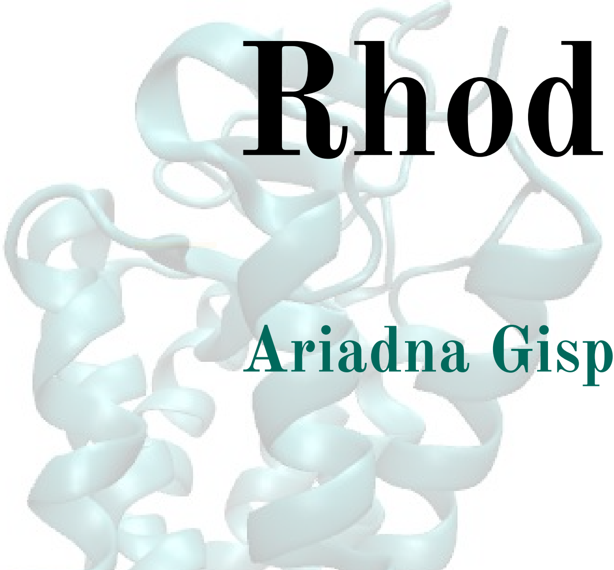
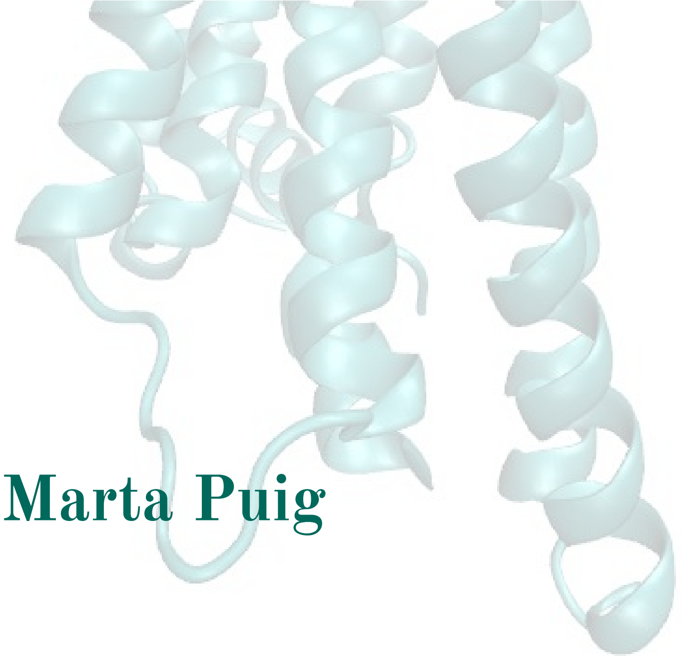




Rhodopsin

Ariadna Gispert, Marina Lleal and Marta Puig

Index

Introduction

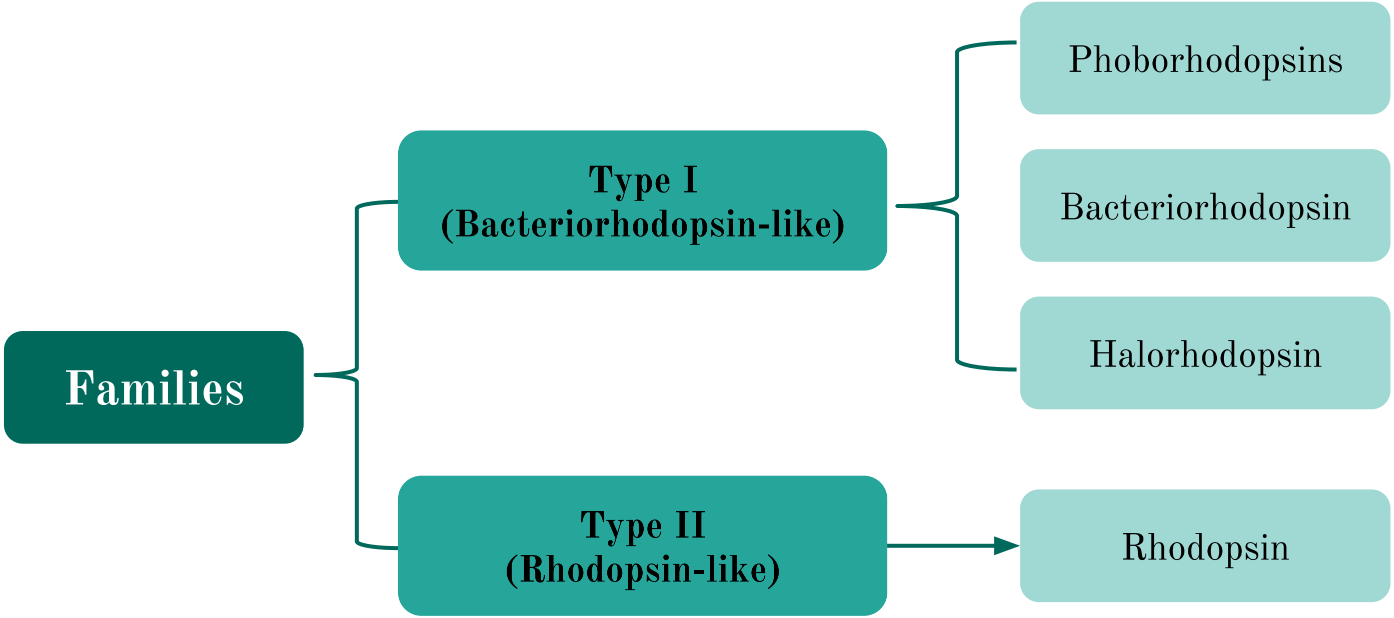
Rhodopsin

- G-protein coupled receptors family (GPCR)
- Membrane protein with 7 transmembrane helices
- 2 parts
  - → Apoprotein (opsin)
  - → Prosthetic group (cromophore) → RETINAL
- Function → Phototransduction of the visual perception
- Found in rod cells

SCOP Classification

Class	Membrane and cell surface proteins and peptides
Fold	A G-coupled receptor-like
Superfamily	Family A G-coupled receptor-like
Family	Rhodopsin-like
Protein	Rhodopsin

Classification



Families

Type I (Bacteriorhodopsin-like)

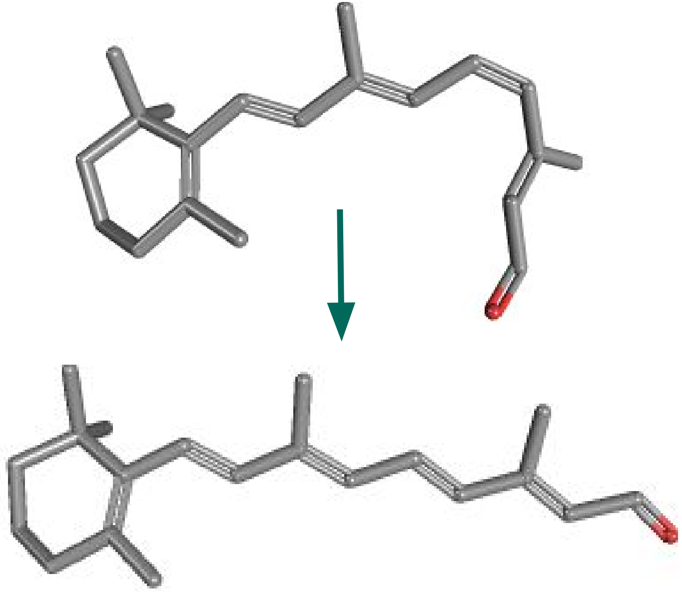
- Phoborhodopsins
- Bacteriorhodopsin
- Halorhodopsin

Type II (Rhodopsin-like)

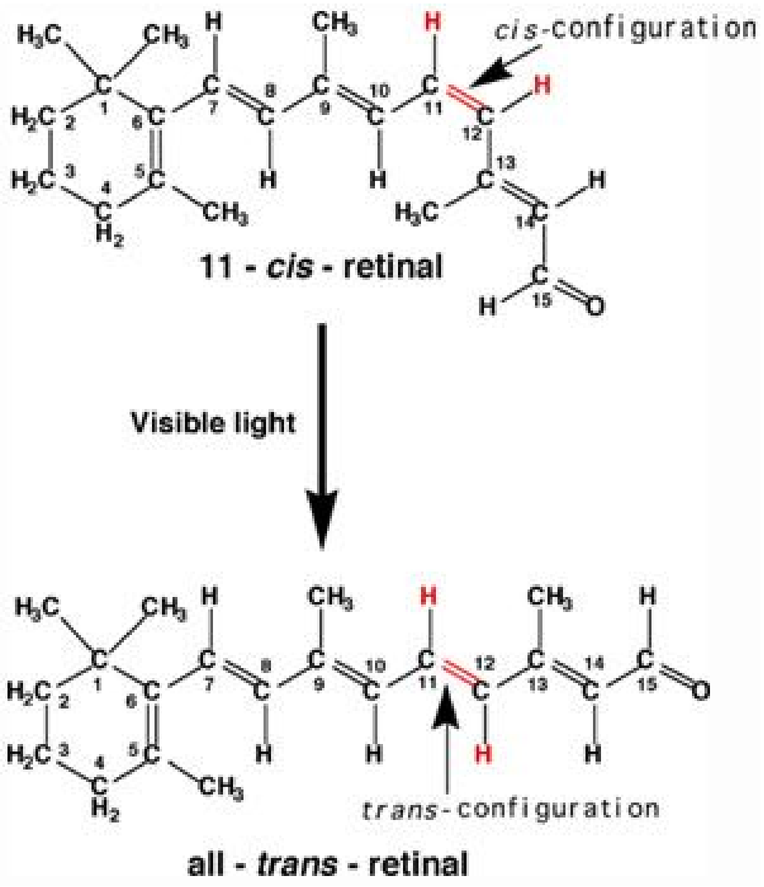
- Rhodopsin

Rhodopsin ligand: retinal

Upon light absorption, a cis-trans isomerization occurs. This isomerization changes the retinal:

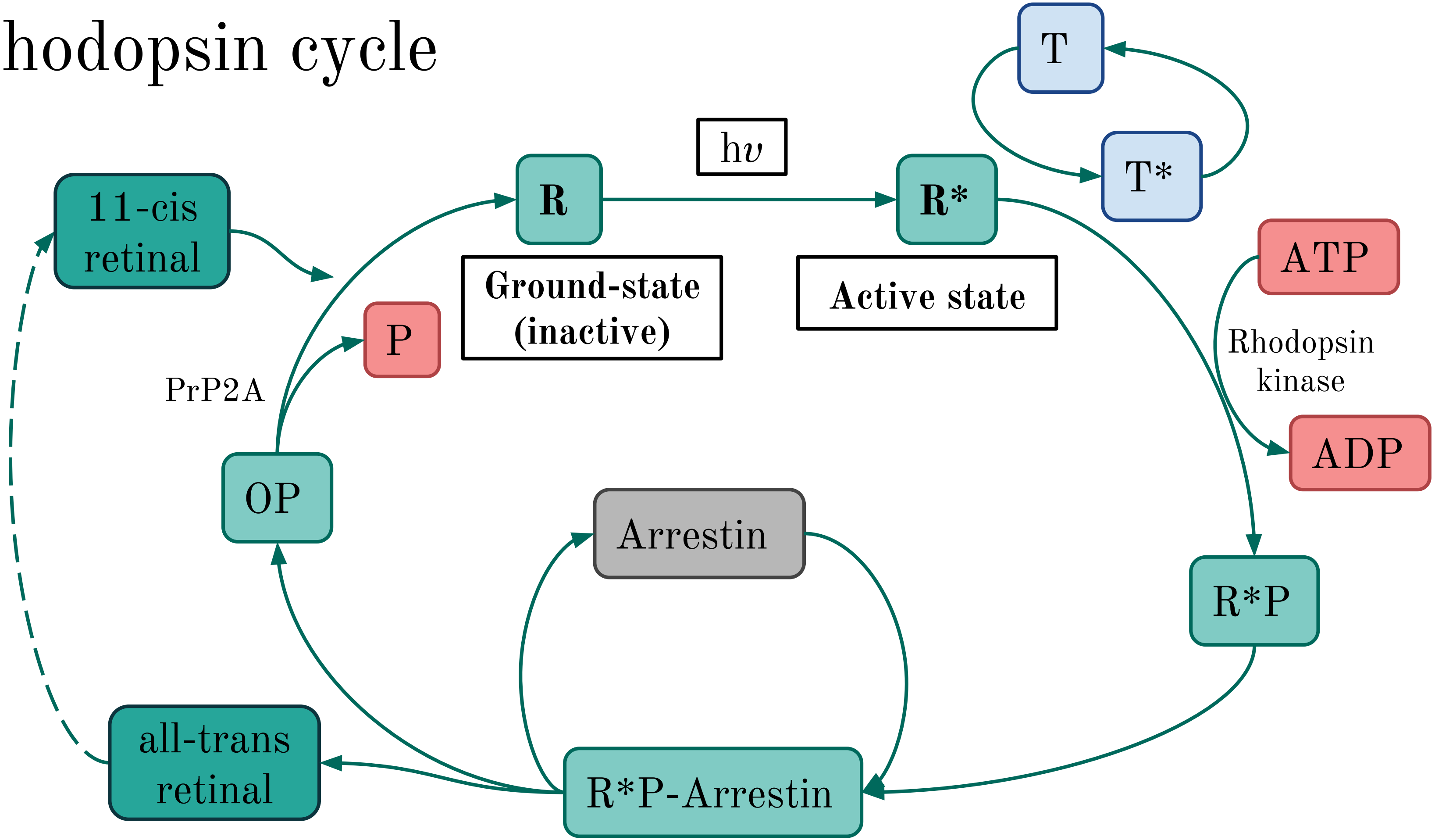


Images from PubChem



Extracted from Cassiday R, Frey, R, 2000

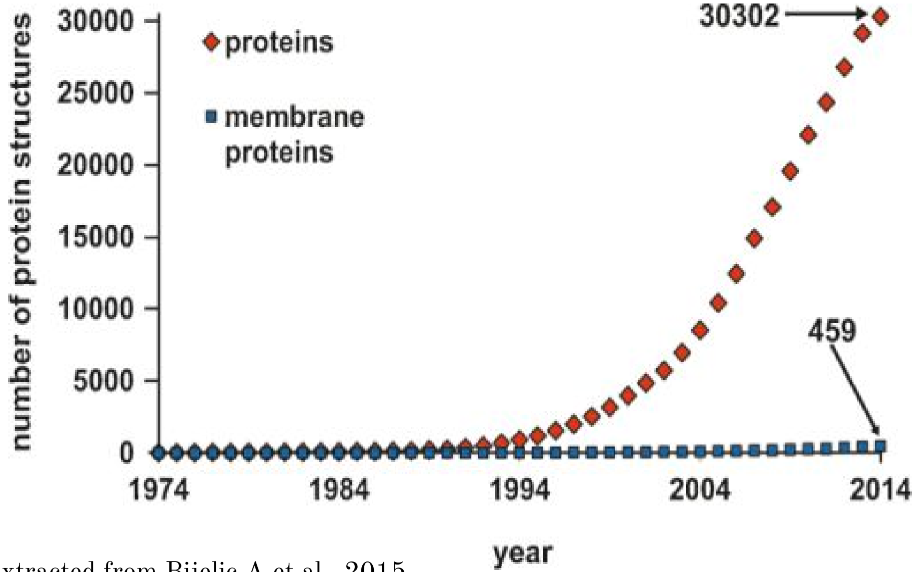
Rhodopsin cycle



Difficulties in rhodopsin crystallisation

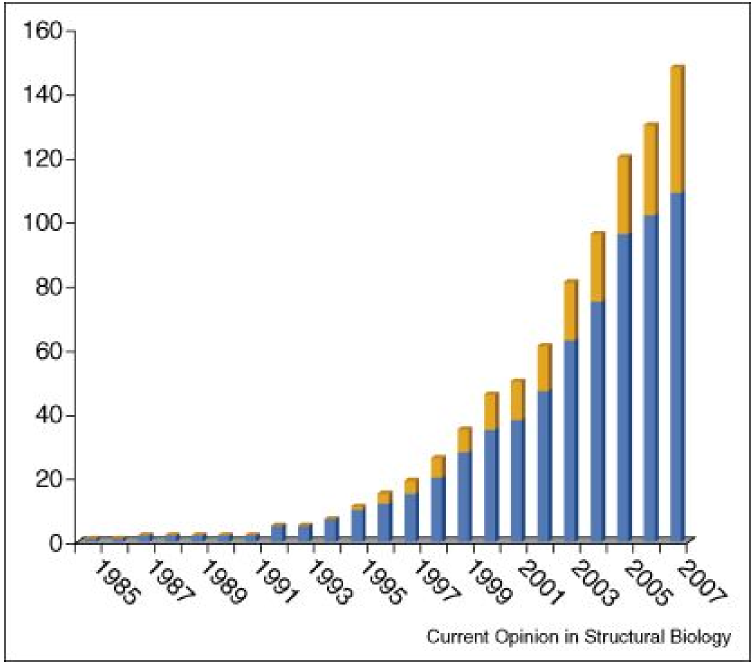
Low number of available structures

Number of total proteins in PDB vs. membrane proteins



Extracted from Bijelic A et al., 2015

Prokaryotic (blue) vs. eukaryotic (yellow) membrane proteins in PDB

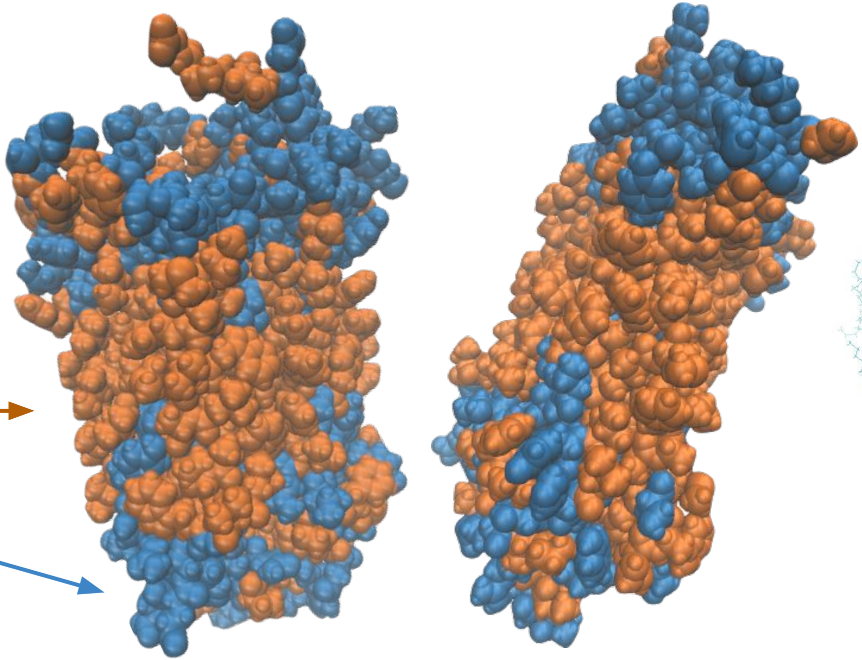


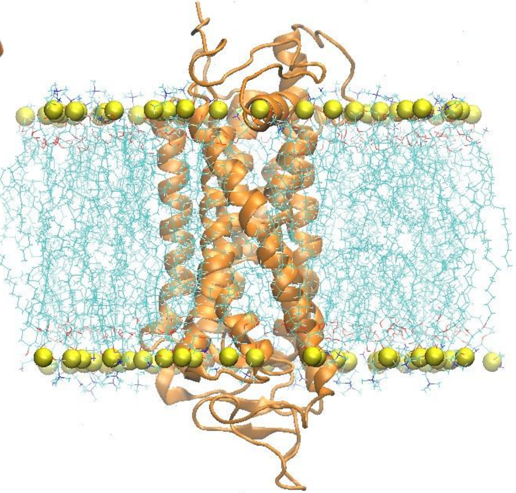
Extracted from Carpenter EP et al., 2008

Rhodopsin structure: hydrophobicity

Hydrophobic residues

Hydrophilic residues





Extracted from Madej, BD, et al, 2014

Rhodopsin structure: X-ray crystallisation

PDB structures:

1U19
INACTIVE

3PQR
ACTIVE

↓

Bovine rhodopsin

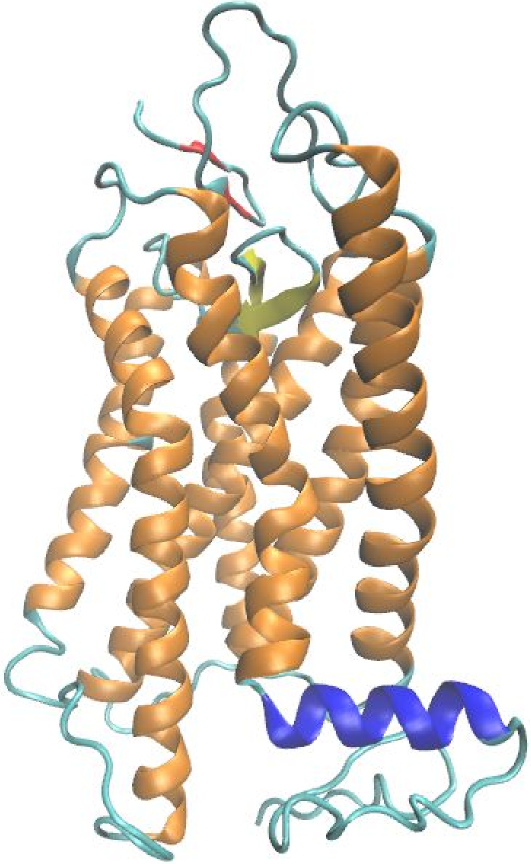
Extracellular N-terminus

3 extracellular loops

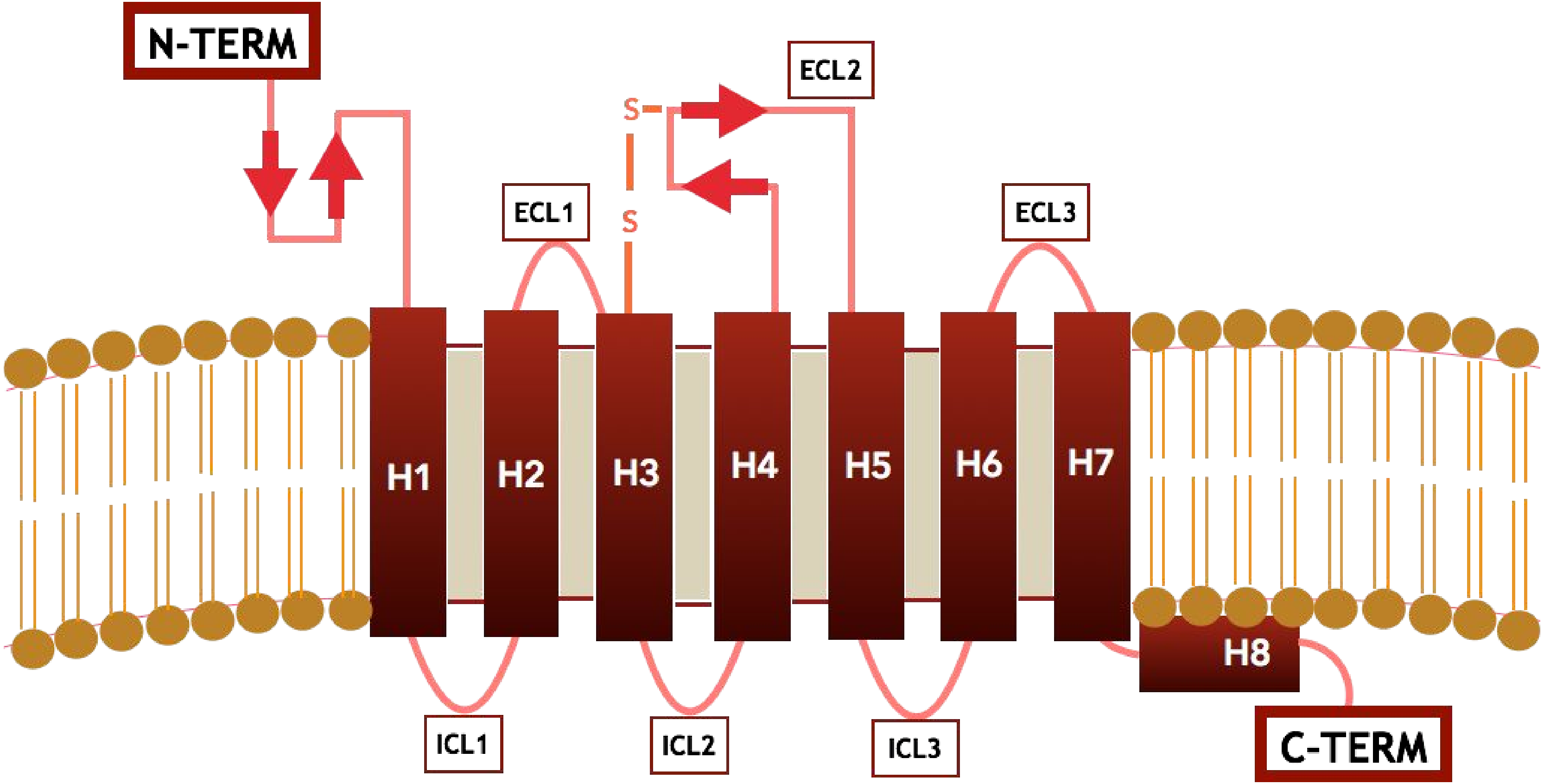
7 transmembrane helices

3 intracellular loops

Intracellular C-terminus
with a short helix



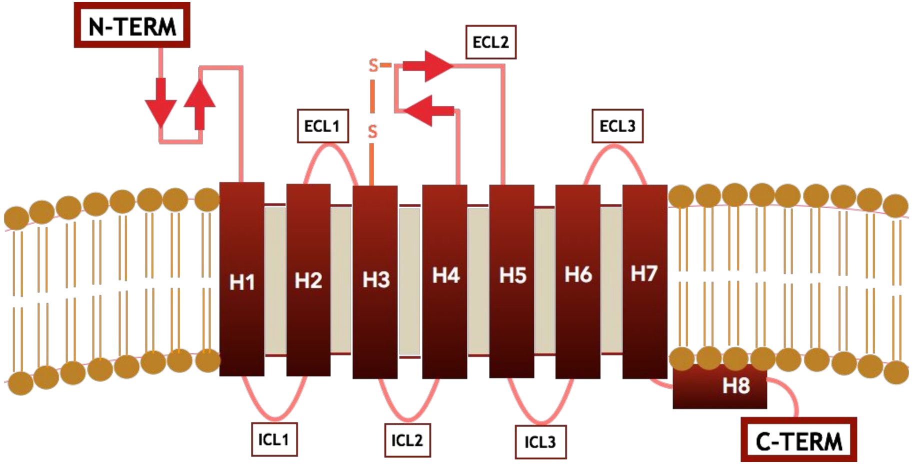
Rhodopsin structure: 2D representation



Rhodopsin structure: outline

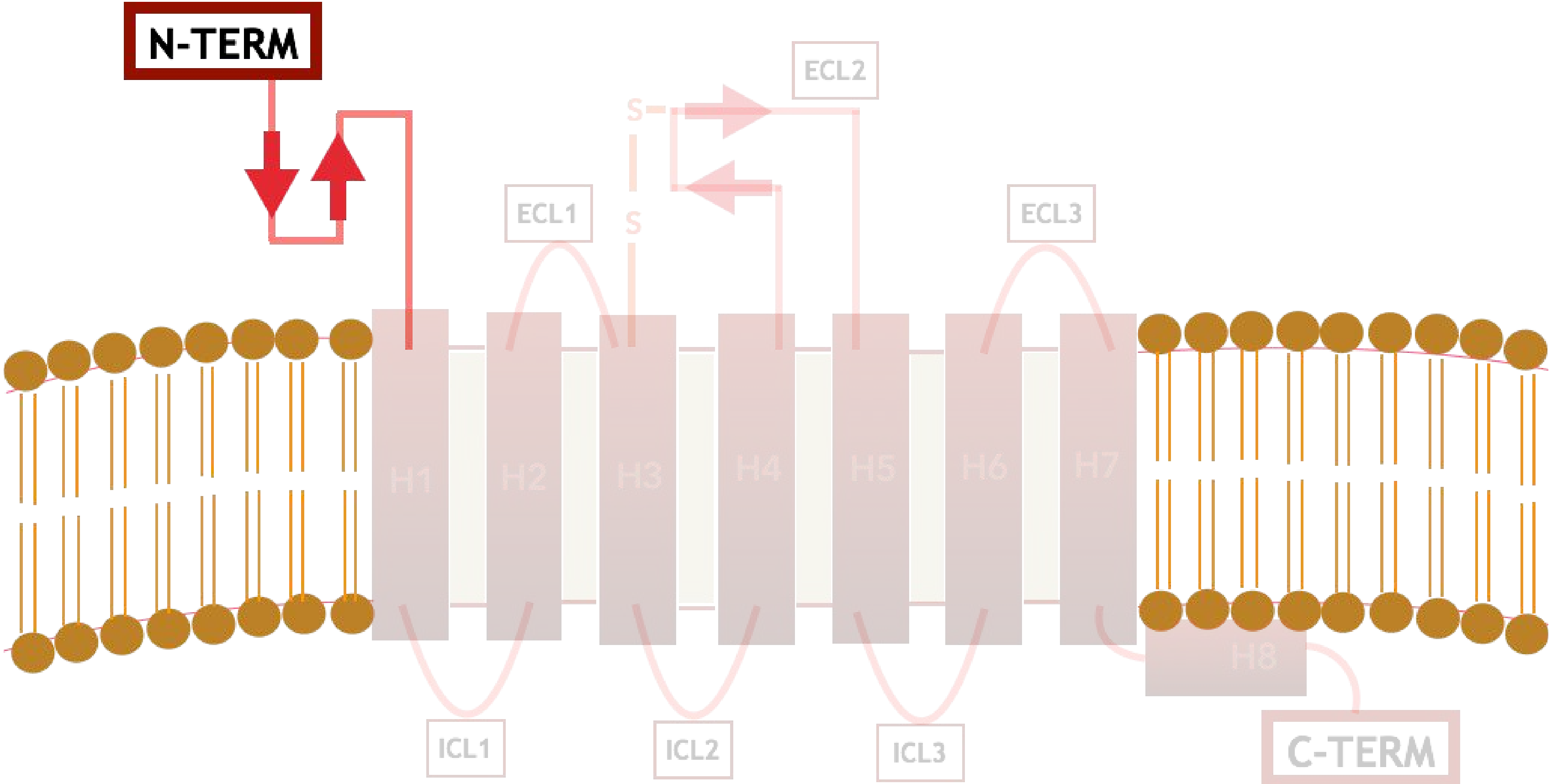
Most relevant areas:

- N-terminus
- **Second extracellular loop (ECL2)**
- **Ligand binding site**
- **7TM domain**
- Third intracellular loop (ICL3)
- C-terminus



Comparison: INACTIVE vs ACTIVE

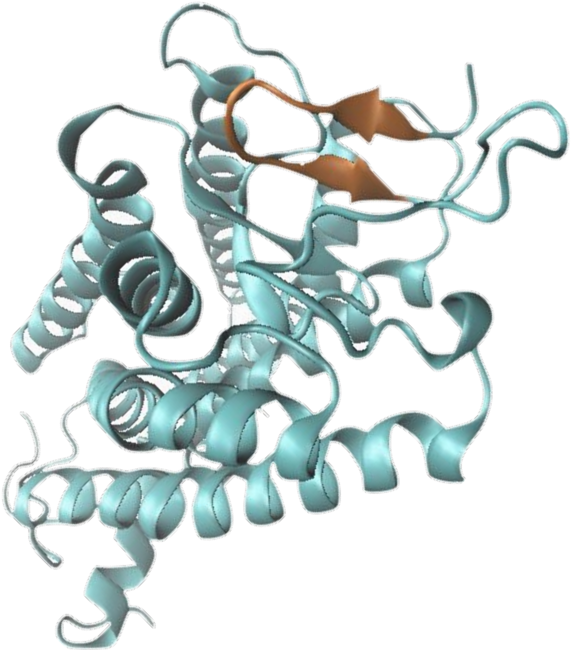
N-terminus

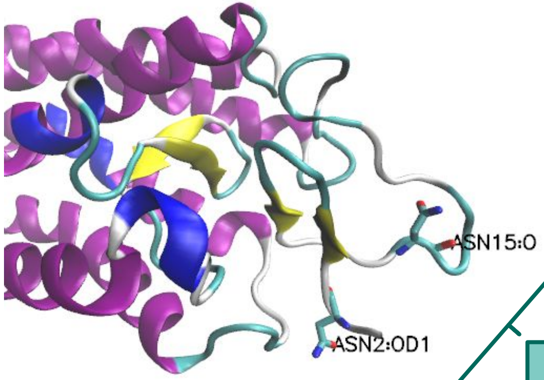

N-TERM
ECL2
S
S
ECL1
ECL3
H1
H2
H3
H4
H5
H6
H7
H8
ICL1
ICL2
ICL3
C-TERM

N-terminus

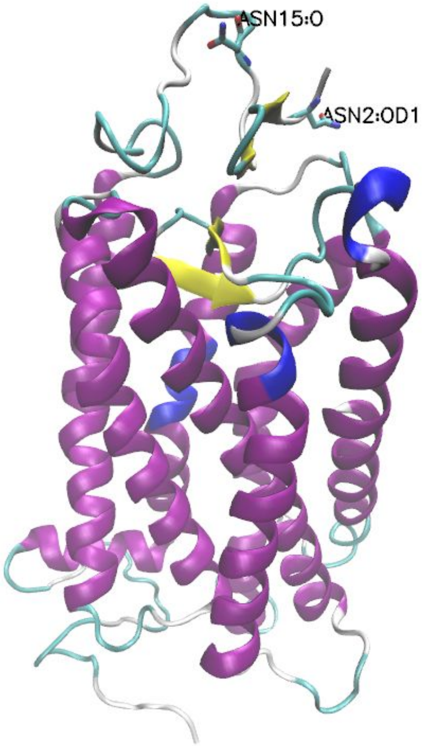
- Extracellular side
- Two-stranded β sheet
- Glycosylation sites → Asn2 and Asn15

Essential role in function

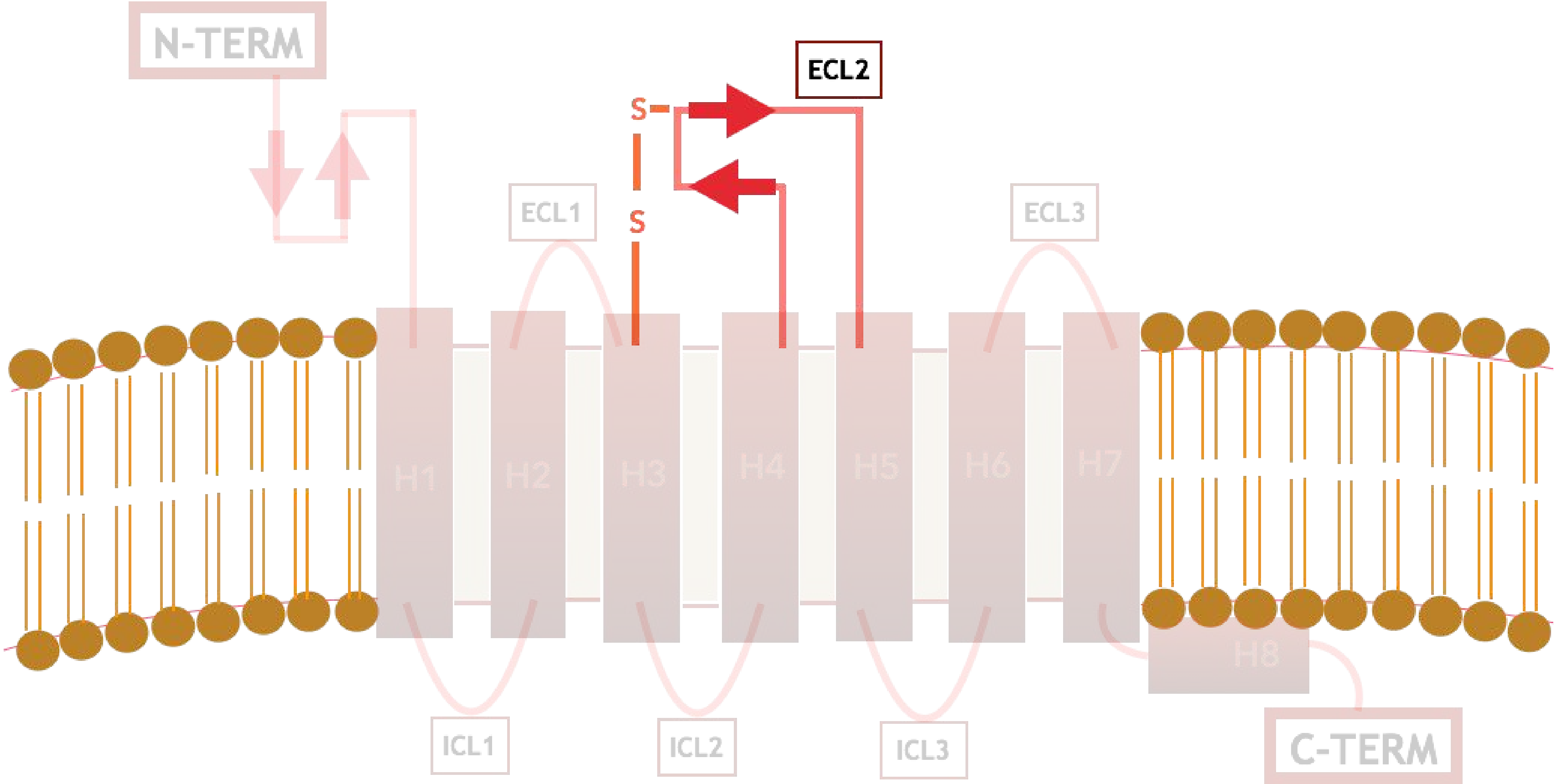




Glycosylation sites

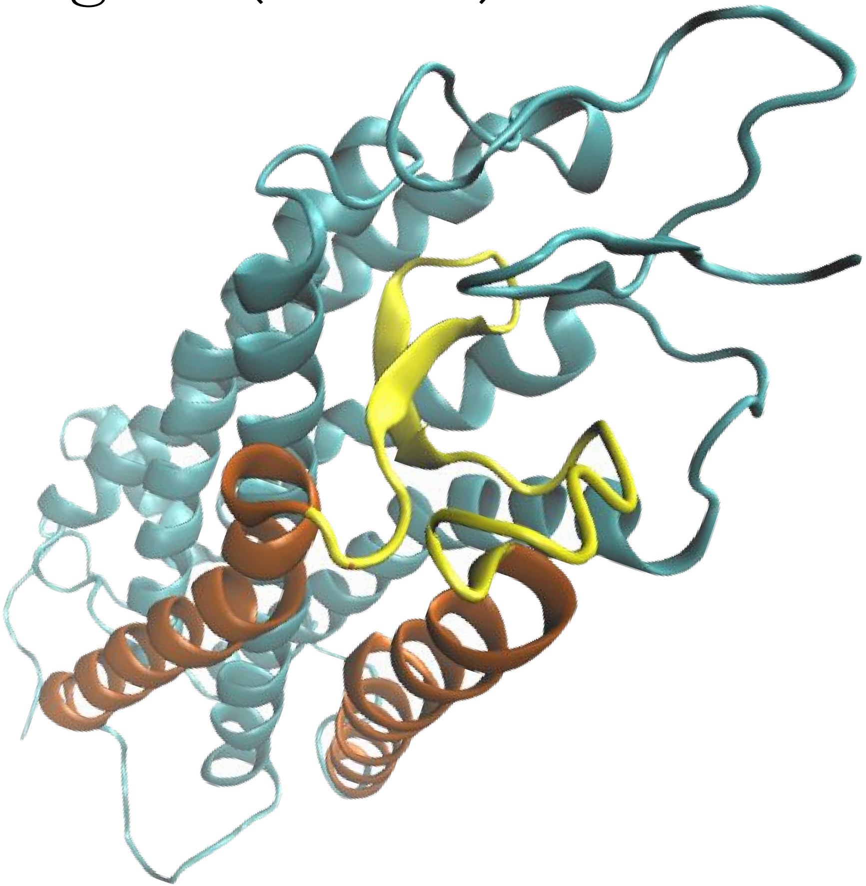


Second Extracellular Loop (ECL2)



Second Extracellular Loop region (ECL2)

- Essential for
  - Folding
  - Ligand binding
  - Biological function
- Connects H4 and H5
- Twisted two-stranded β-sheet
- Blocks rapid exit of the ligand (retinal) from the binding site

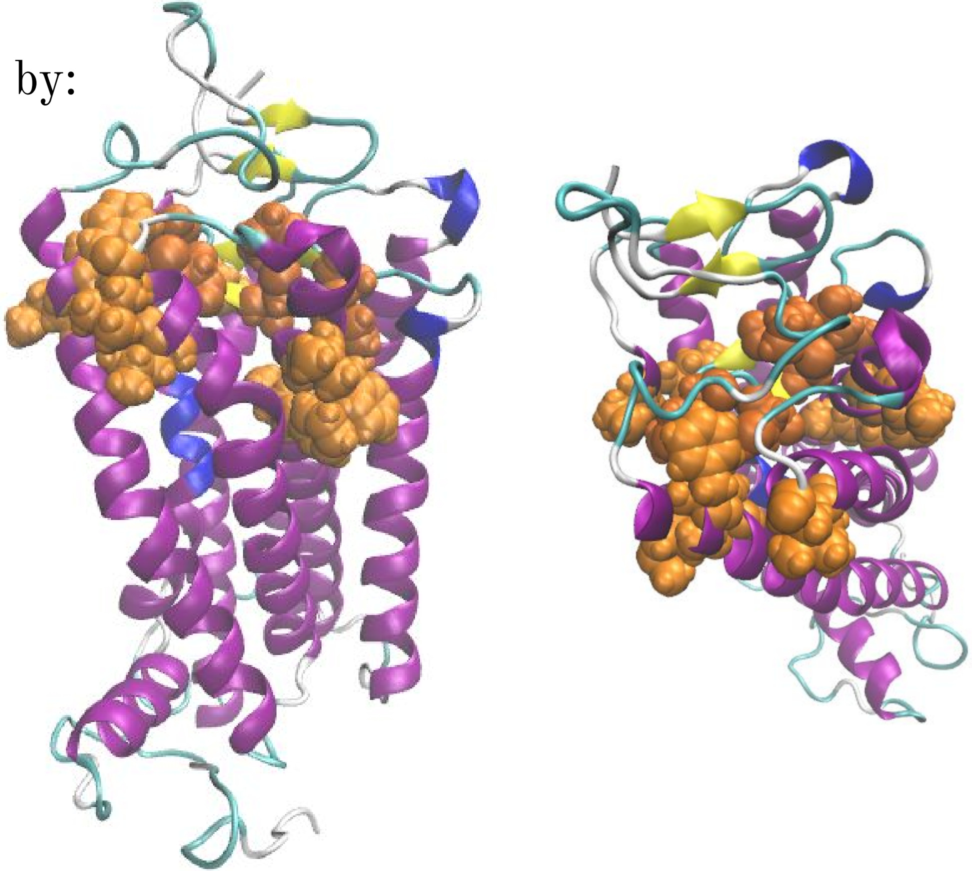


Second Extracellular Loop (ECL2)

The position of this β-sheet is stabilized by:

- **Hydrophobic interactions**
- Disulfide bond
- Hydrogen bonding network
- Salt bridge

Surrounding residues of the helix bundle, especially those from H1, H2, H3, and H7



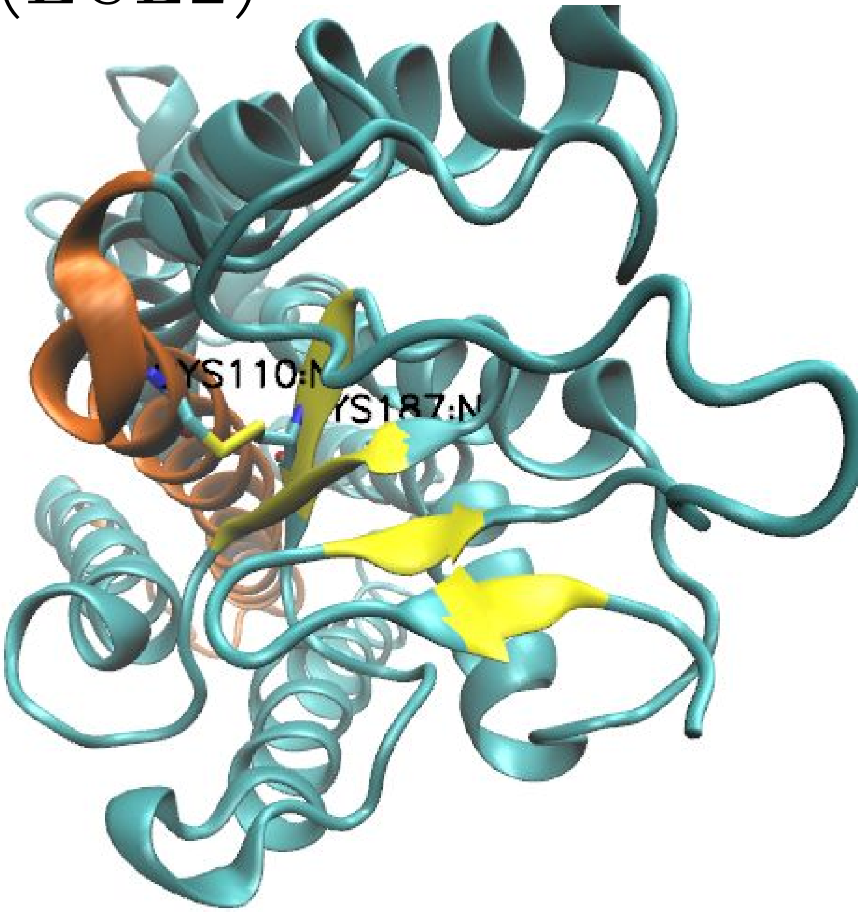
Second Extracellular Loop (ECL2)

The position of this β-sheet is stabilized by:

- Hydrophobic interactions
- **Disulfide bond** → Highly conserved
- Hydrogen bonding network
- Salt bridge

Cys110 - Cys187

H3 — ECL2



Second Extracellular Loop (ECL2)

The position of this β-sheet is stabilized by:

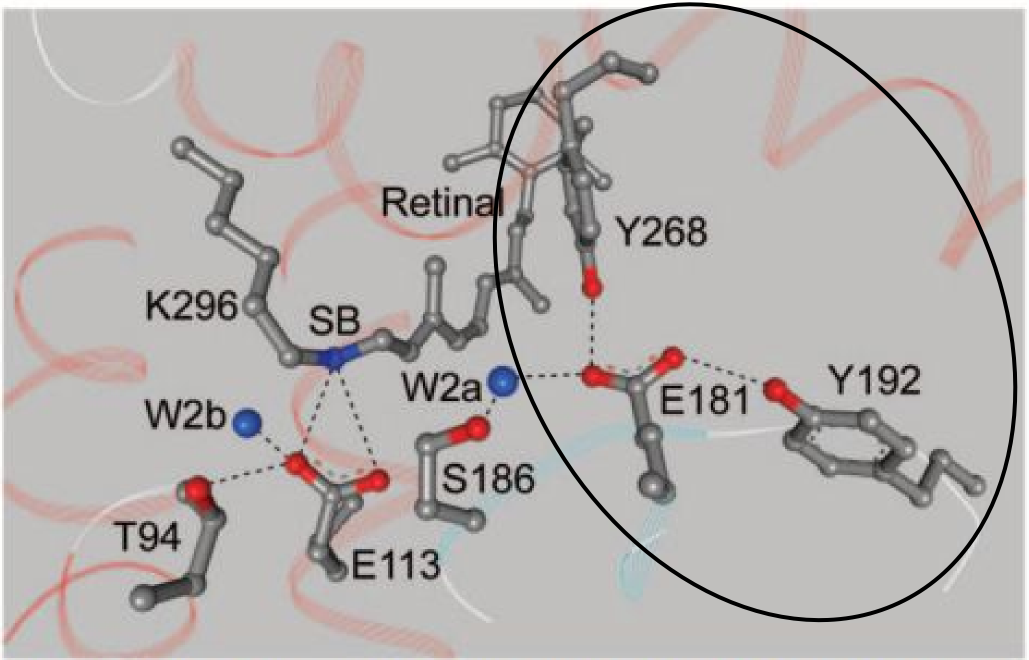
- Hydrophobic interactions
- Disulfide bond
- **Hydrogen bonding network**
- Salt bridge

Glu181 - Tyr192

ECL2

Tyr268

H6



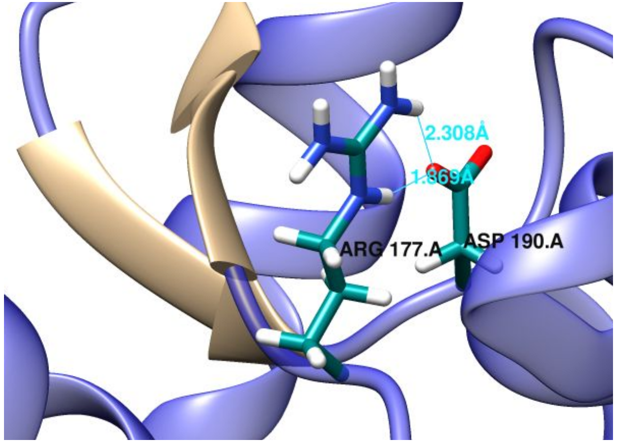
Extracted from Janz JM et. al., 2004

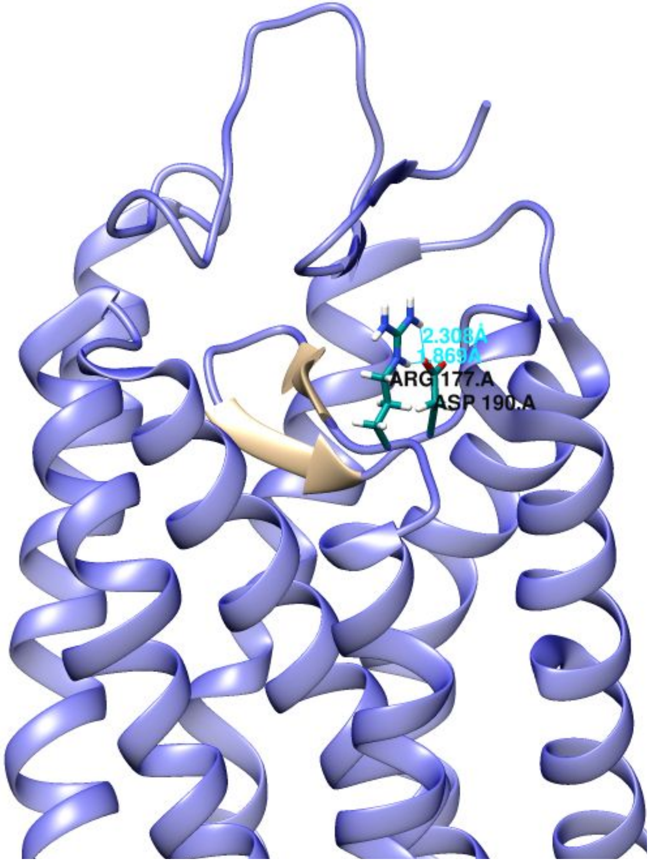
Second Extracellular Loop (ECL2)

The position of this β-sheet is stabilized by:

- Hydrophobic interactions
- Disulfide bond
- Hydrogen bonding network
- **Salt bridge**

Arg177 - Asp190





Second Extracellular Loop (ECL2): ACTIVE

ECL2 is part of the retinal binding site

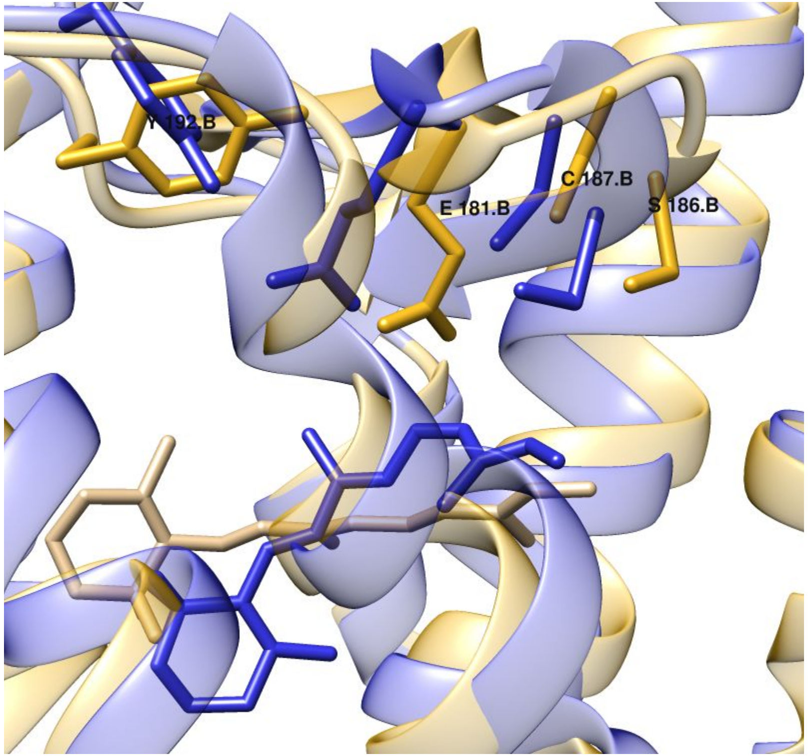
- Conformational changes in key residues:

Glu 181 **Ser 186**

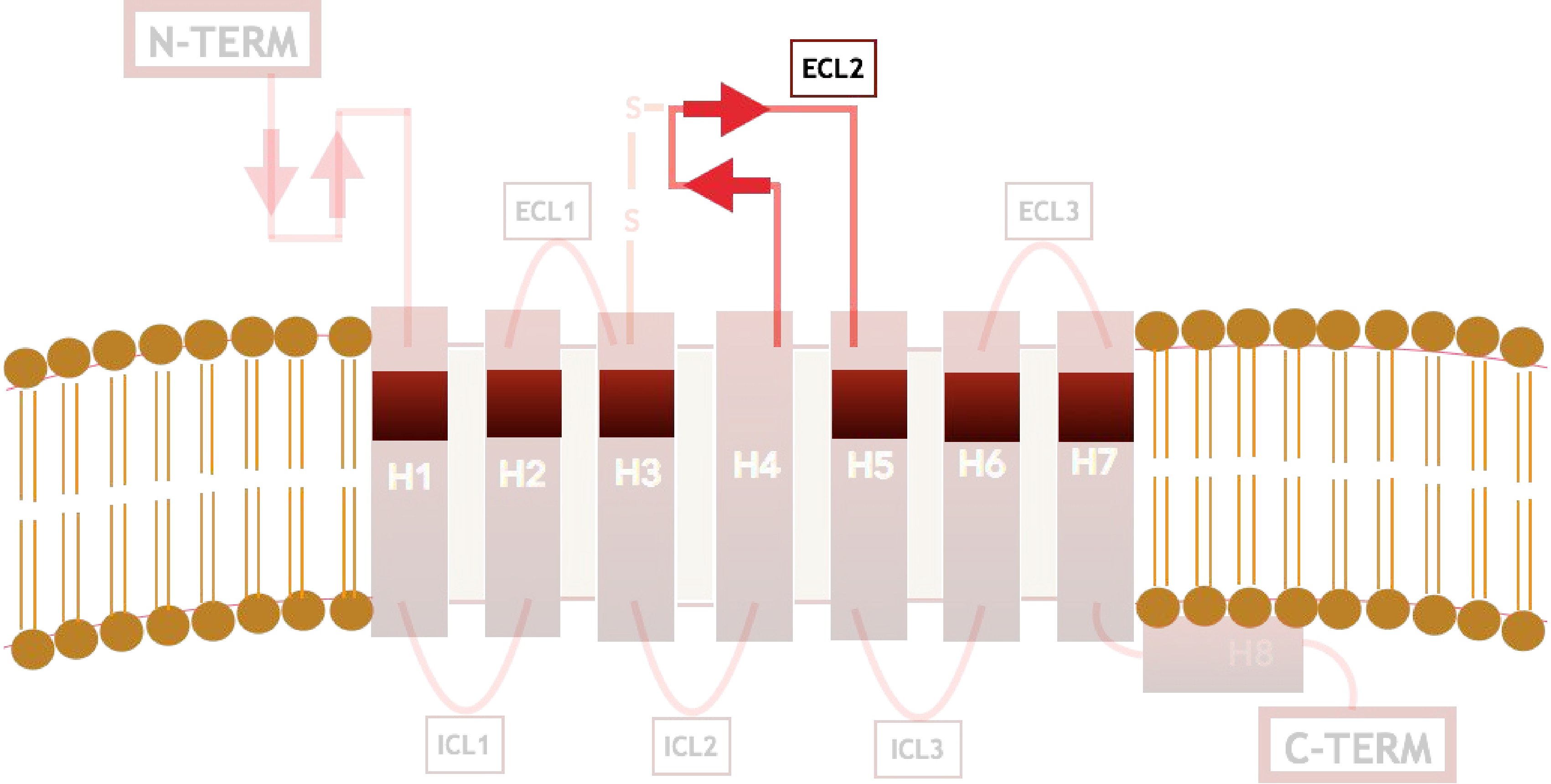
Cys 187 **Tyr 192**

These residues are in contact and covering the retinal molecule

ACTIVE INACTIVE

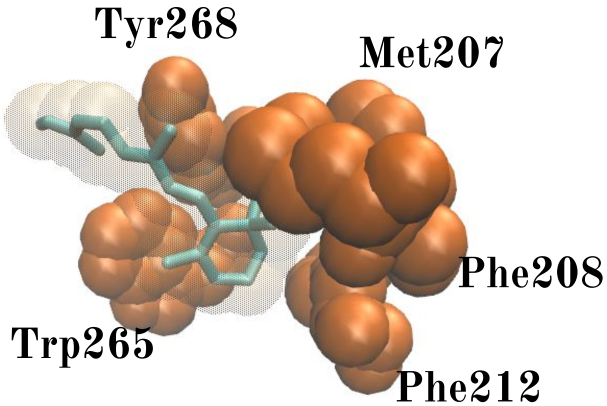


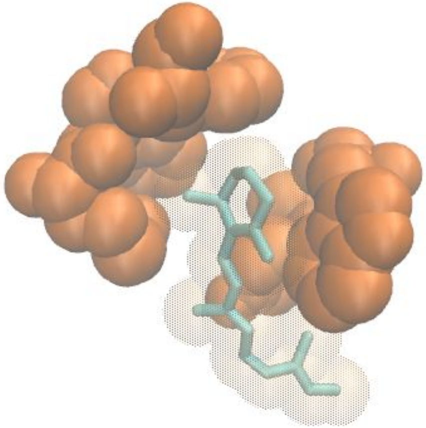
Ligand binding site

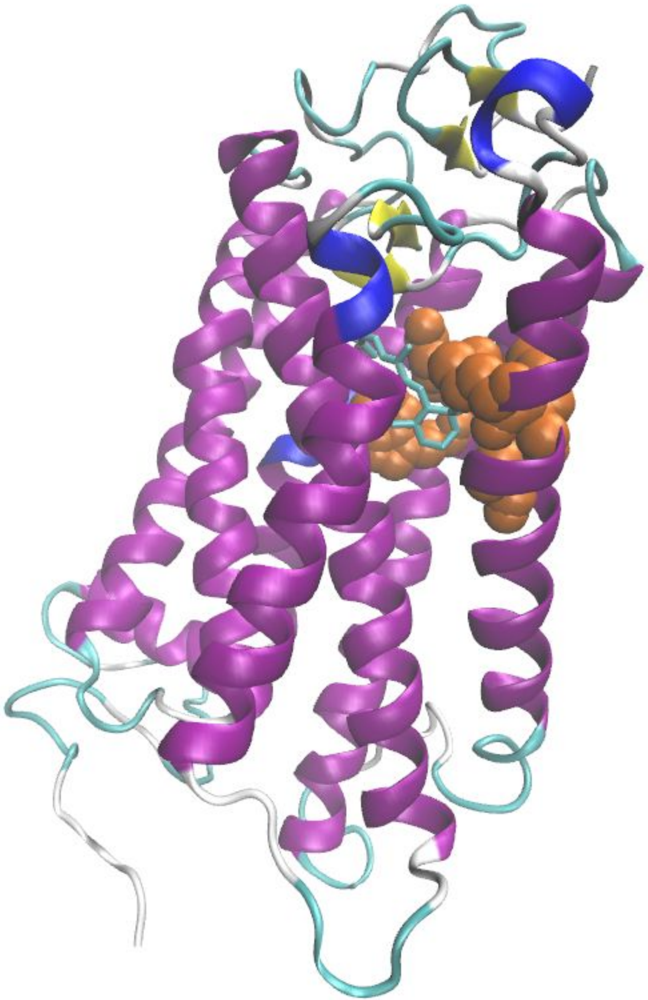


Ligand binding site

- Extracellular side of the transmembrane bundle
- Mostly surrounded by hydrophobic residues
- Stabilizes retinal
- Dynamic changes during photoactivation



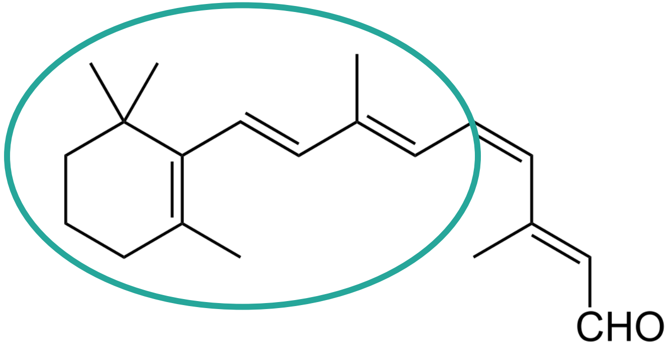


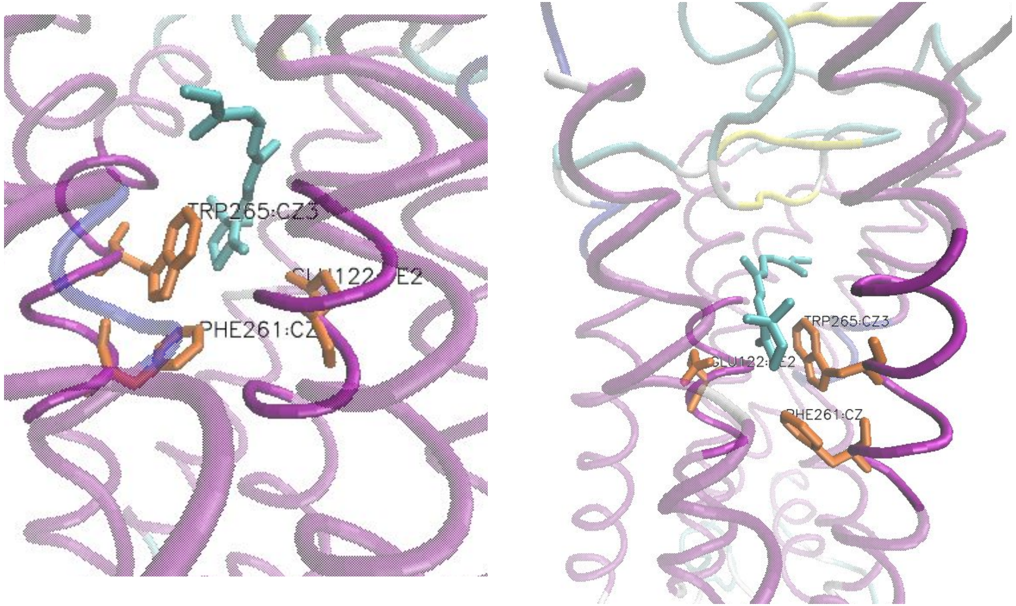


Ligand binding site

Retinal: cis-conformation

β-ionone ring





Position of β-ionone ring:
→ **covered** from the cytoplasmic side by residues from H3 and H6

→ **Glu122, Phe261, and Trp265**

Ligand binding site

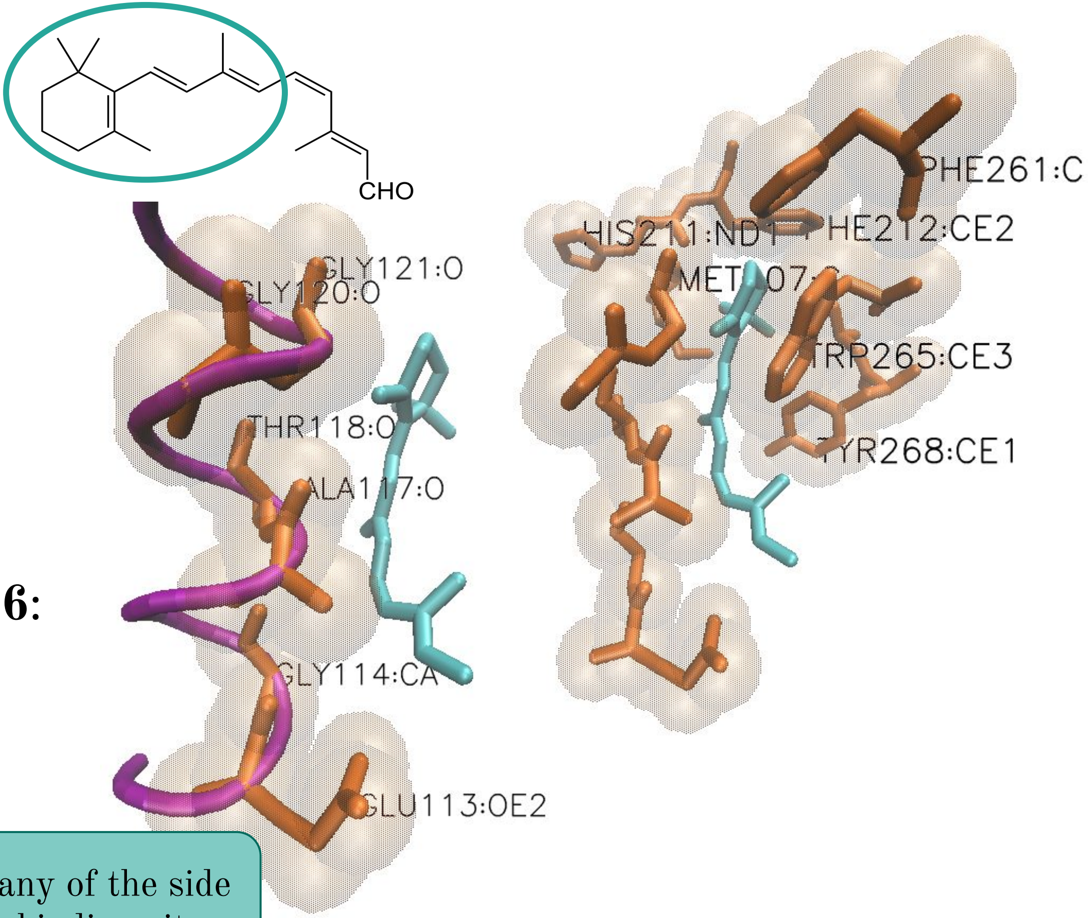
Retinal: cis conformation

β-ionone ring

From the β-ionone ring to C11, the retinal runs **parallel to H3**

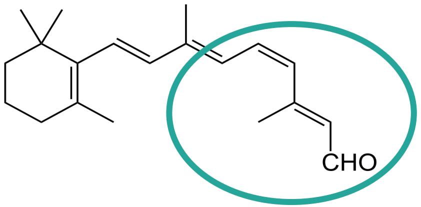
Side chains, mostly from **H5 and H6**:

- Surround the β-ionone ring
- Restrict its orientation



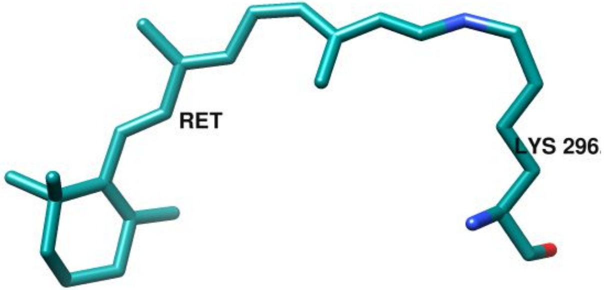
H3 provides many of the side chains for the binding site

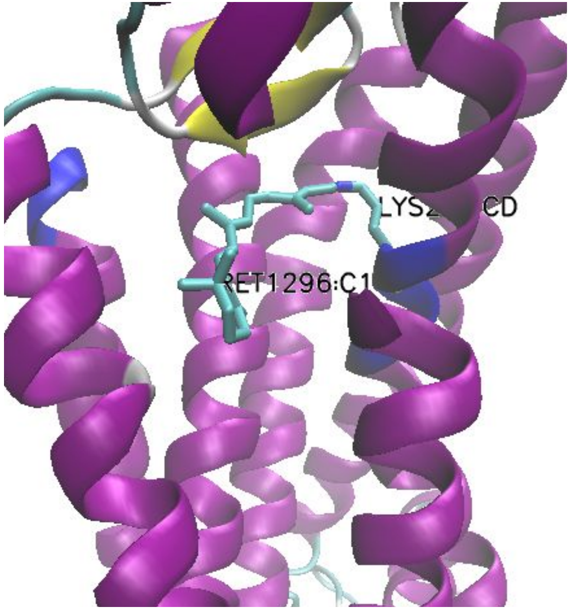
Ligand binding site

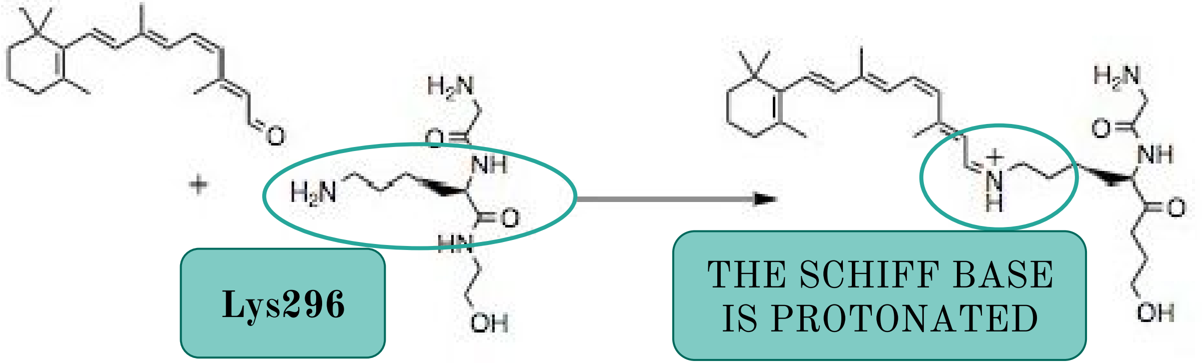


Schiff base

Retinal binds covalently to Lys296 (H7)







Lys296

THE SCHIFF BASE IS PROTONATED

Ligand binding site

Schiff base: hydrophobic interactions

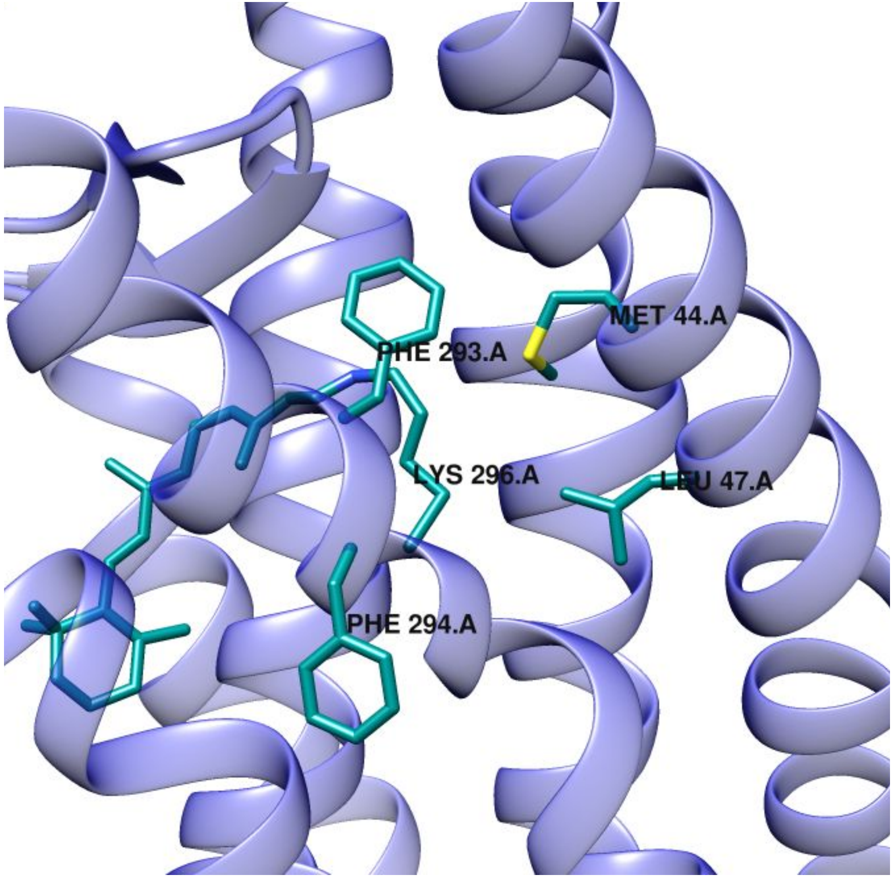
Side chain direction of Lys296 is supported by:

- A nearby peptide bond

  Phe293 and Phe294

- Hydrophobic side chains in H1

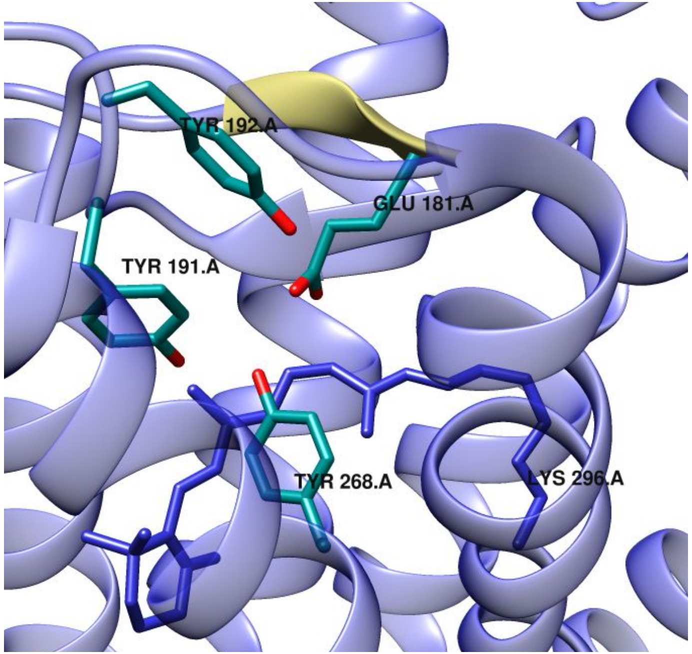
  Met44 and Leu47

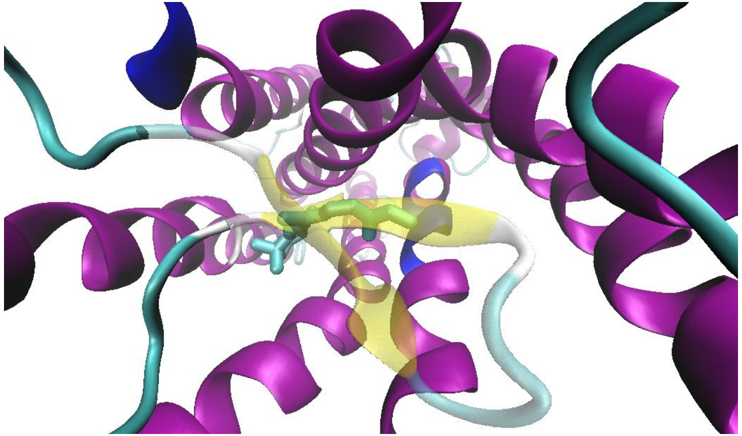


Ligand binding site

Schiff base: extracellular site

→ covered by $\beta4$ sheet from ECL2





Glu181

($\beta3$ sheet)

→ Surrounded by a cluster of 3 polar residues

Tyr191 **Tyr192** **Tyr268**

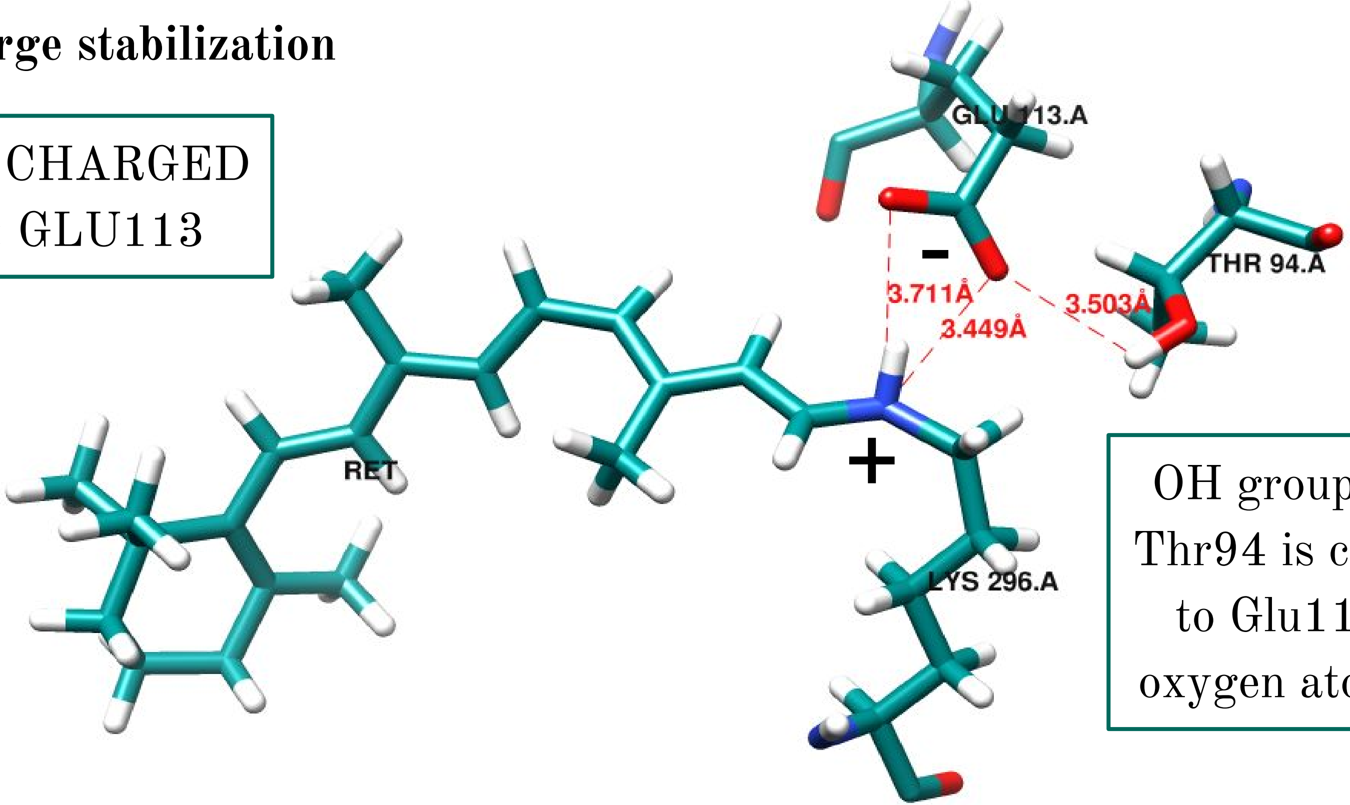
Their OH groups cover part of the retinal binding site

→ Side chain points towards the retinal

Ligand binding site

Schiff base: charge stabilization

NEGATIVELY CHARGED COUNTERION: GLU113



OH group of Thr94 is close to Glu113 oxygen atoms

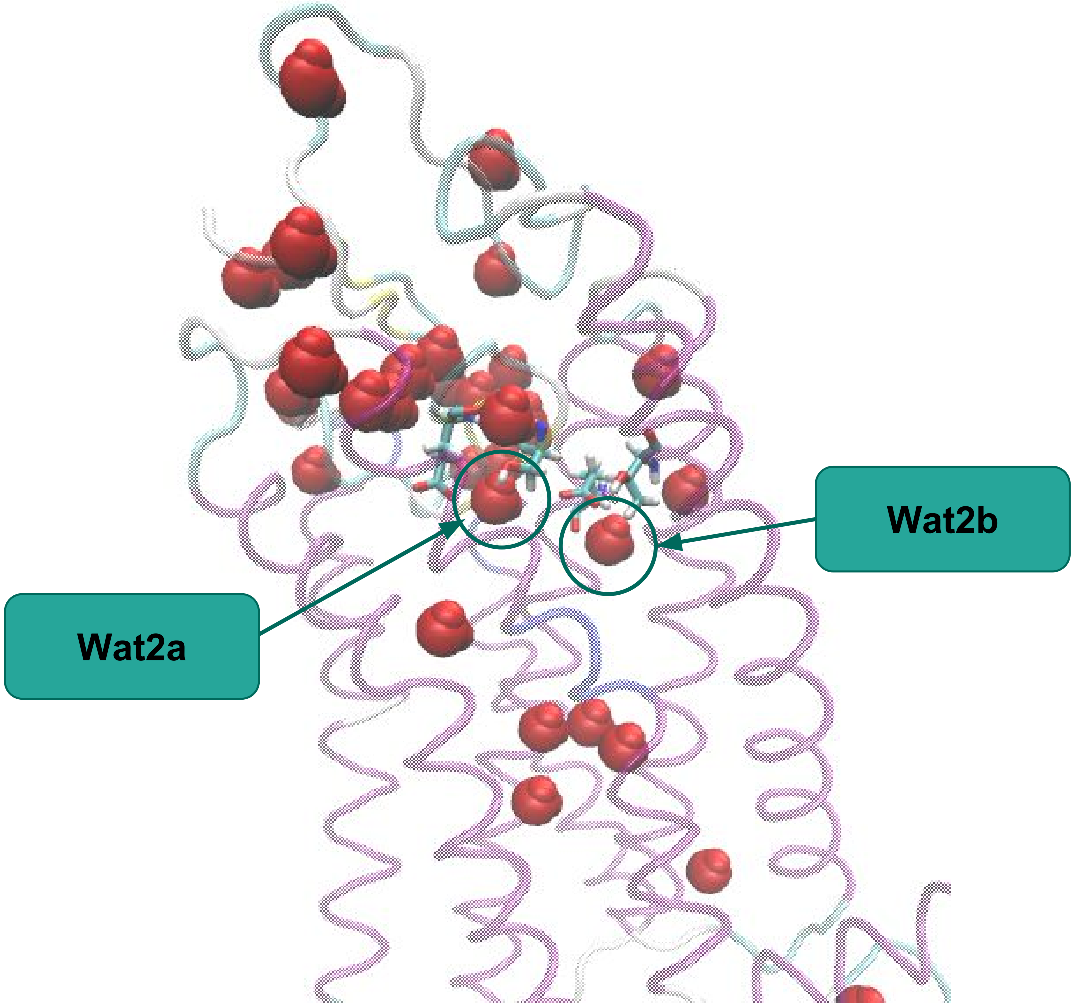
Ligand binding site

Hydrogen bonding network

↓

Important water molecules

Same position in all crystals

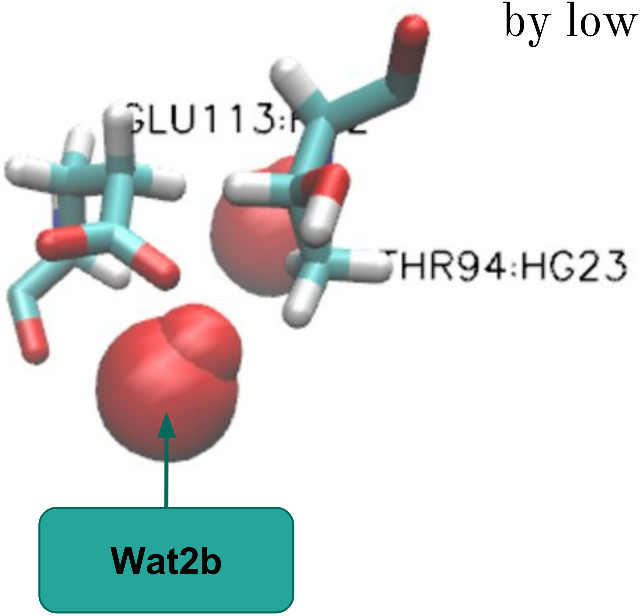


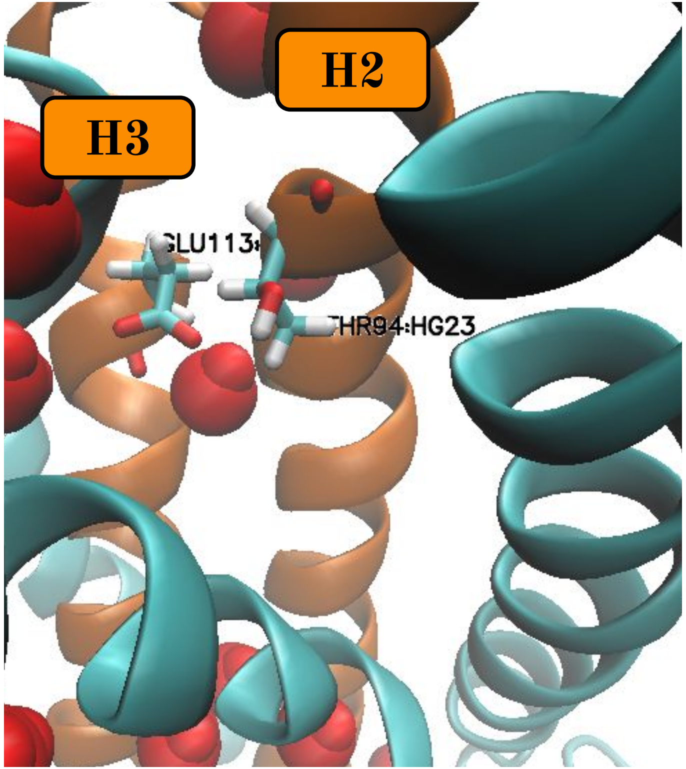
Ligand binding site

Fills a spatial gap between H2 and H3 at the retinal-binding site

Hydrogen bonding network

Wat2b → Near Glu113: stabilizes salt bridge by lowering its pKa

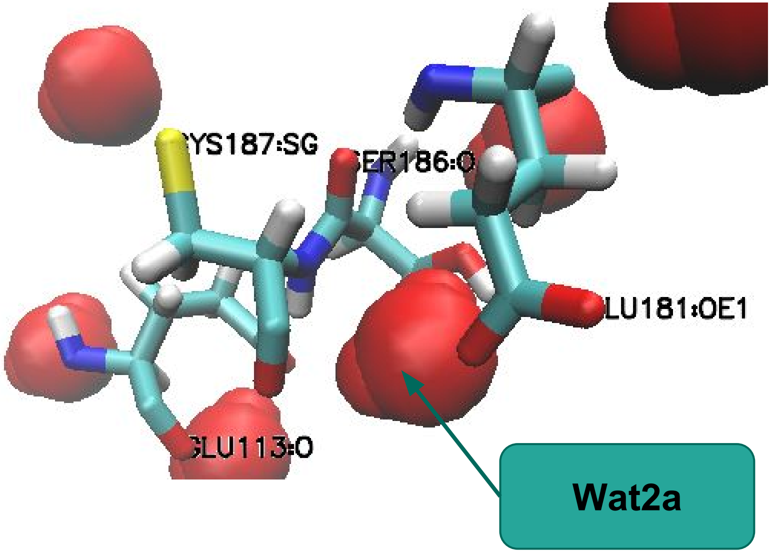




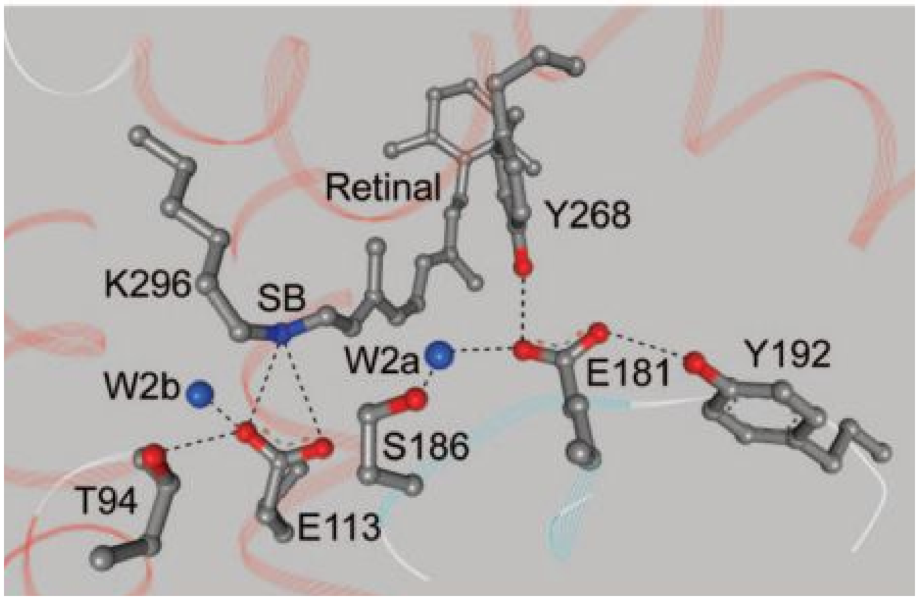
Ligand binding site

Hydrogen bonding network

Wat2a → H bonds: Ser186, Cys187 and Glu181



H-bonding network postulated on the basis of QM/MM modelling and MD simulations



Extracted from Janz JM et. al., 2004

Ligand binding site

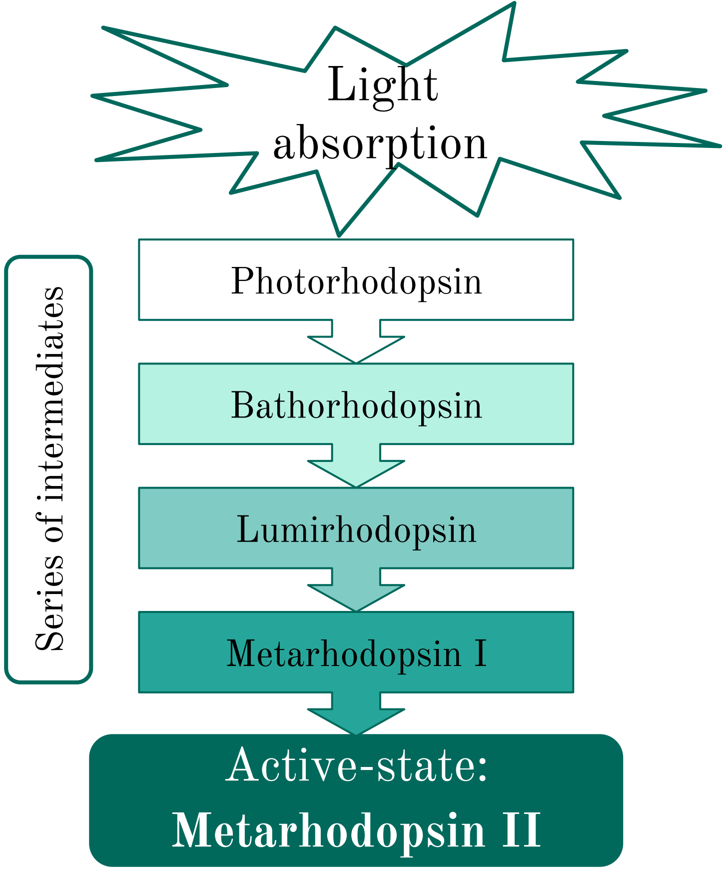
Factors contributing to the stability of the protonated Schiff base:

- Negatively charged Glu113 at a very short distance from the Schiff base
- Hydrogen bonding network:
  - Reduces Glu113 basicity
  - Thr94 and Wat2b involved

The proton is kept at the Schiff base nitrogen atom rather than at the carboxylate oxygen atom

CHANGES IN PHOTOACTIVATION

Ligand binding site: **ACTIVE**



Light absorption

Series of intermediates

Photorhodopsin

Bathorhodopsin

Lumirhodopsin

Metarhodopsin I

Active-state:
Metarhodopsin II

Photon-induced cis-trans isomerisation of retinal

The retinal flips 180º around the double bond

The β-ionone ring tilts towards the space between helices 5 and 6

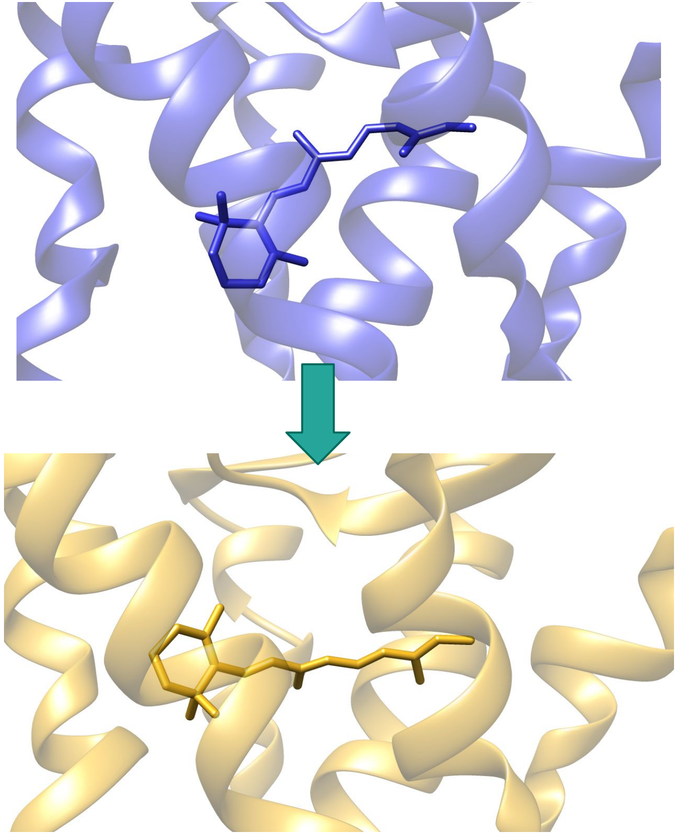
Deprotonation of the Schiff base

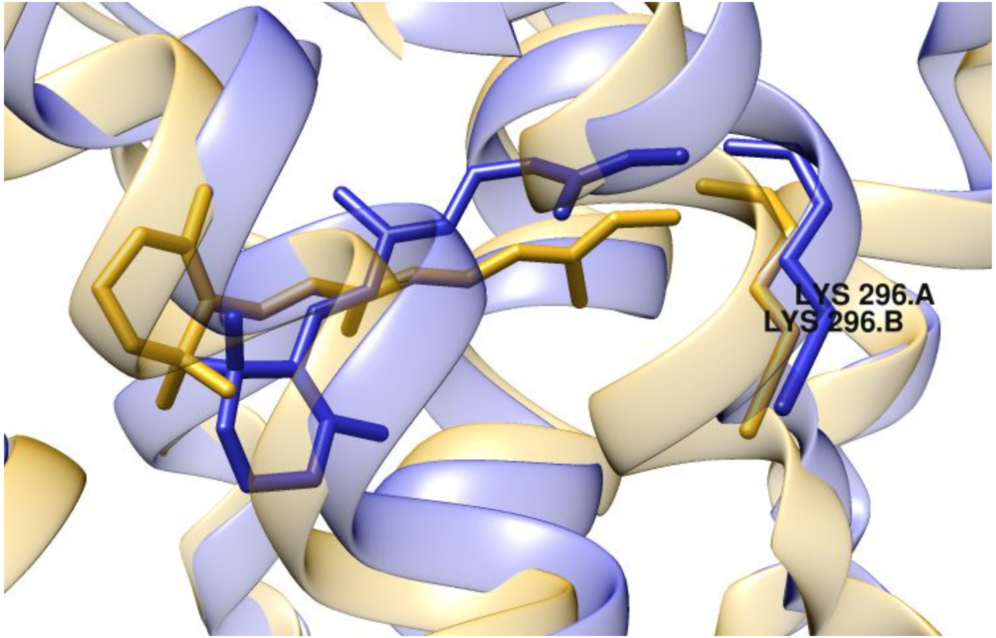
Protonation of Glu113

Ligand binding site: ACTIVE

ACTIVE

INACTIVE





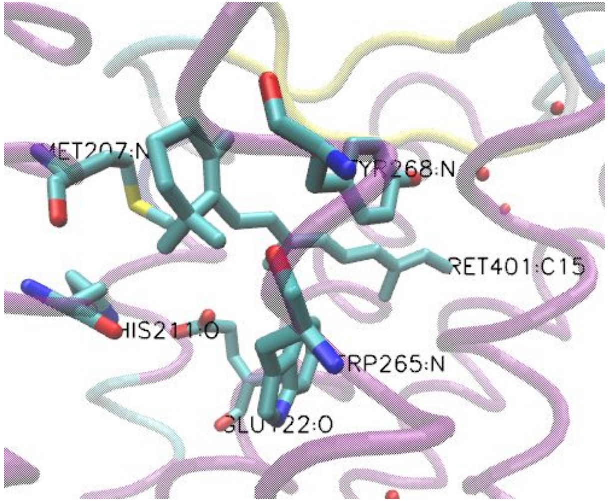
Ligand binding site: ACTIVE

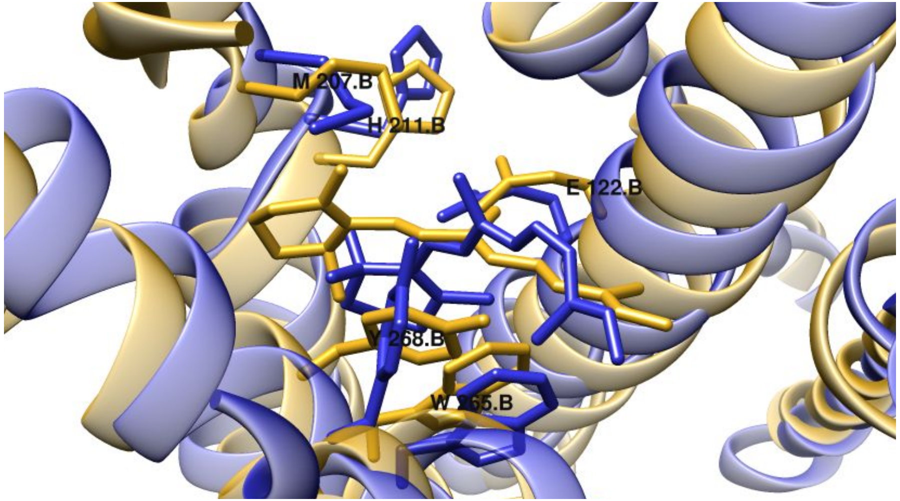
ACTIVE INACTIVE

The arrangement around the Schiff base is of particular interest in terms of understanding the mechanism of the photoactivation of rhodopsin.

Presence of retinal causes adjustment of side chains:

Met207 **His211** **Glu122** **Trp265** **Tyr268**





Ligand binding site: ACTIVE

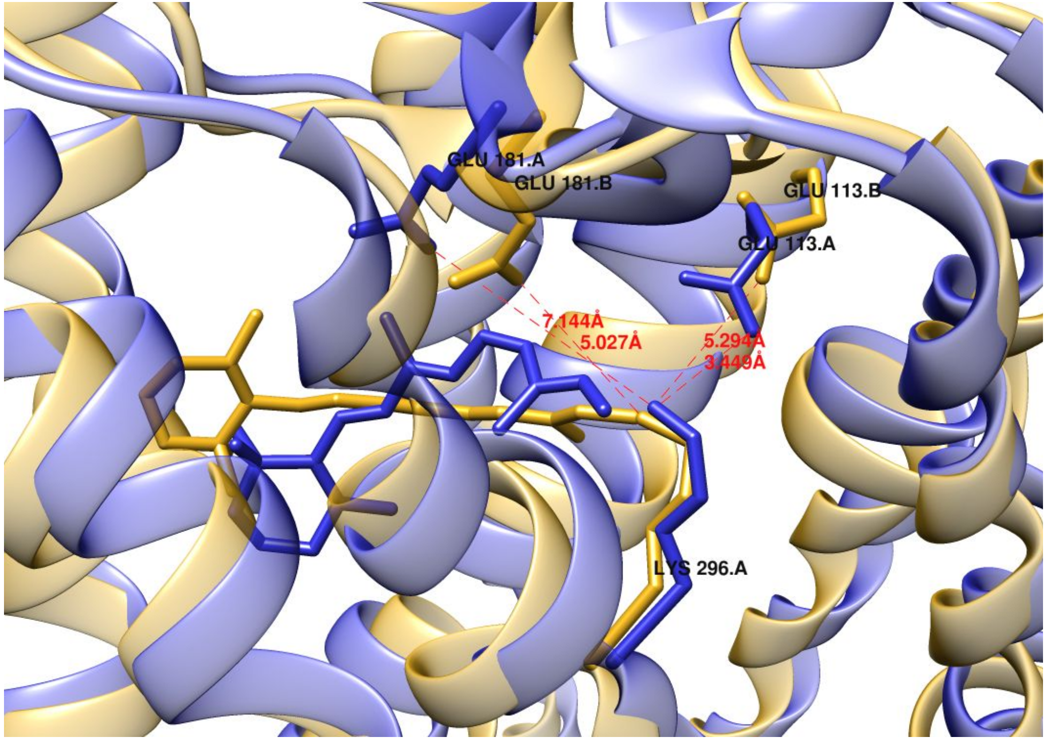
ACTIVE INACTIVE

↑ Distance between Schiff base and side chain carboxyl group of Glu113

3,4 to 5,3Å

↓ Distance between Schiff base and Glu181

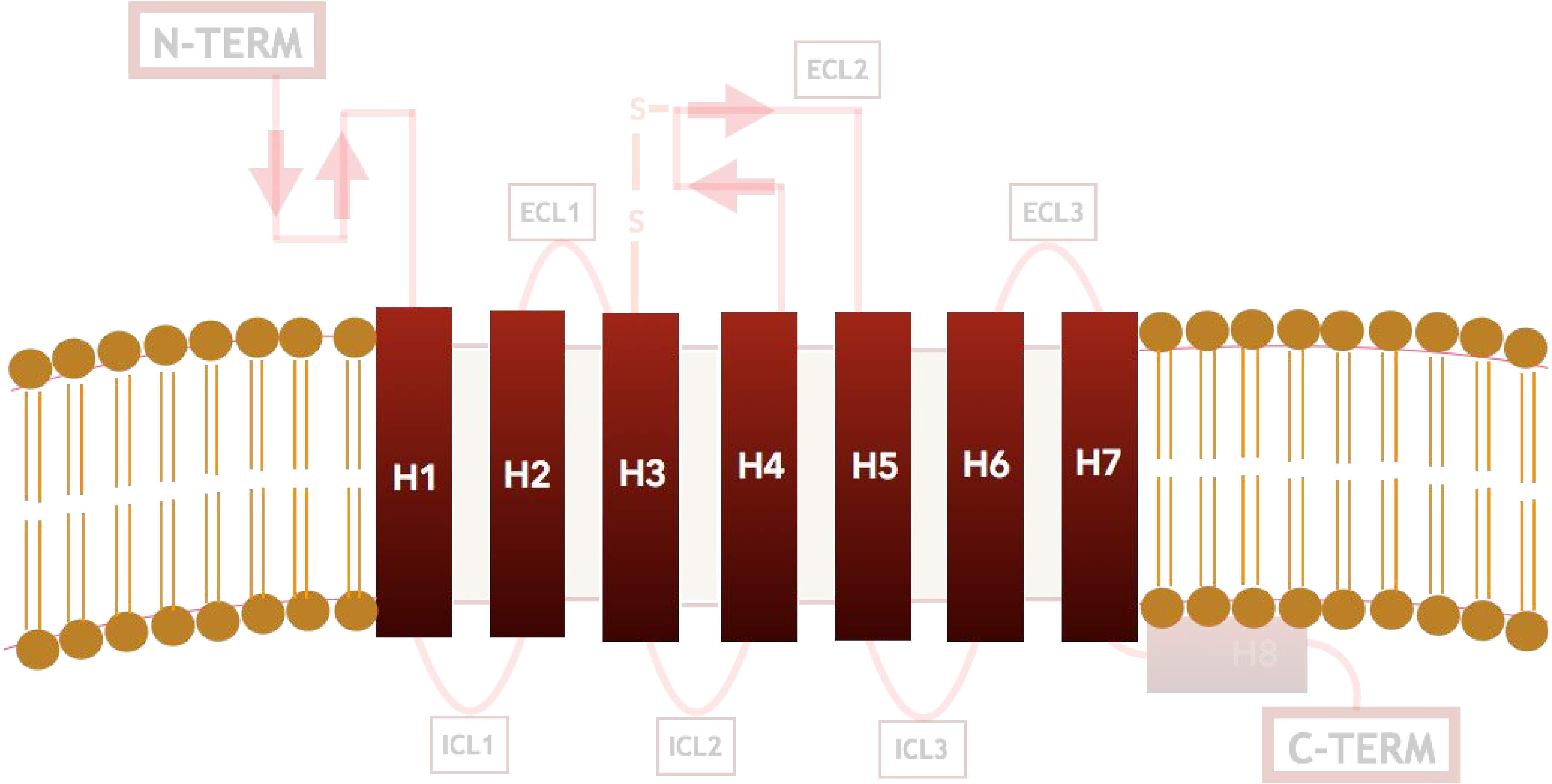
7,1 to 5,0Å



Ligand binding site: INACTIVE vs. ACTIVE

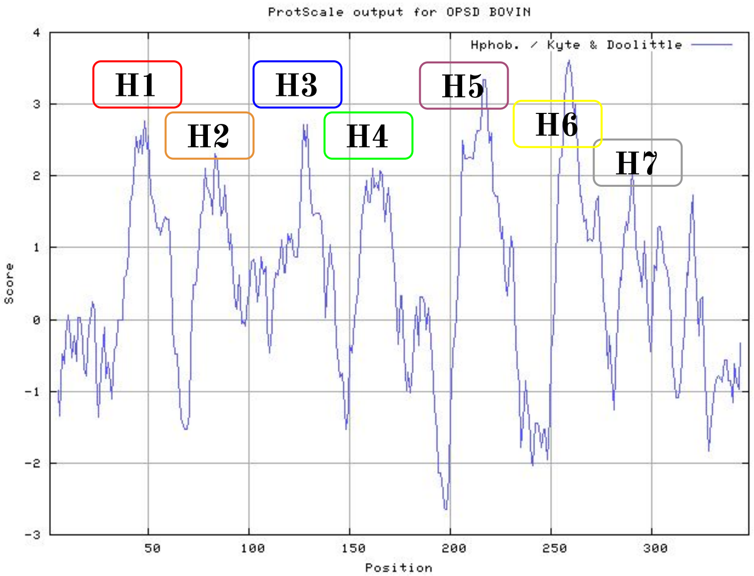
INACTIVE	ACTIVE
→ Protonated Schiff base	→ Deprotonated Schiff base
→ Short distance between Schiff base and Glu113 (counterion)	→ Elongation and torsional motions of retinal polyene chain and β-ionone ring
→ Complex hydrogen bonding network around ligand binding site	→ Changes in the H-bonding network

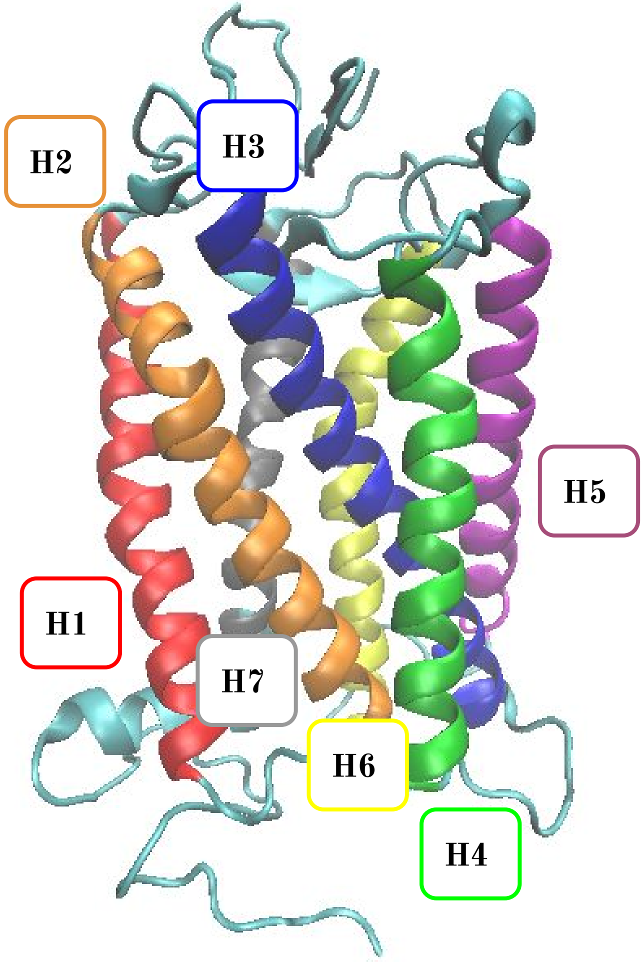
7TM domain


N-TERM
ECL2
S
S
ECL1
ECL3
H1
H2
H3
H4
H5
H6
H7
H8
ICL1
ICL2
ICL3
C-TERM

7TM domain

- Common in all GPCR
- 7 transmembrane helices



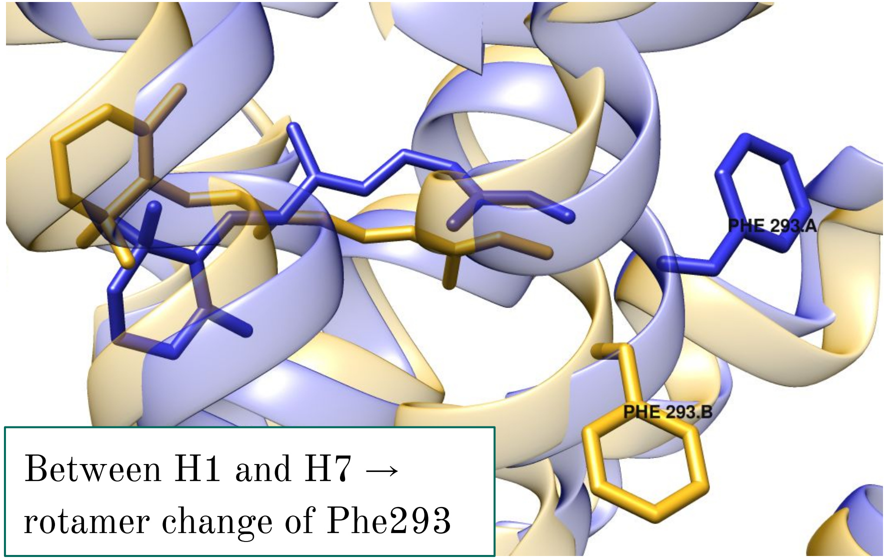


7TM domain: ACTIVE

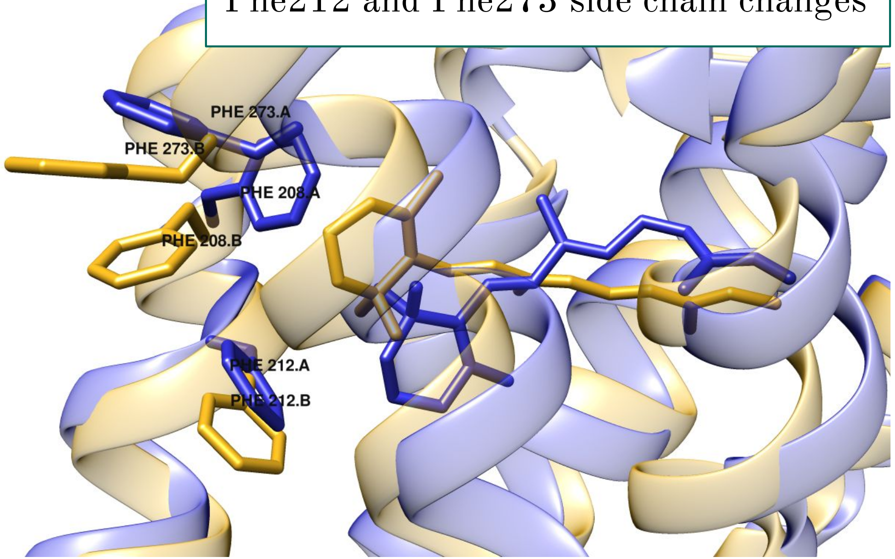
ACTIVE INACTIVE

Conformational changes after photoactivation

Two openings of the retinal binding site:



Between H1 and H7 → rotamer change of Phe293



Between H5 and H6 → Phe208, Phe212 and Phe273 side chain changes

7TM domain: ACTIVE

ACTIVE **INACTIVE**

Conformational changes after photoactivation

Pro267 acts as a hinge in H6

↓

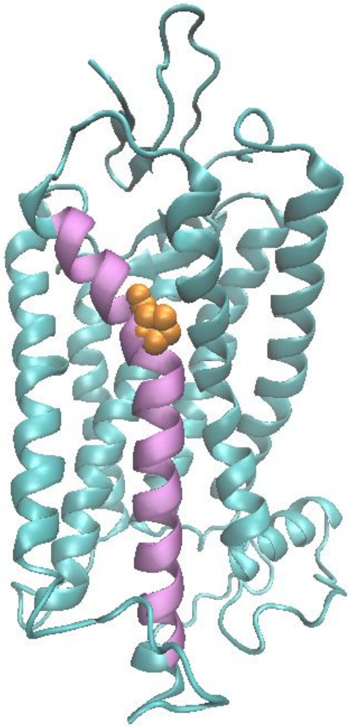
H6 bends away from the core

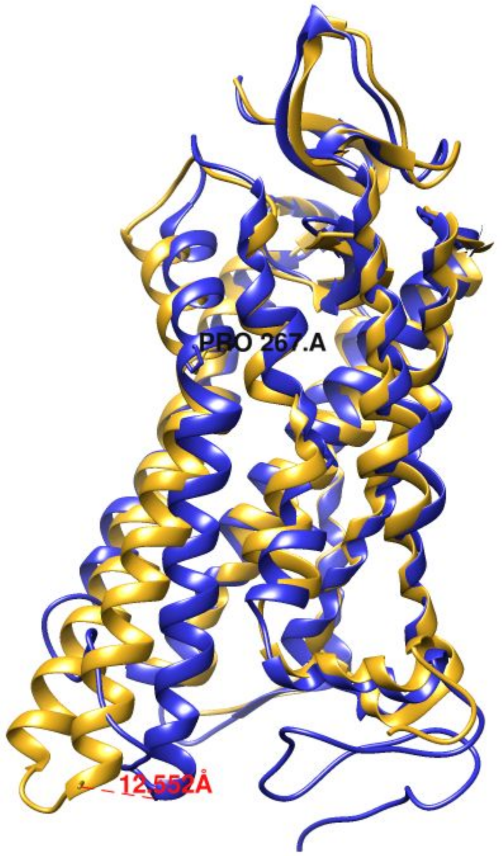
↓

A crevice in the cytoplasmatic side is created

↓

G-protein interaction and signal transduction





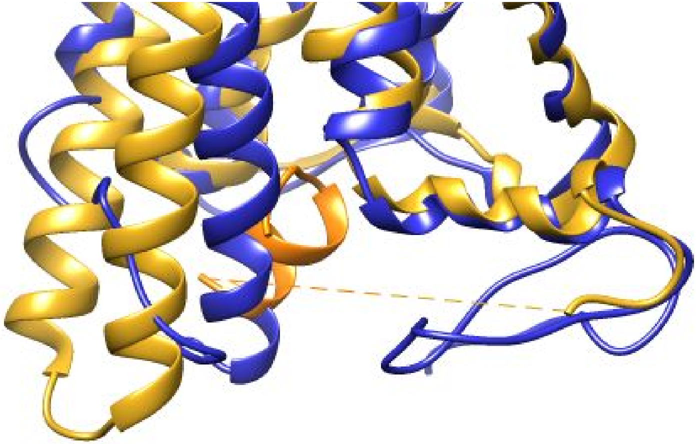
7TM domain: ACTIVE

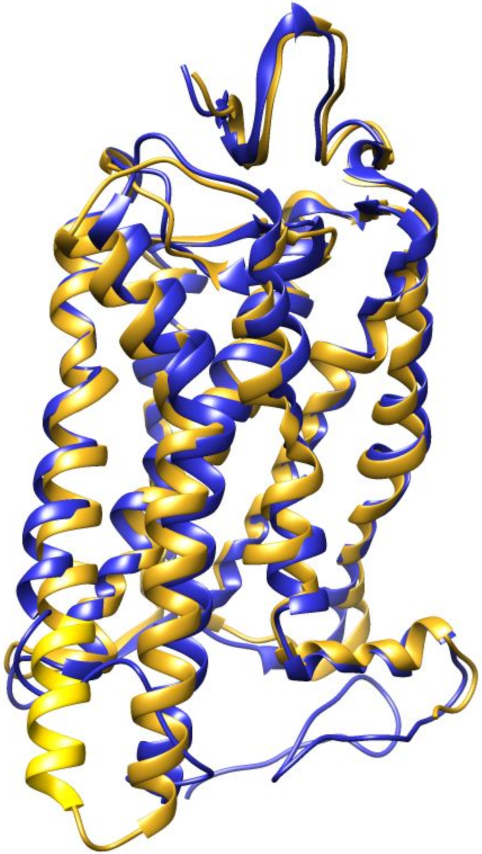
ACTIVE

INACTIVE

Conformational changes after photoactivation

- 8-residue elongation of H5
- Expansion of the interface for G protein interaction
- Higher binding affinity for G protein

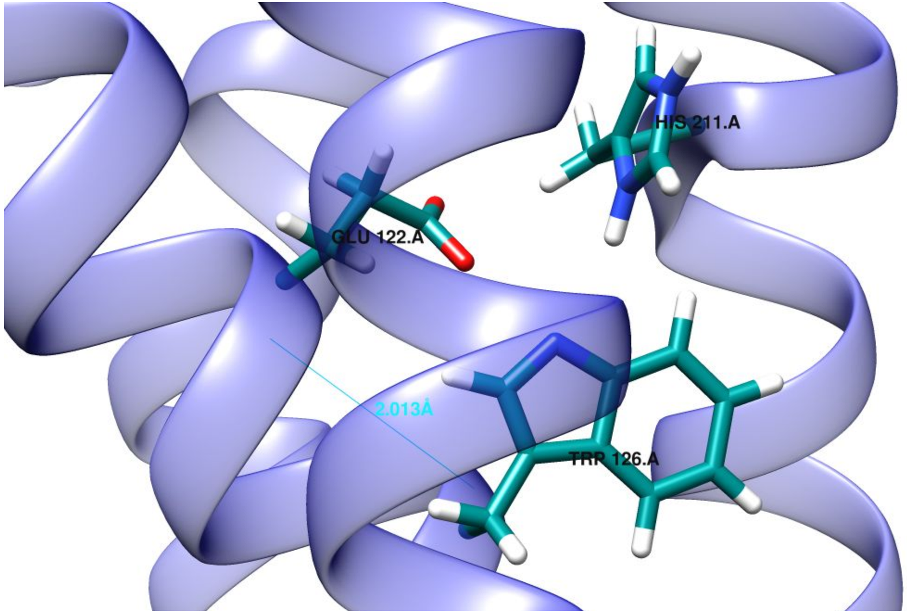


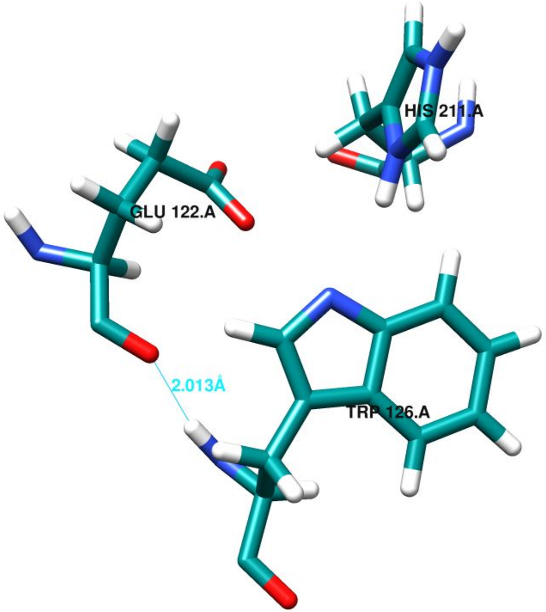


7TM domain

Conformational changes after photoactivation

Motion of H5 towards H6 → changes in the H3-H5 H bonding network





Glu122 - Trp126

His211

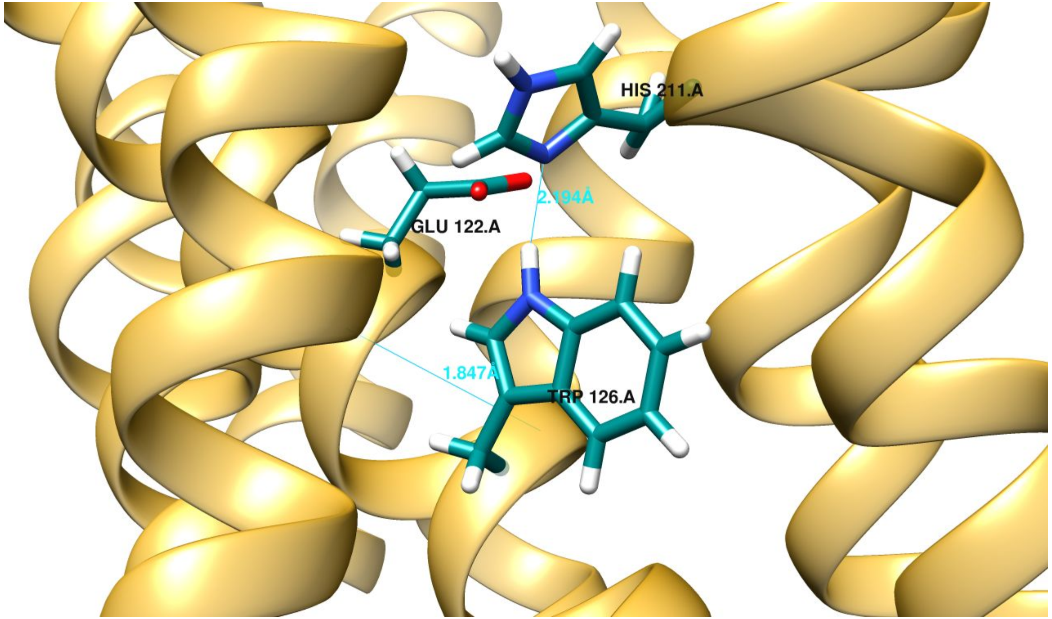
H3

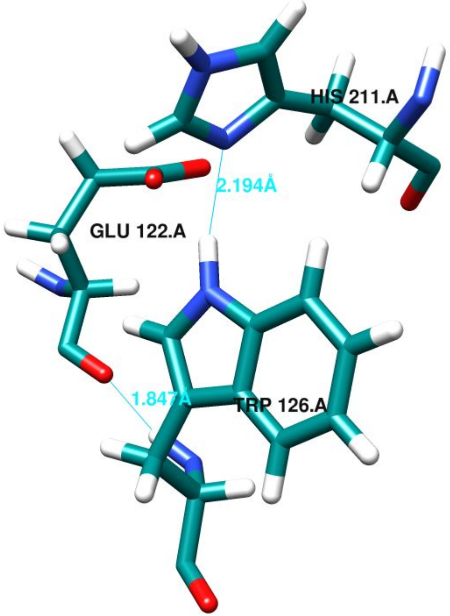
H5

7TM domain: ACTIVE

Conformational changes after photoactivation

Motion of H5 towards H6 → changes in the H3-H5 H-bonding network





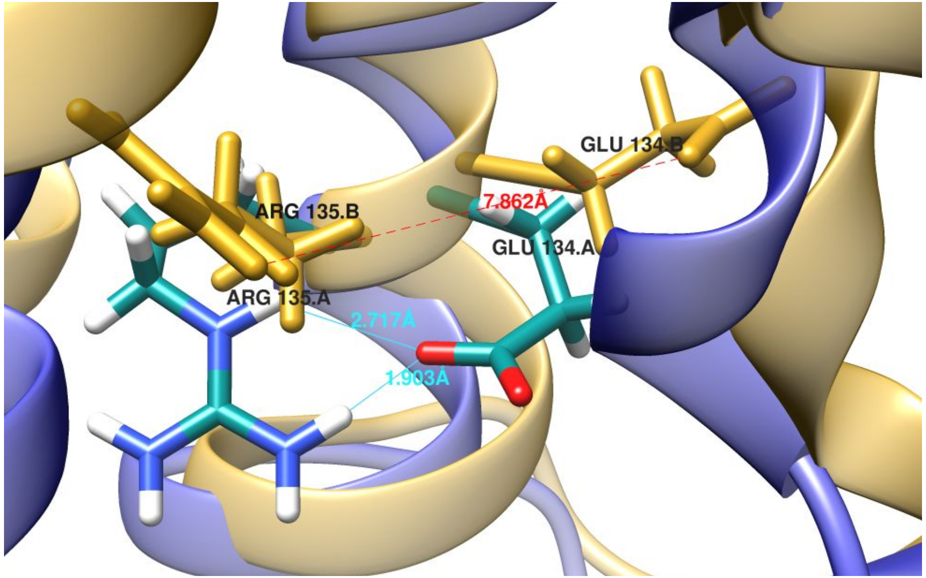
Glu122 - Trp126 - His211

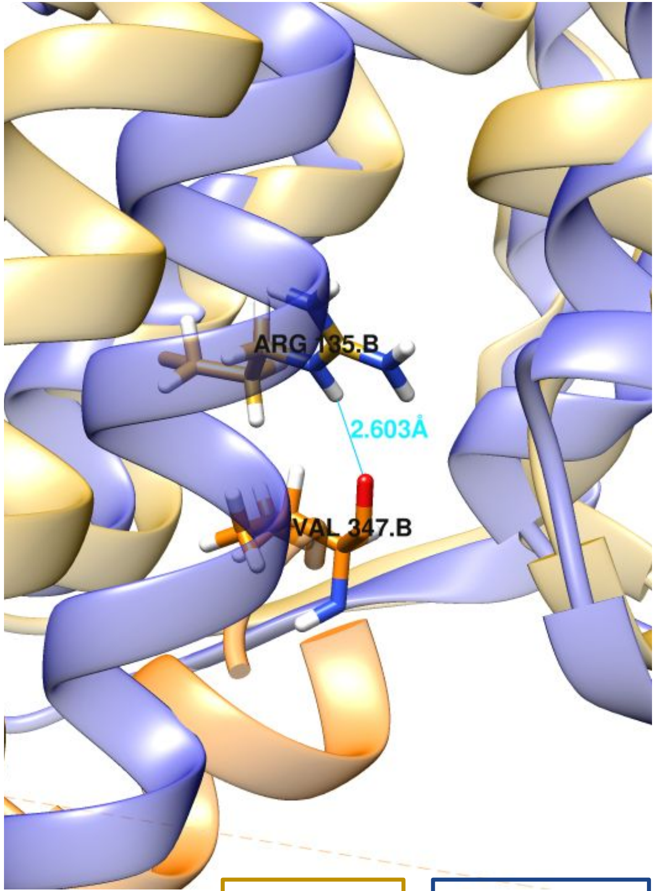
H3 — H5

7TM domain: ACTIVE

Conformational changes after photoactivation

- Glu134-Arg135 interaction in H3 is lost (ERY motif)
- H bond between Arg135 and Gα C-term (Val)

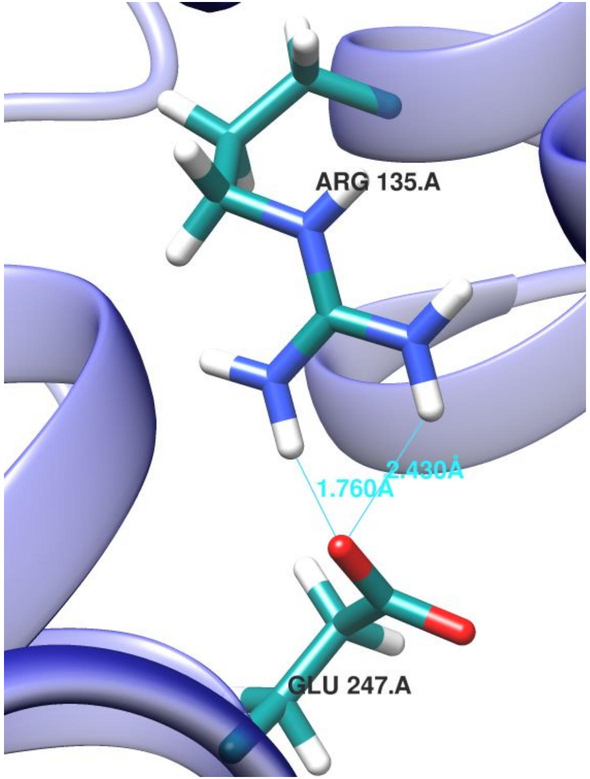




ACTIVE

INACTIVE

Ionic lock



Salt bridge

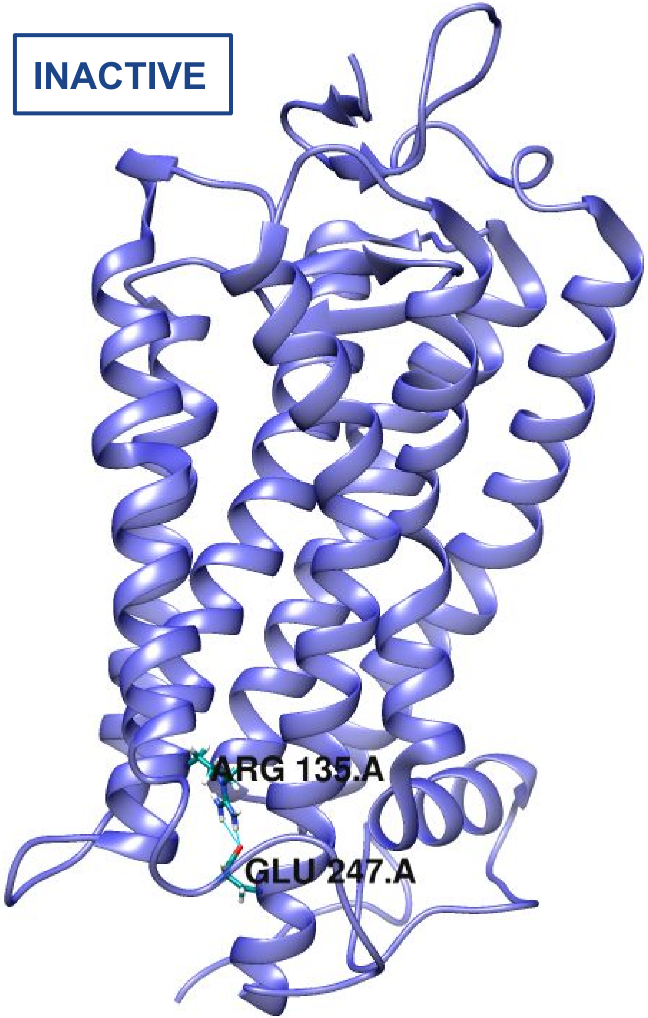
Arg135 - Glu247

H3 — H6

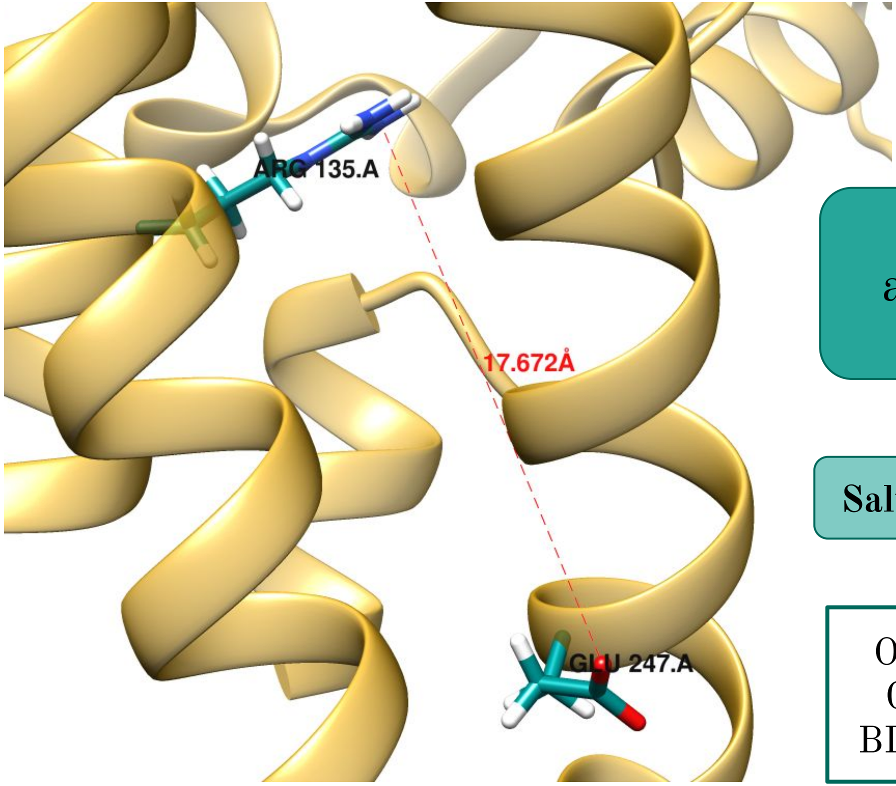
G-PROTEIN
BINDING SITE
IS BLOCKED

E(D)RY motif + E247:
Conserved in GPCRs

INACTIVE



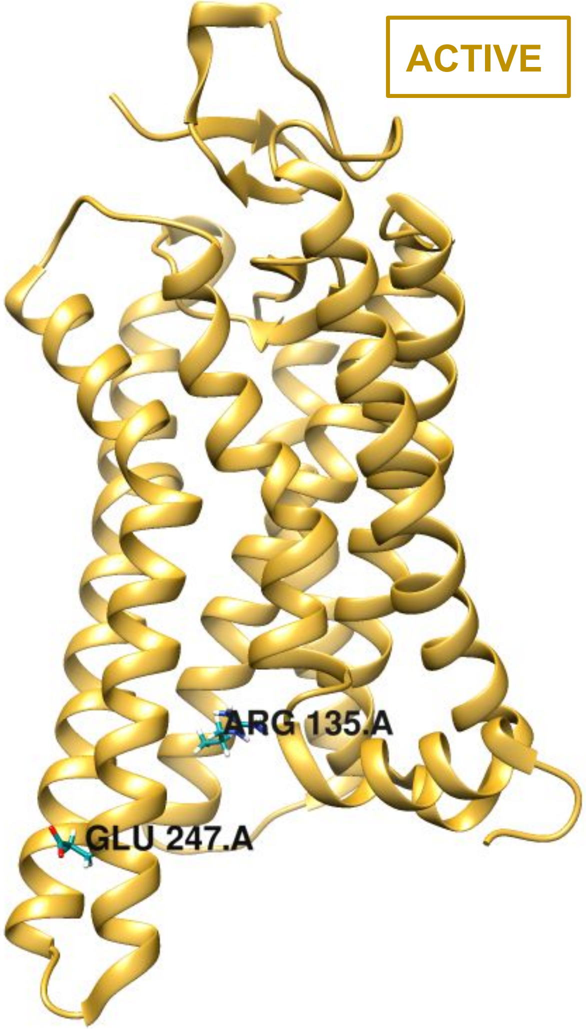
Ionic lock: ACTIVE



H6 bends away from the core

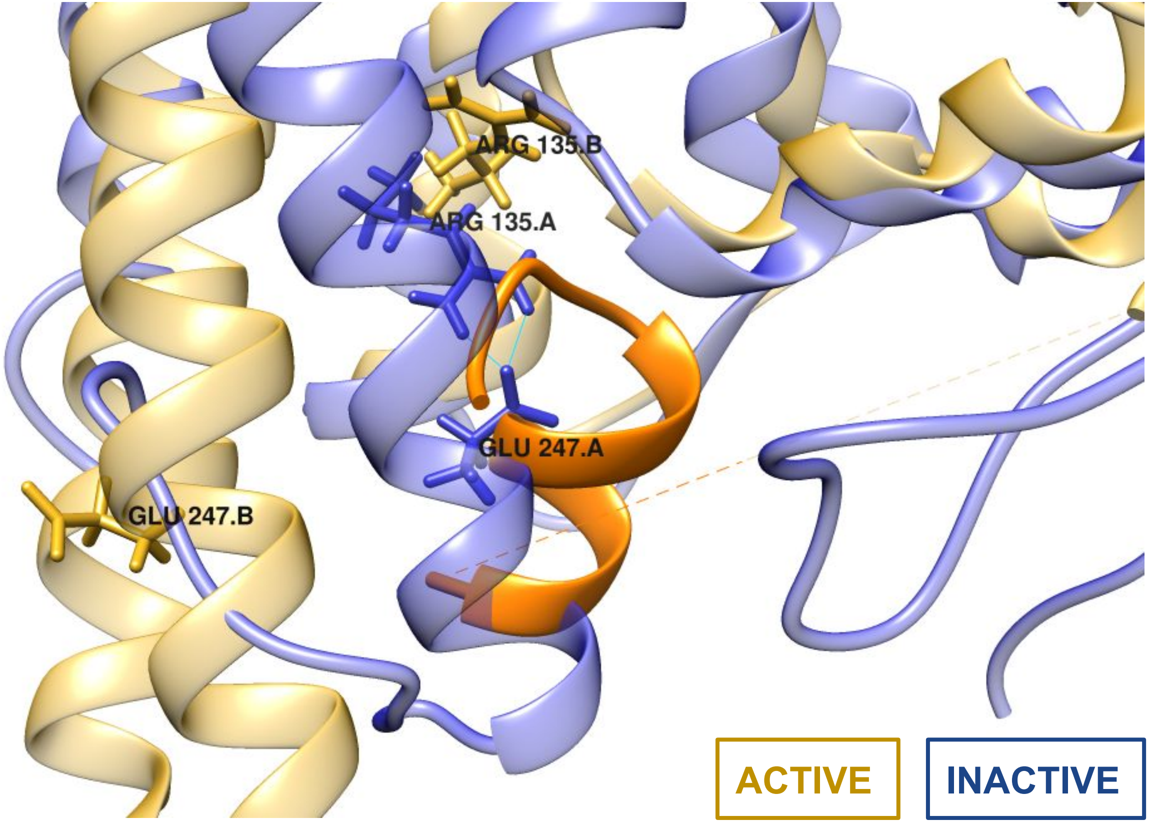
Salt bridge breaks

OPENING OF G-PROTEIN BINDING SITE



ACTIVE

Ionic lock: **INACTIVE** vs. **ACTIVE**



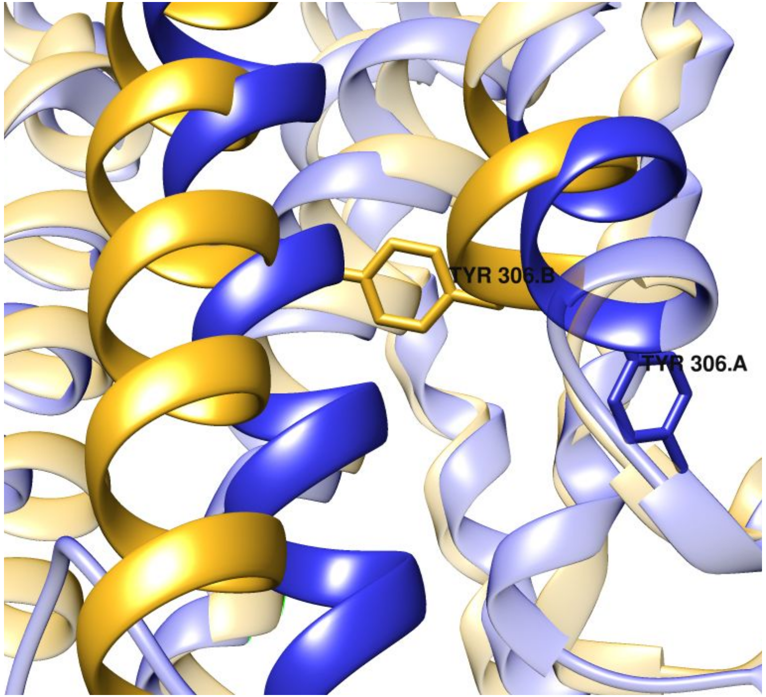
NPXXY motif

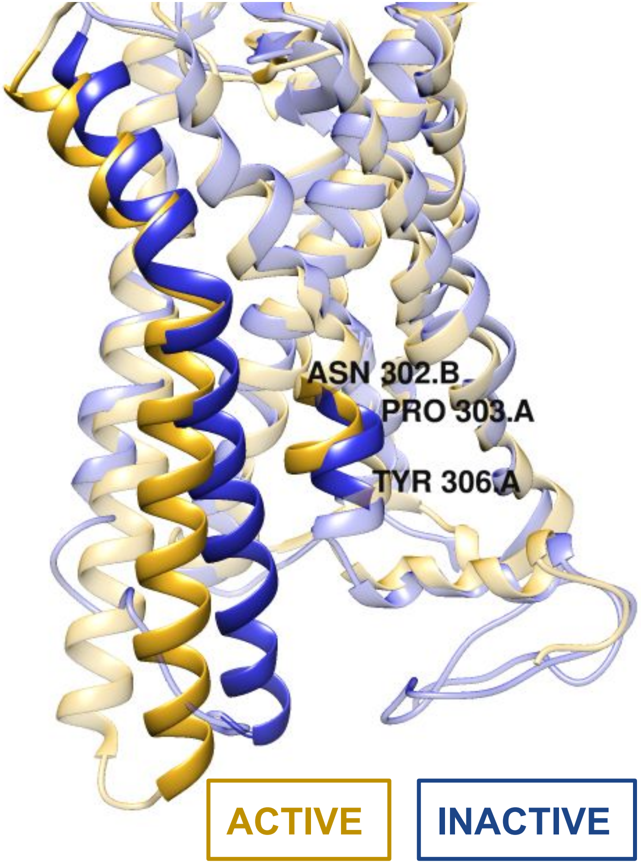
Conformational changes after photoactivation:

Tyr306 (H7) shifts towards H6

Pushes H6 away from the transmembrane bundle

Helps breaking the ionic lock





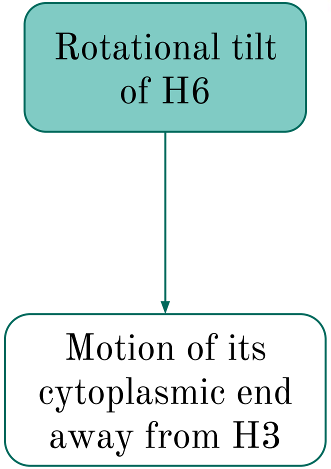
Common to all GCPRs

7TM domain and ionic lock: ACTIVE

ACTIVE

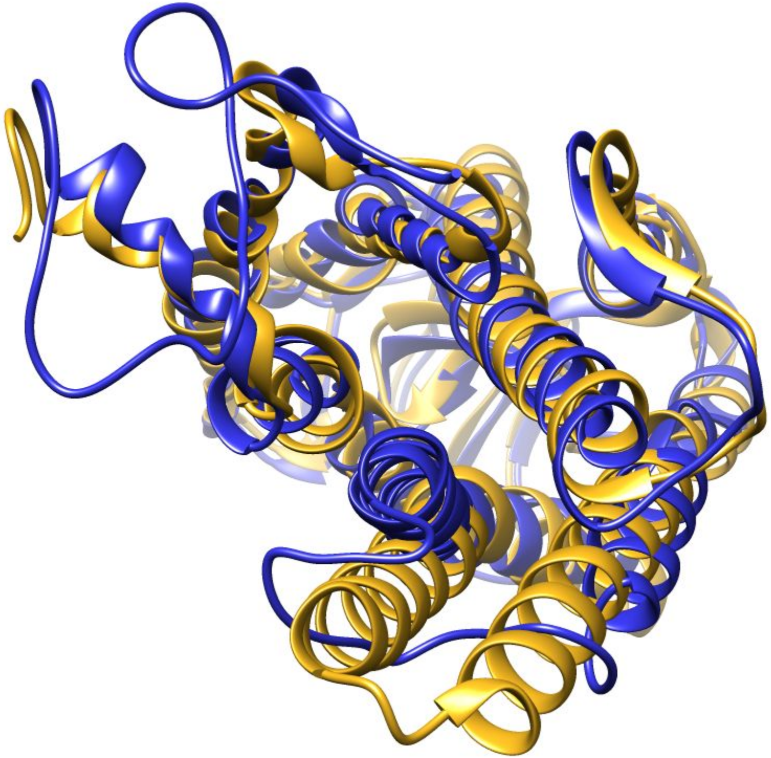
INACTIVE

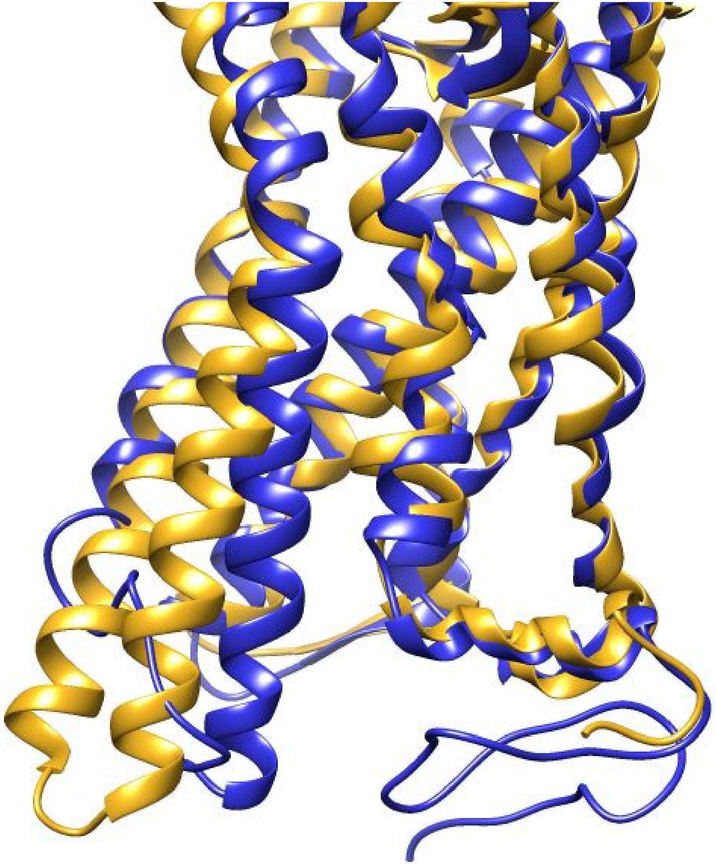
Conformational changes after photoactivation



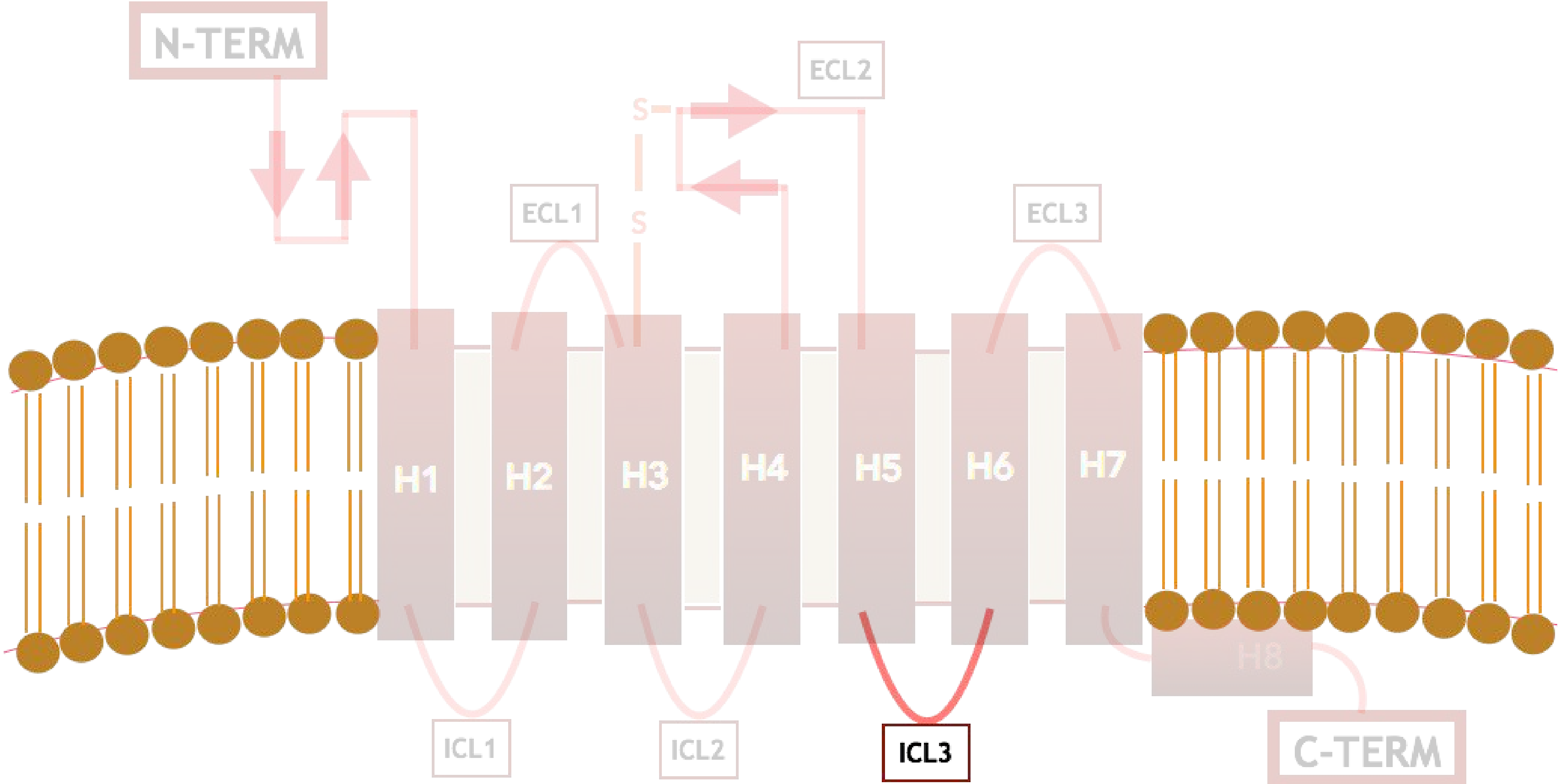
Rotational tilt of H6

Motion of its cytoplasmic end away from H3



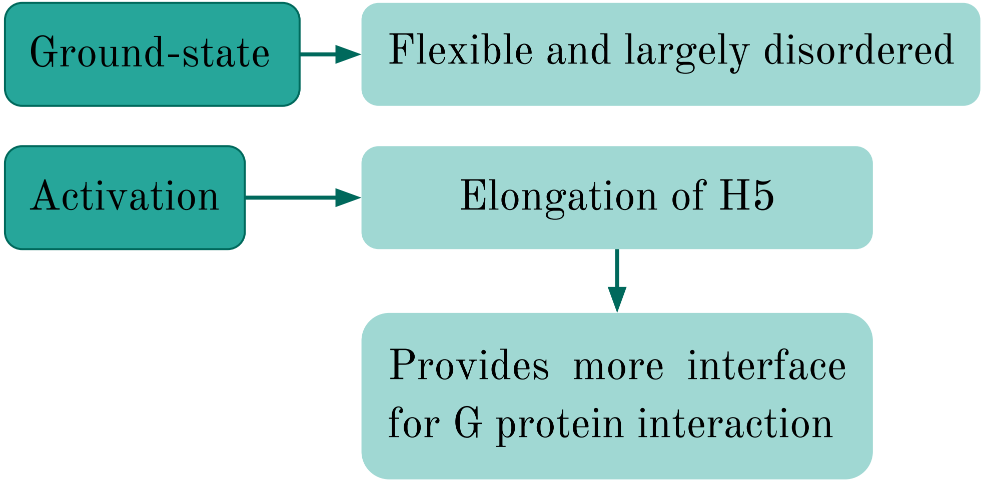


Third Intracellular Loop (ICL3)



Third Intracellular loop (ICL3)

Loop region between helices 5 and 6

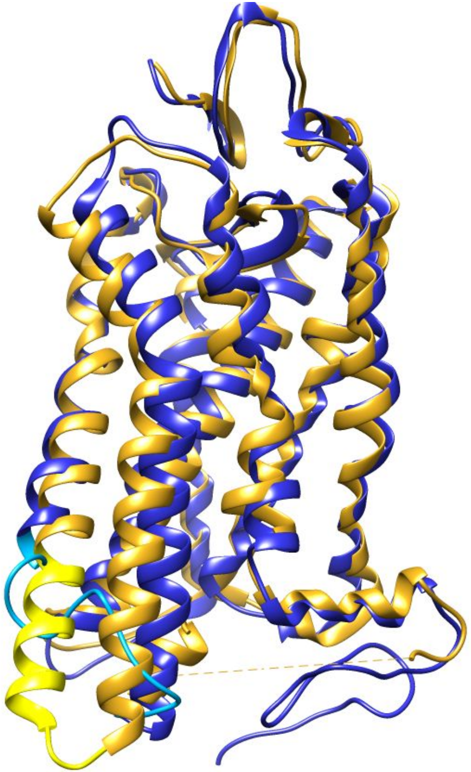


Ground-state → Flexible and largely disordered

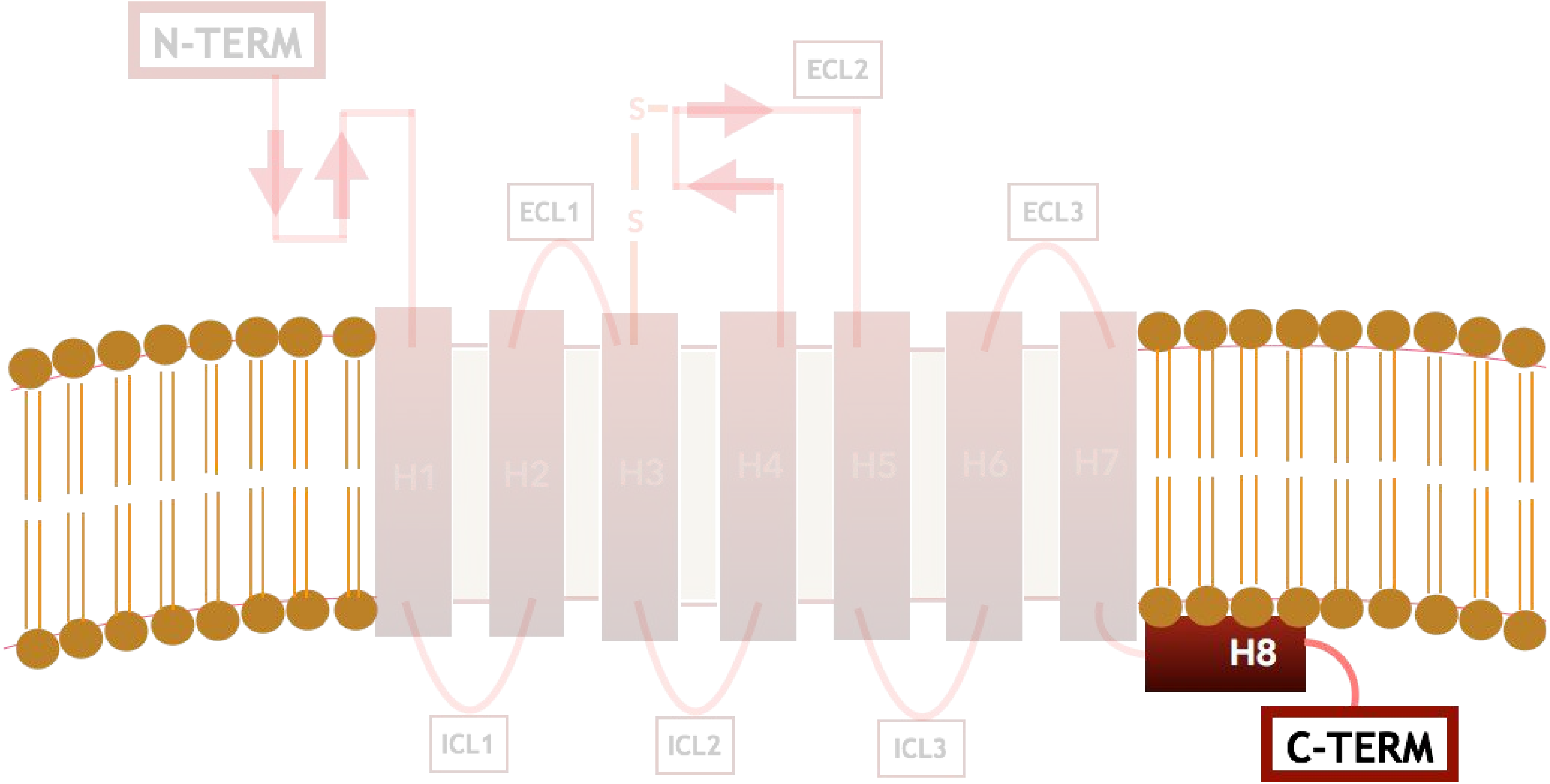
Activation → Elongation of H5 → Provides more interface for G protein interaction

ACTIVE

INACTIVE

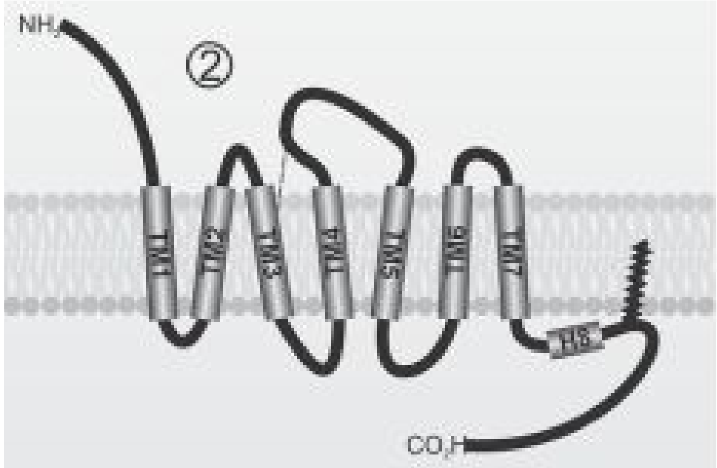


C-terminus

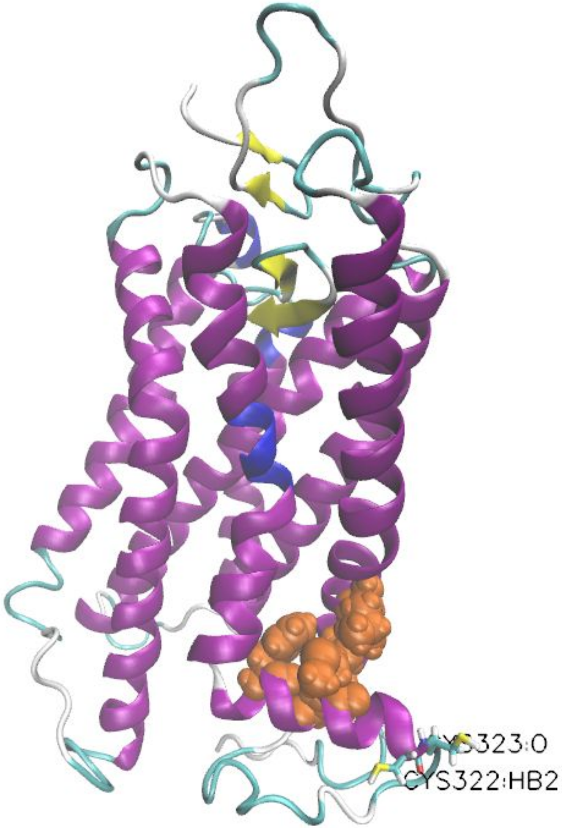

N-TERM
ECL2
S
S
ECL1
ECL3
H1
H2
H3
H4
H5
H6
H7
H8
ICL1
ICL2
ICL3
C-TERM

C-terminus

- On the cytoplasmic side
- Short amphipathic helix (H8) perpendicular to H7
  - Hydrophobic interactions with H1 and its following loop
- Palmitoylation of 2 Cys → anchored to membrane



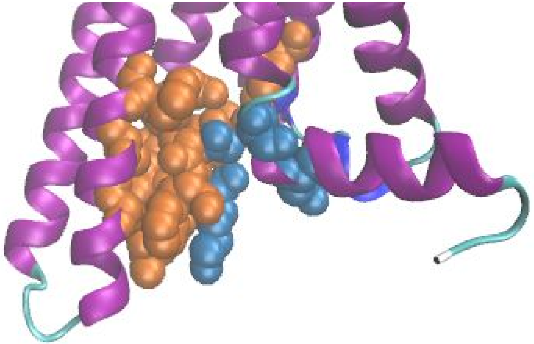
Extracted from Adams MN et. al., 2011

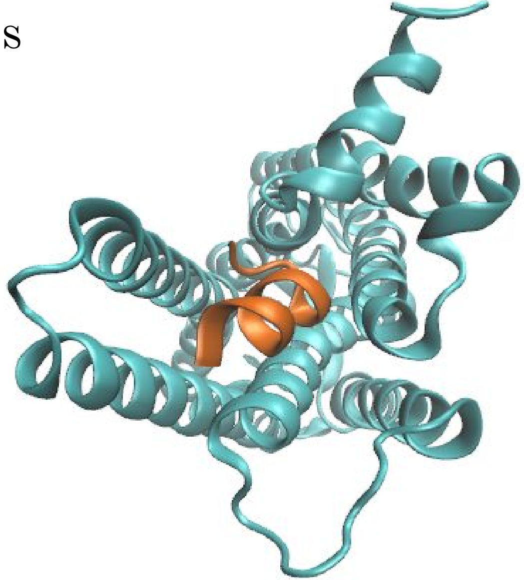


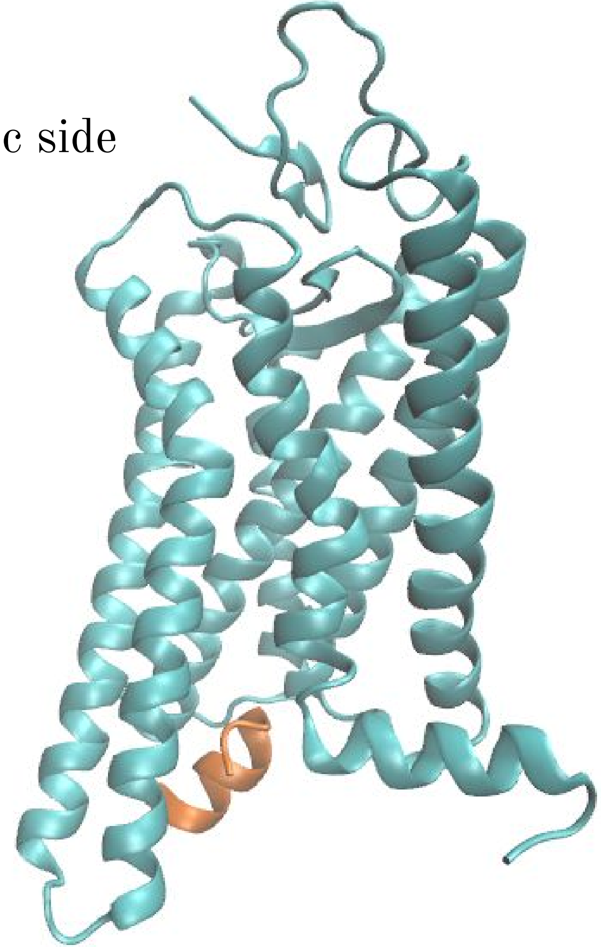
G protein interaction

Binding of the Gα peptide of G protein on the cytoplasmic side of rhodopsin facilitated by two sets of interactions:

1. Hydrophobic interactions
2. Polar interactions







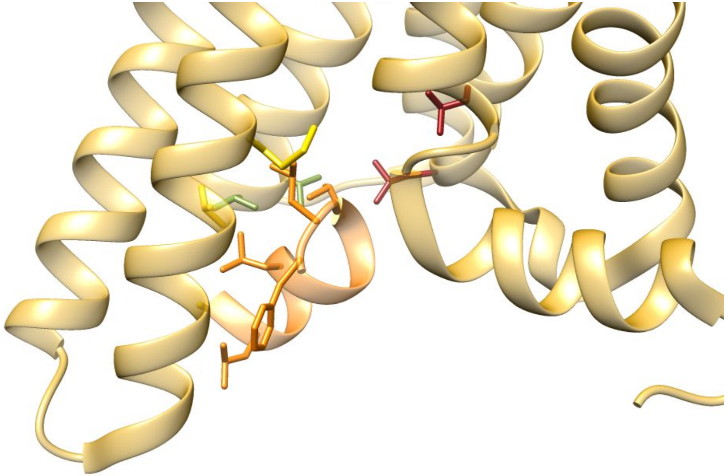
G protein interaction

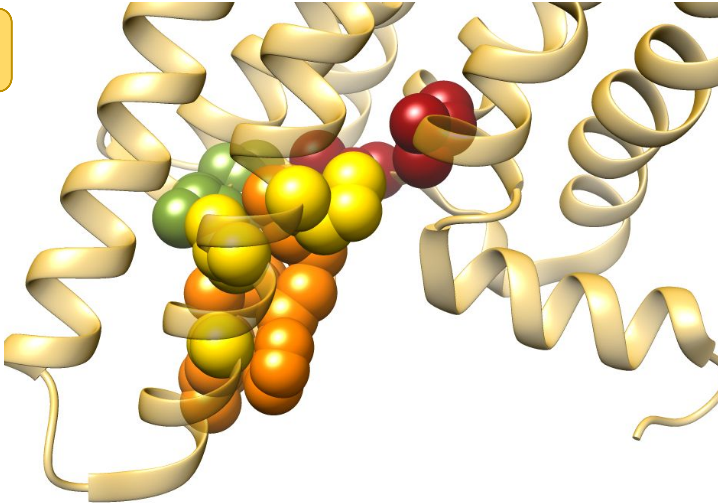
1. Hydrophobic interactions between:

G protein residues: **Leu 341** **Leu 344** **Val 347** **Phe 350** **Leu 349**

Rhodopsin side-chains: **Ala 246 (H6)** **Val 250 (H6)** **Met 253 (H6)** **Val 138 (H3)** **Val 139 (H3)**

Rhodopsin backbone: **Leu 72 (H2)** **Leu 76 (H2)**



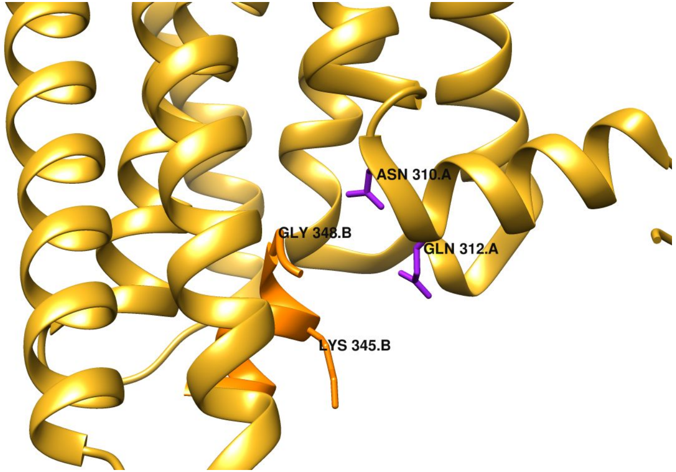


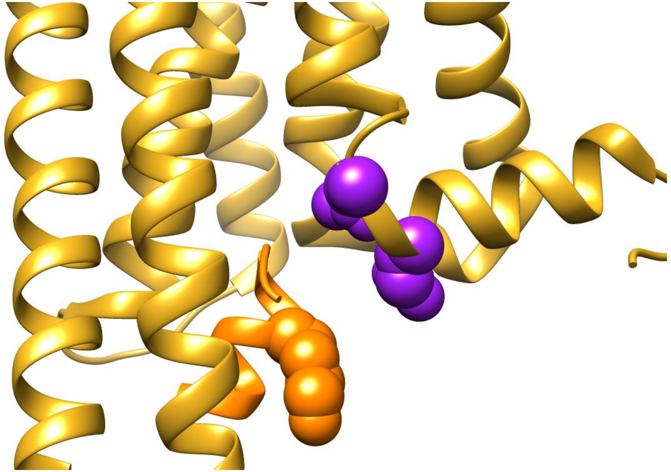
G protein interaction

2. Polar interactions between:

G protein carbonyl groups: **Lys 345** **Gly 348**

Rhodopsin residues: **Asn 310 (H8)** **Gln 312 (H8)**





Rhodopsin: sequence alignment

Rhodopsin-like family

Human (*Homo sapiens*)

Bovine (*Bos taurus*)

Mouse (*Mus musculus*)

Chicken (*Gallus gallus*)

Zebrafish (*Danio rerio*)

African clawed frog (*Xenopus laevis*)

Common fruit fly (*Drosophila melanogaster*)

Japanese flying squid (*Todarodes pacificus*)

Tiger wandering spider (*Cupiennius salei*)

Whiteleg shrimp (*Litopenaeus vannamei*)

Bacteriorhodopsin-like family

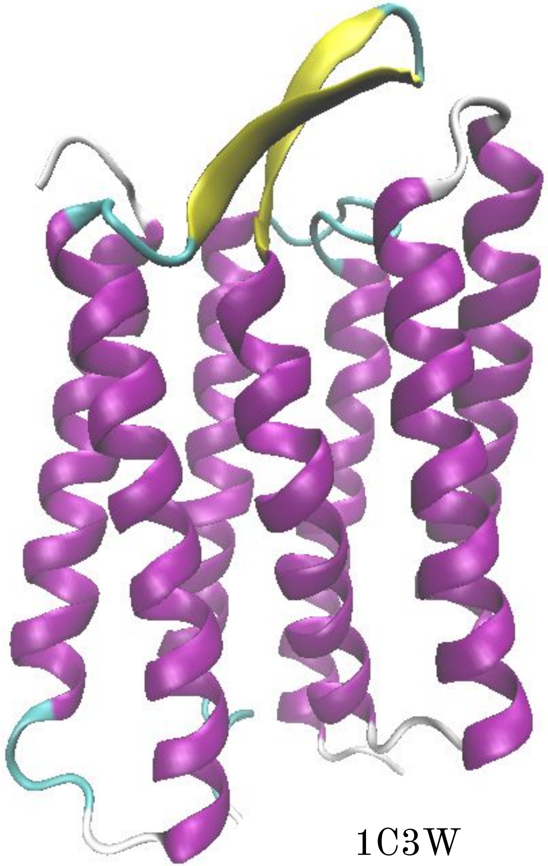
Halobacterium salinarum

Nostoc sp.

Sequence alignment with PFAM 7TM domain HMM profile

Bacteriorhodopsin

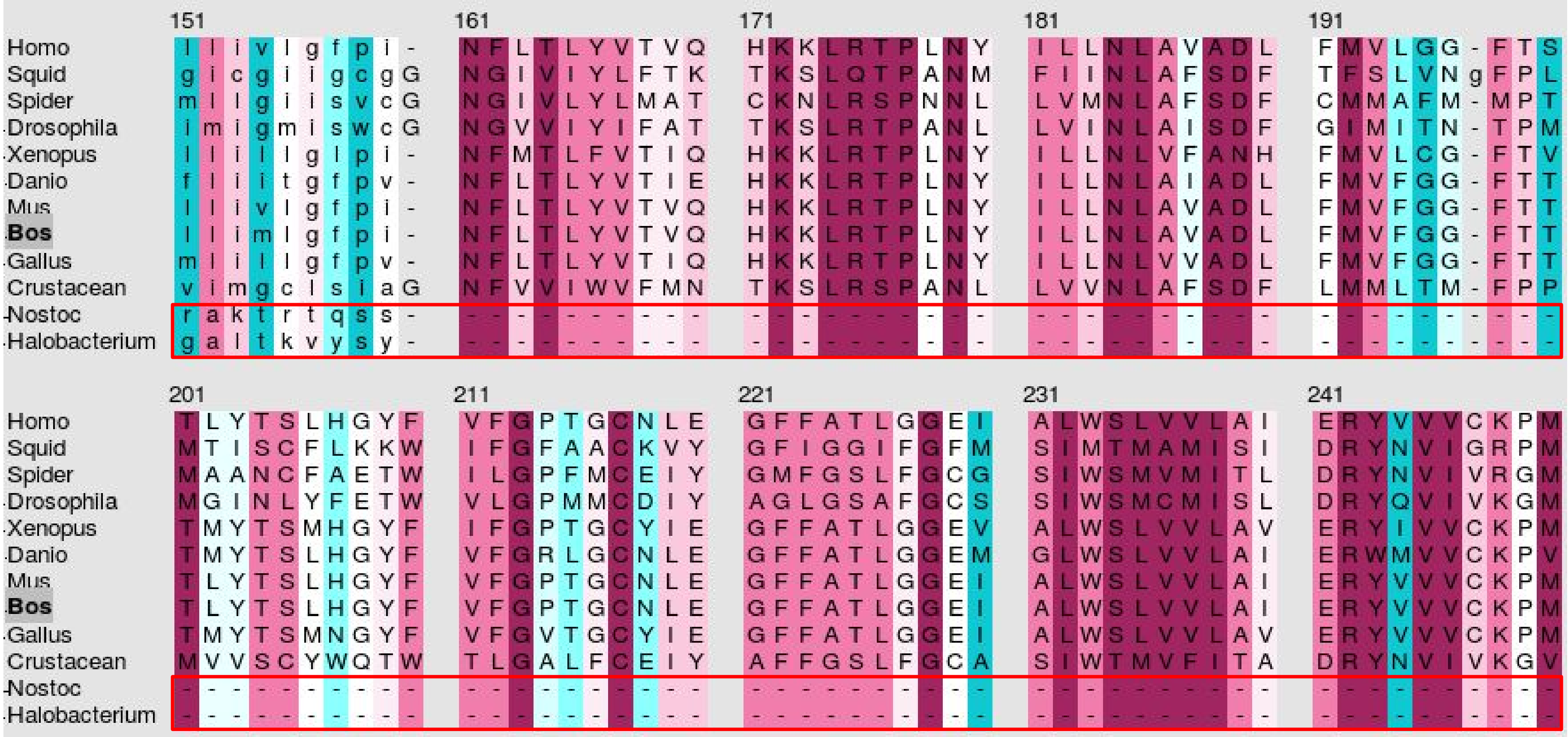
- Light driven ion pump
- Retinal changes from 13-cis to all-trans conformation
- First crystallised in the late 90s
- Now more than 100 structures in PDB
- 7 transmembrane alpha helices



1C3W

Rhodopsin: sequence alignment

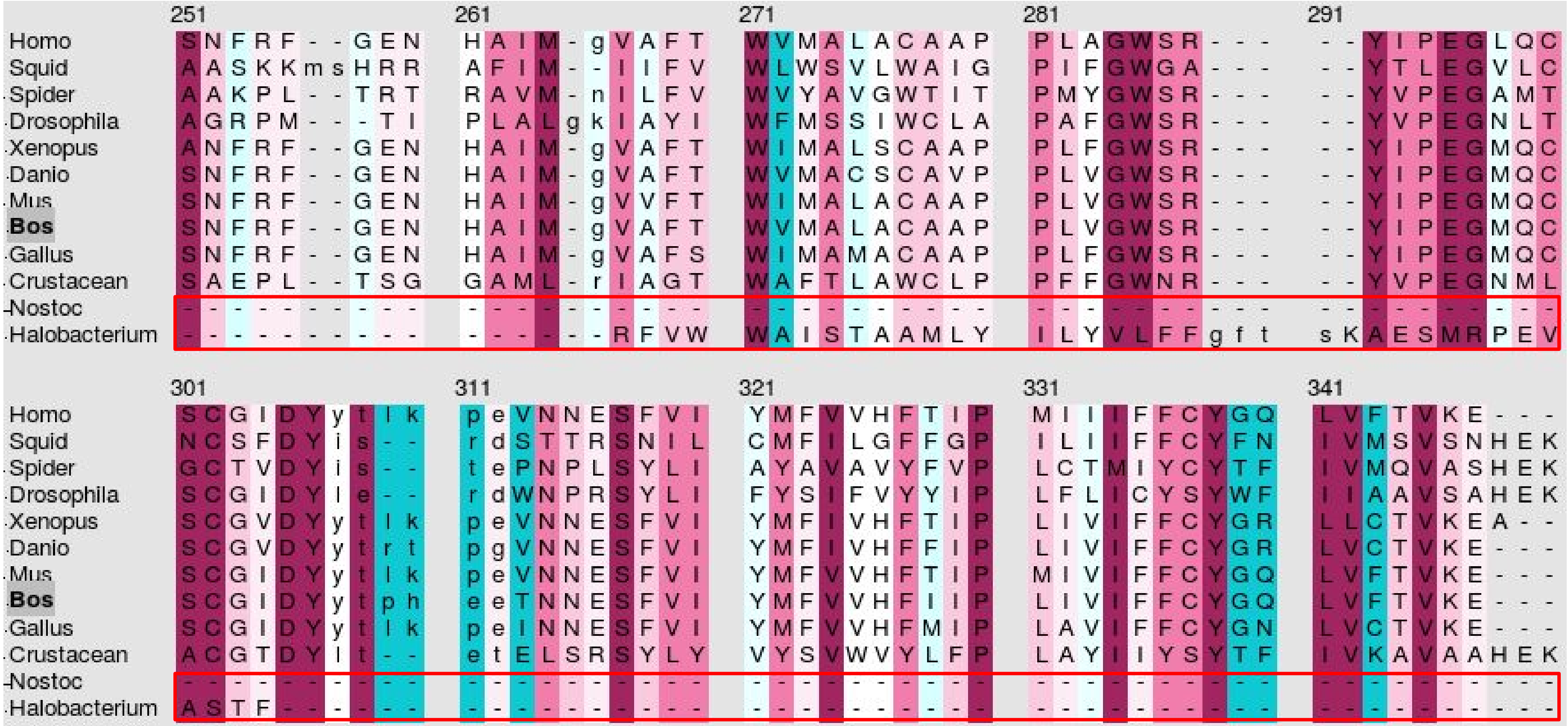
1	2	3	4	5	6	7	8	9
Variable			Average			Conserved		



	151	161	171	181	191
Homo	l l i v l g f p i -	N F L T L Y V T V Q	H K K L R T P L N Y	I L L N L A V A D L	F M V L G G - F T S
Squid	g i c g i i g c g G	N G I V I Y L F T K	T K S L Q T P A N M	F I I N L A F S D F	T F S L V N g F P L
Spider	m l l g i i s v c G	N G I V L Y L M A T	C K N L R S P N N L	L V M N L A F S D F	C M M A F M - M P T
Drosophila	i m i g m i s w c G	N G V V I Y I F A T	T K S L R T P A N L	L V I N L A I S D F	G I M I T N - T P M
Xenopus	l l i l l g l p i -	N F M T L F V T I Q	H K K L R T P L N Y	I L L N L V F A N H	F M V L C G - F T V
Danio	f l i i t g f p v -	N F L T L Y V T I E	H K K L R T P L N Y	I L L N L A I A D L	F M V F G G - F T T
Mus	l l i v l g f p i -	N F L T L Y V T V Q	H K K L R T P L N Y	I L L N L A V A D L	F M V F G G - F T T
Bos	l l i m l g f p i -	N F L T L Y V T V Q	H K K L R T P L N Y	I L L N L A V A D L	F M V F G G - F T T
Gallus	m l i l l g f p v -	N F L T L Y V T I Q	H K K L R T P L N Y	I L L N L V V A D L	F M V F G G - F T T
Crustacean	v i m g c l s i a G	N F V V I W V F M N	T K S L R S P A N L	L V V N L A F S D F	L M M L T M - F P P
Nostoc	r a k t r t q s s -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -
Halobacterium	g a l t k v y s y -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -

	201	211	221	231	241
Homo	T L Y T S L H G Y F	V F G P T G C N L E	G F F A T L G G E I	A L W S L V V L A I	E R Y V V V C K P M
Squid	M T I S C F L K K W	I F G F A A C K V Y	G F I G G I F G F M	S I M T M A M I S I	D R Y N V I G R P M
Spider	M A A N C F A E T W	I L G P F M C E I Y	G M F G S L F G C G	S I W S M V M I T L	D R Y N V I V R G M
Drosophila	M G I N L Y F E T W	V L G P M M C D I Y	A G L G S A F G C S	S I W S M C M I S L	D R Y Q V I V K G M
Xenopus	T M Y T S M H G Y F	I F G P T G C Y I E	G F F A T L G G E V	A L W S L V V L A V	E R Y I V V C K P M
Danio	T M Y T S L H G Y F	V F G R L G C N L E	G F F A T L G G E M	G L W S L V V L A I	E R W M V V C K P V
Mus	T L Y T S L H G Y F	V F G P T G C N L E	G F F A T L G G E I	A L W S L V V L A I	E R Y V V V C K P M
Bos	T L Y T S L H G Y F	V F G P T G C N L E	G F F A T L G G E I	A L W S L V V L A I	E R Y V V V C K P M
Gallus	T M Y T S M N G Y F	V F G V T G C Y I E	G F F A T L G G E I	A L W S L V V L A V	E R Y V V V C K P M
Crustacean	M V V S C Y W Q T W	T L G A L F C E I Y	A F F G S L F G C A	S I W T M V F I T A	D R Y N V I V K G V
Nostoc	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -
Halobacterium	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -

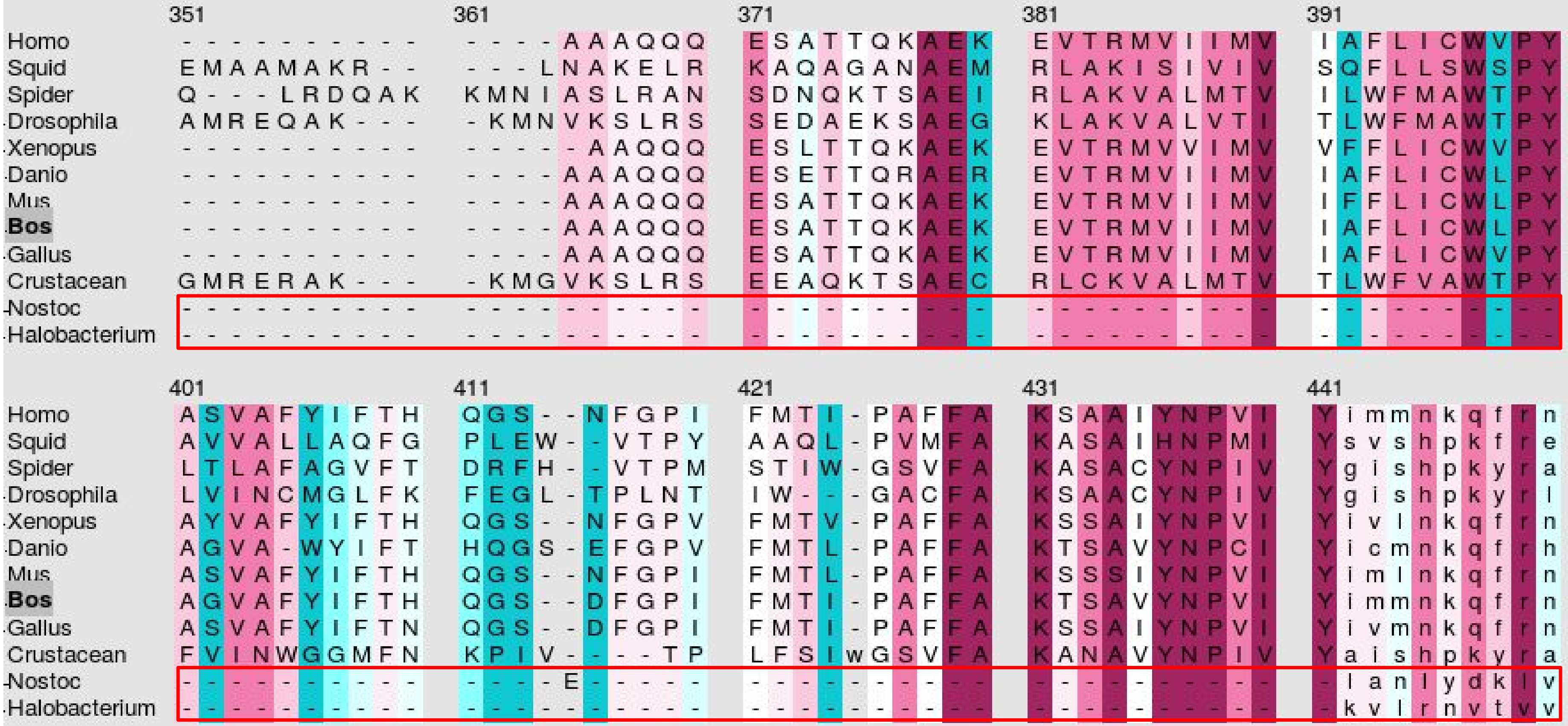
Rhodopsin: sequence alignment



	251	261	271	281	291
Homo	SNFRF--GEN	HAIM-gVAFT	WVMALACAAP	PLAGWSR---	--YIPEGLQC
Squid	AASKKmsHRR	AFIM--IIFV	WLWSVLWAIG	PIFGWGA---	--YTLEGVLC
Spider	AAKPL--TRT	RAVM-nILFV	WVYAVGWTIT	PMYGWSR---	--YVPEGAMT
Drosophila	AGRPM---TI	PLALgkIAYI	WFMSSIWCLA	PAFGWSR---	--YVPEGNLT
Xenopus	ANFRF--GEN	HAIM-gVAFT	WIMALSCAAP	PLFGWSR---	--YIPEGMQC
Danio	SNFRF--GEN	HAIM-gVAFT	WVMACSCAVP	PLVGWSR---	--YIPEGMQC
Mus	SNFRF--GEN	HAIM-gVVFT	WIMALACAAP	PLVGWSR---	--YIPEGMQC
Bos	SNFRF--GEN	HAIM-gVAFT	WVMALACAAP	PLVGWSR---	--YIPEGMQC
Gallus	SNFRF--GEN	HAIM-gVAFS	WIMAMACAAP	PLFGWSR---	--YIPEGMQC
Crustacean	SAEPL--TSG	GAML-rIAGT	WAFTLAWCLP	PFFGWNR---	--YVPEGNML
Nostoc	----------	----------	----------	----------	----------
Halobacterium	----------	------RFVW	WAISTAAMLY	ILYVLFFgft	sKAESMRPEV

	301	311	321	331	341
Homo	SCGIDYytlk	peVNNESFVI	YMFVVHFTIP	MIIIFFCYGQ	LVFTVKE---
Squid	NCSFDYis--	rdSTTRSNIL	CMFILGFFGP	ILIIFFCYFN	IVMSVSNHEK
Spider	GCTVDYis--	tePNPLSYLI	AYAVAVYFVP	LCTMIYCYTF	IVMQVASHEK
Drosophila	SCGIDYle--	rdWNPRSYLI	FYSIFVYYIP	LFLICYSYWF	IIAAVSAHEK
Xenopus	SCGVDYytlk	peVNNESFVI	YMFIVHFTIP	LIVIFFCYGR	LLCTVKEA--
Danio	SCGVDYytrt	pgVNNESFVI	YMFIVHFFIP	LIVIFFCYGR	LVCTVKE---
Mus	SCGIDYytlk	peVNNESFVI	YMFVVHFTIP	MIVIFFCYGQ	LVFTVKE---
Bos	SCGIDYytph	eeTNNESFVI	YMFVVHFIIP	LIVIFFCYGQ	LVFTVKE---
Gallus	SCGIDYytlk	peINNESFVI	YMFVVHFMIP	LAVIFFCYGN	LVCTVKE---
Crustacean	ACGTDYlt--	etELSRSYLY	VYSVWVYLFP	LAYIIYSYTF	IVKAVAAHEK
Nostoc	----------	----------	----------	----------	----------
Halobacterium	ASTF------	----------	----------	----------	----------

Rhodopsin: sequence alignment

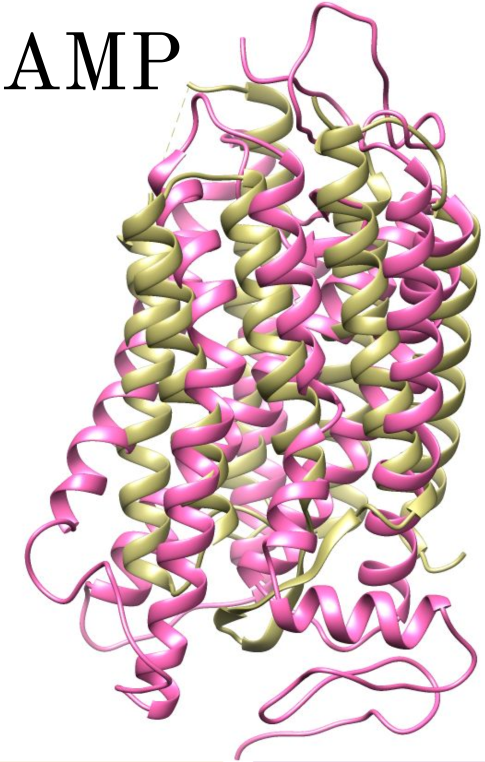


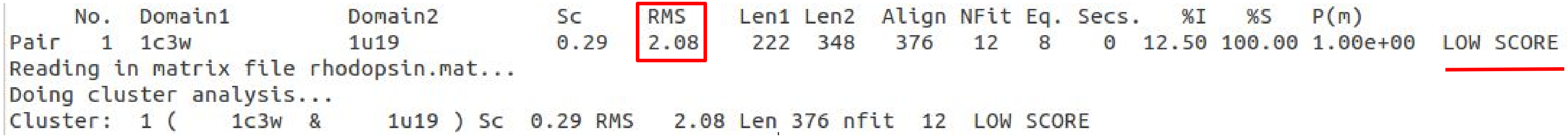
	351	361	371	381	391
Homo	- - - - - - - - - -	- - - - A A A Q Q Q	E S A T T Q K A E K	E V T R M V I I M V	I A F L I C W V P Y
Squid	E M A A M A K R - -	- - - L N A K E L R	K A Q A G A N A E M	R L A K I S I V I V	S Q F L L S W S P Y
Spider	Q - - - L R D Q A K	K M N I A S L R A N	S D N Q K T S A E I	R L A K V A L M T V	I L W F M A W T P Y
Drosophila	A M R E Q A K - - -	- K M N V K S L R S	S E D A E K S A E G	K L A K V A L V T I	T L W F M A W T P Y
Xenopus	- - - - - - - - - -	- - - - - A A Q Q Q	E S L T T Q K A E K	E V T R M V V I M V	V F F L I C W V P Y
Danio	- - - - - - - - - -	- - - - A A A Q Q Q	E S E T T Q R A E R	E V T R M V I I M V	I A F L I C W L P Y
Mus	- - - - - - - - - -	- - - - A A A Q Q Q	E S A T T Q K A E K	E V T R M V I I M V	I F F L I C W L P Y
Bos	- - - - - - - - - -	- - - - A A A Q Q Q	E S A T T Q K A E K	E V T R M V I I M V	I A F L I C W L P Y
Gallus	- - - - - - - - - -	- - - - A A A Q Q Q	E S A T T Q K A E K	E V T R M V I I M V	I A F L I C W V P Y
Crustacean	G M R E R A K - - -	- K M G V K S L R S	E E A Q K T S A E C	R L C K V A L M T V	T L W F V A W T P Y
Nostoc	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -
Halobacterium	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -

	401	411	421	431	441
Homo	A S V A F Y I F T H	Q G S - - N F G P I	F M T I - P A F F A	K S A A I Y N P V I	Y i m m n k q f r n
Squid	A V V A L L A Q F G	P L E W - - V T P Y	A A Q L - P V M F A	K A S A I H N P M I	Y s v s h p k f r e
Spider	L T L A F A G V F T	D R F H - - V T P M	S T I W - G S V F A	K A S A C Y N P I V	Y g i s h p k y r a
Drosophila	L V I N C M G L F K	F E G L - T P L N T	I W - - - G A C F A	K S A A C Y N P I V	Y g i s h p k y r l
Xenopus	A Y V A F Y I F T H	Q G S - - N F G P V	F M T V - P A F F A	K S S A I Y N P V I	Y i v l n k q f r n
Danio	A G V A - W Y I F T	H Q G S - E F G P V	F M T L - P A F F A	K T S A V Y N P C I	Y i c m n k q f r h
Mus	A S V A F Y I F T H	Q G S - - N F G P I	F M T L - P A F F A	K S S S I Y N P V I	Y i m l n k q f r n
Bos	A G V A F Y I F T H	Q G S - - D F G P I	F M T I - P A F F A	K T S A V Y N P V I	Y i m m n k q f r n
Gallus	A S V A F Y I F T N	Q G S - - D F G P I	F M T I - P A F F A	K S S A I Y N P V I	Y i v m n k q f r n
Crustacean	F V I N W G G M F N	K P I V - - - - T P	L F S I w G S V F A	K A N A V Y N P I V	Y a i s h p k y r a
Nostoc	- - - - - - - - - -	- - - - E - - - - -	- - - - - - - - - -	- - - - - - - - - -	- l a n l y d k l v
Halobacterium	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- k v l r n v t v v

Rhodopsin vs. bacteriorhodopsin: STAMP

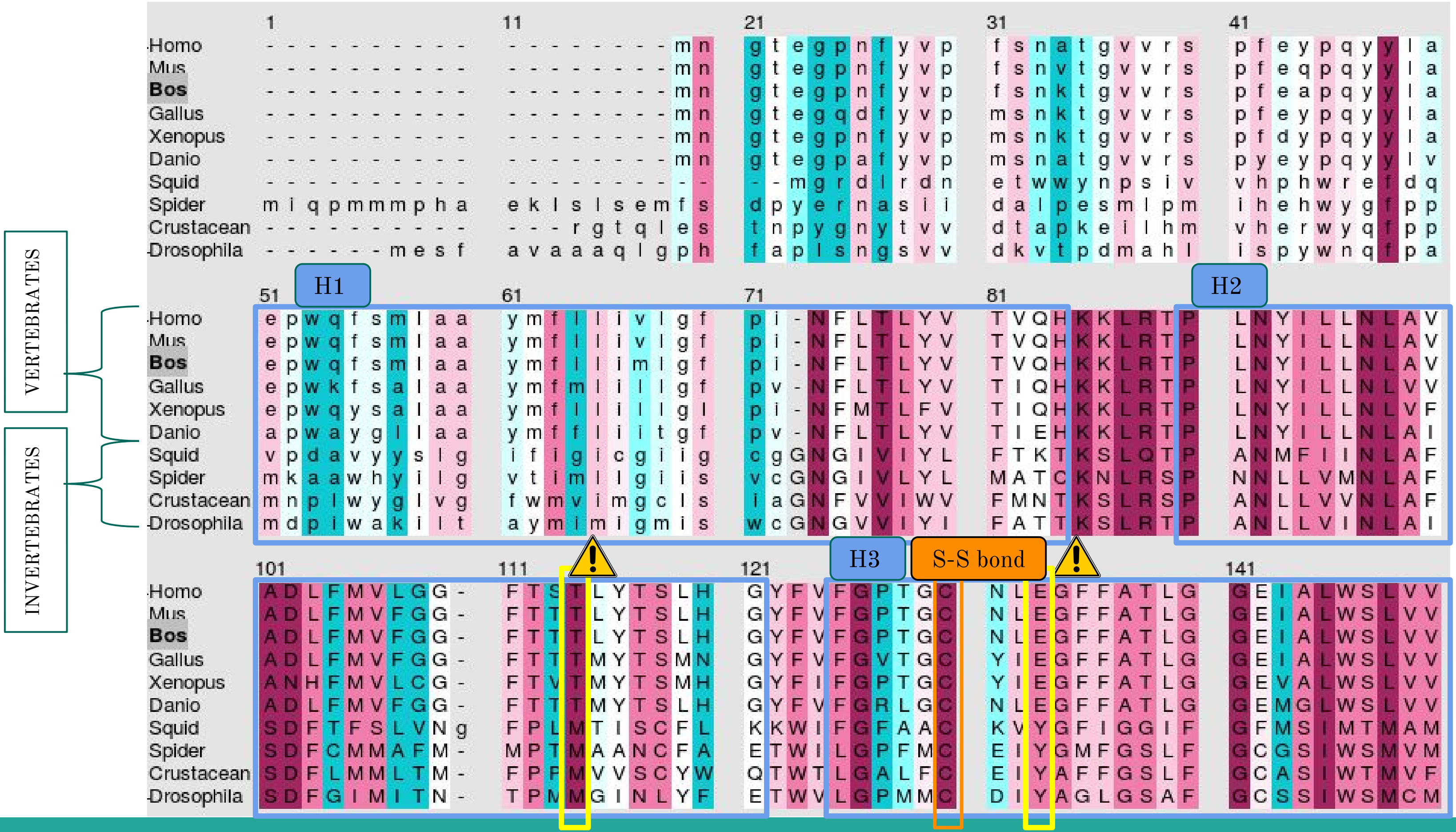
```
1c3w       TGRPEWIWLALGTALMGLGTLYFLVKGMGVSDPDAK---KFY-AITT-LVPA-IAF-TMY
1u19       -MNGTEGPNFYVPFSNKTGVVRSPFEA-PQYYL--AEPWQ-FSML-AAY-MFLL-IMLGF

1c3w       LSMLLGYG-LTM-----VPFGGEQN-P-I--YWA-RYADWLFTTPLLLLDLALLVDADQG
1u19       PINFL--TLYVTVQHKKL----RTPLNYILLNLAVA-D----------------------

1c3w       TILALVGADGIMIGTGLVGALTKVYSYRFVWWAISTAAMLYILYVLFFGFSMRPEVASTF
1u19       ------------------------------------------------------------

1c3w       KVL------------------------------RNV-TVVL-WSAYPVVWLIGSEG----
1u19       ---LFMVFGGFTTTLYTSLHGYFVFGPTGCNLEG-FFAT-LGGEIALWSLVV--LAIERY

1c3w       ---------------AGIV-PLNI-ETLL-FMVL-DVSAKVG-F------GLILLRSRAI
1u19       VVVCKPMSNFRFGENHAIMGV--AFT-WVMA-LACA-A----PPLVGWSRY--I----PE

1c3w       -F----------------------------------------------------------
1u19       GMQCSCGIDYYTPHEETNNESFVIYMFVVHFIIPLIVIFFCYGQLVFTVKEAAAQQQESA

1c3w       -----------------------------------------G------------------
1u19       TTQKAEKEVTRMVIIMVIAFLICWLPYAGVAFYIFTHQGSDFGPIFMTIPAFFAKTSAVY

1c3w       -----------------------------------------------
1u19       NPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTTVSKTETSQVAPA
```





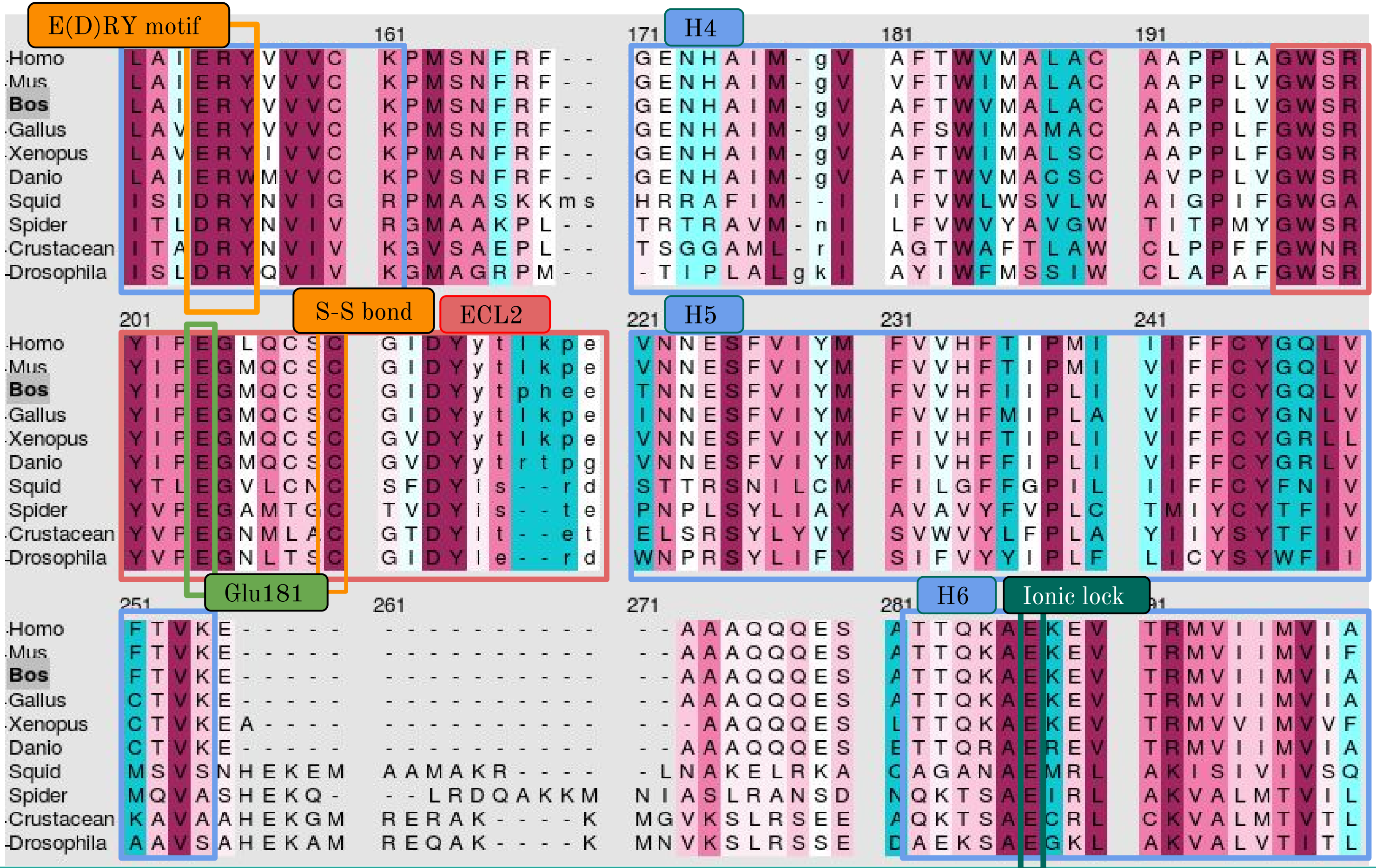
```
     No.  Domain1         Domain2         Sc      RMS     Len1 Len2  Align NFit Eq. Secs.    %I    %S    P(m)
Pair   1  1c3w            1u19            0.29    2.08     222  348   376    12   8     0  12.50 100.00 1.00e+00   LOW SCORE
Reading in matrix file rhodopsin.mat...
Doing cluster analysis...
Cluster:  1 (    1c3w   &     1u19 ) Sc  0.29 RMS   2.08 Len 376 nfit  12  LOW SCORE
```

Rhodopsin: conservation in sequence alignments



```
            1          11         21         31         41
Homo        ---------- --------mn gtegpnfyvp fsnatgvvrs pfeypqyyla
Mus         ---------- --------mn gtegpnfyvp fsnvtgvvrs pfeqpqyyla
Bos         ---------- --------mn gtegpnfyvp fsnktgvvrs pfeapqyyla
Gallus      ---------- --------mn gtegqdfyvp msnktgvvrs pfeypqyyla
Xenopus     ---------- --------mn gtegpnfyvp msnktgvvrs pfdypqyyla
Danio       ---------- --------mn gtegpafyvp msnatgvvrs pyeypqyylv
Squid       ---------- ---------- --mgrdlrdn etwwynpsiv vhphwrefdq
Spider      miqpmmmpha eklslsemfs dpyernasii dalpesmlpm ihehwygfpp
Crustacean  ---------- ---rgtqles tnpygnytvv dtapkeilhm vherwyqfpp
Drosophila  ------mesf avaaaqlgph faplsngsvv dkvtpdmahl ispywnqfpa

            51         61         71         81
Homo        epwqfsmlaa ymfllivlgf pi-NFLTLYV TVQHKKLRTP LNYILLNLAV
Mus         epwqfsmlaa ymfllivlgf pi-NFLTLYV TVQHKKLRTP LNYILLNLAV
Bos         epwqfsmlaa ymfllimlgf pi-NFLTLYV TVQHKKLRTP LNYILLNLAV
Gallus      epwkfsalaa ymfmlillgf pv-NFLTLYV TIQHKKLRTP LNYILLNLVV
Xenopus     epwqysalaa ymfllillgl pi-NFMTLFV TIQHKKLRTP LNYILLNLVF
Danio       apwaygllaa ymffliitgf pv-NFLTLYV TIEHKKLRTP LNYILLNLAI
Squid       vpdavyyslg ifigicgiig cgGNGIVIYL FTKTKSLQTP ANMFIINLAF
Spider      mkaawhyilg vtimllgiis vcGNGIVLYL MATCKNLRSP NNLLVMNLAF
Crustacean  mnplwyglvg fwmvimgcls iaGNFVVIWV FMNTKSLRSP ANLLVVNLAF
Drosophila  mdpiwakilt aymimigmis wcGNGVVIYI FATTKSLRTP ANLLVINLAI

            101        111        121                   141
Homo        ADLFMVLGG- FTSTLYTSLH GYFVFGPTGC NLEGFFATLG GEIALWSLVV
Mus         ADLFMVFGG- FTTTLYTSLH GYFVFGPTGC NLEGFFATLG GEIALWSLVV
Bos         ADLFMVFGG- FTTTLYTSLH GYFVFGPTGC NLEGFFATLG GEIALWSLVV
Gallus      ADLFMVFGG- FTTTMYTSMN GYFVFGVTGC YIEGFFATLG GEIALWSLVV
Xenopus     ANHFMVLCG- FTVTMYTSMH GYFIFGPTGC YIEGFFATLG GEVALWSLVV
Danio       ADLFMVFGG- FTTTMYTSLH GYFVFGRLGC NLEGFFATLG GEMGLWSLVV
Squid       SDFTFSLVNg FPLMTISCFL KKWIFGFAAC KVYGFIGGIF GFMSIMTMAM
Spider      SDFCMMAFM- MPTMAANCFA ETWILGPFMC EIYGMFGSLF GCGSIWSMVM
Crustacean  SDFLMMLTM- FPPMVVSCYW QTWTLGALFC EIYAFFGSLF GCASIWTMVF
Drosophila  SDFGIMITN- TPMMGINLYF ETWVLGPMMC DIYAGLGSAF GCSSIWSMCM
```

Rhodopsin: conservation in sequence alignments

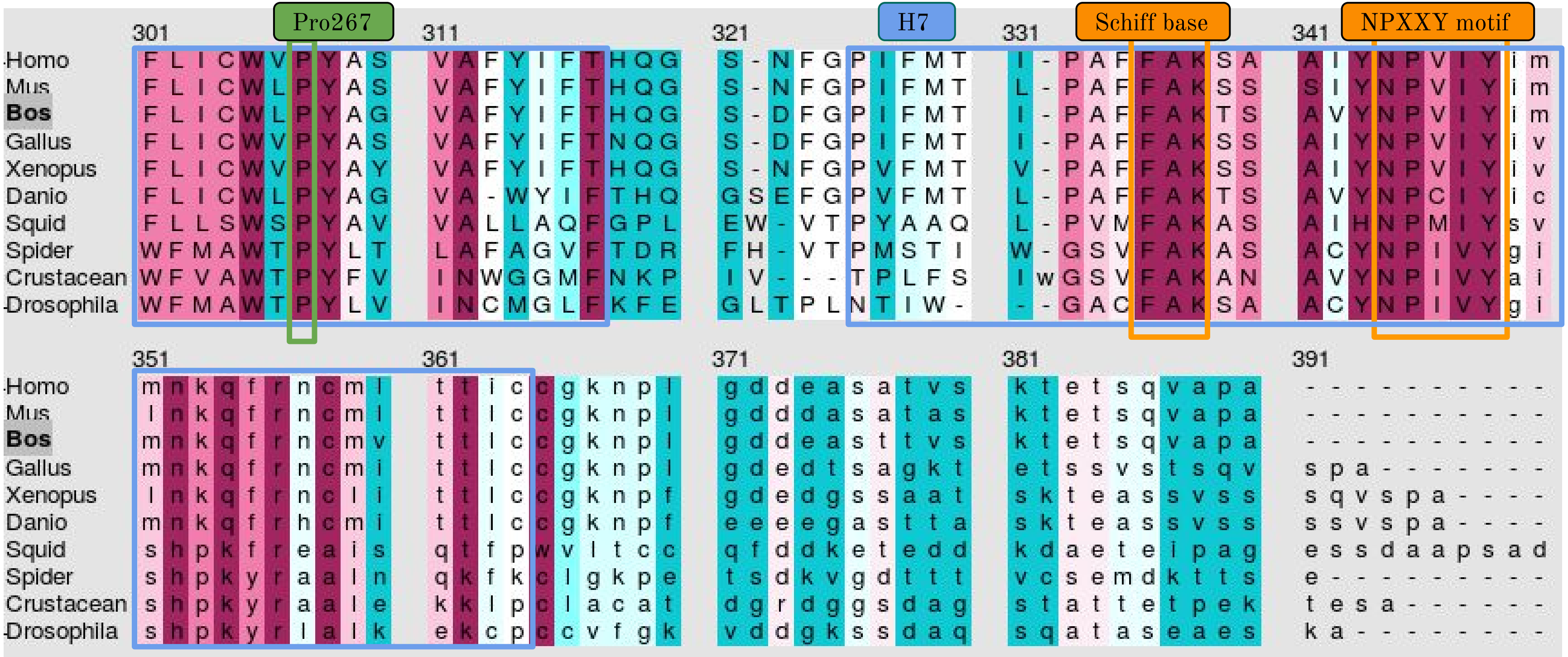


		161	171	181	191
Homo	L A I E R Y V V V C	K P M S N F R F - -	G E N H A I M - g V	A F T W V M A L A C	A A P P L A G W S R
Mus	L A I E R Y V V V C	K P M S N F R F - -	G E N H A I M - g V	V F T W I M A L A C	A A P P L V G W S R
Bos	L A I E R Y V V V C	K P M S N F R F - -	G E N H A I M - g V	A F T W V M A L A C	A A P P L V G W S R
Gallus	L A V E R Y V V V C	K P M S N F R F - -	G E N H A I M - g V	A F S W I M A M A C	A A P P L F G W S R
Xenopus	L A V E R Y I V V C	K P M A N F R F - -	G E N H A I M - g V	A F T W I M A L S C	A A P P L F G W S R
Danio	L A I E R W M V V C	K P V S N F R F - -	G E N H A I M - g V	A F T W V M A C S C	A V P P L V G W S R
Squid	I S I D R Y N V I G	R P M A A S K K m s	H R R A F I M - - I	I F V W L W S V L W	A I G P I F G W G A
Spider	I T L D R Y N V I V	R G M A A K P L - -	T R T R A V M - n I	L F V W V Y A V G W	T I T P M Y G W S R
Crustacean	I T A D R Y N V I V	K G V S A E P L - -	T S G G A M L - r I	A G T W A F T L A W	C L P P F F G W N R
Drosophila	I S L D R Y Q V I V	K G M A G R P M - -	- T I P L A L g k I	A Y I W F M S S I W	C L A P A F G W S R

	201		221	231	241
Homo	Y I P E G L Q C S C	G I D Y y t l k p e	V N N E S F V I Y M	F V V H F T I P M I	I I F F C Y G Q L V
Mus	Y I P E G M Q C S C	G I D Y y t l k p e	V N N E S F V I Y M	F V V H F T I P M I	V I F F C Y G Q L V
Bos	Y I P E G M Q C S C	G I D Y y t p h e e	T N N E S F V I Y M	F V V H F I I P L I	V I F F C Y G Q L V
Gallus	Y I P E G M Q C S C	G I D Y y t l k p e	I N N E S F V I Y M	F V V H F M I P L A	V I F F C Y G N L V
Xenopus	Y I P E G M Q C S C	G V D Y y t l k p e	V N N E S F V I Y M	F I V H F T I P L I	V I F F C Y G R L L
Danio	Y I P E G M Q C S C	G V D Y y t r t p g	V N N E S F V I Y M	F I V H F F I P L I	V I F F C Y G R L V
Squid	Y T L E G V L C N C	S F D Y i s - - r d	S T T R S N I L C M	F I L G F F G P I L	I I F F C Y F N I V
Spider	Y V P E G A M T G C	T V D Y i s - - t e	P N P L S Y L I A Y	A V A V Y F V P L C	T M I Y C Y T F I V
Crustacean	Y V P E G N M L A C	G T D Y l t - - e t	E L S R S Y L Y V Y	S V W V Y L F P L A	Y I I Y S Y T F I V
Drosophila	Y V P E G N L T S C	G I D Y l e - - r d	W N P R S Y L I F Y	S I F V Y Y I P L F	L I C Y S Y W F I I

	251	261	271	281	91
Homo	F T V K E - - - - -	- - - - - - - - - -	- - A A A Q Q Q E S	A T T Q K A E K E V	T R M V I I M V I A
Mus	F T V K E - - - - -	- - - - - - - - - -	- - A A A Q Q Q E S	A T T Q K A E K E V	T R M V I I M V I F
Bos	F T V K E - - - - -	- - - - - - - - - -	- - A A A Q Q Q E S	A T T Q K A E K E V	T R M V I I M V I A
Gallus	C T V K E - - - - -	- - - - - - - - - -	- - A A A Q Q Q E S	A T T Q K A E K E V	T R M V I I M V I A
Xenopus	C T V K E A - - - -	- - - - - - - - - -	- - - A A Q Q Q E S	L T T Q K A E K E V	T R M V V I M V V F
Danio	C T V K E - - - - -	- - - - - - - - - -	- - A A A Q Q Q E S	E T T Q R A E R E V	T R M V I I M V I A
Squid	M S V S N H E K E M	A A M A K R - - - -	- L N A K E L R K A	C A G A N A E M R L	A K I S I V I V S Q
Spider	M Q V A S H E K Q -	- - L R D Q A K K M	N I A S L R A N S D	N Q K T S A E I R L	A K V A L M T V I L
Crustacean	K A V A A H E K G M	R E R A K - - - - K	M G V K S L R S E E	A Q K T S A E C R L	C K V A L M T V T L
Drosophila	A A V S A H E K A M	R E Q A K - - - - K	M N V K S L R S S E	D A E K S A E G K L	A K V A L V T I T L

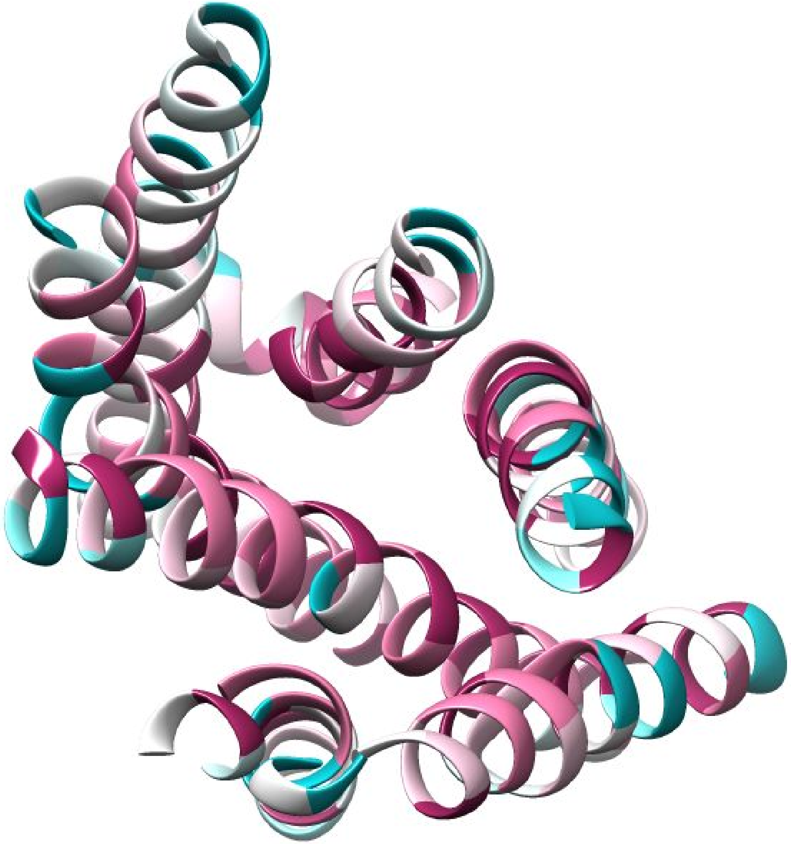
Rhodopsin: conservation in sequence alignments

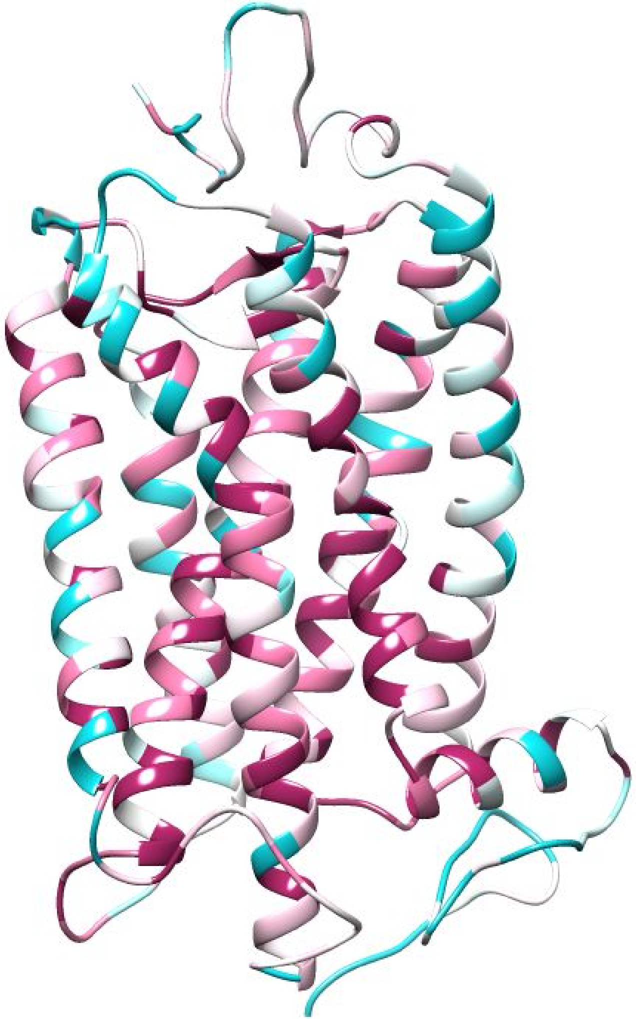


	301	311	321	331	341
Homo	F L I C W V P Y A S	V A F Y I F T H Q G	S - N F G P I F M T	I - P A F F A K S A	A I Y N P V I Y i m
Mus	F L I C W L P Y A S	V A F Y I F T H Q G	S - N F G P I F M T	L - P A F F A K S S	S I Y N P V I Y i m
Bos	F L I C W L P Y A G	V A F Y I F T H Q G	S - D F G P I F M T	I - P A F F A K T S	A V Y N P V I Y i m
Gallus	F L I C W V P Y A S	V A F Y I F T N Q G	S - D F G P I F M T	I - P A F F A K S S	A I Y N P V I Y i v
Xenopus	F L I C W V P Y A Y	V A F Y I F T H Q G	S - N F G P V F M T	V - P A F F A K S S	A I Y N P V I Y i v
Danio	F L I C W L P Y A G	V A - W Y I F T H Q	G S E F G P V F M T	L - P A F F A K T S	A V Y N P C I Y i c
Squid	F L L S W S P Y A V	V A L L A Q F G P L	E W - V T P Y A A Q	L - P V M F A K A S	A I H N P M I Y s v
Spider	W F M A W T P Y L T	L A F A G V F T D R	F H - V T P M S T I	W - G S V F A K A S	A C Y N P I V Y g i
Crustacean	W F V A W T P Y F V	I N W G G M F N K P	I V - - - T P L F S	I w G S V F A K A N	A V Y N P I V Y a i
Drosophila	W F M A W T P Y L V	I N C M G L F K F E	G L T P L N T I W -	- - G A C F A K S A	A C Y N P I V Y g i

	351	361	371	381	391
Homo	m n k q f r n c m l	t t i c c g k n p l	g d d e a s a t v s	k t e t s q v a p a	- - - - - - - - - -
Mus	l n k q f r n c m l	t t l c c g k n p l	g d d d a s a t a s	k t e t s q v a p a	- - - - - - - - - -
Bos	m n k q f r n c m v	t t l c c g k n p l	g d d e a s t t v s	k t e t s q v a p a	- - - - - - - - - -
Gallus	m n k q f r n c m i	t t l c c g k n p l	g d e d t s a g k t	e t s s v s t s q v	s p a - - - - - - -
Xenopus	l n k q f r n c l i	t t l c c g k n p f	g d e d g s s a a t	s k t e a s s v s s	s q v s p a - - - -
Danio	m n k q f r h c m i	t t l c c g k n p f	e e e e g a s t t a	s k t e a s s v s s	s s v s p a - - - -
Squid	s h p k f r e a i s	q t f p w v l t c c	q f d d k e t e d d	k d a e t e i p a g	e s s d a a p s a d
Spider	s h p k y r a a l n	q k f k c l g k p e	t s d k v g d t t t	v c s e m d k t t s	e - - - - - - - - -
Crustacean	s h p k y r a a l e	k k l p c l a c a t	d g r d g g s d a g	s t a t t e t p e k	t e s a - - - - - -
Drosophila	s h p k y r l a l k	e k c p c c v f g k	v d d g k s s d a q	s q a t a s e a e s	k a - - - - - - - -

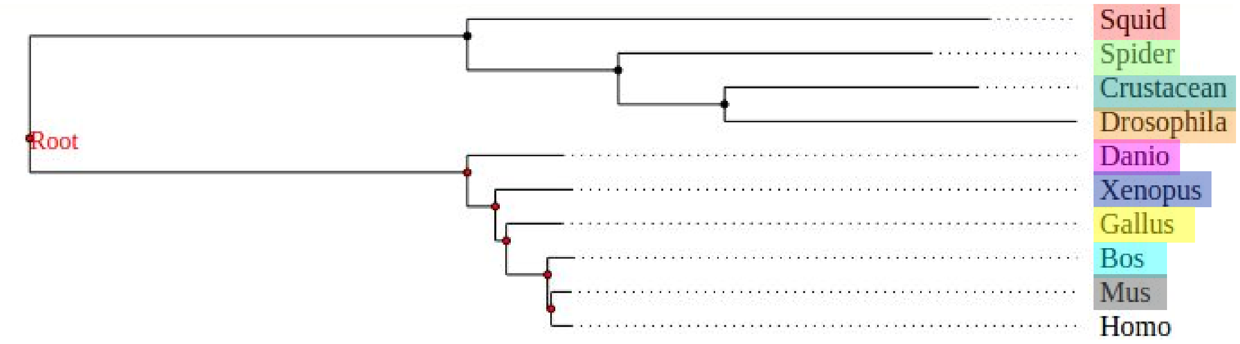
Rhodopsin: conserved residues



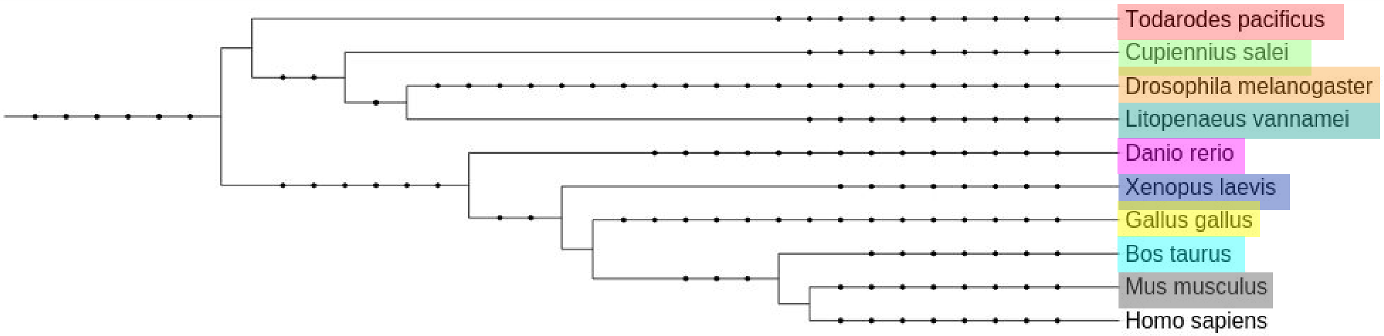


Rhodopsin: sequence alignment

Phylogenetic tree obtained from rhodopsins alignment



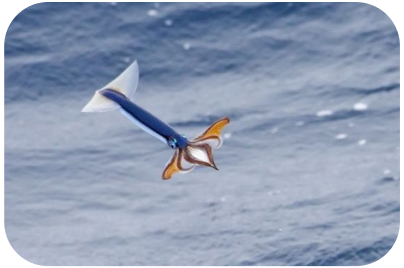
Phylogenetic tree obtained from iTOL (interactive tree of life)



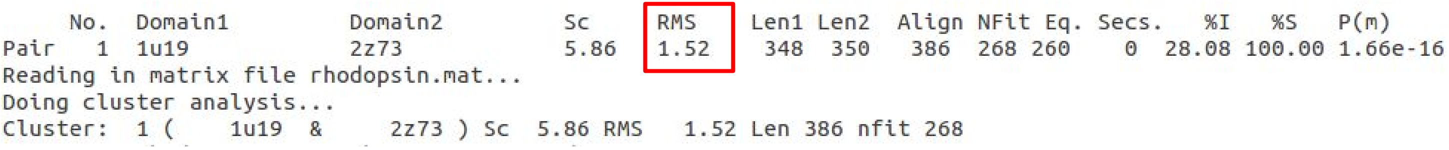
Rhodopsin orthologues: bovine vs. squid

Only another GPCR-rhodopsin crystallised → Japanese flying squid (2Z73)

Bovine rhodopsin	Squid rhodopsin
348 aa	448 aa
7 TM helices	7 TM helices
Activated by light	Activated by light
Ligand: 11-cis/all-trans retinal	Ligand: 11-cis/all-trans retinal
Coupled to Gt protein → cGMP signalling cascade	Coupled to Gq protein → IP3 signalling cascade



Bovine vs. squid rhodopsin: STAMP

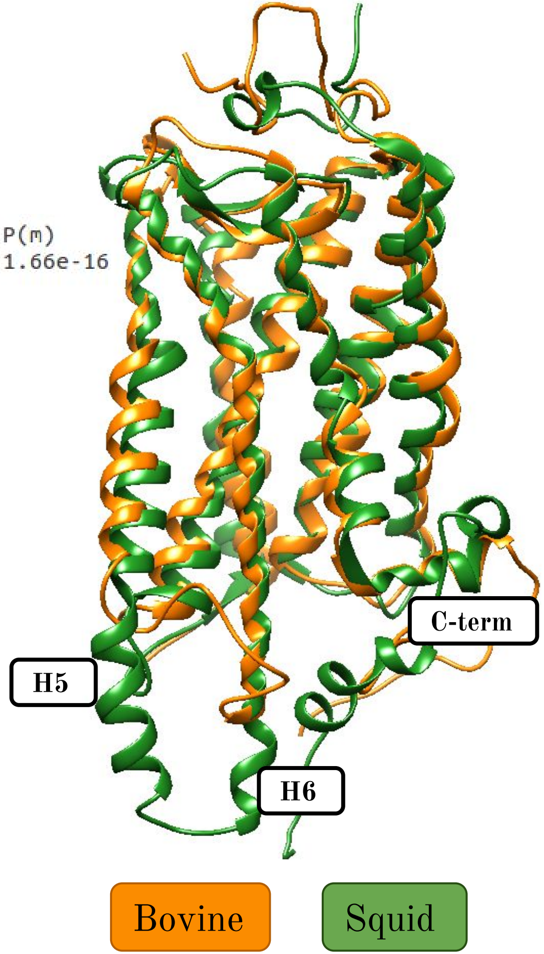


```
     No.  Domain1          Domain2          Sc     RMS    Len1 Len2  Align NFit Eq. Secs.    %I    %S    P(m)
Pair   1  1u19             2z73             5.86   1.52    348  350    386  268 260     0  28.08 100.00 1.66e-16
Reading in matrix file rhodopsin.mat...
Doing cluster analysis...
Cluster:  1 (    1u19  &      2z73 ) Sc  5.86 RMS   1.52 Len 386 nfit 268
```

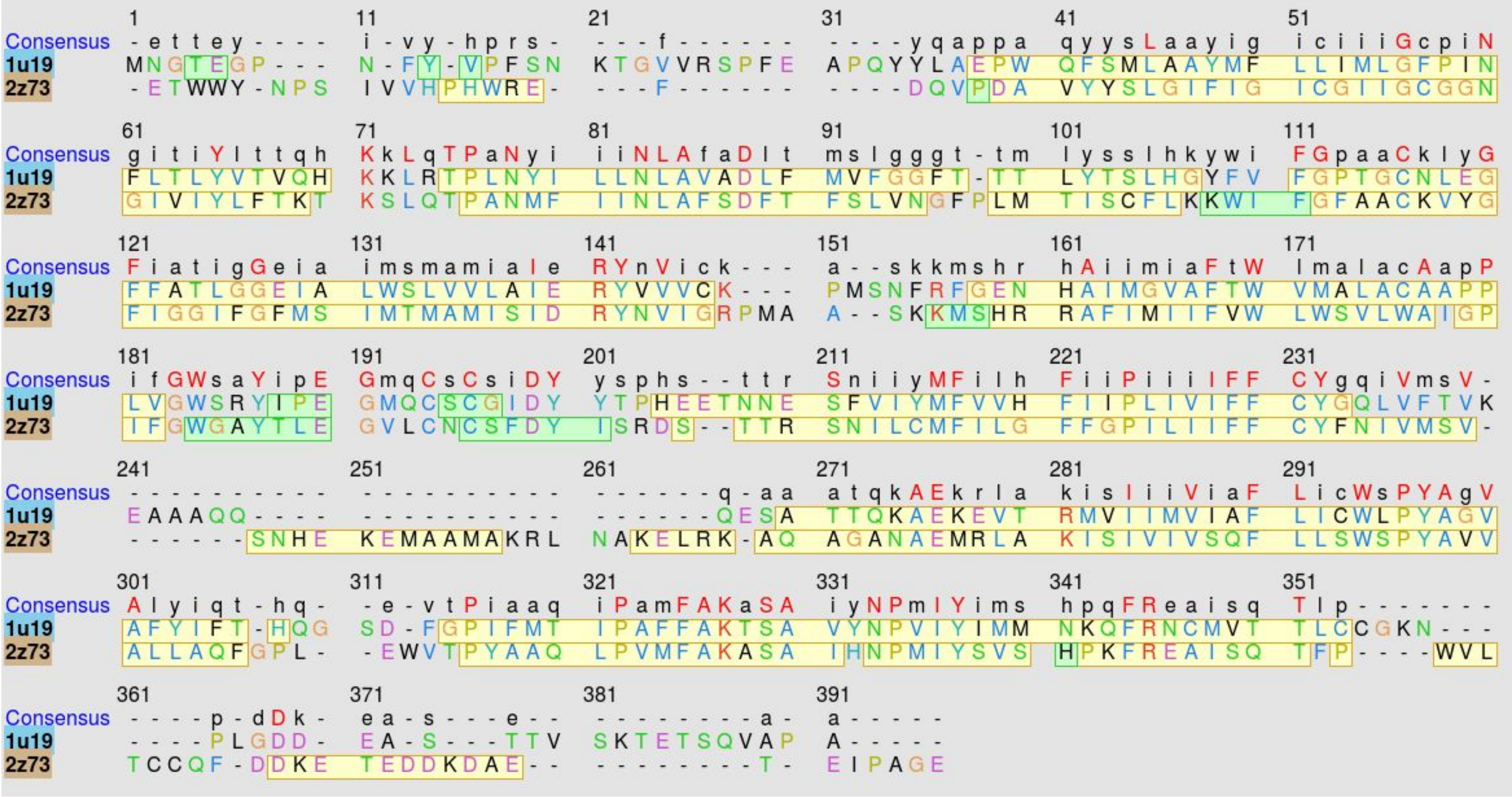
Low RMSD value → High structural similarity

- Conserved 7TM domain
- H5 and H6 extended to the cytoplasmic side
- Extended C-term

By means of its longer sequence



Bovine vs. squid rhodopsin: structure alignment



```
           1          11         21         31         41         51
Consensus  -ettey---- i-vy-hprs- ---f------ ----yqappa qyysLaayig iciiiGcpiN
1u19       MNGTEGP--- N-FY-VPFSN KTGVVRSPFE APQYYLAEPW QFSMLAAYMF LLIMLGFPIN
2z73       -ETWWY-NPS IVVHPHWRE- ---F------ ----DQVPDA VYYSLGIFIG ICGIIGCGGN

           61         71         81         91         101        111
Consensus  gitiYlttqh KkLqTPaNyi iiNLAfaDlt mslgggt-tm lysslhkywi FGpaaCklyG
1u19       FLTLYVTVQH KKLRTPLNYI LLNLAVADLF MVFGGFT-TT LYTSLHGYFV FGPTGCNLEG
2z73       GIVIYLFTKT KSLQTPANMF IINLAFSDFT FSLVNGFPLM TISCFLKKWI FGFAACKVYG

           121        131        141        151        161        171
Consensus  FiatigGeia imsmamiale RYnVick--- a--skkmshr hAiimiaFtW lmalacAapP
1u19       FFATLGGEIA LWSLVVLAIE RYVVVCK--- PMSNFRFGEN HAIMGVAFTW VMALACAAPP
2z73       FIGGIFGFMS IMTMAMISID RYNVIGRPMA A--SKKMSHR RAFIMIIFVW LWSVLWAIGP

           181        191        201        211        221        231
Consensus  ifGWsaYipE GmqCsCsiDY ysphs--ttr SniiyMFilh FiiPiiiIFF CYgqiVmsV-
1u19       LVGWSRYIPE GMQCSCGIDY YTPHEETNNE SFVIYMFVVH FIIPLIVIFF CYGQLVFTVK
2z73       IFGWGAYTLE GVLCNCSFDY ISRDS--TTR SNILCMFILG FFGPILIIFF CYFNIVMSV-

           241        251        261        271        281        291
Consensus  ---------- ---------- ------q-aa atqkAEkrla kisIiiViaF LicWsPYAgV
1u19       EAAAQQ---- ---------- ------QESA TTQKAEKEVT RMVIIMVIAF LICWLPYAGV
2z73       ------SNHE KEMAAMAKRL NAKELRK-AQ AGANAEMRLA KISIVIVSQF LLSWSPYAVV

           301        311        321        331        341        351
Consensus  Alyiqt-hq- -e-vtPiaaq iPamFAKaSA iyNPmIYims hpqFReaisq Tlp-------
1u19       AFYIFT-HQG SD-FGPIFMT IPAFFAKTSA VYNPVIYIMM NKQFRNCMVT TLCCGKN---
2z73       ALLAQFGPL- -EWVTPYAAQ LPVMFAKASA IHNPMIYSVS HPKFREAISQ TFP----WVL

           361        371        381        391
Consensus  ----p-dDk- ea-s---e-- --------a- a-----
1u19       ----PLGDD- EA-S---TTV SKTETSQVAP A-----
2z73       TCCQF-DDKE TEDDKDAE-- --------T- EIPAGE
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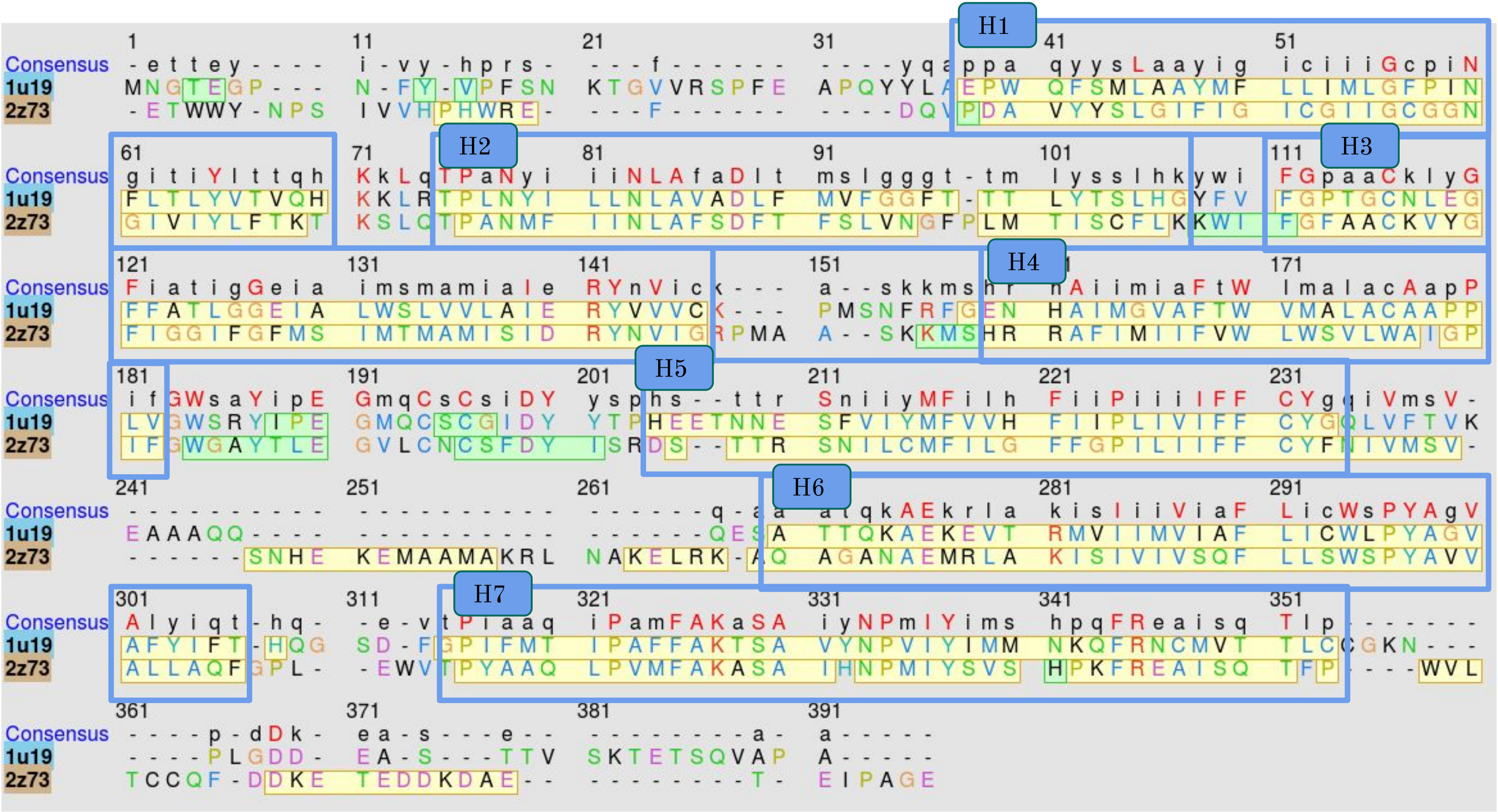
Bovine vs. squid rhodopsin: structure alignment



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           1          11         21         31         41         51
Consensus  -ettey---- i-vy-hprs- ---f------ ----yqappa qyysLaayig iciiiGcpiN
1u19       MNGTEGP--- N-FY-VPFSN KTGVVRSPFE APQYYLAEPW QFSMLAAYMF LLIMLGFPIN
2z73       -ETWWY-NPS IVVHPHWRE- ---F------ ----DQVPDA VYYSLGIFIG ICGIIGCGGN

           61         71         81         91         101        111
Consensus  gitiYlttqh KkLqTPaNyi iiNLAfaDlt mslgggt-tm lysslhkywi FGpaaCklyG
1u19       FLTLYVTVQH KKLRTPLNYI LLNLAVADLF MVFGGFT-TT LYTSLHGYFV FGPTGCNLEG
2z73       GIVIYLFTKT KSLQTPANMF IINLAFSDFT FSLVNGFPLM TISCFLKKWI FGFAACKVYG

           121        131        141        151        161        171
Consensus  FiatigGeia imsmamiale RYnVick--- a--skkmshr nAiimiaFtW lmalacAapP
1u19       FFATLGGEIA LWSLVVLAIE RYVVVCK--- PMSNFRFGEN HAIMGVAFTW VMALACAAPP
2z73       FIGGIFGFMS IMTMAMISID RYNVIGRPMA A--SKKMSHR RAFIMIIFVW LWSVLWAIGP

           181        191        201        211        221        231
Consensus  ifGWsaYipE GmqCsCsiDY ysphs--ttr SniiyMFilh FiiPiiiIFF CYgqiVmsV-
1u19       LVGWSRYIPE GMQCSCGIDY YTPHEETNNE SFVIYMFVVH FIIPLIVIFF CYGQLVFTVK
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1u19       ----PLGDD- EA-S---TTV SKTETSQVAP A-----
2z73       TCCQF-DDKE TEDDKDAE-- --------T- EIPAGE
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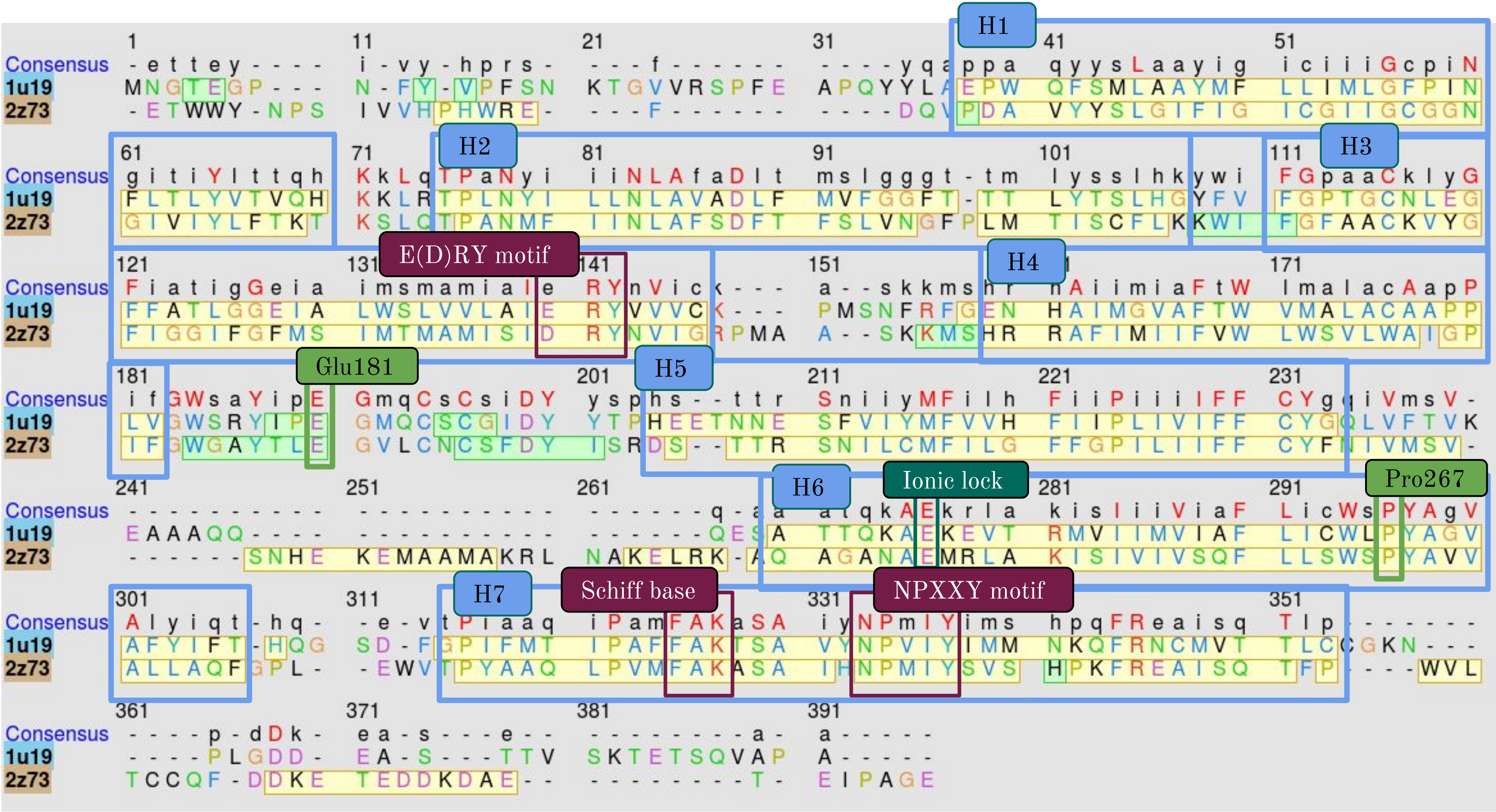

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           1                    11                   21                   31                   41                   51
Consensus  - e t t e y - - - -  i - v y - h p r s -  - - - f - - - - - -  - - - - y q a p p a  q y y s L a a y i g  i c i i i G c p i N
1u19       M N G T E G P - - -  N - F Y - V P F S N  K T G V V R S P F E  A P Q Y Y L A E P W  Q F S M L A A Y M F  L L I M L G F P I N
2z73       - E T W W Y - N P S  I V V H P H W R E -  - - - F - - - - - -  - - - - D Q V P D A  V Y Y S L G I F I G  I C G I I G C G G N

           61                   71                   81                   91                   101                  111
Consensus  g i t i Y l t t q h  K k L q T P a N y i  i i N L A f a D l t  m s l g g g t - t m  l y s s l h k y w i  F G p a a C k l y G
1u19       F L T L Y V T V Q H  K K L R T P L N Y I  L L N L A V A D L F  M V F G G F T - T T  L Y T S L H G Y F V  F G P T G C N L E G
2z73       G I V I Y L F T K T  K S L Q T P A N M F  I I N L A F S D F T  F S L V N G F P L M  T I S C F L K K W I  F G F A A C K V Y G

           121                  131                  141                  151                  161                  171
Consensus  F i a t i g G e i a  i m s m a m i a l e  R Y n V i c k - - -  a - - s k k m s h r  n A i i m i a F t W  l m a l a c A a p P
1u19       F F A T L G G E I A  L W S L V V L A I E  R Y V V V C K - - -  P M S N F R F G E N  H A I M G V A F T W  V M A L A C A A P P
2z73       F I G G I F G F M S  I M T M A M I S I D  R Y N V I G R P M A  A - - S K K M S H R  R A F I M I I F V W  L W S V L W A I G P

           181                  191                  201                  211                  221                  231
Consensus  i f G W s a Y i p E  G m q C s C s i D Y  y s p h s - - t t r  S n i i y M F i l h  F i i P i i i l F F  C Y g q i V m s V -
1u19       L V G W S R Y I P E  G M Q C S C G I D Y  Y T P H E E T N N E  S F V I Y M F V V H  F I I P L I V I F F  C Y G Q L V F T V K
2z73       I F G W G A Y T L E  G V L C N C S F D Y  I S R D S - - T T R  S N I L C M F I L G  F F G P I L I I F F  C Y F N I V M S V -

           241                  251                  261                  271                  281                  291
Consensus  - - - - - - - - - -  - - - - - - - - - -  - - - - - - q - a a  a t q k A E k r l a  k i s l i i V i a F  L i c W s P Y A g V
1u19       E A A A Q Q - - - -  - - - - - - - - - -  - - - - - - Q E S A  T T Q K A E K E V T  R M V I I M V I A F  L I C W L P Y A G V
2z73       - - - - - - S N H E  K E M A A M A K R L  N A K E L R K - A Q  A G A N A E M R L A  K I S I V I V S Q F  L L S W S P Y A V V

           301                  311                  321                  331                  341                  351
Consensus  A l y i q t - h q -  - e - v t P i a a q  i P a m F A K a S A  i y N P m l Y i m s  h p q F R e a i s q  T l p - - - - - - -
1u19       A F Y I F T - H Q G  S D - F G P I F M T  I P A F F A K T S A  V Y N P V I Y I M M  N K Q F R N C M V T  T L C C G K N - - -
2z73       A L L A Q F G P L -  - E W V T P Y A A Q  L P V M F A K A S A  I H N P M I Y S V S  H P K F R E A I S Q  T F P - - - - W V L

           361                  371                  381                  391
Consensus  - - - - p - d D k -  e a - s - - - e - -  - - - - - - - - a -  a - - - - -
1u19       - - - - P L G D D -  E A - S - - - T T V  S K T E T S Q V A P  A - - - - -
2z73       T C C Q F - D D K E  T E D D K D A E - -  - - - - - - - - T -  E I P A G E
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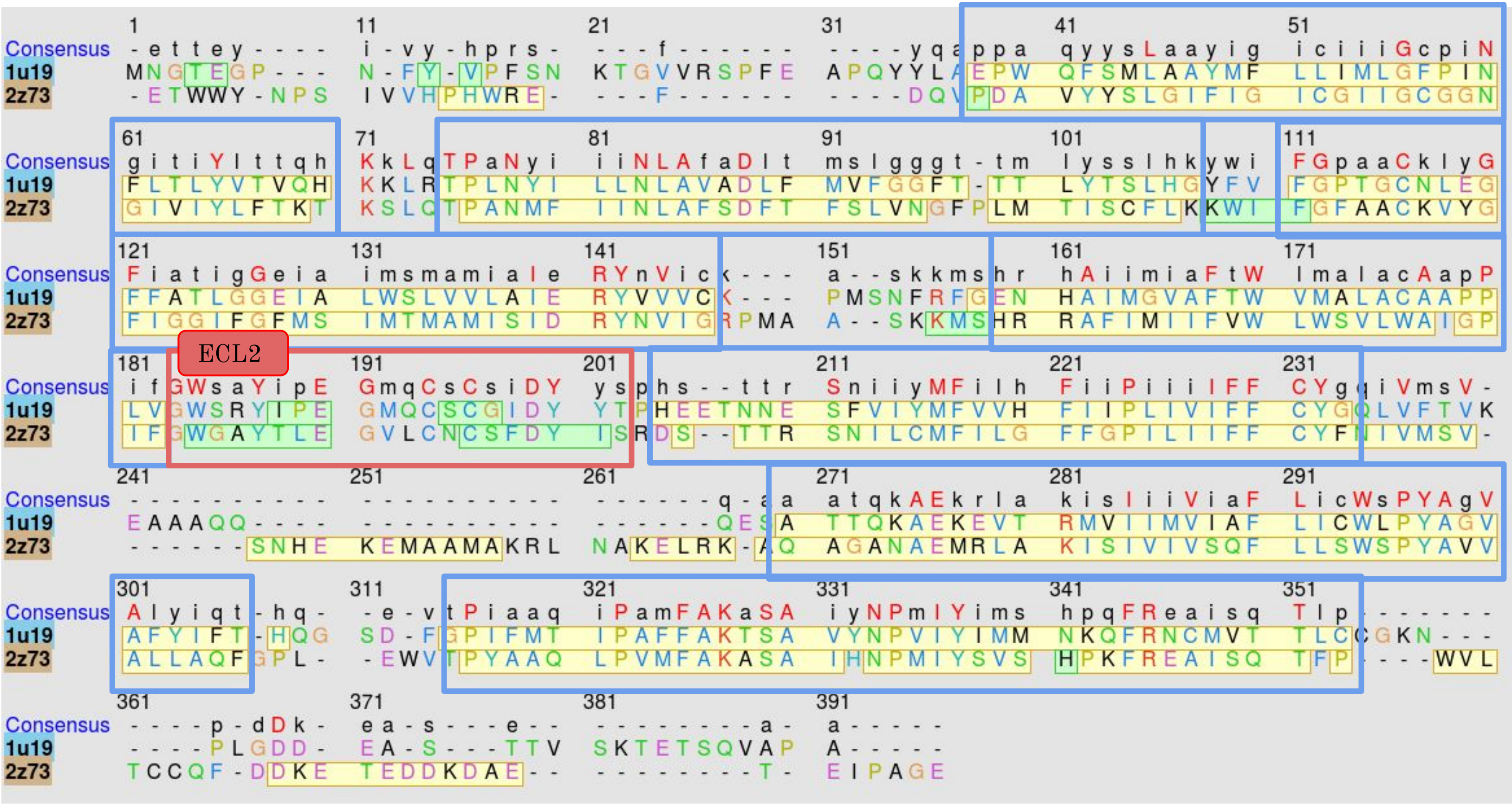

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           1           11          21          31          41          51
Consensus  -ettey----  i-vy-hprs-  ---f------  ----yqappa  qyysLaayig  iciiiGcpiN
1u19       MNGTEGP---  N-FY-VPFSN  KTGVVRSPFE  APQYYLAEPW  QFSMLAAYMF  LLIMLGFPIN
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           121         131         141         151         161         171
Consensus  Fiatiggeia  imsmamiale  RYnVick---  a--skkmshr  hAiimiaFtW  lmalacAapP
1u19       FFATLGGEIA  LWSLVVLAIE  RYVVVCK---  PMSNFRFGEN  HAIMGVAFTW  VMALACAAPP
2z73       FIGGIFGFMS  IMTMAMISID  RYNVIGRPMA  A--SKKMSHR  RAFIMIIFVW  LWSVLWAIGP

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           361         371         381         391
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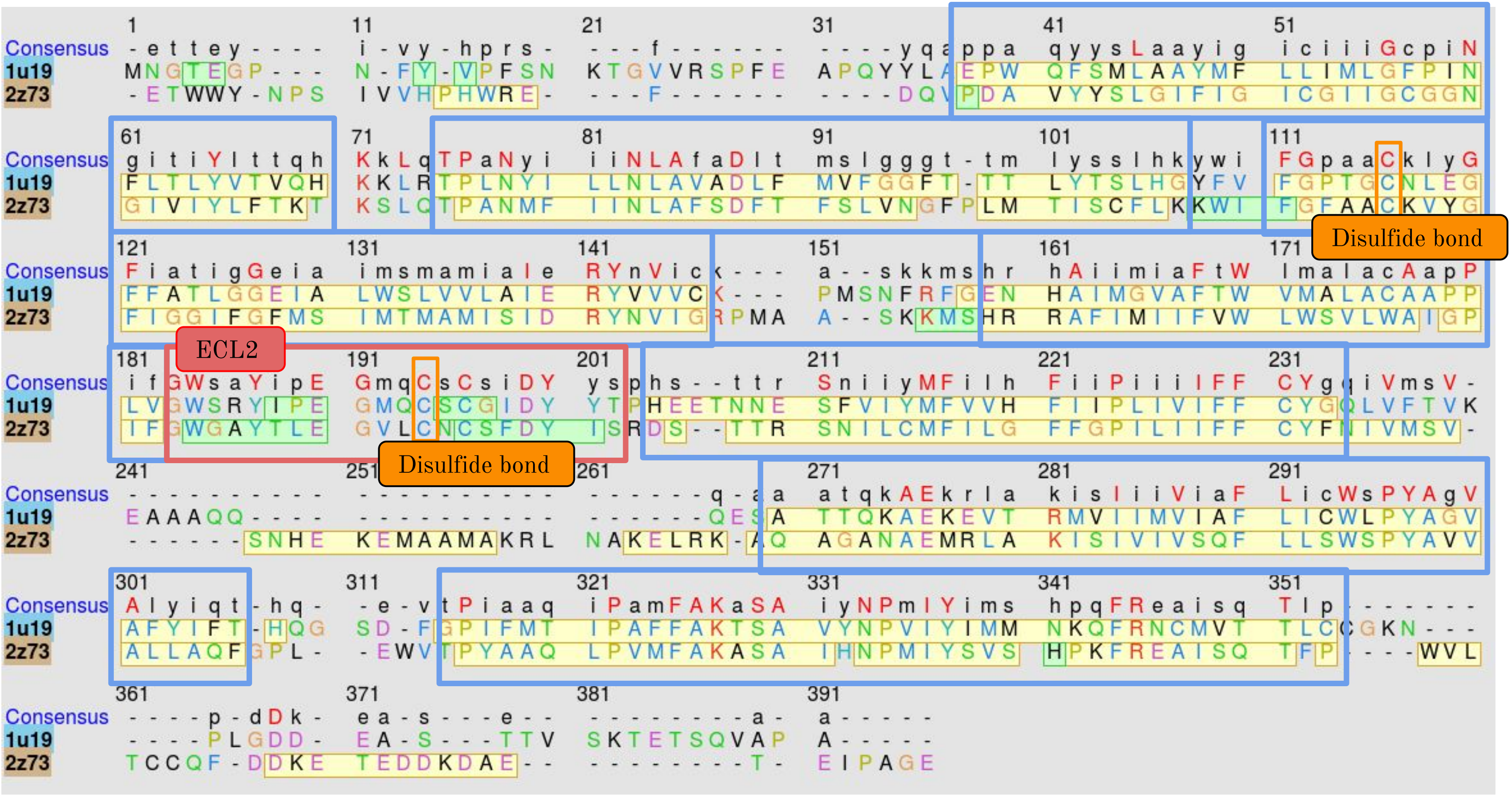

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2z73       TCCQF-DDKE TEDDKDAE-- --------T- EIPAGE
```

Conclusions

- Since transmembrane proteins are difficult to crystallize, bovine rhodopsin is one of the few structures that exist
- Bovine rhodopsin is used as a model to study rhodopsin structure and function due to:
    - Difficulties in transmembrane protein crystallization
    - Particularities of the extraction procedure
- Upon photoactivation, retinal undergoes a cis/trans isomerization and the Schiff base is deprotonated
- Light induces conformational changes in rhodopsin structure:
    - Bending of H6 → opening of a crevice → G protein binding and activation
- Most of the residues involved in the interaction with G protein are conserved
- The binding of retinal to the Lys forming the Schiff base is conserved

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Multiple Choice Questions

1. How is rhodopsin activated?
    a. By ligand binding
    b. By light exposure
    c. It is always active
    d. By a second messenger
    e. By dimerisation
2. Identify the correct sentences
    a. Rhodopsin is a membrane receptor with 7 transmembrane helices
    b. Membrane proteins are difficult to crystallize
    c. a and b are correct
    d. Rhodopsin signals through a G protein
    e. a, b and d are correct

Multiple Choice Questions

3. Regarding rhodopsin structure

   a. It contains 5 domains
   b. It does not have beta-sheets
   c. It has 7 transmembrane helices
   d. It is highly polar
   e. It is a globular protein

4. After light exposure, which protein does rhodopsin activate?

   a. G protein
   b. MAP Kinase
   c. Cycline
   d. Phosphatase
   e. Peptidase

Multiple Choice Questions

5. Which of the following sentences are true?

a. There are not many rhodopsin PDB structures
b. It is not located in the eyes
c. a and b are true
d. The formation of a salt bridge between Phe235 and Phe236 is crucial for its activation
e. a, b and d are true

6. Which of the following sentences are false?

a. There are 2 SCOP families: Bacteriorhodopsin-like and Rhodopsin-like
b. Bacteriorhodopsin is an ion pump
c. Bacteriorhodopsin has been crystallized
d. Bacteriorhodopsin is highly similar to bovine rhodopsin
e. All of them are false

Multiple Choice Questions

7. Regarding rhodopsin structure

   a. Its characteristic fold is a TIM barrel
   b. Water molecules are not relevant for its structure
   c. Retinal is bound to rhodopsin through a Schiff base
   d. Helix 8 is a transmembrane helix
   e. There are not glycosylation sites in rhodopsin

8. Identify the correct sentences:

   a. Light inactivates rhodopsin
   b. The creation of a crevice is necessary to allow G protein binding
   c. a and b are correct
   d. Arg134-Lys256 interaction is very conserved
   e. a, b and d are correct

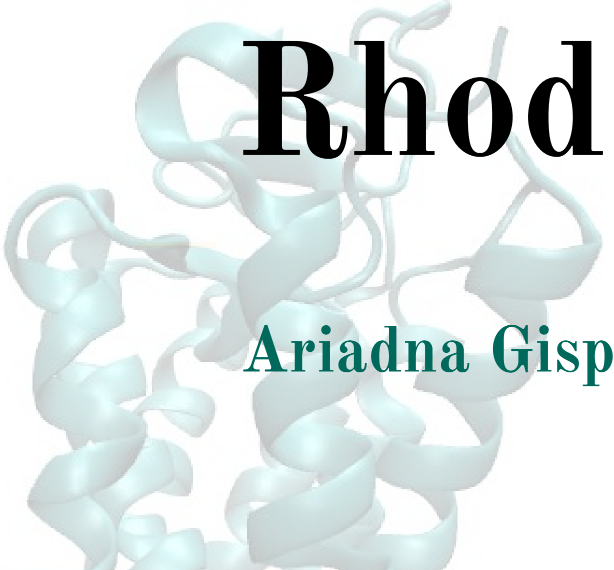
Multiple Choice Questions

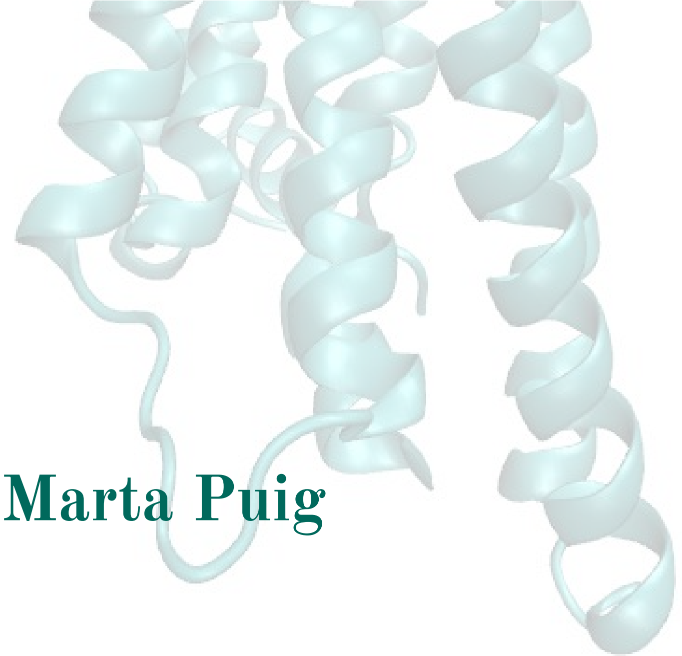
9. Choose the correct sentence

    a. G protein is always bound to rhodopsin
    b. The cis conformation of retinal activates rhodopsin
    c. The ligand binding site is located at the cytoplasmic side of rhodopsin
    d. In rhodopsin, loops are only found in the extracellular side
    e. The Lys involved in Schiff base formation in the ligand binding site is conserved

10. Choose the correct sentence

    a. Rhodopsin has 8 alpha-helices
    b. Rhodopsin has 2 two-stranded beta-sheets
    c. A Schiff base is involved in retinal binding to rhodopsin
    d. The retinal isomerization produced by light activates rhodopsin
    e. All of them are correct





Rhodopsin

Ariadna Gispert, Marina Lleal and Marta Puig