ment with measured rates. At any moment, each channel contains no more than a single, incomplete file of water molecules. The molecules diffuse through the channel in a highly cooperative fashion. In this respect, the motion is similar to water transport through carbon nanotubes, which has been studied in another recent "reality simulation" (*7*). In the hydrophobic carbon nanotube, hydrogen-bonded chains of water molecules move through the tube in a pulse-like fashion. In the aquaporin channel, water molecules hydrogen bond with each other and with a line of carbonyl oxygens of the protein backbone.

By analyzing a long, reliable simulation, validated by correct prediction of permeation rates, insights into the details of the permeation mechanism can be gained. The aquaporin channel contains two "filters": One is a positive charge and the other the NPA motif (a positive region between two helices that are oriented such that their dipoles point toward the region). These filters confer water specificity and block the passage of positive ions and protons. A strong electric field changes direction at the NPA motif, causing the water molecules to rotate during passage and thereby enhancing specificity. Such a real-time simulation is like reality television: One observes details on the spot without having to add unwarranted interpretations.

A high-resolution x-ray structure of aquaporin-1 is not (yet) available. In an earlier paper (*8*), the authors overcame this problem by exploiting the homology of aquaporin with the glycerol facilitator, GlpF. They used the known x-ray structure of the latter to computationally refine electron microscopy data of aquaporin. De Groot and Grubmüller also simulate water transport through GlpF, starting from the x-ray structure obtained in the presence of glycerol. During the simulation, the structure hardly changes, but water permeation through GlpF steadily decreases, indicating that adaptations in the channel due to removal of the crystallographic glycerol are not completed after 10 ns. Recent simulations of GlpF by Schulten and co-workers (*9*) have shown how glycerol may move through the channel, although the through-channel glycerol transport could not be followed in the available time.

As system sizes and simulation times continue to increase, detailed real-time simulations will become available for many biological processes. These simulations may be used to check the validity of approximations made to study events on much longer time scales. One example is the slow passage of individual water molecules or other small molecules through lipid membranes. Such rates can be predicted with standard rate theory and relatively short simulations (*10*, *11*), but the theory is based on assumptions regarding the permeation mechanism. When motion is cooperative, as in membrane channels, the theory must be modified. This and many other approximate theories can now be checked against "reality."

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PERSPECTIVES: THERMODYNAMICS

Glassy Water

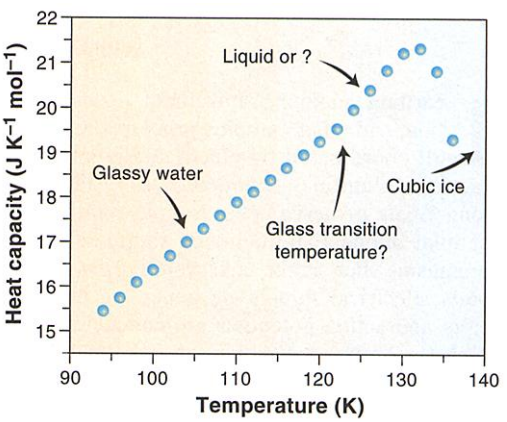
Dennis D. Klug

Few known simple molecular systems can rival the complexity of the water phase diagram. Water boasts numerous solid phases and may even form two different liquid phases at low temperatures. But pinning down the exact nature of the different phases and the transitions between them has proven difficult. One of the intractable properties of water is the temperature at which water changes from a liquid to a glassy state. On page 2335 of this issue, Velikov *et al.* (*1*) show that this transition may occur 30 K above the currently accepted value. If proven correct, this report will require a rethinking of the water phase diagram.

The glass transition temperature is usually defined as the point at which the liquid becomes very viscous and essentially a quenched liquid upon cooling or as the temperature at which the solidlike glass transforms to a liquid upon heating. This is an operational rather than a thermodynamic definition because the glass transition temperature depends, for example, on the rate at which the liquid is cooled. On time scales of a picosecond, even liquid water at room temperature is quite hard.

The glass transition temperature for liquid water is of interest for several reasons, beyond the fundamental desire to determine another well-defined point in the phase diagram of this important liquid. Experimental studies of the liquid and amorphous phases of water suggest a highly complex behavior. Several theories (*2*) suggest the possible existence of two distinct liquid water phases, a liquid-liquid phase transition upon cooling and a liquid-liquid critical point in the low-temperature region of the phase diagram. The glass transition may occur in one of these liquid forms of water if the theories are correct; its location defines the region where one can search for the low-temperature liquid, the liquid-liquid transition, and the proposed second critical point. The glass transition of water is also of interest in the context of cryoprotection processes and biological organisms at low temperature (*3*).

The determination of the glass transition temperature would seem to be a rather straightforward exercise. In water, however, it has proven to be very difficult to



Heat capacity of low-density amorphous ice. The sample was obtained by annealing high-density amorphous ice produced through pressurization of hexagonal ice. The vertical arrow identifies the glass transition temperature as measured with a slow scan heat-flow calorimeter. The heat capacity decreases above 132 K because of the large exothermic heat of crystallization to form cubic ice.

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track down. The main difficulty lies perhaps in the preparation and characterization of the sample itself. There are many ways to prepare what has been called "glassy water," including the deposition of water vapor on a cold substrate (*4*), the preparation of an amorphous solid by applying pressure to crystalline ice at low temperatures (which yields a high-density form of amorphous ice) and then annealing this material to obtain a low-density amorphous ice (*5*), and the very rapid cooling (at rates of ~10^6°C/s) of liquid water to obtain a glassy solid (*6*). The glass transition temperature can be determined by warming these samples and observing either thermodynamic behavior characteristic of the glass transition (*7*) (see the figure) or measurable liquidlike behavior.

Experimental measurement techniques include calorimetry, diffusion, and macroscopic flow measurements (*8*). In these data, it is often difficult to distinguish a glass transition from other phenomena occurring in the solid phase. Theoretical studies typically use molecular dynamics studies to identify a liquidlike diffusion constant from mean-squared displacements of particles. Here, the main difficulty is that the glass transition depends on the interatomic potential used to describe interactions between water molecules at low temperatures. Another more fundamental problem is that the materials made by vapor deposition or annealing of pressurized materials may not be related to a glass at all (*9*). Also, different forms of the amorphous ice can yield different values for the glass transition temperature (*10*).

From their analysis of thermodynamic data, Velikov *et al.* (*1*) conclude that the glass transition temperature in water is almost 30 K above the currently accepted value of about 136 K. The authors analyze data on hyperquenched glassy water obtained from the use of a differential scanning calorimeter. This is a very reasonable choice because this sample is most likely a true glass. On the basis of a simple replotting of the calorimetric data of Johari *et al.* (*10*), the authors suggest that if the glass transition temperature for water is the accepted value of ~136 K, liquid water data would be quite anomalous compared with a number of other glasses. Only when the transition temperature is about 165 K do the replotted data for water look normal.

These results may have a substantial impact on the study of the phase behavior of water. If the transition temperature must indeed be relocated to 165 K, then studies of the glass transition will become even more difficult because crystallization is harder to avoid at this temperature. Furthermore, there are suggestions in the literature that water undergoes a transition from a "strong" to a "fragile" liquid (*11*) around 140 K. For a strong liquid, the logarithm of the viscosity changes linearly with the inverse of temperature, whereas the viscosity of a fragile liquid shows a steep drop over a narrow temperature range. If water is not a liquid at 140 K, then this picture will change substantially. Our understanding of the behavior of water in microporous materials and in contact with biological materials at low temperatures would also change.

If Velikov *et al.*'s results are correct, then there is a new region in the water phase diagram where the material differs from the low-temperature glass and the high-temperature liquid. Their analysis will likely be controversial and stimulate new research on the search for the elusive glass transition temperature in water. For example, the use of pressure may help to avoid crystallization and identify the "true" glass transition.

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PERSPECTIVES: PHYSIOLOGY

A One-Domain Voltage-Gated Sodium Channel in Bacteria

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Learning, memory, movement, sensation, and other complex processes are all coordinated by electrical signals (action potentials) conducted along the long fibers of nerve cells. In both multicellular animals and complex single-cell organisms such as the eukaryote *Paramecium*, electrical signals are generated locally and action potentials are conducted globally through the activity of a family of voltage-gated sodium channels. This ion channel family was not thought to exist in bacteria, the jellyfish being the simplest organism in which it had been found (*1*). On page 2372 of this issue, Ren *et al.* (*2*) surprise us with their discovery of the first voltage-gated ion channel in a prokaryote. They describe the structure and function of a sodium channel in the salt-loving bacterium *Bacillus halodurans*.

The voltage-gated sodium (Na_v) channels of eukaryotes are complex proteins composed of more than 2000 amino acid residues. The large pore-forming α subunit comprises four homologous domains containing six transmembrane α helices (see the figure) (*3*). It is bell-shaped (*4*) and associates with smaller β subunits (*3*). As Ren *et al.* report (*2*), the bacterial sodium channel (NaChBac) is much simpler, consisting of a single domain with 274 amino acid residues and six transmembrane α helices (see the figure). This structure resembles voltage-gated potassium (K_v) channels, which are homotetramers (composed of four identical subunits) (*5*). The primary structure of NaChBac raises provocative questions about the three most important tasks of sodium channels—selective ion conductance, voltage-dependent activation, and fast inactivation.

In vertebrates, an ion selectivity filter enables voltage-gated sodium channels to conduct sodium ions 15 to 50 times as rapidly as potassium and calcium ions, the other prevalent cations in physiological fluids (*6*). The transmembrane pore of these ion channels is formed by the S5 and S6 segments of the α subunit and the pore loop segment between them [reviewed in (*3*)]. Ion selectivity is thought to be determined by a small number of amino acid residues in the second half of the pore loop, which forms a narrow opening into the extracellular end of the pore (*7*). As discussed by Ren *et al.* (*2*), preferential sodium selectivity in Na_v channels is thought to require an asymmetric pore structure with different amino acid residues in key positions in the pore loops of each of the four domains. At one critical position, the amino acid residues in the pore loops of the four domains of

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