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Functional Dynamics of Ion Channels: Modulation of Proton Movement by Conformational Switches

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Abstract: Detailed comparative studies of proton relay in native and chemically modified gramicidin channels provide a unique opportunity to uncover the structural basis of biological proton transport. The function of ion channels hinges on their ability to provide surrogate solvation in narrow pore filters so as to overcome the dielectric barrier presented by biological membranes. In the potassium channel KcsA and in the cation channel gramicidin, permeant selectivity and mobility are determined by the proteinaceous matrix via hydrogen bonding, charge-dipole, and dipole−dipole interactions. In particular, main-chain carbonyl groups in these pore interiors play an essential role in the solvation of alkali ions and of protons. In this study, molecular dynamics simulations reveal how the translocation of H^+ is controlled by nanosecond conformational transitions exchanging distorted states of the peptidic backbone in the single-file region of a dioxolane-linked analogue of the gramicidin dimer. These results underline the functional role of channel dynamics and provide a mechanism for the modulation of proton currents by fluctuating dipoles.

Introduction

Understanding the molecular determinants of proton movement in membrane proteins is necessary in order to elucidate biological energy conversion.[1] In Grotthuss relay mechanisms,[2] the rapid long-range transport of protons is mediated by small fluctuations in the arrangement of relay groups forming hydrogen-bonded networks.[3] Heavy-atom displacements of the order of 0.01−0.1 nm result in H^+ translocation over distances of 1 nm in nanosecond time scales. This process of structural diffusion is beginning to be understood in bulk water[4,5] and in the array of water molecules (proton wires) embedded in the gramicidin channel.[6,7]

Gramicidin is a pentadecapeptide that assembles as a noncovalent head-to-head dimer in lipid bilayers to form a channel permeable to small monovalent cations.[8] Its main chain adopts a right-handed β-helical structure resulting in a narrow cylindrical pore lined with peptide bonds.[9,10] The lumen accommodates a single file of up to eight water molecules (water wire) that mediates the passive conduction of protons via a hop-and-turn Grotthuss mechanism.[11,12] Structural fluctuations of the hydrogen-bonded network involving water molecules and the channel backbone give rise to the translocation of an ionic defect (movement of the excess proton via successive transfers or hops of H nuclei) and of a bonding or turning defect (reorientation of the water chain) restoring the polarization of the wire prior to the passage of another proton in the same direction.[7] The local environment primes the wire for both hop and turn steps. Hydrogen-bond donation by each water molecule in the wire to carbonyl O atoms of the channel helps solvate both elementary forms of the hydrated proton, namely, the hydronium (OH_3^+) and Zundel ($O_2H_5^+$) cations, and catalyzes the reorganization of the network.[6]

Chemical modifications of gramicidin A (gA) offer a unique avenue to refine our understanding of structural diffusion at the molecular level. The insertion of the five-member ring dioxolane between the two formylated N-termini of gramicidin results in covalently linked dimers that form channels.[13] The two diastereoisomeric forms of the linked dimers, respectively, SS and RR, differ in their proton permeation properties.[14] The proton conductance, g_H, of the RR channel is reduced by a factor of 2 to 4 compared to that of native and SS dimers. Detailed atomic models of the linked channels suggest that functional differences are a consequence of structural distortions in the middle of the channel.[15] Here, we investigate the physical basis for the modulation of proton currents through a comparative study of the hop-and-turn mechanism in native, SS, and RR dimers. To this end, we use molecular dynamics simulations extending to several tens of nanoseconds (see Methods). Because this time scale well exceeds that of structural diffusion in the lumen,[7]

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this study provides unprecedented insight into the functional role of channel dynamics. Free energy profiles characterize the interplay between structural fluctuations of the water-filled channels and the translocation of a bonding defect and of an excess proton in the water wire.

Methods

The molecular system consisted of the dimers together with eight water molecules in the single file region, two at the interface, and cylindrical caps of water molecules at each mouth of the channel as in earlier studies.[6] New parameters for the dioxolane linkers based on ab initio calculations were developed[15] and combined with the CHARMM22 force field[16] used for the polypeptide. The conventional TIP3P water model[17] was used to represent the wire in the turn step, whereas the PM6 model,[18,19] a polarizable and dissociable empirical force field consisting of O^{2-} and H^+ moieties, was used as before in both turn and hop steps.[6] Matching caps of 14 water molecules were used for the turn step, whereas caps of 36 TIP3P water molecules were employed for the hop step. No cutoff was employed in the calculation of nonbonded interactions, and a dielectric constant of 1 was used throughout. A previous study of proton translocation in native gA suggested that caps of 36 water molecules are sufficiently large to capture the primary influence of outlying water on the distribution of the excess proton in the single-file region.[6] As described elsewhere,[6] artificial restraints were applied to the eight Trp indole rings of the channel, to prevent unfolding in the absence of the lipid bilayer, and to water molecules, to prevent diffusion and evaporation. Despite the neglect of the lipid bilayer and the use of artificial restraints, the average tilts of peptide planes and their dynamic fluctuations were shown in an earlier study of the dimers in vacuo[15] to be commensurate with experimental data obtained in NMR studies of native gA in lipid. Results reported below for turn and hop steps in the native gA dimer were obtained, respectively, from a previous study[6] and from its extension by 6 ns. The initial structures of the SS and RR dioxolane-linked dimers were taken from a previous study of the empty channels.[15]

Langevin integration with a friction coefficient of 5 ps^{-1} imposed on heavy atoms was propagated at 300 K with the CHARMM program[20] to generate molecular dynamics trajectories for each of the two linked channels, successively without and with an excess proton. Each simulation of the unprotonated linked dimers with the TIP3P water model was carried out with an integration time step of 2 fs and extended to a total of at least 20 ns of equilibration and 36 ns of production. All the simulations with the PM6 model used a time step of 1 fs. They extended to 12 and to at least 36 ns for the unprotonated SS and RR dimers, respectively. In the latter case, umbrella simulations were employed to improve the sampling efficiency along μ_z, the axial component of the total dipole moment of the water wire. Biasing potentials of the form $U(\mu_z) = {}^1/_2 k_\mu(\mu_z - \mu_z^\circ)^2$ were imposed, with a force constant $k_\mu = 2.0$ kcal/mol e^{-2} $Å^{-2}$ and μ_z° ranging from 0 to 9 e Å in increments of either 0.5 or 1 e Å. A set of umbrella calculations was also performed for each of the four conformers **1** to **4** with additional harmonic restraints on the RR linker's backbone torsions θ and θ' (see ref 15) with a force constant of 3.28 kcal/mol/rad^2 and reference $(\theta,\theta')_0 = (-105.0,-18.5)$, $(-36.0, -36.0)$, $(-18.5,-105.0)$, and $(-108.0, -108.0)$, respectively, for conformers **1**, **2**, **3**, and **4**. The system was reequilibrated as before[7] following the addition of an excess proton between two water molecules near $z = 0$, and hydrogen nuclei were subsequently free to move in the single-file chain without any bias on proton position or channel backbone. Simulations of the protonated wire in the linked dimers comprised 36 and 60 consecutive 1 ns trajectories, respectively, for the SS and RR channels.

Results

Both SS and RR linked channels conserve the overall secondary structure of native gA, with peptide planes nearly aligned with the helical axis (Figure 1B).[15] In all three channels, most peptide planes undergo rapid (picosecond to nanosecond) librations about the axis defined by the two adjacent C_α atoms, with an rms amplitude of $\pm 8°$ to $\pm 15°$ on average. Such plasticity of the channel backbone, which is commensurate with experimental data,[15] is thought to play a functional role: when they tilt toward the inside of the channel lumen, carbonyl groups help solvate alkali ions permeating through the pore via charge-dipole interactions.[8,21] In native and SS dimers, librations of the two N-terminal peptide planes, like those of all other peptide planes, are unimodal and centered near zero, which corresponds to alignment with the axis of the channel (Figure 1A,B). The only significant difference presented by the RR channel lies in the structure and fluctuations of the backbone next to the linker.[15] Distortions due to the chirality of the RR linker force the two peptide planes flanking the dioxolane ring to tilt out of alignment with the helix axis by 30° on average. Thermally activated transitions exchange the topology of each of the two central CO groups with respect to the channel lumen. Four conformers henceforth labeled **1** through **4** are characterized by these two carbonyl groups pointing (in, out), (out, out), (out, in), and (in, in), respectively (Figure 1C). In the absence of an excess proton, conformational switching across free energy activation barriers of 2.1 to 2.8 kcal/mol results in an equilibrium between the four RR conformers on a nanosecond time scale. Addition of an excess proton to the water wire displaces the equilibrium distribution by destabilizing (out, out) conformations. A similar effect is also visible in native and SS dimers. The relative preference for asymmetric conformers **1** and **3** is exacerbated by the presence of H^+.

The reorientation of water molecules results in the translocation of a bonding defect or turn step of the Grotthuss mechanism.[3,7] Comparison of potential of mean-force (PMF) profiles to invert the polarity of the unprotonated wire (Figure 2A) shows that the polarization of water molecules in the middle of the pore is highly sensitive to the topology of carbonyl groups at the dimer junction. This effect is highly consistent for both water models used in the study. Although the wire is polarized in native gA,[6] there is no such preference in the SS dimer, and each conformer of the RR channel gives rise to a different profile. The reversible thermodynamic work required to reorient the water wire is determined by the topology of the CO groups adjacent to the RR dioxolane ring. When a CO points out, a substantial activation energy barrier (similar to that of native gA) opposes the reorientation of the three innermost water molecules in the same monomer moiety, whereas when the CO points in, the process is activationless (as in the SS dimer) (Figure 2B). These results underline the role of dipole−dipole as well as hydrogen-bonding interactions between water molecules and the channel in the migration of a bonding defect.

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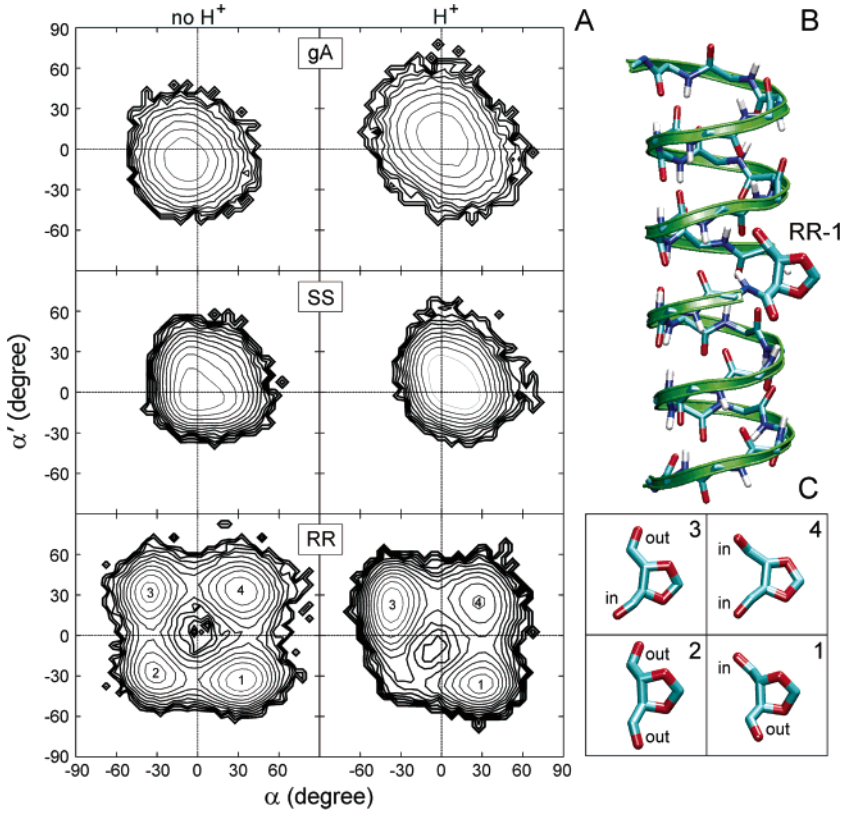

Figure 1. (A) Potential of mean force for backbone fluctuations, respectively, (top) in native gA and (middle) in SS and (bottom) RR linked dimers. The orientation of the two N-terminal peptide planes is characterized by the tilt angles α and α′, which are defined as zero when the mean plane of the amide group is collinear with the channel axis and positive when the carbonyl group points into the lumen. Left: with water only. Right: with water and an excess proton in the channel lumen. Contour spacing is 0.5 kcal/mol. The small deviation from symmetry with respect to the $\alpha = \alpha'$ diagonal suggests adequate statistical convergence. (B) Representative snapshot of the backbone of the RR dimer emphasizing the linker and its two flanking CO groups, which are displayed with thicker bonds. The two carbonyl groups bonded to the dioxolane ring in the top and bottom gramicidin monomers are, respectively, in and out of the lumen (conformer **1**). (C) Representative snapshots of the RR linker in this and subsequent figures highlighting the topology of carbonyl groups in conformers **1** through **4**. Structural representations in this and subsequent figures were prepared with the program VMD.[32]

These results also suggest that the activation energy barrier for water reorientation in native gA is due to the significant preference for an outward tilt of both N-terminal CO groups in the absence of H^+ (see Figure 1A).

Backbone distortions of the RR linked channel modulate the equilibrium distribution of the excess proton (Figure 3). The effect of linkage depends on the chirality of the dioxolane ring (Figure 3A). The relative proton affinity of the eight single-file water molecules in the SS channel is essentially identical to that in native gA, with a broad distribution peaking near the dimeric interface (water molecules #5 and #6). By contrast, the average proton distribution peaks in each monomer moiety of the RR channel. The mean position of the excess proton strongly depends on the conformation of the RR linker. In each of the asymmetric conformers **1** and **3**, H^+ is preferentially hosted in the monomer in which one of the two CO groups tilts into the lumen, by or near a water molecule aligned with the amide dipole moment (Figure 3A,B). In confomer **4**, where both CO groups point in, the sum of the two amide dipole moments is aligned with the dimer interface and the excess proton is preferentially shared by the two central water molecules in a Zundel cation.

The nature of the coupling between proton movement and conformational isomerization of the RR linker is revealed by the two-dimensional free energy surface correlating the position of the excess proton to the linker conformation (Figure 3C). Each of the three channel conformations **4**, **3**, and **1** corresponds to a trough in the free energy surface. The fact that the position of the two ridges separating these three valleys is essentially independent of the proton position (i.e., they are aligned with the ordinate axis) indicates that the isomerization of the channel backbone controls the progress of the ionic defect, not the other way around. The movement of the excess proton from one end of the single-file region to the other requires conformational isomerization of the linker, whereas conformational isomerization does not require proton movement. Thus, an excess proton starting from the upper part of the channel (top right corner region of Figure 3C) dwells in the top monomer in conformation **1**, with the top CO pointing in; following an activated transition flipping the bottom CO from *out* to *in* (activation free energy of 1.8 ± 0.2 kcal/mol), the proton becomes localized in the middle of the channel with both COs pointing in (conformer **4**). This conformation constitutes an intermediate state in the preferred translocation pathway. From there, the channel backbone rapidly decays either back to **1** or on to **3**, following the flip of the top CO from *in* to *out*. In the latter case, the excess proton completes its journey past the middle turn of the helix and on to the lower monomer (bottom left corner).

Discussion

These results highlight the role of backbone carbonyl groups and of nanosecond fluctuations of the polar environment in proton transport mechanisms. It was recognized early that rapid

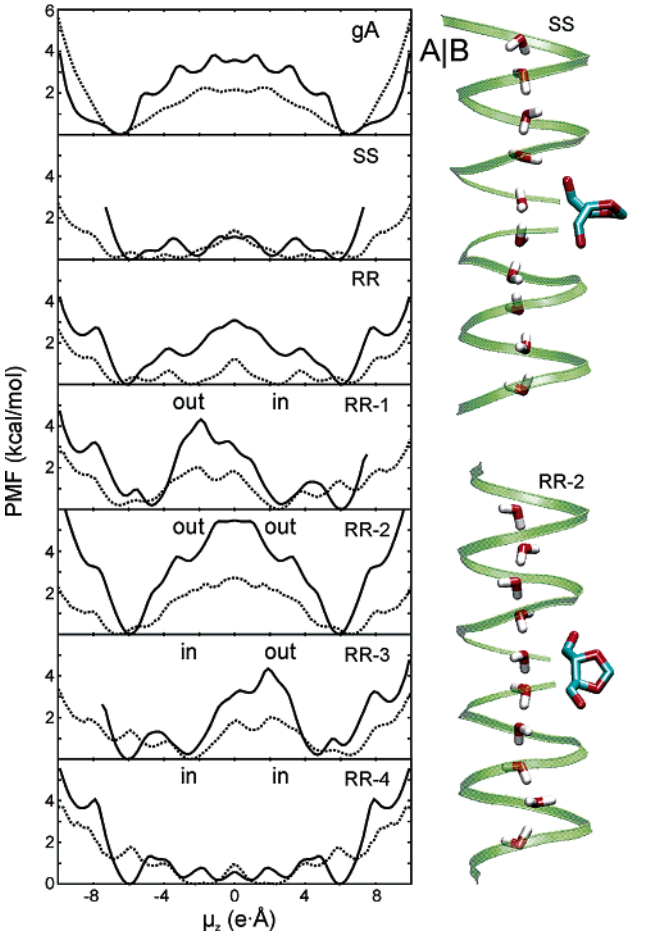

Figure 2. (A) Potential of mean-force profiles for the reorientation of water molecules in gA, SS, and RR channels and in RR conformers **1** through **4**. μ_z is the projection of the total molecular dipole of the single-file water chain along the channel axis. Results obtained with the PM6 and TIP3P water models are shown in solid and dashed lines, respectively. In the latter case, μ_z was scaled by 0.417, the partial charge of H atoms in the TIP3P model, for direct comparison with the PM6 model, in which the formal charge of H atoms is 1. The two water models yield qualitatively consistent profiles. Local minima and inflection points mark the progression of the bonding defect among the 10 water molecules from mouth to mouth. (B) Representative conformations of linked channels and their lumen contents. (Top) Half-oriented water chain in the SS channel; the bonding defect is on the fourth water molecule from the top, which donates a hydrogen to one of the two CO groups adjacent to the linker. (Bottom) Polarized water chain in conformer **2** of the RR channel; in that snapshot, the bonding defect is on the ninth water molecule.

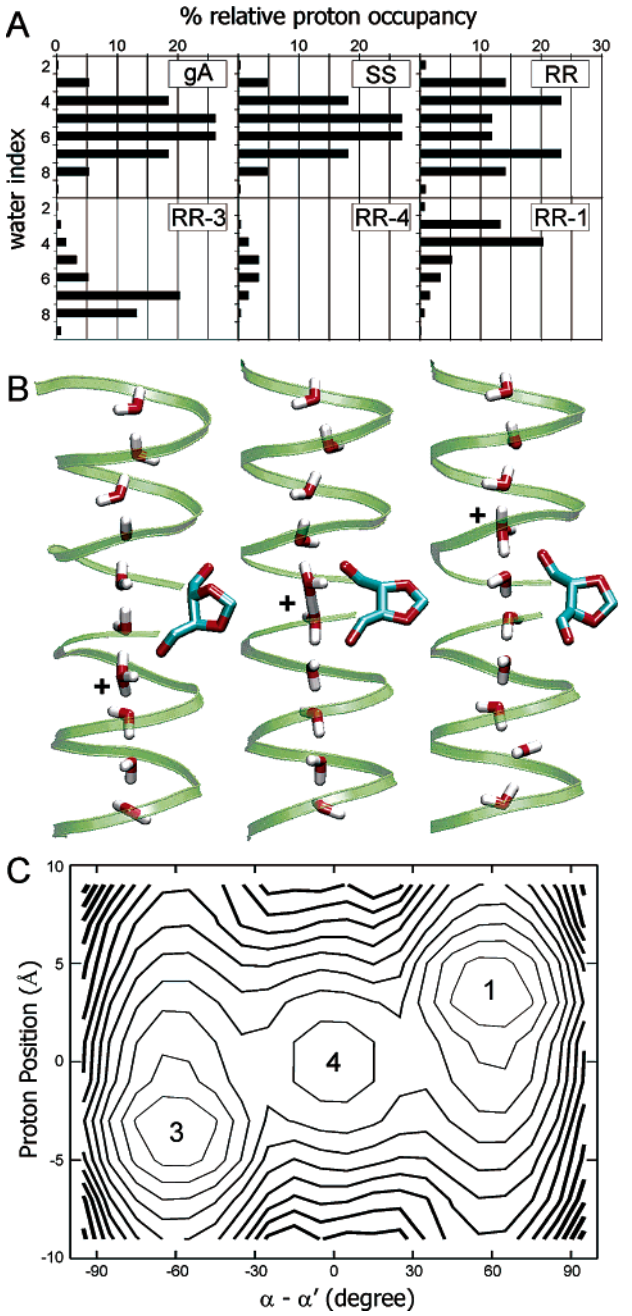

Figure 3. (A) Equilibrium distribution of the excess proton among the eight innermost single-file water molecules (indices 2 to 9) in native gA, in SS and RR channels, the latter of which is also shown broken down into RR conformers **3**, **4**, and **1**. The O atom hosting H^+ at any given time is determined as that for which the distance to the third-nearest H nucleus is shortest among the single-file water molecules. Escape of the excess proton from the channel was precluded by construction.[6] (B) Representative conformations of RR conformers **3**, **4**, and **1**, respectively, with H^+ hosted by water #7 as a hydronium ion, shared by waters #5 and #6 as a Zundel cation, and hosted by water #4 as hydronium. Note how the location of the excess charge is aligned with the dipole moment of the CO group(s) tilted into the lumen. (C) Potential of mean force for the position z_{H+} of an excess proton along the axis of the RR channel as a function of channel conformation. In the symmetric conformation **4**, the difference between the tilt angles of the two CO groups, $\alpha - \alpha'$, is near zero, whereas, in the two asymmetric conformations **1** and **3**, the difference is, respectively, large and positive and large and negative. Contour spacing is 0.5 kcal/mol. The lowest free energy pathway for the translocation of H^+ from end to end of the single file involves successive conformational switches exchanging the topology of the two central CO groups.

(picosecond) librations of the peptidic backbone play a role in the mobility of the lumen contents[22] and that a moderate inward tilt of carbonyl groups provides stabilization of alkali cations so as to make up for their partial desolvation in the narrow pore interior.[8] All the peptide carbonyl groups in the native and the SS-linked channels, and all but two of them in the RR channel, undergo such oscillations freely with an average tilt of 0° to ±20°.[15] The only significant difference between the three channels is that, in the RR linker, the tilt of two peptide planes is more pronounced and presents a bimodal character, with thermally activated transitions governing the exchange between inward- and outward-facing conformations of the carbonyl groups. Together, these two peptides appear to act as "push switches" that control the passage of protons.

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By comparing the molecular mechanism in highly analogous systems, we are able to ascribe experimentally measured functional differences to the structural and dynamic properties of the channel. While conformational switching modulates both hop and turn steps of the Grotthuss mechanism, its effect on the turn step is unlikely to control the overall rate of proton permeation through the pore. On one hand, proton conductance of the SS dimer is similar to that of native gA[14] despite the dramatic drop in the height of the activation free energy barrier opposing water reorientation (Figure 2). On the other hand, the proton distribution is nearly identical in native and SS channels but differs in the RR dimer. The asymmetric conformers of the RR linker are unique in their ability to promote the localization of H^+ in one monomer through charge-dipole interactions, suggesting a mechanism for the modulation of proton conduction, whereby activated switching of one of the two central peptides from outward to inward tilt limits the progress of the excess proton.

This work opens the way to comparative studies of structure−function relationships in proton ducts. More remains to be done to confront the results presented here to experimental conductance data using a detailed kinetic model.[23] It has been proposed that the exit of protons from the lumen is the rate-limiting step for proton transport in gA.[24] The present study focuses on the single-file region of the channel and does not exclude that the rate of proton entrance and/or exit could be modified indirectly by RR linkage, either through a change in the proton affinity of the channel relative to that of the bulk solution or through modulation of collective motions of the channel in its lipid environment. The effect of linkage on the transport properties of gA channels embedded in a lipid bilayer will be the object of further studies. Nevertheless, the above results afford unprecedented insight into the effect of nanosecond channel dynamics on proton movement.

Short- and long-range dipolar interactions play an essential role in biological ion transport. In gramicidin, solvation of protonated water molecules and of bonding defects is achieved locally by hydrogen bonds.[6,7] In the single-file region of aquaporin and water−glycerol channels,[25−27] permeating water molecules likewise form hydrogen bonds with backbone carbonyl O atoms, yet these channels are impermeable to H^+. Proton exclusion could be due in part to the adverse polarization of the water chain induced by dipole−dipole interactions with α helices lining the lumen.[28] The dipole moment of four α helices was shown to control charge selectivity in the potassium channel KcsA.[29] In the diffusion of K^+ ions through the selectivity filter of KcsA[30] and of alkali metal ions through gramicidin,[8] backbone carbonyl groups directly solvate the permeating ion via charge-dipole interactions. In the RR linked gramicidin dimer, the dipole moment of the channel backbone is modulated by thermal fluctuations. In the present work, we show how these dynamic effects interfere with the flow of protons, revealing the interplay between structural diffusion and structural fluctuations of the proton conduit and underlining the central importance of a fluctuating polar environment in charge translocation. The biological significance of this result extends to protein-mediated transport of protons in energy transduction. This work demonstrates that the control of proton translocation can be achieved by subtle modulations of the structure and dynamics of the proteic matrix. Such effects could have significant implications to molecular mechanisms of proton pumping, where coupling between thermodynamic control of proton movement by changes in the charge distribution in the enzyme and kinetic control by structural rearrangement of proton pathways[31] is paramount.

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