The Development and Application of Computational Methods for the Prediction of G-Protein Coupled Receptor Structures

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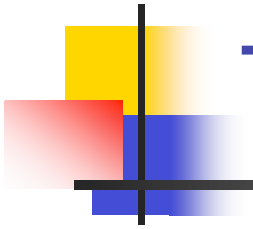


Talk Preview

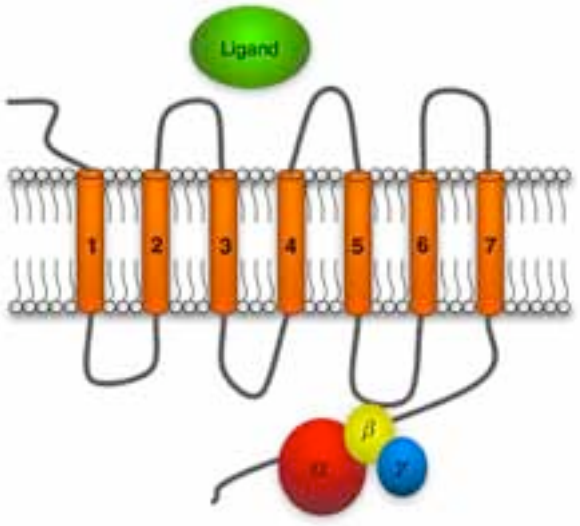
- Intro to GPCRs
- Computational Methods
- Validation of Methods
- Structure Prediction of the Orphan GPCR GPR88

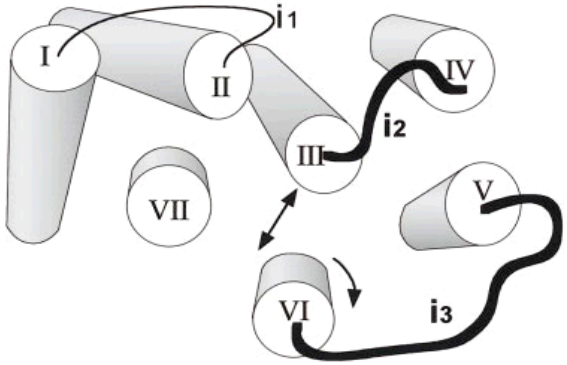
G-Protein Coupled Receptors

- Superfamily of transmembrane (TM) proteins that regulate signal transduction
- Targets of 50% of recently released drugs and 25 of top 100 best-selling drugs
- Structures of only four are known experimentally

GPCR Structure and Function

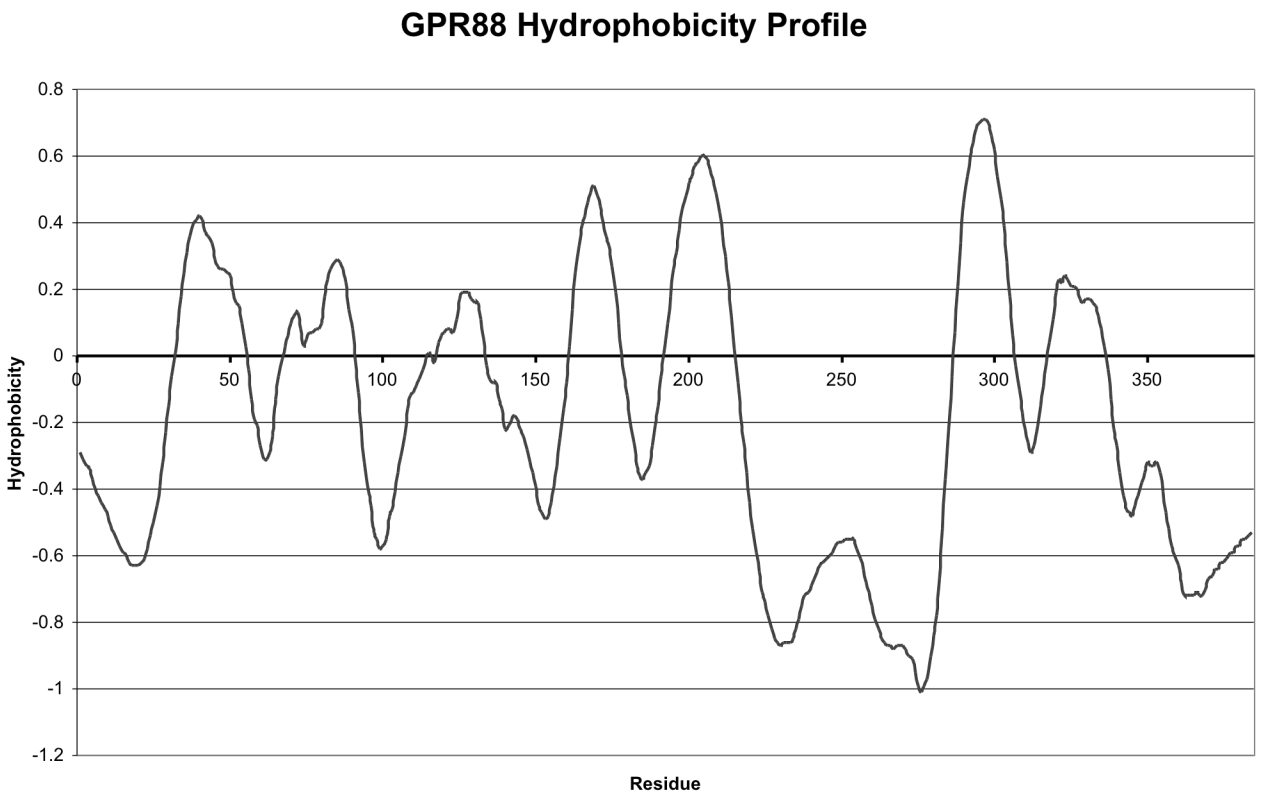
- All GPCRs have seven TM helices
- Extracellular signals activate GPCRs to interact with G-protein heterotrimers





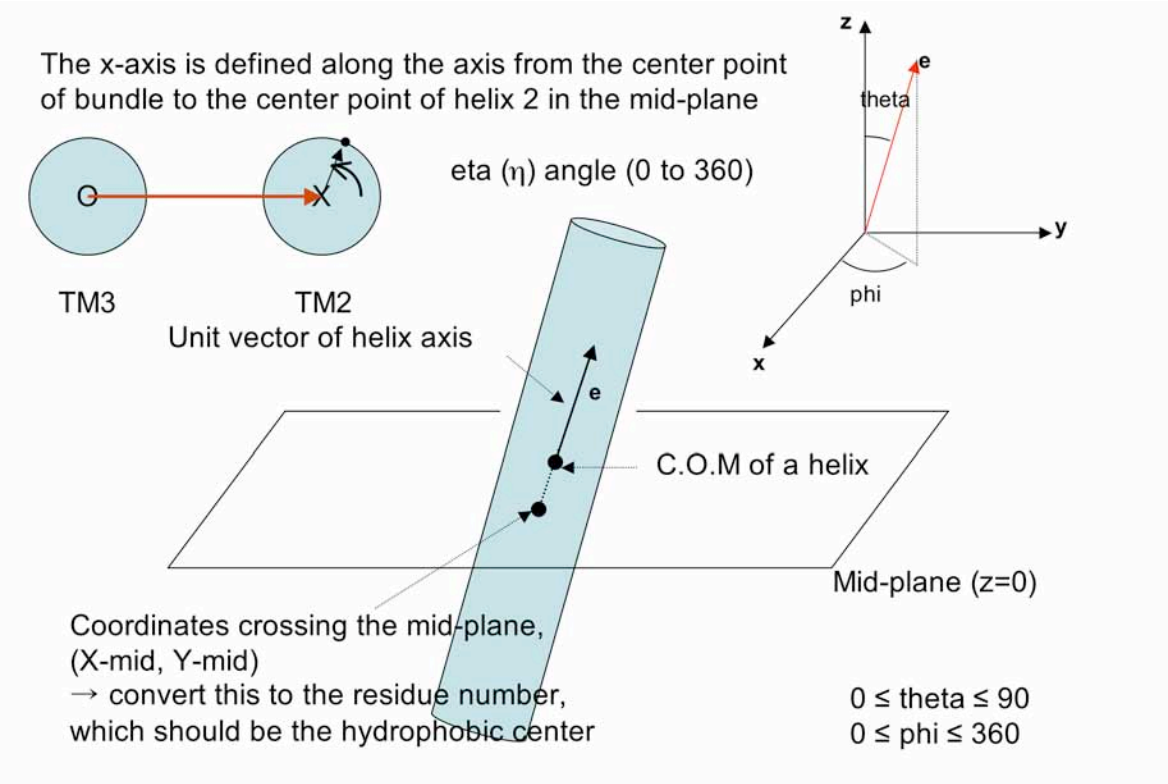
TM Helix Prediction

Hydrophobic membrane environment governs GPCR structure.



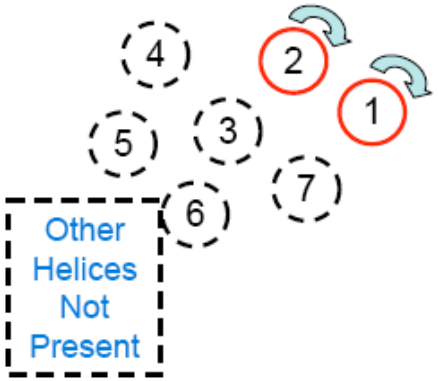
Aligning Helices to Templates

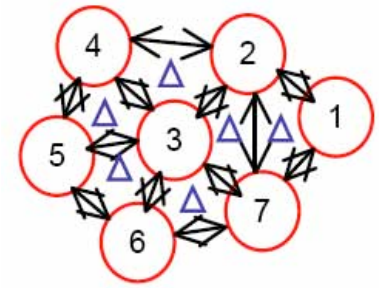
Align homology helices for each experimental template



BiHelix Sampling

- Determines most energetically favorable rotation (eta) of each helix around its own helical axis.





H1_H2 H1_H3 H1_H7
H2_H3 H2_H7 H3_H4
H3_H5 H3_H6 H3_H7
H4_H5 H5_H6 H6_H7

- Generate 144 (12 x 12 with 30^{o} rotations) combinations for each interacting helix pair
- Sum pairwise energies to generate best bundle combinations - allows sampling of all $12^{7} \approx 35$ million conformations.

Total Bihelical Bundle Energy

- The bihelical energies are divided into intrahelical and interhelical components to ensure no one helix biases the total bundle energies

$$E_{intra}\left(\overline{\eta},\overline{\theta},\overline{\phi}\right) = \sum_{i=1}^{7} \frac{1}{N_i} \sum_{j=J_{i,1},j>i}^{J_{i,N_i}} E_{i,intra}^{ij}\left(\eta_i,\eta_j,\theta_i,\theta_j,\phi_i,\phi_j\right)$$

$$E_{inter}\left(\overline{\eta},\overline{\theta},\overline{\phi}\right) = \sum_{i=1}^{7} \sum_{j=J_{i,1},j>i}^{J_{i,N_i}} E_{inter}^{ij}\left(\eta_i,\eta_j,\theta_i,\theta_j,\phi_i,\phi_j\right)$$

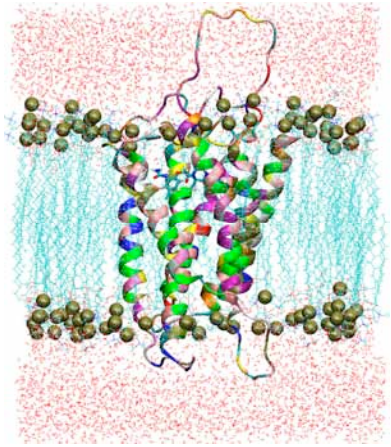
$$E_{total}\left(\overline{\eta},\overline{\theta},\overline{\phi}\right) = E_{intra}\left(\overline{\eta},\overline{\theta},\overline{\phi}\right) + E_{inter}\left(\overline{\eta},\overline{\theta},\overline{\phi}\right)$$

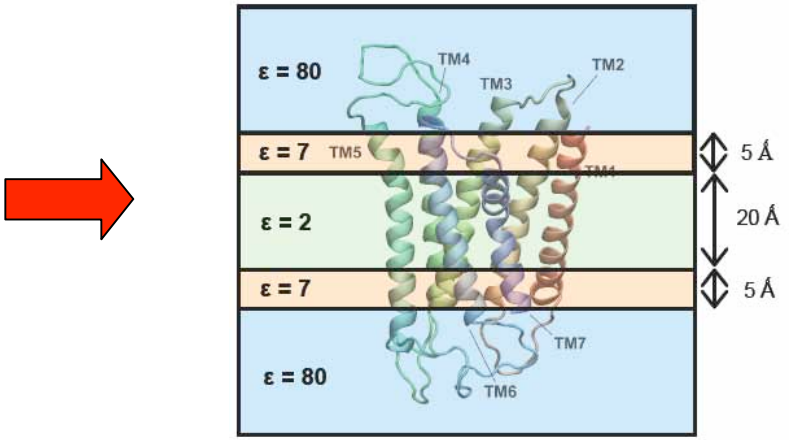
$$\overline{\eta} = \left(\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7\right)$$
$$\overline{\theta} = \left(\theta_1,\theta_2,\theta_3,\theta_4,\theta_5,\theta_6,\theta_7\right)$$
$$\overline{\phi} = \left(\phi_1,\phi_2,\phi_3,\phi_4,\phi_5,\phi_6,\phi_7\right)$$

- N_i is the number of helices interacting with helix i, and $J_{i,k}$ is the kth neighbor of helix i

CombiSCREAM TM Bundle Building

- Take top 1000 combinations from BiHelix analysis and build each bundle.
- Reassign the sidechains, evaluate membrane solvation energy, then total energy.





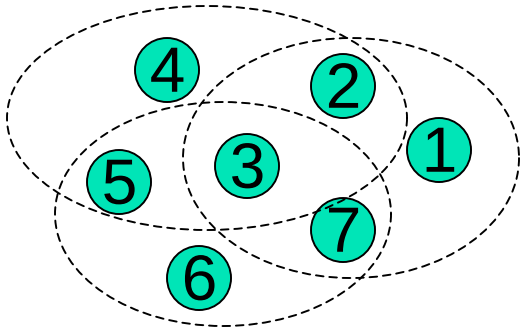
- Choose the lowest energy structure for SuperBiHelix sampling.

SuperBiHelix Sampling

- Samples the tilts and sweep angle of each helix in addition to the rotation of each helix around its own axis.
- Makes structure prediction less dependant on template, leading to more accurate prediction of receptors dissimilar to available experimental structures.
- Leads to an ensemble of low-lying structures
- Sampling 3 θ values, 5 ϕ values and 5 η values for each helix leads to 5625 bihelical conformations, and 10^{13} possible seven-helix bundles

SuperBiHelix Analysis

- Calculate bihelical energies of 3 quadhelix bundles: 1-2-3-7, 2-3-4-5, 3-5-6-7



- Output top 2000 structure by energy for each quadhelix
- Rank conformations for each helix, alternating conformations from each applicable quadhelix

SuperBiHelix Analysis

- Take top 36 conformations for each helix and calculate the total bihelical energies for all $36^7 = 8 \times 10^{11}$ seven helix bundles.
- Output the top 2000 structures from this analysis by total energy for further analysis in CombiSCREAM

Validation of BiHelix and CombiSCREAM for Rhodopsin

Structure	TotalE	
0_0_0_0_0_330_0	-237	
0_0_0_0_300_330_0	-234	
0_0_0_0_180_330_0	-231	
0_0_0_0_90_330_0	-220	
0_0_0_0_210_330_0	-215	
0_0_0_0_60_330_0	-213	
0_0_0_0_0_0_0	-210	XTAL
0_0_0_0_330_330_0	-203	
0_0_0_0_90_0_0	-195	
0_0_0_0_180_0_0	-189	
0_0_0_0_60_0_0	-175	

H1	H2	H3	H4	H5	H6	H7	Total	
0	0	0	0	0	0	0	-55.1	XTAL
0	0	0	0	210	0	0	-20.6	
0	0	0	0	90	0	0	-16.7	
0	0	0	0	0	0	330	-4.1	
0	0	0	0	180	0	0	3.1	
0	0	0	0	330	0	0	23	
0	0	0	0	210	0	330	30.5	
0	0	0	0	210	330	0	33.2	
0	0	0	0	300	330	0	36.7	

- BiHelix method shows Bovine Rhodopsin XTAL packing at number 7 out of possible 35 million choices.
- Top 100 combinations are used to build TM bundles and side-chains optimized for each.
- XTAL packing is the best.
- Other low energy packings are likely involved in conformational changes during activation:
  - Helix 5 highly flexible rotationwise.
  - Helices 6 and 7 show an alternate packing involving anti-clockwise 30° rotation.

Validation of SuperBiHelix and CombiSCREAM for A_{2A} Adenosine Receptor

SuperBiHelix

θ	H1	H2	H3	H4	H5	H6	H7	ϕ	H1	H2	H3	H4	H5	H6	H7	η	H1	H2	H3	H4	H5	H6	H7	Energy (kcal/mol)	RMSD (Å)
	0	0	0	-10	10	0	0		0	0	15	-30	0	0	0		0	0	0	-30	30	0	0	392.6	1.3
	0	0	0	0	0	0	0		0	0	0	0	0	0	0		0	0	0	0	0	0	0	396.9	0
	0	0	0	-10	0	0	0		0	0	15	-30	0	-15	0		0	0	0	-30	-15	0	0	398.3	1
	0	0	0	-10	10	0	0		0	0	15	-30	0	0	0		0	0	15	-30	30	0	0	400.6	1.3
	0	0	0	-10	0	0	0		0	0	15	-30	0	0	0		0	0	0	-30	0	0	0	400.7	0.9
	0	0	0	-10	0	0	0		0	0	15	-30	0	0	0		0	0	0	-30	-15	0	0	401.2	0.9
	0	0	0	-10	10	0	0		0	0	15	-30	-15	0	0		0	0	0	-30	15	0	0	401.3	1.3
	0	0	0	0	0	0	0		0	0	0	15	0	0	0		0	0	0	0	0	0	0	402.4	0.4
	0	0	0	-10	10	0	0		0	-15	15	-30	0	0	0		0	0	0	-30	30	0	0	402.4	1.3
	0	0	0	-10	0	0	0		0	0	15	-30	-15	0	0		0	0	0	-30	0	0	0	402.8	1

SuperComBiHelix

θ	H1	H2	H3	H4	H5	H6	H7	ϕ	H1	H2	H3	H4	H5	H6	H7	η	H 1	H2	H3	H4	H5	H6	H7	Energy (kcal/mol)	RMSD (Å)
	0	0	0	0	0	0	0		0	0	0	0	-15	0	0		0	0	0	0	0	0	0	59.1	0.4
	0	0	0	0	0	0	0		0	0	-15	0	0	0	0		0	0	0	0	0	0	0	71.2	0.4
	0	0	0	0	0	0	0		0	0	-15	0	0	15	0		0	0	0	0	0	0	0	74.7	0.6
	0	0	0	0	0	0	0		0	0	-15	0	0	-15	0		0	0	0	0	0	0	0	76.3	0.7
	0	0	0	0	0	0	0		0	0	0	-15	0	-15	0		0	0	0	0	0	0	0	77	0.6
	0	0	0	0	0	0	0		0	0	0	0	0	0	0		0	0	0	0	0	0	0	78	0
	0	0	0	0	10	0	0		0	0	0	0	0	-15	0		0	0	0	0	15	0	0	79.1	1
	0	0	0	0	0	0	0		0	0	0	0	0	-15	0		0	0	0	0	0	0	0	85.8	0.5
	0	0	0	0	0	0	0		0	0	0	0	0	-15	-15		0	0	0	0	0	0	0	86.2	0.6
	0	0	0	0	0	0	0		0	0	-15	0	0	15	15		0	0	0	0	0	0	0	87.8	0.6

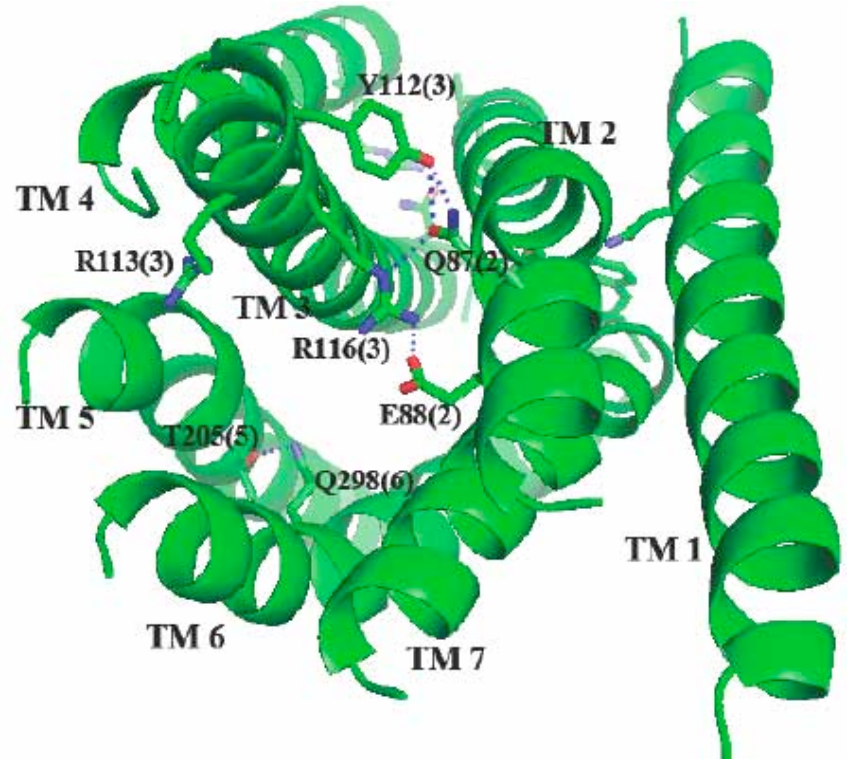
SuperBiHelix Validation Conclusions

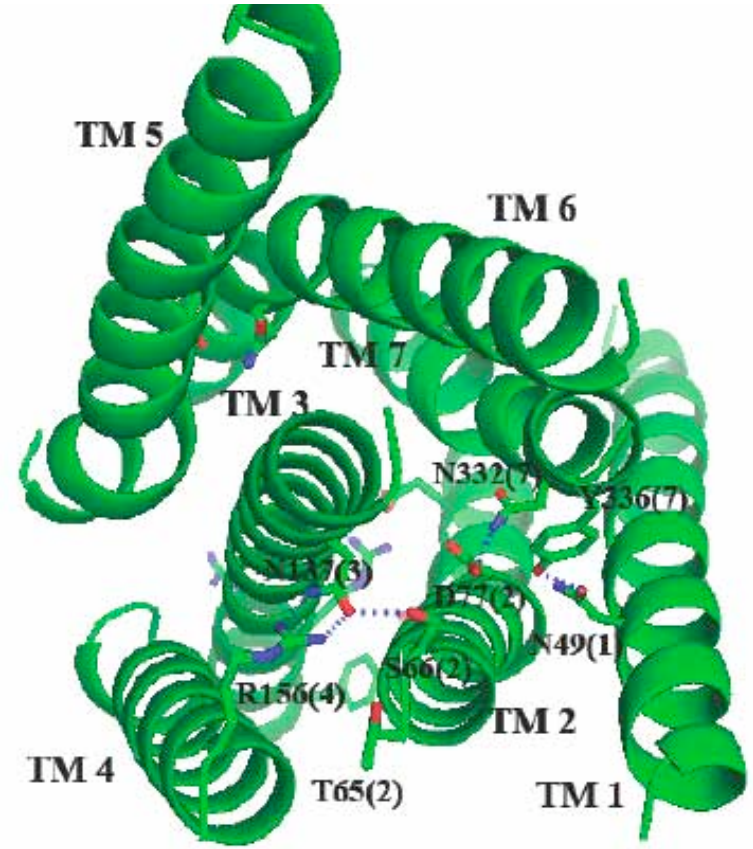
- SuperBiHelix identifies crystal structures when starting with a crystal structure
- SuperBiHelix is successful at improving structures when helices are placed in the incorrect template
- SuperBiHelix will make it possible to predict structures dissimilar to any experimental crystal structure

Application of SuperBiHelix: Orphan GPCR GPR88

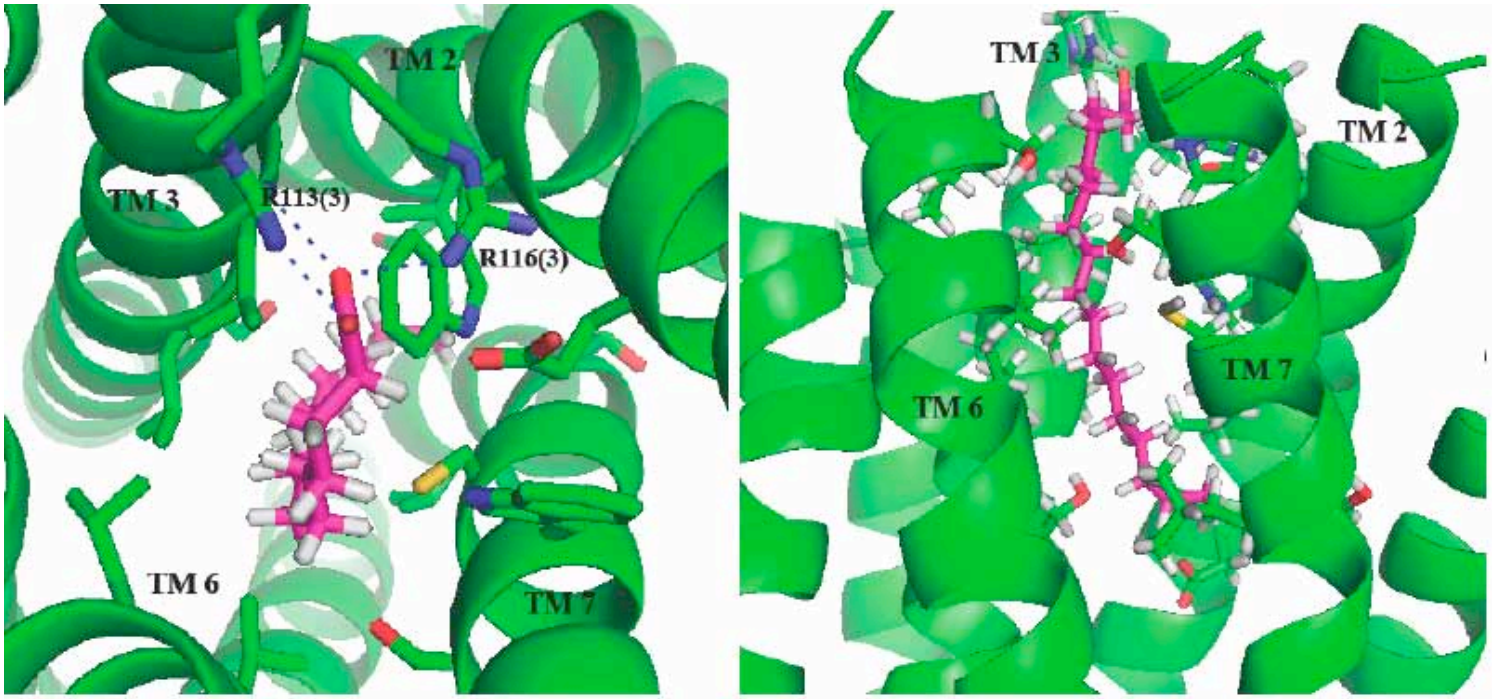
- Orphan receptor with no know function or ligand binding
- Found in adult mouse striatum
- Schizophrenia-like phenotypes displayed by GPR88 knock-out mice
- Antidepressant treatments affect levels of GPR88 expression
- Implicated in bipolar disorder

Best Energy GPR88 Structure





GPR88 Lipid Binding Pocket



GPR88 Structure Prediction Conclusions

- Lipids will bind to GPR88, with the head group docking to R113 and R116 on TM3 and the tail group in the 2-3-6-7 aliphatic pocket
- Residues in the binding region can be tested experimentally to validate the predicted structure and binding sites
- SuperBiHelix is a promising method to use for the prediction of GPCR structures that are dissimilar to any experimental structure

Acknowledgements

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