Structural mechanisms for α-conotoxin activity at the human α3β4 nicotinic acetylcholine receptor

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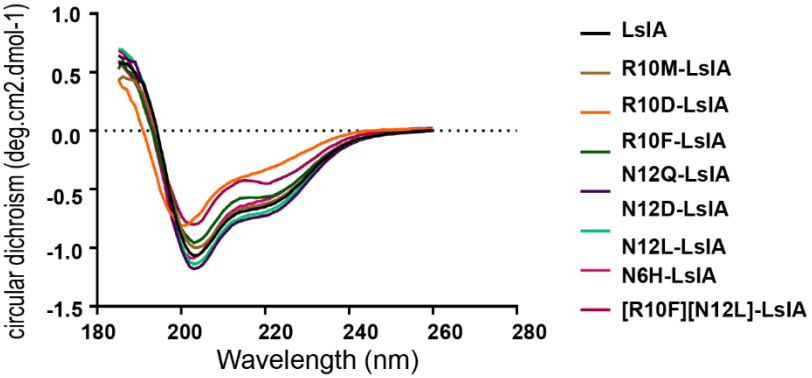
Supplementary information



Supplementary figure S1: CD spectra for synthetic LsIA analogues. LsIA analogues with modifications to position 6, 10 and 12 were chemically synthesised. R10M, R10D and R10F were synthesised as analogues of position 10. N12Q, N12D and N12L as analogues for position 12. CD spectra for LsIA analogues confirmed that all the analogues preserved the α-helical fold, similar to the native peptide with the exception of R10D-LsIA where the peak at 222 nm was shallower and the peak at 204 nm shifted to 200 nm as compared to LsIA. Suggesting that R10D-LsIA conformation might differ from the conformation of native LsIA, possibly due to global structural changes in the peptide induced by this modification.

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Ls-AChBP      ----------LDRADILYNIRQTSRPDVIPTQRDRPVAVSVSLKFINILEVNEITNEVDV  50
hα3           --------AEHRLFERLF--EDYNEIIRPVANVSDPVIIHFEVSMSQLVKVDEVNQIMET  50
rα3           --------AEHRLFQYLF--EDYNEIIRPVANVSHPVIIQFEVSMSQLVKVDEVNQIMET  50

Ls-AChBP      VFWQQTTWSDRTLAWNSSHSP--DQVSVPISSLWVPDLAAYNAIS-KPEVLTPQLARVVS  107
hα3           NLWLKQIWNDYKLKWNPSDYGGAEFMRVPAQKIWKPDIVLYNNAVGDFQVDDKTKALLKY  110
rα3           NLWLKQIWNDYKLKWKPSDYQGVEFMRVPAEKIWKPDIVLYNNADGDFQVDDKTKALLKY  110

Ls-AChBP      DGEVLYMPSIRQRFSCDVSGVD-TESGATCRIKIGSWTHHSREISVDPTTENSDDSEYFS  166
hα3           TGEVTWIPPAIFKSSCKIDVTYFPFDYQNCTMKFGSWSYDKAKIDLVLIGSSMNLKDYW-  169
rα3           TGEVTWIPPAIFKSSCKIDVTYFPFDYQNCTMKFGSWSYDKAKIDLVLIGSSMNLKDYW-  169

Ls-AChBP      QYSRFEILDVTQKKNSVTYSCCPEAYEDVEVSLNFRKKGRSEIL-210
hα3           ESGEWAIIKAPGYKHDIKYNCCEEIYPDITYSLYIRRLPLFYTIN214
rα3           ESGEWAIIKAPGYKHEIKYNCCEEIYQDITYSLYIRRLPLFYTI-213
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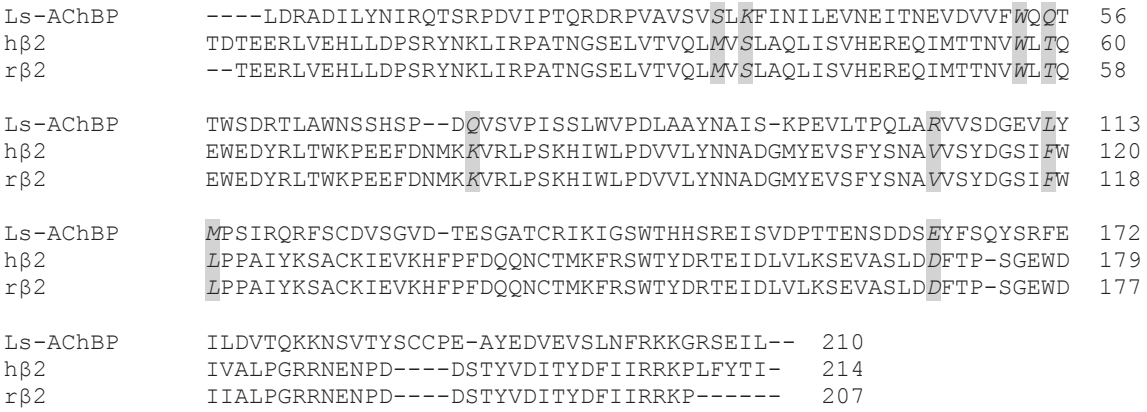
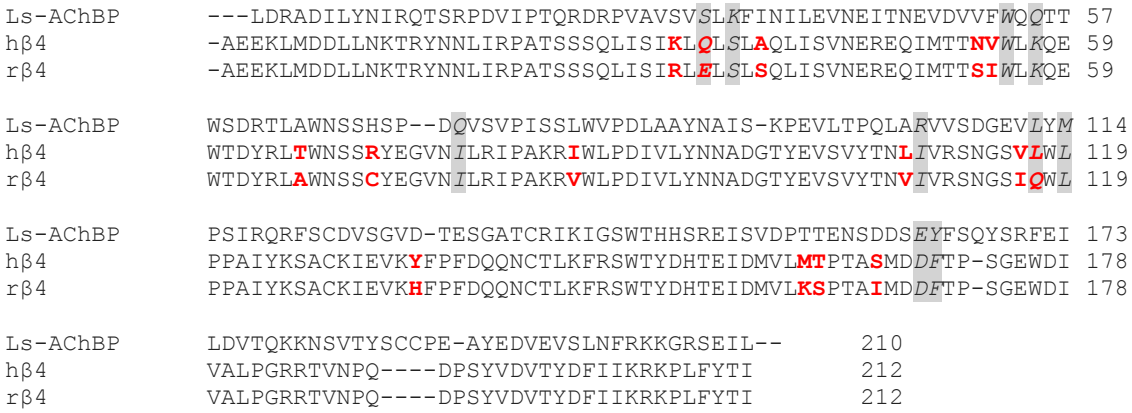
Supplementary figure S2: Sequence alignment for the human and rat α3 subunit ligand binding domain. The human (Uniprot: P32297) and rat (Uniprot: P04757) α3 are aligned with the Ls-AChBP. The α3 subunit from the two species share 95% sequence identity with an E value of $1x10^{-150}$. The residues varying between the two species are highlighted in red. The residues participating in receptor-ligand interactions are bold with grey back ground.



Supplementary figure S3: Sequence alignment for the human and rat β2 subunit ligand binding domain. The human (Uniprot: P11787) and rat (Uniprot: P12390) β2 are aligned with the Ls-AChBP. The β2 subunit from the two species share 99% sequence identity with an E value of $2x10^{-158}$. The residues participating in receptor-ligand interactions are highlighted with grey back ground.



Supplementary figure S4: Sequence alignment for the human and rat β4 subunit ligand binding domain. The human (Uniprot: P30926) and rat (Uniprot: P12392) β4 are aligned with the Ls-AChBP. The β4 subunit from the two species share 93% sequence identity with an E value of $4x10^{-154}$. The residues varying between the two species are highlighted in red. The residues participating in receptor-ligand interactions are highlighted in grey back ground.

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Ls-AChBP      --LDR---ADILYNIRQTSRPDVIPTQRDRPVAVSVSLKFINILEVNEITNEVDVVFWQQ 55
hα7           GEFQRKLYKELVKNYNPLERP---VANDSQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQ 57
rα7           GEFQRRLYKELVKNYNPLERP---VANDSQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQ 57

Ls-AChBP      TTWSDRTLAWNSSHSP--DQVSVPISSLWVPDLAAYNAIS-KPEVLTPQLARVVSDGEVL 112
hα7           MSWTDHYLQWNVSEYPGVKTVRFPDGQIWKPDILLYNSADERFDATFHTNVLVNSSGHCQ 117
rα7           MSWTDHYLQWNMSEYPGVKNVRFPDGQIWKPDILLYNSADERFDATFHTNVLVNASGHCQ 117

Ls-AChBP      YMPSIRQRFSCDVSGV-DTESGATCRIKIGSWTHHSREISVDPTTENSDDSEYFSQYSRF 171
hα7           YLPPGIFKSSCYIDVRWFPFDVQHCKLKFGSWSYGGWSLDLQM--QEADISGYIP-NGEW 174
rα7           YLPPGIFKSSCYIDVRWFPFDVQQCKLKFGSWSYGGWSLDLQM--QEADISSYIP-NGEW 174

Ls-AChBP      EILDVTQKKNSVTYSCCPEAYEDVEVSLNFRKKGRSEIL     210
hα7           DLVGIPGKRSERFYECCKEPYPDVTFTVTMRRRT-----     208
rα7           DLMGIPGKRNEKFYECCKEPYPDVTYTVTMRRRT-----     208
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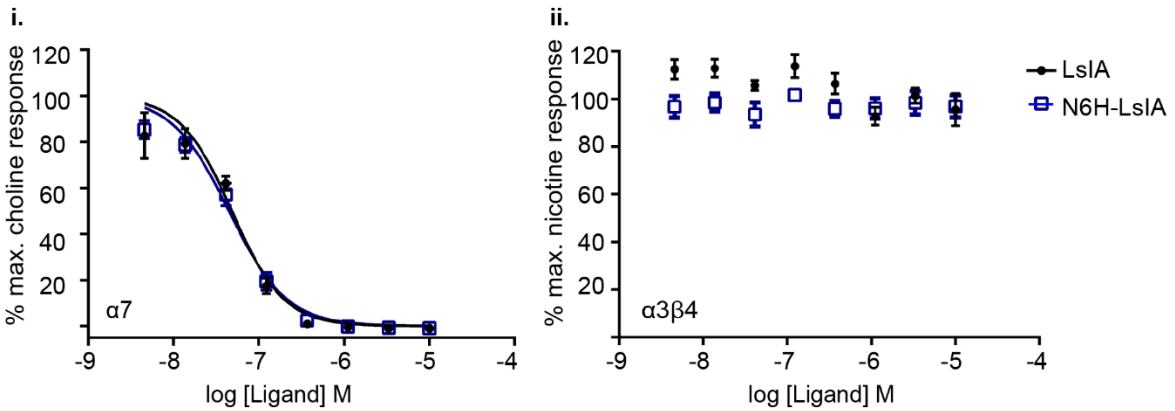
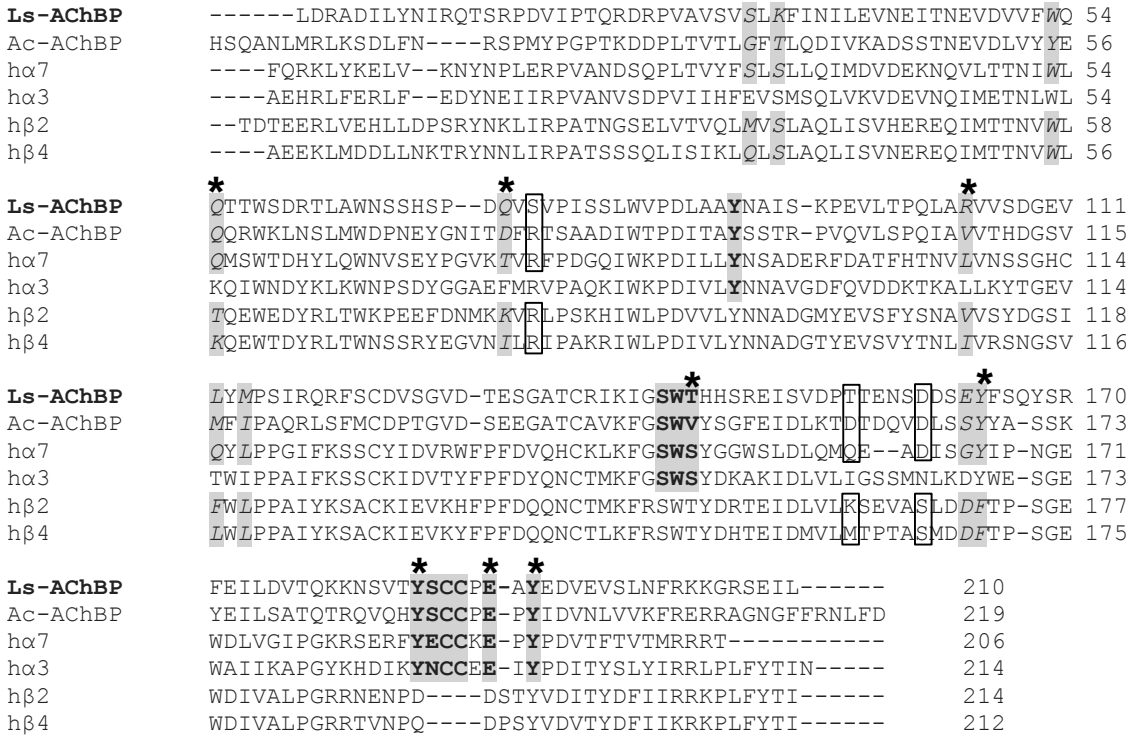
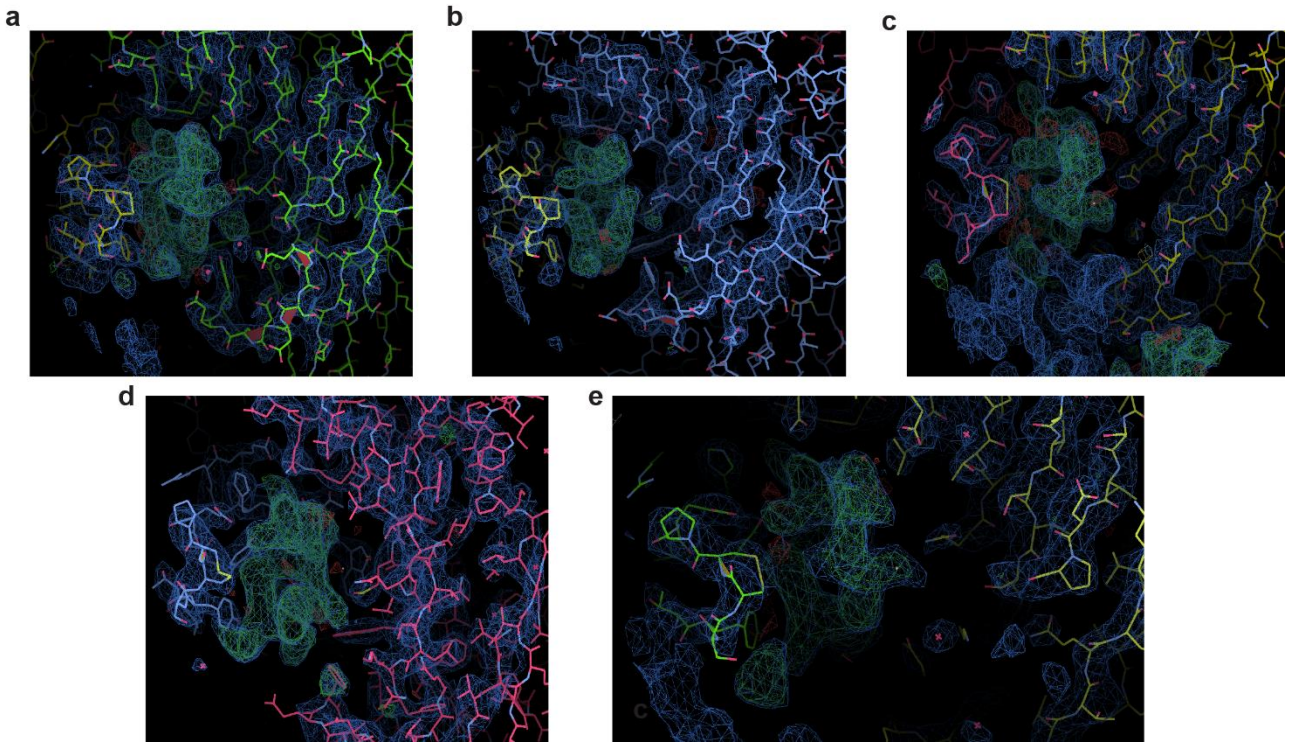
Supplementary figure S5: Sequence alignment for the human and rat α7 subunit ligand binding domain. The human (Uniprot: P30926) and rat (Uniprot: P12392) α7 are aligned with the Ls-AChBP. The α7 subunit from the two species share 95% sequence identity with an E value of $5x10^{-156}$. The residues varying between the two species are highlighted in red. The residues participating in receptor-ligand interactions are highlighted in bold with grey back ground (residues of the principle face) and in *italics* with grey background (residues of the complimentary face).



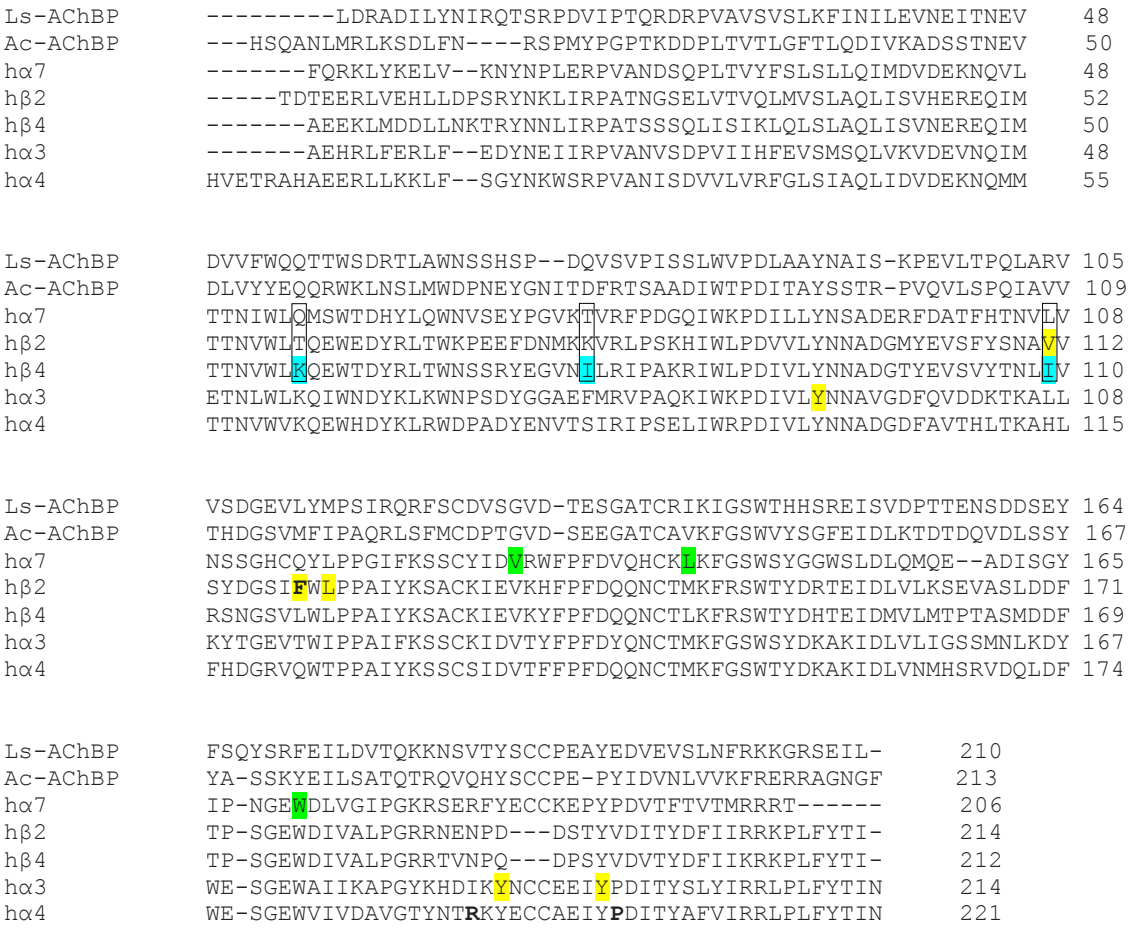
Supplementary figure S6: Characterisation of LsIA-N6H analogue. A histidine residue at position 6 is a common feature for α-conotoxins that antagonise the α3β4 nAChR. LsIA N6 was substituted with histidine. The N6H analogue had a functional profile similar to LsIA at α7 and α3β4 subtypes. This suggests that the histidine residue is not critical for the activity and selectivity of antagonists at the α3β4 nAChR.



Supplementary figure S7: Multiple sequence alignment of the Ac- , Ls-AChBPs, human α7, α3,β2 and β4 subunits. The alignment highlights residues involved in receptor-ligand interactions. Principle side contacts are in bold with grey background. Complimentary side contacts are in italics with grey background. Residues involved in hydrogen bond are indicated with (*), Residues not in direct contact, but are a part of the interacting surface are boxed.



Supplementary figure S8: Electron density maps of the LsIA/Ls-AChBP co-crystal structure. Shown are the Fo-Fc (3.0 σ) and 2Fo-Fc (1.0 σ) maps for the co-crystal structure before peptide building. The ligand binding pocket shows clear electron density for LsIA. The five interfaces are shown as (a) Subunits A-E interface (b) Subunits B-D (c) Subunits C-A (d) Subunits D-C (e) Subunits E-B.



Supplementary figure S9: Residues modulating α-conotoxin activity at nAChRs. Residues influencing α-conotoxin activity at the α7[1] (green), α3β2[1,2] (yellow), α4β2[3,4] (bold)and α3β4[this study] (blue) are shown. Distinct sets of residues influence α-conotoxin activity at the different subtypes. A comparison of residues equivalent to the α3β4 pharmacophore in other nAChRs (boxed) reveal some sequence variations. Therefore, in addition to being primary determinants of α3β4 activity, the β4 triad identified is likely to influence the selectivity of α-conotoxins for different nAChR subtypes.

Supplementary Table S1: Data collection and refinement statistics.

	Ls-AChBP - LsIA
Data collection	
Space group	C 2 2 2_1
Cell dimensions, Å	a=115.8Å, b=124.5Å, c=154.2Å
Cell dimensions, °	$\alpha=90^0$, $\beta=90^0$, $\gamma=90^0$
Resolution, Å	51.40-2.80 (2.95 - 2.80)
Rsym	0.137 (0.934)
$I/I\sigma$	14.6 (2.6)
Completeness (%)	96.9 (90.7)
Multiplicity	11.4 (11.2)
Total no. of reflections	306906 (40559)
Unique reflections	26929 (3618)
Refinement	
Resolution, Å	48.44-2.8
Rwork/Rfree	0.2176 / 0.2451
rmsd bond distance, Å	0.008
rmsd bond angle, Å	1.06
Average B-factor	70.0

Supplementary table S2: Receptor-ligand interactions seen in LsIA/Ls-AChBP co-crystal structure.

LsIA	**Ls AChBP**	
	Principal subunit (+)	Complimentary subunit (-)
Ser1	Ser186#* Cys187*	
Gly2	**Tyr185#**	
Cys3	Cys187 Cys188	-
Cys4		**Tyr164*#**
Ser5		Glu163#*
Asn6	**Tyr185#**	
Pro7	Tyr89* Trp143	Trp53* Met114*
Ala8	**Tyr192#** Ser142 Trp143	
Cys9	Cys187 Cys188 Tyr192#	
Arg10		**Tyr164*** Ser32* **Gln55*** Lys34*
Val11		Leu112
Asn12	**Thr144* (backbone)**	**Gln73* Arg104***
Asn13	**Glu190 Tyr192***	
Pro14		Leu112*
Asn15		
Ile16	Cys187	
Cys17		

(#) interactions seen in ≤ 3 pockets.
(**Bold**) Hydrogen bonds (*) Interactions unique to LsIA/Ls-AChBP complex.

Supplementary references

1 Hopping, G. *et al.* Hydrophobic residues at position 10 of α-conotoxin PnIA influence subtype selectivity between α7 and α3β2 neuronal nicotinic acetylcholine receptors. *Biochemical pharmacology* **91**, 534-542 (2014).

2 Lin, B. *et al.* From crystal structure of α-conotoxin GIC in complex with Ac-AChBP to molecular determinants of its high selectivity for α3β2 nAChR. *Scientific reports* **6** (2016).

3 Beissner, M. *et al.* Efficient binding of 4/7 α-conotoxins to nicotinic α4β2 receptors is prevented by Arg185 and Pro195 in the α4 subunit. *Molecular pharmacology* **82**, 711-718 (2012).

4 Dutertre, S., Nicke, A. & Lewis, R. J. β2 subunit contribution to 4/7 α-conotoxin binding to the nicotinic acetylcholine receptor. *Journal of Biological Chemistry* **280**, 30460-30468 (2005)