Force-Dependent Adhesion of Malaria-Infected Red Blood Cells with ICAM-1 and CD36

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1 Introduction

1.1 Background

Patients of malaria show abnormally high levels of infected red blood cell (iRBC) adhesion to the membrane proteins expressed in the endothelial vessels. This is one of the main causes that lead to severe disease pathology in malarial patients like multiple organ failure and cerebral malaria.[1] In this study, the authors have taken a biophysical approach to study the interactions between iRBCs and specific endothelial receptor proteins - CD36 (cluster of differentiation 36) and ICAM-1 (intercellular adhesion molecule 1). The reason behind choosing these two proteins was that these two proteins have been found to be present in high amounts in the patients' vasculature system.[2] Also, upregulated levels of ICAM-1 in brain microvasculature are found to be correlated with cases of cerebral malaria. [2]

1.2 Experimental Setup

In this study, the researchers have studied the protein-ligand interaction systems in two different regimes - single molecule and single cell levels. At the single molecule level, Atomic Force Microscopy (AFM) experiments were performed, where the AFM tip, attached with ligand (CD36 or ICAM1) molecule, were brought in contact with single iRBC cell and the two molecules were made to bind to each other using a ramping force. After the ramping, a constant force is applied that causes protein-ligand bond to break after a certain time, known as bond lifetime.

At the single cell level, flow experiments were performed, where individual iRBC cells were flowed through artificial microchannels functionalized with the ligand protein (CD36 or ICAM-1). After the cell is attached to the ligand deposit, a flow of constant sheer stress is applied that translates to a constant force being applied on the cell. After a certain time period, the cell again detaches from the surface and gets washed with the flow.

1.3 Theoretical Models

Individual models are used to describe the 2 systems in 2 regimes, i.e. a total of 4 models. The interaction of a single iRBC-CD-36 bond is described with what is known as a slip model or Bell's model:

$$\tau_s^N = \frac{1}{k_s} e^{-\beta F x_0} \tag{1}$$

where τ_s is the mean lifetime of the bond, k_s is the dissociation rate, F is the constant force the system is pulled at, x_0 is the transition distance and, β is 1/(K_bT), K_b being Boltzmann's constant and T being the temperature of the system. On the other hand, the interaction of a single iRBC-ICAM-1 bond is described with what is known as a catch model:

$$\tau_c = \frac{1}{k_c} e^{-\beta (F - F_0)^2 \xi} \tag{2}$$

where now the stuff inside the exponential is described as a spring system, with ξ being the spring constant and F_0 being the offset equilibrium Force value.

At the single cell level, multiple bonds are formed between iRBC cell and the ligand deposit. The dynamics of detachment are then described as follows:

$$(\mathrm{N}) \underset{k_{\text{on}}}{\overset{k_{\text{off}}}{\rightleftharpoons}} (\mathrm{N}-1) \underset{k_{\text{on}}}{\overset{k_{\text{off}}}{\rightleftharpoons}} (\mathrm{N}-2) \underset{k_{\text{on}}}{\overset{k_{\text{off}}}{\rightleftharpoons}} (2) \underset{k_{\text{on}}}{\overset{k_{\text{off}}}{\rightleftharpoons}} (1) \xrightarrow{k_{\text{off}}} (0) \tag{3}$$

where each index (i) represents the state of number of bonds of the system, and once the system reaches state (0) it will be considered detach. So we want to estimate the mean lifetime of going from (N) to (0). A master equation type analysis results in the following formula:

$$\tau_s^N = \frac{1}{k_s} \times \frac{(N-1)!}{N} \times \left(\frac{k_{\text{on}}^s}{k_{\text{s}}}\right)^{N-1} \times \text{Exp}\left[-\beta F x_0 \sum_{i=1}^{N} \frac{1}{i}\right] \tag{4}$$

$$\tau_c^N = \frac{1}{k_c} \times \frac{(N-1)!}{N} \times \left(\frac{k_{\text{on}}^c}{k_{\text{c}}}\right)^{N-1} \times \text{Exp}\left[-\beta (F \sum_{i=1}^{N} \frac{1}{i} - F_0)^2 \xi\right] \tag{5}$$

Thus Eq(1), (2), (4) and (5) together describe the 4 models of our system (respectively) :

- Single bond - Slip
- Single bond - Catch
- Multiple Bonds - Slip
- Multiple Bonds - Catch

1.4 Questions Probed in this study and their significance

The goal of this study is to quantitatively describe the protein-ligand interaction strength in the 4 cases, by deriving the parameter estimates of the theoretical model. For example, if k_s is found to be significantly larger than k_c, we will know that the dissociation rates of the iRBC-CD36 are very high compared to ICAM-1 and thus will not complications to the extext that ICAM-1 can cause. By studying the catch bond of iRBC-ICAM-1, we can study the regime of forces where catch bond gets in action, i.e. the strength of the bond increases as the force is increased. As different microvasculature maintain different ranges of sheer stress, this can further help to study why the adhesion levels of iRBC-ICAM-1 in organs like brain are high. Thus, the results of this study can overall help us better understand the strategies employed by the parasite's infection to form multiple sites of adhesion in different organs of the host, and not get washed away in the flow.

2 Methods

2.1 Data and Distribution of Data

In this study, we asked the authors of the paper to get access to the data, and they were very kind to give us the access. The data they have shared with us comprises of 4 different datasets, each corresponding to one of the 4 different regimes described above. All the datasets, however, are in the form of Force vs Lifetime data, i.e. for a system being pulled at F force, how much does it take for the molecule or the cell to detach. For the AFM studies i.e. single molecule level, we have constant force values in pN and and the corresponding mean lifetime in seconds. For the flow experiment study, we have constant values of sheer stress and the lifetime values. We manually convert each sheer stress value to force before using it in Eq. (4) and (5), using the following formula derived using stokes law:

$$F = \frac{28.3\pi r^3}{\sigma abc}\text{Sheer Stress} \tag{6}$$

where r is the effective radius of the RBC cell and σabc is the effective number of bonds times the width of the rupture area.

For the single molecule study of slip bond, we first used two different distributions - Gaussian and exponential. An AIC analysis of the models with 2 different distributions, showed that exponential is much more preferable over Gaussian, see next section for results. Thus, for the rest of our analysis we assumed the data to be exponentially distributed. This is also supported by the fact that our data consists of events (detachment) with a constant average rate, i.e. probability of detachment is same at all times.

2.2 Methods of Analysis

To estimate model parameters, we performed an MLE analysis for each of the model with their corresponding dataset. We also verified that a model is the most supported for the corresponding dataset using AIC analysis. For example, verifying that the data for catch model actually requires the extra degree of freedom when determining its optimal fit. The optimal fit resulted in the parameter estimates with confidence interval. However, we ran into some problems in the case of fitting multiple bond model. First, since we wanted number of bonds N to be a positive integer, we had to separately run MLE analyses to find the optimal value of N. Second, since we had already found confidence intervals of single bond model parameters, we had to use those extremes to find the confidence interval of k_{on} (I will describe this in detail in the results section).

3 Results

3.1 Single Bond - CD36 Slip Bond

As shown in Table 1, we found that exponential distribution fits our data much better than Gaussian distribution. Thus, we have done all rest of the analysis after assuming exponential distribution.

Distribution - Slip Model	dAIC	DoF
Exponential Distribution	0.0	2
Gaussian Distribution	625.1	3

Table 1: AIC analysis of Exponential vs Gaussian Distribution models on single-slip data.

The model parameter estimates of Eq. (1), i.e. single molecule slip-bond study, are shown in Table 2. We have also generated confidence interval and the profiles of confidence intervals (see Fig. 3) where we assumed ch-square distribution and found quantiles for 0.025 and 0.975. The parameter values reported in the main paper [3] lie in our confidence interval range.

Parameter	Value reported in paper	Our Estimate	Our Confidence Interval (95%)
x_0 (nm)	0.0613	0.0564	0.0146 - 0.0974
k_s (s^{-1})	0.3095	0.3078	0.2666 - 0.3548

Table 2: Paramter Estimate of single molecule - slip model Eq(1). The table shows both our estimates and the estimates reported in the main paper [3]

Finally, to qualitatively observe the force-lifetime curves fitted with the model, see Fig. 1 and 2. Fig 1 Left shows all the data for single molecule slip bond study and the model overlaid over them. However, in the figure published in the main paper, they have done binning of force values as replicated in Fig.2. Therefore to see the similarity we also performed the binning and then estimated standard error of mean (SEM) corresponding to each bin as the error

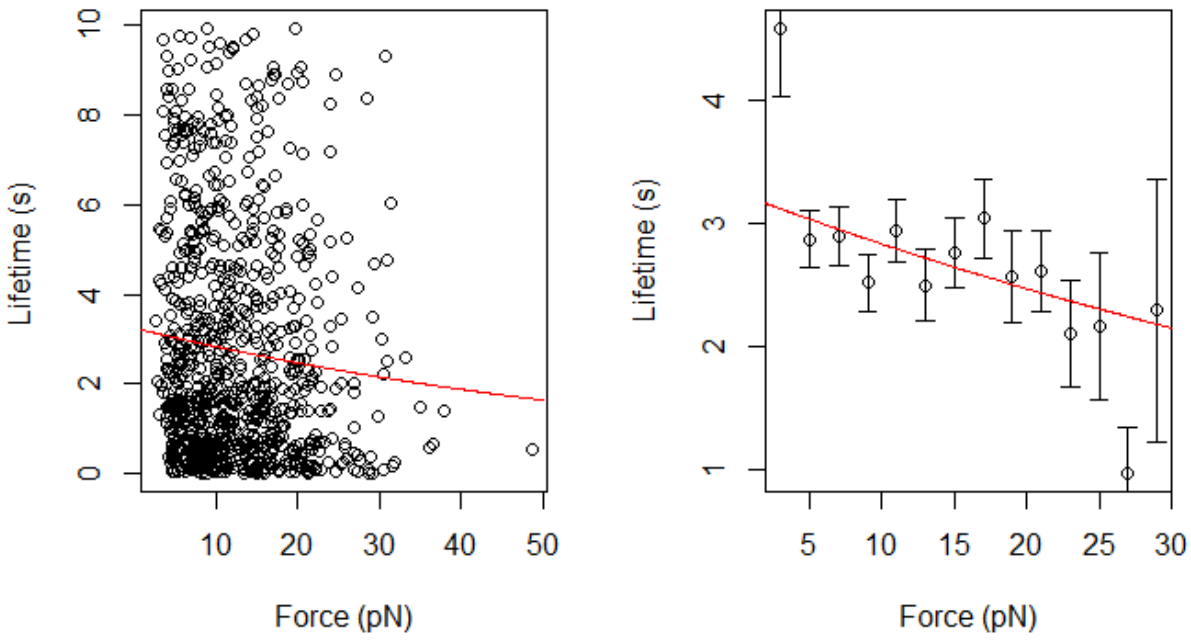

Figure 1: Single-Slip Model fitted on single slip data. Left: All data points are shown with the model fit overlaid as a red curve. Right: Binning of data is performed to be compared with the resulting figures of the original paper. See next Fig

bars in Fig. 1 Right.

3.2 Single Bond - ICAM-1 Catch Bond

A similar analysis has been performed for single molecule catch bond data. The parameter estimates of Eq (2) are shown in Table 3 with their 95 % confidence intervals. The profile of the confidence intervals for the three parameters k_c F_0 and ξ (written as 'ksi') are also shown in Fig. 6. The qualitative picture of the fitting are shown in Fig 4 and Fig 5. Just like last case, Fig 4 left shows all the data points and estimated model overlaid, while Fig 4 right shows the average taken after binning Force data. The error bars in Fig 4 right again indicate SEM corresponding to each force bin and can be compared to the original figure of the main paper, replicated in Fig 5. [3]

Parameter	Value reported in paper	Our Estimate	Our Confidence Interval (95%)
k_c (s^{-1})	0.3192	0.3169	0.2898 - 0.3458
F_0 (pN)	13.87	13.80	12.88 - 15.01
ξ (nm·pN^{-1})	0.0263	0.0276	0.0170 - 0.0379

Table 3: Paramter Estimate of single molecule - catch model Eq(2). The table shows both our estimates and the estimates reported in the main paper [3]

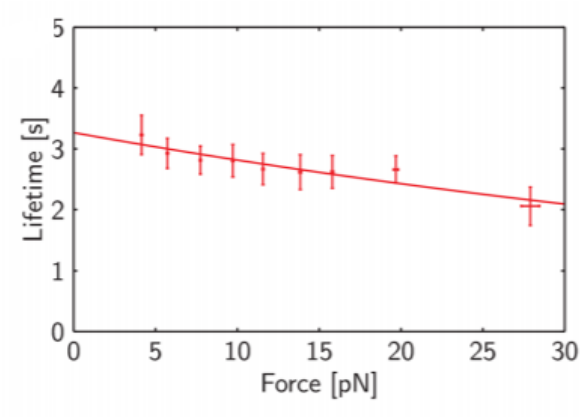

Figure 2: Single-Slip Model fitted on single slip data after binning Force data. Error bars indicate Average ± SEM. Taken from original paper [3]

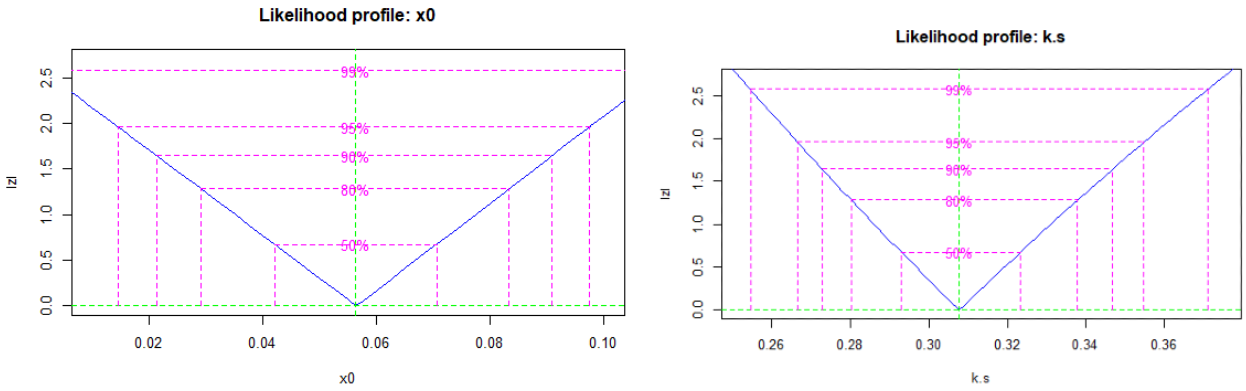

Figure 3: Confidence Interval profiles of single-slip model parameter estimates.

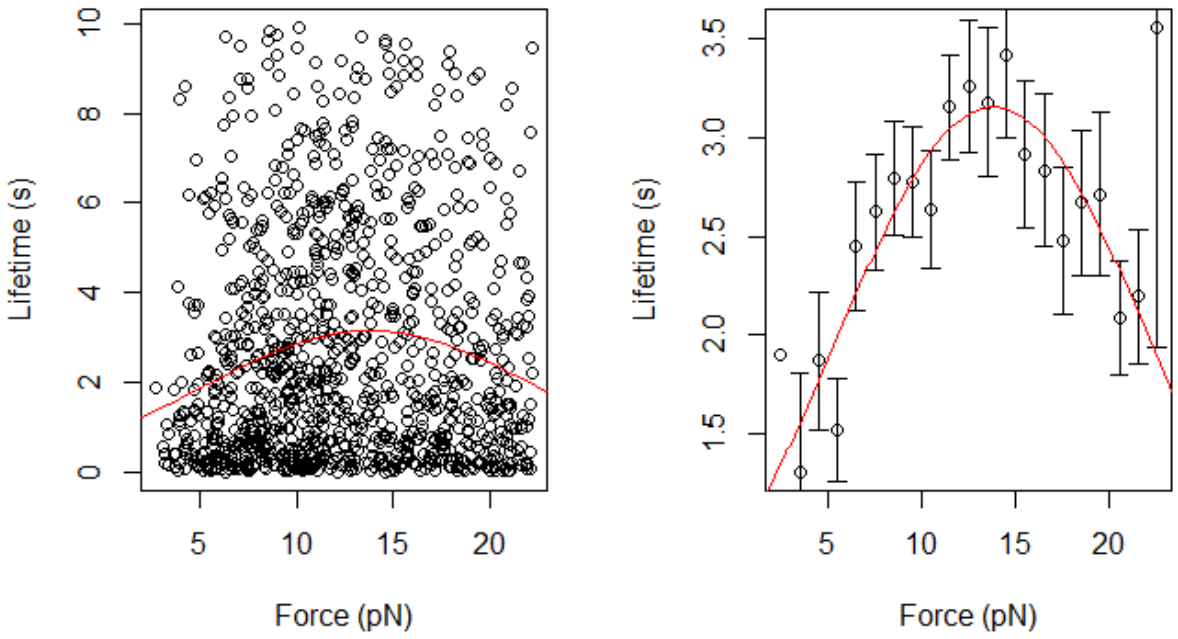

Figure 4: Single-Catch Model fitted on single catch data. Left: All data points are shown with the model fit overlaid as a red curve. Right: Binning of data is performed to be compared with the resulting figures of the original paper. See next Fig

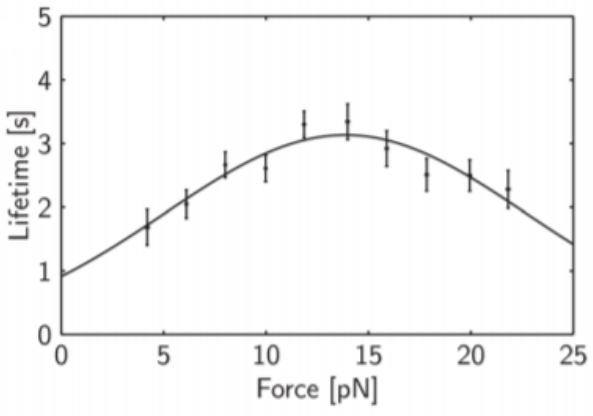

Figure 5: Single-Catch Model fitted on single slip data after binning Force data. Error bars indicate Average $\pm$ SEM. Taken from original paper [3]

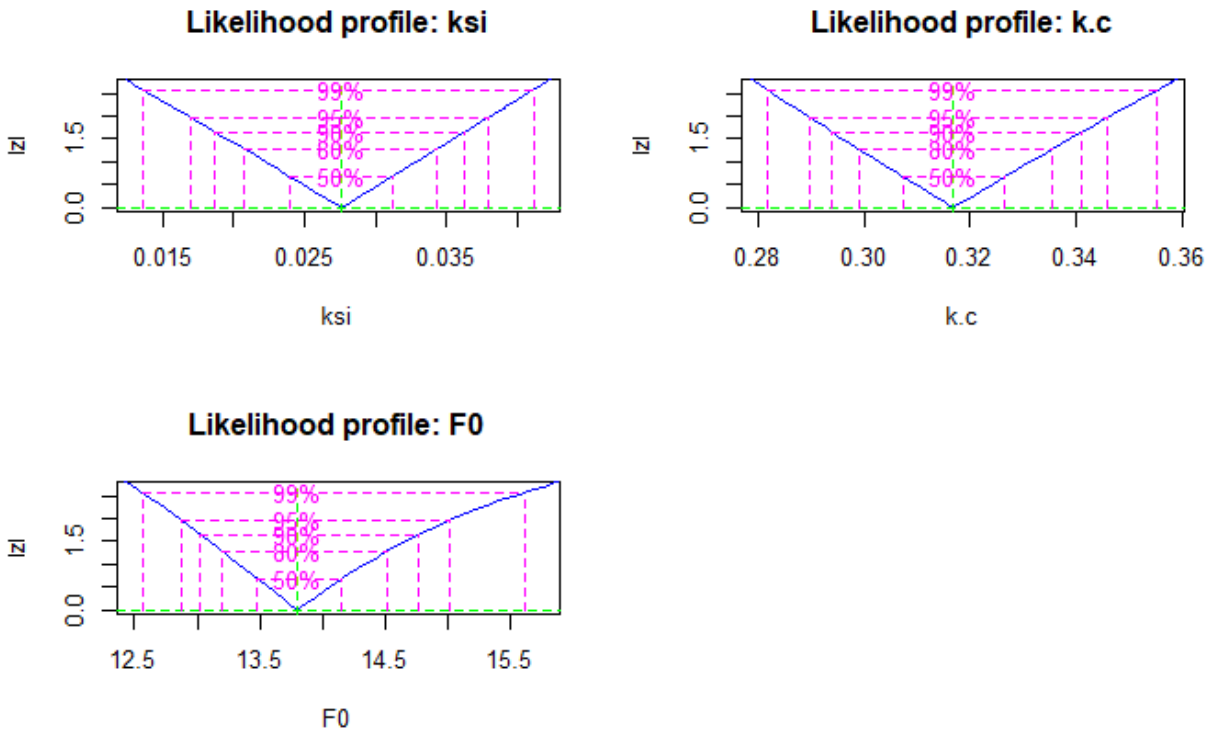

Figure 6: Confidence Interval profiles of single-catch model parameter estimates.

Finally, Table 4 shows that the extra degree of freedom is justified in using catch model with single bond ICAM-1 data. The dAIC of using a slip model to fit ICAM-1 data is 23.4, hence is much less supported relative to the catch model.

Model	dAIC	Dof
Slip data fit on Slip Bond model	0.0	2
Slip data fit on Catch Bond model	6.9	3
Catch data fit on Catch Bond model	0.0	3
Catch data fit on Slip Bond model	23.4	2

Table 4: AIC analysis to show whether the extra degree of freedom is justified when using catch model on catch data

3.3 Multiple bonds - CD36 slip bond

For the multiple bond cases, since we wanted to fix N to be an integer value, we manually created a for loop varying N fro 2 to 10 and determined to optimal N value. The result of the AIC analysis is shown in Table 5. As it can be seen from the Table 5, the optimally best fit occurs when N = 2. Our main paper has also reported N=2 as the number of bonds found in CD-36 slip data. We then estimated model parameters for Eq (4) and the result has been summarized in Table 6. We were not able to generate the automatic confidence interval profiles for the multiple bond cases as the

log-likelihood values remained the same even after a single parameter is varied, while keeping the other parameters same. However since 2 of the parameters are repeated, k_s and x_0, we know their confidence interval range from the single molecule study. To estimate the confidence interval for k_{on^s} we ran the MLE at the two extremes of the confidence interval ranges of k_s and x_0. The results are summarized in Table 6.

Model : value of N	dAIC	weight
2	0.0	0.8205
3	3.2	0.1628
4	7.9	0.0155
5	13.1	0.0012
>6	>18.3	<0.001

Table 5: AIC analysis that compares different multiple bond - slip model with different values of N>1

Parameter	Value reported in paper	Our Estimate	Our Confidence Interval (95%)
x_0 (nm)	0.0613	0.0555	0.0146 - 0.0974
k_s (s^{-1})	0.3095	0.3194	0.2666 - 0.3548
k_{on}^s (s^{-1})	1.22	1.22	0.055 - 53.4

Table 6: Paramter Estimate of multiple bonds - slip model Eq(4). The table shows both our estimates and the estimates reported in the main paper at N=2. [3]

The plots are shown in Fig 7 and 8 for multiple bond CD36 interactions. We kept N=2 and estimated the model curve fit which is shown in red in Fig 7. The left panel of Fig 7 shows the average data with SEM error bars, while the right panel shows all the data points with the model fit. To compare with the original plot, we have replicated the figure from main paper in Fig 8.

3.4 Multiple bonds - ICAM-1 catch bond

A similar analysis was repeated for single cell ICAM-1 case. Table 7 shows the AIC analysis for different N values. As it can be seen from Table 7, while N=6 gives the optimally best fit, N=7 has a dAIC =0.9 with N=7, hence can not be rejected. The paper reports N=7 with no explanation for rejecting N=6. Hence our results show discrepancy with the original paper. In the further analysis, we have used N=6, while the corresponding results of the main paper have used N=7. The parameter values of the model fit of Eq.(5) are shown in Table 8 with their confidence intervals. Fig 9 and 10 show the plot for multiple bonds iRBC-ICAM-1 interaction.

3.5 Highlighting biological conclusions inferred from the results

One of the main results is that the rebinding rate k_{on^s} for ICAM-1 is about 6-8 times higher than k_{on^c} for CD-36, as shown in Table 6 and Table 8. This result may potentially be able to explain the correlation of upregulation of only ICAM-1 and not CD-36 in cases of cerebral malaria. This higher adhesion levels of iRBCs to ICAM-1 can further be

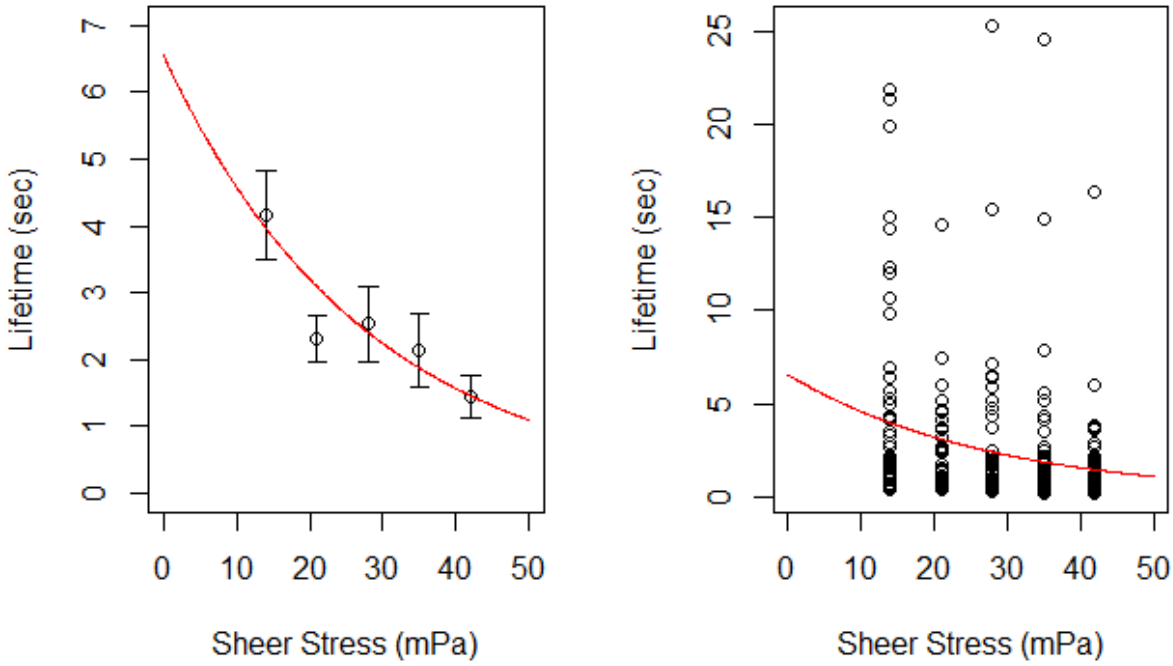

Figure 7: Multiple-Slip Model fitted on multiple bonds slip data, when N=2. Left: Average of the data is fitted with the model shown in red curve. The error bars indicate the SEM associated with each data point. Right: All data points are shown with the model fit overlaid as a red curve.

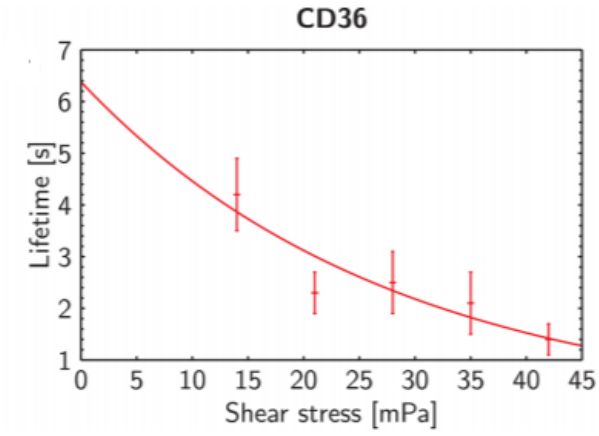

Figure 8: Multiple-Slip Model fitted on multiple bonds slip data. Error bars indicate Average $\pm$ SEM. Taken from original paper [3]

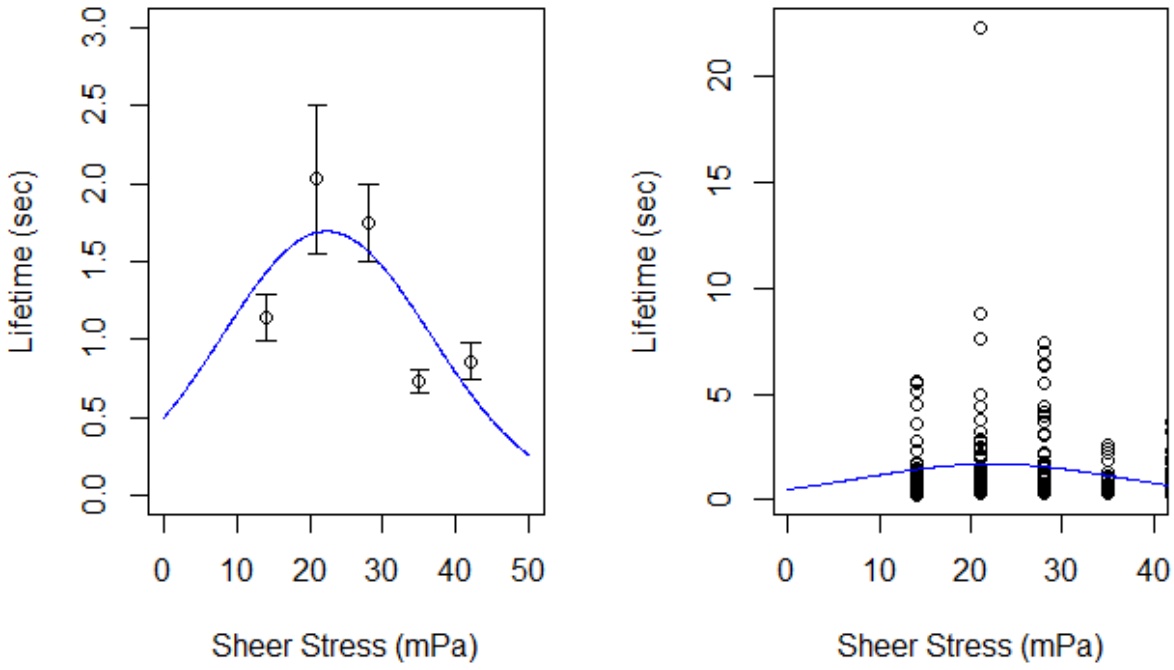

Figure 9: Multiple-Catch Model fitted on multiple bonds catch data, when N=6. Left: Average of the data is fitted with the model shown in blue curve. The error bars indicate the SEM associated with each data point. Right: All data points are shown with the model fit overlaid as a blue curve.

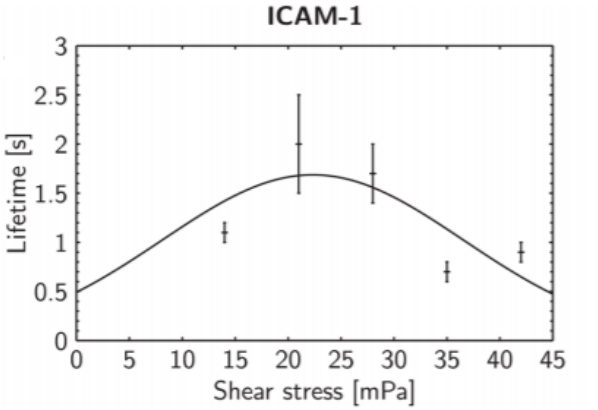

Figure 10: Multiple-Catch Model fitted on multiple bonds catch data. Error bars indicate Average $\pm$ SEM. Taken from original paper [3]

Model : value of N	dAIC	weight
6	0.0	0.5288
7	0.9	0.3377
5	3.3	0.1006
8	5.7	0.0304
4	11.0	0.0022
otherwise	>14	<0.001

Table 7: AIC analysis that compares different multiple bond - catch model with different values of N>1

Parameter	Value reported in paper	Our Estimate	Our Confidence Interval (95%)
k_c (s^{-1})	0.3192	0.4565	0.2898 - 0.5458
F_0 (pN)	13.87	13.87	12.88 - 15.01
ξ (nm·pN^{-1})	0.0263	0.0228	0.0170 - 0.0379
k_{on}^c (s^{-1})	0.133	0.201	0.019 - 1.75

Table 8: Paramter Estimate of multiple bomds - catch model Eq(5). The table shows both our estimates and the estimates reported in the main paper. [3]

supported using Fig 9, which show that the overall behavior of ICAM-1 in multiple bond remains catch-like. Thus the adhesion levels can further be supported by high sheer rates in the flow as compared to CD-36 slip bond in Fig 7, where increasing the force monotonically decrease the adhesion lifetimes of the cells. Thus, even though the dissociation rates k_s and k_c are almost similar in magnitude (see Table 6 and 8), the adhesion levels of ICAM-1 turn out to be much more sever as compared to those of CD-36 interactions.

4 Discussion

In this paper, we studied two different protein-ligand interactions: receptor proteins on iRBC cells with ICAM-1 and CD-36 molecules. Experimental research have shown a correlation between high adhesion rates of ICAM-1 and the cases of cerebral malaria. [2] However, they have found similar expression levels of other proteins like CD-36, CD-31, E-Selectin and V-Selectin, which are not found to be correlated with cerebral malaria. [2] Our results are in alignment with the experimental data as we have found that, even though the dissociation rates of ICAM-1 and CD-36 are similar to each other in magnitude, the catch-bond like behavior and a high re-association rates of ICAM-1 make its adhesion to iRBCs much more significant.

The method described in this paper is a very elegant techniques that is used to simultaneously study protein-ligand adhesive interactions at single-molecule and single-cell scale. The theoretical mapping from single bond to multiple bonds allow us to obtain parameter estimates of the model at different scales. Our parameter estimates and the values reported in the main paper all fall in our confidence interval ranges. Our graph fits are also in agreement with the main paper and show the enhanced adhesion of ICAM-1 with iRBCs in sheer flow.

References

[1] Turner, G. D. et al. (1994), An immunohistochemical study of the pathology of fatal malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. *The American journal of Pathology 145* (5) Pages 1057–1069.

[2] Newbold, C. et al. (1997), Receptor-specific adhesion and clinical disease in Plasmodium falciparum *The American Society of Tropical Medicine and Hygiene 57* Pages 389–398.

[3] Lim Y. B., Thingna J., Cao J. & Lim C. T. (2017), Single molecule and multiple bond characterization of catch bond associated cytoadhesion in malaria *Scientific Reports 7* (1).