

Entropic elasticity controlled dissociation and energetic elasticity controlled rupture induce catch to slip bonds in cell-adhesion molecules

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Abstract

We develop a physical model to describe the kinetic behavior in cell-adhesion molecules. Unbinding of non-covalent biological bonds is assumed to occur by both bond dissociation and bond rupture. Such a decomposition of debonding processes is a space decomposition of the debonding events. Dissociation under thermal fluctuation is non-directional in a 3-dimensional space, and its energy barrier to escape is not influenced by a tensile force but the microstates which could lead to dissociation are changed by the tensile force; rupture happens along the tensile force direction. An applied force effectively lowers the energy barrier to escape along the loading direction. The lifetime of the biological bond, due to the two concurrent off-rates, may grow with increasing tensile force to moderate amount and decrease with further increasing load. We hypothesize that a catch-to-slip bond transition is a generic feature in biological bonds. The model also predicts that catch bonds in compliant molecular structure have longer lifetimes and need less force to be fully activated.

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I. INTRODUCTION

Based on the kinetic theory of the strength of solids by Zhurkov [1], Bell [2] showed that the lifetime of biological bonds shortens exponentially with increasing tensile force. The model has been used broadly to depict the weakening of biological bonds [3, 4], and such a bond behavior is usually termed as a ‘slip bond’.

In the last few years, progress in experimental techniques has enabled the mechanical activation of chemical bonds to be studied on both an individual basis [5–7] and in a cluster composed of multiple bonds. Experiments have revealed that a small tensile force could strengthen bonds of adhesion molecules in the sense that bond lifetimes are prolonged. Such a binding behavior is termed as a ‘catch bond’, and was first predicted by Dembo [8, 9]. The prolonging of the lifetime of a bond cluster in response to tensile force was first observed by Thomas et al. [10]. They found that the adhesion of *Escherichia coli* bacteria binding to mannose coated surface via the adhesin FimH was enhanced by moderate amount of shear force. The same trend was found on a single cell-adhesion molecule by Marshall et al. [7]. Their study revealed that bonds between P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) display a biphasic relationship between bond lifetime and applied force, whereby lifetime first increases and then decreases with increasing force. More recent work, including contact and separation tests on microspheres coated with a PSGL-1 ligand and P-selectin separately [11], flow chamber experiments [12–16], and laser trap tests [17], have all observed catch-to-slip transition in biological bonds.

Several phenomenological models are proposed to interpret the transition from ‘catch’ to ‘slip’ bonds. Evans et al. [11] assumed that bond failure originates from two possible bound states with different dissociation pathways. Catch bonds are then explained by switching between the two pathways. Barsegov and Thirumalai [18] suggested that the observed catch-slip behavior in specific protein-protein complexes can be captured in general by using an energy landscape that allows for two bound states: one force-free state and another force-stabilized bound state. External forces redistribute the population in these two states and give rise to the catch-slip bond behavior. A common feature to these two models is that two bound states (or two energy wells) are assumed. External force changes the respective off-rates of the two bound states, and gives rise to a catch-slip transition. Pereverzev et al. [19] suggested a four-parameter and two-pathway model for the catch-slip transition in biological

adhesion. In their model, there is only one energy well in a biological bond but ligands can escape receptor binding sites via two alternative routes. An applied force will modify the possibilities of escaping via the two routes and results in a catch-slip transition.

The aforementioned models, more or less, are based on the conjecture that structure mechanisms play the critical role for the observed ‘catch-slip’ transition in selectin-ligand bonds. This concept, first hypothesized by Konstantopoulos et al. [20], suggests that conformational changes may alter either the population of different bound states or off-rates along distinct pathways.

Recent experiments by Phan et al. [14] and Lou et al. [15] seem to support the viewpoint that conformational change induces catch bonds [21]. Their studies were motivated by differences in the unliganded versus the liganded crystal structures of the N-terminal calcium-dependent lectin domain and an epidermal growth factor (EGF)-like domain of P-selectin [22]. The unliganded P-selectin has a relatively stiff “bent” conformation, while the ligand-bound structure shows an flexible “extended conformation [21, 22]. Phan et al. [14] added a glycosylation site between the two domains to wedge the inter-domain open and stabilize P-Selectin in the extended conformation. They investigated altered binding properties that resulted from such conformational changes in P-selectin and showed that the extended selectin conformation has higher affinity for ligands. The authors also predicted that adhesion via L-selectin would be enhanced by a mutation (N138G) in the inter-domain region through the elimination of a hydrogen bond to favor the bent confirmation. Lou et al. [15] confirmed that eliminating a hydrogen bond to increase the flexibility of the inter-domain in L-selectin increases tethering and prolongs the lifetime of the selectin-ligand bond. They postulated that this conformational change plays an important role in regulating the kinetic on/off-rate of selectin-ligand interaction as an applied tensile force is sufficient to switch the P-selectin from low- to higher-affinity conformations.

The experiments by these two groups have clearly established that the inter-domain substantially influences the affinity of selectins for ligands. It remains hypothetical whether the original bend conformation could be shifted toward the extended conformation by small external forces alone. The observed catch bonds in single molecule bonds for P-selectin with monomeric sPSGL-1 and PSGL-1 are in the force region of 5-10pN and 10-25pN respectively [7]; the lifetime for a single actomyosin bond also shows a catch bond in the force range about 1-6pN [17]. Note that catch bonds occur in a force region of no more than 3-fold of the force

due to thermal fluctuation (4-10pN, [7]). More importantly, experiments by Lou et al. [15] for both the low- (bent conformation) and high-affinity (extended conformation) bonds show catch-slip transitions, which may suggest either that there is further conformational change in high-affinity bonds, or that the catch-slip transition is a general behavior in the L-selectin and ligand interactions. We hypothesize that catch-slip bond transition is a feature in protein-protein complexes in general, and suggest a simple mechanism model to explain the observed mechanical behavior in non-covalent biological bonds. The most significant difference of our model from others is that debonding occurs via both bond dissociation and bond rupture through a space decomposition, as detailed in the next section.

II. THEORY

Taking a sphere s around the bonding pocket of a bound molecule with sphere radius λ_s being the width of the energy well of the bond, the off-rate k of the bound molecule is the summation of all debonding events found in the sphere during a unit time. It can be expressed as

$$k = k_0 \oint_s \exp\left(\frac{\lambda_s \mathbf{f} \cdot \mathbf{n} - \varepsilon}{k_B T}\right) ds \quad (1)$$

where k_0 is a constant off-rate, ε is the energy barrier of bond dissociation in the absence of external force, \mathbf{n} is the surface normal of ds – a unit vector, and \mathbf{f} is the applied force and also a vector with f being its magnitude, k_B the Boltzman’s constant and T the absolute temperature. Eqn. 1 is consistent with the Kramers’ theory [23] of chemical reactions in a field of force.

The whole surface in the integral (Eqn. 1) is divided into a “dissociation space” and a “rupture space” such that each sub-space is dominated by one debonding mechanism. In the dissociation space, debonding in the “dissociation space” is dominated by bond dissociation, which is non-directional and its energy barrier for escape is not influenced by a tensile force. However, the microstates which could lead to dissociation are changed by the tensile force. In the “rupture space”, its surface normal almost parallels to the loading direction. Bond rupture happens mainly along the tensile force direction and the applied force effectively lowers the energy barrier to escape along the loading direction.

The integrated off-rate in the “dissociation space” in Eqn. 1 depends on the total number of possible conformations Ω of a molecule under a given loading f . This statement

is based on the factor that dissociation prefers to occur in certain conformations, as shown by experiments by Zhang et al. [24] and molecular dynamics simulations by Lou and Zhu [25]. To connect the off-rate by dissociation with total accessible micro-states of the molecule, we make a simple assumption that bond dissociation rate k_d is proportional to Ω , i.e.,

$$k_d = k_c \Omega \quad (2)$$

where k_c is a referential off-rate. We proceed to construct the relationship for the applied force, entropy and Ω .

For a polymer chain, the first law of thermodynamics states that

$$dU = dQ - dW \quad (3)$$

where dU is the change in the system's internal energy, and dQ and dW are the heat and work exchanged between the system and its surroundings as the system undergoes differential change. In the specific case of uniaxial tensile force applied to a molecule, work done is given by force multiplied by distance, so the work done by a uniaxial force f (the scalar of \mathbf{f}) is given by

$$dW = -f dl \quad (4)$$

where dl is the extension of the molecule due to the force f . Further, assuming that the deformation process occurs reversibly in a thermodynamic sense, we obtain

$$dQ = T dS \quad (5)$$

where dS is the differential change in entropy. For uniaxial tension with V and T constant, combining the above equations gives

$$dU = T dS + f dl \quad (6)$$

and leads to the expression of the tensile force

$$f = \left(\frac{dU}{dl}\right)_{T,V} - T \left(\frac{dS}{dl}\right)_{T,V} \quad (7)$$

The first term on the right hand side in Eqn. 7 is the energy contribution to the tensile force, or energetic elasticity, see e.g. Rubinstein and Colby [26]. The second term is the entropy contribution to the tensile force, or entropic elasticity. When the molecule is extended with

small forces, the change in length (and energy) comes almost entirely from a change in conformation. Therefore, at constant temperature, it can be approximated that the internal energy of the bonds does not change, i.e. $dU = 0$ and that leads to

$$f = -T(dS/dl) \quad (8)$$

Thus, a tensile force applied to a molecule will elongate the molecule and reduce its entropy.

Based on the worm-like chain model, the entropy change in a molecule subjected to a uniaxial tensile force is given by

$$S - S_0 = -\frac{1}{2}Nk_B\left(\frac{2}{\lambda} + \lambda^2 - 3\right) \quad (9)$$

where N is the number of chain segments of the molecule, λ the nominal stretch (current chain length l normalized by the original length L), and S_0 the initial entropy. From Eqns. 8 and 9, we obtain

$$f = \frac{Nk_B T}{L}\left(\lambda - \frac{1}{\lambda^2}\right) \quad (10)$$

We further linearize Eqns. 9 and 10 to get a simple relationship between f and $(S - S_0)$ when the stretch is small. Assuming $\lambda = 1 + x$ with $x \ll 1$ and using Taylor expansion, we can get the relationship between entropy reduction and the tensile force f . After simple algebra, we arrive at

$$(S - S_0)T = -\frac{f^2}{2K} \quad \text{with} \quad K = \frac{3k_B T N}{L^2} \quad (11)$$

where K is the stiffness of the molecule in the linear entropic elasticity regime. From Eqn. 2, Eqn. 11, and the general relation

$$S = k_B \ln(\Omega) \quad (12)$$

we have the off-rate by bond dissociation which is given as

$$k_d = k_c \exp\left(\frac{S_0}{k_B}\right) \exp\left(-\frac{f^2}{2Kk_B T}\right) \quad (13)$$

Lower entropy in the molecule means that there is less chance for the bond to dissociate under thermal fluctuation (see Fig. 1a), and results in a slower off-rate. Isberg and Barnes [27] made an analogy between FimH adhesion as a catch-bond and a piece of flexible rope caught in a hook. If one holds both ends of the rope and pull it, the rope cannot be removed from the hook; when we stop pulling, the rope can be freed from the hook by an environment perturbation like the wind. This is the low-end limit. On the other hand, the high-end limit

is that when we pull so hard that either the hook or the rope can be broken. Returning to the adhesion problem, a binding could be knocked off by Brownian motion in the absence of external forces. A uniaxial force reduces the entropy (degrees of freedom) of the molecule and the chance of bond dissociation triggered by thermal fluctuations gets smaller, and prolongs bond lifetime.

Concurrently, in the “rupture space”, the applied force can increase elastic energy in the bound pocket and so reduce the energy barrier and accelerate bond rupture, as predicted by the Bell model. Bond rupture under the influence of applied forces is demonstrated in Fig. 1b. The bond has an initial high energy barrier. While f increases, the energy barrier decreases and the probability of bond rupture by thermal fluctuation increases. Note that while bond dissociation can occur along any direction, rupture of the bond is mainly due to bond separation along the tensile loading direction, as shown in Eqn. 1. As predicted by the Bell model, in the rupture regime, the off-rate k_r of biological non-covalent bonds subjected to a tensile force f follows

$$k_r = k_s \exp\left(\frac{f\lambda_s}{k_B T}\right) \quad (14)$$

where k_s is a referential rupture rate.

Based on Eqn. 1, unbinding of a bond occurs by both dissociation and rupture. Considering that we are collecting debonding events in the “dissociation space” and “rupture space”, overall off-rates is a natural superimposition of bond dissociation (Eqn. 13) and bond rupture (Eqn. 14) and is given as

$$k = k_c \exp\left[-\frac{f^2}{2Kk_B T}\right] + k_s \exp\left[\frac{f\lambda_s}{k_B T}\right] \quad (15)$$

The precoefficient term $k_c \exp(S_0/k_B)$ in Eqn. 13 is for simplicity written as k_c in the above equation, which depends on the intrinsic structure of a bound molecule. If we assume that the rebinding rate is small in the presence of an applied force, the lifetime $\langle \tau \rangle$ of a bond is approximated as the reciprocal of k . When $f \rightarrow 0$, the dissociation rate in Eqn. 15 has the regular meaning as that in a bonding reaction system, as described by Eqn. 1. The rupture rate at zero external force can interpret as the dissociation rate in a particular region where its surface normal is almost parallel to the loading direction. Hence it should be much less than the dissociation rate of regular meaning. Indeed, that is the case as shown in our application (data listed in Table 1 and 2).

In Eqn. 15, k_c and k_s need to be determined quantitatively by experiments. As discussed in [1], the strongest influence on λ_s are the properties of the bond pocket like bond angles and bond affinity. The physical meaning of λ_s , in our understanding, is the width of the energy well corresponding to a specific bond (the characteristic length between the minimum energy and the nearby saddle point). The high sensitivity of λ_s to various structural changes in a bond pocket makes it difficult to precisely quantify this parameter. We will fit Eqn. 15 to experimental data and get the estimates of these parameters in the next section.

III. APPLICATION

We apply the model to representative published experimental data for P-selectin adhesion complexes with monomeric sPSGL-1, dimeric PSGL-1, and antibody G1. The model parameters in Eqn. 15 were obtained by fitting the theoretical curves of $\langle \tau \rangle$ vs. f to the experimental data by Marshall et al. [7]. The results shown in Fig. 2 were obtained by using the model parameters listed in Table I.

With Eqn. 15, the theoretical parameters (listed in Table I) for the P-selectin with sPSGL-1 and PSGL-1 are $k_s = 0.2 \sim 0.45s^{-1}$ and $\lambda_s = 0.2 \sim 0.45$ nm, which are in the range suggested by experiments [7, 13, 28, 29]. The stiffnesses fitted using Eqn. 15 for P-selectin with monomeric sPSGL-1 and dimeric PSGL-1, are about 2.5 pN/nm and 10 pN/nm respectively, which are in the range of the stiffness for proteins 1~40 pN/nm [30]. The difference in K also reflects the structural change in monomeric sPSGL-1 and dimeric PSGL-1, the latter is about four times stiffer than the former. Based on Eqn. 15, the off-rate due to bond dissociation at zero external forces k_c is about two orders of magnitude faster than k_s . This reflects that in the absence of or at very small forces, bond lifetime is dominated by dissociation. As f increases, bond rupture takes over gradually and becomes the primary mechanism that controls bond lifetime. The competition of these two off-rates gives rise to the observed catch-slip transition in noncovalent biological bonds.

The model is also applied to experimental results for L-selectin with sPSGL-1, PSGL-1, and DREG56. The off-rate k versus f reported by Sarangapani et al. [13], and fitted curves using our theory are shown in Fig. 3 with fitted parameters as listed in Table II. Eqn. 15 can fit experimental data quite well. Also, the stiffnesses of bond complex for L-selectin with monomeric sPSGL-1 (13pN/nm) and dimeric PSGL-1 (42pN/nm) indicate that the

latter is stiffer.

We use Eqn. 15 to show the influence of conformational change of a bond complex on bond lifetime. For L-selectin or P-selectin, the crystal structures show ‘bent’ and ‘extended’ confirmation. Experiments by [14, 15] have shown an extended selectin conformation can strengthen selectin-ligand bonds. The extended structure of the bond complex, in contrast to its bent status, should be more compliant, as demonstrated in Fig. 4a. We simply reduce the stiffness K in Eqn. 15 to reflect a bond complex conformation change from bent to extended. Fig. 4b shows several curves of bond lifetime versus force for different stiffness K of the bonding complex based on model parameters used in Fig. 2b. In the case where stiffness in a complex is lower, catch bonds have longer lifetimes and require less force to be fully activated. Such a trend is consistent with observations by Lou et al. [15], as shown in their Fig. 4. Experiments and analysis by Nguyen-Duong et al. [31] have also shown that stiffer force probe can reduce the lifetime of molecular complex dramatically.

IV. CONCLUSION

In this article we have developed a theoretical model for the analysis of the mechanical activation of chemical bonds between specific molecules. Unbinding of biological bonds is assumed to take place through dissociation and bond rupture. Both dissociation and rupture are thermally activated escapes over a transition state barrier. The difference lies in that dissociation is non-directional, and its energy barrier for escape is not influenced by a tensile force but the microstates which could lead to dissociation are changed by the tensile force. On the other hand, rupture happens along the tensile force direction. The applied force effectively lowers the energy barrier to escape along the loading direction. The lifetime of the biological bond, due to the combination of both off-rates, grows with increasing tensile force and decreases with further increasing force. We hypothesize that a catch-to-slip bond transition is a common feature in biological bonds. We showed that modification on the stiffness K of the bond complex changed the effect of force on bond lifetimes: reduced K could form catch bonds with longer lifetimes and it doesn’t require as much force to be fully activated. The features predicted by the model are consistent with the experimental observations by Lou et al. [15] and Nguyen-Duong et al. [31].

V. FIGURES AND TABLES

TABLE I: Model parameters for bonds of P-selectin with specific ligand sPSGL-1, PSGL-1, and G1, obtained by fitting the lifetime versus force curve reported by Marshall et al. [7], with $T = 300$ K.

	k_c	K	k_s	λ_s
	1/s	pN/nm	1/s	nm
sPSGL-1	20	2.5	0.45	0.4
PSGL-1	25	10	0.25	0.2
G1 ^a			0.2	0.45

^aOnly the bond rupture term in Eqn. 15 is used to fit the experimental data for P-selectin with G1.

TABLE II: Model parameters for interactions of L-selectin with specific ligand sPSGL-1, PSGL-1, or DREG56, obtained by fitting the off-rates versus force curve reported by Sarangapani et al. [13], with $T = 300$ K.

	k_c	K	k_s	λ_s
	1/s	pN/nm	1/s	nm
sPSGL-1	50	15	2	0.1
PSGL-1	40	40	4	0.05
DREG56 ^a			0.8	0.3

^aOnly the bond rupture term in Eqn. 15 is used to fit the experimental data for L-selectin with DREG56.

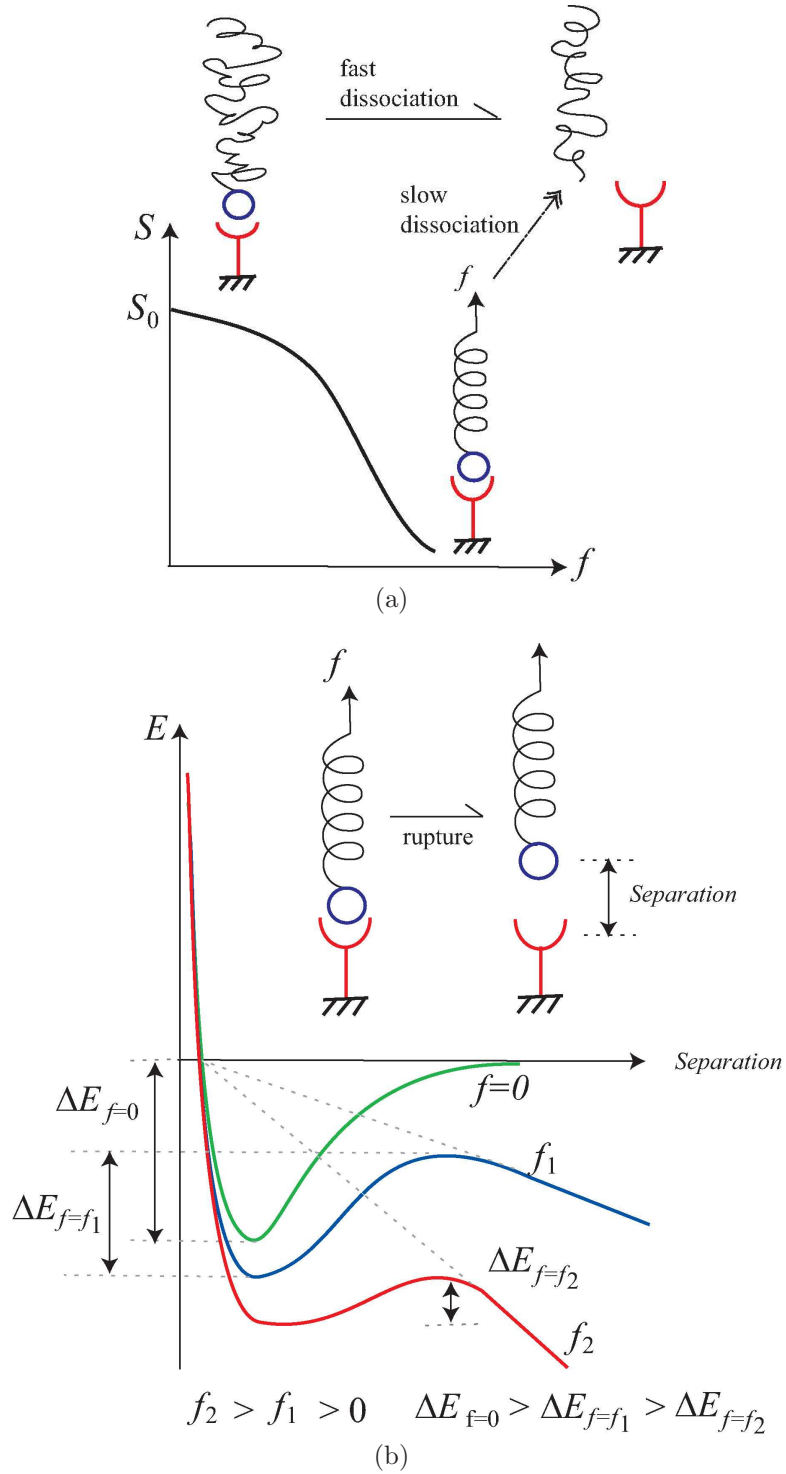


FIG. 1: (Color online) Schematic of the dependence of bond dissociation and bond rupture on external force f : (a) the applied force will reduce the bond configuration (controlled by entropic elasticity) and slow down bond dissociation; (b) energy increase in the bond pocket due to the applied force reduces the energy barrier for bond rupture, and shortens bond life time.

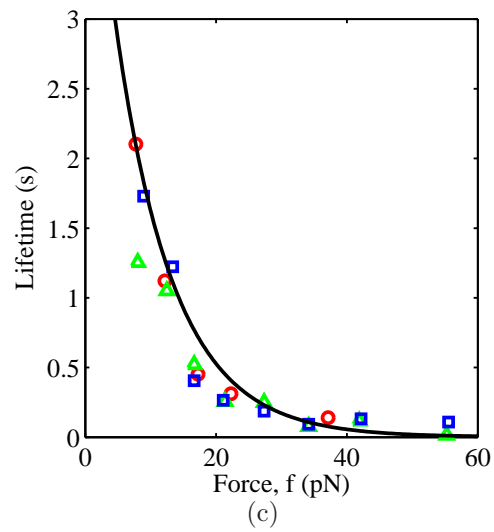
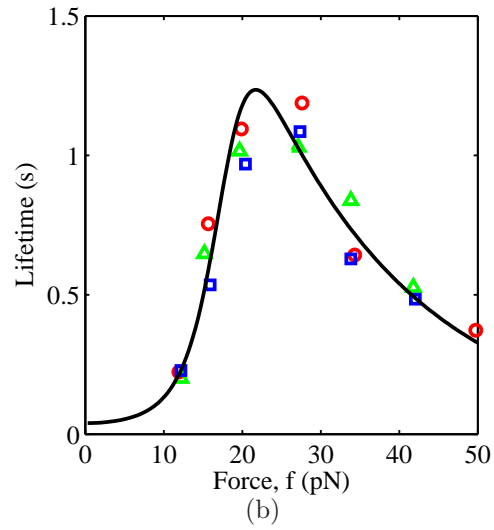
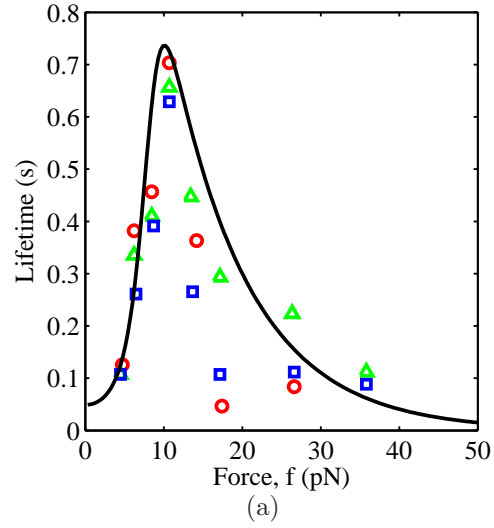


FIG. 2: (Color online) The life time versus force for bonds of dimeric P-selectin with monomeric sPSGL-1 (a), PSGL-1 (b), and G1 (c). The experimental data on lifetimes shown here are determined by Marshall et al. [7] with three types of definition: mean lifetime $\langle \tau \rangle$ (blue squares), standard deviation of the lifetime (green triangles), and inverse negative slopes for the off-rate (red cycles). The solid line is fitted generated from Eqn. 15 with parameters listed in Table I.

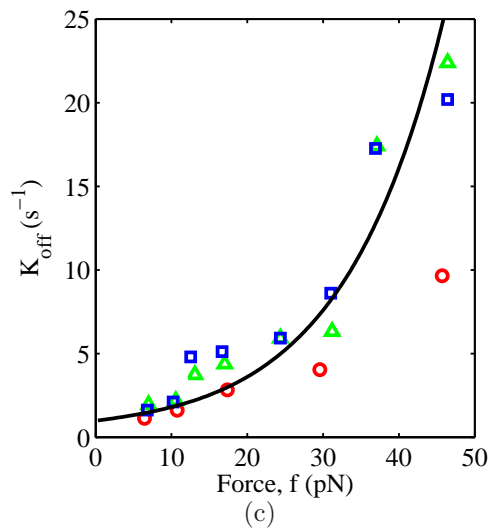
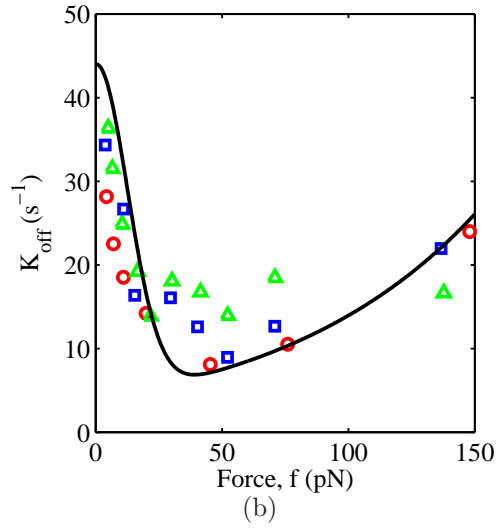
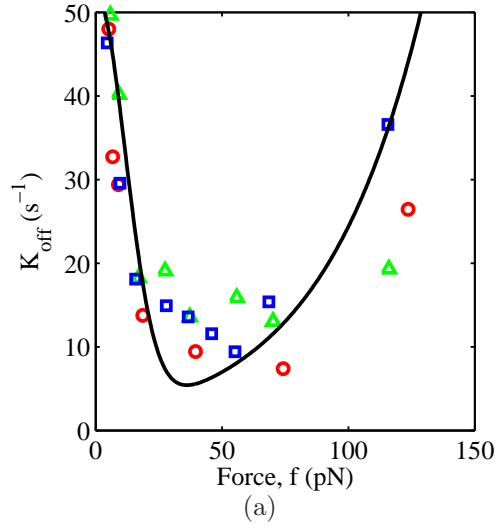


FIG. 3: (Color online) Off-rates as a function of the applied force for bonds of L-selectin with monomeric sPSGL-1 (a), PSGL-1 (b), and DREG56 (c). The experimental data on off-rates are obtained from Sarangapani et al. [13]. Off-rates are estimated from the reciprocal mean lifetime $\langle \tau \rangle$ (blue squares), the reciprocal standard deviation of the lifetime (green triangles), and negative slopes for the off-rate (red cycles). The solid line is the fitted curve generated from Eqn. 15 with parameters listed in Table II.

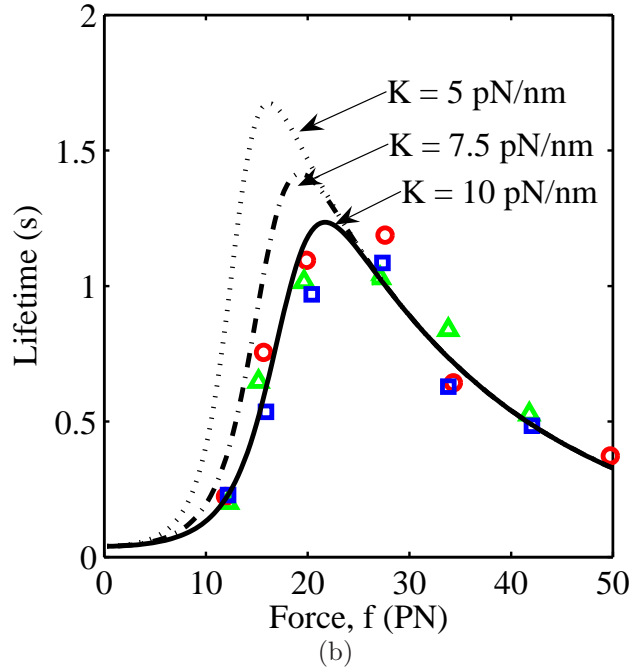
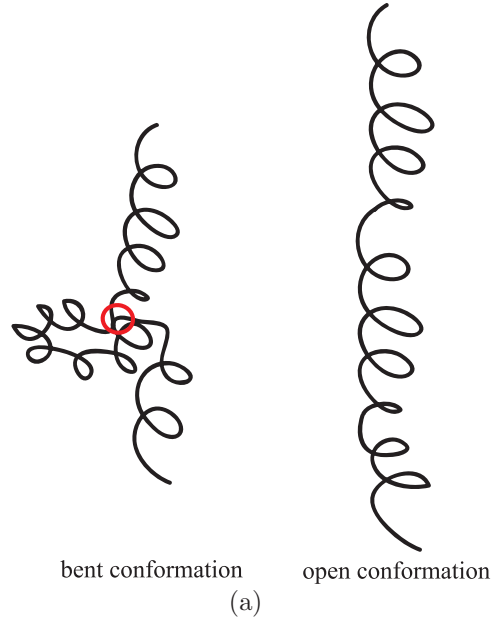


FIG. 4: (Color online) The influence of conformational change in a complex on its bond lifetime: (a) the diagram shows a structure with a hinge (red cycle) between two domains (bent conformation), as well as the same structure without the hinge (extended conformation). We expect that the structure in the extended conformation is more compliant than that of the bent conformation; (b) model prediction for the effect of bond complex stiffness to the bond lifetime. As stiffnesses decrease, catch bonds have longer lifetimes and require less force to be fully activated. Such a trend is consistent with observations by Lou et al. [15]. Other parameters used in Equ. 15 for these curves are the same as those in Fig. 2b for the P-selectin-PSGL-1 interaction.

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