doesn't seem unreasonable. It would also be interesting to look at the behavior of probe diffusion when the net charge on the probe is opposite that of DNA or other host macromolecule. (BSA and DNA are both negatively charged.) Such a system would be more sensitive to intermolecular electrostatic interactions since the probe and host species would, on the average, be in closer proximity. In any case, there is little doubt that the article by Dwyer and Bloomfield opens the door on using Brownian dynamics to study diffusional phenomena in crowded or congested media.

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Testing the Limits of Flagellar Motors

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What happens when you push the bacterial rotary motor past redline? Do its main bearings fail, or does it burn a piston? And how fast can it turn before internal processes become rate-limiting and its torque begins to drop? To answer these questions requires a means of controlling the speed of the motor over a wide range, while measuring the torque it produces. The method that best answers the need is electrorotation (Iwazawa et al., 1993, Washizu et al., 1993); this method appears to have reached its apotheosis in the elegant study of Berg and Turner (1993).

The bacterial flagellar motor rotates, using energy derived from the transmembrane proton gradient. Considering the very small size of the motor—its diameter is only about 50 nm—it is remarkable how precisely its performance can be measured. For almost 20 years, it has been possible to measure the torque of individual motors operating at slow speeds. These measurements take advantage of the fact that flagellar filaments, which under ordinary circumstances rotate to produce the thrust that propels a cell, can be fixed to a stationary substrate (a coverslip, for example), so that the motor forces the entire cell body to spin. These "tethered" cells are easy to observe under the light microscope, and by measuring their rotation speed and size it is possible to compute the torque produced by the motor. However, the viscous drag-resisting movement of a tethered cell body is much greater than that on the slender flagellar filament, so motors turning tethered cells are subject to an abnormally large load. The result is that the motors turn slowly (about 10 Hz is typical), at a speed dictated by the load rather than by internal rate limitations.

In contrast, motors rotating the filaments of free-swimming cells can turn at more than 300 Hz, and the speed appears to be governed by the internal dynamics of the motor (Lowe et al., 1987). But under those circumstances, the several filaments on each cell coalesce into a bundle, acting jointly to propel the cell. Since the bundle is driven by several motors, the torque of individual motors is difficult to estimate. Also, while the load on the flagellar bundle can be adjusted by changing the medium viscosity, it is not possible to span the full load-range of interest in this way.

The method of electrorotation furnishes a means for exploiting the simple geometry of tethered cells while at the same time reducing or even reversing the load so that the motor can turn very fast. In this method, a rapidly rotating electric field is applied to the cell. In Berg and Turner's experiments, the field was rotated at 2.25 MHz. Washizu et al. (1993) also have reported experiments in which the field was rotated very fast (0.5 MHz). In both cases, the field rotation was much faster than the rotation of the cell—torque is applied not because the field "grabs" the cell but because it induces dipolar charge distributions in the cell and surrounding medium which, owing to the finite time required for charge movement, are slightly out of phase with the applied field. This method using high-frequency fields should not be confused with the very different method of Iwazawa et al. (1993), in which the field was rotated slowly (about 60 Hz) and the cells were entrained to the field.

Berg and Turner faced a number of experimental challenges in order to take full advantage of the electrorotation method. When applying strong electric fields, a major concern is to avoid cooking the cells by Joule heating. To ensure that heat buildup was not prohibitive, they tethered cells to sapphire, whose thermal conductance is about 30 times that of glass. When large torques are applied to tethered cells they often tear loose from their moorings and, to the experimenter's chagrin, drift away. That problem was avoided by covalently linking the filaments to the sapphire, so they remained fast under torques greatly exceeding the normal motor torque.

These innovations have made it possible to measure the torque of the flagellar motor of *E. coli* across a very broad range of speeds, including negative speeds where rotation is in the direction opposite to the motor torque. It turns out to be surprisingly easy to push the motor beyond its safe limits—when a torque is applied so as to oppose rotation, the motor resists until, at about three times the normal running torque, it breaks. Breakage can be either catastrophic and irreversible as if the drive shaft were sheared, or incremental and more easily reversed as if multiple components successively fell off or were damaged. Since many genes specifying motor proteins have been isolated and their expression can be

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controlled, it should be possible to identify the vulnerable components by determining how the rate of repair is affected when various parts are supplied in excess.

It will be important to extend the electrorotation experiments to other bacterial species. Washizu et al., working with *S. typhimurium,* did not encounter the resistance to reversed rotation or the motor breakage noted by Berg and Turner. While it is often assumed that the motors of *E. coli* and *S. typhimurium* are identical in all important respects, that might not be true. Likewise, experiments with motile *Streptococcus* cells will be invaluable, because they can be starved and reenergized by artificial proton gradients.

The torque-speed characteristic of the flagellar motor, now known much better than before, must be accounted for by any viable model for torque generation. In contrast to an earlier suggestion that motor torque decreases steadily with increasing speed (Lowe et al., 1987), we now can say that the motor torque remains nearly constant up to about 100 Hz, and decreases more or less linearly above that speed, until more complex behaviors are encountered at very high speeds. The question is, what causes the torque to decrease in this way? The new data, and analogous data obtained using D_2O or motor mutants, should furnish some clues to the number and character of the processes that become limiting at high speeds. Why is the torque constant across such a broad range? Berg and Turner suggest the existence of a mechanical stop in the motor that limits the torque. Alternative explanations appear possible. The torque in this range might just reflect the total amount of energy available from the proton gradient; to explain the constancy, one would only have to assume that internal processes are fast enough not to dissipate significant amounts of energy across this range of speeds. In any event, the new data place severe constraints on plausible mechanisms, and more than ever it will be essential that new mechanistic proposals include calculations of steady-state performance that will permit them to be evaluated quantitatively.

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To Fuse or Not to Fuse?

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That was the question addressed by David Siegel in his paper "The Energetics of Intermediates in Membrane Fusion: Comparison of Stalk and Inverted Micellar Intermediate Mechanisms." This theoretical analysis of the fusion of pure phospholipid bilayers, and of the mechanisms underlying this process, poses a legitimate question: should one spend one's time on this problem? Is it a *jeau d'esprit* of a mathematician, or has it some bearing on physical chemistry or on real biological phenomena? We believe that answer is "yes" to all three questions.

The cellular membrane evolved as a wall separating the cell's contents from the external medium. This barrier function was conveniently assigned to a lipid bilayer, an ingenious biological invention combining the remarkable property of self-organization with unhindered lateral movement of membrane components. This leakproof shell maintains its integrity even in the toughest situations; rupture of the membrane is a catastrophic event leading to the loss of important cellular components and potentially to a cell's death.

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Despite the importance of membrane integrity, a number of crucial physiological events require the breakdown of two apposing membranes and their subsequent reconnection to one another. Examples include fertilization, cell division, endocytosis, exocytosis, and the entry of enveloped viruses into cells. Although membrane fusion has long been a focus of attention, even recent reviews inevitably come to the conclusion that "the physical and molecular mechanisms of membrane fusion remain obscure."

There is no doubt that, in many important cases of membrane fusion, specific proteins initiate the process. Then why study fusion of pure phospholipid bilayers? This approach is appropriate because any structural rearrangement of membranes must involve lipid bilayers. Besides, membrane fusion can be observed in pure phospholipid systems which have important practical applications per se and hence deserve special attention. These model systems have taught us important lessons about fusion (Rand and Parsegian, 1986) by defining the different stages of this process: membrane apposition, triggering, contact, local destabilization, membrane coalescence, and final restabilization. Different physical forces are involved at various stages of the overall process. One can assign various priorities to these stages, but the most crucial are breakdown of the apposing membranes and their reconnection. The intermediate structures involved in these processes stimulated intensive discussion in the literature and led to the emergence of two models. The first proposal, that fusion proceeds by way of inverted micelles, was initially advanced and strongly advocated by Verkleij and his colleagues (Verkleij et al., 1979a, b). The opposing model, which favors a stalk as the intermediate structure, was analyzed in detail by Russian biophysicists led by Chizmadzhev (Markin et al., 1984; Chernomordik et al., 1985, 1987; Leikin et al., 1987; Kozlov et al., 1989).

The theoretical analysis of the intermediate structures exploits a simple physical concept, the elasticity of membranes. Biological membranes resist