DISCUSSION

Session Chairman: Hans Frauenfelder *Scribe*: James B. Matthew

KALLENBACH: Dr. Wüthrich finds the Δ volume to be 60.0 $Å^3$ and proposes a model in which you have a sphere swept out inside a hydrophobic cluster. If you relax the protein in stages, what will be the apparent ΔV of activation? Will it seem to give the same sphere if you do it in increments?

KARPLUS: The effective activation volume for tyrosine rotations in the interior of the protein has two contributions: One arises from the fact that protein atoms have to get out of the way when the ring rotates; the other is due to the collisions between protein atoms and the aromatic ring. Both effects tend to increase as the pressure increases, so that their sum is observed experimentally as the activation volume.

The calculated effective activation volume you get by not having a completely empty sphere the size of the aromatic ring is smaller than the value Dr. Wüthrich mentioned. This does not mean that the measured effective volume is smaller, because the collisional damping term, corresponding to a change in viscosity, is expected to be important. There are cases in simple systems (e.g. butane isomerizations) where the viscosity dependent term (i.e. the collisional term) is larger than the activation volume calculated by standard techniques.

WÜTHRICH: It is interesting that the environment of these rings is compressible with hydrostatic pressure. It was suggested early on that high pressure leads to protein denaturation because the protein itself isn't compressible but H_2O gets pushed inside, ripping it apart. Dr. Karplus, when you do molecular dynamic calculations you want to use conditions which are at thermodynamic equilibrium to see how the protein responds to temperature at this equilibrium condition. Can you say if your calculations have ever been at equilibrium conditions? In order to test this you'd probably have to calculate over a much longer time scale.

KARPLUS: It is true that we do not know that in the protein dynamic simulation the system under study is really an equilibrium system. What one does is to integrate the equations of motions over an equilibration period after which the system seems to be thermally stable— the average kinetic energy and therefore the average temperature is essentially constant. Over the time period used for analysis of the dynamics the system behaves as if it were in equilibrium; that is, average properties are nearly independent of time. If the calculation is done several times, the same average and fluctuation properties are obtained, indicating that the detailed initial conditions are not important. Also, to test whether doing the calculation for a protein in vacuum has a large effect, we immersed the protein in a box of solvent (neon) atoms with the diameter and packing of water. The protein stayed closer to its average x-ray structure than in the absence of solvent, but, looking at the interior of the protein, there were very small changes in the calculated behavior in the absence and presence of solvent.

BLOOMFIELD: Don't Lakowicz' experiments on the wavelength dependence of fluorescence show lifetimes of nanosecond for thermal fluctuations to decay inside proteins.?

KARPLUS: If you assign a uniform distribution of velocities to the atoms of a protein, after a picosecond you have a Gaussian distribution of velocities; thus, on an atom-by-atom basis, there is a very fast transfer of energy. If there is a large amount of excess energy in a given region of the protein, it takes longer to achieve a redstribution of this energy.

LAKOWICZ: I can guess at the actual relaxation times around excited state indole moities. It is a matter of whether we are on the low or high temperature side of the effect. I think we're on the rapid side, so probably .3–.5 ns is the right time.

FRAUENFELDER: We have a question from a referee, David Chandler: "By relaxing the surrounding protein from its rigid crystal structure, the authors find a significant spreading of the torsional angle distribution function and an enormous lowering of the activation barrier for ring rotations. These results suggest an important degree coupling of the torsional angle to all the other dynamical degrees of freedom. Indeed, the analysis of a Langevin model leads the authors to conclude that the motion of the torsional angle is close to being overdamped. In view of these conclusions, I am puzzled by the apparent validity of transition state theory for describing the rates of barrier crossings for the torsional angle.

Recall that the transition state theory estimate of the rate for an activated process would be exact if there were no multiple crossing for trajectories that pass through the surface separating two configurational states. The coupling of a reaction coordinate to other degrees of freedom can reverse trajectories that are passing slowly over a barrier, and

thereby lead to a violation of the transition state approximation. In the limit of very strong coupling, the reaction coordinate performs a Brownian motion on the barrier. A consequence of this limiting behavior is Kramers' result: the rate constant is proportional to (1/f), where f is the friction constant.

The authors report the results of seven barrier crossings. In each one, the inertia succeeded in carrying the reaction coordinate over the barrier with no recrossing. This inertial behavior is surprising to me in light of the authors' other conclusions which indicate the importance of coupling. Notice that in three of the seven reaction trajectories, the torsional angle became almost stationary for a period of time. During such periods, the torsional of freedom. Perhaps it is a statistical coincidence that these trajectories were not reversed, and that further sampling would produce non-transition state theory trajectories."

In other words damping occurs at a few centipoise, meaning the viscosity of water at room temperature. Any solvent one uses is in the high damping region where rate is proportional to 1/f.

KARPLUS: It is certainly true that we did not do enough trajectories to determine a rate constant and that in a statistically more complete set, it is likely that some would be found that are reflected in the transition region. The main point of the calculations was to determine the general nature of activated trajectories in proteins, and to show that collisional damping does exist. The fluctuations in interior of the protein and the calculated tyrosine motions near the minimum suggest that the viscosity in the interior of a protein is lower than that of water; in fact, it appears to be closer to that of a hydrocarbon liquid.

PRENDERGAST: For motion in proteins we need a scale which ranges from vibrational motion to rapid large motions, allowing O_2 or acylamide to diffuse inside. We think in terms of rate or amplitudes. Can you correlate the frequencies of fluctuations with their amplitudes? Does packing imply a particular rate or amplitude?

KARPLUS: You would expect that high frequency motions would have small amplitudes and low frequency modes would have large amplitudes.

PRENDERGAST: Can we quantitate amplitudes? Given a rate, can we estimate the amplitude for a motion? We are trying to deduce structure from O_2 quenching data.

KARPLUS: The detailed correlations are not simple. Looking at some of the activated events suggests that a simple approach neglecting correlation is not applicable. There are qualitative generalizations that can be made, but if one wishes to understand a specific process it will have to be examined in detail.

Hydrogen exchange mechanisms span the spectrum of almost everything suggested today: variation with the system, with temperature, with pressure, and with pH.

HENDRICKSON: Do you find in the calculation of aromatic ring flips that the tendency to flip is given away by the general rotation?

KARPLUS: If you look at the calculated amplitude of fluctuations of the tyrosine ring near equilibrium (as described in the text), you do not get information about what happens far from equilibrium. For temperature factors and small fluctuations in the neighborhood of the equilibrium position, there is a correlation with the effective potentials It would be nice to take the potential near equilibrium and determine the barrier height. In a system like ethane, for which the form of the potential is known, this is possible to do approximately, but in a protein it is probably not possible.

SANDER: Dynamic calculations are for the moment a first approximation to physical reality, with a lot of hard work yet to be done. One task is to remove the vacuum; another would be to consider bond frequency. At room temperature they are not excited and best treated quantum mechanically. When you freeze out bond angle vibrations, what is the highest remaining frequency in the simulation? Do you agree that when quantum mechanical vibrations are frozen out that energy transfer in the molecule is slowed, and thus equilibration is slower?

KARPLUS: When you speak of frequencies large compared to kT in a protein, if you forget about hydrogens, you are talking about frequencies of 1,000 cm^{-1} or less. Ideally, a semiclassical approach would be used for these higher frequencies. In principle this is possible, but in practice it is too difficult for a system as large as a protein, though it has been done in smaller systems.

Saying that a semiclassical treatment is appropriate does not mean that we want to freeze out these modes. Because they are high frequency modes they will relax adiabatically to the minimum energy position for what the low frequency modes are trying to do. Calculations indicate that it is the correct approach to include the relaxation of the modes. If you are following a slow motion (e.g. rotation of a dihedral angle) you have to let the bond angle, which has a higher frequency, relax to its minimum energy position for each dihedral angle value. Thus, if you are not interested

in questions like the contribution to the heat capacity of the system from these high frequency modes, the dynamics of low frequency modes is given correctly as a first approximation by treating the whole system classically.

As to the question of what happens when you freeze out the high frequency modes involving the bond lengths and bond angles, we have done a constrained calculation. If the bond lengths are fixed at some average value, there is a relatively small effect on the dynamics; i.e., the amplitudes of the fluctuations are unchanged. It is a better approximation to include the bond length fluctuations, but their variation has a small effect and if you fix them, the fluctuations are reduced by a factor of two.

The final question was what are the highest frequencies that remain if you fix the bond lengths and bond angles; they are on the order of 350 cm^{-1}.

LLINAS: Would it be possible to treat the protein as a single entity trying to find the quantum spectrum of the whole system, and from there deduce the large amplitude and low frequency components of the protein fluctuations? They could be highly related to hydrogen exchange models.

KARPLUS: There has been some work which treats the protein as an elastic body. deGennes and also Go have looked at this problem but they have examined only the lowest low frequency mode. The term "breathing" which is related to this work thus refers to "spherical" breathing. Going beyond that into the imtermediate frequency range, which is the more important one and involves some localization of the modes, will not give a significant answer without the introduction of structural details. If we consider the BPTI β-sheet, which has local modes, that will never appear in the continuum treatment. Geophysicists use concentric shell models with different elastic constants and perhaps they could be applied to large proteins. My feeling is that such an approach, though interesting, will not solve the problem if we are asking detailed questions of the type we have been considering at this meeting. However, it should be said that the magnitudes of the calculated fluctuations are in accord with expectations for an elastic system of this type.