Supplementary Information

Does Proton Conduction in the Voltage-Gated H^+ Channel hHv1 Involve Grotthuss-like Hopping via Acidic Residues?

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Supplementary Tables

Table S1: Summary of the equilibration classical MD simulations.

#	*Description*	*Length [ns]*	*Starting point*
1	Equilibration	488.2	Homology Model
2	Equilibration in the presence of a 100 mV transmembrane potential	462.8	Started from 113.7 ns of simulation 1

Table S2: Summary of the classical MD simulations performed, involving a single proton, using the H_3O^+ model.

#	*Proton Localization*	*Description*	*Length [ns]*	*Starting point*
3	I	Equilibration	93.5	H_3O^+ placed in bottom cavity
4		Under a 100 mV transmembrane potential	156.4	Started from end of simulation 3
5		FEP	4.0	Started from end of simulation 3
6	C	Equilibration	423.3	H_3O^+ placed in the top cavity, just above C
7		Under a 100 mV transmembrane potential	463.3	Started from 152.8 ns of simulation 6
8		Metadynamics	884.9	Started from 91.1 ns of simulation 6
9		Metadynamics, second instance	333.0	Started from 91.1 ns of simulation 6
10		FEP	4.0	Started from 91.1 ns of simulation 6
11	E	Equilibration	130.0	Started from 38.0 ns of simulation 8
12		FEP	4.0	Started from 38.0 ns of simulation 8

Table S3: Summary of the classical MD simulations performed, involving a single proton, using the Gaussian potential proton model.

#	*Proton Localization*	*Description*	*Length [ns]*	*Starting point*
13	I	Equilibration	185.0	GPot placed in bottom cavity
14	C	Equilibration	120.0	GPot placed between D112 and D185
15		Equilibration, second instance	120.0	GPot placed between D112 and D185
16	E	Equilibration	120.0	GPot placed in top cavity

Table S4: Summary of the classical MD simulations performed, involving a single proton, using protonated aspartic acid or glutamic acid models.

#	*Proton Localization*	*Description*	*Length [ns]*	*Starting point*
17	I	Protonated D174	320.5	
18		Protonated D174, in the presence of a 100 mV transmembrane potential	279.3	started from 111.3 ns of 17
19		Protonated E153	325.1	
20		Protonated E153, in the presence of a 100 mV transmembrane potential	278.4	started from 108.72 ns of 19
21	C	Protonated D185	331.9	
22		Protonated D185, in the presence of a 100 mV transmembrane potential	147.2	Started from beginning of 21
23		Protonated D185, in the presence of a 100 mV transmembrane potential, second instance	122.0	started from 295.9 ns of 21
24		Protonated D185, second instance	387.1	
25		Protonated D112	84.0	
26		Protonated D112, second instance	183.8	

Table S5: Summary of the classical MD simulations involving two protons, using the H_3O^+ model.

#	*Proton Localization*	*Description*	*Length [ns]*	*Starting point*
27	I+C	Equilibration	130.9	H_3O^+ placed in the bottom cavity and just above C
28		Metadynamics	609.3	Started from 16.0 ns of simulation 27
29		Under a 100 mV transmembrane potential	286.3	Started from 16.0 ns of simulation 27
30	I+C	I proton bridging E153 and D112	140.0	Snapshot extracted from 18
31		I proton bridging E153 and D112, in the presence of a 100 mV transmembrane potential	76.6	Started from 104.6 ns of simulation 30
32	C+E	E proton bridging D185 and E119	96.5	Snapshot extracted from 18
33		E proton bridging D185 and E119, in the presence of a 100 mV transmembrane potential	107.2	Started from 95.0 ns of simulation 32

Table S6: Summary of all the QM/MM MD simulations performed.

Simulation ID	Description	Length (ps)	Initiation	Box Size (Å^3)
Q.1	H_3O^+ in I	14.4	#3	88x74x108
Q.2	H_3O^+ in C	6.7	#6	97x67x108
Q.3	H_3O^+ in C (second instance)	7.2	#6	96x67x108
Q.4	H_3O^+ in Ext.	9.0	#11	81x82x107
Q.5	H_3O^+ bridging E153 and D112	8.7	#30	97x68x107
Q.6	H_3O^+ bridging D185 and E119	9.0	#32	95x69x106

Table S7: pKa estimates of the various protonatable residues located in a transmembrane position. 100 snapshots were extracted from the classical MD simulations and their average pKa is reported.

		pKa	**St.d**
GLU	**119**	5.9	0.3
ASP	**185**	4.1	0.5
ASP	**112**	3.4	0.6
GLU	**153**	5.2	0.8
ASP	**174**	3.9	0.5
LYS	**157**	11.3	1.0
ARG	**205**	12.3	0.3
ARG	**208**	14.5	0.6
ARG	**211**	11.9	0.4

Table S8: Classical Model hydronium Parameters

Partial charge on oxygen [e]	-0.899	
Partial charge on hydrogen [e]	0.633	
Lennard-Jones parameters for oxygen	σ [kcal/mol]	$R_{min}/2$ [Å]
	-0.1521	1.7682
Lennard-Jones parameters for hydrogen	σ [kcal/mol]	$R_{min}/2$ [Å]
	-0.0460	0.2245
Bond (O-H) Parameters	K_b [kcal/mol/ Å ^{2}]	b_0 [Å]
	450.0	0.96884
Angle (H-O-H) Parameters	K_θ [kcal/mol/rad^2]	θ_0 [deg]
	55.0	113.07286

Supplementary Figures

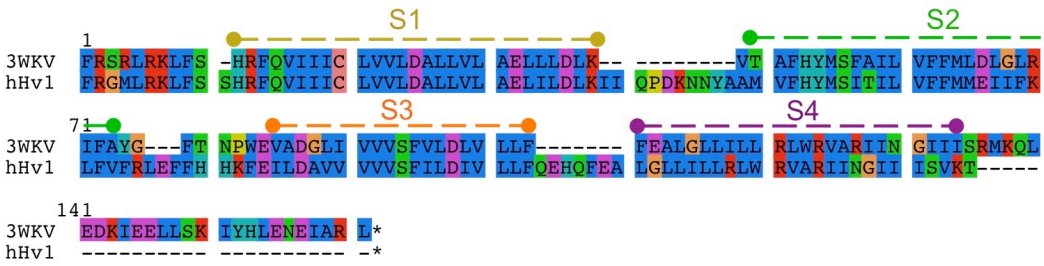

Figure S1: Alignment between the template sequence (mHv1 chimera, PDB ID: 3WKV) and hHv1 in the activated-open state.

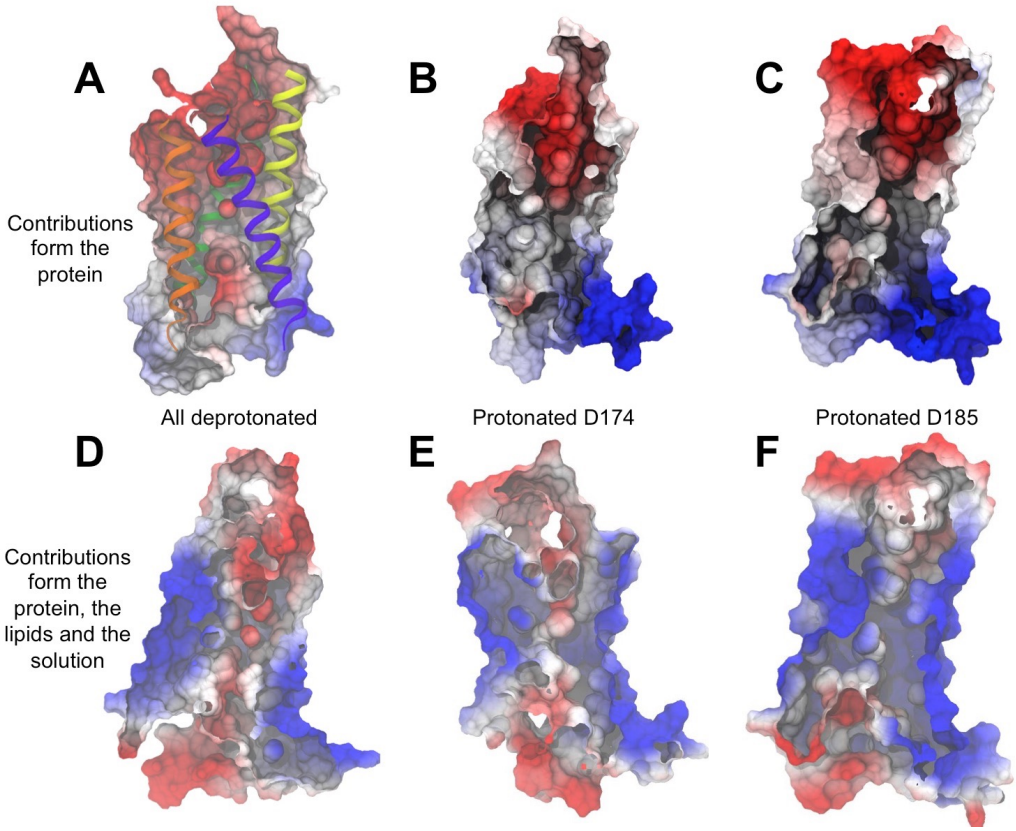

Figure S2: Electrostatic potential maps calculated over the last 100 ns of simulations of three different protonation states, projected onto a cut-through of the protein surface. The electrostatic potential arising from the contributions arising from the protein atoms are presented on the top row, for (A) the system in which all the glutamates and aspartates are considered in the deprotonated form (#2) ,(B) for the system where D174 is protonated (#27) and (C) for the system where D185 is protonated (#26). The electrostatic potential arising from contributions from the entire system are presented in the bottom row for the same systems, i.e. (D) for the system in which all the glutamates and aspartates are considered in the deprotonated form (#2), (E) for the system where D174 is protonated (#27) and (F) for the system where D185 is protonated (#26).) Positive potentials are shown in red and negative potentials are represented in blue.

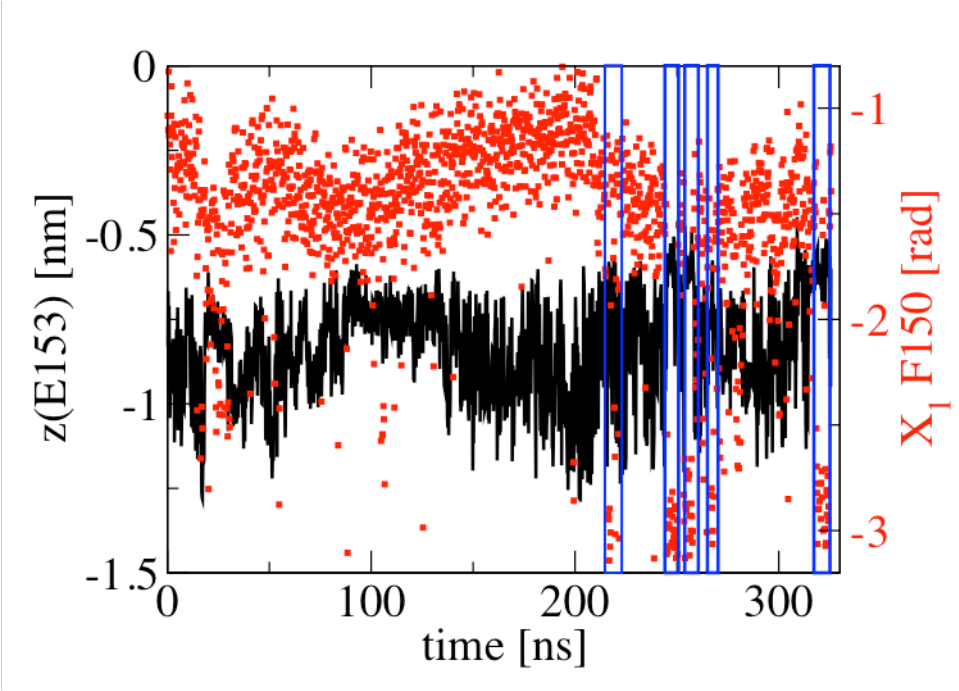

Figure S3: Conformational reorganizations along simulation #29. The time evolution of the X_1 dihedral of F150,the conserved phenylalanine residue lining the permeation pore, and the position of the side chain of protonated E153 are represented. The five blue boxes highlight the opening events occurring after 215 ns simulation time.

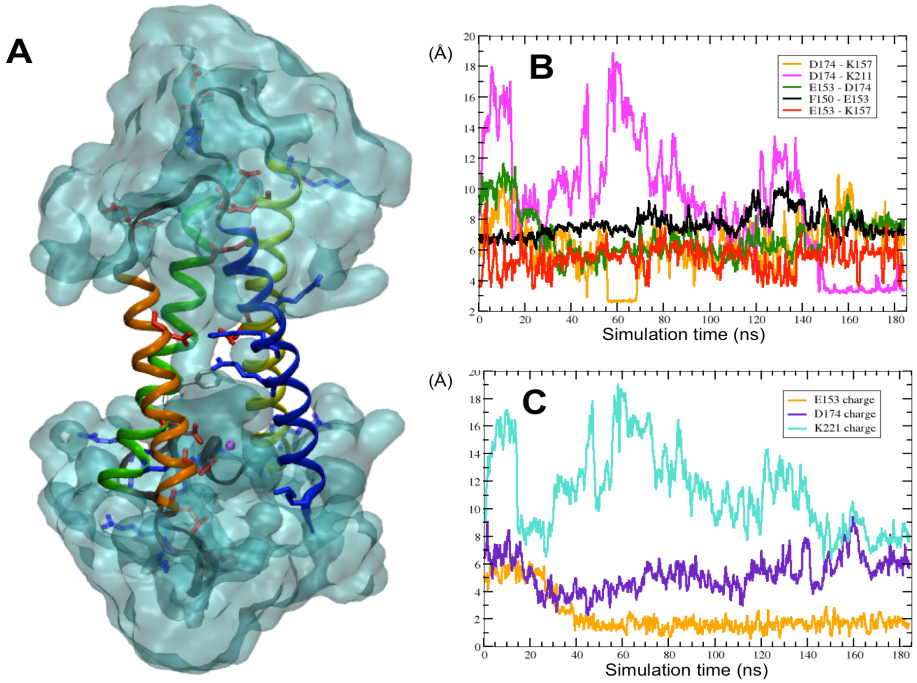

Figure S4. Charge localization at the intracellular site. (A) View of the Hv1 proton channel. S1 Helix is colored in yellow; helix in green; S3 helix in orange; S4 helix in blue. Water hydrating the site is represented in transparent quick-surf mode, colored in light blue. Charged residues facing the lumen are shown; positively and negatively charged residues in red and blue, respectively. The external charge is represented as a purple sphere. (B) & (C) Distances between amino acid pairs and amino acid/charge are plotted.

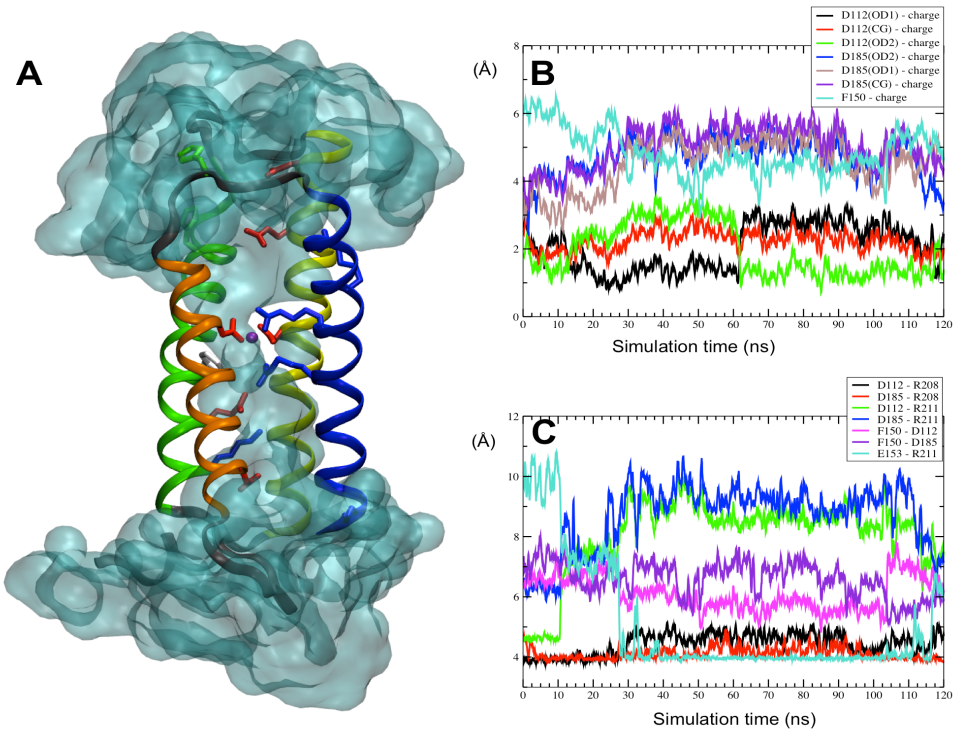

Figure S5. Charge localization at the core site. (A) View of the Hv1 proton channel. S1 Helix is colored in yellow; S2 helix in green; S3 helix in orange; S4 helix in blue. Water hydrating the site is represented in transparent quick-surf mode, colored in light blue. Charged residues facing the lumen are shown; positively and negatively charged residues in red and blue, respectively. The external charge is represented as a purple sphere. (B) & (C) Distances between amino acid pairs and amino acid/charge are plotted.

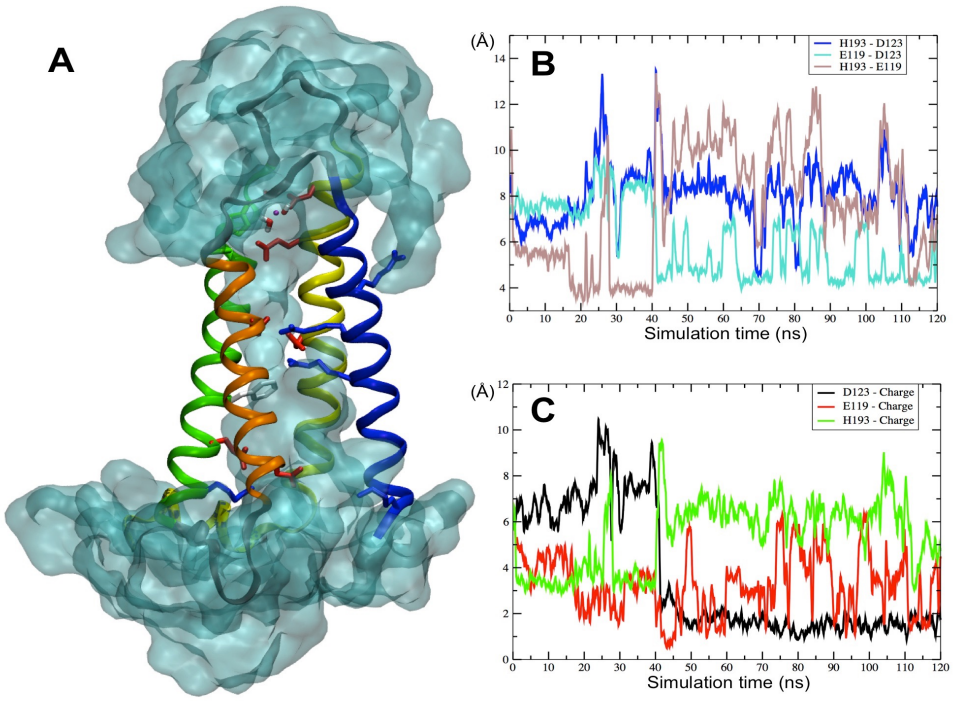

Figure S6. Charge localization at the extracellular site. (A) View of the Hv1 proton channel. S1 Helix is colored in yellow; S2 helix in green; S3 helix in orange; S4 helix in blue. Water hydrating the site is represented in transparent quick-surf mode, colored in light blue. Charged residues facing the lumen are shown; positively and negatively charged residues in red and blue, respectively. The external charge is represented as a red sphere. (B) & (C) Distances between amino acid pairs and amino acid/charge are plotted.

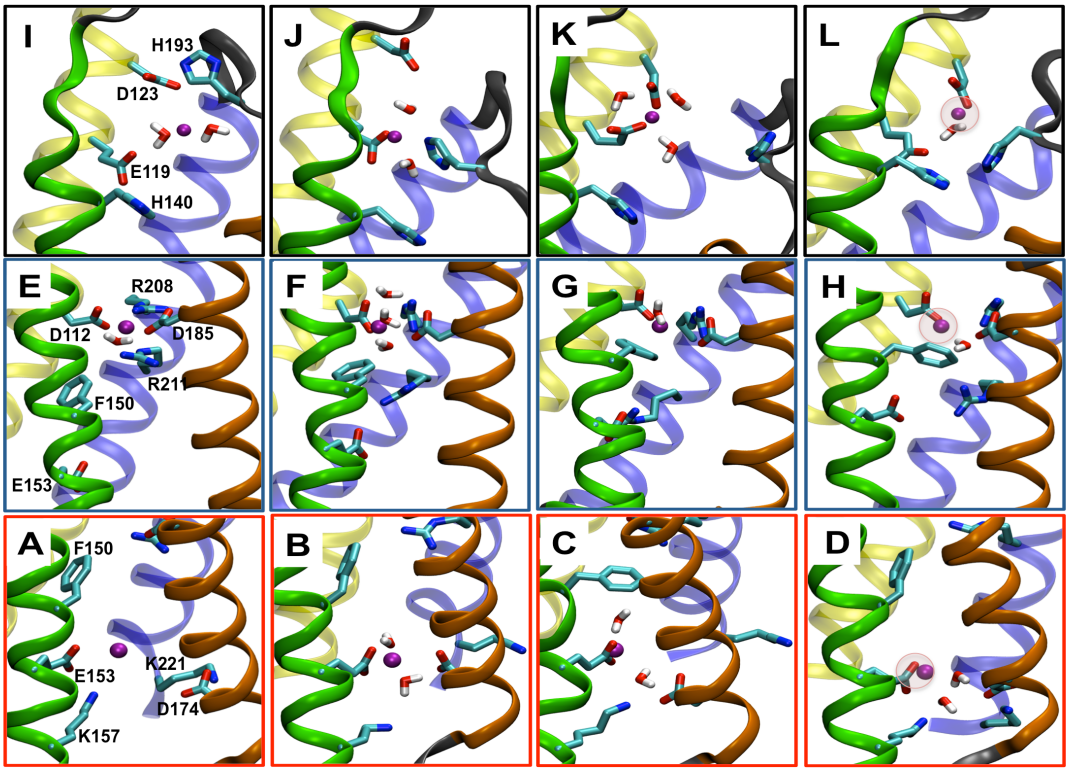

Figure S7:. Effects of placing one external charge along the pathway of proton transport. Simulation #13 (185 ns), red boxes: the charge is localized at the intracellular site (A) through (D). Simulation #14 (120 ns), blue boxes: the charge is localized at the core site (E) through (H). Simulation #16 (120 ns), black boxes: the charge is localized at the extracellular site (I) through (L).

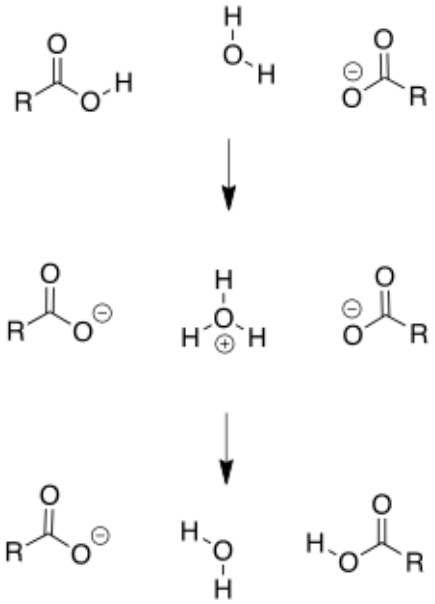

Figure S8: Scheme of proton hopping from an acidic residue to the next through a water molecule bridging the two acidic moieties.