Supplementary Information: Phenotypic models of T cell activation

Melissa Lever[†], Philip K. Maini[‡], P. Anton van der Merwe[†], Omer Dushek[†,‡,¶]

[†]Sir William Dunn School of Pathology, University of Oxford, Oxford, Oxfordshire, United Kingdom
[‡]Wolfson Centre for Mathematical Biology, Mathematical Institute, University of Oxford, Oxford, Oxfordshire, United Kingdom
[¶]Corresponding author

Contents

1 Generation and parameter values for main text figures

1.1 Figure 2

The occupancy, kinetic proofreading, limited signalling and sustained signalling models were calculated using the analytical results derived in the next section. The dose-response plots and plots showing activation as a function of τ were normalised by T_T, so that activation was in the range $[0,\ 1]$. Parameters: number of TCRs $T_T = 1.5708 \times 10^4$, $k_{\text{on}} = 3.1831 \times 10^{-5}$ s^{-1} , $k_{\text{p}} = 1$ s^{-1}, $N = 10$, $\phi = 0.09$ s^{-1}, λ = 0.001 s^{-1}.

1.2 Figure 3

The negative feedback model is calculated as described by Francois et al (1). The dose-repsonse plot and the plot showing activation as a function of τ were calculated using the Matlab (Mathworks, MA) function *ode15s*. The plots were normalised by the most stimulating ligand in the plot, to give activation in the range of $[0,\ 1]$. Parameters: number of TCRs $T_T = 3 \times 10^4$, $S_T = 6 \times 10^5$, $k_{\text{on}} = 1 \times 10^{-4}$ s^{-1}, $N = 10$, $k_{\text{p}} = 0.252$ s^{-1}, $b = 0.04\ s^{-1}$, $\gamma = 4.4 \times 10^{-4}$.

1.3 Figure 4

The kinetic proofreading with limited signalling was calculated for the shown dissociation times and then a transformation of either 4 c) a threshold and switch or 4 d) threshold was applied to the TCR signal and the resultant activation plotted as a function of ligand dose. Parameters: number of TCRs $T_T = 1.5708 \times 10^4$, $k_{\text{on}} = 3.1831 \times 10^{-5}$ s^{-1} , $k_{\text{p}} = 1$ s^{-1}, $N = 10$, $\phi = 0.09$ s^{-1}.

1.4 Figure 6

The relative change in activation when a second ligand is co-presented compared to the first ligand alone was calculated for the occupancy, kinetic proofreading, limited signalling and sustained signalling models analytically using the results from the derivations in the next section. The negative feedback model was numerically calculated using the Matlab (Mathworks, MA) function *ode15s*. The first ligand has τ = 10 s and is presented at 10^3 ligands per cell. The second ligand has a dissociation time and ligand number denoted by the axes and is presented at 3×10^3 ligands per cell. Parameters for the occupancy, kinetic proofreading, kinetic proofreading with limited and sustained signaling: number of TCRs $T_T = 1.5708 \times 10^4$, $k_{\text{on}} = 3.1831 \times 10^{-5}$ s^{-1} , $k_{\text{p}} = 1$ s^{-1}, $N = 10$, $\phi = 0.09$ s^{-1}, λ = 0.001 s^{-1}. Parameters for the negative feedback model: number of TCRs $T_T = 3 \times 10^4$, $S_T = 6 \times 10^5$, $k_{\text{on}} = 1 \times 10^{-4}$s^{-1}, $k_{\text{p}} = 0.252$s^{-1}, $N = 10$, $b = 0.04$s^{-1}, $\gamma = 4.4 \times 10^{-4}$.

1.5 Figure S1

The effect of thresholds and switches was found for the occupancy, kinetic proofreading and sustained signalling models by calculating the models at the shown dissociation times and then applying a transformation of either c) a threshold and switch or d) a threshold to the TCR signal and the resultant activation plotted as a function of ligand dose. Parameters: number of TCRs $T_T = 1.5708 \times 10^4$, $k_{\text{on}} = 3.1831 \times 10^{-5}$ s^{-1} , $k_{\text{p}} = 1$ s^{-1}, $N = 10$, $\phi = 0.09$ s^{-1}, λ = 0.001 s^{-1}.

1.6 Figure S2

a) - e) These plots show the change in the level of T cell activation when a second ligand is co-presented compared to the first ligand alone. The first ligand has τ = 10 s and is presented at 10^3 ligands. The second

ligand has a dissociation time and ligand number denoted by the axes. The occupancy, kinetic proofreading, limited signalling and sustained signalling models were all calculated analytically using the results from the derivations in the next section. The negative feedback model was numerically calculated using the Matlab (Mathworks, MA) function *ode15s*. A heat map was applied to the matrices that contained the relative change in activation when the second ligand was co-presented. The same colorbar was used for all plots so that the models could be directly compared. Parameters for the occupancy, kinetic proofreading, kinetic proofreading with limited and sustained signaling: number of TCRs $T_T = 1.5708 \times 10^4$, $k_{\text{on}} = 3.1831 \times 10^{-5}$ s^{-1} , $k_{\text{p}} = 1$ s^{-1}, $N = 10$, $\phi = 0.09$ s^{-1}, λ = 0.001 s^{-1}. Parameters for the negative feedback model: number of TCRs $T_T = 3 \times 10^4$, $S_T = 6 \times 10^5$, $k_{\text{on}} = 1 \times 10^{-4}$s^{-1}, $k_{\text{p}} = 0.252$s^{-1}, $N = 10$, $b = 0.04$s^{-1}, $\gamma = 4.4 \times 10^{-4}$.

f) The percentage of TCR occupied by the second ligand was calculated using the analytical result for co-presentation under the occupation model, which is in the next section. The first ligand has τ = 10 s and is presented at 10^3 ligands. The second ligand has a dissociation time and ligand number denoted by the axes. The plot shows the percentage of TCRs occupied by the second ligand as a fraction of the total amount of bound TCRs. Parameters are the same as for a).

2 Model derivations

Each model of T cell activation described below has a different predictor of T cell activation, which we define to be R. We define $E_{\max}$ to be the value of R in the limit of excess pMHC ligand (e.g. $E_{\max} = \lim_{P_T \to \infty} R$) and define EC_{50} as the concentration of pMHC ligand that produces half-maximal R (e.g. value of P_T that satisfies $R = E_{\max}/2$).

2.1 Occupancy (Fig. 2A-C)

The occupancy model describes T-cell activation as being proportional to the concentration of bound TCR, which is defined as C and therefore in this model $R = C_T$. In this model pMHCs (P) can reversibly bind TCRs (T) to form a TCR-pMHC (C), see Fig. 2A. Using the principle of mass action, the kinetics are governed by the equation,

$$\frac{dC}{dt} = k_{\text{on}} PT - k_{\text{off}} C. \tag{1}$$

At equilibrium, $\frac{dC}{dt} = 0$ and

$$PT = K_{\text{D}} C, \tag{2}$$

where $K_{\text{D}} = k_{\text{off}}/k_{\text{on}}$. The total amount of peptide P_T and total amount of TCR T_T are conserved quantities given by the following conservation equations:

$$P_T = P + C \tag{3}$$
$$T_T = T + C. \tag{4}$$

Inserting the conservation equations into equation (2) gives,

$$C = \left(P_T + T_T + K_{\text{D}} - \sqrt{((P_T + T_T + K_{\text{D}})^2 - 4P_T T_T)}\right)/2. \tag{5}$$

2.1.1 Calculating $E_{\max}$ and EC_{50}

In this model $R = C$ and therefore $E_{\max}$ is the value of C in the limit of excess ligand (P_T). This can be found by non-dimensionalising the variables,

$$\hat{P} = \frac{P}{P_T}, \quad \hat{T} = \frac{T}{T_T}, \quad \hat{C} = \frac{C}{T_T},$$

and substituting these non-dimensionalised variables into the equations above to obtain,

$$\hat{P}\hat{T} = \frac{K_{\text{D}}}{P_T}\hat{C} \tag{6}$$
$$\hat{P} + \frac{T_T}{P_T}\hat{C} = 1 \tag{7}$$
$$\hat{T} + \hat{C} = 1 \tag{8}$$

Taking the limit of large P_T we obtain,

$$\lim_{P_T \to \infty} \hat{C} = 1$$

or in dimensional form,

$$E_{\max} = \lim_{P_T \to \infty} R = T_T.$$

To determine EC_{50} the concentration of P_T that satisfies the following relationship is determined,

$$R = E_{\max}/2$$
$$C = T_T/2.$$

By substituting the solution for C and solving for P_T we find that this concentration (defined as EC_{50}) is,

$$EC_{50} = K_{\mathrm{D}} + T_T/2 \tag{9}$$

2.2 Kinetic proofreading (Fig. 2D-F)

In this model a pMHC (P) can reversibly bind to a TCR (T) to form a complex (C_0). Upon forming a complex a series of chemical modifications can take place (C_i) that eventually produce a productive signaling complex (C_N). In this model, T cell activation is determined by the concentration of productive signaling complexes and therefore $R = C_N$. The parameters governing this model are the bimolecular binding rate (k_{on}), the unbinding rate (k_{off}), the modification rate (k_{p}), and the number of intermediate states (N). In this canonical kinetic proofreading model it is assumed that dissociation of pMHC from the TCR immediately reverses all TCR modifications.

This model is described by the following set of ODEs,

$$\partial P/\partial t = -k_{\mathrm{on}} PT + k_{\mathrm{off}} \sum_{i=0}^{N} C_i \tag{10}$$

$$\partial T/\partial t = -k_{\mathrm{on}} PT + k_{\mathrm{off}} \sum_{i=0}^{N} C_i \tag{11}$$

$$\partial C_0/\partial t = k_{\mathrm{on}} PT - (k_{\mathrm{off}} + k_{\mathrm{p}}) C_0 \tag{12}$$

$$\partial C_i/\partial t = k_{\mathrm{p}} C_{i-1} - (k_{\mathrm{p}} + k_{\mathrm{off}}) C_i \qquad \big(1 \leq i < N-1\big) \tag{13}$$

$$\partial C_N/\partial t = k_{\mathrm{p}} C_{N-1} - k_{\mathrm{off}} C_N \tag{14}$$

As before, we have the following conservation for pMHCs and TCRs,

$$P_T = P + C_T \tag{15}$$

$$T_T = T + C_T \tag{16}$$

where $C_T = \sum_{i=0}^{N} C_i$. We find an expression for C_T by substituting the conservation equations (15) and (16) into the steady-state of the ODE system,

$$C_T = \big(P_T + T_T + K_{\mathrm{D}} - \sqrt{(P_T + T_T + K_{\mathrm{D}})^2 - 4P_T T_T}\big)/2. \tag{17}$$

The next step is to relate C_N to C_T so that the magnitude of T-cell activation can be expressed as a function of P_T, T_T and K_{D}. At steady-state equation (14) becomes,

$$C_N = \frac{k_{\mathrm{p}}}{k_{\mathrm{off}}} C_{N-1}$$
$$= \frac{\alpha^N}{1-\alpha} C_0 \tag{18}$$

where $\alpha = k_{\mathrm{p}}/(k_{\mathrm{p}} + k_{\mathrm{off}})$. Next we can relate C_T to C_N using $C_i = \alpha C_{i-1}$ (equation 13 at steady-state),

$$\begin{aligned} C_T &= \sum_{i=0}^{N-1} C_i + C_N \\ &= \sum_{i=0}^{N-1} \alpha^i C_0 + C_N \\ \textit{(using geometric sum)} \qquad &= \Big(\frac{1-\alpha^N}{1-\alpha}\Big) C_0 + C_N \\ &= \frac{1}{\alpha^N} C_N. \end{aligned} \tag{19}$$

It follows that,

$$\mathrm{R} = C_N = \alpha^N C_T.$$

2.2.1 Calculating the $E_{\max}$ and EC_{50}

As before, we non-dimensionalise using $\hat{P} = P/P_T$, $\hat{T} = T/T_T$ and $\hat{C_T} = C_T/T_T$. The non-dimensionalised conservation equations are,

$$1 = \hat{P} + \frac{T_T}{P_T}\hat{C_T} \tag{20}$$

$$1 = \hat{T} + \hat{C_T}. \tag{21}$$

By substituting (20) and (21) into the steady-state non-dimensional form of equation (10) we get,

$$-k_{\mathrm{on}} P_T \hat{P}\hat{T} + k_{\mathrm{off}}\hat{C_T} = 0 \tag{22}$$

$$\hat{C_T} = \frac{P_T \hat{P}}{K_{\mathrm{D}} + P_T \hat{P}}. \tag{23}$$

When $P_T \to \infty$, we can see from equation (23) that,

$$\lim_{P_T \to \infty} \hat{C_T} = 1$$

and therefore,

$$E_{\max} = \lim_{P_T \to \infty} R = \alpha^N T_T.$$

To determine EC_{50} we solve for P_T in the following equation,

$$R = E_{\max}/2 \tag{24}$$

$$\alpha^N C_T = \alpha^N T_T/2. \tag{25}$$

and find that the value of P_T is,

$$EC_{50} = K_{\mathrm{D}} + T_T/2. \tag{26}$$

2.3 Kinetic proofreading with limited signalling (Fig. 2G-I)

To include limited signaling the kinetic proofreading model is modified to include a state whereby the productively signaling TCR-pMHC complex (C_N) may undergo a modification (with rate ϕ) that renders it non-signaling (C_N^-).

The ODE system governing this model is,

$$\partial P/\partial t = -k_{\text{on}}PT + k_{\text{off}}C_T \tag{27}$$
$$\partial T/\partial t = -k_{\text{on}}PT + k_{\text{off}}C_T \tag{28}$$
$$\partial C_0/\partial t = k_{\text{on}}PT - (k_{\text{off}} + k_{\text{p}})C_0 \tag{29}$$
$$\partial C_i/\partial t = k_{\text{p}}C_{i-1} - (k_{\text{p}} + k_{\text{off}})C_i \quad 1 \leq i < N-1 \tag{30}$$
$$\partial C_N/\partial t = k_{\text{p}}C_{N-1} - (k_{\text{off}} + \phi)C_N \tag{31}$$
$$\partial C_N^-/\partial t = \phi C_N - k_{\text{off}}C_N^- \tag{32}$$

where $C_T = \sum_{i=0}^{N} C_i + C_N^-$. As before, T cell activation is determined by C_N (i.e. $R = C_N$). At steady-state it can be seen that,

$$\begin{aligned} C_T &= \sum_{i=0}^{N-1} C_i + C_N + C_N^- \\ &= \frac{1-\alpha^N}{1-\alpha}C_0 + C_N + \frac{\phi}{k_{\text{off}}}C_N. \end{aligned} \tag{33}$$

By combining (28) with (29) at steady-state it can be seen that,

$$C_T = \frac{1}{1-\alpha}C_0. \tag{34}$$

Substituting this expression into equation (33) gives

$$C_N = \frac{k_{\text{off}}}{k_{\text{off}} + \phi}\alpha^N C_T. \tag{35}$$

2.3.1 Calculating the $E_{\max}$ and EC_{50}

As in the kinetic proofreading model, we find that $C_T \to T_T$ as $P_T \to \infty$. It follows that,

$$E_{\max} = \frac{k_{\text{off}}}{k_{\text{off}} + \phi}\alpha^N T_T. \tag{36}$$

Solving for P_T when $R = E_{\max}/2$ we find,

$$\text{EC}_{50} = K_{\text{D}} + T_T/2. \tag{37}$$

2.4 Kinetic proofreading with sustained signalling (Fig. 2J-L)

To include sustained signaling we modify the basic kinetic proofreading model by allowing TCRs that have reached the productively signaling state (C_N) to continue signaling even when pMHC dissociates (T^*). The rate that these productively signaling but unbound TCRs return to the unmodified state is determined by λ.

In this model, T cell activation is determined by both C_N and T^* and therefore $R = C_N + T^*$.

This model is described by the following system of ODEs,

$$\partial P/\partial t = -k_{\text{on}}PT + k_{\text{off}}\sum_{i=0}^{N} C_i - k_{\text{on}}PT^* \tag{38}$$

$$\partial T/\partial t = -k_{\text{on}}PT + k_{\text{off}}\sum_{i=0}^{N-1} C_i + \lambda T^* \tag{39}$$

$$\partial T^*/\partial t = k_{\text{off}}C_N - k_{\text{on}}PT^* - \lambda T^* \tag{40}$$

$$\partial C_0/\partial t = k_{\text{on}}PT - (k_{\text{off}} + k_{\text{p}})C_0 \tag{41}$$

$$\partial C_i/\partial t = k_{\text{p}}C_{i-1} - (k_{\text{p}} + k_{\text{off}})C_i \tag{42}$$

$$\partial C_N/\partial t = k_{\text{p}}C_{N-1} - k_{\text{off}}C_N + k_{\text{on}}PT^* \tag{43}$$

Using equation (40) at steady-state we can express the magnitude of T cell activation as a function of C_N,

$$T^* = \frac{k_{\text{off}}C_N}{k_{\text{on}}P + \lambda}.$$

and therefore,

$$\begin{aligned} R &= C_N + T^* \\ &= \left(\frac{k_{\text{on}}P + \lambda + k_{\text{off}}}{k_{\text{on}}P + \lambda}\right)C_N \\ &= \left(\frac{k_{\text{on}}(P_T - C_T) + \lambda + k_{\text{off}}}{k_{\text{on}}(P_T - C_T) + \lambda}\right)C_N. \end{aligned}$$

The parameter C_N must then be related to C_T so that T cell activation can be determined in terms of the variables T_T, P_T, and K_{D}. This can be done by substituting (41) into (39) to give,

$$-(k_{\text{off}} + k_{\text{p}})C_0 + k_{\text{off}}\sum_{i=0}^{N-1} C_i + \lambda T^* = 0. \tag{44}$$

Using the result,

$$C_T - C_N = \sum_{i=0}^{N-1} C_i = \left(\frac{1-\alpha^N}{1-\alpha}\right)C_0,$$

C_0 can be eliminated from (44) to eventually give,

$$C_N = \left(\frac{k_{\text{on}}P + \lambda}{\lambda + k_{\text{on}}P\alpha^N}\right)\alpha^N C_T. \tag{45}$$

T cell activation can now be determined as a function of C_T:

$$R = \left(\frac{k_{\text{on}}P + k_{\text{off}} + \lambda}{\lambda + \alpha^N k_{\text{on}}P}\right)\alpha^N C_T \tag{46}$$

$$= \left(\frac{k_{\text{on}}(P_T - C_T) + k_{\text{off}} + \lambda}{\lambda + \alpha^N k_{\text{on}}(P_T - C_T)}\right)\alpha^N C_T \tag{47}$$

where $C_T = \left(P_T + T_T + K_{\text{D}} - \sqrt{(P_T + T_T + K_{\text{D}})^2 - 4P_T T_T}\right)/2$.

2.4.1 Calculating the $E_{\max}$ and EC_{50}

As before, the non-dimensionalised variables are $\hat{P} = P/P_T,\ \hat{T} = T/T_T,\ \hat{T^*} = T^*/T_T,\ \hat{C}_i = C_i/T_T$. The non-dimensionalised conservation equations become,

$$1 = \hat{P} + \frac{T_T}{P_T}\hat{C_T} \tag{48}$$

$$1 = \hat{T} + \hat{T^*} + \hat{C_T}. \tag{49}$$

To calculate the $E_{\max}$, equation (46) can be written in terms of non-dimensionalised variables,

$$\begin{aligned} R &= \left(\frac{k_{\text{on}}P_T\hat{P} + k_{\text{off}} + \lambda}{\lambda + \alpha^N k_{\text{on}}P_T\hat{P}}\right)\alpha^N T_T\hat{C_T} \\ &= \left(\frac{k_{\text{on}}P_T\hat{P} + k_{\text{off}} + \lambda}{\lambda + \alpha^N k_{\text{on}}P_T\hat{P}}\right)\alpha^N P_T(1-\hat{P}) \\ &= \left(\frac{k_{\text{on}}P_T\hat{P} + k_{\text{off}} + \lambda}{\lambda + \alpha^N k_{\text{on}}P_T\hat{P}}\right)\alpha^N P_T(1 - \frac{-(K_{\text{D}} - P_T + T_T) + \sqrt{\left(K_{\text{D}} - P_T + T_T\right)^2 + 4P_T K_{\text{D}}}}{2P_T}) \end{aligned}$$

In the limit of excess P_T we find,

$$E_{\max} = \lim_{P_T \to \infty} R = T_T$$

The concentration of P_T at half the maximal response can be found by solving for P_T in the equation $R = T_T/2$. This leads to,

$$T_T/2 = \left(\frac{k_{\text{on}}(P_T - C_T) + k_{\text{off}} + \lambda}{\lambda + \alpha^N k_{\text{on}}(P_T - C_T)}\right)\alpha^N C_T,$$

where $C_T = \left(P_T + T_T + K_{\text{D}} - \sqrt{(P_T + T_T + K_{\text{D}})^2 - 4P_T T_T}\right)/2$, which can be rearranged to give,

$$P_T = \frac{\alpha^N C_T(k_{\text{off}} + \lambda - k_{\text{on}}C_T + k_{\text{on}}T_T/2) - T_T\lambda/2}{\alpha^N k_{\text{on}}(T_T/2 - C_T)}, \tag{50}$$

Note that this expression implicitly depends on P_T through C_T and therefore to determine the EC_{50} this equation must be numerically solved for P_T.

2.5 Summary of analytical results

Definitions: $\alpha = k_p/(k_p + k_{off})$, T_T is the total concentration of TCR, P_T is the total concentration of pMHC, and C_T is the total concentration of bound TCR and is given by $C_T = \left(P_T + T_T + K_D - \sqrt{(P_T + T_T + K_D)^2 - 4P_T T_T}\right)/2$ where $K_D = k_{off}/k_{on}$.

Occupancy (Fig. 2A-C)
T-cell activation is proportional to the concentration of bound TCR-pMHC complexes ($R = C_T$).

$$R = C_T$$
$$EC_{50} = K_D + T_T/2$$
$$E_{max} = T_T$$

Kinetic proofreading (Fig. 2D-F)
T-cell activation is proportional to the concentration of competently signaling TCRs ($R = C_N$).

$$R = \alpha^N C_T$$
$$EC_{50} = K_D + T_T/2$$
$$E_{max} = \alpha^N T_T$$

Kinetic proofreading with limited signalling (Fig. 2G-I)
T-cell activation is proportional to the concentration of competently signaling TCRs ($R = C_N$).

$$R = \left(\frac{k_{off}}{k_{off}+\phi}\right)\ \alpha^N C_T$$
$$EC_{50} = K_D + T_T/2$$
$$E_{max} = \frac{k_{off}}{k_{off}+\phi}\ \alpha^N T_T$$

Kinetic proofreading with sustained signalling (Fig. 2J-L)
T-cell activation is proportional to the concentration of competently signaling TCRs ($R = C_N + T^*$).

$$R = \left(\frac{k_{on}(P_T - C_T) + k_{off} + \lambda}{\lambda + \alpha^N k_{on}(P_T - C_T)}\right) \alpha^N C_T$$
$$EC_{50} = \frac{\alpha^N C_T (k_{off} + \lambda - k_{on} C_T + k_{on} T_T/2) - T_T \lambda/2}{\alpha^N k_{on}(T_T/2 - C_T)}$$
$$E_{max} = T_T$$

3 Co-presentation of peptides

The phenotypic models described above can be modified to predict T cell activation when two different pMHC are presented (Fig. 5). Note that explicit expressions for $E_{\max}$ and EC_{50} are not determined but instead we numerically solve for the predictor of T cell activation (R) in each case.

3.1 Occupancy model

When two pMHC are co-presented (denoted as P_1 and P_2), we have the following equilibrium equations,

$$P_1T = k_{1D}C_{1T} \tag{51}$$
$$P_2T = k_{2D}C_{2T}. \tag{52}$$

and conservation equations:

$$P_{1T} = P_1 + C_{1T} \tag{53}$$
$$P_{2T} = P_2 + C_{2T} \tag{54}$$
$$T_T = T + C_{1T} + C_{2T} \tag{55}$$
$$C_T = C_{1T} + C_{2T}. \tag{56}$$

Equations (51) and (52) can be combined to give:

$$k_{1D}k_{2D}(C_{1T} + C_{2T}) = k_{1D}P_2(T) + k_{2D}P_1(T) \tag{57}$$
$$k_{1D}k_{2D}C_T = k_{1D}P_2(T_T - C_T) + k_{2D}P_1(T_T - C_T). \tag{58}$$

Equations (51), (53) and (55) can be used to give the expression,

$$P_i = \Big(\frac{k_{iD}}{k_{iD} + T_T - C_T}\Big)P_{iT}. \tag{59}$$

This can be substituted into (58) to eventually give:

$$(k_{1D} + T_T - C_T)(k_{2D} + T_T - C_T)C_T =$$
$$(k_{1D} + T_T - C_T)(T_T - C_T)P_{2T} + (k_{2D} + T_T - C_T)(T_T - C_T)P_{1T}.$$

This can be simplified as follows,

$$\begin{aligned} &C_T^3 - \big(P_{1T} + P_{2T} + 2T_T + k_{1D} + k_{2D}\big)C_T^2 \\ &+ \big((k_{1D} + T_T)(k_{2D} + T_T) + P_{2T}(2T_T + k_{1D}) + P_{1T}(2T_T + k_{2D})\big)C_T \\ &+ P_{2T}(k_{1D}T_T + T_T^2) + P_{1T}(k_{2D}T_T + T_T^2) = 0. \end{aligned} \tag{60}$$

To determine C_T we numerically solve this cubic equation using the Matlab (Mathworks, MA) function *fsolve*. In order to check this derivation, it can be seen that when $P_{2T} = 0$ equation (60) becomes:

$$\begin{aligned} &C_T^3 - \big(P_{1T} + 2T_T + k_{1D} + k_{2D}\big)C_T^2 + \big((k_{1D} + T_T)(k_{2D} + T_T) + P_{1T}(2T_T + k_{2D})\big)C_T + P_{1T}(k_{2D}T_T + T_T^2) \\ &= (C_T - k_{2D} - T_T)(C_T^2 - (k_{1D} + P_{1T} + T_T)C_T + T_TP_{1T}) \\ &= 0. \end{aligned}$$

Since $C_T \leq T_T$ we can see that $C_T = k_{2D} + T_T$ cannot be a solution and therefore,

$$C_T^2 - (k_{1D} + P_{1T} + T_T)C_T + T_TP_{1T} = 0, \tag{61}$$

which is the equation for C_T for a single peptide, as in (17).

3.2 Kinetic proofreading

Now that an expression C_T as a function of P_{1T}, P_{2T}, k_{1D}, k_{2D} and T_T has been found, it can be seen using (51) and (55) that:

$$C_{iT} = \frac{P_{iT}(T_T - C_T)}{k_{iD} + T_T - C_T}. \tag{62}$$

And so from (19), it can be seen that the amount for each peptide in the final signalling state C_{iN} is,

$$C_{1N} = \alpha_1^N C_{1T} \tag{63}$$
$$C_{2N} = \alpha_2^N C_{2T} \tag{64}$$

where $\alpha_i = \frac{k_{\mathrm{p}}}{k_{\mathrm{p}}+k_{\mathrm{offi}}}$. Therefore, T cell activation in this model can be determined as follows,

$$R = C_{1N} + C_{2N}. \tag{65}$$

3.3 Kinetic proofreading with limited signalling

Assuming that the rate ϕ of transferring from the productively signaling state (C_N) to the inert state (C_N^-) is the same for both pMHCs, then following from (35) and (62) it can be seen that,

$$C_{1N} = \frac{k_{\mathrm{off1}}}{k_{\mathrm{off1}} + \phi}\alpha_1^N C_{1T} \tag{66}$$
$$C_{2N} = \frac{k_{\mathrm{off2}}}{k_{\mathrm{off2}} + \phi}\alpha_2^N C_{2T}. \tag{67}$$

and as before, T cell activation in this model can be calculated using,

$$R = C_{1N} + C_{2N}. \tag{68}$$

3.4 Kinetic proofreading with sustained signalling

In this model, T cell activation is determined as follows,

$$R = C_{1N} + C_{2N} + T^*. \tag{69}$$

The expressions for C_{1N} and C_{2N} are,

$$C_{1N} = \left(\frac{k_{\mathrm{on}}(P_{1T} - C_{1T}) + \lambda}{\lambda + \alpha_1^N k_{\mathrm{on}}(P_{1T} - C_{1T})}\right)\alpha^N C_{1T} \tag{70}$$
$$C_{2N} = \left(\frac{k_{\mathrm{on}}(P_{2T} - C_{2T}) + \lambda}{\lambda + \alpha_2^N k_{\mathrm{on}}(P_{2T} - C_{2T})}\right)\alpha^N C_{2T}. \tag{71}$$

In order to find the expression for T^* we use the equivalent of equation (40) when two pMHC are presented,

$$\partial T^*/\partial t = k_{\mathrm{off1}}C_{1N} + k_{\mathrm{off2}}C_{2N} - k_{\mathrm{on}}(P_1 + P_2)T^* - \lambda T^*.$$

At steady-state we find,

$$T^* = \frac{k_{\mathrm{off1}}C_{1N} + k_{\mathrm{off2}}C_{2N}}{k_{\mathrm{on}}(P_{1T} - C_{1T} + P_{2T} - C_{2T}) + \lambda}. \tag{72}$$

References

1. P. Francois, G. Voisinne, E. D. Siggia, G. Altan-Bonnet, and M. Vergassola. PNAS Plus: Phenotypic model for early T-cell activation displaying sensitivity, specificity, and antagonism. *Proceedings of the National Academy of Sciences*, Feb. 2013.

Supplementary Figure

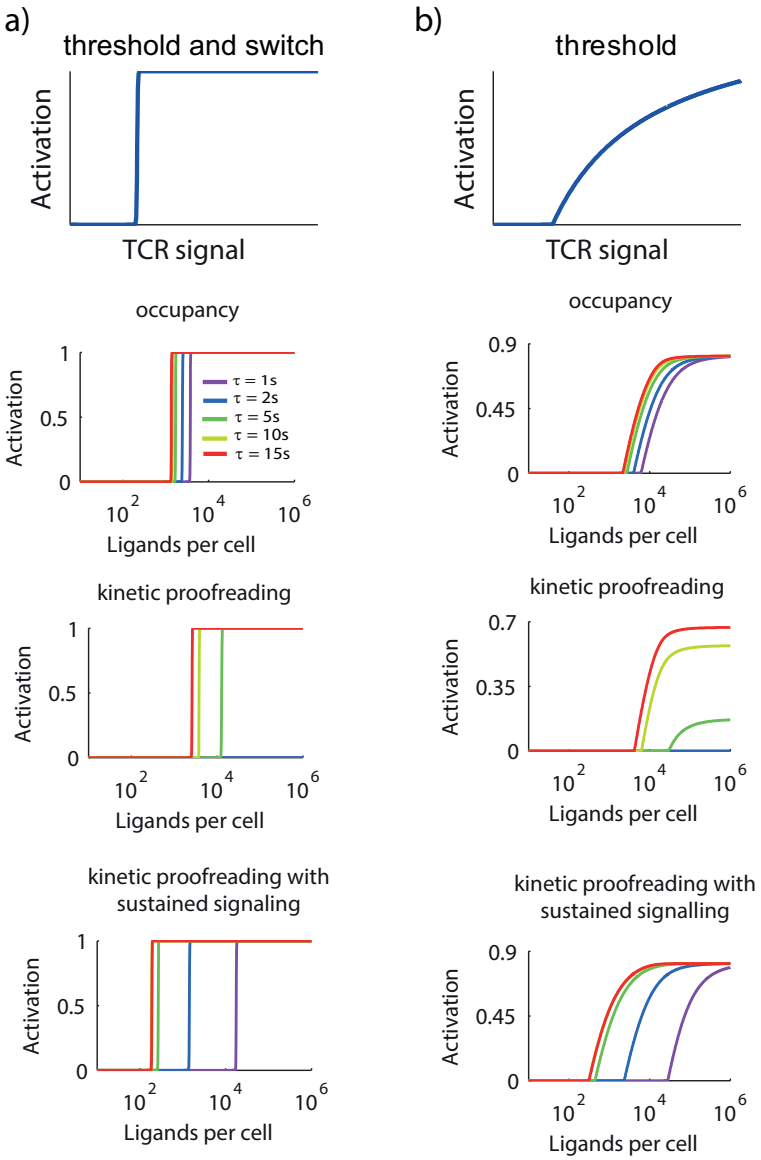

Figure S1: Effect of thresholds and switches on phenotypic models not shown in Fig. 4.

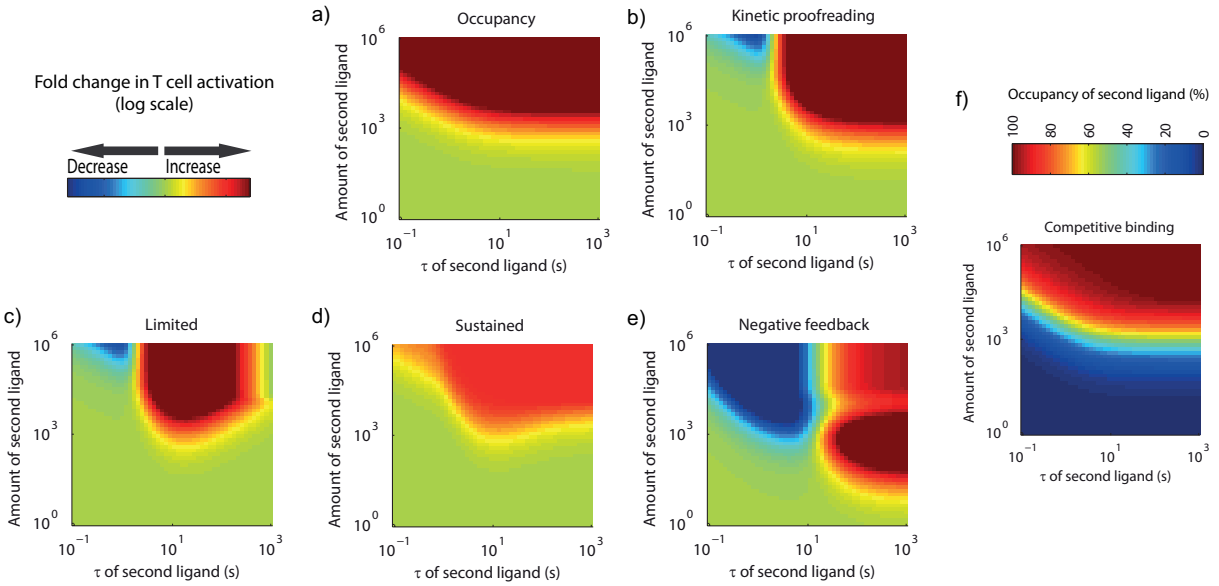

Figure S2: Effect of co-presentation of a second pMHC on T cell activation. A-E) Shown are heat maps of the fold change in T cell activation (on a log-scale) over the dissociation time (x-axis) and the number of ligands (y-axis) for the second pMHC. The first ligand is presented at a fixed number of 1000 per cell with a dissociation time of 10 s. F) Shown is the percentage of TCRs occupied by the second ligand. The calculations shown in Fig 6 are slices of these heat maps when the second pMHC is presented at 1000 ligands per cell.