Time scales and pathways for kinetic energy relaxation in solvated proteins: Application to carbonmonoxy myoglobin

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Using a novel method for determining the coordinate friction for systems that possess bounded diffusion, the rates of kinetic energy partitioning for various elements of solvated carboxy-myoglobin were calculated. Energy redistribution within the heme group and solvent is found to be rapid compared with energy redistribution within the protein. Within the protein, charged residues exhibit much more rapid dispersal than neutral residues. The results suggest that a possible doorway for energy release from the photolyzed heme involves the interaction of its isopropionate groups with the neighboring solvent molecules. The results are analyzed as a function of atom type, protein residue and residue group (charged, polar, aliphatic, and aromatic) leading to general observations relating to the inherent inhomogeneity in the spatially dependent relaxation rate of the solvated protein. The computational results are used to analyze a variety of estimates of the internal friction, viscosity or damping invoked to interpret experimental measures of protein dynamics. The concluding discussion includes speculations on the origin of internal viscosity in proteins. © *2000 American Institute of Physics.* [S0021-9606(00)52141-2]

I. INTRODUCTION AND BACKGROUND

The concept of ''friction'' is one that is often used at both the macroscopic and microscopic level to describe the dissipative effects of a generally defined ''bath'' on the detailed dynamics of a well defined ''system.'' In the dynamics of a protein, the *system* may be the stretching vibration of a CO ligand molecule in the heme pocket of myoglobin. The *bath* may be defined as the atoms of the surrounding protein and solvent. If one wishes to study the rate at which an initially hot CO molecule transfers energy to the surrounding protein and solvent, one can reduce the complex Newtonian dynamics of the CO/protein/solvent system of thousands of atoms to the simple Langevin dynamics of two atoms—the CO—in a dissipative bath characterized simply in terms of an average background potential and a ''friction.''

At the macroscopic level, the friction is defined as a proportionality constant relating the dissipative force F_{diss} felt by an object of mass m moving with velocity v as $F_{\mathrm{diss}} = -\gamma_0 m v$. At a microscopic level, the friction is fundamentally defined through the generalized Langevin equation (GLE) as

$$m\ddot{x} + \left(\frac{\partial V}{\partial x}\right) = -\int_0^t d\tau m\,\gamma(\tau)\dot{x}(t-\tau) + R(t), \tag{1}$$

where x is the solute coordinate, m the mass, $V(x)$ the potential of mean force, and $\gamma(t)$ the time dependent friction that is assumed to be spatially independent.[1,2] The fluctuating random force $R(t)$ acting on the x coordinate is related to the time dependent friction $\gamma(t)$ through the fluctuation dissipation theorem

$$\gamma(t) = \frac{1}{m k_B T}\langle R(t)R(0)\rangle. \tag{2}$$

For a single atom or the center of mass of a molecule in a liquid, it is in principle possible to estimate the zero frequency friction

$$\gamma_0 = \int_0^\infty \gamma(t)dt \tag{3}$$

through the Einstein relation for the translational diffusion constant

$$D = \frac{k_B T}{m\gamma_0}. \tag{4}$$

The diffusion constant may be known from experiment or it may be computed using either the asymptotic dependence of the mean-square displacement as a function of time,

$$D = \lim_{t\to\infty}\frac{1}{6t}\langle \Delta\mathbf{x}(t)\Delta\mathbf{x}(0)\rangle \tag{5}$$

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or the associated Kubo relation for the velocity autocorrelation function,

$$D=\frac{1}{3}\int_0^\infty \langle \mathbf{v}(t)\mathbf{v}(0)\rangle. \tag{6}$$

It may seem that the problem of determining the friction γ_0 is solved. In theory, all that needs to be done is to calculate the mean square displacement or velocity autocorrelation function of the particle and through these relations determine the friction.

However, due to the length scales—on the order of 1–10 Å—and time scales—on the order of 0.1 ps–1 ns—for thermal relaxation processes in proteins, molecular dynamics simulations are ideally suited to explore the times scales and pathways for energy transfer in biomolecular systems. However, in practice this can be a very difficult task. If we were interested in determining the friction acting on only one degree of freedom, many trajectories over many time steps would be needed in order to achieve convergence. Furthermore, if the motion is bounded the mean square displacement, after an initial rise, approaches a constant value in time making it difficult to use the linear relation in Eq. (5) to extract the friction. Nevertheless, there has been some success using Langevin models[3–5] and temperature echoes[6,7] in characterizing the magnitude of the intrinsic friction acting in proteins.

Another possibility is to directly compute the time dependent friction by solving the memory function equation,

$$\frac{d\langle \mathbf{v}(t)\mathbf{v}(0)\rangle}{dt}=-\int_0^t \gamma(t)\langle \mathbf{v}(t-\tau)\mathbf{v}(\tau)\rangle d\tau \tag{7}$$

relating the time dependent friction to the velocity autocorrelation function through the associated GLE.[2] Calculation of $\langle \mathbf{v}(t)\mathbf{v}(0)\rangle$ and a subsequent inversion of Eq. (7) delivers $\gamma(t)$.[8,9] However, this approach is sensitive to the quality of the velocity autocorrelation function. Numerical instabilities result if the velocity autocorrelation function being inverted is poorly characterized.

However, as a result of these limitations on the standard methods developed to derive $\gamma(t)$, the task of determining the friction is commonly limited to the study of simple diatomics such as CO, CN^-, or HgI.[10–13] (A notable exception is the application to the case of CO diffusion in leghemoglobin by Elber and co-workers.[14]) While it is true that the calculation of friction along bonds within some simple polyatomic molecules has been accomplished, the methods employed either reduced the molecule to a simple diatomic using an extended atom approach[15] or involved the incorporation of normal modes, a method that can have serious size limitations.[16] An alternative approach is to characterize the friction acting on probes of a protein using a combination of experimental and modeling studies.

In this paper, we present a method for the computation of the rate of kinetic energy relaxation that makes use of the kinetic energy metric of the ergodic measure. Through a Langevin dynamical model, the rate of kinetic energy relaxation is related to the intrinsic friction γ_0 acting on a specific atom of the protein. This method not only provides a direct and accurate means of computing the intrinsic atomic friction but also provides a means of separating the contributions of collisions and activated barrier crossing to the overall ''friction'' acting on atoms of the protein.

II. THE ERGODIC MEASURE

The ergodic measure is a method for determining the time scale for the self-averaging of properties in many body systems.[17] The fluctuation metric of the ergodic measure is

$$\Omega(t)=\sum_j^N [f_j(t)-\overline{f}(t)]^2, \tag{8}$$

where $f_j(t)$ is the time average for atom j of property F of the system,

$$f_j(t)=\frac{1}{t}\int_0^t ds F_j(s) \tag{9}$$

and $\overline{f}(t)$ is the average over all N atoms of property F at time t,

$$\overline{f}(t)=\frac{1}{N}\sum_j^N f_j(t). \tag{10}$$

If the system is self-averaging, the function $\Omega(t)$ in Eq. (8) decays to zero as

$$\Omega(t)\sim\frac{\Omega(0)}{Dt}\to 0. \tag{11}$$

The slope of $\Omega(t)$ is proportional to the generalized diffusion constant D for the observable F that can be written

$$D\Omega(0)=\frac{l^2}{\tau}, \tag{12}$$

where $\Omega(0)$ is the mean square fluctuation of the property F and τ is the time scale for taking a ''step'' of generalized mean square length $l^2=\Omega(0)$ in sampling the fluctuations of the property F. Using the kinetic energy metric in this way, the degree of damping on subsets of the protein and solvent atoms within the system can be investigated.

If we take F_j to be the atomic kinetic energy of the jth atom then $\Omega_{\mathrm{KE}}(t)$ is the kinetic energy fluctuation metric. $\Omega_{\mathrm{KE}}(t)$ can be written[17]

$$\begin{aligned}\Omega_{\mathrm{KE}}(t)&=\frac{1}{t^2}\int_0^t ds_1\int_0^t ds_2\,\frac{1}{N}\sum_j \delta f_j(s_1)\delta f_j(s_2)\\&=\frac{1}{t^2}\int_0^t ds_1\int_0^t ds_2 C_{\mathrm{KE}}(s_1-s_2).\end{aligned} \tag{13}$$

The function $C_{\mathrm{KE}}(s_1-s_2)$ is the equilibrium autocorrelation function for the fluctuations in the kinetic energy given by

$$C_{\mathrm{KE}}(t)=\frac{m^2}{4}[\langle v^2(t)v^2(0)\rangle-\langle v^2\rangle^2]. \tag{14}$$

Following the standard derivation for the translational diffusion constant[2] from the time correlation function of the mean square displacement[2] one writes

$$\Omega_{KE}(t)=\frac{2}{t^2}\int_0^t (t-y)C_{KE}(y)dy. \tag{15}$$

At long times this is approximated by

$$\Omega_{KE}(t)\approx\frac{2}{t}\int_0^\infty C_{KE}(y)dy \tag{16}$$

so that the generalized diffusion constant D_{KE} can be related to the mean square fluctuation in the kinetic energy and the equilibrium time autocorrelation function of the kinetic energy as

$$\frac{1}{D_{KE}}=\frac{2}{\Omega_{KE}(0)}\int_0^\infty C_{KE}(y)dy. \tag{17}$$

A. The Gaussian random variable approximation

What is needed to build a connection between the friction and the rate of convergence of the kinetic energy metric is a relation between the velocity autocorrelation function and the kinetic energy autocorrelation function. Suppose that we assume that the velocity is a Gaussian random variable. The joint probability of having velocity $v=v(t)$ at time t and $v_0=v(0)$ at $t=0$ is[18]

$$P[v(t),v(0)]=\left[\frac{m}{2\pi k_B T(1-\psi^2(t))}\right]^{3/2}\times\exp\left[\frac{m}{2k_BT}\frac{(v^2+v_0^2-2vv_0\psi^2(t))}{(1-\psi^2(t))}\right], \tag{18}$$

where $\psi(t)=\langle v(t)v(0)\rangle/\langle v^2\rangle$ is the velocity autocorrelation function. In that case, the kinetic energy metric can be expressed in terms of the velocity autocorrelation function[18,19] that in turn is related to the diffusion constant. Berne, Pechukas, and Harp showed that the joint probability distribution given by Eq. (18) for a Gaussian random variable also results if the information entropy corresponding to the probability of having $v(t)$ at time t and $v(0)$ at time 0 is maximized.[20]

Given the conditional probability $P[v(t),v(0)]$, the autocorrelation function for any higher moments of the velocity may be calculated. Predictions for the second, fourth, and eighth moments of the linear and angular momentum of a diatomic fluid were compared with direct calculations from computer simulations by Berne and Harp.[18] For their study the assumption that the velocity is a Gaussian random variable was found to be valid.

The joint probability distribution Eq. (18) may be used to calculate the autocorrelation function for the fluctuations of the kinetic energy[18] about its equilibrium average value in terms of $\psi(t)$,

$$C_{KE}(t)=\langle\delta K(t)\,\delta K(0)\rangle=\tfrac{3}{2}(k_BT)^2\psi^2(t). \tag{19}$$

By combining Eqs. (17) and (19) we can determine the generalized diffusion constant D_{KE} from the slope of $\Omega_{KE}(0)/\Omega_{KE}(t)$ for a particular model of $\psi(t)$ as

$$\frac{1}{D_{KE}}=\frac{2}{\Omega_{KE}(0)}\int_0^\infty C_{KE}(y)dy=2\int_0^\infty\psi^2(t)dt \tag{20}$$

using the fact that $\Omega_{KE}(0)=3(k_BT)^2/2$.

B. Evaluation of D_{KE} for a Langevin dynamical model

The object of this work is to compute the friction associated with the individual groups in the protein whose motion is bounded. Let us assume that the relaxation of the kinetic energy occurs along an undetermined and directional coordinate. An approximate equation of motion for any atom with position x and mass m experiencing a constant friction η can be derived from the Langevin equation for a harmonic potential with force constant κ,

$$m\dot{v}+\kappa x=-m\gamma_0 v+R(t), \tag{21}$$

where $v=\dot{x}$. The autocorrelation function of the random force $R(t)$ satisfies the second fluctuation-dissipation theorem $\langle R(t)R(0)\rangle=2mk_BT\gamma_0\delta(t)$. For this mode the velocity autocorrelation function is[3]

$$\langle v(t)v(0)\rangle=\frac{k_BT}{m}e^{-\gamma_0 t/2}\left[\cos(at)-\frac{\gamma_0}{2a}\sin(at)\right] \tag{22}$$

with $a^2=\omega^2-\gamma_0^2/4$ and $\omega^2=\kappa/m$. When the force constant $\kappa=0$, the self diffusion constant D for the mean square displacement of x is given by the Einstein relation $D=k_BT/m\gamma_0$. When the force constant $\kappa>0$, the motion of x is bounded and the diffusion constant $D=0$. This can be clearly seen through the Kubo relation relating the diffusion constant to the velocity autocorrelation function of Eq. (6).[21] The integral over all time of the velocity autocorrelation function for $\kappa=0$ is $1/\gamma_0$ but for $\kappa>0$ is 0.

For the kinetic energy metric, using Eqs. (17) and (19) we can determine the generalized associated diffusion constant of $\Omega_{KE}(t)/\Omega_{KE}(0)\rightarrow 1/D_{KE}t$ which is

$$D_{KE}=\gamma_0 \tag{23}$$

for all $\kappa\geqslant 0$. It follows that at long times

$$\text{slope}\left[\frac{\Omega_{KE}(0)}{\Omega_{KE}(t)}\right]=\gamma_0. \tag{24}$$

Through a determination of the generalized diffusion constant for the kinetic energy metric, the friction acting on the motion of a particle may be determined *even for the motion of a bounded system* when the diffusion constant is zero.

III. COMPUTATIONAL MODEL AND METHODS

We have applied our approach to the computation of the kinetic energy relaxation rates and friction coefficients for the atoms of the solvated carboxymyoglobin protein. We used the 260K x-ray structure of carbonmonoxy myoglobin (MbCO) reported by Kuriyan *et al.*[22] for the initial configuration. The MbCO molecule was introduced into a $56.570\times56.570\times37.712$ Å^3 box of equilibrated TIP3P water molecules and simulated using the CHARMM program.[23] Any of the equilibrated water molecules lying within 2.5 Å of the protein molecule were removed, while the original water molecules of the x-ray structure were kept. The excess po-

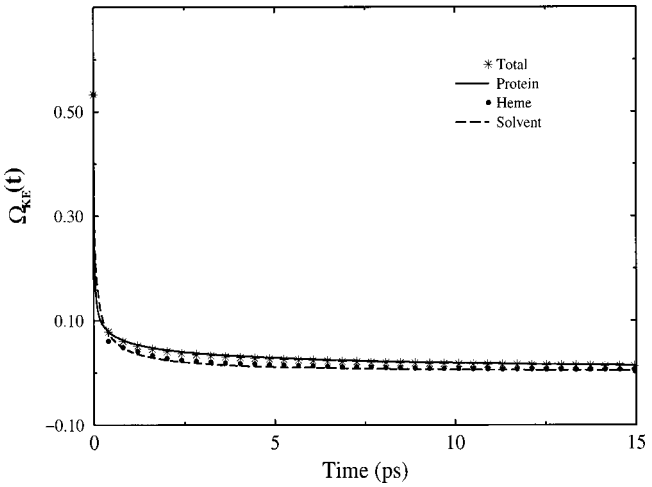

FIG. 1. $\Omega_{KE}(t)$ plotted as a function of time for the solvated myoglobin system, the protein, the heme, and the water solvent. The criterion for ergodicity requires $\Omega_{KE}(t)$ to decay to zero with time. The calculation was done at 300 K.

tential energy due to bad contacts and strain was then reduced using the steepest descent method. This resulted in 2534 protein and ligand atoms and 2946 water molecules for a total of 11372 atoms.

Using classical molecular dynamics the system temperature was gradually raised to 300 K. Once a temperature of 300 K was achieved, molecular dynamics was run for 20 ps and the temperature was monitored. If a drift was detected, the velocities were resampled according to the Maxwell distribution. During the last 10 ps of the run such velocity reassignment was not necessary indicating that the system had relaxed to a near equilibrium state. The molecular dynamics time step was 1.0 fs using the Verlet algorithm.[24] A switching function was used to taper the intermolecular potential to zero from 9.5 Å to 11.5 Å.

Beginning from the equilibrium structure obtained using the above protocol a 50 ps trajectory was generated. During this trajectory the velocities were resampled every 2.5 ps and a configuration was saved every 5 ps giving 10 configurations. Each of these ten configurations was run for 15 ps, resampling the velocities every 3 ps for the first 13 ps. The remaining 2 ps were run without temperature resampling to allow the system to relax before the data were collected. From each of the configurations obtained in this way a 30 ps trajectory was run during which the coordinates and velocities of the system were saved every 2 fs.

IV. RESULTS

In Fig. 1, the function $\Omega_{KE}(t)$ for the solvated myoglobin system is plotted as a function of time. It is clear from the figure that $\Omega_{KE}(t)$ decays to zero for each of the components listed. Using a cutoff of 0.01 for the decay of $\Omega_{KE}(t)$ as an indicator of sufficient convergence,[25] the time scales for ergodic convergence were assigned.

The averaged values of the relaxation times are listed in Table I. The time scales for this redistribution are similar for both the heme and solvent molecules, while the time scale for the protein is roughly twice as long. The rate at which the kinetic energy self-averages is remarkably quick. Each component of the system reaches thermalization within a few picoseconds. Furthermore, the data show that the kinetic energy is more rapidly redistributed within the heme and the solvent, than within the protein.

TABLE I. Time scales for the ergodic convergence of the kinetic energy in solvated myoglobin at 300 K. Total signifies the complete system of the protein myoglobin, heme, and water. The criterion for convergence of the kinetic energy fluctuation metric is that it decay to 1% of its original value.

Region	Time scale (ps)
Total	1.25
Protein	1.44
Heme	0.72
Solvent	0.75

In Fig. 2, several forms of $\Omega_{KE}(0)/\Omega_{KE}(t)$ for the solvated myoglobin system are plotted. In addition, the plots for the protein, the heme, and the solvent alone are displayed. These data illustrate the relative amount of damping felt by the atoms within the indicated subsets of the solvated myoglobin system. The frictional forces felt by the heme and solvent molecules are considerably higher than those on the remainder of the system. In fact, the friction constant for the heme is approximately three times larger than that of the protein itself. The kinetic energy metrics discussed thus far describe energy redistribution within the moiety itself and do not necessarily indicate a transfer of energy from one moiety to another.

The concept of rapid intramolecular vibrational relaxation (IVR) within the heme is not new.[26,27] The heme group has a large manifold of states, making it easy for energy to redistribute quickly. The heme group and its host protein share only one covalent bond—that between the proximal histidine and the iron atom. The heme is otherwise kept in place by roughly 90 van der Waals contacts with surrounding

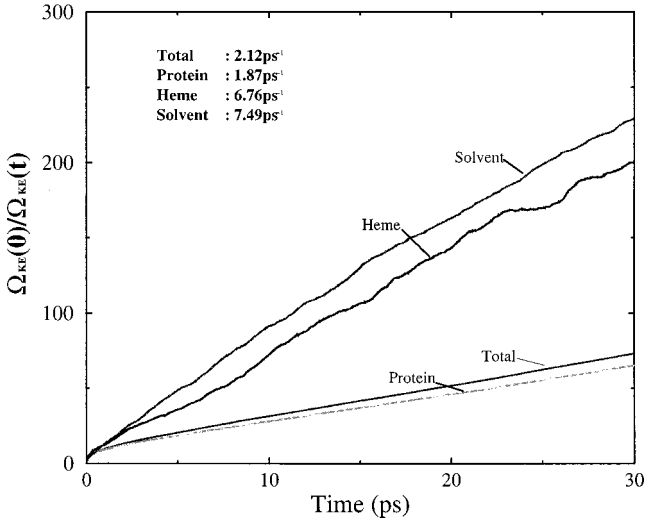

FIG. 2. $\Omega_{KE}(0)/\Omega_{KE}(t)$ as a function of time for the total system, the protein, the heme, and the water solvent. The simulations were run at 300 K. The data were averaged over ten 30 ps trajectories.

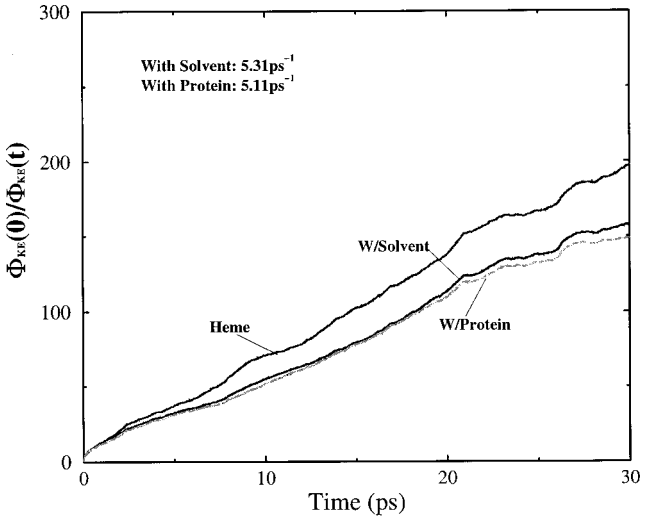

FIG. 3. $\Phi_{KE}(0)/\Phi_{KE}(t)$ as a function of time for heme using Eq. (8) in looking at the fluctuations within the heme itself and using Eq. (25) for the fluctuation between the heme and the solvent and protein. The simulations were run at 300 K. The data were averaged over ten 30 ps trajectories.

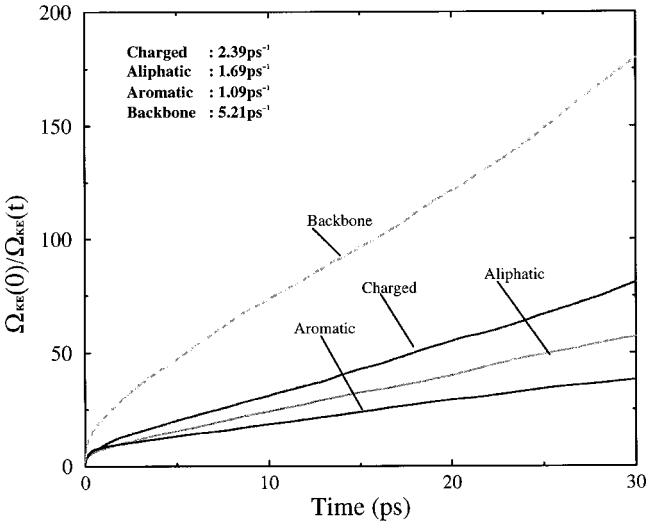

FIG. 4. $\Omega_{KE}(0)/\Omega_{KE}(t)$ as a function of time for charged (Asp,Asn, Glu,Gln,Lys), aliphatic (Gly,Ala,Val,Leu,Ile), and aromatic (His,Tyr, Phe,Trp) sidechains. The simulations were run at 300 K. The data were averaged over ten 30 ps trajectories.

protein atoms. In that sense, the environment of the heme is not unlike that of a molecule in solution.

The extent of this isolation can be brought to light using a modified form of the ergodic measure of Eq. (8), where

$$\Phi_{KE}(t)=\sum_{j=1}^{N_{heme}}[f_j(t)-\overline{f}_{ref}(t)]^2, \tag{25}$$

where f_j is the time average of the kinetic energy of the jth atom of the N_{heme} atoms while and $\overline{f}_{ref}(t)$ is the average over all atoms of the reference system which may, for example, be the heme alone, the protein alone or the protein and solvent bath. The results of this analysis are shown in Fig. 3. It is clear from the figure that energy redistribution within the heme itself occurs on a faster time scale than the thermalization of the heme with its environment. This indicates that the rate limiting step in heme relaxation following photolysis is the doorway between the heme and its surroundings.

The relatively small friction felt by the protein is also of interest, begging the question as to whether or not particular parts of the protein experience more friction than others or if the environment within the protein is fairly homogeneous. The data in Fig. 4 suggest that energy redistribution throughout the protein may occur primarily through the protein backbone. For those backbone atoms, the friction constant is much closer in magnitude to that experienced by the heme. In contrast, the protein side-chains are significantly less efficient in redistributing their kinetic energy.

The data for the convergence of the kinetic energy fluctuation metric for the different types of backbone atoms of the protein are displayed in Fig. 5. The data show that the hydrogen atoms feel very little friction relative to the other atoms of the backbone. Typically, the vibrational frequency of bonds involving hydrogen atoms is much higher than the frequencies of bonds involving the heavier atoms of the backbone and side chains. The relative mismatch in frequency of the vibrations reduces the importance of V–V transfer and results in the hydrogen stretching motion being a good normal mode. The fact that the frequency of the hydrogen stretching motions is absolutely high makes V–T transfer inefficient. The weak coupling between the surroundings is the cause of the small values of the rate of convergence of the kinetic energy equipartitioning for the hydrogen atoms.

The situation is quite different for the oxygen and nitrogen atoms that display a marked increase in the rate of kinetic energy equipartitioning compared to that of the hydrogen atoms. These backbone atoms are involved in hydrogen bonding interactions that serve to lower their frequency and increase the surrounding protein's influence on their motion. Explaining the behavior of the carbonyl carbons relative to the α-carbons is more difficult. One might be inclined to attribute this difference to the relative mobility of α and

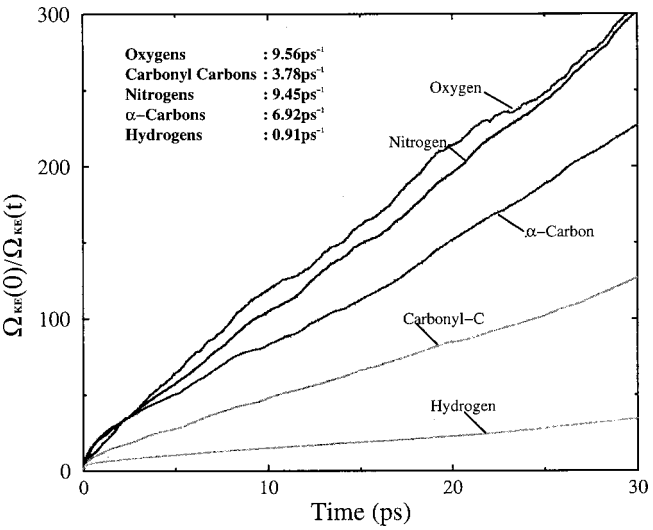

FIG. 5. $\Omega_{KE}(0)/\Omega_{KE}(t)$ as a function of time for the atoms of the protein backbone, oxygen, nitrogen, hydrogen, α-carbons, and carbonyl carbon. The simulations were run at 300 K. The data were averaged over ten 30 ps trajectories.

TABLE II. A summary of values of the average static friction γ_0 computed for separate atom and chemical type.

	Region	γ_0 (ps^{-1})
System	Total	2.12
	Protein	1.87
	Heme	6.76
	Solvent	7.49
Backbone		
	Oxygens	9.56
	Nitrogens	9.45
	α-Carbons	6.92
	Carbonyl carbons	3.78
	Hydrogens	0.91
Residues		
	Charged	2.39
	Aliphatic	1.69
	Aromatic	1.09

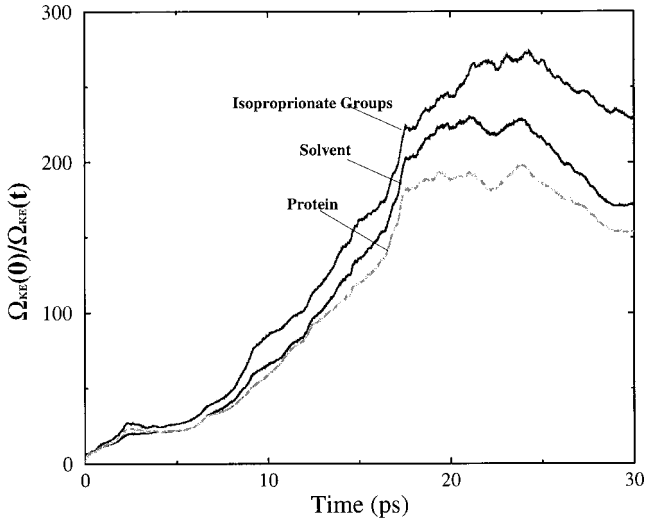

FIG. 6. $\Omega_{KE}(0)/\Omega_{KE}(t)$ as a function of time for the atoms of the protein solvent and heme isopropionate groups. The simulations were run at 300 K. The data were averaged over ten 30 ps trajectories.

carbonyl carbon atoms or the number of nonbonded contacts. However, since a majority of the myoglobin molecule consists of relatively rigid α-helices, neither the α-carbon nor the carbonyl carbon atoms exhibit much orientational mobility. The origin of the different average rates of kinetic energy relaxation may be the fact that the carbonyl carbon is engaged in a relatively high frequency vibration that is a good normal mode and weakly coupled to the surrounding protein through covalent bonds, V–V or V–T transfer. However, the carbonyl oxygens do not display a similarly reduced rate of kinetic energy relaxation. In fact, they equipartition the kinetic energy more rapidly than any other type of backbone atom.

The relative roles of residues based on their type—charged, aliphatic, or aromatic—can be seen in Fig. 4. The results for the averaged rates of kinetic energy relaxation are summarized in Table II. It is clear that charged groups could play an important role in the redistribution of excess kinetic energy within the protein. This observation has interesting repercussions when one considers the relaxation of myoglobin following photolysis. The heme group is in direct contact with solvent water molecules. This contact is made with the charged isopropionate groups that lie on the periphery of the heme. It is possible that the contact of the heme with the solvent through these groups is an important *doorway* for excess vibrational energy to leave the photolysis site. To investigate this possibility further, $\Omega_{KE}(t)$ was calculated for the isopropionate side chains using the protein and the solvent as reference systems. The results are plotted in Fig. 6. While the statistics are not sufficient to assign a quantitative number to the rate of kinetic energy relaxation, the data suggest that the isopropionate groups form an important link with the solvent for the dissipation of kinetic energy from the heme moiety.

Interestingly, the aliphatic side chains show a more rapid rate of kinetic energy equipartitioning than the aromatic side chains. It is believed that this results from the fact that the intraside chain vibrational modes of the aromatic side chain are relatively high compared with the vibrations of the aliphatic side chains. As a result, the harmonic bond force constants of the CHARMM potential for the bonds forming the aromatic rings are significantly greater than those used for the aliphatic side chains.

In our earlier work, we examined the rate of equipartitioning of the kinetic energy at seven temperatures for the S-peptide and the RNase A enzyme complex.[28] In those results, computed for the peptide and protein in vacuum, at short times there was a rapid convergence while at longer times there was a linear convergence characteristic of a diffusive process. The rate of kinetic energy equipartitioning increased linearly with the temperature for the S-peptide and the RNase A enzyme/product complex. Using a Langevin model the data were used to estimate the friction constant to be, averaged over all atoms, $\gamma_0 = (0.5-3)\,ps^{-1}$ over the range of temperature studied for both the enzyme/product complex and the S-peptide. At room temperature, the average velocity relaxation time was found to be $1/\gamma_0 = 0.5$ ps.

Relaxation times for atomic fluctuations have been calculated for bovine pancreatic trypsin inhibitor (BPTI) in vacuum and solvent. McCammon, Wolynes, and Karplus[3] have estimated the friction acting on a single dihedral angle degree of freedom of Tyr 21 in BPTI and found a relaxation time of 0.2 ps which is significantly faster than our average value. Assuming an exponential decay of the atomic displacement correlation function the relaxation time was estimated to be approximately 1.7 ps for the main chain atoms in both vacuum and solvent at room temperature.

In conclusion, an essential point made in this and earlier work is that there exists a strong inhomogeneity in the distribution of rates of kinetic energy relaxation that correlates with the details of the protein structure. Average values of the relaxation rate cannot be used to characterize the rates of relaxation for specific protein modes. The method presented here provides a straightforward means of estimating the friction for all atoms of the protein in a way that allows for a global analysis of the dominant pathways for kinetic energy relaxation.

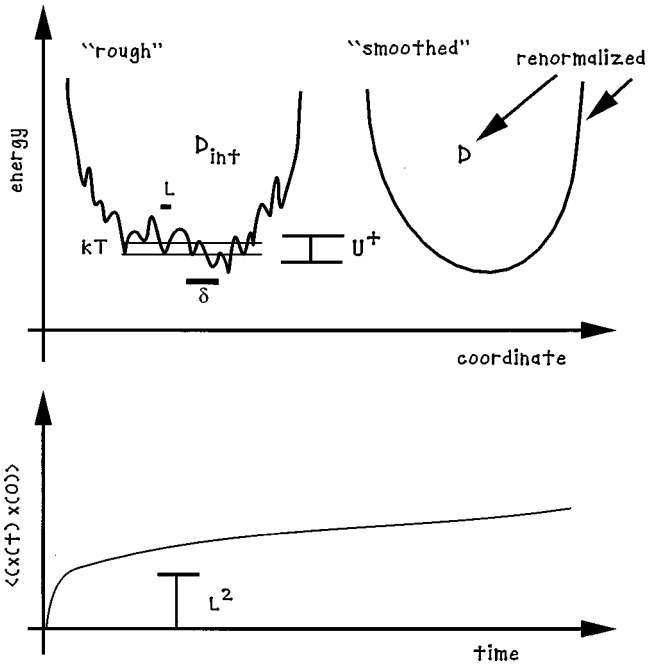

FIG. 7. A rough potential where motion is characterized by the intrinsic diffusion constant $D_{\rm int}$ is plotted above the smoothed potential energy function where motion is characterized by a renormalized diffusion constant D.

V. LENGTH SCALE DEPENDENCE OF FRICTION

Recall from the Introduction that the magnitude of the static friction acting on a given coordinate may be determined from the Einstein relation if the translational diffusion constant is known. However, the value of that static friction constant is dependent on the exact definition of (1) the coordinate that is diffusing *and* (2) the length (or time) scale over which the motion is examined. Consider the case of the diffusive motion of a particle in a one dimensional rough potential, such as that shown in Fig. 7, considered by Zwanzig.[29] One way to describe the motion of that coordinate is in terms of a Smoluchowski equation where the coordinate moves in the rough potential $U(x)$ subject to an intrinsic diffusion constant $D_{\rm int}$ that is related to a friction $\gamma_{\rm int}$ as $D_{\rm int}=kT/m\gamma_{\rm int}$. In terms of the relaxation of the velocity autocorrelation function, the initial decay time will be $1/\gamma_{\rm int}$ which will lead to a mean square displacement as a function of time that will initially increase over a length scale L with a strong slope $2kT/\gamma_{\rm int}$ (see Fig. 7). That time scale will characterize the relaxation of the velocity *within* a basin of width L of the rough potential.

On a longer time scale, it will also be possible for the coordinate to move over barriers and between basins separated by a length scale $\delta>L$. If the frequency of the basin is taken to be ω_0 and that of the barrier is ω_B, the time scale for the activated barrier crossing,

$$\tau=\frac{\gamma_{\rm int}}{\omega_0\omega_B}e^{U^\dagger/kT}, \tag{26}$$

is inversely proportional to the probability of gaining the activation energy $U^\dagger$. That activation time scale will be much longer than the time scale for the initial velocity relaxation in the potential energy basin. Therefore, when the mean square displacement is followed to longer length scales $\Delta x \gg L$ over longer times $t\gg\tau$, a second and weaker convergence with a slope on the order of $2\delta^2/\tau$ will emerge (see Fig. 7).

If we think of the initial relaxation within a basin, we would characterize the friction as being on the order of $\gamma_{\rm int}$. We would then identify a ''friction'' as being on the order of $\gamma=kT\tau/\delta^2\ll\gamma_{\rm int}$. As Zwanzig demonstrated,[29] motion on a longer length scale is characterized by an effective diffusion constant for the ''coarse grained'' motion in a smoothed potential that has been averaged over a length scale δ. The ''renormalized'' diffusion constant is $D=kT/m\gamma=\delta^2/\tau$. The weakly damped dynamics within a basin and hopping between basins is replaced by the strongly damped diffusion on a length scale δ. Therefore, the exact value of the friction will depend not only on the choice of the coordinate but the time scale of the motion that is considered.

In this work, we have presented a method for determining the *intrinsic* friction $\gamma_{\rm int}$. Experimental or computational methods may be used to define the diffusive motion on a longer length and time scale providing an estimate of γ. The two frictions will be related by

$$\gamma=\gamma_{\rm int}\frac{kT}{\omega_0\omega_B\delta^2}e^{U^\dagger/kT}. \tag{27}$$

In the case that $\omega_0\propto\omega_B$ we find that

$$U^\dagger=kT\ln\frac{\gamma}{\gamma_{\rm int}}. \tag{28}$$

This result is identical to that found by Zwanzig for the case of one-dimensional motion when the barriers were of an identical energy $U^\dagger$.

In a multidimensional system, there will be many potential pathways connecting each pair of basins. Some pathways will contribute little to the flux between basins because they involve crossing a high energy barrier. Other pathways that involve crossing lower energy barriers will be dominant and correspond to pathways of maximum flux for the diffusive motion between basins. The value of $U^\dagger$ that emerges when Eq. (28) is applied to a complex systems will be that of the *highest* or rate limiting barrier along the pathway of *maximum* flux.

Let us consider the application of this idea to the case of diffusive motion in liquid water. There the translational diffusion constant is roughly $D=2.2\times10^{-5}\,{\rm cm}^2/{\rm s}$ at 300 K corresponding to a $\gamma=2.7\times10^{13}\,{\rm s}^{-1}$. We have applied the method presented in this paper to find that for the same system the intrinsic friction $\gamma_{\rm int}=7.3\times10^{12}\,{\rm s}^{-1}$. Therefore, using Eq. (28) we find that the characteristic ''roughness'' energy scale for the essential rate-limiting motion in the translational diffusion of water molecules is

$$U^\dagger=1.2\,{\rm kcal/mol}, \tag{29}$$

which is roughly on the order of the dissociation energy for a hydrogen bond. This agrees with our intuition that the rate limiting step for diffusion in water will be the breaking of hydrogen bonds in the instantaneous hydrogen bond network. This application demonstrates that the method pre-

sented here is capable of extracting the characteristic energy scale for the essential motions followed in the relaxation of complex systems.

Equation (7) may be applied to diffusive motions of proteins in a similar manner. An experimental determination of γ through the Einstein or Stokes–Einstein relation may be combined with a computational evaluation of the intrinsic friction $\gamma_{\rm int}$ and used to extract a ''roughness'' scale $U^{\dagger}$ for motion on the rugged energy surface.

VI. INTERPRETING EXPERIMENTAL MEASURES OF PROTEIN FRICTION

There are several experimental techniques designed to investigate energy relaxation processes that offer an indirect measure of the frictional forces acting along the relaxing degree of freedom. A measured relaxation time can be interpreted using a microscopic model of the local environment about the mode of interest that captures the essential dynamics of the process. These techniques include time-resolved IR spectroscopy, reaction kinetics experiments, transient phase grating spectroscopy, and resonance Raman spectroscopy.

A. Interpretation of reaction kinetics in hemoglobin using Kramers reaction rate theory

Using nanosecond lasers, Ansari *et al.*[30] fit the rate of conformational change of carbonmonoxy myoglobin following ligand photolysis to the Kramers reaction rate theory expression for unimolecular processes in the high friction limit,

$$k \sim \frac{\omega_0 \omega_B}{\gamma_0} e^{-\beta E^{\ddagger}}, \tag{30}$$

where k is the reaction rate, ω_0 and ω_B are the harmonic estimates of the reactant well and barrier frequencies, $E^{\ddagger}$ is the activation energy, and γ_0 is the static friction with units of inverse time. The dissipative force was related to the static friction γ_0 and the bulk viscosity of the surrounding bath η as

$$F_{\rm diss} = -m\gamma_0 v = -6\pi\eta a_H v, \tag{31}$$

where m is the effective mass of the reaction coordinate, v is the velocity of that coordinate, and a_H is the effective hydrodynamic radius where it was assumed that the viscous drag on the reaction coordinate could be modeled as a sphere of radius a_H moving in a viscous medium of bulk viscosity η. Ansari *et al.* decomposed the total viscosity acting on the reaction coordinate as the sum of the solvent and protein viscosities $\eta_{\rm solvent} + \eta_{\rm protein}$ with $\eta_{\rm protein}$ being the internal viscosity of the protein.[31] This decomposition was found to give the best fit to the data which was their rationalization for invoking the internal protein viscosity. In doing so, they assumed that (1) the changes in the reaction rate k were due to the changes in the effective friction, (2) the effective friction was proportional to the viscosity, and (3) there were no changes in the effective activation energy due to changes in the solvent used to vary the viscosity. Taking the solvent viscosity to be the 0.01 Poise of room temperature water, they estimated the contribution of the protein to the total viscosity to be approximately 0.04 Poise. The viscosity can be interpreted in terms of a friction on the reaction coordinate through the Stokes–Einstein relation only after the ratio a_H/m for the reaction coordinate is specified.

Unfortunately, the reaction coordinate for the process studied is not well characterized making it difficult to make an accurate estimate of the friction. However, if we assume that the increase in internal viscosity arises from barriers associated with roughness within a single basin, then we can write

$$\eta_{\rm protein} \simeq \eta_{\rm solvent} \exp(U^{\dagger}/kT). \tag{32}$$

A barrier on the order of kT is sufficient to give enhanced values for $\eta_{\rm protein} \simeq 3 \times \eta_{\rm solvent}$. A similar observation was made by Go who noted that the presence of the solvent can influence the shape of the protein potential energy surface, producing local minima separated by low-energy barriers.[4]

B. Transient phase grating spectroscopy and thermal diffusion in heme proteins

Other probes have at their core the monitoring of the migration of thermal energy. Transient phase grating experiments[32] monitor the change in the index of refraction of the solvent due to the thermal expansion and changes in the density that accompany ligand photolysis. In a pioneering application of the technique to biomolecular systems, Miller and co-workers have determined the time scale for heme relaxation and transfer of energy from the protein to the water bath to be less than 20 ps.[32,33] Their work has raised a number of important questions related to the directionality of energy transfer following photolysis in heme proteins.

In this work, we have demonstrated that there are strongly preferred pathways for kinetic energy relaxation. The nature of the pathway will depend on the details of the protein structure, the protein or heme composition, the degree of compactness or local density, and the degree of exposure to the solvent. In the heme region of myoglobin, the result is fast kinetic energy relaxation through the heme and isopropionate groups into the solvent. Such directional mechanisms for energy transfer could be important in preserving the protein's structure in the aftermath of a protein quake following ligand rebinding by effectively funneling excess kinetic energy into the solvent bath and avoiding a sharp increase in the local temperature of the protein.

C. Resonance Raman probes of vibrational relaxation in heme proteins

Resonance Raman experiments have also been used to study thermal relaxation processes in heme proteins by Kitagawa and co-workers.[26] They probed the relaxation of the photolyzed heme in myoglobin. Monitoring the time dependence of the relaxation process, they concluded that the heme relaxation exhibited a biphasic decay. Earlier analysis of Hochstrasser and co-workers[34] suggested that the biphasic decay may have its origin in the fast relaxation of a ''collective mode'' and slower collisional relaxation of the heme through the protein to the solvent.

Perhaps the simplest method, yet the one that most closely models experiment, involves the excitation of the re-

laxing moiety, often by adding excess kinetic energy, and the subsequent monitoring of its vibrational and/or kinetic energy. Using that direct method, Henry and co-workers[27] found that the directly simulated relaxation times for thermal equilibration of an excited heme in myoglobin were in good agreement with experiment for the process of energy relaxation of modes in the heme group of myoglobin to the surrounding protein and solvent. No identification of friction acting on atoms or normal modes resulted from this study.

However, in this work we have shown the dominant pathways for kinetic energy relaxation to be rapid funneling of excess kinetic energy through the heme and isopropionate groups to the solvent and slower transfer to the solvent through the protein matrix.[34] Those competing channels are intimately related to the biphasic time relaxation observed in the heme relaxation experiments.

D. Inelastic neutron scattering experiments interpreted by Langevin normal mode models

One of the most direct experimental probes of friction in proteins is quasielastic neutron scattering.[35] Using a Langevin normal mode description of the protein dynamics, the scattering function is computed and fitted by adjusting the distribution of friction constants assigned to the normal modes of the protein. One such model has employed a distribution of friction coefficients that is a Gaussian function of the frequency of the ω normal mode,

$$\gamma(\omega) \simeq \gamma(\omega=0)\exp(-\omega^2/2\sigma^2) \tag{33}$$

so that the greatest frictional damping is assigned to the lowest frequency vibrations that are most strongly coupled to the solvent bath. In the parameterization,[35] it was found that $\gamma(\omega=0)=30\ \mathrm{cm}^{-1}=0.90\ \mathrm{ps}^{-1}$ and $\sigma=15\ \mathrm{cm}^{-1}=0.45\ \mathrm{ps}^{-1}$ resulted in the best fit to the experimental data using the damped Langevin normal mode model.[35]

In this work, we have found that relaxation times for local fluctuations fall on the order of $1–10\ \mathrm{ps}^{-1}$. Our rates of relaxation are somewhat larger than those resulting from the experimental fits. That may be due in part to the fact that the experimental fits were dominated by low temperature data. We have shown that there is a significant temperature dependence in the values of the static friction.[28] A more accurate distribution of friction constants would incorporate that apparently linear temperature dependence in $\gamma(\omega=0)$ and σ.

E. Geminate ligand recombination following photolysis in heme proteins

The rate of ligand rebinding in myoglobin following flash photolysis has been extensively studied beginning with the seminal work of Frauenfelder.[36] The rebinding kinetics has been measured over a wide range of temperature and solvent type. The data have been fitted by a variety of models beginning with the work of Agmon and Hopfield (AH).[37] The AH model reduces the complex ligand/protein/solvent dynamics to the dynamics of two coordinates. The ligand rebinding coordinate represents the motion of the ligand relative to the heme iron. The protein coordinate represents the relaxation of the protein following photolysis. The barrier to ligand rebinding is a sensitive function of the protein coordinate. The protein coordinate is modeled by an overdamped, diffusive dynamics with an associated diffusion constant that depends on temperature and solvent type. That diffusion constant has been interpreted in terms of a temperature dependent friction acting on the protein coordinate. However, the exact nature of the protein coordinate, and as a result the friction, is only vaguely defined.

F. Ligand vibrational energy relaxation in heme proteins

Time resolved IR spectroscopy has been used to measure the time scale of vibrational population relaxation from the first excited vibrational state to the ground vibrational state ($\nu_0 \leftarrow \nu_1$) of CO in heme proteins. Using this tool, relaxation times for both bound[38–40] and photolyzed[10] CO in myoglobin or hemoglobin have been determined. The time scale for ligand vibrational energy relaxation was found to be on the order of 20 ps in the bound case and 600 ps in the unbound case.

The process of vibrational energy relaxation from a photolyzed CO in the heme pocket of myoglobin has been studied using molecular dynamics simulations and theory by Sagnella and Straub.[11] The fluctuating force correlation function, classically related to the time dependent friction of the CO bond stretching vibration, was calculated from direct molecular dynamics simulations. The resulting relaxation time was in good agreement with the value of T_1 experimentally determined by Anfinrud and co-workers.[10] The relaxation rate for the bond stretching relaxation is on the order of $0.05\ \mathrm{ps}^{-1}$ in the bound case and $0.0017\ \mathrm{ps}^{-1}$ in the unbound case which are both significantly larger than the atomic relaxation rates determined in this study. In the case of CO relaxation, the high frequency CO stretch is a ''good normal mode'' that is only weakly coupled to the surrounding protein and heme through bonds or collisions and is isolated from the solvent. Those facts account for the observed slow IVR of the CO oscillator.

This overview of experimental measures of protein friction makes it clear that the assignment of an accurate measure of the friction constant requires a good knowledge of the specific mode on which the friction acts and an accurate experimental probe of the dynamics of that mode. The most direct method for such a determination of the friction comes from the direct molecular dynamics simulation of the dissipative dynamics of a protein.

VII. SUMMARY

The computation of the ergodic measure provides a clean, facile method for the computation of the internal rate of kinetic energy relaxation within proteins. Issues of the rate of kinetic energy relaxation are intimately related to the concept of the ''protein quake'' that follows ligand photolysis in heme proteins.[41] Using this method, various local regions of interest in the protein can be separately examined. This can provide interesting information regarding the local environ-

ment felt by various parts of the protein. Furthermore, the magnitude of the relaxation rates provides insight into possible relaxation pathways within the protein.

In this paper, we have presented data for solvated carbomonoxy myoglobin. The data suggest that the water solvent could be an important channel in the loss of excess kinetic energy from the heme where the coupling is strongest through the isopropionate groups. In addition, the protein backbone may be an effective ''thermal wire'' in the dissipation of excess energy throughout the protein.

The method used to isolate the rate of kinetic energy relaxation may be combined with experimental or computational measures of diffusion over longer length scales and used to isolate the ''roughness'' energy scale for diffusion on a rugged energy landscape.[42] The origin of internal friction in proteins is still not entirely clear. Because we start with x-ray coordinates in our simulations, it is most probable that dissipation of thermal energy arises due to collisions between side chain atoms and backbone atoms that are already in contact in the native state. It is unlikely that loop contacts can explain the source of internal friction for folded proteins.[43] For such a mechanism to be operative, larger scale conformational fluctuations, which may be prevalent under denaturing conditions, are required. These considerations point to a rather complex role for internal friction in proteins. We speculate that protein internal viscosity depends on the extent to which the chain is folded. Thus, in the process of folding internal viscosity is best described by loop entropy considerations,[44] while in the compact and native states local collisions that redistribute the thermal energy may dominate. It is that latter process that is best described by our method.

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