# Supplementary information

## From crystal structure of α-conotoxin GIC in complex with *Ac*-AChBP to molecular determinants of its high selectivity for α3β2 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 | $P2_12_12_1$ |
| **Cell dimensions** | |
| *a*, *b*, *c* (Å) | 78.6, 84.9, 208.6 |
| **α, β, γ (°)** | 90.0, 90.0, 90.0 |
| Resolution (Å) | 50-2.1 |
| $R_{merge}$ (%) | 8.8 (67.7) |
| $I / \sigma I$ | 18.7 (3.5) |
| Completeness (%) | 96.9 (93.5) |
| Redundancy | 6.7 (6.9) |
| **Refinement** | |
| Resolution (Å) | 43.5-2.1 |
| No. Reflections | 85409 |
| $R_{work}$ / $R_{free}$ (%) | 19.7/23.7 |
| **No. atoms** | |
| Protein | 8299 |
| Ligand/ion | 545 |
| Water | 521 |
| **B factor (Å$^2$)** | |
| 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 Å)**

| ***Ac*-AChBP** | **GIC [a]** | **PnIA(A10L, D14K)[b]** | **TxIA(A10L)[c]** | **ImI[d]** | **BulA** |
|---|---|---|---|---|---|
| ***Principal side*** | | | | | |
| **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 | | |
| ***Complementary side*** | | | | | |
| **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 | *Ac*-AChBP | *Ls*-AchBP | hα3β2 | hα3β4 | hα7 | rα3β2 | rα7 |
|---|---|---|---|---|---|---|---|
| GIC | 29 ± 2 [a] | 220 ± 10 [a] | 1 [b] | 755 [b] | | | |
| ImI | 33 ± 5 [c] | 4140 ± 400 [c] | 41 [d] | 3390 [d] | 132 [e] | >5000 [f] | 69 [g] |
| PnIA(A10L,D14K) | 28 ± 6 [h] | 13 ± 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].

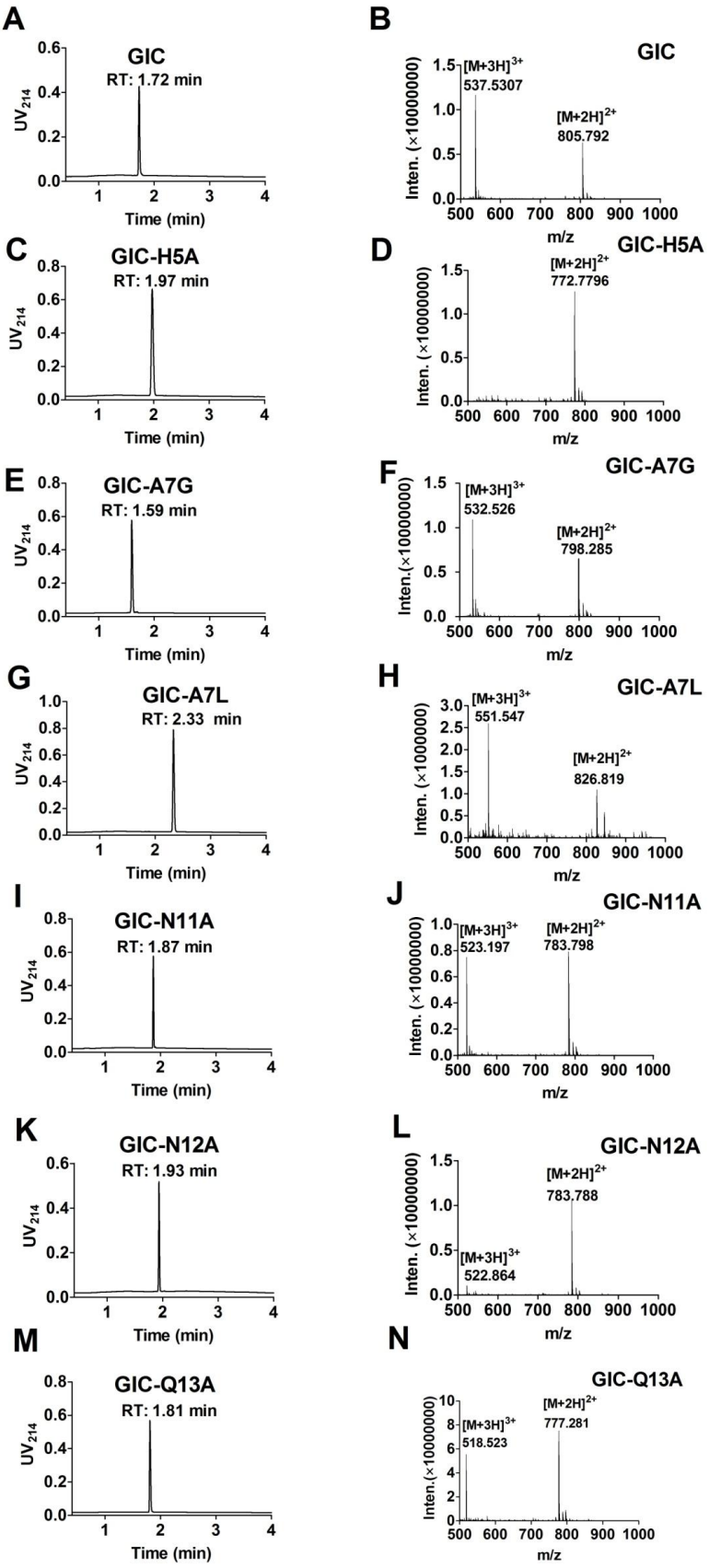


**Figure S1**| HPLC chromatograms with a retention time and ESI-MS data of

α-conotoxin GIC and its analogues. The peptides were analyzed on a waters ACQUITY UPLC BEH C18 column (2.1×50 mm, 1.7 μm) 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 ℃, where A = 0.1% trifluoroacetic acid (TFA) and B = 0.75% TFA, 90% acetonitrile, and the remainder water. Absorbance was monitored at 214 nm.

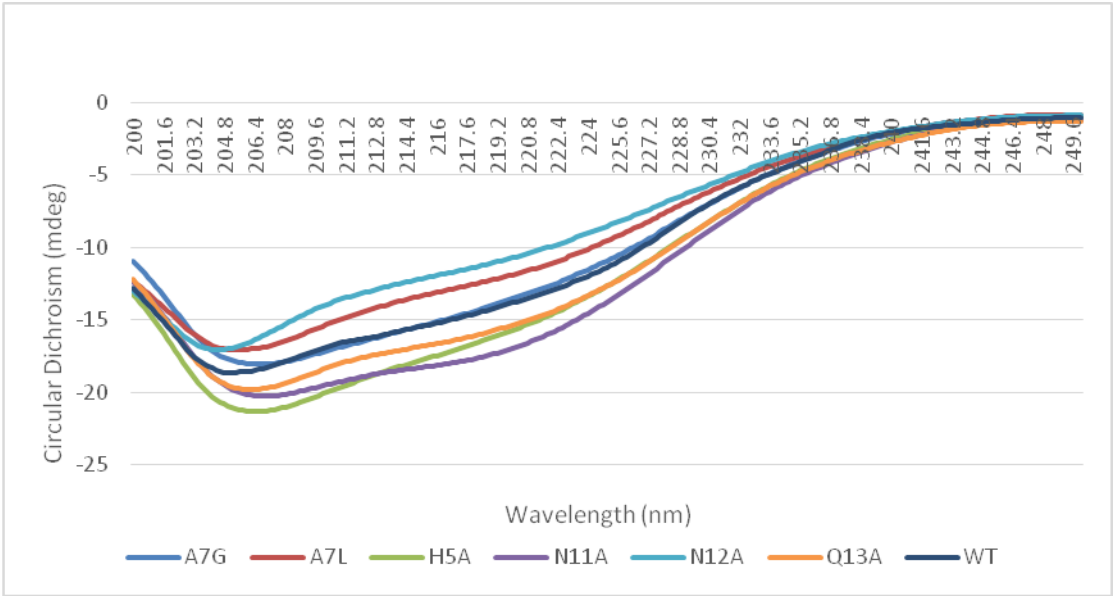


**Figure S2|** Circular dichroism of α-conotoxin GIC and its analogues. CD spectra were recorded on a JASCO J-715 Spectropolarimeter. Spectra was recorded at room temperature under nitrogen atmosphere. Peptides were dissolved to 100 μM. Spectra were recorded over a 200-250 nm range at 25 ℃ using an average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

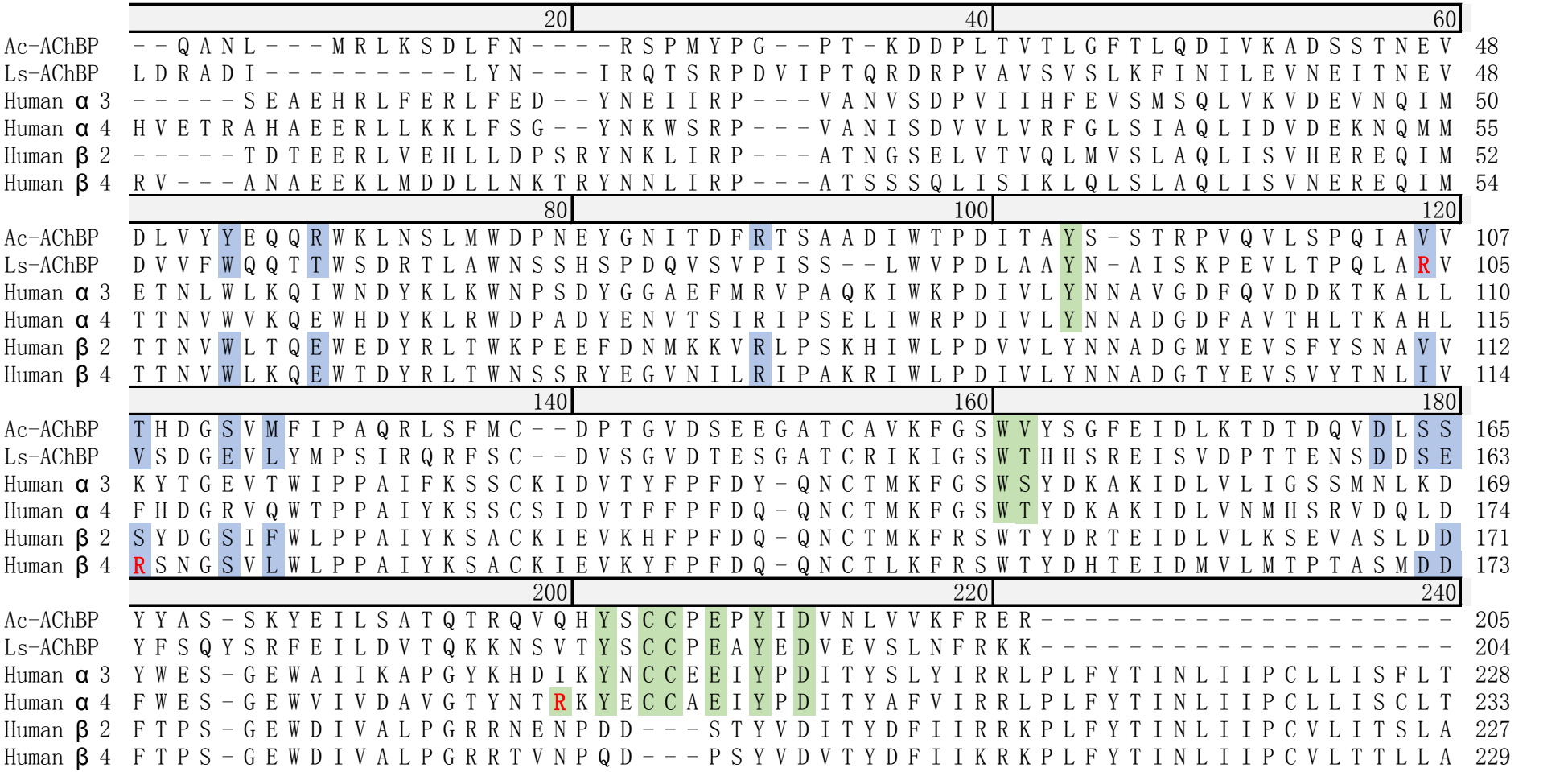


**Figure S3 |** Sequence alignment of Ac-AChBP, Ls-AChBP, and human α3, human α4, human β2, human β4 of nAChR subunits. Each subunit sequence numbering is shown at right side. Residues of the principal binding side that interact with α-conotoxin GIC are shown in light green; residues of the complementary binding side are in light blue. The Arg residues which could be a potential reason of decreased GIC affinity for the Ls-AChBP, human α4β2 nAChR and α3β4 nAChR are typed in red.