2 stoichiometry. It is also noteworthy that the authors of this article (9) could not unambiguously determine whether the lipid present was palmitoleic or palmitic acid. Indeed, the fatty acyl chain modeled in the XWnt8–mFZD8 CRD structure is surprisingly straight (Fig. 5E), rather than kinked as one would expect from a monounsaturated fatty acid, although the resolution is low; if the fatty acid were linear, the presence of such a lipid would preclude FZD CRD dimerization. Nonetheless, the Wnt–FZD CRD 1:2 stoichiometry proposed here suggests that the Wnt protein, through its unsaturated fatty acyl group, could promote FZD CRD dimerization. This model has implications on downstream signaling as it could facilitate signalosome assembly, for example, by triggering PDZ DEP domain swapping (40, 41).
stERIC CLASHES BETWEEN THE TWO WNT-BINDING SITES 1 AND 2 ON FZD CRD. In this context, FZD clustering could be mediated through (i) the transmembrane or C-terminal region of the FZD receptor in a fashion similar to what has been observed with GPCRs (42) or through (ii) the FZD CRD loop–loop dimer interface (Fig. 6C). Both scenarios would result in the formation of Wnt-mediated FZD receptor clusters at the cell surface.

In summary, we report crystal structures for two FZD CRDs in complex with free fatty acids, providing a long-sought-after structural rationale for how FZD CRDs may recognize cis-unsaturated fatty acids on Wnt ligands. Our studies reveal that the lipid-binding cavity is flexible, accommodating a single fatty acid that bridges two CRD monomers by traversing their α-helical dimer interface. Upon reexaming the earlier published structures (25), we found that mFZD8 CRD shares similar structural features with hFZD7 and -5 CRDs, including an α-helical dimer interface and a contiguous U-shaped lipid-binding cavity, leading us to revisit the interpretation of the biological unit in that structure. Together our data suggest a common mechanism among multiple FZD CRDs for recognition of cis-unsaturated fatty acids. The data also provide a molecular model into how Wnts could promote CRD dimerization via the cis-unsaturated fatty acyl group, raising several questions that warrant further investigation. For example, is the integrity of the FZD CRD dimer interface required for signaling? Also, is FZD CRD dimerization driven by fatty-acylated Wnt or is the dimer preformed? If it is preformed, how does Wnt exchange into and out of the dimer? Does Wnt sample different conformations of FZD CRDs, and do free fatty acids modulate Wnt binding at the cell surface? Finally, our findings may facilitate the development of pharmacological strategies to target FZD receptors by taking advantage of the newly discovered CRD dimer interface and the lipid-binding cavity configuration.

MATERIALS AND METHODS

Detailed methods are provided in SI Appendix, Supplemental Materials and Methods. Recombinant proteins were purified from insect cells as described previously (31). Crystals were generated through vapor diffusion, and the structures were solved using molecular replacement. Dimer formation of FZD CRD in the presence of fatty acids was monitored by FSEC.

ACKNOWLEDGMENTS. We thank R. Ferrao for assistance with collecting the crystallographic dataset; the Genentech baculovirus expression group for help with construct generation; R. Ferrao, C. Koth, and D. Whalen for helpful discussions; and N. Thorsteinson (Chemical Computing Group) for guidance on in silico dimer interface calculations.