Calculation of Static ^{19}F Chemical-Shift Span. The rotationally averaged chemical shift span of ^{19}F in 6F-Trp-41 of the M2 TMD was calculated using the following principal values of the chemical shift tensor: δ_{11} = -159 ppm, δ_{22} = -130 ppm, and δ_{33} = -71 ppm (1). The direction of $\hat{\delta}_{11}$ is along the indole normal and the direction of $\hat{\delta}_{22}$ is along the F-C vector (see Fig. S3). Let β be the angle between the external magnetic field and $\hat{\delta}_{11}$, and α be the angle between the projection of the magnetic field into the indole plane and $\hat{\delta}_{22}$. Then the rotationally averaged chemical shift span is (2)

$$\Delta\delta = \delta_{11}\frac{3\cos^2\beta - 1}{2} + \delta_{22}\frac{3\sin^2\beta\cos^2\alpha - 1}{2} + \delta_{33}\frac{3\sin^2\beta\sin^2\alpha - 1}{2} \quad [\mathbf{1}].$$

At a given α, the span is maximal at $\beta = 90°$. At a given β, the span is a symmetric function of α, maximizing at $\alpha = \pm 90°$.

We note that the span can be rewritten as

$$\Delta\delta = \delta_{11}P_2(u_{11}) + \delta_{22}P_2(u_{22}) + \delta_{33}P_2(u_{33}) \quad [\mathbf{2}].$$

where u_{ii} are the direction cosines of the unit vectors $\hat{\delta}_{ii}$ along the external magnetic field. Eq. **2** was used for calculating the chemical shift span. The results were separately averaged over the small- and large-kink populations.

pH-Dependent Conformational Change Proposed by Manor et al. (3). We propose that a main effect of lowering pH is to increase the large-kink population. This proposition is supported by solid-state NMR (1, 4) and disulfide cross-linking (5) experiments. In contrast, the differences in the IR data between high and low pH have been interpreted to support a change in the rotation about the helical axis (3). The interpretation was based on modeling the M2 TMD as a symmetric tetramer comprised of ideal, rigid helices; however, the present and previous (6) simulation studies and a large number of experimental studies (4, 7-12) have suggested that the M2 TMD possesses significant conformational heterogeneity and exhibits helix kinking. No evidence for a significant helix rotation upon pH activation has been presented by any of the other experimental data. Although the IR approach is promising, the structural result was extracted from a limited set of data along the helical backbone. It is also possible that the organic solvent in the sample preparation protocol for this study may have influenced the structure and dynamics, as has been previously shown for the M2 TMD (4). While a great deal of work has gone into the characterization of the ^{15}N chemical shift tensors for solid-state NMR studies (13), the orientation of the vibrational IR transition moment is still a subject of considerable debate (14).

Kinetic Model for Proton Conductance. In our model for proton conductance in the M2 protein (see Fig. S6), protons can bind to an internal site, His-37, from both the virus

exterior and interior. The rate constants are denoted by k_{ex+} and k_{in+}, respectively. A bound proton can be released to either the exterior or interior, with rate constants k_{ex-} and k_{in-}. Let the proton concentrations in the exterior and interior be $[H^+]_{ex}$ and $[H^+]_{in}$ (note that pH = –log[H^+]). The net proton flux is given by (15, 16)

$$I = \frac{(k_{in+}k_{ex-}[H^+]_{in} - k_{ex+}k_{in-}[H^+]_{ex})/(k_{ex-} + k_{in-})}{1 + \frac{k_{ex+}[H^+]_{ex} + k_{in+}[H^+]_{in}}{k_{ex-} + k_{in-}}} \quad [\mathbf{3}].$$

First we present the predictions of Eq. **3** in the absence of a voltage or similar electrochemical gradient across the membrane. We use a superScript "0" to signify the corresponding "intrinsic" rate constants (e.g., k^0_{ex+} for binding from the exterior). They satisfy the constraint

$$\frac{k^0_{ex-}}{k^0_{ex+}} = \frac{k^0_{in-}}{k^0_{in+}} = 10^{-pK_a} \quad [\mathbf{4}].$$

where pK_a denotes the pK_a value of the His-37 residue that undergoes protonation and deprotonation when protons are translocated. Let the resulting proton flux be denoted as I^0. Using Eq. **4** in Eq. **3**, it can be easily seen that $I^0 = 0$ when $[H^+]_{ex} = [H^+]_{in}$; I^0 [lt] 0 (i.e., inward flux) when $[H^+]_{ex}$ [mt] $[H^+]_{in}$; and I^0 [mt] 0 (i.e., outward flux) when $[H^+]_{ex}$ [lt] $[H^+]_{in}$. Moreover, at a given $[H^+]_{in}$, I^0 reaches a maximum magnitude of k^0_{in-} as $[H^+]_{ex} \rightarrow \infty$ (in practice, at low pH). Similarly, at a given $[H^+]_{ex}$, the outward proton flux reaches a maximum of k^0_{ex-} at low pH_{in}.

All of the illustrative calculations here use $k^0_{ex+} = 10^7\ M^{-1}s^{-1}$, $k^0_{ex-} = 10\ s^{-1}$, $k^0_{in+} = 10^8\ M^{-1}s^{-1}$, and $k^0_{in-} = 10^2\ s^{-1}$; they satisfy Eq. **4** with pK_a = 6 (17). Fig. S7 displays the inward proton flux as a function of pH_{ex} when pH_{in} = 7. Chizhmakov et al. (18) fitted their experimental data to a one-site binding function

$$I = \frac{I_{max}([H^+]_{in} - [H^+]_{ex})}{K_{app} + |[H^+]_{in} - [H^+]_{ex}|} \quad [\mathbf{5}].$$

where K_{app} is an apparent dissociation constant. The calculation results in Fig. S7 can be fitted to Eq. **5** extremely well with $I^0_{max} = k^0_{in-}$ and K_{app} = 12.1 μM. For comparison, the same figure also displays the outward proton flux as a function of pH_{in} when pH_{ex} = 7.

If the channel is modeled as a cylindrical pore (with a radius *a*) and the binding site modeled as an absorbing disk (located a distance *d* into the membrane from the exterior side), it was found (19)

$$k_{\mathrm{ex+}}^{0} = \frac{4Da}{1+\eta}$$ [**6**].

where $\eta = 4Dd/\pi D_1 a$, with D and D_1 denoting the proton diffusion constants in bulk solution and in the pore, respectively. If the binding site is located at the channel entrance (i.e., $d = 0$), then the binding rate constant would be $4Da$. By burying the binding site inside the pore, the binding rate constant is reduced by a factor of $1 + \eta$. The corresponding result for $k_{\mathrm{in+}}^{0}$ is obtained when d is replaced by $L - d$, where L is the full thickness of the membrane.

When the interior is maintained at an electric potential V_0 while the exterior is at zero potential, the electric potential within the membrane can be assumed to depend linearly on the depth into the membrane. The electric potential at the binding site is

$$V = V_0 d / L$$ [**7**].

The cross-membrane voltage perturbs the binding and unbinding rate constants. They now satisfy new constraints:

$$\frac{k_{\mathrm{ex-}}}{k_{\mathrm{ex+}}} = 10^{-\mathrm{p}K_{\mathrm{a}}} \exp(eV / RT)$$ [**8**]

$$\frac{k_{\mathrm{in-}}}{k_{\mathrm{in+}}} = 10^{-\mathrm{p}K_{\mathrm{a}}} \exp[-e(V_0 - V) / RT]$$ [**9**],

where e is the proton charge, R is the gas constant, and T is the absolute temperature. For the cylindrical-pore model, it can be shown (19)

$$\frac{k_{\mathrm{ex+}}}{k_{\mathrm{ex+}}^{0}(1+\eta)} = \frac{eV / RT}{eV / RT + \eta[\exp(eV / RT) - 1]}$$ [**10**].

The corresponding expression for $k_{\mathrm{in+}}$ is obtained when V is replaced by $-(V_0 - V)$ and d replaced by $L - d$. Eqs. **8** and **9** then allow the unbinding rate constants to be determined. In Fig. S8, we display the voltage-proton flux relation at $\mathrm{pH_{in}} = 7$ and $\mathrm{pH_{ex}} = 5$ (values of the intrinsic rate constants are given above; the other parameters are $d/L = 0.6$, $\eta = 10$, and $T = 298$ K). This curve is qualitatively similar to corresponding experimental plots of Chizhmakov et al. (18) (see, e.g., their figures 2*B* and 6*C*).

In our structural model for the activation of the M2 proton channel by pH (see Fig. 5), the protein is assumed to rapidly exchange between two populations, with small and large helix-kink angles, in both the protonated and unprotonated states. In each population, proton binding and unbinding can occur. Eq. **3** can be extended to account for such population exchanges (15). If the exchanges are fast relative to proton binding and unbinding, as assumed for the M2 protein, then the proton flux is still given by Eq. **3**, but

the effective binding and unbinding rate constants are given by the weighted averages of the two populations:

$$k_{ex+} = f^{0S}k_{ex+}^{0S} + f^{0L}k_{ex+}^{0L}; \quad k_{in+} = f^{0S}k_{in+}^{0S} + f^{0L}k_{in+}^{0L} \quad [\mathbf{11}]$$

$$k_{ex-} = f^{1S}k_{ex-}^{1S} + f^{1L}k_{ex-}^{1L}; \quad k_{in-} = f^{1S}k_{in-}^{1S} + f^{1L}k_{in-}^{1L} \quad [\mathbf{12}].$$

where, e.g., f^{0S} is the fraction of the small-kink population in the unprotonated state, and k_{in+}^{0L} is the rate constant for binding a proton from the interior side while the protein adopts a large-kink conformation. Beyond the slowing down factor of $1 + \eta$ due to the burial of the binding site in the channel pore (see Eq. **6**), at least two mechanisms can further reduce the rate constants. The first is a constriction, such as presented by Val-27 on the N-terminal side of the pore (20), that disappears only some of the time to allow the passage of protons. The second mechanism arises when only one of the populations, such as the large-kink population, is efficient for proton uptake or release. In this case $k_{in+} \approx f^{0L}k_{in+}^{0L}$ and $k_{in-} \approx f^{1L}k_{in-}^{1L}$. That the large-kink population is a minority in both the protonated and unprotonated states (i.e., f^{0L} and $f^{1L} \ll 1$) can significantly limit these rate constants.

The M2 protein is a tetramer with four symmetric His-37 residues; two of the pK_a values are much higher than 6 and one much lower than 6 (17). Eq. **3** can be extended to such a four-site model (16); the results can be reproduced well by the one-site model with slight adjustment of parameters.

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