# nAChRs: from primary to quaternary structures

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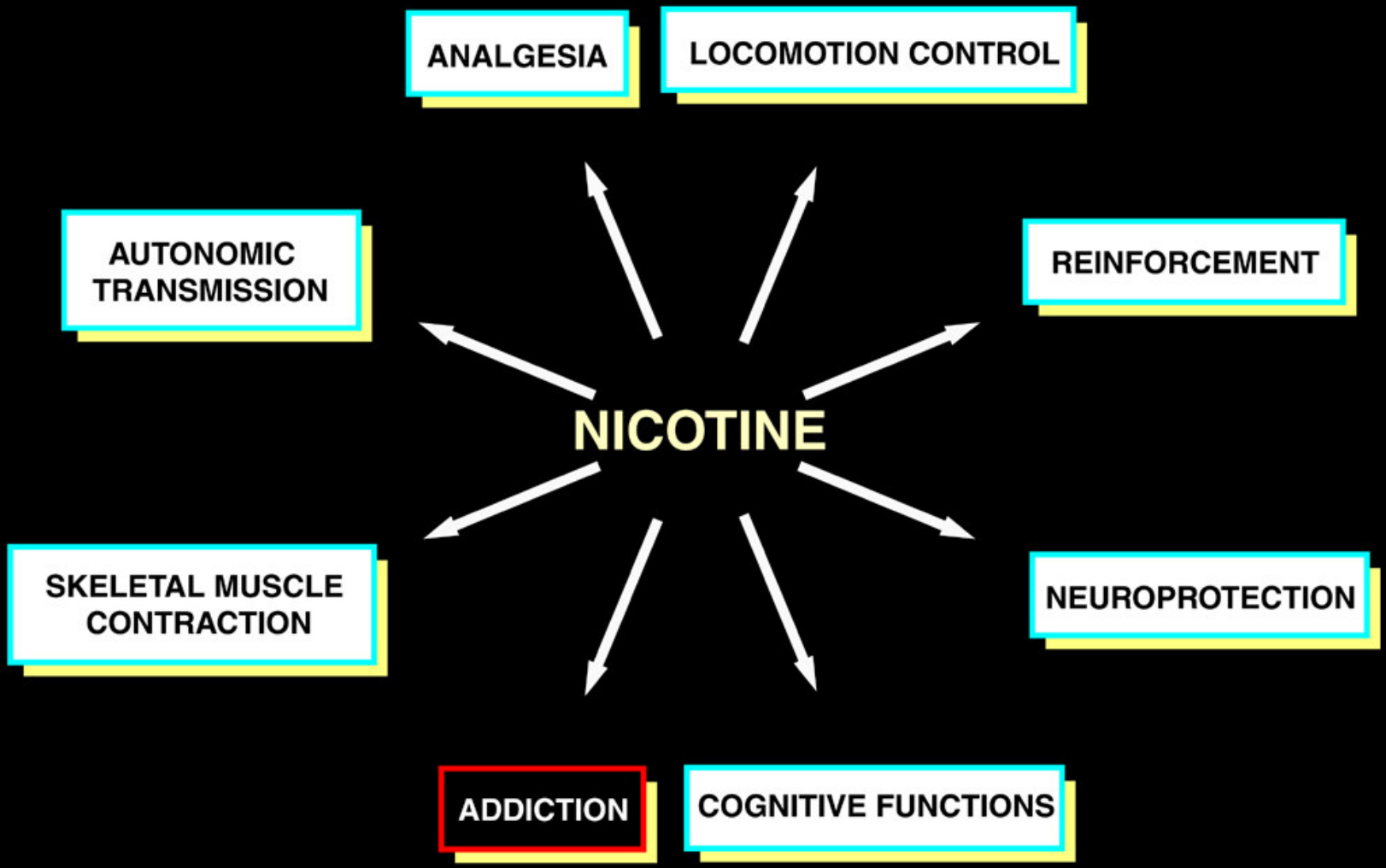
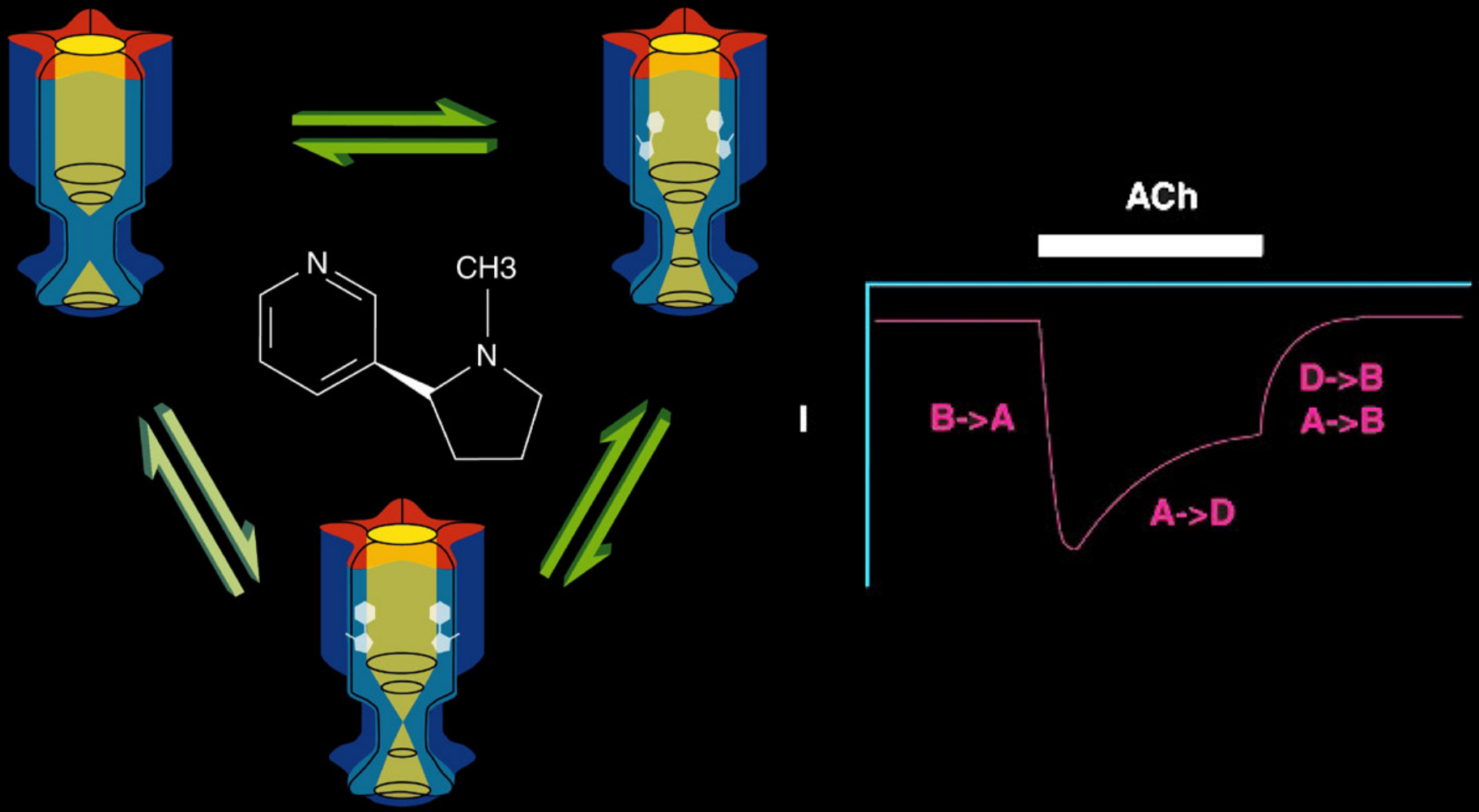
**Institut Pasteur**, Paris

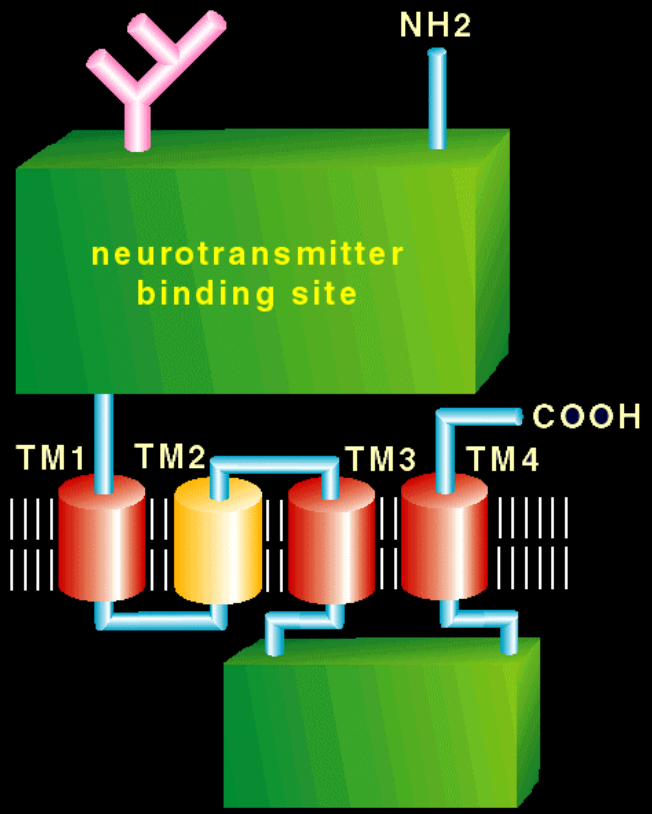
# Pleiotropic effects of nicotine



# An allosteric protein

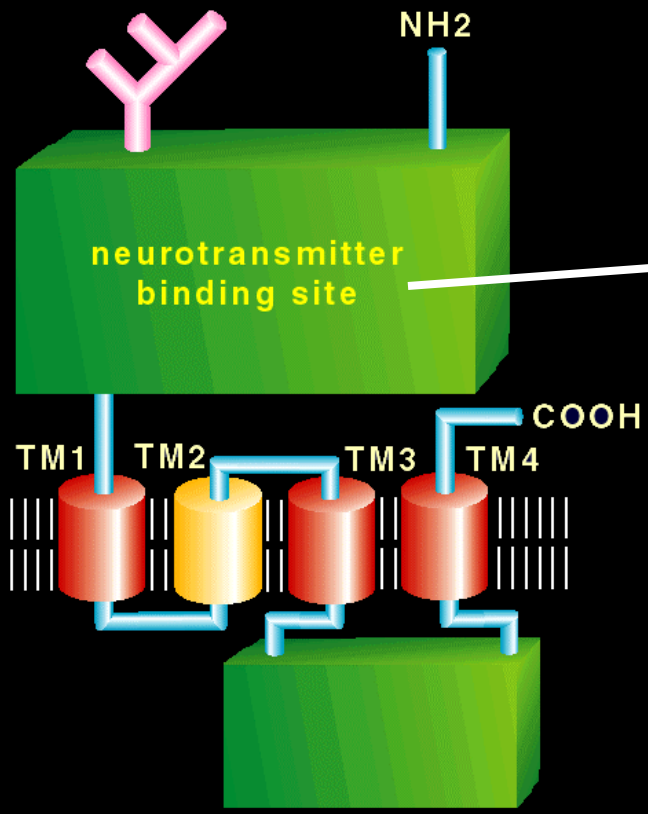


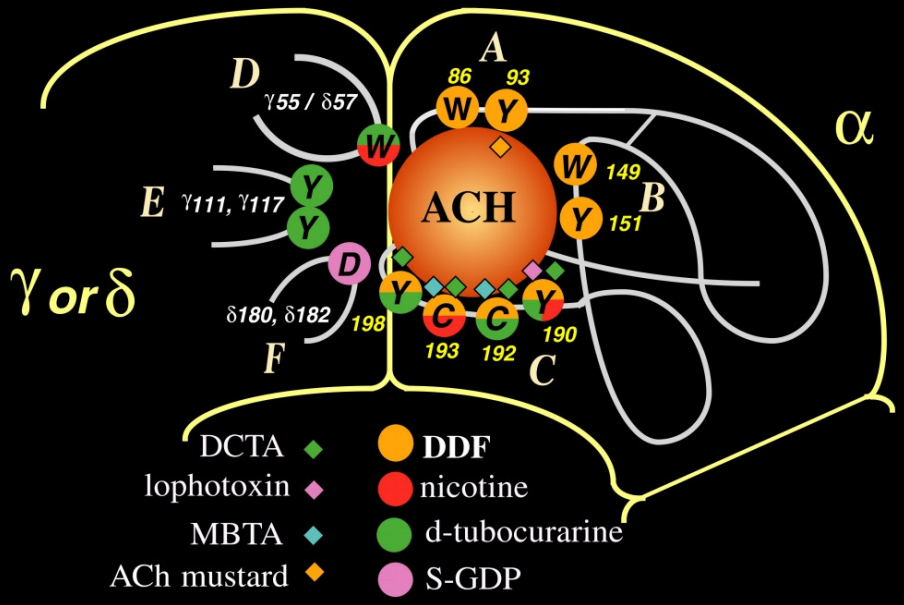
# Indirect structural evidences




Review in **CORRINGER** *et al.* (2000)
Annu. Rev. Pharm. Tox.

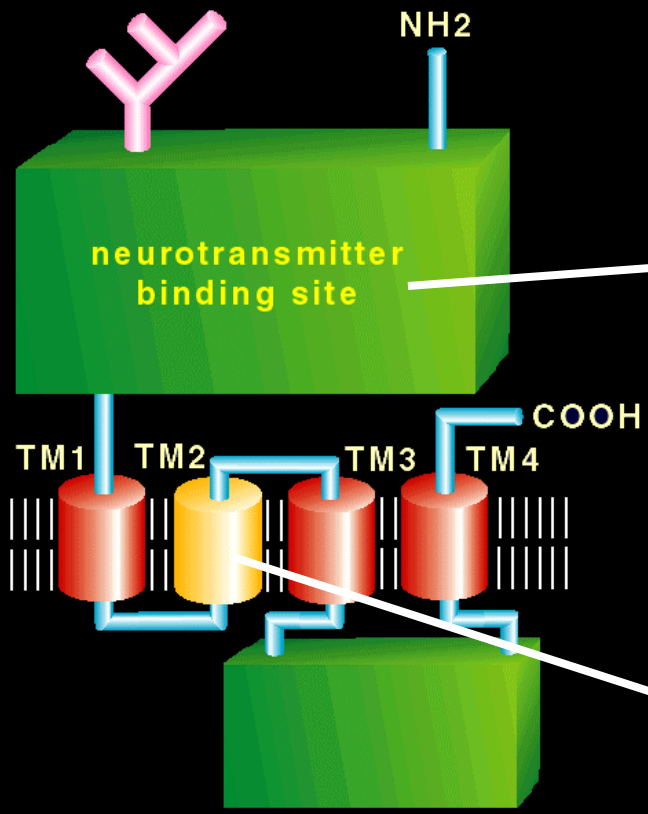
# Indirect structural evidences

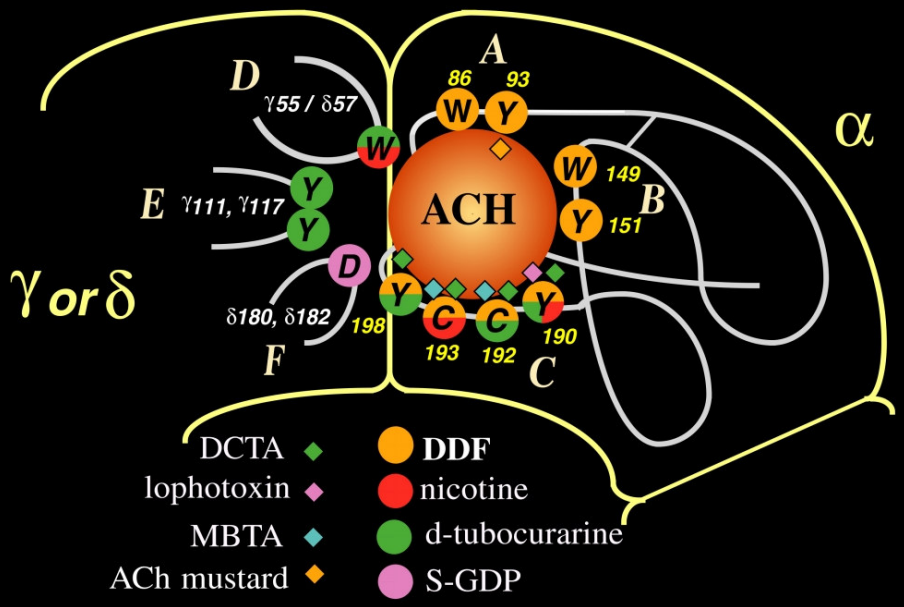


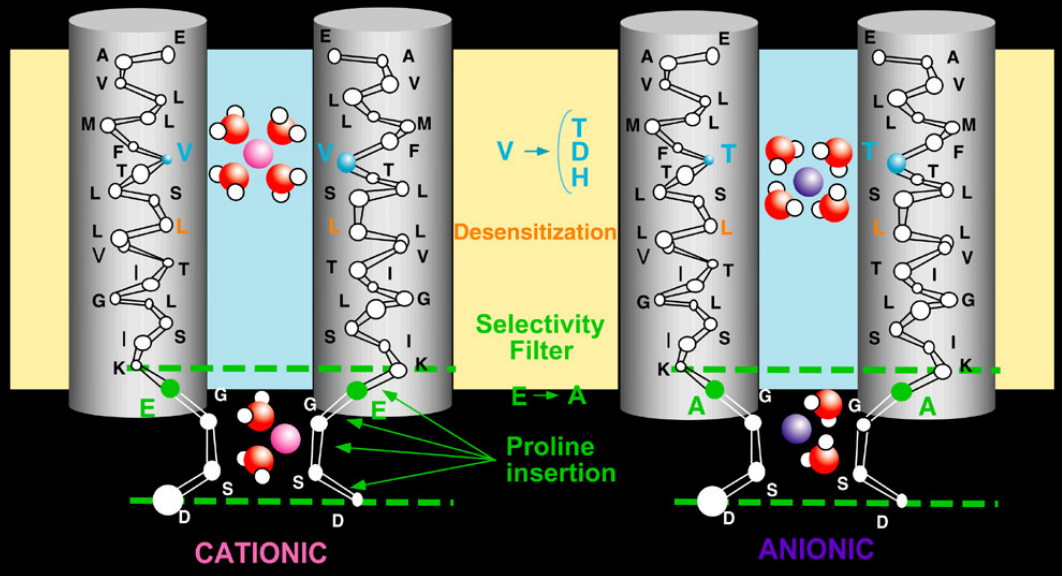




Review in **CORRINGER** *et al.* (2000)
Annu. Rev. Pharm. Tox.

# Indirect structural evidences




Review in **CORRINGER** *et al.* (2000)
Annu. Rev. Pharm. Tox.

# nAChRS subunits => multigene family

Mammals:

- "Muscles": α1, β1, γ, δ, ε
- "Neurones": α2, α3, α4, α5, α6,
  α7, α9, α10,
  β2, β3, β4

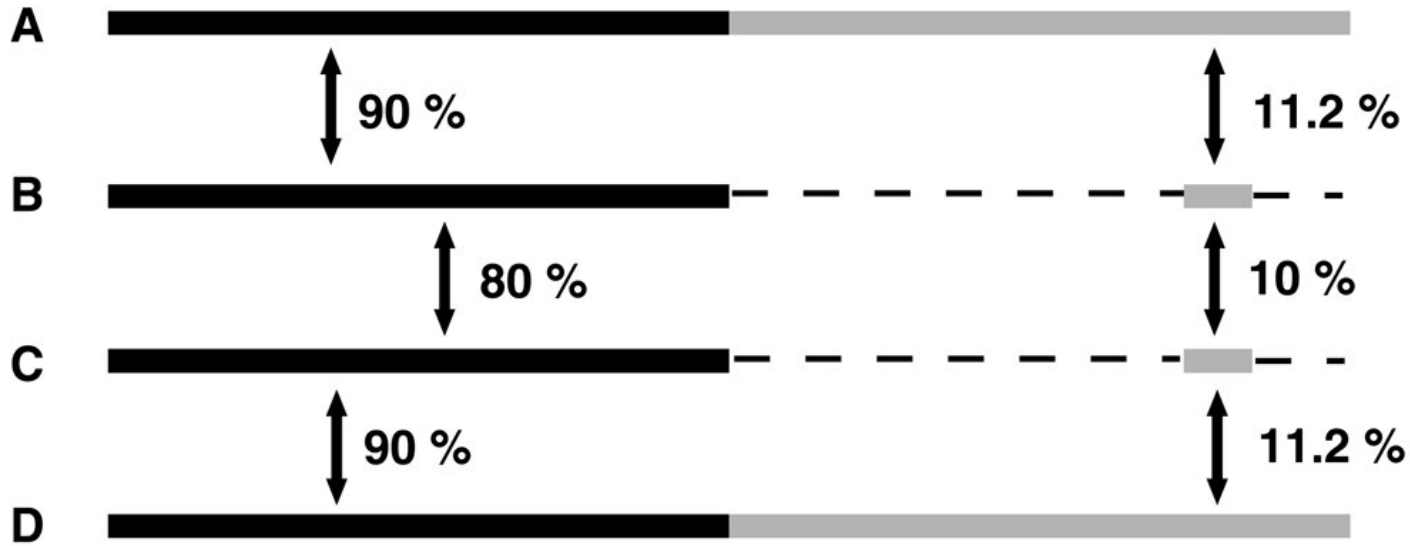
Sequence relationships of subunits

?

functional similarity of receptors

# Phylogeny of nAChR subunits

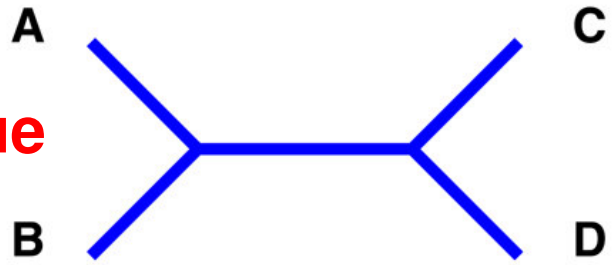
# Bias due to sequence variability

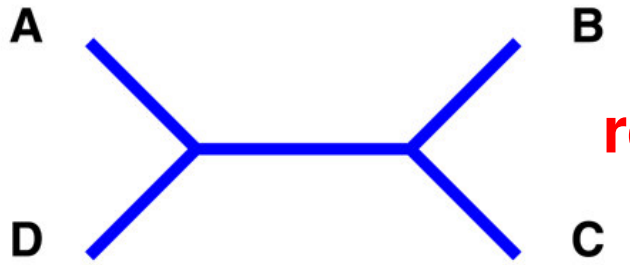



$$d(A,B) = ( 0.5 \times 90 ) + ( 0.5 \times 11.2 ) = 50.6\ \%$$

$$d(B,C) = ( 5/6 \times 80 ) + ( 1/6 \times 10 ) = 68\ \%$$

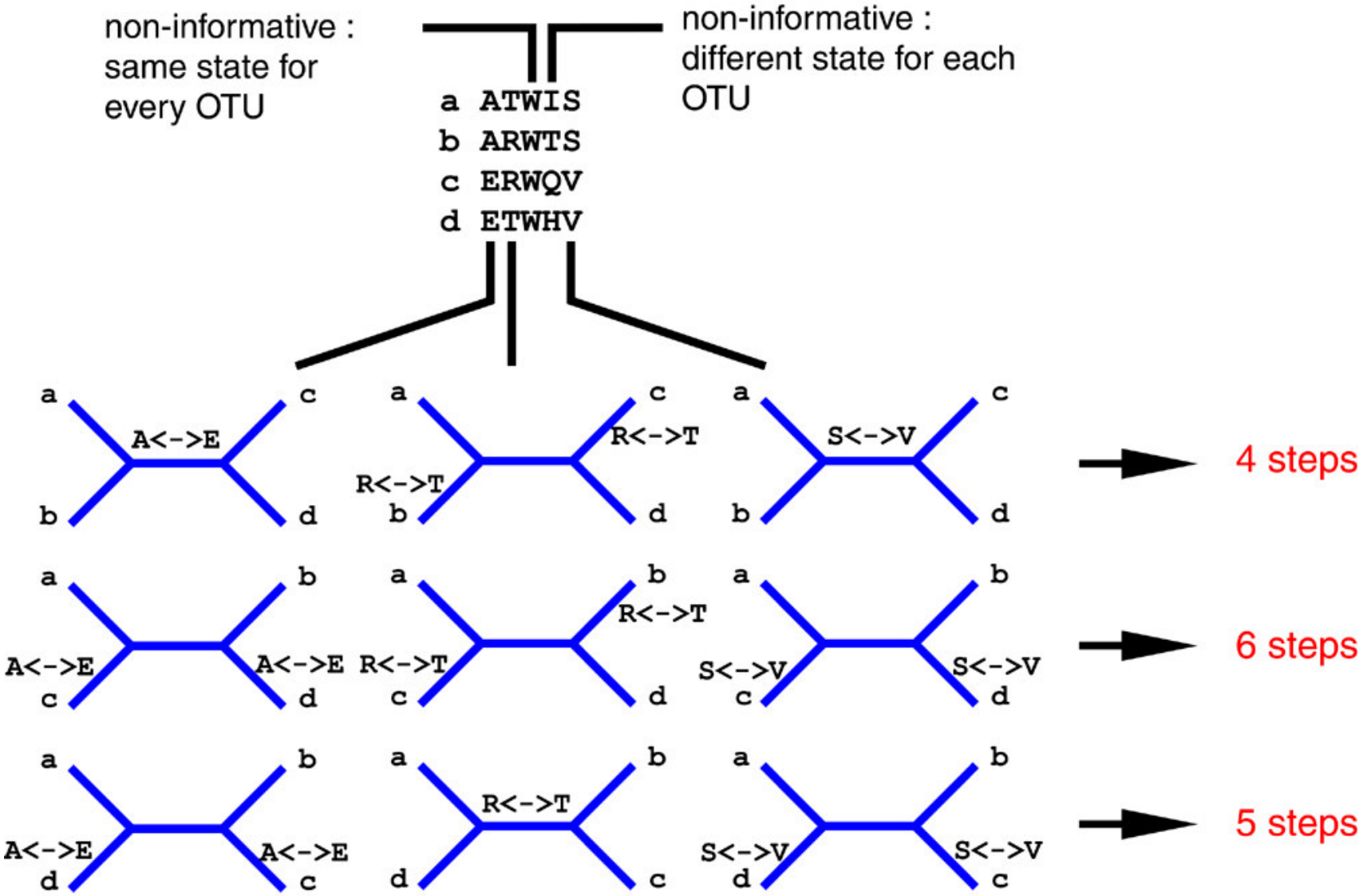
True



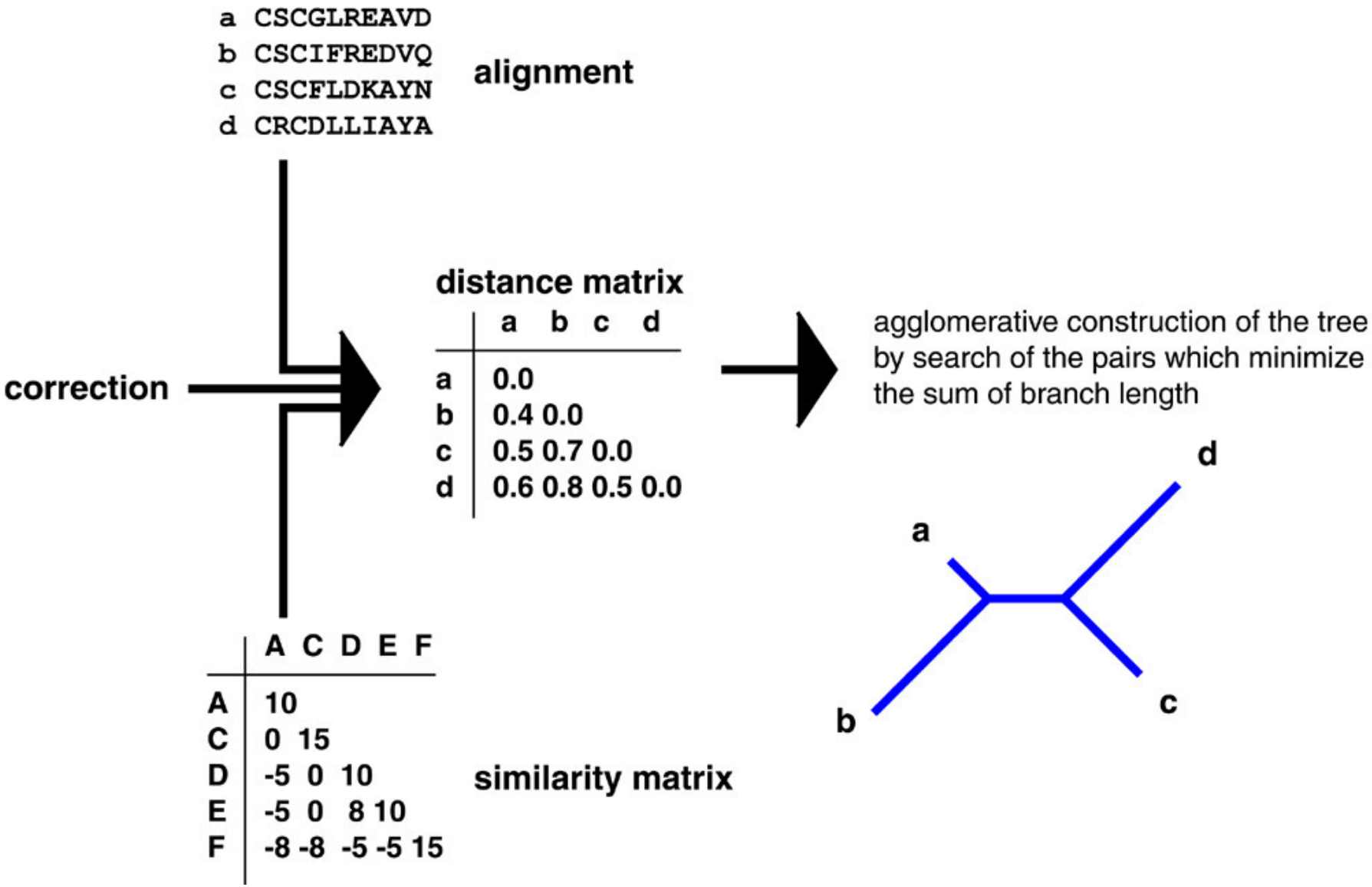




result

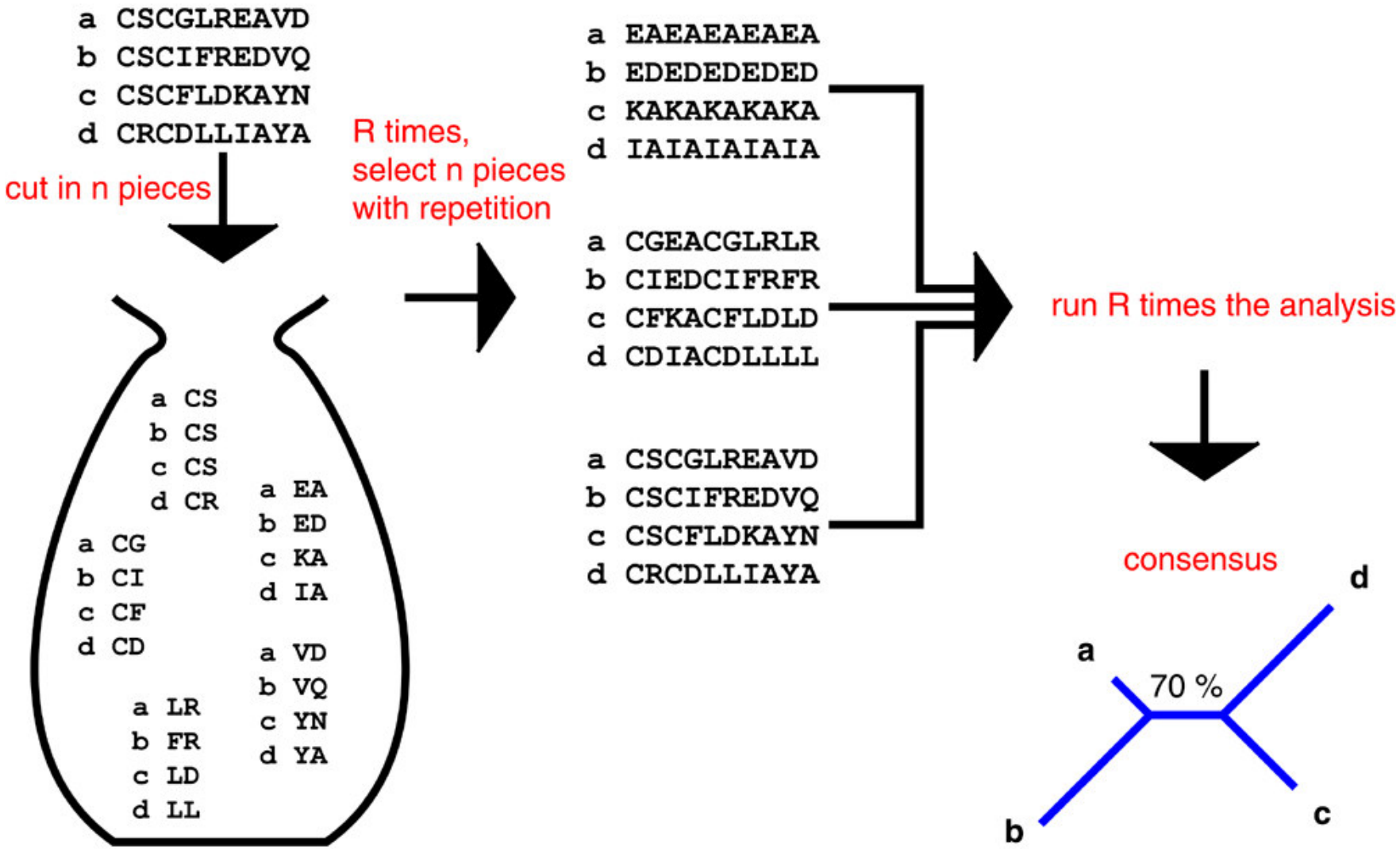
# Maximum of parcimony



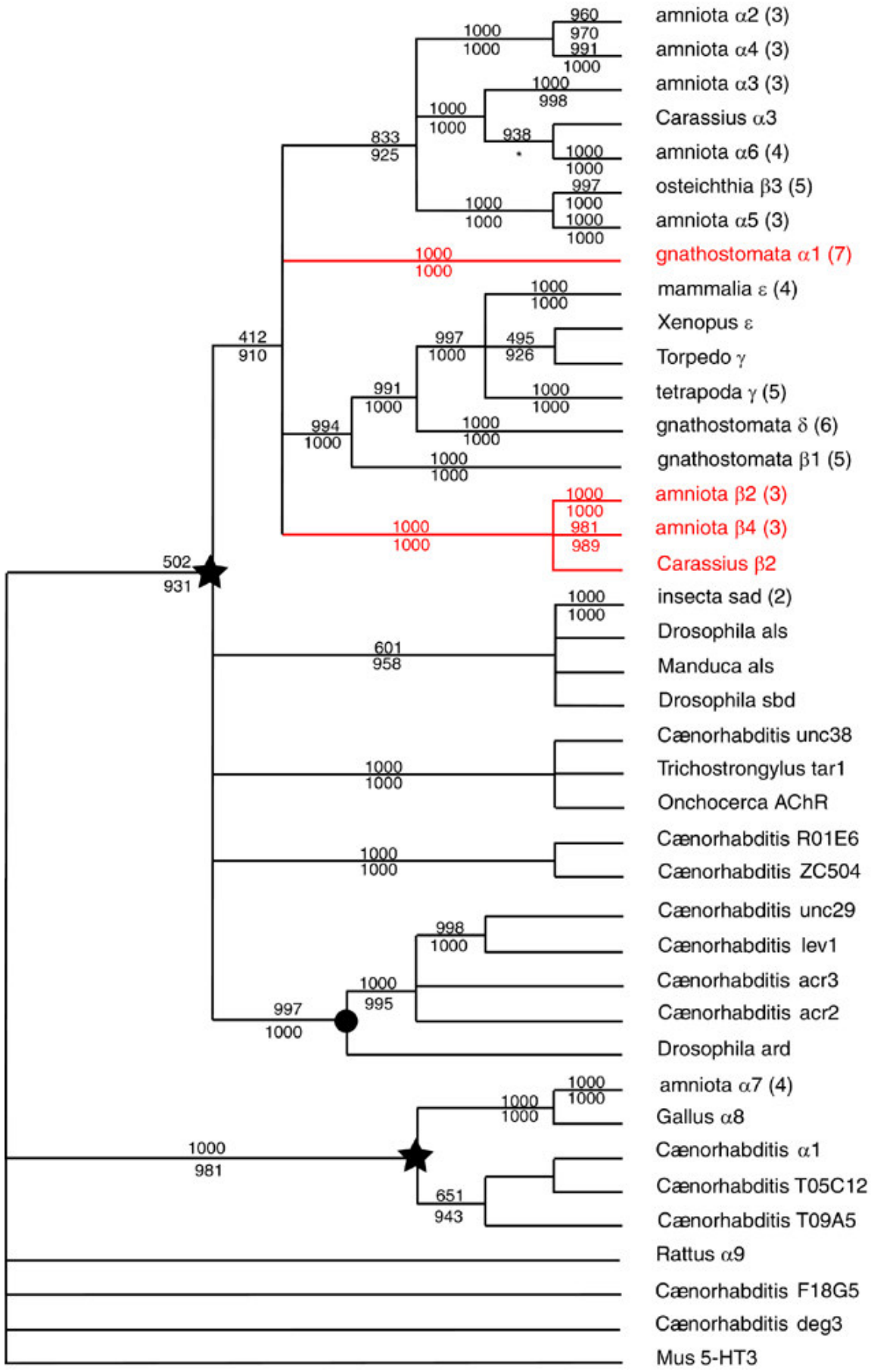
# Neighbor-joining



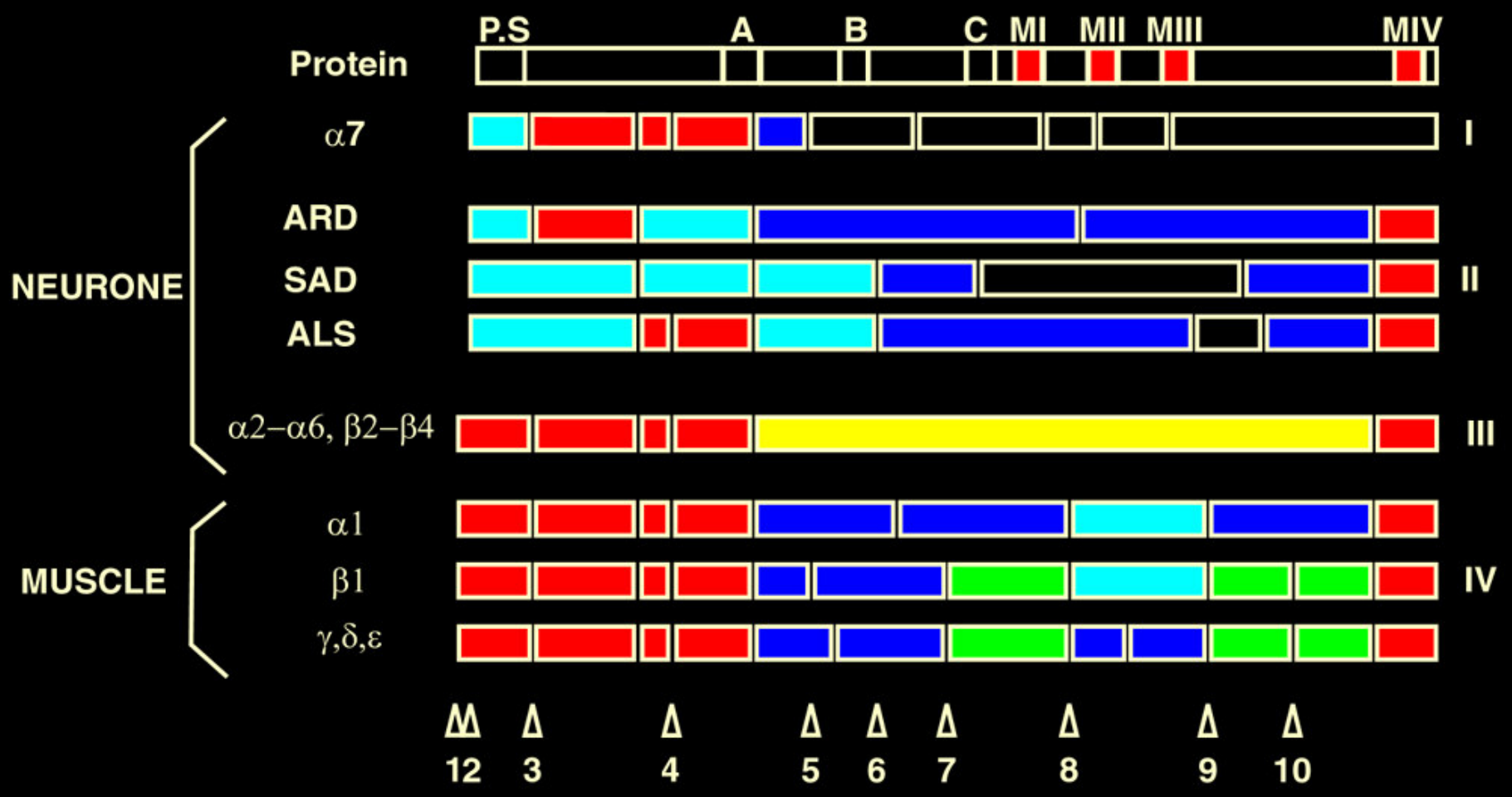
# Statistical support: Bootstrap



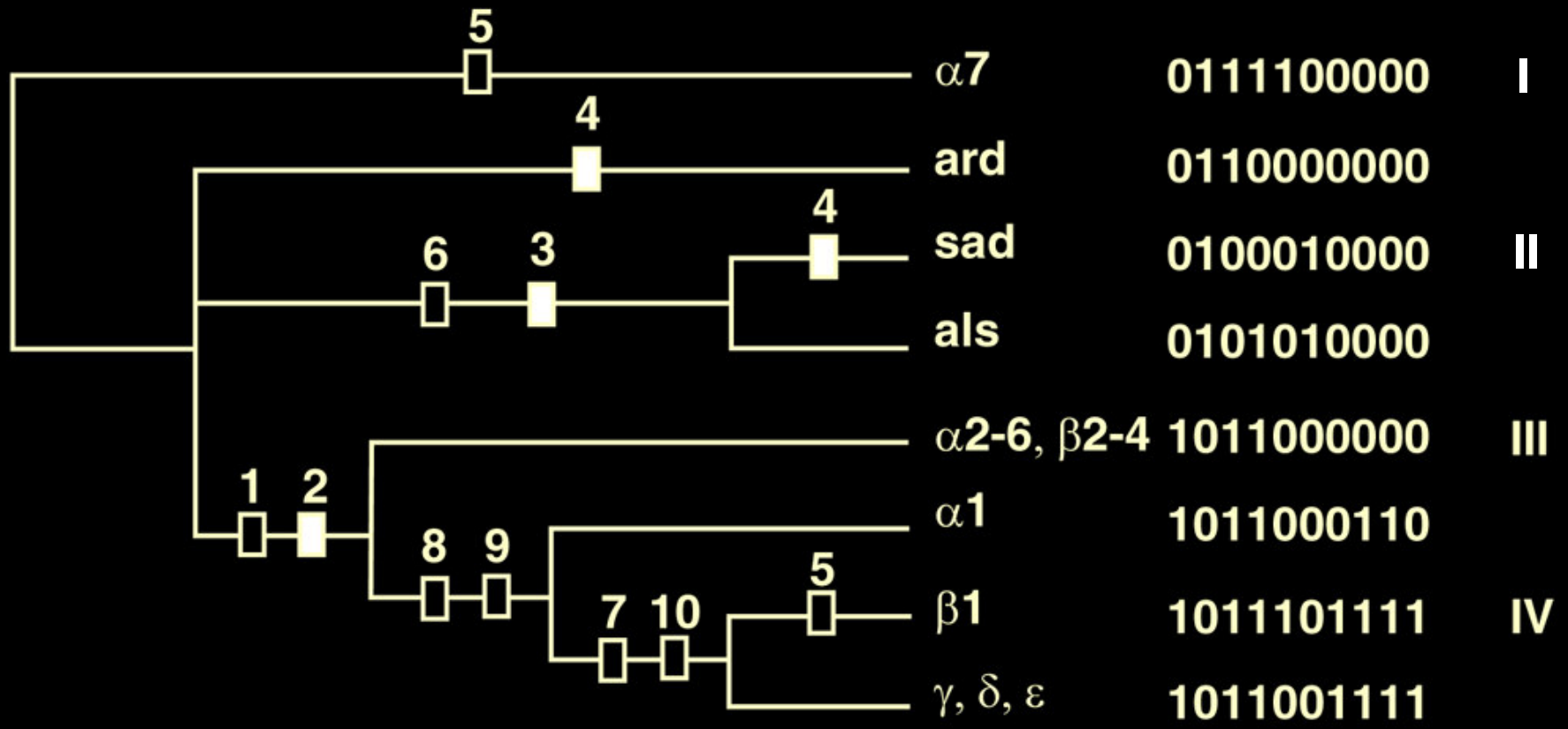
# Raw results


amniota α2 (3)
amniota α4 (3)
amniota α3 (3)
Carassius α3
amniota α6 (4)
osteichthia β3 (5)
amniota α5 (3)
gnathostomata α1 (7)
mammalia ε (4)
Xenopus ε
Torpedo γ
tetrapoda γ (5)
gnathostomata δ (6)
gnathostomata β1 (5)
amniota β2 (3)
amniota β4 (3)
Carassius β2
insecta sad (2)
Drosophila als
Manduca als
Drosophila sbd
Cænorhabditis unc38
Trichostrongylus tar1
Onchocerca AChR
Cænorhabditis R01E6
Cænorhabditis ZC504
Cænorhabditis unc29
Cænorhabditis lev1
Cænorhabditis acr3
Cænorhabditis acr2
Drosophila ard
amniota α7 (4)
Gallus α8
Cænorhabditis α1
Cænorhabditis T05C12
Cænorhabditis T09A5
Rattus α9
Cænorhabditis F18G5
Cænorhabditis deg3
Mus 5-HT3

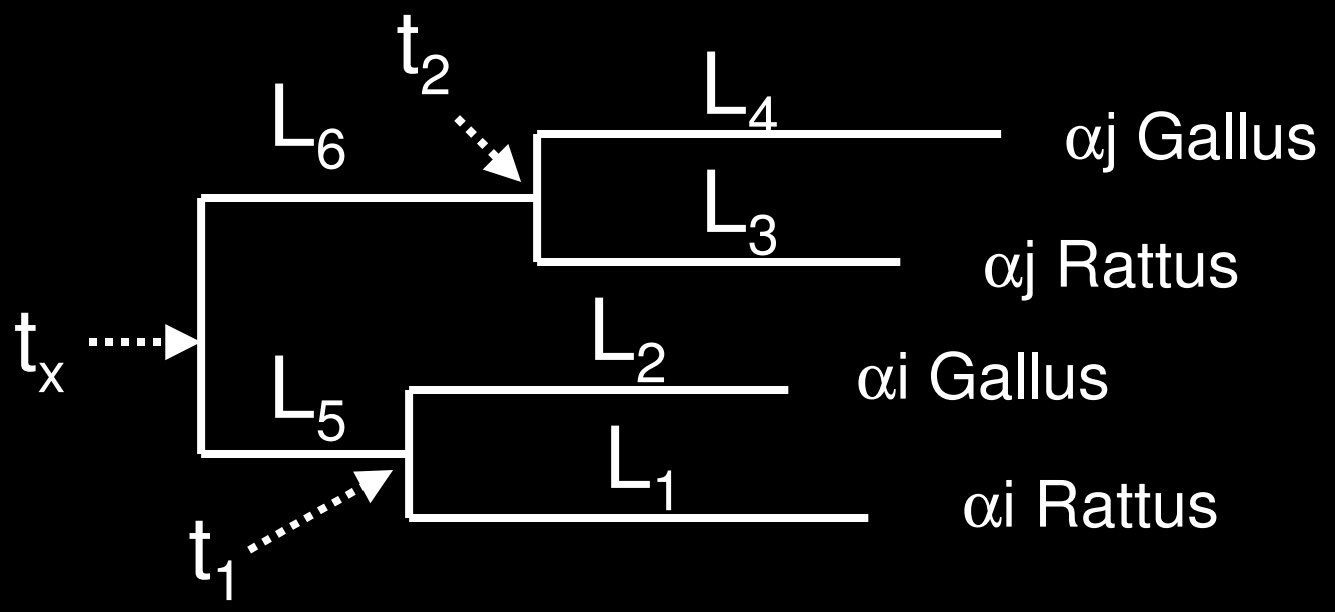
# Exon-intron structures



# Cladistique analysis of gene structures

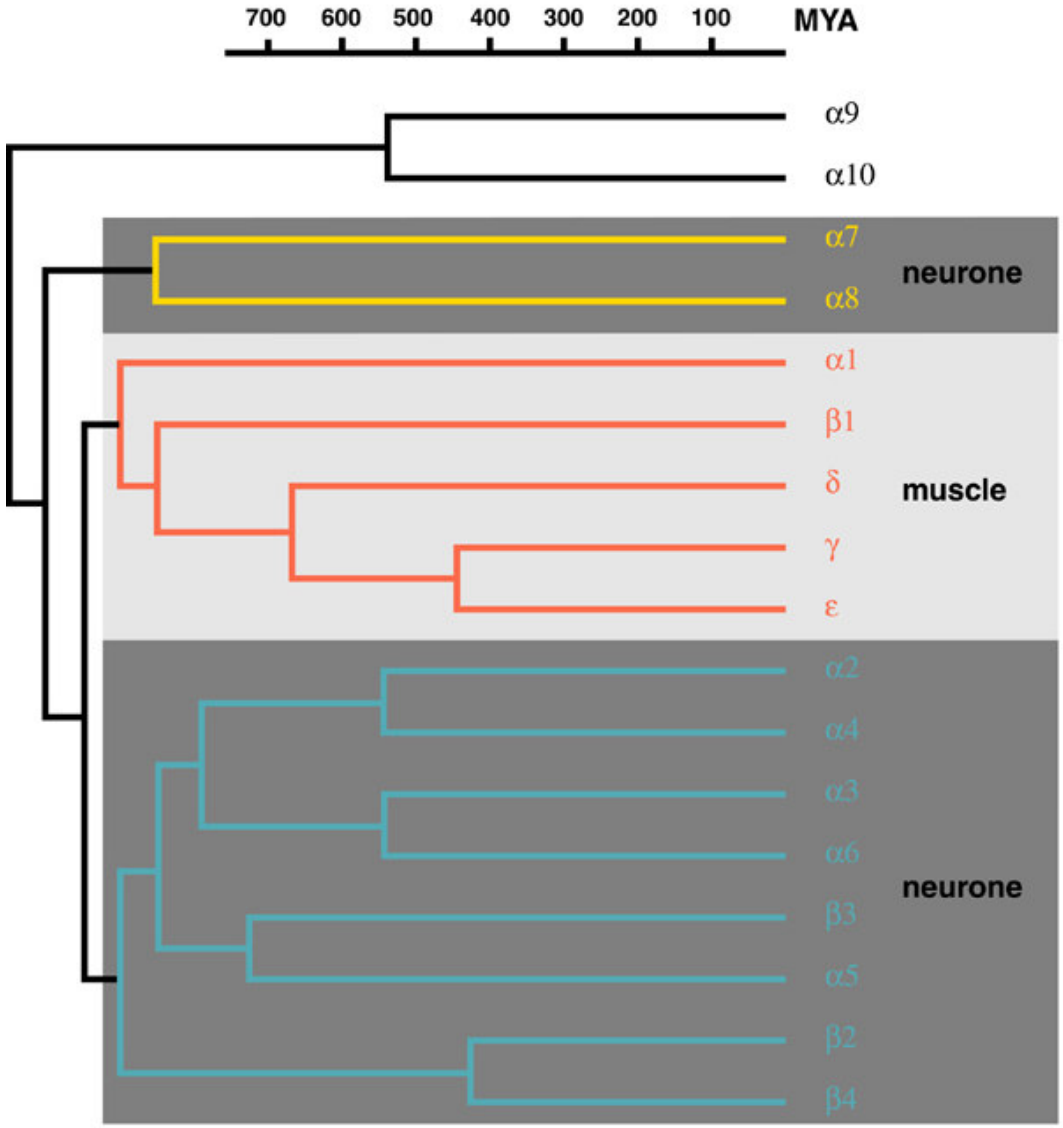


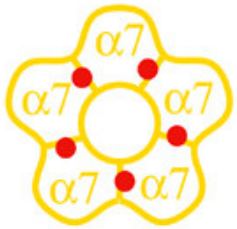
# Date of gene duplication



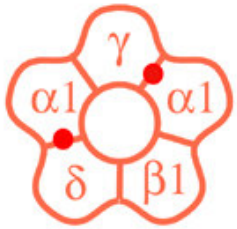

$$t_x = \frac{1}{2}\left[\frac{t_1}{L_1+L_2}(2L_5+L_1+L_2) + \frac{t_2}{L_3+L_4}(2L_6+L_3+L_4)\right]$$

# Phylogeny constrains physiology

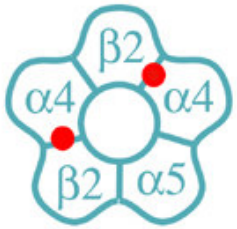



α-Bgt +
pCa/Na ~10




α-Bgt +
pCa/Na ~0.2



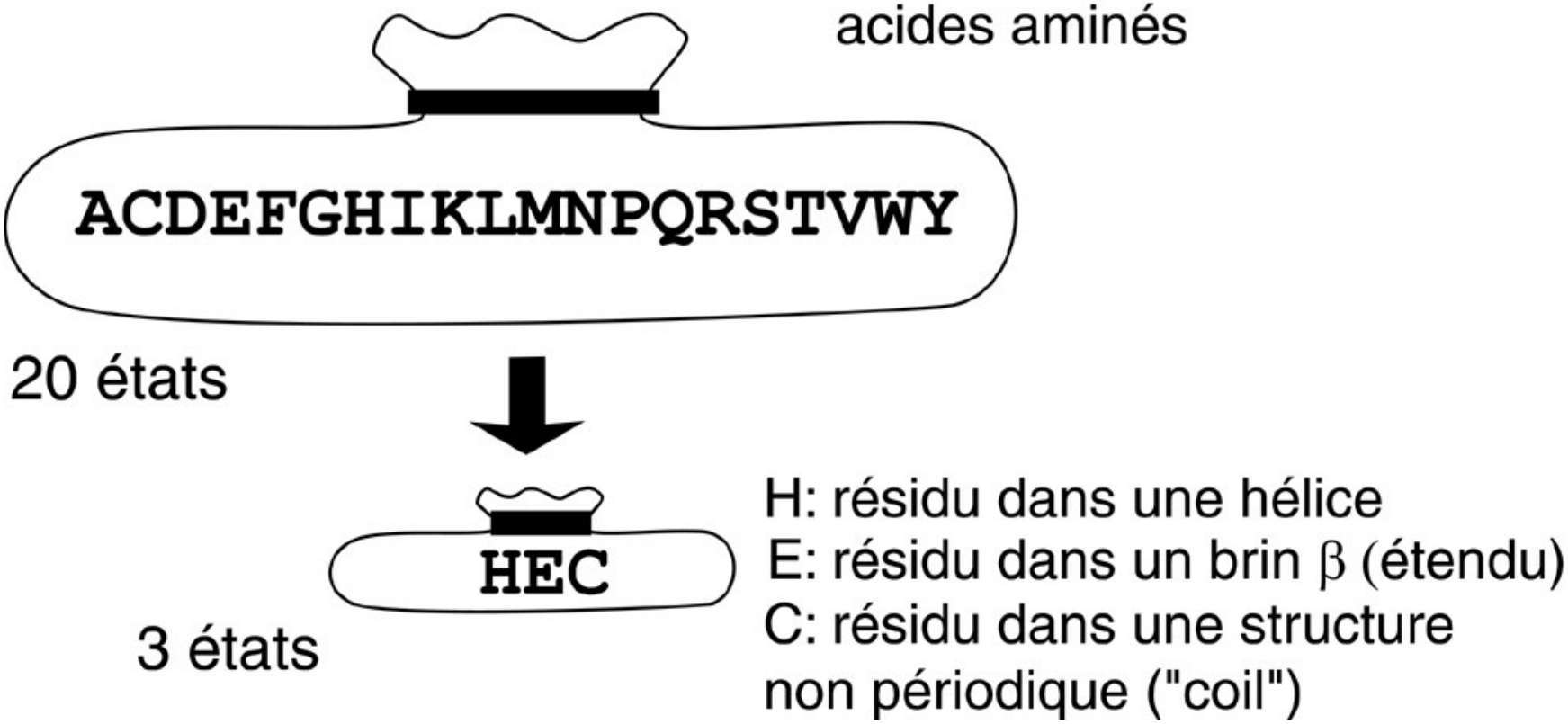

α–Bgt –
pCa/Na ~1.3

β2 (α4β2): nic > cyt
slow desens
β4 (α3β4): cyt > nic
fast desens

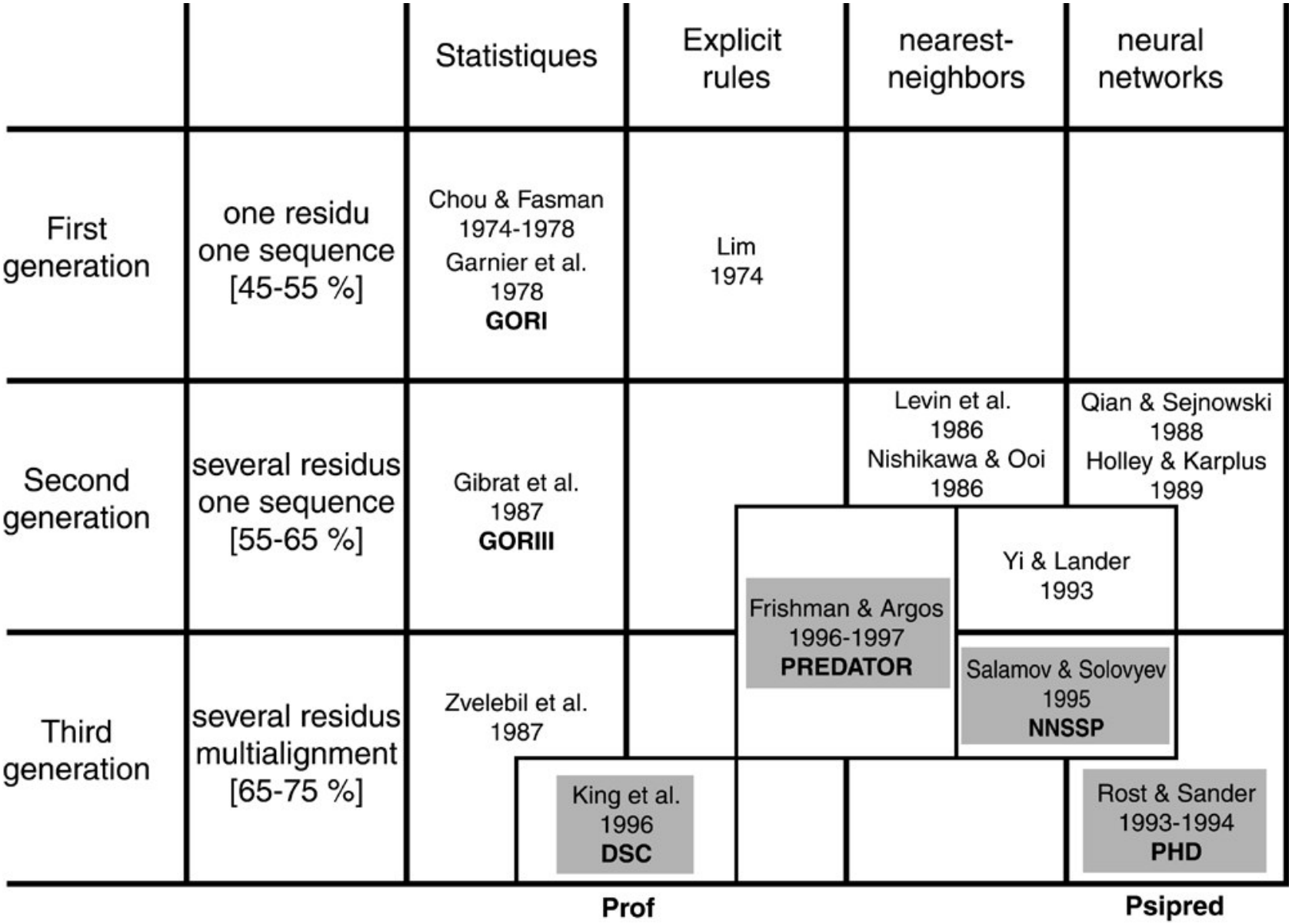
LE NOVÈRE and CHANGEUX (1995) *J. Mol. Evol.*, 40: 155-172

# Prediction of secondary structure

# Objective of secondary structure prediction



# History of secondary structure prediction



# Explicit physical properties

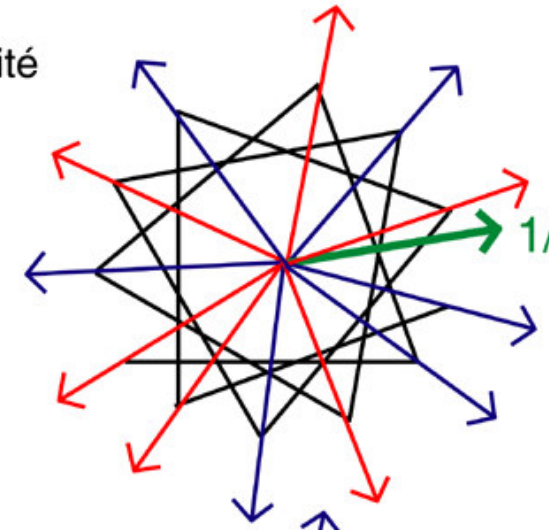
-Moments
d'hydrophobicité

Hydrophobicité :


-Transformée
de Fourier

Freq = 1/2 => E

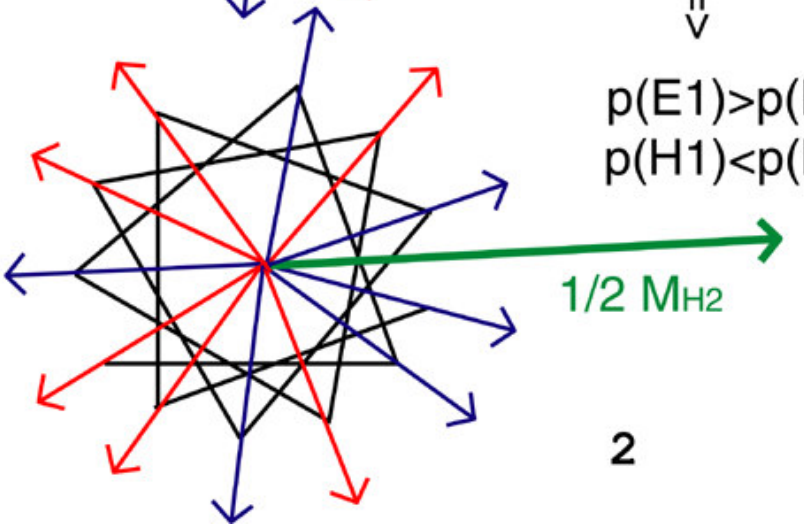

Freq = 1/4 => H




1

P(E) α 1/MH α ME

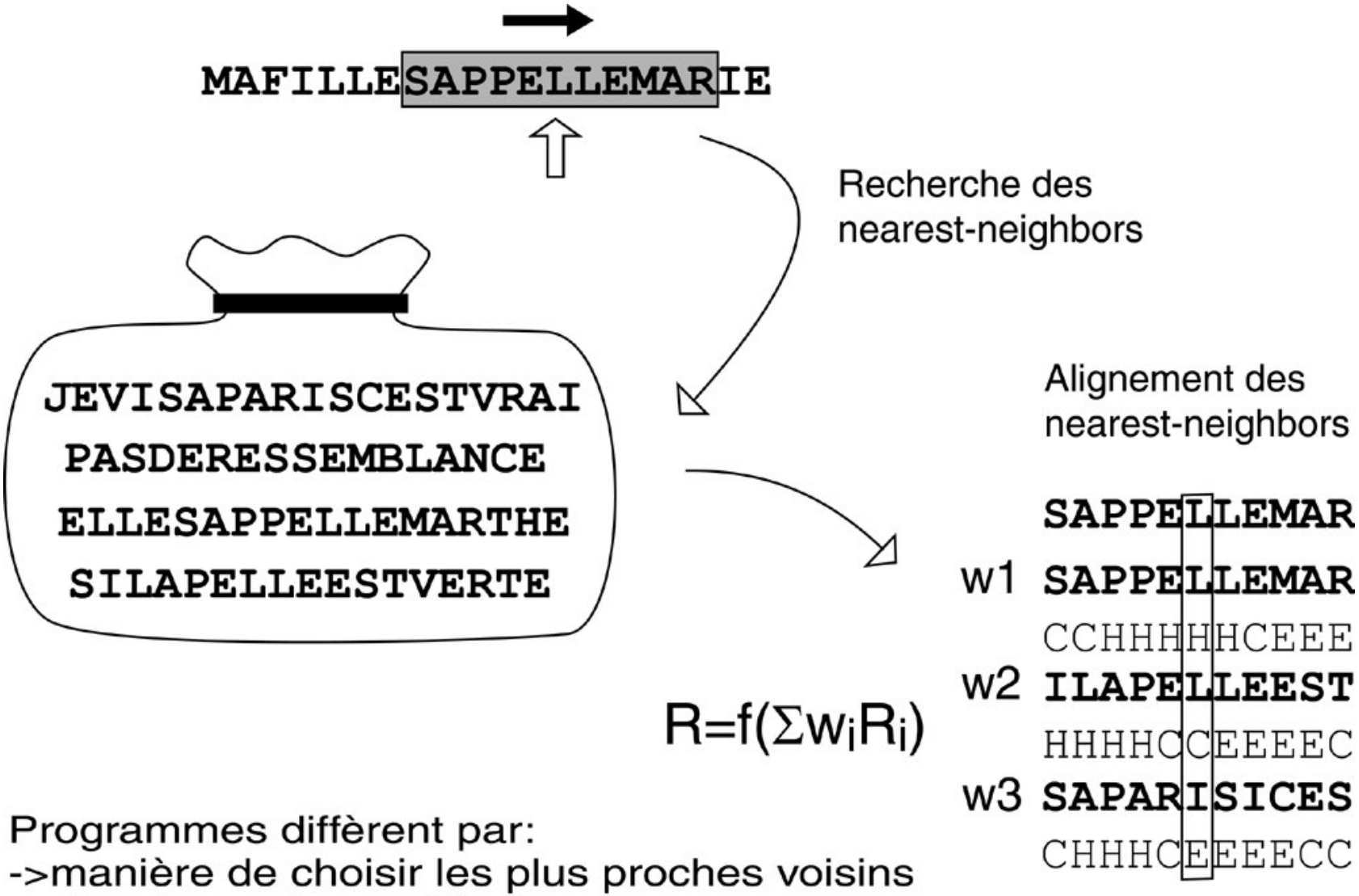
P(H) α MH




p(E1)>p(E2)
p(H1)<p(H2)

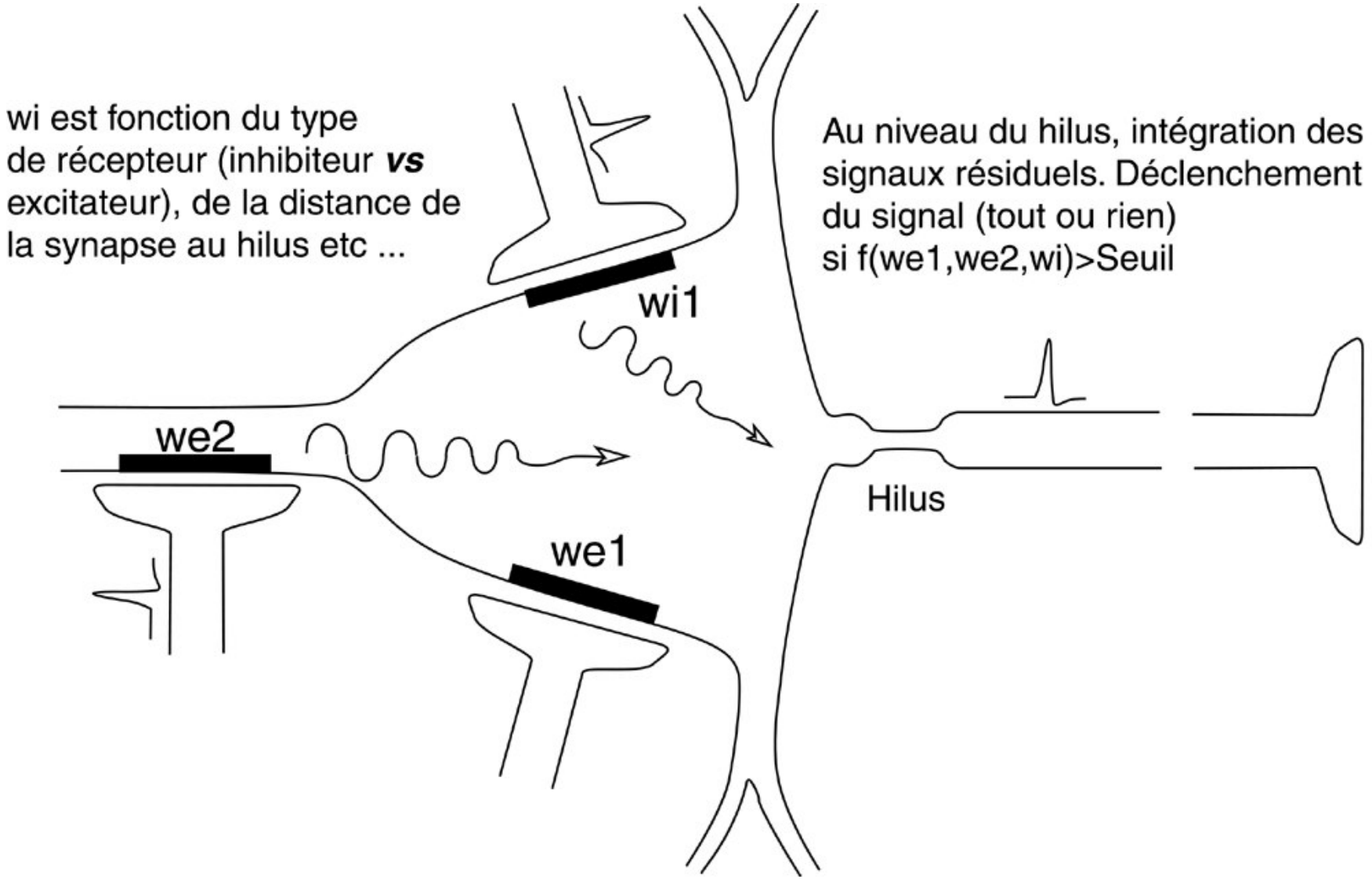
2

# Nearest-neighbor approach

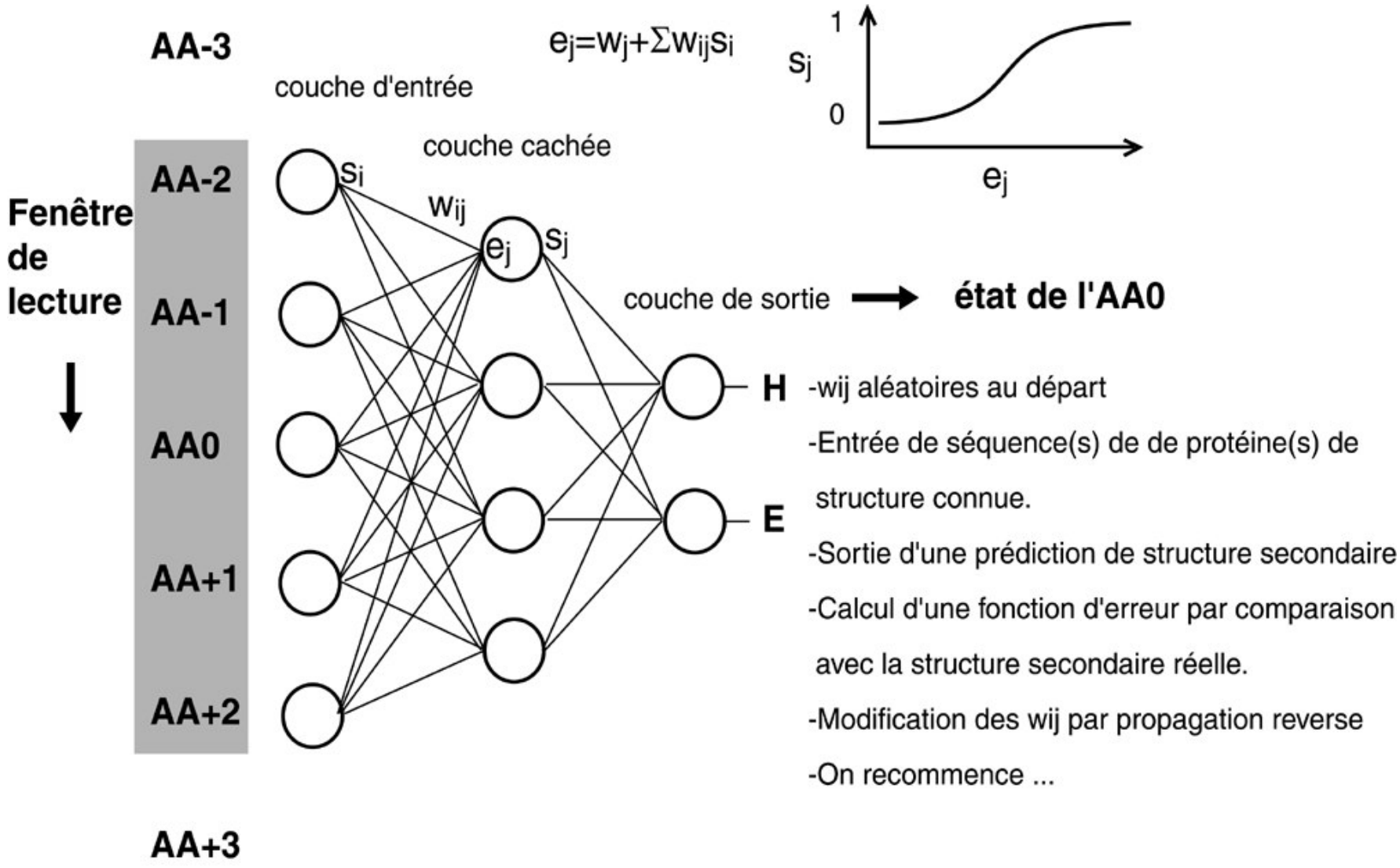



Programmes diffèrent par:
->manière de choisir les plus proches voisins
->algorithme de pondération

# The biological neuron



# The formal neural network




-wij aléatoires au départ

-Entrée de séquence(s) de de protéine(s) de structure connue.

-Sortie d'une prédiction de structure secondaire

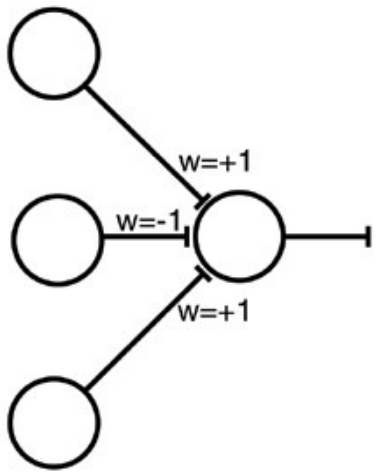
-Calcul d'une fonction d'erreur par comparaison avec la structure secondaire réelle.

-Modification des wij par propagation reverse

-On recommence ...

# Example of a simple neural network

| Cas | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| Pos1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 |
| Pos2 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| Pos3 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |




A: 0

B: 1

C: -1

D: 1

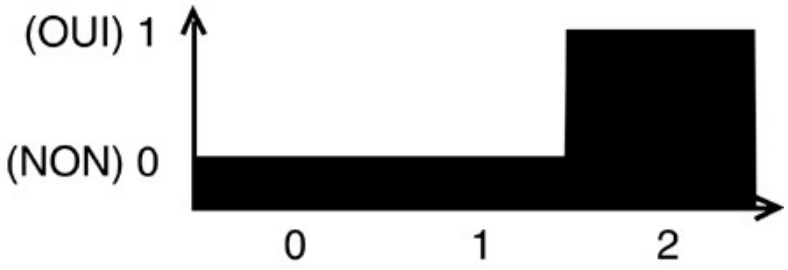
E: 0

F: 0

G: 2

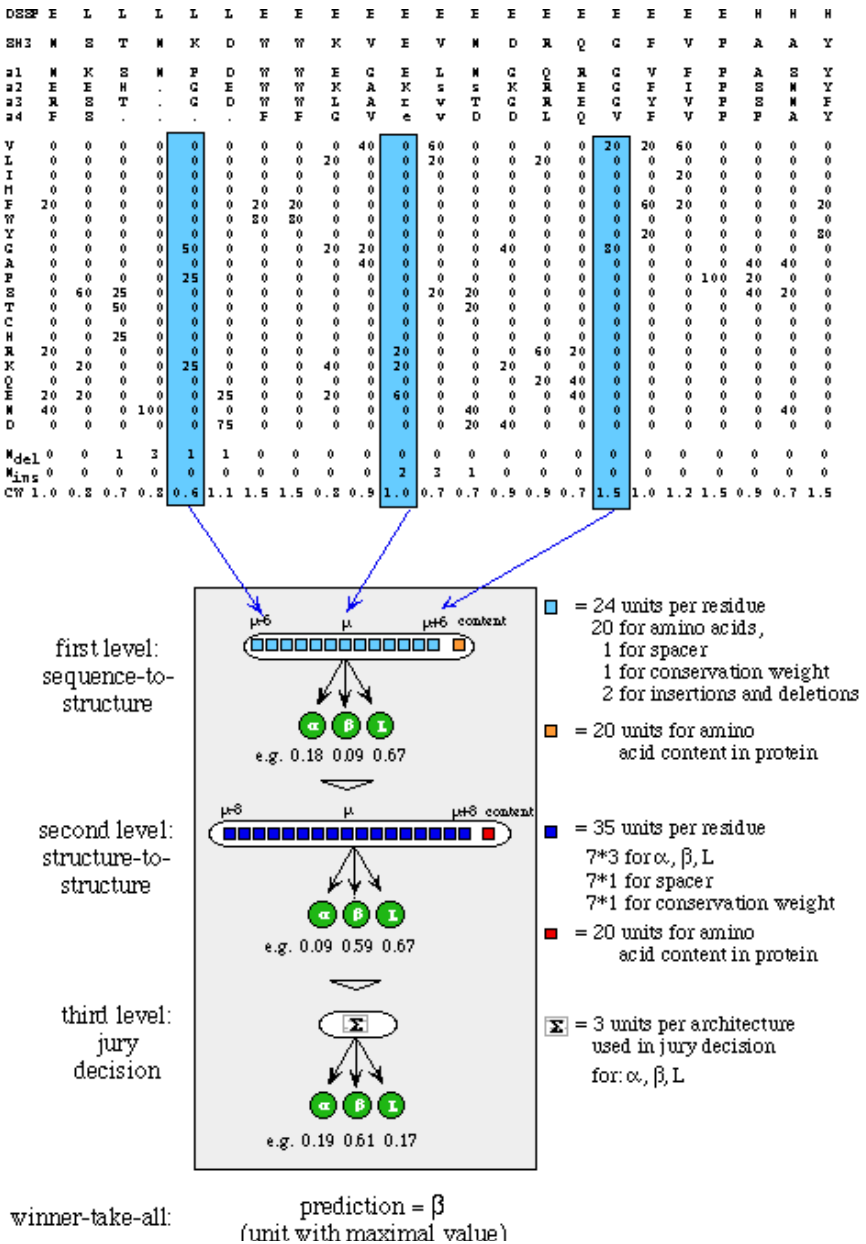
H: 1

**reconnaissance du motif "101"**
**dans une chaîne binaire**

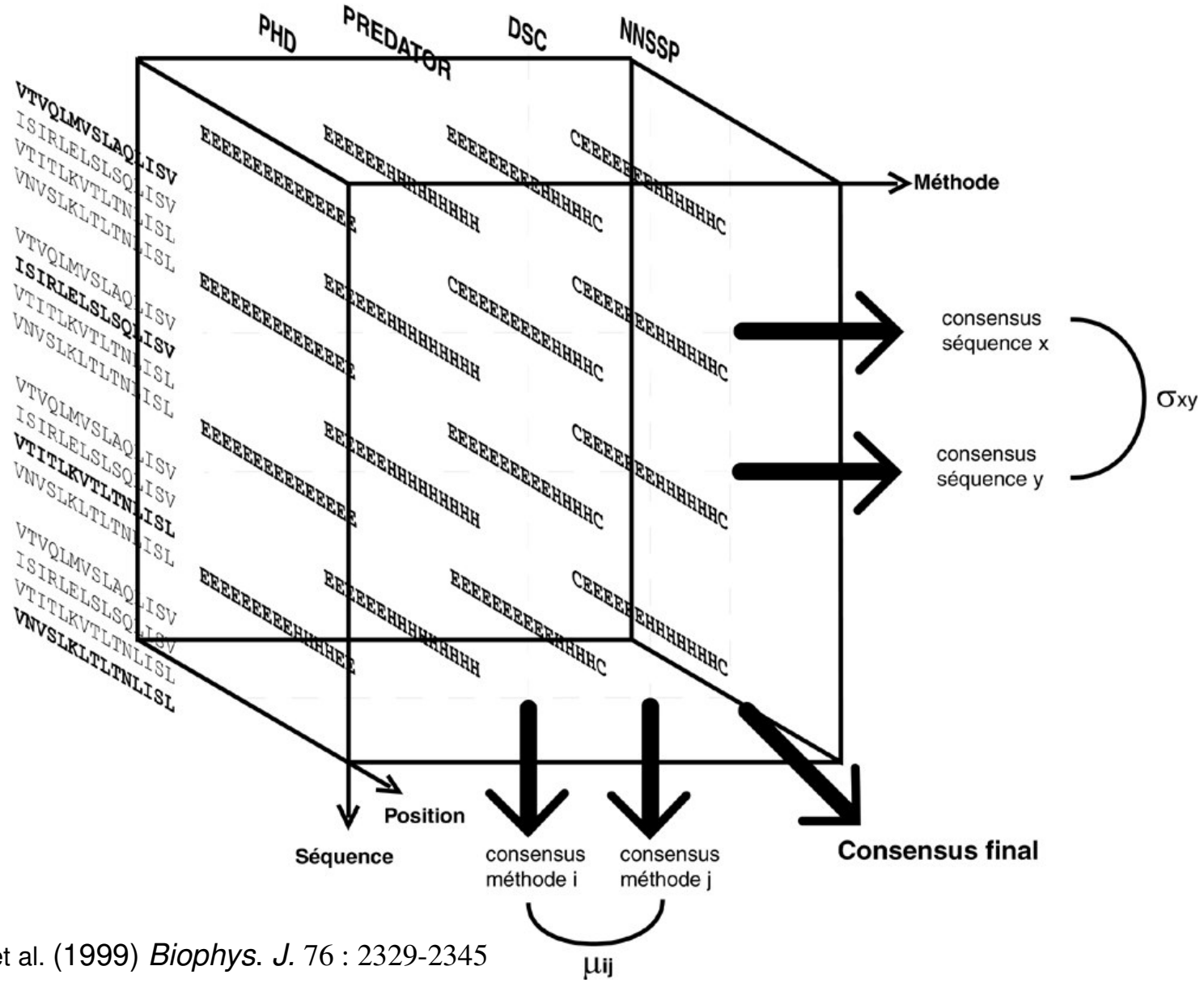


# Cascading neural networks

**Example of PHDsec**

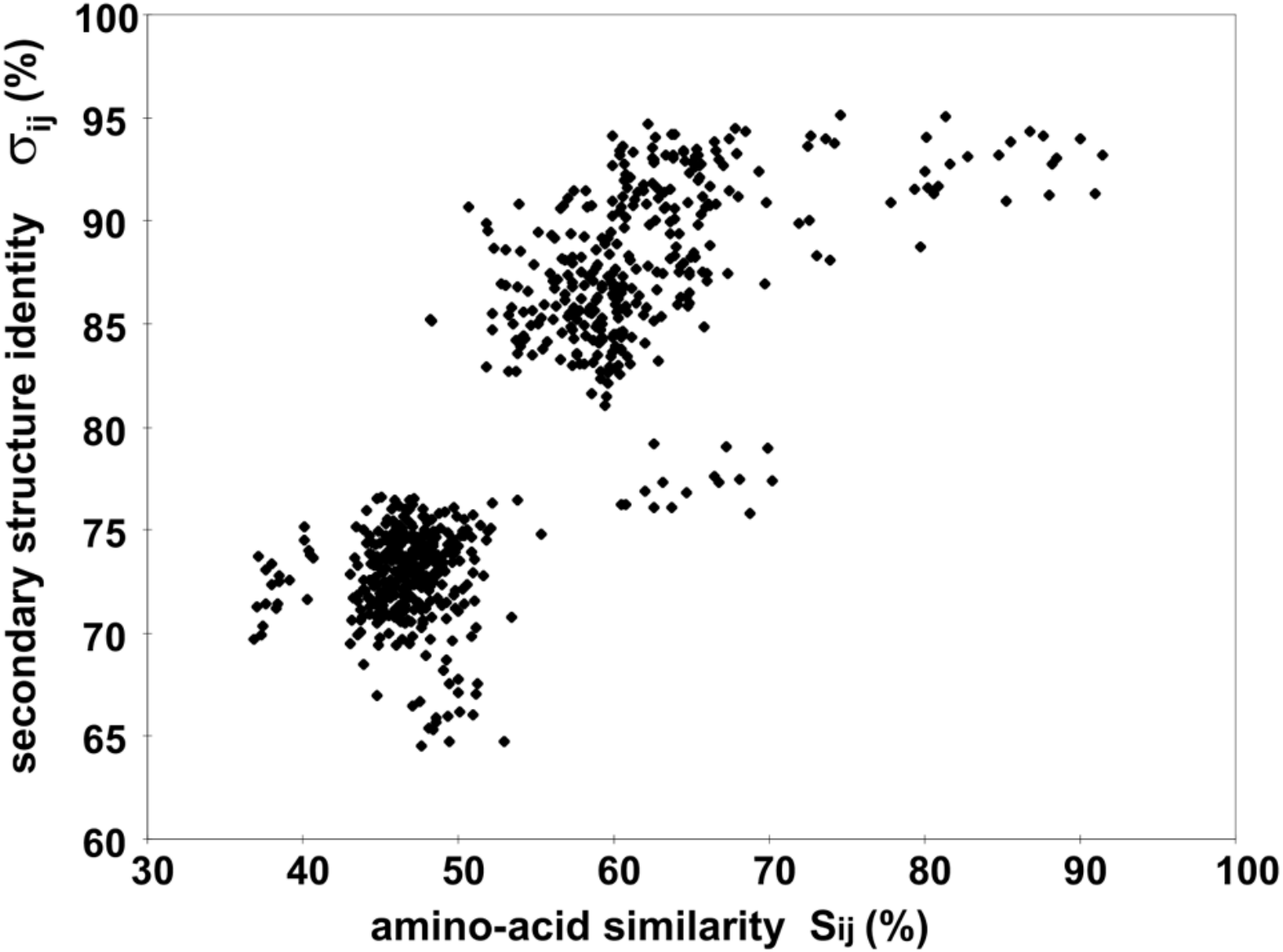


# Algorithm of consensus

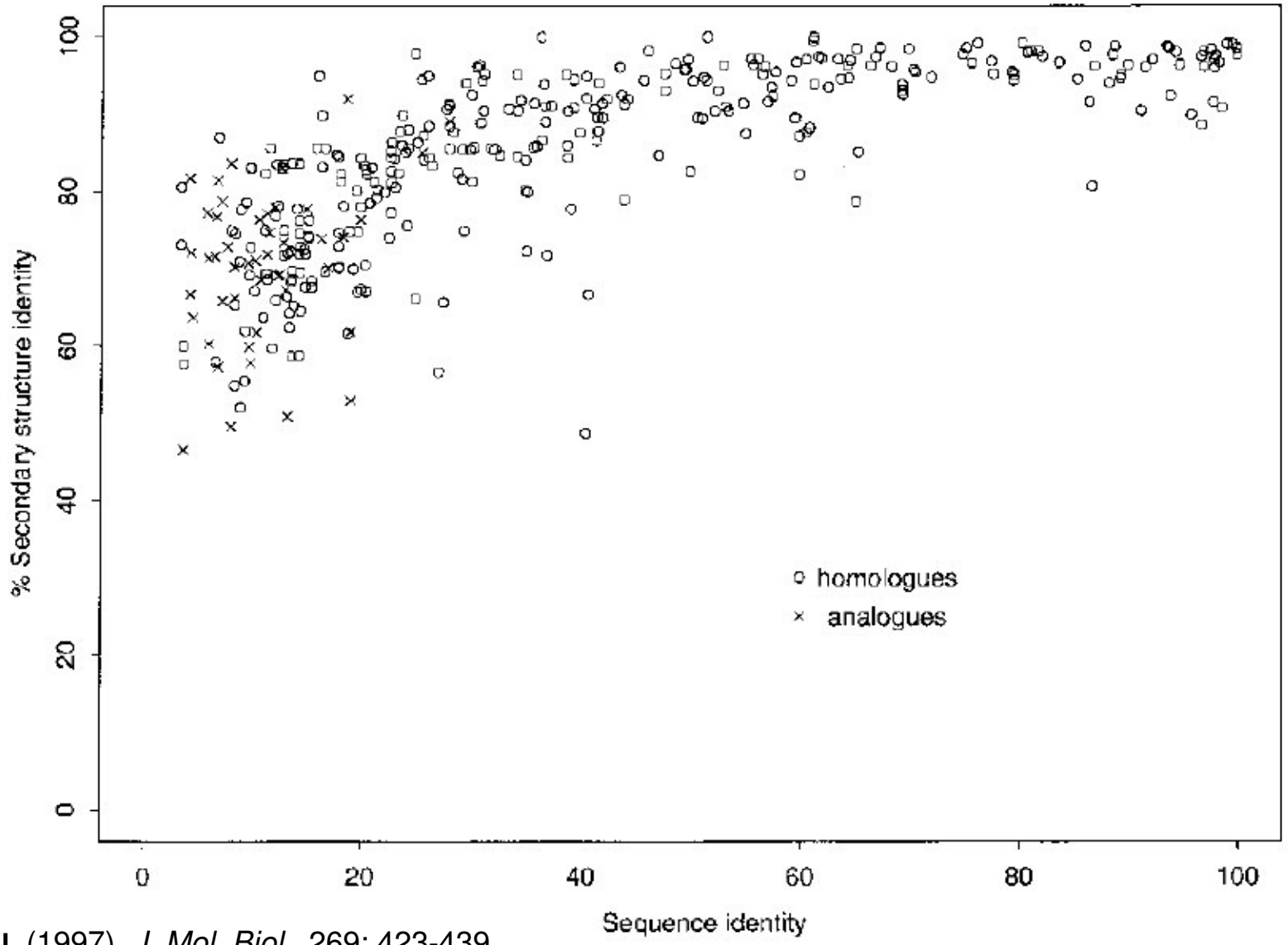



LE NOVÈRE et al. (1999) *Biophys. J.* 76 : 2329-2345

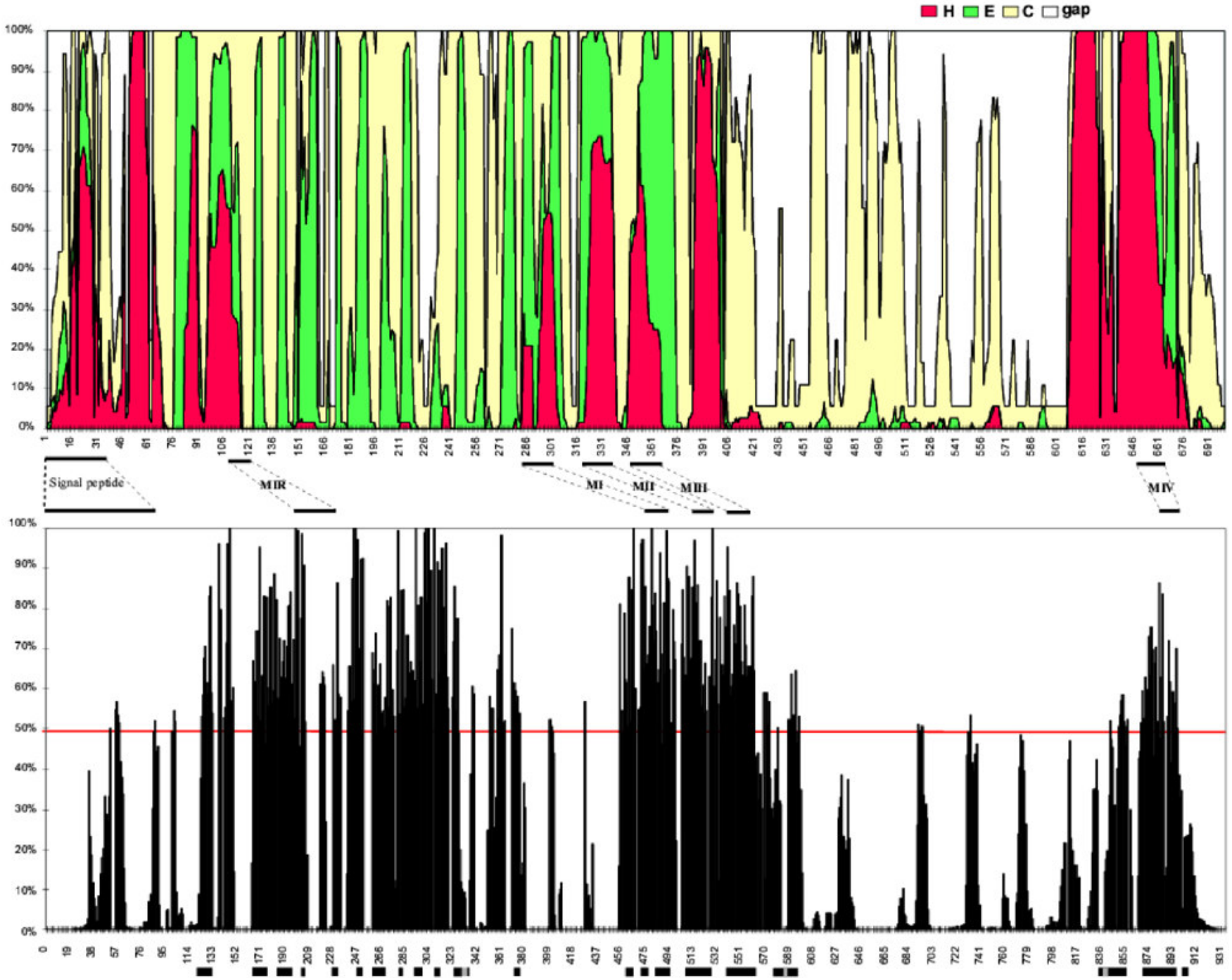
# Predicted SS identity Vs. sequence identity



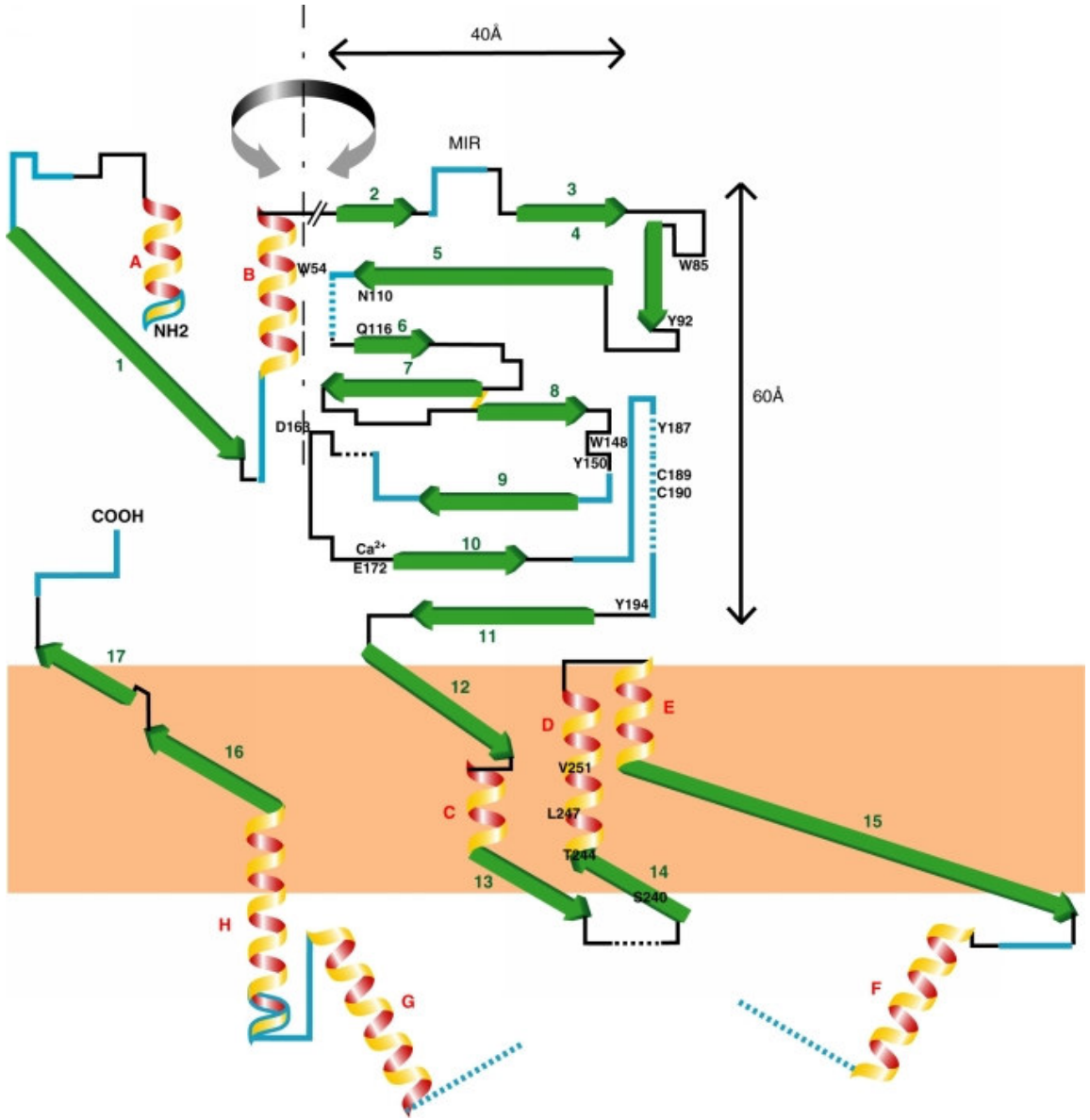
# Observed SS identity Vs. sequence identity




R**USSEL** (1997). *J. Mol. Biol.*, 269: 423-439

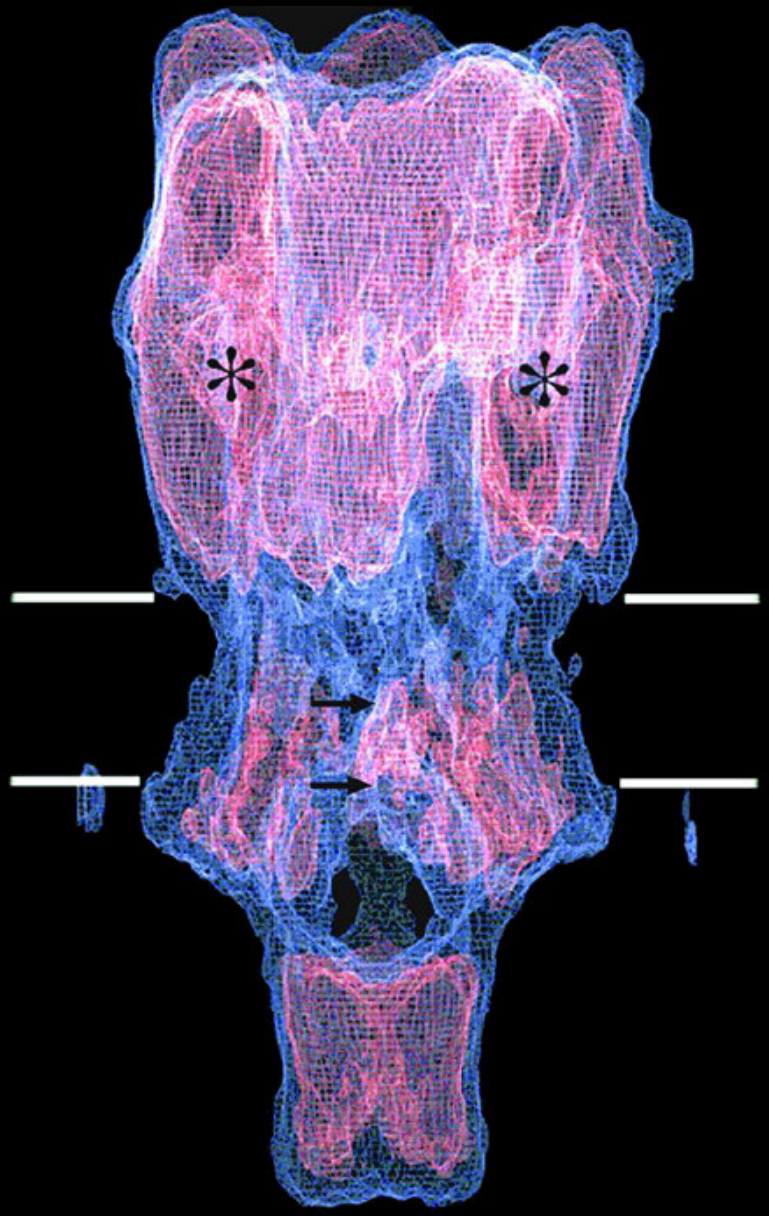

H
E
C
gap
Signal peptide
MIR
MI
MII
MIII
MIV

# Integration of different predictions



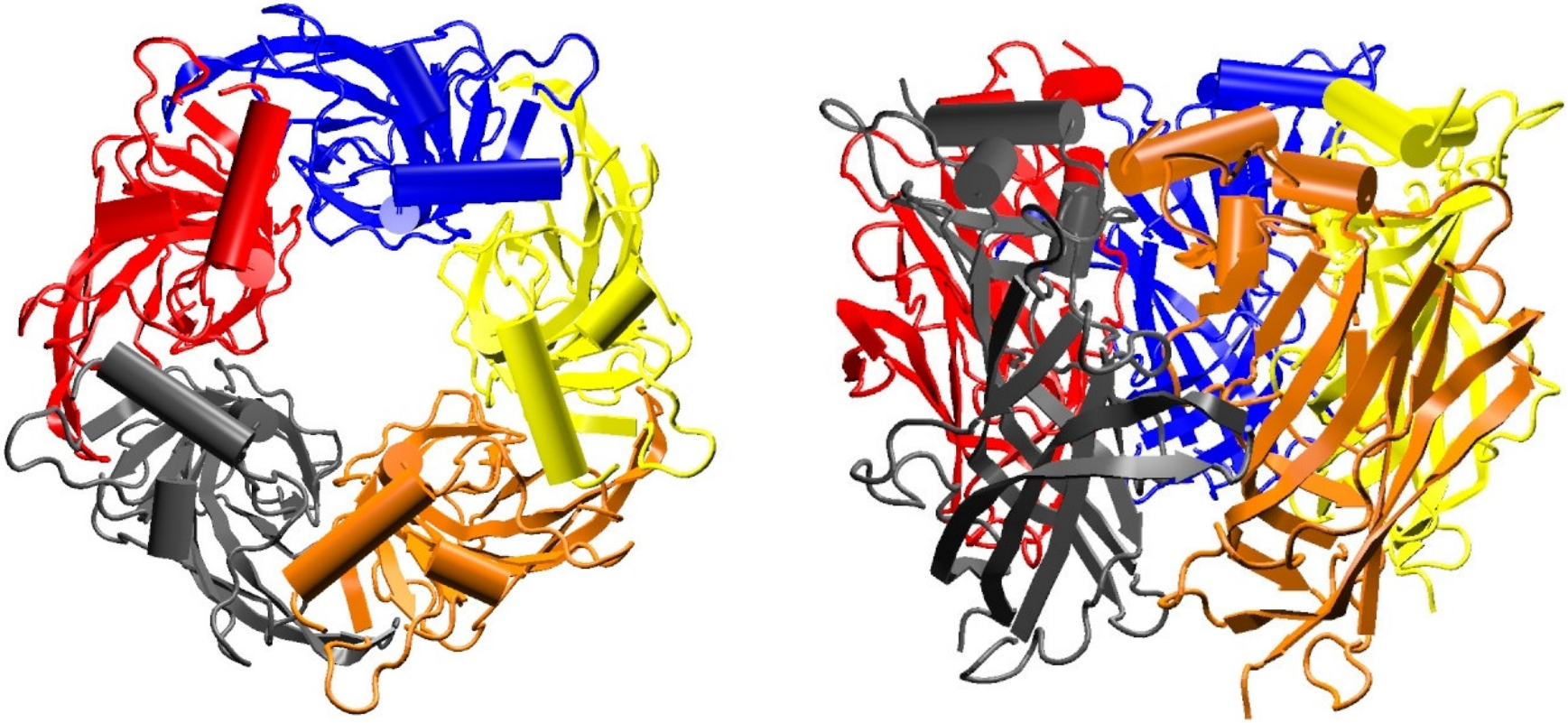
# Limited resolution of electron diffraction

UNWIN (2000)
*Phil. T. Roy. Soc. B*
355 : 1813-1829



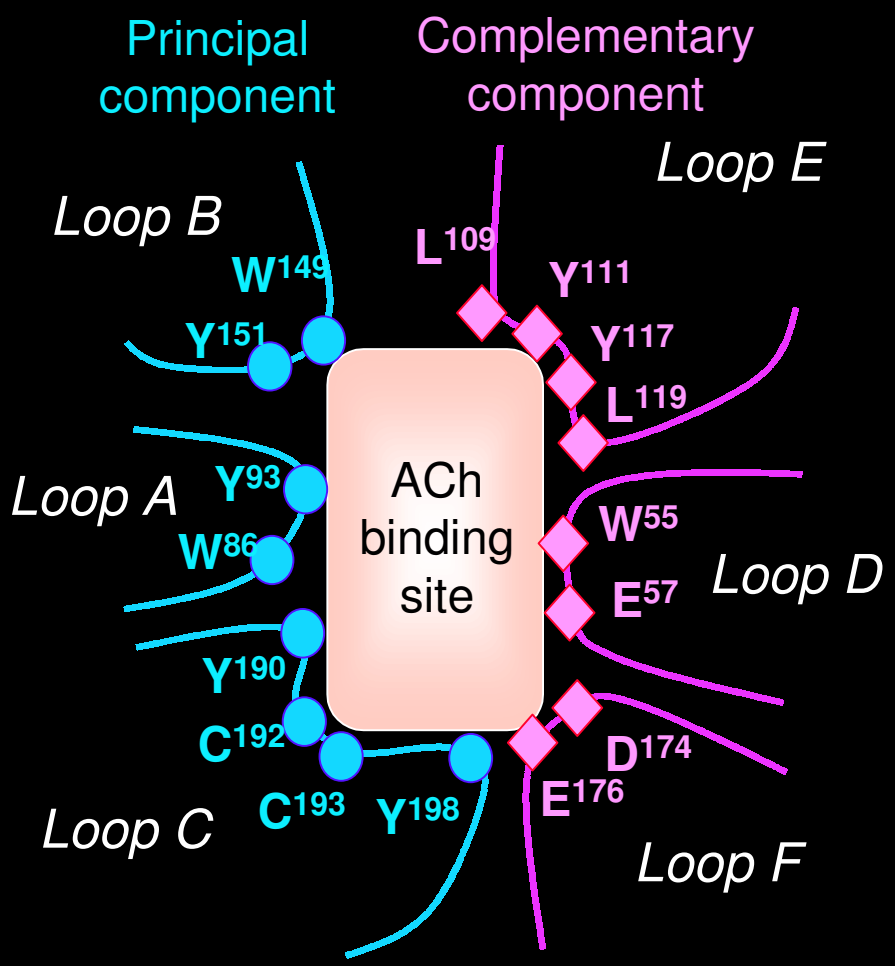
# AChBP, a partial molluscan homolog

BREJC *et al.* (2001) *Nature* 411 : 269-276

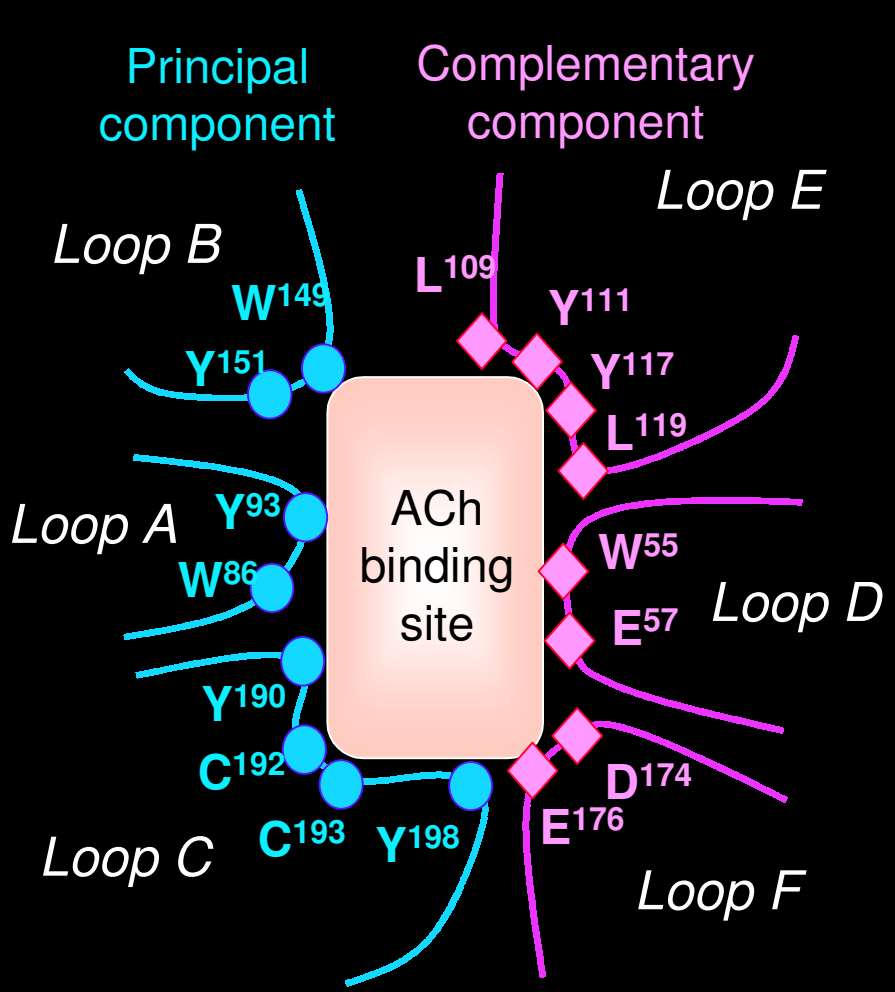


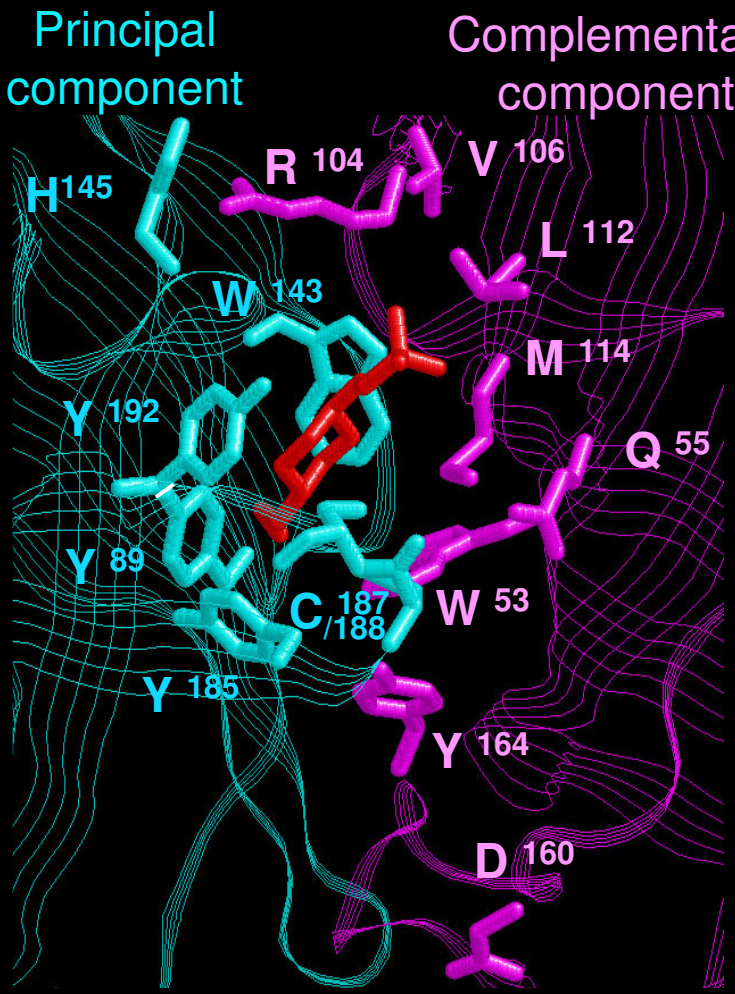
- 20-25 % sequence identity with extra-cellular portion of nAChR subunits;
- AChBP secondary structure 61 % identical to prediction of nAChR subunits;
- Immunoglobulin-like fold as predicted for extra-cellular portion of nAChR.

# nAChR Vs AChBP ACh sites



# nAChR Vs AChBP ACh sites



# Accuracy of 2nd structure prediction

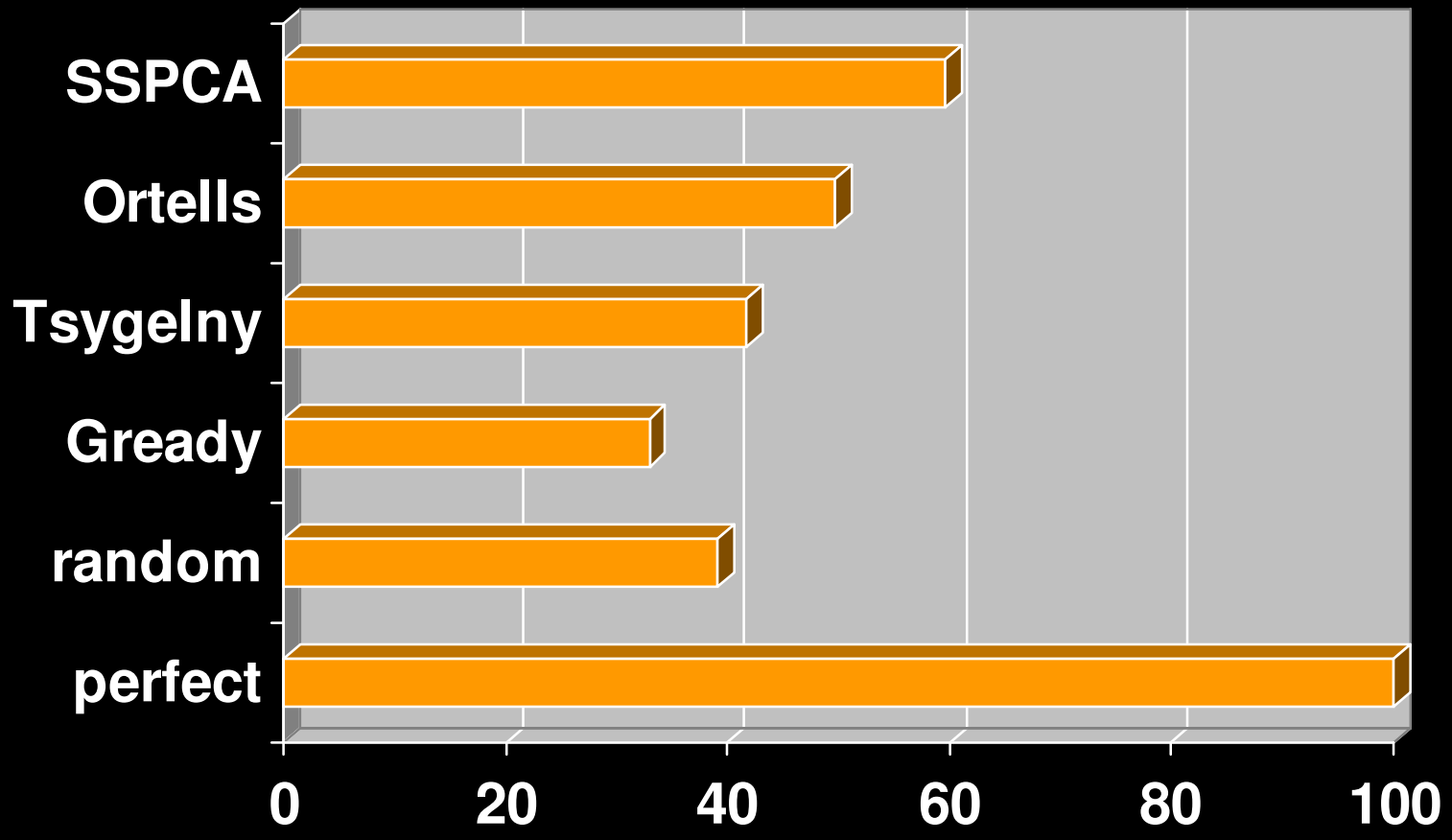
```
ACHBP -FDRADILYNIRQTSRPDVIPTQRD-RPVAVSVSLKFINILEVNEITNEVDVVFWQQTTWSDRTLA
struc -..HHHHHHHHHHH..........-..EEEEEEEEEEEEEEEE...EEEEEEEEEEEEEEEE....
pred  .HHHHHHHHHH....-..........EEEEEEEEEEEEE......HHHHHHHHHHHHHHH..EEE
α7gg  EFQRKLYKELLKNYN-PLERPVANDSQPLTVYFTLSLMQIMDVDEKNQVLTTNIWLQMYWTDHYLQ

ACHBP WNSSHSP--DQVSVPISSLWVPDLAAYNAISKP-EVLTPQLARVVSDGEVLYMPSIRQRFSCDVSGVD
struc .......__EEEEEEE......EEEEEEEE...-EEE..EEEEEEE.EEEEEEEEEEEEEEE......
pred  .........EEEE..........EEEE.....EEEEEEEEEEEEE....EEE..........EEEEEE
α7gg  WNVSEYPGVKNVRFPDGLIWKPDILLYNSADERFDATFHTNVLVNSSGHCQYLPPGIFKSSCYIDVRW

ACHBP TESGA-TCRIKIGSWTHHSREISVDPTTENSDDSEYFSQYSRFEILDVTQKKNSVTYSCCPE
struc ....E-EEEEEEEEE.....EEEEE................EEEEEEEEEEEEEE.......
pred  .........EEEE.........EEEEEE..--.........-..EEEEE..............
α7gg  FPFDVQKCNLKFGSWTYGGWSLDLQMQE--ADISGYISN-GEWDLVGIPGKRTESFYECCKE
```

Identité de séquence = 25 % → Identité de structure secondaire = 80 %

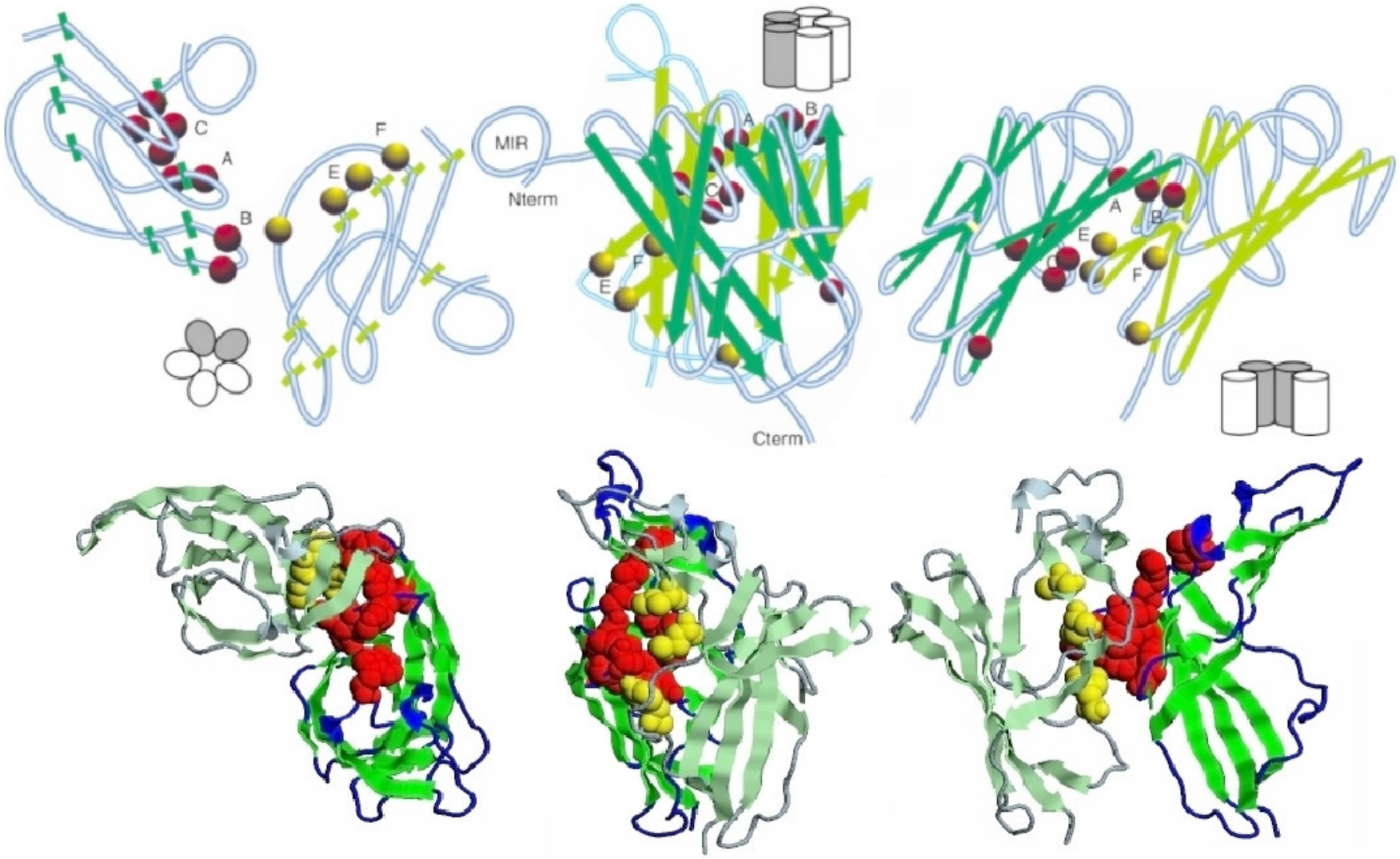
61 % des positions prédites pour α7 sont identiques à la structure d 'AChBP. En l'absence de biais cela représente une justesse de prédiction supérieure à 76 % pour la structure d 'α7 .

# Accuracy of 2nd structure predictions

(accuracy computed on the portion present in all studies)



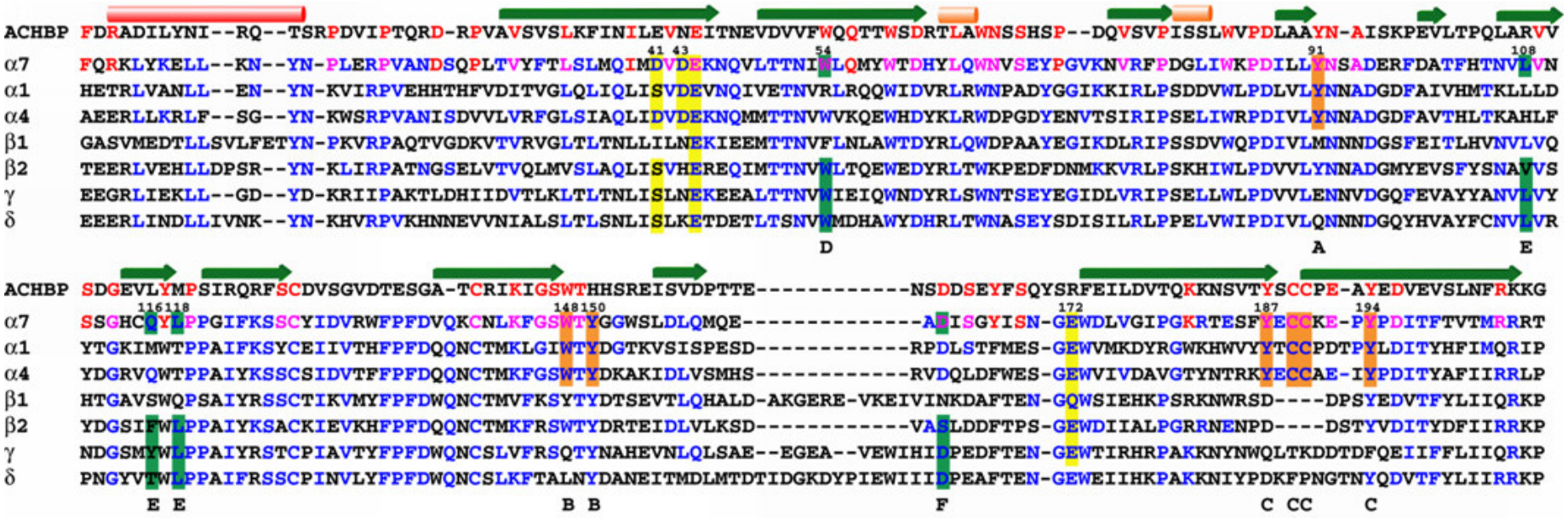
# Validity of the proposed fold




C**ORRINGER** et coll. (2000) *Annu. Rev. Pharmacol. Toxicol.* 40: 431-458

# Comparative modelling

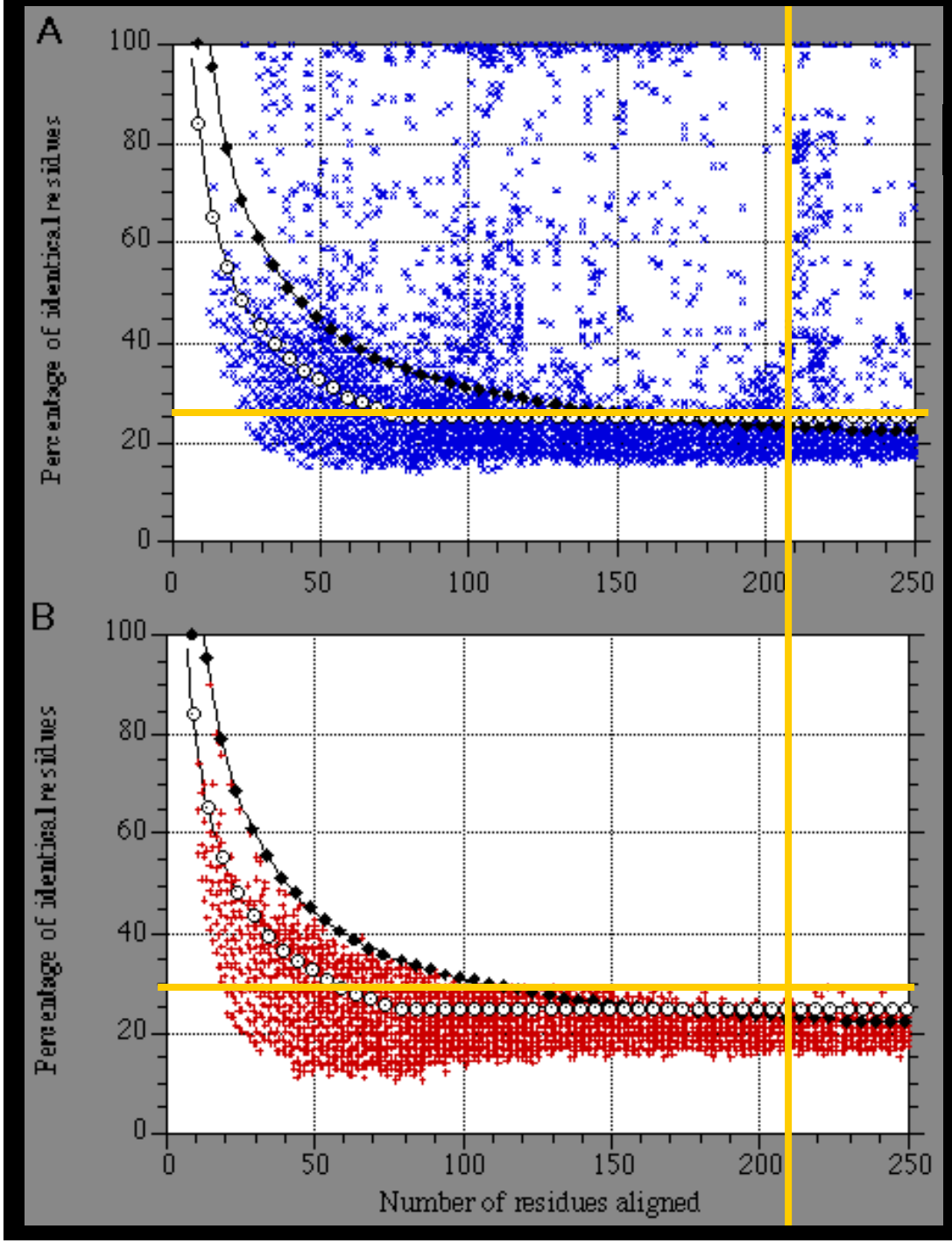
# AChBP and nAChR subunits are similar




LE NOVÈRE *et al.* (2002) *Proc. Natl. Acad Sci. USA,* 99: 3210-3215

# Uneven sequence identities

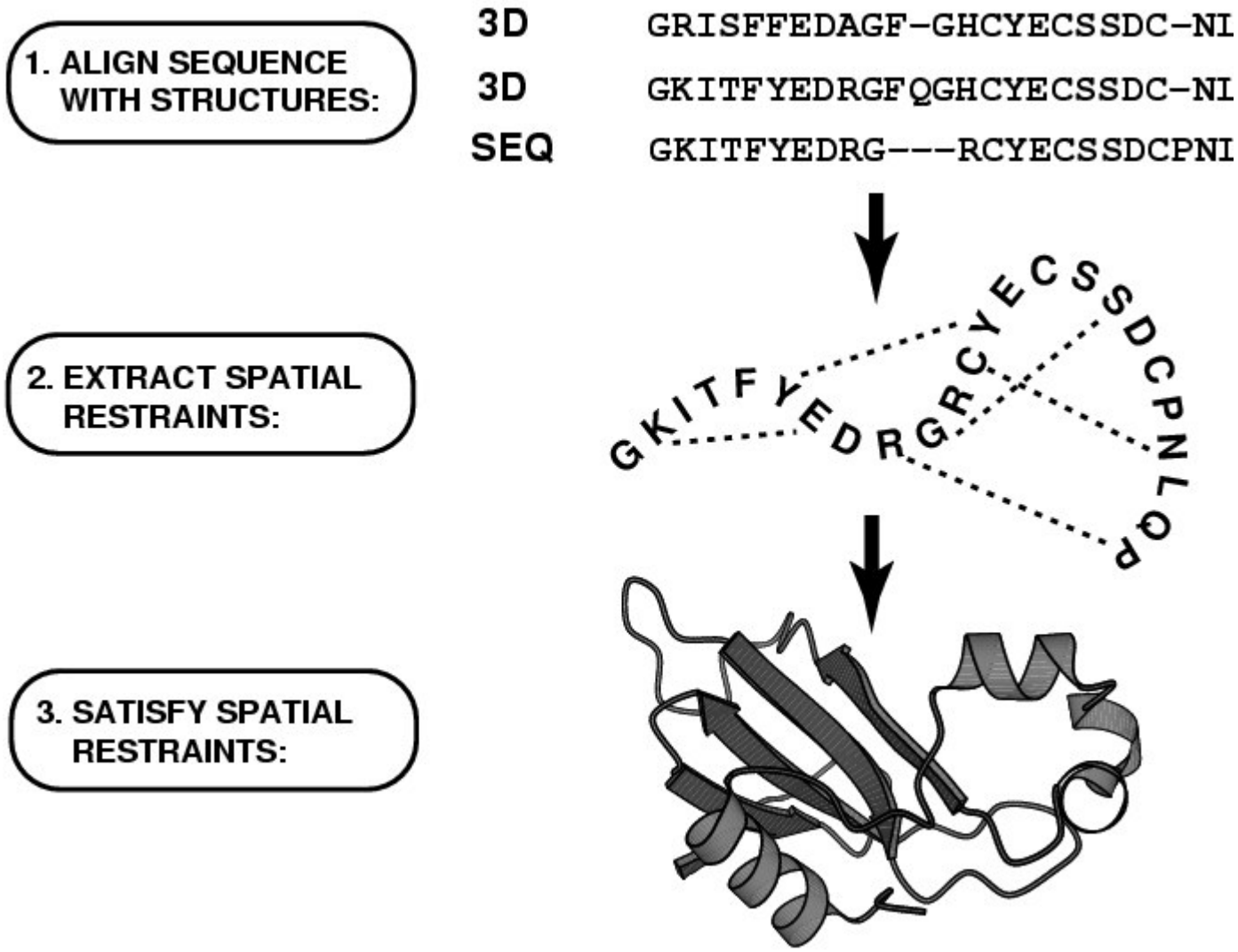
| | AChBP | α7gg | α4rn | β2rr | αltc | γtc | δtc |
|---|---|---|---|---|---|---|---|
| α7gg | 26.4 | | | | | | |
| α4rn | 21.7 | 45.6 | | | | | |
| β2rr | 19.7 | 40.6 | 54.7 | | | | |
| αltc | 20.8 | 38.3 | 52.7 | 49.3 | | | |
| γtc | 20.9 | 34.8 | 39 | 47.5 | 39.3 | | |
| δtc | 22.7 | 35 | 39.4 | 44 | 38.3 | 53.9 | |
| β1tc | 22.6 | 35.1 | 41.7 | 43 | 40.5 | 49.5 | 47.0 |




# What are homologs for the structural biologist?

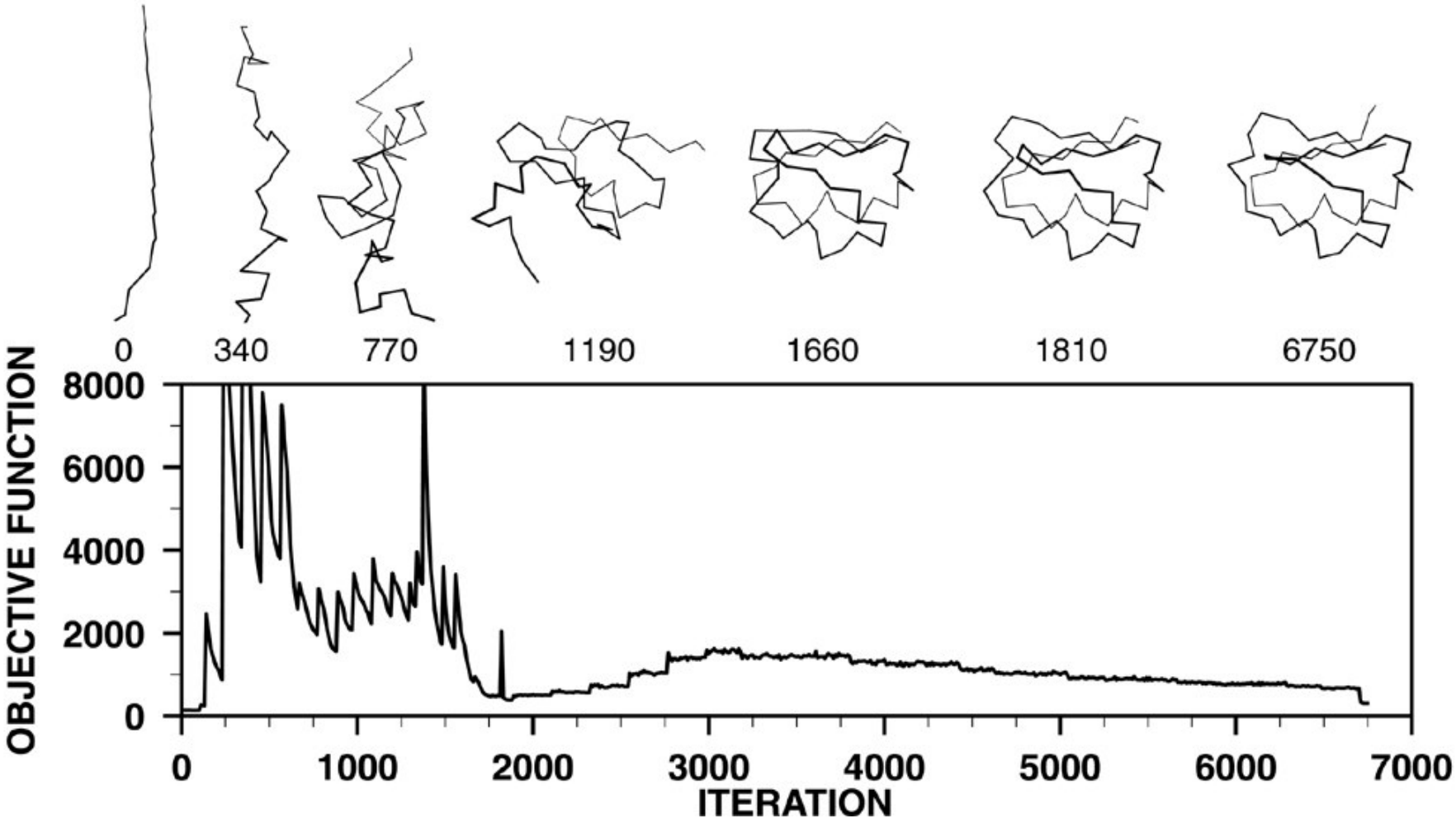
ROST (1999). *Protein Eng.*, 12: 85-94

# MODELLER algorithm (1)

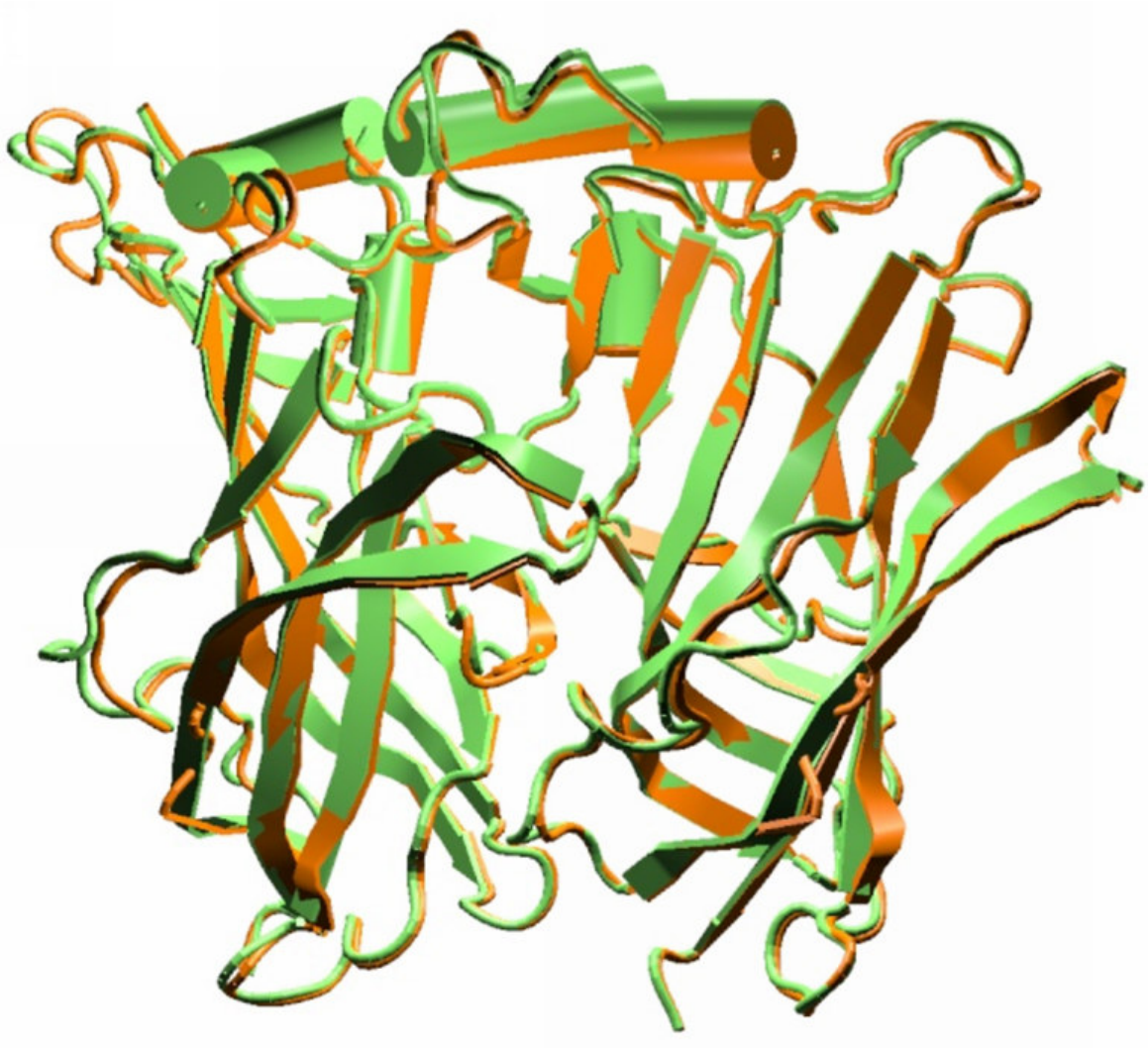



SALI (1993). *J. Mol. Biol.*, 234: 779-815

# MODELLER algorithm (2)

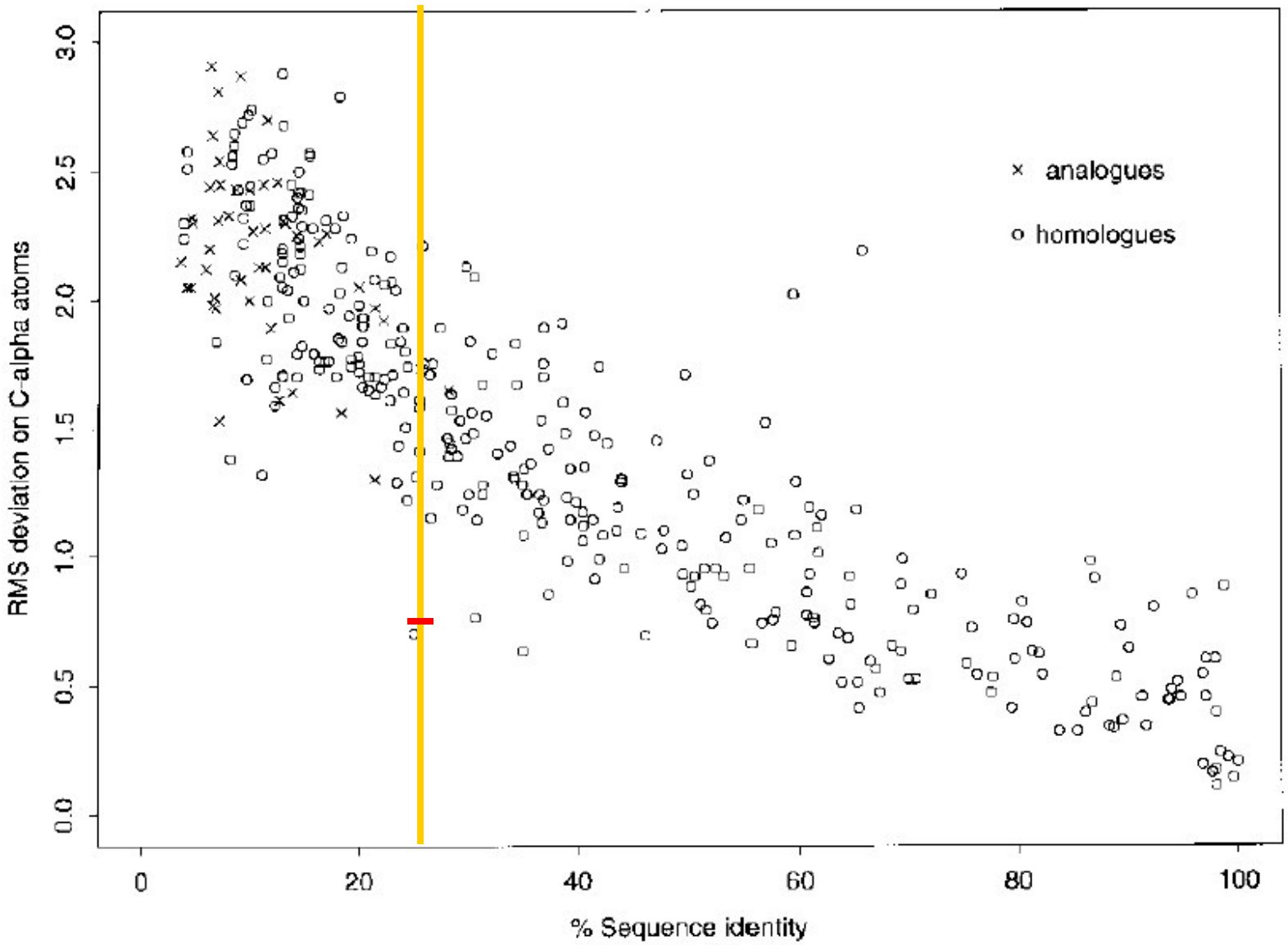


# AChBP structure / model of nAChR α7



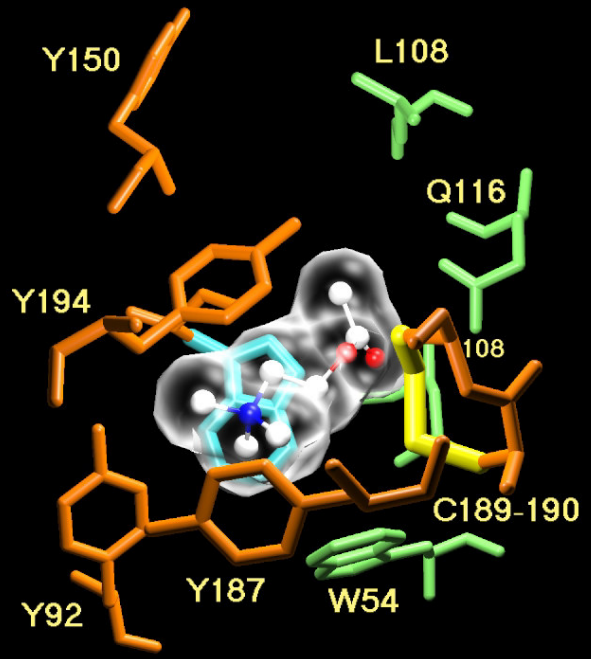
LE NOVÈRE *et al.* (2002) *Proc. Natl. Acad Sci. USA,* 99: 3210-3215

# RMSD Vs sequence identity



# Docking of ACh, Nicotine, and Epibatidine

Autodock3: M**ORRIS** et al. (1998) J Comp Chem, 19: 1639-1662




| | | | |
|---|---|---|---|
| **Exp** : | $2.5\ 10^{-5}$ M | $0.55\ 10^{-6}$ M | $1.9\ 10^{-7}$ M |
| **Comp** : | $4.2\ 10^{-5}$ M | $4.2\ 10^{-6}$ M | $5.6\ 10^{-7}$ M |

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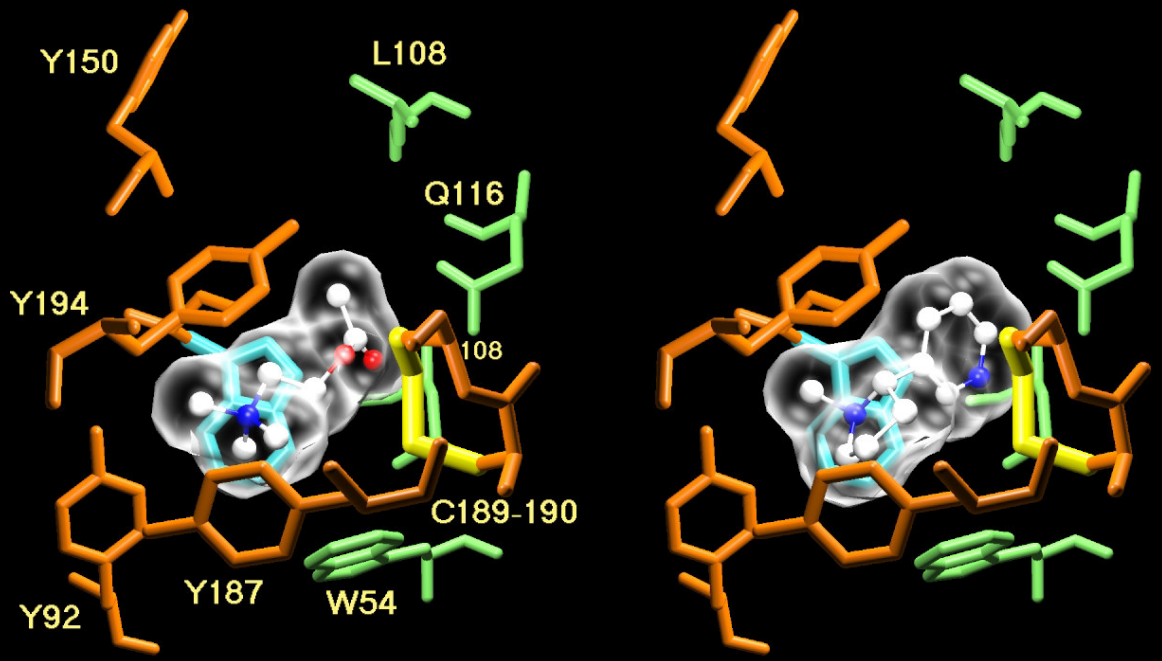


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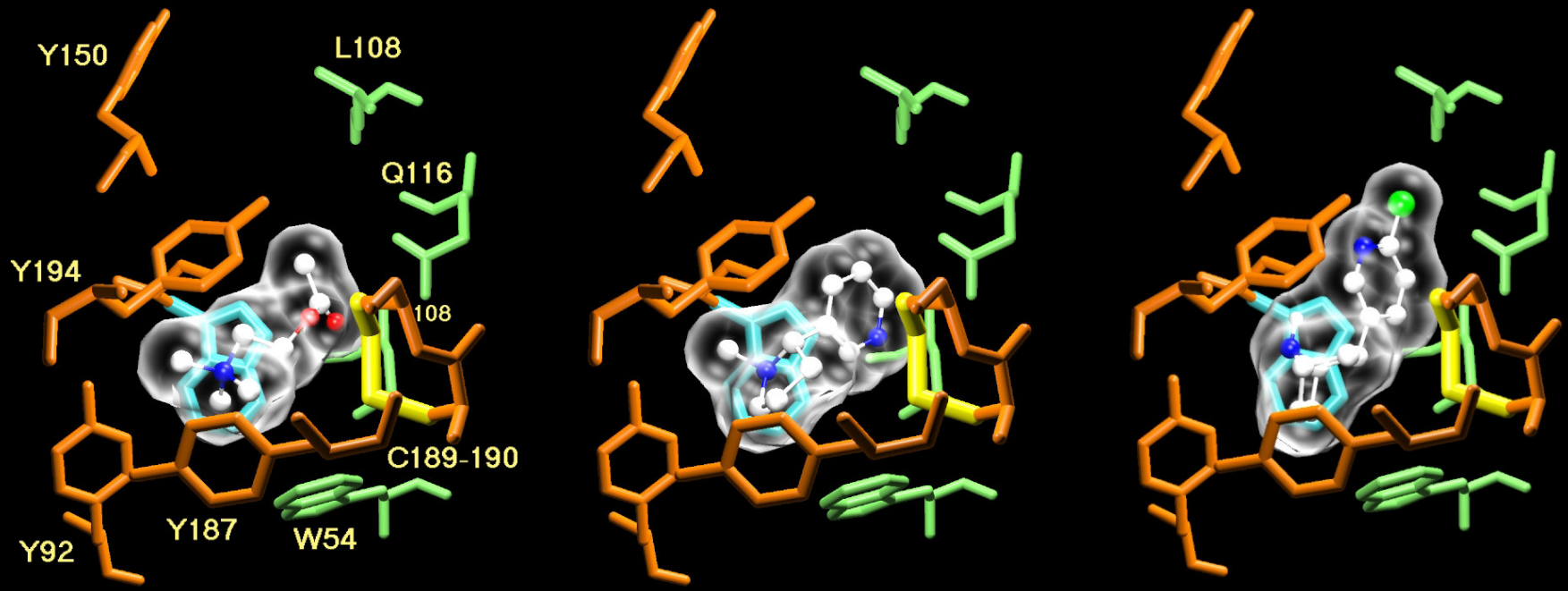


| | | | |
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# α-Bungarotoxin on α7 model

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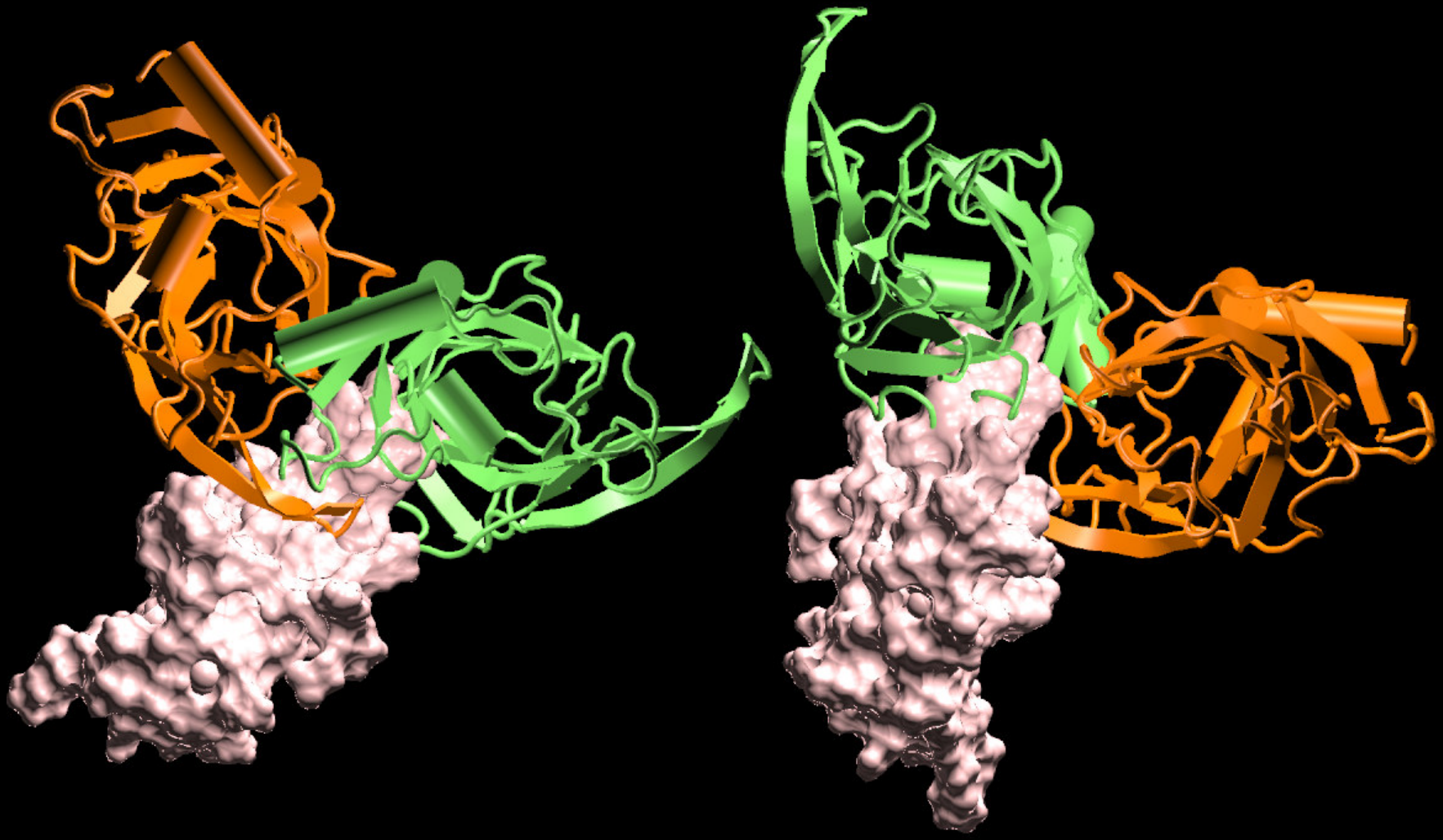
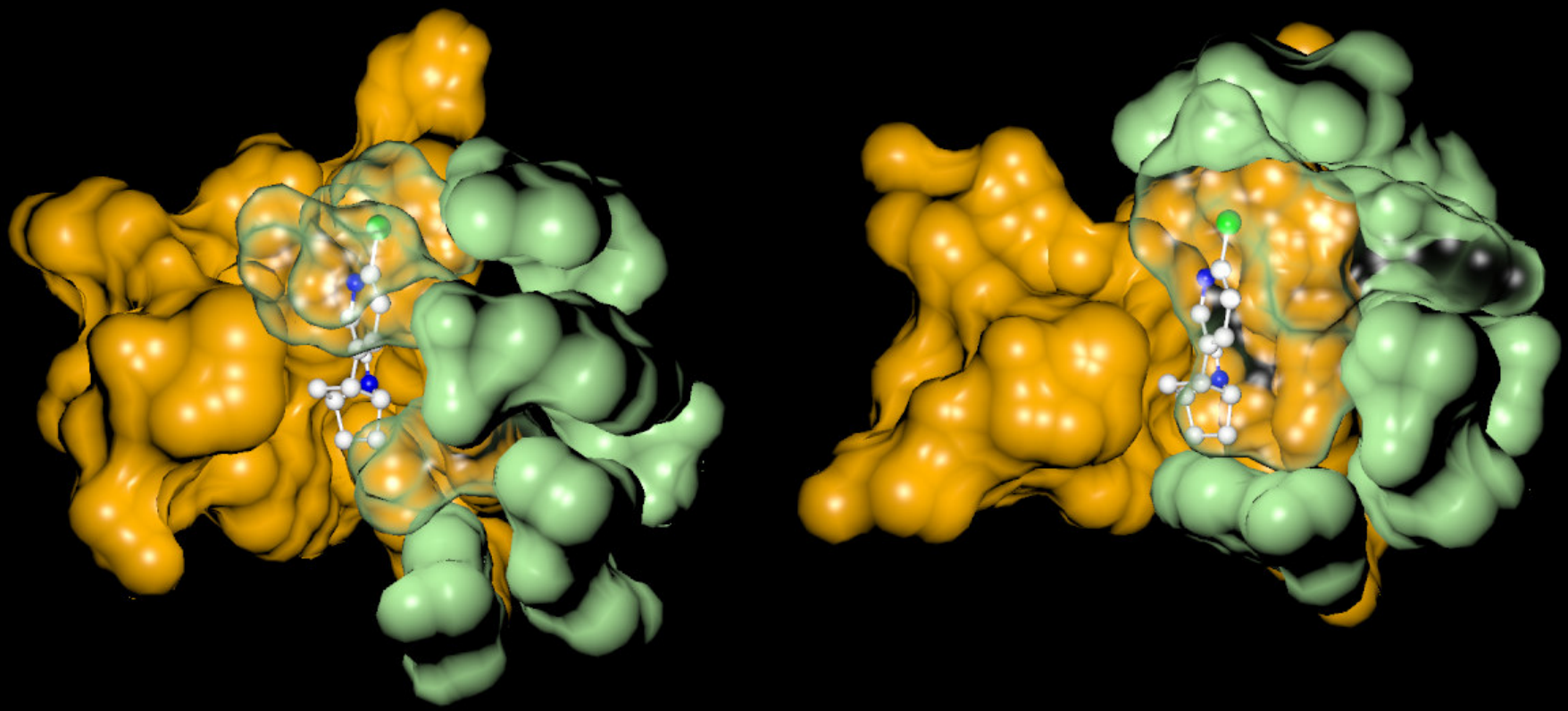


# Epibatidine in models of different α7 states

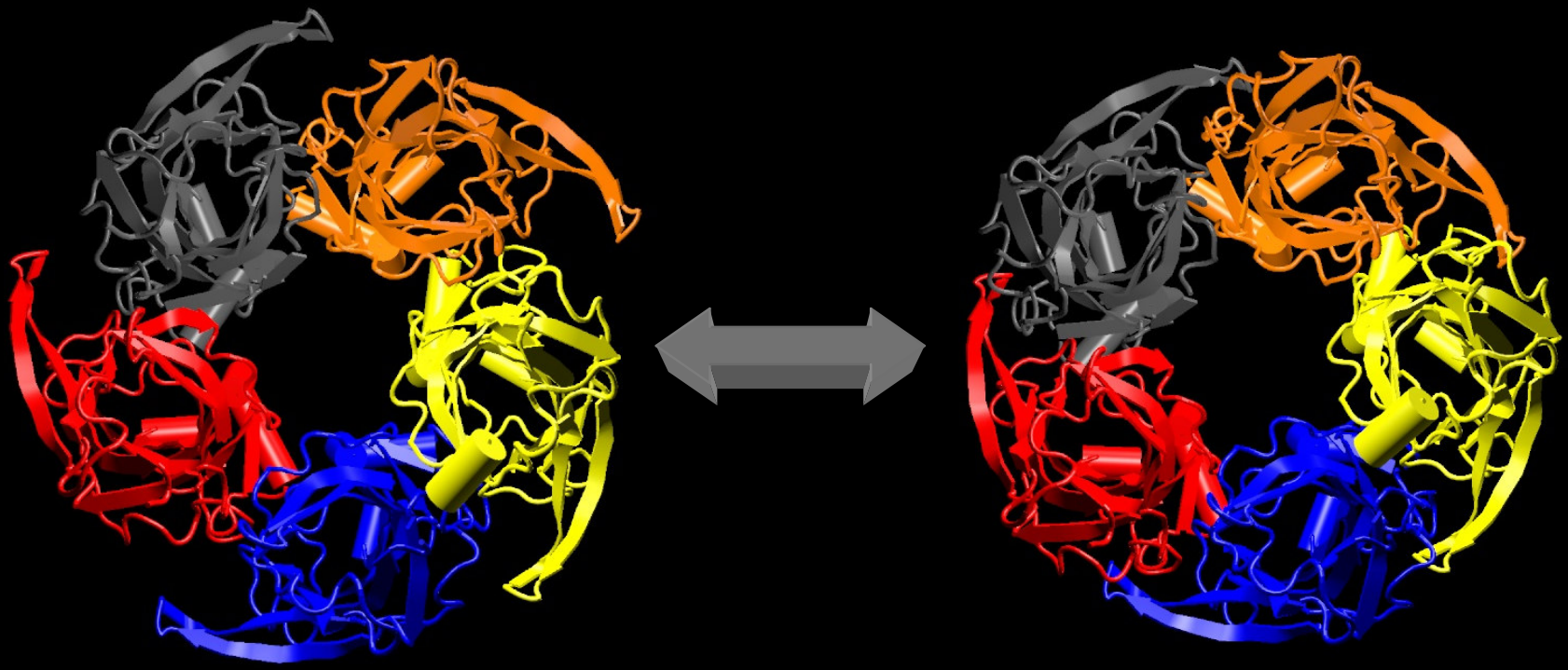


**Basal state: Ki = 1.1 $10^{-5}$ M**

**Desensitized state: Ki = 5.6 $10^{-7}$ M**

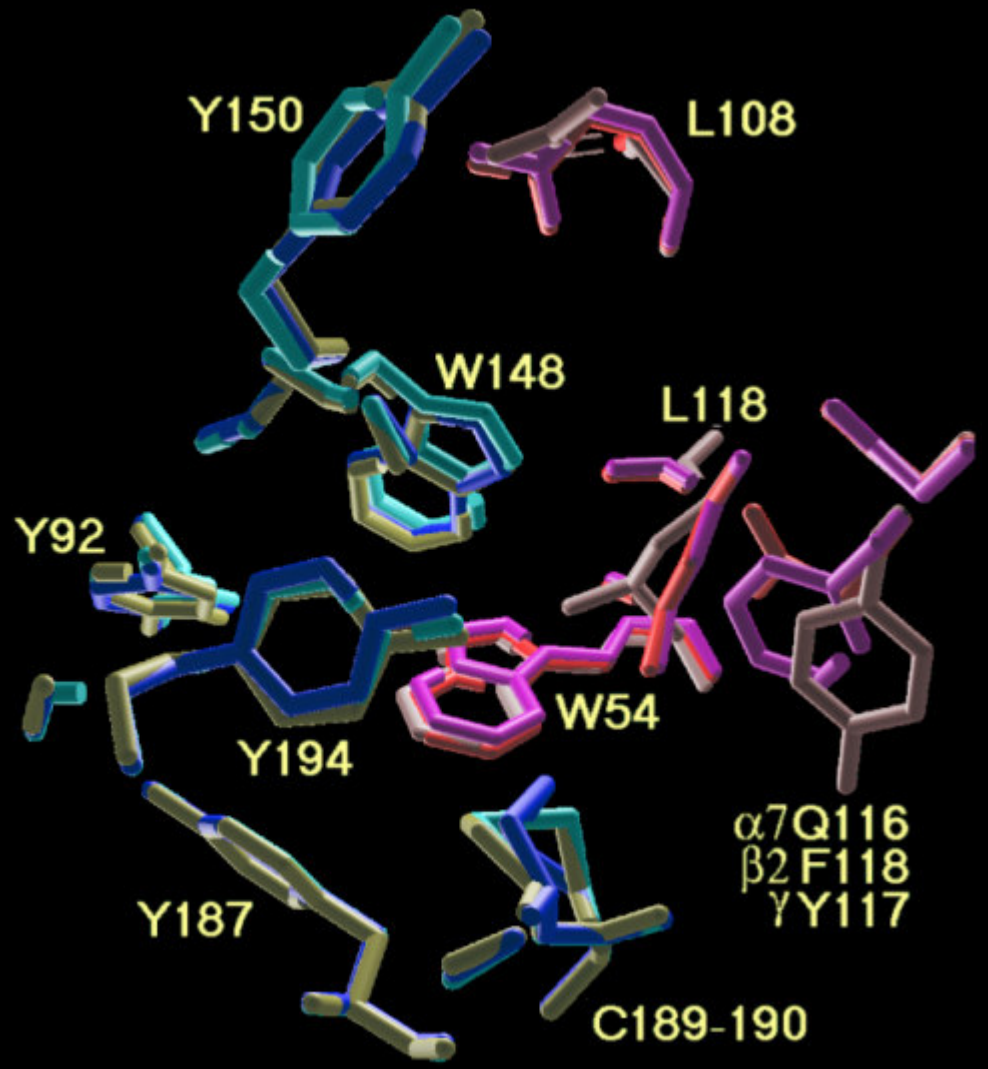
FRUCHART-GAILLARD *et al.* (2002) *Proc. Natl. Acad Sci. USA,* 99: 3216-3222

# A model of concerted transitions

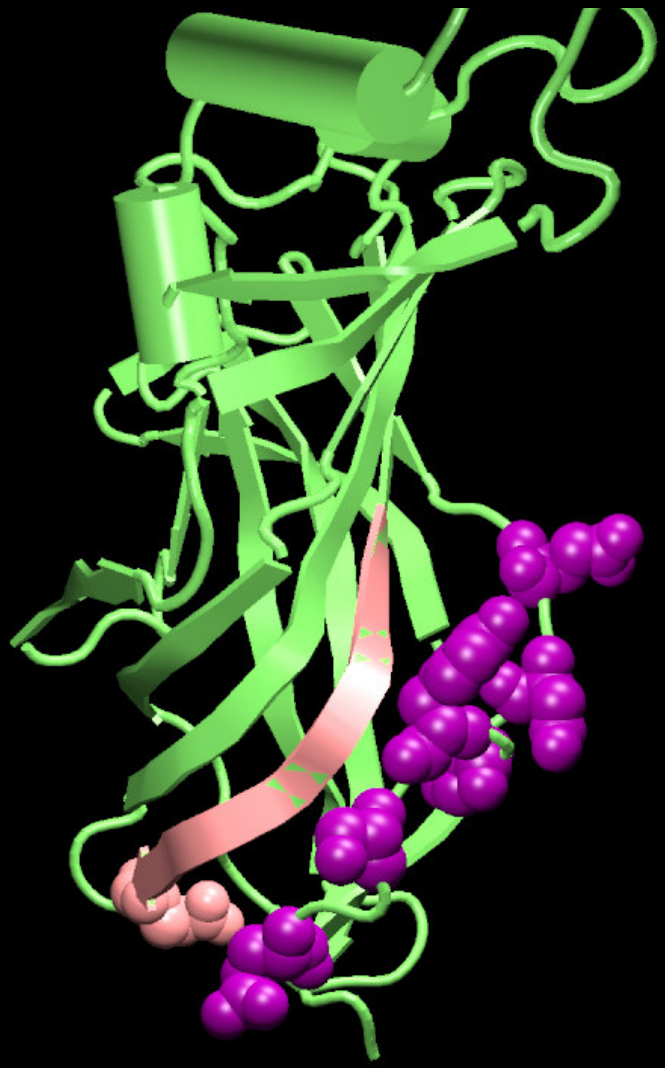


GRUTTER et CHANGEUX. (2001) *Trends Biochem Sci.* 26: 459-463

# ACh binding sites of α7:α7, α4:β2 and α1:γ



# Calcium sites predicted by mutagenesis

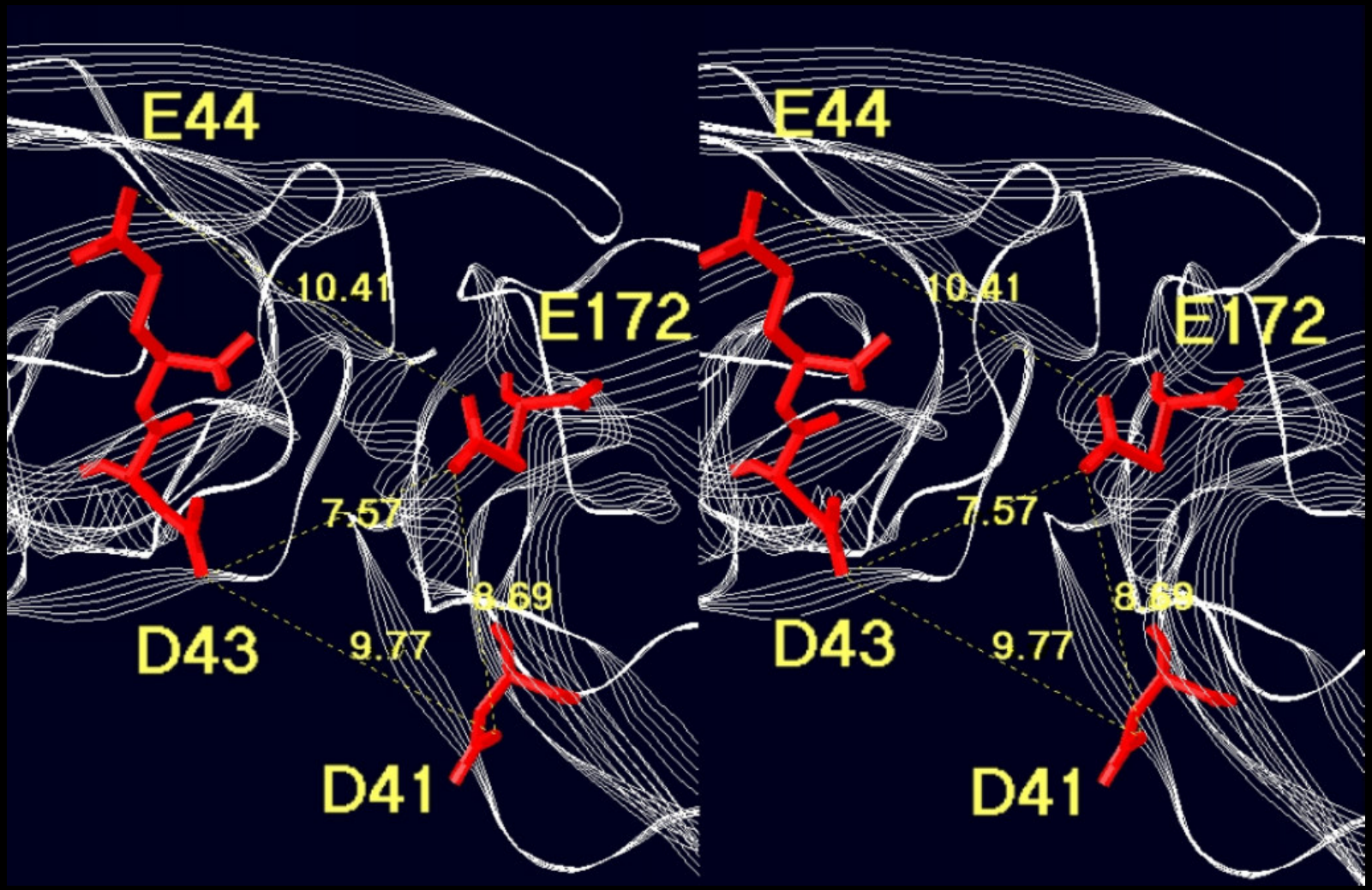


GALZI *et al.* (1996) *EMBO J.*, 15: 5824-5832

# Calcium sites predicted by mutagenesis

GALZI *et al.* (1996) *EMBO J.*, 15: 5824-5832

# Inter-subunit $Ca^{++}$ Binding site



# Perspectives

nAChRs can now be modelled at the atomic level under various conformations; We can envision to understand the structural basis of pharmacological specificity, and even the mechanisms of allosteric transitions.

Program:

- Modelisation of the various state of the extracellular portion;
- Modelisation of the transmembrane domain;
- Mechanism of the coupling between the two domains.

# LGICdb

**http://www.pasteur.fr/recherche/banques/LGIC/LGIC.html**

- three different superfamilies
  - Cys-loop superfamily
  - Glutamate cationic superfamily
  - ATP P2x superfamily
- 402 entries
- 36 species
- sequences
  - Proteins, transcripts, genomic;
  - FASTA, Genbank, EMBL;
- alignments; phylogeny; structures;

# Acknowledgments


## Jean-Pierre Changeux

- Structural biology
  - Daniel Bertrand
  - Sonia Bertrand
  - **Pierre-Jean Corringer**
  - Anne Devillers-Thiéry
  - **Jean-Luc Galzi**
  - Thomas Grutter
- Molecular Modelling
  - Catherine Fruchart-Gaillard
  - **Bernard Gilquin**
  - André Ménez
  - Denis Servent