

# HOW MUCH BOVINE RHODOPSIN CRYSTAL STRUCTURE IS USEFUL FOR MODELING HUMAN GPCRS?

## $\beta$ 2-Adrenergic Receptor as a Test Case

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**Abstract:** Availability of realistic models for human G-Protein Coupled Receptors (hGPCRs) will aid structure-based drug design (SBDD), thus shortening the time period needed for drug development and minimizing cross-reactivity of drugs with other hGPCRs. Many researchers have constructed models for hGPCRs with homology modeling techniques based on the X-ray structure of bovine rhodopsin and recently to  $\beta$ 2-adrenergic receptor which are the only two GPCRs that have high resolution crystal structures. In this study, we evaluate the usefulness of the bovine rhodopsin crystal structures for modeling hGPCRs by analysis of large database of human G-protein coupled receptors that are members of family A (rhodopsin family). The recently released structure of  $\beta$ 2-adrenergic receptor was used as a test case for validation purposes of our findings. From pair-wise sequence alignment of each of the receptors in the database to bovine rhodopsin, we come to the conclusion that only for few hGPCRs, X-ray structure of rhodopsin could be used as a template for modeling the trans-membrane domains (TMDs). The detailed analysis of the whole database shows that in general, similarity to bovine rhodopsin is found more in the middle/endoplasmic part than in the exoplasmic part. The shift in the cytoplasmic end of TMD-6 that has been seen in the recently released crystal structure of  $\beta$ 2-adrenergic receptor could be understood well from our bioinformatics study. On the basis of our results from this research, we propose to regard specific parts from the endoplasmic domain of the rhodopsin helices as appropriate template for constructing models of other GPCRs, while most of the exoplasmic parts of GPCRs in this family require other techniques for their modeling, due to the low sequence similarity between the receptors and rhodopsin in that region.

## 1 INTRODUCTION

G-protein coupled receptors (GPCRs) are membrane embedded proteins that have a typical structural topology: seven transmembrane helices (7TMH) connected by intracellular and extracellular loops, with an extracellular N-terminal and an intracellular C-terminal (Gether, 2000). GPCRs derive their name from their ability to recruit and to regulate the activity of intracellular heterotrimeric G-proteins. Their main role is to transfer (transduce) a signal across the cell membrane. Such signals emerge from interactions of GPCRs with extracellular agents, which are highly diverse entities (e.g., ions, biogenic

amines, nucleosides, lipids, peptides, proteins, and even light). These agents are called “ligands” or “agonists” and ligand binding is followed by a change in the state of a GPCR to one with decreased affinity to G-proteins. Thus, the meeting between such agonists and GPCRs results in the conversion of “extracellular events” to intracellular responses (Nurnberg et al., 1995).

GPCRs are implicated in a very wide range of body functions and processes, including cardiovascular, nervous, endocrine, and immune systems. Also, their involvement in many disease conditions such as asthma, cardiovascular disease, central nervous system disorders, pain and others has been proven or suspected and they are considered to be the single largest group of drug targets. It has been estimated that GPCRs comprise ~45% of drug targets (Drews, 2000) and more than

Abbreviations: hGPCR (human G-Protein Coupled Receptor), TMD (Trans Membrane Domain).

50% of current drugs are directed to GPCRs (Nambi and Aiyar, 2003).

The number of known GPCRs is in the thousands, and many more are being discovered as a result of recent advances in genomics and proteomics. To be useful for drug design, structures of these drug targets should be elucidated, in order to employ them by methods of “Structure Based Drug Design” (SBDD). The structural aspects of GPCRs are however a source of constant debate in recent years (Bissantz et al., 2003).

Direct experimental study of GPCR structures is currently too complicated due to their native membrane environment. Until November 2007, only a single G-protein-coupled receptor, bovine rhodopsin, has been studied by high-resolution crystallography (Palczewski et al., 2000; Okada and Nakamichi, 2004).  $\beta$ 2-adrenergic receptor was the second GPCR to solve and its structure revealed fair similarity to the model obtained based on rhodopsin as a template (Rasmussen et al., 2007; Cherezov et al., 2007). The prospects for elucidating the structures of other GPCR are not very high, and await a major breakthrough. With no other structures at hand, rhodopsin and/or  $\beta$ 2-adrenergic receptor are considered to be the prototypes of the main family of GPCRs, of type A.

Due to the lack of experimental 3D-structures of other GPCRs, one could hope to gain from approximations based on molecular models. While “ab initio” modeling is not practical yet for any protein, “homology” or “comparative” modeling are quite established methods (Rayan et al., 2000) and are expected to be especially successful in the GPCR subfamily A, that is considered to have the general features of rhodopsin. Indeed, many GPCR structures have been modelled recently, based on the template of bovine rhodopsin/ $\beta$ 2-adrenergic receptors, by using its backbone coordinates and adding the appropriate side chains of each sequence (Eszter and Zsolt, 2008). Such “homology” or “comparative” modeling of GPCRs has been aided mainly by experimental information from point mutations and other experimental resources (<http://www.gpcr.org/7tm/>, 2006). The length of helices in the TMD remain similar in the modelled GPCRs to those of the template rhodopsin, and loops are not included in the template construction, except in those rare cases where loop lengths are similar to those of rhodopsin. But other approaches for constructing models of GPCRs suggest that GPCRs could differ in their structure from rhodopsin even though their general features are similar (Oliveira et al., 2002).

There are a few indications to justify such deviations from the rhodopsin structure, in constructing models for other GPCRs. A review by Baker and Sali (Schacham et al., 2001) has shown that a homology model for a protein at medium size at least and with sequence identity of less than 30% to the template crystal structure is unreliable. The averaged sequence identity of bovine rhodopsin to hGPCRs is less than 20%, meaning a homology-based approach is unlikely to provide a reliable structure to be used for making predictions. Others in the community think that this “rule” is correct in globular proteins and it is doubtful if this “rule” could be extended to membrane proteins. Also, this rule does not specify how identity should be distributed along a sequence. As much as the GPCRs super family is united by an overall structural topology and an ability to recruit and regulate the activity of G proteins, sequence identity between super family members, even in the more conserved transmembrane cores is too low. Significant sequence conservation is found, however, within several subfamilies of GPCRs. The subfamily of rhodopsin-like GPCRs is by far the largest (more than 85% of GPCRs) and is characterized by the presence of some 35 (out of ~190) highly conserved residue positions in the TMD, that may be involved in binding and/or in activation (Baler and Sali, 2001).

The conserved positions along the TM sequences constitute less than 20%. In contrast, the intracellular and extracellular loops and the N- and C- terminals of GPCRs vary in their lengths and therefore they pose an alignment problem. Palczewski, K. and his colleagues (Baldwin et al., 1997) via investigation of sequence analysis of the TMD of GPCRs demonstrated that “... the extracellular domain is the least conserved, while GPCRs display considerable conservation toward the endoplasmic side...” While this is an important observation, it lacks specific quantitative character. The conclusions of that study concentrated on individual residue conservation and on microenvironment conservation, and have thus detected the most conserved residues in the TMD. The authors concluded by suggesting that “It is reasonable to speculate that the overall fold of these receptors is highly conserved”. One of the implications of that study are thus, that it is reasonable to use the overall structure of rhodopsin to model the TMD of other GPCRs.

Therefore, the question remains open, to what extent is the structure of rhodopsin useful as a template for constructing models of other GPCRs? A quantitative measure of conservation in that family

of GPCRs could be helpful for deciding upon the exact parts of rhodopsin that could be used as templates for such comparative modeling, and those that should better be excluded. Should we use the full extent of TM helices, some of the helices, or stretches of sequences along helices? It was already noticed earlier that endoplasmic parts of the TMD are more conserved than exoplasmic parts (Baldwin et al., 1997). But what are the quantitative aspects of that conservation and how do they impinge on the most important decision, which is - how much of the rhodopsin structure may be used to model other GPCRs?

Between the two extreme approaches, to use the full crystallographic structure of the TMD of rhodopsin or to employ none of it, we propose an alternative. From our quantitative analysis, we assign the parts of the structure of rhodopsin that may be used as a template, and suggest to construct the rest by other methods that allow deviations from the crystal structure of the template.

## 2 METHODS

In this study, we hope to examine if there is a quantitative basis for modeling the TMDs of hGPCRs based on the X-ray structure of bovine rhodopsin. A database of 951 rhodopsin like hGPCRs were achieved from RAND Biotechnologies Ltd company. They have used in-house software called GPCR-scanner to screen the protein database of human species composed of 63125 proteins (Ensembl human database). Trans-membrane domains allocations and multiple sequence alignments were performed by applying Intelligent Learning Engine technology (Mirzadegan et al., 2003) from RAND Biotechnologies Ltd company. Some of the 951 receptors are identical in the TMDs and differ only in length of the protein sequence or in the rest of the structure – the two terminals and/or the extracellular and/or the intracellular loops. Higher similarity in sequences means better chance to have close three-dimensional structures and high confidence to obtain reliable model for the query protein.

### Sequence Alignments

Stretches of helical sequences for each of the GPCRs have been determined by TMDs-Scanner (Rayan and Raiyn, 2008), and were subsequently aligned with those of rhodopsin in the crystal structure. The length of each helix was imposed by

the rhodopsin template (TMDs) and is 194 residues in total. No insertions or deletions were considered.

The calculation of cumulative similarity of sequences to bovine rhodopsin or any other receptor  $C_{ij}$  is expressed by the average of conservation scores for single sequences positions:

$$C_{ij} = \frac{n_{ij}}{k} * 100\% \quad (1)$$

Where  $l$  is the number of amino acid positions in the sequence of a helix in the TMD and  $C_j$  is the score of the bovine rhodopsin/or of any other receptor amino acid at position  $j$  and can adopt a value of 1 if the residues are identical and a value of 0 if the residues are not identical. This score was calculated in order to evaluate the similarity for the seven TMDs separately as well as the lower endoplasmic part (G-protein binding) and the upper exoplasmic part (ligand binding) of the TMDs or over certain windows along the helices.

### Optimization of Windows' Positions

After a window width was determined, the first residue in the helix starts the window and the identity percentage to rhodopsin was evaluated for a certain hGPCR. The window was then shifted by one amino acid all along the helix as well as the other helices. The evaluation has been performed for all the hGPCRs in our database. The analysis was done in a few windows of widths between 7-14 residues. We have concentrated on the results of windows of 11 residues, which are close to about three such turns, respectively.

## 3 RESULTS AND DISCUSSION

### Conserved Residues in the TMDs

Looking on the frequencies of individual residues in particular positions along the TMDs (unpublished data) reveals that large number of positions are enriched with certain type of amino acid. Very low variability in specific position contents could mean importance in signal transduction pathway or in structural fold. Those residues are mostly found in the endoplasmic half of the TMDs or interacting with the membrane or phospholipids head groups in the edges of the membrane. The frequencies in some case are different from those reported by Tara Mirzadegan et al (Baldwin et al., 1997). For example, in helix I, Gly20, Leu23 and Val24 were

found 79.2% instead of 68%; 50.2% instead of 60% and 36.6% instead of 66%, respectively. Position 9 in helix II is occupied by Leu in 92.9% while the other amino acid types are mostly very hydrophobic like Ile, Met or Phe. This position could be important to determine the height of the helix by fixing this hydrophobic moiety in interaction with the membrane. Position 16 is occupied in 43.9% by Ser or Thr which properly interact with Trp from helix IV. Basic residues are dominant in the first two positions of helix IV and helix VII. Those residues and others could play important role in determining the orientation of the GPCR relative to the membrane.

### Entire Similarity in the TMDs of the hGPCRs

To check the entire similarity between all members in our database, the receptors were clustered by requiring that clusters should be dissimilar at least by  $x\%$  (with  $x$  ranging between 1-100). For example, assume that the threshold for clustering is  $x\%$ , then, if receptor A has sequence identity with receptor B less than the particular threshold, the two receptors are considered one cluster.

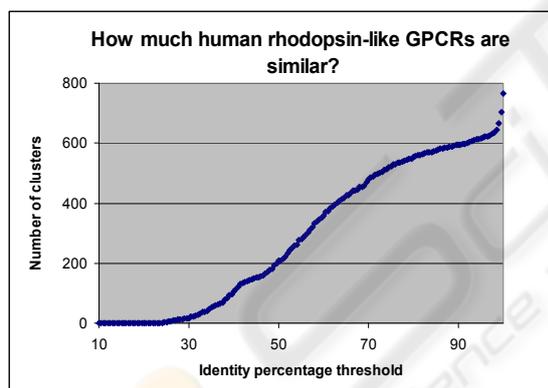


Figure 1: Each cluster should have at least one pair of receptors sharing percentage of identity within the TMD above a certain threshold. Number of clusters converge to one near 25% of identity. The horizontal axis shows the sequence identity threshold while the vertical one shows the number of clusters.

The process is continued until all pairs of receptors are evaluated. Each receptor in each cluster should share sequence identity less than  $x\%$  with at least one other receptor. The number of the clusters in each threshold and the shape of the obtained graph could be an index for the cumulative sequence identity within the family or subfamily. If the number of clusters converge to 1 in high threshold, then we should conclude that the cumulative

sequence identity is high. Number of clusters converge to one near 25% of identity in TMDs of human GPCRs (figure 1) while it is in 42% and 37% of identity in amine and peptide subfamilies respectively.

### Similarity with Bovine Rhodopsin

Firstly, similarities within the TMDs were evaluated, and then in order to evaluate the similarities in the upper half (ligand binding domain) and the lower half (G-protein binding domain) separately, each helix was divided at its centre. The averaged similarity of the whole TMDs was 19.7%. While in the endoplasmic half of the TMDs, it was greater than in the exoplasmic part. The average score for the endoplasmic half of the rhodopsin-like hGPCRs is 25.0% while for the exoplasmic half, it is 14.1%.

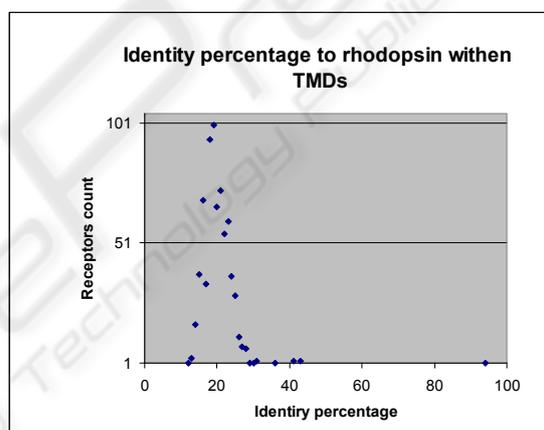


Figure 2: Pair-wise alignment of each family A receptor in the human genome with rhodopsin separately (only TMDs). Only six receptors are above 30% of identity and according to the well-known rules in the field of homology modeling, X-ray structure of the TMDs of rhodopsin could be employed for constructing models with enough confidence.

From pair-wise alignment of all hGPCRs with bovine rhodopsin, we obtained only six receptors with sequence identity above a threshold of 30%. And as depicted in figure 2, most of the receptors have sequence identity around 20%. The need for a detailed analysis of the similarity to bovine rhodopsin stems from the question of usefulness of the rhodopsin model as a template for constructing other GPCRs. Any model construction must relate to sequential parts of the structure and not to individual positions in space. Therefore, it is important to record the change in the similarity along each one of the helices and to realize which parts may be

considered to be “stable” enough so that a variation of sequence will not affect their structures. The conservation of sequence stretches of different length was calculated. Each stretch begins from N to C.

In this study, we employed a conservation scoring of segments in order to examine the extent of the single known GPCR structure of bovine rhodopsin which should probably not be “copied” in modeling of other GPCRs. It was shown previously that most of the conservation takes place in the endoplasmic parts of the TMD, but quantitative evaluations were limited to the conservations of single residues. In our study, we focused on cumulative conservation, because structural templates can not be constructed of isolated residues that are disconnected. By computing the similarity along stretches of residues, thus constructing a “cumulative similarity”, we demonstrated the quantitative aspects of the differences in conservation between the more conserved endoplasmic regions of most TM helices in rhodopsin-like hGPCRs and the exoplasmic parts. This has been attributed to the more prominent structural roles of the endoplasmic parts, or to their very similar function, to transmit a signal to intracellular G-proteins. The high variability of the exoplasmic parts probably reflect the need to interact and to be specific to a wide range of ligands.

There are certainly other possibilities for dividing the lengths of the transmembrane helices, and these may be useful for further refinement. We have shown that it is possible to determine the exact number of residues in a “stretch” whose averaged similarity to bovine rhodopsin does not exceed a certain threshold. We have also employed the “windows” method and found that then we could have better chances to model hGPCRs based on bovine rhodopsin than employing the whole set of residues in the endoplasmic half (see figure 3a-3g).

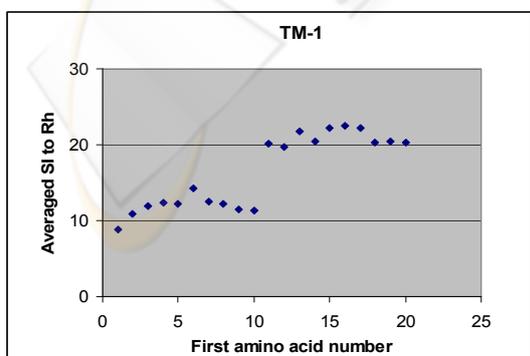


Figure 3a

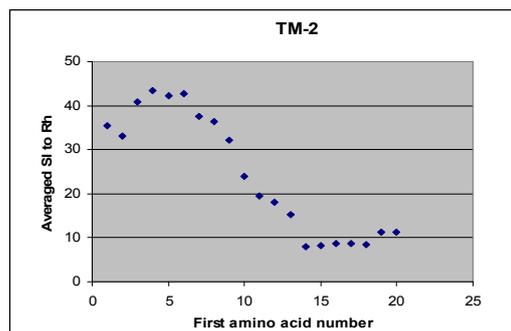


Figure 3b

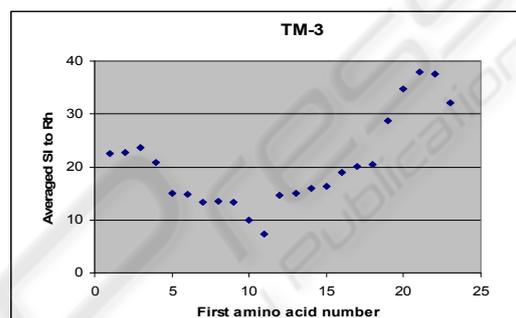


Figure 3c

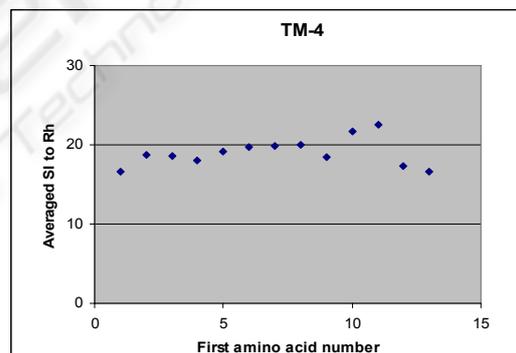


Figure 3d

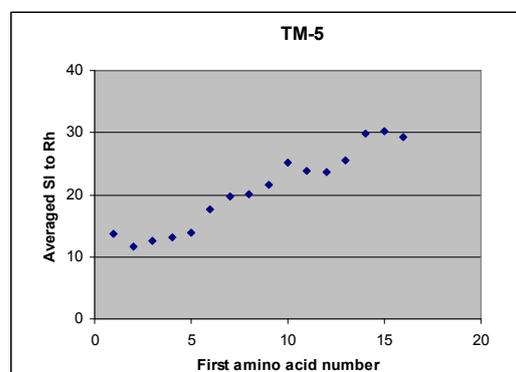


Figure 3e

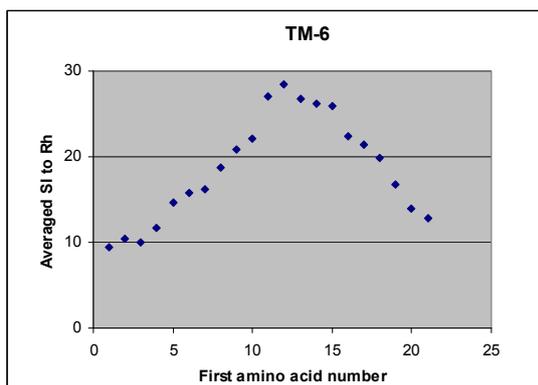


Figure 3f

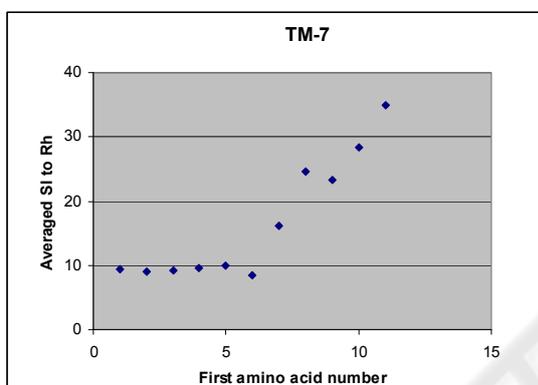


Figure 3g

Figure 3a-3g: Averaged identity scores to bovine rhodopsin (equation 1) of the seven TMDs of all family A hGPCRs averaged over window of 11 residues. Horizontal axis presents initial window positions. Y-axis is partial conservation. The direction in each helix goes from the N-terminal side to the C-terminal side.

### Modeling of $\beta$ 2-Adrenergic Receptor based on Bovine Rhodopsin as a Test Case

Since X-ray structure of  $\beta$ 2-adrenergic receptor was released recently, we have used it to validate our findings that were obtained in this bioinformatics study. In figure 4 we find the pairwise sequence alignment of the transmembranal domains of  $\beta$ 2-adrenergic receptor and Bovine Rodopsin, while in figure 5, the structural alignment is presented. The best core segments that were selected according to the findings as depicted in figure 3 gives backbone RMSD equal 1.39 Å (see figure 6).

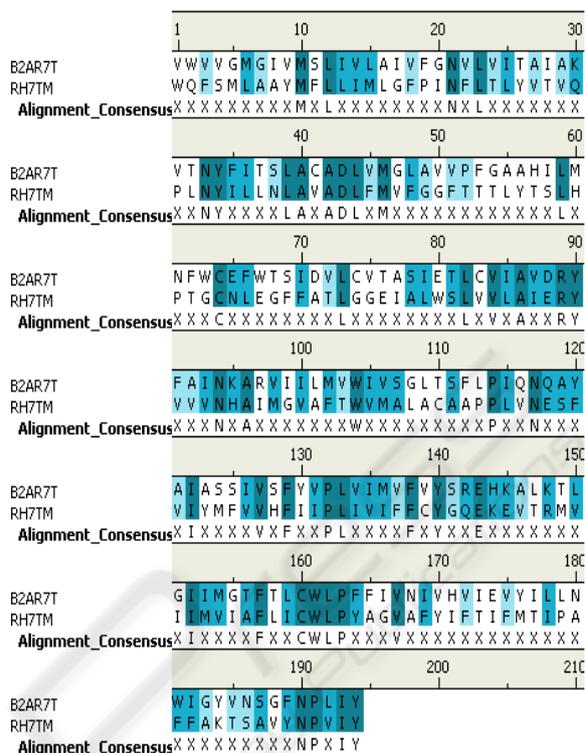


Figure 4: Pair-wise alignment of TMD of bovine rhodopsin with  $\beta$ 2-adrenergic receptor.



Figure 5: Superposition of TMD 3D structures,  $\beta$ 2-adrenergic receptor (2RH1) with Bovine Rodopsin (1F88). The backbone RMSD is equal 2.05 Å. In general, the upper half is more deviated than the lower half (mainly the first three turns of transmembrane-1, left side shown in the picture above).

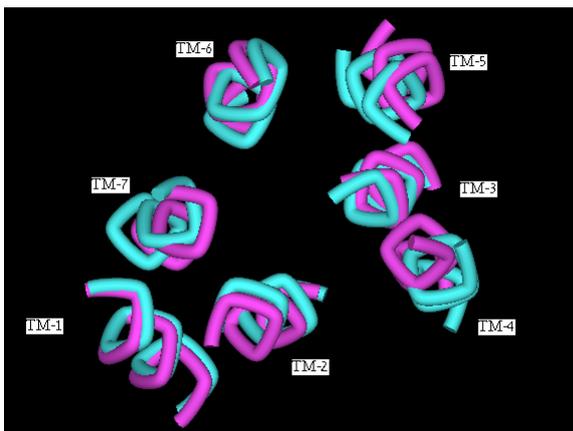


Figure 6: Superposition of the 7-transmembranal segments composed of 11 residues each, that were selected based on the bioinformatics analysis,  $\beta$ 2-adrenergic receptor (2RH1) with Bovine Rhodopsin (1F88). The backbone RMSD is equal 1.39 Å.

The shift in the cytoplasmic end of TMD-6 that has been seen in the crystal structure of  $\beta$ 2-adrenergic receptor (Rasmussen et al., 2007) could be explained by graph 3f. The segment of TMD-6 to be used for modeling  $\beta$ 2-adrenergic receptor based on bovine rhodopsin in lying on the middle of the helix.

Pair-wise alignment of the TMDs of family A hGPCRs with  $\beta$ 2-adrenergic receptor is shown in figure 7. 103 receptors are above 30% of identity and many others with identity less than 20%. We will further test if we could obtain better models while combining segments from the two crystal structures (bovine rhodopsin and  $\beta$ 2-adrenergic receptor).

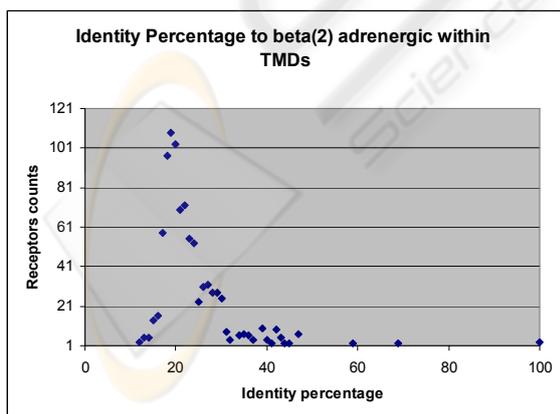


Figure 7: Pair-wise alignment of each family A receptor in the human genome with  $\beta$ 2-adrenergic receptor separately (only TMDs). 103 receptors are above 30% of identity.

## 4 CONCLUSIONS

We present in this study a qualitative and a quantitative analysis of family A hGPCRs database and tested the usefulness of employing crystal structure of bovine rhodopsin as a template for modeling the TMDs of other receptors from the same family. In most cases, as shown in figures 3a-3g, helix terminals display a smaller conservation than other parts of the helices. This is also found in most of the endoplasmic, more conserved parts (except for helix VI that has a larger conservation value at the middle). These variations could be connected to the structural changes from helix to loop at both the endoplasmic and exoplasmic terminals. Structural analysis of the recently released structure of  $\beta$ 2-adrenergic receptor and superposition of certain parts from the transmembranal domains with Bovine Rhodopsin backed our findings that were obtained by this study.

Since we are using only a partial template from the TM helical region of bovine rhodopsin or  $\beta$ 2-adrenergic receptor, there still persists an immense problem of determining the rest of the helical coordinates. Based on the information extracted from this study, we plan to use Molecular Dynamics (MD), Simulated Annealing (SA) or Iterative Stochastic Elimination (ISE) (<http://www.pdb.org/pdb/explore.do?structureId=1F88>) in order to construct better models for GPCRs, starting with a partial template of rhodopsin and/or  $\beta$ 2-adrenergic receptor.

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