Stability of heterogeneous parallel-bond adhesion clusters under load

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To study the stability of heterogeneous adhesion clusters of receptor-ligand pairs, a theoretical model for a parallel adhesion bond cluster under constant loading is extended to multiple bond populations. The stability of entire cluster can be tuned by changing densities of different bond populations as well as their extensional rigidity and binding properties. Interestingly, an optimal stability is generally achieved when the total cluster load is shared such that loads on distinct bond populations are equal to their individual critical rupture forces. We also show that cluster heterogeneity can drastically affect cluster lifetime.

Heterogeneity is a part of many essential aspects of biological systems, including gene expression, cellular metabolism and function \textsuperscript{1}\textsuperscript{13}. A prominent example is the adhesion of biological cells to other cells or to a substrate (focal adhesion) via receptor-ligand bonds, which is a pre-requisite for various cellular phenomena such as cell migration, tissue development, etc. \textsuperscript{4}\textsuperscript{6} Such adhesive interactions are often very heterogeneous and may involve more than one type of receptor-ligand pairs, which differ in their local density, adhesion strength, and/or bond kinetic rates. For instance, leukocytes before extravasation first bind to and roll at an endothelial cell layer, then show a firm adhesion at the surface \textsuperscript{7}\textsuperscript{9}. This process is facilitated by the ability of P-selectin glycoprotein (PSGL-1) at the surface of leukocytes to bind to both selectin and integrin molecules expressed at endothelial cells. Another example is the adhesion of malaria-infected red blood cells to the endothelium, in order to avoid their removal in the spleen \textsuperscript{10}\textsuperscript{12}. Here, Plasmodium falciparum erythrocyte membrane receptor (PIEMP-1) can bind to multiple ligands (e.g. CD36, ICAM-1, and CSA molecules) at the surface of endothelial cells \textsuperscript{13}\textsuperscript{15}. Even though it is hypothesized that they act synergistically \textsuperscript{16}, the exact roles of different receptor-ligand pairs remain largely unknown.

Focal adhesions were first studied theoretically by a mean-field approach that describes the stability of parallel adhesion-bond cluster under constant loading \textsuperscript{17}. Recently, a stochastic model for parallel bond clusters has been developed \textsuperscript{18}\textsuperscript{20}, which shows that the stability and lifetime of a heterogeneous cluster can be tuned by changing the fractions of different bond populations and their extensional rigidity and binding properties. We start with the original model for parallel bond cluster under a constant loading \textsuperscript{17}, \textsuperscript{18}. The system contains $N^t$ adhesion sites, where $N(t) \leq N^t$ (time) bonds with an extensional rigidity $k$ and a dimensionless rebinding rate $\gamma = \kappa^{\text{on}} / \kappa^{\text{off}}$ can stochastically form under an external force $F$. From the stability analysis \textsuperscript{17}, with the assumption that each spring shares the same force $F/N$, there exists a critical force $f^c$ below which the cluster equilibrates to an average number of bonds $\langle N \rangle$ and above which the cluster is unstable and disassoci-

Note that the ratio $\kappa^{\text{on}} / \kappa^{\text{off}}$ represents a Boltzmann factor related to the energy change due to bond formation, and therefore, it characterizes binding strength. The off-rate expression above describes the so-called slip bond, whose lifetime increases with increasing $F$ \textsuperscript{21}. Some biological bonds may behave differently, so-called catch bonds, such that their lifetime increases first with increasing $F$ until a certain threshold, and then decreases with increasing $F$ similar to a slip bond. The catch-bond behavior was first predicted theoretically \textsuperscript{22}, \textsuperscript{23} and later discovered for leukocytes experimentally \textsuperscript{23}. The parallel bond-cluster model for slip bonds has also been adapted to the case of catch bonds \textsuperscript{24}\textsuperscript{25}.

In this Letter, we extend the parallel-bond-cluster model to multiple bond populations and show that the stability and lifetime of a heterogeneous cluster can be tuned by changing the fractions of different bond populations and their extensional rigidity and binding properties. We start with the original model for parallel bond cluster under a constant loading \textsuperscript{17}, \textsuperscript{18}. The system contains $N^t$ adhesion sites, where $N(t) \leq N^t$ (time) bonds with an extensional rigidity $k$ and a dimensionless rebinding rate $\gamma = \kappa^{\text{on}} / \kappa^{\text{off}}$ can stochastically form under an external force $F$. From the stability analysis \textsuperscript{17}, with the assumption that each spring shares the same force $F/N$, there exists a critical force $f^c$ below which the cluster equilibrates to an average number of bonds $\langle N \rangle$ and above which the cluster is unstable and disassoci-

FIG. 1. Heterogeneous parallel bond cluster with two different bond populations indicated by blue and red colors. $\gamma_1$ and $\gamma_2$ are rebinding rates of the 1st and 2nd bond types, respectively. Similarly, $k_1$ and $k_2$ are the corresponding spring rigidities. The unstressed off-rate $\kappa_0$ is assumed to be the same for both bond types and $1/\kappa_0$ sets a basic timescale.
rates, i.e. \( N = 0 \). The critical force \( f^c \) and critical number \( N^c \) of bonds are given by

\[
\frac{f^c}{f_d} = N^c \frac{\ln(\gamma/e)}{N^c} = N^c \frac{\ln(\gamma/e)}{1 + \ln(\gamma/e)}, \tag{1}
\]

where \( \ln(a) \) is the product logarithm function which solves the equation \( x e^x = a \).

We generalize this model to heterogeneous cluster with two different bond populations, characterized by the extensional rigidities \( k_1 \) and \( k_2 \) and the rebinding rates \( \gamma_1 \) and \( \gamma_2 \), see Fig. 1. The total number \( N^s \) of adhesion sites is assumed to be constant, and \( \rho \) determines a fraction of type-1 adhesion sites, such that \( N_1^s = \rho N^s \) and \( N_2^s = (1-\rho)N^s \). We also define a spring-rigidity ratio \( k^s = k_1/k_2 \) and a rebinding ratio \( \gamma^s = \gamma_1/\gamma_2 \). For simplicity, the unstressed off-rate \( \nu_0 \) for both types of bonds is chosen the same; its inverse \( 1/\nu_0 \) sets the basic timescale in the system. At any time \( \tau = t\nu_0 \), the applied force is

\[
F = N_1 k_1 \Delta x + N_2 k_2 \Delta x = (N_1 k^s + N_2) k_2 \Delta x, \tag{2}
\]

where \( \Delta x \) is the extension of bound springs. \( f_1 \) and \( f_2 \) are the forces acting on the corresponding populations of bond types 1 and 2, given by

\[
f_1 = \frac{N_1 F k^s}{N_1 k^s + N_2}, \quad f_2 = \frac{N_2 F}{N_1 k^s + N_2}, \tag{3}
\]

such that \( f_1 + f_2 = F \). Clearly, \( k^s \) can be used to directly control the distribution of forces between the two bond populations. For the case of \( \gamma^s = 1 \) and \( k^s = 1 \), the heterogeneous cluster becomes identical to the homogeneous bond cluster considered previously.

**Mean-field approximation.** The average number of bonds is governed by two rate equations

\[
\frac{dN_1}{d\tau} = -N_1 e^{f_1/(N_1 f_d)} + (N_1 - N_1) \gamma_1, \tag{4}
\]

\[
\frac{dN_2}{d\tau} = -N_2 e^{f_2/(N_2 f_d)} + (N_2 - N_2) \gamma_2, \tag{5}
\]

where \( f_d^1 \) and \( f_d^2 \) are the two force scales which are assumed to be the same, \( f_d^1 = f_d^2 = f_d \). Equations \( 4 \) and \( 5 \) are coupled via the forces \( f_1 \) and \( f_2 \) in Eq. \( 3 \) and are used to deduce the average number \( \langle N \rangle = (N_1 + N_2) \) of bonds and critical force \( F^* \) of the entire cluster. Note that further on all quantities with force dimensions are implicitly normalized by \( f_d \).

**Master equation.** An extension of the mean-field approach is a one-step, two-variable master equation for this system \( 26 \). If \( P_{i,j} \) is the probability of \( i \) type-1 bonds and \( j \) type-2 bonds, then the master equation is

\[
\frac{dP_{i,j}}{d\tau} = r^{i+1,j}_1 P_{i+1,j} + r^{i,j+1}_2 P_{i,j+1} + g^{i-1,j}_1 P_{i-1,j} + g^{i,j-1}_2 P_{i,j-1} - \left[ r^{i,j}_1 + r^{i,j}_2 + g^{i,j}_1 + g^{i,j}_2 \right] P_{i,j}, \tag{6}
\]

where \( r^{i,j}_1 = i \exp[f_1/(i f_d)] \) and \( r^{i,j}_2 = j \exp[f_2/(j f_d)] \) are the reverse rates and \( g^{i,j}_1 = \gamma_1 (N_1^s - i) \) and \( g^{i,j}_2 = \gamma_2 (N_2^s - j) \) are the rebinding rates for type-1 and type-2 bonds, respectively. The cluster lifetime \( T^{i,j} \) can then be computed from this master equation, see Supplemental Material \( 27 \) for details.

**Stochastic simulations.** Another approach for analyzing cluster stability is direct stochastic simulations using the Gillespie’s algorithm \( 28 \). The heterogeneous system is described by four rate equations, two for each type of bonds, representing their association and dissociation (see Supplemental Material \( 27 \)). The bond cluster is advanced in time until a stationary state characterized by a constant average number \( \langle N \rangle \) of bonds is reached.

**Results.** Figure 2a shows typical evolution of \( N(\tau) = N_1(\tau) + N_2(\tau) \) for several bond clusters with various \( k^s \) and \( \gamma^s \), where \( F = 50, \rho = 0.3, N^s = 200 \). The case of \( k^s = 1 \) and \( \gamma^s = 1 \) corresponds to a homogeneous...
cluster for which \( f^c \approx 55.7 \). Even though \( F < f^c \), the stochastic trajectory (red line) shows a complete cluster dissociation due to fluctuations in \( N \) and the condition of \( N(\tau) = 0 \) for simulation termination. Note that cluster dissociation occurs more frequently when the applied force is approaching \( f^c \). The corresponding solution of deterministic Eqs. (1) and (3) shown by the black line converges to a constant \( N \) for large \( \tau \). For \( k^r = 1 \) and \( \gamma^r = 5 \) (blue line), the cluster is very stable because the critical force is much larger than \( F = 50 \), which is evident from Fig. 2(b), where the average number \( \langle N \rangle \) of bonds is presented as a function of \( F \) for different \( \gamma^r \) and \( k^r \). In contrast, the cluster with \( k^r = 5 \) and \( \gamma^r = 1 \) quickly dissociates at \( F = 50 \), as it significantly exceeds the critical force. The differences in \( \langle N \rangle \) between stochastic simulations (symbols) and deterministic solutions (lines), as \( F \) approaches \( f^c \) in Fig. 2(b), characterize the fraction of simulations where cluster dissociation has occurred.

Dissociation of a heterogeneous cluster can be thought of as a multistep process. For two bond populations, as the applied force \( F \) is increased, one of the sub-clusters dissociates first, followed by the detachment of the other. Thus, depending on how \( F \) is shared between two sub-clusters [see Eq. (3)], there exist two possibilities

\[
\begin{align*}
\text{(i)} & \quad f_1 = f_1^c, \quad N_1 = N_1^c & f_2 \leq f_2^c, \\
\text{(ii)} & \quad f_2 = f_2^c, \quad N_2 = N_2^c & f_1 \leq f_1^c,
\end{align*}
\]

where \( f_1^c = N_1^c \text{phn}(\gamma_1/e) \), \( f_2^c = N_2^c \text{phn}(\gamma_2/e) \), \( N_1^c \), and \( N_2^c \) are the corresponding critical forces and numbers of bonds of the two sub-clusters separately. The combination of Eq. (7) or (8) with Eqs. (3), (4), and (5) allows the calculation of the applied force \( F^c \) required to dissociate one of the sub-clusters for selected \( k^r \) and \( \gamma^r \), see Supplemental Material [27] for details. Note that the critical force \( f^c \) for rupturing the entire cluster must necessarily satisfy \( f^c \geq f_{1,2}^c = \max(f_1^c, f_2^c) \). Thus, \( f^c = F^c \) if \( F^c \geq f_{1,2}^c \), and \( f^c = f_{1,2}^c \) otherwise.

Figure 3(a) shows the critical force map as a function of \( k^r \) and \( \gamma^r \) for a heterogeneous cluster with \( \rho = 0.3 \). Note that for any fixed \( \gamma^r \), there exists a maximum \( f^c \), which corresponds to the special case with \( f_1 = f_1^c \) & \( f_2 = f_2^c \). The ratio \( f_1/f_2 = f_1^c/f_2^c = N_2^c k^r/N_2^c \) allows the calculation of optimal \( k^r \) values for cluster stability as

\[
k_{\text{opt}}^r = \frac{1}{1 + \text{phn}(\gamma_1/e)}. \quad (9)
\]

Thus, for a fixed \( \gamma^r \), the largest \( f^c \) is achieved when the forces on individual bond sub-clusters (controlled by \( k^r \)) are equal to the corresponding critical forces. Surprisingly, \( k_{\text{opt}}^r \) depends only on the rebinding rates, and is independent of \( \rho \). The dashed line in Fig. 3(a) represents \( k_{\text{opt}}^r \) and separates the \( f^c \) map into two regions. Region on the right side from the dashed line corresponds to Eq. (7), where the first sub-cluster dissociates first. Consequently, the region on the left side corresponds to

\[
\rho \text{max} = 2, \quad \text{which is located on the } k_{\text{opt}}^r \text{ line at a } \gamma^r \text{ value determined by the equality } f_1^c = f_2^c. \quad (10)
\]

which describes the effect of a weaker sub-cluster (i.e. the sub-cluster with a smaller critical force \( \min(f_1^c, f_2^c) \)) on \( f^c \) in comparison with the critical force \( \max(f_1^c, f_2^c) \) of the strongest sub-cluster. Figure 3(b) shows the stability enhancement \( \chi \) by a weaker sub-cluster as a function of \( k^r \) and \( \gamma^r \). It can be shown analytically [27] that the maximum possible enhancement is \( \chi_{\text{max}} = 2 \), which is located on the \( k_{\text{opt}}^r \) line at a \( \gamma^r \) value determined by the equality \( f_1^c = f_2^c \). Thus, maximum enhancement of \( f^c \) by a weaker sub-cluster is achieved when critical forces
of individual sub-clusters are equal. Furthermore, large or small values of $\gamma^r$ and $k^r$ (compared to unity) generally result in $\chi \approx 1$, and therefore, nearly no stability enhancement by the addition of a weaker sub-cluster.

Furthermore, changes in the fraction $\rho$ of the first bond population do not affect the $\chi_{\text{max}}$ value, but alter its $\gamma^r$ position on the $k^r_{\text{opt}}$ line, as can be seen in Figs. S1 and S2 [27]. For example, $\chi_{\text{max}}$ lies at $\gamma^r \simeq 3.5$ for $\rho = 0.3$ and at $\gamma^r \simeq 9$ for $\rho = 0.2$. For a fixed $\gamma^r$, the optimal fraction $\rho_{\text{opt}}$, such that $\chi_{\text{max}}$ lies at $\gamma^r$, can be found from the equality $f_1^r = f_2^r$ as [27]

$$
\rho_{\text{opt}} = \frac{\text{pln}(\gamma_2/e)}{\text{pln}(\gamma_1/e) + \text{pln}(\gamma_2/e)}. \tag{11}
$$

Thus, the fraction $\rho$ can also be tuned to control $f^c$ and maximize $\chi$, see Fig. S3 [27]. Note that $\rho_{\text{opt}}$ is independent of $k^r$, which is consistent with no dependence of $k^r_{\text{opt}}$ on $\rho$ in Eq. (4). In case of three different bond populations, $\chi_{\text{max}} = 3$ [27], suggesting that $\chi_{\text{max}}$ is equal to the number of bond populations. However, such a maximum in $\chi$ may not easily be achieved in biological systems, as it requires simultaneous regulation of multiple parameters, including intrinsic properties and densities of different bond populations.

Cluster lifetime. For a single bond with vanishing rebinding rate, its lifetime is simply $T(F) = 1/\kappa_{\text{eff}}(F)$. In the mean-field description, when the applied force is less than $f^c$, the cluster lifetime is infinite. However, stochastic fluctuations may result in cluster dissociation within a finite time. The lifetime of a heterogeneous cluster can be obtained from the master equation (6) [27], and also directly from stochastic simulations. Figure 4 presents cluster lifetimes for different model parameters, with an excellent agreement between the results from Eq. (4) and stochastic simulations for $N^t = 10$. Clearly, cluster lifetimes are finite even when $F < f^c$. $T$ increases drastically with increasing $\gamma^r$ for a given $\rho$. Furthermore, the cluster lifetime strongly increases with increasing $\rho$ for a fixed $\gamma^r > 1$. Differences in $T$ for various $k^r$ are nearly negligible, as can be seen from Fig. S4 [27]. We have employed a relatively small value of $N^t = 10$ because the lifetime increases exponentially with $N^t$, so that direct stochastic simulations do not permit the calculation of $T$ for large $N^t$. Figure 4 also shows that the lifetime for $N^t = 200$ (magenta color) rapidly increases when the applied force becomes smaller than the critical force.

Conclusions. Heterogeneity is essential in biological systems because it allows an adaptation to diverse environments and conditions. Especially in cell adhesion, different types of receptors or ligands play an important role in distinct cellular processes and functions. In this Letter, we have studied a simple theoretical model for focal adhesion clusters with multiple types of receptor-ligand pairs under a constant load. Different bond populations within a heterogeneous cluster can be used to tune the stability and lifetime of entire cluster by changing densities of different bond populations as well as their extensional rigidity and binding properties. The critical force of the entire cluster increases drastically with the rebinding ratio $\gamma^r$, while the dependence of $f^c$ on the ratio $k^r$ of spring extensional rigidities is rather weak. Interestingly, even though a large spring rigidity makes the corresponding sub-cluster to carry a large load, this leads to a fast rupture rate due to its exponential dependence on the applied force. There exists an optimal spring-rigidity ratio $k^r_{\text{opt}}$, which weakly depends on the rebinding ratio and results in the maximum critical force for a given $\gamma^r$. Noteworthy, $k^r_{\text{opt}}$ represents a load sharing where the forces on individual bond sub-clusters are equal to their corresponding critical forces. The lifetime of a heterogeneous cluster is nearly independent of $k^r$, but has a strong dependence on $\gamma^r$.

Another important parameter is the fraction $\rho$ of the first bond population, which can be tuned to control the critical force and lifetime of a heterogeneous cluster. This has direct biological relevance as cells can regulate receptor density, while $\gamma^r$ and $k^r$ are intrinsic properties of bond populations within the cluster. Furthermore, when $f^c$ is compared to the maximum critical force of individual sub-clusters, a stability enhancement by weaker sub-clusters up to a factor of $m$ can theoretically be achieved, where $m$ is the number of different bond populations. The presented model can be used for the interpretation of cell-adhesion measurements and better understanding of the role of different bond populations within heterogeneous adhesion clusters.
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[27] See Supplemental Material at [URL by publisher] for further details about the master equation, stochastic simulations, the derivation of maximum stability enhancement, and additional contour plots of the critical force for various $\rho$.
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