Energy Transfer Efficiency Distributions in Polymers in Solution During Folding and Unfolding

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Abstract

Distribution of fluorescence resonance energy transfer (FRET) efficiency between the two ends of a Lennard-Jones polymer chain both at equilibrium and during folding and unfolding has been calculated, for the first time, by Brownian dynamics simulations. The distribution of FRET efficiency becomes bimodal during folding of the extended state subsequent to a temperature quench, with the width of the distribution for the extended state broader than that for the folded state. The reverse process of unfolding subsequent to an upward temperature jump shows different characteristics. The distributions show significant viscosity dependence which can be tested against experiments.

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1 Introduction

Dynamics of polymer folding and unfolding in solution is a problem of much current interest. [1, 2, 3] Although some aspects of polymer folding appears to be understood, detailed experimental study of the folding scenario (especially the initial part) is still not available. Given the complexity of the problem, the computer simulations could consider only relatively simple models, such as a necklace of Lennard-Jones or square-well beads. In addition to its own intrinsic interest, the collapse of polymers from poor solvents has served as a theoretical model of protein folding in the early stages. [4, 5, 6, 7, 8, 9, 10, 11, 12]

In a notable recent development, fluorescence resonance energy transfer (FRET) has been combined with single molecule spectroscopic (SMS) technique to provide a powerful novel approach to study the dynamics of folding. Deniz et al, [13] reported studies of dynamics of protein folding by observing FRET in time domain from a single donor-acceptor (D-A) pair. In the measurement of Deniz et al, the subpopulations [13, 14, 15, 16, 17] of the folded and denatured states of the protein chymotrypsin inhibitor 2 (CI2) were obtained as the concentration of the denaturant (guanidinium chloride) was varied. The most interesting result was that at the intermediate concentration of the denaturant, the distribution of Forster efficiency becomes bimodal. It was concluded that this bimodal distribution is the signature of the "two state" nature of CI2 folding transition. We were curious to know whether such a bimodal distribution can be observed (a) for simple models like collapsing homopolymers and (b) whether it can originate from dynamics alone. To answer
these questions, we have carried out extensive Brownian dynamics (BD) simulations of FRET in single macromolecules. We find, surprisingly, that even such a simple system shows a bimodal distribution in the excitation transfer efficiency, for chosen sets of parameter values. The double peak is similar to the ones observed in experiments.\cite{13} The bimodal distribution is observed not only during folding but also at equilibrium near the $\theta$ temperature. The results obtained here should help in designing future experiments.

The usually assumed mechanism for FRET is the Forster energy transfer (FET).\cite{18,20} The rate of this transfer depends on the separation ($R$) between the energy donor and the energy acceptor. This rate, $k(R)$, can be written as\cite{18},

$$k(R) = k_F \left(\frac{R_F}{R}\right)^6$$  \hspace{1cm} (1)

where $R_F$ is the Forster radius and $k_F$ is the radiative rate, which is typically in the range $10^8$ to $10^9$ sec$^{-1}$ for the commonly used chromophores. The rate of energy transfer becomes equal to $K_F$ when $R = R_F$. For a given D–A pair, $R_F$ can be obtained by the usual method of overlap between the fluorescence and the absorption spectra of the D-A pair.\cite{19} For commonly used chromophores, $R_F$ is fairly large, often as large as 50Å. This means that the rate is very large when the donor-acceptor pair is separated by a short distance. This may actually be a limitation of the Forster expression which is strictly valid when the separation between the $D–A$ pair is much larger than their size. However, the above limitation shall have a minor effect in the present study which is qualitative in nature and aims at exploring the general aspects of the energy transfer efficiency distribution during folding.
In writing Eq.(1), the standard averaging over the orientations of the transition dipole moments has been carried out. In standard FRET experiments, the macromolecule is doped with a D-A pair in suitable locations along the chain.20 Excitation transfer can be monitored by following the fluorescence either from the donor or the acceptor or from both. The time, $\tau_{rxn}$, taken for the excitation transfer to occur depends strongly on the D-A separation $R$, as given by $k(R)$ in Eq.(1). For a polymer (or a protein) in solution, both at equilibrium and during folding/unfolding, $R$ is not only a fluctuating, stochastic function of time, but also varies in a definite way. In such cases, the distribution of the energy transfer efficiency contains non-trivial and useful information. Note that simulating FRET in real proteins is an exceedingly difficult problem – it is not trivial even for a simple homopolymer – a problem that has eluded theoretical description even today.

We define the FRET efficiency ($\Phi_F$) by the following relation,

$$\Phi_F = \frac{k(R)}{k(R) + k_{rn}},$$

(2)

where $k_{rn}$ is the rate which includes the radiative and non-radiative (other than Forster migration) decay rates of the donor-acceptor pair. We next define the FRET efficiency distribution $P(\Phi_F)$ by the following expression

$$P(\Phi_F) = \frac{1}{N} \sum_{i=1}^{N} \delta(\Phi_F - \Phi_F(\tau_{rxn})).$$

(3)

The above equation is to be understood in the following fashion. After choosing a D-A pair at time $t = 0$, the pair is followed till the trajectory gets terminated due to the reaction. We define this time by $\tau_{rxn}$. At this time, the existing end-to-end distance ($R$) is used in Eq. (2) to calculate $\Phi_F$. This was followed for $N$
independent trajectories for pairs chosen from an equilibrium distribution. At the end we distribute the FRET efficiencies into the bins of width 0.1. In this way, a continuous probability distribution \( P(\Phi_F) \) can be obtained from Eq. (3), by taking the \( N \to \infty \) limit - in our case, we get a histogram (figures 1-4). Similarly, we define the probability distribution of reaction times,

\[
P(\tau_{rxn}) = \frac{1}{N} \sum_{i=1}^{N} \delta(t - \tau_{rxn})
\] (4)

In this paper, we present the calculations of the distribution of \( P(\Phi_F) \) and \( P(\tau_{rxn}) \) for Forster migration among polymer ends both at equilibrium and during folding and unfolding. In next section we describe the model and the simulation details. In section III we present the results. We close the paper with a few conclusions in section IV.

2 Simulation details

2.1 The model

The model polymer chain is made of connected Lennard-Jones (LJ) beads. While this model homopolymer does not represent complex richness of a protein, it is known to show interesting folding kinetics.\[2\] The total potential energy can be written as,\[2\]

\[
U = \sum_{i=2}^{N} \sum_{j=1}^{i-1} u_{L,J}(r_{ij}) + \sum_{i=2}^{N} u_b(| r_i - r_{i-1} |)
\] (5)
where $N$ is the number of beads, $r_i$ is the position of bead $i$, $r_{ij} = |r_i - r_j|$, $u_{LJ}(r)$ is the LJ potential,

$$u_{LJ}(r) = \epsilon \left[ \left( \frac{\sigma}{r} \right)^{12} - \left( \frac{\sigma}{r} \right)^6 \right]$$

(6)

$u_b$ is the bonding potential,

$$u_b = \frac{3\kappa k_b T}{2b^2} \sum (|r_i - r_j| - b)^2$$

(7)

where $\sigma$ and $\epsilon$ are the LJ collision-diameter and the well depth, respectively, $k_BT$ is the thermal energy and $\kappa$ represents the stiffness of the spring. Here, we use $\kappa = 9, N = 80,$ and $b = \sigma$. For convenience, we define $\epsilon^* = \epsilon/k_BT$. The unit of time, $\tau$, is $b^2/D_0$. Thus, $\tilde{k}_F \equiv k_F b^2/D_0$ is also dimensionless. In this study we have chosen $\tilde{k}_F = 1$ and 10. This choice of $\tilde{k}_F$ corresponds to the experimentally observed $k_F$ values, a bit biased towards higher values. For example, in a solvent with viscosity ($\eta$) equal 1 cp, the radius of monomer molecules ($R$) equal to $4A_0$, $\tilde{k}_F = 1$ corresponds to a $k_F$ of 0.76 ns while $\tilde{k}_F = 10$ corresponds to that of 7.6 ns.[21]

$R$ is scaled by $b$, the bead diameter, as usual. In viscous solvents, the viscosity can be much higher, and the $\tilde{k}_F$ can be even larger than 10.

The time evaluation of the polymer chain is done according to the following equation of motion,

$$r_j(t + \Delta t) = r_j(t) + F_j(t)\Delta t + \Delta X^G(t)$$

(8)

where $r_j(t)$ is the position of $j$–th bead at time $t$. and the systematic force on $j$ is denoted by $F_j(t)$. The random Brownian displacement, $\Delta X^G(t)$, is taken from a Gaussian distribution with zero mean and $2\Delta t$ variance. The time step, $\Delta t$, is varied from 0.0001$\tau$ to 0.0005$\tau$. The scheme of Noguchi and Yoshikawa[2] has been used
to investigate the polymer folding. In this method, the equilibrium configuration obtained at $\epsilon^* = 0.1$ is quenched by decreasing the temperature instantaneously to different values of $\epsilon^*$, higher than 0.62 which is the $\theta$ temperature in this model. The time dependent total energy, the root mean square end-to-end distance ($R^2$) and the radius of gyration ($R_g^2$) were all monitored to follow the progress of folding. The results presented here are the average over 10,000 of such trajectories with different initial configuration. More details on the simulation scheme can be found elsewhere.[22] To simulate FRET, we have probed many combinations of $R_F$ and $k_F$. We have selected $R_F = 7$, which is near the maximum in $R^2 P(R)$ (we denote it by $R_0$), where $P(R)$ is the end-to-end distribution. Another important parameter which affects FRET is $k_F$. Large $k_F$ values result in the higher efficiency of FRET. In this study we have mostly dealt with $k_F = 10$.

2.2 Time scales

FRET in polymers involves several different time scales. Two time scales, $\tau_{F\text{fold}}$ and $\tau_{F\text{un}}$, are required to describe the average survival probability of FRET in the folded and unfolded states, respectively.[22] These two time scales are widely separated from each other, due to the sensitivity of survival time to the separation between the two ends. The third relevant time in this problem is $\tau_{q\text{fold}}$, the time required for the polymer to fold subsequent to the quench. For FRET to be useful in the study of folding, this $\tau_{q\text{fold}}$ should be intermediate and well-separated from the other two times. Two additional time scales $k_F^{-1}$ and $b^2/D_0$ come from Forster energy transfer and Brownian dynamics, respectively. While $k_F$ is fixed, $b^2/D_0$ can
be varied by changing viscosity ($\eta$).

3 Results and Discussion

Results for the distribution during folding process (subsequent to the quench from $\epsilon^* = 0.1$ to $\epsilon^* = 0.8$) are shown in figure 1. In this figure the simulated probability distribution of FRET efficiency $P(\Phi_F)$ during folding is plotted at $R_F = 7$ and $\tilde{k}_F = 10$. One sees a clear bimodal distribution in the FRET efficiency. The first peak at low efficiencies arise from the extended state while the one at high efficiencies arise from the folded configurations. Note also that the distribution for the extended state is broad while that from the collapsed state is narrow. This is expected on physical grounds and has been observed in experiments. This bimodality is found to depend critically on the value of $k_{rn}$ which is a consequence of several competing time scales in the FRET.

Results presented in figure 1 can be better understood from figure 2 where we have plotted the equilibrium FRET efficiency distribution in the extended (unfolded) and the collapsed (folded) states, at $R_F = 7$ and $\tilde{k}_F = 10$. It is observed that the time taken for FRET is much larger in the unfolded state (Fig. 2a), compared to that in the folded state (Fig. 2b). This is reflected in the (ensemble averaged) survival probability ($S_p(t)$) of D-A pair (not shown), which is extremely fast in the collapsed state and very slow in the extended state. The reason for the observed (nearly) exponential distribution for the unfolded state (Fig. 2a) lies in the choice of $R_F$. Since maximum probability for end-to-end distance (obtained by maximizing
$R^2P(R)$ is at $R \approx 7.3$, for the given temperature and interaction strength. Thus, at $R_F = 7$, there is significant population already at this distance. If we change $R_F$ to small (like $R_F = 1$) or large (like $R_F = 10$) values, the exponential distribution will be replaced by a Gaussian type distribution. This sensitivity of the distribution to $R_F$ can be exploited in experiments. The distribution of reaction efficiencies in the folded state (Fig. 2b), however, is not exponential. The initial fast fall in the probability is followed by a somewhat slower decay.

Both at high ($\bar{\epsilon} \approx 0.1 - 0.3$) and low temperatures ($\bar{\epsilon} \approx 0.8 - 0.9$) the equilibrium $P(\Phi_F)$ shows a single peak at lower and higher FRET efficiencies, respectively. However, at intermediate temperatures ($\bar{\epsilon} \approx 0.5 - 0.6$), the equilibrium FRET efficiency distribution ($P(\Phi_F)$) again shows a bimodal distribution. This is shown in figure 3, where the equilibrium $P(\Phi_F)$ is plotted at $\bar{\epsilon} = 0.5$. The emergence of bimodality in $P(\Phi_F)$, even at equilibrium, is due to the closeness to the $\theta$ temperature ($\bar{\epsilon}_\theta = 0.62$). This indicates that a "two-state" model exists in this simple system of homopolymer chain, near the $\theta$ temperature.

The above observations seem to provide the following interpretation of the observed bimodality in figure 1. As the polymer collapses subsequent to the quench, the polymer passes through a succession of configurations. The initial configurations correspond to the extended state. The polymer passes through the intermediate state rather fast which shows the dearth of population at intermediate $\Phi_F$, giving rise to the bimodality.

The effect of viscosity ($\eta$) can be studied by varying the dimensionless rate $\tilde{k}_F$,
defined as \( k_F b^2 / D_0 \). At constant \( k_F \), large values of \( \tilde{k}_F \) represent solution of high viscosity and vice versa. In figure 4, \( P(\Phi_F) \) is plotted against the FRET efficiency at two very different values of \( \tilde{k}_F \). Open bars show the result for \( \tilde{k}_F = 1 \), while the filled bars represent that for the \( \tilde{k}_F = 10 \). Figure 4 shows that viscosity can have a dramatic effect on FRET efficiency distribution.

During the unfolding (when the temperature instantaneously raised to \( \epsilon^* = 0.1 \) from \( \epsilon^* = 0.8 \)), \( P(\Phi_F) \) shows a large peak at higher efficiencies which is accompanied by a tail (of smaller peaks) towards lower efficiencies. The probability distribution of reaction times (subsequent to a temperature quench) \( P(\tau_{rxn}) \) also shows a bimodal distribution. We have found that one can efficiently study the dynamics of the initial stages of folding by placing the pair near the chain end where the nucleation of the collapse starts in nearly all the cases that we studied. In this case distribution of the reaction efficiency is not as strongly bimodal as in the case previously mentioned.

We have monitored the variation in the mean square radius and the energy during the polymer folding as a function of time. They show somewhat different behavior. \( \langle R^2 \rangle \) and \( \langle R_g^2 \rangle \) starts decaying only after an initial characteristic time delay. The total energy of polymer chain starts decaying immediately after the quench and continues to do so till it reaches the final stable minimum energy configuration. This is because initial decrease of energy does not require change in \( R_0 \) – it occurs by establishing favorable contacts.
4 Conclusion

To conclude, we have shown in this work that a bimodal distribution of excitation transfer efficiency and of reaction times emerge during folding and unfolding of model homopolymers in solution. The distribution looks surprisingly similar to the ones observed recently in the folding of real proteins by single molecule spectroscopy. The extent of bimodality is found to depend on the values of the Forster parameters ($k_F$ and $R_F$), $k_{rn}$ and the value of the diffusion coefficient (that is, the viscosity). Thus, a study like the one performed here can be useful in designing FRET experiments via single molecule spectroscopy. The present study suggests several exciting future problems. Both for folding and unfolding, one can initiate the FRET process after a suitable time lag ($\tau$) following the quench and can thus obtain a two dimensional distribution of $P(\Phi_F, \tau)$, like in NMR or ESR. FRET may also be able to differentiate between different collapsed states, like rod and toroid.\[2, 3\] Simulations of this can be carried out by using a stiff polymer chain.\[2, 3\] Simulation study by using more realistic models and also by incorporating the solvent molecules implicitly, may reveal more information. Further work in these directions is under progress. Finally, simulations of $P(\Phi_F)$ during folding of real proteins remains a challenging task.

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Figure captions:

**Figure 1.** The distribution of FRET efficiency ($P(\Phi_F)$), subsequent to a temperature quench (from $\epsilon^* = 0.1$ to $\epsilon^* = 0.8$), obtained from BD simulations is shown at $R_F = 7$, $\tilde{k}_F = 10$ and $N=80$. This demonstrates the emergence of bimodality in $P(\Phi_F)$. Here $k_{rn}$ is fixed as 0.0001; the bimodality is present at other values also, but sharpness depends on the magnitude of the radiative rate.

**Figure 2.** The equilibrium FRET efficiency ($\Phi_F$) distribution is shown for (a) $\epsilon^* = 0.3$ and (b) $\epsilon^* = 0.8$. $\tilde{k}_F = 10$ and $R_F = 7$ while $k_{rn}$ is fixed as 0.01.

**Figure 3.** The equilibrium FRET efficiency distribution ($P(\Phi_F)$) is shown for $\epsilon^* = 0.5$, which is close to the $\theta$ temperature ($\epsilon^*_\theta = 0.62$). $\tilde{k}_F$, $R_F$ and $k_{rn}$ are same as in figure 2. This figure demonstrates the emergence of bimodality even at equilibrium.

**Figure 4.** FRET efficiency distribution ($P(\Phi_F)$), subsequent to quench (from $\epsilon^* = 0.1$ to $\epsilon^* = 0.8$) is shown for $\tilde{k}_F = 1$ (open bars) and $\tilde{k}_F = 10$ (filled bars). $R_F = 7$ and $k_{rn}$ are same as in figure 2.
Fig. 1  Srinivas & Bagchi
Fig. 2(a) Srinivas & Bagchi
Fig. 3 Srinivas & Bagchi
Fig. 4  Srinivas & Bagchi