Supplemental Information

Open-Channel Structures of the Human Glycine Receptor α1 Full-Length Transmembrane Domain

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SUPPLEMENTAL DATA

Figure S1. The sequence of the 150-residue protein under study (hGlyR-α1 TM) aligned with the transmembrane (TM) domains of the native human GlyR-α1 subunit (GlyR-α1), the Glutamate Chloride channel from *C. elegans* (GluCl), four representative members in the Cys-loop receptor superfamily ($GABA_A$ α1 subunit, nAChR α1 and α4 subunits, and $5HT_3$ α subunit), and two bacterial homologues (ELIC and GLIC). An artificial loop between TM3 and TM4 domains and a 6-His tag at the C-terminal are shaded in light purple. Solid lines below six sequences mark the experimentally determined TM helices. Residues believed to be part of the ion selectivity filter are highlighted in red rectangle boxes. Sequence alignment was performed using Clustal X version 2.0 (Larkin et al., 2007).

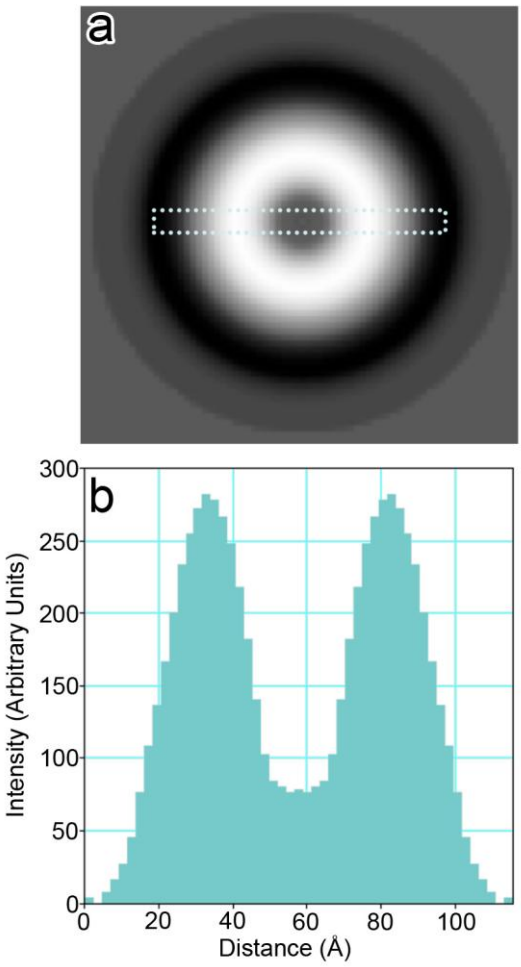

Figure S2. Radial intensity profiling of circular averaged pentameric particles in negatively stained EM images. (**a**) Radial averaging of pentameric particles. (**b**) Density line profile of the box region in (a). Peak to peak distance is ~45 Å. See also Figure 1.

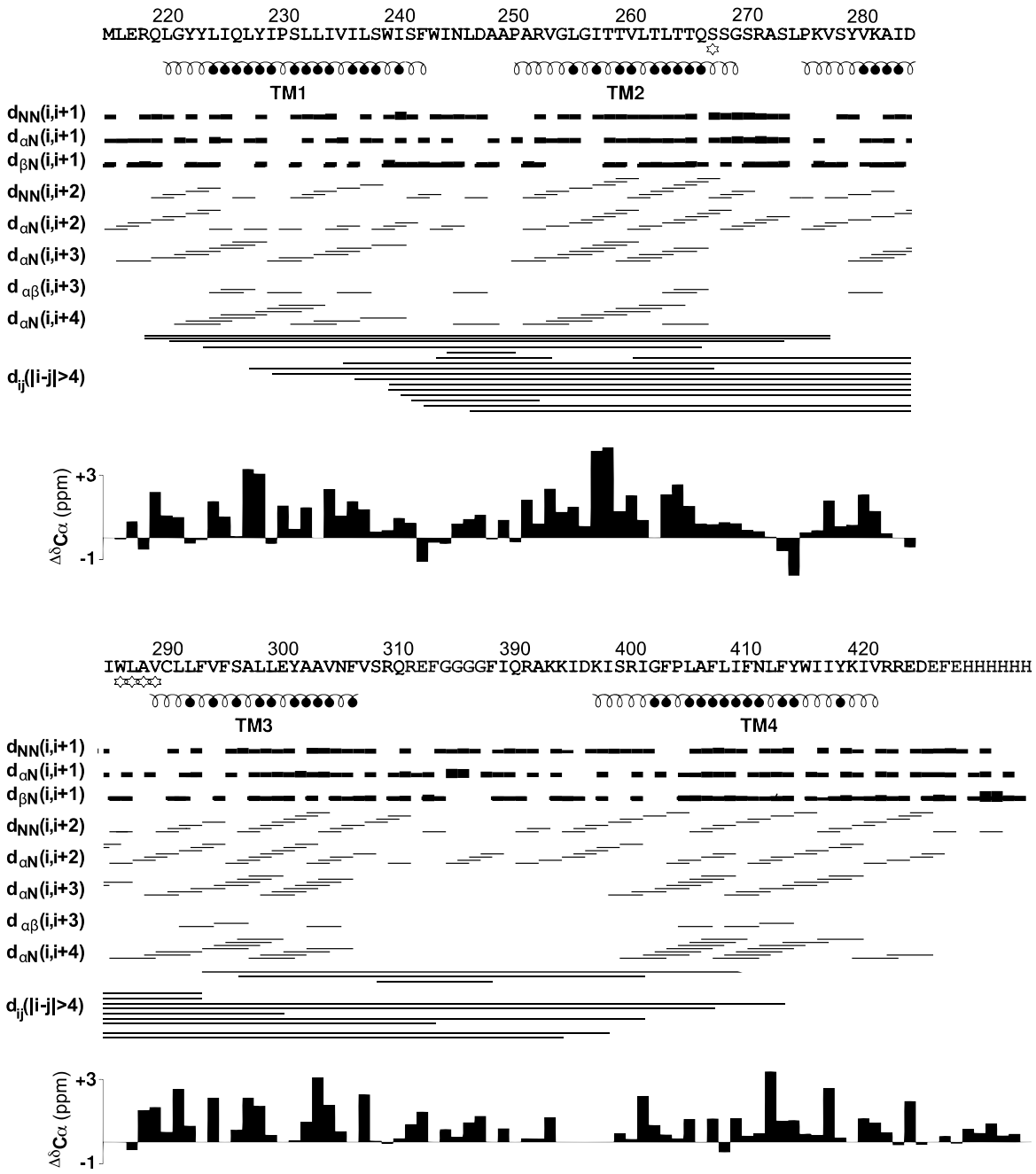

Figure S3. The NOE connectivity and Cα chemical shift index for the hGlyR-α1 TM domain in LPPG micelles. The line thickness of the NOE connectivity is proportional to the cross-peak intensities. The helical regions observed in the NMR structure (black coils) are shown underneath the sequence for comparison. The filled circles below the sequence mark the residues where backbone hydrogen-bond restraints were imposed on the basis of the temperature dependence of the exchangeable amide protons. The two ends of a segment from TM2 to TM3 that is highly dynamic are marked with stars above the sequence. The artificial linker between TM3 and TM4 and the His-tag in the C-terminus are shown in gray color in the sequence. See also Table 1 and Figure 3.

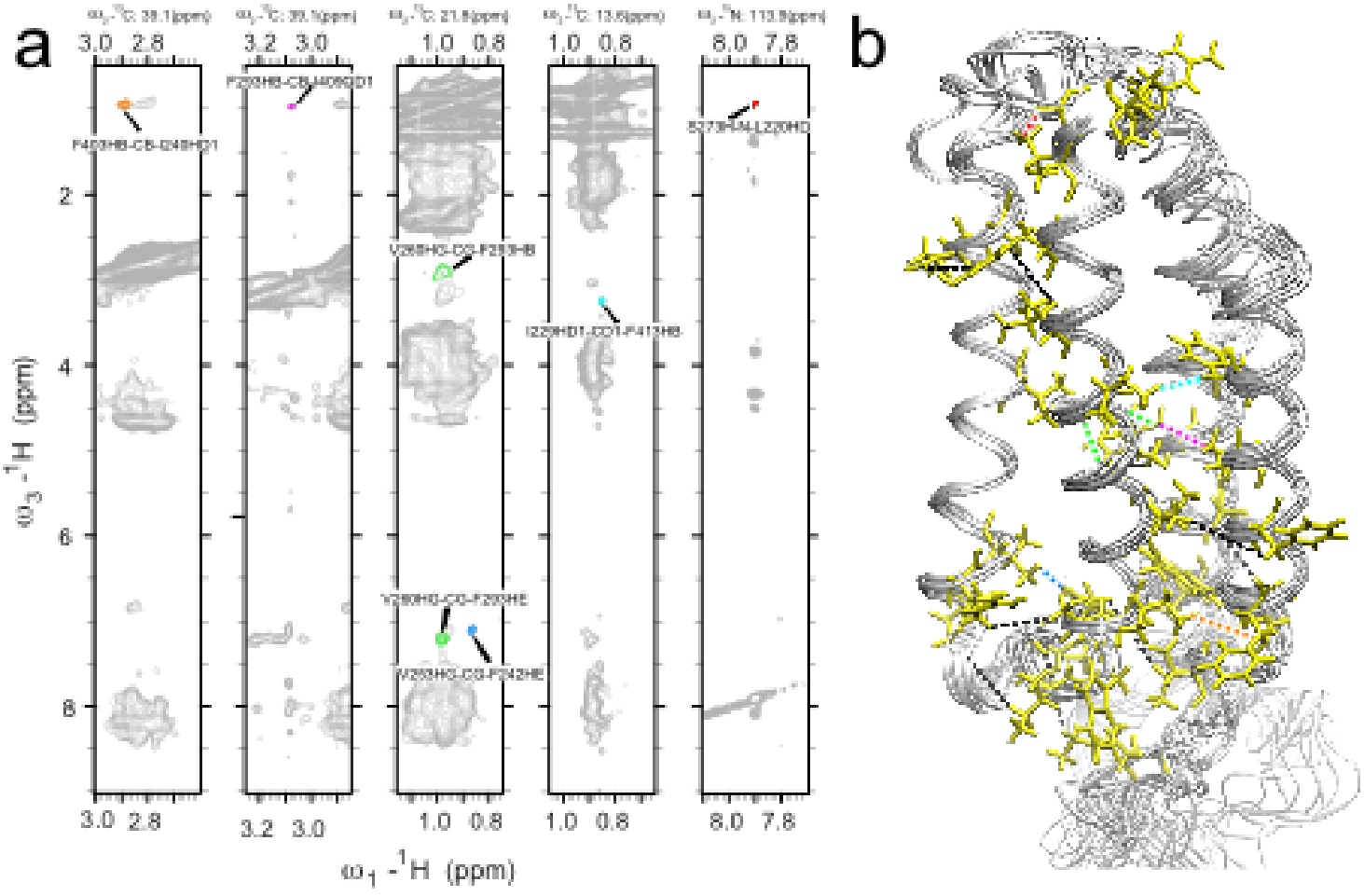

Figure S4. Long-range distance restraints between different TM helices generated from NOESY experiments. (**a**) Representative strip plots of ^{13}C and ^{15}N filtered NOESY spectra showing NOE cross peaks resulting from residues in different TM helices. (**b**) The tertiary structures of the hGlyR-α1 TMD showing all inter-helical NOEs ($d_{ij}|i-j|>4$) demonstrated in Figure S3. The colored dashed lines highlight NOEs corresponding to the colored cross peaks shown in (a). Total numbers of NOESY restraints are reported in Table 1. See also Figure 3.

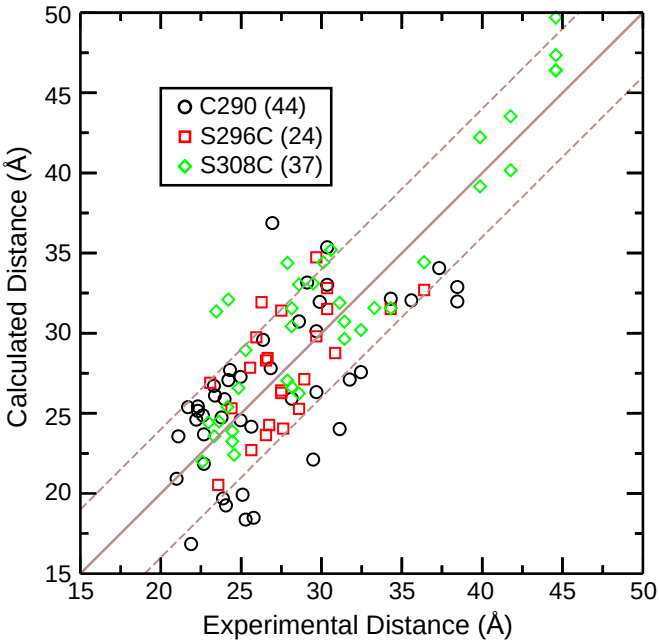

Figure S5. Correlation between the distances measured by the paramagnetic relaxation enhancement (PRE) experiments and the distances calculated from the averaged NMR structure. Three sets of data are from paramagnetic spin labels at C290 (○), S296C/C290S (□), and S308C/C290S (◊). The solid line indicated the ideal case when experimental distance is identical to the calculated distance. The dashed lines mark the ±4 Å upper and lower bounds. The total number of PRE restraints is listed in Table 1. Calculated distances are based on the structures shown in Figure 3.

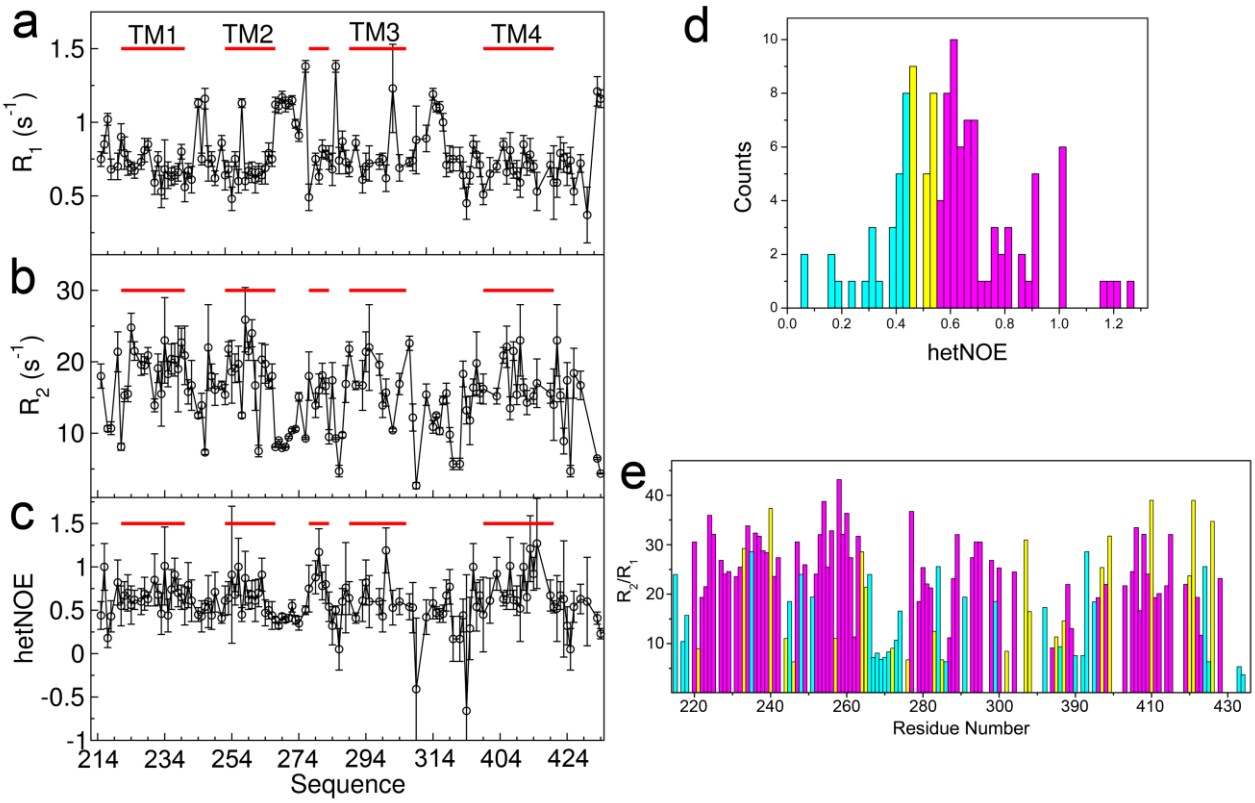

Figure S6. NMR dynamics measurements of the hGlyR-α1 TM domain in LPPG micelles at 40°C and 16.5 T (700 MHz). Data for (**a**) R_1, (**b**) R_2, and (**c**) hetNOE plotted according to residue number. (**d**) Histogram of residue counts as a function of hetNOE values. A few residues had hetNOE value ≥ 1 due to either peak overlapping or low signal-to-noise ratio. Residues are classified into three categories: hetNOE ≥ 0.55 (pink), 0.45 ≤ hetNOE < 0.55 (yellow), and hetNOE < 0.45 (cyan), corresponding to residues in helices, in regions transitioning from helices to loops, and in flexible loops or terminal regions, respectively. (**e**) The R_2/R_1 ratio of individual residues is plotted as a function of residue number. The corresponding hetNOE values are color-coded using the same categorical range as shown in (d). Notice the high flexibility near the end of the TM2 helix (S267, S268, G269, and S270) and the beginning of the TM3 domain (I285, W286, and L287). See also Figure 4.

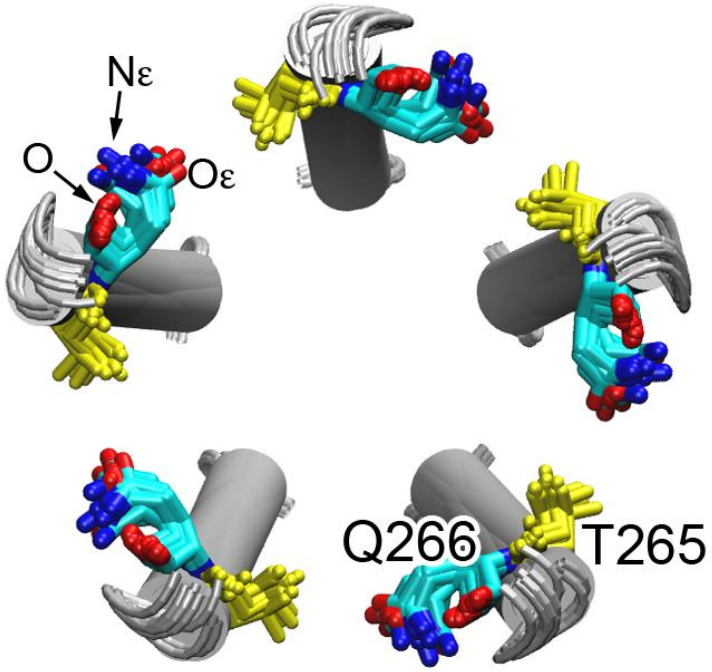

Figure S7. Self-contact of the Q266's side chain amide with the backbone carbonyl oxygen. TM2 helices from the bundle of 15 pentamer structures of the hGlyR-α1 TM domain are shown in cartoon representation. The side chain of Q266 is tangential to the pore. The side chain amide group (blue, labeled Nε) is only ~2Å away from the backbone carbonyl oxygen of Q266 (red, labeled O). Such a self-contact competes with the helical hydrogen bonding and potentially weakens the local helical structure.