



A homology-based model of the human 5-HT_{2A} receptor derived from an *in silico* activated G-protein coupled receptor

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Key words: 5-HT_{2A} receptor, G-protein coupled receptor, Ligand binding, Molecular dynamics, Receptor activation.

Summary

A homology-based model of the 5-HT_{2A} receptor was produced utilizing an activated form of the bovine rhodopsin (Rh) crystal structure [1,2]. *In silico* activation of the Rh structure was accomplished by isomerization of the 11-*cis*-retinal (1) chromophore, followed by constrained molecular dynamics to relax the resultant high energy structure. The activated form of Rh was then used as a structural template for development of a human 5-HT_{2A} receptor model. Both the 5-HT_{2A} receptor and Rh are members of the G-protein coupled receptor (GPCR) super-family. The resulting homology model of the receptor was then used for docking studies of compounds representing a cross-section of structural classes that activate the 5-HT_{2A} receptor, including ergolines, tryptamines, and amphetamines. The ligand/receptor complexes that ensued were refined and the final binding orientations were observed to be compatible with much of the data acquired through both diversified ligand design and site directed mutagenesis.

Abbreviations: rhodopsin (Rh), G-protein coupled receptor (GPCR), serotonin (5-HT), transmembrane (TM), root mean square distance (RMSD), beta-carbon (C β).

Introduction

The super-family of G-protein coupled receptors (GPCRs) encompasses a large portion of present drug targets [3]. These membrane bound receptors receive extracellular signals in the form of photons, peptides, proteins, lipids, eicosanoids, purines, nucleotides, excitatory amino acids, ions, or small molecules such as serotonin (5-HT) and then, following a conformational change of the protein, propagate the signal across the membrane to the intracellular space. Rhodopsin (Rh), a protein integral to vision, contains a retinal chromophore, derived from vitamin A, covalently linked to K₂₉₆ in transmembrane segment 7 (TM7). The chromophore requires interactions with proximal Rh

residues to maintain a bound state. In the inactive, or dark state of this receptor, the chromophore exists as the inverse agonist 11-*cis*-retinal (1) [4]. Upon absorption of a single photon, isomerization of the 11-12 olefinic bond (Figure 1) converts the chromophore into the agonist all-*trans*-retinal (2). Subsequent protein relaxation to accommodate the isomerization-induced potential energy increase renders the protein transiently active. It is the active form of the Rh structure that couples to transducin, an intracellular G-protein, thereby facilitating nucleotide exchange, thus triggering a signaling cascade that ultimately results in vision [4,5]. The isomerized retinal (2) maintains fewer stabilizing interactions in this isomeric form and is subsequently hydrolyzed from K₂₉₆ and dissociates from the protein.

The recent publication of the bovine Rh crystal structure, the first prototypical GPCR structure to be solved at high resolution, has made possible

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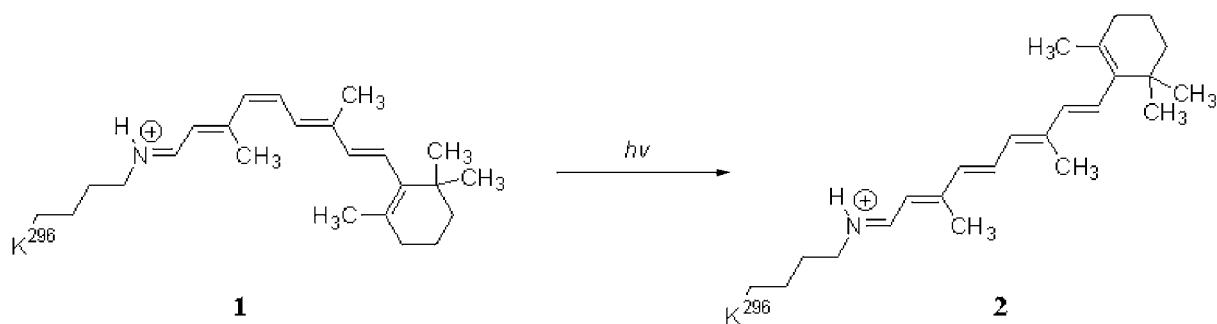


Figure 1. Light induced isomerization of 11-*cis*-retinal (**1**) to all-*trans*-retinal (**2**).

homology-based model production of other GPCRs. While the information obtained from study of this crystal structure is invaluable for understanding GPCR structure and function, the Rh that was crystallized was the *inactive* form – with the inverse agonist 11-*cis*-retinal (**1**) isomer bound [2,4]. Our research objective is to understand the binding orientations of compounds that behave as human 5-HT_{2A} receptor agonists – compounds that alter the equilibrium between the *inactive* and active receptor state in favor of the active form. Development of a homology model of our target receptor based solely on the *inactive* Rh crystal structure as the template would result in a model of the *inactive* state of the receptor. A more appropriate structure for ligand docking studies would be one that was founded on an *active* state GPCR template. At the present time, no such structure is available. Therefore, *in silico* generation of an active state GPCR template based on the Rh crystal structure was accomplished using weighted masses molecular dynamics – a molecular dynamics approach that favors rigid body motion of macromolecular bodies over high frequency atomic motion, thereby synthetically activating the Rh structure in a time efficient manner [6].

Results and discussion

The mechanism by which Rh is transformed from an inactive state to an active state has been the subject of much study by direct experiments of both the wild type protein and site directed mutant proteins using various forms of spectroscopy [7-13]. Collectively, these studies suggest that TM3 and TM6 are subject to some form of rearrangement, possibly rigid body motion, relative to one another following the *cis-trans* photochemical isomerization of the bound chromophore. Further evidence for the role of TM3 and TM6 in Rh

activation comes from the structural interaction of the cytoplasmic end of TM3 with that of TM6. The crystal structure [2] demonstrates the ionic interaction of R₁₃₅ with E₁₃₄, part of the E/DRY motif conserved in TM3 of all GPCRs, as well as with a conserved E₂₄₇ of TM6. These residues, E/DRY in TM3 and E₂₄₇ in TM6, are part of the conserved set of amino acids that define the fingerprint of the GPCR super-family. Rigid body motion of these two TM segments has been hypothesized to result in exposure and protonation of E₁₃₄ upon 11-*cis*- to all-*trans*-retinal isomerization, thus allowing direct coupling of Rh to an intracellular G-protein, in this case, transducin [4,14]. Further investigation of the crystal structure demonstrates an interaction between E₁₂₂ in TM3 and H₂₁₁ in TM5. We believe this interaction to also be involved with the receptor activation mechanism because these two amino acids correspond to sites known to be important for 5-HT_{2A} receptor agonist binding. More specifically, the ionone ring of 11-*cis*-retinal (**1**), based upon our *in silico* receptor activation, appears to disrupt the inter-helical hydrogen bond network in which these latter two residues participate.

To create a model of the activated Rh protein, *in silico* isomerization of the bound chromophore, followed by relaxation of the resulting high energy protein structure was accomplished using a weighted masses molecular dynamics protocol [6]. The process of relaxation was quantified by inter-atomic distance measurement of residues corresponding to the double cysteine mutants in a study by Farrens, et al. [7]. In brief, the Farrens experiments consisted of a series of double cysteine mutants of Rh that were spin labeled and examined in either the absence or presence of light to observe inter-spin distance changes before and after the activation process. Their experiments indicated that after light activation, TM6 tilted and rotated relative to TM3. In our analysis of *in silico*

activation of the native protein, we noted that the majority of protein rigid body motion in the model system did indeed occur in TM3 and TM6 relative to one another. Inter-atomic measurements of key residues, shown in Table 1, indicate that motion in the isomerized model system correlates with the data observed in the Farrens, et al. study. Further, the rigid body motion of these helices has caused a disruption of inter-helical hydrogen bonds between E₁₃₄ and R₁₃₅ of TM3 and E₂₄₇ of TM6 as observed when the activated Rh structure is compared to the initial, inactive structure. These residues have migrated apart during the activation process – a further indication that this model represents an active form of Rh. We feel that, as a first approximation, the end-point of *in silico* activation does explain the observed data from Rh activation studies, however, no assumption is, or should be, made about the activation pathway or intermediate stages of the model system and a relationship to the process that occurs naturally. The specific path of molecular motion during Rh activation and intermediates thereof is currently being investigated by other researchers.

Control experiments were also performed to be certain that the observed rigid body motion of TM3 and TM6 had its origin in the 11-*cis*- to all-*trans*-retinal isomerization and was not simply an artifact of computer simulation. To investigate the effects of molecular dynamics alone on the system, the original crystal structure, less chromophore isomerization, was subjected to identical manipulation and dynamics. Results of this experiment indicated that macromolecular motion was induced by chromophore isomerization because only small fluctuations were observed in this control experiment. To investigate the reversibility of the isomerization-induced macromolecular motion, the final activated Rh structure was modified by reversion of the all-*trans*-retinal (**2**) back to 11-*cis*-retinal (**1**). This structure was then subjected to a similar weighted masses molecular dynamics regime and the results indicated that TM3 and TM6 did migrate back toward their initial placements in the crystal structure. Although the structure did not converge completely with the initial crystal structure (1.22 Å RMSD), it did more closely resemble the product of the previous control experiment (0.83 Å RMSD), the end-point of the protein containing **1** that was subjected to parallel manipulation and dynamics. These experiments demonstrate that the rigid body motion observed during *in silico* Rh activation was not simply a software artifact, but was instead induced by isomerization of the chromophore.

The synthetically activated Rh structure was then utilized as the structural template for homology modeling of the human 5-HT_{2A} receptor. The resultant receptor was used to generate initial placements for molecules representative of several compound classes known to activate the 5-HT_{2A} receptor. These receptor/ligand complexes were then refined and the resulting binding orientations and interactions were examined.

Ergoline binding to the human 5-HT_{2A} receptor (Figure 3)

Our model indicated that LSD (**3**), a partial agonist at the 5-HT_{2A} receptor, bound to the receptor in an orientation that presented the protonated amine of the ligand to D_{3.32(155)} (monoamine receptor numbering scheme described by Ballesteros and Weinstein [15]), a residue conserved across the family of monoamine GPCRs, forming a strong ionic bond [16]. We also observed that the N(1)-hydrogen of the ligand formed a hydrogen bond with the hydroxyl and backbone carbonyl of S_{5.46(242)}. This N(1)-hydrogen/S_{5.46(242)} hydroxyl interaction for ergolines and tryptamines has been suggested by others based on mutational evidence, species-specific receptor binding data and data derived from ligand N(1)-alkylation [17-20]. Further, we observed that the amide carbonyl oxygen of LSD accepts a hydrogen bond from the amide of N_{6.55(343)}, a residue at the extracellular end of TM6. To the best of our knowledge, this interaction has not previously been identified. We hypothesize that the interaction of the ergoline amide carbonyl oxygen substituent with N_{6.55(343)} may be directly involved in 5-HT_{2A} receptor *antagonism*. This strong hydrogen bond with the ergoline may dampen the full range of motion of TM6, thereby preventing normal activation of the receptor. That the ergoline class of drugs are invariably partial agonists or antagonists and *not* full agonists at the 5-HT_{2A} receptor may be explained by this hypothesis.

Tryptamine binding to the human 5-HT_{2A} receptor (Figure 4)

Investigation of the binding orientation of serotonin (5-HT), the endogenous ligand for this receptor, resulted in ambiguous results likely due to conformational flexibility of the ligand, which led to multiple potential docking orientations. Rigid analogues of known drugs have proven valuable for these modeling studies by locking ligand structures

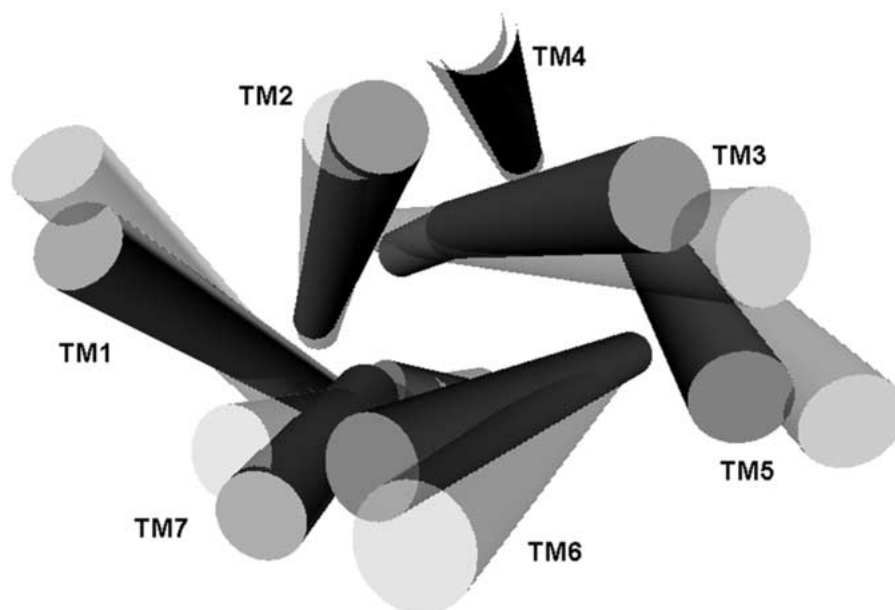


Figure 2. Cartoon depiction of Rh activation. View is from intracellular face of helical bundle. Crystal structure shown as transparent tubes, *in silico* activated Rh as dark grey.

into a rigid conformation – ideally one that is biologically active. Compound rigidity serves molecular modeling by limiting the number of rotameric forms or conformations and therefore reducing ambiguity during the docking phase of studies. To limit the uncertainty of the tryptamine orientation in the binding site, we therefore employed the rigid analogue (*R*)-(+)-3-(*N*-methylpyrrolidin-2-ylmethyl)-5-methoxyindole (**4**). This compound was chosen because it has been fully characterized *in vitro* and it is known which stereochemical isomer is more active.[21] The results of docking **4** were unambiguous and produced only one favorable binding orientation. That interaction is shown in Figure 4 where the protonated amine interacts with $D_{3.32(155)}$ in the same manner as LSD (**3**). The 5-methoxy group of **4** was found to form a hydrogen bond with the hydroxyl groups of both $S_{3.36(159)}$ and $T_{3.37(160)}$ and the indole N(1)-hydrogen was found to interact with the hydroxyl of $S_{5.43(239)}$. This finding indicates that the binding orientation of the indole nucleus of **4** differs from the indole nucleus embedded in the ergoline structure. That 4-hydroxytryptamines (i.e. psilocin) are also active indicates that there must be flexibility in the requirements for hydrogen bond directionality, or donor/acceptor role reversals that would account for the similarity of both the 4-hydroxy and 5-methoxytryptamine pharmacology.

The role of $S_{3.36(159)}$ was of particular interest in this docking experiment. Almaula et al. [22] have shown that mutation of this residue to alanine or cysteine has no effect on binding of LSD and only a small effect on binding of bufotenine (5-hydroxy-*N,N*-dimethyltryptamine), but leads to a marked reduction in affinity for 5-HT. Those authors therefore suggested that $S_{3.36(159)}$ may form an additional hydrogen bond to the protonated amino group of 5-HT, which cannot occur due to steric effects if the amino group is substituted with alkyl groups, such as in bufotenine. By contrast, our results provide an attractive alternate explanation, where the 5-oxygen function of the indole accepts a hydrogen bond from $S_{3.36(159)}$.

Other studies of site-directed mutants and species-specific binding data, however, do not fit a single binding orientation model for the tryptamine drug class. Instead, it appears that there may be at least two divergent orientations for tryptamine ligands to bind and activate the 5-HT_{2A} receptor. Studies have shown that N(1)-alkyl substituted tryptamines exhibit species selectivity parallel to N(1)-alkyl substituted ergolines.[17-19] These findings suggest a direct interaction of the N(1)-substituent of both compound classes with $S_{5.46(242)}$ – the lone amino acid difference in the putative binding domain of the human and rat 5-HT_{2A} homologues – an alanine in the 5-HT_{2A} rat homologue. The studies indicated that N(1)-

Table 1. Weighted masses molecular dynamics results. Inter-atomic measured and inter-spin[7] measured distances.

Measured amino acid pair	Change in inter-atomic distance from initial structure; C β of first residue to C β of second, Å ^a					Change in inter-spin distance from distance from initial structure; Å ^b
	Dynamics simulation time					
	0 ps	250 ps	500 ps	750 ps	1000 ps	
V ₁₃₉ -K ₂₄₈	0.0	2.50	1.54	1.71	2.44	+11 (1)
V ₁₃₉ -E ₂₄₉	0.0	0.65	0.39	0.46	1.06	0 (2.5)
V ₁₃₉ -V ₂₅₀	0.0	2.40	0.71	-0.47	0.06	-4.5 (2.5)
V ₁₃₉ -T ₂₅₁	0.0	0.37	-0.07	1.11	1.48	+11 (1)
V ₁₃₉ -R ₂₅₂	0.0	-2.49	-0.84	1.35	1.56	+6.5 (2.5)

^aChange in RMS distance compared to t = 0 ps structure from the C β of residue 139 to C β of the residue listed.

^bEstimated values from Farrens et al. [7] double cysteine mutant inter-spin labeling studies. Spin label movements found in the Farrens, et al., study are of greater magnitude than the dynamics simulation possibly because the inter-spin measured distances occur further from the protein backbone than the current measure of inter-atomic C β distances.

alkyl substitution of ergolines and tryptamines led to enhanced affinity for the rat receptor compared to N(1)-unsubstituted compounds, supporting a binding orientation that is similar to that of LSD (**3**) shown in Figure 3. Further support for an ergoline-like binding orientation for some tryptamines comes from a study by Shih, et al. [23] in which it was observed that psilocin binds with 15-fold higher affinity to the human 5-HT_{2A} receptor compared to the rat 5-HT_{2A} receptor. This species-dependent difference in binding affinity for psilocin indicates that some part of the ligand must interact, either directly or indirectly, with S_{5.46(242)}. This same study, however, reported that bufotenine binds with comparable affinity to both species homologues, suggesting a binding orientation for this compound in which the importance of S_{5.46(242)} is diminished.

Indeed, using our receptor model we have found that psilocin may adopt a binding orientation that resembles either the embedded tryptamine moiety within the ergoline compound class shown in Figure 3 or an orientation in which the 4-hydroxyl of psilocin interacts directly with S_{5.46(242)} similar to the tryptamine shown in Figure 4. Thus, it seems plausible that tryptamines can bind in either of the two modes illustrated in Figures 3 and 4, and that minor structural variations in the ligand may lead to changes in the docking orientation. The observation of multiple, divergent binding modes during the docking phase of the native ligand, 5-HT, also supports the dual binding mode hypothesis. Questions that have surfaced

surrounding tryptamine binding orientations will be investigated in future studies.

Phenethylamine binding to the human 5-HT_{2A} receptor (Figure 5)

Following our success to limit the number of possible binding orientations using rigid analogues, the conformationally restricted amphetamine analogue (1*R*,2*S*)-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine (**5**), [24] known to be the most potent of the possible stereoisomers, was docked into our receptor model. The resulting binding orientation of **5** indicated that the protonated amine of the ligand forms an ionic bond with D_{3.32(155)} as was observed in ergoline and tryptamine binding. Further, the 2-methoxy group of **5** was found to interact with the hydroxyl groups of S_{3.36(159)} and T_{3.37(160)}, one helical turn down TM3 from D_{3.32(155)} and the 5-methoxy group was observed to interact with the hydroxyl of S_{5.43(239)} in TM5. The *para*-substituent, in this case a methyl group, known to be important for agonist efficacy, [25,26] was found projecting into a lipophilic pore lined by I_{4.56(206)}, L_{4.65(215)} and G_{5.42(238)}. The interactions of the *para*-substituent of hallucinogenic amphetamines with the receptor have been speculated upon; however, it can now be surmised from this model of binding that this interaction is both of a steric, as well as a lipophilic nature and that it positively effects binding affinity of this compound class.

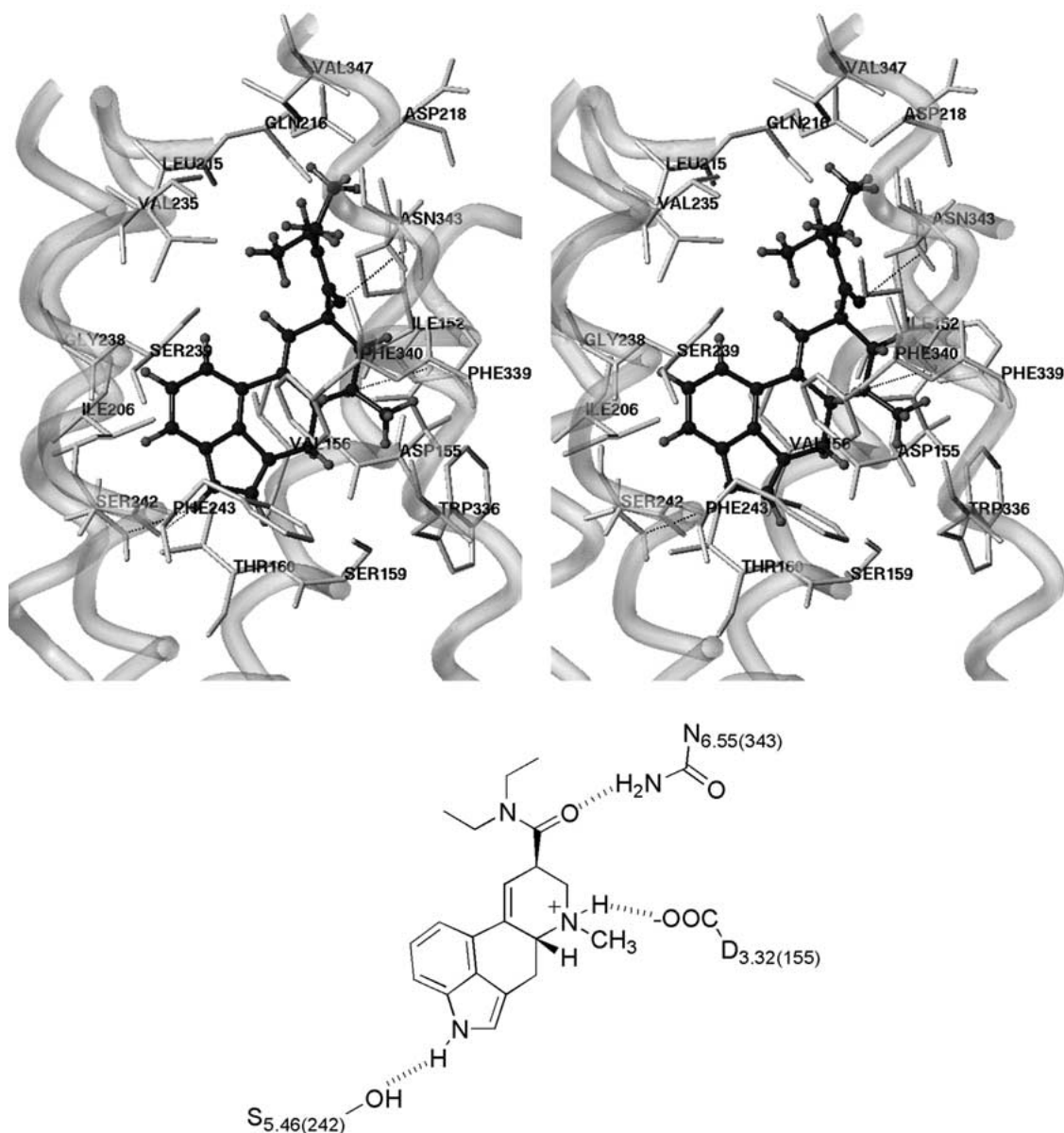


Figure 3. Top: Stereo view (cross-eyed) of LSD (**3**) docked into the 5-HT_{2A} receptor. Hydrogen bonds are shown as dashed lines. Bottom: Schematic representation of LSD in the 5-HT_{2A} receptor model.

The basis for the stereospecific biological activity of the *1R,2S*-enantiomer **5**, when compared with its *1S,2R*-antipode, is not immediately evident, but possibly resides in the inability of the less active enantiomer to adopt a similar binding orientation, due principally to the non-bonded interaction between the CH₂ of the cyclopropane ring and H(6) of the aromatic ring. In the structurally simpler hallucinogenic amphetamines, a non-bonded interaction between the alpha-methyl group and H(6) in the less active *S*-

enantiomer would similarly disfavor a conformation that was superimposable on the ligand in Figure 5, offering a possible explanation for the lower affinity of the *S*-isomer compared to its *R*-antipode in the hallucinogenic amphetamines [27].

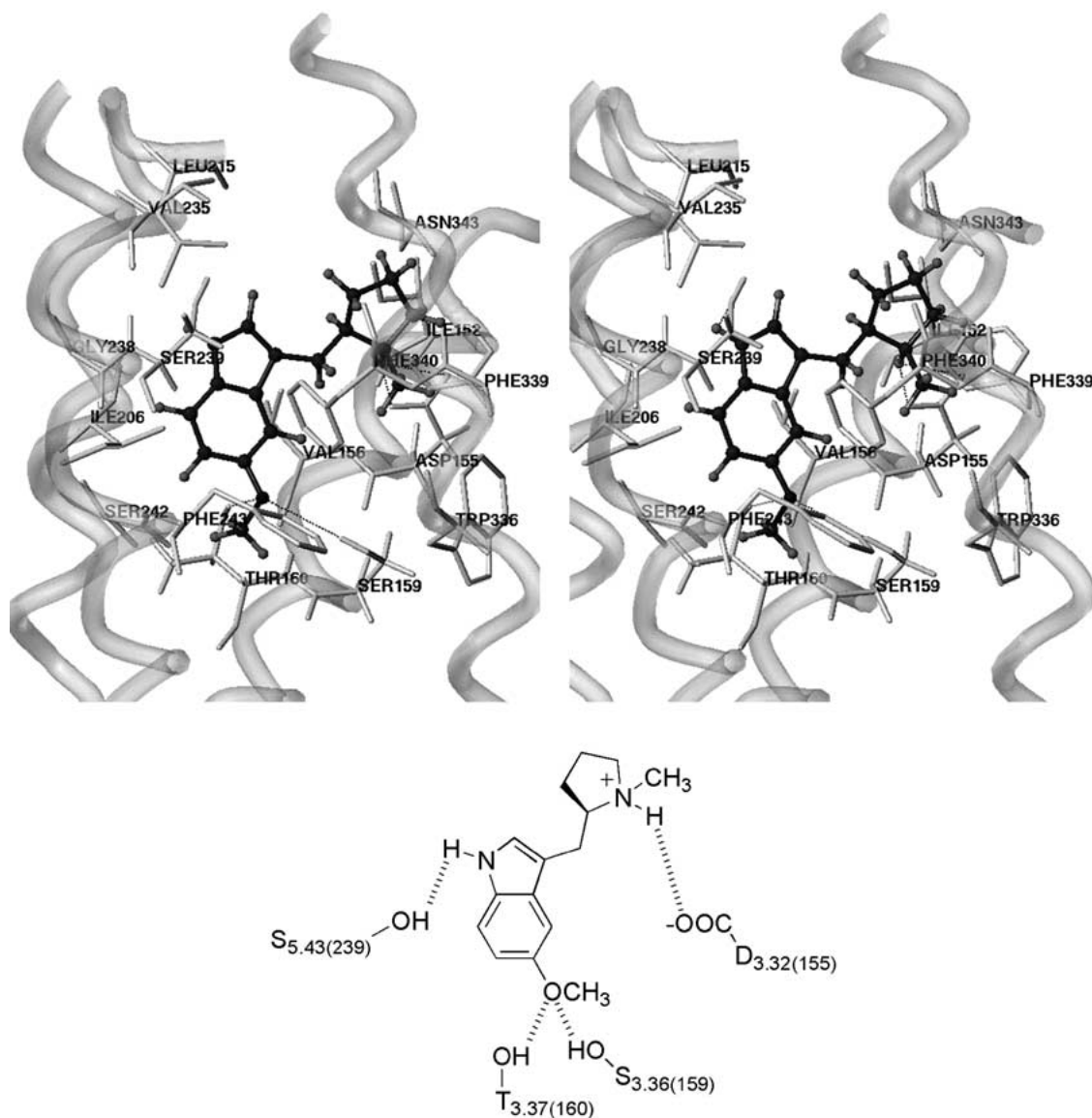


Figure 4. Top: Stereo view (cross-eyed) of (*R*)-(+)-3-(*N*-methylpyrrolidin-2-ylmethyl)-5-methoxyindole (**4**) docked into the 5-HT_{2A} receptor. Hydrogen bonds are shown as dashed lines. Bottom: Schematic representation of **4** in the 5-HT_{2A} receptor model.

Conclusions

Our study of Rh activation has shown that a weighted masses molecular dynamics simulation of GPCR activation is consistent with the body of data gathered from in vitro GPCR activation studies. The advantage of this technique is that total CPU time is significantly decreased because less time is spent calculating high frequency atomic movement (bending, stretching, etc.) and more CPU time is focused on rigid body motion. However, disadvantages of this method

include a possible loss of fidelity in the relationship between the computer-generated model and the real protein system that occurs due to the reduction in the degrees of freedom during dynamic simulations. Support for rigid body motion during Rh activation is rich and our model of this process, while synthetic and purely computational, does explain the empirical data from the end-point of activation. Whereas the fine detail of atomic coordinates is most certainly not accurate in the activated 5-HT_{2A} receptor model, the level of uncertainty should be considered not greater

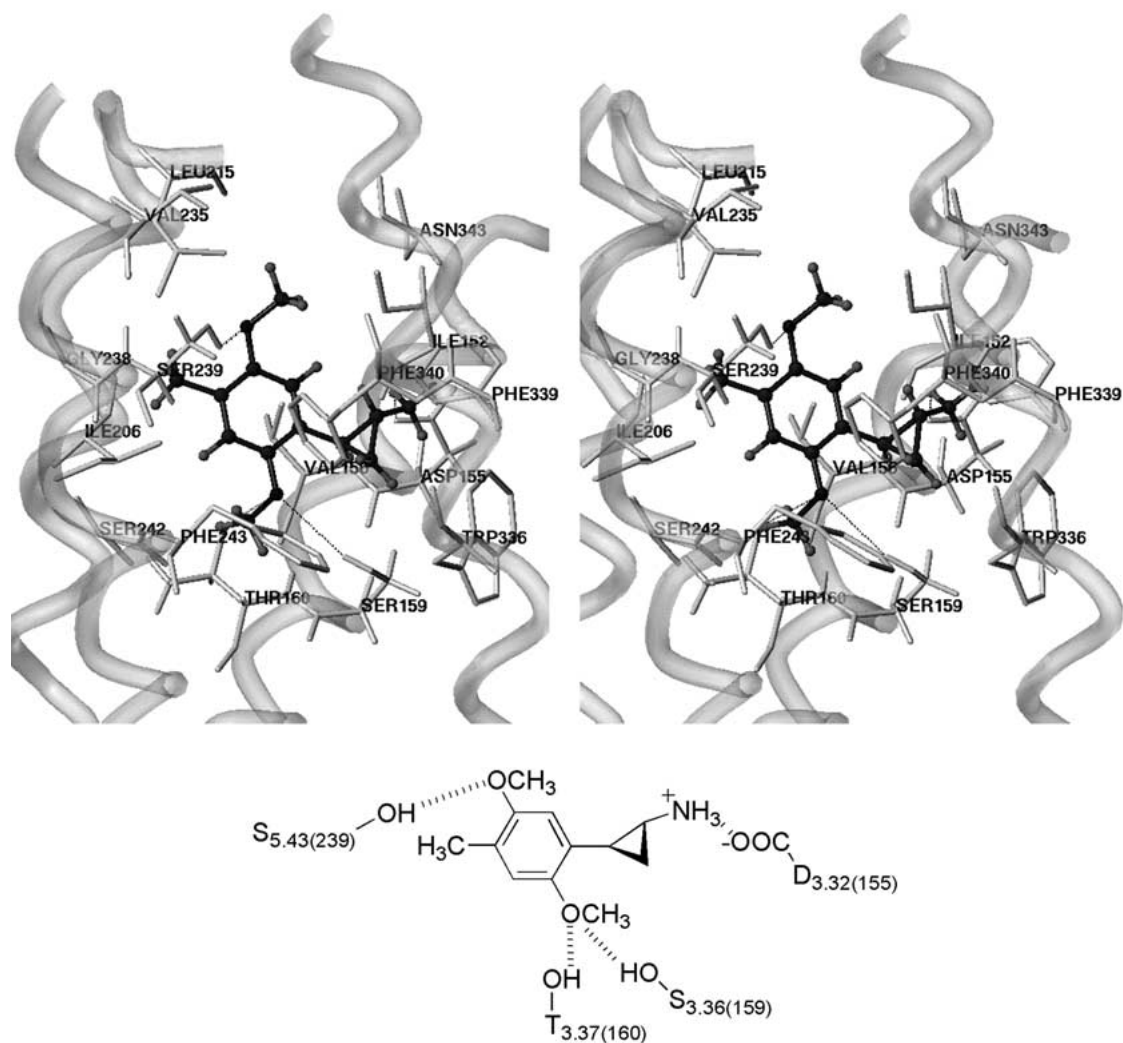


Figure 5. Top: Stereo view (cross-eyed) of (1*R*,2*S*)-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine (**5**) docked into the 5-HT_{2A} receptor. Hydrogen bonds are shown as dashed lines. Bottom: Schematic representation of **5** in the 5-HT_{2A} receptor model.

than any other homology-based GPCR model that is currently in use. In the future, this model will be further refined in an iterative approach, incorporating novel implications that are generated through both site-directed mutagenesis and rigid analogue ligand design. That our 5-HT_{2A} receptor model is able to explain empirical data from diverse ligand pharmacology indicates that, while the technique used to arrive at the model may be somewhat unorthodox, the result is a viable tool for future ligand design and receptor study.

Methods: weighted masses molecular dynamics

In brief, the crystal structure of Rh (PDB code 1F88) [2] was modified by deletion of metal atoms, water, etc. to leave one semi-complete molecule of Rh and bound 11-*cis*-retinal (**1**) remaining. The *N*- and *C*-termini, intra- and extra-cellular loops were deleted (with the exception of the extracellular loop between TM4 and TM5 because of the TM interactions) using Quanta. Specifically, W₃₅-K₆₆, T₇₀-H₁₀₀, T₁₀₈-C₁₄₀, E₁₅₀-V₂₂₇, A₂₄₆-H₂₇₈, and I₂₈₆-C₃₂₂ remained after the original PDB file was truncated. The coordinates of the helices were then used by a parallelized version of CHARMM (version c28a3) [28] on an IBM SP system and neutral caps were applied to the end groups.

N-termini were capped with acetyl groups and *C*-termini were capped with *N*-methylamino groups. Any hydrogens that were missing were added using the HBUILD facility of CHARMM and the model was then saved (referred to as *cis*-Rh). The covalently linked chromophore was then isomerized from 11-*cis*-retinal (**1**) to all-*trans*-retinal (**2**) by modification of torsion angles to situate the ionone ring of the chromophore in the vicinity of T₁₁₈, C₁₆₇, A₁₆₈, P₁₇₁, S₁₇₆, Y₁₇₈, I₁₈₉ and F₂₀₃ and the file was then saved (*trans*-Rh). From this point forward, both *cis*-Rh and *trans*-Rh were treated identically in all operations. Bond lengths, angles and improper dihedral angles were artificially weighted using constraints on the internal coordinate system of 500, 5000, and 5000, respectively.[6] A distance dielectric constant, timestep of 0.001 and non-bonded update every 5 steps was used during all molecular dynamics. To relax the initial structures, 100 steps of steepest descent minimization was performed followed by dynamics using the Verlet leap frog algorithm for 10 ps of heating to 300 K, 10 ps of equilibrium, and 1200 ps of production time. The final 100 ps of dynamics were averaged and the resulting structure was minimized using the Adopted Basis Newton-Raphson minimization protocol for 1000 steps. The final structure of *trans*-Rh was then used as the structural template for homology modeling.

Homology modeling

A sequence alignment of bovine Rh and the human 5-HT_{2A} receptor was generated using ClustalW [29] with the PAM250 scoring matrix, a gap penalty of -15 and an extension penalty of -2. Minor adjustments were performed manually using GeneMine [30] to align known conserved motifs and minimize gaps in putative TM regions to achieve the alignment. A homology model was then generated using Modeler 4.0 [31] on an SGI Octane machine. The output structure was relaxed with 50 steps of steepest descent minimization using CHARMM and the resulting structure was then used as input for docking.

Docking

All hydrogens and lone pairs of electrons were added to the receptor and Kollman All-Atom charges were calculated using the Biopolymer package of Tripos

Sybyl 6.6 [32]. Ligand molecules were generated using Spartan, [33] the basic amines protonated, a formal +1 charge was applied, and semi-empirical geometry optimization (HF/6-31G* basis set) was utilized to generate initial ligand conformations. The ligands were then imported into Sybyl and Gasteiger charges were computed and files saved. Residues within a 12 Å radius sphere of D_{3.32(155)} were used for generation of a molecular surface [34] and spheres [35] and then property grids were calculated using the appropriate bundled software. DOCK [35] was then used to generate preliminary placements for the ligand molecules.

Ligand orientations were chosen based on clustered matches from DOCK and were then optimized using a local minimization with the Tripos force field of Sybyl. Almost invariably, a sample of top-ranking clusters converged on the final low energy structures shown.

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References

1. Chambers, J.J., Nichols, D.E., Great Lakes/Central Regional American Chemical Society Meeting. (2001).
2. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C.A., Motoshima, H., Fox, B.A., Trong, I.L., Teller, D.C., Okada, T., Stenkamp, R.E., Yamamoto, M., and Miyano, M., *Science*, 289 (2000) 739.
3. Gudermann, T., Nurnberg, B., and Schultz, G., *J. Mol. Med.*, 73 (1995) 51.
4. Okada, T., Ernst, O.P., Palczewski, K., and Hofmann, K.P., *Trends Biochem. Sci.*, 26 (2001) 318.
5. Sakmar, T.P., *Prog. Nucleic Acid Res. Mol. Biol.*, 59 (1998) 1.
6. Elamrani, S., Berry, M.B., Phillips, G.N., Jr., and McCammon, J.A., *Proteins*, 25 (1996) 79.
7. Farrens, D.L., Altenbach, C., Yang, K., Hubbell, W.L., and Khorana, H.G., *Science*, 274 (1996) 768.
8. Lin, S.W., Sakmar, T.P., *Biochemistry*, 35 (1996) 11149.
9. Garcia-Quintana, D., Francesch, A., Garriga, P., de Lera, A.R., Padros, E., and Manyosa, J., *Biophys. J.*, 69 (1995) 1077.
10. Salamon, Z., Wang, Y., Brown, M.F., Macleod, H.A., and Tollin, G., *Biochemistry*, 33 (1994) 13706.

11. Farahbakhsh, Z.T., Hideg, K., and Hubbell, W.L., *Science*, 262 (1993) 1416.
12. Resek, J.F., Farahbakhsh, Z.T., Hubbell, W.L., and Khorana, H.G., *Biochemistry*, 32 (1993) 12025.
13. Rothschild, K.J., Cantore, W.A., and Marrero, H., *Science*, 219 (1983) 1333.
14. Cohen, G.B., Yang, T., Robinson, P.R., and Oprian, D.D., *Biochemistry*, 32 (1993) 6111.
15. Ballesteros, J.A., Weinstein, H., *Methods Neurosci.*, 25 (1995) 366.
16. Ho, B.Y., Karschin, A., Branchek, T., Davidson, N., and Lester, H.A., *FEBS Lett.*, 312 (1992) 259.
17. Nelson, D.L., Lucaites, V.L., Audia, J.E., Nissen, J.S., and Wainscott, D.B., *J. Pharmacol. Exp. Ther.*, 265 (1993) 1272.
18. Johnson, M.P., Audia, J.E., Nissen, J.S., and Nelson, D.L., *Eur. J. Pharmacol.*, 239 (1993) 111.
19. Johnson, M.P., Loncharich, R.J., Baez, M., and Nelson, D.L., *Mol. Pharmacol.*, 45 (1994) 277.
20. Almaula, N., Ebersole, B.J., Ballesteros, J.A., Weinstein, H., and Sealfon, S.C., *Mol. Pharmacol.*, 50 (1996) 34.
21. Gerasimov, M., Marona-Lewicka, D., Kurrasch-Orbaugh, D.M., Qandil, A.M., and Nichols, D.E., *J. Med. Chem.*, 42 (1999) 4257.
22. Almaula, N., Ebersole, B.J., Zhang, D., Weinstein, H., and Sealfon, S.C., *J. Biol. Chem.*, 271 (1996) 14672.
23. Shih, J.C., Chen, K., and Gallaher, T.K., *NIDA Res. Monogr.*, 146 (1994) 284.
24. Nichols, D.E., Woodard, R., Hathaway, B.A., Lowy, M.T., and Yom, K.W., *J. Med. Chem.*, 22 (1979) 458.
25. Domelsmith, L.N., Eaton, T.A., Houk, K.N., Anderson, G.M., III, Glennon, R.A., Shulgin, A.T., Castagnoli, N., Jr., and Kollman, P.A., *J. Med. Chem.*, 24 (1981) 1414.
26. Nichols, D.E., Frescas, S., Marona-Lewicka, D., Huang, X., Roth, B.L., Gudelsky, G.A., and Nash, J.F., *J. Med. Chem.*, 37 (1994) 4346.
27. Chambers, J.J., Kurrasch-Orbaugh, D.M., Parker, M.A., and Nichols, D.E., *J. Med. Chem.*, 44 (2001) 1003.
28. Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S., and Karplus, M., *J. Comp. Chem.*, 4 (1983) 187.
29. Higgins, D.G., Thompson, J.D., and Gibson, T.J., *Methods Enzymol.*, 266 (1996) 383.
30. Irizarry, K., Lee, C., *IBM Systems Journal*, 40 (2001) 592.
31. Sali, A., Blundell, T.L., *J. Mol. Biol.*, 234 (1993) 779.
32. Sybyl 6.6 Tripos Inc., 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.
33. Spartan 5.0, Wavefunction, Inc., 18401 Von Karman Ave., Ste. 370, Irvine, California, 92612, USA.
34. Richards, F.M., *Annu. Rev. Biophys. Bioeng.*, 6 (1977) 151.
35. Kuntz, I.D., Blaney, J.M., Oatley, S.J., Langridge, R., and Ferrin, T.E., *J. Mol. Biol.*, 161 (1982) 269.