Emmy Noether Junior Research Group
Cellular Adhesion Clusters under Force

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Physical concepts are essential to understand the functioning of biological cells. For example, the physical properties of cytoskeleton, plasma membrane, adhesion clusters and extracellular matrix strongly influence cell shape, adhesion and migration, which in turn are essential elements of many important physiological processes, including development, inflammation and wound healing. During recent years, a large variety of new experimental tools has been developed in biophysics and materials science which now allow to characterize and control various physical determinants of cellular systems in a quantitative way. On the extracellular side, this includes the use of soft lithography to create biochemically, topographically and mechanically structured surfaces. On the intracellular side, this includes a large variety of novel fluorescence probes, colloidal spectroscopy and microrheology. In parallel to these experimental advances, concepts from statistical mechanics and soft matter physics have been increasingly applied to cellular systems.

In cell adhesion, physical concepts like force and elasticity are particularly important. Cells adhere to each other and to the extracellular matrix through clusters of transmembrane adhesion receptors, which on the intracellular side usually couple to the cytoskeleton. Therefore they usually are under considerable mechanical load. For example, adherens junctions in cell-cell adhesion and focal adhesions in cell-matrix adhesion are mediated by receptors from the cadherin and integrin families, respectively, which both couple to the actin cytoskeleton. In some cases, cell adhesion is determined by the interplay between several receptor systems. One example is the way in which white blood cells, but also stem and cancer cells exit the blood flow, as depicted schematically in **Fig. 1**. In the initial stages, the white blood cells bind to the vessel walls through receptors from the selectin family. Because the selectin bonds break rapidly, the cells start to roll, with new bonds forming at the front and old ones breaking at the back. The main function of rolling adhesion is to slow down the cell in such a way that it can survey the vessel walls for exit signals. If these are present, firm adhesion through long-lived integrin receptors is activated, leading to arrest and subsequent extravasation from the blood vessel. Thus rolling adhesion is characterized by the interplay of selectin and integrin receptors, which both couple to the actin cytoskeleton.

The coupling of adhesion clusters to the cytoskeleton does not only provide structural integrity, it also allows the cell to regulate the internal state of the adhesion cluster by force. For example, it has been shown during recent years that focal adhesion act as mechanosensors, i.e. they convert force into intracellular signalling events **[1,2]**. Mechanical properties of the extracellular environment modulate the build-up of actomyosin-generated force at focal adhesions and therefore can be sensed by cells through force-mediated processes at focal adhesion. Based on this information, cells can for example decide how to position and orient in a mechanically anisotropic environment **[3,4]**.

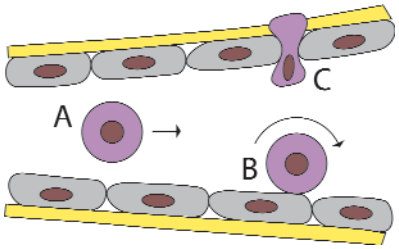

Fig. 1: White blood cells, but also stem and cancer cells travel the body in the blood flow (A). In order to exit the blood flow, they have to interact adhesively with the vessel walls. Initial adhesion is provided by short-lived selectin-bonds, resulting in rolling adhesion (B). Adhesion through long-lived integrin-bonds leads to firm arrest and extravasation (C).

Stochastic Dynamics of Adhesion Clusters

In order to understand these processes in more detail, microscopic models for force-modulated processes at adhesion clusters are required. In general, formation and rupture of adhesion bonds is a stochastic process. In this context, the simplest theoretical model for a biomolecular bond is a one-dimensional energy landscape with a transition state barrier separating the unbound from the bound state. Then the average bond lifetime T_0 can be identified with the mean first passage time to cross the transition state barrier. Kramers theory predicts that T_0 is an exponential function of barrier height in units of thermal energy. The resulting values for T_0 are typically of the order of seconds. Force tilts the energy landscape. For a sharp transition state barrier, Kramers theory predicts that average bond lifetime T under force decreases in an exponential way as function of force, $T = T_0 e^{-f}$, where f is force in units of thermal energy divided by the distance between the bound state and the sharp transition state barrier. The resulting intrinsic force scale typically is of the order of pico-Newtons. In 1978, Bell postulated this relation for single bonds under constant force. In 1997, Evans and Ritchie applied this concept to single bonds under time-dependent forces. They predicted that for a linearly rising force, average bond lifetime becomes a logarithmic function of loading rate. This prediction has been confirmed impressively in subsequent experiments and defined the new field of *dynamic force spectroscopy*. Since force is usually applied through some soft transducer, the bond cannot rebind after rupture due to elastic recoil of the transducer.

Since adhesion bonds in cellular systems usually act in a cooperative way in adhesion clusters, this single molecule effort now has to be extended to multiple bonds. In contrast to the situation with single bonds, now rebinding should be possible as long as at least one closed bonds can ensure spatial proximity of receptors and ligands. In order to investigate the role of force for the stochastic dynamics of adhesion clusters, we studied a one-step master equation for the dynamics of N parallel adhesion bonds under dimensionless force f and with dimensionless rebinding rate γ **[5,6]**. **Fig. 2** schematically shows the situation under consideration. In our model, we neglect spatial aspects and the state of the adhesion cluster is described completely by the number i of closed bonds. There are N+1 possible states ($0 \leq i \leq N$) and the

reverse and forward rates between the different state are $i\, e^{f/i}$ and $\gamma(N-i)$. Here the factor f/i reflects the fact that force is assumed to be shared equally between the closed bonds, leading to non-trivial cooperativity between the different bonds. For finite force, this model is highly non-linear and therefore difficult to solve. Nevertheless exact solutions can be found for several special cases, including f=0, γ=0 and N=2. In the general case of arbitrary N, f and γ, the master equation can be solved by computer simulations, for example by adapting the Gillespie algorithm for exact stochastic simulations. Computer simulations are also essential to reveal the nature of single rupture trajectories. For most of the time, these trajectories follow the smooth time course of the first moment. The final stages of rupture however are characterized by rather abrupt decay which results from the Arrhenius factor $e^{f/i}$ in the reverse rate: if the number of closed bonds i fluctuates to a smaller value, force on the remaining closed bonds and therefore their dissociation rate increase, leading to a positive feedback loop for rupture. Our analysis also shows that there is a threshold in force beyond which rupture is increased strongly. Moreover our master equation can be used to study the case of a linearly rising force **[7]**, a situation which is relevant for dynamic force spectroscopy on adhesion clusters.

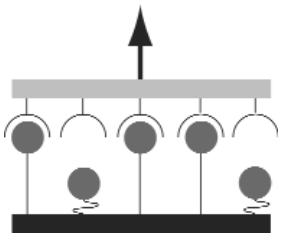

Fig. 2: Schematic representation of an adhesion cluster under force: in this cartoon, there are N=5 identical receptor-ligand bonds, of which i=3 are closed and equally share the dimensionless force f. At the same time, N-i=2 bonds are open and can rebind with the dimensionless rebinding rate γ.

For experimental purposes, the quantity of largest interest is the average cluster lifetime T as a function of the model parameters N, f and γ. This quantity can be identified with the mean first passage time to reach the completely dissociated state. For constant force, it can be calculated exactly from the adjoint master equation for arbitrary model parameters. In the case N=2, we find

$$T = \frac{T_0}{2}\left(e^{-f/2} + 2e^{-f} + \gamma e^{-3f/2}\right).$$

This two-bond equation can be understood as the generalization of Bell's single bond equation $T = T_0\, e^{-f}$. For arbitrary N, we find that average cluster lifetime T is always exponentially suppressed by force f and that the stabilizing contribution due to rebinding is a polynomial in γ of rank N-1.

Adhesion Clusters in Rolling Adhesion

In a collaboration with immunologists from the Weizmann Institute in Israel, we used these results to evaluate flow chamber data for white blood cells adhering under shear flow **[8,9]**. The red line with circles in **Fig. 3** shows the measured dissociation rate as a function of shear rate for single cells transiently tethered to the bottom of the flow chamber sparsely coated with ligands for L-selectin. At low shear, the dissociation rate plateaus at a value of 250 Hz, which most likely is the intrinsic dissociation rate of single L-selectin bonds. The force acting on the cell due to viscous drag from the hydrodynamic flow can be calculated from the Stokes equation. Combined with Bell's equation, this leads to the light blue line in **Fig. 3**, which clearly does not agree with the experimental data. However, this calculation neglects the fact that at low shear, both intrinsic dissociation and loading occur on the same time scale of milliseconds. Correcting Bell's equation for initially linear loading leads to the green line in **Fig. 3**, which is much closer to the experimental result. Most importantly, **Fig. 3** shows that at a shear rate of 40 Hz, the cellular dissociation rate suddenly drops by a factor of 14. This dramatic stabilization can be argued to result from multiple bond formation due to increased transport at higher shear. Several lines of reasoning suggest that the dominating event is the formation of two-bond clusters. The dark blue lines in **Fig. 3** are plots of the two-bond equation for different values for the rebinding rate γ. The value γ=40 (10^4 Hz in dimensional units) agrees best with the experimental data, suggesting that L-selectin mediated tethering in shear flow is characterized by unusual fast rebinding. Although these results represent only a small step toward a complete understanding of the complicated process of rolling adhesion, they show how quantitative evaluation of experimental data can help to dissect complex cellular systems.

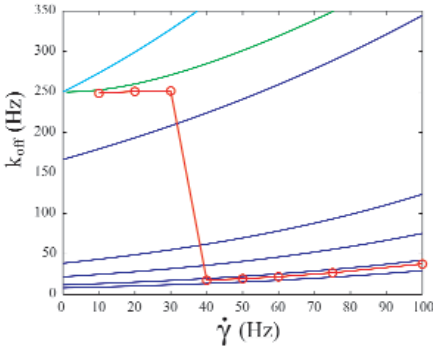

Fig. 3: Red line with circles: experimental data for cellular dissociation rate as a function of shear rate as measured in flow chambers for white blood cells adhering through L-selectin. Light blue line: single bond dissociation as predicted by Bell's equation with immediate loading. Green line: Bell's equation corrected for finite loading rate. Dark blue lines: two-bond equation for different values of the rebinding rate γ (γ=0, 10, 20, 40 and 60 from top to bottom). Agreement with the experimental data is best for γ=40.

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