

# Molecular mechanisms of T cell sensitivity to antigen

Jesús A. Siller-Farfán<sup>†</sup>, Omer Dushek<sup>†¶</sup>

<sup>†</sup>Sir William Dunn School of Pathology, University of Oxford, Oxford, UK

<sup>¶</sup>Corresponding author

June 27, 2018

## **Correspondence to:**

Omer Dushek

Sir William Dunn School of Pathology

South Parks Road

University of Oxford

Oxford OX1 3RE

United Kingdom

Telephone: +44 (0) 1865 275576

e-mail: omer.dushek@path.ox.ac.uk

## Abstract

T cells initiate and regulate adaptive immune responses that can clear infections. To do this, they use their T cell receptors (TCRs) to continually scan the surfaces of other cells for cognate peptide antigens presented on major histocompatibility complexes (pMHCs). Experimental work has established that as few as  $\sim 1$ -10 pMHCs are sufficient to activate T cells. This sensitivity is remarkable in light of a number of factors, including the observation that the TCR and pMHC are short molecules relative to highly abundant long surface molecules, such as CD45, that can hinder initial binding and moreover, the TCR/pMHC interaction is of weak affinity with solution lifetimes of  $\sim 1$  seconds. Here, we review experimental and mathematical work that has contributed to uncovering molecular mechanisms of T cell sensitivity. We organise the mechanisms by where they act in the pathway to activate T cells, namely mechanisms that 1) promote TCR/pMHC binding, 2) induce rapid TCR signalling, and 3) amplify TCR signalling. We discuss work showing that high sensitivity reduces antigen specificity unless molecular feedbacks are invoked. We conclude by summarising a number of open questions.

**Keywords:** T cells, T cell receptor, antigen, MHC, sensitivity, lymphocyte signalling, mathematical modelling

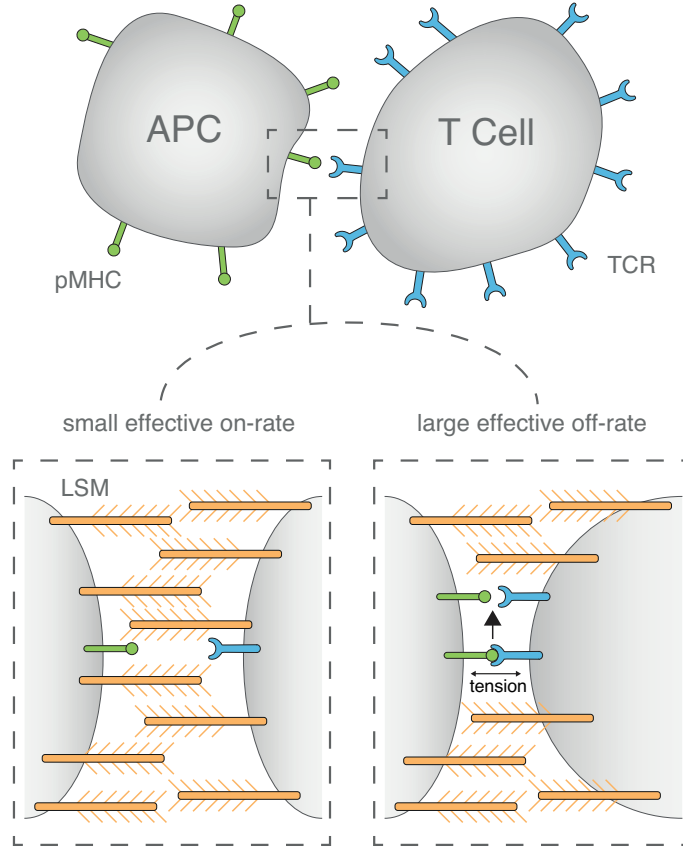
# 1 The Challenge

T cells are important players in adaptive immunity. They are activated to respond when their T cell receptors (TCRs) recognise antigens in the form of short peptides bound to major histocompatibility complexes (pMHCs) on the surfaces of other cells, which are collectively known as antigen presenting cells (APCs). Upon activation, T cells are able to clear infections through multiple mechanisms. For example, CD8<sup>+</sup> T cells can directly kill virally infected cells and CD4<sup>+</sup> T cells provide help to B cells to produce viral-specific antibodies. Through these and other mechanisms, T cells are able to initiate and regulate immune responses that can clear infections.

Experimental work over the past two decades has clearly shown that T cells can become activated when recognising even a single pMHC ligand on the APC surface. This level of sensitivity was indirectly inferred in early studies (1); subsequent microscopy based studies have been able to directly observe this sensitivity at the single cell level. In the case of CD4<sup>+</sup> T cells, it has been observed that a single pMHC can not only produce an early calcium response (2) but can also lead to cytokine secretion after several hours (3). As for CD8<sup>+</sup> T cells, it has been observed that 3 pMHC ligands can induce target cell killing (4). The precise number of pMHC antigens required for T cell activation will likely depend on the affinity of the interaction and other variables such as the life history of the T cell, but it is clear that T cells can be activated in response to  $\sim 1$ -10 pMHC antigens.

The sensitivity displayed by T cells is remarkable in light of a number of observations (*Fig. 1*). First, initial TCR/pMHC binding is unfavourable because the TCR and pMHC are short molecules spanning  $\sim 7$  nm (5). They reside within highly abundant large surface glycoproteins such as the phosphatase CD45, whose extracellular domain extends  $\sim 22$  nm (6). Second, once binding takes place, individual TCR/pMHC interactions are short lived (lifetimes as short as  $\sim 0.1$ -10 s), yet continued TCR/pMHC interactions for minutes to hours can be required for full T cell activation (7, 8). This is made even more remarkable by noting that the TCR/pMHC interaction is subjected to tensile forces at the contact interface (9), which may further reduce the lifetime at interfaces (10, 11). Finally, T cells are able to ignore APCs that express a high abundance of self pMHCs while retaining sensitivity against a few foreign pMHC that may be chemically similar. This ability is known as antigen discrimination and, as we discuss below, it can place constraints on sensitivity (12, 13). Collectively, these observations suggest that mechanisms must be in place to convert a low number of highly improbable and transient pMHC interactions into a signal that can lead to the activation of T cells.

Why are T cells highly sensitive? The most likely explanation is that they have evolved high sensitivity because only a small number of pMHC ligands are presented to them during infections. During a viral infection, a fraction of viral proteins will be degraded into peptides, which will then be presented on the cell surface as a heterogeneous mixture of foreign pMHC ligands. The copy number of any single pMHC is unlikely to be large. This is consistent with a study that found that a recombinant TCR was only able to recognise 8-46 pMHCs after *in vitro* infection with HIV (14). In addition, viruses, such as HIV (15) and CMV (16), are known to downregulate pMHC as an escape mechanism (e.g. CMV can downregulate expression from 10,000 to less than 100 copies per cell (16)). It follows that for efficient detection of infection, T cells must exhibit high



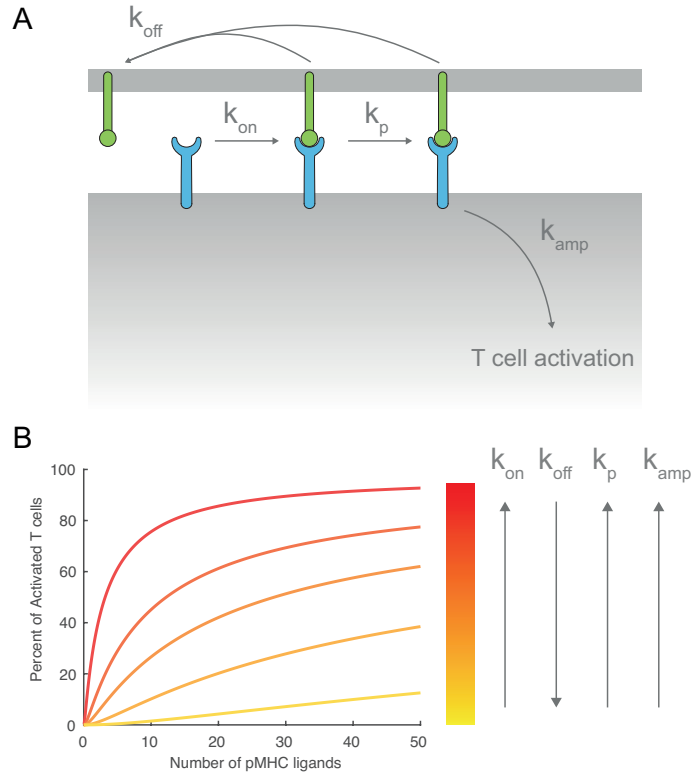
**Figure 1:** T cells are activated by sustained interactions with as few as  $\sim 1$ -10 pMHC ligands on the surfaces of APCs. This sensitivity is difficult to understand because a number of factors seemingly reduce TCR/pMHC interactions. First, the TCR and pMHC are short molecules residing within highly abundant long surface molecules (LSMs). This reduces the probability that the membranes will come into proximity to allow for TCR/pMHC binding (bottom left). Second, the TCR/pMHC interaction off-rate is large, with lifetimes of  $\sim 1$  s when measured in solution using surface plasmon resonance (SPR). Moreover, it can be subjected to tensile forces at the cellular interface that further increase the effective off-rate. Therefore, T cells employ a number of mechanisms to ensure that TCR/pMHC interactions can be sustained and detected.

sensitivity to pMHC antigens.

In this review, we focus on molecular mechanisms that have contributed to our understanding of how T cells are able to recognise a small number of weakly binding pMHC ligands. We then highlight that the requirement of T cells to discriminate between foreign and self pMHC antigens places constraints on sensitivity that can be overcome with feedbacks. We conclude with a number of open questions that would benefit from experiments and mathematical modelling.

## 2 Mechanisms of T cell sensitivity to antigen

There are a large number of diverse mechanisms that contribute to T cell antigen sensitivity. Using a coarse-grained view of signal transduction, we classify them into three categories (*Fig. 2*). We first discuss mechanisms that promote TCR/pMHC binding, which includes molecular processes that effectively increase the on-rate ( $k_{\text{on}}$ ) and/or decrease the off-rate ( $k_{\text{off}}$ ). Second, we discuss mechanisms that are able to quickly induce TCR signalling within the short timescale of a TCR/pMHC interaction (increasing  $k_p$ ). Third, we discuss processes that amplify signalling from a small number of TCRs to allow for T cell activation (increasing  $k_{\text{amp}}$ ).



**Figure 2:** Mechanisms of T cell sensitivity to antigen can be broadly classified into three major categories. A) A simplified signalling schematic that classifies mechanisms of sensitivity into those that 1) promote TCR/pMHC binding by increasing the on-rate ( $k_{\text{on}}$ ) and/or decreasing the off-rate ( $k_{\text{off}}$ ), 2) induce rapid TCR signalling before pMHC unbinds ( $k_p$ ), and 3) amplify TCR signalling allowing for T cell activation by a small number of bound TCRs ( $k_{\text{amp}}$ ). This simplified panel omits feedbacks for clarity. B) A schematic dose-response curve showing that as  $k_{\text{on}}$ ,  $k_p$ , and  $k_{\text{amp}}$  increase or as  $k_{\text{off}}$  decreases, the sensitivity of T cells to pMHC antigens is expected to increase. Arrow indicates direction of increasing kinetic rate.

## 2.1 Mechanisms that promote TCR/pMHC binding

### 2.1.1 Role of close-contacts in promoting TCR/pMHC binding

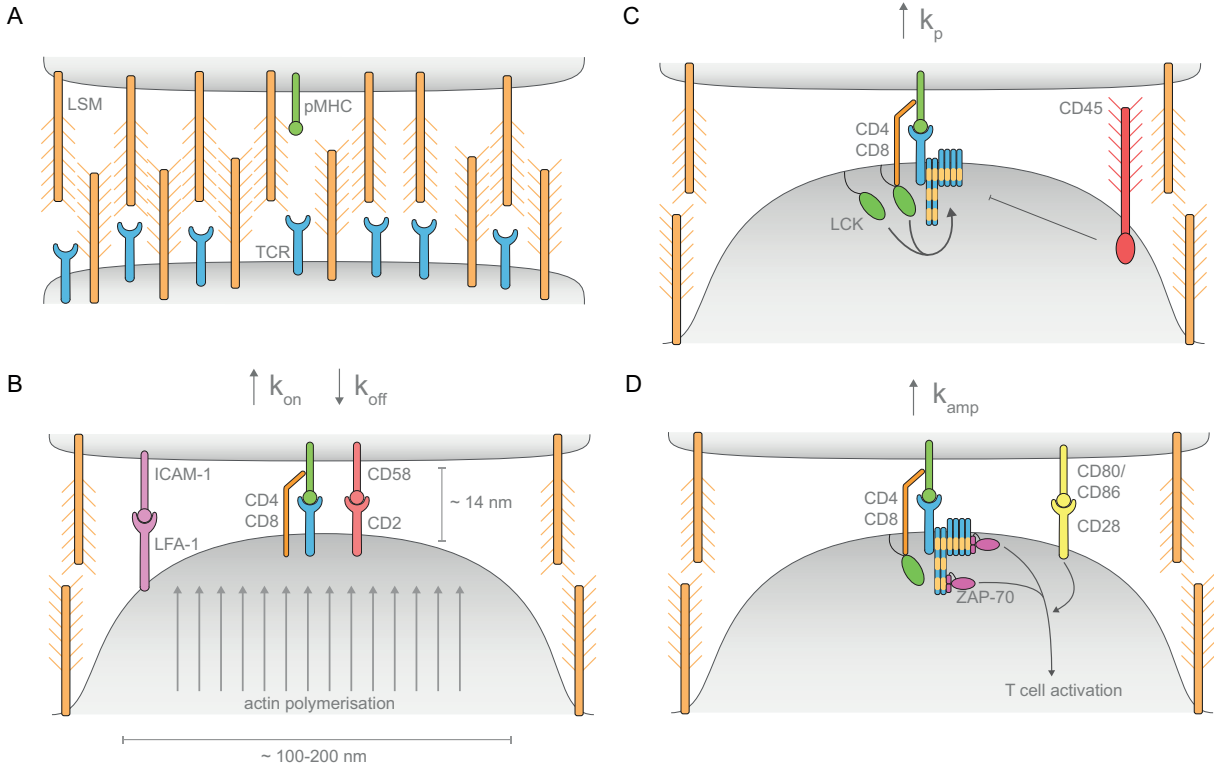
The TCR and pMHC are small molecules whose ectodomains measure  $\sim 7$  nm. Hence, a close contact between the T cell and APC membranes (spanning  $\sim 14$  nm) must be formed for TCR/pMHC interactions (17). However, the surface of both cells contain abundant, negatively charged glycoproteins, such as CD43 and CD45. These large glycoproteins are collectively referred to as long surface molecules (LSMs), and they normally prevent close membrane apposition (*Fig. 3A*). Based on its crystal structure, CD45, one of the most abundant cell surface proteins on T cells (18, 19), has recently been reported to extend  $\sim 22$  nm (6). Therefore, these LSMs reduce the effective on-rate for TCR/pMHC binding and must be segregated and/or compressed for TCR/pMHC binding.

How long would it take for CD45 to be passively (i.e. spontaneously) segregated from a small 100 nm membrane patch? This calculation was recently performed showing that at physiological densities of CD45, it would take an astronomical period of time ( $10^9$  s) (20). A recent *in vitro* reconstitution of passive CD45 segregation showed that it would require  $\sim 15$  min to first observe short receptor/ligand interactions (21). The discrepancy between this experiment and the mathematical model may simply be down to the concentration of CD45, which the model predicts to have a highly nonlinear relationship to the segregation timescale (the model predicts segregation within 5 s with a 4-fold dilution of CD45). Nonetheless, these studies highlight that initial TCR/pMHC binding is unlikely to be driven by passive processes given that TCR signalling is observed within seconds (22).

Instead of waiting for passive close contact formation and hence TCR/pMHC binding, T cells actively produce close contacts. They use structures known as microvilli (23–25) or invadosome-like protrusions (ILPs) (26). These actin-rich 100-200 nm diameter protrusions enable T cells to push on nearby cells in order to achieve close membrane apposition compatible with TCR/pMHC interactions (24), which leads to CD45 segregation (25, 26) (*Fig. 3B*). It is noteworthy that these structures have short ( $\sim 5$ -50 s) lifetimes without antigen (24, 26) but upon antigen recognition they can be stabilised for long ( $\sim 10$  min) timescales (26). This stabilisation may involve TCR signalling leading to sustained actin polymerisation, a phenomenon observed in these protrusions (24, 26).

### 2.1.2 Role of additional receptors in promoting TCR/pMHC binding

A number of additional receptors can modulate TCR/pMHC interactions. The adhesion receptors CD2 and LFA-1 have been shown to increase the sensitivity of T cells to antigens in functional studies (27, 28). Although detailed studies of LFA-1 and CD2 in the context of microvilli protrusions have yet to be reported, it is conceivable that the relatively large LFA-1/ICAM-1 interaction ( $\sim 40$  nm length) (29) paves the way for the smaller CD2/CD58 association. CD2/CD58 are then capable of stabilising a close contact at an intermembrane distance compatible with TCR/pMHC interactions (30, 31) (*Fig. 3B*). Indeed, the CD2/CD58 pair spatially segregates from LFA-1/ICAM-1 complexes (32), which are found surrounding areas of TCR/pMHC interactions (33). Consistent with the role of CD2 in promoting close contact formation and hence TCR/pMHC interactions, it



**Figure 3:** Molecular mechanisms of T cell sensitivity to antigen. A) The small size of TCR and pMHC molecules relative to LSMs means that their basal, effective on-rate is likely to be small. B) A close contact is actively produced by an actin-rich microvilli protrusion that results in the segregation of LSMs. The close contact may be stabilised peripherally by adhesion receptors such as LFA-1, and centrally by CD2. The stabilisation of the close contact allows repeated TCR/pMHC interactions and, potentially, reduced forces on the TCR/pMHC interaction. The CD4/CD8 coreceptors are able to interact with both pMHC and the TCR complex, further promoting TCR/pMHC association. C) The rate at which the TCR is converted into a state where it can signal downstream must be faster or on the same timescale as the TCR/pMHC lifetime for efficient signalling. The net rate of TCR phosphorylation is made large by the fact that the phosphatase that dephosphorylates the TCR (CD45) is an LSM that is segregated from the close contact. However, the kinase Lck is not segregated so the net phosphorylation of the TCR in a close contact is expected to be large. The net phosphorylation may be increased further because coreceptors are associated with Lck. D) Mechanisms that amplify the weak TCR signal include the fact that the TCR is able to potentially recruit multiple cytoplasmic ZAP-70 molecules in order to convert a single pMHC binding event into the activation of multiple enzymes. Costimulation by, for example, CD28 is able to amplify TCR signalling but does not modulate TCR/pMHC binding.

has been shown that human cytomegalovirus can specifically reduce CD58 expression leading to reduced recognition of infected cells by CD8<sup>+</sup> T cells (34). Additionally, knockout of CD58 in cancer cell lines can impair recognition and cytotoxicity by CD8<sup>+</sup> T cells (35).

The coreceptors CD4 and CD8 are well known to improve the sensitivity of T cells to pMHC, particularly when the pMHC has a weak affinity and is presented at low numbers (36–40) (*Fig.*

3B). These transmembrane receptors are able to bind to pMHC at a site that is independent of TCR binding (41, 42) and in addition, their cytoplasmic tails associate with the kinase Lck. In turn, Lck can interact with and phosphorylate the cytoplasmic tails of the TCR complex. It is thought that both the extracellular and intracellular interactions contribute to the ability of CD4/CD8 to improve sensitivity. A striking example of how CD8 can modulate TCR/pMHC association was observed by Zhu and colleagues using a micropipette adhesion assay (43). In that study, a steep increase in the probability of binding was observed, which critically relied on both CD8 binding to pMHC and the catalytic activity of Lck. Therefore, CD8 can be effectively thought of as a bivalent molecule able to bind both pMHC and TCR to increase their association rate. It should be noted that the CD4/MHC-II affinity is substantially lower than the CD8/MHC-I affinity (44) and therefore, modulation of TCR/pMHC binding may be less important for CD4 (45) (see below).

### 2.1.3 Role of serial binding and rebinding in promoting TCR/pMHC binding

Although the TCR/pMHC interaction is short lived, a stable close contact allows for various forms of repeated binding. First, pMHC can serially bind multiple localised TCRs. Mathematical analysis (*Box 1*) of a single pMHC interacting with localised (clustered) TCRs has shown that it can serially bind multiple TCRs before diffusing away (46). While it is well known that serial binding occurs, it is still unclear whether it is required for T cell activation (*Box 2*). Second, mathematical models explicitly accounting for slow membrane diffusion have predicted that upon unbinding, the pMHC is likely to rebind the same TCR multiple times before diffusing away (47). This rebinding hypothesis has been supported by a number of experiments (48–50). Third, the oligomerisation of the TCR has been observed biochemically and suggested to improve sensitivity (51–53). However, microscopy based techniques have failed to observe TCR oligomers instead suggesting that the TCR remains monomeric (54–56). Finally, nanometer-scale clustering of the TCR (57), as distinct from a physically associated oligomeric state, may effectively increase the on-rate for binding by increasing the local concentration of TCR (46, 47).

In summary, the formation and stabilisation of a close contact driven by an active microvilli protrusion combined with adhesion receptors and coreceptors generates an environment that effectively increases the on-rate between TCRs and pMHC. Stabilisation of close contacts is pMHC-dependent (24, 26) and likely involves TCR signalling, implying that feedback is operating to increase the effective TCR/pMHC on-rate. Consistent with such feedback, it has been observed that TCR binding becomes greater over time (58) and that increasing the number of pMHC molecules, and hence TCR signalling, can also increase the effective TCR/pMHC on-rate (59).

### 2.1.4 Role of molecular forces in modulating TCR/pMHC binding

There is now emerging data suggesting that the TCR/pMHC interaction is subjected to ~10-20 pN tensile forces at the contact interface (9). An applied force is generally expected to reduce the interaction lifetime (increase the off-rate) (60), reducing the sensitivity of T cells to antigen (*Fig. 2B*). The source of these forces may include those actively generated by actin polymerisation and/or those passively generated by LSMs pushing on the membrane near TCR/pMHC interactions. A



mathematical model that coupled membrane mechanics to the compressional stiffness of LSMs highlights that, by pushing the membranes away, LSMs effectively generate a time-dependent tension on the TCR/pMHC complex that is predicted to dramatically increase the off-rate in some parameter regimes (61) (*Box 1*).

In order to increase antigen sensitivity, it would seem that T cells need to have mechanisms in place to reduce the force on the TCR/pMHC complex or limit the effect of force on the off-rate. A proposed mechanism is the suggestion that TCR/pMHC interactions do not form a slip bonds but rather catch bonds, whereby an applied force actually increases the lifetime (60). Catch bonds have been observed for the OT-1 TCR interacting with its agonist OVA pMHC (50). However, this phenomenon may not be universal because many activating pMHC variants for the 1G4 TCR displayed slip bond behaviours (62). An additional mechanism is that the applied force is simply shared by multiple receptor/ligand complexes. A mathematical modelling study predicts that forces can be shared provided complexes are within  $\sim 80$  nm (63), which is consistent with the spatial scale of a microvilli protrusion.

In summary, although forces are likely to be generated at the contact interface, mechanisms may be operating to reduce the tension on the TCR/pMHC interaction. Although beyond the scope of this review, it has been suggested that applied forces on the TCR may be required to induce TCR signalling (64), but there is also evidence that TCR signalling can take place without pMHC ligands and hence an applied force (6). The role of force in mechanically transducing signals is beyond the scope of this work and we refer the reader elsewhere (65).

## 2.2 Mechanisms that rapidly induce TCR signalling

The TCR/pMHC interaction is transient, with direct measurements reporting lifetimes of  $\sim 0.1$  s ( $k_{\text{off}} \sim 10$  s $^{-1}$ ) for pMHCs that can activate T cells (10, 11). This measurement implies that TCRs must be converted from their non-signalling state into their activated signalling-state on the order of this timescale. In the context of the simplified schematic (*Fig. 2*) this conversion rate is represented by  $k_p$ , which must be similar to  $k_{\text{off}}$  in order for the TCR to communicate the binding event to the cell.

A large value of  $k_p$  can be obtained if the net phosphorylation rate of the TCR is large upon pMHC binding. This is likely achieved because CD45, which is the primary phosphatase responsible for dephosphorylating the TCR, is a segregated LSM. In contrast, Lck, which is the primary kinase responsible for phosphorylating the TCR, is anchored to the inner leaflet of the plasma membrane and as a result it remains within close contacts (6). Moreover, Lck is constitutively active in T cells (66) and it has been shown to efficiently phosphorylate the TCR signalling chains with a rate of  $k_{\text{cat}} = 6.2$  s $^{-1}$  (or average time of 0.16 s) (67), which is consistent with the short TCR/pMHC lifetime of 0.1 s.

To achieve this high catalytic rate, the TCR would need to experience a concentration of Lck that is larger than the  $K_M$  of  $230$   $\mu\text{m}^{-2}$  reported for this reaction (67). The estimated concentration of Lck in T cells is  $\sim 630$   $\mu\text{m}^{-2}$  (assuming  $2 \times 10^4$  Lck molecules per T cell (68) with a radius of  $5$   $\mu\text{m}$ ), which is indeed larger than  $K_M$ . In addition, the association of Lck with coreceptors may

further increase its concentration at TCR/pMHC complexes. Recently, it has been shown that this association directly increases the intrinsic activity of Lck (69). In fact, mathematical modelling has suggested that the primary mechanism by which CD4 increases T cell sensitivity to antigen is to localise Lck at the TCR/pMHC complex (70).

We note that CD45 can directly dephosphorylate tyrosines on Lck that are inhibiting and activating (71, 72). In this way, segregation of CD45 from close contacts may reduce the activity of Lck. It has been observed that CD45 remains near close contacts (25) and this may facilitate the continued generation of active Lck required for TCR signalling. The complex biochemical regulation of Lck by CD45, combined with their dynamic spatial distributions means that mathematical modelling may be particularly useful in understanding how the net rate of TCR phosphorylation is affected by the formation of a close contact.

We note that the segregation of CD45 from TCR/pMHC interactions at close contacts has been proposed to be the mechanism by which TCR signalling is initiated upon pMHC binding, a process termed receptor triggering. This model of triggering is known as kinetic-segregation and it has been reviewed elsewhere, along with other proposed triggering models (65).

## 2.3 Mechanisms that amplify TCR signalling

In addition to promoting TCR/pMHC binding and to quickly inducing TCR signalling upon binding, antigen sensitivity requires the T cell signalling machinery to efficiently convert a small number of transiently signalling TCRs into a fully activated T cell. This amplification step is denoted by  $k_{\text{amp}}$  in our minimalistic model (*Fig. 2*).

The TCR complex uniquely contains 10 immunoreceptor tyrosine-based activation motifs (ITAMs) distributed on 6 chains (73), which is the largest number of ITAMs found on any receptor (5). It is widely appreciated that multiple ITAMs contribute to signal amplification (73) with some evidence that they preferentially contribute to amplification at low antigen doses (74, 75). Mathematical models have previously suggested that multiple ITAMs may be important for generating switch-like responses by various mechanisms (76, 77) but experimental work has failed to observe this (67, 78).

A large number of ITAMs can amplify TCR signalling because each phosphorylated ITAM generates a docking site for the tyrosine kinase ZAP-70. When bound to the TCR, ZAP-70 can be activated to phosphorylate downstream targets that ultimately lead to T cell activation. Therefore, a single TCR/pMHC binding event can potentially be translated into the activation of multiple ZAP-70 molecules (79, 80). This signalling may also be sustained beyond pMHC binding because it has been suggested that, upon internalisation, TCRs may continue to signal within the T cell (81). As in other signal transduction cascades, phosphorylation by ZAP-70 of downstream targets initiates signalling that involves cascades and feedbacks that can amplify a small number of activated molecules at the TCR into a large number that can lead to T cell activation (82, 83).

In addition to the adhesion receptors discussed above, there are costimulatory receptors that can amplify TCR signalling without directly promoting TCR/pMHC binding. The most heavily

studied costimulatory receptor is CD28 (84), which when ligated by its ligands CD80 or CD86 reduces the number of pMHC ligands required for T cell activation (85). The mechanism of this amplification involves the recruitment of multiple effectors, including Lck (86) and PI3K (87), to the cytoplasmic tail of CD28 that may contribute to amplifying feedbacks with TCR signalling (88). Importantly, unlike CD2 and LFA-1, CD28 amplifies TCR signalling without altering TCR/pMHC binding (27).

### 3 Relationship between antigen sensitivity and specificity

The primary task of T cells is to recognise APCs that express foreign pMHC ligands. Implicit in this task is to ignore APCs that do not express foreign pMHC even though they express large numbers of pMHCs derived from self proteins. This task is known as antigen discrimination (12, 13) and it is difficult to understand because, as a result of thymic positive selection, self pMHC ligands are also able to bind the TCR and generate signals required for T cell survival (89). Consistent with this discrimination, *in vitro* experiments have shown that a small  $\sim 5$ -fold increase in the TCR/pMHC off-rate (e.g. by presenting T cells with altered pMHC ligands) can completely abolish the T cell response even if the altered pMHC is presented at large numbers (90–92). Collectively, these observations imply that T cells are not only highly sensitive to a pMHC ligand, but that they are also highly specific because altered pMHCs (e.g. self pMHC) with a marginally weaker affinity are unable to activate them.

Mathematical models have been instrumental to our understanding of antigen discrimination by T cells. The key mechanism underlying all models is kinetic proofreading, which relies on a delay between initial TCR binding and signalling (93). This delay is a result of a number of biochemical steps that must take place before the TCR is able to signal (e.g. phosphorylation of the TCR by Lck, binding and phosphorylation of ZAP-70 at the TCR, etc). This delay is effectively controlled by the rate of TCR conversion into an active signalling state (denoted by  $k_p$  in Fig. 2). The model shows that with a suitably long delay, there is a nonlinear relationship between the TCR/pMHC off-rate and the probability of TCR signalling. This means that a small increase in the off-rate can abolish the T cell response for a large variation in the pMHC dose. The models also show that, although this long delay preferentially reduces TCR signalling for larger off-rates, it reduces TCR signalling at all off-rates. This means that improving specificity comes at a cost to sensitivity (93, 94). A quantitative analysis suggested that there is no parameter regime where the standard kinetic proofreading model can explain the observed discrimination (92).

Given that T cells are both specific and sensitive, modifications of the basic kinetic proofreading model were required. Experimental data suggested that an intracellular negative feedback involving the phosphatase SHP-1 that can attenuate TCR proximal signalling can be overcome by a positive feedback involving ERK (95). Deterministic and stochastic models that modified kinetic proofreading to include these molecular feedbacks were able to explain antigen discrimination (92, 96). However, subsequent experimental work has questioned these feedbacks because positive feedback with ERK was not observed using an ERK inhibitor (97) and moreover, the key serine thought to be regulated by ERK to reduce SHP-1 activity did not impact antigen discrimi-

nation (98). A recent report has provided evidence for another TCR proximal negative feedback mediated by the negative regulation of ZAP-70 by a MAPK (99). In contrast to previously proposed feedbacks, this feedback would apply only after several proofreading steps and it would therefore be important to understand how this feedback impacts antigen discrimination.

In addition to intracellular feedbacks, a modification of kinetic proofreading to include induced rebinding has been shown to improve specificity whilst maintaining sensitivity (13). In this mechanism, a long delay in kinetic proofreading is used to produce specificity but sensitivity is maintained because the final signalling state is effectively sustained by a large probability of pMHC rebinding. The molecular mechanism(s) underlying this increase may include the signalling-dependent processes that stabilise the close contact to allow for TCR/pMHC interaction (see above).

## 4 Concluding remarks

T cells employ a diverse set of molecular mechanisms to achieve their high sensitivity to pMHC antigens. These mechanisms can be broadly classified into those that promote TCR/pMHC binding, induce rapid TCR signalling, and amplify TCR signalling (*Fig. 2A*). The requirement of T cells to discriminate antigens means that additional mechanisms, such as feedbacks, must be in place to ensure that their high sensitivity to foreign pMHC does not impact their specificity.

The sensitivity of T cells to antigens is also known to be regulated by the state of the T cell. For example, previously activated T cells are known to have higher antigen sensitivities (100) whereas T cells with a higher average affinity for self pMHC can exhibit reduced antigen sensitivities (101). It is also noteworthy that there is experimental evidence that different T cell responses may require a different number of pMHC ligands to be induced, with a general agreement that cytotoxicity exhibits higher antigen sensitivities compared to cytokine production (102–105).

In addition to recognising virally infected cells, T cells are also known to recognise cancerous cells when they express, for example, neoantigens (i.e. altered self pMHC as a result of genetic mutations) or developmental antigens. Here, the number of pMHC antigens expressed on the cancer cell is also expected to be small (106). The additional challenge of recognising these few pMHC antigens on cancer cells is that the affinity of the TCR for cancer pMHCs is weaker than for viral pMHCs (107), which is likely a result of thymic selection.

As we have highlighted, mathematical models have been instrumental in our understanding of various aspects of T cell sensitivity and specificity. These models have largely been formulated based on a static contact interface but it is now appreciated that T cells locally probe APC surfaces using dynamic microvilli. To understand the impact of microvilli probing and close contact formation, it would be interesting to revisit previously published mechanical models that examined the passive spatial redistribution of the TCR based on size (108–111) to include forces generated by microvilli.

Although the sensitivity of T cells to pMHC ligands is well documented, it is unclear how this sensitivity compares to the ligand sensitivity of other receptors. In particular, the TCR belongs to

a large group of receptors known as non-catalytic tyrosine-phosphorylated receptors (NTRs) (5). Given that NTRs share many properties, it is interesting to speculate that other ITAM-containing NTRs also exhibit high ligand sensitivity. A detailed comparison of ligand sensitivities between NTRs may shed light on the mechanisms that specifically contribute to the high sensitivity of T cells.

The sensitivity of T cells to antigens is well documented but a number of open questions remain concerning the underlying molecular mechanisms (*Box 3*). An improved understanding of these mechanisms may be useful when designing therapies with, for example, chimeric antigen receptors to re-direct T cells to kill infected or cancerous cells (112).

## Boxes

### Box 1: Mathematical models

A variety of mathematical models have been used to study various aspects of T cell activation. Below we describe just two of the many approaches that have been previously used. We refer the reader to the primary sources referenced in the main text for details of other models.

#### Models for serial binding

First-passage time calculations were originally used to estimate the number of TCRs that a single pMHC can serially bind in the micrometer-scale contact between T cells and APCs (113). This calculation was subsequently adapted to the  $\sim 100$  nanometer-scale clusters of TCR (46)

These calculations proceed by directly solving for the mean first-passage time of the pMHC sojourn in the area of interest (e.g. cluster of TCR), subject to reversible binding with TCRs,

$$\begin{aligned} D_1 \nabla^2 t_1 - k_{\text{on}}[\text{TCR}]t_1 + k_{\text{on}}[\text{TCR}]t_2 &= -1 \\ D_2 \nabla^2 t_2 - k_{\text{off}}t_2 + k_{\text{off}}t_1 &= -1, \end{aligned}$$

where  $t_i$  is the mean first passage-time with  $i = 1$  denoting the free pMHC state and  $i = 2$  denoting the bound pMHC state. The first term of each equation represents the diffusion of pMHC in that state with the next two terms representing reversible binding. The form of these equations is not intuitive (e.g. the fact that  $k_{\text{on}}$  appears twice in the first equation but not in the second) but they can be intuitively derived from an underlying discrete random walk (see Appendix A in (114)). Assuming adsorbing boundary conditions it is possible to obtain analytical solutions for specific geometries (46, 113). The number of TCR binding events is determined by dividing the mean passage-time by the cycle time for a single TCR binding event ( $1/k_{\text{on}}[\text{TCR}] + 1/k_{\text{off}}$ ).

Stochastic spatial simulations of TCR/pMHC interactions based on the Gillespie algorithm revealed that in addition to serial binding of different TCRs, there can be substantial rebinding of the same TCR (47). Rebinding is not captured in standard deterministic models, such as the

first passage time calculations described above. However, rebinding can be explicitly accounted for using a simplified two-state ordinary-differential-equation model where binding (second state) requires the pMHC to arrive within proximity of the TCR (first state) (48). The rate of entering the first state is determined by the diffusion-limited on-rate on the membrane ( $k_+ = 2\pi D/\log(b/s)$ , where  $b$  is the mean distance between TCR and  $s$  is the reaction radius). This two-state model has been used not only to study TCR/pMHC interactions (48, 49, 115) but also enzymatic reactions (76, 116).

## Models for spatial segregation

Mechanical models were originally used to explain the micrometer-scale spatial segregation of surface molecules (108–111) and more recently, mechanical models have been used to study how nanometer-scale segregation modulates the tensile force on the TCR/pMHC interaction (61, 63).

As an example, we describe a mechanical model used to examine the nanometer-scale spatial redistribution of the LSM CD45 upon TCR/pMHC binding (61). The partial-differential-equation that tracked the spatial distribution of LSMs ( $P$ ) included a diffusion term and an advection term,

$$\frac{\partial P}{\partial t} = D\nabla^2 P + \mu\nabla \cdot (P\nabla E_P),$$

where advection captures the tendency of LSMs to avoid being in membrane regions that compress/bend their large extracellular domain. The elasticity of LSMs is modelled as a Hookean spring with preferred height of  $z_p$ ,

$$E_P = \frac{1}{2}k_p(z_p - z(r, t))^2.$$

To determine  $z(r, t)$ , the total mechanical energy of the membrane is minimized,

$$E = \iint \frac{1}{2}\kappa_M (\nabla^2 z)^2 + PE_P dA,$$

where the first term captures the membrane bending stiffness and the second term captures the deformation of the LSM. Therefore, the model couples membrane bending with LSM compression. It follows that by pushing the membranes apart, LSMs are able to generate a tensile force ( $f(t)$ ) on the shorter TCR/pMHC interaction (located at  $r = r_0$ ) and this key output of the model is calculated as follows,

$$f(t) = \partial E / \partial z(r, t)|_{r=r_0}.$$

Calculation of  $f(t)$  revealed that although the TCR/pMHC interaction experiences a large  $\sim 50$  pN force initially upon binding, the spatial segregation of LSMs can reduce this force to below  $\sim 20$  pN within 1 s. It was proposed that a time-dependent force can contribute to antigen discrimination by preferentially accelerating the dissociation of short-lived TCR/pMHC interactions (61).

Recently, a stochastic version of this model was formulated to investigate the effects of membrane fluctuations on the TCR/pMHC interaction in the context of catch bonds (63). In both models, the applied force on the TCR/pMHC interaction is produced exclusively by membrane deformations resulting from size-based segregation. Future work is required to understand the contribution of actin polymerisation to molecular forces on the TCR/pMHC interaction.

## Box 2: Serial binding in T cell sensitivity to antigen

A number of studies have invoked serial binding as a mechanism of T cell sensitivity to pMHC ligands. The concept is that a single pMHC can amplify its own signal by engaging multiple TCRs.

There is overwhelming evidence that a single pMHC can serially bind multiple TCRs. First, the TCR/pMHC lifetime is short ( $\sim 0.1$ -10 s) as measured by recombinant proteins in solution (48, 117) and by direct measurements at contact interfaces (10, 11, 59, 79). This implies that a single pMHC is able to engage multiple TCRs, which is supported by mathematical modelling (46, 113). Second, quantitative analysis of TCR downregulation has shown that many more TCRs are downregulated than presented pMHCs suggesting that a single pMHC engages multiple TCRs (118).

Therefore, the key question is not whether serial binding takes place but whether it is necessary for T cell activation. In order for serial binding to be necessary, there must either be a penalty for TCR/pMHC interactions with long lifetimes or an advantage for short lifetimes (119). A penalty may arise if the TCR is only able to signal for a limited period of time even though pMHC remains bound. At a molecular level, this may happen if the TCR becomes ubiquitinated (120) and/or enters a membrane environment incompatible with signalling. An advantage may arise if the TCR is able to sustain signalling even without pMHC binding. In this case, a single pMHC can generate a large number of signalling TCRs. At a molecular level, this may happen if downregulated TCRs continue to signal (81).

A prediction of these limited signalling and sustained signalling models is that TCR/pMHC interactions with longer lifetimes (high affinities) would reduce signalling and therefore T cell activation. There is now abundant evidence that this is the case across different TCR/pMHC systems (121–124). However, T cell activation is generally only reduced with supra-physiological lifetimes. For example, we have systematically examined the T cell response to a 1-million fold variation in pMHC affinity (124). We found that reduced T cell responses at low pMHC doses can be observed but only when the lifetime exceed  $\sim 60$  s ( $K_D \lesssim 0.01 \mu\text{M}$ ), which is larger than the lifetime for physiological TCR/pMHC interactions. We note that in addition to this optimal lifetime of  $\sim 60$  s at low pMHC doses, optimal lifetimes were also observed at high pMHC doses in the physiological range of  $\sim 1$  s.

In summary, there is substantial evidence for serial binding of multiple TCRs by a single pMHC. Although there is also evidence that serial binding is necessary for T cell activation, the current evidence suggests that this is only relevant when 1) the TCR/pMHC affinity is supra-physiological and 2) the number of pMHC presented is low. Therefore, available data suggests that serial binding may not be necessary for physiological interactions but may be important to consider when designing high affinity therapeutic receptors.

## Box 3: Open questions in T cell sensitivity to antigen

- How unique is the sensitivity of the TCR compared to other NTRs (5)?

- Is serial binding of multiple TCRs by pMHC necessary for T cell activation? See Box 1.
- What is the applied force on the TCR/pMHC interaction at physiological close contact interfaces?
- How universal are catch bonds in TCR/pMHC interactions?
- What is the relative contribution to sensitivity of the intracellular and extracellular domains of coreceptors?
- What molecular feedbacks are responsible for producing T cell specificity and how do they impact sensitivity?
- What role do microvilli play in T cell sensitivity to antigen and how do they relate to microclusters? See Pettmann *et al.* (125) for a detailed discussion.

## Acknowledgements

We thank P. Anton van der Merwe and Jesse Goyette for discussions and a critical reading of the manuscript. O.D. is supported by a Wellcome Trust Senior Research Fellowship (grant 207537/Z/17/Z). J.A.S.-F. acknowledges funding from the University of Oxford, the EPSRC & BBSRC Centre for Doctoral Training in Synthetic Biology (grant EP/L016494/1), the CONACyT-I<sup>2</sup>T<sup>2</sup> consortium (grant 440334) and the Amelia Jackson Scholarship Fund.

## Conflict of interest

The authors declare no conflict of interest.

## References

1. Sykulev Y, Joo M, Vturina I, Tsomides TJ, Eisen HN (1996) Evidence that a Single Peptide–MHC Complex on a Target Cell Can Elicit a Cytolytic T Cell Response. *Immunity* 4:565–571.
2. Irvine DJ, Purbhoo MA, Krogsaard M, Davis MM (2002) Direct observation of ligand recognition by T cells. *Nature* 419:845–849.
3. Huang J, Brameshuber M, Zeng X, et al (2013) A Single Peptide-Major Histocompatibility Complex Ligand Triggers Digital Cytokine Secretion in CD4+ T Cells. *Immunity* 39:846–857.



4. Purbhoo MA, Irvine DJ, Huppa JB, Davis MM (2004) T cell killing does not require the formation of a stable mature immunological synapse. *Nature Immunology* 5:524–530.
5. Dushek O, Goyette J, van der Merwe PA (2012) Non-catalytic tyrosine-phosphorylated receptors. *Immunological Reviews* 250:258–276.
6. Chang VT, Fernandes RA, Ganzinger KA, et al (2016) Initiation of T cell signaling by CD45 segregation at 'close contacts'. *Nature Immunology* 17:574–582.
7. Iezzi G, Karjalainen K, Lanzavecchia A (1998) The duration of antigenic stimulation determines the fate of naive and effector T cells. *Immunity* 8:89–95.
8. Huppa JB, Gleimer M, Sumen C, Davis MM (2003) Continuous T cell receptor signaling required for synapse maintenance and full effector potential. *Nature immunology* 4:749–55.
9. Liu Y, Blanchfield L, Ma VPy, et al (2016) DNA-based nanoparticle tension sensors reveal that T-cell receptors transmit defined pN forces to their antigens for enhanced fidelity. *Proceedings of the National Academy of Sciences* 113:5610–5615.
10. Huppa JB, Axmann M, Mörtelmaier M, et al (2010) TCR–peptide–MHC interactions in situ show accelerated kinetics and increased affinity. *Nature* 463:963–967.
11. Huang J, Zarnitsyna VI, Liu B, et al (2010) The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness. *Nature* 464:932–6.
12. Feinerman O, Germain RN, Altan-Bonnet G (2008) Quantitative challenges in understanding ligand discrimination by alphabeta T cells. *Molecular immunology* 45:619–31.
13. Dushek O, van der Merwe PA (2014) An induced rebinding model of antigen discrimination. *Trends in Immunology* 35:153–158.
14. Yang H, Buisson S, Bossi G, et al (2016) Elimination of latently HIV-infected cells from antiretroviral therapy-suppressed subjects by engineered immune-mobilizing T-cell receptors. *Molecular Therapy* 24:1913–1925.
15. Dirk BS, Pawlak EN, Johnson AL, et al (2016) HIV-1 Nef sequesters MHC-I intracellularly by targeting early stages of endocytosis and recycling. *Scientific Reports* 6:1–13.
16. Lemmermann NAW, Gergely K, Böhm V, Deegen P, Däubner T, Reddehase MJ (2010) Immune evasion proteins of murine cytomegalovirus preferentially affect cell surface display of recently generated peptide presentation complexes. *Journal of virology* 84:1221–36.
17. Choudhuri K, Wiseman D, Brown MH, Gould K, van der Merwe PA (2005) T-cell receptor triggering is critically dependent on the dimensions of its peptide-MHC ligand. *Nature* 436:578–582.
18. Hermiston ML, Xu Z, Weiss A (2003) CD45: a critical regulator of signaling thresholds in immune cells. *Annual review of immunology* 21:107–37.

19. Evans EJ, Hene L, Sparks LM, et al (2003) The T cell surface—how well do we know it? *Immunity* 19:213–23.
20. Newby J, Allard J (2016) First-Passage Time to Clear the Way for Receptor-Ligand Binding in a Crowded Environment. *Physical Review Letters* 116:1–5.
21. Carbone CB, Kern N, Fernandes RA, et al (2017) In vitro reconstitution of T cell receptor-mediated segregation of the CD45 phosphatase. *Proceedings of the National Academy of Sciences* p 201710358.
22. Huse M, Klein LO, Girvin AT, et al (2007) Spatial and temporal dynamics of T cell receptor signaling with a photoactivatable agonist. *Immunity* 27:76–88.
23. Jung Y, Riven I, Feigelson SW, et al (2016) Three-dimensional localization of T-cell receptors in relation to microvilli using a combination of superresolution microscopies. *Proceedings of the National Academy of Sciences* p 201605399.
24. Cai E, Marchuk K, Beemiller P, et al (2017) Visualizing dynamic microvillar search and stabilization during ligand detection by T cells. *Science* 356:eaal3118.
25. Razvag Y, Neve-Oz Y, Sajman J, Reches M, Sherman E (2018) Nanoscale kinetic segregation of TCR and CD45 in engaged microvilli facilitates early T cell activation. *Nature Communications* 9:732.
26. Sage PT, Varghese LM, Martinelli R, et al (2012) Antigen recognition is facilitated by invadosome-like protrusions formed by memory/effector T cells. *Journal of immunology (Baltimore, Md. : 1950)* 188:3686–99.
27. Bachmann MF, McKall-Faienza K, Schmits R, et al (1997) Distinct Roles for LFA-1 and CD28 during Activation of Naive T Cells: Adhesion versus Costimulation. *Immunity* 7:549–557.
28. Bachmann MF, Barner M, Kopf M (1999) CD2 sets quantitative thresholds in T cell activation. *Journal of Experimental Medicine* 190:1383–1392.
29. Springer TA (1990) Adhesion receptors of the immune system. *Nature* 346:425–434.
30. Wild MK, Cambiaggi A, Brown MH, et al (1999) Dependence of T cell antigen recognition on the dimensions of an accessory receptor-ligand complex. *The Journal of experimental medicine* 190:31–41.
31. Dustin ML, Cooper JA (2000) The immunological synapse and the actin cytoskeleton: molecular hardware for T cell signaling. *Nature Immunology* 1:23–29.
32. Dustin ML, Olszowy MW, Holdorf AD, et al (1998) A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. *Cell* 94:667–677.
33. Tane AH, Sakuma M, Ike H, et al (2016) Micro – adhesion rings surrounding TCR micro-clusters are essential for T cell activation. pp 1–17.

34. Wang ECY, Pjechova M, Nightingale K, et al (2018) Suppression of costimulation by human cytomegalovirus promotes evasion of cellular immune defenses. pp 1–6.
35. Patel SJ, Sanjana NE, Kishton RJ, et al (2017) Identification of essential genes for cancer immunotherapy. *Nature Publishing Group* 548.
36. Purbhoo MA, Boulter JM, Price DA, et al (2001) The Human CD8 Coreceptor Effects Cytotoxic T Cell Activation and Antigen Sensitivity Primarily by Mediating Complete Phosphorylation of the T Cell Receptor Chain. *Journal of Biological Chemistry* 276:32786–32792.
37. Holler PD, Kranz DM (2003) Quantitative analysis of the contribution of TCR/pepMHC affinity and CD8 to T cell activation. *Immunity* 18:255–64.
38. Hutchinson SL, Wooldridge L, Tafuro S, et al (2003) The CD8 T cell coreceptor exhibits disproportionate biological activity at extremely low binding affinities. *The Journal of biological chemistry* 278:24285–93.
39. Li QJ, Dinner AR, Qi S, et al (2004) CD4 enhances T cell sensitivity to antigen by coordinating Lck accumulation at the immunological synapse. *Nature immunology* 5:791–799.
40. Laugel B, Van Den Berg HA, Gostick E, et al (2007) Different T cell receptor affinity thresholds and CD8 coreceptor dependence govern cytotoxic T lymphocyte activation and tetramer binding properties. *Journal of Biological Chemistry* 282:23799–23810.
41. Wyer JR, Willcox BE, Gao GF, et al (1999) T Cell Receptor and Coreceptor CD8 Bind Peptide-MHC Independently and with Distinct Kinetics. *Immunity* 10:219–225.
42. Xiong Y, Kern P, Chang HC, Reinherz EL (2001) T Cell Receptor Binding to a pMHCII Ligand Is Kinetically Distinct from and Independent of CD4. *Journal of Biological Chemistry* 276:5659–5667.
43. Jiang N, Huang J, Edwards LJ, et al (2011) Two-stage cooperative T cell receptor-peptide major histocompatibility complex-CD8 trimolecular interactions amplify antigen discrimination. *Immunity* 34:13–23.
44. Jönsson P, Southcombe JH, Santos AM, et al (2016) Remarkably low affinity of CD4/peptide-major histocompatibility complex class II protein interactions. *Proceedings of the National Academy of Sciences* 113:5682–5687.
45. Hamad AR, O’Herrin SM, Lebowitz MS, et al (1998) Potent T cell activation with dimeric peptide-major histocompatibility complex class II ligand: the role of CD4 coreceptor. *The Journal of experimental medicine* 188:1633–40.
46. Dushek O, Coombs D (2008) Analysis of serial engagement and peptide-MHC transport in T cell receptor microclusters. *Biophysical journal* 94:3447–60.
47. Dushek O, Das R, Coombs D (2009) A role for rebinding in rapid and reliable T cell responses to antigen. *PLoS computational biology* 5:e1000578.

48. Aleksic M, Dushek O, Zhang H, et al (2010) Dependence of T cell antigen recognition on T cell receptor-peptide MHC confinement time. *Immunity* 32:163–74.
49. Govern CC, Paczosa MK, Chakraborty AK, Huseby ES (2010) Fast on-rates allow short dwell time ligands to activate T cells. *Proceedings of the National Academy of Sciences* 107:8724–9.
50. Liu B, Chen W, Evavold BD, Zhu C (2014) Accumulation of Dynamic Catch Bonds between TCR and Agonist Peptide-MHC Triggers T Cell Signaling. *Cell* 157:357–68.
51. Schamel WW, Arechaga I, Risueño RM, et al (2005) Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response. *The Journal of Experimental Medicine* 202:493–503.
52. Schamel WWa, Risueño RM, Minguet S, Ortíz AR, Alarcón B (2006) A conformation- and avidity-based proofreading mechanism for the TCR-CD3 complex. *Trends in immunology* 27:176–82.
53. Kumar R, Ferez M, Swamy M, et al (2011) Increased Sensitivity of Antigen-Experienced T Cells through the Enrichment of Oligomeric T Cell Receptor Complexes. *Immunity* 35:375–387.
54. James JR, White SS, Clarke RW, et al (2007) Single-molecule level analysis of the subunit composition of the T cell receptor on live T cells. *Proceedings of the National Academy of Sciences of the United States of America* 104:17662–7.
55. James JR, McColl J, Oliveira MI, et al (2011) The T cell receptor triggering apparatus is composed of monovalent or monomeric proteins. *Journal of Biological Chemistry* 286:31993–32001.
56. Brameshuber M, Kellner F, Rossboth BK, et al (2018) Monomeric TCRs drive T cell antigen recognition. *Nature Immunology* 19.
57. Paegeon SV, Tabarin T, Yamamoto Y, et al (2016) Functional role of T-cell receptor nanoclusters in signal initiation and antigen discrimination. *Proceedings of the National Academy of Sciences* 113:E5454–E5463.
58. Zarnitsyna VI, Huang J, Zhang F, Chien YH, Leckband D, Zhu C (2007) Memory in receptor-ligand-mediated cell adhesion. *Proceedings of the National Academy of Sciences of the United States of America* 104:18037–42.
59. Pielak RM, O'Donoghue GP, Lin JJ, et al (2017) Early T cell receptor signals globally modulate ligand:receptor affinities during antigen discrimination. *Proceedings of the National Academy of Sciences* p 201613140.
60. Bell G (1978) Models for the specific adhesion of cells to cells. *Science* 200:618–627.
61. Allard JF, Dushek O, Coombs D, Anton van der Merwe P (2012) Mechanical Modulation of Receptor-Ligand Interactions at Cell-Cell Interfaces. *Biophysical Journal* 102:1265–1273.

62. Robert P, Aleksic M, Dushek O, Cerundolo V, Bongrand P, van der Merwe PA (2012) Kinetics and Mechanics of Two-Dimensional Interactions between T Cell Receptors and Different Activating Ligands. *Biophysical Journal* 102:248–257.
63. Pullen RH, Abel SM (2017) Catch Bonds at T Cell Interfaces: Impact of Surface Reorganization and Membrane Fluctuations. *Biophysical Journal* 113:120–131.
64. Feng Y, Brazin KN, Kobayashi E, Mallis RJ, Reinherz EL, Lang MJ (2017) Mechanosensing drives acuity of  $\alpha\beta$  T-cell recognition. *Proceedings of the National Academy of Sciences of the United States of America* 114:E8204–E8213.
65. van der Merwe PA, Dushek O (2011) Mechanisms for T cell receptor triggering. *Nature Reviews Immunology* 11:47–55.
66. Nika K, Soldani C, Salek M, et al (2010) Constitutively active Lck kinase in T cells drives antigen receptor signal transduction. *Immunity* 32:766–77.
67. Hui E, Vale RD (2014) In vitro membrane reconstitution of the T-cell receptor proximal signaling network. *Nature Structural & Molecular Biology* pp 1–12.
68. Hukelmann JL, Anderson KE, Sinclair LV, et al (2015) The cytotoxic T cell proteome and its shaping by the kinase mTOR. *Nature Immunology* 17.
69. Liaunardy-Jopeace A, Murton BL, Mahesh M, Chin JW, James JR (2017) Encoding optical control in LCK kinase to quantitatively investigate its activity in live cells. *Nature structural & molecular biology* 24:1155–1163.
70. Artyomov MN, Lis M, Devadas S, Davis MM, Chakraborty AK (2010) CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. *Proceedings of the National Academy of Sciences of the United States of America* 107:16916–21.
71. Acuto O, Di Bartolo V, Michel F (2008) Tailoring T-cell receptor signals by proximal negative feedback mechanisms. *Nature Reviews Immunology* 8:699–712.
72. McNeill L, Salmond RJ, Cooper JC, et al (2007) The differential regulation of Lck kinase phosphorylation sites by CD45 is critical for T cell receptor signaling responses. *Immunity* 27:425–37.
73. Love PE, Hayes SM (2010) ITAM-mediated signaling by the T-cell antigen receptor. *Cold Spring Harbor perspectives in biology* 2:a002485.
74. Holst J, Wang H, Eder KD, et al (2008) Scalable signaling mediated by T cell antigen receptor-CD3 ITAMs ensures effective negative selection and prevents autoimmunity. *Nature immunology* 9:658–66.
75. James JR (2018) Tuning ITAM multiplicity on T cell receptors can control potency and selectivity to ligand density. *Science Signaling* 11.
76. Dushek O, van der Merwe PA, Shahrezaei V (2011) Ultrasensitivity in multisite phosphorylation of membrane-anchored proteins. *Biophysical journal* 100:1189–97.

77. Mukhopadhyay H, Cordoba SP, Maini PK, van der Merwe PA, Dushek O (2013) Systems model of T cell receptor proximal signaling reveals emergent ultrasensitivity. *PLoS computational biology* 9:e1003004.
78. Mukhopadhyay H, Wet BD, Clemens L, et al (2016) Multisite phosphorylation of the T cell receptor  $\zeta$ -chain modulates potency but not the switch-like response. *Biophys. J.* 110:1896–1906.
79. O'Donoghue GP, Pielak RM, Smoligovets AA, Lin JJ, Groves JT (2013) Direct single molecule measurement of TCR triggering by agonist pMHC in living primary T cells. *eLife* 2:e00778–e00778.
80. Katz ZB, Novotná L, Blount A, Lillemeier BF (2017) A cycle of Zap70 kinase activation and release from the TCR amplifies and disperses antigenic stimuli. *Nature Immunology* 18:86–95.
81. Willinger T, Staron M, Ferguson SM, De Camilli P, Flavell RA (2015) Dynamin 2-dependent endocytosis sustains T-cell receptor signaling and drives metabolic reprogramming in T lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America* 112:4423–8.
82. Altan-Bonnet G, Germain RN (2005) Modeling T cell antigen discrimination based on feedback control of digital ERK responses. *PLoS Biology* 3:1925–1938.
83. Das J, Ho M, Zikherman J, et al (2009) Digital signaling and hysteresis characterize ras activation in lymphoid cells. *Cell* 136:337–51.
84. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone JA (2016) CD28 Costimulation: From Mechanism to Therapy.
85. Viola A, Lanzavecchia A (1996) T cell activation determined by T cell receptor number and tunable thresholds. *Science (New York, N.Y.)* 273:104–6.
86. Dobbins J, Gagnon E, Godec J, et al (2016) Binding of the cytoplasmic domain of CD28 to the plasma membrane inhibits Lck recruitment and signaling. *Science Signaling* 9:1–13.
87. Andreotti AH, Schwartzberg PL, Joseph RE, Berg LJ (2010) T-cell signaling regulated by the Tec family kinase, Itk. *Cold Spring Harbor perspectives in biology* 2:a002287.
88. Yang W, Pan W, Chen S, et al (2017) Dynamic regulation of CD28 conformation and signaling by charged lipids and ions. *Nature Structural and Molecular Biology* 24:1081–1092.
89. Surh CD, Sprent J (2008) Homeostasis of Naive and Memory T Cells. *Immunity* 29:848–862.
90. Matsui K, Boniface JJ, Steffner P, Reay PA, Davis MM (1994) Kinetics of T-cell receptor binding to peptide/I-Ek complexes: correlation of the dissociation rate with T-cell responsiveness. *Proc Natl Acad Sci U S A* 91:12862–12866.

91. Kersh GJ, Kersh EN, Fremont DH, Allen PM (1998) High- and low-potency ligands with similar affinities for the TCR: The importance of kinetic in TCR signaling. *Immunity* 9:817–826.
92. Altan-Bonnet G, Germain RN (2005) Modeling T cell antigen discrimination based on feedback control of digital ERK responses. *PLoS biology* 3:e356.
93. McKeithan TW (1995) Kinetic proofreading in T-cell receptor signal transduction. *Proceedings of the National Academy of Sciences of the United States of America* 92:5042–6.
94. Stark J, George A, Chan C (2003) T cell sensitivity and specificity - kinetic proofreading revisited.
95. Stefanová I, Hemmer B, Vergelli M, Martin R, Biddison WE, Germain RN (2003) TCR ligand discrimination is enforced by competing ERK positive and SHP-1 negative feedback pathways. *Nature immunology* 4:248–54.
96. Francois P, Voisinne G, Siggia ED, Altan-Bonnet G, Vergassola M (2013) Phenotypic model for early T-cell activation displaying sensitivity, specificity, and antagonism. *Proceedings of the National Academy of Sciences*.
97. Vogel R, Erez A, Altan-Bonnet G (2016) Dichotomy of cellular inhibition by small-molecule inhibitors revealed by single-cell analysis. *Nature communications* 7.
98. Paster W, Bruger AM, Katsch K, et al (2015) A THEMIS:SHP1 complex promotes T-cell survival. *The EMBO journal* 34:393–409.
99. Giardino Torchia ML, Dutta D, Mittelstadt PR, et al (2018) Intensity and duration of TCR signaling is limited by p38 phosphorylation of ZAP-70T293 and destabilization of the signalosome. *Proceedings of the National Academy of Sciences* p 201713301.
100. Fahmy TM, Bieler JG, Edidin M, Schneck JP (2001) Increased TCR avidity after T cell activation: a mechanism for sensing low-density antigen. *Immunity* 14:135–43.
101. Persaud SP, Parker CR, Lo WL, Weber KS, Allen PM (2014) Intrinsic CD4 + T cell sensitivity and response to a pathogen are set and sustained by avidity for thymic and peripheral complexes of self peptide and MHC. *Nature Immunology* 15:266–274.
102. Valitutti BS, Miiuer S, Dessing M, Lanzavecchia A (1996) Different Responses Are Elicited in Cytotoxic T Lymphocytes by Different Levels of T Cell Receptor Occupancy. 183:1917–1921.
103. Itoh, Y; Germain R (1997) Single cell analysis reveals regulated hierarchical T cell antigen receptor signaling thresholds and intracloal heterogeneity. *The Journal of experimental medicine* 186.
104. Hemmer B, Stefanova I, Vergelli M, Germain RN, Martin R (1998) Relationships among TCR ligand potency, thresholds for effector function elicitation, and the quality of early signaling events in human T cells. *Journal of Immunology* 160:5807–5814.

105. van den Berg Ha, Ladell K, Miners K, et al (2013) Cellular-level versus receptor-level response threshold hierarchies in T-cell activation. *Frontiers in immunology* 4:250.
106. Purbhoo MA, Sutton DH, Brewer JE, et al (2006) Quantifying and Imaging NY-ESO-1/LAGE-1-Derived Epitopes on Tumor Cells Using High Affinity T Cell Receptors. *The Journal of Immunology* 176:7308–7316.
107. Aleksic M, Liddy N, Molloy PE, et al (2012) Different affinity windows for virus and cancer-specific T-cell receptors: Implications for therapeutic strategies. *European Journal of Immunology* 42:3174–3179.
108. Qi SY, Groves JT, Chakraborty AK (2001) Synaptic pattern formation during cellular recognition. *Proceedings of the National Academy of Sciences* 98:6548–6553.
109. Weikl TR, Groves JT, Lipowsky R (2002) Pattern formation during adhesion of multicomponent membranes. *Europhysics Letters* 59:916–922.
110. Burroughs NJ, Wülfing C (2002) Differential segregation in a cell-cell contact interface: The dynamics of the immunological synapse. *Biophysical Journal* 83:1784–1796.
111. Coombs D, Dembo M, Wofsy C, Goldstein B (2004) Equilibrium Thermodynamics of Cell-Cell Adhesion Mediated by Multiple Ligand-Receptor Pairs. *Biophysical Journal* 86:1408–1423.
112. Sadelain M, Rivière I, Riddell S (2017) Therapeutic T cell engineering. *Nature* 545:423–431.
113. Wofsy C, Coombs D, Goldstein B (2001) Calculations show substantial serial engagement of T cell receptors. *Biophysical journal* 80:606–12.
114. Goldstein B, Griego R, Wofsy C (1984) Diffusion-limited forward rate constants in two dimensions. Application to the trapping of cell surface receptors by coated pits. *Biophysical Journal* 46:573–586.
115. Jansson A (2010) A mathematical framework for analyzing T cell receptor scanning of peptides. *Biophysical Journal* 99:2717–2725.
116. Lawley SD, Keener JP (2016) Including Rebinding Reactions in Well-Mixed Models of Distributive Biochemical Reactions. *Biophysical Journal* 111:2317–2326.
117. Dushek O, Aleksic M, Wheeler R, et al (2011) Antigen potency and maximal efficacy reveal a mechanism of efficient T cell activation. *Science Signaling* 4:ra39.
118. Valitutti S, Müller S, Cella M, Padovan E, Lanzavecchia A (1995) Serial triggering of many T-cell receptors by a few peptide–MHC complexes. *Nature* 375:148–151.
119. Lever M, Maini PK, van der Merwe PA, Dushek O (2014) Phenotypic models of T cell activation. *Nature Reviews Immunology* 14:619–629.
120. Yang H, Gou X, Wang Y, Fahmy TM, Leung AYH, Lu J, Sun D (2015) A dynamic model of chemoattractant-induced cell migration. *Biophysical journal* 108:1645–51.



121. Kalergis AM, Boucheron N, Doucey MA, et al (2001) Efficient T cell activation requires an optimal dwell-time of interaction between the TCR and the pMHC complex. *Nature immunology* 2:229–34.
122. Thomas S, Xue SA, Bangham CRM, Jakobsen BK, Morris EC, Stauss HJ (2011) Human T cells expressing affinity-matured TCR display accelerated responses but fail to recognize low density of MHC-peptide antigen. *Blood* 118:319–29.
123. Irving M, Zoete V, Hebeisen M, et al (2012) Interplay between T cell receptor binding kinetics and the level of cognate peptide presented by major histocompatibility complexes governs CD8+ T cell responsiveness. *The Journal of biological chemistry* 287:23068–78.
124. Lever M, Lim HS, Kruger P, et al (2016) Architecture of a minimal signaling pathway explains the T-cell response to a 1 million-fold variation in antigen affinity and dose. *Proceedings of the National Academy of Sciences of the United States of America* 113:E6630–E6638.
125. Pettmann J, Santos AM, Dushek O, Davis SJ (2018) Membrane ultrastructure and T-cell activation. *Frontiers in Immunology* (Submitted).