Supplementary Information for Estimating the probability of polyreactive antibodies 4E10 and 2F5 disabling a gp41 trimer after T cell-HIV adhesion

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Supplementary Information

SI1: Relation of measured rate constants to rate constants used in the model

In Figure 1 three binding schemes are shown. It is important to know how the rate constants in the three panels are related to the rate constants determined from SPR binding experiments. SPR data for Fab binding to HIV-1(Fig. 1 in Alam et al. \cite{10}) shows a very rapid off rate constant indicating that the binding from solution to the lipid membrane rapidly equilibrates. The transport of the bound Fab to the epitope should also be very rapid since once an Fab is bound to a lipid binding site it should diffuse with a diffusion coefficient that is comparable to that of the lipid \((10^{-8} - 10^{-9}) \text{ cm}^2/\text{s})\). Such rapid transport will result in the Fab-epitope binding being reaction limited. Making these two quasi-equilibrium approximations we can write the chemical rate equation for binding steps 1-3 as follows:

\[
\frac{dE_1}{dt} = (K_L A)(K_D)3\kappa_{+1}E_0 - \kappa_{-1}E_1 \tag{S1}
\]

where \(K_D = k_+/k_-\) is the diffusion limited equilibrium constant.

Noting that \(A_1 = K_L L A\), the above equation becomes

\[
\frac{dE_1}{dt} = 3K_L L K_D(\kappa_{+1})E_0 A - \kappa_{-1}E_1 \tag{S2}
\]

We also have that

\[
\frac{dE_1}{dt} = 3k_{+e}AE_0 - k_{-e}E_1 \tag{S3}
\]

In the experiments of Alam et al. \cite{4} \(k_{+e}\) is determined for Fab binding to peptide-liposome conjugates. The peptides on the liposome surfaces are not in the form of trimers. Therefore \(k_{+e}\) is the rate constant for binding to a single peptide binding site. For this reason, in Eq. (S3), there is a factor of three in the expression for the forward rate for binding of an Fab to a spike.

Comparing Eqs. (S2) and (S3) we find

\[
k_{+e} = K_L L K_D \kappa_{+1} \tag{S4}
\]

Since \(L\) is the concentration of free lipid binding sites on HIV, in theory \(L\) changes with the concentration of Fab. The higher the Fab concentration in solution the more bound sites on the verion are taken up by Fab. However, we assume that the binding of Fab takes up a negligible fraction of lipid binding sites, i.e., we treat \(L\) as a constant equal to the total concentration of lipid binding sites. When we consider the binding of the antibodies 2F5 and 4E10 we make the same assumption.

We note that

\[
\tilde{k}_{+1} = K_D \kappa_{+1} \tag{S5}
\]

and therefore

\[
k_{+e} = \tilde{k}_{+1} K_L L \tag{S6}
\]
Because we are in the reaction limit we also have that

\[ k_{-e} = \kappa_{-1} = \bar{k}_{-1} \]  \hspace{1cm} (S7)

In the reaction limit diffusion away from the bound complex and dissociation from the lipid attachment are much faster than dissociation from the MPER.

**SI2: Solving equation 4-6**

To solve Eqs. (4)-(6) we will use properties of Laplace Transforms (LTs) that are listed below, taking the transform variable to be \( s \) and the LT of \( f(t) \) to be \( \tilde{f}(s) \). This can only be done because \( A \) is a constant which makes the set of ODEs linear.

\[
\begin{align*}
    f(t) & \rightarrow \tilde{f}(s) \\
    \frac{df(t)}{dt} & \rightarrow s\tilde{f}(s) - f(0) \\
    \int_0^t f(u)\,du & \rightarrow \frac{1}{s}\tilde{f}(s) \\
    \lim_{t \to \infty} f(t) & = \lim_{s \to 0} s\tilde{f}(s)
\end{align*}
\]  \hspace{1cm} (S8-S11)

Before solving the ODEs we note the following result which follows from Eq. (1) and the first, third and fourth properties of LT above.

\[
E_i(\infty) = \lambda \lim_{t \to \infty} \int_0^t E_0(t')dt' = \lambda \lim_{s \to 0} s\tilde{E}_0(s) = \lambda\tilde{E}_0(0)
\]  \hspace{1cm} (S12)

Therefore the probability of incapacitating an epitope is

\[
p_b = 1 - \frac{\lambda\tilde{E}_0(0)}{E_0(0)}
\]  \hspace{1cm} (S13)

Let us now return to our set of ODEs, Eqs. (4)-(6), and solve them for \( \tilde{E}_0(0) \). We start by taking LTs of the ODEs, noting that at \( t = 0 \), \( E_0 = \tilde{E}_0(0) \), \( E_1 = 0 \) and \( E_T = 0 \).

\[
\begin{align*}
    s\tilde{E}_0 - \tilde{E}_0(0) & = -(\lambda + 3k_{+e}A)\tilde{E}_0 + k_{-e}\tilde{E}_1 \\
    s\tilde{E}_1 & = 3k_{+e}A\tilde{E}_0 - (k_{-e} + \kappa_+d)\tilde{E}_1 + \kappa_-d\tilde{E}_T \\
    s\tilde{E}_T & = \kappa_+d\tilde{E}_1 - (\kappa_-d + d_T)\tilde{E}_T
\end{align*}
\]  \hspace{1cm} (S14-S16)

We next set \( s = 0 \) and solve these equations for \( \tilde{E}_0 \). We start by defining the following determinants.

\[
\det D_1 = 
\begin{vmatrix}
    -\tilde{E}_0(0) & k_{-e} & 0 \\
    0 & -(k_{-e} + \kappa_+) & \kappa_-d \\
    0 & \kappa_+d & -(\kappa_-d + d_T)
\end{vmatrix}
\]

\[
\det D = 
\begin{vmatrix}
    -\lambda + 3k_{+e}A & k_{-e} & 0 \\
    3k_{+e}A & -(k_{-e} + \kappa_+) & \kappa_-d \\
    0 & \kappa_+d & -(\kappa_-d + d_T)
\end{vmatrix}
\]

We now solve for \( \tilde{E}_0(0) \).

\[
\tilde{E}_0(0) = \frac{\det D_1}{\det D}
\]  \hspace{1cm} (S17)

\[
\tilde{E}_0(0)/E_0(0) = \frac{\kappa_-d(k_{-e} + d_T(k_{-e} + \kappa_+d))}{\lambda\kappa_-d(k_{-e} + d_T(\lambda k_{-e} + \kappa_+d(\lambda + 3k_{+e}A)))}
\]  \hspace{1cm} (S18)

With this result we can obtain the probability of disabling an epitope from Eq. (S13). Upon substitution and rearranging terms, Eqs. (7) and (8) are obtained.
SI3: Model equations and their solutions for any Fab concentration

Figure 2 shows the states that can arise when up to three Fabs can bind to a trivalent gp41. The reactions between the states and their rate constants are indicated in the figure as well. The equations that describe the model when \( d_1 = 0 \) are:

\[
\begin{align*}
\frac{dE_0}{dt} &= -(\lambda + 3\tilde{k}_{+1} A_1)E_0 + \tilde{k}_{-1} E_1 \\
\frac{dE_1}{dt} &= 3\tilde{k}_{+1} A_1 E_0 - (\tilde{k}_{-1} + \kappa_{+d} + 2\tilde{k}_{+1} A_1)E_1 + 2\tilde{k}_{-1} D_0 + \kappa_{-d} E_T \\
\frac{dE_T}{dt} &= \kappa_{+d} E_1 - (\kappa_{-d} + 2\tilde{k}_{+1} A_1 + d_T)E_T + \tilde{k}_{-1} D_1 \\
\frac{dD_0}{dt} &= 2\tilde{k}_{+1} A_1 E_1 - (2\tilde{k}_{-1} + 2\kappa_{+d} + \tilde{k}_{+1} A_1)D_0 + \kappa_{-d} D_1 + 3\tilde{k}_{-1} T_0 \\
\frac{dD_1}{dt} &= 2\tilde{k}_{+1} A_1 E_T + 2\kappa_{+d} D_0 - (\tilde{k}_{+1} A_1 + \tilde{k}_{-1} + \kappa_{-d} + \kappa_{+d} + d_T)D_1 + 2\kappa_{-d} D_2 + 2\tilde{k}_{-1} T_1 \\
\frac{dD_2}{dt} &= \kappa_{+d} D_1 - (\tilde{k}_{+1} A_1 + 3\kappa_{-d} + 2d_T)D_2 + \tilde{k}_{-1} T_2 \\
\frac{dT_0}{dt} &= \tilde{k}_{+1} A_1 D_0 - (3\tilde{k}_{-1} + 3\kappa_{+d})T_0 + \kappa_{-d} T_1 \\
\frac{dT_1}{dt} &= \tilde{k}_{+1} A_1 D_1 + 3\kappa_{+d} T_0 - (\kappa_{-d} + 2\tilde{k}_{-1} + 2\kappa_{+d} + d_T)T_1 + 2\kappa_{-d} T_2 \\
\frac{dT_2}{dt} &= \tilde{k}_{+1} A_1 D_2 + 2\kappa_{+d} T_1 - (\tilde{k}_{-1} + 3\kappa_{-d} + \kappa_{+d} + 2d_T)T_2 + 3\kappa_{-d} T_3 \\
\frac{dT_3}{dt} &= \kappa_{+d} T_2 - 3(\kappa_{-d} + d_T)T_3
\end{align*}
\]

We solve Eqs (S19)-(S28) in the limit as \( t \to \infty \) and \( d_T \to \infty \) as we previously did in SI2 for the three equations that described the model in the low concentration limit. We take LT of these ODEs, converting them to a set of algebraic equations, set the transform variable equal to zero (the \( t \to \infty \) limit), and solve these algebraic equations using Mathematica. We find that

\[
\tilde{E}_0/E_0(0) = \frac{(2\kappa_{+d} + 2\tilde{k}_{-1} + \kappa_{+d} (6\tilde{k}_{-1} + 5A_1\tilde{k}_1) + \kappa_{+d} (6\tilde{k}_{-1} + 5A_1\tilde{k}_1 + 2A_1^2\tilde{k}_2))}{2\kappa_{+d} + 2\tilde{k}_{-1} + \kappa_{+d} (6\tilde{k}_{-1} + 5A_1\tilde{k}_1 + 5A_1\tilde{k}_1 + 5A_1^2\tilde{k}_2 + 2A_1^2\tilde{k}_2)}
\]

The quantity \( \tilde{E}_0(0) \) has the following form

\[
\tilde{E}_0(0) = \frac{\gamma E_0(0)}{\gamma \lambda + \beta}
\]

where

\[
\begin{align*}
\gamma &= 2(\kappa_{+d} + \tilde{k}_{-1})^3 + 5(\tilde{k}_{+1} A_1)\kappa_{+d}(\kappa_{+d} + \tilde{k}_{-1}) + 2\kappa_{+d}(\tilde{k}_{+1} A_1)^2 \\
\beta &= 6\kappa_{+d}(\kappa_{+d} + \tilde{k}_{-1})^2(\tilde{k}_{+1} A_1) + 3\kappa_{+d}(5\kappa_{+d} + 4\tilde{k}_{-1})(\tilde{k}_{+1} A_1)^2 + 6\kappa_{+d}(\tilde{k}_{+1} A_1)^3
\end{align*}
\]

and

\[
p_b = 1 - \lambda \tilde{E}_0(0) = \frac{(\beta/\gamma)}{(\lambda + (\beta/\gamma))}
\]
We would like to know over what Fab concentration range Eqs. (7) and (10), that result from the simple three equation model, hold. To do this we expand $p_b$ in a power series in $(\bar{k}+1A_1)$ and see when the second term in the expansion becomes comparable to the first. When this is done we have that

$$p_b = \frac{3\kappa+d}{\kappa+d + k-1} \bar{k}+1A_1 \left(1 + \frac{2\bar{k}-1}{(\kappa+d + k-1)^2} \bar{k}+1 + o(\bar{k}+1A_1^2)\right)$$  \hspace{1cm} (S34)

We want to know when

$$1 \gg \frac{2\bar{k}-1}{(\kappa+d + k-1)^2} \bar{k}+1 = \frac{2\bar{k}-1}{(\kappa+d + k-1)^2} k_e A$$  \hspace{1cm} (S35)

or equivalently

$$A \ll \frac{(\kappa+d + k-1)^2}{2k-1k_e}$$  \hspace{1cm} (S36)

We have assumed that it takes only one Fab bound to an MPER to disable an epitope composed of three gp41. If this is not the case, and it takes two or three bound Fab to disable an epitope, it is straightforward to modify the model. For example, if the binding of two Fab is required, then $d_T$ should be removed from Eqs. (S21) and (S23). If the binding of three Fab are required then $d_T$ should be removed from Eqs. (S24), (S26) and (S27) and only be present in Eq. (S28). When two or three bound Fab are required to disable an gp41 trimer, at low Fab concentrations, $p_b$ will be proportional to $A^2$ and $A^3$ respectively.

**SI4: The model equations for IgG binding**

Figure 4 of the manuscript illustrates the surface reactions that polyreactive antibodies can undergo in the model. We assume that these antibodies cannot cross-link MPERS on either the same spike or on different spikes. However, the antibodies can bind bivalently in the ways shown in the figure. The model expands from three to five ODEs since there are now two new states, $E_T^1$ and $E_T^*$, that involve the binding of antibodies to MPERS. $E_T^*$ is the concentration of MPERS that have not undergone a conformational change and that have an antibody bound that has its second site bound to lipid. $E_T^*$ is the concentration of those MPERS that have undergone a conformational change and that have an antibody bound that has its second site bound to lipid. The set of ODEs now become

$$\frac{dE_0}{dt} = -\lambda E_0 - 3\bar{k}+1A_1E_0 - 6\bar{k}+1A_2E_0 - \bar{k}-1E_1 - \bar{k}-E_1^*$$  \hspace{1cm} (S37)

$$\frac{dE_1}{dt} = 3\bar{k}+1A_1E_0 - \bar{k}-1E_1 - (\bar{k}+2LE_1 - \bar{k}-2E_1^*) - \kappa+dE_1 + \kappa-dE_T - d_1E_1$$

$$\frac{dE_1^*}{dt} = 6\bar{k}+1A_2E_0 - \bar{k}-1E_1^* + (\bar{k}+2LE_1 - \bar{k}-2E_1^*) - \kappa+dE_1^* + \kappa-dE_T^* - d_1E_1^*$$

$$\frac{dE_T}{dt} = \kappa+dE_1 - \kappa-dE_T - (\bar{k}+2LE_T - \bar{k}-2E_T^*) - d_TE_T$$

$$\frac{dE_T^*}{dt} = \kappa+dE_1^* - \kappa-dE_T^* + (\bar{k}+2LE_T - \bar{k}-2E_T^*) - d_TE_T^*$$

However, we can reduce the number of equations back to three by adding equations two and three together and equations four and five together. Calling $\bar{E}_1 = E_1 + E_1^*$ and $\bar{E}_T = E_T + E_T^*$

$$\frac{dE_0}{dt} = -\lambda E_0 - 3\bar{k}+1(2A_2 + A_1)E_0 + \bar{k}-1\bar{E}_1$$  \hspace{1cm} (S38)

$$\frac{d\bar{E}_1}{dt} = 3\bar{k}+1(2A_2 + A_1)E_0 - \bar{k}-1\bar{E}_1 - \kappa+d\bar{E}_1 + \kappa-d\bar{E}_T - d_1\bar{E}_1$$  \hspace{1cm} (S39)

$$\frac{d\bar{E}_T}{dt} = \kappa+d\bar{E}_1 - \kappa-d\bar{E}_T - d_T\bar{E}_T$$  \hspace{1cm} (S40)

Note that the term in Eqs. (S38) and (S39)

$$3\bar{k}+1(2A_2 + A_1)E_0 = 3\bar{k}+1A_1(K_2L + 1)E_0 = 6\bar{k}+1K_L(K_2L + 1)AE_0$$  \hspace{1cm} (S41)
From the argument presented in S1, for the bivalent antibody
\[ 3k_{t+c}^{\text{IgG}} = 6k_{+1}KL(K_2L + 1) \] (S42)
SI5: Fitting methods used to determine $N$ and the IC$_{50}$ values

We fit four neutralization experiments simultaneously to determine the best fit values of $N$ and four different IC$_{50}$s. As described in the Methods section, the best fit values were obtained using a program (FUI) based on the Levenberg-Marquardt algorithm for solving nonlinear least squares problems. We found $N = 0.64 \pm 0.07$, where the error estimate is the 95% confidence interval obtained from 500 simulations using the bootstrap method [46]. Because the model we used to derive $p_b$ is only exact at low mAb concentrations we refit a reduced data set, dropping the two data points at the highest mAb concentrations from each of the five neutralization experiments. To two places the value we obtained for $N$ was the same. We also used the commercial software pro Fit (http://www.quansoft.com/) with the robust fitting option and found $N = 0.62$. When we fit each experiment separately $N$ ranged from 0.45 to 1.15 with an average $N = 0.77 \pm 0.15$.

Because we were expecting $N \geq 1$, we were concerned that these methods may have not found a global minimum. We therefore carried out the following search procedure in parameter space. We evaluated the sum of squares of the theoretical values of $p_{cell}$ minus the experimental values of $p_{cell}$ over parameter space. The evaluations were done for every value of $N$ in the range $N = 0.01 - 3.01$ with a step size of 0.01. At each $N$ the four IC$_{50}$ values were randomly sample $1 \times 10^6$ times from a uniform distribution between zero and ten. All the $N$, IC$_{50}$ and sum of squares were recorded generating a text file of 13GB. In Table 1 we list the ten parameter sets having the lowest values of the sum of squares. All are within the 95% confidence interval we obtained for $N$ using the Levenberg-Marquardt algorithm and the bootstrap method.
Table 1. Listed are the ten sets of parameter values (black print) that had the lowest sum of squares in our search over parameter space. The parameter values and the sum of squares in red were obtained using a program (FUI) based on the Levenberg-Marquardt algorithm.
Figure 1. Kinetic binding schemes for a polyreactive Fab binding from solution to an epitope on a HIV membrane. A. 1. The Fab binds from solution to a lipid on the membrane with rate constants $k_+L$ and $k_−L$; 2. The Fab bound to lipid diffuses with diffusion limited rate constants $k_+$ and $k_−$ to within a distance where it can react with an epitope; 3. The Fab binds to an epitope with chemical rate constants $κ_+1$ and $κ_−1$. B. The surface reactions 2. and 3. are incorporated into a single surface reaction with rate constants $\bar{k}_+1$ and $\bar{k}_−1$, where $\bar{k}_+1 = κ_+1K_D$ and $K_D = k_+/k_−$. C. Binding from solution to the membrane, diffusion to the epitope, and reaction with the epitope are combined into a single reaction with the rate constants $k_+e$ and $k_−e$. This corresponds to the first reaction in the induced conformational fit model [4,15,17].
Figure 2. Binding scheme for a polyreactive Fab binding to a trimer of gp41 epitopes. The brown boxes represent unbound gp41, the grey boxes bound gp41, and the grey elliptical boxes represent gp41 bound to Fab that have undergone a conformational change.