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# Structure and Function Reveal Insights in the Pharmacology of 5-HT Receptor Subtypes

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### Summary

The purpose of this review is to examine experimental information concerning the structure and function of the G protein-coupled serotonin receptors in the three-dimensional context provided by the structure of rhodopsin. A critical examination of the suitability of rhodopsin as a template for serotonin receptor modeling from the level of sequence alignment to interpretation of biochemical experiments of relevance to the issues of structure-function relationships is presented.

**Key Words:** G Protein-coupled receptors; serotonin receptors; rhodopsin; sequence homology; molecular models; protein structure.

### 1. Introduction

The six G protein-coupled (GPCR) 5-HT receptor classes consist of the 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>4</sub>, 5-HT<sub>5</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub>, which are further divided into a total of 13 subfamilies. GPCRs have long been known to consist of a single polypeptide chain having extracellular, intracellular, and membrane embedded domains. The membrane domain consists of seven  $\alpha$ -helical segments connected by extracellular and intracellular loops with the N-terminus located extracellularly and the C-terminus located intracellularly. Signal transduction is initiated by ligands that bind in a site defined by the transmembrane helices and, perhaps, components of the extracellular loops. The intracellular domains are thought to interact with cytoplasmic proteins sensitive to agonist-induced conformational changes in receptor structure, ultimately giving rise to signaling that modulates cell function (*see ref. 1* for a recent review).

From: *The Receptors: The Serotonin Receptors:  
From Molecular Pharmacology to Human Therapeutics*  
Edited by: B. L. Roth © Humana Press Inc., Totowa, NJ

## 2. Structure–Function Relationships

Both direct and indirect methods can be used to explore structure–function relationships in GPCR systems. Often, indirect sequence-based observations can identify target residues, the role of which can be investigated by mutagenesis or other biochemical experiments. Multiple sequence alignments can reveal residues and sequence motifs that are conserved throughout a family of GPCRs. Such conserved features could be important in maintaining structure, thus determining function. Discrete differences in sequence between subtypes or species might point to residues that may be responsible for functional differences such as ligand selectivity. Sequence-based secondary structure prediction as well as location of and periodicity of amino-acid-residue physiochemical properties can assist in the identification of structural domains. For example, segments corresponding to the transmembrane domains of GPCRs have been identified as sequence segments with high hydrophobicity occurring in an amphiphilic pattern.

Site-directed mutagenesis is a widely used experimental method that can help directly establish the identity of residues particularly important for receptor structure and function. Mutagenesis studies have been used to identify residues lining the ligand-binding site, to determine the disposition of residues with respect to the aqueous pore and lipid layer, and to detect proximity of multiple residues to each other (2,3). In such studies, single-point mutations that affect receptor properties (ligand affinity, activation, constitutive activity, etc.) are often presumed to be directly responsible for the effects observed; it is inferred that these residues line the ligand-binding site, making contact with bound ligand, or directly mediate conformational change reflecting the activation state. However, the possibility that mutated residues may indirectly affect receptor properties can seldom be conclusively ruled out. The demonstration that changes in properties induced by a single-point mutation that can be reversed or eliminated by complementary changes in a structurally associated feature, either ligand or second receptor residue (reciprocal mutations), provides strong evidence that the observed effects are the result of direct interaction between target residues rather than being an indirect consequence of long-range perturbation of the structure. Evaluation of the effects of native or engineered cysteine disulfide bond crosslinking can provide evidence for the proximity of the studied residues. Evaluation of the effects of metal ions ( $\text{Zn}^{2+}$ ) on receptors with native or engineered ion-binding sites can also provide evidence for the proximity of the coordinating residues to a receptor histidine. Site-directed spin labeling can introduce nitroxide labels by disulfide bond formation with native cysteines or residues mutated to cysteine. The distance between labeled residues and changes in the distance between residues can be estimated by electron paramagnetic resonance spectroscopy. Amino acid

residues are individually mutated to cysteine in the substituted cysteine accessibility method (SCAM). The disposition of the targeted residue (solvent accessible, lipid accessible) is estimated by disulfide bond formation with water- and/or lipid-soluble labeling reagents. The location of a residue in the ligand-binding site can be further inferred from ligand protection studies. Several recent reviews provide compilations of the results of such studies with numerous GPCRs (2–4).

### 3. Structure of the 5-HT Receptors

Data from structure-related experiments has little meaning in the absence of a structural hypothesis (i.e., a three-dimensional receptor model that allows the evaluation of experimental observations in a structural context). Most early 5-HT receptor model construction was based on the experimental structure of bacteriorhodopsin, although not a GPCR, and later on the low-resolution projection structures of rhodopsin (reviewed in ref. 5). The solution of the crystal structure of the visual pigment bovine rhodopsin has opened new horizons in modeling GPCR structures (6). There has been considerable discussion concerning the suitability of the rhodopsin structure as a template for the construction of models of other GPCRs (2,3,7–9). The purpose of this review is to examine experimental information concerning the structure and function of the GPCR 5-HT receptors in the three-dimensional context provided by the structure of rhodopsin, a central issue being the suitability of rhodopsin as a template for 5-HT receptor modeling.

### 4. Rhodopsin as a Template for 5-HT Receptor Model Construction

Although several 5-HT receptors have been subjected to extensive structural studies (reviewed in ref. 10), this review will focus on those of particular relevance to the validity of rhodopsin as a homology modeling template for hypothetical 5-HT receptor models. Establishing an alignment between the sequences of templates of known structure with sequences of the model protein is the first and most crucial step in any homology modeling exercise. Although sequence homology between the GPCRs and rhodopsin is very low, there are several highly conserved residues and motifs in the transmembrane helices that are common to both rhodopsin and nearly all other GPCRs (Fig. 1) (11). Thus, if it is assumed that the number of residues in the corresponding helical segments of rhodopsin and the model target are identical, models of the transmembrane helices can be readily constructed. By far, most model building studies start with the assumption that there are no differences in the lengths of the transmembrane (TM) segments. This may not always be the case since irregularities

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5HT5A_HUMAN -----MDLPVNLTSFSLSTP-----SPLETN 21
5H7_HUMAN MMDVNSSGRPDLYGHLRSFLLPEVGRGLPDLSPDGGADPVAGSWAPHLLSEVTASAPTW 60
5H1B_HUMAN -----MEEPGAQCAPPFPAGSETWVQANLSSA 28
5H1D_HUMAN -----MSPLNQSAEGLPQE--ASN 17
5HT1F_RAT -----MDFLNS 6
5HT6_HUMAN -----MDFLNS 6
5HT1E_HUMAN -----MNITNC 6
5H1A_HUMAN -----MDVLSPPGQGNNTTSP 15
5H2A_HUMAN -----MDILCEENTSLSSTTNSLMQLNDDRLYSNDFNSGEANTSDAFNW 45
5H2C_HUMAN -----MVNLRNAVHSFLVHLIGLLVWQCDSVSP-----VAAIVTDIFN- 39
5H2B_HUMAN -----MALSRYRVSELQSTIPEHILQSTFVHVIS-----SNWSGLQTE 37
5H4_HUMAN -----MDKLD 6
RHODOPSIN_BOV -----MNGTEGPNFYVFPFSNK 16

                                1.30                1.50
                                |                |
5HT5A_HUMAN HSLGKDDLRPSSPLLX-----VXGVLIITLLGFLVAATFAWNLLVLATILRVRTF 71
5H7_HUMAN DAPPDNASGCGEQINYG-----RVEKVVIGSILTLITLLTIAGNCLVVISVCFVKKL 112
5H1B_HUMAN PSQNCSAKDYIYQDSIS-----LPWKVLLVMLLALITLATLSNAPVIATVYTRKL 80
5H1D_HUMAN RSLNATETSEAWDPRTL-----QALKISLAVVLSVITLATVLSNAPVLTITLLTRKL 69
5HT1F_RAT SDQNLTSSELLNR-----MPSKILVSLTSLGLALMTTINCLVITAIIVTRKL 54
5HT6_HUMAN SDQNLTSSELLNR-----MPSKILVSLTSLGLALMTTINSLVIAIIVTRKL 54
5HT1E_HUMAN T-TEASMAIRPKT-----ITEKMLICMTLVVITLTLLNLAVIMAIGTTKKL 53
5H1A_HUMAN PAPFETGGNTTGISDVT-----VSQVITSLLLGTLIFCAVLGNACVVAIAIALERSL 67
5H2A_HUMAN TVDSENRTNLSCEGLSPSCSLHLHQEKNWSALLTAVVILIITAGNILVIMAVSLEKKL 105
5H2C_HUMAN TSDG-GRFKFPDG-----VQNPALSIWIIIMTIGGNILVIMAVSMEKKL 84
5H2B_HUMAN SIPEEMKQVVEEQG-----NKLHWAALLILMVIIPTIGNTVLILAVSLEKKL 85
5H4_HUMAN NVSSEEGFG-----SVEKVLLTFLSTVILMAILGNLLVMVAVCWDRQL 50
RHODOPSIN_BOV TGVVRSPEEAPQYYLAEP-----WQFSMLAAYMFLMLGFPINFLTLYVTQVHKKL 68

                                :                *      :      :      :
                                |-----TM1-----|-----i1-----

                                2.38                2.50                3.22
                                |                |                |
5HT5A_HUMAN HRV-PHNLVASMADVSLVLAALVMPLSLVHLSGRRWQLGRRLCQLWIACDVLCTASIW 130
5H7_HUMAN RQP-SNYLIVSLALADLSVAVAVMPFVSVDLIGGKWI FGHFFCNVFIAMDVMCCTASIM 171
5H1B_HUMAN HTP-ANYLIASLAVTDLLVSLVMPISITMYTVTGR-WTLGQVCDLWSSDITCCTASIL 138
5H1D_HUMAN HTP-ANYLIGSLATDLLVSLVMPISIAYTITHT-WNFGQILCDIWLSSDITCCTASIL 127
5HT1F_RAT HHP-ANYLICSLAVTDFLVAVLVMPFSIVYIVRES-WIMGQGLCDLWLSVDIICCTCSIL 112
5HT6_HUMAN HHP-ANYLICSLAVTDFLVAVLVMPFSIVYIVRES-WIMGQVCDLWLSVDITCCTCSIL 112
5HT1E_HUMAN HQP-ANYLICSLAVTDLLVAVLVMPLSIIYIVMDR-WKLG YFLCEVWLSVDMTCCTCSIL 111
5H1A_HUMAN QNV-ANYLIGSLAVTDLMSVSLVLPMAALYQVLNK-WTLGQVTCDFLIALDVLCTSSIL 125
5H2A_HUMAN QNA-TNYFLMSLAIAIDMLGLVMPVSMILTILYGYRWPLPSKLCAYWILYDLVLFSTASIM 164
5H2C_HUMAN HNA-TNYFLMSLAIAIDMLVGLVMPLSLLAILYDYVWPLPRYLCPVWISLDVLFSTASIM 143
5H2B_HUMAN QYA-TNYFLMSLAVADLLVGLFVMPIALLTIMFEAMWPLPLVLCPAWFLDLVLFSTASIM 144
5H4_HUMAN RKIKTNYFIVSLAFADLLVSVLVMPFGAIELVQDI-WIYGEVCLVRTSLDVLTTASIF 109
RHODOPSIN_BOV RTP-LNYILLNLAVADLFMVFGGFTTTLTYSLHG Y-FVFGPTGCNLEGGFATLGEIAIW 126

:      :      :      :      *      :      :      :      :      :      :
--|-----TM2-----|-----e1-----|-----TM3-----

                                3.50                4.38                4.50
                                |                |                |
5HT5A_HUMAN NVTIAIALDRYWSIT-RHMEYTLRTRKCVSNVMIALT WALXTVISLAPLLFGWGET----- 184
5H7_HUMAN TLCVISIDRYLGIT-RPLTYPVQRNGKCMAMKILSVWLLSASITLPP-LFGWAQN----- 224
5H1B_HUMAN HLCVIALDRYWAIT-DAVEYSAKRTPKRAAVMIALVWVFSISISLPPFF-WRQAK----- 191
5H1D_HUMAN HLCVIALDRYWAIT-DALEYSKRRTAGHAATMIAI VWAISICISIPPLF-WRQAK----- 180
5HT1F_RAT HLSAIALDRYRAIT-DAVEYARKRTPRHAGITITTVWVISV FVISVPPLF-WRHQG----- 165
5HT6_HUMAN HLSAIALDRYRAIT-DAVEYARKRTPKHAGIMITIVW IISVFISMPPLEF-WRHQG----- 165
5HT1E_HUMAN HLCVIALDRYWAIT-NAIEYARKRTAKRAALMILT VWTISIFISMPPLEF-WRSHRR----- 165
5H1A_HUMAN HLCVIALDRYWAIT-DPIDYVNRKTPRAAALISL TWLIGFLISIPPLMGWRTE----- 179
5H2A_HUMAN HLCVIALDRYVAIQ-NPIHHSRFRNSRTKAF LKIAVWTVISVGLSMPIPVFGLQDSSKV-- 221
5H2C_HUMAN HLCVIALDRYVAIR-NPIEHSRFRNSRTKAIMK IAI VWAISIGSVSVPPIPVIGLRDEEKV-- 200
5H2B_HUMAN HLCVIALDRYIAIK-KPIQANQYNSRATAFI KITVWVLISIGIAIPVPKGIETDVDN-- 201
5H4_HUMAN HLCVIALDRYVAICQPLVYRNKMTPLRIALML GGCWVPTFTISFPI MQGWNINIGIDL 169
RHODOPSIN_BOV SLVVLAIERIYVVVC-KPMNSNFRF-GENHAIMGVAF TWMALACAAPPVGVGWSRYIEG-- 182

:      :      :      :      :      :      :      :      :      :      :
-----i2-----|-----TM4-----|-----


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**Fig. 1.** CLUSTAL W (1.82) multiple sequence alignment for serotonin receptors and rhodopsin.

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                                5.35                5.50
                                |                |
5HT5A_HUMAN  -----YSEGSEECQVSREPSYAVFSTVGAFYLP LCVLVFVYWKIYKATKFRVGSRKTN 237
5H7_HUMAN    -----VND-DKVC LISQDFGYTIYSTAVAFYIPMSVLMFMYQIYKAARKSAAKHKFP 276
5H1B_HUMAN   -----AEEEVSECVVNTDHLITYTVYSTVGAFYFPTLLLI ALYGR IYVEARSRILKQTPN 245
5H1D_HUMAN   -----AQEEMSDCLVNTSQISYTIYSTCGAFYIPSVLLI ILYGR IYRAARNRILNP-PS 233
5HT1F_RAT    -----NSRDDQCI IKHDHIVSTIYSTFGAFYIPLVLILIL IYKYIYRAARTLYHKRQAS 218
5HT6_HUMAN   -----TSRDDECI IKHDHIVSTIYSTFGAFYIPLALILIL IYKYIYRAAKTLYHKRQAS 218
5HT1E_HUMAN  -----LSPPPSQCTIQHDHVIYTIYSTLGAFYIPLTLILIL IYRIYHAAKSLYQKRGS 219
5H1A_HUMAN   -----DRSDPDACTISKDHGYTIYSTFGAFYIPLLLMLVL YGRIFRAARFRIRKTVKK 232
5H2A_HUMAN   -----FKE-GSCLLA---DDNFVLIGSFVSFFIPLTIMVITY FTLIKSLQKEATLCVSD 271
5H2C_HUMAN   -----FVNNTTCVLN---DPNFVLIGSEFAFFIPLTIMVITY C LTIYVLRQALMLLHG 251
5H2B_HUMAN   -----PNN-ITCVLTKERFGDFMLFGSLAAFFTPLAIMIVTY FTLIHALQKKAYLVKNK 254
5H4_HUMAN    IEKRKFNQNSNSTYCVFMVNKPYAITCSVAFYIPLLMVLAYRIYVTAKEHAHQIQML 229
RHODOPSIN_BOV --MQCSCGIDYYPHEETNNESFVIYMFVVFHFIPLIVIFFCYGQLVFTVKEAAAQQQES 240
                                :      *      *      *      :
-----e2-----|-----TM5-----|-----

5HT5A_HUMAN  SVSPIS--EAVEVKDSAQQPQMVFTRHA---TVTFQPEG-DTCREQ----- 278
5H7_HUMAN    GFPRVEPDSVIALNGIVKLQKEVEECANL----SRLLKHERKNISIFK----- 320
5H1B_HUMAN   RTGKRLTRAQLITDSPGTSSTVTSINSRV---PDVPSES--GSPVYVNVQVVRVSD- 296
5H1D_HUMAN   LYGKRFTTAHLITGSAG--SSLCSLNSSL---HEGHSLSA-GSPLFFNHVKIKLAD-- 283
5HT1F_RAT    RMIKEELNGQVLLESGEKSIKLVSTSYML----EKSLSDPSTDFDRIHSTVKS PRSE-- 271
5HT6_HUMAN   RIAKEEVNGQVLLESGEKSTKSVTSYVL----EKSLSDPSTDFDKIHSTVRSLSRE-- 271
5HT1E_HUMAN  RHLNRSRSTDS---QNSFASCKLTQTFCVS---DFSTSDPTTEFEKFHASIRIPPFD-- 269
5H1A_HUMAN   VEKTGADTRHGASAPAPQPKSVNGESGSR---NWLGVESKAGGALCANGAVRQGDGA 288
5H2A_HUMAN   LGTRAKLASFSFLP-----QSSLSEKLFQRSIHREPGSYTGR-- 309
5H2C_HUMAN   HTEEPGLSLDLFLCKCKRNTAE-----EENSANPNQDQNARRKKKERRPR-- 297
5H2B_HUMAN   PPQRLTWTVTSTVFQRDETPCSSPEKVAMLDGSRKDKALPNSGDETLMR--TSTIGK--- 310
5H4_HUMAN    QRAGASSESR-----PQSDQHSHTHRMR----- 252
RHODOPSIN_BOV ATTQK-----i3----- 246

                                6.30
                                |
5HT5A_HUMAN  -----KEQRPALMVG 288
5H7_HUMAN    -----REQKAATTLG 330
5H1B_HUMAN   -----ALLEK-----KKLMAARERKATKTLG 317
5H1D_HUMAN   -----SALER-----KRISAARERKATKILG 304
5HT1F_RAT    -----LKHEKSWRR---QKISGTREKKAATTLG 296
5HT6_HUMAN   -----FKHEKSWRR---QKISGTREKKAATTLG 296
5HT1E_HUMAN  -----NLDLHPGER---QQISGTREKKAATILG 294
5H1A_HUMAN   ALEVIEVHRVGNSEKHLPLPSEAGPTPCAPASFERKNERNAEAKRKMALAREKTVKTLG 348
5H2A_HUMAN   -----RTMQSISNEQKACKVLG 326
5H2C_HUMAN   -----GTMQAINNERKASKVLG 314
5H2B_HUMAN   -----KSVQTSISNEQKASKVLG 327
5H4_HUMAN    -----TETKAAKTLC 262
RHODOPSIN_BOV -----AEKEVTRMVI 255
                                *      .      :
                                |-----|-----

                                6.50                7.33                7.50
                                |                |                |
5HT5A_HUMAN  ILIGVFVLCWIPFFLTTELISPLCS---CDIPA IWKSI FLWLGYNSSFNPLIYTA FNKN 344
5H7_HUMAN    IIVGATVVCWLPPFLLSTARPFCIGTSCSCIPLWVERTFLWLGYANSLINPFIYAFFNRD 390
5H1B_HUMAN   IILGAFIVCWLPPFIISLVMPICKD--ACWFHLAIFDFFTWLGYNSLINPFIYTM SNE 375
5H1D_HUMAN   IILGAFIICWLPPFVVSVLPICRD--SCWIHPALDFFTWLGYNSLINPFIYTVFNEE 362
5HT1F_RAT    LILGAFVICWLPPFVKELVNVNICE---KCKISEEMS NFLAWLGYNSLINPFIYTFINED 353
5HT6_HUMAN   LILGAFVICWLPPFVKELVNVNCD---KCKISEEMS NFLAWLGYNSLINPFIYTFINED 353
5HT1E_HUMAN  LILGAFILSWLPFFIKELIVGLS---IYTVSSEVADFTWLGYNSLINPFLYTSFNED 350
5H1A_HUMAN   IIMGTFILCWLPPFII VALVLPFCES---SCHMPTLLGAIINWLGYNSLINPVIYAYFNKD 406
5H2A_HUMAN   IVFFFLVVMWCPFFITNIMAVICKESCNEDVIGALLNVFVWIGYLS SAVNPLVYTLFNKT 386
5H2C_HUMAN   IVFFFLIMWCPFFITNILSVLCEKSCNQKLEKLLNVFVWIGYVCSG INPLVYTLFNKI 374
5H2B_HUMAN   IVFFFLMLMWCPFFITNITLVLCDS-CNQTTQLQMLEIFVWIGYVSSG VNPLVYTLFNKT 386
5H4_HUMAN    IIMGCFCCLWAPFFVTVNIDPFIDY---TVPGQVWTAFLWLGYNSGLNPFYAF LNK 318
RHODOPSIN_BOV IMVIAFLICWLPPYAGVAFYIFTHQG---SDFGPIEMTIPAFFAKTSAYVNPVIYIMMNKQ 312
:::  *      *      *      :      :      :      *      *      *
-----TM6-----|-----e3-----|-----TM7-----|-----

```

**Fig. 1. (continued)**

5HT5A_HUMAN	YNSAFKNFFSRQH-----	357
5H7_HUMAN	LRTTYRSLQCCQYRNINRKLSAAGMHEALKLAERPERPEFVLRACR-----RVLL	441
5H1B_HUMAN	EKQAFHKLIRFKCTS-----	390
5H1D_HUMAN	FRQAFQKIVPFRKAS-----	377
5HT1F_RAT	FKKAFQKLVRCRN-----	366
5HT6_HUMAN	FKKAFQKLVRCRC-----	366
5HT1E_HUMAN	FKLAFKKLIRCReHT-----	365
5H1A_HUMAN	FQNAFKKIICKKFCRQ-----	422
5H2A_HUMAN	YRSAFSRYIQCYKENKKP-LQLILVNTIPALAYKSSQLQMGQKK-----NSKQ	434
5H2C_HUMAN	YRRAFSNYLRCNYKVEKKPPVRQIPRVAATALSGRELVNVIYRHT-----NEPV	423
5H2B_HUMAN	FRDAFGRYITCNYRATKSVKTLRKRSSKIYFRNPMaENSKFFKKHGIRNGINPAMYQSPM	446
5H4_HUMAN	FRRAFLIILCCDDERYRRPSILGQTVPCS-----TTTI	351
RHODOPSIN_BOV	FRNCMVTTLCCKGNPLGDDEASTTVSKTETSQVAPA-----	348
	<div> <div></div> <div> <div></div> <div></div> </div> </div>	
	--TM8--- -----	
5HT5A_HUMAN	-----	
5H7_HUMAN	RPEKRPPVSVVWLQSPDHHNLADKMLTTVEKKVMHMD	479
5H1B_HUMAN	-----	
5H1D_HUMAN	-----	
5HT1F_RAT	-----	
5HT6_HUMAN	-----	
5HT1E_HUMAN	-----	
5H1A_HUMAN	-----	
5H2A_HUMAN	DAKTTDNDCSMVALGKHSEESKDNSDGVNEKVSVCV-	471
5H2C_HUMAN	IEKASDNEPGIEMQ--VENLELPVNPSSVVSERISSV-	458
5H2B_HUMAN	RLRSSTIQSSSIIL--LDTLLLTENEGDKTEEQVSYV-	481
5H4_HUMAN	NGSTHVLRLDAVECGGQWESQCHPPATSPLVAAQPSDT-	388
RHODOPSIN_BOV	-----	

Fig. 1. (continued)

in the three-dimensional structure of the  $\alpha$ -helices of rhodopsin ( $\pi$ - and  $3_{10}$ -helix segments in TM5 and TM7, respectively) could accommodate single amino acid insertions or deletions in modeled helices without perturbing the overall dimensions of the segment (12,13). A major limitation to the use of rhodopsin as a template for modeling nonhelical domains of 5-HT receptors is the large differences in the lengths of corresponding structural elements. Fortunately, the largest discrepancies in sequence length occur in the N-terminus and C-terminus and intracellular loops, which are less likely to affect the structure of the ligand-binding site than the helices and extracellular loops. Where differences in segment length are too great to be modeled directly from the rhodopsin structure, investigators usually elect to generate a candidate loop structures, not from the rhodopsin structure but from the database of known protein structure using geometric (e.g., end-to-end distance) and sequence homology criteria. Unfortunately, there are usually no existing substructures with even modest homology to the 5-HT receptor sequences being modeled. Thus, loops created in this fashion are of questionable validity, particularly when the target sequences are significantly longer than those corresponding to rhodopsin. A final limitation in the existing structural data is that only the “dark” or inactive form of rhodopsin has been determined. The 5-HT receptor models derived from this structure are

thought to represent the inactive, resting, or antagonist occupied form of the receptor. The active form of rhodopsin would probably be the most appropriate template for the active or agonist occupied form of the GPCRs. Even though an experimental structure has not been determined directly, numerous biophysical approaches have provided insight into the nature of the conformational changes that rhodopsin undergoes on conversion to the light or activated form of the receptor (14). To date, there has been at least one computational investigation directed toward simulating conformational changes on rhodopsin activation and  $\beta$ -adrenergic receptor activation based on distance constraints derived from biophysical experiments using constrained molecular dynamics simulations (4). A similar approach might ultimately prove useful for generating models of the active form of 5-HT receptors.

The following is an examination of each structural element of rhodopsin and its suitability as 5-HT receptor model building templates. Figure 2 shows a graphic model of the 5-HT<sub>2A</sub> receptor constructed from the rhodopsin template.

## 5. Extracellular Domains

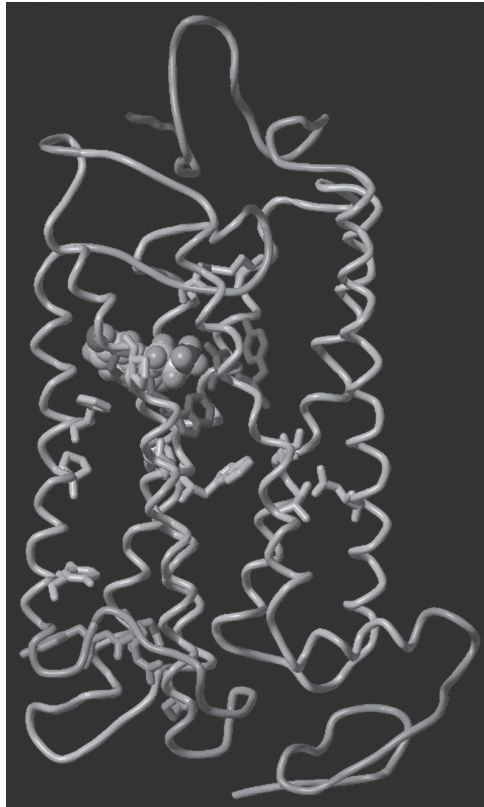
### 5.1. N-Terminus

The N-terminal segment of GPCRs is highly variable in length (11–879 residues) (15). The 5-HT receptor N-termini lengths range from 16 to 78 residues in length with very little conservation of sequence, with the possible exception of the first few residues in each sequence (Fig. 1). Given the total differences in length, it is difficult to envision a common N-terminus three-dimensional (3D) structure or function among the 5-HT receptors and rhodopsin. The rhodopsin N-terminus consists of a compact structure roughly parallel to the membrane that lies on top of the extracellular loops. If directly incorporated into 5-HT receptor models, this structure could limit the flexibility and disposition of the remaining extracellular loops. Deletion of the N-terminus from the 5-HT<sub>2A</sub> receptor does not appear to affect receptor function (16). Although no systematic mutagenesis studies have appeared, several single-nucleotide polymorphisms (SNPs) have been identified in the N-terminus of the 5-HT<sub>1A</sub> (17) and 5-HT<sub>2A</sub> (18) receptors, none of which affect receptor function. Together these observations suggest that explicit consideration of the N-terminal segment might not be necessary or desirable in the generation of 5-HT receptor models.

### 5.2. Extracellular Loop 1 (e1)

The first extracellular loop of rhodopsin directly connects TM2 and TM3 with a very short linker (six residues). With the exception of a single tryptophan, there is little sequence homology among the e1 loops of the 5-HT receptors. Fortunately, the lengths of the 5-HT receptor e1 segments are similar (six to seven residues) to the rhodopsin e1 loop. Thus, the rhodopsin e1 loop is





**Fig. 2.** A 5-HT<sub>2A</sub>-receptor model constructed from the rhodopsin crystal structure. Serotonin is shown as a space-filled structure. Magenta colored backbone traces represent modeled structures of low reliability (N-terminus, C-terminus, and i3) as well as segments generated by insertion into or deletion of rhodopsin residues (e1, e2, e3, and i3 loops). Stick structures of the side chains of conserved residues (yellow), ligand-binding site residues (red), and residues involved in receptor activation are shown. (Illustration appears in color in insert that follows p. 240.)

likely to be a viable template for 5-HT receptor model structures. Even if the structure of the rhodopsin e1 loop is not considered explicitly, it is likely that protein database search-based generation of this segment will likely produce geometrically acceptable results for all of the 5-HT receptors.

### 5.3. Extracellular Loop 2 (e2)

One of the most striking features of the rhodopsin structure is the complexity and compactness of a helix bundle “cap” or “plug” formed from the extracellular interhelix loops and N-terminal segment (19). Together, the N-terminal



sequence and the three loops e1 (TM2 to TM3), e2 (TM4 to TM5), and e3 (TM6 to TM7) form a layered, interlocking structure consisting partly of  $\beta$ -sheet loops. The bottommost of these is the e2  $\beta$ -hairpin, which traverses the entire helix aggregate, extending from its origin at TM4 and its terminus at TM5 toward TM1 and TM7. The e2 loop is tethered to TM3 (Cys3.26) via a disulfide bond between the two highly conserved cysteines. The e2 loop of rhodopsin comes within the van der Waals distance of the covalently bound retinal chromophore forming the bottom of the plug, equivalent to the top of the retinal binding site. Thus, the relevant question becomes, is there an analogous structure that forms part of the ligand-binding site of neurotransmitter G protein-coupled receptors? It has been suggested that such a complete enclosure of the ligand-binding site is not likely for receptors that, unlike rhodopsin, must reversibly associate with the ligand (7). With the exception of the 5-HT<sub>4</sub> receptor (30 residues), most 5-HT receptors have relatively short e2 loops (16–21 residues) compared to the rhodopsin e2 loop (26 residues). The difference in lengths of the corresponding 5-HT and rhodopsin segments makes it difficult to model e2 structures directly from the rhodopsin template. However, the existence of the disulfide bond between TM3 and e2 does provide a geometric constraints that might help eliminate irrelevant structures generated by searching structural databases. It has been argued that the e2 loop of the 5-HT<sub>2A</sub> receptor might not encroach as severely into the ligand-binding site as the corresponding structure for rhodopsin (20) because of the shorter length of the 5-HT<sub>2A</sub> e2 loop. There have been no systematic mutagenesis studies conducted with the e2 loops of the 5-HT receptors. The P184L mutation did not affect 5-HT<sub>1A</sub> receptor function in response to a wide range of agents (17). Evaluation of chimeras between 5-HT<sub>1B</sub> and 5-HT<sub>1D</sub> has led to the suggestion that the e2 loop might contribute to ligand selectivity (21) for some but not all investigated ligand (10) receptors. A recent SCAM study of the short e2 loop (approx 15 residues) supports the notion that the loop does in fact contribute to the ligand-binding site of the D2 receptor (22). Thus, with caveats, the e2 loop of rhodopsin is a reasonable model of, if not a directly transferable template for, the general structural features of the e2 loops of 5-HT receptors.

#### 5.4. Extracellular Loop 3 (e3)

The eight residue e3 loop of the rhodopsin receptor spans TM6 and TM7 on the periphery of the helical aggregate. The lengths of the e3 loop for the 5-HT receptors (7–11 residues) range from 1 fewer to 3 greater than the number of residues in the e2 loop of rhodopsin. Given the relatively remote disposition of the loop and the similarities in sequence length, it is expected that the rhodopsin e3 loop will be a reasonably good template for homology modeling. There have been no mutations of this segment reported for 5-HT receptors.

## 6. Intracellular Domains

### 6.1. Intracellular Loop 1 (i1)

The rhodopsin structure places the i1 loop adjacent to the short eighth membrane-embedded  $\alpha$ -helix. With the exception of the 5-HT<sub>4A</sub> receptor (six residues), the 5-HT receptors have the same length as the rhodopsin loop (seven residues). Interestingly a XKKLXXX motif is conserved between the rhodopsin sequence and the majority of the 5-HT sequences, suggesting that the i1 loops of rhodopsin and the 5-HT receptors could have a common structure. Systematic mutagenesis studies have not been conducted.

### 6.2. Intracellular Loop 2 (i2)

The second intracellular loop of rhodopsin consists of 11 residues that span TM3 and TM4 peripherally, extending into the membrane away from the helix aggregate. All 5-HT receptors are one residue longer, with the exception of 5-HT<sub>4</sub>, which contains two additional residues compared to the rhodopsin sequence. Even in the absence of any sequence homology, the similarities in length suggest that the i2 loop of rhodopsin is at least a reasonable starting point for 5-HT receptor modeling.

### 6.3. Intracellular Loop 3 (i3)

The i3 loop has been shown to be a major site of receptor–G protein interaction and might have a potential role in mediating interhelical interactions. The large difference in sequence lengths in the i3 loop of 5-HT receptors and rhodopsin suggests that this feature represents the largest structural divergence between rhodopsin and the 5-HT receptors. The rhodopsin loop is relatively short (21 residues) and traverses the perimeter of the helix aggregate, extending slightly into the membrane near the membrane surface (away from the bundle). The 5-HT i3 loops are much longer, ranging from 57 to 121 residues. Because of the differences in sequence length, this feature cannot be modeled using rhodopsin as a template with any accuracy. Because there are no protein database structures with any degree of sequence homology to the GPCR loop, it is unlikely that this loop can be modeled with any accuracy. In one approach reported for 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor models, the (113-residue) i3 loop was constructed in a stepwise fashion from segments with predicted  $\alpha$ -helices (2 helices, 8 and 19 residues long) and  $\beta$ -conformations (two segments, 8 and 13 residues long) (23). Backbone structures of segments predicted to be of random conformation were selected from sequences retrieved from a protein database search. It is not clear, however, how the tertiary structure of the loop was determined during this process. An alternative solution has been to omit the i3 loop from 5-HT (and other GPCR) models rather than explicitly including a structure that is most certainly wrong.

#### 6.4. C-Terminus

The C-terminus of rhodopsin consists of 399 residues, only the first 18 of which are included in the crystal structure. A short eighth  $\alpha$ -helical segment (approx 12 residues) initiates the C-terminus near the C-terminus of TM7. TM8 is approximately parallel to the membrane surface and perpendicular to the helix aggregate and is at the cytoplasm–lipid interface. The rhodopsin TM8 is preceded by the NPXXY(X)<sub>5,6</sub>F motif of TM7, a motif shared with 5-HT receptor sequences. Disruption of hydrophobic interaction between Y306/F313 alters H8 conformation and allows activation, suggesting that TM8 of rhodopsin acts as a membrane-dependent conformational switch-mediating activation (24). The importance of an analogous interaction for 5-HT<sub>2C</sub> receptors has been evaluated experimentally and results are consistent with models based on the rhodopsin structure (25). It is likely that the N-terminus of rhodopsin including the eight helices is a reasonable 5-HT receptor model-building template.

#### 6.5. Transmembrane Helix 1 (TM1)

The 3D structure of rhodopsin shows a kink in TM1 on the intracellular half of the helix. Rhodopsin has a proline at the 1.48 position (P53) that results in displacement of the extracellular portion of the helix toward and between TM2 and TM7 referenced from the extracellular side. There is no corresponding proline residue in any of the 5-HT receptors, suggesting that this helix irregularity might not be conserved. However, six subtypes (5-HT<sub>7</sub>, 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>2B</sub>, and 5-HT<sub>4</sub>) have either a glycine residue at 1.48, or 1.49, or both. Because glycine residues are often the site of irregularity in helices, the kink might very well be conserved in at least some these receptors. Because the cytoplasmic end of TM1 is tied to the cytoplasmic end of TM2 by the very short i2 loop in both the 5-HT and rhodopsin sequences, removal of the kink by replacement with an idealized helix in models of non-glycine-containing subtypes (5-HT<sub>1A</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub>, 5-HT<sub>1F</sub>, 5-HT<sub>5</sub>, and 5-HT<sub>6</sub>) would displace the intracellular portion of TM1 away from TM2 and TM7. Interestingly, it has been pointed out with reference to other A-type GPCRs (2) that the region of helix irregularity might be a site of dynamic flexibility. None of the TM1 residues has been strongly implicated as being part of the ligand-binding site. Because TM1 is basically on the outside of the aggregate formed by the remaining helices, the precise nature of the geometry of TM1 might not be important in 5-HT receptor model generation, at least with respect to the ligand-binding domain. The role of 5-HT residues at 1.48 and 1.49 have not been investigated by site-directed mutagenesis to date.

#### 6.6. Transmembrane Helix 2 (TM2)

The rhodopsin structure shows a helix irregularity in the extracellular half of TM2, placing it toward TM1, which is initiated by a pair of glycine residues

(2.56, 2.57) followed by three threonine residues (2.59–2.61). The threonine sequence appears to stabilize the helix bend with side chains hydrogen-bonding to the i-4 residue backbone carbonyl atoms (2). Although none of the 5-HT receptor sequences have the threonine repeat, each has a conserved proline at 2.59, suggesting that this helix irregularity might be a structurally conserved feature of the 5-HT receptors. Several TM2 residues have been evaluated using site-directed mutagenesis. Mutation of the conserved aspartate (D2.50) to alanine abolished agonist affinity (26) for 5-HT<sub>1A</sub> receptors. Mutation to asparagine in 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors has been shown to selectively affect ligand affinity in an unpredictable fashion (27). In a separate study (28), the D2.50N mutation of the 5-HT<sub>2A</sub> receptor did not affect agonist or antagonist affinity but reduced G protein coupling. The effect was reversed by a rescuing TM7 mutation N7.49D, which strongly suggest that residues at 2.50 and 7.49 are directly interacting with each other. The rhodopsin structure places both residues at positions within interacting distance, suggesting that the orientation of TM2 and TM7 represented by rhodopsin might also apply to 5-HT receptors.

### 6.7. Transmembrane Helix 3 (TM3)

The TM3 helix of rhodopsin deviates slightly from an ideal  $\alpha$ -helix in that its axis has a slight precession creating a subtle S-shape. Backbone hydrogen-bonding residues at 3.33 and 3.43 (T118 and S127) might be responsible for the slight precession. All of the 5-HT receptors have multiple backbone hydrogen-bonding residues (Cys/Ser/Thr) at various positions (not conserved), suggesting that similar helix irregularities might be present in the 5-HT receptors. Whereas differences in structure of 5-HT TM3 segments might vary only slightly from rhodopsin, significant changes in function could result from changing the immediate environment by several structurally important residues, particularly TM3 D3.32 and TM3 D3.49. Numerous mutagenesis studies have supported the role of D3.32 and the ammonium ion counterion (27,29–33). In addition, interaction of D3.32 with other receptor residues has been proposed for several GPCRs. In particular, interaction of D3.32 with a TM7 asparagine (N7.36) was proposed to maintain the receptors in the inactive state. Disruption of the interaction by ligand association (a “salt-bridge switch”) was proposed to result in activation (33) for the adrenergic receptor. The observation that D3.32A, -E, -Q, and -N all result in receptors with decreased rather than increased constitutive activity is contradictory to the “salt-bridge” proposal but was consistent with an early 5-HT<sub>2A</sub> receptor model (30). Examination of the rhodopsin crystal structure indicates that residues at the 3.32 and 7.36 positions are in fact not within interacting distance.

The demonstration of potential interactions between the arginine (R3.50) of the highly conserved D(E)RY motif near the intracellular end of TM3 and a

glutamate (E6.30) near the intracellular end of TM6 is of particular importance to 5-HT<sub>2A</sub> receptor structure and activation. The fact that this interaction stabilizes the inactive conformation of the receptor is suggested by the observation that interruption of the interaction by mutagenesis results in a high constitutive activity (34). It has been suggested that activation of the 5-HT<sub>2A</sub> receptor involves the relative displacement of the intracellular end of TM3 and TM6, as has been proposed on the basis of mutagenesis and biophysical studies for a variety of GPCRs. It was further suggested the displacement of TM6 is mediated at the TM6 kink located at the highly conserved P6.50 (35) for the adrenergic receptor. Residues and the 3.50 and 6.30 positions are in fact within interacting distance in rhodopsin, further suggesting that the rhodopsin TM3 is a viable template for 5-HT receptor models.

#### **6.8. Transmembrane Helix (TM4)**

The TM4 helix of rhodopsin is a short helix on the outer face of a helix “wall” around the ligand-binding site formed by the aggregate of TM2, TM3, and TM5. TM4 is a regular helix from the intracellular end up to a highly conserved proline at (P4.59) that results in the remaining few helix turns being displaced away from the TM2 and TM3 helix ends toward lipid. Contrary to the assumption that highly conserved residue might be important for ligand binding, a highly conserved tryptophan residue (W4.50) faces the TM2/TM3 interface shielded from direct contact with the ligand-binding site in rhodopsin-based models. The relatively remote location of the rhodopsin TM4 is consistent with the observation that most 5-HT receptor mutations in TM4 have little effect on ligand affinity (reviewed in ref. 10).

#### **6.9. Transmembrane Helix 5 (TM5)**

It has been suggested because sequence variability of all transmembrane helices among all GPCRs is greatest for TM5, much of the specificity of GPCRs could be attributable to TM5 properties (13). All of the 5-HT receptors and rhodopsin share a highly conserved proline (P5.50) residue near mid-helix. The rhodopsin TM5 is relatively “straight” despite the proline residue, but it shows a significant deviation from ideal helical periodicity in that it has a single “underwound”  $\pi$ -helix turn (residues 5.44–5.49) near P5.50. Although relatively rare in general, it has been suggested that a  $\pi$ -helix might be a universal feature of all class A GPCRs because of the highly conserved nature of P5.50 (13). In addition, there is some evidence that  $\pi$ -helices are frequently associated with ligand-binding domains (13,36,37). Many experimental studies have suggested that portions of TM5 interact with the ligand. A hydrogen bond donating serine is present at either the 5.42 or 5.43 position in all 5-HT receptors. With the exception of the 5-HT<sub>2</sub> subtypes, a hydrogen bond donating residue is present at

both the 5.42 and 5.43 positions. The rhodopsin structure places these residues in a ligand-accessible position. Although not entirely self-consistent, mutation data for the 5-HT<sub>2A</sub> suggest that a hydrogen bond donor might interact with a ligand having a hydrogen bond acceptor in a position analogous to that of the 5-OH group of 5-HT (reviewed in ref. 19). If the helical “bulge” were replaced with a idealized helix, positions 5.42 and 5.43 would be rotated clockwise, placing both residues more toward the TM4–TM5 interface inaccessible to the ligand-binding site, suggesting that this helix irregularity might well be conserved in the 5-HT and other class A GPCRs. A phenylalanine at the 5.47 position is conserved in all 5-HT receptors. The neighboring 5.48 position is occupied by either a phenylalanine or tyrosine residue. The structure of rhodopsin places the 5.47 in a ligand-accessible orientation, but a 5.48 residue is clearly in lipid or at the TM5–TM6 interface. Mutation of each of these residues to alanine has been shown to selectively affect ligand affinity and both mutations reduce potency and efficacy of 5-HT-stimulated PI hydrolysis (38), partially inconsistent with the lipid orientation of position 5.48. The partial discrepancy between the orientations of side chains dictated by the rhodopsin structure and mutagenesis data have led to the hypothesis that helix flexibility at the conserved proline 5.50 position and/or ligand-induced helix rotation might account for the inconsistencies (2,10).

### 6.10. Transmembrane Helix 6 (TM6)

Rhodopsin TM6 has a pronounced kink at the uniformly conserved proline and position 6.50, tilting the extracellular half toward TM5. Evidence points to a major conformational change in this helix (particularly with respect to TM3) in the conversion of the inactive to the active state of rhodopsin. Spin-labeling and cysteine crosslinking studies in rhodopsin are consistent with a displacement of the cytoplasmic side of TM6 away from TM3 upon rhodopsin activation (39,40). In addition, rhodopsin activation is prevented in a rhodopsin-containing engineered metal-ion-binding site (41). It has been proposed that the conserved proline at this position represents a conformationally flexible hinge, the bending of which mediates TM6 conformational change on activation. An alanine scanning mutagenesis study of residues 6.28 through 6.40 on the intracellular half of TM6 of the 5-HT<sub>2A</sub> receptor suggests that none has a direct effect on ligand binding (34) and all of these residues are distant from the ligand the ammonium ion-binding residue D3.32 of TM3. Single and reciprocal mutagenesis studies of E3.60 and R3.50 support the hypothesis that a salt bridge between these TM6 and TM3 residues might mediate receptor activation, as discussed earlier (34). The rhodopsin structure is consistent with the potential presence of such an interaction in the inactive state. Mutagenesis studies of conserved residues on the extracellular half (W6.48 and F6.52) of the receptor suggest that these



residues might be important for ligand binding for the 5-HT<sub>2A</sub> receptor (42). The aromatic side chains of both are in the proposed ligand-binding site in proximity to D3.32 of TM3 in a rhodopsin-based 5-HT<sub>2A</sub> model (20).

### 6.11. Transmembrane Helix 7 (TM7)

The 5-HT and rhodopsin receptors contain a highly conserved asparagine/proline pair at position 7.49 and 7.50. In addition to inducing a pronounced kink at this position, an unusual helix conformation occurs on the intracellular side of the kink consisting of a tightly wound 3<sub>10</sub>-helix (7.43–7.46). This feature of the rhodopsin structure might be a consequence of retinal covalently bound to K296 (7.43), which is obviously not a property shared with the 5-HT receptors. Double-revertant studies of the 5-HT<sub>2A</sub> receptor residues D2.50 on TM2 and N7.49 suggest that disruption of a hydrogen bond between these residues might be important for activation. Potential interaction between these two residues is evident in the rhodopsin structure and is consistent with a rhodopsinlike abnormal helix in this region (28). In addition, a SCAM study of the D<sub>2</sub> receptor is more consistent with a 3<sub>10</sub>-helix than a more regular, kinked  $\alpha$ -helix (43). Mutation of the highly conserved Y7.53 of NPXXY motif led to constitutively active 5-HT<sub>2C</sub> receptors, suggesting that this locus has a role in receptor activation (44). Further investigations of double-revertant Y/F pairs suggest that activation might, in part, be dependant on disruption of a direct interaction of Y7.53 and Y7.60 of the C-terminal helix 8 (25), as has been proposed for rhodopsin. Such an interaction is readily apparent in the rhodopsin structure.

## 7. Conclusion

With a few notable exceptions, the rhodopsin structure appears to be a useful starting point for the generation of 3D models of the serotonin receptors that can be used to aid further receptor structure–function studies. Segments that almost certainly cannot be generated accurately from the rhodopsin structure include the N-terminus and i3 loop. Segments that almost certainly can be generated from the rhodopsin structure include the e1, e2, e3, and i1 loops, the TM2, TM3, TM6, and TM7 helices, and the C-terminus. Available data suggest that structural correspondence between rhodopsin and 5-HT receptor segments are not entirely consistent for TM1, TM4, and TM5.

Numerous 5-HT receptor models have been constructed from the experimental crystal rhodopsin structure with various levels of fidelity to the template. To date, models have been reported for 5-HT<sub>1A</sub> (23,45–47), 5-HT<sub>2A</sub> (20,23,30,34, 38,48–50), 5-HT<sub>2B</sub> (31,50), 5-HT<sub>2C</sub> (50), 5-HT<sub>4</sub> (51), and 5-HT<sub>6</sub> (52,53) receptors. Not unexpectedly, each of the models presented has been useful in providing insight into the potential structural origins of the specific experimental feature being investigated (a limited set of mutations, SCAM of a



particular feature of the studied receptor). There have been very few systematic attempts to evaluate the usefulness of models for the generation of new ligands. Perhaps the most stringent and meaningful test of overall validity of GPCR homology models for this purpose is the application of virtual screening paradigms. Large databases of compounds with known activities (both active and inactive) are docked with receptor models in an automated fashion and the resulting complexes are scored. Overall model “fitness” can be determined statistically by comparing predicted affinity with the experimental results. Success would suggest that hypothetical models encode enough useful receptor structure information to allow prediction of ligand affinity for a large range of structural types of potential ligand; the models could be useful for structure-based ligand design. Encouraging results of this kind have been recently reported for the dopaminergic (54) and adrenergic (55) receptor models. A major limitation in the use of rhodopsin as a model template for screening purposes is that only the structure of the inactive form of the receptor is available. Thus, one would expect virtual screening using rhodopsin-based models to produce antagonists only. A second potential confounding factor in the use of rhodopsin as an explicit template for the generation of GPCR models has very seldom been mentioned explicitly. Ligand-binding sites of GPCR models based on the helix backbone of rhodopsin closely resemble the original retinal binding site (a “ghost site”) even in the absence of sequence homology and even when the model side-chain geometries are constructed independent of the rhodopsin side-chain geometries (20). Imprintation of a more realistic site has been accomplished by molecular mechanics minimization of receptor–ligand complexes or receptor–ligand ensemble complexes generated from manually docked orientations consistent with known site-directed mutagenesis studies (20,54). The obvious liability of this approach is that the steric and electronic attributes of the imprinted or created site is highly dependent on the particular ligand(s) and ligand orientations selected. Simulations in which a ligand is randomly “solvated” with N- and C-blocked amino acids produces an artificial site quite effective in retrieving similar ligands from databases virtually screened in the automated docking algorithm. In fact, similar approaches have been used to generate explicit atomic models originally referred to as pseudoreceptors (56,57). Although the use of GPCR models as virtual screening templates shows promise for both model validation and structure-based ligand design, results should be interpreted with appropriate skepticism.

## Acknowledgment

This work was supported by United States Public Health Service Grant MH057969.

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**The Serotonin Receptors**

From Molecular Pharmacology to Human Therapeutics

Roth, B.L. (Ed.)

2006, XVIII, 618 p. 82 illus., 5 illus. in color., Hardcover

ISBN: 978-1-58829-568-2

A product of Humana Press