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Homology Model of the Human 5–HT$_{1A}$ Receptor Using the Crystal Structure of Bovine Rhodopsin

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Homology Model of the Human 5–HT$_{1A}$ Receptor Using the Crystal Structure of Bovine Rhodopsin [#]

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Abstract

Motivation. The 5–HT$_{1A}$ receptor, a member of class A GPCRs, is associated with psychiatric disorders like depression and anxiety, thus representing an important target for developing new drugs. In the absence of availability of X–ray crystal structure of the receptor, alternative approaches must be used for building 3D models of the receptor. Bacteriorhodopsin was previously used as a template for homology modeling of GPCRs. However bacteriorhodopsin is not coupled to G protein and exhibits very low sequence homology with human GPCRs.

Method. The homology model of the human 5–HT$_{1A}$ receptor was constructed using the published X–ray crystal structure of bovine rhodopsin (PDB code: 1F88) with MOE 2003.04 (Chemical computing group, Canada). Due to low overall sequence identity, a segmented approach was used for model building. The loops, C–terminal and the N–terminal regions were modeled separately and attached to the transmembrane region. The model was validated by docking serotonin, an endogenous 5–HT$_{1A}$ receptor ligand.

Results. The model retains global arrangement of GPCRs and is energetically and geometrically consistent. After docking, the environment of serotonin in the receptor model is consistent with reported SAR data for 5–HT$_{1A}$ ligands.

Conclusions. This work has provided a first complete model of the human 5–HT$_{1A}$ receptor for further drug development. This model can serve as basis for future development of 5–HT$_{1A}$ receptor ligands.

Keywords. G–protein coupled receptors; 5–hydroxytryptamine$_{1A}$ receptor; homology modeling; docking.

Abbreviations and notations

GPCR, G–protein coupled receptor	PDB, protein data bank
MD, molecular dynamics	5–HT$_{1A}$R, 5–hydroxytryptamine$_{1A}$ receptor

1 INTRODUCTION

GPCRs are the most abundant receptors among the membrane bound protein receptors. They transduce signals in response to a wide variety of stimuli [1]. Many diseases involve malfunctioning of these receptors, making them important targets for the drug development [2]. However, despite their importance, there is insufficient structural information about GPCRs [3]. Due to these

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[#] Dedicated to Professor Lemont B. Kier on the occasion of the 75th birthday.
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difficulties, great importance has been placed on indirect structural evidence obtained from a variety of biophysical techniques, as well as detailed sequence analysis and molecular modeling studies. These data and computational techniques have been used to create and refine an almost limitless number of published and unpublished theoretical three–dimensional (3D) models over the past decade [4].

The 5–HT$_{1A}$R belongs to class of family A of GPCRs. The members of this class have a number of characteristic amino acids in common [5]. It is coupled to different effector systems: an enzymatic one that produces adenylate cyclase inhibition and two ionic effectors (*i.e.*, potassium channel activation and a calcium channel inhibition) [6]. It is associated with the psychiatric disorders like depression and anxiety [7]. Potential therapeutic applications for 5–HT$_{1A}$R antagonists are evaluated in depression, anxiety and cognition disorder [8,9]. Therefore modulation of the 5–HT$_{1A}$R activity will be an important therapeutic approach in the treatment of these disorders. Many early homology models for 5–HT$_{1A}$R were generated using bacteriorhodopsin as a structural template. However validity of a bacteriorhodopsin as structural template was questioned as it is not coupled to G protein and exhibits very low sequence homology with human GPCRs [10–12].

The recent publication of the first high–resolution crystal structure for rhodopsin at 2.8 Å provides the option of homology modeling to generate 3D models based more firmly on detailed structural information [3] whose 7–TM motif has been proven valid for other receptors in its family as well [13,14]. Given the relative simplicity of the helical fold and ~30% sequence identity with rhodopsin in the transmembrane core region, it should be possible to generate models where ~80% of the Cα atoms are within 3.5 Å of their correct positions [15]. Protein models obtained at such resolution can be correctly used to predict the location of binding sites and the size of the ligands as well as to provide strong evidence for validating the structural model for understanding the structure and function [15]. These rhodopsin models have the potential to significantly improve structure–based approaches to GPCR drug discovery of other GPCRs [16].

2 MATERIALS AND METHODS

The comparative homology modeling of human 5–HT$_{1A}$R was carried out with the HOMOLOGY functionality in MOE 2003.04 (Chemical Computing Group, Canada) on Pentium IV workstation (2.66GHZ Processor) [17]. The amino acids sequence for the human 5–HT$_{1A}$ receptor, consisting of 422 amino acids residues, was obtained from the Swissprot database (SP08908) in FASTA format. The X–ray crystalline structure of bovine rhodopsin (PDB code: 1F88) with 2.8 Å resolution served as a template for modeling 3D structure of 5–HT$_{1A}$R.

2.1 Sequence Alignment

Due to considerable homology between TMS in various GPCRS, the TMS of 5–HT$_{1A}$ Receptor were aligned with corresponding TM region of bovine rhodopsin without introduction of gap using segmented alignment approach [18,19]. The boundaries for transmembrane segments (TM1 to TM7) of 5–HT$_{1A}$R were assigned based on previously published data [12]. The PAM250 substitution matrix was used for evaluating amino acid similarity. The percentage similarity between the 5–HT$_{1A}$ receptor and bovine rhodopsin for each of the seven transmembrane regions were as follows: TM1 47%; TM2 52%; TM3 46%; TM4 67%; TM5 59%; TM6 58%; TM7 64%. The largest loop IC3 consisting of 128 amino acid residues was separately aligned on protein fragments obtained from NCBI PSI–BLAST algorithm [20] and modeled. Other loops and the end terminals were aligned manually on the crystal structure of bovine rhodopsin or built in extended conformation using '*Create sequence*' functionality in the MOE.

```
TM1   1F88    42  AYMFLLIMLGFPINFLTLYVTVQ  64
     5-HT₁ₐR   38  ITSLLLGTLIFCAVLGNACVVAA  60
                    ●**  * * ●  ●   ● * ●●
TM2   1F88    73  ILLNLAVADLFMVFGGFTTTLYT  97
     5-HT₁ₐR   72  LIGSLAVTDLMVSVLVLPMAALY  96
                   ●   ●***●**  ●  ●   ●  ●●
TM3   1F88   110  CNLEGFFATLGGEIALWSLVVLAI 133
     5-HT₁ₐR  109  CDLFIALDVLCCTSSILHLCAIAL 132
                   *●*    ●   *   ●●   * ● *●
TM4   1F88   152  HAIMGVAFTWVMALACAAPPLVGW 175
     5-HT₁ₐR  152  RAAALISLTWLIGFLISIPPMLGW 175
                    *●● ●●●*●  ●    ● ** ●**
TM5   1F88   203  FVIYMFVVHFIIPLIVIFFCYG  224
     5-HT₁ₐR  195  YTIYSTFGAFYIPLLLMLVLYG  216
                   ●  **      * ***●● ●● **
TM6   1F88   254  VIIMVIAFLICWLPYAGVAFYIFT 277
     5-HT₁ₐR  347  LGIIMGTFILCWLPFFIVALVLPF 370
                   ●  *    ●* ●*****  **● ●
TM7   1F88   287  FMTIPAFFAKTSAVYNPVIYIMMNK 311
     5-HT₁ₐR  381  LGAIINWLGYSNSLLNPVIYAYFNK 405
                   ●  ●*  ●●●    ●● *****●   **
```

Figure 1. Segmented alignment of the transmembrane regions of Bovine rhodopsin and 5–HT$_{1A}$ receptor. The identical amino acids are indicated with '*', where as the 'conserved' amino acid residues, which meet the criteria of either highly conservative or semi conservative substitution as defined by PAM250 substitution matrix are indicated with "·". Underlined amino acid residues play important role in agonist or antagonist binding.

Conserved residues Asp83 (residue number in the rhodopsin PDB file) and Asp82 (residue number in the 5–HT$_{1A}$ sequence), Cys110 and Cys109, Trp161 and Trp161, Pro215 and Pro207, Pro267 and Pro360, Pro303 and Pro397 were employed in the alignment of rhodopsin and human 5–HT$_{1A}$R transmembrane sequences [21]. The MOE –Constraint tool was used to apply constraints to the conserved residues. The alignment of 5–HT$_{1A}$ receptor with 1F88 is shown in Figure 1.

```
Score = 24.6 bits (52), Expect = 6.1, Identities = 17/49 (34%), Positives = 21/49 (42%)
5-HT₁ₐ  233  VEKTGADTRHGASPAPQPKKSVNGESGSRNWRLGVESKAGGALCANGAV  281
1K23    300  VNAHGTSTPAGDKAEAQAVKTIFGEAASRVLVSSTKSMTGHLLGAAGAV  348
             *    *   *    *     *  *●●  **●  **      ●*   *   *  *  ***
Score = 28.1 bits (61), Expect = 0.55, Identities = 15/58 (25%), Positives = 27/58 (50%)
5-HT₁ₐ  282  RQGDDGAALEVIEVHRVGNSKEHLPLPSEAGPTPCAPASFERK-NE  326
1WPM     84  KDIEEVQVLEVIDHHRIANFETAEPLYYRAEPVGCTATILNKMYKE  129
             ●   ●●    ****●  **●  *  ●     **     *  *   *       ●    *
5-HT₁ₐ  327  RNAEAKRKMA  336
1WPM    130  NNVKIEKEIA  139
                *  ●  ●●●●●*
```
Figure 2. Alignment for third Intracellular loop with the protein fragments obtained from BLAST search.

2.2 Building Homology Models

Homology model of the 5–HT$_{1A}$ receptor was constructed based on the assumption that GPCRs share similar TM boundaries and overall topology [22].

2.2.1 Modeling of the transmembrane helices

The helical parts of bovine rhodopsin (PDB 1F88) were isolated from the X–ray coordinates and used as a template. The helical boundaries for bovine rhodopsin and 5–HT$_{1A}$ receptor were taken from the literature [3,12]. A total of 10 intermediate homology models of individual transmembranes were generated along with minimized average model using MOE–HOMOLOGY, which was the result of the permutational selection of side chain rotamers. The Best Cartesian Average and Best Intermediate methods were employed using the MOE Alignments [23].

2.2.2 Modeling of the extracellular N–terminal and intracellular C–terminal

The N–terminus and C–terminus were modeled as separate molecular entities using rhodopsin templates. The N–terminal was modeled using the Bovine rhodopsin template. The C–terminal was modeled in extended conformation using '*create sequence*' functionality in MOE. Both the termini were stitched using 'Join_ProteinChains' algorithm available in MOE.

2.2.3 Modeling of the loops

The variable regions were modeled by either directly copying from template when the Structurally Variable Regions (SVRs) in question have the same number of residues in the template and target or identifying a suitable segment from a known structure from protein data bank (PDB) [24]. Peptide segments corresponding to the N– and C–terminal halves of each loop were constructed in extended conformation using '*Create sequence*' functionality in the MOE and were attached to the ends of the appropriate transmembrane helices using '*JoinProtein_chains*' algorithm. In the 5–HT$_{1A}$ receptor, the third Intracellular loop (IC3) consists of 128 amino acids residues. The whole loop was submitted to the PSI–BLAST algorithm in NCBI server [25]. Two hits obtained for IC3 with sequence identities of 50% for 1WPN (Crystal structure of the N–

terminal core of *Bacillus subtilis* Inorganic Pyrophosphates) and 42% for 1K23 (Beta–ketoacyl carrier protein). The alignment for IC3 is shown in Figure 2. These fragments were stitched together with the remaining fragment, generated in extended conformation with the use of '*Create sequence*' functionality, and finally the whole loop was inserted between TM5 and TM6 using 'JoinProtein_chains' algorithm.

2.2.4 Conserved disulphide bond

The disulphide bond is conserved across all GPCR families. Two highly conserved cysteines are important for proper protein folding probably by forming the disulphide bond between the Cys109 in TM3 and Cys187 in EC2 [26]. The disulphide bond was created manually using '*Builder*' functionality in MOE. It was further refined using an energy minimization protocol.

2.2.5 Proline induced helical bending

Proline induced helical bending was taken into account in the model building procedure. Proline can induce helical bending in α helices [27]. Prolines containing helical kinks are due to lack of hydrogen bond donor capacity of proline. These proline residues have an important structural or dynamic function and play a role in signal transduction. In $5–HT_{1A}R$, almost all the proline residues were conserved except in TM2 (91) and TM6 (369). Proline residues in TM–1 and TM–7 of rhodopsin template were also capable of inducing proline kinks in the target $5–HT_{1A}R$. Therefore, proline kinks in these regions were adjusted manually by modifying the Φ *and* φ backbone angles of residues at positions $i − 1$ and $i − 2$ relative to proline, so as to adopt the values observed in a detailed analysis of proline kinks in a collection of high resolution protein crystal structures [28].

2.2.6 Assignment of coordinates and side chain rotamer search

The coordinates for the residues in the N–terminus and transmembrane helices (TM1 to TM7) were assigned from the N–terminus and corresponding TMs of the bovine rhodopsin crystal structure. The coordinates for the ECs (EC1 to EC3), ICs (IC1 to IC3) and the C– terminal were extracted either from the crystal structure or from the loops identified in the PDB database [25] or were constructed with the MOE–Create sequence functionality and "Join_ProteinChains" algorithm. Finally side chains of all residues other than those conserved were explored for their optimal conformation and those with minimum steric clashes were assigned to the model using rotamer explorer functionality in MOE.

2.3 Refinement of $5–HT_{1A}$ R Homology Model

To remove distortion in geometry, the generated homology models were refined by successive iterations of molecular dynamics followed by energy minimization using Amber'89 all atom force field in MOE [29]. The energy minimization is carried out with the protocol involving initial minimization with steepest descent followed by conjugate gradient method and finally by Newton–

Raphson method to a gradient convergence of 0.001 kcal/mol/ Å, keeping backbone atoms of helices fixed. The simulated annealing was then carried out wherein all degrees of freedom for these regions were allowed to relax, but the heavy atoms of all other residues (TM1 to TM7) were held rigid. The protocol used for simulated annealing involve a slow heating to 600 K in 100 ps, equilibrated for 150 ps at 600 K, cooled to 300 K at 200 ps, and equilibrated to 300 K over 200 ps interval. The lowest energy structure was then taken from the 300K trajectories and subjected to final round of minimization with all heavy atoms tethered by a force constant of 100 kcal/mole/ Å^2. This procedure allowed the arbitrarily positioned amino acid side chains to adopt an energetically favorable conformation. All models were evaluated for structural integrity.

2.4 Stereochemical Evaluation of Homology Model

The model structures were analyzed using MOE–ProEval, which implements the PROCHECK suite of stereochemical measurements [30]. The criteria used in the analysis include bond lengths, bond angles, dihedrals, side chain contacts and chirality of alpha carbon atoms. The phi–psi map, Ramachandran plot, Chi plot and distance matrix plot of the model were constructed.

2.5 Validation of the Homology Model

Serotonin, the endogenous ligand for the receptor, was docked into the homology model. The minimum energy conformation of serotonin was generated using systemic search functionality in MOE. Multiple low temperature molecular dynamics trajectories were then run for each docked complex to identify stable receptor–ligand interactions using CHARMm22 force field with MOE [31]. The protonated amine of serotonin was placed near Asp116 in TM3, and all receptor–ligand complexes maintained the charge–reinforced hydrogen bond during dynamics. The catechol, or catechol–equivalent end of the serotonin, was oriented toward TM5, in the pocket formed by TM3 and TM5 (see Figure 3). The final models were evaluated for binding orientations using scoring svl functionality available in MOE which is used to visualize intermolecular contacts like direct hydrogen bonds, water–mediated hydrogen bonds, transition metal interactions and hydrophobic interactions. It also computes predicted pKi based on ligand–receptor interaction.

3 RESULTS AND DISCUSSION

Due to low overall percentage sequence identity of 27% between bovine rhodopsin and 5–HT$_{1A}$R, direct sequence alignments is not possible. However, significantly higher sequence homology in transmembrane region prompted us to perform alignment using segmented alignment approach in which corresponding transmembrane regions of bovine rhodopsin were aligned with that of 5–HT$_{1A}$R. It has been postulated that in GPCRs, by structural mimicry, a common ancestor could diverge sufficiently to develop selectivity necessary to interact with diverse ligands but still

maintain a similar overall fold [32]. Taking this into account, a segmented alignment was performed using PAM250 as a substitution matrix thus taking care of the problems arising due to low overall sequence identity. The PDB structure 1F88 with the 2.8 Å resolutions was chosen as the reference protein for the 5–HT$_{1A}$ receptor modeling the TMs and N–terminal. The loops and the C–terminal region were modeled separately.
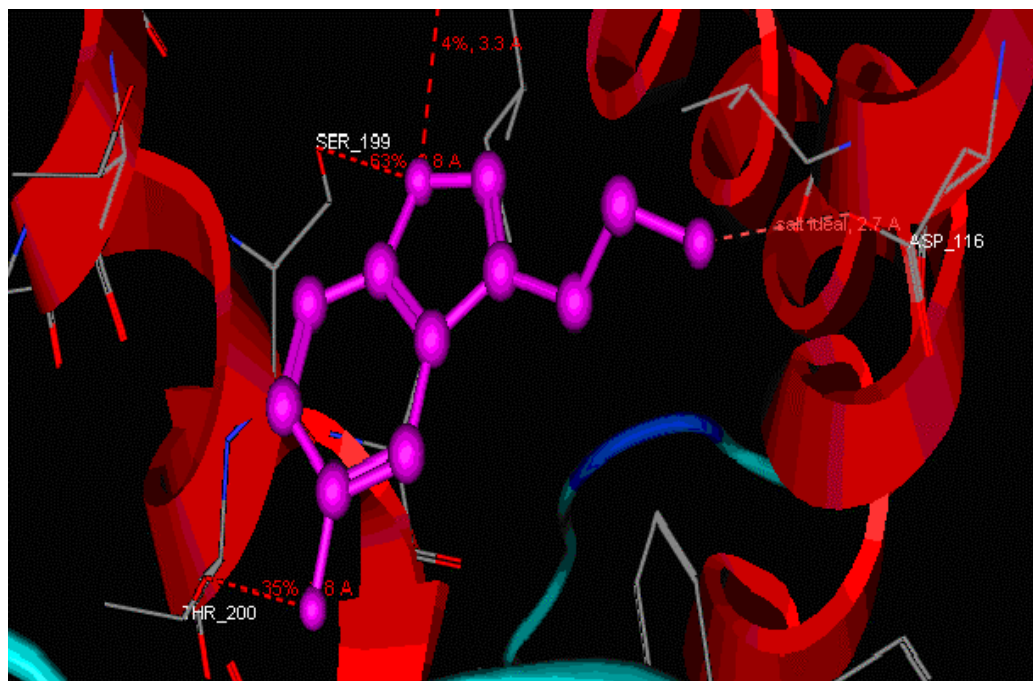
Figure 3. Interaction of serotonin with 5–HT$_{1A}$ homology model generated using segmented alignment method.

3.1 General Model Characteristics

The topology of the developed homology model of 5–HT$_{1A}$ receptor is shown in Figure 4. This homology model closely matches the rhodopsin backbone conformation, with an RMS deviation for backbone atoms of 0.6 Å, and has acceptable stereochemical parameters and side–chain packing densities. 95% of the residues were found in the allowed region of Ramachandran plot for this model. The N–terminal region in 5–HT$_{1A}$ receptor model (although extracted from the template structure) does not adopt a β–sheet configuration like 1F88 after refinement with simulated annealing. The rhodopsin structure has a short α–helical segment extending from the cytosolic end of TM7 that lies parallel to the lipid bilayer surface. Sequence alignment results suggest that 5–HT$_{1A}$ receptors may also possess this helical segment, so it was generated manually using the corresponding region on the rhodopsin structure as a template. The resulting helix segment places Gln422 in positioned favorable for interaction with the cytosolic membrane phospholipids head groups.

The difficulty in obtaining structural data of the loop regions based on sequence homology

makes it arduous to structurally characterize these regions. However they play pivotal role in complete characterization of the receptor. The intracellular loops embedded in the cytoplasmic region of the cell have crucial structures as they are linked to the G–protein to whom the message must be conveyed for elucidation of the final response. The IC3, consisting of 128 amino acid residues, was refined using simulated annealing protocol as discussed above. It is situated far from the active sites and does not directly interact with the ligand investigated in the study. The antiparallel β–sheet found between segments Tyr178 to Glu181 and Ser186 to Ile189 in EC2 in 1F88, does not exist in EC2 of the 5–HT$_{1A}$ model even the loop are of equal length.
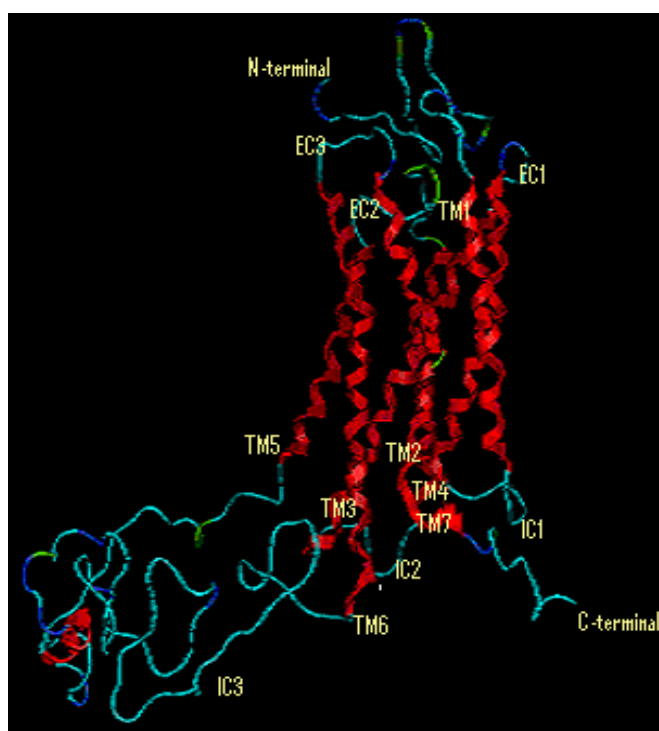
Figure 4. The topology of human 5–HT$_{1A}$ receptor model generated by homology modeling technique using segmented alignment approach and PAM250 as substitution matrix. The transmembrane helices are shown in red and the rest of structure (ICs, ECs, N–and C–terminals) is shown in Cyan.

GPCRs are thought to exhibit a conserved disulfide link between the top of TM3 and the second extracellular loop (EC2). This was indeed shown to be the case with the rhodopsin crystal structure [3]. Like in most GPCRs, 5–HT$_{1A}$ receptors contain two highly conserved cysteine residues at the top of the helix III, at the extracellular side and Cys187 in the second extracellular loop. This is important for the proper folding of the receptor. The conformational restriction imposed by this short tether, positions several loop residues in close contact with the ligand–binding site, giving additional structure to the top of the binding pocket. But this "roof" does not seem to affect small agonists as they bind in lower pocket (see Figure 5).
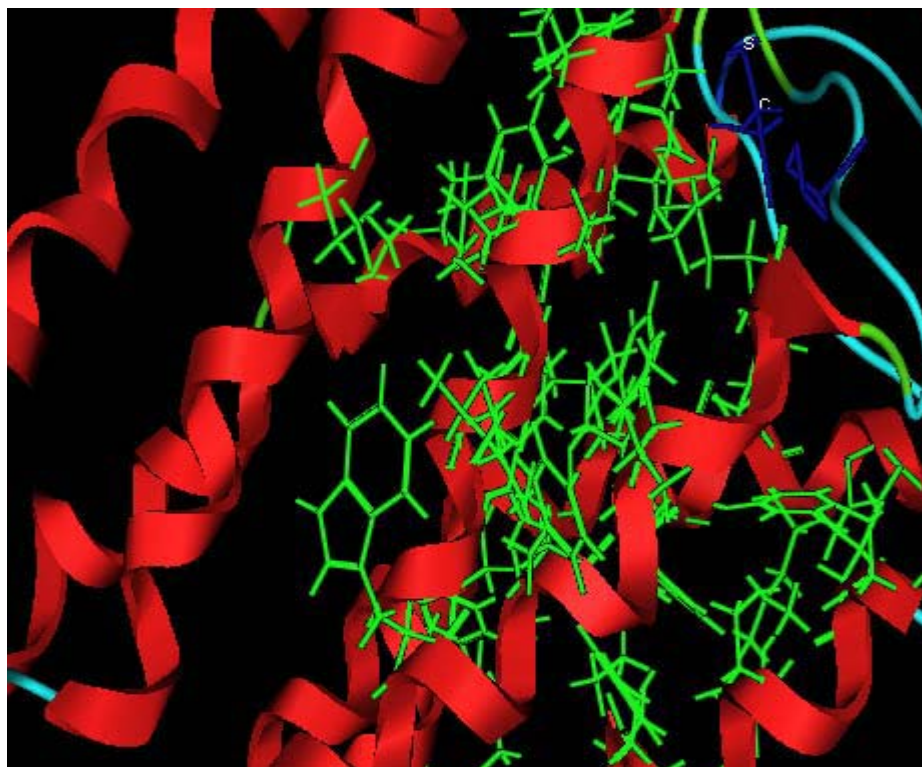
Figure 5. The active site cavity generated with alpha site finder functionality in MOE is depicted here. The active site residues are shown in Green. The disulphide linkage is shown in blue between Cys109 and Cys187 which is the characteristic of the 5–HT$_{1A}$ receptor.

Highly conserved proline residues in GPCRs are likely to induce notable kinks in the transmembrane helices and may serve important functional roles in signal transduction. Unlike the globular proteins, proline may induce only slight curving in the alpha helical backbones of membrane bound proteins. Only two of the proline residues were not identical with the transmembrane region of bovine rhodopsin. These prolines are modeled using manual adjustment of their *phi–psi* angles. Superimposition with bovine rhodopsin structure (Figure 6) shows that global arrangement of transmembrane is roughly maintained.

All of these local helical adjustments introduced some bad steric contacts and reoriented some important ligand–binding residues slightly away from the putative ligand–binding pocket. Stringent energy minimization and molecular dynamics protocol was followed to bring the amino acid residues into the energetically favorable conformation. Weak harmonic constraints were then applied during the course of short, low temperature MD simulations to close the loop segments, forming a trans amide bond at the ligation site.

3.2 Comparison with Other Models

Many homology models previously reported in the literature [33–35] were based on bacteriorhodopsin, which is not coupled to G protein and shows less homology with the GPCRs. Thus it is not possible to build a 3–dimensional model using straightforward sequence alignment. In

our model, the structure of bovine rhodopsin at resolution of 2.8 Å was used to model the 5–HT$_{1A}$ receptor. The previous models modeled the α helices only, leaving the highly variable loop regions. In our homology model of the 5–HT$_{1A}$ receptor, we have made an attempt to model the entire receptor including the α helices, loops and both the terminals so as to better characterize ligand receptor interaction in the presence of the complete receptor. We compared our model with previously reported models to confirm that model is precise in order to explain SAR data. Although various previously reported models had used different templates and different alignment procedure, serotonin was found to interact with the same conserved residues and is part of the proposed binding sites of different receptor models. The hydrogen bond of indole is missing in some of the published models [12,35]. The present model clearly shows this H–bonding interaction with Ser199 and Thr200.
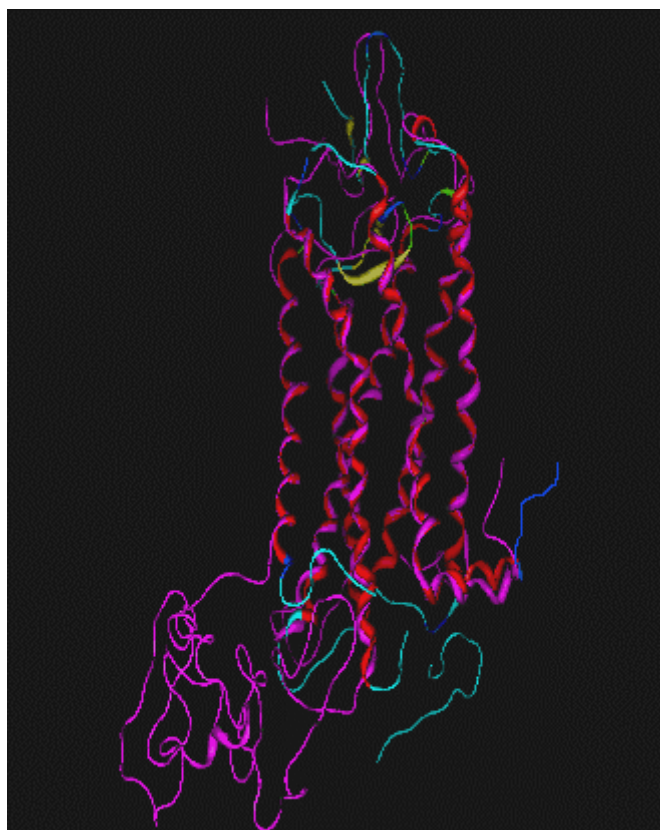
Figure 6. Superposition of the human 5–HT$_{1A}$R model (magenta) and the bovine rhodopsin (red) crystal structure.

3.3 Agonist Binding Sites

The position of serotonin is almost fully determined by the three point interaction with Ser199, Thr200 and Asp116 (see Figure 3). The complex was then refined with limited energy minimization and short, low–temperature molecular dynamics simulations to relieve any residual bad steric contacts. Harmonic restraints were applied to all backbone atoms in the transmembrane domain to minimize potential distortion of helices during *in vacuo* structural refinement. The free space

available between the helices III, VI and VII can accommodate large substituents at the phenolic oxygen or the basic nitrogen. The 2nd position of the indole ring is close to the backbone of helix V. All these observation associated with the present model are consistent with the literature reported SAR data stating that these groups can be substituted without loss of activity. The 5–OH group and Indole–NH of serotonin was found to form hydrogen bond with Thr200 and Ser199. The negatively charged Asp116 forms an ionic hydrogen bond with the protonated nitrogen of serotonin. The predicted pKi value found using scoring svl functionality available in MOE was used to compare the pKi value with experimentally determined value reported in literature [12]. The predicted pKi value for serotonin was found to be 7.9 which compares well with biologically or experimentally determine pKi value of 8.4.

4 CONCLUSIONS

The discovery of 3D structure of Rhodopsin, a member of GPCR super family, is part of allure for GPCR research for understanding ligand–GPCR interaction and characterization of GPCRs at atomic level. The usefulness of these homology models greatly depends on their ability to explain and predict the binding of their endogenous ligands and to efficiently aid the discovery of new synthetic compounds. We have developed the homology model of human $5–HT_{1A}R$ based on crystal structure of bovine rhodopsin. The model was refined using simulated annealing coupled with stringent energy minimization protocols to remove steric clashes as well as to preclude side chain contacts. The complete model was assessed for geometry and energetics. The *phi* and *psi* dihedrals of all residues in the $5–HT_{1A}$ receptor are within the allowed region of the Ramachandran plot. The three dimensional models of endogenous agonist serotonin complex with $5–HT_{1A}R$ are also presented which are consistent with the reported data. We have presented comprehensive model of human $5–HT_{1A}$ receptor which can be used to rationalize distinction between its agonists and antagonists and to know the qualitative structure activity relationship for its ligand. This model can serve as platform for designing of selective ligands for $5–HT_{1A}R$. As more data are available to better characterize the details of $5–HT_{1A}$ receptor complexes with ligands, we will be able to resolve these issues more definitively.

Supplementary Material

Coordinates for model will be available on request.

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