SIMULATION Provides Key to the Mystery of MOLECULAR Machines

Voltage-gated ion channels constitute an important class of proteins associated with the cell membrane. Scientists have long struggled to understand how these complex nanoscale devices work, but the enormous time and energy scales have made computations infeasible. Now, using the computational power of the Cray XT and the IBM Blue Gene leadership-class computers, researchers are gaining new insights into the mechanisms of these electro-mechanical marvels.

The cell membrane represents the physical and functional boundary between living organisms and their environment. Membrane-associated proteins play an essential role in controlling the bidirectional flow of material and information. As such, they can be viewed as nanoscale molecular machines able to accomplish complex tasks. Ion channels, a highly specific class of such proteins, regulate the permeation of small ions in and out of the cell. These proteins can switch between different conformational states—a process known as "gating"—thereby allowing or blocking the passage of ions across the cell membrane in response to various cellular signals (figure 1).

Voltage-gated potassium channels, or Kv channels, are involved in the generation and spread of electrical signals in neurons, muscle, and other excitable cells. To open the gate of a channel, the electric field across the cellular membrane acts on specific charged amino acids that are strategically placed in the protein in a region called the voltage sensor. In humans, malfunction of these proteins, sometimes owing to the misbehavior of only a few atoms, can result in neurological diseases.

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A long-term endeavor of research in biophysics is to understand the workings of these ion channels and to predict their function. A wealth of experimental data exists from a wide range of approaches, but its interpretation is complex. One must ultimately be able to visualize atom by atom how these tiny mechanical devices move and change their shape as a function of time while they perform. This goal has long remained elusive.

Recently, however, a team of researchers from Argonne National Laboratory, the University of Chicago, the University of Illinois–Chicago, and the University of Wisconsin has used high-performance computing to break new ground in understanding how these membrane proteins work. Exploiting state-of-the-art developments in molecular dynamics and protein modeling, the team has constructed models of Kv voltage-gated channels and run them on the Cray XT and IBM Blue Gene leadership-class computers.

"Being able to run on these top computers is essential for this work," says Dr. Benoit Roux, a computational scientist who holds a joint appointment at Argonne and the University of Chicago. "The time

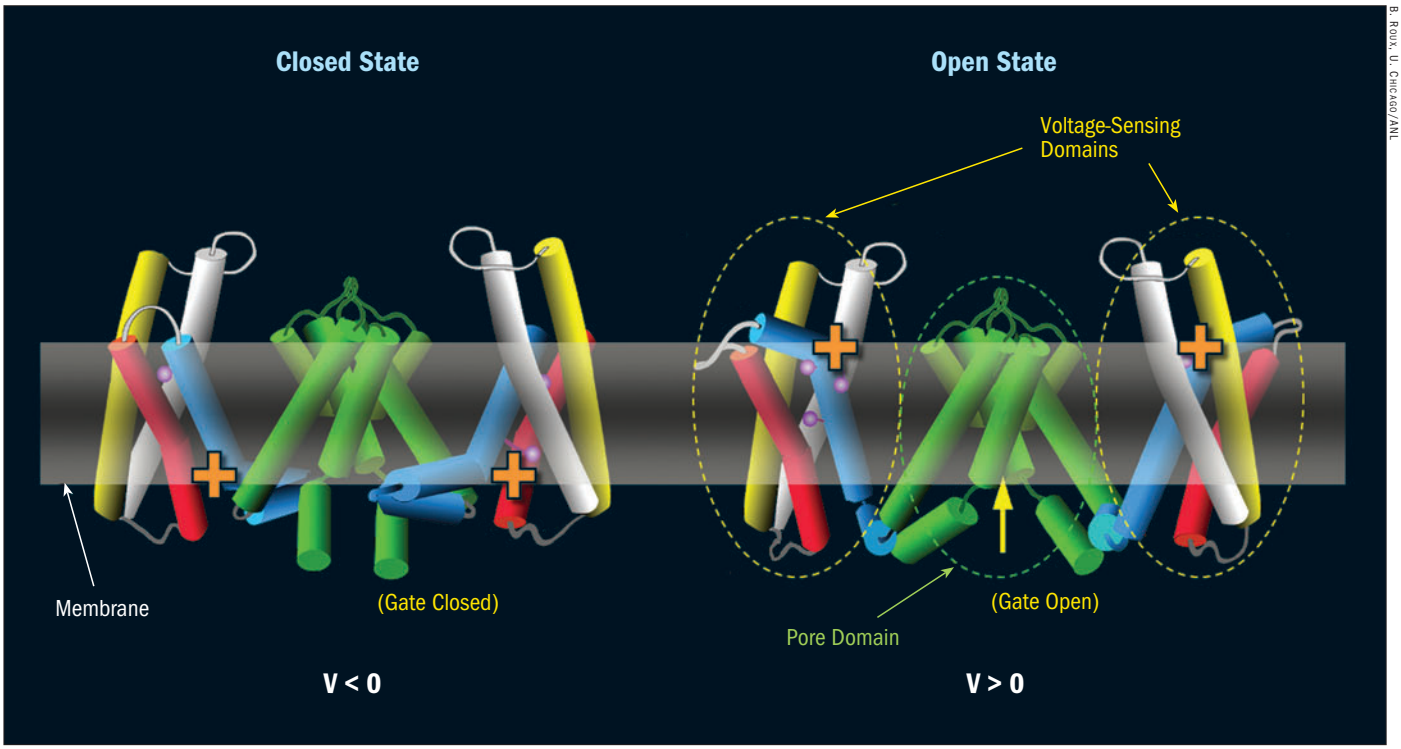

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Figure 1. *Schematic of the function of a voltage-gated ion channel. At rest, the normal cellular potential of a living cell is negative and the potassium channels are closed. When the cellular membrane is depolarized and the potential becomes positive, the channel is activated and can conduct K^+ ions (represented by the yellow arrow, right). The conformation change from the closed to the open state is driven by the movement of the positively-charged amino acids (orange "+" symbols) located in a region of the protein called the voltage sensor surrounding the central ionic pore.*

and energy scales of the underlying molecular processes are just within reach of the computational capabilities of such leadership-class computers."

Enabling New Science

In earlier work the team had assessed a model of Kv channels in mammalian cells. Under a grant from the U.S. Department of Energy Innovative and Novel Computational Impact on Theory and Experiment (INCITE) program in 2007, the researchers were able to extend this work to more complex membrane proteins, taking advantage of the computational power of the 5.7-teraflop Blue Gene/L (BG/L) at the Argonne Leadership Computing Facility (ALCF) and the 100-teraflop Cray XT3 at Oak Ridge National Laboratory.

Their study has already produced exciting results. For example, the researchers have, for the first time, confirmed the hypothesis that the electric field controlling the voltage-gating is focused over a particular area, rather than spread throughout the whole thickness of the cellular membrane. As a result of these initial successes, Dr. Roux recently received an allocation of five million hours under the 2008 INCITE program to continue this research on the Blue Gene/P at Argonne and the Cray at Oak Ridge.

The practical applications of this work are significant. For example, the research in ion channel mechanisms may help identify strategies for treating cardiovascular disorders such as long-QT syndrome, which causes irregular heart rhythms and is associated with more than 3,000 sudden deaths each year in children and young adults in the United States. Moreover, the studies may help researchers find a way to switch or block the action of toxins—such as those emitted by scorpions and bees—that plug the ion channel pores in humans.

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An Integrated Approach

One of the team's first steps in elucidating the voltage-gating mechanism was to develop models of a Kv channel in both the closed (resting) and open (activated) states. Of special importance in this work was experimental data, reported in the literature, on the x-ray crystallographic structure of a voltage-gated potassium channel, the Kv1.2 channel from rat brain, which provided the first atomic resolution structure of a voltage-gated potassium channel in the open state. A previous study had determined the x-ray structure of the potassium channel KvAP from an archaebacterium, although it was soon realized that the protein was distorted in those crystals.

The researchers also drew on recent major advances in high-resolution *de novo* prediction of the three-dimensional structure of proteins from amino acid sequences. In particular, the Rosetta membrane method was extended and used by Dr. V. Yarov-Yarovoy, one of the team collaborators at the University of Washington, for modeling the voltage-sensing domain conformations of Kv

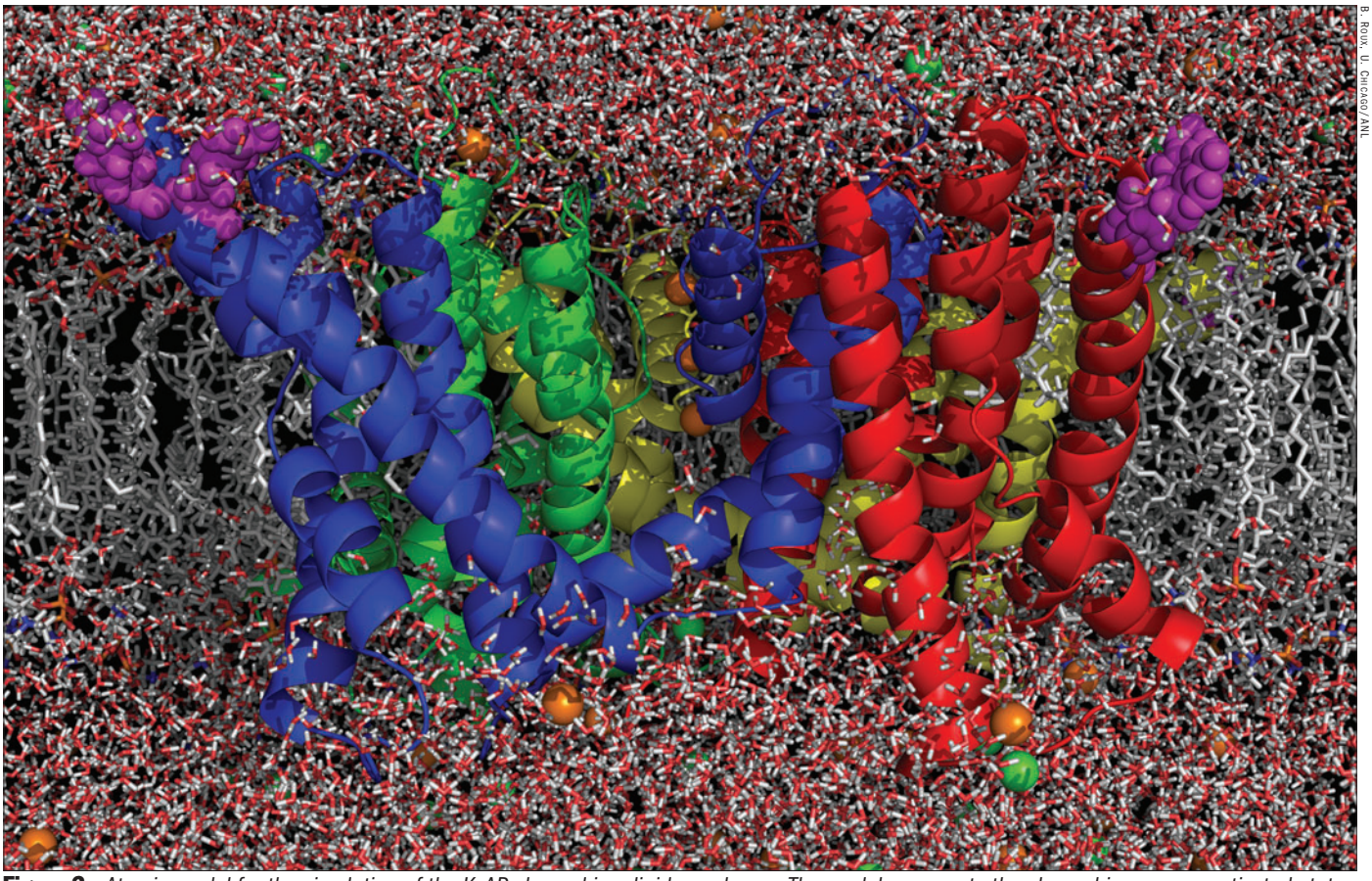

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Figure 2. *Atomic model for the simulation of the KvAP channel in a lipid membrane. The model represents the channel in an open activated state as determined by electron paramagnetic resonance experiments from the laboratory of Eduardo Perozo (University of Chicago). The atomic model comprises 964 amino acids, 302 lipid molecules, 12,046 water molecules, and 53 K^+ and Cl^- ion pairs. In total there are 112,798 atoms in the system. The positively charged arginine residues of the voltage sensors are colored in magenta. The simulations of about 50 ns were performed by using the NAMD package with up to 512 processors on the BG/L at the ALCF.*

channels. This method is based on the assumption that the native state of a protein is at the global free energy minimum. A large-scale search of conformational space for protein tertiary structures is carried out to select structures that are especially low in free energy for a given amino acid sequence.

The team also capitalized on advances in molecular dynamics. This computational approach involves simulating the dynamical motions of all the atoms of a system as a function of time (sidebar "Advanced Computational Techniques"). Introduced in the late 1950s for studies of simple liquids of hard spheres, molecular dynamics has matured and grown rapidly in complexity over the years, with simulations of liquid water, proteins, lipid bilayer membranes, and in recent years, even a complete viral life form.

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Large-Scale Simulations

Armed with this structure–prediction algorithm and with advanced modeling methodology, the team constructed a complete model of the KvAP voltage-gated potassium channel. To correct for the distortion observed in the x-ray structure of earlier studies, the model exploited electron paramagnetic resonance experimental data measured in the laboratory of the University of Chicago's Department of Biochemistry and Molecular Biology.

The researchers ran their simulated system, which consisted of the channel embedded in a lipid bilayer surrounded by an aqueous salt solution, on the 2,048-processor BG/L at the ALCF (figure 2). The simulations were generated by using the parallel program Nanoscale Molecular Dynamics (NAMD), a parallel code developed at the University of Illinois–Urbana-Champaign in the lab of Dr. Klaus Schulten, one of the team's researchers. The code is designed for high-performance classical simulation of large biomolecular systems and can scale up to thousands of processors on high-end parallel platforms such as BG/L. The optimized version of NAMD used on the BG/L at Argonne was provided by IBM. Preliminary analysis indicated that the native arginine (a common amino acid) residues in the voltage sensor are more hydrated and located farther from the center of the membrane than previously indicated by the use of traditional spin labels and electron paramagnetic resonance spectroscopy.

Advanced Computational Techniques

Molecular dynamics (MD) constructs an atomic model of the macromolecular system, represents the microscopic forces between atoms with potential functions, and integrates Newton's classical equation $F = mA$ to generate a trajectory—a "movie" of the dynamical motions of all the atoms as a function of time. Many biological studies are amenable to MD studies, including protein biochemistry, pharmaceutical binding, protein conformational changes, assembly of macromolecular machines, and molecular transport. With today's fast and reliable numerical algorithms, current MD methodologies have reached the point where one can realistically model the dynamic atomic models of complex biological channel membrane systems. In addition, the predictive power of simple MD trajectories can be extended considerably with statistical mechanics concepts.

Consider the reaction coordinate, the conceptual reaction coordinate or pathway for making progress during a biochemical process, from beginning to end. One of the key concepts in the dynamics of microscopic processes is the potential of mean force (PMF). First introduced by J. G. Kirkwood in 1935, it corresponds to the average reversible thermodynamic work function W done by the mean force $\langle F \rangle$ along a chosen reaction coordinate such as the position along the ion channel axis Z:

$$W(z_1) = W(z_0) - \int_{z_0}^{z_1} \mathrm{d}z' \, \langle F(z') \rangle$$

Note that the PMF can be generalized to multidimensional cases. Hence the PMF constitutes the "free energy surface" on which the long-time dynamics of the system is evolving.

Specific computational techniques have been designed to calculate the PMF from simulations. For example, in umbrella sampling, simulations are first performed in the presence of an external time-independent biasing potential to enhance the statistical sampling of the most relevant configurations of the system. The bias introduced by this potential is then rigorously removed in post-analysis, thus enabling the characterization of the true unbiased free energy surface of the system. An alternative technique, steered MD and interactive MD, uses a non-equilibrium calculation during which the system is submitted to an external time-dependent "pulling" biasing force. Again, the bias introduced by this perturbation can be rigorously removed in post-analysis by using a fundamental identity derived by C. Jarzynski in 1997. Both techniques are powerful computational approaches to characterize quantitatively the free energy landscape governing the dynamics of complex biomolecular systems.

Furthermore, approaches to determine the complete pathway for large conformational transitions are likely to play an important role in the characterization of macromolecular machines. A transition path is represented by a "string" of configurations. One strategy, recently introduced by A. C. Pan, D. Sezeer, and B. Roux, consists of refining a putative transition path in the multidimensional space by following the average dynamic drift of a set of chosen variables. This drift is estimated on the fly via "swarms" of short unbiased trajectories started at different points along the transition path. Successive iterations of this algorithm, which can be easily distributed over many computer nodes, refine an initial trial path toward the most probable transition path between two stable conformational states (sidebar "String Method," p27).

It is also possible to monitor the dynamical motions in the presence of imposed external forces to reproduce some aspect of the physical environment, such as membrane surface tension, hydrostatic pressure gradient, or transmembrane voltage. In some cases external pulling forces can even be generated interactively for MD trajectories, for example, to probe channel selectivity. In addition, free energy perturbation, in which one molecular species is "alchemically" converted into another one by using an altered potential (unphysical in its intermediate states), is an important technique to directly address questions about thermodynamic stability. Altogether, these MD techniques play a key role in investigating the microscopic factors controlling ion selectivity.

Encouraged by these novel results, the team also ran a reduced, or truncated, model of the Kv1.2 voltage-gated potassium channel in an explicit membrane (figure 3, p26) on a 350-node Linux cluster at Argonne National Laboratory. These simulations were generated using the program CHARMM developed by a community of international scientists. One important result of these simulations concerns the properties of the electric field responsible for the voltage activation. The calculations show that this electric field is indeed more intense than at other equivalent position across the membrane far away from the protein.

"It's analogous to the familiar electrostatic problem of a charged capacitor with two parallel conducting metallic plates," explains Dr. Roux. The membrane and the protein act as an insulator separating the intracellular and extracellular aqueous solutions. The intensity of the electric field varies roughly like the inverse of the thickness of the insulating region between these two conducting solutions. The channel increases the intensity of the field by making the insulating region as thin as possible. The simulations show that many regions of the Kv1.2 channel on the intracellular side are penetrated by the "conducting" aqueous solution, leaving only a thin "insulating" region from the protein. Rather than being spread over the whole thickness of the cellular membrane, the electric field is effectively focused over the outer half of the bilayer, in the region of the most important charged residues of the voltage sensor. "These findings have important functional implications because an intense electric field is better to cause the charges of the voltage sensor to move," says Dr. Roux.

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ILLUSTRATION: A. TOVEY SOURCE: B. ROUX, U. CHICAGO/ANL

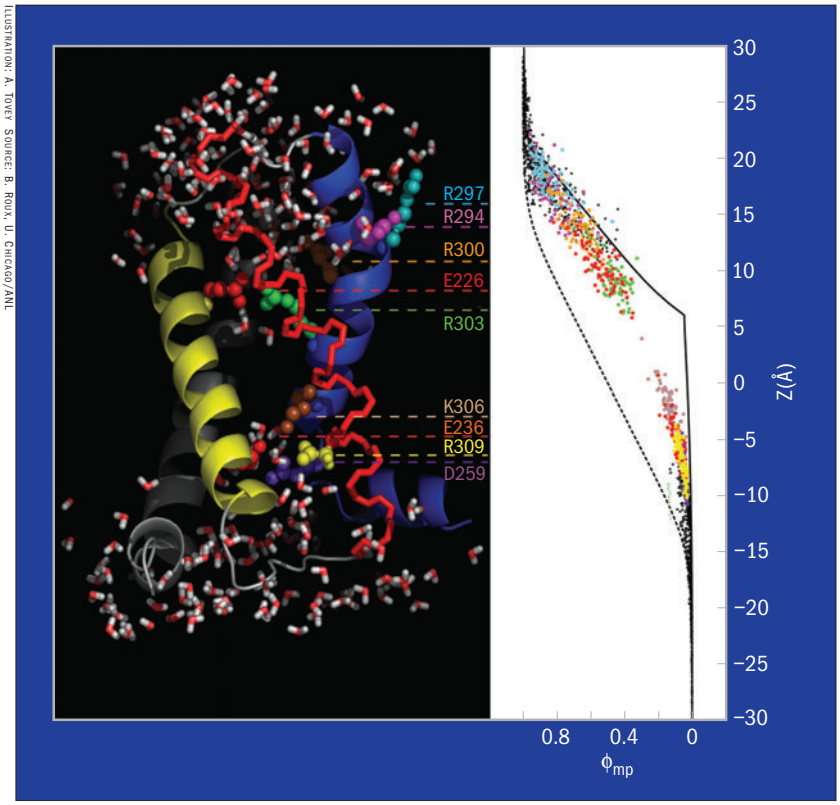

Figure 3. *Organization of the charged residues in the voltage sensor of the Kv1.2 channel and their relationship to the transmembrane potential profile calculated from the Poisson–Boltzmann voltage theory. On the left is a view of the aqueous crevices that develop rapidly during the simulation. On the right is the transmembrane potential sensed by the charged residues in the voltage sensor as a function of their average position along the z-axis. The calculations were done using the program CHARMM on the Jazz cluster at Argonne National Laboratory.*

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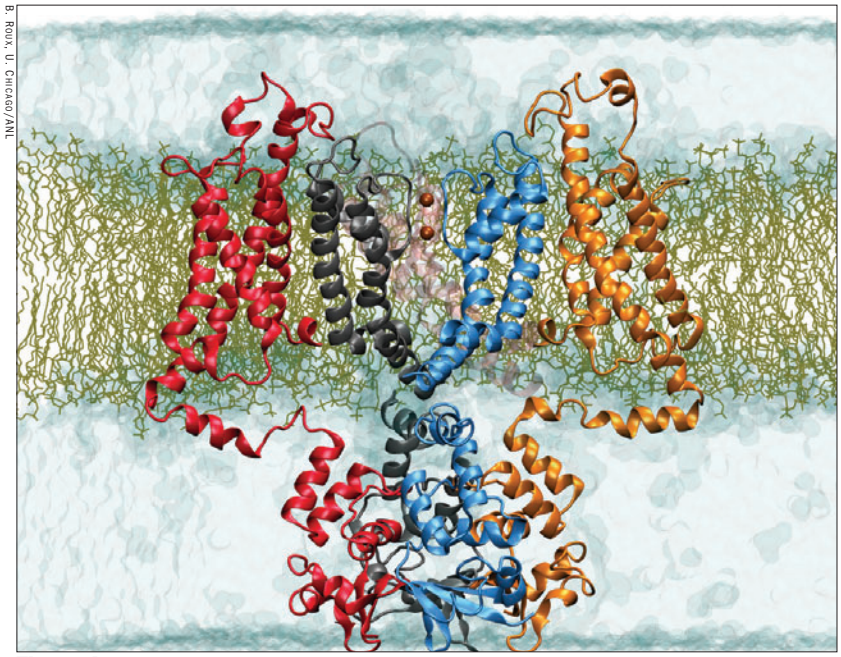

Figure 4. *Complete model of the Kv1.2 channel assembled using the Rosetta method. The atomic model comprises 1,560 amino acids, 645 lipid molecules, 80,850 water molecules, and ~300 K^+ and Cl^- ion pairs. In total there are more than 350,000 atoms in the system. The simulations were generated using NAMD and up to 2,000 CPUs on the Cray XT (Jaguar) at Oak Ridge National Laboratory.*

The researchers have also developed complete models of the Kv1.2 channels in the open and closed states using the structure prediction algorithm of the Rosetta program. The simulations—comprising more than 350,000 atoms (figure 4)—were run using NAMD on the Cray XT (Jaguar) at Oak Ridge. These extensive simulations were made possible through the 2007 INCITE grant from the DOE Office of Science. The results show that complete atomic models of the channel in a bilayer submitted to a membrane voltage are very stable. Equally exciting is the fact that preliminary results indicate that the researchers should be able to compute the actual magnitude of the gating charge, which couples the channel to the membrane voltage. Work performed by the membrane voltage is first converted into molecular motion and then encoded into information-carrying physiological signals by a membrane protein. For this reason, the mechanism of voltage-gating has far-reaching implications in our understanding of energy transduction in bacterial systems.

Challenges Ahead

The tight integration of experiment, modeling, and simulation on massive leadership-class computers is providing new insights into voltage-gated membrane channels. Because these channels are functional electro-mechanical devices, they could be used in the design of artificial switches in various nanotechnologies. Similar voltage-driven molecular motions, occurring on a relatively slow timescale, are also essential for the function of many important membrane proteins such as transporters, pumps, and channels.

But although Kv channels are among the most well-characterized molecular machines experimentally, many outstanding issues remain before scientists fully understand the gating transition of a K^+ channel. One such issue focuses on the open and closed conformations of the channel. The gating charge tells how those conformations are energetically coupled to the transmembrane potential. Dr. Roux and his colleagues are already refining models of the open and closed state, running large-scale simulations on DOE's leadership-class computers as part of a INCITE 2008 award. The next challenge will be the conformational pathway for the open and closed gating transition of the channel. Advanced and novel strategies will be essential here in determining the reaction pathway by describing the transition process through a chain of states (sidebar "String Method").

Given the encouraging results to date, and the dramatic computing power now available thanks to the INCITE allocations, Dr. Roux is confident of making progress, with the team's studies serving as a roadmap for simulating, visualizing, and elucidating the inner workings of molecular nanomachines. ●

String Method

The string method is a novel approach inspired by the work of Dr. Eric Vanden-Eijnden from the Courant Institute at NYU. The string method represents the conformation transition pathway as a chain of states, called images, in the multidimensional space of the set of collective variables *Z*. These collective variables are chosen to capture all the putative "slow" variables in the system, while leaving out the fast degrees of freedom. The motions of dihedral angles, gyrations of some subunits, and many sliding motions are constituents of slow variables. Fast variables include motions like vibrations along covalent bonds and changes in the angles where two atoms are covalently bound to a third atom. Hence the set of slow variables is weakly coupled to the fast variables.

The iterative process for relaxing and improving an initial guess for the transition pathway is illustrated schematically in figure 5. Beginning with a trial transition pathway (lower-right white line in figure 5, left panel), a swarm of short trajectories are run from each image along the transition path (dots). The natural motion of these trajectories in the vicinity of each of the images (red lines in figure 5, right panel) reports faithfully on local dynamical properties, giving information about how each image should be updated during the next iteration. For example, if all the trajectories tend to run "downhill" to a certain region, then this would indicate that the local image is unstable and should evolve toward this more stable point where all the trajectories have gone. These swarms of short trajectories can be easily parallelized over many compute nodes to estimate true dynamical quantities such as diffusion tensors as well as the mean force around each image.

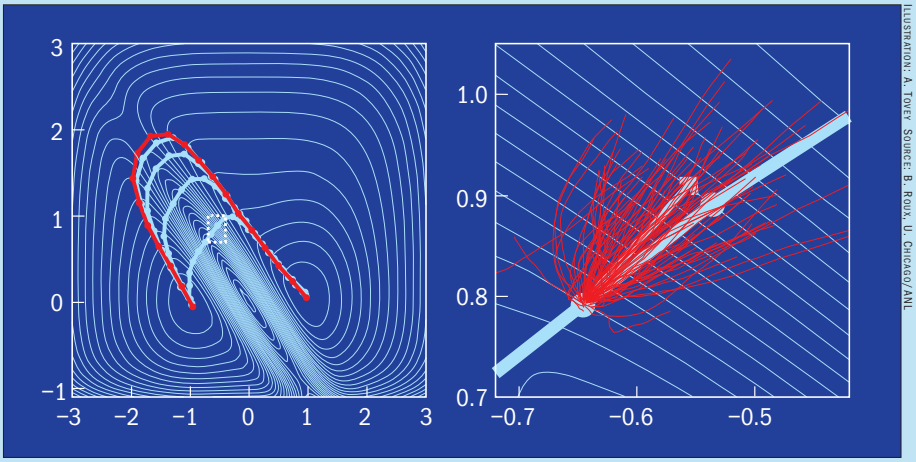

ILLUSTRATION: A. TOVEY. SOURCE: B. ROUX, U. CHICAGO/ANL

Figure 5. *Schematic illustration of the transition path refinement obtained from the string method with swarms of trajectories. On the left, a putative path is progressively refined toward the most probable transition path (red). On the right, at each "image," a number of short, unbiased trajectories are launched, and the average drift* $\langle \Delta Z \rangle$ *is then used to refine the path (blowup from the small rectangle in the left panel).*

An important step in the evolution of the pathway is to reparametrize periodically to prevent images from pooling into stable states. In this way, important regions of high free energy, like transition states, can always be well resolved. Reparametrization is done most simply by interpolating a line through all the collective variables representing the images and then spacing the images equally along this line from one stable state to another.

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Further Reading

Y. Jiang, A. Lee, J. Chen, V. Ruta, M. Cadene, B. T. Chait, and R. MacKinnon. 2003. X-ray structure of a voltage-dependent K+ channel. *Nature,* **423:** 33–41.

V. Jogini and B. Roux. 2007. Dynamics of the Kv1.2 voltage-gated K+ channel in a membrane environment. *Biophysical J.*, **93:** 3070–3082.

A. D. MacKerell, B. Brooks, C. L. Brooks III, L. Nilsson, Y. Won, B. Roux, and M. Karplus. 1998. CHARMM: The energy function and the program, in *The Encyclopedia of Computational Chemistry,* P. V. R. Schleter, N. L. Allinger, T. Clark, J. Gasteiger, P. A. Kollman, H. F. Schaefer III, and P. R. Schreiner (eds.). John Wiley & Sons, Chichester.

M. M. Pathak, V. Yarov-Yarovoy, G. Agarwal, B. Roux, P. Barth, S. Kohout, F. Tombola, and E. Y. Isacoff. 2005. Closing in on the resting state of the shaker K+ channel. *Neuron,* **56:** 124–140.

J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kale, and K. Schulten. 2005. Scalable molecular dynamics with NAMD. *J. Comput. Chem.*, **26:** 1781–1802.

B. Roux and K. Schulten. 2004. Computational studies of membrane channels. *Structure,* **12:** 1343–1351.

V. Vasquez, M. Sotomayor, D. M. Cortes, B. Roux, K. Schulten and E. Perozo. 2007. Three dimensional architecture of MscS N-terminus and transmembrane segments in the closed state. *Biophysical J.*: **114A**.

V. Yarov-Yarovoy, D. Baker, and W. A. Catterall. 2006. Voltage sensor conformations in the open and closed states in ROSETTA structural models of K(+) channels. *Proc. Natl. Acad. Sci. USA,* **103:** 7292–7297.

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