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.

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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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monitoring the height fluctuations of lysozyme in the presence of a substrate (Radmacher et al., 1994). The Bustamante group has identified new morphologies for chromatin that have implications for the role of histone H1 (Zlatanaova et al., 1994), and L. Chang, F. S. Frank, P. Flicker, and D. Keller (personal communication) have used AFM to provide rather compelling evidence that both right and left handed forms of F-actin exist. Using a clever statistical analysis, Williams et al. (1994) have shown how aggregates can be analyzed to yield highly accurate particle size data. Thus, even rather poor images can be used to obtain valuable information on biologically important surfaces in realistic conditions.

The atomic force microscope is presently the instrument of choice for biological applications but, as discussed in a recent review I wrote for this journal (*Biophysical Journal* 67:937), the scanning tunneling microscope (STM) has a role in very high resolution imaging and in probing electronic effects. And, just to quell any impression that this is now a mature technology, the STM has surprises in store. The group of Guckenberger has provided dramatic illustrations of the way in which the STM, nominally limited to imaging good conductors, can, in fact, image on insulating surfaces in certain conditions (Guckenberger et al. (1994) and other material still in preprint form).

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Unraveling the Ryanodine Receptor

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Skeletal muscle activity is initiated by depolarization of the transverse (T) tubules, followed by release of calcium from the sarcoplasmic reticulum (SR). The ryanodine receptor (RyR), a 30S homotetramer, has been identified as the SR calcium release channel. The sequence of the single polypeptide predicts that 80% of the molecule at the N terminal side is hydrophilic and the remaining 20% forms the intramembrane channel. Thus, the unusually large size of the RyR (~2,200 kDa) is because of its cytoplasmic assembly, formed by the four subunits. The square shape of the cytoplasmic assemblies of RyRs have allowed their identification with the feet, structures located in the gap between SR and T tubules. In skeletal muscle, the voltage sensors of e-c coupling, the dihydropyridine receptors (DHPRs), are probably linked to the RyRs, so that a molecular interaction between the two is possible. Another calcium release channel of the endoplasmic reticulum (the IP3 receptor) has sequence homology to the RyR, and an analogous structure, but it does not interact with the surface membrane. Two recent papers have added important new information on the ryanodine receptor by providing a high resolution image of the channel, and identfying a ligand binding site.

Understanding of the control of RyR permeability during activation of muscle contraction and of The RyR's response to various ligands requires knowledge of the protein configuration. A paper in press in the *Journal of Cell Biology* (Radermacher et al., 1994) and a report in this issue of *Biophysical Journal* by Wagenknecht et al. give a 3-D reconstruction of the channel at ~3 nm resolution, and the location of the calmodulin binding site. The purified, detergent solubilized channel was imaged in the EM. Three powerful approaches were used: cryoEM, which allows structural preservation to high levels of resolution without stains or fixatives, image averaging, and conical tilt reconstruction, which permits three dimensional reconstruction from a random field of molecules, using only a pair of images. In both papers, correspondence analysis allowed classification of the images into classes and averaging of information from a number of structurally homogeneous molecules selected in an unbiased fashion. The data provide a framework, which will be the basis for an atomic model of the RyR.

The reconstructed RyR (Radermacher et al., 1994) shows a clear demarcation between the channel and cytoplasmic assemblies, both with fourfold symmetry. The cytoplasmic assembly is tall, thus providing a physical link between SR and T tubules. Several details of its structure are noteworthy. It is highly hydrated, with the architecture of a scaffolding designed to provide a mechanical linkage between the two membranes while allowing excellent opportunity for flow of calcium from the channel to the myofibrils. In addition, a possible direct pathway for calcium exit is seen near the junction of the transmembrane and cytoplasmic assemblies. The four corners of the molecule have a complex outline with grooves that seem appropriate for interdigitating with neighboring molecules, as seen when the molecule forms arrays in situ. The three highest domains of the cytoplasmic assembly, near its corners, are likely to be those interacting with the DHPRs. Finally, the most important consideration is the link between the cytoplasmic domain, which presumably has binding sites for regulators of the channel properties (e.g., DHPRs and calmodulin, see below) and the channel that is being regulated. Only one connection is seen between the cytoplasmic and transmembrane domains, making it a somewhat tenuous link. However, a central plug-like mass in the channel is sufficiently close to this connecting domain that an interaction is possible. As usual, details of the intramembrane region of the molecule

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