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New and Notable

Virus Dynamics Lead to Structure, by George!

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The interplay between macromolecular structure, dynamics, and function is an increasingly important theme in modern day biophysics. An understanding of function demands an exquisite appreciation of macromolecular dynamics be it on the picosecond time scale, as for bacterial reaction centres, or millisecond time scale, as for many enzyme reactions. The preceding statement will not ruffle the readers' feathers, but the notion that a virus particle may have interesting dynamical properties seems somewhat strange at first. However, George Thomas and coworkers in "Raman Dynamic Probe of Hydrogen Exchange in Bean Pod Mottle Virus: Base-specific Retardation of Exchange in Packaged ss RNA" have demonstrated that it is possible to probe dynamical events occurring in a virus and, moreover, that these events provide structural insight into RNA-protein interactions.

The so-called middle component of bean pod mottle virus (BPMV) consists of a protein capsid encapsulating a single strand of RNA (the RNA is $\sim 2 \times 10^6$ Da, which is about 30% of the mass of the entire particle). The crystal structure provides detail of the protein capsid, but only 20% of the RNA is sufficiently ordered to appear as electron density at the threefold vertices of the capsid. The structure shows that the ordered nucleic acid maintains a backbone stereochemistry approximating one strand of A-form RNA and that the nucleotides are in contact with hydrophilic protein side-chains. Obviously any approach that can shed further light on the nature of the RNA-protein packing will be of high interest.

The present work uses are a favorite strategem of scientists studying macromolecular dynamics, namely, observing the time scale of replacement of labile hydrogen atoms by deuterium atoms from the bulk solvent (Englander and Kallenbach, 1984). The concept goes back to the pioneering work of Linderstrøm-Lang in the 1950s and carries with it the assumption that hydrogen atoms, e.g., in the peptide NH bonds, that are tied up in rigid "fixed" interactions within macromolecules will undergo H to D exchange far less readily than those exposed to solvent (either permanently by being on the outside of the protein or periodically exposed due to fluctuations in the protein structure). Thus, by following and characterizing the exchange one can probe macromolecular dynamics.

The paper by Li, Johnson, and Thomas in this volume is the latest of a seminal series of studies using Raman spectroscopy to probe virus properties. Li et al. play two of Raman spectroscopy's trump cards to great effect namely, experimental flexibility, and the ability to acquire specific information. They design and use a simple apparatus to observe the effect of hydrogen to deuterium exchange on the Raman spectrum of the virus. Second, they employ separate Raman spectral features associated with protein amide linkages and the RNA's uracil, cytosine, guanine, and adenine populations to follow H to D exchange as a function of time.

The new sampling apparatus is simplicity itself. Li and colleagues use as their sample a drop of virus solution in H_2O inside a glass capillary. The laser beam is focussed into the drop and the Raman photons collected at right angles. They perform H_2O to D_2O exchange by threading a small dialysis tube through the top of the drop and running D_2O through the tube. They achieve complete bulk solvent exchange in about 2 mins, which represents the time resolution of their set-up. Parenthetically, it is instructive to compare the cost of this equipment, a few dollars for a time resolution of 120 s, with picosecond or femtosecond time-resolved spectroscopy equipment sometimes costing hundreds of thousands of dollars (and which would be of no use for the present experiment). Obviously, time, or lack of it, is money.

Li et al's results are easy to comprehend. When BPMV is exposed to D_2O for up to 10 min there is no measurable exchange of amide NH groups of the protein capsid indicating that more than 90% of these groups are engaged in relatively rigid hydrogen bonds. They do, however, see exchange occurring in the RNA bases but, and here is the crux, exchange in the bases occurs with different time scales! In fact base-specific retardation of exchange is seen in the packaged RNA. Retardation is greatest for the uracil residue population, for which the first-order exchange rate constant is 40% lower than for cytosine. The guanine labile hydrogens also exchange more slowly than adenine. These observations lead the authors to point out that a structural feature shared by uracil and guanine, but not by cytosine and adenine, is the location of three contiguous hydrogen-bonding sites along a single edge of the heterocycle. They propose that the 4CO-3NH-2CO network of uracil and 6CO-1NH-2CNH_2 network of guanine may form more extensive and presumably more rigid contacts with capsid subunits. There is some support for these ideas from analysis of the RNA sequence.

Usually one starts with the structure of a macromolecule and goes on the discuss dynamics. In Li et al's study however, measurement of dynamical properties are starting to emphasize key structural elements. As the authors point out, the way to the future lies in improving both their time-resolution and photon detection system. Raman spectroscopy will provide information on aspects quite inaccessible to other techniques. Time resolved studies on viruses, by George!

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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