

HOMOLOGY MODELING OF FRIZZLED GPCR THROUGH GPCR-MOD SIM

Kumud Pant^{1*}, Reshu Nautiyal¹, and Bhasker Pant²

¹Department of Biotechnology, Graphic Era University, Dehradun, India

² Department of IT, Graphic Era University, Dehradun, India

*Corresponding author: Email: pant.kumud@gmail.com, Tel: +91-9319348009

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ABSTRACT:

52% of all medicines available today act on GPCRs which are the most important biological signal processing molecules. Despite their importance in all physiological processes very less 3D structures of GPCRs are available in various data bases. Only 1% of the structures deposited in PDB are of GPCRs.

Due to ease, less time consumption and high reproducibility of results most of the laboratories in the world are turning towards in-silico modeling of these molecules. Many of dedicated web servers and softwares have been developed for this purpose. In this paper we are trying to model 3 dimensional structure of Frizzled GPCRs through one such server GPCR MOD-Sim. The results obtained were of high quality and showed more than 90% residues in most favorable region. This model generated can provide reference point for scientist to probe deeper into the structure and function of these important class of GPCRs and also aid pharmaceutical industries in designing new drugs and inhibitors for them.

Keywords: *Frizzled GPCRs, Homology Modeling, GPCR Mod-Sim*

[I] NTRODUCTION

GPCRs are one of those proteins which have immense importance not only in pharmaceutical industry but also from research perspective. G-Protein Coupled Receptors (GPCRs) form hottest target class for therapeutics and drug development and understanding their 3D protein structures is very important for future drug development [1].

Since GPCRs are membrane embedded proteins and face difficulties of over expression, purification and concentration of membrane proteins therefore very less experimentally

determined structures are available for ex. bovine rhodopsin, which is elucidated at resolution of 3.5 Å X-ray crystallography and deposited in Protein Data Bank (PDB). Very less number of GPCRs are homologous to Bovine Rhodopsin and show divergence in sequence, still all have similar structure with seven trans membrane regions (as 7 α -helices), three intracellular loops and three intracellular loops besides N-terminal and C-terminal regions [2]. Now with recent crystallization of many GPCRs the homology

modeling of these proteins has started at an even faster pace.

Taking advantage of the above mentioned fact various laboratories of the world have developed dedicated servers for GPCR modeling. In 2004 Trabanino RJ et.al. developed the first principles computational method for predicting the three-dimensional structure of GPCRs, MembStruk [3]. Recently another dedicated web server GPCR Mod-Sim for GPCR 3-D structure prediction has been developed by Rodríguez, D et.al. which can model any GPCR belonging to any class. It offers for the first time full atom MD simulations for GPCR molecular modeling [4]. Here using the same server we are developing homology model of Frizzled GPCR class member with ID Q55CD6 in Uniprot. Frizzled proteins also play key roles in governing cell polarity, embryonic development, formation of neural synapses, cell proliferation, and many other processes in developing and adult organisms [6, 7]. Their normal body function is to serve as receptor in Wnt signaling pathway. But alteration or mutation in this pathway has also been implicated in cancer, cardiac hypertrophy, familial exudative vitreoretinopathy and schizophrenia, both in humans and in animal models [5]. But many aspects of frizzled signal transduction and pharmacology are still unclear therefore we are attempting to build 3 dimensional model of one of the member of this class using GPCR Mod-Sim server so as to provide a reference point for researchers to probe even further into their structure and functioning.

[II] MATERIALS AND METHODS

2.1. Frizzled and smoothened-like protein B

On a thorough search of www.uniprot.org it was found that reviewed protein with accession ID Q55CD6 which is a Frizzled and smoothened-like protein B has not been modeled. Therefore this protein has been chosen for our study [8].

2.2. GPCR Mod-Sim server

GPCRModSim has been developed by Rodriguez D et.al. and provides with full MD simulation. It uses Modeller as a back end modeling tool and once the UniProt ID or fasta of the protein is loaded it provides the best template on the basis of sequence alignment with the trans membrane domain. It is a complete package which provides with the facility of loop refinement using loopmodel routines) and the MD simulation of your simulated model in an atomistic model of the membrane (using GROMACS routines) [4].

[III] RESULTS

3.1. Best Alignment

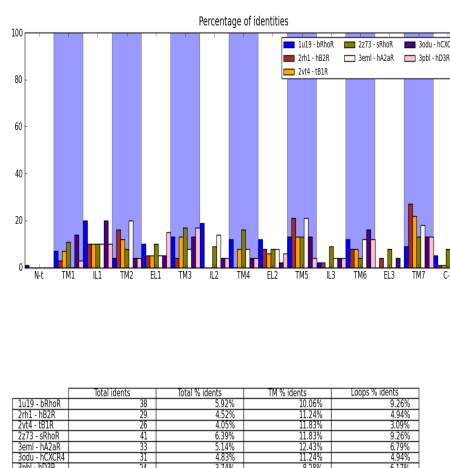


Figure1: Resume chart showing the different homology percentages in each of the 13+2 regions of a GPCR structure.

As can be seen from figure 1 above our query protein has been aligned against following templates Iu19, 2rh1, 2vt4, 2z73, 3eml, 3odu and 3pbl. The template 3eml with the Trans-Membrane identity of 12.43% has been selected as the best template.

3.2 Generated Models

After loop refinement and molecular dynamics 10 models were generated, out of which best model showed following structural validation checks with SAVES Meta Server [9]. The result with ProCheck showed 98.4% bond lengths within limits, 93.3% bond angles within limits and 100% planar groups within limits. The Ramachandran plot showed 91.9% residues within core region, 5.4% residues in allowed region, 1.7% in generously allowed region and 1% residues in disallowed region. The best values of Ramachandran plot were shown by the top 5

models. As another check the WHATIF analysis was also used according to which 'RMS Z-score given below is expected to be around 1.0 for a normally restrained data set and more common values are around 1.55'.

For our models protein the following results were obtained which shows good model quality.

RMS Z-score for bond angles: 1.274

RMS-deviation in bond angles: 2.280

The Z-score average packing quality of generated model is -4.21 which also indicated good model.

The complete statistics for all the 10 generated models is shown below in table1.

[Table-1]. Results with PROCHECK for all the 10 models generated with 3e1 as template

S. No.	% of residues in core region	% of residues in allowed region	% of residues in disallowed region	% of bond lengths within limits	% of bond angles within limits	% of planar groups within limits
1	91.9	5.4	1.0	98.4	93.3	100
2	90.5	7.8	1.2	98.5	91.4	99.6
3	90.1	7.3	1.2	98.5	93.0	100
4	90.1	7.1	0.5	98.5	93.8	100
5	91.0	6.6	0.7	98.2	94.4	100
6	89.6	8.0	1.2	98.6	94.0	100
7	88.9	8.3	1.0	98.3	93.7	100
8	91.2	5.9	1.0	98.3	92.9	99.6
9	90.6	7.6	0.5	98.4	92.6	99.6
10	89.8	8.5	0.9	98.5	91.7	99.6

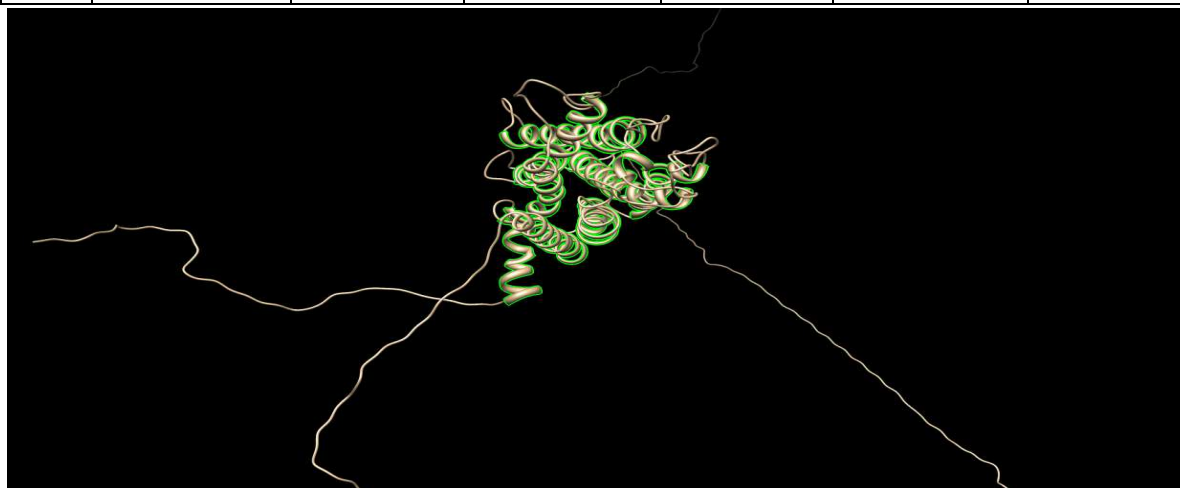


Figure 2. the 3d structure of GPCR generated through GPCR modsim server and visualized through Chimera

[IV] CONCLUSION

With the help of various freely available on-line softwares we are attempting to develop accurate three dimensional models of GPCR. The models

can help to understand various structural variations as well provide reference point to model other structures as well.

The only requisite is availability of templates so as to design new structures based on them. The technique can be applied to other proteins with unmodelled structures which will to quiet an extent help in solving problem of piled up unannotated data.

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