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The effect of harmonic conformational trajectories on protein fluorescence and lifetime distributions

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Proteins change their structure constantly due to their conformational flexibility and dynamic nature. The structure of the molecule follows a trajectory in conformational space determined by the hierarchy of conformational substates of the protein. These conformational trajectories may differ in the ground and excited states. The molecule constant structural changes exposes the fluorescent protein residues to continuously mutating environments in a predetermined sequence given by the molecule conformational path. The absorption extinction coefficient and the emission decay rate of a given fluorescent residue, dependent upon the instantaneous localized environment of the residue, are modulated by the conformational dynamics of the molecule. A simple harmonic conformational trajectory describes the behavior of traditional, time invariant, fluorescence lifetime distributions with temperature. The lifetime distribution describes at any given time the distribution of excited residues in the protein conformational space. Consequently, dynamic conformational trajectories require the use of time dependent lifetime distributions. The evolution of uniform lifetime distributions modulated by harmonic conformational trajectories are illustrated. The results presented here open the way to the description of complex conformational dynamics in terms of structural harmonic fluctuations.

I. INTRODUCTION

Traditionally, the temporal fluorescence of proteins is assumed to decay as the sum of few exponential functions. The physical interpretation being that the number and relative contribution of the exponential components can be related to the number and quantity of the different species present in the protein sample under examination. For example, a double exponential fit of the decay of a protein containing a single fluorophore has been assumed to suggest the existence of two conformations of the protein. However, the inference regarding a number of unique conformations, in this instance two, is biased by the assignment of a bi-exponential decay and by the experimental uncertainty. It is usually difficult to rationalize the physical structure of the protein with the existence of few (two to four) unique conformations even for the indole moiety only, especially in view of experimental evidence of the dynamic nature of the protein molecule.[1-3] The nonexponential and complex decay of the fluorescence from single tryptophan residue proteins, in the context of the dynamic nature of the protein molecule, has led to the formulation of distributions of exponential functions (fluorescence-lifetime distributions)[4-10] to account for the temporal fluorescence of single fluorescent residue proteins. The physical interpretation being that the amplitude and lifetime of each exponential in the continuous distribution is related to the set of conformational states and dynamics of the protein molecule. The use of arbitrary, time invariant, lifetime distributions like Gaussian, Lorentzian and rectangular functions has provided a measure of the complexity of the protein fluorescence decay. The derivation of fluorescence lifetime distribution functions in terms of residue-protein densities of energy states has correlated the complexity of the decay, or width of the lifetime distribution function, with the residue flexibility in the protein.[9] The higher the density of energy states, the higher the residue conformational flexibility, and the broader the lifetime distribution. Tryptophan residues with high degree of conformational flexibility yield wider lifetime distributions.[9,10]

The exponential model, both discrete and continuous, does not take into consideration any dependence of the hierarchical nature of the dynamics of the protein molecule. In accordance with the conformational hierarchy of the molecule, the protein must follow a path (or perhaps many paths) in conformational space to travel between major conformational states.[11-18] The path is determined by the hierarchy of the protein conformational substates.

It has been shown that both, the absorption[19] and the fluorescence[10,20-26] of the protein residues are dependent on the residue localized environment. Conformational trajectories connect the absorption and emission characteristics of the protein fluorescent residues to the dynamics of the molecule as presented in this article. This paper focuses on the study of excited protein samples undergoing harmonic conformational fluctuations. It is shown that the effect of a conformational trajectory becomes relevant if the fluorescent residue excited state lifetime is dependent on conformational coordinate. The frequency of the dynamic fluctuations modify the fluorescence decay curve only when the fluctuation period is of the same order of magnitude, or longer, than the natural fluorescence lifetime of the protein residue. It is also shown that the effect of conformational trajectories become even more relevant if the residue absorption of excitation is also conformational coordinate dependent. The results of this paper will allow the representation of complex con-

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formational dynamics of proteins as superpositions of harmonic structural fluctuations.

II. THEORY

Let us consider the case of a fluorescent residue in a protein matrix which fluctuates in a continuum conformational space, in the ground state, denoted by **R**. Let **r** be a particular location or conformational coordinate of the residue-protein-matrix system in **R**. Similarly, let **R*** be the conformational space of the system in the excited state and **r*** be its location in **R***. Depending upon the nature of the particular system under study, **R** and **R*** may (or may not) be the same, or may (or may not) overlap.

At the time of excitation $t=0$ the residue-protein-matrix system changes conformational spaces from **R** to **R*** and conformational location from **r**(0) to **r***(0). Once the system returns to the ground state its dynamics also returns to **R**. In what follows assume that **R*** and **r*** map into **R** and **r**, such that for each excited state conformational coordinate corresponds a ground state conformational coordinate.

The system probability of survival in the excited state when excited into conformation $\mathbf{r}^*(0)=\mathbf{r}_0^*$, denoted by $\mathscr{N}(t,\mathbf{r}_0^*)$, is determined by

$$-\frac{d\mathscr{N}(t,\mathbf{r}_0^*)}{dt}=K(\mathbf{r}^*)\mathscr{N}(t,\mathbf{r}_0^*),\quad \mathbf{r}^*(t), \tag{1}$$

where $K(\mathbf{r}^*)$ is the decay rate of the system in conformational coordinate **r***. Equation (1) states that (i) the conformation of the excited residue changes with time and (ii), its decay rate constant is a function of conformational coordinate $K(\mathbf{r}^*)$. $\mathscr{N}(t,\mathbf{r}_0^*)$, given by

$$\mathscr{N}(t,\mathbf{r}_0^*)=\mathscr{N}(0,\mathbf{r}_0^*)e^{-\int_0^t K(\mathbf{r}^*(t))dt} \tag{2}$$

is the residue probability of remaining in the excited state at time t and conformational location $\mathbf{r}^*(t)$, when excited at $t=0$ at location **r**(0). Similarly, $\mathscr{N}(0,\mathbf{r}_0^*)$ yields the probability of creating an excited the residue at location $\mathbf{r}_0^*$. It is proportional to the residue absorption cross-section σ (or extinction coefficient ϵ) in the protein matrix given by:

$$\mathscr{N}(0,\mathbf{r}_0^*)=\sigma(\mathbf{r}_0)\frac{E}{A}=\epsilon(\mathbf{r}_0)\frac{E}{A}\ln(10), \tag{3}$$

where $\sigma(\mathbf{r}_0)$ (or $\epsilon(\mathbf{r}_0)$) is a function of the ground state conformational coordinate of the fluorescent residue in the protein at the time of excitation. E is the average number of excitation photons per unit time distributed uniformly over the beam area A. Alternatively, $\mathscr{N}(0,\mathbf{r}_0^*)$ also represents the probability of creating an excited residue in location $\mathbf{r}^*(0)$ along the excited state conformational trajectory $\mathbf{r}^*(t)$ in **R***:

$$\mathscr{N}(0,\mathbf{r}_0^*)=\sigma^*(\mathbf{r}_0^*)\frac{E}{A}, \tag{4}$$

where $\sigma^*(\mathbf{r}_0^*)$ is the cross section of producing an excited residue in conformational location $\mathbf{r}_0^*$ at the time of excitation.

The probability of emitting a photon by our excited residue $\mathscr{I}(t,\mathbf{r}_0^*)$ at a given time t and location $\mathbf{r}^*(t)$, when excited at time zero into location $\mathbf{r}^*(0)$, is proportional to $\mathscr{P}(t,\mathbf{r}_0^*)$.

$$\mathscr{I}(t,\mathbf{r}_0^*)\propto\mathscr{P}(t,\mathbf{r}_0^*) \tag{5}$$

$\mathscr{I}(t,\mathbf{r}_0^*)$ is also proportional to the radiative component of the decay rate at conformational coordinate $\mathbf{r}^*(t)$. At each location in **R*** the overall decay $K(\mathbf{r}^*)$ is determined by the superposition of all radiative and all nonradiative processes with overall decay rate functions $K_{\text{rad}}(\mathbf{r}^*)$ and $K_{\text{n-}rad}(\mathbf{r}^*)$ respectively, such that

$$K(\mathbf{r}^*)=K_{\text{rad}}(\mathbf{r}^*)+K_{n\text{-rad}}(\mathbf{r}^*). \tag{6}$$

Non-radiative processes increase the value of $K(\mathbf{r}^*)$. K_{rad} is, in this discussion, a constant determined by the minimum value of $K(\mathbf{r}^*)$:

$$K_{\text{rad}}=K(\mathbf{r}^*)_{\min}=\text{const.} \tag{7}$$

The excited residue relative quantum yield Q_{rel} in the protein, as a function of conformation, is given by the following ratio

$$Q_{\text{rel}}=\frac{K(\mathbf{r}^*)_{\min}}{K(\mathbf{r}^*)} \tag{8}$$

and its luminescence as a function of time, when excited into location $\mathbf{r}^*(0)$, is then

$$\mathscr{I}(t,\mathbf{r}_0^*)=K_{\text{rad}}\mathscr{P}(t,\mathbf{r}_0^*). \tag{9}$$

Consider the fluorescence from a single residue in a protein sample. Alternatively, consider the fluorescence from a single fluorescent residue protein (e.g., single tryptophan residue samples). The luminescence observed is given by the superposition of excited residues, excited at time zero, distributed throughout the entire conformational trajectory traveled by the particular protein matrix-residue system in **R**. The density of ground state residues along **r**, $\rho(\mathbf{r})$ is inversely proportional to the residue speed $|\mathbf{v}|=v$ at location **r**:

$$\rho(\mathbf{r})\propto\frac{1}{v},\quad v=\frac{d|\mathbf{r}|}{dt}. \tag{10}$$

Let $\rho(\mathbf{r}_0)$ be the density of ground state residues in **R** at the time of excitation where **r**(0) is denoted by $\mathbf{r}_0$. Let $\rho^*(\mathbf{r}_0^*)$ be the corresponding excited residue density in **R*** such that

$$\rho(\mathbf{r}_0)d\mathbf{r}_0=\rho^*(\mathbf{r}_0^*)d\mathbf{r}_0^*. \tag{11}$$

The normalized natural luminescence of a single residue in a protein is then determined by

$$I(t)=\frac{\int_{\mathbf{r}_0^*}\mathscr{I}(t,\mathbf{r}_0^*)\rho^*(\mathbf{r}_0^*)d\mathbf{r}_0^*}{\int_{\mathbf{r}_0^*}\rho^*(\mathbf{r}_0^*)d\mathbf{r}_0^*} \tag{12}$$

where the integral extends over all the excited state residue conformations excited at time zero $\mathbf{r}_0^*$. In cases when the residue-protein-matrix is excited along the entire ground state conformational trajectory Eq. (12) can be written in the following form:

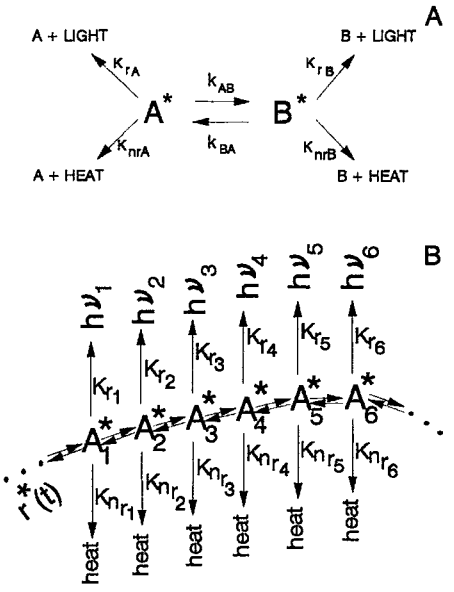

FIG. 1. (A) Discrete state systems assume residues in the protein matrix fluctuating between discontinuous conformational states (two shown). The residue decays only when it reaches one of the separate conformational states regardless of the interconversion time. The equilibrium constant determines the relative state occupation. (B) Conformational trajectories assume a continuum of states with negligible time of travel between adjacent conformations. The excited residue may decay at each instant and conformational location along its dynamic trajectory. The relative density of conformational occupation is determined by the localized conformational speed.

$$I(t)=\frac{\oint_{\mathbf{r}^*}\mathscr{T}(t,\mathbf{r}_0^*)\rho^*(\mathbf{r}_0^*)d\mathbf{r}_0^*}{\oint_{\mathbf{r}^*}\rho^*(\mathbf{r}_0^*)d\mathbf{r}_0^*}. \tag{13}$$

Similarly, the overall fluorescence decay of a protein sample with N fluorescent residues, each of them with excited state trajectories $\mathbf{r}_i^*$ in their respective $\mathbf{R}_i^*$, with conformational dependent decay rate functions $K(\mathbf{r}_i^*)$ and initial densities of ground state residues $\rho_i^*(\mathbf{r}_{0,i}^*)$, is given by

$$I(t)=\frac{\sum_{i=1}^{N}\int_{\mathbf{r}_{0_i}^*}\mathscr{T}(t,\mathbf{r}_0^*)_i\rho_i^*(\mathbf{r}_{0,i}^*)d\mathbf{r}_{0i}^*}{\sum_{i=1}^{N}\int_{\mathbf{r}_{0_i}^*}\rho_i^*(\mathbf{r}_{0,i}^*)d\mathbf{r}_{0,i}^*}, \tag{14}$$

where $\rho_i^*(\mathbf{r}_{0,i}^*)$ is the density of excited residues along $\mathbf{r}^*$ created at the time of excitation and $\mathbf{r}_{0_i}^* = \mathbf{r}^*(0)_i$.

Knowledge of $\mathscr{P}(0,\mathbf{r}_0^*)$, $K(\mathbf{r}^*)$ and $\mathbf{r}_i^*(t)$ is sufficient, for a given protein, to characterize the temporal fluorescence of its samples in terms of the protein excited state molecular dynamics. The evolution of the excited residue ensemble in time can also be determined given $\mathscr{P}(0,\mathbf{r}_0^*)$, $K(\mathbf{r}^*)$ and $\mathbf{r}_i^*(t)$, as illustrated in a subsection below.

Discrete state systems versus conformational trajectories. The discrete state systems, like the two state system illustrated in Fig. 1(A), assumes the residue in the protein matrix fluctuating between discrete conformational states. The residue may decay radiatively or nonradiatively only when it reaches one of the discrete states, regardless of the interconversion time taken between states. The residue relative occupation state is determined by the equilibrium constant.

Conformational trajectories may be regarded as a continuous state system with negligible time of travel between adjacent conformations as illustrated in Fig. 1(B). Conformational trajectories allow the excited residue to decay at each instant and conformational location along its dynamic trajectory. The equilibrium relative density of conformational occupation, in this case, is determined by the localized conformational speed.

III. THE FIRST ORDER APPROXIMATION

Let us consider the case in which the natural decay rate of the excited residue $\mathscr{K}_0$ is perturbed by its location in the conformational trajectory $\mathbf{r}^*$:

$$K(\mathbf{r}^*)=\mathscr{K}_0+\mathscr{K}_1 r^*+\mathscr{K}_2 r^{*2}+\mathscr{K}_3 r^{*3}+\cdots, \tag{15}$$

where r^* is the magnitude of $\mathbf{r}^*$ ($r^*=|\mathbf{r}^*|$) and $\mathscr{K}_1,\mathscr{K}_2,\mathscr{K}_3,\ldots$, are the coefficients of the Taylor series expansion of $K(\mathbf{r}^*)$. For small perturbations $K(\mathbf{r}^*)$ can be well approximated by the linear term

$$K(\mathbf{r}^*)\simeq\mathscr{K}_0+\mathscr{K}_1 r^*. \tag{16}$$

$\mathscr{K}_1 r^*$ modifies the first order differential equation that yields the exponential function. [Eq. (1)].

IV. DECAY RATES PERTURBED BY EQUILIBRIUM HARMONIC FLUCTUATIONS

Consider the case of an excited residue that fluctuates in the protein excited state conformational space $\mathbf{R}^*$ undergoing, on the average, a harmonic motion around an equilibrium conformational coordinate $\mathbf{r}_{eq}^*$ given by

$$\mathbf{r}^*(t)=\mathbf{r}_{eq}^*+\mathbf{r}_a^*\cos(\phi^*-\omega t), \tag{17}$$

where ϕ^* establishes the location of the residue at the time of excitation $\mathbf{r}^*(0)$. $\mathbf{r}_{eq}^*$ and $\mathbf{r}_a^*$ give the equilibrium coordinate and the amplitude of the fluctuation, respectively. $\omega=2\pi/P$ is the effective circular frequency of the excited residue fluctuation at the temperature of the sample. P is the fluctuation period. $\mathbf{r}^*(t)$ describes a residue moving in the direction of increasing ϕ^* in Eq. (17). We do not need to be concerned with trajectories in the direction of decreasing ϕ^* at this point. Eq. (16) yields

$$K(\mathbf{r}^*)=K_0+K_1\cos(\phi^*-\omega t), \tag{18}$$

where

$$K_0=\mathscr{K}_0+\mathscr{K}_1 r_{eq}^* \quad \text{and} \quad K_1=\mathscr{K}_1 r_a^* \tag{19}$$

such that $r_{eq}^*=|\mathbf{r}_{eq}^*|$ and $r_a^*=|\mathbf{r}_a^*|$. Consequently,

$$K(\mathbf{r}^*)_{\min}=\mathscr{K}_0+\mathscr{K}_1(r_{eq}^*-r_a^*)>0. \tag{20}$$

Equation (1) then becomes

$$-\frac{d\mathscr{P}(t,\phi^*)}{dt}=[K_0+K_1\cos(\phi^*-\omega t)]\mathscr{P}(t,\phi^*). \tag{21}$$

The term $K_1\cos(\phi^*-\omega t)$ in Eq. (21) constitutes a perturbation to the first order differential equation that yields the exponential function. K_1, ω, and ϕ^* characterize such a per-

turbation. The probability of survival $\mathscr{P}(t,\phi^*)$ at a later time t, for the residue excited in conformational coordinate ϕ^* at time $t=0$, is given by

$$\mathscr{P}(t,\phi^*)=\mathscr{P}(0,\phi^*)\exp-(K_0t+(K_1/\omega)[\sin(\phi^*) - \sin(\phi^*-\omega t)]). \quad (22)$$

$\mathscr{P}(0,\phi^*)$ is the probability distribution function of creating a residue at location ϕ^* along the harmonic trajectory $\mathbf{r}^*$ of Eq. (17).

The probability of this particular residue of emitting a photon $I(t,\phi)$ is then given by

$$I(t,\phi)=K_{\rm rad}(\mathbf{r}^*)\mathscr{P}(t,\phi^*). \quad (23)$$

The relative quantum yield of the excited residue in the harmonic trajectory of Eq. (17) is given by

$$Q_{\rm rel}(\phi^*)=\frac{K_0-K_1}{K_0+K_1\cos\phi^*} \quad (24)$$

and

$$I(t,\phi)=(K_0-K_1)\mathscr{P}(t,\phi^*). \quad (25)$$

The residue distribution or density in phase space, for harmonic fluctuations (ω=constant), is constant throughout the coordinate ϕ^* (or ϕ):

$$\rho^*(\phi^*)=\rho(\phi)=\frac{1}{2\pi}. \quad (26)$$

Equation (12) yields the following result:

$$I(t)=\frac{K_0-K_1}{2\pi}e^{-K_0t}\int_0^{\pi}\mathscr{P}(0,\phi^*)(\exp-(K_1/\omega)[\sin\phi^* - \sin(\phi^*-\omega t)]+\exp-(K_1/\omega)[-\sin\phi^* + \sin(\phi^*+\omega t)])d\phi^*. \quad (27)$$

The two exponential functions in Eq. (27) correspond to residues moving in the directions of increasing and decreasing ϕ^*, respectively. $\mathscr{P}(0,\phi^*)$ is dependent only on conformational coordinate and not on direction of conformational travel of the excited residue-protein-matrix system. This assumption is based on the fact that the absorption process is much faster than any molecular conformational fluctuations. The protein molecule may be regarded as static during the absorption process.

Equation (27) explains the nonexponential nature of protein fluorescence and the behavior of time invariant fluorescence lifetime distributions of proteins with temperature.[10] Such behavior is characterized by

(1) the observed reduction of the average value of protein fluorescence lifetime distributions with increasing temperatures;
(2) the decrease of the lifetime distribution width at higher temperatures;
(3) the improvement of the distribution fit goodness as temperature increases.

Consider the following limits of Eq. (27):

(1) When the lifetime of the residue becomes independent of conformational coordinate ($\mathscr{K}_1=0$) then Eq. (27) reduces to a single exponential decay with the natural (longest lived) lifetime of the residue:

$$I(t)=\left(\frac{\mathscr{K}_0}{2\pi}\oint_{\phi^*}\mathscr{P}(0,\phi^*)d\phi^*\right)e^{-\mathscr{K}_0t}. \quad (28)$$

(2) When the dynamics of the system becomes much faster than the lifetime of the excited residue $\omega\gg 2\pi K_{\max}$ (or $P\ll 1/K_{\max}$) Eq. (27) reduces to a short lived single exponential

$$I(t)=\left(\frac{K_0-K_1}{2\pi}\oint_{\phi^*}\mathscr{P}(0,\phi^*)d\phi^*\right)e^{-(\mathscr{K}_0+\mathscr{K}_1r^*_{\rm eq})t}. \quad (29)$$

Equation (29) states that in the limit when the temporal window of the fluorescence becomes too long compared to the dynamics of the protein, the residue fluorescence decay becomes insensitive to the dynamics of the protein. A related effect has been observed in the study of the fluorescence of single tryptophan residue proteins in terms of fluorescence lifetime distributions: in some instances, for sufficiently high temperatures, the width of the lifetime distribution becomes indistinguishable from the width of a single exponential.[8,10]

(3) In the limit when the dynamics of the system is turned off $\omega\to 0$ (frozen sample) Eq. (27) produces the most complex decay with relative long lived average lifetimes:

$$I(t)=\frac{K_0-K_1}{2\pi}\oint_{\phi^*}\mathscr{P}(0,\phi^*)e^{-K_1t\cos\phi^*}d\phi^*. \quad (30)$$

In terms of fluorescence lifetime distributions, Eq. (30) yields the broadest distributions with the longest average lifetime value and the worst fit goodness, as observed in the case of single tryptophan residue proteins.[10]

Figure 2 illustrates the above results with the particular values of $K_0=0.20$ ($\tau_0=5$ ns) and $K_1=0.18$ ($\tau_1=5.56$ ns). With these values the residue excited state lifetime varies between 2.6 ns and 50 ns throughout the harmonic trajectory. In Fig. 2 all residues are excited with equal probability [$\mathscr{P}(0,\phi^*)$=constant] throughout the conformational trajectory $\mathbf{r}^*(t)$ of Eq. (17). Figure 2(A) shows the Gaussian lifetime distribution when $P=2\pi/\omega=5$ ns. The decay for $P=5$ ns approaches the single exponential given by Eq. (28). Faster dynamics (shorter values of P) yield this exponential decay. The decay curve begins to deviate from the exponential function with decreasing dynamic speeds. Figure 2(B) shows the gaussian lifetime distribution obtained when the effective harmonic fluctuation period is 50 ns. In this case the average lifetime obtained is 8.65 ns with a width of 5.77 ns. The duration of the transient fluorescence increases with decreasing dynamics until the limit is reached in which the dynamic period is about two orders of magnitude longer than $1/K_0$. Figure 2(C) shows the lifetime distribution obtained when the effective harmonic fluctuation period is 500 ns. In this case the average lifetime obtained is 16.5 ns with a width of 26.7 ns. The decay curve remains invariant for

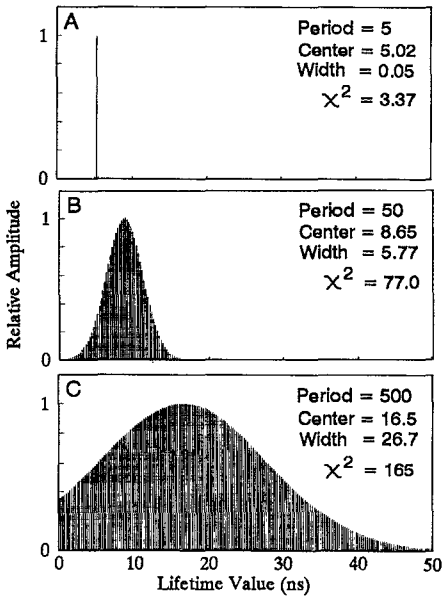

FIG. 2. Gaussian lifetime distributions of a residue undergoing harmonic structural fluctuations. The distribution center, width and χ^2 increase with decreasing fluctuation periods. The same behavior is observed in the lifetime distributions of protein fluorescence with temperature.

larger values of P. Figure 2 also presents the relative values of the χ^2 fits of Gaussian distributions to fluorescence decays modulated by harmonic fluctuations [Eq. (27)].

Figure 3 shows the behavior of typical symmetric (Gaussian, Lorentzian and uniform) lifetime distribution fits to the excited residue-protein matrix harmonic oscillator of Eq. (27). Figure 3(A) shows the average value of the distributions as functions of the oscillator period P. Figure 3(B) shows the behavior of the distribution widths as functions of P. Figure 3(C) shows the relative goodness of these symmetric distribution fits (χ^2) as functions of the oscillator dynamics.

The behavior of lifetime distributions of harmonic conformational fluctuations resembles the behavior of fluorescence lifetime distributions of single tryptophan residue proteins.[4,10]

The effect of conformation dependent extinction coefficients. The absorption of excitation is in general coupled to the localized environment of organic molecules. This has been demonstrated by the difference between the absorption spectra of proteins in the native and denaturated states.[19]

In turn, the dependence of $\mathscr{P}(0,r_0^*)$ with conformational coordinate also affects the protein fluorescence decay curve as illustrated in Fig. 4 in the case of the harmonic conformational trajectory. Figure 4 illustrates three extreme cases. Curve A shows a decay in which the fluorescent residues are excited with equal probability [$\mathscr{P}(0,\phi^*)$=constant] throughout the harmonic conformational trajectory of Eq. (17). Curve B shows the decay curve when the residue can only be excited at one point, e.g., $\mathbf{r}^* = \mathbf{r}_{eq}^* + \mathbf{r}_a^*[\mathscr{P}(0,\phi^*) = \delta(\phi^* - 0)]$. Curve C illustrates the decay when the sample residues are excited uniformly along a limited path of the trajectory, e.g., $2\pi/5<\phi^*<4\pi/5$. No excitation takes place outside of these values. An unperturbed exponential

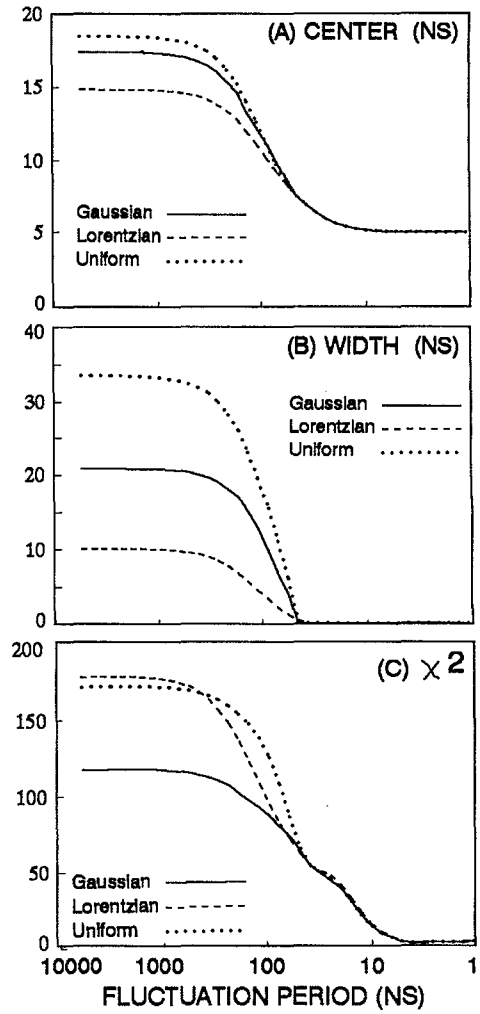

FIG. 3. Behavior of Gaussian, Lorentzian and uniform lifetime distributions as functions of the oscillation period of harmonic conformational fluctuations. (A) Distribution average values, (B) distribution widths, and (C) relative χ^2 of distribution fits.

decay is shown in the figure for reference. In general, the excitation distribution function $\mathscr{P}(0,\phi^*)$ [or more generally $\mathscr{P}(0,\mathbf{r}_0^*)$ in Eq. (2)] determines the form of the protein fluorescence decay provided, (i) the excited residue lifetime is dependent on protein conformation and (ii) the dynamic speed of the protein molecule fluctuations is of the same order of magnitude or longer than the fluorescence lifetime.

V. MOLECULAR DYNAMICS, FLUORESCENCE AND LIFETIME DISTRIBUTIONS

A lifetime distribution, created at the time of excitation, provides a measure of the environment diversity of the initial ensemble of excited residues. The shape of the distribution may remain invariant in the case of "frozen" samples that do not significantly alter their characteristics during the fluorescence process. However, in the most general case, the initially excited residue population will evolve during the excited state lifetime. The path of this evolution is determined

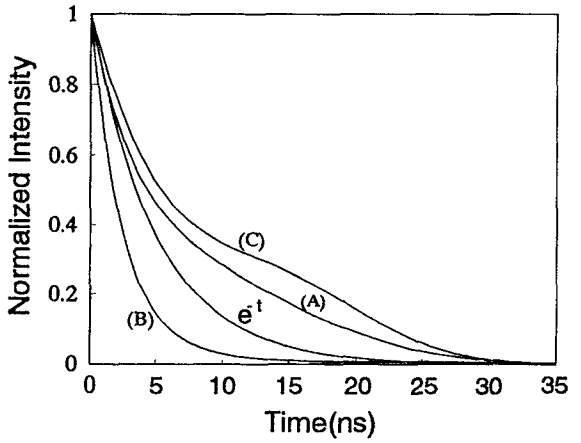

FIG. 4. Illustration of the dependence of the fluorescence decay curve on excitation absorption. (A) Decay curve of fluorescent residues excited with equal probability [$\mathcal{I}(0,\phi^*)$=constant] throughout the harmonic conformational trajectory. (B) Decay curve of residue excited at one point $\mathbf{r}^* = \mathbf{r}^*_{eq} + \mathbf{r}^*_a(\mathcal{I}(0,\phi^*) = \delta(\phi^* - 0))$. (C) Decay of residues uniformly excited along a limited path of the trajectory: $2\pi/5<\phi^*<4\pi/5$. A exponential decay is shown in the figure for reference. In the figures K_0=0.20 (τ_0=5 ns) and K_1=0.18 (τ_1=5.56 ns).

by the conformational trajectories and velocities followed by the excited residue ensemble in the protein matrix. The concept of mutating lifetime distributions due to hierarchical conformational dynamics is illustrated in Figs. 5 and 6. These figures show the evolution of a sample initially excited

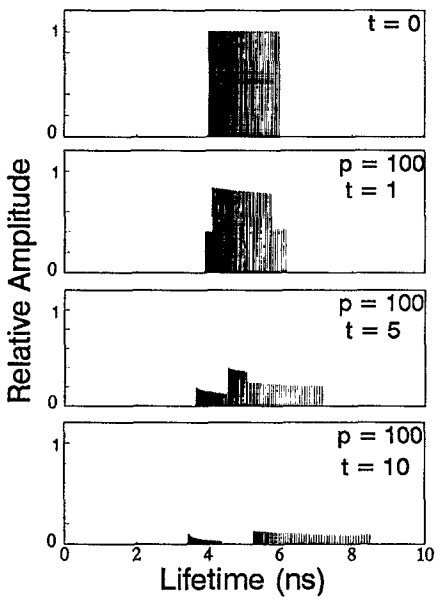

FIG. 5. Time variant lifetime distributions of harmonic conformational trajectory with 100 ns fluctuation period. A uniform lifetime distribution created a the time of excitation evolves during the residue excited state lifetime due to harmonic conformational dynamics of the protein molecule. The lifetime of each residue in the ensemble is given by its instantaneous conformational location. Distributions are shown at 0, 1, 5, and 10 ns. The relative density of excited residues in ϕ^* space is indicated by the line separation of the distribution in lifetime space.

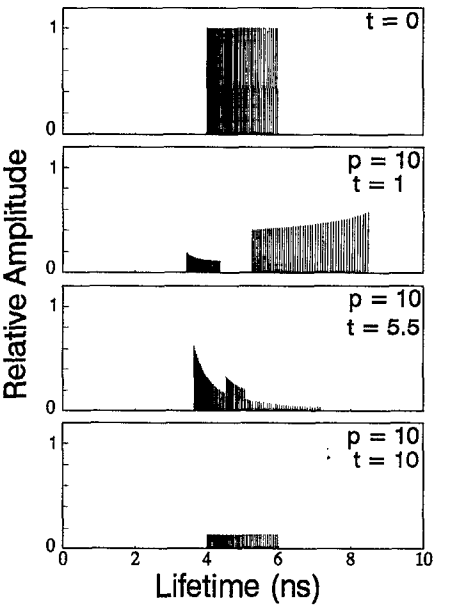

FIG. 6. Time variant lifetime distributions of harmonic conformational trajectory with 10 ns fluctuation period. A uniform lifetime distribution created a the time of excitation oscillates during the residue excited state lifetime. After 10 ns the distribution returns to is uniform (attenuated) shape. Distributions are shown at 0, 1, 5.5, and 10 ns. The relative density of excited residues in ϕ^* is indicated by the line separation of the distribution in lifetime space.

with a uniform lifetime distribution throughout the harmonic conformational trajectory determined by Eq. (17). The initial distribution has a center value of 5 ns and a width of 2 ns. The relative density of excited residues in ϕ^* space is indicated by the line separation of the lifetime distribution in lifetime space. In Fig. 5 the effective oscillation period is 100 ns. The lifetime distribution is given at times 0, 1, 5, and 10 ns, respectively. Similarly, Fig. 6 illustrate the evolution of a uniform distribution following a harmonic conformational trajectory with P=10 ns. The distribution is given at 0, 1, 5.5, and 10 ns. In Figs. 5 the excited state lifetime distribution decays within the duration of the harmonic fluctuation while in Fig. 6 the excited ensemble decays over several oscillations.

The time varying lifetime distributions of Figs. 5 and 6 may be represented in terms of traditional time invariant distributions (e.g., Gaussian, uniform, Lorentzian). In the case of Fig. 5 the result will be a broader distribution with larger average value and with poorer fit goodness than in the case of Fig. 6. The causes of these differences have their origins in the decrease of sensitivity of fluorescence with molecular dynamic speed. This also limits the spatial resolution provided by fluorescence of more complex protein structural fluctuations.[27]

VI. CONCLUSIONS

The concept of conformational fluctuation of the protein assumes that the structural fluctuations of the molecule occur in sequential and hierarchical manner.

The excitation absorption and the fluorescence decay of protein residues, which are modulated by the localized environment of the residue, change continuously as functions of the residue-protein matrix conformational coordinate. Consequently, for a given excited residue, its probability of remaining in the excited state, at any time, is dependent upon the residue previous dynamic history.

The computed fluorescence decay of a protein sample, composed of an ensemble of excited residues undergoing harmonic fluctuations, behaves in accordance with experimental results. Specifically, the behavior of fluorescence lifetime distributions with temperature, the dependence of protein excitation absorption, and fluorescence decay dependent upon protein residue environment are in agreement with the models here presented.

Lifetime distributions, in this view, are representations of the conformational coordinate occupancy of the excited residue population at a given time. Therefore, protein conformational trajectories in the excited state imply time variant fluorescence lifetime distributions in which not only the distributions amplitudes but also the distribution lifetime values (e.g., average value and width) change during the time span of the fluorescence.

The results of this paper will allow the representation of complex conformational dynamics of proteins as superpositions of harmonic structural fluctuations.

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