About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 500 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

OMICS International is a pioneer and leading science event organizer, which publishes around 700+ open access journals and conducts over 500+ Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS International has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

From Peptide and Protein Neurotoxins to Receptor Structure-Function and New Drugs.

Victor Tsetlin

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

Protein Engineering, Chicago , October 26-28 2015

Introduction

and a bit of history

Receptor



Electric ray ***Torpedo californicais*** **"stuffed" with**

Toxin



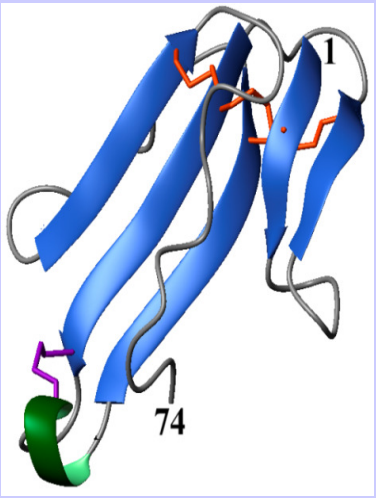
Krait ***Bungarus multicinctus contains*** ***α-bungarotoxin*** ***which binds specifically and tightly***

nicotinic acetylcholine receptor (nAChR)

Karlsson E et al., FEBS Lett.28, 107-111 (1972)

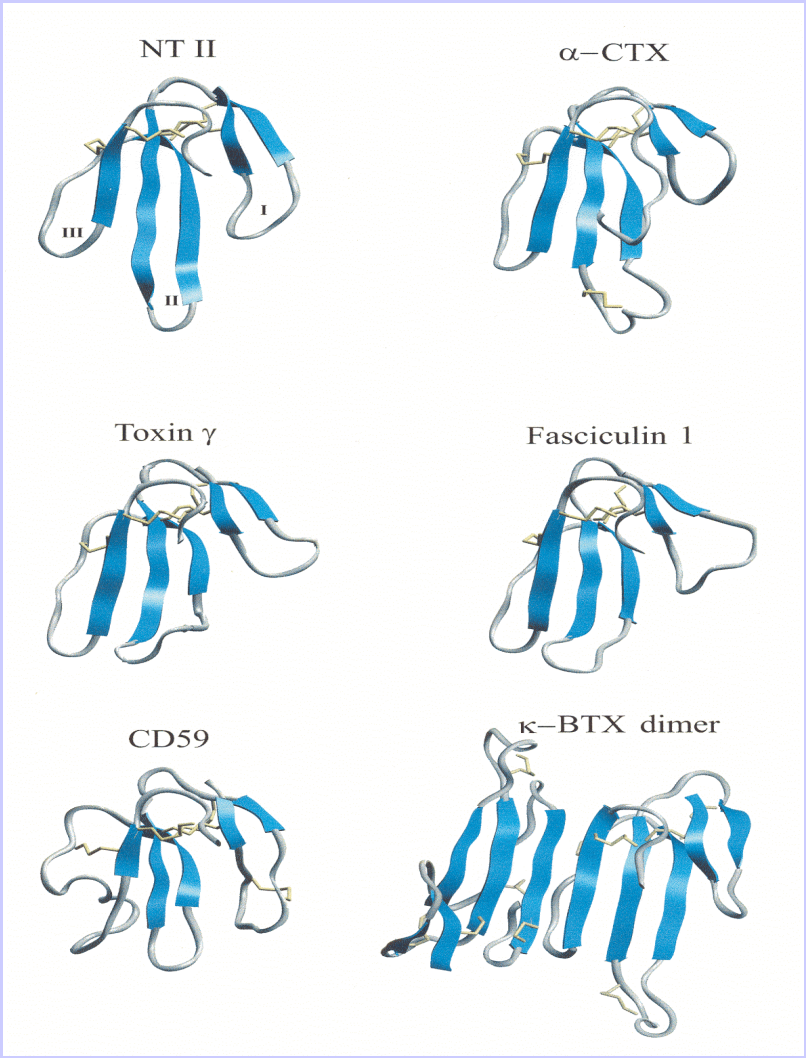
Different groups of three-finger proteins from snake venoms

α-bungarotoxin



Muscle-type nAChRs

Muscle-type and neuronal α7 and α9 nAChRs



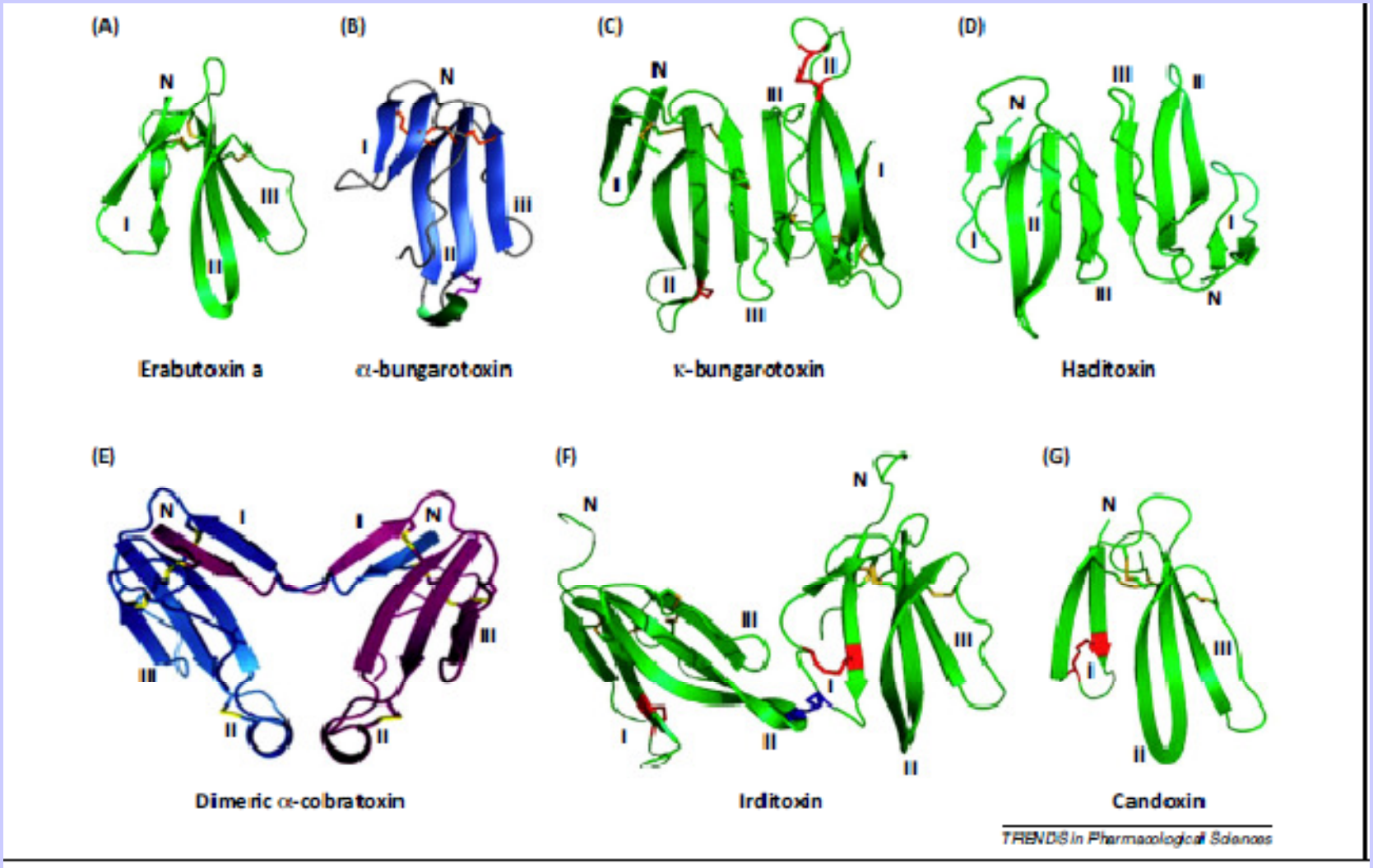
Nonspecific cleavage of membranes

Acetylcholinesterase

Ly6 proteins: not from snakes !

Neuronal α3β2 nAChRs

Three-finger α-neurotoxins acting on nicotinic acetylcholine receptors (nAChRs)



Conotoxins

C.geographus

(the geography cone)

C. striatus

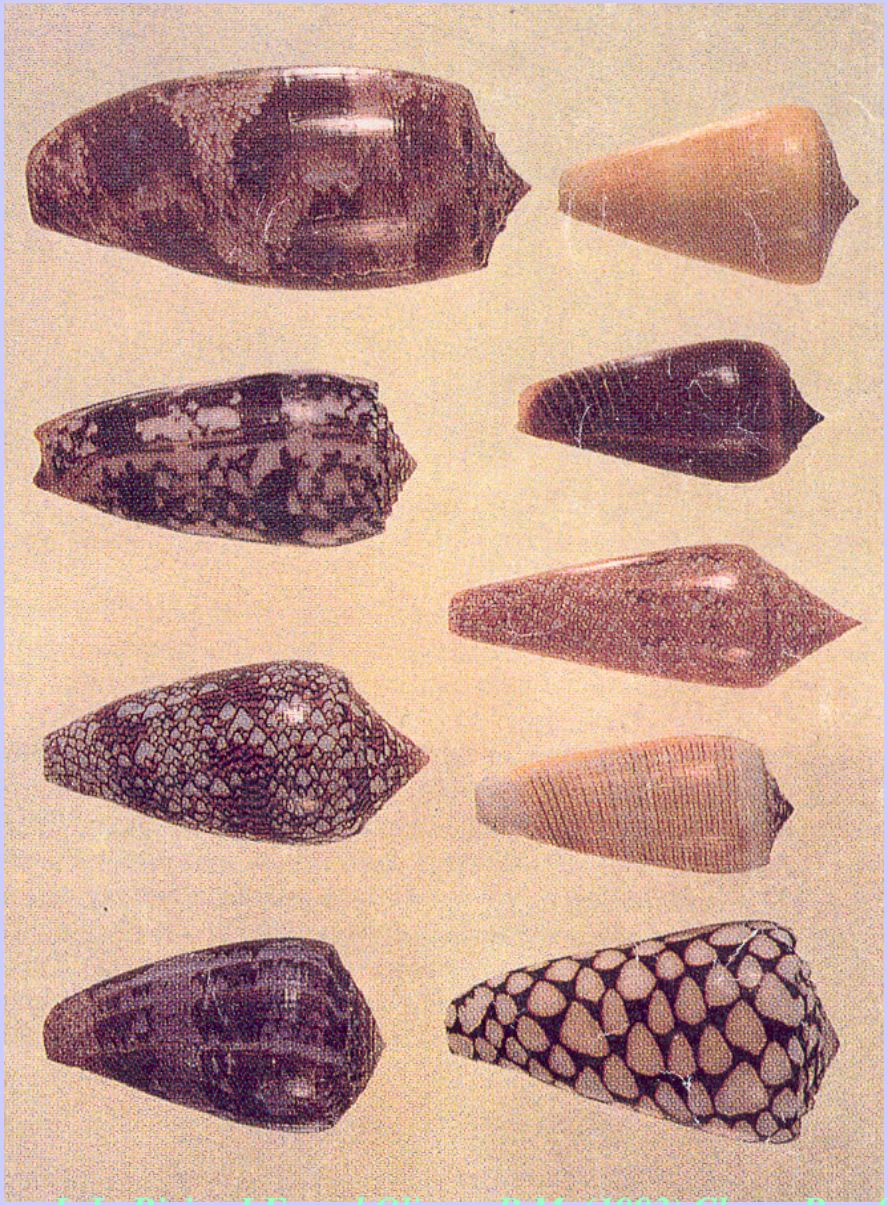
(the striated cone)

C.textile

(the cloth-of-gold cone)

C.purpurascens

*(*the purple cone)



C.quercinus

(the oak cone)

C.radiatus

(the radial cone)

C.gloriamaris

(the glory-of-the – sea)

C.magus

*(*the magus cone)

C.marmoreus

(the marble cone)

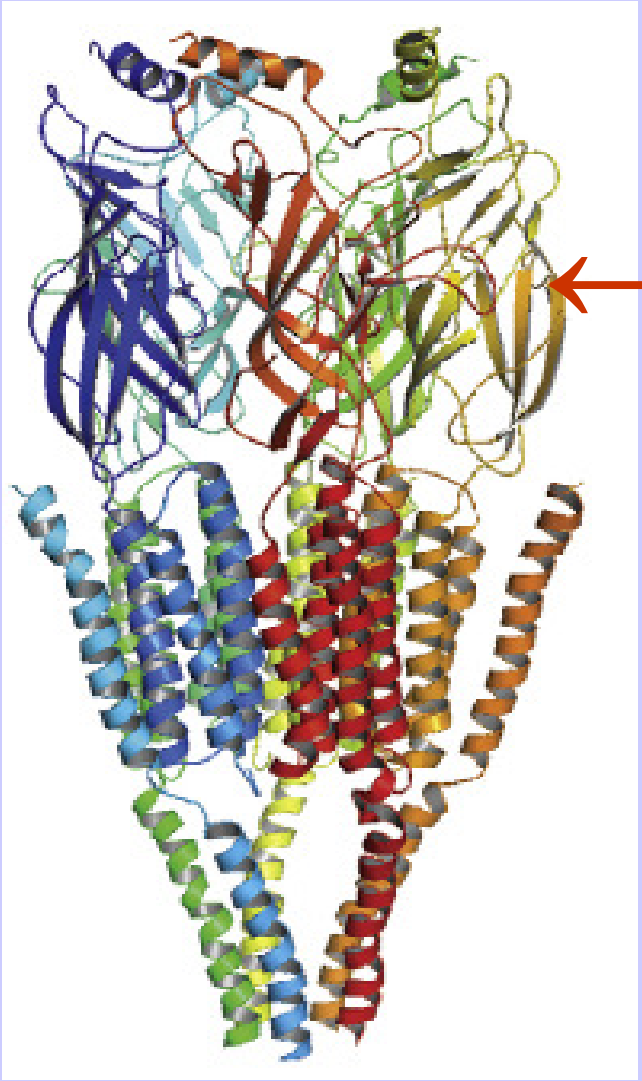
Myers R.A., Cruz L.J., Rivier J.E. and Olivera B.M. (1993) Chem. Rev. 93, 1923-1936

Family of nicotinic acetylcholine receptors

LBD

TMD

CID



agonists;

antagonists: ***exogenous neurotoxins*** **from snake and snail venoms,**

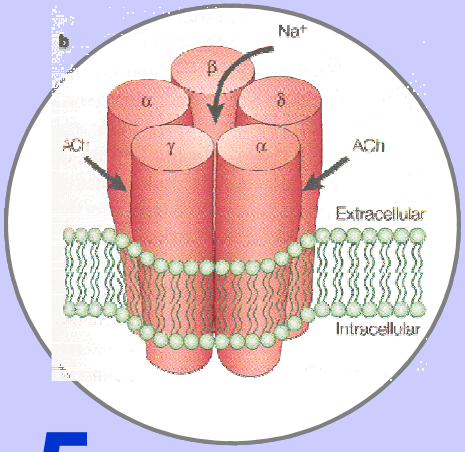
endogenous prototoxin Lynx1 (***binds here and somewhere else***)

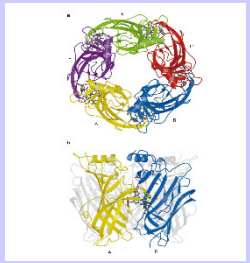
Cationic excitatory ligand-gated ion channel

Cryo-EM structure of *Torpedo* nAChR,

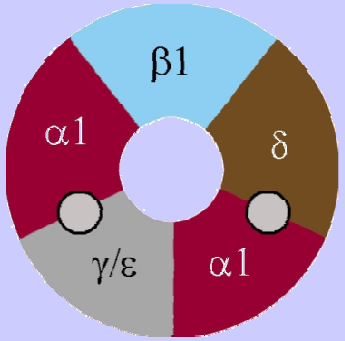
Unwin (2005)

Structural diversity of nicotinic acetylcholine receptors





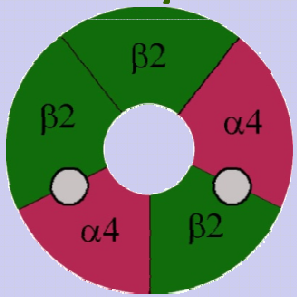
muscle subunits
α1, β1, γ or ε, δ



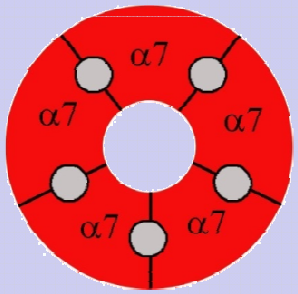
5 ***subunits***

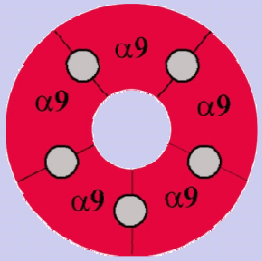
neuronal *subunits :*
α2 α3
α4 α5

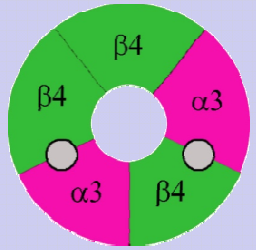
α4 β2



α7

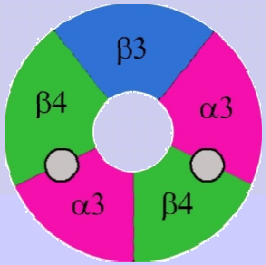


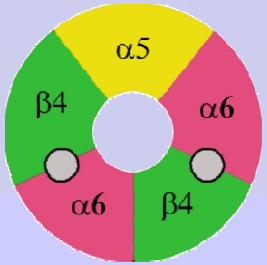


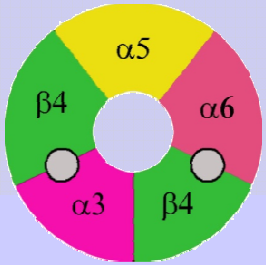


Affinity for nicotine ***6 nM*** ***2000 nM***

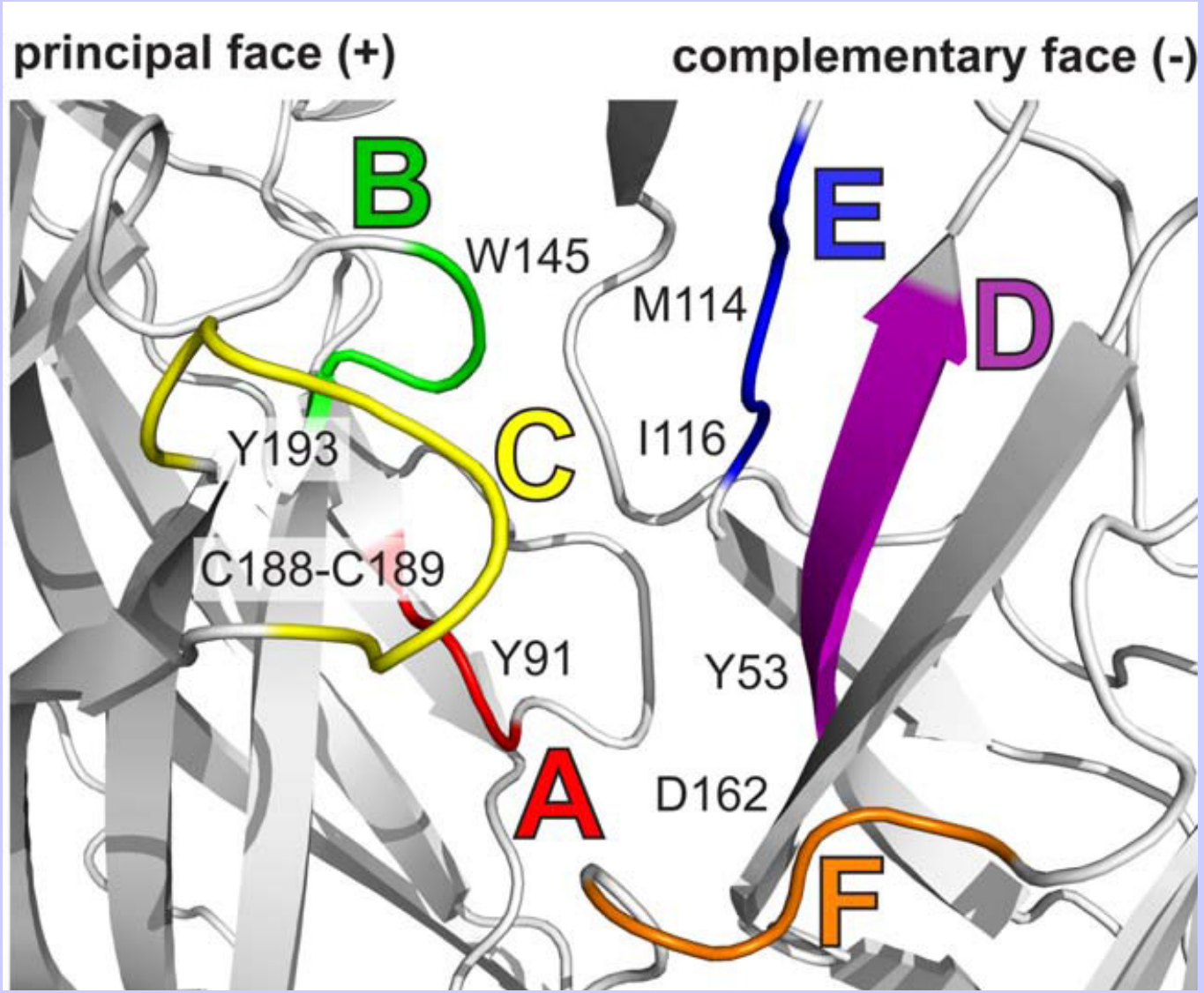
α6 α7
α8 α9 α10
β2, β3, β4,



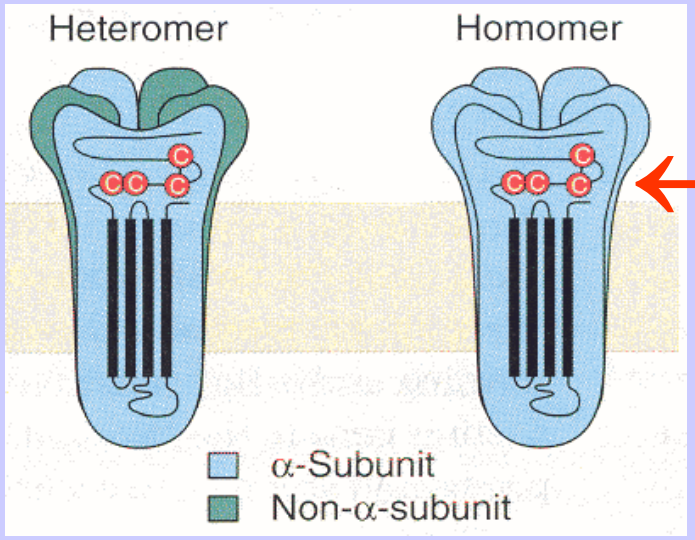


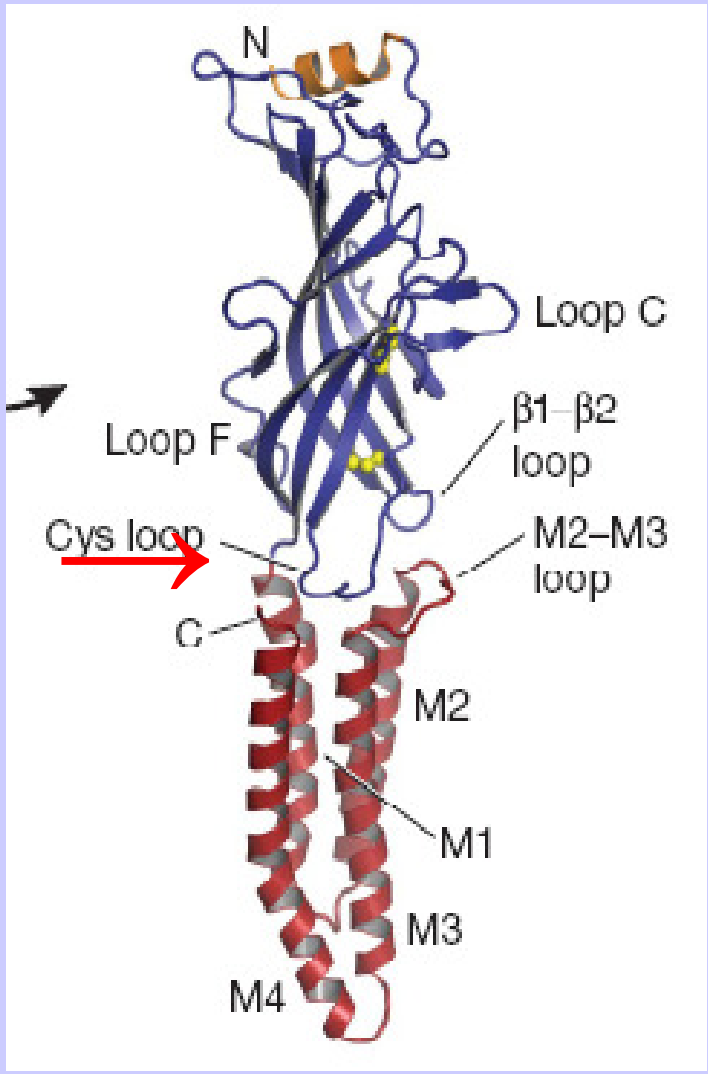


Fragments of principal ("front') and complementary ("back") sides of the subunits at the interface



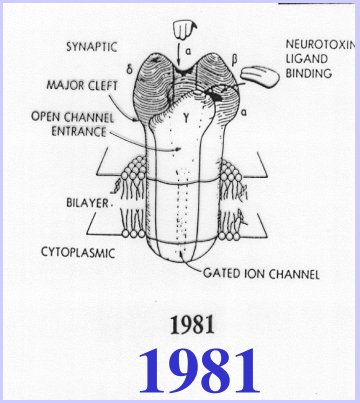
Cys-loop ligand-gated ion channels



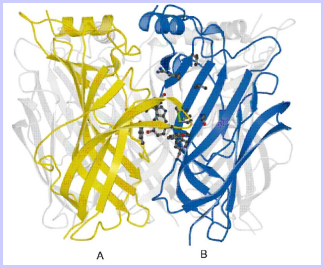


1. **Nicotinic acetylcholine receptors рецепторы**
2. **5HT3 serotonin receptors**
3. **Glycine receptors**
4. **Ionotropic GABA-A receptors**
5. **Bacterial ligand –gated channels (ELIC, GLIC)**
6. **Glutamate receptor *C.elegans***

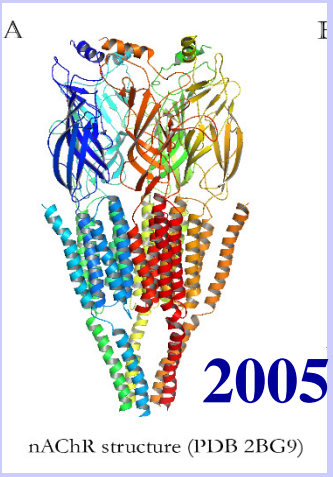
3D structures in 3 Decades

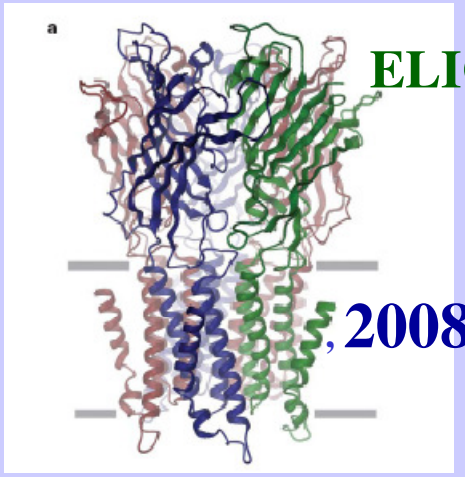


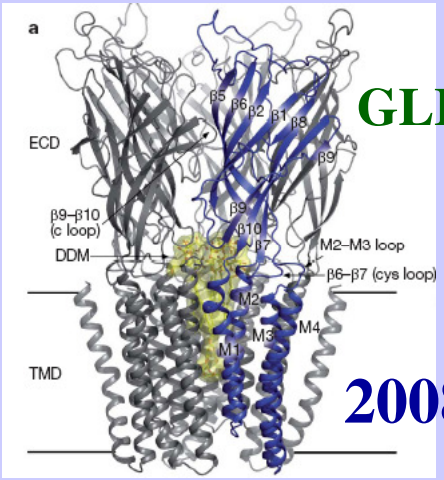
1981

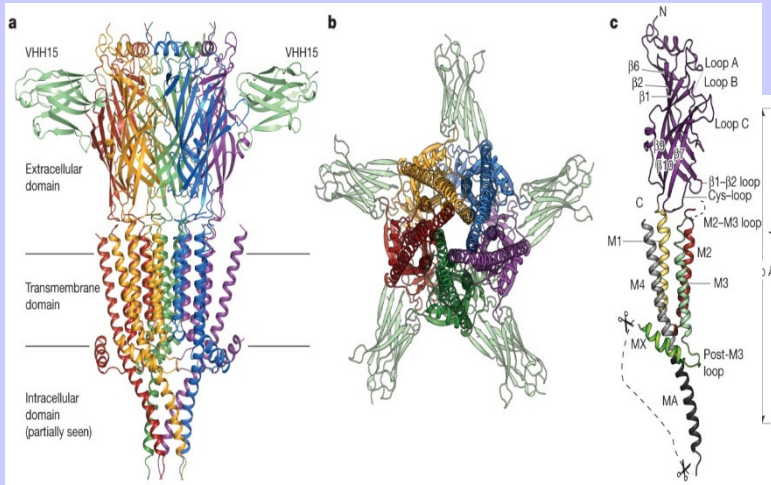


2001

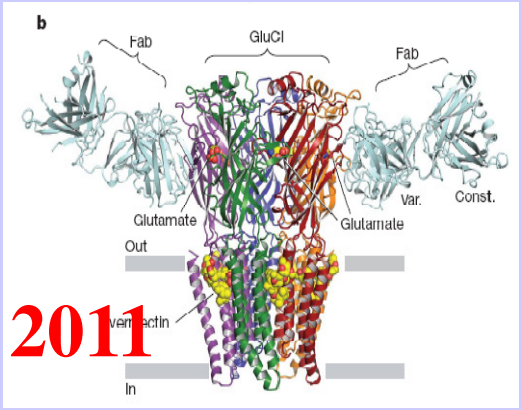








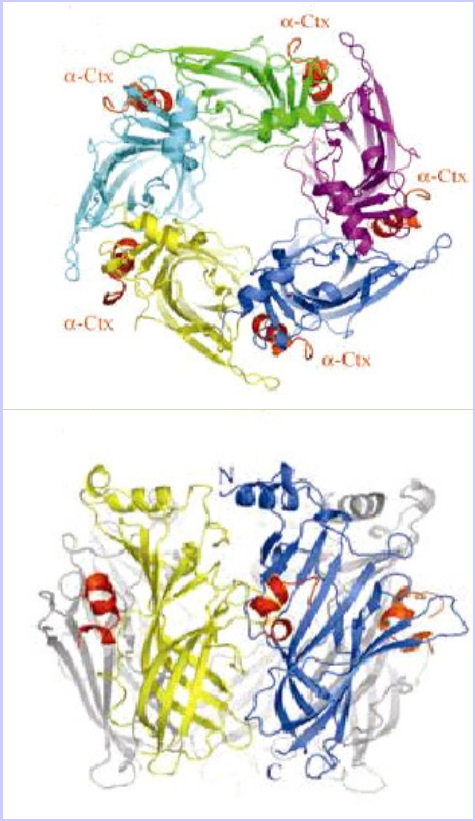
rat 5-HT3



. human GABAA R-β3

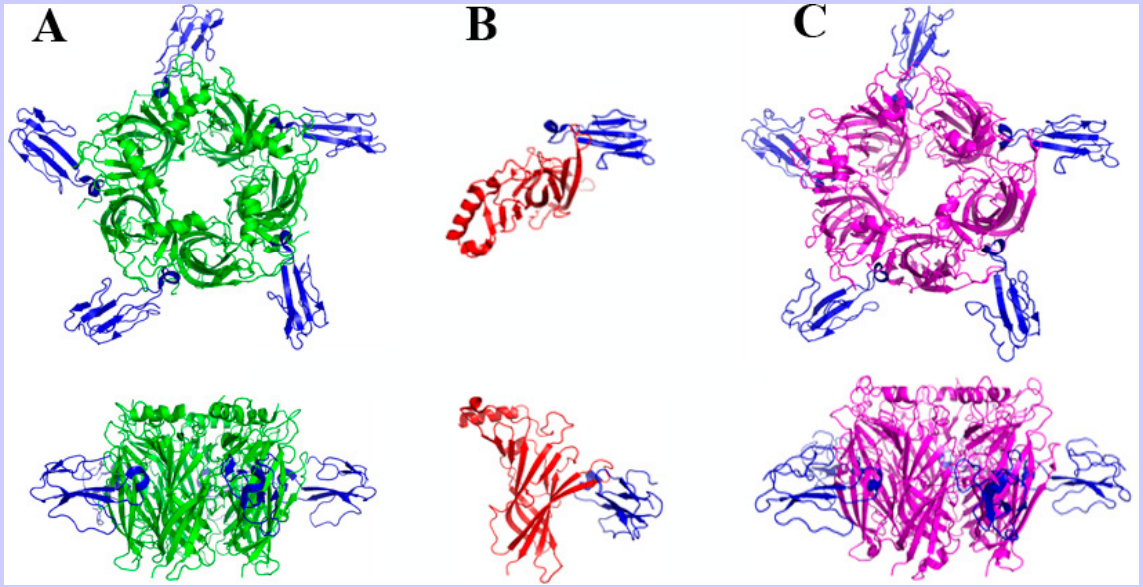
Crystal structures of α-conotoxin and α- neurotoxin complexes with nAChR models

α-conotoxin



Celie, Kasheverov *et al.*, 2005

α –cobratoxin or α-bungarotoxin



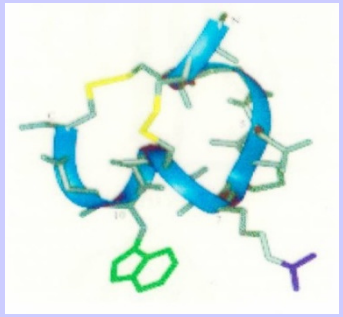
A: Bourne *et al.*, 2005
B: Dellisanti *et al.*, 2007
C: Huang et al., 2013
Zouridakis et al., 2014 (α9)

Our work

Objects (targets)

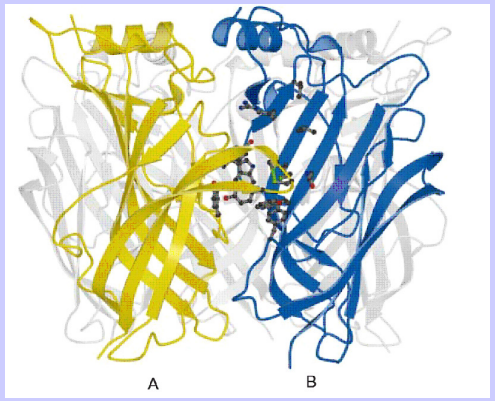
Tools



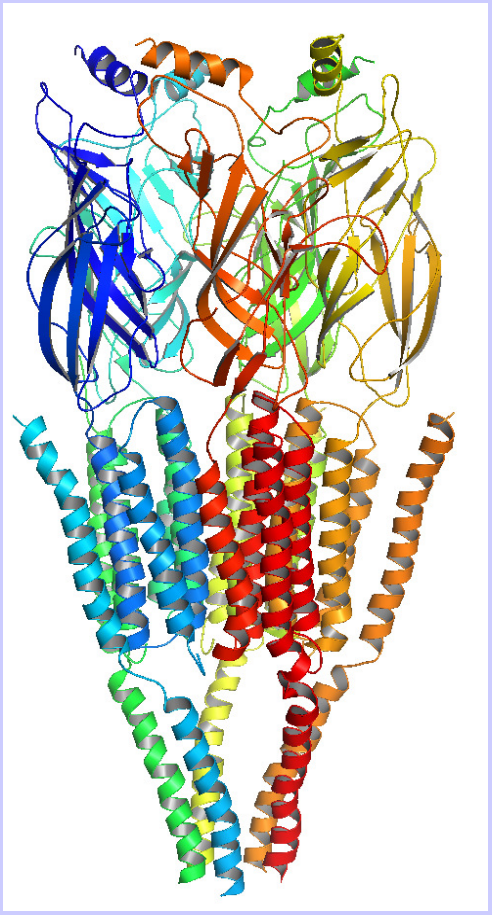


α-conotoxins

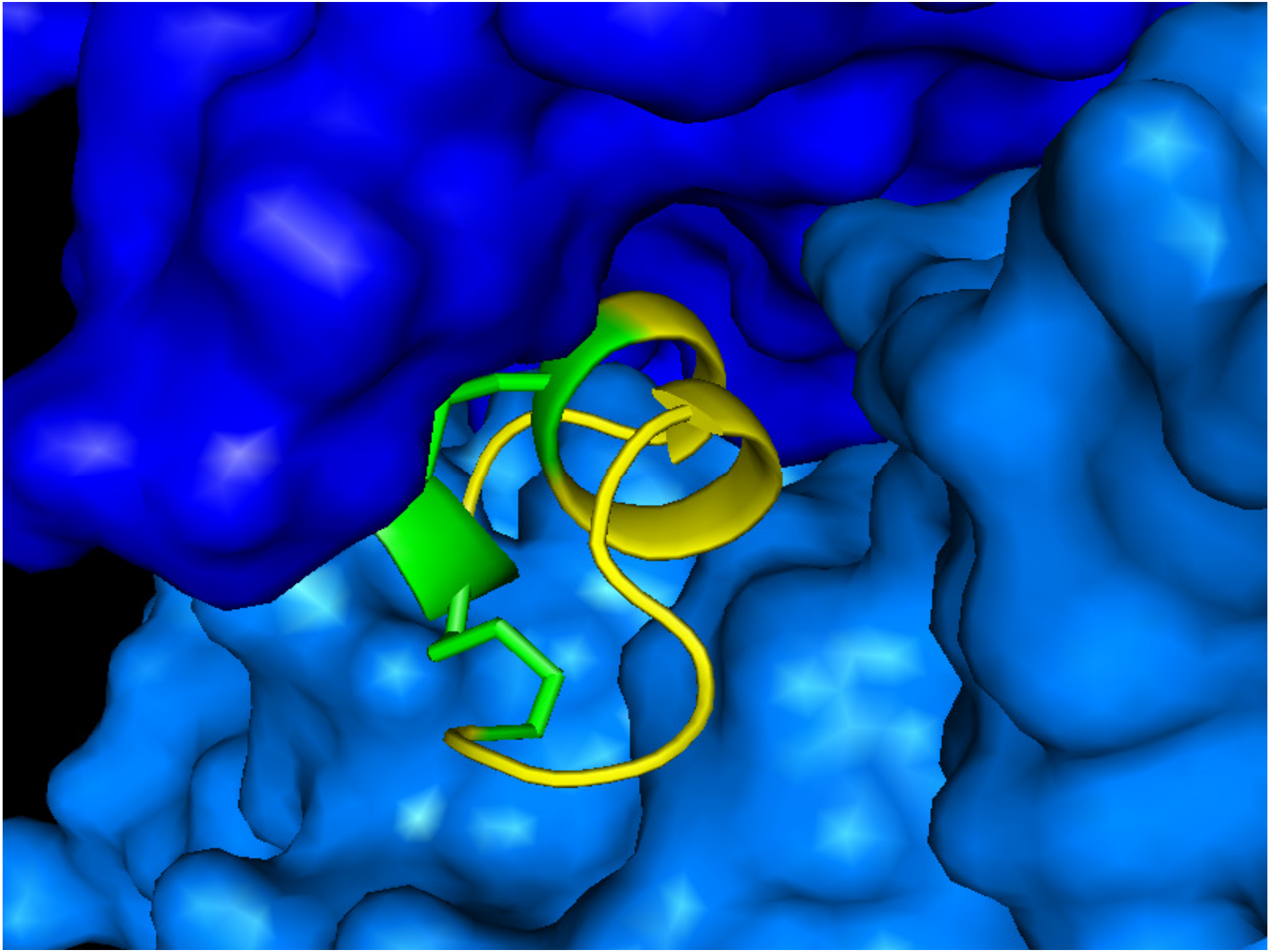
α-neurotoxins



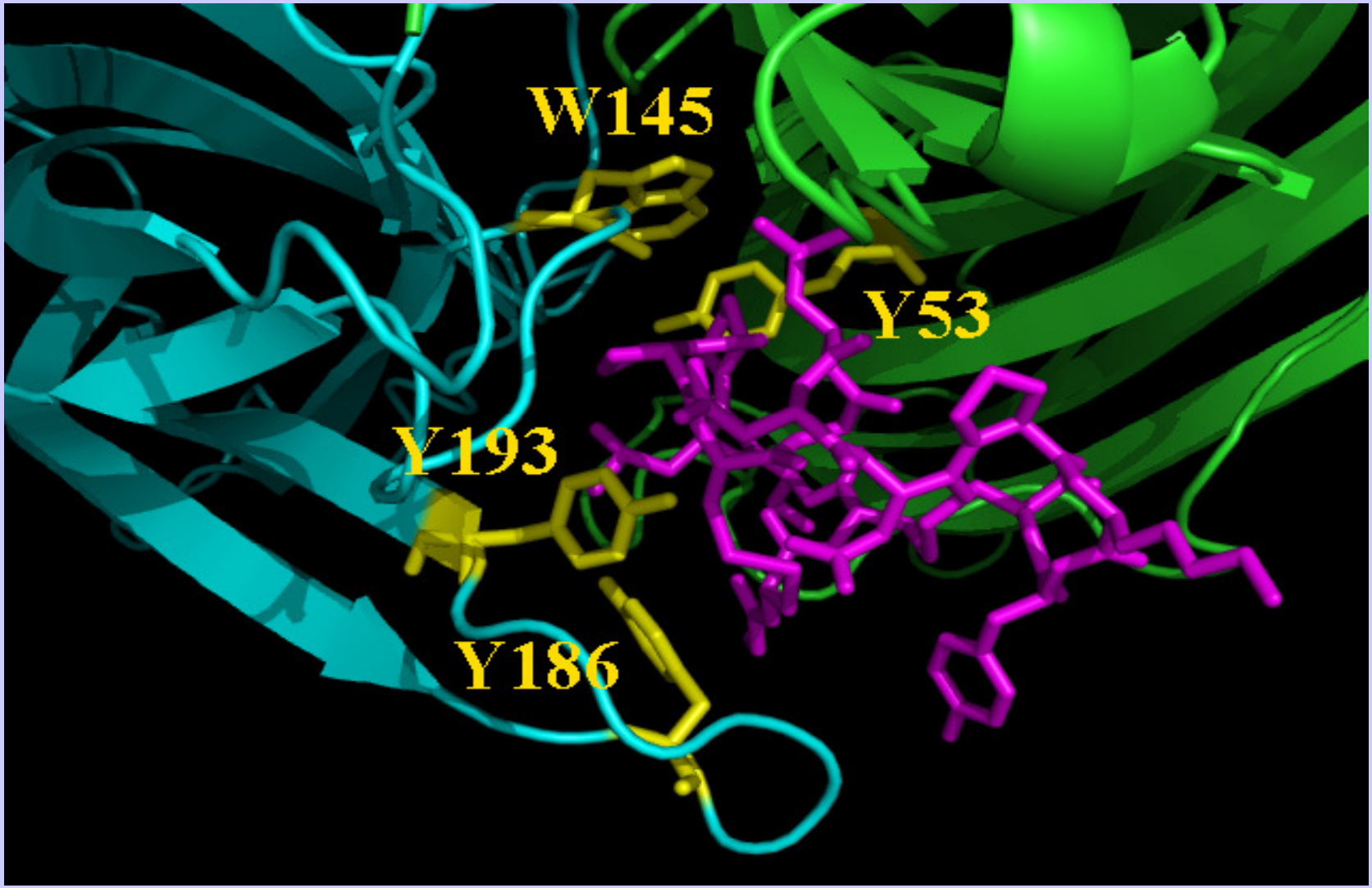
AChBPs



nAChRs



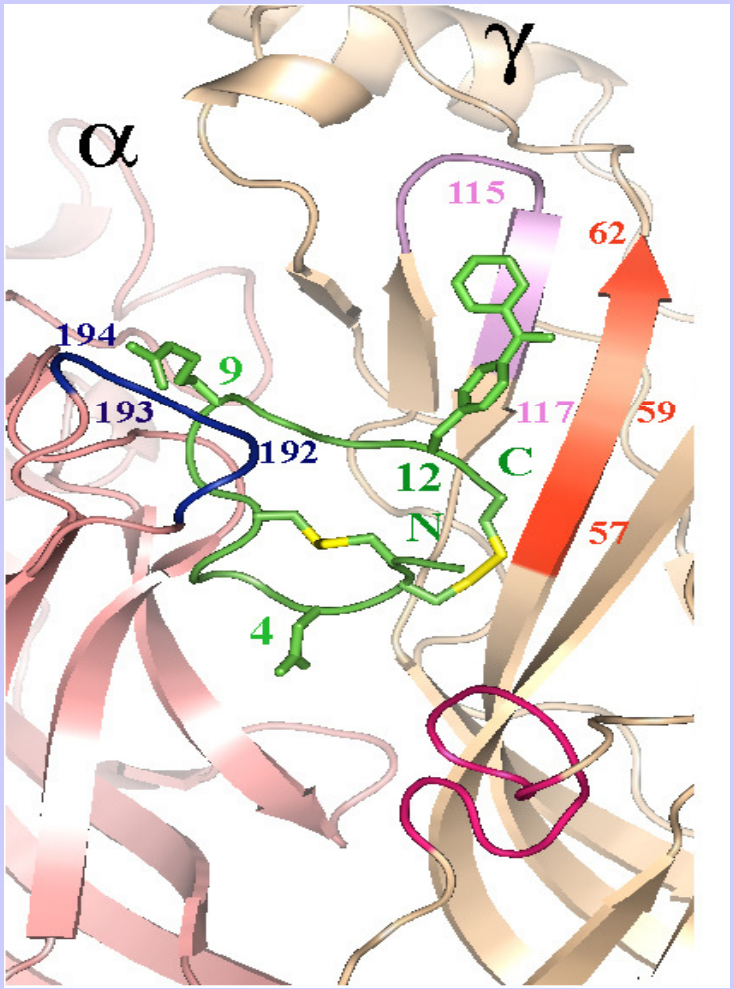
Aromatic box and loop C in the binding site for agonists and competitive antagonists

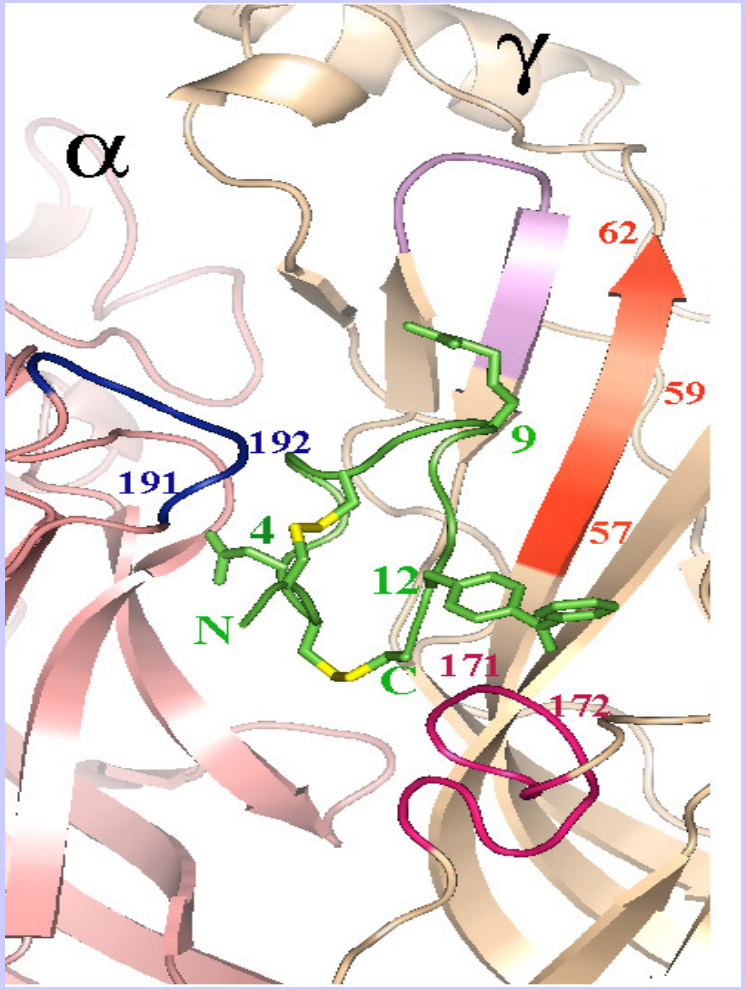


Celie P.H.N., Kasheverov I.E. et al, Nature Str.&Mol.Biol. 12, 582-588 (2005)

Orientation of photoactivatable α-conotoxin GI (Bpa12) at the *Torpedo* nAChR binding sites

(combination of "wet biochemistry" and computer modeling)





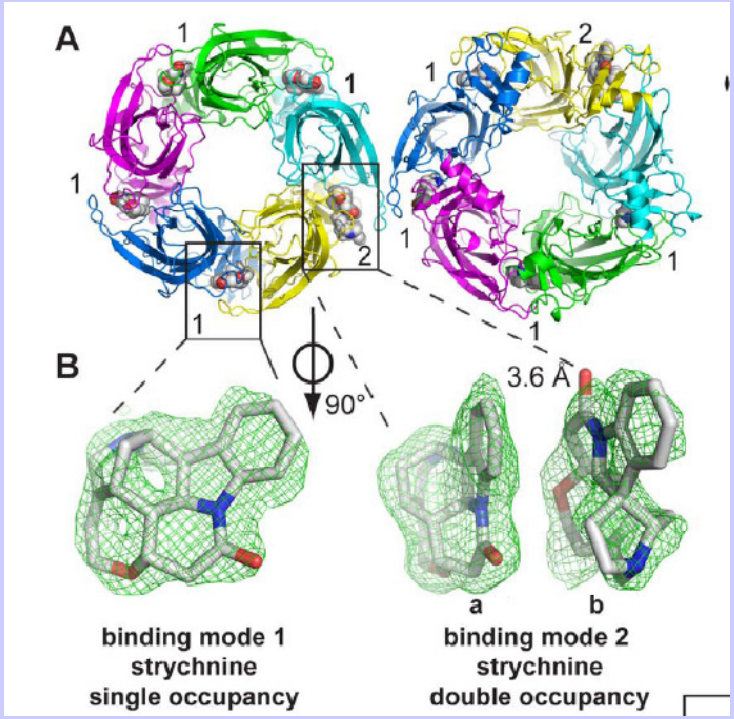
Kasheverov IE et al. FEBS J. 273,1373-1388 (2006).

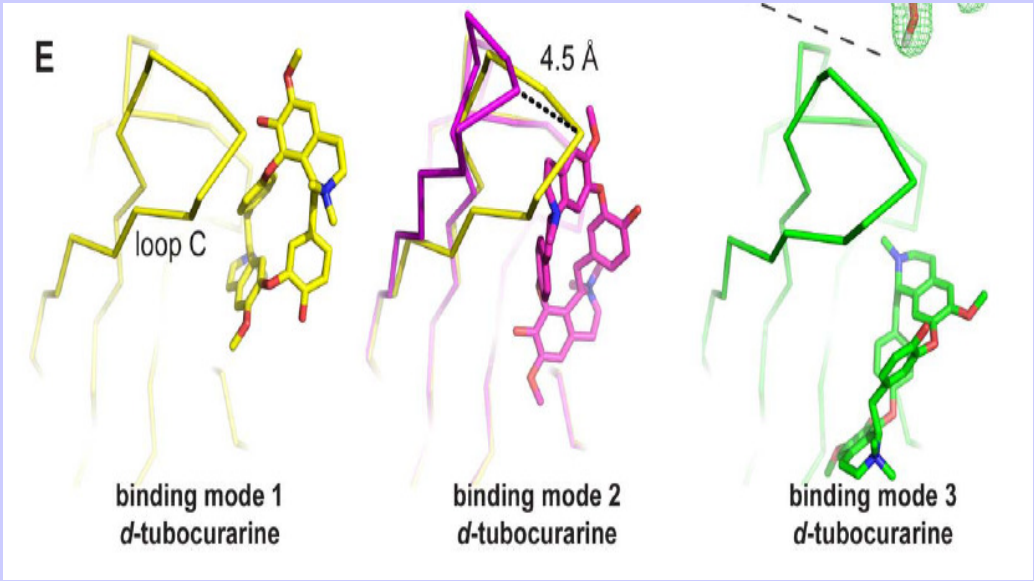
OPEN ACCESS Freely available online

PLoS BIOLOGY

A Structural and Mutagenic Blueprint for Molecular Recognition of Strychnine and *d*-Tubocurarine by Different Cys-Loop Receptors

Marijke Brams[1☯], Anshul Pandya[2☯], Dmitry Kuzmin[3], Rene van Elk[4], Liz Krijnen[1], Jerrel L. Yakel[2], Victor Tsetlin[3], August B. Smit[4], Chris Ulens[1*]





different orientations and double occupancies were found in 5 binding sites of one AChBP molecule

Design of more potent α-conotoxin PnIA analogs

Kasheverov et al ., Marine Drugs (2015), Toxins (2015)

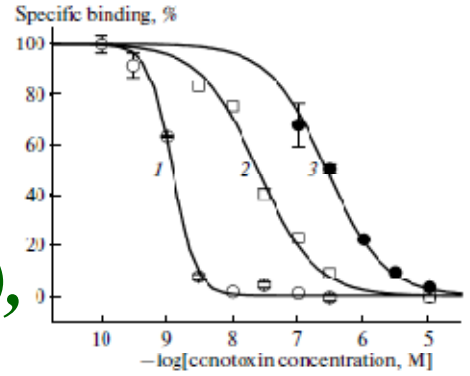


Fig. 1. Inhibition curves of the most acive analog for (*1*) AChBPs from *L. stagnalis* (PnIA[R5, R9, L10, R14]), (*2*) AChBPs from *A. californica* (PnIA[R5, R9, L10, R14]), and (*3*) human α7 nAChR (PnIA[R9, L10]). The calculated IC_{50} values are 1.22 ± 0.04, 24 ± 2, and 270 ± 10 nM, respectively.

Table 1. Primary structures of α-conotoxin PnIA and its proposed mutant analogs

α-Conotoxin PnIA and its analogs	Amino acid sequence	MH^+ molecular weight, Da	
		Theor	Measur
PnIA	GCCSLPPCAANNPDYC-NH_2	1623.8	—
PnIA[R9]	GCCSLPPCRANNPDYC-NH_2	1708.9	1708.1
PnIA[R9, L10]	GCCSLPPCRLNNPDYC-NH_2	1751.0	1750.2
PnIA[R5, R9, L10, R14]	GCCSRPPCRLNNPRYC-NH_2	1835.1	1834.3

All peptides have amidated C end. Disulfide bonds in all peptides were formed between Cys2–Cys8 and Cys3–Cys16, analogously to most of the natural α]-conotoxins. The introduced substitutions are marked gray. The calculated (Theor) and measured (Measur) by MALDI mass spectrometry molecular weights are shown.

- **Fundamental problem :**

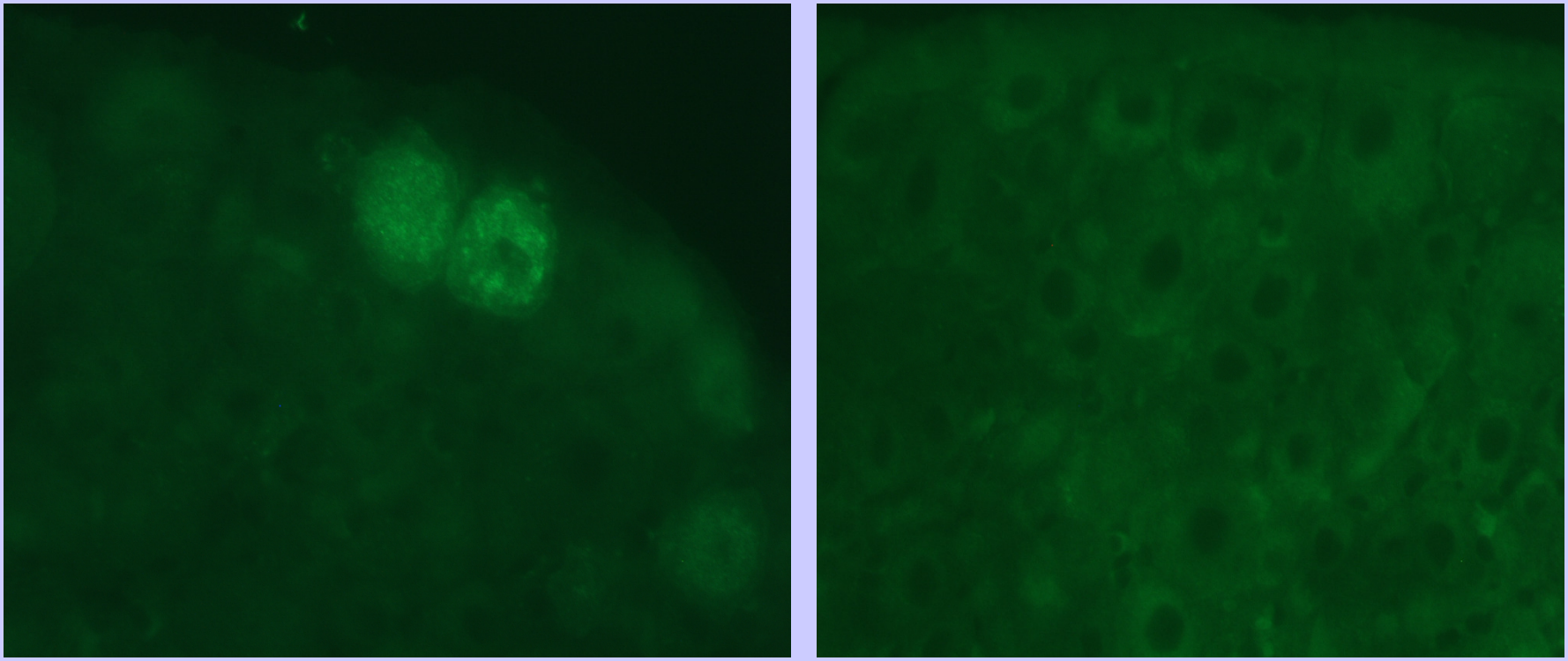
principles of ligand-receptor recognition

- **Practical tasks :**

identification of distinct nAChR subtypes and their quantification at normal state and pathologies

(known and new toxins employed as tools)

Identification of α7 nAChRs in mouse dorsal root ganglion with the aid of fluorescent α-bungarotoxin



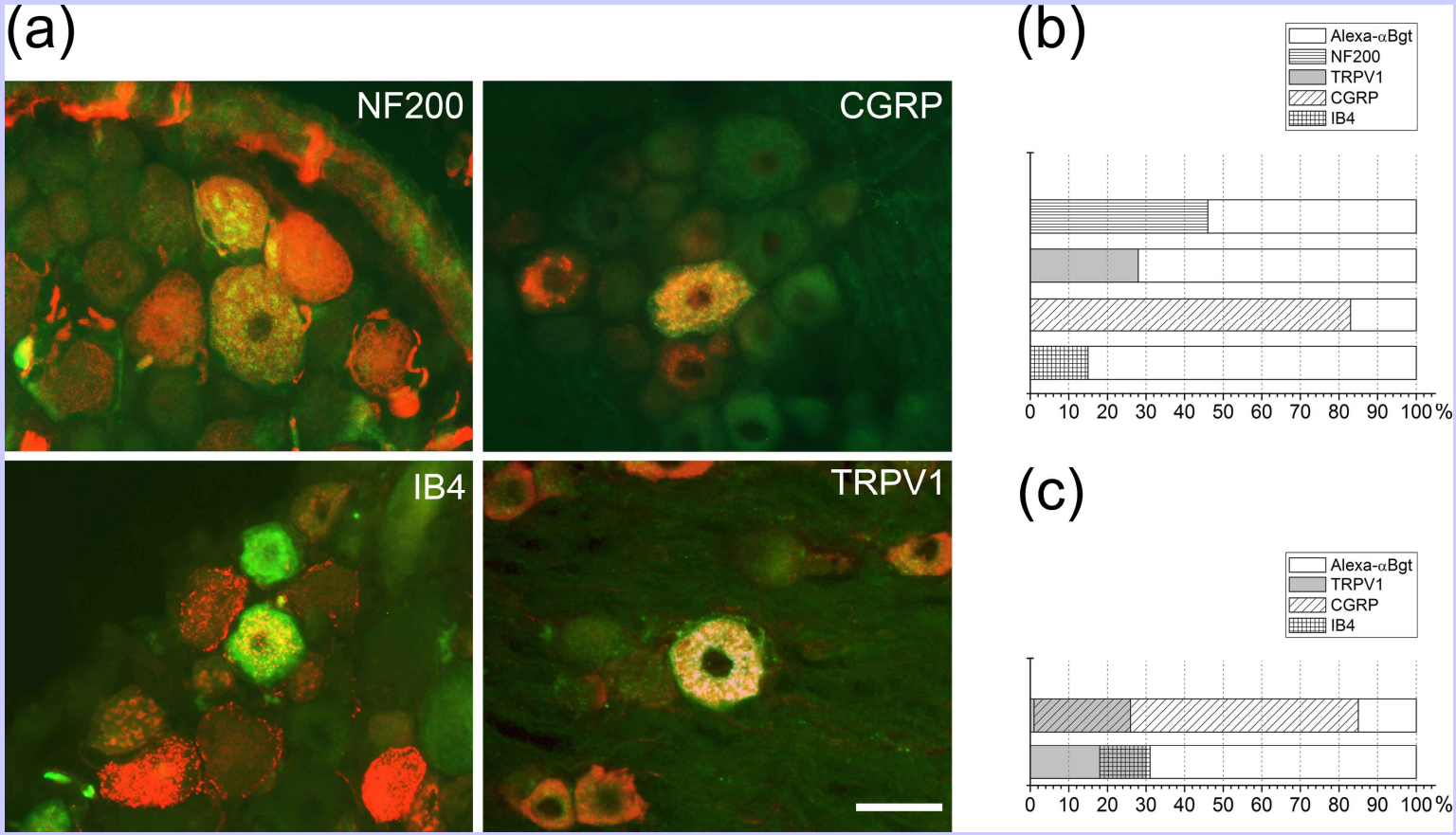
Alexa 488 Bgt

wild type **α7 -/-**

Shelukhina et al. (2009) J. Neurochem. 109, 1087-1095.

Colocalization of α7 nAChRs

in dorsal root ganglion neurons with pain signal peptide CGRP and nociceptive receptor TRPV1



Shelukhina et al., Brain Str. Function, 2014

New toxins



Proteomic studies



Herpetologist
Vlad Starkov

Azemiopsin

from the *Azemiops feae* viper venom

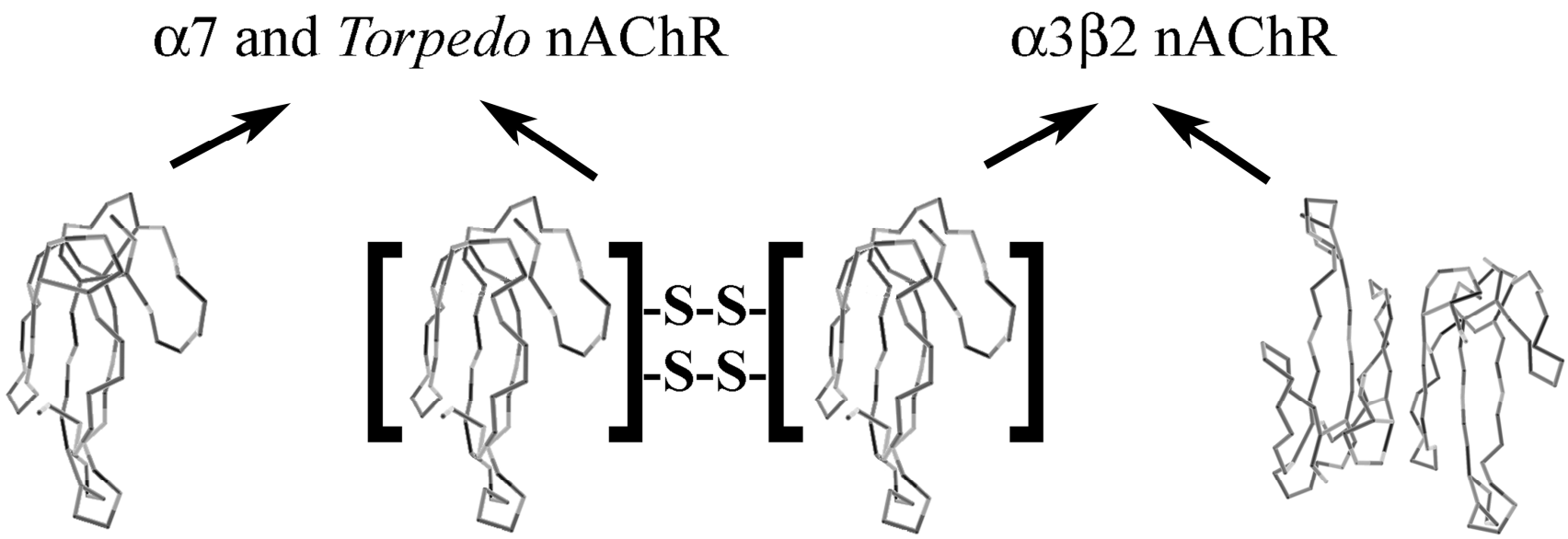
DNWWPKPPHQGPRPPRPRPKP

the first peptide from animal venoms which blocks muscle nicotinic receptor but does not contain S-S bridges

By shortening and modifications (Ala-scanning) we came to a 5-member peptide retaining high activity.

Utkin Yu. *et al.*, J Biol Chem. 287, 27079-86 (2012).

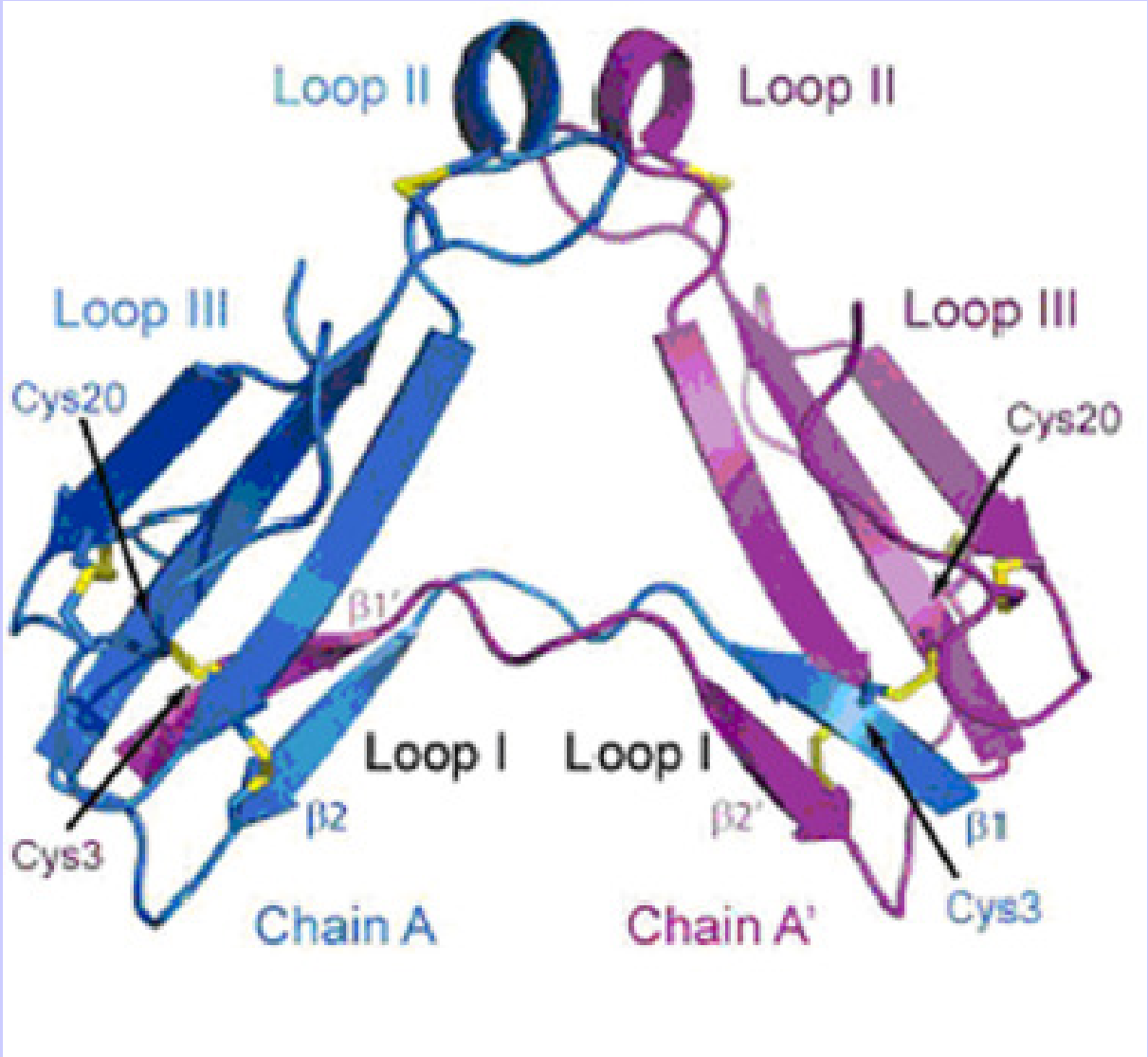
Dimeric S-S-bound α-cobratoxin



Dimerization is essential for 3-finger α-neurotoxins to recognize a heteromeric nAChR

Osipov A.V. *et al,* J.Biol.Chem.283, 14571 (2008)

Crystal structure of dimeric α-cobratoxin



Osipov, Rucktooa *et al,* J.Biol.Chem. 287 , 6725-6734 (2012).

Action of polypeptide and peptide toxins on

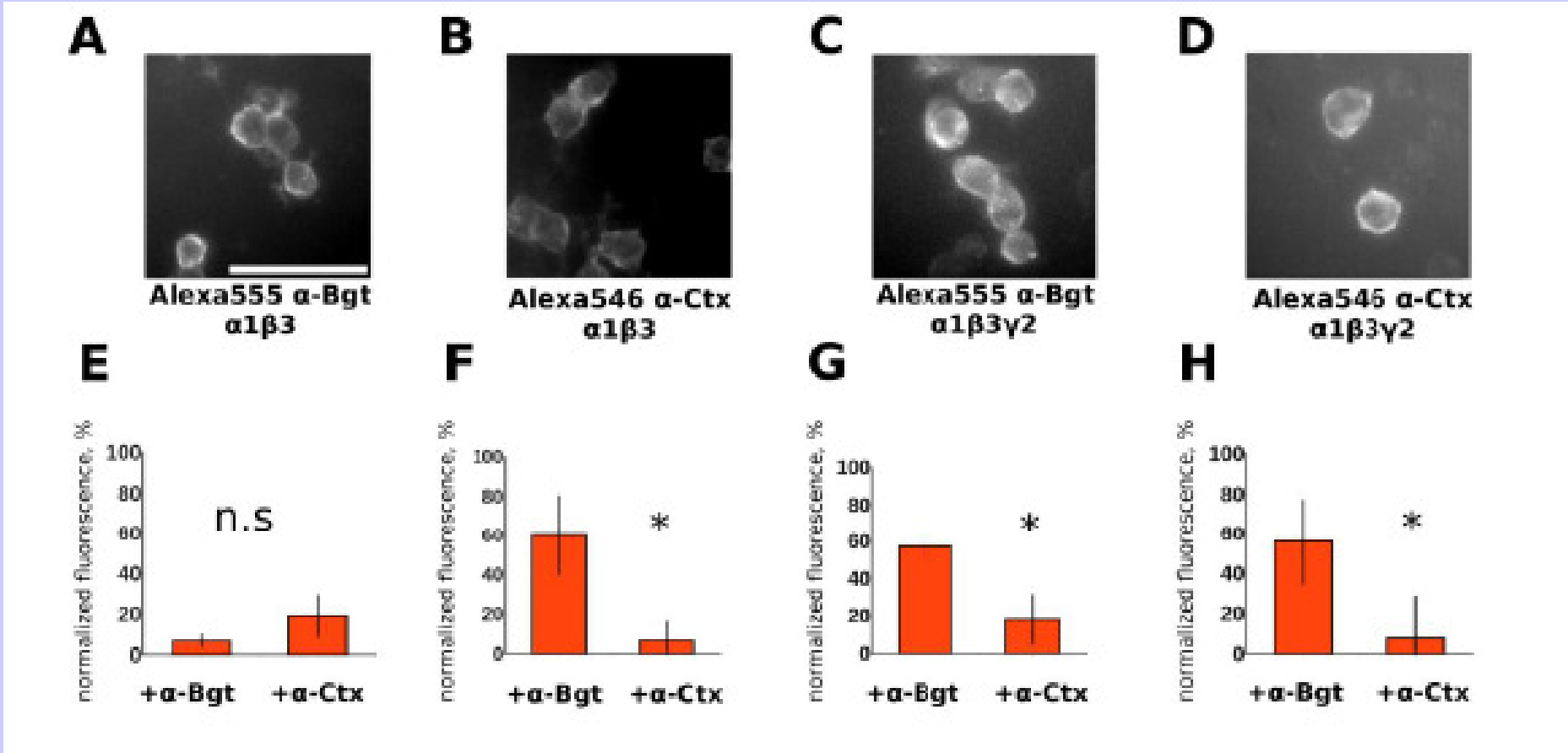
other Cys-loop receptors

Interaction of α-neurotoxins and other protein and peptide neurotoxins with distinct subtypes of GABA-A receptors

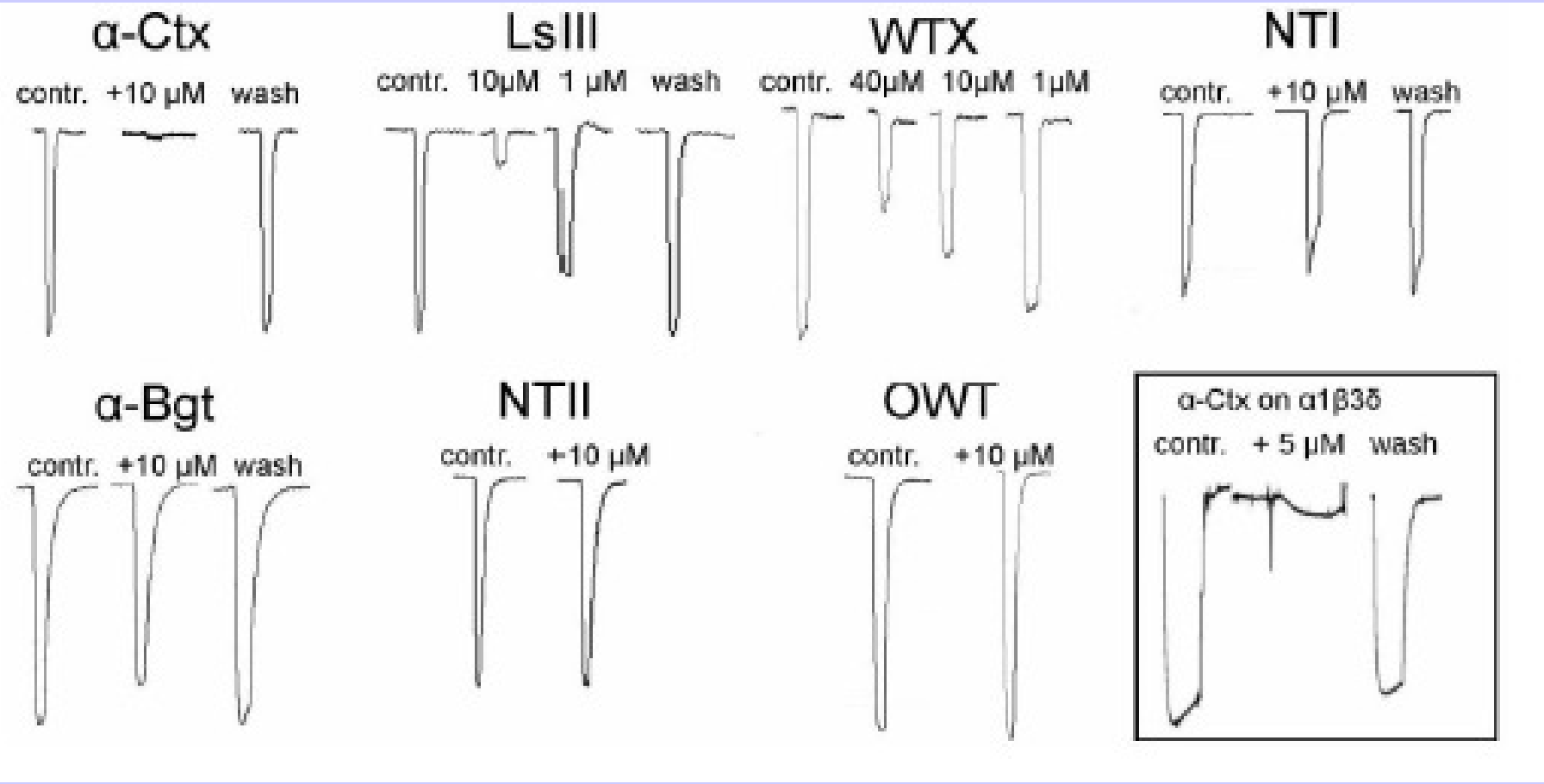
Kudryavtsev et al. J.Biol.Chem. 2015 (Epub)

McCann, C. M., Bracamontes, J., Steinbach, J. H., and Sanes, J. R. The cholinergic antagonist alpha-bungarotoxin also binds and blocks **a subset of GABA receptors**. ***Proc. Natl. Acad. Sci. U. S. A.* 103, 5149–5154 (2006)**

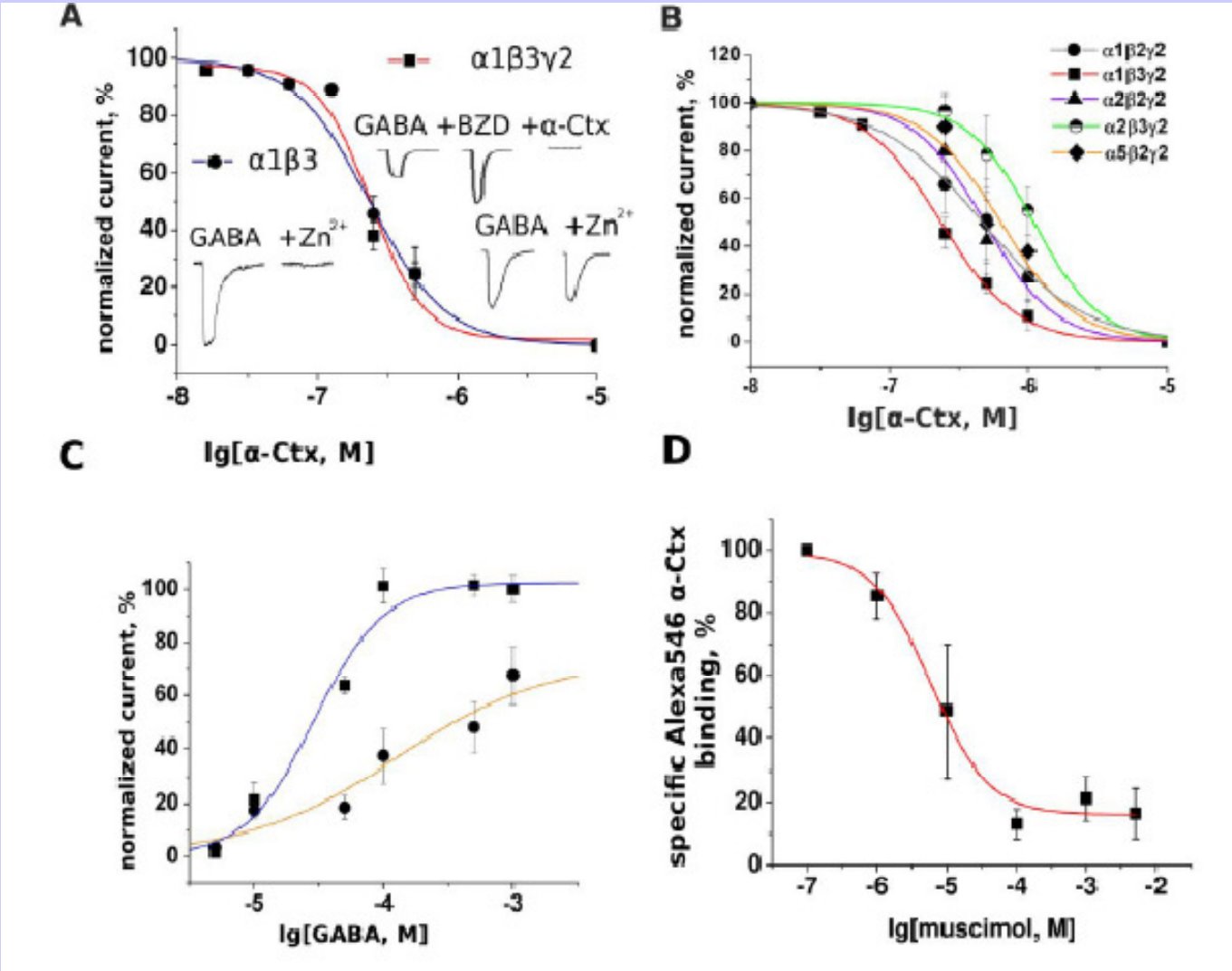
Comparison of of α-bungarotoxin and α-cobratoxin interaction with different subtypes of GABA-A receptors



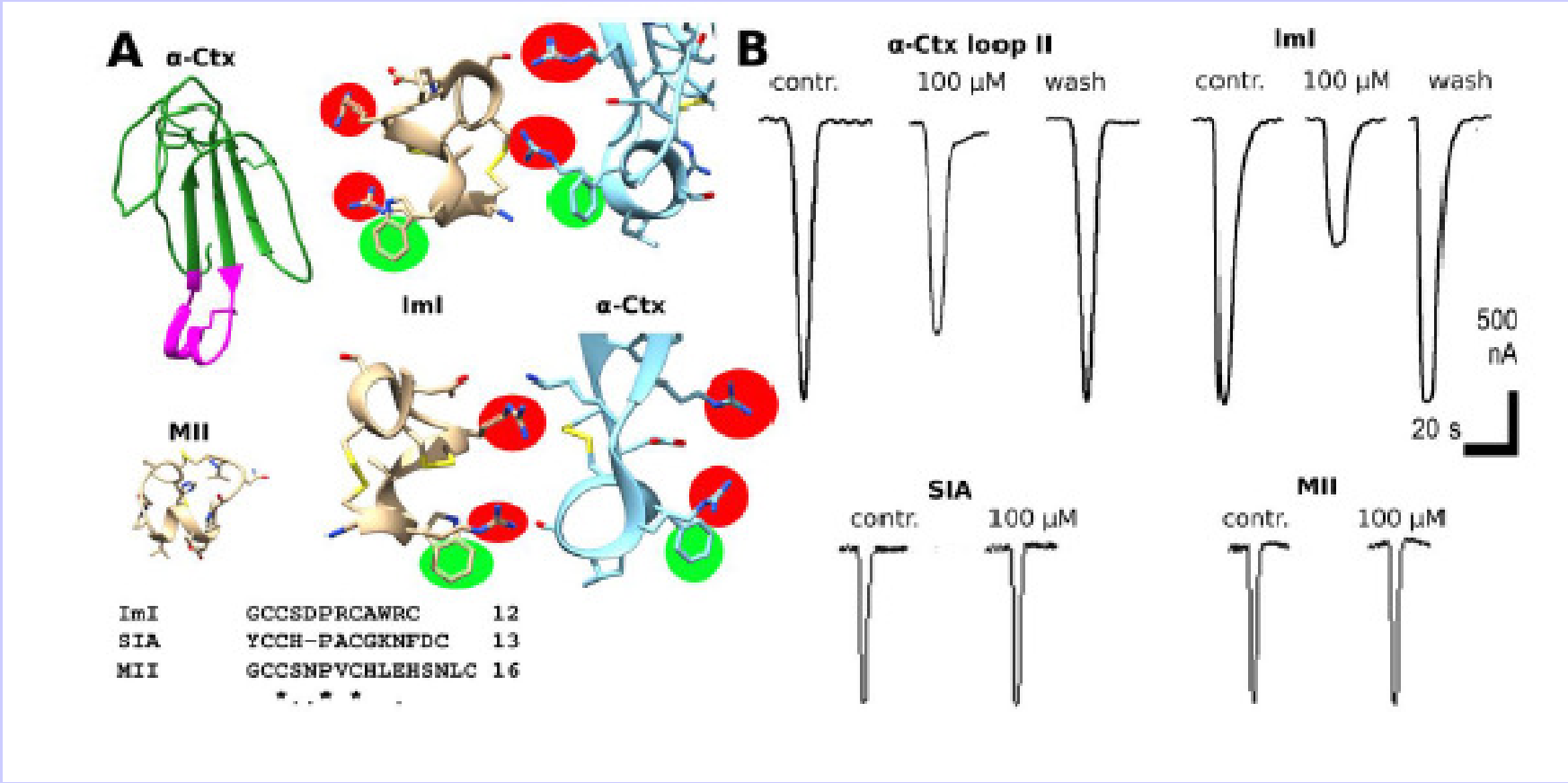
Some long–chain α-neurotoxins and "non-conventional" toxins inhibit α1β3γ2 GABA-A receptor and some not.



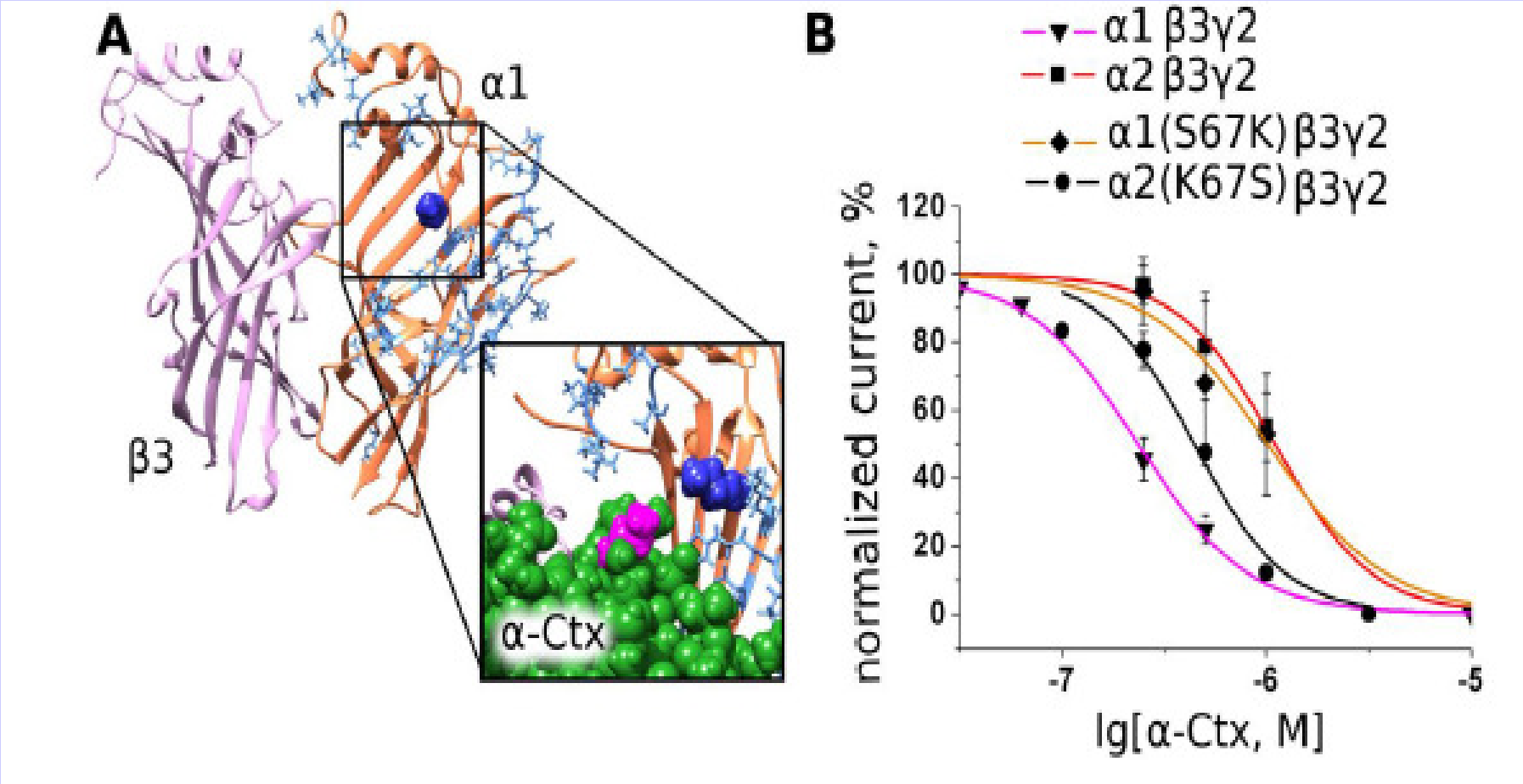
Electrophysiology (C) and fluorescence (D) measurements reveal non-competitive/competitive mode for α-Ctx inhibition ofα1β3γ2 GABA-A receptor



Tip of the α-cobratoxin loop II is essential for inhibition of α1β3γ2 GABA-A receptor



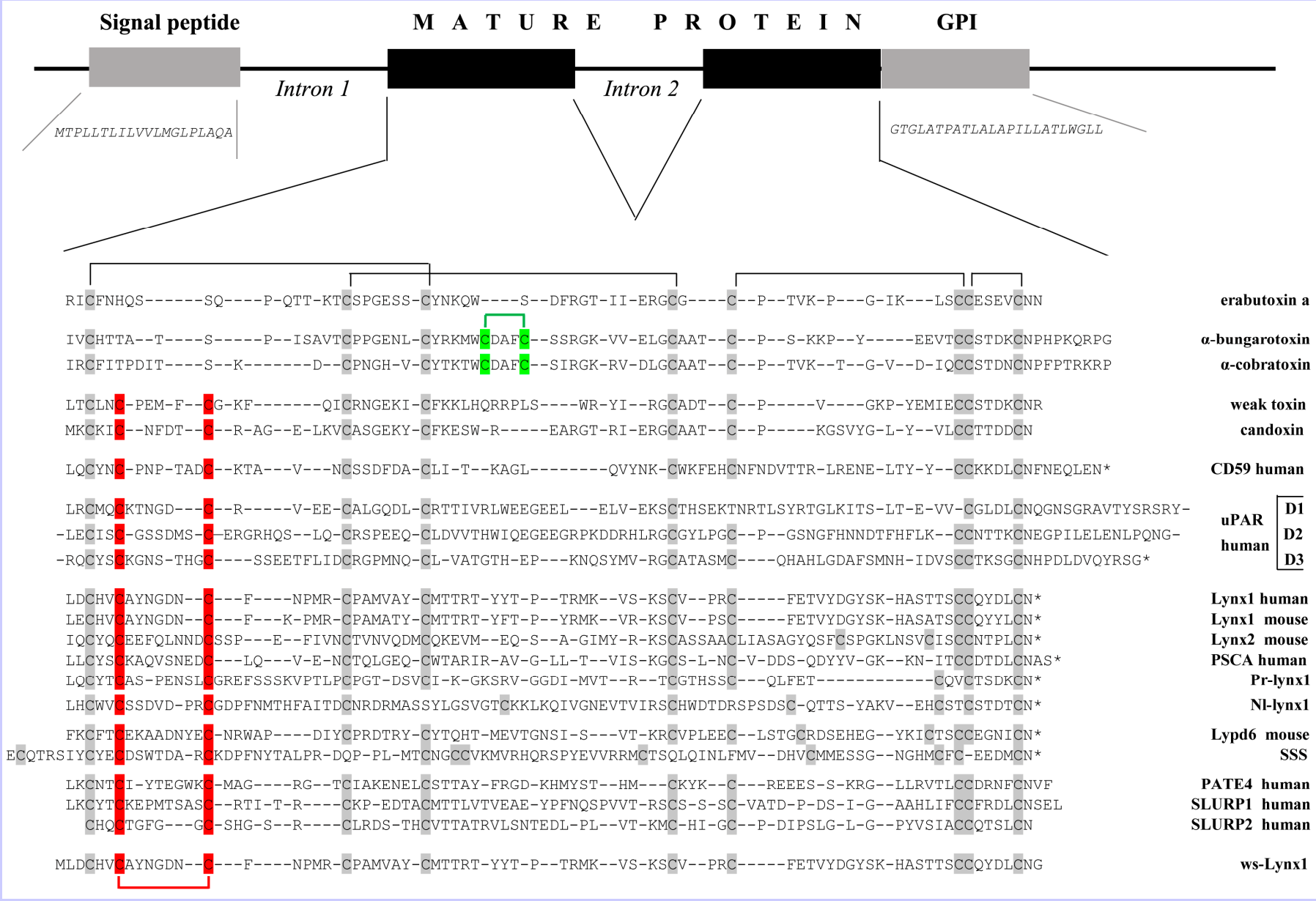
Influence of single point mutations in α1 and α2 subunits of GABA-A receptor on inhibition by α-cobratoxin



Family of non-toxic three-finger Ly6 proteins:

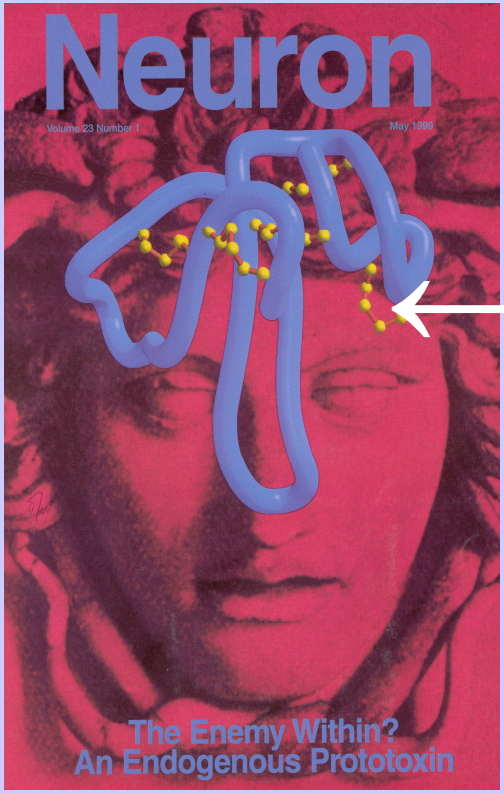
from insects to mammals

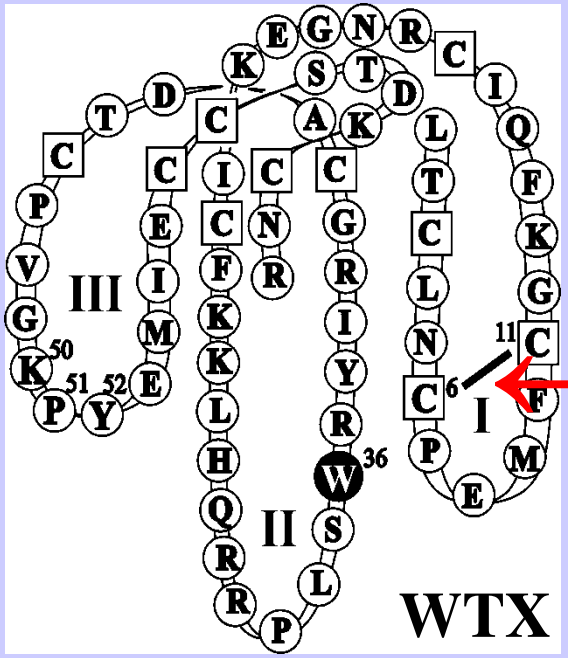
3F neurotoxins and endogenous proteins

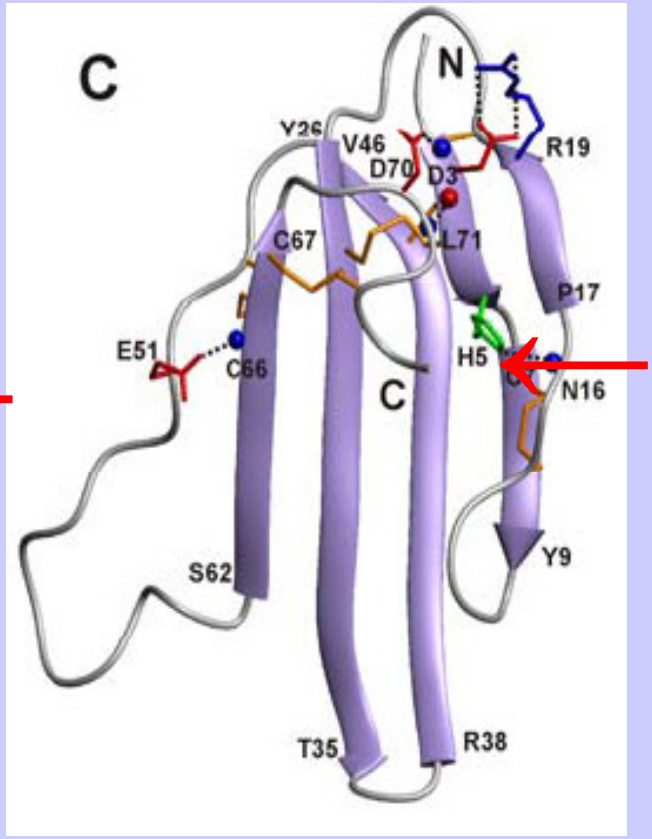


Tsetlin V. Trends Pharm. Sci.36, 109-123(2015)

Lynx 1 - endogenous "prototoxin", a modulator of the nAChR activity







Utkin et al.
J.Biol.Chem.276,
15810-15815 ***(2001)***

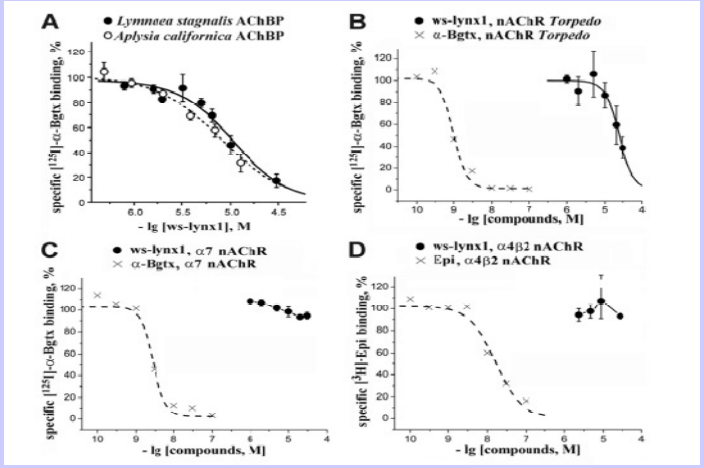
Miwa et al.
Neuron 23,
105-14 ***(1999)***

ws-Lynx1

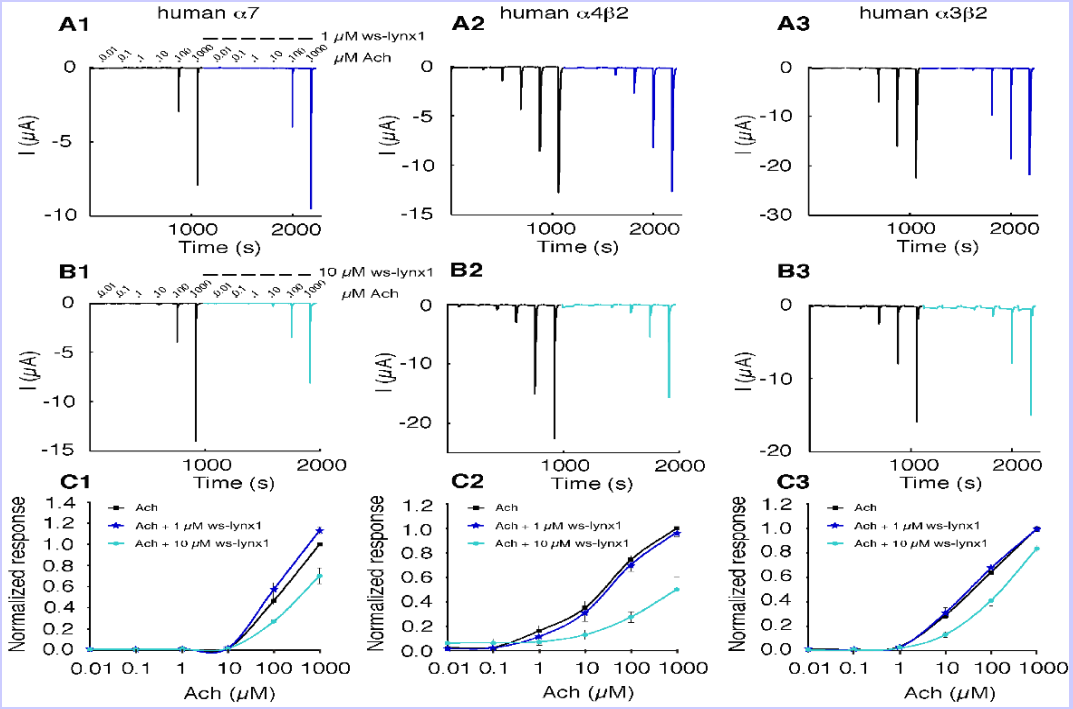
Lyukmanova et al. J. Biol.Chem. 286,
10618-10627 ***(2011)***

Ws-Lynx1 and its mutants bind with μM affinity competitively to AChBP and muscle nAChR, but non-competitively to neuronal nAChRs

Radioligand analysis



Electrophysiology in Xenopus oocytes



Lyukmanova *et al.*, J.Biol.Chem (2011, 2013)

Thomsen *et al.*, Brain Str.and Function (2014)

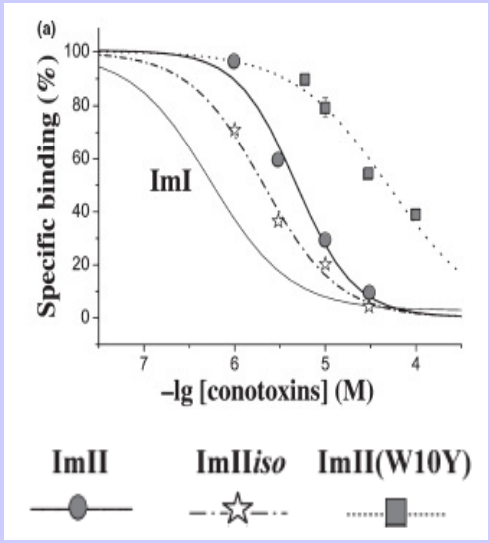
Tsetlin Trends Pharm Sci. (2015)

α-Conotoxin ImII binds at different sites at neuronal and muscle nicotinic receptors

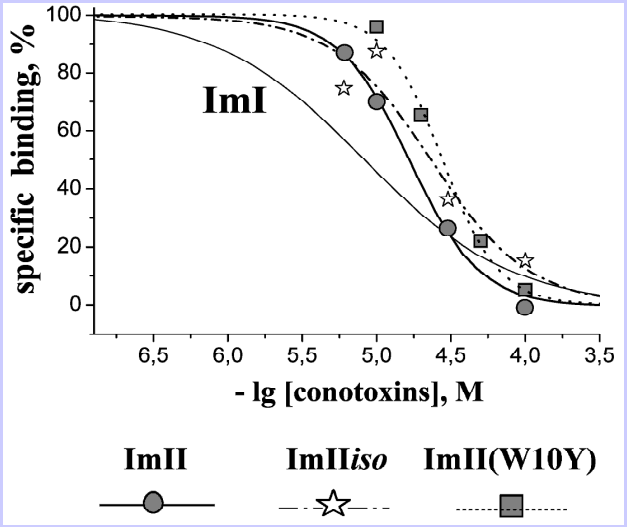
α-conotoxin ImI GCCSDPRCAWRC*

α-conotoxin ImII ACCSDRRCRWRC*

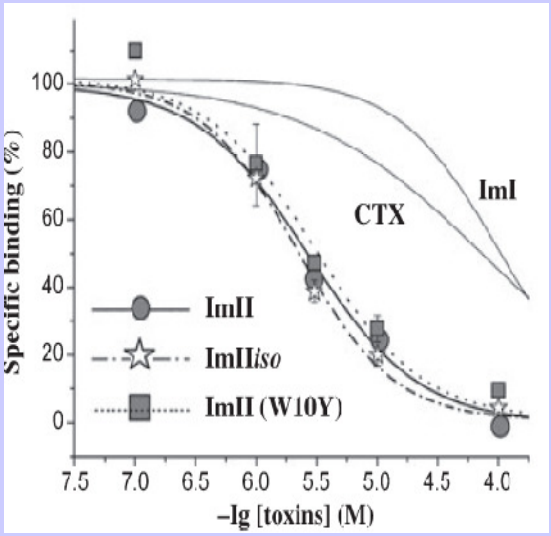
(Ellison et al, J.Biol.Chem., 2003)



AChBP *L.stagnalis*



α7 nAChR



Torpedo nAChR

Binding at the classical site **Binding outside**

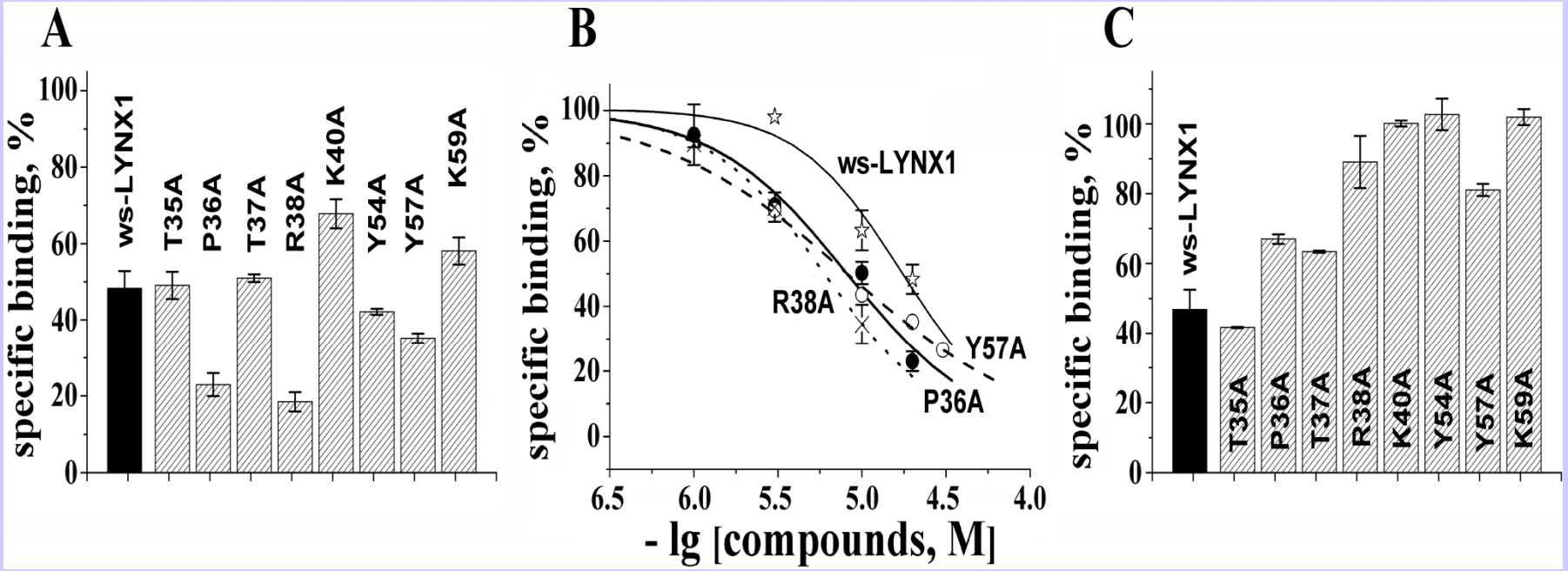
Kasheverov et al . J.Neurochem. 111,934-944 (2009)

Why do α-neurotoxins and Lynx1 have so different modes of action?

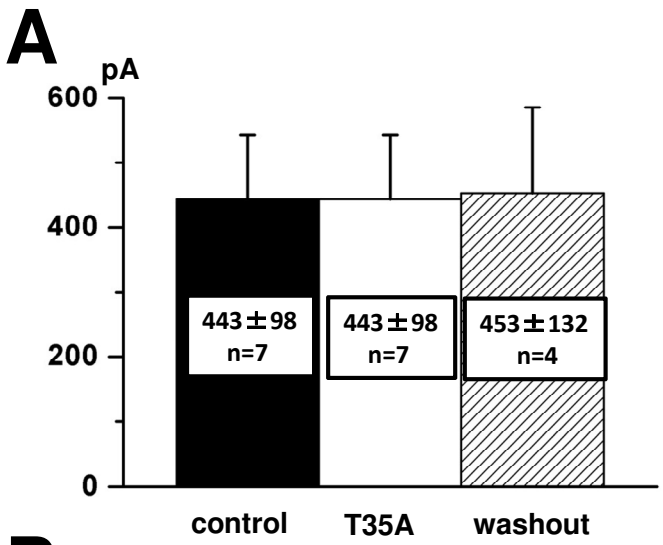
α-Neurotoxins are blocking nAChRs at nM concentrations and virtually irreversibly , whereas ws-lynx1 is active at μM concentrations and can be washed out quickly . This might explain a milder modulating activity of Lynx1 and its homologs.

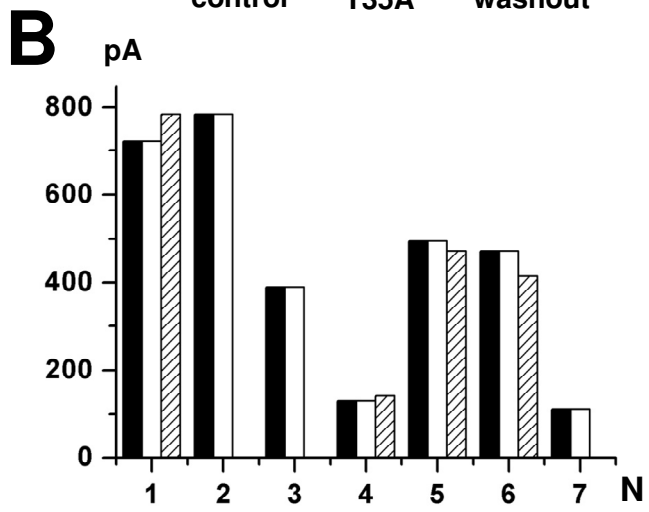
Competition of ws-lynx1 and its mutants with [^{125}I]-α-bungarotoxin for binding to:

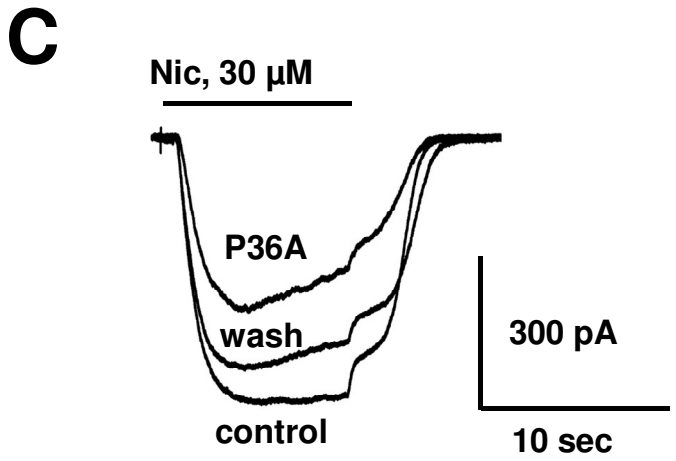
L.stagnalis AChBP *T.californica* nAChR

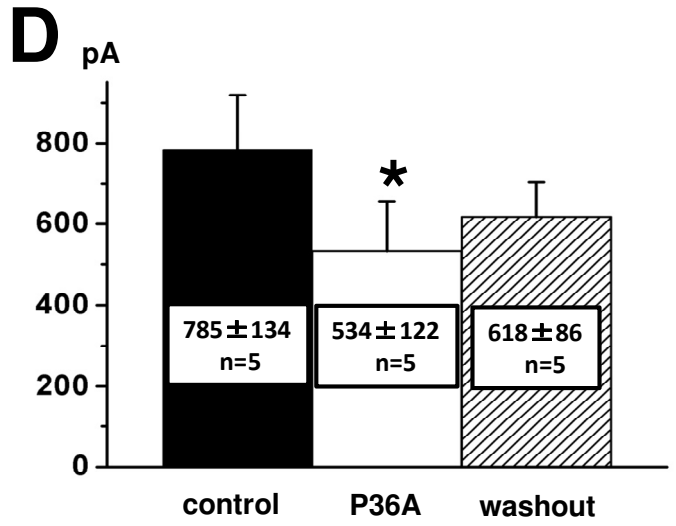


Electrophysiology: analysis of ws-lynx1 mutants effects on α7/GlyR chimera

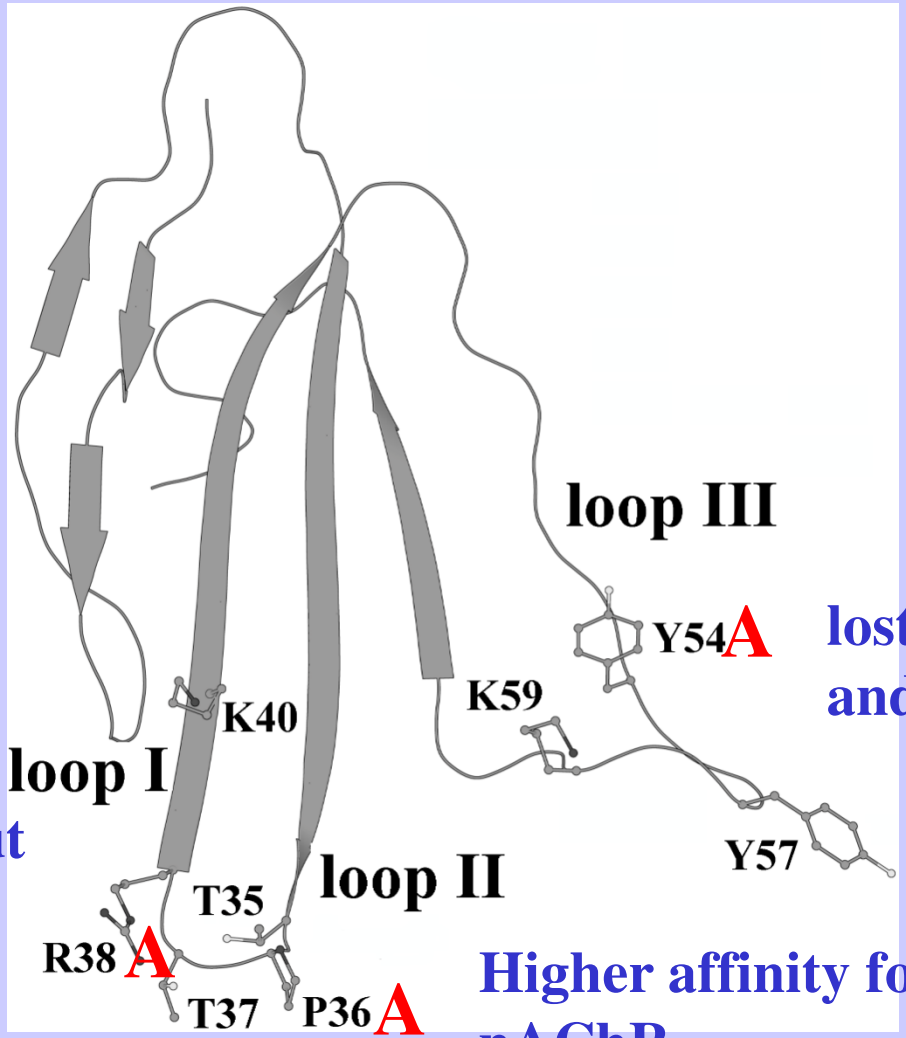








Similar or opposite effects of mutations on binding to individual targets



lost interaction with α7 and *Torpedo* nAChR

higher affinity for AChBP, but lost activity against α7 nAChR

Higher affinity for AChBP and α7 nAChR

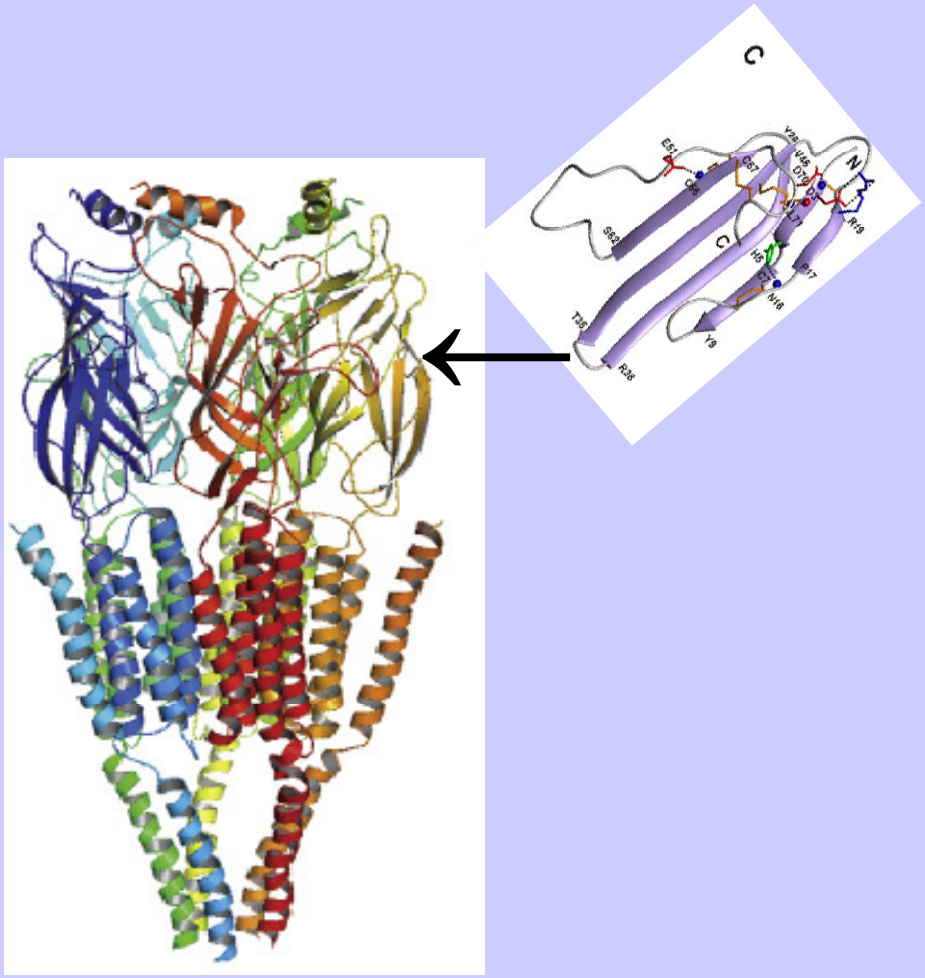
Lyukmanova et al., J Biol Chem. 288, 15888-15899 (2013)

Naturally-occurring molecules and their models, membrane-bound or soluble

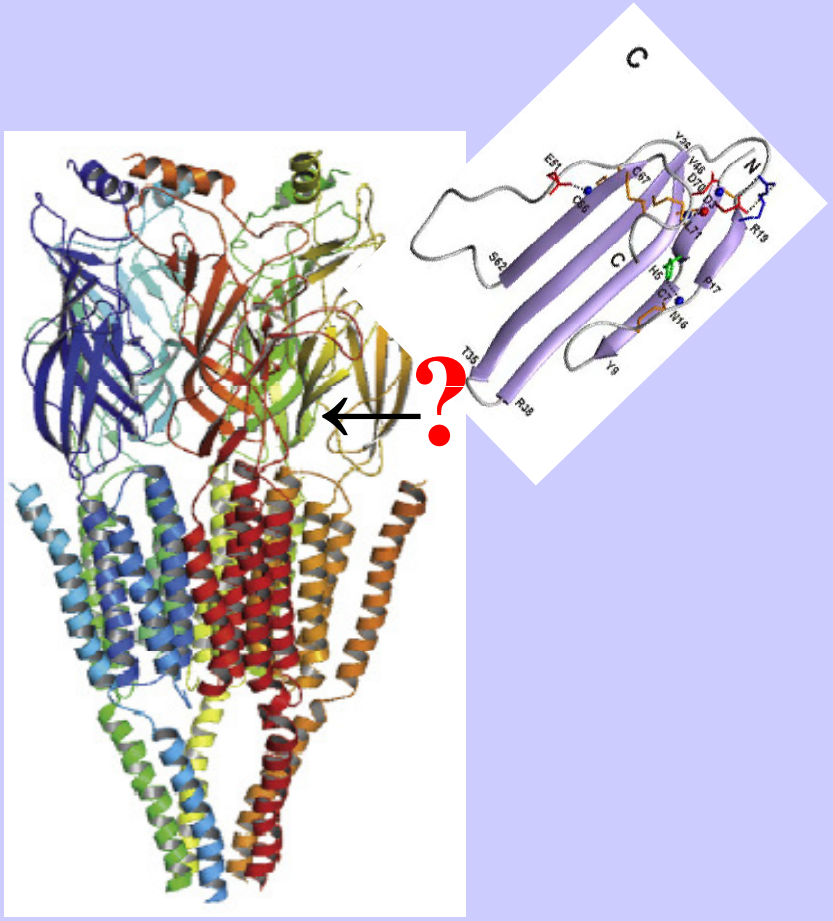
nAChRs ---------- AChBPs
Lynx1 (*GPI-tethered*) ----- ------ws-Lynx1
SLURP-1 and SLURP-2 (*no GPI*) --*secreted*

Similar action of ws-Lynx1 and SLURP-1 on α7 nAChRs (unpublished)

Where is the enigmatic binding center on neuronal nAChRs ?



Muscle-type nAChR



Neuronal α7 nAChR

Dep. Molecular Basis of Neurosignaling



Dr. E. Kryukova
immunology



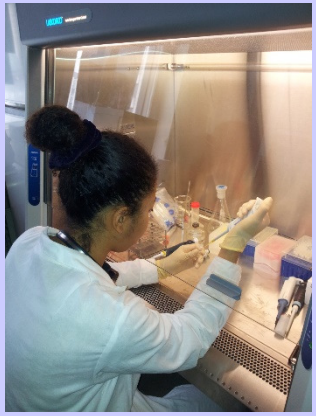
Dr. I. Kasheverov
radioligand analysis



Prof. Yu. Utkin
proteomics of venoms



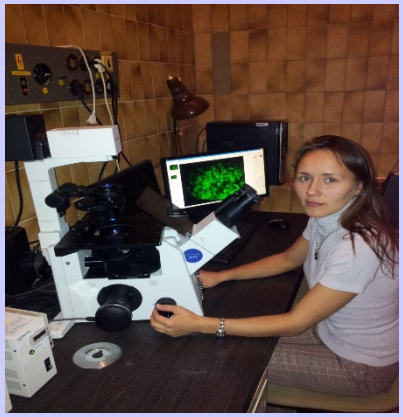
Dr. M. Zhmak
peptide synthesis



Ph.D. student
L. Odzhomoko
molecular biology



Ph.D. student
D.Kudryavtsev
electrophysiology



Dr. I. Shelukhina
neurochemistry

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FP7 grant "Neurocypres"



Kraits are venomous snakes of genus Bungarus from family Elapidae. Krait venoms are generally neurotoxic, but toxicity strongly depends on the particular species and regional origin of snakes. We analyzed the proteomes of Vietnamese Bungarus multicinctus and Bungarus fasciatus venoms both qualitatively and quantitatively. It should be noted that no proteomic data for B. multicinctus venom existed so far. We have found that in this venom, almost half (45%) of the proteins by weight was represented by β-bungarotoxins, followed by three finger toxins (28%) and phospholipases A_2(16%), other proteins being present at the level of 1-3%. In B. fasciatus venom, phospholipase $A_2$was the main component (71%), followed by oxidase of l-amino acids (8%), acetylcholinesterase (5%) and metalloproteinases (4%). Unexpectedly, extremely low amount of three finger toxins (1%) was found in this venom. Interestingly, the presence of complement depleting factor was observed in both venoms. Although our data showed the presence of the same toxin families in Vietnamese krait venoms as those found earlier in the venoms of kraits from other geographic regions, their relative ratio is completely different. This concerns especially B. fasciatus venom with predominant content of phospholipases A_2 and very low amount of three finger toxins.

Quantitative proteomic analysis of Vietnamese krait venoms: Neurotoxins are the major components in Bungarus multicinctus and phospholipases A2 in Bungarus fasciatus.

Ziganshin RH, Kovalchuk SI, Arapidi GP, Starkov VG, Hoang AN, Thi Nguyen TT, Nguyen KC, Shoibonov BB, Tsetlin VI, Utkin YN.

Latest news about Ly6–nAChR interactions …

Lynx1 shifts nAChR stoichiometry to low sensitivity **(α4)3(β2)2** pentamers **through the interaction in the endoplasmic reticulum,** rather than via direct modulation of activity on the plasma membrane

Nichols et al., J.Biol.Chem.(2014)

SLEEPLESS (SSS)

1. induces sleep in *Drosophila* inhibiting nAChR or by potentiating Shaker type potassium channel
2. **Lynx1** in the cell culture binds to both these targets

Wu et al., Current Biology (2014)

Ws-Lynx1 interacts with M3 muscarinic acetylcholine receptor

Lyukmanova et al. J.Biol.Chem, 2011

Prototoxin Ly6h reduces trafficking of the α7 nAChRs to the cell surface and potentiation of glutamate signaling

Puddifoot C. et al., J.Neurosci.35, 3420(2015)

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