Selectivity Mechanism of the Voltage-gated Proton Channel, H\textsubscript{V}1

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Voltage-gated proton channels, H\textsubscript{V}1, trigger bioluminescence in dinoflagellates, enable calcification in coccolithophores, and play multifarious roles in human health. Because the proton concentration is minuscule, exquisite selectivity for protons over other ions is critical to H\textsubscript{V}1 function. The selectivity of the open H\textsubscript{V}1 channel requires an aspartate near an arginine in the selectivity filter (SF), a narrow region that dictates proton selectivity, but the mechanism of proton selectivity is unknown. Here we use a reduced quantum model to elucidate how the Asp–Arg SF selects protons but excludes other ions. Attached to a ring scaffold, the Asp and Arg side chains formed bidentate hydrogen bonds that occlude the pore. Introducing H\textsubscript{3}O\textsuperscript{+} protonated the SF, breaking the Asp–Arg linkage and opening the conduction pathway, whereas Na\textsuperscript{+} or Cl\textsuperscript{−} was trapped by the SF residue of opposite charge, leaving the linkage intact, thus preventing permeation. An Asp–Lys SF behaved like the Asp–Arg one and was experimentally verified to be proton-selective, as predicted. Hence, interacting acidic and basic residues form favorable Asp\textsuperscript{H}_{2}O\textsuperscript{−}–H\textsuperscript{2}O\textsuperscript{−}–Arg\textsuperscript{+} interactions with hydronium but unfavorable Asp\textsuperscript{−}–X/\textsuperscript{−}–Arg\textsuperscript{+} interactions with anions/cations. This proposed mechanism may apply to other proton-selective molecules engaged in bioenergetics, homeostasis, and signaling.

The voltage-gated proton channel, H\textsubscript{V}1, has been implicated in numerous biological functions in humans\textsuperscript{4}: charge compensation during the respiratory burst of phagocytes killing bacteria\textsuperscript{2,3}, pH homeostasis in airway epithelia\textsuperscript{4}, histamine secretion by basophils\textsuperscript{5}, and triggering sperm capacitation\textsuperscript{6}. It is a desirable and novel drug target\textsuperscript{7} due to its involvement in various inflammatory pathologies and its exacerbation of diseases such as ischemic stroke\textsuperscript{8}, breast cancer\textsuperscript{9}, and chronic lymphocytic leukemia\textsuperscript{10}. In other species H\textsubscript{V}1 channels play diverse roles including mediating action potentials that trigger bioluminescence in dinoflagellates\textsuperscript{11} and enabling biogenic calcite production by coccolithophores as part of the global carbon cycle\textsuperscript{12}. The ability of H\textsubscript{V}1 to perform its functions would fail if its proton selectivity were not perfect, due to the low concentration of protons in biological fluids. A conserved aspartate (Asp112 in humans) in the middle of the S1 transmembrane helix is an essential part of the H\textsubscript{V}1 selectivity filter (SF)\textsuperscript{11,13}. This Asp consistently interacts with the second\textsuperscript{14,15} or third\textsuperscript{16,17} Arg in the S4 segment in homology models of human H\textsubscript{V}1 in a protein (conduction) conformation. However, it is seen to interact with the second Arg in the crystal structure of a closely related voltage-sensing phosphatase in the active conformation\textsuperscript{18}. Classical molecular dynamics (MD) simulations indicate that charge compensation (e.g., an intact salt bridge) appears essential\textsuperscript{18}, but do not reveal the mechanism by which proton selectivity...
occurs. Might selectivity result from obligatory protonation and deprotonation of a titratable group\textsuperscript{13,20} lining the SF? How does an Asp in a constricted SF select protons, while rejecting other cations/anions?

Takeshita et al.\textsuperscript{21} have determined a 3.45 Å structure of a chimeric murine H\textsubscript{V1} channel in a probable closed conformation. This structure shows that the SF Asp is located in a hydrophobic layer comprising two conserved Phe residues, which might prevent water penetration. Presumably, this hydrophobic region prevents conduction of any ions including protons in closed channels. We adopt the nearly universal assumption that channel opening involves a protein conformational change. Opening allows \( \text{H}_3\text{O}^+ \) to access the SF from either side of the membrane. Since no 3D structure of H\textsubscript{V1} in an open conformation has been solved, hypotheses on proton selectivity and conduction have been based on homology models derived from the open-state structures of voltage-gated sodium or potassium channels, which share only 13–19% sequence identity with hH\textsubscript{V1}\textsuperscript{12}. MD simulations of hH\textsubscript{V1} using as templates the open-state structures of the K\textsubscript{AP} (1ORS)\textsuperscript{23} and the K\textsubscript{1.2–K\textsubscript{2.1}} paddle chimera (2R9R)\textsuperscript{24} potassium channels predict a stable water wire in the open channel. It is widely accepted that protons can be conducted efficiently along a hydrogen-bonded water chain\textsuperscript{14–28}. However, MD simulations of the same hH\textsubscript{V1} channel derived from multiple templates (1ORS, 2R9R, and 3RVT)\textsuperscript{24} show that the Asp–Arg interaction, which interrupts the water wire, is only occasionally broken, yielding a transient water wire. Likewise, in simulations of \textit{Ciona intestinalis} H\textsubscript{V}1\textsuperscript{17}, which is homologous to hH\textsubscript{V1} with 52% sequence identity, the average lifetime of a continuous water wire in an open-state model was only 6 ps. An ephemeral water-wire is suggestive of proton permeation involving titratable residues.

Whether proton selectivity could result from protonation/deprotonation of a titratable group can be answered only by considering explicit protonation/deprotonation reactions using all-electron quantum mechanical calculations, as done here. The lack of an open, proton-bound X-ray structure of hH\textsubscript{V1} prohibits accurate evaluations of multi-ion free energy profiles for ion permeation. Thus, we evaluated selectivity by comparing the binding affinity of \( \text{H}_3\text{O}^+ \), \( \text{Na}^+ \), \( \text{Cl}^- \), and \( \text{H}_2\text{O} \) in the SF, assuming that the hH\textsubscript{V1} would be selective to the permeating ion that binds with higher affinity in the SF. A reduced SF model was devised to capture the essential chemical processes underlying proton selectivity. It was designed to maximize resemblance to the open H\textsubscript{V1} SF and was constructed on the basis of the following considerations: At the narrowest, relatively dry region of the pore\textsuperscript{4}, the SF is lined by an aspartate (Asp112 in hH\textsubscript{V1}), which is conserved in all known and putative H\textsubscript{V1}\textsuperscript{1}. This Asp interacts almost continuously with one of the three Arg residues in the S4 transmembrane segment in the open channel from MD simulations based on different homology models\textsuperscript{14–17,29}. Even when the Asp was moved by double mutation from position 112 to 116 (D112V/V116D), it still interacted with one or two Arg residues with an intact or a broken salt-bridge in MD simulations\textsuperscript{19}. Intriguingly, a positive point charge pulled through this double mutant in the broken configuration encountered a 10 kcal/mol barrier, but no barrier in the intact salt-bridge configuration\textsuperscript{19}. These findings indicate that the Asp–Arg interaction is essential to proton selectivity, hence it was incorporated into the SF model. Ions such as \( \text{Na}^+ \), \( \text{OH}^- \), and \( \text{Cl}^- \) were assumed to be dehydrated since the SF pore is purported to be narrow\textsuperscript{14,21}. Ions in bulk solution were not included in the SF model, since H\textsubscript{V1} channels are notoriously indifferent to ionic strength\textsuperscript{13}, cations such as \( \text{Ca}^{2+} \) or \( \text{Mg}^{2+} \)\textsuperscript{30,31}, or anion species\textsuperscript{31}.

To address whether proton selectivity arises from protonation and deprotonation of a titratable group, the interactions between the permeating ions and H\textsubscript{V1} SF ligands, which play a key role in the competition between the native proton and its rivals, were treated explicitly using density functional theory to account for electronic effects such as polarization of the participating entities and differential amounts of ligand→ion charge transfer, while the region inside the SF was represented by a continuum dielectric. The proton was modeled as \( \text{H}_3\text{O}^+ \), while the Asp\textsuperscript{6}, Arg\textsuperscript{2}, Ala, His, and Lys\textsuperscript{7} side chains were modeled as \(-\text{CH}_2\text{-COO}^-, \)-CH\textsubscript{2}-NH-C(NH\textsubscript{2})\textsubscript{2}+, -CH\textsubscript{2}-CH\textsubscript{3}, -CH\textsubscript{2}-imidazole, and -CH\textsubscript{2}-NH\textsubscript{3}+, respectively. The SF ligands were attached to a ring scaffold (see Methods), and the resulting complex was subject to all-electron geometry optimization without any constraints. The fully optimized SF geometries were then used to compute the ion-binding/exchange reactions in the H\textsubscript{V1} pore characterized by an effective dielectric constant, ɛ. Since MD simulations of the open-state hH\textsubscript{V1} model\textsuperscript{14} show that the SF is not in a bulk water environment but is relatively dry (see above), we employed ɛ ranging from 4 to 30\textsuperscript{32} to reflect a solvent-inaccessible or a partially solvent-exposed binding site, respectively, in order to encompass the actual value in the SF (see Methods). In interpreting results, we focus not on the absolute free energies, but on the change in ion-binding/exchange free energies with increasing ɛ. The approach outlined above has yielded structures and free energy trends in model SFs of various ion channels that are consistent with experimental findings\textsuperscript{32–36}. The distance found here between the charge centers of the SF Asp and Arg (3.7 Å) agrees with that (3.8–4.6 Å) in MD simulations of the open hH\textsubscript{V1}\textsuperscript{14,19}. The free energy trends in the model H\textsubscript{V1} SF found herein are also consistent with experimental findings.

**Results**

**Binding of \( \text{H}_3\text{O}^+ \) in the Asp–Arg SF.** The ion-free Asp–Arg SF adopted two closed conformations that differ by <1 kcal/mol: an ion-pair conformation where the Asp and Arg side chains formed a bidentate salt bridge (Fig. 1a) and a hydrogen-bonded pair conformation where Arg protonated Asp, forming two hydrogen bonds (Fig. 1b). An Arg-carboxylate structural motif identified in several enzymes is thought to ensure rapid equilibrium between protonated and deprotonated Arg\textsuperscript{27}. To see how the SF could accommodate passing ions, \( \text{H}_3\text{O}^+ \) was placed between Asp and Arg, above the hydrogen-bond
network plane (Fig. 1c), mimicking the transient breaking of the Asp–Arg linkages, allowing H$_3$O$^+$ into the SF. The positioning of H$_3$O$^+$ between a deprotonated acid and a base has been observed spectroscopically. In the final, fully optimized structure (Fig. 1d), the Asp and Arg side chains moved apart, breaking the two hydrogen bonds, thus opening the permeation pathway to accommodate the permeating H$_3$O$^+$, which transferred a proton to the SF leaving a water bridging AspH$^0$ and Arg$^+$. Binding of H$_3$O$^+$ to the Asp–Arg SF is thermodynamically favorable throughout the range of dielectric constant explored (negative $\Delta G^i$, Fig. 1e).

**Figure 1.** Binding of H$_3$O$^+$ to the Asp–Arg SF. Fully optimized B3-LYP/6-31+G(3d,p) structures of (a) ion-free Asp$^-$–Arg$^+$ SF, (b) Asp$^0$–Arg$^0$ SF, (c) initial configurations of the SF–H$_3$O$^+$ complex and (d) final configuration of the SF–H$_3$O$^+$ complex, AspH$^0$–H$_2$O–Arg$^+$ with H in grey, C in green, N in blue and O in red. A dashed line denotes a hydrogen bond, which is defined by a donor–acceptor distance $\leq 3.5$ Å and a H–acceptor distance $\leq 2.5$ Å. The reaction between SF and H$_3$O$^+$ is depicted in (e) with free energies given in kcal/mol; $\Delta G^i$ is the binding free energy in the gas phase, whereas $\Delta G^4$ and $\Delta G^{30}$ are the corresponding free energies in the SF characterized by an effective dielectric constant of 4 and 30, respectively.

**Binding of Cl$^-$ and Na$^+$ to the Asp–Arg SF.** The Asp–Arg SF responded quite differently to the introduction of the proton's competitors, Cl$^-$ and Na$^+$. We started from the ‘open’ pore structure, where the Asp and Arg side chains were separated, and placed the incoming ion between them (Fig. 2, left). Such a configuration was not favorable as during geometry optimization, the introduced ion was ejected from the pore, away from the residue bearing the same charge and became trapped by the residue...
carrying the opposite charge: Arg$^+$ for Cl$^-$ and Asp$^-$ for Na$^+$ (Fig. 2, right). In contrast to the open starting structures, the hydrogen-bond network between Asp and Arg was partially restored in the final optimized structures, closing the SF aperture and excluding other ions.

The above results highlight the importance for proton selectivity of electrostatic interactions between the SF and permeating ions. The SF Asp–Arg pair intrinsically selects protons and rejects other cations and anions: the only species that can bind favorably to both Asp$^-$ and Arg$^+$ in an “open” state is H$_3$O$^+$ (Fig. 1e). Cl$^-$ and Na$^+$ are not permeable, as they do not promote pore opening (Fig. 2).

H$_2$O vs. H$_3$O$^+$ Binding in the Asp–Arg SF. Although the Asp112–Arg208 pair is broken only 10% of the time in MD simulations of a homology model of hHv1 in an open conformation, this transient disruption allows formation of a water wire that could last for 1 ns$^{14}$. Would a water molecule be even more stable than H$_3$O$^+$ in the Hv1 SF? In other words, can H$_3$O$^+$ displace water bound to the Asp–Arg pair? To address this question, we placed H$_2$O in between the Asp–Arg pair and optimized the structure. The fully optimized structure in Fig. 3 (left) shows that a water molecule, unlike H$_3$O$^+$, cannot fully dissociate the Asp–Arg pair, as a hydrogen bond remains between the two residues. Furthermore, H$_3$O$^+$ can easily displace water bound to the Asp–Arg pair and protonate Asp (Fig. 3, right): The computed free energies ($\Delta G^x$, $x = 1$–30) for H$_3$O$^+$ to displace H$_2$O from the Asp–Arg pair are all favorable (negative $\Delta G^x$, Fig. 3). The positive free energies for the reverse reaction imply that a water molecule cannot readily displace H$_3$O$^+$ bound to the Asp–Arg pair.

Figure 2. Binding of Cl$^-$ and Na$^+$ to Asp–Arg SF. Ball and stick diagrams of the initial (left) and final (right) structures of SF complexes with (a) Cl$^-$ and (b) Na$^+$. 

Figure 3. Free energies (in kcal/mol) for replacing H$_2$O bound in Asp–Arg SF with H$_3$O$^+$. See Fig. 1 legend.
The Arg208Lys Mutant is Predicted to be Proton-selective. Replacing the Lys lining the pore of voltage-gated Na\(^+\) channels with Arg nearly abolishes the channel’s selectivity for Na\(^+\) over K\(^+\). Is Arg in the H\(_v\)1 SF likewise indispensable for proton selectivity? To address this question, we replaced the SF Arg by Lys and evaluated its proton selectivity. Lys behaved like its Arg counterpart: in the ion-free state, Lys protonated Asp forming a hydrogen bond (Fig. 4a, left); however, because Lys has a lower \(pK_a\) than Arg, a stable Asp–Lys\(^+\) ion pair minimum could not be found. In the ion-bound state, H\(_3\)O\(^+\), which was initially placed between the protonated Asp and neutral Lys, transferred a proton to the SF leaving a water molecule to bridge AspH\(^0\) and Lys\(^+\) (Fig. 4a, right). The AspH\(^0\)–H\(_2\)O–Lys\(^+\) complex formation free energies remain thermodynamically favorable, although slightly less so than those for the wild-type Asp–Arg SF (compare numbers in Figs. 1e and 4a). As in the wild-type SF, during geometry optimization, Cl\(^–\) and Na\(^+\) were repelled by the SF residue of the same net charge and moved towards the SF residue with the opposite charge. In the final optimized structures, Asp\(^–\) and Lys\(^+\) formed a hydrogen bond, prohibiting the competing Cl\(^–\) and Na\(^+\) ions from passing through the pore (Figs. 4b and 4c).

Why D112A and D112H Mutants are Chloride-selective. Mutagenesis studies\(^1^3\) show that replacing Asp112 in the SF with a neutral residue such as Ala or the weak base His converts the channel into an anion-selective pore. Why? To address this question we modeled two types of SF mutants: Ala\(^0\)–Arg\(^+\) (Fig. 6a,b) and His\(^0\)–Arg\(^+\) (Fig. 6c). Replacing anionic Asp112\(^–\) with neutral Ala or His leaves the positive charge on the SF Arg\(^+\) uncompensated, which disfavors H\(_3\)O\(^+\) binding to the SF due to the like charge repulsion between H\(_3\)O\(^+\) and Arg\(^+\). On the other hand, strong attractive forces between the permeating OH\(^–\)/Cl\(^–\) and Arg\(^+\) stabilize the OH\(^–\)/Cl\(^–\)–SF complexes, and thus favor binding of the anion. To verify that the AlаБ112 and His112 mutants would be anion-selective, we computed the free energy for replacing H\(_3\)O\(^+\) in the mutant SFs with Cl\(^–\). In line with the experimental observations, the Ala\(^0\)–Arg\(^+\) SF is...
highly Cl–-selective in both solvent-inaccessible and exposed pores (negative $\Delta G^*$, Fig. 6a). It is predicted
to be even more selective for OH– (more negative $\Delta G^*$ in Fig. 6b than in Fig. 6a), in accord with the
experimental finding that the Asp112Ala mutant is more permeable to OH– than to Cl–13. This is likely
so because the SF Arg can protonate OH–, yielding a neutral Ala0–H2O0–Arg0 complex.

Like the Ala0–Arg+ mutant, the His0–Arg+ SF is predicted to be also anion-selective provided the
narrow pore has limited solvent accessibility (negative $\Delta G^*$), which is seen in the 3.45 Å crystal struc-
ture of a mouse HV1 chimeric channel (PDB 3WKV)21 and in simulations of open-state H2O1 models14,17.
However, it is predicted to be less Cl–-selective than the Ala0–Arg+ filter (less negative $\Delta G^*$ in Fig. 6c
than in Fig. 6a), which is also consistent with experiment13. This is largely because H3O+ protonated the
His–Arg SF, stabilizing the His+–H2O–Arg+ “reactant” complex (Fig. 6c, left), but no such stabilization
can occur in the Ala0–H3O+–Arg+ “reactant” complex (Fig. 6a, left).

Discussion

Previous studies16,23 have proposed that a water wire might conduct protons through H2O1, but this does
not explain how other ions are excluded and why an aspartate (Asp112 in humans) in the H2O1 pore is
essential for proton selectivity11,13. This work shows that the H2O1 Asp–Arg SF selects protons by transfer-
rating a proton from H3O+ to the SF, highlighting the importance of quantum effects (charge transfer and
polarization). Although a water molecule can be inserted between Asp and Arg, it is readily displaced
by H3O+ (Fig. 3), which then transfers its extra proton to the SF.

This work suggests the following proton selectivity mechanism in the H2O1 SF: On a time-scale of
seconds, the channel helices, S4 in particular18,40, move from a closed conformation that does not allow
conduction to an open one that does. For other ion channels, opening produces a continuous water-filled
pore, through which water and ions pass, often in single-file through the narrowest region41,42. For H2O1,
channel opening produces instead a relatively dry pore that is constricted by two hydrogen bonds formed
by the SF Asp and Arg14 (Fig. 1a,b). Thermal fluctuations could transiently break the Asp–Arg linkage,
allowing ions or water to approach the narrow SF (Fig. 1c, Figs. 2 and 3, left). The permeating H3O+ pro-
tonates the SF Asp, resulting in favorable AspH+–H2O0–Arg+ interactions (Fig. 1d), thus “opening” the
pore to enable its own permeation, whereas anions (X–) or cations (X+) encounter unfavorable Asp–X–
Arg+ or Asp–X+–Arg+ interactions, and are ejected, restoring the Asp–Arg linkage (Fig. 2, right). Hence,
the H2O1 Asp–Arg SF intrinsically selects protons by virtue of its ability to “close” its pore when H3O+ is
absent, to “open” its pore by accepting a proton when H3O+ enters, while rejecting other cations and
anions though electrostatic repulsion. In the absence of permeating ions, the SF residues form hydrogen
bonds that occlude the pore. Among cations, H3O+ is uniquely able to protonate the SF ligands, permeate
as neutral H2O, and then retrieve the excess proton (Fig. 7).

The mechanism for proton selectivity found herein may also apply to other molecules. For example,
if Asp112 from human H2O1 is superimposed on Asp61 of the F0F1-type H+–ATPase, Arg210 aligns with
Arg208 of Hv1 (Fig. 8). Asp61 and Arg210 are located in the proton pathway of this H+–ATPase and are
the only two amino acids that are absolutely required for function43.
Several other proteins, which have Asp–Arg/Lys pairs thought to be critical to proton transport, also exhibit distances between the charge centers similar to the pair in Hv1. Examples of such proteins and the distances between charge centers include Na\(^+\)phosphatase, 3.9 Å\(^{-}\); H\(^+\)phosphatase, 4.0 Å\(^{-}\); and the glucose H\(^+\)symporter XylE, 4.1 Å\(^{-}\). In the Asp–Arg motif common to several proton pumps, a function of Arg is thought to be electrostatic ejection of the proton at the appropriate moment in the pump cycle\(^{43,47}\). This interacting charge pair may help enforce proton selectivity in these molecules, as in Hv1.

Conversely, we searched for Asp–Arg pairs in pores of non-proton channels, where such linked acid-base pairs should not exist. We examined 60 ion channels and transporters (including various cation and anion channels, aquaporin, and organic cation transporters) for which X-ray structures exist (see Supplementary Table S6). Following criteria for a proton SF established previously\(^{19}\), we searched for a pore-facing Asp/Glu in hydrogen-bond contact with a single Arg/Lys, located in a narrow region of the pore in an open conformation. We found no counterexample contradicting our hypothesis.

Although the interactions between ions and the known SF ligands (notably, both amino acids directly implicated in selectivity by mutation studies) have been treated in detail using all-electron quantum mechanical calculations, the contributions from other segments of the pore and ions have not been modeled explicitly in the absence of a high-resolution structure of the open-state Hv1 channel. Consequently, the present results, which are in line with experimental observations, are limited to explaining proton selectivity in the constricted, relatively dry Asp-Arg SF. How the proton leaves this SF is not explicitly dealt with here. Perhaps an incoming H\(_3\)O\(^{+}\) (or another cation) could dislodge H\(_2\)O\(^{+}\) from the SF.
as in the classical “knock-on” mechanism for K⁺ channels proposed by Hodgkin and Keynes. MD simulations of the open hH₂₁ channel derived from multiple templates show that the SF is located at the extracellular end of a narrow constriction ~10 Å long with a hydrophobic region surrounding Phe150–Arg211 at the inner end. Thus, another question is how protons pass through this second hydrophobic zone. However, in a recent computational study, H₃O⁺ positioned at the entrance to a hydrophobic pore was found to induce water entry, creating its own water wire and lowering the free energy barrier for proton permeation. Such a mechanism may transiently hydrate the Phe-Arg bottleneck, enabling proton hopping from one water molecule to the next. When the open H₂₁ channel structure becomes available, the contributions of non-SF residues, proton coupling, and kinetic barriers to proton selectivity could be assessed from computed charge-transfer free energy profiles.

**Methods**

**SF Model and Justification.** Models of the hH₂₁ SFs were built using GaussView version 3.09, following the guidelines from our previous work. The SF ligating groups were coordinated to the permeating ion or water and attached to a carbon–hydrogen ring scaffold via flexible methylene spacers (see Figures). The ring scaffold prevents the metal ligands from drifting away or assuming unrealistic geometries.
pore-occluding positions during geometry optimization. However, the shape and the C–H orientations of the ring do not obstruct the pore lumen. Moreover, the ion-ligating groups and their connection to the ring are flexible enough to allow them to optimize their positions upon ion/water binding.

**Geometry Optimization of the SF Model.** In previous studies,

the B3-LYP/6-31+G(3d,p) method was shown to be the most efficient among the various methods tested in reproducing experimentally determined molecular properties and structural characteristics of model ligands and metal complexes (see Supplementary Table S1). Hence, it was used to optimize the geometry of each model SF without any constraints and to compute the electronic energies, $E_{el}$, using the Gaussian 09 program. It was also used to compute the frequencies of each optimized structure. No imaginary frequency was found in any of the optimized structures.

**Free Energy Calculations.** The binding of $\text{H}_3\text{O}^+$ to a model SF to yield $[\text{H}_3\text{O}^+\text{-SF}]$ is described by the following reaction

$$\text{H}_3\text{O}^+ + \text{SF} \rightarrow [\text{H}_3\text{O}^+\text{-SF}]$$  \hspace{1cm} (1)

Binding of $\text{H}_3\text{O}^+$ to the wild-type or mutant Hv1 SF is thermodynamically favorable only if the binding free energy for eq 1 is negative. Following Eisenman's equilibrium theory of ion selectivity,

the filter's selectivity can be expressed in terms of the free energy $\Delta G^x$ for replacing the native $\text{H}_3\text{O}^+$ bound inside a model SF, $[\text{H}_3\text{O}^+\text{-SF}]$, with a rival ligand such as water, $\text{Na}^+$, $\text{Cl}^-$ or $\text{OH}^-$ (denoted as X)

$$X + [\text{H}_3\text{O}^+\text{-SF}] \rightarrow [X\text{-SF}] + \text{H}_3\text{O}^+$$  \hspace{1cm} (2)

The native $\text{H}_3\text{O}^+$ is preferred to the rival ligand X in the wild-type or mutant Hv1 SF if $\Delta G^x$ for eq 2 is positive or if $\Delta G^x$ for the reverse reaction, $[X\text{-SF}] + \text{H}_3\text{O}^+ \rightarrow X + [\text{H}_3\text{O}^+\text{-SF}]$, is negative. $\text{Na}^+$ or $\text{Cl}^-$ in the SF was unstable and was found near the side chain of opposite charge in the final optimized structures, precluding determination of its binding affinity.

The reaction in eq 1 or 2 was modeled to occur in vicinity of the SF so that the dielectric environment $\varepsilon$ was assumed to be uniform for all participating entities; the respective free energy was computed using the following thermodynamic cycle:

$$\Delta G^x = \Delta G^1 + \Delta \Delta G_{\text{solv}}^x$$  \hspace{1cm} (3)

Thus, the free energy for eq 1 or 2 can be computed as a sum of the gas-phase free energy $\Delta G^1$ and the solvation free energy $\Delta \Delta G_{\text{solv}}^x$ difference between the products and reactants; i.e.,

$$\Delta G^x = \Delta G^1 + \Delta \Delta G_{\text{solv}}^x$$  \hspace{1cm} (4)

The gas-phase free energy, $\Delta G^1$, was computed from the electronic energy ($\Delta E_{el}$), thermal energy ($\Delta E_{th}$), work term ($\Delta PV$), and entropy differences between products and reactants,

$$\Delta G^1 = \Delta E_{el} + \Delta E_{th} + \Delta PV - T \Delta S$$  \hspace{1cm} (4)

The thermal energies including zero-point energy and entropies were computed from the B3-LYP/6-31+G(3d,p) frequencies scaled by an empirical factor of 0.96135.

The solvation energy, $\Delta G_{\text{solv}}^x$, was estimated by solving Poisson's equation with the MEAD program54 using natural bond orbital atomic charges52 and the following effective solute radii (in Å): $R_{\text{H}} = 1.50$, $R_{\text{Na(H}_3\text{O}^+)} = 1.05$, $R_{\text{Na(OH}^-)} = 1.72$, $R_{\text{C}} = 1.95$, $R_{\text{N}} = 1.75$, $R_{\text{O(H}_2\text{O)}} = 1.85$, $R_{\text{O(H}_3\text{O}^+)} = 1.65$, $R_{\text{O(HO}^-)} = 1.64$, $R_{\text{O(COO}^-)} = 1.56$, and $R_{\text{C}} = 2.30$. The computed hydration free energies of the cations and ligands could reproduce the experimental values32,34,53 (Supplementary Table S2).

**Validation against Experimental Free Energies.** The methodology used to compute $\Delta G^x$ has been validated against experimental ion exchange free energies between biogenic metal cations ($\text{Na}^+$, $\text{K}^+$, and $\text{Ca}^{2+}$) in crown ethers, which resemble SF pores52, and in systems containing carboxylic ligands (nitritolriacetic acid)34. The computed metal exchange free energies can reproduce the corresponding experimental values to within 1 kcal/mol (Supplementary Table S3)32,34,53. The methodology has yielded trends in the free energy changes that are in accord with experimental findings32,36,53–56. It has also yielded calculated pore aperture areas in good agreement with experimental estimates (Supplementary Table S4).
References

1. DeCoursey, T. E. Voltage-gated proton channels: molecular biology, physiology, and pathophysiology of the Hv family. *Physiol. Rev.* 93, 599–652, doi:10.1152/physrev.00011.2012 (2013).

2. DeCoursey, T. E., Morgan, D. & Cherry, V. V. The voltage dependence of NADPH oxidase reveals why phagocytes need proton channels. *Nature* 422, 531–534, doi:10.1038/nature01523 (2003).

3. Henderson, L. M., Chappell, J. B. & Jones, O. T. G. The superoxide-generating NADPH oxidase of human neutrophils is electrogenic and associated with an H+ channel. *Biochem. J.* 236, 325–329 (1987).

4. Iwamatsu, A., Ikle, B. & Fischer, H. Function of the HvC21 proton channel in airway epithelia and a naturally occurring mutation, M91T. *J. Gen. Physiol. 136*, 35–46, doi:10.1085/jgp.200910379 (2010).

5. Musset, B. et al. A pH-stabilizing role of voltage-gated proton channels in IgE-mediated activation of human basophils. *Proc. Natl. Acad. Sci. USA* 105, 11020–11025, doi:10.1073/pnas.0800886105 (2008).

6. Lishko, P. V., Botchkina, I. L., Fedorenko, A. & Kirichok, Y. Acid extrusion from human spermatozoa is mediated by flagellar voltage-gated proton channel. *Cell* 140, 327–337, doi:10.1016/j.cell.2009.12.053 (2010).

7. Seredenina, T., Demaurex, N. & Krause, K. H. Voltage-gated proton channels as novel drug targets: From NADPH oxidase regulation to sperm biology. *Antioxid. Redox Signal* in press, doi:10.1089/ars.2013.5806 (2014).

8. Wu, L. J. et al. The voltage-gated proton channel Hv1 enhances brain damage from ischemic stroke. *Nat. Neurosci.* 15, 565–573, doi:10.1038/nn.3059 (2012).

9. Wang, Y., Li, S. J., Wu, X., Che, Y. & Li, Q. Clinicopathological and biological significance of human voltage-gated proton channel Hv1 over-expression in breast cancer. *J. Biol. Chem.* 287, 13877–13888, doi:10.1074/jbc.M112.345280 (2012).

10. Hondares, E. et al. Enhanced activation of an amino-terminally truncated isoform of the voltage-gated proton channel HvC21 enriched in malignant B cells. *Proc. Natl. Acad. Sci. USA* 111, 18078–18083 (2014).

11. Smith, S. M. E. et al. Voltage-gated proton channel in a dinoflagellate. *Proc. Natl. Acad. Sci. USA* 108, 18162–18167, doi:10.1073/pnas.1115405108 (2011).

12. Taylor, A. R., Chracchi, A., Wheeler, G., Goddard, H. & Brownlee, C. A voltage-gated H+ channel underlying pH homeostasis in calcifying coccidophores. *PLoS Biol.* 9, e1001085, doi:10.1371/journal.pbio.1001085 (2011).

13. Musset, B. et al. Aspartate 112 is the selectivity filter of the human voltage-gated proton channel. *Nature* 480, 273–277, doi:10.1038/nature10551 (2011).

14. Kulleperuma, K. et al. Construction and validation of a homology model of the human voltage-gated proton channel HvH1. *J. Gen. Physiol. 141*, 445–465, doi:10.1085/jgp.201210856 (2013).

15. Chamberlin, A., Qu, F., Wang, T., Noskov, S. Y. & Larsson, H. P. Mapping the gating and permeation pathways in the voltage-gated proton channel Hv1. *J. Mol. Biol. 417*, 131–145, doi:10.1016/j.jmb.2014.11.018 (2015).

16. Wood, M. L. et al. Water wires in atomistic models of the Hv1 proton channel. *Biochim. Biophys. Acta* 1818, 286–293, doi:10.1016/j.bbamem.2011.07.045 (2012).

17. Chamberlin, A. et al. Hydrophobic plug functions as a gate in voltage-gated proton channels. *Proc. Natl. Acad. Sci. USA* 111, E272–282, doi:10.1073/pnas.1318018111 (2014).

18. Li, Q. et al. Structural mechanism of voltage-dependent gating in an isolated voltage-sensing domain. *Nat. Struct. Mol. Biol.* 21, 244–252 (2014).

19. Morgan, D. et al. Peregirination of the selectivity filter delineates the pore of the human voltage-gated proton channel HvH1. *J. Gen. Physiol. 142*, 625–640, doi:10.1085/jgp.201311045 (2013).

20. DeCoursey, T. E. & Cherry, V. V. Voltage-activated hydrogen ion currents. *J. Membr. Biol.* 141, 203–223, doi:10.1007/BF00235130 (1994).

21. Takeshita, K. et al. X-ray crystal structure of voltage-gated proton channel. *Nat. Struct. Mol. Biol.* 21, 352–357, doi:10.1038/nsmb.2783 (2014).

22. Pupo, A., Baet-Nieto, D., Martinez, A., Latorre, R. & González, C. Proton channel models. *Channels (Austin)* 8, 180–192, doi:10.4161/chan.28665 (2014).

23. Ramsey, I. S. et al. An aqueous H+ permeation pathway in the voltage-gated proton channel Hv1. *Nat. Struct. Mol. Biol.* 17, 869–875, doi:10.1038/nsmb.2865 (2010).

24. Levy, D. G., Elias, S. R. & Hautman, J. M. Number of water molecules coupled to the transport of sodium, potassium and hydrogen ions via gramicidin, nonactin or valinomycin. *Biochim. Biophys. Acta* 512, 436–451, doi:10.1016/0005-2766(78)90266-3 (1978).

25. DeCoursey, T. E. & Hosler, J. Philosophy of voltage-gated proton channels. *J. R. Soc. Interface* 11, 20130799, doi:10.1098/rsif.2013.0799 (2014).

26. Musset, B. et al. Zinc inhibition of monomeric and dimeric proton channels suggests cooperative gating. *J. Physiol.* 588, 1435–1449, doi:10.1113/physiol.2010.183318 (2010).

27. Byerly, L., Meech, R. & Moody, W., Jr. Rapidly activating hydrogen ion currents in perfused neurons of the snail. *Lymnaea stagnalis. J. Physiol.* 351, 199–216 (1984).

28. DeCoursey, T. E. Voltage-gated proton channels and other proton transport pathways. *Physiol. Rev.* 83, 475–579, doi:10.1152/physrev.00028.2002 (2003).

29. Dudev, T. & Lim, C. Determinants of K+ vs. Na+ selectivity in potassium channels. *J. Am. Chem. Soc.* 131, 8092–8101 (2009).

30. Dudev, T. & Lim, C. Factors governing the Na+ vs. K+ selectivity in sodium ion channels. *J. Am. Chem. Soc.* 132, 2321–2332 (2010).

31. Dudev, T. & Lim, C. Competition among Ca2+, Mg2+, and Na+ for ion channel selectivity filters: Determinants of metal ion selectivity. *J. Phys. Chem. B* 116, 10703–10714 (2012).

32. Dudev, T. & Lim, C. Importance of metal hydration on the selectivity of Mg2+ vs. Ca2+ in magnesium ion channels. *J. Am. Chem. Soc.* 135, 17200–17208, doi:10.1021/ja087769 (2013).

33. Dudev, T. & Lim, C. Evolution of eukaryotic ion channels: Principles underlying the conversion of Ca2+-selective to Na+-selective channels. *J. Am. Chem. Soc.* 136, 3535–3539, doi:10.1021/ja087769 (2014).

34. Guillén Schlippe, Y. V. & Hedstrom, L. A twisted base? The role of arginine in enzyme-catalyzed proton abstractions. *Arch. Biochem. Biophys.* 433, 266–278 (2005).

35. Mohammed, O. F., Pines, D., Dreyer, J., Pines, E. & Nibbering, E. T. Sequential proton transfer through water bridges in acid-base reactions. *Science* 310, 83–86, doi:10.1126/science.1117756 (2005).

36. Favre, L., Moczydlowski, E. & Schmid, L. On the structural basis for ionic selectivity among Na, K and Ca in the voltage-gated sodium channel. *Biophys. J.* 71, 3110–3125 (1996).
40. Gonzalez, C., Rebolledo, S., Perez, M. E. & Larsson, H. P. Molecular mechanism of voltage sensing in voltage-gated proton channels. *J. Gen. Physiol.* **141**, 275–285, doi:10.1085/jgp.201210857 (2013).
41. Dani, J. A. & Levitt, D. G. Water transport and ion-water interaction in the gramicidin channel. *Biophys. J.* **35**, 501–508 (1981).
42. Hodgkin, A. L. & Keynes, R. D. The potassium permeability of a giant nerve fibre. *J. Physiol.* **128**, 61–88 (1955).
43. Miller, M. J., Oldenburg, M. & Fillingame, R. H. The essential carboxyl group in subunit c of the F1F0 ATP synthase can be moved and H+-translocating function retained. *Proc. Natl. Acad. Sci. USA* **87**, 4900–4904 (1990).
44. Luoto, H. H., Nordbo, E., Baykov, A. A., Lahti, R. & Malinen, A. M. Membrane Na+-pyrophosphatases can transport protons at low sodium concentrations. *J. Biol. Chem.* **288**, 35489–35499, doi:10.1074/jbc.M113.510905 (2013).
45. Lin, S. M. *et al.* Crystal structure of a membrane-embedded H+-translocating pyrophosphatase. *Nature* **484**, 399–403 (2012).
46. Reed, A., Weinstock, R. & Weinhold, F. Natural population analysis. *J. Chem. Phys.* **83**, 735–746 (1985).
47. Reed, T. & Lim, C. Bidentate vs. monodentate carboxylate coordination modes in magnesium and calcium proteins: What are the basic principles? *J. Am. Chem. Soc.* **127**, 4091–4103 (2005).
48. Dudev, T. & Lim, C. Competition between Li+ and Mg2+ in Metalloproteins. Implications for Lithium Therapy. *J. Am. Chem. Soc.* **133**, 9506–9515 (2011).

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**Author Contributions**

T.D. performed the calculations. B.M., D.M., and V.C. conducted patch-clamp studies and analyzed results. S.M.E.S. provided constructs. S.M.E.S. and K.M. performed PDB data analysis. T.D., S.M.E.S. and K.M. prepared figures, T.E.D. and C.L. designed the project and discussed results. T.D., T.E.D., and C.L. participated in writing the manuscript.

**Additional Information**

Supplementary information accompanies this paper at http://www.nature.com/srep

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