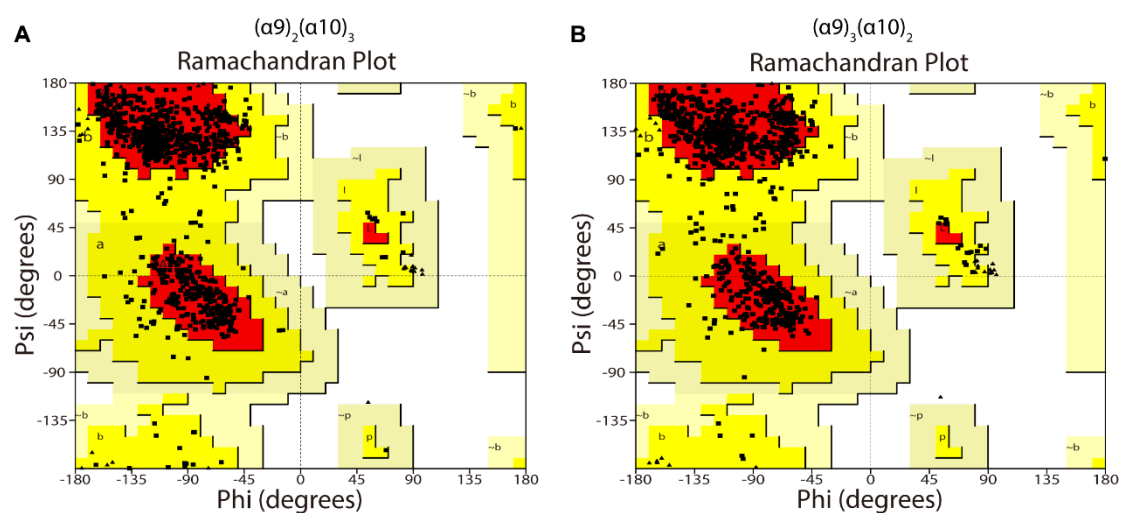
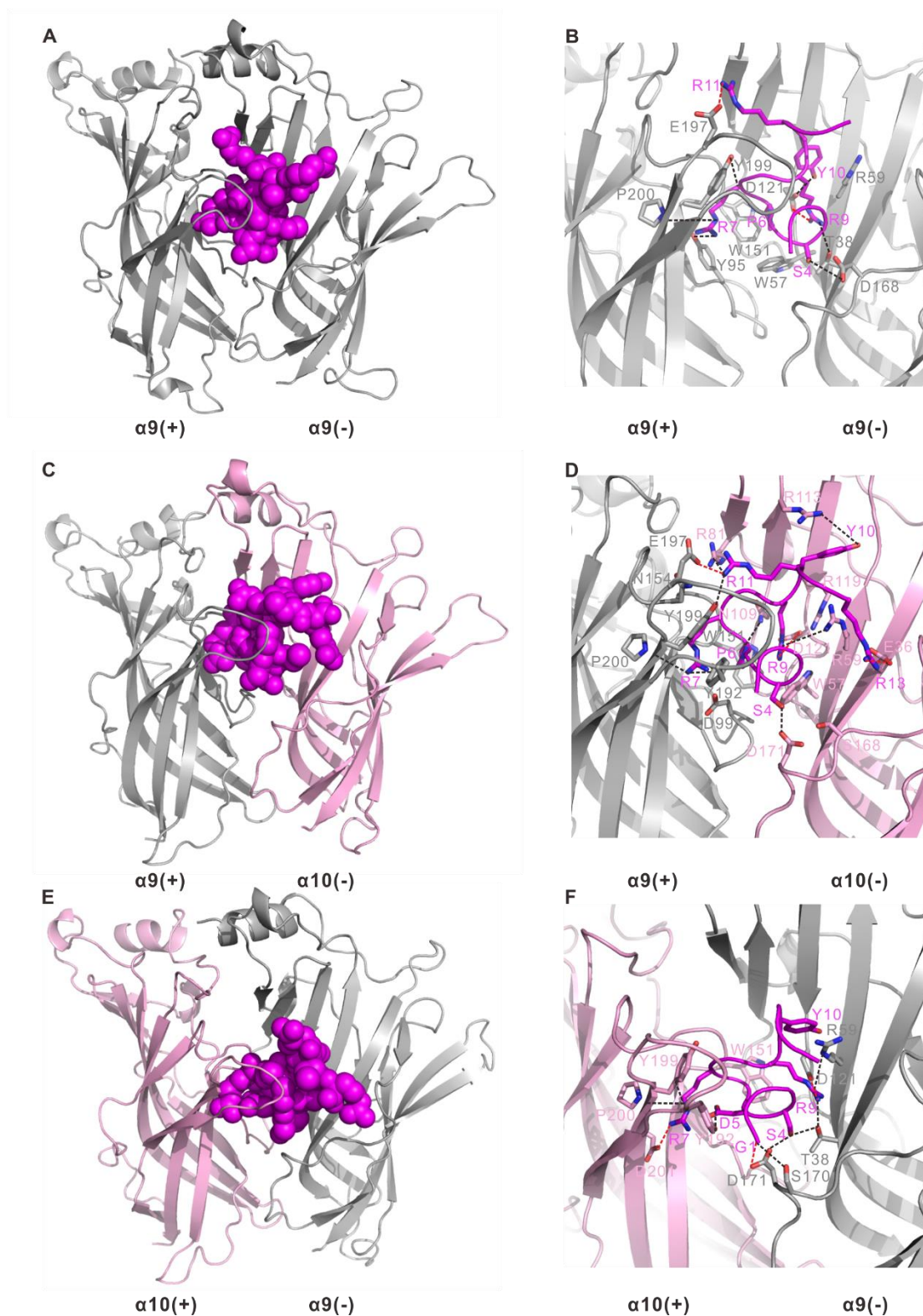


**Figure S1. HPLC and mass spectra profiles of RgIA/RgIA4.** A, C HPLC chromatograms of RgIA and RgIA4, respectively. B, D Electrospray ionization mass spectrometry (ESI-MS) data for RgIA and RgIA4, respectively.



**Figure S2. Ramachandran plot of the homology models of two possible stoichiometries  $(\alpha 9)_2(\alpha 10)_3$  (A) and  $(\alpha 9)_3(\alpha 10)_2$  (B).** Red: Residues in most favored region; Dark yellow: Residues in additional allowed regions; Pale yellow: Residues in generously allowed regions.



**Figure S3. Molecular dynamics models of human  $\alpha 9\alpha 10$  nAChR bound to RgIA.** A, B RgIA bound to the  $\alpha 9(+)/\alpha 9(-)$  interface. C, D RgIA bound to the  $\alpha 9(+)/\alpha 10(-)$  interface. E, F RgIA bound to the  $\alpha 10(+)/\alpha 9(-)$  interface. hydrogen bonds and salt bridges were represented by black dashed lines and red dashed lines, respectively.



**Figure S4. Comparison of different  $\alpha$ -CTxs bound by *Ac*-AChBP.** **A** Comparison of *Ac*-AChBP with four conotoxins. RgIA is showed in magenta, RgIA4 in green, GIC in pink, and LvIA in yellow. **B** Multiple sequence alignment of RgIA, RgIA4, GIC and LvIA. Disulfide bridges are showed in Cys1-Cys3 and Cys2-Cys4.

**Table S1. Data collection and refinement statistics.**

	<b>RgIA-Ac-AChBP</b>	<b>RgIA4-Ac-AChBP</b>
<b>Data collection</b>		
Beamline	SSRF BL17U	SSRF BL17U
Wavelength	0.9796 Å	0.9796 Å
Space group	C 1 2 1	C 1 2 1
Cell dimensions		
a, b, c (Å)	206.271, 135.955, 132.067	206.149, 136.529, 131.111
$\alpha$ , $\beta$ , $\gamma$ (°)	90, 101.162, 90	90, 100.566, 90
Resolution (Å)	50.00-2.50	50.00-2.60
<sup>a</sup> $R_{merge}$	0.110(0.792)	0.204(1.418)
<sup>b</sup> $R_{pim}$	0.063(0.466)	0.083(0.580)
<sup>c</sup> CC <sub>1/2</sub> of the highest resolution shell	0.992(0.672)	0.987(0.648)
I/ $\sigma$ I	10.980(1.804)	11.694(2.290)
Completeness (%)	98.36 (99.83)	98.20 (93.27)
Redundancy	3.9	7.0
<b>Refinement</b>		
Resolution (Å)	35.4 - 2.503 (2.592-2.503)	48.26 – 2.611 (2.704 - 2.611)
No. reflections	121025 (11626)	106333 (10042)
R-work	0.1727 (0.2565)	0.1840 (0.2475)
R-free	0.2214 (0.3164)	0.2251 (0.3073)
No. atoms	17680	17319
macromolecules	17164	16872
ligands	10	76
solvent	506	371
Wilson B-factor (Å <sup>2</sup> )	35.34	36.94
Average B-factors (Å <sup>2</sup> )	41.74	39.48
macromolecules	41.79	39.36
ligands	42.97	70.64
solvent	39.78	38.42
r.m.s. deviations		
Bond lengths (Å)	0.008	0.010
Bond angles (°)	1.03	1.38
Ramachandran plot (%)		
Favored	96.42	95.80
Allowed	3.30	3.67
Outlier	0.28	0.53