ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2012, *57*(7-8), 763-768

Dedicated to Professor Victor-Emanuel Sahini on the occasion of his 85th anniversary

3D HOMOLOGY MODEL OF THE α_{2C}-ADRENERGIC RECEPTOR SUBTYPE

Liliana HALIP,* Alexandra T. GRUIA, Ana BOROTA, Maria MRACEC, Ramona F. CURPAN and Mircea MRACEC

Chemistry Institute Timişoara of Roumanian Academy, 24, M.Viteazul Av., RO-300223, Timişoara, Roumania

Received October 22, 2010

Comparative modeling of the three-dimensional structure of the human α_{2C} adrenoceptor (α_{2C}-AR) based on the high-resolution X-ray structure of the human β_2-AR (2RH1, PDB file) is reported. The sequence of the α_{2C}-AR subtype was aligned with the sequence of the 2RH1 template and the 3D homology model of the α_{2C}-AR was built using the Modeller software. The stereochemical quality of the 3D homology model was checked by using PROCHECK software and its accuracy was established by docking the endogen ligand, norepinephrine.

INTRODUCTION

α_{2C}-Adrenergic receptor (α_{2C}-AR) belongs to class A of the large family of G protein-coupled receptors (GPCRs). The progress in experimental studies on distribution, polymorphism, cloning, function, physiology, pharmacology and clinical applications of α_2-ARs is discussed in several recently published reviews.[1-5]

The α_{2C}-AR is present in the adrenal medulla, where it mediates the epinephrine release and in the central nervous system (CNS), where it participates with α_{2A}-AR in the presynaptic inhibition of norepinephrine release.[6] The α_{2C}-AR is also expressed in kidney and cutaneous blood vessels where it is supposed to contribute to cold-induced vasoconstriction.[7] Like α_{2A}-AR, the α_{2C}-AR has a contribution to the spinal nociception and opioid synergy induced by moxonidine.[8] The α_{2C}-AR has a distinct inhibitory role in various CNS-mediated behavioral and physiological responses such as amphetamine-induced locomotor hyperactivity and aggressive behavior.[9] In the last years the number of potential therapeutic uses of the α_{2C}-AR increased rapidly and led to a stringent need for potent and selective α_{2C}-AR agonists and antagonists. The most rapid and less expensive manner of approaching such a challenging task is the computer-aided drug design (CAMD). A common approach is virtual screening based on similarity criteria and docking conformers of ligands from large libraries. When the experimentally determined 3D structure of the target protein is not known, accurate models of the 3D structure now can be obtained either by *ab initio*, or by homology modeling methods. The progress in the methodological approaches and structural features of the 3D homology models of α-ARs was recently published.[10]

One of the most important steps in homology modeling technique is the identification of a good template structure. Such a structure must fulfill some requirements: the resolution of its X-Ray spectrum must be equal or better than 2.5 Å, the sequence identity with the target protein must be high (at least 30%), and the biological function of the two proteins must be similar or at least related.

* Corresponding author: lili_ostopovici@gmail.com

The first template used for building 3D homology models of α-ARs was the structure of bacteriorhodopsin. The 3D models[11,12] based on this template were followed by those[12-17] based on a true GPCR template, namely the X-ray structure of bovine rhodopsin obtained at different resolutions by Palczewski[18], Teller[19] and Okada.[20] Recently, other GPCR structures have been solved: the human β2-AR[21], the turkey β1-AR[22], the squid rhodopsin[23] and the human adenosine A2A receptor.[24] Thanks to these findings new insights into ligand binding and the structural changes required to accommodate catecholamine agonists have been provided.

In this paper we report the building, refinement and geometrical characteristics of a 3D homology model of α_{2C}-AR based on a high-resolution (2.4 Å) crystal structure of the human β_2-AR[21] crystallized with the inverse agonist carazolol. Despite the missing of the third intracellular loop, the β2-AR-T4L chimera protein was shown to retain near-native pharmacologic properties of β2-AR.[21]

METHODS

The sequence of 2RH1 and human α_{2C}-AR were extracted from Protein Data Bank (PDB) and SwissProt database respectively.[25] The two sequences have been automatically aligned with T-coffee[26,27] and manually refined. The final alignment was used to generate the homology model of α_{2C}-AR using Modeller software[28,29] based on the X-ray structure at 2.4 Å resolution of the human β_2-AR, PDB code file 2RH1.[21] The resulted 3D model was first stereochemically evaluated using the Procheck software[30,31] and then, refined with Protein Preparation Wizard[32] included in the Schrödinger suite 2009. After refinement the model was validated by docking its endogen ligand, norepinephrine, using Induced Fit Docking module from the Schrödinger software. In the process of docking, both ligand and receptor were flexible. The primary binding site of α_{2C}-AR was identified by using SiteMap application[33] implemented in Schrödinger suite.

Norepinephrine was prepared for docking using LigPrep 2.1 application[34]. For this purpose, the ionization state was set according to physiological conditions (pH range 7±0.2). Finally, the geometry was optimized using OPLS2005 force field.

RESULTS

The Ballesteros-Weinstein convention[35] was used for amino acid numbering in the sequences of the two proteins. In this convention the most conserved amino acid on a certain helix is arbitrarily noted with 50 preceded by the number of the helix, n. The rest of the amino acids on a helix are numbered relative to n.50 position. For example on helix 3 the most conserved amino acid is an Arg residue noted Arg3.50. The precedent amino acid is Asp3.49 and the following amino acid after Arg3.50 is a Tyr3.51. This sequence Asp-Arg-Tyr (or DRY in one letter notation of amino acids) is one of the most conserved sequences in the GPCR family.

The alignment of the α_{2C}-AR and 2RH1 sequences revealed the absence of the following fragments in the 3D structure of the β_2-AR: Asn1 – Arg28, Gln231 – Ser262, Arg343 – Leu413. The sequence Pro230 – Arg363 from the third intracellular loop of the α_{2C}-AR has no correspondent sequence in the 2RH1 template, because it corresponds to the T4 lysozyme (T4L). Therefore, the third intracellular loop of the α_{2C}-AR was deleted from Thr241 to Val372. This action does not influence the interactions from the binding pocket, which is located in the transmembrane (TM) region. In the final alignment the proper alignment of the highly conserved residues was checked.[36] In the α_{2C}-AR sequence the following conserved amino acids (numbered according to the Ballesteros-Weinstein convention) were identified: on the TM1 Gly1.49, Asn1.50 and Val1.53, on TM2 Ser2.45, Leu2.46, Ala2.47, Ala2.49 and Asp2.50, on TM3 Ser3.39, Leu3.43, Ile3.46, Ser3.47 (Ala3.47 on 2RH1), Asp3.49, Arg3.50, Tyr3.51, Val3.54 (Ile3.54 on 2RH1), on TM4 Trp4.50, Ser4.53 and Pro4.60, on TM5 Phe5.47, Pro5.50, Ile5.53, Tyr5.58 and Ile5.61 (Val5.61 on 2RH1), on TM6 Arg6.32 (Lys6.32 on 2RH1) Phe6.44, Cys6.47, Trp6.48, Pro6.50, on TM7 Asn7.45, Ser7.46, Asn7.49, Pro7.50, Tyr7.53, Phe7.60 and Arg7.61. Other conserved amino acids, such as Asp131 (Asp3.32 on TM3), Ser214 (Ser5.42 on TM5), and Ser218 (Ser5.46 on TM5), are implicated in interaction with α_{2C}-AR agonists and antagonists. The final alignment after deletion of the amino acids from the third intracellular loop is shown in Fig. 1.

The model building was carried out using Modeller software[28,29] based on the final alignment shown in Fig. 1.

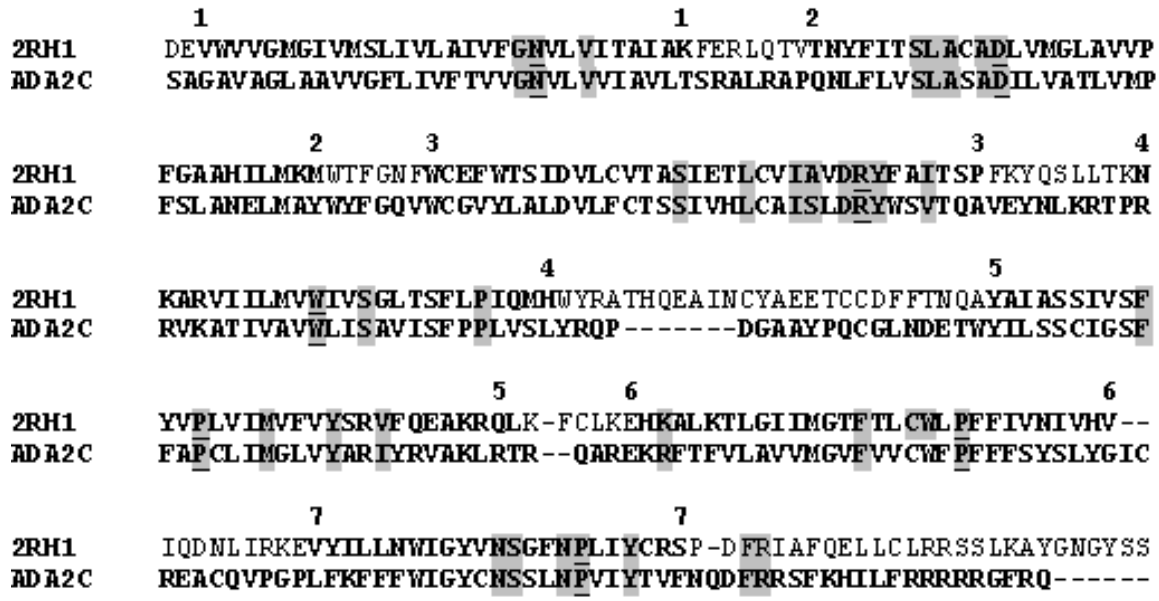

Fig. 1 – Sequence alignment of the hα_{2C}-AR and hβ_2-AR (PDB 2RH1) sequences.
Bold characters – alpha helices; gray background – highly conserved amino acids in the GPCR family; underlined – the n.50 amino acid on the helix 'n' in the Ballesteros-Weinstein convention. The helix number is marked above the first and the last residue from that helix.

From stereochemical point of view, a satisfactory homology model should have over 90% residues in the most favored regions of the Ramachandran plot.[37] Therefore, the initial 3D model of the α_{2C}-AR was refined in several steps. To avoid close contacts and to correct some distorted bond angles or planarity of some aromatic rings signaled by PROCHECK the geometrical parameters of certain residues have been optimized with AMBER99 force field. The geometry of the final model had all geometry parameters in the normal limits.

The refined 3D model of α_{2C}-AR contained 228 residues in the most favored regions (91.6 % in A, B, L regions of the Ramachandran plot), 21 residues in the additional allowed regions (8.4 % in a, b, l, p regions) and no residues in each of generously allowed (0.0 % in ~a, ~b, ~p, ~l regions), and in disallowed regions (0.0 % in white area). The Ramachandran map for the refined 3D model of the α_{2C}-AR based on 2RH1 template is displayed in Fig. 2.

Using the Procheck software the main-chain parameters (peptide bond planarity, bad non-bonded interactions, Cα distortion, overall G-factor), main-chain bond length distributions, main-chain bond angle distributions, and side-chain parameters of residues were also verified and found to be in the admitted error limits or better. The homology model of α_{2C}-AR, represented as a solid ribbon is displayed in Fig. 3.

Mutagenesis studies[38] performed on α_{2A}-AR showed that amino acid residues important for ligand binding and receptor activation by agonists are Asp3.32 and Ser5.46. The sequence identity between α_{2A}-AR and α_{2C}-AR is extremely high (over 85%) and the Asp3.32 and Ser5.46 residues are being conserved within α_2-ARs. Therefore a similar binding mode of norepinephrine in the α_{2C}-AR binding site is expected. To check the model accuracy with regard to experimental data, the endogen ligand of all adrenergic receptors, norepinephrine, was docked in the α_{2C}-AR binding site using InducedFit protocol. A grid that included the ligand binding domain resulted from Site Map[33] was used for docking the norepinephrine. The best pose of the α_{2C}-norepinephrine complex obtained from docking is displayed in Fig. 4.

As one can see in Fig. 4, the protonated NH_3^+ group of norepinephrine is favorably placed and oriented to electrostatically interact (salt bridge formation) with the negatively charged carboxylic group from Asp3.32 (Asp131) side chain (3.3Å). In addition, the hydroxyl groups found in *meta* and *para* positions of the catecholic ring form hydrogen bonds with the hydroxyl groups from Ser 5.42 and Ser5.46 side-chain. The output of docking the endogen ligand in α_{2C}-AR supports the experimental data obtained through site-directed mutagenesis and provides evidence that homology model is reliable and it can be used in further studies.

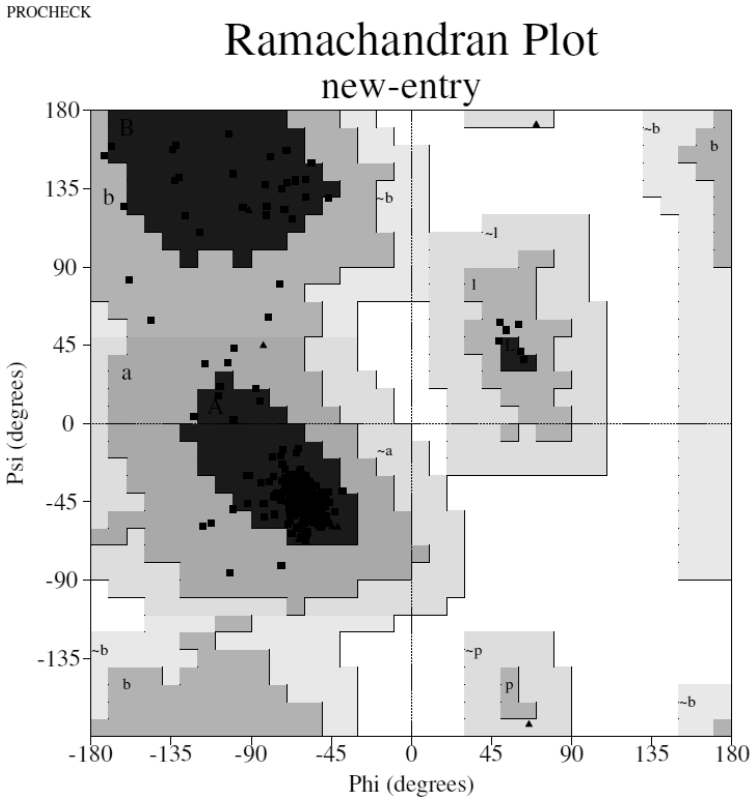

Fig. 2 – Ramachandran map for α_{2C}-AR after refinement steps.

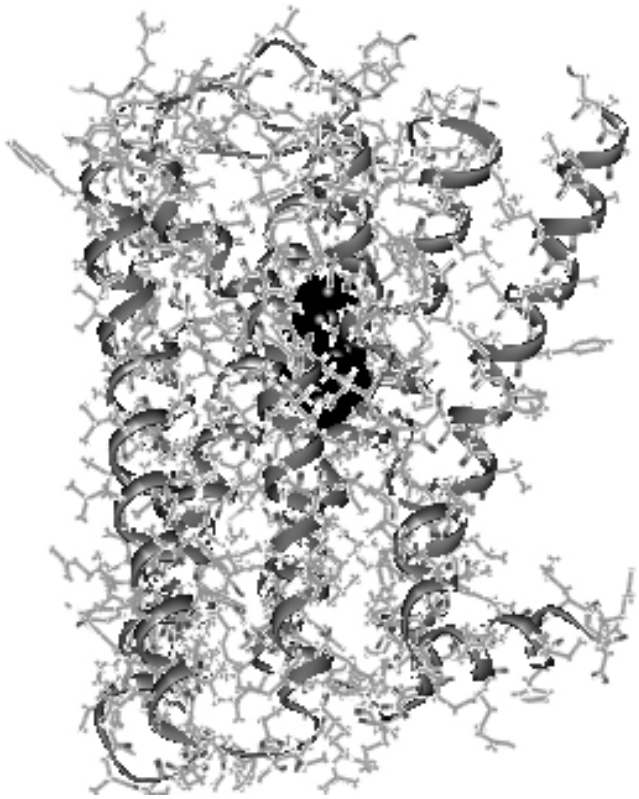

Fig. 3 – The 3D-structure of α_{2C}-AR obtained by homology modeling using the 2RH1 structure as template. Norepinephrine binding pocket is highlighted with dark black surface.

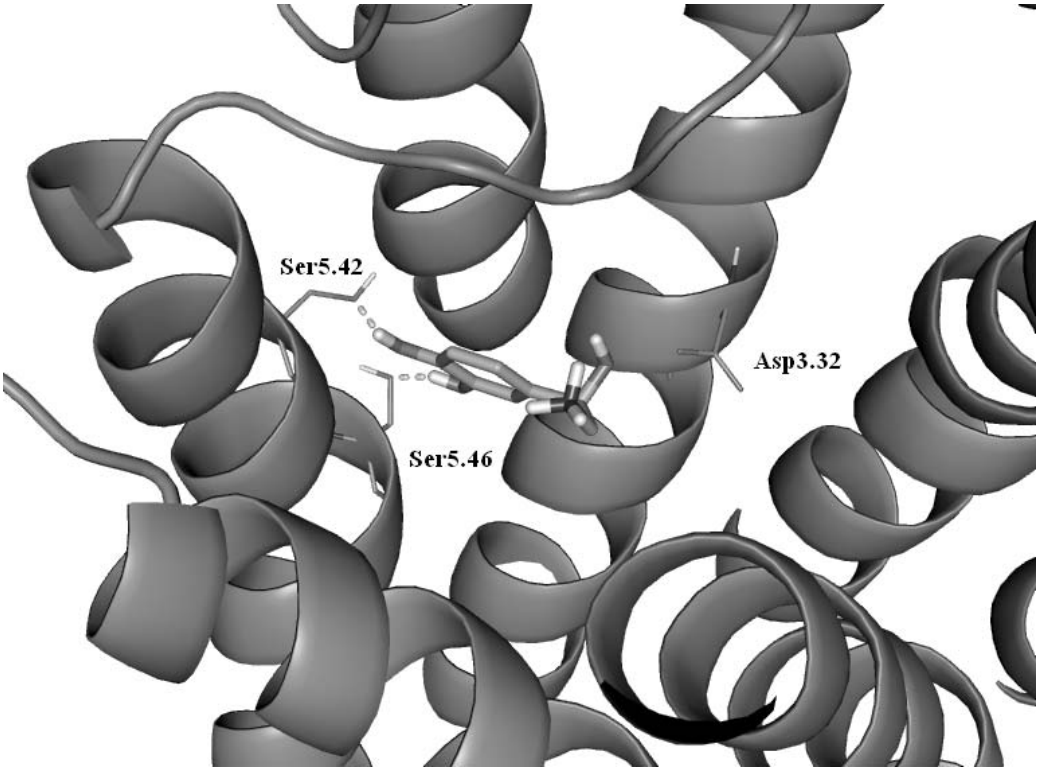

Fig. 4 – Norepinephrine docked into the α_{2C}-AR binding site. The residues involved in norepinephrine binding are Asp3.32, Ser5.42 and Ser5.46. Hydrogen bonds are depicted with dashed lines.

CONCLUSIONS

The 3D model of the human α_{2C}-AR was developed based on the crystal structure of human β2-AR as an attempt to have a better view of the ligand behavior in the α_{2C}-AR binding pocket. The homology modeling of the human α_{2C}-AR was possible due to 1) the human α_{2C}-AR structural pattern (seven transmembrane helices) similar to the one of the β_2-AR and 2) the highly conserved residues in the rhodopsin family. Identification of the ligand binding domain and docking the norepinephrine to the α_{2C}-AR led to the conclusion that the obtained model is accurate enough to be used in further studies as a useful tool for the design of new receptor mutants and hopefully, it might serve to design compounds with better binding affinity and selectivity for the α_{2C}-AR subtype.

Acknowledgments: This work was supported by the Romanian National Council for Scientific Research (CNCSIS), Grant PN-II-PCE-ID nr 1268/2007.

REFERENCES

1. P.-A. Crassous, C. Denis, H. Paris and J.M. Sénard, *Curr. Top. Med. Chem.*, **2007**, *7*, 187-194.
2. F. Gentili, M. Pigini, A. Piergentili and M. Giannella, *Curr. Top. Med. Chem.*, **2007**, *7*, 163-186.
3. 5. K. Gyires, Z.S. Zádori, T. Török and P. Mátyus, *Neurochem. Int.*, **2009***, 55*, 447-453.
4. J. P Hieble, *Curr. Top. Med. Chem.*, **2007**, *7*, 129-134.
5. S Schaak, J Mialet-Perez, C Flordellis and H. Paris, *Curr. Top. Med. Chem.*, **2007**, *7*, 217-231.
6. M. Brede, G. Nagy, M. Philipp, J.B. Sorensen, M.J. Lohse and L. Hein, *Mol.Endocrinol.*, **2003**, *17*, 1640-46.
7. M.A. Chotani, S. Flavahan, S. Mitra, D. Daunt and N.A. Flavahan, *Am. J. Physiol. Heart Circ.Physiol.*, **2000**, *278*, 1075-83.
8. C.A. Fairbanks, L.S. Stone, K.F. Kitto, H.O. Nguyen, I.J. Posthumus and G.L. Wilcox, *J Pharmacol Exp Ther.*, **2002**, *300*, 282-290.
9. M. Scheinin, J. Sallinen and A. Haapalinna, *Life Sci*., **2001**, *68*, 2277-2285.
10. A. Carrieri and A. Fano, *Curr. Top. Med. Chem*., **2007**, *7*, 195-205.
11. J.A. Bikker, S. Trumpp-Kallmeyer and C. Humble, *J.Med. Chem*., **1998**, *41*, 2911-2927.
12. T. Salminen, M. Varis, T. Nyrönen, M. Pihlavisto, A.M. Hoffrén, T. Lönnberg, A. Marjamäki, H. Frang, J.M. Savola, M. Scheinin and M.S. Johnson, *J. Biol.Chem.*, **1999**, *274*, 23405-13.
13. J.M. Peltonen, T. Nyronen, S. Wurster, M. Pihlavisto, A.M. Hoffren, A. Marjamaki, H. Xhaard, L. Kanerva, J.M. Savola, M.S. Johnson and M. Scheinin, *Br. J. Pharmacol.*, **2003**, *140*, 347-358.
14. H. Xhaard, T. Nyronen, V.V. Rantanen, J.O. Ruuskanen, J. Laurila, T. Salminen, M. Scheinin and M.S. Johnson, *J. Struct. Biol*., **2005**, *150*, 126-143.

15. F. Gentili, F. Ghelfi, M. Giannella, A. Piergentili, M. Pigini, W. Quaglia, C. Vesprini, P.A.; Crassous, H. Paris and A. Carrieri, *J. Med. Chem*., **2004**, *47*, 6160-73.
16. B. Balogh, Cs. Hetenyi, M.G. Keseru and P. Matyus, *Chem. Med. Chem*., **2007**, *2*, 801-805.
17. B. Balogh, A. Szilágyi, K. Gyires, D. Bylund and P. Mátyus, *Neurochem. Int*., **2009**, *55*, 355-361.
18. K. Palczewski, T. Kumasaka, T. Hori, C.A. Behnke, H. Motoshima, B.A. Fox, I. Le Trong, D.C. Teller, T. Okada, R.E. Stenkamp, M. Yamamoto and M. Miyano, *Science*, **2000**, *289*, 739-745.
19. D.C. Teller, T. Okada, C.A. Behnke, K. Palczewski and R.E. Stenkamp, *Biochemistry*, 2001, *40*, 7761-72.
20. T. Okada, M. Sugihara, N. Bondar, M. Elstner, P. Entel and V.J. Buss, *J. Mol. Biol*., **2004**, *342*, 571-583.
21. V. Cherezov, D.M. Rosenbaum, M.A. Hanson, S.G.F. Rasmussen, F.S. Thian, T.S. Kobilka, H.-J. Choi, P. Kuhn, W.I. Weis, B.K. Kobilka and R.C. Stevens, *Science*, **2007**, *318*, 1258-65.
22. A. Warne, M.J. Serrano-Vega, J.G. Baker, R. Moukhametzianov, P.C. Edwards, R, Henderson, A.G.W. Leslie, C.G. Tate and G.F.X. Schertler, *Nature,* **2008**, *454,* 486-91.
23. M. Murakami and T. Kouyama, *Nature* **2008**, *453*, 363-367.
24. V.P. Jaakola, M.T. Griffith, M.A. Hanson, V. Cherezov, E.Y. Chien, J.R. Lane, A.P. Ijzerman and R.C. Stevens, *Science* **2008**, *322*, 1211-1217.
25. http://www.expasy.org/sprot/sprot-top.html
26. C. Notredame, D.G. Higgins and J. Heringa, *J. Mol. Biol*., **2000,** *302,* 205-217.
27. O. Poirot, E. O'Toole and C. Notredame, *Nucl. Acids Res*., **2003**, *31*, 3503-3506.
28. Sali and T.L. Blundell. *J. Mol. Biol.*, **1993**, *234*, 779-815.
29. N Eswar, M.A. Marti-Renom, B. Webb, M.S. Madhusudhan, D. Eramian, M. Shen, U. Pieper, and A. Sali, *Current Protocols in Bioinformatics*, **2007**, *15*, 561-5630.
30. T. Schwede, J. Kopp, N. Guex and M.C. Peitsch, *Nucl. Acids Res.*, **2003**, *31*, 3381-5.
31. N. Guex and M.C. Peitsch, *Electrophoresis*, **1997**, *18*, 2714-2723.
32. Protein Preparation Wizard; Schrödinger LLC, New York, NY 2007.
33. SiteMap version 2.1; Schrödinger LLC, New York, NY 2007.
34. LigPrep version 2.1; Schrödinger, LLC, New York, NY 2007.
35. J. Ballesteros, H. Weinstein. *Methods Neurosci* **1995**, *25*, 366-428.
36. J.M, Baldwin, G.F.X. Schertler, V.M. Unger, *J Mol Biol.,* **1997**, *272*, 144-164.
37. G.N. Ramachandran, C. Ramakrishna and V. Sasisekharan, *J.Mol.Biol.*, **1963**, *7*, 95-9.
38. C.-D. Wang, M.A. Buck and C.M. Fraser, *Mol. Pharmacol.*, **1991**, *40*, 168-179.