make the basic capability demonstrated here even more powerful.

One of the main questions still to be addressed is the effect of the substrate and the imaging tip on the movements of target molecules. This is especially crucial when the process of interest involves loosely bound or mobile molecules, as in the diffusion of a protein along a DNA molecule. In the experiments of van Noort et al., photolyase was in some cases observed to diffuse in the direction opposite that of the of the AFM tip, suggesting that the effects of imaging forces may not be large. But this is a point that must be carefully tested with well-understood systems before quantitative conclusions can be drawn.

Real-time, single-molecule movies have been demonstrated only rarely in the past, mostly with nucleic acids. For example, Guthold showed that individual cuts of surface-bound DNA by the Bal 31 restriction nuclease could be followed by AFM (Bustamante et al., 1994). And in perhaps the most spectacular demonstration of real-time imaging, Kasas et al. were able to directly observe transcription by *Escherichia coli* RNA polymerase on a DNA template by AFM (Kasas et al., 1997). Collectively, these experiments and others point the way to a general AFM-based approach to studying protein-nucleic acid interactions of many kinds. One particularly intriguing long-term possibility is the use of AFM to perform "in vitro motility assays" for molecules like the RNA and DNA polymerases. In the molecular motor field, "gliding filament" assays, combined with site-specific mutagenesis, have played a crucial role in sorting out the important structural features of myosins and other motors, and in testing hypothetical mechanisms of force generation. If it can be demonstrated that neither the substrate nor the imaging tip interferes significantly with movement, the AFM may make similar experiments possible for polymerases.

REFERENCES

Bustamante, C., D. A. Erie, and D. Keller. 1994. Biochemical and structural applications of scanning force microscopy. *Curr. Opin. Struct. Biol.* 4:750–760.

Coppin, C. M., J. T. Finer, J. A. Spudich, and R. D. Vale. 1996. The detection of sub-8 nm movements of kinesin by high resolution optical trap microscopy. *Proc. Natl. Acad. Sci. USA.* 93:1913–1917.

Kasas, S., N. H. Thompson, B. L. Smith, H. G. Hansma, X. S. Zhu, M. Guthold, C. Bustamante, E. T. Kool, M. Kashlev, and P. K. Hansma. 1997. *Escherichia coli* RNA polymerase activity observed using atomic force microscopy. *Biochemistry.* 36:461–468.

Kellermayer, M. S. Z., S. B. Smith, H. L. Granzier, and C. Bustamante. 1997. Folding-unfolding transitions in single titin molecules characterized with laser tweezers. *Science.* 276:1112–1116.

Rief, M., M. Gautel, F. Oesterhelf, J. M. Fernandez, and H. E. Gaub. 1997. Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science.* 276:1109–1112.

Molecular Dynamics Simulations of Ion Channels: How Far Have We Gone and Where Are We Heading?

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In this issue of the *Biophysical Journal,* Tieleman and Berendsen report the results of a molecular dynamics (MD) simulation of the pores formed by an *Escherichia coli* porin in a fully hydrated explicit POPE bilayer. The microscopic system includes the full OmpF trimer, 318 lipids (POPE), and 12,992 water molecules for a total of 65,898 atoms. After an equilibration period, the trajectory is generated for more than 1 nanosecond. By all standards, this is a monumental calculation of an important biological system.

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The publication of the paper of Tieleman and Berendsen is a good opportunity to pause and look back at the impressive progress accomplished in computer simulations of biomolecular systems over the years. Since the first dynamical calculation of a simple liquid of hard spheres (Alder and Wainwright, 1957), MD simulations have grown rapidly in complexity: first molecular dynamics of liquid water (Rahman and Stillinger, 1971), of a protein (McCammon et al., 1977), of an ion channel (McKay et al., 1984), of a bilayer membrane (Egbert and Berendsen, 1988), and of an ion channel in a membrane (Woolf and Roux, 1994). The present work by Tieleman and Berendsen offers a striking example of how current MD simulations have reached the point where atomic models can provide realistic representations of complex biological systems. In the present paper, OmpF, a large transmembrane ion channel, was simulated in a realistic model of a bilayer membrane. In particular, the simulation shows the properties of the pore and its water content. Around the pore constriction zone, the water dipoles are highly ordered perpendicular to the channel axis; the diffusion coefficients of water molecules inside the pore is greatly reduced.

Porins represent an important model system for studying ion channels at the microscopic levels. Several aspects of the function of OmpF have not been entirely elucidated and will probably require a combination of experiments and calculations. In principle, MD simulations based on detailed realistic atomic models can help to understand better the function of these systems. Nonetheless, despite the progress in computer simulations, theoretical investigations of ion channels are still faced with particularly difficult and serious problems.

A first problem arises from the magnitude of the interactions involved. The large hydration energies of ions, around -400 kJ/mol for Na^+, contrast with the activation energies deduced from experimentally observed ion fluxes, which generally do not exceed 10 k_BT. This implies that the energet-

ics of ion transport results from a delicate balance of very large interactions. This raises the question of the potential function and the influence of induced polarization, which is usually neglected in current calculations. A second problem arises from the time scales involved. The passage of one ion across a channel takes place on a microsecond time scale and realistic simulations of biological systems, which typically do not exceed a few nanoseconds, are insufficiently short. Straight molecular dynamics still cannot account for the time scales of ion permeation, and specialized simulation methods must be used to investigate these systems. A last difficulty is the translation of the results obtained from a microscopic model into macroscopic observables such as channel conductance and current-voltage relations (IV). How to go effectively from MD to IV curves remains a fundamentally unresolved question.

Future progress in theoretical studies of ion transport will come from efforts to push forward the limits in three directions: improving the potential function, developing appropriate simulation methods, and formulating useful theoretical frameworks for establishing a link between detailed trajectory and macroscopic quantities that are measured experimentally. An essential prerequisite for undertaking meaningful studies based on atomic models is the availability of a high resolution structure. The present work was made possible because the structure of OmpF was determined by x-ray crystallography (Cowan et al., 1992). The very recent determination of the structure of the K channel from *Streptomyces lividans* will provide another very exciting system to investigate ion permeation (Doyle et al., 1998). Meanwhile, it is stimulating to read about this impressive calculation.

REFERENCES

Alder B. J., and T. E. Wainwright. 1957. Phase transition for a hard sphere system. *J. Chem. Phys.* 27:1208–1209.

Cowan S. W., T. Schirmer, G. Rummel, M. Steiert, R. Gosh, R. A. Pauptit, and J. N. Jansonius. 1992. Crystal structures explain functional properties of two E. coli porins. *Nature*. 358:727–733.

Doyle D. A., J. M. Cabral, R. A. Pfuetzner, A. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, and R. MacKinnon. 1998. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science*. 280: 69–77.

Egberts E., and H. J. C. Berendsen. 1988. Molecular dynamics of a smectic liquid crystal with atomic detail. *J. Chem. Phys.* 89: 3718–3732.

McCammon J. A., B. R. Gelin, and M. Karplus. 1977. Dynamics of folded proteins. *Nature*. 267:585–590.

Mackay D. H., P. H. Berens, K. R. Wilson, and A. T. Hagler. 1984. Structure and dynamics of ion transport through gramicidin A. *Biophys. J.* 46:229–248.

Rahman A., and F. H. Stillinger. 1971. Molecular dynamics study of liquid water. *J. Chem. Phys.* 55:3336–3359.

Woolf T. B., and B. Roux. 1994. Molecular dynamics simulation of the gramicidin channel in a phospholipid bilayer. *Proc. Natl. Acad. Sci. USA*. 91:11631–11635.

Cells Use the Singular Properties of Different Channels to Produce Unique Electrical Songs

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A challenge of membrane biophysics is to determine how the gating properties of the ionic channels expressed in a cell contribute to the electrical activity pattern of that cell. Acting as a conductor, the cell chooses which channels are expressed and modifies those channels so that the electrical notes of each class of channels combine to form a unique song. In this issue of *Biophysical Journal*, Richmond et al. (1998) examined how the unique gating properties of cardiac Na^+ channels enable the channels to remain excitable in the setting of repetitive long-duration cardiac action potentials.

We have some understanding of how the gating properties and distribution of Na^+ channels enable different activity patterns in mature innervated skeletal muscle fibers (Ruff, 1996). Fast twitch skeletal muscle fibers fire action potentials at relatively high frequencies but are active briefly. In contrast, slow twitch fibers fire at relatively slow rates and are tonically active (Hennig and Lømo, 1985). Fast twitch fibers have a high density of Na^+ channels. The high channel density reduces the refractory period for action potential generation, which enable fast twitch fibers to fire at a high rate. The resting potentials of fast twitch fibers are close to the operating voltage ranges for fast and slow inactivation. Therefore, action potential activity and membrane depolarization produced by accumulation of extracellular potassium inactivate Na^+ channels in fast twitch fibers and prevent fast twitch fibers from firing continuously. In slow twitch fibers, the resting potential is separated from the operating ranges for fast and slow inactivation by a relatively large margin, which enables slow twitch fibers to fire tonically. The low density of Na^+ channels on slow twitch fibers forces the slow twitch fibers to fire at a slow rate. Consequently, variations in the distribution and gating properties of skeletal muscle Na^+ channels enable fast and slow twitch fibers to have distinctive activity patterns (Ruff, 1996).

Cardiac cells have very different activity patterns compared with skeletal muscle cells. Extremely long-duration cardiac action potentials would inactivate skeletal muscle Na^+ channels. Natural firing rates of cardiac cells are slow enough to permit Na^+ channels to recover from fast inactivation. However, if cardiac cells were populated with skeletal muscle Na^+ channels, the tardy recovery from slow inactivation would prevent cardiac cells from firing at rates $\geq$1 Hz. In skeletal muscle, slow inactivation regulates the popula-

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