Homology modelling of PORCN structure

Homology modeling was performed using the MODELLER (Webb and Sali, 2016) program from trRosetta (Yang et al., 2020) and $(PS)^2$ (Huang et al., 2015) by query-template alignment. Two recently published human MBOAT proteins structures, DGAT1 (PDB 6VP0) and ACAT1 (PDB 6P2P), were chosen as templates to guide the modelling with the human PORCN amino acid sequence (Unipro:Q9H237-1/NCBI Q9H237.2, also known as splice isoform D, 461 amino acids), using the T-Coffee homology extension (PSI-coffee) algorithm (Tommaso et al., 2011). To refine the PORCN simulation, I-Tasser (Yang and Zhang, 2015b) was used by assigning the template generated from trRosetta and $(PS)^2$ with specified secondary structure for the C-terminal 41 amino acids (Val421 to Gly461). Lastly, the simulated structure was refined by GalaxyRefine2 from GalaxyWEB (Afgan et al., 2018; Boekel et al., 2015) to generate the final predicted structure.

Protein tunnels were identified and assessed using Caver 3.03 (Pavelka et al., 2015) as a Pymol Plugin using a probe radius of 2 Å, shell radius of 4 Å, and a shell depth of 3 Å. The clustering threshold was set at 3.5. The starting point was defined as the point between His341, Asn306, and Phe257.

Molecular Docking of Palmitoleoyl-CoA, WNT8A and PORCN inhibitors

System preparation and receptor-ligand docking calculations were performed using the Schrödinger Suite package (version 2020–4), using default parameters unless otherwise noted. The homology model of human PORCN as a receptor was first prepared using the Protein Preparation Wizard (Sastry et al., 2013). The atom and bond types were corrected and the protonation states of ionizable species adjusted to pH 7.4 by Epik (Greenwood et al., 2010). During receptor grid generation, the ligand to be docked was confined to an enclosing box centered on the middle of the tunnel, using Ser262, Glu300, Asn315 and His341 to set the box center with an external box of 36 Å for PAM-CoA docking, and a ligand-sized box centered on Glu293, Val302 and His409 for inhibitor docking. The PAM-CoA structure and PORCN inhibitors were generated from PubChem, prepared using the LigPrep module to convert the 2D sdf format into 3D molecular structures, and docked to the orthosteric site of PORCN with the Glide program (Friesner et al., 2006) using the standard precision (SP) mode. The best binding pose predicted for human PORCN-PAM-CoA complex and PORCN-inhibitors docking was saved for further analysis for molecular interactions.

To predict protein-protein interactions, the structure of WNT8A was extracted from the WLS/WNT8A structure (PDB 7KC4) (Nygaard et al., 2021) and the ClusPro 2.0 Server (Kozakov et al., 2017) was used to predict its interaction with PORCN. We used the hydrophobic-favored potential in the server. A near-native state of protein conformations was chosen during protein-protein docking.

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