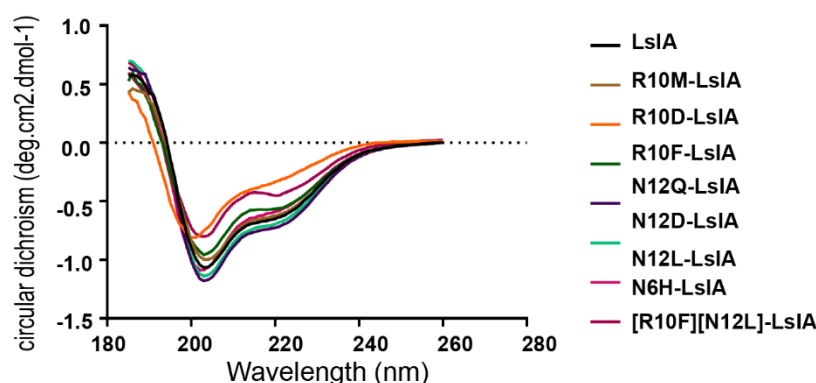


Structural mechanisms for α -conotoxin activity at the human $\alpha 3\beta 4$ nicotinic acetylcholine receptor

**Nikita Abraham, Michael Healy, Lotten Ragnarsson, Andreas Brust,
Paul Alewood, Richard Lewis***

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.

Ls-AChBP	-----LDRADILYNIHQTSRPDVIPTQRDRPVAVSVSLKFINILEVNEITNEVDV	50
h α 3	-----AEHRLF ER LF--EDYNEIIRPVANVSDPVI I HFEVSMSQLVKVDEVNQIMET	50
r α 3	-----AEHRLF QY LF--EDYNEIIRPVANVSH PV II Q FEVSMSQLVKVDEVNQIMET	50
Ls-AChBP	VFWQQTTWSDRTLAWNSSHSP--DQVSVPISSLWVPDLAAY N NAIS-KPEVLTQPQLARVVS	107
h α 3	NLWLKQIWNDYKLLK W NPSDY GGA EFMRVPAQKIWKPDIVLY N NAVGFQVDDKTKALLKY	110
r α 3	NLWLKQIWNDYKLLK K PSDY QGV EFMRVPAEKIWKPDIVLY N NADGDFQVDDKTKALLKY	110
Ls-AChBP	DGEVLYMPSIRQRFSCDVSQVD-TESGATCRIKIG SWT HHSREISVDPTTENSDDSEYFS	166
h α 3	TGEVTWIPPAIFKSSCKIDVTYFPFDYQNTMKFG SWS YDKAKIDLVLIGSSMNLKDYW-	169
r α 3	TGEVTWIPPAIFKSSCKIDVTYFPFDYQNTMKFG SWS YDKAKIDLVLIGSSMNLKDYW-	169
Ls-AChBP	QYSRFEILDVTVQKKNVTV YSCCEIAY EDVEVSLNFRKKGRSEIL- 210	
h α 3	ESGEWAIKAPGYKH DIKYNCC E I Y PDITYSLYIRRLPLFYTI- 214	
r α 3	ESGEWAIKAPGYKH EIKYNCC E I Y QDITYSLYIRRLPLFYTI- 213	

Supplementary figure S2: Sequence alignment for the human and rat α 3 subunit ligand binding domain. The human (Uniprot: P32297) and rat (Uniprot: P04757) α 3 are aligned with the Ls-AChBP. The α 3 subunit from the two species share 95% sequence identity with an E value of 1×10^{-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.

Ls-AChBP	----LDRADILYNIRQTSRDPVIPTQRDRPVAVSVSLKFINILEVNEITNEVDVVF ^W QQT	56
hβ2	TDTEERLVEHLLDPSRYNKLIRPATNGSELVTVQLMVSLAQLISVHEREQIMTTNV ^W L ^{TQ}	60
rβ2	--TEERLVEHLLDPSRYNKLIRPATNGSELVTVQLMVSLAQLISVHEREQIMTTNV ^W L ^{TQ}	58
Ls-AChBP	TWSDRTLAWNSSHSP--DQVSVPISSLWVPDLAAYNAIS-KPEVLTPQLARVVSDGEVLY	113
hβ2	EWEDYRLTWKPEEFDNMKKVRLPSKHIWLPDVVLYNNADGMYEVSFYSNAVWSYDGSIF ^W	120
rβ2	EWEDYRLTWKPEEFDNMKKVRLPSKHIWLPDVVLYNNADGMYEVSFYSNAVWSYDGSIF ^W	118
Ls-AChBP	MPSIRQRFSCDVSGVD-TESGATCRIKIGSWTHHSREISVDPTTENSDDSEYFSQYSRFE	172
hβ2	LPPAIYKSACKIEVKHFPPDQQNCTMKFRSWTYDRTEIDLVLKSEVASLDDFTP-SGEWD	179
rβ2	LPPAIYKSACKIEVKHFPPDQQNCTMKFRSWTYDRTEIDLVLKSEVASLDDFTP-SGEWD	177
Ls-AChBP	ILDVTQKKNSVTYSCCPE-AYEDVEVSLNFRKKGRSEIL--	210
hβ2	IIVALPGRRNENPD----DSTYVDITYDFIIRKPLFYTI-	214
rβ2	IIVALPGRRNENPD----DSTYVDITYDFIIRKPLFYTI-	207

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 2×10^{-158} . The residues participating in receptor-ligand interactions are highlighted with grey back ground.

Ls-AChBP	---LDRADILYNIRQTSRDPVIPTQRDRPVAVSVSLKFINILEVNEITNEVDVVF ^W QQT	57
hβ4	-AEEKLMDLLNKTRYNNLIRPATSSSSQLISIKLQLSLAQLISVNEREQIMTTNV ^W LKQE	59
rβ4	-AEEKLMDLLNKTRYNNLIRPATSSSSQLISIRLELSLSQLISVNEREQIMTT ^{SI} W ^L KQE	59
Ls-AChBP	WSDRTLAWNSSHSP--DQVSVPISSLWVPDLAAYNAIS-KPEVLTPQLARVVSDGEVLYM	114
hβ4	WTDYRLTWNSSRYEGVNLIRIPAKR ^I WLPDIVLYNNADGTYEVS ^V YTN ^L IVRSNGSV ^L W ^L	119
rβ4	WTDYRLAWNSSCYEGVNLIRIPAKR ^V WLPDIVLYNNADGTYEVS ^V YTN ^V IVRSNGSI ^Q W ^L	119
Ls-AChBP	PSIRQRFSCDVSGVD-TESGATCRIKIGSWTHHSREISVDPTTENSDDSEYFSQYSRFEI	173
hβ4	PPAIYKSACKIEVK ^Y FPPDQQNCTLKFRSWTYDHTEIDMVL ^{MT} PTAS ^{MD} FTP-SGEWDI	178
rβ4	PPAIYKSACKIEVK ^H FPPDQQNCTLKFRSWTYDHTEIDMVL ^{KS} PTA ^I MD ^D FTP-SGEWDI	178
Ls-AChBP	ILDVTQKKNSVTYSCCPE-AYEDVEVSLNFRKKGRSEIL--	210
hβ4	VALPGRRTVNPQ----DPSYVDVITYDFIIRKPLFYTI	212
rβ4	VALPGRRTVNPQ----DPSYVDVITYDFIIRKPLFYTI	212

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 4×10^{-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.

```

Ls-AChBP      --LDR---ADILYNIRQTSRPDVIPTQRDRPVAVSVSLKFINILEVNEITNEVDVVFWQO 55
hα7           GEFQRKLYKELVKYNPLERP---VANDSQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQ 57
rα7           GEFQRRLYKELVKYNPLERP---VANDSQPLTVYFSLSLLQIMDVDEKNQVLTTNIWLQ 57

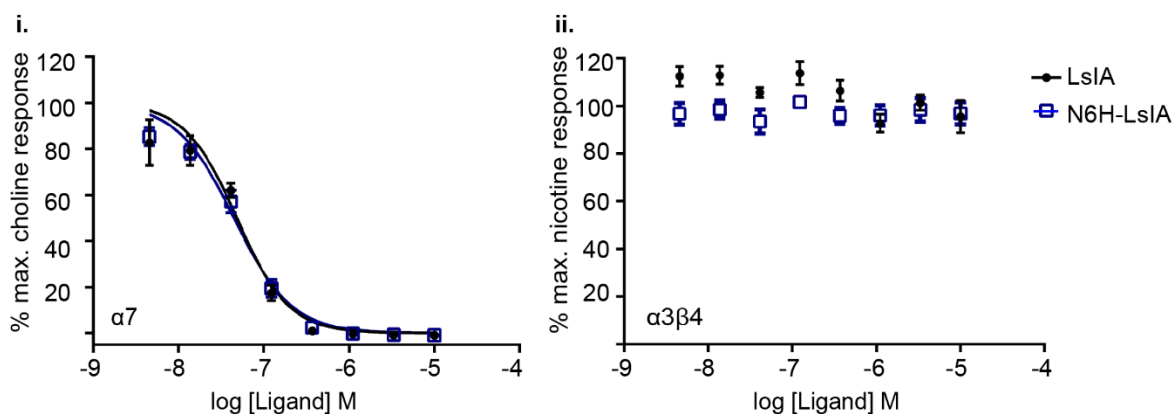
Ls-AChBP      TTWSDRTLAWNSSHSP--DQVSVPISSLWVPDLAAYNAIS-KPEVLTPQLARVVDGEVL 112
hα7           MSWTDHYLQWNVSEYPGVKTVRFPDQGIWKPDILLYNSADERFDATFHTNVLVNSSGHCQ 117
rα7           MSWTDHYLQWNMSEYPGVKNVRFPDQGIWKPDILLYNSADERFDATFHTNVLVNASGHCQ 117

Ls-AChBP      YMPSIRQRFSCDVSGV-DTESGATCRIKIGSWTHHSREISVDPTTENSDDSEYFSQYSRF 171
hα7           YLPPGIFKSSCYIDVRWFPPDVQHCKLKFGSWSYGGWSLDLQM--QEADISGYIP-NGEW 174
rα7           YLPPGIFKSSCYIDVRWFPPDVQQCKLKFGSWSYGGWSLDLQM--QEADISSYIP-NGEW 174

Ls-AChBP      EILDVTQKKNSVTYSCCPEAYEDVEVSLNFRKKGRSEIL 210
hα7           DLVGIPGKRSERFYECCKEPYPDVTFTVTMRRRT----- 208
rα7           DLMGIPGKRNEKFYECCKEPYPDVTYTVTMRRRT----- 208

```

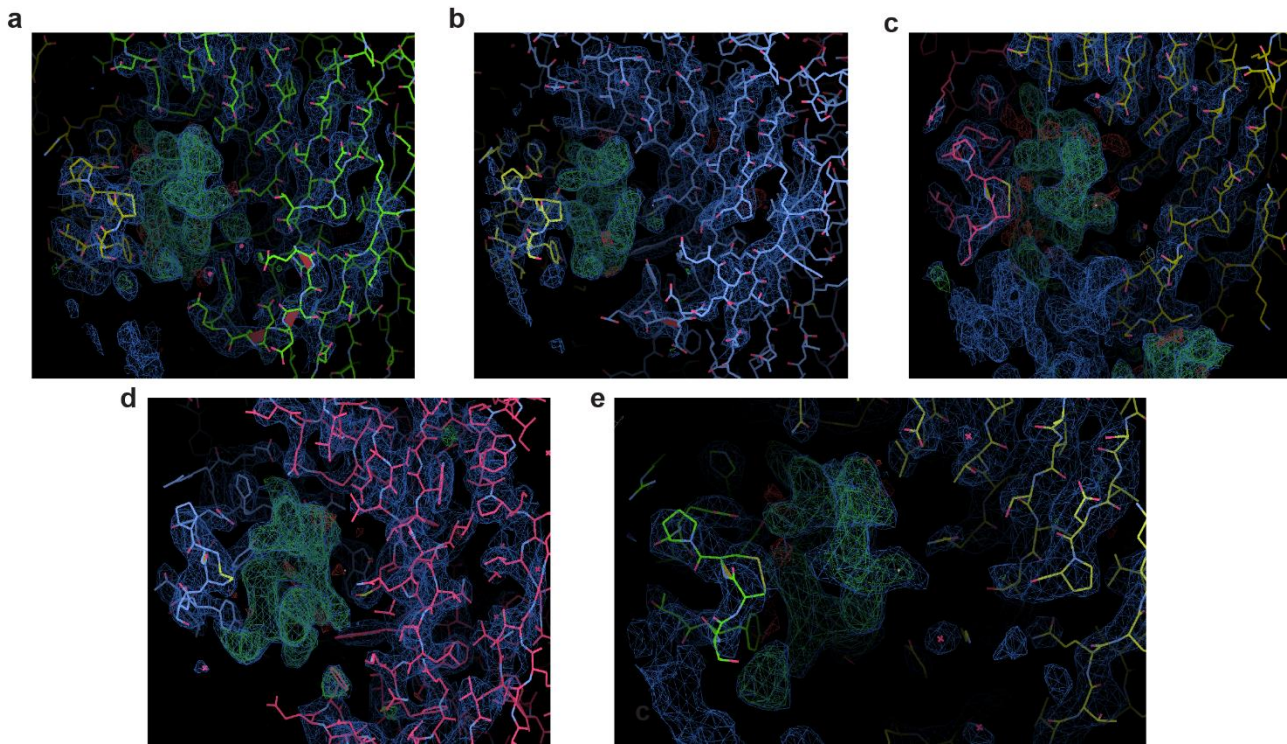
Supplementary figure S5: Sequence alignment for the human and rat $\alpha 7$ subunit ligand binding domain. The human (Uniprot: P30926) and rat (Uniprot: P12392) $\alpha 7$ are aligned with the Ls-AChBP. The $\alpha 7$ subunit from the two species share 95% sequence identity with an E value of 5×10^{-156} . 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 $\alpha 3\beta 4$ nAChR. LsIA N6 was substituted with histidine. The N6H analogue had a functional profile similar to LsIA at $\alpha 7$ and $\alpha 3\beta 4$ subtypes. This suggests that the histidine residue is not critical for the activity and selectivity of antagonists at the $\alpha 3\beta 4$ nAChR.

Ls-AChBP	-----LDRADILYNIHQTSRPDVIPTQRDRPVAVSVSLKFINILEVNEITNEVDVVF	WQ	54
Ac-AChBP	HSQANLMRLKSDLFN----RSPMPGPTKDDPLTVTLGFTLQDIVKADSSSTNEVDLVYYE		56
h α 7	----FQRKLYKELV--KNYNPLERPVANDSQPLTVYFSLSLQIMDVDEKNQVLTNI	WL	54
h α 3	----AEHRLFERLF--EDYNEIIRPVANVSDPVIHFEVSMSQLVKVDEVNQIMETNLWL		54
h β 2	--TDTEERLVEHLLDPSRYNKLIRPATNGSELVTVQLMVSLAQLISVHEREQIMTNNV	WL	58
h β 4	----AEEKLMDLLNKTRYNNLIRPATSSSQLISIKLQLSLAQLISVNEREQIMTNNV	WL	56
Ls-AChBP	* QTTWSDRTLAWNSSHSP--DOVSVPISSSLWVPDLAAYNAIS-KPEVLTPQLARVSDGEV		111
Ac-AChBP	QQRWKLNSLMWDPNEYGNITDEFRTSAADIWTPDITAYSSSTR-PVQVLSPQIAVWTHDGSV		115
h α 7	QMSWTDHYLQWNVSEYPGVKTVRFPPDQGIWKPDILLYNSADERFDATFHTNVLVNSSGHC		114
h α 3	KQIWNNDYKLLWNPSDYGGAEFMRVPAQKIWKPDIVLYNNAVGFQVDDKTKALLKYTGEV		114
h β 2	TQEWEDYRLTWKPEEFDNMKVRRLPSKHIWLPDVVLYNNADGMYEVSFYNSNAVSYDGS		118
h β 4	KQEWTDYRLTWNSSRYEGVNTLRLPAKRIWLPDIVLYNNADGTYEVSVYTNLTVRSNGSV		116
Ls-AChBP	LYMPSIRQRFSCDVSQVD-TESGATCRIKIG SWT HHSREISVDPFTENSDDSEYFSQYSR		170
Ac-AChBP	MEIPAQRLSFMCDPTGVD-SEEGATCAVKFG SWV YSGFEIDLKTDIDQVDLSYYA-SSK		173
h α 7	QYLPPGIFKSSCYIDVRWFPPFDVQHCKLKFG SWS YGGWSLDLQME--ADISGYIP-NGE		171
h α 3	TWIPPAIFKSSCKIDVTYFFFDYQNCTMKFG SWS YDKAKIDLVLIGSSMNLKDYWE-SGE		173
h β 2	FWLPPAIYKSACKIEVKHFPPDQONCTMKFRSWTYDRTEIDLVLKSEVASLDDFTP-SGE		177
h β 4	LWLPPAIYKSACKIEVKYFFPDQONCTLKFERSWTYDHTEIDMVLMTPTASMDFTP-SGE		175
Ls-AChBP	FEILDVTQKKNSVT YSCCPE -AYEDVEVSLNFRKKGRSEIL-----		210
Ac-AChBP	YEILSATQTRQVQH YSCCPE -PYIDVNLVVKFRERRAGNGFFRNLF		219
h α 7	WDLVGIPGKRSERF YECCKE -PYPDVTFTVTMRRRT-----		206
h α 3	WAIKAPGYKHDIK YNCCEE -IYPDITYSLYIRRLPLFYTI-----		214
h β 2	WDIVALPGRRNENPD----DSTYVDITYDFIIRKPLFYTI-----		214
h β 4	WDIVALPGRRTVNPQ----DPSYVDVITYDFIIRKPLFYTI-----		212

Supplementary figure S7: Multiple sequence alignment of the Ac-, Ls-AChBPs, human α 7, α 3, β 2 and β 4 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 (3.0σ) and 2Fo-Fc (1.0σ) 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.

Ls-AChBP	-----LDRADILYNIRQTSRDPVIPTQRDRPVAVSVSLKFINILEVNEITNEV	48
Ac-AChBP	---HSQANLMRLKSDLFN----RSPMPYGPPTKDDPLTVTLGFTLQDIVKADSSSTNEV	50
h α 7	-----FQRKLYKELV--KNYNPLERPVANDSQPLTVYFSLSLQIMDVDEKNQVL	48
h β 2	-----TDTEERLVEHLLDPSRYNKLIRPATNGSELVTVQLMVSLAQLISVHEREQIM	52
h β 4	-----AEEKLMDLLNKTRYNNLIRPATSSSQQLISIKLQLSLAQLISVNEREQIM	50
h α 3	-----AEHRLFERLF--EDYNEIIRPVANVSDPVI IHFEVSMSQLVKVDEVNQIM	48
h α 4	HVETRAHAERLLKRLF--SGYNKWSRPVANISDVVLVRFGLSIAQLIDVDEKNQMM	55
Ls-AChBP	DVVFQQTTWSDRTLAWNSSHSP--DQVSVPISSLWVPDLAAYNAIS-KPEVLTPQLARV	105
Ac-AChBP	DLVYEEQQRWKLNSLMWDPNEYGNITDFRTSAADIWTPDITAYSSTR-PVQVLSQPQIAVV	109
h α 7	TTNIWLQMSWDTHYLQWNVSEYPGVKITVRFDPDGIWKPDILLNSADERFDATFHTNVTLV	108
h β 2	TTNVWLTQEWEDYRLTWKPEEFDNMKKVRLPSKHIWLPDVVLYNNADGMYEVSFYSNAYV	112
h β 4	TTNVWLTQEWEDYRLTWNSSRYEGVNI LRIPAKRIWLPDIVLYNNADGTVEVSVYTNLIV	110
h α 3	ETNLWLKQIWN DYK LKWNPSDYGGAEFMRVPAQKIWKPDIVLYNNAVGDVQVDDKTKALL	108
h α 4	TTNVVVKQEWHDYKLRWDPADYENVTSIRIPSELIWRPDI VLYNNADGDFAVTHLTKAHL	115
Ls-AChBP	VSDGEVLYMPSIRQRFSCDVSGVD-TESGATCRIKIGSWTHHSREISVDPTTENSDDSEY	164
Ac-AChBP	THDGSVMFIPAQRLSFMCDPTGVD-SEEGATCAVKFGSWVYSGFEIDLKTDTDQVDLSSY	167
h α 7	NSSGHCQYLPPGIFKSSCYIDRWFPPFDVQHCKIKFGSWSYGGWSLDLQMQE--ADISGY	165
h β 2	SYDGSIFWLP PAIYKSACKIEVKHFPFDQQNCTMKFRSWTYDRTEIDLVLKSEVASLDDF	171
h β 4	RSNGSVLWLPPAIYKSACKIEVKYFPFDQQNCTLKRFSWTYDHT EIDMVLMTPTASMDDF	169
h α 3	KYTGEVTVIPPAIFKSSCKIDVTYFPFDYQNC TMKFGSWSYDKAKIDLVLIGSSMNLKDY	167
h α 4	FHDGRVQWTPPAIYKSSCSIDVTFPPFDQQNCTMKFGSWTYDKAKIDLVMHRSRVDQLDF	174
Ls-AChBP	FSQYSRFEILDVTQKKN SVTYSCPEAYEDVEVSLNFRKKGRSEIL-	210
Ac-AChBP	YA-SSKYEILSATQTRQVQHYSCCPE-PYIDVNLVVKFRERRAGNGF	213
h α 7	IP-NGEIDLVGIPGKRSERFYECCKEYPDVTFVTMRRRT-----	206
h β 2	TP-SGEWDIVALPGRRNENPD---DSTYVDITYDFIIRRKPLFYTI-	214
h β 4	TP-SGEWDIVALPGRRTVNPO---DPSYVDVITYDFIIRRKPLFYTI-	212
h α 3	WE-SGEWAI IKAPGYKHDIKYNCCEIY PDITYSLYIRRLPLFYTIN	214
h α 4	WE-SGEWIVDAVGTYNTRKYECCEAIY PDITYAFVIRRLPLFYTIN	221

Supplementary figure S9: Residues modulating α -conotoxin activity at nAChRs. Residues influencing α -conotoxin activity at the α 7¹ (green), α 3 β 2^{1,2} (yellow), α 4 β 2^{3,4} (bold) and α 3 β 4^{this study} (blue) are shown. Distinct sets of residues influence α -conotoxin activity at the different subtypes. A comparison of residues equivalent to the α 3 β 4 pharmacophore in other nAChRs (boxed) reveal some sequence variations. Therefore, in addition to being primary determinants of α 3 β 4 activity, the β 4 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</i> / σ	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 $\alpha 7$ and $\alpha 3\beta 2$ 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 $\alpha 3\beta 2$ nAChR. *Scientific reports* **6** (2016).
- 3 Beissner, M. *et al.* Efficient binding of 4/7 α -conotoxins to nicotinic $\alpha 4\beta 2$ receptors is prevented by Arg185 and Pro195 in the $\alpha 4$ subunit. *Molecular pharmacology* **82**, 711-718 (2012).
- 4 Dutertre, S., Nicke, A. & Lewis, R. J. $\beta 2$ subunit contribution to 4/7 α -conotoxin binding to the nicotinic acetylcholine receptor. *Journal of Biological Chemistry* **280**, 30460-30468 (2005)