TARGETS FOR MAJORITY OF DRUGS: G PROTEIN-COUPLED RECEPTORS – – THEIR STRUCTURE AND INTERACTIONS WITH BIOLIGANDS

JERZY CIARKOWSKI[1], CEZARY CZAPLEWSKI[1]
AND MARTA PASENKIEWICZ-GIERULA[2]

[1]*Faculty of Chemistry, University of Gdansk, Sobieskiego 18, 80-952 Gdansk, Poland*
[2]*Department of Biophysics, Jagiellonian University, A. Mickiewicza 3, 31-120 Cracow, Poland*

Abstract: G protein-coupled receptors (GPCRs) are the most frequent targets for many drugs. They form the largest superfamily of integral membrane proteins, of which more than 1000 members have the following common features: (i) All GPCRs form 7 hydrophobic α-helices of length ~38Å (25 amino acids, 7 turns) along a single chain. The consecutive helices alternatively cross the membrane, starting from the extracellular side, so that they form a heptahelical transmembrane domain interwoven with 6 loops, of which the even ones plus the N-terminus create the receptor's extracellular domain while the odd ones plus the C-terminus form its intracellular domain. (ii) All GPCRs are stimulated by diverse extracellular (primary) signals. (iii) Stimulated GPCRs convey the primary signals via their transmembrane and intracellular domains to the cytosolic peripheral heterotrimeric GTP-binding proteins (G proteins), mediating the signal's further transduction to various cellular second messenger systems. A current status of structural studies on GPCRs, consisting of low ~7.5Å resolution experimental structures and supplementary molecular modeling, is outlined. Subsequently, some results of authors' own work on studying essential interactions of the V2 vasopressin renal receptor (V2R) with its agonist [Arg^8]Vasopressin (AVP) and selected antagonists are presented, as well as their possible impact on the biological signal transduction is discussed. Finally, perspectives for future developments are sketched.

Keywords: G protein-coupled receptor, molecular modeling, GPCR/bioligand interaction, molecular dynamics, membrane

1. Biological Signal Transduction via G Protein-Coupled Receptors and G Proteins

A typical career in medicinal chemistry will run across a G protein-coupled receptor (GPCR) as a drug target. GPCRs form the biggest known (~2000 sequences reported to date [1]) superfamily of homological proteins, integral to the membranes in most cells of any vertebrate, where they serve as transducers of a bewildering array of incoming extracellular signals. These signals, after being processed within the receptor and conveyed across the membrane, are picked up by the cytosolic GTP-binding proteins (G proteins) to initiate chains of intracellular

processes virtually controlling all cell activities, see Figure 1. Accordingly, GPCRs, also known as heptahelical transmembrane (7TM), or serpentine receptors, are *de facto* the most frequent drug targets. Ironically, despite their absolutely fundamental physiological roles, little is known about GPCR structure as, being typical integral membrane proteins, they are crystallization-resistant and thus immune against structural elucidation by X-ray.

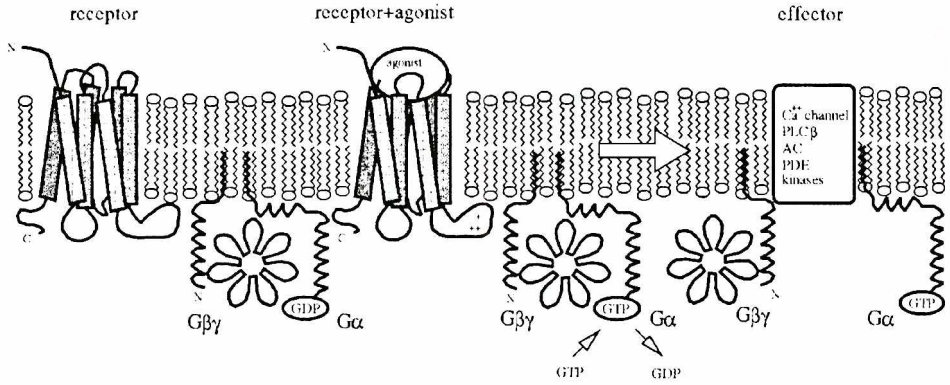


Figure 1. *GPCR signal transduction system. The activated receptor catalyses the exchange of GDP for GTP in the $G_{\alpha\beta\gamma}$ heterotrimer. This triggers the dissociation of the heterotrimer into G_{α}.GTP and $G_{\beta\gamma}$ which activate effectors: enzymes and/or ion channels. Native G_{β} never separates of helical G_{γ} and has a structure reminiscent of a 7-blade propeller [10] as reflected on the scheme.*

Until now, only low-resolution (≥ 6 Å) structures of the light receptor, bovine [2] and frog [3] rhodopsin, are known from the electron diffraction in cryo-microscopic measurements. Together with the multi-sequence analysis [4, 5], they have confirmed the structural concensus agreed upon, and schematically shown in Figure 1. Thus, any GPCR consists of a hydrophobic heptahelical transmembrane domain (7TM), interlaced with the 6 alternating extracellular and cytosolic loops (EL1-EL3 and IL1-IL3, respectively), creating with the N-terminus and with the C-terminus the extracellular and intracellular domains, respectively. Accordingly, 7TM is composed of seven transmembrane helices TM1-TM7, aligned one after the other counterclockwise (if viewed from the extracellular space) into a kidney-like shape [1, 6]. The amino acid sequences of respective TM1-TM7 helices, within the most abundant rhodopsin-like GPCR family, are homological to ~20% level, thus supporting a hypothesis on common functionality and structure of their 7TMs. On the contrary, both the extracellular and intracellular domains exhibit no mutual sequence homology, unless among very closely related GPCRs. This diversity is reflected in the enormous wealth of primary signals for GPCRs, ranging from a photon via metal ions, gustatory substances, odorants, biogenic amines, neurotransmitters, to small, medium-size and large peptide/non-peptide/glycoprotein hormone ligands [1, 6, 7, 8].

Interestingly, this first-messenger diversity merges into a uniform signal-transduction path, embodied within the receptor and at the GPCR/G-protein interface into a putative common mechanism [1, 6-8, 9]. A contribution to the

elucidation of the G protein role in this mechanism was appreciated with the Nobel price in physiology and medicine, awarded in 1994 to G.M. Rodbell and A. Gilman. Thus, the G protein is stimulated through the cytosolic domain of a ligand-activated GPCR, see Figures 1 and 2. At this instant, it is a heterotrimer consisting of the G_α

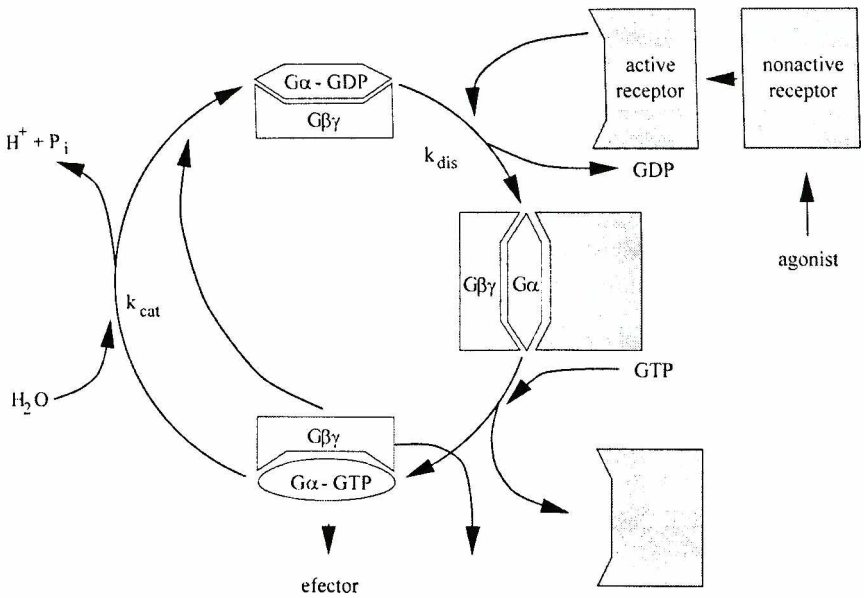


Figure 2. *G protein cycle. The intracellular signal is proportional to the GDP dissotiation rate constant k_{dys} and inversely proportional to the GTP hydrolysis rate constant k_{cat}.*

with GDP (guanosine diphosphate) bound, G_β and G_γ subunits, in short $G_{\alpha\beta\gamma}$.GDP. Over 20 isoforms of G_α, 5 isoforms of G_β and 11 isoforms of G_γ are known [8, 10], yielding > 1100 theoretical $G_{\alpha\beta\gamma}$ combinations, a figure of an order consistent with the number of various GPCRs.

Since an activated GPCR changes its conformation, the G_α subunit of $G_{\alpha\beta\gamma}$ receives this as a signal to exchange GDP for omnipresent cellular GTP (guanosine triphosphate). A nascent complex $G_{\alpha\beta\gamma}$.GTP first dissociates off the receptor and simultaneously (subsequently?) splits into the funtional G_α.GTP and $G_{\beta\gamma}$, see Figure 2, capable of stimulating intracellular efector/second messenger systems. These, depending of primary signal/GPCR/G protein systems, may consist of ion channels and/or various enzymes/second messengers, controlling cellular behavior and function. As the G_α.GTP is simultaneously a slow-acting Mg^{2+}-dependent GTPase, it gradually hydrolyses GTP to GDP. Restored G_α.GDP provides a signal for the $G_{\alpha\beta\gamma}$.GDP to reassociate, synonymous with the deactivation of the G protein (as both G_α.GTP and $G_{\beta\gamma}$ dissappear). Thus, the G protein working cycle closes, see Figure 2. A single activation of a GPCR may evoke a few hundred to a few thousand of the G protein cycles; this being a measure of the amplification of a primary signal at this stage.

2. GPCR Structure

Whereas the G protein structures at various stages of the G protein cycle [11, 12, 13, 14, 15, 16, 17], likewise the mechanism of hydrolysis of G_α.GTP to G_α.GDP [13, 14], are known, the conformation of GPCRs and our knowledge on what happens at the GPCR/G protein interface are really misty, as the only GPCR structural information available are the low-resolution electron cryo-microscopic images of rhodopsins [2, 3], see above. These images at 6-7Å resolution parallel and 16-17Å resolution perpendicular to the membrane surface are good enough for the reassessment of the match of the specific helices with the three-dimensional low-resolution 7TM image [2, 3, 18]. They are, however, much worse for the prediction of mutual inter-helical arrangements, involving helical rotations, tilts and kinks [4, 19], and they are totally incapable of locating atomic positions. It is only known from the mutagenesis, photoaffinity and spin labeling experiments that some 20 amino acid-long N- and C-terminal G_α sequences [13] are involved in possible interactions with the IL2, IL3 and possibly C-terminal fragments [7, 8] of GPCRs. Thus, a state-of-the-art molecular modeling is currently the only approach to study the structure of GPCRs and their interactions with bioligands.

Early modeling schemes used the low-resolution structure of bacteriorhodopsin [20], another 7TM — albeit not a GPCR — integral membrane protein, as a template for homology modeling. Interestingly, despite no homology between the transmembrane sequences of bacteriorhodopsin and GPCRs, this modeling resulted in a huge database of GPCR 7TM templates [21]. Using this scheme numerous bioligand-interaction models for various GPCRs have been developed [22]. However, recent comparisons of high-resolution structures of bacteriorhodopsin [23, 24, 25] with low-resolution structures of rhodopsin [2, 3] clearly indicate that the latter, despite a lower resolution, would make a much better generic GPCR 7TM template, as being dissimilar to the former and simultaneously having its 7TM at ~20% homological with other GPCRs. Thus, current molecular modeling of GPCRs consists of rhodopsin-based 7TM templates onto which specific GPCR sentences are threaded.

```
Multalin version 5.3.3
Copyright I.N.R.A. France 1989, 1991, 1994, 1996
Published research using this software should cite
Multiple sequence alignment with hierarchical clustering
F. CORPET, 1988, Nucl. Acids Res., 16 22, 10881-10890
Symbol comparison table: blosum62
Gap weight: 12
Gap length weight: 2
Consensus levels: high=90% low=50%
Consensus symbols:
 ! is anyone of IV
 $ is anyone of LM
 % is anyone of FY
 # is anyone of NDQEBZ

 MSF:    659    Check:    0          ..
 Name: sp|P02699|OPSD_BOVIN Len:   659  Check: 4601  Weight:  1.19
 Name: sp|P25103|NK1R_HUMAN Len:   659  Check: 7852  Weight:  1.10
 Name: sp|P21555|NY1R_RAT   Len:   659  Check: 4036  Weight:  1.10
 Name: sp|P41143|OPRD_HUMAN Len:   659  Check: 3951  Weight:  0.99
 Name: sp|P41145|OPRK_HUMAN Len:   659  Check: 9850  Weight:  0.99
```

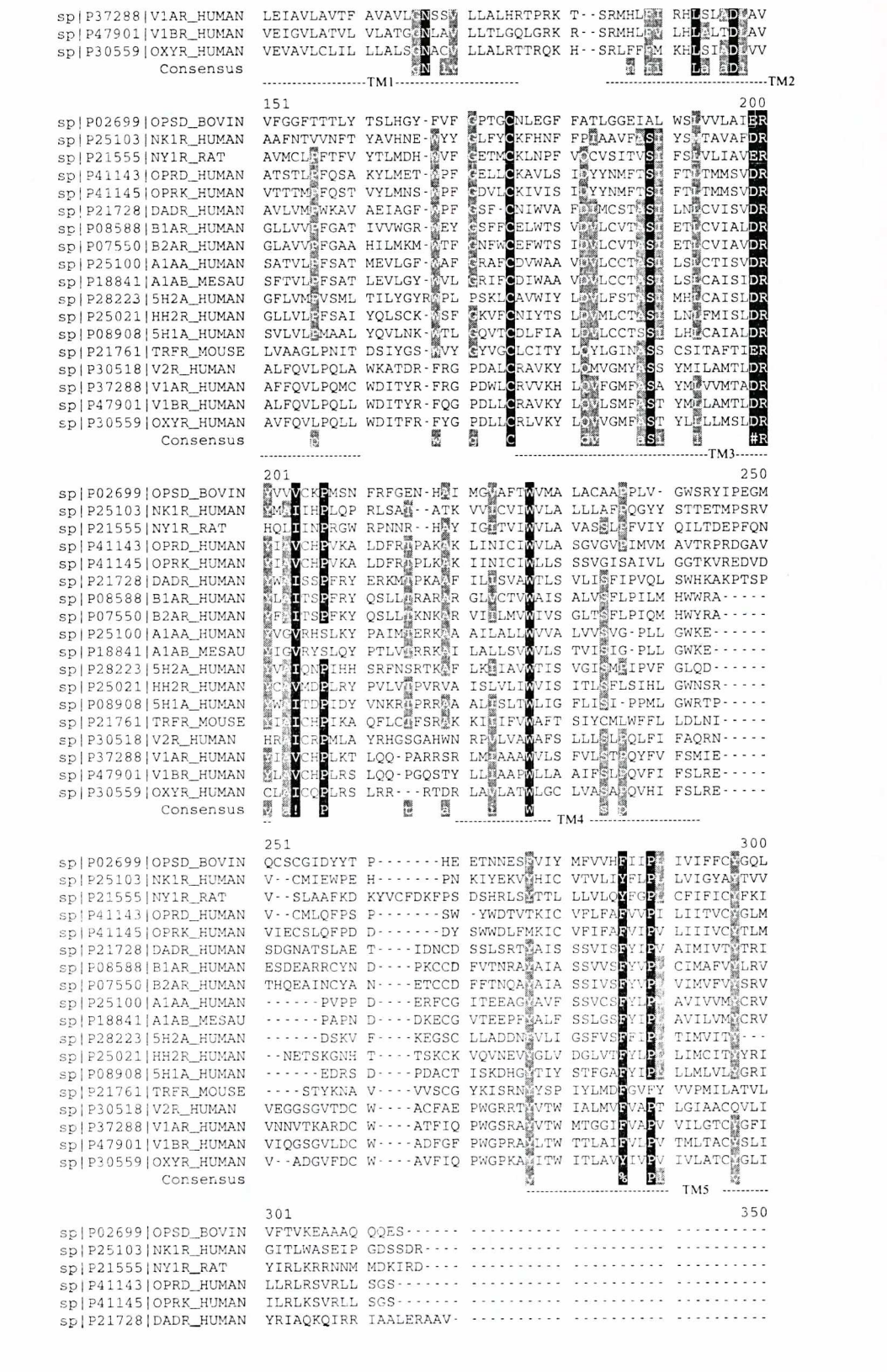
```
 Name: sp|P21728|DADR_HUMAN Len:   659  Check: 4069  Weight:  1.00
 Name: sp|P08588|B1AR_HUMAN Len:   659  Check: 8391  Weight:  0.92
 Name: sp|P07550|B2AR_HUMAN Len:   659  Check: 9981  Weight:  0.92
 Name: sp|P25100|A1AA_HUMAN Len:   659  Check: 3098  Weight:  0.82
 Name: sp|P18841|A1AB_MESAU Len:   659  Check: 9868  Weight:  0.82
 Name: sp|P28223|5H2A_HUMAN Len:   659  Check: 8336  Weight:  0.99
 Name: sp|P25021|HH2R_HUMAN Len:   659  Check: 2329  Weight:  1.08
 Name: sp|P08908|5H1A_HUMAN Len:   659  Check: 2393  Weight:  1.10
 Name: sp|P21761|TRFR_MOUSE Len:   659  Check:  965  Weight:  1.15
 Name: sp|P30518|V2R_HUMAN  Len:   659  Check:  242  Weight:  1.03
 Name: sp|P37288|V1AR_HUMAN Len:   659  Check: 4017  Weight:  0.93
 Name: sp|P47901|V1BR_HUMAN Len:   659  Check: 6899  Weight:  0.93
 Name: sp|P30559|OXYR_HUMAN Len:   659  Check: 9637  Weight:  0.96
 Name:       Consensus      Len:   659  Check: 9692  Weight:  18.00

//

                          1                                                50
sp|P02699|OPSD_BOVIN
sp|P25103|NK1R_HUMAN
sp|P21555|NY1R_RAT
sp|P41143|OPRD_HUMAN                                                      ME
sp|P41145|OPRK_HUMAN                                                M ESPIQIFRGE
sp|P21728|DADR_HUMAN
sp|P08588|B1AR_HUMAN                                                  MGAGVLVLG
sp|P07550|B2AR_HUMAN
sp|P25100|A1AA_HUMAN  MTFRDLLSVS FEGPRPDSSA GGSSAGGGGG SAGGAAPSEG PAVGGVPGGA
sp|P18841|A1AB_MESAU
sp|P28223|5H2A_HUMAN                          MDILC EENTSLSSTT NSLMQLNDDT
sp|P25021|HH2R_HUMAN
sp|P08908|5H1A_HUMAN
sp|P21761|TRFR_MOUSE
sp|P30518|V2R_HUMAN
sp|P37288|V1AR_HUMAN                                                      MR
sp|P47901|V1BR_HUMAN
sp|P30559|OXYR_HUMAN
          Consensus

                         51                                               100
sp|P02699|OPSD_BOVIN                MNGTEGPN FYVPFSNKTG VVRSPFEAPQ YYLAEPWQFS
sp|P25103|NK1R_HUMAN                    MDNV LPVDSDLSPN ISTNTSEPNQ FVQPAWQIVL
sp|P21555|NY1R_RAT              M NSTLFSRVEN YSVHYNVSEN SPFLAFENDD CHLPLAVIFT
sp|P41143|OPRD_HUMAN  PAPS-AGAEL QPPLFANASD AYPSAFPSAG ANASGPPGPG SASSLALAIA
sp|P41145|OPRK_HUMAN  PGPTCAPSAC LPPNSSAWFP GWAEPDSNGS AGSEDAQLEP AHISPAIPVI
sp|P21728|DADR_HUMAN                              MRTL NTSAMDGTGL VVERDFSVRI
sp|P08588|B1AR_HUMAN  ASEPGNLSSA APLPDGAATA ARLLVPASPP ASLLPPASES PEPLSQQWTA
sp|P07550|B2AR_HUMAN                MGQPGNGS AFLLAPNRSH A----PDHDV TQQRDEVWVV
sp|P25100|A1AA_HUMAN  GGGGGVVGAG SGEDNRSSAG EPGSAG-AGG DVNG--TAAV GGLVVSAQGV
sp|P18841|A1AB_MESAU    MNPDLDTG HNTSAPAQWG ELKDANFTGP NQTS--SNST LPQLDVTRAI
sp|P28223|5H2A_HUMAN  RLYSNDFNSG EANTSDAFNW TVDSENRTNL SCEGCLSPSC LSLLHLQEKN
sp|P25021|HH2R_HUMAN                                MAPNGTASS FCLDSTACKI
sp|P08908|5H1A_HUMAN                MDVLSPG QGNNTTSPPA PFETGGNTTG ISDVTVSYQV
sp|P21761|TRFR_MOUSE                        MENDTV SEMNQTELQP QAAVALEYQV
sp|P30518|V2R_HUMAN                 MLMASTTS AVPGHPSLPS LPSNSSQERP LDTRDPLLAR
sp|P37288|V1AR_HUMAN  LSAGPDAGPS GNSSPWWPLA TGAGNTSREA EALGEGNGPP RDVRNEELAK
sp|P47901|V1BR_HUMAN                MDSGPLWDAN PTPRGTLSAP NATTPWLG-- ---RDEELAK
sp|P30559|OXYR_HUMAN              ME GALAANWSAE AANASAAPPG AEGNRTAGPP R--RNEALAR
          Consensus

                         101                                              150
sp|P02699|OPSD_BOVIN  MLAAYMFLLI MLGFPI[NFLT LYVTVQHKKL RT--PL[NYIL LNL[VV[D[FM
sp|P25103|NK1R_HUMAN  WAAAYT-VIV VTSVV[NVVV MWIILAHKRM RT--VT[YYL VNL[FL[BASM
sp|P21555|NY1R_RAT    LALAYG-AVI ILGVS[NLAL IIIILKQKEM RN--VT[NIL VNL[SFSD[LV
sp|P41143|OPRD_HUMAN  ITALYS-AVC AVGLL[NVLV MFGIVRYTKM KT--AT[NIY FNL[LL[DA-L
sp|P41145|OPRK_HUMAN  ITAVYS-VVF VVGLV[NSLV MFVIIRYTKM KT--AT[NIY FNL[LL[DA-L
sp|P21728|DADR_HUMAN  LTACFLSLLI LSTLL[NTLV CAAVIRFRHL RSKVT-[NFY ISL[VS[DLV
sp|P08588|B1AR_HUMAN  GMGLLMALIV LLIVA[NVLV IVAIAKTPRL QT-LT-[NLY MSL[VS[DLVM
sp|P07550|B2AR_HUMAN  GMGIVMSLIV LAIVF[NVLV ITAIAKFERL QT-VT-[NYF TSL[VC[DLVM
sp|P25100|A1AA_HUMAN  GVGVFLAAFI LMAVA[NLLV ILSVACNRHL QT-VT-[NYF VNL[VV[DLLL
sp|P18841|A1AB_MESAU  SVGLVLGAFI LFAIV[NILV ILSVACNRHL RT-PT-[NYF VNL[IV[DLLL
sp|P28223|5H2A_HUMAN  WSALLTAVVI ILTIA[NILV IMAVSLEKKL QN-AT-[NYF MSL[IL[DLLL
sp|P25021|HH2R_HUMAN  TITVVLAVLI LITVA[NVVV CLAVGLNRRL RN-LT-[NCF VSL[IT[DLLL
sp|P08908|5H1A_HUMAN  ITSLLLGTLI FCAVL[NACV VAAIALERSL QN-VA-[NYF GSL[VT[DFMV
sp|P21761|TRFR_MOUSE  VTILLVVIIC GLGIV[NILV VLVVMRTKHM RT-PT-[NCF VSL[VA[DFMV
sp|P30518|V2R_HUMAN   AELALLSIVF VAVALS[NGLV LAALARRGRR GHWAPIHV[F GHL[CL[DLAV
```


sp|P37288|V1AR_HUMAN
sp|P47901|V1BR_HUMAN
sp|P30559|OXYR_HUMAN
Consensus
TM1
TM2
151
200
sp|P02699|OPSD_BOVIN
sp|P25103|NK1R_HUMAN
sp|P21555|NY1R_RAT
sp|P41143|OPRD_HUMAN
sp|P41145|OPRK_HUMAN
sp|P21728|DADR_HUMAN
sp|P08588|B1AR_HUMAN
sp|P07550|B2AR_HUMAN
sp|P25100|A1AA_HUMAN
sp|P18841|A1AB_MESAU
sp|P28223|5H2A_HUMAN
sp|P25021|HH2R_HUMAN
sp|P08908|5H1A_HUMAN
sp|P21761|TRFR_MOUSE
sp|P30518|V2R_HUMAN
TM3
201
250
TM4
251
300
TM5
301
350

```
sp|P08588|B1AR_HUMAN  FREAQKQVKK IDSCERRFLG GPARPPSPSP SPVPA----- ----------
sp|P07550|B2AR_HUMAN  FQEAKRQLQK IDKSEGRF-- ---------- ---------- ----------
sp|P25100|A1AA_HUMAN  YVVARSTTRS LEAGVKRERG --------KA SEVVL----- ----------
sp|P18841|A1AB_MESAU  YIVAKRTTKN LEAGVMKEMS --------NS KELTL----- ----------
sp|P28223|5H2A_HUMAN  FLTIKSLQKE ATLCVSDLGT --------RA KLASF----- ----------
sp|P25021|HH2R_HUMAN  FKVARDQAKR IN-------- ---------- ---------- ----------
sp|P08908|5H1A_HUMAN  FRAARFRIRK TVKKVEKTGA DTRHGASPAP QPKKSVNGES GSRNWRLGVE
sp|P21761|TRFR_MOUSE  YGFIARILFL NPIPSDPKEN ---------- ---------- ----------
sp|P30518|V2R_HUMAN   FREIHASLVP GPSERPGGRR ---------- ---------- ----------
sp|P37288|V1AR_HUMAN  CYNIWCNVRG KT-ASRQSKG ---------- ---------- ----------
sp|P47901|V1BR_HUMAN  CHEICKNLKV KTQAWRVGGG ---------- ---------- ----------
sp|P30559|OXYR_HUMAN  SFKIWQNLRL KTAAAAAAEA ---------- ---------- ----------
           Consensus

                      351                                                400
sp|P02699|OPSD_BOVIN  ---------- ---------- ---------- ---------- ----------
sp|P25103|NK1R_HUMAN  ---------- ---------- ---------- ---------- ----------
sp|P21555|NY1R_RAT    ---------- ---------- ---------- ---------- ----------
sp|P41143|OPRD_HUMAN  ---------- ---------- ---------- ---------- ----------
sp|P41145|OPRK_HUMAN  ---------- ---------- ---------- ---------- ----------
sp|P21728|DADR_HUMAN  ---------- ---------- ---------- ---------- -HAKNCQTTT
sp|P08588|B1AR_HUMAN  ---------- ---------- ---------- ---PAPPPGP PRPAAAAATA
sp|P07550|B2AR_HUMAN  ---------- ---------- ---------- ---------- -HVQNLSQVE
sp|P25100|A1AA_HUMAN  ---------- ---------- ---------- ---RIHCRGA ATGADGAHGM
sp|P18841|A1AB_MESAU  ---------- ---------- ---------- ---RIHSKNF H---EDTLSS
sp|P28223|5H2A_HUMAN  ---------- ---------- ---------- ---SFLPQSS LS--SEKLFQ
sp|P25021|HH2R_HUMAN  ---------- ---------- ---------- ---------- ----------
sp|P08908|5H1A_HUMAN  SKAGGALCAN GAVRQGDDGA ALEVIEVHRV GNSKEHLPLP SEAGPTPCAP
sp|P21761|TRFR_MOUSE  ---------- ---------- ---------- ---------- -SKMWKNDSI
sp|P30518|V2R_HUMAN   ---------- ---------- ---------- --------RG RRTGSPGEGA
sp|P37288|V1AR_HUMAN  ---------- ---------- ---------- --------AE QAGVAFQKGF
sp|P47901|V1BR_HUMAN  ---------- ---------- ---------- --------GW RTWDRPSPST
sp|P30559|OXYR_HUMAN  ---------- ---------- ---------- --------PE GAAAGDGGRV
           Consensus

                      401                                                450
sp|P02699|OPSD_BOVIN  ---------- -----ATTQK AEKEVTRMVI IMVIAFLICW LP---YAGVA
sp|P25103|NK1R_HUMAN  ---------- ----YHEQVS AKRKVVKMMI VVVCTFAICW LPFHIFFLLP
sp|P21555|NY1R_RAT    ---------- ----SKYRSS ETKRINVMLL SIVVAFAVCW LPLTIFNTVF
sp|P41143|OPRD_HUMAN  ---------- -----KEKDR SLRRITRMVL VVVGAFVVCW APIHIFVIVW
sp|P41145|OPRK_HUMAN  ---------- -----REKDR NLRRITRLVL VVVAVFVVCW TPIHIFILVE
sp|P21728|DADR_HUMAN  GNGKPVECSQ PESSFKMSFK RETKVLKTLS VIMGVFVCCW LPFFILNCIL
sp|P08588|B1AR_HUMAN  PLANGRAGKR RPS--RLVAL REQKALKTLG IIMGVFTLCW LPFFLANVVK
sp|P07550|B2AR_HUMAN  QDGRTGHGLR RSS--KF-CL KEHKALKTLG IIMGTFTLCW LPFFIVNIVH
sp|P25100|A1AA_HUMAN  RSAKGHTFRS SLSVRLLKFS REKKAAKTLA IVVGVFVLCW FPFFFVLPLG
sp|P18841|A1AB_MESAU  TKAKGHNPRS SIAVKLFKFS REKKAAKTLG IVVGMFILCW LPFFIALPLG
sp|P28223|5H2A_HUMAN  RSIHREPGSY TGRRTMQSIS NEQKACKVLG IVFFLFVVMW CPFFITNIMA
sp|P25021|HH2R_HUMAN  ---------- HISSWKAATI REHKATVTLA AVMGAFIICW FPYFTAFVYR
sp|P08908|5H1A_HUMAN  ASFERKNERN AEAKRKMALA RERKTVKTLG IIMGTFILCW LPFFIVALVL
sp|P21761|TRFR_MOUSE  HQNKNLNLNA TNRCFNSTVS SRKQVTKMLA VVVILFALLW MPYRTLVVVN
sp|P30518|V2R_HUMAN   ---------- ------HVSA AVAKTVRMTL VIVVVYVLCW APFFLVQLWA
sp|P37288|V1AR_HUMAN  LLAPC----- -VSSVKSISR AKIRTVKMTF VIVTAYIVCW APFFIIQMWS
sp|P47901|V1BR_HUMAN  LAATTRGLPS RVSSINTISR AKIRTVKMTF VIVLAYIACW APFFSVQMWS
sp|P30559|OXYR_HUMAN  ALA------- RVSSVKLISK AKIRTVKMTF IIVLAFIVCW TPFFFVQMWS
           Consensus                          k  kml  !v  %  cW  Pff
                                        ------------ TM6 ------------

                      451                                                500
sp|P02699|OPSD_BOVIN  FYIF--THQG SDFGPIFMTI PAFFAKTSAV YNPVIYIMMN KQ-FRNCMVT
sp|P25103|NK1R_HUMAN  YINP--DLYL KKFIQQVYLA IMWLAMSSTM YNPIIYCCLN DR-FRLGFKH
sp|P21555|NY1R_RAT    DWNH--QIIA TCNHNLLFLL CHLTAMISTC VNPIFYGFLN KN-FQRDLQF
sp|P41143|OPRD_HUMAN  TLVD--IDRR DPLVVAALHL CIALGYANSS LNPVLYAFLD EN-FKRCFRQ
sp|P41145|OPRK_HUMAN  ALGS--TSH- STAALSSYYF CIALGYTNSS LNPILYAFLD EN-FKRCFRD
sp|P21728|DADR_HUMAN  PFCGSGETQP FCIDSNTFDV FVWFGWANSS LNPIIY-AFN AD-FRKAFST
sp|P08588|B1AR_HUMAN  AF------HR ELVPDRLFVF FNWLGYANSA FNPIIY-CRS PD-FRKAFQG
sp|P07550|B2AR_HUMAN  VI------QD NLIRKEVYIL LNWIGYVNSG FNPLIY-CRS PD-FRIAFQE
sp|P25100|A1AA_HUMAN  SLFP--QLKP SE---GVFKV IFWLGYFNSC VNPLIYPCSS RE-FKRAFLR
sp|P18841|A1AB_MESAU  SLFS--TLKP PD---AVFKV VFWLGYFNSC LNPIIYPCSS KE-FKRAFMR
sp|P28223|5H2A_HUMAN  VICK--ESCN EDVIGALLNV FVWIGYLSSA VNPLVYTLFN KT-YRSAFSR
sp|P25021|HH2R_HUMAN  GL-----RGD DAINEVLEAI VLWLGYANSA LNPILYAALN RD-FRTGYQQ
sp|P08908|5H1A_HUMAN  PFC----ESS CHMPTLLGAI INWLGYSNSL LNPVIYAYFN KD-FQNAFKK
sp|P21761|TRFR_MOUSE  SF-----LSS PFQENWFLLF CRICIYLNSA INPVIYNLMS QK-FRAAFRK
```

```
sp|P30518|V2R_HUMAN   AWDP--EAPL EGA---PFVL LMLLASLNSC TNPWIYASFS SSVSSE-LRS
sp|P37288|V1AR_HUMAN  VWDP--MSVW TESENPTITI TALLGSLNSC CNPWIYMFFS GHLLQDCVQS
sp|P47901|V1BR_HUMAN  VWDK--NAPD EDSTNVAFTI SMLLGNLNSC CNPWIYMGFN SHLLPRPLRH
sp|P30559|OXYR_HUMAN  VWDA--NAPK EAS---AFII VMLLASLNSC CNPWIYMLFT GHLFHELVQR
            Consensus                        lgy ns   NP iy       f  f
                                          ---------- TM7 ----------
                      501                                              550
sp|P02699|OPSD_BOVIN  TLCGGKNPLG DDEASTTVSK TETSQVAPA
sp|P25103|NK1R_HUMAN  AFRSCPFISA GDYEGLEMKS TRYLQTQGSV YKVSRLETTI STVVGAHEEE
sp|P21555|NY1R_RAT    FFNFCDFRSR DDDYETIAMS TMHTDVSKTS LKQASPVAFK KISMNDNEKI
sp|P41143|OPRD_HUMAN  LCRKPCGRPD PSSFSRPREA TARERVTACT PSDGPGGGRA A
sp|P41145|OPRK_HUMAN  FCFPLKMRME RQSTSRVRNT VQDPAYLRDI DGMNKPV
sp|P21728|DADR_HUMAN  LLGCYRLCPA TNNAIETVSI NNNGAAMFSS HHEPRGSISK ECNLVYLIPH
sp|P08588|B1AR_HUMAN  LLCCARRAAR RRHATHGDRP RASGCLARPG PPPSPGAASD DDDDDVVGAT
sp|P07550|B2AR_HUMAN  LLCLRRSSLK ----AYGNGY SSNGNTGEQ- ---SGYHVEQ EKENKLLCED
sp|P25100|A1AA_HUMAN  LLRCQCR--R RRRRRPLWRV YGHHWRASTS GLRQDCAPSS GDAPPGAPLA
sp|P18841|A1AB_MESAU  ILGCQCRSGR RRRRRRRLGA CAYTYRPWTR GGSLERSQSR KDSLDDSGSC
sp|P28223|5H2A_HUMAN  YIQCQYKENK KPLQLILVNT IPALAYKSSQ LQMGQKKNSK QDAKTTDNDC
sp|P25021|HH2R_HUMAN  LFCCRLANRN SHKTSLRSNA SQLSRTQSRE PRQQEEKPLK LQVWSGTEVT
sp|P08908|5H1A_HUMAN  IIKCKFCRQ
sp|P21761|TRFR_MOUSE  LCNCKQKPTE KAANYSVALN YSVIKESDRF STELEDITVT DTYVSTTKVS
sp|P30518|V2R_HUMAN   LLCCARGRTP PSL-GPQDES CTTASSSLAK DTSS
sp|P37288|V1AR_HUMAN  FPCCQNMKEK FNK-EDTDSM --SRRQTFYS NNRSPT---- NSTGMWKDSP
sp|P47901|V1BR_HUMAN  LACCGGPQPR MRR-RLSDGS LSSRHTTLLT RSSCPATLSL SLSLTLSGRP
sp|P30559|OXYR_HUMAN  FLCCSASYLK GRRLGETSAS KKSNSSSFVL SHRSSSQRSC SQPSTA
            Consensus    C

                      551                                              600
sp|P02699|OPSD_BOVIN
sp|P25103|NK1R_HUMAN  PEDGPKATPS SLDLTSNCSS RSDSKTMTES FSFSSNVLS
sp|P21555|NY1R_RAT
sp|P41143|OPRD_HUMAN
sp|P41145|OPRK_HUMAN
sp|P21728|DADR_HUMAN  AVGSS-EDLK KEEAAGIARP LEKLSPALSV ILDYDTDVSL EKIQPITQNG
sp|P08588|B1AR_HUMAN  PPARLLEPWA GCNGGAAADS DSSLDEPCRP GFASESKV
sp|P07550|B2AR_HUMAN  LPGT--EDFV GHQGTVPSDN ID
sp|P25100|A1AA_HUMAN  LTALPDPDPE PPGTPEMQAP VASRRKPPSA FREWRLLGPF RRPTTQLRAK
sp|P18841|A1AB_MESAU  MSGSQRTLPS ASPSPGYLGR GAQPPLELCA YPEWKSGALL SLPEPPGRRG
sp|P28223|5H2A_HUMAN  SMVALGKQHS EEASKDNSDG VNEKVSCV
sp|P25021|HH2R_HUMAN  APQGATDR
sp|P08908|5H1A_HUMAN
sp|P21761|TRFR_MOUSE  FDDTCLASEN
sp|P30518|V2R_HUMAN
sp|P37288|V1AR_HUMAN  KSSKSIKFIP VST
sp|P47901|V1BR_HUMAN  RPEESPRDLE LADGEGTAET IIF
sp|P30559|OXYR_HUMAN
            Consensus

                      601                                              650
sp|P02699|OPSD_BOVIN
sp|P25103|NK1R_HUMAN
sp|P21555|NY1R_RAT
sp|P41143|OPRD_HUMAN
sp|P41145|OPRK_HUMAN
sp|P21728|DADR_HUMAN  QHPT
sp|P08588|B1AR_HUMAN
sp|P07550|B2AR_HUMAN
sp|P25100|A1AA_HUMAN  VSSLSHKIRA GGAQRAEAAC AQRSEVEAVS LGVPHEVAEG ATCQAYELAD
sp|P18841|A1AB_MESAU  RLDSGPLFTF KLLGEPESPG TE-GDASNGG CDATTDLANG QPGFKSNMPL
sp|P28223|5H2A_HUMAN
sp|P25021|HH2R_HUMAN
sp|P08908|5H1A_HUMAN
sp|P21761|TRFR_MOUSE
sp|P30518|V2R_HUMAN
sp|P37288|V1AR_HUMAN
sp|P47901|V1BR_HUMAN
sp|P30559|OXYR_HUMAN
            Consensus
```

```
                                651
sp|P02699|OPSD_BOVIN
sp|P25103|NK1R_HUMAN
sp|P21555|NY1R_RAT
sp|P41143|OPRD_HUMAN
sp|P41145|OPRK_HUMAN
sp|P21728|DADR_HUMAN
sp|P08588|B1AR_HUMAN
sp|P07550|B2AR_HUMAN
sp|P25100|A1AA_HUMAN  YSNLRETDI
sp|P18841|A1AB_MESAU  APGHF
sp|P28223|5H2A_HUMAN
sp|P25021|HH2R_HUMAN
sp|P08908|5H1A_HUMAN
sp|P21761|TRFR_MOUSE
sp|P30518|V2R_HUMAN
sp|P37288|V1AR_HUMAN
sp|P47901|V1BR_HUMAN
sp|P30559|OXYR_HUMAN
          Consensus
```

Figure 3. *A typical multiple sequence alignment. 18 various GPCRs were aligned using the Multalin program; see the header in the listing for reference. The SWISS-PROT-encoded sequences decypher into the following human (unless otherwise stated) GPCRs in the descending order: bovine rhodopsin, NK1 receptor, rat neuropeptide Y(1) receptor, opioid δ receptor, opioid κ receptor, D(1a) dopamine receptor, adrenergic β 1a receptor, adrenergic β 2a receptor, adrenergic α1a receptor, rabbit adrenergic α1b receptor, serotonin 2A receptor, histamine H2 receptor, serotonin 1A receptor, mouse thyroliberin receptor, V2R, V1aR, V1bR and OTR. Invariant residues are black and conservative residues are gray. The trasmembrane helices TM1-TM7 are underlined.*

The following three schemes for 7TM building are our favorite:

(i) The older scheme of Baldwin [4], based on a critical alignment, see Figure 3, of ~200 GPCR sequences. Through an extensive examination of distributions along the putative helices of polar/non-polar and conservative/non-conservative residues, the method enables a rational choice of the helical sequences TM1-TM7 and their unique threading onto a low-resolution structure of rhodopsin. This 7TM model was subsequently refined to the self-consistency of 1.67Å, by the inclusion of all experimentally available distance, positional and orientational constraints typical of bovine rhodopsin [26]. The automated GPCR-modeling server, based on this scheme, is available via Internet [27].

(ii) The most recent scheme of Baldwin et al [19] using the same rationale as the original one [4] yet for as many as ~500 sequences and therefore enabling refinements such as variable helical lengths, relative TM shifts, and kinks in TM5 and TM6, all features affirmed by recent experimental data. A relevant 7TM template is available from the authors upon request.

(iii) The *ab initio* model of Mosberg et al [28]. This model also uses multi-sequence alignment for the choice of the 7TM helices, followed by a distance-geometry optimization applied simultaneously to 410 GPCR sequences. First, putative hydrogen bonds between polar/charged residues within the GPCR interior are

singled out. Subsequently, they are used collectively and simultaneously for many overlapping GPCRs as constraints in the iterative distance-geometry procedure, aimed at the optimization of *an averaged* 7TM bundle.

All three methods have in common an extensive use of multi-sequence analysis for making choices of TM1 through TM7. Ones the helices singled out, the methods [(i) and (ii)] probe the TM1-TM7 mutual arrangement either by a rule-based threading them onto the foggy shape of rhodopsin, upon an assumption that the sequence homology legitimates 7TM 3-dimensional structure homology, or [Method (iii)] by a rule-based *ab initio* 7TM arrangement optimization. Methods (i) and (ii) work only at the C^{α}-trace level and are strictly limited to modeling the 7TM bundle, while Method (iii) in principle can also be used for modeling EL and IL loops. These are non-conservative among the receptor types and much more obscure for modeling than the 7TM domain. All three methods give similar averaged shape of the 7TM bundle, Figure 4. Interestingly, the resulting bundles from (ii) and (iii) better overlap than (i) and (ii), despite close methodological relationship between the latter two.

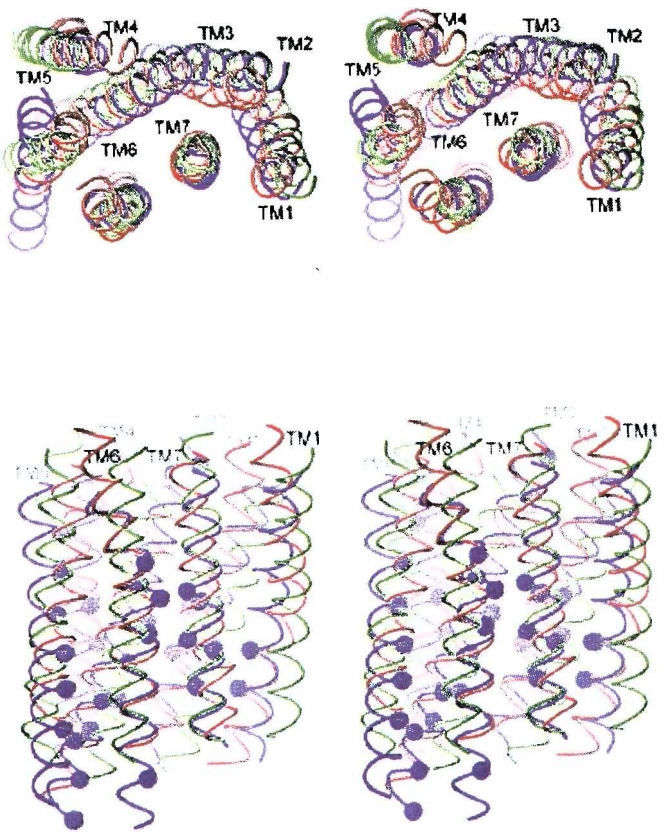


Figure 4. *Stereodiagram of overlapping TM bundles resulting from procedure: (i) green, (ii) blue and (iii) red. Extracellular view onto the membrane surface (TOP) and lateral view (BOTTOM). The conservative residues as indicated in Ref. [19] are marked with balls in Model (ii).*

3. Biological Signal Transductuion via Vasopressin V2 Receptor

Vasopressin (AVP) and oxytocin (OT) are two similar nonapeptide hormones produced in the neurophysis and released to the blood in the pituitary posterior lobe. They differ only on the two amino acids **X** and **X'** in their otherwise common sequence: CY**X**QNCP**X'**G-NH_2, where in AVP (X, X') = (F, R) and in OT (X, X') = (I, L). Major AVP activities consist of blood pressure control via the V1a receptors (V1aR) in blood vessels and urine concentration (antidiuresis) via the V2 receptors (V2R) in the kidney. Oxytocin controls labor and lactation in mammalian females via a common oxitocin receptor (OTR) in the uterus and the mammalian gland, respectively. V1aR, V2R and OTR, being 370-400 amino acid long, are typical members of the rhodopsin family of GPCRs, compare Figure 3. For the best-studied AVP/V2R system a number of mutations were identified, giving rise to the hereditary X-linked (i.e. carried by women but affecting boys) nephrogenic diabetes insipidus, consisting in a disability to concentrate the urine, resulting in an extensive diuresis and, possibly, death of dehydratation [29]. Other variants of nephrogenic diapetes insipidus, resulting from pathological deficiency of AVP, are cured by administration of V2R-selective AVP superagonist, desamino-[D-Arg^9]AVP (DDAVP, desmopressin®) [29]. Both, the pathological mutations and model biochemical experiment, using mutagenesis [30, 31, 32] as a tool for studying V2R structure-activity relationships, warrant molecular modeling of V2R and its interaction with bioligands.

Our initial V2R model was obtained using Method (i), see above. The loops EL1-EL3, IL1-IL3 and the N- and the C-termini were added using protein loop-building tools inherent in Sybyl suite of programs [33]. Initial ligand docking was attained in several ways, always respecting a complementarity in the electrostatic potentials of the V2R cleft, see below, and the ligand. The systems were

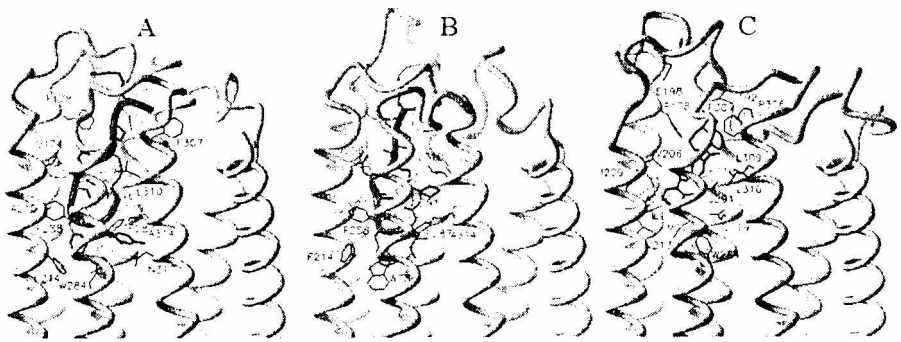


Figure 5. *Optimized V2R/bioligand complexes. Only the extracellular parts are shown. V2R is gray-shaded and the ligands are black. The receptor's interacting residues are labeled and their side chains exposed. A. V2R/AVP; B. V2R/selective peptide antagonist desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP; C. V2R/selective nonpeptide antagonist OPC-31260.*

equilibrated using a Constrained Simulated Annealing (CSA) protocol, with all but the 7TM C^{α} atoms free to move. Optimal ligand docking modes were chosen using the ligand/receptor interaction energies and structure-activity data [34, 35] as the selection criteria. Our studies consisted of systems' relaxation using CSA in vacuo in the earlier works [36, 37, 38], and the molecular dynamics for the systems immersed in the fully hydrated phospholipid bilayer in the recent works [39, 40]. Details of computing and analyses are described elsewhere [36, 40].

From Figure 5 it is seen that any GPCR modeled to the RD template [26, 27] has a ~21Å deep cavity on the extracellular side, surrounded by TM3-TM7, with a narrower extension towards TM2. The cavity ends up on a floor from the hydrophobic residues TM3:M123, TM4:L170, TM5:V213,F214 and TM6:W284, F287,F288 in V2R. The cleft is large enough to accommodate the pressin ring (CYFQNC) of AVP and even more so to fit the OPC-31260 non-peptide antagonist. Most of the simulations, whether with a peptide ligand or not, converged to the docking modes typical of V2R/AVP [36]. However, OPC-31260 as much thinner than the AVP pressin ring, cannot fill up the entire V2R cleft and it adheres to the front side of the TM3-TM7 cavity in its most preferred arrangements, see Figure 5C [37].

In Figure 5 all V2R interacting residues are marked so that the significant receptor-ligand interactions could be seen. Both the V2R/peptide complexes develop a number of polar and nonpolar interactions with the cleft walls. Major interactions, common to both AVP and its peptide antagonist desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP involve on the V2R part TM3:C112,V115-K116,Q119,M123; TM4:Q174; TM5:V206,A210,V213; TM6:W284,F287,F288,Q291 and TM7:F307,L310,A314,N317; see Figs. 5A and 5B. The Mca* β,β-pentamethylene moiety fits snugly a hydrophobic pocket formed by TM3:V115 and TM7:L310 and A314. The non-peptide antagonist OPC-31260 orients itself typically so that its long axis is nearly vertical and its $HN(CH_3)_2^+$ is involved in an (bifurcated) ion bridge with one (two) of the numerous negatively charged Asp and/or Glu residues in ELs, see Figure 5. With this regard, it is interesting to notice that EL2 contains three carboxylates in V2R and two in V1aR, which may bear on the increased V2R/V1aR selectivity of the OPC-31260* analogs with a cationic group in the equivalent place [35].

The tendency for all three ligands to dock within the same compartment of the V2R extracellular cavity, suggests a simple competitive mechanism for the antagonism toward V2R by both desGly^9-[Mca^1,D-Ile^2,Ile^4]AVP and OPC-31260. The V2R amino acid residues, involved in ligand binding, are invariant or conservative for the subfamily, or even invariant over the whole GPCR superfamily (TM3:C112, TM4:Q174, TM6:W284,F287 and TM7:N317). The invariant (conservative) residues within the subfamily may be pertinent to ligand binding while those invariant over the whole GPCR superfamily may have to do with the

* Abbreviations: **Mca** β,β-cyclopenta-methylene-β-mercaptopropionyl; **OPC-31260** [5-dimethylamino-1-{4-(2-methyl-benzoyl-amino)- benzoyl}-2,3,4,5-tetrahydro-1H-benzazepine.

signal transduction, putatively universal for the whole GPCR superfamily. Our results on agonist docking agree with those obtained by Mouillac et al for a related AVP/V1aR system. Furthermore, some of the equivalent V1aR residues have already been found critical for the ligand affinity [41].

In our most recent work [40] we performed comparative unconstrained molecular dynamics of the AVP/V2R complex and an empty V2R in the fully hydrated lecithine (dimyristoylphosphatidilcholine, DMPC) bilayer. In Figure 6 the interiors of the intracellular sides of empty V2R and its V2R/AVP complex are compared. Both images represent well-relaxed structures, resulting from averaging the last 300 ps of ~1500 ps total simulation in each case. It is seen that in the V2R/AVP complex there is a contiguous network of internal polar receptor residues, extending from the ligand to the cytosolic domain, whereas in the empty V2R a similar network is interrupted. The most prominent difference regards the TM3:Thr134-TM7:Tyr325 contact, present in the V2R/AVP complex but absent in the empty V2R. Interestingly, both residues belong to those universally conserved over the GPCR superfamily [4, 19], see Figure 3. Thus, the relaxed structures are compatible with the active and passive forms of V2R respectively, and possibly indicative of details of an allosteric signal transduction mechanism.

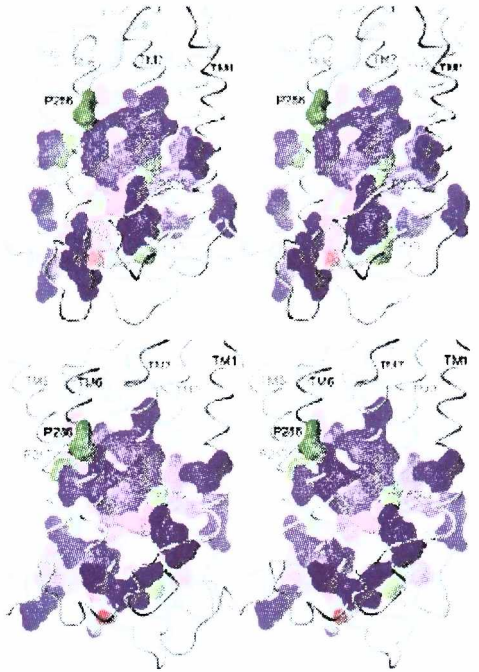


Figure 6. *Space-filled networks of polar residues (blue) and prolines (green), spanning interior of V2R from AVP (thick gray backbone) to the conservative and important in signal transduction (see Figure 3) DRH sequence at the C-terminus of TM3 (standard colors: C,H gray, O red and N blue): in V2R/AVP complex (TOP) and in empty V2R (BOTTOM). It is seen that a contiguous network only preserves in the receptor-ligand complex. Note that the TM3:Thr134-TM7:Tyr325 (pink) contact is preset in the V2R/AVP complex while it is absent in the empty V2R.*

4. Perspectives

Contemporary protein modeling is not advanced enough for full credibility. Rather, it hopefully may guide as to specific mutagenesis/affinity studies aimed at the verification of arising hypotheses. The afore-mentioned V2R residues, appearing to be responsible for ligand binding, should be among the first candidates for experiments of this kind. Progress in GPCR modeling may be expected from the following areas:

(1) The Protein Structure Database at Brookhaven [42] is growing exponentially, now exceeding 7000 objects totally and 1000 having unique folds [43], thus providing a basis for analyses and taxonomy of structural protein motifs. The analysis of the accumulation rate of these motifs optimistically indicates that in 5-10 years, more or less simultaneously with the completion of the human genome project, the collection of the structural motifs may approach the state of saturation [43, 44], which in turn forecasts well for homology modeling.

(2) Progress continues in the electron cryo-microscopy going down to lower and lower temperatures, towards the liquid helium. This will dramatically reduce a destructive effect of the electron beam thus improving the number of collected structure factors and eventually the resolution. A measure of success in this field may be the first high-resolution structure solution of bacteriorhodopsin (another 7TM integral membrane protein yet not a GPCR) [25], giving hope that other TM proteins will follow soon.

(3) A new era for X-ray (micro)crystallography is coming, implementing cubic lipid phase [45]. This, together with the third-generation synchrotronic sources, providing for enormously strong X-ray radiation, forecast a possible break-through in the X-ray crystallography of integral membrane proteins [46, 47]. Two recent high-resolution structure solutions, using these new techniques for the most studied landmark protein bacteriorhodopsin [24, 48], argue in favor of this optimism.

If a break-through in the accumulation rate of the transmembrane protein motifs to the Protein Structure Database were indeed around the corner, then the basis for an increasingly rationalized homology modeling of GPCRs would grow rapidly.

Acknowledgement

The work was supported by the Polish Scientific Research Committee (KBN), grant DS/8245-4-0124-8, by the Academic Computer Centre in Gdańsk TASK and by the Interdisciplinary Center for Mathematical and Computational Modeling (ICM) at the University of Warsaw. C. C. is a recipient of the fellowship from Foundation for Polish Science. Partial support for M.P. by a grant from the Foundation for Polish Science (Grant BiMOL 103/93) is acknowledged.

References

[1] Ji T. H., Grossman M., Ji I., *G protein-coupled receptors: I. Diversity of receptor-ligand interactions*, J. Biol. Chem. **273**: 17299-17302 (1998)

[2] Unger V. M., Schertler G. F. X., *Low resolution structure of bovine rhodopsin determined by electron cryo-microscopy*, Biophys. J., **68**: 1776-1786 (1995)

[3] Schertler G. F. X., Hargrave P. A., *Projection structure of frog rhodopsin in two crystal forms*, Proc. Natl. Acad. Sci. **92**: 11578-11582 (1995)

[4] Baldwin J. M., *The probable arrangements of the helices in G protein-coupled receptors*, EMBO J. **12**, 1693-1703 (1993)

[5] Baldwin J. M., *Structure and function of receptors coupled to G proteins*, Curr. Opinion. Cell Biol. **6**: 180-190 (1994)

[6] Bikker J. A., Trumpp-Kallmeyer S., Humblet C., *G-protein coupled receptors: models, mutagenesis, and drug design*, J. Med. Chem. **41**: 2911-2927 (1998)

[7] Iismaa T. P., Biden T. J., Shine J., *G Protein-Coupled Receptors*, Springer-Verlag, Heidelberg, Germany, 1995

[8] Watson S., Arkinstall S., *The G-protein linked receptor*, Facts Book, Academic Press Ltd., London 1994

[9] Gether U., Kobilka B., *G protein-coupled receptors: II. Mechanism of agonist activation*, J. Biol. Chem. **273**: 17979-17982 (1998)

[10] Sprang S. R., *G protein mechanisms: insights from structural analysis*, Annu. Rev. Biochem. **66**: 639-678 (1997)

[11] Lambright D. G., Noel J. P., Hamm H. E., Sigler P. B., *Structural determinants for activation of the α-subunit of a heterotrimeric G protein*, Nature **369**: 621-628 (1994)

[12] Mixon M. B., Lee E., Coleman D. E., Berghuis A. M., Gilman A. G. Sprang S. R., *Tertiary and quaternary structural changes in $G_{i\alpha 1}$ induced by GTP hydrolysis*, Science **270**: 954-960 (1995)

[13] Noel J. P., Hamm H. E., Sigler P. B., *The 2.2Å crystal structure of transducin-α complexed with GTPγS*, Nature **366**: 654-663 (1993)

[14] Coleman D. E., Berghuis A. M., Lee E., Linder M. E., Gilman A. G., Sprang, S. R., *Structure of active conformations of $G_{i\alpha 1}$ and the mechanism of GTP hydrolysis*, Science **265**: 1405-1412 (1994)

[15] Sondek J., Bohm A., Lambright D. G., Hamm H. E., Sigler P. B., *Crystal structure of a G protein βγ dimer at 2.1Å resolution*, Nature **379**: 369-374 (1996)

[16] Lambright D. G., Sondek J., Bohm A., Skiba N. P., Hamm H. E., Sigler P. B., *The 2.0Å crystal structure of heterotrimeric G protein*, Nature **379**: 311-319 (1996)

[17] Wall M. A., Coleman D. E., Lee E., Iñiguez-Lluhi J. A., Posner B. A., Gilman A. G., Sprang S. R., *The structure of the G protein heterotrimer $G_{i\alpha 1\beta 1\gamma 2}$*, Cell **83**: 1047-1058 (1996)

[18] Unger V. M., Hargrave P. A., Baldwin J. M., Schertler G. F. X., *Arrangement of rhodopsin transmembrane α-helices*, Nature, **389**: 203-206 (1997)

[19] Baldwin J. M., Schertler G. F. X., Unger V. M., *An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors*, J. Mol. Biol. **272**: 144-164 (1997)

[20] Henderson R., Baldwin J. M., Ceska T. A., Zemlin F., Beckman E., Downing K. H., J. Mol. Biol., **213**: 899 (1990)

[21] Vriend G., GPCRDB ©1996 (http://swift.embl-heidelberg.de/7tm/)

[22] Trumpp-Kallmeyer S., Hoflack J., Bruinvels A., Hibert M., *Modeling of G-protein-coupled receptors: application to dopamine, adrenaline, serotonin, Acetylcholine, and mammalian opsin receptors*, J.Med.Chem. **35**: 3448-3462 (1992)

[23] Grigorieff N., Ceska T. A., Downing K. H., Baldwin J. M., Henderson R., *Electron-crystallographic refinement of the structure of bacteriophodopsin*, J. Mol. Biol. **259**: 393-421 (1996)

[24] Pebay-Peyroula E., Rummel G., Rosenbusch J. P., Landau E. M., *X-ray structure of bacteriorhodopsin at 2.5 Angstroms from microcrystals grown in lipidic cubic phases*, Science **277**: 1676-1681 (1997)

[25] Kimura Y., Vassylyev D. G., Miyazawa A., Kidera A., Matsushima M., Mitsuaka K., Murata K., Hirai T., FujioshiY., *Surface of bacteriorhodopsin revealed by high-resolution electron crystallography*, Nature **389**: 206-211 (1997)

[26] Herzyk P., Hubbard R. E., *Automated method for modeling seven-helix transmembrane receptors from experimental data*, Biophys. J. **69**: 2419-2442 (1995)

[27] Peitch M. C., Herzyk P., Wells T. N. C., Hubbard R. E., *Swiss-Model, an automated knowledge-based protein modeling server at Glaxo Wellcome in Geneva*, (http:// expasy.hcuge.ch/swissmod/SWISS-MODEL.html)

[28] Pogozheva I. D., Lomize A. L., Mosberg,H. I., *The transmembrane 7-α-bundle of rhodopsin: distance geometry calculation with hydrogen bonding constraints*, Biophys. J. **70**: 612-634 (1997)

[29] Gross, P., Richter D., Robertson G. L., Eds., *Vasopressin*, John Libbey Eurotext, 1993, Paris

[30] Postina R., Kojro E., Fahrenholz F., *Separate agonist and peptide antagonist binding sites of the oxytocin receptor defined by their transfer into the V2 vasopressin receptor*, J.Biol.Chem. **271**: 31593-31601 (1996)

[31] Kojro E., Eich P., Gimpl G., Fahrenholz F., *Direct identification of an extracellular agonist binding site in the renal V2 vasopressin receptor*, Biochemistry **32**: 13537-13544 (1993).

[32] Liu J., Wess J., *Different single receptor domains determine the distinct G protein coupling profiles of members of the vasopressin receptor family*, J. Biol. Chem. **271**: 8772-8778 (1996)

[33] *SYBYL*, v. 6.1, Tripos Inc., St. Louis, MO, U.S.A., 1994

[34] Manning M., Sawyer W. H., *Design, synthesis and some uses of receptor-specific agonists and antagonists of vasopressin and oxytocin*, J. Recept. Res. **13**: 195-214 (1993)

[35] Ogawa H., Yamashita H., Kondo K., Yamamura Y., Miyamoto H., Kan K., Kitano K., Tanaka M. Nakaya K., Nakamura S., Mori T., Tominaga M., Yabuuchi Y., *Orally active, nonpeptide vasopressin V2 receptor antagonists: a novel series of 1-[4-(benzoylamino)benzoyl]-2,3,4,5-tetrahydro-1H-benzazepines and related compounds*, J. Med. Chem. **39**: 3547-3555 (1996)

[36] Czaplewski C., Kaźmierkiewicz R., Ciarkowski J., *Molecular modeling of the human vasopressin V2 receptor/agonist complex*, J. Comp.-Aided Molec. Design, **12**: 275-287 (1998)

[37] Czaplewski C., Kaźmierkiewicz R., Ciarkowski J., *Vasopressin V2 receptor/bioligand interactions*, Lett. Peptide Sci **4**: (1998)

[38] Czaplewski C., Kaźmierkiewicz R., Ciarkowski J., *Molecular modelling of the vasopressin V2 receptor/ antagonists interactions*, Acta Biochim. Pol **45**: 19-26 (1998)

[39] Czaplewski C., Pasenkiewicz-Gierula M., Ciarkowski J., *Molecular dynamics of a vasopressin V2 receptor in a phospholipid bilayer membrane*, J. Rec. Signal Transduction Res **19**: in press (1999)

[40] Czaplewski C., Pasenkiewicz-Gierula M., Ciarkowski J., *G protein-coupled receptor - bioligand interactions modeled in a phospholipid bilayer*, Int. J. Quantum Chem.Biophys. Quart.

[41] Mouillac B., Chini B., Balestre M. N., Elands J., Trumpp-Kallmeyer S., *Hoflack J., Hibert M., Jard S., Barberis C., The binding site of neuropeptide vasopressin V1a receptor*, J.Biol.Chem. **270**: 25771-25777 (1995)

[42] Bernstein F. C., Koetzle T. F., Williams G. J., Meyer E. E. J., Brice D. M., Rodgers J. R., Kennard O., Shimanouchi T., Tasumi M., *Protein Data Bank: a computer based archival file for macromolecular structures*, J.Mol.Biol. **112**: 535-542 (1977)

[43] Holm L., Sander C., *Mapping the protein universe*, Science **273**: 595-602 (1996); Holm, L., Sander, C., *New structure - novel fold?* Structure **5**: 165-171 (1997)

[44] Brenner S. E., Chothia C., Hubbard T. J. P., *Population statictics of protein structures: lessons from structural classifications*, Curr. Opinion Struct. Biol. **7**: 369-376 (1997)

[45] Gouauc E., *It's not just a phase: crystallization and X-ray structure determination of bacteriorhodopsin in lipidic cubic phases*, Structure **6**: 5-10 (1998)

[46] Service R. F., *Brightness speeds search for structure great and small*, Science **277**: 1217-1219 (1997)

[47] Moffat A. S., *Opening the door to more membrane protein structures*, Science **277**: 1607-1608 (1997)

[48] Luecke H., Richter H. T., Lanyi J. K., *Proton transfer pathways in bacteriorhodopsin at 2.3Å resolution*, Science **280**: 1934-1937 (1998)