Supplementary Information-

Title- Crystal Structures of the Ligand Binding Domain of a α_7 Nicotinic Receptor Chimera and its Complex with Agonist

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Table S1- Data collection and refinement statistics

	Apo Chimera (PDB ID 3SQ9)	Epi/Chimera(PDB ID 3SQ6)
a. Data collection		
Space group	$P2_1$	$P2_1$
Unit cell		
a, b, c (Å)	79.123 144.558 131.115	81.237 141.069 130.207
α, β, γ (^{o})	90.000 102.462 90.000	90.000 99.649 90.000
Resolution (Å)	50-3.1 (3.21-3.10)[a]	50-2.8 (2.90-2.80)[a]
R_{sym}	0.086 (0.425)	0.111(0.538)
I/σ (I)	11.1 (1.7)	10.7/(1.8)
Completeness (%)	90.9 (54.1)	86.4(55.9)
Redundancy	3.4 (2.4)	3.9(2.3)
Number of unique Reflections	47134	61567
b. Refinement		
Rwork/Rfree (%)	0.265/0.290	0.235/0.260
Number of non-H atoms		
Protein	16470	16572
Ligands		
Epi		140
NAG	280	210
Water		16
Average B factors (Å^2)		
Protein	110.0	62.9
ligands		
Epi		39.2
NAG	147.7	104.0
Water		28.9
R.M.S. deviations		
Bond lengths (Å)	0.008	0.009
Bond angles (^{o})	1.3	1.6
Ramachandran plot		
Favored region (%)	88.0	90.0
Allowed region (%)	8.8	6.0
Outlier region (%)	3.3	4.0

[a] Highest resolution shell shown in parentheses.

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Table S2- Structure-based mutational analysis of the α_7 AChR expressed in 293 HEK cells: agonist competition against the initial rate of ^{125}I-α-bungarotoxin binding.

Mutant α_7AChR	Agonist	Kd apparent, nM	n_H
Wild type α_7	Epibatidine	17.3 ± 0.4	2.82 ± 0.17
W53F	Epibatidine	67.8 ± 6.6	1.22 ± 0.14
Y191F	Epibatidine	195 ± 3.8	2.43 ± 0.11
Y91F	Epibatidine	1,245 ± 53	2.22 ± 0.19
Y184F	Epibatidine	4,446 ± 34	2.73 ± 0.37
W145F	Epibatidine	38,280 ± 673	2.47 ± 0.10
Wild type α_7	ACh	5,400 ± 130	2.58 ± 0.14
R182V	ACh	887 ± 69	2.39 ± 0.25
E185Q	ACh	543 ± 15	2.53 ± 0.14
E181S	ACh	141 ± 13	1.18 ± 0.14
Q37N	ACh	369 ± 31	1.53 ± 0.17

Parameters and standard errors were obtained by non-linear least-squares fits of the Hill equation to the data in Fig. 8, main text, using GraphPad Prism 5.0 software. The following mutations of the α_7 AChR showed undetectable or very low ^{125}I-α-bungarotoxin binding: R179A, F196A, N108A, D160A.

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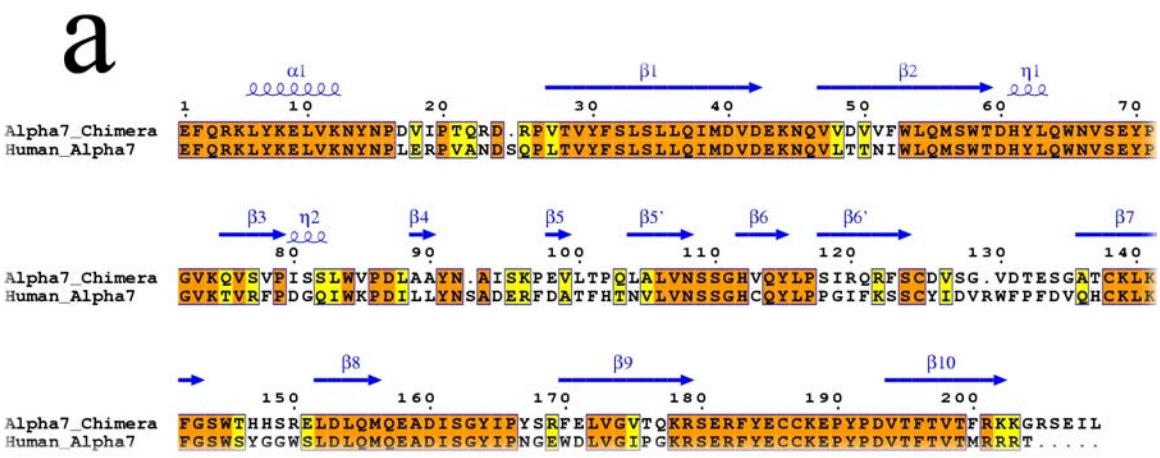
Fig. S1- Construction of the α_7/AChBP chimera. (a) Sequence of the α_7/AChBP chimera aligned with that of human α_7 AChR. Identical residues are highlighted in orange, homologous residues in yellow. Secondary structural elements are shown above the sequences. (b) Regions of α_7 AChR (blue) and AChBP (orange) are mapped on the subunit structure of the chimera.



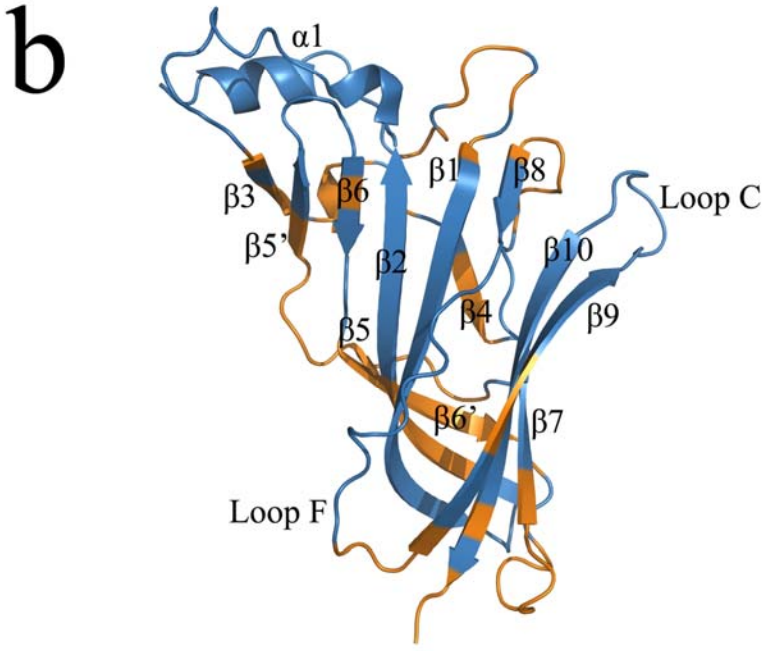


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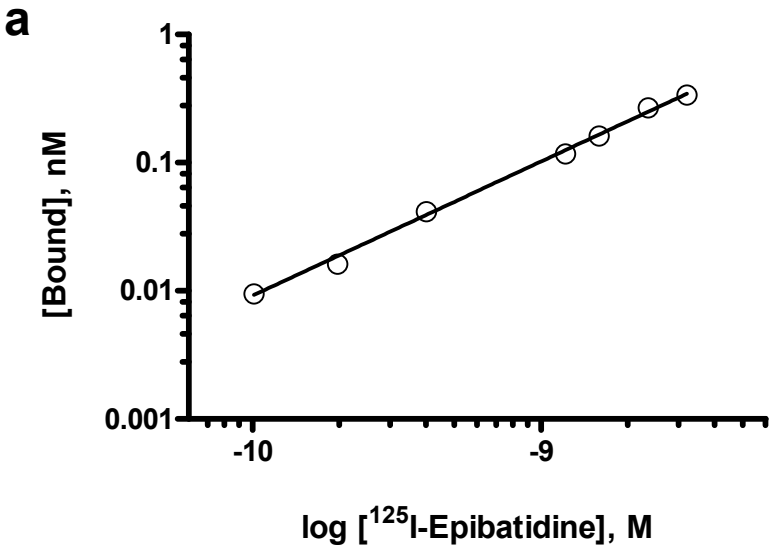
Fig. S2- Epibatidine binding to the α_7/AChBP chimera. Purified α_7/AChBP chimera was bound to anti-MF2 agarose and steady state binding of ^{125}I-epibatidine (panel a) or competition of unlabeled +/-epibatidine against ^{125}I-epibatidine (panel b) was measured using the method in ref. 36 of the main text. For panel b, a concentration of 3 nM ^{125}I-epibatidine was used. Non-specific binding was determined in the presence of 10 μM methyllcaconitine (TOCRIS Bioscience). In panel a, the line is a least squares fit to the log-log data with a slope 1.05 ± 0.05 (SE). In panel b, the curve is a non-linear fit of the Hill equation to the data with Kd=1.3 ± 0.19 μM and n_H= 1.38 ± 0.11. Note that the Kd for epibatidine obtained for the α_7/AChBP chimera is greater than that obtained the α_7 AChR (Fig. 8, main text), but apparent affinities for the two proteins may not be comparable because the chimera lacks a pore domain allosterically linked to the binding domain.



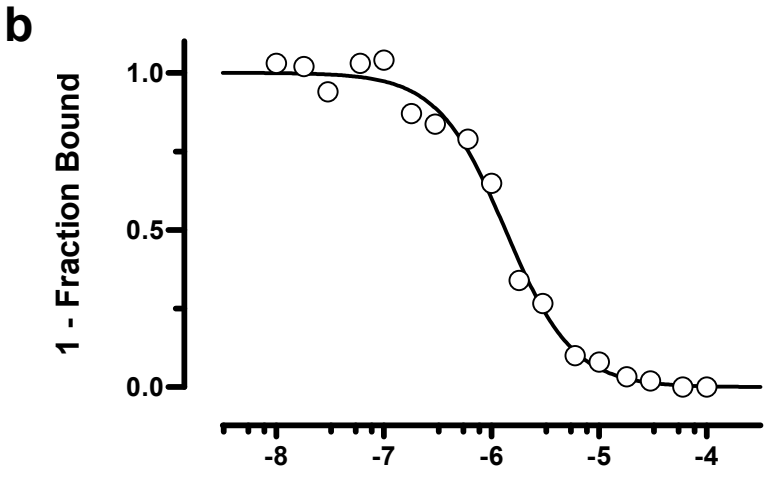


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Fig. S3- Representative regions of the Apo structure showing well-defined electron density. **a**. Simulated annealing omit map calculated for the loop C region contoured at 3.0σ level. **b**. 2Fo-Fc electron density map for loop F and the surrounding β-strand 9, contoured at 1.0σ level. Key residues are labeled.

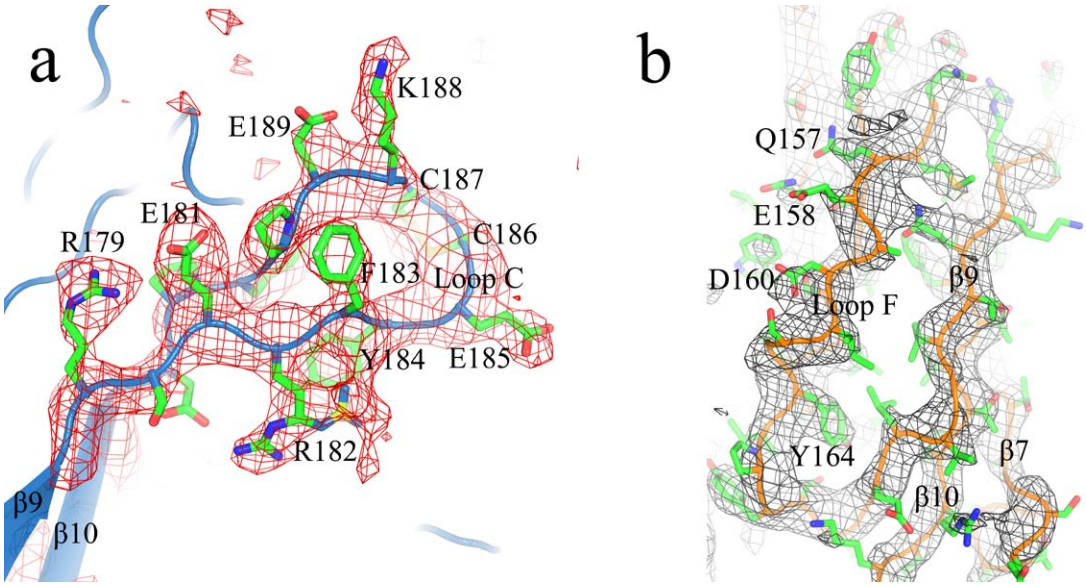

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Fig. S4- Representative regions of the Epi structure showing well-defined electron density. **a**. Simulated annealing omit map calculated for the loop C region contoured at 3.0σ level. **b**. 2Fo-Fc electron density map for β-strand 10 and part of the inner β-sheet, contoured at 1.0σ level. Key residues are labeled.

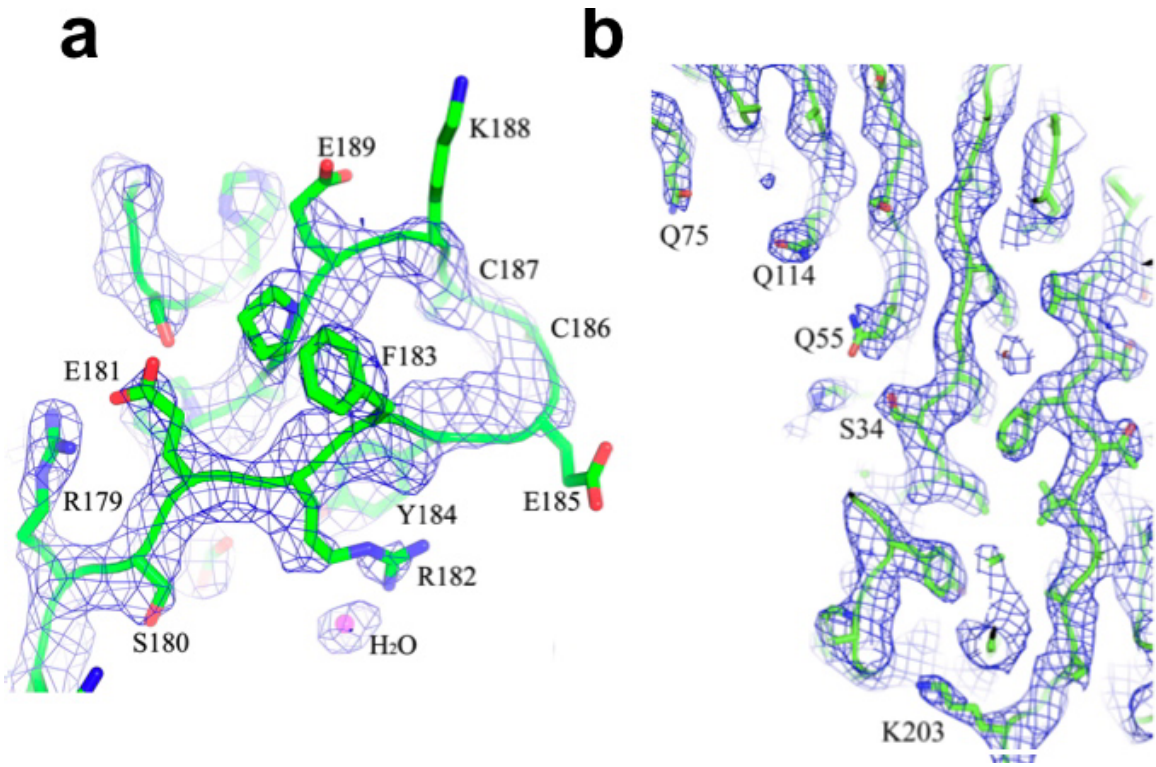

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Fig. S5- Structure superposition of the α_7/AChBP chimera (blue) and AChBP (orange) shows that the N-terminal helix (α1) is positioned differently with respect to the β2-β3 loop in the two structures.

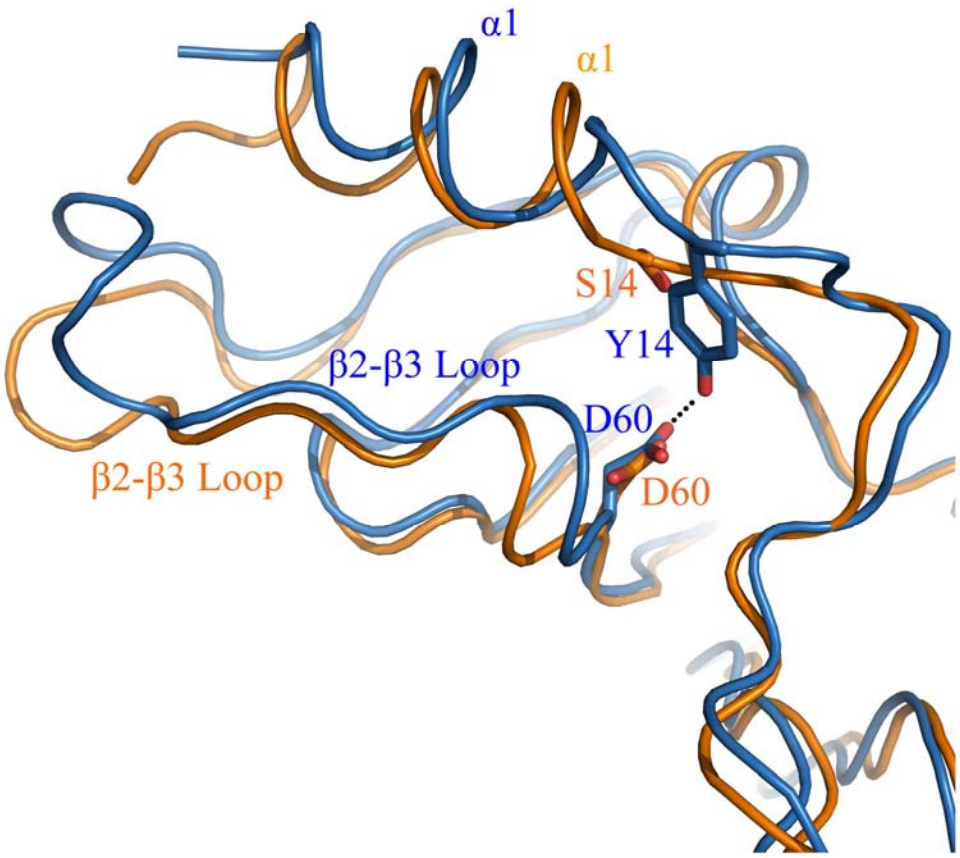

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Fig. S6- Structure superposition between the α_7/AChBP chimera (blue) and AChBP (orange) viewed from the bottom. This comparison shows the different positions of the β1-β2 loop and the F loop in the two structures.

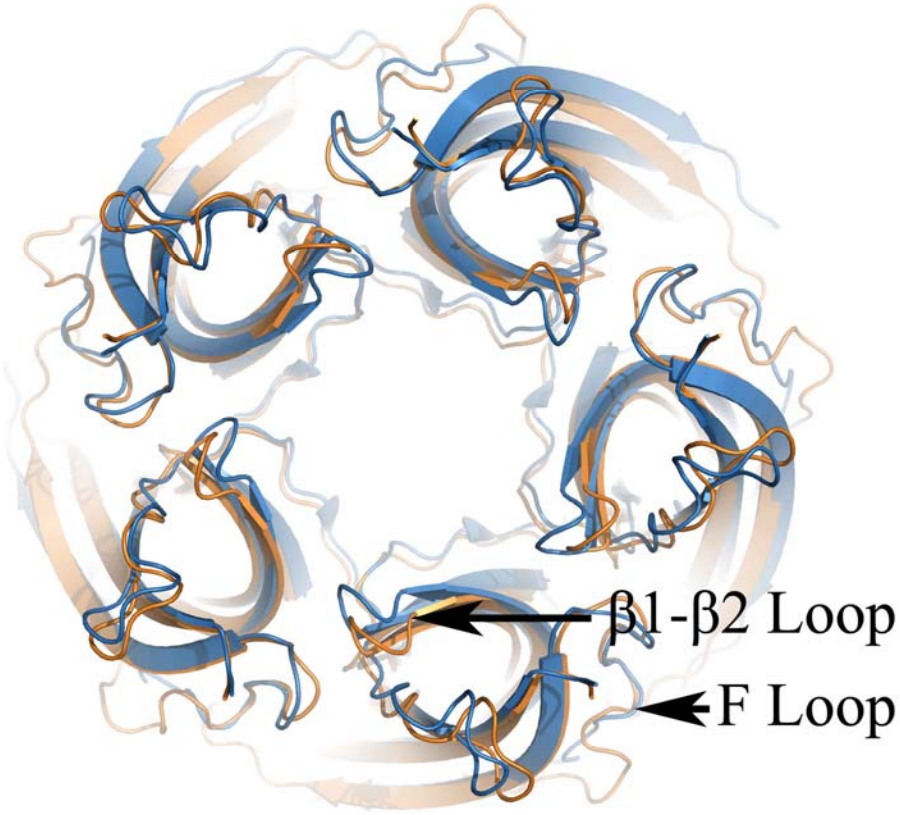

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Fig. S7- Comparison of epibatidine binding by AChBP (pdb code 2BYQ) and the α_7/AChBP chimera (blue) in stereo. The structures are superimposed by aligning the β-sandwich core. Key residues of both structures are labeled for reference.

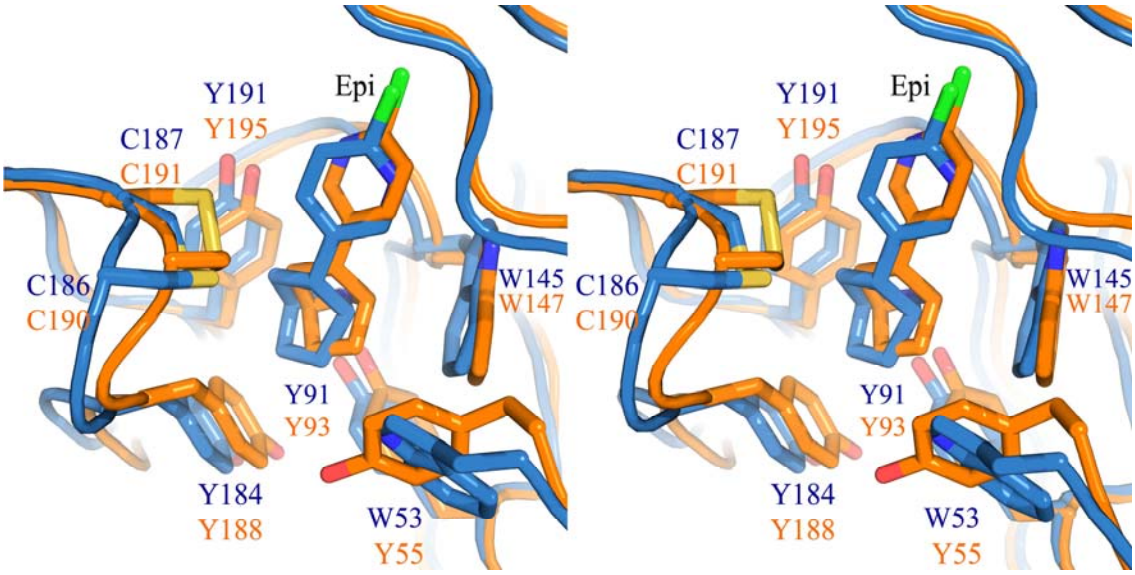

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Fig. S8- Comparison of ligand binding pockets of the α_7/AChBP chimera and AChBP in stereo. The α_7/AChBP chimera is shown in blue and AChBP in yellow. Only residues from the chimera are labeled. The 2Fo-Fc electron density map is contoured at 3.0σ level. A strong density (red mesh) coincides with the position of the quaternary ammonium moiety of carbamylcholine (CCh) bound to AChBP[2].

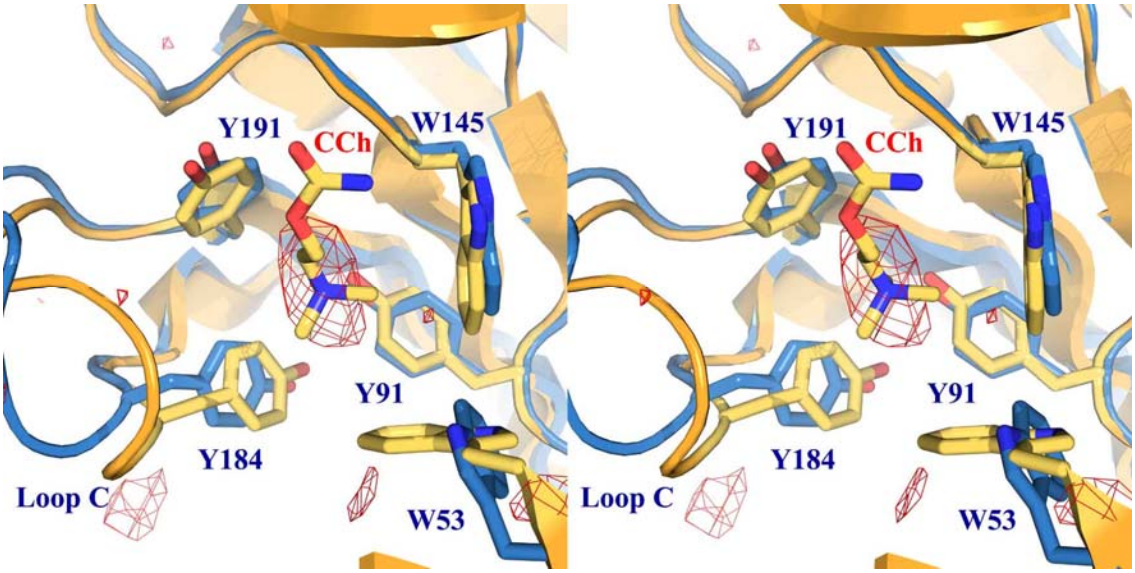

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Fig. S9- The binding of epibatidine induces a concerted, counterclockwise rotation of the α1-β1 loop, loop B and loop C at the top part of the subunit when viewed down the pentamer axis. The Apo structure is in orange while the Epi structure is in blue. This rotation alters the interface interaction with the adjacent subunit, leading to the stabilization of the N-terminus of helix α1 on the complementary subunit.

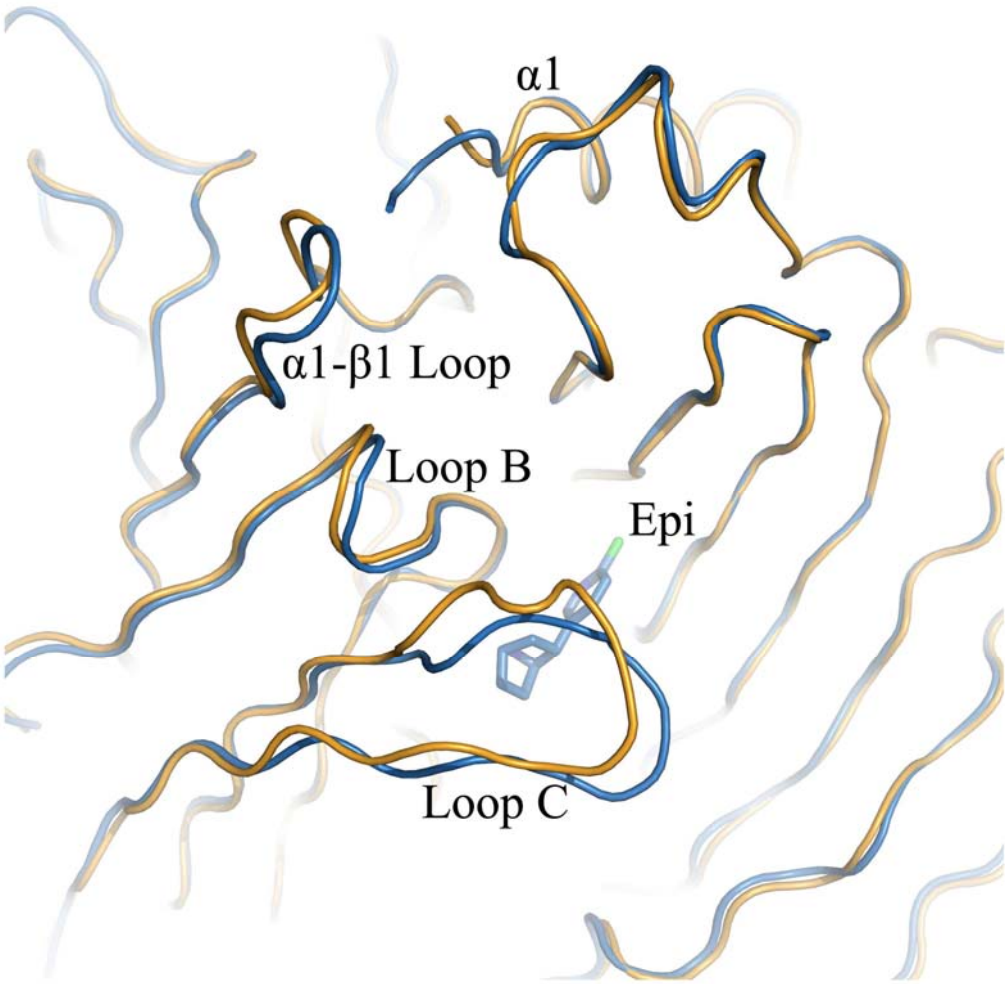

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Fig. S10- Electron density for the highly ordered assembly beneath loop C in the Epi structure. The 2Fo-Fc electron density map is contoured at 1.5σ level. Key residues including Tyr184, Tyr91, Lys141, and Arg182 are labeled.

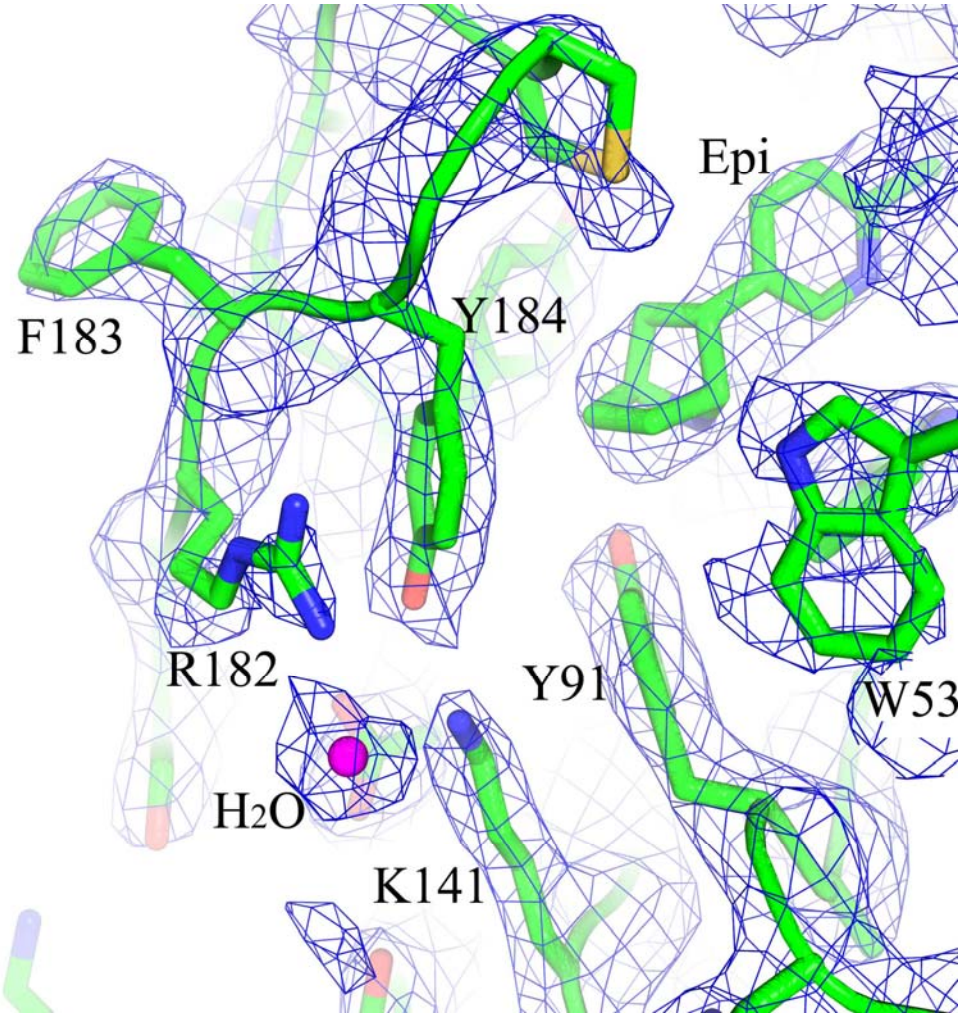

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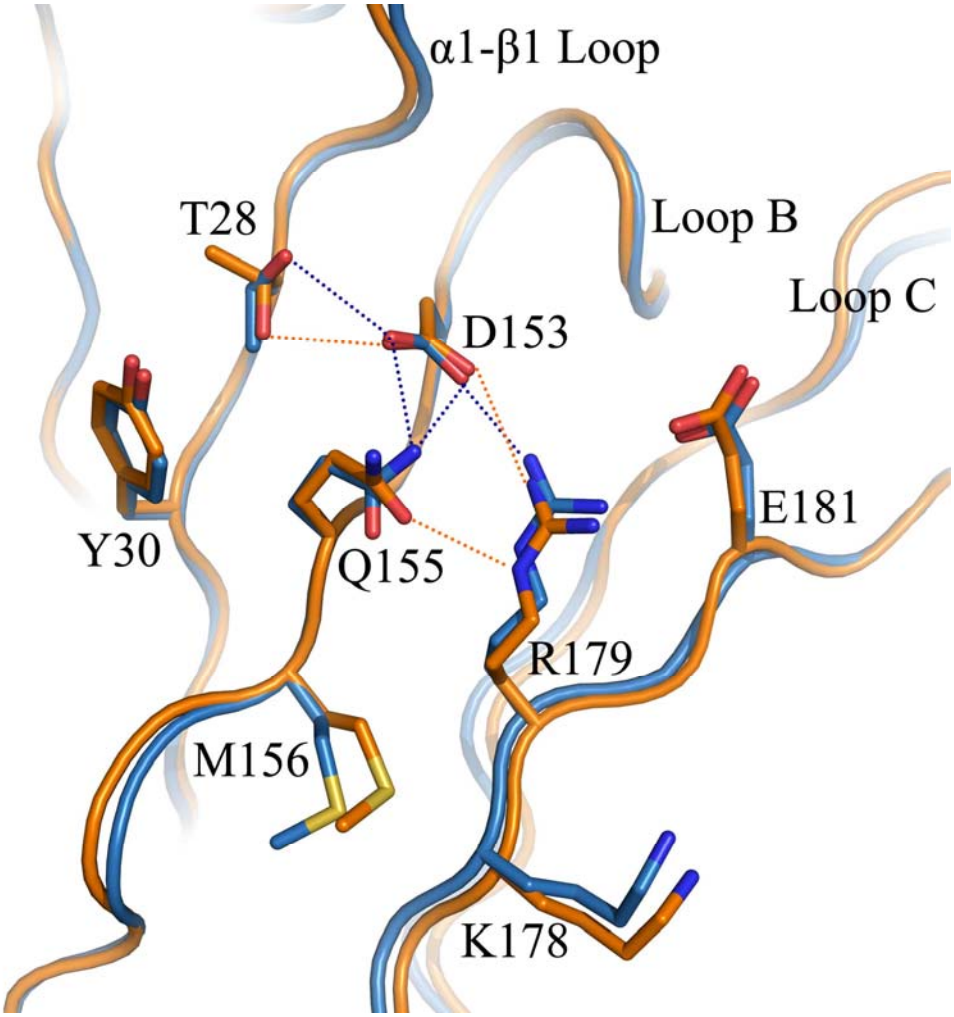
Fig. S11- Interactions at the linkage region between loops C and F within the same subunit are largely maintained between the Apo (orange) and Epi (blue) structures.



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