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Is the cyclic imine core common to the marine macrocyclic toxins sufficient to dictate nicotinic ACh receptor antagonism? (BSI 2023, Session "Neurons", Poster P80)

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Yves Bourne, Gerlind Sulzenbacher, Laurent Chabaud, Rómulo Aráoz, Zoran Radić, et al.. Is the cyclic imine core common to the marine macrocyclic toxins sufficient to dictate nicotinic ACh receptor antagonism? (BSI 2023, Session "Neurons", Poster P80). BSI 2023 - 3rd edition of the Integrative Structural Biology Meeting, Nov 2023, Marseille, France. . hal-04752741

HAL Id: hal-04752741

<https://hal.science/hal-04752741v1>

Submitted on 24 Oct 2024

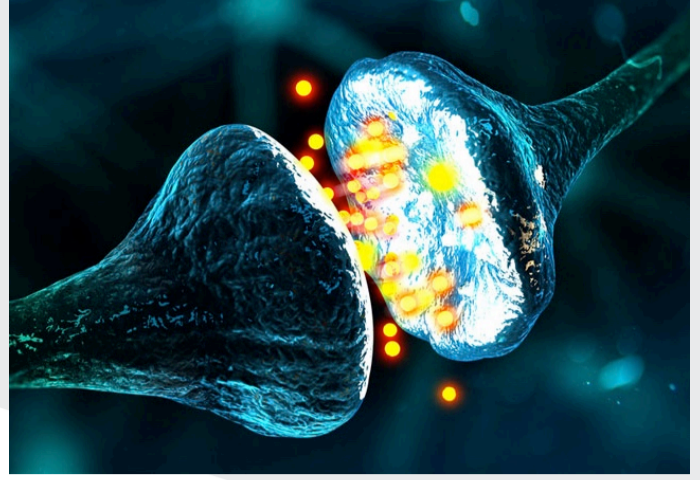
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Is the cyclic imine core common to the marine macrocyclic toxins sufficient to dictate nicotinic acetylcholine receptor antagonism?

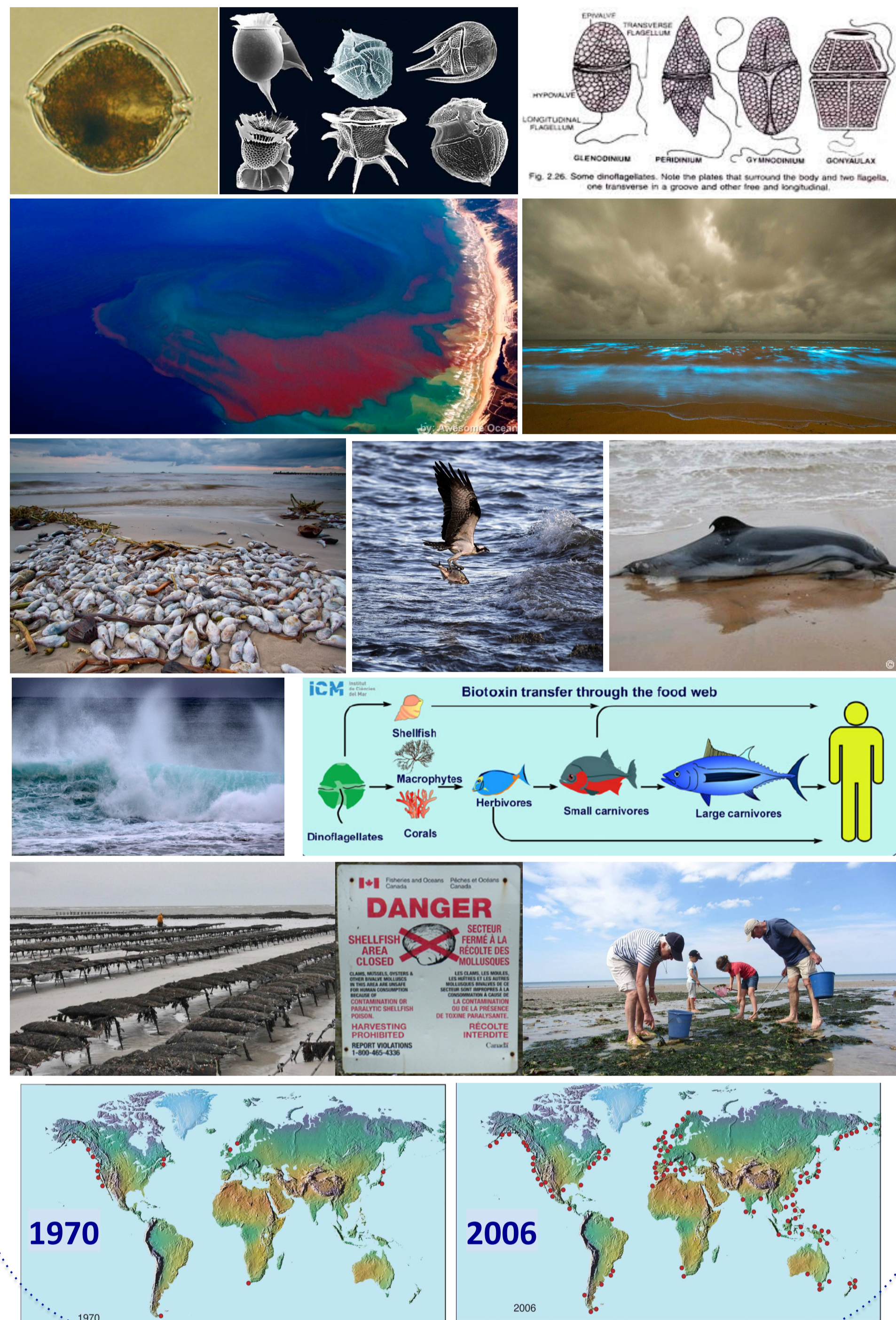


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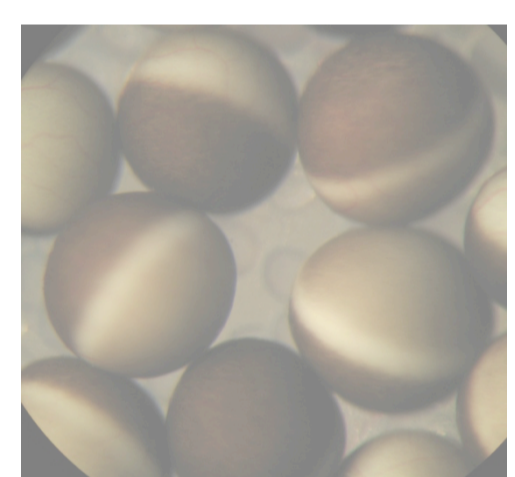
1- Marine phycotoxins from dinoflagellate organisms



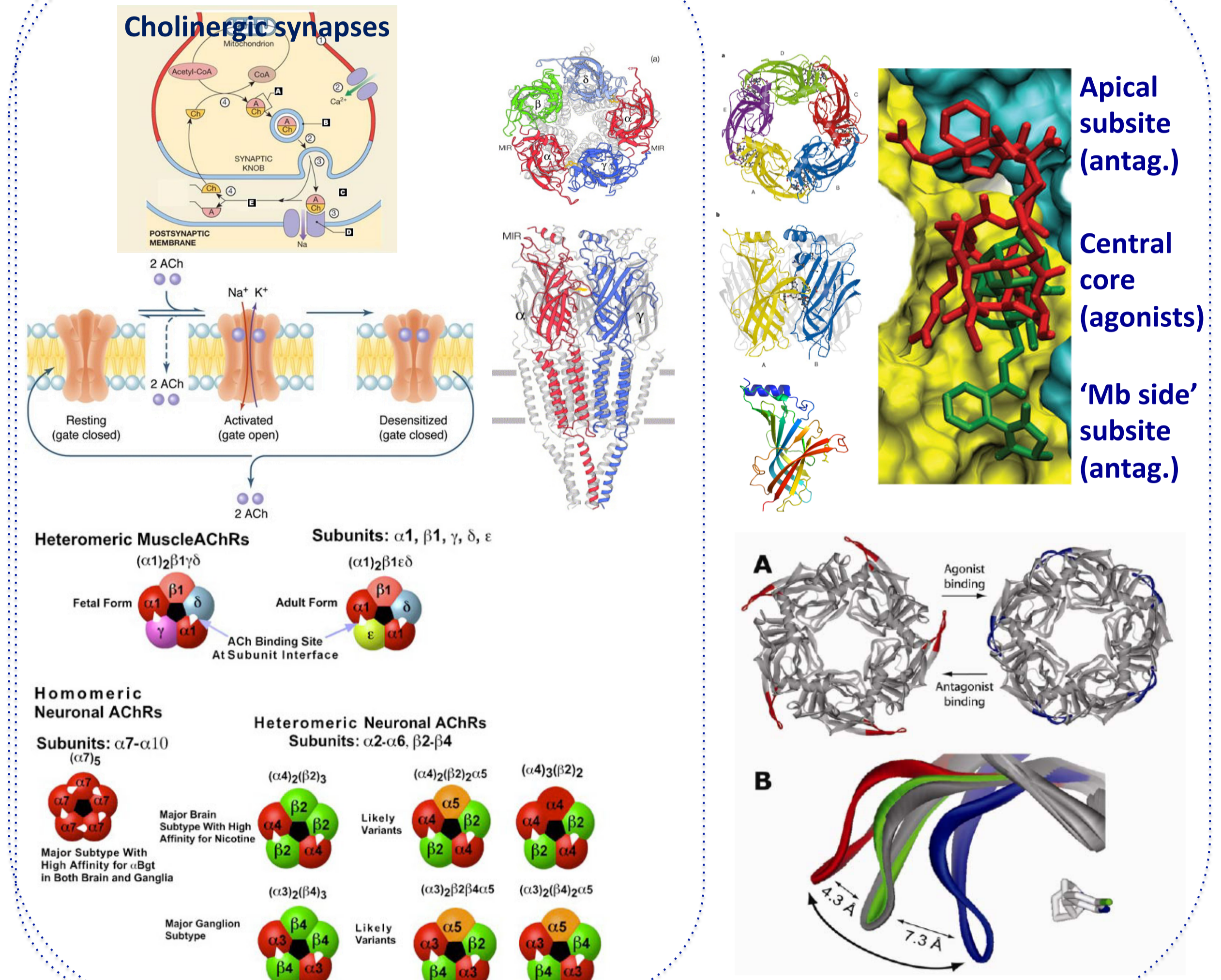
2- A combination of experimental approaches

- Identify the toxins and their chemical structure
- Identify the toxins' pharmacological target(s)
- Synthesize toxins and analogs with controlled iso/enantiomeric properties
- Define mode of action, affinity, and subtype specificity for nAChR subtypes* in the membrane state (transfected oocytes), using voltage-clamp electrophysiology
- Record affinity and subtype specificity by competition binding at equilibrium to nAChR subtypes* in the membrane state (HEK cells)
- Record kinetics and affinities for binding to soluble AChBP subtypes and mutants
- Crystallize (by hand!) the preformed AChBP complexes and solve their crystal structures
- Correlate functional and structural data to explain the toxins' mode of action

*muscle-type $\alpha 1_2\beta\gamma\delta$ from *Torpedo* electrocyte
neuronal $\alpha 7$, $\alpha 4\beta 2$, $\alpha 3\beta 4$ from human cDNAs

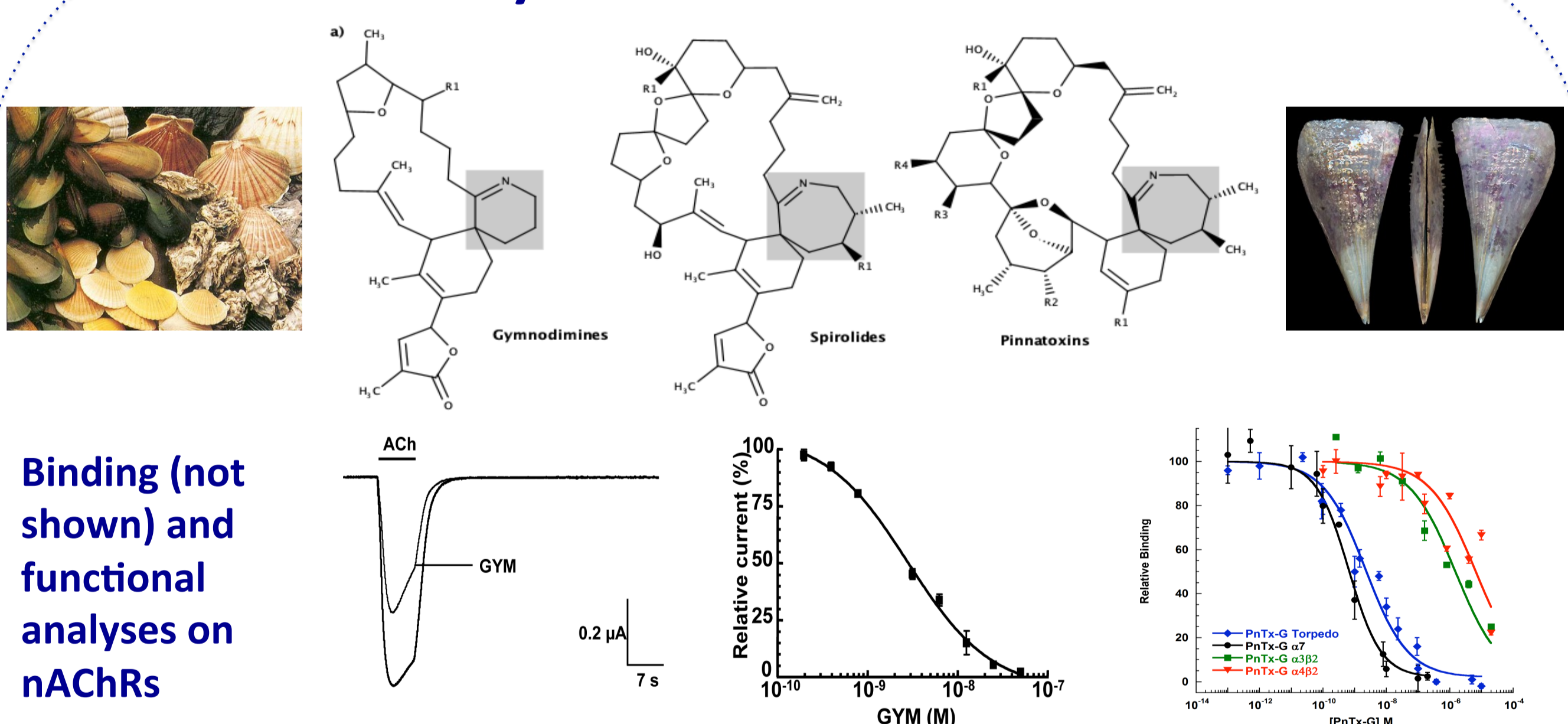


3- nAChRs versus AChBPs



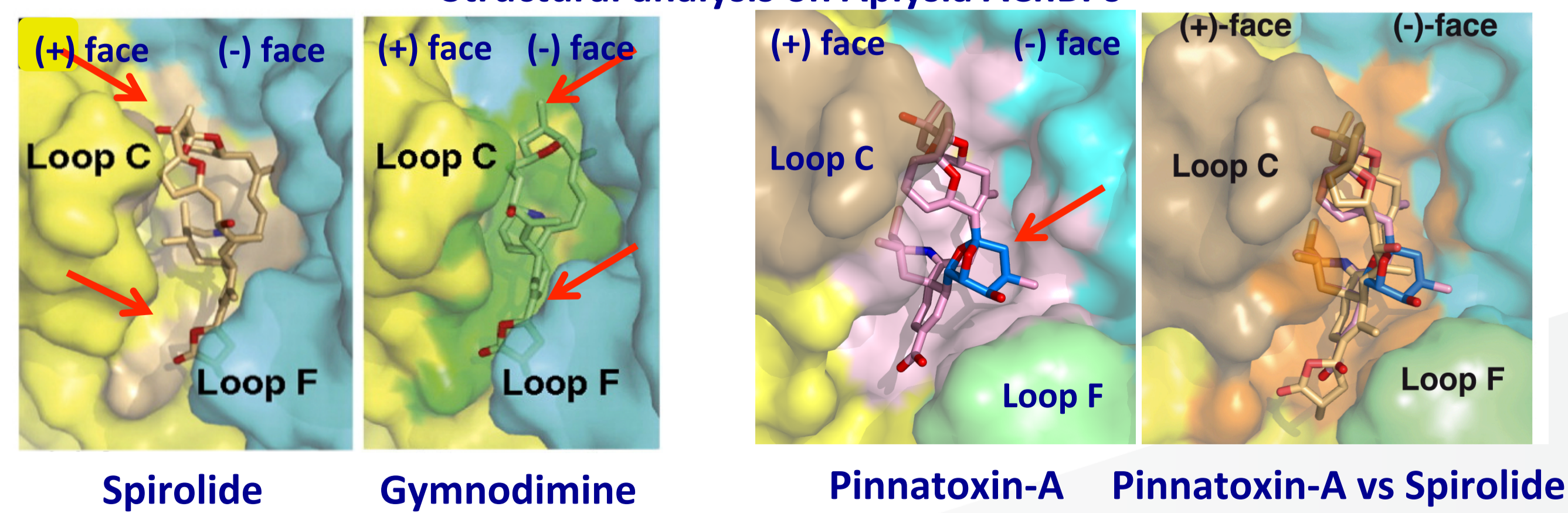
4- YES, the imine core is sufficient for dictating nicotinic antagonism!

4- Macrocyclic imine toxins from shellfish



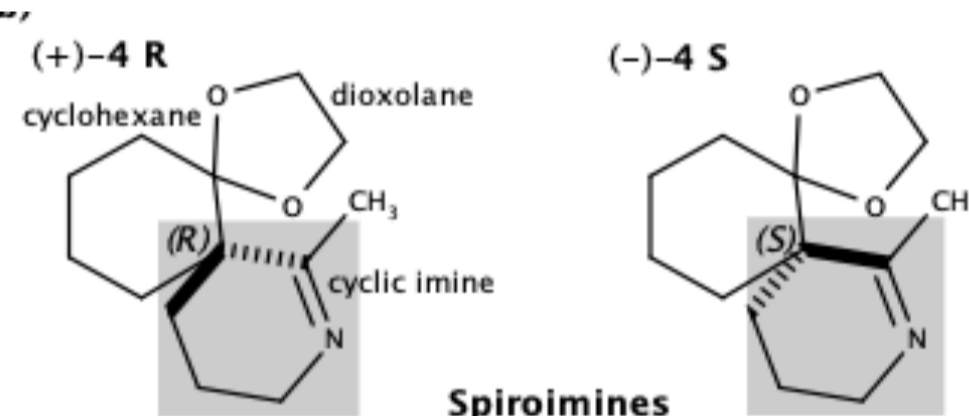
Display high affinities (nM range) for the nAChRs and AChBPs and either low (gymnodimines & spirolides) or high (pinnatoxins) nAChR subtype specificities

Structural analysis on Aplysia AChBPs



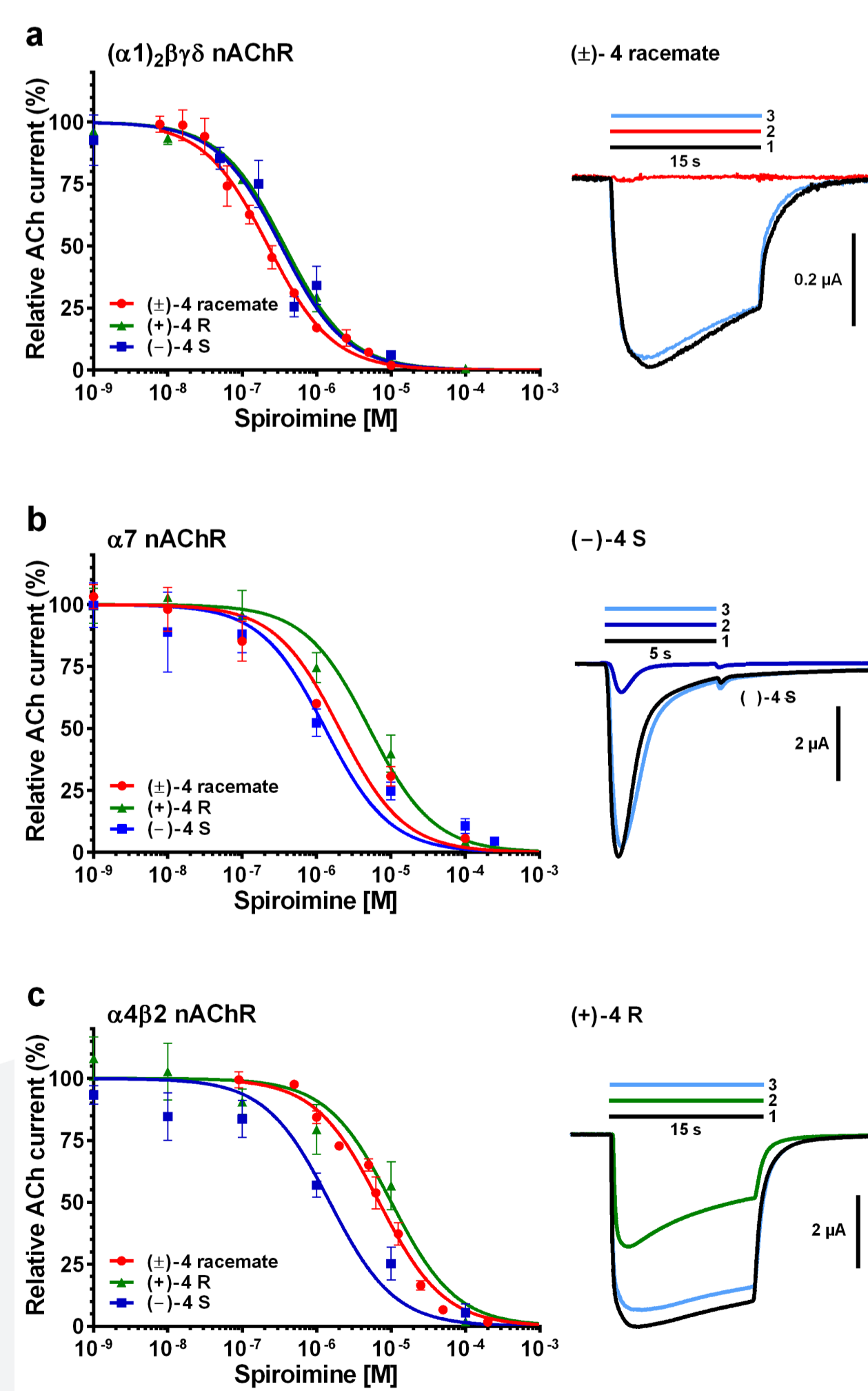
- Main anchoring point for the cyclic imine: central to the subunit interface
- Additional anchoring points in the apical and "mb" directions: promote high-affinity binding
- The sequence-variable loop F hardly contributes to toxin binding --> low nAChR subtype specificity
- The bridged ketal group specific to the pinnatoxins extends radially toward the edge of the subunit interface and loop F
- The sequence-variable loop F largely contributes to toxin binding and governs high nAChR subtype specificity

(+)-4 R and (-)-4 S spiroimines



Functional analysis on nAChRs

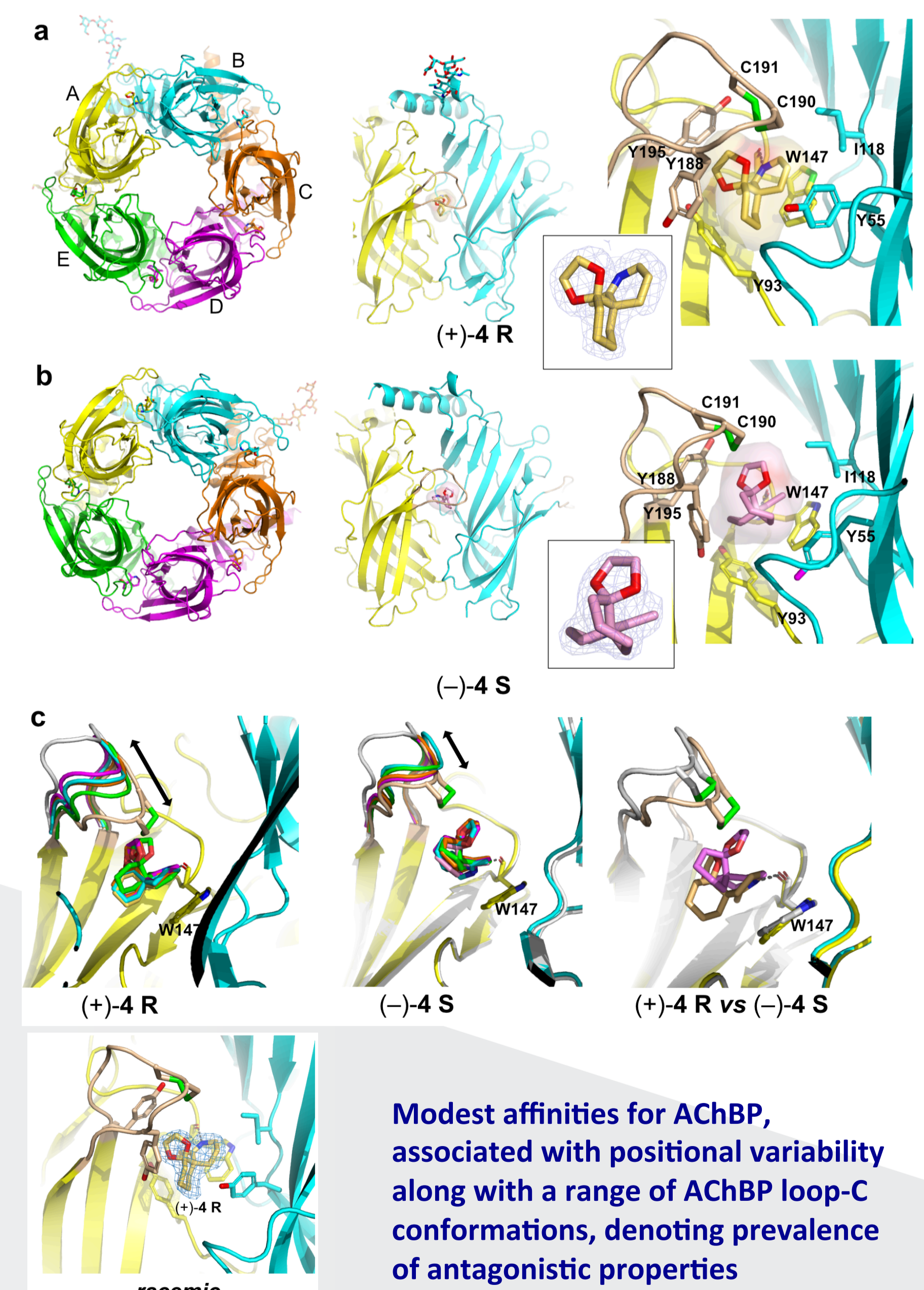
nAChR subtype	(±)-4	(+)-4 R	(-)-4 S
<i>Torpedo</i> $\alpha 1_2\beta\gamma\delta$	0.22	0.37	0.33
Human $\alpha 7$	2.04	5.15	1.31
Human $\alpha 4\beta 2$	6.93	10.09	1.50



Compared with the parental macrocyclic imine toxins: the spiroimines display preserved antagonism for both enantiomers, despite lower nAChR subtype specificity and binding affinities associated with faster reversibility.

Binding & structural analyses on Aplysia AChBP

Kinetic/equilibrium parameters	(±)-4		
	(±)-4	(+)-4 R	(-)-4 S
k_{on} ($10^9 M^{-1}min^{-1}$)	6.3	7.4	6.1
k_{off} ($10^3 min^{-1}$)	18	16	26
k_{off}/k_{on} ($10^3 min^{-1}$)	15	14	24
$K_d/k_{off}/k_{on}$ (μM)	2.9	2.2	4.3
K_d/S_{Eq} (μM)	4.2	3.1	5.4
K_d/SP_{Aeq} (μM)	2.4	2.3	2.4



This study identifies a minimal set of functional pharmacophores and binding determinants as templates for designing new antagonists targeting disease-associated nAChR subtypes.