Using computational modeling to optimize the design of antibodies that trap viruses in mucus

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Supporting Information

- 8 pages
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Probability distribution of times for virus freely diffusing vs. interacting with mucins

We derive probability distributions for the time that virions spend interacting with mucins and for the time that the virions spend freely diffusing. We model these interactions by assuming a rate of Ab binding to mucin, $m_{on}[M]$, where $[M]$ is the concentration of mucin proteins. For brevity, we assume a uniform mucin concentration and abbreviate the forward reaction rate as m_{on}, with units of s^{-1}. Similarly, Ab unbind from the mucin network at a rate m_{off} (s^{-1}).

With attachments and detachments following a Poisson process, the probability density function (pdf) for the waiting time of a free Ab to associate with the mucin network is

$$h_{free}(t) = m_{on}e^{-m_{on}t}. \tag{1}$$

Similarly, the waiting time for an Ab fixed to the mucin network to dissociate is

$$h_{\text{interact}}(t) = m_{off}e^{-m_{off}t}. \tag{2}$$

We assume that Ab already bound to virus also interact with mucins with the same binding/unbinding rates m_{on} and m_{off}, with the exception of the first binding, which has rate $m^{first}m_{on}$, where $m^{first} = \frac{1}{30}$. We assume that virions are freely diffusing if and only if all of its bound Ab are free from the mucin network. Thus, it is of interest to calculate the waiting time distributions $h_{\text{free,n}}(t)$ for the time a virion with n Ab remains freely diffusing and the distribution $h_{\text{interact,n}}(t)$ of time it is fixed to mucins.

The term m^{first} simply modifies the freely diffusing distribution, without affecting the virion-mucin interaction distribution. The distribution for the time a virion with n Ab is freely diffusing is the distribution of the minimum of n exponential variables with rate $m^{first}m_{on}$. It is a standard result that this is again an exponential distribution but with rate $nm^{first}m_{on}$ [1]:

$$P(\min(z_1,...,z_n) < t) = 1 - P(z_1 > t, z_2 > t..., z_n > t) = 1 - P(z_1 > t)^n = 1 - e^{-nm^{first}m_{on}t}, \tag{3}$$

so that the freely diffusing density function is given by

$$h_{\text{free,n}}(t) = nm^{first}m_{on}e^{-nm^{first}m_{on}t}. \tag{4}$$

For the density function of the time interacting with mucin, we represent the number of Ab associated with mucin over time as a stochastic process $\{X_t\}$ with $X_0 = 1$, with jump times $\{T_k\}_{k\geq 1}$ (when an Ab-mucin event takes place), and calculate the stopping time τ such that $X_\tau = 0$. For notational convenience, we also define the process $\{Y_k\}_{k\geq 1}$ by the relation $Y_1 = X_0$ and $Y_k = X_{T_k}$. Thus, $\{Y_k\}$ is a discrete Markov chain representing state changes in Ab-mucin interactions.

The transition probabilities from each state depend only on the number of Ab r currently associated with mucin. That is, suppose $X_t = r$, and let z_i denote the waiting time for the i^{th} Ab associated with mucin to dissociate, and let w_j denote the waiting time for the j^{th} Ab not currently associated with mucin to associate. Then the probability of the next event being a new Ab association is: $P_r(X_t = r+1) = P(\min(z_1,...,z_{n-r}) < \min(w_1,...,w_{n-r}))$.

Since z_i are all independent and identically distributed, $\min(z_1,...,z_{n-r})$ has density function $(n-r)m_{on}e^{-(n-r)m_{on}t}$. Similarly, $\min(w_1,...,w_{n-r})$ has density function $rm_{off}e^{-rm_{off}t}$.

Then

$$P(\min(z_1,...,z_{n-r}) < \min(w_1,...,w_r)) = \int_0^\infty \int_0^w (n-r)m_{on}e^{-(n-r)m_{on}z} rm_{off}e^{-rm_{off}w} dzdw$$
$$= \frac{(n-r)m_{on}}{(n-r)m_{on} + rm_{off}} \tag{5}$$

We can also derive the waiting time distribution for the next Ab-mucin interaction, given that the next event is dissociation:

$$P(\min(z_1,...,z_{n-r}) < t \mid \min(z_1,...,z_{n-r}) < \min(w_1,...,w_r))$$
$$= \frac{P(\min(z_1,...,z_{n-r}) < t, \min(z_1,...,z_{n-r}) < \min(w_1,...,w_r))}{P(\min(z_1,...,z_{n-r}) < \min(w_1,...,w_r))} \tag{6}$$

The numerator is

$$\int_0^\infty \int_0^t (n-r)m_{on}e^{-(n-r)m_{on}z} rm_{off}e^{-rm_{off}w} dzdw - \int_0^t \int_w^t (n-r)m_{on}e^{-(n-r)m_{on}z} rm_{off}e^{-rm_{off}w} dzdw$$
$$= \frac{(n-r)m_{on}}{(n-r)m_{on} + rm_{off}} \left[1 - e^{-[(n-r)m_{on} + rm_{off}]t}\right]. \tag{7}$$

So

$$P(\min(z_1,...,z_{n-r}) < t \mid \min(z_1,...,z_{n-r}) < \min(w_1,...,w_r)) = 1 - e^{-\left[(n-r)m_{on} + rm_{off}\right]t}. \tag{8}$$

Similarly, we can show that the waiting time distribution given that the next event is association is the same; that is, the distribution of $T_{k+1} - T_k$, given $X_t = r$ is:

$$P(\min(z_1,...,z_{n-r}) < t) = 1 - e^{-\left[(n-r)m_{on} + rm_{off}\right]t}. \tag{9}$$

Now we derive the pdf for the total time that a virion interacts with mucin. To do this, it suffices to take a weighted sum of the waiting time distributions over all paths $\{Y_k\}$ of the Ab-mucin interaction state. The total probability $P(\{Y_k\})$ of a given path is the product of the probabilities $P_{Y_k}(Y_{k+1})$ of taking the corresponding path at each point:

$$P(\{Y_k\}) = \Pi_{k\geq 1} P_{Y_k}(Y_{k+1}), \tag{10}$$

where

$$P_{Y_k}(Y_{k+1}) = \begin{cases} \dfrac{(n-Y_k)m_{on}}{(n-Y_k)m_{on} + Y_k m_{off}} & \text{if } Y_{k+1} = Y_k + 1 \\ \dfrac{Y_k m_{off}}{(n-Y_k)m_{on} + Y_k m_{off}} & \text{if } Y_{k+1} = Y_k - 1. \\ 0 & \text{otherwise} \end{cases}$$

And the total waiting time for a path $h(\{Y_k\})$ is given by a convolution of the waiting times $h_{Y_{k+1}|Y_k}$ along the path:

$$h(\{Y_k\}) = \prod_{k\geq 1}^{*} h_{Y_{k+1}|Y_k}(t), \tag{11}$$

where

$h_{Y_{k+1}|Y_k} = [(n-Y_k)m_{on} + Y_k m_{off}]e^{-[(n-Y_k)m_{on}+Y_k m_{off}]t}$, and $\prod_{k\geq 1}^{*} h_{Y_{k+1}|Y_k}(t)$ represents the convolution $h_{Y_1|Y_0} * h_{Y_2|Y_1} ... * h_{Y_k|Y_{k-1}}(t)$.

Then the waiting time distribution for Ab-mucin interaction is

$$h_{\text{interact,n}}(t) = \sum_{\{Y_k\}} P(\{Y_k\})h(\{Y_k\}), \tag{12}$$

Equation (12) can further be simplified since it is the convolution of exponential variables, with exactly n different rates. Since the convolution of exponential variables with the same rate parameter is a gamma distribution, the waiting time for a given path can be written simply as the convolution of n gamma density functions.

For example, when $n = 2$, the formula reduces to

$$\sum_{k=0}^{\infty}\left(\frac{m_{on}}{m_{on}+m_{off}}\right)^{k+1} h_{1\to 2}^{*k} * h_{2\to 1}^{*k} * h_{1\to 0}(t) = \sum_{k=0}^{\infty}\left(\frac{m_{on}}{m_{on}+m_{off}}\right)^{k+1} \Gamma(k+1, m_{on}+m_{off}) * \Gamma(k, 2m_{off}). \tag{13}$$

Numerical examples of the waiting time distributions

We conclude with a few examples of the freely diffusing and interaction distributions for common numbers of Ab.

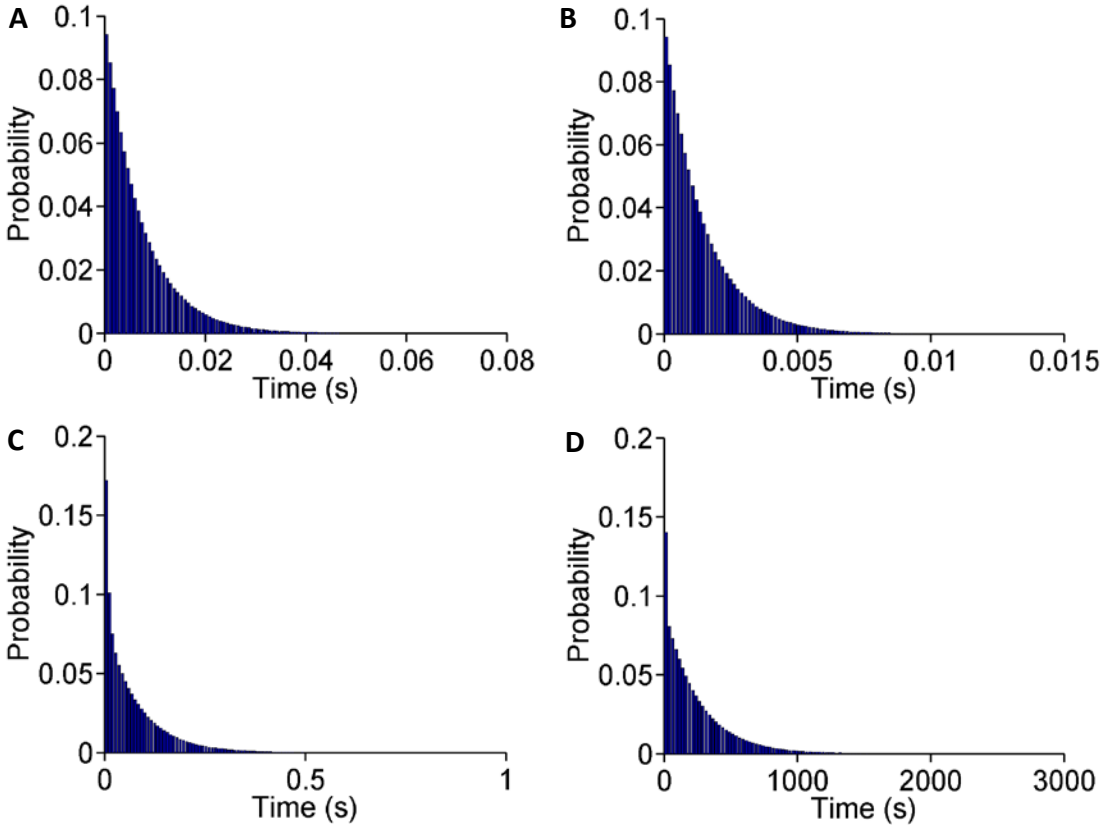

Figure S1. Distribution of time virus spends **(A,B)** freely diffusing and **(C,D)** interacting with mucin for virions with **(A,C)** 2 associated Ab and **(B,D)** 10 associated Ab.

Theoretical estimate of IgG-mucin bond rates & times

From the Smoluchowski equation, the diffusional flux to a flat sink of radius "a" at steady state is given by $F_{steady\ state}$ = 4DaCo. Since the radius of reactive sites for many diffusion-limited enzymes is found to be ~1 Angstrom i.e. 10^{-4} μm, assuming a = 10^{-4} μm, an average IgG concentration of ~500 μg/mL in CVM [2] and diffusivity of ~40 $\mu m^2/s$, the flux of IgG to the site would be on the order of 30 IgG/s. We assume binding sites on mucins are not saturated, based on the previous finding that addition of exogenous IgG to CVM can quickly immobilize HSV [2], that the trapping potency of exogenous IgG is indistinguishable to endogenous HSV-binding IgG [2], and that the total amount of IgG in CVM appears substantially less than the total binding sites available (see below). If the binding sites on mucins are not saturated, by definition the bond times between IgG and mucins at steady state, which is equal to $1/m_{off}$, must be less than 1/ (30 s^{-1}) = 0.03 s. This estimate is consistent with observations that IgG-mucin interactions appear to be weak and exceedingly transient during Fluorescence Recovery After Photobleaching (FRAP) experiments, where complete recovery is observed within seconds [3].

Alternatively, we consider the possibility that IgG binds selectively to different parts of mucins, either directly to an entire mucin molecule (or 1 binding site per mucin), naked protein domains on mucins, or to select glycans on the mucins (10% of all glycan chains). Mucins possess a MW of ~0.5 MDa, are ~80% by weight sugar, and possess ~4 naked protein domains (NPDs) per mucin. At a mucin concentration of 2% w/v (typically ~1-5% w/v in various mucus secretions [4]), this is equivalent to 40 μM of mucins in CVM, 160 μM of NPD, or ~1 mM of glycan binding sites. At ~500 μg/mL total IgG in CVM (~3.3 μM), and assuming up to 20% of IgG associates with mucins at any moment in time (the ratio of diffusivity in mucus to that in water, Dm/Dw, typically ranges between 0.8-1 [3]), this would imply that at steady state, up to 1 in 60 binding sites are occupied if there is only 1 binding site per mucin, 1 in ~300 binding sites are occupied if IgG binds to NPD on mucins, and 1 in ~2000 binding sites are occupied if IgG binds to 10% of the glycan chains on mucins. For equilibrium binding, the fraction of sites with a ligand bound is given by $1/(1+ K_D/[IgG])$. Hence, the K_D for IgG-mucin interactions ranges from ~200 μM if there is only 1 binding site per mucin to ~800 μM if IgG binds to NPD to ~5 mM if IgG binds to 10% of the glycan chains. Arrhenius had derived a very simple relationship between the time a bound particle remains bound, Tb, and the binding energy Δε, by assuming that the bound particle has average kinetic energy by equipartition of energy. Hence ½ mv^2 along the escape axis equals ½ kT. If the particle is constrained within the bond to bounce back and forth a distance Δx, then it will collide with the barrier (of magnitude Δε) and escape with a frequency of v/2Δx. The odds it will have enough energy to escape per collision is given by $e^{-(1/2)kT/\Delta\varepsilon}$. Thus, Tb ~ $\Delta x(m/kT)^{1/2}e^{\Delta\varepsilon/kT}$. The corresponding bond times for K_D in the 200 μM to 5 mM range would be in the 10^{-6} to 10^{-4} s range.

From both approaches, the estimated bond times between IgG and mucins are exceedingly short (i.e. IgG readily unbinds, thus high m_{off} at rates likely substantially exceeding 1 s^{-1}). This supports our assumption that the binding rates are exceptionally fast, and justifies our use of partial differential equations in our model. Unfortunately, such rapid binding and unbinding rates also pose a very difficult challenge to make such measurements experimentally, which remains unresolved to date.

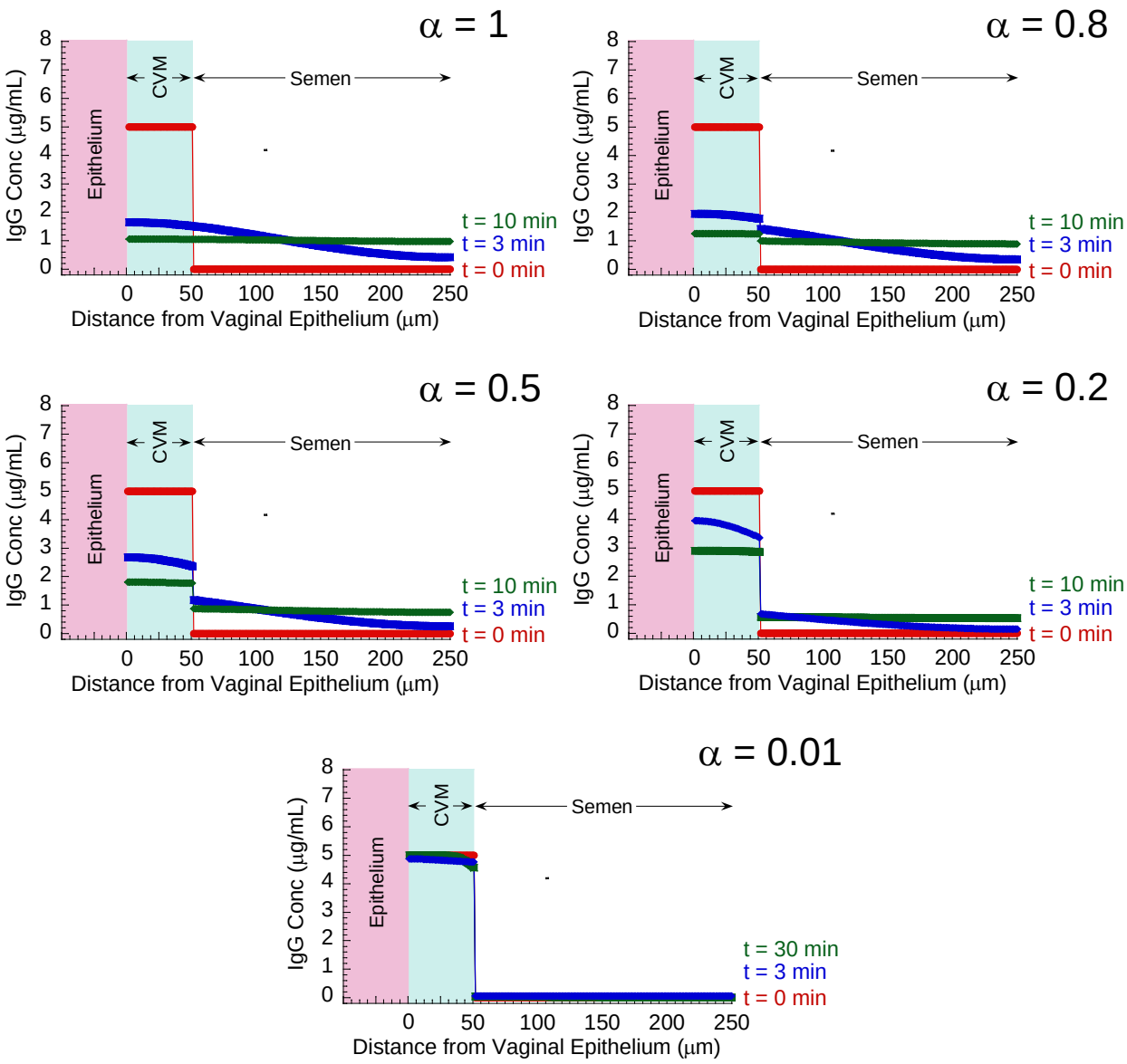

Figure S2. IgG concentration profiles in genital secretions overlaying the vaginal epithelium over time for IgG with distinct affinity to mucins (represented by α).

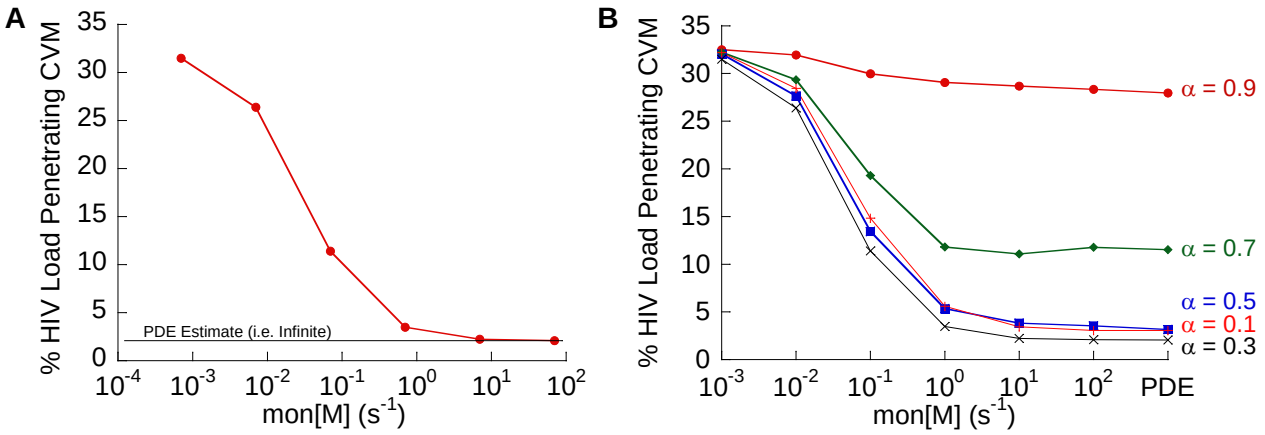

Figure S3. Comparison of m_{on} and m_{off} dynamics to the deterministic reaction-diffusion system. **(A)** Predicted fraction of HIV load initially in semen that can diffuse across CVM containing NIH45-46 over the first two hours post-deposition, at various values of $m_{on}[M]$, with m_{off} adjusted so that $m_{off}/(m_{on} + m_{off}) = 0.3$. The solid black line indicates the predicted HIV load for the PDE system at $\alpha = 0.3$. **(B)** Predicted HIV load diffusing across CVM for various values of $\alpha = m_{off}/(m_{on}[M] + m_{off})$, with results for the equivalent PDE system at the rightmost point.

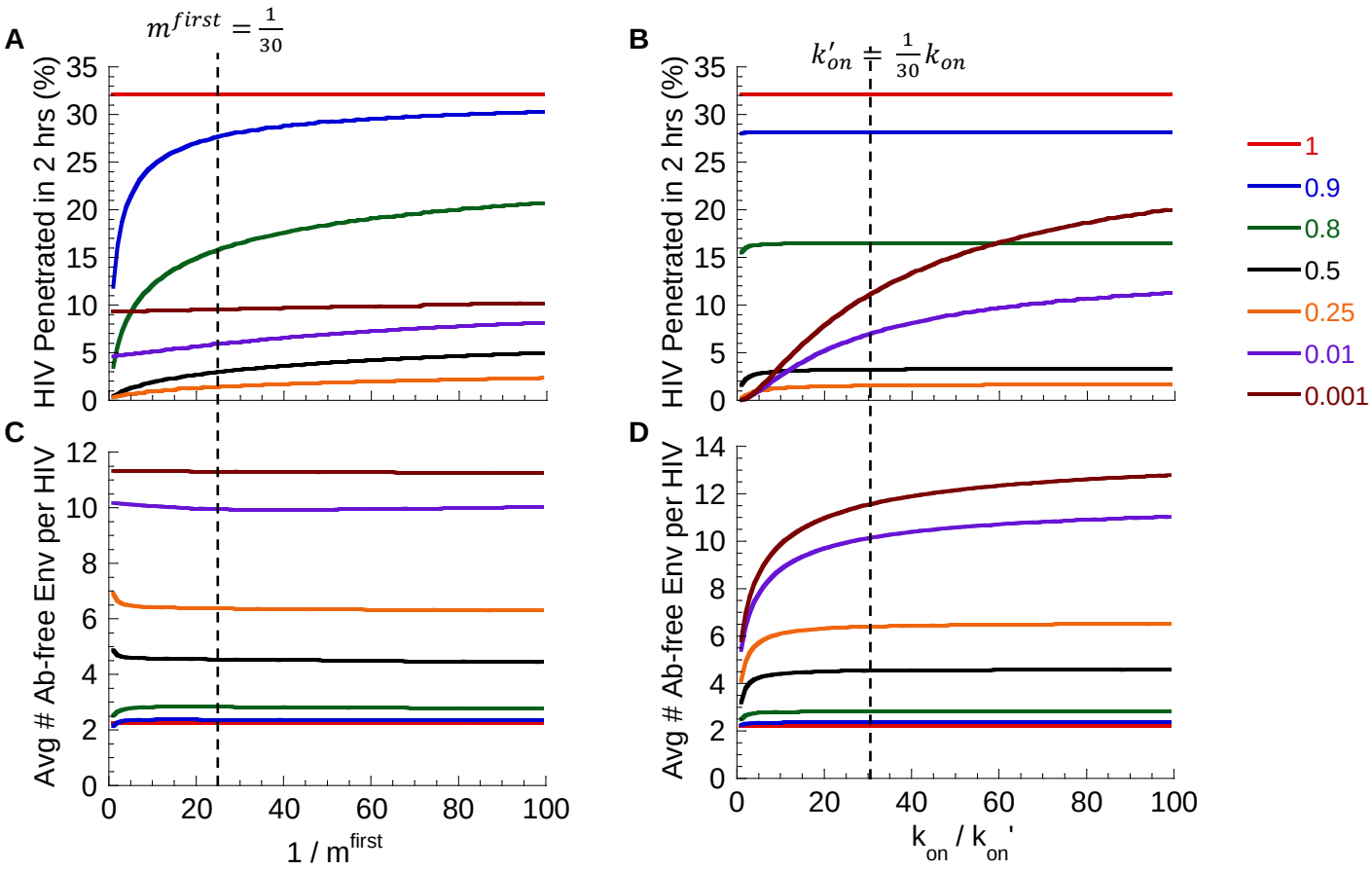

Figure S4. Comparison of how differences in **(A,C)** m^{first} and **(B,D)** k_{on}' can affect **(A,B)** the predicted fraction of HIV load initially in semen that can diffuse across CVM containing NIH45-46 over the first two hours post-deposition as well as **(C,D)** the average number of Ab-free Env trimer on HIV arriving the vaginal epithelium for Ab with different mucin-affinity (i.e. α). Dashed line represents the value chosen in the model to minimize over-estimating the protective efficacy of Ab-mediated trapping in mucus.

References

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