These anomalies should be investigated further to discern how replication is initiated at these sites.

Both groups fine-tuned their techniques with the well-characterized *S. cerevisiae* genome, and both used knowledge of existing *oris* to enhance their data analysis. Detailed information about the replication of yeast chromosomes III and VI made this possible. How, then, might these technologies be applied to discover *oris* in, for example, human chromosomes where the location of *oris* has not been well researched? It is clear that neither technique alone would convincingly point to the location of *oris* in a naïve genome, but when combined, sites of overlap would strongly suggest (but not prove) potential *ori* locations. For the chromosomes of metazoan species, we need better techniques to map the genetic elements that determine where *oris* occur, and better antibody reagents for chromatin precipitation.

Recently, CHIP experiments with anti-ORC and anti-MCM antibodies suggested the locations of pre-RC components near known sites of replication initiation in the Epstein-Barr virus genome (*10*). For mammalian genomes, ordered bacterial artificial chromosome (BAC) arrays or representation arrays will prove useful for mapping *oris* with both the replication density shift and CHIP-array methods.

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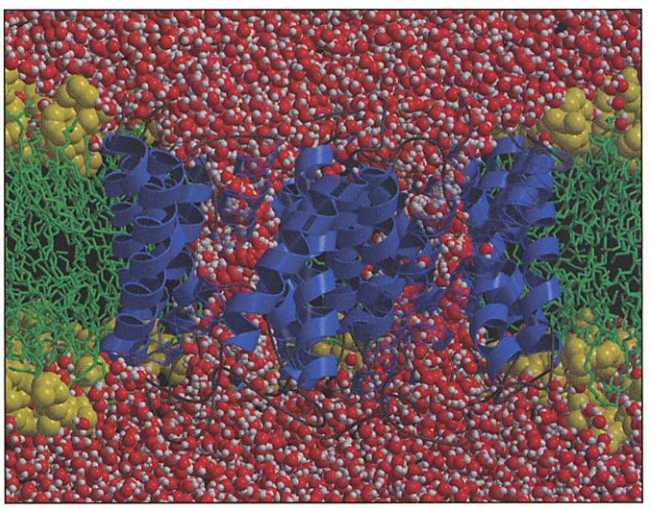
PERSPECTIVES: BIOINFORMATICS

Reality Simulation—Observe While It Happens

Herman J. C. Berendsen

Since the first simulation of the dynamics of a small protein in 1976 (*1*, *2*), considerable insights have been gained into the dynamics of biological macromolecules and membranes on time scales of nanoseconds. But progress toward simulations on biologically relevant time scales has been slow (*3*, *4*). Decades of method development, together with rapid growth in computational power, are now beginning to enable simulation of complex biological processes on realistic time scales, as demonstrated by de Groot and Grubmüller on page 2353 of this issue (*5*).

Biomolecular processes present three problems for simulation on the atomic level. First, they involve hundreds of thousands of atoms, often in intricate interactions that are difficult to simplify. Second, they span a wide range of time scales: Primary events (for example, in vision or photosynthesis) occur within picoseconds, enzymatic and regulatory processes take milliseconds, and protein folding and structural reorganizations may exceed seconds. Third, the small driving forces that cause molecular changes result from large, opposing energetic effects. This requires careful fine-tuning of the force fields that describe interatomic interactions. Current simulations are limited to system sizes of about 100,000 atoms, time scales of about 100 ns, and classical dynamics with simple, pair-additive, interactions. Quantum mechanics can also be incorporated, but at the expense of system size or time scale.



Water permeation through aquaporin-1. From a 10-ns molecular dynamics simulation (*5*). During the simulation, water molecules (red/white, shown as an overlay of 100 snapshots) permeate the four pores of the tetramer (blue), which is embedded in a lipid bilayer (yellow: hydrophilic head groups; green: hydrophobic tails).

The future will see rapid progress in all three areas. Within existing limitations, however, most biological processes cannot be simulated in "real time." Even for processes that do occur on a 100-ns time scale, it is not sufficient to simulate an event—say, the folding of a peptide into a helix—once. Statistics must be collected under varying conditions. In practice, one can either simulate processes that do occur on the time scale attainable for simulations with full atomic detail, or one can resort to approximations in system size, detail of description, or accuracy of dynamics. The latter approach is the only possible way to proceed for most processes but does involve approximations that need thorough testing. There is also a danger that the chosen approximations bias the results.

De Groot and Grubmüller describe a state-of-the-art example of the first approach (*5*). They have simulated a biological process that occurs on the nanosecond time scale: the motion of water through the water-specific membrane-channel protein, aquaporin-1. Aquaporins are essential for maintaining osmotic balance in cells. They are embedded in cell membranes and transport water molecules at rates of more than 10^9 molecules per second. Other molecules or ions, including hydronium ions and protons, cannot pass through the channel. Because of the high permeation rate, several complete permeation events can be observed in a simulation.

De Groot and Grubmüller's system consists of about 100,000 atoms, which form an aquaporin tetramer embedded in a bilayer of lipid molecules and surrounded by water molecules (see the figure). They use a full lattice summation method for computing electrostatic interactions, which is computationally expensive but essential for reliable results, and use one of the fastest (if not the fastest) molecular dynamics programs for biomolecular simulation (*6*).

The authors observe 16 full permeation events within 10 ns, in reasonable agree-

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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

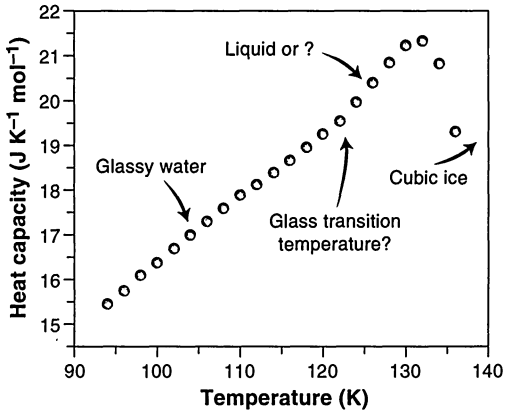
Dennis D. Klug

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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