Supplementary information for Activated TCRs remain marked for internalization after dissociation from pMHC

The results in the paper are based on a model that starts with binding of T cell receptors (TCRs) to peptide-major histocompatibility complexes (pMHC) in the immunological synapse between a T cell and an antigen presenting cell. Engagement is followed by a sequence of biochemical modifications to the TCR; these are reversed if the TCR-pMHC bond breaks before the final modification. If the modifications are completed before the bond breaks, the TCR is "activated" and subject to internalization. We compare predictions of alternative versions of the model, a "dimer model" in which TCR-pMHC must form dimers before the biochemical modifications can proceed, and a "monomer model" in which bound monomers undergo the biochemical steps leading to activation and internalization. Here, in Section 1, we present the equations for the monomer model and in Section 2, the equations for the dimer model. Section 3 documents the parameter values used in the simulations. Section 4 documents the results presented in the paper on initial rates of activation and internalization, for the monomer model, under different assumptions regarding the TCR states that are subject to internalization.

1. The monomer model

A sketch of the geometry and a schematic diagram of the reactions in the model are shown in the paper (**Fig. 1** and **Fig. 2**). The model is described by a system of partial differential equations, boundary conditions, and initial conditions for the concentrations of unbound pMHC (P), unbound TCR in the basal state (T), unbound TCR in the activated state (T^*), bound TCR-pMHC in the basal state (B_0), and a sequence of states of bound TCR-pMHC (B_1, B_2, $\cdots$, B_N) that have undergone 1 to N successive modifications. The equations give concentrations as functions of both position (r) and time (t). Diffusion coefficients for free pMHC, free TCR, and bound TCR-pMHC are denoted by D_P, D_T, and D_B, respectively. Association and dissociation rate constants for pMHC binding to TCR are $\bar{k}_{\text{on}}$ and k_{off}. Rate constants for all biochemical modifications are taken to be the same and are denoted by k_p. The rate constant for the internalization of bound activated TCR (B_N) is denoted by λ_B. The rate constant for the internalization of unbound activated TCR (T^*) is denoted by λ_T. Free activated TCR decay to the basal inactive state with rate constant μ_1 inside the contact region and μ_2 in the transition region.

In the contact region ($r \leq a$), the equations for cell surface concentrations are

$$\partial P/\partial t \;=\; D_P \nabla^2 P - \bar{k}_{\text{on}}(T + T^*)P + k_{\text{off}} \sum_{i=0}^{N} B_i + \lambda_B B_N \tag{1a}$$

$$\partial T/\partial t \;=\; D_T \nabla^2 T - \bar{k}_{\text{on}} TP + k_{\text{off}} \sum_{i=0}^{N-1} B_i + \mu_1 T^* \tag{1b}$$

$$\partial B_0/\partial t \;=\; D_B \nabla^2 B_0 + \bar{k}_{\text{on}} TP - (k_{\text{off}} + k_p)B_0 \tag{1c}$$

$$\partial B_i/\partial t \;=\; D_B \nabla^2 B_i + k_p B_{i-1} - (k_{\text{off}} + k_p)B_i \qquad i = 1, \cdots, N-1 \tag{1d}$$

$$\partial B_N/\partial t \;=\; D_B \nabla^2 B_N + \bar{k}_{\text{on}} T^* P + k_p B_{N-1} - (k_{off} + \lambda_B)B_N \tag{1e}$$

$$\partial T^*/\partial t \;=\; D_T \nabla^2 T^* - \bar{k}_{\text{on}} T^* P + k_{\text{off}} B_N - \lambda_T T^* - \mu_1 T^* \tag{1f}$$

where ∇^2 is the two-dimensional diffusion operator. In the transition region ($a < r \leq 2a$) there are no bound complexes (*i.e.*, all $B_i = 0$). The equations for concentrations of free pMHC and TCR are

$$\partial P/\partial t \;=\; D_P \nabla^2 P \tag{2a}$$

$$\partial T/\partial t = D_T \nabla^2 T + \mu_2 T^* \tag{2b}$$

$$\partial T^*/\partial t = D_T \nabla^2 T^* - \mu_2 T^* - \lambda_T T^* \tag{2c}$$

The number of TCRs internalized up to time t, $T_I(t)$ satisfies the equation

$$dT_I/dt = 2\pi \int_0^a r(\lambda_T T^*(r,t) + \lambda_B B_N(r,t))dr + 2\pi \int_a^{2a} r\lambda_T T^*(r,t)dr \tag{2d}$$

Beyond the transition region (*i.e.*, for $r > 2a$), concentrations of bound states and T^* are 0. T and P are taken to be uniform in r for $r \geq 2a$. Their values at time t are denoted $T(t)_{out}$ and $P(t)_{out}$ and are determined by conservation laws for the total number of TCRs on the T cell initially, before internalization, and the total number of pMHC on the APC. The initial, uniform concentrations of TCR and pMHC are denoted T_T and P_T. $A1$ is the area of the T cell and $A2$ is the area of the APC. The areas of the T cell and APC outside the contact and transition areas are denoted by $A1_{out}$ and $A2_{out}$ (*i.e.*, $A1_{out} = A1 - 4\pi a^2$, $A2_{out} = A2 - 4\pi a^2$). The conservation laws that determine $T(t)_{out}$ and $P(t)_{out}$ are

$$A1T_T = 2\pi \int_0^a r \left(T(r,t) + T^*(r,t) + \sum_{i=0}^N B_i(r,t) \right) dr$$

$$+ 2\pi \int_a^{2a} r \left(T(r,t) + T^*(r,t) \right) dr + T_I(t) + A1_{out}T(t)_{out} \tag{3a}$$

$$A2P_T = 2\pi \int_0^a r \left(P(r,t) + \sum_{i=0}^N B_i(r,t) \right) dr + 2\pi \int_a^{2a} rP(r,t)dr + A2_{out}P(t)_{out} \tag{3b}$$

The boundary conditions include the requirement that all concentrations remain finite at $r = 0$. At $r = a$, the boundary of the contact area, concentrations of free pMHC and TCR (P, T, and T^*) are continuous. Concentrations of bound complexes (B_i) satisfy reflecting (*i.e.*, no flux) boundary conditions at $r = a$. At $r = 2a$, the outer boundary of the transition region, $T = T_{out}$ (Eq. 3a), $P = P_{out}$ (Eq. 3b), and $T^* = 0$ (*i.e.*, P, T, and T^* are taken to be continuous across the outer boundary of the transition region).

Standard initial conditions for the simulations are $T = T_T$ and $P = P_T$ at $t = 0$, for all r. All other concentrations are 0 initially.

We solve the system of partial differential equations numerically, subject to the boundary conditions and initial conditions, by discretizing the system of equations and integrating the difference equations, using a Crank-Nicolson scheme[1].

2. The dimer model

The model consists of a system of partial differential equations, boundary conditions, and initial conditions for the concentrations of unbound pMHC (P), unbound TCR in the basal state (T), unbound TCR in the activated state (T^*), monomeric TCR-pMHC in the basal state (B), monomeric TCR-pMHC in the activated state (B^*), and a sequence of dimer states (D_0, D_1, $\cdots$, D_N) with successive biochemical modifications. The total dimer concentration is denoted by D, *i.e.*, D$=\sum_{i=0}^N D_i$. The equations give concentrations as functions of both position (r) and time (t). Diffusion coefficients for free pMHC, free TCR, bound TCR-pMHC monomers, and TCR-pMHC dimers are denoted D_P, D_T, D_B and D_D, respectively. Association and dissociation rate constants for pMHC binding to TCR are $\bar{k}_{on}$ and k_{off}. Forward and reverse rate constants for dimer formation are k_{+2} and k_{-2}. Rate constants for all modifications of dimers are taken to be the same and are denoted by k_p. The rate constants for internalization of TCR monomers and dimers in the three active states (T^*, B^*, and D_N) are denoted by λ_T, λ_B, and λ_D, respectively. Free activated TCRs decay to the basal inactive state with rate constant μ_1 inside the contact region and μ_2 in the transition region.

In the contact region ($r \leq a$), the equations for cell surface concentrations are

$$\partial P/\partial t = D_P \nabla^2 P - \bar{k}_{on}(T + T^*)P + k_{off}(B + B^*) + 2k_{off}D + \lambda_B B^* + 2\lambda_D D_N \tag{4a}$$

$$\partial T/\partial t = D_T \nabla^2 T - \bar{k}_{on}TP + k_{off}B + 2k_{off}(D - D_N) + \mu_1 T^* \tag{4b}$$

$$\partial B/\partial t = D_B \nabla^2 B + \bar{k}_{on}TP - k_{off}B - k_{+2}B^2 + 2(k_{-2} + k_{off})(D - D_N) \tag{4c}$$

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$$\partial D_0/\partial t = D_D \nabla^2 D_0 + k_{+2}B^2/2 - (k_{-2} + 2k_{\text{off}} + k_p)D_0 \tag{4d}$$

$$\partial D_i/\partial t = D_D \nabla^2 D_i + k_p(D_{i-1} - D_i) - (k_{-2} + 2k_{\text{off}})D_i \qquad i = 1, \cdots, N-1 \tag{4e}$$

$$\partial D_N/\partial t = D_D \nabla^2 D_N + k_p D_{N-1} - (k_{-2} + 2k_{\text{off}} + \lambda_D)D_N \tag{4f}$$

$$\partial T^*/\partial t = D_T \nabla^2 T^* - \bar{k}_{\text{on}}T^*P + k_{\text{off}}B^* + 2k_{\text{off}}D_N - \lambda_T T^* - \mu_1 T^* \tag{4g}$$

$$\partial B^*/\partial t = D_B \nabla^2 B^* + \bar{k}_{\text{on}}T^*P - k_{\text{off}}B^* + 2(k_{-2} + k_{\text{off}})D_N - \lambda_B B^* \tag{4h}$$

In the transition region ($a < r \leq 2a$) there are no bound complexes (*i.e.*, B, B^*, D_i are all 0). The equations for concentrations of free pMHC and TCR are

$$\partial P/\partial t = D_P \nabla^2 P \tag{5a}$$

$$\partial T/\partial t = D_T \nabla^2 T + \mu_2 T^* \tag{5b}$$

$$\partial T^*/\partial t = D_T \nabla^2 T^* - \mu_2 T^* - \lambda_T T^* \tag{5c}$$

The number of TCRs internalized up to time t satisfies the equation

$$dT_I/dt = 2\pi \int_0^a r(\lambda_T T^*(r,t) + \lambda_B B^*(r,t) + 2\lambda_D D_N(r,t))dr + 2\pi \int_a^{2a} r\lambda_T T^*(r,t)dr \tag{5d}$$

Beyond the transition region (*i.e.*, for $r > 2a$), all concentrations are 0 except for P and T, the concentrations of free pMHC and free, inactive TCR. T and P are taken to be uniform in r for $r \geq 2a$. Their values at time t are denoted $T(t)_{out}$ and $P(t)_{out}$ and are determined by conservation laws for the total number of TCRs on the T cell initially, before internalization, and the total number of pMHC on the APC. The initial, uniform concentrations of TCR and pMHC are denoted T_T and P_T. $A1$ is the area of the T cell and $A2$ is the area of the APC. The areas of the T cell and APC outside the contact and transition areas are denoted by $A1_{out}$ and $A2_{out}$ (*i.e.*, $A1_{out} = A1 - 4\pi a^2$, $A2_{out} = A2 - 4\pi a^2$). The conservation laws that determine $T(t)_{out}$ and $P(t)_{out}$ are

$$A1T_T = 2\pi \int_0^a r\left(T(r,t) + B(r,t) + T^*(r,t) + B^*(r,t) + 2D(r,t)\right)dr$$

$$+ 2\pi \int_a^{2a} r\left(T(r,t) + T^*(r,t)\right)dr + T_I(t) + A1_{out}T(t)_{out} \tag{6a}$$

$$A2P_T = 2\pi \int_0^a r\left(P(r,t) + B(r,t) + B^*(r,t) + 2D(r,t)\right)dr$$

$$+ 2\pi \int_a^{2a} rP(r,t)dr + A2_{out}P(t)_{out} \tag{6b}$$

The boundary conditions, initial conditions, and method of solution given for the monomer model apply to the dimer model as well. Concentrations of the additional bound states, *i.e.*, B^* and D_i, satisfy reflecting (no flux) boundary conditions at $r = a$.

3. Estimation of parameters for simulations

The parameter values used for the simulations are estimated from a variety of published data. In the simulations the T cell has 3×10^4 TCRs[2,3] and the radius of the contact area is[4] 4.7×10^{-4} cm. We have taken the surface areas of the T cell and APC to be the same and equal to 5×10^{-6} cm^2.

Measurements by Grakoui *et al.*[4] in two experimental systems were used to estimate binding and dissociation rate constants for TCR interacting with agonist pMHC, as described in Wofsy *et al.*, Table 1[5]. In particular, for six peptides the forward rate constant for binding was in the range $\bar{k}_{\text{on}} = 5 \times 10^{-11} - 1 \times 10^{-9}$ cm^{2}s^{-1}. In the simulations, $\bar{k}_{\text{on}} = 1 \times 10^{-10}$ cm^{2}s^{-1}.

The kinetic proofreading parameters used in the simulations, *i.e.*, the number of modifications, $N = 6$, and the modification rate constant, $k_p = 0.25$, were chosen so that Eq. 1 in the paper is satisfied when the optimal value for the dissociation rate constant is that of a full agonist, $k_{\text{off}}^{\text{max}} = 0.05$.

Estimates of the diffusion coefficient for pMHC on various cell lines[6−9] lie in the range $D_P = 1 \times 10^{-10} - 4 \times 10^{-10}$ cm^{2}s^{-1}. We have taken $D_P = 3 \times 10^{-10}$ cm^{2}s^{-1}. The TCR diffusion coefficient, D_T, was determined

on unstimulated Jurkat cells[10] and TCR-ζ transfected stimulated HeLa cells[11] to be respectively 1.2×10^{-9} cm^{2}s^{-1} and 1.1×10^{-10} cm^{2}s^{-1}. In the simulations we have used an intermediate value, $D_T = 3 \times 10^{-10}$ cm^{2}s^{-1}. For the monomer model we have taken the bound state to be immobile, $i.e.$, $D_B = 0$. For the dimer model, TCR-pMHC monomers must be mobile if they are to interact and form dimers. We have taken $D_B = D_P D_T/(D_P + D_T) = 1.5 \times 10^{-10}$ cm^{2}s^{-1} and taken the dimer to be immobile.

There is no information about the rate constants for dimer formation and dissociation, denoted k_{+2} and k_{-2}. In the simulations $k_{+2} = 5 \times 10^{-10}$ cm^{2}s^{-1}, which is about a factor of two below the diffusion limit, and $k_{-2} = 1 \times 10^{-4}$ s^{-1}. This low value for k_{-2} is chosen so that the dominant mode of dimer dissociation is through the opening of a bond, which occurs with rate constant $2k_\text{off}$.

For the simulations shown in **Fig. 5**, the decay rate constants for unbound activated receptors inside and outside the contact region are equal and are varied as specified in the figure caption. For other simulations, we have taken $\mu_1 = 0$ s^{-1} and $\mu_2 = 0.1$ s^{-1} so that an activated TCR remains active while in the contact region and reverts back to the basal state with a half-life of 6.9 s after diffusing out of the contact region.

We have compared predictions based on alternative assumptions about which TCR states are subject to internalization, considering cases where $\lambda_B = 0$ or $\lambda_T = 0$. For states subject to internalization, we have taken the rate constant for internalization equal to 3×10^{-3} s^{-1}. This is faster than the reported estimates, $1 \times 10^{-4} - 9 \times 10^{-4}$ s^{-1}, for T cell hybridomas and naive CD4$^+$ T cells[12] but these values may be underestimates. Different T cell clones and hybridomas show wide variation in TCR down regulation under similar conditions[3] and we expect that part of this variation arises from variation in the rate constant for internalization.

4. Derivations of initial rates of activation and internalization

To estimate the rates of TCR activation and internalization per peptide for the monomer model, in an initial period after formation of the immunological synapse when pMHC and TCR are still essentially uniformly distributed in the immunological synapse, we use the expressions

$$\text{activation rate} \quad = \quad (\text{hits/s}) \times (\text{fraction activated}) \tag{7}$$

$$\text{internalization rate} \quad = \quad (\text{hits/s}) \times (\text{fraction internalized}) \tag{8}$$

Under different assumptions, there will be different forms for the "hitting rate" (hits/s), $i.e.$, the rate at which a single pMHC engages TCRs, and the fraction of peptide-bound TCR that complete all the steps leading to activation or internalization.

In the absence of internalization, the rate of serial engagement, per peptide, is[5]:

$$\text{hits/s} \approx \frac{\bar{k}_\text{on} T}{1 + \bar{K} T} = \frac{k_\text{off} \bar{K} T}{1 + \bar{K} T} \tag{9}$$

where $\bar{k}_\text{on}$ is the effective two-dimensional forward rate constant for the binding of pMHC to TCR, within the contact area between an APC and T cell, k_off is the reverse rate constant, and $\bar{K} = \bar{k}_\text{on}/k_\text{off}$. T denotes the concentration of unbound TCR in the contact area. An assumption underlying Eq. 9 is that the density of specific pMHC that can interact with the TCR is small relative to the density of TCR. Then the pMHC act independently, in that they do not compete for TCRs. The fraction of peptide-bound TCRs that go through all the steps leading to activation before the TCR and pMHC dissociate is[13]

$$\text{fraction activated} \quad = \quad \left(\frac{k_p}{k_p + k_\text{off}}\right)^N \tag{10}$$

where N is the number of successive, irreversible biochemical modifications a bound TCR must complete to become activated and k_p is the rate constant for each of the modifications. Then

$$\text{activation rate} \quad \approx \quad \left(\frac{k_\text{off} \bar{K} T}{1 + \bar{K} T}\right) \left(\frac{k_p}{k_p + k_\text{off}}\right)^N \tag{11}$$

The activation rate has a maximum value as a function of k_off. The value of k_off where the activation rate is maximal is given approximately by $k_\text{off}^\text{max} \approx k_p/(N-1)$ (Eq. 1 in the paper).

The McKeithan model can be modified in different ways to include internalization.

Case 1: Only bound, activated TCRs are subject to internalization
For a pMHC in the immunological synapse, the number of TCR hits per second = 1/(mean time between hits). The time between successive engagements is the time the pMHC remains bound to the first TCR, *i.e.* the time until dissociation or internalization, plus the time for the free pMHC to bind to the next TCR. For Case 1 we find that

$$\text{hits/s} \approx \left[\frac{1}{k_{\text{off}}} \left(1 - \left(\frac{k_p}{k_p + k_{\text{off}}} \right)^N \left(\frac{\lambda_B}{k_{\text{off}} + \lambda_B} \right) \right) + \frac{1}{\bar{k}_{\text{on}} T} \right]^{-1} \tag{12}$$

where λ_B is the rate constant for internalization. In this version of the model, internalization acts on a TCR-pMHC with N modifications, internalizing the TCR and leaving the pMHC free on the surface of the APC. The fraction of bound TCRs that are internalized is

$$\text{fraction internalized} = \left(\frac{k_p}{k_p + k_{\text{off}}} \right)^N \left(\frac{\lambda_B}{k_{\text{off}} + \lambda_B} \right) \tag{13}$$

Examination of the derivative of the internalization rate (*i.e.* the product of the expressions in Eqs. 12 and 13) shows that internalization is a decreasing function of k_{off}.

In contrast, there may or may not be an optimal k_{off} for activation, depending on the value of the rate constant for internalization, λ_B, relative to the parameters governing binding and modification. Consideration of the initial activation rate in this case:

$$\text{activation rate} = \left[\frac{1}{k_{\text{off}}} \left(1 - \left(\frac{k_p}{k_p + k_{\text{off}}} \right)^N \left(\frac{\lambda_B}{k_{\text{off}} + \lambda_B} \right) \right) + \frac{1}{\bar{k}_{\text{on}} T} \right]^{-1} \left(\frac{k_p}{k_p + k_{\text{off}}} \right)^N \tag{14}$$

shows that activation goes through a maximum as a function of k_{off} only if

$$\lambda_B^2 < \left(\frac{1}{\bar{k}_{\text{on}} T} \times \frac{N}{k_p} + \frac{N(N-1)}{2k_p^2} \right)^{-1} \tag{15}$$

Otherwise, the initial rate of activation decreases monotonically as a function of k_{off}.

Case 2: Activated TCRs are subject to internalization only after dissociation
Now suppose activated TCRs remain active for a period after dissociation (**Fig. 2** of the paper, state T^*) and are subject to internalization only during this period (*i.e.*, $\lambda_B = 0$). We also assume an activated free TCR can revert to the basal state, with rate constant μ. Then the initial rates of TCR activation and internalization are

$$\text{activation rate} = \left(\frac{k_{\text{off}} \bar{K} T}{1 + \bar{K} T} \right) \left(\frac{k_p}{k_p + k_{\text{off}}} \right)^N \tag{16}$$

$$\text{internalization rate} = \left(\frac{k_{\text{off}} \bar{K} T}{1 + \bar{K} T} \right) \left(\frac{k_p}{k_p + k_{\text{off}}} \right)^N \left(\frac{\lambda_T}{\mu + \lambda_T} \right) \tag{17}$$

Both activation and internalization have a maximum as a function of k_{off}.

Case 3: Activated TCRs are subject to internalization in both the bound and unbound states
For the general case shown in **Fig. 2** of the paper, the initial activation rate is the same as in Case 1 (Eq. 14), and therefore the condition for activation to have a maximum as a function of k_{off} is given by Eq. 15. The initial internalization rate is

$$\text{internalization rate} = \text{activation rate} \times \left(\frac{\lambda_B}{\lambda_B + k_{\text{off}}} \right) \left(1 + \frac{k_{\text{off}} \lambda_T}{\lambda_B (\lambda_T + \mu)} \right) \tag{18}$$

Like activation, internalization may or may not have a maximum. In the special case when the rate constant for internalization of activated TCRs is the same for bound and free TCRs ($\lambda_B = \lambda_T$) and there is negligible

decay of activated TCRs to the basal state ($\mu = 0$), the rates of activation and internalization are equal and the condition for the existence of an optimal k_{off} for TCR internalization is given by Eq. 15.

Rate of activation for responses that require fewer than N modifications
So far, we have presented the rate of activation in the limited setting where a TCR is considered to be activated if it has undergone the N biochemical modifications that target it for internalization. In fact the derivations apply more generally, to other TCR states with fewer modifications ($M < N$) that might be considered to be "activated" in the sense that they are capable of generating T cell responses other than TCR internalization. For Case 1, where only bound TCR with N modifications are subject to internalization, the analogue of Eq. 14 is

$$\text{activation rate} \ = \left[\frac{1}{k_{\text{off}}} \left(1 - \left(\frac{k_p}{k_p + k_{\text{off}}} \right)^N \left(\frac{\lambda_B}{k_{\text{off}} + \lambda_B} \right) \right) + \frac{1}{\bar{k}_{\text{on}} T} \right]^{-1} \left(\frac{k_p}{k_p + k_{\text{off}}} \right)^M \tag{19}$$

This expression depends indirectly, through the hitting rate, on the full pathway to internalization because internalization frees peptides to engage additional TCRs. In Case 2, where activated TCRs are subject to internalization only after dissociation, Eq. 16 holds with N replaced by M.

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