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

Received for publication 1 April 1998 and in final form 2 April 1998.

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0006-3495/98/06/2743/02 $2.00

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.

Received for publication 20 April 1998 and in final form 21 April 1998.

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0006-3495/98/06/2744/02 $2.00

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-