Supplementary Material

for

A Computational Study of the Closed and Open States of the Influenza A M2 Proton Channel

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This supplementary material contains 1 figure (Figure S1) and three tables (Tables S1-S3) as follows.

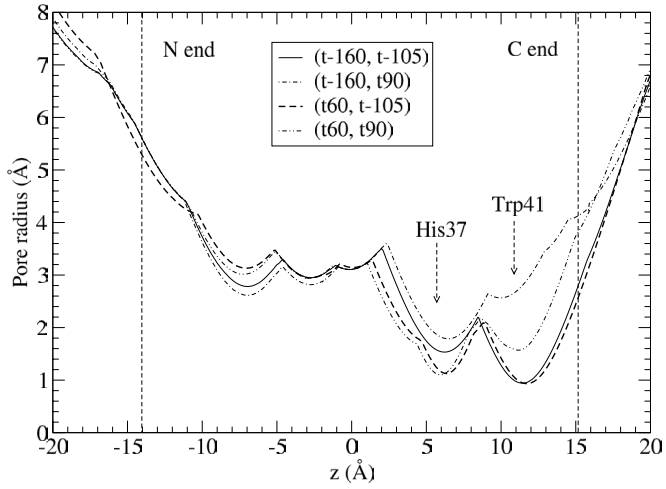

Figure S1. The pore radius profiles of the representative structures of the four different rotameric states of the M2-TMP channel, as calculated using the HOLE program (1).

Table S1. The different structures as used in Table S2.

Structure [*]	d_{SS} (Å) [†]	d_{GG} (Å) [‡]	d_{RR} (Å) [¶]	tilt (°)	ρ_0 (°)
1	17.71	7.26	14.77	38	-43
2	17.82	7.29	14.36	38	-43
3	17.76	7.15	14.30	38	-43
4	15.25	7.4	12.98	30 [§]	-33 [‖]

[*] The PDB files are provided for structure 1, 2, 3, and 4 as "1.pdb", "2.pdb", "3.pdb", and "4.pdb".

[†] The distance between the alpha-carbon atoms of the Ser23 residues from two neighboring helices.

[‡] The distance between the alpha-carbon atoms of the Gly34 residues from two neighboring helices.

[¶] The distance between the alpha-carbon atoms of the Arg45 residues from two neighboring helices.

[§] This value is within the range of the experimental values (30°~40°) (2-5)

[‖] This value is within the *uncertainty of the experimental value (43±10°)* (5).

main 1

Table S2. Estimated dipolar coupling for different rotameric states in slightly different overall structures, showing that a small uncertainty of the overall structure can have dramatic effects on the estimated $^{15}N_{\delta}$-$^{13}C_{\gamma}$ dipolar coupling such that the (t-160, t-105), (t-160, t90), and (t60, t90) states cannot be distinguished.

Structure *	Rotameric states †	Dihedral angles (°)				$d_{\delta-\gamma}$ (Å)	$^{15}N_{\delta}$-$^{13}C_{\gamma}$ dipolar (Hz) ¶	^{1}H-$^{15}N_{\delta}$ dipolar (kHz) §
		His37		Trp41				
		χ_1	χ_2	χ_1	χ_2			
1	(t-160, t-105)	170	170	150	276	3.718826	54.083694	-1.629635
		170	170	150	274	3.718827	54.083652	-1.629588
		170	170	150	272	3.718828	54.08363	-1.629534
		170	170	150	270	3.718828	54.083611	-1.629478
		170	170	150	268	3.718829	54.083569	-1.629423
	(t-160, t90)	168	150	150	100	3.429518	69.743729	-4.502992
		168	150	150	98	3.432061	69.590584	-4.488073
		168	150	152	96	3.463875	67.83094	-4.503251
		168	152	150	100	3.462075	67.80069	-4.303906
		168	152	150	98	3.462078	67.800591	-4.303829
	(t60, t-105)	150	70	164	282	3.406589	12.773658	4.294664
		150	70	162	284	3.377845	12.352449	4.294896
		150	70	162	286	3.377843	12.352428	4.294965
		150	68	170	272	3.528294	12.310157	4.25356
		150	68	166	278	3.468083	11.889872	4.254378
	(t60, t90)	150	110	172	100	3.186779	48.69199	0.450094
		154	110	178	100	3.218281	48.355915	-0.536775
		152	110	176	100	3.216668	48.092655	-0.04869
		150	110	174	100	3.219711	47.444675	0.449933
		150	108	172	100	3.187747	47.009987	0.790515

(Table S2 continued)

Structure *	Rotameric states †	Dihedral angles (°)				$d_{\delta-\gamma}$ (Å)	$^{15}N_{\delta}$-$^{13}C_{\gamma}$ dipolar (Hz) ‡	^{1}H-$^{15}N_{\delta}$ dipolar (kHz) ¶
		His37		Trp41				
		χ_1	χ_2	χ_1	χ_2			
2	(t-160, t-105)	168	190	152	288	3.541911	48.985104	-0.849826
		168	190	152	290	3.541911	48.985104	-0.849795
		168	190	154	284	3.572264	48.348343	-0.849866
		168	190	154	286	3.572264	48.348343	-0.849917
		168	190	154	288	3.572264	48.348343	-0.849947
	(t-160, t90)	168	166	180	94	3.590394	53.714424	-4.02628
		168	166	180	100	3.591619	53.655464	-4.02639
		168	166	180	98	3.592683	53.614838	-4.009851
		168	166	180	96	3.593921	53.555267	-4.009737
		168	168	180	100	3.619942	52.427109	-3.807992
	(t60, t-105)	154	60	160	286	3.419081	6.889967	3.111437
		154	60	160	284	3.419083	6.889906	3.111368
		152	60	160	288	3.469284	6.739545	3.49054
		152	60	160	286	3.469286	6.739475	3.49042
		152	60	160	284	3.469286	6.739394	3.490354
	(t60, t90)	150	110	180	100	3.246675	53.192245	0.346519
		152	110	182	100	3.256448	52.257214	-0.14916
		150	108	180	100	3.247219	51.662849	0.688154
		150	110	182	100	3.282577	51.139545	0.346508
		152	110	184	100	3.292739	50.12907	-0.149172

(Table S2 continued)

Structure *	Rotameric states †	Dihedral angles (°)				$d_{\delta-\gamma}$ (Å)	$^{15}N_{\delta}$-$^{13}C_{\gamma}$ dipolar (Hz) ‡	^{1}H-$^{15}N_{\delta}$ dipolar (kHz) ¶
		His37		Trp41				
		χ_1	χ_2	χ_1	χ_2			
3	(t-160, t-105)	166	190	154	286	3.499382	59.375362	-0.848227
		164	190	150	288	3.478864	59.100632	-0.960065
		166	190	156	284	3.532406	58.134926	-0.8482
		164	190	152	284	3.511996	57.940395	-0.960017
		164	190	152	286	3.511996	57.940395	-0.960008
	(t-160, t90)	168	178	172	98	3.573428	59.77853	-2.483876
		168	178	172	100	3.573428	59.77853	-2.483868
		168	178	172	94	3.57343	59.778473	-2.483879
		168	178	172	96	3.57343	59.778473	-2.483882
		168	180	170	100	3.569917	59.740204	-2.206609
	(t60, t-105)	150	54	160	282	3.365268	14.540196	3.350254
		150	54	158	284	3.336036	14.07232	3.350242
		150	52	162	278	3.431287	13.822364	3.132428
		150	52	160	280	3.401546	13.45107	3.132361
		150	52	160	282	3.401546	13.45107	3.132383
	(t60, t90)	158	110	160	100	3.163814	76.683212	-2.374692
		156	110	160	98	3.186831	74.55468	-1.974108
		156	110	160	100	3.186831	74.55468	-1.974109
		156	108	160	98	3.171492	74.510368	-1.638909
		156	108	160	100	3.171492	74.510368	-1.63891

(Table S2 continued)

Structure [*]	Rotameric states [†]	Dihedral angles (°)				$d_{\delta-\gamma}$ (Å)	$^{15}N_{\delta}$-$^{13}C_{\gamma}$ dipolar (Hz) [‡]	^{1}H-$^{15}N_{\delta}$ dipolar (kHz) [¶]
		His37		Trp41				
		χ_1	χ_2	χ_1	χ_2			
4	(t-160, t-105)	170	170	150	276	3.718826	54.083694	-1.629635
		170	170	150	274	3.718827	54.083652	-1.629588
		170	170	150	272	3.718828	54.08363	-1.629534
		170	170	150	270	3.718828	54.083611	-1.629478
		170	170	150	268	3.718829	54.083569	-1.629423
	(t-160, t90)	168	150	150	100	3.429518	69.743729	-4.502992
		168	150	150	98	3.432061	69.590584	-4.488073
		168	150	152	96	3.463875	67.83094	-4.503251
		168	152	150	100	3.462075	67.80069	-4.303906
		168	152	150	98	3.462078	67.800591	-4.303829
	(t60, t-105)	150	60	150	276	3.318503	28.651745	5.424517
		150	58	154	270	3.396334	27.440521	5.374725
		150	58	152	272	3.371465	27.191692	5.374712
		150	58	150	274	3.347325	26.831198	5.374703
		150	58	150	276	3.347325	26.831198	5.374708
	(t60, t90)	158	110	160	100	3.163814	76.683212	-2.374692
		156	110	160	98	3.186831	74.55468	-1.974108
		156	110	160	100	3.186831	74.55468	-1.974109
		156	108	160	98	3.171492	74.510368	-1.638909
		156	108	160	100	3.171492	74.510368	-1.63891

(Table S2 continued)

* The difference between the structures is given by Table S1.

† The rotamer nomenclature of the Penultimate Rotamer Library (6) is used here.

‡ These are $^{15}N_{\delta}$-His37-$^{13}C_{\gamma}$-Trp41 dipolar couplings calculated using the same method as in (7). The experimental value is 51.6~74.6 Hz (7). For each state, only the five largest $^{15}N_{\delta}$-$^{13}C_{\gamma}$ dipolar coupling are presented.

¶ These are ^{1}H-$^{15}N_{\delta}$-His37 dipolar coupling calculated using the same method as in (7). The experimental (absolute) value is 1±0.5 kHz (7).

Table S3. Rotamers, structures, and energies of the His-Trp adduct with different conformations.

	Initial							B3LYP final						
		His37		Trp41		$d_{\delta-\gamma}$	Energy		His37		Trp41		$d_{\delta-\gamma}$	Energy
MPS	Rotamers	χ_1 (°)	χ_2 (°)	χ_1 (°)	χ_2 (°)	(Å)	(kcal)	Rotamers	χ_1 (°)	χ_2 (°)	χ_1 (°)	χ_2 (°)	(Å)	(kcal)
ε	(t-160, t-105)	170	190	160	280	3.6	93.4	same	161	200	200	308	4.6	1.6
ε	(t-160, t90)	170	180	170	90	3.6	111.9	(t-160, m95)	168	200	292	103	4.5	0
ε	(t60, t-105)	150	50	160	280	2.6	90.9	same	151	96	193	264	3.6	4.2
ε	(t60, t90)	150	100	210	100	3.8	86.5	(t60, m-90)	156	98	248	243	4.1	3.1
δ	(t-160, t-105)	160	200	160	280	3.9	98.1	same	157	196	195	257	4.6	8.7
δ	(t-160, t90)	170	180	170	90	3.6	120.4	(t-160, t-105)	150	214	187	248	4.9	2.5
δ	(t60, t-105)	150	50	150	280	3.6	6.7	same	160	20	204	312	4.3	2.5
δ	(t60, t90)	150	100	210	100	3.8	89.6	same	147	45	187	97	4.0	3.5

MPS stands for mono-protonation state. Symbols ε and δ indicate that the histidine is mono-protonated at its ε- and δ-nitrogen, respectively. $D_{\delta-\gamma}$ denotes the distance between the histidine δ-nitrogen and the tryptophan γ-carbon atoms. The rotamer nomenclature of the Penultimate Rotamer Library (6) is used here. In each rotamer pair, the first one is for His37 and the second for Trp41. The word "same" in the table indicates that the rotamers are not changed after optimization. All structures were optimized at the B3LYP level of theory with the 6-31G** basis set. The energies are relative to the lowest value.

REFERENCES

1. Smart, O.S., J. Breed, G.R. Smith, and M.S. Sansom. 1997. A novel method for structure-based prediction of ion channel conductance properties. *Biophys J* 72:1109-1126.

2. Kovacs, F.A., and T.A. Cross. 1997. Transmembrane four-helix bundle of influenza A M2 protein channel: structural implications from helix tilt and orientation. *Biophysical Journal* 73:2511-2517.

3. Kukol, A., P.D. Adams, L.M. Rice, A.T. Brunger, and T.I. Arkin. 1999. Experimentally based orientational refinement of membrane protein models: A structure for the Influenza A M2 H^+ channel. *Journal of Molecular Biology* 286:951-962.

4. Song, Z., F.A. Kovacs, J. Wang, J.K. Denny, S.C. Shekar, J.R. Quine, and T.A. Cross. 2000. Transmembrane domain of M2 protein from influenza A virus studied by solid-state ^{15}N polarization inversion spin exchange at magic angle NMR. *Biophysical Journal* 79:767-775.

5. Wang, J., S. Kim, F. Kovacs, and T.A. Cross. 2001. Structure of the transmembrane region of the M2 protein H^+ channel. *Protein Science* 10:2241-2250.

6. Lovell, S.C., J.M. Word, J.S. Richardson, and D.C. Richardson. 2000. The penultimate rotamer library. *PROTEINS: Structure, Function, and Genetics* 40:389-408.

7. Nishimura, K., S. Kim, L. Zhang, and T.A. Cross. 2002. The closed state of a H^+ channel helical bundle combining precise orientational and distance restraints from solid state NMR. *Biochemistry* 41:13170-13177.