

Supplementary information

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

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Running Title: Co-crystal structure of α -conotoxin GIC and acetylcholine binding proteins

Table S1|Crystal diffraction data collection and structural refinement statistics

Data collection	
Beamline	SSRF BL17U
Wavelength	0.9796 Å
Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	78.6, 84.9, 208.6
α , β , γ (°)	90.0, 90.0, 90.0
Resolution (Å)	50-2.1
<i>R</i> _{merge} (%)	8.8 (67.7)
<i>I</i> / σI	18.7 (3.5)
Completeness (%)	96.9 (93.5)
Redundancy	6.7 (6.9)
Refinement	
Resolution (Å)	43.5-2.1
No. Reflections	85409
<i>R</i> _{work} / <i>R</i> _{free} (%)	19.7/23.7
No. atoms	
Protein	8299
Ligand/ion	545
Water	521
B factor (Å²)	
Protein	41.1
Ligand/ion	45.3
Water	42.5
r.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	1.104

Table S2 | Contacts between *Ac*-AChBP and α -conotoxins ($d < 4 \text{ \AA}$)

<i>Ac</i> -AChBP	GIC ^a	PnIA(A10L, D14K) ^b	TxIA(A10L) ^c	ImI ^d	BuIA
<i>Principal side</i>					
Lys-23				Arg-11	
Tyr-91	His-5	Leu-5, Pro-7	Arg-5, Pro-6, Pro-7	Arg-7	Pro-7
Ser-144		Pro-7	Pro-7	Arg-7	Pro-7
Trp-145	Pro-6, Ala-7	Pro-6, Pro-7	Pro-6, Pro-7	Pro-6, Arg-7	Pro-6, Pro-7
Val-146	Ala-7	Leu-10, Asn-11	Pro-7, Leu-10	Arg-7	Pro-7
Tyr-147			Pro-7	Arg-7	Pro-7
Ser148	Asn-11	Asn-11	Asn-11		
Glu151		Asn-11			
Tyr-186	Gly-1, Cys-2, His-5, Cys-8	Gly-1, Cys-2, Leu-5, Cys-8	Gly-1, Cys-2, Arg-5	Gly-1,Cys-2, Asp-5	Gly-1, Cys-2, Thr-5
Cys-188	Cys-2	Cys-2, Tyr-15	Cys-2	Cys-2	Cys-2
Cys-189	Asn-12	Cys-2, Asn-12	Cys-2, Asn-12	Cys-2	Cys-2, Tyr-12
Glu-191	Asn-11, Asn-12	Asn-12	Asn-11, Asn-12	Arg-11	Leu-11, Tyr-12
Tyr-193	His-5, Ala-7, Cys-8, Asn-11, Asn-12	Leu-5, Pro-7, Cys-8, Asn-11, Asn-12	Arg-5, Pro-7, Cys-8, Asn-11, Asn-12	Arg-7, Cys-8	Pro-7, Cys-8, Tyr-12
Ile-194				Arg-7	
Asp-195			Arg-5		
<i>Complementary side</i>					
Thr-34	Ser-4		Cys-3		Cys-3

Tyr-53	Ser-4, Pro-6	Pro-6	Ser-4, Pro-6	Ser-4	Ser-4
Gln-55	Ala-9, Cys-16		Ile-9	Cys-3	Cys-3, Ala-9, Cys-13
Arg-57	Gln-13,	Pro-13	Pro-13		Cys-13
Asp-75				Trp-10	
Arg-77	Asn-11	Asn-11	Asn-11	Trp-10	
Ile-104		Leu-10			
Val-106	Gly-10, Gln-13	Leu-10	Leu-10	Trp-10	Val-10
Thr-108	Gln-13			Trp-10	
Asp-110		Lys-14			
Ser-112	Gln-13	Lys-14			
Met-114	Ala-9,Gly-10, Gln-13	Ala-9, Leu-10, Pro-13	Ile-9, Leu-10, Pro-13	Trp-10	Val-10, Leu-11
Ile-116	Pro-6, Ala-9	Pro-6, Ala-9, Leu-10,	Leu-10	Pro-6, Ala-9	Ala-9, Val-10
Asp-157	Cys-16				
Asp-162	Ser-4	Ser-4	Cys-3	Ser-4	Cys-3
Ser-164	Ser-4	Ser-4	Ser-4	Gly-1, Ser-4	Ser-4
Ser-165	Ser-4		Ser-4		Ser-4

Table S3 | Affinity (as K_i or IC_{50} s, nM) of four α -conotoxins for *Ac*-AChBP, AChBP from *Lymnaea stagnalis* (*Ls*-AChBP) and different human or rat nAChR subtypes

α -conotoxins	<i>Ac</i> -AChB	<i>Ls</i> -AChBP	h α 3 β 2	h α 3 β 4	h α 7	r α 3 β 2	r α 7
	P						
GIC	29 \pm 2 ^a	220 \pm 10 ^a	1 ^b	755 ^b			
ImI	33 \pm 5 ^c	4140 \pm 400 ^c	41 ^d	3390 ^d	132 ^e	>5000 ^f	69 ^g
PnIA(A10L,D14K)	28 \pm 6 ^h	13 \pm 2 ^h			260 ^h		
TxIA(A10L)		1.1 ⁱ				2.0 ⁱ	39 ⁱ

Data from ^a this work, ^b ref. ¹¹, ^c ref. ⁹, ^d ref. ²¹, ^e ref. ²⁸, ^f ref. ²², ^g ref. ³⁰, ^h ref. ⁷ and ⁱ ref. ¹⁰.

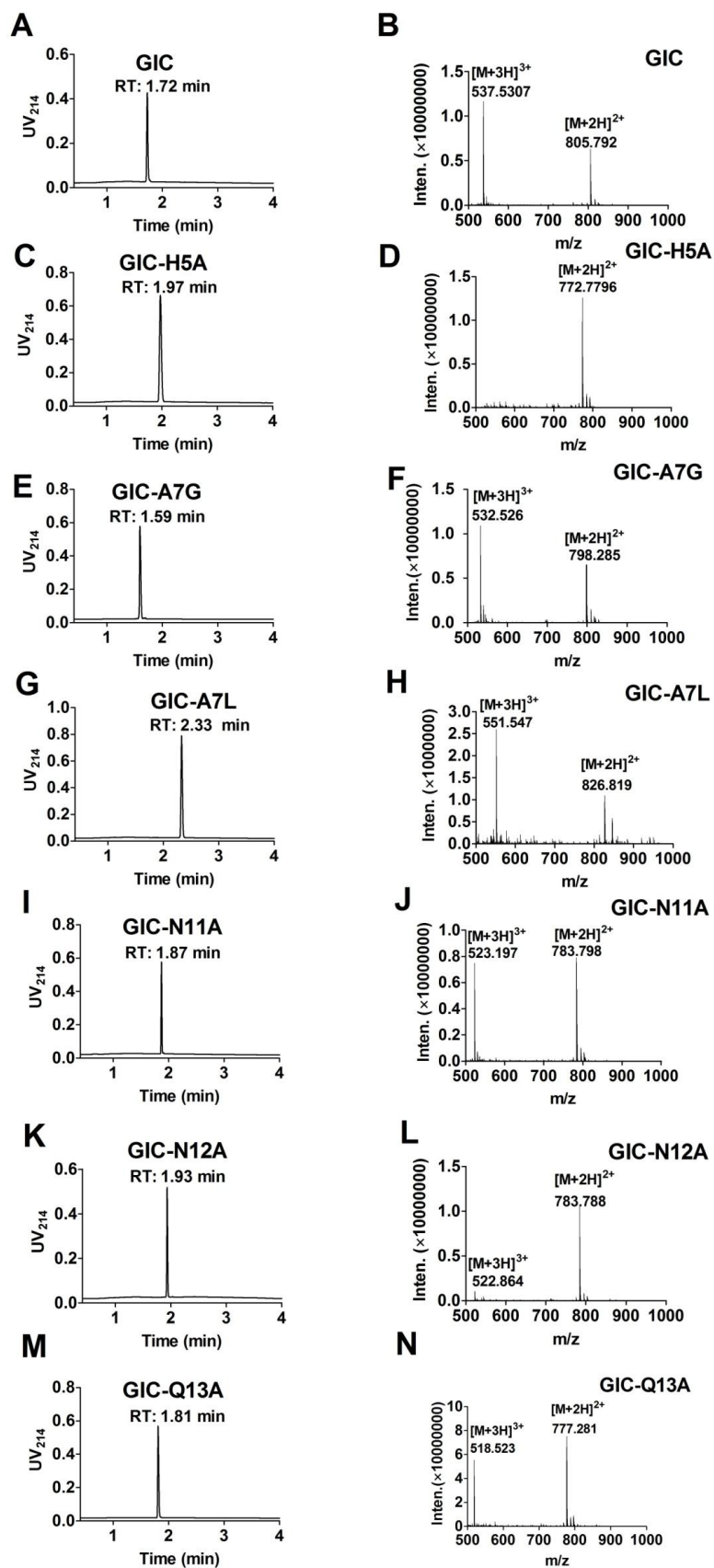


Figure S1 | HPLC chromatograms with a retention time and ESI-MS data of

α -conotoxin GIC and its analogues. The peptides were analyzed on a waters ACQUITY UPLC BEH C18 column (2.1×50 mm, 1.7 μ m) using a linear gradient of 5% Buffer B to 40% Buffer B over 3.5 min with flow rate of 0.5 ml/min at temperature of 40 °C, where A = 0.1% trifluoroacetic acid (TFA) and B = 0.75% TFA, 90% acetonitrile, and the remainder water. Absorbance was monitored at 214 nm.

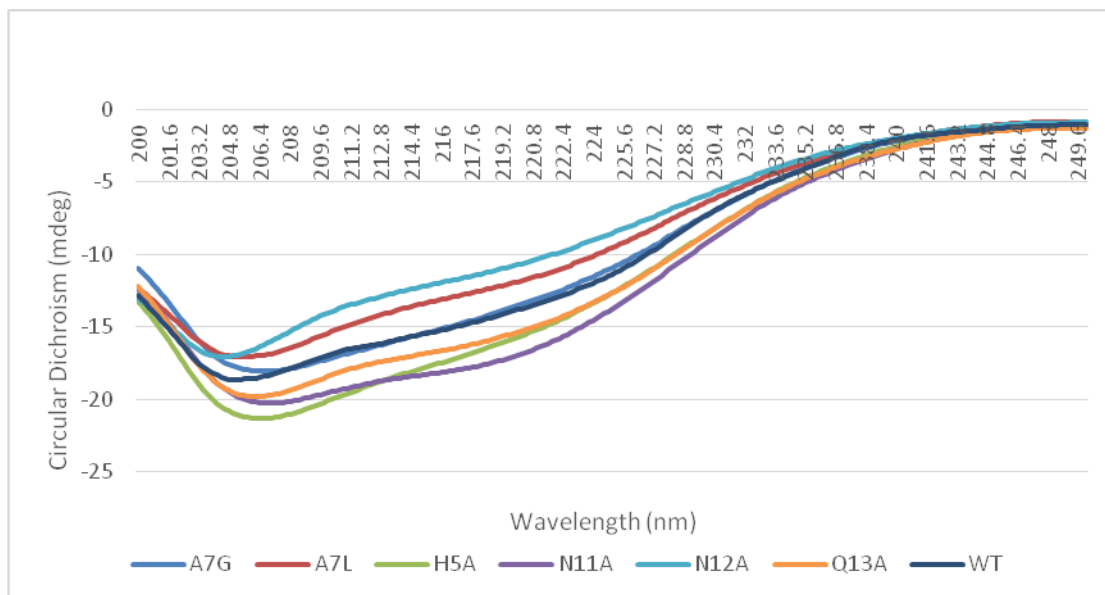


Figure S2| Circular dichroism of α -conotoxin GIC and its analogues. CD spectra were recorded on a JASCO J-715 Spectropolarimeter. Spectra was recorded at room temperature under nitrogen atmosphere. Peptides were dissolved to 100 μ M. Spectra were recorded over a 200-250 nm range at 25 °C using an average of 3 scans (scan speed of 200 nm/min).

	20	40	60																	
Ac-AChBP	--QANL---	MRLKSDLFN---	RSPMYPG--	PT-KDDPLT	VTLGFTLQ	DIVKADSS	STNEV	48												
Ls-AChBP	LDRADI---	---	LYN---	IRQTSRPD	VIP	TQRDRPV	AVSVSL	KFINILEV	NEITNEV	48										
Human α 3	-----	SEAEHRL	FERLFED--	YNEIIRP--	---	VANVSDP	VIHF	FEVSM	SQLVKV	DEVNQIM	50									
Human α 4	HVETRA	HAEEER	LLKKL	FSG--	YKWSRP--	---	VANISD	VVLV	RFGLS	IAQLID	VDEK	NQMM	55							
Human β 2	-----	TDTEER	LVEHLL	DPSRY	NKLIRP--	---	ATNGSE	LVTV	QLMV	SLAQLI	SVH	EREQIM	52							
Human β 4	RV---	ANAEEK	LMDDDL	LNKTR	YNNLIRP--	---	ATSSSQ	LISIKL	QLSLA	QLISV	NERE	QIM	54							
	80	100	120																	
Ac-AChBP	DLVY	YEQQR	WKLNS	LMSWDP	NEYGNIT	DFRT	SAADI	WTPDI	ITAY	S-STR	RPVQ	VLSP	QIAV	V	107					
Ls-AChBP	DVVFW	QQTT	WSDRT	LAWNS	SHSPD	QVSVPI	SS--	LWVPD	LAA	YN-A	ISKPE	VLTP	QLA	R	V	105				
Human α 3	ETNL	WLKQI	WNDY	KLKWN	PSDY	GGA	EFM	RVPA	QKI	WKPD	IVLY	NNAV	GDFQ	VDDK	TKALL	110				
Human α 4	TTNV	WVKQE	WHDY	KLRWD	PADY	ENV	TSIR	IPSEL	IWRP	IVLY	NNAD	GDF	AVTH	LTK	AHL	115				
Human β 2	TTNV	WLTQE	WEDY	RLTWK	PEE	FDNM	KKVR	LP	SKHI	WLPD	VVLY	NNAD	GMYE	VSVF	YSNA	VV	112			
Human β 4	TTNV	WLKQE	WTDY	RLTWN	SSRY	EGV	NILR	IPAK	RIWLP	DIVLY	NNAD	GTYE	VSVY	TNLI	IV	114				
	140	160	180																	
Ac-AChBP	THDG	SVMF	IPAR	LSFMC--	DPTG	VDS	EEG	ATCA	VKFGS	WVYS	GFE	IDL	KTDT	DQV	D	LS	SS	165		
Ls-AChBP	VSDG	EVL	YMPS	IRQRF	SC--	DVSG	VDTE	SGAT	CR	IKIGS	WTH	HRS	REIS	VDPT	TENS	D	SE	163		
Human α 3	KYTGE	VTW	IPPA	IFKSS	CKID	VTY	FPFD	Y-Q	NCTM	KFGS	WSY	DKAK	IDLV	LIGS	SMNL	KD	169			
Human α 4	FHDGR	VQW	TPPA	IYKSS	CSID	VTF	FPFD	Q-Q	NCTM	KFGS	WTY	DKAK	IDLV	NMHS	RV	DQLD	174			
Human β 2	SYDG	SIF	WLPPA	IYKSA	CKIE	VKH	FPFD	Q-Q	NCTM	KFRS	WTY	DRTE	IDLV	LKSE	VASL	DD	171			
Human β 4	R	SNG	SVL	WLPPA	IYKSA	CKIE	VKY	FPFD	Q-Q	NCTL	KFRS	WTY	DHTE	IDM	VLM	TP	TASMD	DD	173	
	200	220	240																	
Ac-AChBP	YYAS-	SKYE	ILSAT	QTRQ	VQH	YS	CCPE	PEYI	DVNL	VVK	FRER	-----	-----	-----	-----	-----	-----	205		
Ls-AChBP	YFSQ	YSRFE	ILDVT	QKKN	SVT	YS	CCPE	EA	YED	VEV	SLN	FRKK	-----	-----	-----	-----	-----	204		
Human α 3	YWES-	GEWA	IKAPG	YKHD	IKY	N	CC	EEI	YPDI	TYSL	YIR	RLPL	FYT	INLI	I	PCLL	ISFLT	228		
Human α 4	FWES-	GEWV	IVDA	VGT	YNT	R	K	YE	CCAE	IY	PDIT	YAF	VIR	RLPL	FYT	INLI	I	PCLL	ISCLT	233
Human β 2	FTPS-	GEWD	IVAL	PGR	RNEN	PD	---	STY	VDIT	YDFI	IR	KPL	FYT	INLI	I	PCVL	ITSLA	227		
Human β 4	FTPS-	GEWD	IVAL	PGR	RTV	NP	QD	---	PSY	VDV	TYDFI	IK	KPL	FYT	INLI	I	PCVL	TTLLA	229	

Figure S3 | Sequence alignment of Ac-AChBP, Ls-AChBP, and human α 3, human α 4, human β 2, human β 4 of nAChR subunits. Each subunit sequence numbering is shown at right side. Residues of the principal binding side that interact with α -conotoxin GIC are shown in light green; residues of the complementary binding side are in light blue. The Arg residues which could be a potential reason of decreased GIC affinity for the Ls-AChBP, human α 4 β 2 nAChR and α 3 β 4 nAChR are typed in red.