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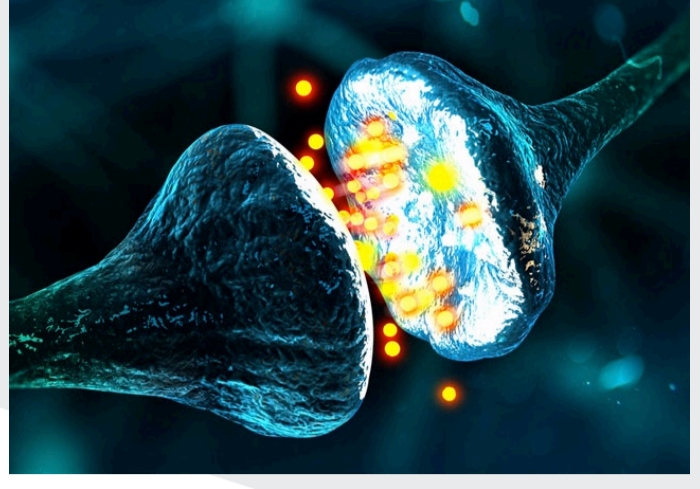
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Is the cyclic imine core common to the marine macrocyclic toxins sufficient to dictate nicotinic acetylcholine receptor antagonism?



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1- Marine phycotoxins from dinoflagellate organisms

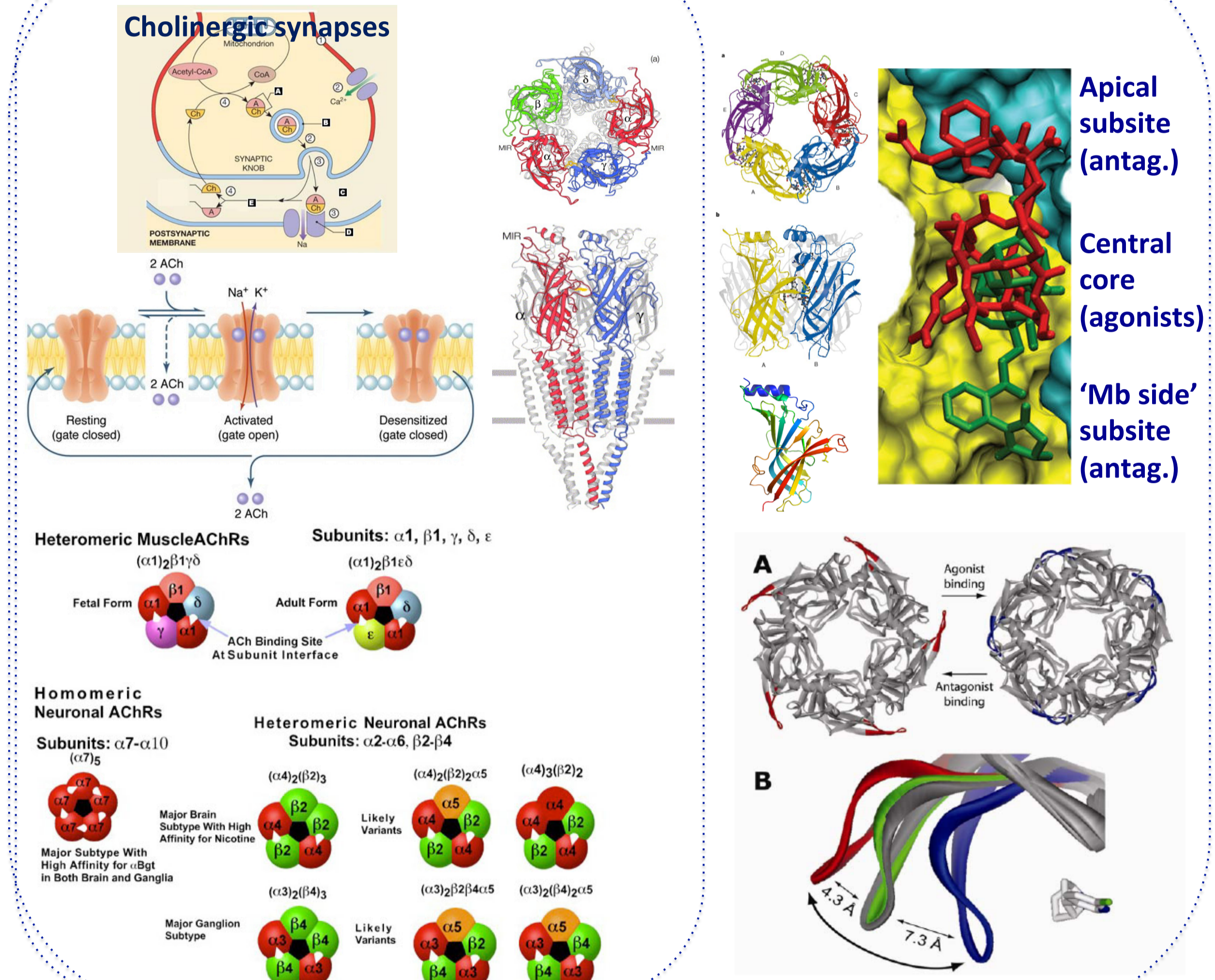


2- A combination of experimental approaches

- 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

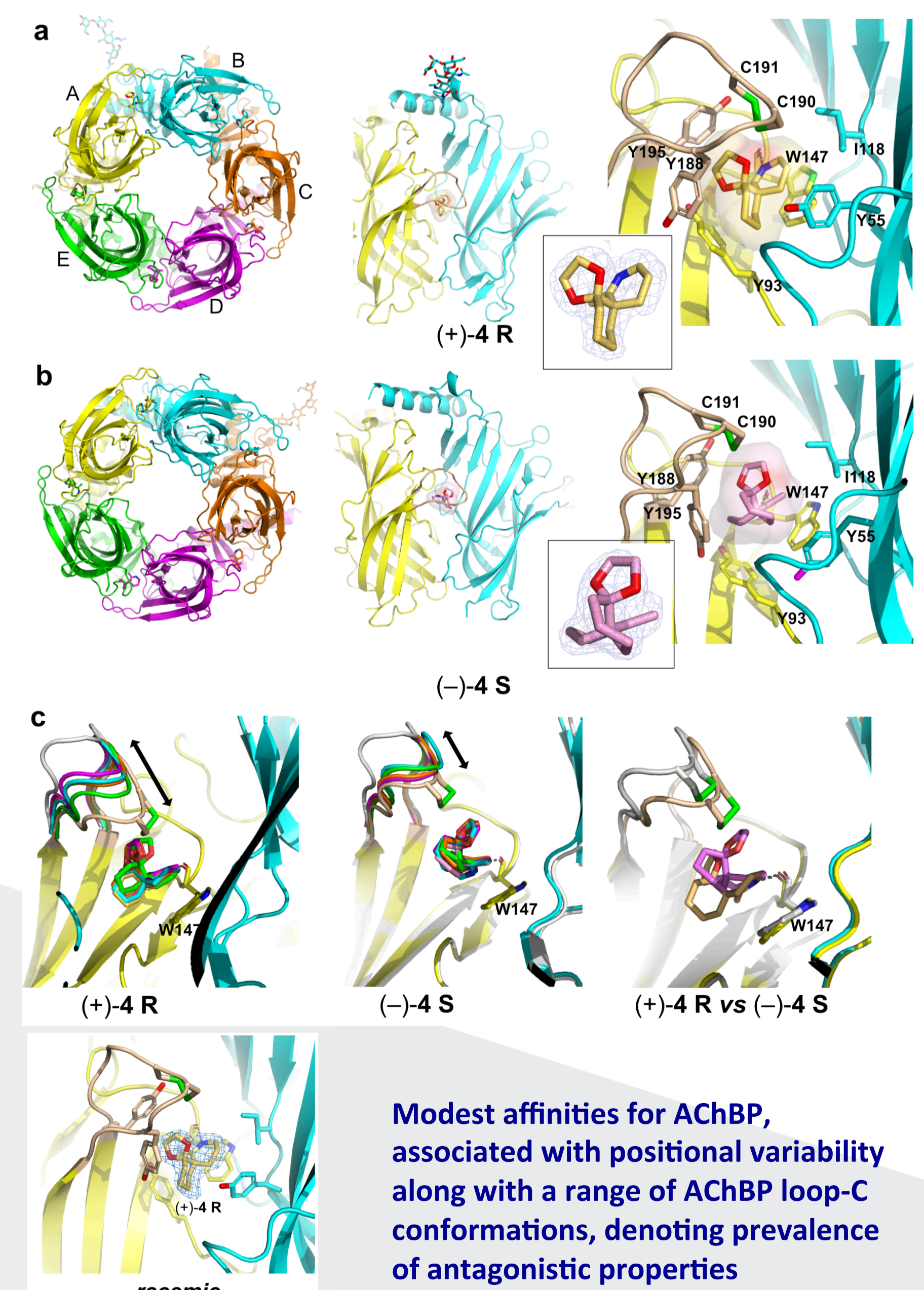
3- nAChRs versus AChBPs



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

Binding & structural analyses on *Aplysia* AChBP

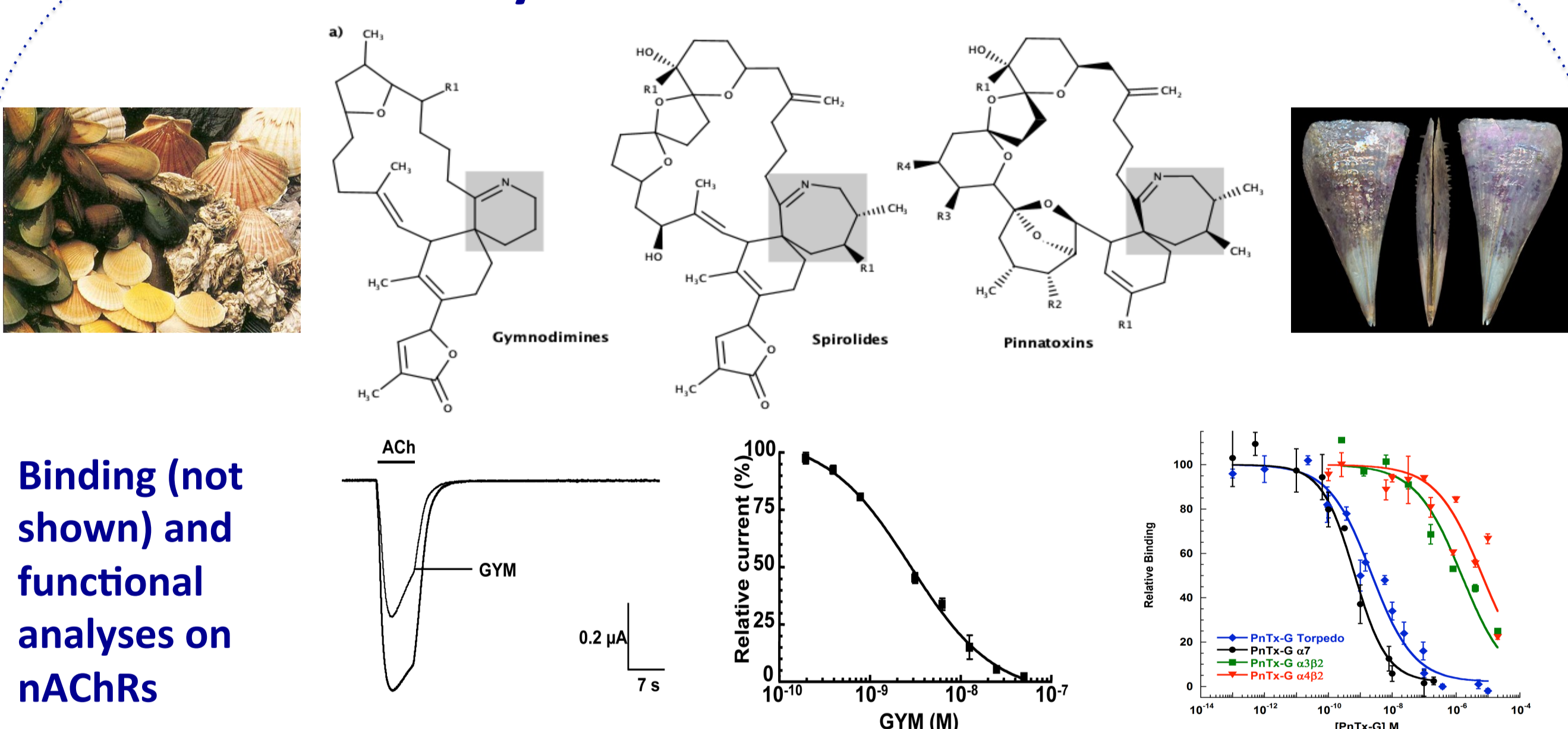
Kinetic/equilibrium parameters	(±)-4	(+)-4 R	(-)-4 S
	k_{on} ($10^9 M^{-1}min^{-1}$)	6.3	7.4
k_{off} ($10^3 min^{-1}$)	18	16	26
k_{off}/k_{on} ($10^3 min^{-1}$)	15	14	24
K_d (μM)	2.9	2.2	4.3
K_d/S_{Eq} (μM)	4.2	3.1	5.4
K_d/SP_{Aeq} (μM)	2.4	2.3	2.4



Modest affinities for AChBP, associated with positional variability along with a range of AChBP loop-C conformations, denoting prevalence of antagonistic properties

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

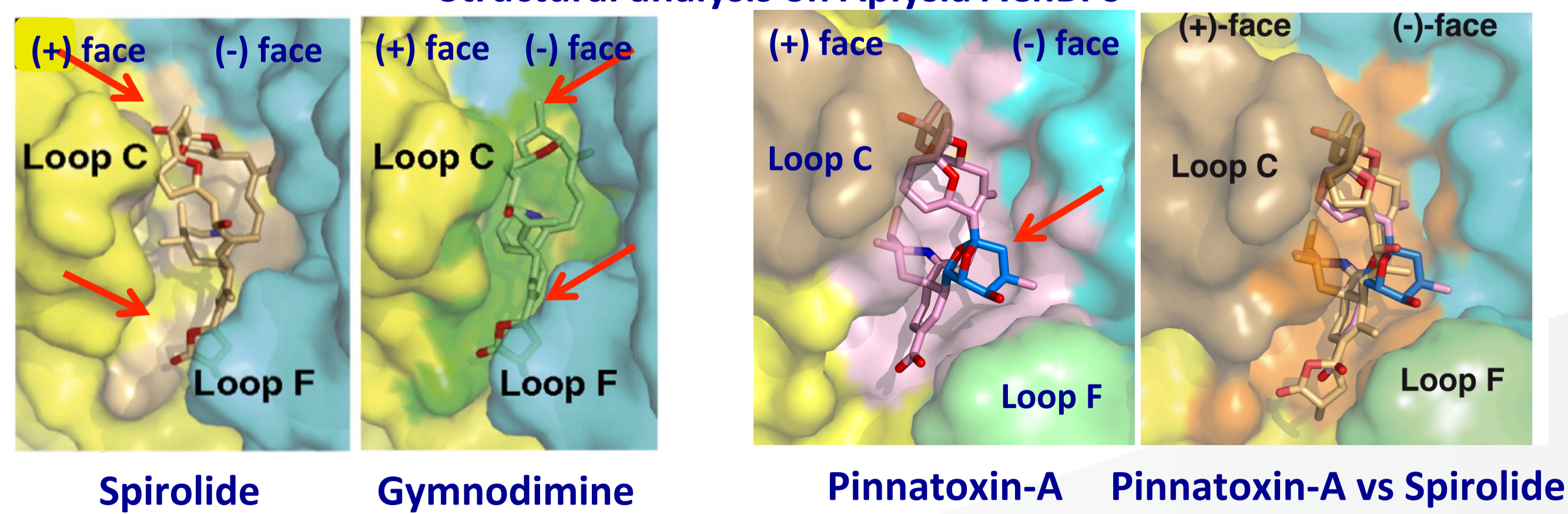
4- Macrocyclic imine toxins from shellfish



Binding (not shown) and functional analyses on nAChRs

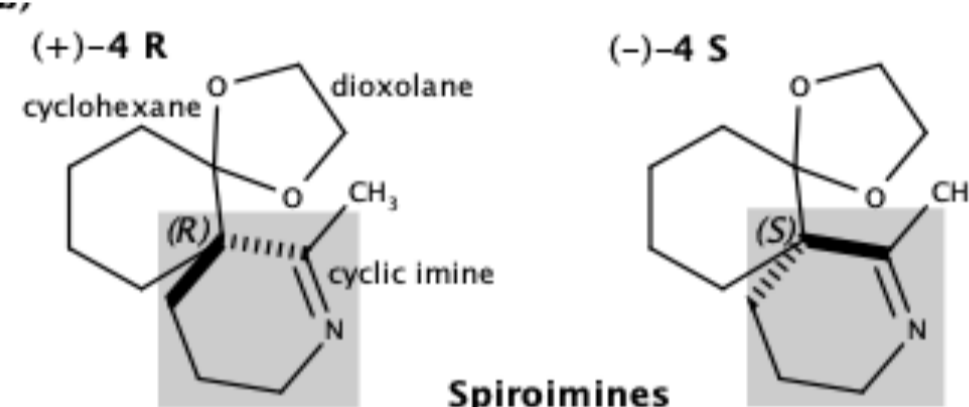
Display high affinities (nM range) for the nAChRs and AChBPs and either low (gymnodimines & spirolides) or high (pinnatoxins) nAChR subtype specificities

Structural analysis on *Aplysia* AChBPs



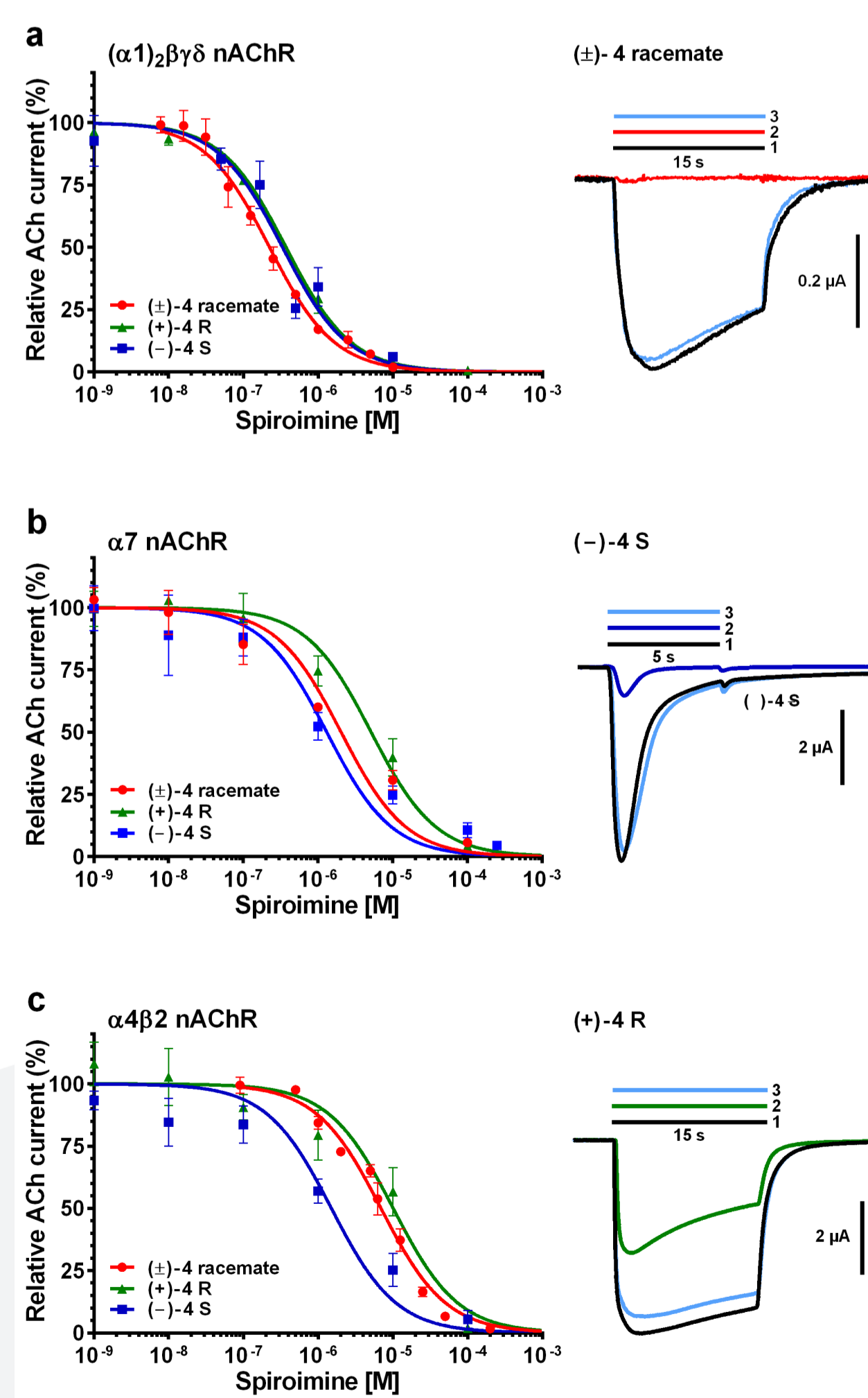
- Main anchoring point for the cyclic imine: central to the subunit interface
- Additional anchoring points in the apical and "mb" directions: promote high-affinity binding
- The sequence-variable loop F hardly contributes to toxin binding --> low nAChR subtype specificity
- The bridged ketal group specific 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	IC ₅₀ (μM)		
	(±)-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.