Dielectric Dispersion Interpretation of Single Enzyme Dynamic Disorder, Spectral Diffusion, and Radiative Fluorescence Lifetime†

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A formulation based on measurable dielectric dispersion of enzymes is developed to estimate fluctuations in electrostatic interaction energy on time scales as long as milliseconds to seconds at a local site in enzymes. Several single molecule experimental obsevations occur on this time scale, currently unreachable by real time computational trajectory simulations. We compare the experimental results on the autocorrelation function of the fluctuations of catalysis rate with the calculations using the dielectric dispersion formulation. We also discuss the autocorrelation functions of the fluorescence lifetime and of spectral diffusion. We use a previously derived relation between the observables and the electric field fluctuations and calculate the latter using dielectric dispersion data for the proteins and the Onsager regression hypothesis.

I. Introduction

Recent advances in single molecule spectroscopy allow the observation of real time trajectories of individual molecules. With these experimental techniques, several novel observations were made on single proteins: on−off switching of fluorescence in proteins,[1−3] oscillations of green fluorescent proteins between neutral and anionic conformations at a near-denatured condition,[4,5] spectral diffusion of the chromophore in an enzyme,[6] fluctuations of fluorescence lifetime in enzymes,[7,8] and the fluctuations in the rates of enzyme catalysis.[6,9−11] All of these observations have been regarded as reflecting the dynamics of the enzyme between different conformational substates on the milliseconds time scale. For example, several enzyme-catalyzed reactions occur typically on the time scale of milliseconds to seconds, and the millisecond conformational dynamics of enzymes is considered as an important contributor to the functional dynamics of the enzyme.[12] Fluctuations in catalysis rate observed in single enzymes were also interpreted as being due to fluctuations in the conformation of the enzyme.[9,10]

The ensemble experimental data on enzyme catalysis have often been studied theoretically with the aid of computer simulations: for example, see refs 13−26. In these studies, electrostatic interactions have played a key role in enzyme catalysis.[13,16−28] Earlier, a relation between three different observables, catalysis rate fluctuations ($\delta k(t)$), spectral diffusion ($\delta\omega_0(t)$), and radiative fluorescence lifetime fluctuations ($\delta\gamma_r^{-1}(t)$), was derived on the basis of fluctuations of the electrostatic interaction energy ($\delta E(t)$).[29] Computer simulations of protein dynamics in real time are currently limited to tens of nanoseconds. Accordingly, a detailed dynamical analysis for the estimation of $\delta E(t)$ on the millisecond time scale is not analyzable by current real-time trajectory computational methods, and a resort to other methods must be made. As a step toward estimating the $\delta E(t)$, we model the autocorrelation function of $\delta E(t)$ by relating it to another experimental observable, the frequency-dependent dielectric response function $\epsilon(\omega)$ of the protein. The experimentally observable $\epsilon(\omega)$ makes possible the comparison of experimental and theoretical autocorrelation functions of fluctuations in δk, $\delta\omega_0$, and $\delta\gamma_r^{-1}$ that are observables in single molecule experiments. This comparison of experiment and theoretically based relations is the essence of the present paper.

The paper is organized as follows. Equations for the autocorrelation of δk, $\delta\omega_0$, and $\delta\gamma_r^{-1}$ in terms of those in $\delta E(t)$ are summarized in section II using results derived previously,[29] and the autocorrelation function for $\delta E(t)$ is also given in terms of the dielectric dispersion of the protein. The comparison of experimental and theoretical results is given in section III, and some general remarks on the treatment are given in section IV, together with suggested further experimental tests of eqs 1e and 7.

II. Dielectric Dispersion and Fluctuations in Electrostatic Interaction

A. Relation Among Observables. In a previous article on the observables in single molecule experiments,[29] the fluctuations in the rate of catalysis of a substrate by the enzyme ($\delta k(t)$), spectral diffusion of the fluorescence emission ($\delta\omega_0(t)$), and the radiative part of the fluorescence lifetime ($\delta\gamma_r^{-1}(t)$) of a chromophore in the enzyme were treated as arising from the fluctuations of electrostatic interaction energy $\delta E(t)$ at the local site in the enzyme. On the basis of this assumption, a relation was derived for the autocorrelation functions of each of these quantities in terms of the autocorrelation function $C_E(t)$ of fluctuations in electrostatic interactions at that active site, $\delta E(t)$. The latter correlation function is defined by

$$C_E(t) = \frac{\langle \delta E(t)\delta E(0)\rangle}{\langle \delta E(0)^2\rangle} \tag{1a}$$

For the autocorrelation of the catalysis rate fluctuations, $C_k(t)$, we had[29]

$$C_k(t) = \frac{\langle \delta k(t)\delta k(0)\rangle}{\langle \delta k(0)\delta k(0)\rangle} \approx C_E(t) \tag{1b}$$

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for the autocorrelation of the spectral difusion, $C_{\omega_0}(t)$,

$$C_{\omega_0}(t) = \frac{\langle \delta\omega_0(t)\delta\omega_0(0)\rangle}{\langle \delta\omega_0(0)^2\rangle} \approx C_E(t) \tag{1c}$$

and for the autocorrelation function of the radiative component of the fluorescence lifetime, $C_{\gamma^{-1}}(t)$

$$C_{\gamma_r^{-1}}(t) = \frac{\langle \delta\gamma_r^{-1}(t)\delta\gamma_r^{-1}(0)\rangle}{\langle \delta\gamma_r^{-1}(0)\delta\gamma_r^{-1}(0)\rangle} \approx C_E(t) \tag{1d}$$

From eqs 1b−d, we have

$$C_k(t) = C_{\omega_0}(t) = C_{\gamma^{-1}}(t) = C_E(t)$$

Although eq 1e was derived treating the δE as causal for the other fluctuations, an alternative view would be that all four quantities may have a common cause, fluctuations in protein conformation. We consider next a way of evaluating $C_E(t)$ approximately in terms of the overall dielectric dispersion $\epsilon(\omega)$ for the protein and then relate the experimental observables to the calculations based on these equations. While the protein itself is heterogeneous, we use its averaged property in the form of $\epsilon(\omega)$ as a first approximation and then compare predictions from the model with experiments.

B. Autocorrelation of $\delta E(t)$. Using the Onsager regression hypothesis,[32] we find the autocorrelation function of the fluctuations of electrostatic interaction energy $E(t)$ about the equilibrium value is related to the decay of interaction energy in a nonequlibrium process following an initial excitation

$$\frac{\langle \delta E(t)\delta E(0)\rangle}{\langle \delta E(0)\delta E(0)\rangle} = \frac{E(t) - E(\infty)}{E(0) - E(\infty)} \tag{2}$$

Electrostatic fluctuations in the interaction energy $E(t)$ can be estimated by approximating the enzyme as a homogeneous dielectric with a frequency dependent dielectric constant $\epsilon(\omega)$ and the reactants/chromophore as a dipole embedded in a spherical cavity of dielectric constant ϵ_c in the enzyme. By using eq 2, the autocorrelation function of the equilibrium fluctuations of E can be studied by considering a model nonequilibrium system formed by the creation of a dipole $\Delta\mu(t) = \Delta\mu\theta(t)$ in the cavity at $t = 0$, where $\theta(t)$ is the unit step function. $\Delta\mu$ is the dipole moment created by electronic excitation of the chromophore in the case of spectral diffusion and the dipole moment difference between the transition state and the reactants in enzymatic catalysis. In the case of single enzyme experiments on catalysis rate,[10] the rate is obtained by averaging the turnover times for several cycles of the enzyme over which the enzyme is assumed to be in the same conformation. This $\Delta\mu$ for the reaction contributes to an electrostatic interaction for this conformation of the enzyme. On longer time periods, there are changes in conformations, resulting in fluctuations in this energy difference associated with fluctuations in the electric field and thereby leading to fluctuations in the rate constant for the enzymatic catalysis.

The time-dependent interaction energy $E(t)$ of dipole in a spherical cavity of radius r_0 following an initial creation of the dipole is given in terms of the time-dependent reaction field $\mathbf{R}(t)$ because of the protein environment acting on the dipole $\Delta\mu(t)$ and the response function tensor $\mathbf{r}(t)$ as[33]

$$\mathbf{R}(t) = \int_0^t \mathbf{r}(t - t')\cdot\Delta\mu(t')\,\mathrm{d}t' \tag{3}$$

where the integration is from $t = 0$ because of the unit step function $\theta(t)$ in $\Delta\mu(t)$. The Fourier−Laplace transform, $\mathcal{L}$ defined as $F(\omega) = \mathcal{L}(f(t)) = \int_0^\infty \exp(-i\omega t)f(t)\,\mathrm{d}t$, of the response function $\mathbf{r}(t)$ is given as[33]

$$\mathbf{r}(\omega) = \frac{2}{r_0^{\,3}}\frac{\epsilon(\omega) - \epsilon_c}{2\epsilon(\omega) + \epsilon_c}\underset{\sim}{\boldsymbol{I}} \tag{4}$$

where $\underset{\sim}{\boldsymbol{I}}$ is a unit tensor.

The time-dependent component of the interaction energy defined as

$$E(t) = -\Delta\mu(t)\cdot\mathbf{R}(t) \tag{5}$$

is obtained using the above relations as[33]

$$E(t) = \frac{2\Delta\mu^2}{r_0^{\,3}}\mathcal{L}^{-1}\left[-\frac{1}{i\omega}\frac{\epsilon(\omega) - \epsilon_c}{2\epsilon(\omega) + \epsilon_c}\right] \tag{6}$$

Combining eqs 2 and 6 and using the Laplace transform identities $f(t = 0) = \lim_{\omega\to\infty} i\omega F(\omega)$ and $f(t \to \infty) = \lim_{\omega\to 0} i\omega F(\omega)$, the autocorrelation $C_E(t)$ is given as

$$C_E(t) = \frac{\mathcal{L}^{-1}\left[\frac{1}{i\omega}\frac{\epsilon(\omega) - \epsilon_c}{2\epsilon(\omega) + \epsilon_c}\right] - \left[\frac{\epsilon_s - \epsilon_c}{2\epsilon_s + \epsilon_c}\right]}{\left[\frac{\epsilon_\infty - \epsilon_c}{2\epsilon_\infty + \epsilon_c}\right] - \left[\frac{\epsilon_s - \epsilon_c}{2\epsilon_s + \epsilon_c}\right]} \tag{7}$$

C. Dielectric Dispersion of Proteins. The continuum dielectric response of proteins has been modeled in the literature[34,35] using the Havriliak−Negami behavior[36] with a and b in the range [0,1]:

$$\frac{\epsilon(\omega) - \epsilon_\infty}{\epsilon_s - \epsilon_\infty} = \frac{1}{[1 + (i\omega t_0)^a]^b} \tag{8}$$

This dielectric response becomes a Cole−Cole dispersion when $b = 1$ and a Cole-Davidson dispersion when $a = 1$.

Dielectric dispersion measurements of some proteins are available for a frequency range corresponding to the time scale of milliseconds to seconds (Hz−kHz).[34,35,37] Dielectric properties of proteins were also used to study protein denaturation.[38] One interest in the dielectric relaxation measurements of proteins in the millisecond time scale has been in the possible relation to biological activity.[37]

In the case of hemoglobin, a Cole−Cole behavior with $a = 0.7$, $b = 1$ in eq 8 was observed for $2\pi/\omega$ in the range of milliseconds to seconds.[34] For hydrated lysozyme powder, the imaginary part of the dielectric response was found to behave as $\epsilon''(\omega) \sim 1/\omega^\alpha$, α varying from 0.3 to 0.7 when temperature was changed from 260 to 280 K.[37] This imaginary part $\epsilon''(\omega)$ can correspond to $a = 0.3-0.7$ and $b = 1$ in eq 8. Other dielectric measurements in this time range yield a decreasing from 0.50 to 0.36 for candida antarctica lipase B and lysozyme, and $b = 0.3$ for both these enzymes[35] as the temperature is increased from approximately 195 to 255 K. The parameter a in the above experiments becomes a constant value at temperatures higher than 243 K (personal communication with J. Mijovic and Y. Bian). Among glassy materials, Cole−Cole behavior with $\epsilon''(\omega) = \omega^{-1/2}$ (which can correspond to $a = 0.5$, $b = 1$) in the milliseconds range is commonly observed.[39]

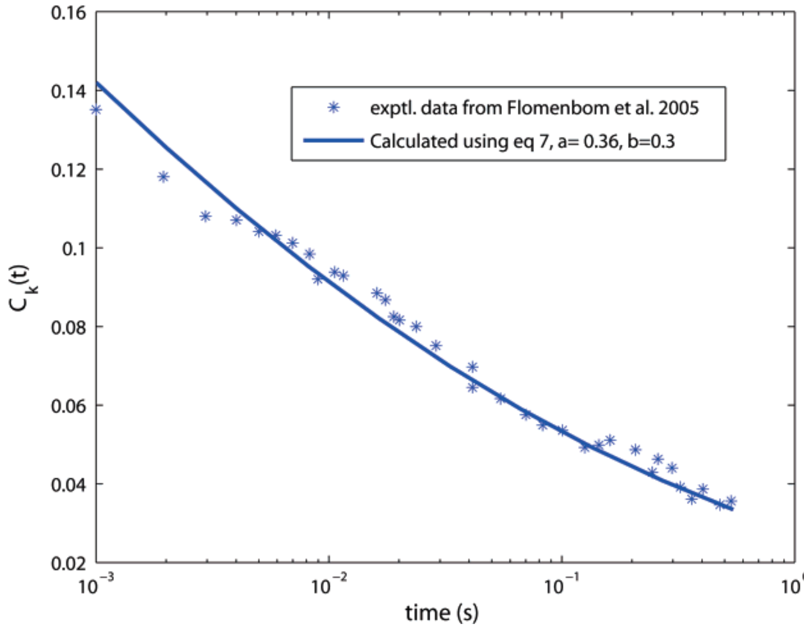

Figure 1. Comparison of $C_k(t)$ of the experimental data of candida antarctica lipase B from Figure 4A of ref 9 with $C_k(t)$ calculated using the dielectric dispersion data on candida antarctica lipase B from ref 36.

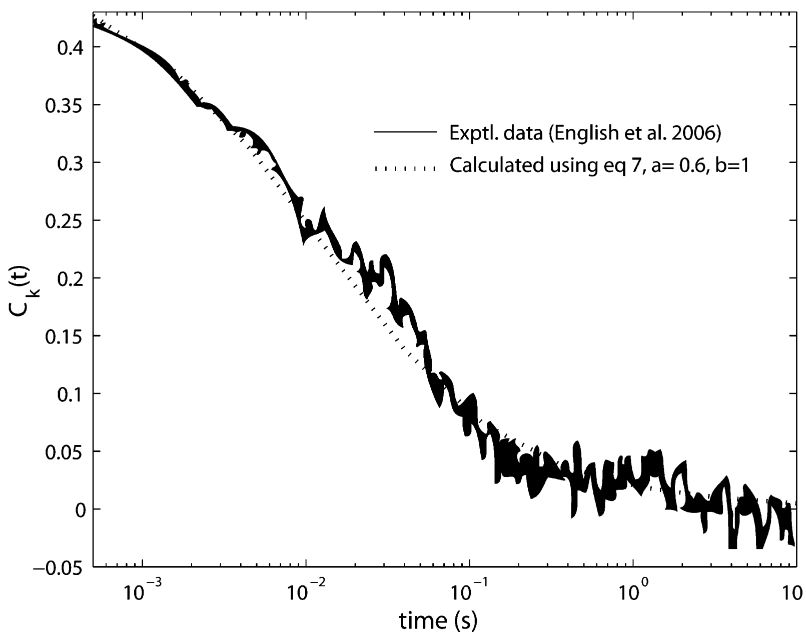

Figure 2. Comparison of experimental data from ref 10 and calculated correlation using Cole−Cole relation using $a = 0.60$, $b = 1$ in eq 8. The data points from Figure S7 of ref 10 were extracted using Adobe Illustrator.

III. Comparison with Experiments

A. Catalysis Rate Fluctuations. As noted in section II C, the dielectric dispersion behavior of candida antarctica lipase B shows $b = 0.3$ and a saturation in parameter a at 0.36 at temperatures higher than 243 K.[35] The autocorrelation $C_k(t)$ in eq 1b calculated with these parameters in eq 8 and using eqs 2 and 6 is compared in the present Figure 1 with the experimental data of ref 8 (Figure 4A there). Numerical inversion of the Laplace transform was performed using the method in ref 41. In the calculation, ϵ_c of the cavity is assumed to be 2. The parameters ϵ_s, ϵ_∞, and τ were not given in ref 36. For the present, we assume $\epsilon_s = 40$ and $\epsilon_\infty = 4$, similar to the parameters observed experimentally for lysozyme.[37] For those values of ϵ_s and ϵ_∞, a calculation using eq 2 with $\tau = 1$ s in eq 8 gives agreement with the $C_k(t)$ in Figure 1 for the candida antarctica lipase B over the time range considered. With a different choice of ϵ_s and ϵ_∞, the calculations can again be matched with experiment by choosing an appropriate value for τ. While a was taken from the above $\epsilon(\omega)$ data, the calculation of $C_k(t)$ is not very sensitive to a. Using a in the range 0.25−0.4 fits the experimental data equally well.

The experimental data on the autocorrelation function $C_k(t)$ for β-galactosidase[10] are compared with the calculated $C_k(t)$ using eq 2 in Figure 2. The calculation was performed with $a = 0.6$ and $b = 1$ in eq 8, again assuming $\epsilon_0 = 40$ and $\epsilon_\infty = 4$. The dielectric dispersion data on β-galactosidase are not yet presently available. However, the parameters a and b needed in eq 8 for the fit in Figure 2 are close to those available for hemoglobin[34] and lysozyme.[37]

B. Fluctuations in Fluorescence Lifetime. The fluorescence lifetime of a chromophore in the protein, γ^{-1}, depends upon the rate constants of radiative (γ_r) and nonradiative (γ_{nr}) decays

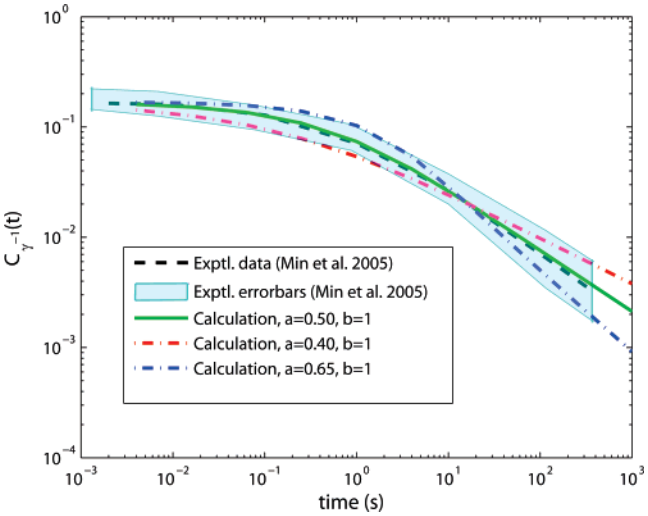

Figure 3. Comparison of the experimental data from ref 8 along with the error bars and calculated correlation using Cole−Cole relation using $a = 0.40, 0.50, 0.65$ and $b = 1$ in eq 8. The normalized autocorrelation $C_{\gamma^{-1}}(t)$ was obtained by using eq 3 of ref 8 and $C_x(t)$ from Figure 4 of ref 8.

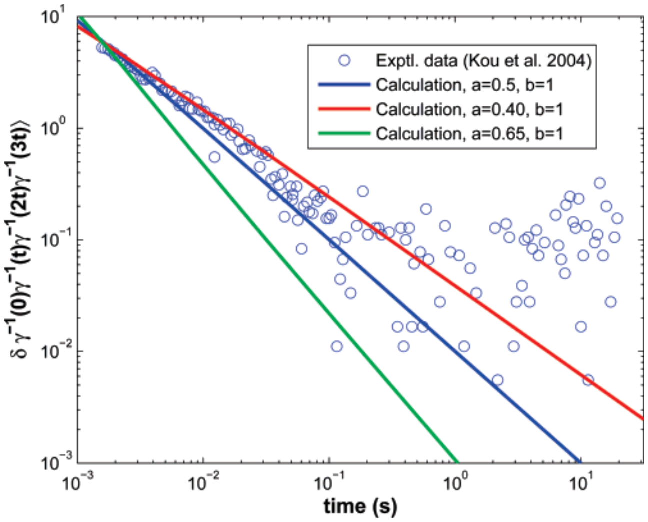

Figure 4. Comparison of fourth order correlation from the experimental data of ref 48 with the calculated correlation using Cole−Cole relation using $a = 0.40, 0.50, 0.65$, $b = 1$ in eq 8, and Wick's theorem.

of fluorescence, with possible fluctuations in either or both of these:

$$\gamma^{-1} = (\gamma_{nr} + \gamma_r)^{-1} \tag{9}$$

Only if fluctuations in γ_{nr}^{-1}, γ_r^{-1}, and $E(t)$ have a common origin (e.g., fluctuations in enzyme conformations) can both the fluctuations in the radiative and those in the nonradiative components be represented by the fluctuations in electrostatic interactions $\delta E(t)$, thereby yielding $C_{\gamma^{-1}}(t) = C_{\gamma r^{-1}}(t) = C_E(t)$. Only then could the results of the previous section be applied to the experimentally observed $C_{\gamma^{-1}}$.[7,8]

However, although it is not known whether distance fluctuations and the energy fluctuations have a common origin in enzyme conformational fluctuations, it is nevertheless useful to compare the calculated autocorrelation for $C_{\gamma^{-1}}(t)$ with the experimentally observed ones from ref 8, we use eq 1d and $a = 0.5$ and $b = 1$ for the Cole−Cole exponents in eq 8. A comparison of the experimental data on $C_{\gamma^{-1}}$ antifluorescein from ref 8 with the calculated $C_E(t)$ is shown in Figure 3. To test the sensitivity of the calculated correlation to the value of a, Laplace inversion was performed numerically for the cases $b = 1$ and $a = 0.40-0.65$ in eq 8, and the results are shown in Figure 3. The value of $a = 0.5$ and $b = 1$ gives the best agreement with the experiment on γ^{-1} fluctuations. From the discussion in section II C, this use of a Cole−Cole exponent of $a = 0.5$ and $b = 1$ can be replaced by the experimental values when dielectric dispersion data on the enzyme become available.

When $\epsilon_\infty = \epsilon_c$, an analytical relation can be derived from eqs 2 and 6 for the autocorrelation function $C_E(t)$ as

$$C_{\gamma^{-1}}(t) = \exp(t/t_0)\ \mathrm{erfc}(\sqrt{t/t_0}) \qquad (a = 0.5, b = 1) \tag{10}$$

where erfc is the complementary error function, $\mathrm{erfc}(u) = (2/\sqrt{\pi}) \int_u^\infty \exp(-v^2)\, \mathrm{d}v$, and $t_0 = \tau(\epsilon_\infty + 1)^2/(\epsilon_0 + 1)^2$. Equation 10 is functionally the same as the experimental fluorescence lifetime autocorrelation function reported in the literature.[7,8] A test is proposed later to see whether the radiative component yields a correlation function similar to that of $\delta\omega_0(t)$ and $\delta k(t)$.

Since the Gaussian nature of protein fluctuations is assumed in studies of solvation of chromophores by protein dynamics[43] and is also seen in computer simulations,[44] the higher order correlations of lifetime fluctuations can be immediately calculated from the second-order ones. They are obtained directly, for example, using Wick's theorem[45] and will be the same as the experimental observations. Results for the fourth-order correlation for $a = 0.43, 0.5, 0.65$ are compared with the experimental data on flavin reductase from ref 48 in Figure 4, to test the sensitivity of the calculation to the parameter a.

C. Memory Kernel. The Havriliak-Negami dielectric response as in eq 8 has been modeled as a dynamics with a memory kernel of $K(t)$ whose Fourier−Laplace transform is given as[47]

$$K(\omega) = \frac{i\omega}{[1 + (i\omega)^a]^b - 1} \tag{11}$$

For $a = 0.5$ and $b = 1$, this expression gives a memory kernel $K(t)$ of

$$K(t) \sim \frac{1}{\sqrt{t}} \tag{12}$$

Thus, an alternative way of representing the Cole−Cole relaxation mathematically for the present problem, when $a = 0.5$ and $b = 1$, involves a diffusive dynamics of the protein structure with a memory kernel of $1/\sqrt{t}$. This memory kernel is the same as that used in ref 8 to fit the correlation to the observed fluorescence lifetime autocorrelation data on $\delta\gamma_{nr}^{-1}$. It has been shown[8] that this memory kernel approach provides a useful mathematical model for summarizing the data although the origin of the memory kernel remains to be addressed. One feature in modeling fluctuations using dielectric dispersion is that a memory kernel can be obtained from the measurable $\epsilon(\omega)$, even though that feature does not indicate its molecular origin.

The Cole−Cole relaxation, with $b = 1$ and arbitrary a, has been mathematically cast into the formalism of continuous time random walk (CTRW), a diffusion with an associated memory kernel.[48,49] The difference between the usual random walk and CTRW is that in CTRW there is a distribution of times at which the random walker can take a step, unlike in conventional random walk where each step happens at a regular interval.[49]

This distribution of waiting times leads to a memory function in the anomalous diffusive dynamics in CTRW.[49]

IV. Discussion

A. General Remarks. In the present work, a relation between the dynamic disorder,[50] observed as catalysis rate fluctuations, and another experimental observable $\epsilon(\omega)$ is developed. The derivation itself was based on electrostatic interactions in enzymatic catalysis.[13,16−28] The relations derived previously[29] between various observables in single molecule experiments were used to relate the autocorrelation function of all these observables to $\epsilon(\omega)$ on the Hertz to kiloHertz frequency range.

Different kinds of dynamics in a protein have their intrinsic time scales,[51] ranging from vibrations in the hundreds of femtoseconds to global motions involving dynamics of large domains in the proteins that are in the milliseconds. The $\epsilon(\omega)$ measurements in kHz range thus capture the global dynamics of the domains involving polar groups in the protein. A Debye dielectric response with $a = 1$, $b = 1$ in eq 8 represents one characteristic time scale associated with the dynamics of the protein. On the other hand, the Havriliak−Negami dielectric dispersion as noted in section II C has been attributed to cooperative dynamics in the protein,[34] possibly arising from a contribution from several different domains in the protein each responding over a different time scale. Possible origins of non-Debye behavior of $\epsilon(\omega)$ in proteins and glasses have been discussed extensively in the literature.[34,35,37−39,42]

The conformational dynamics in enzymes can affect the catalysis rate through electric fields, steric effects, changes of reactants' bond lengths in the transition state, and hydrogen bond interactions with the reactants.[13] Since electrostatic interactions are usually considered to be a key factor (e.g., ref 13) in determining the rate of catalysis, we focused on the catalysis rate fluctuations arising from δE. This aspect of the conformational dynamics resulting in δE was treated using $\epsilon(\omega)$. Other features of the conformational dynamics such as those leading to the donor−acceptor distance (r_{DA}) fluctuations reflected as fluctuations in the electron-transfer rate may or may not be the same as those in δE. A way of testing the presence of a common origin for these two aspects of conformational dynamics is given later.

The nonradiative decay due to electron transfer (γ_{nr}) depends upon the nuclear reorganization energy (λ), free energy difference between donor and acceptor states (ΔG), and on any factors that affect the electronic coupling, such as the donor−acceptor distance (r_{DA}),[52]

$$\gamma_{nr} = \gamma_{nr}^{0}\, e^{-((\lambda+\Delta G)^2)/4\lambda k_B T}\, e^{-\beta r_{DA}} \tag{13a}$$

When ΔG is small compared with λ, the following approximation can be made

$$\gamma_{nr} = {\gamma_{nr}}^{0}\, e^{-\lambda/4k_B T}\, e^{-\Delta G/2k_B T}\, e^{-\beta r_{DA}} \tag{13b}$$

ΔG depends upon the electrostatic interaction energy E of the dipole (μ) formed by electron transfer from donor to acceptor. This gives an exponential dependence of γ_{nr} on E. However, when the system is in the activationless regime, with $\Delta G \approx -\lambda$, γ_{nr} will be insensitive to the changes in E and will depend only on the electronic coupling of the two reactants, and hence on r_{DA}.

$$\gamma_{nr} \approx {\gamma_{nr}}^{0}\, e^{-\beta r_{DA}} \tag{13c}$$

In the general case, γ_{nr} can depend upon fluctuations in δE and on electronic coupling-dependent fluctuations such as in δr_{DA}. If δE and δr_{DA} have a common origin in conformational fluctuations of the enzyme, the analysis using δE fluctuations for $\delta\gamma_r$ can be immediately extended to $\delta\gamma$. Otherwise, an analysis that includes both sources γ_r and γ_{nr} is needed.

A relation between the autocorrelation function of γ^{-1} (taken to be γ_{nr}^{-1}) and that of r_{DA} was derived in eq 3 of ref 8: $C_{\gamma nr}^{-1}(t) = e^{\beta^2 C_{r_{DA}}(t)} - 1$. For the range of parameters involved, it can be verified using a perturbation expansion that the autocorrelations $C_{\gamma_{nr}^{-1}}(t)$ and $C_{r_{DA}}(t)$ are the same after normalization. Because of this similarity, the functional form of the autocorrelation $C_{r_{DA}}(t)$ shown in ref 8 is the same, to within a normalization constant, to that of $C_{\gamma^{-1}}(t)$ which in turn is equal to the present eq 10.

Other potential approaches interpreting the fluorescence lifetime autocorrelation function without invoking a memory kernel can be explored. In one of them, a polymer dynamics model was used for the primary chain of the protein.[53,54] However, it was shown[54] that the nanosecond time scale for the transition of $C_{\gamma}^{-1}(t)$ to $1/\sqrt{t}$ behavior in this model is not consistent with the experimental millisecond time scale.

B. Suggested Experiments. 1. Measurements of the fluctuations $\delta k(t)$, $\delta\gamma^{-1}(t)$, and $\delta\omega_0(t)$ for the same enzyme would permit a test of the present expressions, eq 1e. Measurements of dielectric dispersion data on the milliseconds to seconds time scale for that protein would be useful, for example, for β-galactosidase and cholesterol oxidase enzymes on which $\delta k(t)$ were observed[6,10] and on flavin reductase and antifluorescein for which $\delta\gamma^{-1}(t)$ was observed.[7,8]

2. When fluorescence lifetime measurements are performed removing the quencher, for example, Tyr35 in flavin reductase,[7] measurement of the autocorrelation function in the absence of the quencher will be helpful in interpreting the relative contributions of the radiative and nonradiative components to $\delta\gamma^{-1}$, as discussed in ref 29. If there is a common origin of both components (e.g., fluctuations in protein conformation), the autocorrelation functions of $\delta\gamma_{nr}^{-1}$ and $\delta\gamma_r^{-1}$ will both be similar, but otherwise differ. The suggested experiment will help in examining the commonality of conformational changes responsible for δE and δr_{DA}.

V. Conclusion

The local fluctuations in the electrostatic interactions occurring in the milliseconds to seconds time scale in enzymes are modeled using the dielectric dispersion of the proteins. This model provides a formalism for interpreting the fluctuations in the observables on these timescales, which is presently not readily addressed using real time computational methods. Using the formulation presented earlier[29] relating various observables to the electrostatic interactions, several correlation functions can be modeled using a Cole−Cole or more general dielectric behavior. Experiments on the dielectric dispersion and on the various correlation functions for the same system would be useful in presenting a broad picture of the fluctuation phenomena in proteins.

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References and Notes

(1) Dickson, R. M.; Cubitt, A. B; Tsien, R. Y.; Moerner, W. E. *Nature* **1997**, *388*, 355.

(2) Habuchi, S.; Ando, R.; Dedecker, P.; Verheijen, W.; Mizuno, H.; Miyawaki, A.; Hofkens, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 9511.

(3) Garcia-Parajo, M. F.; Segers-Nolten, G. M. J.; Veerman, J. A.; Greve, J.; van Hulst, N. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 7237.

(4) Baldini, G.; Cannone, F.; Chirico, G. *Science* **2005**, *309*, 1096.

(5) Baldini, G.; Cannone, F.; Chirico, G.; Collini, M.; Campanini, B.; Bettati, S.; Mozzarelli, A. *Biophys. J.* **2007**, *92*, 1724.

(6) Lu, H. P.; Xun, L.; Xie, X. S. *Science* **1998**, *282*, 1877.

(7) Yang, H.; Luo, G. B.; Karnchanaphanurach, P.; Louie, T. M.; Rech, I.; Cova, S.; Xun, L. Y.; Xie, X. S. *Science* **2003**, *302*, 262.

(8) Min, W.; Luo, G.; Cherayil, B, J.; Kou, S. C.; Xie, X. S. *Phys. Rev. Lett.* **2005**, *94*, 198302.

(9) Flomenbom, O.; Velonia, K.; Loos, D.; Masuo, S.; Cotlet, M.; Engelborghs, Y.; Hofkens, J.; Rowan, A. E.; Nolte, R. J. M.; Van der Auweraer, M.; de Schryver, F. C.; Klafter, J. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 2368.

(10) English, B. P.; Min, M.; van Oijen, A. M.; Lee, K. T.; Luo, G.; Sun, H.; Cherayil, B. J.; Kou, S. C.; Xie, X. S. *Nat. Chem. Bio.* **2006**, *2*, 87.

(11) Edman, L.; Földes-Papp, Z.; Wennmalm, S.; Rigler, R. *Chem. Phys.* **1999**, *247*, 11.

(12) Benkovic, S. J.; Hammes-Schiffer, S. *Science* **2003**, *301*, 1196.

(13) Warshel, A.; Sharma, P. K.; Kato, M.; Xiang, Y.; Liu, H.; Olsson, M. H. M. *Chem. Rev.* **2006**, *106*, 3210.

(14) Gao, J.; Truhlar, D. J. *Ann. Rev. Phys. Chem.* **2002**, *53*, 467.

(15) Agarwal, P. K.; Billeter, S. R.; Rajagopalan, P. T. R.; Benkovic, S. J.; Hammes-Schiffer, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2794.

(16) Warshel, A.; Levitt, M. *J. Mol. Bio.* **1976**, *103*, 227.

(17) Pickersgill, R. W.; Goodenough, P. W.; Sumner, I. G.; Collins, M. E. *Biochem. J.* **1988**, *254*, 235.

(18) Dardenne, L. E.; Werneck, A. S.; Neto, M. D.; Bisch, P. M. *Proteins Struct. Funct. Genet.* **2003**, *52*, 236.

(19) Wong, K. F.; Watney, J. B.; Hammes-Schiffer, S. *J. Phys. Chem. B* **2004**, *108*, 12231.

(20) Benach, J.; Winberg, J.-O.; Svendsen, J.-S.; Atrian, S.; Gonzalez-Duarte, R.; Ladenstein, R. *J. Mol. Biol.* **2005**, *345*, 579.

(21) Ranaghan, K. E.; Ridder, L.; Szefczyk, B.; Sokalski, W. A.; Hermann, J. C.; Mulholland, A. J. *Org. Biomol. Chem.* **2004**, *2*, 968.

(22) Warshel, A. *J. Biol. Chem.* **1998**, *273*, 27035.

(23) Garcia-Viloca, M.; Gao, J.; Karplus, M.; Truhlar, D. G. *Science* **2004**, *303*, 186.

(24) Marti, S.; Andres, J.; Moliner, V.; Silla, E.; Tunon, I.; Bertran, J. *Theo. Chem. Acc.* **2001**, *105*, 207.

(25) Garcia-Viloca, M.; Truhlar, D, G.; Gao, J. L. *J. Mol. Bio.* **2003**, *327*, 549.

(26) Bjelic, S.; Aqvist, J. *Biochemistry* **2006**, *45*, 7709.

(27) Kienhofer, A.; Kast, P.; Hilvert, D. *J. Am. Chem. Soc.* **2003**, *125*, 3206.

(28) Wolfenden, R.; Snider, M. J. *Acc. Chem. Res.* **2001**, *34*, 938.

(29) Prakash, M. K.; Marcus, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 15982.

(30) Lu, H. P.; Xie, X. S. *Nature* **1997**, *385*, 143.

(31) Vallee, R. A. L.; Tomczak, N.; Kuipers, L.; Vansco, G. J.; van Hulst, N. F. *Chem. Phys. Lett.* **2004**, *384*, 5.

(32) Chandler, D. *Introduction to modern statistical mechanics;* Oxford University Press: New York, 1987.

(33) Hsu, C. P.; Song, X. Y.; Marcus, R. A. *J. Phys. Chem. B* **1997**, *101*, 2546.

(34) Jansson, H.; Bergman, R.; Swenson, J. *J. Phys. Chem. B* **2005**, *109*, 24134.

(35) Mijovic, J.; Bian, Y.; Gross, R. A.; Chen, B. *Macromolecules* **2005**, *38*, 10812.

(36) Havriliak, S.; Negami, S. *Polymer* **1967**, *8*, 161.

(37) Careri, G.; Consolini, G. *Phys. Rev. E* **2000**, *62*, 4454.

(38) Taylor, K. M.; van der Weide, D. W. *IEEE Trans. Microw. Theory Tech.* **2005**, *53*, 1576.

(39) Dyre, J. C. *Europhys. Lett.* **2005**, *71*, 646.

(40) Reichardt, C. *Solvents and solvent effects in organic chemistry;* Wiley-VCH: New York, 2003.

(41) Abate, J.; Valko, P. P. *Int. J. Numer. Methods Eng.* **2004**, *60*, 979. The *Mathematica* routine developed by Abate and Valko is available at http://library.wolfram.com/infocenter/MathSource/5026/.

(42) Lindsey, C. P.; Patterson, G. D. *J. Chem. Phys.* **1980**, *73*, 3348.

(43) Jordanides, X. J.; Lang, M. J.; Song, X. Y.; Fleming, G. R. *J. Phys. Chem. B* **1999**, *103*, 7995.

(44) Simonson, T. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 6544.

(45) Frisch, U.; Bourret, R. *J. Math. Phys.* **1970**, *11*, 64.

(46) Kou, S. C.; Xie, X. S. *Phys. Rev. Lett.* **2004**, *93*, 180603.

(47) de la Fuente, M. R.; Jubindo, M. A. P.; Solier, J. D.; Tello, M. J. *J. Phys. C* **1985**, *18*, 6547.

(48) Weron, K.; Kotulski, M. *Physica A* **1996**, *232*, 180.

(49) Coffey, W. T.; Kalmykov, Yu. P.; Titov, S. V. *J. Chem. Phys.* **2002**, *116*, 6422.

(50) Zwanzig, R. *Acc. Chem. Res.* **1990**, *23*, 148.

(51) McCammon, J. A.; Harvey, S. C. *Dynamics of Proteins and Nucleic Acids*; Cambridge University Press: New York, 1987.

(52) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265.

(53) Debnath, P.; Min, W.; Xie, X. S.; Cherayil, B. J. *J. Chem. Phys.* **2005**, *123*, 204903.

(54) Tang, J.; Marcus, R. A. *Phys. Rev. E* **2006**, *73*, 022102.