Supporting Text
L-selectin mediated leukocyte tethering in shear flow is controled by multiple contacts and cytoskeletal anchorage facilitating fast rebinding events

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Model

We consider a cluster with a constant number N of parallel bonds under constant force F. At any time t, i bonds are closed and $N-i$ bonds are open ($0 \leq i \leq N$). Closed bonds are assumed to rupture according to the Bell equation [1]:

$$k_{off} = k_0 e^{F/F_b} \ . \tag{1}$$

For convenience, we introduce dimensionless variables: dimensionless time $\tau = k_0 t$, dimensionless dissociation rate k_{off}/k_0, and dimensionless force $f = F/F_b$. The i closed bonds are assumed to share force f equally, that is, each closed bond is subject to force f/i. Thus the dimensionless dissociation rate is $e^{f/i}$. As long as the receptors are held in proximity to the ligands, rebinding of open bonds can occur. Therefore we assume that single open bonds rebind with the force-independent association rate k_{on}. The dimensionless rebinding rate is defined as $\gamma = k_{on}/k_0$.

The stochastic dynamics of the bond cluster can be described by a one-step Master equation [2]

$$\frac{dp_i}{d\tau} = r_{i+1}p_{i+1} + g_{i-1}p_{i-1} - [r_i + g_i]p_i \ , \tag{2}$$

where $p_i(\tau)$ is the probability that i closed bonds are present at time τ. The reverse and forward rates between the different states i follow from the single molecule rates as

$$r_i = ie^{f/i} \ , \quad g_i = \gamma(N-i) \ . \tag{3}$$

Once the completely dissociated state $i = 0$ is reached, the cell will be carried away by shear flow and the cluster cannot regain stability. This corresponds to an absorbing boundary at $i = 0$, which can be implemented by setting $r_0 = g_0 = 0$. Cluster lifetime T is identified with the mean time to reach the absorbing state $i = 0$.

Lifetime of two-bonded cluster

For a cluster with two bonds, $N = 2$, cluster lifetime T can be calculated exactly in the following way. At $\tau = 0$, the cluster starts with the initial condition $i = 2$. Next it moves to state $i = 1$ with probability 1, after the mean time $1/r_2$. From there, it rebinds to state $i = 2$ with probability $w_R = g_1/(r_1 + g_1)$ or dissociates with probability $w_D = r_1/(r_1 + g_1)$. The mean time for this part is $1/(r_1 + g_1)$. Thus after two steps, the system has reached state $i = 0$ with probability w_D or returned to state $i = 2$ with probability w_R, with $w_D + w_R = 1$. In detail, the probabilities and mean times for both processes are

$$w_D = \frac{e^f}{e^f + \gamma}, \qquad t_D = \frac{1}{2e^{f/2}} + \frac{1}{e^f + \gamma} \ , \tag{4}$$

$$w_R = \frac{\gamma}{e^f + \gamma}, \qquad t_R = t_D \ . \tag{5}$$

Different paths to dissociation differ only in the number of rebinding events j to state $i = 2$:

$$w_j = w_D w_R^j, \qquad t_j = t_D + jt_R \ . \tag{6}$$

We first check normalization:

$$\sum_{j=0}^{\infty} w_j = w_D \frac{1}{1 - w_R} = 1 \tag{7}$$

and then calculate cluster lifetime:

$$T = \sum_{j=0}^{\infty} t_j w_j = t_D + t_R w_D \sum_{j=0}^{\infty} j w_R^j \tag{8}$$

$$= t_D + t_R w_D \frac{w_R}{(1 - w_R)^2} = \frac{t_D}{1 - w_R} \tag{9}$$

$$= \frac{1}{2}\left(e^{-f/2} + 2e^{-f} + \gamma e^{-3f/2}\right) \ . \tag{10}$$

This formula is given in dimensional form as Eq. 1 in the main text.

Cluster size versus rebinding

For arbitrary cluster size N, cluster lifetime T can be obtained from the adjoint Master equation [2, 3]. In the case of vanishing force, $f = 0$, the solution can also be found by using Laplace transforms [2]:

$$T = \frac{1}{(1+\gamma)}\left(\sum_{i=1}^{N} \binom{N}{i} \frac{\gamma^i}{i} + \frac{1}{i}\right) \ . \tag{11}$$

For $\gamma = 0$, this equation reduces to

$$T = \sum_{i=1}^{N} \frac{1}{i} = H_N \ , \tag{12}$$

where H_N are the harmonic numbers. An expansion for large N gives

$$H_N = \Gamma + \ln N + \frac{1}{2N} + O(\frac{1}{N^2}) \ . \tag{13}$$

Here $\Gamma = 0.577$ is the Euler constant. This formula is rather good already for small values of N. Eq. (12) is easy to understand: for $\gamma = 0$, dissociation is simply a sequence of Poisson decays with mean times $1/r_i = 1/i$. The overall mean time for dissociation is the sum of the mean times of the subprocesses. We conclude that for vanishing rebinding, T grows only weakly (logarithmically) with N, and very large cluster sizes are required to achieve long lifetimes [4, 5]. Therefore rebinding is essential to achieve stabilization for small cluster sizes.

Effect of finite loading rate

Loading and dissociation of single L-selectin bonds occur on the same time scale. As a cell is captured from shear flow and comes to a stop, force rises from cero and plateaus at a finite value. We model the initial rise as linear, with loading rate r. Therefore $f = \mu\tau$ until time τ_0, followed by constant loading $f = f_0$, where $\mu = r/k_0F_b$ is dimensionless loading rate. Because $\mu = f_0/\tau_0$, there are only two independent parameters, τ_0 and f_0. The mean lifetime can be calculated in the usual way [5, 6]. We find

$$T = \frac{e^{\frac{1}{\mu}}}{\mu}\left(E(\frac{1}{\mu}) - E(\frac{e^{\mu\tau_0}}{\mu}) + \frac{\mu}{e^{f_0}}e^{-\frac{e^{\mu\tau_0}}{\mu}}\right) , \qquad (14)$$

where $E(x)$ is the exponential integral. For $\tau_0 \to 0$, we find the result for constant loading, $T = 1/e^{f_0}$. For $\tau_0 \to \infty$, we find the result for linear loading, $T = e^{1/\mu}E(1/\mu)/\mu$ [5]. Eq. (14) is used in the section on single bond loading and for the plot of the dash-dotted line in Fig. 3.

Simulations

In the presence of diffusion with diffusion constant D, the single-molecule association rate becomes a function of time t that has passed since unbinding. In this paper, we use the approximation

$$k_{on}(t) = k_{on}\left(1 - e^{-s^2/4Dt}\right) , \qquad (15)$$

where s is the capture radius. Because analytical solutions are intractable in this case, the Master equation, Eq. (2), has to be solved numerically. The standard methods to do so are Monte Carlo simulations. Unfortunately, the Gillespie algorithm for exact stochastic simulations [7] cannot be used in this case, because it does not track the identity of different bonds [8]. Therefore, we simulate the Master equation by discretizing time τ in small steps $\Delta\tau$. For each time step, random numbers are drawn to decide how the system evolves according to the rates defined for the different processes. In detail, in the time interval $[\tau, \tau + \Delta\tau]$, each closed bond has the probability $e^{f/i}\Delta\tau$ to rupture, and each open bond has the probability $\gamma(1 - e^{-k/(\gamma\tau)})\Delta\tau$ to rebind. Here $k = k_{on}s^2/4D$ is the dimensionless ratio of the time-scales set by diffusion and rebinding. Our model for stochastic cluster dynamics was implemented in the programming environment MATLAB (The MathWorks Inc., Natick, Mass.). A typical run simulates $5,000$ tethers (larger tether numbers give better statistics for long time behavior, but similar results), each comprising N bonds. Results from different runs are binned into histograms for the number of tethers dissociating in the time interval $[\tau, \tau + \Delta\tau]$. In Fig. 4, we plot the natural logarithm of the fraction of tethers that last longer than dimensional time t as a function of t, as is common for the analysis of experimental data. The slope of this curve is identified with the dissociation rate. Although this procedure involves numerical integration of the probability distribution for dissociation and therefore leads to loss of information, its smoothing effect is essential to obtain reliable estimates for the dissociation rate in the presence of noisy data. In Fig. 4 Inset, the dissociation rates obtained in this way are plotted as function of shear rate (that is force) and diffusion constant (which determines the dimensionless parameter k).

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