Phenotypic models of T cell activation

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Online Summary

- Many studies have aimed to relate the TCR-pMHC binding parameters and the pMHC concentration to the degree of T cell activation. This extensive work has produced a wealth of often conflicting data.
- To make sense of conflicting data, a variety of verbal and mathematical models have been proposed.
- It is unclear which model(s) are consistent and inconsistent with data because comparisons between the models has been difficult, in part, because they have been formulated in different frameworks.
- We re-formulate published models into five distinct phenotypic models that can be directly compared.
- We provide a look-up figure showing the predicted T cell activation for each model as a function of pMHC concentration and TCR-pMHC binding parameters.
- We suggest that a kinetic proofreading model that is modified to include limited signaling is consistent with the majority of experimental data but highlight that additional data is required.

Abstract

T cell activation is a critical checkpoint in adaptive immunity and their activation depends on the binding parameters governing the interactions between T cell receptors (TCRs) and peptide bound to major- histocompatibility-complexes (pMHCs). Despite extensive experimental studies, the relationship between the TCR-pMHC binding parameters and T cell activation remains controversial. In order to make sense of conflicting experimental data, a variety of verbal and mathematical models have been proposed. However, it is presently unclear which model(s) are consistent and inconsistent with experimental data. A key problem is that a direct comparison between the models has not been performed, in part because they have been formulated in different frameworks. In this analysis we re-formulated published models of T cell activation into phenotypic models, which allowed us to directly compare them. We find that a kinetic proofreading model that is modified to include limited signaling is consistent with the majority of published data. This model makes the intriguing prediction that the stimulation hierarchy of two different pMHC (or two different TCRs specific to the same pMHC) may reverse at different pMHC concentrations.

Introduction

T cells initiate and regulate adaptive immune responses to infections and cancer and play crucial roles in allergy, autoimmunity, and transplant rejection[1]. These T cell functions critically rely on productive binding between T cell antigen receptors (TCRs) and antigens, typically short peptides bound to major-histocompatibility-complexes (pMHCs), displayed on the surface of a variety of cells referred to as antigen-presenting-cells (APCs). Upon activation, T cells may proliferate, differentiate, release cytokines, kill target cells, and carry out other effector functions. By measuring these functional T cell responses to a variety of pMHC ligands, experiments have established that T cell activation is determined by the TCR-pMHC binding parameters. Despite extensive experimental and mathematical work, a model relating the TCR-pMHC binding interaction to T cell activation that is consistent with published data remains elusive.

Experiments using panels of different TCRs and pMHCs have reported various relationships between the TCR-pMHC binding parameters and T cell activation, as measured by downstream functional readouts such as cytokine secretion[2–16] (Fig. 1). Several studies have reported that T cell activation exhibits an optimum when plotted over the TCR-pMHC dissociation time (τ)[7–9], which is the reciprocal of the off-rate ($k_{\text{off}} = 1/\tau$), and this optimum has been observed *in vivo*[12,17]. Interestingly, one study suggested that the optimum is lost at high pMHC doses[11]. Experiments using detailed pMHC titrations have shown that the potency (EC_{50}, defined as the concentration of pMHC producing half-maximal response) does not exhibit an optimum and is directly correlated to the TCR-pMHC dissociation constant, K_{D} ($K_{\text{D}} = k_{\text{off}}/k_{\text{on}}$)[2,4–6,13]. In these dose-response assays it was found that the maximal efficacy ($E_{\max}$, defined as the maximal response) exhibits a negative correlation with k_{off}[6,13,16].

These experimental studies highlight that the relationship between the TCR-pMHC binding parameters and T cell activation may be complex (Fig. 1). Over the years, these intriguing observations have, in part, motivated the formulation of a variety of models that aimed to reproduce these observed T cell activation phenotypes[8,11,13,18–24]. However, it is presently unclear which model best describes the published experimental data. One reason for this is that these models have been formulated using different mathematical frameworks, making different biochemical assumptions, and as with the experiments, have provided different readouts of T cell activation. This means that when experimental data are generated it is often unclear which model(s) are consistent and which are inconsistent with the data.

In this analysis we compared all the published models that aimed to relate the TCR-pMHC binding parameters to T cell activation. We find that the model most consistent with published experimental data is a kinetic proofreading model that includes limited signalling. The work highlights the need for additional quantitative experimental data.

Phenotypic models

In what follows we performed a synthesis of published models that aimed to predict the quantitative T cell response to antigens of varying affinities. In order to directly compare these published models, we re-cast them using the same mathematical framework into phenotypic models, which are models aimed at making a minimal set of assumptions in order to capture a cellular phenotype (Box 1). The mathematical framework is based on standard well-mixed biochemical reactions at steady-state, which is a reasonable assumption for comparisons with prolonged (> 4 hours) T cell activation assays (see Supplementary Information). Five distinct models were identified including the basic occupancy and kinetic proofreading models and 3 modified kinetic proofreading models.

Occupancy.

The occupancy model (also known as the affinity model) posits that T cell activation is proportional to the number of TCRs occupied by pMHC (Fig. 2A). In this model, the TCR is assumed to achieve a competent signaling state immediately upon pMHC binding. The fraction of bound TCR over the dissociation time and ligand number (Fig. 2B,C) highlights that pMHC with small dissociation times can produce responses identical to those with large dissociation times provided they can be presented at sufficiently high concentrations. The model predicts that pMHC potency (EC_{50}) is directly related to the dissociation time and therefore to K_{D} (Fig. 2C). The majority of evidence in support of the occupancy model has come from studies that have found a strong correlation between EC_{50} and K_{D}[2,4,5,20]. The model predicts that $E_{\max}$ is independent of the TCR-pMHC binding parameters, such as the dissociation time (Fig. 2C), which is inconsistent with experimental data[13].

Kinetic proofreading.

The kinetic proofreading model[18] posits that T cell activation is proportional, not to the total number of occupied TCRs, but to the fraction of TCRs that have been bound by pMHC for sufficient duration to allow the TCR to achieve a competent signalling state (Fig. 2D). In this model, biochemical modifications to the TCR required to achieve the competent signalling state (e.g. tyrosine phosphorylation by Lck, binding by ZAP-70) are initiated upon pMHC binding and are immediately reversed upon pMHC unbinding. The delay between pMHC binding and TCR signalling allows T cells to discriminate pMHC ligands based on their dissociation time for the TCR. The fraction of TCR in the competent signaling state over the dissociation time and ligand number (Fig. 2E,F) highlights that $E_{\max}$ will be dependent on the dissociation time, which has been experimentally observed[6,13,16]. It follows that the model predicts that antigens with small dissociation times cannot attain responses equivalent to antigens with large dissociation times by simply increasing their concentrations. Interestingly, the kinetic proofreading model also predicts the correlation between EC_{50} and K_{D} (Fig. 2I) and therefore all evidence supporting the occupancy model also supports the kinetic proofreading model.

Kinetic proofreading with limited signalling.

As a result of assuming reversible binding between TCR and pMHC, both models considered so far allow for a single pMHC to serially bind multiple TCRs. Why then does the present kinetic proofreading model not exhibit an optimum dissociation time for T cell activation as reported by serial triggering models[13,19,25–28]? The two models make identical biochemical assumptions but differ in the predictor used for T cell activation. Serial triggering models assume that T cell activation is proportional to the *rate* of forming competent signaling TCRs rather then simply their concentration. This assumption translates into assuming that each TCR can only produce a single packet of signaling per pMHC binding event and therefore prevents continuous signaling by pMHC with large dissociation times. Put another way, serial triggering models implicitly assumes that signaling through individual TCRs is limited.

The kinetic proofreading with limited signalling model is an extension of the kinetic proofreading model that posits that TCRs that have reached the competent signalling state produce signalling for a limited period of time (Fig. 2G). Mechanistically, this assumption is consistent with the observation that TCR signaling is limited to their journey from the periphery to the centre of the synapse[29] Cite: Lee KH *et al*. The immunological synapse balances T cell receptor signaling and degradation. *Science* **302** 1218-1222 and/or that the TCRs cease to signal once they are tagged for removal from the T cell surface[30–32].

In this model, the predicted activation exhibits an optimum when plotted over the dissociation time (Fig. 2H) even at high ligand concentrations, which is exemplified by the optimum in $E_{\max}$ (Fig. 2I). Why does an optimum dissociation time persist at high concentrations? In this model, limited signaling means that in order to maintain continuous (steady-state) signaling, which is required for T cell activation, pMHC must serially bind TCRs. It

follows that pMHC ligands with large dissociation times will ultimately remain bound to non-signaling TCRs after producing only transient signaling, at all pMHC concentrations. This is the underlying mechanism for the optimum T cell activation in serial triggering models[13,19,25–28].

Kinetic proofreading with sustained signalling.

The kinetic proofreading with limited signaling predicts that there will be an optimal dissociation time for T cell activation at all pMHC concentrations. This is inconsistent with some modified kinetic proofreading models that predict an optimum at low but not high pMHC concentrations[8,11]. Instead of assuming that signaling is limited, these models make the assumption that competently signaling TCRs can sustain signaling upon pMHC unbinding.

The kinetic proofreading with sustained signalling model is an extension of the kinetic proofreading model that allows competent signalling TCRs to sustain signalling, for a prescribed period of time, even after pMHC unbinding (Fig. 2J). The possibility of a sustained signalling state was inferred from experimental data[8,11] and may be mechanistically related to the idea that TCRs and associated complexes (signalosomes) continue to signal upon pMHC unbinding until phosphatases dephosphorylate the signalling competent TCRs or they are internalized.

The fraction of competent signaling TCR over the dissociation time reveals a concentration-dependent optimum (Fig. 2K). At low concentrations the balance between serial binding and kinetic proofreading means that there will be an optimal dissociation time for a single pMHC to produce multiple TCR with sustained signaling. Since signaling is not limited in this model there is no requirement for serial binding and therefore at high concentrations even pMHC with large dissociation time produce maximal signaling. Put differently, the appearance of the optimum is not a result of requiring serial triggering but a by-product of it. Animations comparing this model to the previous three models underlines this difference (Supplementary Videos 1-3).

The fraction of competent signaling TCR over the number of ligands (Fig. 2L) reveals that, like the occupancy model, this model predicts that $E_{\max}$ will be independent of the dissociation time. This puzzling result can be understood as a breakdown in kinetic proofreading. Although the rate of producing competently signaling TCRs by pMHC with small dissociation times is slow (as a result of kinetic proofreading) many such TCRs can be produced when the concentration of pMHC is sufficiently high. Sustained signaling prevents TCRs to return to their basal state resulting in a breakdown of kinetic proofreading.

Kinetic proofreading with negative feedback.

The kinetic proofreading with negative feedback is an extension of the kinetic proofreading model that allows for TCRs at intermediate steps and/or TCRs at the final competent signalling state to modulate the modification rate of the TCR. Mechanistically it is thought that negative feedback may involve SHP-1 being phosphorylated and recruited to the TCR by active Lck that is associated with the phosphorylated TCR[33]. We note that other phosphatases (e.g. SHP-2, DUSP6, and others that are under the control of miR-181a) may also be involved in this feedback[34]. Unlike all models discussed so far, this model predicts that T cell activation will exhibit an optimum as a function of pMHC dose (Fig. 3). The maximum response and the pMHC concentration producing half-maximal response in this model are modulated by τ. The model was initially formulated with both positive and negative feedbacks using deterministic calculations[23] and subsequently investigated using stochastic simulations[35,36]. Recently, a phenotypic reformulation of the model was performed, which we have used in the present work, illustrating that the main feature of the model can be reproduced with a single negative feedback loop[24].

Experimental support for phenotypic models.

In some form or another, there is experimental support for all proposed phenotypic models of T cell activation. Experiments have shown correlations between EC_{50} and K_{D} in a number of systems[4–6,13], which is consistent with all proposed phenotypic models (Fig. 2). It follows that EC_{50}-K_{D} correlations cannot be used to discriminate between models. On the other hand, some of these experiments have shown that $E_{\max}$ depends on the pMHC binding parameters, including correlations between $E_{\max}$ and τ (or k_{off})[6,9,13,16], which cannot be explained by the occupancy model nor by the kinetic proofreading with sustained signalling model, suggesting that these two models are incomplete. However, there have also been reports of an optimal τ for T cell activation[7–9,11], which cannot be explained by the occupancy or the kinetic proofreading model. Taken together, this would suggest that the only model that cannot be rejected is the kinetic proofreading with limited signalling.

However, Gonzalez et al[11] performed experiments at low and high antigen doses and found that an optimal τ disappeared at high doses. This result is inconsistent with the limited signalling model but is consistent with the sustained signalling model. We note that only a single study found a dose-dependent optimum. Given these limited data in support of the sustained signalling model and the large datasets that are inconsistent with it[6,9,13,16], we conclude that the majority of published data support the kinetic proofreading with limited signalling model.

Evidence for an optimal dissociation time has also come from naturally occurring TCRs and *in vivo* studies[10,12,17]. Ueno et al[10] studied two T cell clones isolated from an HIV-infected patient that recognize a Pol-derived peptide showing that the T cell bearing the higher affinity TCR exhibited impaired functional responses. By expressing the TCRs from these clones in other primary T cells they show that the decreased response is not related to the state of the isolated clones but is likely a generic feature of TCR signaling. In the work of Corse et al[12] immunization with an intermediate affinity peptide produced the maximal immune response as measured by, for example, the number of antigen specific responding cells. Interestingly, in vitro experiments did not detect an optimum affinity. One possibility to reconcile these results is that signaling was not limited in vitro but was in vivo, which may reflect a change in the T cell signaling machinery which could arise as a result of other receptor-ligand interactions.

The kinetic proofreading with limited signaling predicts that the maximal response but not potency will exhibit an optimum (Fig. 2I). This implies that the dose-response curves predicted by this model may intersect for specific dissociation times (not shown). This would mean that at a low dose one antigen will outperform another at activating a T cell while at a high dose their performance would be reversed (see Fig. 4 in[13]). Although not explicitly stated, previous work does suggest that dose-response curves may intersect so that the relative activity of antigens does not simply depend on their binding properties but also on the dose at which they are presented[2,6,37].

The kinetic proofreading with negative feedback, in its present formulation, is unable to reproduce an optimal dissociation time but does predict an optimal pMHC dose for T cell activation (Fig. 3). There is some experimental evidence for an optimal antigen dose[24,38,39] but additional work with antigens of varying affinities is needed.

Extensions, modifications, and applications of phenotypic models

Effect of thresholds and switch-like responses

We have so far assumed that the fraction of competently signaling TCR in each model directly determines the extent of T cell activation in individual T cells. However, TCR signals are processed by the complex cellular signaling machinery[1] (Fig. 4A,B), which ultimately determines the extent of T cell activation. Given that cellular signaling is known to exhibit thresholds and switches[23,40–42] we examine the consequences of such signal processing on

phenotypic model predictions.

There is evidence for 'digital' signaling in T cells whereby the concentration of phosphorylated ERK (ppERK) in individual T cells appears to exist in only two modes, namely fully desphosphorylated or fully phosphorylated[23,41]. Mechanisms for producing such all-or-none responses often involve feedback between signaling proteins[41,43]. Assuming that cellular signaling is all-or-none produces a good threshold and a good switch and ultimately changes the predicted dose-response from phenotypic models (Fig. 4C). T cell activation is now predicted to be highly sensitive to the number of ligands producing steep dose-response curves. Such highly sensitive dose-response curves have been experimentally observed for various functional T cell responses, such as IL-2, TNF-α, IFN-γ, and certain functional readouts, such as CD69, appear perfectly all-or-none[6,13,41,44].

A key drawback with all-or-none cellular signaling is that it cannot explain the differential activation states of certain functional responses in individual T cells, which is further exemplified by the fact that $E_{\max}$ appears independent of the dissociation time (Fig. 4C). This is inconsistent with experimental data showing that the amount of IFN-γ produced by individual T cells directly depends on the pMHC concentration and dissociation time[13,45] and recently, it has been shown that the rate of IL-2 production is proportional to antigen dose[46].

One possible way to reconcile these observations is to assume that the cellular signaling pathway for these functional responses exhibits a threshold but not a switch (Fig. 4D). A simple signaling mechanism to produce a good threshold but a poor switch is multisite phosphorylation[47]. Under this assumption, we find that T cell activation is more sensitive to ligand number whilst maintaining differential activation states for individual T cells as experimentally observed. Interestingly, experiments measuring the number of triggered TCRs (a proxy for TCR signaling) as a function of IFN-γ have produced this precise relationship[40].

Effect of a second pMHC ligand

Experimental work has also revealed intriguing effects on T cell activation (induced by an agonist pMHC) by the co-presentation of a second pMHC (Fig. 5). The presentation of a second pMHC can actually decrease the T cell response to an agonist; such ligands have been termed antagonists[14,33,48–53]. These antagonist ligands are typically altered peptide ligands of the agonist with a smaller dissociation time[50,52,53]. Interestingly, self pMHC, which are expected to have smaller dissociation times compared to antagonists, have been suggested to act synergistically with the agonist leading to enhanced T cell responses[54,55]. It is reasonable to assume that in the limit of small dissociation times the pMHC will no longer interact with the TCR and such null pMHC are expected to have no effect on T cell activation. Collectively this work points to a complex regulation of T cell activation by the presentation of multiple pMHC (Fig. 5).

The precise mechanisms by which antagonist pMHC decrease and self pMHC increase T cell activation is controversial. It has been reported that antagonist stimulation results in incomplete patterns of ζ-chain phosphorylation and a failure to activate ZAP-70[33,48,49,51], which is consistent with a kinetic proofreading model whereby antagonist binding leads to some but not all TCR modifications. A study by Carreno et al[53] found that antagonists were able to attract the polarisation of the T cell Golgi away from dendritic cells presenting agonist ligands, indicating that the dissociation time necessary for immunological synapse assembly is smaller than that needed for activation. The ability of antagonists to dominate the polarisation of the TCR signalling machinery and initiate incomplete signalling raises the question of whether antagonism is mediated simply by antagonists outcompeting agonists for TCR occupancy or if antagonists produce an inhibitory signal. The question has been investigated with experiments involving T cell hybridomas expressing two independent TCRs to determine if the stimulatory activity of an agonist pMHC that binds one TCR can be reduced by an antagonist pMHC that binds the other TCR. In this system, some investigators did not find evidence for cross-antagonism[56,57], while others did[58,59]. It has been argued that a reason for the discrepant results could be that the expression level and spatial separation of the TCRs mean that a local inhibitory signal from one TCR may not affect the second[60]. There is no clear consensus

on the precise mechanism of antagonism, but signalling-dependent theories have suggested that proteins could associate with the incompletely phosphorylated ζ-chains through single SH2 domains[48,60]. These proteins would be displaced during full T cell activation by ZAP-70 which has a stronger binding to fully phosphorylated ITAMs through its tandem SH2 domains. In agreement with this, the cytoplasmic tyrosine-phosphatase SHP-1 has been found to be associated with both TCRs during a dual TCR experiment that showed cross-antagonism[58].

The effect of a second pMHC (presented at 3000 ligands per cell) on T cell activation for all phenotypic models is shown in Fig. 6. We find that only the kinetic proofreading with negative feedback predicts the possibility of antagonism as a result of the initiation of negative feedback which inhibits the response from the agonist. Antagonism is observed for the kinetic proofreading and the kinetic proofreading with limited signaling models at very high concentrations of the second pMHC but the decreased response in this case is mediated by the antagonist ligand out-competing the agonist for TCR occupancy (Fig. S2). As discussed above, experimental data on antagonism is controversial and in many studies a very high concentration of the antagonistic pMHC is required to observe inhibition. This means that it is difficult to reject models on the basis of whether the model exhibits antagonism. Note that none of the current phenotypic models are able to reproduce the qualitative observation of co-presentation (Fig. 5).

Effect of co-receptors

The effect of co-receptors on the relationship between the TCR-pMHC binding parameters and T cell activation has also been investigated. A study by Holler & Kranz[4] showed that CD8 generally increased the pMHC potency (reduced EC_{50}). At the extremes, high affinity pMHC were found to be sufficiently stimulatory without CD8 and pMHC with low affinities (K_{D} >3 μM) require CD8 in order to stimulate T-cells[4,61]. These effects are not a result of cooperative binding as it has been shown that the binding of TCR and co-receptors to pMHC are independent[62,63]. Instead, it is likely that co-receptors increase T cell sensitivity by facilitating the formation of a ternary TCR-pMHC-coreceptor complex, which is stabilized by an interaction between the TCR and co-receptor through Lck and/or ZAP-70[64,65] consistent with structural data[66]. Co-receptors have been found to modulate the properties of ligands that on their own do not produce a T cell response. Such ligands can act as antagonists when presented to cells that lack CD8 but can act as co-agonists in the presence of CD8[14]. This is thought to be caused by the recruitment of CD8 to the immunological synapse in a peptide-independent but MHC-dependent manner[55]. Similar results have been found with CD4[51]. In addition to modulating the effective TCR-pMHC kinetics, co-receptors, by virtue of their association with Lck, may also alter the rate of kinetic proofreading.

The formation of a ternary complex coupled to modification of kinetic proofreading introduces a large number of unknowns, which may explain why the implementation of co-receptors has differed between mathematical models[28,67–69]. Additional work is needed to independently determine the contribution of co-receptors to modulating the TCR-pMHC binding kinetics and to altering the kinetic proofreading process before accurate predictions can be made.

Application to T cell differentiation

Signaling downstream of the TCR following antigen stimulation has also been implicated in T cell differentiation. In the case of CD4 T cells, it is generally accepted that a unique cytokine profile will determine their differentiation into several T helper cell lineages[70] (e.g. IFN-γ and IL-12 producing Th1 cells and IL-4 producing Th2 cells with the same antigenic stimulation). However, studies have also shown that the dose of antigen can influence differentiation both *in vitro*[71–73] and *in vivo*[74], with high and low antigen doses producing Th1 and Th2 cells, respectively. Given that TCR signaling is determined by both antigen dose and the TCR-pMHC binding parameters (Fig. 2), it is no surprise that the TCR-pMHC binding kinetics may also influence differentiation, with low affinity ligands favouring a Th2 response[75,76]. Low dose antigenic stimulation has also been shown to favour regulatory

T cell induction[77,78].

It is interesting to consider how phenotypic models of T cell activation, which directly predict the TCR signal, can be modified or directly applied to the study of cell differentiation. The kinetic proofreading with limited signaling, for example, would suggest that pMHC with both small and large dissociation times (affinities) will result in Th2 differentiation whereas Th1 differentiation will exhibit an optimum as a function of dissociation time. The kinetic proofreading with negative feedback would predict that both low and high doses of antigen can induce Th2 differentiation, which has been experimentally observed[71]. We note that it is unclear what information the pMHC dissociation time provides to the immune system that would make it beneficial to mount a Th1 vs Th2 type of immune response. The present analysis highlights that coupling differentiation experiments with titration of pMHC of varying affinities can be used to infer phenotypic models of differentiation.

Effect of 2D TCR/pMHC interactions

Development of T cell activation models has relied almost exclusively on relating functional T cell responses to TCR/pMHC binding parameters determined when at least one of the proteins is in solution (e.g. surface plasmon resonance based measurements). However, the TCR and pMHC are confined to membranes and, like many other receptor-ligand interactions, interact at the interface between two cells. The relationship between the membrane (or 2D) binding parameters and the solution (or 3D) binding parameters remains controversial. To develop models of T cell activation, it has been implicitly assumed that the measured 3D binding parameters are linearly related to the 2D binding parameters. In this section we discuss two processes that may impact this assumption.

Rebinding may influence 2D dissociation times. Bimolecular reactions between proteins confined to the plasma membrane are thought to be limited by diffusion because the membrane diffusion coefficient is small (generally < 1 μm^2/s). This means that upon unbinding, proteins confined to membranes can have a high probability of rebinding (instead of diffusing apart) and this process is predicted to be rapid (sub-millisecond). Given that rebinding has been theoretically predicted[79] and experimentally observed[80] for cytosolic proteins, it is expected to be even more pronounced when both proteins are confined to membranes.

The implication of this for the TCR is that intervals between rebinding events may not be detected and therefore the effective 2D dissociation time is approximately equal to the 3D dissociation time multiplied by the number of rebinding events[15,81,82]. Since the number of rebinding events is determined, in part, by the on-rate, the 2D dissociation time may exhibit a dependency on k_{on}. Although direct evidence for TCR/pMHC rebinding has not been reported, the rebinding-corrected 2D dissociation time has been shown to be a better predictor of T cell activation compared to the 3D dissociation time[13,15,82].

It is worth noting that rebinding can also be enhanced by the clustering of the TCR, membrane alignment, and conformational changes in the TCR. Recently, induced rebinding has been proposed to improve antigen discrimination[83].

Force may influence 2D dissociation times. Multiple processes have been proposed to impart tension on the TCR-pMHC complex at the T cell-APC interface. Highly abundant long surface molecules ($\approx$50 nm; e.g. CD45, CD148, and CD43) are predicted to indirectly produce tension on short TCR-pMHC interaction ($\approx$13 nm) and a mechanical model has predicted this tension to be in the range of 10 pN[84]. Other, cytoskeletally-driven sources of force include the relative movements of cell membranes and lateral transport of cell surface molecules within the membrane[85].

Precisely how the dissociation time of TCR-pMHC interaction will depend on force remains an open question. A study utilizing a flow chamber assay reports that nearly all interactions subjected to a force exhibit smaller dissociation times characteristic of slip bonds[86]. It is worth noting that a single pMHC exhibited larger dissociation

times under force, which is characteristic of catch bonds (e.g. some integrins are known to form catch bonds[87]). A recent study by Zhu and colleagues[88] found that agonists for the OT-1 TCR exhibited catch bond behaviour using the biomembrane force probe assay.

The significance of catch bonds is that the 3D dissociation time (measured without an applied force) may not exhibit a positive correlation to the 2D dissociation time (measured with an applied force). Future work is needed to determine whether these results can be generalised to other TCRs. We speculate that all categories of pMHC ligands (e.g. agonists, antagonists, null, etc) may exhibit catch bond behaviour but that ultimately the effective 2D dissociation time will determine the functional T cell response.

Direct measurements of 2D binding parameters. Measurements of 2D binding parameters at cell interfaces are challenging. Recently, published studies have used FRET-based[89] and an adhesion-based[90] assay to examine the 2D binding parameters. Both studies report that the 2D dissociation time is shorter than the 3D dissociation time, consistent with the slip bonds under tension. In support of this inhibitors of the actin cytoskeleton increase the 2D dissociation time[89]. More recently a study using indirect single-particle tracking based assays to measure 2D binding parameters reported no changes between the 3D and 2D dissociation times[91]. This conclusion is consistent with slip bonds under tension combined with rebinding.

All mathematical models of T cell activation require 2D TCR-pMHC binding parameters. It follows that incorporation of rebinding and/or force into the models involves transforming the 3D binding parameters into 2D binding parameters or by directly measuring the 2D binding parameters. It is important to note that correlations between 3D binding parameters (without an applied force) and the functional T cell response have been very high and therefore it is likely that the relationships between the 3D and 2D binding parameters are monotonic (i.e. increasing or decreasing).

Conclusion

In this analysis, we have reformulated models of T cell activation into simple phenotypic models (Box 1) which allowed us to directly compare the predicted T cell response for pMHC at different concentrations and binding parameters. We have found that the phenotypic model most compatible with experimental data is the kinetic proofreading with limited signalling. However, we emphasize that the published data are incomplete with experiments typically using only a small panel of TCRs (or pMHCs) with a limited range of affinities and a single or just a few different doses. The study of co-presentation of pMHC, co-receptors, differentiation, and other co-stimulatory/co-inhibitory molecules are often limited to qualitative studies involving pMHCs whose affinities are unknown and whose concentration is often fixed. Phenotypic models of T cell activation can be used to guide the design of modified T cell receptors and chimeric antigen receptors for adoptive T cell based therapies[92–94]. We note that all phenotypic models we have considered make no explicit assumptions about the mechanism by which pMHC binding generates intracellular signaling, a process termed TCR triggering, and these phenotypic models are largely consistent with all mechanisms of TCR triggering (van der Merwe P.A & Dushek, O. Mechanisms for T cell receptor triggering. *Nat. Rev. Immunol* **11** 47-55). The present analysis highlights that detailed dose-response experiments using many TCR/pMHC pairs with a large range of affinities can be used to dramatically constrain, reject, and formulate models of T cell activation.

ACKNOWLEDGEMENTS. ML is supported by a Doctoral Training Centre Systems Biology studentship from the Engineering and Physical Sciences Research Council (EPSRC). OD is supported by a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (Grant Number: 098363).

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Highlighted references

[18] The first application of kinetic proofreading to T cell receptor signaling, which has formed the basis of all models of T cell signaling and activation.

[19] The first study to propose that a trade-off between serial binding and kinetic proofreading will produce an optimal dissociation time for T cell activation.

[7] The first study to provide experimental evidence in support of an optimal dissociation time for T cell activation.

[11] Provided the only known experimental evidence for a concentration-dependent optimum and constructed a modified kinetic proofreading model that can explain this result.

[13] Resolved the long standing debate between occupancy and kinetic proofreading based models by showing that the maximal response depends on the dissociation time at the population and single-cell level.

[24] Formulated a simple phenotypic model of kinetic proofreading with a single negative feedback that exhibits improved antigen discrimination and predicts an optimum in the dose-response.

[23] Modified kinetic proofreading to include both positive and negative feedbacks showing improved antigen discrimination and the ability to predict a bimodal (digital) ERK response.

[40] Provided evidence that IFN-γ exhibits a good threshold but a poor switch to proximal TCR signaling.

[33] Provided mechanistic evidence for a SHP-1 mediated negative feedback and an ERK mediated positive feedback.

[89] [90] These studies report direct measurements of the physiological TCR-pMHC kinetics at 2D membrane interfaces showing smaller dissociation times compared to 3D solution measurements.

Figures

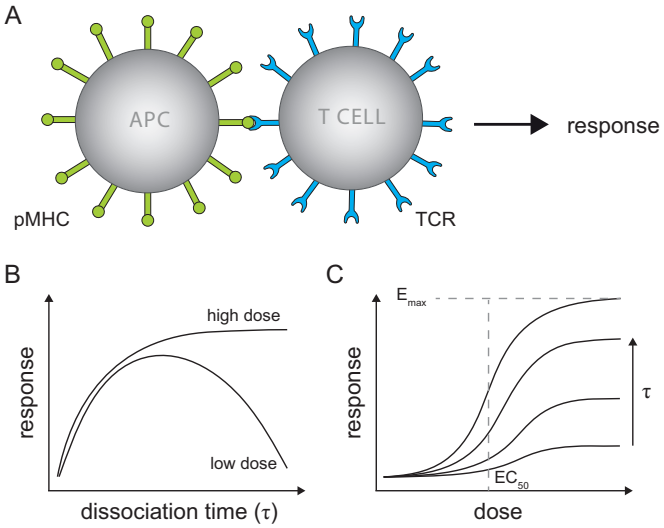

Figure 1: Relationship between TCR-pMHC binding parameters and T cell activation. A) Schematic illustrating typical T cell activation assays whereby a functional T cell response (e.g. cytokine production) is measured after several hours of interaction with antigens presented on APCs (or an APC surrogate). B) Experiments have suggested that the T cell response exhibits a maximum when plotted over the dissociation time (τ) so that antigens that bind with long dissociation times (large affinities) lead to poor activation of T cells[7–9,12,17] with one study suggesting that this optimum may only exist at low antigen doses[11]. C) Experiments using detailed antigen titration have also shown that the dissociation time determines both the potency (EC_{50}) and maximal efficacy ($E_{\max}$) so that antigens with short dissociation times can not produce the same maximal response as antigens with long dissociation time[6,13]. Recent data have also suggested that an optimal antigen dose for T cell activation (not shown)[38,39]. Note that panels B-C are schematics.

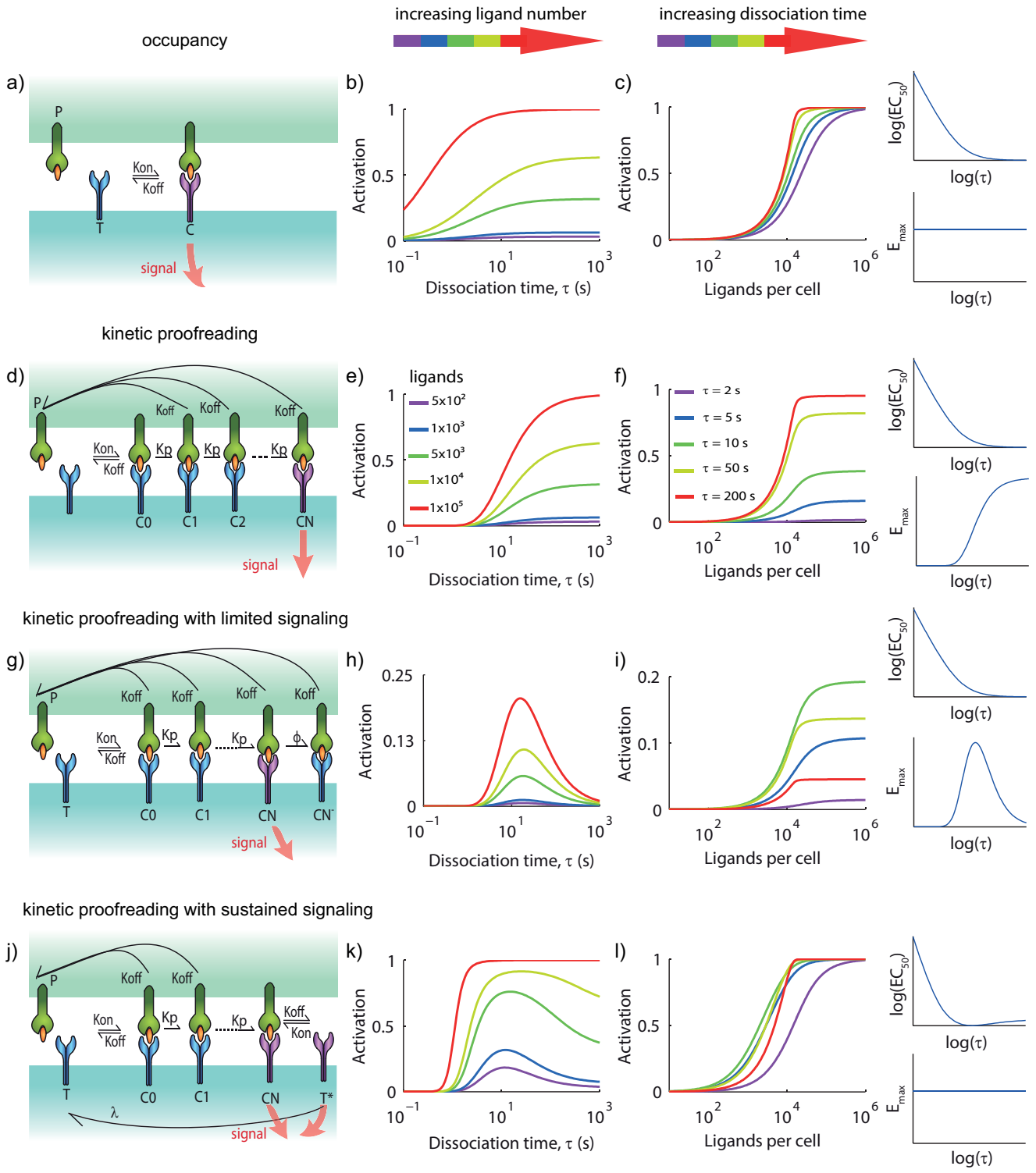

Figure 2: Phenotypic models of T cell activation. Model schematics (left), predicted T cell activation over the dissociation time (centre) and over pMHC number (right) are shown for the indicated model on each row. Each model makes a qualitatively different prediction in a dose-response assay yet differs by few (or even one) reaction. All calculations are performed with identical k_{on} values and the indicated dissociation time ($\tau = 1/k_{\text{off}}$). The inverse relationship between EC_{50} and τ translates into a direct relationship between EC_{50} and K_{D} since $K_{\text{D}} = k_{\text{off}}/k_{\text{on}}$. Included as Supplementary Videos 1-3 are animations illustrating the models. See Supplementary Information for details on model formulations, calculations, and parameter values. A summary of the predictors of EC_{50} and E_{max} can be found in the Supplementary Information.

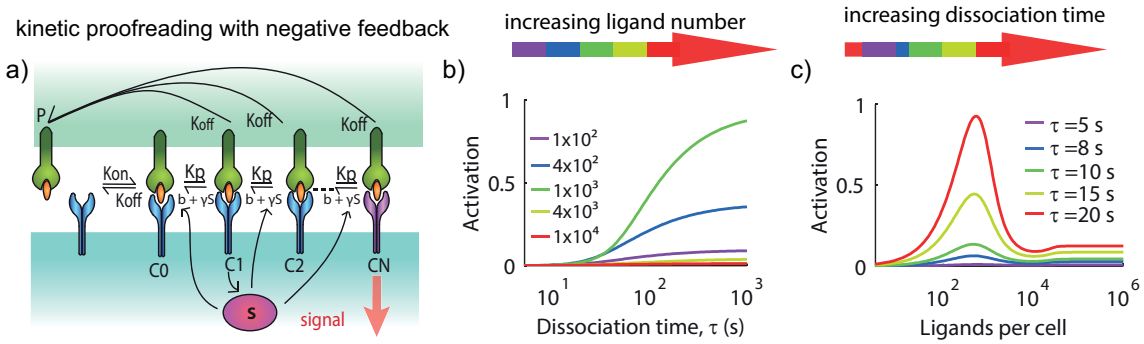

Figure 3: Kinetic proofreading with negative feedback. Shown is a schematic of the model (left), the predicted T cell activation over the dissociation time (centre) and pMHC number (right). See Supplementary Information for details on model formulation, calculation, and parameter values.

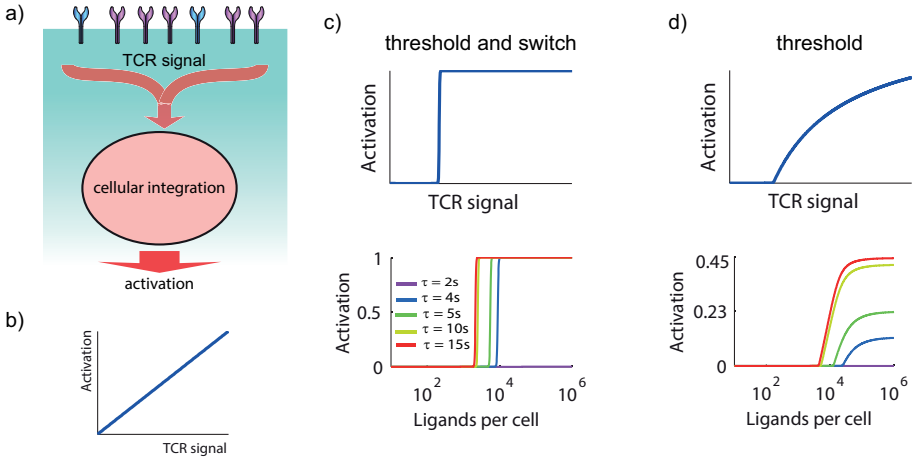

Figure 4: Effects of thresholds and switches in cellular signaling on T cell activation. A) The cellular signaling machinery integrates signals from competent signaling TCRs and relays this information into the degree of T cell activation. B) All models presented so far have assumed that the cellular signaling machinery linearly relates the TCR signal into T cell activation. C-D) Predicted T cell activation of kinetic proofreading with limited signaling when the cellular signaling machinery produces C) good thresholds and switches (e.g. digital signaling) and/or D) good thresholds and poor switches. Similar results are found with other phenotypic models (Fig. S1).

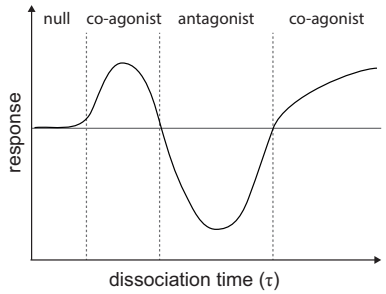

Figure 5: Modulation of T cell activation by co-presentation of a second pMHC. The presentation of an agonist pMHC is known to elicit T cell responses (horizontal grey line). However, the co-presentation of a second pMHC is known to modulate this response (wavy black line) and this modulation depends on the dissociation time of the second pMHC (x-axis). Both self, altered-self (cancer), and foreign pMHC may induce co-agonist and antagonist effects. Presently, no model of T cell activation can reproduce these results.

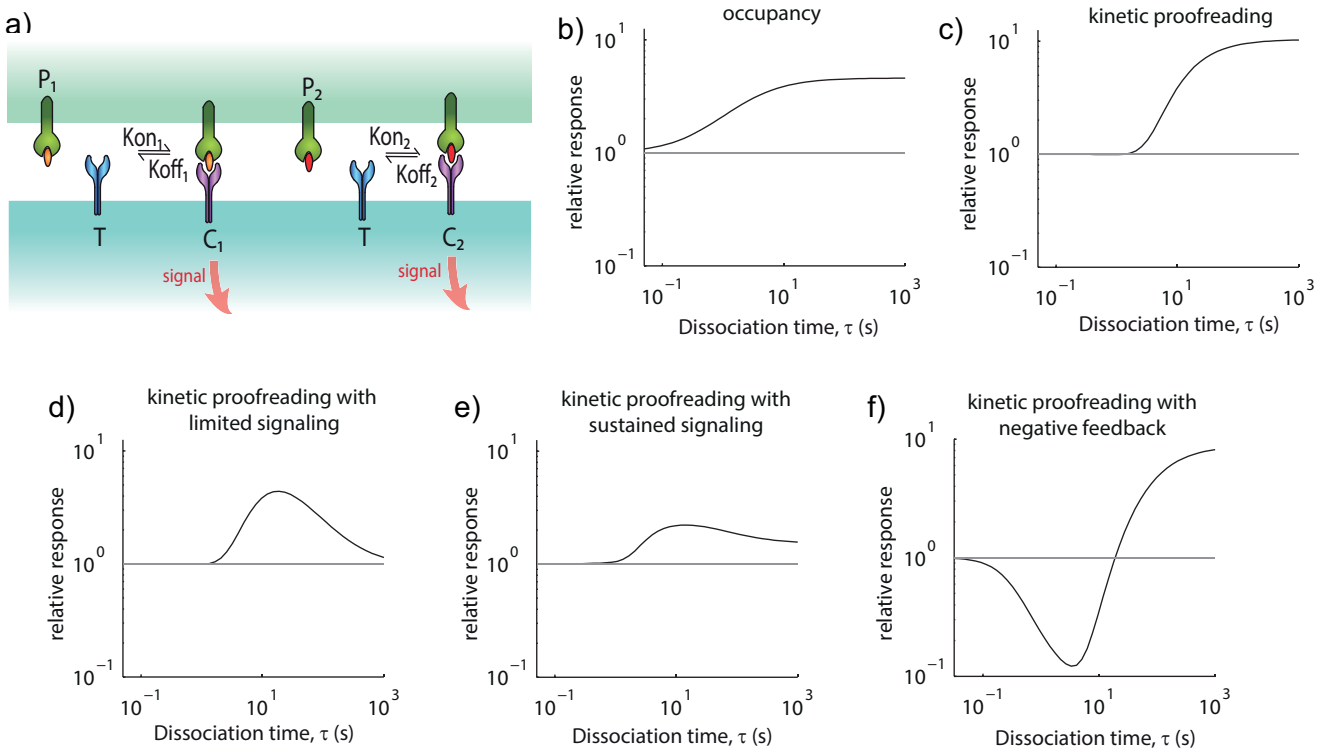

Figure 6: Co-presentation of a second pMHC is predicted to inhibit T cell activation in the kinetic proofreading with negative feedback. A) Schematic of binding reactions when two pMHC are present. B-F) The fold-change in T cell activation when the second pMHC is presented at 3000 ligands per cell with the indicated dissociation time (x-axis). The first pMHC is assumed to have a dissociation time of 10 s and be presented at 1000 ligands per cell. Effects of changing the concentration of the second pMHC is shown in Fig. S2.

Box 1. Phenotypic models.

Mechanistic models of T cell activation that capture signalling events from T cell receptor triggering to transcriptional regulation are based on many assumptions. These assumptions include which proteins are involved in the process, how they interact with one another, and a variety of parameter values, such as reaction rate constants and protein concentrations. Ultimately, this means that predictions using these models may have high uncertainty. This is the elephant in the room and a consensus for how to solve this problem does not presently exist.

In contrast to mechanistic models, phenotypic models aim to reproduce experimental data based on a minimal set of assumptions. Since they do not capture all signalling events they are deemed effective models. By virtue of making only a few assumptions, phenotypic models have only a few unknown parameters. A key advantage of these models is that it is often obvious (and intuitive) which model assumption is responsible for a particular phenotype. A recent article has highlighted the utility of such minimal models[95]. Interestingly, these minimal phenotypic models have been able to reproduce the quantitative T cell phenotypes despite the large and complex T cell signalling machinery[13,24].

Glossary.

Bimolecular on-rate ($k_{\mathbf{on}}$). The rate constant of TCR-pMHC binding (with typical units of $\mu\mathrm{M}^{-1}\mathrm{s}^{-1}$ for 3D solution measurements and typical units of $\mu\mathrm{m}^{2}\mathrm{s}^{-1}$ for 2D membrane measurements).

Off-rate ($k_{\mathbf{off}}$). The rate of TCR-pMHC unbinding (with typical units of s^{-1}).

Dissociation time (τ). The characteristic duration of a TCR-pMHC binding interaction ($\tau = 1/k_{\mathrm{off}}$ with typical units of s).

Dissociation constant ($K_{\mathbf{D}}$). The characteristic strength of binding ($K_{\mathrm{D}} = k_{\mathrm{off}}/k_{\mathrm{on}}$ with typical units of μM for 3D solution measurements and typical units of $\mu\mathrm{m}^{-2}$ for 2D membrane measurements).

Maximal efficacy ($E_{\max}$). The maximal T cell response achieved at saturating pMHC concentrations (with units provided by the functional assay).

Potency (EC_{50}). The concentration/dose of pMHC ligand producing half-maximal response (with units provided by the ligand dose).

Slip bonds. These are bonds whose dissociation time decreases under tension.

Catch bonds. These are bonds whose dissociation time increases under tension.

Analogue signalling. A mode of cellular signalling whereby the concentration of a signalling protein in individual cells is found in a continuum of states.

Digital signalling. A mode of cellular signalling whereby the concentration of a signalling protein in individual cells is confined to discrete states (e.g. all protein is either fully phosphorylated or fully dephosphorylated in a cell)

Deterministic models. A form of mathematical models whereby the mean behaviour of a biochemical reaction network is directly calculated, often using ordinary-differential-equations. All mathematical models in the present analysis are of this type.

Stochastic models. A form of mathematical model whereby the behaviour of a biochemical reaction network is simulated based on reaction probabilities. Each simulation produces a different result but the mean of many such simulations often (but not always) agrees with the mean directed calculated in deterministic models.

Supplementary Video Captions

Animations depict the signaling outcome for the occupancy, kinetic proofreading, kinetic proofreading with limited signaling, and the kinetic proofreading with sustained signaling for a pMHC ligand with A) small, B) intermediate, and C) large dissociation time. Animations are shown for a low concentration of pMHC.

Figure S1:

Figure S2: