New and Notable

Making Movies of Molecular Motions

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Traditionally, biomolecules have been viewed as chemical entities, to be characterized and understood in terms of their thermodynamic properties or by the kinetics of their chemical reactions. But in recent years, with the growing body of high-resolution structural information from crystallography, and new, detailed mechanisms of large "mechanochemical" molecules such as the molecular motors, F_1F_o ATP synthases, type II topoisomerases, RNA polymerases, and others, it has become fashionable to think of living things as collections of tiny machines, each carrying out a specific task in the overall process of growth and cell division. Most standard biophysical methods require large numbers of molecules and measure ensemble-averaged properties. This has the distinct advantage that signals that depend on the number of molecules are greatly amplified, making the experimentalist's job easier. But many of the most important properties of any machine (small or large) do not scale up with numbers, and many of the most interesting puzzles cannot be easily solved by ensemble-averaged measurements. It seems reasonable to expect that small machines are best studied the same way we would naturally study big machines: by observing what they do in well-defined situations one at a time. Considerable effort has been devoted in recent years toward developing methods for doing exactly that. Examples include direct measurements of forces and stepping distances on individual molecular motors (Coppin et al., 1996), measurements of stretching and protein unfolding forces in titin (Rief et al., 1997; Kellermayer et al., 1997), and the direct observation of transcription by RNA polymerase (Kasas et al., 1997), among many others.

Now, in the paper by van Noort et al. on page 2840 of this issue of *Biophysical Journal*, this "single molecule manipulation and measurement" approach is taken to a new level of sophistication. The authors show that by careful attention to detail, and by careful optimization of the parameters that control imaging forces and speed in an atomic force microscopy (AFM) experiment, it is possible to record the detailed movements of DNA molecules and proteins. This includes such difficult-to-detect processes as sliding of nonspecifically bound protein along DNA molecules and transient annealing of sticky ends of DNA molecules previously cleaved by an endonuclease. Perhaps most importantly, the authors have been able to distinguish clearly which regions on any given DNA molecule are firmly pinned to the substrate surface, and which regions are free to move and interact with other molecules. This kind of detailed molecule-by-molecule information goes a long way toward making real-time AFM investigations of protein-nucleic acid interactions both interpretable and routine.

Although it has been clear from almost the beginning that AFM has the potential for this kind of powerful, single-molecule cinematography, several technical problems have hindered it. The essence of movie-making is the ability to quickly take many images of the same field of view. AFM images are formed by measuring the force of interaction between the sample molecules and a sharp probe tip as the tip is scanned over the sample surface. This method of creating an image makes the two requirements for a molecular movie (images must be collected quickly and many images must be collected from the same spot) difficult to satisfy at the same time. On the one hand, interaction forces must be kept small, or the sample will be damaged by repeated scans. On the other hand, each force measurement must be made quickly, or the time resolution of the experiment will suffer. This is an example of the trade-off between sensitivity and bandwidth that is encountered in many high-sensitivity measurements, and is a nearly universal problem in single-molecule experiments of all kinds, no matter what the instrumental setup. In AFM, the main source of noise is the ever-present, irreducible Brownian forces acting on the force sensor. These "thermal fluctuations" are caused purely by the presence of the buffer solution surrounding the sample, and are impossible to distinguish from sample-induced forces without extensive, relatively slow, time averaging.

The central result of the experiments by van Noort et al. is that with existing instrumentation it is possible to take high-quality movies involving many images (25 or more) of the same field of view with reasonable time resolution (~1 min/frame, or 5000 image pixels/s) and minimal disturbance to the sample molecules. Furthermore, by aligning and averaging many frames together, areas of loosely attached, rapidly moving DNA could be distinguished from areas strongly bound to the substrate. When molecules of photolyase, a DNA repair enzyme, are introduced, they bind and diffuse in the rapidly moving regions of the DNA, but stop at the bound regions. This is the most systematic demonstration so far that detailed, almost real-time dynamical information can be obtained by in situ AFM imaging. Moreover, as the authors themselves point out, AFM is still a relatively young technique, and breakthrough improvements are likely in the near future. This can only

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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.

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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.

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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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tion of excitable Na^+ channels. Disruption of slow inactivation in mutant skeletal muscle Na^+ channels potentiates the ability of the mutant channels to produce depolarization-induced paralysis in disorders such as hyperkalemic periodic paralysis (Hayward et al., 1997). While slow inactivation may act as governor for membrane excitability in skeletal muscle, slow inactivation would prevent the electrical activity pattern characteristic of cardiac cells. Richmond et al. (1998) demonstrate that cardiac cells circumvent the problem presented by the presence of slow inactivation in skeletal muscle Na^+ channels by using a different Na^+ channel. Slow inactivation reduces cardiac Na^+ currents by only 40% in response to prolonged depolarizations. Cardiac Na^+ channels manifest complete fast inactivation. However, the rapid kinetics for recovery from fast inactivation enables the cardiac Na^+ channels to regain excitability in sufficient time to permit cardiac cells to have repetitive long-duration action potentials at 1 $\geq$Hz firing rates.

The unique properties of cardiac Na^+ channels help to explain some electrical patterns observed in immature skeletal muscle fibers and in denervated fibers. Early in development and after denervation, skeletal muscle cells express cardiac Na^+ channels (Trimmer, 1990). Immature and denervated skeletal muscle fibers are depolarized compared with mature innervated muscle fibers. The presence of the cardiac Na^+ channel isoform may enable immature fibers to be electrically excitable. Cardiac Na^+ channels expressed in denervated skeletal muscle fibers probably enable the denervated muscle fibers to be electrically excitable and to manifest spontaneous action potentials called fibrillation potentials. Spontaneous electrical activity in denervated skeletal muscle fibers may be important in slowing the rate of disuse atrophy. The spontaneous electrical activity in denervated fibers could enable the fibers to survive until reinnervation occurs.

By selecting the appropriate Na^+ channels a striated muscle cell can produce the brief staccato song of the fast twitch skeletal muscle cell or the slow persistent song of the cardiac myocyte.

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