

SUPPLEMENTARY MATERIAL

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μOR MDSSAAPTNASNCTDALAYSSCPSPAPSPGWSVNLSHLDGNLSDPCGNRNTDLGGRD SLCPTTSPSPMITAITIMALYSIVCVVGLFGNFLVMYVIVRYTKMKTAT
δOR MEP--APSAGAEIQPPLFANASDAYPS-----ACPSAGANASGPPGAR-----SASSLALAIITALYSAVCAVGLLGNVLVMFGIVRYTKMKTAT
κOR MESP-IQIFRGEPEGPTCAPSACLPPNSSAWFPGWAEPSNGSAGSEDAQLEP-----AHISPAIPVITAVYSVVFVVLVGNLSLVMFVIIRYTKMKTAT
β2-AR ---PGNGSAFLLAPNRSHAPDHDVTQQR-----DEVVWVGMGIVMSLIVLAIIVFGNVLVITAIKFERLQTVT
β1-AR -----MGDGLWLPDCGPHNRSGGGGATAAPTGRSQRVSAELL-----SQQWEAGMSLLMALVLLVIVAGNVLVIAAIGRTQRLQTLT
A2aR -----MPIMGSSVYITVELAIIVLAILGNVLVLCWAVWLNSNLQNVIT
Rhod -----MNGTEGPNFYVFPFSNKTGVVRSPEAP-----QYLLAEFPQFSMLAAYMFLILMLGFPINFLTLTYVTVQHKKLRTPL

μOR NIYIFNLALADALATS-TLPPQSVNYLMGTWPFPGTIICKIVISIDYNNMFTSIFTLCTMSVDRYIAVCHPVKALDFRTPRNAKINVCNWLSSAIGLPVFMFAT
δOR NIYIFNLALADALATS-TLPPQSAKYLMETWPFGEIICKAVLSIDYNNMFTSIFTLTMMSVDRYIAVCHPVKALDFRTPAKAKLINICIWVLSAGVGPIMVMAV
κOR NIYIFNLALADALVTT-TMPFQSTVYLMNSWPFPGDVCKIVISIDYNNMFTSIFTLTMMSVDRYIAVCHPVKALDFRTPKAKIINICIWLLSSSVGISAIVLGG
β2-AR NYFITSLACADLVMLAVVPPGAHILMKMWTFGNFWCEFWTSIDVLCVTASIE TLCVIAVDRYFAITSPPKYQSLTKNKARVILMVWIVSGLTSPFPIQMHW
β1-AR NLFITSLACADLVMLLVVPPGATLVVRGTWLVGSPLCECWTSIDVLCVTASIE TLCVIAIDRYLAITSPPFRYQSLMTRARAKVIICTVWVAISALVSPFIMMHW
A2aR NYFVVSLLAAADI AVGLVLAIPFAITISTG--FCAACHGCLFIACFVVLVTQSSIFSLLAIAIDRYIAIRIPLRYNGLVTGTRAKGIIAICVWLSFAIGLTPMLGWN
Rhod NYILLNLAVADLFMVFGGFTTLYTSLHGYFVFGPTGCNLEGGFFATLGGIEALWLVVLAERYVVVCKPMSNFRFG-ENHAIMGVAFTVMVALACAAAPLV-GW

μOR TKYRQGS-----IDCTLTFSSHPT---WYWENLLKICVFI FAFIMPVLIITVICYGLMILRLKSVRMLSGSKE-----
δOR TRPRDGA-----VVCMLQFPSPS---WYWDVTVKICVFLFAFVVPVLIITVICYGLMLLRLRSVRLLSGSKE-----
κOR TKVREDV---DVIECSLQFPDDDY--SWWDLFMKICVFI FAFVIVPVLIIIVCYTLMILRLKSVRRLSGSRE-----
β2-AR YRATHQE---AINCYANETCDF---FTNQAYAIASSIVSFYVPLVIMVVFYSRVFQEAQRQLQKIDKSEGRFHVQN-LSQVE-----QDGRGTGHGLRRSS
β1-AR WRDEDPQ---ALKCYQDPGCDF---VTNRAYAIASSIISFYIPLLIMIFVYLRVYREAKEQIRKIDRCEGRFYGSQEQQPPPLPQHQP ILGNRASKRKTSR
A2aR NCGQPKEGKNHSGGCGEGQVACLFEDVVPNMNYMVFNFACVLVPLLLMLGVYLRIFLAARRQLKQMESQP-----LPPERAR
Rhod SRYIPEG---MQCSGIDYYTPHEE--TNNESFVIYMFVVHFIPLIVIFFCYQQLVFTVKEAAAAQQQESA-----

μOR -KDRNLRRITRMVLVVAVFVCWTPPIHIYVIIKALVTIP-ETTFQTVSWHFCIALGYTNSCLNPVLYAFLDENFKRCFRE-FCIPTSSNI-----
δOR -KDRSLRRITRMVLVVGAFAVCVAPIHIFIVVTLVDI DRDPLVVAALHLCIALGYANSSLNPVLYAFLDENFKRCFRQ-LCRKPCGRP-----
κOR -KDRNLRRITRLVLVVAVFVCVTPPIHIFILVEALGSTS-HSTAALSSYYFCIALGYTNSSLNPIYAFLDENFKRCFRD-FCFPLKMRM-----
β2-AR KFCLKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVIQDNL---IRKEVYILLNWIGYVNSGFNPLIYCRSDFRIAFQELL--CLRSS---LKAYGN-----
β1-AR VMAMREHKALKTLGIIMGVFTLCWLPFFLVNIVNVFNRLD---VPDWLFVFFNWLYANSFANPIYCRSPDFRKAFAKRL--CFPRKADRRLLHAGGQPAPLPGG
A2aR STLQKEVHAAKSLAIVGLFALCWLPLHIINCPTFFCPDC--SHAPLWLMYLAIVLSHTNSVNVNPFIYAYRIRERFRQTFRKIIRSHVLRQEPFKAAG-----
Rhod TQKAEKEVTRMVIIMVIAPLICWLPYAGVAFYIFTHQS---DFGPIEMTIPAFFAKTSAVYNPVIYIMNKQFRNCMVTTLCCGKNPLG-----

μOR -----EQQNSTRIRQNTDRHPSTANTVDRTNHQLENLEAETAPLP-----
δOR -----DPSSFSRAREATARERTACTPS-----DGGGGAAA-----
κOR -----ERQSTSRVNTVQDPAYLR-----DIDGMNKPV-----
β2-AR -----GYSSNGTGEQSGYHVEQEK-----ENKLLCED--LPGTEDFVGHQG-----TVP--SDNIDSQGRNCSTNDSL--
β1-AR FTSTLGSPEHSPGGTWSDCNNGTRGGSESSLERHSKTSRSESKMEREKNTLATTRFYC TPLGNGDKAVFCTVIRIVKIFRDATACTCPHCHKIKMKWRPFKQHA-
A2aR -----TSARVLAAGSDGEQVSLRLNGHPGWANGSAPHERRPNGYALGLVSGGSAQESQGNLGLPVELLSHELKGVCEPPEGLDDPLAQDGAGVS-
Rhod -----DDEASTTVSKTETSQVAPA-----
    
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Fig. (1). The alignment of sequences of three opioid receptors and other GPCRs with known structures (rhodopsin, β<sub>1</sub>- and β<sub>2</sub>-adrenergic receptors, and adenosine receptor A<sub>2a</sub>R). The sequence of transmembrane helices of rhodopsin is underlined and highlighted in blue. The conserved residues forming switches are colored in green. Two disulfide bridges in β<sub>1</sub>- and β<sub>2</sub>-AR are bracketed. All cysteines superimposed in crystal structures with the disulfide bridge in rhodopsin are colored in red. Additional information can be found in Methods section.

**Animations**

Fragments of molecular dynamics simulations of investigated complexes of  $\delta$ OR and  $\kappa$ OR models with ligands. Three white dashed lines inform about selected distances (not always bonds) (numbers in Å): ligand(C<sub>3</sub>)OH-Y3.33, ligand(C<sub>3</sub>)OH-D6.52 and D3.32-Y7.43. Transmembrane helices are colored according to rainbow color scheme: TM1 in blue, TM2 in light blue, TM3 in green, TM4 in yellow-green, TM5 in yellow, TM6 in orange, TM7 in red.

**Animation 1.** A complex of NTI- $\delta$ OR.

**Animation 2.** A complex of norBNI- $\kappa$ OR.

**Animation 3.** A complex of NTX- $\delta$ OR.

**Animation 4.** A complex of NTX- $\kappa$ OR.

**Animation 5.** A complex of butorphanol- $\kappa$ OR.

**Animation 6.** A complex of NTX- $\delta$ OR. NTX(C<sub>3</sub>)OH-H6.52 distance is restrained.

**Animation 7.** A complex of NTX- $\kappa$ OR. NTX(C<sub>3</sub>)OH-H6.52 distance is restrained.