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# T cell activation: Kinetic proofreading, serial engagement and cell adhesion

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## Abstract

There is a fundamental difference in complexity between signaling initiated by ligands on the surface of one cell binding to receptors on the surface of another cell and ligands in solution binding to these receptors. The fact that two cells must approach each other and form a number of intercellular bonds of different types, all within the restricted geometry of the intercellular contact region, introduces the possibility of complex spatio-temporal dynamics of surface receptors that is not present otherwise. Mathematical modelling of these dynamics is in its early stages. However, useful tools have emerged. The purpose of this paper is to describe some of these mathematical tools, indicating their strengths and shortcomings, and suggest some directions for future theoretical studies.

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## 1. Introduction to T cell activation

### 1.1. Basics of antigen presentation and T cell recognition

The two key cell types of the adaptive immune system are T cells (which mature in the thymus) and B cells (which mature in the bone marrow). The basic role of T cells is the detection and destruction

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of intracellular pathogens, while B cells are principally concerned with the detection and destruction of extracellular pathogens. Intracellular pathogens include some bacteria (for instance, those causing tuberculosis and leprosy) and all viruses. T cells operate by scanning the surfaces of cells for markers of infection. Upon detection, the T cell becomes activated, and appropriate responses to the pathogen begin. T cells are divided into two main classes according to their principle response to markers of infection: *cytotoxic* or *effector* T cells kill the offending cell, while *helper* T cells provide important signals to other parts of the immune system (including B cells). In order for this arm of the immune system to work safely, T cells must be able to reliably distinguish infected from non-infected cells. Inappropriate responses to uninfected cells lead to autoimmune diseases such as insulin-dependent diabetes mellitus, rheumatoid arthritis and multiple sclerosis.

The T cell is only able to scan cell surfaces. In order to present information about their cellular contents, a number of cells contain machinery that can cut proteins into peptide fragments, transport these to the cell surface and present them for surveillance. If the cell contains a pathogen, signature peptide groups (antigens) will be presented. However, the cellular machinery responsible for peptide presentation is not specific to antigen. In fact, the great majority of presented peptides on a given antigen presenting cell are believed to be self-peptides that must be ignored. In effect, the T cells search for a signal (recognised antigens) against a (noisy) background of self peptides.

The peptide fragments are bound to specialised cell surface molecules, called major histocompatibility complex molecules (MHC). We shall abbreviate the whole ligand complex of peptide and MHC to pMHC henceforth. pMHC are the ligands of T cell receptors (TCR). Each mature T cell bears many identical copies ( $10^4$ – $10^5$ ) of a single TCR on its cell surface. It is observed that a particular T cell will respond to only a small set of presented pMHC. This *specificity* is clearly essential to prevent inappropriate T cell responses. The mechanism whereby T cells bearing receptors recognising self peptides are destroyed before reaching maturity is complex, but briefly, T cells are generated bearing random TCR, and then undergo a training period in the thymus where they are exposed to circulating cells. Those T cells that react strongly are programmed for apoptosis. Therefore, T cells reaching maturity should not be activated by interactions with self pMHC.

As described above, T cells are classified into cytotoxic and helper varieties. These two varieties are activated by peptides bound to different kinds of MHC. MHC class I molecules present peptides (length 8–10 amino acids) derived from pathogens in the cytosol (such as viruses) to cytotoxic T cells. Cytotoxic T cells bear the CD8 coreceptor for MHC I. MHC class II molecules are principally present on “professional” antigen-presenting cells, dendritic cells, macrophages and B cells. Macrophages and B cells actually take up stray proteins from the extracellular environment, hence allowing surveillance by T cells of extracellular pathogens as well as of certain bacteria that reproduce within macrophages. MHC class II molecules present peptides derived from these sources (more than 13 amino acids long) to helper T cells that bear the CD4 coreceptor for MHC II.

An excellent textbook describing most of immunology is [34]. Reviews of the general application of modelling to immunology are also presented in [56] and [51], but neither includes substantial discussion of the details of T cell activation. A review that focus on signaling mediated by immune recognition receptors including the TCR is presented in [19]. An interesting discussion of successful methods for modelling immunology is given in [75]. In this review, we focus on three general areas of modeling, all concerning the interaction between a single T cell and a single antigen-presenting-cell (APC) bearing an antigen recognised by the T cell. Important effects occur at a range of scales in both space and time, and these are outlined here, and in more detail afterwards.

### 1.2. Individual TCR signaling

At the nanometer scale, TCR–pMHC binding leads to transduction of a signal from the cell surface to the interior of the cell. The signal is carried by a network of biochemical reactions. Modelling signal transduction in complex chemical networks is a growth area that is beyond the immediate scope of this review [1,4,24,45,53]. Here we shall consider only how the first signal may cross the cell membrane, reviewing simple ways to model the likelihood of cell signaling given the kinetics of a particular TCR–pMHC bond.

### 1.3. Rates of engagement of TCR

Although many different pairs of ligands and receptors may bind within the intercellular contact area, the only specific signal that the T cell receives is from the engagement of pMHC with TCR. The rate constants governing formation and destruction of this bond can be determined experimentally. It is found that TCR–pMHC bonds are transient, typically with lifetimes in the range of seconds, and therefore a single pMHC may presumably engage many TCR during the lifetime of the cellular contact. If each bond formed is capable of producing a signal quantum, it is natural to ask how signaling can be affected by the population dynamics of TCR, pMHC and TCR–pMHC bonds.

### 1.4. Cell adhesion

It is observed that the two cells form a stable region of contact, and this region can persist for many hours. The stable region is termed the “immunological synapse” (IS) [22] or “supra-molecular activation complex” (SMAC) [50]. The region of close contact is the only region within which TCR–pMHC bonds can form, and many studies elucidating different aspects of its formation and dynamics have been undertaken. Since under most conditions lymphocytes do not stick together, formation of the IS raises the question of how, and under what conditions, specific ligand–receptor interactions induce cell–cell adhesion. Recent review articles addressing the function of the IS are [30,33,12].

### 1.5. Energetics and kinetics of membrane-bound ligand–receptor interactions

A brief aside on the kinetics of chemical reactions with membrane-bound participants is a valuable preamble to the rest of this review. Consider a ligand  $L$  and receptor  $R$  that form a bound complex:  $L + R \leftrightarrow B$  with solution kinetic parameters  $k_{\text{on}}$  and  $k_{\text{off}}$ , and solution affinity  $K = k_{\text{on}}/k_{\text{off}}$ . We now consider these proteins to be diffusing within closely apposed lipid membranes, as would be the case for TCR and pMHC during the formation of an IS. We assume that the necessary binding domains are exposed so the basic energetics of binding are the same as in solution. For instance, suppose the binding domains of each player are bound to the lipid membrane by a flexible linker region. Then the chemistry of binding between a single ligand and receptor should follow the same law of mass action except that the rate constants  $k_{\text{on}}$  and  $k_{\text{off}}$  may have changed. In particular,  $k_{\text{on}}$  is now a two-dimensional forward rate constant (with different units). The heuristic procedure to convert a measured three-dimensional forward rate constant to its two-dimensional analogue is to divide by a confinement length of the same order as the distance between the two membranes [14,73,21].

The question of what happens when the distance between the two membranes varies was considered in [3] and subsequently in [13]. The bond is modelled as a spring following Hooke’s law. Basic

thermodynamics then predicts that the two-dimensional affinity of an elastic bond (spring constant  $\kappa$  and preferred length  $\zeta$ ) forming between two membranes at separation  $z$  is

$$K(z) = K_A \exp\left(-\frac{\kappa(z - \zeta)^2}{k_B T}\right), \quad (1)$$

where  $K_A$  is the maximal (two-dimensional) affinity, achieved at  $z = \zeta$ . The separation-dependent kinetic constants must now obey  $k_{\text{on}}(z)/k_{\text{off}}(z) = K(z)$ . Different choices of functional forms are possible. Dembo et al. [13] proposed a model depending on *two* effective elastic constants:

$$k_{\text{on}}(z) = k_{\text{on}}(\zeta) \exp\left(-\frac{\kappa'(z - \zeta)^2}{2k_B T}\right), \quad (2)$$

$$k_{\text{off}}(z) = k_{\text{off}}(\zeta) \exp\left(+\frac{(\kappa - \kappa')(z - \zeta)^2}{2k_B T}\right). \quad (3)$$

The two elastic constants  $\kappa$  and  $\kappa'$  give a description of how elastic stress leads to a change in chemical kinetics. If  $\kappa' > \kappa$  then stretched bonds are more stable than unstretched bonds, termed ‘catch bonds’. Bonds for which  $\kappa' \geq \kappa$  are thus called ‘slip bonds’.

## 2. Individual TCR signaling

As we have seen, the basic signal to a T cell is the engagement of a TCR by a pMHC ligand. The details of how a signal crosses the cell membrane are still the subject of intense investigation. The key feature of the process, however, is its ability to discriminate between different pMHC. In fact, modification of the peptide at a single site is sufficient [64,38]. Fundamentally, we can ask what the TCR might measure in making distinctions.

Much attention has been paid to how the duration of a particular binding event could be important, leading to models under the general heading of “kinetic proofreading” or “kinetic discrimination”. Considerable experimental evidence has been amassed showing correlations between the average time of pMHC binding ( $1/k_{\text{off}}$ ) and various cellular responses, for instance see [47,37,38].

It is clear from experiments that  $k_{\text{off}}$  is not the only parameter governing the efficacy of T cell stimulation. Remaining within the general kinetic framework described above, attention is also paid to the binding affinity  $K$  [27].  $K$  will control the equilibrium binding of populations of presented pMHC to the T cell, and also acts as a measure of the change in free energy associated with binding [48].

A second question of interest is to what extent oligomerisation of TCR is responsible for signaling. It has been shown that stimulation of TCR using soluble dimers to TCR is sufficient to induce activation (soluble monomers are insufficient) [5,9], but that the binding domains of the dimers cannot be too far apart [67]. However, the recent finding that a single agonist pMHC on an APC was sufficient to achieve limited signaling [31] indicates that close TCR apposition may be sufficient but is not necessary for some signaling events.

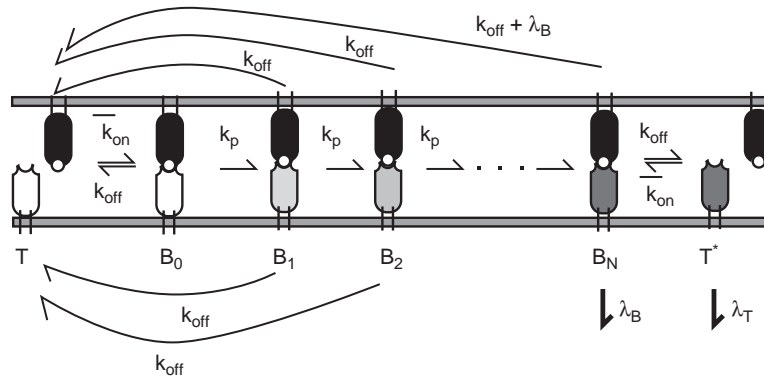


Fig. 1. Kinetic proofreading model.

### 2.1. Kinetic proofreading

McKeithan introduced the kinetic proofreading model [28,54] to T cell activation [49]. The key idea of a sequence of biochemical modifications is illustrated in Fig. 1. The original model made three hypotheses: (i) The initial inactive receptor species undergoes a sequence of  $N$  modifications leading to the activated state via a sequence of intermediaries. Details of the modifications are not specified, but might include recruitment of scaffolding components to the receptor, or (de)phosphorylation of tyrosines within the receptor complex. (ii) Dissociation of pMHC from the TCR leads to reversal of these modifications. (iii) Reversal of these modifications is quick in that the receptor is always in the basal (unactivated) state when it binds a pMHC. Consistent with the predictions of this model, Kersh et al. [37] found that pMHC with different half-lives of binding to TCR generate different phosphorylated forms of CD3- $\zeta$ , a molecule that associates with TCR as part of the signaling complex (although the details of this consistency have been challenged [8]).

In his initial formulation of the model, McKeithan took the rate constants for movement down the signaling pathway to be all the same, and therefore the following system of ODEs describes the dynamics:

$$\frac{dT}{dt} = -k_{on}PT + k_{off} \sum_i B_i, \tag{4}$$

$$\frac{dP}{dt} = -k_{on}PT + k_{off} \sum_i B_i, \tag{5}$$

$$\frac{dB_0}{dt} = k_{on}PT - k_{off}B_0 - k_pB_0, \tag{6}$$

$$\frac{dB_i}{dt} = k_p(B_{i-1} - B_i) - k_{off}B_i \quad i = 1, \dots, N - 1, \tag{7}$$

$$\frac{dB_N}{dt} = k_pB_{N-1} - k_{off}B_N. \tag{8}$$

Here  $P$  represents the concentration of the pMHC in question and the  $B_i$  are the complex of  $T$  and  $P$ , where the TCR is in the  $i$ th activation state.  $k_p$  represents the forward rate constant in the signaling cascade. A mathematical analysis of steady states of these equations was given by Sontag [65]. Signaling is assumed to arise according to the concentration of  $B_N$ . Then at steady state, the fraction of activated complexes follows

$$\frac{B_N}{\sum_i B_i} = \left( \frac{k_p}{k_p + k_{\text{off}}} \right)^N, \quad (9)$$

where the quantity  $k_p/(k_p + k_{\text{off}})$  is the probability that any step  $i$  ( $i \neq N$ ), a modification will occur before dissociation of the pMHC. Now supposing that  $k_p$  is set by the chemistry of the T cell and that  $k_{\text{off}}$  is the only parameter with which to distinguish peptides, then (for quite modest values of  $N$ ), the concentration of complexes in the activated form becomes sensitive to small variations in  $k_{\text{off}}$ . As McKeithan observed, however, this power of discrimination comes only at the expense of overall sensitivity to specific stimuli. Therefore, a method was proposed to achieve higher selectivity without reducing sensitivity, by assuming a different rate of dissociation of the complex once the fully modified state has been reached (so  $k_{\text{off}}$  is replaced by  $k_{\text{off}}^* < k_{\text{off}}$  in Eq. (8) and with appropriate modifications in Eqs. (4) and (5)). This modification means that fully modified complexes build up over time, and at steady state, higher sensitivity is achieved. However, there is no evidence for such a modification. Further, as we will see when we discuss triggering of populations of TCR, this would inhibit a pMHCs ability to activate (serially engage) multiple TCR.

## 2.2. Limitations of kinetic proofreading

A recent reanalysis of kinetic proofreading [8] explicitly quantified the concepts of sensitivity and specificity using the notions of true and false signaling events and true and false negative (lack of signaling) events. Using TP, FP, TN and FN to describe these events, the standard definitions are sensitivity = TP/(TP + FN) and specificity = TP/(TP + FP). The trade-off between specificity and sensitivity as a function of  $N$  can then be found exactly. Subsequently, this paper examines kinetic proofreading at the level of a single bound TCR by recasting the process as a sequence of probabilistic events and calculating the mean and variance of the time to activation. This leads to the conclusion that the specificity of the system is maximised by choosing that each step have the same rate constant. A problem, however, is that the kinetic proofreading model does not prescribe how to determine  $N$  or  $k_p$  from experiment so the relation of these two abstract quantities to well defined physical quantities that characterise signaling cascades is unknown.

## 2.3. Modified kinetic proofreading models

Modifications of kinetic proofreading to address different situations are presented in [25] and [26] to model receptors that must be dimerised by a ligand dimer before kinetic proofreading steps can begin and the extension for the case where receptor dimers must also recruit an extrinsic membrane-bound kinase for signaling to occur. However, the main thrust of these models was to explain how some responses could be under the control of kinetic proofreading while other responses seemed to escape kinetic proofreading [61,46,16]. It should be noted, however, that these models do not escape the trade-off between specificity and sensitivity that exists in McKeithan's original formulation.

## 2.4. Antagonism

Certain pMHC are known to act as antagonists, in that T cells exposed to them can lose sensitivity to otherwise potent stimuli. Rabinowitz et al. showed how a modification of McKeithan's model to allow for incomplete signals could be used to describe antagonism [58]. In terms of Eqs. (4)–(8), their model (termed “kinetic discrimination”) uses  $N = 2$  and also keeps track of concentrations of unbound TCR in the first two modified states. The partially modified receptor (first modified state) is taken to provide a negative signal while the fully modified receptor provides a positive signal. Another model examining how sensitivity and specificity can be achieved over a population of T cells, with special regard to possible effects of ligand antagonism, is described in [70].

## 2.5. The future of kinetic proofreading

The kinetic proofreading model [49] has had a major impact in stimulating experimental investigation in TCR mediated signaling. Its major contribution has been to show how T cells can distinguish pMHC on the basis of a single kinetic parameter,  $k_{\text{off}}$ . There are some pMHC that do not fit the model [40], but recognising these peptides as exceptional has been an important contribution of the model as well. Although we anticipate that additional extensions of the kinetic proofreading model will be forthcoming, there is now a need for models that are less abstract and capture more of the details of the events triggered by activated TCR. One such model has been presented for the early signaling events mediated by the immune recognition receptor that binds IgE with high affinity, FcεRI [17]. This model exhibits kinetic proofreading but the quantitative description provided by the model of McKeithan only poorly approximates the behaviour predicted by the detailed model (Goldstein, unpublished result).

## 3. Engagement and triggering of populations of receptors

### 3.1. Downregulation of TCR as a measure of serial triggering

The phenomenon of serial triggering of TCR was proposed in [69]. It was found there that a single pMHC was able to serially engage and trigger as many as  $\sim 200$  peptides over the course of a prolonged cell–cell interaction. The experimental measure was the downregulation of TCR from the cell surface over time. This measure was shown to reflect some general level of cellular activation by the experimental finding of a strong correlation between downregulation and production of the cytokine IFN- $\gamma$ . It was also shown that only triggered TCR were downregulated by producing T cell clones bearing two distinct populations of receptors, only one of which was found to be downregulated. Downregulation of about 100 and about 10 TCR/pMHC was later found for a human and a mouse T cell clone, respectively [32]. However there is growing evidence that a bystander effect can occur where some TCR are downregulated without binding to the pMHC [35].

### 3.2. TCR downregulation models

Simple models for TCR downregulation following binding to pMHC were proposed by Bachmann et al. [2]:





Here  $T_I$  represents downregulated TCR. The goal of this paper was to distinguish dimer and monomer models for TCR activation, therefore a second model of the form



was also introduced. Here  $D$  is a dimer of ligand–receptor pairs. Using this simple model, which does not contain any notions corresponding to kinetic proofreading, the question of whether measured down-regulation curves could be better fit by a monomer or dimer model was addressed. It was concluded that TCR dimerisation (or possibly trimerisation) was essential for TCR internalisation. Unfortunately, a subsequent study by Sousa and Carneiro [66] applied the same model to the data of Valitutti et al. [69], but could not reach the same conclusion. Sousa and Carneiro presented a set of generalisations of the monomer and dimer models each of which was able to fit the data. The generalisations were (i) imposition of a ligand-independent TCR turnover due to a background rate of TCR synthesis and downregulation, (ii) breaking the single triggering/downregulation rate of model (10) into separate rates for these two processes, (iii) altering the order of the chemical kinetics and (iv) considering a pool of TCR with no access to pMHC that is in dynamic equilibrium with the regular TCR pool (for example, TCR within and outside the immunological synapse). Each of these extensions can be justified on the basis of the biology, although each also introduces more fitting parameters.

### 3.3. Diffusive transport of pMHC

Downregulation per pMHC available is a natural measure of the efficiency of activation by a limited number of triggering pMHC. An interesting question that can be addressed mathematically is the number of encounters a diffusing pMHC would have during its sojourn within the close contact region of the IS. This problem was analysed mathematically in [73]. A calculation was presented of the time a diffusing pMHC, initially at a random position within a circular contact area, would take to reach the edge. The pMHC was taken to diffuse with a diffusion coefficient  $D_P$  while unbound, and with a slower diffusion coefficient  $0 \leq D_B \leq D_P D_T / (D_P + D_T)$  while bound, where the upper bound comes from the Einstein relation with  $D_T$  the diffusion rate for free TCR. In the extreme cases, the mean residence time  $\langle t \rangle$  simplifies to

$$\langle t \rangle = \frac{a^2}{8D_P} \quad \text{if } D_B = D_P, \quad (12)$$

$$\langle t \rangle = \frac{a^2}{8D_P} (1 + KT) \quad \text{if } D_B = 0. \quad (13)$$

Here  $T$  is the concentration of TCR (assumed constant) and  $K$  is the two-dimensional affinity of the TCR–pMHC interaction. Using these results, the mean number of binding encounters undergone by a single pMHC (“hits”) is

$$\text{hits} = \frac{k_{\text{off}} K T}{1 + K T} \langle t \rangle. \quad (14)$$

Hence the rate at which pMHC encounter TCR,  $\text{hits}/\langle t \rangle$  depends only on the kinetic rate coefficients and the TCR concentration, and is independent of the diffusion constants and the geometry of the synapse



area. Using characteristic parameters for T cells and APCs and the pMHC–TCR set described in [22], it was shown that substantial serial engagement should occur. Further, an interesting possibility was raised: the pMHC giving the highest predicted number of hits functioned as an *antagonist* (see above) with  $k_{\text{off}} = 5.0 \text{ s}^{-1}$ . This can be viewed as evidence for one proposed mechanism of antagonism: depletion of an initiating kinase in TCR signaling by repeated unproductive association with pMHC [68].

### 3.4. Combining kinetic proofreading and serial engagement models

As discussed above, there seems to be an effective minimum lifetime of the TCR–pMHC bond leading to productive signaling and eventual TCR downregulation. However, in order for a few pMHC to successfully trigger many TCR, there must be reasonably rapid turnover of bonds following triggering. Clearly, these arguments together suggest that there must be an optimal  $k_{\text{off}}$  for the TCR–pMHC bond, in terms of achieving a maximum rate of TCR activation and downregulation. Experiments confirming the existence of such a window have been performed by producing point mutations in the TCR of T cell clones [36,11].

A reaction–diffusion model describing dynamics of TCR and pMHC on their respective cell surfaces was described in [11]. TCR and pMHC were assumed to freely diffuse in and out of a contact region, within which they were able to bind. Bound TCR were assumed to undergo a kinetic proofreading sequence of modifications leading to an activated state. Different assumptions regarding how an activated TCR becomes internalised led to different predictions of how the rate of down-regulation depends on  $k_{\text{off}}$ . Distinct internalisation rates were introduced, permitting the following possibilities: (i) TCR bound to pMHC which have been fully modified (the  $B_N$  state in kinetic proofreading) are internalised with rate  $\lambda_B$ . (ii) If an activated TCR separates from the pMHC, it becomes internalised at a rate  $\lambda_T$ , as long as it remains in the contact region. Outside the contact region, it reverts to an inactivated state at a given rate. It may also rebind with a free pMHC when it returns to the contact region.

Using this model, conditions for a maximum in internalisation could be found. If TCR may be internalised only after unbinding ( $\lambda_B = 0$ ), then a maximum is guaranteed for both internalisation and activation of TCR. If  $\lambda_B \neq 0$  then the existence of the experimentally observed maxima depends on other parameters of the problem. If  $\lambda_T = 0$  then no maximum in internalisation can exist. This is essentially because the internalisation provides a way to free pMHC, thus increasing the efficiency of serial engagement. The prediction made is that activated TCR must remain subject to internalisation for a period after they unbind from pMHC. It was shown, using physiological parameters, that this period must be at least 2–3 min.

### 3.5. Receptor crosstalk

Chan et al. [7] investigated possible effects on TCR triggering caused by the activation state of its neighbours. The mathematical approach used was to perform Monte Carlo simulation on a lattice of TCR. Each TCR existed in one of four “activation states”: empty (no pMHC bound), occupied (pMHC bound), partly activated (after around 3 s of binding) and fully activated (after a further 5 s of binding). Binding and activation of the receptor were implemented analogously to the kinetic proofreading model. Receptor crosstalk is included in the model by rules for signal spreading (inhibitory or protecting) from receptors binding agonists and antagonists. It was found that the specificity of a given interaction can be significantly improved if the response of a particular TCR depends on the history of binding of nearby TCR. This assumes a rule that partial activation of a TCR leads to a local inhibitory effect. Further, they

determined that a “spreading protection” effect whereby ligands binding agonist protect others nearby from inhibition caused by later binding of antagonists leads to increased sensitivity for agonist. Clearly, rule-based approaches such as this have great potential for studies of complex and competing effects, especially where spatial effects must be integrated into the model.

#### 4. T Cell-APC adhesion and the immunological synapse

The T cell and its target APC are not held together solely by the TCR–pMHC interaction. In fact, several other ligand–receptor pairs act to stabilise the region of close contact. An important secondary interaction is that between the adhesion molecules LFA-1 (presented on the T cell) and ICAM-1 (on the antigen presenting cell). An important feature is that this bound pair of molecules are longer (42 nm) than the TCR–peptide–MHC pair (14 nm). Therefore, coexistence of the two binding pairs in a spatially localised region requires local bending of the cell membranes, and one observes separation of the two in experiments as described below. This separation is termed “kinetic segregation”. Single molecule fluorescent labeling of cell surface molecules permits detailed visualisation of the configuration of ligands and receptors on cell surfaces. A groundbreaking series of experiments, beginning in the late 1990s, showed dynamic reorganisation and spatial patterning of certain important ligand–receptor pairs. Importantly, the ligands presented along with pMHC are generically independent of the presented peptides, and hence the key interaction from the point of view of signaling remains that of the TCR and pMHC. However, some of these ligands are known to act as costimulatory molecules, increasing the likelihood of signaling in a nonantigen-specific way.

##### 4.1. Spatial patterning within the IS

Monks et al. [50] demonstrated that fluorescently labeled LFA-1 organises around the periphery of the cell–cell contact area, with a region of labeled TCR within. A similar experiment [22] replaced the antigen presenting cell with a planar bilayer, to which was bound only labeled peptide–MHC and ICAM-1. In each case, the formation of patterns was observed when a T cell reached the cell/bilayer, recognised the presented peptide–MHC, and formed a long-lasting attachment. Further, regions of closer apposition between the cell and the bilayer could be observed, and these correlated well with high concentrations of peptide–MHC (the shorter of the two binding molecules). The dynamics of patterning are as one might expect: as the T cell approaches the cell/planar bilayer from above, the first contacts made are between the longer (LFA-1–ICAM-1) binding pairs. Fluctuations locally bring the TCR–peptide–MHC pairs into contact, forming bonds with relatively high affinity. The formation of these bonds leads to regions of close membrane/bilayer apposition, and thus steep (energetically unfavourable) gradients in the position of the cell membrane. After 3–5 min, an approximately disc-shaped region of shorter bonds is found towards the centre of the nascent synapse, surrounded by an approximate annulus of longer bonds. This bulls-eye configuration then persists for hours. This is now understood as the generic description of the IS: subsequent experiments showed variations [42,52,18,76].

##### 4.2. Kinetic segregation: modeling approaches

Kinetic segregation and spatial patterning in the IS are clearly interesting physical phenomena, relying on an interplay between binding and unbinding of a ligand–receptor pair (described by chemical kinetics)

Table 1  
Symbols used in this paper

Description	Symbol
$T, P$	Concentration of free, unmodified TCR and free pMHC
$B, D$	Concentration of bound TCR–pMHC complexes and dimers thereof
$B_i$	TCR–pMHC complex with the TCR in the $i$ th activation state
$k_p$	Forward rate constant in kinetic proofreading model
$T_I$	Number of internalised TCR
$K$	TCR–pMHC binding affinity
$k_{\text{on}}, k_{\text{off}}$	Kinetic rates for TCR–pMHC binding
$a$	Radius of immunological synapse
$D_T, D_P, D_i$	Diffusion constants for free TCR, free pMHC and bound complex of type $i$
$\lambda_T, \lambda_B$	Rate constants for internalisation of free and bound TCR
$\kappa, \zeta$	Bond spring constants and preferred lengths
$\beta, \gamma$	Cell-surface bending and stretching moduli
$\Gamma(z)$	Cell–cell interaction potential

and physical properties of the cell membrane. As such, it is not surprising that several groups have presented models describing kinetic segregation with specific application to T cells.

### 4.3. PDE approaches

In [57], a PDE model was presented that described the variation over time and space of the numbers of TCR–pMHC bonds, numbers of LFA-1–ICAM-1 bonds and spatial position of the cell membrane during an experiment analogous to that performed by Grakoui et al. [22]. This model was developed further and analysed in a series of papers from the same group [29,44,43,59].

The heart of the model developed in these papers is described by a set of PDEs describing the dynamics of concentrations of TCR, pMHC, LFA-1 and ICAM-1. The pMHC and ICAM-1 are taken to diffuse on a fixed membrane at  $z = 0$  while the TCR and LFA-1 are presented on a cell that is held above the membrane, with position given by  $z(x, y, t)$ . The TCR and LFA-1 may also diffuse on the cell surface. The adhering cell is modeled as an elastic membrane with a given bending stiffness and surface tension, opposing bending and stretching deformations.

The PDE describing the time evolution of bound complexes  $C_i$  (where  $i = 1$  represents TCR–pMHC bonds and  $i = 2$  represents LFA-1–ICAM-1 bonds) has the form (see Table 1 for notation)

$$\frac{\partial C_i}{\partial t} = D_i \nabla \cdot (\nabla C_i) + k_{\text{on}}^i(z) C - k_{\text{off}}^i C. \tag{15}$$

An identical equation with different parameters describes the dynamics of the LFA-1–ICAM-1 bonds. Here  $D_i$  is the diffusion constant for bound complexes of type  $i$ , and  $k_{\text{on}}^i(z)$  and  $k_{\text{off}}^i$  are rate constants for the formation and dissolution of bond  $i$ . The two-dimensional forward binding rates  $k_{\text{on}}^i(z)$  are taken in [57] to be Gaussian functions centred at the preferred bond length  $\zeta_i$ , and with widths taken to be between 5 and 13 nm. Taking  $k_{\text{off}}$  to be independent of membrane separation is the case of “slip bonds” in the description of Eqs. (2)–(3) above. A slight generalisation of that description is also present, in that the spring constant for the binding energy well is distinguished from the width of the Gaussian in Eq. (2).

The authors model the adhering cell as an elastic membrane at a distance  $z(x, y, t)$  from the supported bilayer. The energy of the cell membrane is approximated (in the small-angle approximation) by a bending and a stretching term, as follows [62]:

$$E_{\text{mem}} = \int \beta \nabla^2 z + \gamma (\nabla z)^2 \, dA, \quad (16)$$

where the integral is taken over the whole computational domain and  $\beta$  and  $\gamma$  are the bending and stretching moduli, respectively. A second integral term describes the energy associated with the deformation of bound complexes from their preferred lengths:

$$E_{\text{bond}} = \sum_i \frac{\kappa_i}{2} \int C_i (z - \zeta_i) \, dA. \quad (17)$$

These energies are then associated with the time evolution of the membrane separation in the obvious way:

$$\frac{\partial z}{\partial t} = -M \frac{\delta(E_{\text{mem}} + E_{\text{bond}})}{\delta z}, \quad (18)$$

where  $M$  is a constant linking the membrane responsiveness to free energy changes (conceptually, a drag coefficient). It should be noted that taking the functional derivative of  $E_{\text{mem}}$  produces a PDE that is fourth-order in space, requiring care for efficient numerical solution. Finally, the model presented in [57] includes terms for TCR downregulation following productive engagement by pMHC.

A second group presented a model with essentially the same physics in 2002 [6]. In this model, an additional energy term models nonspecific repulsion between two cells:

$$E_{gc} = \int \Gamma(z) \, dA. \quad (19)$$

The functional form used for  $\Gamma$  is a quadratic potential well. Therefore, in this model, in the absence of bridging bonds, the two surfaces will find an equilibrium distance. A second novel term was included to examine the important observation that, on disruption of the T cell cytoskeleton, kinetic segregation is observed, but not reorganisation of domains into the bulls-eye pattern [22]. In the PDEs, this term took the form of a simple advection of TCR to the centre of the domain.

#### 4.4. Boundary conditions

As always with a PDE model, boundary conditions must be specified. In the model of Qi et al. [57], the T cell membrane was taken to be held at a fixed distance (much greater than the ligand binding distance) from the supported bilayer (i.e.,  $z = \text{constant}$ ), far from the centre of the computational domain. Varying this distance was found to make no qualitative difference to the results. In the alternative formulation of Burroughs and Wülfing [6], periodic boundary conditions were used so effectively a larger region of cell–cell or cell–membrane contact was studied.

#### 4.5. *Equilibrium model*

Given that the IS is stable for a considerable time after it forms, it is possible to directly ask which states of the system are thermodynamically stable. This has the advantage of speed of computation, permitting the investigation of wide ranges of parameter space. This approach was taken in Coombs et al. [10]. Briefly, within a radially symmetric geometry for the synapse, and assuming only a single boundary between TCR–pMHC and LFA-1/ICAM-1 rich regions of the contact area, an energy function of several variables was constructed. Essentially, this energy function contained contributions from binding, intercellular repulsion, and a line tension penalising boundaries between long- and short-receptor rich regions. The line tension (units of energy per length) was calculated by integrating across minimisers of the elastic energy given in Eq. (16). Minimisation of this function revealed thermodynamically stable states for the system. Of particular interest were sharp boundaries between qualitatively different synapse formations in parameter space. The existence of these boundaries suggests mechanisms for the cells involved to control the formation or destruction of the IS.

#### 4.6. *Lattice-based approaches*

In [72], the pattern formation questions were reformulated in terms of a lattice-based model. An effective energy of a given configuration of binding and membrane position across the lattice was computed, and then used to produce dynamics via a Monte Carlo algorithm. The energy includes three terms (similar to the model of [10]), due to binding, intercellular interactions and elastic energy of the membranes. The curvature and surface tensions terms in Eq. (16) were reformulated in terms of their discrete analogues (like a finite difference scheme) [71]. Rather than using a distance-dependent affinity of binding for each binding pair, a square well potential was used: bonds may only form if the distance is within 5 nm of the preferred length. Also, the glycoproteins that may be responsible for much of cell–cell repulsion were explicitly included in the model, in that there is an energetic cost to bond formation in the presence of glycoproteins. The glycoproteins diffuse, so over time they are excluded from the synapse and this effect becomes less important. An extension to the model includes a term for directed motion of TCR towards the centre of the contact region (included as an energy penalty proportional to the distance each TCR is from the synapse centre).

#### 4.7. *Results*

The models described so far are all successful in describing the segregation of short (TCR–pMHC) and long (LFA-1/ICAM-1) bonds within the IS. Further, the dynamic models produce qualitatively similar dynamics for segregation with the correct time scales. However, issues regarding the importance of directed motion of TCR to the synapse centre remain. In both the PDE model of Burroughs et al. [6] and the lattice dynamics of Weikl and Lipowsky [72], central aggregation of TCR is not seen unless a central force is imposed. Understanding how patterning and spatial segregation of cell-surface receptors occurs under membrane and cytoskeletal forces is an ongoing challenge. The basic physics of how bound receptors of different lengths spontaneously segregate in the central synapse (Eqs. (16) and (17)) is common to these models and is now well understood. As described below, a serious future challenge lies in linking this picture with detailed signaling models and the important effects of the cell cytoskeleton.

#### 4.8. Energy well analysis and thermal fluctuations

The model presented in [57] contained additive thermal noise terms in each PDE. However, it was argued in that paper that thermal noise is small compared to the other energetic scales in the problem, and it was therefore neglected. This question was revisited in the context of low TCR expression levels (where the scale of thermal noise is greater compared to the other scales of the problem) in [43]. The biological question at issue was the formation of short lived, multifocal regions of TCR binding during thymocyte selection. Thymocytes are immature T cells. Experiments showed substantial differences between thymocyte synapses and those formed by mature T cells [23,60], and that thymocytes present substantially lower levels of TCR than mature T cells. Specifically, TCR are not observed to stably aggregate in the IS centre, rather forming transient patches. The proposal of [43] was that thermal fluctuations become significant if the energetic cost for formation of a TCR–pMHC bond rich region within a synapse dominated by longer bonds becomes small.

An analysis of the steady, homogeneous system is presented in [6]. The steady state criteria are that, for each species  $i$ , the chemical kinetics are at steady state (and thus are governed only by the affinity  $K(z)$ ) and that the membrane separation balances the attractive force of the bonds with the repulsive force of the intercellular potential.

$$L_i R_i K(z) = B_i, \quad (20)$$

$$\sum_i \kappa_i (z - \zeta_i) B = \Gamma'(z). \quad (21)$$

In the absence of an intercellular potential, of course, all bonds are unstressed in this analysis. In this case, for low values of  $\kappa_i$ , Eq. (21) has a single solution for  $z$ , found between  $\zeta_1$  and  $\zeta_2$ . As  $\kappa_i$  are increased, two stable solutions emerge close to the preferred bond lengths  $\zeta_i$ .

An examination of the stability of the full PDE model was presented in [29] and advanced in [59]. An effective free energy is written, revealing a competition between diffusion of different species (reducing chemical gradients) and sharpening terms due to an effective repulsion between short- and long-bond rich regions. In terms of the wavenumber of the patterns formed  $q$ , these terms have the form  $q^2$  and  $(\gamma q^2 + \beta q^4 + c)^{-1}$  for a constant  $c$ . There is therefore the possibility of a band of stable modes of low wavenumber, corresponding to synapse-like behaviour. An interesting connection can also be made with an earlier model of Komura and Andelman [39]. In that paper, phase separation of adhering membranes into regions with close binding and loose binding was examined, when only a single length of ligand–receptor bond is present. These membrane bound “stickers”, were shown to exhibit lateral phase separation, provided stickers on the same membrane have an attractive interaction or repulsion. As might be expected, the mathematical descriptions of phase separation in the two systems are quite similar.

#### 4.9. Modeling TCR signaling at the synapse

The main goal of the studies described so far has been the physical description of bond segregation by length. The next step will be to model the cell signaling events that occur after initial contact between an APC and a T cell and this will require coupling a model for the dynamics of IS formation [6,43,57] with a model for TCR mediated cell signaling. This is a formidable challenge. Recently, an agent based model that makes a start at incorporating some of the details of the signaling cascade initiated by pMHC–TCR



bound formation has been used to investigate how spatial organisation within the synapse influences signaling [41]. The model starts with an IS and investigates how TCR density in the contact region influences signaling. The T cell is replaced by a  $101 \times 100 \times 100$  lattice with the contact region between APC and T cell being  $100 \times 100$ . Surface dynamics outside the contact region are ignored. Initially, 120 TCR are randomly distributed on the T cell surface and 12 pMHC on the APC surface. Cytosolic components of the TCR signaling cascade are distributed in the three-dimensional lattice. One problem with such agent based models is that it can be difficult to make a direct correspondence between the parameters and the outputs of the models and experimentally determined quantities. For example, the time scale in the model output of Lee et al. [41] is in units of  $10^9$  Monte Carlo steps.

Recall that TCR signaling is initiated when Lck, a Src tyrosine kinase (PTK) that is membrane associated, phosphorylates specific tyrosines on the  $\zeta$  chains of the TCR. Each of the two  $\zeta$  chains of the TCR has three copies of a shared sequence, the immunoreceptor tyrosine-based activation motif (ITAM), that contains two critically spaced tyrosines. When both tyrosines in an ITAM are phosphorylated, a high affinity docking site is created for the cytosolic PTK ZAP70 ( $\zeta$ -chain-associated protein of 70 kDa), resulting in recruitment of ZAP70 to the plasma membrane. The model [41] introduces a simplified picture of the  $\zeta$  chain, allowing only three states of phosphorylation: unphosphorylated (TCR $\zeta$ ), phosphorylated once by Lck (TCR $\zeta$ 1) and doubly phosphorylated by Lck (TCR $\zeta$ 2). All three states are subject to internalisation but only TCR $\zeta$ 2 is subject to degradation once internalised. TCR $\zeta$ 2, but not TCR $\zeta$ 1, can initiate a signaling cascade. If TCR $\zeta$ 2 signaling is above some threshold then a force is turned on that biases TCR motion toward the centre of the contact region.

The model was used to analyse experiments comparing signaling in wild-type (WT) T cells and in T cells from CD2AP-deficient mice, which do not form mature synapses with well defined bulls eye patterns [15]. Compared to WT, the CD2AP-deficient T cells showed delayed but prolonged tyrosine phosphorylation of the  $\zeta$  chain of the TCR and of ZAP70 and enhanced proliferation [41]. In WT T cells, tyrosine phosphorylation rose, peaked and then dropped to baseline. In the model the force that causes TCR to move toward the centre of the contact area is only turned on in the WT cells. For the parameters used in the model, the increased TCR density in the WT cells increases pMHC–TCR serial engagement. The increased density also allows Lck to serially phosphorylate TCR. Initially this leads to a rapid rise in TCR $\zeta$ 2 but because this state is subject to degradation, signaling rapidly decreases. In this way, the model explains the rapid downregulation of TCR signaling that is observed in WT and is due to enhanced degradation.

## 5. Discussion

The study of T cell signaling has led modelers to adopt a variety of approaches. This is as a result of the biological phenomena occurring at a variety of length scales: individual binding events at the nanometer scale, aggregation of receptors and co-signaling at the scale of tens of nanometers, and the overall organisation of the synapse and populations of receptors and ligands at the scale of microns. A number of directions for future study are suggested at each scale and linking the scales.

### 5.1. Cell signaling networks

A single TCR/CD3 complex has ten ITAMs each containing two tyrosines that can be phosphorylated or not. Phosphorylated ITAMs on the cytoplasmic domains of the TCR/CD3 complex act as reversible



nucleation sites that recruit signaling molecules and bring enzymes and substrates together. Critical steps in the initiating of signaling are the recruitment and activation of ZAP70 and the phosphorylation of multiple tyrosines on the transmembrane protein LAT (linker for activated T cells) by recruited ZAP70 (reviewed in [63]). A large part of the signaling cascade involves construction projects carried out just below the plasma membrane where the cytoplasmic domains of the receptor, LAT and other proteins act as the scaffolds, and kinases, phosphatases and adapters that are recruited, as the building blocks. However, the structures that form are ephemeral with components going on and off rapidly. Because the structures (distinct chemical complexes) that form are composed of multiple components that can be modified in multiple ways, the number of possible complexes that can form is enormous. Further, for any complex there are multiple ways it can be constructed and multiple ways it can be modified. Thus, the signaling cascade that is initiated by a pMHC binding to a TCR is essentially a network with a huge number of nodes (the chemically distinct species that may form). Building and analysing network models for TCR signaling is a daunting challenge. For a simpler ITAM-bearing immunoreceptor, the high affinity receptor for IgE (Fc $\epsilon$ RI), a systematic construction of the signaling network is underway, but only the ligand, receptor and two enzymes have been incorporated into the model [17,20]. The problems in modeling ligand-induced receptor aggregation are not unique to the immunoreceptors [24].

Simple models, such as the kinetic proofreading model, have given us considerable insight into certain aspects of cell signaling. However, the kind of data now being acquired demands detailed models that incorporate specific interactions among the domains of the signaling proteins. The ultimate understanding of ligand-induced receptor signaling rests on our ability to build such models.

### 5.2. Mesoscale modelling

Closely related to the questions of TCR signaling, but distinguished by spatial distribution, are questions of how close aggregations enhances signaling. As mentioned above, soluble dimers that bind TCR lead to signaling only if the linker region is sufficiently short. Further, Wülfing et al. [74] showed that the presence of nonagonist pMHC (that otherwise do not cause T cell signaling) can enhance the signaling ability of recognised pMHC. Therefore, there is considerable interest in understanding the role of TCR dimerisation and/or formation of small aggregates of TCR. These questions can be viewed as bridging the scale from single TCR triggering to understanding population dynamics of TCR on the cell surface.

### 5.3. Directed motion in the IS

The phenomena of kinetic segregation of receptors seems to be understood from a variety of points of view. However, dynamics of the cellular cytoskeleton form a key part of IS formation, and the balance of importance between these two phenomena remains unclear. Given progress in modeling the cytoskeleton in other contexts [55] it would be of interest to try to link events downstream of TCR signaling to communication with the cytoskeleton. Success in this area could also indicate to what extent IS formation requires active expenditure of energy by the conjugating cells.

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