Plasticity of hydrogen bond networks regulates mechanochemistry of cell adhesion complexes

Shaon Chakrabarti, Michael Hinczewski, and D. Thirumalai

Biophysics Program, Institute For Physical Science and Technology, University of Maryland, College Park, MD 20742

Edited* by Arieh Warshel, University of Southern California, Los Angeles, CA, and approved May 13, 2014 (received for review March 22, 2014)

Mechanical forces acting on cell adhesion receptor proteins regulate a range of cellular functions by formation and rupture of noncovalent interactions with ligands. Typically, force decreases the lifetimes of intact complexes (“slip bonds”), making the discovery that these lifetimes can also be prolonged (“catch bonds”) a surprise. We created a microscopic analytic theory by incorporating the structures of selectin and integrin receptors into a conceptual framework based on the theory of stochastic equations, which quantitatively explains a wide range of experimental data (including catch bonds at low forces and slip bonds at high forces). Catch bonds arise due to force-induced remodeling of hydrogen bond networks, a finding that also accounts for unbinding in structurally unrelated integrin–fibronectin and actomyosin complexes. For the selectin family, remodeling of hydrogen bond networks drives an allosteric transition resulting in the formation of the maximum number of hydrogen bonds determined only by the structure of the receptor and independent of the ligand. A similar transition allows us to predict the increase in the number of hydrogen bonds in a particular allosteric state of αβ1 integrin–fibronectin complex, a conformation which is yet to be crystallized. We also make a testable prediction that a single point mutation (Tyr51Phe) in the ligand associated with selectins (14, 15) and integrins (16, 17). In these systems, the rupture rate of the ligand from the receptor depends on an angle between two domains in the receptor molecule (Fig. 1). Conformations with smaller angles detach more slowly than those with large angles. In the case of integrins, multiple conformations at varying angles have been crystallized (17), whereas for selectins, only two (Fig. 1) have been found so far in the crystal structures (14). In the absence of an external force, the molecule fluctuates between conformations corresponding to a variety of angles, including the larger angles from which the ligand can rapidly detach. With the application of force, the two domains increasingly align along the force direction, restricting the system to small angles and longer lifetimes, until large forces again reduce the barrier to rupture. Previously, theories based on kinetic models with the assumption of a phenomenological Bell-like coupling of rates to force (18–21) have been used to explain catch-bond behavior. However, the parameters extracted from these kinetic models cannot be easily related to microscopic physical processes in specific catch-bond systems. More importantly, such models merely rationalize the experimental data, and do not have predictive power. The large scale of catch-bond lifetimes, ~10−104 ms, makes it impossible to directly observe unbinding in a realistic all-atom simulation, much less the macroscopic consequences of mutations.

Here, we solve the difficulties alluded to above by creating a new theoretical approach. By building on the insights from the structures of cell adhesion complexes, we introduce a microscopic theoretical model that captures the essential physics of (AFM) experiments in the last decade (9–11), along with examples from other load-bearing cellular complexes like actomyosin bonds (12) and microtubule–kinetochore attachments (13). The catch-bond lifetime exhibits nonmonotonic biphasic behavior—increasing up to a certain critical force and decreasing at larger forces. The structural mechanisms leading to catch-bond behavior have largely been elusive, although experiments have provided key insights for selectins (14, 15) and integrins (16, 17). In these systems, the rupture rate of the ligand from the receptor depends on an angle between two domains in the receptor molecule (Fig. 1). Conformations with smaller angles detach more slowly than those with large angles. In the case of integrins, multiple conformations at varying angles have been crystallized (17), whereas for selectins, only two (Fig. 1) have been found so far in the crystal structures (14). In the absence of an external force, the molecule fluctuates between conformations corresponding to a variety of angles, including the larger angles from which the ligand can rapidly detach. With the application of force, the two domains increasingly align along the force direction, restricting the system to small angles and longer lifetimes, until large forces again reduce the barrier to rupture.

Previously, theories based on kinetic models with the assumption of a phenomenological Bell-like coupling of rates to force (18–21) have been used to explain catch-bond behavior. However, the parameters extracted from these kinetic models cannot be easily related to microscopic physical processes in specific catch-bond systems. More importantly, such models merely rationalize the experimental data, and do not have predictive power. The large scale of catch-bond lifetimes, ~10−104 ms, makes it impossible to directly observe unbinding in a realistic all-atom simulation, much less the macroscopic consequences of mutations.

Here, we solve the difficulties alluded to above by creating a new theoretical approach. By building on the insights from the structures of cell adhesion complexes, we introduce a microscopic theoretical model that captures the essential physics of

Significance

Selectins and integrins are receptor proteins on cell surfaces responsible for adhesion to extracellular biomolecules, a critical component of physiological processes like white blood cell localization at sites of inflammation. The bonds which the receptors form with their targets are regulated by mechanical forces (for example due to blood flow). The bond lifetimes before rupture increase with force before decreasing. Based on crystal structures of selectin and integrin, we created a general analytic theory which for the first time, to our knowledge, relates microscopic structural rearrangements at the receptor–ligand interface to macroscopic bond lifetimes. We quantitatively explain experimental data from diverse systems spanning four decades of lifetime scales, and also predict the outcome of mutations in specific residues.

Author contributions: S.C., M.H., and D.T. designed research, performed research, contributed new reagents/analytic tools, analyzed data, and wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

1To whom correspondence should be addressed. E-mail: thirum@umd.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1405384111/-/DCSupplemental.
the angle-dependent detachment, and its implications for catch-bond behavior. Taking a cue from the crystal structures of selectin and integrin, we construct a coarse-grained energy function for receptor–ligand interactions. The model yields an analytic expression for the bond lifetime as a function of force, which gives excellent fits to a broad range of experimental data on a number of systems. The extracted parameters have clear structural interpretations, and their values provide predictions for energetic and structural features like strength of hydrogen bonding networks at the receptor–ligand interface. Where estimates of these properties can be directly obtained from crystal structures, our predictions are in remarkable agreement. The energy scales identified through the model are specific enough to allow predictions for structures not yet crystallized, and suggest mutation experiments that would modify catch-bond behavior in quantifiable ways. For the selectins, we predict how a specific mutation in the PSGL-1 ligand will alter its unbinding from P-selectin under force, and provide new interpretation of data from L-selectin mutants (20). Interestingly, the experimental fits suggest that both P- and L-selectin have a characteristic, ligand-independent energy scale, determined by the chemistry of their binding interfaces. For integrins, we predict the strength of extra interactions that scale, determined by the chemistry of their binding interfaces.

**Theory**

**Structural Basis of Catch-Bond Model.** We now define our model using the structures of P-selectin, which has been crystallized in two conformations: a “bent” (Fig. 1A) and “extended” state (Fig. 1B) (14). The two states differ by the angle which the epidermal growth factor (EGF) domain (green in Fig. 1 A and B) assumes with respect to the lectin domain (gray–beige). Although ligands can bind to lectin in both conformations, cocrystallization with the ligand (the truncated N-terminal portion of the glycoprotein PSGL-1) was achieved only for the extended state. In the extended state, there are two major regions of the lectin domain (B₀ and B₁, colored purple in Fig. 1 A–D) that form substantial hydrogen bond networks with the ligand, thereby stabilizing the complex. Based on alignments of the lectin domain in the bent and extended states (Fig. 2A), it is believed that the binding interface is remodeled in the bent conformation (14, 22). The region B₁ (a loop between Asp82 and Glu88, shown in Fig. 2A, Inset) rotates so that it can no longer engage the ligand. This angle-dependent rearrangement results in weaker ligand attachment, and hence explains the shorter bond lifetimes in the bent vs. the extended conformation.

Our minimal model, which captures the structure-based angle-dependent dissociation, describes the ligand–receptor interaction through an effective spring with bond vector \( r \equiv (r, \theta, \phi) \) between a pivot point within the receptor and a point in the ligand (Fig. 1 C and D). The pivot point is fixed at the origin, and the ligand is under an external force \( F \) along the \( z \) axis. The energy associated with the spring is given by the potential

\[
U(r, \theta) = \frac{1}{2} (k_0 + k_1 (1 + \cos \theta))(r - r_0)^2 - Fr \cos \theta, \tag{1}
\]

with \( k_0, k_1 > 0 \). The first term is an elastic energy, where \( r_0 \) is the natural length of the bond magnitude \( r \), and \( k_0 + k_1 (1 + \cos \theta) \equiv k(\theta) \) is an angle-dependent spring constant. The second term is the contribution due to the mechanical force \( F \). The bond ruptures if \( r \geq b \), where \( b \equiv r_0 + d \) and \( d \) is the transition state distance. The structural inspiration of our model naturally leads to a multidimensional potential energy, dependent on both \( r \) and \( \theta \), which is a key requirement for a physically sensible description of catch-bond behavior. Any one-dimensional potential with rupture defined by a single cutoff in some reaction coordinate will always exhibit monotonic lifetime behavior as a function of force. We assume that the time evolution of the vector \( r \) follows a Fokker–Planck equation, describing diffusion on the potential surface \( U(r, \theta) \) with a diffusion constant \( D \). We define the lifetime of the bond \( \tau(F) \) at a given force \( F \) as the mean first passage time from \( r_{\text{ruin}}(F) \), the position of the minimum in \( U \), to any \( r \) with \( r = b \). Implicit in this diffusive picture is the assumption that the angle \( \theta \) can change continuously. This is a reasonable approximation even if the receptor–ligand complex fluctuates between several discrete angular states (17), assuming the energy barriers between the states are such that the interconversion between states happens on much faster timescales than \( \tau(F) \). The presence of the barriers...
would in this case be incorporated through a renormalization of the effective diffusion constant $D$ (23).

We show in Fig. 1E a representative zero-force potential energy surface $U(r, \theta)$, with the energy at rupture $U(b, \theta)$ highlighted in red. The form of $k(\theta)$ makes it energetically favorable for bond rupture at $\theta = \pi$ (the bent state), with a cost $E_0 = k_d d^2 / 2$ to dislodge the ligand to the failure point. In the opposite limit of $\theta = 0$ (the extended state), energy for rupture is highest, with a cost $E_0 + E_1$, where $E_1 = k_D d^2$. The values of $E_0$ and $E_1$ correspond to the stabilization energies associated with the ligand in the two allosteric (bent and extended) states. However, as $F$ is increased, the bond aligns along $z$, and the minimum in $U(r, \theta)$ shifts toward $\theta = 0$ (Fig. 1F), biasing the system toward the extended state. Thus, we expect the lifetime $\tau(F)$ to initially increase with $F$ and eventually decrease at forces sufficiently large to reduce the rupture barrier.

Although the schematic diagram of the model in Fig. 1C and D draws the vector $r$ between a pivot at the EGF–lectin interface to the tip of the ligand, one should note that the actual ligand–lectin complex does not behave like a perfectly rigid object rotating about a hinge, nor does it cover the entire angular range $[0, \pi]$. Because proteins are deformable, the pivot location and the length $r_0$ will depend on the compliance of the specific domains involved in reorientation. Hence, we expect $r_0$ to be of the order of, or less than, the size of the localized domains that rotate to become restructured under force. It therefore follows that the structures of the complex in different allosteric states provide valuable insights into their response to force. The length scale $d$ reflects the brittleness of the bonding interactions (24), with larger $d$ indicating a malleable bond interface which can be deformed over longer distances before the complex falls apart.

The two energy scales $E_0$ and $E_1$ also have physical interpretations, with $E_0$ being roughly the total strength of noncovalent interactions between the region $B_0$ and the ligand, whereas $E_1$ is the additional contribution from the region $B_1$ in the extended conformation.

Results and Discussion

Mean Bond Lifetime. By assuming that $\tau(F)$ is much longer than the local equilibration time around $r_{\text{min}}(F)$, the lifetime of the complex is approximately given by

$$\tau(F) \approx \frac{\sqrt{\pi} r_0(E_1 - 2F(d + r_0))e^{(E_0 + dF)}(\alpha^2)F_{0} - 1}{4D(jE_0)^{1/2}F(1 + r_0/d)^2(1 - e^{(2F(d + r_0) - E_0)})}$$  \hspace{1cm} [2]

where $\beta = 1/k_BT$, $k_B$ is the Boltzmann constant, and $T$ is the temperature. The full details of the derivation are in SI Text. The central result of this work in Eq. 2 provides an analytic expression for the mean first passage time in terms of the microscopic energy and length scales covering both the catch- and slip-bond regimes. To set a reasonable scale for $D$, we make it equal to the diffusivity of a sphere of radius $r_0$: $D \approx k_BT/6\pi \eta r_0$, where $\eta$ is the viscosity of water. (A prefactor in $D$ due to molecular shape can be absorbed as a small logarithmic correction to the energy scale $E_0$.) With this assumption, Eq. 2 becomes an equation with four parameters: $E_0, E_1, d$, and $r_0$. We validated the approximation underlying Eq. 2 by comparison with Brownian dynamics simulations of diffusion on $U$ (details in SI Text), which showed excellent agreement with our analytical theory.

For $\beta d \gg 1$, $\tau(F)$ decays exponentially in a manner similar to the standard Bell model for systems exhibiting slip bonds, $\tau(F) \sim \exp(-\beta d F)$. The decay rate is controlled by the transition state distance $d$. The characteristic catch-bond behavior occurs at smaller $F$, where we see a biphasic $\tau(F)$ peaked at $F = F_p$,

$$F_p \approx \frac{AE_1}{2(r_0 + d)}$$  \hspace{1cm} [3]

with a prefactor $A \sim O(1)$. The ratio of the peak height $\tau(F_p)$ to the lifetime $\tau(0)$ at zero force, which is a measure of the strength of the catch bond, scales like

$$\frac{\tau(F_p)}{\tau(0)} \approx \frac{4\eta(\alpha^2)(d + r_0)}{E_1^-} \sinh \left( \frac{E_1}{2} \right) \sinh \left( \frac{r_0E_1^-}{2(d + r_0)} \right),$$  \hspace{1cm} [4]

with a prefactor $A' \sim O(1)$. From Eqs. 3 and 4 we see that $E_1 \to 0$ leads to $F_p \to 0$ and $\tau(F_p)/\tau(0) \to 1$. In this limit, the model predicts only slip-bond behavior, where the lifetime decreases monotonically with force. Thus, our model interpolates between catch-bond and slip-bond regimes by varying the energy scale $E_1$.

Analysis of Experimental Data. We first establish the efficacy of the theory by analyzing experimental data for $\tau(F)$ for a variety of complexes. The fits in Fig. 3 (selectins) and Fig. 4 (nonselectin complexes) show that there is excellent agreement between the analytical theory and measurements, which is remarkable because our microscopic model shows that only a small number of fitting parameters suffice to fit nine complexes with vastly differing architectures. These experiments involve applying force to molecular complexes either through AFM or optical traps, with the force initially ramped from zero to a given value $F$. Bonds which survive the ramp are then held at constant $F$ until rupture. If the initial ramp is sufficiently slow such that the system always remains quasidiabatically in equilibrium at the instantaneous applied force (25), the subsequent duration of the bond while at constant $F$, averaged over many trials, provides an accurate estimate of $\tau(F)$. [Extremely high ramp speeds may lead to nonequilibrium artifacts (26).]
To establish that our theory is general, we analyzed experimental results from both selectin systems [P-selectin (10); L-selectin (20)], and others [fibronectin dissociating from a truncated construct of α5β1 integrin (11) and myosin unbinding from actin (12)]. Details of the maximum-likelihood procedure for obtaining the best-fit parameter values (Table 1) are in SI Text. All of the systems in Figs. 3 and 4 exhibit catch bonds at low forces except in Fig. 3B, which for comparison shows P-selectin forming a slip bond ($E_0 = 0$ arises naturally by fitting the theory to experimental data) with the antibody G1.

**Selectin family.** Fig. 3A includes data for P-selectin with two different forms of PSGL-1 ligand: sPSGL-1, which is a monomer interacting with single lectin domains, and PSGL-1, which is a dimer capable of simultaneously forming two bonds with two neighboring lectin domains (10). (All other selectin complexes, including L-selectin/PSGL-1 (20) in Fig. 3C and D, involve only monomeric interactions.) We fit both curves in Fig. 3A with the same set of parameters, using $\tau(F)$ from Eq. 2 for the monomeric case, and in the dimer case $\tau_{\text{dim}}(F) \equiv \tau(F/2) + \tau(F/2)(1 + k_1 \tau(F/2)/2)$. When the dimer is intact, each bond feels a force $F/2$. When one of the bonds breaks, the intact bond still feels a force approximately equal to $F/2$, due to the large stiffness and roughly constant displacement of the AFM cantilever (27). In the latter case, the broken bond can reform with some rate $k_r$, which adds one fitting parameter, $\tau_{\text{dim}}(F)$ is a model that accounts for all these possibilities. The resulting fits in Fig. 3A show that a total of five parameters ($k_r \sim 1.1 \pm 0.3 \text{ s}^{-1}$, the rest listed in Table 1) can simultaneously capture the general lifetime behaviors of both data sets.

**Physical meaning of the parameters.** A sine qua non of a valid theory of any phenomenon is that the extracted parameters must have sound physical meaning. To provide a structural interpretation of the extracted parameters for the selectin systems, it is instructive to compare the resulting energy and length scales to what we know about selectin bonds independent of the model. From the crystal structure of the P-selectin–PSGL-1 complex in Fig. 1B, we estimated that the regions B1 and B2 involve, respectively, 14 and 6 ligand–lectin hydrogen bonds (the B1 bonds are shown in Fig. 2A). We used the software PyMol (28) to count hydrogen bonds, with a distance cutoff of 0.35 nm for the heavy atoms. The corresponding energy scales from Table 1 are $E_1 = 17 \text{ kBT}$ and $E_2 = 9 \text{ kBT}$, which gives an enthalpy of $-1.2\text{--}1.5 \text{ kBT}$ per hydrogen bond. This range is consistent with earlier estimates of the strength of hydrogen bonds in proteins (29). The distance from the EGF domain–lectin interface to the lectin–ligand interface is $\sim 3 \text{ nm}$. Because the crystal structures suggest that restructuring of hydrogen bonds in this region leads to catch-bond behavior, $r_0$ should be $\sim 3 \text{ nm}$ or less. The fitted values of $r_0 = 0.2\text{--}2.0 \text{ nm}$ for L- and P-selectin lie well within this estimate. The transition distances $\delta$ vary between $-0.1$ and 0.6 nm, which is the range typical for proteins (30). Given the realistic values for all of the fitted parameters, our theoretical model is an accurate coarse-grained description of selectin-type systems.

The sum $E_0 + E_1$ is essentially constant for a given selectin receptor, independent of the ligand; $E_0 + E_1 \sim 27 \text{ kBT}$ for P-selectin and $\sim 31 \text{ kBT}$ for L-selectin. This suggests that the maximum number of possible interactions is fixed by the interactions associated with the receptor interface. For each ligand there is a different partitioning of these interactions among those that contribute to $E_0$ and $E_1$. The values of $E_0$ and $E_1$ can be estimated from the structures alone using $E_0 \sim n_b h_b n_p h_p$ and $E_1 \sim n_r h_b n_p h_p$, where $n_b$, $n_p$, and $n_r$ are the number of hydrogen bonds in the bent and extended states, respectively, and $h_b$ is the strength of a hydrogen bond. For the catch-bond complexes, $E_1 \sim 7\text{--}10 \text{ kBT}$, or roughly $5\text{--}8$ noncovalent bonds. For P-selectin and G1 (Fig. 3B), all interactions contribute to $E_0$, and we get slip-bond behavior instead; G1 is a blocking monoclonal antibody for P-selectin. In this case the binding is so strong, involving all possible interactions at the interface, that there is no room for additional stabilization under alignment ($E_1 = 0$). The finding that the ligands achieve nearly the same value of $E_0 + E_1$ means that in the aligned state
Estimated to be a decrease in binding affinity of fibronectin fragments lacking the is also known that additional interactions that can be ascertained from the crystal structure, it Asp forms a salt bridge with the ligand residue Arg. Beyond the range, and is therefore consistent with the structural analysis. We will return to this case below in discussing a mutant of the ligand PSGL-1.

**Integrin.** In the case of the integrin–fibronectin complex, we took as an example AFM data for a truncated integrin (only the headpiece of $\alpha_\beta_3$) binding to fibronectin FNIII7–10 (fibronectin fragment comprising the 7–10th type III repeats) (11). There is ample evidence for an angle-dependent detachment of ligand in the integrin headpiece (16), where the $\beta$-hybrid domain swings out from the $\alpha$-subunit via multiple intermediate states (17). Our model is well suited to describe these structural changes, and the quality of fit to experimental data (Fig. 4A) shows that the physics governing the effect of force on selectin complexes also holds for the complex involving integrin. We can compare some of the fitted parameters with a recently obtained crystal structure of the $\alpha_\beta_3$ headpiece complexed with fibronectin (only the arginine-glycine-aspartic acid (RGD) peptide portion of fibronectin is resolved in the structure; Fig. 2B) (31).

Because the structure shows the integrin headpiece in a closed (large-angle) conformation, we can directly compare the number of hydrogen bonds with the parameter $E_0$. As shown in Fig. 2B, there are nine hydrogen bonds formed between the headpiece domain and the RGD peptide. In addition, the acidic residue Asp forms a salt bridge with the ligand residue Arg. Beyond the interactions that can be ascertained from the crystal structure, it is also known that additional “synergy” sites in the ligand, not visible in the structure, play a role in binding. From the measured decrease in binding affinity of fibronectin fragments lacking the synergy sites, their contribution to the binding energy can be estimated to be $\sim$2–4 $k_B T$ (31). Combining this with the hydrogen bonds and salt bridges seen in the structure [using our earlier range of 1.2–1.5 $k_B T$ per hydrogen bond, and 4–5 $k_B T$ for the salt bridge (32)], we get an estimated total of $E_0 = 17$–26 $k_B T$. Our fitted result $E_0 = 23$ $k_B T$ from the model falls in this range, and is therefore consistent with the structural analysis. The fitted value of $r_0$ is also reasonable, given that the longest axis of the hybrid domain is $\sim$4 nm. The parameter $d$ is again well within the range of transition state distances expected in proteins. Our model predicts that $E_1 = 12$ $k_B T$, the extra interaction strength that would be gained in an open conformation of the $\alpha_\beta_3$–fibronectin complex. This prediction can be verified once crystal structures of the open conformation become available.

**Actomyosin.** Finally, in the case of actomyosin catch bonds (Fig. 4B), no crystal structures exist for the complex and an angle-dependent lifetime has not been established. However, we can use our theory to propose the origins of catch-bond behavior in these complexes based on experimental data. There is strong evidence that the upper 50K and lower 50K domains surrounding the major cleft in the motor head behave like pinners—binding to actin tightly in the ADP and rigor states, thereby forming a tight complex (33). Once ATP binds, the pinners move apart (the upper 50K domain breaks contact with actin) by an allosteric mechanism (34), thus allowing the motor head to unbind from actin faster. While in the ADP–rigor state, if an external force is applied through the lever arms of myosin, local rearrangements and rotations would cause the N-terminal domain and the two 50K domains to align with the direction of force. Along with these local reorientations, the force would also stretch the domains, causing narrowing of the major cleft, and facilitating increased interactions of both 50K domains with actin. This mechanism would lead to catch-bond behavior, in a manner similar to the FinH–mannose adhesions in *Escherichia coli* (35). Our fitted value of $r_0$ shows that the alignments occur over a length scale $\sim$2.4 nm, which agrees well with single molecule results showing that the cross-bridge compliance resides only locally in the actin-motor domain of the actomyosin complex (36).

**Predictions for Mutations in Selectin Complexes.** Because the energy scales in our model correspond to the strengths of noncovalent bonding networks, we can use our theory to predict and explain the impact of mutations on the bond lifetime, thus providing a framework for engineering catch bonds with specific properties. We will consider two examples, one a modification of the ligand, the other of the receptor in selectin systems. A recent study (37) considered a PSGL-1 mutant where Tys51, a sulfated tyrosine that makes one hydrogen bond with Arg85 in the B1 region of P-selectin (Fig. 2), is replaced by phenylalanine (Phe), which cannot form the hydrogen bond. Kinetic assays showed that the mutant has a weaker binding affinity to P-selectin, but a zero-force off rate that remains virtually unchanged from the wild type. The lifetime under force has not yet been measured, but our model predicts that removing one hydrogen bond from B1 should decrease $E_1$ by $\sim$1.3 $k_B T$. Using the reduced value for $E_1$, and all other parameters the same as in the wild type (first row of Table 1), we predict that the $r(F)$ curve (dashed red line labeled Y51F in Fig. 3A) should be dramatically different from the wild type. Relative to the wild type, the peak is decreased by a factor of 3.4, and shifted slightly (from 24 to 21 pN). Because effects of a mutation in $E_1$ are most relevant to alignment under force, the low force behavior is relatively unperturbed, similar to the kinetic assay results: $r(F)$ of the mutant for $F < 2$ pN differs less than 20% from the wild type.

The second example, where experimental $r(F)$ data are available, involves two receptor mutations performed on L-selectin (20). The authors compared the $r(F)$ behavior of wild-type L-selectin to a mutant where Asn138 was changed to Gly. The mutation effectively breaks a hydrogen bond in the hinge region, between Tyr37 and Asn138. Two different ligands (PSGL-1 and 6-sulfoSL6E) both showed the same trends: the peak in the $r(F)$ curve for the mutant was shifted up and toward smaller forces, relative to the wild type [Fig. 3C and D]. To determine the minimal perturbation in the parameters that would produce this shift, we simultaneously fit the wild-type and mutant data sets for each ligand, allowing only a subset of parameters to change for the mutant. The most likely subset, determined using the Akaike information criterion (see SI Text for details), involved only changes in the energy scales $E_0$ and $E_1$. The fit results are shown in Table 1. Both ligands show a similar pattern: $E_1$ decreased by $\sim$1.5–1.7 $k_B T$ in the mutant, whereas $E_0$ increased by $\sim$1.5–1.6 $k_B T$. The magnitudes of the energy changes suggest that the enthalpy loss due to a single hydrogen bond contributing to $E_1$ in the wild type is compensated by an increase in $E_0$. The mutation added flexibility to the lectin domain, allowing it to bind the ligand more effectively in both the bent and extended conformations. Thus, a contact between the ligand and receptor in B1 (Fig. 1) that forms only at small angles in the wild type is present at all angles in the mutant.

### Table 1. Best-fit parameter values of the catch-bond model for the various experimental complexes shown in Figs. 3 and 4

<table>
<thead>
<tr>
<th>Complex</th>
<th>$E_0$, $k_B T$</th>
<th>$E_1$, $k_B T$</th>
<th>$d$, nm</th>
<th>$r_0$, nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psel/(s)PSGL-1</td>
<td>9.3(2)</td>
<td>17.2(3)</td>
<td>0.56(2)</td>
<td>2.0(1)</td>
</tr>
<tr>
<td>Psel/GI</td>
<td>0.0</td>
<td>26.73(4)</td>
<td>0.51(3)</td>
<td>2.0*</td>
</tr>
<tr>
<td>Lsel/PSGL-1</td>
<td>10.2(7)</td>
<td>21.0(6)</td>
<td>0.14(4)</td>
<td>0.38(7)</td>
</tr>
<tr>
<td>LselN138G/PSGL-1</td>
<td>8.7(6)</td>
<td>21.8(5)</td>
<td>0.14(4)</td>
<td>0.38(7)</td>
</tr>
<tr>
<td>Lsel/6-sulfo-sLe*</td>
<td>8.7(7)</td>
<td>22.7(4)</td>
<td>0.17(4)</td>
<td>0.23(5)</td>
</tr>
<tr>
<td>LselN138G/6-sulfo-sLe*</td>
<td>7.0(7)</td>
<td>24.3(3)</td>
<td>0.17(4)</td>
<td>0.23(5)</td>
</tr>
<tr>
<td>Integrin–fibronectin</td>
<td>0.2(1)</td>
<td>23(1)</td>
<td>0.71(1)</td>
<td>0.5(2)</td>
</tr>
<tr>
<td>Actin–myosin (ADP)</td>
<td>4.1(3)</td>
<td>18.2(5)</td>
<td>0.47(4)</td>
<td>2.6(5)</td>
</tr>
<tr>
<td>Actin–myosin (rigor)</td>
<td>3.9(4)</td>
<td>18.4(8)</td>
<td>0.50(5)</td>
<td>2.7(7)</td>
</tr>
</tbody>
</table>

Parentheses denote uncertainties in the least significant digit.

*For this Psel slip-bond system, the lack of data at small forces prevents independent fitting of $r_0$, so its value is set to the $r_0$ for Psel/(s)PSGL-1.
Conclusions

The general principle for the formation of catch bonds emerging from experiments and theory is an increase in stabilizing interactions as a result of topological rearrangements of protein domains under force (19, 22). Whereas we quantitatively established the mechanism for certain classes of protein complexes in this work, recent computational studies on a knotted protein (38) and a long α-helix (39) suggest that the same principle could lead to nonmonotonic unfolding lifetimes in single proteins as well. In the former, the protein thymidine kinase was studied, where a threaded loop is surrounded by a knotting loop, forming a slipknot (38). At intermediate forces, the knotting loop shrinks faster than the threaded loop, effectively leading to increased interactions between the loops and hence an increased barrier to unfolding. At smaller forces, the threaded loop shrinks faster and slips out of the knotting loop before any extra interactions can form. In the beta–myosin helix studied using molecular simulations with the milestone algorithm (39), at intermediate forces broken hydrogen bonds from the native alpha helix secondary structure reform to create a longer-lived force-stabilized pi helix structure, thereby leading to a catch-bond-like effect. More generally, we suggest that if the number of hydrogen bond or side chain interactions can be increased in single-domain proteins by force-induced structural rearrangements, then such systems should exhibit catch-bond behavior. This is likely to be the case in mammalian prions which have a number of unsatisfied hydrogen bonds in the functional state (40). Thus, it is increasingly becoming clear that diverse force-induced topological rearrangements are used by nature as a mechanism to modulate bond lifetimes. At the larger scale of protein complexes, one can ask whether the rearrangements responsible for catch bonds among different biomolecule families share common features. From structure-based observations in selectin and integrin systems, we have shown that a model based on force-dependent rotation of protein domains, facilitating enhanced interactions with their binding partner, explains experimental observations remarkably well. Our precise analytical theory quantitatively reproduces data on a variety of structurally unrelated complexes with lifetimes spanning nearly four orders of magnitude. More importantly, the key parameters of the theory are linked to the formation (or disruption) of a network of hydrogen bonds and/or salt bridges. Because the strength of these interactions can be estimated, our theory can be readily used to predict the effects of mutations, as demonstrated for the selectin complexes. Interestingly, analysis of experimental data allowed us to predict the strength of additional hydrogen bonds that form in the open αβ4 integrin–fibronectin complex. The specificity of our model, with very few parameters, lays a foundation for synthetic mechanochemistry (41): designing and fine-tuning catch-bond adhesion complexes with a desired set of load-bearing characteristics.

ACKNOWLEDGMENTS. This work was supported by the National Institutes of Health through Grant GM 089685 and the National Science Foundation (CHE 1361946).