STUDY OF CYS-LOOP FAMILY

ISABEL FERNÁNDEZ
ELENA ESCUBEDO

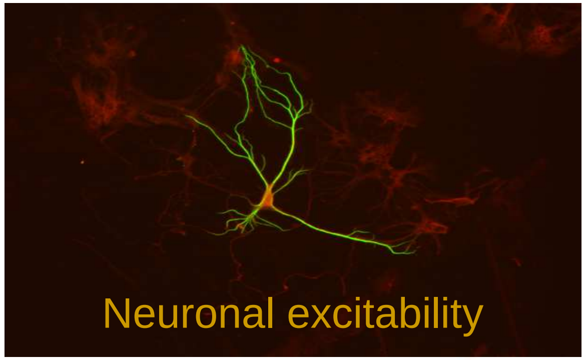
STUDY OF CYS-LOOP FAMILY: INDEX

- CYS-LOOP FAMILY
- THE NICOTINIC RECEPTOR
- ALIGNMENTS
- STUDY OF CONSERVED RESIDUES IN NICOTINIC $\alpha 1$ SUBUNIT
- EVOLUTION OF α SUBUNITS IN CYS-LOOP FAMILIES
- STUDY OF PUNCTUAL MUTATIONS IN NICOTINIC $\alpha 1$ SUBUNIT

CYS-LOOP FAMILY

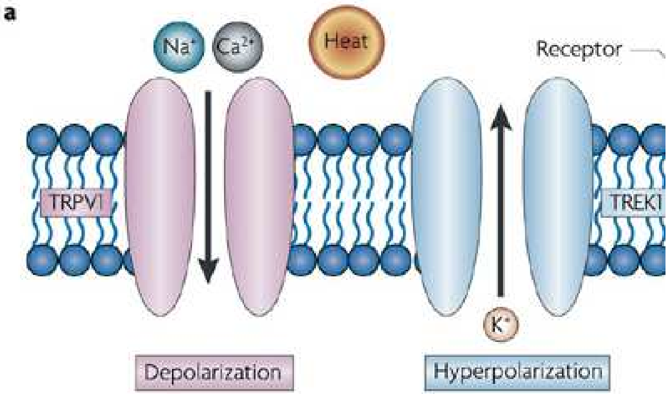
- SYNAPTIC TRANSMISSION
- ION CHANNELS
- CYS-LOOP RECEPTORS: ANIONIC CHANNELS
- CYS-LOOP RECEPTORS: CATIONIC CHANNELS
- NICOTINIC ACETYLCHOLINE RECEPTORS
- CLASSIFICATION
- DOMAINS

SYNAPTIC TRANSMISSION

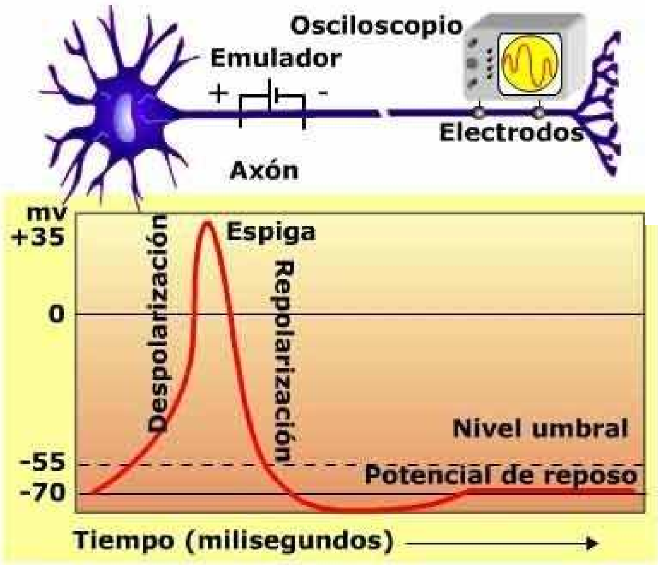


ACTION POTENTIAL

OUTSIDE CELL

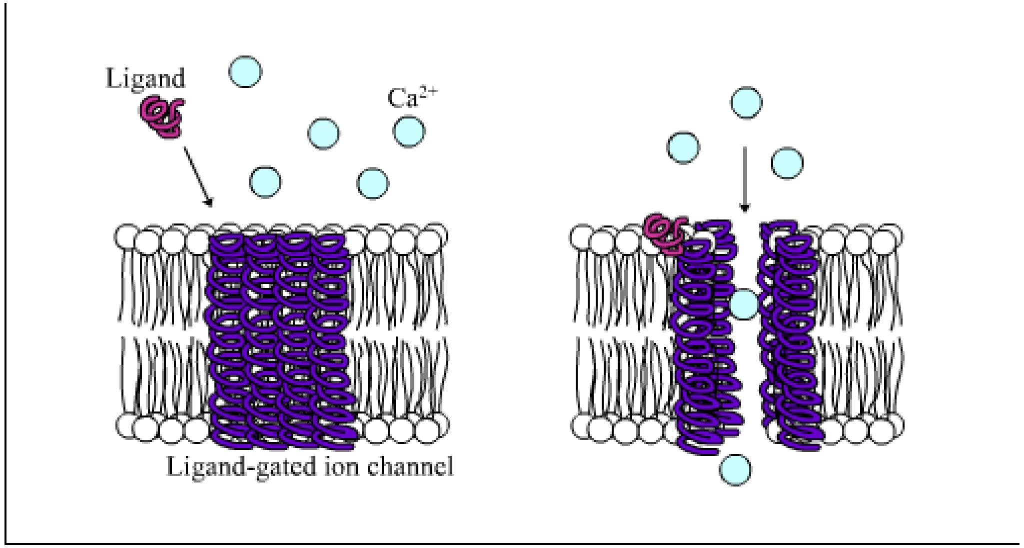


INSIDE CELL



Cuando el axón se depolariza hasta –55 mv Aproximadamente, se inicia el potencial de acción.

A) -Voltage-gated ion channels
B) -Ligand-gated ion channels (LGIC)

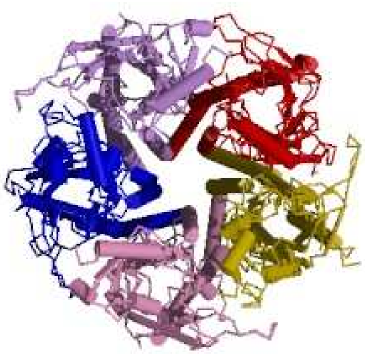


Selectivity

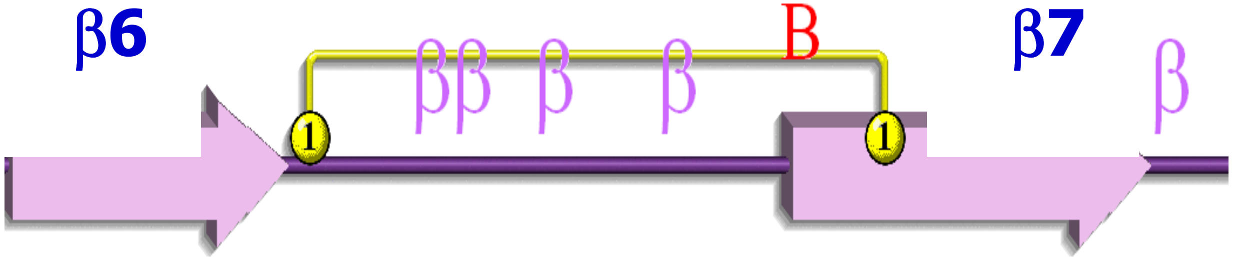
Convert chemical signal

A.- Ionotropic glutamate receptors. Form tetramers
B.- ATP-gated channels . They form trimers.
C.- **Cys-loop receptors**

CYS-LOOP RECEPTORS



PENTAMERIC.

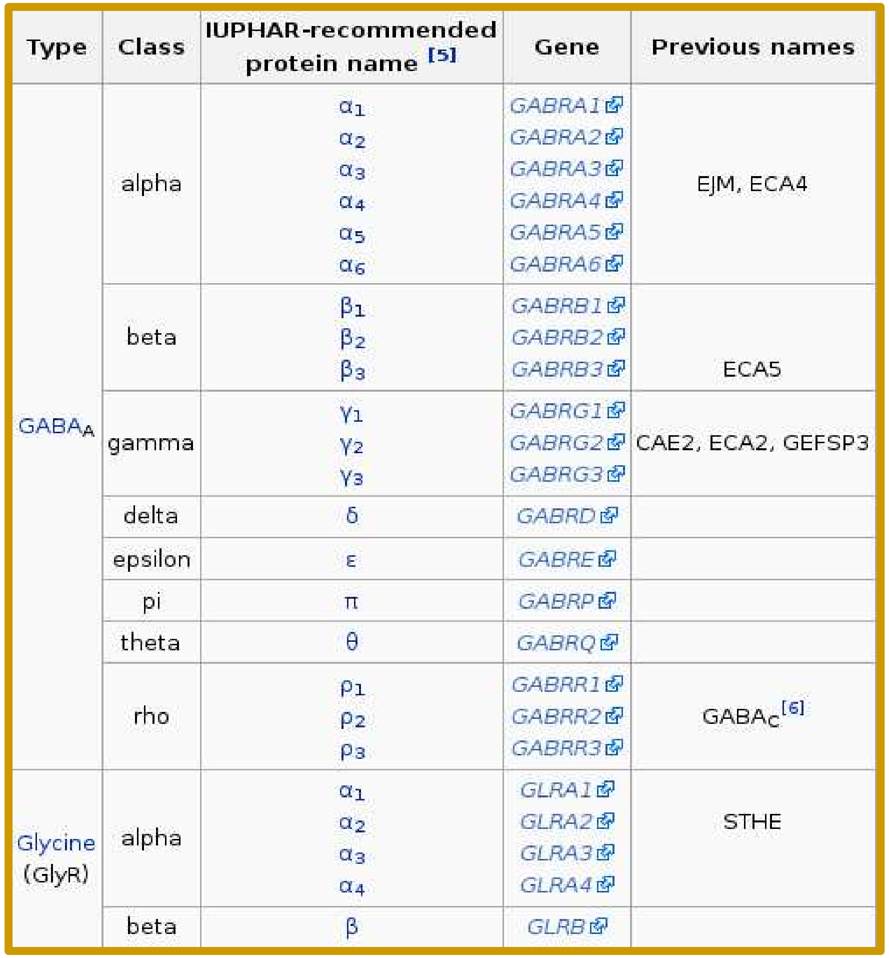


CYS_LOOP : DISULFIDE BOND

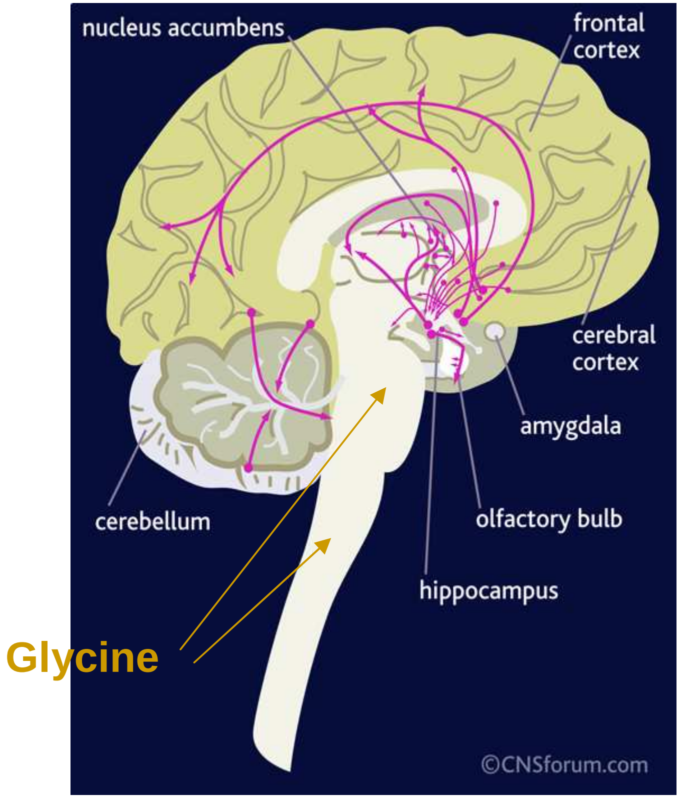
Subdivided: type of ion and the endogenous ligand. Subunits of the cys-loop superfamily :

- one containing the subunits forming **anionic channels (GABA$_A$ and Glycine receptors)**
- one containing the subunits forming **cationic channels (5-HT$_3$ and nicotinic receptors)**

ANIONIC CHANNELS



Type	Class	IUPHAR-recommended protein name [5]	Gene	Previous names
GABA$_A$	alpha	α_1 α_2 α_3 α_4 α_5 α_6	*GABRA1* *GABRA2* *GABRA3* *GABRA4* *GABRA5* *GABRA6*	EJM, ECA4
	beta	β_1 β_2 β_3	*GABRB1* *GABRB2* *GABRB3*	ECA5
	gamma	γ_1 γ_2 γ_3	*GABRG1* *GABRG2* *GABRG3*	CAE2, ECA2, GEFSP3
	delta	δ	*GABRD*	
	epsilon	ε	*GABRE*	
	pi	π	*GABRP*	
	theta	θ	*GABRQ*	
	rho	ρ_1 ρ_2 ρ_3	*GABRR1* *GABRR2* *GABRR3*	GABA$_C$[6]
Glycine (GlyR)	alpha	α_1 α_2 α_3 α_4	*GLRA1* *GLRA2* *GLRA3* *GLRA4*	STHE
	beta	β	*GLRB*	



CATIONIC CHANNELS

Cationic

Type	Class	IUPHAR-recommended protein name [5]	Gene	Previous names
Serotonin (5-HT)	5-HT$_3$	5-HT3A	HTR3A	5-HT$_{3A}$
		5-HT3B	HTR3B	5-HT$_{3B}$
		5-HT3C	HTR3C	5-HT$_{3C}$
		5-HT3D	HTR3D	5-HT$_{3D}$
		5-HT3E	HTR3E	5-HT$_{3E}$
** Nicotinic acetylcholine (nAChR)	alpha	α_1	CHRNA1	ACHRA, ACHRD, CHRNA, CMS2A, FCCMS, SCCMS
		α_2	CHRNA2	
		α_3	CHRNA3	
		α_4	CHRNA4	
		α_5	CHRNA5	
		α_6	CHRNA6	
		α_7	CHRNA7	
		α_9	CHRNA9	
		α_{10}	CHRNA10	
	beta	β_1	CHRNB1	CMS2A, SCCMS, ACHRB, CHRNB, CMS1D EFNL3, nAChRB2
		β_2	CHRNB2	
		β_3	CHRNB3	
		β_4	CHRNB4	
	gamma	γ	CHRNG	ACHRG
	delta	δ	CHRND	ACHRD, CMS2A, FCCMS, SCCMS
	epsilon	ε	CHRNE	ACHRE, CMS1D, CMS1E, CMS2A, FCCMS, SCCMS
Zinc-activated ion channel (ZAC)		ZAC	ZACN	ZAC1, L2m LGICZ, LGICZ1

nAChR: fast synaptic transmission and neuromuscular junction

PDB: 2BG9,……….Staphylococcus, snails, mouse (truncated)

NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR)

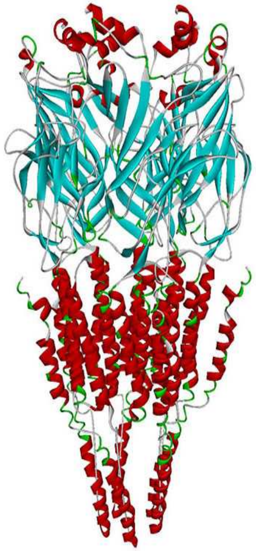
Neuronal-type					Muscle-type
I	II	III			IV
		1	2	3	
α9, α10	α7, α8	α2, α3, α4, α6	β2, β4	β3, α5	α1, β1, δ, γ, ε

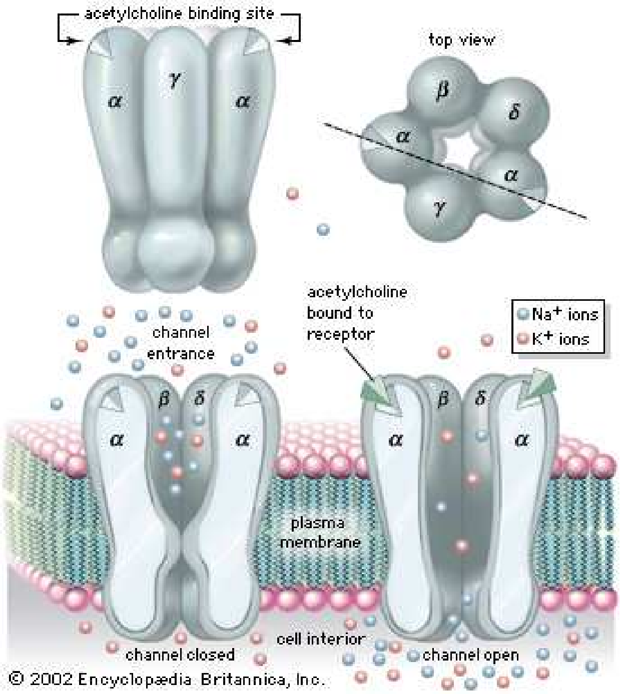
17

NEURONAL-TYPE

Heteromeric: 3α:2β

Homomeric: 5α

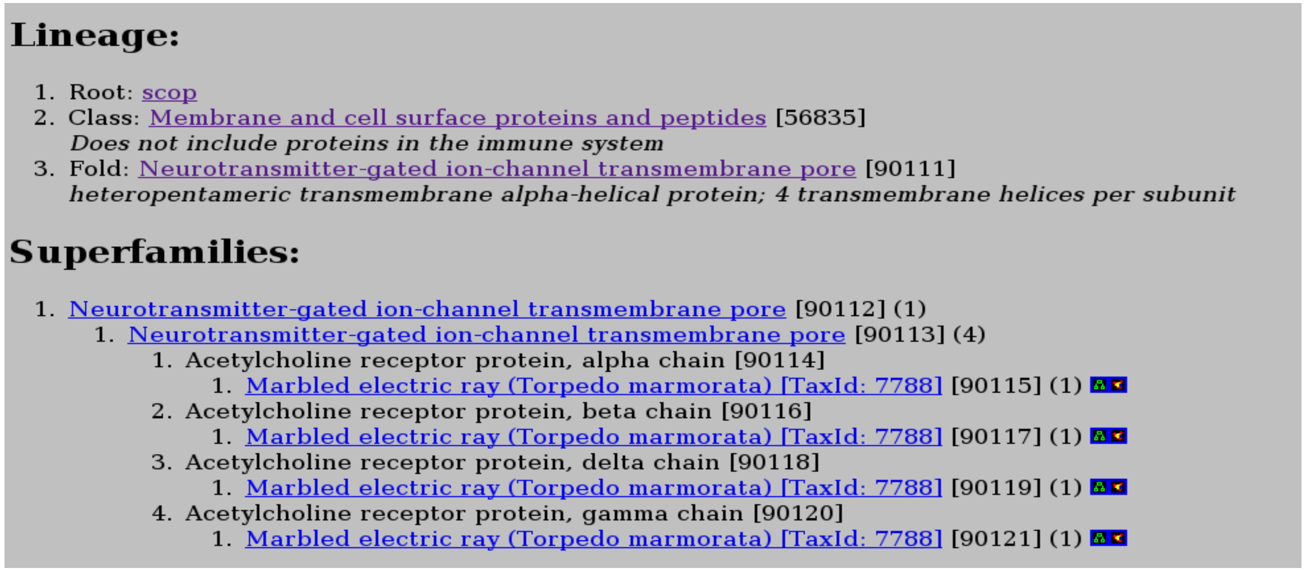




© 2002 Encyclopædia Britannica, Inc.

MUSCLE- TYPE

2 α : β : γ : δ/ε

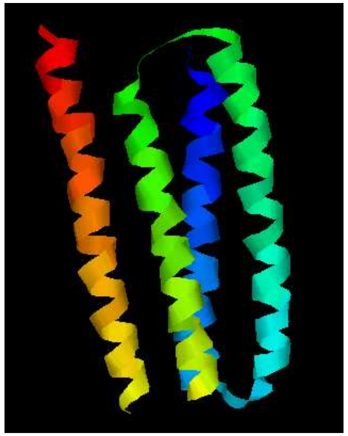


Lineage:

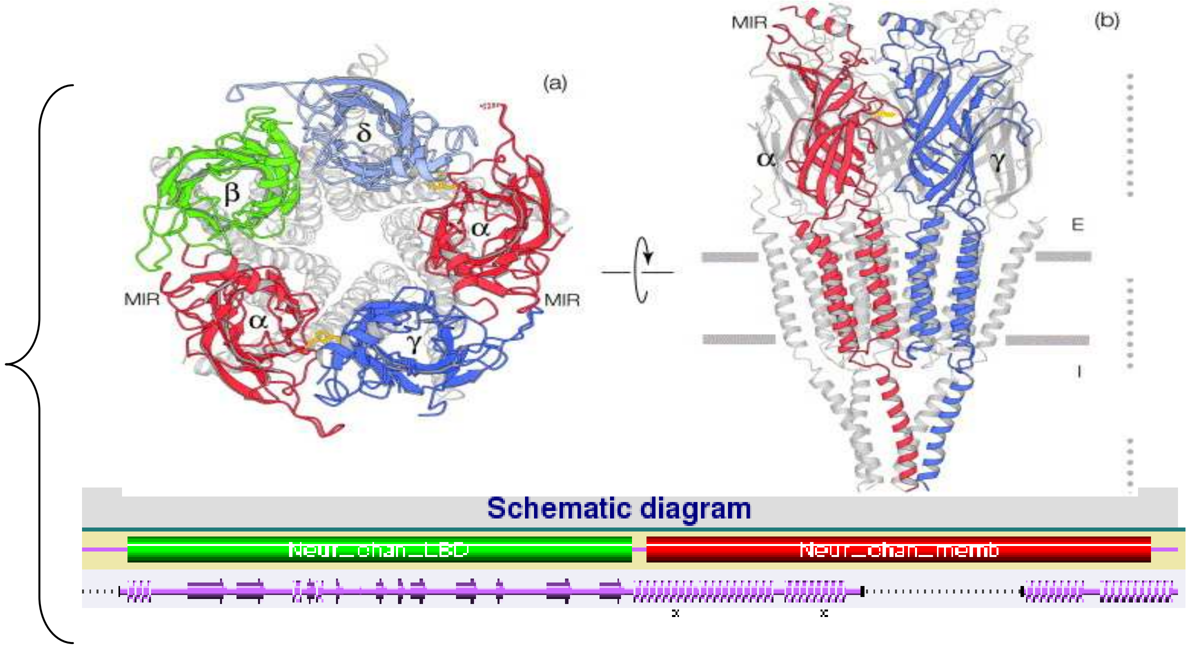
1. Root: scop
2. Class: Membrane and cell surface proteins and peptides [56835]
   Does not include proteins in the immune system
3. Fold: Neurotransmitter-gated ion-channel transmembrane pore [90111]
   heteropentameric transmembrane alpha-helical protein; 4 transmembrane helices per subunit

Superfamilies:

1. Neurotransmitter-gated ion-channel transmembrane pore [90112] (1)
    1. Neurotransmitter-gated ion-channel transmembrane pore [90113] (4)
        1. Acetylcholine receptor protein, alpha chain [90114]
            1. Marbled electric ray (Torpedo marmorata) [TaxId: 7788] [90115] (1)
        2. Acetylcholine receptor protein, beta chain [90116]
            1. Marbled electric ray (Torpedo marmorata) [TaxId: 7788] [90117] (1)
        3. Acetylcholine receptor protein, delta chain [90118]
            1. Marbled electric ray (Torpedo marmorata) [TaxId: 7788] [90119] (1)
        4. Acetylcholine receptor protein, gamma chain [90120]
            1. Marbled electric ray (Torpedo marmorata) [TaxId: 7788] [90121] (1)



PFAM



LBD

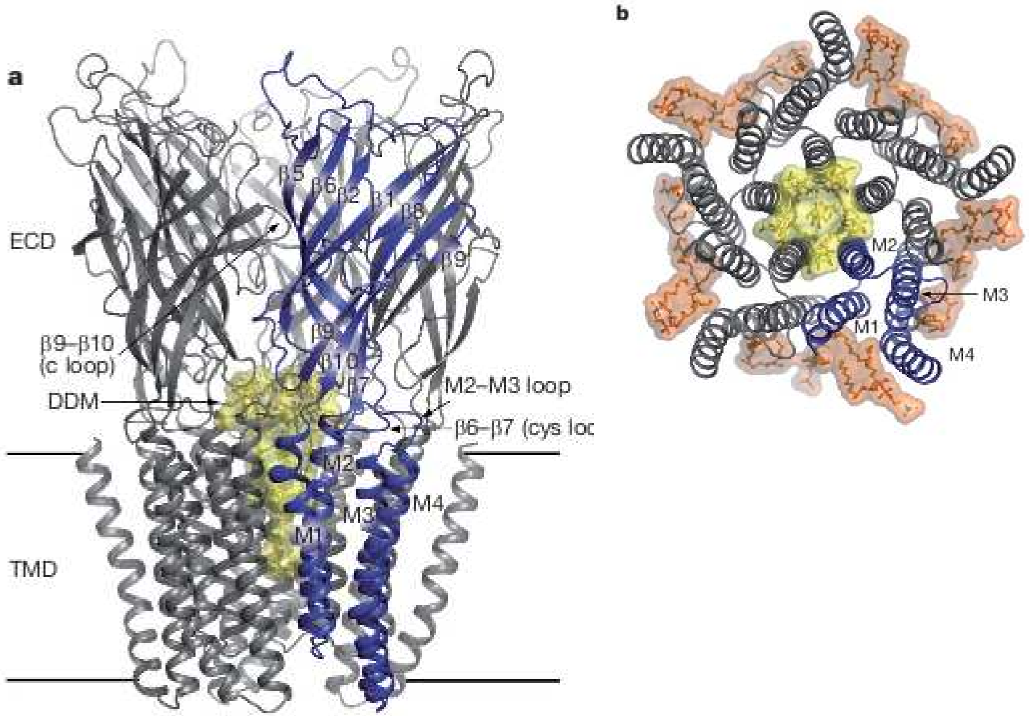
TMD

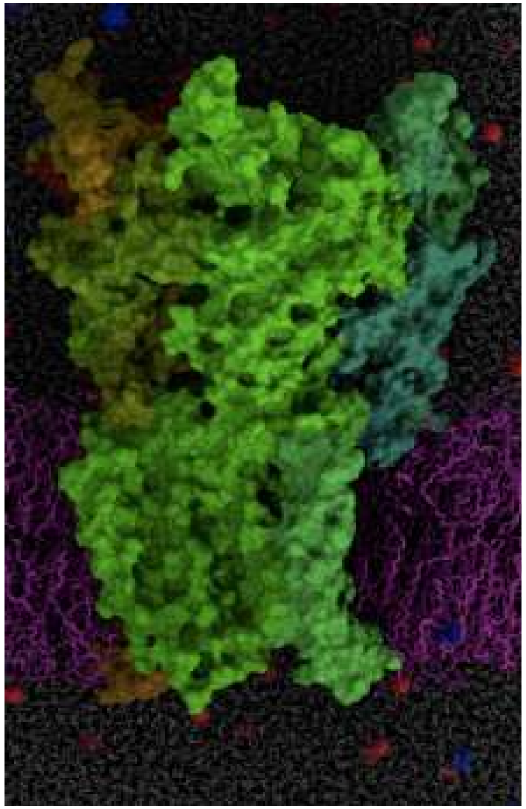
THE NICOTINIC RECEPTOR
GENERAL STRUCTURE

- A PENTAMER
- TOPOLOGY DIAGRAM OF LBD
- LBD
- TMDs

A PENTAMER

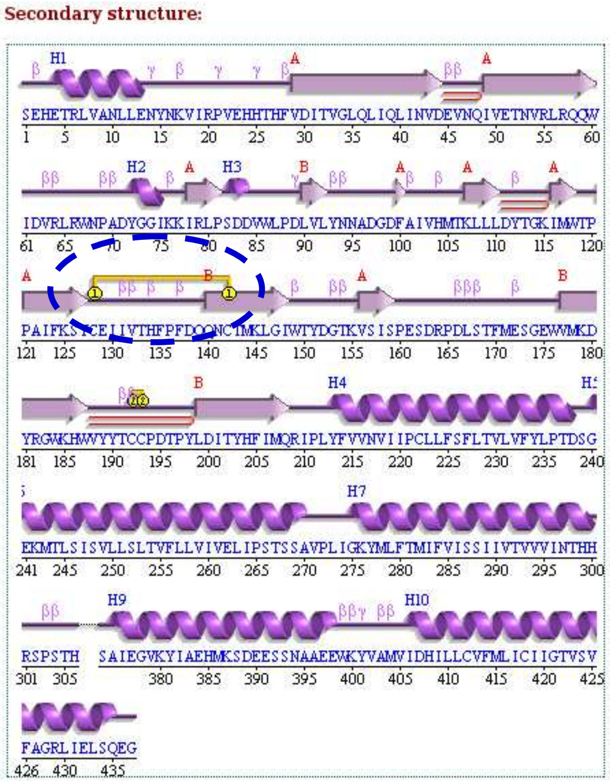
Five subunits arranged in a barrel-like manner





TOPOLOGY DIAGRAM

Fold : α+ β protein and all α (9AH, 12BS)



Secondary structure:

SEHETRLVANLLENYNKVIRPVEHHTHFVDITVGLQLIQLINVDEVNQIVETNVRLRQQW
1 5 10 15 20 25 30 35 40 45 50 55 60

IDVRLRWNPADYGGIKKIRLPSDDVWLPDLVLYNNADGDFAIVHMTKLLLDYTGKIMWTP
61 65 70 75 80 85 90 95 100 105 110 115 120

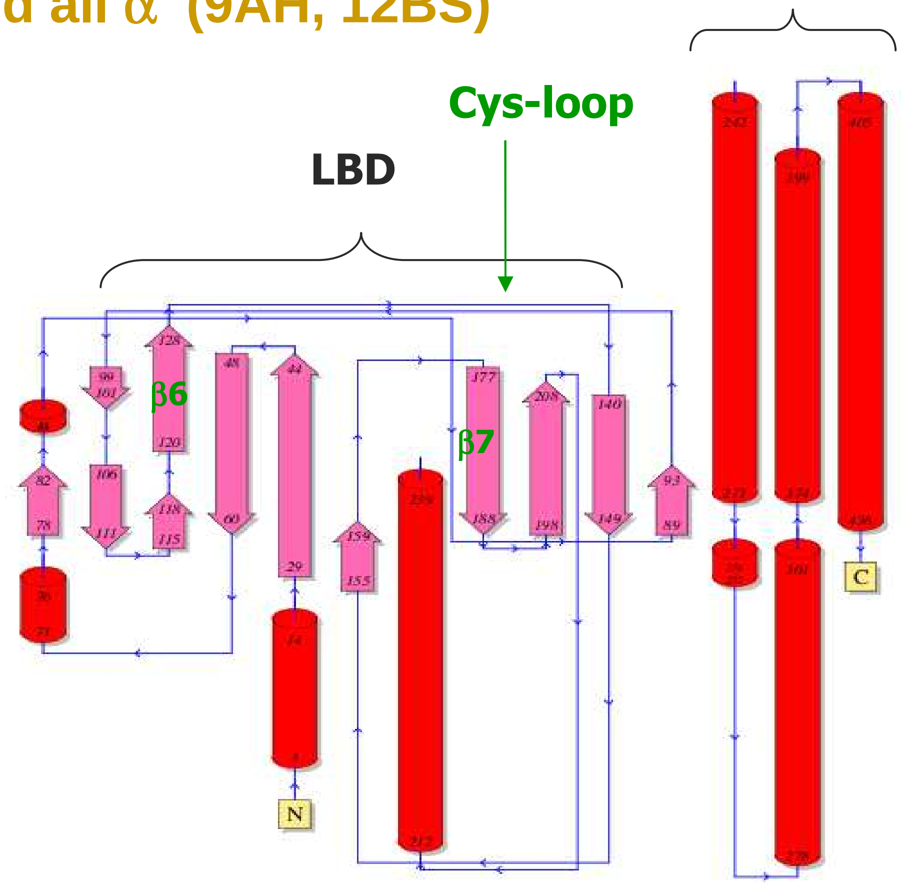
PAIFKSYCEIIVTHFPFDQQNCTMKLGIWTYDGTKVSISPESDRPDLSTFMESGEWVMKD
121 125 130 135 140 145 150 155 160 165 170 175 180

YRGWKHWVYYTCCPDTPYLDITYHFIMQRIPLYFVVNVIIPCLLFSFLTVLVFYLPTDSG
181 185 190 195 200 205 210 215 220 225 230 235 240

EKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMIFVISSIIVTVVVINTHH
241 245 250 255 260 265 270 275 280 285 290 295 300

RSPSTH SAIEGVKYIAEHMKSDEESSNAAEEWKYVAMVIDHILLCVFMLICIIGTVSV
301 305 380 385 390 395 400 405 410 415 420 425

FAGRLIELSQEG
426 430 435



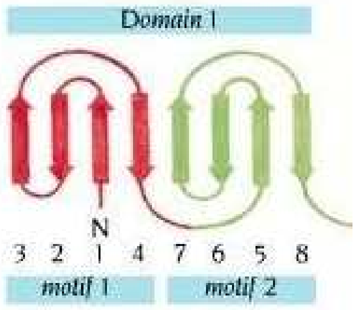
TMD

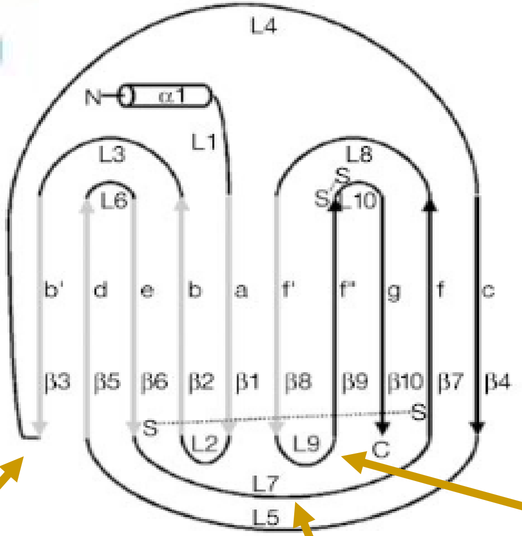
LBD

Cys-loop

TOPOLOGY DIAGRAM OF THE LBD

- β-sheet (from β1 to β10),
- a short helix (H1) at the N terminus.
- organized in 2 sets of β strands forming a “greek key”
- linked together through the cys-loop disulphide bound
- folded into a curled β-sandwich.
- β-sandwich 5 inner 3 outer strands





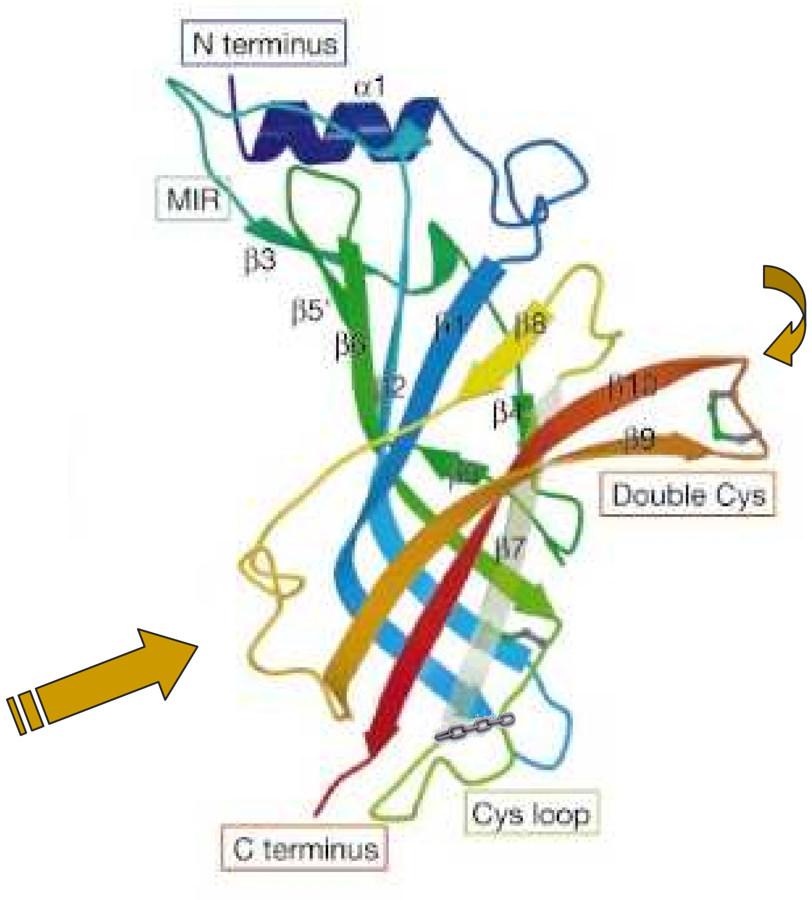
the additional strand (b')

Cys-loop

hairpin (f'±f'')

LIGAND BINDING DOMAIN

- β9 (**C loop**) β10 → LBS (between adjacent subunits).
- At the base of the LBD, inserted in between the tops of the four TM helices, is the conserved **Cys loop** from which the superfamily was named.
- **S-S** in cys loop: identity
- S-S in C loop
- β8 - β9 : sequence variation in the LBD.

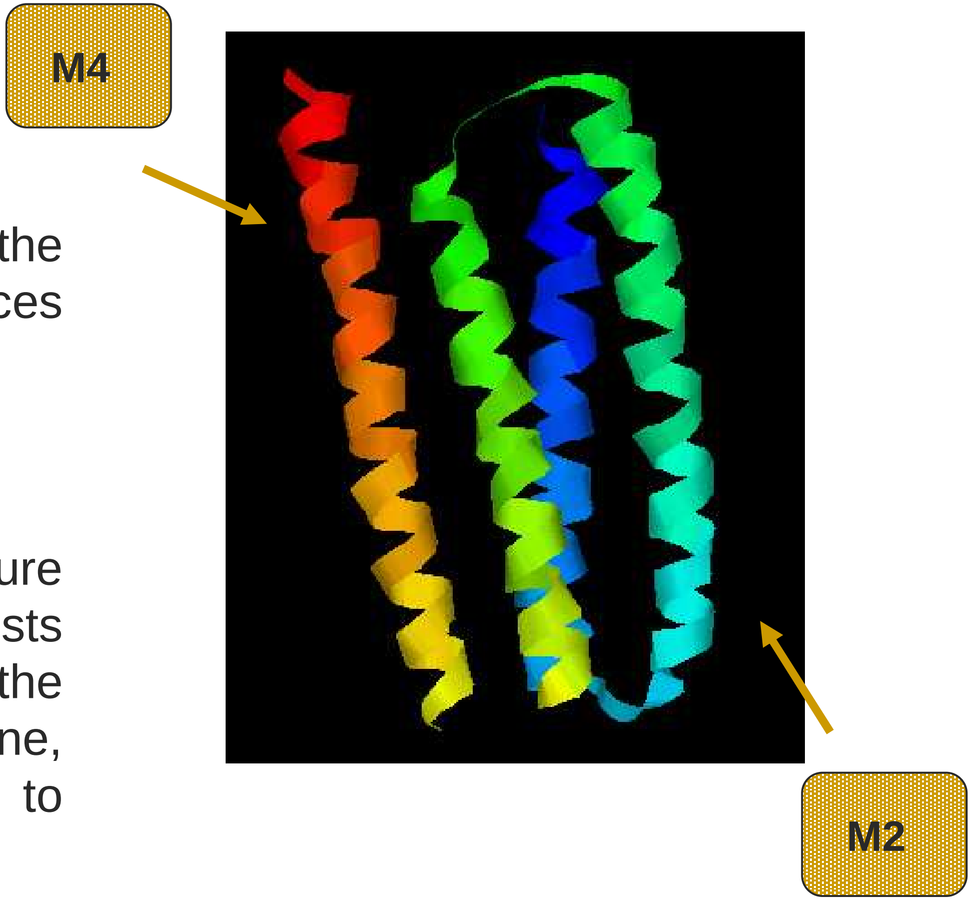


TRANSMEMBRANE DOMAIN

TMD:

- N and C termini extracellular
- The protein passing through the membrane 4 times as α-helices (M1 to M4).
- M2 is the pore-lining helix.

Because of the lack of a structure for the vestibule domain that exists between M3 and M4, on the cytoplasmic side of the membrane, M4 is not covalently connected to the rest of the protein.



ALIGNMENTS

- TORPEDO MARMORATA
- HUMAN NICOTINIC α SUBUNITS
- HUMAN α SUBUNITS (EXTENDED)

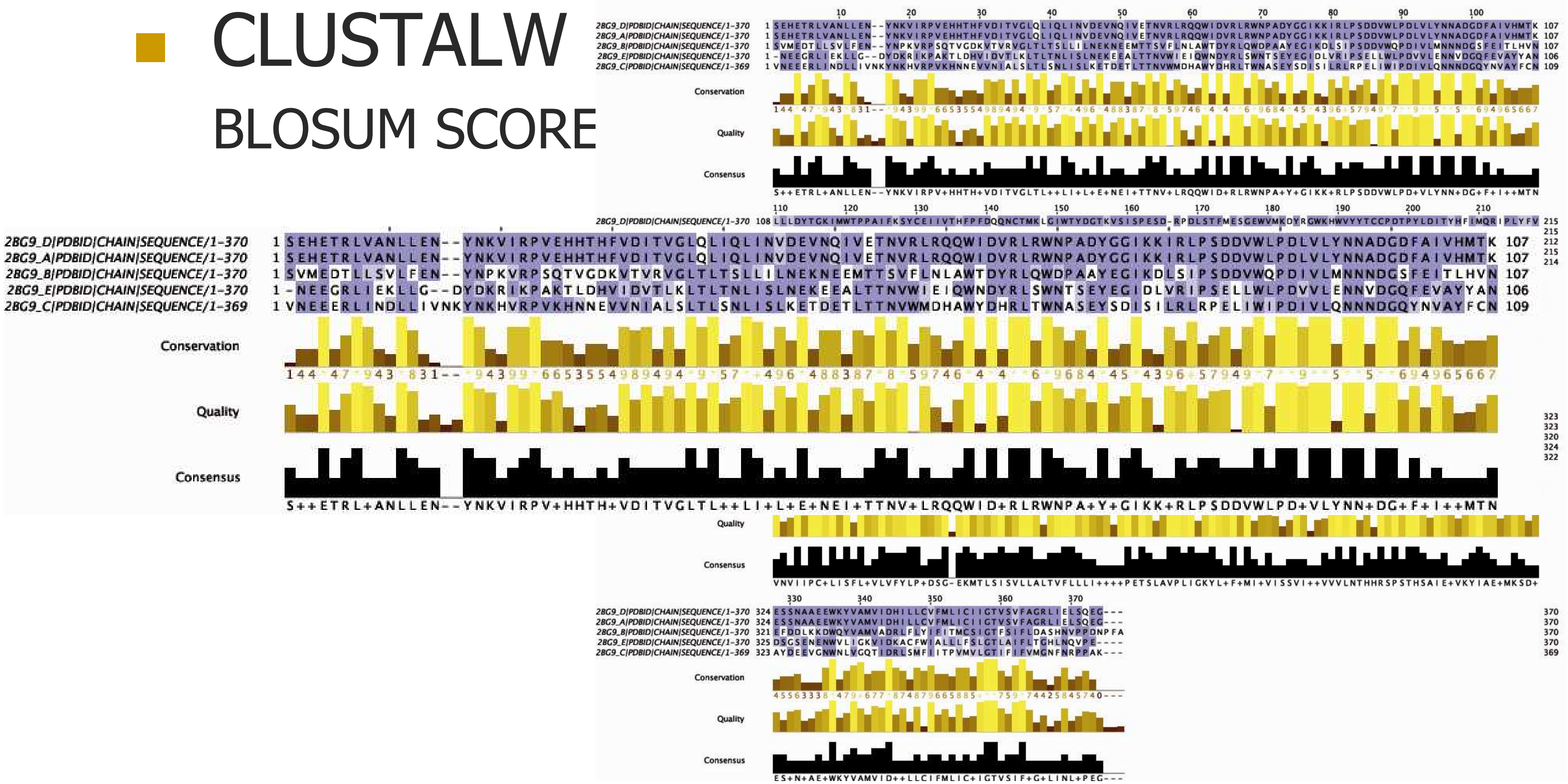
ALIGNMENTS: TORPEDO MARMORATA

- CLUSTALW
- TCOFEE
- HMM
  - PFAM MATRIX (NEUCHAN)
  - CUSTOM MATRIX
- STAMP

ALIGNMENTS: TORPEDO MARMORATA

- CLUSTALW

  BLOSUM SCORE

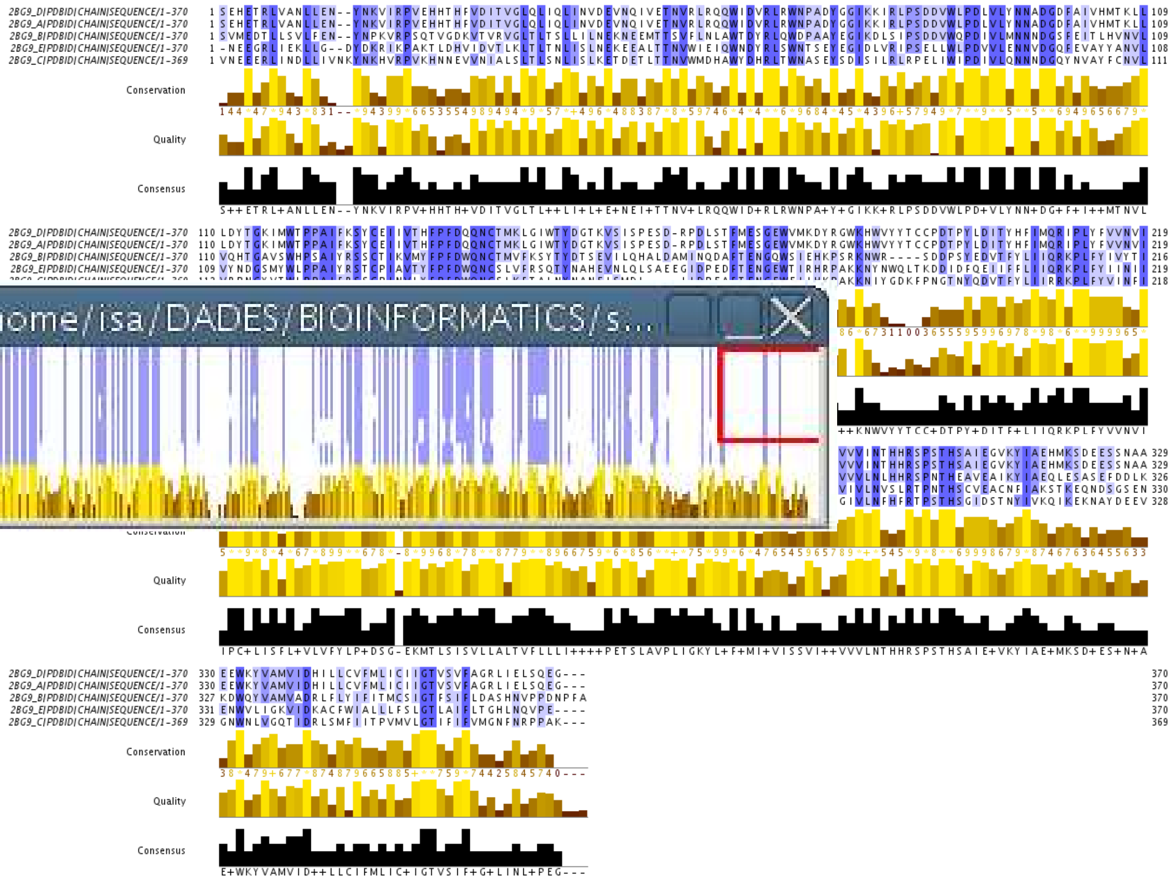


```
2BG9_D|PDBID|CHAIN|SEQUENCE/1-370  1 SEHETRLVANLLEN--YNKVIRPVEHHTHFVDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPADYGGIKKIRLPSDDVWLPDLVLYNNADGDFAIVHMTK 107
2BG9_A|PDBID|CHAIN|SEQUENCE/1-370  1 SEHETRLVANLLEN--YNKVIRPVEHHTHFVDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPADYGGIKKIRLPSDDVWLPDLVLYNNADGDFAIVHMTK 107
2BG9_B|PDBID|CHAIN|SEQUENCE/1-370  1 SVMEDTLLSVLFEN--YNPKVRPSQTVGDKVTVRVGLTLTSLLILNEKNEEMTTSVFLNLAWTDYRLQWDPAAYEGIKDLSIPSDDVWQPDIVLMNNNDGSFEITLHVN 107
2BG9_E|PDBID|CHAIN|SEQUENCE/1-370  1 -NEEGRLIEKLLG--DYDKRIKPAKTLDHVIDVTLKLTLTNLISLNEKEEALTTNVWIEIQWNDYRLSWNTSEYEGIDLVRIPSELLWLPDVVLENNVDGQFEVAYYAN 106
2BG9_C|PDBID|CHAIN|SEQUENCE/1-369  1 VNEEERLINDLLIVNKYNKHVRPVKHNNEVVNIALSLTLSNLISLKETDETLTTNVWMDHAWYDHRLTWNASEYSDISILRLRPELIWIPDIVLQNNNDGQYNVAYFCN 109

Conservation
                                     144*47*943*831--*94399*665355498949 4*9*57*+496*488387*8*59746*4*4**6*9684*45*4396+57949*7**9**5**5**694965667

Quality

Consensus
                                     S++ETRL+ANLLEN--YNKVIRPV+HHTH+VDITVGLTL++LI+L+E+NEI+TTNV+LRQQWID+RLRWNPA+Y+GIKK+RLPSDDVWLPD+VLYNN+DG+F+I++MTN
```

ALIGNMENTS: TORPEDO MARMORATA

- CLUSTALW

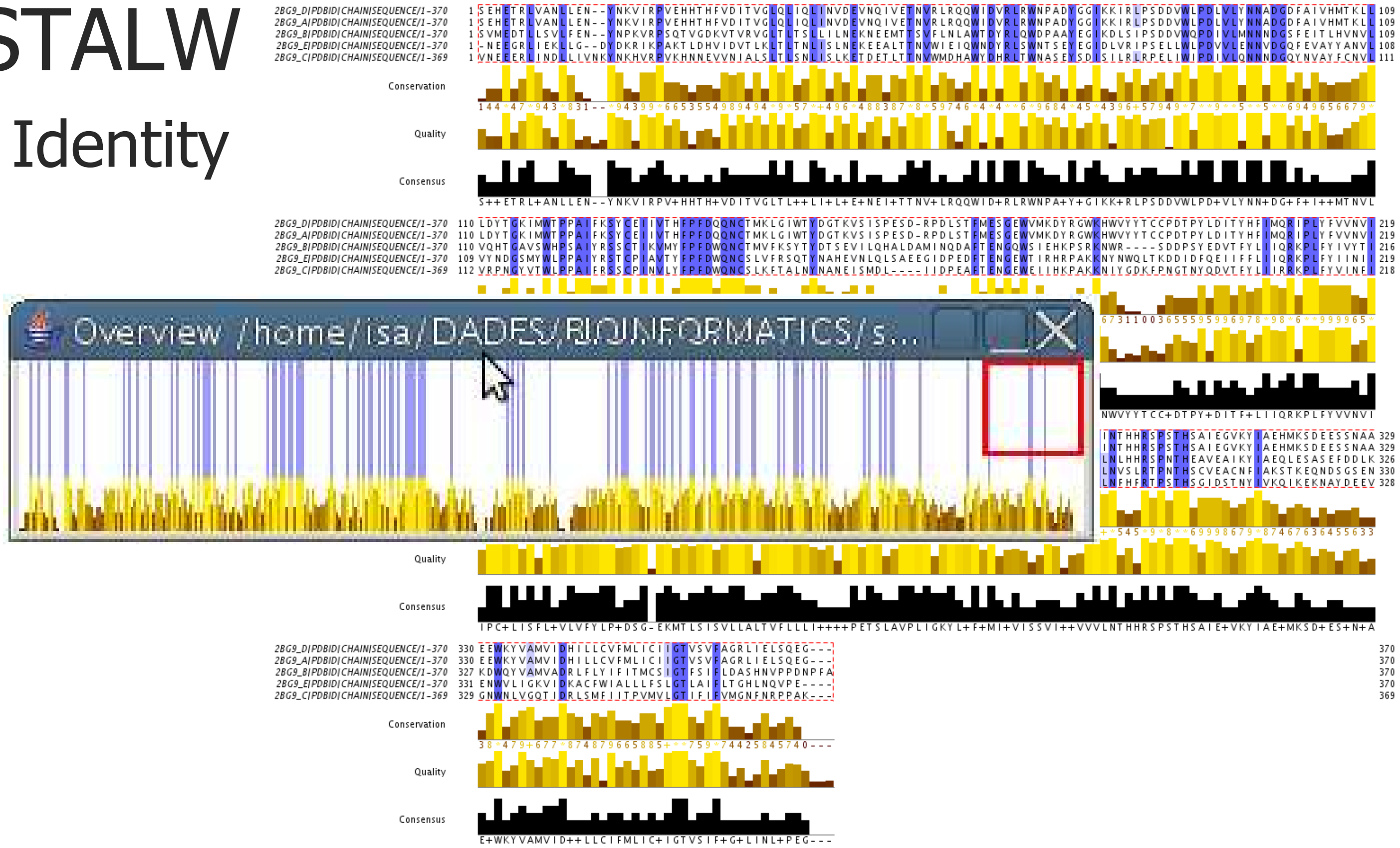
  70% Identity



ALIGNMENTS: TORPEDO MARMORATA

- CLUSTALW

  100% Identity



```
2BG9_D|PDBID|CHAIN|SEQUENCE/1-370   1 SEHETRLVANLLEN--YNKVIRPVEHHTHFVDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPADYGGIKKIRLPSDDVWLPDLVLYNNADGDFAIVHMTKLL 109
2BG9_A|PDBID|CHAIN|SEQUENCE/1-370   1 SEHETRLVANLLEN--YNKVIRPVEHHTHFVDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPADYGGIKKIRLPSDDVWLPDLVLYNNADGDFAIVHMTKLL 109
2BG9_B|PDBID|CHAIN|SEQUENCE/1-370   1 SVMEDTLLSVLFEN--YNPKVRPSQTVGDKVTVRVGLTLTSLLILNEKNEEMTTSVFLNLAWTDYRLQWDPAAYEGIKDLSIPSDDVWQPDIVLMNNNDGSFEITLHVNVL 109
2BG9_E|PDBID|CHAIN|SEQUENCE/1-370   1 -NEEGRLIEKLLG--DYDKRIKPAKTLDHVIDVTLKLTLTNLISLNEKEEALTTNVWIEIQWNDYRLSWNTSEYEGIDLVRIPSELLWLPDVVLENNVDGQFEVAYYANVL 108
2BG9_C|PDBID|CHAIN|SEQUENCE/1-369   1 VNEEERLINDLLIVNKYNKHVRPVKHNNEVVNIALSLTLSNLISLKETDETLTTNVWMDHAWYDHRLTWNASEYSDISILRLRPELIWIPDIVLQNNNDGQYNVAYFCNVL 111
                        Conservation
                                      144*47*943*831--*94399*6653554989494*9*57*+496*488387*8*59746*4*4**6*9684*45*4396+57949*7**9**5**5**6949656679*
                             Quality
                           Consensus
                                      S++ETRL+ANLLEN--YNKVIRPV+HHTH+VDITVGLTL++LI+L+E+NEI+TTNV+LRQQWID+RLRWNPA+Y+GIKK+RLPSDDVWLPD+VLYNN+DG+F+I++MTNVL

2BG9_D|PDBID|CHAIN|SEQUENCE/1-370 110 LDYTGKIMWTPPAIFKSYCEIIVTHFPFDQQNCTMKLGIWTYDGTKVSISPESD-RPDLSTFMESGEWVMKDYRGWKHWVYYTCCPDTPYLDITYHFIMQRIPLYFVVNVI 219
2BG9_A|PDBID|CHAIN|SEQUENCE/1-370 110 LDYTGKIMWTPPAIFKSYCEIIVTHFPFDQQNCTMKLGIWTYDGTKVSISPESD-RPDLSTFMESGEWVMKDYRGWKHWVYYTCCPDTPYLDITYHFIMQRIPLYFVVNVI 219
2BG9_B|PDBID|CHAIN|SEQUENCE/1-370 110 VQHTGAVSWHPSAIYRSSCTIKVMYFPFDWQNCTMVFKSYTYDTSEVILQHALDAMINQDAFTENGQWSIEHKPSRKNWR----SDDPSYEDVTFYLIIQRKPLFYIVYTI 216
2BG9_E|PDBID|CHAIN|SEQUENCE/1-370 109 VYNDGSMYWLPPAIYRSTCPIAVTYFPFDWQNCSLVFRSQTYNAHEVNLQLSAEEGIDPEDFTENGEWTIRHRPAKKNYNWQLTKDDIDFQEIIFFLIIQRKPLFYIINII 219
2BG9_C|PDBID|CHAIN|SEQUENCE/1-369 112 VRPNGYVTWLPPAIFRSSCPINVLYFPFDWQNCSLKFTALNYNANEISMDL----IIDPEAFTENGEWEIIHKPAKKNIYGDKFPNGTNYQDVTFYLIIRRKPLFYVINFI 218
                                      67311003655595996978*98*6**999965*
                                      NWVYYTCC+DTPY+DITF+LIIQRKPLFYVVNVI

                                      INTHHRSPSTHSAIEGVKYIAEHMKSDEESSNAA 329
                                      INTHHRSPSTHSAIEGVKYIAEHMKSDEESSNAA 329
                                      LNLHHRSPNTHEAVEAIKYIAEQLESASEFDDLK 326
                                      LNVSLRTPNTHSCVEACNFIAKSTKEQNDSGSEN 330
                                      LNFHFRTPSTHSGIDSTNYIVKQIKEKNAYDEEV 328
                                      +*545*9*8**69998679*87467636455633
                             Quality
                           Consensus
                                      IPC+LISFL+VLVFYLP+DSG-EKMTLSISVLLALTVFLLLI++++PETSLAVPLIGKYL+F+MI+VISSVI++VVVLNTHHRSPSTHSAIE+VKYIAE+MKSD+ES+N+A

2BG9_D|PDBID|CHAIN|SEQUENCE/1-370 330 EEWKYVAMVIDHILLCVFMLICIIGTVSVFAGRLIELSQEG---   370
2BG9_A|PDBID|CHAIN|SEQUENCE/1-370 330 EEWKYVAMVIDHILLCVFMLICIIGTVSVFAGRLIELSQEG---   370
2BG9_B|PDBID|CHAIN|SEQUENCE/1-370 327 KDWQYVAMVADRLFLYIFITMCSIGTFSIFLDASHNVPPDNPFA   370
2BG9_E|PDBID|CHAIN|SEQUENCE/1-370 331 ENWVLIGKVIDKACFWIALLLFSLGTLAIFLTGHLNQVPE----   370
2BG9_C|PDBID|CHAIN|SEQUENCE/1-369 329 GNWNLVGQTIDRLSMFIITPVMVLGTIFIFVMGNFNRPPAK---   369
                        Conservation
                                      38*479+677*874879665885+**759*74425845740---
                             Quality
                           Consensus
                                      E+WKYVAMVID++LLCIFMLIC+IGTVSIF+G+LINL+PEG---
```

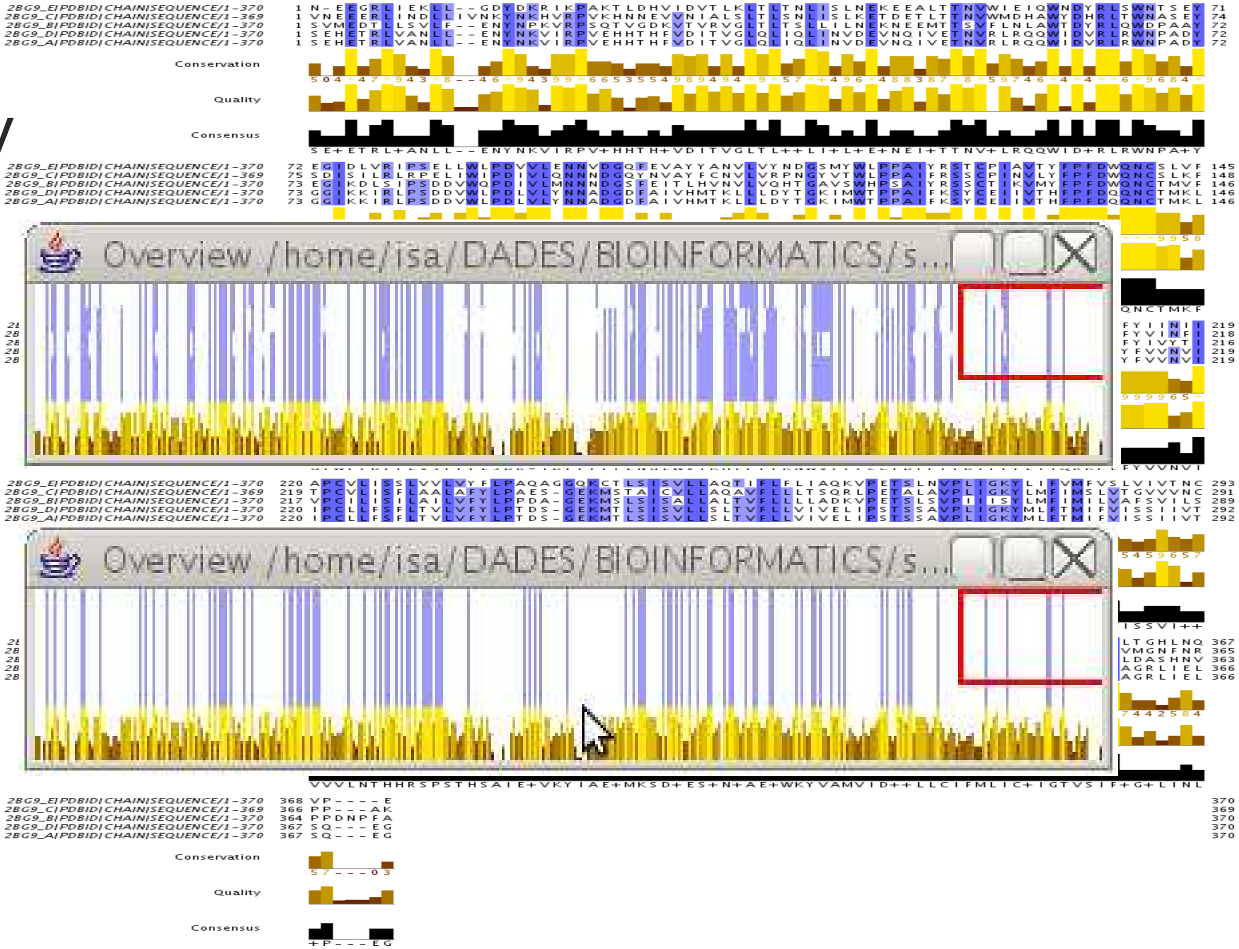
ALIGNMENTS: TORPEDO MARMORATA

- TCOFFEE

70% Identity

70% Identity

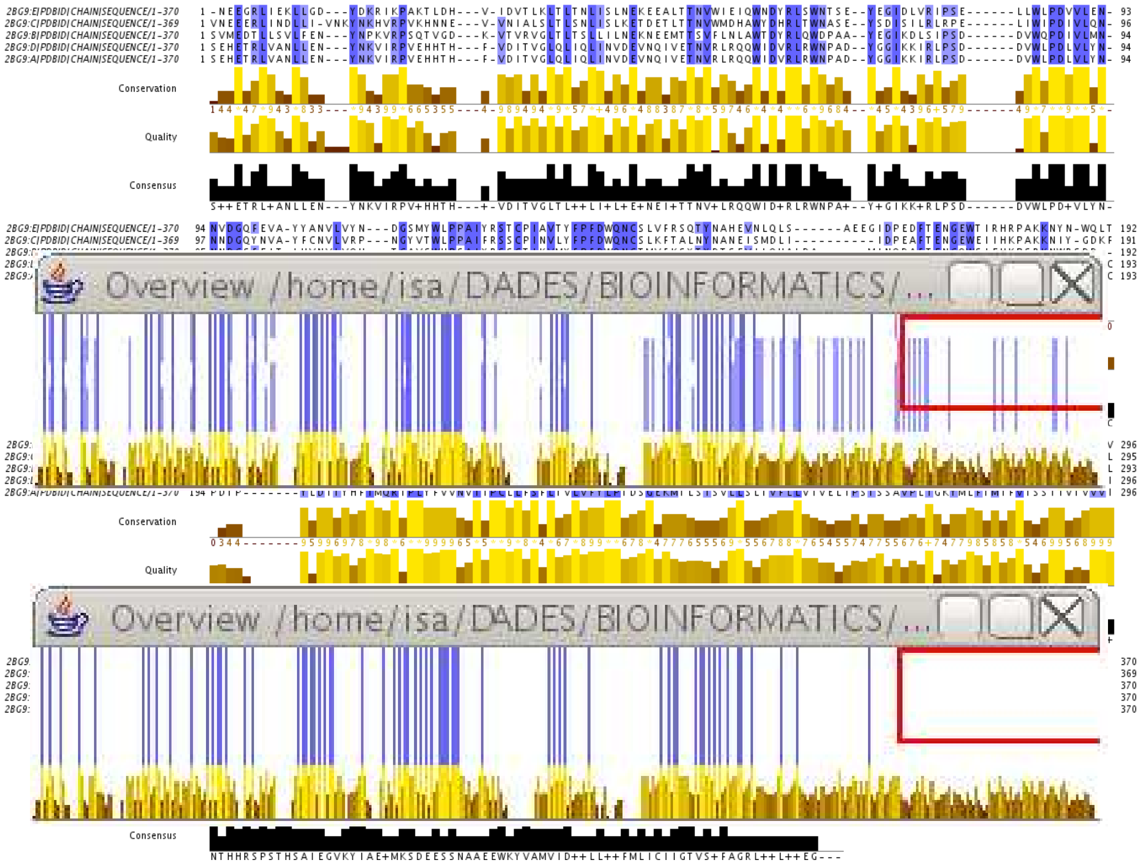
100% Identity



ALIGNMENTS: TORPEDO MARMORATA

- HMM-PFAM

70% Identity



```
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370   1 -NEEGRLIEKLLGD---YDKRIKPAKTLDH---V-IDVTLKLTLTNLISLNEKEEALTTNVWIEIQWNDYRLSWNTSE--YEGIDLVRIPSE------LLWLPDVVLEN-  93
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369   1 VNEEERLINDLLI-VNKYNKHVRPVKHNNE---V-VNIALSLTLSNLISLKETDETLTTNVWMDHAWYDHRLTWNASE--YSDISILRLRPE------LIWIPDIVLQN-  96
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370   1 SVMEDTLLSVLFEN---YNPKVRPSQTVGD---K-VTVRVGLTLTSLLILNEKNEEMTTSVFLNLAWTDYRLQWDPAA--YEGIKDLSIPSD------DVWQPDIVLMN-  94
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370   1 SEHETRLVANLLEN---YNKVIRPVEHHTH---F-VDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPAD--YGGIKKIRLPSD------DVWLPDLVLYN-  94
2BG9:A|PDBID|CHAIN|SEQUENCE/1-370   1 SEHETRLVANLLEN---YNKVIRPVEHHTH---F-VDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPAD--YGGIKKIRLPSD------DVWLPDLVLYN-  94

                     Conservation
                                      144*47*943*833---*94399*665355---4-989494*9*57*+496*488387*8*59746*4*4**6*9684--*45*4396+579------49*7**9**5*-

                          Quality

                        Consensus
                                      S++ETRL+ANLLEN---YNKVIRPV+HHTH---+-VDITVGLTL++LI+L+E+NEI+TTNV+LRQQWID+RLRWNPA+--Y+GIKK+RLPSD------DVWLPD+VLYN-

2BG9:E|PDBID|CHAIN|SEQUENCE/1-370  94 NVDGQFEVA-YYANVLVYN---DGSMYWLPPAIYRSTCPIAVTYFPFDWQNCSLVFRSQTYNAHEVNLQLS------AEEGIDPEDFTENGEWTIRHRPAKKNYN-WQLT 192
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369  97 NNDGQYNVA-YFCNVLVRP---NGYVTWLPPAIFRSSCPINVLYFPFDWQNCSLKFTALNYNANEISMDLI----------IDPEAFTENGEWEIIHKPAKKNIY-GDKF 191
2BG9:                                                                                                                       - 192
2BG9:                                                                                                                       C 193
2BG9:                                                                                                                       C 193
```

Overview /home/isa/DADES/BIOINFORMATICS/...

```
2BG9:                                                                                                                       V 296
2BG9:                                                                                                                       L 295
2BG9:                                                                                                                       L 293
2BG9:                                                                                                                       I 296
2BG9:A|PDBID|CHAIN|SEQUENCE/1-370                                                                                           I 296

                     Conservation
                                      0344-------95996978*98*6**999965*5**9*8*4*67*899**678*4777655569*556788*76545574775567 6+7477985858*546995 68999

                          Quality
```

Overview /home/isa/DADES/BIOINFORMATICS/...

```
2BG9:                                                                                                                         370
2BG9:                                                                                                                         369
2BG9:                                                                                                                         370
2BG9:                                                                                                                         370
2BG9:                                                                                                                         370

                        Consensus
                                      NTHHRSPSTHSAIEGVKYIAE+MKSDEESSNAAEEWKYVAMVID++LL++FMLICIIGTVS+FAGRL++L++EG---
```

70% Identity

100% Identity

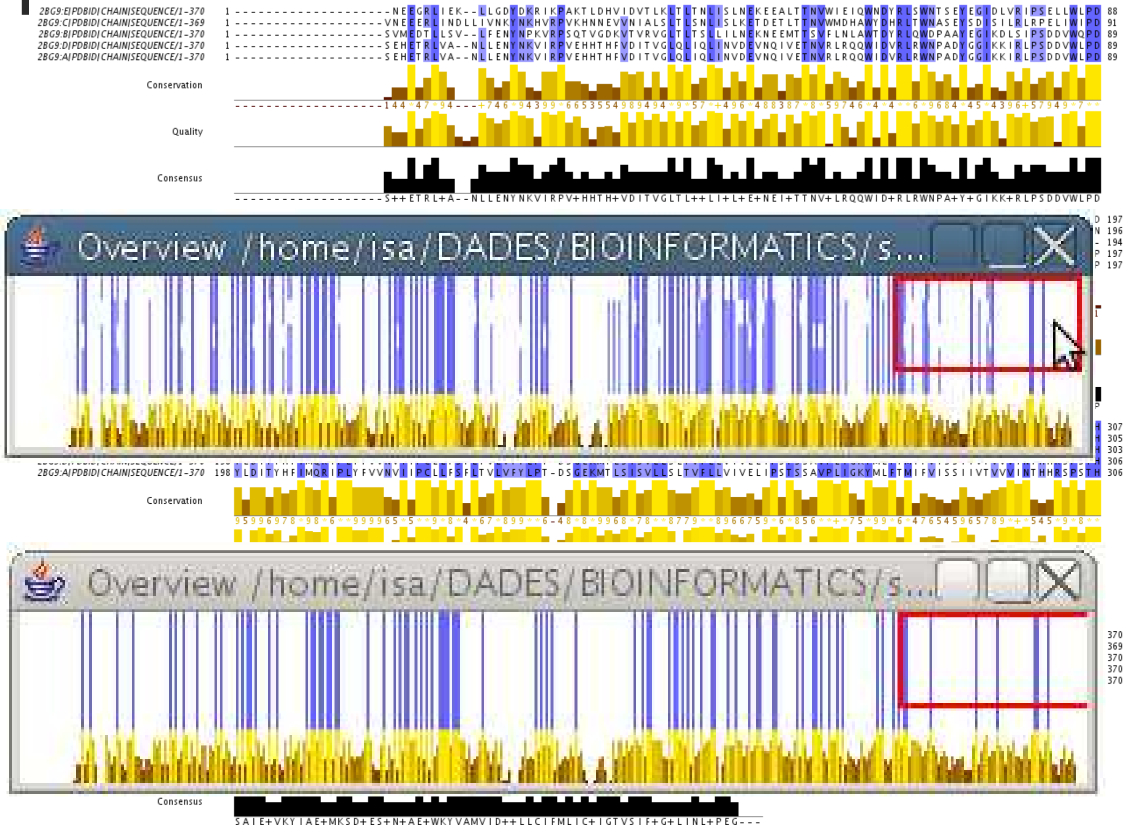
ALIGNMENTS: TORPEDO MARMORATA

- HMM-CUSTOM

70% Identity

70% Identity

100% Identity

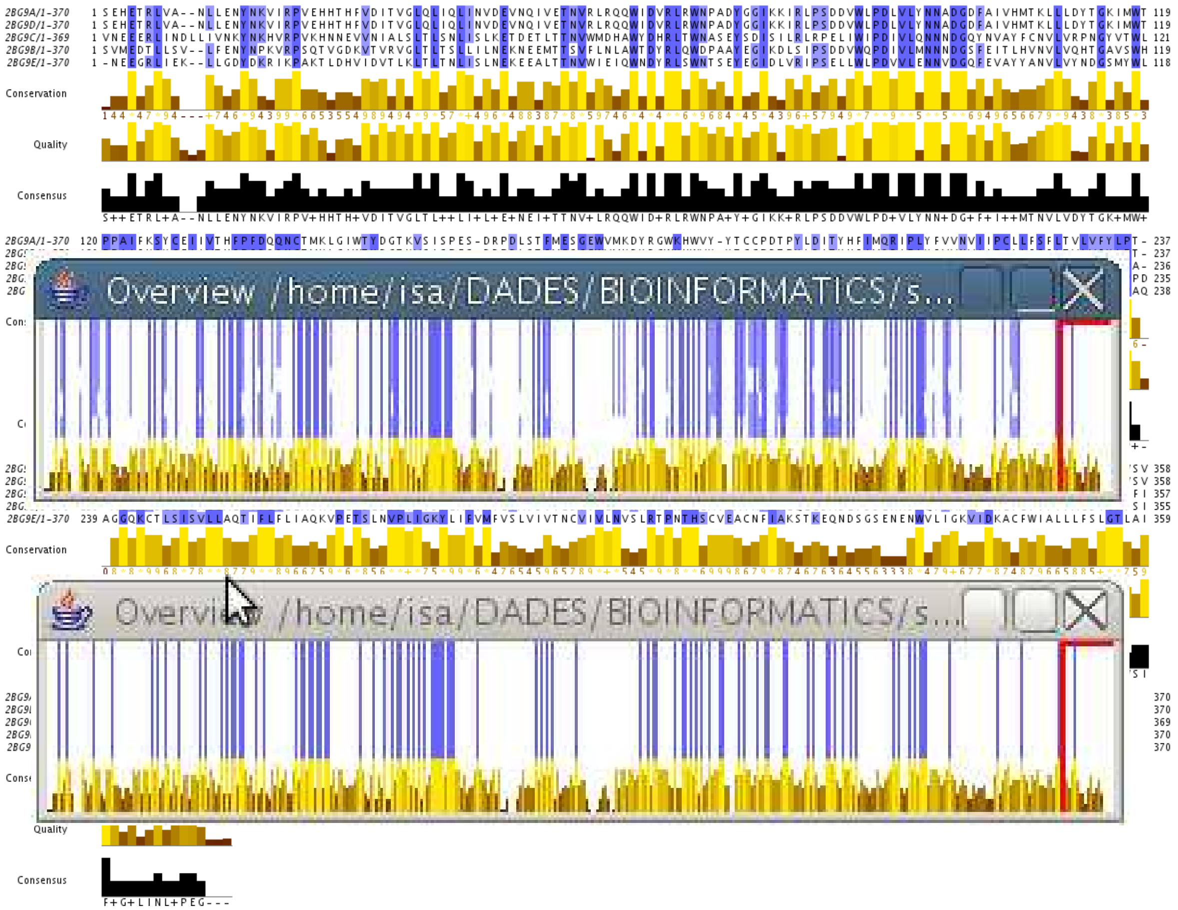


```
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370  1 --------------------NEEGRLIEK--LLGDYDKRIKPAKTLDHVIDVTLKLTLTNLISLNEKEEALTTNVWIEIQWNDYRLSWNTSEYEGIDLVRIPSELLWLPD 88
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369  1 -------------------VNEEERLINDLLIVNKYNKHVRPVKHNNEVVNIALSLTLSNLISLKETDETLTTNVWMDHAWYDHRLTWNASEYSDISILRLRPELIWIPD 91
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370  1 -------------------SVMEDTLLSV--LFENYNPKVRPSQTVGDKVTVRVGLTLTSLLILNEKNEEMTTSVFLNLAWTDYRLQWDPAAYEGIKDLSIPSDDVWQPD 89
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370  1 -------------------SEHETRLVA--NLLENYNKVIRPVEHHTHFVDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPADYGGIKKIRLPSDDVWLPD 89
2BG9:A|PDBID|CHAIN|SEQUENCE/1-370  1 -------------------SEHETRLVA--NLLENYNKVIRPVEHHTHFVDITVGLQLIQLINVDEVNQIVETNVRLRQQWIDVRLRWNPADYGGIKKIRLPSDDVWLPD 89

Conservation
                                     -------------------144*47*94---+746*94399*6653554989494*9*57*+496*488387*8*59746*4*4**6*9684*45*4396+57949*7**
Quality

Consensus
                                     -------------------S++ETRL+A--NLLENYNKVIRPV+HHTH+VDITVGLTL++LI+L+E+NEI+TTNV+LRQQWID+RLRWNPA+Y+GIKK+RLPSDDVWLPD

D 197
N 196
- 194
P 197
P 197

H 307
H 305
H 303
H 306
2BG9:A|PDBID|CHAIN|SEQUENCE/1-370  198 YLDITYHFIMQRIPLYFVVNVIIPCLLFSFLTVLVFYLPT-DSGEKMTLSISVLLSLTVFLLVIVELIPSTSSAVPLIGKYMLFTMIFVISSIIVTVVVINTHHRSPSTH 306

Conservation
                                     95996978*98*6**999965*5**9*8*4*67*899**6-48*8*9968*78**8779**8966759*6*856**+*75*99*6*476545965789*+*545*9*8**

370
369
370
370
370

Consensus
                                     SAIE+VKYIAE+MKSD+ES+N+AE+WKYVAMVID++LLCIFMLIC+IGTVSIF+G+LINL+PEG---
```

ALIGNMENTS: TORPEDO MARMORATA

- STAMP

70% Identity

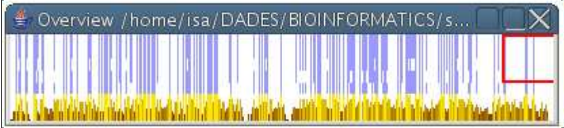
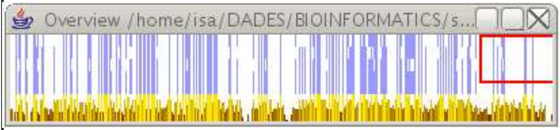
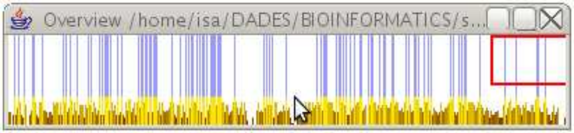
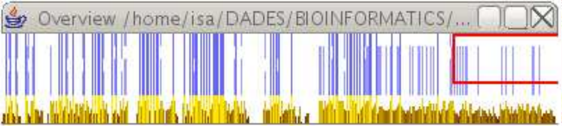
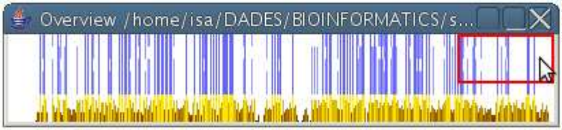
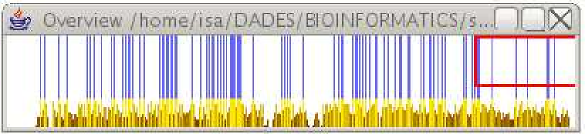
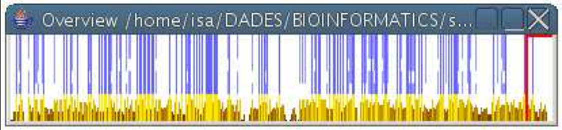
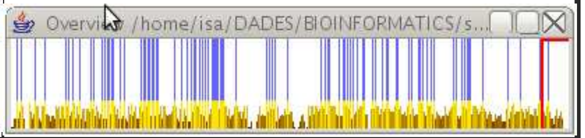


70% Identity

100% Identity

ALIGNMENTS: TORPEDO MARMORATA

COMPARISON

	70% Identity	100 % Identity
CLUSTALW		
TCOFFEE		
HMM-PFAM		
HMM-CUSTOM		
STAMP		

ALIGNMENTS: TORPEDO MARMORATA

COMPARISON

70% Identity

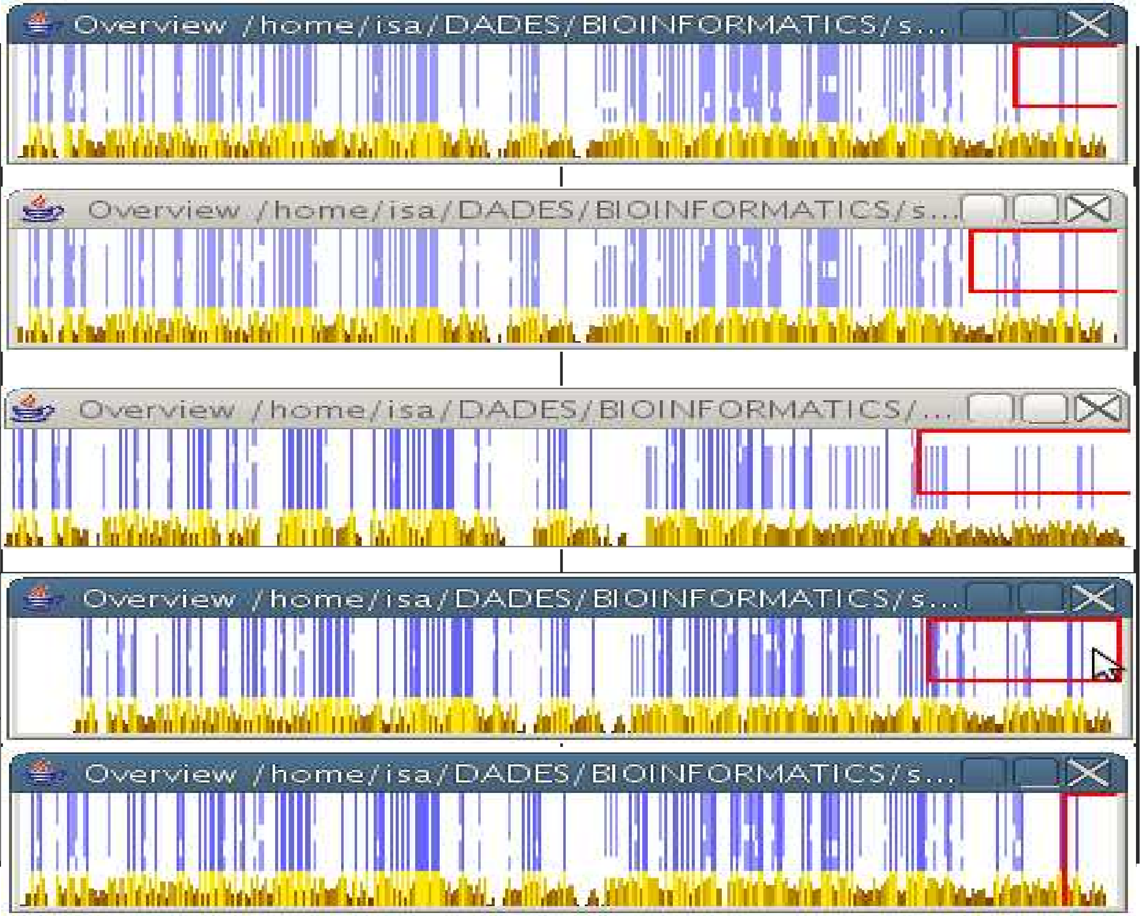
CLUSTALW

TCOFFEE

HMM-PFAM

HMM-CUSTOM

STAMP



ALIGNMENTS: TORPEDO MARMORATA

COMPARISON

100 % Identity

CLUSTALW

TCOFFEE

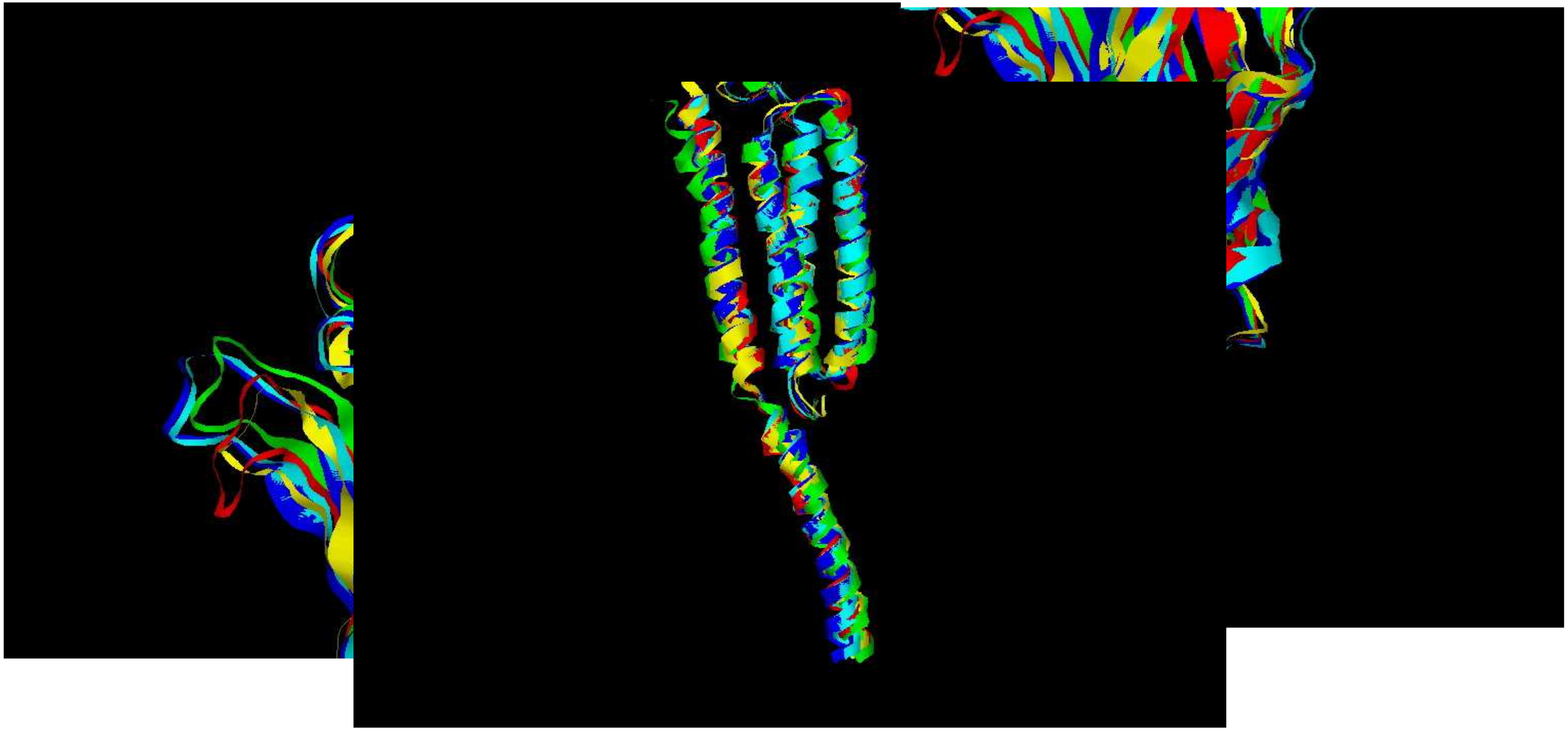
HMM-PFAM

HMM-CUSTOM

STAMP

ALIGNMENTS: TORPEDO MARMORATA

Structures’ superposition



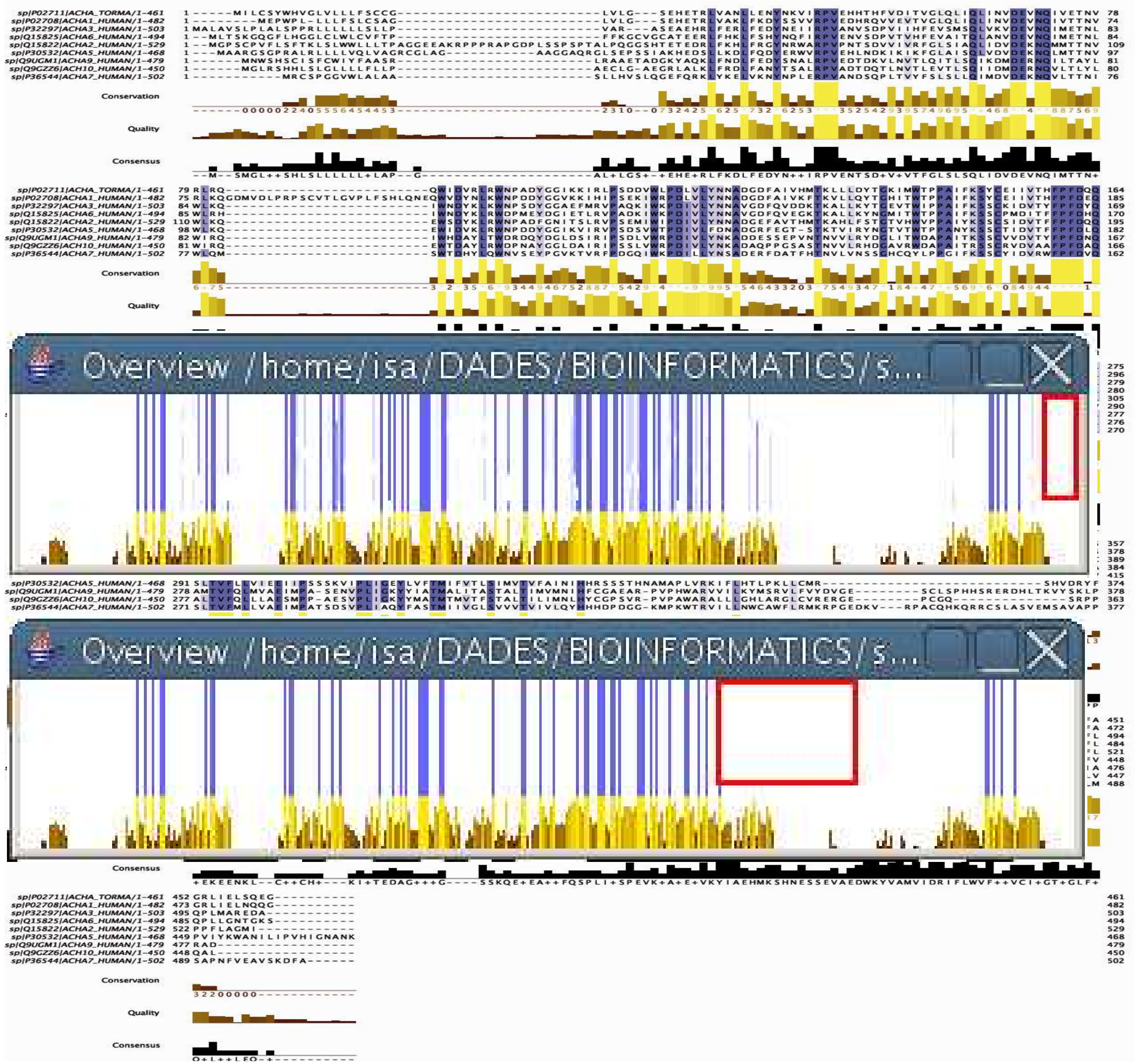
ALIGNMENTS: HUMAN α SUBUNITS

- CLUSTALW
- TCOFFEE
- HMM
    - PFAM MATRIX (NEUCHAN)
    - CUSTOM MATRIX

ALIGNMENTS: HUMAN α SUBUNITS

- CLUSTALW

70% Identity



70% Identity

100% Identity

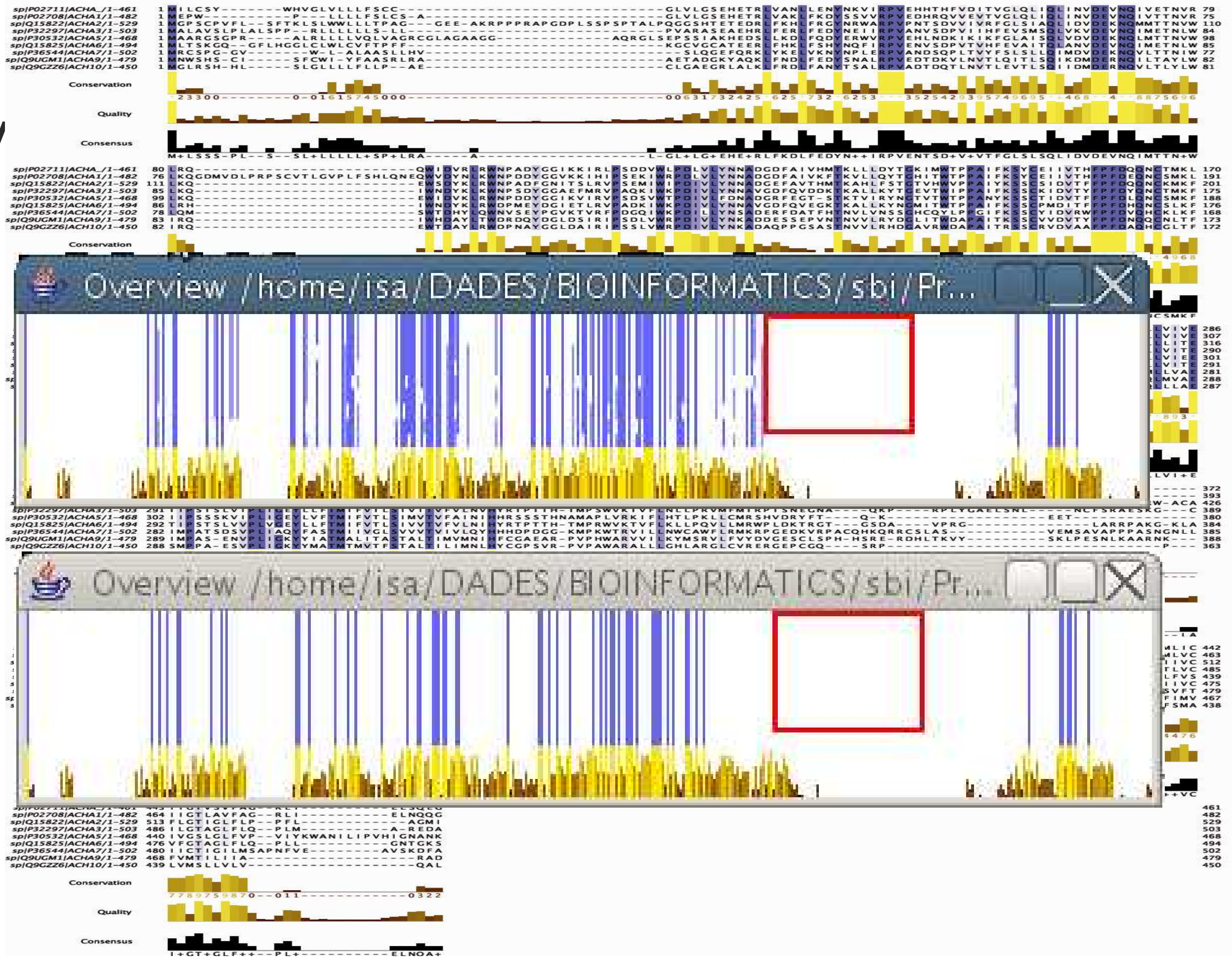
ALIGNMENTS: HUMAN α SUBUNITS

- TCOFFEE

70% Identity

70% Identity

100% Identity



ALIGNMENTS: HUMAN α SUBUNITS

- HMM-PFAM

  70% Identity

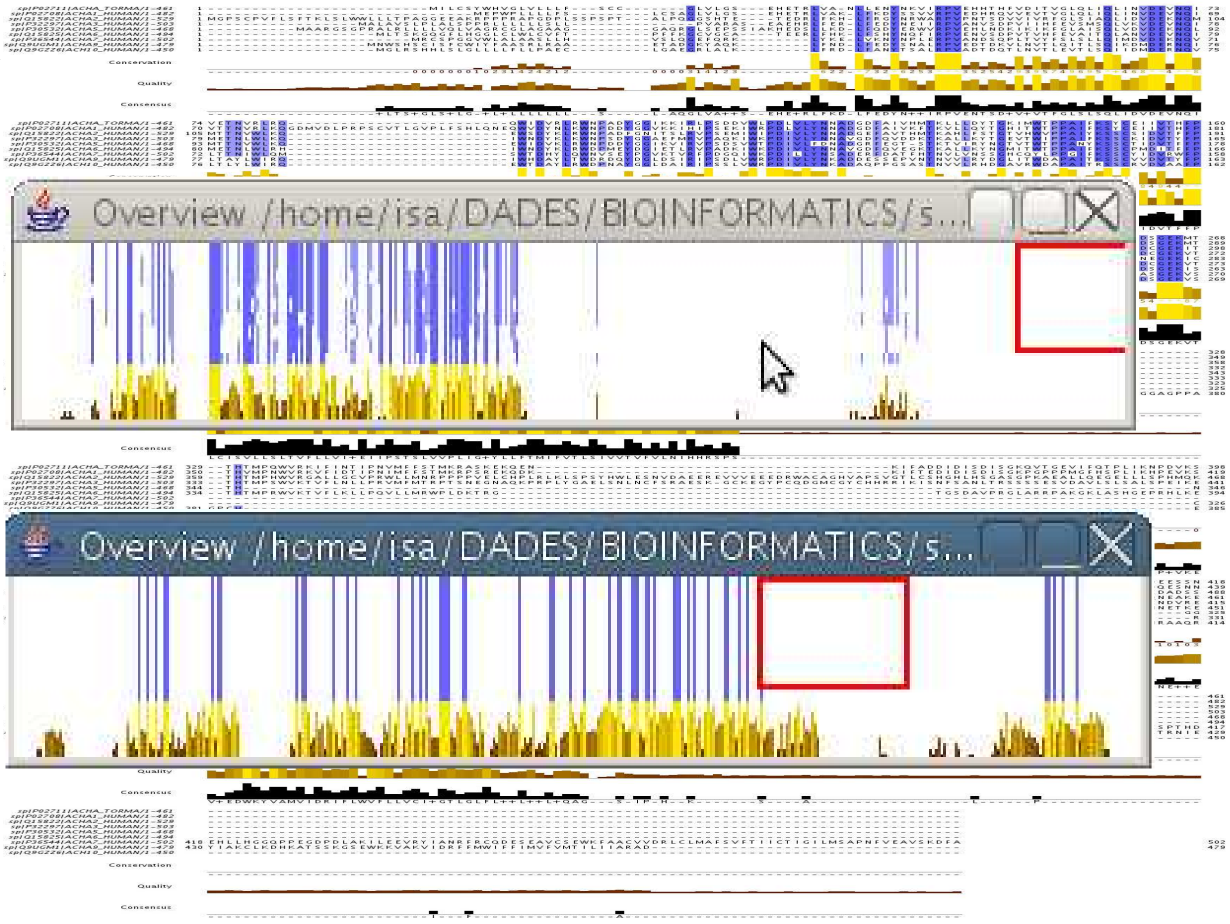
70% Identity

100% Identity

ALIGNMENTS: HUMAN α SUBUNITS

- HMM-CUSTOM

  70% Identity

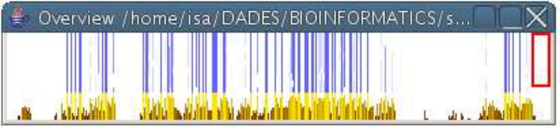
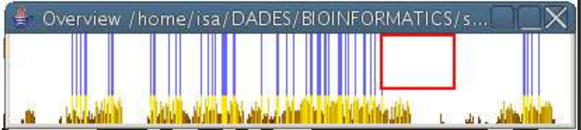
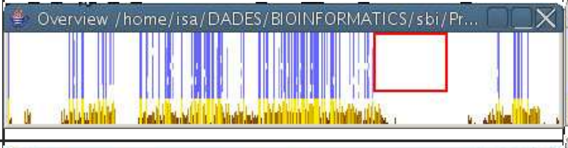
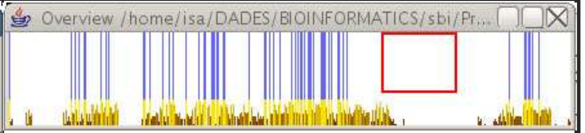
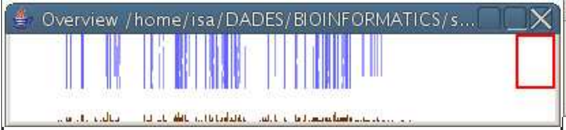
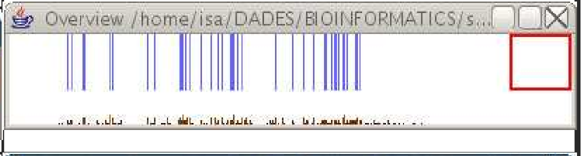
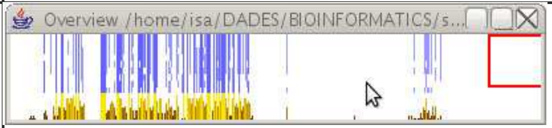
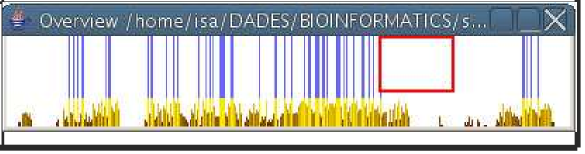


70% Identity

100% Identity

ALIGNMENTS: HUMAN α SUBUNITS

COMPARISON

	70% Identity	100 % Identity
CLUSTALW		
TCOFFEE		
HMM-PFAM		
HMM-CUSTOM		

ALIGNMENTS: HUMAN α SUBUNITS

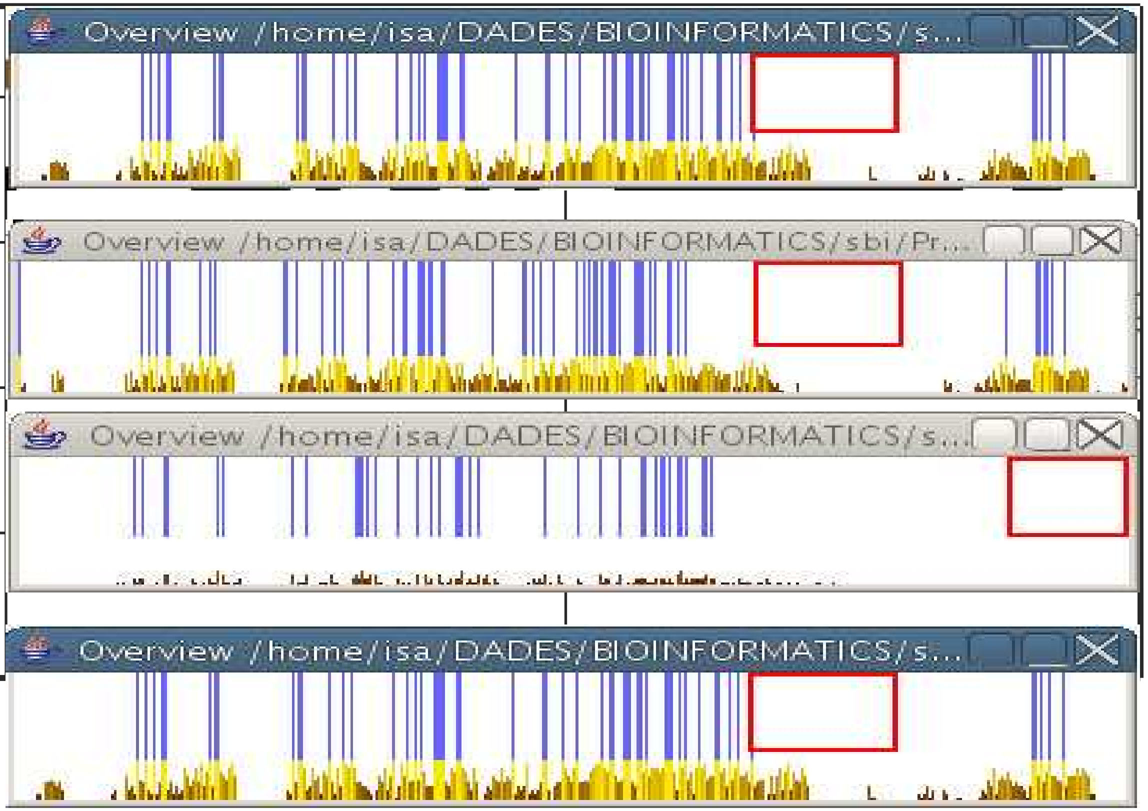
COMPARISON

70% Identity
CLUSTALW
TCOFFEE
HMM-PFAM
HMM-CUSTOM

ALIGNMENTS: HUMAN α SUBUNITS

COMPARISON

100 % Identity
CLUSTALW
TCOFFEE
HMM-PFAM
HMM-CUSTOM



Overview /home/isa/DADES/BIOINFORMATICS/s...

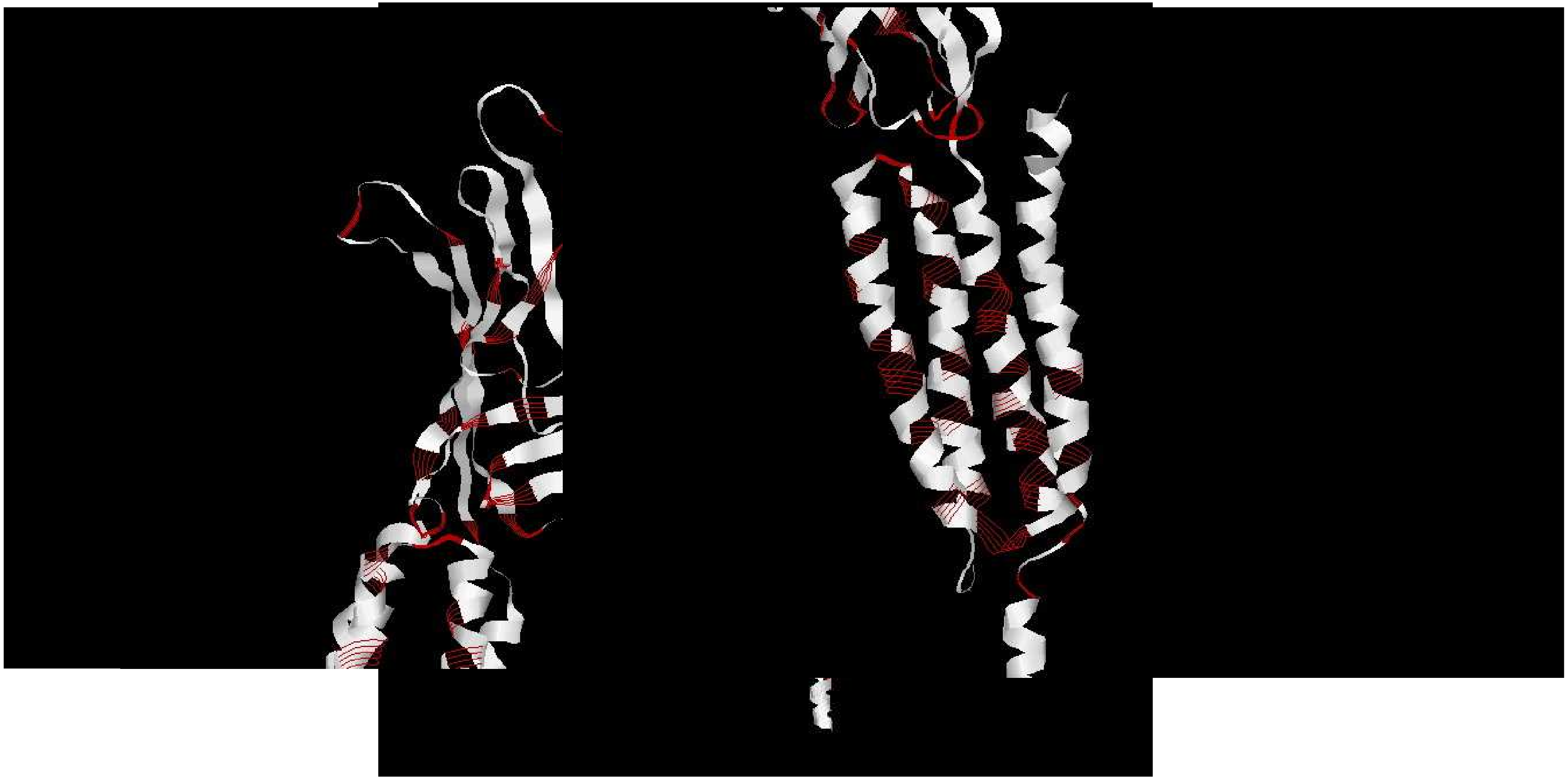
Overview /home/isa/DADES/BIOINFORMATICS/sbi/Pr...

Overview /home/isa/DADES/BIOINFORMATICS/s...

Overview /home/isa/DADES/BIOINFORMATICS/s...

ALIGNMENTS: HUMAN α SUBUNITS

100% Identity



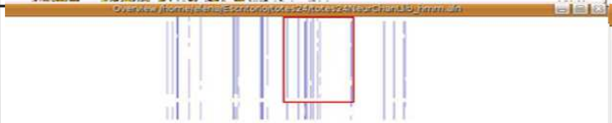
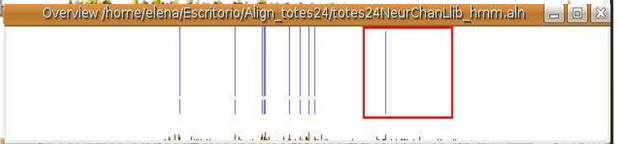
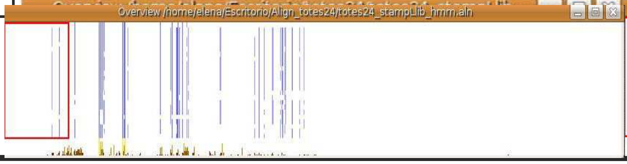
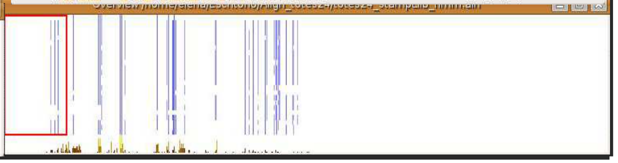
ALIGNMENTS: HUMAN α SUBUNITS (EXTENDED)

COMPARISON BLOSUM 62

CLUSTALW	
TCOFFEE	
HMM-PFAM	
HMM-CUSTOM	
STAMP	

ALIGNMENTS: HUMAN α SUBUNITS (EXTENDED)

COMPARISON

	70% Identity	100 % Identity
CLUSTALW		
TCOFFEE		
HMM-PFAM		
HMM-CUSTOM		
STAMP		

ALIGNMENTS:HUMAN α SUBUNITS (EXTENDED)

COMPARISON - 70%

CLUSTALW

TCOFFEE

HMM-PFAM

HMM-CUSTOM

STAMP

ALIGNMENTS:HUMAN α SUBUNITS (EXTENDED)

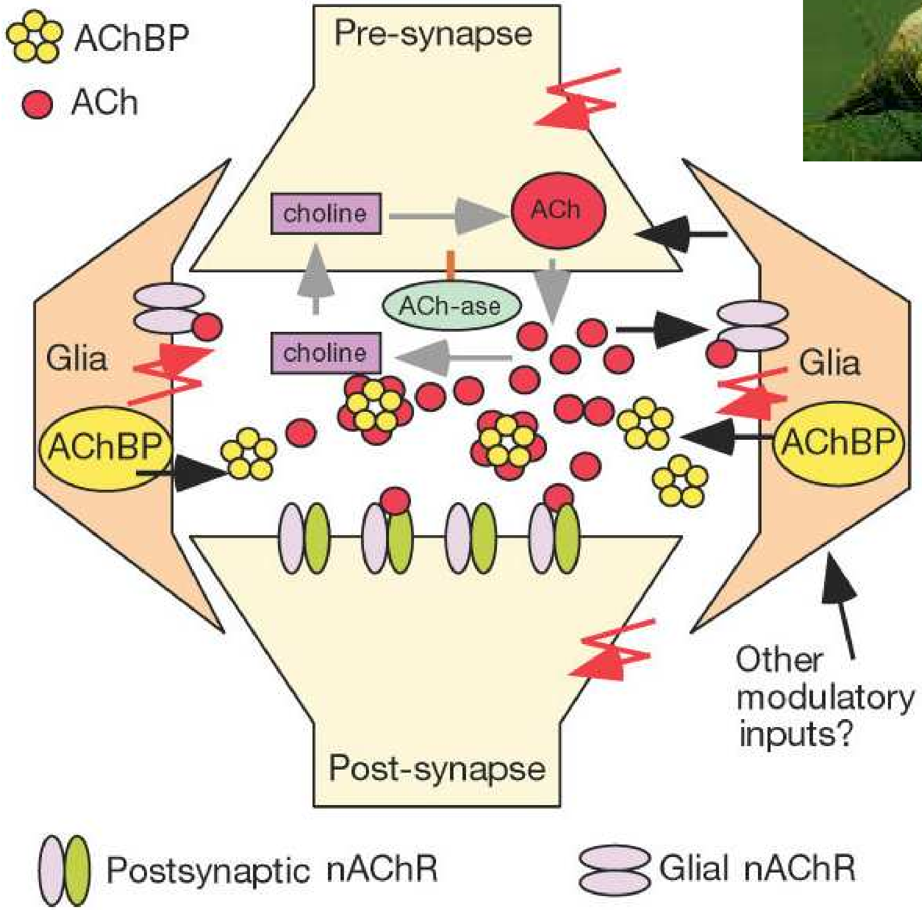
COMPARISON - 100%

CLUSTALW	
TCOFFEE	
HMM-PFAM	
HMM-CUSTOM	
STAMP	

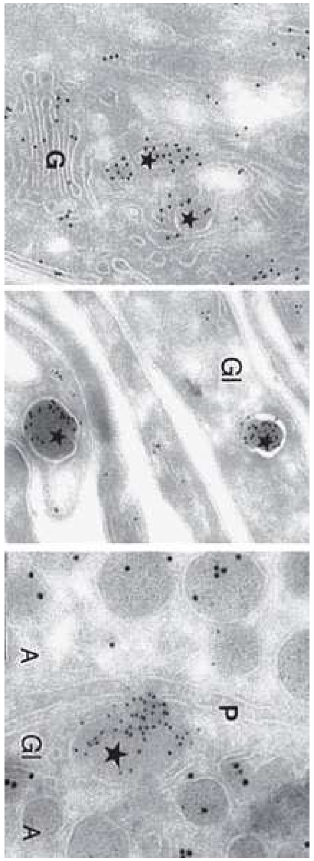
STUDY OF CONSERVED RESIDUES IN NICOTINIC α SUBUNITS

- Acetylcholine binding protein
- Ligand-binding domain (LBD)
- Transmembrane domain (TMD)

ACETYLCHOLINE BINDING PROTEIN



Lymnaea stagnalis

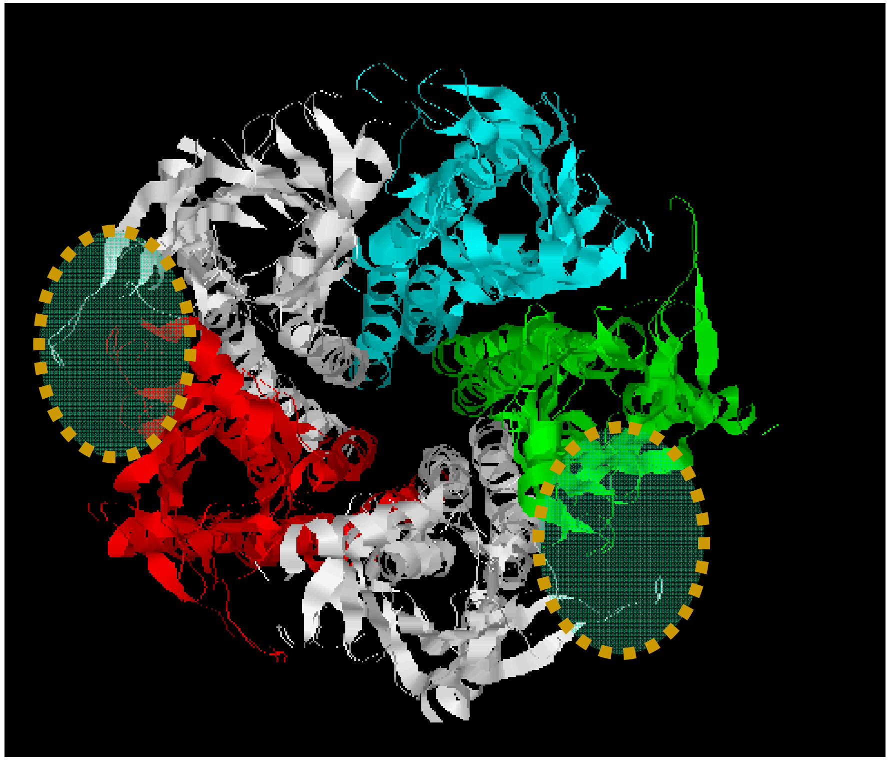


Example of LBD of α nicotinic subunits

LBD: Conservation - function

LBS for nicotinic agonists and competitive antagonists subunit interface :

- α_1 / γ
- α_1 / δ

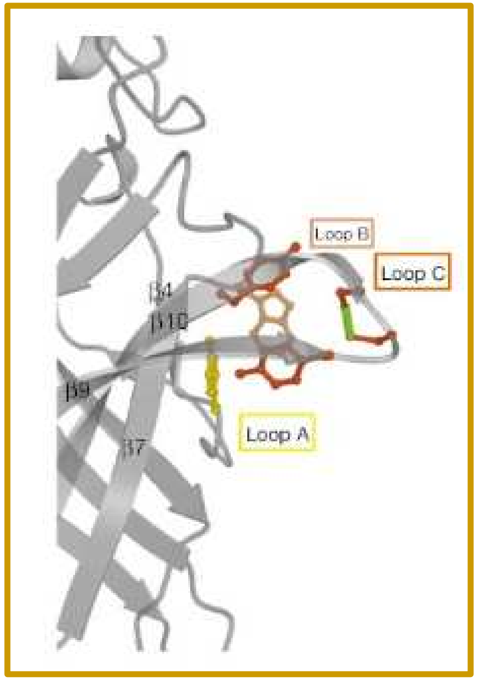


It was proposed to refer to the α1 subunits as carrying the "principal component," and the δ and γ subunits as contributing to the "complementary component" of the nicotinic binding site

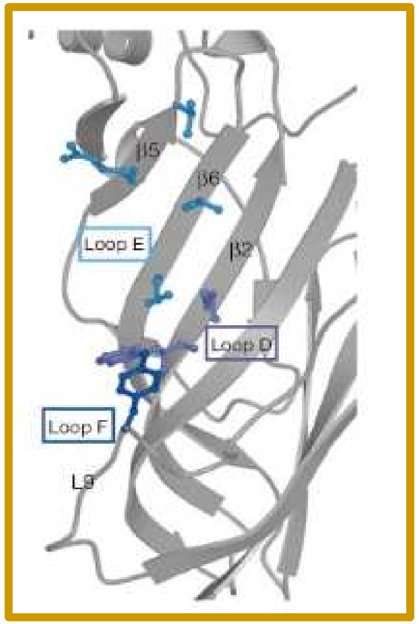
LDB: LIGAND-BINDING SITE

Muscle nAChR (heteromeric) contains two different ligand-binding sites (α/γ , α/δ)

Homopentameric α7/9 receptor contains 5 identical ligand-binding sites.

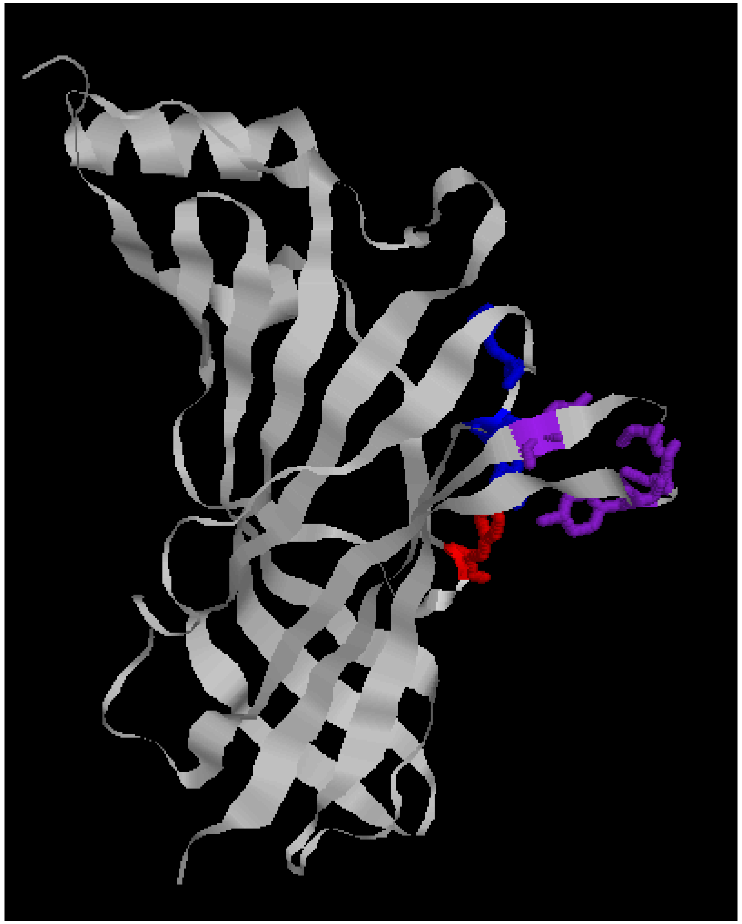


The principal part - α-subunit : `loops' A, B and C



complementary part : ‘loops' D, E and F

LIGAND BINDING POCKET-**AROMATIC BOX**

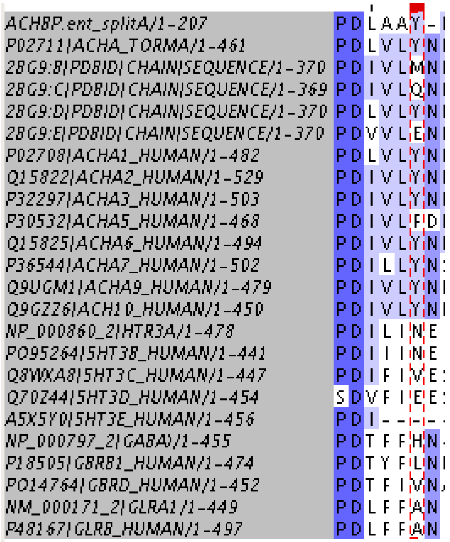


Plus side of the α/γ interface:

- **loop A** (Tyr A89)
- **loop B** (Trp A143, Tyr A145) and
- **loop C** (Tyr A185, the double cysteine A187, A188 and Tyr A192).

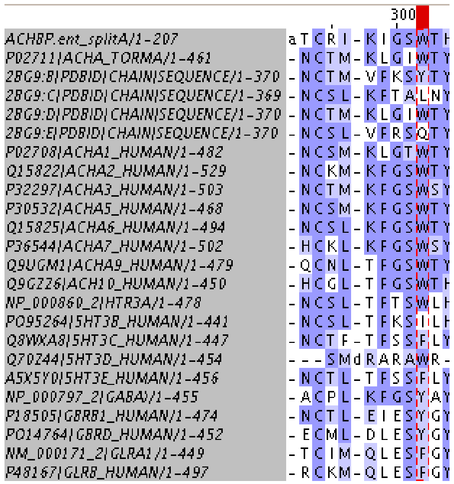
LIGAND BINDING POCKET-**AROMATIC BOX**

- **loop A** (Tyr A89),
- **loop B** (Trp A143, Tyr A145) and
- **loop C** (Tyr A185, the double cysteine A187,A188 and Tyr A192).

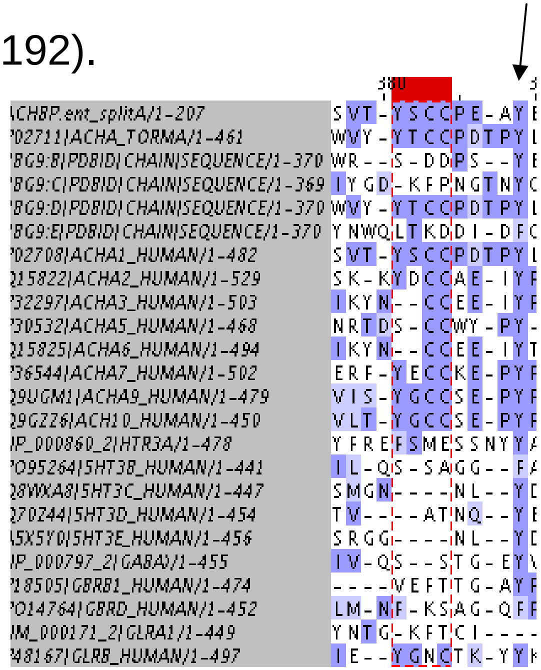


```
ACHBP.ent_splitA/1-207                PDLAAY-
P02711|ACHA_TORMA/1-461               PDLVLYN
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370     PDIVLMN
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369     PDIVLQN
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370     PDLVLYN
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370     PDVVLEN
P02708|ACHA1_HUMAN/1-482              PDLVLYN
Q15822|ACHA2_HUMAN/1-529              PDIVLYN
P32297|ACHA3_HUMAN/1-503              PDIVLYN
P30532|ACHA5_HUMAN/1-468              PDIVLFD
Q15825|ACHA6_HUMAN/1-494              PDIVLYN
P36544|ACHA7_HUMAN/1-502              PDILLYN
Q9UGM1|ACHA9_HUMAN/1-479              PDIVLYN
Q9GZZ6|ACH10_HUMAN/1-450              PDIVLYN
NP_000860_2|HTR3A/1-478               PDILINE
PO95264|5HT3B_HUMAN/1-441             PDIIINE
Q8WXA8|5HT3C_HUMAN/1-447              PDIFIVE
Q70Z44|5HT3D_HUMAN/1-454              SDVFIEE
A5X5Y0|5HT3E_HUMAN/1-456              PDI----
NP_000797_2|GABA)/1-455               PDTFFHN
P18505|GBRB1_HUMAN/1-474              PDTYFLN
PO14764|GBRD_HUMAN/1-452              PDTFIVN
NM_000171_2|GLRA1/1-449               PDLFFAN
P48167|GLRB_HUMAN/1-497               PDLFFAN
```

Conservation in nicotinic receptor



```
                                                 300
ACHBP.ent_splitA/1-207                aTCRI-KIGSWT
P02711|ACHA_TORMA/1-461               -NCTM-KLGIWT
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370     -NCTM-VFKSYT
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369     -NCSL-KFTALN
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370     -NCTM-KLGIWT
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370     -NCSL-VFRSQT
P02708|ACHA1_HUMAN/1-482              -NCSM-KLGTWT
Q15822|ACHA2_HUMAN/1-529              -NCKM-KFGSWT
P32297|ACHA3_HUMAN/1-503              -NCTM-KFGSWS
P30532|ACHA5_HUMAN/1-468              -NCSM-KFGSWT
Q15825|ACHA6_HUMAN/1-494              -NCSL-KFGSWT
P36544|ACHA7_HUMAN/1-502              -HCKL-KFGSWS
Q9UGM1|ACHA9_HUMAN/1-479              -QCNL-TFGSWT
Q9GZZ6|ACH10_HUMAN/1-450              -HCGL-TFGSWT
NP_000860_2|HTR3A/1-478               -NCSL-TFTSWL
PO95264|5HT3B_HUMAN/1-441             -NCSL-TFKSIL
Q8WXA8|5HT3C_HUMAN/1-447              -NCTF-TFSSFL
Q70Z44|5HT3D_HUMAN/1-454              ---SMdRARAWR
A5X5Y0|5HT3E_HUMAN/1-456              -NCTL-TFSSFL
NP_000797_2|GABA)/1-455               -ACPL-KFGSYA
P18505|GBRB1_HUMAN/1-474              -NCTL-EIESYG
PO14764|GBRD_HUMAN/1-452              -ECML-DLESYG
NM_000171_2|GLRA1/1-449               -TCIM-QLESFG
P48167|GLRB_HUMAN/1-497               -RCKM-QLESFG
```



```
                                     380
CHBP.ent_splitA/1-207               SVT-YSCCPE-AY
02711|ACHA_TORMA/1-461              WVY-YTCCPDTPY
BG9:B|PDBID|CHAIN|SEQUENCE/1-370    WR--S-DDPS--Y
BG9:C|PDBID|CHAIN|SEQUENCE/1-369    IYGD-KFPNGTNY
BG9:D|PDBID|CHAIN|SEQUENCE/1-370    WVY-YTCCPDTPY
BG9:E|PDBID|CHAIN|SEQUENCE/1-370    YNWQLTKDDI-DF
02708|ACHA1_HUMAN/1-482             SVT-YSCCPDTPY
15822|ACHA2_HUMAN/1-529             SK-KYDCCAE-IY
32297|ACHA3_HUMAN/1-503             IKYN--CCEE-IY
30532|ACHA5_HUMAN/1-468             NRTDS-CCWY-PY
15825|ACHA6_HUMAN/1-494             IKYN--CCEE-IY
36544|ACHA7_HUMAN/1-502             ERF-YECCKE-PY
9UGM1|ACHA9_HUMAN/1-479             VIS-YGCCSE-PY
9GZZ6|ACH10_HUMAN/1-450             VLT-YGCCSE-PY
P_000860_2|HTR3A/1-478              YFREFSMESSNYY
O95264|5HT3B_HUMAN/1-441            IL-QS-SAGG--F
8WXA8|5HT3C_HUMAN/1-447             SMGN----NL--Y
70Z44|5HT3D_HUMAN/1-454             TV----ATNQ--Y
5X5Y0|5HT3E_HUMAN/1-456             SRGG----NL--Y
P_000797_2|GABA)/1-455              IV-QS--STG-EY
18505|GBRB1_HUMAN/1-474             ----VEFTTG-AY
O14764|GBRD_HUMAN/1-452             LM-NF-KSAG-QF
M_000171_2|GLRA1/1-449              YNTG-KFTCI---
48167|GLRB_HUMAN/1-497              IE--YGNCTK-YY
```

LIGAND BINDING POCKET:

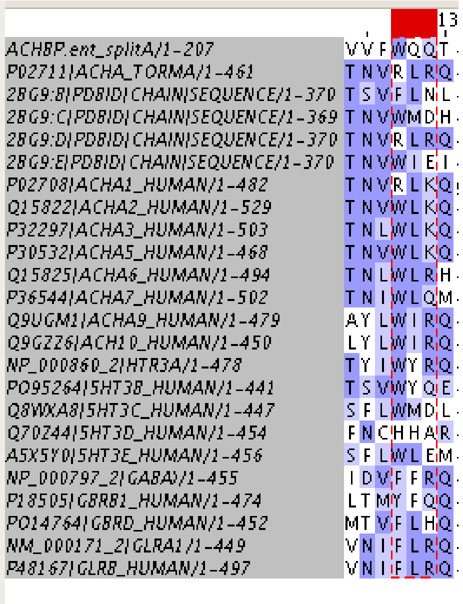
The complementary part

Complementary:

- **loop D** (Trp B53, Gln B55)
- **loop E** (Arg B104, Val B106, Leu B112 and Met B114) and
- **loop F** (Tyr B164).

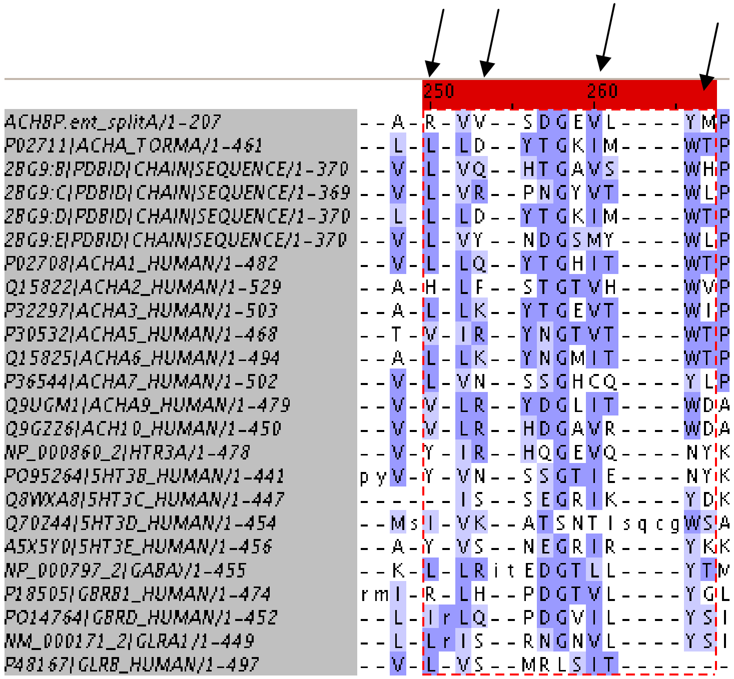
LIGAND BINDING POCKET:

The complementary part

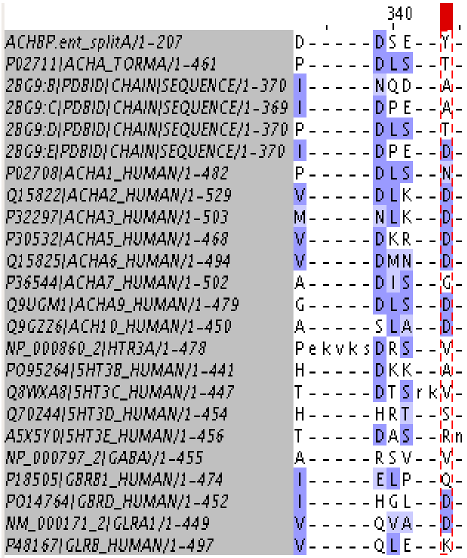


```
                                         13
ACHBP.ent_splitA/1-207             VVFWQQT
P02711|ACHA_TORMA/1-461            TNVRLRQ
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370  TSVFLNL
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369  TNVWMDH
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370  TNVRLRQ
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370  TNVWIEI
P02708|ACHA1_HUMAN/1-482           TNVRLKQ
Q15822|ACHA2_HUMAN/1-529           TNVWLKQ
P32297|ACHA3_HUMAN/1-503           TNLWLKQ
P30532|ACHA5_HUMAN/1-468           TNVWLKQ
Q15825|ACHA6_HUMAN/1-494           TNLWLRH
P36544|ACHA7_HUMAN/1-502           TNIWLQM
Q9UGM1|ACHA9_HUMAN/1-479           AYLWIRQ
Q9GZZ6|ACH10_HUMAN/1-450           LYLWIRQ
NP_000860_2|HTR3A/1-478            TYIWYRQ
PO95264|5HT3B_HUMAN/1-441          TSVWYQE
Q8WXA8|5HT3C_HUMAN/1-447           SFLWMDL
Q70Z44|5HT3D_HUMAN/1-454           FNCHHAR
A5X5Y0|5HT3E_HUMAN/1-456           SFLWLEM
NP_000797_2|GABA)/1-455            IDVFFRQ
P18505|GBRB1_HUMAN/1-474           LTMYFQQ
PO14764|GBRD_HUMAN/1-452           MTVFLHQ
NM_000171_2|GLRA1/1-449            VNIFLRQ
P48167|GLRB_HUMAN/1-497            VNIFLRQ
```

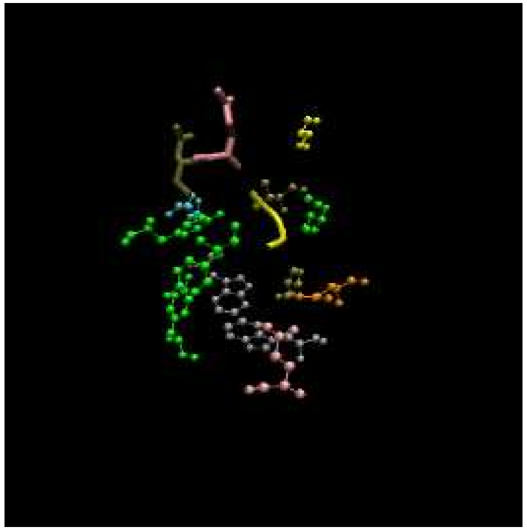
loop D (Trp B53), **loop E** (Arg B104, Val B106, Leu B112 and Met B114) and **loop F** (Tyr B164).



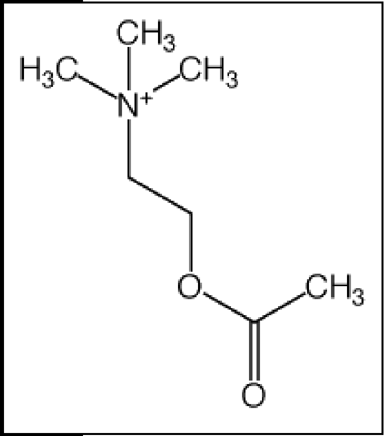
```
                                       250       260
ACHBP.ent_splitA/1-207             --A-R-VV--SDGEVL----YMP
P02711|ACHA_TORMA/1-461            --L-L-LD--YTGKIM----WTP
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370  --V-L-VQ--HTGAVS----WHP
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369  --V-L-VR--PNGYVT----WLP
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370  --L-L-LD--YTGKIM----WTP
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370  --V-L-VY--NDGSMY----WLP
P02708|ACHA1_HUMAN/1-482           --V-L-LQ--YTGHIT----WTP
Q15822|ACHA2_HUMAN/1-529           --A-H-LF--STGTVH----WVP
P32297|ACHA3_HUMAN/1-503           --A-L-LK--YTGEVT----WIP
P30532|ACHA5_HUMAN/1-468           --T-V-IR--YNGTVT----WTP
Q15825|ACHA6_HUMAN/1-494           --A-L-LK--YNGMIT----WTP
P36544|ACHA7_HUMAN/1-502           --V-L-VN--SSGHCQ----YLP
Q9UGM1|ACHA9_HUMAN/1-479           --V-V-LR--YDGLIT----WDA
Q9GZZ6|ACH10_HUMAN/1-450           --V-V-LR--HDGAVR----WDA
NP_000860_2|HTR3A/1-478            --V-Y-IR--HQGEVQ----NYK
PO95264|5HT3B_HUMAN/1-441          pyV-Y-VN--SSGTIE----NYK
Q8WXA8|5HT3C_HUMAN/1-447           ------IS--SEGRIK----YDK
Q70Z44|5HT3D_HUMAN/1-454           --MsI-VK--ATSNTIsqcgWSA
A5X5Y0|5HT3E_HUMAN/1-456           --A-Y-VS--NEGRIR----YKK
NP_000797_2|GABA)/1-455            --K-L-LRitEDGTLL----YTM
P18505|GBRB1_HUMAN/1-474           rmI-R-LH--PDGTVL----YGL
PO14764|GBRD_HUMAN/1-452           --L-IrLQ--PDGVIL----YSI
NM_000171_2|GLRA1/1-449            --L-LrIS--RNGNVL----YSI
P48167|GLRB_HUMAN/1-497            --V-L-VS--MRLSIT-------
```

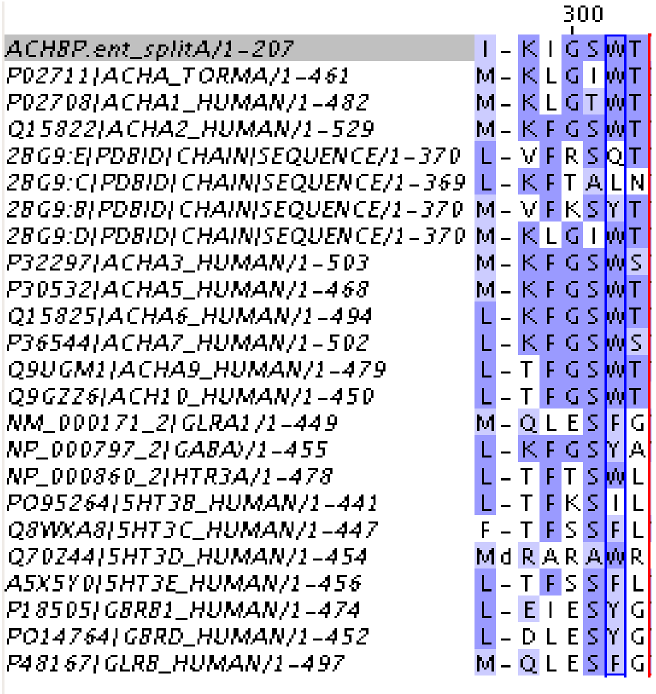


```
                                          340
ACHBP.ent_splitA/1-207             D-----DSE--Y-
P02711|ACHA_TORMA/1-461            P-----DLS--T-
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370  I-----NQD--A-
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369  I-----DPE--A-
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370  P-----DLS--T-
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370  I-----DPE--D-
P02708|ACHA1_HUMAN/1-482           P-----DLS--N-
Q15822|ACHA2_HUMAN/1-529           V-----DLK--D-
P32297|ACHA3_HUMAN/1-503           M-----NLK--D-
P30532|ACHA5_HUMAN/1-468           V-----DKR--D-
Q15825|ACHA6_HUMAN/1-494           V-----DMN--D-
P36544|ACHA7_HUMAN/1-502           A-----DIS--G-
Q9UGM1|ACHA9_HUMAN/1-479           G-----DLS--D-
Q9GZZ6|ACH10_HUMAN/1-450           A-----SLA--D-
NP_000860_2|HTR3A/1-478            PekvksDRS--V-
PO95264|5HT3B_HUMAN/1-441          H-----DKK--A-
Q8WXA8|5HT3C_HUMAN/1-447           T-----DTSrkV-
Q70Z44|5HT3D_HUMAN/1-454           H-----HRT--S-
A5X5Y0|5HT3E_HUMAN/1-456           T-----DAS--Rn
NP_000797_2|GABA)/1-455            A-----RSV--V-
P18505|GBRB1_HUMAN/1-474           I-----ELP--Q-
PO14764|GBRD_HUMAN/1-452           I-----HGL--D-
NM_000171_2|GLRA1/1-449            V-----QVA--D-
P48167|GLRB_HUMAN/1-497            V-----QLE--K-
```



POSITION 143: W



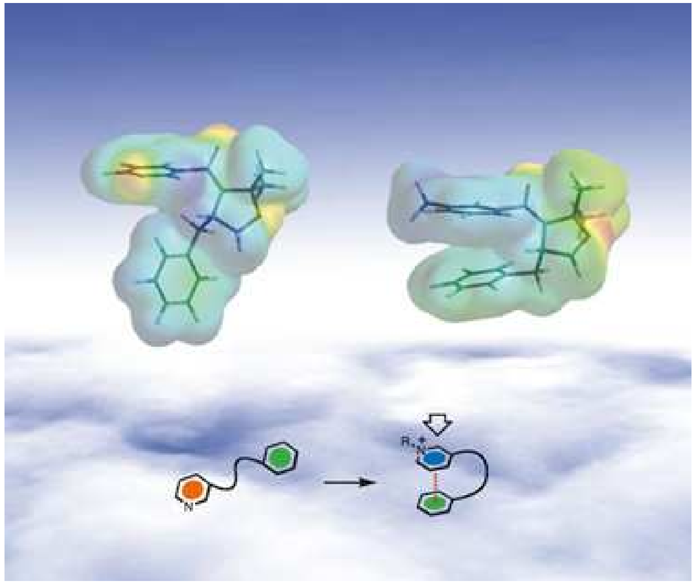


```
                                                    300
                                                     |
ACHBP.ent_splitA/1-207                       I - K I G S W T
P02711|ACHA_TORMA/1-461                      M - K L G I W T
P02708|ACHA1_HUMAN/1-482                     M - K L G T W T
Q15822|ACHA2_HUMAN/1-529                     M - K F G S W T
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370            L - V F R S Q T
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369            L - K F T A L N
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370            M - V F K S Y T
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370            M - K L G I W T
P32297|ACHA3_HUMAN/1-503                     M - K F G S W S
P30532|ACHA5_HUMAN/1-468                     M - K F G S W T
Q15825|ACHA6_HUMAN/1-494                     L - K F G S W T
P36544|ACHA7_HUMAN/1-502                     L - K F G S W S
Q9UGM1|ACHA9_HUMAN/1-479                     L - T F G S W T
Q9GZZ6|ACH10_HUMAN/1-450                     L - T F G S W T
NM_000171_2|GLRA1/1-449                      M - Q L E S F G
NP_000797_2|GABA)/1-455                      L - K F G S Y A
NP_000860_2|HTR3A/1-478                      L - T F T S W L
PO95264|5HT3B_HUMAN/1-441                    L - T F K S I L
Q8WXA8|5HT3C_HUMAN/1-447                     F - T F S S F L
Q70Z44|5HT3D_HUMAN/1-454                     M d R A R A W R
A5X5Y0|5HT3E_HUMAN/1-456                     L - T F S S F L
P18505|GBRB1_HUMAN/1-474                     L - E I E S Y G
PO14764|GBRD_HUMAN/1-452                     L - D L E S Y G
P48167|GLRB_HUMAN/1-497                      M - Q L E S F G
```

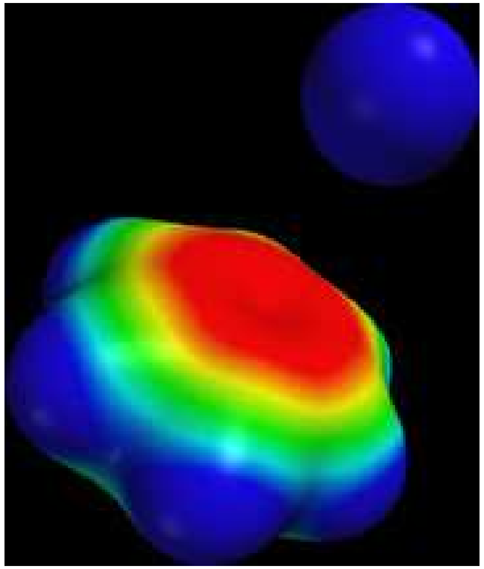
cation – pi interaction also occurs at tyrosine residues

Large interaction between a cation and the high electronic density face of an aromatic ring.

CATION - Π



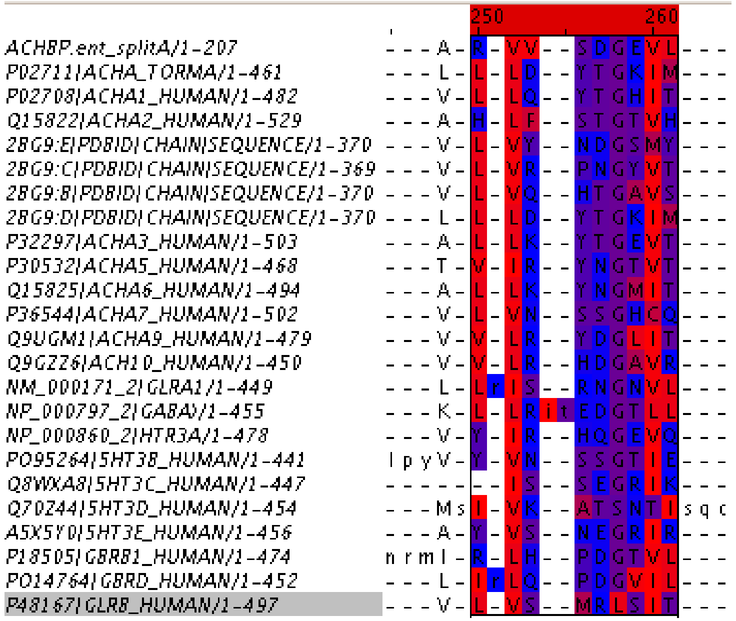
The interaction occupies different faces of the aromatic box, suggesting that the role of the agonist is to serve less as a ‘key in a lock’ and more like a ‘wedge’



The aromatic box is a general ‘capture’ area; activation becomes constitutive if appropriate groups are fixed at any of several positions in the box.

Hydrophobocity in LBD

- The hydrophobic parts of Arg B104, Val B106 and Leu B112 form the top of the binding site.
- Conservation of hydrophobicity in certain positions



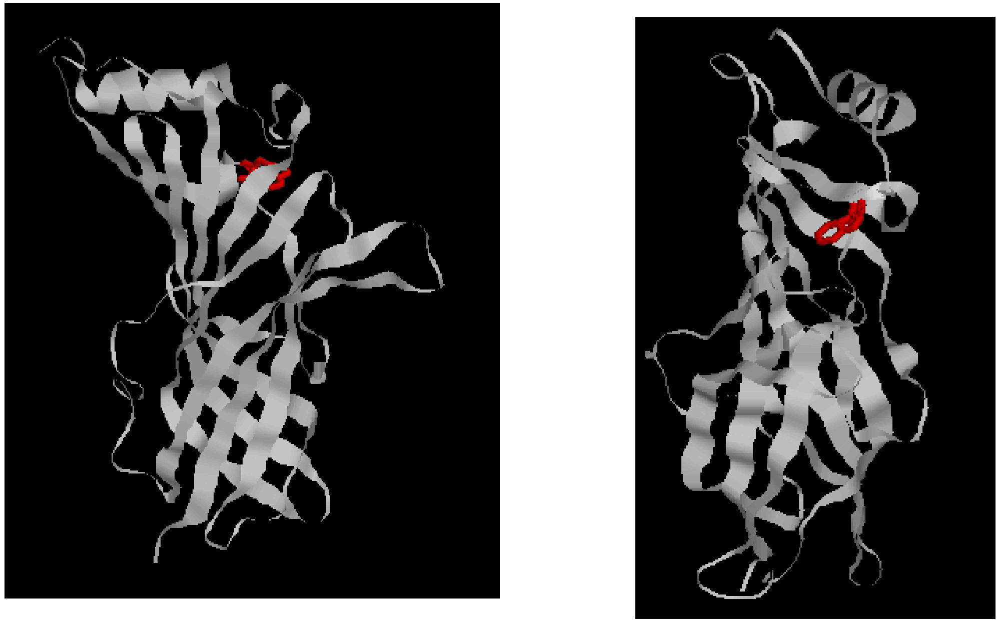
```
                                        250       260
ACHBP.ent_splitA/1-207               ---A-R-VV--SDGEVL---
P02711|ACHA_TORMA/1-461              ---L-L-LD--YTGKIM---
P02708|ACHA1_HUMAN/1-482             ---V-L-LQ--YTGHIT---
Q15822|ACHA2_HUMAN/1-529             ---A-H-LF--STGTVH---
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370    ---V-L-VY--NDGSMY---
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369    ---V-L-VR--PNGYVT---
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370    ---V-L-VQ--HTGAVS---
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370    ---L-L-LD--YTGKIM---
P32297|ACHA3_HUMAN/1-503             ---A-L-LK--YTGEVT---
P30532|ACHA5_HUMAN/1-468             ---T-V-IR--YNGTVT---
Q15825|ACHA6_HUMAN/1-494             ---A-L-LK--YNGMIT---
P36544|ACHA7_HUMAN/1-502             ---V-L-VN--SSGHCQ---
Q9UGM1|ACHA9_HUMAN/1-479             ---V-V-LR--YDGLIT---
Q9GZZ6|ACH10_HUMAN/1-450             ---V-V-LR--HDGAVR---
NM_000171_2|GLRA1/1-449              ---L-LrIS--RNGNVL---
NP_000797_2|GABA)/1-455              ---K-L-LRitEDGTLL---
NP_000860_2|HTR3A/1-478              ---V-Y-IR--HQGEVQ---
PO95264|5HT3B_HUMAN/1-441            lpyV-Y-VN--SSGTIE---
Q8WXA8|5HT3C_HUMAN/1-447             --------IS--SEGRIK---
Q70Z44|5HT3D_HUMAN/1-454             ---MsI-VK--ATSNTIsqc
A5X5Y0|5HT3E_HUMAN/1-456             ---A-Y-VS--NEGRIR---
P18505|GBRB1_HUMAN/1-474             nrml-R-LH--PDGTVL---
PO14764|GBRD_HUMAN/1-452             ---L-IrLQ--PDGVIL---
P48167|GLRB_HUMAN/1-497              ---V-L-VS--MRLSIT---
```

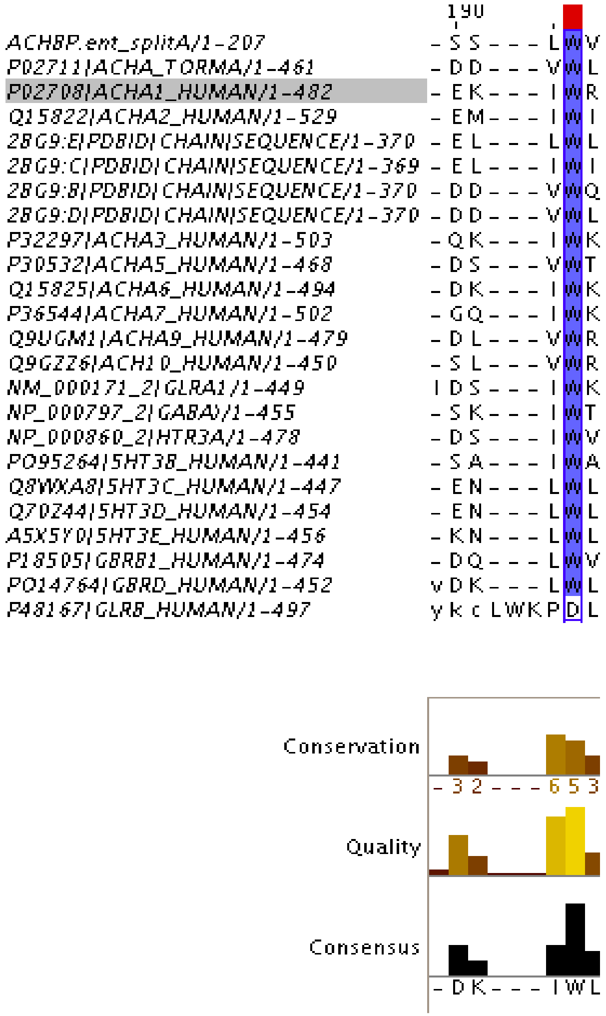
Hydrophobicity in LBD

Trp A82, -hydrophobic core formation- far from the pocket.

It is well conserved.

Binding large ligands (toxins and other neurotransmmitters):





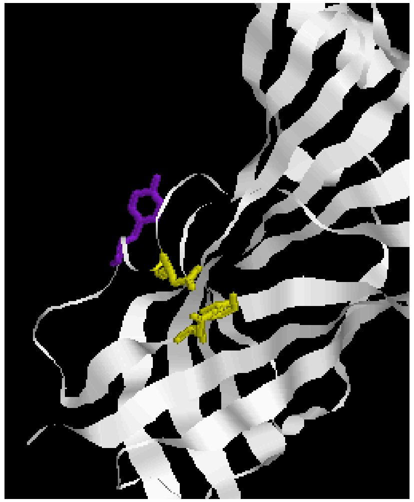
```
                                       190
ACHBP.ent_splitA/1-207                 - S S - - - L W V
P02711|ACHA_TORMA/1-461                - D D - - - V W L
P02708|ACHA1_HUMAN/1-482               - E K - - - I W R
Q15822|ACHA2_HUMAN/1-529               - E M - - - I W I
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370      - E L - - - L W L
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369      - E L - - - I W I
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370      - D D - - - V W Q
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370      - D D - - - V W L
P32297|ACHA3_HUMAN/1-503               - Q K - - - I W K
P30532|ACHA5_HUMAN/1-468               - D S - - - V W T
Q15825|ACHA6_HUMAN/1-494               - D K - - - I W K
P36544|ACHA7_HUMAN/1-502               - G Q - - - I W K
Q9UGM1|ACHA9_HUMAN/1-479               - D L - - - V W R
Q9GZZ6|ACH10_HUMAN/1-450               - S L - - - V W R
NM_000171_2|GLRA1/1-449                I D S - - - I W K
NP_000797_2|GABA)/1-455                - S K - - - I W T
NP_000860_2|HTR3A/1-478                - D S - - - I W V
PO95264|5HT3B_HUMAN/1-441              - S A - - - I W A
Q8WXA8|5HT3C_HUMAN/1-447               - E N - - - L W L
Q70Z44|5HT3D_HUMAN/1-454               - E N - - - L W L
A5X5Y0|5HT3E_HUMAN/1-456               - K N - - - L W L
P18505|GBRB1_HUMAN/1-474               - D Q - - - L W V
PO14764|GBRD_HUMAN/1-452               v D K - - - L W L
P48167|GLRB_HUMAN/1-497                y k c L W K P D L

Conservation
                                       - 3 2 - - - 6 5 3
Quality

Consensus
                                       - D K - - - I W L
```

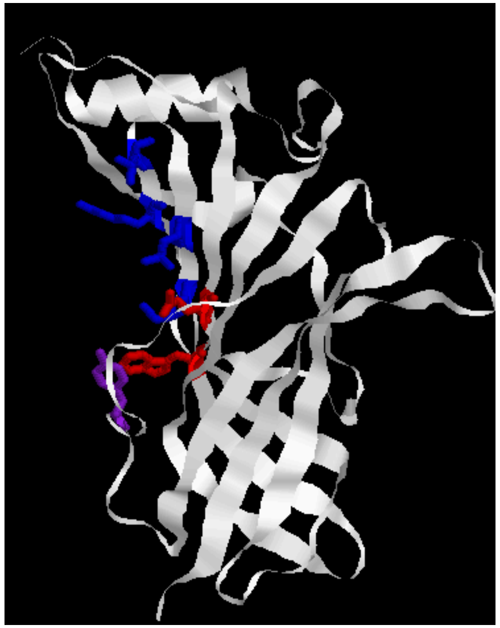
Calcium binding site

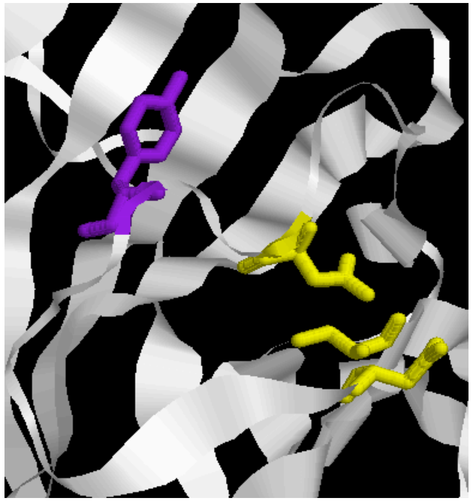
Complementary site (LF. Y164):

Asp(D) B161, Asp B175 and the main chain of B176 as ligands.

Bound Ca2+ → Tyr B164

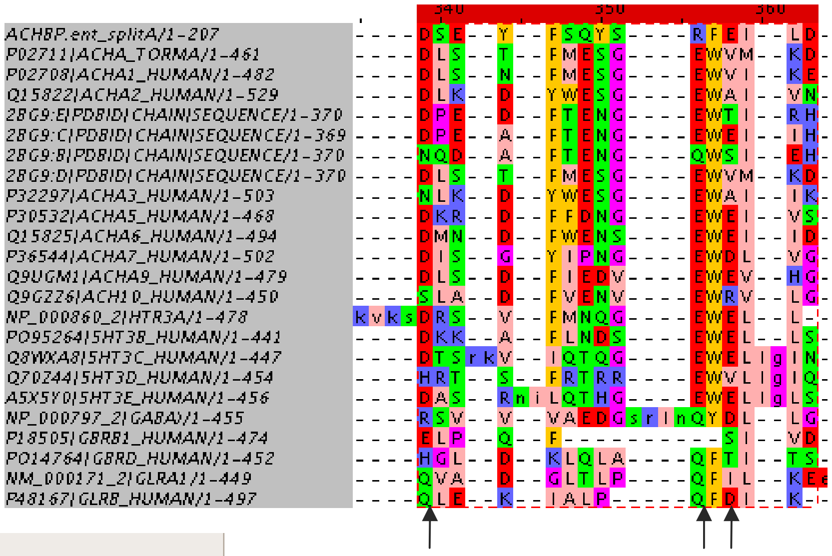






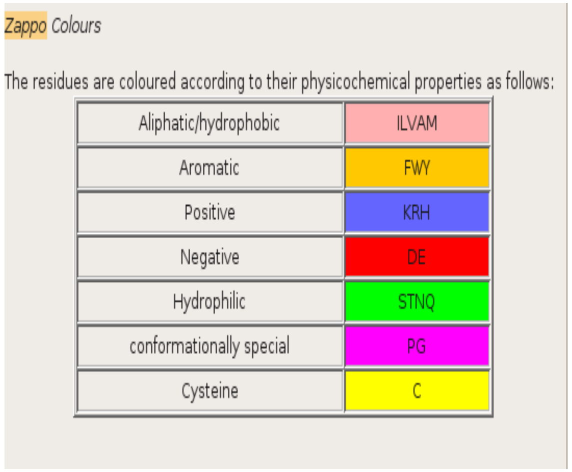
Calcium binding site

Calcium-binding sites have been identified in a homologous region (residue range 161±172) of the neuronal α7-receptor.



Asp(D)161

Glu(E) 170/2



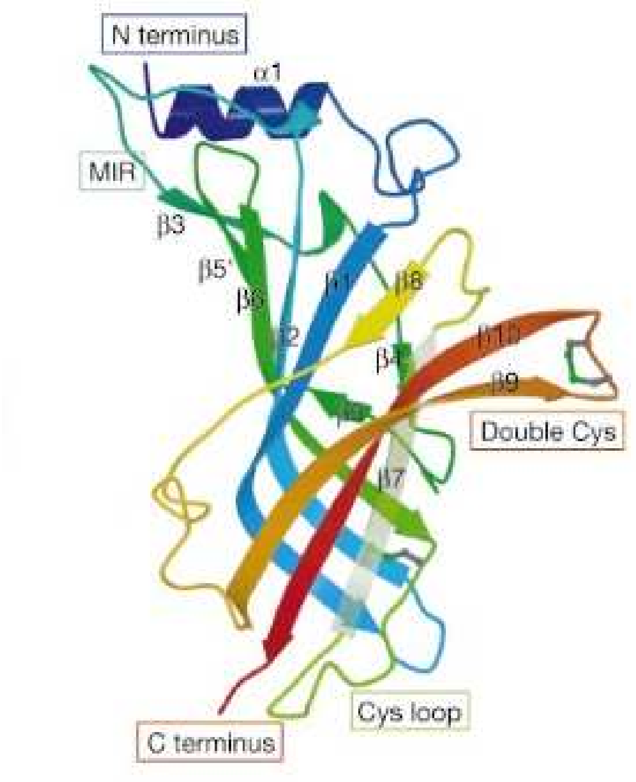
Zappo Colours

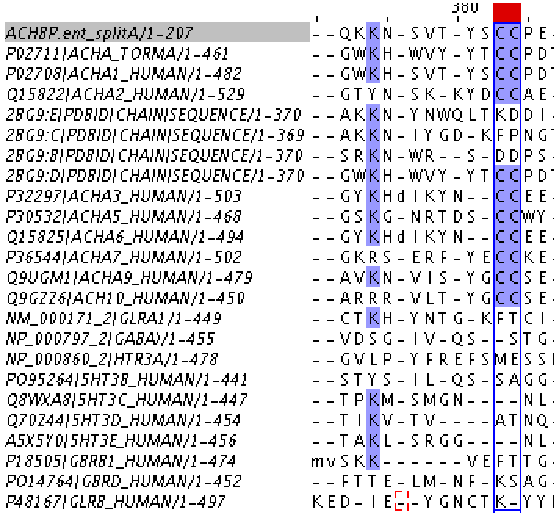
The residues are coloured according to their physicochemical properties as follows:

Aliphatic/hydrophobic	ILVAM
Aromatic	FWY
Positive	KRH
Negative	DE
Hydrophilic	STNQ
conformationally special	PG
Cysteine	C

Acces routes to LBS

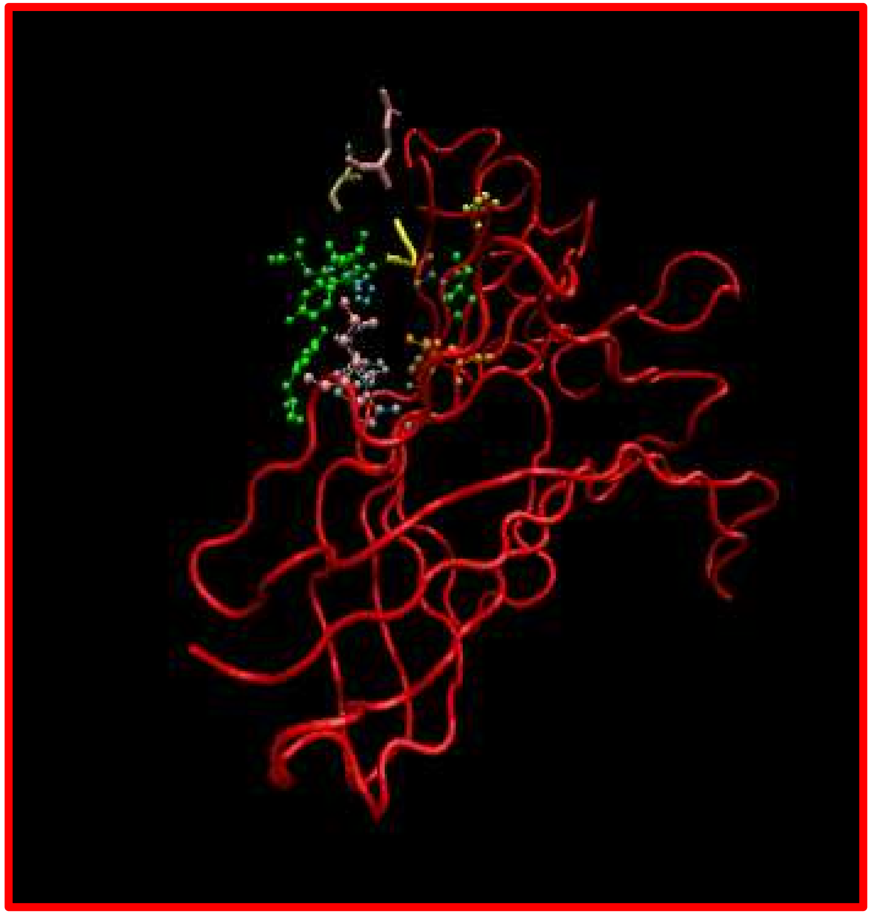
The most likely access routes to the ligand-binding sites are from above or below the double-cysteine-containing loop C (conserved in all α-nicotinic subunit)





```
                                                 380
ACHBP.ent_splitA/1-207                   --QKKN-SVT-YSCCPE
P02711|ACHA_TORMA/1-461                  --GWKH-WVY-YTCCPD
P02708|ACHA1_HUMAN/1-482                 --GWKH-SVT-YSCCPD
Q15822|ACHA2_HUMAN/1-529                 --GTYN-SK-KYDCCAE
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370        --AKKN-YNWQLTKDDI
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369        --AKKN-IYGD-KFPNG
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370        --SRKN-WR--S-DDPS
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370        --GWKH-WVY-YTCCPD
P32297|ACHA3_HUMAN/1-503                 --GYKHdIKYN--CCEE
P30532|ACHA5_HUMAN/1-468                 --GSKG-NRTDS-CCWY
Q15825|ACHA6_HUMAN/1-494                 --GYKHdIKYN--CCEE
P36544|ACHA7_HUMAN/1-502                 --GKRS-ERF-YECCKE
Q9UGM1|ACHA9_HUMAN/1-479                 --AVKN-VIS-YGCCSE
Q9GZZ6|ACH10_HUMAN/1-450                 --ARRR-VLT-YGCCSE
NM_000171_2|GLRA1/1-449                  --CTKH-YNTG-KFTCI
NP_000797_2|GABA)/1-455                  --VDSG-IV-QS--STG
NP_000860_2|HTR3A/1-478                  --GVLP-YFREFSMESSI
PO95264|5HT3B_HUMAN/1-441                --STYS-IL-QS-SAGG
Q8WXA8|5HT3C_HUMAN/1-447                 --TPKM-SMGN----NL
Q70Z44|5HT3D_HUMAN/1-454                 --TIKV-TV----ATNQ
A5X5Y0|5HT3E_HUMAN/1-456                 --TAKL-SRGG----NL
P18505|GBRB1_HUMAN/1-474                 mvSKK-------VEFTTG
PO14764|GBRD_HUMAN/1-452                 --FTTE-LM-NF-KSAG
P48167|GLRB_HUMAN/1-497                  KED-IE--YGNCTK-YYI
```

Acces routes to LBS



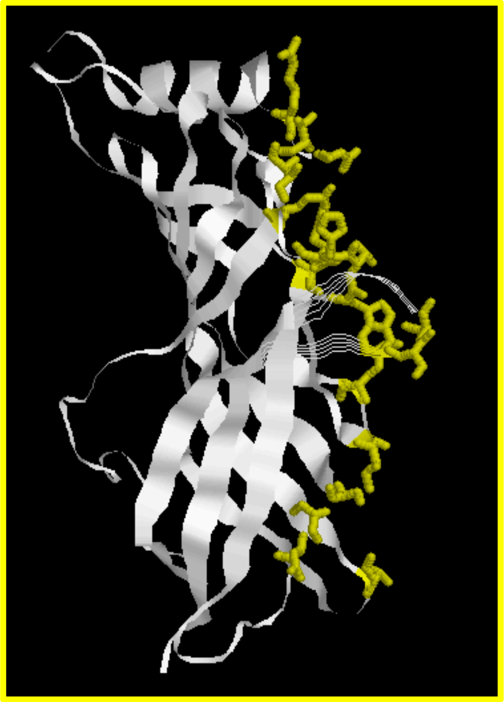
This region buries the ligand-binding site from the solvent, preventing access from the outside.

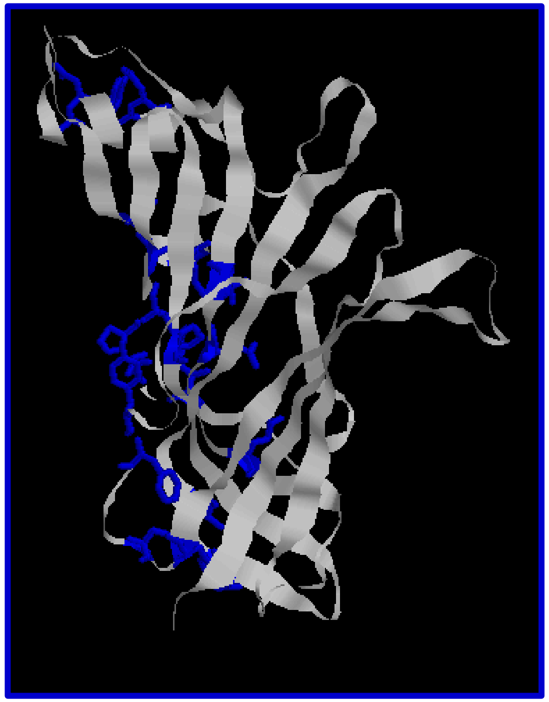
PENTAMER INTERFACE

Large surface area with a mainly uncharged character.

- A single bifurcated salt bridge (Asp A149 with Arg B3 and Arg B104).
- Convoluted surface,
- shape complementarity may be important in pentamer formation.
- The interface residues are not well conserved

PENTAMER INTERFACE

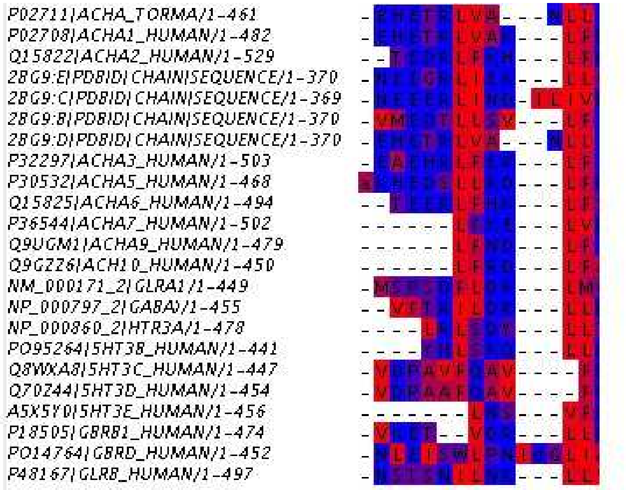




Plus side	Minus side
L1 (Arg A15, Asp A17, Val A18, Ile A19)	α1, β3 (Arg B3, Ala B4, Leu B7, Tyr B8, Pro B77)
L2 (Thr A45)	L9 (Arg B170)
L4 (Asp A85)	β5 (Pro B100, Leu B102)
L5 (Ile A92, Ser A93, Lys A94)	β1, β5, β6 (Leu B39, Glu B96, Leu B98, Arg B118)
L7 (Ser A122, A123, Asp A124)	β1, L9 (Asn B37, Tyr B168)
L8 (Trp A143, ThrA 144, His A145, His A146, Glu A149)	α1, β2, β3, β5, β6 (Arg B3, Trp B53, Ser B75, Thr B99, Arg B104, B114)
L10 (Tyr A185, Ser A186, Cys A187)	L9 (Glu B163, Tyr B164)

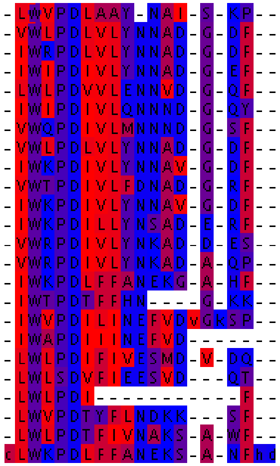
The actual interactions are not conserved in any pentameric LGIC interface, but in all receptors these topological regions are likely to form the interface.

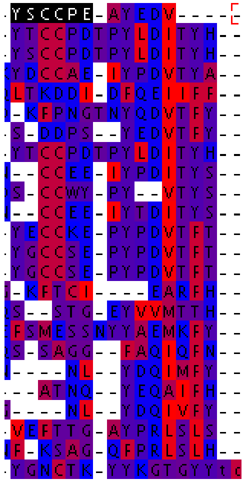
HYDROPHOBICITY: conservation



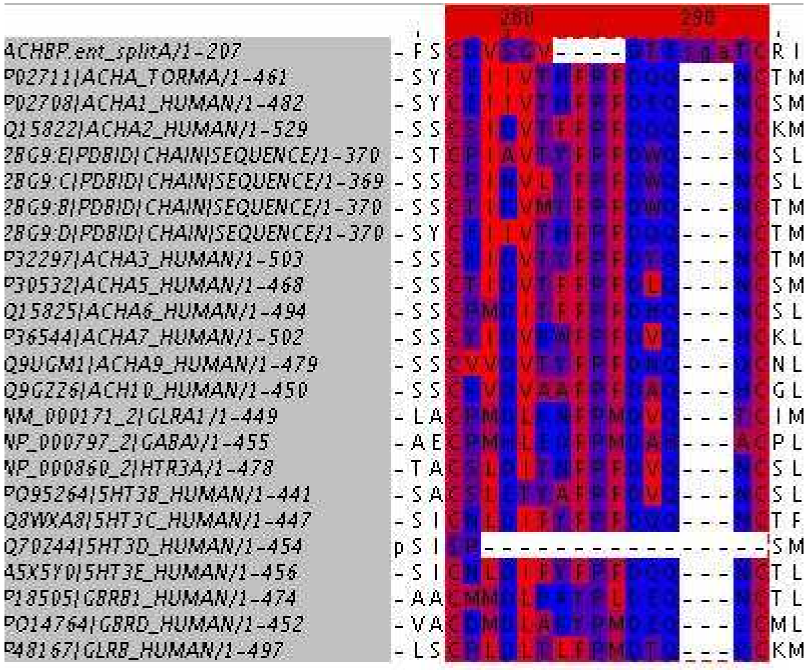
Hydrophobic residues (red) help to maintain the hydrophobic core of the α or acceptor subunit, grouped into three clusters.

- 1.- Packing of the N-terminal α helix
- 2.- Situated in the upper half of the β-core region
- 3.- Located at the lower end of the β-sandwich.

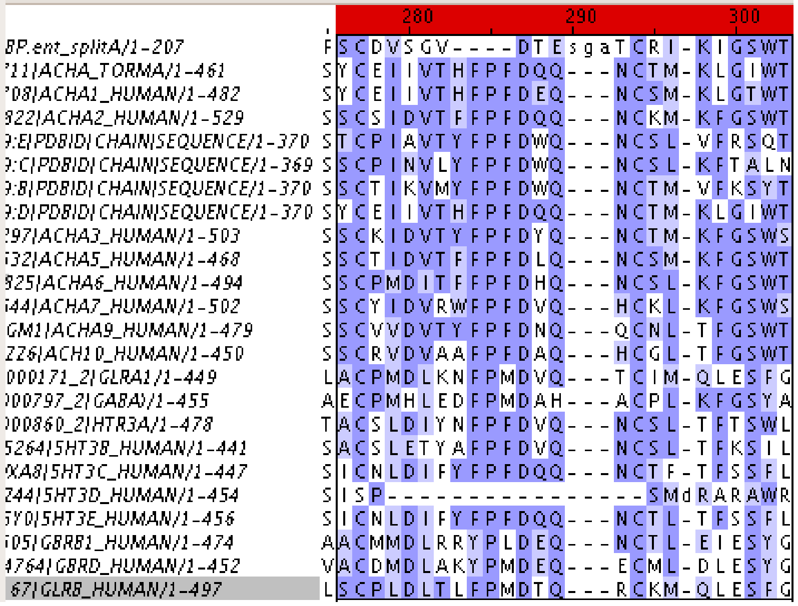




FEW HYDROPHILIC RESIDUES CONSERVED



Cys-loop hydrophibocity

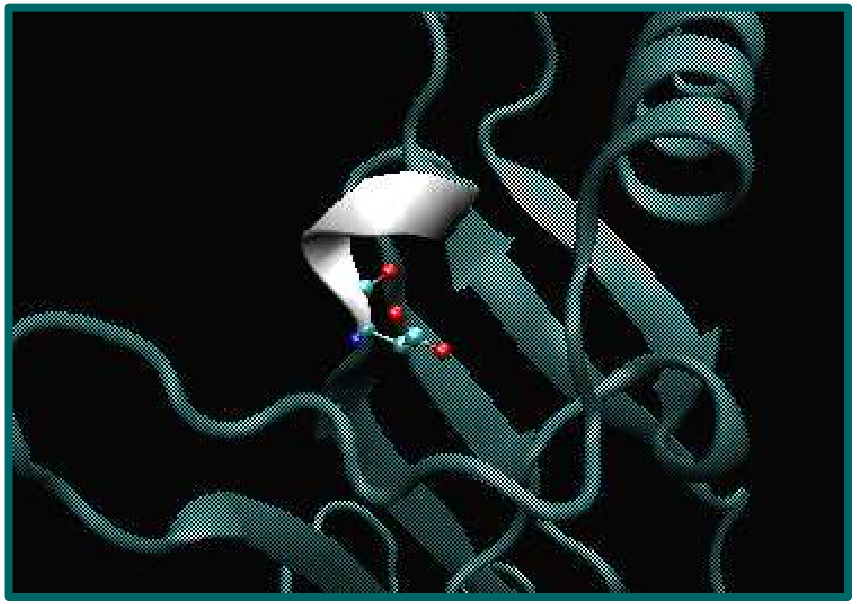


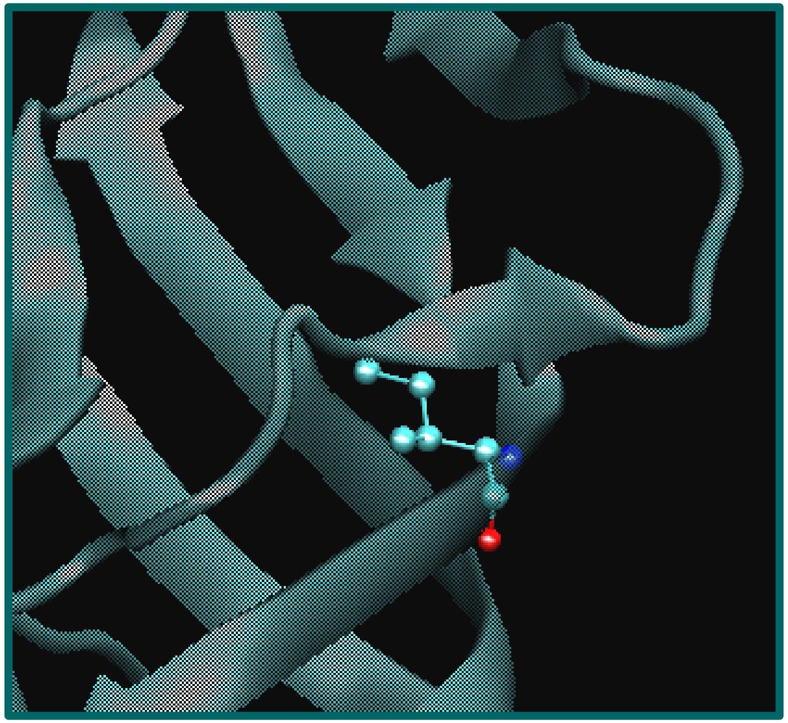
```
                                  280       290       300
8P.ent_splitA/1-207              F SCDVSGV----DTEsgaTCRI-KIGSWT
'11|ACHA_TORMA/1-461             S YCEIIVTHFPFDQQ---NCTM-KLGIWT
'08|ACHA1_HUMAN/1-482            S YCEIIVTHFPFDEQ---NCSM-KLGTWT
822|ACHA2_HUMAN/1-529            S SCSIDVTFFPFDQQ---NCKM-KFGSWT
9:E|PDBID|CHAIN|SEQUENCE/1-370   S TCPIAVTYFPFDWQ---NCSL-VFRSQT
9:C|PDBID|CHAIN|SEQUENCE/1-369   S SCPINVLYFPFDWQ---NCSL-KFTALN
9:B|PDBID|CHAIN|SEQUENCE/1-370   S SCTIKVMYFPFDWQ---NCTM-VFKSYT
9:D|PDBID|CHAIN|SEQUENCE/1-370   S YCEIIVTHFPFDQQ---NCTM-KLGIWT
297|ACHA3_HUMAN/1-503            S SCKIDVTYFPFDYQ---NCTM-KFGSWS
32|ACHA5_HUMAN/1-468             S SCTIDVTFFPFDLQ---NCSM-KFGSWT
825|ACHA6_HUMAN/1-494            S SCPMDITFFPFDHQ---NCSL-KFGSWT
44|ACHA7_HUMAN/1-502             S SCYIDVRWFPFDVQ---HCKL-KFGSWS
GM1|ACHA9_HUMAN/1-479            S SCVVDVTYFPFDNQ---QCNL-TFGSWT
ZZ6|ACH10_HUMAN/1-450            S SCRVDVAAFPFDAQ---HCGL-TFGSWT
000171_2|GLRA1/1-449             L ACPMDLKNFPMDVQ---TCIM-QLESFG
000797_2|GABA)/1-455             A ECPMHLEDFPMDAH---ACPL-KFGSYA
000860_2|HTR3A/1-478             T ACSLDIYNFPFDVQ---NCSL-TFTSWL
5264|5HT3B_HUMAN/1-441           S ACSLETYAFPFDVQ---NCSL-TFKSIL
XA8|5HT3C_HUMAN/1-447            S ICNLDIFYFPFDQQ---NCTF-TFSSFL
Z44|5HT3D_HUMAN/1-454            S ISP----------------SMdRARAWR
Y0|5HT3E_HUMAN/1-456             S ICNLDIFYFPFDQQ---NCTL-TFSSFL
505|GBRB1_HUMAN/1-474            A ACMMDLRRYPLDEQ---NCTL-EIESYG
4764|GBRD_HUMAN/1-452            V ACDMDLAKYPMDEQ---ECML-DLESYG
67|GLRB_HUMAN/1-497              L SCPLDLTLFPMDTQ---RCKM-QLESFG
```

S122-C123-C136-R137-S142-T144

As the conserved residues mainly contribute to the overall structure formation, it is clear that all pentameric LGIC N-terminal domains will have the same three-dimensional structure.

FEW HYDROPHILIC RESIDUES CONSERVED

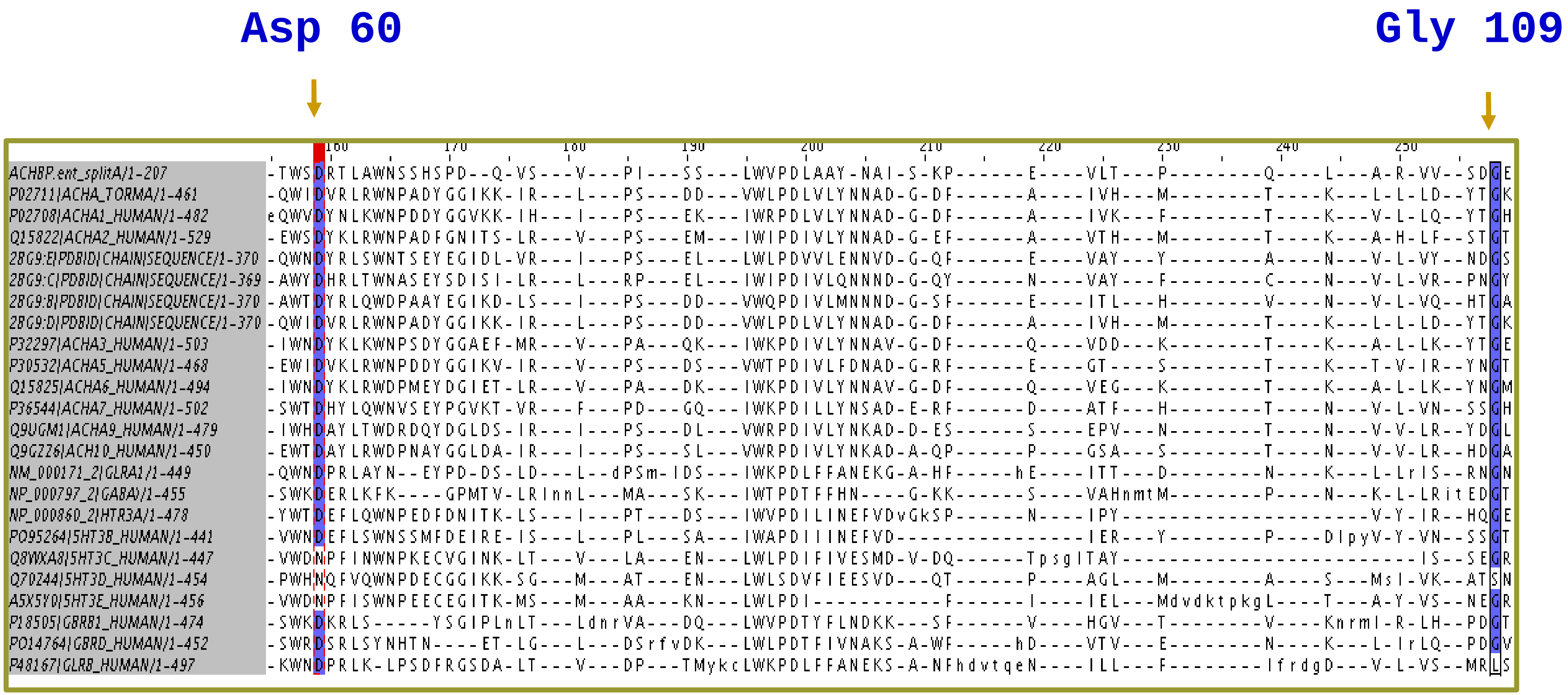




- **Asp (D) 60** stabilizes the N terminus of a small 3rd helix and

- **Gly 109** enables tight-turn formation.

FEW HYDROPHILIC RESIDUES CONSERVED

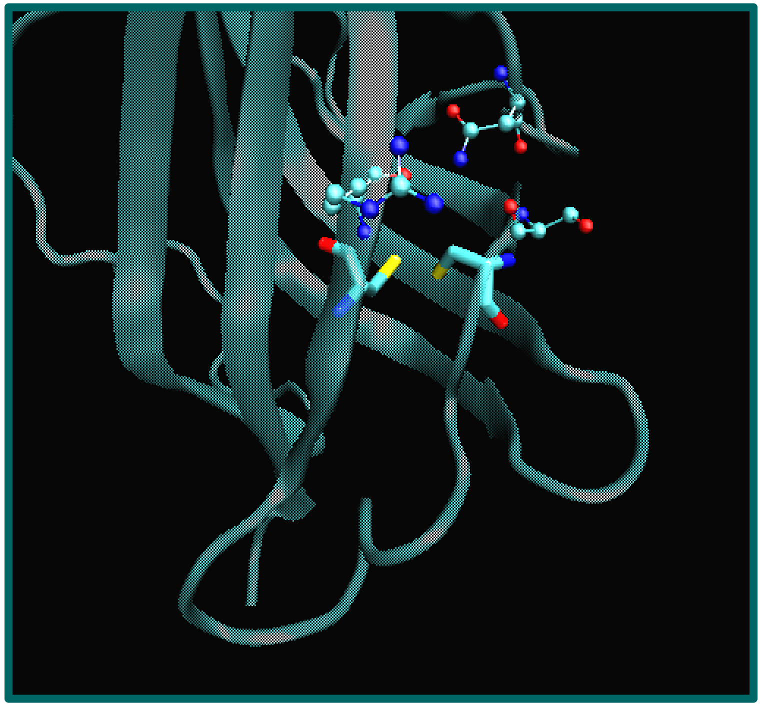


```
                                     160       170       180       190       200       210       220       230       240       250
ACHBP.ent_splitA/1-207               -TWSDRTLAWNSSHSPD--Q-VS---V---PI---SS---LWVPDLAAY-NAI-S-KP------E----VLT---P--------Q----L---A-R-VV--SDGE
P02711|ACHA_TORMA/1-461              -QWIDVRLRWNPADYGGIKK-IR---L---PS---DD---VWLPDLVLYNNAD-G-DF------A----IVH---M--------T----K---L-L-LD--YTGK
P02708|ACHA1_HUMAN/1-482             eQWVDYNLKWNPDDYGGVKK-IH---I---PS---EK---IWRPDLVLYNNAD-G-DF------A----IVK---F--------T----K---V-L-LQ--YTGH
Q15822|ACHA2_HUMAN/1-529             -EWSDYKLRWNPADFGNITS-LR---V---PS---EM---IWIPDIVLYNNAD-G-EF------A----VTH---M--------T----K---A-H-LF--STGT
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370    -QWNDYRLSWNTSEYEGIDL-VR---I---PS---EL---LWLPDVVLENNVD-G-QF------E----VAY---Y--------A----N---V-L-VY--NDGS
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369    -AWYDHRLTWNASEYSDISI-LR---L---RP---EL---IWIPDIVLQNNND-G-QY------N----VAY---F--------C----N---V-L-VR--PNGY
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370    -AWTDYRLQWDPAAYEGIKD-LS---I---PS---DD---VWQPDIVLMNNND-G-SF------E----ITL---H--------V----N---V-L-VQ--HTGA
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370    -QWIDVRLRWNPADYGGIKK-IR---L---PS---DD---VWLPDLVLYNNAD-G-DF------A----IVH---M--------T----K---L-L-LD--YTGK
P32297|ACHA3_HUMAN/1-503             -IWNDYKLKWNPSDYGGAEF-MR---V---PA---QK---IWKPDIVLYNNAV-G-DF------Q----VDD---K--------T----K---A-L-LK--YTGE
P30532|ACHA5_HUMAN/1-468             -EWIDVKLRWNPDDYGGIKV-IR---V---PS---DS---VWTPDIVLFDNAD-G-RF------E----GT----S--------T----K---T-V-IR--YNGT
Q15825|ACHA6_HUMAN/1-494             -IWNDYKLRWDPMEYDGIET-LR---V---PA---DK---IWKPDIVLYNNAV-G-DF------Q----VEG---K--------T----K---A-L-LK--YNGM
P36544|ACHA7_HUMAN/1-502             -SWTDHYLQWNVSEYPGVKT-VR---F---PD---GQ---IWKPDILLYNSAD-E-RF------D----ATF---H--------T----N---V-L-VN--SSGH
Q9UGM1|ACHA9_HUMAN/1-479             -IWHDAYLTWDRDQYDGLDS-IR---I---PS---DL---VWRPDIVLYNKAD-D-ES------S----EPV---N--------T----N---V-V-LR--YDGL
Q9GZZ6|ACH10_HUMAN/1-450             -EWTDAYLRWDPNAYGGLDA-IR---I---PS---SL---VWRPDIVLYNKAD-A-QP------P----GSA---S--------T----N---V-V-LR--HDGA
NM_000171_2|GLRA1/1-449              -QWNDPRLAYN--EYPD-DS-LD---L--dPSm-IDS---IWKPDLFFANEKG-A-HF-----hE----ITT---D--------N----K---L-LrIS--RNGN
NP_000797_2|GABA)/1-455              -SWKDERLKFK----GPMTV-LRInnL---MA---SK---IWTPDTFFHN----G-KK------S----VAHnmtM--------P----N---K-L-LRitEDGT
NP_000860_2|HTR3A/1-478              -YWTDEFLQWNPEDFDNITK-LS---I---PT---DS---IWVPDILINEFVDvGkSP------N----IPY---------------------V-Y-IR--HQGE
PO95264|5HT3B_HUMAN/1-441            -VWNDEFLSWNSSMFDEIRE-IS---L---PL---SA---IWAPDIIINEFVD----------------IER---Y--------P----DlpyV-Y-VN--SSGT
Q8WXA8|5HT3C_HUMAN/1-447             -VWDNPFINWNPKECVGINK-LT---V---LA---EN---LWLPDIFIVESMD-V-DQ------TpsgITAY------------------------IS--SEGR
Q70Z44|5HT3D_HUMAN/1-454             -PWHNQFVQWNPDECGGIKK-SG---M---AT---EN---LWLSDVFIEESVD---QT------P----AGL---M--------A----S---MsI-VK--ATSN
A5X5Y0|5HT3E_HUMAN/1-456             -VWDNPFISWNPEECEGITK-MS---M---AA---KN---LWLPDI-----------F------I----IEL---MdvdktpkgL----T---A-Y-VS--NEGR
P18505|GBRB1_HUMAN/1-474             -SWKDKRLS-----YSGIPLnLT---LdnrVA---DQ---LWVPDTYFLNDKK---SF------V----HGV---T--------V----KnrmI-R-LH--PDGT
PO14764|GBRD_HUMAN/1-452             -SWRDSRLSYNHTN----ET-LG---L---DSrfvDK---LWLPDTFIVNAKS-A-WF-----hD----VTV---E--------N----K---L-IrLQ--PDGV
P48167|GLRB_HUMAN/1-497              -KWNDPRLK-LPSDFRGSDA-LT---V---DP---TMykcLWKPDLFFANEKS-A-NFhdvtqeN----ILL---F--------IfrdgD---V-L-VS--MRLS
```

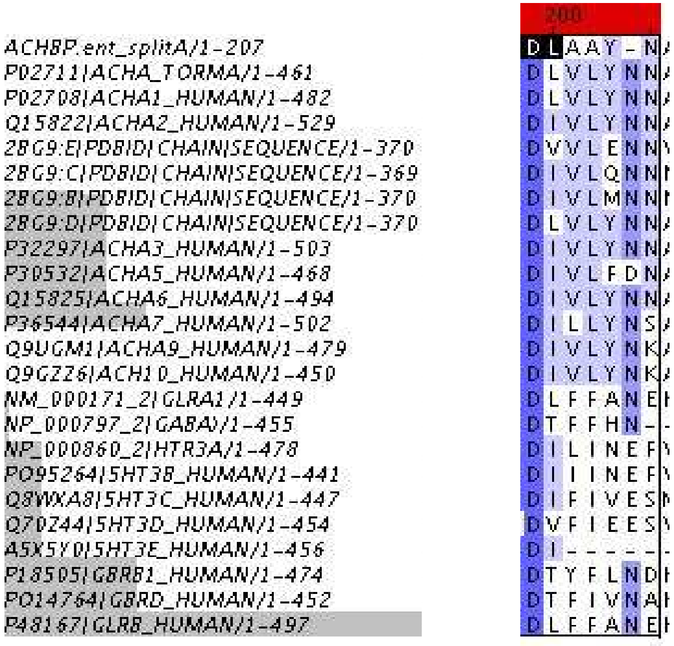
FEW HYDROPHILIC RESIDUES CONSERVED

Packing of the β–sheets:

- **Asn 90** brings together the main-chain oxygens of Ser 122 and Arg 137, enabling disulphide-bond formation of the nearby absolutely conserved disulphide bond (123±136)



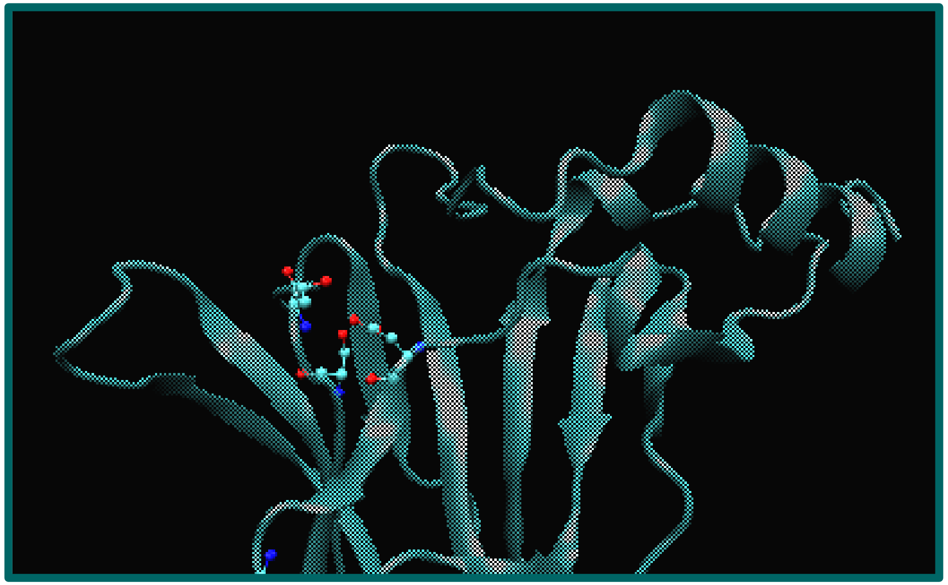
FEW HYDROPHILIC RESIDUES CONSERVED



	200
ACHBP.ent_splitA/1-207	D L A A Y - N
P02711|ACHA_TORMA/1-461	D L V L Y N N
P02708|ACHA1_HUMAN/1-482	D L V L Y N N
Q15822|ACHA2_HUMAN/1-529	D I V L Y N N
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370	D V V L E N N
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369	D I V L Q N N
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370	D I V L M N N
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370	D L V L Y N N
P32297|ACHA3_HUMAN/1-503	D I V L Y N N
P30532|ACHA5_HUMAN/1-468	D I V L F D N
Q15825|ACHA6_HUMAN/1-494	D I V L Y N N
P36544|ACHA7_HUMAN/1-502	D I L L Y N S
Q9UGM1|ACHA9_HUMAN/1-479	D I V L Y N K
Q9GZZ6|ACH10_HUMAN/1-450	D I V L Y N K
NM_000171_2|GLRA1/1-449	D L F F A N E
NP_000797_2|GABA)/1-455	D T F F H N -
NP_000860_2|HTR3A/1-478	D I L I N E F
PO95264|5HT3B_HUMAN/1-441	D I I I N E F
Q8WXA8|5HT3C_HUMAN/1-447	D I F I V E S
Q70Z44|5HT3D_HUMAN/1-454	D V F I E E S
A5X5Y0|5HT3E_HUMAN/1-456	D I - - - - -
P18505|GBRB1_HUMAN/1-474	D T Y F L N D
PO14764|GBRD_HUMAN/1-452	D T F I V N A
P48167|GLRB_HUMAN/1-497	D L F F A N E

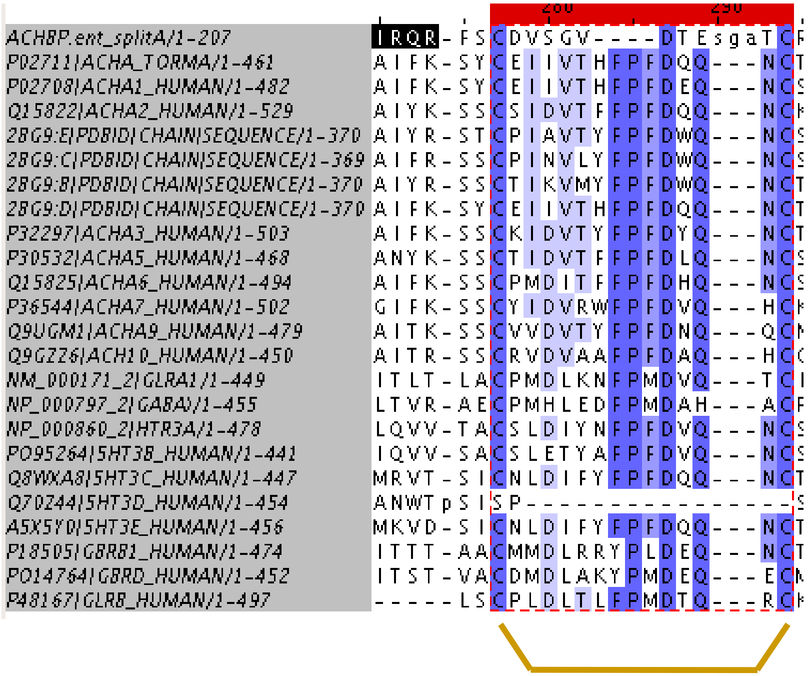
Asp(D)85-Asn(N)90

- **Asp 85** forms hydrogen bonds to the highly conserved Ser 142 and Thr 144

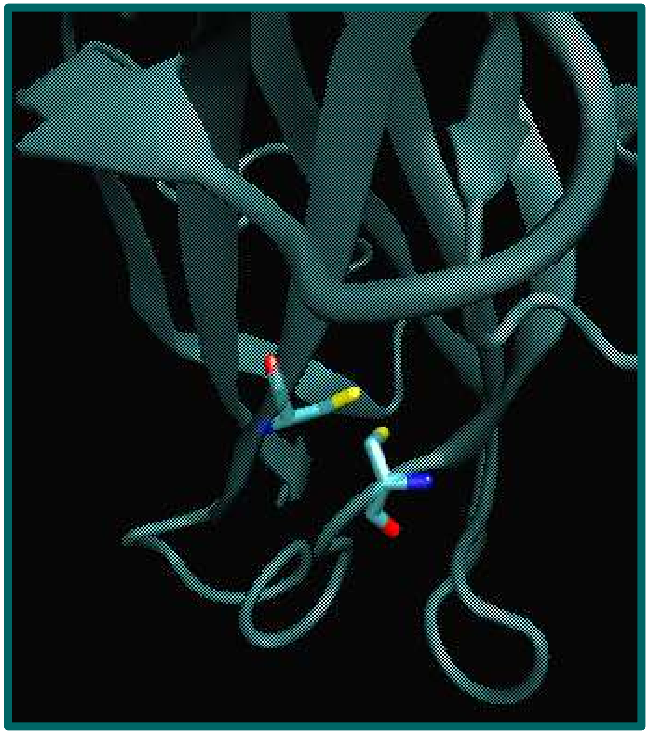


DISULPHIDE BOND

- It links the two sets of β-sheets together.
- For preservation of subunit conformational stability and complete nAChR assembly.



```
                                            280      290
ACHBP.ent_splitA/1-207               IRQR-FSCDVSGV----DTEsgaTC
P02711|ACHA_TORMA/1-461              AIFK-SYCEIIVTHFPFDQQ---NC
P02708|ACHA1_HUMAN/1-482             AIFK-SYCEIIVTHFPFDEQ---NC
Q15822|ACHA2_HUMAN/1-529             AIYK-SSCSIDVTFFPFDQQ---NC
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370    AIYR-STCPIAVTYFPFDWQ---NC
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369    AIFR-SSCPINVLYFPFDWQ---NC
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370    AIYR-SSCTIKVMYFPFDWQ---NC
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370    AIFK-SYCEIIVTHFPFDQQ---NC
P32297|ACHA3_HUMAN/1-503             AIFK-SSCKIDVTYFPFDYQ---NC
P30532|ACHA5_HUMAN/1-468             ANYK-SSCTIDVTFFPFDLQ---NC
Q15825|ACHA6_HUMAN/1-494             AIFK-SSCPMDITFFPFDHQ---NC
P36544|ACHA7_HUMAN/1-502             GIFK-SSCYIDVRWFPFDVQ---HC
Q9UGM1|ACHA9_HUMAN/1-479             AITK-SSCVVDVTYFPFDNQ---QC
Q9GZZ6|ACH10_HUMAN/1-450             AITR-SSCRVDVAAFPFDAQ---HC
NM_000171_2|GLRA1/1-449              ITLT-LACPMDLKNFPMDVQ---TC
NP_000797_2|GABA)/1-455              LTVR-AECPMHLEDFPMDAH---AC
NP_000860_2|HTR3A/1-478              LQVV-TACSLDIYNFPFDVQ---NC
PO95264|5HT3B_HUMAN/1-441            IQVV-SACSLETYAFPFDVQ---NC
Q8WXA8|5HT3C_HUMAN/1-447             MRVT-SICNLDIFYFPFDQQ---NC
Q70Z44|5HT3D_HUMAN/1-454             ANWTpSISP----------------
A5X5Y0|5HT3E_HUMAN/1-456             MKVD-SICNLDIFYFPFDQQ---NC
P18505|GBRB1_HUMAN/1-474             ITTT-AACMMDLRRYPLDEQ---NC
PO14764|GBRD_HUMAN/1-452             ITST-VACDMDLAKYPMDEQ---EC
P48167|GLRB_HUMAN/1-497              -----LSCPLDLTLFPMDTQ---RC
```

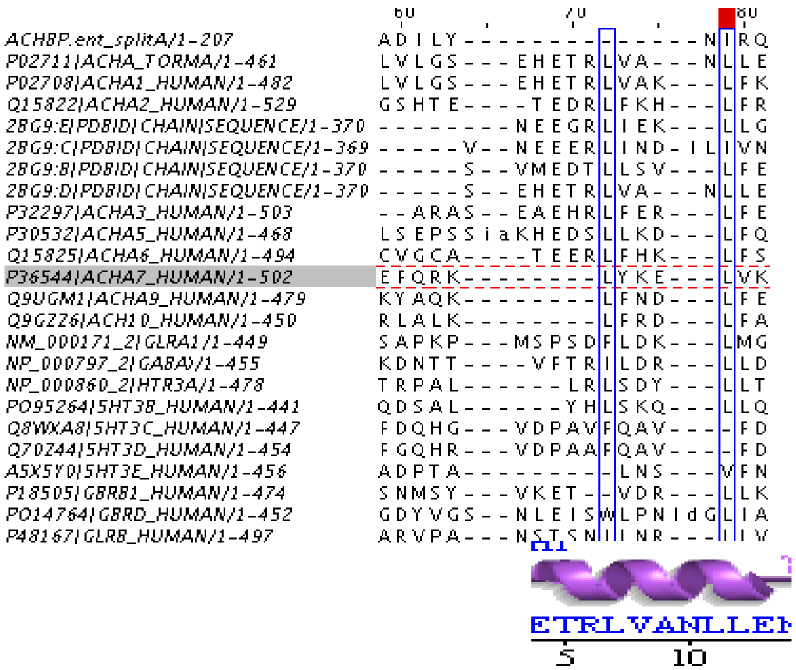


CYS-LOOP

- Close to the dimer interface.
- Hydrophobicity
- Interact with the TM region ??

α-HELIX AT THE N-TERMINUS

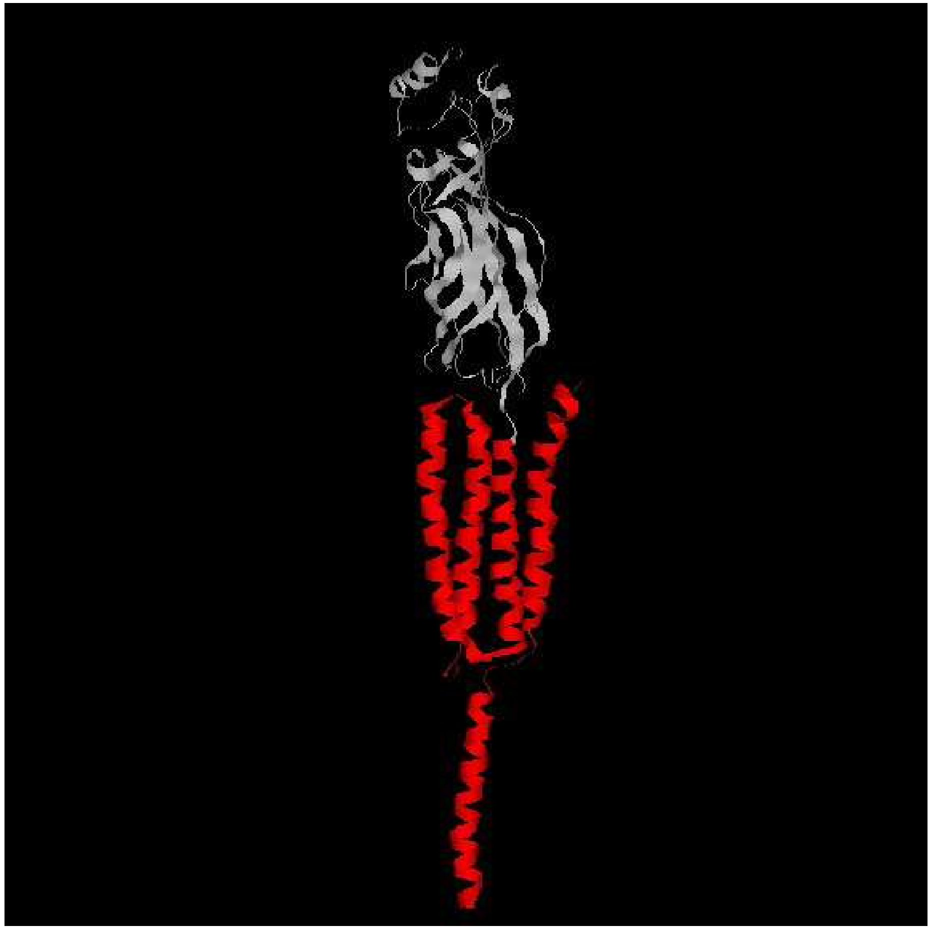
Leu 7 and Leu 11



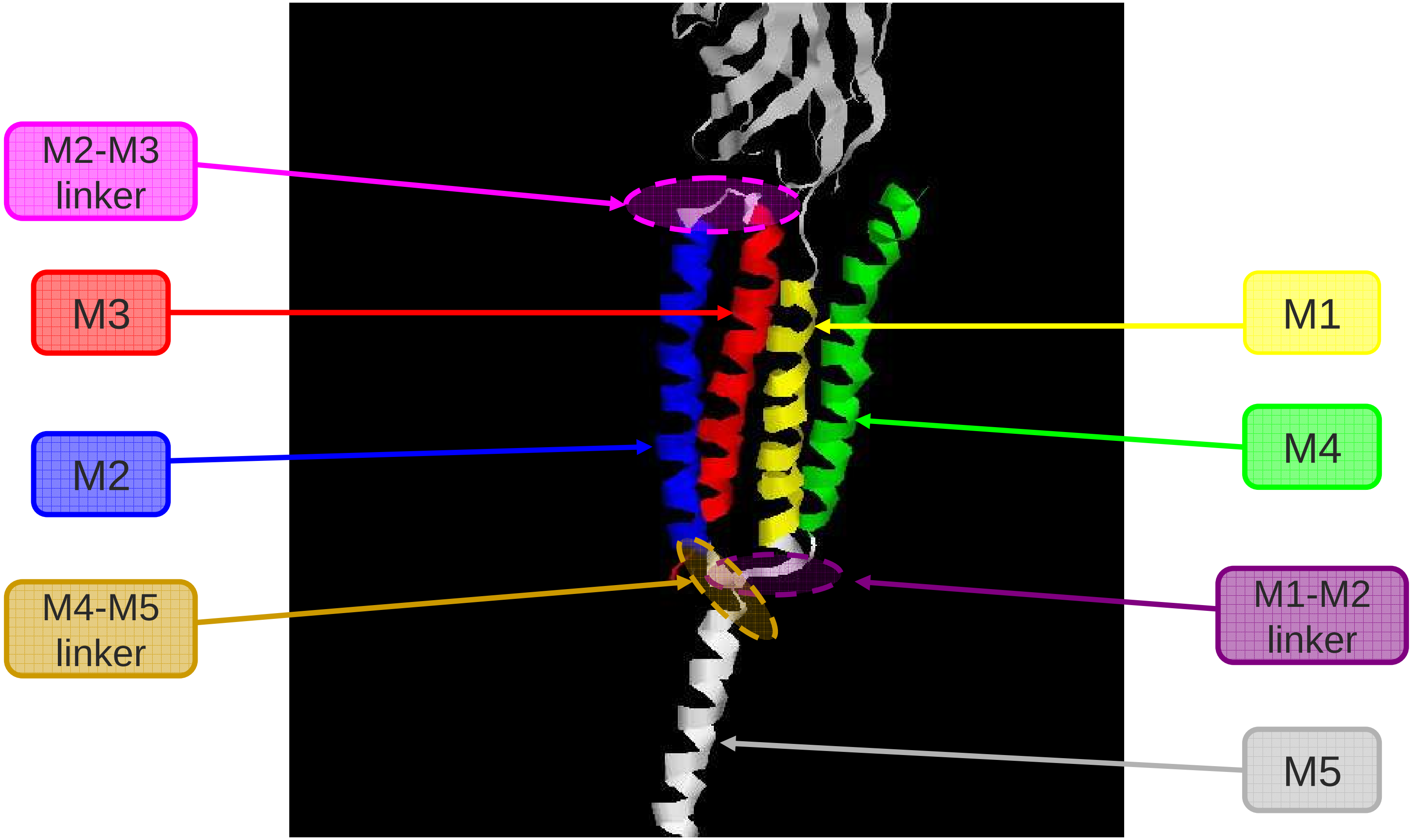
```
                                          60        70        80
ACHBP.ent_splitA/1-207                    ADILY--------------NIRQ
P02711|ACHA_TORMA/1-461                   LVLGS---EHETRLVA---NLLE
P02708|ACHA1_HUMAN/1-482                  LVLGS---EHETRLVAK---LFK
Q15822|ACHA2_HUMAN/1-529                  GSHTE----TEDRLFKH---LFR
2BG9:E|PDBID|CHAIN|SEQUENCE/1-370         --------NEEGRLIEK---LLG
2BG9:C|PDBID|CHAIN|SEQUENCE/1-369         -----V--NEEERLIND-ILIVN
2BG9:B|PDBID|CHAIN|SEQUENCE/1-370         -----S--VMEDTLLSV---LFE
2BG9:D|PDBID|CHAIN|SEQUENCE/1-370         -----S--EHETRLVA---NLLE
P32297|ACHA3_HUMAN/1-503                  --ARAS--EAEHRLFER---LFE
P30532|ACHA5_HUMAN/1-468                  LSEPSSiaKHEDSLLKD---LFQ
Q15825|ACHA6_HUMAN/1-494                  CVGCA----TEERLFHK---LFS
P36544|ACHA7_HUMAN/1-502                  EFQRK--------LYKE---LVK
Q9UGM1|ACHA9_HUMAN/1-479                  KYAQK--------LFND---LFE
Q9GZZ6|ACH10_HUMAN/1-450                  RLALK--------LFRD---LFA
NM_000171_2|GLRA1/1-449                   SAPKP---MSPSDFLDK---LMG
NP_000797_2|GABA)/1-455                   KDNTT----VFTRILDR---LLD
NP_000860_2|HTR3A/1-478                   TRPAL------LRLSDY---LLT
PO95264|5HT3B_HUMAN/1-441                 QDSAL------YHLSKQ---LLQ
Q8WXA8|5HT3C_HUMAN/1-447                  FDQHG---VDPAVFQAV----FD
Q70Z44|5HT3D_HUMAN/1-454                  FGQHR---VDPAAFQAV----FD
A5X5Y0|5HT3E_HUMAN/1-456                  ADPTA---------LNS---VFN
P18505|GBRB1_HUMAN/1-474                  SNMSY---VKET--VDR---LLK
PO14764|GBRD_HUMAN/1-452                  GDYVGS--NLEISWLPNIdGLIA
P48167|GLRB_HUMAN/1-497                   ARVPA---NSTSNILNR---IIV
```

Deletion of Leu11 in α_7-subunits abolished expression of nAChRs at the membrane (Castillo et al., J. Neurochem., 2009).

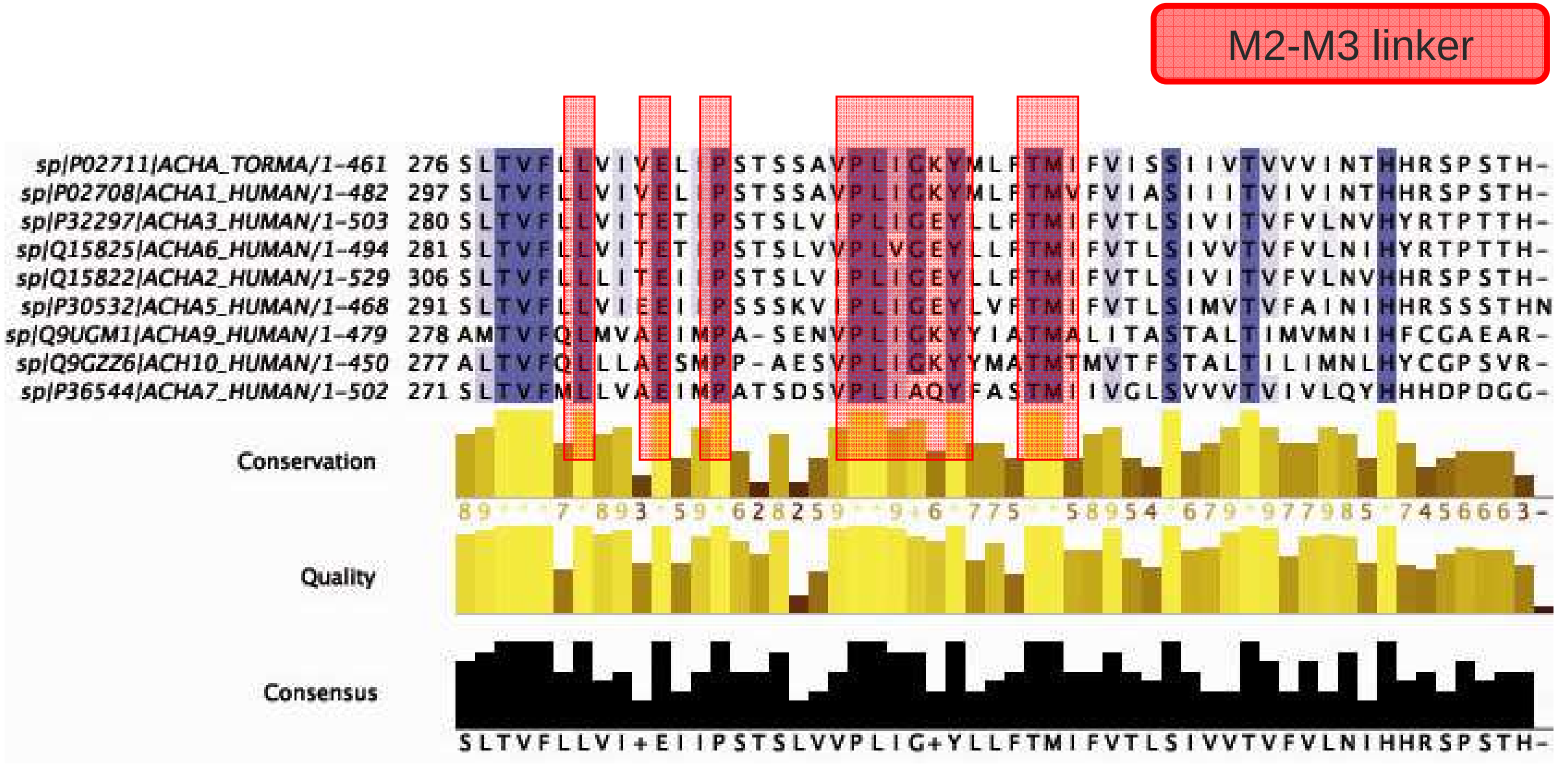
TRANSMEMBRANE DOMAIN



TMD



M2-M3 linker region



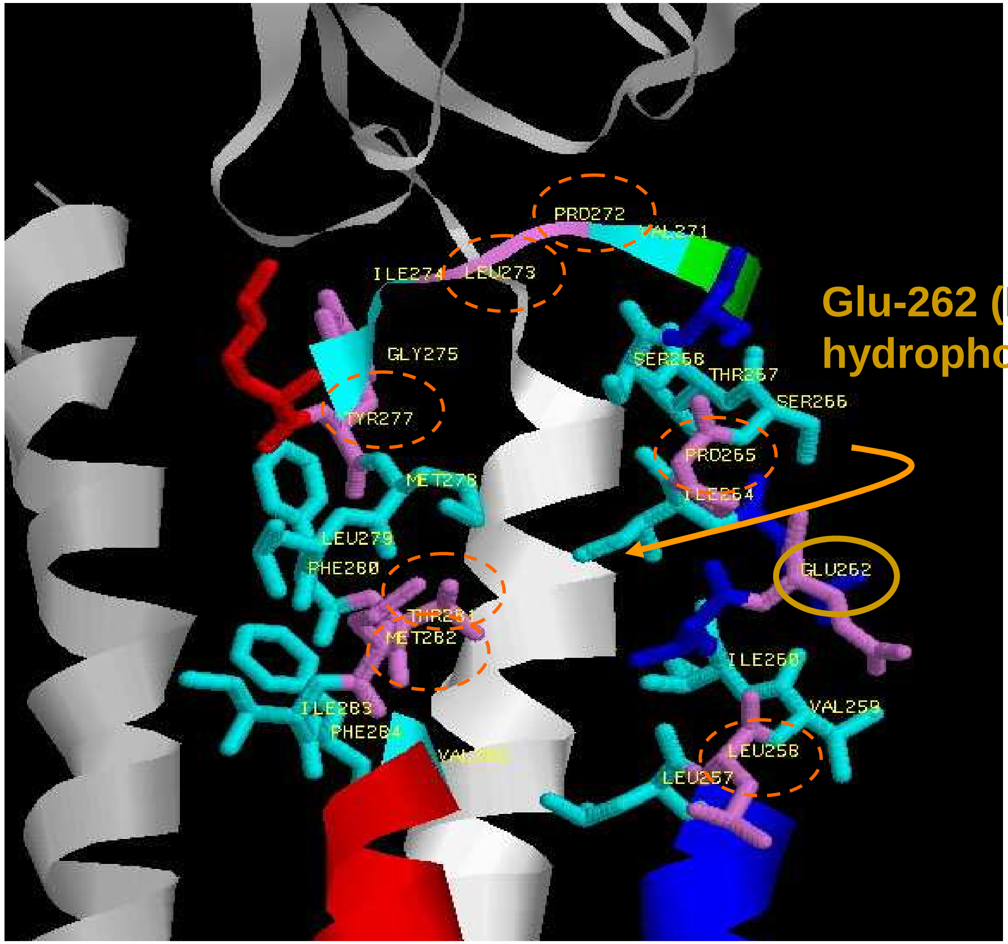
M2-M3 linker

```
sp|P02711|ACHA_TORMA/1-461  276 S L T V F L L V I V E L I P S T S S A V P L I G K Y M L F T M I F V I S S I I V T V V V I N T H H R S P S T H -
sp|P02708|ACHA1_HUMAN/1-482 297 S L T V F L L V I V E L I P S T S S A V P L I G K Y M L F T M V F V I A S I I I T V I V I N T H H R S P S T H -
sp|P32297|ACHA3_HUMAN/1-503 280 S L T V F L L V I T E T I P S T S L V I P L I G E Y L L F T M I F V T L S I V I T V F V L N V H Y R T P T T H -
sp|Q15825|ACHA6_HUMAN/1-494 281 S L T V F L L V I T E T I P S T S L V V P L V G E Y L L F T M I F V T L S I V V T V F V L N I H Y R T P T T H -
sp|Q15822|ACHA2_HUMAN/1-529 306 S L T V F L L L I T E I I P S T S L V I P L I G E Y L L F T M I F V T L S I V I T V F V L N V H H R S P S T H -
sp|P30532|ACHA5_HUMAN/1-468 291 S L T V F L L V I E E I I P S S S K V I P L I G E Y L V F T M I F V T L S I M V T V F A I N I H H R S S S T H N
sp|Q9UGM1|ACHA9_HUMAN/1-479 278 A M T V F Q L M V A E I M P A - S E N V P L I G K Y Y I A T M A L I T A S T A L T I M V M N I H F C G A E A R -
sp|Q9GZZ6|ACH10_HUMAN/1-450 277 A L T V F Q L L L A E S M P P - A E S V P L I G K Y Y M A T M T M V T F S T A L T I L I M N L H Y C G P S V R -
sp|P36544|ACHA7_HUMAN/1-502 271 S L T V F M L L V A E I M P A T S D S V P L I A Q Y F A S T M I I V G L S V V V T V I V L Q Y H H H D P D G G -

Conservation
                                8 9 * * * 7 * 8 9 3 * 5 9 * 6 2 8 2 5 9 * * 9 + 6 * 7 7 5 * * 5 8 9 5 4 * 6 7 9 * 9 7 7 9 8 5 * 7 4 5 6 6 6 3 -
Quality

Consensus
                                S L T V F L L V I + E I I P S T S L V V P L I G + Y L L F T M I F V T L S I V V T V F V L N I H H R S P S T H -
```

M2-M3 linker region

Legend:

- M2
- M3
- Linker
- Fully conserved
- 70% Identity

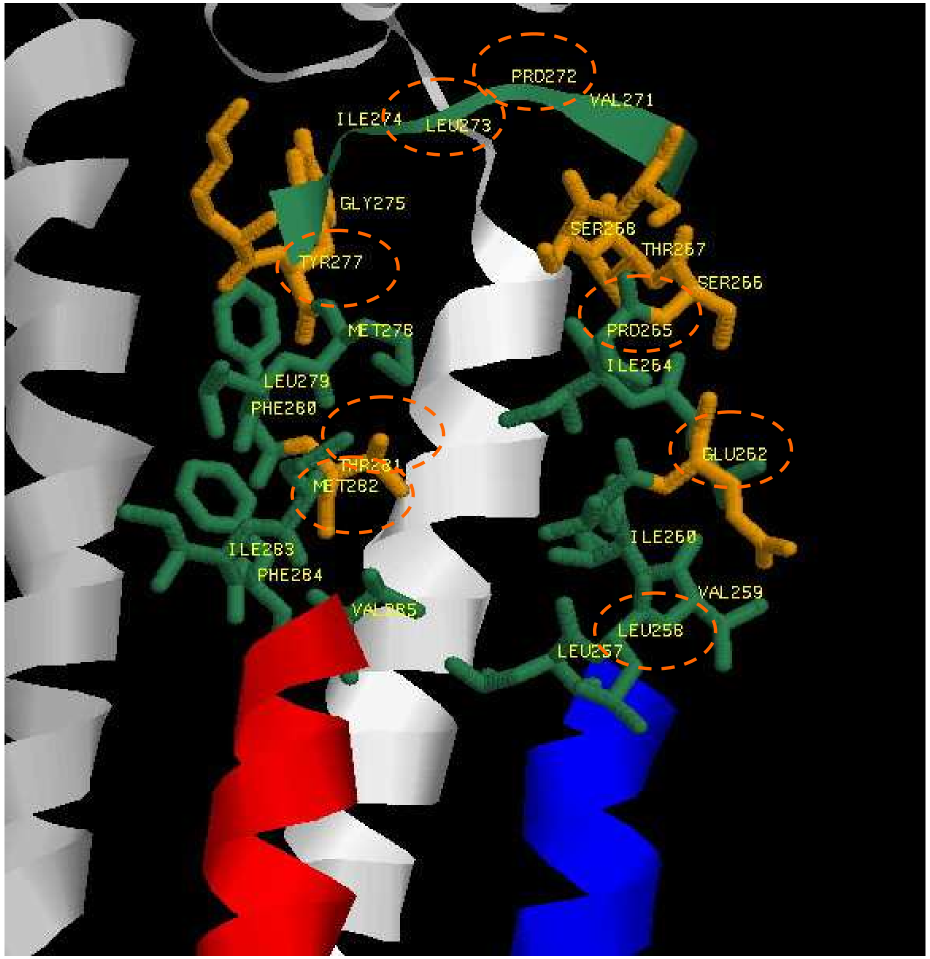


Glu-262 (M2) in contact with hydrophobic residues at M3

M2-M3 linker region

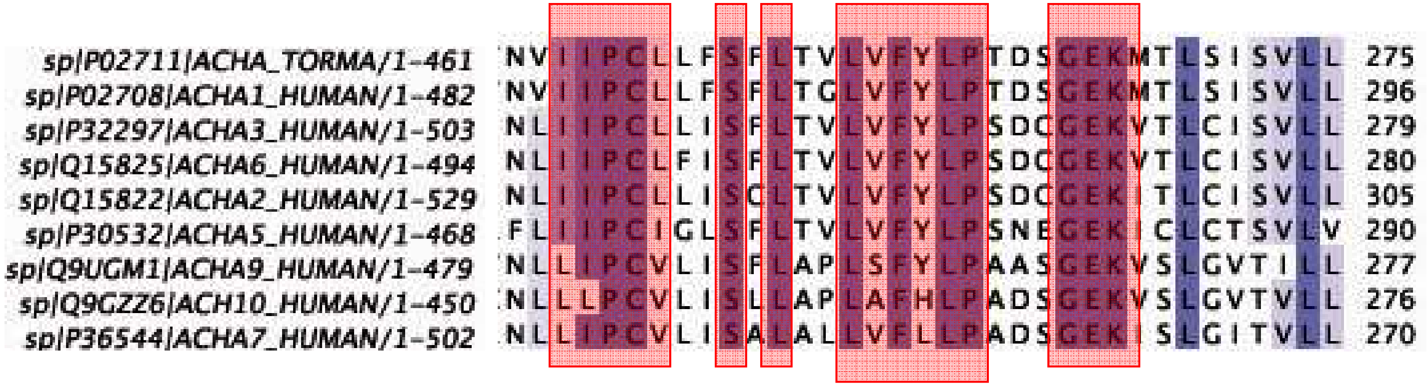
Legend:

- M2
- M3
- Polar
- Hydrophobic



M1-M2 linker region

M1-M2 linker

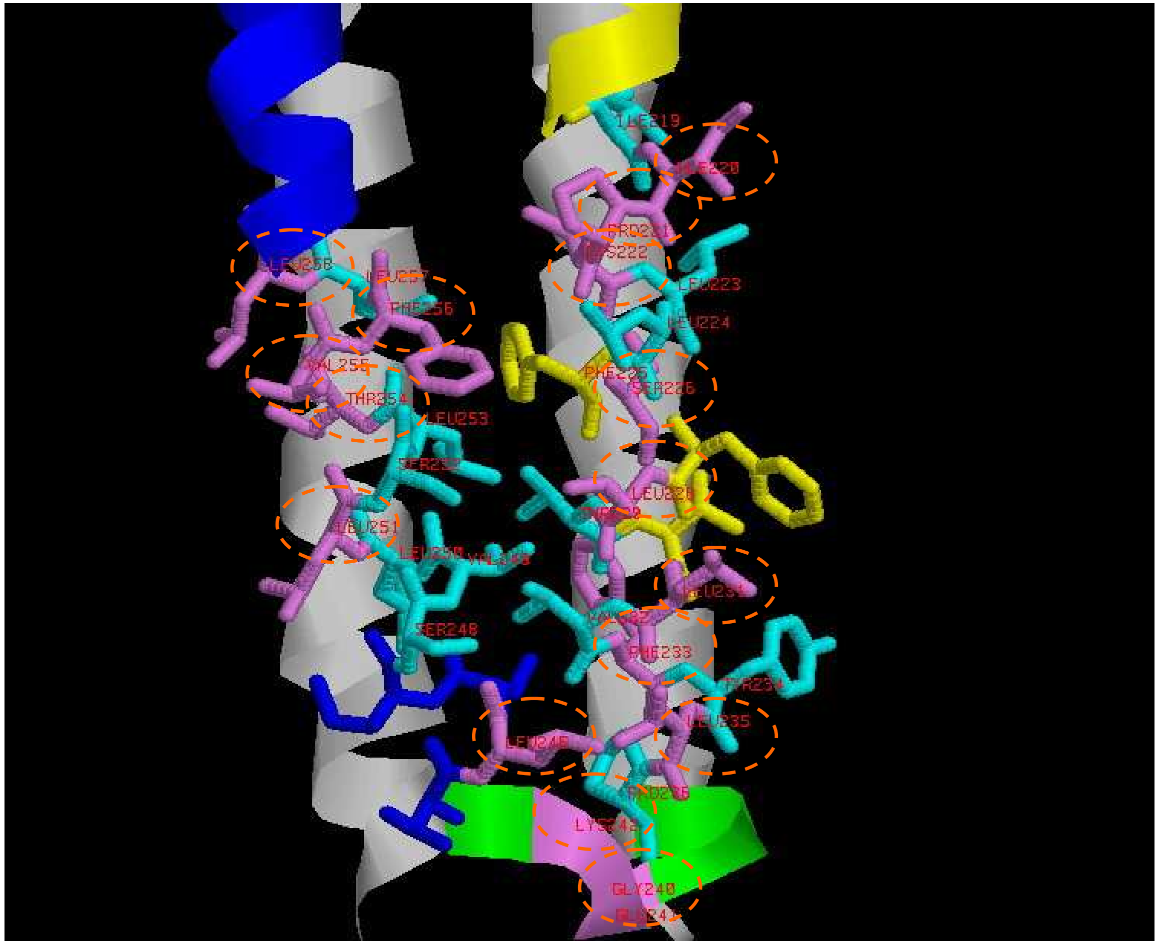


```
sp|P02711|ACHA_TORMA/1-461   NVIIPCLLFSFLTVLVFYLPTDSGEKMTLSISVLL  275
sp|P02708|ACHA1_HUMAN/1-482  NVIIPCLLFSFLTGLVFYLPTDSGEKMTLSISVLL  296
sp|P32297|ACHA3_HUMAN/1-503  NLIIPCLLISFLTVLVFYLPSDCGEKVTLCISVLL  279
sp|Q15825|ACHA6_HUMAN/1-494  NLIIPCLFISFLTVLVFYLPSDCGEKVTLCISVLL  280
sp|Q15822|ACHA2_HUMAN/1-529  NLIIPCLLISCLTVLVFYLPSDCGEKITLCISVLL  305
sp|P30532|ACHA5_HUMAN/1-468  FLIIPCIGLSFLTVLVFYLPSNEGEKICLCTSVLV  290
sp|Q9UGM1|ACHA9_HUMAN/1-479  NLLIPCVLISFLAPLSFYLPAASGEKVSLGVTILL  277
sp|Q9GZZ6|ACH10_HUMAN/1-450  NLLLPCVLISLLAPLAFHLPADSGEKVSLGVTVLL  276
sp|P36544|ACHA7_HUMAN/1-502  NLLIPCVLISALALLVFLLPADSGEKISLGITVLL  270
```

M1-M2 linker region

Legend:

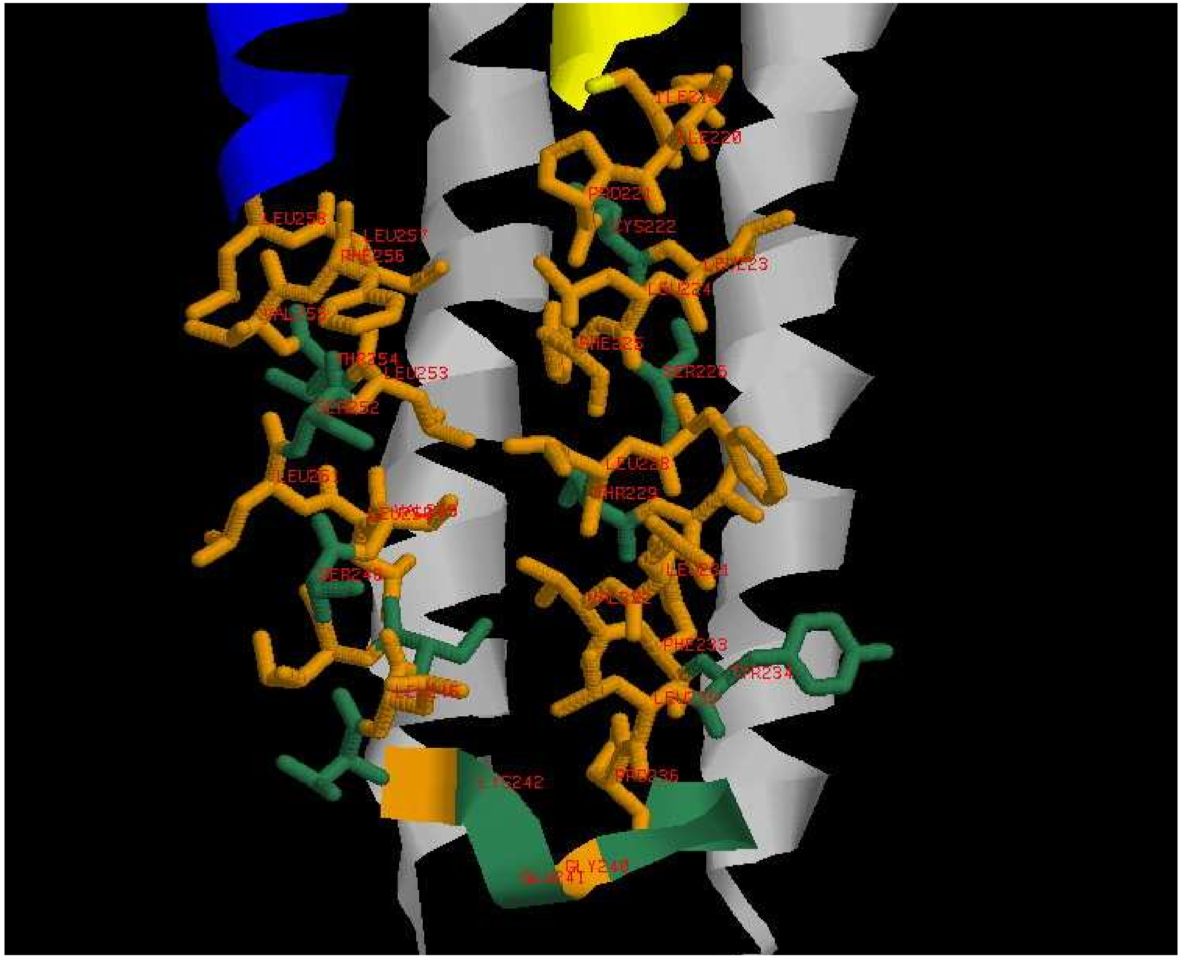
- M1
- M2
- Linker
- Fully conserved
- 70% Identity



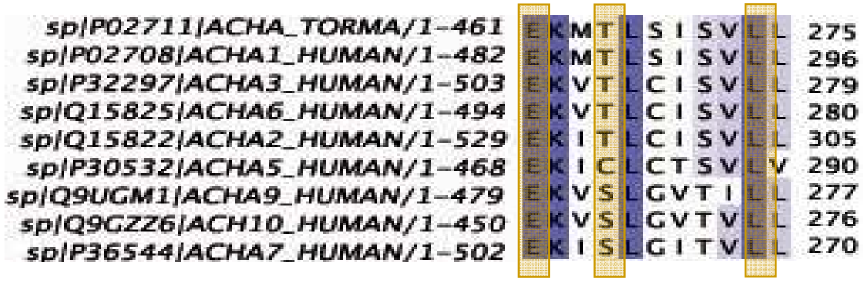
M1-M2 linker region

Legend:

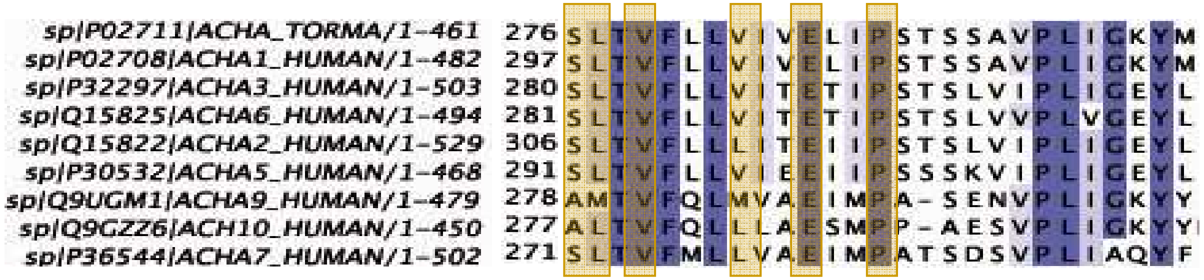
- M1
- M2
- Linker
- Polar
- Hydrophobic



THE ION CONDUCTION PATH



```
sp|P02711|ACHA_TORMA/1-461   EKMTLSISVLL 275
sp|P02708|ACHA1_HUMAN/1-482  EKMTLSISVLL 296
sp|P32297|ACHA3_HUMAN/1-503  EKVTLCISVLL 279
sp|Q15825|ACHA6_HUMAN/1-494  EKVTLCISVLL 280
sp|Q15822|ACHA2_HUMAN/1-529  EKITLCISVLL 305
sp|P30532|ACHA5_HUMAN/1-468  EKICLCTSVLV 290
sp|Q9UGM1|ACHA9_HUMAN/1-479  EKVSLGVTILL 277
sp|Q9GZZ6|ACH10_HUMAN/1-450  EKVSLGVTVLL 276
sp|P36544|ACHA7_HUMAN/1-502  EKISLGITVLL 270
```



```
sp|P02711|ACHA_TORMA/1-461   276 SLTVFLLVIVELIPSTSSAVPLIGKYM
sp|P02708|ACHA1_HUMAN/1-482  297 SLTVFLLVIVELIPSTSSAVPLIGKYM
sp|P32297|ACHA3_HUMAN/1-503  280 SLTVFLLVITETIPSTSLVIPLIGEYL
sp|Q15825|ACHA6_HUMAN/1-494  281 SLTVFLLVITETIPSTSLVVPLVGEYL
sp|Q15822|ACHA2_HUMAN/1-529  306 SLTVFLLLITEIIPSTSLVIPLIGEYL
sp|P30532|ACHA5_HUMAN/1-468  291 SLTVFLLVIEEIIPSSSKVIPLIGEYL
sp|Q9UGM1|ACHA9_HUMAN/1-479  278 AMTVFQLMVAEIMPA-SENVPLIGKYY
sp|Q9GZZ6|ACH10_HUMAN/1-450  277 ALTVFQLLLAESMPP-AESVPLIGKYY
sp|P36544|ACHA7_HUMAN/1-502  271 SLTVFMLLVAEIMPATSDSVPLIAQYF
```

Ion conduction path

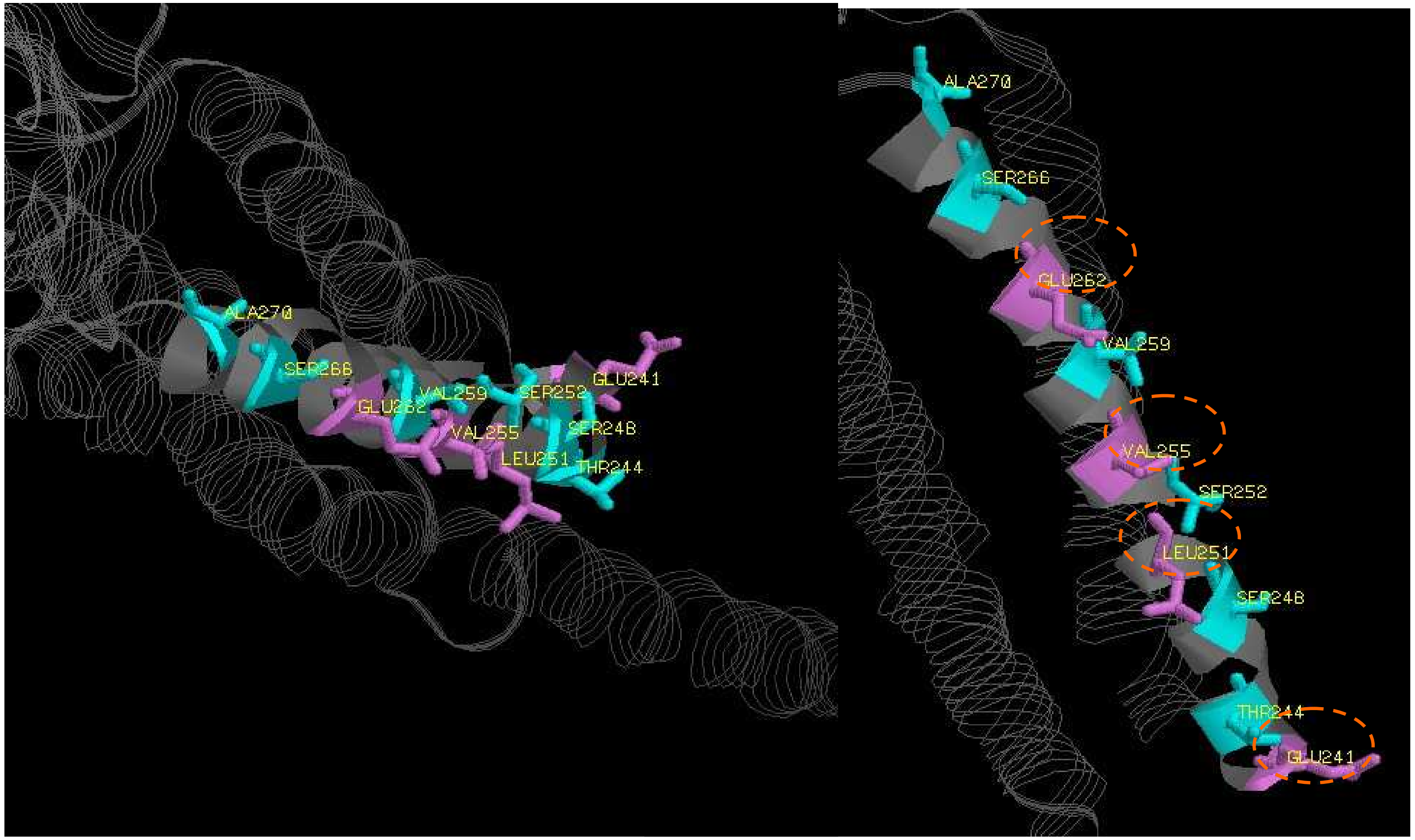
THE ION CONDUCTION PATH

Legend:

M2

Fully conserved

70% Identity



THE ION CONDUCTION PATH

AA facing the lumen of the pore are mostly non-polar. BUT

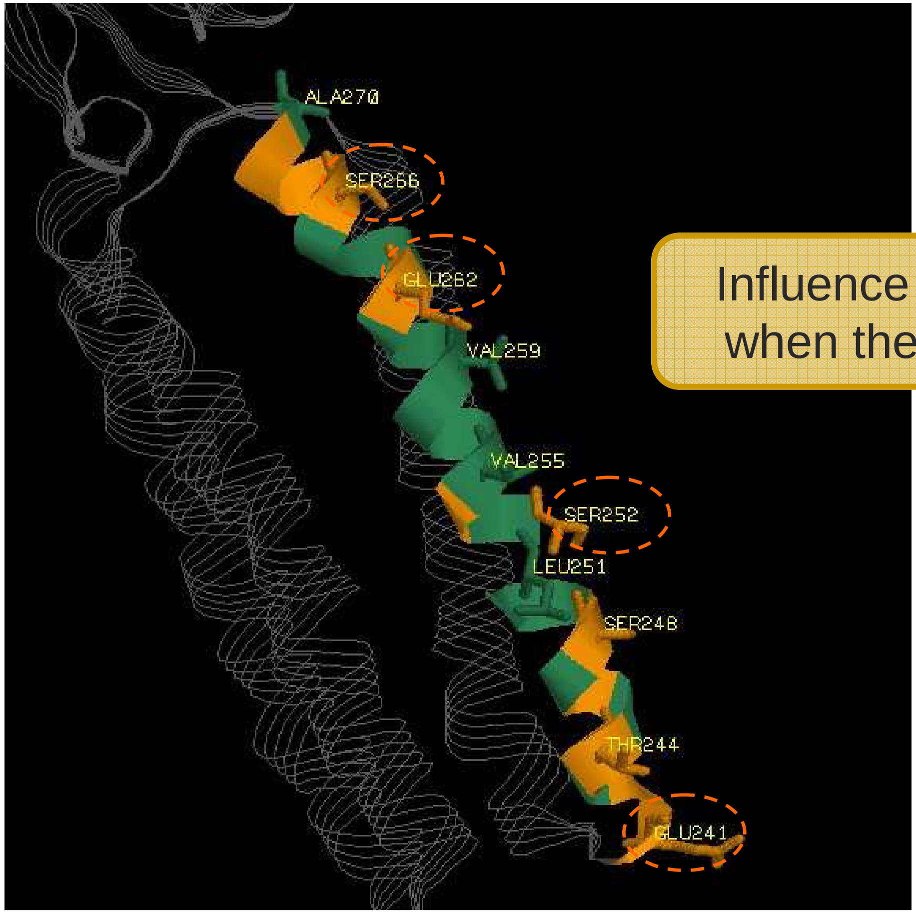
Ser-266, Glu-262, Ser-252 & Glu-242 are polar

Legend:

M2

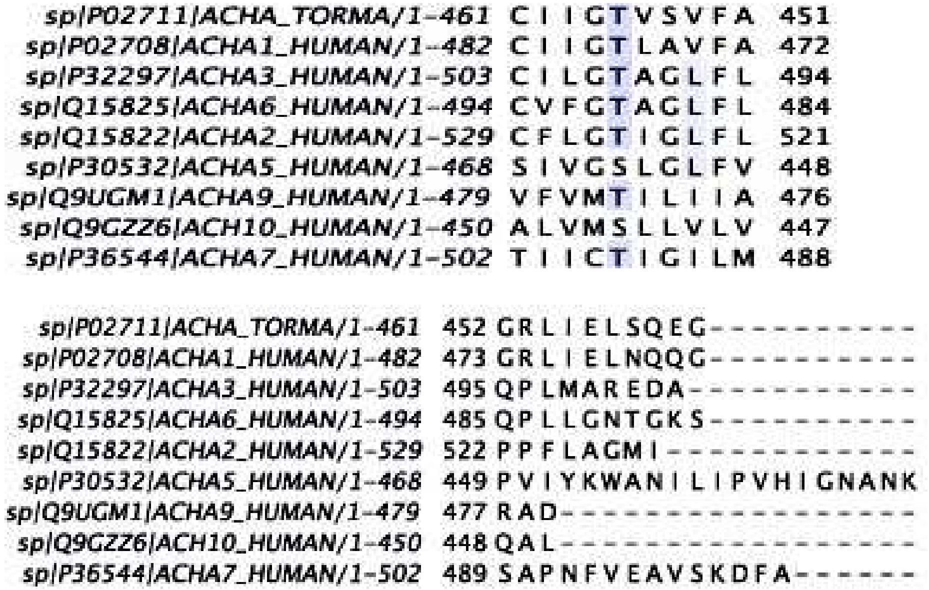
Polar

Hydrophobic



Influence the transport when the pore is open

M4



```
sp|P02711|ACHA_TORMA/1-461  C I I G T V S V F A  451
sp|P02708|ACHA1_HUMAN/1-482 C I I G T L A V F A  472
sp|P32297|ACHA3_HUMAN/1-503 C I L G T A G L F L  494
sp|Q15825|ACHA6_HUMAN/1-494 C V F G T A G L F L  484
sp|Q15822|ACHA2_HUMAN/1-529 C F L G T I G L F L  521
sp|P30532|ACHA5_HUMAN/1-468 S I V G S L G L F V  448
sp|Q9UGM1|ACHA9_HUMAN/1-479 V F V M T I L I I A  476
sp|Q9GZZ6|ACH10_HUMAN/1-450 A L V M S L L V L V  447
sp|P36544|ACHA7_HUMAN/1-502 T I I C T I G I L M  488

sp|P02711|ACHA_TORMA/1-461  452 G R L I E L S Q E G----------
sp|P02708|ACHA1_HUMAN/1-482 473 G R L I E L N Q Q G----------
sp|P32297|ACHA3_HUMAN/1-503 495 Q P L M A R E D A-----------
sp|Q15825|ACHA6_HUMAN/1-494 485 Q P L L G N T G K S----------
sp|Q15822|ACHA2_HUMAN/1-529 522 P P F L A G M I------------
sp|P30532|ACHA5_HUMAN/1-468 449 P V I Y K W A N I L I P V H I G N A N K
sp|Q9UGM1|ACHA9_HUMAN/1-479 477 R A D-----------------
sp|Q9GZZ6|ACH10_HUMAN/1-450 448 Q A L-----------------
sp|P36544|ACHA7_HUMAN/1-502 489 S A P N F V E A V S K D F A------
```

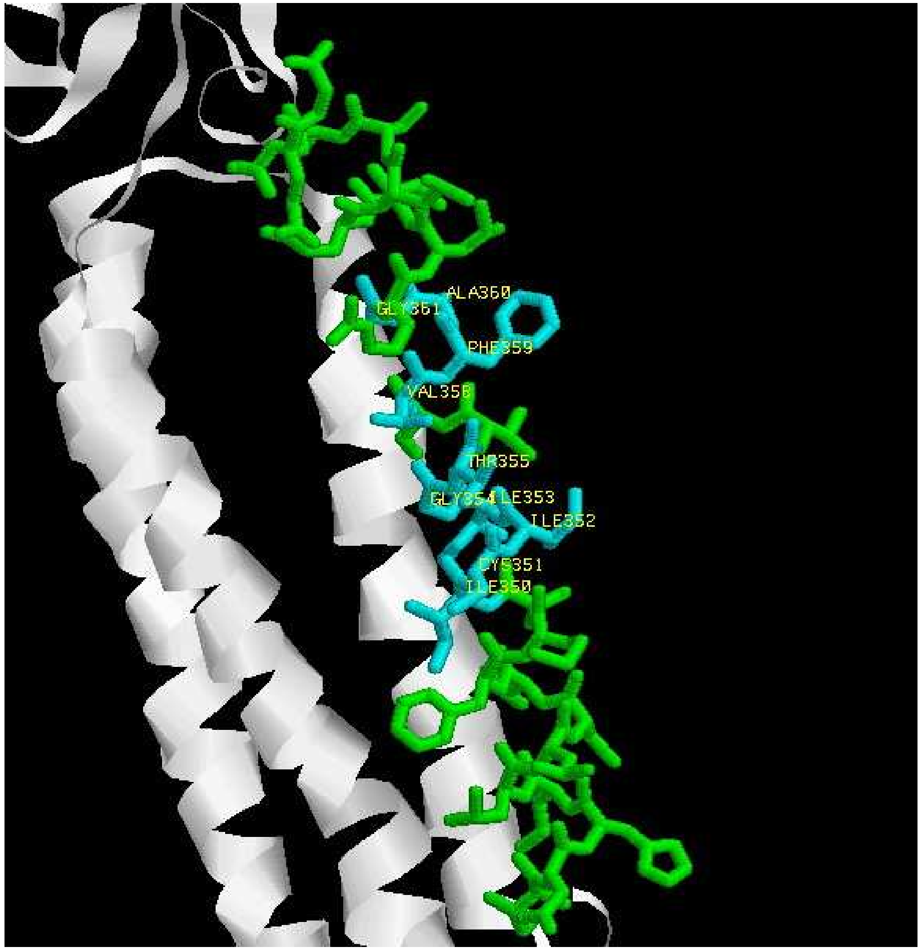
M4

M4

Legend:

🟩 M4

🟦 70% Identity



M4-M5 linker

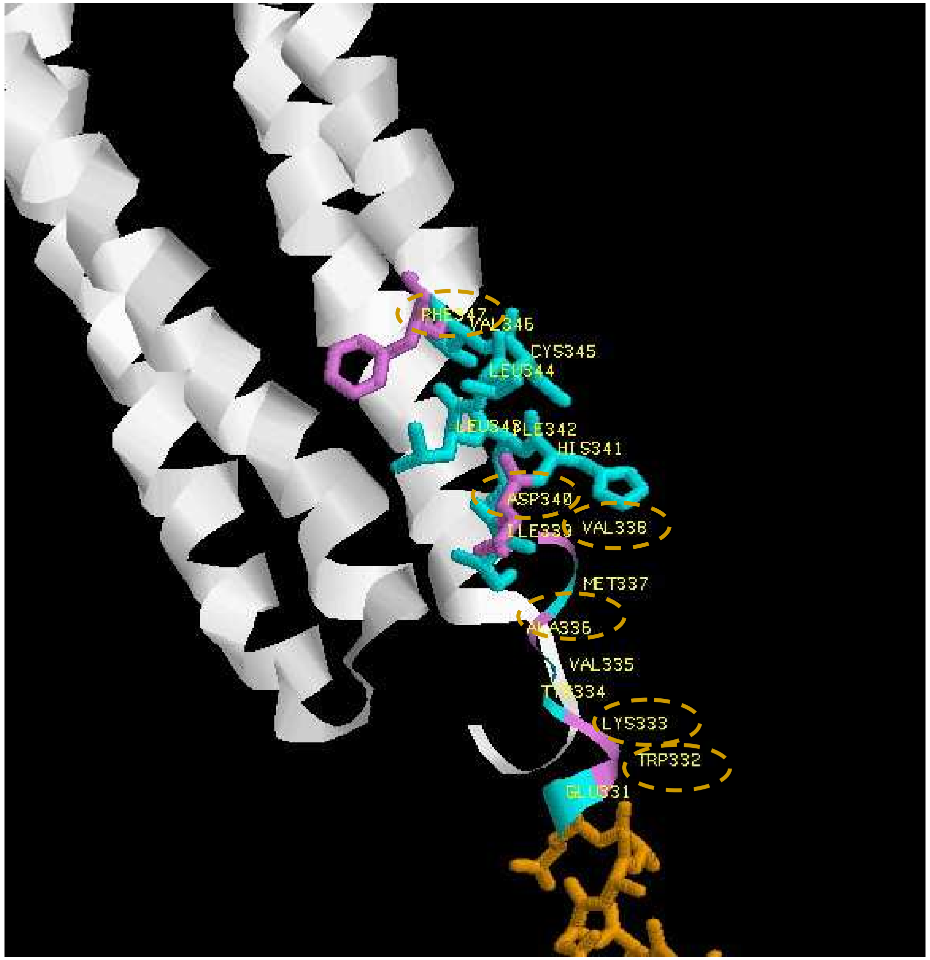
Legend: M5 M4-M5 linker

```
sp|P02711|ACHA_TORMA/1-461   EGVKYIAEHMKSDEESSNAAEEWKYVAMVIDHILLCVFMLICII
sp|P02708|ACHA1_HUMAN/1-482  EGIKYIAETMKSDQESNNAAAEWKYVAMVMDHILLGVFMLVCII
sp|P32297|ACHA3_HUMAN/1-503  QSVKYIAENMKAQNEAKEIQDDWKYVAMVIDRIFLWVFTLVCIL
sp|Q15825|ACHA6_HUMAN/1-494  NSVQFIAENMKSHNETKEVEDDWKYVAMVVDRVFLWVFIIVCVF
sp|Q15822|ACHA2_HUMAN/1-529  EGVHYIADHLRSEDADSSVKEDWKYVAMVIDRIFLWLFIIVCFL
sp|P30532|ACHA5_HUMAN/1-468  DSIRYITRHIMKENDVREVVEDWKFIAQVLDRMFLWTFLFVSIV
sp|Q9UGM1|ACHA9_HUMAN/1-479  RNIEYIAKCLKDHKATSSKGSEWKKVAKVIDRFFMWIFFIMVFV
sp|Q9GZZ6|ACH10_HUMAN/1-450  HHVATIANTFRSHRAAQRCHEDWKRLARVMDRFFLAIFFSMALV
sp|P36544|ACHA7_HUMAN/1-502  EEVRYIANRFRCQDESEAVCSEWKFAACVVDRLCLMAFSVFTII
```

M4-M5 linker

Legend:

- M5
- 70% Identity
- Fully conserved



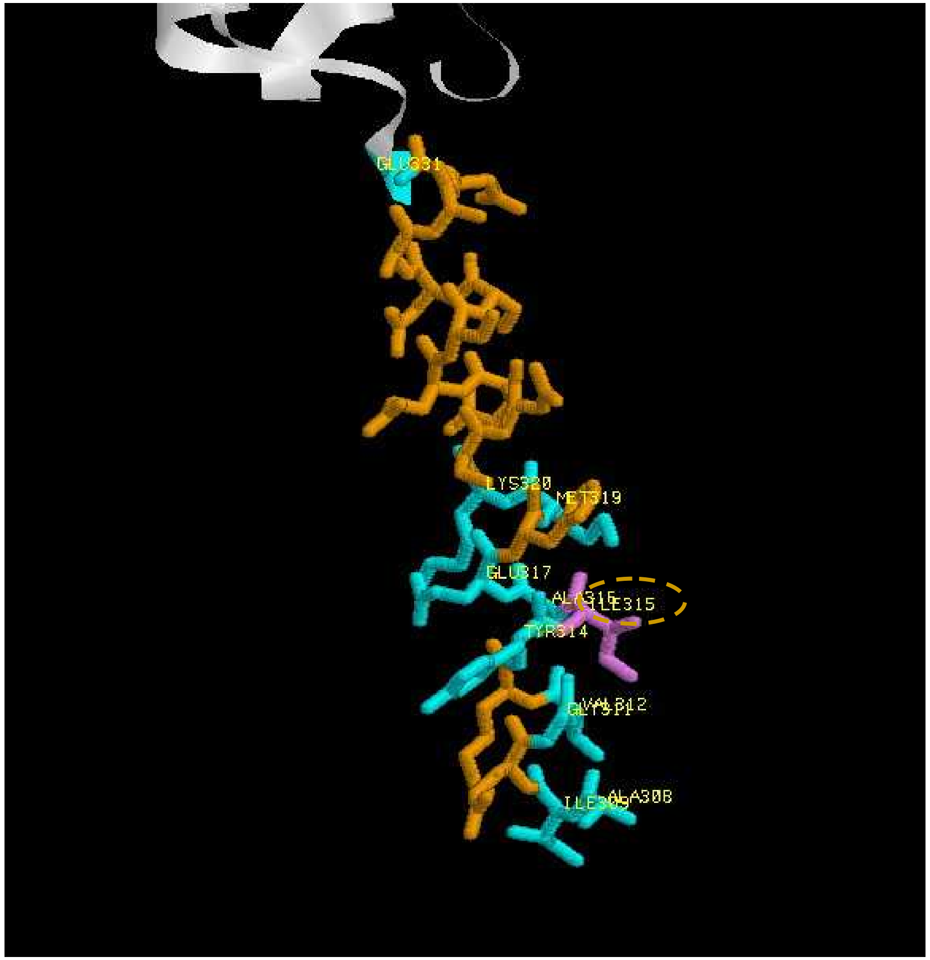
M5

Legend:

M5

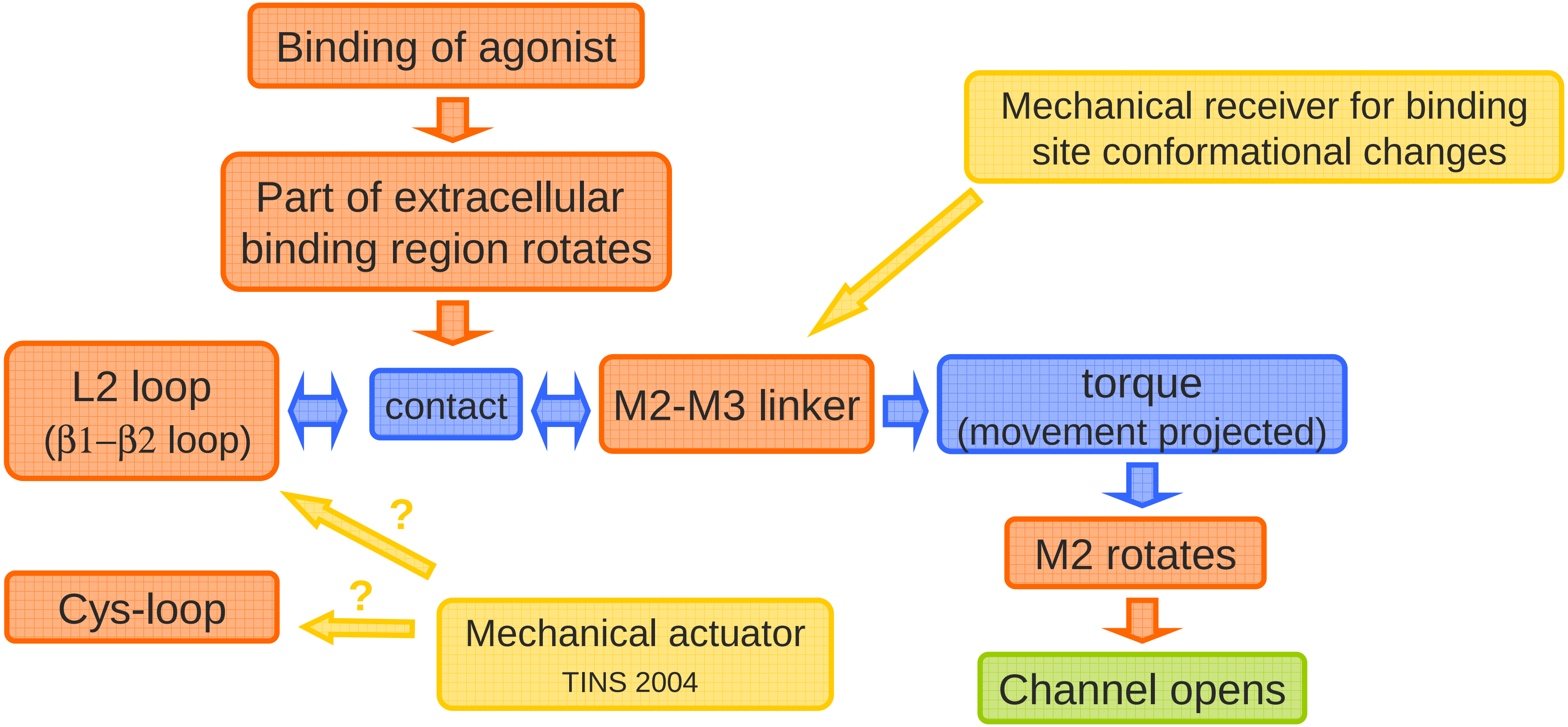
70% Identity

Fully conserved



ACTIVATION MECHANISM

LBD – TMD link



Binding of agonist

Part of extracellular binding region rotates

Mechanical receiver for binding site conformational changes

L2 loop (β1–β2 loop)

contact

M2-M3 linker

torque (movement projected)

M2 rotates

Channel opens

Cys-loop

?

?

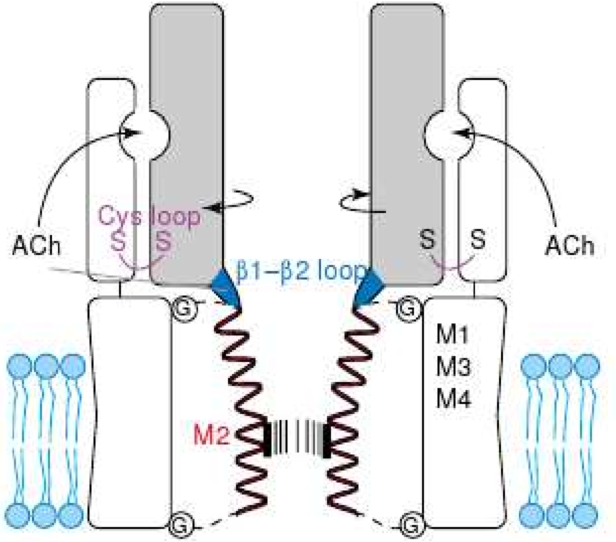
Mechanical actuator

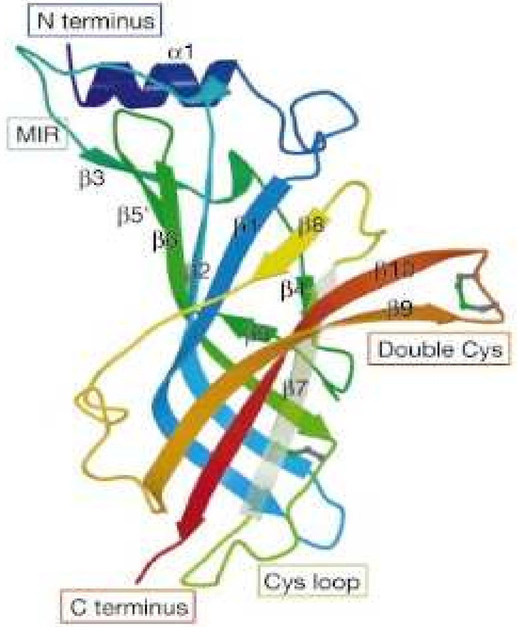
TINS 2004

ACTIVATION MECHANISM

Direct rotation of the subunit · through a pivoting point at the ligand-binding site

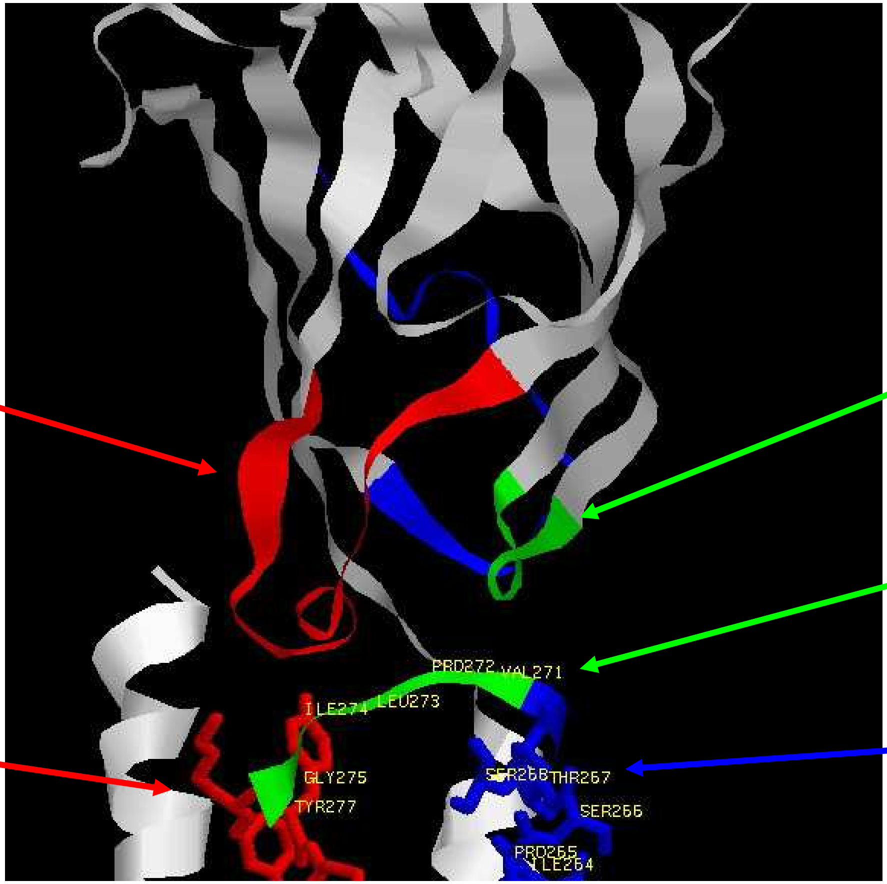
- Hypothesis: agonist binding induces a contraction of the ‘aromatic box’ and the inner β-sheet, consisting of β-strands 1, 2, 3, 5, 6 and 8, to undergo a rotation of 15 degrees.





ACTIVATION MECHANISM

LBD – TMD link



Cys-loop

L2 loop

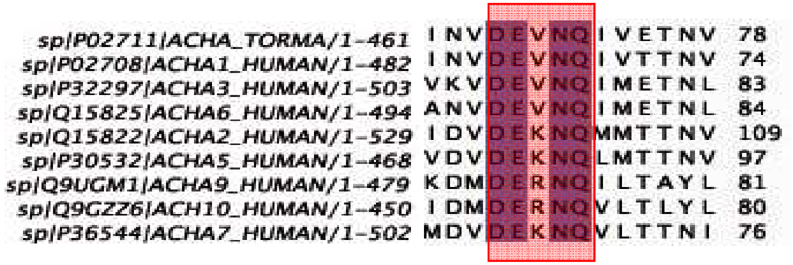
M2-M3 Linker

M3

M2

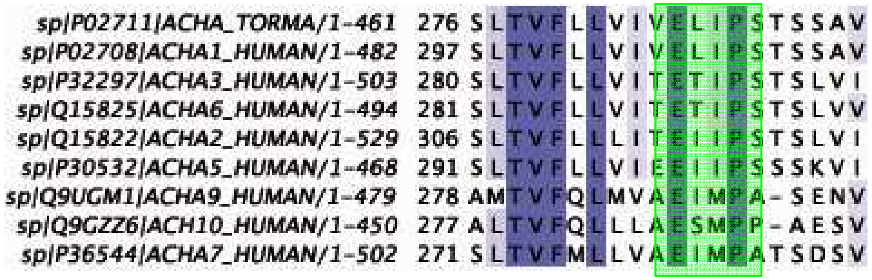
ACTIVATION MECHANISM

L2 – M2/M3 linker contact



```
sp|P02711|ACHA_TORMA/1-461   INVDEVNQIVETNV  78
sp|P02708|ACHA1_HUMAN/1-482  INVDEVNQIVTTNV  74
sp|P32297|ACHA3_HUMAN/1-503  VKVDEVNQIMETNL  83
sp|Q15825|ACHA6_HUMAN/1-494  ANVDEVNQIMETNL  84
sp|Q15822|ACHA2_HUMAN/1-529  IDVDEKNQMMTTNV  109
sp|P30532|ACHA5_HUMAN/1-468  VDVDEKNQLMTTNV  97
sp|Q9UGM1|ACHA9_HUMAN/1-479  KDMDERNQILTAYL  81
sp|Q9GZZ6|ACH10_HUMAN/1-450  IDMDERNQVLTLYL  80
sp|P36544|ACHA7_HUMAN/1-502  MDVDEKNQVLTTNI  76
```

L2 loop



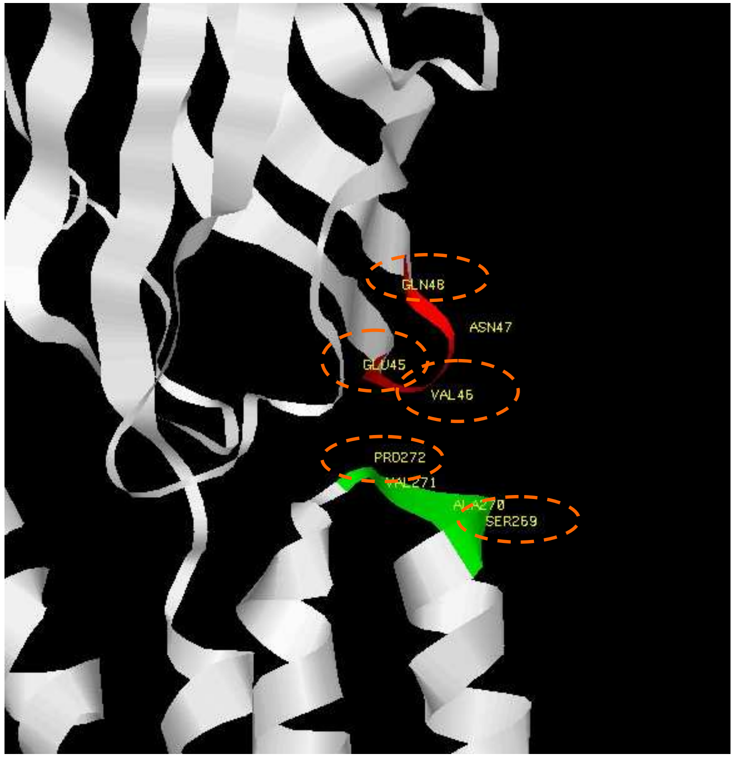
```
sp|P02711|ACHA_TORMA/1-461   276 SLTVFLLVIVELIPSTSSAV
sp|P02708|ACHA1_HUMAN/1-482  297 SLTVFLLVIVELIPSTSSAV
sp|P32297|ACHA3_HUMAN/1-503  280 SLTVFLLVITETIPSTSLVI
sp|Q15825|ACHA6_HUMAN/1-494  281 SLTVFLLVITETIPSTSLVV
sp|Q15822|ACHA2_HUMAN/1-529  306 SLTVFLLLITEIIPSTSLVI
sp|P30532|ACHA5_HUMAN/1-468  291 SLTVFLLVIEEIIPSSSKVI
sp|Q9UGM1|ACHA9_HUMAN/1-479  278 AMTVFQLMVAEIMPA-SENV
sp|Q9GZZ6|ACH10_HUMAN/1-450  277 ALTVFQLLLAESMPP-AESV
sp|P36544|ACHA7_HUMAN/1-502  271 SLTVFMLLVAEIMPATSDSV
```

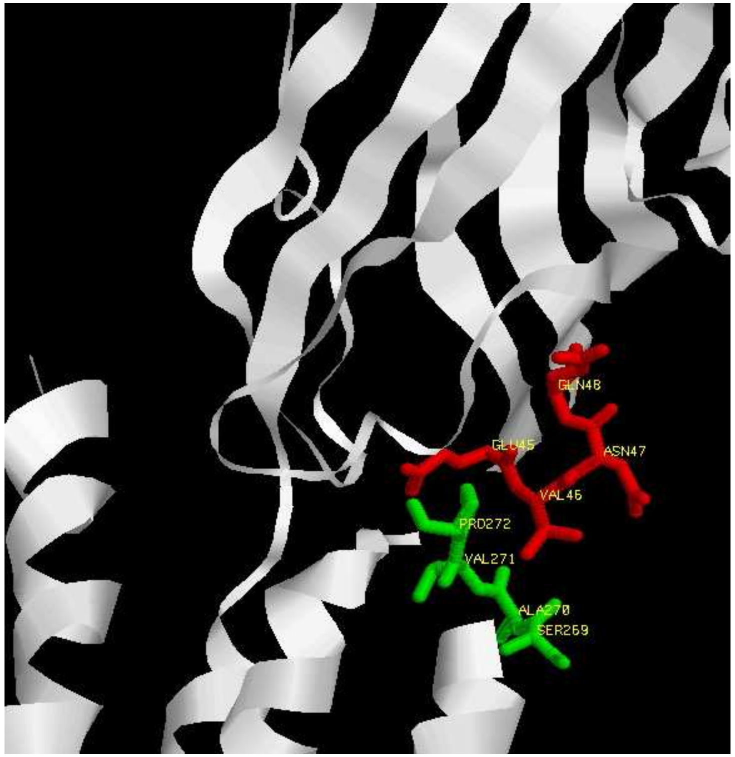
M2/M3Linker

ACTIVATION MECHANISM

L2 – M2/M3 linker contact

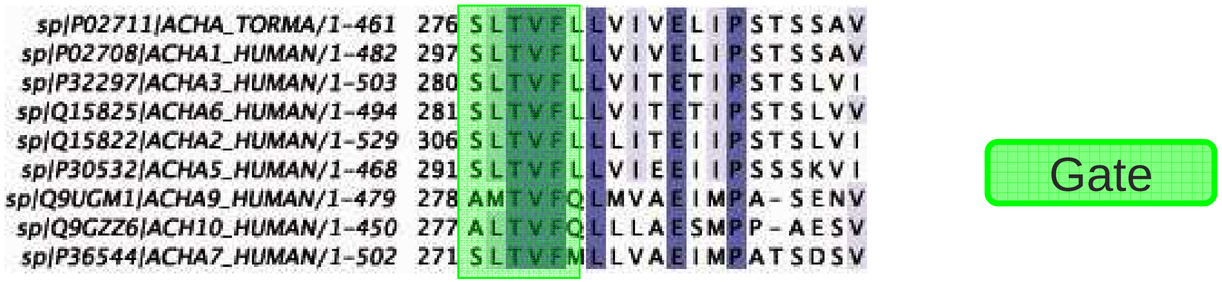
Legend: L2 M2/M3Linker





ACTIVATION MECHANISM

The Gate

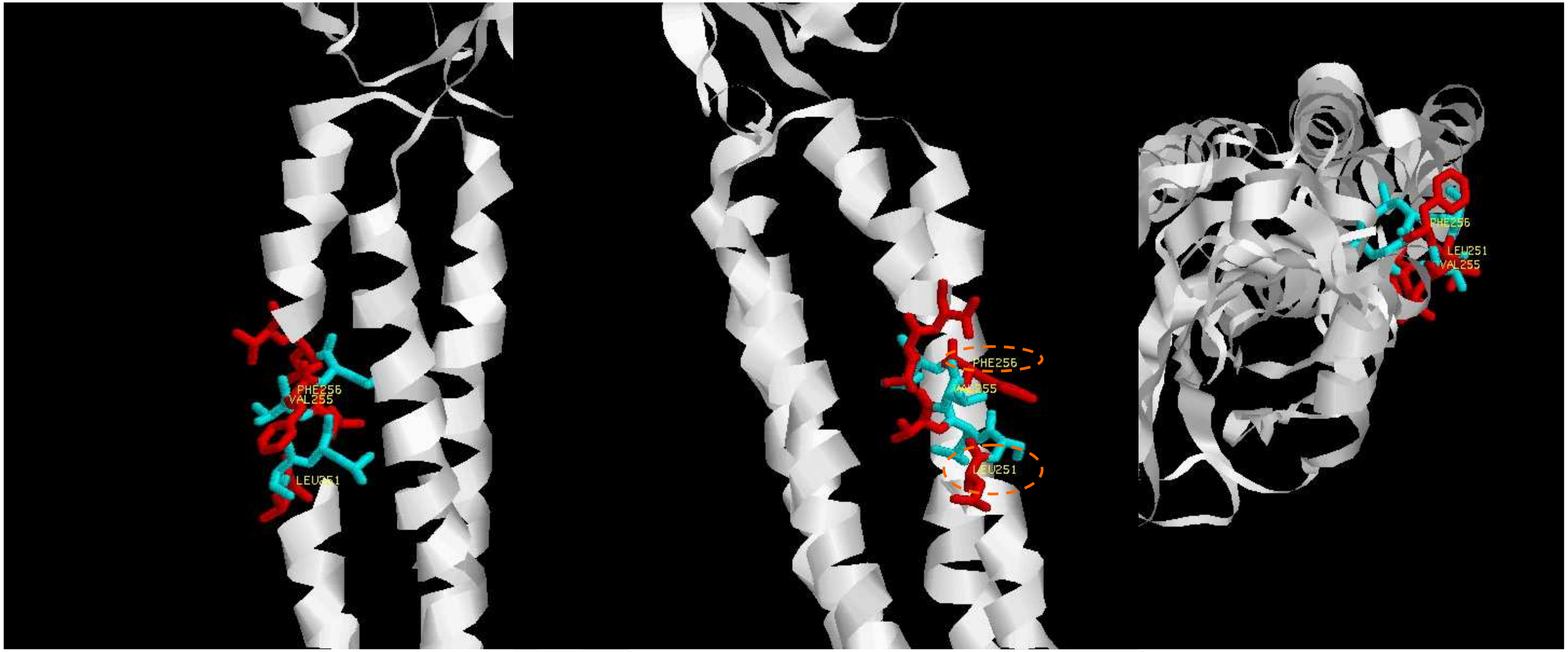


```
sp|P02711|ACHA_TORMA/1-461   276 SLTVFLLVIVELIPSTSSAV
sp|P02708|ACHA1_HUMAN/1-482  297 SLTVFLLVIVELIPSTSSAV
sp|P32297|ACHA3_HUMAN/1-503  280 SLTVFLLVITETIPSTSLVI
sp|Q15825|ACHA6_HUMAN/1-494  281 SLTVFLLVITETIPSTSLVV
sp|Q15822|ACHA2_HUMAN/1-529  306 SLTVFLLLITEIIPSTSLVI
sp|P30532|ACHA5_HUMAN/1-468  291 SLTVFLLVIEEIIPSSSKVI
sp|Q9UGM1|ACHA9_HUMAN/1-479  278 AMTVFQLMVAEIMPA-SENV
sp|Q9GZZ6|ACH10_HUMAN/1-450  277 ALTVFQLLLAESMPP-AESV
sp|P36544|ACHA7_HUMAN/1-502  271 SLTVFMLLVAEIMPATSDSV
```

ACTIVATION MECHANISM

The Gate

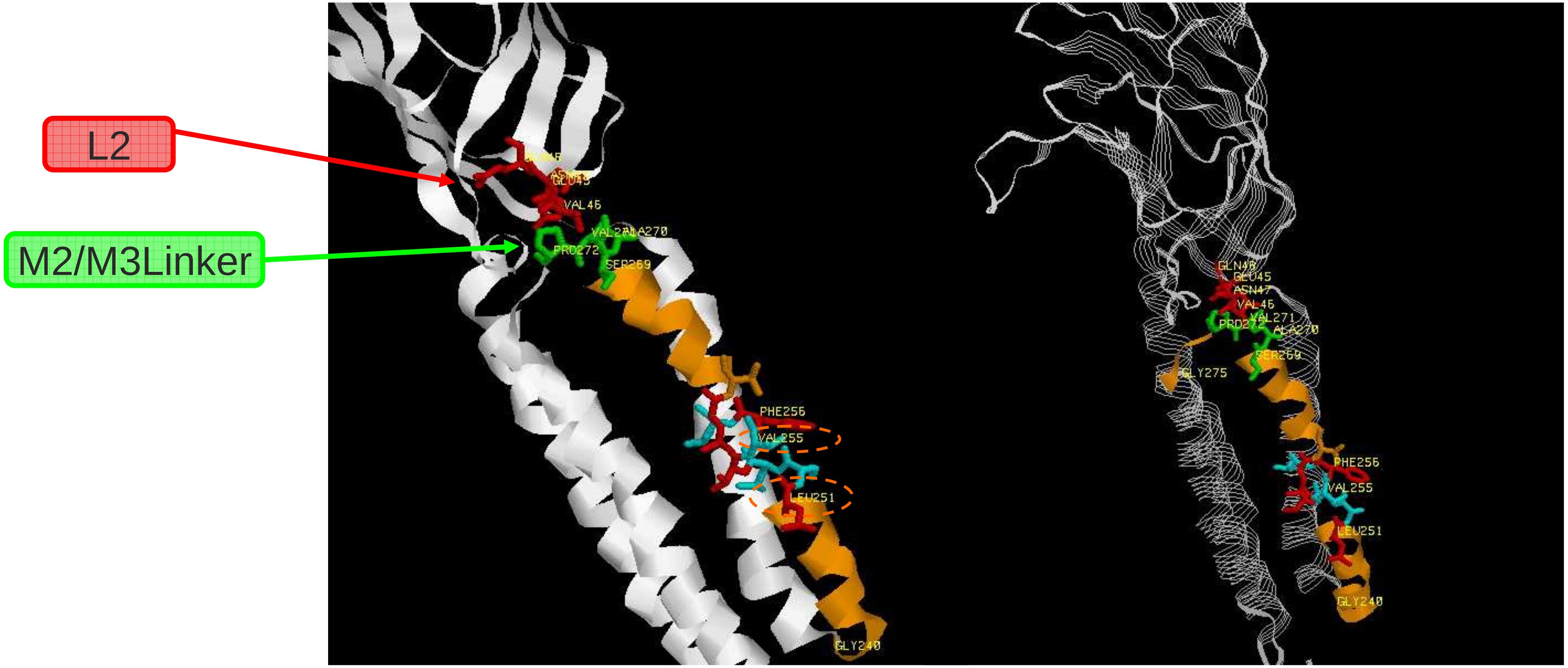
Legend: Fully conserved 70% Identity



ACTIVATION MECHANISM

Full View

Legend: 🟥 Fully conserved 🟦 70% Identity



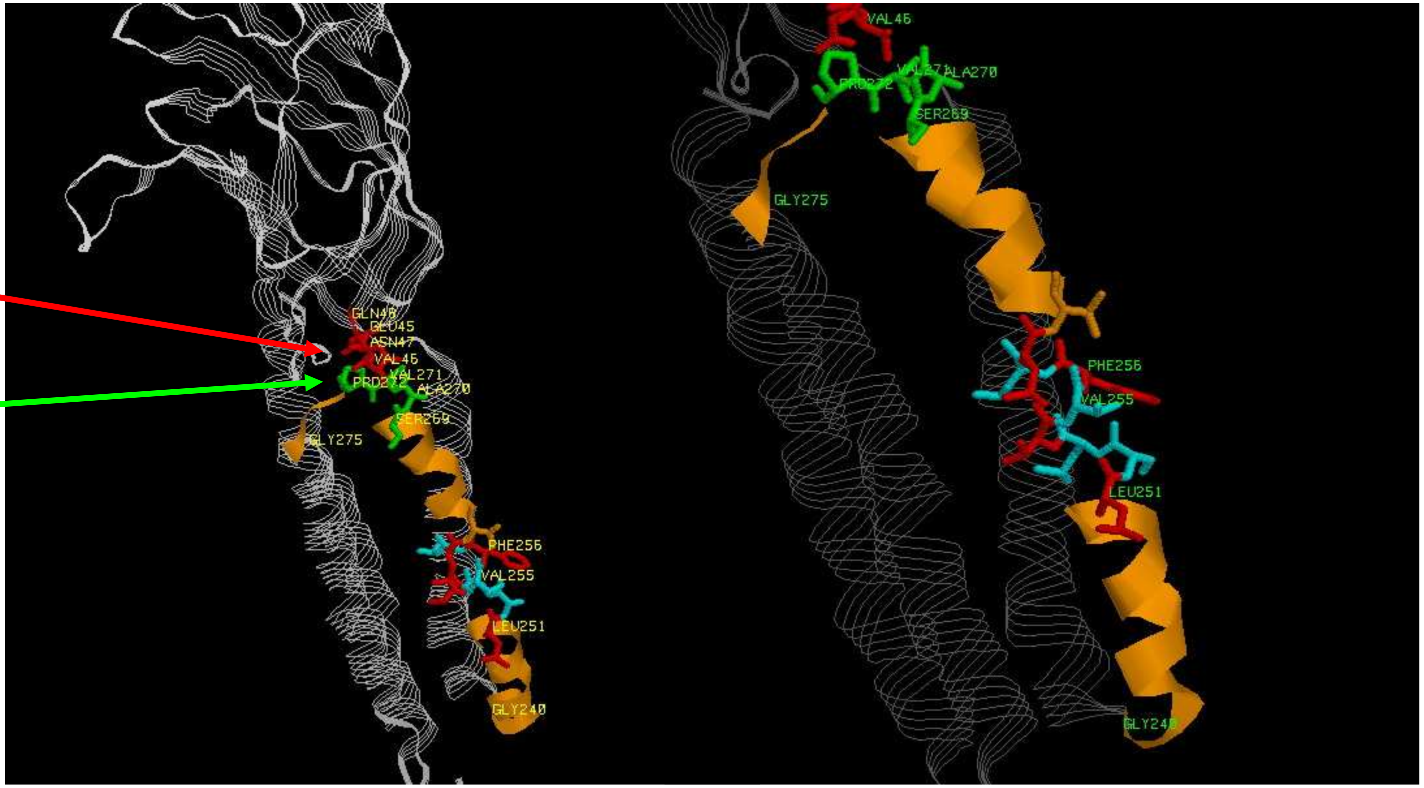
L2

M2/M3Linker

ACTIVATION MECHANISM

Full View

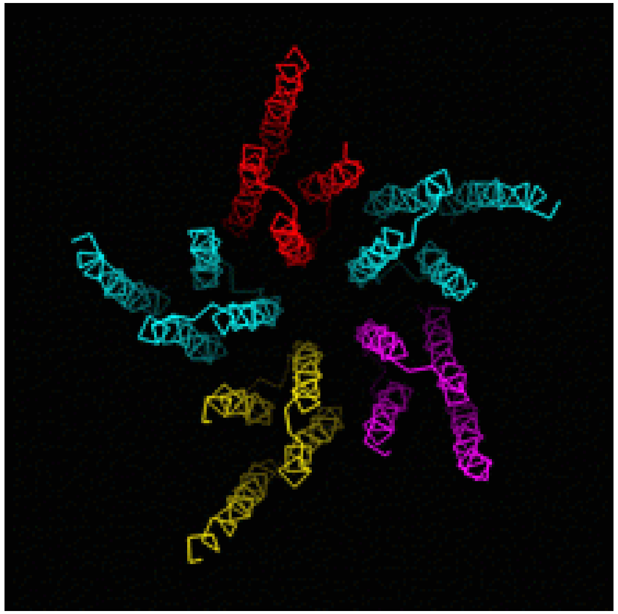
Legend: 🟥 Fully conserved 🟦 70% Identity

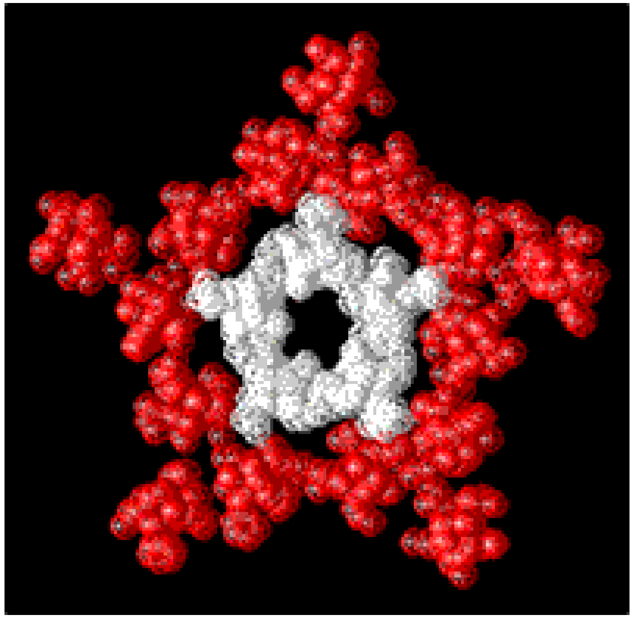


L2

M2/M3Linker

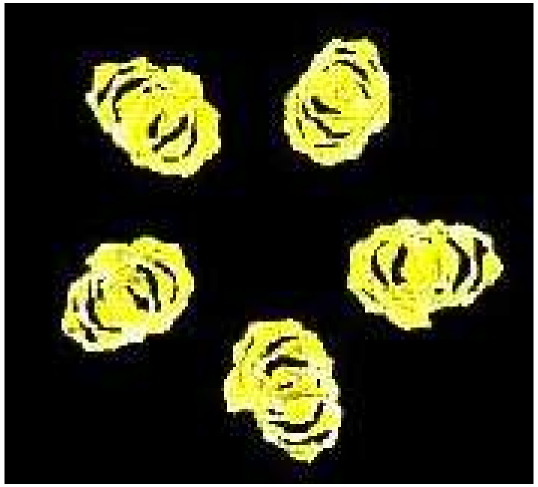
OPEN STATE OF THE CHANNEL





Nigel Unwin Lab – Pictoral Model of channel opening

OPEN STATE OF THE CHANNEL



EM of M2 Helices
(closed)

Model of M2 Helices
(Open)