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ABSTRACT

Water is at the heart of almost all biological phenomena, without which no life that we know of would have been possible. It is a misleadingly complex liquid that exists in near coexistence with the vapor phase under ambient conditions. Confinement within a hydrophobic cavity can tip this balance enough to drive a cooperative dewetting transition. For a nanometer-scale pore, the dewetting transition leads to a stable dry state that is physically open but impermeable to ions. This phenomenon is often referred to as hydrophobic gating. Numerous transmembrane protein ion channels have now been observed to utilize hydrophobic gating in their activation and regulation. Here, we review recent theoretical, simulation, and experimental studies that together have started to establish the principles of hydrophobic gating and discuss how channels of various sizes, topologies, and biological functions can utilize these principles to control the thermodynamic properties of water within their interior pores for gating and regulation. Exciting opportunities remain in multiple areas, particularly on direct experimental detection of hydrophobic dewetting in biological channels and on understanding how the cell may control the hydrophobic gating in regulation of ion channels.

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I. INTRODUCTION

Water is the “matrix of life,” without which no life, that we know of, would be possible.1 With unique characteristics as a polar, protic, and amphoteric substance, water can act as both a reagent and a solvent in biological processes. Its dual nature as both proton donor and acceptor has facilitated many vital reactions and biomolecular recognition events. The relatively high dielectric constant of water gives rise to complex heterogeneous environments for electrostatic interactions of biomolecules. Strong polar interactions that exist among water molecules also underlie the hydrophobic interaction between nonpolar solutes, one of the key forces that derive biomolecular interactions and assemblies. These general physicochemical properties of water and their critical importance to the biomolecular structure, dynamics, and interactions are well appreciated.2-5 At the same time, water is a complex liquid with unique dynamic and structural properties.5-7 It is now recognized that the thermodynamic properties of water are also crucial to many biological functions,7 beyond merely providing an environment or “passive matrix” for facilitating the structure and motion of the biological molecules. Biomolecules can control and harness the unique water structure and dynamics under complex biological environments to enable functions, such that water should be considered an “active constituent” of biology.7

The cell is highly concentrated with small and large solutes, and water makes up only ~70% of the total weight. The average separation distance between macromolecules is only about 1 nm, or a few water layers. As a result, much of water in the cell is confined or constrained; it displays structure and dynamics that can be significantly different from those of the bulk liquid water.7 For example, there is a significant reduction (~10-fold) in the reorientational dynamics of the cell water in a hydration shell of a charged solute or in a bound state with other polar molecules. Highly concentrated biomolecules also reduce the freedom in forming the water hydrogen bond network, further restricting water orientational dynamics. In addition, the presence of nm-scale hydrophobic surfaces of macromolecules significantly perturbs the water hydrogen bond network.7 Importantly, under physiological temperatures and normal pressure, liquid and vapor phases of water are near coexistence and the balance can be readily shifted in either direction with very small free energy...
costs. As a result, the density fluctuation near a hydrophobic surface is significantly elevated. This Perspective briefly reviews how this unique thermodynamic property of water can give rise to complex phase dynamics in confinement and discusses recent progresses in understanding how transmembrane (TM) protein ion channels may control phase transitions of confined water within their conductive pathways for channel gating and regulation. We will end with a discussion of remaining questions and possible future directions.

II. “LIFE AT THE EDGE”: WATER’S UNIQUE DYNAMICS UNDER CONFINEMENT

Under ambient conditions, liquid water is near coexistence with its vapor state. The balance of this liquid–vapor coexistence near a surface can be readily perturbed by the geometric and physical properties of the surface. Confined water, in particular, shows distinctive behaviors, the nature of which is drastically different from its corresponding bulk properties. The effect of confinement is, to some extent, equivalent to an increase in density or pressure, which reduces the average number of hydrogen bonds formed per water molecule. The thermodynamic properties of confined water are hypersensitive to the surface properties of the confining walls. A hydrophobic surface, in particular, can cause a repelling effect, resulting in stronger interactions between water molecules and enhancing water density fluctuations. Within a spherical or cylindrical hydrophobic pore or between two extended hydrophobic surfaces, there exists a critical pore radius or separation distance in the nm range, below which pressure imbalance is created in the trapped water and destabilizes the liquid–vapor phase coexistence. The imbalance pushes the confined water toward the edge of its vapor phase and leads to the so-called capillary evaporation, a cooperative “dewetting” or drying transition where liquid water completely exits the pore region. This phenomenon is often referred to as “hydrophobic dewetting”; it is highly sensitive to the environmental conditions. Subtle changes in the surface hydrophobicity and hydrophilicity or the pore size and shape can tip this liquid–vapor balance back toward the liquid phase, leading to “rewetting.”

The theoretical basis of hydrophobic dewetting dates back to 1973 when Stillinger proposed that water could coexist with its vapor phase at the interface of a sufficiently large nonpolar surface. More recently, Lum, Chandler, and Weeks described a quantitative theory for the solvation of nonpolar species and predicted that depletion of hydrogen bonding between water molecules near extended nonpolar surfaces would result in a “crossover” to a vapor layer producing a dry vapor phase near the surface. Importantly, the theory predicts the free energy cost of creating spherical cavities in water scales with the volume for small cavities but with the surface area for larger ones. The crossover occurs once the radius of about 1 nm is reached under ambient conditions. The reason is that water can form fluid clathrate-like hydrogen-bonding structures around the smaller hydrophobic solute without dewetting. Subsequently, dewetting-based hydrophobic aggregations can minimize the entropic penalties water faces in close proximity to larger hydrophobic substances. The theoretical prediction of depletion of water near the extended hydrophobic surface has been verified by high-energy x-ray reflectivity measurements. These experiments showed that the depletion layer was on the order of one water molecule (~2 Å–4 Å) in thickness and independent of whether or not the water was degassed. While these experiments did not directly detect density fluctuations, molecular dynamics (MD) simulations have largely confirmed the existence of nm-scale density fluctuations near various hydrophobic model surfaces and provided important insights into how non-ideal surface properties such as the presence of hydrophilic groups and roughness modulated the liquid–vapor coexistence. Hydrophobic dewetting likely creates a major driving force that facilitates various biophysical processes including protein folding, binding, and assembly. For example, atomistic simulations were able to directly observe partial and complete dewetting transitions during the folding of a multidomain protein and self-assembly of the melittin tetramers, respectively.

III. HYDROPHOBIC DEWETTING IN MODEL NANOPORES

Determining the structural and dynamical behavior of water under confinement within the complex and heterogeneous biological context is highly nontrivial due to its sensitivity to detailed features of the confining surfaces including hydrophobicity, roughness, and topology. Studies of simplified model systems such as carbon nanotubes and model nanopores with well-defined topological and surface features have provided crucial insights into deriving the principles for predicting water dynamics in complex confined environments. For example, MD simulations of cylindrical nanopores with a central hydrophobic region revealed the importance of pore dimensions in driving the liquid–vapor balance of water within the pore. Drying transitions do not occur until the radius of a hydrophobic pore is less than ~7 Å, remain intermittent for the radius of ~6 Å–4.5 Å, and only become stable below this threshold radius [Figs. 1(a) and 1(b)]. It is notable that liquid water can go from stable to no longer stable in hydrophobic cavities with only ~3 Å reduction in radius. Further studies show that the pore radius threshold for triggering dewetting transitions is greatly reduced as the pore surface becomes less hydrophobic and completely disappears for hydrophilic pores [Fig. 1(b)]. The vapor region created by the hydrophobic constriction generates a high dielectric barrier for ions due to large free energy penalties required for dehydration of ions (the solvation free energies for Na+ and K+ ions are about ~391 kJ mol−1 and ~308 kJ mol−1, respectively). Indeed, MD simulations confirm that a dry pore is impermeable to ions, even though it is physically open and wide enough to accommodate the ion [Figs. 1(c) and 1(d)]. Such a mechanism for turning on and off the ion permeation pathway is referred to as “hydrophobic gating” or a “vapor-lock” gate. It is noteworthy that synthetic nanopores have been engineered based on these principles, where the pore-lining characteristics can be modulated through external stimuli such as electric field and light to trigger the pore wetting and dewetting. Given that the onset of “crossover” for triggering drying transitions occurs in the nm regime, which is pertinent to the interior cavities found in biological structures, there has been great interest in the last couple decades in understanding how biology may have explored hydrophobic gating in functional controls of biomolecules, particularly in gating and regulation of TM protein ion channels.
IV. HYDROPHOBIC GATING IN BIOLOGICAL ION CHANNELS

TM protein ion channels are key regulators of the flow of ions through cell membranes in response to various chemical and electrical signals. These channels usually consist of oligomers that enclose a central pathway (or pore) for ion permeation.\textsuperscript{35-39} The pathway generally contains a selectivity filter, which determines the types of permeating ions, and gating elements, which turn on and off the ion flow in response to various electrical and chemical signals. The central region of the permeation pathway of TM ion channels is often highly hydrophobic and has a diameter around or below the nm range (e.g., see Fig. 2). There is a clear parallel line between these pores and model nanopores to suggest the likelihood of hydrophobic gating in biological ion channels. Indeed, this idea has found support in an increasing number of computational and experimental studies in the last 15 years. Many of these channels were discussed in depth by Aryal, Sansom, and Tucker in their seminal review in 2015.\textsuperscript{8} Here, we summarize the key emerging principles and then focus on recent studies that together have greatly enriched our understanding of various ways biology has exploited to control hydrophobic dewetting to regulate channel activities.

TM protein channels that have been identified to utilize hydrophobic gating possess a wide range of functional, topological, and structural characteristics. They can be activated with different external stimuli such as chemical, mechanical, and electrical signals. In terms of protein size and topology, they expand a wide range from the dimeric two-pore domain TWIK-1 K2P\textsuperscript{44} to the pentameric nAChR\textsuperscript{30} and heptameric bacteria mechanosensitive channels.\textsuperscript{39} Strikingly, the pore size and lengths in these channels are also not in the same range as one might expect for channels sharing similar gating mechanisms. For example, while atomistic structures of the putative closed states reveal that the pore of nAChR is \( \sim 3 \) Å in radius at its narrowest section,\textsuperscript{51} that of BK channels is...
as large as ~7 Å in radius.\textsuperscript{55,56} Despite the functional and structural diversity, these channels seem to share important similarities in how hydrophobic dewetting is controlled within their interior pores. These pores are lined with large patches of hydrophobic residues and generally lack polar or charged side chains, which is expected from studies of the model nanopores.\textsuperscript{20,21,26} These hydrophobic motifs are often well conserved within the same class of ion channels.\textsuperscript{8,30,45,49,51} Coupled with the narrowness of the pore, the hydrophobic constriction region can readily undergo cooperative dewetting transitions (e.g., see Fig. 3), leading to a dry state that persists for extended periods of time and closes the channel for ion permeation. Atomistic simulations and structural studies further revealed

FIG. 2. Ion conduction pathways in four different potassium channels. Shown is a longitudinal section of the pore central region with carbon and sulfur atoms in yellow and the hydrophilic atoms in red. The position of the channel within the membrane has been pinpointed by dotted lines. The selectivity filters are located at narrowest portions, below which are nm-scale interior pore cavities (circled). The figure is obtained with permission from P. Aryal, M. S. P. Sansom, and S. J. Tucker, J. Mol. Biol. \textbf{427}, 121 (2015). Copyright 2015 Author(s), licensed under a Creative Commons Attribution 3.0 Unported License.

FIG. 3. Hydrophobic gating of the 5-HT3 receptor. (a) Cross section of the starting structure emphasizing the hydrated state of the pore region (red circle) (PDB ID: 4PIR). Protein (gray surface) is embedded in the membrane lipid, and only two subunits are shown for visual clarity. Water oxygen atoms are shown as blue spheres and lipid in liquorice. Only the TM domain has been shown. (b) Snapshots of the dewetted pore observed during the MD simulation showing lack of physical constriction. (c) Density of water in the pore region during the MD simulation showing the persistent and fast depletion of water in a ~2-nm region marked by the red arrow. The figure is obtained with permission from Klesse et al., J. Mol. Biol. \textbf{431}, 3353 (2019). Copyright 2019 Author(s), licensed under a Creative Commons Attribution 4.0 license.
another general feature of hydrophobic gating—there is a limited change in the pore geometry even though the channel conductance changes dramatically. As a result, structures of these channels solved in the presumably closed state often fail to reveal physical occlusion of the pore, leading to uncertainties in assigning the functional states captured in structural studies.

Several mechanisms have been observed on how the channels may be exploited to modulate the surface and geometric properties of the pore to control the hydrophobic gating. For example, the pore hydrophobicity could be manipulated by side chain reorientation of pore-lining residues, which would lead to either burial of the previously exposed hydrophilic residues or pore exposure of new hydrophobic side chains. For example, it was shown that polar groups such as glycine have been buried in the narrow closed conformations of the bacteria mechanosensitive channel but exposed to the pore in the open state of the channel. Modulation of the hydrophobic character of the pore-lining surface without substantial changes in the pore size and shape has particularly been observed in the TRPV1 channel. TRPV1 contains a narrow hydrophobic pore (minimal radius ~2 Å), and the motion of the polar side chain of Asn seems to be sufficient to trigger hydrophobic dewetting and lead to a stable inactive state.

More substantial reorientations of the pore-lining helices are required to modulate the pore hydrophobicity and size in order to trigger hydrophobic gating in many other channels. For this, tilting and twisting of the inner pore helices are frequently observed, whereby the hydrophobic residues are rotated away/toward the pore causing a shift in the balance of the wet and dry states. Specific molecular mechanisms of inner pore helix tilting and twisting are highly diverse. One of the most prevalent ones is helix bending and rotation facilitated by proline or glycine hinges, such as in the 5-HT3 receptor, bacterial mechanosensitive channels, and Kv1.2 and BK channels. These hinge motifs are usually located in the middle of the helix and highly conserved within the family of these channels. Depending on the channel conformational arrangements, tilting could result in an iris-like motion of the pore-lining surface.
helices and change in the pore's hydrophobic characteristics. For example, the pentameric GLICs undergo a two-stage tilting of the inner pore helices, resulting in a cooperative pore drying followed by an iris-like closing of the channel pore [e.g., see Fig. 4(b)].

The rearrangement of helices changes the polar angle of the pore-lining atoms and alters their distances from the symmetry axis. Iris-like rotations have also been observed in other pentameric and heptameric ion channels such as the magnesium channel CorA and bacteria mechanosensitive channels. Rearrangement of pore-lining helices could also be triggered by a π-helix to α-helix transition. This mechanism has been observed in the PKD2 channel (polycystin-2 or TRPP2), a member of the TRP family, leading to twisting and splaying the hydrophobic residues, albeit with a moderate structural resolution of 3.5 Å. Located in the middle of the inner pore helices, the π-helix to α-helix transition is accompanied by expansion of the hydrophobic constriction and thus channel activation. In general, it has been shown that the flexing of the inner pore helices not only modulates the pore hydrophobicity but also brings the pore size closer to the threshold, which can increase the thermodynamic possibility of undergoing a dewetting transition.

Recent studies of BK channels highlight how nature has given rise to an extremely effective strategy to control hydrophobic dewetting for channel gating and regulation. The long-awaited high-resolution structures of BK channels were first determined using cryo-EM in 2017. The structures reveal that the large pore under the selectivity filter remains widely open and has a radius of ∼7 Å even in the Ca$^{2+}$-free and presumably deactivated state.
disappointing, this observation is actually consistent with previous studies showing that the BK pore is accessible to organic molecules in both open and closed states.\textsuperscript{54,62} The prevailing hypothesis has thus been that BK channels may be gated at the selectivity filter itself.\textsuperscript{8-10} Closer inspection of the Ca\textsuperscript{2+}-bound and Ca\textsuperscript{2+}-free structures later revealed that Ca\textsuperscript{2+} binding led to bending and twisting of the pore-lining S6 helices, which projected two conserved acidic residue side chains (E321 and E324) away from the pore and at the same time exposed a hydrophobic patch (e.g., V319 and I323) to the pore [Fig. 5(a)]. As a result, the pore becomes longer, narrower, and, importantly, much more hydrophobic in the Ca\textsuperscript{2+}-free state [Fig. 5(b)]. Atomistic simulations show that this significant remodeling of the pore allows it to readily undergo hydrophobic dewetting transitions [Fig. 5(c)].\textsuperscript{36} The dry state of the pore gives rise to a large barrier for ion permeation but remains accessible to small organic molecules such as channel blockers and side chain modifying reagents with minimal free energy barriers. Thus, the hydrophobic gating mechanism provides a simple and direct explanation for how the pore can remain physically open and accessible to relatively large molecules but not permeable to ions. It is also consistent with a systematic scanning mutagenesis study that revealed a striking correlation between the hydration tendency of the side chain and the channel opening probability [e.g., Fig. 5(d)].\textsuperscript{57,58} Nonetheless, the discovery of hydrophobic gating in BK channels is somewhat surprising because the pore is near or above the nm-scale limit generally believed to be necessary for hydrophobic dewetting (such as radius) or some overall hydrophobicity measures are prone to errors in predicting hydrophobic gating. This reflects a wide variety of pore size, shape, hydrophobicity, and hydrophilicity that exist in biological channels.

It has been recognized that a more reliable proxy to predict the hydrophobic gate in ion channels is the pore dehydration itself.\textsuperscript{8} This property could be extracted from short atomistic simulations of 10 nanoseconds (ns), with the channel restrained during the simulations to prevent significant conformational drift from the experimentally determined structures.\textsuperscript{8,9} An automatic simulation and analysis pipeline named Channel Annotation Package (CHAP) has been developed to simultaneously analyze pore radius and hydrophobicity as well as the free energy of water along the channel permeation pathway using short MD simulations for more accurate functional annotation of channel structures.\textsuperscript{34} Leveraging CHAP, Rao and co-workers selected 200 representative channel structures spanning over different classes of ion channels and analyzed their pore geometry, surface properties, and hydration free energy profiles.\textsuperscript{34} Using machine learning-assisted analysis, they further derived a simulation-free heuristic model that could rapidly and accurately predict if a given channel structure contains a hydrophobic gate. Interestingly, the final model, as illustrated in the schematic of Fig. 6, shows that the pore radius and overall hydrophobicity together largely predict the existence of a hydrophobic gate. However, there is substantial variance in the intermediate regions between the limits of small, hydrophobic pores and large, hydrophilic ones that may still require atomistic simulations for

V. PREDICTION OF HYDROPHOBIC GATING THROUGH STRUCTURAL AND FUNCTIONAL ANNOTATIONS

The emergence of cryo-EM has led to an explosion of the high-resolution structures of TM protein channels in different conformational states in recent years.\textsuperscript{54,62} As noted above, the pores of these channels can differ dramatically in size, topology, and surface properties. The assignment of the functional state (e.g., open vs partially open vs closed) is often ambiguous. There is thus an important need to rapidly and reliably assess the conductive state of the pore. A first inspection is to determine the physical dimension and geometry of the pore, which can be readily calculated using several tools including HOLE,\textsuperscript{71} HOLLOW,\textsuperscript{84} and CAVER\textsuperscript{75,86} (e.g., see Fig. 2). The calculated pore profile provides important insight into the potential structural basis of ion permeation and gating. For example, these pore radius profiles can be used to identify potentially closed states, in which a physical constriction narrower than the radius of a water molecule (∼1.5 Å) has been commonly considered the location of the gate.\textsuperscript{15} However, the prevalence of hydrophobic gating in biological channels can lead to important complications. A pore with a physical opening large enough to accommodate the hydrated ions may not necessarily be in a conductive state. This could lead to mislabeling the functional state of a (static) structure. Existence of a “hydrophobic plug/belt” located in the deep pore regions of the ion channels could strongly suggest a “hydrophobic gating”-like mechanism, specifically if mutation of these residues would drastically alter the gating properties of the channel.\textsuperscript{53,61,70,76} Yet, although frequently correlated with a dewetting transition, the presence of large connected hydrophobic areas within the pore by itself is not sufficient to predict one.\textsuperscript{15} As such, simple criteria relying on either the pore geometry (such as radius) or some overall hydrophobicity measures are prone to errors in predicting hydrophobic gating. This reflects a wide variety of pore size, shape, hydrophobicity, and hydrophilicity that exist in biological channels.

FIG. 6 A simulation-free heuristic model for rapid identification of hydrophobic gates in channel structures. The hydration free energy (G), largely a function of the minimal pore radius and overall pore hydrophobicity, predicts the likely existence of hydrophobic gates. The figure is obtained with permission from Rao et al., Proc. Natl. Acad. Sci. U. S. A. 116, 13989 (2019). Copyright 2019 Author(s), licensed under a Creative Commons Attribution 4.0 license.
more reliable annotation, which could be readily achieved using CHAP. Critically, the heuristic model does not consider the plasticity of the protein structure. The consequence of structural plasticity can be difficult to predict because it requires much more extensive simulations, often on the order of microseconds or longer, to capture potential significant conformational relaxation that may be associated with a dewetting transition. This is particularly true for larger ion channels such as BK channels discussed above (e.g., see Fig. 5), where the pore may further collapse to support a stable dry state. This is also true for the GLIC, which has shown an iris-like movement of the inner pore helices during the dewetting transition.

VI. CONCLUDING DISCUSSION AND FUTURE DIRECTIONS

Water as the solvent of life is a complex liquid with many fascinating physicochemical and thermodynamic properties. These properties have been thoroughly exploited by nature and deeply integrated in the organization, dynamics, and function of biomolecules. A particularly unique property is that the liquid and vapor phases of water are near coexistence under biologically relevant conditions. Confinement in a hydrophobic pore could shift the equilibrium toward the vapor phase, leading to a cooperative dewetting transition of the pore. The vapor phase created a large energy barrier to block the passage of ions through the pore, which is known as hydrophobic gating. Theoretical studies and molecular simulations together have now firmly established the prevalence of hydrophobic gating in protein ion channels of a wide range of sizes, topologies, and biological functions. These predictions have been supported mainly through site-directed mutagenesis, which demonstrate that replacing key hydrophobic residues with polar or charge ones would destabilize the dewetting transitions and drastically shift the gating behavior. The systematic scanning mutagenesis study of the human BK channel arguably provides one of the most convincing validations of hydrophobic gating. All pore-lining residues have been replaced with all possible amino acids and the gating voltage was measured. What emerged from this extraordinary effort is an unambiguous and strong correlation with the pore hydration propensity and channel opening probability [e.g., see Fig. 5(d) for a position located in the middle of the BK pore]. Cysteine mutations coupled with side chain chemical modification experiments can reveal accessibility to the deep pore regions by various reagents even in the closed state, lending support to the possibility of hydrophobic gating.

Nonetheless, the experimental support for hydrophobic gating in biological channels so far is largely indirect. Techniques such as nuclear magnetic resonance (NMR), dielectric relaxation spectroscopy, neutron scattering, and neutron diffraction have been used to study the dynamics of water near confined surfaces. Yet, direct measurement of density fluctuations inside a nm-scale pore within the heterogeneous protein and membrane environment is a formidable task. The discovery of channels with large interior pores that can undergo stable dewetting transitions, such as BK channels, may provide new opportunities for direct experimental validation of hydrophobic gating. Other techniques such as interfacial-force microscopy have been developed to measure the long-range hydrophobic attractive forces between superhydrophobic surfaces. Nanosecond bubbles have been observed, leading to sudden strong attractive forces due to evaporation or cavitation of the intervening, confined water at certain tip-to-substrate separations. Visualization of the air layer formed at the superhydrophobic surfaces has also been achieved using laser scanning confocal microscopy combined with atomic force microscopy (AFM) measurements, where the formation of gaseous menisci in water between hydrophobic surfaces can be captured using optical microscopy and multi-beam interferometry (e.g., see Fig. 7). Evidence of the phase transition from liquid water to vapor at the superhydrophobic surfaces has also been reported using confocal Raman spectroscopy, which is a technique proven successful for depth profiling of different materials including living cells. It remains to be seen if any of these techniques could be adopted to directly detect liquid–vapor transitions within a large biological protein pore such as that of the BK channel. Hydrophobic dewetting depends on any solution conditions that change the water activity, including the presence of salt and other solutes, cosolvents, and even external pressure. Studies of how channel activation properties depend on water activity will provide another strategy for detecting and confirming hydrophobic gating. For example, gating of squid axon K+ channels has been observed to depend on the osmolarity of the buffer, where increasing the hyperosmotic stress decreases the open probability. This can be elegantly explained by a hydrophobic gating mechanism as previously predicted by atomistic simulations. The presence of glycerol and sucrose has also been shown to increase the activation voltage of BK channels, even though this was attributed to reduced protein flexibility due to increased solvent viscosity. The sensitivity of the activities of channels equipped with hydrophobic gates to the solution condition could also have important implications in biology. The cell is highly concentrated with ions, small molecules (such as metabolites and glucose), and macromolecules. Properties of cytosolic water near a particular channel protein molecule could vary depending on the intracellular localization as well as the cell type and cell cycle. As a result, the activation properties of the same channel could have
nontrivial spatiotemporal, cell type, and tissue dependence. It will be fascinating to see if hydrophobic gating may allow previously unrecognized strategies that the cell may have access to for regulation of the function of various ion channels.

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DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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