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DISCUSSION

Session chairman: Adrian Parsegian
Scribes: Gary A. Griess and Eric T. Baldwin

BLOOMFIELD: The general technique of Brownian dynamic simulation is an attractive procedure that gets into the range of times where we do most of our experiments. What does diffusional simulation leave out (that molecular dynamics would include if it could be carried out), that would be important for analysis of experiments on the nanosecond and longer time scale? Also, can you estimate a lower bound to the time scale of Brownian dynamics? Does leaving out the velocity make a difference?

ALLISON: I think you can get to all times by overlapping molecular dynamics and Brownian dynamics. For example, molecular dynamics can get out to a couple hundred picoseconds. Brownian dynamics is applicable on time scales longer than the momentum relaxation times of the solvent, and this is on the order of a few tenths of a picosecond. Hence the two methods overlap. As for what is left out, Brownian dynamics replaces the solvent with a bath of random noise. The potentials are not real potentials but potentials of mean force. So you lose the detailed atomic description of the solvent when you go to Brownian dynamics.

BLOOMFIELD: What is your sense of the consequences of that particular omission for the valid analysis of physical situations?

ALLISON: When you ignore momentum relaxation but correct for it using random numbers to represent stochastic displacements, you must remember that the dynamics are being generated in a statistical rather than deterministic sense.

BLOOMFIELD: Macromolecular interactions depend strongly on water structure and its adjustment to the polymer's approach. Do you see any way of incorporating solvent into Brownian dynamics? What might its neglect leave out? The effective dielectric constant for electrostatic interactions is a related problem.

ALLISON: In Brownian dynamics, simulation of the diffusion-controlled reaction between the enzyme and the substrate the dielectric constant was set at 78, and this would certainly not be true if you were looking at the effective dielectric constant between two groups inside a protein. However, in this case, over much of the diffusional process the enzyme and substrate are separated by a fairly thick layer of water. To assume a bulk dielectric constant of water would be fairly accurate when enzyme and substrate are far apart. Presently, the Warwicker-Watson model, where you model the protein as one dielectric and the water as another dielectric, is being used to develop a more realistic model for this problem. This work is just getting under way, and we have no results yet. Our philosophy is to start with the simplest model and develop more and more sophisticated models. If the simple model works, that model should be used.

EISENBERG: In regard to the flexible DNA worm-like chain, I would like to know whether you can interpret some experiments which are well established (Kam, Borochov, and Eisenberg. 1981. *Biopolymers*. 20:2671–2690). The apparent diffusion constant, D_{app}, from quasielastic light scattering, yields the translational diffusion constant at low values of the scattering vector **q**, but increases in sigmoidal fashion with increasing values of **q**. If you stiffen up the molecule, can you see changes in the predicted relaxation times?

ALLISON: Yes, you can carry out the simulations over both high and low scattering vectors. Different experiments correspond to different averages over the internal coordinates of the worm-like chain. You would have to carry out the average over the appropriate physical quantity. I have done that for the 30 subunit worm-like chains, but the results are not particularly interesting. Polarized light scattering is not very sensitive to internal motions of 30 subunit worm-like chains unless the scattering vector is very large. You get a diffusion constant corresponding to that of the overall molecule. Different relaxation times depend on chain conformation and not on internal bending.

POTSCHKA: In macromolecules the location of the target of a reaction-diffusion process is usually quite different from the center of the molecule. Compared to the properties of the target the remainder of the molecule most often has only second-order influence via rotational diffusion. You interpret differences between a simple charge vs. five charge centers by the importance of multipole moments. Intuitively this should be a matter of radial distance away from the reaction center. Wouldn't a simpler model centered in the target do equally well?

ALLISON: The model of SOD enzyme has two active patches. If you put a charge at the center you have a charge monopole model. The five-charge model has charges pulled back inside the enzyme. Now, if you keep the quadrupole moment constant and move quadrupolar charges farther out ($\mathbf{q}a^2$ = constant, where **q** is the quadrupole charge and a the charge separation) the electrostatic potential doesn't change appreciably, as long as the quadrupolar charges are kept within the protein interior.

LEE: I would like to elaborate on Victor Bloomfield's question. Adrian Parsegian, Donald Rau, and I have measured a hydration force that

depends on perturbation of water out to ~10 Å from the surface of DNA double helices. When you have electrostatic forces, the perturbation giving the hydration force must be included in any electrostatic picture, effectively changing the dielectric constant of water.

ALLISON: They may well be important. How can you improve the model to account in a better way for the dielectric constant for the water?

PARSEGIAN: The preoccupation with the dielectric constant as the right fudge factor may be erroneous. There may be another kind of physics of interaction, the physics of dehydration. So it is not just a matter of manipulating the ϵ or D when you want to study the interactions between bodies. Don't stick to old parameters; there might be new ones to worry about.

TAINER: I have two related points regarding superoxide dismutase (SOD). First, if you consider the enzyme as a sphere it seems to me you are already taking for granted local steering effects because the active site is actually located ~14 Å beneath the surface of your sphere. So you are assuming that any time the superoxide reaches the sphere above the active site you have a productive collision. Second, I am bothered that your five-charge model works so well, and I wonder what that is telling us. There is no evidence of different rates among the SOD enzymes from different sources. By your arguments, it seems that the rate should vary considerably as the charge changes. Local charges must be important, because the the overall charge changes, and, as far as I know, the evidence is that the rate is constant.

ALLISON: The objective was to see if the charge distribution does steer superoxide into the active site. We are not claiming that the particular numbers we are getting are quantitatively accurate, but we think that on the basis of the model we are using, we can say that the charge distribution is guiding the superoxide into the active site of the molecule. The rate constants are three to four times larger than experimental rates, so our models are still too simple. There are a variety of ways to improve that. We are assuming that once superoxide gets inside a patch it reacts. That is probably not true because undoubtedly some SO gets in and then escapes.

GLUCKSMAN: Going back to DNA, how can you extrapolate from a naked piece of DNA, 30 bases long, to a piece of chromatin coated with histones?

ALLISON: These chains are ~920 Å long. Each subunit is ~30 Å in diameter. This is the model of Hagerman and Zimm (reference 31) who found that a bead model could reproduce the overall hydrodynamics of a continuous cylinder model of a wormlike chain, provided two criteria were met: first, that the lengths were the same, and second, that the volume of the continous cylinder and discrete bead models were the same. That is where the 31.8 Å comes up for size of these subunits. Each of these is not a single base pair but a hydrodynamic element.

BLOOMFIELD: The apparent hydrated diameter of DNA from sedimentation measurements is ~27 Å. The bare P–P distance is 20 Å. You are a little broader than that, but it is in the same ballpark. I wanted to get back to Heini Eisenberg's discussion because a misconception may have been propagated with regard to time scales. When you are in that low angle-low $\mathbf{q}^2$ plateau region, it is by no means in the nanosecond time range, but in milliseconds for translational diffusion. When you go up the plateau to higher $\mathbf{q}^2$, as Eisenberg says, the mechanism of that transition is not terribly well understood. One explanation is that you're looking at some internal modes. Mickey Schurr has suggested that you are looking at segmental diffusion within the DNA coil. Those time scales are probably microseconds. My question is, to what extent are your capabilities up to that type motion of segmental flex? This again would be polarized scattering, not depolarized scattering.

ALLISON: At Georgia State the computer capabilities on a mainframe UNIVAX 1100 are ~1%, the capacity of a supercomputer (cyber 205). We get out to ~200 nanoseconds. We can't get out to microseconds. This is in a Brownian dynamic simulation for 30 subunit wormlike chains with pre-averaged hydrodynamic interactions. On a supercomputer you could do microsecond simulations of polarized light scattering for 30 subunit wormlike chains, but much longer chains would be difficult. One way to extend this would be to use something like a Harris-Hearst model instead of a discrete wormlike chain of touching beads in which larger subunits are used. In this model you have bending forces and stretching forces. Now you are going to lose some of the rapid internal motion when you go to those lower resolution models, but I have a feeling that for polarized light scattering you are not going to lose much, because those experiments are going to be insensitive to extremely rapid motion.

SCHOENBORN: Dielectric constants are macroscopic constants which you are using on an atomic scale (microcanonical ensemble). What does a dielectric constant mean on a 2 Å scale in a protein or DNA?

ALLISON: Because temperature and not total energy are constant the ensemble is canonical.

BLUM: In regard to SOD and the charge distribution, and the dielectric constant, I have a feeling that when you looked at the multipole solutions you made a very large simplification in your picture of charge distribution. The incoming superoxide anions see on the globular surface of SOD the whole pattern of clusters of charges superimposed on the pattern of hydrophilic and hydrophobic patches on the surface. Only by mapping the surface charge distribution can you really approach the situation. To look at multipole charges you must look on a gradient of dielectric constants between four and 78 in the outer shell of protein globule.

The other interesting thing in your approach is the choice of SO anion, because it is a small charged particle that can see details on the protein surface. From the point of view of the solvent, you are treating SO anion with the solvent value of the dielectric constant, but when it comes close to the surface of the protein you cannot ignore the change of dielectric constant. Do you agree?

ALLISON: For the process we are looking at, the rate constant for association, I believe it is the long-range interactions that are dominating that process.

BLUM: As long as you look at one charge, you are right. When you look at the multipole, it is different.

ALLISON: You are saying that the multipole polarizes the surface charge on the enzyme. We are starting to modify the calculations to use different dielectric constants inside and outside the sphere. Perhaps some of these effects will be incorporated in future simulations.

SALEMME: We have found that the major factor in the rate enhancement is the resolution of the solid angle that has to be sampled to form a

reactive complex. The actual reaction rate between two colliding molecules with localized reactive surfaces depends on the probability of the two sites interacting. Because the reactive sites are typically only a few percent of the total surface area, any forces that direct oriented collisions dramatically enhance the reaction rate. The details of how the physics of the interactions are treated are important, but do not radically affect the computed enhancement of rates. This factor is the dominant term irrespective of the detailed nature of the computed interaction potential in the simulation.

NORTHRUP: What Ray Salemme is saying is true. We've done some preliminary calculations in our studies of cytochrome-like proteins reacting with a charged partner, and we've used a dipole moment corresponding to horse cytochrome. We find if you restrict the electron transfer region on cytochrome down to a 10° patch size, you can lower the rate down to 6% of the case where the whole surface of cytochrome is reactive. But when you put a dipole moment on, which steers the species into productive orientation on the surface, the dipole on cytochrome raises the rate of electron transfer back to about six times what it would be if those forces were absent. We see the same kind of steering mechanism Stuart Allison mentions, seeming to compensate for the fact that you have strong stereochemical constraints to the reactions.

SHARNOFF: The rate constant discussion has really focused on charge-induced charge effects . One could anticipate that a SO molecule approaching the SOD surface would induce its own localized charge distributions. One might regard the superoxide dismutase from the same conceptual standpoint as was addresed by Gary Ackers, namely one of local perturbations. In this case the perturbations are caused not by mutation but some other physical effect. Then, of course, the question arises as to whether there is any cooperativity involved in the structurally induced multipole in the protein that comes from the redistribution of charge by the approaching SO molecule.

ALLISON: I think you could put polarizability into the simulation, but I'm not sure how you would design it.

SHARNOFF: Normally, in referring to a dielectric constant one is referring to macroscopic behavior. You have to use an individual polarizability that in the long run would have to be referenced to the tertiary structure of the protein as well.

PARSEGIAN: I want to pick up a point Victor Bloomfield made about time scales. There is a history of recognition of force fields in aerosols and colloidal systems in solving many problems analytically where particles diffuse, and stick or don't stick. How do your methods supplement or reduce to those analytic systems? How about calibrating against the analytic solutions that have been in the literature for thirty years?

ALLISON: We've done that. Before we try to apply it to something as complicated as superoxide dismutase, we simulate known analytic problems to test the simulations. A good example is a uniformly reactive sphere with a charge embedded in it or a Solc-Stockmayer model where there are no direct forces but one molecule has a reactive path and the other is uniformly reactive.

POLLARD: I doubt that we are going to come back to diffusion, so I would like to add a worrisome note at this point. Most people who think about macromolecular assembly reactions assume that the subunit molecules are free to diffuse, but this may not actually be the case in some systems. Sato, Schwarz and I (Sato et al. 1985. *J. Biol. Chem.* 260:8585–8592) recently reported that solutions of actin molecules at low concentrations (1 mg/ml) in buffers where no filaments form are a viscoelastic solid. Thus at least part of the molecules must form some sort of a continuous network, even though they are a homogeneous population of monomers, judging from hydrodynamic measurements. This discrepancy may be due to two factors. First, standard hydrodynamic methods involve enough mechanical shearing to break the weak bonds between these molecules. Second, formation of these solids takes a long time, 10 or more hours. Subsequently, we have found that profilin and tubulin form viscoelastic solids. Solutions of cytochrome C are Newtonian fluids. Ovalbumin forms a weak viscoelastic liquid. Consequently, it is worth considering the possibility that nonfilamentous proteins may contribute to the mechanical properties of the cytoplasm. Also, one must be aware that at equilibrium some protein molecules may not diffuse as freely as suggested by hydrodynamic methods. This could influence macromolecular assembly processes that are usually thought to involve some diffusion-limited reactions of the subunits.