Probing Protein-Protein Interactions by Dynamic Force Correlation Spectroscopy

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(Received 6 April 2005; published 10 October 2005)

We develop a formalism for single molecule dynamic force spectroscopy to map the energy landscape of protein-protein complex (P_1P_2). The joint distribution $P(\tau_1, \tau_2)$ of unbinding lifetimes τ_1 and τ_2, measurable in a compression-tension cycle, which accounts for the internal relaxation dynamics of the proteins under tension, shows that the histogram of τ_1 is not Poissonian. The theory is applied to the forced unbinding of protein P_1, modeled as a wormlike chain, from P_1P_2. We propose a new class of experiments which can resolve the effect of internal protein dynamics on the unbinding lifetimes.

DOI: 10.1103/PhysRevLett.95.168302 PACS numbers: 82.37.Rs, 87.14.−g, 87.15.Aa

Many biological functions are mediated by interactions between biomolecules under mechanical stress. Protein-DNA interactions involve force-induced motion of proteins [1,2]. Similarly, specific protein-protein interaction in cell-protein complexes are important in molecular recognition [3]. Dynamic force spectroscopic techniques probe these interactions by forced unbinding of protein-protein complexes using forces in the 1–100 pN range [1,2,4–8]. Atomic force microscopy (AFM) has been employed in the studies of protein-protein interactions involving immunoglobulins [9], molecular motors [7,10], and cell adhesion complexes [3,6,8].

In constant force-induced unbinding of single protein-protein complexes, the histograms of unbinding lifetimes are fit using the Poisson distribution

$$P_u(\tau; f_{\rm ext}) = k_1(f_{\rm ext}) \exp[-k_1(f_{\rm ext})\tau]. \quad (1)$$

The dependence of the unbinding rate constant $k_1 = 1/\tau_u$ (τ_u is the lifetime of the complex) on the external force $f_{\rm ext}$ is given by the Bell model [11], $k_1(f_{\rm ext}) = k_{10} \times \exp[f_{\rm ext}\sigma/k_BT]$. The parameter σ is the maximum protein-protein bond extension before rupture, and k_{10} is the force-free unbinding rate of the bound complex P_1P_2. Because the Poisson approximation ignores the intrinsic dynamics of proteins (i.e., conformational motions and rearrangements), this analysis can only be used when τ_u exceeds the time scale of internal protein motion, τ_R. Lifetime measurements of a single P-selectin receptor with specific ligand PSGL-1 show that τ_u varies between milliseconds and few seconds depending on the magnitude of $f_{\rm ext}$ [3,6]. Because the lifetimes of the protein-protein complex under force become comparable to τ_R, the interpretation of the unbinding data is complicated by protein motion. Thus, Eq. (1) cannot be used to describe experimental histograms of the lifetimes. To account for the competing time scales (τ_R and τ_u) a theoretical framework that probes correlations between intrinsic relaxation and unbinding dynamics is needed to analyze experimental data.

In typical AFM experiments, the cantilever tip coated with protein P_1 is brought into contact with the surface-attached protein P_2, and allowed to interact for a time Δt so that the complex P_1P_2 can form (compression cycle). The tip is then retracted to a prescribed distance which results in the complex feeling a constant force $\mathbf{f} = f_{\rm ext}\mathbf{x}$ in the direction $\mathbf{x}$ perpendicular to the surface (tension cycle). The lifetime τ at which P_1P_2 bond breaks is recorded. However, if $\tau_u \sim \tau_R$, there is a finite time ($\sim\tau_R$) for propagation of the constant tension from the pulled terminus of P_1 to the binding interface of the P_1P_2 complex. Thus, the average time τ_u to break the P_1P_2 bond (assuming that cantilever spring constant is stiff compared with the noncovalent linkages that stabilize P_1 and P_2) is enhanced by τ_R resulting in the "apparent" lifetime $\tau \approx \tau_R + \tau_u$ of the complex.

In this Letter we propose a novel theoretical methodology for describing forced unbinding which allows for accurate estimation of protein-protein interaction parameters. The approach is based on analyzing not only the distribution of single lifetimes $P(\tau)$ but also the joint distribution $P(\tau_1, \tau_2; \Delta t)$ of lifetimes τ_1 and τ_2 separated by compression time Δt. The distribution $P(\tau_1, \tau_2; \Delta t)$ is measurable by constructing the joint histogram of lifetimes using current experimental methods. Because in current AFM assays Δt can be as short as microseconds [12], Δt can be varied by changing the frequency of the compression cycle; $P(\tau_1, \tau_2; \Delta t)$ can be utilized to resolve τ_R which in turn can be used to obtain τ_u, and free-energy landscape parameters σ and k_{10}. The theory describes protein-protein complexes that obey $P_1 + P_2 \rightleftharpoons P_1P_2$, and can be extended to more elaborate kinetic and protein models.

Basic concepts.—Typically, for specific protein-protein complexes the binding rate for $P_1 + P_2 \rightarrow P_1P_2$ is fast, and Δt is controlled by the duration of the compression cycle. Because of the conformational fluctuations of P_1, the binding interface experiences a restoring force $f(X, t)$ which tends to decrease the end-to-end distance $X(t)$. As t increases, the unbinding force along the coordinate X increases so that $f(X, t) \rightarrow f_{\rm ext}$ as $t \rightarrow \infty$, and $X(t)$ approaches the equilibrium force-dependent value $\langle X(f_{\rm ext})\rangle$. Because of the conformational dynamics of the proteins, the unbinding rate, $k_1(X, t) = k_{10} \exp[\sigma f(X, t)/k_BT]$, is a

stochastic variable that depends on X through $f(X)$. When application of $f_{\rm ext}$ does not result in complete stretching of P_1 ($X = L$), the instantaneous value of force along the P_1P_2 bond is equal to the restoring force

$$f(X,\tau) = -k_BT\frac{1}{P(X,\tau)}\frac{\partial P(X,\tau)}{\partial X}, \quad (2)$$

where the probability that P_1 has end-to-end distance X at time t is given by $P(X,t) = \frac{1}{N(t)}\int_0^L dX_0 4\pi X_0^2 G_0(X,t;X_0)\times \psi_{\rm eq}(X_0)$ and $N(t)$ is a normalization constant. When $f_{\rm ext}$ is large to fully stretch P_1, the force felt by P_1P_2 bond spikes up to $f_{\rm ext}$ at $X = L$, i.e., $f = f(X,\tau)h(L-X) + f_{\rm ext}h(X-L)$, where $h(X)$ is the Heaviside step function. We only consider $f_{\rm ext}$ that does not exceed the unfolding force threshold. The unbinding time distribution is given by the convolution of unbinding kinetics and dynamics of X, i.e.,

$$P(\tau, f_{\rm ext}) - \frac{1}{N_1}\int_0^L dX_1 4\pi X_1^2 \int_0^L dX_0 4\pi X_0^2 P_u(X,\tau)G_{f_{\rm ext}}(X_1,\tau;X_0)\psi_{\rm eq}(X_0), \quad (3)$$

where N_1 is a normalization constant. In Eqs. (2) and (3), $G_0(X_1,t;X_0)$ and $G_{f_{\rm ext}}(X_1,t;X_0)$ are, respectively, the force-free and force-dependent conditional probability of X at time t and $\psi_{\rm eq}(X)$ is the equilibrium distribution of X. The unbinding probability $P_u(X,t)$ depends on X through k_1, i.e., $P_u(X,t) = k_1(X,t)\exp[-k_1(X,t)t]$. The above equation is a generalization of Eq. (1) for force exerted on P_1P_2 bond that continuously evolves from zero to $f = f_{\rm ext}$ over time τ_R. In the limit $\tau_R \ll \tau_u$, $P(\tau, f_{\rm ext})$ reduces to $P_u(\tau, f_{\rm ext})$ given by Eq. (1).

A model application.—To illustrate the consequences of the stochastic nature of $k_1(X,t)$, we assume that a thermally fluctuating wormlike chain (WLC) (P_1) is in contact with the immobile P_2. Upon application of force $f_{\rm ext}$, extension of P_1 results in unbinding. The Hamiltonian for P_1 is

$$\begin{aligned} H = {} & \frac{3k_BT}{2l_p}\int_{-L/2}^{L/2} ds\left(\frac{\partial \mathbf{r}(s,t)}{\partial s}\right)^2 + \frac{3l_pk_BT}{8}\int_{-L/2}^{L/2} ds\left(\frac{\partial^2 \mathbf{r}(s,t)}{\partial s^2}\right)^2 \\ & + \frac{3k_bT}{4}\left[\left(\frac{\partial \mathbf{r}(-L/2,t)}{\partial s}\right)^2 + \left(\frac{\partial \mathbf{r}(-L/2,t)}{\partial s}\right)^2\right] \\ & + \mathbf{f}\int_{-L/2}^{L/2} ds\left(\frac{\partial \mathbf{r}(s,t)}{\partial s}\right), \end{aligned} \quad (4)$$

where l_p is the protein persistence length and $\mathbf{r}(s,t)$ is the location of monomer s ($-L/2 \le s \le L/2$) at time t. The end-to-end vector is $\mathbf{X}(t) = \mathbf{r}(L/2,t) - \mathbf{r}(-L/2,t)$, where L is the protein contour length. The statistics of X can be represented by a large number of independent modes when $L/l_p \gg 1$. Thus, it is reasonable to assume that $G_0(X,t;X_0)$ is a Gaussian. In the overdamped limit, when $f_{\rm ext}$ exceeds the unfolding threshold force, stretching of P_1 is smooth and thus, preserves Gaussian statistics,

$$G_0(X,t;X_0) = \left(\frac{3}{2\pi\langle X^2\rangle}\right)^{3/2}\frac{1}{[1-\phi^2(t)]^{3/2}} \times \exp\left[-\frac{3[X-\phi(t)X_0]^2}{2\langle X^2\rangle[1-\phi^2(t)]}\right] \quad (5)$$

specified by the mean value $\langle X(t)\rangle = \phi(t)X_0$ and variance $\sigma^2 = \langle X^2\rangle - \langle X\rangle^2$, where the correlation function $\phi(t) = \langle X(t)X(0)\rangle/\langle X^2\rangle$. To construct $G_0(X,t;X_0,0)$ we compute $\langle X(t)X(0)\rangle$ and $\langle X^2\rangle = \lim_{t\to\infty}\langle X(t)X(0)\rangle$ with $f_{\rm ext} = 0$. By using Eq. (4) and assuming that the dynamics of the wormlike chain in the overdamped random media is described by a stochastic force $\xi(s,t)$ with white noise statistics, $\langle\xi_\alpha(s,t)\rangle = 0$ and $\langle\xi_\alpha(s,t)\xi_\beta(s',t')\rangle = 2\gamma k_BT\delta_{\alpha\beta}\delta(s-s')\delta(t-t')$, where $\alpha = x, y, z$ and γ is the friction coefficient, we arrive at the Langevin equation:

$$\gamma\frac{\partial}{\partial t}\mathbf{r}(s,t) + \epsilon\frac{\partial^4}{\partial s^4}\mathbf{r}(s,t) - 2\nu\frac{\partial^2}{\partial s^2}\mathbf{r}(s,t) = \xi(s,t), \quad (6)$$

where $\epsilon = 3l_pk_BT/4$ and $\nu = 3k_BT/2l_p$. We solve Eq. (6) for $\mathbf{r}(s,t)$ with boundary conditions $[2\nu\frac{\partial}{\partial s}\mathbf{r} - \epsilon\frac{\partial^3}{\partial s^3}\mathbf{r}]_{\pm L/2} = 0$, $[2\nu_0\frac{\partial}{\partial s}\mathbf{r} \pm \epsilon\frac{\partial^2}{\partial s^2}\mathbf{r}]_{\pm L/2} = 0$, where $\nu_0 = 3k_BT/4$ to yield [13]:

$$\langle X(t)X(0)\rangle_0 = 12k_BT\sum_{n=1}^{\infty}\frac{1}{z_n}\psi_n^2(L/2)e^{-z_nt/\gamma}, \quad n = 1, 3, \ldots, 2q+1, \quad (7)$$

where the odd eigenfunctions are [13]

$$\psi_n(s) = \sqrt{c_n/L}\left(\frac{\alpha_n}{\cos[\alpha_nL/2]}\sin[\alpha_ns] + \frac{\beta_n}{\cosh[\beta_nL/2]}\sinh[\beta_ns]\right) \quad (8)$$

with normalization constant c_n. The eigenvalues $z_n = \epsilon\alpha_n^4 + 2\nu\alpha^2$ and the constants α_n, β_n are obtained by solving $\alpha_n\sin[\frac{\alpha_nl}{2}]\cosh[\frac{\beta_nl}{2}] - \beta_n^3\cos[\frac{\alpha_nL}{2}]\sinh[\frac{\beta_nL}{2}] - \frac{1}{l_p}(\alpha_n^2+\beta_n^2)\cos[\frac{\alpha_nL}{2}]\cosh[\frac{\beta_nL}{2}] = 0$ and $\beta_n^2 - \alpha_n^2 = \frac{1}{l_p^2}$. In the limit, $L/l_p \to \infty$, we arrive at the Rouse chain model describing the stretching modes $\psi_n^R = \sqrt{2L}\sin(n\pi s/L)$ with eigenvalues $z_n^R = 3n^2\pi^2k_BT/2l_pL^2$. To construct force-dependent propagator $G_{f_{\rm ext}}(X,t;X_0)$, we integrate Eq. (6) with $f_{\rm ext}\mathbf{x}$ added to $\xi(s,t)$ to obtain $\langle X^2\rangle_{f_{\rm ext}} = \langle X^2\rangle_0 + f_{\rm ext}^2\sum_{n=1}^{\infty}\psi_n^2(L/2)/z_n^2$.

We computed $P(\tau, f_{\rm ext})$ by integrating Eq. (3) at room temperature. The parameters L, l_p, and $\gamma = k_BT/DL$ determine the time scale of protein motion $\tau_R \approx \max\{\gamma/z_n\}$. We set $k_{10} = 0.1\ \mu s^{-1}$, $\sigma = 1.0$ nm, $L = 80$ nm, $l_p = 0.4$ nm, and $D = 10^{-8}$ cm^2/s. The largest eigenvalue $z_1/\gamma = 0.2\ \mu s^{-1}$ determines the longest relaxation time scale $\tau_R \approx 5\ \mu$s. In left panels of Fig. 1 we compare $P(\tau, f_{\rm ext})$ for WLC and Rouse model [Eq. (3)] with the Poisson approximation $P_u(\tau, f_{\rm ext})$ [Eq. (1)] for $f_{\rm ext} = 1$ pN,

3 pN, and 10 pN. At $f_{\rm ext} = 3$ pN and 10 pN, $P(\tau, f_{\rm ext})$ for WLC model is in good agreement with $P(\tau, f_{\rm ext})$ computed for the Rouse model. A slight overestimate in $P(\tau)$ at short τ's and lower $f_{\rm ext} = 1$ pN is due to faster relaxation of the Rouse modes. For $k_1 \sim z_1/\gamma$, Poisson approximation $P_u(\tau)$ deviates noticeably from $P(\tau)$. Deviations grow as $f_{\rm ext}$ is increased from 1 pN to 10 pN; $P_u(\tau)$ overestimates $P(\tau)$ at shorter τ and underestimates $P(\tau)$ at longer τ, predicting shorter lifetimes. Therefore, in cases when protein conformational relaxation and unbinding dynamics occur on similar time scales the use of Poisson approximation leads to inaccurate estimates of k_{10} and σ. In the right panels of Fig. 1 we compare $P(\tau, f_{\rm ext})$ for the WLC and Rouse modes with Poisson approximation $P_u(\tau, f_{\rm ext})$ for $z_1/\gamma = 2\ \mu{\rm s}^{-1} \gg k_{10}$. A tenfold increase in z_1/γ corresponds to less overdamped environment with larger $D = 10^{-7}$ cm^2/s (the other parameters are same as in left panels). Because it now takes an order of magnitude shorter time to propagate $f_{\rm ext}$ from the pulled end of P_1 to the P_1P_2 interface, Poisson distribution P_u follows closely $P(\tau, f_{\rm ext})$ at lower $f_{\rm ext} = 1$ pN and 3 pN. However, P_u deviates from $P(\tau, f_{\rm ext})$ at higher $f_{\rm ext} =$ 10 pN due to rapid force-induced increase in the unbinding rate k_1. Thus, even when propagation of tension is rapid there are substantial deviations from Poisson distribution of bond lifetimes at higher $f_{\rm ext}$.

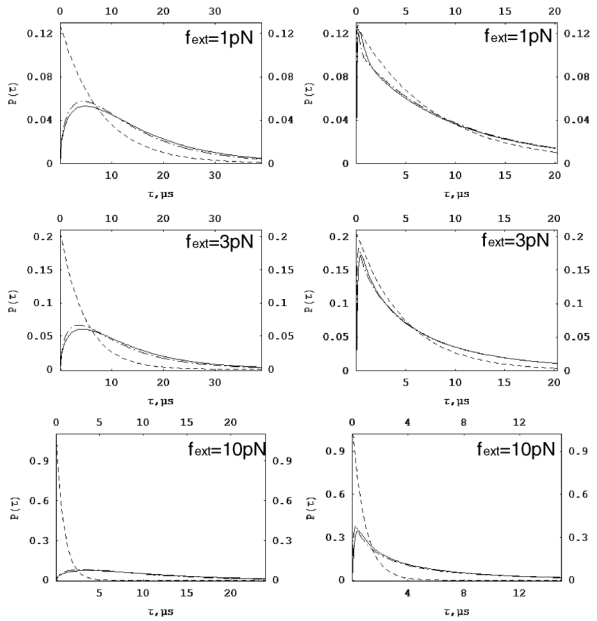

FIG. 1. The distribution of unbinding times $P(\tau, f_{\rm ext})$ for WLC (solid line) and Rouse model (dash-dotted line) of protein and Poisson approximation $P_u(\tau, f_{\rm ext})$ (dashed line) for $f_{\rm ext} = 1$ pN, 3 pN, and 10 pN computed for $k_1 \sim z_1/\gamma$ (left panels) and $k_1 \ll z_1/\gamma$ (right panels).

A practical methodology that can be used in conjunction with experimental data to accurately estimate k_{10} and σ is required. Dynamical signatures of protein motion can be assessed by computing the joint distribution $P(\tau_1, \tau_2; \Delta t)$ of consecutive unbinding times, τ_1 and τ_2, separated by compression time Δt,

$$P(\tau_1, \tau_2; \Delta t, f_{\rm ext}) = \frac{1}{N_2} \int_0^L dX_3 4\pi X_3^2 \int_0^L dX_2 4\pi X_2^2 \int_0^L dX_1 4\pi X_1^2 \int_0^L dX_0 4\pi X_0^2 P_u(X_3, \tau_2) \times G_{f_{\rm ext}}(X_3, \tau_2; X_2) P_b(X_2, \Delta t) G_0(X_2, \Delta t; X_1) P_u(X_1, \tau_1) G_{f_{\rm ext}}(X_1, \tau_1; X_0) \psi_{\rm eq}(X_0), \quad (9)$$

where $P_b(t)$ is the binding probability for $P_1 + P_2 \rightarrow P_1P_2$ and N_2 is a normalization constant. In Eq. (9), $G_0(X_2, t; X_1)$ is the force-free propagator representing correlations of two interaction events decaying over τ_R. When $\tau_R > \Delta t$, $P(\tau_1, \tau_2; \Delta t)$ is a sensitive measure of protein motion and thus, can be employed to estimate τ_R. When $\tau_R \ll \Delta t$, unbinding events are independent, $\lim_{\Delta t \rightarrow \infty} G_0(X_2, \Delta t; X_1) \rightarrow \psi_{\rm eq}(X_2)$, and hence, $P(\tau_1, \tau_2) \rightarrow P(\tau_1)P(\tau_2)$.

We computed $P(\tau_1, \tau_2; \Delta t)$ for $\Delta t = 1\ \mu{\rm s} \ll \gamma/z_1$, $\Delta t = 10\ \mu{\rm s} \sim \gamma/z_1$, and $\Delta t = 500\ \mu{\rm s} \gg \gamma/z_1$ for $f_{\rm ext} =$ 3.0 pN and $k_{10} = 0.1\ \mu{\rm s}^{-1}$, $\sigma = 1.0$ nm, $L = 80$ nm, $l_p = 0.4$ nm, and $z_1/\gamma = 0.01\ \mu{\rm s}^{-1}$ (Fig. 2). We assumed that protein binding ($P_1 + P_2 \rightarrow P_1P_2$) is independent of the dynamics of X; i.e., once P_1 reached the vicinity of binding interface of P_2 it binds, and set $P_b(X, \Delta t) = P_b = 1$ in Eq. (9). A short $\Delta t = 1\ \mu$s and 10 μs peak in $P(\tau_1, \tau_2)$ (top and middle panels) is washed out at longer $\Delta t =$ 500 μs (bottom). Striking asymmetry of the contour plots at short Δt is due to the dependence of shorter τ_2 events on longer τ_1 events. During the first interaction the constant force felt by P_1P_2 bond is ramped up from $f = 0$ to $f = f_{\rm ext}$ following the restoring force $f(X, t)$ thus, prolonging τ_1. When $\Delta t \ll \tau_R \sim \gamma/z_1$, the next binding event takes place (at $t = \Delta t$ after the first unbinding) when P_1 is partially or fully stretched. As a result, the binding interface experiences nonvanishing restoring force from the beginning of the second interaction event and $\tau_2 < \tau_1$. Contour plots of $P(\tau_1, \tau_2)$ become more symmetric as Δt is increased to 10 μs which implies growing statistical independence of unbinding events. At $\Delta t = 500\ \mu{\rm s} \gg \tau_R$, $P(\tau_1, \tau_2)$ is symmetric density, which results in factorization $P(\tau_1, \tau_2) = P(\tau_1)P(\tau_2)$. Thus, to obtain *statistically meaningful distributions of uncorrelated unbinding times*, unbinding events must be separated by much longer Δt compared to τ_R whose *a priori* determination is difficult.

Application to Experiments.—Using $D(\tau_1, \tau_2; \Delta t) = P(\tau_1, \tau_2; \Delta t) - P(\tau_1)P(\tau_2)$, correlations between τ_1's and τ_2's can be probed in AFM experiments. If $D \neq 0$, the unbinding events are influenced by conformational fluctuations of the protein. For the model parameters in Fig. 2 we show in Fig. 3 $D(\tau_1 = \tau_2; \Delta t)$ for $\Delta t = 1\ \mu$s, 10 μs, and 500 μs. The peak of $D(\tau, \Delta t)/D(\tau, 0)$, which signifies the amplitude of correlations between the unbinding events, decays to zero as Δt is increased from $1\ \mu{\rm s} \ll \gamma/z_1$ to $500\ \mu{\rm s} \gg \gamma/z_1$. An accurate statistical analysis of unbinding lifetimes can be made using the following steps.

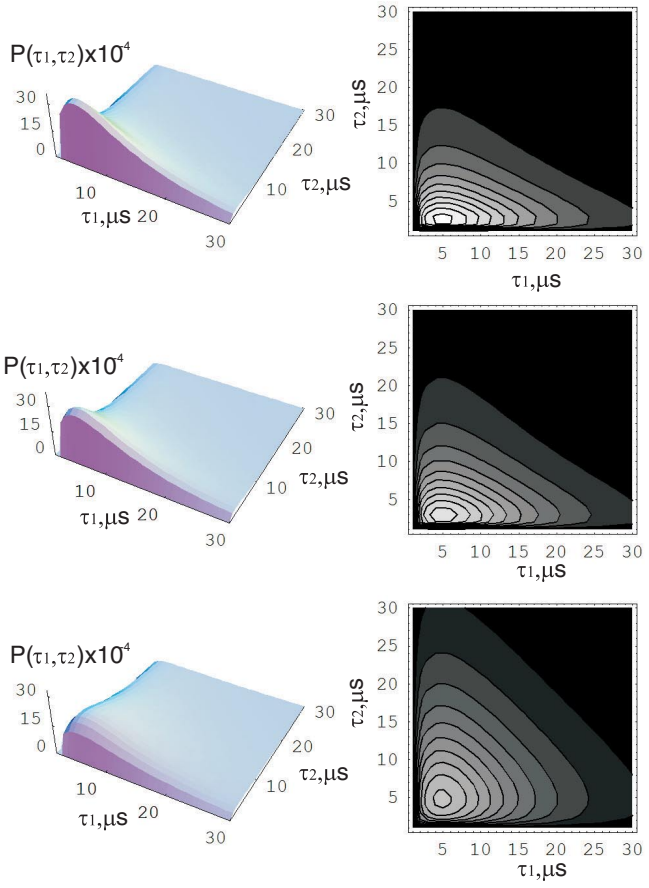

FIG. 2 (color online). The joint distribution $P(\tau_1, \tau_2; \Delta t, f_{\text{ext}})$ of lifetimes τ_1 and τ_2 separated by $\Delta t = 1$ μs (top panel), 10 μs (middle panel), and 500 μs (bottom panel) for $f_{\text{ext}} = 3$ pN. The contour plots of $P(\tau_1, \tau_2; \Delta t, f_{\text{ext}})$ are shown on the right.

From the unbinding time histogram $P(\tau, f_{\text{ext}})$ and the apparent mean lifetime τ_{app} the joint histogram $P(\tau_1, \tau_2; \Delta t)$ for $\Delta t \ll \tau_{\text{app}}$ is computed. The difference $D(\tau_1, \tau_2; \Delta t)$ is evaluated using $P(\tau, f_{\text{ext}})$ and the *experimentally determined* $P(\tau_1, \tau_2; \Delta t, f_{\text{ext}})$. If $D \approx 0$, the unbinding events are uncorrelated, and k_1 can be estimated by fitting Eq. (1) to $P(\tau, f_{\text{ext}})$. If $D > 0$, the unbinding and protein motions are correlated. In this case the lifetime measurements must be repeated for longer Δt. Using the new data, new distributions $P(\tau_1)$, $P(\tau_1, \tau_2; \Delta t)$, and $D(\tau_1, \tau_2; \Delta t)$ can be calculated. The process is iterated until the requirement $D \approx 0$ is satisfied for the compression cycle of duration, say, Δt^*. The protein relaxation time τ_R is the minimum value of $\Delta t = \Delta t^*$ at which $D \approx 0$. Uncorrelated lifetimes collected for $\Delta t \gg \tau_R \approx \Delta t^*$ can then be binned to obtain the final histogram $P(\tau)$. If $\tau_R \ll \tau_{\text{app}} = \tau_R + \tau_u$ then $\tau_{\text{app}} \approx \tau_u$, and k_{10} and σ can be estimated by fitting Eq. (1) to $P(\tau, f_{\text{ext}})$. However, if $\tau_R \sim \tau_{\text{app}}$, $P(\tau, f_{\text{ext}})$ must be analyzed using Eq. (3) for given f_{ext}, L, $\gamma = k_B T/DL$, and estimated τ_R. Thus, the theory presented here suggests a novel dynamic force correlation spectroscopy in which measurements of $P(\tau_1, \tau_2; \Delta t)$ for varying Δt can be used to account for the influence of internal protein dynamics on unbinding of proteins.

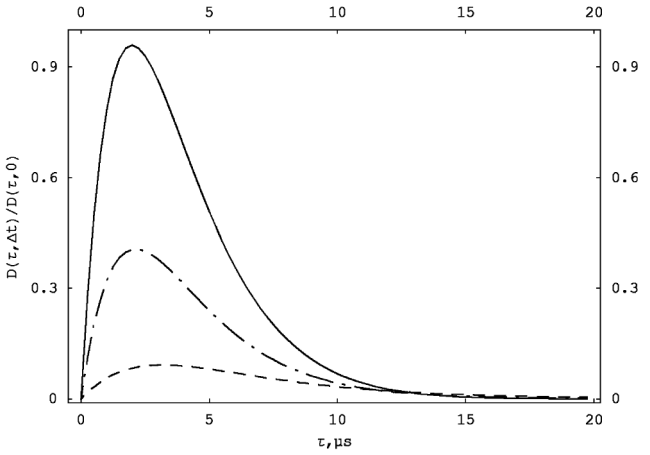

FIG. 3. Normalized correlation amplitude $D(\tau, \Delta t)/D(\tau, 0)$ of equal lifetimes $\tau = \tau_1 = \tau_2$ separated by $\Delta t = 1$ μs (solid line), 10 μs (dash-dotted line), and 500 μs (dashed line) for $f_{\text{ext}} = 3$ pN.

This work was supported by National Science Foundation Grant No. NSFCHE-05-14056.

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