Steered molecular dynamics simulations are combined with analytic theory in order to gain insights into the properties of the P-selectin/PSGL-1 catch–slip bond at the atomistic level of detail. The simulations allow us to monitor the conformational changes in the P-selectin/PSGL-1 complex in response to an external force, while the theory provides a unified framework bridging the simulation data with experiment over 9 orders of magnitude. The theory predicts that the probability of bond dissociation by the catch mechanism is extremely low in the simulations; however, a few or even a single trajectory can be sufficient for characterization of the slip mechanism. Theoretical analysis of the simulation data shows that the bond responds to the force in a highly nonlinear way, with the bond stiffness changing considerably as a function of the force ramp rate. The Langevin description of the simulation provides spring constants of the proteins and the binding interaction and gives the friction coefficient associated with the receptor–ligand motion in water. The estimated relaxation time shows that the simple probabilistic description is accurate for the experimental regime and remains approximately valid for the high ramp rates used in simulations. The simulations establish that bond deformation occurs primarily within the P-selectin receptor region. The two interaction sites within the binding pocket dissociate sequentially, raising the possibility of observing these independent rupture events in experiment. The stronger interaction that determines the overall properties of the bond dissociates first, indicating that the experimental data indeed capture the main rupture event and not the secondary weaker site rupture. The main rupture event involves the interaction between the calcium ion of the receptor and the ligand residue FUC-623. It is followed by new interactions, supporting the sliding–rebinding behavior observed in the earlier simulation [Lou, J.; Zhu, C. Biophys. J. 2007, 92, 1471–1485]. The weaker binding site shows fewer interaction features, suggesting that the sliding–rebinding behavior may be determined by the unique properties of the calcium site. The agreement between simulation and experiment provided by the two-pathway and deformation models, each containing only four parameters, indicates that the essential physics of the catch–slip bond should be relatively simple and robust over a wide range of pulling regimes.

1. Introduction

Many biological functions rely on a series of adhesions between proteins and ligands. An example of this is the inflammatory response that is characterized by an immune cascade—a series of biochemical events that allow the removal of an invading species from infected tissue. Within this multistep process, selectin proteins remove leukocytes from the blood flow via tethering and rolling. Initially, endothelial cells exocytose P-selectin in response to inflammatory signals released by surrounding tissue. Concurrently, P-selectin glycoprotein ligand (PSGL-1) is produced by the leukocytes. Tethering occurs when P-selectin binds with PSGL-1. The leukocyte then rolls until it can bind with another P-selectin. This tethering and rolling continues until the leukocyte reaches the site where it will enter the inflamed tissue, i.e., extravasation. As more blood flows to the area of inflammation, increasing hemodynamic forces stress the protein–ligand bond. If the bond lifetime is too short, the leukocyte will detach before rolling to the next P-selectin, thus preventing it from moving to the site of inflammation. An understanding of how the bond lifetime is affected by changes in shear force can be developed using experimental results, analytic models, and computer simulations.

Several experimental studies of this system have been conducted including atomic force microscopy (AFM) and flow chamber techniques. The AFM experiments involved applying a force to the protein–ligand bond by pulling on the ligand until dissociation occurred. The shear force is replicated in flow-chamber experiments by allowing solution to flow past protein-coated beads and detecting the number of ligands that are removed. The results from these experiments reveal a unique response of the system to low forces resulting in the so-called catch–slip bond transition. Specifically, as the applied force to the ligand is increased, the bond lifetime increases until some critical force is reached, at which point the bond lifetime decreases. It is thought that the development of the catch bond prevents the premature release of the leukocyte, thus enabling proper rolling. The counterintuitive catch-binding behavior has been observed with several other bonds and may occur quite commonly in biological systems, which are often subjected to external forces.

Biological adhesion is a complicated dance between protein–ligand binding and unbinding influenced by such things as thermal fluctuations and conformational changes. The simplest of these considers the transition between two states, i.e., bound and unbound states, being driven by thermal fluctuations. The molecular system stochastically diffuses across the energy barrier in response to these fluctuations. The rate constant of
this diffusion can be represented as $\sim e^{-AE/k_BT}$, where $k_BT$ is thermal energy and $\Delta E$ is the energy barrier that must be overcome to change states. George Bell is recognized for postulating that the rate at which a biological bond will dissociate rises exponentially with the applied force.\textsuperscript{30} This implies that an external force applied to a system lowers the energy barrier thus decreasing the bond lifetime. This phenomenon is called a slip bond.

However, the behavior seen in the P-selectin/PSGL-1 adhesion experiments indicates that a slip-bond model is not adequate and has to be extended in order to describe the catch–slip bond transition. Several mechanisms have been proposed to explain this behavior, relying on a framework of simple analytic models. A two-state model\textsuperscript{7} describes a conceptual energy landscape composed of a low-impedance pathway and a high-impedance pathway driving the catch–slip bond behavior of this complex. If a slow steadily increasing force that begins at zero is used to dissociate the ligand, the bonds are broken rapidly at small forces. However, if the force is changed to a fast-ramping force, the bonds become stronger and the forces required to break them are proportional to the logarithm of the loading rate. Interestingly, if the system is ramped quickly to a finite force, the low-impedance pathway is blocked and the dissociation occurs only along the high-impedance pathway even if the ramp rate is subsequently lowered. A further refinement of this two-state model considers that the complex experiences conformational fluctuations between two initial states.\textsuperscript{14} Dissociation can then occur from either conformational state and each can be described via a Bell model. The external force influences which of the conformational states the dissociation originates from as well as the ease at which this dissociation occurs. Comparison of results from this model to the previously mentioned experimental results shows good agreement. Also, this particular model successfully describes the double-exponential decay behavior for the catch-bond property of the biological adhesion between FinH and mannosse.\textsuperscript{19}

In our previous work, we developed the two-pathway analytic model\textsuperscript{31–33} that readily replicates the catch–slip transition using a minimal number of parameters. In particular, this model describes the ligand escaping the receptor binding site characterized by one rather than two bound states as in the two-state model.\textsuperscript{7,14,19} The dissociation occurs via two alternative routes: a catch pathway that is opposed by the applied force and a slip pathway that is promoted by the force. Good quantitative fit to the experimental data for the P-selectin/PSGL-1 complex is obtained with the two-pathway model in the constant force\textsuperscript{25,31} and time-dependent jump-ramp regimes.\textsuperscript{32}

This two-pathway model has led to a remarkable prediction for the behavior of the P-selectin/PSGL-1 bond experiencing a periodic-shear hemodynamic force.\textsuperscript{34} This prediction is biologically relevant because the rhythmic beating of the heart creates such a pulsing force. The theoretical analysis indicates that a periodically driven P-selectin/PSGL-1 catch–slip bond is unusually sensitive to the driving frequency within a physiologically relevant range. In particular, the bond properties drastically change as the periodicity of blood flow grows from 0 to 30 beats per minute and shows little change upon further frequency increases.

Further study of the P-selectin/PSGL-1 system has led to the development of a deformation model of the force-induced dissociation of biological bonds.\textsuperscript{35} Within the binding pocket, the relative positions of the protein and ligand residues influence the binding interaction. The application of an external force to the bound complex would lead to deformations of the molecules, thus directly impacting the binding interactions. Structural deformations far from the binding pocket can affect the bond via the allosteric mechanism.\textsuperscript{19} The deformation model\textsuperscript{35} differs from the two-pathway one-state model\textsuperscript{11–13} and the two-pathway two-state model\textsuperscript{7,14,19} in that it quantitatively describes the catch–slip transition using only one bound state and one dissociation pathway. Additionally, the deformation model reconciles the experimental data obtained with a number of biological catch and ordinary slip bonds at zero and finite forces.

Both the experimental and analytic results give insight into the magnitude and duration of the forces involved in dissociation of the ligand from the protein. However, a picture of what is happening in the system at the molecular level would be extremely useful to better understand the properties of the bond. For example, the complex may experience conformational changes at the critical forces offering an atomistic explanation for the catch bond. One such explanation, the sliding–rebinding mechanism, has been posited by Zhu et al.\textsuperscript{16,17} In this mechanism, a change in the angle between the epithelial growth factor (EGF) and lectin domains allows an increase in bond lifetimes that creates the catch bond. Initially, under low force, this angle remains unchanged and dissociation readily occurs. However, as the force is increased, the interdomain angle increases, and the binding site becomes aligned with the pulling direction. The dissociation would then progress as a series of formations and ruptures of attractions as the ligand slides along the binding pocket. This would delay the dissociation because of the increased number of attractions. Eventually, the pulling force becomes large enough to overcome any re-binding and the more intuitive slip-bond behavior describes the dissociation.

Atomistic models representing conformational changes in the system are most easily produced by classical molecular dynamics (MD). This method determines the time-dependent trajectory of all the atoms within the molecule in response to the ensemble of forces experienced by said atoms. Knowing the positions of the atoms at any given time allows us to monitor conformational changes. To study the dissociation of PSGL-1 from P-selectin, the simulations need to also incorporate the external force that represents the hemodynamic force. The use of steered molecular dynamics (SMD) achieves this by allowing the analyst to add an external force to the system.\textsuperscript{36} An atom or group of atoms is fixed in space while another atom or group of atoms is pulled, typically with either a constant force or a constant velocity. The trajectory can then be analyzed to identify the dissociation of the ligand as well as any major changes in the structure of the system in response to the external force.

The paper is organized as follows. The section below presents the technical details of the SMD simulations. It also derives an expression for the force acting on the ligand including the effect of protein deformation. Section 3 applies the two-pathway and deformation models to the P-selectin/PSGL-1 catch bond, in order to relate the experimental and SMD data which correspond to very different pulling regimes due to the inherent limitations of simulation and experiment. An important conclusion is obtained that the bond-rupture probability density becomes a very narrow function of time for the large loading rates used in simulations. The beginning of section 4 compares the probability densities computed using the two-pathway and deformation models for the loading rates used in experiments and simulations. The comparison demonstrates that the probability shifts from the catch to the slip regime with accelerated loading.

Section 4.2 presents the simulated trajectories of the force acting on the bond, while section 4.3 analyzes these trajectories using Langevin dynamics. It is shown that in contrast
to experiments, analysis of the simulated trajectories should include the spring constant of the proteins as well as the friction force acting on the moving ligand. Section 4.4 discusses the conformational changes taking place during the simulated bond rupture. The features of the bond revealed by the comparison of the analytic theory and simulation are accentuated, complementing the SMD simulation data that have led to the sliding-rebinding catch-bond mechanism. Finally, we discuss and summarize the key results of the present work.

2. SMD Simulation Details

The initial coordinates for the protein–ligand complex were downloaded from the RCSB Protein Data Bank entry 1G1S. The crystal structure file contained the dimer form of the complex and included strontium for the cation in the binding pocket. For the purposes of our analysis, the dimer was divided into the monomeric form (sP-selectin and sPSGL-1) and the strontium ion was replaced with a calcium ion (Figure 1). This substitution was based on the biological role that the calcium ion plays in the behavior of the complex.

Two software packages were used for the analysis of the complex: VMD for solvation and visualization, and NAMD for MD analyses. The complex was solvated with TIP3P water molecules, creating a cubic box with sides measuring 100 Å to ensure that the water bath was large enough to contain the complex throughout the simulations. Energy minimization of the solvated system was performed using a conjugate gradient and line search algorithm. Subsequently, the system was heated to 310 K and equilibrated using Langevin dynamics for an additional time period ranging from 50 ps to 1.1 ns. The SMD trajectories showed little dependence on the equilibration time. A 1 fs time step was used. Constant pressure was maintained by dynamically adjusting the size of the unit cell and rescaling all atomic coordinates during the simulation, as implemented in the NAMD code. The response of the system to a constant velocity force applied to a ligand atom was used in SMD simulation in order to mimic the AFM experiments.

Two different types of simulations were performed, and in both scenarios a carbon atom (VAL-27) located at the center of mass (COM) of the protein was fixed. For one set of simulations, the position of the pulled carbon atom (LEU-608) was near the COM simulations. For the second set of simulations, the position of the pulled oxygen atom (GBL-624) was near the end of the ligand located near

Figure 1. Chemical structure of the complex between the P-selectin receptor (blue) and PSGL-1 ligand (red). The atom located near COM of the receptor is noted in purple. The ligand atom that is pulled during the COM simulations is noted in orange, and the ligand atom pulled in the END simulations is noted in green. The inset provides a closer view of the binding pocket. The ligand interacts with two distinct regions within the binding pocket of the receptor. The TYS-607 residue is attracted to the SER-46 loop. The FUC-623 residue is attracted to the calcium ion and nearby ARG-85 loop.
the calcium ion of the protein (END simulations). The END scenario corresponds more closely to the experimental situation, while the COM scenario allowed us to study the properties of the bond associated with the two separate receptor—ligand interaction points (Figure 1).

The force was applied to the ligand atoms via a spring attached to a dummy atom. The spring constant, \( k \), was equal to 6.95 pN Å\(^{-1}\), and the dummy atom had a constant velocity of 0.1 Å/ps. The coordinates of the atoms were collected every 100 fs, and the force experienced by the pulled ligand atom was collected every 10 fs. Note that the spring constant used in the simulation is 2 orders of magnitude higher than the corresponding experimental value.\(^7\)

In jump-ramp experiments, the force applied to the bond grows linearly with time, \( f(t) = rt \). In our simulations, the ramp rate \( r \) is determined by the product of the dummy atom’s velocity \( v \) and the stiffness \( k \) of the spring attached to the dummy atom: \( r = kv \). However, this expression should be modified, since the complex can stretch in response to the applied force. This functional dependence can be easily found theoretically by neglecting the frictional force acting on the ligand. In this case, the force acting on the bond is \( f(t) = k[\nu t - \Delta x(t)] \), where \( \Delta x(t) \) is the displacement of the pulled ligand atom relative to the fixed receptor atom. Defining the stiffness of the complex by the force constant \( k_1 \), the ligand displacement can be rewritten as \( \Delta x(t) = f(t)/k_1 \), and the equation for the time-dependent force can be expressed as

\[
f(t) = \frac{r}{1 + r/k_1} = k_1 \exp \left( \frac{r}{k_1} \right) \quad (1)
\]

where \( r_{eff} = r(1 + r/k_1) \) is the effective ramp rate accounting for the flexibility of the receptor—ligand complex. \( r_{eff} < r \) for any finite positive value of \( k_1 \). The deviation between the force applied to the dummy atom \( f_0(t) = rt \) and the force experienced (eq 1), by the bond can be used to determine the stiffness of the complex \( k_1 \). The generalization of eq 1 to include the effect of the frictional force acting on the ligand is discussed in section 4.3 below.

3. Analytic Models of the P-Selectin/PSGL-1 Bond

Biological bonds and the P-selectin/PSGL-1 bond under investigation, in particular, are typically characterized by lifetimes on the order of 1 s. At the same time, atomistic MD studies are computationally expensive and are limited to the timescales on the order of \( 10^{-3} \) s. Therefore, it is not possible to simulate the experimental situation directly. Realization of events such as breaking of ligand/protein bonds on a computer is possible only with forces that are significantly higher than those achieved in experiment. While simulation and experiment cannot be compared directly, they can be related by use of analytic models of bond dissociation, which are applicable for all force ranges. In addition, analytic models can provide useful insights into the simulation results. The modeling has already proven extremely valuable for the analysis of the experimental data. Similarly, the analytic modeling can help analyze MD simulations, which can be regarded as a computational experiment.

The simulation results will be analyzed using the two-pathway\(^{31-33}\) and deformation\(^{35}\) catch-bond models, which have shown good agreement with the experimental data. These models contain few parameters and, therefore, are particularly amenable for analytic techniques and can be easily applied to a variety of situations. Below we summarize the key features of the models for the linearly increasing force regime realized in both simulation and experiment.

3.1. Two-Pathway Model. The two-pathway model\(^{31-33}\) extends the standard single-pathway model used to describe dissociation of slip bonds by introducing a second dissociation pathway, which accounts for the catch behavior. Only one bound state is assumed. The two-state, two-pathway model\(^{3,14,15}\) also introduced to describe the catch bond reduce to two-pathway model in certain limits.

The time-dependent bond dissociation probability density is available experimentally and can be obtained from simulation. The following analytic expression can be obtained for the linearly ramped force:\(^{32}\)

\[
p(t) = \left[ k_c \exp \left( \frac{-x_r t}{k_B T} \right) + k_s \exp \left( \frac{x_r t}{k_B T} \right) \right] \exp \left( \frac{k_B T}{x_r} \left[ \exp \left( \frac{-x_r t}{k_B T} \right) - 1 \right] \right) - \left( \frac{k_B T}{x_r} \right) \exp \left( \frac{x_r t}{k_B T} - 1 \right) \quad (2)
\]

where \( k_c \) and \( k_s \) are the dissociation rate constants for the catch and slip pathways at zero force, \( x_r \) and \( x_s \) are the distances from the bound-state minimum to the catch and slip barrier maxima, and \( r \) is the force ramp rate. The opposite signs in the exponents for the slip and catch pathways are essential and describe the increasing rate of escape along the slip pathway and decreasing rate of escape along the catch pathway under the action of the force. The model parameters that reproduce the P-selectin/PSGL-1 experimental results\(^3\) for the whole range of ramp rates \( r \) used in the experiments are \( k_c = 0.34 \pm 0.09 \) s\(^{-1}\), \( x_r = 2.1 \pm 0.1 \) Å, \( k_s = 20 \pm 10 \) s\(^{-1}\), and \( x_s = 3.8 \pm 1.1 \) Å.\(^{31}\)

Equation 2 is valid if the observation times \( t \) used in experiments or simulations to detect bond dissociation are larger than the system relaxation time \( \tau_1 \). The analysis of the corresponding Langevin equation performed below in section 3.4 shows that this condition is always fulfilled in the AFM experiments. The detection time of our simulation is longer than the relaxation time, however, the difference is not very large. For instance, the simulation ramp rate \( r = 0.695 \) pN/ps gives \( t \tau_1 \approx 2.8 \) and still, eq 2 can be approximately used. In particular, application of the two-pathway model with the above parameters to the SMD simulation ramp rate indicates that atomistic MD simulations cannot directly reveal the properties of the catch pathway. The model predicts that the probability density (eq 2) obtained by setting \( k_s \) to zero is nearly identical to that obtained with the finite \( k_s \) value. See ref 32 for a more detailed analysis.

Setting \( k_s \) in eq 2 to zero, one can easily derive the expression for the time \( t_m \) at which \( p(t) \) is maximized, \( t_m = [k_B T/x_r][\ln(x_r/k_B T)] \). This is the time at which the P-selectin/PSGL-1 bond is most likely to be broken. Using the fact that the ratio \( k_B T/k_s x_r \ll 1 \), we can also determine the maximum value of the probability density \( p(t_m) = x_r/k_B T \). The result indicates that the maximum value of the probability density increases linearly with the ramp rate. Further, since the integrated probability density is normalized to 1, the maximum will become narrower as its height is increased. In particular, the width of the maximum \( \Delta t \) is inversely proportional to the ramp rate: \( \Delta t \sim k_BT/x_r \). The maximum is extremely narrow if \( \ln(x_r/k_B T) \gg 1 \). The above analysis indicates that with ramp rates available in SMD simulations the bonds will be breaking within a very narrow time interval. Thus, few or even a single SMD simulation should
be sufficient in order to establish the characteristic bond-dissociation time.

Note that our analysis is valid for sufficiently large forces and assumes that the receptor–ligand binding potential in the slip-bond regime is dominated by a single barrier, regardless of a specific bond-dissociation protocol. A potential containing several barriers can generate a multilogarithmic dependence of the rupture time on the ramp rate.\textsuperscript{42}

### 3.2. Deformation Model

The deformation model of the catch bond\textsuperscript{35} has been developed in order to limit the number of dissociation pathways to one. Rather that introducing a second dissociation pathway, the deformation model describes the catch behavior by a force-induced bond deformation that changes the receptor–ligand interaction energy. The deformation idea is not limited to the catch bond and describes a wide variety of situations. Similarly to the two-pathway model, the deformation model contains only four parameters.

The reaction rate is defined in the deformation model as

\[
k(t) = k_0 \exp \left\{ \frac{-\alpha [1 - \exp(-rt f_0)] - x_{10} r t}{k_B T} \right\}
\]

(3)

where \(k_0\) is the dissociation rate constant in the absence of the force, \(x_{10}\) is the distance between the bound state minimum and the dissociation barrier maximum, \(\alpha\) is the bond deformation energy, and \(f_0\) is the value of the applied force at which the deformation saturates. The intrinsic properties of the bond induced by its deformation stop changing at \(f > f_0\). The values of the parameters for deformation model\textsuperscript{35} that best reproduce the experimental results\textsuperscript{2} are \(k_0 = 20.34\) s\(^{-1}\), \(\alpha = 185.36\) pNÅ, \(f_0 = 18\) pN, and \(x_{10} = 2.43\) Å. Note that the magnitudes of \(k_0\) in the deformation model and \(k_1\) in the two-pathway model are very similar, since both describe bond dissociation in the absence of the force. The characteristic barrier widths \(x_{10}, x_{10}\) are also similar in the two models, since they describe the behavior of the system in the slip regime, subjected to large forces.

### 4. Results and Discussion

In this section we first consider the bond behavior that we can expect in the SMD simulations based on the analysis of the analytic models described above. In particular, we use the parameter values obtained by fitting the two-pathway and deformation models to the experimental data and calculate the bond dissociation probability density for the ramp rate used in the simulations. Then we discuss in detail the SMD simulation results.

#### 4.1. Predictions of the Two-Pathway and Deformation Models Regarding the SMD Simulation

Figure 2 shows the bond dissociation probability density obtained using the analytic models (eqs 2 and 3) for the relatively low ramp rate \(r = 500\) pN/s, which is representative of the forces applied experimentally. The solid and dashed lines represent the two-pathway and deformation models, respectively. The slight difference between the two models is primarily due to the different fitting procedures used to obtain the model parameters.

The time-dependent probability density shows two maxima originating due to the catch and slip-bond regimes.\textsuperscript{36} Since the force grows linearly with time (eq 1), the force is small at short times, and the bond dissociates primarily by the catch mechanism: through the catch pathway in the two-pathway model or subject to a bond deformation that increases the receptor–ligand interaction in the deformation model. The force is large at longer times. As a result, the second maximum originates by the slip mechanism. The bond dissociates via the slip pathway in the two-pathway model. According to the deformation model, the force-induced deformation of the bond has already saturated by the time the second maximum in the probability density is formed, and the P-selectin/PSGL-1 complex behaves as an ordinary slip bond. The minimum between the two maxima in Figure 2 corresponds to the maximum bond lifetime, characterizing the catch–slip transition. The probability density decays to zero at long times, since all bonds have already dissociated.

In order to evaluate the probability of the P-selectin/PSGL-1 bond dissociating by the catch and slip mechanisms at the experimental ramp rates, we integrate the probability density shown in Figure 2 over the respective time intervals and obtain \(f_{10}^0 p(t) dt = 0.382\) for the catch region and \(f_{10}^1 p(t) dt = 0.556\) for the slip region. Thus, the catch-bond phenomenon occurs in approximately 40% of the experimental measurements.

Next, consider the bond dissociation probability density computed by the two-pathway and deformation models for the much larger ramp rate that is used in the simulations, \(r = 0.695 \times 10^{12}\) pN/s. The result obtained using the same parameters as above is shown in Figure 3. Once again, the solid line represents the two-pathway model and the dashed line describes the deformation model. Increasing the ramp rate has three major impacts on the probability density. First, the bond dissociates significantly faster with the larger ramp rate: compare the time scales in Figures 2 and 3. Second, the maximum becomes significantly narrower and higher: compare the probability-density scales. Third, the probability density is almost entirely shifted to the slip region, and the data are accurately represented by the simplified eqs 2 and 3 in \(k_s = 0\) and \(f_0 = 0\) limits.
Properties of the P-Selectin/PSGL-1 Complex

respectively. The insert in Figure 3 shows that the first maximum originating due to the catch regime does exist; however, its magnitude is negligible relative to the slip maximum. Once again, integrating the density over these two time regimes we obtain the probabilities for the bond to dissociate in the catch regime, \( f_0 \times 10^{-10} \) \( p(t) \, dt = 2.3 \times 10^{-5} \), and in the slip regime, \( f_0 \times 10^{-10} \) \( p(t) \, dt = 0.98 \). Thus, for very large loading rates the bond rupture probability density approaches the Dirac \( \delta \)-function.

Two important conclusions regarding the SMD simulations follow from the above analysis and the data shown in Figure 3. The conclusions are a consequence of the very large ramp rates that are used in simulations. First, it is highly unlikely to obtain an example of catch behavior by simulation. Second, at the same time, few or even one SMD trajectory should be sufficient to characterize the slip behavior, since the slip maximum is very narrow.

4.2. Comparison of SMD Simulation and Experiments.

The generated SMD trajectories allowed us to monitor the structure of the P-selectin/PSGL-1 complex subjected to the dissociating force. Changes in the interaction between residues were monitored together with the conformational changes within larger regions of the protein.

Analysis of the crystal structure of the P-selectin/PSGL-1 complex\(^{38}\) indicates that the ligand strongly interacts with the receptor in two different locations within the binding pocket (Figure 1). Specifically, the TYS-607 residue of the ligand is attracted to the receptor loop containing residue SER-46. At the other side of the binding pocket, the FUC-623 residue of the ligand is attracted to the calcium ion that binds to the receptor. In order to investigate the properties of both binding sites, two sets of simulations were performed as described in the SMD Simulation Details section.

Figure 4 presents the magnitude of the applied force between the spring and the ligand as a function of time for representative COM and END simulations. In either case, the force grew approximately linearly with time, reached a maximum, and then linearly decreased. The force in the COM simulations had its maximum at approximately 400 ps with a magnitude of 240 pN. The END simulations reached maximum force at around 800 ps with a larger magnitude of 460 pN. Visualization of the trajectories indicated that the maxima in the force time dependence seen in Figure 4 occurred when a protein—ligand attraction was broken. In the COM simulations, residue TYS-607 dissociated from the loop containing residue SER-46. The end of the ligand that contains residue TYS-607 continued to move away from the binding site for the duration of the simulations. The END simulations showed the same type of behavior for the other binding site: residue FUC-623 dissociated from the calcium ion. The difference in the rupture forces for the COM and END simulations demonstrates that the receptor—ligand binding facilitated by the calcium ion is about twice stronger than the binding at the other site. This is to be expected since the doubly positively charged calcium ion creates a strong electrostatic interaction.

The dissociation process expected in vivo is represented by the END simulations. It shows that the two binding sites located in the receptor—ligand binding pocket dissociate sequentially. Moreover, the stronger bound site containing the calcium ion dissociates first. The force required to break the weaker of the two interactions is about twice smaller (Figure 4). Following the dissociation of the calcium site, the bond undergoes a conformational change, completes the bond rearrangement, and exerts a force on the weaker site. This can be seen even in the SMD simulations with the force ramp rate that is orders of magnitude faster than the experimental ramp rates. Thus, in experiments the weaker site should dissociate independently of the calcium site and at its intrinsic value of the rupture force. One may expect to see these two rupture events in experiment. Most importantly, since the stronger site dissociates first, it is responsible for the observed properties of the bond. The scenario in which the experimental data are dominated by a partial bond rupture associated with dissociation of only the weaker site can be eliminated on the basis of our calculations.

The analytic models of the catch bond allow us to relate experiment and simulation. This is a unique opportunity, since it is not possible to use the same force values and compare the two situations directly. Using the model parameters determined from the experimental data and the analysis performed in section 4.3, we can calculate the rupture forces corresponding to the ramp rate of the simulations. The two-pathway and deformation models give 491 and 472 pN, respectively. This is in surprisingly good agreement with 460 pN obtained from the simulations (Figure 4). We would like to emphasize once again that the comparison between experiment and simulation pertains only
to the slip regime. Still, it is quite encouraging that experiment, theory, and simulation agree over 9 orders of magnitude.

4.3. Analysis of Bond Elasticity and Friction. The rupture forces for the two binding sites are slightly lower than the ideal force values computed as $f_0(t) = rt$, in which the ramp rate $r$ determined by the product of the dummy atom’s velocity $v$ and the force constant $k$ of the spring connecting the dummy atom to the ligand atom, $r = vk$. The force determined by $f_0(t)$ is plotted in Figure 4 as a dashed line. This simulation result demonstrates that the force acting between the ligand atom and the spring changes more slowly than may be expected due to the elasticity of the complex and the frictional forces acting on the system.

In order to model the time dependence of the applied force observed in the simulations, we will use the Langevin equation, which accounts for both elastic and frictional forces:

$$\zeta \frac{dx}{dt} = -k_1x + k(\nu t - x)$$  \hspace{1cm} (4)

Here, $\zeta$ is the friction coefficient and $k_1$ is the effective spring constant characterizing the two sequentially connected springs, which describe the receptor and ligand proteins, $k_p$ and their interaction that creates the bond, $k_r$, $1/k_1 = 1/k_p + 1/k_r$. By replacing the true receptor–ligand interaction profile with the harmonic approximation, which is approximately valid prior to the bond rupture event, we are able to use this simple form of the Langevin equation. By writing eq 4 we assumed that the inertial relaxation time $\tau_1 = m/\zeta$, where $m$ is mass of the ligand, is significantly shorter than the relaxation time of the system associated with conformational changes

$$\tau_1 = \zeta/(k_1 + k)$$  \hspace{1cm} (5)

The solution of eq 4 subject to the initial condition $x(t=0) = 0$ is

$$x(t) = \frac{\tau_1^2 k \nu}{\zeta} [\exp(-t/\tau_1) + t/\tau_1 - 1]$$  \hspace{1cm} (6)

The force plotted in Figure 4 is generated by stretching the spring acting on the dummy atom. According to eq 4, it is equal to

$$f(t) = k(\nu t - x(t))$$  \hspace{1cm} (7)

where $x(t)$ is given by eq 6. Generally, it is possible to consider two independent approximations to eqs 6 and 7, describing the two limiting regimes $t/\tau_1 \ll 1$ and $t/\tau_1 \gg 1$. In particular, $t/\tau_1 \gg 1$ transforms eq 7 to eq 1. Our analysis shows that neither regime can properly describe the whole simulated time interval from $t = 0$ to bond rupture. Therefore, we will use the general solution given by eqs 6 and 7. The solution contains two undetermined parameters: the relaxation time $\tau_1$ and the friction coefficient $\zeta$.

The theoretical model (eq 7) is compared with the SMD simulation data for the END case, which corresponds to the experimental setup. The fit of the SMD data according to eq 4 is shown in Figure 4 by the solid green line. The relaxation time and the friction coefficient fitted to the SMD data are $\tau_1 = 282.3 \text{ ps}$ and $\zeta = 8561.5 \text{ pN/ps/Å}$, respectively. The well-known relationship between the friction coefficient $\zeta$ and viscosity $\eta$,

$$\zeta = 6\pi a \eta$$

allows us to estimate the size of the ligand $a$. Using the viscosity of water, we obtain $a = 45.9 \text{ Å}$, which agrees quite well with the direct estimate of the PSGL-1 dimension.\(^{45}\) It should be noted that the coordinate $x(t)$ is more properly interpreted as the displacement of the front side of the ligand, while $k_r$ is an effective spring constant representing both receptor and ligand simultaneously. This is particularly true in the present case, since the size of PSGL-1 is quite large and significant relative to the size of the receptor.\(^{45}\) Equation 5 gives the spring constant $k_1 = 23.35 \text{ pN/Å}$.

The Langevin equation allows us to estimate the displacement of the ligand at the bond rupture time that maximizes the applied force, $t_m = 780 \text{ ps}$ (Figure 4). The displacement $x(t_m) = 11.8 \text{ Å}$ exceeds appreciably the width of the receptor–ligand interaction barrier $x_{10} = 2.43 \text{ Å}$. Thus, the majority of the displacement occurs due to elastic extension of the receptor and ligand proteins.

The component of the force that stretches the proteins, breaks the bond, and drives the ligand outside of receptor’s binding pocket can be evaluated using eq 4 as

$$f(t) = f(t) - \alpha \frac{dx(t)}{dt}$$  \hspace{1cm} (8)

At the time of bond rupture $f(t_m) = 276.2 \text{ pN}$. This is significantly smaller than the rupture force of 460 pN observed in the simulation directly (Figure 4). The extra force acts to overcome the friction.

Reference 46 estimated the energy of the P-selectin/PSGL-1 bond in the absence of force as $\Delta E = 41 \text{ kJ/mol} = 683 \text{ pN·Å}$. In order to overcome this binding energy at $f(t_m) = 276.2 \text{ pN}$, the distance between the bound state minimum to the top of the barrier must be equal to $x_{10} \approx 2.47 \text{ Å}$. This value obtained from the simulation is close to the corresponding parameters of the two-pathway and deformation models, which were determined by fitting the experimental data. The equation $k_b x_{10} = f(t_m)$ gives $k_b = 111.8 \text{ pN/Å}$. In turn, the relationship defining $k_b$ in terms of $k_p$ and $k_r$ leads to the following value for the spring constant of the proteins, $k_p = 29.5 \text{ pN/Å}$. This is twice smaller than the corresponding value determined in ref 46 and is similar to the protein stiffness reported in refs 47 and 48. The values reported by Marshall et al.\(^{10}\) are even smaller. The difference between our simulations and the experimental data can arise because the simulations fix the protein COM, therefore eliminating the protein domains that extend beyond the COM. These domains can be stiffer or softer than receptor’s binding region.

The total amount of energy stored in the complex prior to bond rupture equals $\int_{0}^{t_m} f(t) \text{d}(x(t)/dt) \text{dt} = 1631.4 \text{ pN·Å}$, which is more than twice larger than the 683 pN·Å binding energy itself. This fact indicates that a significant amount of work, about 948 pN·Å, performed by the applied force is accumulated in the deformation of the protein and the ligand.

We would like to emphasize once again that the estimated relaxation time $\tau_1 = 282.3 \text{ ps}$ is much smaller than AFM measurement times (see for instance Figure 2) and therefore, the left-hand side of eq 4 can be neglected, leading to eq 1. Moreover, comparing the $k_1$ value determined above with the spring constant $k$ reported in ref 7, the $k/k_1 \ll 1$ term in eq 1 can be neglected as well.

4.4. Bond Rupture Details. Large conformational changes are seen in the receptor when comparing the relative orientation of its EGF and lectin domains. In order to establish whether our simulations support the sliding–rebinding behavior,\(^{16,17}\) we
monitored the protein structure in the END simulations. Three atoms were connected to create an angle that spans the EGF and lectin domains. Changes in this angle represented how the relative orientation of these domains evolved in time. The angle increased from 110° to 125° before the subsequent dissociation of the ligand (Figure 5). The angle increase is consistent with the expected interdomain angle opening necessary for the sliding-rebinding mechanism. In addition to the change in angle, our results showed a relaxation in the EGF domain, resulting in a loss of the helical structure with respect to the original crystallographic conformation.

Important details of the P-selectin/PSGL-1 bond dissociation process can be obtained by considering the distances between selected receptor and ligand atoms (Figure 6). The time at which the FUC-623 residue moves away from the calcium ion is easily identified in Figure 6a by a sharp increase in the corresponding distance. The dissociation event is also reflected in the distance between the receptor COM and the FUC-623 residue (Figure 6b). Compared to part a, the change of the distance shown in part b is less abrupt. It develops gradually, starting before the dissociation event, reflecting the fact that the receptor stretches prior to breaking the bond. The extension and contraction of the distance between the receptor COM and the calcium ion characterizes receptor’s flexibility both before and after the dissociation (Figure 6c). Following the dissociation the receptor slowly contracts. The well-defined oscillation of the distance seen in part c at the earlier times defines receptor’s normal modes that are excited by the applied force. The main frequencies in the corresponding Fourier transform occur at 330 and 490 cm⁻¹. The distance between the receptor COM and the pulled atom of the ligand is plotted in Figure 6d. It behaves nearly identically with that depicted in part c, indicating that the bond extension occurs primarily within the receptor. The conformation of the ligand does evolve over time. Following the bond dissociation and after a slight delay, the distance between the pulled atom and the FUC-623 residue contracts, as clearly seen in Figure 6e. The contraction takes about 60 ps. The ligand overcontracts and has to extend a little at the end. The primary normal modes excited in the ligand by the applied force occur at 330 cm⁻¹ as well as between 490 and 870 cm⁻¹.

Further analysis of the conformational changes taking place in the P-selectin/PSGL-1 bond during the dissociation process was carried out by close examination of a shorter time interval centered on the maximum for the END simulation shown in Figure 4. The analysis revealed that the maximum was composed of a series of peaks (Figure 7). The structure of the binding pocket at each of the points labeled in Figure 7 is shown in Figure 8. The maximum located between points A and B is due to the pulling away of the ligand residue SIA-620 from the
structures corresponding to these maxima are depicted in Figure 8. The separation from the calcium ion site in the binding pocket. The sequence of events can be considered a series of formations and ruptures of attractions. This is the type of behavior predicted by the sliding—rebinding model of Zhu and co-workers.16,17 It is quite interesting that the maximum found in the COM simulations shows fewer features (Figure ), suggesting that the sliding—rebinding behavior is much more pronounced at the calcium binding site, which determines the overall properties of the P-selectin/PSGL-1 bond.

5. Discussion and Conclusions

The complexity found in biological adhesions offers a challenge for both experimental and computational analyses. In order to describe the catch—slip bond transition observed in the P-selectin/PSGL-1 complex and other systems in response to an external force, the slip-bond model proposed by Bell29 had to be extended. Two alternative concepts were introduced to explain the catch-binding regime. It was proposed that the catch behavior arises due to a second dissociation pathway.7,14,19,31–34 It was also proposed that the catch behavior could occur within a single dissociation pathway due to a force-induced deformation, which increases the receptor—ligand interaction.35 The domain opening in P-selectin coupled to the changes within the binding pocket16,17 is a likely candidate for such deformation. At this point, neither the experimental data nor the SMD simulations can distinguish between the two types of models and uniquely identify the second dissociation pathway or the bond deformation event.

Still, SMD simulations can provide extremely valuable atomistic understanding of the catch—slip bond phenomenon, even though a huge discrepancy exists between the time and force scales accessible in experiment and simulation. The earlier SMD simulations provided evidence for the P-selectin domain opening and suggested the sliding—rebinding bond dissociation mechanism.16,17 High ramp rates limit the ability of SMD to replicate the experimental observation of the catch-bond behavior. The probability of the bond dissociation by the catch mechanism is minute in the simulations. At the same time, the slip-bond regime is represented by the simulations very well, better than in experiment. The maximum in the bond-dissociation probability originating due to the slip-bond mechanism is much narrower in the simulation force range, indicating that few or even one SMD trajectory is sufficient to characterize the slip behavior.

The SMD simulations reported here show that the two interaction sites located in the receptor—ligand binding pocket dissociate sequentially. The stronger bound site contains the calcium ion, which creates a more effective electrostatic interaction between the receptor and the ligand. The force required to rupture the weaker of the two interactions is about twice smaller than the force required to rupture the calcium site. Determined by the bond geometry, the stronger site dissociates first, and the force experienced by the bond drops rapidly. The simulations indicate that the weaker site should dissociate independently of the calcium site. Therefore, one may expect to see these two separate rupture events in experiment. Most importantly, since the stronger site dissociates first, it must be responsible for the observed properties of the bond. The scenario in which the experimental data is dominated by a partial bond rupture associated with dissociation of only the weaker site can be eliminated on the basis of our simulations. The experimentally determined rupture force indeed corresponds to the stronger interaction. This conclusion is supported by the agreement between the experimental and theoretical rupture force values, which are related through the analytic models.

The simulations indicate that the P-selectin/PSGL-1 complex undergoes a substantial deformation during the dissociation process. The deformation occurs both within the P-selectin receptor and in the PSGL-1 ligand. The simulations allowed us to estimate the stiffness (force constant) describing not only the receptor and ligand proteins but also, separately, the bond itself. We showed that the bond is significantly stiffer than the proteins.

The stronger interaction that determines the properties of the P-selectin/PSGL-1 bond occurs between the ligand residue FUC-623 and the calcium ion of the protein. The rupture of this binding site activates vibrational motions within both receptor and ligand. The vibrations are damped by the new interactions, for instance, between the FUC-623 residue and the ARG-85 loop, supporting the sliding—rebinding behavior observed in the
Fewer interaction maxima and, therefore, fewer sliding—rebinding events are seen in the dissociation of the weaker binding site, suggesting that the calcium site possesses rather unique properties that favor sliding—rebinding.

Finally, we would like to summarize some of the key conclusions that follow from this work. The analysis shows that real-time atomistic simulations are unlikely to capture bond rupture by the catch-binding mechanism and are able to sample only slip binding. At the same time, the extremely high values of ramp rates available in simulation generate bond-rupture probability densities that are nonzero within a very narrow time interval. Therefore, few trajectories are sufficient in order to evaluate the characteristic bond-rupture time and force. The Langevin description of the simulated force evolution allows one to estimate the spring constant of the proteins and the bond between them, and to obtain the friction coefficient associated with the relative motion of the receptor and ligand in aqueous environment. In general, the force-induced deformation of the proteins and the friction force acting on the ligand from the

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**Figure 8.** Snapshots from the typical END trajectory showing the binding pocket at each of the points labeled in Figure 7. Point A: The initial structure of the binding site as the maximum force is approached. Point B: The ligand residue SIA-620 moves away from the binding site. Point C: The ARG-85 loop moves away from the calcium ion creating a more open pocket. Point D: The attraction between FUC-623 and the calcium ion is broken and the FUC-623 becomes more attracted to the ARG-85 loop. Point E: The attraction between FUC-623 and the ARG-85 loop is broken. A new attraction is formed between ARG-85 and SIA-620. Point F: The attraction between ARG-85 and SIA-620 is broken and the end of ligand pulls away from the binding pocket. Note that in all cases the attraction between the TYS-607 residue and the SER-46 loop remains intact.
medium should be taken into account in the analysis of simulations, which use large loading rates and stiff springs. The estimated relaxation time indicates that the simple probabilistic description given by eqs 2 and 3 remains approximately valid even for the ramp rates used in simulations.

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References and Notes


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