Supporting Information for the Biochemistry article entitled:

Photoaffinity Labeling the Propofol Binding Site in GLIC

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Figures S1 & S2) Stereo views of models of the transmembrane domain of pLGIC subunits, with α helices as cylinders. **A**) GLIC helix bundle pocket with AziP*m* (stick format, color coded by atom type: gray, carbon; red, oxygen; light blue, fluorine; blue, nitrogen) docked within the predicted propofol binding site. The 3 colored residues (Met-205, gold; Tyr-254, magenta; and Asn-307, cyan) were photolabeled by AziP*m* in a propofol-inhibitable manner. Also included is Thr-255, a residue that when mutated to alanine increased potency of propofol inhibition (*1*) but when mutated to cysteine demonstrated higher reactivity with MTSEA in the presence of propofol than in the absence (*2*). **B**) nAChR δ subunit from a homology model derived from the GLIC structure (PDB 3P50). **C**) δ subunit from the *Torpedo marmorata* nAChR cryoelectron microscopy structure (PDB 2BG9). Residues in the δ subunit helix bundle pocket photolabeled by nAChR inhibitors are shown in stick format, color coded to match residue labels. Color coding of residues is conserved between models B & C to highlight structural differences. Photolabeled residues include: Phe-232, Cys-236, & Thr-274 labeled by [^{3}H]AziP*m* (*3*); Tyr-228 labeled by [^{14}C]halothane (*4*); Ile-288, Phe-232, Cys-236, Thr-274, & Leu-278 labeled by 3-(trifluoromethyl)-3-(*m*-[^{125}I]iodophenyl)diazirine (TID) (*5-7*); Pro-286, Ile-288, & Phe-232 labeled by [^{3}H]benzophenone (*8*); Phe-232 & Cys-236 labeled by [^{3}H]TFD-etomidate (*9*); and Cys-236 labeled by [^{3}H]azietomidate (*10*). The locations of residues in the M2 and M3 helices in nAChR homology model based on GLIC (**S1B** & **S2B**) are consistent with results of recent study of cross-links between substituted Cys in the nAChR α subunit M2 and M3 helices (*11*). The distances between the δPhe-232, δThr-274, & δIle-288 in the two nAChR models are compared in Table **S1**.

Figure S1: Stereo views from the lipid of the transmembrane domain of pLGIC subunits: A) GLIC (PDB:3P50) and B & C) nAChR δ subunit from a GLIC-derived homology model (B) and the nAChR cryoelectron microscopy structure (C, PDB 2BG9).

A) GLIC subunit transmembrane domain (PDB:3P50)

B) δ subunit transmembrane domain from nAChR homology model based on GLIC (PDB:3P50)

C) δ subunit transmembrane domain from the nAChR structure (PDB:2BG9)

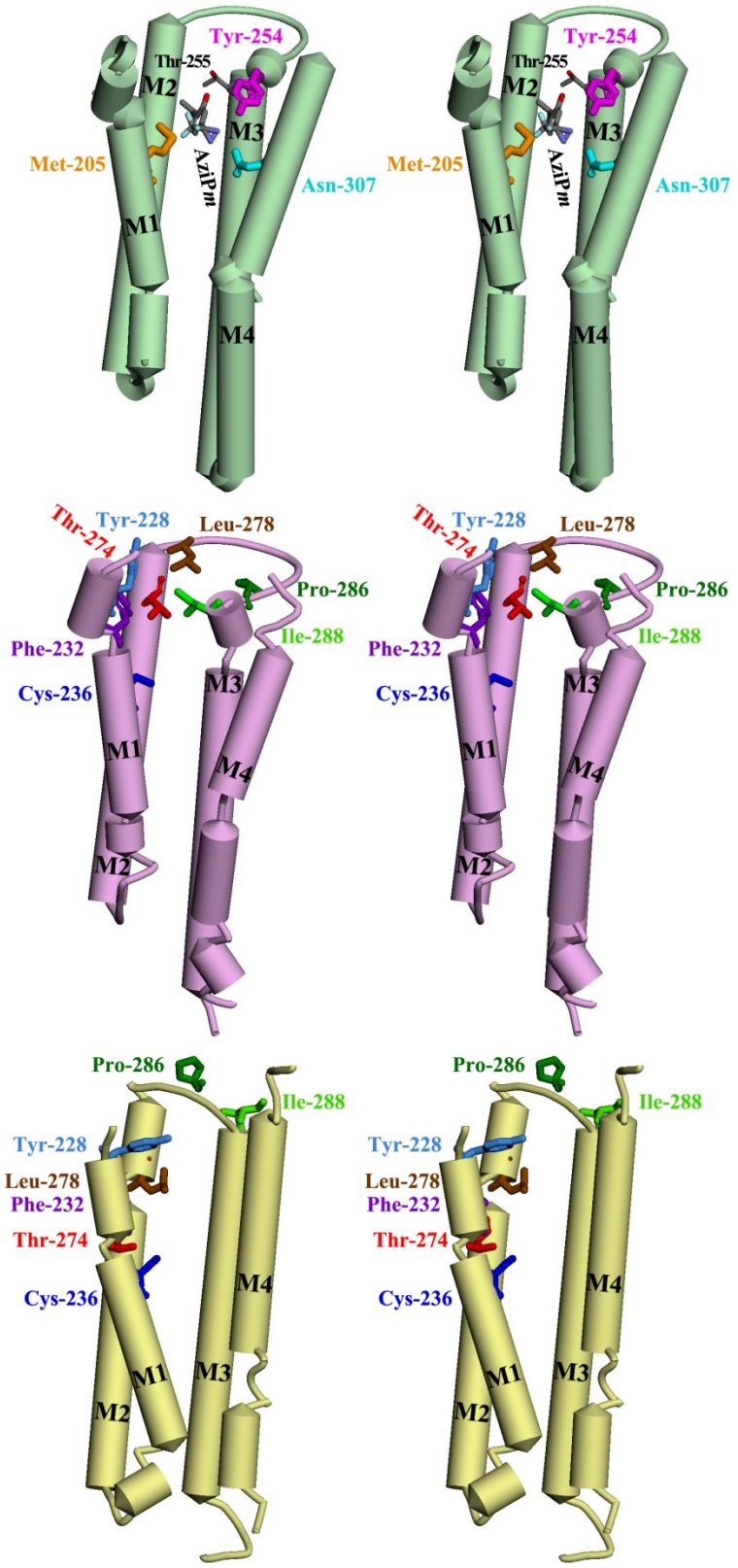

Figure S2: Stereo views from the base of the extracellular domain of the transmembrane domain of pLGIC subunits: A) GLIC (PDB:3P50) and B & C) nAChR δ subunit from a GLIC-derived homology model (B) and the nAChR cryoelectron microscopy structure (C, PDB 2BG9).

A) GLIC subunit transmembrane domain (PDB:3P50)

B) δ subunit transmembrane domain from nAChR homology model based on GLIC (PDB:3P50)

C) δ subunit transmembrane domain from the nAChR structure (PDB:2BG9)

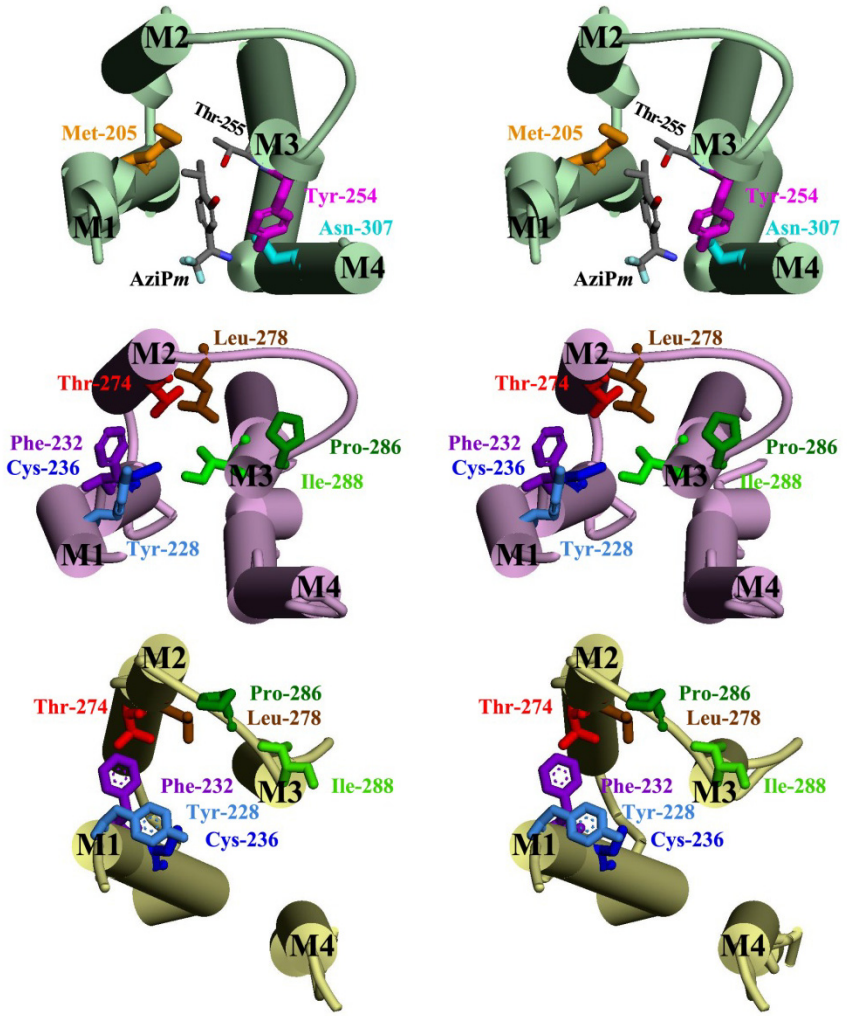

Table S1: Distances in the two nAChR models between photolabeled amino acid side chains in the δ subunit helix bundle pocket (closest non-hydrogen atoms).

	Side Chain Distances: CyroEM nAChR model (PDB:2BG9) (Å)	Side Chain Distances: nAChR homology model based on GLIC (Å)
δPhe-232 (M1) to δThr-274 (M2)	5.4	3.2
δPhe-232 (M1) to δIle-288 (M3)	14.3	5.9
δThr-274 (M2) to δIle-288 (M3)	17.3	4.6

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