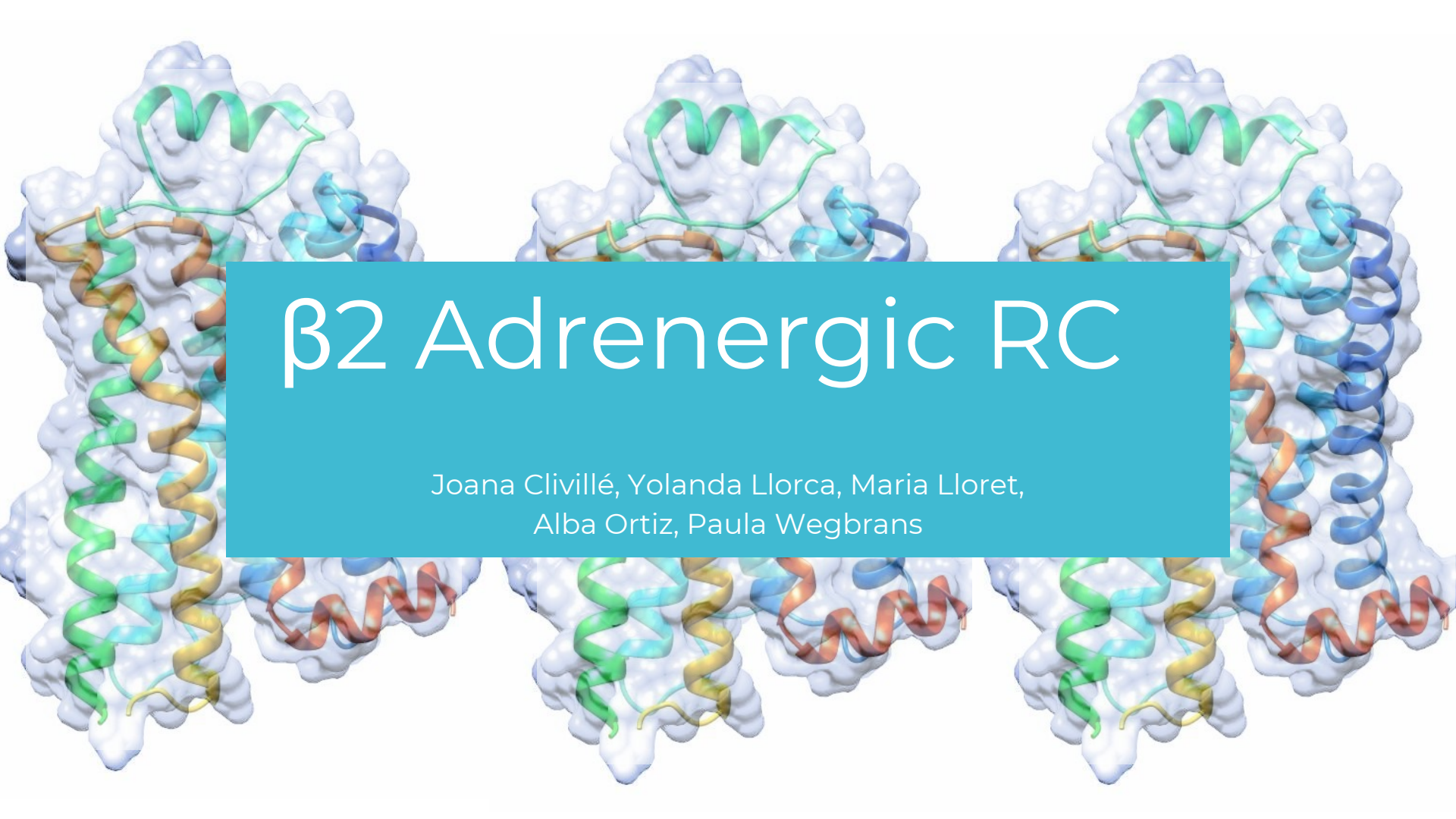

β2 Adrenergic RC

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

Index

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

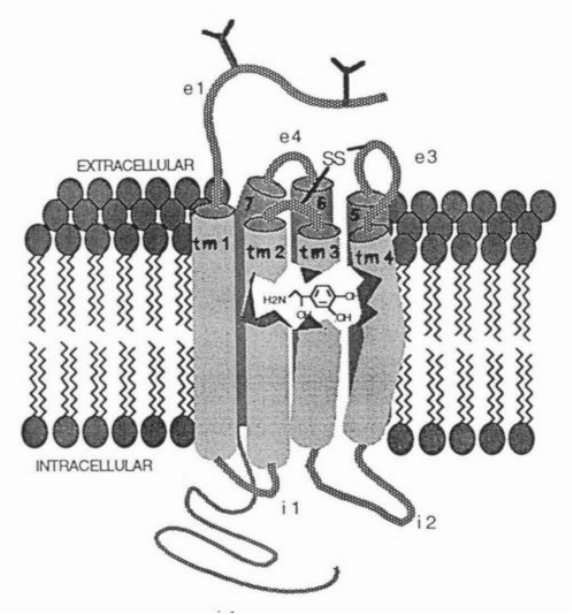

Fig. 1: Strosberg AD, **1991**

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 AMPC

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 GPRC
- **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, bronquial 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, bronquial, gastrointestinal and genitourinary smooth muscle	Gs
	β-3	NA >> AD	Lipolysis in adipose tissue and thermogenesis	Gs

Adrenergic Receptors

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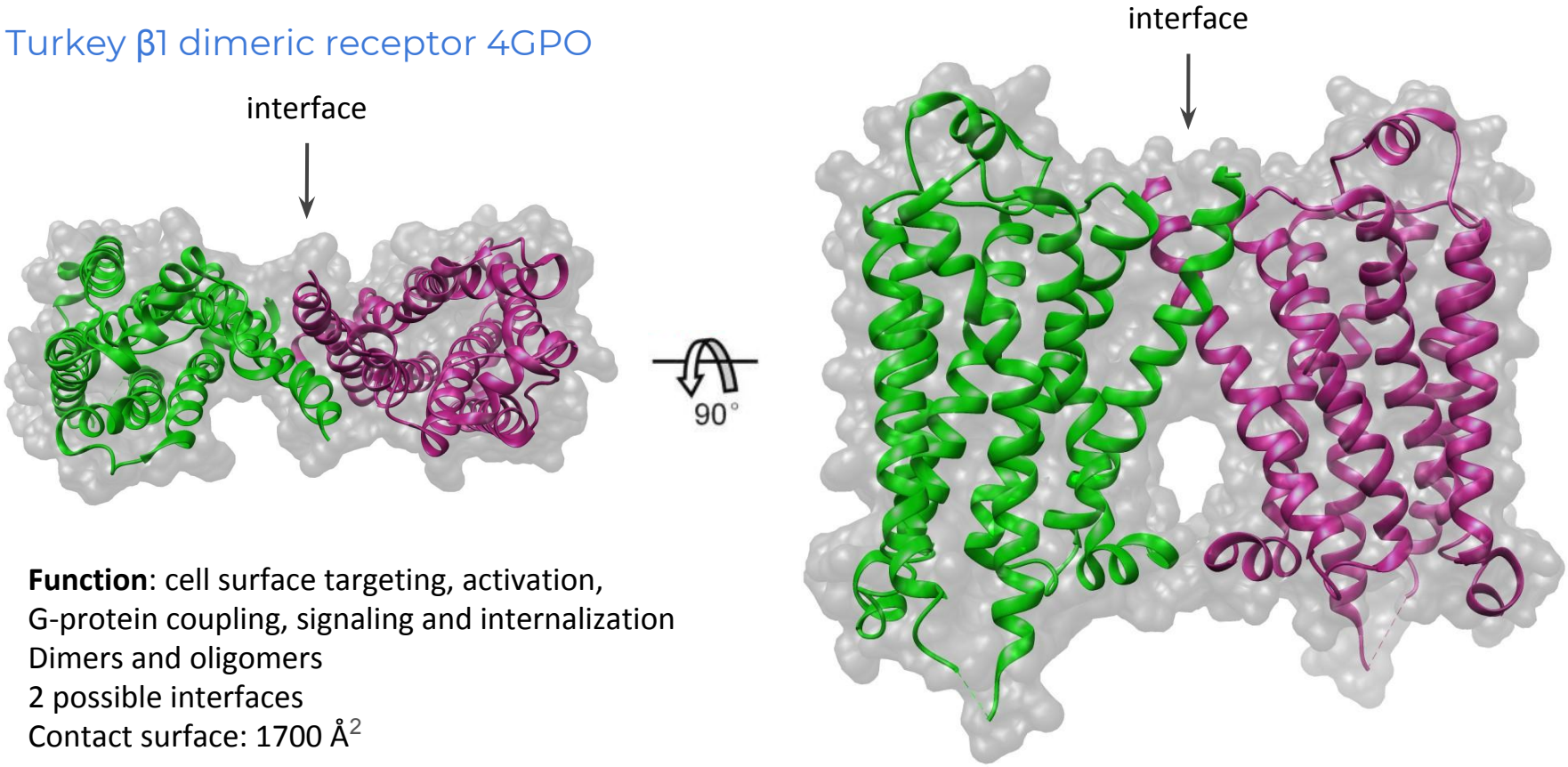
	β-2	ISO > AD >> NA	Relaxation of vascular, bronquial, gastrointestinal and genitourinary smooth muscle	Gs
	β-3	NA >> AD	Lipolysis in adipose tissue and thermogenesis	Gs

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 Å^2

ADRC Dimerization

Turkey β1 dimeric receptor 4GPO

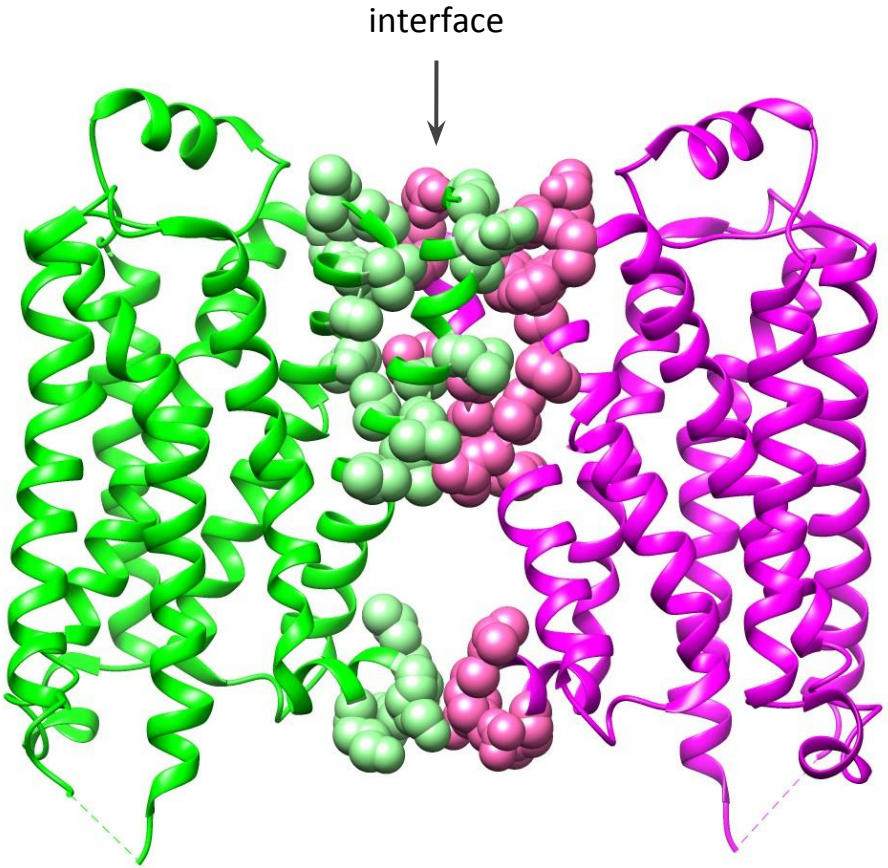
Hydrophobic and Van der Waals interactions

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

TM2: Pro96, Ala99, Thr100 and Val103

ECL1: Thr106, Leu108, and Trp109

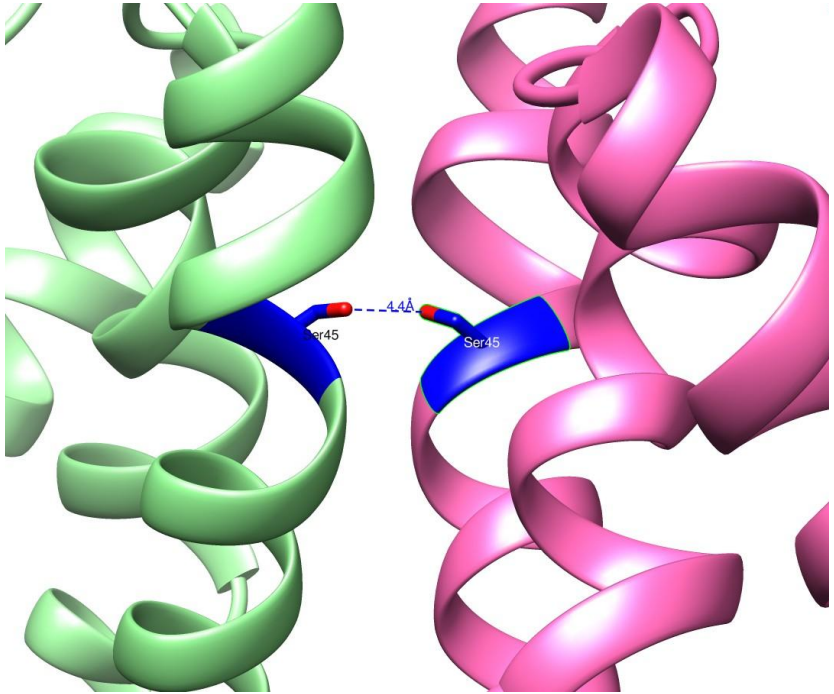
H8: Arg351, Lys354, Arg355 and Leu356



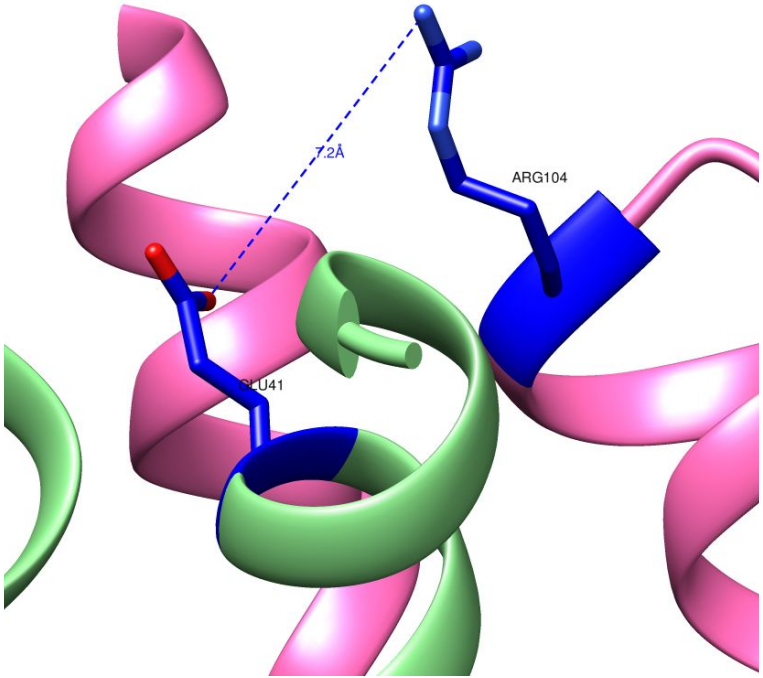
ADRC Dimerization

Turkey β1 dimeric receptor 4GPO

Hydrogen bond formation: Ser45-Ser45 (TM1)

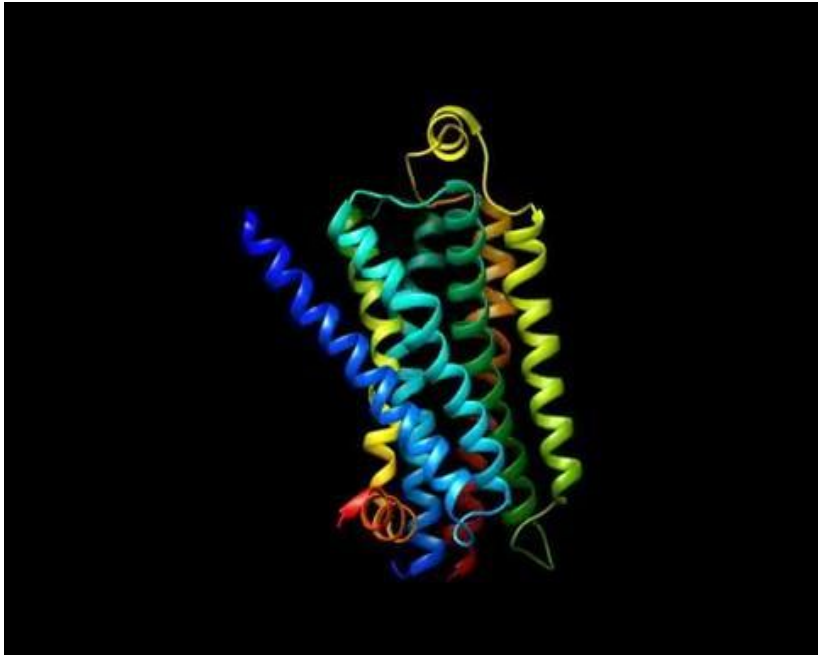


Salt bridge formation: Glu41 (TM1) Arg 104 (TM2)



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

Agonist binding

Gα, β, γ bind to RC

Gα exchanges GDP for GTP and dissociated

- Gα regulates adenylate cyclase
- Gβγ regulate calcium channels

GTP hydrolysis in Gα allows re-binding of Gβγ

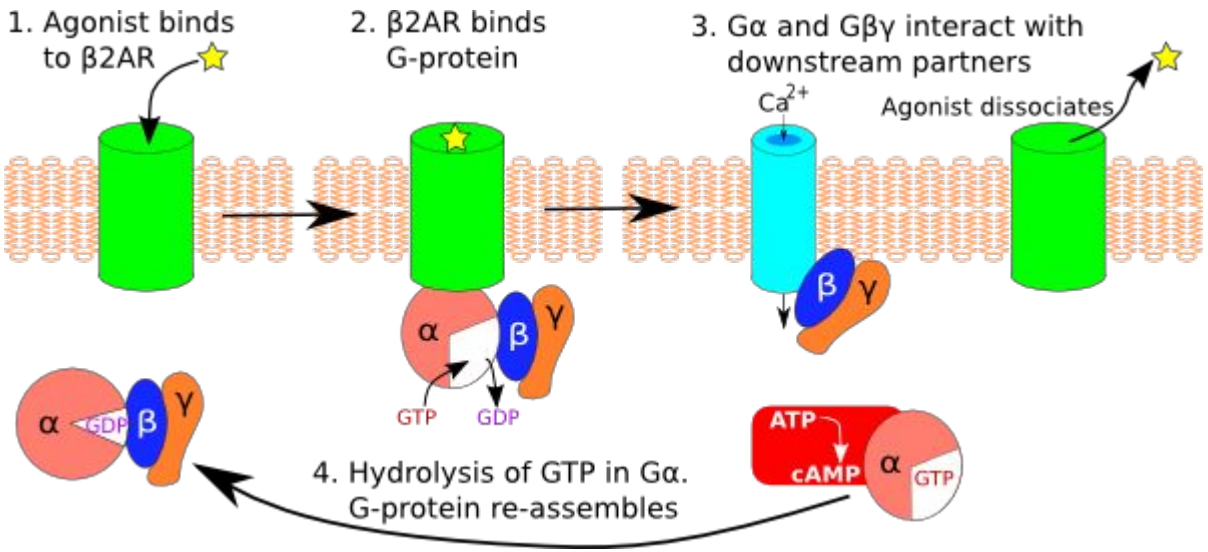


Figure extracted from: http://www.ebi.ac.uk/pdbe/quips?story=B2AR

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

β2-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

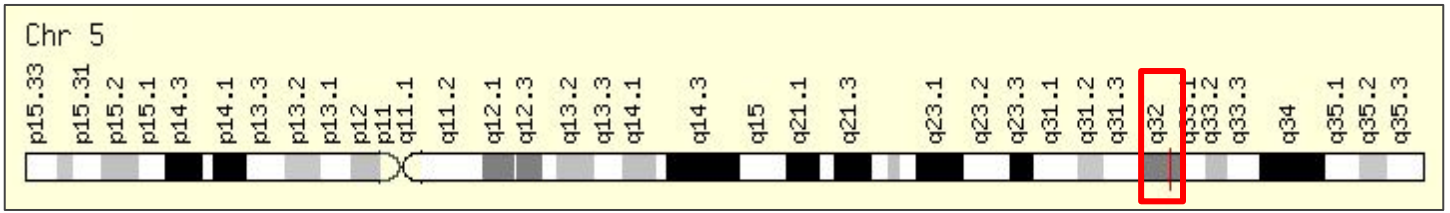
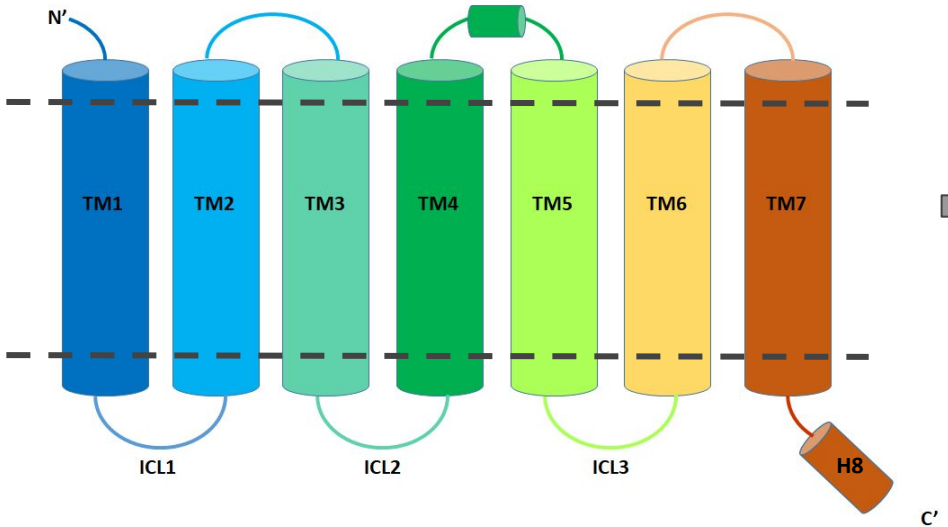


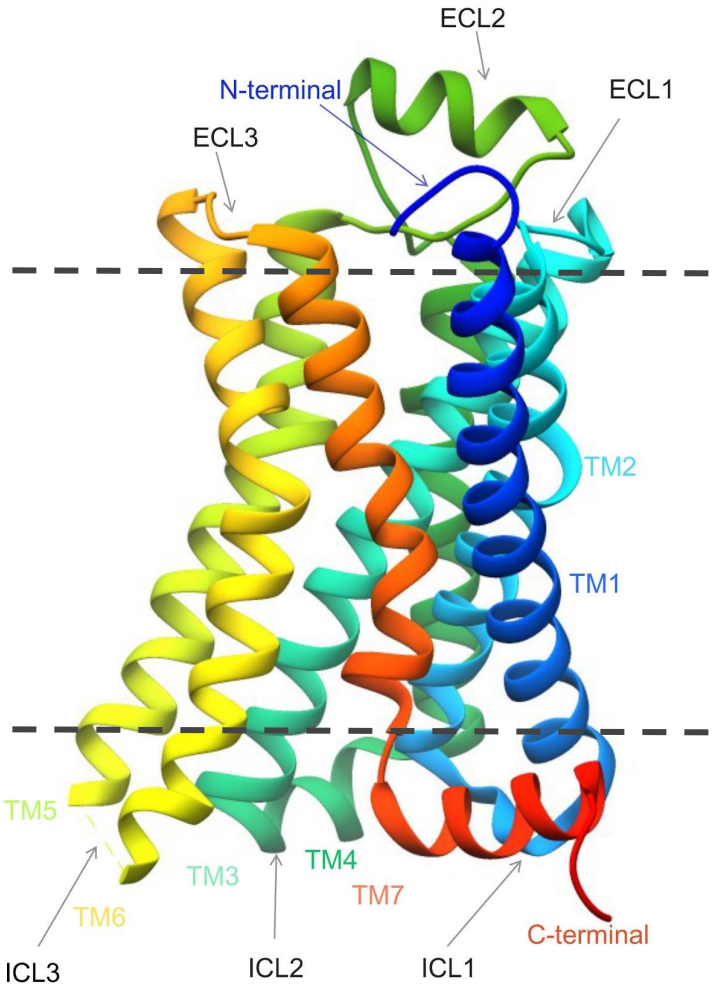
Figure extracted from: https://filizm02.u.hpc.mssm.edu/gpcr-okb/

β2-ADRC Structure

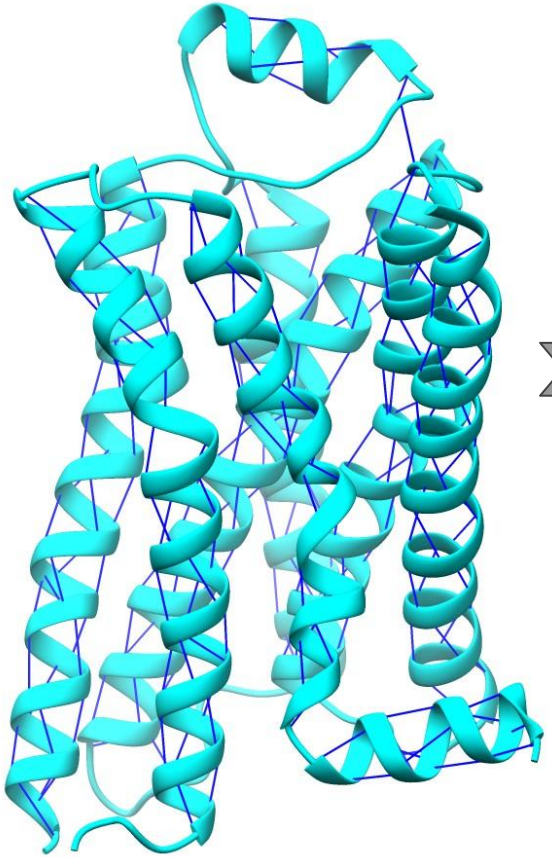
Integral alpha-helical polytopic transmembrane protein



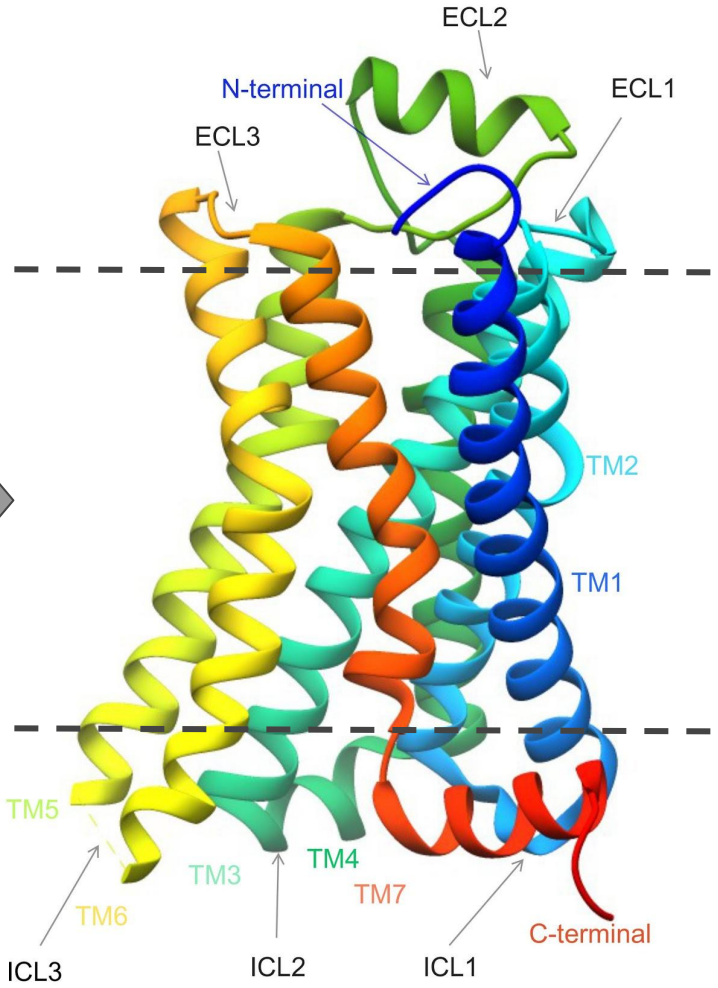
Folding



β2-ADRC Tertiary Structure

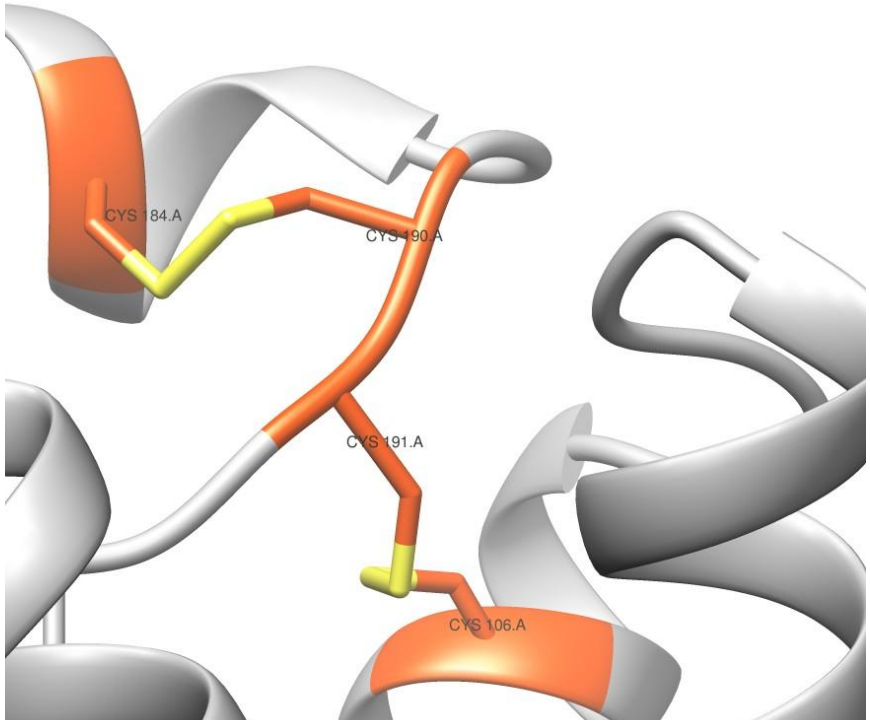


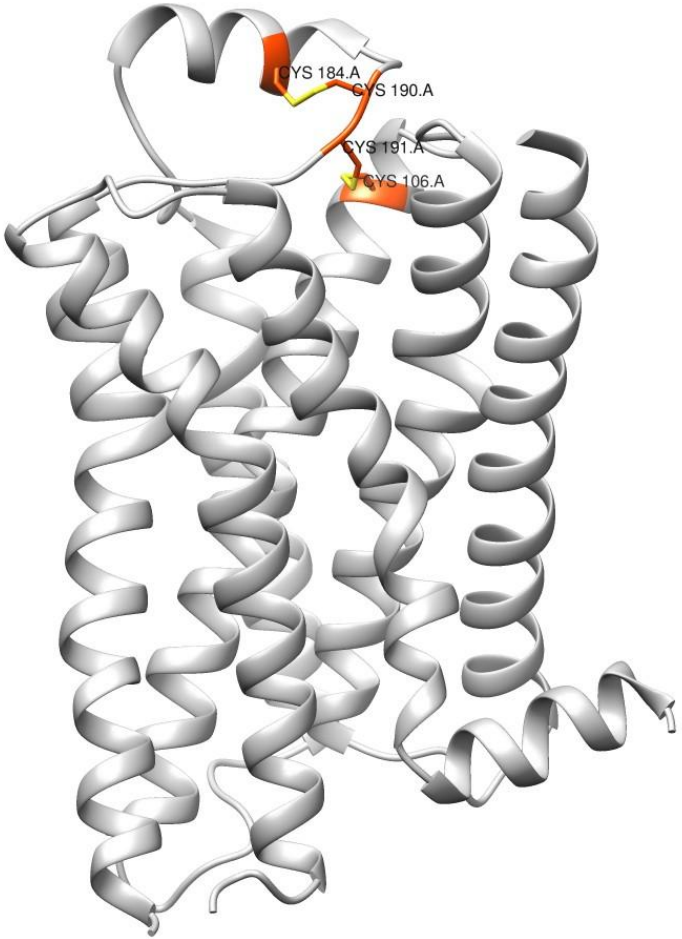
Hydrogen bonds are essential to stabilize the tertiary structure



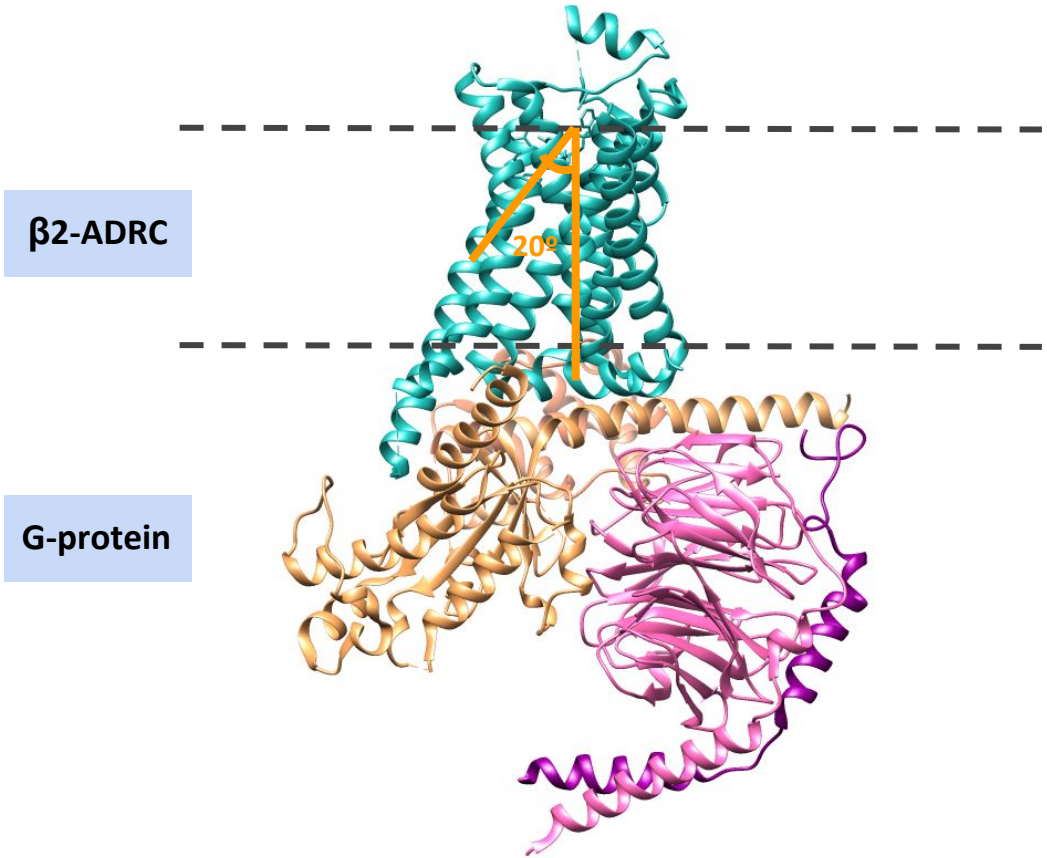
β2-ADRC Tertiary Structure

Disulfide Bridges

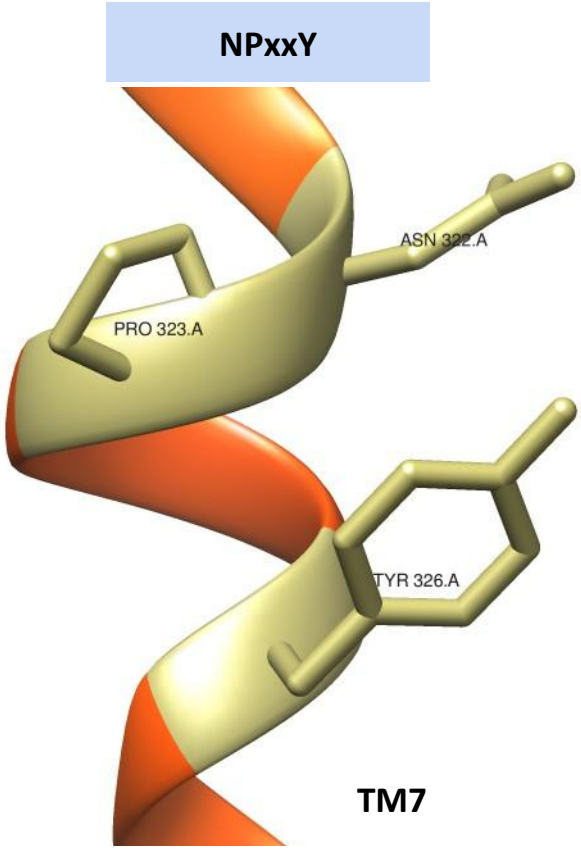


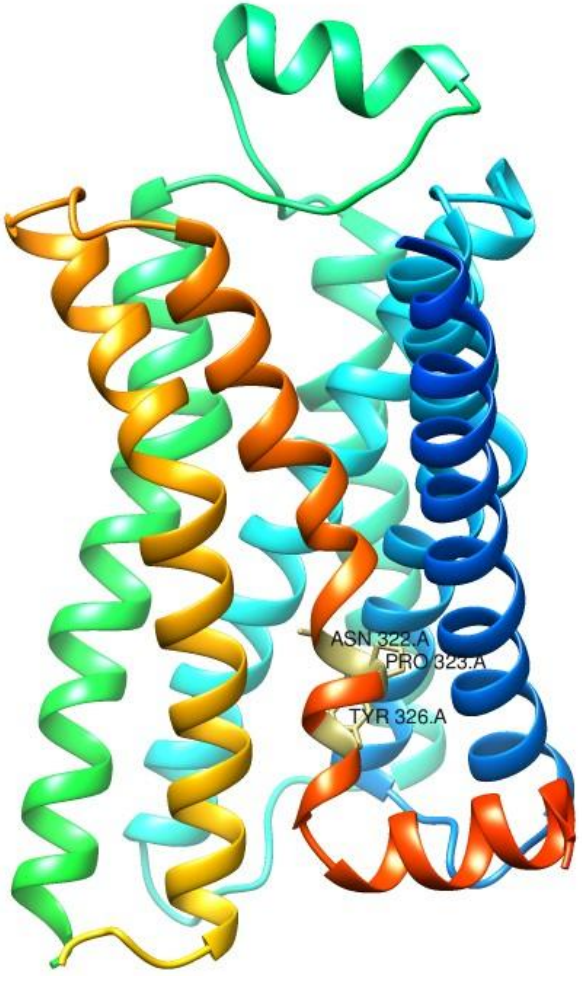


β2-ADRC Quaternary Structure



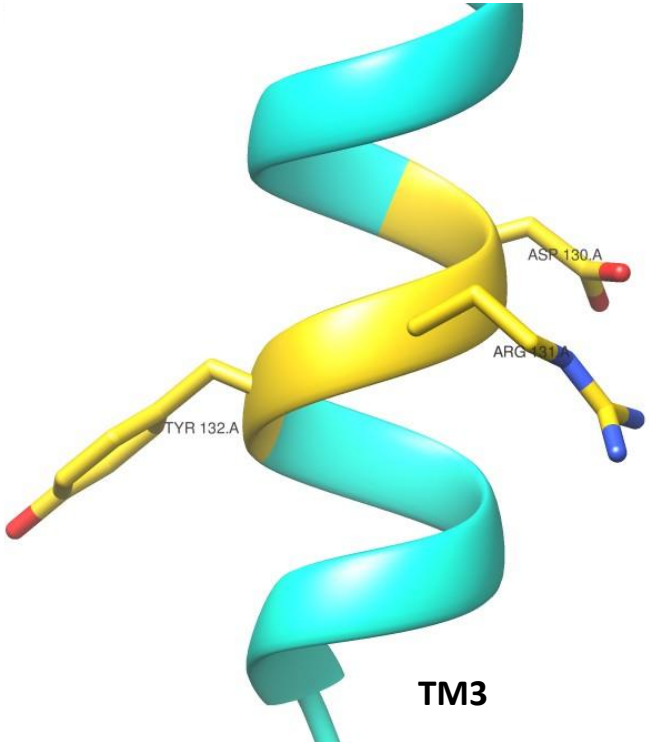
β2-ADRC Conserved Motifs

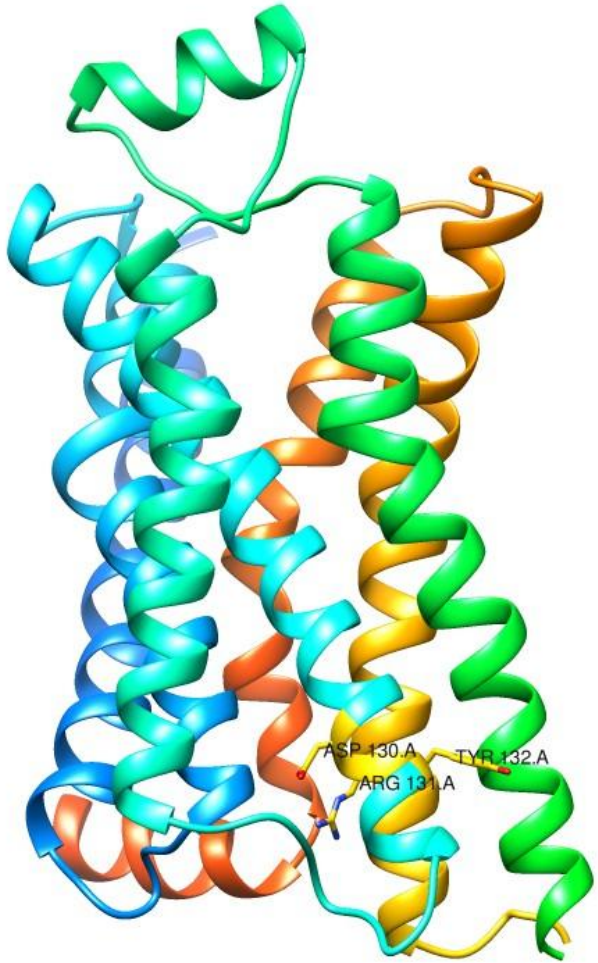




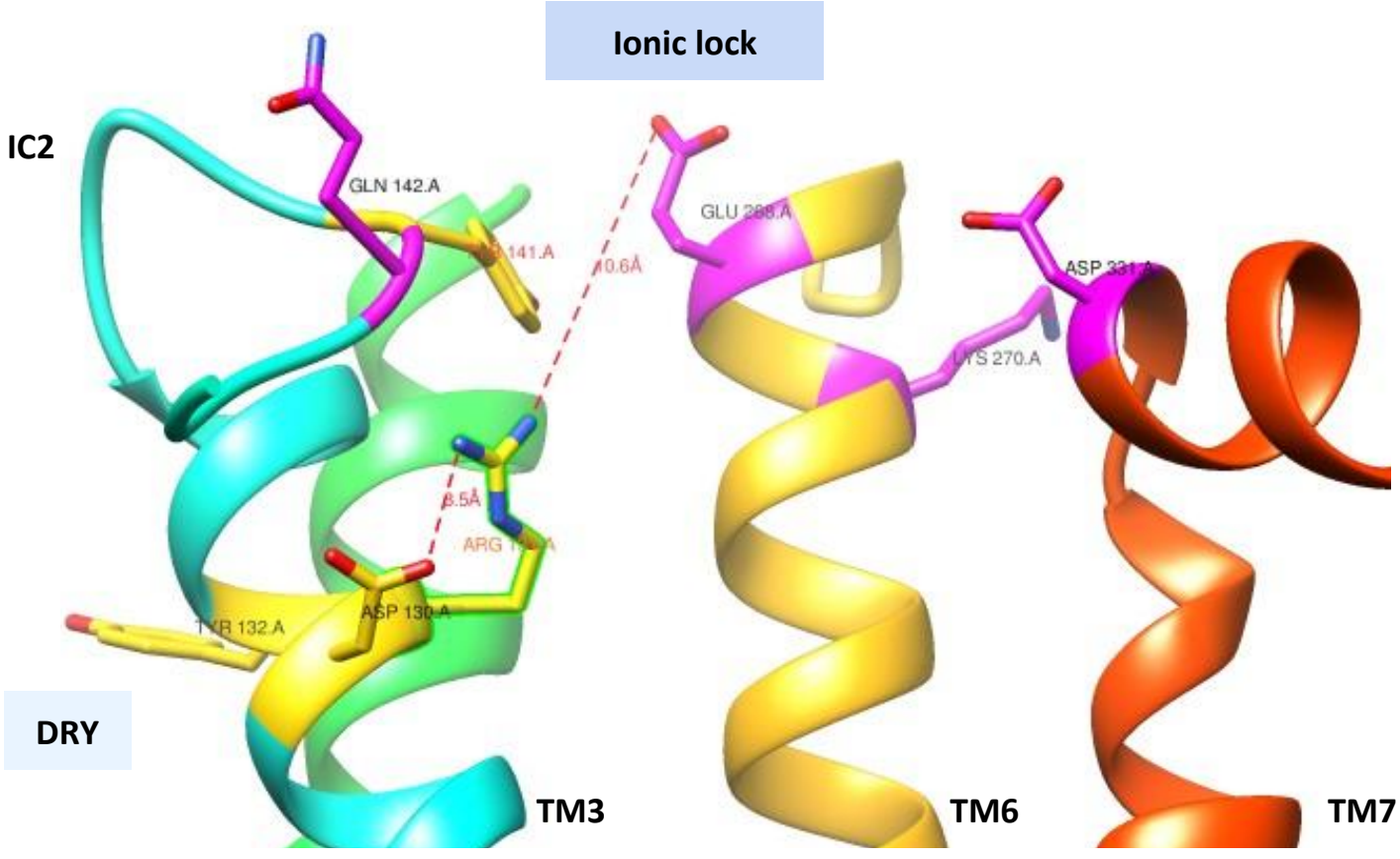
β2-ADRC Conserved Motifs

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



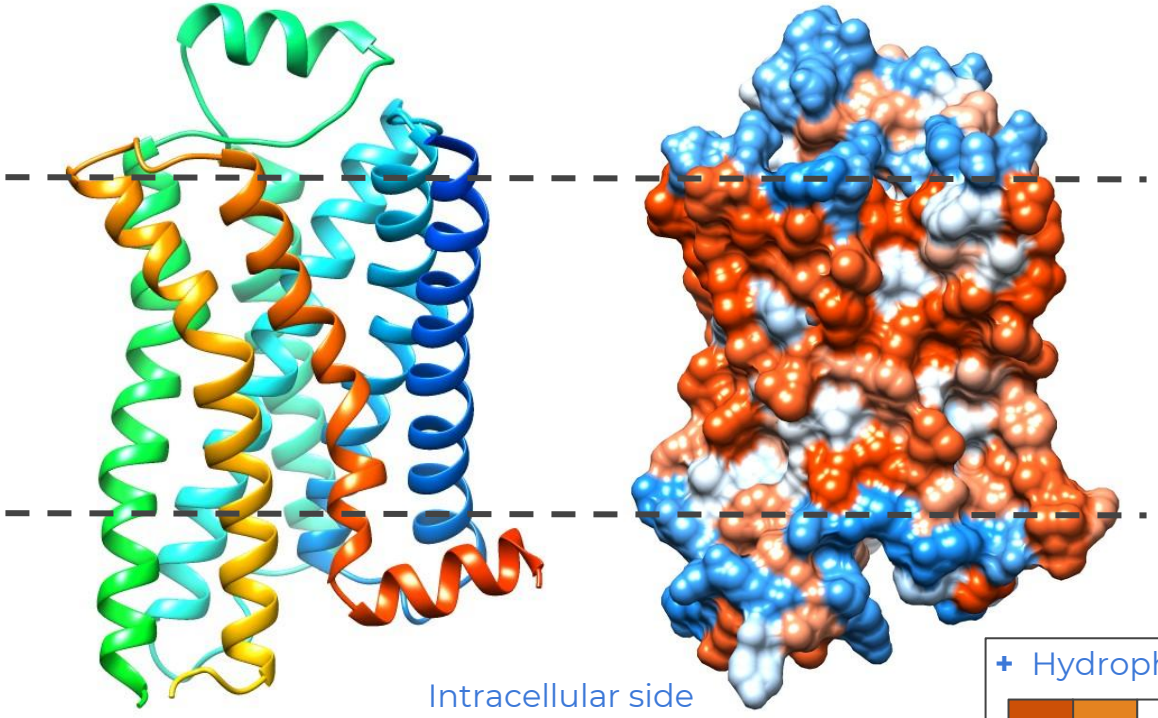


β2-ADRC Conserved Motifs



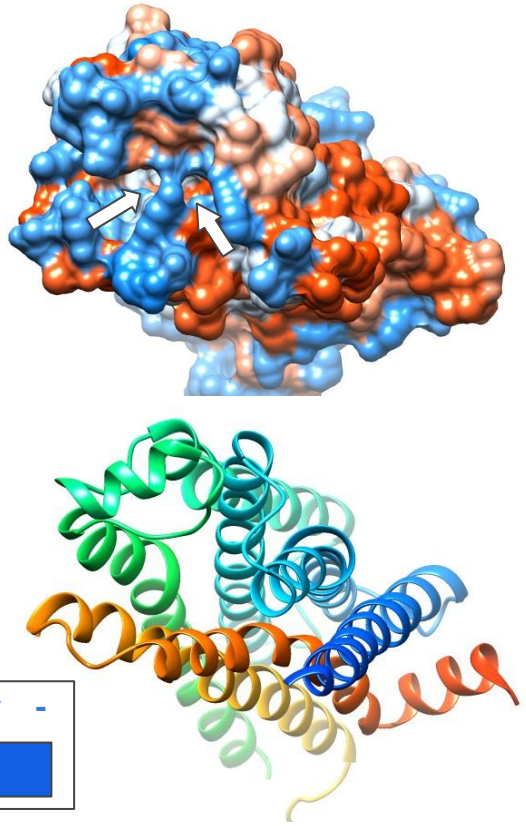
β2-ADRC Hydrophobicity

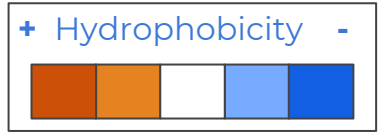
Extracellular side



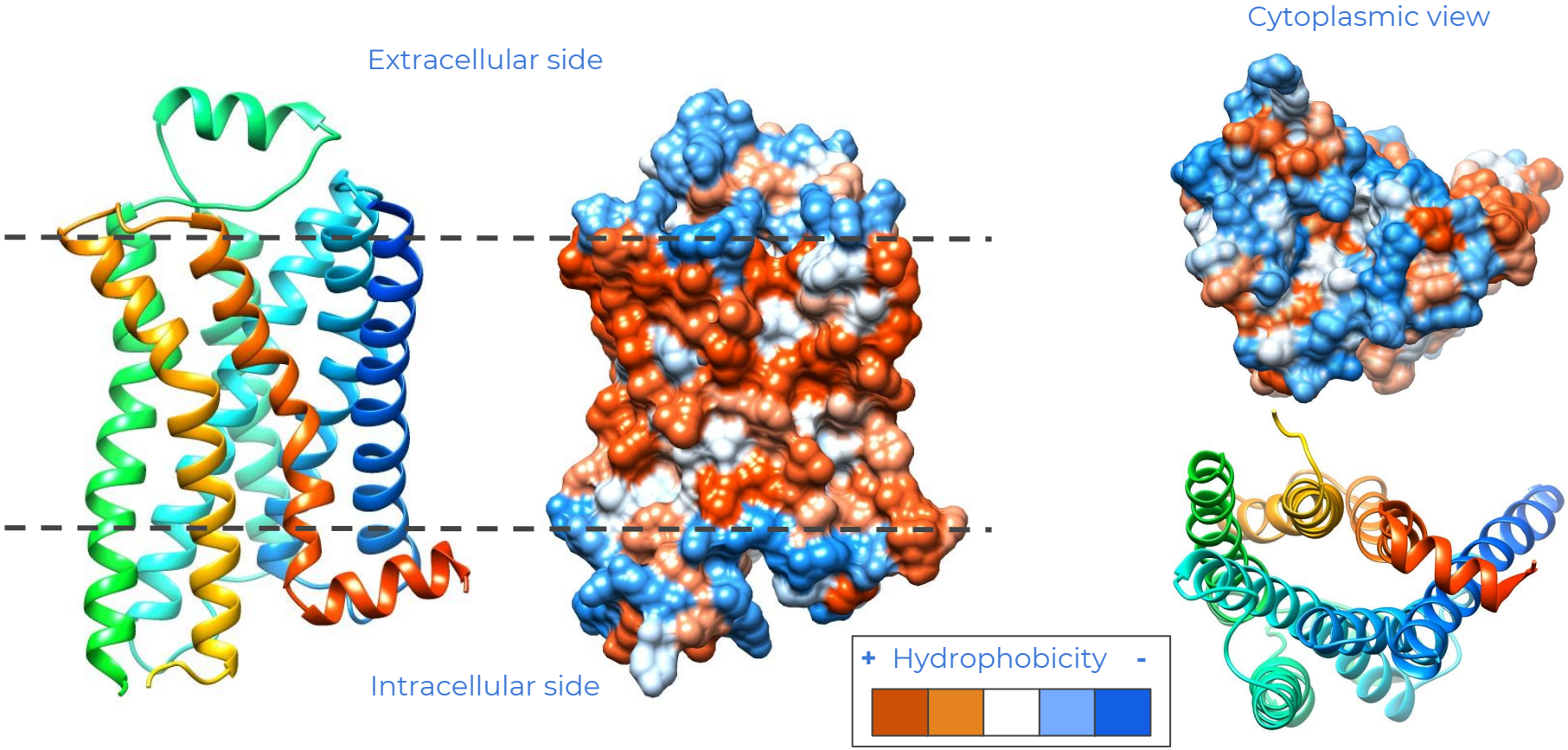
Intracellular side

Extracellular view

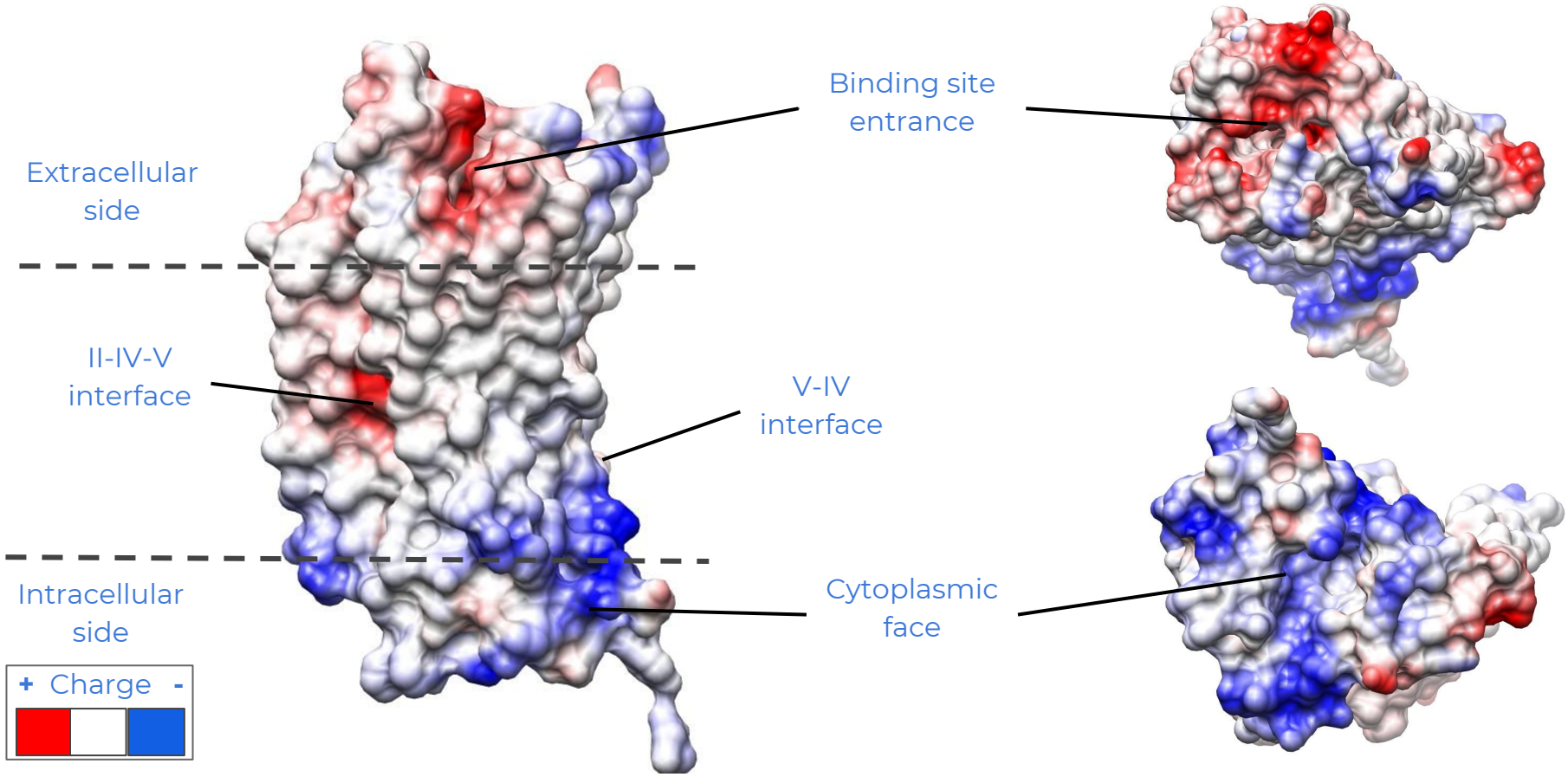




β2-ADRC Hydrophobicity



β2-ADRC Electrostatic Potentials

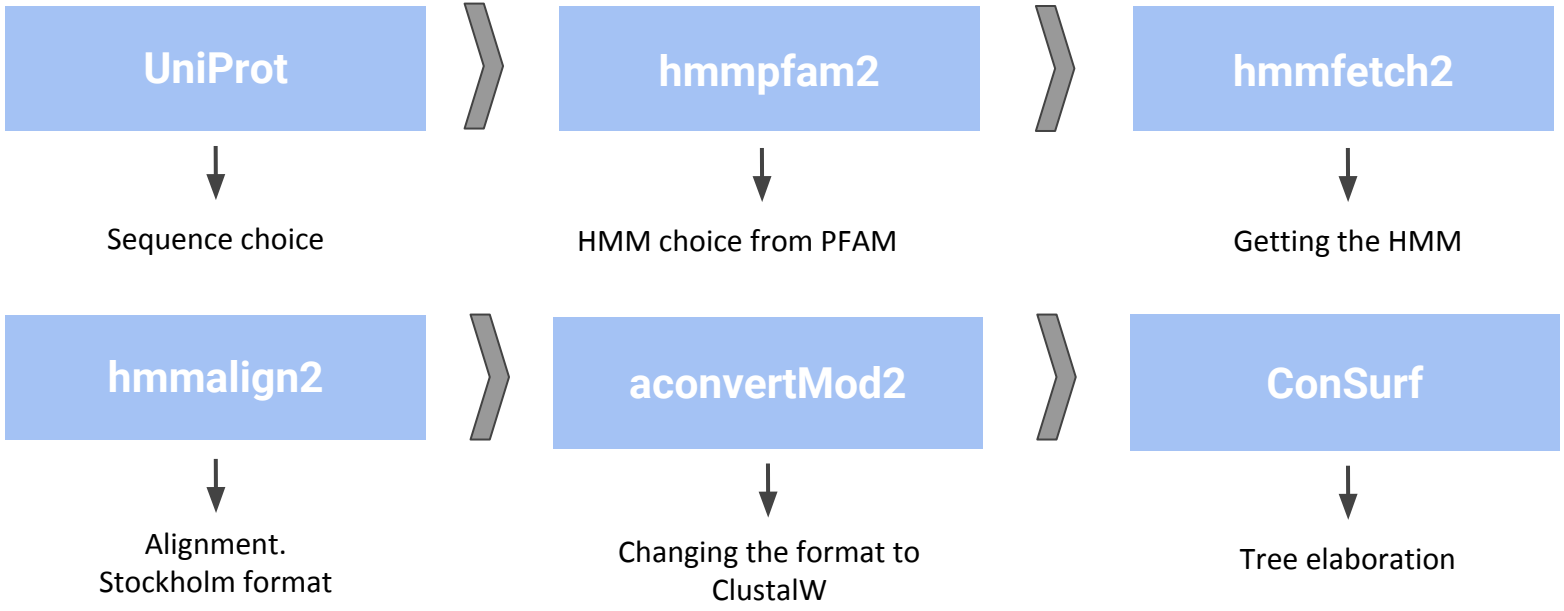


Sequence Evolution

ADRENERGIC FAMILY IN HUMANS

β2 RECEPTORS AMONG DIFFERENT SPECIES

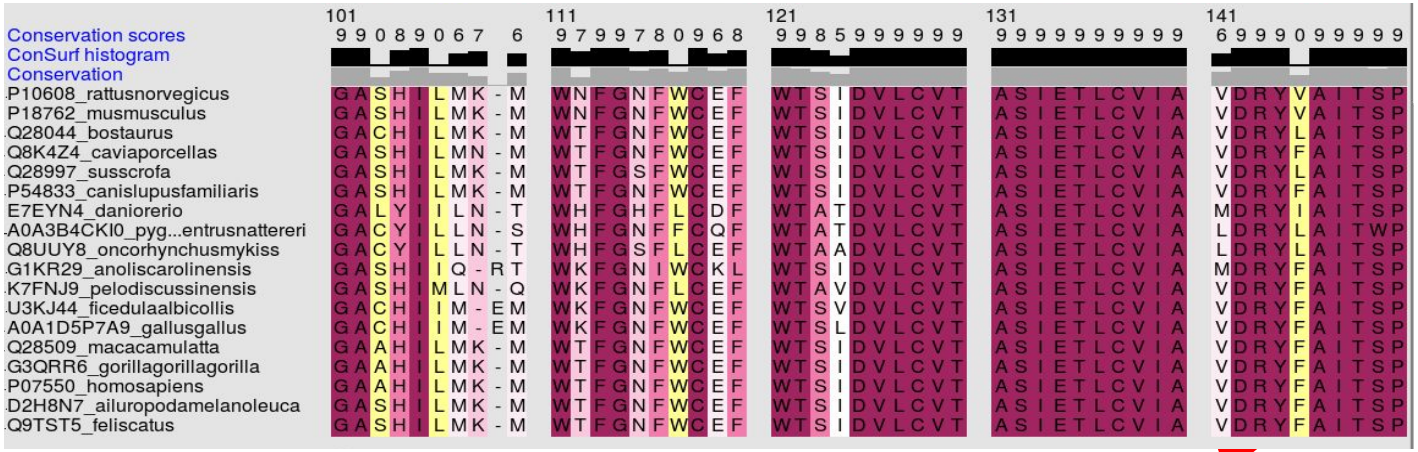
PSIBLAST

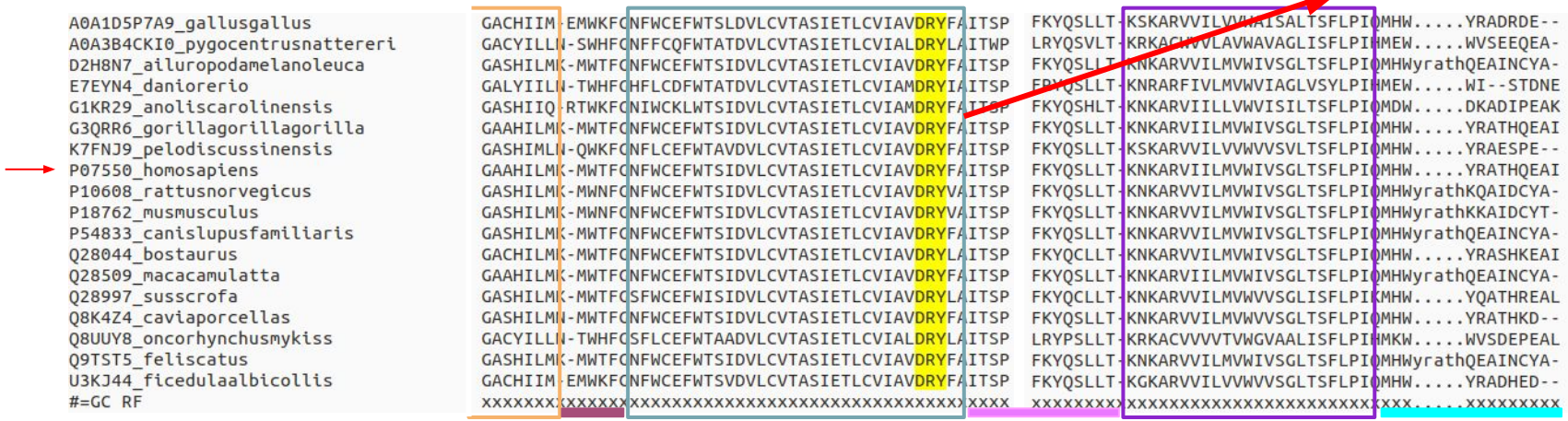


Sequence Evolution of Adrenergic family

Sequence Evolution of β2

Sequence Evolution

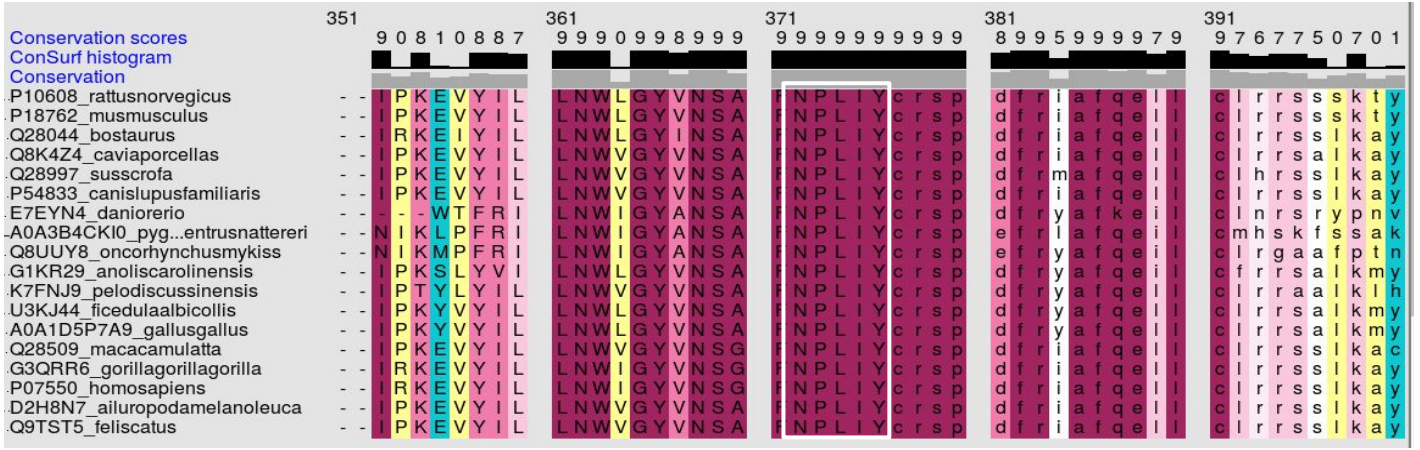


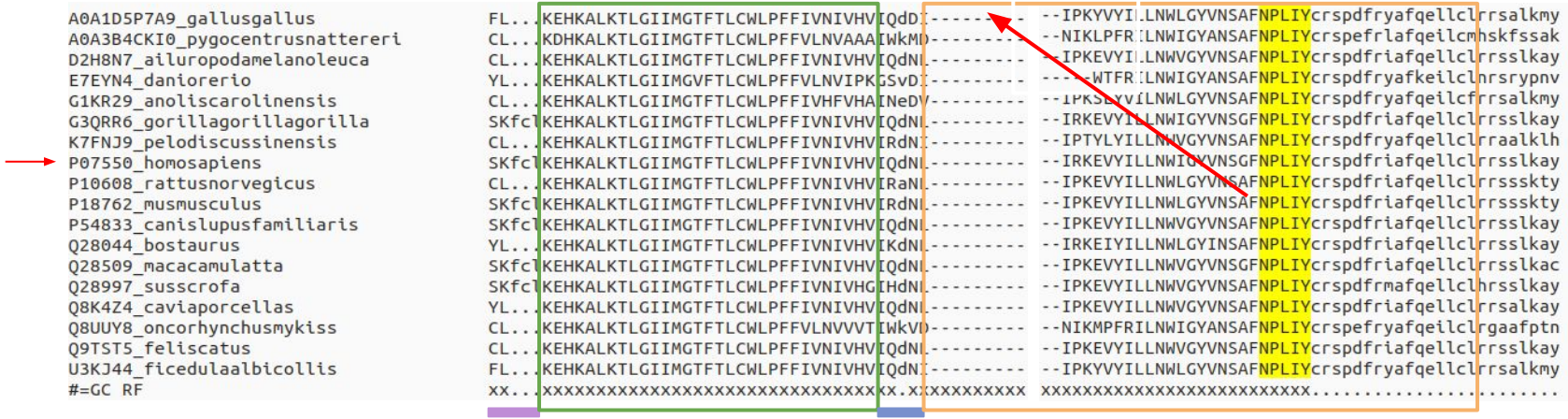


TM1
ICL1
TM2
ECL1
TM3
ICL2
TM4
ECL2

Sequence Evolution

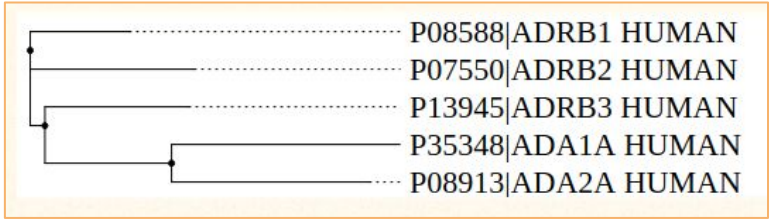
Sequence Evolution

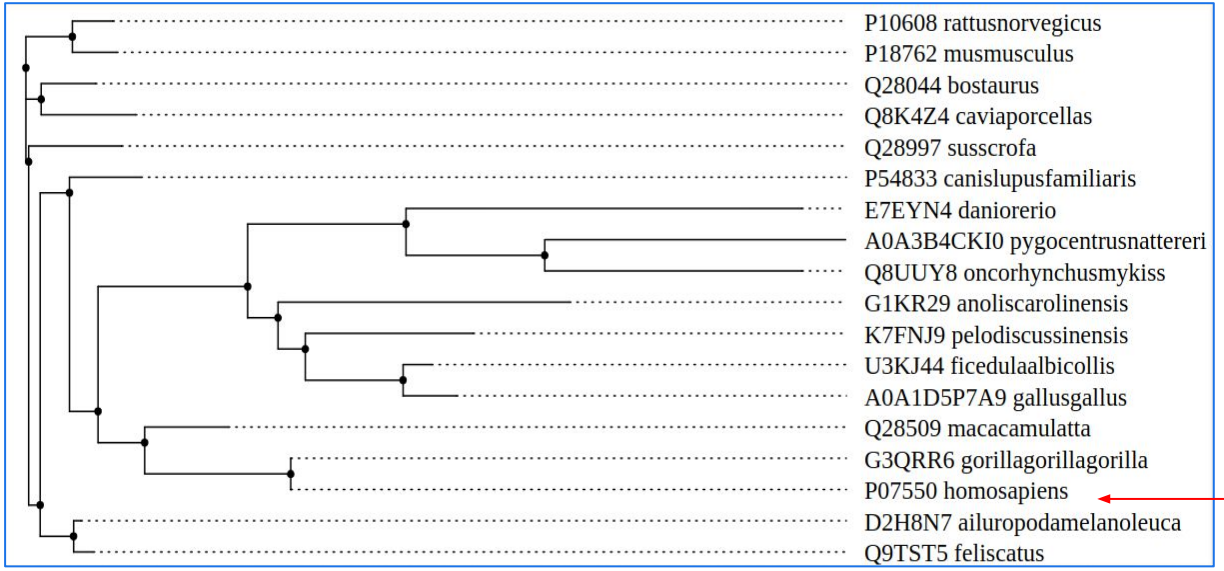




ECL2
TM5
ICL3
TM6
ECL3
TM7

Sequence Evolution



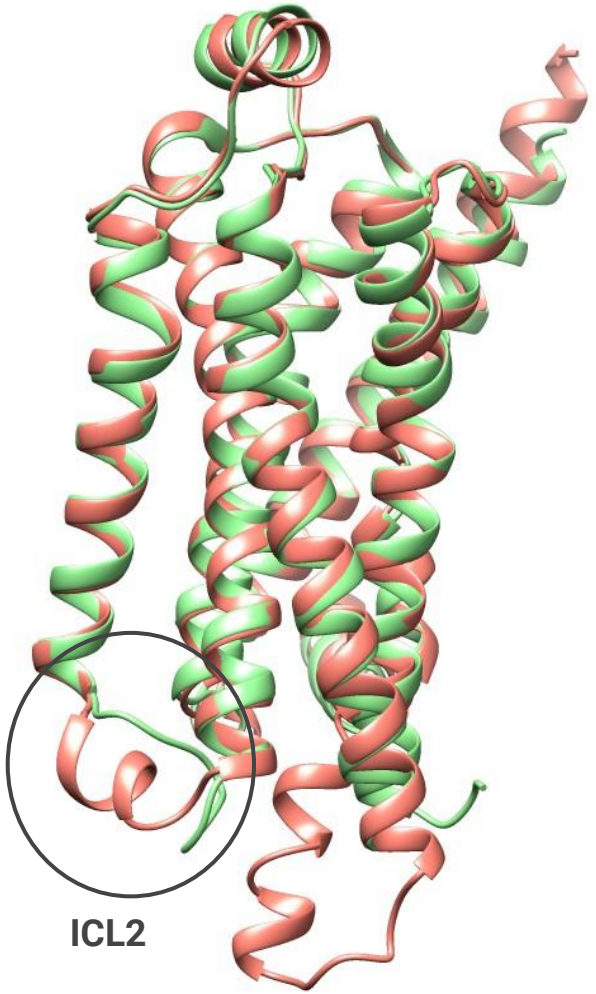


Main conclusions:

- 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

β1ADR - β2ADR comparison

- **S**equence % identity: **67%**
- **S**ubtle differences in EC side, binding site included
- **M**ore differences in IC side
- **ICL2**
  - **α**-helix in **β1** adrenoceptor
  - **β1** Tyr149 (ICL2) forms a HB with Asp138 (DRY, TM3)
    - Important for **β1** activation
    - Existence and relevance of this in **β2** unclear



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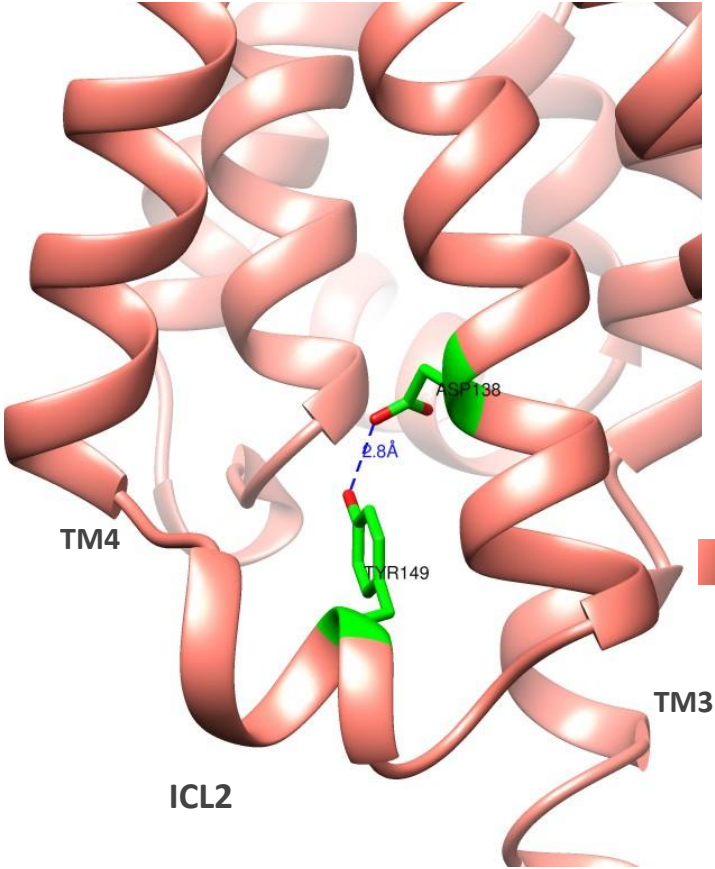


PDB:2YCW carazolol, **β1**, turkey

RMSD: 0.78

Basics on GPCR representation

MODELS USED

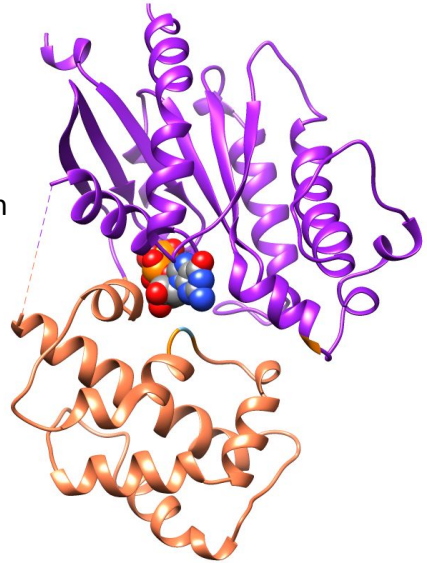
Protein Gs

α Ras domain

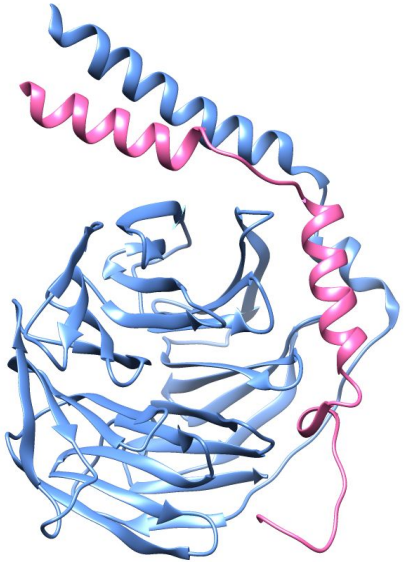
α AH domain

β domain

γ domain



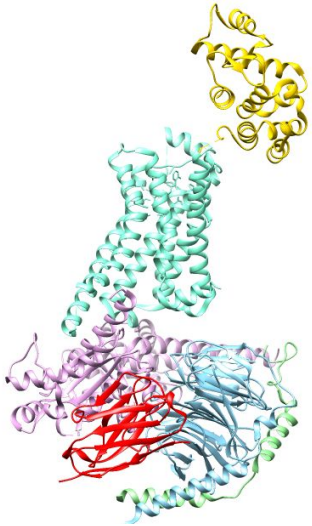
Gs alpha domains + GTPγS
1AZT



Gs beta + gamma domains
1GP2

Ternary complex

agonist + B2AR + Gs protein

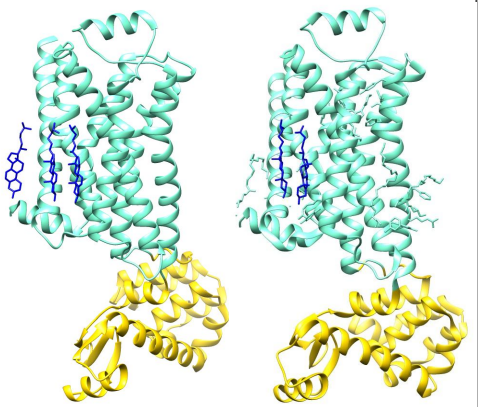


BI167107 + B2AR + T4L + Nb35 + Gs protein ($\alpha\beta\gamma$) **3SN6**

Basics on GPCR representation

MODELS USED

Cholesterol Binding Site

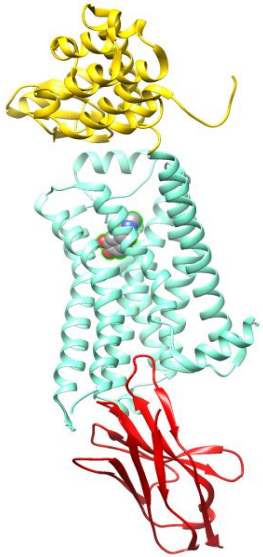


2RH1
Carazolol + T4L

3D4S
Timolol + T4L

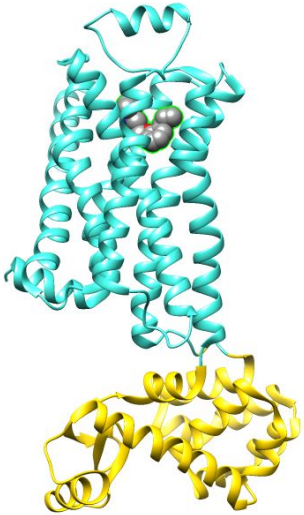
Agonist

Active State B2AR



4LDO
Adrenaline + T4L + Nb80

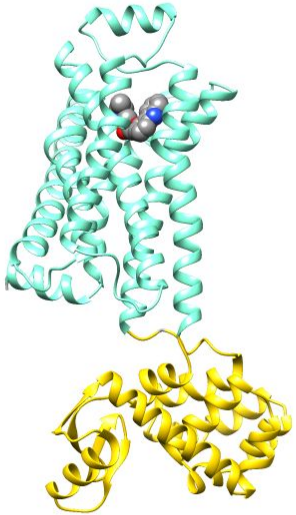
Antagonist



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

→

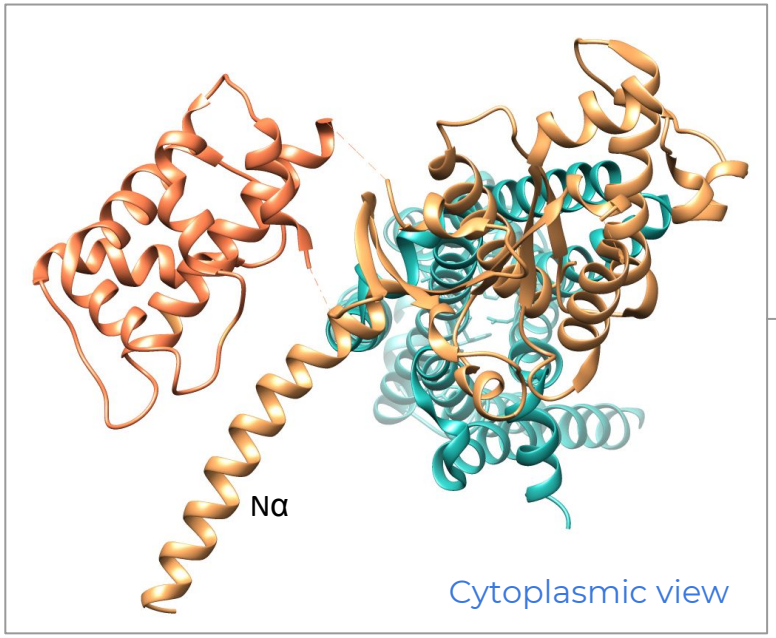
T4L

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

Nb80 Nb35

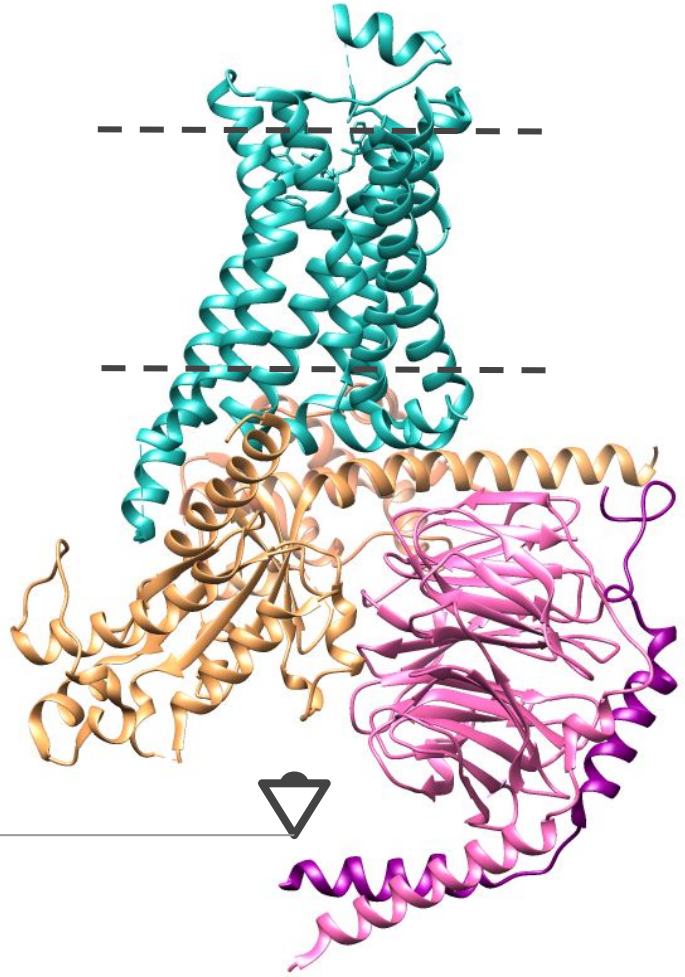
- Single domain fragment of Camelid heavy chain only antibodies.
- Small (15kDa) and stable, has access to clefts of the AR.
- Binds to conformation-specific B2AR epitopes with its long CDR3 loop.

Protein G interactions

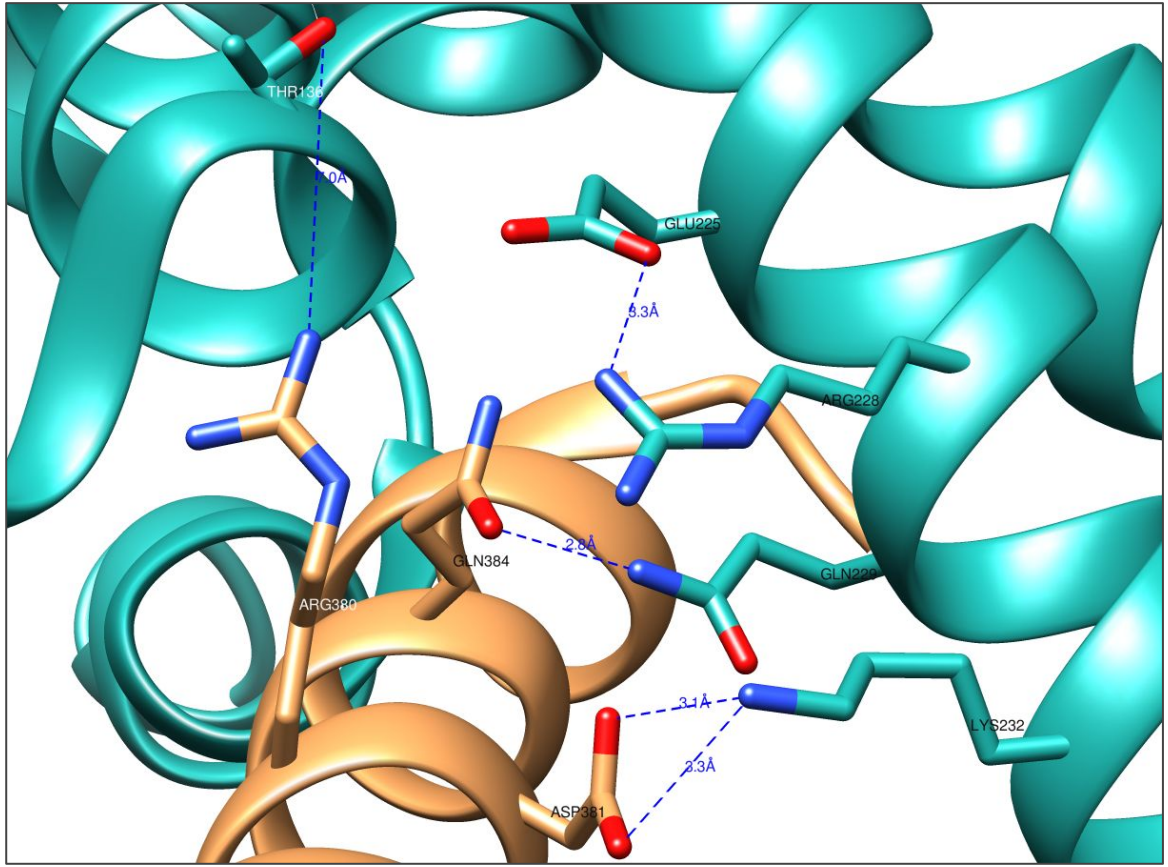


β2A Receptor
α Ras domain
α AH domain
β domain
γ domain

3SN6



Protein G interactions



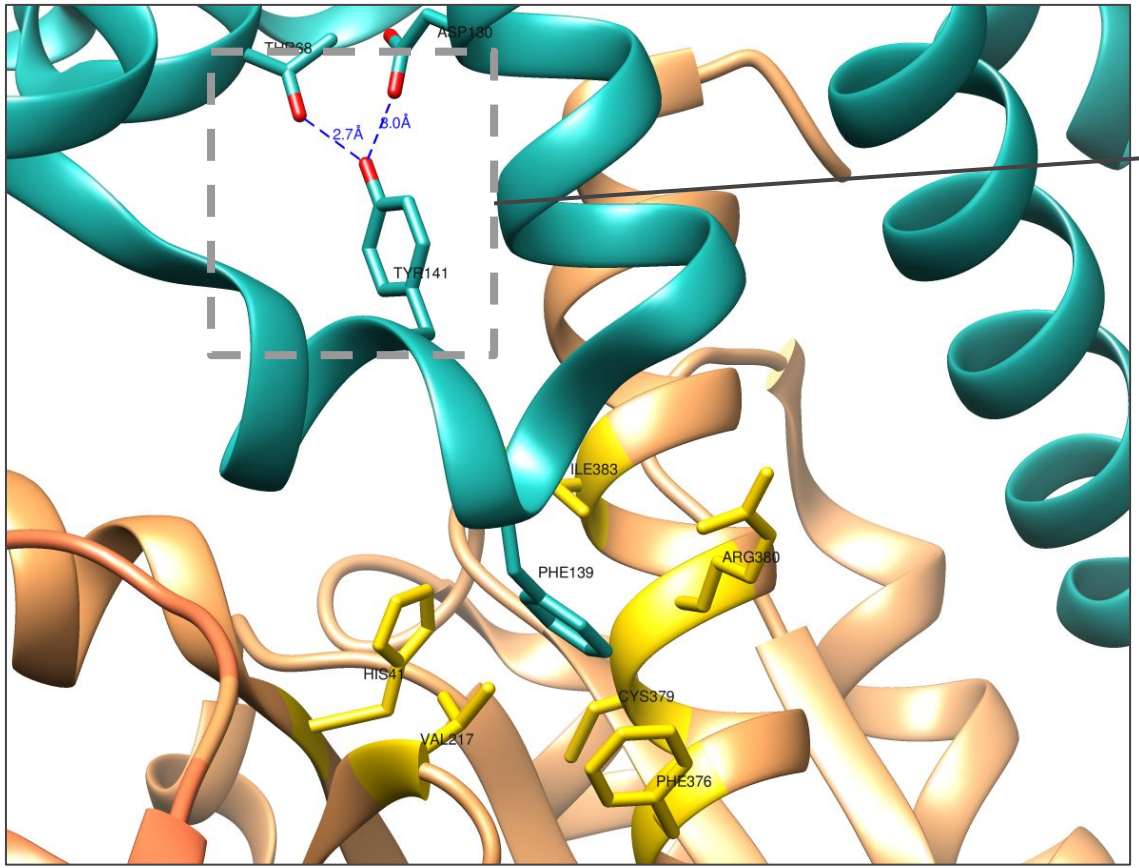
Hydrogen Bonds Contacts		
Atoms β2AR TM3 TM5	**Atoms Gsα α5**	**Distance (Å)**
THR 136 O	ARG 380 NH2	3.0
ILE 135 O	GLN 384 NE2	2.9
GLN 229 NE2	GLN 384 OE1	2.8
LYS 232 NZ	ASP 381 OD1	3.1

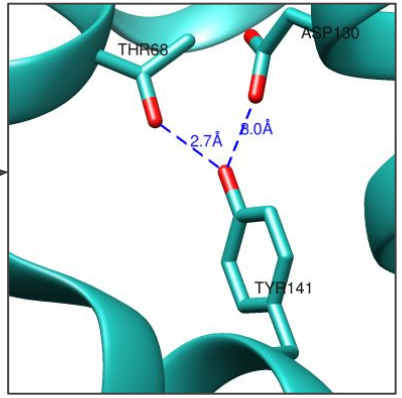
Salt Bridge Contacts		
Atoms β2AR TM5	**Atoms Gsα α5**	**Distance (Å)**
LYS 232 NZ	ASP 381 OD1	3.1

β2A Receptor

α Ras domain

Protein G interactions





Hydrophobic interactions		
Atoms β2AR ICL2	**Atoms Gsα α5**	**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 (β1)	3.6
PHE 139 CB	VAL 217 CG2 (α4)	3.7

β2A Receptor α Ras domain

Protein G activation

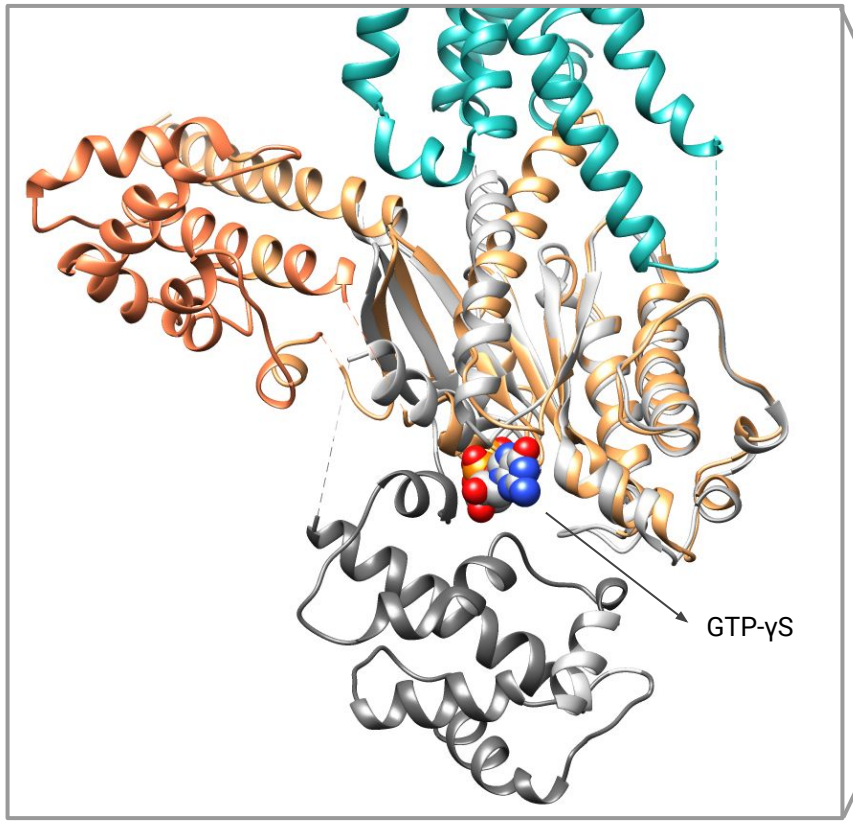
β2A Receptor

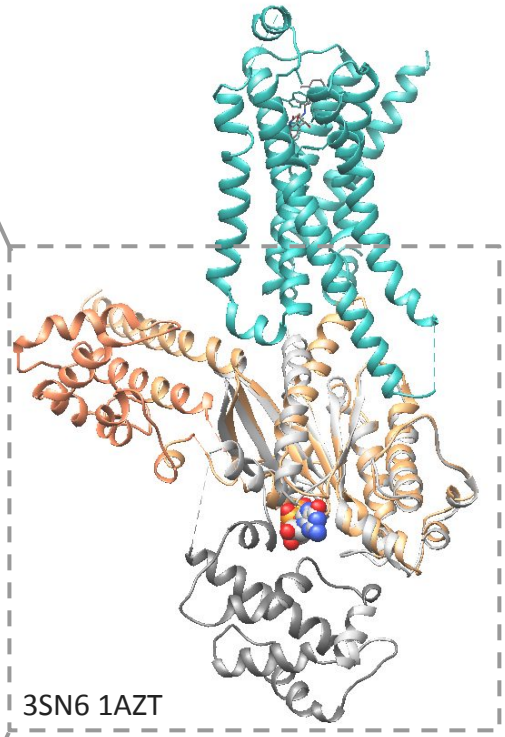
α Ras domain GDP-bound

α AH domain GDP-bound

α Ras domain GTP-bound

α AH domain GTP-bound





Protein G activation

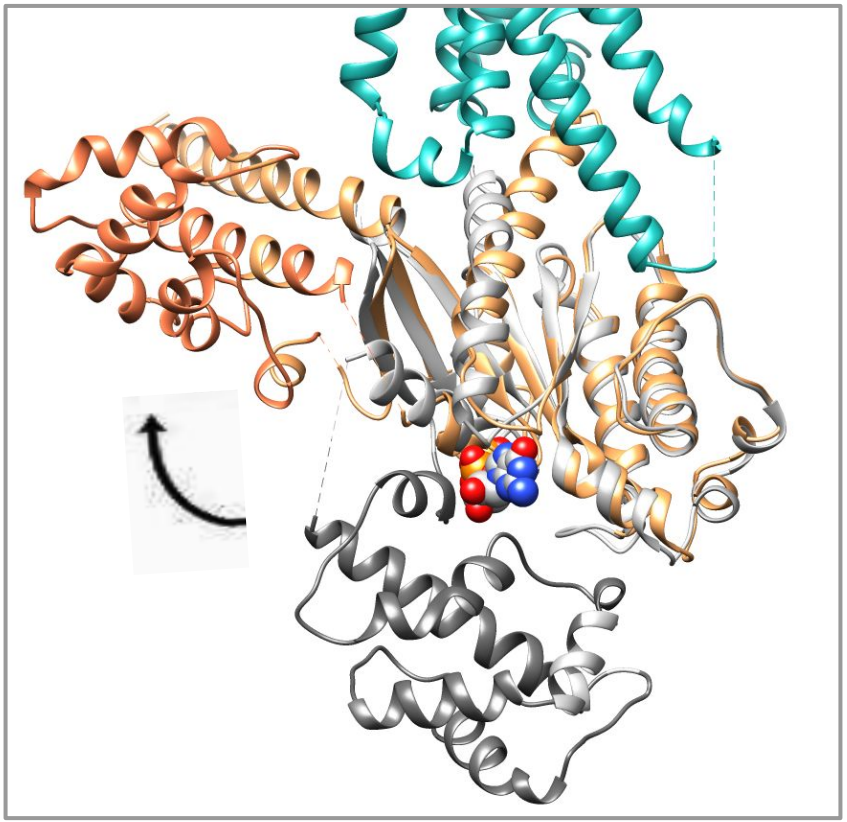
β2A Receptor

α Ras domain GDP-bound

α AH domain GDP-bound

α Ras domain GTP-bound

α AH domain GTP-bound



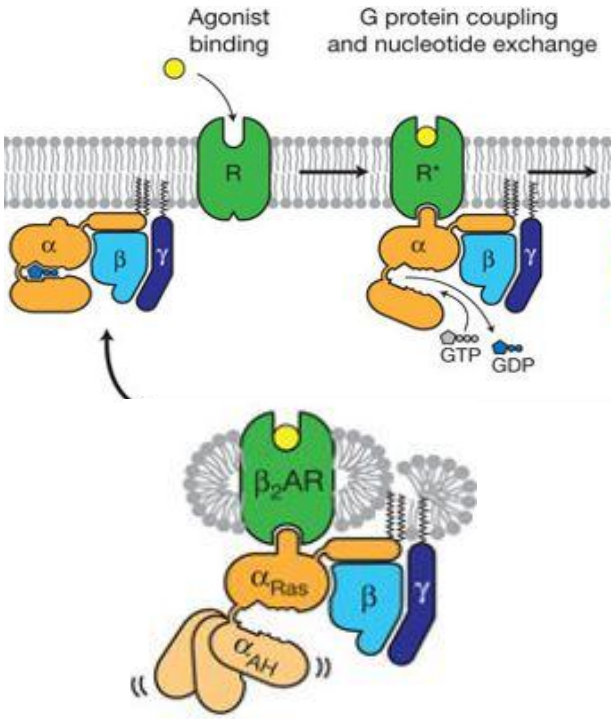


Fig. 4: Rasmussen F. et. al, 2011.

Active-Inactive States

Inactive state

Little changes, cytoplasmic side
Slight change binding site

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

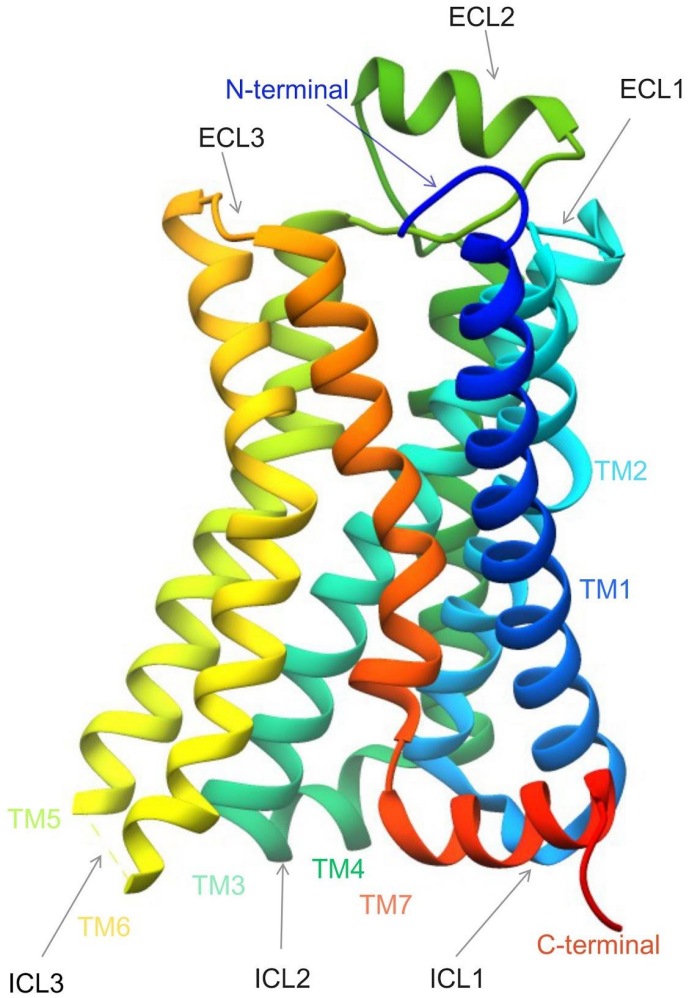
Outwards movement of **TM6** and **TM5**

Distorsion of helix in **TM7**

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

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

Helps holding **TM6**

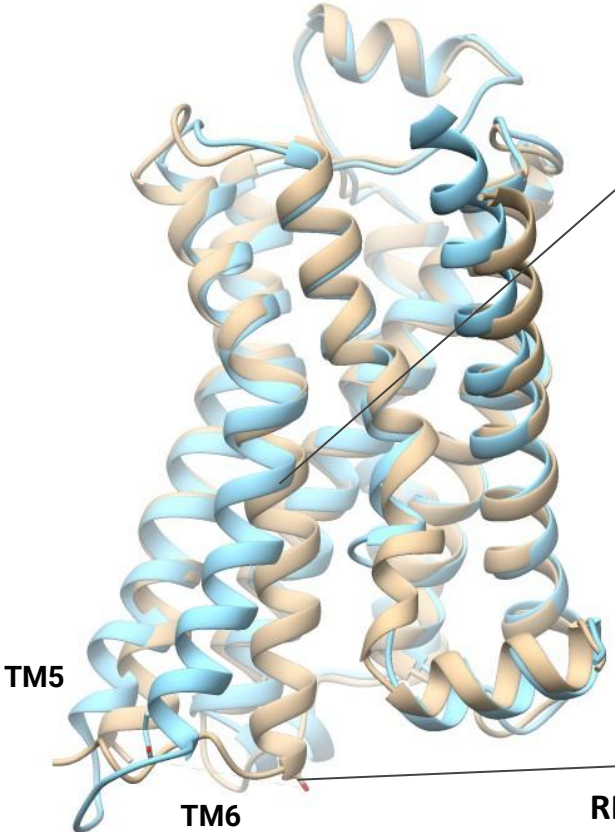


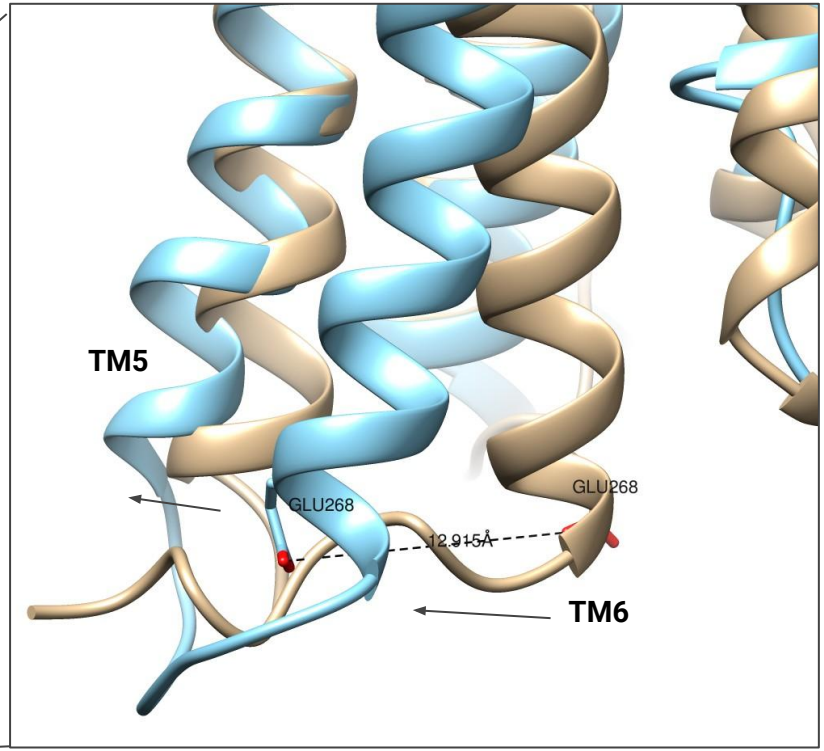
Active-Inactive States

TM5, TM6 (≃14Å) outwards movement

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

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





RMSD: 1.52

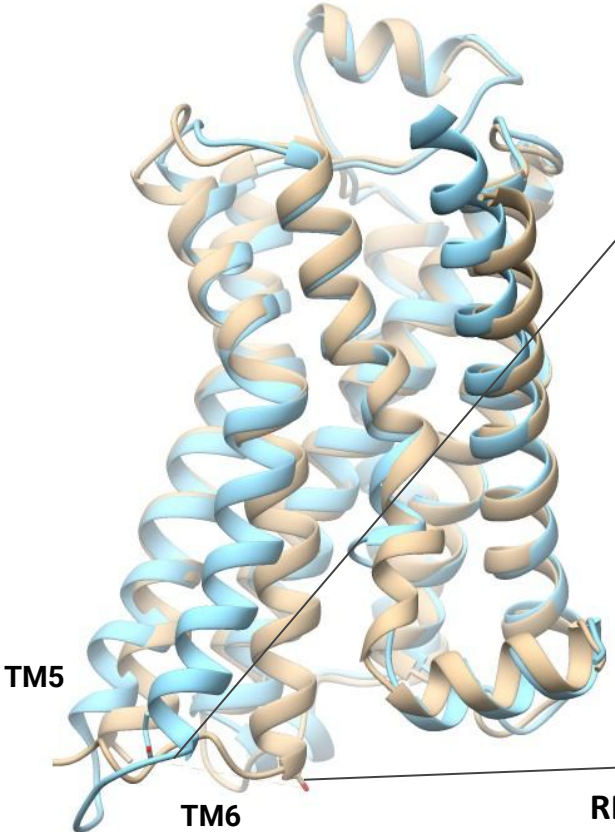
Side view

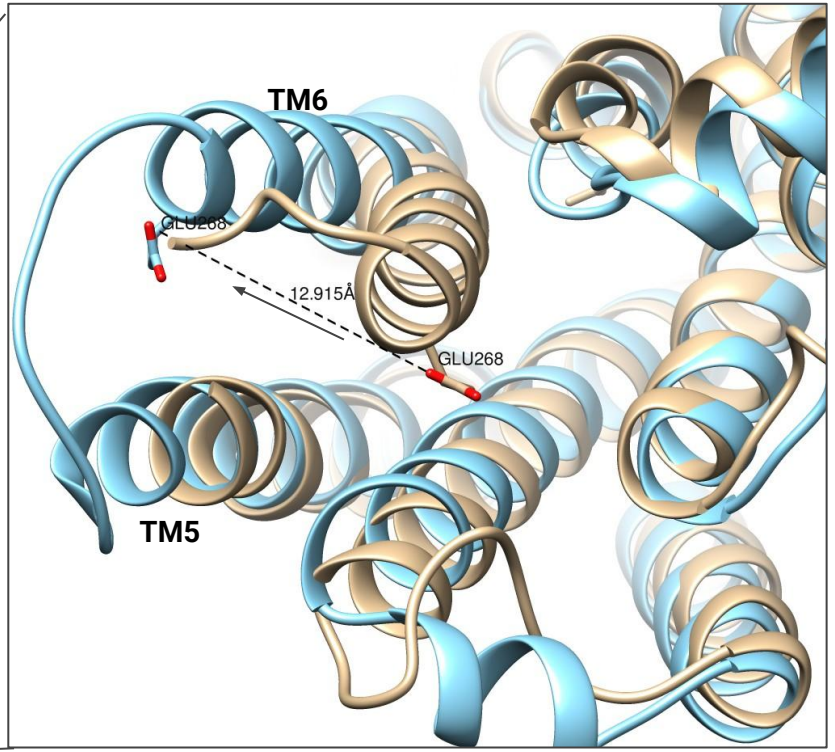
Active-Inactive States

TM5, TM6 (≃14Å) outwards movement

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

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



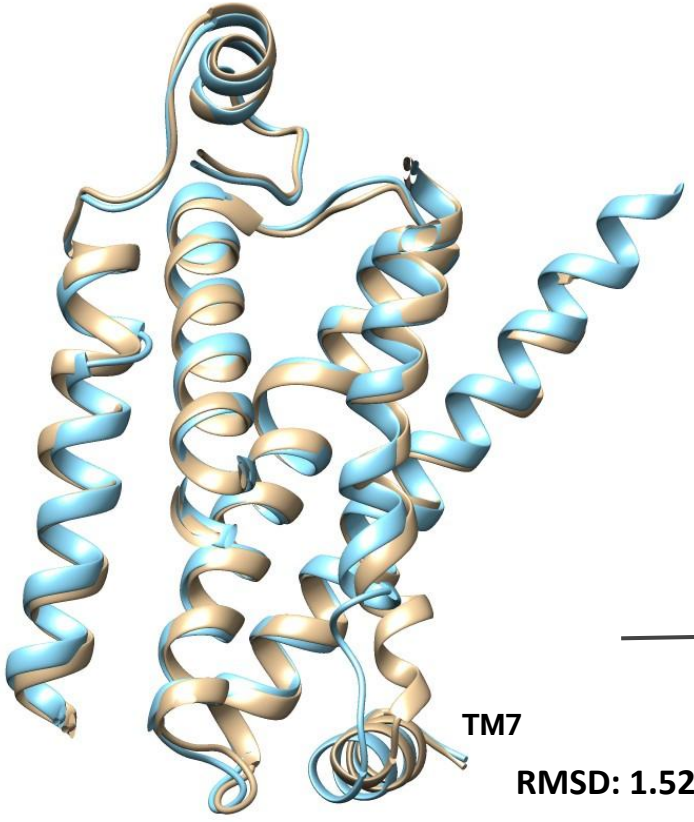


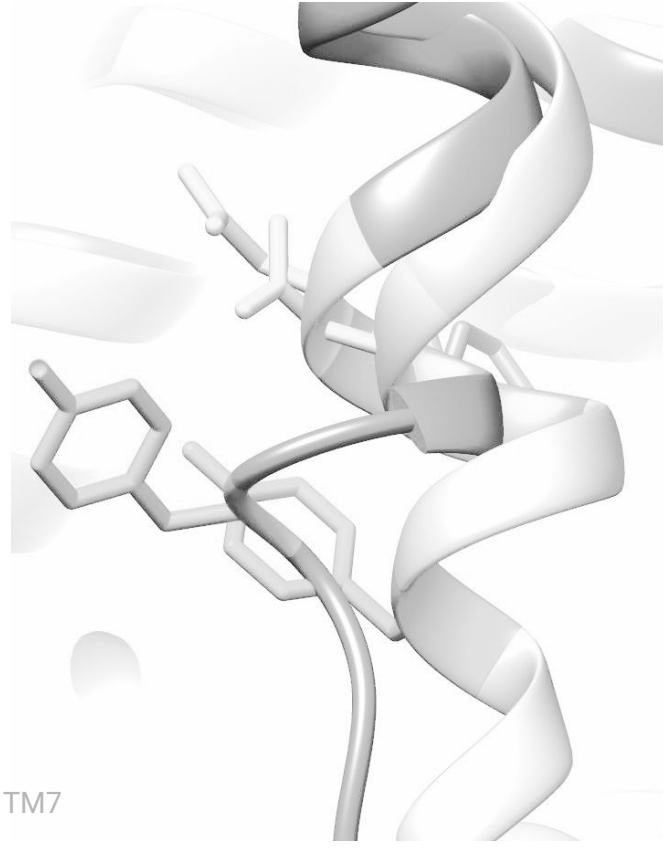
Cytoplasmic view

RMSD: 1.52

Active-Inactive States

TM7 distorted helical configuration → **NPxxY conserved motif in TM7, Y326 disposition changes**





Active-Inactive States

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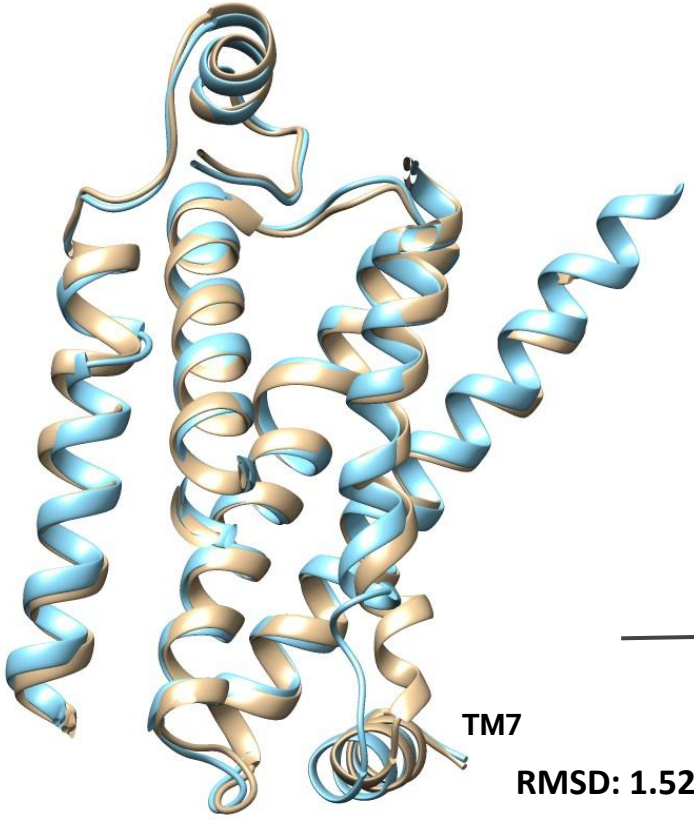
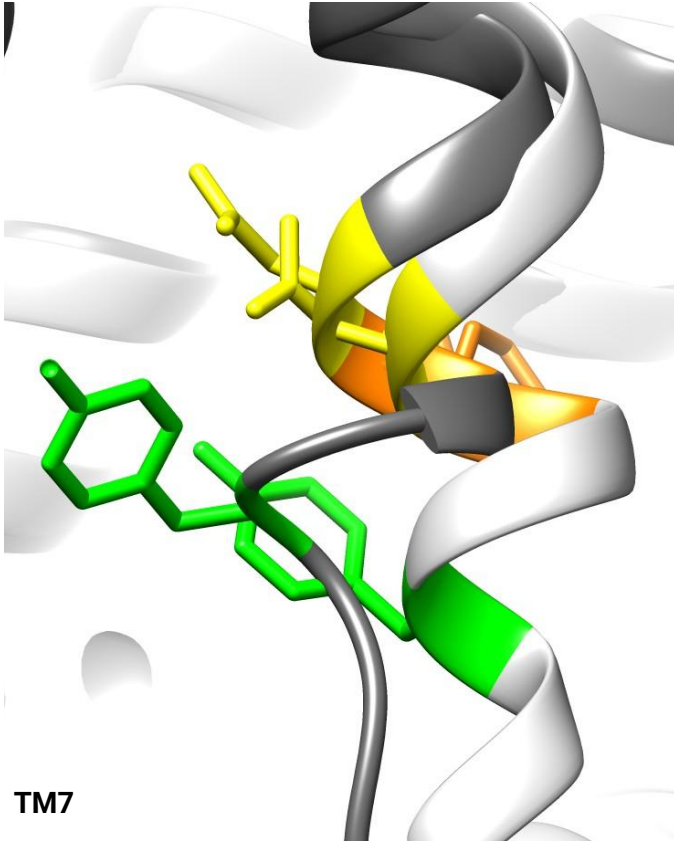
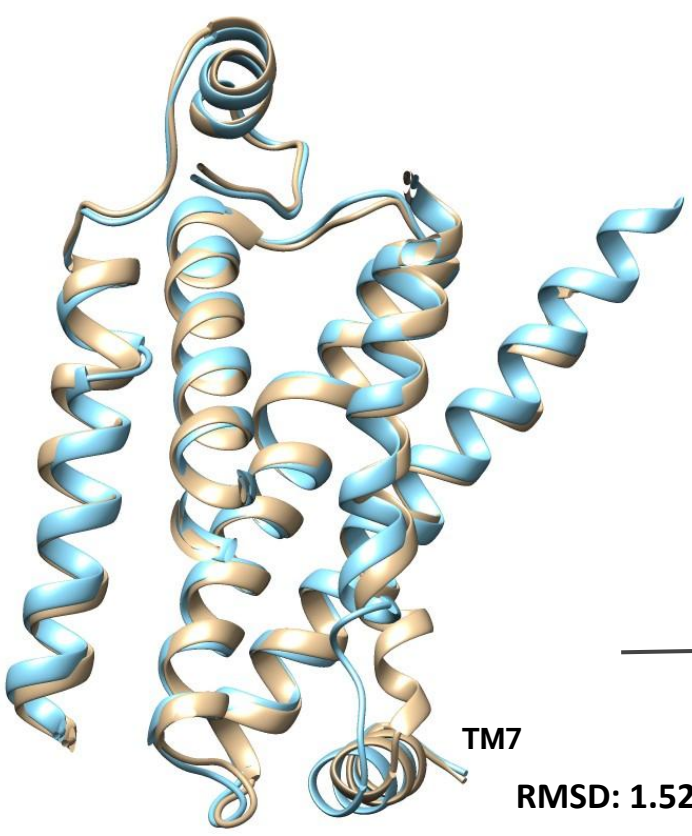
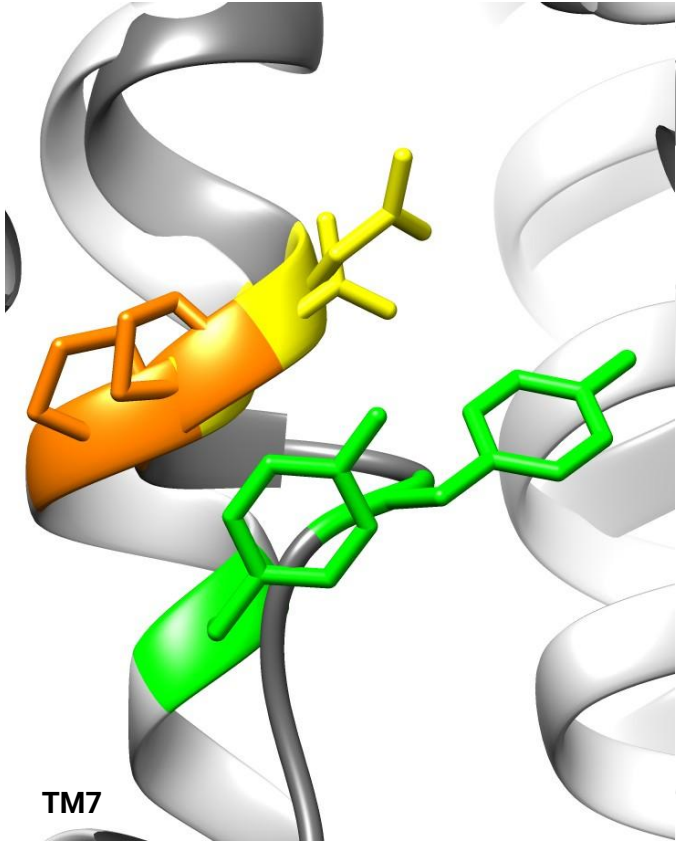




Active-Inactive States

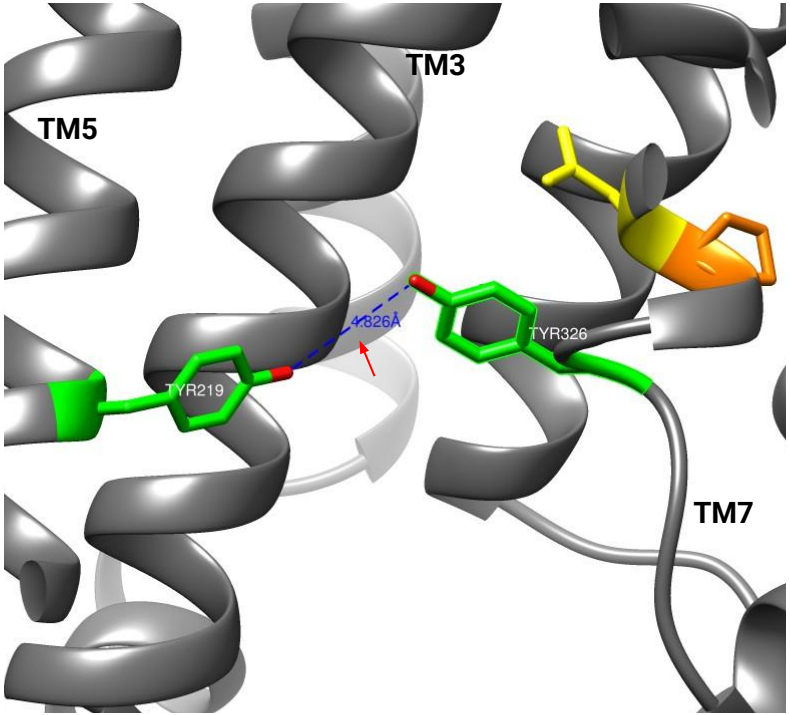
TM7 distorted helical configuration → **NPxxY conserved motif in TM7, Y326 disposition changes**



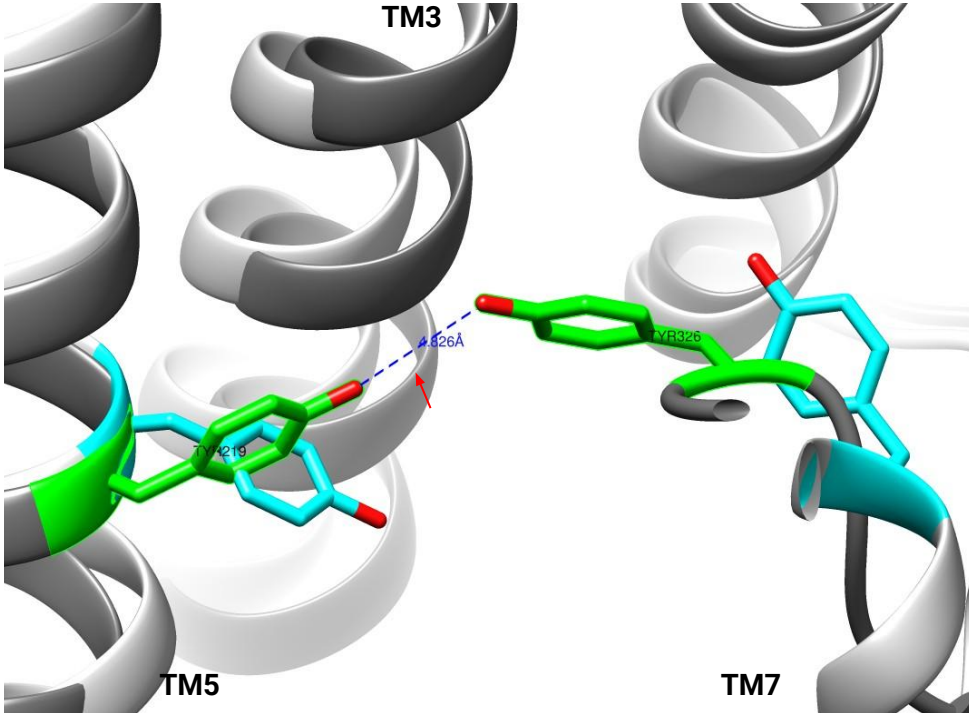


Active-Inactive States

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



RMSD: 1.52

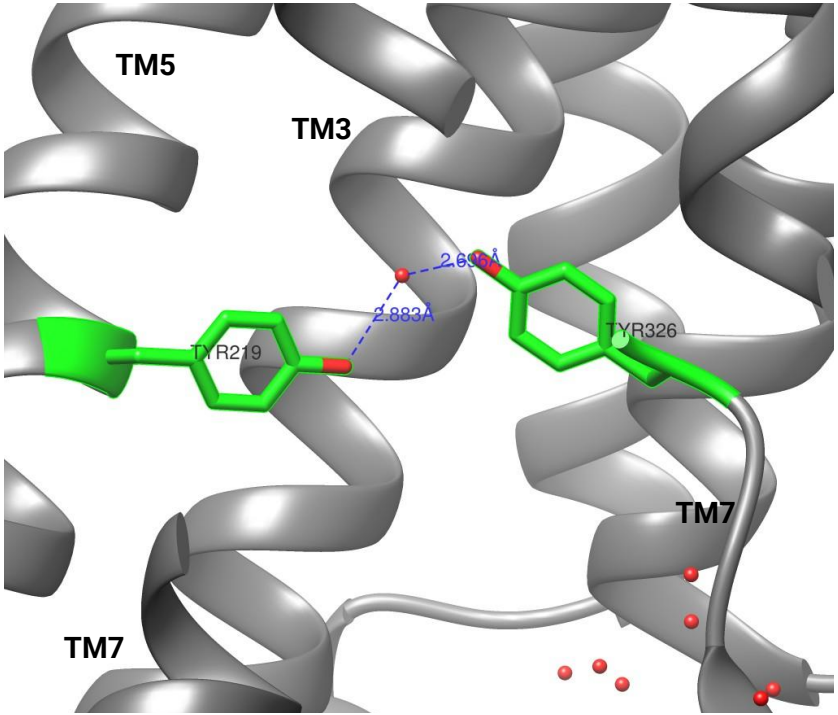


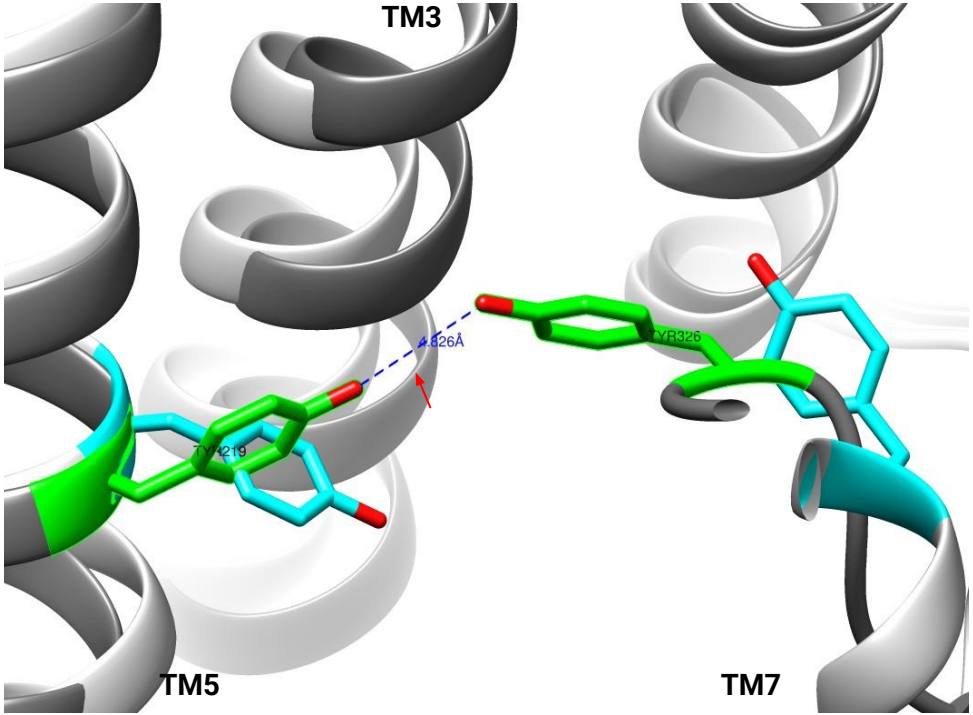
TYR219 & 326 active state

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



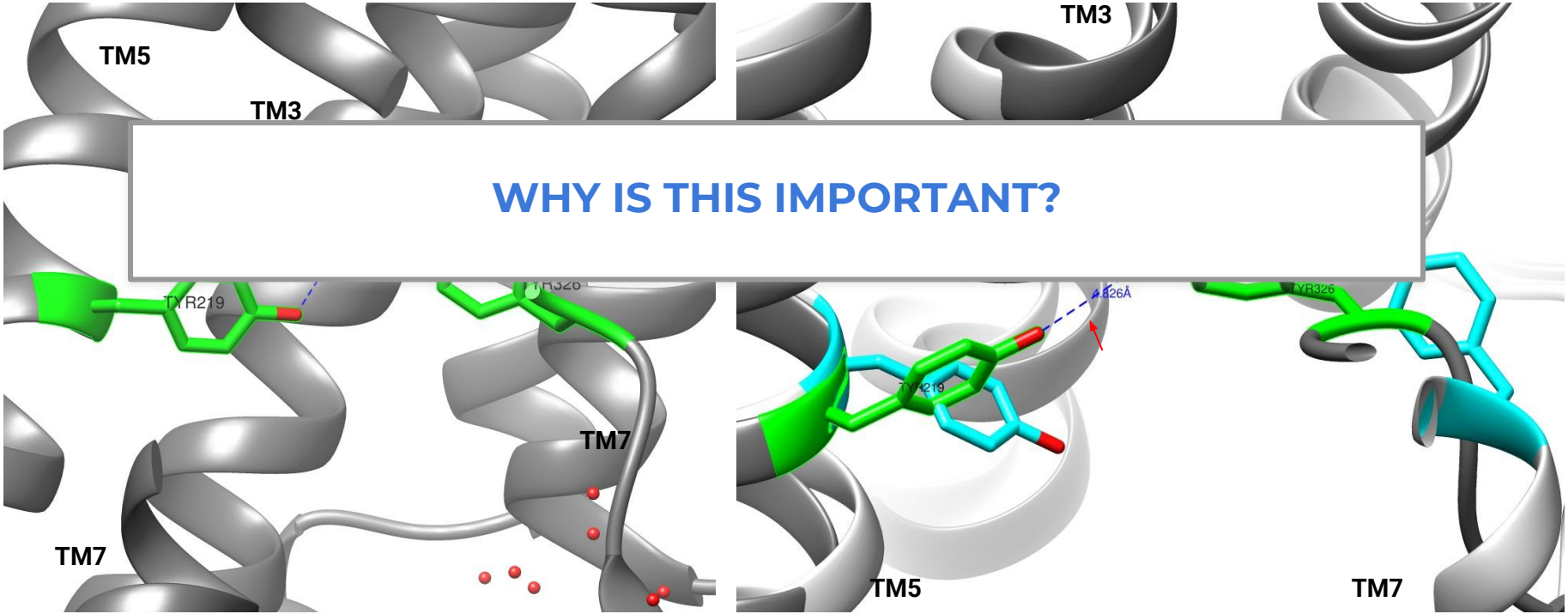


PDB: 4LDE + nanobody, BI167107, agonist

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



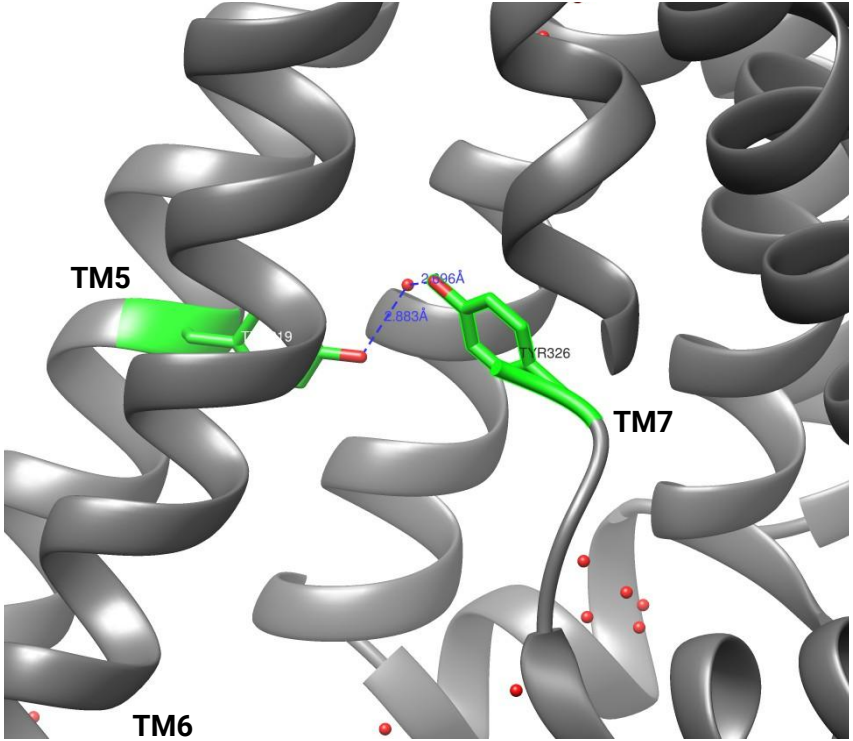
WHY IS THIS IMPORTANT?

PDB: 4LDE + nanobody, BI167107, agonist

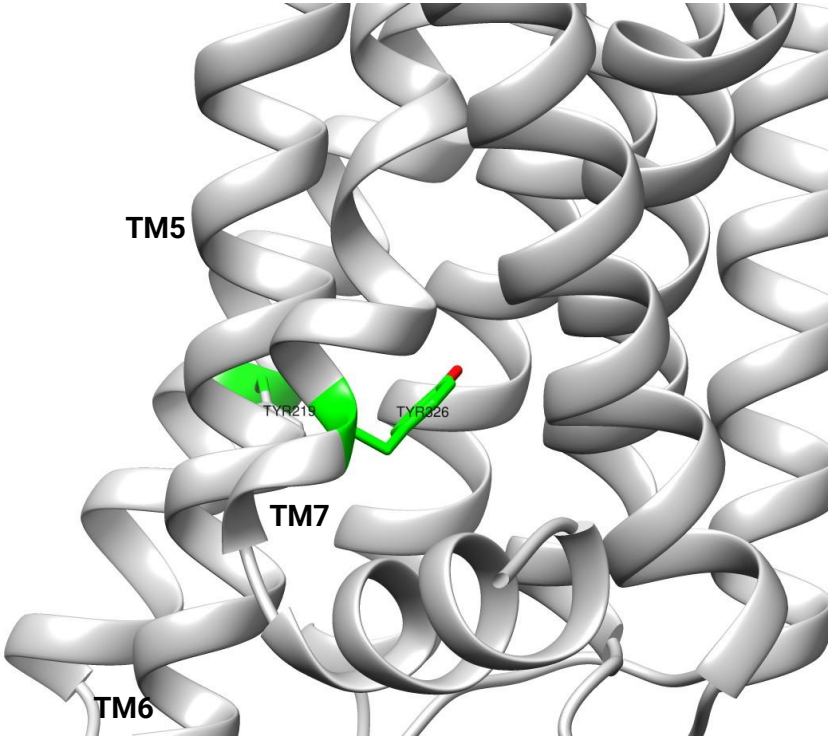
TYR219 & 326 active state TYR219 326 inactive state

Active-Inactive States

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



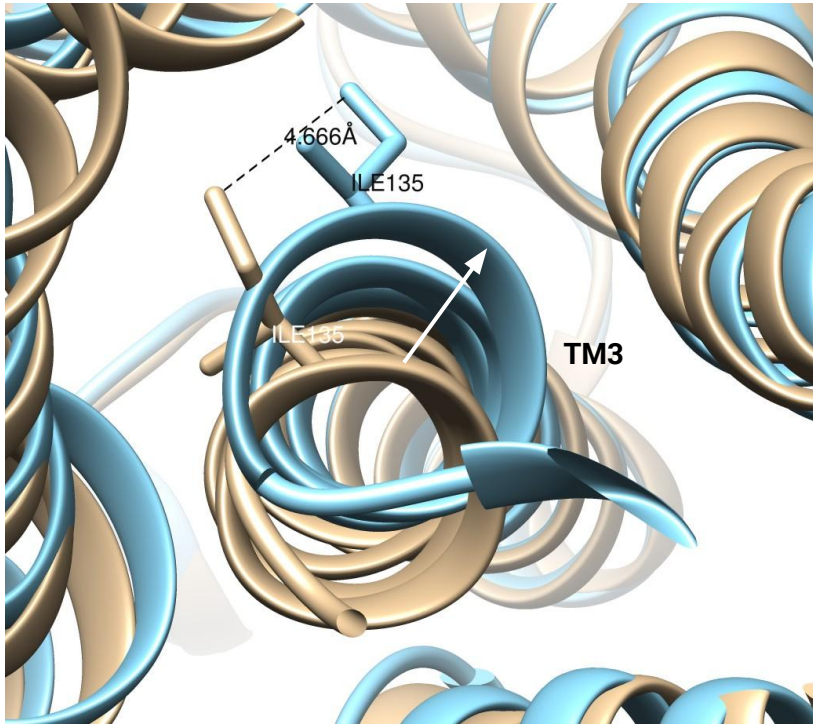
Active state. PDB: **4LDE** + nanobody, BI167107, agonist



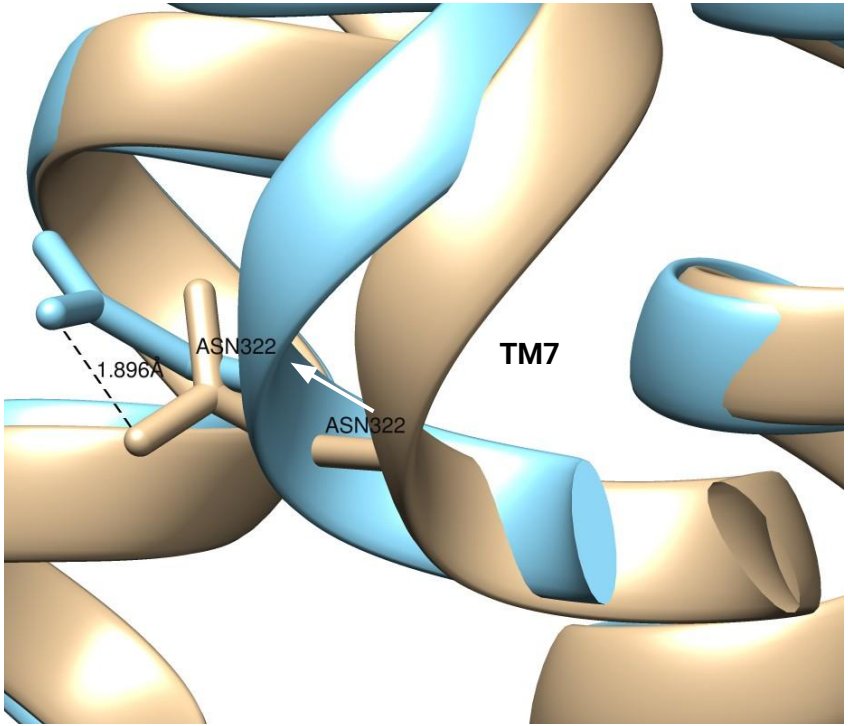
Inactive state

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

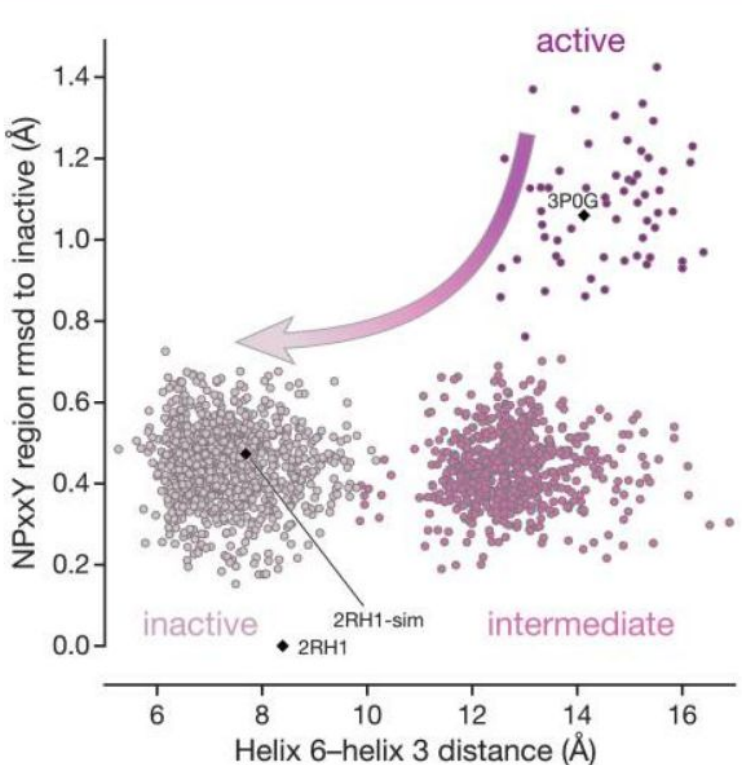


Fig. 5. Shaw DE, et al. 2011

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

Active-Inactive States

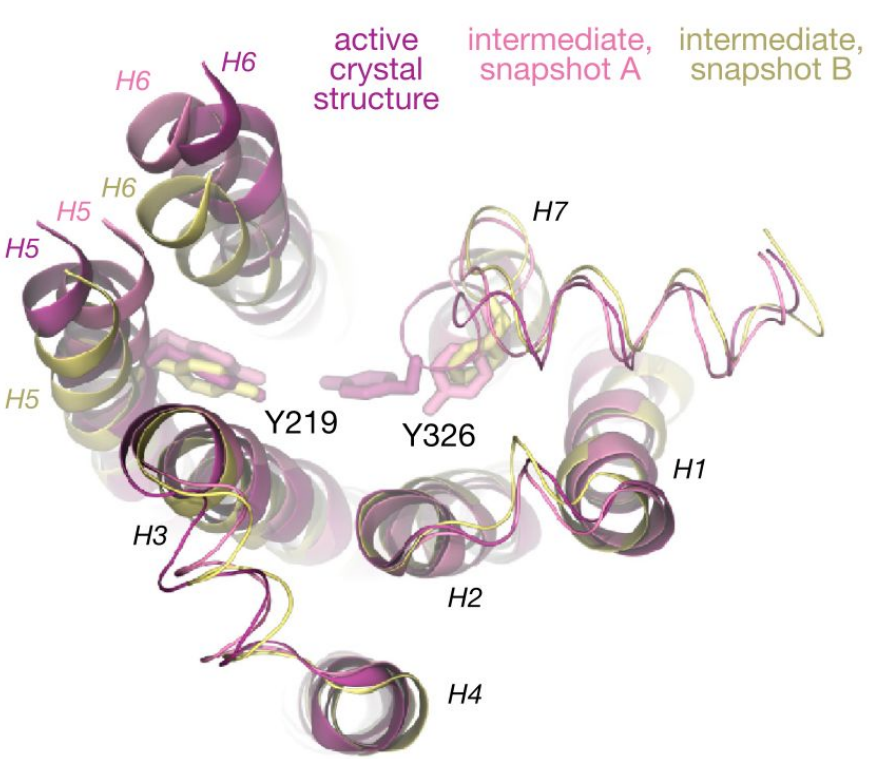


Fig. 6. Shaw DE, et al. 2011

TWO STATES MODEL AS AN APPROXIMATION

Active-inactive ⟶ simplistic

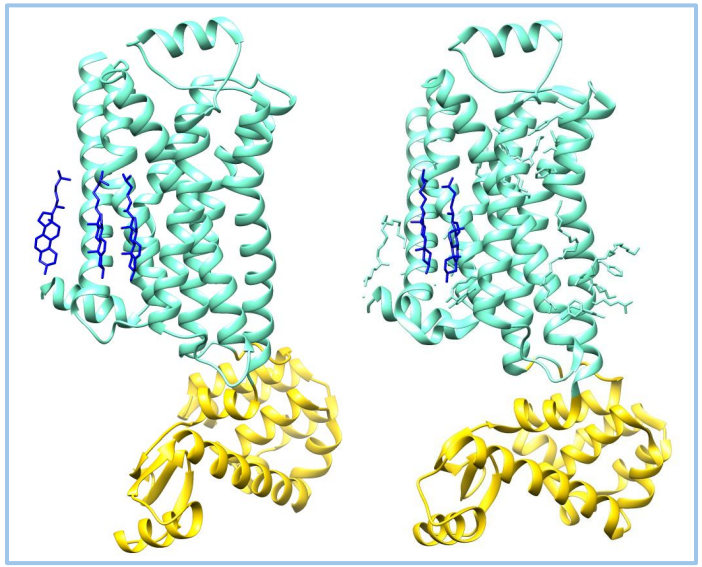
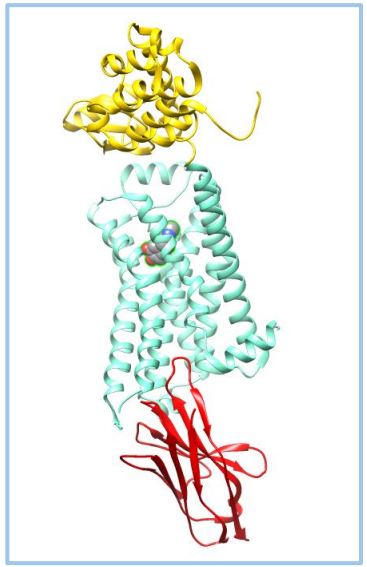
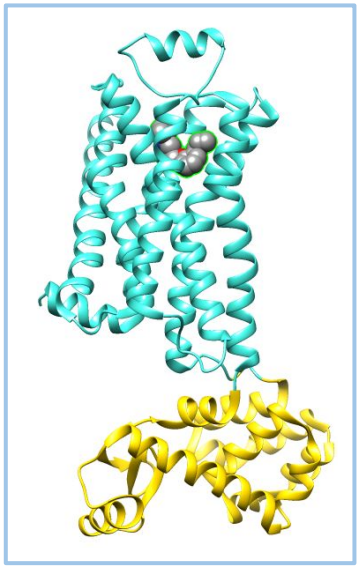
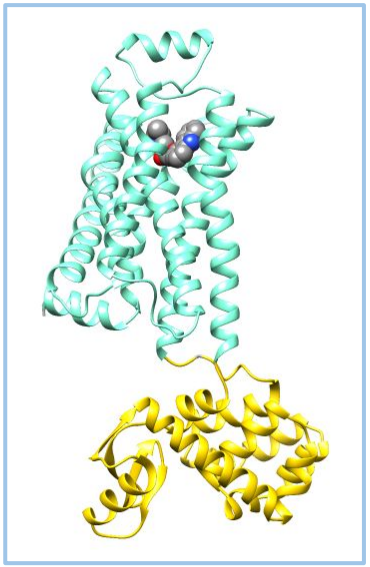
Crystal structures⟶the most energetically stable

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
  - **TM6** maintains separation from TM3
  - **TM5** and **TM6** ⟶ mobility
    - TM6 towards the TM5
    - TM5 towards the TM3

Ligands: Cholesterol 2RH1

Allosteric binding	Ligand binding site		
	Agonist	**Antagonist**	**i-Agonist**
Cholesterol	**Adrenaline**	**Alprenolol**	**Carazolol**

Ligands: Cholesterol 2RH1

Allosteric binding	Ligand binding site		
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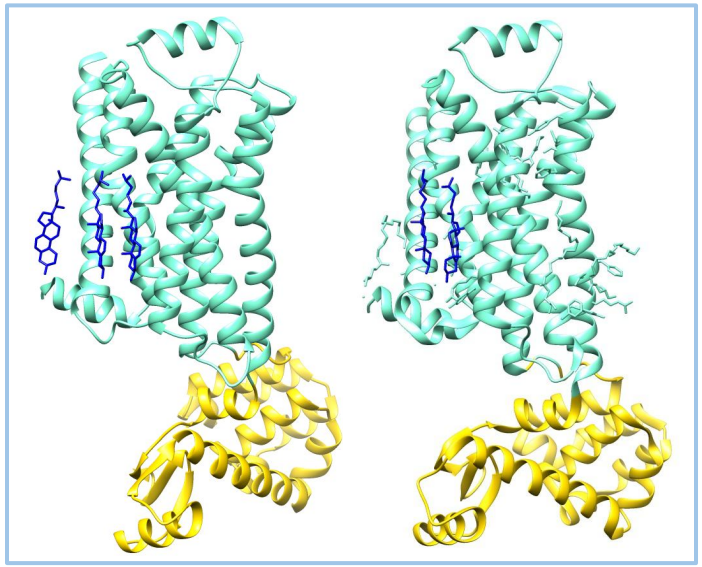
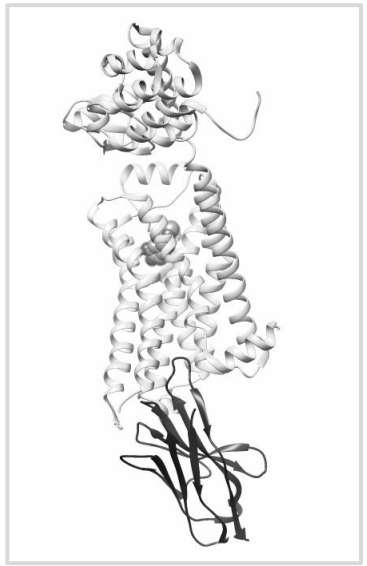
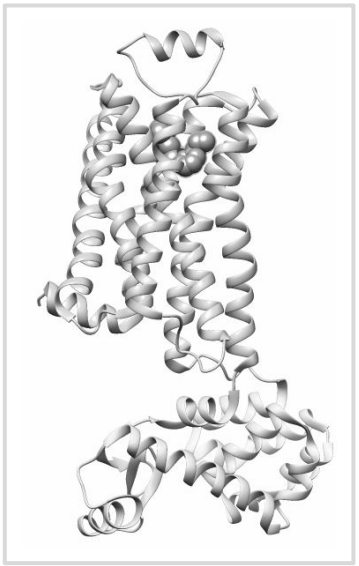
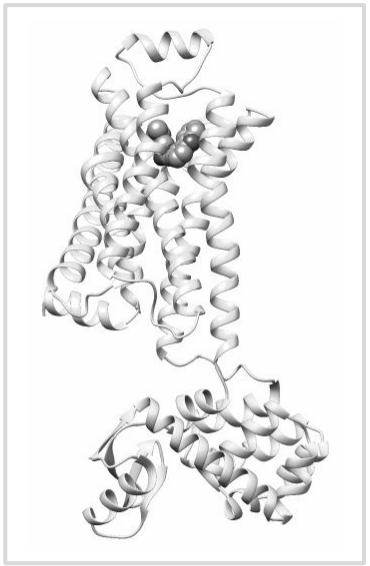


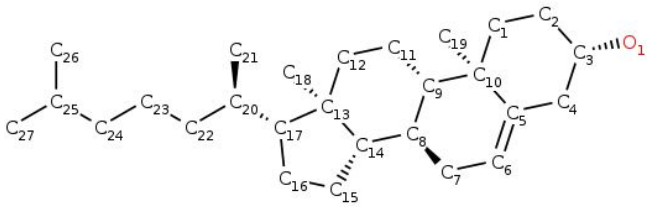




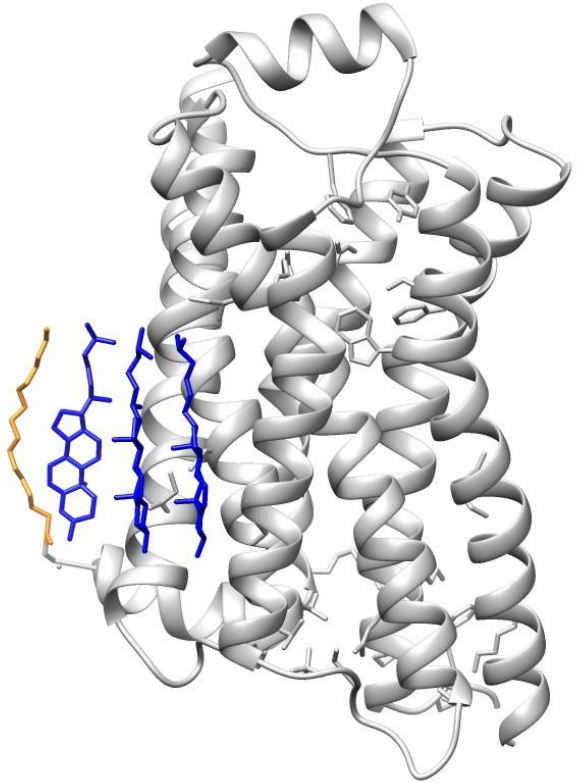


Cholesterol

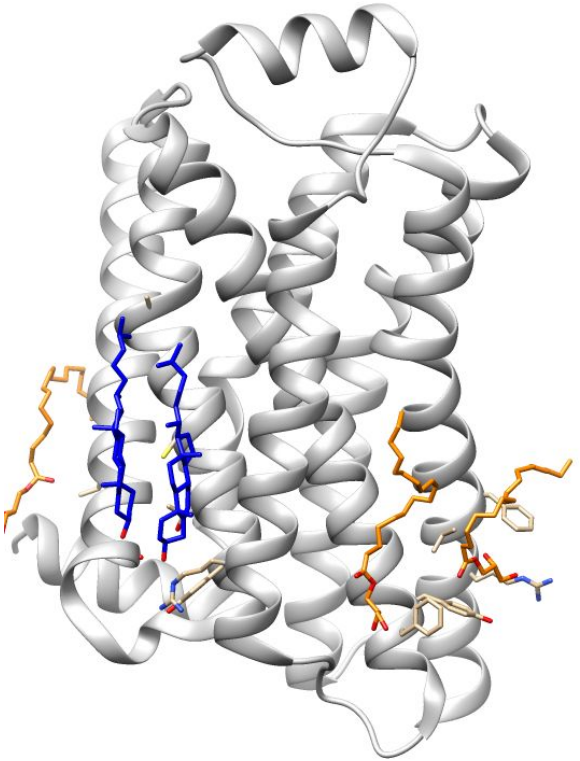
- **Cholesterol** → steroid structure



- **Parallel association** to the β2-AR
- Possible **allosteric regulator**



2rh1



3d4s

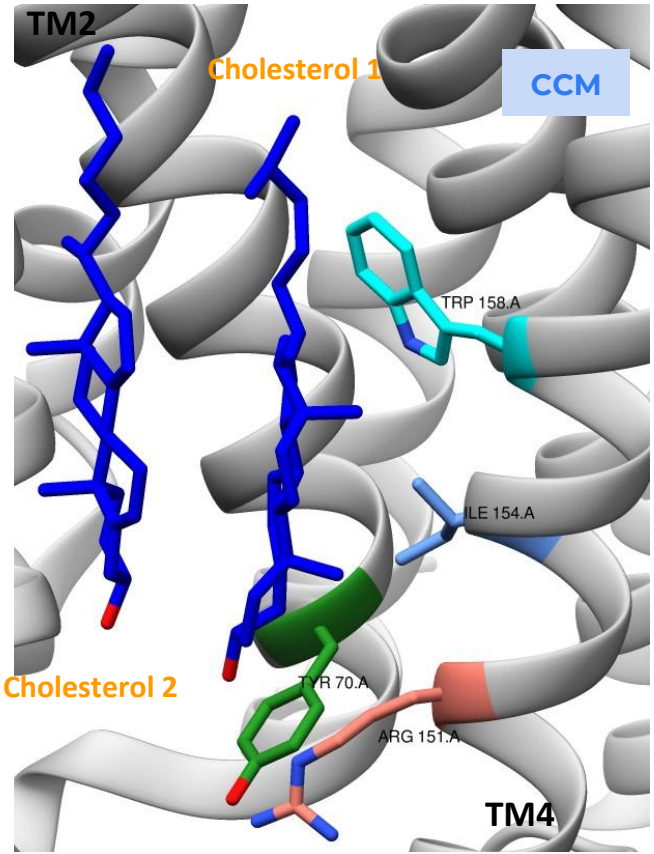
Cholesterol

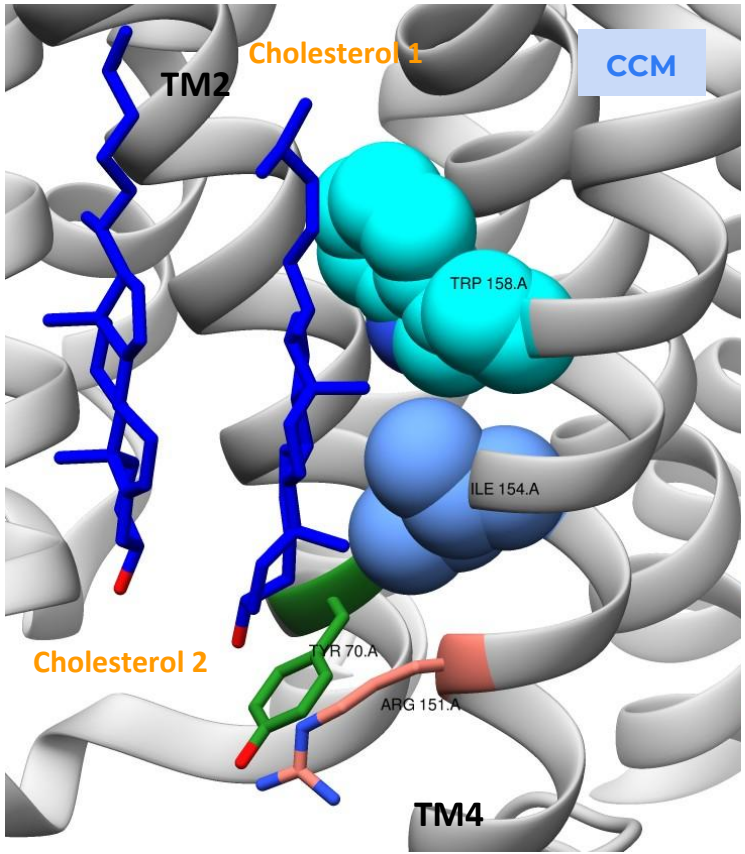
Cholesterol binding site

CCM in TM4 and TM2

Increases the packing interactions for both helix II and IV

3d4s



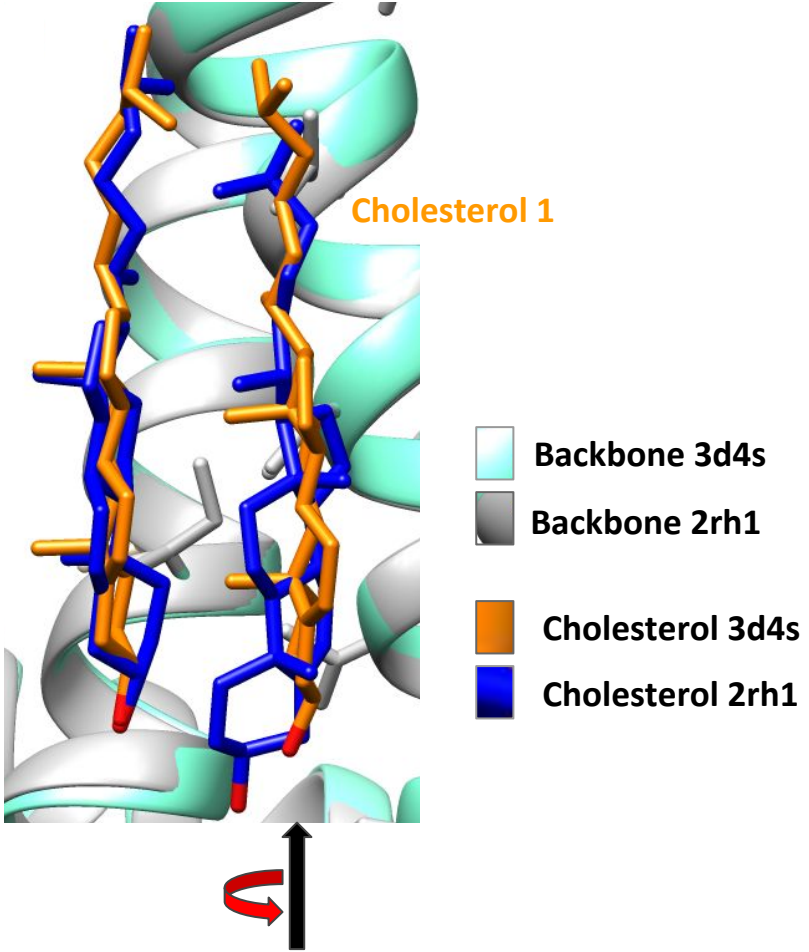


Cholesterol

Cholesterol-RC interaction

- High dynamics
- Additional conformations near the site

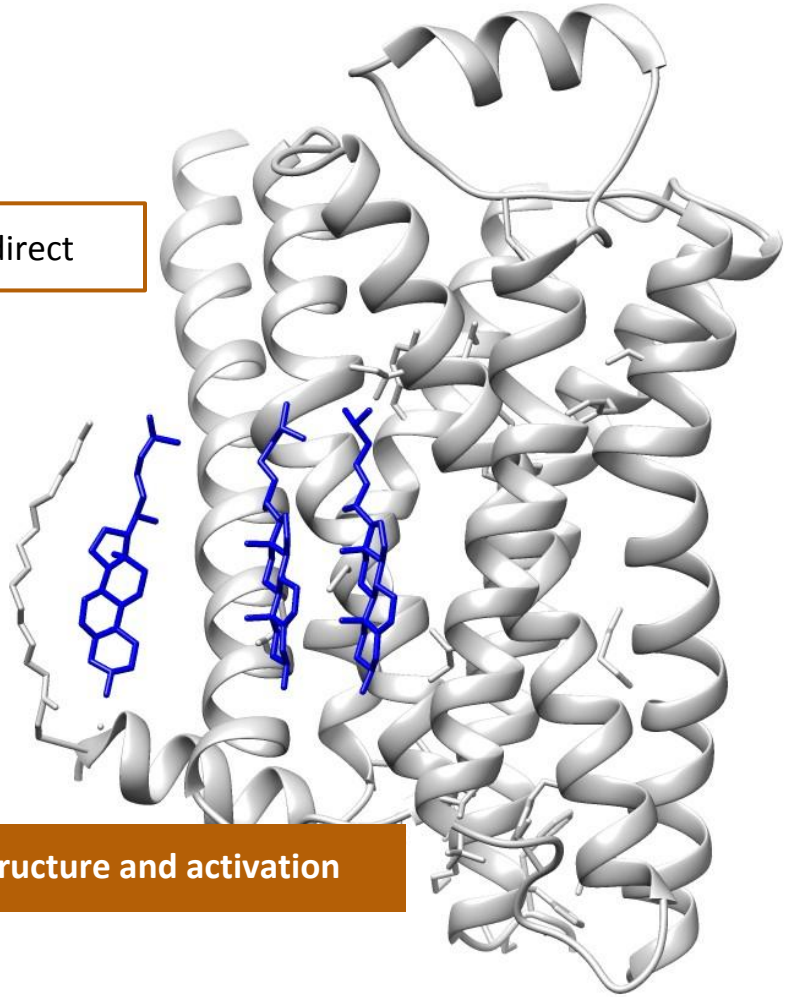
90º rotation and 1.9 Å



Cholesterol

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

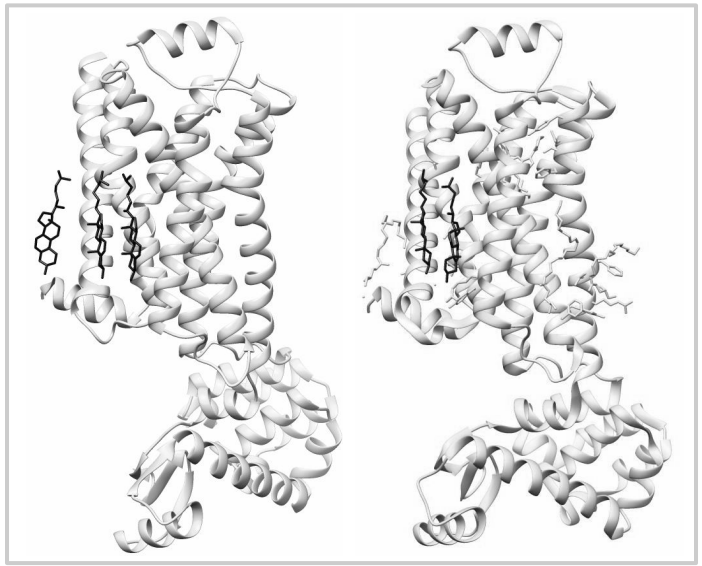
- Improves β2AR **stability**
- **Allosteric site** → restriction of TM6 movement
- Regulate **dimerization** → Cross-talk GPCRs and Cholesterol
- Regulate interactions **β2AR - G proteins**

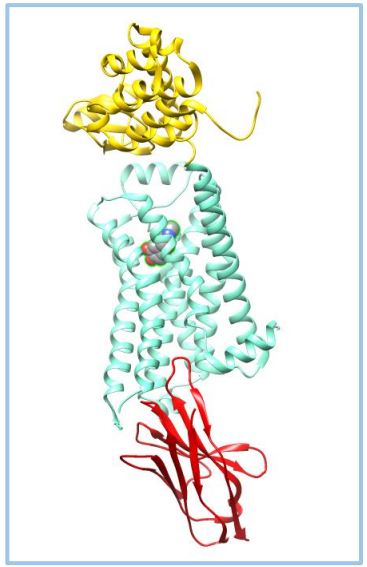


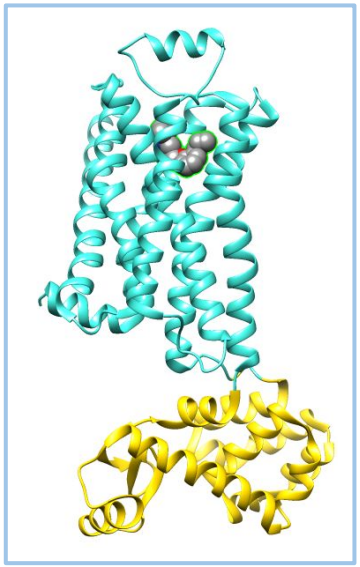
Lipids can be allosteric regulators of membrane protein structure and activation

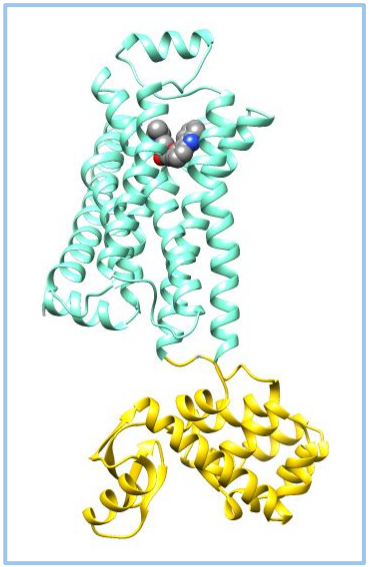
Ligands: Adrenaline 4LDO

Allosteric binding	Ligand binding site		
	Agonist	Antagonist	i-Agonist
Cholesterol	Adrenaline	Alprenolol	Carazolol









Binding Pocket

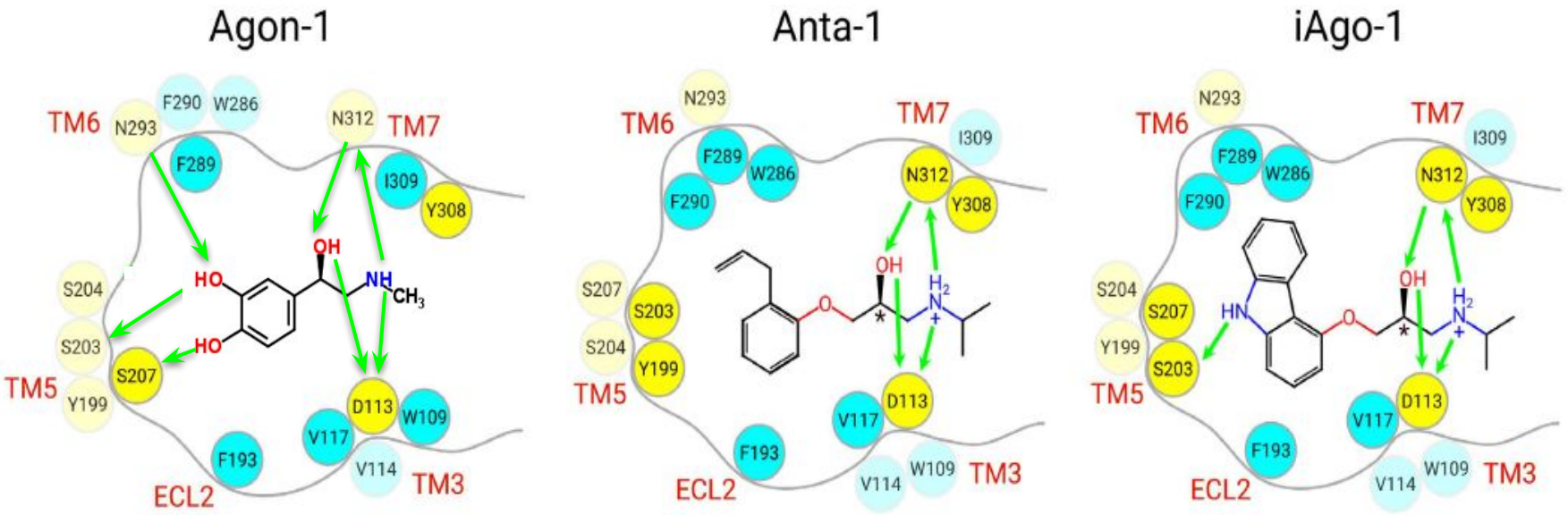
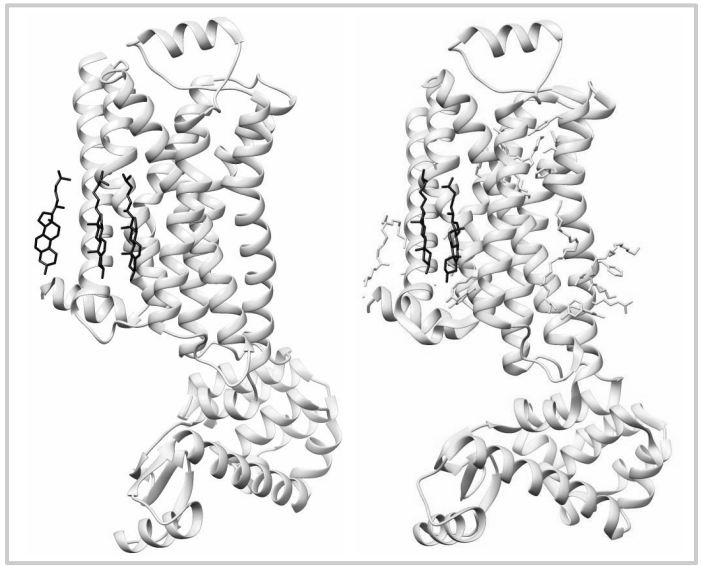
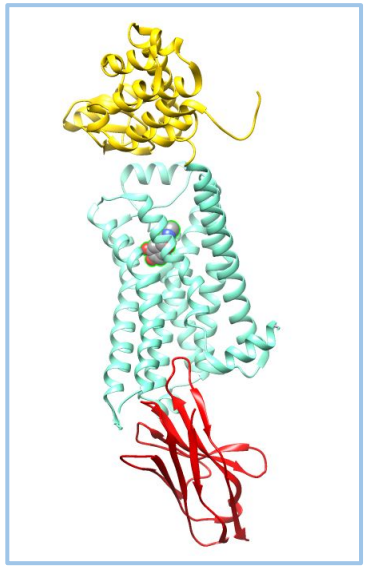


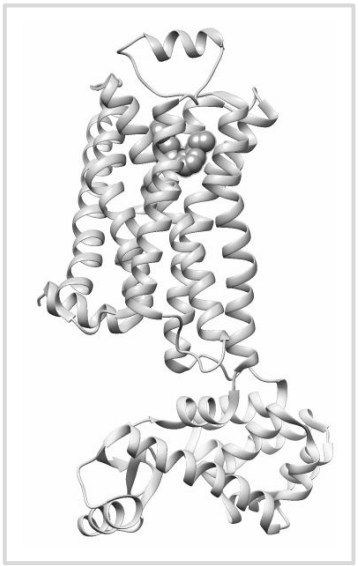
Fig 7. : Adapted from Chan HC et al., 2016

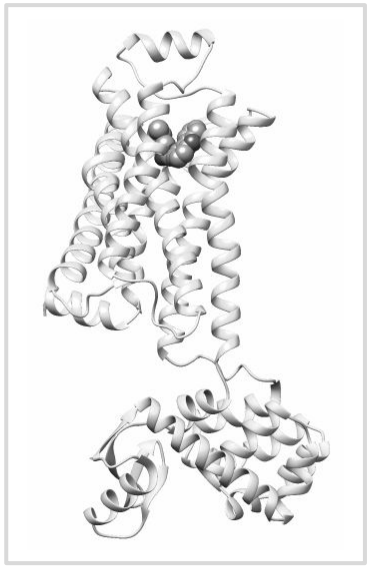
Ligands: Adrenaline 4LDO

Allosteric binding	Ligand binding site		
	Agonist	Antagonist	i-Agonist
Cholesterol	Adrenaline	Alprenolol	Carazolol

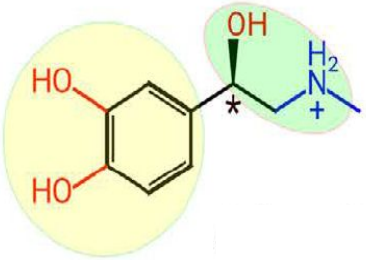


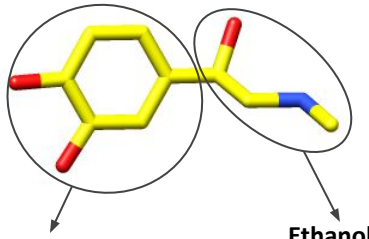


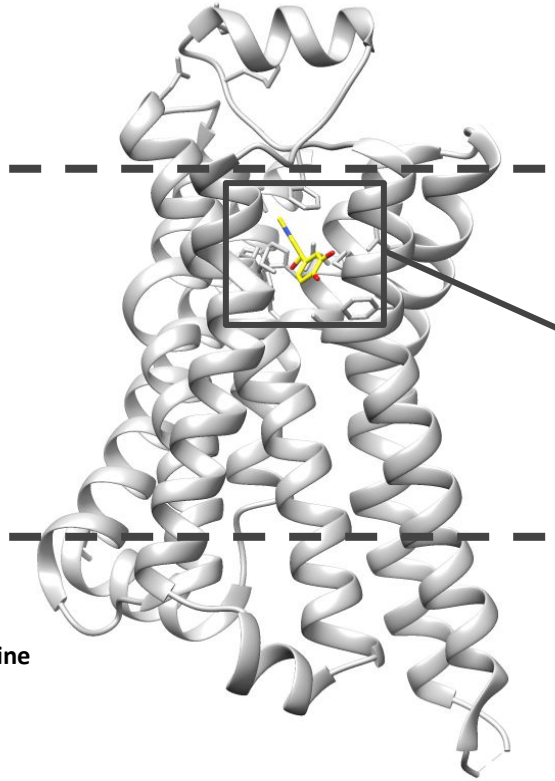


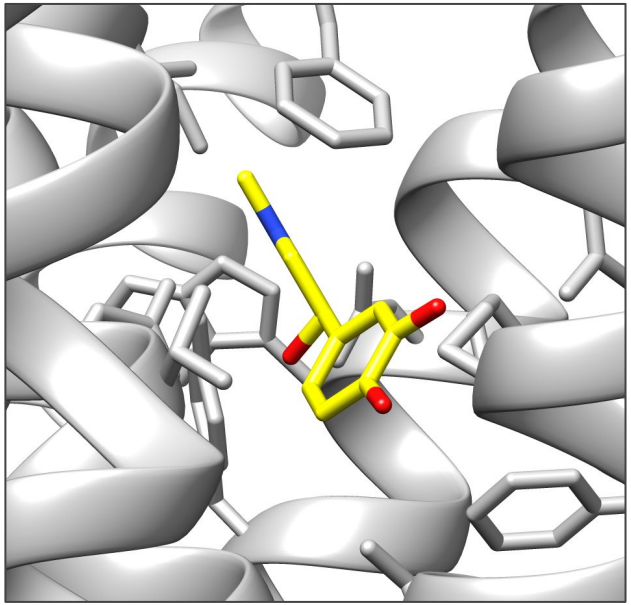


Agonist: Adrenaline



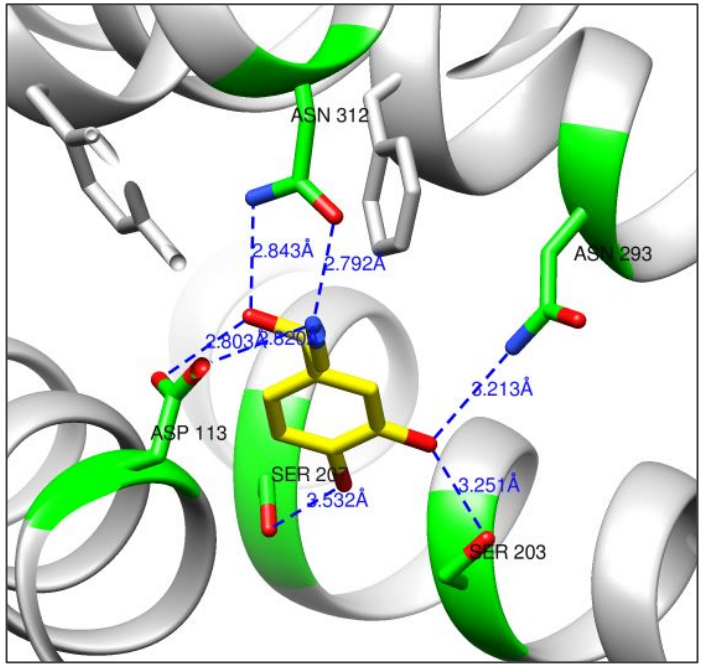


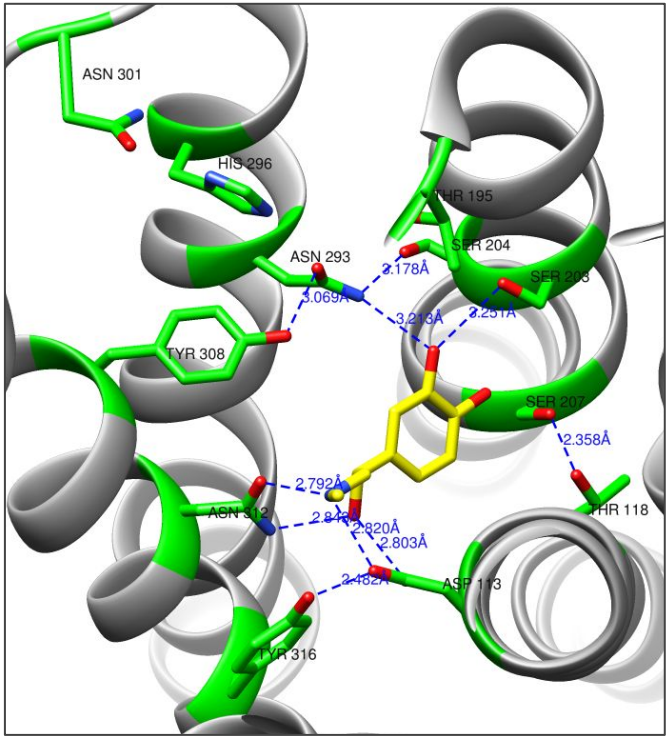




Agonist: Adrenaline

Hydrogen bonds and Salt Bridge contacts of the binding pocket

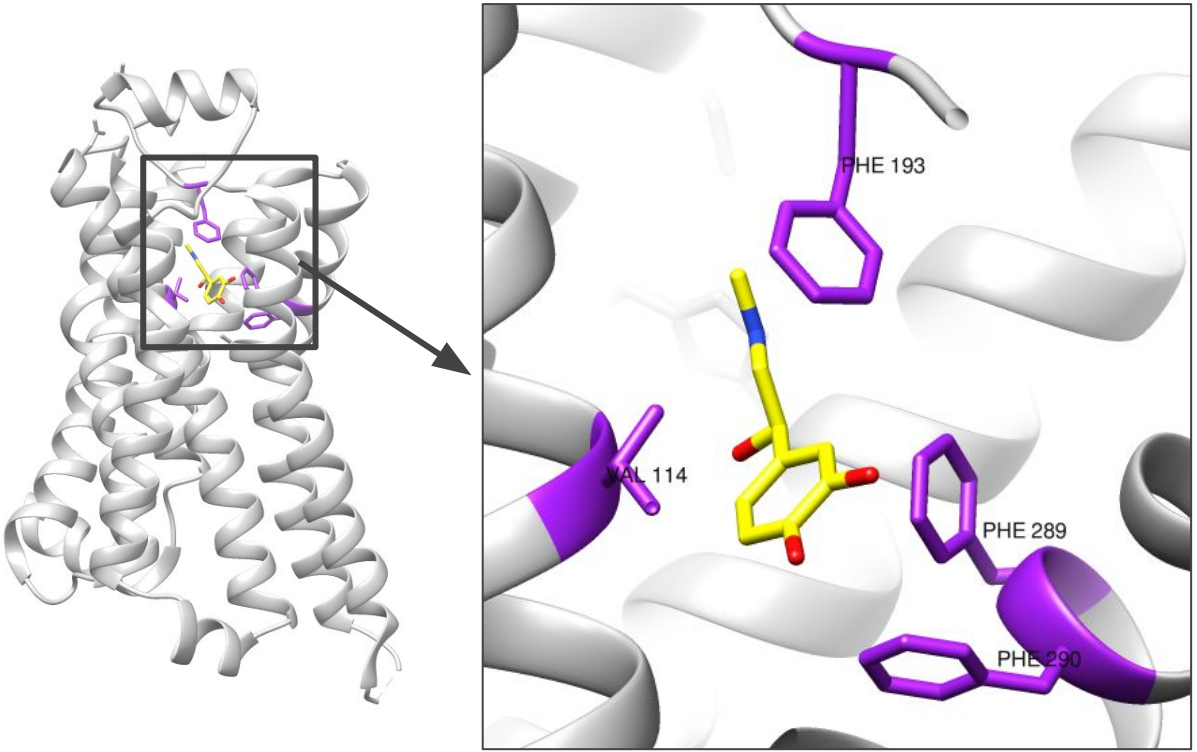




Hydrogen Bonds and Salt Bridge Contacts		
Atoms β2AR	**Atoms Adrenaline**	**Distance (Å)**
ASP 113 OD1	O3	2.8
ASP 113 OD2	N1	2.8
ASN 312 ND2	O3	2.8
ASN 312 OD1	N1	3.8
SER 207 OG	O2	3.5
SER 203 OG	O1	3.3
ASN 293 ND2	O1	3.2

Agonist: Adrenaline

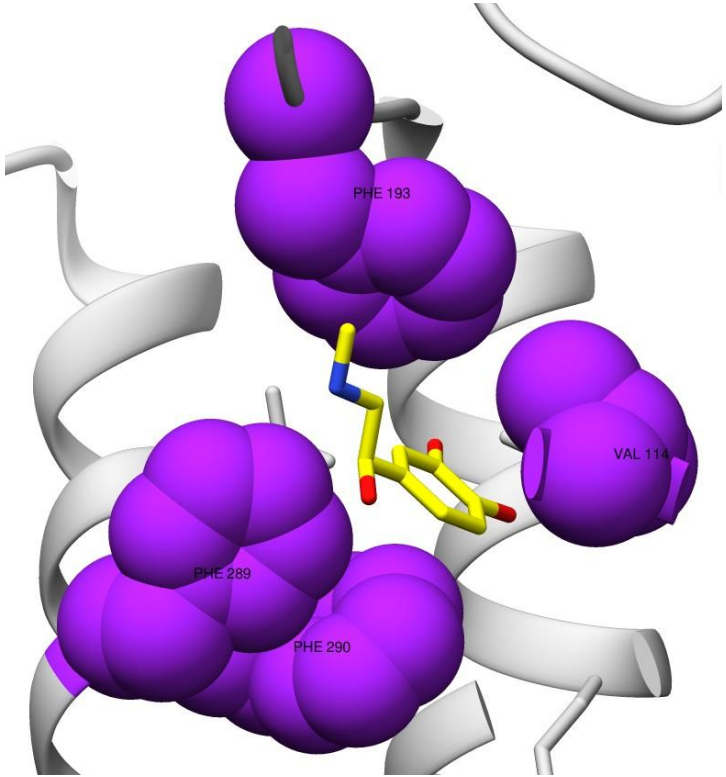
Hydrophobic interactions of the binding pocket



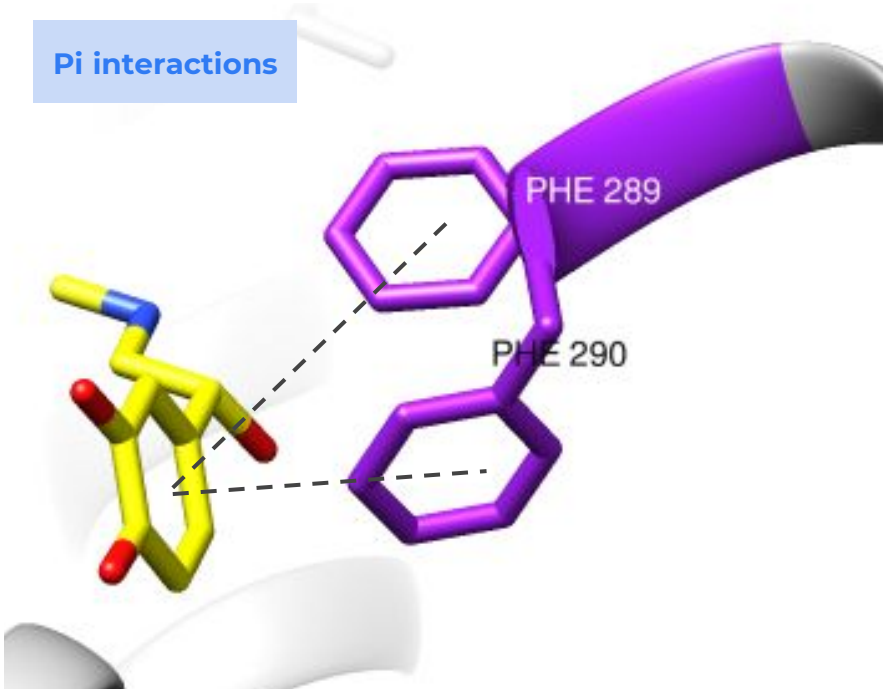
Hydrophobic and Aromatic interactions		
Atoms β2AR	**Atoms Adrenaline**	**Distance (Å)**
PHE 193 CE2	C2	3.9
VAL 114 CG2	C6	4.4
VAL 114 CG1	C5	4.0
PHE 289 CE2	C2	3.7
PHE 290 CE2	C4	4.0
PHE 289 CE2	C1	3.8
PHE 290 CZ	C4	4.0

Agonist: Adrenaline

Aromatic interactions of the binding pocket

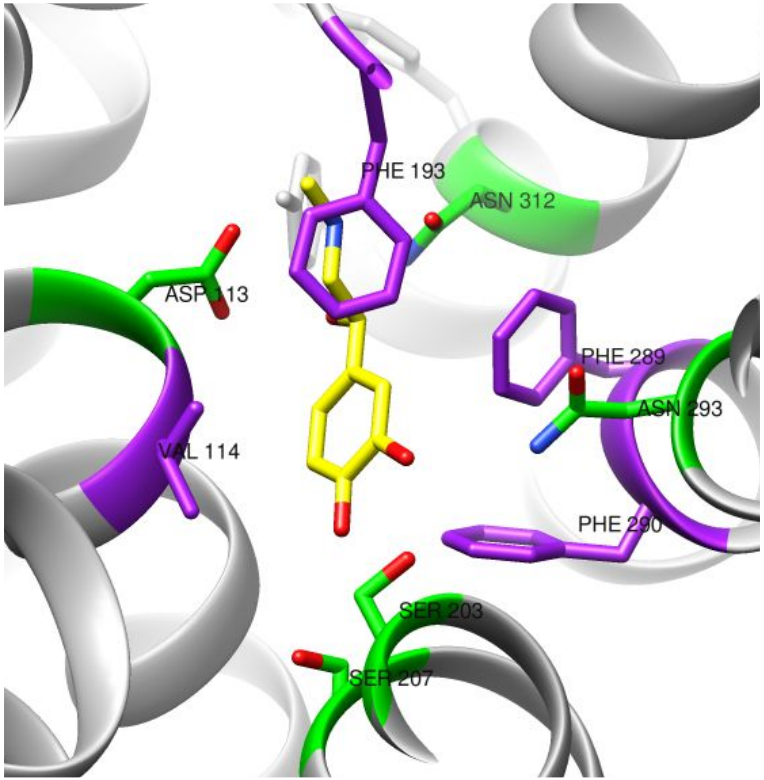


Pi interactions



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

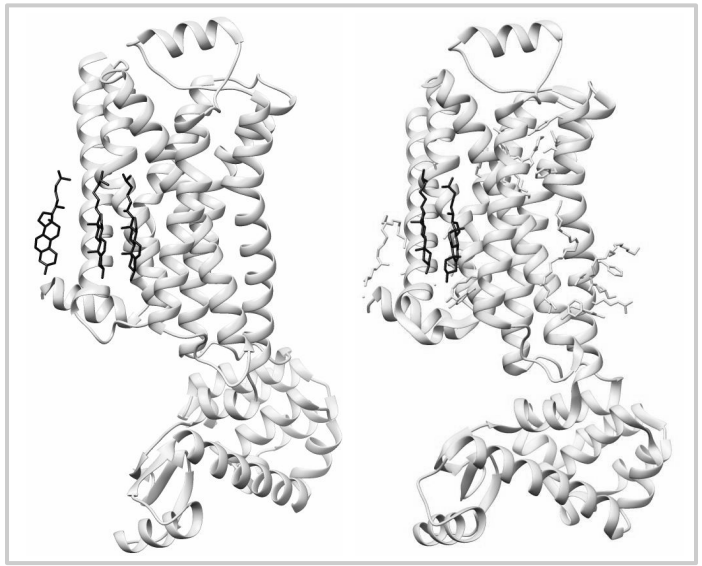
Pi interactions: PHE289, PHE290, catechol moiety

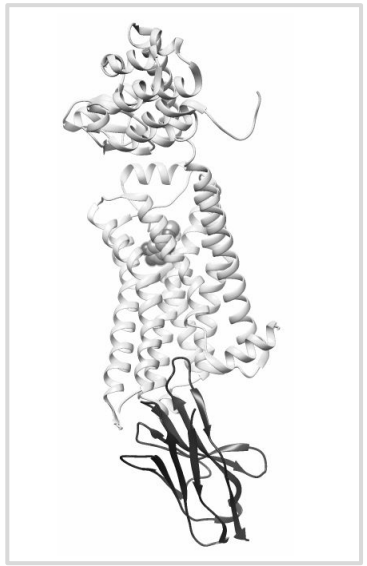
Agonist

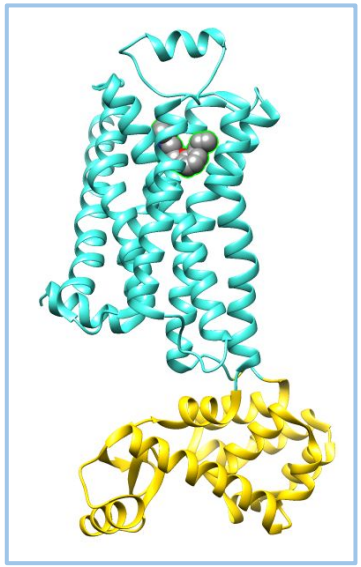
- Activate receptor signaling
- Low affinity

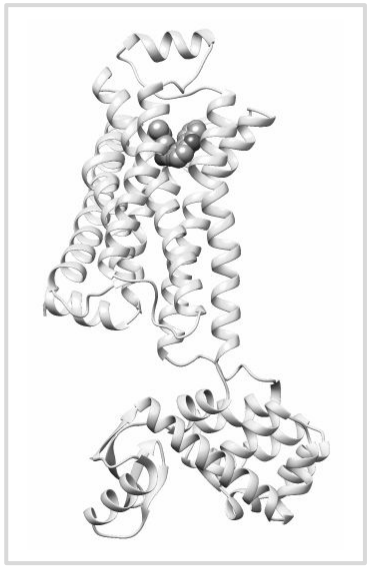
Ligands: Cholesterol 2RH1

Allosteric binding	Ligand binding site		
	Agonist	Antagonist	i-Agonist
Cholesterol	Adrenaline	Alprenolol	Carazolol

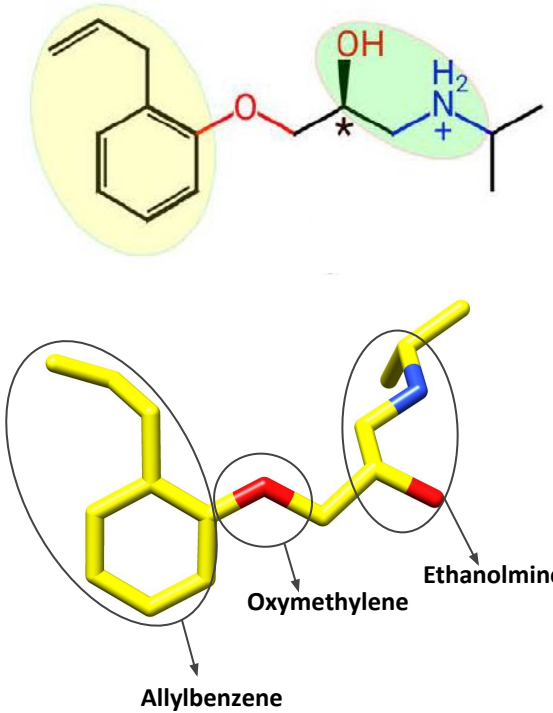


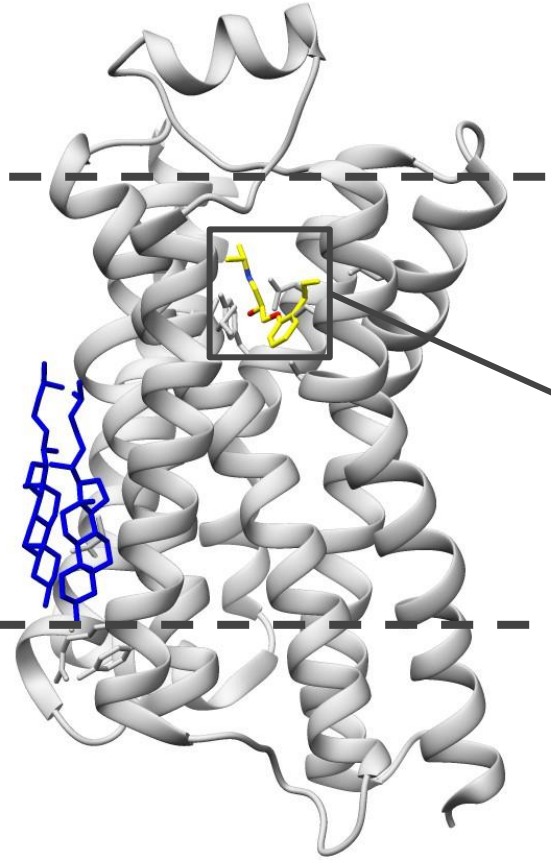


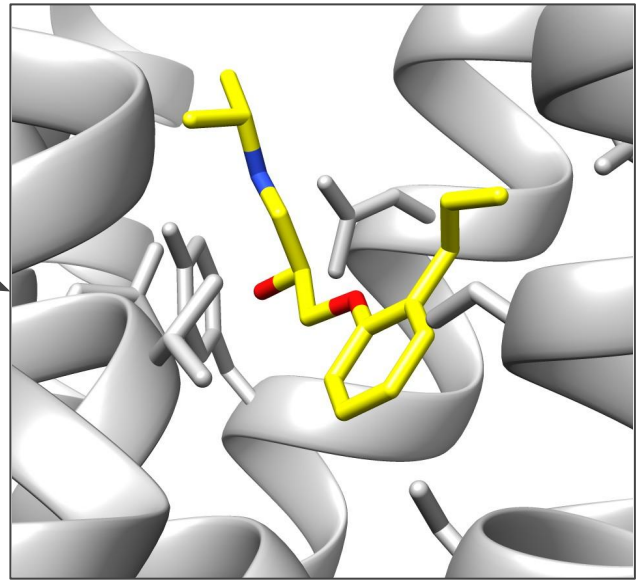




Antagonist: Alprenolol

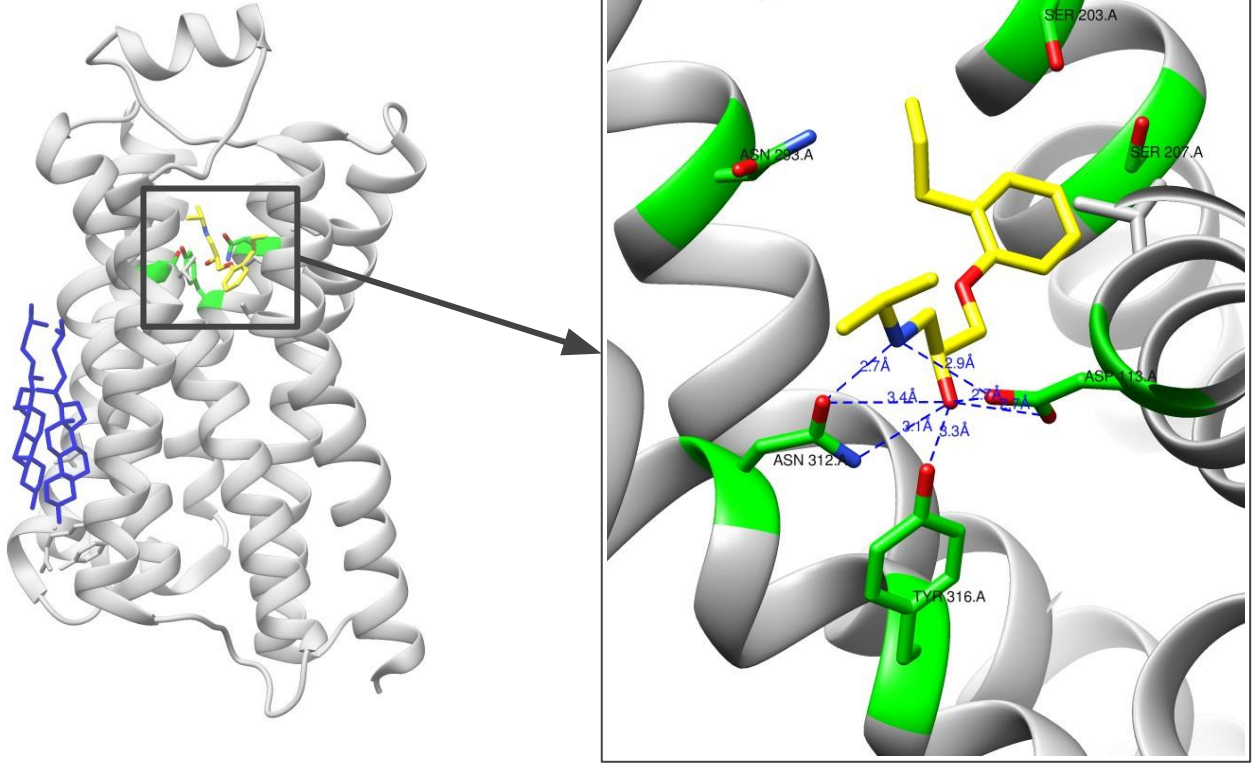






Antagonist: Alprenolol

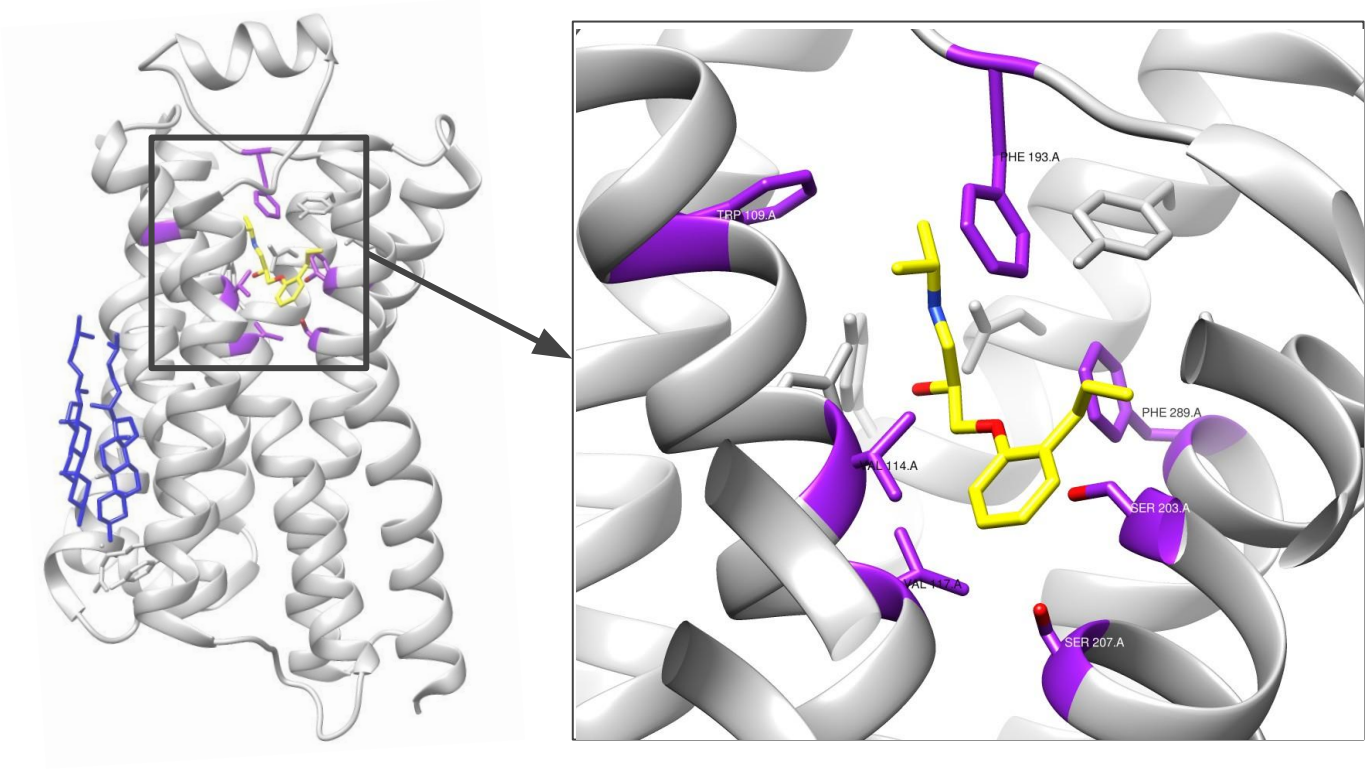
Hydrogen bonds of the binding pocket



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

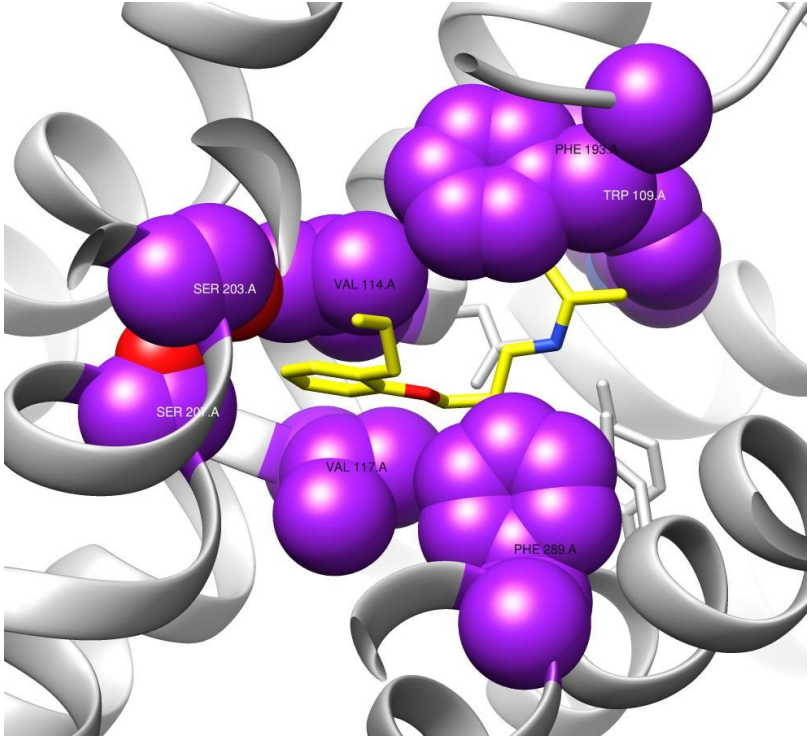
Hydrophobic and Aromatic interactions of the binding pocket

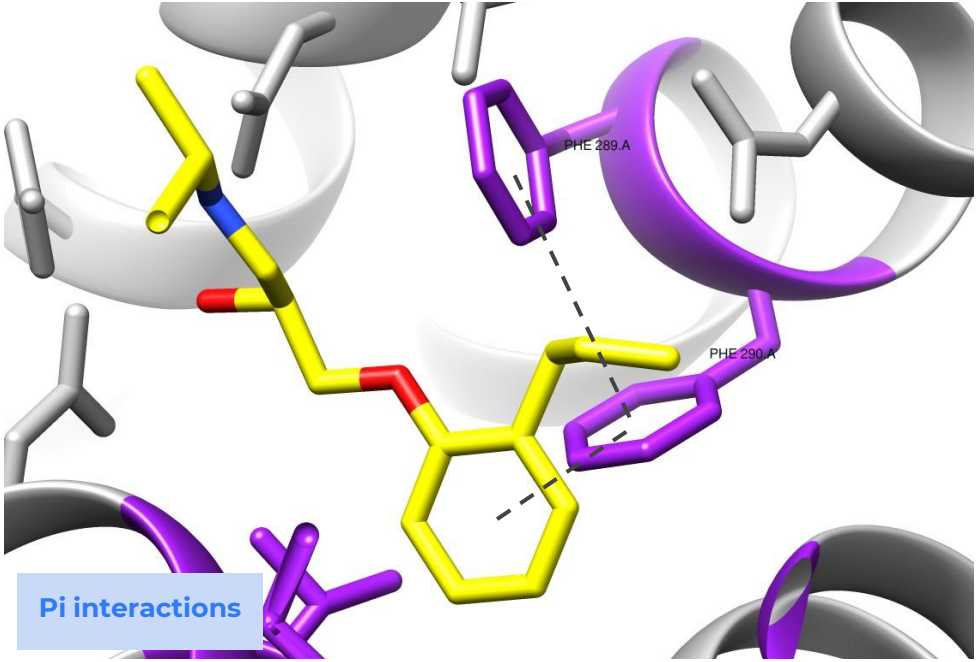


Hydrophobic and Van der Waals interactions		
Atoms β2AR	**Atoms Aprenolol**	**Distance (Å)**
VAL 114 CG1	C9	3.9
VAL 114 CG2	C8	3.6
VAL 117 CB	C8	3.7
TRP 109 CH2	C3	3.6
PHE 193 CE2	C14	3.9
TYR 308 OH	C6	3.6
SER 203 OG	C11	3.4
SER 207 OG	C10	3.6

Antagonist: Alprenolol

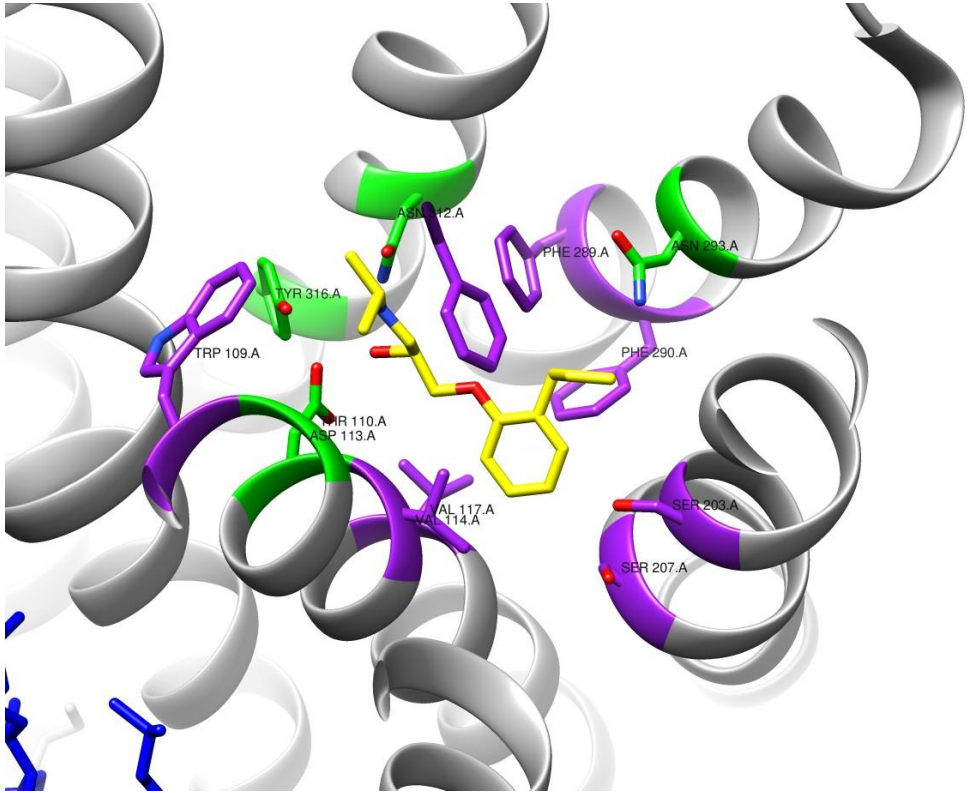
Aromatic interactions of the binding pocket





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

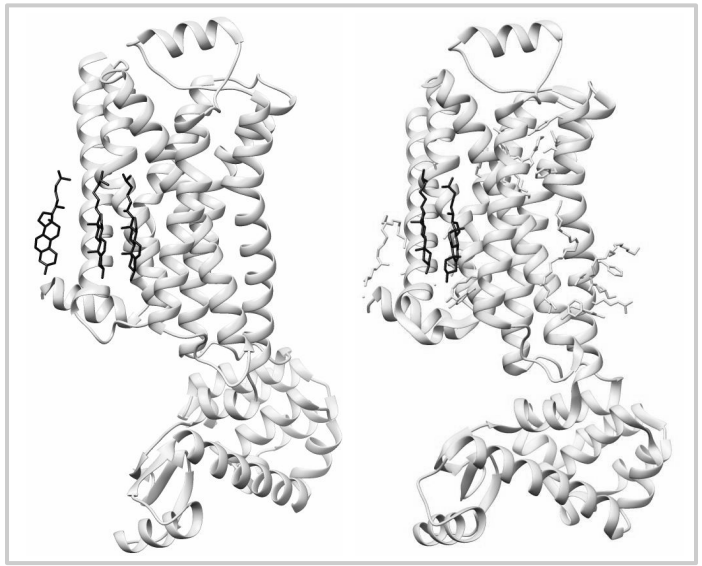
Pi interactions: PHE289, PHE290, allylbenzene moiety

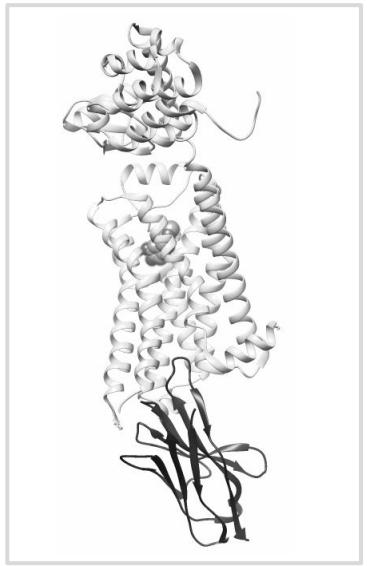
Antagonist

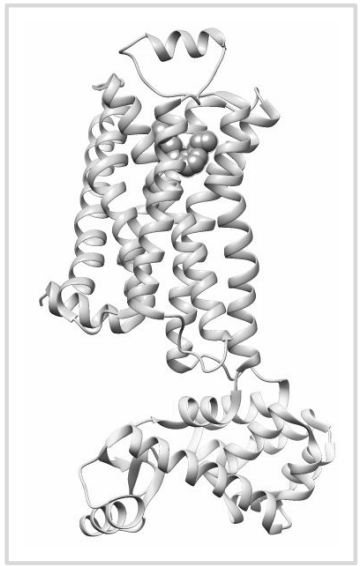
- Blocks agonist signaling
- Micromolar affinity

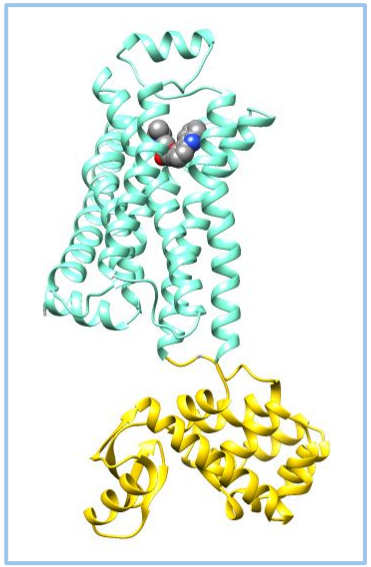
Ligands: Cholesterol 2RH1

Allosteric binding	Ligand binding site		
	Agonist	Antagonist	i-Agonist
Cholesterol	Adrenaline	Alprenolol	Carazolol

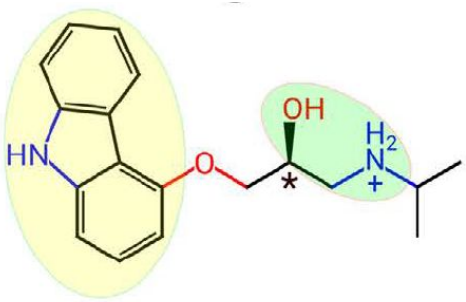


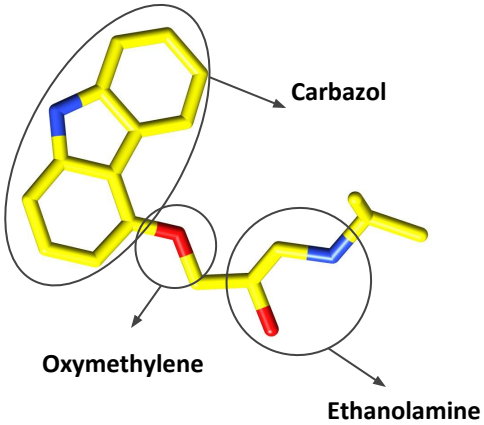


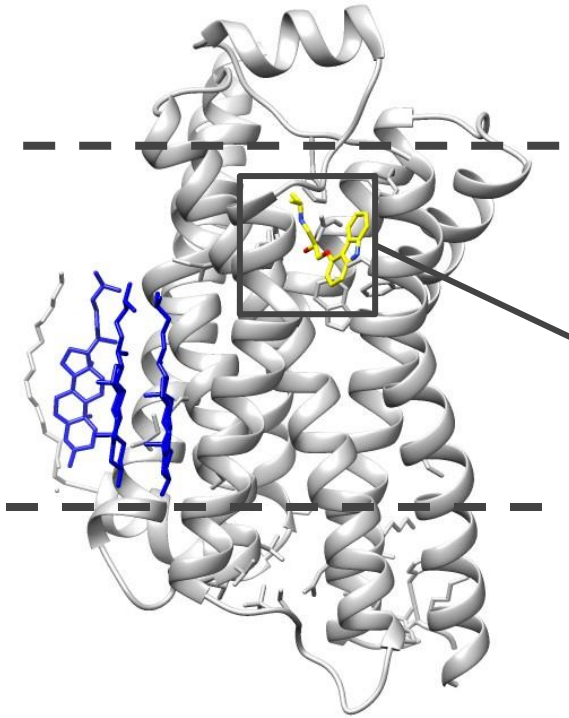


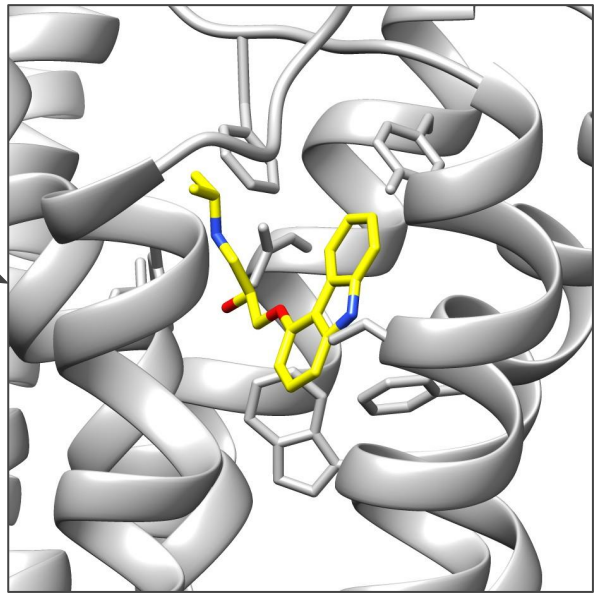


Inverse agonist: Carazolol



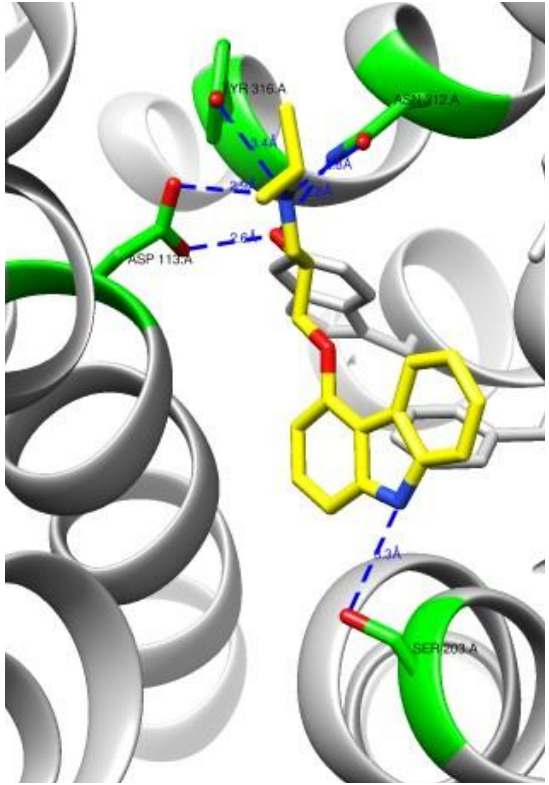


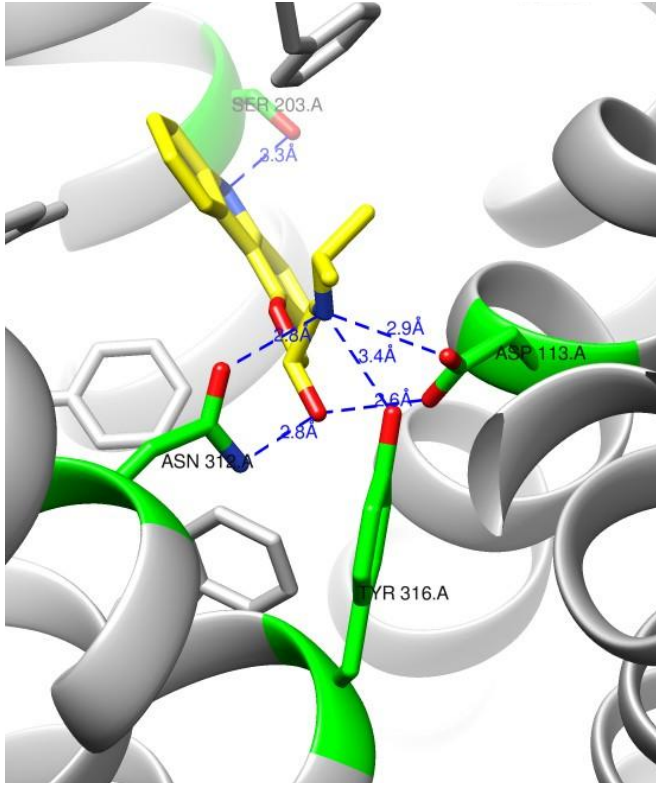




Inverse agonist: Carazolol

Hydrogen bonds and Salt Bridge Contacts

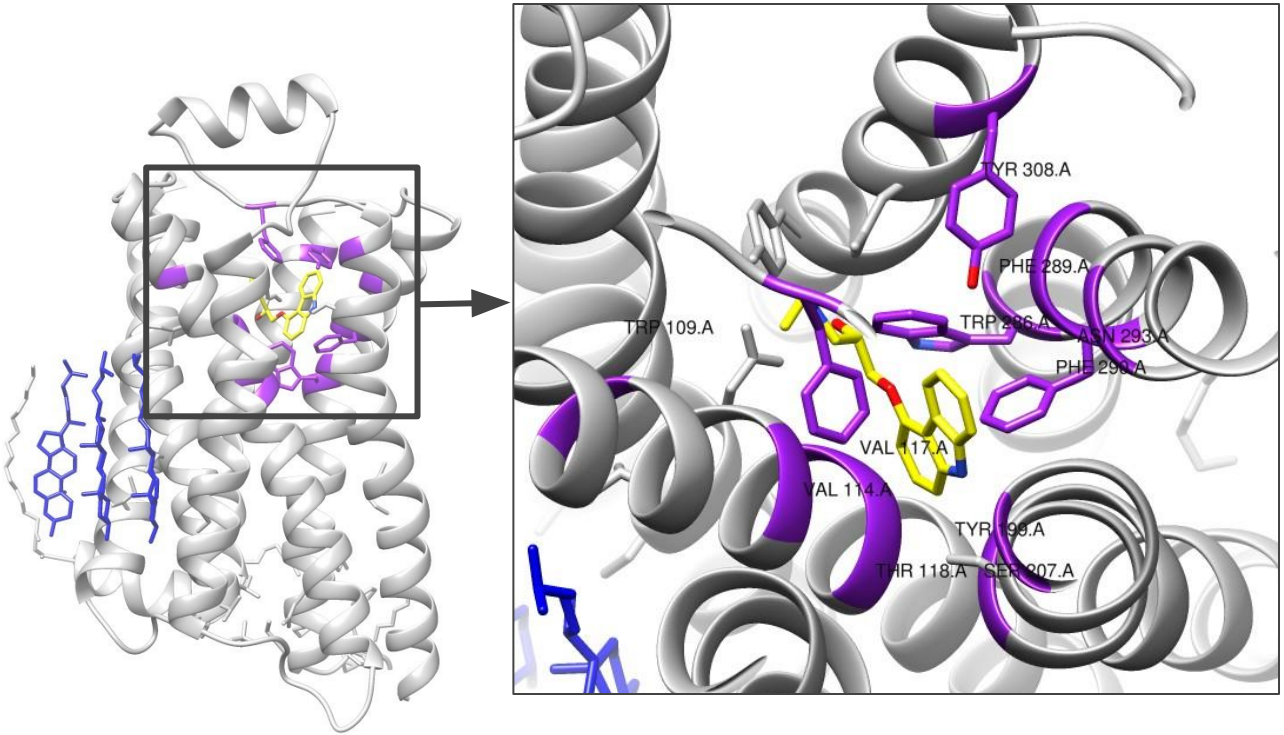




Hydrogen Bonds Contacts and Salt Bridge Contacts		
Atoms β2AR	**Atoms Carazolol**	**Distance (Å)**
SER 203 OG	N7	3.3
ASP 113 OD1	O17	2.6
ASP 113 OD2	N19	2.9
ASN 312 ND2	O17	2.8
ASN 312 OD1	N19	2.8
TYR 316 OH	N19	3.4

Inverse agonist: Carazolol

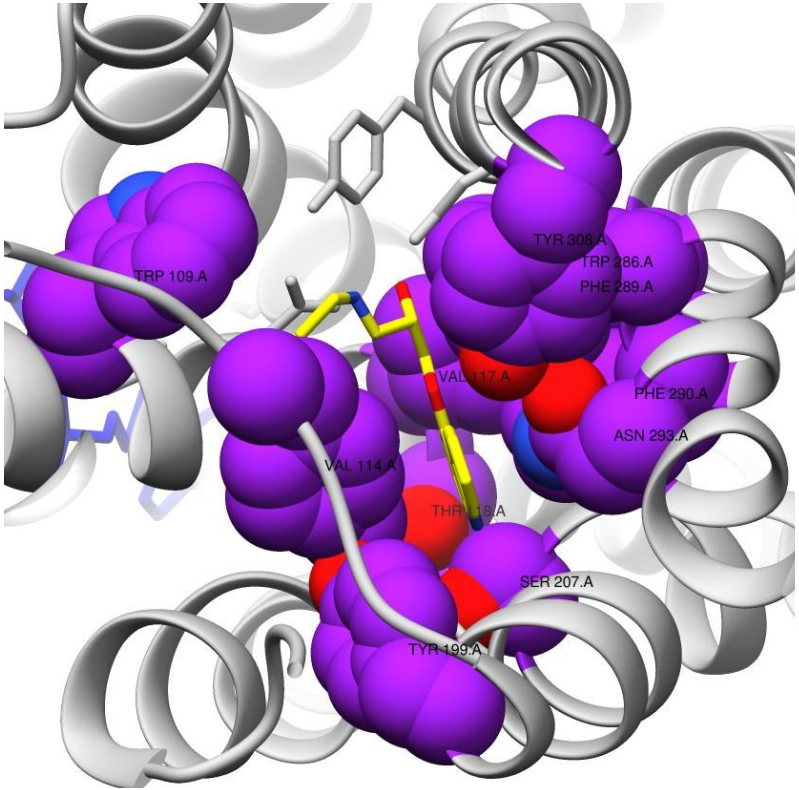
Hydrophobic and Aromatic interactions



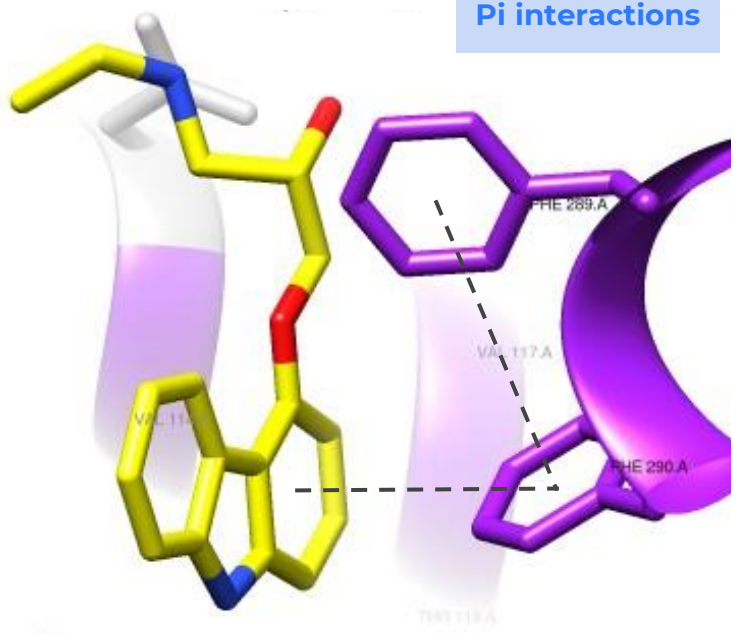
Hydrophobic and Aromatic interactions		
Atoms β2AR	**Atoms Carazolol**	**Distance (Å)**
TRP 109 CH2	C21	3.8
VAL 114 CG1	C11	3.9
VAL 117 CG1	C12	4.0
THR 118 OG1	C11	3.9
PHE 193 CE2	C6	3.5
TYR 199 CE2	C2	3.9
SER 207 CB	C10	3.6
TRP 286 CH2	O17	3.4
PHE 289 CE2	O14	3.7
PHE 290 CZ	C12	3.5
ASN 293 ND2	C5	3.6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

Inverse agonist: Carazolol

Hydrophobic and Aromatic interactions

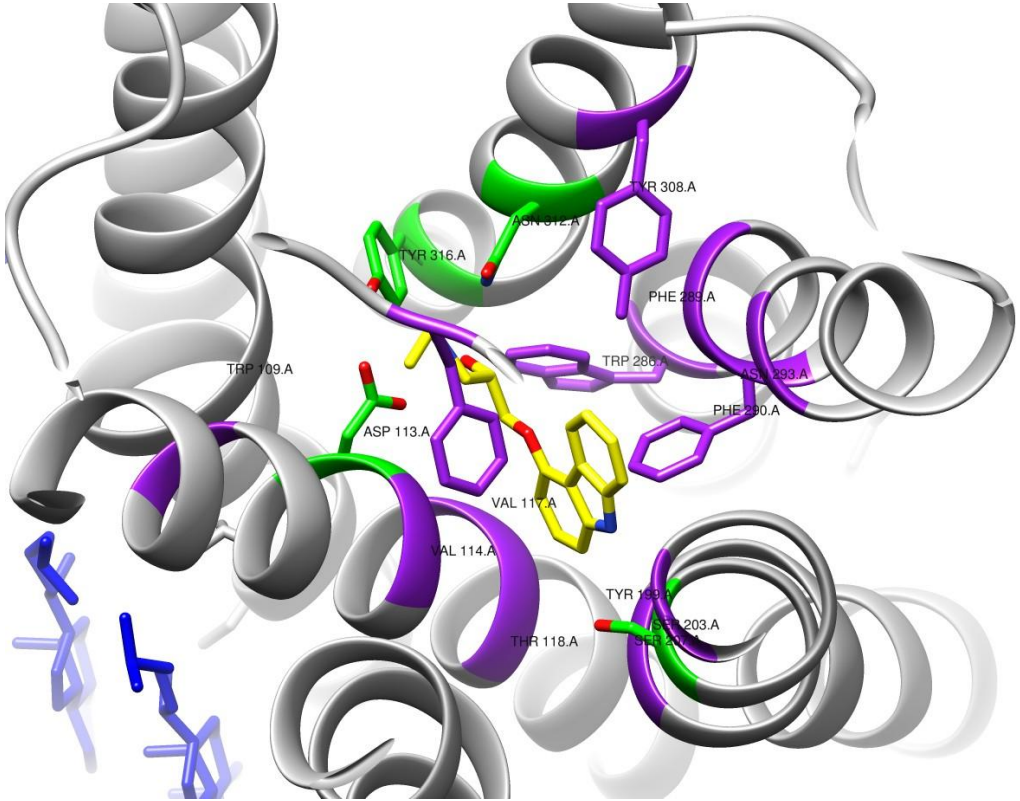


Pi interactions



Inverse agonist: Carazolol

Important residues



SUMMARY

Hydrogen bond network

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

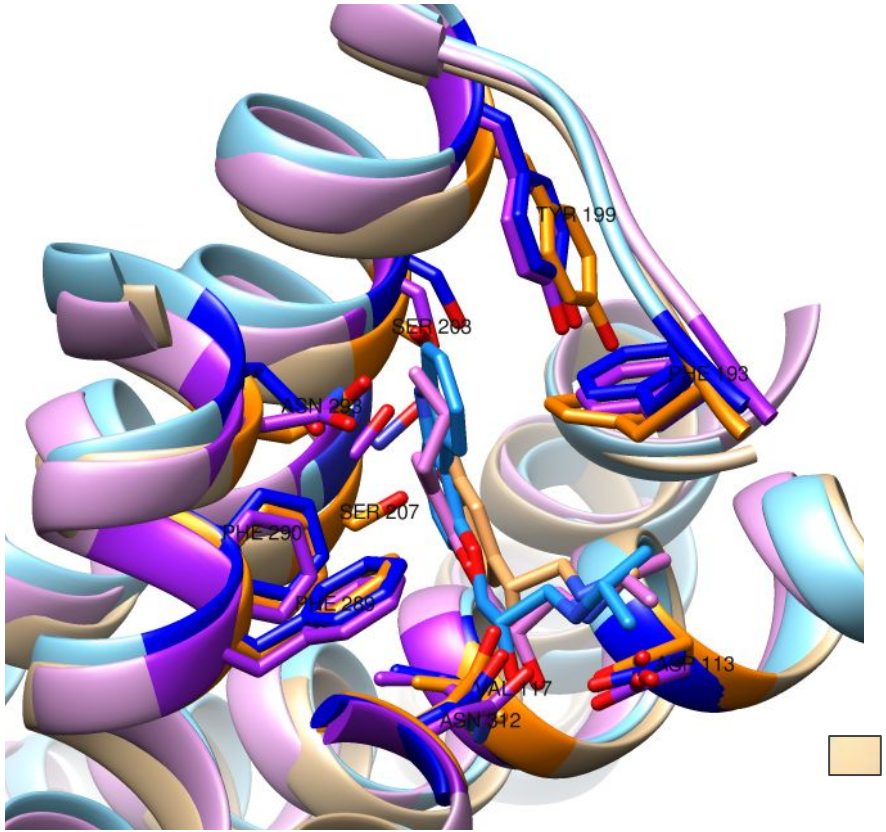
Hydrophobic and aromatic interactions: TMs 3, 5 and 6 and ECL2

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).

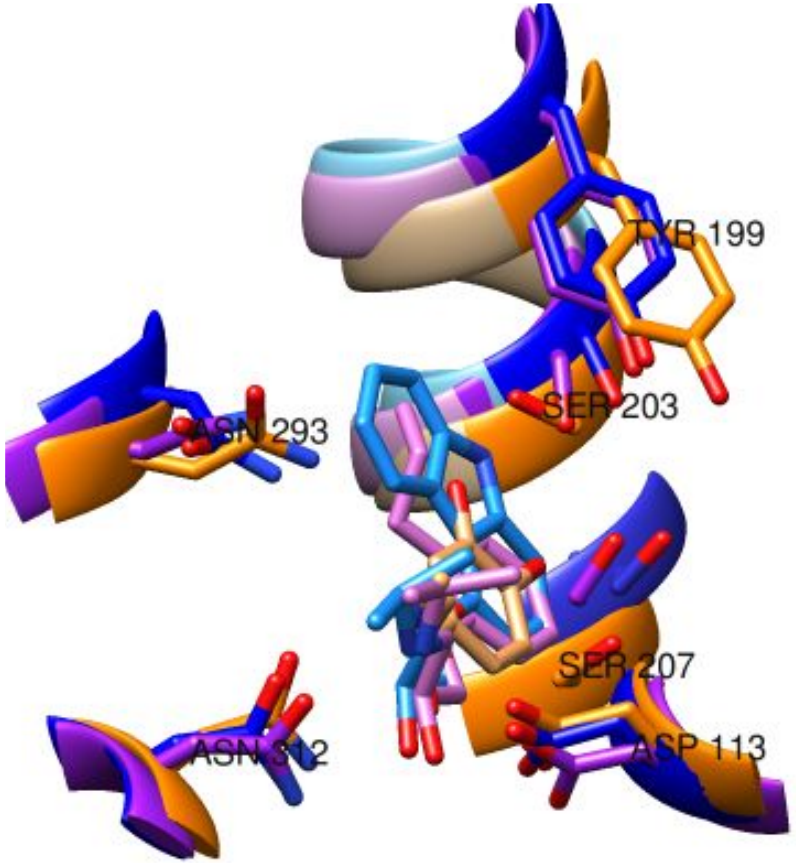
RMSD: 1.54

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 β2AR**
- **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 β2AR 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 β2AR 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

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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.

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[5], [6] Shaw DE, Borhani DW, Xu H, Pan AC, Mildorf TJ, Maragakis P, et al. Activation mechanism of the β2-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.

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2. Chan HC, Filipek S, Yuan S. The Principles of Ligand Specificity on beta-2-adrenergic receptor. *Sci Rep* 2016;6:34736.
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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.
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9. Kobilka BK, Garcia K, Weis W, Enos M, Kruse A, Manglik A, et al. Adrenaline-activated structure of β2-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.
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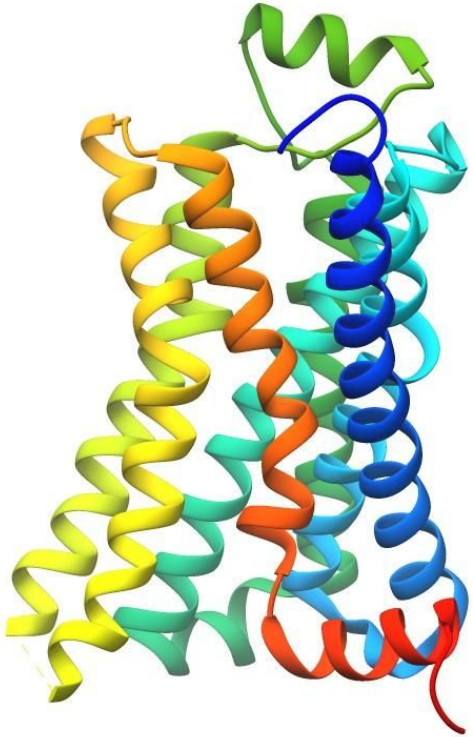
12. Rasmunssen S, Choi H, Rosenbaum D, Kobilka T, Thian F, et al. Crystal structure of the human β2 adrenergic G-protein-coupled receptor. *Nature.*2007 Nov 15; 450:383-387.



THANK YOU FOR YOUR ATTENTION

COMPLEMENTARY INFORMATION

PSI-BLAST- Choosing sequences of β2RC from different species

```
Results from round 5


Query= P07550_homosapiens

Length=413

                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  Value
Sequences used in model and found again:

  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|P18090|ADRB1_RAT Beta-1 adrenergic receptor OS=Rattus norveg...  317     1e-103
```

COMPLEMENTARY INFORMATION

BLAST- Looking for β2RC structure from different species

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

Length=500
                                                                      Score     E
Sequences producing significant alignments:                          (Bits)  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
  3eml_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
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  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
  1jtm_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
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  1g0q_A mol:protein length:164 PROTEIN (LYSOZYME)                    334     4e-114
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  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
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  221l_A mol:protein length:164 T4 LYSOZYME                           334     7e-114
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  123l_A mol:protein length:164 T4 LYSOZYME                           334     7e-114
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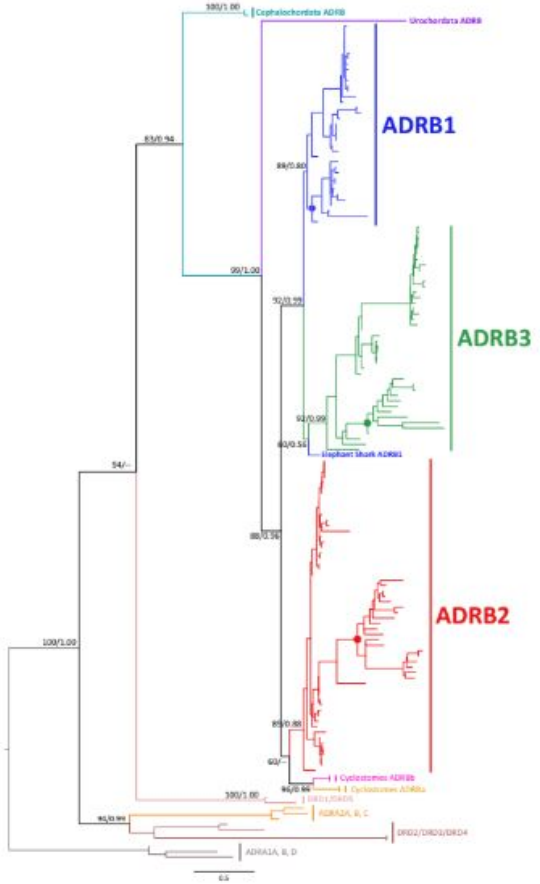
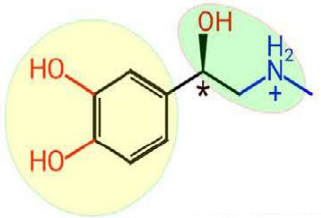
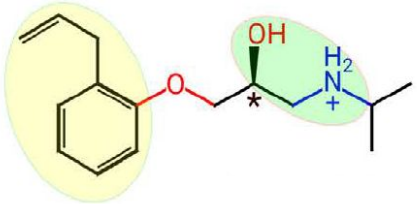
COMPLEMENTARY INFORMATION



COMPLEMENTARY INFORMATION

Category	Ligand	PDB	Structure
Agonist	Adrenaline	4LDO	
Antagonist	Alprenolol	3NYA	
Inverse agonist	Carazolol	2RH1