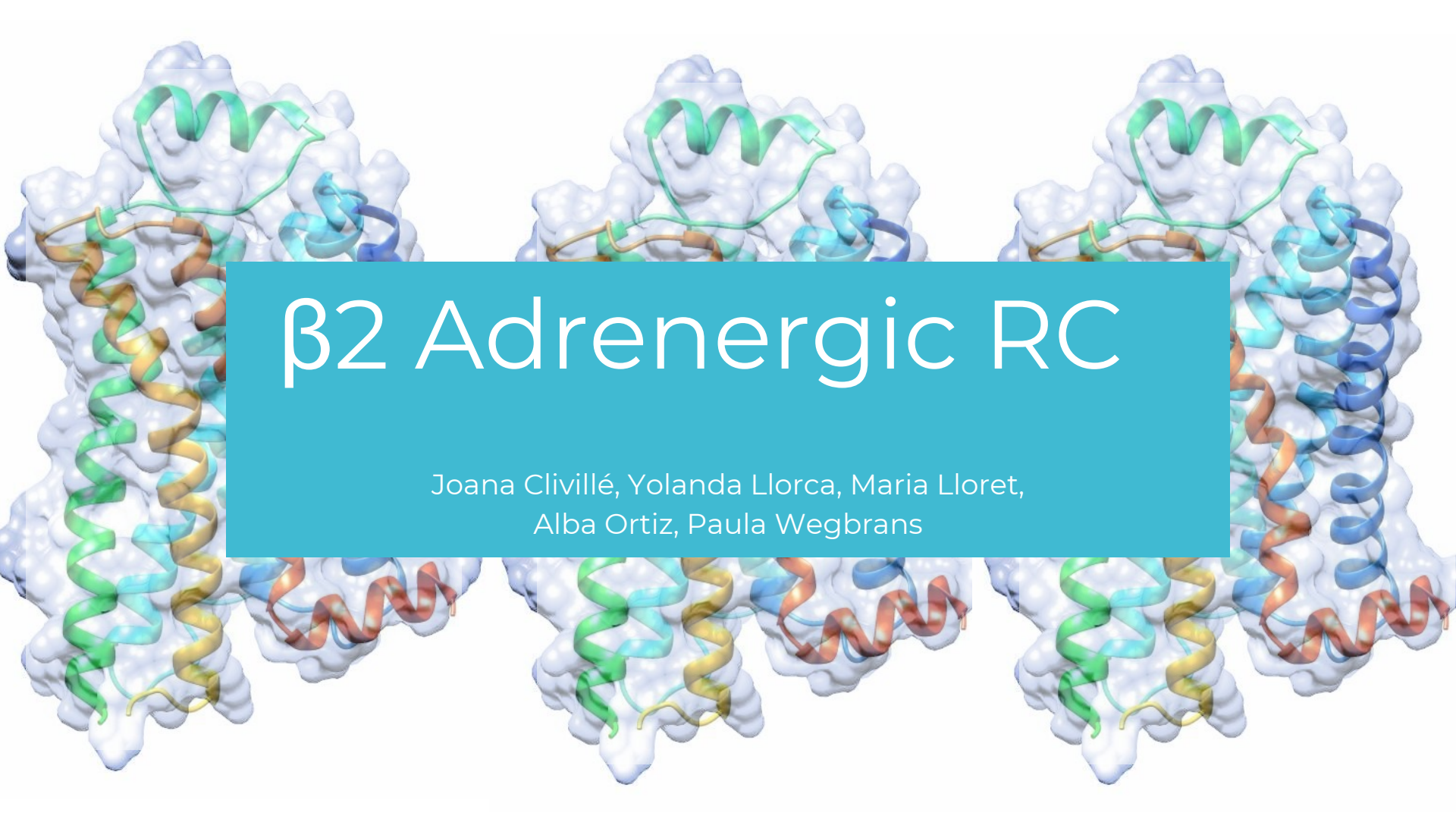




β 2 Adrenergic RC

Joana Clivillé, Yolanda Llorca, Maria Lloret,
Alba Ortiz, Paula Wegbrans

Index

1.

General overview on adrenergic receptors

- 1.1. Adrenergic Receptors
- 1.2. Dimerization

2.

Adrenergic receptor beta2

- 2.1. Characteristics of the β -2 Adrenergic Receptor
- 2.2. Evolution

3.

Basics on GPCRs representations

- 3.1. Models used and crystallization

4.

Function

- 4.1. Protein G interaction
- 4.2. Active/Inactive States

5.

Adrenergic receptor beta2 ligands

- 5.1. Cholesterol
- 5.2. Agonist: adrenaline
- 5.3. Antagonist: alprenolol
- 5.4. Inverse agonist: carazolol

Introduction

The importance of G protein-coupled receptors

Largest family of eukaryotic signal transduction proteins → **800**

2000: first GPCR described → Bovine rhodopsin

Biomedical **targets** → target for 50% of drugs

Constitutively active **basal signaling**

General structural topology →

EL1-3 and IL1-3
7T α-helical bundles
Binding site ↑ ligands

Diversity → Functional specificity

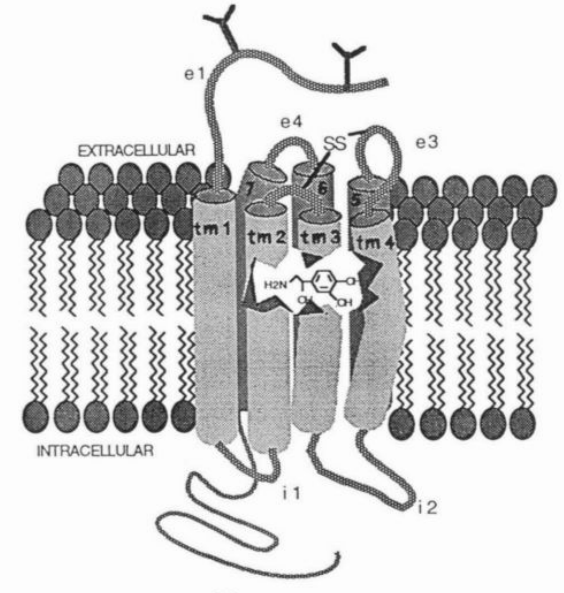


Fig. 1: Strosberg AD, 1991

Introduction

Nobel Prizes in the GPCR area



Granit R, Hartline K, Wald G

for physiological and chemical
processes in photoreception.

Black JW

for the discovery of propranolol
and cimetidine

Buck L, Axel R

for odorant receptors



Sutherland EW
for cyclic AMP

Rodbell M, Gilman AG
for heterotrimeric G-proteins

**Nobel
Prize**

Introduction

Nobel Prizes in the area

GPCRs win 2012 Nobel Prize in Chemistry

2012: Robert J. Lefkowitz and Brian Kobilka, for G-protein coupled receptors



Buck L, Axel R

for odorant receptors



Sutherland EW
for cyclic AMPC

Rodbell M, Gilman AG
for heterotrimeric G-proteins

Nobel Prize

Adrenergic Receptors

Earliest and the most extensively studied group of GPCR

Ligands → peptidic and non peptidic hormones, drugs and NT

Receptor	Subtype	Endogenous ligand	Function	G-type affinity
α-Adrenergic Receptors	α-1	AD ≥ NA >> ISO	Contraction of vascular, bronchial and genitourinary smooth muscle	Gq
	α-2	AD ≥ NA >> ISO	Vascular smooth muscle Presynaptic membrane	Gi
β-Adrenergic Receptors	β-1	ISO > AD = NA	Cardiac stimulator	Gs
	β-2	ISO > AD >> NA	Relaxation of vascular, bronchial, gastrointestinal and genitourinary smooth muscle	Gs
	β-3	NA >> AD	Lipolysis in adipose tissue and thermogenesis	Gs

Adrenergic Receptors

Earliest and the most extensively studied group of GPCR

Ligands → peptidic and non peptidic hormones, drugs and NT

Receptor	Subtype	Endogenous ligand	Function	G-type affinity
α-Adrenergic Receptors	α-1	AD ≥ NA >> ISO	Contraction of vascular, bronchial and genitourinary smooth muscle	Gq
	α-2	AD ≥ NA >> ISO	Vascular smooth muscle Presynaptic membrane	Gi
β-Adrenergic Receptors	β-1	ISO > AD = NA	Cardiac stimulator	Gs
	β-2	ISO > AD >> NA	Relaxation of vascular, bronchial, gastrointestinal and genitourinary smooth muscle	Gs
	β-3	NA >> AD	Lipolysis in adipose tissue and thermogenesis	Gs

ADRC Dimerization

Modulates the **ligand** affinity and the **response** pathway

Dimerization made by an **interface** of conserved residues

Dimer interface involving **TM1** and **H8** has been identified in crystal structures of the **β1-AR** and **β2-AR**

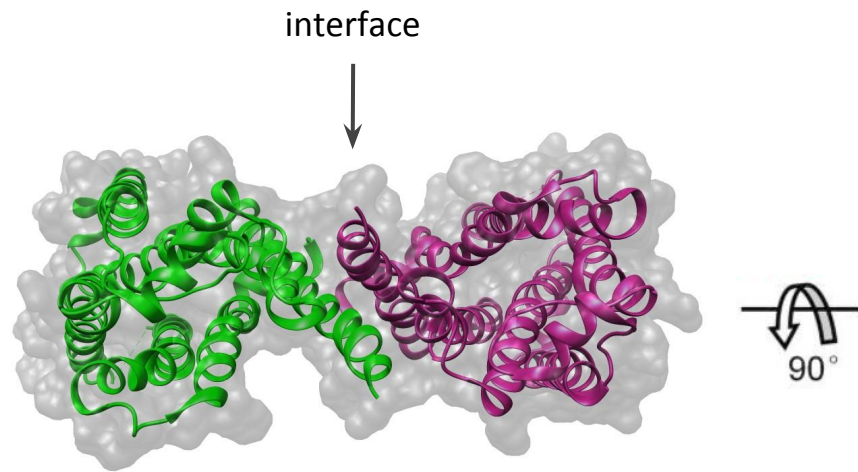
Ortholog lipidic ligands (**cholesterol** and **palmytic acid**) aid establish contact

Highly controversy due to the exclusion of phospholipids in the crystallization conditions

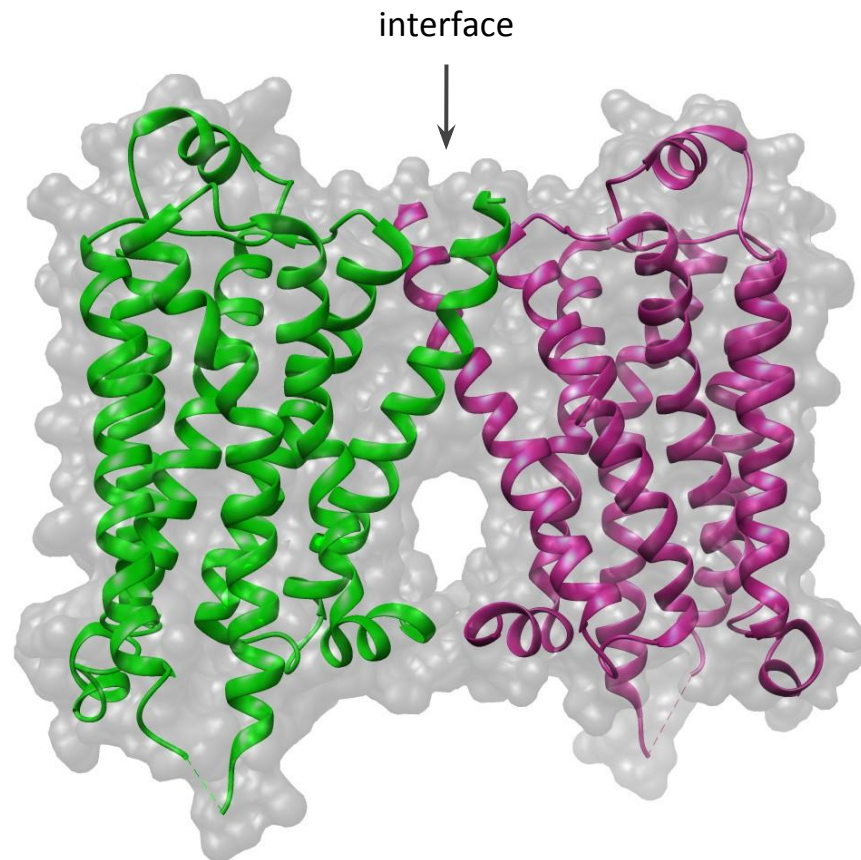
Techniques: co-immunoprecipitation (in vitro) and **BRET** (in vivo)

ADRC Dimerization

Turkey β1 dimeric receptor 4GPO



Function: cell surface targeting, activation,
G-protein coupling, signaling and internalization
Dimers and oligomers
2 possible interfaces
Contact surface: 1700 Å²



ADRC Dimerization

Turkey $\beta 1$ dimeric receptor 4GPO

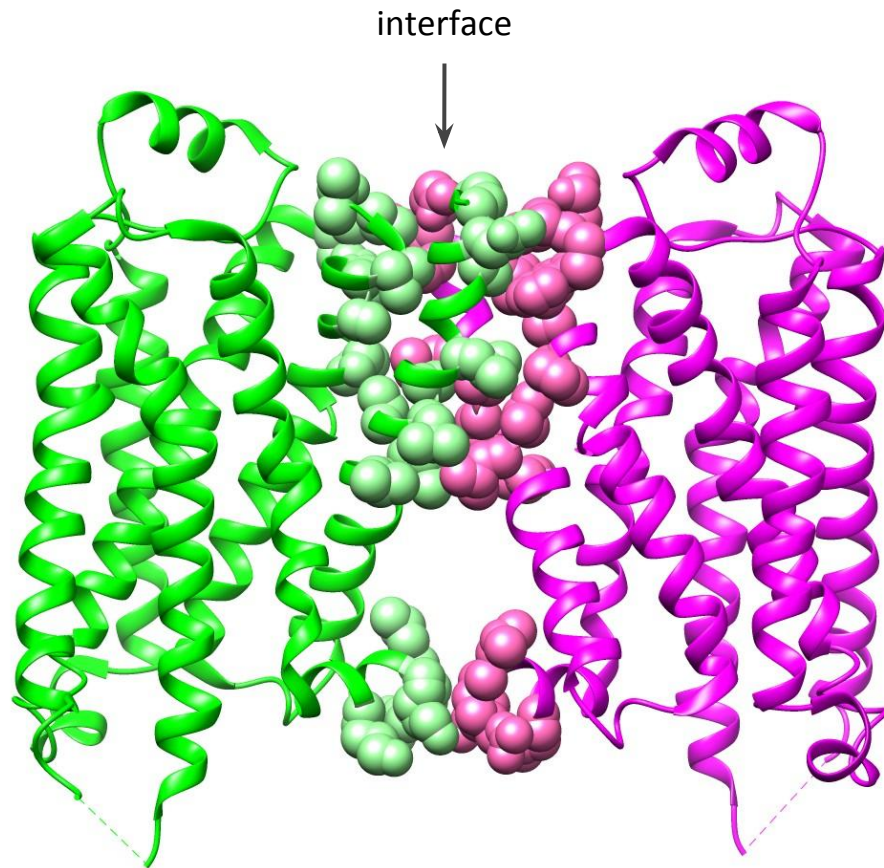
Hydrophobic and Van der Waals interactions

TM1: Gln38, Gln39, Ala42, Leu46, Ala49, Leu50, Val52, Leu53 and Leu54

TM2: Pro96, Ala99, Thr100 and Val103

ECL1: Thr106, Leu108, and Trp109

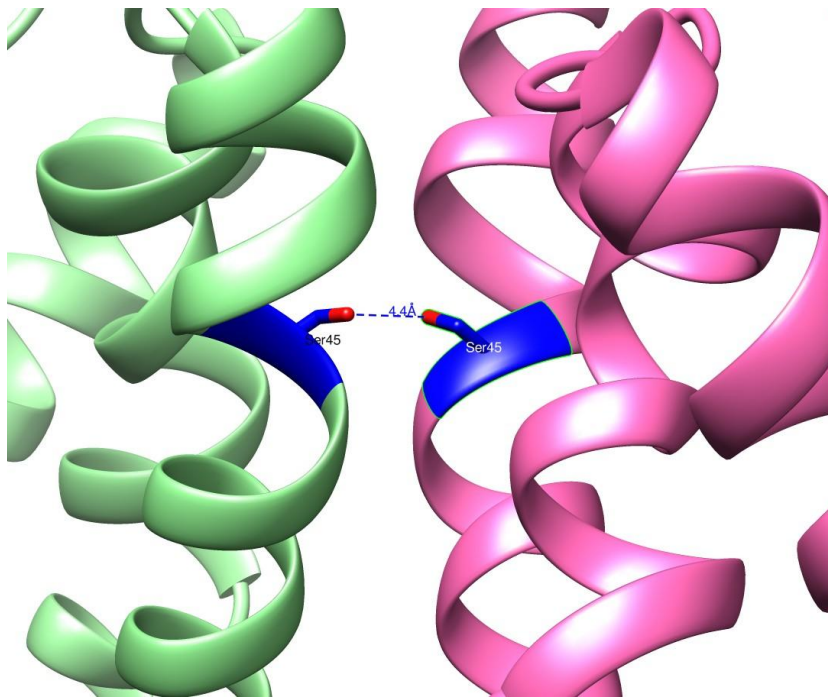
H8: Arg351, Lys354, Arg355 and Leu356



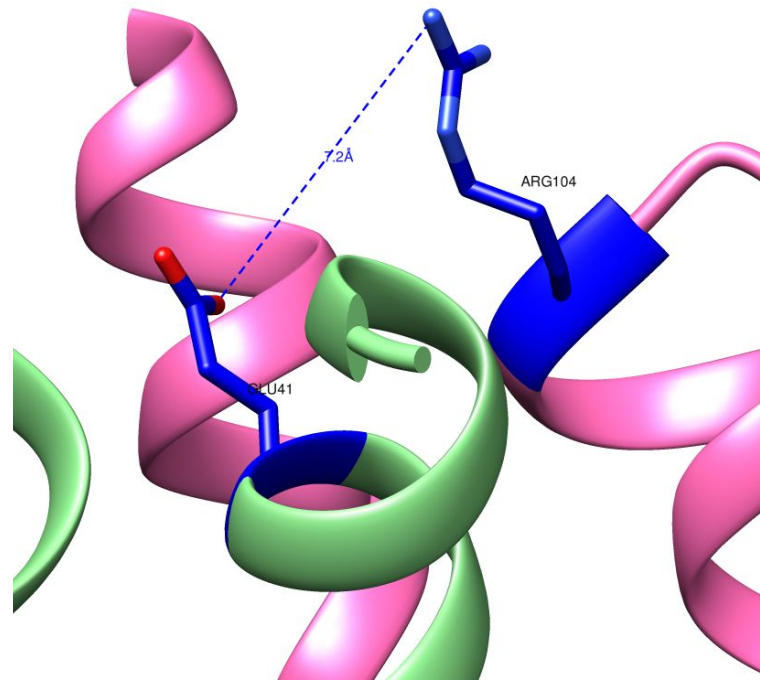
ADRC Dimerization

Turkey $\beta 1$ dimeric receptor 4GPO

Hydrogen bond formation: Ser45-Ser45 (TM1)



Salt bridge formation: Glu41 (TM1) Arg 104 (TM2)



β2-ADR

Excellent prototype → GPCR organization and function

Therapeutic target for asthma and heart failure

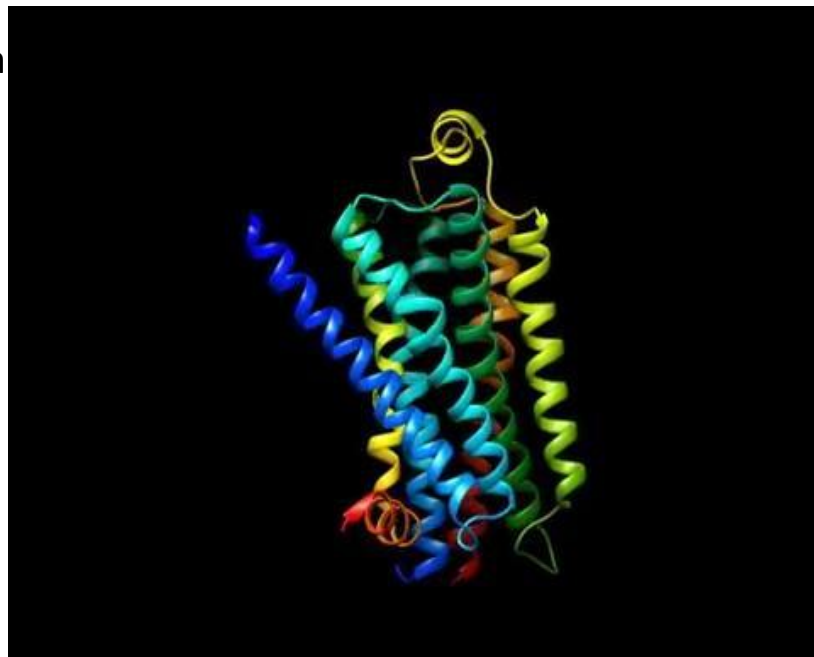
β2-ADRC can exist as **dimers** in vivo (*speculative*)

Active/Inactive states and **basal** active signalling

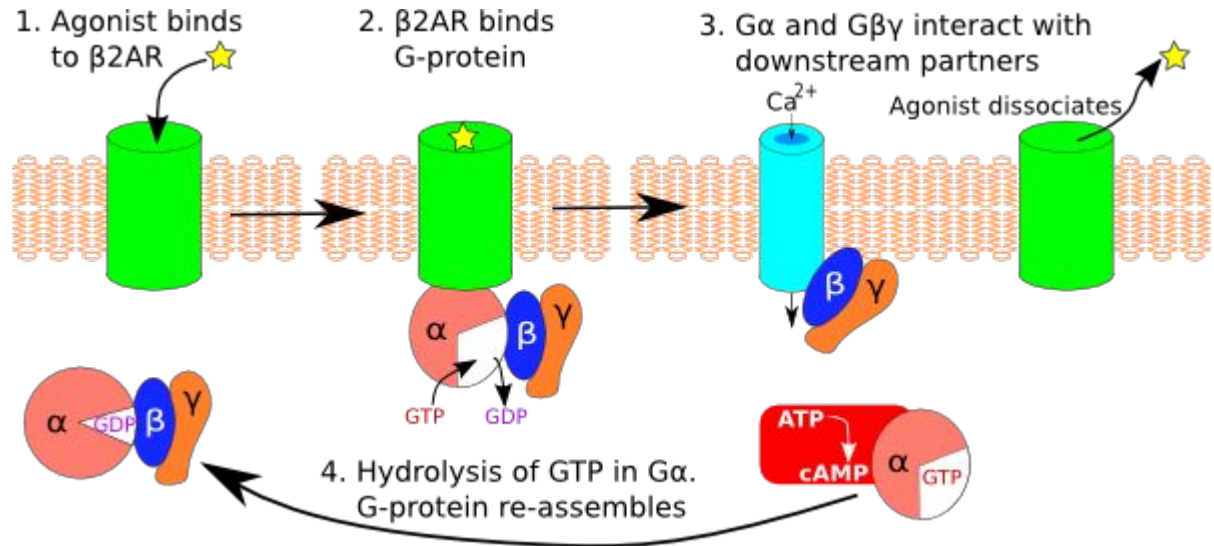
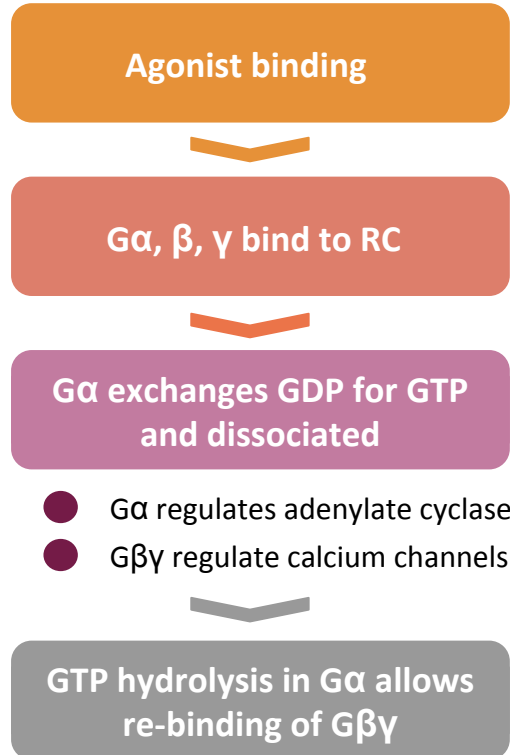
Inverted hydrophobicity

Structure:

All-alpha helixes
7 TM domains
3 ELC and 3 ICL



β2-ADR Signalling Cascade



Adrenergic Receptors: β2-ADRC classification

SCOP CLASSIFICATION

1. **Root:** SCOP
2. **Class:** Membrane and cell surface proteins and peptides
Does not include proteins in the immune system
3. **Fold:** Family A G protein-coupled receptor-like
core: up-and-down bundle of seven transmembrane helices tilted 20 degrees with respect to the plane of the membrane
4. **Superfamily:** Family A G protein-coupled receptor-like
5. **Family:** Rhodopsin-like
Individual TM segments have a number of kinks and distortions

β 2-ADRC Genetics

Gene: ADRB2

5q32, forward
strand



1 Exon

Transcript length: 3,443 bps

Translation length: 413 residues



171 orthologues

19 paralogues

2 phenotypes: Asthma, Obesity

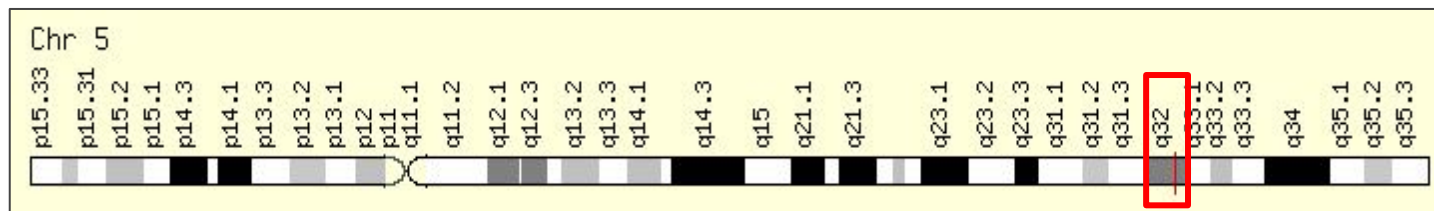
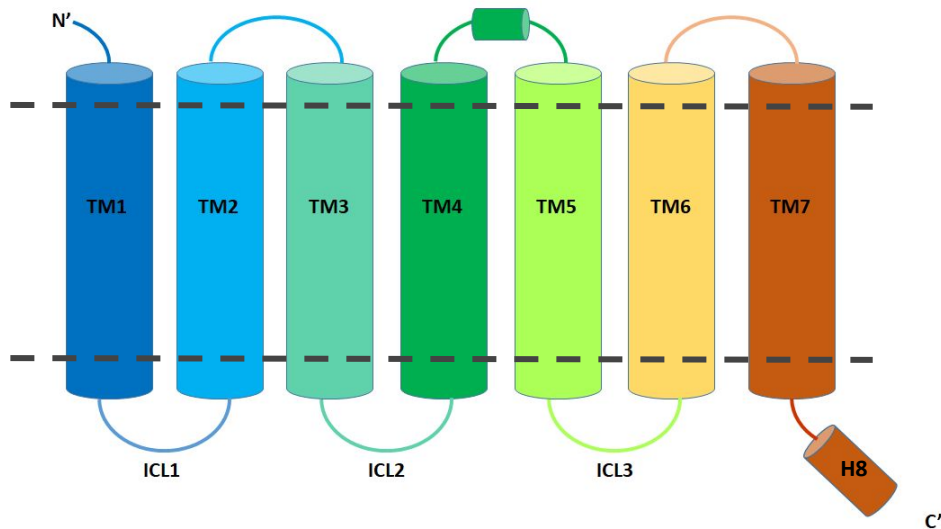


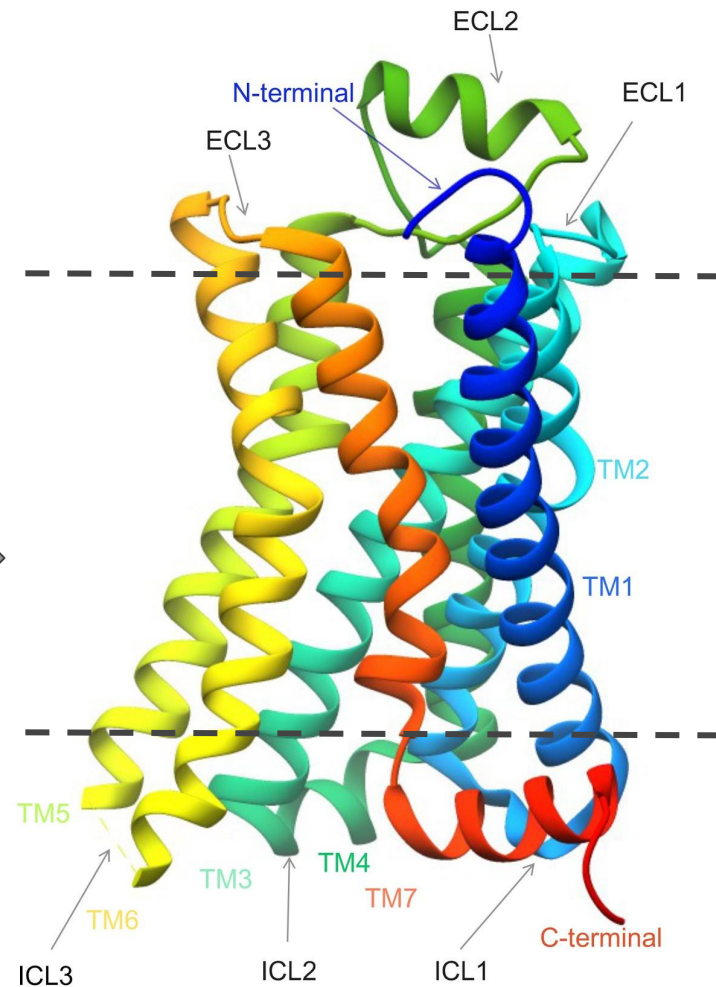
Figure extracted from: <https://filizm02.u.hpc.mssm.edu/gpcr-okb/>

β2-ADRC Structure

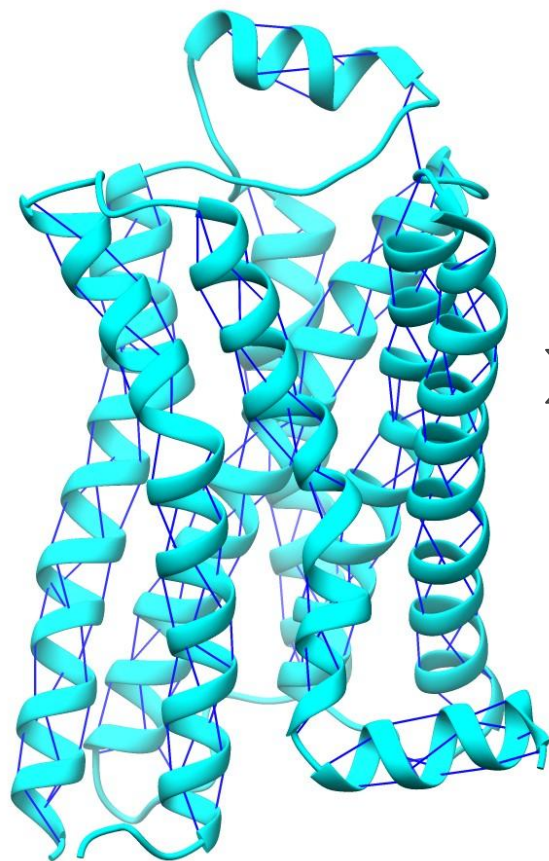
Integral alpha-helical polytopic transmembrane protein



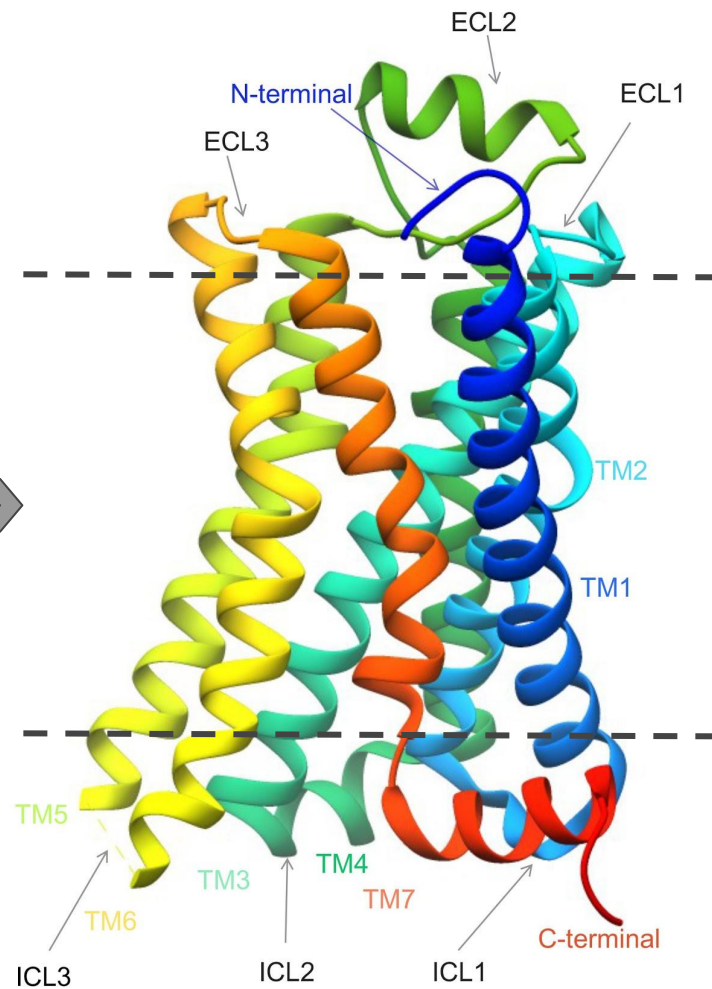
Folding



β2-ADRC Tertiary Structure

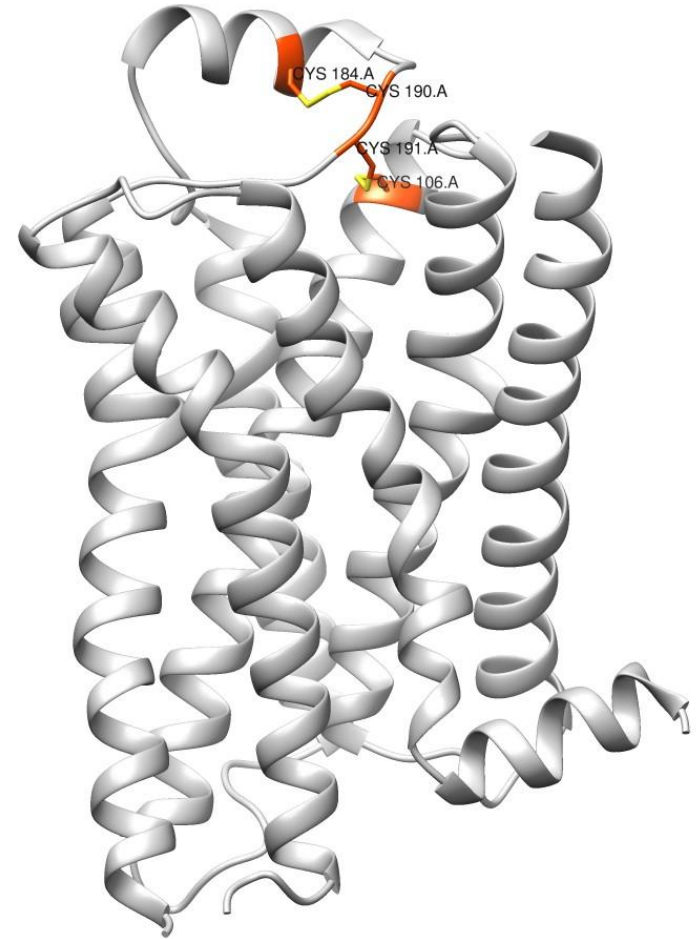
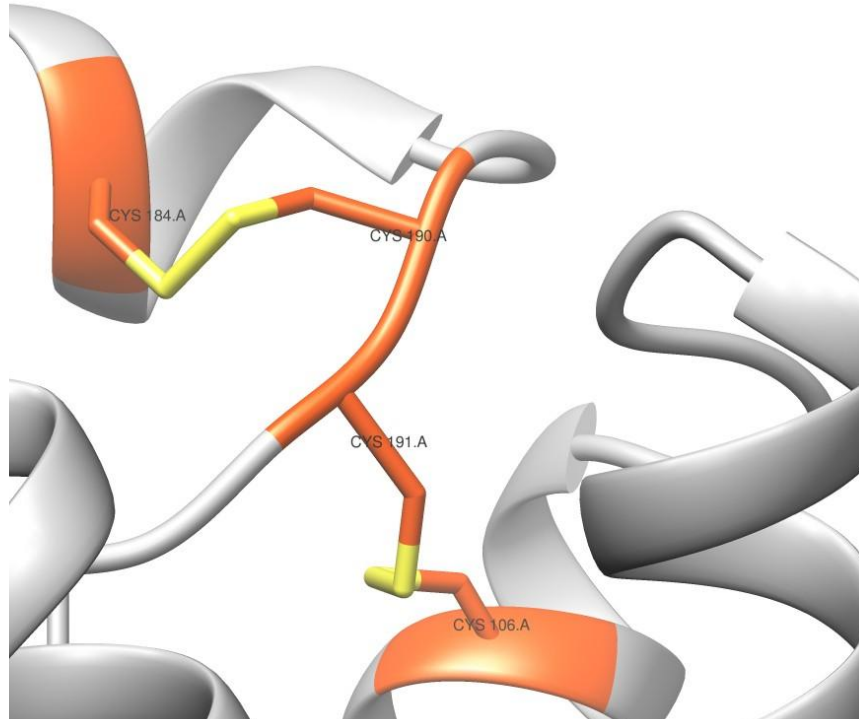


Hydrogen bonds are essential to stabilize the tertiary structure

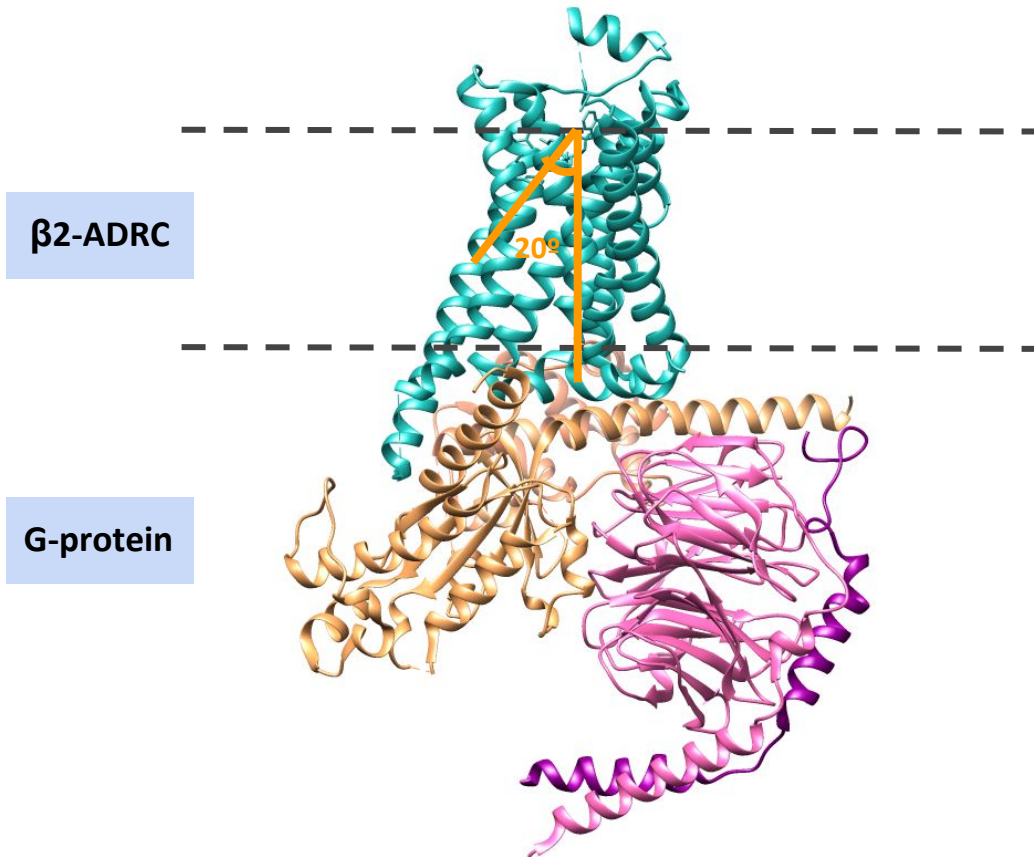


β2-ADRC Tertiary Structure

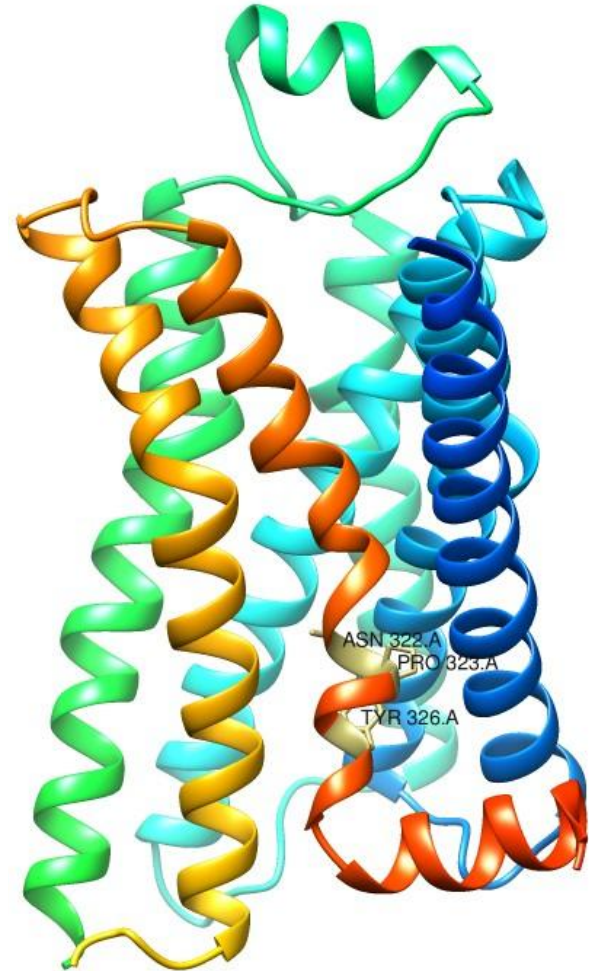
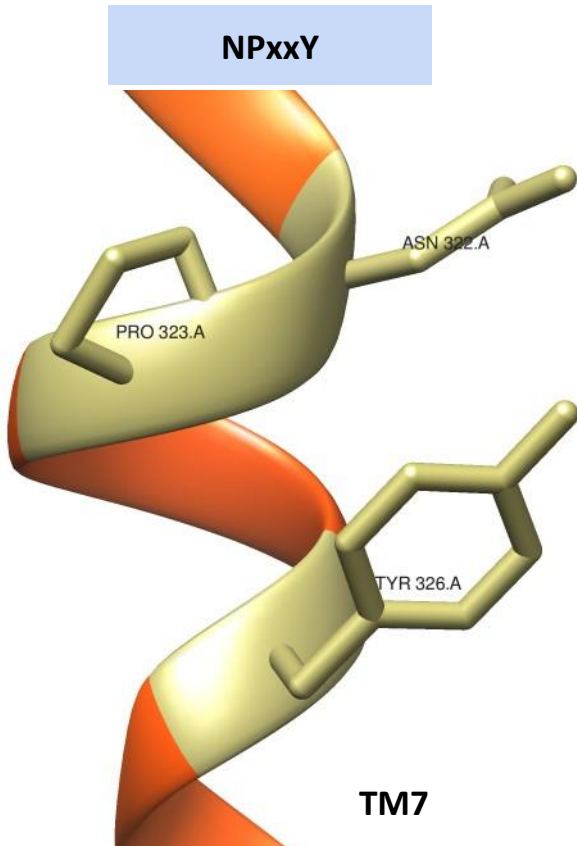
Disulfide Bridges



β2-ADRC Quaternary Structure

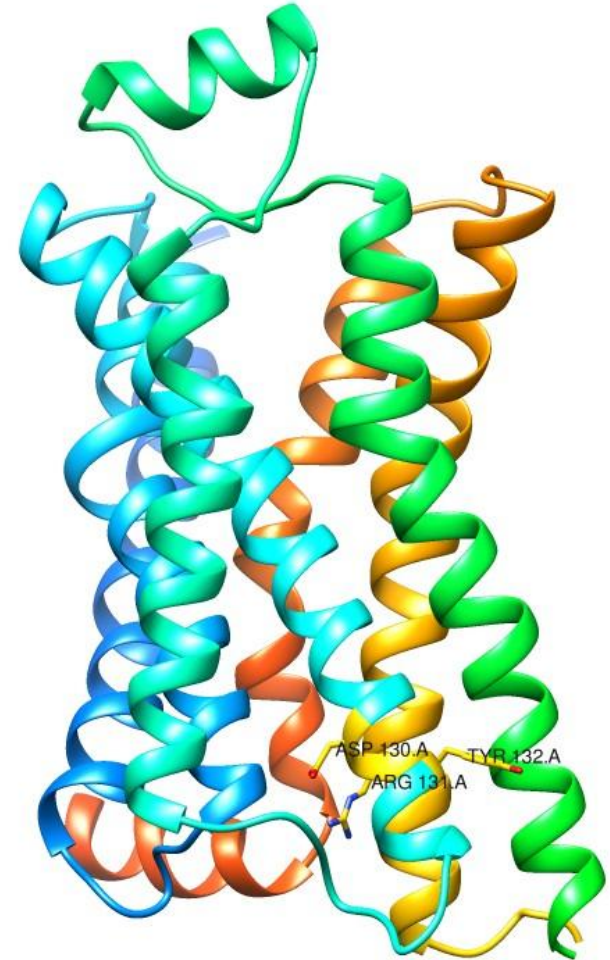
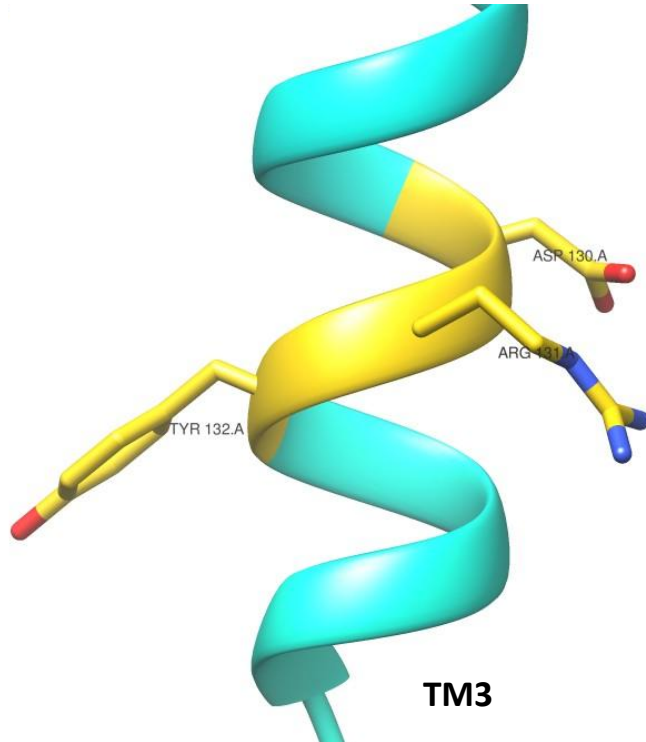


β2-ADRC Conserved Motifs

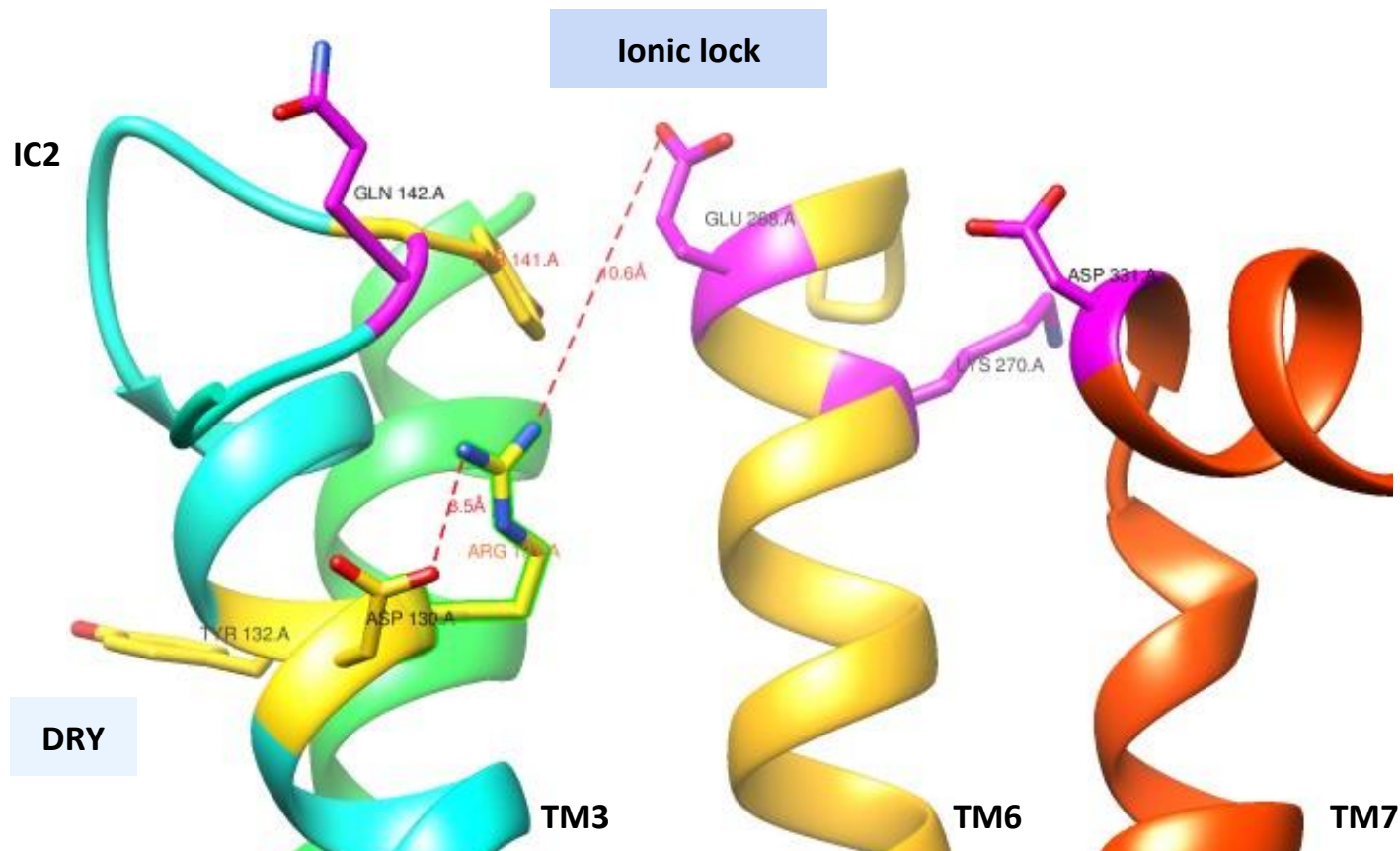


β2-ADRC Conserved Motifs

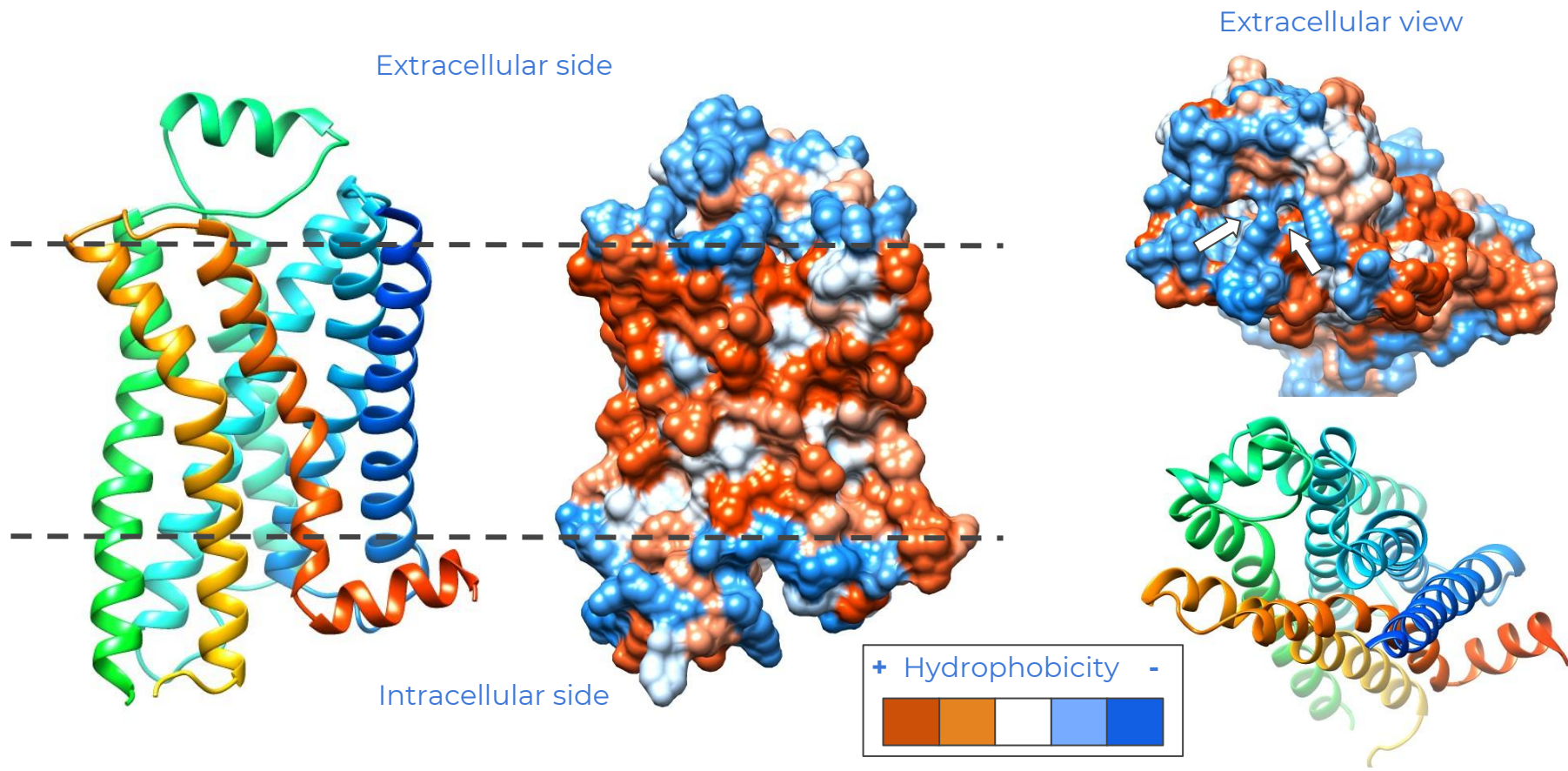
DRY (E/D)R(Y/W)



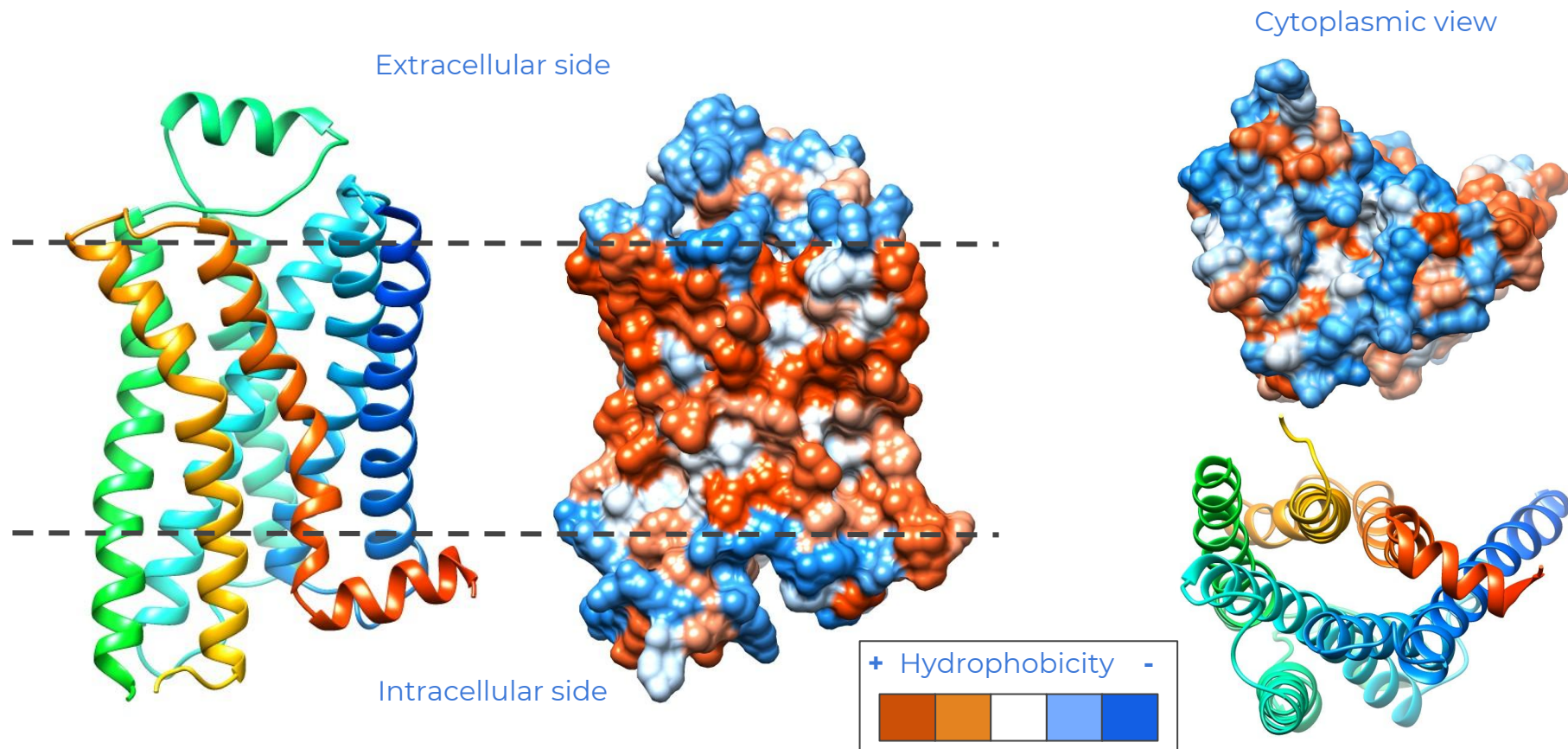
$\beta 2$ -ADRC Conserved Motifs



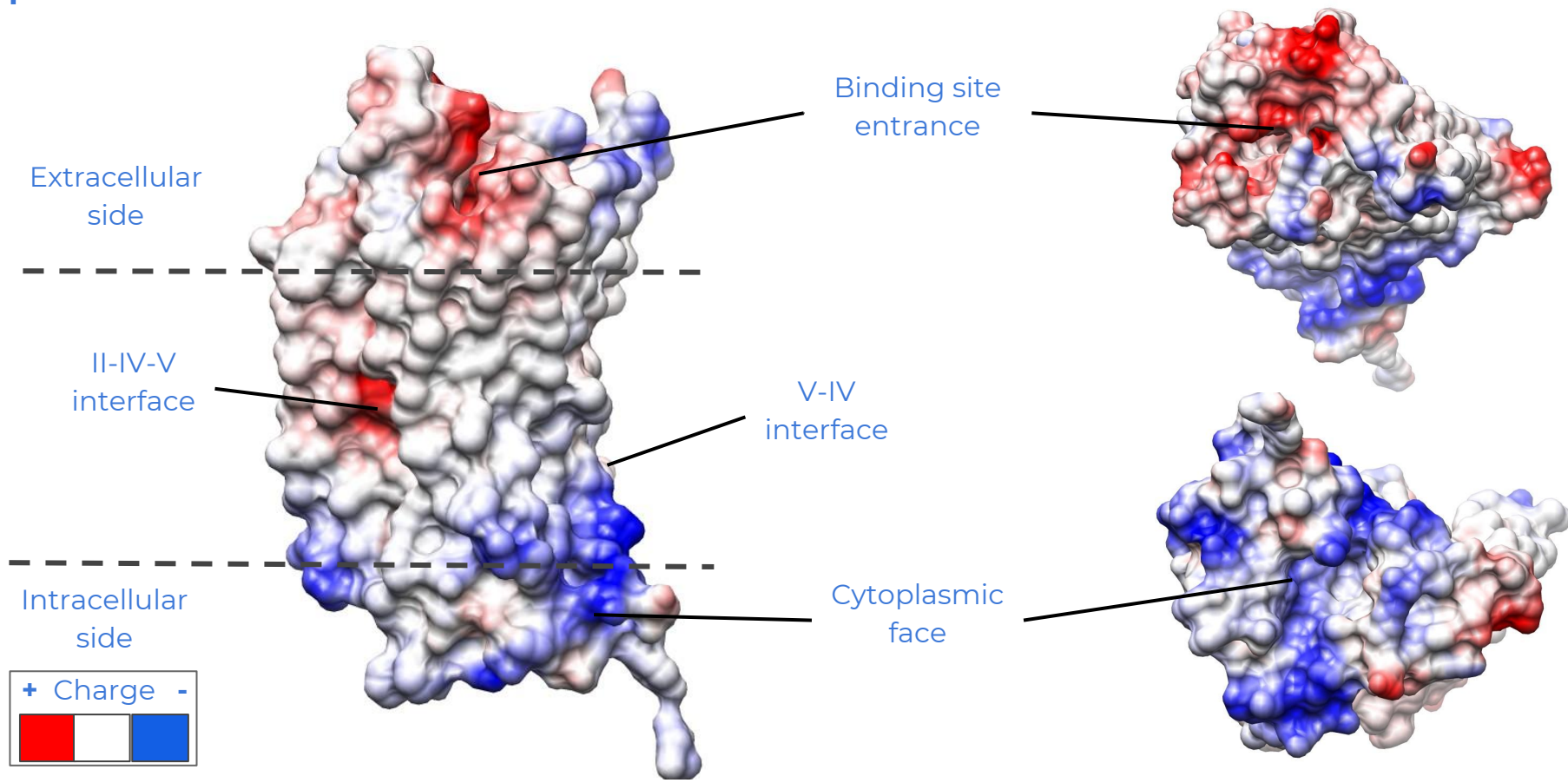
β2-ADRC Hydrophobicity



β2-ADRC Hydrophobicity



$\beta 2$ -ADRC Electrostatic Potentials



Sequence Evolution

ADRENERGIC FAMILY IN HUMANS

β2 RECEPTORS AMONG DIFFERENT SPECIES

PSIBLAST

UniProt



Sequence choice

hmmpfam2



HMM choice from PFAM

hmmfetch2



Getting the HMM

hmmalign2



Alignment.
Stockholm format

aconvertMod2



Changing the format to
ClustalW

ConSurf



Tree elaboration

Sequence Evolution of Adrenergic family

```

P35348|ADA1A_HUMAN      .....mvflsgnasdsnsctqppapvniskatllgvilggllilfgvlgNILVILSVACHRHLHSVTHYIVIVNL
P08913|ADA2A_HUMAN      .....mgsllqpdagnaswngteapgggaratpyslqvtllvclagllmltlvfgNVLVIIAVFTSRALKAPQNLFLVSL
P08588|ADRB1_HUMAN      mgagvlvlgasepgnlssaaplpgdaataarllvpasppasllppasespplsqqwtgmgllmalivllivaGNVLVIAIAKTPRLQTLTNLFIMSL
P07550|ADRB2_HUMAN      .....mgqpngsafllapngshapdhdtgerdevvvgmgivmslivlaivfgNVLVITAIKFERLQTVTNFYFITS
P13945|ADRB3_HUMAN      .....mapwphensslapwpdltlapntantsglpgvpweaalagallalavlatvgNLLVIAIAWTPRLQTMNTNVFVTS
#=GC RF                  .....XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

```

TM1

ICL1

TM2

```

P35348|ADA1A_HUMAN      AVADLLLTSTVLPFSAIFEVLG-YWAFGRVFCNIWAADVLCCTASIMGLCIISIDRYIGVSYPLRYPTIVT-QRRGLMALLCVWALSIVISIGP-LFGW
P08913|ADA2A_HUMAN      ASADILVATLVIPFSLANEVMG-YWYFCKAWCEIYLALDVLCTSSIVHLCAISLDRYWSITQAI EYNLKRTPRRIKAIITVWVISAVISFPPLISIE
P08588|ADRB1_HUMAN      ASADLVMGLLVVPFGATIVVWG-RWEYCSFFCELWTSVDVLCVTASIELTCVIALDRYLATISPFYQSLLT-RARARGLVCTVWASISALVSFLPILMHW
P07550|ADRB2_HUMAN      ACADLVMGLAVVPFGAAHILMK-MWTFCNFWCFWTSIDVLCVTASIELTCVIAVDRYFAITSPFKYQSLLT-KNKARVILMVWIVSGLTSFLPIQMHW
P13945|ADRB3_HUMAN      AAADLVMGLLVPPAATLALTG-HWPLCATGCELWTSVDVLCVTASIELTCALAVDRYLAVTNPLRYGALVT-KRCARTAVVLVWVSAAVSFAPIMSQW
#=GC RF                  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

```

ECL1

TM3

ICL2

```

P35348|ADA1A_HUMAN      RQPAPED---...-ETICQIN--EEP-----GYVLFSAIGSYFLPLAIILVMYCRVYVAKRESRGLKSgltktdksdseqvtlrihrkn.....
P08913|ADA2A_HUMAN      KKGGGGG---...-PQPAERPRCEIN-----DQKWYVISSCIGSFAPCLIMILVYVRIYQIAKRRT--VPsrrgpdavaappggterrpnglger
P08588|ADRB1_HUMAN      WRAESDE---...-ARRCYNDPKCCD---FVTN-RAYAIASSVSFYVPLCIMAFAVYLRVFREAQKQVKKIDScerrflggparppspspvpapapp.
P07550|ADRB2_HUMAN      YRATHQEAINcyANETCCDFFTNQA-----YAIASSIVSFYVPLVIMVFVYSRVFQEAQRQLQKIDKsegrfhvqnls.....
P13945|ADRB3_HUMAN      WRVGADAE---...-AQRCHSNPRCCA---FASN-MPYVLLSSVSFYFLPLLVMFLVYARVFFVATRQLRLRGelgrfppeesppapsrslap.....
#=GC RF                  XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

```

TM4

ECL2

TM5

```

P35348|ADA1A_HUMAN      hfsvrLLKFS...REKKAAKTLGIVVGCFLVCLWLPFFFLVMPIGSFFpDf-----KPSETVFKIVFWLGYLNSCINPIIYpcssqefkafqnvlr
P08913|ADA2A_HUMAN      kasrwRGRQN...REKRFTFVLAVVIGVFVVCWFPPFFFTYTLTAVGcSV-----PRTLKFFFFWFGYCNSSLNPVIYtfnhdfrfakfkilc
P08588|ADRB1_HUMAN      krtpsRLVAL...REQKALKTLGIIMGVFTLCWLPPFLANVVKAFHrEL-----VPDRLFVFFNWLGYNASAFNPIIYcrspdfkrqfllcc
P07550|ADRB2_HUMAN      tghgLRSSKfcLKEHKALKTLGIIMGTFTLCWLPPFFIVNIHVHIQdNL-----IRKEVYILLNWIGYVNSGFNPLIYcrspdfriaqfllcl
P13945|ADRB3_HUMAN      grrpaRLLPL...REHRALCTGLIMGTFTLCWLPPFLANVLRALGgPS-----LVPGPAFLALNWLGYNASAFNPLIYcrspdfsafrllcr
#=GC RF                  .....XXXXX.....XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.....

```

ICL3

TM6

ECL3

TM7

Adrenergic Rc	β2	General features	Evolution	Representation	Function	Ligands
---------------	-----------	------------------	------------------	----------------	----------	---------

Sequence Evolution of $\beta 2$

A0A1D5P7A9_gallusgallus
A0A3B4CKI0_pygocentrusnattereri
D2H8N7_ailuropodamelanoleuca
E7EYN4_daniorerio
G1KR29_anoliscarolinensis
G3QRR6_gorillagorillagorilla
K7FNJ9_pelodiscussinensis
P07550_homosapiens
P10608_rattusnorvegicus
P18762_musmusculus
P54833_canislupefamiliares
Q28044_bostaurus
Q28509_macacamulatta
Q28997_susscrofa
Q8K4Z4_caviaporcellas
Q8UUY8_oncorhynchusmykiss
Q9TST5_feliscatus
U3KJ44_ficedulaalbicollis
#=GC RF

ECL2

Sequence Evolution

A0A1D5P7A9_gallusgallus
 A0A3B4CKI0_pygocentrusnattereri
 D2H8N7_ailuropodamelanoleuca
 E7EYN4_dantiorerio
 G1KR29_anoliscarolinensis
 G3QR6_gorillagorillagorilla
 K7FNJ9_pelodiscussinensis
 P07550_homosapiens
 P10608_rattusnorvegicus
 P18762_musmusculus
 P54833_canislupefamiliaris
 Q28044_bostaurus
 Q28509_macacamulatta
 Q28997_susscrofa
 Q8K4Z4_caviaporcellas
 Q8UUY8_oncorhynchusmykiss
 Q9T5T5_feliscatus
 U3KJ44_ficedulaalbicollis
 #=GC RF

-...-AILCYEKDCCD---FFTNQAYAIASSIISFYPLVVMVFVY
 QdcLKN.AYCCDFNTNAP-----YAVASSIVSFYIPLVIMVFVY
 -...-.KETCCDFFTNQA-----YAIASSIVSFYPLVVMVFVY
 TlccYN.NTYCCFDITYS-----YAISSISFYIPLVIMVFVY
 K...CYKDENCDDFFINGG-----YAISSIVSFYPLVVMVFVY
 Nc.YAN.ETCCDFFTNQA-----YAIASSIVSFYVPLVIMVFVY
 -...-.ALECYNNASCH---FFTNQAYAITSSIIISFYPLVVMVFVY
 Nc.YAN.ETCCDFFTNQA-----YAIASSIVSFYVPLVIMVFVY
 -...-.KETCCDFFTNQA-----YAIASSIVSFYVPLVVMVFVY
 -...-.EETCCDFFTNQA-----YAIASSIVSFYVPLVVMVFVY
 -...-.KETCCDFFTNQA-----YAIASSIVSFYPLVVMVFVY
 N..cYA.KETCCDFFTNQ-----YAIASSIVSFYPLVVMVFVY
 -...-.KETCCDFFTNQA-----YAIASSIVSFYVPLVIMVFVY
 N..cYA.EEACDDFFTNQ-----YAIASSIVSFYPLVVMVFVY
 -...-.AINCAYEETCCD---FFTNQAYAIASSIVSFYPLVVMVFVY
 Sc.LED.AHCCDFNTNAA-----YAVASSVVSFYIPLAVMAFVY
 -...-.KETCCDFFTNQA-----YAIASSIVSFYPLVVMVFVY
 -...-.AIRCYEDEMCCD---FFTNQAYAIASSIISFYPLVVMVFVY
 X...XX.XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

ARVFQVAKKQLQKIDQsegrfhiqnke.....qdqngkaghrssSKF
 GRVFQEARRQLQKIDRvegrirtqsfg.....tqdgnevrnrkTRF
 SRVFQVQAQRQLQKIDKsegrfhaqnlsqv.....eqdgrsgghrrsASKF
 SRVFQEARRQLKINKtegrfhaqksnsrn..qdvannhsemrstkkAKF
 SRVFQVARKQLKKIDKcegrfhqqsnnq.....eqnagragnttrvSKF
 SRVFQEAKRQLQKIDKsegrfhvqnls.....qveqdgtrtghlLRRS
 AKVFQVAKKQLKKIDKsegrfhnnqnnq.....qepngrashrrtsSKF
 SRVFQEAKRQLQKIDKsegrfhvqnls.....qveqdgtrtghlLRRS
 SRVFQVAKRQLQKIDKsegrfhaqnlsqv.....eqdgrsgghlrrssSKF
 SRVFQVAKRQLQKIDKsegrfhaqnls.....qveqdgtrsgghlLRRS
 SRVFQVQAQRQLQKIDRsegrfhaqnls.....qveqdgtrsgghrRRS
 SRVFQVAKRQLQKIDKsegrfhaqnvsqv.....eqdgrsglgqrrtsSKF
 SRVFQEAKRQLQKIDKsegrfhaqnls.....qveqdgtrtghlLRRS
 SRVFQVARRQLQKIDKsegrfhaqnls.....qaeqdgtrsgpgghRRS
 SRVFQVAKKQLQKIDRsegrfhtqnlsqv.....eqdgrsgghlrrssSKF
 GRVFQEARKQLEKIRGsegrfhaqmldnnqgqdgddgsgggggngkrPKF
 SRVFQVQAQRQLQKIDKsegrfhaqnlsqv.....eqdgrsgghrrsASKF
 ARVFQVAKKQLQKIDRsegrfhtqnke.....qeqkggaghrssSKF
 XX

ECL2

TM5

ICL3

TM6

ECL3

TM7

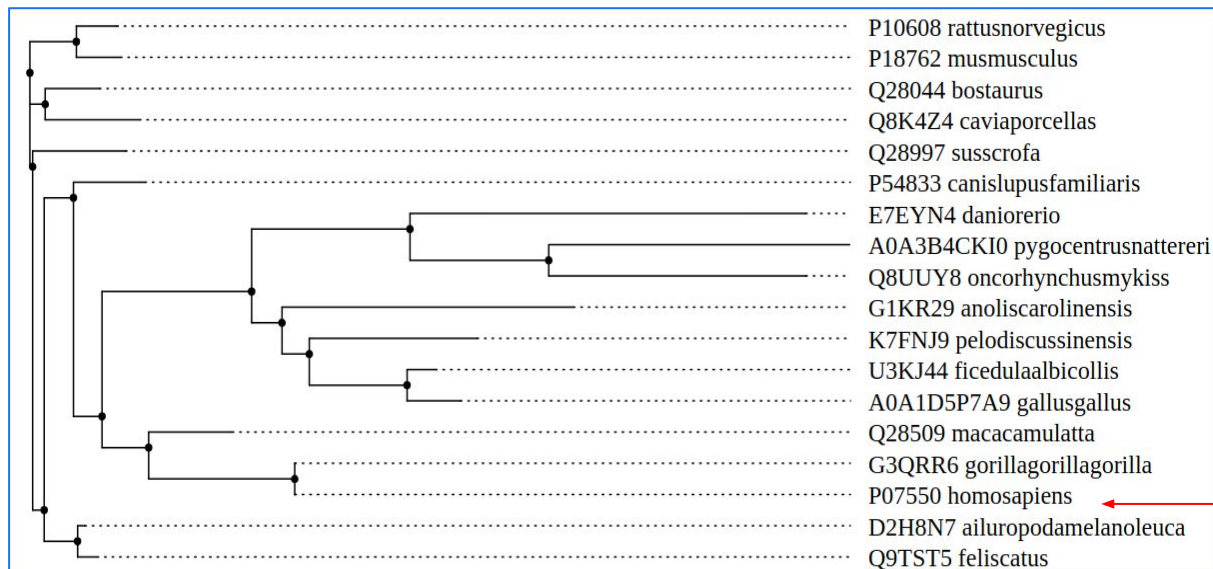
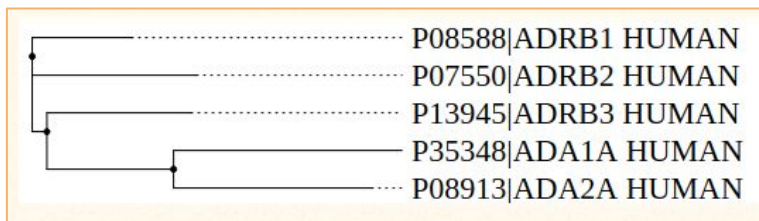
A0A1D5P7A9_gallusgallus
 A0A3B4CKI0_pygocentrusnattereri
 D2H8N7_ailuropodamelanoleuca
 E7EYN4_dantiorerio
 G1KR29_anoliscarolinensis
 G3QR6_gorillagorillagorilla
 K7FNJ9_pelodiscussinensis
 P07550_homosapiens
 P10608_rattusnorvegicus
 P18762_musmusculus
 P54833_canislupefamiliaris
 Q28044_bostaurus
 Q28509_macacamulatta
 Q28997_susscrofa
 Q8K4Z4_caviaporcellas
 Q8UUY8_oncorhynchusmykiss
 Q9T5T5_feliscatus
 U3KJ44_ficedulaalbicollis
 #=GC RF

FL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 CL...KDHKALKTLGIIMGTFTLCWLPFFVLNVAAAwkM-----
 CL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 YL...KEHKALKTLGIIMGTFTLCWLPFFVLNVIPKGSvd-----
 CL...KEHKALKTLGIIMGTFTLCWLPFFIVHVFHAnedN-----
 SKfcKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 CL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 CL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 SKfcKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 CL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 SKfcKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 YL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 SKfcKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 SKfcKEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 YL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 CL...KEHKALKTLGIIMGTFTLCWLPFFVLNVVVTkwV-----
 CL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 FL...KEHKALKTLGIIMGTFTLCWLPFFIVNIVHVtQdN-----
 XX...XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

--IPKYVYILLNWLGYVNSAFNPLIYcrspdfryafqellclrrsalkmy
 --NIKLPFRILNWIGYANSAFNPLIYcrspefrlafqellclrrsskssak
 --IPKEVYILLNWLGYVNSAFNPLIYcrspdfriafqellclrrsslkay
 ----WTFRILNWIGYANSAFNPLIYcrspdfryafqellclrrsrypnv
 --IPKSLYVILLNWLGYVNSAFNPLIYcrspdfryafqellclrrsalkmy
 --IRKEVYILLNWLGYVNSGFNPLIYcrspdfriafqellclrrsslkay
 --IPTLYVILLNWLGYVNSAFNPLIYcrspdfryafqellclrraalklh
 --IRKEVYILLNWLGYVNSGFNPLIYcrspdfriafqellclrrsslkay
 --IPKEVYILLNWLGYVNSAFNPLIYcrspdfriafqellclrrssskty
 --IPKEVYILLNWLGYVNSAFNPLIYcrspdfriafqellclrrsslkay
 --IPKEVYILLNWLGYVNSAFNPLIYcrspdfriafqellclrrsslkay
 --IRKEIYILLNWLGYNSAFNPLIYcrspdfriafqellclrrsslkay
 --IPKEVYILLNWLGYVNSGFNPLIYcrspdfriafqellclrrsslkac
 --IPKEVYILLNWLGYVNSAFNPLIYcrspdfriafqellclrrsslkay
 --IPKEVYILLNWLGYVNSAFNPLIYcrspdfriafqellclrrsalkay
 --NIKMPFRILNWIGYANSAFNPLIYcrspefrlafqellclrrgaafptn
 --IPKEVYILLNWLGYVNSAFNPLIYcrspdfriafqellclrrsslkay
 --IPKYVYILLNWLGYVNSAFNPLIYcrspdfryafqellclrrsalkmy
 XX

[illegible]

Sequence Evolution



Main conclusions:

- Relationships observed among the whole family: α Rc are shown in the same cluster
- β2RC sequence (specifically TM regions) is highly conserved among evolution
- Primates' β2RC are closely located in the tree

$\beta 1$ ADR - $\beta 2$ ADR comparison

Sequence % identity: **67%**

Subtle differences in EC side, binding site included

More differences in IC side

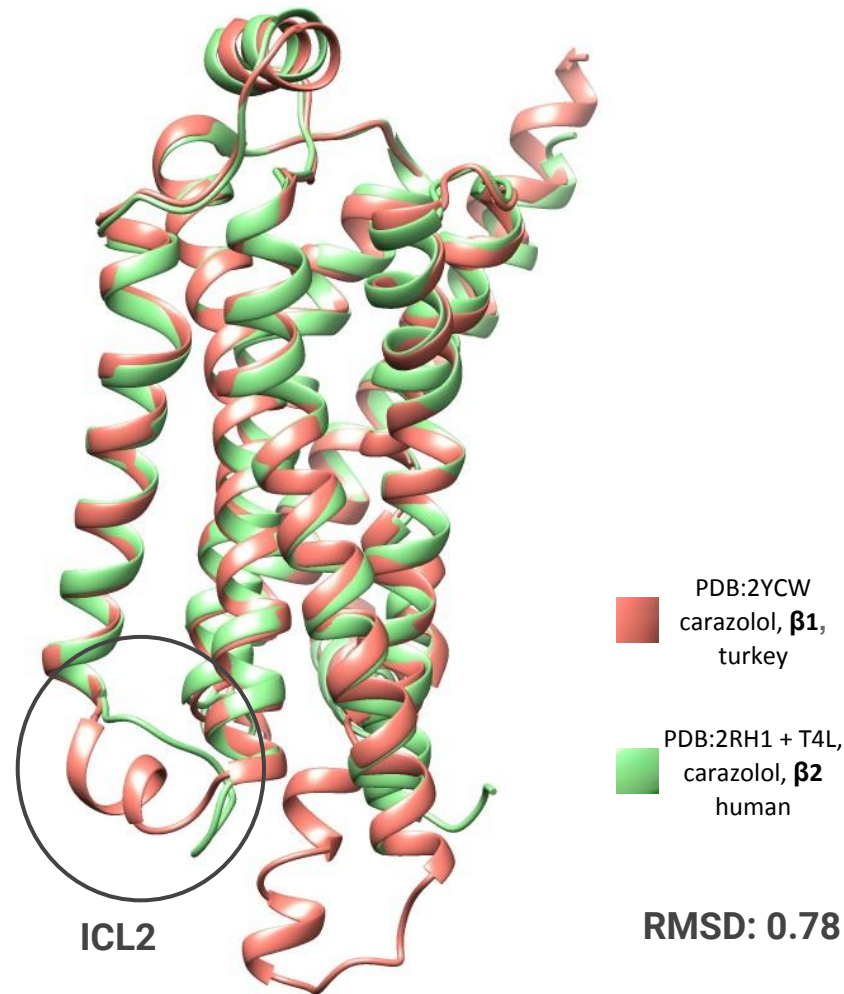
ICL2

α -helix in **$\beta 1$** adrenoceptor

$\beta 1$ Tyr149 (ICL2) forms a HB with Asp138 (DRY, TM3)

Important for **$\beta 1$** activation

Existence and relevance of this in **$\beta 2$** unclear



$\beta 1$ ADR - $\beta 2$ ADR comparison

Sequence % identity: **67%**

Subtle differences in EC side, binding site included

More differences in IC side

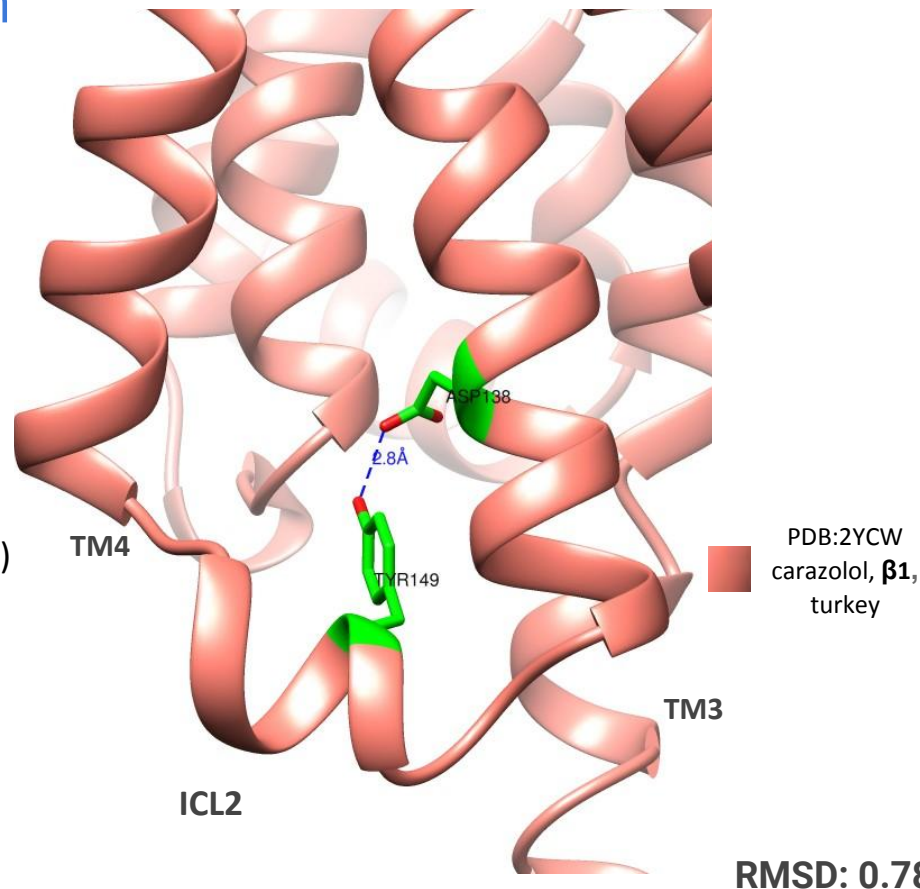
ICL2

α -helix in **$\beta 1$** adrenoceptor

$\beta 1$ Tyr149 (ICL2) forms a HB with Asp138 (DRY, TM3)

Important for **$\beta 1$** activation

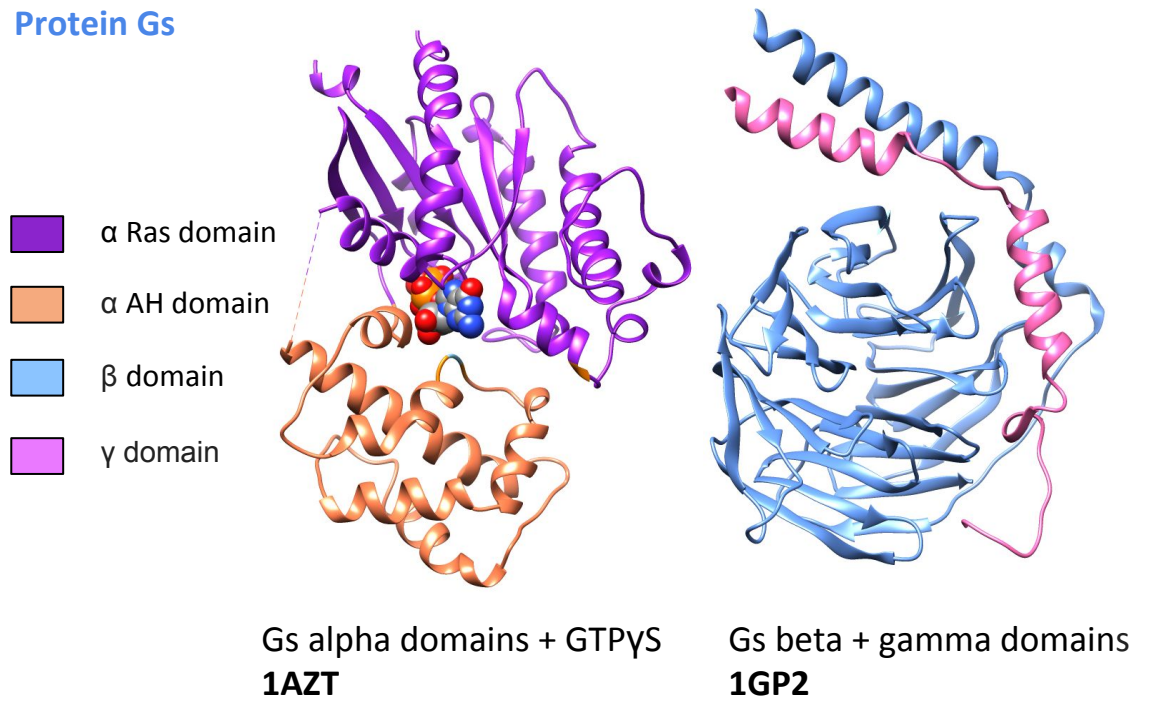
Existence and relevance of this in **$\beta 2$** unclear



Basics on GPCR representation

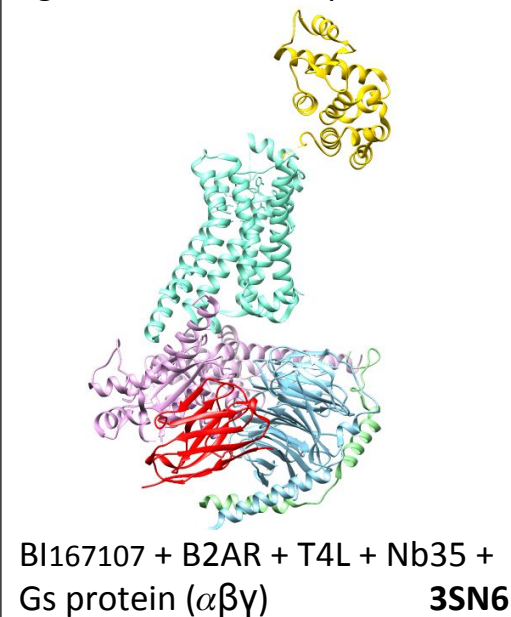
MODELS USED

Protein Gs



Ternary complex

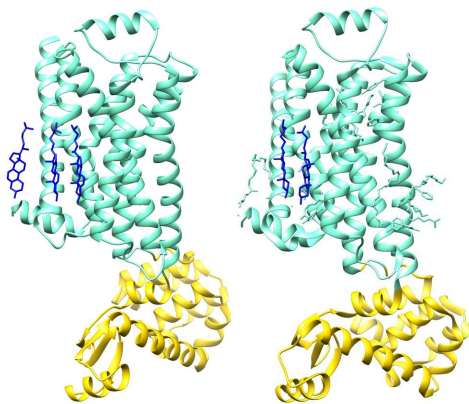
agonist + B2AR + Gs protein



Basics on GPCR representation

MODELS USED

Cholesterol Binding Site

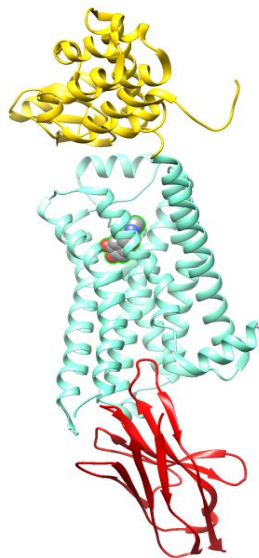


2RH1
Carazolol +
T4L

3D4S
Timolol +
T4L

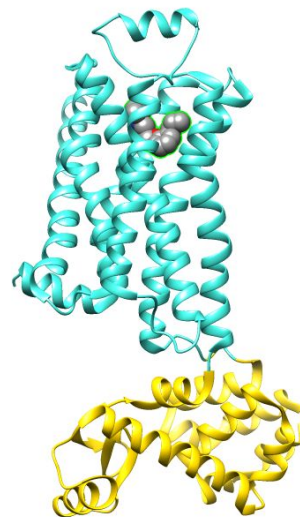
Agonist

Active State B2AR



4LDO
Adrenaline + T4L + Nb80

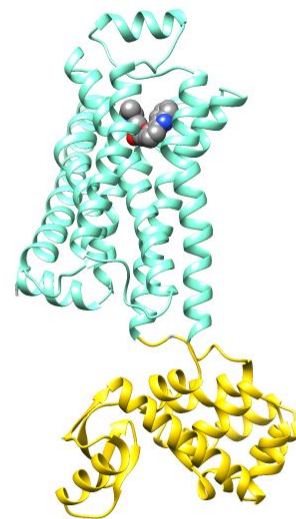
Antagonist



3NYA
Alprenolol + T4L

Inverse Agonist

Inactive State B2AR



2RH1
Carazolol + T4L

Basics on GPCR representation

CHALLENGES OF CRYSTALLISATION

B2AR is conformationally complex:

1. Intramolecular bonds in inactive state are essential for structural integrity
2. Agonist-independent **basal activity**
3. **Structural instability** of its active state
4. Most unstructured regions:
C-terminal and ICL2

Water-soluble crystallization strategy

Removing most **unstable regions**

Essential for intracellular protein interaction

GPCR crystallisation strategy

Minimal interchain contacts

+

Important crystal packing interactions

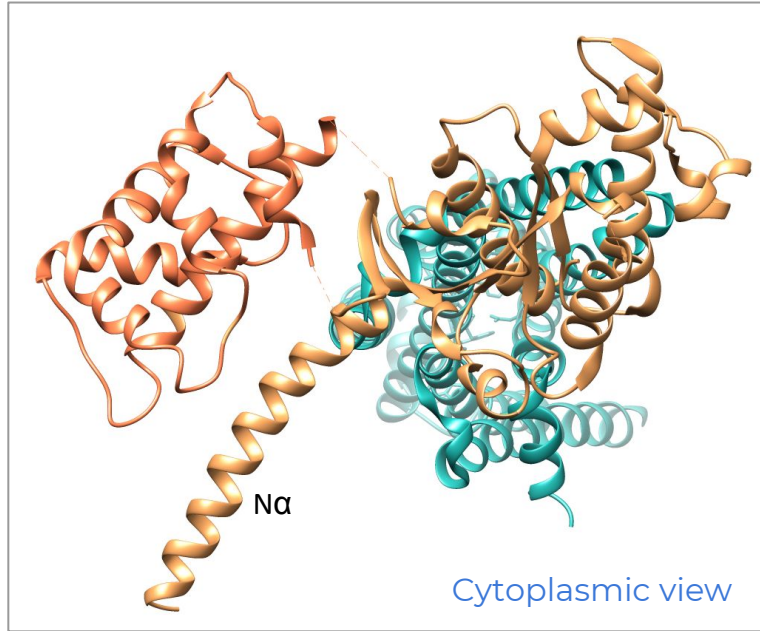
T4L

- Glycoside hydrolase of the T4 bacterial phage.
- Fused between T5 and T6 (ICL3) or at N-terminal.

**Nb80
Nb35**

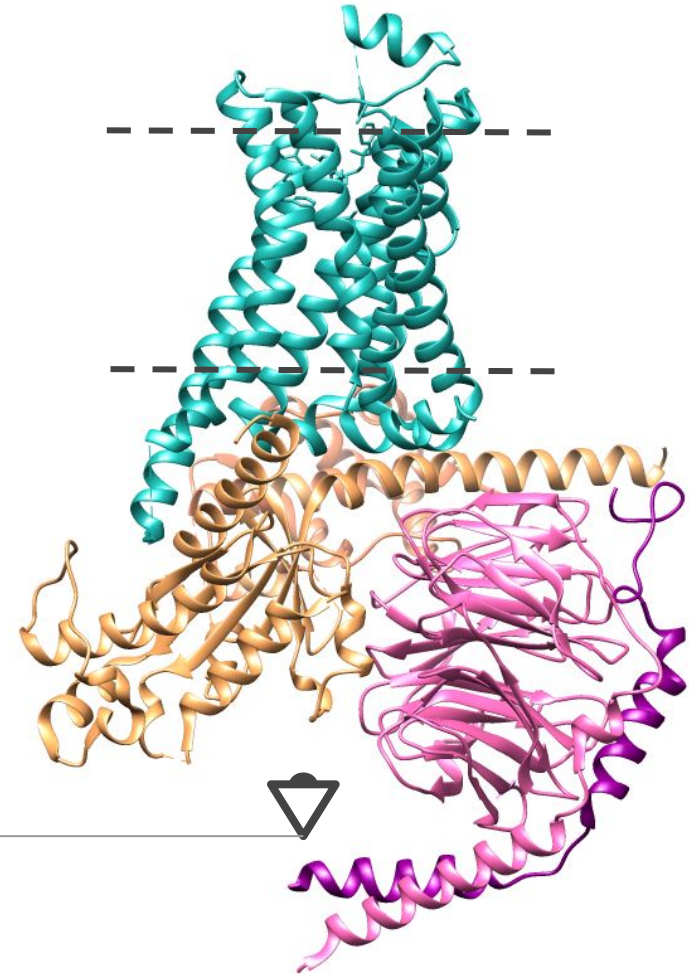
- Single domain fragment of Camelid heavy chain only antibodies.
- Small (15kDa) and stable, has access to clefts of the AR.
- Binds to conformation-specific B2AR epitopes with its long CDR3 loop.

Protein G interactions

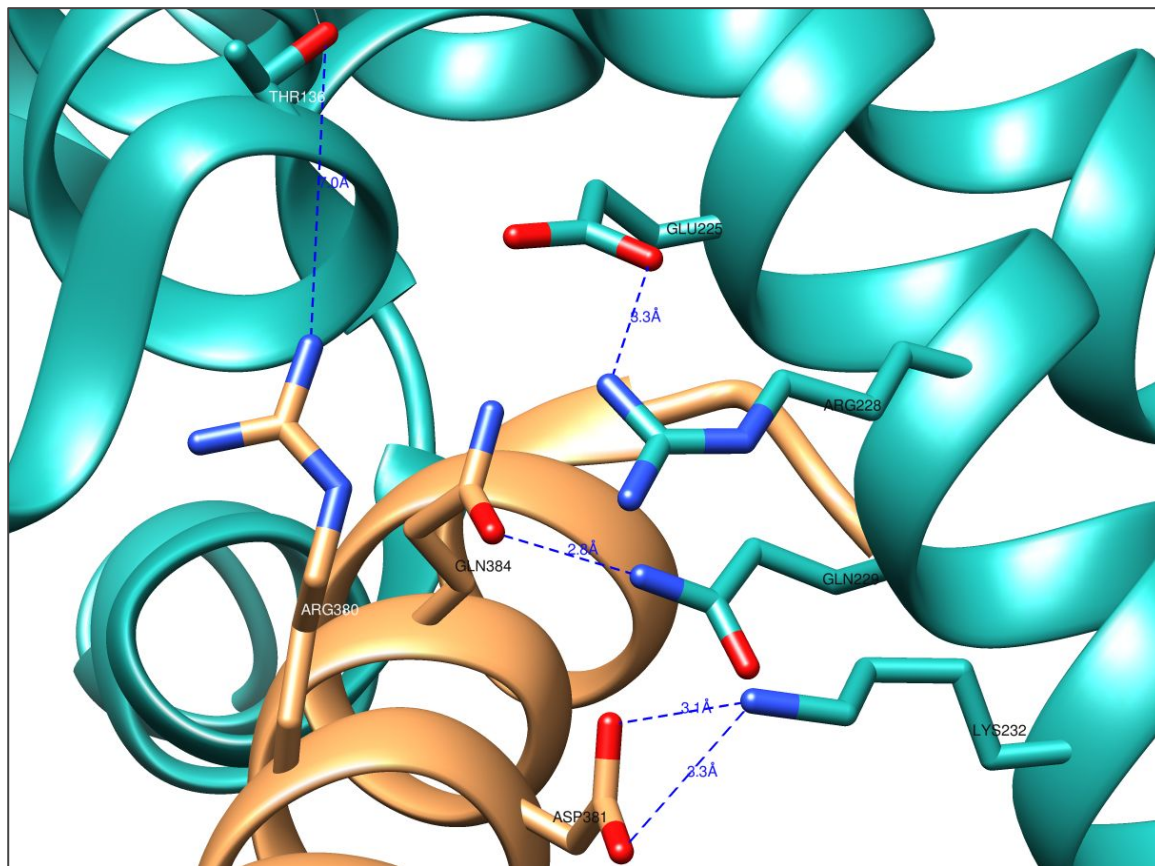


- β2A Receptor
- α Ras domain
- α AH domain
- β domain
- γ domain

3SN6



Protein G interactions



Hydrogen Bonds Contacts

Atoms $\beta 2AR$ TM3 TM5	Atoms $Gs\alpha$ $\alpha 5$	Distance (Å)
THR 136 O	ARG 380 NH2	3.0
ILE 135 O	GLN 384 NE2	2.9
GLN 229 NE2	GLN 384 OE1	2.8
LYS 232 NZ	ASP 381 OD1	3.1

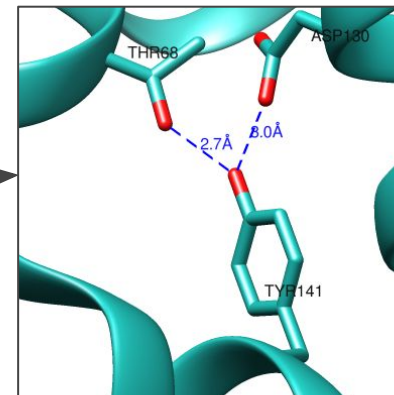
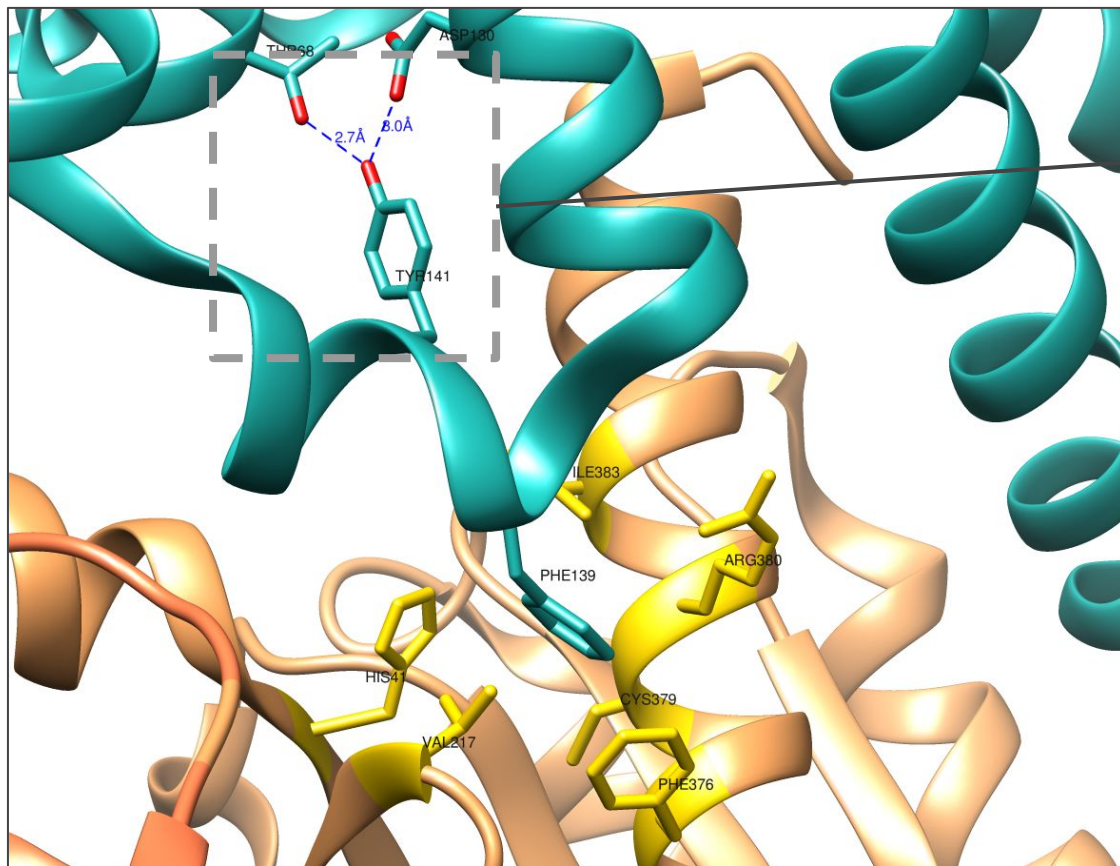
Salt Bridge Contacts

Atoms $\beta 2AR$ TM5	Atoms $Gs\alpha$ $\alpha 5$	Distance (Å)
LYS 232 NZ	ASP 381 OD1	3.1

 $\beta 2A$ Receptor

 α Ras domain

Protein G interactions



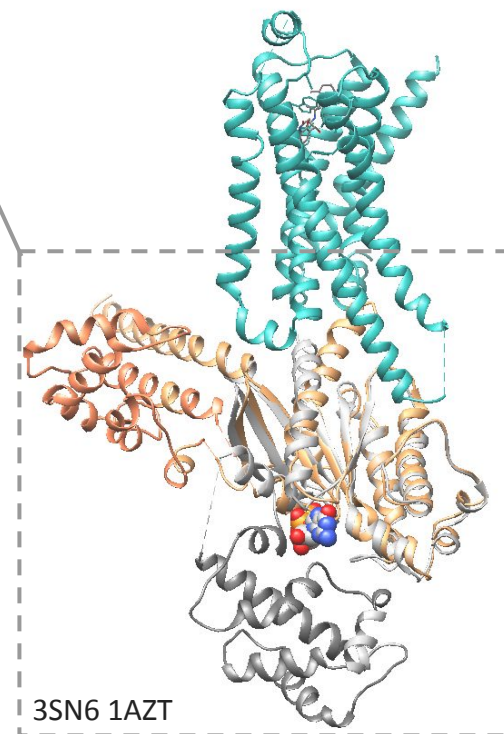
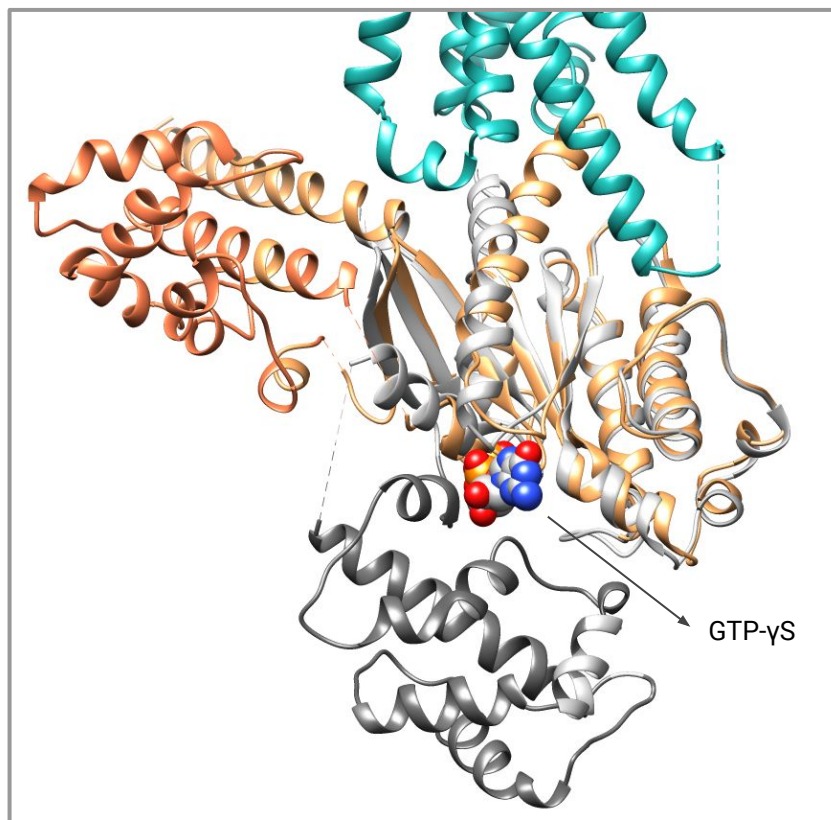
Hydrophobic interactions

Atoms $\beta 2AR$ ICL2	Atoms $Gs\alpha$ $\alpha 5$	Distance (Å)
PRO 138 O	ILE 383 CD1	3.4
PHE 139 CE1	PHE 376 CZ	3.3
PHE 139 CZ	CYS 379 C	3.9
PHE 139 CZ	ARG 380 N	3.2
PHE 139 CD2	HIS 41 NE ($\beta 1$)	3.6
PHE 139 CB	VAL 217 CG2 ($\alpha 4$)	3.7

■ $\beta 2A$ Receptor ■ α Ras domain

Protein G activation

- β2A Receptor
- α Ras domain GDP-bound
- α AH domain GDP-bound
- α Ras domain GTP-bound
- α AH domain GTP-bound



Protein G activation

-  $\beta 2A$ Receptor
-  α Ras domain GDP-bound
-  α AH domain GDP-bound
-  α Ras domain GTP-bound
-  α AH domain GTP-bound

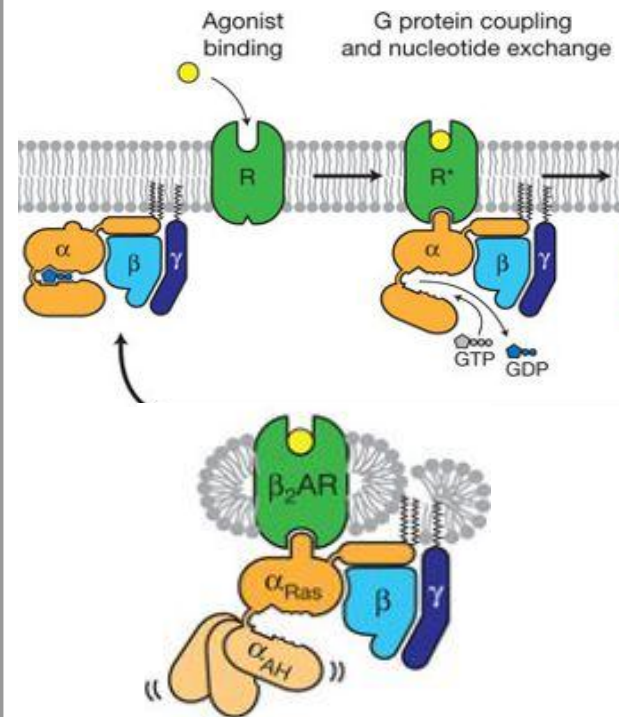


Fig. 4: Rasmussen F. et. al, 2011.

Active-Inactive States

Inactive state

Little changes, cytoplasmic side
Slight change binding site

Inwards movement of **TM3** and **TM7**

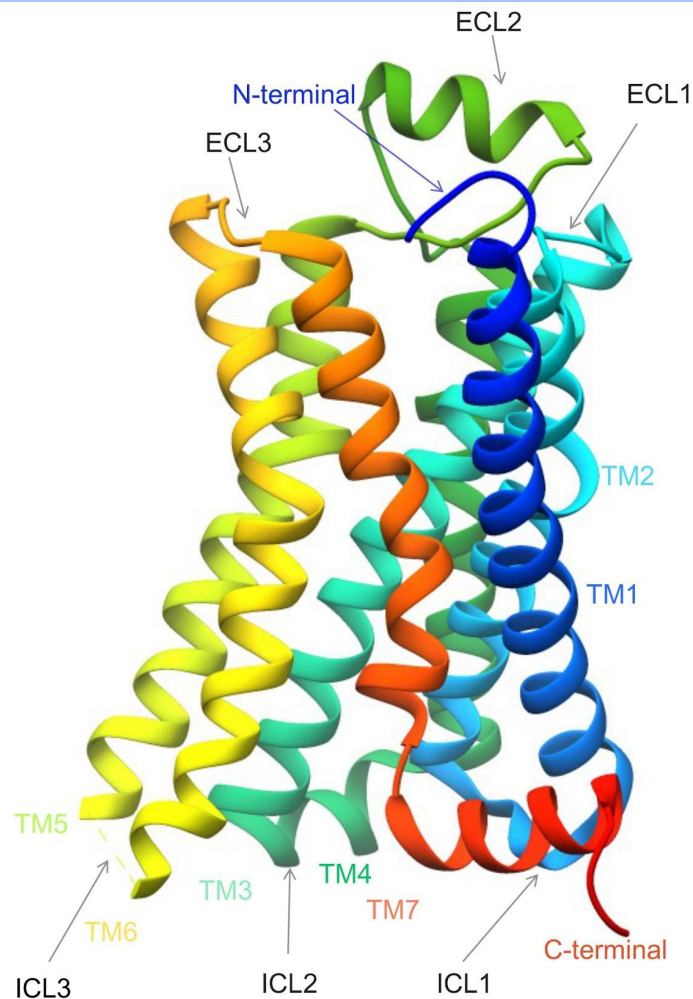
Outwards movement of **TM6** and **TM5**

Distorsion of helix in **TM7**

Different disposition of Tyr326 in **TM7**
(NPxxY domain)

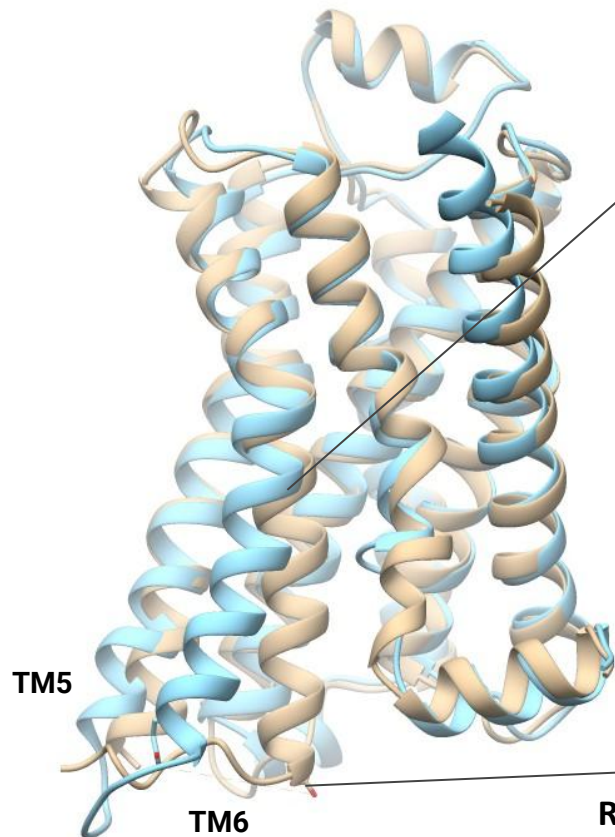
Hydrogen bond between Tyr326 (**TM7**)
and Tyr219 (**TM5**)

Helps holding **TM6**



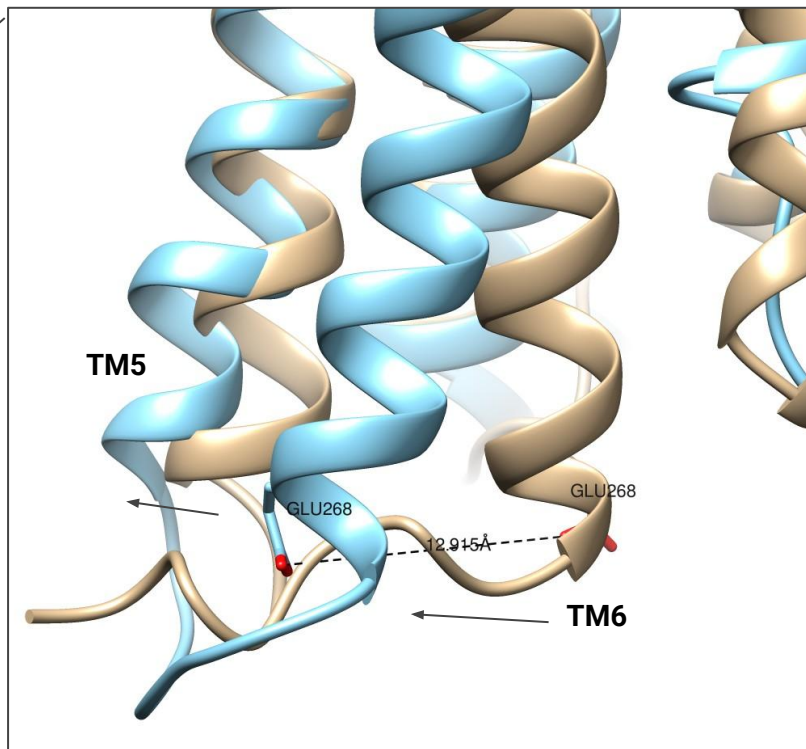
Active-Inactive States

TM5, TM6 ($\approx 14\text{\AA}$) outwards movement



Inactive state. PDB
code: 2RH1 + T4L,
carazolol

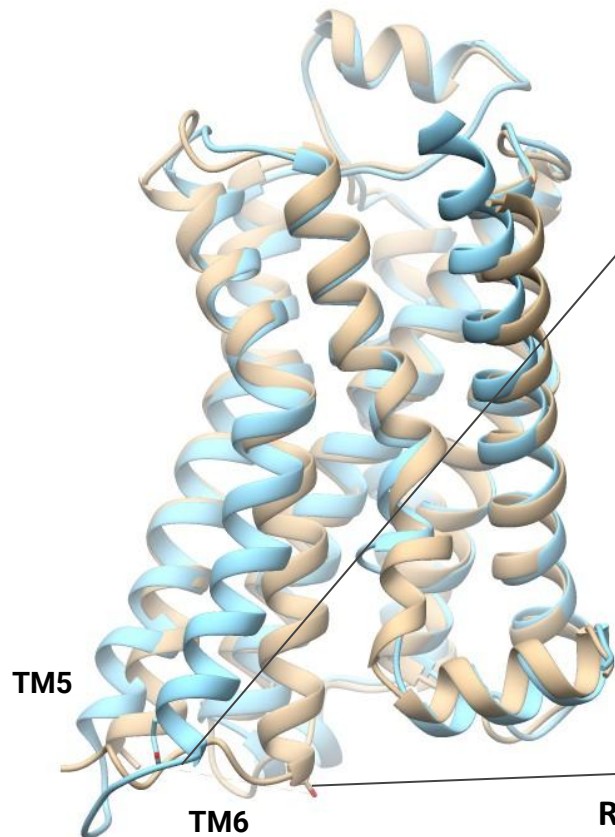
Active state. PDB
code: 4LDO, +
Nb6B9, adrenaline



Side view

Active-Inactive States

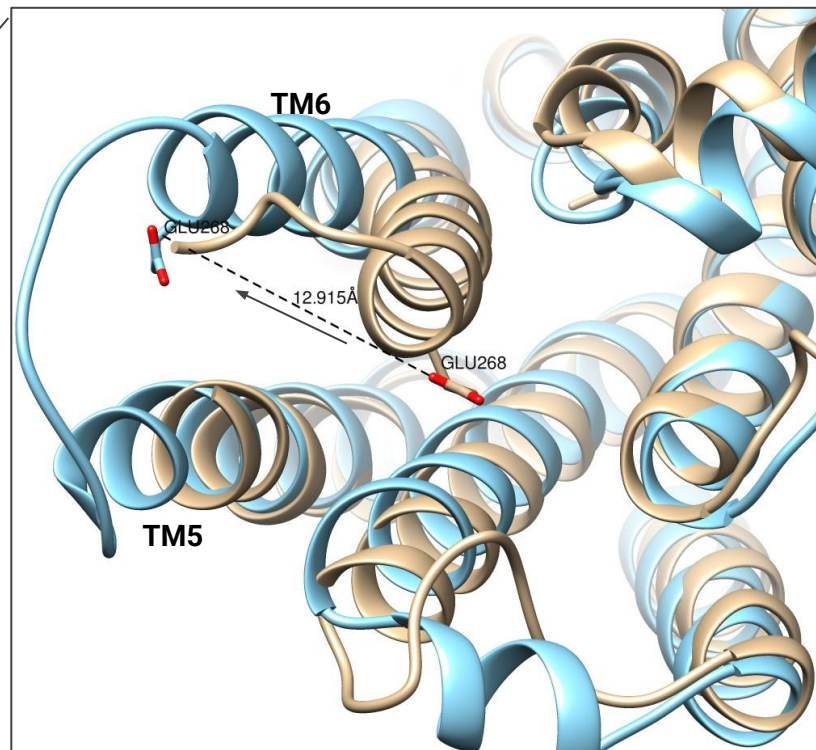
TM5, TM6 (≈14Å) outwards movement



RMSD: 1.52

Inactive state. PDB
code: 2RH1 + T4L,
carazolol

Active state. PDB
code: 4LDO, +
Nb6B9, adrenaline



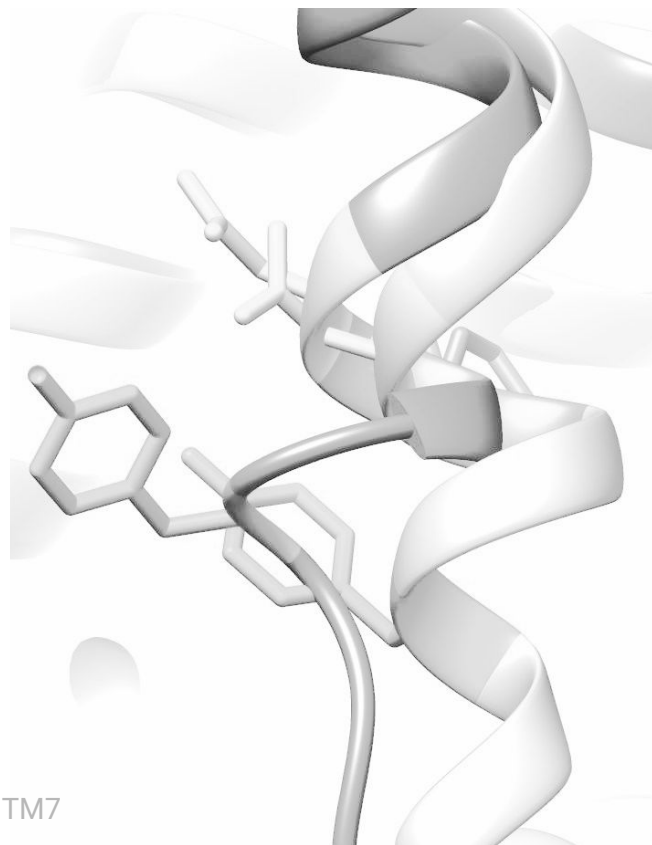
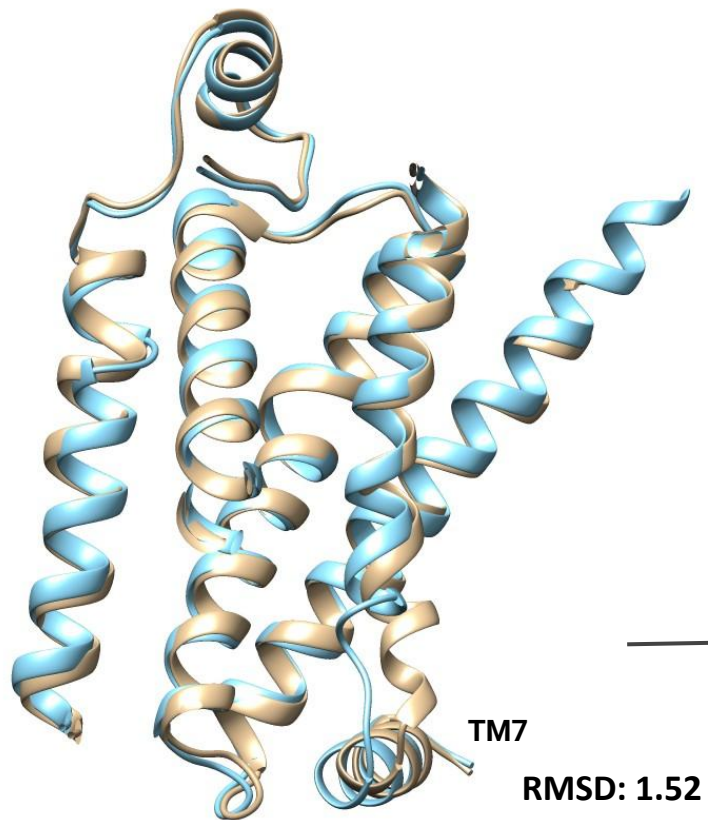
Cytoplasmic view

Active-Inactive States

TM7 distorted helical configuration



NPxxY conserved motif in TM7, Y326 disposition changes



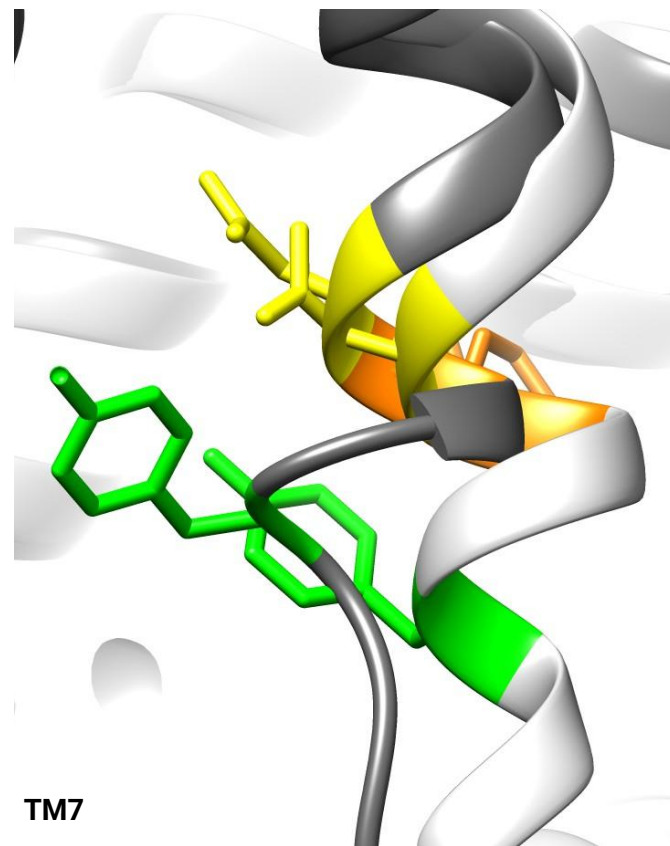
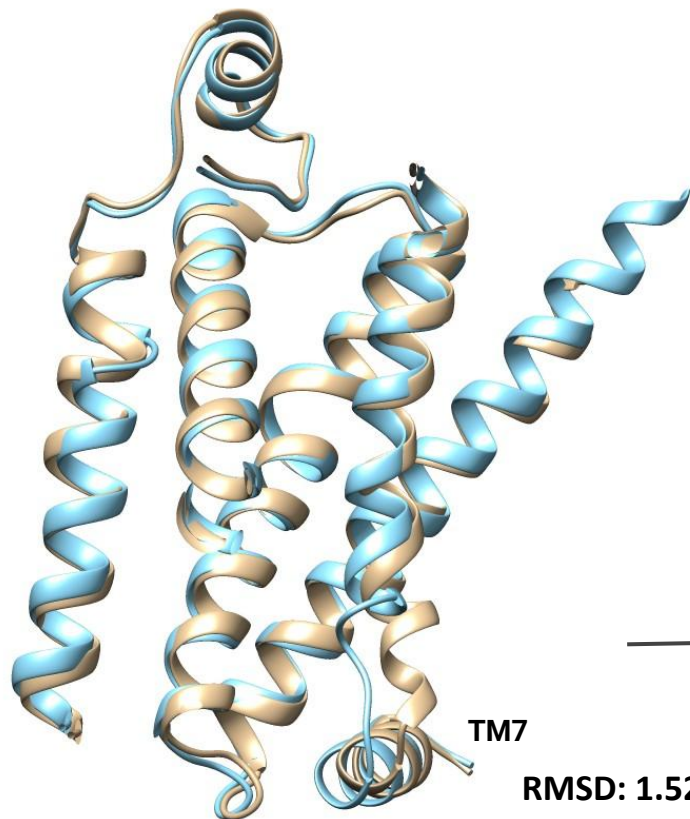
- Inactive state
- Active state
- Asparagine 322
- Proline 323
- Tyrosine 326

Active-Inactive States

TM7 distorted helical configuration



NPxxY conserved motif in TM7, Y326 disposition changes



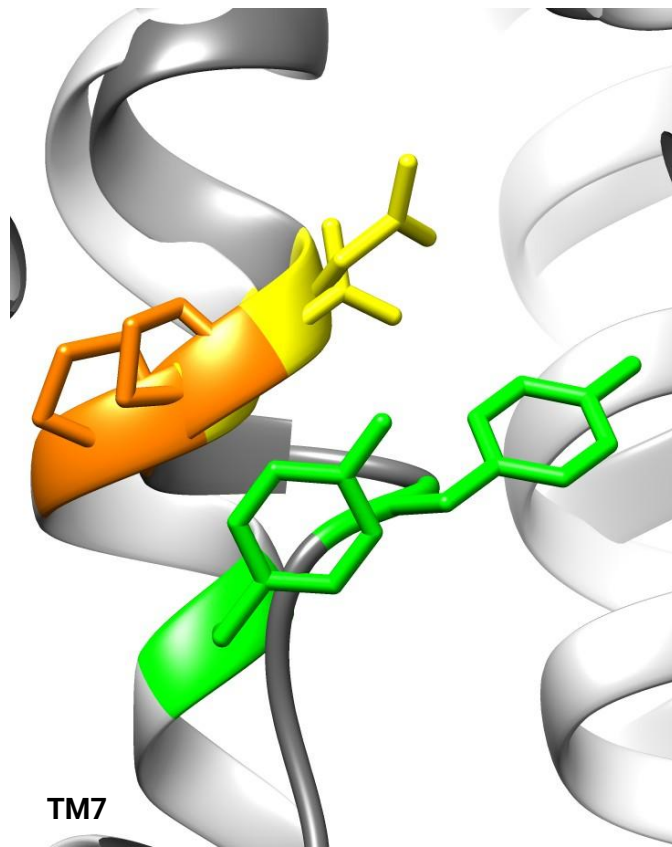
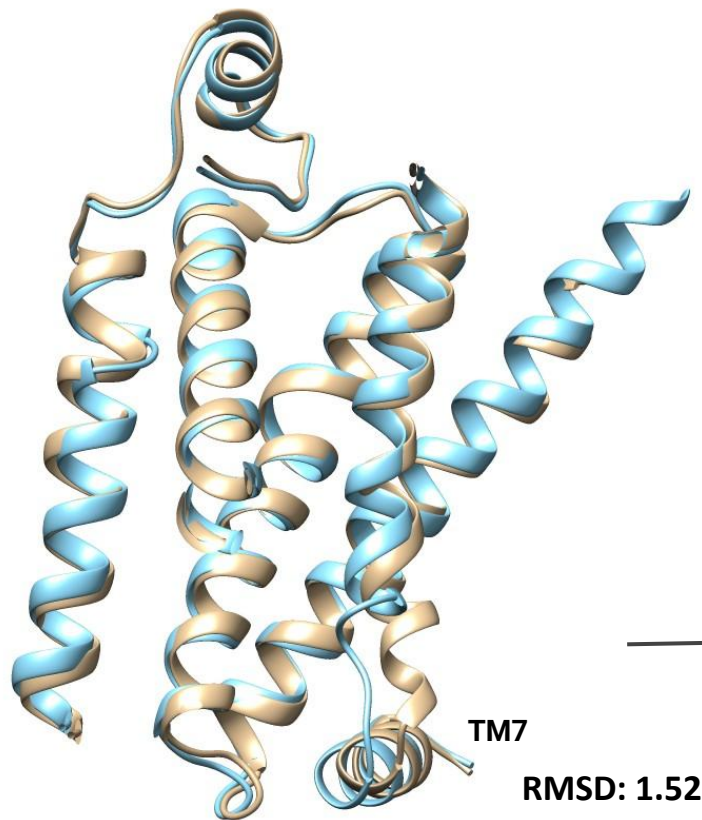
- Inactive state
- Active state
- Asparagine 322
- Proline 323
- Tyrosine 326

Active-Inactive States

TM7 distorted helical configuration



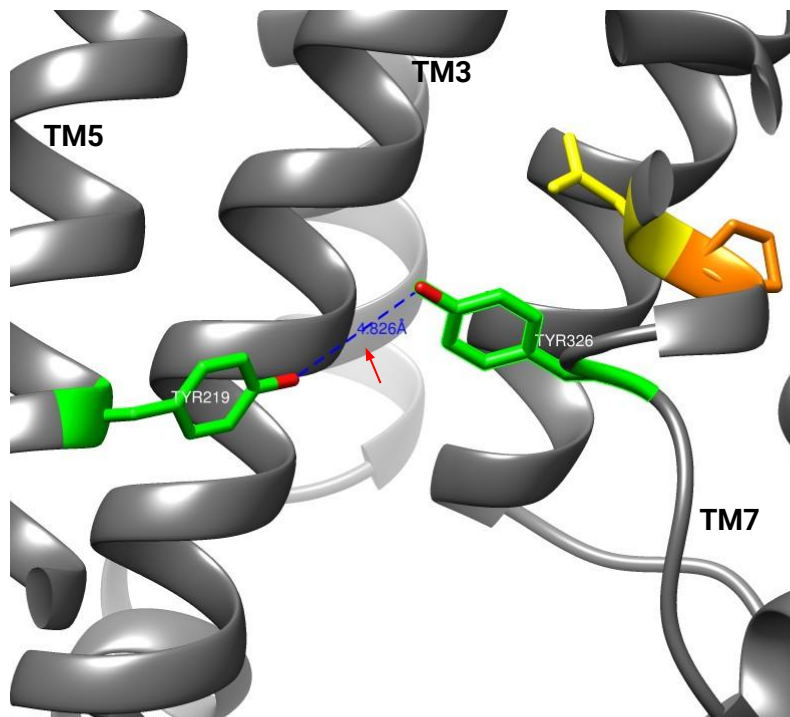
NPxxY conserved motif in TM7, Y326 disposition changes



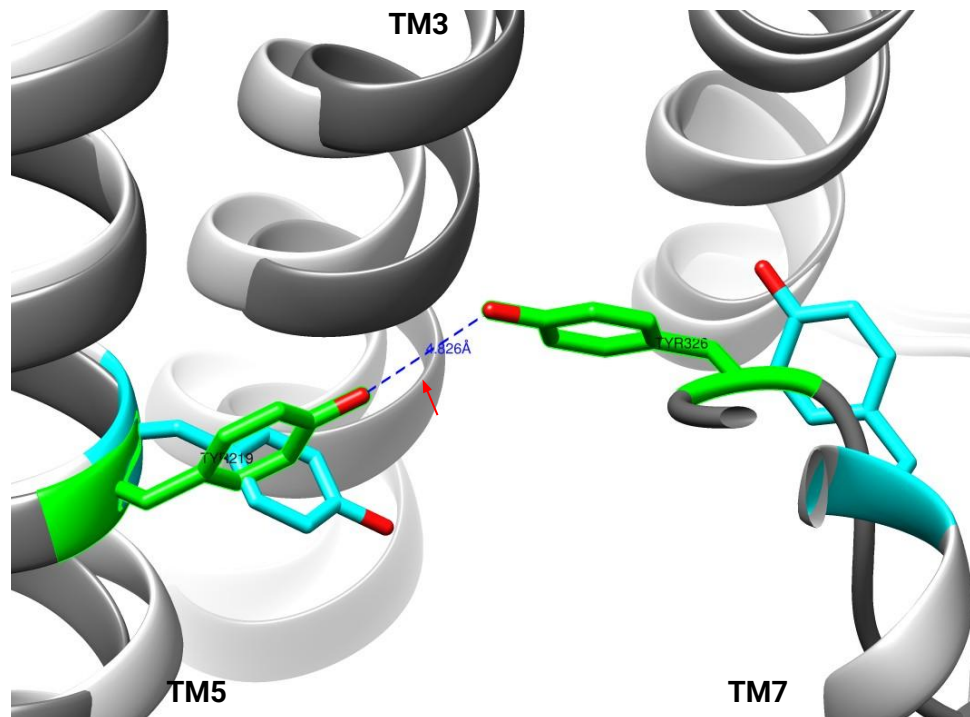
- Inactive state
- Active state
- Asparagine 322
- Proline 323
- Tyrosine 326

Active-Inactive States

Hydrogen bond between Y326 (TM7) and Y219 (TM5) and mediated by a water molecule is just present in the active state



RMSD: 1.52

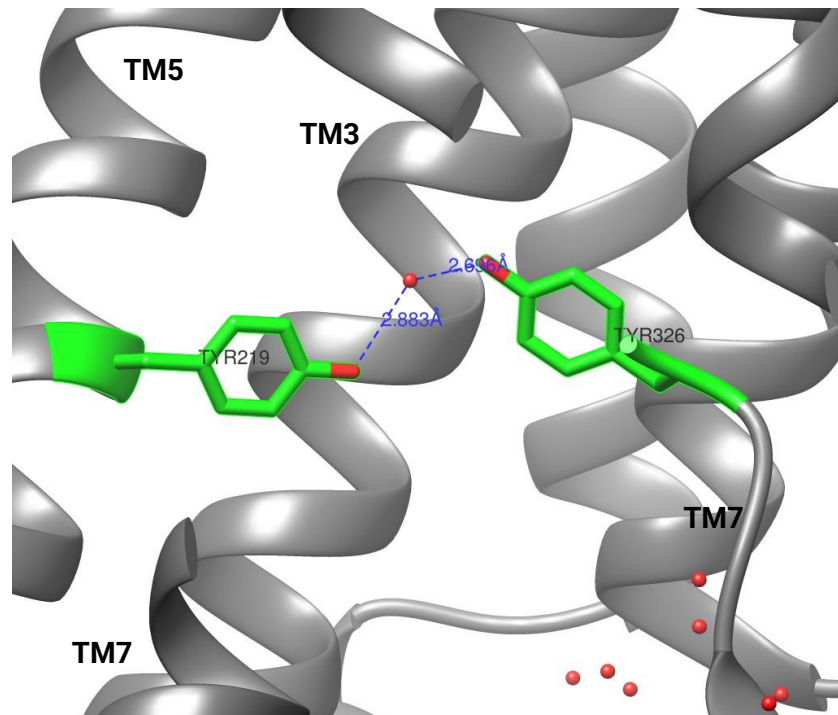


■ → TYR219 & 326 active state

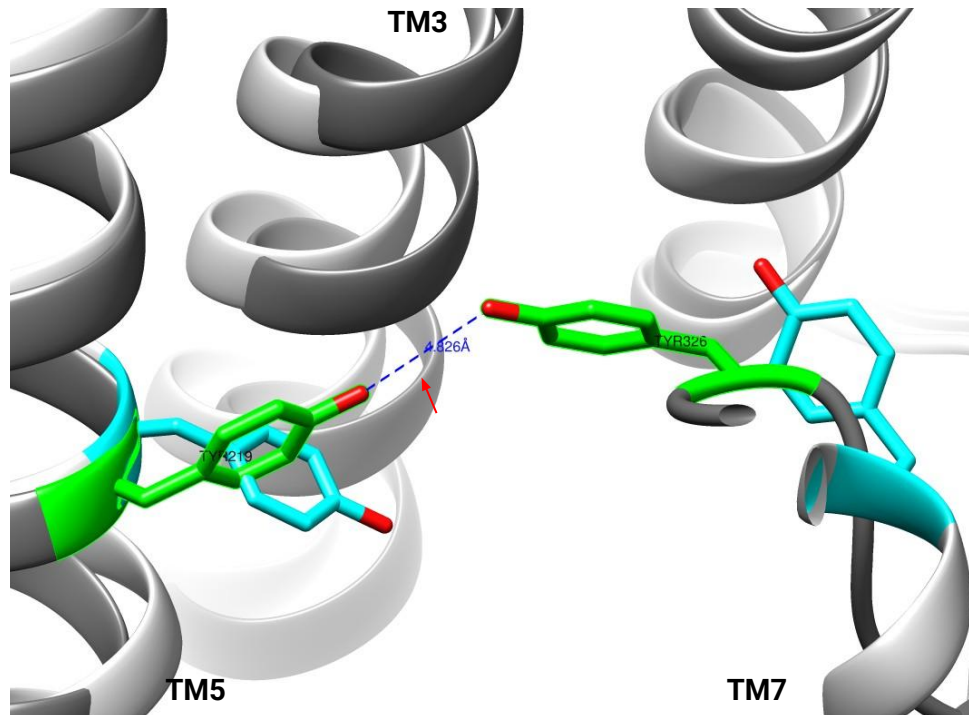
■ → TYR219 326 inactive state

Active-Inactive States

Hydrogen bond between Y326 (TM7) and Y219 (TM5) and mediated by a water molecule is just present in the active state



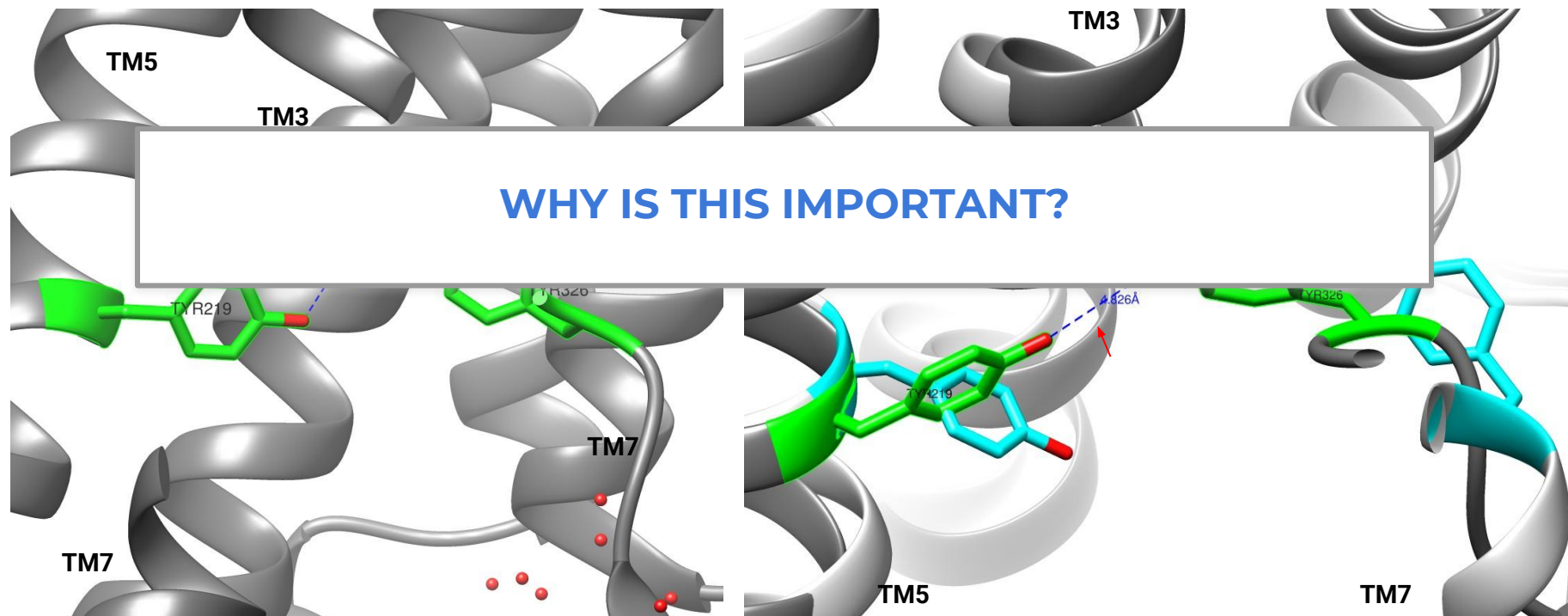
PDB: 4LDE + nanobody, BI167107, agonist



■ → TYR219 & 326 active state ■ → TYR219 326 inactive state1

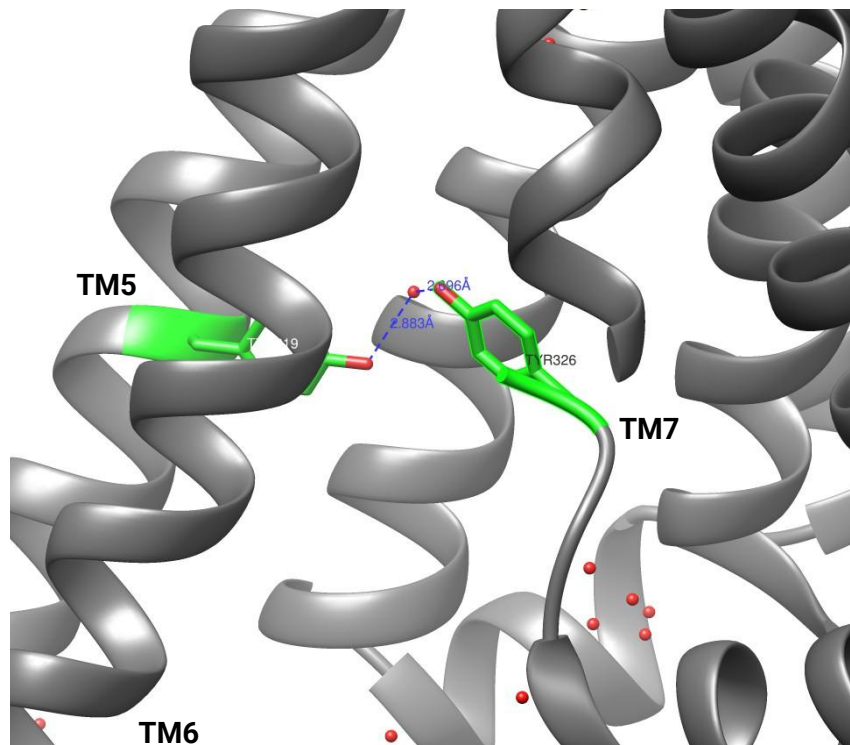
Active-Inactive States

Hydrogen bond between Y326 (TM7) and Y219 (TM5) and mediated by a water molecule is just present in the active state

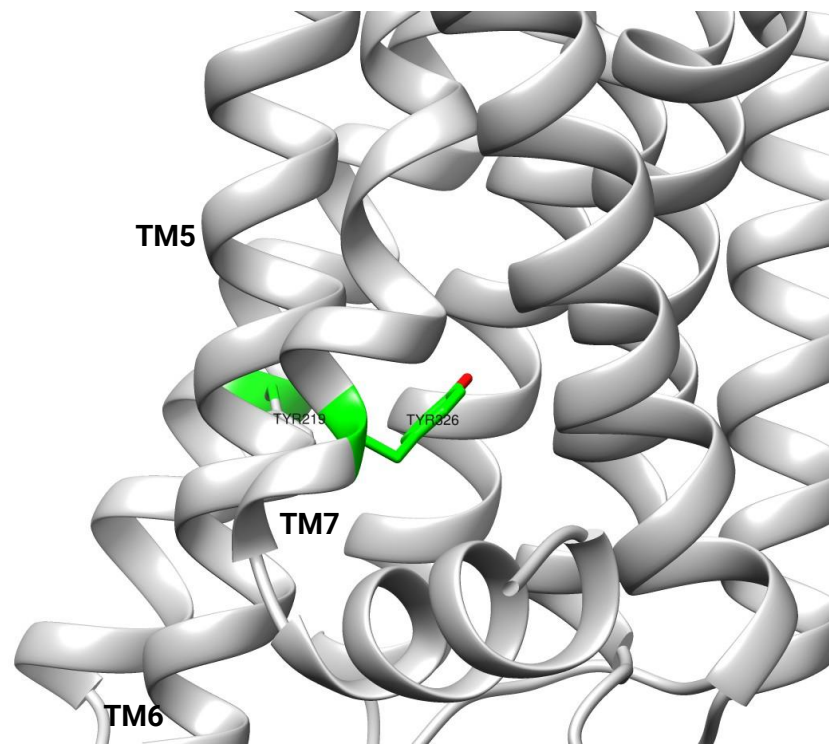


Active-Inactive States

This water-mediated hydrogen bond holds helix 6 in the outward position



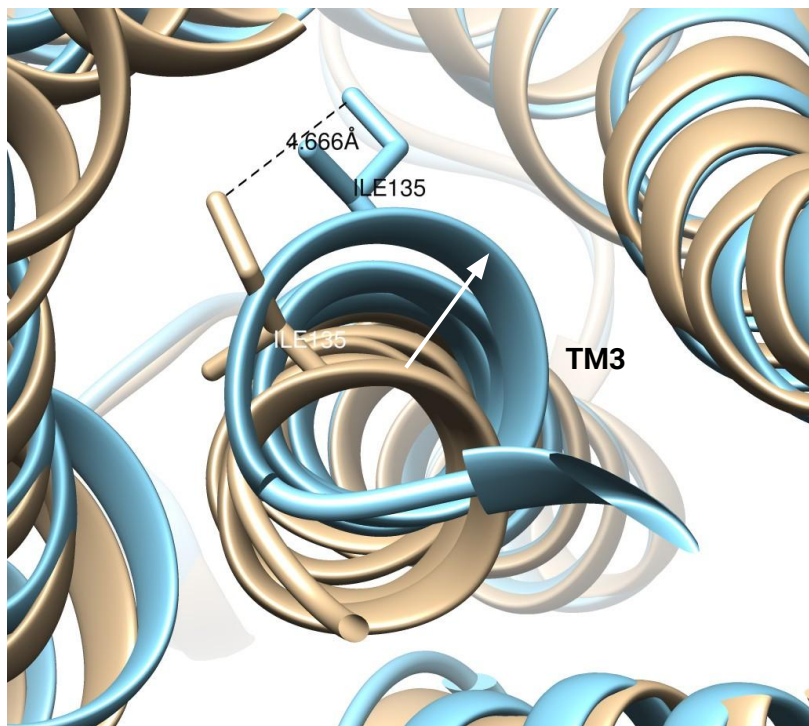
Active state. PDB: **4LDE** + nanobody, BI167107, agonist



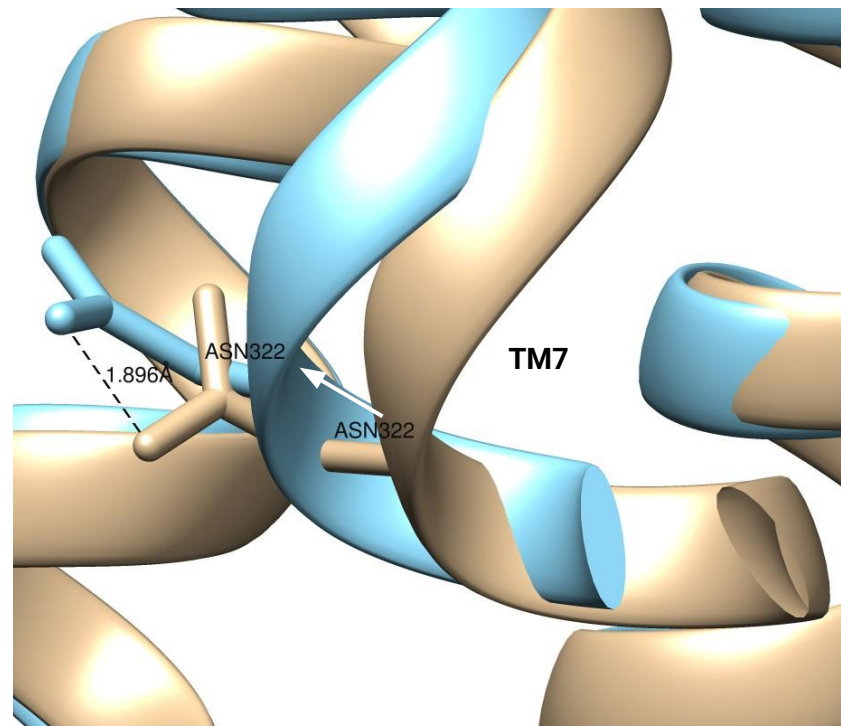
Inactive state

Active-Inactive States

TM3 inwards movement in the active state (4Å)

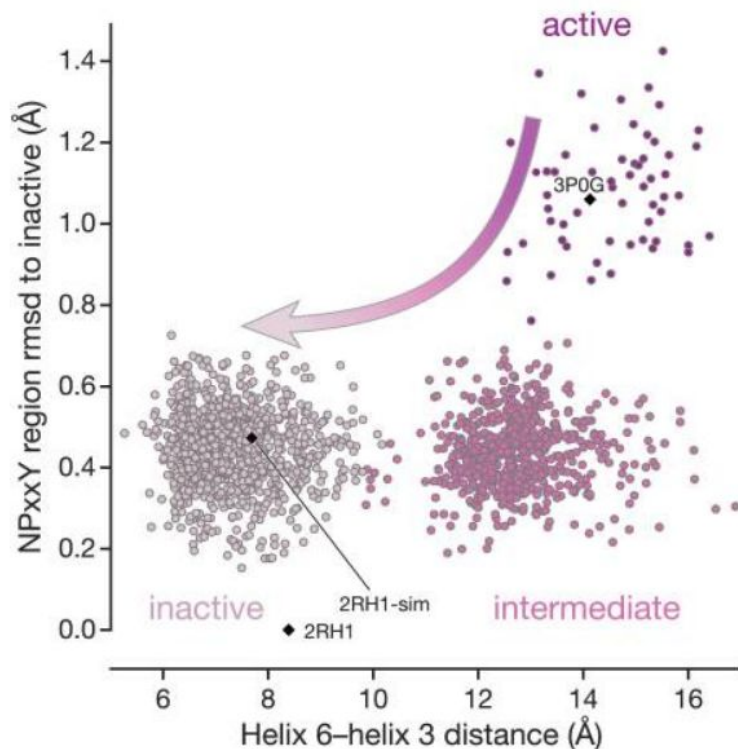


TM7 inwards movement in the active state (2Å)



RMSD: 1.52

Active-Inactive States



TWO STATES MODEL AS AN APPROXIMATION

Active-inactive → simplistic

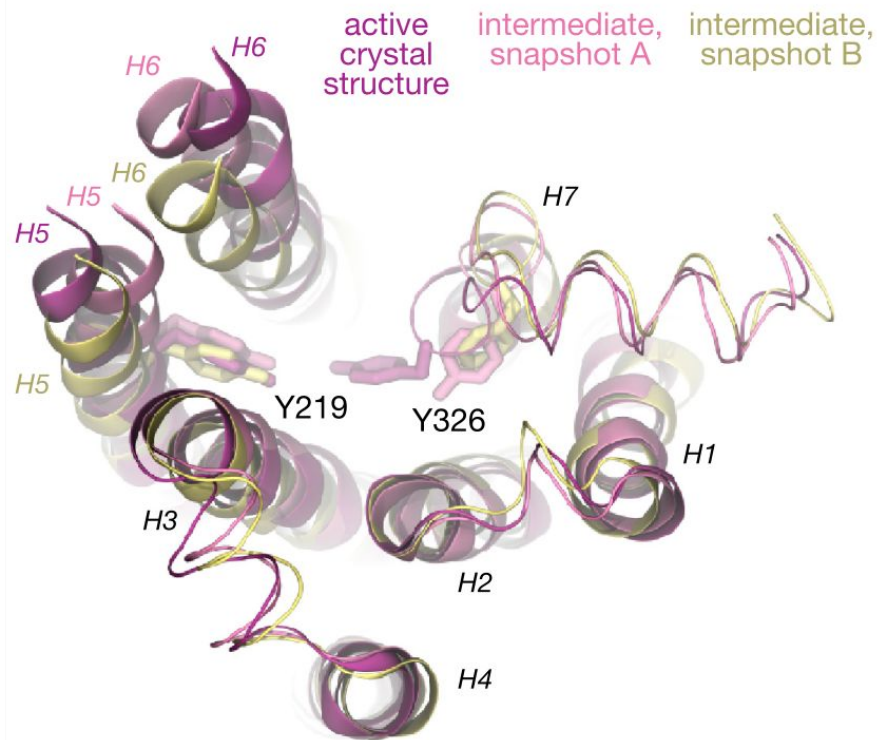
Crystal structures → the most energetically stable

Accelerated MD simulations starting from active state revealed: **intermediate structure.**

- **TM7** moves into its inactive conformation
- **TM7** moves away from TM3,6
- **TM3, 5, 6** → active-like conformation

Fig. 5. Shaw DE, et al. 2011

Active-Inactive States



TWO STATES MODEL AS AN APPROXIMATION

Active-inactive $\rightarrow$ simplistic

Crystal structures $\rightarrow$ the most energetically stable

Accelerated MD simulations starting from active state revealed: **intermediate structure**.

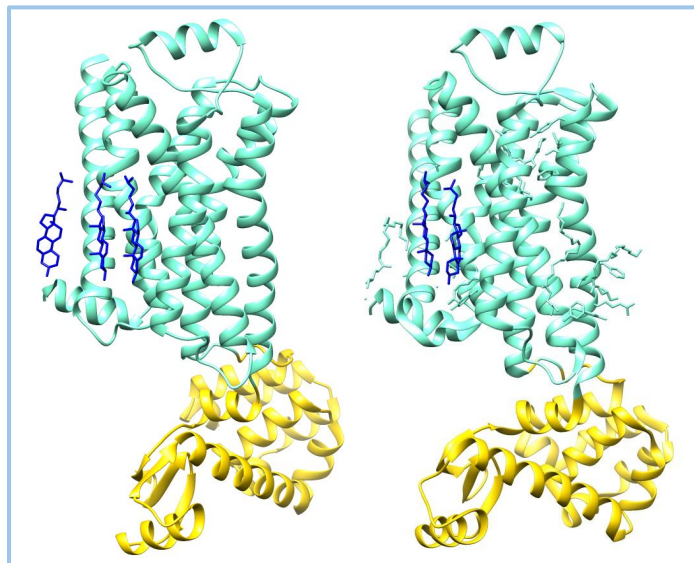
- **TM7** moves into its inactive conformation
- **TM7** moves away from TM3,6
- **TM3, 5, 6** $\rightarrow$ active-like conformation
 - **TM6** maintains separation from TM3
 - **TM5** and **TM6** $\rightarrow$ mobility
 - TM6 towards the TM5
 - TM5 towards the TM3

Fig. 6. Shaw DE, et al. 2011

Ligands: Cholesterol 2RH1

Allosteric binding

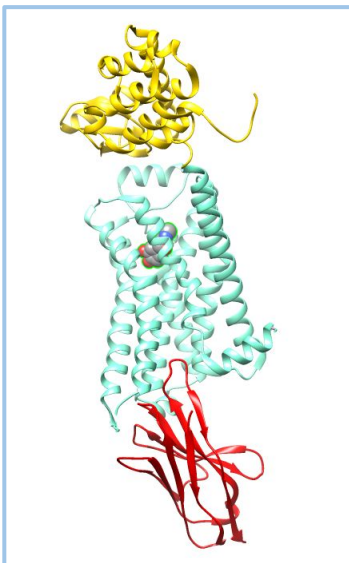
Cholesterol



Ligand binding site

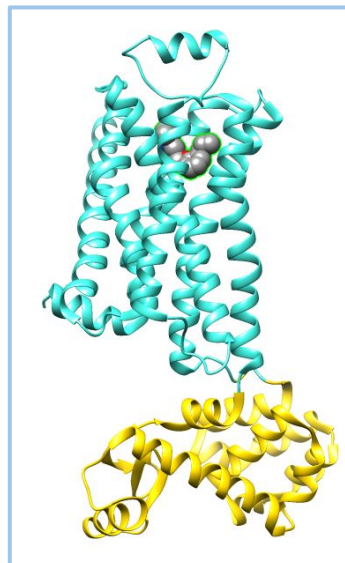
Agonist

Adrenaline



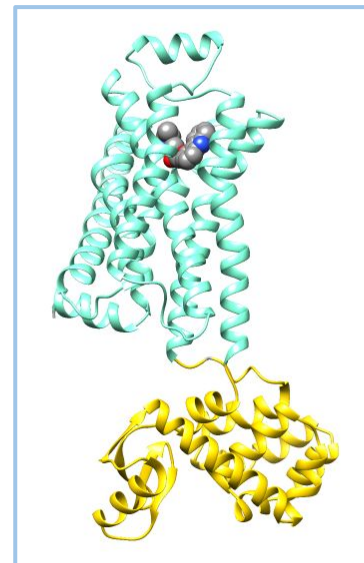
Antagonist

Alprenolol



i-Agonist

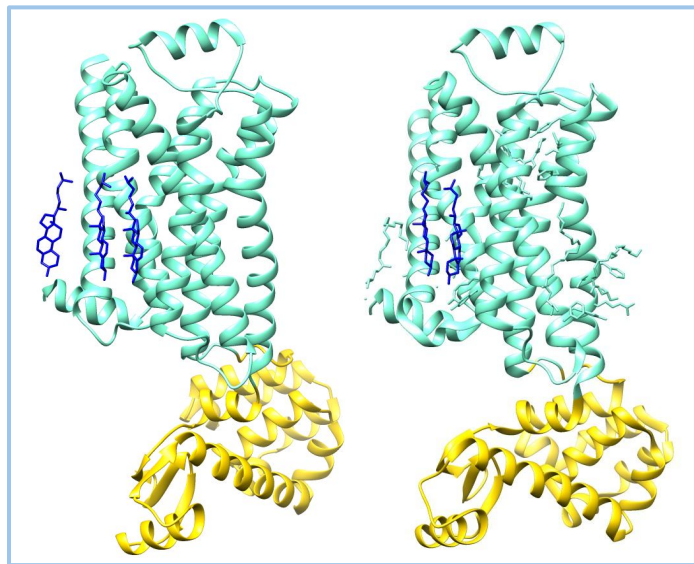
Carazolol



Ligands: Cholesterol 2RH1

Allosteric binding

Cholesterol



Ligand binding site

Agonist

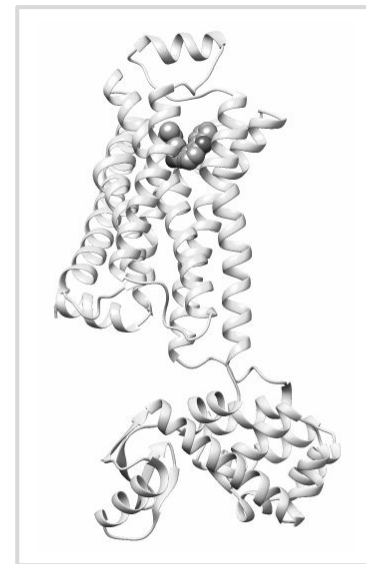
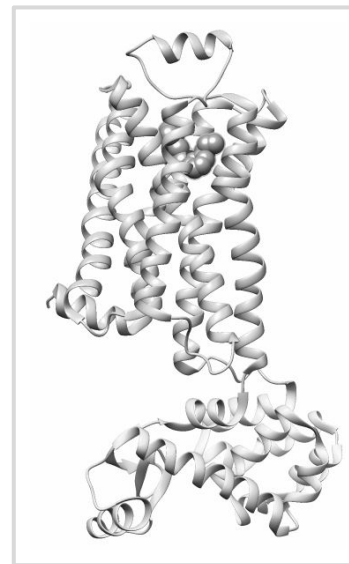
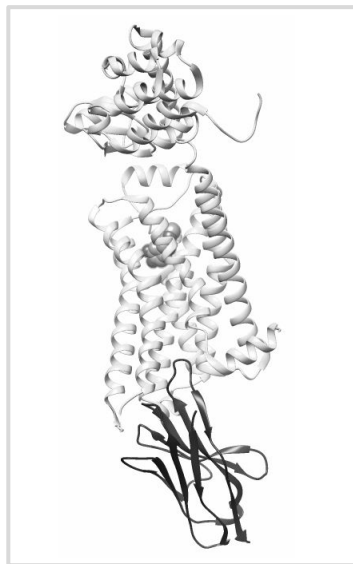
Antagonist

i-Agonist

Adrenaline

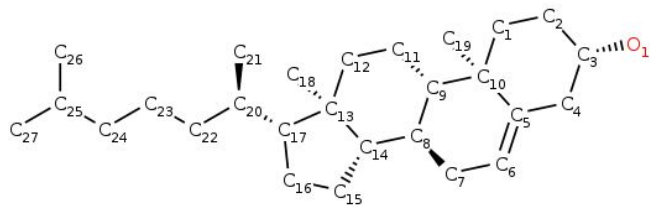
Alprenolol

Carazolol

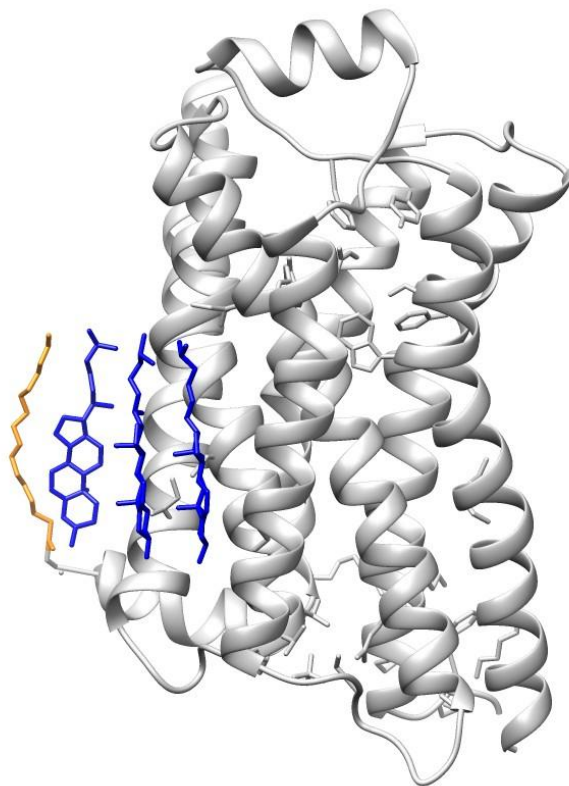
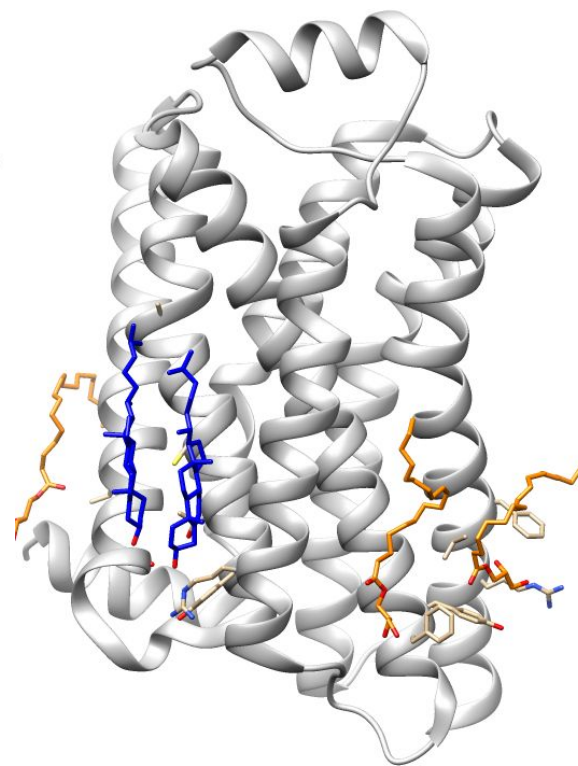


Cholesterol

- **Cholesterol** → steroid structure



- **Parallel association** to the β 2-AR
- Possible **allosteric regulator**

**2rh1****3d4s**

Cholesterol

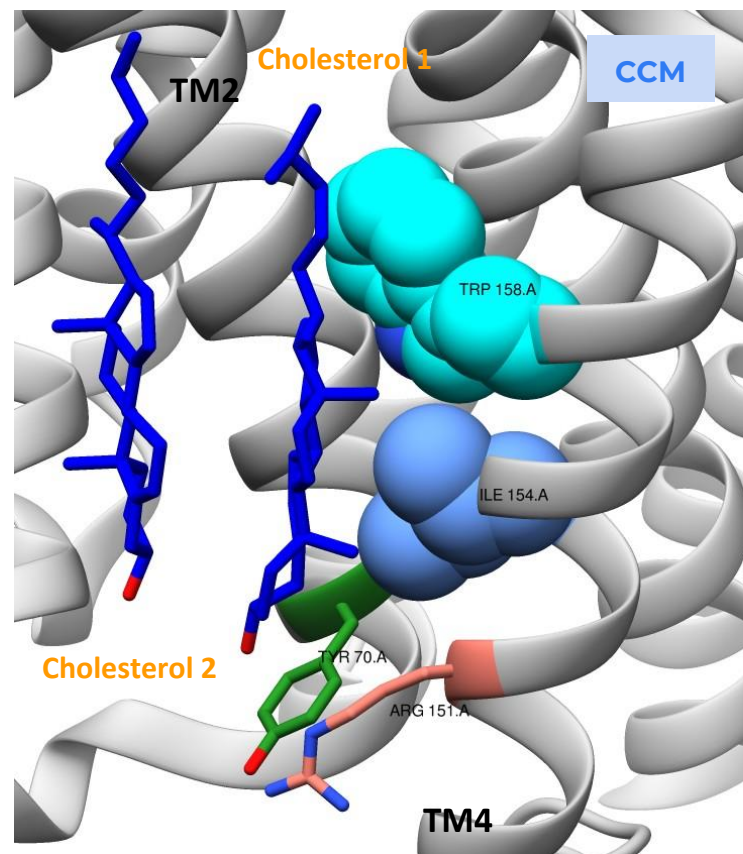
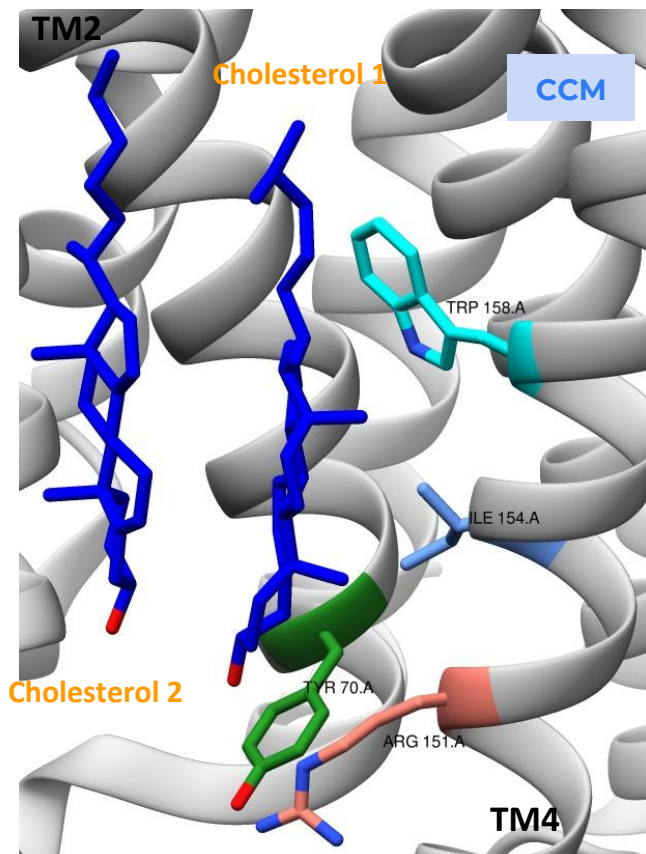
Cholesterol binding site

CCM in TM4 and TM2



Increases the packing interactions for both helix II and IV

3d4s



Cholesterol

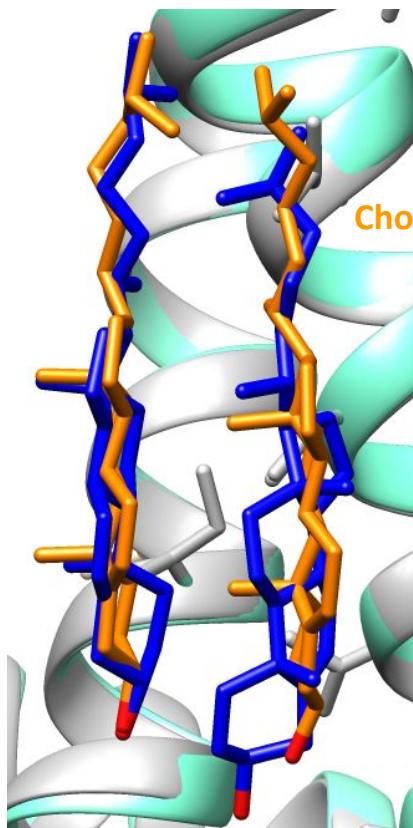
Cholesterol-RC interaction

- High dynamics
- Additional conformations near the site

90° rotation and 1.9 Å

Cholesterol 2

Cholesterol 1



Backbone 3d4s

Backbone 2rh1

Cholesterol 3d4s

Cholesterol 2rh1

Cholesterol

Influence **organization, stability and function of GPCRs** → direct/indirect

Improves **β2AR stability**

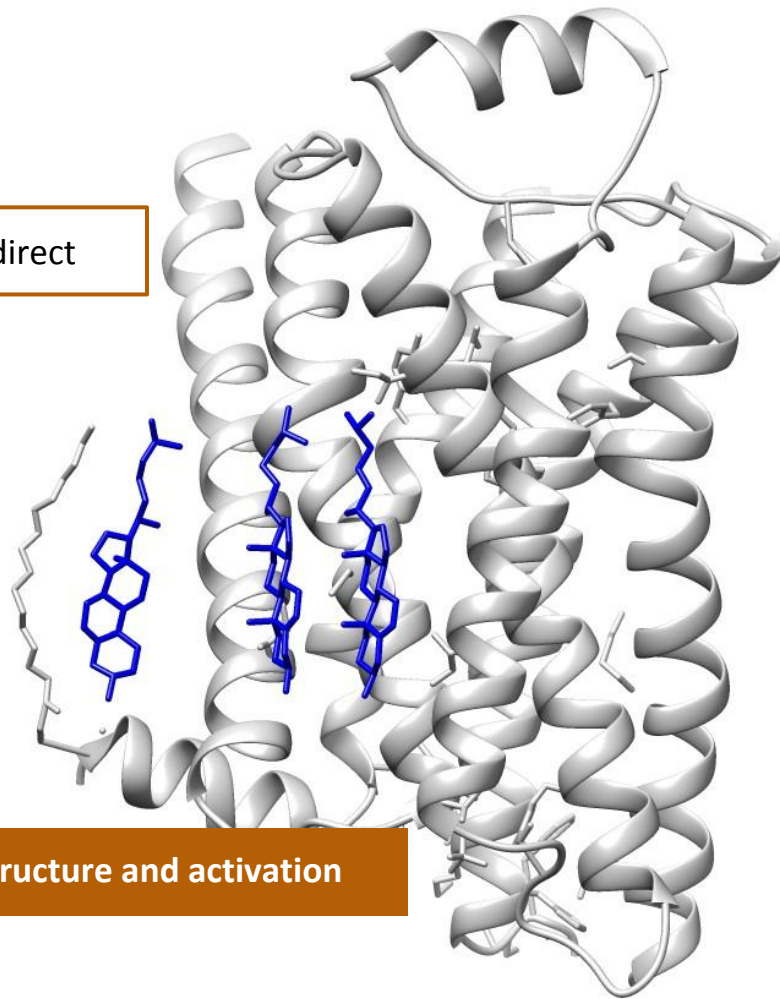
Allosteric site → restriction of TM6 movement

Regulate **dimerization** → Cross-talk GPCRs and Cholesterol

Regulate interactions **β2AR - G proteins**



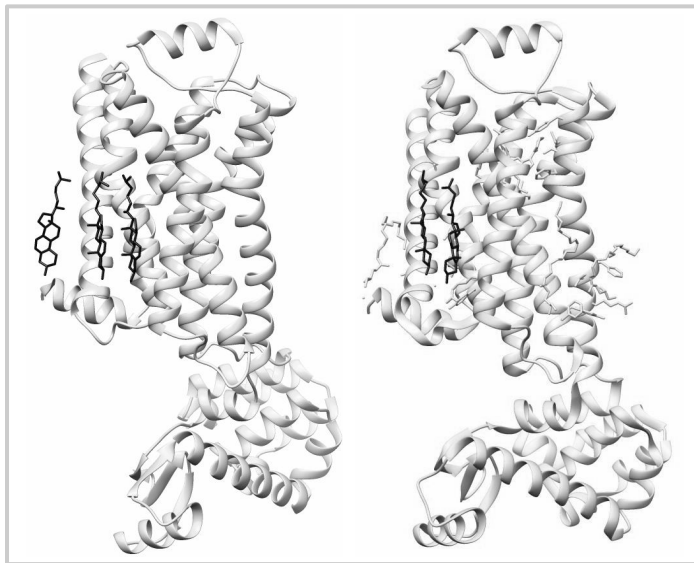
Lipids can be allosteric regulators of membrane protein structure and activation



Ligands: Adrenaline 4LDO

Allosteric binding

Cholesterol



Ligand binding site

Agonist

Antagonist

i-Agonist

Adrenaline

Alprenolol

Carazolol

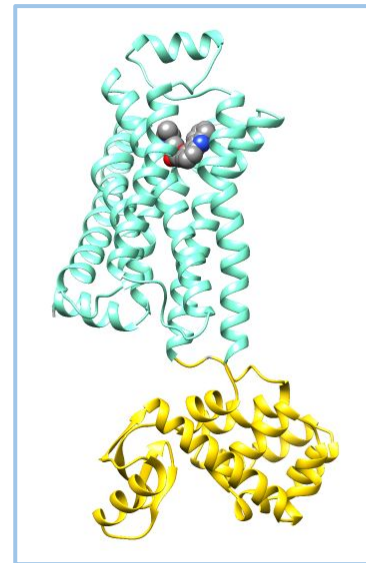
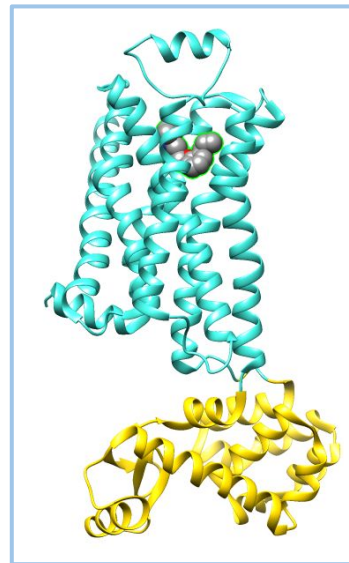
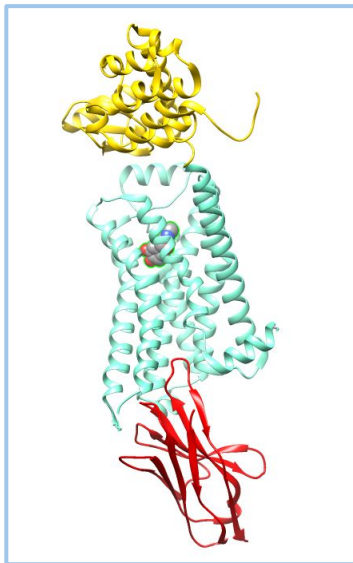
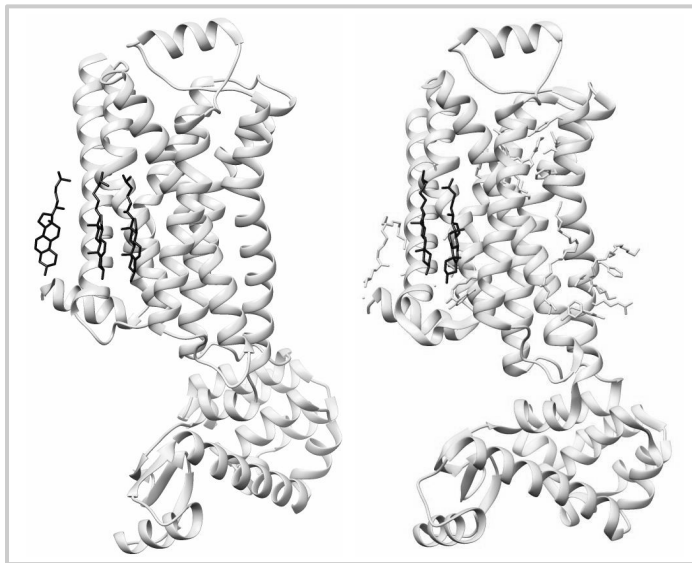


Fig 7.: Adapted from Chan HC et al., 2016

Ligands: Adrenaline 4LDO

Allosteric binding

Cholesterol



Ligand binding site

Agonist

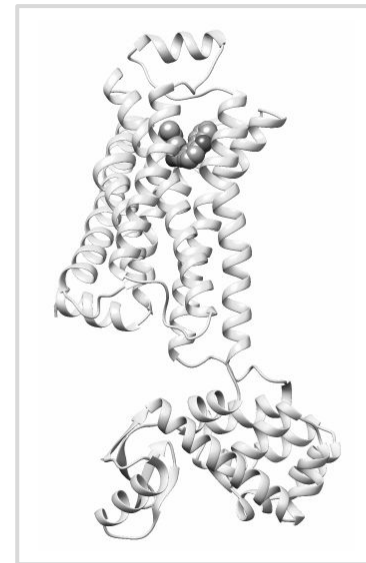
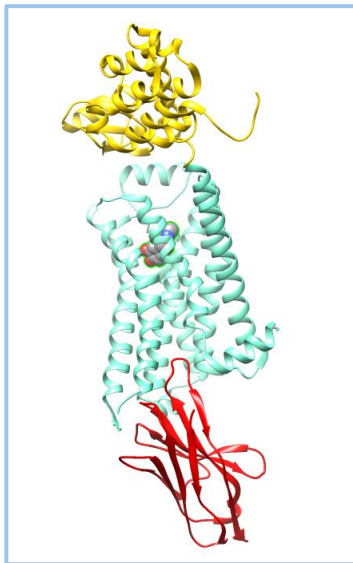
Antagonist

i-Agonist

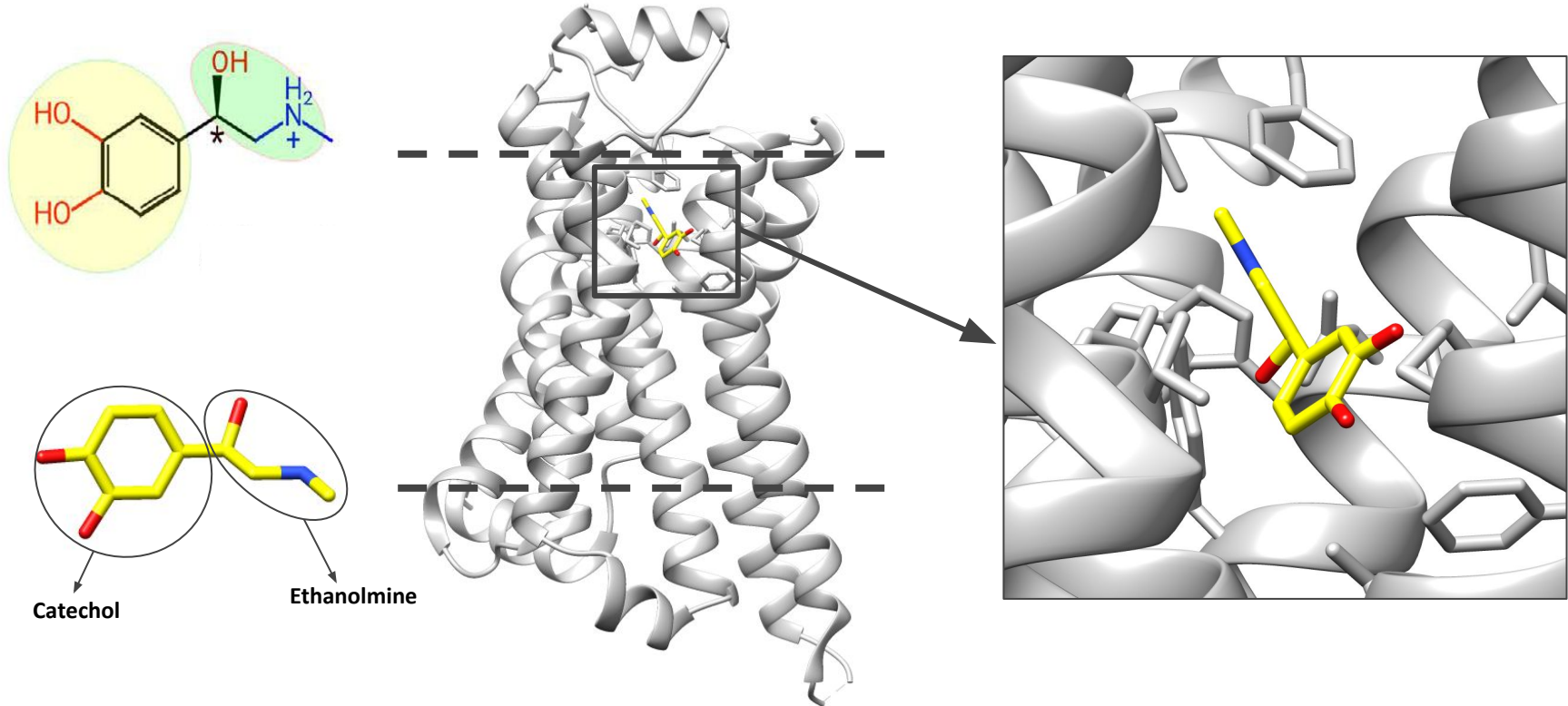
Adrenaline

Alprenolol

Carazolol



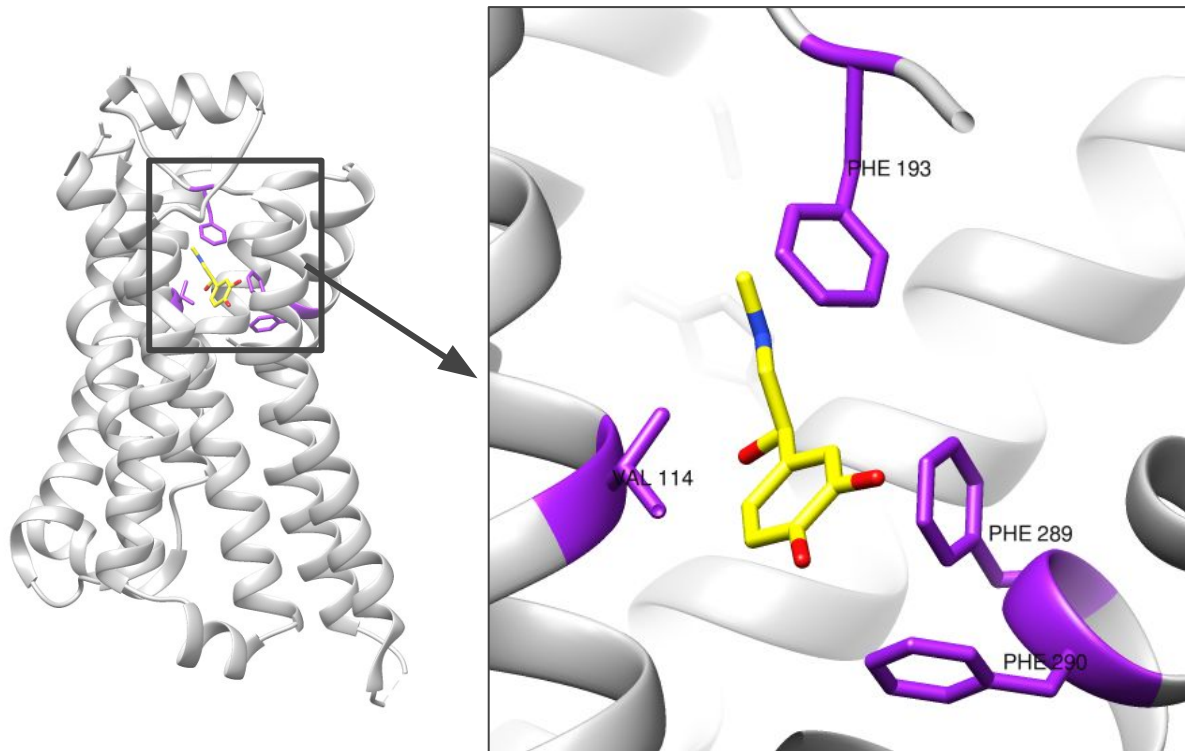
Agonist: Adrenaline



Hydrogen bonds and Salt Bridge contacts of the binding pocket

Agonist: Adrenaline

Hydrophobic interactions of the binding pocket

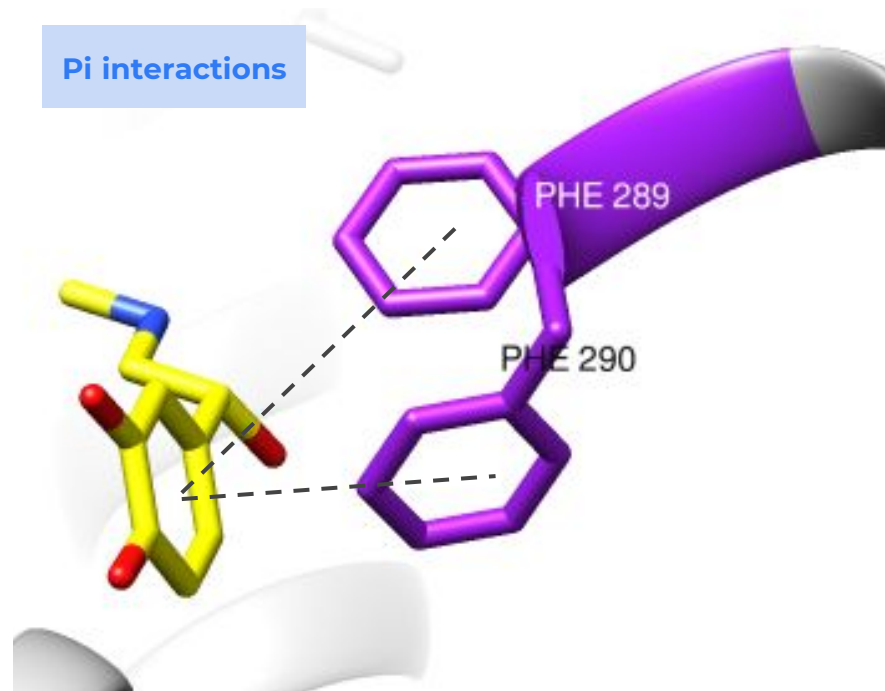
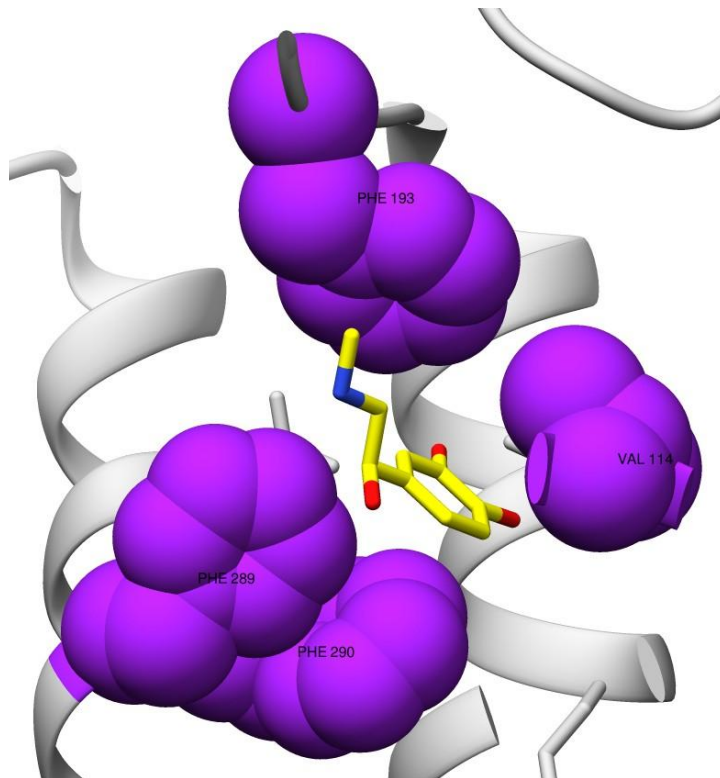


Hydrophobic and Aromatic interactions

Atoms β 2AR	Atoms Adrenaline	Distance (Å)
PHE 193 CE2	C2	3.9
VAL 114 CG2	C6	4.4
VAL 114 CG1	C5	4.0
PHE 289 CE2	C2	3.7
PHE 290 CE2	C4	4.0
PHE 289 CE2	C1	3.8
PHE 290 CZ	C4	4.0

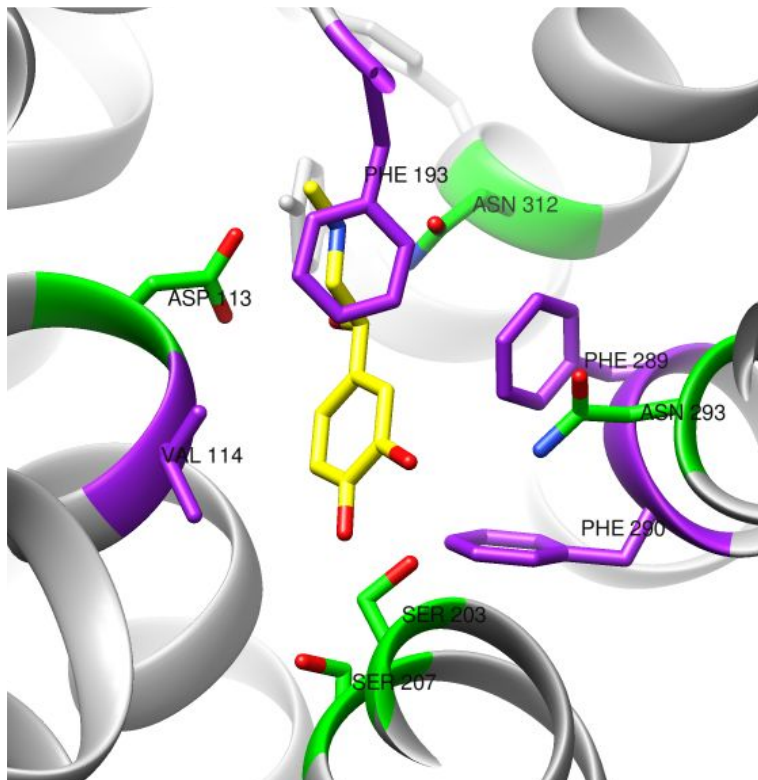
Agonist: Adrenaline

Aromatic interactions of the binding pocket



Agonist: Adrenaline

Important residues



SUMMARY

Hydrogen bond network

- Common: ASP113, ASN312
 - Specific: SER203, SER207, ASN293
- Polar network: SER204

Hydrophobic and aromatic interactions: TMs 3 and 6 and ECL2

Pi interactions: PHE289, PHE290, catechol moiety



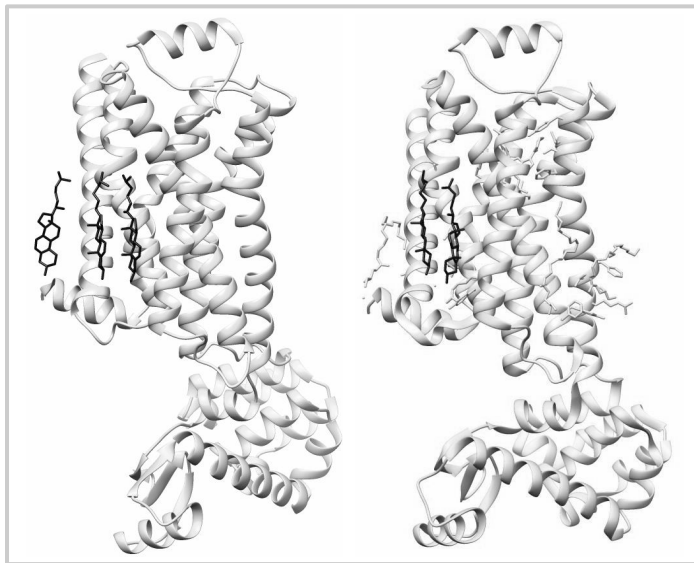
Agonist

- Activate receptor signaling
- Low affinity

Ligands: Cholesterol 2RH1

Allosteric binding

Cholesterol



Ligand binding site

Agonist

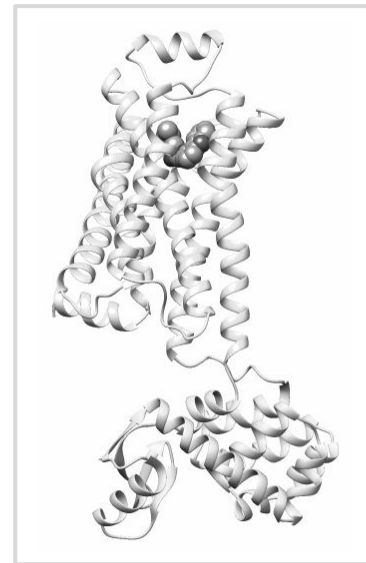
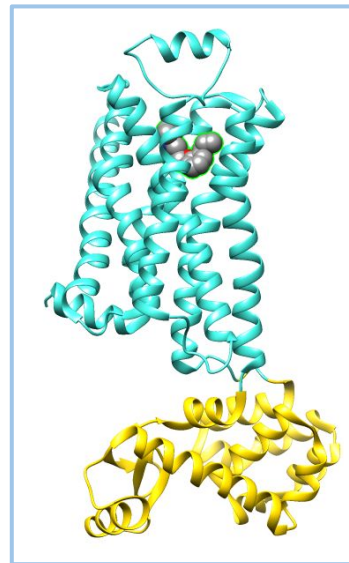
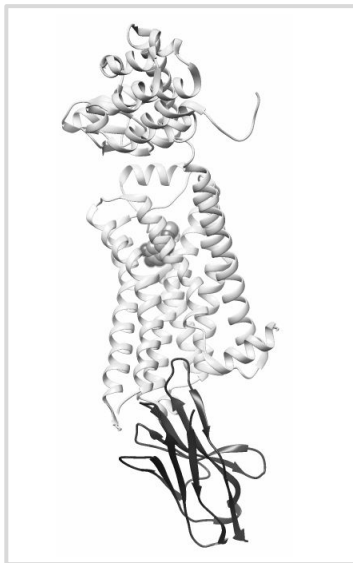
Antagonist

i-Agonist

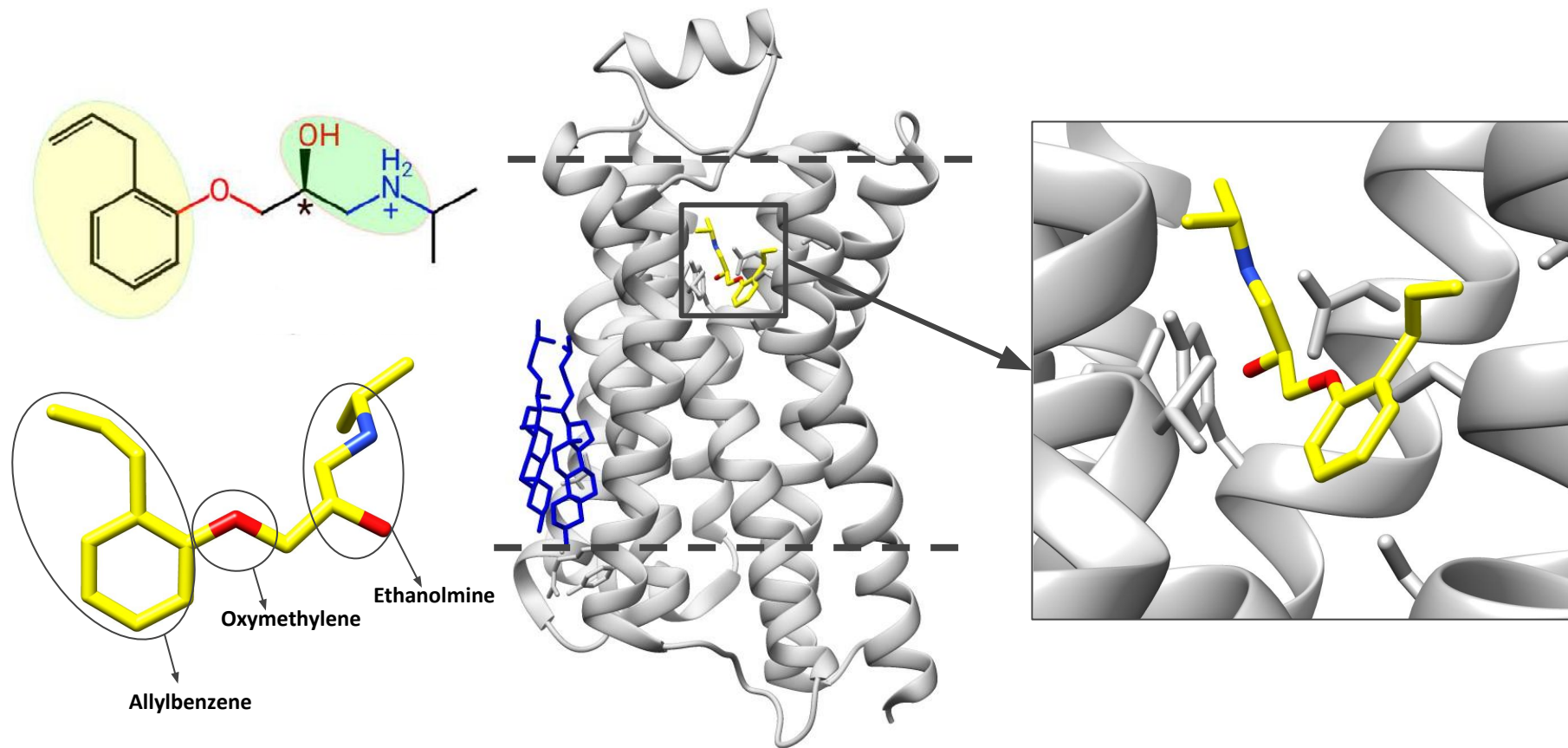
Adrenaline

Alprenolol

Carazolol

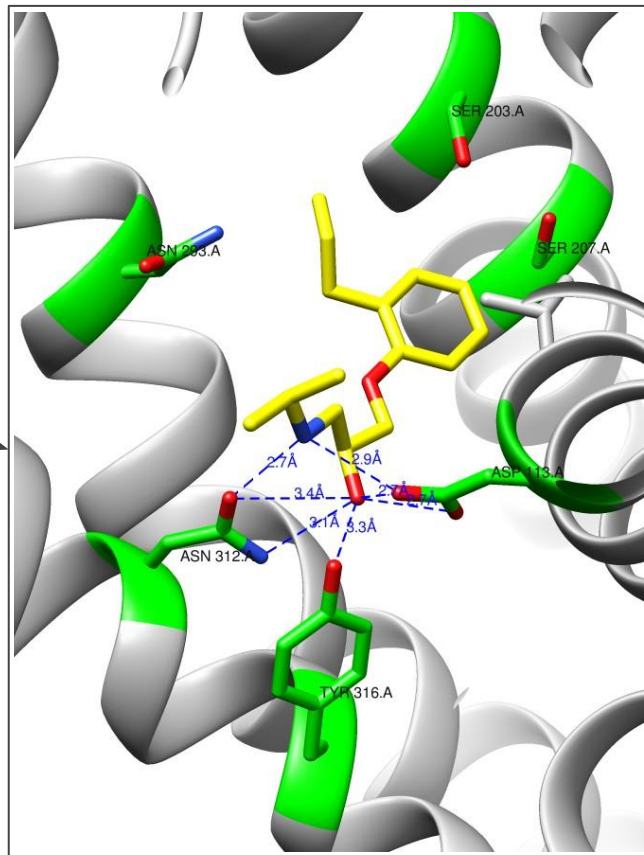
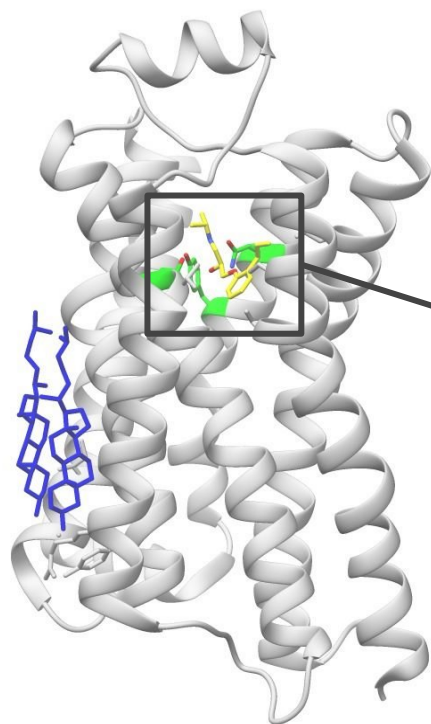


Antagonist: Alprenolol



Antagonist: Alprenolol

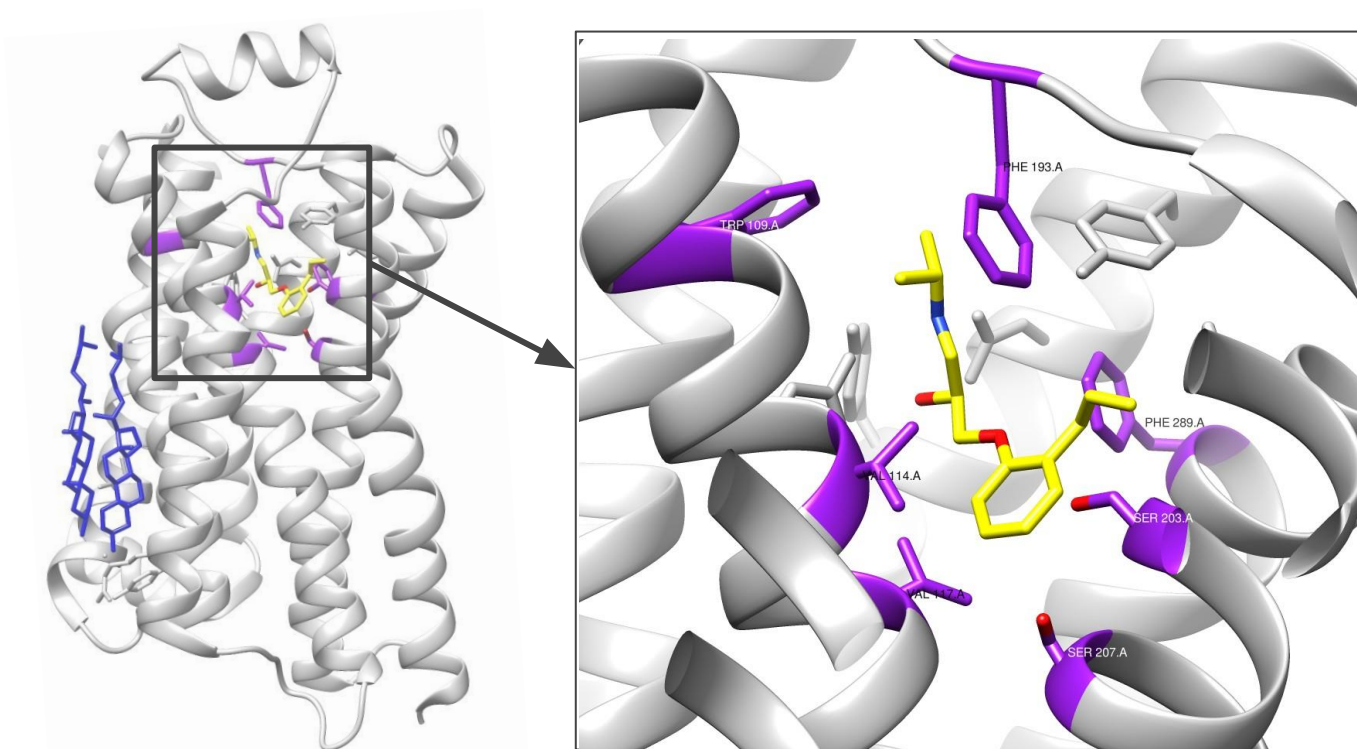
Hydrogen bonds of the binding pocket



Hydrogen Bonds and Salt Bridge Contacts		
Atoms β2AR	Atoms Alprenolol	Distance (Å)
ASP 113 OD1	O1	2.7
ASP 113 OD2	O1	2.7
ASP 113 OD2	N1	2.9
ASN 312 OD1	O1	3.4
ASN 312 OD1	N1	2.7
ASN 312 ND2	O1	3.1
TYR 316 OH	O1	3.3

Antagonist: Alprenolol

Hydrophobic and Aromatic interactions of the binding pocket

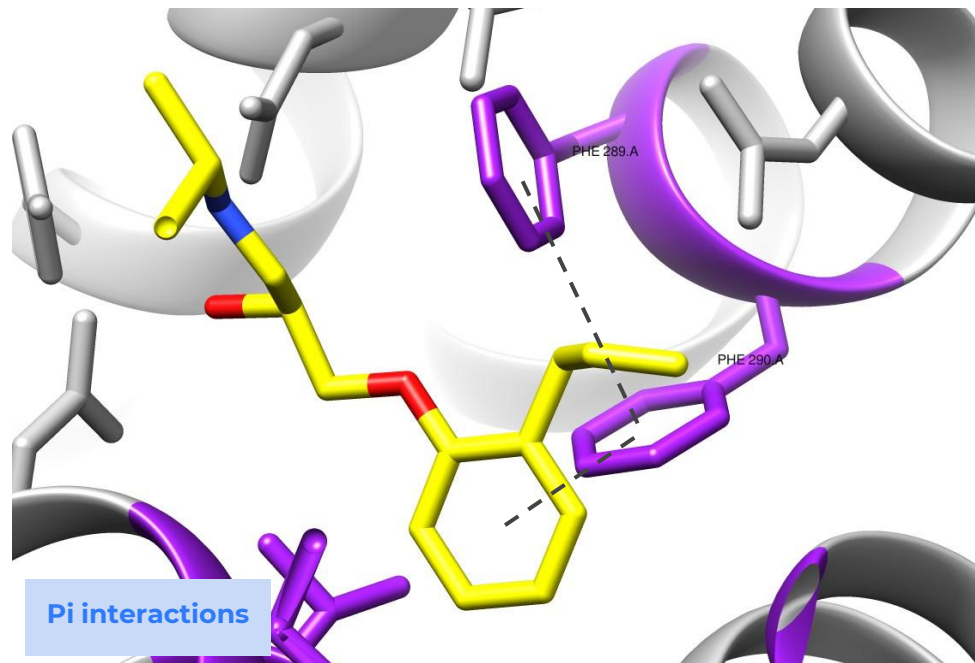
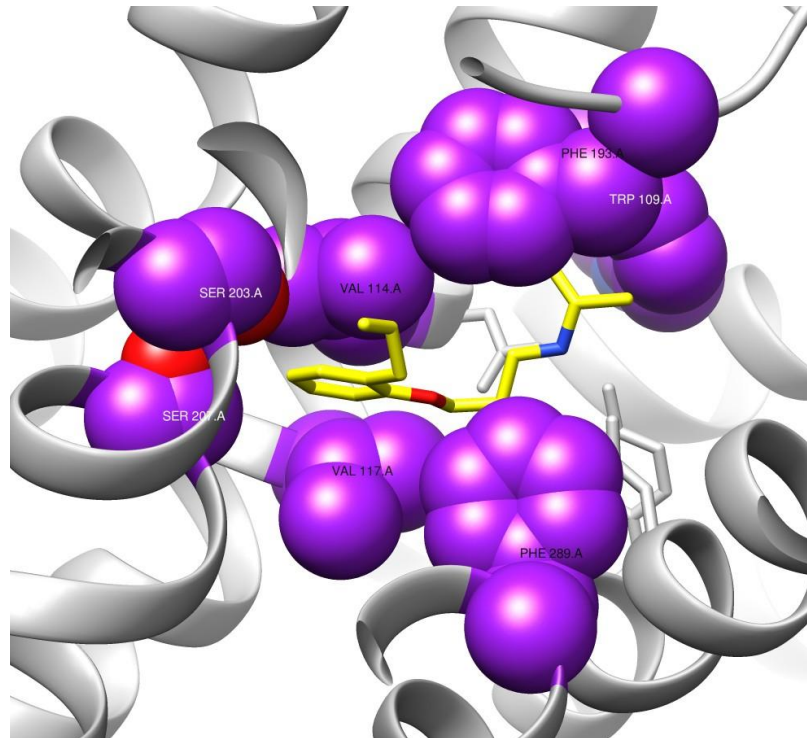


Hydrophobic and Van der Waals interactions

Atoms β2AR	Atoms Alprenolol	Distance (Å)
VAL 114 CG1	C9	3.9
VAL 114 CG2	C8	3.6
VAL 117 CB	C8	3.7
TRP 109 CH2	C3	3.6
PHE 193 CE2	C14	3.9
TYR 308 OH	C6	3.6
SER 203 OG	C11	3.4
SER 207 OG	C10	3.6

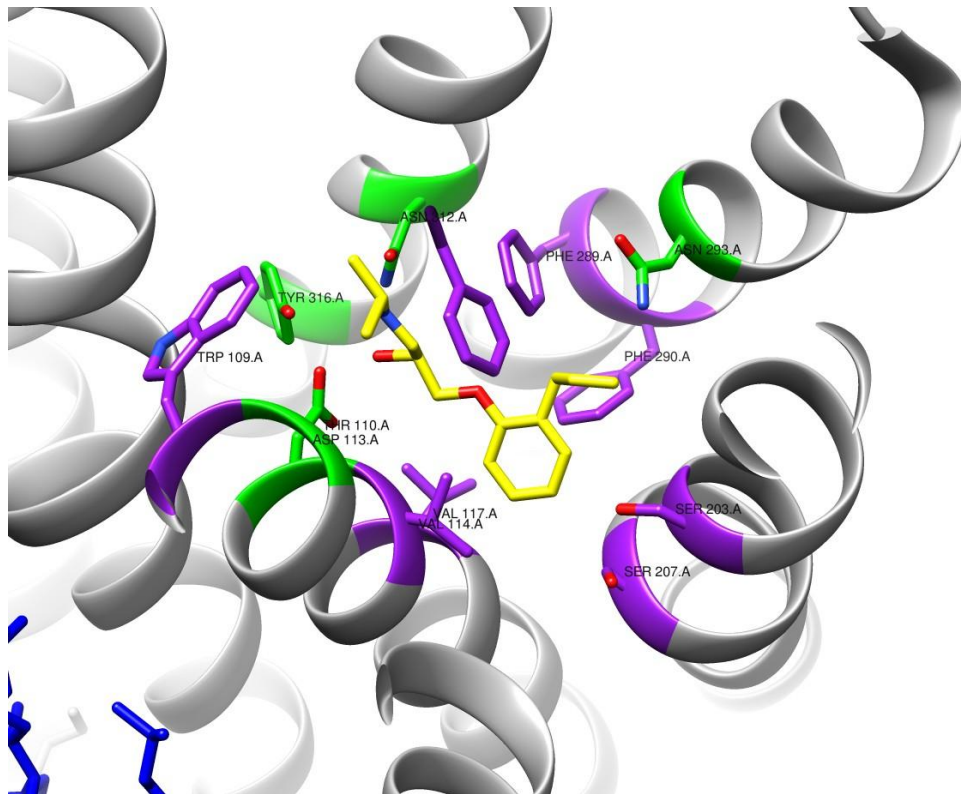
Antagonist: Alprenolol

Aromatic interactions of the binding pocket



Antagonist: Alprenolol

Important residues



SUMMARY

Hydrogen bond network

- Common: ASP113, ASN312
- Specific: TYR316, aromatic ring system

Hydrophobic and aromatic interactions: TMs 3, 5 and 6

Pi interactions: PHE289, PHE290, allylbenzene moiety



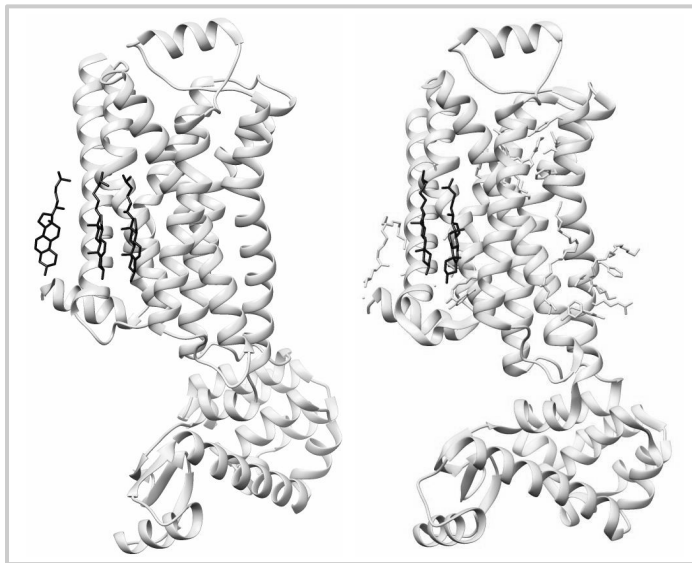
Antagonist

- Blocks agonist signaling
- Micromolar affinity

Ligands: Cholesterol 2RH1

Allosteric binding

Cholesterol



Ligand binding site

Agonist

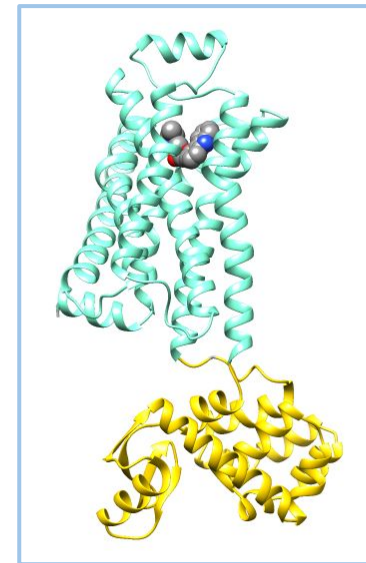
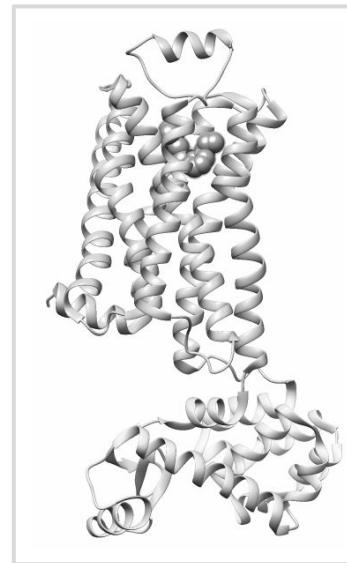
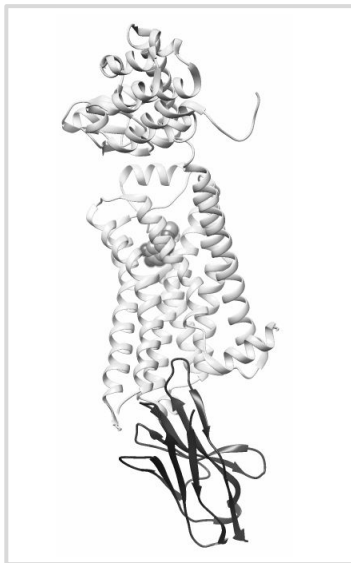
Antagonist

i-Agonist

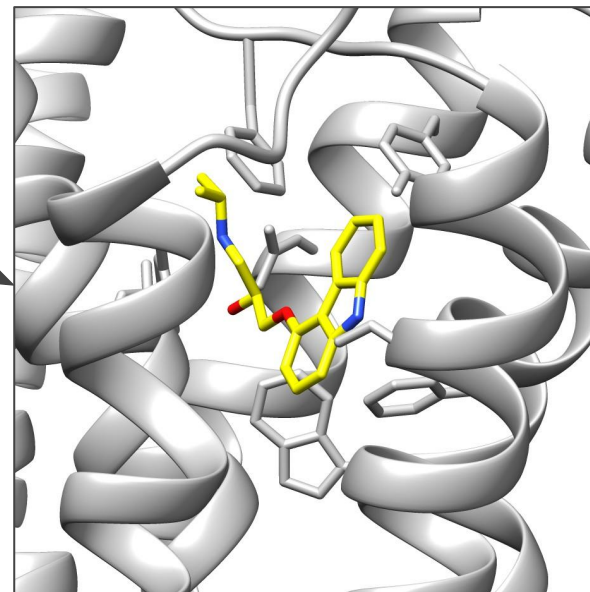
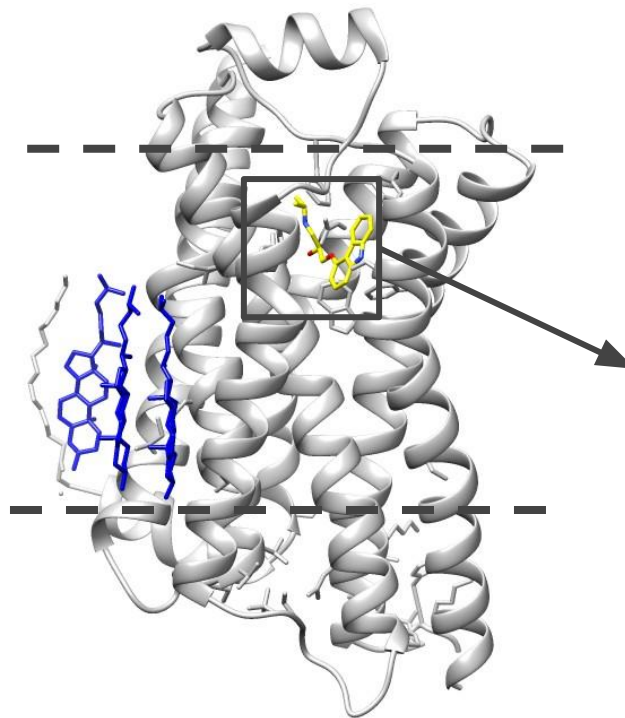
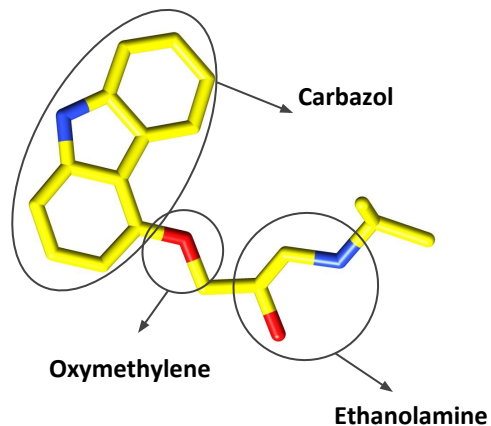
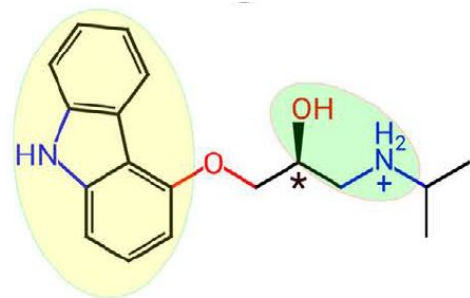
Adrenaline

Alprenolol

Carazolol

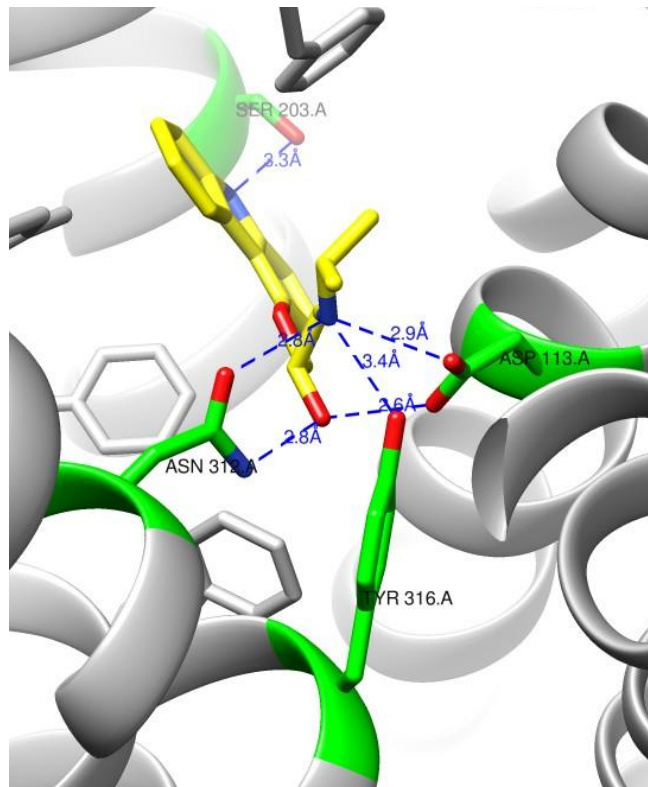
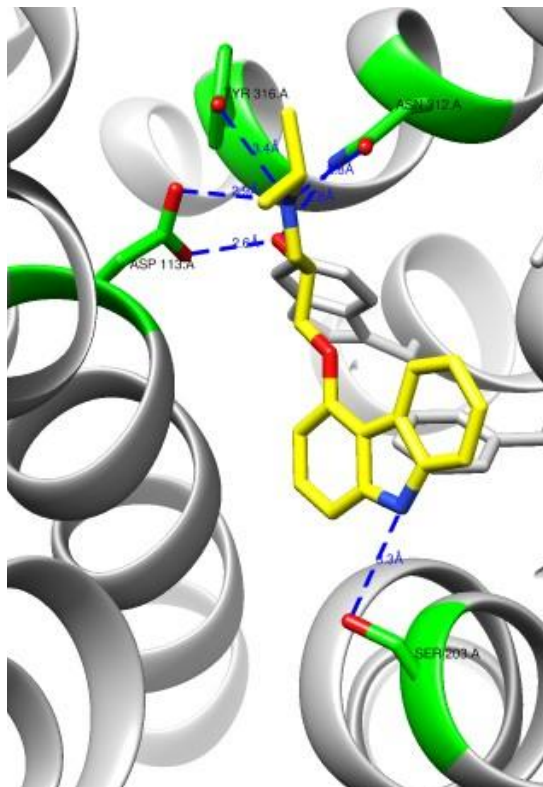


Inverse agonist: Carazolol



Inverse agonist: Carazolol

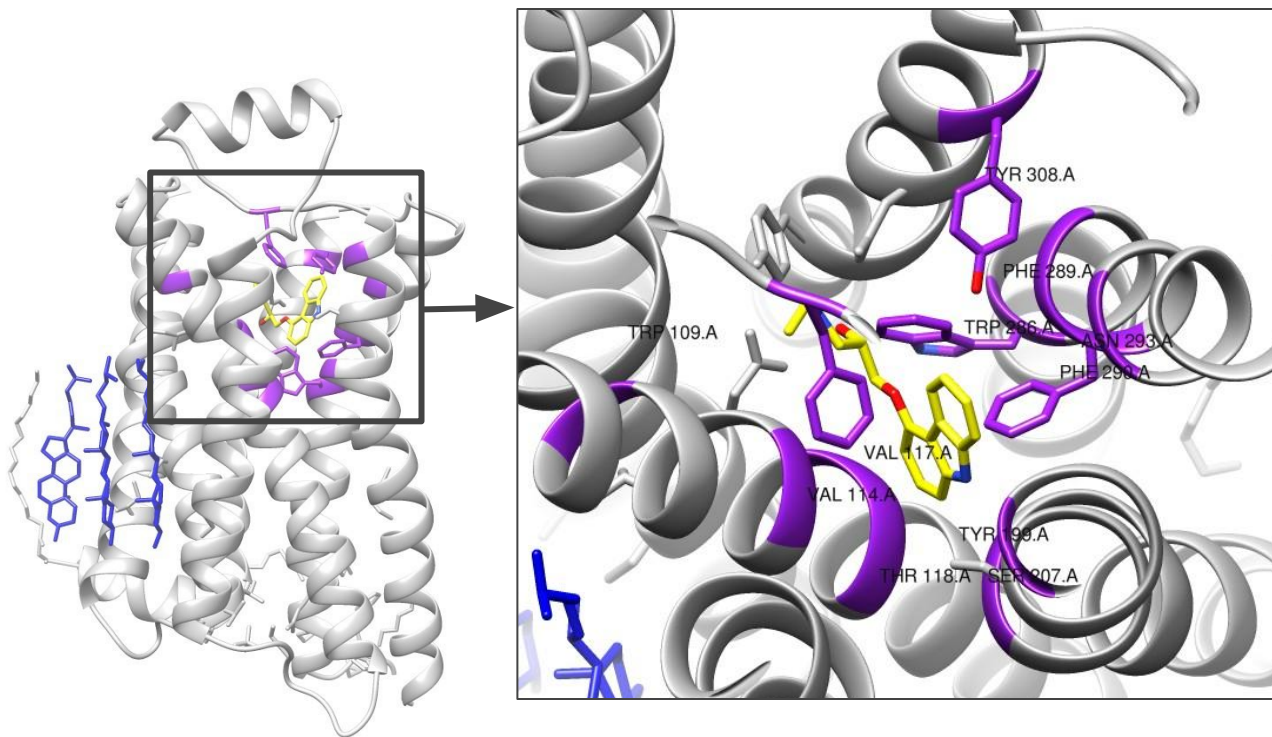
Hydrogen bonds and Salt Bridge Contacts



Hydrogen Bonds Contacts and Salt Bridge Contacts		
Atoms β2AR	Atoms Carazolol	Distance (Å)
SER 203 OG	N7	3.3
ASP 113 OD1	O17	2.6
ASP 113 OD2	N19	2.9
ASN 312 ND2	O17	2.8
ASN 312 OD1	N19	2.8
TYR 316 OH	N19	3.4

Inverse agonist: Carazolol

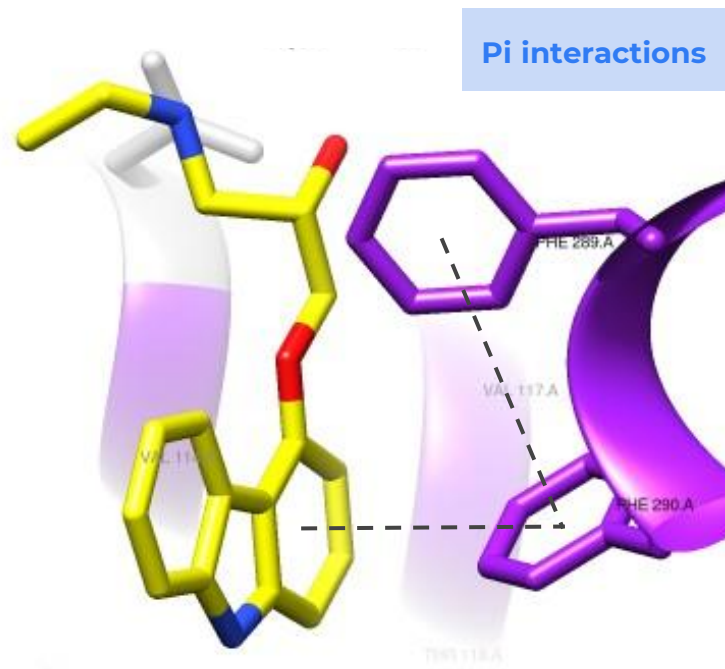
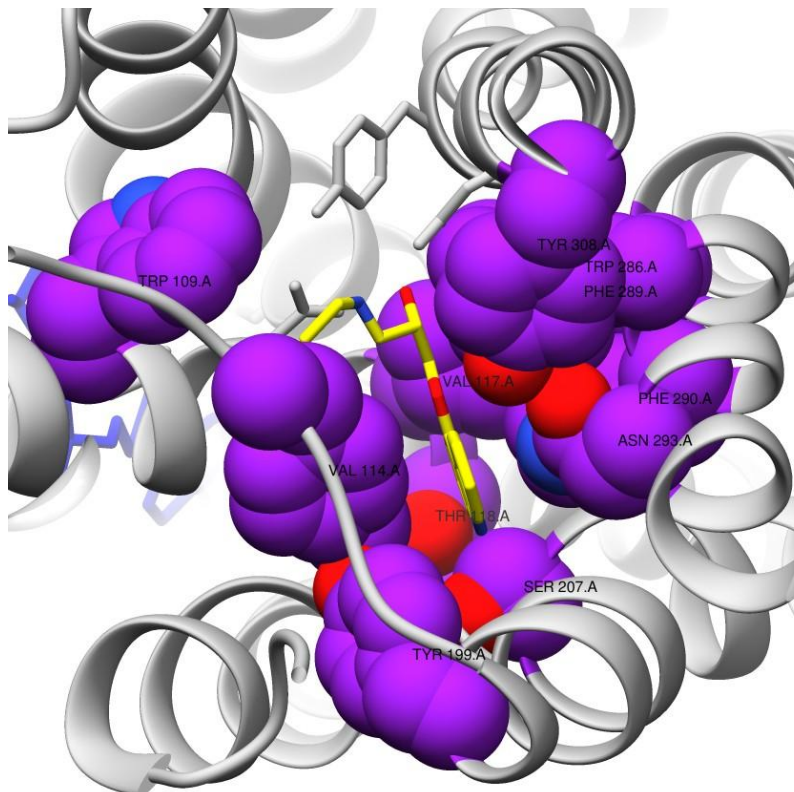
Hydrophobic and Aromatic interactions



Hydrophobic and Aromatic interactions		
Atoms β2AR	Atoms Carazolol	Distance (Å)
TRP 109 CH2	C21	3.8
VAL 114 CG1	C11	3.9
VAL 117 CG1	C12	4.0
THR 118 OG1	C11	3.9
PHE 193 CE2	C6	3.5
TYR 199 CE2	C2	3.9
SER 207 CB	C10	3.6
TRP 286 CH2	O17	3.4
PHE 289 CE2	O14	3.7
PHE 290 CZ	C12	3.5
ASN 293 ND2	C5	3.6
TYR 308 OH	C6	3.6

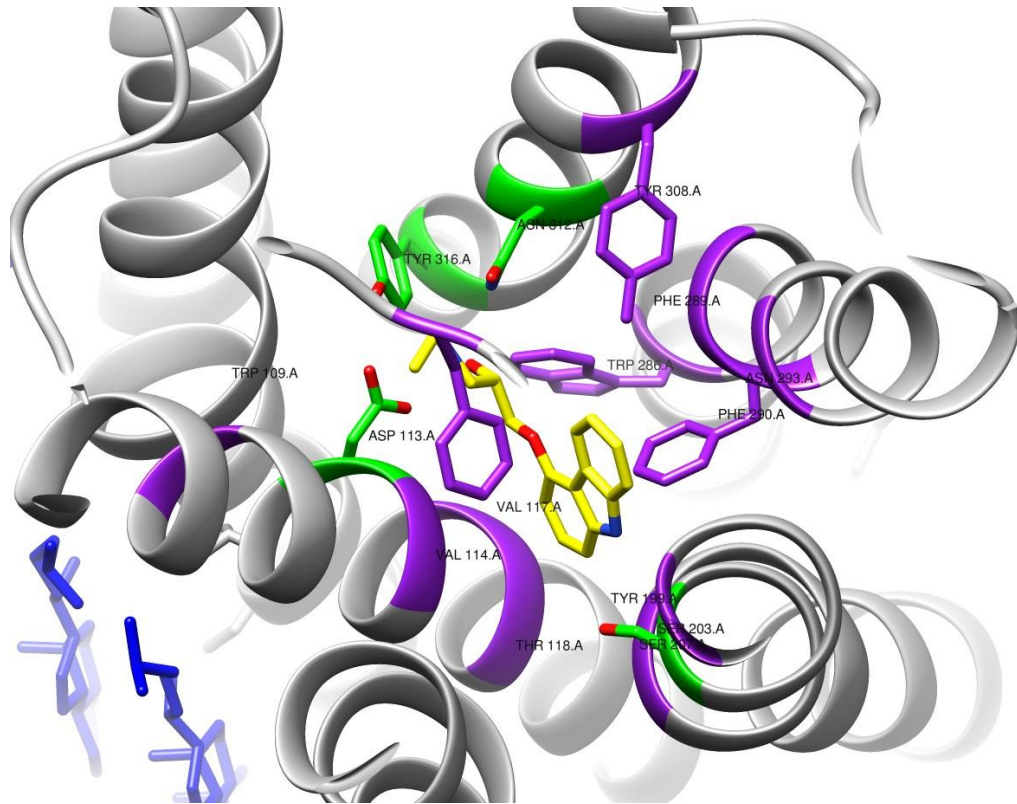
Inverse agonist: Carazolol

Hydrophobic and Aromatic interactions



Inverse agonist: Carazolol

Important residues



SUMMARY

Hydrogen bond network

- Common: ASP113, ASN312
- Specific: SER203, TYR316

Hydrophobic and aromatic interactions: TMs 3, 5 and 6 and ECL2

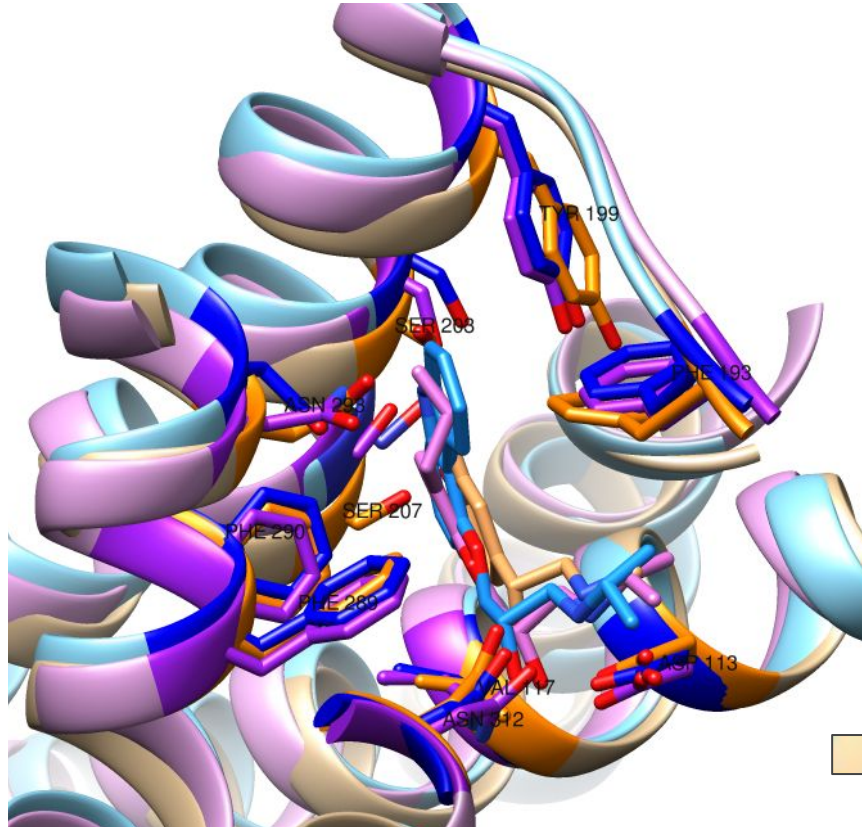
Pi interactions: PHE289, PHE290, carbazol moiety



Partial inverse agonist

- Lowers the basal activity
- Picomolar affinity

Superimposition



Key residues: ASN 312 (TM7) and ASP 113 (TM3)

Hydrophobic: VAL 114, PHE 193, PHE 289 and PHE 193

Agonist: SER 203 and SER 207 in TM5 (HB)

Antagonist and inverse agonists: TYR 199, SER 203, SER 207, TRP, PHE 290 (Hydrophobic).

RMSD: 1.54



Agonist:
PDB code: 4LDO
Adrenaline

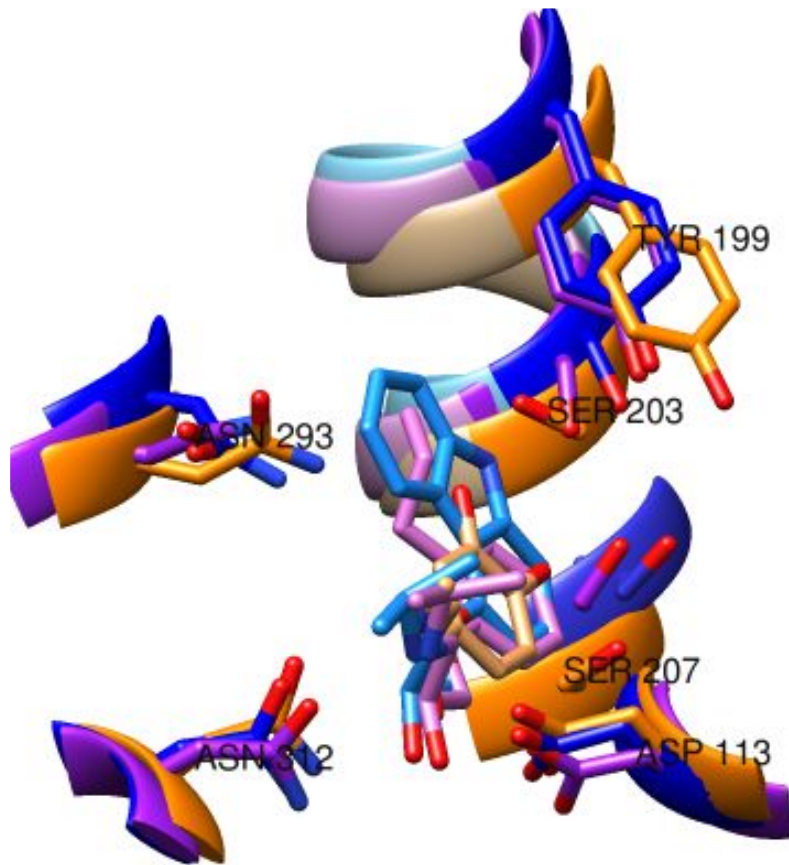


Inverse Agonist:
PDB code: 2RH1
Carazolol



Antagonist:
PDB code: 3NYA
Alprenolol

Superimposition



The main shifts are found on SER203-207 (TM5)

Polar interactions between agonist and residues of TM5 are key to activate the signalling cascade

Hydrophobic interactions have an important role to stabilize antagonist and inverse agonist ligands

RMSD: 1.54

Agonist:
PDB code: 4LDO
Adrenaline

Inverse Agonist:
PDB code: 2RH1
Carazolol

Antagonist:
PDB code: 3NYA
Alprenolol

CONCLUSIONS

50% of drugs target GPCRs → cardiological and respiratory diseases

Controversial **dimerization**

Structural instability causes **agonist-independent basal activity in $\beta 2AR$**

Different active states exist in GPCRs

Main change Active/Inactive states → outwards movement of TM6.

Cholesterol has an important role regarding stabilization and regulation of $\beta 2AR$ function

Comparison between **different ligands** → main differences SER203-207

QUESTIONS

1. How many transmembrane segments do the Adrenergic Receptors have?

- a. 2
- b. 3
- c. 7
- d. 5
- e. 10

2. Which one of these conserved motives can be found in the Adrenergic Receptors?

- a. TATA box
- b. DRY motif
- c. The two previous ones
- d. Zinc Finger
- e. None of the above

3. What particularity have the transmembrane proteins such as the adrenergic receptors?

- a. They never have Serines
- b. Their loops are very short
- c. The two previous ones
- d. Most hydrophobic residues are in the surface, whereas the polar ones are in the core
- e. All of the above

4. Why are the adrenergic receptors structurally unstable?

- a. Because they are wrongly oriented in the membrane
- b. Because they don't have interactions with membrane components
- c. The two previous ones
- d. Because they have ligand-independent basal activity
- e. All of the above

5. Which kinds of ligands can the adrenergic receptors have?

- a. Agonists
- b. Inverse Agonists
- c. The two previous ones
- d. Antagonists
- e. All of the above

6. What kind of signalling do the adrenergic receptors have?

- a. G-protein coupling
- b. Tyrosine kinase
- c. The two previous ones
- d. MAP kinase
- e. None of the above

7. Which kind of ligand is Alprenolol?

- a. Agonist
- b. Inverse Agonist
- c. The two previous ones
- d. Antagonist
- e. All of the above

8. Select the correct option about active-inactive states of beta2 adrenoceptors:

- a. helix 6 movement outwards is the main difference
- b. there are almost no changes in the extracellular side
- c. The two previous ones
- d. Intermediate structures have been observed using molecular dynamics
- e. All of the above

9. To which protein class belongs the β_2 AR receptor?

- 1. Alpha + Beta
 - 2. All Beta
 - 3. Alpha/Beta
 - 4. All Alfa
-
- a. 1,2,3
 - b. 1,3
 - c. 2,4
 - d. 4
 - e. 1,2,3,4

10. Choose the correct sentence:

- a. GPCR crystallisation is difficult to achieve
- b. There are methods that crystallize transmembrane GPCRs in polar environments
- c. There are PDB models of the adrenergic receptors for all the species that have them
- d. The methods to crystallize GPCRs are the standard procedures
- e. No GPCR has been crystallized

QUESTIONS

1. How many transmembrane segments do the Adrenergic Receptors have?

- a. 2
- b. 3
- c. **7**
- d. 5
- e. 10

2. Which one of these conserved motives can be found in the Adrenergic Receptors?

- a. TATA box
- b. **DRY motif**
- c. The two previous ones
- d. Zinc Finger
- e. None of the above

3. What particularity have the transmembrane proteins such as the adrenergic receptors?

- a. They never have Serines
- b. Their loops are very short
- c. The two previous ones
- d. **Most hydrophobic residues are in the surface, whereas the polar ones are in the core**
- e. All of the above

4. Why are the adrenergic receptors structurally unstable?

- a. Because they are wrongly oriented in the membrane
- b. Because they don't have interactions with membrane components
- c. The two previous ones
- d. **Because they have ligand-independent basal activity**
- e. All of the above

5. Which kinds of ligands can the adrenergic receptors have?

- a. Agonists
- b. Inverse Agonists
- c. The two previous ones
- d. Antagonists
- e. **All of the above**

6. What kind of signalling do the adrenergic receptors have?

- a. **G-protein coupling**
- b. Tyrosine kinase
- c. The two previous ones
- d. MAP kinase
- e. None of the above

7. Which kind of ligand is Alprenolol?

- a. Agonist
- b. Inverse Agonist
- c. The two previous ones
- d. **Antagonist**
- e. All of the above

8. Select the correct option about active-inactive states of beta2 adrenoceptors:

- a. helix 6 movement outwards is the main difference
- b. there are almost no changes in the extracellular side
- c. The two previous ones
- d. Intermediate structures have been observed using molecular dynamics
- e. **All of the above**

9. To which protein class belongs the β 2AR receptor?

- 1. Alpha + Beta
 - 2. All Beta
 - 3. Alpha/Beta
 - 4. All Alfa
-
- a. 1,2,3
 - b. 1,3
 - c. 2,4
 - d. **4**
 - e. 1,2,3,4

10. Choose the correct sentence:

- a. **GPCR crystallisation is difficult to achieve**
- b. There are methods that crystallize transmembrane GPCRs in polar environments
- c. There are PDB models of the adrenergic receptors for all the species that have them
- d. The methods to crystallize GPCRs are the standard procedures
- e. No GPCR has been crystallized

External figures

[1] Strosberg AD. Structure/function relationship of proteins belonging to the family of receptors coupled to GTP-binding proteins. *Eur J Biochem.* 1991 Feb 26;196(1):1-10.

[2] Extracted from: <https://filizm02.u.hpc.mssm.edu/gpcr-okb/>

[3] Transmembrane view of High resolution crystal structure of human B2-adrenergic G protein-coupled receptor.

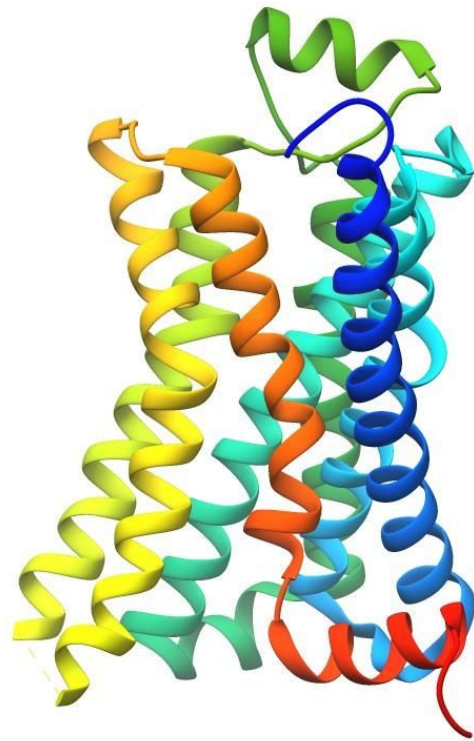
[4] Rasmussen S., DeVree B., Kruse A., Young K., et al. Crystal structure of the β 2 adrenergic receptor–Gs protein complex *Nature* 2011; 477: 549–555.

[5], [6] Shaw DE, Borhani DW, Xu H, Pan AC, Mildorf TJ, Maragakis P, et al. Activation mechanism of the β 2-adrenergic receptor. *Proc Natl Acad Sci U S A.* 2011;108(46):18684-9.

[7] Chan HC, Filipek S, Yuan S. The Principles of Ligand Specificity on beta-2-adrenergic receptor. *Sci Rep* 2016;6:34736.

Bibliography

1. Angers S, Salahpour A, Bouvier M. Biochemical and biophysical demonstration of GPCR oligomerization in mammalian cells. *Life Sciences* 2001; 69: 2243-2250.
2. Chan HC, Filipek S, Yuan S. The Principles of Ligand Specificity on beta-2-adrenergic receptor. *Sci Rep* 2016;6:34736.
3. Nemoto W, Toh H. Prediction of interfaces for oligomerization of G-protein coupled receptors. *Proteins* 2004; 58: 644-660.
4. Rasmussen S, DeVree B, Kruse A, Young K, et al. Crystal structure of the β 2 adrenergic receptor-Gs protein complex *Nature* 2011; 477: 549-555.
5. Wacker D, Fenalti G, Brown MA, et al. Conserved binding mode of human beta2 adrenergic receptor inverse agonists and antagonist revealed by X-ray crystallography. *J Am Chem Soc.* 2010;132(33):11443-5.
6. Strosberg AD. Structure, function, and regulation of adrenergic receptors. *Prot Sci.* 1993; 2: 1198-1209.
7. Prasanna X, Chattopadhyay A, and Sengupta D. Cholesterol Modulates the Dimer Interface of the β 2-Adrenergic Receptor via Cholesterol Occupancy Sites. *Biophys J.* 2014 Mar 18; 106(6): 1290-1300.
8. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen S, Thian FS, Kobilka TS, et al. High Resolution Crystal Structure of an Engineered Human β 2-Adrenergic G protein-Coupled Receptor. *Science.* 2007 Nov 23; 318(5854): 1258-1265.
9. Kobilka BK, Garcia K, Weis W, Enos M, Kruse A, Manglik A, et al. Adrenaline-activated structure of β 2-adrenoceptor stabilized by an engineered nanobody. *Nature.* 2013;502(7472):575-579.
10. Schertler G, Tate C, Leslie A, Henderson R, Edwards P, Moukhametzianov R, et al. Structure of a beta1-adrenergic G-protein-coupled receptor. *Nature.* 2008;454(7203):486-91.
11. Bang I, Choi HJ. Structural features of β 2 adrenergic receptor: crystal structures and beyond. *Mol Cells.* 2014;38(2):105-11.
12. Rasmussen S, Choi H, Rosenbaum D, Kobilka T, Thian F, et al. Crystal structure of the human β 2 adrenergic G-protein-coupled receptor. *Nature.* 2007 Nov 15; 450:383-387.



THANK YOU FOR YOUR ATTENTION

COMPLEMENTARY INFORMATION

PSI-BLAST- Choosing sequences of β 2RC from different species

Results from round 5

Query= P07550_homosapiens

Length=413

Sequences producing significant alignments:

Sequences used in model and found again:

Score E
(Bits) Value

sp P07550 ADRB2_HUMAN	Beta-2 adrenergic receptor	OS=Homo sapien...	433	2e-149
sp Q28509 ADRB2_MACMU	Beta-2 adrenergic receptor	OS=Macaca mula...	427	2e-147
sp Q9TST5 ADRB2_FELCA	Beta-2 adrenergic receptor	OS=Felis catus...	424	5e-146
sp P54833 ADRB2_CANFA	Beta-2 adrenergic receptor	OS=Canis famil...	416	4e-143
sp Q4KWL2 ADRB2_TSCTR	Beta-2 adrenergic receptor	OS=Tscherskia ...	415	2e-142
sp Q28997 ADRB2_PIG	Beta-2 adrenergic receptor	OS=Sus scrofa GN...	415	2e-142
sp P04274 ADRB2_MESAU	Beta-2 adrenergic receptor	OS=Mesocricetu...	414	5e-142
sp Q28044 ADRB2_BOVIN	Beta-2 adrenergic receptor	OS=Bos taurus ...	413	7e-142
sp P18762 ADRB2_MOUSE	Beta-2 adrenergic receptor	OS=Mus musculu...	411	5e-141
sp Q8K4Z4 ADRB2_CAVPO	Beta-2 adrenergic receptor	OS=Cavia porce...	409	3e-140
sp P10608 ADRB2_RAT	Beta-2 adrenergic receptor	OS=Rattus norveg...	405	9e-139
sp P07700 ADRB1_MELGA	Beta-1 adrenergic receptor	OS=Meleagris g...	361	1e-120
sp P43141 ADB4C_MELGA	Beta-4C adrenergic receptor	OS=Meleagris ...	336	2e-111
sp O42574 ADRB1_XENLA	Beta-1 adrenergic receptor	OS=Xenopus lae...	332	1e-110
sp P79148 ADRB1_CANFA	Beta-1 adrenergic receptor	OS=Canis famil...	333	1e-109
sp Q28927 ADRB1_SHEEP	Beta-1 adrenergic receptor	OS=Ovis aries ...	329	2e-108
sp Q9TT96 ADRB1_BOVIN	Beta-1 adrenergic receptor	OS=Bos taurus ...	328	8e-108
sp Q28998 ADRB1_PIG	Beta-1 adrenergic receptor	OS=Sus scrofa GN...	328	1e-107
sp Q9TST6 ADRB1_FELCA	Beta-1 adrenergic receptor	OS=Felis catus...	327	4e-107
sp P53452 DRD1L_TAKRU	D(1)-like dopamine receptor	OS=Takifugu r...	326	8e-107
sp P08588 ADRB1_HUMAN	Beta-1 adrenergic receptor	OS=Homo sapien...	323	1e-105
sp P47899 ADRB1_MACMU	Beta-1 adrenergic receptor	OS=Macaca mula...	321	8e-105
sp P34971 ADRB1_MOUSE	Beta-1 adrenergic receptor	OS=Mus musculu...	319	3e-104
sp P18841 ADA1B_MESAU	Alpha-1B adrenergic receptor	OS=Mesocrice...	320	4e-104
sp P53454 DRD5L_TAKRU	D(5)-like dopamine receptor	OS=Takifugu r...	318	6e-104
sp P15823 ADA1B_RAT	Alpha-1B adrenergic receptor	OS=Rattus norv...	319	1e-103
sp P18841 ADRB1_RAT	Beta-1 adrenergic receptor	OS=Rattus norveg...	317	1e-103

COMPLEMENTARY INFORMATION

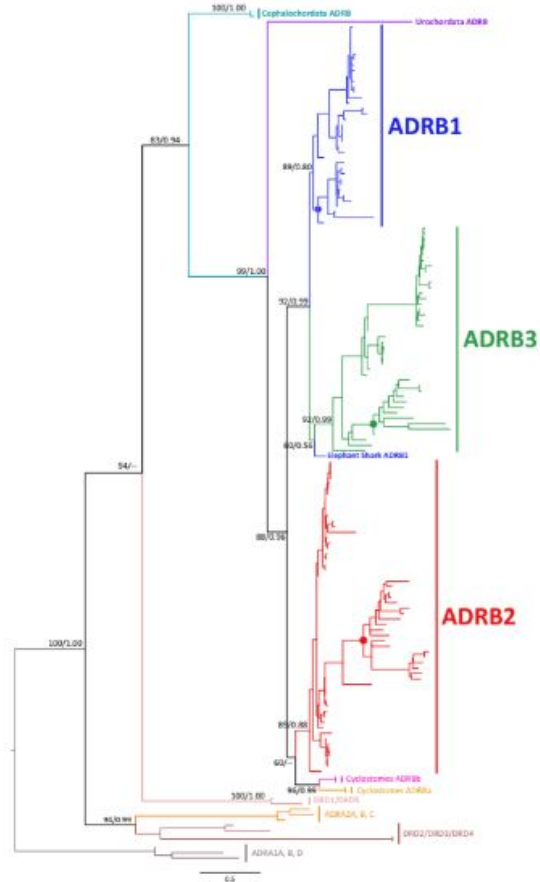
BLAST- Looking for β 2RC structure from different species

Query= 2RH1:A|PDBID|CHAIN|SEQUENCE

Length=500

Sequences producing significant alignments:				Score (Bits)	E Value
2rh1_A	mol:protein	length:500	beta-2-adrenergic receptor/T4-lys...	1042	0.0
3d4s_A	mol:protein	length:490	Beta-2 adrenergic receptor/T4-lys...	996	0.0
2r4r_A	mol:protein	length:365	Beta-2 adrenergic receptor	476	7e-167
3enl_A	mol:protein	length:488	Human Adenosine A2A receptor/T4 l...	461	4e-159
2r4s_A	mol:protein	length:342	Beta-2 adrenergic receptor	423	2e-146
212l_A	mol:protein	length:168	T4 LYSOZYME	335	2e-114
2oea_X	mol:protein	length:164	Lysozyme	335	3e-114
2oe9_X	mol:protein	length:164	Lysozyme	335	3e-114
2oe7_X	mol:protein	length:164	Lysozyme	335	3e-114
2oe4_X	mol:protein	length:164	Lysozyme	335	3e-114
219l_A	mol:protein	length:164	T4 LYSOZYME	335	3e-114
1lw9_A	mol:protein	length:164	LYSOZYME	335	3e-114
1l63_A	mol:protein	length:164	LYSOZYME	335	3e-114
1c6t_A	mol:protein	length:164	PROTEIN (LYSOZYME)	335	3e-114
1c6q_A	mol:protein	length:164	PROTEIN (LYSOZYME)	335	3e-114
1c6p_A	mol:protein	length:164	PROTEIN (LYSOZYME)	335	3e-114
1p56_A	mol:protein	length:176	PROTEIN (Lysozyme)	335	4e-114
1jtn_B	mol:protein	length:178	LYSOZYME	335	4e-114
1jtn_A	mol:protein	length:178	LYSOZYME	335	4e-114
1jtn_A	mol:protein	length:178	LYSOZYME	335	4e-114
2l78_A	mol:protein	length:164	T4 LYSOZYME	334	4e-114
1p7s_A	mol:protein	length:164	LYSOZYME	334	4e-114
1p2l_A	mol:protein	length:164	LYSOZYME	334	4e-114
1g0q_A	mol:protein	length:164	PROTEIN (LYSOZYME)	334	4e-114
1l93_A	mol:protein	length:164	T4 LYSOZYME	334	5e-114
1d3j_A	mol:protein	length:164	LYSOZYME	334	5e-114
1cv5_A	mol:protein	length:164	LYSOZYME	334	5e-114
1cv4_A	mol:protein	length:164	LYSOZYME	334	5e-114
1cv3_A	mol:protein	length:164	LYSOZYME	334	5e-114
1cu5_A	mol:protein	length:164	LYSOZYME	334	5e-114
1cu2_A	mol:protein	length:164	LYSOZYME	334	5e-114
1p36_A	mol:protein	length:164	LYSOZYME	334	5e-114
1p2r_A	mol:protein	length:164	LYSOZYME	334	5e-114
224l_A	mol:protein	length:164	T4 LYSOZYME	334	7e-114
221l_A	mol:protein	length:164	T4 LYSOZYME	334	7e-114
206l_A	mol:protein	length:164	LYSOZYME	334	7e-114
125l_A	mol:protein	length:164	T4 LYSOZYME	334	7e-114
123l_A	mol:protein	length:164	T4 LYSOZYME	334	7e-114
122l_A	mol:protein	length:164	T4 LYSOZYME	334	7e-114
120l_A	mol:protein	length:164	T4 LYSOZYME	334	7e-114
119l_A	mol:protein	length:164	T4 LYSOZYME	334	7e-114
118l_A	mol:protein	length:164	T4 LYSOZYME	334	7e-114
1p0q_A	mol:protein	length:164	Lysozyme	333	7e-114

COMPLEMENTARY INFORMATION



COMPLEMENTARY INFORMATION

Category	Ligand	PDB	Structure
Agonist	Adrenaline	4LDO	The chemical structure of Adrenaline (Epinephrine) is shown. It features a benzene ring with two hydroxyl groups (HO-) at the 3 and 4 positions. Attached to the 1 position of the ring is a side chain consisting of a chiral carbon atom (marked with an asterisk) bonded to a hydroxyl group (OH), a hydrogen atom, and a secondary amine group (-CH₂-NH₂⁺). The benzene ring and the side chain are highlighted with a yellow oval and a green oval, respectively.
Antagonist	Alprenolol	3NYA	The chemical structure of Alprenolol is shown. It features a benzene ring with a vinyl group (-CH=CH₂) at the 1 position and an ether linkage (-O-) at the 3 position. The ether linkage connects to a side chain consisting of a chiral carbon atom (marked with an asterisk) bonded to a hydroxyl group (OH), a hydrogen atom, and a secondary amine group (-CH₂-NH₂⁺). The benzene ring and the side chain are highlighted with a yellow oval and a green oval, respectively.
Inverse agonist	Carazolol	2RH1	The chemical structure of Carazolol is shown. It features a benzimidazole ring system with an ether linkage (-O-) at the 2 position. The ether linkage connects to a side chain consisting of a chiral carbon atom (marked with an asterisk) bonded to a hydroxyl group (OH), a hydrogen atom, and a secondary amine group (-CH₂-NH₂⁺). The benzimidazole ring system and the side chain are highlighted with a yellow oval and a green oval, respectively.