A number of biological bonds show dramatically increased lifetimes at zero-force conditions, compared to lifetimes when even a small tensile force is applied to the ligand. The discrepancy is so great that it cannot be explained by the traditional receptor–ligand binding models. This generic phenomenon is rationalized here by considering the interaction of water with the receptor–ligand complex. It is argued that the water–protein interaction creates an energy barrier that prevents the ligand unbinding in the absence of the force. The properties of the interaction are such that even application of a relatively low force results in a dramatic drop of the bond lifetime due to the alteration of the water–receptor and water–ligand interaction network. The phenomenon is described by the presence of a second shallow interaction energy minimum for the bound ligand followed by a wide receptor–ligand dissociation barrier. The general analysis is applied quantitatively to the actin–myosin system, which demonstrates the gigantic drop of the bond lifetime at small forces and catch behavior (an increase in the lifetime) at moderate forces. The base hypothesis proposed to explain the small-force abnormal drop in the bond lifetime suggests that the majority of biological bonds may exhibit this phenomenon irrespectively whether they behave as slip or catch-slip bonds.

2. General Theoretical Analysis

In accordance with the above hypothesis and the earlier theoretical works,8–10 we suggest that the receptor–ligand interaction in aqueous environment can be described by a potential with two minima (Figure 2). The first deep minimum arises due to the intrinsic receptor–ligand interaction and corresponds to the state with the ligand located deep inside the receptor binding pocket. The residues of the ligand directly interact with the residues of the receptor. Once the ligand overcomes the intrinsic interaction barrier and leaves the first minimum, it encounters the second minimum, which is separated from the free, unbound state by a wide barrier. The second, shallow minimum and the wide barrier arise due to the water–protein surface tension.

The small depth of the second minimum and the large width of the corresponding barrier guarantee that exposure of the receptor–ligand complex to a small force eliminates the additional barrier, as depicted by dashes in Figure 2, reducing the two-minima model to the traditional one-minimum model. By defining the critical force $f_c$, which is sufficient to suppress the water–protein tension and to eliminate the second barrier, we consider the following two force regimes: (A) $0 < f \leq f_c$, where both states “1” and “2” are present, and (B) $f > f_c$, where state “2” is absent. In case B the ligand escaping from state 1 immediately dissociates away from the receptor, and the system can be described by a standard receptor–ligand binding model. It is the transition from regime A to regime B that is responsible for the gigantic discrepancy in the bond lifetimes observed in the experiments.1–7

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As detailed in the Appendix, the lifetime of the receptor–ligand complex in case A is given by the following expression:

\[
\tau_A(f) = \frac{k_{21}(f)}{k_2(f) k_{12}(f)}, \quad 0 < f < f_c
\]  

where \(k_{12}(f)\) is the rate constant for transition from state 1 to state 2, \(k_2(f)\) is the rate constant for the reverse process, and \(k_{12}(f)\) describes the transition from state 2 to the free state. All rate constants, present in eq 1, are functions of the applied force \(f\). The analysis takes the common point-of-view that the reaction coordinate, defined by the direction along which the ligand escapes from the binding pocket, is not significantly changed by the applied force.

The Appendix also shows that in regime A the time dependence of the number of bound receptor–ligand complexes, as measured for instance in SPR experiments, is characterized by two lifetimes, fast and slow. Such fast and slow components were observed with the P-selectin/PSGL-1 bond in ref 2, providing support for our two-state model. The approximate integrated lifetime \(\tau_A(f)\), eqs 1 and S5, corresponds to the slow component.

The bond lifetime in case B with \(f \geq f_c\) and only a single state 1 equals

\[
\tau_B(f) = 1/k_{12}(f), \quad f \geq f_c
\]

where \(k_{12}(f)\) is the same as in eq 1, since the properties of the first barrier, determined by the intrinsic receptor–ligand interaction, are independent of state 2, determined by the protein–water interaction. Thus, the bond lifetime below the critical force \(f_c\) that overcomes the protein–water surface tension is described by eq 1, while the bond lifetime above the critical force is described by eq 2.

Expression 1 was obtained under the following two assumptions: (1) minimum 2 is significantly more shallow than minimum 1, giving \(k_{21}(f) \gg k_{12}(f)\); and (2) the second maximum is very wide relative to the other extrema of the potential, giving \(k_{21}(f) \gg k_2(f)\) (see the following section and the Appendix).

The analysis presented below indicates that within the interval \(0 < f < f_c\), both \(k_{21}(f)\) and \(k_2(f)\) are essentially independent of the applied force: \(k_{21}(f) \approx k_{21}(0)/k_{12}(0) k_{12}(f)\). Therefore, eq 1 can be presented in the form \(\tau_A(f) \approx k_{21}(0)/k_{21}(0) k_{12}(f)\). Now comparison with eq 2 leads to the following two important conclusions. First, the ratio of the bond lifetimes in the limit \(f \rightarrow f_c\) achieved in regimes A and B is much greater than one, since \(\tau_A(f_c)/\tau_B(f_c) \approx k_{21}(0)/k_2(0) \gg 1\). Second, the type of the receptor–ligand binding, for instance catch vs slip discussed below, is determined by the force dependence of the rate constant \(k_{12}(f)\). That is, the binding type is established by the intrinsic receptor–ligand interaction and is independent of the interaction with the surrounding water.

To recapitulate, in the absence of the water–protein interfacial tension eq 2 remains valid for the whole force interval \(f \geq 0\). The interfacial tension and the resulting secondary minimum in the receptor–ligand interaction potential create a gigantic increase in the bond lifetime at small forces, according to eq 1. The generality of the water–protein interaction suggests that the low-force anomaly should be seen with the majority of receptor–ligand bonds.

3. actin–Myosin Complex

The theoretical analysis presented above is illustrated here with the actin–myosin complex,7 whose lifetime demonstrates not only the enormous decrease at very low forces (regime A) but also the catch–slip phenomenon at moderate forces (regime B). The catch and slip terminology arises when studying the dependence of the bond lifetime as a function of the applied force. Slip bonds constitute the majority of biological bonds and describe the typical situation, in which a force applied to break the bond decreases the bond lifetime. The catch bond is
a rare and counterintuitive phenomenon, in which the bond lifetime increases rather than decreases with growing force. A few biological systems, including the actin–myosin complex, exhibit the catch-bond phenomenon at moderate forces.

In the actin–myosin complex the critical force separating regime A, governed by the protein–water interface, from regime B is taken to be equal to \( f_c = 1.5 \) pN, which is the smallest force reported in the AFM measurements. The actin–myosin complex is the third system found to exhibit the catch–slip behavior. The other two catch–slip systems are the complexes of P, L-selectins with PSGL-1 \(^{1,3,4,17}\) and FilM with mannose.\(^{18}\)

In the force interval \( f = 1.5–6 \) pN, the actin–myosin bond shows increasing bond lifetime with increasing force. This catch-bond phenomenon requires a nontrivial treatment of the intrinsic receptor–ligand interaction described by the rate constant \( k_{12}(f) \).

The current treatment provides quantitatively descriptive of the experimental data for the actin–myosin bond over the whole force interval,\(^7\) including both low-force and catch–slip anomalies. The properties and parameters defining the \( k_{12}(f) \) rate constant that arises due to the intrinsic receptor–ligand interaction can be most easily derived in regime B, which shows the catch–slip anomaly.

### 3.1. Catch–Slip Anomaly

The catch–slip anomaly has been described theoretically by several models employing different potential energy landscapes for the description of the receptor–ligand interaction. One of the models\(^{19–21}\) uses a potential with two energy minima and two dissociation pathways. Another model\(^{22}\) uses only one potential energy minimum and two dissociation pathways. The authors\(^7\) have used the latter, simpler model in order to describe the experimental data generated with forces above 1.5 pN. Reference 23 proposed a conceptually different model of the catch–slip behavior. Called the deformation model, it develops the idea of the force-induced deformation of the interacting receptor and ligand. The catch behavior arises in the deformation model in the cases, in which the deformation improves the contact between the receptor and the ligand, thereby increasing the binding energy. If the deformation can be regarded as a discrete process, one can view the initial and deformed conformations as two states, providing a connection to the two-state models.\(^{19,20}\) Compared to the other models,\(^{19–22}\) the deformation model requires only one dissociation pathway, which is in agreement with schematic representations shown in Figures 1 and 2, and which may prove advantageous when interpreting the catch behavior at the atomistic level. It is interesting to note that that receptor–ligand binding can affect mechanical stability of the receptor.\(^{24,25}\) This effect can be regarded as inverse to the mechanically induced changes in binding and may also be explained using the deformation concept. The current work uses the deformation model\(^{23}\) in order to describe the catch–slip properties of the intrinsic interaction between actin and myosin,\(^7\) reflected in the rate constant \( k_{12}(f) \).

Thus, the rate constant for the thermally driven escape from the bound state 1 across the first barrier subject to pulling force \( f \) is given by the following expression:

\[
k_{12}(f) = k_{12}(0) \exp \left[ -\Delta E_d(f) + x_{12} f \right] / k_B T \tag{3}
\]

as described by the deformation model.\(^{23}\) Here, \( x_{12} \) is the characteristic bond length related to the distance between the minimum and maximum of the potential, \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, and \( k_{12}(0) \) is the dissociation rate constant in the absence of the applied force.

The term \( \Delta E_d(f) \) in eq 3 represents the change in the binding energy, i.e., in the depth of the potential well in Figure 2, induced by the deformation. As shown in ref 23, the functional dependence \( \Delta E_d(f) \) should be linear for small \( f \). Since any system can be deformed only to a certain extent, it is valid to assume that the deformation energy \( \Delta E_d(f) \) should reach a limit at a certain force \( f_0 \). Once \( f > f_0 \), the force stops affecting the receptor–ligand interaction, for instance, if the system resists further deformation or if the deformation continues outside the binding pocket. To retain the linear dependence at small forces and achieve the limit at large forces, the change in the bond energy due to the force-induced deformation was postulated to have the following functional form:\(^{23}\)

\[
\Delta E_d(f) = \alpha \left[ 1 - \exp(-f/f_0) \right]
\tag{4}
\]

where \( \alpha \) is the largest possible change in the binding energy due to the bond deformation and \( f_0 \) is the force value at which the effect of the receptor–ligand deformation on the binding energy saturates. The second term in the exponent in eq 3 is the standard Bell term,\(^{26}\) describing the lowering of the potential energy barrier by the applied force, common to the slip bond.

Figure 3 shows the experimental data\(^7\) and the deformation model fit of the actin–myosin bond lifetime, eqs 2–4. The four fit parameters of the deformation model are \( k_{12}(0) = 0.34 \) ms\(^{-1}\), \( x_{12} = 4.59 \) Å, \( f_0 = 2.43 \) pN, and \( \alpha = 134.97 \) pN Å.

### 3.2. Small-Force Anomaly

To describe the actin–myosin bond anomaly associated with the gigantic drop in the bond lifetime observed at the very low forces \( f \approx 0 \), one has to use eq 1 derived for regime A, \( 0 < f < f_c \). Since ref 7 reports only a single experimental point characterizing the small-force anomaly, we need to decrease the number of independent parameters and choose arbitrarily, but based on \( k_{12}(f) \gg k_{21}(f) \) derived above, that \( k_{12}(0)/k_{21}(0) = 10^{-2} \). Using the available experimental data point\(^7\) \( \tau(f=0.07\text{pN}\approx 0) = 2700 \) ms, eq 1 applied for zero force gives the following value for the rate constant describing the escape from the shallow minimum to the free state \( k_{21}(0) = 0.037 \) ms\(^{-1}\). This value of agrees with the proposed wide shape of the added barrier, as argued below. Recall\(^27\) that the Kramer’s reaction rates at zero force are given in the diffusive regime by

\[
k_{12}(0) = \frac{\omega_1 \omega_{12}}{2 \pi \zeta} \exp \left( -\frac{\Delta E_{12}^0}{k_B T} \right), \quad k_{21}(0) = \frac{\omega_2 \omega_{12}}{2 \pi \zeta} \times \exp \left( -\frac{\Delta E_{21}^0}{k_B T} \right)
\tag{5}
\]

Here, \( \Delta E_{12}^0, \Delta E_{21}^0, \Delta E_{21}^0 \) are the heights of the activation energy barriers for transitions from state 1 to 2, from state 2 to 1, and from state 2 to the free state, respectively. The frequencies \( \omega_1, \omega_2 \) and \( \omega_{12}, \omega_{21} \) characterize the curvatures in the two minima and the two maxima of potential. In the Kramer’s approach, \( \zeta, \zeta' \) are phenomenological friction coefficients for the diffusion of
the ligand over the first and second barriers, respectively. Assuming that three of the four frequencies describing the curvatures of the two minima and the maximum between them are similar, i.e., \( \omega_1 = \omega_2 = \omega_{12} \), and taking into account that the second minimum is much more shallow than the first, for instance, \( \Delta E_{12}^0 = 0.1 \Delta E_{12}^0 \) and \( k_{12}(0)/k_{21}(0) = 10^{-2} \) as before, we obtain \( \Delta E_{12}^0 = 208 \) pN Å and \( \Delta E_{21}^0 = 20.8 \) pN Å. This estimate allows us to obtain the preexponential factors in the first two equations of eq 5: \( \omega_2 \gamma/(2 \pi \zeta) = 57.29 \) ms\(^{-1}\). Note that at room temperature, \( k_B T = 40.57 \) pN Å. Assuming further that the barriers from state 2 to both state 1 and the free state are approximately the same, i.e., \( \Delta E_{12}^f = \Delta E_{21}^f \), we can evaluate the preexponential factor for \( k_{21}(0) \): \( \omega_2 \omega_3/(2 \pi \zeta^2) = 0.06 \) ms\(^{-1}\).

Provided that the ligand diffusing over the first and second barriers experiences similar friction, i.e., \( \zeta = \zeta' \), the second barrier must have a significantly smaller curvature than the first: \( \omega_2/\omega_1 \approx 10^{-3} \). The above analysis shows that the small value of the reaction rate \( k(0) \) is determined by the smallness of the corresponding preexponential factor and is associated with the low curvature of the barrier connecting the second minimum with the free state. These arguments support our interpretation of the small-force anomaly observed in the actin–myosin complex and other systems. The small-force anomaly occurs due to the additional shallow minimum, separated from the free state by a very wide barrier, Figure 2.

How will the bond lifetime of the actin–myosin complex will change in the 0–1.5 pN force interval? Current experiments cannot answer this question. Provided that our interpretation of the small-force anomaly is correct, we can easily reconstruct the entire picture. The force dependence of the bond lifetime \( \tau_A(f) \) in this force interval is governed by \( k_{12}(f) \), which depends on force through the deformation-induced change in the depth of the first minimum, eq 4. The distance parameter \( \alpha f_0 \approx 55.5 \) Å that determines the extent of the bond deformation is significantly larger than all barrier widths present in eq 1 and responsible for the changes in the bond lifetime according to the Bell mechanism.\(^{26}\) The width \( x_{12} \) of the first barrier in Figure 2 was established above. Now we will investigate the remaining barriers. The reverse process from 2 to 1 proceeds against the applied force, and its rate constant decreases with applied force according to the Bell expression \( k_{21}(f) = k_{21}^0 \exp(-x_{21} f/k_B T) \).

Since ref 7 reports only one experimental point in the 0–1.5 pN force interval, it is not possible to determine all parameters of the model uniquely. Therefore, we assume that the characteristic distance between the first maximum and the second minimum \( x_{21} \) is about half of the distance between the first maximum and the first minimum \( x_{12} \), i.e., \( x_{21} = 2.29 \) Å. To estimate the distance \( x_{2f} \) from the second minimum to the second maximum, consider the condition that defines \( f_c \). The critical force determines when the height \( \Delta E_{2f}(f) \) of the barrier separating the shallow minimum from the free state becomes zero. Using the Bell expression for the force dependence of the barrier height \( \Delta E_{2f}(f) = \Delta E_{2f}^0 - x_{2f} f \), we obtain the relationship \( \Delta E_{2f}^0 - x_{2f} f_c = 0 \). Using this equation and taking as before \( f_c = 1.5 \) pN, we estimate the characteristic distance describing the wide barrier as \( x_{2f} = 13.87 \) Å. This is about three times larger than the corresponding distance \( x_{12} \) characterizing the first barrier and is consistent with our model. According to the above estimates of the barrier widths, we conclude that within the force interval \( 0 < f < f_c \), the rate constants \( k_{21}(f) \) and \( k_{2f}(f) \) are essentially independent of force, \( k_{21}(f) \approx k_{21}(0) \), \( k_{2f}(f) \approx k_{2f}(0) \), and, therefore, the overall force dependence of the bond lifetime in eq 1 is governed by the behavior of \( k_{12}(f) \).

Using the above parameter values, we can use eq 1 to plot the force dependence of the bond lifetime in the small-force regime \( 0 < f < f_c \), Figure 4. The lifetime increases with applied force, indicating a catch-bond regime. As the force reaches the critical value \( f_c \), the shallow barrier disappears, and the lifetime rapidly drops to the beginning value \( \tau_0(f_c) \) in Figure 3. One can suppose that instead of a sudden change in the bond lifetime around the critical force, both experiment and a more sophisticated theory will give a more gradual change from regime A to regime B, as depicted by the dashed line in Figure 4.

3.3. actin–Myosin Summary. Summarizing the behavior of the lifetime of the actin–myosin bond over the whole range of applied force, Figures 3 and 4, we observe the following behavior. In the small-force regime \( 0 < f < 1.5 \) pN the lifetime grows according to eq 1, demonstrating catch behavior. This catch-bond regime is associated with the combination of the catch feature of the intrinsic receptor–ligand interaction reflected in the force dependence of \( k_{12}\) at small forces and the protein–water surface tension creating the additional barrier. The lifetime reaches its maximum at the critical force \( f \to f_c \), \( \tau_A(f_c) = 5831 \) ms. At this point, the shallow minimum disappears and the lifetime drops to \( \tau_0(f_c) = 11.4 \) ms. Then, the bond lifetime evolves according to eq 2, demonstrating further catch behavior within \( 1.5 \) pN < \( f < 6 \) pN. Finally, above 6 pN, the bond becomes the ordinary slip bond, and the lifetime decreases with applied force. It should be emphasized that the above values of the critical force and the bond lifetime in the small-force interval should be interpreted qualitatively, since they rely on a number of assumptions made necessarily due to the limited amount of experimental data.

4. Discussion and Conclusions

The current work presented a quantitative explanation of the large discrepancy between the biological bond lifetimes measured with and without an applied force. The discrepancy was rationalized by the presence of a secondary, shallow minimum in the receptor–ligand interaction potential, separated from the free state by a wide barrier, Figure 2. The secondary minimum appears in addition to the primary minimum arising due to the intrinsic receptor–ligand interaction. The bond dissociation rate is extremely slow in the absence of the force, because the escape from the secondary minimum to the free state is prevented by the wide barrier. The combination of the shallow minimum and wide barrier creates the situation in which the barrier can be eliminated by application of a small force.

The model was applied to the actin–myosin bond, which demonstrates two anomalies in the bond lifetime as a function of the applied force. Both anomalies were quantitatively
described by extending the deformation model of the catch bond with an additional shallow minimum and a broad barrier. In the small-force regime A the bond lives very long due to the new broad barrier. As soon as the applied force suppresses the broad barrier, the lifetime dramatically drops, switching to a new broad barrier. As soon as the applied force suppresses the small-force regime A the bond lives very long due to the effective size of the ligand can be larger in the second case, since it is exposed to more water and some of the water molecules can be tightly bound to the ligand surface.

In our hypothesis regarding the physical origin of the second barrier in the binding potential is true, Figure 1, the small-force anomaly in the bond lifetime should be general to any receptor–ligand bond. Our study focused on the biological catch bond, in which the catch–slip transition is seen with further force increase, in addition to the small-force anomaly. If the discussed phenomenon is indeed produced by the water network, the majority of the more common slip bonds should also exhibit the small-force anomaly. The lifetime of most slip bonds at zero force should be greatly enhanced relative to the finite-force lifetime, as already documented in some cases.5,6 According to eq 1 and in contrast to catch bonds, Figure 4, the lifetime of slip bonds will decrease from zero force up to a critical force at which point the lifetime would sharply drop to the range typically observed with finite forces and associated with the intrinsic receptor–ligand interaction rather than with the surrounding water. Our analysis leads to an intriguing prediction that most biological bonds should show a large drop in the bond lifetime at very weak forces. One may suppose that the wide and low barrier induced by the protein–water tension keeps biological bonds in a “dormant” state and that a small perturbation induced by a mechanical contact, a concentration gradient, and an electric signal, etc.31–33 activates receptor–ligand systems.

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Appendix

The general expression for the lifetime of a receptor–ligand complex \( \tau_{\Lambda} = \int_{0}^{\infty} t p(t) \, dt \) is applied to regime A governed by the protein-water tension with the interaction potential characterized by the two minima, Figure 2, in the following way. The probability density distribution \( p(t) \) is defined via the probabilities \( P_{1}(t) \) and \( P_{2}(t) \) for the bond to be in state “1” and “2” as \( p(t) = -\partial P_{1}(t)/\partial t \). Integrating the above expression for \( \tau_{\Lambda} \) by parts and taking into account that as \( t \to \infty \) the probability for the complex to exist in the bound state vanishes, we obtain

\[
\tau_{\Lambda} = \int_{0}^{\infty} [P_{1}(t) + P_{2}(t)] \, dt \tag{S1}
\]

The probabilities evolve according to the kinetic equations
\[
\frac{dP_1(t)}{dt} = -k_{12}(f)P_1(t) + k_{21}(f)P_2(t)
\]
\[
\frac{dP_2(t)}{dt} = -(k_{21}(f) + k_{23}(f))P_2(t) + k_{12}(f)P_1(t)
\] (S2)

where \(k_{12}(f)\) is rate constant for the transition from state “1” to state “2”, \(k_{21}(f)\) is the rate constant for the reverse process, and \(k_{23}(f)\) describes the transition from state “2” to the free state. Solving eqs S1 and S2, one finds
\[
\tau_A(f) = \frac{k_{12}(f) + k_{21}(f) + k_{23}(f)(1 - P_2(0))}{k_{12}(f)k_{23}(f)}
\] (S3)

Since the complex is initially bound, the sum of the probabilities at \(t = 0\) equals to one, \(P_1(0) + P_2(0) = 1.\) Assuming initial equilibrium between states “1” and “2”, one finds that
\[
P_2(0) = k_{12}(f)/[k_{21}(f) + k_{23}(f)]
\] (S4)

Since the minimum “2” is shallow relative to “1” and the barrier between state “2” and the free state is very wide, \(k_{23}(f) \gg k_{12}(f)k_{21}(f)\), and \(P_2(0)\) is much smaller than 1. Then, eq S3 simplifies to
\[
\tau_A(f) = \frac{k_{21}(f)}{k_{12}(f)k_{23}(f)}
\] (S5)

For forces greater than the critical force \(f \geq f_c\), corresponding to regime B with a single minimum in the interaction potential, dashed line in Figure 2, the expression for the force dependence of the bond lifetime \(\tau(f)\) can be formally found from eq S3 in the limit of \(P_2(0) = 0\) and \(k_2 \rightarrow \infty\)
\[
\tau_B(f) = 1/k_{12}(f)
\] (S6)

Finally, it follows from eq S2 and the ratio of the rate constants in regime A) that the time dependence \(P_1(t) + P_2(t)\) in eq S1 is a sum of two exponential functions. The fast component is characterized by the lifetime \(\sim 1/k_{23}(f)\), while the slow component is determined by \(\sim k_{21}(f)/k_{12}(f)k_{23}(f)\). The slow component dominates the integrated lifetime eq S5. Such fast and slow regimes were seen, for instance, in the SPR experiments.²

References and Notes


(22) Pereverzev, Y. V.; Prezhdo, O. V.; Forero, M.; Sokurenko, E. V.; Thomas, W. E. Biophys. J. 2005, 89, 1446.


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