Supplementary information

Identification of a possible secondary picrotoxin binding site on the $GABA_A$-Receptor

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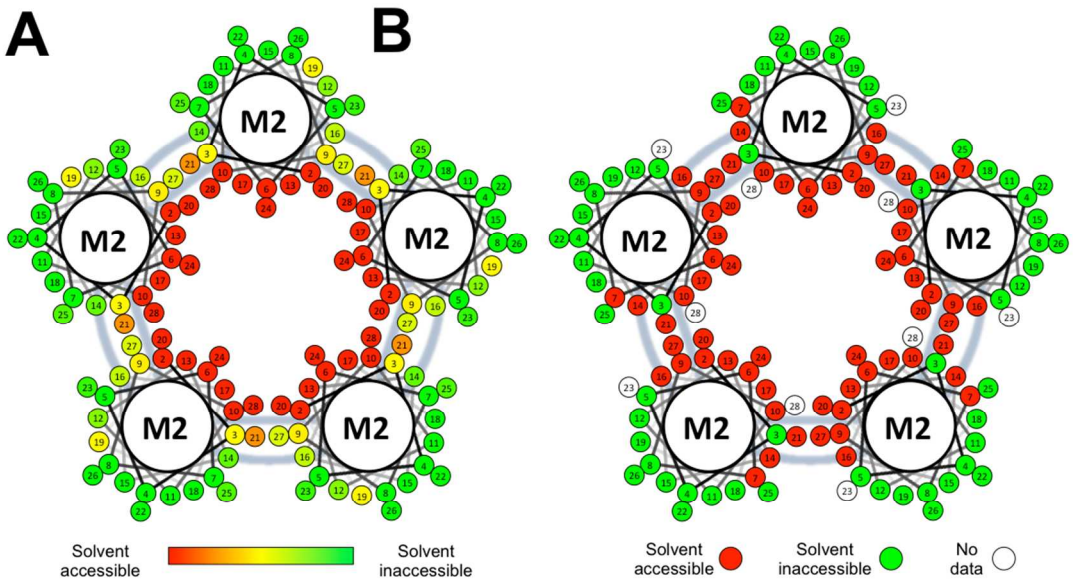

Figure S1

The average percentage solvent accessible surface area (SASA) is measured for the apo M2 residues. These values are normalized, attributed to a green→red color scale and assembled onto a pentameric helix-wheel *(A)*. The positions of these residues match the equivalent representation generated from experimental data *(B)*. These experimental results were classified as either solvent accessible or *not* solvent accessible, rather than a sliding scale.

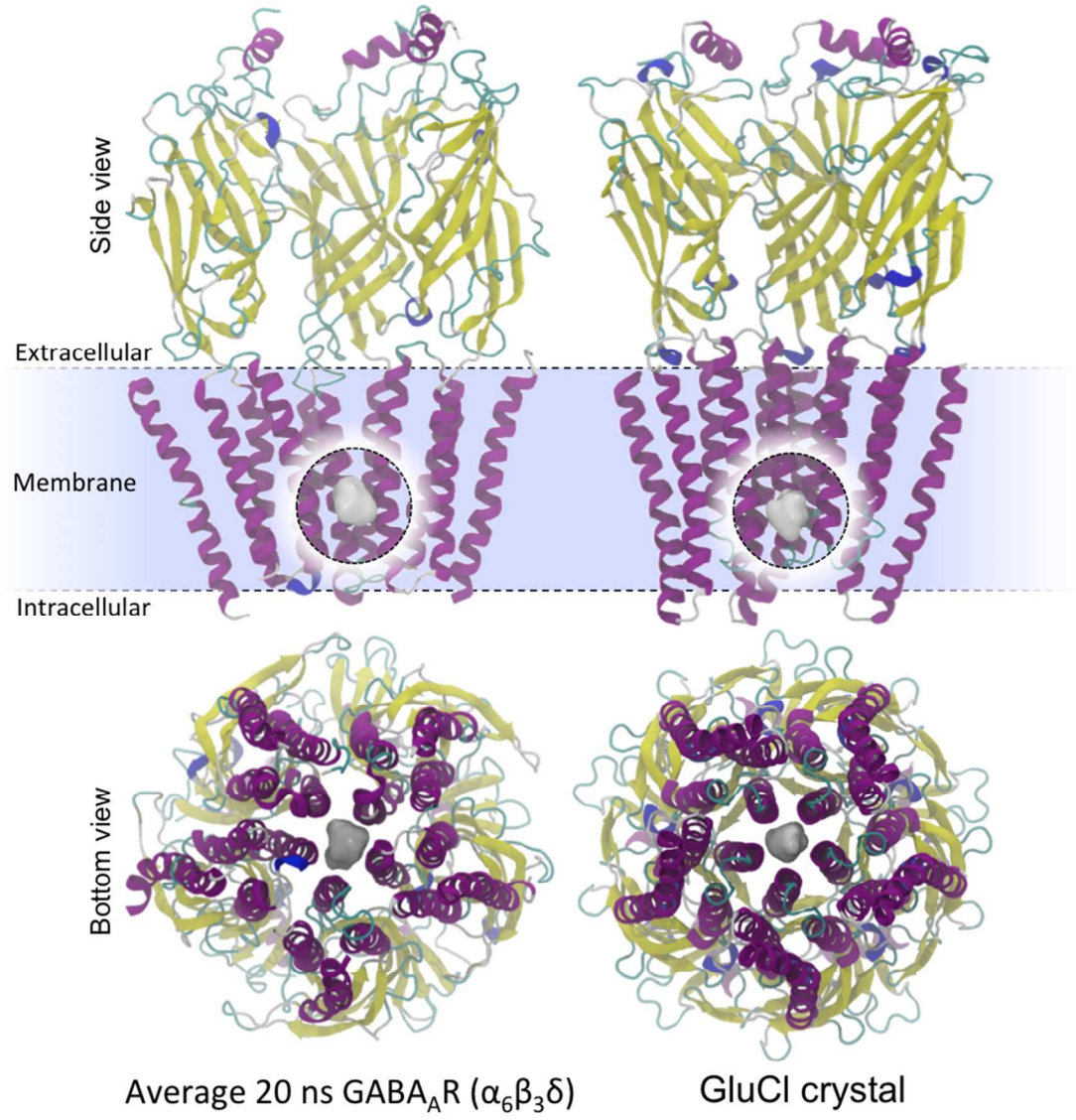

Figure S2

The density of PTX in the pore of our $\alpha_6\beta_3$ GABA$_A$R model averaged over 20 ns of simulation (left) very closely matches the electron density of PTX observed in the crystal structure of the GluCl receptor (right).

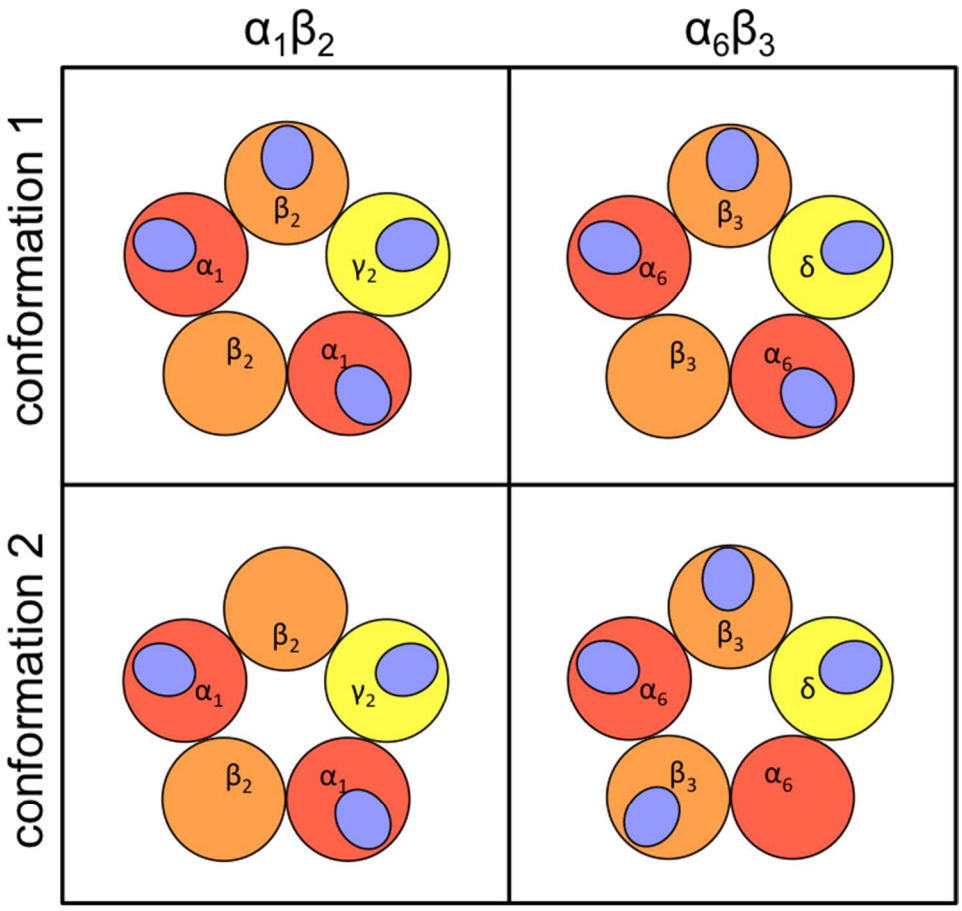

Figure S3

The binding pocket is located at the interface between the TM domain and the LBD and is lined by residues from the cys-loop, the top of the M1 helix, the M2-M3 loop, and the top of the M4 helix. This binding pocket is found within seven of the eight α-subunits (four α_1, three α_6), four of eight β-subunits (three β_3, and only one β_2), and all four γ/δ-subunits. A subunit that had PTX docked to it is represented with a blue ellipse.

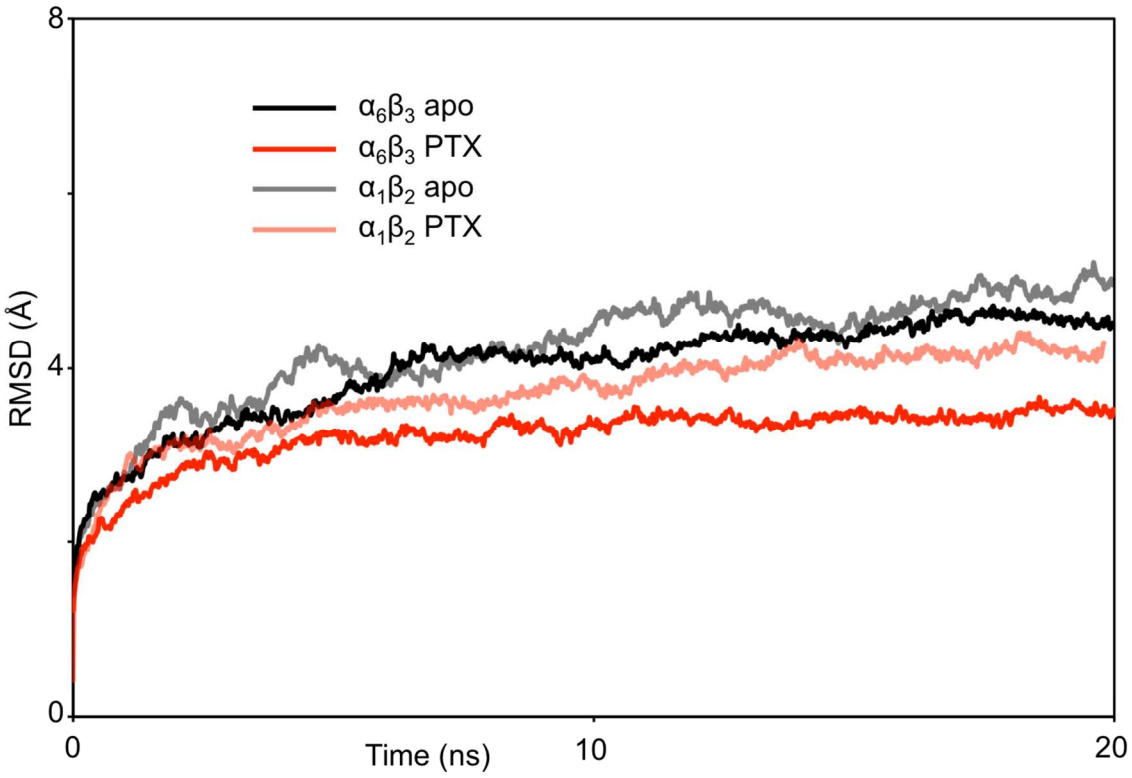

Figure S4

The RMSDs for all the simulations (PTX bound and apo) have plateaued after ~10 ns to average values of ~4.5 and 4.8 Å for the apo $\alpha_6\beta_3$ (black) and $\alpha_1\beta_2$ (gray) systems, respectively. The average plateau values for the PTX-bound $\alpha_6\beta_3$ (red) and $\alpha_1\beta_2$ (pink) systems are ~3.5 and 4.2 Å, respectively.

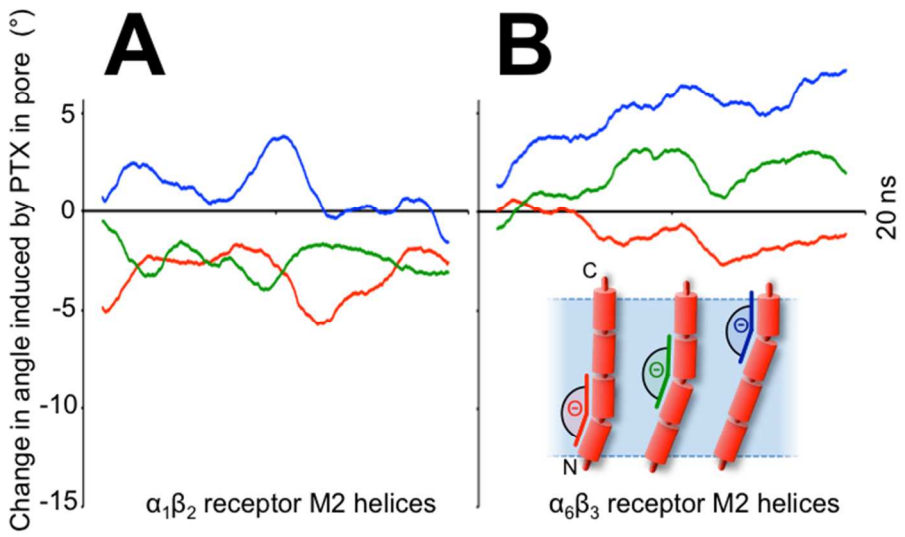

Figure S5

Control simulations for each receptor system were carried out with PTX just docked into the pore of the protein. The change in M2 helix kink is shown for the control simulations relative to the apo for the $\alpha_1\beta_2$ receptor *(A)* and the $\alpha_6\beta_3$ receptor *(B)*. There is no change for the helices in the $\alpha_1\beta_2$ receptors. For the $\alpha_6\beta_3$ receptors, the helices appear to become *less* kinked compared to the apo simulations. However, this lack of kinking has no effect on the pore profile (Fig S6).

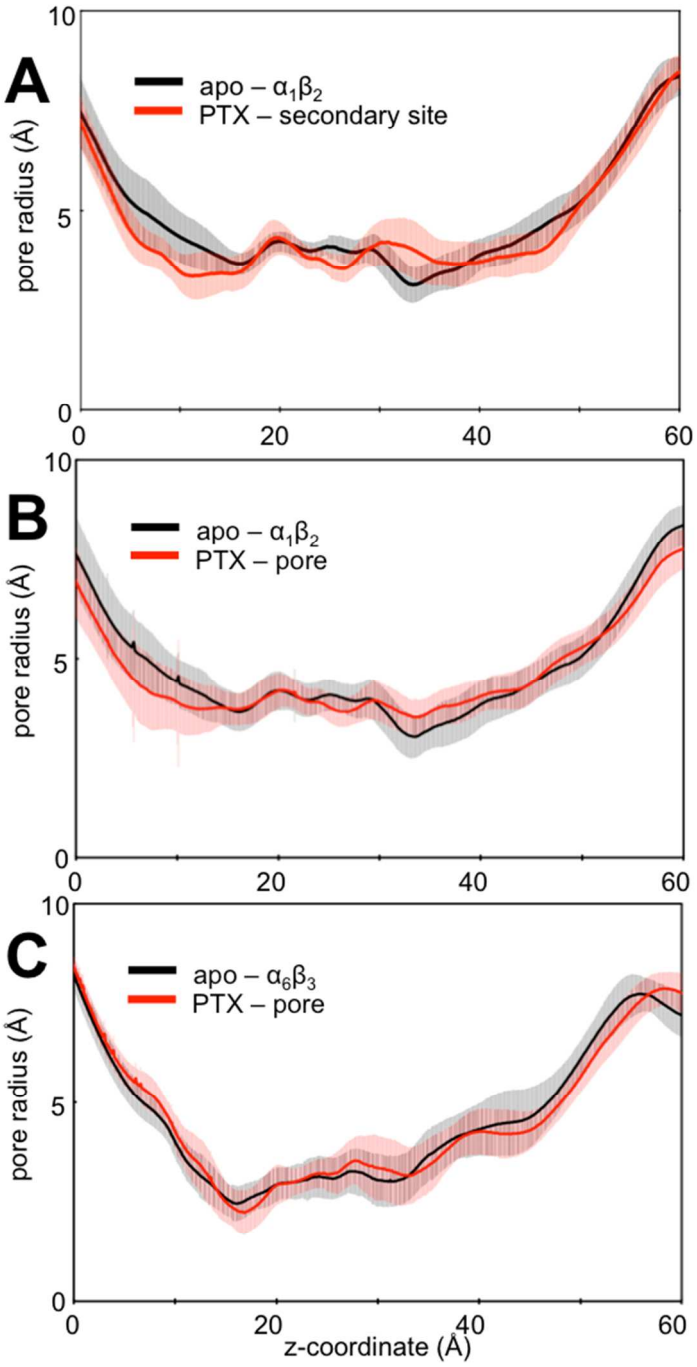

Figure S6

The average pore radius profile is shown for the apo (black line) and PTX-bound (red line) simulations for the $\alpha_1\beta_2$ receptor with PTX docked to the secondary sites *(A)*, for the $\alpha_1\beta_2$ receptor with PTX docked to the pore site *(B)*, and for the $\alpha_6\beta_3$ receptor with PTX docked to the pore site *(C)*. The overlap of the error bars indicates that there is no PTX-induced change in the pore profile for any of the systems shown.

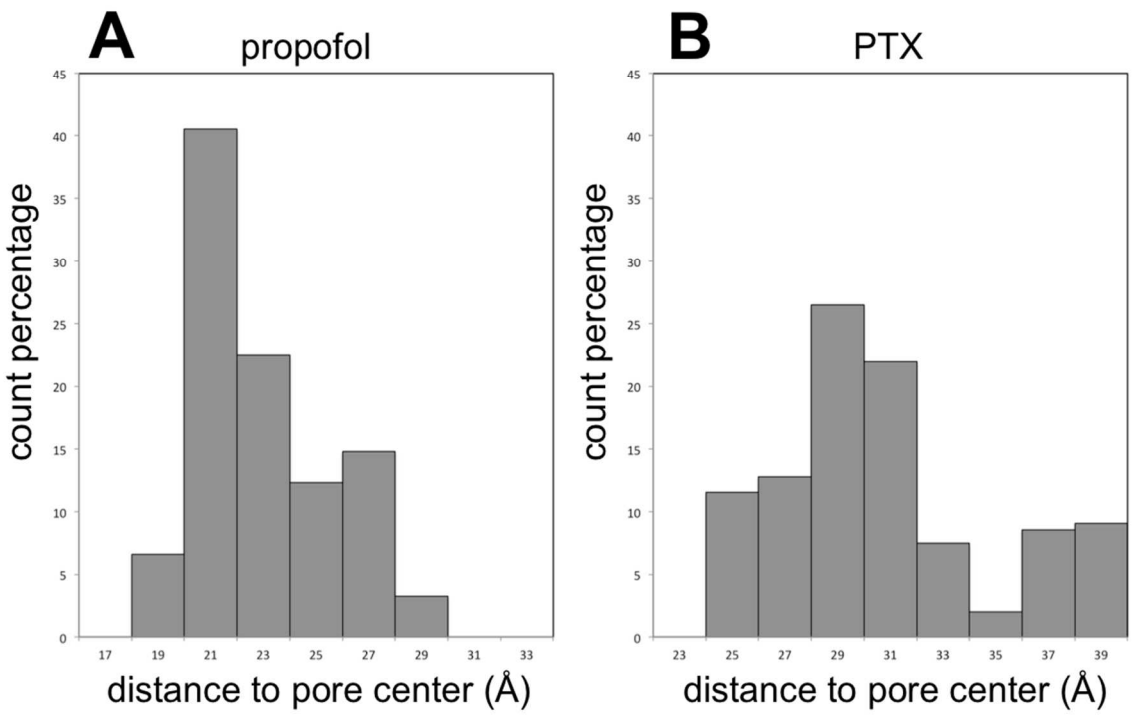

Figure S7

The distribution of distances between the propofol center of mass and the pore center (*A*), adapted from Nury *et al*[1], and the equivalent distance distributions for PTX from our $\alpha_6\beta_3$ simulations (*B*). The distribution range is similar (10-12 Å for propofol, 14 Å for PTX) between the two molecules. The slightly wider range, and greater distance to the pore center are to be expected for the much larger PTX molecule, which cannot fit into the deeper recesses of the pocket that are closer to the pore center.

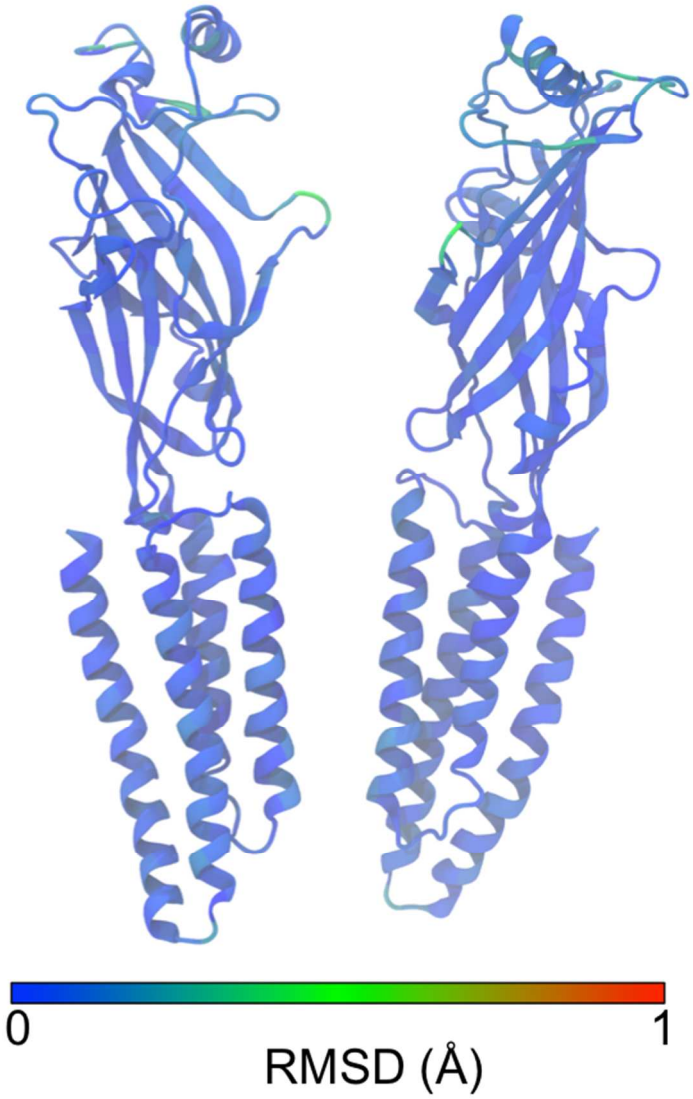

Figure S8

Two of the GluCl crystal structures (one apo, and one with PTX present in the pore) have been colored according to the RMSD between them. The average backbone Cα atom RMSD between the two protein structures is only 0.10 Å. The average for the M2-M3 region involved in the interface pocket is just 0.08 Å, indicating that binding of PTX into the protein channel produces no conformational change in the M2-M3 region.

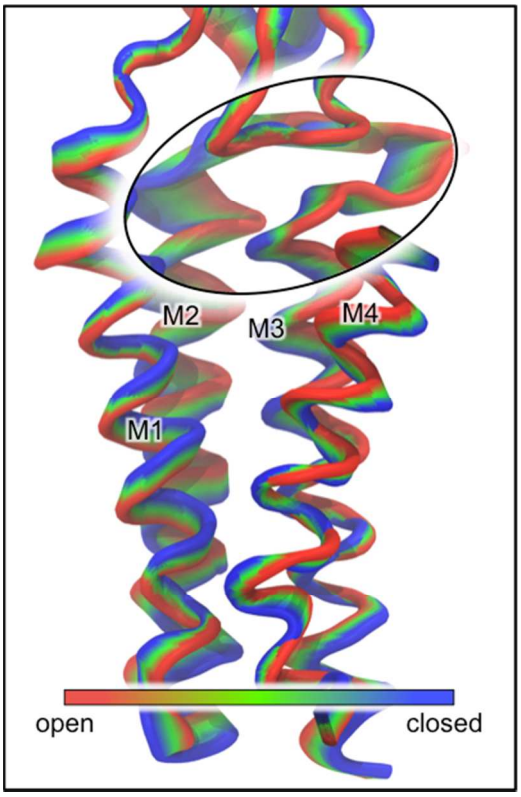

Figure S9

Linear interpolation between ELIC/closed (blue) and GLIC/open (red) structures show that the M2-M3 region (circled), which correlates to the PTX binding site, undergoes the largest movement of any part of the protein upon channel gating. This is in contrast to the negligible change in structure seen in this region upon PTX binding to the pore (Fig S2).

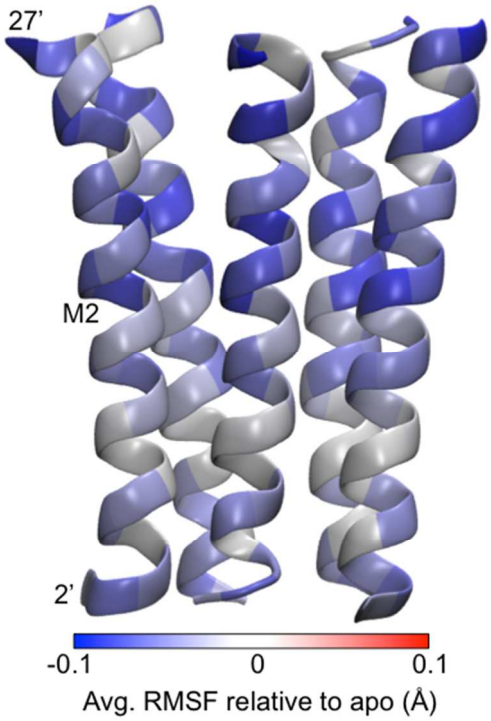

Figure S10

The average root mean squared fluctuation (RMSF) of the Cα-atom positions of the M2 helices in the $\alpha_6\beta_3$ PTX-bound simulation relative to the apo simulation. A darker blue color indicates a residue fluctuates less and is thus stabilized by the presence of PTX.

References

1. Nury, H., Van Renterghem, C., Weng, Y., Tran, A., Baaden, M., Dufresne, V., Changeux, J. P., Sonner, J. M., Delarue, M., and Corringer, P. J. (2011) X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel. *Nature 469*, 428-31.