Cecchi, G., P. J. Griffiths, and S. Taylor. 1982. Muscular contraction: kinetics of crossbridge attachment studied by high-frequency stiffness measurements. *Science.* 217:70–72.

Cooke, R., H. White, and E. Pate. 1994. A model of the release of myosin heads from actin in rapidly contracting muscle fibers. *Biophys. J.* 66:778–788.

Finer, J. T., R. M. Simmons, and J. A. Spudich. 1994. Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature.* 368:113–119.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1977. Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *J. Physiol.* 269:441–515.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1981. The relation between stiffness and filament overlap in stimulated frog muscle fibres. *J. Physiol.* 311:219–249.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1986. Tension transients during the rise of tetanic tension in frog muscle fibres. *J. Physiol.* 372:595–609.

Harada, Y., K. Sakurada, T. Aoki, D. D. Thomas, and T. Yanagida. 1990. Mechanochemical coupling in actomyosin energy transduction studied by *in vitro* movement assay. *J. Mol. Biol.* 216:49–68.

Higuchi, H., and Y. E. Goldman. 1991. Sliding distance between actin and myosin filaments per ATP molecule hydrolysed in skinned muscle fibres. *Nature.* 352:352–354.

Huxley, A. F. 1973. A note suggesting that the cross-bridge attachment during muscle contraction may take place in two stages. *Proc. Roy. Soc. Lond. B.* 183:83–86.

Huxley, A. F. 1974. Review lecture. Muscle contraction. *J. Physiol.* 243:1–43.

Huxley, A. F., and R. M. Simmons. 1971. Proposed mechanism of force generation in striated muscle. *Nature.* 233:533–538.

Huxley, A. F., and R. M. Simmons. 1972. Mechanical transients and the origin of muscular force. *Cold Spring Harbor Symp. Quant. Biol.* 37:669–680.

Huxley, H. E., A. Stewart, H. Sosa, and T. Irving. 1994. x-ray diffraction measurements of the extensibility of actin and myosin filaments in contracting muscle. *Biophys. J.* 67:2411–2421.

Irving, M. 1987. Muscle mechanics and probes of the crossbridge cycle. *In* Fibrous Protein Structure. J. M. Squire and P. J. Vibert, editors. Academic Press, San Diego. 495–528.

Ishijima, A., Y. Harada, H. Kojima, T. Funatsu, H. Higuchi, and T. Yanagida. 1994. Single-molecule analysis of the actomyosin motor using nano-manipulation. *Biochem. Biophys. Res. Commun.* 199:1057–1063.

Julian, F. J., and D. L. Morgan. 1981. Tension, stiffness, unloaded shortening speed and potentiation of frog muscle fibres at sarcomere lengths below optimum. *J. Physiol.* 319:205–217.

Kojima, H., A. Ishijima, and T. Yanagida. 1994. Direct measurement of stiffness of single actin filaments with and without tropomyosin using *in vitro* nano-manipulation. *PNAS.* In press.

Lombardi, V., and G. Piazzesi. 1990. The contractile response during steady lengthening of stimulated frog muscle fibres. *J. Physiol.* 431: 141–171.

Lombardi, V., G. Piazzesi, and M. Linari. 1992. Rapid regeneration of the actin-myosin power stroke in contracting muscle. *Nature.* 355: 638–641.

Naber, N., M. Lorenz, and R. Cooke. 1994. The orientation of spin-probes attached to cys374 on actin in oriented gels. *J. Mol. Biol.* 236: 703–709.

Naber, N., E. M. Ostap, D. D. Thomas, and R. Cooke. 1993. Orientation and rotational dynamics of spin-labeled phalloidin bound to actin in muscle fibers. *Proteins.* 17:347–354.

Nishizaka, T., T. Yagi, Y. Tanaka, and S. Ishiwata. 1993. Right-handed rotation of an actin filament in an *in vitro* motile system. *Nature.* 361:269–271.

Oosawa, F. 1977. Actin-actin bond strength and the conformational change of *F*-actin. *Biorheology.* 14:11–19.

Ostap, E. M., T. Yanagida, and D. D. Thomas. 1992. Orientation distribution of spin-labeled actin oriented by flow. *Biophys. J.* 63:966–975.

Wakabayashi, K., Y. Sugimoto, H. Tanaka, Y. Ueno, Y. Takezawa, and Y. Amemiya. 1994. x-ray diffraction evidence for the extensibility of actin and myosin filaments during muscle contraction. *Biophys. J.* 67:2422–2435.

Single Molecule Spectroscopies and Imaging Techniques Shed New Light on the Future of Biophysics

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Starting the mid-1970s, the development of the patch-clamp technique revolutionized molecular neurophysiology by allowing investigators to observe directly the gating of individual (i.e., single molecule) transmembrane ion channels both in vitro and in vivo. However, other fields of biomedical research could not actively participate in this revolution because the sensitivity needed to examine other classes of biomolecules did not exist. This has now changed. Technological advances in the fields of video enhanced differential interference contrast microscopy (Gelles et al., 1988; Schafer et al., 1991; Berliner et al., 1994), microscopic optical interferometry (Denk and Webb, 1990; Svoboda et al., 1994), optical trapping (Block, 1992; Kuo and Sheetz, 1993; Finer et al., 1994), ultrasensitive fluorescence detection (Bustamante, 1991), atomic force microscopy (Radmacher et al., 1994), and near-field scanning optical microscopy (Betzig and Chichester, 1993; Trautman et al. 1994; Hess et al., 1994; Xie et al., 1994; Ambrose et al., 1994) has now ushered in an age where single molecule experiments on a wide variety of biomolecules are now possible (note: reference listings are neither inclusive nor historical, simply a sampling of recent interesting results). Single-molecule studies (kinetic and structural) have numerous advantages over conventional biochemical techniques, which are restricted to examination of the population averaged properties of large molecular ensembles. It is clear that for a detailed understanding of the mechanistic steps of many molecular machines (e.g., actin-myosin and kinesin power strokes, DNA replication, DNA transcription, microtubule assembly, etc.) individual molecular events need to be monitored.

In a paper appearing in this issue, Yin et al. (1994) describe kinetic measurements of mRNA transcript elongation by a single *Escherichia coli* RNA polymerase molecule. In these experiments, biotinated DNA is tethered to a 0.23 μm polystyrene microsphere coated with avidin. Video enhanced differential interference contrast microscopy directly images the polystyrene bead. Stalled transcription complexes of *E. coli* RNA polymerase is adsorbed onto the surface of a specially treated coverslip. These stalled complexes are then incubated with avidin coated beads, which will attach to the end of the DNA. The DNAs can be made such that the bead is either "upstream" or "downstream" from the stalled site. When located upstream, the bead is drawn downward toward the coverslip as transcription proceeds, whereas for the downstream location, the length of the DNA tether increases and the bead moves farther away from the coverslip (see Fig. 1 *b* of Schafer et al. (1991) for a schematic representation of these experiments).

All of the nanometer displacements in this system are obtained by observing the extent of Brownian motion of

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the tethered particle on the end of the DNA. Larger tethers will have more extensive Brownian motion and will thus appear more "blurry" on successive images obtained with the DIC. Bead position is accurately located by nonlinear fitting of DIC images using a difference function of two two-dimensional Gaussians with their centers offset by a fixed distance along the direction of the shear axis of the DIC microscope. Previous work has had to utilize untested numerical simulation of tethered bead motion to calibrate distance changes. In this work, a range of DNA tethers are examined from 308 to 1915 base pairs. These experiments yielded a linear calibration relating the observed Brownian motion and tether length that allowed a full description of the limits affecting the accuracy of measuring single molecule motion along a DNA primer/template. An instrumentational limit of ± 10 base pairs is obtained in the limit of very small DNA tethers.

Application of this technique to the study of proteins that move in a directed fashion on DNA are just beginning. As such, this article is "setting-the-stage" for many additional future studies. Systems as wide ranging as DNA helicases, DNA polymerases, DNA and RNA exonucleases (etc.) could be characterized using this approach. This group has also recently measured DNA looping induced by the binding of two lactose repressor molecules (Finzi and Gelles, submitted for publication). These studies reveal that this methodology should also prove useful for the examination of long-range topological changes (i.e., DNA looping) associated with multi-component protein complexes binding upstream of gene start sites. As more and more single molecule methodologies (see above) reach maturation, it could very well occur that biophysics (in the next decade) could become dominated by single molecule techniques. All of this goes to show that perhaps "one" is not such a lonely number after all.

REFERENCES

Ambrose, W. P., P. M. Goodwin, J. C. Martin, and R. A. Keller. 1994. Alterations of single molecule fluorescence lifetimes in near-field optical microscopy. *Science*. 265:364.

Berliner, E., H. K. Mahtani, S. Karki, L. F. Chu, J. E. Cronan, and J. Gelles. 1994. Microtubule movement by a biotinated kinesin bound to a streptavidin-coated surface. *J. Biol. Chem.* 269:8610–8615.

Betzig, E., and R. J. Chichester. 1993. Single Molecules observed by near-field scanning optical microscopy. *Science*. 262:1422–1425.

Block, S. M. 1992. Making light work with optical tweezers. *Nature*. 360:493–495.

Bustamante, C. 1991. Direct observation and manipulation of single DNA molecules using fluorescence microscopy. *Annu. Rev. Biophys. Biophys. Chem.* 20:415–446.

Denk, W., and W. W. Webb. 1990. Optical measurement of picometer displacements of transparent microscopic objects. *Applied Optics*. 29:2382–2391.

Finer, J. T., R. M. Simmons, and J. A. Spudich. 1994. Single myosin molecule mechanics: piconewton forces and nanometre steps. *Nature*. 368:113–113–119.

Gelles, J., B. J. Schnapp, and M. P. Sheetz. 1988. Tracking kinesin-driven movements with nanometre-scale precision. *Nature*. 331:450–453.

Hess, H. F., E. Betzig, T. D. Harris, L. N. Pfeiffer, and K. W. West. 1994. Near-field spectroscopy of the quantum constituents of a luminescent system. *Science*. 264:1740–1745.

Kuo, S. C., and M. P. Sheetz. 1993. Force of single kinesin molecules measured with optical tweezers. *Science*. 260:232–234.

Radmacher, M., M. Fritz, H. G. Hansma, and P. K. Hansma. 1994. Direct observation of enzyme activity with the atomic force microscope. *Science*. 265:1577–1579.

Schafer, D. A., J. Gelles, M. P. Sheetz, and R. Landick. 1991. Transcription by single molecules of RNA polymerase observed by light microscopy. *Nature*. 352:444–448.

Svoboda, K., C. F. Schmidt, B. J. Schnapp, and S. M. Block. 1994. Direct observation of kinesin stepping by optical trapping interferometry. *Nature*. 365:721–727.

Trautman, J. K., J. J. Macklin, L. E. Brus, and E. Betzig. 1994. Near-field spectroscopy of single molecules at room temperature. *Nature*. 369: 40–42.

Xie, X. S., and R. C. Dunn. 1994. Probing single molecule dynamics. *Science*. 265:361–364.

Yin, H., R. Landick, and J. Gelles. 1994. Tethered particle motion method for studying transcript elongation by a single RNA polymerase molecule. *Biophys. J.* 2468–2478.

Biological Scanning Probe Microscopy Comes of Age

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Not so long ago, a referee of one of our papers opined that "atomic force microscopy (AFM) is only convenient, as the authors claim, if one has access to such a microscope. Generally, the electron microscope is to be preferred." Despite its low cost, and obvious potential, the new kid on the block was not universally welcomed by the establishment. Now a group (based at a renowned center for electron microscopy) has produced a study (Schabert and Engel, 1994) that goes well beyond the quick and pretty demonstrations characteristic of some early work. It shows how, once the uncertainties that plague a new technology are worked out, sophisticated analysis can be used to great advantage.

On page 000 of this issue, Schabert and Engel describe an atomic force microscopy (AFM) study of crystalline membranes reconstituted from *Escherichia Coli* OmpF porin and phospholipids. The work is notable not only for the careful procedures and excellent (sub-nanometer) resolution, but also for the introduction of image analysis techniques that have long been used in the electron microscope community. High resolution topographs of both the periplasmic and extracellular sides of the porin were obtained and a novel crystal packing was observed. But the paper has an appeal that goes beyond what is added to the literature on porin. It is a model of clarity in describing sample preparation, microscope operation and image analysis. New workers in the field would do well to read this paper carefully, for it delineates the steps that need to be taken for high resolution imaging in biologically relevant conditions. The pedagogical value of the paper is enhanced by clear descriptions of the limitations of the technique and some of the difficulties encountered by the authors.

The introduction of the paper refers to a number of recent reports of high resolution AFM imaging of (even soluble) proteins in water. The stage is set for major discoveries, not least because the new technology is now widely available, and at a fraction of the cost of electron microscopes.

This is not the only area in which biological scanning probe microscopy is making an impact. The Hansma group has recently shown how enzymatic activity can be monitored in-situ by

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