

# Forced dissociation of a biomolecular complex under periodic and correlated random forcing

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The dissociation of a biomolecular complex under the action of periodic and correlated random forcing is studied theoretically. The former is characterized by the period  $\tau_p$  and the latter by the correlation time  $\tau_r$ . The rupture rates are calculated by overdamped Langevin dynamics and three distinct regimes are identified for both cases by comparison to local relaxation time  $\tau_R$  and bond lifetime  $\langle T \rangle$ . For periodic forcing, the adiabatic approximation cannot be applied in the regime  $\tau_p \ll \tau_R$  and the bond lifetime is determined by the average pulling. As  $\tau_R \ll \tau_p \ll \langle T \rangle$ , the rupture rate is enhanced by periodic forcing but is  $\tau_p$  independent. Analytical expressions are obtained for small and large force amplitudes. As  $\langle T \rangle \ll \tau_p$ , the rupture rate depends on the phase lag and the process behaves like it is under constant force or loading rate. The result of correlated random forcing is similar to that of periodic forcing. Since the fluctuating forces greater than the average force  $\langle F \rangle$  contribute more than the fluctuating forces less than  $\langle F \rangle$ , the force fluctuations enhance the rupture rate. As  $\langle T \rangle < \tau_r$ , the pulling felt by the bond before rupture cannot follow the random forcing protocol and, thus, force fluctuations decline with increasing  $\tau_r$ . © 2008 American Institute of Physics. [DOI: 10.1063/1.2841404]

## I. INTRODUCTION

The noncovalent biomolecular bond, such as receptor-ligand interaction, mediates many of life's functions in cells. It also plays an important role in applications such as biosensors and biomaterials. Lately, the physical nature of weak noncovalent bonds has been experimentally explored at the single-molecule level.<sup>1,2</sup> In these experiments, such as ligand-receptor dissociation and protein unfolding, the externally applied force (in the piconewton range) is exerted on an anchored molecular complex. Direct measurements of single bond strength have been conducted with three types of ultrasensitive force-probing techniques: Atomic force microscopy (AFM), biomembrane force probe, and laser optical tweezer. In general, the pulling spring is moved away from the anchored molecule at a constant velocity. Hence, the force is slowly increased until bond rupture occurs. In contrast to equilibrium binding properties, the bond-rupture force used to describe bond strength is not constant but, instead, is dependent on the rate of force increment (loading rate).

The natural lifetime of a ligand-receptor complex is long (milliseconds to seconds). This fact indicates that the thermally activated escape (unbinding at zero force) must cross an energy barrier. The lifetime is stochastic in nature due to

kinetic escape. Therefore, the unbinding process of the bound complex along the reaction coordinate on the free energy landscape can be regarded as the escape of an overdamped particle from a kinetic trap.<sup>3–8</sup> When an external force ( $F$ ) is applied, the kinetic escape across the barrier is assisted mechanically and, thereby, the escape time is reduced. In fact, the main goal of performing single-molecule pulling experiments is to extract the intrinsic kinetic parameters, including the intrinsic rate constant ( $k_0$ ), the reaction range ( $a$ ), and the energy barrier ( $E_a$ ). Because of the pulling, the critical force ( $F_c$ ), which represents the maximum slope of the trapped potential, also plays a key role in determining the rupture rate. When pulling is absent ( $F=0$ ), the bound complex dissociates eventually. This thermally activated escape characterizes the kinetic limit. On the contrary, as  $F > F_c$ , the dissociation is controlled by mechanical pulling. Under constant pulling  $F < F_c$ , the free energy surface is altered and the energy barrier is lowered. Consequently, the mean first passage time (bond lifetime) associated with the forced escape from a kinetic trap decreases with increasing  $F$ .

A given bond under any level of pulling will break ultimately if the force is applied over a sufficient period of time. When the applied pulling is ramped up with time, the interplay between mechanic pulling and kinetic escape leads to loading rate dependent behavior of the bond-rupture force ( $F_u$ ).<sup>3–6</sup> When the loading rate [ $\dot{F}_t = F(t)/t$ ] is very slow, the time is long enough so that the rupture occurs mainly by

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thermal fluctuations and low rupture forces are resulted. In contrast, if the loading rate is very fast, then no time is available for accumulated thermal actions. Thus, the rupture force approaches the maximum, intrinsic binding force ( $F_c$ ). The rupture force is clearly not an intrinsic property associated with the bound complex and extracting useful information from pulling experiments under steady speed detachment needs an appropriate relation between the mean rupture force and the loading rate  $F_u(\dot{F}_r)$ , which must involve kinetic properties as well.

The mean bond-rupture time (inverse of rate constant) under constant force condition, i.e.,  $1/k(F)$ , instead of bond-rupture force under constant loading rate condition, is more direct and is desirable for extracting kinetic information. In fact, from the theoretical viewpoint,  $F_u(\dot{F}_r)$  can only be derived based on knowing  $k(F)$ . In AFM experiments, the constant tensile force can be applied to the biomolecular bond as the cantilever is retracted a prescribed distance, after the cantilever tip bearing ligands was brought into contact with receptors.<sup>9</sup> Nonetheless, the constant force is susceptible to thermal fluctuations because Brownian effects of cantilever in fluids have long been a source of noise contamination in AFM experiments.<sup>8,10</sup> The random motion of ligand and receptor causes the tensile force fluctuation as well. As a result, the dissociation rate associated with the constant force rupture experiment may be altered by the force fluctuation.

In addition to constant loading (linear ramp) and constant force experiments, recently, periodic loading (periodic ramp) was proposed to improve the quality of data obtained by single-molecule pulling experiments.<sup>11</sup> It is found that the reconstructed free energy profile by the periodic ramp is far better than the linear ramp. Obviously, each forcing protocol has its own advantages and its dissociation characteristics will be different from others. In order to obtain the kinetic information from the rupture experiments under various forcing protocols, fundamental understanding and theoretical models are required to analyze experimental results. In this study, we investigate the rupture rate under a mean constant force subjected to periodic changes or correlated random noises. The former is characterized by the period  $\tau_p$ , while the latter is characterized by the correlation time  $\tau_r$ .

On the basis of overdamped Langevin dynamics, we will show that the bond lifetime varies with  $\tau_p$  for periodic forcing and changes with  $\tau_r$  for correlated random forcing. The behavior of the rupture rate can be generally classified into three types: (i) Dependent on average force only, (ii) enhanced by fluctuating forces, and (iii) initial forcing dominant. In the first regime, the adiabatic approximation cannot be applied to the fluctuating forces. In the second regime, analytical expressions are obtained for periodic forcing by asymptotic analysis of Kramers rate theory based on Bell's expression for small force amplitude and Garg's form for large amplitude. In the third regime, the behavior is rupture like under constant force.

## II. FORCED KRAMERS RATE THEORY

The force-assisted dissociation process of the biomolecular complex is equivalent to the escape of an over-

damped particle from a kinetic trap  $U(x)$  under the action of force  $F$ , where  $x$  is the reaction coordinate. The dynamics of this particle is described by the Langevin equation

$$M\gamma \frac{dx}{dt} = -\frac{d}{dx}[U(x) - F(t)x] + \xi(t), \quad (1)$$

where  $M\gamma$  is the friction coefficient and is related to the diffusivity  $D$  by  $M\gamma = k_B T/D$ .  $M\gamma$  comes from the dissipation of kinetic energy into internal degrees of freedom; therefore,  $M\gamma$  in trapped state may be significantly greater than that in escaped state. In this case, rebinding events can be neglected. The effect of thermal fluctuations is represented by a random force with zero mean and  $\langle \xi(t)\xi(t') \rangle = 2M\gamma k_B T \delta(t-t')$ .

The free energy landscape associated with  $U(x)$  is characterized by three parameters: The interaction range  $a = x_+^0 - x_-^0$ , which denotes the distance from the metastable well  $x_-^0$  to the barrier  $x_+^0$ , the intrinsic energy barrier  $E_a^0 = U(x_+^0) - U(x_-^0)$ , and the maximum slope of the trapped potential, i.e., the critical force,  $F_c = U'(x_c)$ . Typically,  $E_a^0 \sim \mathcal{O}(10k_B T)$ ,  $a \sim \mathcal{O}(1 \text{ nm})$ , and  $F_c \sim \mathcal{O}(10 \text{ pN})$ . When  $F=0$ , the biomolecular complex dissociation occurs due to thermal fluctuations. For  $E_a \gg k_B T$ , the escape rate can be evaluated by Kramers rate theory<sup>12</sup>

$$k = \tau^{-1} \exp(-\beta E_a) \quad \text{with} \quad \tau = \frac{2\pi\gamma}{\omega_- \omega_+}. \quad (2)$$

Here,  $\omega_{\pm} = [\mp U''(x_{\pm})/M]^{1/2}$ . This thermally activated escape denotes the *kinetic* limit. On the contrary, when  $F > F_c$ , the binding force can be overcome without any help of thermal fluctuations. The dissociation is dominated by mechanical pulling, this is the *mechanical* limit. When  $0 < F < F_c$ , the external force alters the effective trapped potential to  $V(x) = U(x) - Fx$ . Thus, the well and saddle positions are determined by  $U'(x_{\pm}) = F$ . The barrier height becomes  $E_a(F) = V[x_+(F)] - V[x_-(F)]$ ,  $\tau$  becomes  $\tau(F)$ , and the rate constant at  $F=0$  is  $k_0 = \tau_0^{-1} e^{-\beta E_a^0}$ . Because the energy barrier is lowered, the bond lifetime is reduced with increasing  $F$ .

The kinetics of bond-rupture modeled by the escape of an overdamped particle from an energy well can be depicted by a phenomenological formalism if the Brownian particle is adjusted to the apparent potential,  $U(x) - F(t)x$ , instantaneously (adiabatic approximation). The survival probability  $P_s(t)$  satisfies the first-order rate equation with a time-dependent rate constant  $k[F(t)]$ ,

$$\frac{dP_s}{dt} = -k(t)P_s(t). \quad (3)$$

The solution is given by

$$P_s(t) = \exp \left[ - \int_0^t k(t') dt' \right]. \quad (4)$$

The probability distribution of lifetime is  $-dP_s/dt$ . Thus, the mean bond lifetime is

$$\langle T \rangle = \int_0^\infty t \left( -\frac{dP_s}{dt} \right) dt. \quad (5)$$

Inserting Eq. (4) into Eq. (5) yields

$$\langle T \rangle = \int_0^\infty t \left\{ k(t) \exp \left[ - \int_0^t k(t') dt' \right] \right\} dt. \quad (6)$$

Once the forcing protocol associated with the external pulling is known, one is able to obtain the mean bond lifetime  $\langle T \rangle$  or the rate constant ( $\langle k \rangle = \langle T \rangle^{-1}$ ) from Eqs. (2) and (6).

### III. ASYMPTOTIC ANALYSIS OF PERIODIC FORCING

For the dissociation of a single bond under time-periodic force, we consider the simplest sinusoid case without loss of generality,  $F(t) = F_0 \cos(2\pi t/\tau_p)$ . The external pulling is characterized by the force amplitude  $F_0$  and period  $\tau_p$ . The rate constant  $k(F)$  becomes periodic because the effective energy barrier varies with time periodically. Simple analytical expressions for the bond lifetime can be obtained in the asymptotic limits, just like other pulling protocols such as constant force and constant loading rate.<sup>7</sup> Under periodic forcing, the asymptotic limits are small amplitude ( $F_0/F_c \ll 1$ ) and large amplitude ( $1 - F_0/F_c \ll 1$ ) when  $\langle T \rangle \gg \tau_p$ .

The primary role of external pulling in the dissociation kinetics is lowering the energy barrier  $E_a$ . Both Bell's expression and the Garg's form are adopted to quantitatively describe the effect of external pulling on the energy barrier. A recent study<sup>7</sup> has shown that Bell's expression provides a good approximation for the bond dissociation at  $F/F_c \ll 1$ , while the dissociation rate is better described by the generalized Garg's form for  $1 - F/F_c \ll 1$ . When  $\langle T \rangle \gg \tau_p$ , Bell's expression,<sup>13</sup>  $E_a = E_a^0 - F_a$ , is valid in the small amplitude regime ( $F_0/F_c \ll 1$ ). Therefore, the energy barrier height  $E_a$  and intrinsic time constant  $\tau$  are given by

$$E_a[F(t)] = E_a^0 - F_0 a \cos(2\pi t/\tau_p) \quad \text{and} \quad \tau[F(t)] = \tau_0. \quad (7)$$

Inserting Eq. (2) with Eq. (7) into Eq. (6) gives

$$\langle T \rangle = k_0 \int_0^\infty t \exp[\beta F_0 a \cos(2\pi t/\tau_p)] \times \exp \left\{ -k_0 \int_0^t \exp[\beta F_0 a \cos(2\pi t'/\tau_p)] dt' \right\} dt, \quad (8)$$

where  $k_0$  denotes the rate constant in the absence of external forces. First, the integration within the exponential term can be performed by letting  $\theta = \omega t'$  with  $\omega = 2\pi/\tau_p$ . Since  $d\theta = \omega dt'$  and  $t \gg \tau_p$ , one has

$$\begin{aligned} \int_0^t \exp[\beta F_0 a \cos(2\pi t'/\tau_p)] dt' \\ = \frac{1}{\omega} \int_0^{\omega t} e^{\beta F_0 a \cos \theta} d\theta = I_0(\beta F_0 a) t, \end{aligned}$$

where  $I_0(z) = (2\pi)^{-1} \int_0^{2\pi} e^{z \cos \theta} d\theta > 1$ . Now, the mean bond lifetime becomes

$$\langle T \rangle = k_0 \int_0^\infty t \exp[\beta F_0 a \cos(\omega t)] \exp[-k_0 I_0(\beta F_0 a) t] dt. \quad (9)$$

Note that  $\omega \gg k_0$  because of  $\langle T \rangle \gg \tau_p$ . The exponential term involving  $\cos(\omega t)$  can be expanded in a Taylor series due to small value of  $\beta F_0 a$ . As a result, the integration in Eq. (9) can be carried out,

$$\begin{aligned} \langle T \rangle &\cong k_0 \int_0^\infty \left[ t + (\beta F_0 a) t \cos(\omega t) \right. \\ &\quad \left. + \frac{1}{2} (\beta F_0 a)^2 t (\cos \omega t)^2 \right] \exp[-k_0 I_0(\beta F_0 a) t] dt \\ &= k_0^{-1} \frac{1 + \frac{(\beta F_0 a)^2}{4}}{I_0^2(\beta F_0 a)}. \end{aligned} \quad (10)$$

For higher value of  $\beta F_0 a$ , one can keep higher order terms in the Taylor expansion and finds

$$\begin{aligned} \langle T \rangle &\cong k_0^{-1} \frac{1 + \frac{(\beta F_0 a)^2}{4} + \frac{(\beta F_0 a)^4}{64} + \frac{(\beta F_0 a)^6}{2304} + \dots}{I_0^2(\beta F_0 a)} \\ &= [k_0 I_0(\beta F_0 a)]^{-1}, \end{aligned} \quad (11)$$

where  $I_0(z)$  denotes the modified Bessel function,

$$I_0(z) = \sum_{k=0}^{\infty} \frac{(\frac{1}{4} z^2)^k}{(k!)^2}.$$

In general, Eq. (11) is valid for  $\beta F_0 a \leq 4$ .

For strong pulling ( $1 - F/F_c \ll 1$ ), the generalized Garg's form<sup>7,14</sup> is a reasonable approximation for the force-altered energy barrier,  $E_a(F) = E_a^* (1 - F/F_c)^{(n+1)/n}$  and  $\tau(F) = \tau_0^* (1 - F/F_c)^{-(n-1)/n}$ . This result is obtained because the energy well  $U(x)$  can be expanded near  $x_c$  and, therefore, approximated by  $U(x) \cong U(x_c) + F_c b/2 [y - y^{n+1}/(n+1)]$ , where  $y = (x - x_c)/(b/2)$  and  $b = 2[-n! F_c / U^{(n+1)}(x_c)]^{1/n}$  is the characteristic length of  $U$  in this limit.<sup>7</sup> When  $U^{(3)}(x_c)$  is nonvanishing,  $n=2$ . Otherwise,  $n$  is the smallest even integer for which  $U^{(n+1)}(x_c)$  is nonvanishing but  $U^{(m)}=0$  for all  $m$  less than or equal to  $n$ . On the basis of the generalized Garg's form, one has

$$\begin{aligned} \int_0^t k(t') dt' &= \int_0^t \frac{1}{\tau_0^*} \left( 1 - \frac{F_0 \cos \omega t'}{F_c} \right)^{(n-1)/n} \\ &\quad \times \exp \left[ -\beta E_a^* \left( 1 - \frac{F_0 \cos \omega t'}{F_c} \right)^{(n+1)/n} \right] dt' \\ &= \frac{k(F_0)}{\sqrt{2\pi \frac{n+1}{n} \beta E_a^* \left( 1 - \frac{F_0}{F_c} \right)^{(n-1)/n} \left( \frac{F_0}{F_c} \right)}} t = Kt \end{aligned} \quad (12)$$

where  $k(F_0) = 1/\tau(F_0) \exp[-\beta E_a(F_0)]$  denotes the rate constant associated with constant pulling  $F_0$ . Equation (12) is a Laplace integral and the integration is performed by Laplace method. The asymptotic expansion of the Laplace integral

$\int_0^{\tau_p} f(t) e^{x\phi(t)} dt$  for large  $x(\beta E_a^* \gg 1)$  is carried out with the maximum of  $\phi(t)$  at  $t=0$  in the first period. Note that there are  $t/\tau_p$  periods.

Now, the mean bond lifetime can be written as

$$\langle T \rangle = \int_0^\infty tk(F_0 \cos \omega t) \exp(-Kt) dt. \quad (13)$$

When  $K^{-1} \gg \tau_p$ , the integration can be conducted for each period  $\tau_p$ , i.e.,  $\int_{j\tau_p}^{(j+1)\tau_p} dt \rightarrow \tau_p/2\pi \int_0^{2\pi} d\theta$ . Equation (13) can then be summed up period by period and becomes

$$\begin{aligned} \langle T \rangle &\cong \sum_{j=0}^{\infty} e^{-K(j\tau)} \\ &\times \left[ \sqrt{2\pi}(j\tau_p) \frac{k(F_0)}{\sqrt{\frac{n+1}{n} \beta E_a^* \left(1 - \frac{F_0}{F_c}\right)^{(n-1)/n} \left(\frac{F_0}{F_c}\right)}} \right] \\ &= \sum_{j=0}^{\infty} K(j\tau_p) e^{-K(j\tau)} \cong \int_0^\infty Kt \exp(-Kt) dt = K^{-1}. \end{aligned} \quad (14)$$

In the large amplitude regime,  $1 - F_0/F_c \ll 1$ , the lifetime is obtained by applying the generalized Garg's form,

$$\langle T \rangle \cong \frac{1}{k(F_0)} \sqrt{2\pi \frac{n+1}{n} \beta E_a^* \left(1 - \frac{F_0}{F_c}\right)^{(n-1)/n} \left(\frac{F_0}{F_c}\right)}. \quad (15)$$

Note that in both asymptotic limits, the bond lifetime  $\langle T \rangle$  decreases with the force amplitude  $F_0$  but is independent of the period  $\tau_p$  as  $\langle T \rangle \gg \tau_p$  and the adiabatic assumption is valid.

#### IV. OVERDAMPED LANGEVIN DYNAMICS

Equation (1) can be solved numerically by the overdamped Langevin equation,

$$\hat{x}' = \hat{x} + \Delta \hat{t} \left[ -\frac{\partial \hat{U}}{\partial \hat{x}} + \hat{F}(t) \right] + \sqrt{2\Delta \hat{t}} Z, \quad (16)$$

where  $Z$  denotes a Gaussian distribution with zero mean and unit variance. The position, time, and force are scaled by  $a$ ,  $a^2/D$ , and  $k_B T/a$ , respectively. Typically, the integration time step is  $\Delta \hat{t} = 10^{-4}$ . The mean bond lifetime  $\langle T \rangle$  is calculated by an average over  $2 \times 10^4$  runs. We consider periodic forcing and correlated random forcing with an average pulling  $\langle F \rangle$  for  $F(t)$ . A general periodic forcing can be expressed by

$$F(t) = \langle F \rangle + F_0 \cos\left(2\pi \frac{t}{\tau_p} + \alpha\right).$$

The mean bond lifetime  $\langle T \rangle$  is thus a function of mean force  $\langle F \rangle$ , amplitude  $F_0$ , period  $\tau_p$ , and phase lag  $\alpha$ . A correlated random forcing can be represented by the force variance and exponentially correlated random number,

$$F(t) = \langle F \rangle + \sigma_F r_n,$$

where  $r_n$  denotes a correlated random distribution and  $\sigma_F$  is force variance.  $r_n$  can be generated by

$$r_n = fr_{n-1} + \sqrt{1-f^2} Z_n,$$

where  $f = \exp(-1/\tau_r)$  with the correlation time  $\tau_r$  and  $Z_n$  is Gaussian random number. Under a given  $\langle F \rangle$ ,  $\langle T \rangle$  may vary with the force variance and correlation time.

To examine our analysis, we consider a model potential

$$U(x) = \frac{E_a^0}{2} \left[ 1 - \cos\left(\frac{x}{a}\right) \right], \quad 0 \leq x \leq a, \quad (17)$$

where  $\beta E_a^0 = 12$  for all Langevin dynamics simulations. The advantage of this potential is that the escape rate  $k(F)$  under constant force  $F$  can be obtained analytically by Kramers rate theory [Eq. (2)] without resorting to Bell's expression and the generalized Garg's form.<sup>7</sup> The energy barrier and the intrinsic time constant are given by  $E_a(F) = E_a^0 [1 - (F/F_c)^2 - (F/F_c) \cos^{-1}(F/F_c)]$  and  $1/\tau(F) = \tau_0^{-1} [1 - (F/F_c)^2]^{1/2}$ , respectively. These results associated with the forced Kramers escape are valid only when the adiabatic assumption is justified. That is, the Brownian particle is adjusted to the apparent potential,  $U(x) - F(t)x$ , instantaneously. This approximation is valid when the escape time  $\langle T \rangle$  is large compared to the local relaxation time in the absence of noise and driving  $\tau_R$ .<sup>15</sup> As implied in Eq. (1), the local relaxation time is  $\tau_R \approx \tau_0 \approx (a^2/D)/\beta E_a^0$ . In general,  $\langle T \rangle \sim \tau_0 \exp(\beta E_a) \gg \tau_R$  and, thus, the adiabatic approximation is justified.

#### V. RESULTS AND DISCUSSION

The dissociation kinetics of a ligand-receptor complex has been experimentally investigated by ultrasensitive force-probing techniques at the single-molecule level. A periodic forcing protocol can be used to extract the kinetic information. Moreover, the thermal fluctuations inherent in the system may influence the dissociation characteristics of a constant force rupture experiment. By using overdamped Langevin dynamics, we investigate the effects of periodic forcing and correlated random forcing on the rupture rate.

##### A. Periodic forcing

There exists three regimes, (i)  $\tau_p \ll \tau_R \ll \langle T \rangle$ , (ii)  $\tau_R \ll \tau_p \ll \langle T \rangle$ , and (iii)  $\tau_R \ll \langle T \rangle \ll \tau_p$ , as shown in Fig. 1, for different values of  $\langle F \rangle$  and  $\alpha$ . At very small  $\tau_p$ , the bond lifetime approaches a constant value independent of the oscillating period. In this regime, the period is small compared to the local relaxation time. In other words, the pulling force oscillates so fast that the Brownian particle cannot respond to the pulling force in time. The adiabatic approximation is thereby invalid and the forced Kramers theory breaks down for the fast oscillating force. As illustrated in Figs. 1–3, the bond lifetime  $\langle T \rangle$  is independent of the oscillating period  $\tau_p$ , force amplitude  $F_0$ , and phase lag  $\alpha$  but varies with the average pulling force  $\langle F \rangle$ . That is, the Brownian particle feels mainly the average pulling force and the influence of the color noise is insignificant for the condition  $\tau_p \ll \tau_R$ . Nonetheless, this regime is not of practical experimental interest.

At very large  $\tau_p$ , the bond lifetime approaches another constant, which depends on the phase lag. In this regime, the bond lifetime is small compared to the oscillating period,  $\tau_p \gg \langle T \rangle$ . Therefore, the bond is effectively under constant



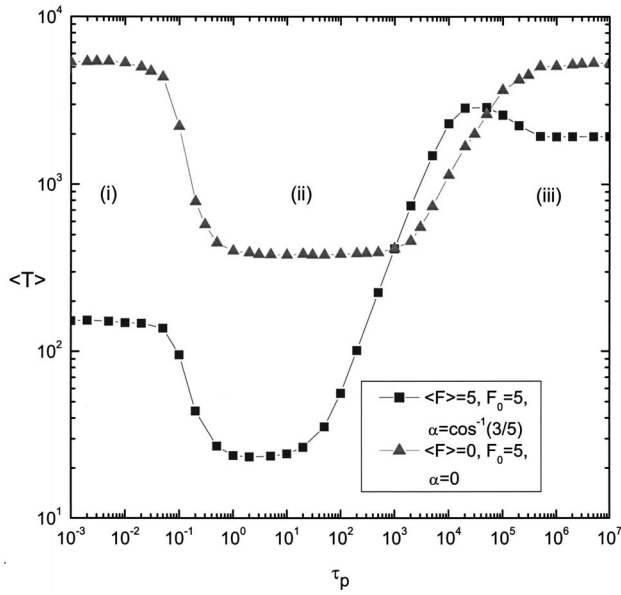


FIG. 1. The variation of the bond lifetime with the time period for periodic forcing. Two sets of  $\{\langle F \rangle, F_0, \alpha\}$  are considered. Three regimes can be identified.

force  $\langle F \rangle + F_0 \cos \alpha$  when it breaks, as demonstrated in Figs. 1–3. Figure 2 clearly shows that at  $\langle F \rangle = 0$ , the rupture rate is highest at  $\alpha = 0$  and lowest at  $\alpha = -\pi/2$ . The former case represents the constant pulling  $F = F_0 = 10$ , while the latter case corresponds to the absence of pulling  $F = 0$ . Note that  $T_0 = k_0^{-1}$  denotes the bond lifetime estimated by the Kramers rate theory in the absence of external force. The theoretical value is about twice greater than that obtained from Langevin simulation. For  $\alpha = -\pi/2$ , the pulling force with  $\langle F \rangle = 0$  is actually  $F(t) = F_0 \sin 2\pi t / \tau_p$ . At very large  $\tau_p$ ,  $F(t) \approx F_0(2\pi / \tau_p)t$ , which corresponds to the typical protocol for

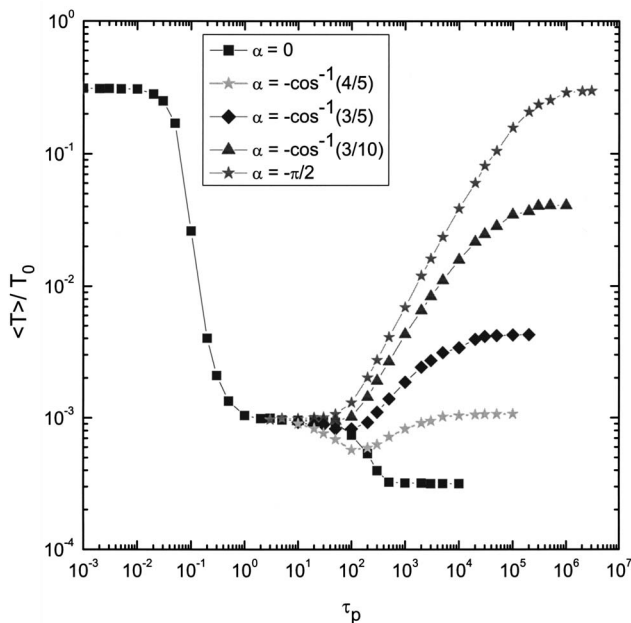


FIG. 2. The variation of the bond lifetime with the period is plotted for different values of phase lag at  $\langle F \rangle = 0$ .  $T_0 = k_0^{-1}$  is the bond lifetime calculated by the Kramers rate theory without pulling.

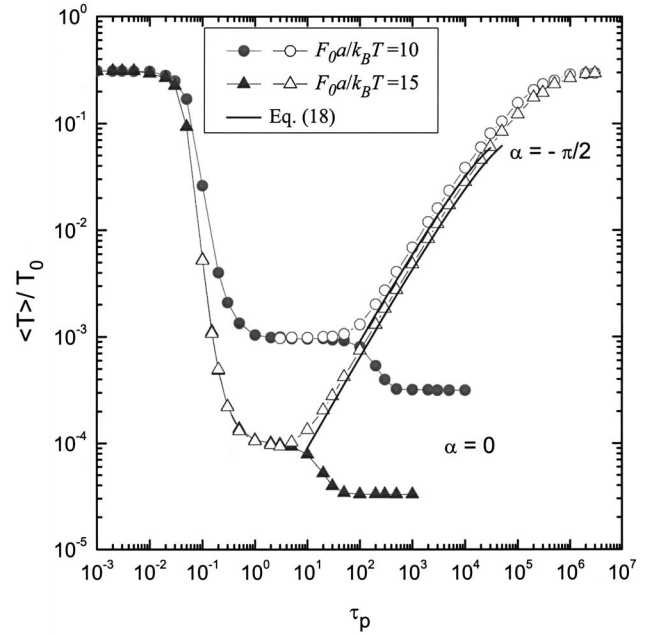


FIG. 3. The variation of the bond lifetime with the period is plotted for different force amplitudes and phase lag at  $\langle F \rangle = 0$ . The solid lines denote the prediction based on the forced Kramers rate theory under constant loading rate [Eq. (18)].

pulling experiments, the constant loading rate condition with  $\dot{F}_t = 2\pi F_0 / \tau_p$ . The analytical expression of the bond lifetime under constant loading rate has been obtained<sup>6,7</sup>

$$\langle T \rangle \equiv \frac{F_c}{\dot{F}_t} \left\{ 1 - \left[ \frac{-\ln(\beta b \dot{F}_t \tau_* (\beta E_a^*)^{n-2/n+1})}{\beta E_a^*} \right]^{n/n+1} \right\} \quad (18)$$

for the fast pulling regime  $1 - \dot{F}_t \langle T \rangle / F_c \ll 1$ . Figure 3 shows that the crossover from regime (ii) to regime (iii) with increasing  $\tau_p$  can be well represented by the pulling under constant loading rate [Eq. (18)]. Eventually, the effect of linear loading can be neglected as  $2\pi F_0 \langle T \rangle / \tau_p \rightarrow 0$ .

In the intermediate  $\tau_p$  where the adiabatic approximation is valid and still  $\langle T \rangle \gg \tau_p$ , there is indeed a region where  $\langle T \rangle$  is independent of  $\tau_p$  and Eq. (11) or (15) can be applied depending on the magnitude of  $F_0$ . The lifetime is independent of the frequency of the force for the following reason. In each period, the escape probability is largest in the vicinity of  $F_0$ , and this escape window is  $\sim \tau_p$ . Since the occurring frequency of this window is proportional to  $\tau_p^{-1}$ , the total escape window over many periods is independent of  $\tau_p$  as long as  $\langle T \rangle \gg \tau_p$ . Figure 4 shows the rupture rate  $\langle k \rangle = \langle T \rangle^{-1}$  as a function of  $F_0$  for intermediate  $\tau_p$ -independent region simulations. Simulation results are compared to results from numerical solution of Kramers rate theory and asymptotic Bell's and Garg's expressions. The inset demonstrates the validity of Bell's expression in small force amplitude regime. Equation (15), based on a general Garg's form, describes  $\langle k \rangle$  with high precision at large  $F_0$  and agrees with simulation results over a wide range of force amplitudes, while Eq. (11), based on Bell's expression, describes  $\langle k \rangle$  well at small  $F_0$ .

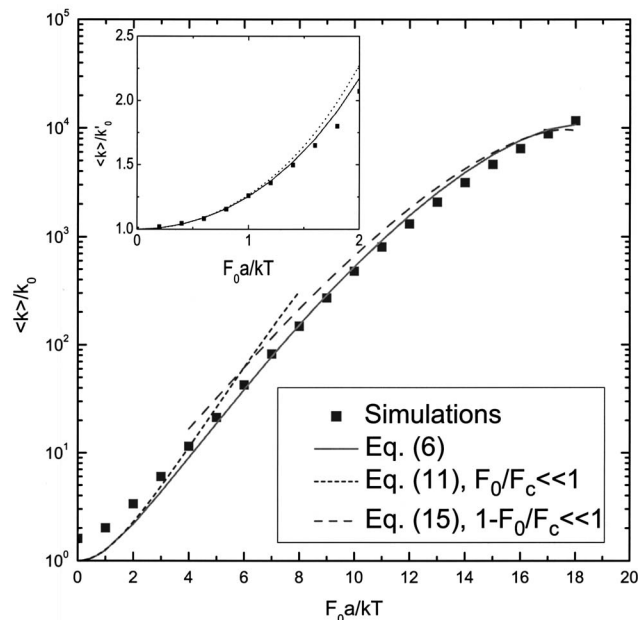


FIG. 4. The variation of the escape rate with the force amplitude for  $\langle F \rangle = 0$ ,  $\tau_p = 1.0$ , and  $\alpha = 0$ . The lines represent numerical solution of Kramers rate theory and asymptotic expressions. The inset shows the escape rate under small force amplitude, where Bell's expression is valid.

This consequence indicates that periodic forcing experiments allow one to extract  $k_0$  and  $a$  at small  $F_0$ , and  $F_c$  and  $E_a^*$  at large  $F_0$ .

It should be noted that a very weak linker is commonly used in unbinding experiments by AFM. It will make periodic forcing problematic. One of the possible ways to overcome this problem is to perform periodic forcing experiments by biomembrane force probe, which can monitor the force required to break bonds between pairs of receptor/ligand, antigen/antibody, etc., without the linker.<sup>1</sup>

## B. Correlated random forcing

In order to study the effect of force fluctuations on the rupture rate, an exponentially correlated random forcing  $\sigma_F \tau_r$  is added into an average force  $\langle F \rangle$ . The correlated random forcing is characterized by  $\tau_r$ , which is always large compared to the characteristic time of the random forcing associated with thermal fluctuations. Similar to periodic forcing escape, there also exists three regimes, (i)  $\tau_r \ll \tau_R \ll \langle T \rangle$ , (ii)  $\tau_R \ll \tau_r$ , and (iii)  $\tau_R \ll \langle T \rangle \ll \tau_r$ , as shown in Fig. 5, for different values of the mean force  $\langle F \rangle$ , force amplitude  $\sigma_F$ , and initial random forcing  $r_0$ .

When the correlation time of the fluctuating forcing  $\tau_r$  is small compared to the local relaxation time of the Brownian particle in the well  $\tau_R$ , the pulling fluctuates so rapid that the Brownian particle cannot respond to the fluctuating force in time. The adiabatic approximation cannot be applied to the random forcing but the average pulling. As a result, the particle feels essentially the average pulling and is not sensitive to the random forcing, just like periodic forcing. Nevertheless, the effect of correlated random force is like rising temperature (white noise). Figure 5 shows that the bond lifetime  $\langle T \rangle$  in this regime is slightly less than that associated with

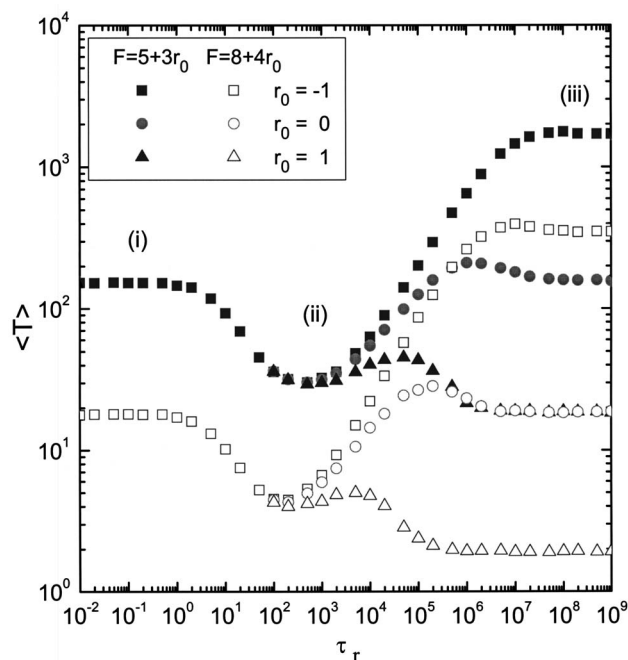


FIG. 5. The variation of the bond lifetime with the correlation time is plotted for correlated random forcing. Two sets of  $\{\langle F \rangle, \sigma_F, r_0\}$  are considered. Three regimes can be identified.

large  $\tau_r$  and  $r_0 = 0$  (very weak white noise). Moreover, as illustrated in Fig. 6, the mean rupture force  $\langle F_u \rangle$  is slightly greater than  $\langle F \rangle$  but is also  $\tau_r$  independent. Figure 7 demonstrates that the standard deviation of the rupture force  $\langle \sigma_F \rangle_u$  equals to that of the random forcing  $\sigma_F$ . This result reveals that the pulling force does fluctuate fully according to the random forcing protocol before escape. However, the pulling force at the instant of bond rupture contributes to the rupture process insignificantly. The contributions of fluctuating forces actually cancel out each other and lead to an average pulling.

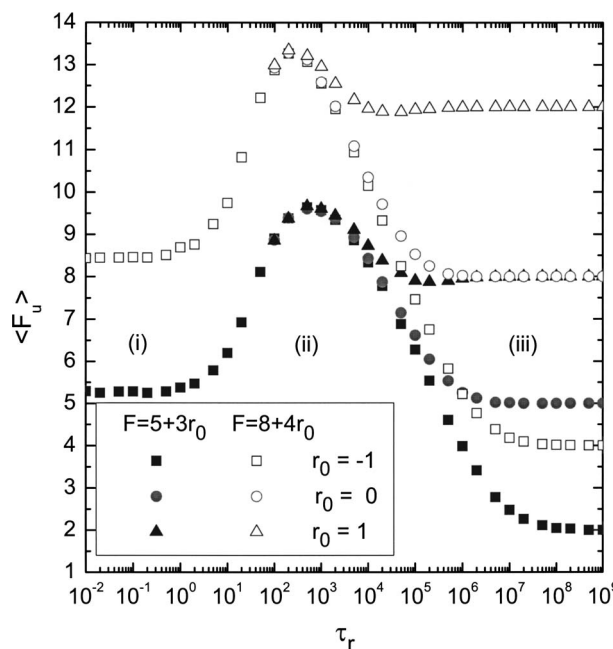


FIG. 6. The variation of the rupture force with the correlation time for correlated random forcing is plotted for two sets of  $\{\langle F \rangle, \sigma_F, r_0\}$ .

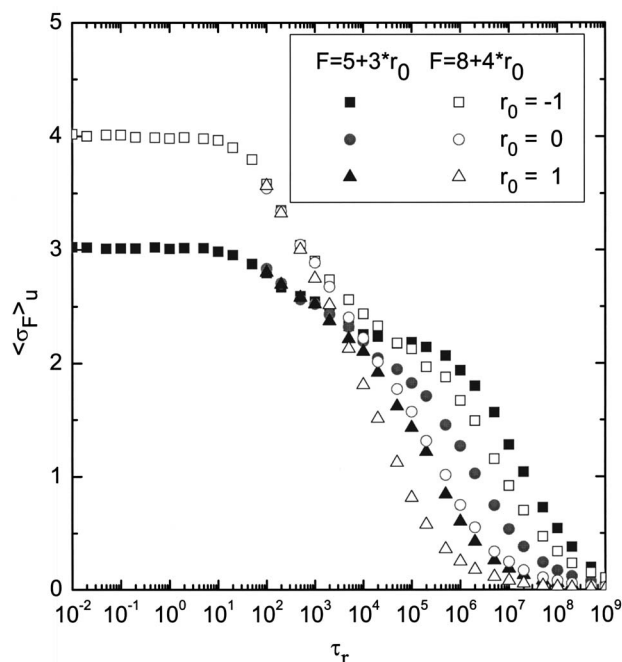


FIG. 7. The variation of the standard deviation of the rupture force with the correlation time for correlated random forcing is plotted for two sets of  $\{\langle F \rangle, \sigma_F, r_0\}$ .

As the correlation time is increased and becomes much greater than the relaxation time,  $\tau_R \ll \tau_r$ , the adiabatic approximation is valid and the random forcing contributes to the rupture kinetics. On the basis of Bell's expression for weak pulling  $\beta \langle F \rangle a \leq 1$ , one has

$$\langle k \rangle \cong k_0 \exp \left[ \beta \langle F \rangle a + \frac{(\beta a)^2 \sigma_F^2}{2} \right]. \quad (19)$$

This result shows that the fluctuations of the pulling force speed up the rupture process.<sup>8</sup> However, Eq. (19) is valid for  $\tau_R \ll \tau_r \ll \langle T \rangle$  because the particle must have enough time to feel the complete random forcing before bond rupture. Figure 6 illustrates that the mean rupture force is always highest in regime (ii). This consequence indicates that the rupture rate enhanced by the fluctuating forces greater than  $\langle F \rangle$  is more than that impeded by the force less than  $\langle F \rangle$ . Moreover, the fact that  $\langle \sigma_F \rangle_u < \sigma_F$  in this regime, as illustrated in Fig. 7, reveals that the pulling force felt by the bond does not follow the random forcing protocol completely. In fact, the bond lifetime is less than the correlation time,  $\langle T \rangle < \tau_r$ , and the rupture takes place during the period  $F > \langle F \rangle$ .

When the correlation time is large compared to the bond lifetime,  $\langle T \rangle \ll \tau_r$ , the escape process behaves like that under constant force and the initial random forcing becomes dominant. The effective pulling can be regarded as  $F = \langle F \rangle + \sigma_F r_0$  and the bond lifetime becomes  $\tau_r$  independent, as illustrated in Fig. 5. Consequently, the rupture force is also expected to follow  $\langle F_u \rangle = \langle F \rangle + \sigma_F r_0$ , as confirmed in Fig. 6. Although the pulling force is essentially constant, it still fluctuates primarily according to the Gaussian distribution, unlike periodic forcing. Figure 7 shows that the standard deviation of the rupture force declines with increasing  $\tau_r$  and approaches zero variance since the fluctuating force felt by the bond fails to

follow the random forcing protocol before rupture. Moreover, the faster the escape of the Brownian particle, the lower the value of  $\langle \sigma_F \rangle_u$  because there is less time for the pulling force to fluctuate without time correlation.

## VI. CONCLUSION

In single-molecule pulling experiments, various forcing protocols can be used to extract kinetic information of the weak bond. By using overdamped Langevin dynamics, we investigate the kinetics of a Brownian particle escaping from a trap under the periodic forcing and correlated random forcing. The former is characterized by the period  $\tau_p$ , while the latter is characterized by the correlation time  $\tau_r$ . By comparison to the local relaxation  $\tau_R$  and bond lifetime  $\langle T \rangle$ , three regimes can be identified for periodic forcing. In the regime  $\tau_p \ll \tau_R$ , the adiabatic approximation cannot apply to the periodic forcing and the bond lifetime is determined by the average pulling force  $\langle F \rangle$ . In the regime  $\tau_R \ll \tau_p \ll \langle T \rangle$ , the adiabatic condition is satisfied and the periodic forcing enhances the rupture rate which, however, is  $\tau_p$  independent. Analytical expressions have been obtained by the asymptotic analysis based on Bell's expression for small force amplitude [Eq. (11)] and generalized Garg's form for large amplitude [Eq. (15)]. In the regime  $\langle T \rangle \ll \tau_p$ , the bond lifetime depends on the phase lag (initial forcing). The rupture process behaves essentially like under the forcing condition of constant force or constant loading rate [Eq. (18)].

We have shown that periodic forcing can lead to the nonmonotonic behavior of the lifetime associated with a biomolecular complex. Although periodic forcing does not give additional information, it allows one to extract complete kinetic parameters accurately by choosing suitable force amplitude or frequency. It is recently reported that periodic forcing could also be useful in modulating enzymatic activity. A similar nonmonotonic dependency of enzymatic activity on the forcing frequency was observed for a single enzyme manipulated by an external harmonic force.<sup>16</sup> This study also revealed that one can selectively obtain information on the reaction rates by choosing an oscillation in a suitable frequency range.

For correlated random forcing, there exists three regimes as well. In the regime  $\tau_r \ll \tau_R$ , the fluctuating force is too fast to be followed by the Brownian particle during the escape process. The bond lifetime is thus insensitive to  $\tau_r$  and is decided by  $\langle F \rangle$ , similar to the periodic forcing. In the regime  $\tau_R \ll \tau_r$ , the pulling force felt by the bond before rupture does not follow the random forcing protocol completely. The dissociation rate enhanced by the fluctuating force greater than  $\langle F \rangle$  is more than that hindered by the fluctuating force less than  $\langle F \rangle$ . As a result, the force fluctuation enhances the bond-rupture rate. In the regime  $\langle T \rangle \ll \tau_r$ , the pulling force felt by the bond fails to follow the random forcing protocol completely and the initial forcing becomes dominant. Although the rupture process behaves like under constant force, there exists force fluctuations which, however, diminish with increasing  $\tau_r$ .

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<sup>1</sup>E. Evans, *Annu. Rev. Biophys. Biomol. Struct.* **30**, 105 (2001).

<sup>2</sup>E. L. Florin, V. T. Moy, and H. E. Gaub, *Science* **264**, 415 (1994).

<sup>3</sup>G. Hummer and A. Szabo, *Biophys. J.* **85**, 5 (2003).

<sup>4</sup>O. K. Dudko, A. E. Filippov, J. Klafter, and M. Urbakh, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 11378 (2003).

<sup>5</sup>H.-Y. Chen and Y.-P. Chu, *Phys. Rev. E* **71**, 010901(R) (2005).

<sup>6</sup>Y.-J. Sheng, S. Jiang, and H.-K. Tsao, *J. Chem. Phys.* **123**, 091102 (2005).

<sup>7</sup>H.-J. Lin, H.-Y. Chen, Y.-J. Sheng, and H.-K. Tsao, *Phys. Rev. Lett.* **98**, 088304 (2007).

<sup>8</sup>H.-J. Lin, Y.-J. Sheng, H.-Y. Chen, and H.-K. Tsao, *J. Phys. Chem. B* **111**, 6493 (2007).

<sup>9</sup>B. T. Marshall, M. Long, J. W. Piper, T. Yago, R. P. McEver, and C. Zhu, *Nature (London)* **423**, 190 (2003).

<sup>10</sup>R. J. Clarke, O. E. Jensen, J. Billingham, A. P. Pearson, and P. M. Williams, *Phys. Rev. Lett.* **96**, 050801 (2006).

<sup>11</sup>O. Braun, A. Hanke, and U. Seifert, *Phys. Rev. Lett.* **93**, 158105 (2004).

<sup>12</sup>H. A. Kramers, *Physica (Amsterdam)* **7**, 284 (1940).

<sup>13</sup>G. I. Bell, *Science* **200**, 618 (1978).

<sup>14</sup>A. Garg, *Phys. Rev. B* **51**, 15592 (1995).

<sup>15</sup>P. Hänggi, P. Talkner, and M. Borkovec, *Rev. Mod. Phys.* **62**, 251 (1990).

<sup>16</sup>M. A. Lomholt, M. Urbakh, R. Metzler, and J. Klafter, *Phys. Rev. Lett.* **98**, 168302 (2007).