Gonzalez-Gutierrez et al., https://doi.org/10.1085/jgp.201711803

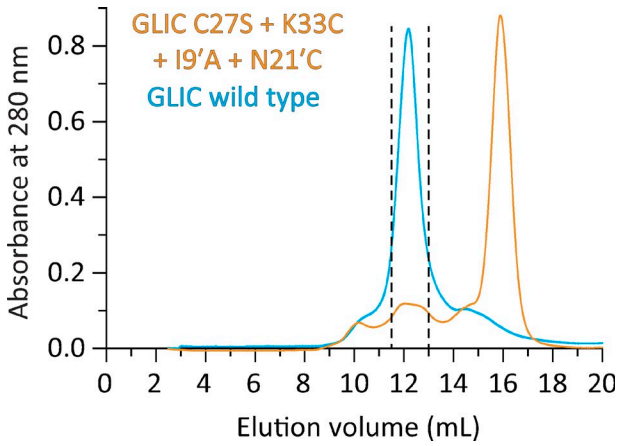

Figure S1. **Decreased pentameric stability of GLIC C27S + K33C + I9′A + N21′C mutant in *n*-dodecyl-β-D-maltopyranoside (DDM).** Representative size-exclusion chromatography profiles of WT GLIC and the quadruple mutant; the y-axis is not normalized. The chromatography column was a Superdex 200 10/300 GL (GE Healthcare Life Sciences). The vertical dashed lines indicate the pooled fractions used for x-ray crystallography.

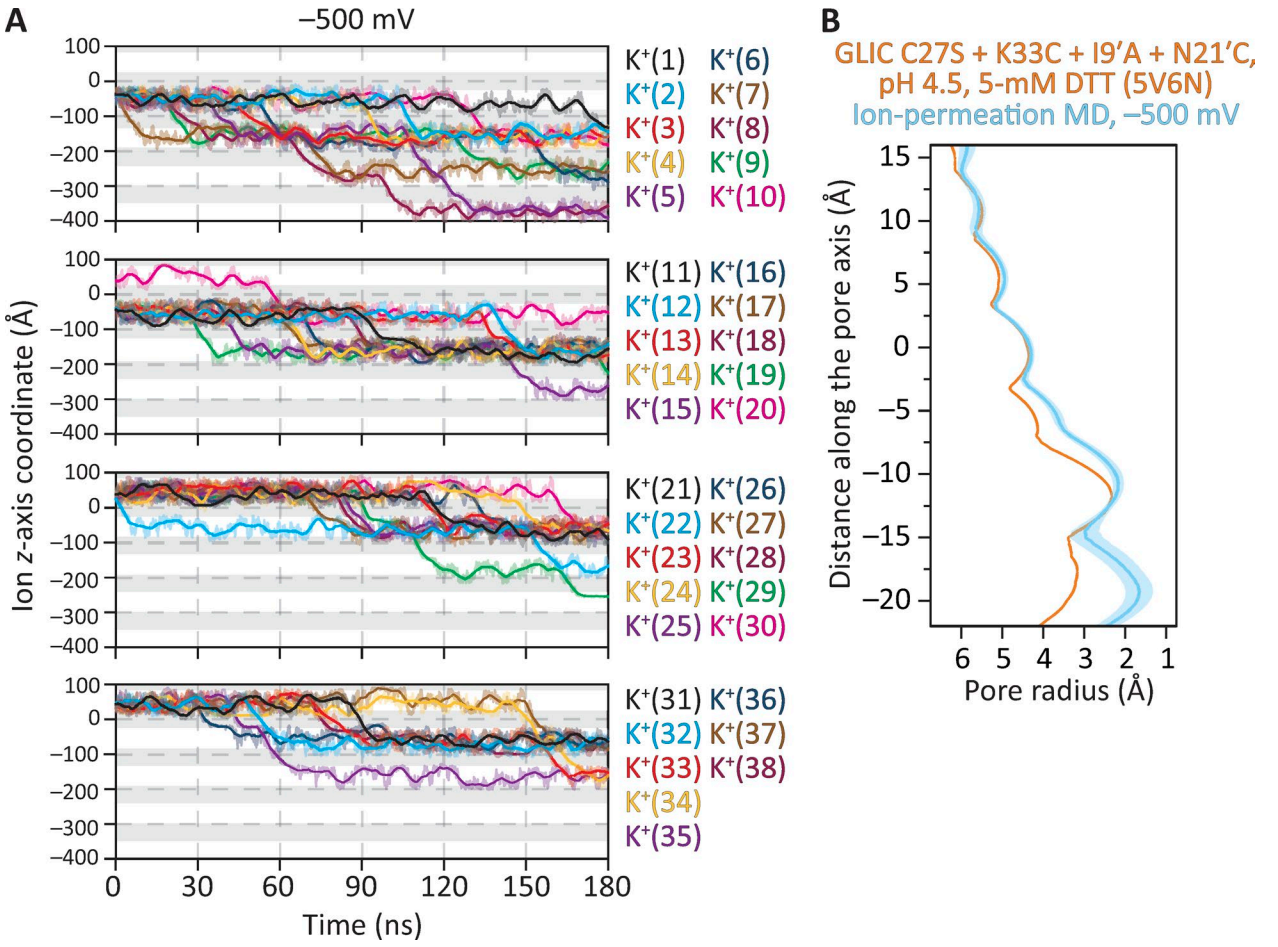

Figure S2. **MD simulation of ion permeation through the structural model of the disulfide-reduced GLIC C27S + K33C + I9′A + N21′C mutant at −500 mV.** The membrane was bathed by symmetrical 150 mM KCl, and the temperature was 37°C. (A) Ion trajectories. For clarity of display, the trajectories of the 38 K^+ that crossed the membrane at least once in the simulated ∼180 ns were displayed in four separate panels. Of these 38 K^+, 27 crossed the membrane inward once, 8 did so twice, and 3 did so three times. Because no Cl^- crossings were observed during the simulated time, these 52 net K^+ inward crossings represent an inward current of ∼−46 pA. Only the trajectories of ions that crossed the membrane are plotted. The gray areas indicate the regions occupied by the membrane in the periodic-simulation system, and downward transitions of the ion trajectories through these regions represent inward crossings. The darker lines are running averages of the data, which are displayed in a lighter shade. The length of the simulation box along the z-axis was 108 Å. (B) Pore-radius profiles (estimated using HOLE [Smart et al., 1996]) of structural models of the disulfide-reduced quadruple mutant. The profile of the crystal-structure model (PDB ID code 5V6N; this work) is compared with that computed during the ion-permeation MD simulation illustrated in A. The latter is the mean profile—displayed as the mean (darker lines) ± 1 SD (lighter shade)—calculated from the different frames of the simulation; the vertical axis extends, approximately, between M2 positions −3′ (bottom) and 21′ (top). Side-chain rotamers were optimized using SCWRL4 (Krivov et al., 2009) before the ion-permeation simulation was run.

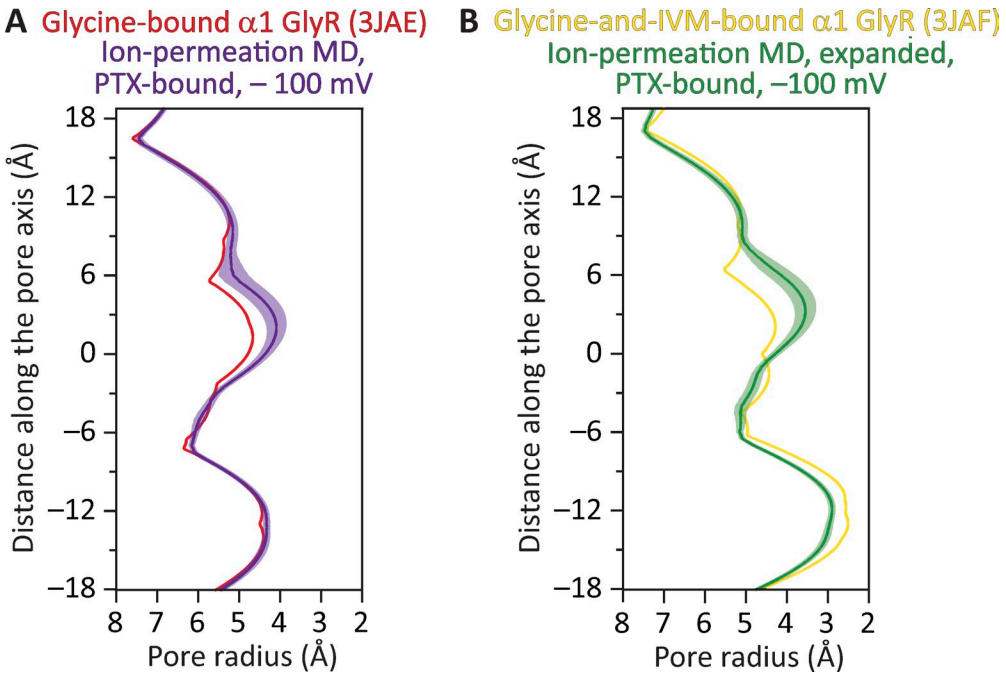

Figure S3. **Pore-radius profiles of picrotoxinin-bound models of the α1 GlyR.** (A) The profile of the cryo-EM-structure model of the glycine-bound GlyR (PDB ID code 3JAE) is compared with that computed during the MD simulation of ion permeation through the 3JAE model with a molecule of picrotoxinin placed in the pore (see Fig. 9, D and E). PTX, picrotoxinin. (B) The profile of the cryo-EM-structure model of the glycine-and-ivermectin–bound GlyR (PDB ID code 3JAF) is compared with that computed during the MD simulation of ion permeation through the expanded 3JAF model with a molecule of picrotoxinin placed in the pore (see Fig. 10 E). IVM, ivermectin. In A and B, ion-permeation-MD pore-radius profiles are mean profiles—displayed as the mean (darker lines) ± 1 SD (lighter shade)—calculated from the different frames of each simulation upon removing the modeled molecule of picrotoxinin; the vertical axes extend, approximately, between M2 positions −3′ (bottom) and 21′ (top). Side-chain rotamers were optimized using SCWRL4 (Krivov et al., 2009) before the ion-permeation simulations were run.

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

Parameter	GLIC E−2′I + I9′A pH 4.5	GLIC C27S + K33C + I9′A + N21′C pH 4.5, 5 mM DTT
Data collection		
Wavelength, Å	0.97857	0.97857
Space group	C2	C2
Cell dimensions		
a, b, c, Å	180.99, 134.28, 159.94	177.18, 134.06, 159.68
α, β, γ, °	90, 102.06, 90	90, 101.13, 90
Resolution, Å	105.98–3.12	106.16–3.36
R_{sym}	0.162 (1.090)	0.244 (1.433)
R_{meas}	0.192 (1.300)	0.262 (1.535)
R_{pim}	0.089 (0.602)	0.095 (0.549)
CC1/2	0.995 (0.764)	0.979 (0.743)
I/σ(I)	8.69 (1.3)	9.6 (1.9)
Completeness, %	99.9 (99.5)	99.9 (100.0)
Multiplicity	4.2 (4.3)	7.7 (7.8)
Refinement		
Resolution, Å	70.44–3.12 (3.23–3.12)	59.76–3.36 (3.47–3.36)
No. unique reflections	65,330 (6,485)	52,507 (5,228)
R_{work}	0.240	0.231
R_{free}	0.279	0.284
RMSD values		
Bond lengths, Å	0.011	0.012
Bond angles, °	1.55	1.63
No. atoms		
Protein	12,583	12,560
Ligand/ions	128	86
B-factors, Å²		
Protein/ligand/ions	67.4	67.7
Ramachandran plot		
Favored, %	98.0	96.0
Allowed, %	1.9	3.0
MolProbity score	1.87	2.26
PDB ID code	5V6O	5V6N

Highest-resolution shell values are shown in parentheses.

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Smart, O.S., J.G. Neduvelil, X. Wang, B.A. Wallace, and M.S. Sansom. 1996. HOLE: a program for the analysis of the pore dimensions of ion channel structural models. *J. Mol. Graph.* 14:354–360, 376. https://doi.org/10.1016/S0263-7855(97)00009-X