DIHYDROFOLATE REDUCTASE

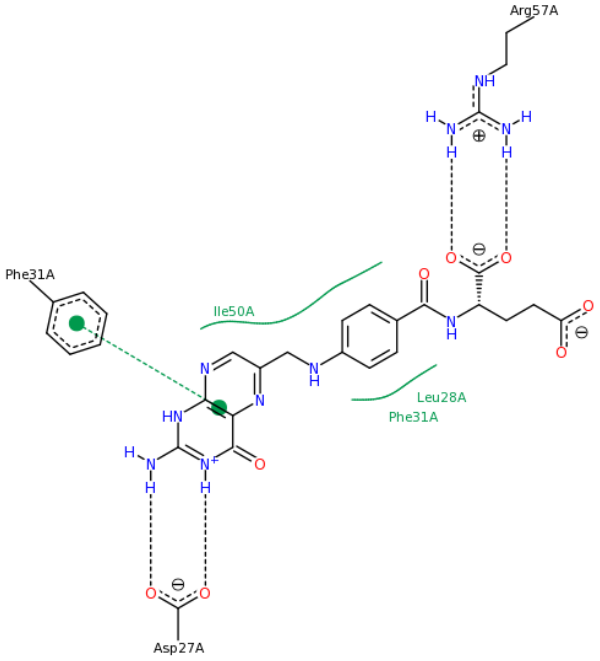
1RX2 DIHYDROFOLATE REDUCTASE (E.C.1.5.1.3) COMPLEXED WITH WITH FOLATE AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (OXIDIZED FORM)

Loop and subdomain movements in the mechanism of *Escherichia coli* dihydrofolate reductase: crystallographic evidence.

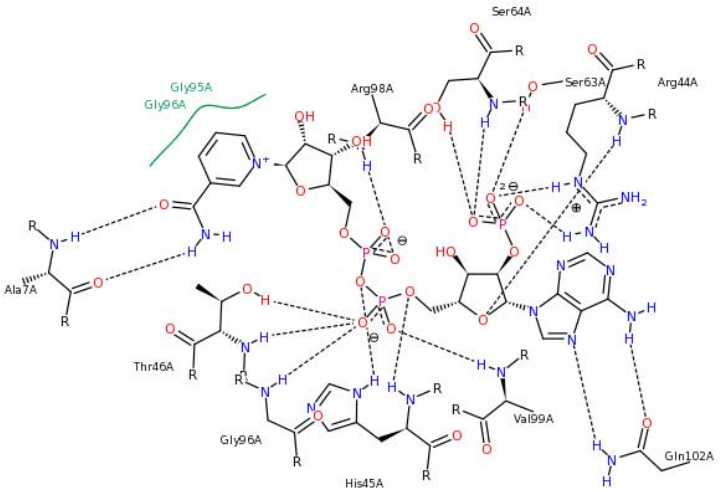
Sawaya, M.R., Kraut, J.; *Biochemistry* 36: 586-603, (**1997**); DOI: 10.1021/bi962337c.

Abstract:
The reaction catalyzed by *Escherichia coli* dihydrofolate reductase (ecDHFR) cycles through five detectable kinetic intermediates: holoenzyme, Michaelis complex, ternary product complex, tetrahydrofolate (THF) binary complex, and THF.NADPH complex. Isomorphous crystal structures analogous to these five intermediates and to the transition state (as represented by the methotrexate-NADPH complex) have been used to assemble a 2.1 A resolution movie depicting loop and subdomain movements during the catalytic cycle (see Supporting Information). The structures suggest that the M20 loop is predominantly closed over the reactants in the holoenzyme, Michaelis, and transition state complexes. But, during the remainder of the cycle, when nicotinamide is not bound, the loop occludes (protrudes into) the nicotinamide-ribose binding pocket. Upon changing from the closed to the occluded conformation, the central portion of the loop rearranges from beta-sheet to 3(10) helix. The change may occur by way of an irregularly structured open loop conformation, which could transiently admit a water molecule into position to protonate N5 of dihydrofolate. From the Michaelis to the transition state analogue complex, rotation between two halves of ecDHFR, the adenosine binding subdomain and loop subdomain, closes the (p-aminobenzoyl)glutamate (pABG) binding crevice by approximately 0.5 A. Resulting enhancement of contacts with the pABG moiety may stabilize puckering at C6 of the pteridine ring in the transition state. The subdomain rotation is further adjusted by cofactor-induced movements (approximately 0.5 A) of helices B and C, producing a larger pABG cleft in the THF.NADPH analogue complex than in the THF analogue complex. Such movements may explain how THF release is assisted by NADPH binding. Subdomain rotation is not observed in vertebrate DHFR structures, but an analogous loop movement (residues 59-70) appears to similarly adjust the pABG cleft width, suggesting that these movements are important for catalysis. Loop movement, also unobserved in vertebrate DHFR structures, may preferentially weaken NADP+ vs NADPH binding in ecDHFR, an evolutionary adaptation to reduce product inhibition in the NADP+ rich environment of prokaryotes.

Ligand: FOLIC ACID (real charge= -2)



Cofactor: NADP, NICOTINAMIDE-ADENINE-DINUCLEOTIDE PHOSPHATE (real charge= -3)

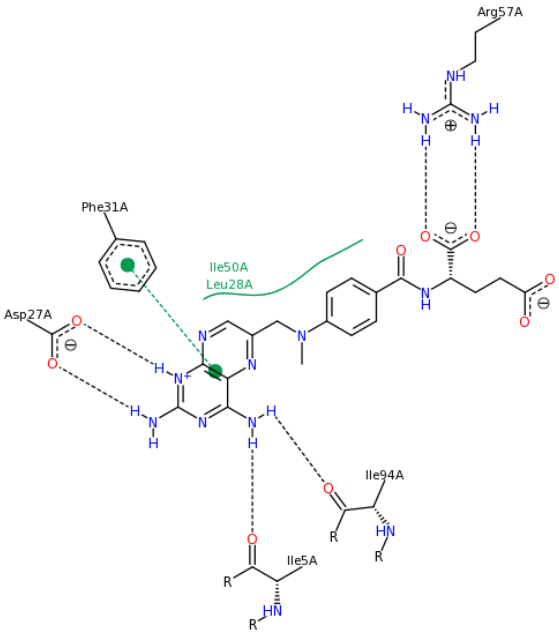


1RB3 DIHYDROFOLATE REDUCTASE COMPLEXED WITH METHOTREXATE AND NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (OXIDIZED FORM)

Loop and subdomain movements in the mechanism of *Escherichia coli* dihydrofolate reductase: crystallographic evidence.

Sawaya, M.R., Kraut, J.*; Biochemistry,* 36: 586-603, (**1997**); DOI: 10.1021/bi962337c.

Ligand: FOLIC ACID (real charge= -2)



HUMAN ESTROGEN RECEPTOR

1ERE: HUMAN ESTROGEN RECEPTOR LIGAND-BINDING DOMAIN IN COMPLEX WITH 17BETA-ESTRADIOL

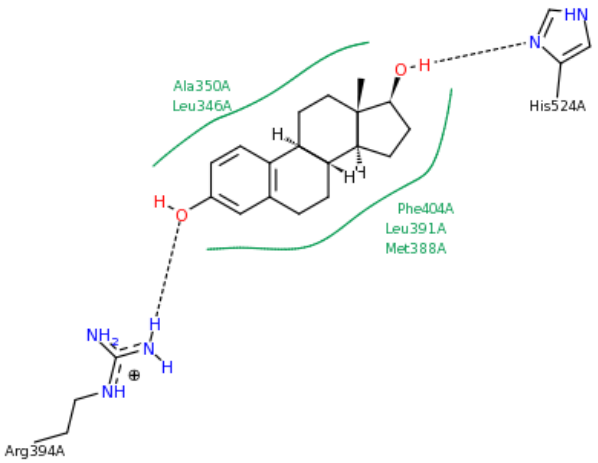
Molecular basis of agonism and antagonism in the oestrogen receptor.

Brzozowski, A.M., Pike, A.C., Dauter, Z., Hubbard, R.E., Bonn, T., Engstrom, O., Ohman, L., Greene, G.L., Gustafsson, J.A., Carlquist, M.; *Nature* 389: 753-758; (**1997**), DOI: 10.1038/39645.

Abstract:
Oestrogens are involved in the growth, development and homeostasis of a number of tissues. The physiological effects of these steroids are mediated by a ligand-inducible nuclear transcription factor, the oestrogen receptor (ER). Hormone binding to the ligand-binding domain (LBD) of the ER initiates a series of molecular events culminating in the activation or repression of target genes. Transcriptional regulation arises from the direct interaction of the ER with components of the cellular transcription machinery. Here we report the crystal structures of the LBD of ER in complex with the endogenous oestrogen, 17beta-oestradiol, and the selective antagonist raloxifene, at resolutions of 3.1 and 2.6 A, respectively. The structures provide a molecular basis for the distinctive pharmacophore of the ER and its catholic binding

properties. Agonist and antagonist bind at the same site within the core of the LBD but demonstrate different binding modes. In addition, each class of ligand induces a distinct conformation in the transactivation domain of the LBD, providing structural evidence

Ligand: ESTRADIOL



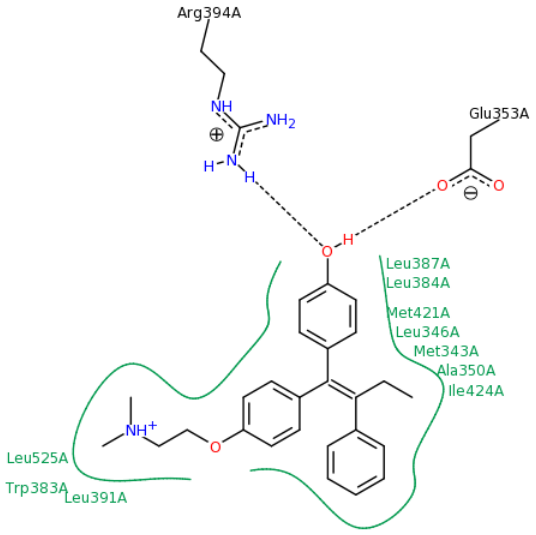
2ERT : HUMAN ESTROGEN RECEPTOR ALPHA LIGAND-BINDING DOMAIN IN COMPLEX WITH 4-HYDROXYTAMOXIFEN

The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen.

Shiau, A.K., Barstad, D., Loria, P.M., Cheng, L., Kushner, P.J., Agard, D.A., Greene, G.L.; *Cell*(Cambridge,Mass.) 95: 927-937, (**1998**).

Abstract:
Ligand-dependent activation of transcription by nuclear receptors (NRs) is mediated by interactions with coactivators. Receptor agonists promote coactivator binding, and antagonists block coactivator binding. Here we report the crystal structure of the human estrogen receptor alpha (hER alpha) ligand-binding domain (LBD) bound to both the agonist diethylstilbestrol (DES) and a peptide derived from the NR box II region of the coactivator GRIP1 and the crystal structure of the hER alpha LBD bound to the selective antagonist 4-hydroxytamoxifen (OHT). In the DES-LBD-peptide complex, the peptide binds as a short alpha helix to a hydrophobic groove on the surface of the LBD. In the OHT-LBD complex, helix 12 occludes the coactivator recognition groove by mimicking the interactions of the NR box peptide with the LBD. These structures reveal the two distinct mechanisms by which structural features of OHT promote this "autoinhibitory" helix 12 conformation.

Ligand: 4-HYDROXYTAMOXIFEN



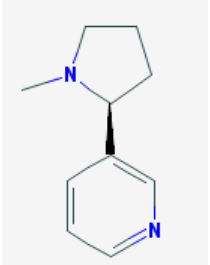
1UW6: X-RAY STRUCTURE OF ACETYLCHOLINE BINDING PROTEIN (ACHBP) IN COMPLEX WITH NICOTINE

Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures.

Celie, P.H.N., Van Rossum-Fikkert, S.E., Van Dijk, W.J., Brejc, K., Smit, A.B., Sixma, T.K.; *Neuron* 41: 907, (**2004**).

Abstract:
Nicotinic acetylcholine receptors are prototypes for the pharmaceutically important family of pentameric ligand-gated ion channels. Here we present atomic resolution structures of nicotine and carbamylcholine binding to AChBP, a water-soluble homolog of the ligand binding domain of nicotinic receptors and their family members, GABAA, GABAC, 5HT3 serotonin, and glycine receptors. Ligand binding is driven by enthalpy and is accompanied by conformational changes in the ligand binding site. Residues in the binding site contract around the ligand, with the largest movement in the C loop. As expected, the binding is characterized by substantial aromatic and hydrophobic contributions, but additionally there are close contacts between protein oxygens and positively charged groups in the ligands. The higher affinity of nicotine is due to a main chain hydrogen bond with the B loop and a closer packing of the aromatic groups. These structures will be useful tools for the development of new drugs involving nicotinic acetylcholine receptor-associated diseases.

Ligand: (S)-3-(1-METHYLPYRROLIDIN-2-YL)PYRIDINE



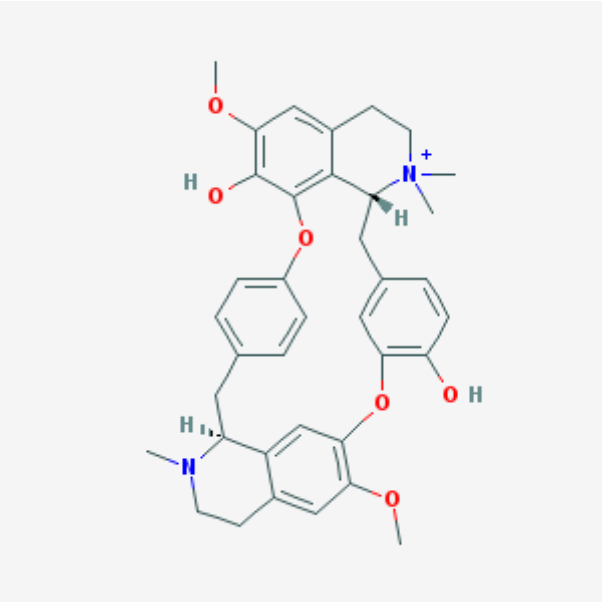
2XYT: CRYSTAL STRUCTURE OF *APLYSIA CALIFORNICA* ACHBP IN COMPLEX WITH D-TUBOCURARINE

A Structural and Mutagenic Blueprint for Molecular Recognition of Strychnine and d-Tubocurarine by Different Cys-Loop Receptors.

Brams, M., Pandya, A., Kuzmin, D., Van Elk, R., Krijnen, L., Yakel, J.L., Tsetlin, V., Smit, A.B., Ulens, C.; *Plos Biol.* 9: E1001-e1001034, (34) ; DOI: 10.1371/journal.pbio.1001034

Abstract:
Cys-loop receptors (CLR) are pentameric ligand-gated ion channels that mediate fast excitatory or inhibitory transmission in the nervous system. Strychnine and d-tubocurarine (d-TC) are neurotoxins that have been highly instrumental in decades of research on glycine receptors (GlyR) and nicotinic acetylcholine receptors (nAChR), respectively. In this study we addressed the question how the molecular recognition of strychnine and d-TC occurs with high affinity and yet low specificity towards diverse CLR family members. X-ray crystal structures of the complexes with AChBP, a well-described structural homolog of the extracellular domain of the nAChRs, revealed that strychnine and d-TC adopt multiple occupancies and different ligand orientations, stabilizing the homopentameric protein in an asymmetric state. This introduces a new level of structural diversity in CLRs. Unlike protein and peptide neurotoxins, strychnine and d-TC form a limited number of contacts in the binding pocket of AChBP, offering an explanation for their low selectivity. Based on the ligand interactions observed in strychnine- and d-TC-AChBP complexes we performed alanine-scanning mutagenesis in the binding pocket of the human α1 GlyR and α7 nAChR and showed the functional relevance of these residues in conferring high potency of strychnine and d-TC, respectively. Our results demonstrate that a limited number of ligand interactions in the binding pocket together with an energetic stabilization of the extracellular domain are key to the poor selective recognition of strychnine and d-TC by CLRs as diverse as the GlyR, nAChR, and 5-HT(3)R.

Ligand: D-TUBOCURARINE



DNA

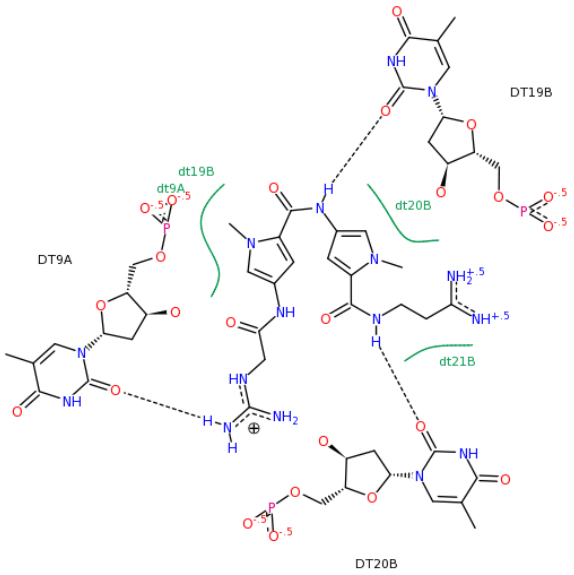
121D: MOLECULAR STRUCTURE OF THE A-TRACT DNA DODECAMER D(CGCAAATTTGCG) COMPLEXED WITH THE MINOR GROOVE BINDING DRUG NETROPSIN

Molecular structure of the A-tract DNA dodecamer d(CGCAAATTTGCG) complexed with the minor groove binding drug netropsin.

Tabernero, L., Verdaguer, N., Coll, M., Fita, I., van der Marel, G.A., van Boom, J.H., Rich, A., Aymami, J.; *Biochemistry* 32: 8403-8410; (**1993**)

Abstract:
The molecular structure of the complex between the minor groove binding drug netropsin and the dodecamer d(CGCAAATTTGCG) has been solved and refined by X-ray diffraction analysis to an R-factor of 19.8% and 2.2-A resolution. The drug lies in the narrow minor groove of the B-DNA fragment, covering five of the six A.T base pairs (from A5.T20 to T9.A16). The long six A.T base pair tract allows the drug to bind in a position that optimizes its contacts with the DNA, establishing hydrogen bonds with O2 of thymines and N3 of adenines. The DNA molecule shows a high propeller twist only at the A6.T19 step of the A-tract. Two three-centered hydrogen bonds are observed in the major groove at half of the A-tract.

Ligand: NETROPSIN



8BNA : BINDING OF HOECHST 33258 TO THE MINOR GROOVE OF B-DNA

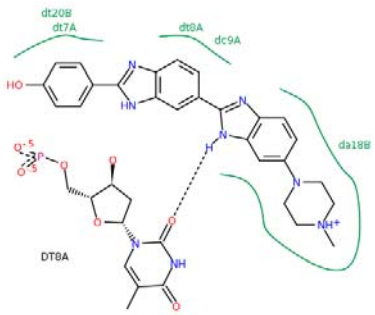
Binding of Hoechst 33258 to the minor groove of B-DNA.

Pjura, P.E., Grzeskowiak, K., Dickerson, R.E.; *J.Mol.Biol.* 197: 257-271, (**1987**)

Abstract:
An X-ray crystallographic structure analysis has been carried out on the complex between the antibiotic and DNA fluorochrome Hoechst 33258 and a synthetic B-DNA dodecamer of sequence C-G-C-G-A-A-T-T-C-G-C-G. The drug molecule, which can be schematized as: phenol-

benzimidazole-benzimidazole-piperazine, sits within the minor groove in the A-T-T-C region of the DNA double helix, displacing the spine of hydration that is found in drug-free DNA. The NH groups of the benzimidazoles make bridging three-center hydrogen bonds between adenine N-3 and thymine O-2 atoms on the edges of base-pairs, in a manner both mimicking the spine of hydration and calling to mind the binding of the auti-tumor drug netropsin. Two conformers of Hoechst are seen in roughly equal populations, related by 180 degrees rotation about the central benzimidazole-benzimidazole bond: one form in which the piperazine ring extends out from the surface of the double helix, and another in which it is buried deep within the minor groove. Steric clash between the drug and DNA dictates that the phenol-benzimidazole-benzimidazole portion of Hoechst 33258 binds only to A.T regions of DNA, whereas the piperazine ring demands the wider groove characteristic of G.C regions. Hence, the piperazine ring suggests a possible G.C-reading element for synthetic DNA sequence-reading drug analogs.

Ligand: 2'-(4-HYDROXYPHENYL)-5-(4-METHYL-1-PIPERAZINYL)- 2,5'-BI-BENZIMIDAZOLE



GPCR

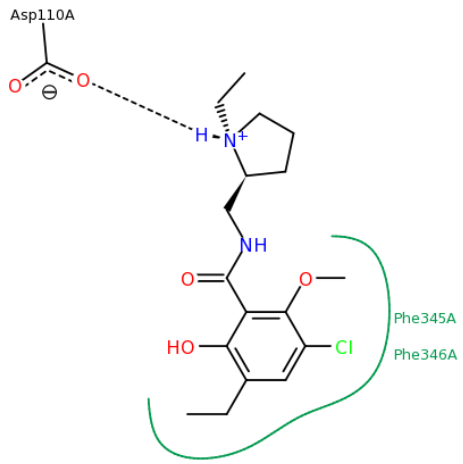
3PBL: STRUCTURE OF THE HUMAN DOPAMINE D3 RECEPTOR IN COMPLEX WITH ETICLOPRIDE

Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist.

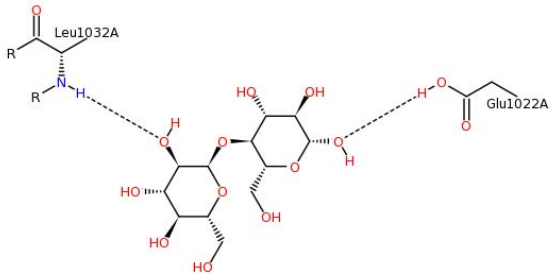
Chien, E.Y., Liu, W., Zhao, Q., Katritch, V., Han, G.W., Hanson, M.A., Shi, L., Newman, A.H., Javitch, J.A., Cherezov, V., Stevens, R.C.; Science 330: 1091-1095, (**2010**); DOI: 10.1126/science.1197410

Abstracts
Dopamine modulates movement, cognition, and emotion through activation of dopamine G protein-coupled receptors in the brain. The crystal structure of the human dopamine D3 receptor (D3R) in complex with the small molecule D2R/D3R-specific antagonist eticlopride reveals important features of the ligand binding pocket and extracellular loops. On the intracellular side of the receptor, a locked conformation of the ionic lock and two distinctly different conformations of intracellular loop 2 are observed. Docking of R-22, a D3R-selective antagonist, reveals an extracellular extension of the eticlopride binding site that comprises a second binding pocket for the aryl amide of R-22, which differs between the highly homologous D2R and D3R. This difference provides direction to the design of D3R-selective agents for treating drug abuse and other neuropsychiatric indications.

Ligand: 3-chloro-5-ethyl-N-{[(2S)-1-ethylpyrrolidin- 2-yl]methyl}-6-hydroxy-2-methoxybenzamide



Cofactor: MALTOSE



2YCW : TURKEY BETA1 ADRENERGIC RECEPTOR WITH STABILISING MUTATIONS AND BOUND ANTAGONIST CARAZOLOL

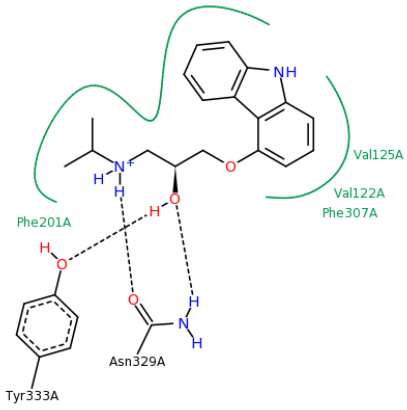
Two distinct conformations of helix 6 observed in antagonist-bound structures of a {beta}1-adrenergic receptor.

Moukhametzianov, R., Warne, T., Edwards, P.C., Serrano-Vega, M.J., Leslie, A.G., Tate, C.G., Schertler, G.F.; Proc.Natl.Acad.Sci.USA 108: 8228, (**2011**); DOI: 10.1073/pnas.1100185108

Abstract:
The β(1)-adrenergic receptor (β(1)AR) is a G-protein-coupled receptor whose inactive state structure was determined using a thermostabilized mutant (β (1)AR-M23). However, it was not thought to be in a fully inactivated state because there was no salt bridge between Arg139 and Glu285 linking the cytoplasmic ends of transmembrane helices 3 and 6 (the R(3.50) - D/E(6.30) "ionic lock"). Here we compare eight new structures of β(1)AR-M23, determined from crystallographically independent molecules in four different crystals with three different antagonists bound. These structures are all in the inactive R state and show clear electron density for cytoplasmic loop 3 linking transmembrane helices 5 and 6 that had not been seen previously. Despite significantly different crystal packing interactions, there are only two

distinct conformations of the cytoplasmic end of helix 6, bent and straight. In the bent conformation, the Arg139-Glu285 salt bridge is present, as in the crystal structure of dark-state rhodopsin. The straight conformation, observed in previously solved structures of β -receptors, results in the ends of helices 3 and 6 being too far apart for the ionic lock to form. In the bent conformation, the R(3.50) - E(6.30) distance is significantly longer than in rhodopsin, suggesting that the interaction is also weaker, which could explain the high basal activity in β (1)AR compared to rhodopsin. Many mutations that increase the constitutive activity of G-protein-coupled receptors are found in the bent region at the cytoplasmic end of helix 6, supporting the idea that this region plays an important role in receptor activation.

Ligand :(2S)-1-(9H-Carbazol-4-yloxy)-3-(isopropylamino)propan- 2-ol



Cofactor :HEGA-10