Homology Modeling of Human α_{2A}-Adrenoceptor

Jignesh Patel, Gourja G, Vivek Kumar, Yogesh Narkhede, Shikhar Gupta, C. Gopi Mohan*

Center for Pharmacoinformatics
National Institute of Pharmaceutical Education and Research (NIPER),
Sector 67, S.A.S Nagar, Mohali, Punjab-160062, INDIA.
E-Mail: cmohan@niper.ac.in

INTRODUCTION

⇒ α_{2A}-adrenoceptor (ADA2A) is a membrane bound receptor which has been classified as the member of larger superfamily G-protein coupled receptors (GPCRs); also known as seven transmembrane (7-TM) domains receptor

⇒ Around 50% drugs currently available in the market exert their effects through GPCRs

⇒ Membrane proteins are difficult to crystallize as compared to soluble proteins

⇒ There is a need of 3D-structure of ADA2A to understand binding modes of various agonists and antagonists and hence homology model of ADA2A was developed

⇒ Homology model of ADA2A constructed based on crystal structure of β_2-adrenoceptor

⇒ The crystal structure of β_2-adrenoceptor (PDB ID: 2RH1) was used as template, which has good sequence identity and higher resolution (2.4 Å) and models were generated using MODELLER9v7, among them some models were selected based on molpdf and DOPE score

⇒ The built homology model was evaluated using various programs like ERRAT, PROCHECK, PROSA2003, and WHAT_IF

⇒ The built homology model can be useful for designing more potent subtype selective antagonists or/and agonists and can provide guidance for mutagenesis studies

OBJECTIVES

⇒ Development of homology model of α_{2A}-adrenoceptor

⇒ Validate the built homology model using different software

METHODOLOGY

⇒ Sequence of α_{2A}-adrenoceptor was retrieved from UniProtKB/Swiss-Prot

⇒ Query sequence was subjected to BLASTP against Protein Data Bank database

⇒ Four top hits were obtained viz. 2RH1, 3D4S, 2R4R and 2R4S

⇒ Between these four top hits 2RH1 was selected as template

⇒ Template sequence and query sequence was aligned using ClustalX program

⇒ 100 models were generated using MODELLER9v7 program

⇒ Based on Discrete Optimized Protein Energy (DOPE), molpdf and GA341 scores, five models were selected

⇒ Selected models were assessed using PROCHECK, ERRAT plot and Verify3D software

⇒ Among these models best model was selected based on the results of various assessment tests

⇒ The best model was further undergone for loop modeling, energy minimization and rotamer search using MODELLER9v7 and MOE2007.09 software

⇒ An optimized model was validated using various software: PROCHECK, PROSA2003, WHAT_IF and VERIFY3D

RESULTS AND DISCUSSION

⇒ BLASTP results gave different top hits as shown in Table-1. Among these 2RH1 was selected due to higher sequence identity and good crystal structure resolution

Table 1: Top hits obtained by BLASTP search

PDB-ID	IDENTITY	SIMILARITY	SCORE	EXPECT	RESOLUTION
2RH1	31	46	179	1.00E-45	2.4 Å
3D4S	31	46	179	2.00E-45	2.8 Å
2R4R	35	52	142	2.00E-34	3.4 Å
2R4S	35	52	142	3.00E-34	3.4 Å

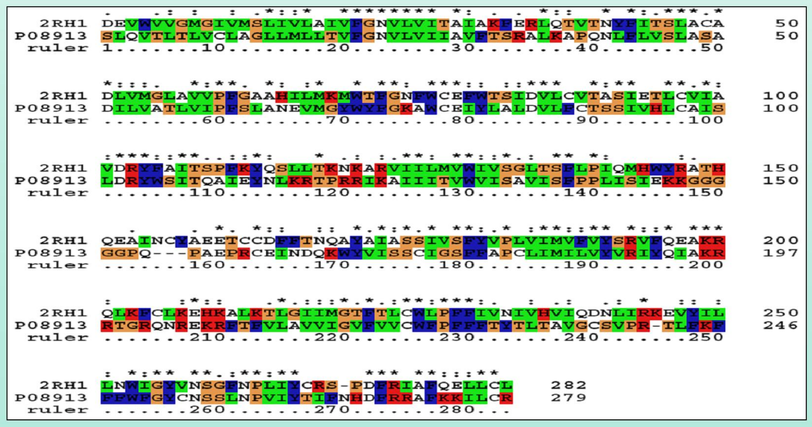

Fig. 1: Sequence alignment of template (2RH1) and target (ADA2A) using ClustalX

⇒ From PROCHEK analysis, Ramachandran plot indicated that all residues phi/psi angle distribution was within core and allowed regions and G-factor score was 0.2 which also showed reliability of developed homology model

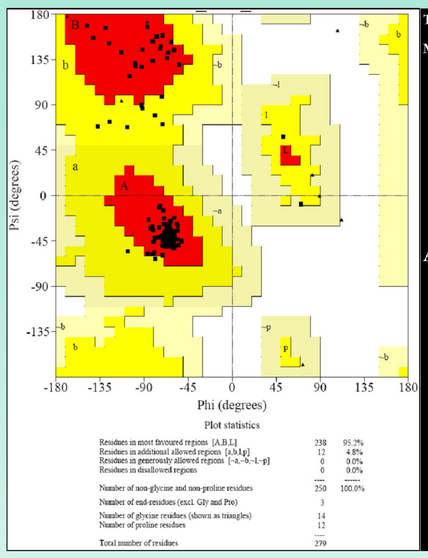

Fig. 2: Ramachandran Plot of the ADA2A homology model

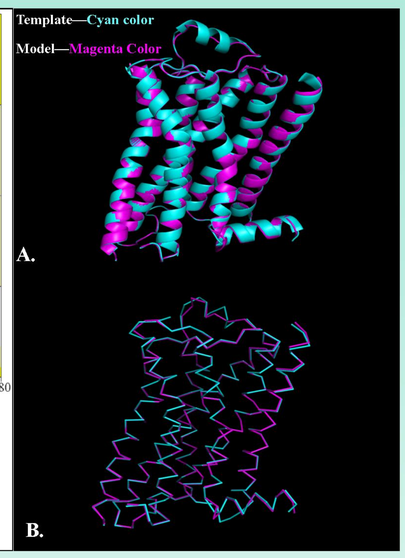

Fig. 3: Superimposition of 2RH1 and ADA2A model

⇒ Superimposition of template and target rendered less difference between C-alpha backbone and root mean square deviation (rmsd) was 0.1518 Å, calculated using Sybyl7.1 software

⇒ Verify3D profile for the model designated that 81.04% of residues of the model had a score over 0.2, a value used as judgment for a good model

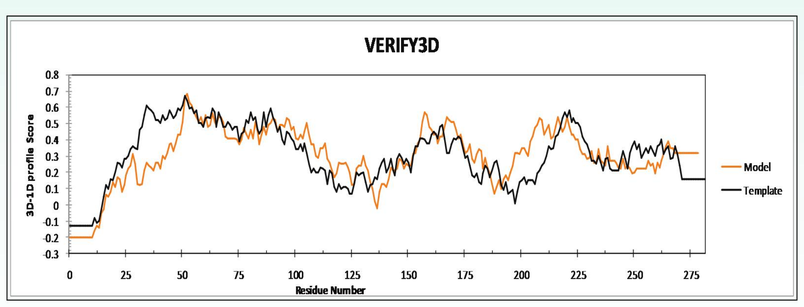

Fig. 4: 3D score distribution of template (2RH1) and model (ADA2A)

⇒ ERRAT plot of final model exhibits overall quality factor 94.07%, which increases the reliability to acceptance of the built homology model of ADA2A protein

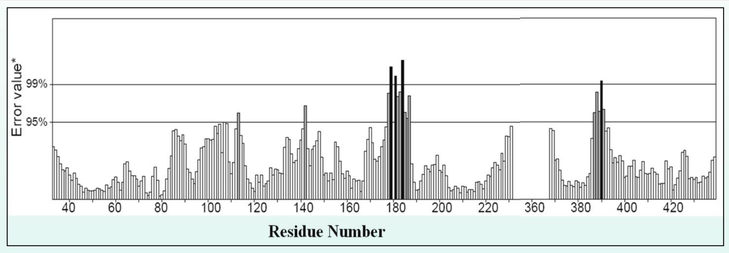

Fig. 5: ERRAT Plot of the built ADA2A homology model

⇒ In WHAT_IF analysis, packing quality score did not show stretches of four or more residues each having a quality control Z-score worse than -1.75 and inside/outside profile score was 1.2 which is within the range of transmembrane protein

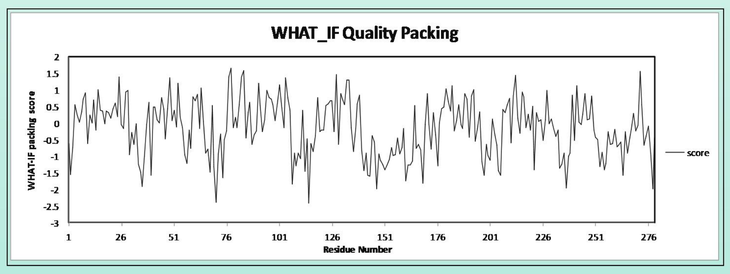

Fig. 6: WHAT_IF quality packing score of the built ADA2A homology model

⇒ PROSA2003 program was used to calculate the residue interaction energy and PROSA Z-score and PROSA energy of the built ADA2A homology model was consistent with a reliable conformation based on its similarity with that of the template 2RH1

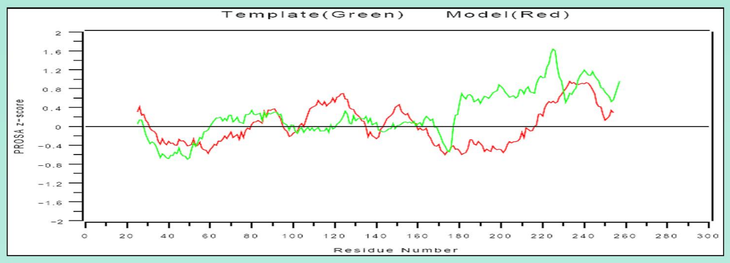

Fig. 7: PROSA Z-score comparison between the template (2RH1) and the built ADA2A homology model

CONCLUSIONS

⇒ The 3D-structure of α_{2A}-adrenoceptor (ADA2A) obtained by homology modeling showed high structural similarity to the template 2RH1 protein

⇒ Results of structure validation using different software: PROCHECK, WHAT_IF, ERRAT plot, Verify3D and PROSA2003 showed that the built homology model have overall good structure quality

⇒ Knowledge based approach such as comparative modeling can be used as an important tool in rational drug design/ docking analysis, mutagenesis studies and structure-based drug design

REFERENCES

1. Li M., Fang H., Du L. *et al.*, Computational studies of the binding site of α_{1A}-adrenoceptor antagonists, J. Mol. Model. 14 (2008) 957-966.

2. Xhaard H., Nyronen T., Rantanen V., *et al.*, Model structures of alpha-2 adrenoceptors in complex with automatically docked antagonist ligands raise the possibility of interactions dissimilar from agonist ligands, J. Struct. Biol. 150 (2005) 126-143.

3. Philipp M., Brede M. and Hein L., Physiological significance of α_2-adrenergic receptor subtype diversity: one receptor is not enough, Am. Physiol. Soc. 283 (2002) 287-295.

Nature Precedings : doi:10.1038/npre.2009.4106.1 : Posted 24 Dec 2009