Computational Model of the Mechanical Behavior of Clusters of E-Selectin Bonds

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ABSTRACT
The relationship between bond kinetics and mechanical properties of bonds between the selectin family of proteins and P-selectin glycoprotein ligand-1 are important in the biological process of leukocyte adhesion. Previous studies have shown that selectin bonds exhibit catch behavior at the single molecule level and ideal behavior when multiple bonds interact in a cluster. However, the internal mechanisms that cause this ideal behavior remains unknown. To explore these mechanisms, a one state two pathway model was developed assuming that the bonds were linear springs and could rebind after rupturing. This model was developed to resolve contradicting conclusions about the behavior of catch bonds on a cluster and aimed to explain the experimentally observed behavior of a catch bonds cluster. However, the model was unable to predict ideal behavior for a catch bond cluster when experimental data from a previous study was fitted for single bonds and bond clusters simultaneously. The results demonstrated that assumptions about tether elasticity and rebinding rates cause a cluster of selectin catch bonds to exhibit catch behavior.

BACKGROUND

Cell interactions with environmental stimuli are a vital life function involved in every physiological mechanism (Hammer et al., 1996). Because of this, the ability to understand the behavior of receptor-ligand binding between cells and extracellular surfaces is critical to the successful development of synthetic molecular biology and biotechnologies. Specific leukocytes such as neutrophils, monocytes, eosinophiles, memory-effector T-like lymphocytes, and T-killer cells have specific sialylated carbohydrates on their cell surface which bind to E-selectin receptors as they roll in our bodies. Thus implying E-selectin is involved in recruitment of cells to inflammatory sites thus indicating it plays a key role in our bodies inflammatory response (Robbins SL, 1999).

The receptor-ligand bonds that form on the surfaces of cells have been experimentally shown to exhibit both catch and slip behaviors in the presence of tensile force (Sun et al., 2012). Slip behavior occurs between receptor-ligand pairs where lifetime decreases when greater tensile forces are applied to the bond and catch behavior is where the lifetime increases with greater tensile force. Catch bonds are a type of bond that display catch behavior up to a critical point where it transitions to slip. Selectins are a group of proteins that exhibit this catch bond behavior and are of particular interest in current studies and models to further understand their behavior as they are essential to leukocyte adhesion (Snook and Guilford, 2012). In physiological conditions selectin bonds are most commonly found in clusters which causes the need for understanding the behavior that clusters of catch bonds exhibit due to rebinding and load sharing mechanisms. Previous attempts at modeling clusters of catch bond have created inconsistent results on this topic.

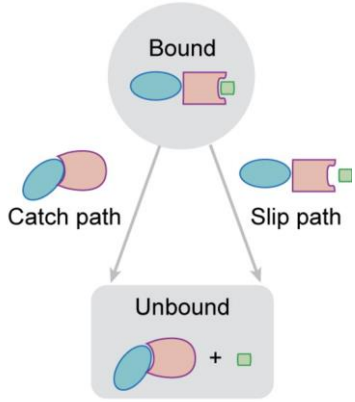


Figure 1. A two pathway model for selectin catch bonds between a ligand(green) and binding domain (pink and teal) (Thomas, 2008). The catch path dominates at lower forces and is characterized by a decrease in unbinding as force increases. The slip path dominates at higher forces - past the critical force where there is the most inhibition of unbinding - and is characterized by an increase in unbinding as force increases.

In previous studies about catch bond behavior in selectin cell adhesion molecules, Pereverzev et al. developed a model that assumes one minimum state and two unbinding pathways. The two unbinding pathways are a combination of slip and catch behavior where at low forces it is dominated by a catch pathway and at higher force is dominated by the alternative slip pathway, as can be seen in **figure 1**(Pereverzev et al., 2005). Furthermore, this model uses a single exponential decay formula for bond survival over time under constant force and omits the rebinding rate of the bonds, claiming that its effect is insignificant.

Building off of the knowledge about single catch bonds in Pereverzev et al., a different theoretical study explored the behavior of catch bonds clusters. Catch bond clusters were modeled by applying single receptor-ligand pair theory to a larger system (Novikova, E.A. and C. Storm, 2013). Novikova *et al.* created a computational model that utilized Evan's two low-energy state, two pathway model, and assumed uniform distributed load on the cluster with a rebinding rate that is unaffected by force. This was different from the previously described one energy state model by including two separate energy domains that the bond can be in in which there is a rapid equilibrium between the two domains. Their computational solutions demonstrated catch bond behavior under these assumptions. However, a different study also based on Evan's two state, two pathway model by Sun *et. al.* showed that catch bond clusters exhibited slip behavior when increased tensile force was applied (Sun et al, 2012). Sun et al., took a different approach when modeling rebinding rates by making them dependent on force because they assumed that the elasticity of the receptor-ligand pairs could be affected by force when considering rebinding rates. The two studies contradicting results raised further questions of how to appropriately model catch bond clusters and what behavior should be seen.

Experimental work done on clusters of E-selectin and sialyl Lewis bonds by Snook and Guilford observed ideal behavior where bond lifetime is unaffected by external tensile force, which contrasts the expected behavior seen in current computational models (Snook and Guilford, 2012). The experiments were conducted by using magnetic tweezers to pull receptor-coated microspheres that bond to ligand-coated surfaces that had low and high density of ligands on their surface. For low-density conditions which were assumed to be single bonds they reconfirmed catch-slip behavior, but in high density which was assumed to be bond clusters they observed ideal behavior. This is speculated by Snook and Guilford to be caused by rebinding and formation of more new bonds even as other bonds rupture when subject to tensile force because of highly available ligands.

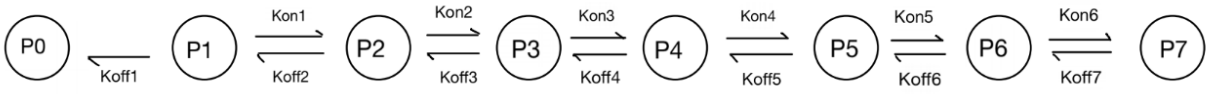
In this study, we proposed a computational model that calculates the mean lifetime of catch bond clusters. This model was developed assuming that rebinding rates depend on the external tensile force and that selectin bonds can be characterized as linear springs in order to replicate the experimental ideal bond behavior in clusters of E-selectin catch bonds (Snook and Guilford, 2012). The model builds on the one state, two pathway model presented in Pereverzev et al. to calculate the unbinding rate for each bond, and introduces the equation theorized for the rebinding rate presented by Sun et al. to describe the change in bonds present for the cluster model. We chose to base the model off the one state two unbinding pathway model due to its simplicity and potential for translating from modeling single bonds to cluster bonds accurately. Implementation of a force sensitive rebinding rate and use of the one state two unbinding pathway model to depict clusters of bonds is a novel approach for explaining catch bond cluster behavior. The model based on these principles was then validated by fitting it to the mean lifetime and standard deviation from data presented in the study by Snook and Guilford. The sensitivity of the parameters produced was tested to determine the effect of each on the results. Through parameter fitting and analysis of the model we determined that the sensitivity to the spring constant and therefore tether

elasticity was extremely low and that the assumptions made did not lead to the creation of a model able of depicting ideal behavior but instead demonstrated catch behavior in a cluster.

METHODS

Here we develop a model for catch bond clusters that incorporates both unbinding and rebinding effects to the behavior of low and high density bond clusters. The effects of the rebinding rates may help us show ideal behavior in high density clusters because the ruptured bonds could rebind and maintain the lifetime of that bond cluster constant with different magnitudes of tensile force.

To analyze the effects of both unbinding and rebinding on catch bond clusters, we considered a collection of 7 ligand-receptor pairs where N_i is the initial number of bound receptor-ligand pairs at time t = 0. 7 bonds were chosen due to the adhesion probabilities of the experimental runs in Snook and Guilford. The probability of having a certain number of closed bonds at any given time, t, was determined by the Kolmogorov forward equations, a system of differential equations that describe time-evolution of probability, **Equations 1** (Feller, 1940). The probabilities were calculated to range from P_0 ,the probability that all bonds have ruptured, to P_7 is the probability that all 7 bonds are formed. These equations required us to assume that each bond rupture and rebinding event is independent of the other. This assumption is physiologically relevant because the spatial separation between different receptor-ligand pairs allows us to safely say that the intermolecular chemical interactions between them are negligible. A different differential equation was derived for the probability of an i number of bonds from 0 to 7 and the unbinding and rebinding rate constants were used to model the transition of the cluster between different closed bond states (**table 1**). The bond cluster is considered fully dissociated when there are no bonds left, at which point no rebinding can occur. When all of the bonds are broken, we assumed that the distance between the receptor and ligand is far too great to allow rebinding.



$$\frac{\partial P_0}{\partial t} = k_{off1}P_1$$
$$\frac{\partial P_1}{\partial t} = 2k_{off2}P_2 - k_{on1}P_1 - k_{off1}P_1$$
$$\frac{\partial P_2}{\partial t} = 3k_{off3}P_3 - 2k_{on2}P_2 - 2k_{off2}P_2 + k_{on1}P_1$$
$$\frac{\partial P_3}{\partial t} = 4k_{off4}P_4 - 3k_{on3}P_3 - 3k_{off3}P_3 + 2k_{on2}P_2$$
$$\frac{\partial P_4}{\partial t} = 5k_{off5}P_5 - 4k_{on4}P_4 - 4k_{off4}P_4 + 3k_{on3}P_3$$
$$\frac{\partial P_5}{\partial t} = 6k_{off6}P_6 - 5k_{on5}P_5 - 5k_{off5}P_5 + 4k_{on4}P_4$$
$$\frac{\partial P_6}{\partial t} = 7k_{off7}P_7 - 6k_{on6}P_6 - 6k_{off6}P_6 + 5k_{on5}P_5$$
$$\frac{\partial P_7}{\partial t} = -7k_{off7}P_7 + 6k_{on6}P_6$$

Equations 1: Equations for the probability that there are a certain number of bonds formed in the system ranging from 7 bonds formed to all receptor ligand-pairs dissociated and zero bonds formed.

We used ode45, MATLAB's ODE solver, to determine an array of probabilities for each individual bond cluster state over time. From this, the probability that one or more bonds were still intact, also known as the cumulative distribution function, was determined by P(t) = (1 - P_0), where P_0 is the probability that all of the bonds are broken (Pereverzev, Y., et al., 2005). Knowing the cumulative distribution function, P(t), it follows that the probability density function is:

$$p(t) = \frac{-dP(t)}{dt},$$

The mean lifetime of the bond cluster can then be calculated with the following function,

$$T(f) = \int_0^\infty tp(t)dt\,.$$

To accurately reflect the experimental setup that was demonstrated by Snook and Guilford, we implemented a poisson distribution of initial conditions that reflect the low and high adhesion probabilities of receptor-ligand bonds, which characterized low and high ligand densities on the surface. λ, which is a parameter of the poisson distribution, was calculated from the adhesion probability A_w with the equation $\lambda = -ln(1 - A_w)$ based on unpublished work by the Thomas lab (**figure 2**). The low site density A_w was experimentally measured to be 0.25 on average, and the high site density A_w was 0.58 on average (Snook and Guilford, 2012). The probability for each initial number of bonds was then multiplied by 1000 pulls, which is a standard for experimental bond lifetime testing based on expert testimony from Dr. Wendy Thomas. The scaled distribution values were then rounded to the nearest integer giving the number of trials to be run with a given number of initial bonds (**figure 3, figure 4**). Because the low site density condition has approximately 80% single catch bonds (**figure 4**), we considered it to be equivalent to simulating a single catch bond. The distribution was calculated over an integer range from 1 bond to 10 bonds, not including the pulls where there were no initial bonds assuming that those trials give outlying lifetime data. The model outlined above was then run with the generated initial conditions and averaged over all of the trials for each of the site densities.

A maximum initial condition of 5 receptor-ligand pairs bound in the system of equations was justified by **figure 2** because the probability of having five bound catch bonds in a cluster was less than 1/1000 for high site density distributions. For 1000 pulls, 5 receptor-ligand pairs bound was the highest initial condition seen to exist theoretically (**figure 3**). We modeled a 7 receptor-ligand pair system because it allows for both unbinding and rebinding from the maximum initial condition of 5 bound pairs.

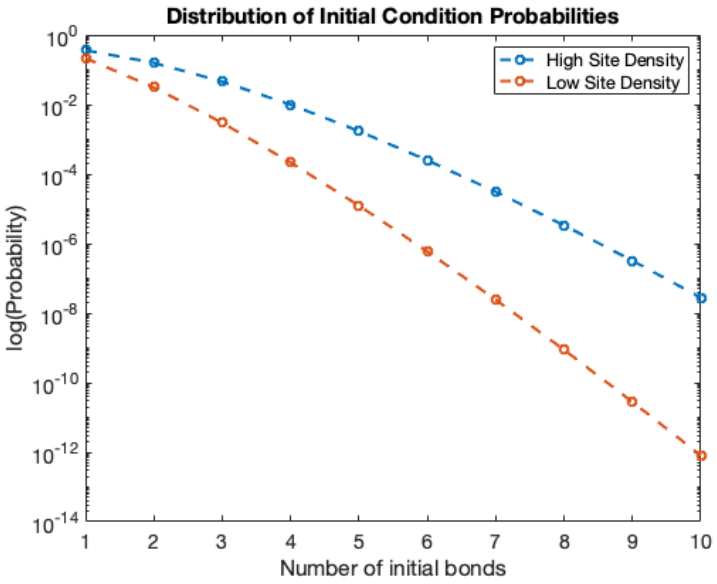


Figure 2: Log Scale of the Poisson distribution of probabilities for different numbers of initial receptor-ligand pairs. Natural log of probability for high site density adhesion frequency is in blue and natural log of probability for low site density adhesion frequency is in orange.

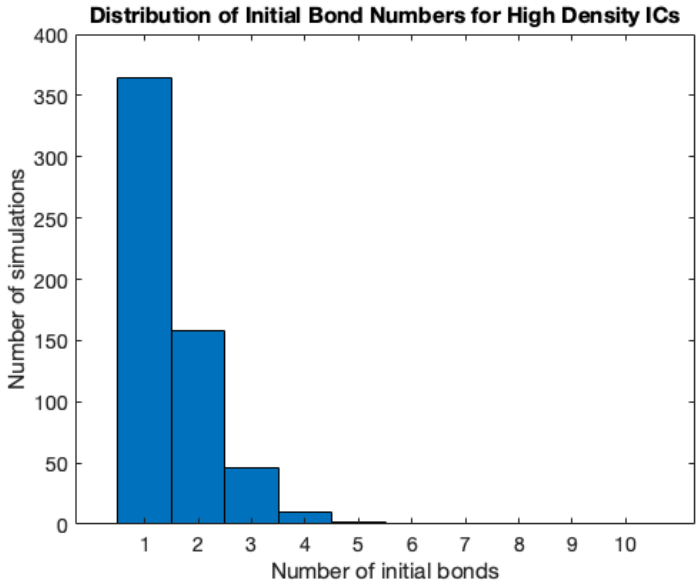


Figure 3: Number of simulations for each initial condition given a high site density adhesion probability. Initial conditions are represented as the starting number of receptor ligand pairs, or initial bonds.

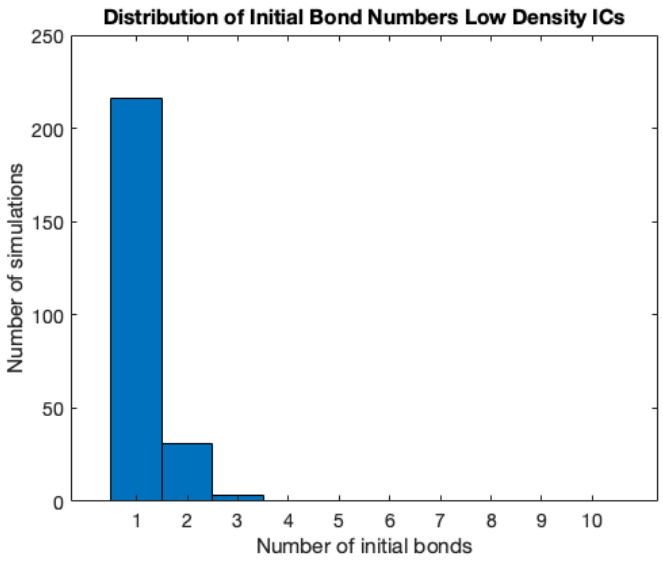


Figure 4: Number of simulations for each initial condition given a low site density adhesion frequency. Initial conditions are represented as the starting number of receptor ligand pairs, or initial bonds.

Rebinding of Receptor-Ligand Bonds

The rebinding rate for receptor ligand pairs is modeled by a distance dependent rate**,**

$$k_{on} = k^0{}_f e^{-(\delta/\delta_b)^2}$$

k_{on} is the distance dependent rebinding rate for receptor-ligand pairs which is determined by $k^0{}_f$, is the rebinding rate coefficient, δ, the separation between receptor-ligand pairs, and δ_b, the characteristic length for bond separation. According to our assumptions and as shown in previous studies, the rebinding of ruptured bonds requires a multiple bond system, and when all bonds are ruptured, no bond can reform (Sun., 2012). In these studies, the rebinding rate k_{on} was found to be dependent on the receptor-ligand separation, δ, which can be nondimensionalized by δ_b. This is a characteristic length introduced to describe the effect of the separation on bond-formation rate. The bond is treated as a linear spring as $\delta = \frac{f}{Nk}$, with k as the spring constant. δ is proportional to the force enacted by the pulling mechanism f, and the number of receptor-ligand pairs N.

Unbinding of Receptor-Ligand Bonds

The unbinding of E-selectin bonds is described by the following force dependent equation.

$$k_{off} = k_c^0 e^{\frac{-f\xi}{kT}} + k_s^0 e^{\frac{f\xi}{kT}}$$

k_{off} is the unbinding rate for receptor-ligand pairs. k_s^0 is the unbinding rate for slip behavior when there is no force on the bond. k_c^0 is the same for catch behavior. *f* is the force applied on that bond. ξ is the characteristic bond length, used for both slip and catch pathways, (Novikova et al., 2013). K is the boltzmann constant, and T is the standard room temperature, 298 degrees Kelvin respectively. The above equation has been derived in previous work to understand the mean

lifetime of a single catch bond system at various forces (Pereverzev et al., 2005; Novikova et al., 2013). The force acting on a single bond is derived as the total force enacted on the bond cluster by the magnetic tweezers divided by the total number of bound receptor-ligand pairs. The separate catch and slip mechanisms are described by the two pathway model described in **figure 1**. This was included in the model because we assumed the bond will follow one or the other unbinding pathway at any given time, so we combined the individual unbinding rates into one rate k_{off}.

Definitions	Equations
Unbinding rate of E-selectin	$k_{off} = k_c^0 e^{\frac{-f\xi}{kT}} + k_s^0 e^{\frac{f\xi}{kT}}$
Rebinding rate of E-selectin	$k_{on} = k^0{}_f e^{-(\delta/\delta_b)^2}$

***Table 1**: Summary of Rebinding and Unbinding rates*

Parameter Value Definitions	
Parameter	**Definition**
κ (N/m)	Spring constant
ξ (m)	Unbinding length for a particular bonds developed from the unbinding rates for the slip pathway and the catch pathway
Ks (s^{-1})	the slip pathway unbinding rate without force applied to a particular bond
Kc (s^{-1})	the catch pathway unbinding rate without force applied to a particular bond
K0r (s^{-1})	Rupture rate when no force is applied to a particular bond
Characteristic Length, δ_b (m)	Describes the effect of the separation on bond rebinding rate

***Table 2:** Summary of parameters in Rebinding and Unbinding rates*

Minimum Bond Lifetime Observed Calculation

In their study, Snook and Guilford developed a high throughput technique that measures the kinetics of single receptor-ligand bonds (Snook and Guilford, 2012). Snook and Guilford used a 0.25 NA 10x objective on a Olympus BH2 microscope with bright field illumination and a digital

CCD camera operating at 90 frames per second to measure the mean lifetime of single bonds and bonds in a cluster. The out of plane resolution of this imaging system set a lower limit to the bond lifetimes that could be detected. The depth of field of the microscope used in the study was approximately 9 μm. Therefore the imaging system was unable to detect the binding state between E-selectin receptors, on a magnetic bead, and sLe^a ligands, on a stationary surface, until the minimum detectable bond lifetime was reached. A bead had to be at least 9 μmoff of the surface of the flow cell inorder to detect that it is no longer bound. The time it takes for the bead to travel this distance is dependent on the force pulling on the bead, therefore, Snook and Guilford used the following formula to calculate the minimum detectable bond lifetime for each tensile force used,

$$t_{min} = 6\pi\eta r z / f_t,$$

where $\eta = 0.001 Ns/m^2$is the fluid viscosity, $r = 1.5E^{-6}\, m^2$is the bead radius, z = 9E^{-6} is the depth of field, and f_t is the applied tensile force.

We calculated the minimum bond lifetimes for each of the simulations we ran to incorporate these experimental limitations. At the smallest force of 5.9519pN the lowest detectable bond lifetime was 0.0428s and this time decreased as the tensile force applied to the bond cluster increased. The smallest lifetime observed was for the greatest force modeled, 39.2560pN, at 0.065s. **Appendix: Table 1** shows the calculated minimum detectable bond lifetimes for each tensile force used in our model model for single bonds and multiple bonds.

These experimental limitations were included in our model to ensure that we calculated physiologically relevant bond cluster lifetimes. Therefore, the mean lifetime calculations made by the model were changed based on the minimum detectable bond lifetime described in **Appendix: Table 1** and the lifetimes that fell below the minimum detectable bond lifetimes for each force were removed from the mean bond cluster lifetime calculations.

Verification of Model

For single bond (low density) conditions, the model is capable of reproducing catch bond behavior as seen in **figure 5**. This plot was produced by simulating a low site density using the parameters shown in **table 3**. These parameters were found by fitting our model to the low density experimental data from Snook and Guilford. This behavior is expected for a single catch bond, and other experimental and theoretical studies have demonstrated it (Pereverzev, Y., et al., 2005). The lifetime of a catch bond increases until a critical point where it reverts to slip behavior. Being able to obtain this single catch bond behavior contributes to the verification of our model. Furthermore, the model resulted in physiologically relevant parameters to depict this behavior. We believe the parameters are physiologically relevant because they are positive, non-zero values. We believe this further contributes to our verification. When the parameters ξ and δ_b, which are responsible for the force sensitive aspect of the unbinding and rebinding rates are set to zero, the model shows force insensitivity (**figure 6**), confirming the expected behavior further verifying the model.

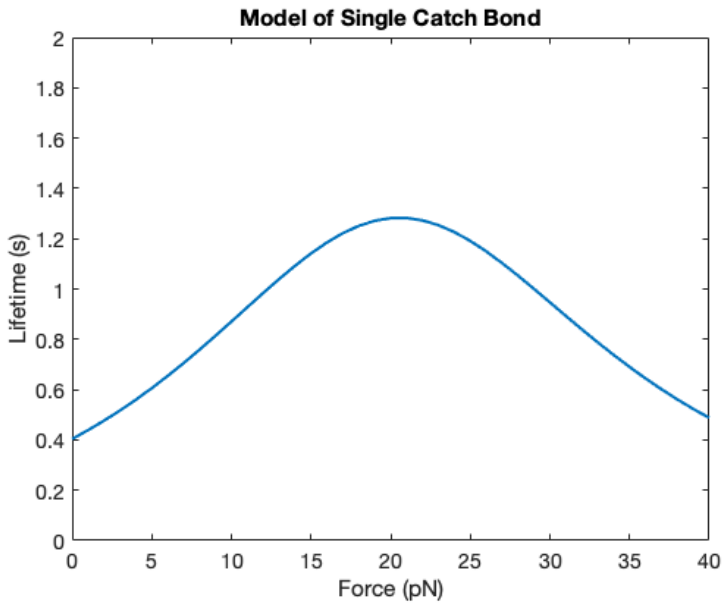


Figure 5: Lifetime of Single Catch Bond with physiologically relevant parameters (**table 3**) simulated at low site density initial conditions.

Fitted Parameters					
κ (N/m)	**ξ (m)**	**k_s (s^{-1})**	**k_c (s^{-1})**	**K0r (s^{-1})**	**Characteristic Length , δ_b(m)**
2.833E-3	3.73E-10	6.45E-2	2.590	6.491E-14	1.319E-09

Table 3: Fitted Parameter values for model in figure 1

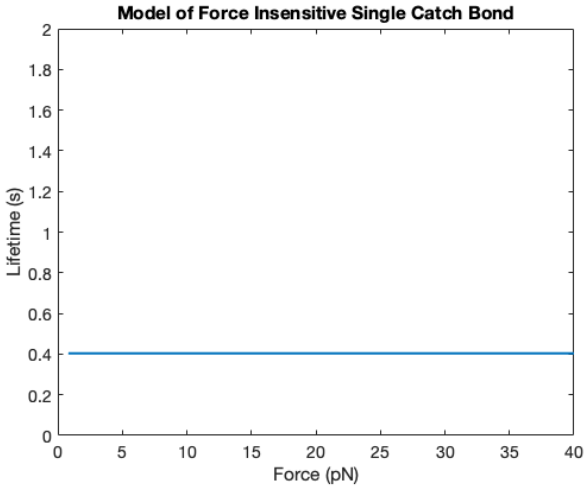


Figure 6: Lifetime of Single Catch Bond with an unbinding length and characteristic length of 0 making the model force insensitive. The model is generated based on the parameters in **table 3**.

The cumulative distribution function showed the overall combined probability never changed from one over time, and the system naturally descended from the initial bounded states to an overall unbound cluster (**figure 7**). Because the system is not perturbed by any outside environmental factors, the probability of the different states always adding up to one verifies the system's closed nature. The system also showed a smooth transition between different states without any unexpected fluctuations or inexplicable variations verifying the transitions between the ODEs. The ODE system was solved with multiple solver algorithms such as ode45, ode15s, and ode23s to ensure reproducibility and the robustness of the model to different methods.

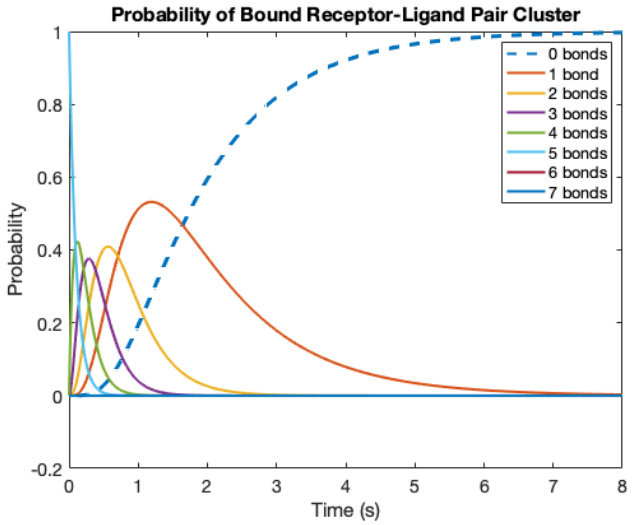


Figure 7: Probability of the different states of bound receptor-ligand pairs for a high density catch bond cluster over time with initial conditions of 5 bound receptor-ligand pairs.

We performed dimensional analysis on the unbinding and rebinding rates successfully (**Appendix: figure 1**). It also worked toward the dimensional analysis of the ODEs because the unitless probabilities in them were simply multiplied by the unbinding and rebinding rates. This contributed to the mathematical verification and consistency of the ODEs and the overall model.

We performed sensitivity analysis on the parameters that impact unbinding in the model with high density initial conditions. This helped verify the model because we know what to expect from these analyses as they were described in various previous studies. The parameters of interest for unbinding verification were k_s, the zero force slip unbinding rate, k_c, the zero force catch unbinding rate, and ξ, the combined unbinding length for a particular bond in the system.

Changes in k_s were measured over a span of 0.001 [1/s] to 100 [1/s], and the corresponding model for selectin catch bonds analyzed in **figure 8**. At the lowest values of k_s, the model exhibited catch behavior at all forces with the lifetime increasing as the applied force increases. This behavior transitioned at $k_s = 1$ where the model exhibited both catch and slip behavior, and at higher values of k_s, the model demonstrated exclusively slip behavior with lifetime decreasing as force increased. This behavior is congruent with the expected unbinding of the function as the slip unbinding behavior varies directly with the value of k_s.

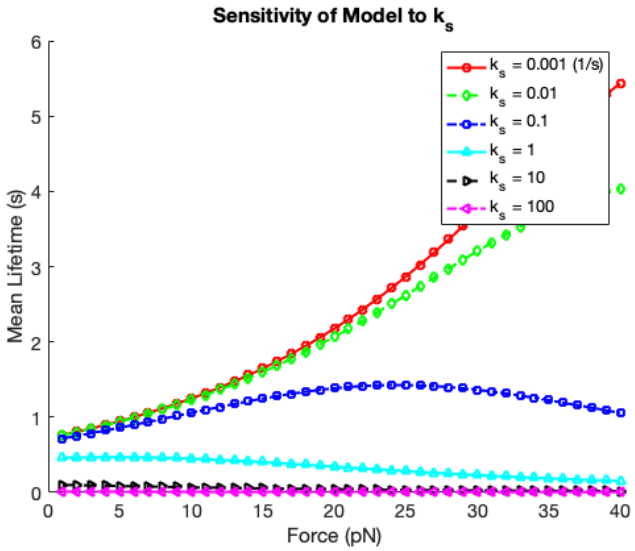


Figure 8: Sensitivity of the model to variations in magnitude of the zero force slip pathway unbinding rate, k_s. The model was plotted using the physiologically relevant parameters in **table 4** and high site density initial conditions**.**

Changes in k_c were similarly applied over a span from 0.001 [1/s] to 100 [1/s] and the response of the model plotted (**figure 9**). At the lowest values of k_c the model showed unbinding exclusively in a slip pattern where within the range of applied forces an increase in force meant a subsequent decrease in mean lifetime. As k_c increased the severity of the slip behavior decreased until $k_c = 1$ where the model demonstrated catch behavior up to the critical force and slip behavior after the critical force. At the highest parameter values the catch unbinding pathway was so heavily weighted that there is little to no visible mean lifetime for the bond cluster. This behavior verifies our model because a proportional increase of catch behavior in the model would be expected with an increase in k_c.

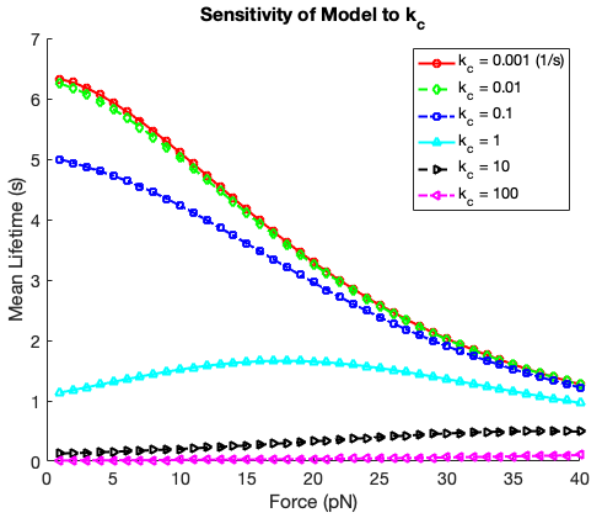


Figure 9: Sensitivity of the model to variations in magnitude of the zero force catch pathway unbinding rate, k_c. The model was plotted using the physiologically relevant parameters in **table 4** and high site density initial conditions**.**

Plotting the changes in the model as ξ from 1E-11 [m] to 50E-11 [m] shows that variations in ξ cause change in the critical force of the model (**figure 10**). As the value of ξ increases the critical force decreases and the transition from catch to slip behavior happens at lower forces. This makes sense within the context of the model as ξ and force are part of a ratio within an exponent: $\frac{f\xi}{TK_B}$. With a higher value of ξ the ratio will reach the critical point for transition between catch and slip behavior at a lower force. Overall sensitivity analysis verified the known components of our model, demonstrating that the parameters are functioning as expected and has been demonstrated in previous work (Pereverez et. al., 2005).

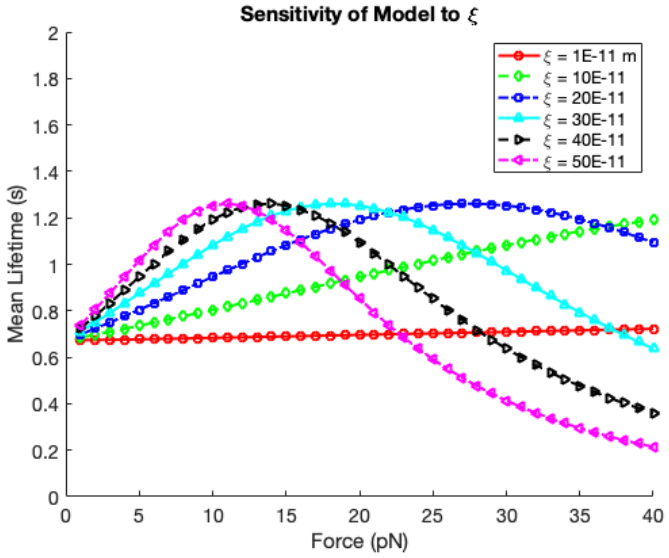


Figure 10: Sensitivity of the model to variations in magnitude of the combined unbinding length, ξ. The model was plotted using the physiologically relevant parameters in **table 4** and high site density initial conditions**.**

Acquiring Experimental Data

Using the digitize 2 function provided by Dr. Thomas, we were able to acquire experimental data directly from graphs produced by Snook and Guilford.

RESULTS

This model aims to demonstrate that a cluster of catch bonds between E-Selectin and sialyl Lewis^a (sLe^a) can exhibit ideal behavior when the bonds are modeled as linear springs and are allowed to rebind. We subject the cluster to a uniformly distributed force, and assume that the magnetic beads in the experimental work by Snook and Guilford were much larger than the ligand-receptor pairs themselves. The bonds are all assumed to be formed in the middle of the sphere without subjecting them to the force variations caused by the bead's curvature.

The results of this model were produced by assuming bond clusters with a high density of ligands and low density of ligands on the binding surface. Therefore multiple bonds in a cluster and single bonds were modeled. The parameters we used in our model were obtained by fitting the parameters $\boldsymbol{\kappa}$, ξ, k_s, k_c, and K0r to the experimental data obtained by Snook and Guilford for single

bonds and clusters of bonds simultaneously (Snook and Guilford, 2012). The parameters shown in **Table 4** were estimated through rigorous optimization of the ODE model described in the methods for the bond probability of the modeled system. Initial guesses of these parameters were made off of physiologically relevant values described by Sun et al., Perverazev et al. and Whitefeild et al. Then the model parameters were fitted to the experimental data of a high density cluster and a low density cluster, as described by Snook and Guilford, by minimizing the following objective function J,

$$J = \Sigma\left(\frac{(data_{HD} - fitted_{HD})^2}{\sigma_{HD}} + \frac{(data_{LD} - fitted_{LD})^2}{\sigma_{LD}}\right)$$

The best parameters were determined by minimizing J through the use of MATLAB's built in fminsearch function with an error model of 1 for both σ_{HD} and σ_{LD} (Sun et al., 2012, Perverazev et al. 2005, Whitefeild et al., 2010) where HD stands for high density and LD for low density. The lowest objective function that was observed after using different initial guesses for the parameters described in **Table 4** was 0.8906. This is of importance because a low J value demonstrated that the best fit of the model to the data was achieved. Therefore, we based our estimated parameter off of this calculation.

Optimal Parameters					
κ (N/m)	**ξ (m)**	**k_s (s^{-1})**	**k_c (s^{-1})**	**K0r (s^{-1})**	**Characteristic Length (m)**
2.4906E-4	2.46E-10	1.29E-2	1.698	1.85E-3	9.0E-09

Table 4: Estimation of physiologically relevant parameters through rigorous optimization of Equations 1 with the high bond density and low bonds density data simultaneously

After the 6 optimized parameters were obtained, they were used to create a plot of our modeled system and determine the behavior of a seven bond cluster given the assumptions we made. The comparison between our model for a bond cluster and the experimental data show that our model does not predict the observed experimental behavior (**figure 11**). The model predicts that E-Selectin bonds in a cluster will exhibit catch behavior at low forces that transitions into slip behavior at high forces. In contrast, the experimental data shows that E-Selectin bonds in a cluster exhibit ideal behavior where the lifetimes stay constant at approximately 1.3s. This behavior was observed for all of the experimental conditions in the experimental data except an outlier that was observed at 18pN of force where the lifetime increases showing that the bonds might be exhibiting catch bond behavior. These results fail to validate the model's ability to characterize the experimental conditions used by Snook and Guilford. In high density conditions our depicts catch bond behavior for the bond cluster and not the ideal behavior seen by Snook and Guilford (Snook and Guilford, 2012). This failure in validation is highly significant because it reveals that the assumptions made by the model cannot explain why ideal bond behavior was observed experimentally, leaving these results unexplained.

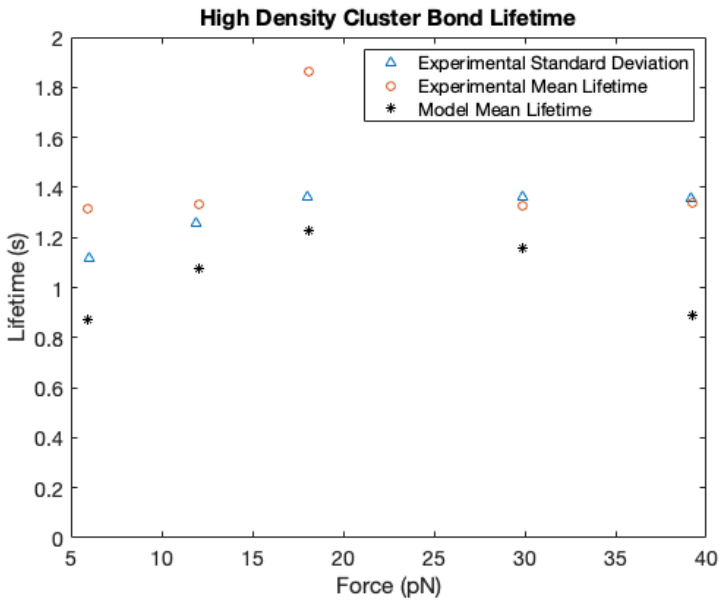


Figure 11: *Mean lifetime of a bond cluster with high density initial conditions. The model was fit to both low density mean lifetimes and high density mean lifetimes.*

These results contradict our hypothesis that a catch bond cluster would present ideal behavior when the individual bonds were treated as a linear spring and able to rebind after rupturing. It was predicted that the rebinding rate of each individual receptor-ligand pair would cause some portion of the pairs on the magnetic bead and ligand-coated surface to remain bound long enough for other pairs to rebind. This would give the cluster a lifetime independent of tensile force because a dynamic between rebinding rates and unbinding rate would occur. However, the results show that assuming that the bonds are linear springs and rebind can occur will cause the system to show catch behavior. This means that rebinding of ruptured bonds is not enough to keep the cluster in place to allow an equal balance of unbinding and rebinding of bonds at different tensile forces.

The same parameters from **Table 4** were used in our model to create a plot of the behavior of a low catch bond density system. The modeled system demonstrates the behavior of a single catch bond (**Figure 12).** These results show that when our model assumed a cluster with low E-selectin receptor density, catch bond behavior was exhibited. Similarly, the experimental data demonstrates that a single E-selectin receptor bound to a sLe^a ligand will exhibit catch behavior as greater tensile force is applied to it (**Figure 12),** validating our model for single bond conditions**.** Based on this comparison, the unbinding assumptions used in our model were appropriate to a capture the catch behavior shown in the low density experiments done by Snook and Gulliford even with the addition of force dependent rebind rates to **Equations 1.**

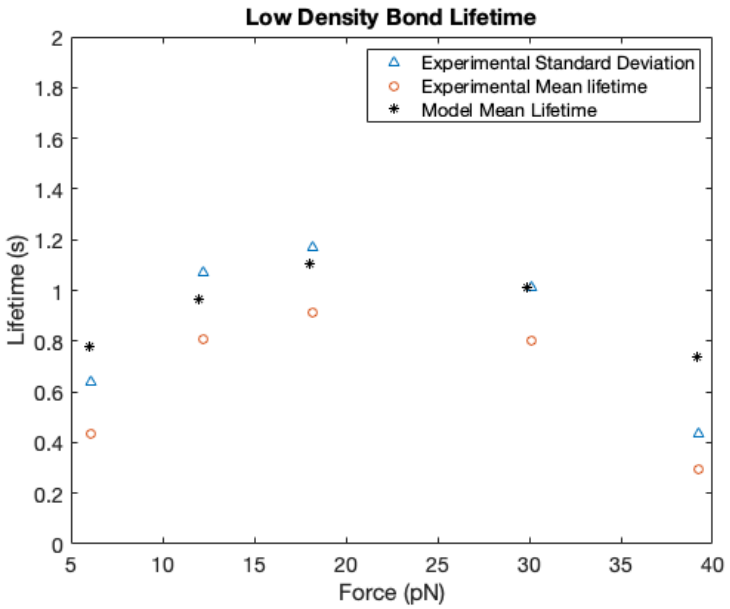


Figure 12: *Mean lifetime of a bond cluster with low density initial conditions. The model was fit to both low density mean lifetimes and high density mean lifetimes.*

This result is physiologically important because in most biological processes the number of receptor ligand pairs is variable but usually more than one bond is present especially in platelet adhesion which is the system that we are modeling. Additionally, this model predicts that the unbinding rate for a single bond can go down either a catch bond pathway or a slip bond pathway. At lower forces, the catch bond pathway is dominant when the tensile force is applied to the system because a conformational change occurs between the E-selectin receptors and sLe^a ligands allowing for longer bond lifetimes. This holds true until a critical force (**figure 12**), after which the slip pathway dominates because the bond remains in the same conformation as when it initially binds together thus this action is easily reversed.

Other analysis of our model included estimating the parameters by minimizing an objective function that just took into account the high density data from the Snook and Guilford (Snook and Guilford, 2012). This was done to ensure all ranges of parameters were analyzed and that the parameters found previously in **Table 4** were not obtained as a result of a local minima. After new parameters were obtained (**Appendix Table 2**), these parameters were used in our model to create the results seen in **figure 13.** While the fit demonstrated ideal behavior, there were many drawbacks to this particular fit. When these parameters were utilized to characterize single bond conditions it showed that the model remained force insensitive contradicting the known behavior for a single catch bond (**figure 14**) (Snook and Guilford, 2012). Therefore, it was concluded that taking only high density data into account creates a physiologically incorrect model. Therefore, it was established that it is important to fit the model to both high and low density data simultaneously to get parameters that are physiologically relevant to the system we are modeling.

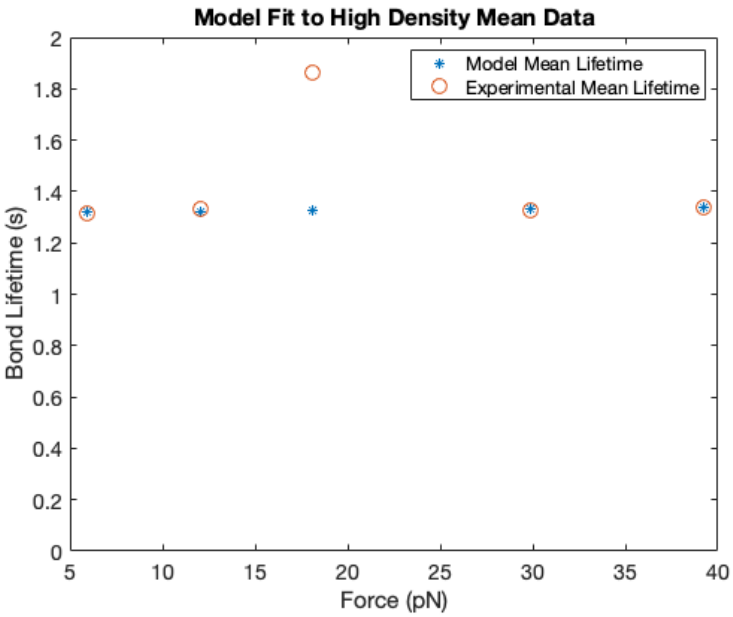


Figure 13: Model fit to only high density mean lifetime data with the parameters in **Appendix: Table 2** and high site density initial conditions. Model is plotted with the high site density experimental results.

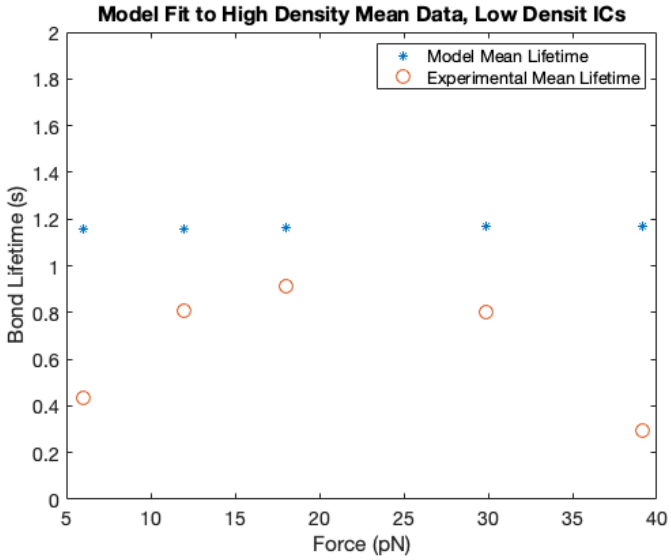


Figure 14: Model fit to only high density mean lifetime data with the parameters in **Appendix: Table 2** and low site density initial conditions. Model is plotted with the low site density experimental results.

After this change the mean lifetime for each simulated bond cluster increased. However even with this assumption included, the model was still unable to describe the experimental data for multiple bonds. Nevertheless, this change increased the validity of the model as it ensured that the calculations for mean lifetime consisted solely of values that were detectable through Snook and Guilford's bond cluster imaging system (Snook and Guilford, 2012).

Snook and Guilford also made the assumption that the time it took for the bead to meet the surface or move away from the surface was not influenced by hydrodynamic interactions (Snook and

Guilford, 2012). Hydrodynamic interactions would slow down the departure of the bead from and to the ligand coated surface. The model implemented these assumptions through a symmetric loading rate at the start of each trial, which implied, not taking into account the time the bead took to reach the surface. Furthermore, the on and off rates used in the model do not take into account electrostatic forces and are only dependent on applied force. With these assumptions modeled, the model showed catch behavior for a single catch bond and multiple catch bonds.

As shown in **Figure 15**, two residual plots are plotted one for the low density model and another for the high density model. The sum of the two residuals plots is close to zero; this demonstrates a normal distribution, indicating that our fitting method has no bias.

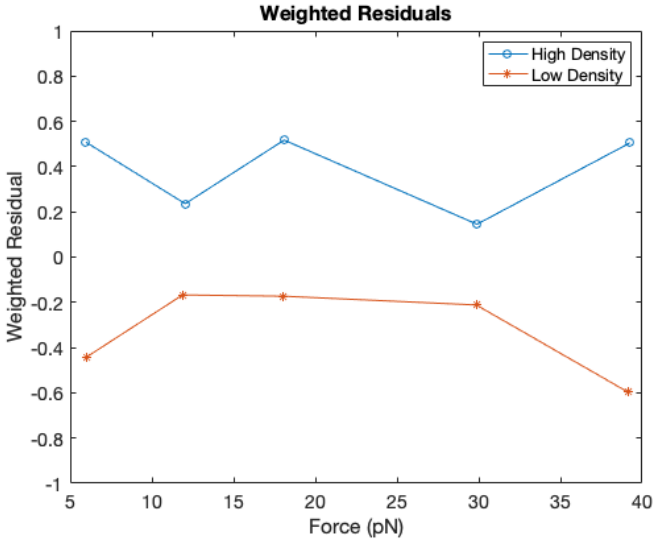


Figure 15: Weighted residual plots of the high density model and the low density model. The normal distribution of the combined residuals around zero demonstrates that there was no bias in our fitting methods.

Sensitivity analysis on high density clusters

To assess the effect that variation in rebinding parameters has on our fit model, we performed sensitivity analysis for each relevant parameter. These graphs were used to analyze the physiological implications of the new rebinding parameters. To determine the impact of κ, the spring constant, on the behavior of the model we plotted the models response to κ at values ranging from 0.001 [N/m] to 100 [N/m]. Over this spectrum, the model was insensitive to variation in κ (**figure 16**). This implies that biologically the ability of a receptor-ligand pair to rebind is independent of the stiffness of the remaining bonds, and that either rebinding depends more on other parameters or a linear spring is an inappropriate approximation of the receptor-ligand pairs.

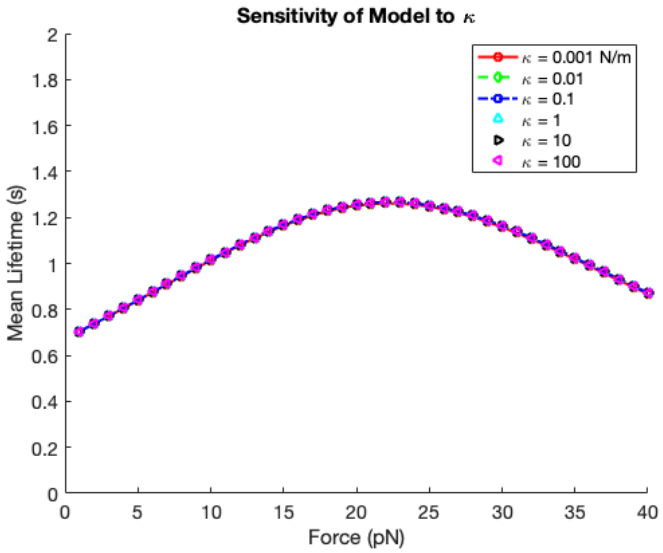


Figure 16: Sensitivity of the model to variations in magnitude of the spring constant, κ. The model was plotted using the physiologically relevant parameters in **table 4** and high site density initial conditions**.**

Further analysis of the rebinding rate involved varying k0r, the zero force rebinding rate, over a span of 0.001 [1/s] to 1000 [1/s] and plotting the behavioral response of the system (**figure 17**). At lower values of k0r there was no visible effect on the model's response to force, however as k0r increased the model's response varied greatly at lower tensile forces. While the exact pattern of the effect of k0r variation is unclear, this variation accounts for the force sensitivity of the rebinding rate because k0r is the only rebinding parameter that affects the rebinding rate, (**figure 18**). The biological significance of this sensitivity is that the ability of receptor ligand pairs to rebind depends on the reaction kinetics of the binding pathway and consequently the bond affinity far more than it does on the mechanical properties of the bond.

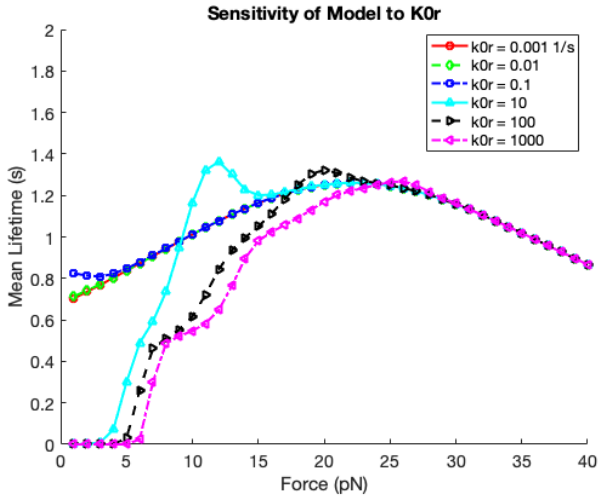


Figure 17: Sensitivity of the model to variations in magnitude of the zero force rebinding rate K0r. The model was plotted using the physiologically relevant parameters in **table 4** and high site density initial conditions**.**

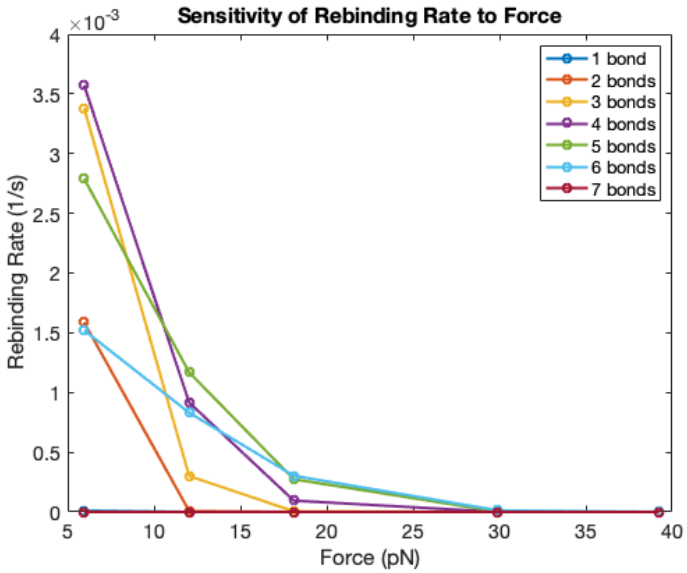


Figure 18: Force sensitivity of the kon rates broken down by bond number. The model was plotted using the physiologically relevant parameters in **table 4** and high site density initial conditions**.**

DISCUSSION

Based on all the model assumptions we made and the resulting verification graphs (**figures 5-10**), we are certain in our model's conceptual and mathematical strength. Our model is comparable to other theoretical models in our field, notably the previous models we referred to in our background section. We drew heavily from these studies, and created our model through the combination of various theories put forth in these studies. Specifically, we used the one-state two-pathway model for the unbinding rates from Pereverzev et al. and combined it with the rebinding rate theorized in Sun et al. to create a unique model of catch bond cluster behavior. These steps give us a high level of certainty in the theoretical aspect of our model. However, the model was not able to pass the validation thus giving us a medium level of certainty that our model is valid at an experimental level.

The significance of our work is that assumptions about rebinding rates and tether elasticity are not enough to cause a cluster of E-selectin bonds to exhibit "ideal" behavior as observed experimentally. Therefore a one state two unbinding pathway model with unbinding rates and rebinding rates dependent on the tensile force used to pull on individual bonds is not an appropriate model to explain the mechanical properties of E-selectin bonds that were displayed in experiments by Snook and Guilford. Incorporating these unbinding pathways into the model may have had different effects on the bond clusters than what was observed experimentally, explaining conflicting observed results. It is possible that as the tensile force, applied to each bond, increased more of the single bonds in the cluster followed the slip pathway which would result in a smaller bond cluster lifetime.These contradictions between the theory and experimental work calls for more work to be done on catch bonds clusters. Future work could include using a magnetic puller and magnetic bead similar to Snook and Guilford to show whether ideal bond behavior can be replicated experimentally because currently their paper is the only one that shows

the ideal behavior. We could also try to produce new data that validates the behavior observed by our model.

In contrast to previous experimental work cotraductions, our results support the catch bond cluster behavior demonstrated by the analytical study of Novikova et al., which found that a cluster of catch bonds exhibits catch behavior when ruptured bonds are allowed to rebind. Taking these two models into account it is possible that a cluster of catch bonds will exhibit catch behavior regardless of the assumptions made about the elasticity and rebinding rates of the bonds, due to the catch bond nature of the individual bonds. This discovery is important because physiologically, clusters of bonds are more relevant than single bonds, thus behavior of catch bonds produced by our model can be used to develop targeted drug treatments or to create new biological adhesives for processes that involve catch bonds. For example, this knowledge can be applied to studies about leukocytes and their role in repairing damaged blood vessels in the coagulation processes. Therefore our model's ability to explain the behavior of catch bonds in a cluster will open the field for other researchers to develop a better molecular understanding of hemostasis and other biological processes that involve catch bonds.

Going forward we would want to try and add complexity to our model to make it more relevant to the experiments by Snook and Guilford and other biological processes. The main addition would be to create asymmetrical loading of the tensile force on the receptor-ligand configuration to make a more physiologically accurate model. Also taking into account the different geometries present of ligand and receptor surfaces. The first geometry that would be significant would be when the ligands are on a microsphere as is the case in the experiment by Snook and Guilford. This could be possible through the adaptation of our model to incorporate the varying distances and angles of binding that would occur for different receptor-ligand pairs depending on the site on the bead and its vicinity to the ligand surface. The effect of the distance and angle could be incorporated into the off and on rates for the receptor-ligand pairs because the different positions would cause unique forces being applied to the different receptor-ligand pairs which in turn would change the rate at which they rebind and unbind in each position. This addition would be a very important step forward with the model to more accurately validate if ideal behavior is possible for clusters on a bead since the geometry of the bead can potentially play a large role in the behavior.

References

D. A. Hammer and M. Tirrell, “Biological Adhesion at Interfaces,” Annual Review of Materials Science, vol. 26, no. 1, pp. 651–691, 1996, doi: 10.1146/annurev.ms.26.080196.003251.

Feller, W. (1940). On the Integro-Differential Equations of Purely Discontinuous Markoff Processes. Transactions of the American Mathematical Society, 48(3), 488–515. JSTOR. https://doi.org/10.2307/1990095

Lou, Jizhong et al. “Flow-enhanced adhesion regulated by a selectin interdomain hinge.” *The Journal of cell biology* vol. 174,7 (2006): 1107-17. doi:10.1083/jcb.200606056

Novikova, E.A. and C. Storm, *Contractile fibers and catch-bond clusters: a biological force sensor?* Biophys J, 2013. 105(6): p. 1336-45.

Pereverzev, Y., et al., *The Two-Pathway Model for the Catch-Slip Transition in Biological Adhesion.* Biophys J, 2005. 89(3): p. 1446-1454.

Robbins SL, Cotran RS, Kumar V, Collins T (1999). *Robbins pathologic basis of disease*. Philadelphia: WB Saunders. ISBN 0-7216-7335-X.

Snook, J.H. and W.H. Guilford, *A High-Throughput Technique Reveals the Load- and Site Density-Dependent Kinetics of E-selectin.* Cellular and Molecular Bioengineering, 2012.5(4): p. 493-503.

Sun, L., et al., *Effect of loading conditions on the dissociation behaviour of catch bond clusters.* J R Soc Interface, 2012. 9(70): p. 928-37.

Thomas, W. (n.d.). *Catch Bonds in Adhesion.* Annual Review of Biomedical Engineering *10*(1), 39-57.

Whitfield, M., Ghose, T., & Thomas, W. (2010). Shear-Stabilized Rolling Behavior of E. coli Examined with Simulations. *Biophysical Journal, 99*(8), 2470-2478.

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APPENDIX

Force (N)	Minimum Detectable Bond Lifetime (ms)
5.9519E-12	0.0428
12.0350E-12	0.0211
18.0890E-12	0.0141
29.8906E-12	0.0085
39.2560E-12	0.0065

Table 1. Minimum bond lifetime that was detected using the imaging system in previous research at a given tensile force.

Optimal Parameters for the Model Fit to High Density Data					
κ (N/m)	**ξ (m)**	**Ks (s^{-1})**	**Kc (s^{-1})**	**K0r (s^{-1})**	**Characteristic Length (m)**
-2.17E-05	1.768E-12	8.5935E-2	0.93917	6.6954	1.49E-08

Table 2. Estimation of physiologically relevant parameters through rigorous optimization of high density cluster experimental data

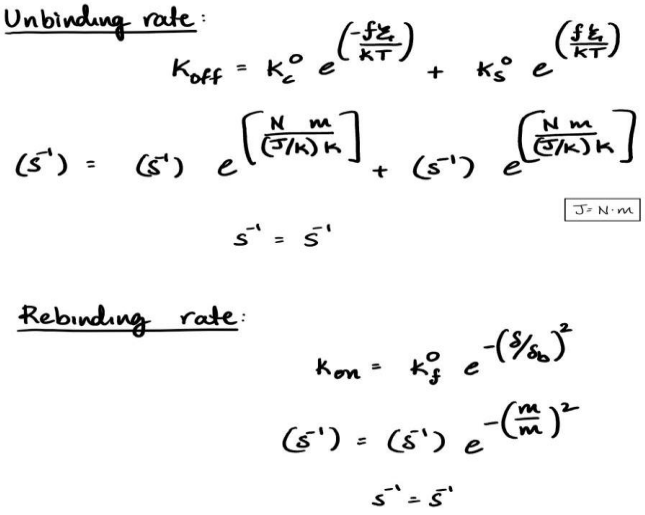

Figure 1: Dimensional Analysis of Unbinding and Rebinding Rates