Lifetime of ligand-receptor clusters under external force

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We present a theory for the lifetime $T(N,f)$ of a ligand-receptor cluster with N bonds under constant external force $F=fN$, where f is force per bond. We show that there exists a critical force per bond f_c such that at large N when $f>f_c$ the lifetime $T(N,f)$ is independent of cluster size N; when $f<f_c$ the lifetime scales as $\ln[T(N,f)]\sim N$ due to a free energy barrier with height $\sim N$ along the dissociation path; when $f=f_c$, the lifetime scales as $T(N,f)\sim N^{1/3}$ due to strong fluctuation of a number of closed bonds. Our study shows that adhesion clusters with more bonds are more stable at small f but respond to external force as fast as smaller adhesion clusters at large f.

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I. INTRODUCTION

Cell adhesions are mediated by specific noncovalent bonds that are reversible and sensitive to external perturbations [1]. The response of adhesion complexes to external force is important in embryo development, cell migration, and immunological response [2], therefore single molecule pulling experiments for adhesion complexes have attracted considerable interest [3]. In biological situations, multiple adhesion complexes are arranged in parallel as adhesion clusters in cell-cell or cell-matrix adhesion. When an external force acts on an adhesion cluster, broken bonds may rebind before the whole adhesion cluster dissociates because the remaining closed bonds still hold two surfaces close to each other. This effect is clearly important for cell adhesion, and it cannot be studied by single molecule experiments. Recently, experiments have started to study the response of multiple parallel bonds to an overall mechanical load [4]. Theoretical study of adhesion clusters under external force begins with the seminal work of Bell [1], in which the lifetime of adhesion clusters under constant external force was studied by a deterministic equation. Later Bell's work was extended to include fluctuation of a number of closed bonds by a stochastic model [5]. Recently, the popularity of mechanical pulling experiments has stimulated theories [6–8] for the rupture force of adhesion clusters under linear load rate. Adhesion clusters under constant displacement have also been studied [9].

As shown schematically in Fig. 1, an adhesion cluster made of ligand-receptor pairs can be modeled as N parallel bonds with N_b of them in the closed state and $N-N_b$ of them in the free state [5–9]. The receptors are fixed on a rigid plate A and the ligands are connected to another rigid plate B by flexible polymer linkers. When external force F is applied to the cluster, all closed bonds share the force equally, therefore at any instance each closed bond experiences an external force $F/N_b \equiv f/n_b$, where $f=F/N$ is force per bond and $n_b=N_b/N$ is the fraction of closed bonds. The energy $U(x)$ of a bond along the reaction coordinate x under external force f/n_b has a bound state at x_b and a free state at x_f separated by a barrier at x_Δ, where $x_b<x_\Delta<x_f$ all depend on f and n_b. The unbinding rate of a closed bond and the rebinding rate of a broken bond can be calculated from the Kramers theory [10,11], i.e.,

$$k_{\text{off}}(f,n_b) = \frac{D}{2\pi k_B T}\sqrt{U''(x_b)|U''(x_\Delta)|}e^{-\beta[U(x_\Delta)-U(x_b)]} \equiv \frac{D}{2\pi k_B T}\sqrt{|U''(x_\Delta)|}e^{-\beta U(x_\Delta)}e^{\beta G_b(n_b,f)} \quad (1)$$

and

$$k_{\text{on}}(f,n_b) = \frac{D}{2\pi k_B T}\sqrt{U''(x_f)|U''(x_\Delta)|}e^{-\beta[U(x_\Delta)-U(x_f)]} \equiv \frac{D}{2\pi k_B T}\sqrt{|U''(x_\Delta)|}e^{-\beta U(x_\Delta)}e^{\beta G_f(n_b,f)}. \quad (2)$$

Here $G_b(n_b,f)$ and $G_f(n_b,f)$ are the free energy associated with the bound state and free state of a single bond for given n_b and f. Equations (1) and (2) also indicate that both k_{off} and k_{on} depend on f and n_b through x_b, x_Δ, and x_p, but they are independent of N. Many previous theoretical studies [7,12–15] have discussed both k_{off} and k_{on} in the context of single bond and adhesion clusters, but theoretical studies on the relations between $T(N,f)$, the lifetime of an adhesion cluster, and N for constant external force have only been carried out by applying certain approximations for k_{off} and k_{on}. For example, Refs. [1,5] assumed that $k_{\text{off}}(f,n_b)=k_0 e^{\beta f x_0/n_b}$ and $k_{\text{on}}=\gamma$, where x_0 is the intrinsic length scale of a ligand-receptor complex, k_0 is the dissociation rate of a complex in the absence of external force, and γ is a constant. The approximations for k_{off}, and especially assuming k_{on} to be a constant, certainly limit the generality of the quantitative predictions in these studies. Other choices of k_{on} [7,16] have been chosen in more recent studies. Thus a question remains to be answered is what properties of adhesion clusters depend on the details of k_{off} and k_{on} and what properties are general features of adhesion clusters that are independent of these details. Therefore in this article we study $T(N,f)$ by constructing the effective free energy $G(N_b,f)$ of the system

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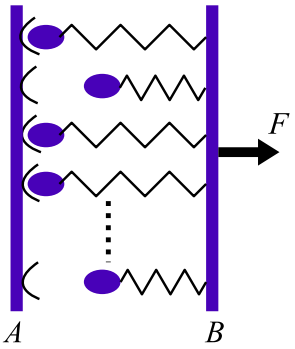

FIG. 1. (Color online) Schematics of an adhesion cluster. There are N parallel bonds with N_b of them in the closed state, $N-N_b$ of them in the free state. The receptors are fixed on a rigid plate A and the ligands are connected to another rigid plate B by flexible polymer linkers. External force F is applied on plate B while plate A is stationary.

from the master equation. We find that, indeed, there are several general features of adhesion clusters under constant external force in the limit of large N that are independent of specific forms of $k_{\rm on}$ and $k_{\rm off}$.

II. EFFECTIVE FREE ENERGY OF AN ADHESION CLUSTER

The probability that under given force F there are N_b closed bonds in the cluster at time t, $P(N_b,t)$, satisfies a one-step master equation

$$\frac{dP(N_b,t)}{dt} = g(f,N_b-1)P(N_b-1,t) - r(f,N_b)P(N_b,t) - g(f,N_b)P(N_b,t) + r(f,N_b+1)P(N_b+1,t) = J(N_b-1\to N_b) - J(N_b\to N_b+1), \tag{3}$$

for $1\leqslant N_b\leqslant N-1$, where $g(f,N_b)=(N-N_b)k_{\rm on}(f,n_b)$, $r(f,N_b)=N_b k_{\rm off}(f,n_b)$. $J(N_b-1\to N_b)=g(f,N_b-1)P(N_b-1,t)-r(f,N_b)P(N_b,t)$ is the probability current from N_b-1 to N_b. The cluster dissociates when $N_b=0$, thus it is an absorbing boundary of the master equation. It is convenient to introduce the effective free energy $G(N_b,f)$ by

$$\frac{r(f,N_b)}{g(f,N_b-1)} = e^{[G(N_b,f)-G(N_b-1,f)]/k_BT}, \tag{4}$$

i.e., when $J(N_b-1\to N_b)=0$, the system reaches a time-independent state with $P(N_b-1)/P(N_b)=\exp\{[G(N_b,f)-G(N_b-1,f)]/k_BT\}$. Note that the system under consideration does not have such a time-independent state due to the absorbing boundary at $N_b=0$. In the limit $N\gg 1$, one can approximate $G(N_b,f)-G(N_b-1,f)$ by $\partial G(N_b,f)/\partial N_b$, thus Eq. (4) can be expressed as

$$\ln\frac{N_b}{N-N_b} + \beta[G_b(n_b,f)-G_f(n_b,f)] = \beta\frac{\partial G(N_b,f)}{\partial N_b} + O\left(\frac{1}{N}\right). \tag{5}$$

Direct integration gives the free energy difference between two different N_b at fixed f,

$$G(N_b,f)-G(N_b',f) = N\left\{\int_{n_b'}^{n_b}\left[G_b(n_b'',f)-G_f(n_b'',f)+k_BT\ln\frac{n_b''}{1-n_b''}\right]dn_b''\right\}, \tag{6}$$

where $n_b=N_b/N$, $n_b'=N_b'/N$. For given f, this expression can be used to construct the free energy landscape of the adhesion cluster. Equation (6) also indicates that $G(N_b,f)\sim N$ in the large N limit, as one expects from elementary thermodynamics.

Next we make a connection between the rate equation for N_b and the free energy of the cluster. The probability current can be reexpressed by direct substitution of Eq. (4) into $J(N_b-1\to N_b)$,

$$\begin{aligned} J(N_b-1\to N_b) &= -N_b k_{\rm off}(f,n_b)[P(N_b,t) - e^{-\beta[G(N_b,f)-G(N_b-1,f)]}P(N_b-1,t)] \\ &\approx -N_b k_{\rm off}(f,n_b)(1-e^{-\beta[G(N_b,f)-G(N_b-1,f)]})P(N_b,t) \\ &\quad -N_b k_{\rm off}(f,n_b)e^{-\beta[G(N_b,f)-G(N_b-1,f)]}\partial_{N_b}P(N_b,t) \\ &\quad +\text{higher order terms}, \end{aligned} \tag{7}$$

where "higher order terms" are negligible when $N_b\gg 1$. The second term in the last expression is the diffusion current and the first term is the current driven by free energy gradient, i.e., $-N_b k_{\rm off}(f,n_b)(1-e^{-\beta[G(N_b,f)-G(N_b-1,f)]})P(N_b,t) = \left\langle\frac{dN_b}{dt}\right\rangle P(N_b,t)$. Thus when the fluctuation of N_b is small, a deterministic rate equation describes the time evolution of N_b,

$$\begin{aligned} \frac{dN_b}{dt} &= -N_b k_{\rm off}(f,n_b)(1-e^{-\beta[G(N_b,f)-G(N_b-1,f)]}) \\ &= -N_b k_{\rm off}(f,n_b) + (N-N_b)k_{\rm on}(f,n_b) \\ &\quad +\text{small corrections} \end{aligned}$$

or

$$\frac{dn_b}{dt} = -n_b k_{\rm off}(f,n_b) + (1-n_b)k_{\rm on}(f,n_b) + \text{small corrections}. \tag{8}$$

Here "small corrections" are negligible when $N_b\gg 1$. It is important to note that the approximations $\langle r(f,N_b)\rangle = r(f,\langle N_b\rangle)$ and $\langle g(f,N_b)\rangle = g(f,\langle N_b\rangle)$ are made when deriving the rate equation from the master equation. Moreover, the rate equation follows a master equation with natural boundary condition at $N_b=0$, not the biological relevant absorbing boundary condition [5]. Therefore the prediction of the rate equation is expected to be nonexact.

Although the rate equation is only an approximation to the dynamics of an adhesion cluster in the limit of small fluctuation in N_b, by comparing the first term on the right hand side of Eq. (8), i.e., number of rupture events per unit time, to the second term, i.e., number of rebinding events per unit time, one still obtains valuable information about the

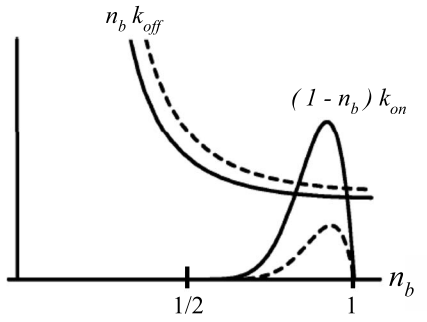

FIG. 2. Schematics of $n_b k_{\rm off}$ and $(1-n_b)k_{\rm on}$ at large f (dashed curves) and small f (solid curves). Because $k_{\rm off}$ increases and $k_{\rm on}$ decreases as f increases, at sufficiently large f, $n_b k_{\rm off} > (1-n_b)k_{\rm on}$ for all $n_b > 0$.

dynamics of an adhesion cluster under given external force. From Eqs. (1) and (2), in general $k_{\rm on}$ decreases and $k_{\rm off}$ increases as f increases or n_b decreases due to change of rebinding and unbinding energy barrier. Thus as shown in Fig. 2, $n_b k_{\rm off}$ increases as f increases or n_b decreases, and $(1-n_b)k_{\rm on}$ at given f has a peak and the height of the peak drops as f increases. From the shapes of $n_b k_{\rm off}$ and $(1-n_b)k_{\rm on}$, Eq. (8) predicts that when f is small, the number of closed bonds in the cluster decreases from $N_b=N$ to $N_b^m = N n_b^m$, where n_b^m is determined by $n_b^m k_{\rm off}(f,n_b^m) = (1-n_b^m)k_{\rm on}(f,n_b^m)$. Furthermore, there exists a critical force per bond f_c such that as force increases to $f > f_c$, the peak of $(1-n_b)k_{\rm on}$ becomes sufficiently small and $n_b k_{\rm off}$ becomes sufficiently large such that the relation $n_b k_{\rm off}(f,n_b) > (1-n_b)k_{\rm on}(f,n_b)$ holds for all nonzero n_b. At $f=f_c$ the curve $n_b k_{\rm off}$ and $(1-n_b)k_{\rm on}$ intersect at only one point n_b^*. Thus f_c and n_b^* are determined by

$$n_b^* k_{\rm off}(f,n_b^*) = (1-n_b^*)k_{\rm on}(f,n_b^*) \tag{9}$$

and

$$\frac{\partial}{\partial n_b}[n_b^* k_{\rm off}(f,n_b^*)] = \frac{\partial}{\partial n_b}[(1-n_b^*)k_{\rm on}(f,n_b^*)]. \tag{10}$$

It is clear that f_c is independent of N, so the critical force for the cluster $F_c = N f_c$ is proportional to the number of bonds in the cluster. When $f > f_c$ the cluster dissociates fast under external force. In the seminal work [1] Bell showed that f_c exists even when $k_{\rm on}$ is independent of f. He also calculated the critical force for $k_{\rm on}=\gamma$ and $k_{\rm off}(f,n_b) = k_0 e^{\beta f x_0/n_b}$.

The existence of a critical force per bond f_c also reveals important information about the shape of $G(N_b,f)$. From Eq. (4), a stable steady state at $n_b^m = N_b^m/N$ in the $N \gg 1$ limit actually means $\partial G(N_b,f)/\partial N_b = 0$ at $N_b = N_b^m$, i.e., at $f < f_c$, $G(N_b,f)$ has a local minimum at $N_b^m = N n_b^m$. Of course in this case the cluster still has a finite lifetime due to an absorbing boundary at $N_b=0$, but this is beyond the regime where the rate equation is applicable for studying $T(N,f)$. When $f > f_c$, there is no stable steady state for any nonzero n_b and $G(N_b,f)$ increases monotonically as N_b increases. The critical force is given by the condition that $G(N_b,f)$ has a inflection point at $N_b^* = N n_b^* < N$.

III. LIFETIME OF AN ADHESION CLUSTER

The above discussion suggests that the lifetime of a cluster under external force in these three regions ($f > f_c$, $f = f_c$, and $f < f_c$) should be analyzed separately. Therefore we have performed Monte Carlo simulations for the master equation with Gillespie algorithm [17] for various f and N and two choices of $k_{\rm on}$ and $k_{\rm off}$. In all simulations the initial number of closed bonds is chosen to be N. Since the goal of this paper is to point out some general properties of adhesion clusters under constant external force, these two models are chosen to illustrate the fact that although they have different $k_{\rm on}$ and $k_{\rm off}$, still the lifetimes of adhesion clusters at given f in these two models have some common properties.

In the "constant $k_{\rm on}$ model," $k_{\rm off}(f,n_b) = k_0 e^{\beta f x_0/n_b}$ and $k_{\rm on} = \gamma$ [5]; here unit time is chosen to be $1/k_0$, unit force is $(\beta x_0)^{-1}$, and $\gamma = 1$. In the "nonconstant $k_{\rm on}$ model," $U(x)$ is chosen to be $U(x)/k_BT = U_0(x)/k_BT + U_{\rm spring}(x)/k_BT = 3(x^{-12} - 8x^{-8} + 8x^{-6}) + \frac{1}{2}(x-x_p)^2$; here unit length and unit time are chosen such that the spring constant $k=1$ and $D=1$, and x_p satisfies $x_p - x_b = f/n_b$ [6,18]. When calculating $k_{\rm on}$ and $k_{\rm off}$ in the "nonconstant $k_{\rm on}$ model" Eqs. (1) and (2) are used and we have taken the values of x_b, x_Δ, and x_f with the following approximation: x_b and x_Δ are taken to be the positions of the bound state and barrier of $U_0(x)$, and $x_f \approx x_p = x_b + f/n_b$ [18,19] for the choices of f in the simulations. Thus $k_{\rm on}$ and $k_{\rm off}$ can be found by substituting U_0 and x_b, x_Δ, x_f into Eqs. (1) and (2), f_c and n_b^* are found from numerical solutions of Eqs. (9) and (10). Since the fact that the shapes of effective free energy of an adhesion cluster at the $N \gg 1$ limit below and above f_c are different is central in our analysis; a discussion on the critical forces in constant $k_{\rm on}$ and nonconstant $k_{\rm on}$ models is included in the Appendix. The important message in the Appendix is that f_c and n_b^* are both model dependent. Different choices of $k_{\rm on}$ and $k_{\rm off}$ give very different f_c and n_b^*.

Figure 3(a) shows that when f is greater than f_c, $T(N,f)$ approaches the lifetime predicted by the rate equation $T_{\rm rate}(f)$ for large clusters for both constant and nonconstant $k_{\rm on}$ models. Here $T_{\rm rate}$ is obtained by solving Eq. (8) numerically, thus it is independent of N. When $f > f_c$, $G(N_b,f)$ increases monotonically as N_b increases, and as a result the probability current in Eq. (8) is dominated by $\left\langle \frac{dN_b}{dt} \right\rangle P(N_b,t)$ term. Therefore $T(N,f)$ in this case agrees well with that in the rate equation except for a small difference due to fluctuations in N_b and different boundary conditions between the rate equation and master equation (as pointed out in the previous section). Since Eq. (8) also indicates that for large N, dn_b/dt is independent of N, thus the lifetime of the cluster at given $f > f_c$ becomes independent of N. The distinct trends of $T(N,f)/T_{\rm rate}(f)$ in two models at small N comes from different choices of $k_{\rm on}$ and $k_{\rm off}$; because our main focus is $T(N,f)$ at $N \gg 1$, we shall not discuss this difference in detail.

Figure 3(b) shows that when $N \gg 1$, $T(N,f)$ scales as $N^{1/3}$ at $f = f_c$. This power law dependence can be understood by the following analysis. As the inset of Fig. 3(b) shows, $G(N_b,f_c)$ is very flat around the inflection point N_b^*, thus the cluster spends most of its lifetime in a region with width $|N_b - N_b^*| \approx \Delta N_b$, where $\langle dN_b/dt \rangle$ is small and the dissociation of the cluster is due to a "random walk" along the N_b

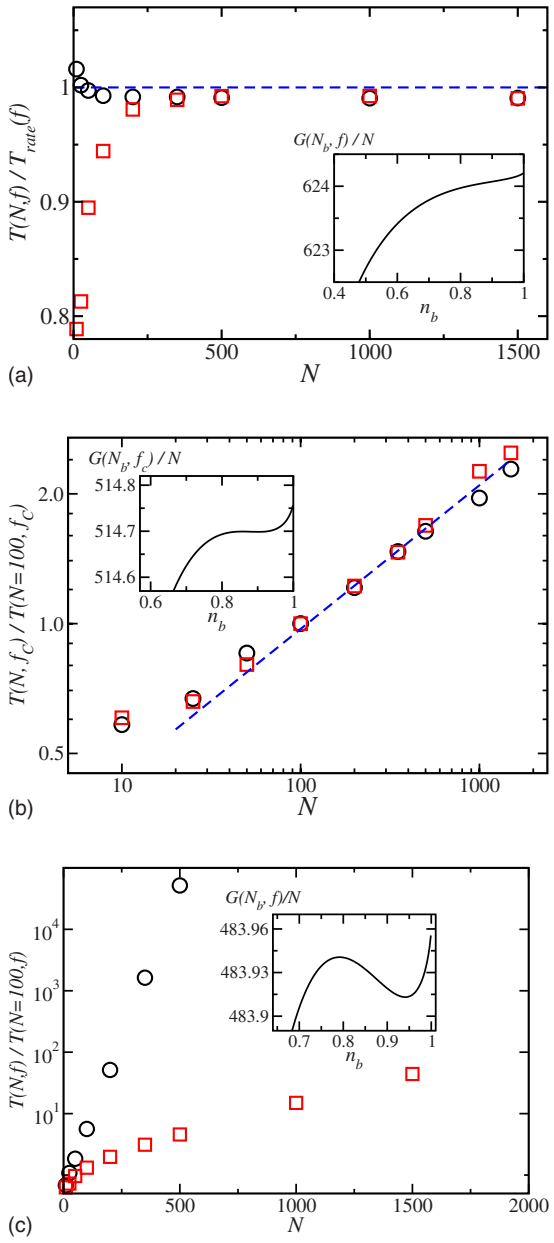

FIG. 3. (Color online) (a) $T(N,f)/T_{\text{rate}}(f)$ for $(f-f_c)/f_c=0.1$. In both the constant k_{on} model (squares) and nonconstant k_{on} model (circles), $T(N,f)$ approaches the prediction of rate equation as N increases. (b) $T(N,f_c)/T(N=100,f_c)$ for the constant k_{on} model (squares) and nonconstant k_{on} model (circles). The dashed line is $T\propto N^{1/3}$. (c) $T(N,f)/T(N=100,f)$ for $(f-f_c)/f_c=-0.03$. Both the constant k_{on} model (squares) and nonconstant k_{on} model (circles) show that $\ln T(N,f)\sim N$ for $N\gg 1$. $G(N_b,f)/N$ for $f<f_c$, $f=f_c$, and $f>f_c$ for the nonconstant k_{on} model are shown in the insets of (a), (b), and (c), respectively.

axis (fluctuation of N_b) with an exit at $N_b-N_b^*\approx-\Delta N_b$. To determine ΔN_b, note that the characteristic time scale of $\langle dN_b/dt\rangle$ at $|N_b-N_b^*|\approx\Delta N_b$, $T_{\text{det}}(\Delta N_b)$, should be roughly the same as $T_{\text{dif}}(\Delta N_b)$, the characteristic time for N_b to "diffuse" around N_b^* for a distance ΔN_b. From Eq. (7), the effective diffusion constant of N_b near N_b^* is simply $Nn_b^*k_{\text{off}}(f_c,n_b^*)$, thus $T_{\text{dif}}\sim\Delta N_b^2/[2Nn_b^*k_{\text{off}}(f_c,n_b^*)]$. From the first line of Eq. (8) one finds that $dN_b/dt\approx-[Nn_b^*k_{\text{off}}(f_c,n_b^*)/k_BT]\frac{\partial G}{\partial N_b}$ when N_b is not far from N_b^*. Since $G(N_b,f_c)-G(N_b^*,f_c)\sim\left[\frac{\partial^3 G}{\partial N_b^3}\right]_{N_b^*}\Delta N_b^3$ when N_b is close to N_b^*, and $\left[\frac{\partial^3 G}{\partial N_b^3}\right]_{N_b^*}\sim N^{-2}$ because $G\sim N$, thus one can write $G(N_b,F_c)=G(N_b^*,F_c)+g_3N^{-2}\Delta N_b^3$ where g_3 is independent of N. It follows that $T_{\text{det}}\sim\Delta N_b|dN_b/dt|^{-1}\sim\{[Nn_b^*k_{\text{off}}(f_c,n_b^*)/k_BT]\Delta N_bN^{-2}\}^{-1}$. Because $T\sim T_{\text{det}}\sim T_{\text{dif}}$, one finds that $\Delta N_b\sim N^{2/3}$ and $T\sim T_{\text{dif}}\sim N^{1/3}$. That is, the 1/3 power law is a result of diffusion in the N_b axis for a distance $\Delta N_b\sim N^{2/3}$ with an effective diffusion constant $\sim N$.

Figure 3(c) shows that when $f<f_c$, $\ln[T(N,f)]$ is linear in N. From the shape of $G(N-b,f)$ shown in the inset of Fig. 3(c), the existence of a metastable well at N_b^m indicates that one may treat cluster dissociation at $f<f_c$ as a barrier crossing process along the N_b axis. Since $G(N_b,f)\sim N$ when $N\gg 1$, at given f the height of the free energy barrier along the N_b axis for cluster dissociation is proportional to N, thus $T(N,f)$ increases exponentially with N. The cluster dissociation rate $k_{\text{cluster}}(N,f)$ can be expressed as the Kramers escape rate from N_b^m to $N_b=0$ [10,11]. Consider a steady state of an ensemble of adhesion clusters maintained by adding an adhesion cluster at $N_b=N$ whenever a cluster dissociates to $N_b=0$. Since $J(N_b-1\to N_b)=J$ is independent of N_b in the steady state, by expressing the steady state probability distribution as $P_s(N_b)=\psi(N_b)e^{-\beta G(N_b,f)}$, J can be expressed as

$$J=-\frac{\psi(N_b^m)}{\sum_{N_b=1}^{N_b=N_b^m}\frac{e^{\beta G(N_b,f)}}{N_bk_{\text{off}}(f,n_b)}}.$$

Furthermore, in the limit when the metastable well is sufficiently deep, the probability for a cluster to have $N_b\approx N_b^m$, $\tilde{P}$, follows Kramers' original approximation

$$\tilde{P}=\sum_{N_b=N_b^M}^{N}\psi(N_b)e^{-\beta G(N_b,f)}\approx\psi(N_b^m)\sum_{N_b=N_b^M}^{N}e^{-\beta G(N_b,f)},$$

where N_b^M is the local maxima of $G(N_b,f)$. Approximating the summations by integrals and evaluating the integrals by the method of steepest decent, and dropping terms of order unity, one finds that

$$k_{\text{cluster}}=-\frac{J}{\tilde{P}}\approx\frac{N_b^Mk_{\text{off}}(f,n_b^M)}{2\pi k_BT}\sqrt{G''(N_b^m,f)|G''(N_b^M,f)|}\times e^{-\beta[G(N_b^M,f)-G(N_b^m,f)]}. \quad (11)$$

That is, the effective kinetic coefficient of the cluster along the N_b axis is $N_b^Mk_{\text{off}}(f,n_b^M)$, i.e., the number of bond rupture events per unit time at the barrier of $G(N_b,f)$. As can be seen from Eq. (6), the barrier height $G(N_b^M,f)-G(N_b^m,f)\sim N$ and the prefactor is $\sim N^0$ because $G''(N_b^m,f)|G''(N_b^M,f)|\sim N^{-2}$. Therefore for large clusters $\ln T(N,f)\sim N$ at given $f<f_c$ due to a barrier of height $\sim N$. It is interesting to note that from the analytical solution of the master equation, Ref. [5] showed that in the constant k_{on} model, when $f<f_c$, $\ln T\sim N$ in the large N limit. In this paper we show that by

studying the effective free energy for given $f<f_c$, $\ln T \sim N$ at large N for general $k_{on}(f,n_b)$ and $k_{off}(f,n_b)$.

IV. CONCLUSION

We have shown that $T(N,f)$, the lifetime of an adhesion cluster under external force, is closely related to the shape of $G(N_b,f)$, the effective free energy of the system. For adhesion clusters under constant force per bond, larger adhesion clusters have much higher stability than smaller adhesion clusters when $f<f_c$ but dissociate as fast as smaller clusters when force per bond exceeds a critical strength. At $f=f_c$ cluster lifetime scales $\sim N^{1/3}$, this is a crossover from N-independent lifetime to exponential N dependence. Similar results have been shown for the "constant k_{on} model" at $f<f_c$ by Erdmann and Schwarz [5]. Our work shows that the scaling laws hold for any adhesion cluster whose ligand-receptor unbinding and binding rates can be expressed as functions of f and n_b. We also show that the critical force per bond f_c and the point of inflection n_b^* are both model dependent: they depend on the details of ligand-receptor interactions. Since there is no scaling relation for $T(N,f)$ in the small N limit, the lifetime of adhesion clusters with a few bonds is also model dependent.

Constant f experiments can be performed by putting ligands and receptors on two plates with constant areal density and applying a force that is proportional to the area of the plates. Since experiments have shown that in cell-matrix adhesion the area of the focal adhesion domain is proportional to the force transmitted from the cell to the substrate [20], it is possible that focal adhesion is under constant force per bond. Thus our theory could be relevant to new cell adhesion experiments and experiments on biomimetic systems. The idea of constructing an effective free energy for multiple-bond systems is quite general: it can be applied to adhesion clusters under constant loading rate [4,21] or constant displacement [9], or other adhesion-related problems such as systems with mobile stickers and variable contact area [22].

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APPENDIX

In this appendix we discuss f_c and n_b^* in both constant k_{on} and nonconstant k_{on} models. It will be shown that f_c and n_b^* in these two models are very different, thus the critical force per bond and the position of inflection point of $G(N_b,f_c)$ depend on the choices of k_{on} and k_{off}.

In the constant k_{on} model, $k_{off}(f,n_b)=k_0 e^{\beta f x_0/n_b}$ and $k_{on}=\gamma$ [5]; here unit time is chosen to be $1/k_0$ and unit force is $(\beta x_0)^{-1}$. The critical force per bond f_c was solved by Bell from Eqs. (9) and (10) [1]; it satisfies

$$Z\exp(Z)=\gamma e^{-1}/k_0, \tag{A1}$$

where

$$Z=\beta f_c x_0 \quad \text{and} \quad n_b^*=\frac{Z}{1+Z}. \tag{A2}$$

We find $f_c=0.2785$ and $n_b^*=0.2178$ when $\gamma=1$. The exponential in Eq. (A1) indicates that for any reasonable values of γ/k_0, Z is not greater than $O(1)$, thus from Eq. (A2), n_b^* is not close to 1 in the constant k_{on} model. We will show in the following that this is very different from the nonconstant k_{on} model.

In the nonconstant k_{on} model, the total energy for a bond is the sum of a "bare ligand-receptor interaction energy" and a spring potential $U(x)/k_BT=U_0(x)/k_BT+U_{spring}(x)/k_BT=3(x^{-12}-8x^{-8}+8x^{-6})+\frac{1}{2}(x-x_p)^2$; here unit length and unit time are chosen such that the spring constant $k=1$ and $D=1$. $U_0(x)$ has a bound state at $x=x_b^{(0)}\approx 0.7574$, a barrier at $x=x_\Delta^{(0)}\approx 1.068$ with $U(x_b^{(0)})\approx -10.30k_BT$, $U(x_\Delta^{(0)})\approx 3.356k_BT$, and $U(x)\to 0$ at $x\to\infty$. In fact, the width of the barrier is of order unity because $U(x)<0.3k_BT$ for $x>2.0$. The simulations are carried out at f close to f_c and we have found that in this regime $x_b\approx x_b^{(0)}$, $x_\Delta\approx x_\Delta^{(0)}$, $x_p=x_b=f/n_b$, and $x_f\approx x_p=x_b+f/n_b$ are all very good approximations [19]. At very small f this approximation breaks down since x_f should not become x_b at $f=0$.

Although in the simulation we solve Eqs. (9) and (10) numerically to find f_c and n_b^* for the nonconstant k_{on} model, to further compare f_c in both models it is convenient to make the following approximation: $U_0(x_f)+U_{spring}(x_f)\approx 0$ when $f=f_c$. This approximation has been shown to give f_c and n_b^* within 0.2% from numerical solutions and allows us to compare f_c and n_b^* in both models [19]. In this approximation, Eqs. (1) and (2) can be expressed as

$$k_{off}(f,n_b)=k_0 e^{f(x_\Delta^{(0)}-x_b^{(0)})/n_b}, \tag{A3}$$

where

$$k_0=\frac{D}{2\pi k_BT}\sqrt{[U_0''(x_b^{(0)})+k]|U_0''(x_\Delta^{(0)})+k|}\times e^{-\{[U_0(x_\Delta^{(0)})-U_0(x_b^{(0)})]+k/2(x_b^{(0)}-x_\Delta^{(0)})^2\}}$$

and

$$k_{on}(f,n_b)=\frac{D}{2\pi k_BT}\sqrt{k|U_0''(x_\Delta^{(0)})+k|}e^{-U_0(x_\Delta^{(0)})}\times\exp\left[-\frac{k}{2}(x_\Delta^{(0)}-x_b^{(0)}-f/kn_b)^2\right]. \tag{A4}$$

Substituting Eqs. (A3) and (A4) into Eqs. (9) and (10), we find

$$f_c=\sqrt{kY}\left(1-\frac{1}{Y}\right), \tag{A5}$$

where $Y=1/(1-n_b^*)$ is the solution of

$$(Y-1)W = e^{-Y/2}, \quad W = \sqrt{\frac{U_0''(x_b^{(0)})+k}{k}} e^{U_0(x_b^{(0)})}. \quad \text{(A6)}$$

The numerical solutions of Eqs. (9) and (10) for our choice of $U_0(x)$ give $f_c \approx 2.525$ (this corresponds to $\beta f_c \Delta x \approx 0.7865$, where $\Delta x = x_\Delta^{(0)} - x_b^{(0)}$), and $n_b^* \approx 0.8788$.

In general $W \ll 1$ because $-U_0(x_b^{(0)})$ is large compared to unity. This leads to $\exp(-Y/2) \ll 1$, i.e., n_b^* is close to 1, thus in general $f_c > 1$. This is very different from the constant k_{on} model, where f_c can be small if γ is chosen to be small and in general n_b^* is not close to 1. The physical consequence of this difference can be seen from Eq. (9). In the nonconstant k_{on} model $n_b^* > 1 - n_b^*$, as a result $k_{off}(f_c, n_b^*) < k_{on}(f_c, n_b^*)$. That is, at critical force, force per bond at $n_b = n_b^*$ is still weak. In general this is not true in the constant k_{on} model. This difference apparently comes from different choices of k_{on} and k_{off} in these two models, thus f_c and n_b^* both depend on the microscopic details of ligand-receptor interactions. However, these different models obey the same scaling laws for $T(N,f)$ at the $N \gg 1$ limit, which strongly supports the main results of this paper.

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[18] $Nf = \Sigma_{i=1}^{N_b} k[x_p(t) - x_i(t)] + \Sigma_{j=1}^{N-N_b} k[x_p(t) - x_j(t)]$, where $x_i(t)$ is the position of the ith closed bond, and $x_j(t)$ is the position of the jth open bond at time t. Since $x_i(t)$ fluctuates around x_b and $x_j(t)$ fluctuates around x_f, thus $f \approx n_b k(x_p - x_b) + (1-n_b)k(x_p - x_f)$. When f is not too small, $x_p \approx x_f$ and the relation between x_p and f is further simplified to $x_p \approx x_b + f/n_b k$ [6].
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