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On Rare Events in T Cell Activation

Janak R. Wedagedera

Department of Mathematics, University of Ruhuna, Matara, Sri Lanka.
Correspondence: *janak@maths.ruh.ac.lk*

Abstract

T cell activation is a stochastic process occurring over a finite time interaction with an antigen presenting cell (APC). The T cell commits to activation based on a finite number of T cell receptor phosphorylation events; the high specificity and sensitivity of T cells indicating that information on ligand quality can be extracted with precision. We examine a mathematical model for T cell activation based on a number of criteria, or strategies, that utilise the history of TCR phosphorylation to discriminate ligands. We justify our model using experimental data in a situation where one of the key co-receptors on the T cell surface, namely the CD4 receptor is blocked. A dual threshold-strategy with a temporal treatment turns out to perform better in discriminating agonist peptides than a single-threshold strategy for T cell's activation. We employ the Wentzell-Friedlin theory for large deviations in stochastic processes [5, 13] to determine the activation probabilitites.

Introduction

Background immunology of T cell activation

The activation of T lymphocyte helper cells through interaction of their T-cell receptors (TCRs) with antigenic peptide bound to major histocompatibility complex (MHC) is a crucial step in adaptive immunity. This still remains unclear at least to the extent of explaining various temporal and spatial functions (events) associated with the signals received by the T cell [7]. The primary focus on this problem to-date is on two related aspects: namely, (i) the spatial organisation of the signalling molecules within the cell-cell interface (i.e. the interface between T cell and the Antigen Presenting Cell (APC)), the immunological synapse [7, 4, 9], and (ii) the recruitment and connectivity structure of the signalling network. What we mean here by *activation* needs to be further clarified: the final goals of the activation with regards to a T helper cell are cell proliferation and differentiation that lead to production of the so called *effector/memory* cells capable of launching attacks on the invading pathogens *specific* to the

T cell. However, these tasks are not achieved as a response to just a single threshold of the signal strength the T cell received as a result of the interaction with the pMHC (the complex of molecules consisted of a peptide and an MHC molecule). Instead, the final states of the functionality could result from a sequence of reactions or perhaps many parallel sequences of reactions that take place upon the initial APC 'T' cell contact. Depending on the strength of the signal the T cell receives in its time course for achieving final goals, there are many other tasks it has to fulfil on its way for the full activation. Any of these tasks or functions can be defined as some kind of activation. If one of the components that assists the T cell to achieve its final goal is absent, activation of some of the functions may be delayed or completely stopped. If the peptide being presented to the T cell is *not specific*, or if it is *partially specific* to the T cell it would de-conjugate from the APC after a certain duration. Thus, we characterise this specific conjugation of the pMHC and the T cell by the *rate of dissociation* of the pMHC (or simply of the peptide) from the bound TCR. When one talks about specific and non-specific peptides this essentially refers to lower or higher values of dissociation rate of the peptides respectively, which we denote by k_{off} (which is inversely proportional to the duration of the conjugation).

Signal transduction in the T cell

Signal transduction refers to the process by which extracellular events or cues are transmitted via a receptor or multiple receptors to the interior of the cell. Many of the current principles in the study of signal transduction have arisen from the study of various growth factor receptors [10]. The main signalling molecule involved in this process is the T cell receptor which functions together with it's integral components CD3, γ, δ, and ε and the TCRζ chain dimer. [12] (see the Figure .1).

The cytosolic components of these molecules contain a unique motif, the ITAM (immunoreceptor based tyrosine activation motif) which contains binding sites namely, tyrosine residues that become rapidly phosphorylated when the T cell receptor binds to a ligand [1]. This results recruitment of additional molecules to ITAM sites such as Fyn, Lck and ZAP70. Phosphorylation of ZAP70 activates several other proteins to bind and activate which finally leads to the activation of the gene-transcription factors such as NF-κ,β, NF-AT which are responsible for cell proliferation and differentiation. For an extensive and overall discussion about the full functionality understood to-date we refer the reader to [8] and the references therein.

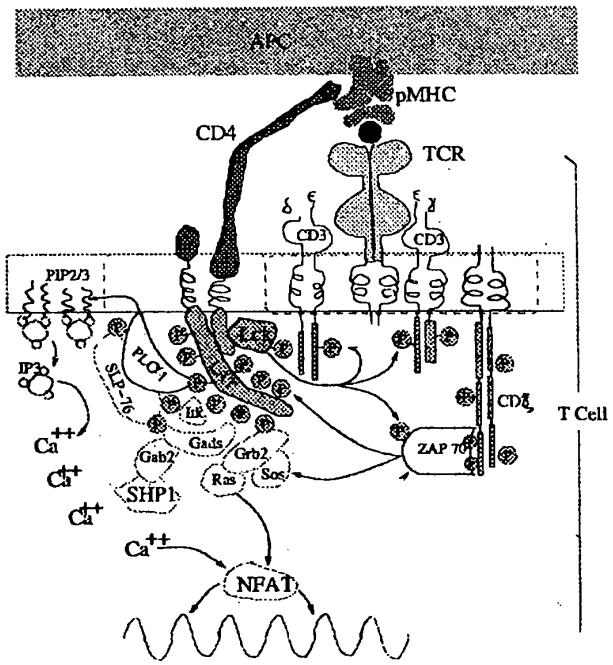


Figure .1: A simplified model for T cell APC interaction and signalling pathways based on various experimental data to-date. The T cell receptor (TCR) consists of the two α and β chains which associate with a $\delta\varepsilon$ dimer, a $\gamma\varepsilon$ dimer and a TCRζ dimer. The circled 'P's represent the ITAM sites that undergo phosphorylation upon TCRs encounter with a peptide. CD4 binding to the MHC-II results the recruitment of SRC kinase Lck to CD4's cytoplasmic tail. Lck in turn interacts with the ITAMs in CD3 dimers which results the recruitment of the ZAP70 to doubly phosphorylated ITAMs on the ζ chains of the TCR-CD3 complex. ZAP70 activates several other proteins those responsible for releasing Ca^{++} (or calcium signals as we call here) which activates the gene transcription factors such as NFAT. As we see even in this highly simplified model, modelling of each and every phosphorylation makes the modelling a tedious task so we simply take all phosphorylations as a sequence of *m* reactions without specifically referring to any of the intermediate components. Furthermore, we assume all these reactions are reversible including binding and unbinding of the pMHC to the TCR.

Role of CD4 receptor

Particularly important is the role of the co-receptor CD4 in signal transduction through the T cell. CD4 enhances the response of specific T cell both by stabilising the pMHC-TCR complex through binding to the MHC-II molecule and by bringing a tyrosine kinase (Lck) into the proximity of the cytoplasmic tails of the CD3 molecule and zeta proteins (ZAP70) (see Figure .1). If the CD4 receptor is blocked as done in the experiments [2], weaker responses from the T cell is to be expected. (It should also be noted here that it is CD4 receptor which provides the docking site for the HIV. T cell activation in such a situation turns out to be a rare event if the number of agonists presented is also too low. This is clear from the results reported in [2] where it is estmated a 10% of probability of activation (say to some function

as a response to the level of the Ca^{++} signal for instance) when the number of agonist pMHC is about 1-10. This ratio increases to about 25% when the agonist pMHC number is increased to 11-25. To achieve a profound response comparable to the CD4 unblocked situation the agonist number has to be increased more than 25 according to these results (see Figure .1). In a situation where the available ligand number is highly limited and when the functionality of the CD4 is also blocked (due to a disease say) then it would be useful to study the temporal behaviour of the T cell's response. An important question to ask is how many *signalling* receptors on average would be required to achieve *an activation* in such a situation.

Defining the activation and specifying the key problem

The main problem we investigate in this paper is the following: *suppose the number of agonists M available to the T cell is a constant. Then what will be the T cell's best strategy for it's activation if it is to use a minimum number of TCRs for signalling and to make a decision within a given duration of time ?*
Moreover, we define the activation of the T cell in the present context as to perform a particular function (e.g. initial synapse formation, recruitment of adaptor proteins, activation of kinases and initial calcium signalling etc) as a response to a certain level of the signal (threshold) it receives.

Results in the present study

It turns out from our analysis that within 12 minutes from the initial APC T cell contact when there are 128 signalling TCRs in the APC T cell interface, the T cell becomes activated with 10% probability in the absence of the CD4 molecule (let us call this situation *strategy* S_1). On the other hand if the number of signalling TCRs is about 118 by the first 12 minutes and if this number continues to be the same or higher for another 1 hour duration, then the T cell may still become activated with the same probability in the absence of the CD4 molecule. (let us call this situation *strategy* S_2). Our main concern in this paper shall be to analyse these two scenarios. The results obtained in the strategy S_1 are of very good agreement with the experimental results reported in [2] (see the Table 1) which is an indication of the validity of our modelling methodology. Strategy S_2,which serves as a prediction of our model, is proven to be a better strategy than S_1.

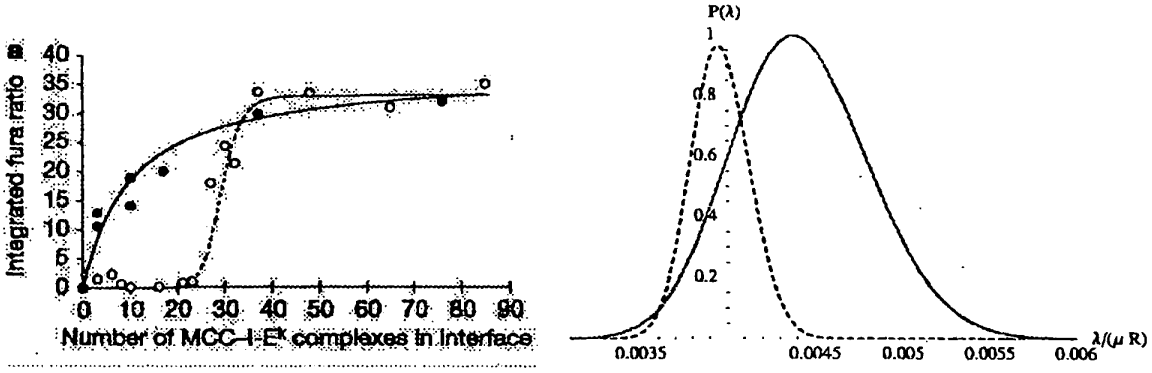


Figure .1: **Left:** Adapted from [2]. Effect of blocking CD4 by antibodies. Integrated fura ratio measures the strength of Ca^{++} signalling. CD4 blocked data are shown in unfilled circles and those unblocked are shown in filled circles. About 30 agonist pMHC are required to achieve the same performance for the CD4 blocked situation whereas this number is between 10-25 when the CD4 is unblocked over the same time course (i.e. about 10-12 minutes). **Right:** Probability of activation as a function of λ which is the rate at which the TCRs 'join' in a pool of signalling TCRs obtained via the present model. The solid line corresponds to the strategy S_1 (after 12 minutes from the initial cell-cell contact) described in the text and the dashed-line to the strategy S_2 (after 1 hour from the initial cell-cell contact). Notice the peaked behaviour in the second case which shows the T cell's highly specific response to agonists that take place after about 1 hour from initial contact with the APC. In contrast the T cell responds to a broad range of agonists within the first 12 minutes.

Condition	Number of agonists	% of activation
CD4 blocked	1-10	0.091
	11-25	0.25
	>25	100.00

Table 1: Results reported in [2] for T cells with CD4 blocked.

Modelling temporal functions

For the sake of convenience in our modelling process, we shall assume the ITAM phosphorylation is an $m+1$ step reaction process in which each step has a forward reaction rate k_{on}. Thus, we do not refer to any specific ITAM in this set-up but treat the TCR-pMHC complex as a whole which undergoes $m+1$ phosphorylations (associated with all relevant ITAMs), which finally will give rise to certain strength (threshold) in the overall signal received by the T cell.

We model T cell's ability to achieve a threshold capable of producing some function (e.g. sustained Ca^{++} signalling) within given time T and furthermore model it's ability to hold the

already attained threshold for some duration of time, say ΔT.

Given the complexity of the activation process, our model clearly cannot address all of the phenomena; however our aim is to generate a broader approach to the discussion of signalling which emphasise the role of the signal history. It should be noted here that we do not analyse the events happening during the first few seconds up-to first 10-12 minutes in this paper as those events usually happens with higher probabilities. (A separate analysis has been done to address these problem by us in [20] and will be published elsewhere).

Suppose the phosphorylated pMHC+TCR complexes dissociate with rate k_{off} and downregulate from the cell surface with rate proportional to the number of triggering TCRs at time t (say $x(t)$). The present approach is to construct a queueing model of TCR triggering, incorporating input of phosphorylated TCRs (from a kinetic proof reading scheme we have a Poisson input rate for this which is a function of the dissociation rate k_{off}), and output via TCR downregulation. This directly accounts for the noise associated with signalling based on a finite number of signalling molecules. Here we use this model (an Erlang queue) to analyse the two activation strategies which we have already discussed.

In this analysis our main tool is a large deviation methodology (path analysis by Wentzell-Freidlin theory) [13] which allows us to determine the most probable way (path) a rare event can happen. We shall compute the *probability of activation* of a certain event A at given time T, denoted by $\mathbb{P}_{\text{act}}[A]$ using this approach. This aids understanding and analysis of the behaviour of the *activation probabilities* with respect to variation of parameters, for instance providing analytically tractable approximations.

Criteria for activation

As it has been explained earlier, we consider the following two scenarios:

(S_1) First achieving a signal strength (a threshold), (say α_1) within the given time T.

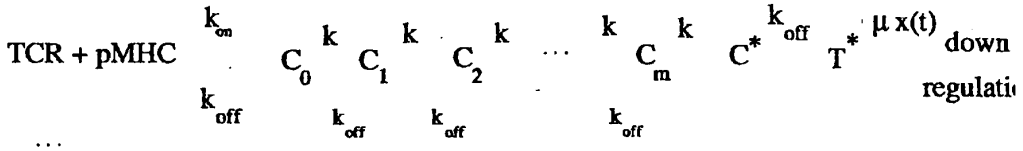
(S_2) Having achieved a threshold (say α_2) within the given time T and then holding the threshold for a further duration ΔT.

By the signal strength we mean the total signal the T cell receives due to the *triggering* TCRs (i.e. signalling TCRs). We denote by T^* a triggering TCR. We assume that in order for a TCR to trigger, a pMHC-TCR complex has to undergo all m phosphorylation reactions end of which a TCR dissociates with rate k_{off} from the pMHC complex and join in a *pool* of

T^*. However, a TCR may dissociate from the complex at any of the $m+1$ steps of phosphorylation reactions. We also analyse T cell's discrimination ability for different peptides characterized by their dissociation rates k_{off} in each of the above strategies. (when we say the dissociation rate of the peptide, this actually means the dissociation rate of the pMHC complex). We wish to determine the above thresholds (cellular level) in a situation where the probability of activation (with respect to these thresholds) are assumed to low (about 0.1) (which is indeed the case as described in the experimental results [2]).

Initiating TCR signalling using a kinetic proof reading scheme for phosphorylation of the ITAMs

Following the kinetic proof reading scheme [11, 6], we consider an $m+1$-step ITAM phosphorylations: TCRs react with pMHC with forward rate k_{on} and backward rate k_{off}. Phosphorylated TCR-pMHC complexes dissociate with rate $\lambda = k_{\text{off}}C^*$ into signalling TCRs denoted by T^* which finally downregulate with rate $\mu x(t)$ ($x(t)$ is the concentration of T^* s accumulated in the final step and $C^*(t)$ denotes the concentration of fully phosphorylated pMHC-TCR complexes at time t). Thus, we have



Assuming the steady-state in the sequence of reactions $C_0, \ldots, C_m$ we readily obtain the total number of p-MHC complexes M_{total} (i.e. free + bound to TCRs and not fully phosphorylated + bound to TCRs and fully phosphorylated):

$$M_{\text{total}} = M + \sum_{i=1}^{m} C_i + C^* \tag{.1}$$

$$= C_0 \left[2\left(k_{\text{off}} + 0.1(m+1)\right) + \frac{1-a^{m+1}}{1-a} + \frac{0.1(m+1)a^m}{k_{\text{off}}} \right] \tag{.2}$$

where $a(k_{\text{koff}}) = \frac{1}{1+\frac{k_{\text{off}}}{k}}$ and, the letters themselves denote the concentrations of the species as well. Following experimental data [16, 14, 17] we take the TCR concentration= $100\mu m^{-2}$, $k = (k_{\text{off}})_{\text{opt}}(m+1)$ with $(k_{\text{off}})_{\text{opt}} = 0.1s^{-1}$ and $k_+ \approx (k_{\text{off}})_{\text{opt}}/20$. Now considering the

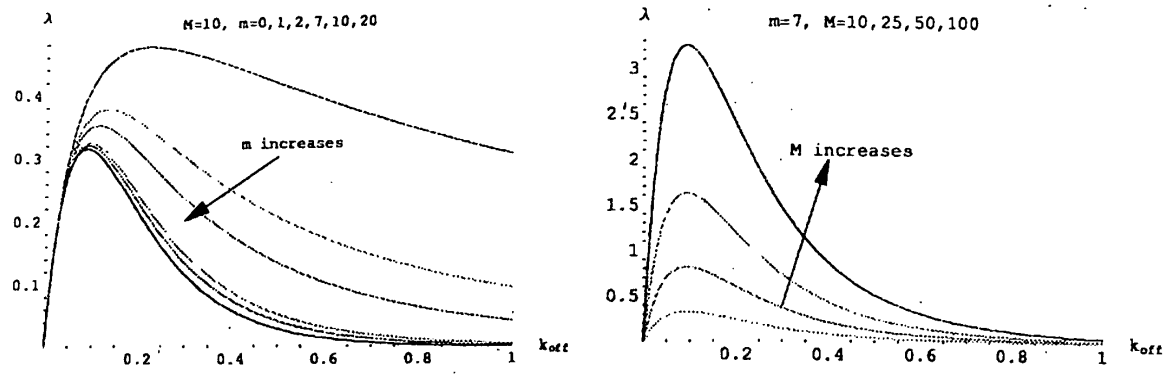


Figure .1: λ vs k_{off}. Left: number of peptide-MHC complexes M is fixed(=10). As the number of reaction steps increases production rate of phosphorylated TCRs, i.e. λ assumes its peak at the chosen optimal value for dissociation rate $k_{\text{off}} = 0.1s^{-1}$. Right: On the other hand with the number of reaction steps m fixed at $m = 7$, more peptide-MHC complexes results a higher production rate at the optimal k_{off}.

reaction $C^* \xrightarrow{k_{\text{off}}} T^*$, we have

$$\lambda = k_{\text{off}} C^* = a^m k C_0, \tag{.3}$$

and so

$$\lambda = \frac{0.1(m+1)a^m M_{\text{total}}}{2\left(k_{\text{off}} + 0.1(m+1)\right) + \frac{1-a^{m+1}}{1-a} + \frac{0.1(m+1)a^m}{k_{\text{off}}}}. \tag{.4}$$

The above calculation for λ is required to determine its appropriate range for the computations in the next section (see Figure .1).

In particular, if we assume the phosphorylation of the ITAMS takes place immediately (with no intermediate steps so $m = 0$), then we have, $\lambda_{m=0} = 0.1M_{\text{total}}/\left(2(k_{\text{off}} + 0.1) + 0.1/k_{\text{off}}\right) \to 0$ as $k_{\text{off}} \to \infty$ which means larger the k_{off} the faster the unbounding as one would expect.

Modelling the signalling TCRs as a queue

After achieving full phosphorylation (C^*), pMHC unbinds from the T*s at rate k_{off}. However, the remaining complexes may also contribute to the signalling, as well as the unbound T*s themselves, implying that for the activation of the T-Cell, at this stage, we have to consider both of these contributions. Nevertheless, we make the (realistic) assumption that $k \ll k_{\text{off}}$ or $C^*(t) \ll x(t)$. Thus, we only consider the signals received by unbound T*s.

Since full phosphorylation of a TCR happens in exponential time, we can consider this as a Poisson event. So, the present scenario is equivalent with these assumption to a queue with Poisson input $\lambda = k_{\text{off}}|C^*|$ ($|C^*|$ =# fully phosphorylated pMHC-TCR complexes) and

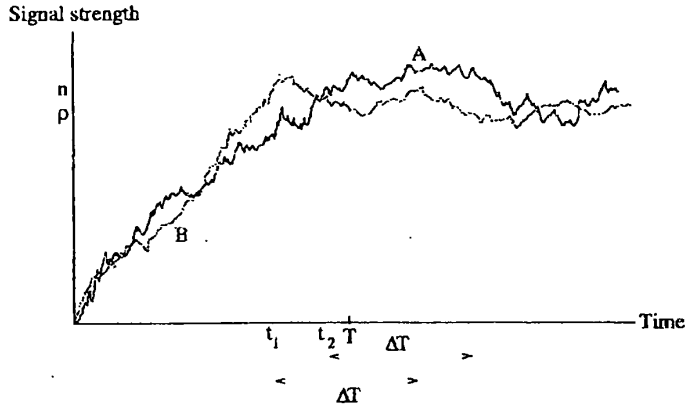


Figure .1: Sample paths for the process $x(t)$. ρ is the equilibrium value of the number of phosphorylated TCRs, n is a threshold which is to be determined for the given probability that the signal strength first exceeds n at time $0 < t \leq T$ for some given T. In relation to the first strategy S_1, path B attains the threshold n within time $t_1 < T$ before the path B does so. On the other hand regarding the strategy S_2, sample path A fails to hold the threshold n for a duration ΔT whereas the path A fulfils both conditions in S_2.

Poisson output with rate $\mu x(t)$:

$$\xrightarrow{\lambda = k_{off}|C^*|} \boxed{x(t)} \xrightarrow{\mu x(t)} \text{down-regulation.}$$

The process $x(t)$ is a jump Markov process which is the difference between two independent one-dimensional Poisson processes with input rate λ with forward jump size $+1$m and output rate $\mu x(t)$ with backward jump size also $+1$. (see Figure .1).

Activation probabilities

Let R be the total number of TCRs (bound and unbound to pMHC complexes) and define $n_1 = \alpha_1 R$, $n_2 = \alpha_2 R$. We rewrite the two strategies S_1 and S_2 in terms of associated probabilities for given T and ΔT:

(S_1) Estimate $\mathbb{P}[x(t) < n_1 \text{ for } t < t', x(t') = n_1 | x(0) = 0, 0 < t' < T]$ which is the *probability of first crossing* the level n_1 which is required to occur within time T.

(S_2) Estimate $\mathbb{P}[x(t) \geq n_2, T \leq t \leq T + \Delta T | x(0) = 0, x(t') = n_2, 0 < t' \leq T]$ which is the probability that the process $x(t)$ first crosses the level n_2 within time T and then being able to stay above n_2 until time $T + \Delta T$.

Since exceeding the equilibrium value ρ of the system is indeeed rare, the T cell activation for a weak agonist can be considered as a rare event if the T cell is required to achieve a threshold

above the equilibrium signalling level ρ. However, rare events occur in the *most probable* way. Thus, we look for the most probable path that $x(t)$ will use to escape from the attractor (i.e. ρ) to reach the level α_i $(i = 1, 2)$. To solve this problem we appeal to Wentzell-Friedlin theory to compute the escape probabilities using large deviation theory [5, 13].
In order to estimate the above probabilities we consider the scaled process $z_R(t)$ defined by $z_R(t) := x(t)/R$. Notice that for the scaled process the incoming rate is $\gamma := \lambda/R$, the outgoing rate is just $z(t)$ where R is the total number of TCRs in the system.]What we have is exactly a standard Erlang queue [13]. So, the scaled process $z_R(t)$ has jump size $+1/R$ with rate γR and jump size $-1/R$ with rate z in the directions indicated by the sign of the jump size. Thus we have $\mathbb{P}[x(t) \leq n_1] = \mathbb{P}[z_R(t) \leq \alpha_i]$ and $\mathbb{P}[x(t) \geq n_2] = \mathbb{P}[z_R(t) \geq \alpha_2]$ to rewrite the strategies S_1 and S_2 for the scaled process.

Large deviation approximation

Large deviation theory tells us that the probabilities associated with rare events decay exponentially with rate given by the so called rate function I (depending on parameters for instance T, α_i, λ, and ΔT in this particular example). It estimates the rare probabilities as [18, 3]

$$\mathbb{P}[z_R(t) \geq \alpha_i] \approx \mathrm{e}^{-RI}. \tag{.1}$$

Thus, we can obtain an estimate for the above probability if we know the rate function I. Applying Friedlin-Wentzell theory to find the most probable path using large deviations we find that the most probable path is the one which minimises the associated cost in reaching the threshold α_i where the cost is defined by $\int l(r(t), r'(t))dt$ for any admissible path $r(t)$. Here $l(x, y)$ is known as the *local rate function* which is given by

$$l(r, r') = r' \log \frac{r' + \sqrt{r'^2 + 4\gamma r}}{2\gamma} + \gamma + r - \sqrt{r'^2 + 4\gamma r}$$

for any admissible path $r(t)$ of the process $x(t)$ (see [19]). Then the minimum cost is obtained via solving the variational problem given by

$$I(\alpha_i) = \min_{r(t)} \int_0^{t_i} l(r(t), r'(t))dt \tag{.2}$$

from which we determine the rate function required in (.1). Here $t_1 = T$ and $t_2 = T + \Delta T$ and $\gamma := \lambda/(\mu R)$. This variational problem is solved in [19].

Estimating the thresholds via most probable paths

We use the following parameter values from experimental data [16, 14, 17] to *calibrate* our model system: $\mu = 0.003(s^{-1})$, $T = 12(min)$, $\Delta T = 1(hour)$, $\mathbb{P}_{\text{act}} = 0.1$ and $R = 30{,}000$.

.1 Strategy S_1

The solution to this part is almost identical to that for the standard Erlang's queueing model given in [13]. However we prove [19]

Proposition 1 *In strategy S_1 the most probable path for $x(t)$ which first attains the threshold n_1 within T is given by*

$$r(t) = k_b(e^t - 1) + \gamma(1 - e^{-t}), \quad k_b = \frac{\alpha_1 - \gamma(1 - e^{-T})}{e^T - 1}. \tag{.1}$$

Since the above path is the minimiser of (.2), substituting this in the same we find

$$I_T(\alpha_1) := I(\alpha_1) = e^{-T}\left(e^{2T} - \gamma\right)\log\left(1 + \frac{e^T k}{\gamma}\right) + \gamma T - k(T + e^T - 1) + (k - \gamma)\log\frac{\gamma}{k + \gamma e^{-T}}. \tag{.2}$$

This enables us to estimate the required probability via (.1). In fact, using $\mathbb{P}_{\text{act}} = 0.1$ we can now determine the required threshold by solving

$$\mathbb{P}_{\text{act}} = 0.1 = \exp\{-RI(\alpha_1)\}$$

numerically for α_1. The numerical solution to this yields $\alpha_1 \approx 0.00426935$ which lies above the equilibrium level of the signal strength as anticipated (see Left graph of Figure .1).

.2 Strategy S_2

In the second strategy S_2, we are interested in finding the most probable path for the system after exiting the level α_2 within time T and reaching a level $\beta \geq \alpha$ at time $T + \Delta T$ with $r(t) \geq \alpha_2$ for all $t \in [T, T + \Delta T]$ with $r(T) = \alpha_2$ and $r(T + \Delta T) = \beta$.

As the system's behaviour after reaching the level α_2 is independent of its behaviour on $[0, T]$ we split the problem into the two domains: solution on $[0, T]$ and the solution on $[T, T + \Delta T]$. The solution on the first half is identical to that in S_1 but with a different threshold α_2. So

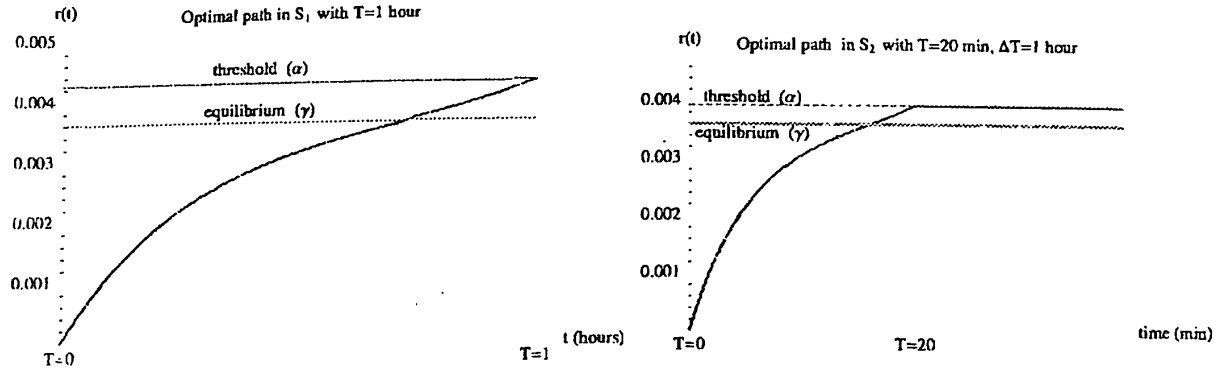


Figure .1: Optimal paths $r_1(t)$ (Left) and $r_2(t)$ (Right) in strategies S_1 and S_2 respectively obtained by numerical solutions. The threshold (which is proportional to the number of triggering TCRs) required in S_1 is about 0.000426935 and that required in S_2 is about 0.0039345 (which gives 128 and 118 as triggering TCR numbers respectively).

we need to determine the optimal path on $[T, T+\Delta T]$. Specifically, we need to solve the variational problem to determine the cost associated on $[T, T+\Delta T]$ given by

$$I_{\Delta T} := \min_{r(t)} \int_T^{T+\Delta T} l(t, r(t), r'(t))dt \tag{.3}$$

with $l(t, r, r')$ being the same rate function found in (.2).
As in the previous case we prove [19]

Proposition 2 *Let $r(t) \geq \alpha_2$ for $t \in [T, T+\Delta T]$ such that $r(T) = \alpha_2$ and $r(T+\Delta T) = \beta$. Then the optimal path for the variational problem (.3) is given by $r(t) = \alpha_2$.*

The second proposition in fact simply says that the minimum cost path to stay above the level α_2 is the one just moves on α_2. This means the total cost associated in the second strategy is given by

$$I(\alpha_2) = I_T(\alpha_2) + I_{\Delta T}(\alpha_2) \tag{.4}$$

where $I_T(\alpha_2)$ is given by (.2) and $I_{\Delta T}(\alpha_2) = \Delta T \left(\sqrt{\alpha_2} - \sqrt{\gamma}\right)^2$.
Taking activation probability $\mathbb{P}_{\text{act}} \approx 0.1$ as before we find

$$\mathbb{P}_{\text{act}} \approx 0.1 = \exp\{-n[I_T(\alpha_2) + I_{\Delta T}(\alpha_2)]\}$$

and the numerical solution to this (for α_2) gives $\alpha_2 \approx 0.0039345$ (see Left graph of Figure .1). Figure .1 shows plots of the optimal paths obtained via Propositions 1 and 2 for the parameter values obtained via the experimental data [2, 15].

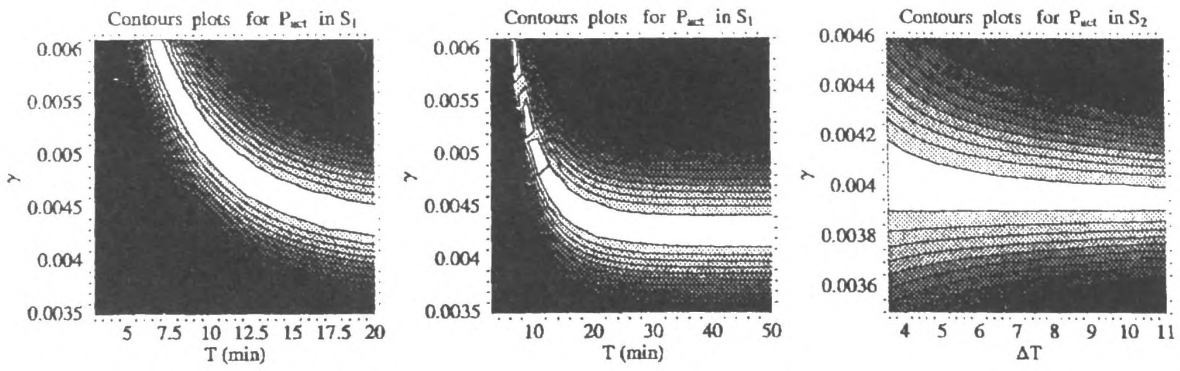


Figure .1: Lighter the area higher the value of the probability. If the T cell is to follow the strategy S_1, it shows a broader response to antigens characterised by the parameter γ with very high probability (almost 1) of activation (Left) and as the T cell waits longer this response however gets narrower (Middle). In contrast, strategy S_2 (Right) shows a very limited response to antigens as the duration the threshold is to hold is further increased. Notice the highly specific response in this case.

Comparison of the two strategies

We compare the two strategies by analysing T cell's response in both cases to antigen variability characterised by γ (which nonlinearly depend on k_{off} via (.4)). We shall consider two measures to compare the specificity, sensitivity and the discrimination. (in fact, how specific the T cell in the two strategies is already clear from the probability contour plots). However, the quantity $-\log(\mathbb{P}_{\text{act}})$ will provide a clear measure for sensitivity and the specificity. A better index to measure the discrimination ability would be the slope of $\log(\mathbb{P}_{\text{act}})$ with respect to the parameter γ. It is clear from the probability contour plots (Figure .1) that the strategy S_2 tends to peak it's response to a very limited range of agonists thus by giving the T cell a switch-like behaviour. This happens as the duration ΔT during which the threshold is uphold, is further increased up-to 1 hour. In contrast to S_1 the strategy S_2 requires less number of triggering TCRs in order to maintain the activation probability of 10% with 10 agonists. Moreover, it is further clear from Figure .2 that the strategy S_2 discriminates agonists better than strategy S_1 and also it is highly specific to agonists characterised by $\gamma \approx 0.0038$ (See Figure .1).

Discussion

The emphasis of this paper is that a single threshold criterion significantly under-performs on peptide discrimination (sensitivity and specificity) relative to the two stage strategy. At

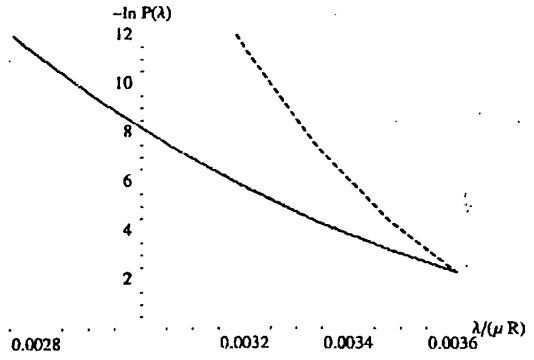


Figure .2: log-probabilities in the two strategies. Steeper the curve better the specificity and the discrimination to agonists in which sense the strategy S_2 performs better.

a simplistic level, the length of the scanning time T determines the discrimination potential. Therefore an initial poorly discriminatory stage can be used to decide if the interaction is of sufficient interest to implement a longer more discriminatory interaction. This two stage process therefore achieves rapid throughput, the majority of cells only have a nonspecific short duration interactions, while allowing for high sensitivity and specificity. Kinetic proof reading based on a single threshold, by contrast, can either have high sensitivity or specificity, but not both.

It should also be noted here that as we are only interested in activation of the T cell under rare circumstances (in a situation where the CD4 is not functioning), a large deviation analysis would be inappropriate to analyse the T cell activation with high probability. This would obviously be the case for events happening during the first minute of the APC and T cell contact. It is evident from data [7, 2, 15] that this is indeed the case, as within first 30s from the initial contact, the T cell would receive signals for initial synapse formation with very high probability (about $85-90\%$) [7, 4, 9] for example. Thus a direct computation of transition probabilities is more appropriate in analysing these very early events as they happen with very high probabilities (80%-95%) which also required extensive computational alorithms. This is the problem we have investigated in [20].

Finally, the results we predict using the present model will be useful in designing further expriments, for instance to have an estimate as to how many TCRs one should expect to be used in flourescent labelling etc. Another obvious use in these models is to design computer simulations for T cell interaction/signalling using exact experimental conditions to have further insight into the problems which are otherwise impossible in the laboratory due to the limitations in direct observation of highly dynamical molecular events for example.

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