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Modeling of Macromolecular Diffusion in Congested Media

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Most in vivo biochemistry occurs in an environment crowded with a wide variety of biomolecules, membranes, and complexes such as the cytoplasm of a cell. Because of this, there have been numerous studies in recent years dealing with the concentration dependence of diffusion constants and other transport properties of macromolecules in both homogeneous and heterogeneous systems. A recent article (Zimmerman and Minton, 1993) reviews both theoretical and experimental aspects of macromolecular crowding. Modeling the dynamics of such systems present a number of difficulties that must be dealt with. First of all, the individual macromolecules themselves are complex. Second, we need to deal with more than just one of them and this includes accounting for their intermolecular interactions. Third, the time scale of interest should be long enough so that relative displacements are at least comparable to the linear dimensions of the biomolecules present. A particle with a hydrodynamic diameter of 30 Å will diffuse about the same distance in 30,000 ps. Molecular dynamics is now used routinely to model the dynamical behavior of individual proteins out to about 100 ps. However, because of the size and time scale considerations discussed above, it is not a viable technique for the problem at hand. From a practical standpoint, it is necessary to model the concentrated system in a manner more simplistic than used in molecular dynamics on the basis of overall size and time step.

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Over the past 10 years, Brownian dynamics has been used successfully to study the dynamical behavior of macromolecules modeled as beads or bead arrays. In the present issue (p. 1810) of *Biophysical Journal*, Dwyer and Bloomfield develop a Brownian dynamics algorithm for simulating probe and self diffusion of concentrated solutions. Most of these studies are based on the Ermak-McCammon algorithm (Ermak and McCammon, 1978) in which the solvent is represented as a bath of random noise which mimics the stochastic displacements of the beads due to impulsive collisions with solvent. Brownian dynamics has two limitations relative to molecular dynamics. First, because solvent averaged potentials are used, one cannot obtain detailed information on solvation structures. Second, one cannot get dynamical information on inertial motions since the underlying diffusion equations describe the average motion of the macromolecule whose motion has been interrupted by at least a few collisions with solvent molecules. For typical biopolymer systems, this will correspond to times larger than about 0.1 ps. In Brownian dynamics, a simplified model is used to represent the actual macromolecule. A short fragment of DNA consisting of several hundred base pairs, for example, might be modeled as a string of beads. Such a representation lacks the detail of an all-atom description used in molecular dynamics, but does exhibit the correct overall translational and rotational behavior of the actual fragment and also accurately mimics the internal dynamics of a semiflexible wormlike chain (Allison and Nambi, 1992). Most Brownian dynamics simulations to date have considered the diffusion of one or two macromolecules.

In the Brownian dynamics algorithm developed by Dwyer and Bloomfield, probe and self diffusion of concentrated solutions containing short DNA fragments (modeled as a string of beads) and the protein bovine serum albumin (BSA) (modeled as a single bead) is simulated. Interaction potentials employed are simple. Electrostatic interactions, for example, are approximated using Debye-Huckel potentials. It is the simplicity of the model that allows it to be used in studying the diffusion of a congested and heterogeneous solution of macromolecules. The question Dwyer and Bloomfield address is whether or not it is also realistic enough to reproduce the experimental transport data which is available for this particular system. At an ionic strength of 0.1 M, the answer is yes. The simulations accurately reproduce the probe diffusion of BSA in DNA over a wide range of DNA concentrations. They also accurately reproduce the self diffusion of BSA over a range of BSA concentrations. At an ionic strength of 0.01 M, the simulations are less successful with regards to the probe diffusion of BSA in DNA. Two possible causes for this discrepancy are inadequacy of the electrostatic model and neglect of hydrodynamic interaction. The authors believe that neglect of hydrodynamic interaction in the simulations is probably the primary reason for the discrepancy. Fitting the simulated diffusion data of BSA to a scaling law gives an ionic strength scaling law exponent, b, in reasonable agreement with b values reported for other systems.

The work of Dwyer and Bloomfield is significant since it shows that a simple model can accurately predict the transport properties of macromolecules in a concentrated and possibly heterogeneous solution under certain conditions at least. One obvious extension of this work would be to include hydrodynamic interaction, but this is problematic as discussed. Perhaps it would not be unreasonable to approximate hydrodynamic interaction using screened mobility tensors (Van Megen and Snook, 1988). This strategy has been criticized (as discussed by Dwyer and Bloomfield) since such screening cannot occur for systems of mobile particles even though it does occur for systems containing a stationary background of particles. In a single time step of a Brownian dynamics simulation, however, the assumption is made that the system is evolving in a stationary force field computed on the basis of its configuration at the beginning of the dynamics step. Consequently, using mobility tensors that are strictly valid only for a stationary background

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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