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Catch Bonds: Physical Models and Biological Functions

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Abstract: Force can shorten the lifetimes of receptor-ligand bonds by accelerating their dissociation. Perhaps paradoxical at first glance, bond lifetimes can also be prolonged by force. This counterintuitive behavior was named catch bonds, which is in contrast to the ordinary slip bonds that describe the intuitive behavior of lifetimes being shortened by force. Fifteen years after their theoretical proposal, catch bonds have finally been observed. In this article we review recently published data that have demonstrated catch bonds in the selectin system and suggested catch bonds in other systems, the theoretical models for their explanations, and their function as a mechanism for flow-enhanced adhesion.

keyword: Kinetics, Off-rate, Lifetimes, Force, Flow-enhanced adhesion.

1 Introduction

Interactions between molecules that generate, exert, or transmit forces are likely regulated by force. Examples may include cell adhesion molecules that physically connect one cell to another or to the extracellular matrix to withstand forces, cytoskeletal molecules that can elongate by polymerization against mechanical loads, and motor molecules that bind their associated cytoskeletal filaments to generate forces and motions. Thus, how externally applied force regulates molecular interactions is a fundamental question in biology. Such interactions are usually described using the framework of chemical reaction kinetics. Therefore, the force regulation of molecular interactions can be treated mathematically by the force dependence or force-history dependence of chemical reaction kinetic rates. Twenty-seven years ago, Bell proposed the first model for force dependence of off-rate of receptor-ligand dissociation, which is expressed as an exponentially increasing function of force [Bell (1978)]. Ten years later, Dembo et al. proposed an alternative model, which assumes off-rate as an exponentially increasing function of the square of force [Dembo, Tourney, Saxman, Hammer (1988)]. As a theoretical possibility, these authors also suggested off-rate to be exponentially decreasing functions of force and of the square of force. Different types of bonds were classified according to how their lifetimes respond to increasing force: slip bonds if lifetimes are shortened, catch bonds if lifetimes are prolonged, and ideal bonds if lifetimes are the same [Dembo, Tourney, Saxman, Hammer (1988)]. It was not until 1995 that the first experimental measurement of off-rate as a function of force was published for P-selectin interacting with P-selectin glycoprotein ligand 1 (PSGL-1); this result was analyzed using the Bell model [Alon, Hammer, Springer (1995)]. Eight years later, the first experimental demonstration of catch bonds was finally published, again for the P-selectin-PSGL-1 interactions [Marshall, Long, Piper, Yago, McEver, Zhu (2003)]. Catch bonds have also been demonstrated for other selectin-ligand interactions [Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004)] and have been suggested for other systems [Chigaev, Buranda, Dwyer, Prossnitz, A. (2003); Forero, Thomas, Bland, Nilsson, Sokurenko, Vogel (2004); Thomas, Nilsson, Forero, Sokurenko, Vogel (2004); Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)]. Several mathematical models describing and analyzing catch-slip bonds have recently been published [Bartolo, Derenyi, Ajdari (2002); Evans, Leung, Heinrich, Zhu (2004); Barsegov, Thirumalai (2005); Pereverzev, Prezhdo, Forero, Sokurenko, Thomas (2005); Pereverzev, Prezhdo, Thomas, Sokurenko (2005); Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)].

Interactions of selectins with their glycoconjugate ligands mediate leukocytes tethering to and rolling on the vascular wall at sites of inflammation and injury. Because these molecules function in a mechanically

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stressful environment, the selectin-ligand interactions serve as excellent model systems for studying the relationship between off-rate and force. Indeed, most of the published data on force dependence of off-rate are for selectin-ligand bonds, including the first slip-bond measurement [Alon, Hammer, Springer (1995)] and all of the catch-bond measurements to date [Marshall, Long, Piper, Yago, McEver, Zhu (2003); Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004); Evans, Leung, Heinrich, Zhu (2004); Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)]. The force dependence of off-rate determines how long a bond lasts under changing forces. Bond lifetimes in turn govern how rapidly and how stably the leukocytes roll and how many of them accumulate on the vessel walls under flow. Hence catch bonds may play an important role in the regulation of the multistep adhesion and signaling cascade of the inflammatory reaction.

In this paper we will summarize recently published data that have demonstrated catch bonds in the selectin system and their biological function as related to flow-enhanced adhesion.[3] We will also review the theoretical models for catch-slip transitional bonds and suggested catch bonds in other systems.

2 Demonstration of catch bonds

Most experiments for measuring the force dependence of off-rates of receptor-ligand interactions have been done in one of two ways. One is to measure the lifetimes of single bonds at a constant force and then vary the force level over a range. The other is to measure the rupture forces of single bonds loaded by a steady ramp, and then vary the ramp rate over a range. The lifetimes have mostly been measured using a parallel-plate flow chamber to set a well-defined flow field. Suspensions of cells or microspheres bearing the receptors of interest are perfused over the chamber floor on which ligands are coated or ligand-expressing cells are cultured. To minimize the possibility of multiple bonds, the densities of receptors and/or ligands are kept very low so that the cells/microspheres tether transiently and re-enter the flow stream upon detachment without rolling. The duration, or lifetime, of each transient tether event is recorded and randomly distributed lifetimes measured at a given wall shear stress are analyzed to obtain various metrics for their distribution. The assumed kinetic model is tested against the data, and the rate parameter(s) in the model is estimated. A lifetime metric vs. force curve is generated by repeating this procedure for a range of wall shear stresses.

The rupture forces of the selectin-ligand bonds have been measured using atomic force microscopy (AFM) [Hanley, McCarty, Jadhav, Tseng, Wirtz, Konstantopoulos (2003); Hanley, Wirtz, Konstantopoulos (2004); Zhang, Bogorin, Moy (2004); Marshall, Sarangapani, Lou, McEver, Zhu (2005)], a biosurface force probe [Evans, Leung, Hammer, Simon (2001); Evans, Leung, Heinrich, Zhu (2004); Heinrich, Leung, Evans (2005)], a microneedle cantilever [Tees, Waugh, Hammer (2001)], and laser tweezers [Rinko, Lawrence, Guilford (2004)]. Again, adhesions are kept infrequent by controlling the densities of the receptors and/or ligands to minimize the possibility of multiple bonds. The rupture forces measured at a given loading rate are analyzed by histogram. The assumed kinetic model is tested against the data, and the rate parameter(s) as function(s) of force is estimated.

Early experiments only found slip bonds, even for selectin-ligand interactions that were later shown to exhibit catch bond behavior by the same experiments as those described in the preceding paragraphs. There could be several reasons for the initial failure to uncover catch bonds. First, earlier flow chamber experiments were mostly performed at forces beyond the regimes where catch bonds were later found. Even in cases where the first few data points measured in the low force regime might have appeared to exhibit catch bond behavior, they were overlooked as the majority of the data points in the high force regime displayed slip bond behavior [Alon, Hammer, Springer (1995)].

Second, early rupture force experiments were analyzed under over-simplified assumptions that precluded catch bonds. A key assumption is the Bell model or multiple Bell models in series, which dictates a special form of slip bonds. This assumption predicts that a plot of the most probable rupture force vs. logarithm of the loading rate – the so-called force spectrum – should have the form of piece-wise line segments [Merkel, Nassoy, Leung, Ritchie, Evans (1999); Evans, Leung, Hammer, Simon (2001); Evans (2001)]. Each segment should correspond to an energy barrier to dissociation. The corresponding Bell model parameters can then be calculated from the slope and y-axis intercept of each line

[3] modified from a recent invited review [Zhu, Lou, McEver (2005)].

segment. All published force spectra appear to have such simple form, suggesting that the Bell model may describe some aspects of the forced dissociation kinetics of receptor-ligand interactions. However, in most publications that measured rupture forces of selectin-ligand bonds only the peak location of a histogram (the most probable rupture force) or the mean force measured at a given loading rate is utilized to construct the force spectrum [Evans, Leung, Hammer, Simon (2001); Tees, Waugh, Hammer (2001); Hanley, McCarty, Jadhav, Tseng, Wirtz, Konstantopoulos (2003); Hanley, Wirtz, Konstantopoulos (2004); Zhang, Bogorin, Moy (2004); Rinko, Lawrence, Guilford (2004)]. The shape of the histogram, which is typically more broadly distributed than the probability density function predicted from the Bell model, contains information regarding other aspects of the bond dissociation characteristics. These may include mixed populations of multiple molecular species and or of multiple binding sites, possible rebinding, and kinetic mechanisms more complex than that of first-order dissociation, such as multiple bound states, multistep, multimeric, and multipathway interactions. Not until recently have some of these aspects been modeled and compared to experimental data [Zhu, Long, Bongrand (2002); Bartolo, Derenyi, Ajdari (2002); Dudko, Filippov, Klafter, Urbakh (2003); Evans, Leung, Heinrich, Zhu (2004); Perret, Leung, Feracci, Evans (2004); Derenyi, Bartolo, Ajdari (2004); Barsegov, Thirumalai (2005); Pereverzev, Prezhdo, Forero, Sokurenko, Thomas (2005); Pereverzev, Prezhdo, Thomas, Sokurenko (2005); Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005); Hukkanen, Wieland, Leckband, Gewirth, Braatz (2005)]. It is possible that dissociation kinetics governed by very different energy landscapes result in very similar rupture force histograms when probed by the simple steady-ramp experiment, thereby preventing a unique interpretation of the rupture force data [Bartolo, Derenyi, Ajdari (2002)]. It is also possible that rupture forces measured in steady ramp did not exhibit catch bonds due to the effect of force history dependence of off-rate, i.e., off-rates exhibit memory [Marshall, Sarangapani, Lou, McEver, Zhu (2005)]. It should be noted that the above variety of possible forms of interactions also complicate lifetime analyses. Most published work assumes the simplest model of single-step first-order irreversible dissociation of single bonds, which predicts an exponential distribution for lifetimes. More complex models usually predict multiple exponentials [Zhu, Long, Bongrand (2002); Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)]. For both lifetime experiments and rupture force experiments, catch bonds could be obscured if data are analyzed using incorrect models.

Finally, and perhaps most importantly, catch bonds are unusual and counter-intuitive. The concept of catch bonds is consistent with at least two counter-intuitive phenomena that have been observed. The first phenomenon is the irreversibility of specific biological adhesion. The work required to peel off a unit area of adhesion (fracture energy density) is much larger than the energy release from forming a unit area of adhesion (adhesion energy density) [Evans, Leung (1984); Evans (1993)]. This is in sharp contrast to many non-biological adhesions that are originated from van der Waals attraction, electric double layer repulsion, entropy-driven repulsion, depletion driven attraction, and other mechanisms [Bongrand, Bell (1984); Evans (1993)]. These nonspecific adhesions can be described by well-established theories of intermolecular and interfacial forces, which express adhesion force and energy as functions of the separation distance between two apposing surfaces [Leckband (2000)]. Such a separation distance is a scalar with no preferable direction; hence the adhesion/fracture energy density is reversible. The irreversible biological adhesion is paradoxical and has not been satisfactorily explained. Although it is consistent with the catch bond hypothesis [Dembo, Tourney, Saxman, Hammer (1988); Bartolo, Derenyi, Ajdari (2002)], the prevailing hypothesis is that peeling pulls the adhesive bonds to concentrate at the edge of the contact zone, which mediates stronger adhesion as peeling proceeds [Evans, Leung (1984); Tozeren, Sung, Chien (1989)]. Alternatively, irreversible adhesion has been attributed to a discrete distribution of adhesive bonds [Evans (1985)].

The second counter-intuitive phenomenon is flow-enhanced adhesion, which has been observed in many systems. For example, selectins require a threshold shear to support cell adhesion [Finger, Puri, Alon, Lawrence, von Andrian, Springer (1996); Alon, Chen, Puri, Finger, Springer (1997); Lawrence, Kansas, Kunkel, Ley (1997)]. As shear drops below the threshold level, fewer flowing cells tether, and the cells roll more rapidly and begin to detach. Platelets require arterial flow rates to adhere to von Willebrand factor (vWF) on damaged vascular surfaces [Savage, Saldivar, Ruggeri (1996); Doggett,

Girdhar, Lawshe, Schmidtke, Laurenzi, Diamond, Diacovo (2002); Doggett, Girdhar, Lawshe, Miller, Laurenzi, Diamond, Diacovo (2003); Li, Li, Moake, Lopez, McIntire (2004)]. In addition, many enteric bacteria require a minimum flow rate to adhere to intestinal epithelia, which likely prevents pathological attachments to bladder mucosa during stasis [Brooks, Trust (1983); Brooks, Trust (1983); Li, Mohamed, Ross (2000); Mohamed, Rainier, Ross (2000); Thomas, Trintchina, Forero, Vogel, Sokurenko (2002)]. These flow-enhanced adhesion phenomena seem counter-intuitive because increasing flow elevates the dislodging force applied to adhesive bonds. In spite of their consistence with the threshold shear requirement, catch bonds were rejected as a possible explanation for flow-enhanced selectin-mediated leukocyte adhesion because other more intuitive mechanisms are also possible and early measurements of force dependence of off-rate failed to reveal catch bonds [Finger, Puri, Alon, Lawrence, von Andrian, Springer (1996); Alon, Chen, Puri, Finger, Springer (1997); Lawrence, Kansas, Kunkel, Ley (1997)]. The flow-enhanced bacteria adhesion was interpreted in terms of a force-induced conformational change in the lectin-like receptor FimH by the authors [Thomas, Trintchina, Forero, Vogel, Sokurenko (2002)] and of catch bonds by commentators [Isberg, Barnes (2002)], but other possible interpretations were not excluded. The initial observations of catch bonds by direct measurements [Piper (1997)] were doubted and did not overcome the intensive scrutiny required for publication in top journals.

The definitive demonstration of catch bonds was obtained using a custom-made AFM capable of measuring single bond lifetimes at forces as low as a few piconewtons [Marshall, Long, Piper, Yago, McEver, Zhu (2003)]. It was found that P-selectin formed catch bonds with monomeric soluble PSGL-1 (sPSGL-1) at forces below $\sim$10 pN, which transitions to slip bonds at larger forces. Bonds of P-selectin with dimeric native PSGL-1 and with monomeric sPSGL-1 were compared to show that the PSGL-1 curve was shifted rightward and upward relative to the sPSGL-1 curve, doubling the time and force without changing the shape of the curve (Fig. 1A). Thus, catch-slip transitional bonds were observed in P-selectin interacting with both sPSGL-1 and PSGL-1 in a self-consistent manner indicative of monomeric interactions for the former and dimeric interaction for the latter. The P-selectin-(s)PSGL-1 inter-

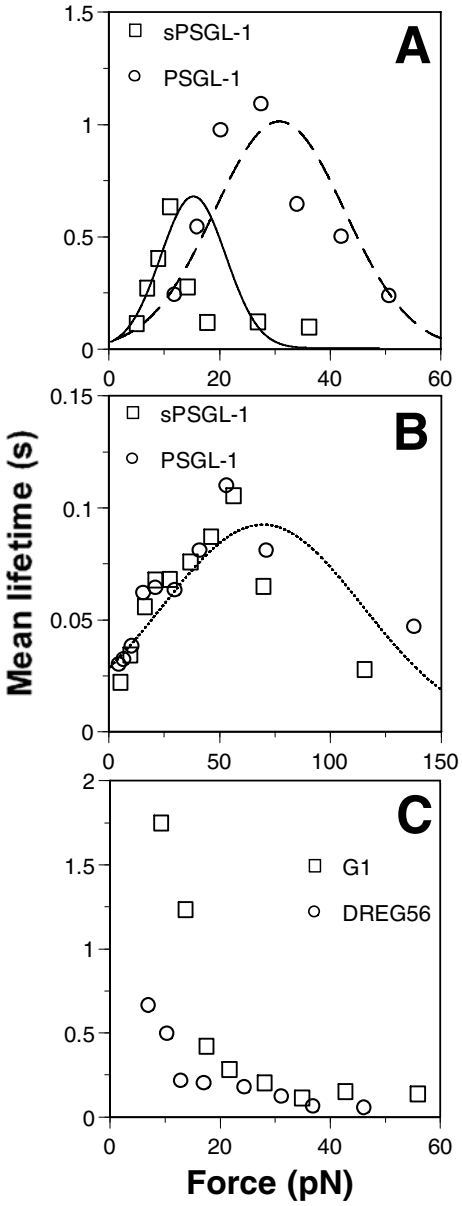

Figure 1 : Demonstration of selectin catch bonds. Single bond lifetimes were averaged and plotted vs. the level of constant force at which lifetimes were measured. (A) Bonds of P-selectin interacting with monomeric sPSGL-1 and dimeric PSGL-1. (B) Bonds of L-selectin interacting with monomeric sPSGL-1 and dimeric PSGL-1. (C) Bonds of P-selectin interacting with anti-P-selectin antibody G1 and L-selectin interacting with anti-L-selectin antibody DREG56. Data are replotted from Refs. [Marshall, Long, Piper, Yago, McEver, Zhu (2003); Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004)].

actions were contrasted to the P-selectin-antibody interactions, which exhibited only slip bond behavior (Fig. 1C). These comparisons establish that the lifetime vs. force curve is specific for the molecules used; in particular, the observed catch bonds are specific for selectin-ligand interactions. They also rule out the possibility that the observed catch bonds are artifacts of the AFM experiment. To obtain confirmation from an independent experiment, a flow chamber was used to measure the force dependence of lifetimes of transient tethers of cells and microspheres, which obtained similar results. The consistent observations from two separate experiments have thus definitively demonstrated the catch-slip bonds for P-selectin-PSGL-1 interactions [Marshall, Long, Piper, Yago, McEver, Zhu (2003)]. Similar approaches were employed to demonstrate L-selectin-PSGL-1 and L-selectin-endoglycan catch bonds [Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004)]. The L-selectin-PSGL-1 catch bonds have much shorter lifetimes but span a much broader force range than the P-selectin-PSGL-1 catch bonds before transitioning into slip bonds, and the mean lifetime vs. force curves are the same regardless of the form of PSGL-1 used, suggesting that, unlike the dimeric P-selectin, L-selectin is monomeric and can only form monomeric bonds with either monomeric sPSGL-1 or dimeric PSGL-1 (Fig. 1B). Thus, catch bonds have been demonstrated as a common rather than isolated property of selectin-ligand interactions, but the catch bonds of different selectins have quantitative differences. Similarly, slip bonds have been demonstrated as a common property for antibody-antigen interactions, because, like the anti-P-selectin antibody dissociation from P-selectin, the anti-L-selectin antibody also dissociated from L-selectin progressively faster as force increased, resulting in monotonically decreasing lifetimes [Marshall, Long, Piper, Yago, McEver, Zhu (2003); Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004)] (Fig. 1C).

3 Physical models for catch-slip bonds

The original proposal of catch bonds was derived from the transition state theory based on a spring model of the interaction energies at the bound state and the transition state, which could have different elastic constants and/or different resting lengths [Dembo, Tourney, Saxman, Hammer (1988); Dembo (1994)]. If the transition-state spring and the bound-state spring are assumed to have the same stiffness but the former has a longer resting length than the latter, the Bell model is obtained where the off-rate, k_{off}, as a function of force, f, can be expressed as

$$k_{\rm off}(f) = k^0_{\rm off} \exp[(\lambda_t - \lambda_b) f / k_B T], \tag{1}$$

where $k^0_{\rm off}$ is the zero-force off-rate, k_B is the Boltzmann constant, T is absolute temperature, and the λ's are the resting lengths with the subscript b and t indicating the bound state and transition state that the two springs respectively model. If the transition-state spring and the bound-state spring are assumed to have the same resting length but the former is softer than the latter, another slip bond model is obtained:

$$k_{\rm off}(f) = k^0_{\rm off} \exp[(\kappa_b - \kappa_t) f^2 / 2\kappa^2 k_B T], \tag{2}$$

where the κ's are the spring constants with the subscript b and t indicating the bound state and transition state that the two springs respectively model. Equation 2 is often referred to as the Dembo model or the spring model, as it predicts off-rate to be an exponentially increasing function of the strain energy of a spring.

However, no physical consideration other than the counterintuitive prediction could reject the possibility that the transition-state spring has a shorter resting length than the bound-state spring or the transition-state spring is stiffer than the bound-state spring. Both cases predict catch bonds, such that off-rate decreases exponentially with force for the former case (Eq. 1) and with the square of force for the latter cases (Eq. 2). To provide a physical picture for these then purely theoretical possibilities, the authors constructed a geometrical model – the finger-prison – which is a toy that traps a child's finger. The harder one tries to pull the finger out, the tighter it locks one's finger up [Dembo, Tourney, Saxman, Hammer (1988)].

In their original paper, Dembo et al. assumed the bound-state spring and the transition-state spring to have either the same stiffness or the same resting length; i.e., only one of the two parameters of the two springs were assumed to differ, which resulted in either slip bonds or catch bonds [Dembo, Tourney, Saxman, Hammer (1988)]. It was pointed out that there existed another possibility – one that the authors did not raise – the case in which not only do the elastic constants differ but the

resting lengths are also different for the transition-state spring and the bound-state spring [Piper (1997)]. In this case, off-rate is predicted to depend exponentially on a quadratic function of force:

$$k_{\text{off}}(f) = k^0_{\text{off}} \exp\{[(\lambda_t - \lambda_b)f + (\kappa_b - \kappa_t)f^2/2\kappa^2]/k_B T\}, \quad (3)$$

Depending on the relative magnitudes of the two elastic constants and the two resting lengths of the two springs, either slip-catch transitional bonds [Piper (1997)] or catch-slip transitional bonds can be predicted. For example, the values of k^0_{off}= 45.2 s^{-1}, $\lambda_t - \lambda_b$ = - 0.108 nm, and $(\kappa_b - \kappa_t)/2\kappa^2$= 3.51 $\times$ 10^{-3} nm/pN allow Eq. 3 to fit the P-selectin-sPSGL-1 catch-slip transitional bond data (Fig. 1A, solid curve). Because this is a monomeric interaction, the mean lifetime is reciprocal of the off-rate [Marshall, Long, Piper, Yago, McEver, Zhu (2003)]. The same set of parameters also allow fitting of the dimeric P-selectin-PSGL-1 catch-slip transitional bond data where the mean lifetime is calculated from $1.5/k_{off}(f/2)$ [Pereverzev, Prezhdo, Forero, Sokurenko, Thomas (2005)] (Fig. 1A, dashed curve). Similarly, the values of k^0_{off}= 35.2 s^{-1}, $\lambda_t - \lambda_b$ = - 0.00829 nm, and $(\kappa_b - \kappa_t)/2\kappa^2$= 5.98 $\times$ 10^{-5} nm/pN allow Eq. 3 to fit both the catch-slip bond data of L-selectin interacting with both sPSGL-1 and with PSGL-1 (Fig. 1B, curve).

Under the assumption of a single dissociation pathway, Evans and Ritchie [Evans, Ritchie (1997)] derived the originally empirical Bell equation [Bell (1978)] from Kramers' kinetic rate theory [Kramers (1940)]. Dissociation is treated as thermally activated diffusive escape over an energy barrier at the transition state from an energy well that traps the interacting molecules in the bound state. The length parameter in Eq. 1,$\lambda_t - \lambda_b$, is interpreted as the distance from the bound state to the transition state along the reaction coordinate projected on the force direction. The externally applied force does positive work to lower the energy barrier by pulling the system from the bound state toward the transition state, thereby accelerating dissociation and shortening bond lifetimes. Such a simple physical picture only allows slip bonds if there is only a single bound state and a single pathway for dissociation.

However, it is not the laws of physics, but the oversimplified assumptions, that are incompatible with catch bonds. It is possible that the force pulls the system further away from the transition state by doing negative work, which results in catch bonds as in Dembo's original proposal [Dembo, Tourney, Saxman, Hammer (1988)]. However, a catch bond model of such a simple form is insufficient to describe the published data, because they all show that catch bonds exist at a low force regime that then transition to slip bonds at a high force regime [Marshall, Long, Piper, Yago, McEver, Zhu (2003); Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004); Yago, Wu, Wey, Klopocki, Zhu, McEver (2004); Evans, Leung, Heinrich, Zhu (2004)]. To account for the catch-slip transitional bonds requires the removal of another over-simplified assumption – the single pathway for dissociation. Two-pathway models were first discussed by Bartolo et al. [Bartolo, Derenyi, Ajdari (2002)] before catch bonds were observed and were independently conceived again by those who tried to explain the physical basis of catch-slip transitional bonds [Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004); Evans, Leung, Heinrich, Zhu (2004)].

Four two-pathway models have been proposed and analyzed for their ability to describe the published data [Evans, Leung, Heinrich, Zhu (2004); Barsegov, Thirumalai (2005); Pereverzev, Prezhdo, Forero, Sokurenko, Thomas (2005); Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)], including the single molecule data from jump/hold experiments [Marshall, Long, Piper, Yago, McEver, Zhu (2003); Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004)] and steady ramp and jump/ramp experiments [Evans, Leung, Heinrich, Zhu (2004)] that demonstrated catch-slip bonds as well as the whole cell dissociation data from flow chamber experiments that strongly suggested catch-slip bonds [Thomas, Trintchina, Forero, Vogel, Sokurenko (2002); Thomas, Nilsson, Forero, Sokurenko, Vogel (2004); Forero, Thomas, Bland, Nilsson, Sokurenko, Vogel (2004); Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)]. The simplest of these is a four-parameter model that assumes dissociation from a single bound state either along a slip-bond pathway over a high-energy barrier or along a catch-bond pathway over a low-energy barrier [Pereverzev, Prezhdo, Forero, Sokurenko, Thomas (2005); Pereverzev, Prezhdo, Thomas, Sokurenko (2005)]. The idea is that a bond can dissociate along two pathways – one slow and the other fast – with different off-rates, $k_{off} = p_s + p_f$, whose relative magni-

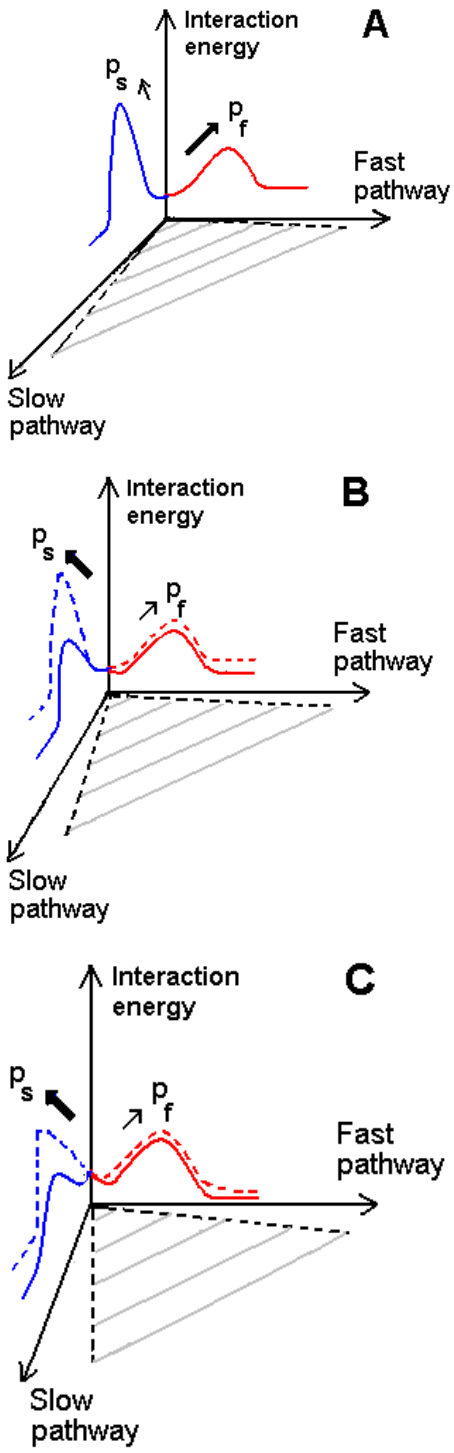

Figure 2 : Two-pathway model of catch-slip transitional bonds. (Adapted from Ref. [Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004)]).

tude is indicated by the thickness of the arrows in Fig. 2 [Sarangapani, Yago, Klopocki, Lawrence, Fieger, Rosen, McEver, Zhu (2004)]. p_s is assumed small at low force but increases with increasing force, i.e., a slip bond along the slow pathway with a small zero-force off-rate. p_f is assumed high at low force but decreases with increasing force, i.e., a catch bond along the fast pathway with a large zero-force off-rate. As a result, dissociation takes place primarily along the fast pathway at low forces but switches to the slow pathway as applied force increases. Increasing force would first slow dissociation because p_f decreases more rapidly than p_s can increase to compensate the decrease in off-rate (Fig. 2 A and B). In other words, a bond trying to dissociate is more likely to get caught along the fast pathway than it can slip out along the slow pathway as force increases. After the fast pathway is shut down by high forces, the slow pathway becomes predominant so a continued increase in force would accelerate dissociation by suppressing the energy barrier on this pathway (Fig. 2C). The interaction energy can be thought of as a three-dimensional surface defined on the $x-y$ plane with the two pathways as axes. The energy barriers on these pathways are depicted in Fig. 2 at low (Fig. 2A), intermediate (Fig. 2B), and high (Fig. 2C) forces. In this figure, the respective configurations at the current and previous force level are indicated by the solid and dashed curves to show how force progressively tilts the energy landscape by adding a mechanical potential (hatched inclined plane) to the system.

The more involved models hypothesize the existence of two bound-states that can interconvert from each other and dissociate along their respective force-accelerated pathways [Evans, Leung, Heinrich, Zhu (2004); Barsegov, Thirumalai (2005); Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)]. This hypothesis allows catch-slip transitional bonds even if all rate parameters – two off-rates describing the respective dissociation from the two bound-states and two transition rates describing the inter conversion between the two bound-states – are slip bonds. However, different assumptions were made regarding how the two bound-states were populated initially. Evans et al. assumed a rapid equilibrium between the two bound states so they were partitioned according to a detailed balance, which reduces the model parameters from nine to five [Evans, Leung, Heinrich, Zhu (2004)]. This model has been tested using not only the conventional steady ramp

force spectroscopy but also a novel jump/ramp force spectroscopy experiment where the bond was first rapidly loaded (i.e., jump) to a predetermined force level and then steadily ramped until rupture [Evans, Leung, Heinrich, Zhu (2004)]. The population of bonds that survived the jump was found to dissociate along the slip bond branch of the biphasic curve only, which could be explained by the fast pathway being switched off by the jump [Evans, Leung, Heinrich, Zhu (2004)]. By comparison, Thomas et al. assumed a slow conversion between the two bound-states, so that their initial partitions were determined by the on-rates of forming bonds in these two bound-states that were assumed to be in thermodynamic equilibrium in the absence of force and to remain the same when force is applied [Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)]. This model is capable of accounting for the double exponential decay of the pause time distribution of *E. coli* bound to mannose-BSA coated surface measured in the flow chamber. The assumption of slow conversion between the two bound-states was supported by the observation of slow conversion from the "stick" mode to the "roll" mode of adhesion in an experiment that rapidly switched the wall shear stress from high to low [Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)].

4 Biological functions of catch bonds

The experimental demonstration of catch bonds begs the question of their biological functions. One answer to this question is the recently demonstrated causal relationship between catch bonds and a phenomenon of known biological relevance – the selectin-mediated flow-enhanced leukocyte rolling [Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)]. As noted earlier, selectins require a threshold shear to support cell adhesion both in vivo [Finger, Puri, Alon, Lawrence, von Andrian, Springer (1996)] and in vitro [Finger, Puri, Alon, Lawrence, von Andrian, Springer (1996); Alon, Chen, Puri, Finger, Springer (1997); Lawrence, Kansas, Kunkel, Ley (1997)], which is particularly pronounced for L-selectin. Rolling can be quantified by rolling velocity and rolling regularity. The latter has many aspects and can be characterized by analyzing the stop-and-go pattern of a large number of rolling steps, which include stop and go frequencies, mean stop times and mean go distances, and fraction of steps that contain stops, as exemplified in

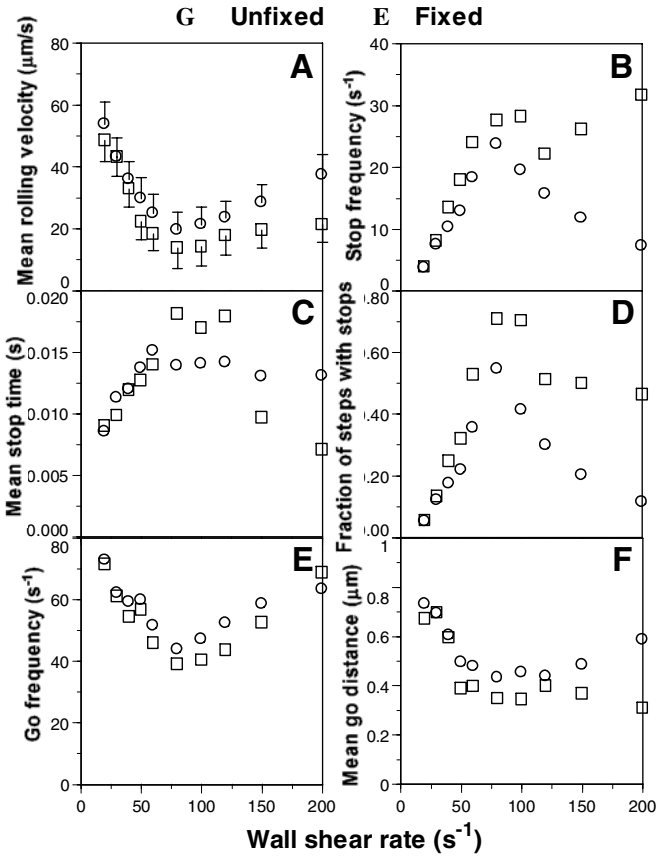

Figure 3 : Parameters of rolling on sPSGL-1 of unfixed (squares) and fixed (circles) neutrophils as functions of wall shear rate. Rolling of 10-15 cells was observed at 250 frames per second for 1 s and rolling velocities were analyzed by an excel macro to segregate the frame-by-frame motions into steps of go cycles of acceleration and deceleration that may or may not contain an intervening stop period. Thousands of such steps were quantified to calculate the mean $\pm$ s.e.m. of rolling velocity (A), the frequency (B) and mean duration (C) of stops, the fraction of steps that contain stops (D), and the frequency (E) and mean distance (F) of go's. Data are replotted from Ref. [Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)].

Fig. 3. Although increasing shear elevates the dislodging force applied to adhesive bonds, cells roll more slowly (Fig. 3A), stop more frequently and for longer times (Fig. 3 B and C), roll with more steps that include full stops (Fig. 3D), and go less frequently and for shorter distance (Fig. 3 E and F), until an optimal shear of $\sim$80 s^{-1} is reached where rolling displays a minimum velocity and maximum regularity. This paradoxical phenomenon

had not been satisfactorily explained. The prevailing hypothesis was that adhesion was enhanced by a flow-dependent increase in the on-rates of bond formation. This might occur by shear rate-dependent transport of adhesion molecules to their ligands so that more new bonds could form before previous bonds dissociated [Finger, Puri, Alon, Lawrence, von Andrian, Springer (1996); Alon, Chen, Puri, Finger, Springer (1997); Chang, Hammer (1999); Chen, Springer (1999)], or by shear stress-dependent enlargement of the cell-surface contact area so that more receptors on the cell could reach the ligands on the surface to form new bonds [Finger, Puri, Alon, Lawrence, von Andrian, Springer (1996); Evans, Leung, Hammer, Simon (2001); Zhao, Chien, Weinbaum (2001)]. As discussed before, the flow-enhanced adhesion phenomenon is also consistent with the catch bond hypothesis such that force slows bond dissociation and lengthens bond lifetimes, thereby stabilizing rolling and reducing rolling velocity. However, the catch bond hypothesis was dismissed because catch bonds were not found in these early experiments [Alon, Chen, Puri, Finger, Springer (1997); Chen, Springer (2001)].

To show that catch bonds govern adhesion through L-selectin at threshold shear, high-speed video microscopy was used to observe tethering and rolling in media of different viscosities of neutrophils of normal and diminished deformability or of L-selectin-coated rigid microspheres of different radii [Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)]. Dimensional analysis was applied to these data to examine the relative importance to rolling of the shear rate $\dot{\gamma}$ (s^{-1}), shear stress σ ($=\mu\dot{\gamma}$, where μ is medium viscosity) (dyn/cm^2), tether force F_t ($\propto r^2\mu\dot{\gamma}$ where r is the microsphere radius) (pN), and cell deformability. Important variables can be identified if data are sensitive to their changes and if multiple data curves measured under different conditions align and collapse into a single curve. Irrelevant mechanisms can be ruled out if data are indifferent to the changes of their controlling variables and if data curves do not align when plotted against their controlling variables.

Using this strategy, the shear stress-dependent enlargement of the cell-substrate contact area [Finger, Puri, Alon, Lawrence, von Andrian, Springer (1996); Evans, Leung, Hammer, Simon (2001); Zhao, Chien, Weinbaum (2001)] has been excluded as the dominant mechanism for flow-enhanced rolling, as the flow-enhanced portions of the rolling parameter curves (wall shear rate

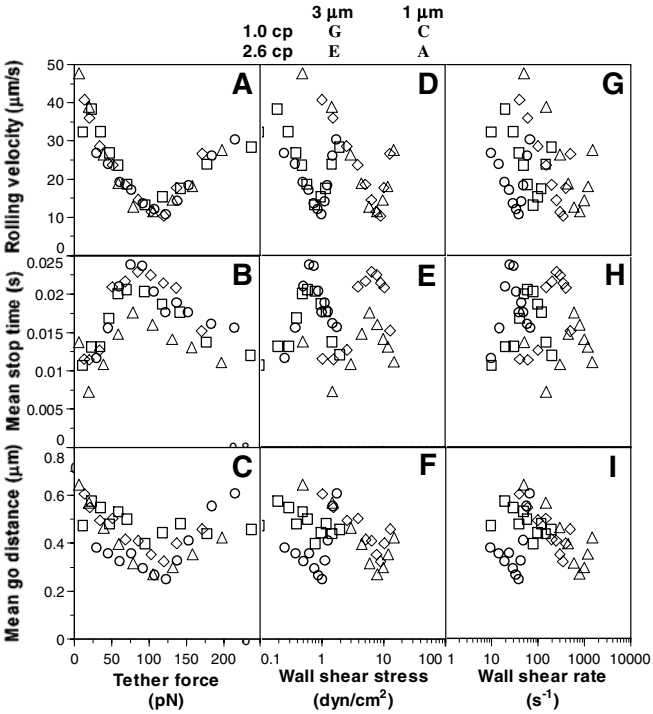

Figure 4 : Tether force governs rolling velocity and regularity below and above the flow optimum. Mean velocity (A, D, G), mean stop time (B, E, H), and mean go distance (C, F, I) of L-selectin-bearing microspheres of 3- or 1-μm radii rolling on sPSGL-1 (140 sites/μm^2) in the absence or presence of 6% Ficoll (corresponding to viscosity of 1 or 2.6 cp) are plotted against tether force, wall shear stress, and wall shear rate. A logarithmic scale is used to plot the broad range of wall shear stresses and wall shear rates, whereas a linear scale is used to plot tether forces. Data are replotted from Ref. [Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)].

< 80 s^{-1}) are insensitive to changes in cell deformability (Fig. 3) and are still present even for rigid microspheres (Fig. 4) [Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)]. The transport of adhesion molecules to their ligands for new bond formation [Finger, Puri, Alon, Lawrence, von Andrian, Springer (1996); Alon, Chen, Puri, Finger, Springer (1997); Chang, Hammer (1999); Chen, Springer (1999)] has also been excluded as an important mechanism for flow-enhanced rolling, as the rolling parameter curves do not align when plotted against wall shear rate (Fig. 4 G-I), which is the parameter that controls transport [Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)].

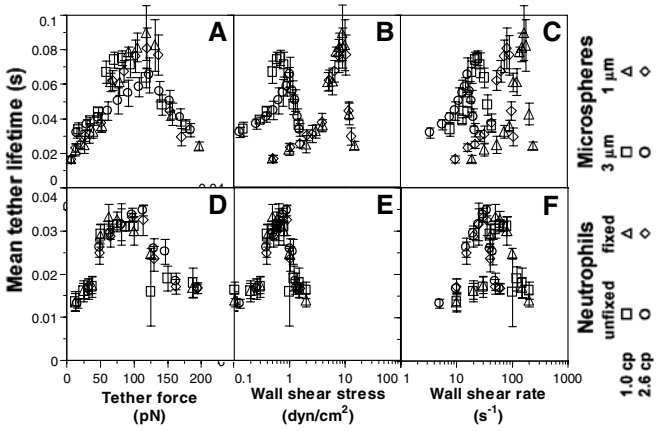

Figure 5 : Tether force governs lifetimes of transient tethers below and above the flow optimum. Lifetimes of transient tethers of L-selectin-bearing microspheres of 3- or 1-μm radii (A-C) or of unfixed or fixed neutrophils (D-F) to low density sPSGL-1 ($<$10 sites/μm^2) in the absence or presence of 6% Ficoll (corresponding to viscosity of 1 or 2.6 cp) were plotted against tether force (A and D), wall shear stress (B and E), and wall shear rate (C and F). A logarithmic scale was used to plot the broad range of wall shear stresses and wall shear rates, whereas a linear scale was used to plot tether forces. The data represent the mean $\pm$ SD of four experiments. Data are replotted from Ref. [Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)].

Multiple lines of evidence have been obtained that establish catch bonds as a mechanism for flow-enhanced cell rolling [Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)]. For every rolling parameter studied (three exemplified in Fig. 4), data align such that multiple curves collapse into a single curve when plotted against tether force (Fig. 4 A-C) but not against wall shear rate (Fig. 4 D-F) or wall shear stress (Fig. 4 G-I). Thus, tether force acts as a similarity variable that incorporates the separate effects on rolling of wall shear stress, wall shear rate, medium viscosity, and microsphere radius. The tether lifetime curves also align and collapse when plotted against tether force (Fig. 5 A and B) but not against wall shear rate (Fig. 5 C and D) or wall shear stress (Fig. 5 E and F) for both microspheres coated with L-selectin (Fig. 5 A-C) and neutrophils expressing L-selectin (Fig. 5 D-F) transiently tethered to sPSGL-1 coated at low density on the flow chamber floor. This is expected because a single molecular bond can only support force, not shear stress or shear rate. Significantly, the biphasic shapes of the rolling parameter vs. tether force curves have the same or reciprocal shapes of the tether lifetime curves (compared Fig. 4 and 5), indicating that these rolling parameters depend on force through the force dependence of off-rate, which exhibit catch-slip transitional bond behavior. Thus, catch bonds enabled increasing force to convert short-lived tethers into longer-lived tethers, which decreased rolling velocities and increased the regularity of rolling steps as shear rose from the threshold to an optimal value. As shear increased above the optimum, transitions to slip bonds shortened tether lifetimes, which increased rolling velocities and decreased rolling regularity.

5 Possible catch bonds in other systems

It is possible that catch bonds also serve as a mechanism for mechanical regulation of molecular interactions in other systems. Although definitive demonstration of catch bonds has yet to be obtained using single bond lifetime measurement, multiple lines of evidence suggest that putative FimH-mannose catch bonds may govern the flow-enhanced adhesion of type 1 fimbriated *E. coli* to mannosylated glycoproteins [Thomas, Trintchina, Forero, Vogel, Sokurenko (2002); Thomas, Nilsson, Forero, Sokurenko, Vogel (2004); Forero, Thomas, Bland, Nilsson, Sokurenko, Vogel (2004); Thomas, Forero, Yakovenko, Nilsson, Vicini, Sokurenko, Vogel (2005)]. The bell-rope model for the activation of αA and βA domains (also named I and I-like domains, respectively) of integrins hypothesizes that an applied force pulling at the C-terminal α helix at the bottom of the α/βA domain results in its downward movement, which allosterically change the conformation of the ligand binding site on the top of the α/βA domain, thereby increasing the binding affinity and bond lifetimes [Shimaoka, Xiao, Liu, Yang, Dong, Jun, McCormack, Zhang, Joachimiak, Takagi, Wang, Springer (2003)]. This model has been supported by both mutational studies [Lu, Shimaoka, Ferzly, Oxvig, Takagi, Springer (2001); Shimaoka, Lu, Palframan, von Andrian, McCormack, Takagi, Springer (2001); Shimaoka, Xiao, Liu, Yang, Dong, Jun, McCormack, Zhang, Joachimiak, Takagi, Wang, Springer (2003)] and molecular dynamics simulations [Jin, Andricioaei, Springer (2004)]. Given the prominent mechanical role in their functions, it seems reasonable to suspect that interactions of motors with

their associated molecules are regulated mechanically. The affinities of kinesin for microtubules and of myosin for actin are allosterically regulated by nucleotides ATP and ADP, and it has been proposed that their binding is regulated by mechanical force due to internal strain [Rosenfeld, Fordyce, Jefferson, King, Block (2003); Rosenfeld, Sweeney (2004)].

It has been proposed that catch bonds may prevent inappropriate leukocyte adhesion [Marshall, Long, Piper, Yago, McEver, Zhu (2003); Yago, Wu, Wey, Klopocki, Zhu, McEver (2004)]. Both L-selectin and PSGL-1 are expressed on the surface of leukocytes, and their interactions mediate tethering and rolling of flowing leukocytes on leukocytes that have already adhered to endothelial cells or platelets at sites of inflammation and injury. Without catch bonds to shorten the bond lifetimes at low forces, the L-selectin-PSGL-1 interactions may induce spontaneous aggregation of free-flowing leukocytes where forces on the bonds are expected to be low. We may further speculate that a similar catch-slip bond mechanism could prevent platelet aggregation. The interaction of platelet glycoprotein Ibα (GPIbα) with the plasma protein vWF mediates the flow-enhanced adhesion of platelets to subendothelial surfaces of disrupted arterial vessels [Savage, Saldivar, Ruggeri (1996); Doggett, Girdhar, Lawshe, Schmidtke, Laurenzi, Diamond, Diacovo (2002); Doggett, Girdhar, Lawshe, Miller, Laurenzi, Diamond, Diacovo (2003)]. Yet platelets do not form aggregates spontaneously in regions of sluggish flows. Interestingly, spontaneous platelet aggregation is a hallmark of the genetic disorders platelet-type and type 2B von Willebrand disease (vWD). The GPIbα molecule in patients with platelet-type vWD and the vWF molecule in patients with type 2B vWD are respectively mutated such that the mutant GPIbα-vWF interactions are of higher affinities and longer lifetimes [Doggett, Girdhar, Lawshe, Schmidtke, Laurenzi, Diamond, Diacovo (2002); Doggett, Girdhar, Lawshe, Miller, Laurenzi, Diamond, Diacovo (2003); Kumar, Dong, Thaggard, Cruz, Lopez, McIntire (2003)]. The functional similarities between the GPIbα-vWF interaction and the L-selectin-PSGL-1 interaction suggests similarities in their kinetic properties, in particular the behavior of catch-slip transitional bonds.

6 Concluding remarks

The proposal of catch bonds was perhaps ahead of its time, for it took 15 years for this seemingly paradoxical phenomenon to be demonstrated. The experimental observation of catch bonds has quickly stimulated renewed interest in how to model such a counterintuitive bond behavior. More experiments are underway and new proposals for catch bonds in different systems are being reported. Perhaps before long, this once unusual observation will become unusually usual.

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References

Alon, R.; Chen, S.; Puri, K. D.; Finger, E. B.; Springer, T. A. (1997): The kinetics of L-selectin tethers and the mechanics of selectin- mediated rolling. *J Cell Biol*, vol. 138, pp. 1169-1180.

Alon, R.; Hammer, D. A.; Springer, T. A. (1995): Lifetime of the P-selectin-carbohydrate bond and its response to tensile force in hydrodynamic flow. *Nature*, vol. 374, pp. 539-542.

Barsegov, V.; Thirumalai, D. (2005): Dynamics of unbinding of cell adhesion molecules: transition from catch to slip bonds. *Proc Natl Acad Sci U S A*, vol. 102, pp. 1835-1839.

Bartolo, D.; Derenyi, I.; Ajdari, A. (2002): Dynamic response of adhesion complexes: Beyond the single-path picture. *Phys. Rev. E*, vol. 65, p. 051910.

Bell, G. I. (1978): Models for the specific adhesion of cells to cells. *Science*, vol. 200, pp. 618-627.

Bongrand, P.; Bell, G. I. (1984): Cell-cell adhesion: Parameters and possible mechanisms. In: Perelson, A. S., C. DeLisi, and F. W. Wiegel, editors. *Cell Surfacee Dynamics: Concept and Models*. New York, Basel: Marcel Dekker, Inc. p 459-493.

Brooks, D. E.; Trust, T. J. (1983): Enhancement of bac-

terial adhesion by shear forces: characterization of the haemagglutination induced by Aeromonas salmonicida strain 438. *J Gen Microbiol*, vol. 129, pp. 3661-3669.

Brooks, D. E.; Trust, T. J. (1983): Interactions of erythrocytes with bacteria under shear. *Ann N Y Acad Sci*, vol. 416, pp. 319-331.

Chang, K. C.; Hammer, D. A. (1999): The forward rate of binding of surface-tethered reactants: effect of relative motion between two surfaces. *Biophys J*, vol. 76, pp. 1280-1292.

Chen, S.; Springer, T. A. (1999): An automatic braking system that stabilizes leukocyte rolling by an increase in selectin bond number with shear. *J Cell Biol*, vol. 144, pp. 185-200.

Chen, S.; Springer, T. A. (2001): Selectin receptor-ligand bonds: Formation limited by shear rate and dissociation governed by the Bell model. *Proc Natl Acad Sci U S A*, vol. 98, pp. 950-955.

Chigaev, A.; Buranda, T.; Dwyer, D. C.; Prossnitz, E. R.; A., S. L. (2003): FRET detection of cellular α_4-Integrin conformational activation. *Biophys J*, vol. 85, pp. 3951-3962.

Dembo, M. (1994): On peeling an adherent cell from a surface. *Lectures on Mathematics in the Life Sciences, Some Mathematical Problems in Biology*. Providence, RI: American Mathematical Society. p 51-77.

Dembo, M.; Tourney, D. C.; Saxman, K.; Hammer, D. (1988): The reaction-limited kinetics of membrane-to-surface adhesion and detachment. *Proc. Royal Soc. London*, vol. 234, pp. 55-83.

Derenyi, I.; Bartolo, D.; Ajdari, A. (2004): Effects of intermediate bound states in dynamic force spectroscopy. *Biophys J*, vol. 86, pp. 1263-1269.

Doggett, T. A.; Girdhar, G.; Lawshe, A.; Miller, J. L.; Laurenzi, I. J.; Diamond, S. L.; Diacovo, T. G. (2003): Alterations in the intrinsic properties of the GPIbalpha-VWF tether bond define the kinetics of the platelet-type von Willebrand disease mutation, Gly233Val. *Blood*, vol. 102, pp. 152-160.

Doggett, T. A.; Girdhar, G.; Lawshe, A.; Schmidtke, D. W.; Laurenzi, I. J.; Diamond, S. L.; Diacovo, T. G. (2002): Selectin-like kinetics and biomechanics promote rapid platelet adhesion in flow: the GPIbα-vWF tether bond. *Biophys J*, vol. 83, pp. 194-205.

Dudko, O. K.; Filippov, A. E.; Klafter, J.; Urbakh, M. (2003): Beyond the conventional description of dynamic force spectroscopy of adhesion bonds. *Proc Natl Acad Sci U S A*, vol. 100, pp. 11378-11381.

Evans, E. (1993): Microscopic-physical determinants in biological adhesion. *Blood Cells*, vol. 19, pp. 401-419.

Evans, E. (2001): Probing the relation between force–lifetime–and chemistry in single molecular bonds. *Annu Rev Biophys Biomol Struct*, vol. 30, pp. 105-128.

Evans, E.; Leung, A. (1984): Adhesivity and rigidity of erythrocyte membrane in relation to wheat germ agglutinin binding. *J Cell Biol*, vol. 98, pp. 1201-8.

Evans, E.; Leung, A.; Hammer, D.; Simon, S. (2001): Chemically distinct transition states govern rapid dissociation of single L-selectin bonds under force. *Proc Natl Acad Sci U S A*, vol. 98, pp. 3784-3789.

Evans, E.; Leung, A.; Heinrich, V.; Zhu, C. (2004): Mechanical switching and coupling between two dissociation pathways in a P-selectin adhesion bond. *Proc Natl Acad Sci U S A*, vol. 101, pp. 11281-11286.

Evans, E.; Ritchie, K. (1997): Dynamic strength of molecular adhesion bonds. *Biophys J*, vol. 72, pp. 1541-1555.

Evans, E. A. (1985): Detailed mechanics of membrane-membrane adhesion and separation. II. Discrete kinetically trapped molecular cross-bridges. *Biophys J*, vol. 48, pp. 185-192.

Finger, E. B.; Puri, K. D.; Alon, R.; Lawrence, M. B.; von Andrian, U. H.; Springer, T. A. (1996): Adhesion through L-selectin requires a threshold hydrodynamic shear. *Nature*, vol. 379, pp. 266-269.

Forero, M.; Thomas, W. E.; Bland, C.; Nilsson, L. M.; Sokurenko, E. V.; Vogel, V. (2004): A catch-bond based nanoadhesive sensitive to shear stress. *Nano Lett.*, vol. 4, pp. 1593-1597.

Hanley, W.; McCarty, O.; Jadhav, S.; Tseng, Y.; Wirtz, D.; Konstantopoulos, K. (2003): Single molecule characterization of P-selectin/ligand binding. *J Biol Chem*, vol. 278, pp. 10556-10561.

Hanley, W. D.; Wirtz, D.; Konstantopoulos, K. (2004): Distinct kinetic and mechanical properties govern selectin-leukocyte interactions. *J Cell Sci*, vol. 117, pp. 2503-2511.

Heinrich, V.; Leung, A.; Evans, E. (2005): Nano- to microscale dynamics of P-selectin detachment from leukocyte interfaces. II. Tether flow terminated by P-

selectin dissociation from PSGL-1. *Biophys J*, vol. 88, pp. 2299-2308.

Hukkanen, E. J.; Wieland, J. A.; Leckband, D. E.; Gewirth, A.; Braatz, R. D. (2005): Multiple-bond kinetics from single-molecule pulling experiments: Evidence for multiple NCAM bonds. *Biophys J*, in press.

Isberg, R. R.; Barnes, P. (2002): Dancing with the host; flow-dependent bacterial adhesion. *Cell*, vol. 110, pp. 1-4.

Jin, M.; Andricioaei, I.; Springer, T. A. (2004): Conversion between three conformational states of integrin I domains with a C-terminal pull spring studied with molecular dynamics. *Structure (Camb)*, vol. 12, pp. 2137-2147.

Kramers, H. A. (1940): Brownian motion in a field of force and the diffusion model of chemical reactions. *Physica (Utrecht)*, vol. 7, pp. 284-304.

Kumar, R. A.; Dong, J. F.; Thaggard, J. A.; Cruz, M. A.; Lopez, J. A.; McIntire, L. V. (2003): Kinetics of GPIbα-vWF-A1 tether bond under flow: effect of GPIbα mutations on the association and dissociation rates. *Biophys J*, vol. 85, pp. 4099-4109.

Lawrence, M. B.; Kansas, G. S.; Kunkel, E. J.; Ley, K. (1997): Threshold levels of fluid shear promote leukocyte adhesion through selectins (CD62L,P,E). *J. Cell Biol.*, vol. 136, pp. 717-727.

Leckband, D. (2000): Measuring the forces that control protein interactions. *Annu Rev Biophys Biomol Struct*, vol. 29, pp. 1-26.

Li, F.; Li, C. Q.; Moake, J. L.; Lopez, J. A.; McIntire, L. V. (2004): Shear stress-induced binding of large and unusually large von Willebrand factor to human platelet glycoprotein Ibalpha. *Ann Biomed Eng*, vol. 32, pp. 961-969.

Li, Z. J.; Mohamed, N.; Ross, J. M. (2000): Shear stress affects the kinetics of Staphylococcus aureus adhesion to collagen. *Biotechnol Prog*, vol. 16, pp. 1086-1090.

Lu, C.; Shimaoka, M.; Ferzly, M.; Oxvig, C.; Takagi, J.; Springer, T. A. (2001): An isolated, surface-expressed I domain of the integrin alphaLbeta2 is sufficient for strong adhesive function when locked in the open conformation with a disulfide bond. *Proc Natl Acad Sci U S A*, vol. 98, pp. 2387-2392.

Marshall, B. T.; Long, M.; Piper, J. W.; Yago, T.; McEver, R. P.; Zhu, C. (2003): Direct observation of catch bonds involving cell-adhesion molecules. *Nature*, vol. 423, pp. 190-193.

Marshall, B. T.; Sarangapani, K. K.; Lou, J.; McEver, R. P.; Zhu, C. (2005): Force history dependence of receptor-ligand dissociation. *Biophys J*, vol. 88, pp. 1458-1466.

Merkel, R.; Nassoy, P.; Leung, A.; Ritchie, K.; Evans, E. (1999): Energy landscapes of receptor-ligand bonds explored with dynamic force spectroscopy. *Nature*, vol. 397, pp. 50-53.

Mohamed, N.; Rainier, T. R., Jr.; Ross, J. M. (2000): Novel experimental study of receptor-mediated bacterial adhesion under the influence of fluid shear. *Biotechnol Bioeng*, vol. 68, pp. 628-636.

Pereverzev, Y. V.; Prezhdo, O. V.; Forero, M.; Sokurenko, E. V.; Thomas, W. E. (2005): The two-pathway model for the catch-slip transition in biological adhesion. *Biophys J*, vol. 89, pp. 1446-1454.

Pereverzev, Y. V.; Prezhdo, O. V.; Thomas, W. E.; Sokurenko, E. V. (2005): Distinctive features of the biological catch bond in the jump-ramp force regime predicted by the two-pathway model. *Phys Rev E Stat Nonlin Soft Matter Phys*, vol. 72, p. 010903.

Perret, E.; Leung, A.; Feracci, H.; Evans, E. (2004): Trans-bonded pairs of E-cadherin exhibit a remarkable hierarchy of mechanical strengths. *Proc Natl Acad Sci U S A*, vol. 101, pp. 16472-16477.

Piper, J. W. (1997): Force Dependence of Cell Bound E-Selectin/ Carbohydrate Ligand Binding Characteristics. Ph.D. Dissertation. School of Mechanical Engineering, Georgia Institute of Technology, Atlanta Gerogia, U. S. A.

Rinko, L. J.; Lawrence, M. B.; Guilford, W. H. (2004): The molecular mechanics of P- and L-selectin lectin domains binding to PSGL-1. *Biophys J*, vol. 86, pp. 544-554.

Rosenfeld, S. S.; Fordyce, P. M.; Jefferson, G. M.; King, P. H.; Block, S. M. (2003): Stepping and stretching. How kinesin uses internal strain to walk processively. *J Biol Chem*, vol. 278, pp. 18550-18556.

Rosenfeld, S. S.; Sweeney, H. L. (2004): A model of myosin V processivity. *J Biol Chem*, vol. 279, pp. 40100-40111.

Sarangapani, K. K.; Yago, T.; Klopocki, A. G.;

Lawrence, M. B.; Fieger, C. B.; Rosen, S. D.; McEver, R. P.; Zhu, C. (2004): Low force decelerates L-selectin dissociation from P-selectin glycoprotein ligand-1 and endoglycan. *J Biol Chem*, vol. 279, pp. 2291-2298.

Savage, B.; Saldivar, E.; Ruggeri, Z. M. (1996): Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell*, vol. 84, pp. 289-297.

Shimaoka, M.; Lu, C.; Palframan, R. T.; von Andrian, U. H.; McCormack, A.; Takagi, J.; Springer, T. A. (2001): Reversibly locking a protein fold in an active conformation with a disulfide bond: integrin alphaL I domains with high affinity and antagonist activity in vivo. *Proc Natl Acad Sci U S A*, vol. 98, pp. 6009-6014.

Shimaoka, M.; Xiao, T.; Liu, J. H.; Yang, Y.; Dong, Y.; Jun, C. D.; McCormack, A.; Zhang, R.; Joachimiak, A.; Takagi, J.; Wang, J. H.; Springer, T. A. (2003): Structures of the alpha L I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. *Cell*, vol. 112, pp. 99-111.

Tees, D. F.; Waugh, R. E.; Hammer, D. A. (2001): A microcantilever device to assess the effect of force on the lifetime of selectin-carbohydrate bonds. *Biophys J*, vol. 80, pp. 668-682.

Thomas, W.; Forero, M.; Yakovenko, O.; Nilsson, L.; Vicini, P.; Sokurenko, E.; Vogel, V. (2005): Catch bond model derived from allostery explains force-activated bacterial adhesion. *Biophys J*, vol., in press.

Thomas, W. E.; Nilsson, L. M.; Forero, M.; Sokurenko, E. V.; Vogel, V. (2004): Shear-dependent 'stick-and-roll' adhesion of type 1 fimbriated Escherichia coli. *Mol Microbiol*, vol. 53, pp. 1545-1557.

Thomas, W. E.; Trintchina, E.; Forero, M.; Vogel, V.; Sokurenko, E. V. (2002): Bacterial adhesion to target cells enhanced by shear force. *Cell*, vol. 109, pp. 913-923.

Tozeren, A.; Sung, K. L.; Chien, S. (1989): Theoretical and experimental studies on cross-bridge migration during cell disaggregation. *Biophys J*, vol. 55, pp. 479-487.

Yago, T.; Wu, J.; Wey, C. D.; Klopocki, A. G.; Zhu, C.; McEver, R. P. (2004): Catch bonds govern adhesion through L-selectin at threshold shear. *J Cell Biol*, vol. 166, pp. 913-923.

Zhang, X.; Bogorin, D. F.; Moy, V. T. (2004): Molecular basis of the dynamic strength of the sialyl Lewis X–selectin interaction. *Chemphyschem*, vol. 5, pp. 1751-82.

Zhao, Y.; Chien, S.; Weinbaum, S. (2001): Dynamic contact forces on leukocyte microvilli and their penetration of the endothelial glycocalyx. *Biophys J*, vol. 80, pp. 1124-1240.

Zhu, C.; Long, M.; Bongrand, P. (2002): Measuring receptor/ligand interaction at the single bond level: Experimental and interpretative issues. *Annals Biomed. Eng.*, vol. 30, pp. 305-314.

Zhu, C.; Lou, J.; McEver, P. R. (2005): Catch bonds: Physical models, structural bases, and rheological relevance. *Biorheology*, vol. 42, pp. 443-462.