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Effects of the γ2 K289M mutation on the structure and function of GABA Type A Receptors: Insights from molecular dynamics simulations

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Abstract

Pentameric ligand-gated ion channels (pLGICs), including GABA type A receptors (GABAARs), play crucial roles in neuronal signaling through their ability to mediate inhibitory neurotransmission. Despite the significant sequence variation among pLGIC subunits, few single nucleotide polymorphisms (SNPs) occur in coding regions, particularly for receptors critical to maintaining neural excitability. The naturally occurring mutation γ2 K289M, linked to generalized epilepsy and febrile seizures, exhibits reduced GABA-evoked current amplitudes and altered receptor kinetics. In this study, we employed molecular dynamics simulations to investigate the structural and functional impacts of the K289M mutation at varying temperatures (300K and 315K). Our findings indicate that the mutation leads to a narrowed pore in the receptor, significantly increasing the energetic barrier for chloride ion conduction, particularly at elevated temperatures. Notably, we observed that the K289M mutation disrupts electrostatic interactions that stabilize the wild-type receptor, suggesting a critical role for charge repulsion in maintaining channel integrity. This research enhances our understanding of the molecular mechanisms underlying epilepsy-associated mutations in GABAARs and underscores the importance of temperature-dependent effects on receptor dynamics.

Keywords: GABA Type A Receptors; γ 2 K289M Mutation; Pentameric Ligand-Gated Ion Channels (pLGICs); Generalized Epilepsy and Febrile Seizures Plus (GEFS+); Epilepsy-Associated Mutations; Adaptive Biasing Force (ABF) Calculations; Thermodynamic Effects on Ion Channels; WTreceptors

1. Introduction

Pentameric Ligand-gated Ion Channels (pLGICs) are essential components of the post-synaptic membrane, serving both inhibitory and excitatory roles. pLGIC sequence varies significantly within and between prokaryotes and eukaryotes, with typical homologies of about 30%. pLGIC function can be quite sensitive to even small differences in sequence, but numerous pLGIC structures have now demonstrated significant structural conservation despite functional variation. This property has made it challenging to isolate the roles of various pLGIC components or sequence variations in subtle functional effects.

Despite the high sequence variation among pLGIC subunits, even for those forming a heteromeric channel, few single nucleotide polymorphisms (SNPs) are found among populations within coding regions for a specific subunit. Mutations causing loss of function in inhibitory receptors or gain of function in excitatory receptors can result in seizures induced by neuron overexcitation. Many naturally occurring mutations are associated with various forms of epilepsy, with several relevant mutations identified even before the use of genome-wide association studies. The molecular mechanisms underlying the effect of nearly all mutations on signaling are unknown.

GABA is the primary inhibitory neurotransmitter in the central nervous system; inhibition is partially transduced by extracellular binding to the type A GABA receptor, an anionic pL GIC. Numerous atoms with calming, anxiolytic, and

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sedative properties are positive modulators of the $GABA_AR$, including neurosteroids, benzodiazepines, and inhalational and intravenous general anesthetics. Negative modulators, such as pregnenolone sulfate, can induce seizures, as can certain mutations. Seizures associated with inherited mutations typically require conditions that are found only infrequently; survival is unlikely in the presence of consistent seizures. $GABA_AR$ receptors with these mutations are therefore known a priori to be functional under typical conditions but dysfunctional under well-defined alternate conditions, making them promising candidates for identifying the role of the mutated residue.

Every subunit comprises of an extracellular agonist-restricting space (ECD) and a transmembrane space containing a four-helix pack with helices named (M1-M4). The M2 helices line the pore, and the M2-M3 circle interfacing the M2 and M3 helices communicates straightforwardly with the ECD. The circle has for quite some time been speculated to" impart" agonist restricting to the transmembrane space, with a few changes reads up showing the significance for agonist responsiveness of short-range alluring electrostatic communications, for example, salt-spans, between the M2-M3 circle and the ECD.

In $GABA_AR$ subunits the M2-M3 loop contains a basic residue appearing at the homologous positions of $\alpha 279$, $\beta 274$, or $\gamma 289$,documented as M2 $24'$ in the excellent numbering plan proposed in. Harrison and colleagues exhibited that charge-inversion of $\alpha 279$ decreased agonist responsiveness (EC50) which was restorable by means of extra charge-inversion of α D57 or αD149, both inside the ECD and expected to be close to the M2-M3 circle. Greatest entire cell current, be that as it may, was diminished by around 1/3 upon the single $\alpha D279K$ change, and further decreased by about a similar sum with the second transformation of α D57K or αD149K, recommending a huge job for $\alpha 279K$ in settling the open state past shaping a salt-span with the ECD. Comparable way of behaving was seen in the nicotinic acetylcholine receptor (nAChR), upon charge-inversion of α R209 in M1 and α E45 in the ECD.

A natural but uncommonly occurring SNP at the homologous residue in the γ subunit ($\gamma 2$ K289), further suggests an additional role for this residue beyond gating, because the γ subunit does not form GABA binding cavities. The γ_2:K289M transformation has been accounted for in families with summed up epilepsy and febrile seizures plus(GEFS+), a summed up aggregate that frequently incorporates just febrile (fever-caused) seizures until about age 11, yet can likewise incorporate less extreme myoclonic, atonic, or nonattendance seizures at typical internal heat level. In $\alpha_1\beta_2\gamma_2$ K289M receptors, GABA-evoked current sufficiency was emphatically diminished comparative with the, while in $\alpha_1\beta_3\gamma_2$K289M receptors the transformation didn't influence current amplitudes yet expanded the deactivation rate. In the last receptors, flows had decreased mean open times, to some extent because of flickering. In hippocampal neurons containing $GABA_AR$ with γ_2:K289M subunits sped up deactivation of inhibitory post synaptic flows was likewise noticed.

Little information has been available regarding the effect of the mutation on $GABA_AR$ structure and dynamics. Using a homology model of the $GABA_AR$ receptor based on the medium resolution cryo electron microscopy structure of the nicotinic Acetylcholine Receptor (nAChR), Brownian Dynamics Simulations of ion conduction were used to suggest that mutant receptors display reduced conductance due to reduced affinity of the ion for the ion channel. However, the recent x-ray structures of eukaryotic and prokaryotic homologs have suggested that alignment of the sequence with the electron density map in the M2 helices is likely incorrect in the structure used for these simulations. Furthermore, these simulations do not contain explicit representations of water or lipid molecules.

The temperature dependence of this mutation suggests a significant role for entropy and conformational fluctuations in determining its effects. Here we conduct molecular dynamics simulations with multiple replicas of the $\gamma 2$ K289 and M289 forms of the receptor, at both lower and higher temperatures. We observe a moderately narrowed pore in the M289 receptor at 300K, and a significantly narrowed pore at 315K. Through adaptive biasing force (ABF) calculations, we demonstrate that the effects at 315K result in a substantially higher barrier for conduction of a chloride ion.

$\gamma 2$ K289 was not observed to form salt bridges with the ECD, and these conformational effects showed no clear correlation to any salt-bridging pattern. We propose instead that the five conserved basic residues at this position form a ring of positive charge that effectively pushes the five M2-M3 loops away from the center, pulling M2 helices with it, and stabilizing the open state. Neutralizing one of the charges as with $\gamma_2 K289M$ reduces this repulsion. When it is combined with a temperature increase that softens the conformational preferences resulting from remaining interactions common to both K and M receptors, the non-temperature dependent change in electrostatic repulsions dominates.

We present a simple variational theory that quantitatively predicts the effect of $\gamma 2$:K289M on the preferred separation of M2-M3 loop charges, using only the mean and standard deviation of the separation in the wild type $\gamma 2$:K289 channel. Temperature dependence appears through both the effect of temperature on the standard deviation and as a linear term

in the theory. The success of the theory supports a critical role for these electrostatic repulsions in stabilizing the wild-type receptor and also in transducing the effects of the mutation.

(A)Side View of EC and TM domain showing γ subunit in blue ; (B) View of TM domain, looking down on the membrane from the extracellular region, where each subunit(colored as in A) comprises of a four helix bundle(M1-M4). M1 is gray, M2 is purple, M3 is pink and M4 is ochre; Side view (C) and view from the top-down to (D) the TM domain showing the mutation K289M in the M2-M3 loop and the LEU residues at the 9' location.

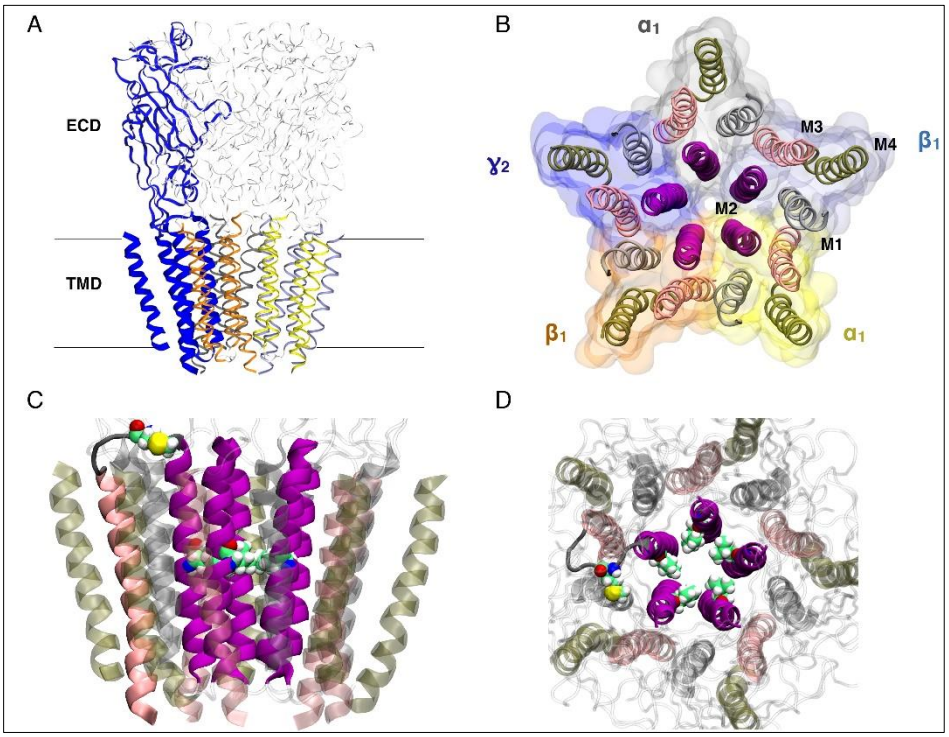


Figure 1 (A) Side View of EC and TM domain showing $\boldsymbol{\gamma}$ subunit in blue; (B) View of TM domain, looking down on the membrane from the extracellular region, where each subunit (colored as in A) comprises of a four-helix bundle(M1-M4). M1 is gray, M2 is purple, M3 is pink and M4 is ochre; Side view (C) and view from the top-down to (D) the TM domain showing the mutation K289M in the M2-M3 loop and the LEU residues at the 9' location

2. Homology Models

A high-resolution structure of a $GABA_AR$ was not available until the recent publication of 3Å resolution structure for a β_3 homopentamer. In the transmembrane domain, homology between $GABA_AR\alpha$ or γ to $GABA_AR\beta$ is not significantly improved relative to homology between $GABA_AR\alpha/\gamma$ subunits and GluClα (need numbers), and as a result homology model of $\alpha\beta\gamma GABA_AR$ built on the $GABA_AR\beta_3$ homopentamer are not expected to be significantly improved relative to those based on GluCl. The framework utilized in this paper compares to Demonstrate 1 - CHOL from Reference, and was worked with GluCl (PDB code: 3RHW) as a layout as well as the arrangements distributed in Ref. Further legitimization and subtleties on this model can be tracked down in Reference

2.1. System Setup

This manuscript considers data from four simulations at 300K and four simulations at 315K, with 2 wild type (termed K1, K2) and 2 mutants (M1, M2). The frameworks were ready as in Ref, by implanting the protein in a lipid bilayer made out of 4:1 phosphatidylcholine (POPC): cholesterol mixture built using CHARMM Membrane builder, with the final system containing 268 POPC and 71 membrane CHOL molecules. In addition to membrane cholesterol, this model includes cholesterol docked to five pseudo-symmetric intersubunit sites, with implications and justification for this decision reported in. The frameworks were solvated utilizing the SOLVATE module in VMD and killing particles were added to carry the framework to a 0.15M salt focus utilizing the AUTOIONIZE module. The last framework contained around 160,000 iotas.

2.2. Simulation Methods

All recreations involved the CHARM22-CMAP force field with torsional remedies for proteins. The CHARMM36 model was utilized for phospholipids, particles, water and cholesterol atoms. Energy minimization and MD reenactments were directed utilizing the NAMD2.9 package. All simulations employed periodic boundary conditions, long-ranged electrostatics were handled with smooth particle mesh Ewald method, and a cutoff of 1.2 nm was used for Lennard-Jones potentials with a switching function starting at 1.0 nm. All simulations were run in the NPT ensemble with weak coupling to Langevin thermostat and a barostat at a respective 300 K/315 K and 1 atm. All bonds to the hydrogen atoms were constrained using the SHAKE/RATTLE algorithm. A multiple time-step rRESPA method was used, and controlled with a high frequency time-step of 2fs and low frequency time-step of 4fs. All the systems were energy minimized for 10000 steps, then simulated for 5 ns with restraints of 1 kcal/mol/Å applied to the Cα atoms of the protein. Restraints were then removed and 195 ns of nearly unrestrained simulation was carried out in every one of the four frameworks. During this time of the reproduction, just symphonious limitations (force steady 0.4 kcal/mol/Å) between the intracellular finishes of the M3 and M4 helices were utilized, to copy the impacts of the intracellular space and keep partition of the M4 helix from the remainder of the group. High temperature (315K) reproductions were run for 500 ns following the 200 ns recreations at lower temperature (300K).

Conformational Analysis: The estimation of pore radii was completed using the Opening software and TCL scripting via VMD. Python scripts were employed to analyze and visualize the hydration of the pore throughout the simulation.

Poisson-Boltzmann Calculations: The conduction profile for Na+ and Cl- ions through the channel was computed using the Poisson-Boltzmann (PB) method, leveraging APBSmem . Pre-generated PQR protein structures were used as input for the electrostatic potential calculations, prepared via the PDB2PQR tool.

SMD Simulations: Steered Molecular Dynamics (SMD) simulations were employed to determine the positions of ions at various points along the channel, providing data for subsequent Adaptive Biasing Force (ABF) calculations. In these simulations, the chloride ion was dragged through the channel at a constant velocity of 10 Å/ns. The force required to maintain this steady pulling speed was calculated, which could, in theory, be used to derive the potential of mean force (PMF) using Jarzynski's equality. However, practically speaking, achieving sufficiently slow pulling speeds is challenging.

ABF Simulations: Adaptive Biasing Force (ABF) calculations were used to derive the PMF, or free energy profile, for the movement of a chloride ion through the $GABA_AR$ ion channel at 315K, for both the wild-type () and mutant () channels. The Collective Variables module in NAMD2.9 facilitated the ABF computations. The pore length was divided into 23 segments, each 5 Å in length. Initial ion positions were sourced from the SMD simulations. Each segment collected 1,000 samples before ABF was applied, ensuring equilibrium effects were minimized. Most segments generated 15 ns of data, though regions near the primary pore barrier required 25 ns of simulation.

3. Theory

The ring of five basic residues can be approximated as five positive charges arranged in a pentamer, each a distance r from the center, which we refer to as the +5 ring. The thermally excited ring may "breathe", causing r to fluctuate, but for simplicity all charges are treated as equidistant from the center. The variation in r is given by the time-average

$$\delta r^2 = \langle (r(t) - \bar{r})^2 \rangle.$$

At equilibrium, the wild-type receptor exhibits normal fluctuations of r around its time average $\bar{r}$. The free energy of the wild-type receptor as a function of the +5-ring radius r can be expanded harmonically as

$$H_K(r) = \frac{k_r (r - \bar{r}_K)^2}{2\bar{r}_K},$$

where the time-average of r is noted by $\bar{r}_K$, and k_r is the temperature-dependent coefficient governing fluctuations:

$$k_r = \frac{RT\ \bar{r}_K}{\langle (r - \bar{r}_K)^2 \rangle},$$

where R is the gas constant and T is the temperature.

The mutation γK289M removes the four long-range repulsive electrostatic interactions involving $\gamma K289$. Shrinking the pentameric ring is therefore less unfavorable in the presence of the mutation, and the free energy as a function of r is reduced by the Coulomb energy of the lost interactions:

$$\Delta U(r) = \frac{-k_e e^2}{r}\left(\frac{1}{\sin 2\pi/5} + \frac{1}{\sin \pi/5}\right) = -\frac{c k_e e^2}{r}$$

where $c \sim 2.75$, e is the electron charge, and \$k_{e} = 332 \mathrm{\AA/kcal/mol}/e^{2}\$ is the Coulomb constant. Note that this simplification is reasonable primarily because all five charges are nearly coplanar in a plane perpendicular to the pore axis. Other electrostatic interactions will also be lost, but it is reasonable to neglect them because they involve residues screened by another oppositely charged residue and/or they do not have a significant radial component. The total free energy for the mutant receptor is therefore

$$\begin{aligned} H_M(r) &= H_K(r) + \Delta U(r) \\ &= \frac{k_r (r - \bar{r}_K)^2}{2\bar{r}_K} - \frac{c k_e e^2}{r} \\ &= k_r \bar{r}_K \left(\frac{(r - \bar{r}_K)^2}{2\bar{r}_K^2} - \frac{\kappa \bar{r}_K}{r}\right), \end{aligned}$$

Where;

$$\kappa \equiv \frac{c\, k_e e^2}{k_r \bar{r}_K^2} = \frac{c}{RT}\frac{k_e e^2}{\bar{r}_K}\frac{\delta r^2}{\bar{r}_K^2}$$

The average radius for the mutant receptor, $\bar{r}_M$, minimizes H_M:

$$\left.\frac{\partial H_M(r)}{\partial r}\right|_{\bar{r}_M} = k_r\left(1 - \frac{\bar{r}_M}{\bar{r}_K} - \kappa\left(\frac{\bar{r}_K}{\bar{r}_M}\right)^2\right) = 0.$$

Defining the ratio between the two mean radii $\alpha \equiv \bar{r}_M/\bar{r}_K$, Equation [eq:minimize] reduces to $1 - \alpha - \kappa/\alpha^2 = 0$. This equation has an exact, real solution for $\kappa < 4/27$ ($\frac{k_e e^2}{k_r r_K^2} < 0.035$), which when expanded around $\kappa = 0$ is

$$\alpha = \frac{\bar{r}_M}{\bar{r}_K} = 1 - \kappa - 2\kappa^2 - 7\kappa^3 + O(\kappa^4).$$

To first order in κ, we predict that

$$\bar{r}_M = \bar{r}_K - \frac{c k_e e^2}{RT}\frac{\delta r_K^2}{\bar{r}_K^2}$$

where $c k_e e^2/R = 8.3 \times 10^5$ Å K.

4. Results and discussion

4.1. Conformational Effects of Mutation

4.1.1. +5 ring

For comparison with the analytical model of the +5 ring presented in Theory, the mean $\bar{r}_K$ and standard deviation $\sqrt{\delta r_K^2}$ of the distance of +5 ring charges from the pore axis (see Figure [fig:diagram]) were measured for the systems at each temperature. Results are in Table [tab:prediction], showing that $\bar{r}_K$ was not sensitive to temperature, while $\sqrt{\delta r_K^2}$ increased with temperature, as expected. These values, as well as Equations [eq:kr] and [eq:kappadef], were used to calculate the parameters k_R and κ for each temperature.

Eq. [eq:predict] was used to generate predictions for $\bar{r}_M$, which were reduced relative to $\bar{r}_K$ at both temperatures, but with a much larger reduction at higher temperatures. Quantitative agreement was very good, especially given the simplicity of the theory; at 300K we predicted a 3.1% reduction upon mutation, but obtained a reduction of 2.5%, while at 315K we predicted an 8.2% reduction but obtained a 6.3% reduction. In both cases, the reduction was overestimated, which may reflect computational limits on equilibration time for the receptor or a higher order contribution to $H_K(r)$ resulting in a steeper free energy cost when $r - \bar{r}_K$ is large.

Observed values and extracted parameters from analysis of +5 ring in receptors, and predicted and observed values upon mutating K289M (yielding +4 ring).

$T(K)$	$\bar{r}_K$(Å)	$\bar{r}_M$(pred,Å)	$\bar{r}_M$(obs, Å)	$\sqrt{\delta r_K^2}$ (Å)	κ	$k_R/\bar{r}_K$ (kcal/mol/Å^2)
300	15.9	15.4	15.5	0.27	0.027	8.5
315	15.8	14.5	14.8	0.42	0.066	3.6

4.1.2. Pore radius

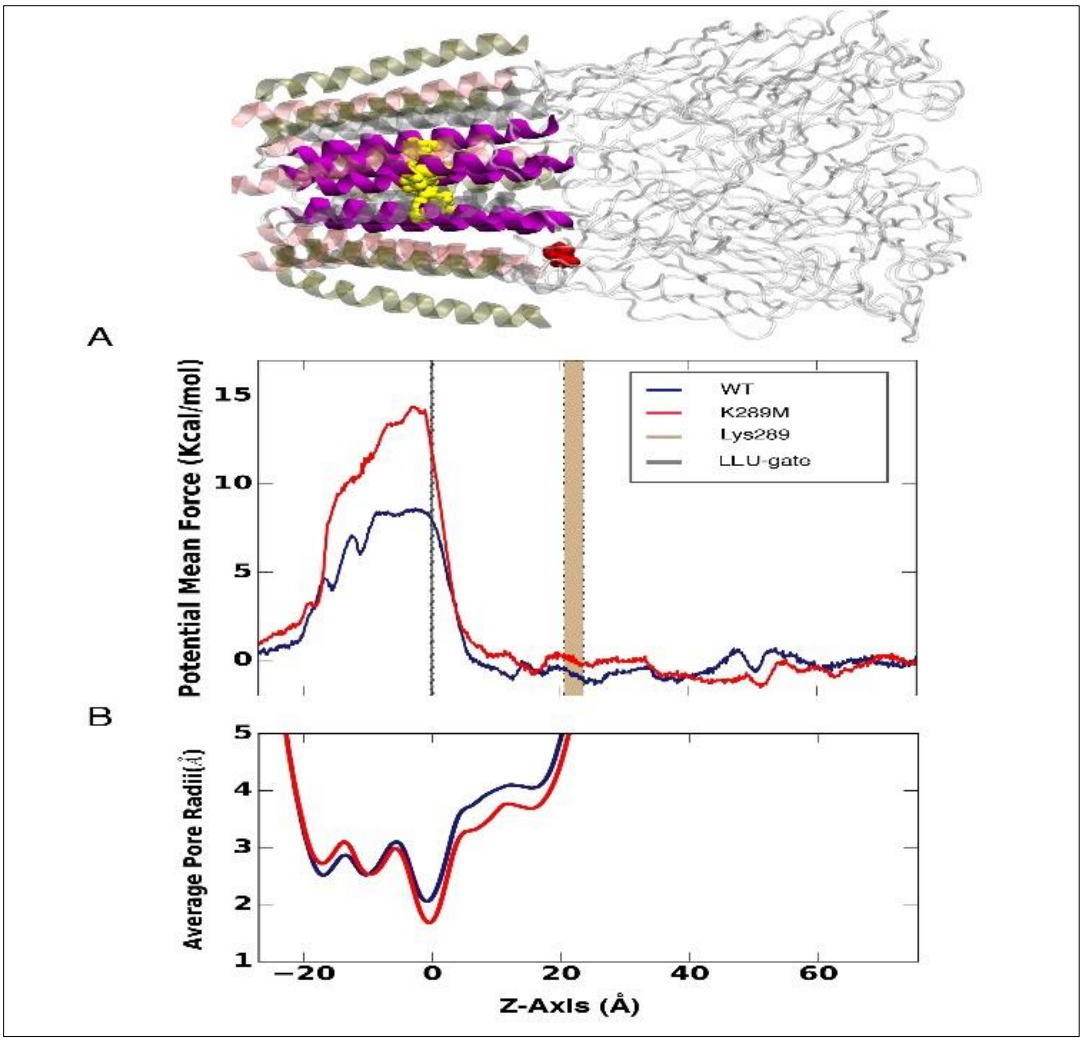
Although the simple electrostatic effects of neutralizing one charge in the +5 ring predict the observed closing of that ring, a functional effect requires that the radius of the +5 ring is coupled to the radius of the pore. The pore radius profile (averaged across two replicas) for the and receptors is shown in Figure 1. The minimum constriction region (flanked by hydrophobic leucine residues) occurs at roughly the same height along the pore axis for the two systems but is substantially tighter for the averaged mutant structure, particularly at higher temperatures.

As shown in Figure 2, overlap between and trajectories (including individual replicas) is substantial at 300K, although the distribution of minimum pore radii is shifted slightly downward (smaller) for the mutant receptor. At 315K, this overlap is substantially reduced, with both replicas yielding conformations with persistently larger pore radii than both replicas. These trends mimic those observed in the +5 ring.

Determining whether a single conformation corresponds to an “open” or “closed” state is not typically possible in MD simulations, but we note here that a Cl- atom has a radius of approximately 1.8Å; at 300K, the minimum pore radius is greater than 1.8Å for 69% () and 43% () of the frames, while at 315K, the minimum pore radius is greater than 1.8Å for 69% () and 26% () of the frames.

All simulations here were done in the absence of GABA or other agonist, which is not stable in the agonist-binding site due to limitations of classical non-polarizable forcefields for capturing cation-π interactions. The presence of agonist would likely alter $\bar{r}_K$ and/or k_R, but would not affect $\Delta U(r)$, which depends only on the protein sequence.

(A) Space-filling models computed from simulations at 315 K, depicting the reduced pore radii of the (red) as compared to that of the (blue). (B) Radii of the transmembrane domain along the Z-axis averaged over all the frames. The pore profile around the 9’ region is more constricted at the higher temperature when compared to that of the lower temperature in both the and the. The space-filling models further compare the significant reduction in the pore radius in the to the fairly open, and the movement of helices compared to their respective initial conformations(gray).



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Smoothed time evolution of the pore minimum constriction, averaged (solid lines) over two replicas (dotted lines) each, at 300 K(A) and 315 K(B). The minimum constriction, formed around the 9' region, is visibly more constricted for the , and this reduction is more pronounced at higher temperatures. The minimum constriction region in falls below the chloride ion radius of 1.8Å, thus driving it to a closed state. The probability distribution further shows a clear shift in the peak of the towards reduced pore radii at a higher temperature.

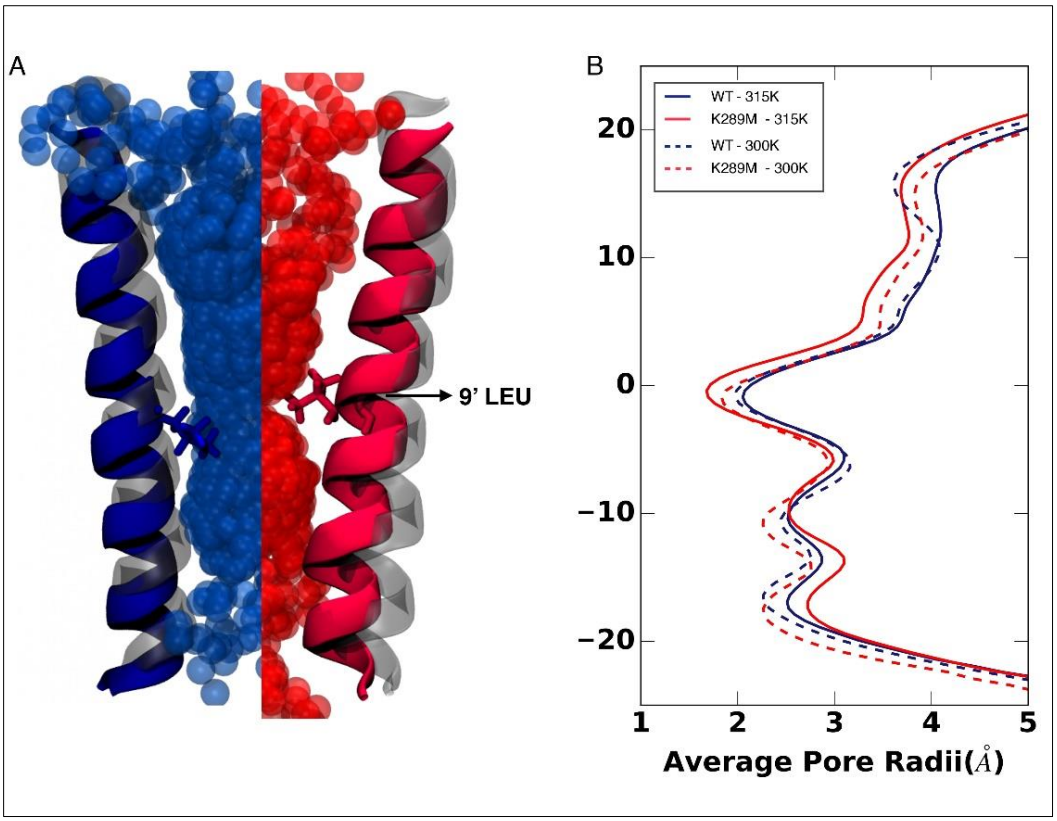

Figure 3 Smoothed time evolution of the pore minimum constriction, averaged (solid lines) over two replicas (dotted lines) each, at 300 K(A) and 315 K(B). The minimum constriction, formed around the 9' region, is visibly more constricted for the , and this reduction is more pronounced at higher temperatures. The minimum constriction region in falls below the chloride ion radius of 1.8Å, thus driving it to a closed state. The probability distribution further shows a clear shift in the peak of the towards reduced pore radii at a higher temperature

4.2. Drying of the pore

To further understand the direct implication of the closing of the channel, we measured the average number of water molecules in the pore channel. Many theoretical studies on water have shown that interfacial drying can be caused by hydrophobic enclosures in the protein. Furthermore, studies have also shown that drying of the pore region could lead to blocking of the channel since water is assumed to facilitate the conduction of ions. As mentioned earlier, the pore-facing residues in $GABA_AR$ are dominated by non-polar residues, and this causes intermittent drying of the channel when the minimum constriction region comes closer to form hydrophobic enclosures. The plot (Figure 3A) shows the density of the water molecules throughout the simulation along the Z-axis. Figure 3B further substantiates the plot by depicting the absence of water in the minimum constriction region of the pore at higher temperatures in the. Thus, such dehydration of the channel could be a mechanism for inhibiting the conduction of the channel.

(A)Number of water particles along the Z-hub found the middle value of over the casings and copies. Presence of water in the narrowing district of the - M2 helices (B) when contrasted with the transitory dryness because of a decrease in pore radii in the - M2 helices(C) at higher temperatures. On a normal, almost zero no. of water particles are found at the 9' district in the framework, portraying the depriving of water particles because of the nook of the hydrophobic residues.

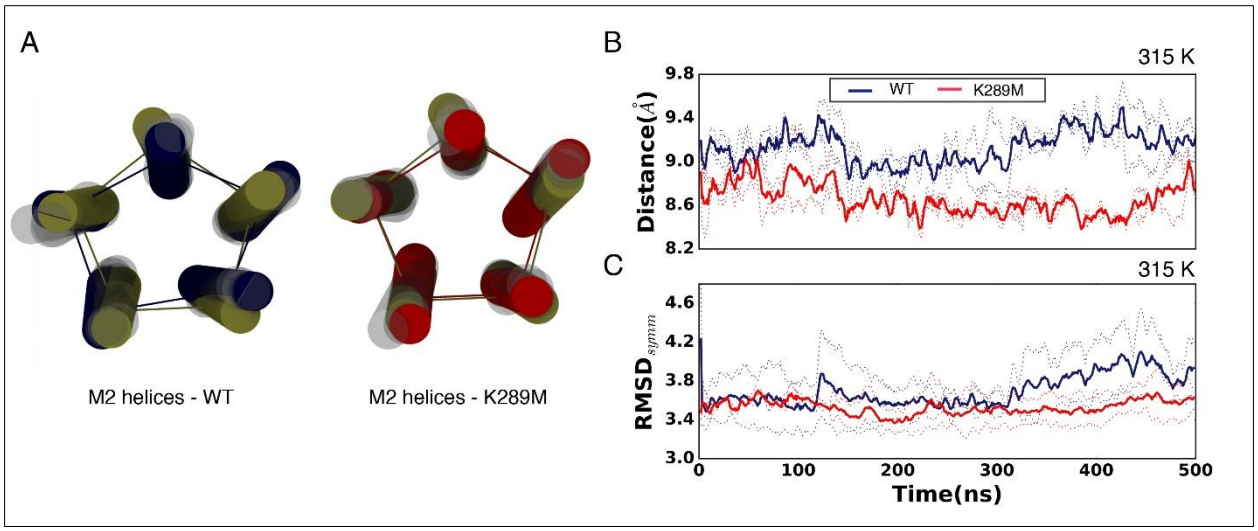

Figure 4 (A) Number of water particles along the Z-hub found the middle value of over the casings and copies. Presence of water in the narrowing district of the - M2 helices (B) when contrasted with the transitory dryness because of a decrease in pore radii in the - M2 helices(C) at higher temperatures. On a normal, almost zero no. of water particles are found at the 9' district in the framework, portraying the depriving of water particles because of the nook of the hydrophobic residues

5. Effects of Mutation on Conduction

5.1. Electrostatic Barriers in the Channel

The impacts of the change on simply electrostatic hindrances for chloride particle movement was evaluated through the Poisson-Boltzmann condition as depicted in Techniques. The transformation from a decidedly charged to unbiased buildup prompted minute changes in the electrostatic profile given indistinguishable beginning designs (as displayed in Valuable Figure S2(A) and Figure S2(B)), recommending that the transformation alone couldn't influence conductance with practically no conformational changes.

The calculation performed on equilibrated structures of and receptors showed a 5-10 kcal/mol (Figure S2(C)) higher electrostatic barrier in, predominantly occurring in the transmembrane domain enclosing the residues containing the minimum pore constriction region. The LEU-gate constriction, in addition to the loss of long-range electrostatic interactions from K289, seems to contribute to the formation of a higher barrier in the. We note that these calculations include electrostatic contributions, but not van der Waals or entropic contributions; these terms are included in the measurement of the potential of mean force via Adaptive Biasing Force calculations as described subsequently.

Potential of mean force profile of chloride ion transport (A) Aligned below the horizontally laid protein figure is the plot showing the potential mean force experienced by the ion as it moves through the channel along the Z-axis. (B) These barriers in the channel are further compared with the average pore radius of the channel's TM region. These comparisons clearly explain that the highest barriers are found in the 9' regions, which form the minimum constriction region. The difference between the barriers at this region is approximately 5 Kcal/mol.

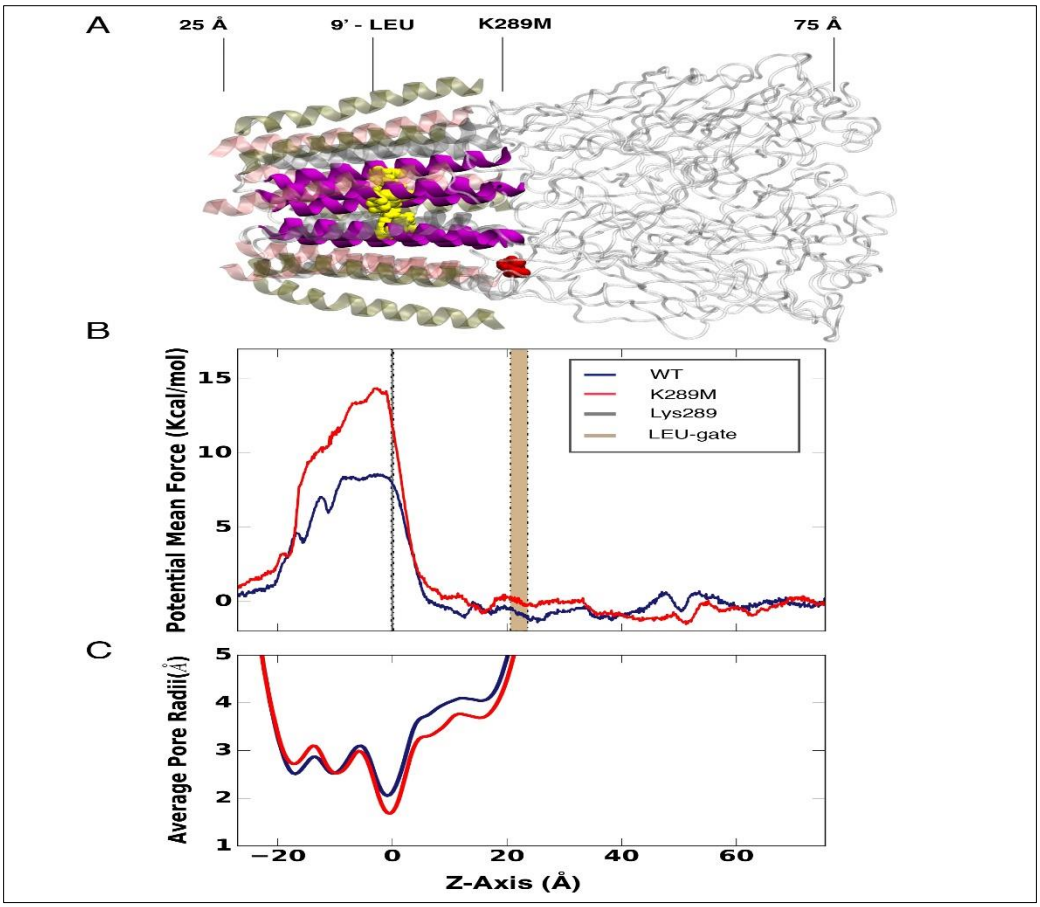

Figure 5 Potential of mean force profile of chloride ion transport (A) Aligned below the horizontally laid protein figure is the plot showing the potential mean force experienced by the ion as it moves through the channel along the Z-axis. (B) These barriers in the channel are further compared with the average pore radius of the channel's TM region. These comparisons clearly explain that the highest barriers are found in the 9' regions, which form the minimum constriction region. The difference between the barriers at this region is approximately 5 Kcal/mol

5.2. Potential of Mean Force

The PMF for chloride particle movement at 315K, estimated utilizing ABF, is displayed in Figure 4. The biggest obstruction happens more proximal to the leucine deposits shaping the most secure narrowing; this hindrance is expanded by 5 kcal/mol for the freak receptors. A slight, broad well (relative to a reference position outside the receptor) is apparent around residue 289 in the PMF for the receptor, while at the same location in the receptor, the PMF is slightly elevated relative to the reference location. However, these differences are slight compared to the effects of the mutation on the primary barrier, indicating that while a change of a decidedly charged to unbiased buildup small affects the partiality of the chloride particle for the locale of the receptor close to the transformation, the prevailing impact of the change on conduction is by means of conformational unsteadiness of the open state.

6. Conclusion

In this work, we investigated the effects of a fever-associated charged-to-hydrophobic mutation in a human ligand-gated ion channel, allowing us to identify the significance of collective, long-range, electrostatic interactions for maintaining the protein's function at higher temperatures. The temperature-dependent structural effect of reducing these electrostatic interactions via substitution of K to M at γ2: M2 24′ can be well-predicted simply by considering Coulombic repulsions between charged residues at M2 24′ in all subunits, as well as a simple variational theory which introduces temperature effects. The phenomenon of unstable activation in γ2 K289M $GABA_AR$, previously observed *in vivo* and *in vitro*, has now been observed *in silico* and *in principio*.

A basic residue at 24′ in the M2-M3 loop is highly conserved across $GABA_AR$ subunits but not across all pLGICs. It is not necessary, however, that charged residues be positioned at 24′ for cross-pore repulsions to stabilize open

conformations, but simply that they be in the same position in each subunit. Crucial collective interactions might therefore be well indicated by the presence of a charged residue that appears at the same position in all pore-sharing species (i.e. all $GABA_AR$ subunits or all GlyR subunits), but which is non-conserved across pLGICs in general.

To maintain the necessary range for cross-pore interactions, it is critical that the charged residues be unscreened. The presence of a nearby oppositely charged residue in one subunit will reduce the charge-charge interaction $(1/r)$ to a charge-dipole interaction $(1/r^2)$, with the presence of an additionally charged residue on the other side yielding a dipole-dipole interaction $(1/r^3)$. Screening may be affected by changes in pH as well as participation in salt bridges, suggesting a mechanism that may be crucial for gating in numerous other pLGICs.

Based on these results, we suggest that the binding of GABA may activate the channel by reducing the screening of residues in the M2 24′ ring. In particular, the results of Harrison and co-workers implicate a critical role for interactions between αD57/D14 and αK279 (M2 24′). While it has often been hypothesized that these residues gate by *forming* a salt-bridge, we speculate here that these residues may gate by *breaking* their salt-bridge, removing screening of charged residues in the +5 ring, increasing cross-pore repulsions, and opening the pore.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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