

Theoretical determination of the strength of soft noncovalent molecular bonds

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The strength of a simple soft bond under a constant loading rate is studied theoretically. There is a scaling regime where rebinding is negligible and the rupture force scales as $\text{const} + [\ln(kv)]^{2/3}$, where kv is the loading rate. The scaling regime is smaller for weaker bonds and broader for stronger bonds. For a loading rate beyond the upper limit of the scaling regime, the bond rupture is deterministic and the thermal effects are negligible. For a loading rate below the lower limit of the scaling regime, the contribution from rebinding becomes important and there is no simple scaling relation between the rupture force and the loading rate. Our theory takes the effect of rebinding in the calculation, therefore we find good agreement between theory and simulation even below the scaling regime.

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Noncovalent molecular bonds in biological systems are soft. Typical binding energy is on the order of $10k_B T$. This extremely weak binding energy enables a fast response to the external stimuli that is preferred by living systems [1]. At the same time, these weak bonds are sensitive to thermal fluctuations, thus the study of the mechanical response of the bonds has to consider thermal effects. Experimentally, the strength of a soft bond is often characterized by the relation between the rupture force and loading rate, i.e., the dynamic force spectroscopy (DFS) [2]. The imposed loading rate in an experiment provides a time scale that can be used to probe the internal dynamics of the bond under study. Therefore DFS experiments of soft bonds have revealed valuable structural and dynamical information for biological soft materials, ranging from the energy landscape of several noncovalent bonds to the unfolding mechanisms of several types of proteins [2].

The theoretical problem of finding the rupture force of a bond under a constant loading rate corresponds to evaluating the escape time of a particle in a time-varying one-dimensional potential well in the presence of thermal fluctuations. This is an interesting extension of the celebrated Kramers escape theory [3–5]. To study experimentally relevant problems, extensions of the theory to systems with multiple bonds in parallel [6] or in series [7], and extensions of the theory to bonds that are described by higher dimensional potentials [8,9] have been studied recently. In the case of multiple bonds, experiments [12] have found good qualitative agreement with theories [6]. In the case of bonds with more than one energy minimum, the theory [9] provides an alternate interpretation for experimental results. Theories that aim at reconstructing the potential energy landscape from the force-extension curve [10] or from DFS of probes with varying spring constants [11] have also been considered and applied to analyze experimental and simulation data.

Despite the successes on both the experimental and theoretical sides, it is surprising that the conventional “linear theory” [2,13,14] for the DFS of a simple one-dimensional bond, the cornerstone of theoretical analysis of most experimental studies, is challenged recently by two different theories that we refer to as harmonic theory [7] and cubic theory [15], respectively. Linear theory assumes that under a con-

stant loading rate the activation barrier for bond dissociation diminishes linearly in time. The resulting rupture force scales like $\ln(kv)$, where kv is the loading rate. In the harmonic theory, Hummer and Szabo [7] treated the bond as a harmonic potential but ignored rebinding and the details of potential shape near the barrier. The rupture force is predicted to scale like $\text{const} + (\ln kv)^{1/2}$. The cubic theory of Dudko *et al.* [15] assumed that bond rupture occurs when the potential energy near the barrier can be approximated by a cubic function. It follows an earlier work by Garg [5], neglects rebinding, and predicts that the rupture force scales like $\text{const} + (\ln kv)^{2/3}$. Reference [7] applied harmonic theory to describe the unfolding of protein titin, and Ref. [15] showed that the scaling prediction in a cubic theory for Morse potential is better than a linear theory by comparing the fitting of rupture force in simulation to $[\ln(kv)]^{2/3}$ and $\ln(kv)$, respectively. Both Refs. [7] and [15] did not provide a quantitative analysis for the effect of rebinding. In another seemingly unrelated field of the physics of the atomic scale friction, linear theory [16] and cubic theory [17] have both been proposed to describe the rupture of bonds (with no rebinding) between two parallel surfaces under relative motion. Although data from a friction experiment [18] is not able to discriminate between linear and cubic theories, it is believed that for atomic friction, linear theory works for weak bonds with a binding energy slightly greater than $k_B T$, and cubic theory is better for bonds with realistic binding energies that are on the order of $10k_B T$ [17,19].

To provide the underlying physical picture, including rebinding, for the rupture of molecular bonds under a constant loading rate, in this paper we present an analysis of simple bond DFS. Unlike Refs. [7] and [15], we do not discuss higher dimensional problems. We focus on simple bonds, and we consider a loading rate beyond the scaling regime. Our result shows that an extended cubic theory that takes rebinding into account is suitable to describe the rupture of simple bonds even at a low loading rate beyond scaling regime.

In a typical DFS experiment, a bond attached to a surface on one end, and a spring on the other end is pulled out by the spring with a constant velocity v until the bond breaks and

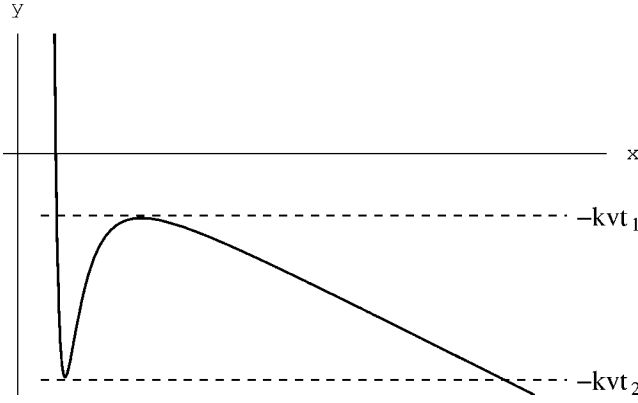


FIG. 1. The solid curve is $\tilde{F}(x)$. When $t_1 < t < t_2$ there are three solutions to the equation $\tilde{F}(x) = -kvt$ and they correspond to the potential wells and the barrier between them.

the spring recoils to its rest position. For a sufficiently simple system, a single reaction coordinate x , usually the distance between the particle and the substrate, is sufficient to describe the dynamics of the system. Let $U_0(x)$ be the potential of the bond and L_0 be its equilibrium position in the absence of external forces. The potential of the spring is $U_{\text{spring}}(x, t) = (k/2)(vt + L_0 - x)^2$ for $t \geq 0$, where k is the spring constant. When the total potential $U(x, t) = U_0(x) + U_{\text{spring}}(x, t)$ is bistable, the minimum at smaller x (due to the bond), at larger x (free state), and the barrier are located at $x_a(t)$, $x_f(t)$, and $x_b(t)$, respectively. $x_a(t)$, $x_b(t)$, and $x_f(t)$ is determined by solving $-kvt = -(dU_0/dx) - k(x - L_0) \equiv \tilde{F}(x)$. As Fig. 1 shows, the solutions to $-kvt = \tilde{F}(x)$ at time t are the crossing points between the stationary curve $y = \tilde{F}(x)$ and the moving horizontal line $y = -kvt$, which scans downward with the constant rate kv . Between t_1 and t_2 , $-kvt = \tilde{F}(x)$ has three solutions and $U(x, t)$ is bistable. We further denote the local minimum (maximum) of $\tilde{F}(x)$ as $x_1(x_2)$, i.e., $-kvt_{1(2)} = \tilde{F}(x_{2(1)})$.

The dynamics of the reaction coordinate is described by the Langevin equation, $dx/dt = -\gamma[\partial U(x, t)/\partial x] + \zeta(t)$. Here γ , assumed to be independent of x for simplicity, is the damping coefficient, ζ is the thermal noise with zero mean and variance $2k_B T \gamma$. The energy unit is chosen to be $k_B T$, L_0 is the unit length, and the unit time is chosen such that $\gamma = 1$. When $U(x, t)$ is bistable, the system is in the bound state if $x(t) < x_b(t)$, otherwise it is in the free state. The probability $\phi(t)$ that the system is in the bound state at time t satisfies the reaction equation

$$\frac{\partial \phi(t)}{\partial t} = -k_{\text{off}}(t)\phi(t) + k_{\text{on}}(t)[1 - \phi(t)], \quad (1)$$

the probability that bond rupture occurs at time t is

$$\begin{aligned} P_b(t) &= \phi(t)k_{\text{off}}(t)e^{-\int_t^t dt' k_{\text{on}}(t')} \\ &= \left(1 - \int_{t_1}^t dt' k_{\text{off}}(t')e^{-\int_{t'}^t [k_{\text{on}}(t'') + k_{\text{off}}(t'')] dt''}\right) \\ &\quad \times k_{\text{off}}(t)e^{-\int_t^t k_{\text{on}}(t') dt'} \end{aligned} \quad (2)$$

and the mean rupture force is

$$\langle k[vt + L_0 - x_a(t)] \rangle = \int_{t_1}^{t_2} dt P_b(t) k[vt + L_0 - x_a(t)]. \quad (3)$$

We assume that the time scales to reach local equilibrium in both bound and free states are much smaller than the time scales of transitions between the two states [20]. Thus Kramers's escape theory [3,4] states that the on and off rates satisfy $k(t) = (1/2\pi) \sqrt{|U'''(x_a, t)| U'''(x_b, t)} e^{-[U(x_b) - U(x_a)]}$, where $U'''(x, t) = \partial^3 U(x, t) / \partial x^2$. For $k(t) = k_{\text{off}}(t)$, $x_a = x_a(t)$; for $k(t) = k_{\text{on}}(t)$, $x_a = x_f(t)$.

Because bond rupture should occur at t closer to t_2 than t_1 , from Fig. 1 it is natural to approximate $\tilde{F}(x)$ by a quadratic function near x_1 , and by a straight line near $x_f(t)$, i.e.,

$$\tilde{F}(x) \approx \begin{cases} \frac{c}{2}(x - x_1)^2 + f_0 & x < x_2 \\ -k(x - L_0) & x > x_2. \end{cases} \quad (4)$$

Here $c = -(d^3 U_0 / dx^3)_{x=x_1}$, and $f_0 = -(dU_0/dx)_{x=x_1} - k(x_1 - L_0)$. Equation (4) corresponds to a potential that is cubic in x near x_1 , and is the spring potential near $x = L_0 + vt$. That is, cubic theory [15] follows naturally from observing the generic shape of $\tilde{F}(x)$. Notice that linear theory assumes that both $x_a(t)$ and $x_b(t)$ are independent of t [2], and harmonic theory assumes that $x_b(t)$ is independent of t and takes $U(x) = -\infty$ for $x > x_b$ so that rebinding can never happen [7]. These theories do not capture the generic shape of $\tilde{F}(x)$. A straightforward calculation leads to Kramers's on and off rates in the cubic theory,

$$\begin{aligned} k_{\text{on}}(t) &= \frac{1}{2\pi} \sqrt{k(2c[-f_0 - kvt])^{1/2}} \exp\left\{-\frac{c}{3} \left(\frac{2}{c}(-f_0 - kvt)\right)^{2/3}\right. \\ &\quad \left. - \frac{k}{2}(x_1 - L_0 - vt)^2 - U_0(x_1)\right\}, \\ k_{\text{off}}(t) &= \frac{1}{2\pi} \sqrt{2c(-f_0 - kvt)} \exp\left\{-\frac{2c}{3} \left(\frac{2}{c}(-f_0 - kvt)\right)^{2/3}\right\}. \end{aligned} \quad (5)$$

At sufficiently large kv rebinding is negligible, and the theory is considerably simpler. In the following we discuss this regime first and consider rebinding later. In this regime,

$$\begin{aligned} P_b(t) &= k_{\text{off}}(t) \exp\left(-\int_{t_1}^t k_{\text{off}}(t') dt'\right) \approx \frac{\sqrt{-2c(f_0 + kvt)}}{2\pi} \\ &\quad \times \exp\left\{-\sqrt{\frac{32}{9c}}(-f_0 - kvt)^{3/2}\right. \\ &\quad \left.- \frac{c}{4\pi kv} e^{-\sqrt{(32/9c)}(-f_0 - kvt)^{3/2}}\right\}. \end{aligned} \quad (6)$$

Neglecting a small term $k[x_1 - \langle x_a(t) \rangle]$, the rupture force has the simple form

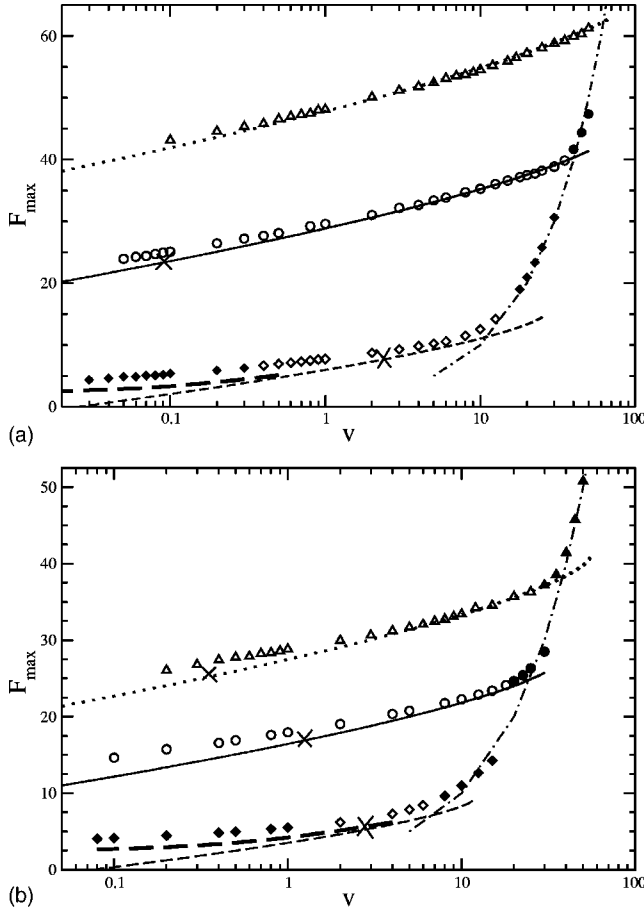


FIG. 2. (a). Rupture force for the Morse potential $U_0(x) = W\{1 - \exp[-2b(x-1)]\}^2 - 1$ with $b=1.5$ from both numerical simulation and theory. (b) The rupture force for a power-law potential $U_0(x) = W(1/2x^6 - 3/2x^2)$ from both numerical simulation and theory. The simulation results for $W=45$ are presented as triangles, $W=30$ as circles, and $W=10$ as diamonds. The filled symbols in the high ν region are where Kramers's rate theory cannot describe bond rupture, the filled symbols in the low ν region are where rebinding is important. Thick dashed lines are from the extended cubic theory, dash-dotted lines are the viscous damping force $\gamma\nu$, and the other lines are from Eq. (7). Cross marks are where $F_{\max} = U'_0(x_1) - (c/2)(x_1 - L_0)^2$. For the Morse potential with $W=45$, $F_{\max} > U'_0(x_1) - (c/2)(x_1 - L_0)^2$ within the range of our simulation.

$$F_{\max} = \left(\frac{dU_0}{dx} \right)_{x=x_1} - \left(\sqrt{\frac{9c}{32}} \ln \frac{c}{4\pi k\nu} \right)^{2/3} \sim \text{const} + [\ln(k\nu)]^{2/3}. \quad (7)$$

This scaling relation has been tested by data collapsing for simulating Morse potential in Ref. [15], and is shown to provide better scaling than the linear theory. Here we provide a direct comparison between simulation and theory for a couple of potentials so that any discrepancy between theory and simulation can be examined critically. Figure 2 compares the rupture force predicted by Eq. (7) and numerical simulations for Morse potential $U_0(x) = W\{1 - \exp[-2b(x - L_0)/L_0]\}^2 - 1$ with $b=1.5$ and a power law potential $U_0(x) = W\{[1/2(x/L_0)^6] - [3/2(x/L_0)^2]\}$ which is taken from

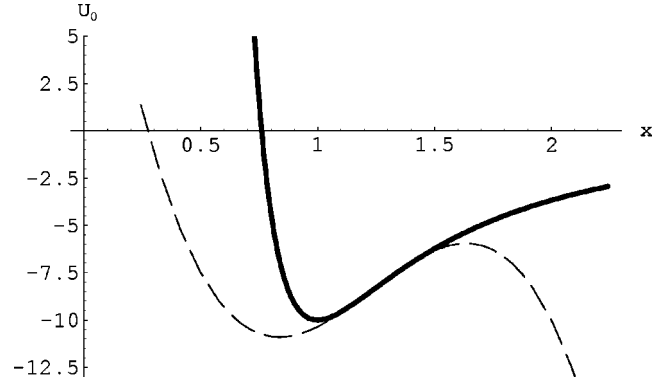


FIG. 3. Solid line: the power-law potential with $W=10$; dashed line: potential predicted by cubic theory. The prediction of the extended cubic theory is not highly accurate when $x_a(\langle t_r \rangle) < L_0 = 1$.

Ref. [6], the spring constant is chosen to be $k=0.8$. Notice that the curves from Eq. (7) on a semilogarithmic plot are not straight lines, they are curved. Our result shows better agreement between Eq. (7) and simulations for stronger bonds, and deviation of simulation from the cubic theory at high and low loading rates.

The deviation of simulation from Eq. (7) at a high loading rate can be understood by noticing that according to Eq. (6), the probability that bond rupture occurs between t_1 and t_2 is $\int_{t_1}^{t_2} P_b(t) dt \approx 1 - e^{-(c/4\pi k\nu)}$, i.e., at a high loading rate with $c/4\pi k\nu \leq 1$, bond rupture is not likely to occur between t_1 and t_2 . This is because from t to t_2 , $x_a(t)$ moves a distance $x_1 - x_a(t) = \sqrt{2k\nu(t_2 - t)}/c$, and the displacement of a particle under free diffusion during the same time interval is $x_d(t) = \sqrt{2(t_2 - t)}$, thus $c/4\pi k\nu \sim \{x_d/[x_1 - x_a(t)]\}^2$ compares free diffusion and the motion of $x_a(t)$ due to the spring. When $c/4\pi k\nu \geq 1$, i.e., $x_1 - x_a(t) < x_d(t)$, free diffusion near $x_a(t)$ is faster than the motion of $x_a(t)$, therefore the particle senses the barrier and the bond rupture is due to barrier crossing, and Kramers's theory is appropriate to describe bond rupture. On the other hand, if $x_1 - x_a(t) > x_d$, free diffusion alone cannot catch up the motion of potential minimum, therefore the particle is located near $x_a(t)$ at all time until the barrier vanishes. That is, the thermal effect is negligible in this case. The filled symbols in the high ν region of Fig. 2 shows where $c/4\pi k\nu < 2$, and that is when Eq. (7) starts to deviate from the simulation result. Notice that at sufficiently high ν , viscous damping force exceeds $(dU_0/dx)_{x=x_1}$, therefore the curves in Fig. 2 approach asymptotically to $F_{\max} = \gamma\nu$ at very high ν .

The deviation of simulation from Eq. (7) at a low loading rate is due to the effect of rebinding. When the chance of observing a rebinding event between the mean rupture time $\langle t_r \rangle$ predicted by Eq. (6) and t_2 is not negligible [21], rebinding has to be taken into account. Filled symbols in the low ν region of Fig. 3 are where $\int_{\langle t_r \rangle}^{t_2} k_{\text{on}}(t) dt \geq 0.1$. For both Morse potential and power-law potential, rebinding is important for weaker bonds. Therefore weaker bonds have smaller upper limits for applying Kramers's rate theory and greater lower limits for neglecting rebinding, i.e., the deviation of Eq. (7) is more serious for weaker bonds. The rupture force F_{\max} in

the regime where rebinding is important can be calculated numerically by extending cubic theory to include nonzero k_{on} in Eq. (5) into Eqs. (2) and (3). The long dashed lines in Fig. 2 show a prediction of this extended cubic theory in regions where rebinding is not negligible. Indeed the extended cubic theory provides a good prediction for the rupture force in a low loading rate regime where Eq. (7) deviates from the simulation result. Notice that the basic assumptions in the linear theory [2] and the harmonic theory [7] are such that the effect of rebinding cannot be taken into account in the calculations, therefore they both fail for weak bonds at a low loading rate.

It is interesting to discuss the small difference between the rupture force in the extended cubic theory and the simulation shown in Fig. 2; it reveals the limit of the theoretical description for DFS. Figure 3 shows a power-law potential with $W=10$, the case where the difference between the extended cubic theory and simulation is the most significant, and the potential predicted by extended cubic theory. The extended cubic theory fits $U_0(x)$ very well near x_1 but deviates from $U_0(x)$ for $x < L_0$. Thus the extended cubic theory does not have a high accuracy when the bond rupture time t predicted by extended cubic theory has $x_a(t) < L_0$, and that is when

$$F_{\text{max}} < U'_0(x_1) - \frac{c}{2}(x_1 - L_0)^2, \quad (8)$$

where F_{max} is calculated from the extended cubic theory. The cross marks in Fig. 2 are where $F_{\text{max}} = U'_0(x_1) - (c/2)(x_1 - L_0)^2$, indeed they mark the limiting loading rates for the high precision prediction of the extended cubic theory. Finally, we note that in analyzing experimental data, the fitting

to the extended cubic theory uses data both inside and outside the high precision region. Therefore the resulting information such as binding energy and k_{on} should be very close to true values. This explains why the data fitting in Ref. [15] is so successful. Thus, the extended cubic theory, an attempt to mimic the potential landscape of a bond near where bond rupture occurs, not only captures the basic physical picture of bond rupture, but also provides good quantitative results for analyzing experimental data.

In summary, the extended cubic theory for the DFS of a simple soft bond, unlike the linear and harmonic theories, is derived from the generic shape of the potential landscape, and is able to take the effect of rebinding into account. Our analysis shows that for simple bonds, rebinding is important at a low loading rate, the thermal effect is negligible at a high loading rate, in the intermediate loading rate the rupture force scales as $\text{const} + [\ln(kv)]^{2/3}$, and the scaling regime is broader for stronger bonds. The numerical simulations for a couple of model potentials with typical binding energy agree with the extended cubic theory even at a low loading rate where rebinding is important. It would very interesting to extend the present model to multiple bonds in series and multiple bonds in parallel, and compare them to the analysis done in previous studies [6,7]. Since rebinding is important for studying multiple bonds in parallel [6], the extended cubic theory would be suitable to study these systems, and that is our future direction.

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- [20] This assumption is reasonable for simple bonds but is likely to fail in more complicated situations. For example, in large proteins the time scale for equilibration within the local potential minimum can be comparable to that of barrier crossing.
- [21] The chance of observing a rebinding event between $\langle t_r \rangle$ and t_2 can be expressed by $\int_{\langle t_r \rangle}^{t_2} k_{\text{on}}(t) dt = (c/4\pi kv) (8k^2/c)^{1/4} e^{-U_0(x_1)} \int_{(c/4\pi kv)^{-1/2}}^1 dw (-\sqrt{(9c/8)\ln w})^{-1/6} e^{(-1/2k)[(-\sqrt{(9c/8)\ln w})^{2/3} - U'_0(x_1)]^2}$.