Multiscale simulations on human Frizzled and Taste2 GPCRs

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Abstract

Recently, molecular dynamics simulations, from all atom and coarse grained to hybrid methods bridging the two scales, have provided exciting functional insights into class F (Frizzled and Taste2) GPCRs (about 40 members in humans). Findings include: (i) The activation of one member of the Frizzled receptors (FZD4) involves a bending of transmembrane helix TM7 far larger than that in class A GPCRs. (ii) The affinity of an anticancer drug targeting another member (Smoothened receptor) decreases in a specific drug-resistant variant, because the mutation ultimately disrupts the binding cavity and affects TM6. (iii) A novel two-state recognition mechanism explains the very large agonist diversity for at least one member of the Taste2 GPCRs, hTAS2R46.

Highlights

- Frizzled receptor's activation may involve a wide conformational change of TM7.
- Smoothened receptor's binding cavity is disrupted in a drug resistant variant.
- A vestibular cavity may explain agonist diversity in a Taste2 receptor.

Introduction

Human G-protein coupled receptors (hGPCRs) are a very large and pharmaceutically relevant superfamily of membrane proteins. Indeed, 108 out of 800 hGPCRs are the target of ~34% of FDA drugs [1,2]. All members share a transmembrane domain (TMD), composed of a bundle of seven transmembrane helices (TM1-7). By binding to their cognate G-proteins, they activate downstream effectors in a myriad of signaling cascades [3,4].

Frizzled/Taste2 (or class F) receptors constitute one of the five hGPCRs families [5-7], the other being the Rhodopsin (or class A), Secretin (or class B1), Adhesion (class B2) and Glutamate (or class C) families. Class F hGPCRs include ten frizzled receptors (hFZD1-10) and one smoothened receptor (hSMO), as well as the twenty-five Taste2 or bitter taste receptors (hTAS2Rs). Nonetheless, this classification has been suggested with reservations [7]. Apart from the conservation of some features in helices TM2, 5 and 7 (which may explain why Frizzled and Taste2 receptors cluster together in the phylogenetic analysis), there are no obvious similarities between these two groups of receptors [7]. Considering the small sequence identity (less than 20%) and differences in architecture (Figure 1), it has recently been suggested that Taste2 receptors could alternatively form a distinct class [8], or even belong to class A [9,10•,11•].

Regarding structural experimental information, there is a big difference between the two branches of this subfamily. As of February 2019, no experimental structures are available for Taste2 receptors, while 30 crystal structures exist for Frizzled (reviewed in reference [12]). The endogenous ligands of hSMO and hFZDs (such as oxysterols and Wnt proteins, respectively) bind in the extracellular N-terminal part of the receptor, the so-called cysteine rich domain (CRD), whereas modulatory ligands bind within the TMD (see Figure 1A-B).

Instead, bitter taste ligands bind in a cavity inside the transmembrane domain (TMD) (Figure 1C) that resembles the canonical orthosteric binding site in class A GPCRs [13,14].

Although class F lacks most of the conserved features found in class A GPCRs, the SMO TMD structure revealed an overall conserved 7TM bundle; only the extracellular loop (ECL) 3 and the TM6 helix are more extended. Moreover, the availability of five different multidomain SMO structures (with or without ligand bound to either the CRD or TMD) has offered clues into the SMO activation mechanism [12]. In contrast, the recently solved FZD4 TMD structure shows a shorter and more tightly packed TM6, together with a more compact and highly hydrophilic binding pocket. Moreover, the TM7 helix features two unusual kinks. This suggests that the ligand binding and activation mechanism of FZD receptors might be different from SMO, as well as from class A GPCRs [12].

Frizzled and Taste2 receptors are relevant pharmaceutical targets [15-19]. Two FDA approved drugs against cancer are hSMO antagonists (vismodegib and sonidegib) [20]. hFZDs have been shown to be associated with cancer and degenerative diseases [21]. hTAS2Rs are expressed in different parts of the body outside the oral cavity, strongly suggesting their association with several diseases, such as chronic rhinosinusitis, asthma, cystic fibrosis, and cancer [17-19].

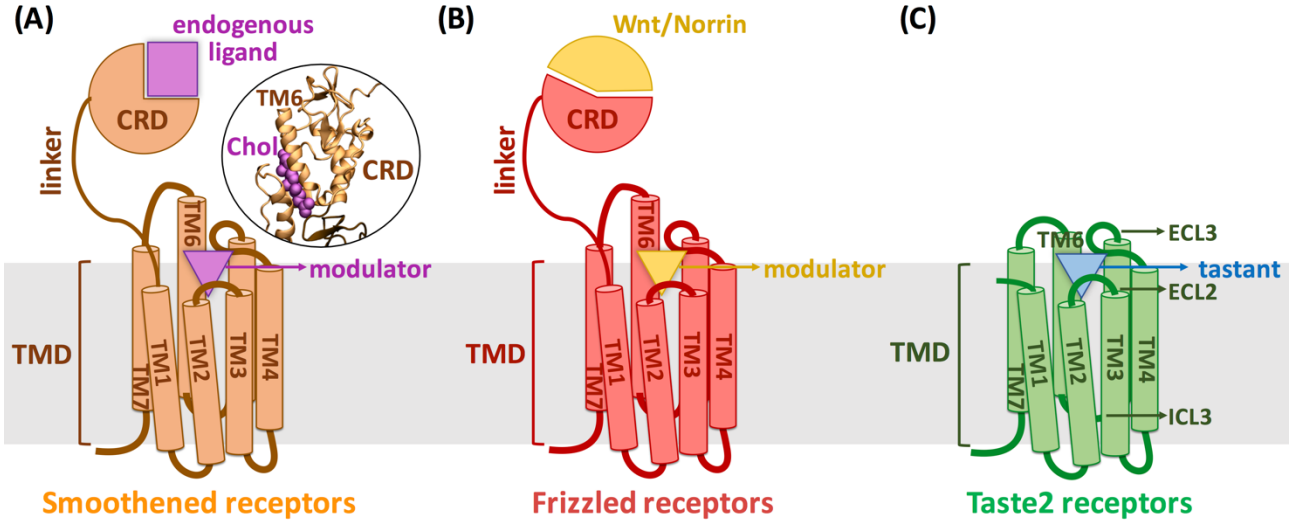

Figure 1. Architecture of class F hGPCRs, composed of 25 hTAS2Rs, 10 hFZDs and 1 hSMO. **(A)** The endogenous ligands of hSMO (such as cholesterol) bind to the extracellular cysteine rich domain (CRD), and modulators bind in the transmembrane domain (TMD). The inset shows the cholesterol binding site of hSMO (PDB code 5L7D) [22••], which involves residues belonging to the long TM6 helix, the linker and the CRD. **(B)** The architecture of hFZD is similar to hSMO, with its endogenous ligands (Wnt proteins and Norrin) binding to the CRD. **(C)** hTAS2R agonists (or tastants) bind in the orthosteric site located inside the transmembrane domain (TMD).

Molecular simulations of Frizzled and Taste2 GPCRs: ligand binding and activation

All atom (AA) and coarse-grained (CG) molecular dynamics (MD) simulations of hFZD and hSMO, based on the available experimental structural information [12], have provided insights into the ligand binding determinants, as well as the effect of mutations on the receptor response to anticancer drugs (Table 1). In addition, these simulations have given important hints on the activation mechanism of these receptors (Table 1).

In contrast, experimental structural information is missing for hTAS2Rs, and hence computational biology is at present the main tool used to provide insights into the molecular basis of ligand binding to hTAS2Rs. Validation against *in vitro* site-directed mutagenesis data is very important to establish the accuracy of the predictions [11•, 23•, 24] (see Table 1). In the next section, we report a detailed discussion on these studies.

Table 1. Molecular dynamics simulation studies on human Frizzled/Taste2 receptors reported in the last five years (in chronological order). For the ligands, the location of the binding site, as well as their agonistic/antagonistic activity is indicated between parentheses. The computational techniques listed are: HM: homology modeling; Dock: molecular docking; VS: virtual screening; MD: molecular dynamics simulations; AA-MD: all atom MD; CG-MD: coarse grained MD; MM/CG-MD: hybrid Molecular Mechanics/Coarse Grained MD; MetaD: metadynamics [25]; ABF: adaptive biasing force [26]; US: Umbrella Sampling [27]; MM/GBSA: Molecular Mechanics with Generalized Born and Surface Area solvation [28].

Frizzled/Smoothened receptors				
Receptor	**Ligand**	**Computational technique**	**Year**	**Reference**
SMO (TMD)	LY2940680 (TMD antagonist)	AA-MD (50 ns) MetaD (ligand rotation free energy) ABF (unbinding free energy)	2014	[29]
SMO (TMD+CRD)	cholesterol (CRD agonist)	CG-MD (1 μs) and AA-MD (10 x 100 ns)	2016	[22••]
SMO (TMD)	–	CG-MD (3 x 1 μs)	2016	[30]
SMO (TMD+CRD)	cholesterol (CRD agonist)	AA-MD (2 x 1000 ns)	2017	[31••]
SMO (CRD)	oxysterols (CRD agonists and antagonists)	HM + Dock + AA-MD (5 ns) MM/GBSA (binding free energy)	2017	[32•]
SMO (TMD)	vismodegib cyclopamine taladegib ZINC12368305 (TMD antagonists)	VS + Dock + AA-MD (300 ns)	2018	[33]
SMO (TMD+CRD)	LDE-225 LEQ-506 (TMD antagonists)	Dock + AA-MD (100 ns) MM/GBSA (binding free energy)	2018	[34•]
FZD7 (CRD)	Wnt2 (CRD agonist)	HM + Dock + AA-MD	2018	[35]
FZD4 (TMD and TMD+CRD)	–	HM + AA-MD (3 x 1.5 μs) AA-MD (3 x 3 μs)	2018	[36••]
SMO (TMD)	cholesterol (TMD allosteric ligand)	CG-MD (8 x 10 μs, 1 x 100 μs) AA-MD (3 x 200 ns) US (unbinding free energy)	2019	[37]

* Additional simulations (not listed here) were performed in this study, using either a different CG model for cholesterol (3 x 10 μs), an alternative conformation of the TMD (8 x 10 μs), or a longer CG simulation time (1 x 100 μs) or a different membrane composition, containing POPE (both leaflets) and anionic phospholipids (PS and PIP_2, inner leaflet) (8 x 10 μs).

Table 1 (continued).

Taste2 receptors				
Receptor	**Ligand**	**Computational technique**	**Year**	**Reference**
TAS2R46	strychnine (TMD agonist)	HM + Dock + MM/CG-MD (3 x 1 μs)	2015	[38••]
TAS2R16	salicin (TMD agonist) probenecid (TMD antagonist)	HM + Dock + AA-MD (2 x 50 ns)	2018	[39]
TAS2R7	quinine dextromethorphan (TMD agonists)	HM + Dock + AA-MD (1 ns)	2018	[40]
TAS2R4	quinine levofloxacin (TMD agonists)	HM + Dock + AA-MD (1 ns)	2018	[41]
TAS2R14	FFA DPH levofloxacin tobramycin (TMD agonists)	HM + Dock + AA-MD (1 ns)	2018	[41]
TAS2R20	cromolyn levofloxacin tobramycin (TMD agonists)	HM + Dock + AA-MD (1 ns)	2018	[41]

Human smoothened receptor

MD studies have focused on (i) antagonists binding to the TMD, (ii) modulators binding to the CRD and (iii) the receptor activation mechanism (Table 1).

LY2940680 is a phase I anticancer drug [42] binding to the hSMO TMD. Bai and coworkers [29] studied the binding determinants of this antagonist using a crystal structure of hSMO (PDB code 4JKV) (Figure 1B). A 50 ns long AA-MD suggested that not only helices TM2, 5, 6 and 7 in the TMD, but also the extracellular loops ECL2 and ECL3, as well as the linker play an important role for ligand binding. In addition, adaptive biasing force (ABF)-based free energy calculations provided insights into the structural determinants and energetics of ligand dissociation. After breaking the interactions with the CRD binding pocket residues, the ligand interacts with residues in the linker and the TM6 helical extension, until it finally escapes the receptor. This information may be important for future pharmacological applications, given that unbinding kinetics, among other factors, affects drug efficacy [43-45].

Other two antagonists targeting the hSMO TMD are LDE-225 (an FDA approved drug, also known as sonidegib or erismodegib) and LEQ-506 (a ligand currently under clinical trials) [15]. The latter is effective not only against the wild-type receptor, but also against the D473H variant found in medulloblastoma patients, which confers resistance to classical TMD inhibitors. Using 100 ns long AA-MD simulations complemented with free energy calculations based on the Molecular Mechanics with Generalized Born and Surface Area

solvation (MM/GBSA) approach, Tu and coworkers [34•] found that the volume of the binding cavity decreases dramatically in the mutated complex with resistant LDE-225. Moreover, the D473H variant was found to disrupt the hydrogen bond network involving residues R400 and Q477, forcing an inward movement of TM6. The simulations also suggest alternative binding residues that could be targeted when designing new non-resistant inhibitors.

Given the appearance of tumors bearing drug-resistant TMD mutations, Sinha and coworkers [33] attempted to find novel hSMO TMD antagonists by using an integrated docking/MD study. First, they used virtual screening to identify a novel drug candidate, ZINC12368305, which was then submitted to an exhaustive docking analysis to confirm that its binding affinity was higher than that of previously described antagonists (such as vismodegib, cyclopamine and talagedib). The best docking pose of the new compound was further tested by running a 300 ns long AA-MD simulation, which showed that this ligand forms a more stable complex than the other aforementioned inhibitors. Moreover, the authors repeated the ligand docking on different protein conformations extracted from the MD trajectory and showed that ZINC12368305 was consistently the inhibitor with the lowest binding energy, giving further support to their proposal. Finally, using protein contact network analysis, they pinpointed a unique protein-ligand interaction responsible for the increased stability (and thus putatively prolonged drug action) of ZINC12368305 compared to the other inhibitors. Based on this information, the candidate compound can be further optimized or used to discover new lead scaffolds.

As an alternative to combat drug-resistant TMD mutations, drug design efforts can focus on antagonists binding to the CRD or allosteric ligands. Cholesterol (and in general oxysterols) are hSMO endogenous ligands binding to the CRD [22••, 46, 47]. Dash and coworkers [32•] explored further these CRD ligands by building a homology model of hSMO (using as template the zebrafish CRD crystal structure, PDB code 4C79) and combining molecular docking, 5 ns AA-MD simulations and MM/GBSA-based binding free energy calculations. They concluded that high affinity oxysterols act as antagonists, whereas low affinity compounds have agonistic activity [32•].

In order to better understand how cholesterol, a lipid present in the cell membrane, activates hSMO, Hedger and coworkers ran CG-MD simulations of the receptor embedded on membranes of different lipidic composition. They showed that the agonist cholesterol binds not only to the CRD, but also to the TMD [37], as observed for class A GPCRs [48-50]. This novel druggable binding site, located at the CRD-membrane interface, opens the way for new drug design strategies.

Besides ligand binding, MD simulations have also been used to study receptor activation in hSMO, in particular how the relative motion between CRD and TMD is connected to activation. A 1 μs CG-MD simulation of the full-length receptor, followed by ten AA-MD simulations of 100 ns each, indicated that the apo CRD is more flexible than the holo CRD (Figure 1C) [22••], suggesting that stabilization of the CRD is associated with activation. A subsequent study, combining X-ray crystallography, hydrogen-deuterium exchange experiments, and 1 μs long AA-MD simulations, has reached similar conclusions [31••].

Human Frizzled receptors

To the best of our knowledge, two MD studies on hFZDs have been reported as of February 2019. On one hand, Kalhor and coworkers [35] built a model of the crystal structure of hFZD7 CRD (PDB accession code 5T44) in complex with a homology model of the Wnt2 protein (its endogenous ligand) by using protein-protein docking. Subsequent 60 ns long AA-MD simulations lead them to propose that the two proteins interact using simultaneously two different binding sites, thanks to their U-shaped topologies.

On the other, Yang and collaborators [36••] performed three 1.5 μs AA-MD simulations of hFZD4 in its apo form to get insights into the receptor activation mechanism. The simulations suggest that activation involves two unusual kinks in transmembrane helix TM7 that are dynamically coupled (Figure 1B). These two kinks are involved in conserved polar networks and impose considerable bending of TM7 and displacement of TM8, as observed during the simulations. This contrasts with class A GPCRs [51], in which the principal displacement upon activation involves TM6.

Human bitter taste receptors

Structural predictions for hTAS2Rs are particularly challenging due to lack of experimental structures. Therefore, insights into their ligand/receptor interactions rely on homology modeling complemented with molecular docking. This computational approach is particularly challenging due to the low sequence identity shared by hTAS2Rs with the available GPCR templates [11•,52,53], which limits the accuracy of the side chain modeling [54-56]. In order to overcome this issue, methods that increase the sampling of the conformational space, such as flexible docking [23,57-61] or molecular dynamics [38-41] can be used. A systematic analysis of all hTAS2Rs for which experimental data are available showed that MD simulations can improve significantly the predictions, even those obtained by using flexible docking, by exploring more extensively the solvated receptor-ligand conformational space [11•], as also suggested previously for GPCR/ligand complexes in general [53].

Subnanosecond AA-MD has been applied to study antibiotic binding to hTAS2R7 [40], as well as hTAS2R4/14/20 [41]. The simulations, validated against mutagenesis experiments, suggest that residues both within the TM bundle and in ECL2 (Figure 1A) are involved in binding [40,41], similar to what is found for class A GPCRs [62,63].

Besides AA-MD, hybrid Molecular Mechanics/Coarse Grained (MM/CG) simulations, used for soluble and membrane proteins [64,65], have been tailored for low resolution GPCR models, such as hTAS2Rs [66,67]. In this case, the ligand, the binding site residues and a water droplet form the MM part, whereas the remaining part of the receptor and the membrane are treated at coarse grained level (Figure 2). This approach reduces the number of degrees of freedom and thus allows to sample longer the conformational space of the receptor-ligand interactions. Moreover, it provides a scaffold that maintains the structural integrity of the receptor, while propagating correctly the fluctuations to and from the MM part.

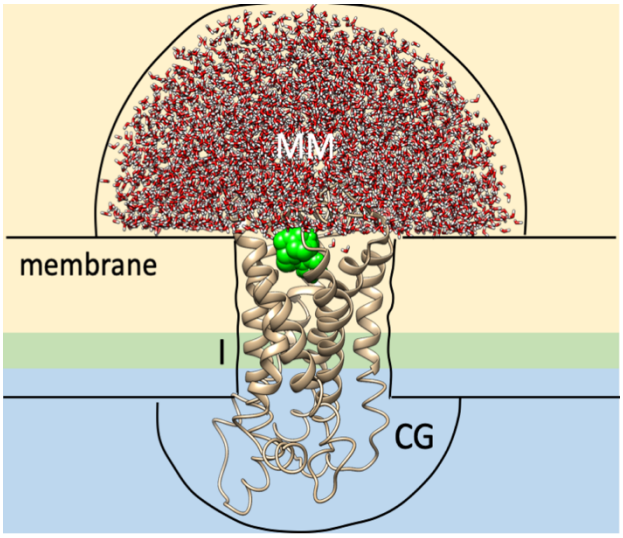

Figure 2. Molecular Mechanics / Coarse Grained (MM/CG) setup of hTAS2R46. The MM (all atom), I (interfacial), and CG (coarse grained) regions are highlighted in yellow, green, and blue, respectively. The receptor is shown as beige cartoon and the ligand (strychnine) as green spheres. The potential walls used to mimic the presence of the membrane and to cap the hemisphere of water molecules are represented as black lines.

This approach has been applied to three ligand/hTAS2R complexes so far [38••,68], clearly improving the quality of the predictions [11•]. A Taste2 receptor characterized by a broad agonist diversity (hTAS2R46) turned out to feature not only the canonical ("orthosteric") binding site, but also a second ("vestibular") site,

located above the orthosteric site. This “two-site” architecture, also present in other hGPCRs [69-72], might play a role as an “access control” [57] for discriminating the highly diverse agonists of hTAS2R46. This mechanism may also be at play for other broad-spectrum bitter receptors and contrasts with what is found for a group-specific bitter taste receptor (hTAS2R38) [73], which appears to use only the canonical orthosteric site [68].

Besides ligand binding, the activation mechanism of one bitter taste receptor, hTAS2R46, was studied by Chen and coworkers [39]. They suggested that a network of interactions at ICL3 (Figure 1A) stabilizes the inactive state of hTAS2R16 while structural changes in the intracellular region are correlated with activation. However, the lack of extensive comparison with experimental data, along with the limited length of these simulations, might limit the predictive power of this study, especially considering that for class A GPCRs several microsecond long AA-MD simulations were needed to provide information about their activation mechanism [69,74].

Future perspectives

A thorough understanding of the molecular determinants underlying GPCR function requires a dynamical insight. During the last decade, a myriad of combined experimental and computational approaches has been used to unravel the exquisite details of ligand binding and activation in class A GPCRs, as nicely reviewed in references [74-79]. Similarly, molecular simulations have provided important insights into ligand binding, drug resistance and activation mechanisms of human Frizzled/Taste2 GPCRs (Figure 3). This is remarkable, considering that the first crystal structures of human Frizzled receptors were reported in 2013* and experimental structure information of hTASR2s is still missing. Future research directions that would be highly interesting to pursue (by no means this list is meant to be exhaustive):
(i) Exploring the flexibility of the CRD in hSMO (Figure 1A), together with virtual screening and simulation of the resulting receptor/ligand complexes, may help identify non-TMD binding ligands to counteracting tumors bearing drug resistant TMD mutations.
(ii) Characterizing the hSMO-lipid interactions could pave the way to new drug design strategies for allosteric ligands. Starting from the recently identified cholesterol binding site TMD [37], simulations could be used to investigate the possible mechanism whereby cholesterol is transported from the membrane to the CRD, possibly through a continuous tunnel within the TMD [80]. Besides cholesterol, preliminary simulations indicated that the receptor activity may be modulated by the anionic phospholipid PIP_2 binding to the TMD-membrane interface [37], as also observed for class A GPCRs [81].
(iii) Unraveling the dimerization mechanism of Frizzled receptors may help understand its connection with receptor activation [12]. An initial CG-MD simulation of a TMD-TMD hSMO dimer has shown a complex network of pi-stacking interactions between TM4 and TM5, similar to class A GPCR dimers [30]. However, other dimeric models were suggested as possible (CRD-CRD or CRD-TMD codimer) [12]) and these alternative dimeric forms are awaiting their study by simulation methods.
(iv) A systematic molecular dynamics study on hTAS2Rs with available experiments could shed light on the markedly different ligand spectrum across bitter taste receptors [73].

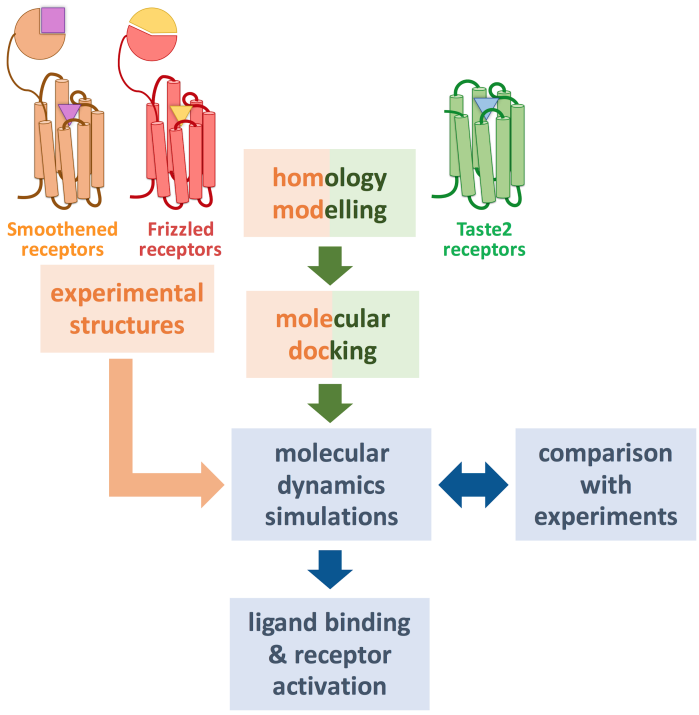

Figure 3. Modeling and simulation protocol for Frizzled/Taste2 receptors.

Acknowledgements
We thank Prof. Meyerhof for many fruitful collaborations over the last five years. M.A.-P. thanks the "Ernesto Illy Foundation" (Trieste, Italy) for financial support. This paper is dedicated to the memory of Ernesto Illy (1925-2008).

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Footnotes

* Here we are not considering two structures of the FZD8 receptor reported in 2001 (PDB code 2IJY) and 2012 (PDB code 4F0A), because they correspond to the mouse ortholog and only include the CRD (either alone or in complex with the zebrafish Wnt8 protein, respectively).