

Stability of Adhesion Clusters under Constant Force

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We solve the stochastic equations for a cluster of parallel bonds with shared constant loading, rebinding, and the completely dissociated state as an absorbing boundary. In the small force regime, cluster lifetime grows only logarithmically with bond number for weak rebinding, but exponentially for strong rebinding. Therefore rebinding is essential to ensure physiological lifetimes. The number of bonds decays exponentially with time for most cases, but in the intermediate force regime a small increase in loading can lead to much faster decay. This effect might be used by cell-matrix adhesions to induce signaling events through cytoskeletal loading.

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Biological systems have to be able to change quickly in response to external stimuli. This is one of the reasons why molecular bonds in biological system are based on noncovalent interactions and have short lifetimes of the order of seconds. In order to achieve long-lived assemblies, cells in multicellular organisms adhere to the extracellular matrix and to each other through clusters of adhesion molecules. The number of receptors in adhesion contacts can range from just a few (e.g., for tethering of leukocytes to vessel walls or in the nascent contacts close to the leading edge of a locomoting cell) to $\sim 10^5$ (e.g., in mature cell-matrix contacts). Most types of adhesion clusters are coupled to the cytoskeleton and have to function under mechanical load, which leads to exponentially increased dissociation rates [1]. Rebinding of broken bonds is often facilitated by the densely packed arrangement of molecular bonds in the cluster. However, if the cluster operates under force, it is likely to be pulled away by some elastic relaxation process in the moment the last bond has been broken. Therefore the cluster usually cannot rebind from the completely dissociated state. Recently, the interest in the role of force at adhesion clusters has strongly increased also in the biological community, since it has been shown that force at cell-matrix adhesions correlates with contact size and intracellular signaling [2].

Quantitative characterization of adhesion bonds has made tremendous progress during the past decade, mainly on the level of single molecules [3–5]. Because of the low binding energies of adhesion bonds, thermal activation is important, and theoretical models are required to interpret experimental data [6]. In order to make contact with situations of biological interest, the quantitative effort now has to be extended to clusters of adhesion bonds. Clusters also allow one to study the effect of rebinding, which is difficult to address on the level of single molecules [7]. Recently, micropipette techniques have been used to study cluster dissociation under a linear ramp of force [8], in good agreement with a theoretical analysis by Seifert [9]. However, physiological loading of

adhesion clusters is usually more or less constant on the time scale of cluster lifetime. The stability of adhesion clusters under constant force has been first modeled by Bell [1], but his treatment was based on a simplifying deterministic equation for the mean number of bonds.

In this Letter, we present exact and simulation results for a stochastic version of the Bell model. In contrast to the deterministic model, the stochastic one allows one to treat the completely dissociated state as an absorbing boundary. Moreover, it includes fluctuation and nonlinear effects which are important for small adhesion clusters. The main objective of this work is to obtain analytical results for generic features of adhesion clusters. We present several new formulas for cluster lifetime as a function of cluster size, rebinding rate, and force, which now can be used for quantitative analysis of adhesion experiments. Although we do not address the specific features of cell-matrix adhesions, our model suggests an appealing mechanism by which cells might induce signaling events through cytoskeletal loading.

Following Bell [1], we consider a cluster with a constant number N_0 of parallel bonds. At any given time, each of the different bonds can be either open or closed. The constant force F applied to the cluster is assumed to be shared equally between the i closed bonds ($0 \leq i \leq N_0$). We assume that a single bond under force F ruptures with the dissociation rate $k = k_0 e^{F/iF_b}$ introduced by Bell [1], which can be rationalized by modeling bond rupture as a thermally activated escape over a sharp transition state barrier [6]. In this framework, the force scale $F_b = k_B T/x_b$ is set by thermal energy $k_B T$ and the distance x_b between the potential minimum and the transition state barrier along the reaction coordinate of rupture. For typical values $x_b \sim 1$ nm and $T \sim 300$ K, we find the typical force scale $F_b \sim 4$ pN. For the single bond association rate k_{on} , we assume that it is independent of force. Physiological values for both k_0 and k_{on} are expected to be in the $1/s$ range. For the following, it is useful to introduce dimensionless time $\tau = k_0 t$, dimensionless rebinding rate $\gamma = k_{on}/k_0$, and dimensionless overall force

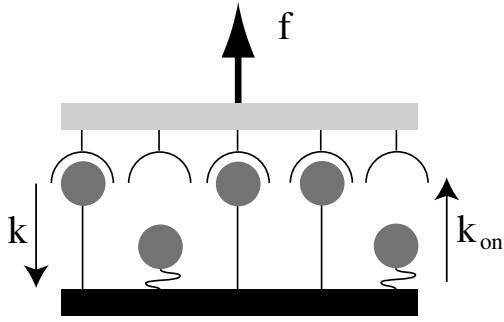


FIG. 1. Schematic representation of an adhesion cluster under constant force: There are $N_0 = 5$ receptor-ligand pairs, of which $i = 3$ are closed and equally share the constant dimensionless force f . Single closed bonds rupture with dissociation rate $k = k_0 e^{f/i}$ and single open bonds rebind with force-independent association rate k_{on} . Our model has three parameters: cluster size N_0 , dimensionless rebinding rate $\gamma = k_{\text{on}}/k_0$, and dimensionless force f .

$f = F/F_b$. Figure 1 shows a schematic representation of our model. Since bond rupture is a discrete process, the stochastic dynamics of the bond cluster can be described by a one-step master equation [10]

$$\frac{dp_i}{d\tau} = r(i+1)p_{i+1} + g(i-1)p_{i-1} - [r(i) + g(i)]p_i, \quad (1)$$

where $p_i(\tau)$ is the probability that i bonds are closed at time τ . The reverse and forward rates between the possible states i are

$$r(i) = i e^{f/i}, \quad g(i) = \gamma(N_0 - i). \quad (2)$$

Depending on the experimental setup, rebinding from the completely dissociated state ($i = 0$) might be possible (*reflecting boundary*) or not (*absorbing boundary*). For $f = 0$ and a reflecting boundary at $i = 0$, we deal with natural boundaries, that is, given reasonable initial conditions, no special equations are needed to treat the boundaries. For finite f , $r(0) = 0$ is required to prevent i from becoming negative. An absorbing boundary at $i = 0$ requires $g(0) = 0$. The mean number of closed bonds N as a function of time τ is $N = \langle i \rangle = \sum_{i=0}^{N_0} i p_i$. From the master Eq. (1), one can derive

$$\frac{d\langle i \rangle}{d\tau} = -\langle r(i) \rangle + \langle g(i) \rangle. \quad (3)$$

If r and g are both linear functions in i , Eq. (3) becomes an ordinary differential equation for N . This suggests to study the following deterministic equation:

$$\frac{dN}{d\tau} = -N e^{f/N} + \gamma(N_0 - N), \quad (4)$$

as has been done by Bell [1] for constant loading and by Seifert [9] for linear loading. However, for finite force f , the solution of Eq. (4) does not give the correct result for

the first moment, since then the rate r defined in Eq. (2) is nonlinear in i and the average in Eq. (3) cannot be taken. More importantly, a differential equation such as Eq. (4) follows from the stochastic equations only in the case of natural boundaries. In order to treat the biologically relevant case of an absorbing boundary, one therefore has to study the stochastic description Eq. (1).

A full stochastic solution amounts to finding the set of state probabilities $p_i(\tau)$ as a function of the three dimensionless parameters N_0 , γ , and f . For force $f = 0$ and a reflecting boundary, the solution results from the generating function given by McQuarrie [11]:

$$p_i(\tau) = \binom{N_0}{i} \frac{(\gamma + e^{-(1+\gamma)\tau})^i (1 - e^{-(1+\gamma)\tau})^{N_0-i}}{(1+\gamma)^{N_0}}. \quad (5)$$

Here and in the following we use the initial condition $N(0) = N_0$. If also rebinding $\gamma = 0$, then we deal with the simple case of independently decaying bonds. Figure 2(a) shows that, in this case, a cluster with ten bonds decays from $i = 10$ to 0 by visiting each of the intermediate states to an appreciable degree. In order to stabilize the cluster, one has to introduce rebinding. Then there is fast relaxation to a stable stationary state, as shown in Fig. 2(b) for $\gamma = 1$. For the biologically relevant case of an absorbing boundary, a stable stationary state does not exist and the cluster will always dissociate on the long run. In this case, one has to solve the first passage problem of reaching the state $i = 0$ for the first time. This can be done semianalytically by using Laplace transforms, where the last backtransform has to be done numerically. Alternatively, one can solve the master equation numerically by the Monte Carlo method (most efficiently with the Gillespie algorithm [12]), as we always do in the

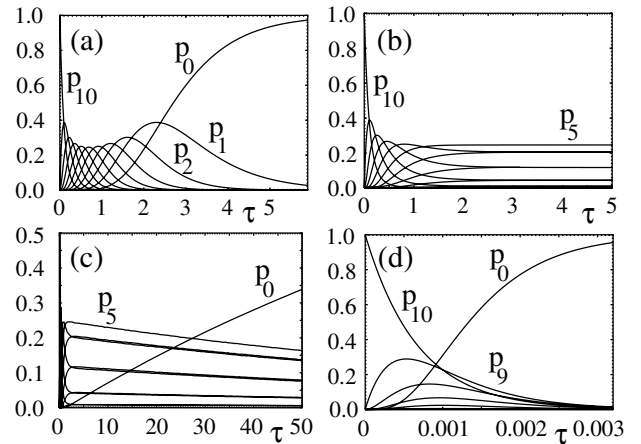


FIG. 2. Solution of the master equation gives the state probabilities p_i for i bonds being closed at time τ ($0 \leq i \leq N_0$). Here they are plotted for $N_0 = 10$. (a) $\gamma = 0$ and $f = 0$. (b) $\gamma = 1$ and $f = 0$ for a reflecting boundary at $i = 0$. (c) Same for absorbing boundary. (d) $\gamma = 0$ and $f = 50$. (a) and (b) follow from Eq. (5), (c) is obtained from Monte Carlo simulations, and (d) follows from Eq. (6).

general case, when both rebinding γ and force f are finite. Figure 2(c) shows that, in this case, the plateaus from Fig. 2(b) tilt downward, while p_0 increases steadily with time τ . Stability further decreases if force f is turned on. For very large force, rebinding (including the boundary type at $i = 0$) becomes irrelevant, because the reverse rate r dominates the forward rate g . Using a recursive scheme to construct p_i from p_{i-1} , for $\gamma = 0$ we find

$$p_i(\tau) = \left(\prod_{j=i+1}^{N_0} r(j) \right) \sum_{j=i}^{N_0} \left\{ \prod_{\substack{k=i \\ k \neq j}}^{N_0} \frac{e^{-r(j)\tau}}{r(k) - r(j)} \right\}. \quad (6)$$

The probability for cluster dissociation at time τ is $p_1(\tau)r(1)$. Setting $i = 1$ in Eq. (6) and using Eq. (2), one obtains a formula which has been given before in Ref. [13]. Figure 2(d) shows, for the case $f = 50$, that now the cluster decays very rapidly, with only a few of the intermediate states being visited to an appreciable degree.

Once the set of state probabilities $p_i(\tau)$ is known, one can calculate any quantity of interest, in particular, the mean number of closed bonds N as a function of time τ . We find that $N(\tau)$ usually decays exponentially. This is demonstrated in Fig. 3(a) for the case $f = 0$. The origin of the exponential decay can be understood as follows: First, the system equilibrates into a binomial distribution peaked around $N_{\text{eq}} = \gamma N_0 / (1 + \gamma)$ as described by the result for the reflecting boundary, that is Eq. (5). The lower tail of this distribution then “leaks” into the state $i = 0$ due to the absorbing boundary. The smaller N_0 or γ , the larger the tail contribution at $i = 1$ and the faster the systems lose realizations to the absorbing boundary. The resulting decay can be approximated by $N(\tau) \approx N_{\text{eq}} e^{-a\tau}$ with $a \approx p_1(\infty)$ from Eq. (5). For the values of N_0 used in

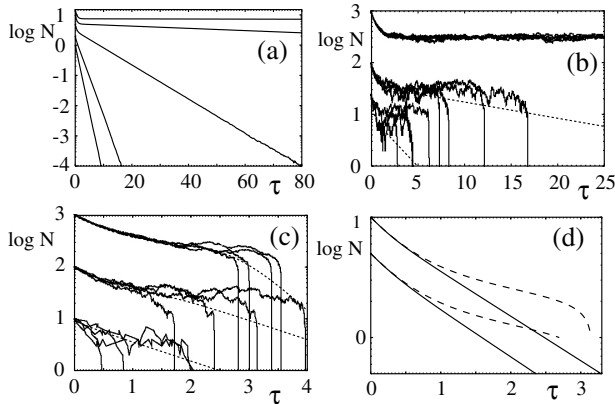


FIG. 3. (a) Simulation results for the mean number of closed bonds N at time τ for $f = 0$, $\gamma = 1$, and $N_0 = 1, 2, 5, 10$, and 15 (lower to upper lines). (b) Four typical simulation trajectories for each of the cases $N_0 = 10, 100$, and 1000 for $\gamma = 1$ and $f/N_0 = 0.25$. Dotted lines are $N(\tau)$. (c) Same for $f/N_0 = 0.3$. (d) Comparison of stochastic (solid lines) and deterministic (dashed lines) results for $N(\tau)$ for $N_0 = 5$ and 10 for $\gamma = 1$ and $f/N_0 = 0.3$.

Fig. 3(a), one finds $a \approx 4.6 \times 10^{-4}, 9.7 \times 10^{-3}, 0.16$ and 0.5 for $N_0 = 2, 5, 10$, and 15 . Numerically, we find $a = 2.5 \times 10^{-4}, 8.5 \times 10^{-3}, 0.13$ and 0.6 ; thus, the leakage estimate is rather good.

In order to assess the role of fluctuations, it is instructive to study single simulation trajectories. Since we use the Gillespie algorithm for exact stochastic simulations [12], they are expected to resemble experimental trajectories. Figure 3(b) shows that, for large cluster size and small force, typical trajectories fluctuate around a plateau value close to N_{eq} . However, for small cluster sizes, fluctuations to smaller bond numbers lead to fast loss of realizations to the absorbing boundary. Final decay is rather abrupt due to force-accelerated rupture for decreasing bond numbers. Figure 3(c) shows that, for sufficiently large force, also the large clusters decay quickly. The loss of stability for any cluster size follows from Bell’s stability analysis of the deterministic Eq. (4), which yields a critical force $f_c = N_0 \text{plog}(\gamma/e)$ [1], where the product logarithm $\text{plog}(a)$ is defined as the solution x of $xe^x = a$. For typical values of N_0 and γ , f_c belongs to the intermediate force regime, $1 < f < N_0$. For $\gamma = 1$, we have $f_c/N_0 = 0.278$. Figures 3(b) and 3(c) are below and above the critical force, respectively. In contrast to Bell’s continuum analysis, our stochastic analysis shows that for small clusters a small increase in loading can lead to the fast decay characteristic for the case without rebinding also for forces below f_c . Figure 3(d) compares $N(\tau)$ as obtained from simulations to $N(\tau)$ as obtained from numerical integration of the deterministic Eq. (4). This shows that stochastic and deterministic results differ also on the level of the first moment.

The quantity of largest practical interest is cluster lifetime T as a function of the model parameters N_0 , γ , and f . In general, T can be calculated from the adjoint master equation [10]. For $N_0 = 2$ and 3 , the solutions can also be found by directly summing with appropriate weights over all possible dissociation paths, each of which is a sequence of Poisson processes. For $N_0 = 2$, we find

$$T = \frac{1}{2}(e^{-f/2} + 2e^{-f} + \gamma e^{-3f/2}). \quad (7)$$

For $N_0 \geq 4$, the direct procedure becomes intractable. However, in the case of vanishing rebinding ($\gamma = 0$), there is only one dissociation path and the exact solution is simply $T = \sum_{i=1}^{N_0} 1/r(i)$ for all values of N_0 [13]. In Fig. 4(a), we plot T as a function of f/N_0 for different cluster sizes N_0 . In the small force regime, $f < 1$, T plateaus at the value $H_{N_0} = \sum_{i=1}^{N_0} 1/i \approx \ln N_0 + 1/(2N_0) + \Gamma$. Here H_{N_0} are the harmonic numbers and $\Gamma = 0.577$ is Euler’s constant. In this regime, T depends only weakly (logarithmically) on N_0 and large cluster sizes are required to achieve long lifetimes [13,14]. In the intermediate force regime, $1 < f < N_0$, we find $T \approx H_{N_0} - H_f \approx \ln(N_0/f)$. Here the effective cluster size is reduced to N_0/f , because the cluster dissociates very

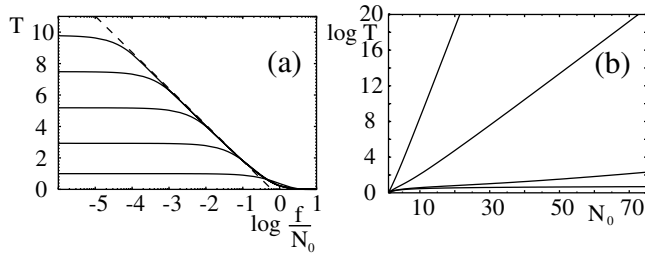


FIG. 4. Analytical results for cluster lifetime T . (a) T as a function of f/N_0 for $\gamma = 0$ and $N_0 = 1, 10, 100, 1000$, and $10\,000$ (lower to upper solid lines). The dotted curve is the approximation $T = \ln[0.61(N_0/f)]$. (b) T as a function of cluster size N_0 for $f = 0$ and $\gamma = 0.0, 0.1, 1.0$, and 10.0 (lower to upper lines).

rapidly for $i < f$. In the high force regime, $f > N_0$, only the term with $i = N_0$ contributes: If the first bond breaks, all remaining bonds break within no time. The destabilizing effect of force can be counteracted by rebinding. In the case of vanishing force ($f = 0$), the solution can also be found by using Laplace transforms [10]. We find

$$T = \frac{1}{1 + \gamma} \left[\sum_{n=1}^{N_0} \left\{ \binom{N_0}{n} \frac{\gamma^n}{n} \right\} + H_{N_0} \right]. \quad (8)$$

For $\gamma = 0$, we recover the result $T = H_{N_0}$ from above. For $N_0 = 2$, we get the result $T = (3 + \gamma)/2$ following from Eq. (7). In general, T scales $\sim \gamma^{N_0-1}$ with rebinding rate. In Fig. 4(b), we plot T as a function of N_0 for different values of γ . For $\gamma < 1$, the logarithmic dependence of T on N_0 is valid over a wide range of cluster size. However, for very large clusters, lifetime starts growing exponentially with N_0 . For $\gamma > 1$, this strong increase of T with N_0 is found for any value of N_0 . Therefore increasing rebinding is much more effective than increasing cluster size in achieving cluster stability, and essential to ensure physiological cluster lifetimes with reasonable numbers of bonds. For example, in the absence of both force and rebinding and if the lifetime of each bond was 1 s ($k_0 = 1$ Hz), Eq. (8) predicts that the astronomical number of $\sim 10^{40\,000}$ independent bonds would be needed to achieve a cluster lifetime of one day ($T \sim 10^5$ s). In contrast, for $k_0 = 1$ Hz ($\gamma = 1$), the same cluster lifetime T is achieved by $N_0 = 20$. If rebinding is 10 times slower than unbinding ($\gamma = 0.1$), cluster lifetime T is down to 7 s and one needs $N_0 = 150$ bonds to regain a cluster lifetime of one day. In this way, knowing cluster lifetime and two out of the three parameters N_0 , γ , and f allows to estimate the unknown one.

Our model is also relevant for cell adhesion if initial loading is much faster than cluster lifetime (otherwise the assumption of constant force is not valid) and if cluster decay is much faster than potential reinforcement process (otherwise the assumption of constant cluster size is not

valid). One example which might satisfy these conditions is L-selectin mediated leukocyte tethering in shear flow [15]. Our assumptions do certainly not hold for cell-matrix adhesions, which have been shown to grow rather than to decay under the effect of force [2]. Although the specific processes at work at cell-matrix processes are not the subject of this work, our model suggests that the stress constant ~ 5.5 nN/ μm^2 recently measured on elastic substrates for the physiological loading of cell-matrix contacts through the cell's own contractile machinery [16] might be close to the critical force $f_c = N_0 p \log(\gamma/e)$, because then small changes in cytoskeletal loading would result in strongly accelerated cluster decay. Increased force on a subset of bonds might in turn induce signaling events (possibly through mechanical opening up of protein domains) leading to subsequent recruitment of additional bonds. Recent single molecule experiments for activated $\alpha_5\beta_1$ -integrin binding to fibronectin gave $k_0 = 0.012$ Hz and $F_b = 9$ pN [17]. Setting $F_c = 5.5$ nN and using $N_0 = 10^4$, we predict $\gamma = 0.2$, corresponding to a rebinding rate $k_{\text{on}} = 0.002$ Hz.

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