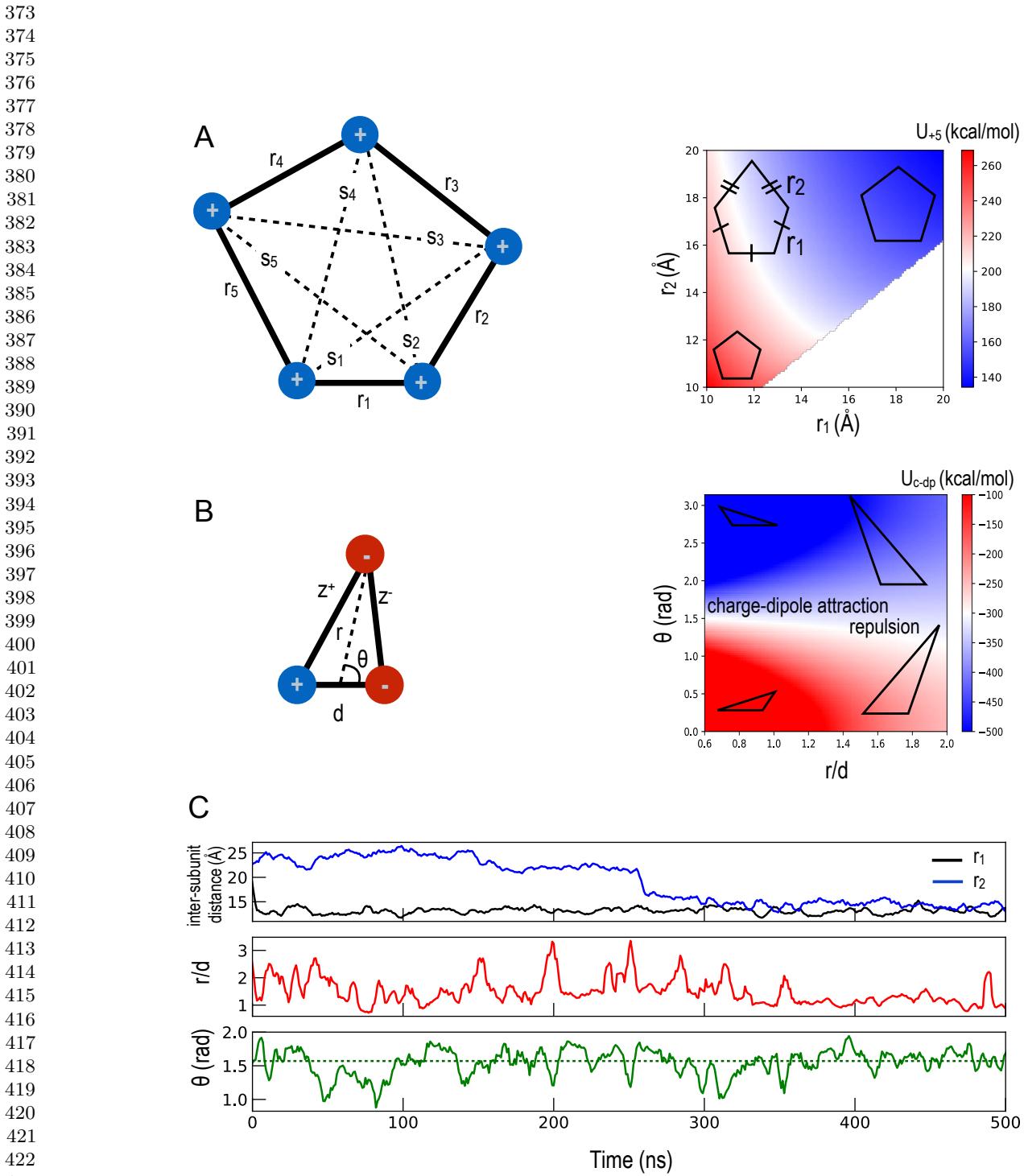


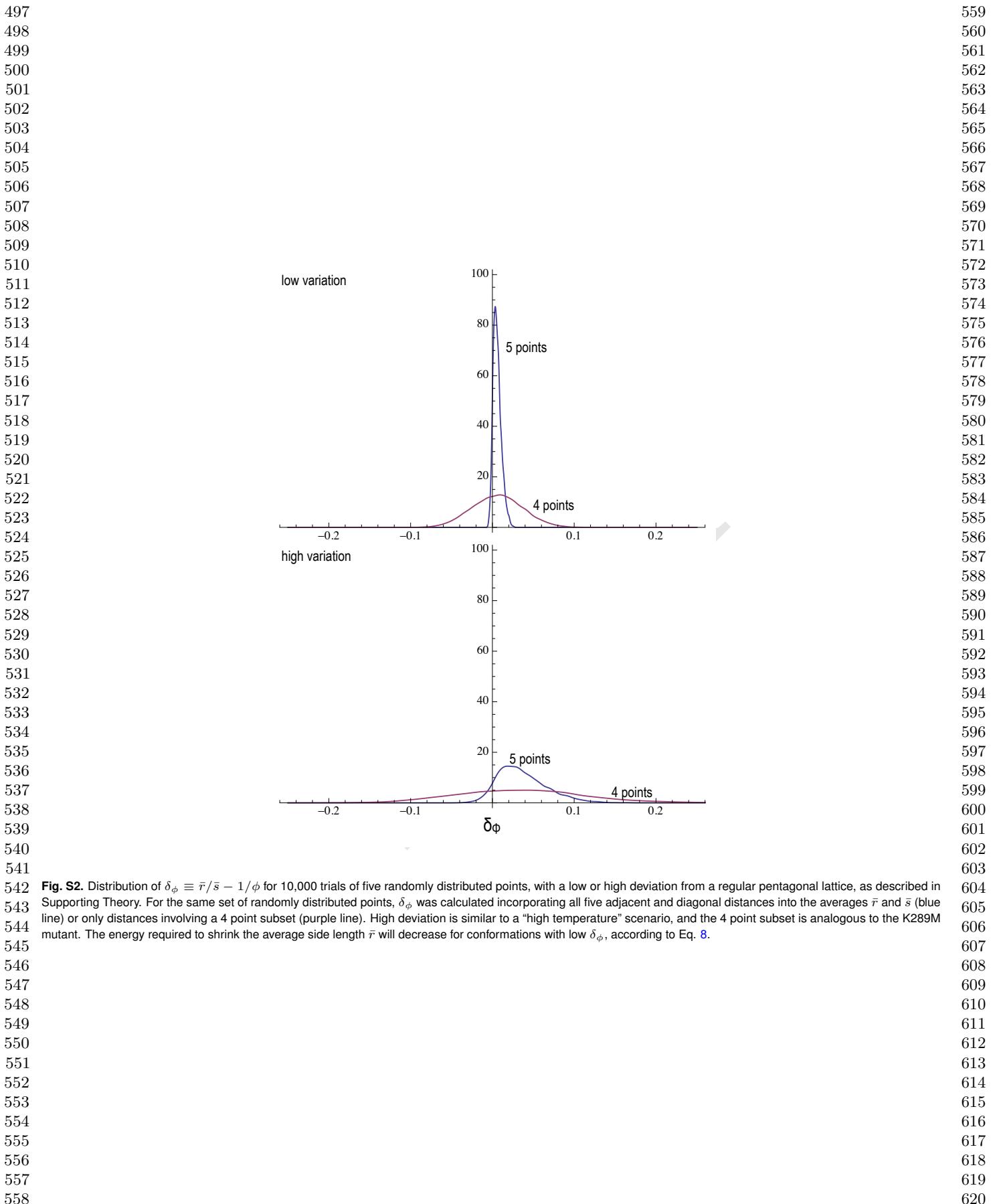
1	<b>Supporting Methods</b>	63
2	The systems were solvated using the SOLVATE plugin in VMD(1) and neutralizing ions were added to bring the system to a	64
3	0.15M salt concentration using the AUTOIONIZE plugin. The final system contained about 160,000 atoms. MUTATE plugin	65
4	was used to introduce the K289M mutation in the $\gamma$ subunit of GABA <sub>A</sub> R receptor.	66
5	All bonds to the hydrogen atoms were constrained using the SHAKE/RATTLE algorithm. A multiple time-step rRESPA	67
6	method was used, and controlled with a high frequency time-step of 2fs and low frequency time-step of 4fs. All the systems	68
7	were energy minimized for 10000 steps, then simulated for 5 ns with restraints of 1 kcal/mol/Å applied to the C <sub>α</sub> atoms of the	69
8	protein. Restraints were then removed and 495 ns of nearly unrestrained simulation was carried out in all four systems at	70
9	low temperature. During this period of the simulation, only harmonic restraints (force constant 0.4 kcal/mol/Å) between the	71
10	intracellular ends of the M3 and M4 helices were used, to mimic the effects of the intracellular domain and prevent separation	72
11	of the M4 helix from the rest of the bundle.	73
12		74
13	<b>Long-range electrostatics.</b> The simulations here used the prescribed cutoff value of 12 Å for the CHARMM forcefield, with a	75
14	switching function past 10Å, combined with PME and a grid size of about 1Å. The distances between charged residues in	76
15	the interfacial band are similar to this cutoff distance, and it is not uncommon to use cutoffs less than 10Å(as in (2)). This	77
16	may cause a significant accumulated error in simulations of any proteins with repeated interactions near the cutoff/switching	78
17	distance, not just pLGICs. In pLGICs, it can reduce the energetic cost of shrinking the interfacial band, leading to an increased	79
18	likelihood of closed states even in WT systems. By recalculating energies using direct Coulomb electrostatics just for interfacial	80
19	band and pore oscillator residues, from a trajectory generated using PME, we found PME reduced the energetic difference	81
20	between elongated and regular conformations by about 5 kcal/mol.	82
21		83
22	<b>SMD Simulations.</b> Steered Molecular Dynamics (SMD) simulations (3, 4) were used to obtain favorable positions of the ion at	84
23	different positions along the channel, for later use in Adaptive Biasing Force (ABF) calculations. The chloride ion was pulled	85
24	along the pore of the channel at a constant velocity of 10Å/ns. The force required to pull at constant velocity is also calculated,	86
25	and can, in principle, be used to calculate a potential of mean force (PMF) using Jarzynski's equation (5, 6), but in practice it	87
26	is challenging to achieve a sufficiently slow pulling speed.	88
27		89
28	<b>ABF Simulations.</b> Adaptive biasing force calculations (ABF)(7–10) were used to measure the potential of mean force (PMF) of	90
29	a chloride ion translocating the GABA <sub>A</sub> R ion channel at 315K, for both the WT and K289M channels. ABF was performed	91
30	using the Collective Variables module(11) of NAMD2.9. The pore axis was divided into 23 bins of each 5Å length.	92
31	Initial coordinates for the ion were obtained from SMD simulations (as described in SI). One thousand samples were collected	93
32	in each bin prior to the application of ABF to avoid undesired non-equilibrium effects on the dynamics. Fifteen ns of trajectory	94
33	were generated in most bins, while bins near the primary barrier in the pore contained 25 ns.	95
34		96
35	<b>Pore Analysis.</b> Measurement and analysis of the pore radii has been carried out using the HOLE software (12) and TCL	97
36	scripting through VMD(1). Python scripts have been used to analyze and visualize the hydration of the pore throughout the	98
37	simulation.	99
38		100
39	<b>Poisson-Boltzmann Calculations.</b> The Poisson-Boltzmann (PB) profile for conduction of both a Na <sup>+</sup> and Cl <sup>-</sup> through the ion	101
40	channel was calculated using APBSmem(13). The pre-generated PQR format of the proteins using PDB2PQR(14) tool was	102
41	used as the input for the electrostatic potential calculations.	103
42	These calculations were performed for initial non-equilibrated structures of the protein, as well as for conformations extracted	104
43	from the last 50 ns of both the 300K and 315K MD simulations (for Cl <sup>-</sup> ).	105
44		106
45	<b>Graphs and images.</b> All plots were calculated and drawn using Python and Tcl scripts. In Figure 2 and the similar supplementary	107
46	figures S5-S11, the series of curves depicting the pore-opening events were further smoothed using a digital filter(Butterworth)	108
47	with a order of the filter value, 2, and a critical frequency value, 0.02, as implemented in the SciPy python module. The time	109
48	derivative of the minimum pore radius was calculated using the gradient function implemented in the numpy python module.	110
49	VMD(1) was used for visualization and for creating molecular images and movies.	111
50		112
51	<b>Supporting Theory</b>	113
52	We consider an irregular pentagon with five side lengths $r_i$ and five diagonal lengths $s_i$ (Figure S1A). The total Coulomb	114
53	energy for the charged ring is given by	115
54		116
55	$U_{+5} = k_e e^2 \sum_i^5 \frac{1}{r_i} + \sum_i^5 \frac{1}{s_i}$	[1]
56		117
57	where $e$ is the electron charge, $k_e = 332\text{Å}/\text{kcal/mol}/e^2$ is the Coulomb constant. Writing each distance as a perturbation from	118
58	the average : $r_i = \bar{r}(1 + \delta r_i)$ and $s_i = \bar{s}(1 + \delta s_i)$ , where the average adjacent length $\bar{r} = \sum_i^5 r_i/5$ and the average diagonal	119
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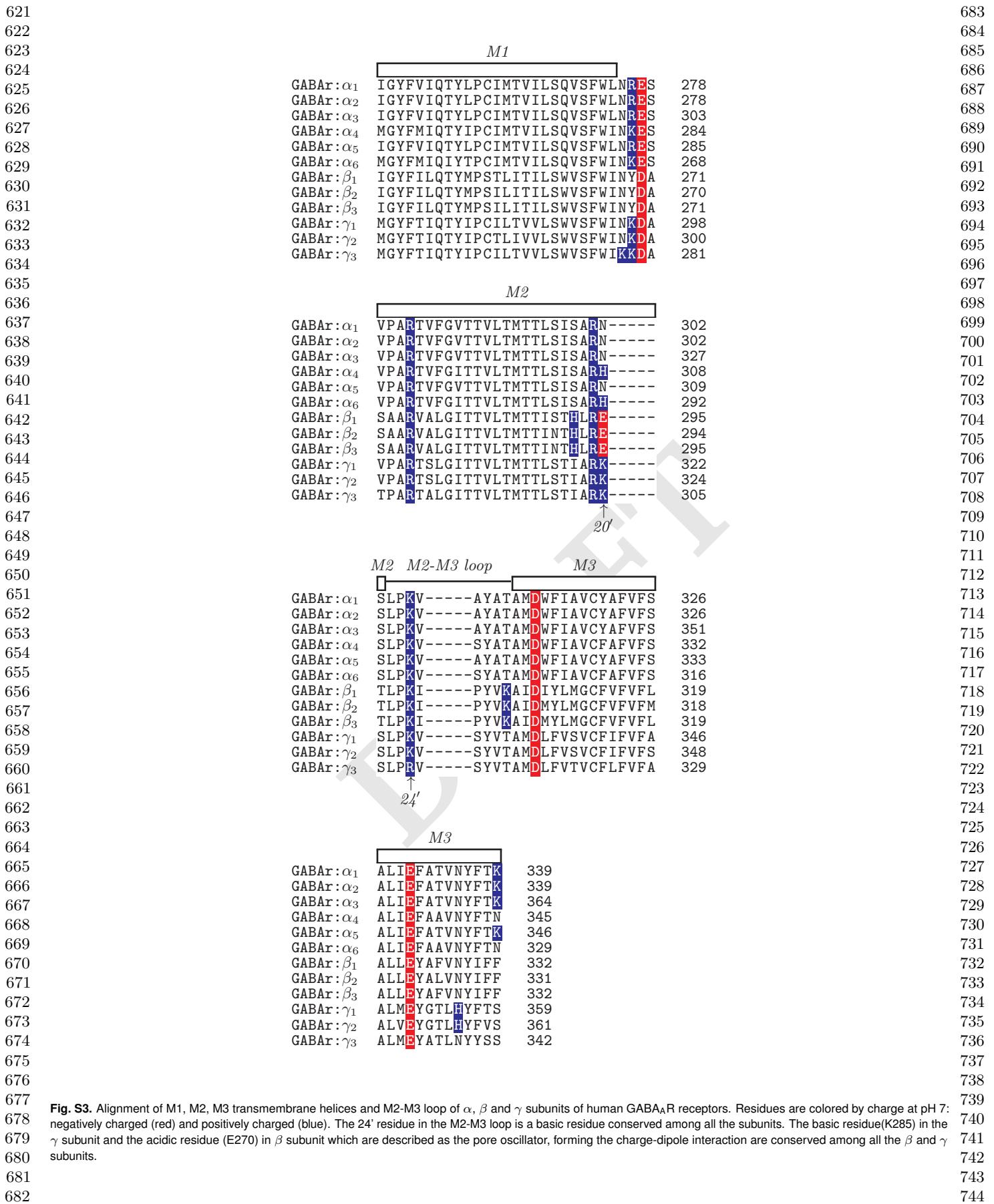
125 length  $\bar{s} = \sum_i^5 s_i/5$ . Expanding in powers of  $\delta s_i$  and  $\delta r_i$ , 187  
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$$\sum_j^5 \frac{1}{r_j} = \sum_j^5 \frac{1}{\bar{r}(1 + \delta r_i)} = \frac{1}{\bar{r}} \sum_j^5 (1 - \delta r_i + O(\delta r_j^2))$$
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 128 [2] 189  
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 130  $= \frac{5}{\bar{r}} (1 + O(\bar{\delta r}^2))$  190  
 131 [3] 191  
 132 and similarly, 192  
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$$\sum_j^5 \frac{1}{s_j} = \frac{5}{\bar{s}} (1 + O(\bar{\delta s}^2))$$
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 135 [4] 194  
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 137 where we have used  $\sum_j^5 \delta s_j = \sum_j^5 \delta r_j = 0$ , and  $\bar{\delta r}^2$  and  $\bar{\delta s}^2$  are the variance in  $r$  and  $s$  across the five sides of the pentagon 195  
 138 respectively. Therefore, 196  
 139  
 140 
$$U_{+5} = k_e e^2 \left( \sum_i^5 \frac{1}{r_i} \right) \left( 1 + \frac{\sum_j^5 \frac{1}{s_j}}{\sum_j^5 \frac{1}{r_j}} \right)$$
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 141 [5] 198  
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 143  $= k_e e^2 \left( \frac{5}{\bar{r}} (1 + O(\bar{\delta r}^2)) \right) \left( 1 + \frac{\frac{5}{\bar{s}} (1 + O(\bar{\delta s}^2))}{\frac{5}{\bar{r}} (1 + O(\bar{\delta r}^2))} \right)$  199  
 144 [6] 200  
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 146  $= \frac{5k_e e^2}{\bar{r}} \left( 1 + \frac{\bar{r}}{\bar{s}} \right) + O(\bar{\delta r}^2) + O(\bar{\delta s}^2)$  201  
 147 [7] 202  
 148  
 149 For a symmetric pentagon  $\bar{s} = \phi \bar{r}$  where  $\phi \equiv (1 + \sqrt{5})/2 \sim 1.62$  is a geometric constant usually called the “golden ratio”, 203  
 150 representing the ratio between the lengths of a pentagon diagonal and side, and with the convenient property  $1/\phi = \phi - 1 = 0.62$ . 204  
 151 We define  $\delta_\phi$  as the deviation of  $\frac{\bar{r}}{\bar{s}}$  from  $1/\phi = \phi - 1 = 0.62$ , so  $\delta_\phi \equiv \frac{\bar{r}}{\bar{s}} - (\phi - 1)$ , and 205  
 152  
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$$U_{+5} = \frac{5k_e e^2 \phi}{\bar{r}} \left( 1 + \frac{\delta_\phi}{\phi} \right) + O(\bar{\delta r}^2) + O(\bar{\delta s}^2)$$
 206  
 154 [8] 207  
 155 The linear term in  $\delta_\phi$  reflects the effects of asymmetry on the relative contributions of diagonal and adjacent distances. 208  
 156 Second-order terms given by  $\bar{\delta r}^2$  and  $\bar{\delta s}^2$  reflect variance in the adjacent and diagonal distances respectively. According to 209  
 157 Eq. 8, positive values of  $\delta_\phi$  (in which diagonal distances are shorter than expected in a regular pentagon) will increase the 210  
 158 overall energy of the interfacial band, provided the average distance between adjacent residues ( $\bar{r}$ ) is kept constant. This 211  
 159 asymmetry-induced increase in energy can be offset by an overall increase in the size of the interfacial band :  $\delta_\phi > 0$  will 212  
 160 stabilize a larger  $\bar{r}$ . Similarly, negative  $\delta_\phi$  will decrease the overall energy of the interfacial band and allow it to decrease the 213  
 161 average separation  $\bar{r}$  with reduced penalty. 214  
 162 Any reduction in  $\delta_\phi$  will thus destabilize the open state. We ran simple numerical calculations to determine how increased 215  
 163 random noise would affect the distribution of  $\delta_\phi$ . Five points were generated with random polar angles corresponding to 216  
 164  $\{0 + \zeta_1, 2\pi/5 + \zeta_2, 4\pi/5 + \zeta_3, 6\pi/5 + \zeta_4, 8\pi/5 + \zeta_5\}$  where  $\zeta_i$  was a random value taken from a flat distribution in the range  $\pm 3\pi/40$  217  
 165 (low variation) or in the range  $\pm 5\pi/40$  (high variation), and the radial values were  $\{1.5 + \rho_1, 1.5 + \rho_2, 1.5 + \rho_3, 1.5 + \rho_4, 1.5 + \rho_5\}$  218  
 166 where  $\rho_i$  was a random value from a flat distribution in the range  $\pm 0.375$  (low variation) or  $\pm 0.625$  (high variation). Although 219  
 167 these are the specific values used in Figure S2 and are given for completeness, the overall trends were not particularly sensitive 220  
 168 to the precise values chosen. 221  
 169  
 170 **S1 Movie:** 222  
 171  
 172 Pore opening event in WT GABA(A) receptor at 315K. View from the extracellular domain showing only the M2 and M3 223  
 173 helices and M2-M3 loop. Charged groups in basic sidechains at M2 24' are shown in blue; orange lines are for visualization 224  
 174 of the interfacial band but do not represent covalent bonds. Charged groups in basic and acidic sidechains at M2 20' are 225  
 175 shown in cyan and red, respectively; white lines are for visualization of the shape of the ‘pore-oscillator’ and do not represent 226  
 176 covalent bonds. Leucine residues at M2 9' forming the hydrophobic gate are shown in gray space-filling representation. A fully 227  
 177 annotated version is in S2 Movie. 228  
 178  
 179 **S2 Movie:** 229  
 180 Fully annotated version of S1 Movie. Pore opening event in WT GABA(A) receptor at 315K. View from the extracellular 230  
 181 domain showing only the M2 and M3 helices and M2-M3 loop. Charged groups in basic sidechains at M2 24' are shown in 231  
 182 blue; orange lines are for visualization of the interfacial band but do not represent covalent bonds. Charged groups in basic 232  
 183 and acidic sidechains at M2 20' are shown in cyan and red, respectively; white lines are for visualization of the shape of the 233  
 184 ‘pore-oscillator’ and do not represent covalent bonds. Leucine residues at M2 9' forming the hydrophobic gate are shown in 234  
 185 gray space-filling representation. 235  
 186

249	<b>S3 Movie:</b>	311
250	Dynamics of the interfacial band, pore oscillator, and hydrophobic gate in K289M GABA(A) receptors at 315K. View from the	312
251	extracellular domain showing only the M2 and M3 helices and M2-M3 loop. Charged groups in basic sidechains at M2 24' are	313
252	shown in blue; orange lines are for visualization of the interfacial band but do not represent covalent bonds. Charged groups in	314
253	basic and acidic sidechains at M2 20' are shown in cyan and red, respectively; white lines are for visualization of the shape of	315
254	the 'pore-oscillator' and do not represent covalent bonds. Leucine residues at M2 9' forming the hydrophobic gate are shown in	316
255	gray space-filling representation.	317
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**Fig. S1.** (A) Adjacent and diagonal distances for the pentagonal Interfacial Band used to calculate averages for Eq. 8, and the associated electrostatic energy for the special case of a pentagon in which three sides are identical and two adjacent sides are also identical, but may differ from the other three. This special case is similar to that observed for the symmetrization step in Figure 2. (B) Definition of terms for the charge-dipole interaction that is formed by three residues in the pore oscillator , as well as associated energy. At around  $\theta = \pi/2$ , the potential energy shifts from decreasing with increasing distance (repulsive) to increasing with increasing distance (attractive). C) Trajectory for defined angles and distances for the K315 replica explored in Figure 2; curves shown here are smoothed much less than in Figure 2 and retain significantly more high frequency noise.

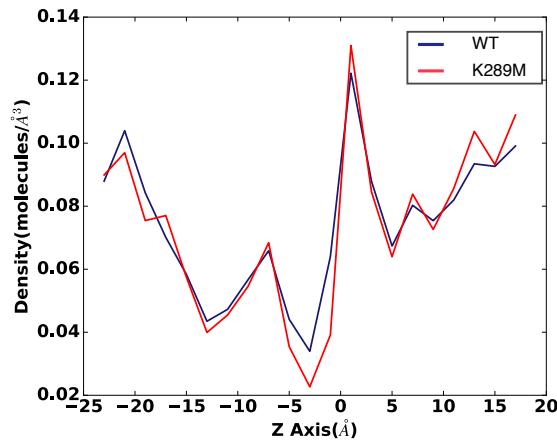




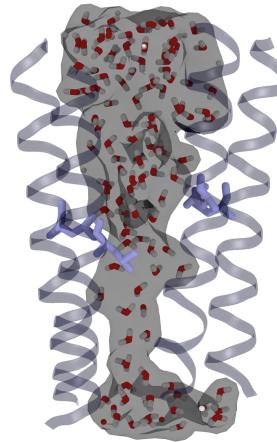
**Fig. S3.** Alignment of M1, M2, M3 transmembrane helices and M2-M3 loop of α, β and γ subunits of human GABA<sub>A</sub>R receptors. Residues are colored by charge at pH 7: negatively charged (red) and positively charged (blue). The 24' residue in the M2-M3 loop is a basic residue conserved among all the subunits. The basic residue(K285) in the γ subunit and the acidic residue (E270) in β subunit which are described as the pore oscillator, forming the charge-dipole interaction are conserved among all the β and γ subunits.

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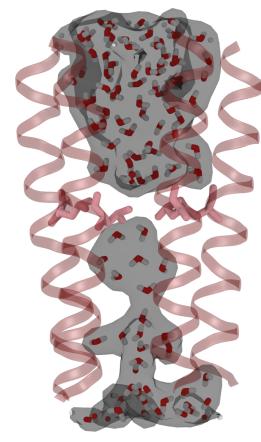
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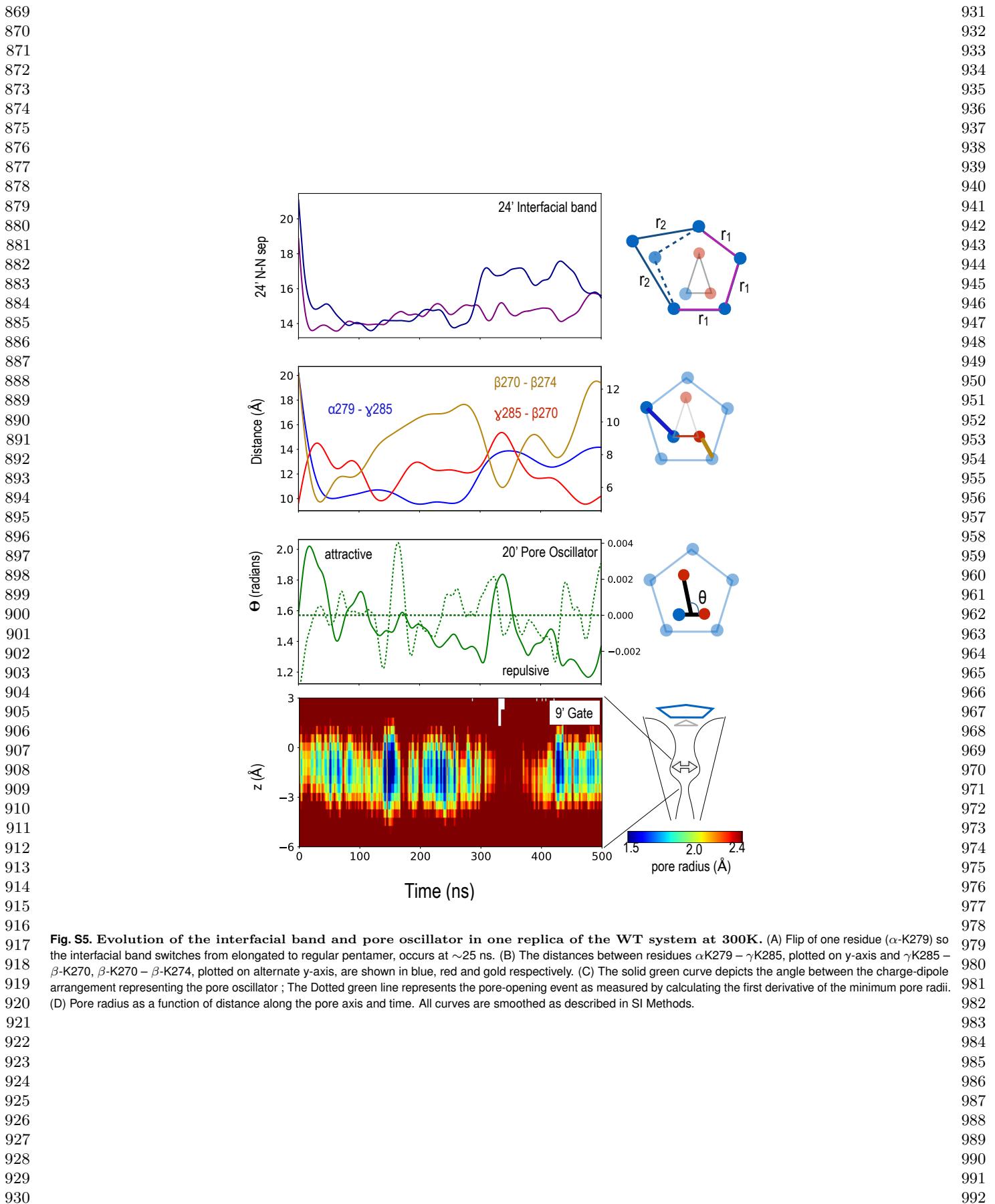


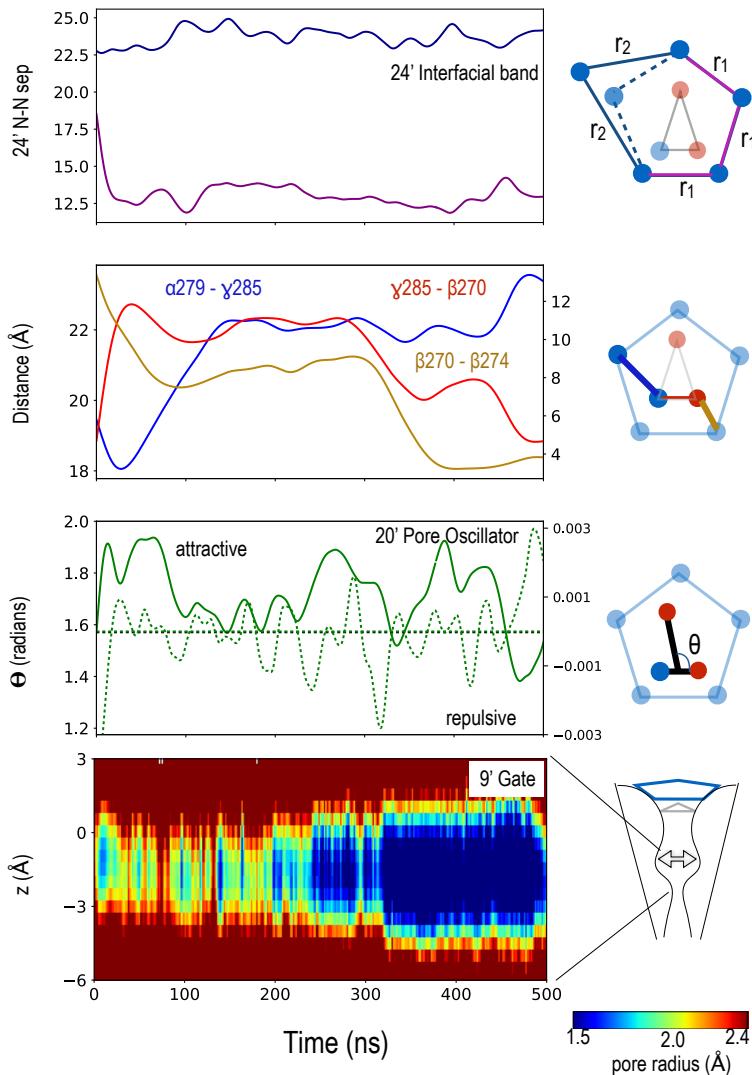
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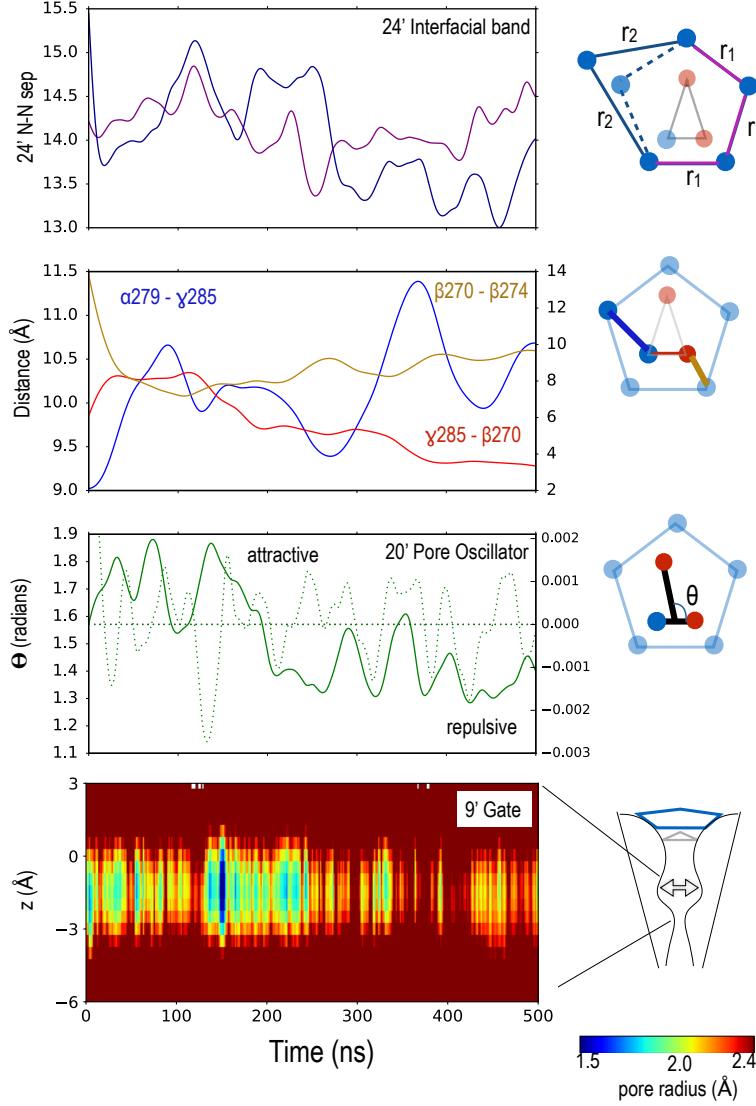
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785 **Fig. S4.** (A)Number of water molecules along the Z-axis averaged over the frames and replicas. Presence of water in the constriction region of the WT - M2 helices (B) as  
786 compared to the temporary dryness due to reduction in pore radii in the K289M - M2 helices(C), at higher temperature.  
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**Fig. S6.** Evolution of the interfacial band and pore oscillator in second replica of the WT system at 300K. (A) Flip of one residue ( $\alpha$ -K279) does not occur and the interfacial band remains in elongated pentamer form. (B) The distances between residues  $\alpha$ K279 –  $\gamma$ K285, plotted on y-axis and  $\gamma$ K285 –  $\beta$ -K270,  $\beta$ -K270 –  $\beta$ -K274, plotted on alternate y-axis, are shown in blue, red and gold respectively. (C) The solid green curve depicts the angle between the charge-dipole arrangement representing the pore oscillator ; The Dotted green line represents the pore-opening event as measured by calculating the first derivative of the minimum pore radii. (D) Pore radius as a function of distance along the pore axis and time. All curves are smoothed as described in SI Methods.

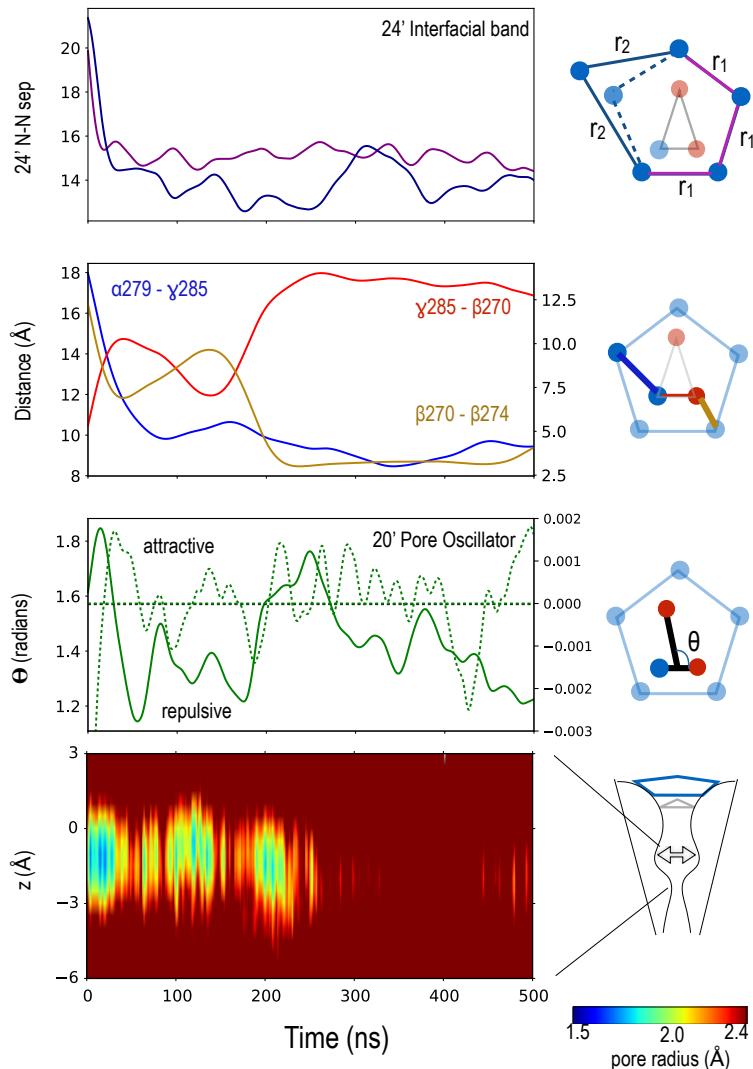


**Fig. S7. Evolution of the interfacial band and pore oscillator in one replica of the WT system at 315K.** (A) Residue ( $\alpha$ -K279) remains flipped from 300K simulations so the interfacial band remains in a regular pentamer form. (B) The distances between residues  $\alpha$ K279 –  $\gamma$ K285 –  $\beta$ -K270,  $\beta$ -K270 –  $\beta$ -K274, plotted on alternate y-axis, are shown in blue, red and gold respectively. (C) The solid green curve depicts the angle between the charge-dipole arrangement representing the pore oscillator ; The Dotted green line represents the pore-opening event as measured by calculating the first derivative of the minimum pore radii. (D) Pore radius as a function of distance along the pore axis and time. All curves are smoothed as described in SI Methods.

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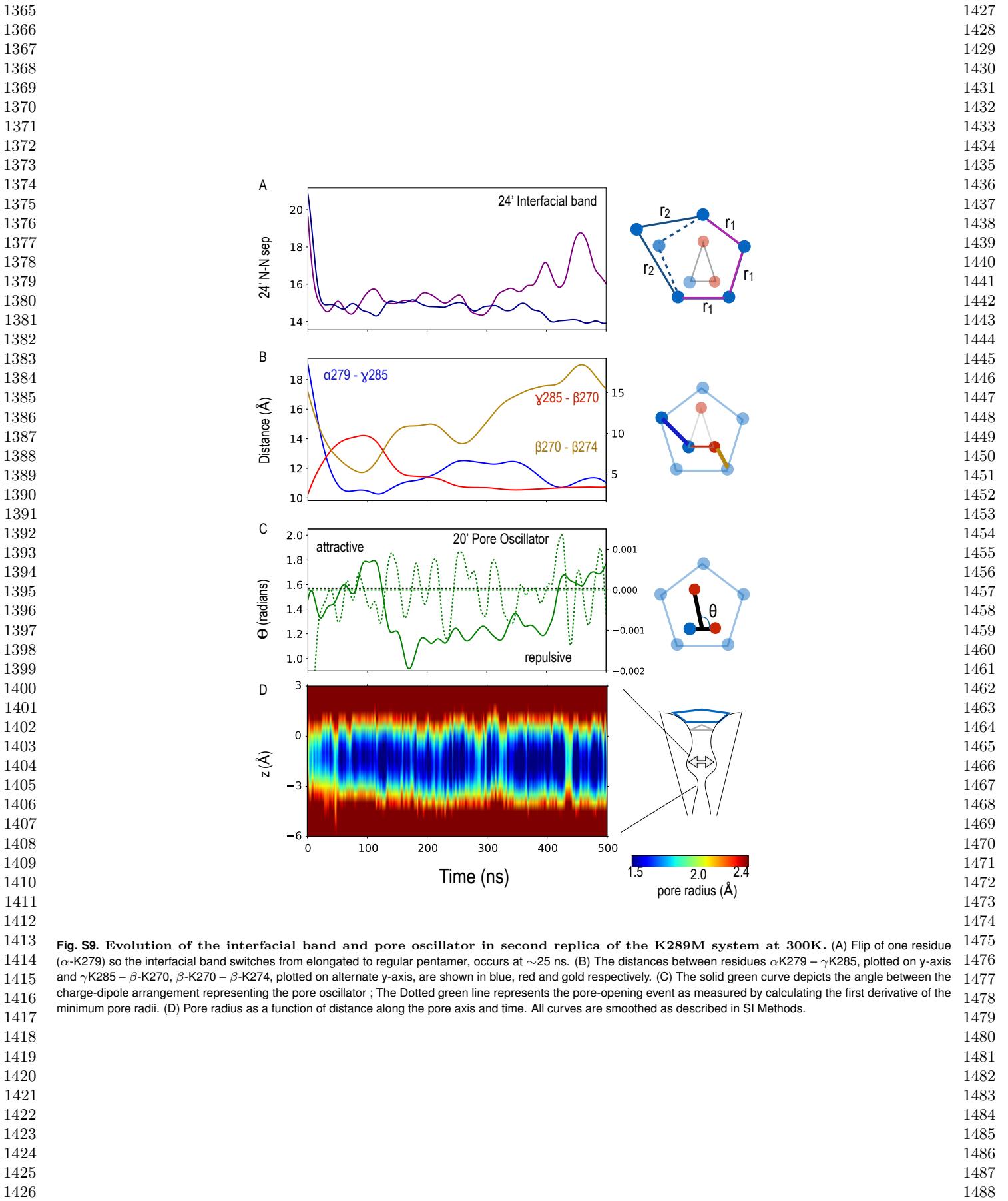
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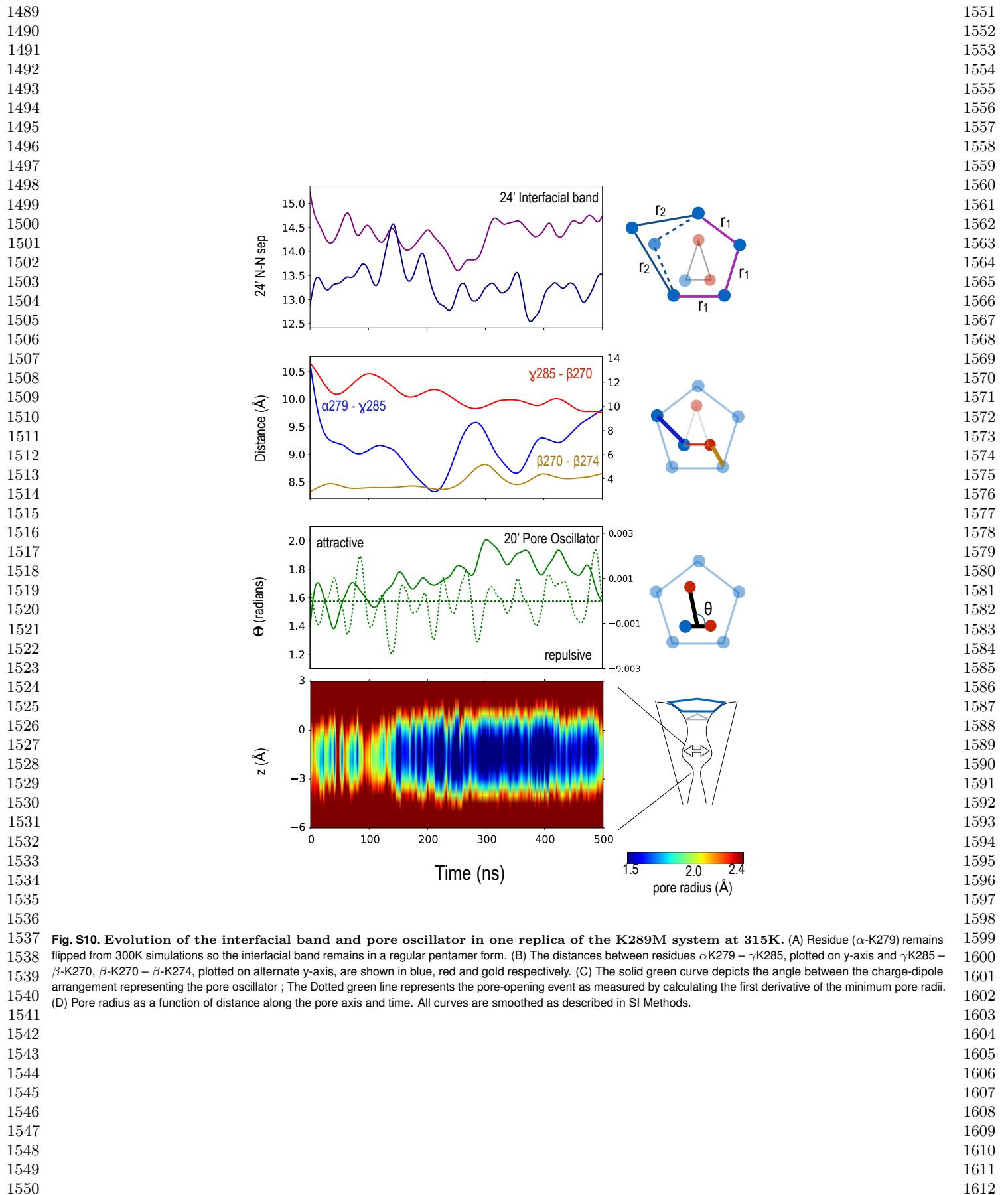
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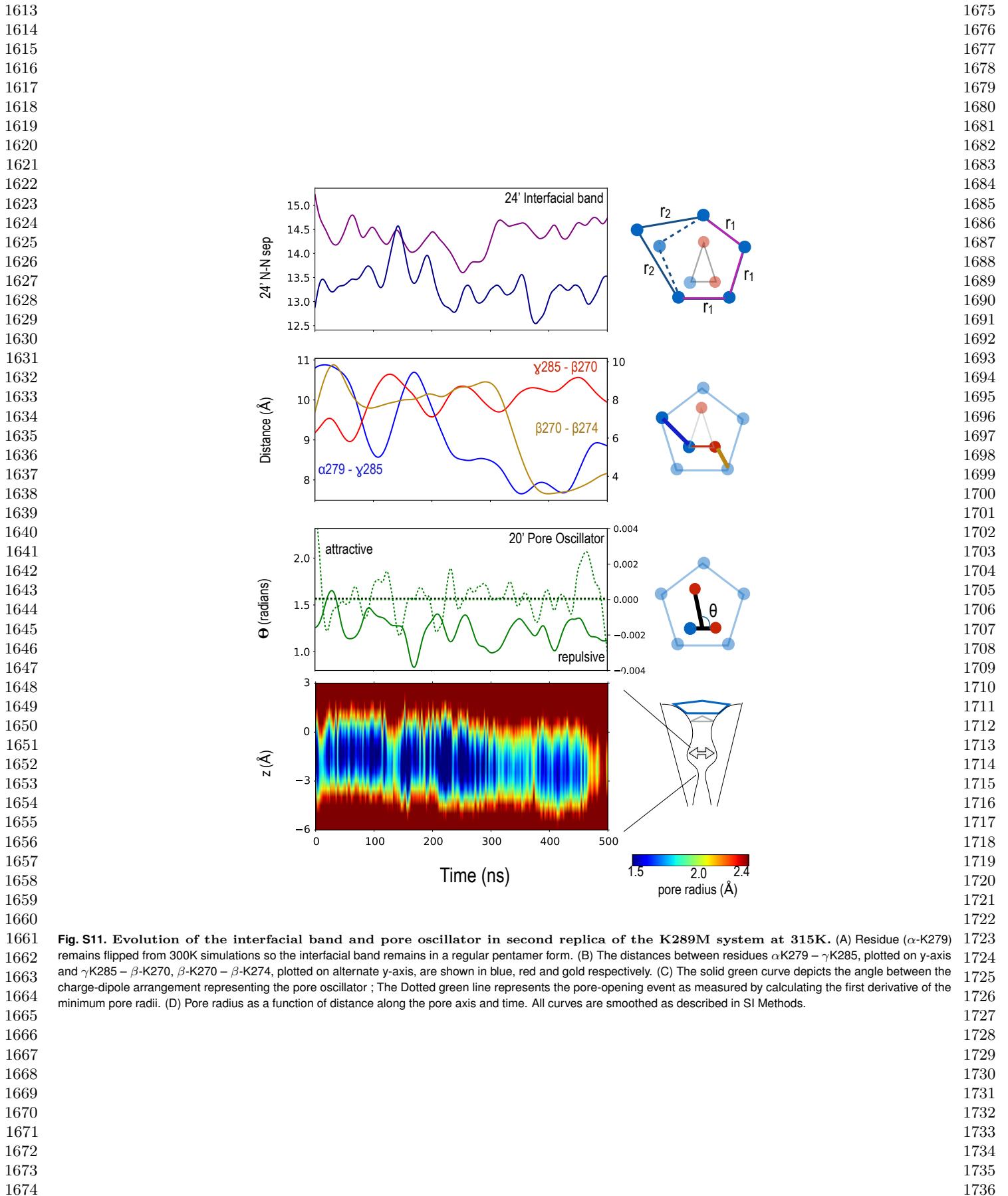


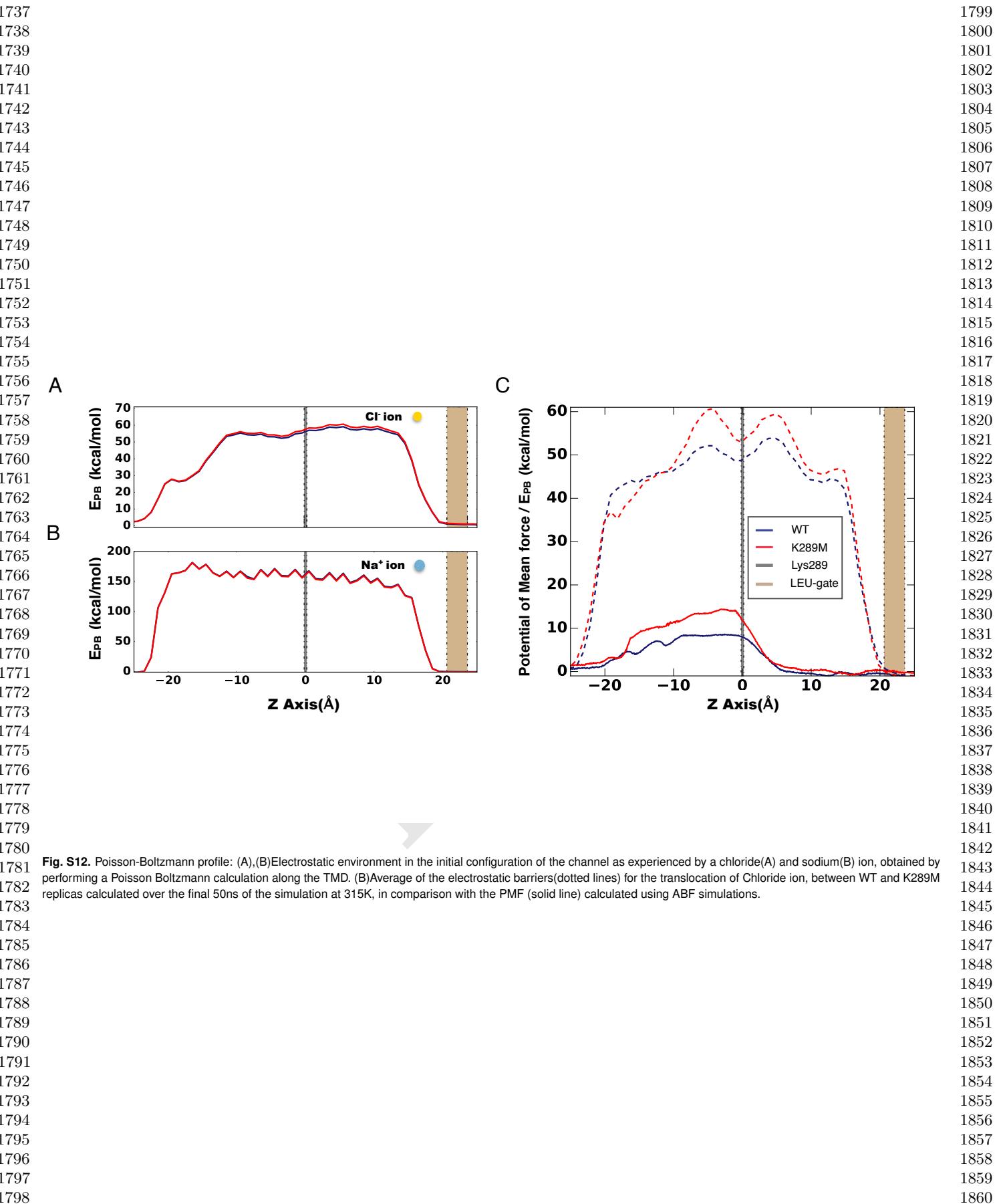
**Fig. S8.** Evolution of the interfacial band and pore oscillator in one replica of the K289M system at 300K. (A) Flip of one residue ( $\alpha$ -K279) so the interfacial band switches from elongated to regular pentamer, occurs at  $\sim 25$  ns. (B) The distances between residues  $\alpha$ -K279 –  $\gamma$ -K285,  $\beta$ -K270 –  $\beta$ -K274,  $\gamma$ -K285 –  $\beta$ -K270, plotted on alternate y-axis, are shown in blue, red and gold respectively. (C) The solid green curve depicts the angle between the charge-dipole arrangement representing the pore oscillator ; The Dotted green line represents the pore-opening event as measured by calculating the first derivative of the minimum pore radii. (D) Pore radius as a function of distance along the pore axis and time. All curves are smoothed as described in SI Methods.

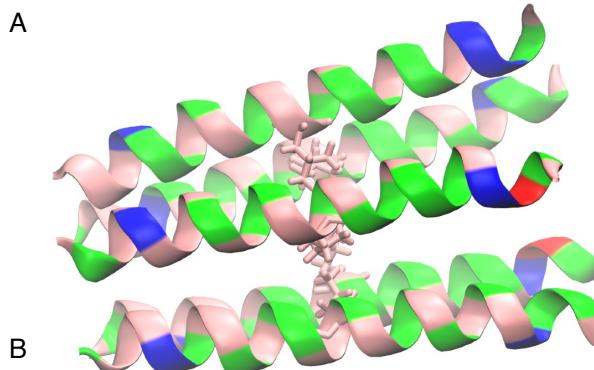
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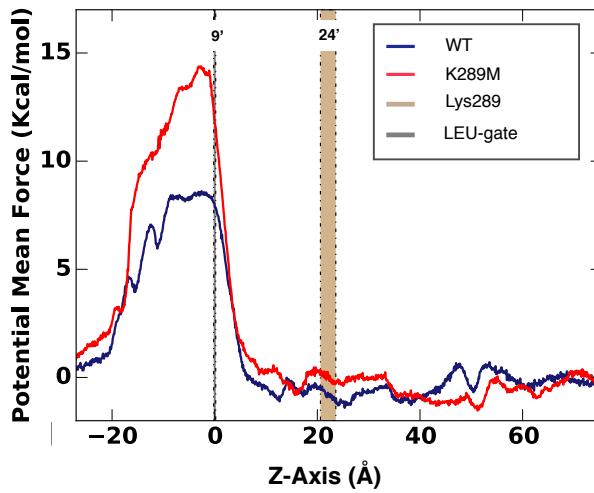




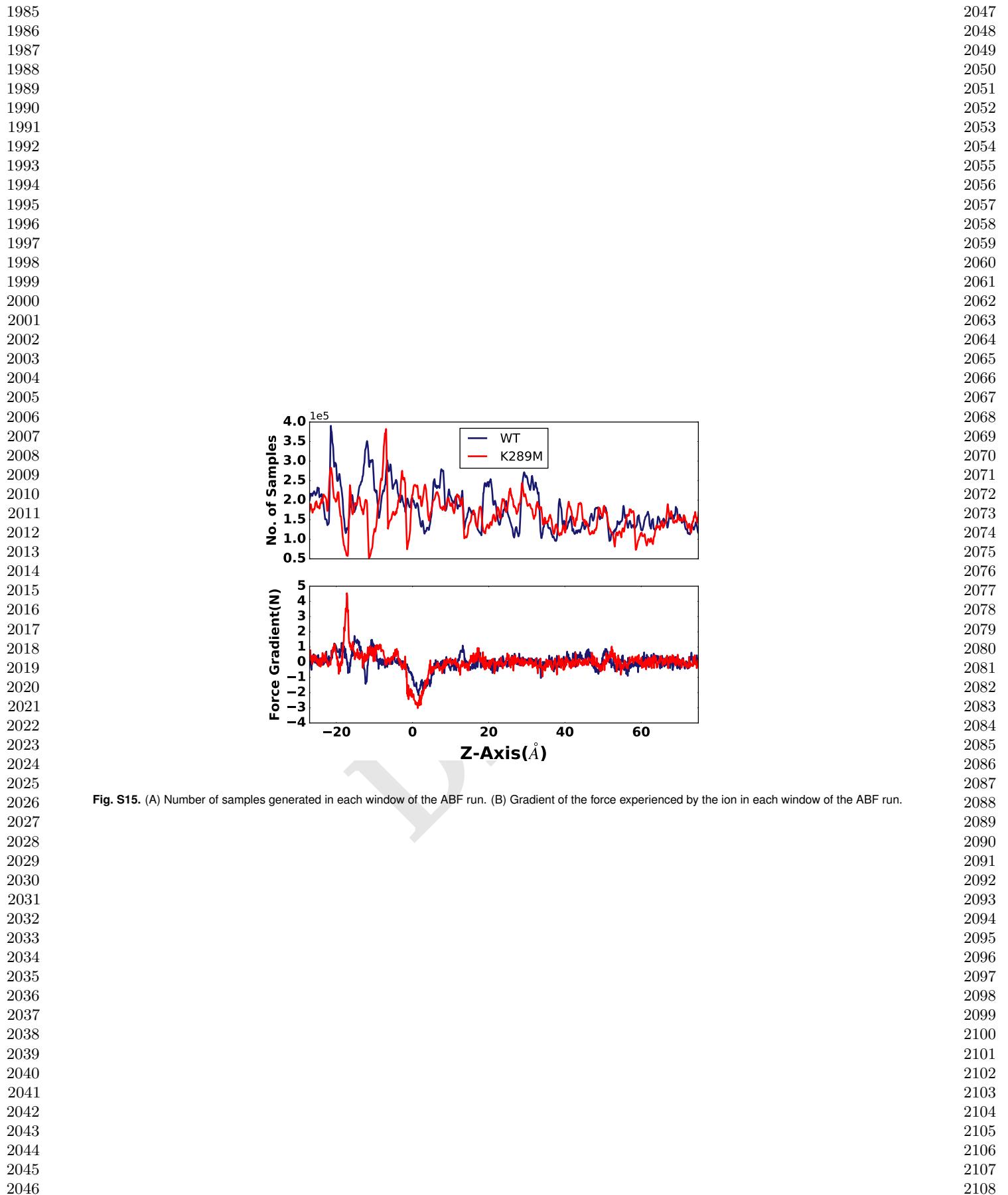




**Fig. S13.** (A) Snap-shot depicting the M2-helices (laid horizontally) showing the minimum constriction region flanked by LEU residues.(B) The force experienced by the ion as a function of position in the channel along the Z axis(TM domain), calculated by performing SMD on a Chloride passing along the pore of the channel.



**Fig. S14.** (A) Potential of mean force profile of a chloride ion crossing the ion channel, calculated at 315K.



**Fig. S15.** (A) Number of samples generated in each window of the ABF run. (B) Gradient of the force experienced by the ion in each window of the ABF run.

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