Published in final edited form as:
Mol Immunol. 2012 October ; 52(3-4): 155–164. doi:10.1016/j.molimm.2012.05.004.

T cell antigen recognition at the cell membrane

Jun Huang[a,*], **Christina Meyer**[b], and **Cheng Zhu**[c]

[a]Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA

[b]Program in Immunology, Stanford University School of Medicine, Stanford, CA 94305, USA

[c]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA 30332, USA

Abstract

T cell antigen receptors (TCR) on the surface of T cells bind specifically to particular peptide bound major histocompatibility complexes (pMHC) presented on the surface of antigen presenting cells (APC). This interaction is a key event in T cell antigen recognition and activation. Most studies have used surface plasmon resonance (SPR) to measure the *in vitro* binding kinetics of TCR-pMHC interactions in solution using purified proteins. However, these measurements are not physiologically precise, as both TCRs and pMHCs are membrane-associated molecules which are regulated by their cellular environments. Recently, single-molecule förster resonance energy transfer (FRET) and single-molecule mechanical assays were used to measure the *in situ* binding kinetics of TCR-pMHC interactions on the surface of live T cells. These studies have provided exciting insights into the biochemical basis of T cell antigen recognition and suggest that TCRs serially engage with a small number of antigens with very fast kinetics in order to maximize TCR signaling and sensitivity.

Keywords

TCR; pMHC; single-molecule *in situ* kinetics; TCR antigen recognition

T cell antigen recognition plays an essential role in both humoral and cellular immunity. The TCR is responsible for recognizing foreign pMHCs presented on the APC or target cell surface. T cell antigen recognition is mediated by multiple receptor-ligand interactions between a T cell and an APC, among which the TCR-pMHC interaction is most important. T cell activation also requires the participation of a diverse group of accessory or co-stimulatory molecules that interact across, within, and beneath the cell membrane to trigger and relay signals (Fig. 1). Here we first review these molecular interactions, then present the methods for their measurements, and finally discuss findings obtained by these new methods.

*Corresponding author at: 279 Campus Dr., Stanford, CA 94305, USA. junhuanggt@gmail.com.

1. Key molecular interactions and cellular environment

The αβ TCR is a heterodimer composed of disulfide-linked α and β chains. Each TCR chain is composed of variable and constant Ig-like domains, followed by a transmembrane segment and a short cytoplasmic tail. The variable domain is generated from rearranging variable (V), diversity (D) and joining (J) gene segments (D is only present in the β chain) (Gascoigne et al., 2001; Janeway et al., 1999; Rudolph et al., 2006). This rearrangement creates an antigen recognition surface capable of binding a tremendous range of pMHC ligands. A TCR only recognizes a pMHC complex, but not peptide or MHC alone. TCRs on $CD8^+$ cells recognize pMHC-I and TCRs on $CD4^+$ T cells recognize pMHC-II. Thus, $CD8^+$ T cells are said to be 'MHC-I restricted' and $CD4^+$ T cells are 'MHC-II restricted' on the basis of the MHC class that their receptors recognize (Gascoigne et al., 2001; Janeway et al., 1999). Surface αβ TCR is assembled with the invariant chains of CD3 that include δε and γε heterodimers and a ζζ homodimer. The association of these three CD3 signaling dimers with the αβ TCR is driven by highly specific polar interactions among transmembrane domains that are uniquely favorable in the lipid environment (Call et al., 2002; Call and Wucherpfennig, 2005). Though the structure of αβ TCR itself has no intrinsic signaling capacity, the CD3 components have immunoreceptor tyrosine-based activation motifs (ITAM) that become phosphorylated after TCR engagement with pMHC and recruit other molecules to initiate a signaling cascade (Gascoigne et al., 2001; Janeway, 1992; Kersh and Allen, 1996; Kersh et al., 1998).

In addition to the TCR-pMHC interaction, the engagement of other molecules is required for effective T cell activation, including those of co-receptors CD4/8 and co-stimulatory molecules. Co-receptors CD4/8 bind to the invariant domain of a pMHC away from the TCR interacting site, and facilitate the TCR antigen recognition process. They are also linked with Lck and associated with the TCR/CD3 complex via their cytoplasmic domain, enabling signaling during early T cell triggering upon TCR antigen recognition (Gascoigne et al., 2001; Turner et al., 1990; Weiss and Littman, 1994). Co-receptor binding to pMHC is a requirement for normal T cell selection and activation (Janeway et al., 1999; Xu and Littman, 1993). Absence or blockage of co-receptors results in poor TCR recognition sensitivity and requires much more pMHC (10~100-fold) for T cell triggering (Davis et al., 2003; Irvine et al., 2002; Purbhoo et al., 2004). Normal T cell activation also requires signals provided by co-stimulatory molecule engagements including those of LFA-1, CD28, CTLA-4, CD2 and CD45. Activation of T cells without co-stimulation may lead to T cell anergy, T cell deletion or development of immune tolerance (Alegre et al., 2001; Davis et al., 2003).

T cell activation and function are actively regulated by the cytoskeleton and lipid rafts. T cell is highly mobile and dynamic, and depends on the cytoskeleton for determining its shapes and performing its functions. A T cell must go through several cytoskeleton-dependent processes to efficiently scan target cells and take appropriate actions to facilitate TCR antigen recognition. T cell processes reliant on cytoskeletal motility include TCR-pMHC interaction, immunological synapse formation, accessory receptor stimulation, cellular polarization, receptor sequestration and signaling (Billadeau et al., 2007; Dustin, 2007; Huppa and Davis, 2003). The cytoskeleton provides the T cell with a dynamic cellular framework to rapidly remodel itself against the target cell and reorient its cellular organelles to the interface between the T cell and the APC (Fuller et al., 2003; Huppa and Davis, 2003). The dynamic cytoskeleton rearrangements are crucial for T cell migration, antigen detection and immune function execution. Treatment with inhibitors of actin polymerization abolishes many T cell functions (Fuller et al., 2003). Lipid rafts may also play a critical role in TCR recognition, signaling and stimulation. Lipid rafts are known as glycosphingolipid-enriched microdomains or detergent resistant microdomains, and are formed by hydrophobic

interactions between saturated fatty acid residues of their main lipid constituent sphingomyelin and glycosphingolipids. TCR engagement promotes aggregation of lipid rafts, which subsequently facilitates colocalization and interaction between TCR and signaling proteins such as Lck, ZAP70 and LAT. This lipid raft-mediated clustering may initiate protein tyrosine phosphorylation and amplify downstream signaling (Brdickova et al., 2003; Harder, 2004; Harder and Engelhardt, 2004; He et al., 2005; Magee et al., 2002).

2. TCR antigen recognition characteristics

MHC molecules are continuously being loaded with peptides derived from cytosolic or endocytosed proteins. At any given time, the vast majority of pMHCs are loaded with endogenous self-peptides. TCRs possess a remarkable ability to quickly and specifically recognize a small number of foreign pMHCs from this large excess of self-pMHCs presented on the APC surface. Here we will briefly review three important characteristics of T cell antigen recognition.

Sensitivity

T cells have extraordinary sensitivity in antigen detection (Demotz et al., 1990; Ebert et al., 2008; Harding and Unanue, 1990; Irvine et al., 2002; Purbhoo et al., 2004; Sykulev et al., 1996). Both $CD4^+$ and $CD8^+$ T cells can initiate transient intracellular calcium flux upon recognition of as few as a single agonist pMHC. This calcium signal reaches a plateau level once about ten agonist pMHCs are recognized (Irvine et al., 2002; Purbhoo et al., 2004). Two agonist pMHCs can promote APC contact and subsequent apoptosis in reactive thymocytes (Ebert et al., 2008), and cytotoxic T cells can initiate killing upon recognition of only three pMHCs without synapse formation (Purbhoo et al., 2004).

Specificity

T cells are able to specifically recognize one foreign pMHC in the presence of a large excess of self-pMHCs. T cells have diverse functions and degrees of responsiveness that are highly specific to the recognized antigens. An individual T cell is capable of recognizing multiple possible pMHCs with varying degrees of functional outcomes, each particular to a recognized ligand. Small differences in the presented peptide may lead to large differences in the functional outcome. TCRs can discriminate the difference of even a single amino acid substitution and elicit distinct functional responses (Evavold and Allen, 1991; Sloan-Lancaster et al., 1993). A given pMHC may lead to full or partial activation of the T cell—a strong or weak agonist—or it may have no effect—a null pMHC. A single amino acid variation in the peptide sequence can convert an agonist into an antagonist or null peptide to shut down T cell proliferation (Kersh and Allen, 1996; Sloan-Lancaster and Allen, 1996).

Speed

$CD4^+$ T cells signal with remarkable speed. After the TCR-pMHC engagement, Huse et al. observed LAT phosphorylation in 4 seconds, diacylglycerol production and calcium flux in 6–7 seconds, and T cell cytoskeleton polarization within 2 min (Huse et al., 2007). Our data also suggested that $CD8^+$ T cell Src kinase signaling occurs ~1 second after TCR engagement (Jiang et al., 2011).

3. Kinetic measurements of TCR-pMHC interactions

The specific interaction between TCR and pMHC triggers the initial T cell signaling that leads to T cell activation and the cellular immune response. Upon pMHC binding to the TCR, the T cell becomes activated with a sustained elevation of calcium and begins to form an immunological synapse with the APC (Grakoui et al., 1999; Monks et al., 1998). Then

the T cell will either secrete cytokines to stimulate other immune cells ($CD4^+$ T cells) or induce death of the target cell ($CD8^+$ T cells) (Davis et al., 2003). T cells have a wide range of functional capabilities; coupled with the broad range of TCR recognition capabilities that trigger these functions, this makes the study of the dynamic binding between TCR and pMHC a fruitful field. This has been borne out, for example, in thymocyte selection where recognition of different peptides results in either positive or negative selection—drastically different fates for the TCR-expressing thymocyte (Janeway et al., 1999). Extensive studies have been undertaken to elucidate the connection between TCR-pMHC binding properties and the resulting T cell functionality in the immune response. The binding kinetics of TCR-pMHC interactions have been mainly measured by two methods: *In vitro* three-dimensional (3D) binding kinetics in solution (Fig. 2) and *in situ* two-dimensional (2D) binding kinetics at the cell membrane (Fig. 3).

3.1 *In vitro* 3D kinetics of TCR-pMHC interactions

Much of our early understanding of TCR-pMHC interaction kinetics came from studies using SPR to measure binding between soluble TCR and pMHC molecules. These experiments typically involved immobilizing either the TCR or the pMHC on a sensor chip and flowing the other binding partner over the chip at various concentrations. SPR employs total internal reflection to monitor binding to the sensor chip. Changes in mass due to binding result in a change in refractive index on the chip's surface. This changes the angle for total internal reflection and allows quantification of binding over time from which kinetic information is derived (Altschuh et al., 1992; Cullen et al., 1987; Stenberg et al., 1991) (Fig. 2).

There are many variations in setting up these experiments, some of which can impact the resulting findings. For instance, TCR can either be coupled directly to the sensor chip surface by covalent amine linkages (Lofas and Johnsson, 1990), indirectly coupled using an covalently linked antibody, or covalently linked using a free cysteine in the Cβ region (Catimel et al., 1997; Klonisch et al., 1996). These variable setups can result in different orientations of the TCR and thus variations in surface activity—the percentage of the surface on the sensor chip that is available for binding. Lyons et al. reported that when immobilizing the 2B4 TCR through the free Cβ cysteine, the surface activity improved and that this also led to a concomitant increase in on-rate, which they hypothesized was due to the increase in surface activity (as compared to immobilization by random amine linkages) (Lyons et al., 1996). Variations such as these are important to consider when comparing SPR experiments, since the surface activity will vary between experiments depending on the immobilization mechanism.

Results from kinetic SPR measurements can be confirmed by reversing the binding partners, and most SPR studies of TCR-pMHC binding employ this strategy. Competition assays can also be performed with SPR, and these can also be used to validate values from direct binding assays. Lyons et al. demonstrated that immobilized pMHC could bind TCR flowing over the sensor chip, and that this binding decreased in a dose-dependent fashion when competitor pMHC was included with the soluble TCR. Based on the variations in concentration of the TCR and the competitor pMHC, they were able to calculate the binding affinity, which agreed with the results without competition (Lyons et al., 1996).

Studying the kinetics of the TCR-pMHC interaction has been of interest because triggering the TCR by different pMHC ligands can result in vastly different T cell fates. In the thymus, recognition of certain peptides can lead to T cell deletion and negative selection, while others lead to positive selection. For many T cell clones isolated from peripheral blood, altered peptide ligands have been identified, including strong and weak agonists and antagonists, which vary by a few amino acids yet lead to drastically different signaling

outcomes. This is a unique situation in biology, in which a single receptor is able to bind multiple, subtly different ligands, while still conveying dramatic differences in downstream signaling. The mechanism behind the TCR's specificity and sensitivity has been an intriguing puzzle for some time. SPR studies of TCR interactions with a range of related pMHCs have revealed some correlation between the binding kinetics and functional outcome (reviewed in Davis et al., 1998; Gascoigne et al., 2001). However, results across many studies proved inconsistent, requiring various models to explain these findings, which are described in detail below.

Most data support a model in which kinetic differences play a role in the differentiation of TCR activation outcome, though there has been some debates as to which aspect of the TCR-pMHC binding kinetics plays the most important role (Davis et al., 1998; Gascoigne et al., 2001). A more recent study attempted to reconcile these seemingly discrepant models. By testing a larger library of pMHC ligands, a large enough data set was obtained to enable mathematically determine a correlation between kinetic factors and stimulatory capacity. By including parameters for the tendency of the TCR and pMHC to rebind after the initial binding event, the authors were able to reconcile both models, making an argument for the importance of on-rate as well as the rate at which the two molecules diffuse apart on the two cell surfaces. This is a factor which is not accounted for in SPR, as one binding partner is fixed and the other is constantly flowing over the surface, without any opportunity for rebinding (Aleksic et al., 2011).

3D kinetic measurements have produced enlightening information on the dynamics and mechanisms of TCR-pMHC interactions, and those results were summarized in two review papers (Davis et al., 1998; Gascoigne et al., 2001). However, there are significant limitations to this method in approximating the *in vivo* conditions of this interaction. Some of these issues can be addressed by examining *in situ* TCR-pMHC interactions by 2D kinetic methods at the single-molecule level.

3.2 *In situ* 2D kinetics of TCR-pMHC interactions

Because TCRs and pMHCs are membrane proteins associated with other signaling molecules and the cytoskeleton, ideally their interactions should be measured *in situ*, in a way that can faithfully reveal the physiological TCR antigen recognition process. Compared to *in vitro* 3D measurements in solution, the *in situ* TCR-pMHC interactions occur in 2D, as TCRs and pMHCs anchor to the T cell membrane and can only diffuse two dimensionally. Recent technological developments enable us to measure cell surface molecular interactions at the single-molecule and the single-cell level. 2D *in situ* kinetics of TCR-pMHC interactions have been measured by a single-molecule FRET assay for $CD4^+$ T cells (Huppa et al., 2010) and by two single-molecule mechanical assays for $CD8^+$ T cells (Huang et al., 2010). Those measurements enable us to directly visualize or monitor *in situ* TCR-pMHC interactions at the single molecule level, compared to the *in vitro* 3D kinetics measured at the ensemble level (Fig. 2).

3.2.1 Single-molecule FRET—Single-molecule FRET was first introduced to study DNA conformational changes under nonaqueous conditions (Ha et al., 1996). Single-molecule FRET relies on the transfer of energy between donor and acceptor fluorescent tags on different parts of the same molecule or between two different molecules to reveal conformational changes or interactions during biological processes. FRET is observed when these changes or interactions bring the FRET partners into close proximity. The single-molecule FRET method has been developed to study replication, transcription, catalysis, refolding, conformational change, signal transduction and more (Roy et al., 2008). Huppa et al. developed a new type of single-molecule FRET to meet the challenge of measuring TCR-

pMHC interactions on live T cell surfaces *in situ* (Huppa et al., 2010). This method enabled scientists to directly visualize TCR-pMHC interactions at the single-molecule level for the first time. According to the spatial information of the TCR-pMHC complex structure, TCR and pMHC were site-specifically labeled with a FRET donor (Cy3) and a FRET acceptor (Cy5) respectively or vice versa. The TCR-pMHC interaction brings the fluorescent donor and acceptor into close proximity to enable FRET. In this method, the occurrence and disappearance of FRET signal directly denotes the TCR-pMHC association and dissociation (Fig. 3A). By fitting the FRET disappearance events versus time using a simple single-step dissociation model, Huppa et al. measured the *in situ* TCR-pMHC off-rates. The 2D off-rate measured in this way is significantly increased (4–12 fold) compared with the 3D off-rate measured in solution using SPR. Based on the FRET signals and the fluorescent intensities of donors and acceptors, the 2D affinity was estimated by calculating the concentrations of TCR/pMHC complexes, TCRs, and pMHCs in representative synapses at different temperatures for agonist pMHCs. Huppa et al. found that the *in situ* 2D TCR-pMHC affinity was significantly elevated—roughly 100-fold—compared to the *in vitro* 3D affinity, and the high 2D binding affinity is driven by the fast on-rates of TCR-pMHC interactions (Huppa et al., 2010).

The single-molecule FRET method is elegant; however, it is relatively hard to directly apply or tailor this method to other molecular systems. Each molecular system must have its own unique FRET design, which requires detailed structural information of the molecules of interest and tremendous biochemical work to make the required reagents. It also presents technical challenges, because the onset of FRET is highly geometrically restricted. The donor and acceptor fluorescent tags must be specifically labeled to precise sites on the two interacting molecules so that FRET signals can faithfully reveal the *in situ* molecular interactions. A fine-tuned optical system is also required to sensitively capture the weak single-molecule FRET signals.

3.2.2 Single-molecule mechanical measurements—Single-molecule mechanical assays have also been used to measure *in situ* 2D TCR-pMHC interactions. Based on early versions of ultrasensitive force techniques (Evans et al., 1991; Evans et al., 1995), Zhu et al. developed an adhesion frequency assay (Chesla et al., 1998) and a thermal fluctuation assay (Chen et al., 2008a) to measure the *in situ* 2D kinetics of cell surface molecular interactions. Although both assays are mechanically based, they measure force-free binding kinetics. Detection of receptor-ligand interactions is accomplished by using a red blood cell (RBC) as a mechanical force sensor, which can detect < 2 pN of force. The adhesion frequency assay measures a binary score of an adhesion event between a T cell and a surrogate APC for each test; the test is then repeated to estimate an adhesion frequency (Fig. 3B). After collecting a series of adhesion frequencies at different contact durations and surface molecular densities, 2D kinetics are obtained by fitting the data with a probabilistic model that is valid for low number of bonds but does not require single bond (Chesla et al., 1998).

The thermal fluctuation assay measures the TCR-pMHC bond association and dissociation by directly monitoring the reduction and resumption of thermal fluctuation of a pMHC-coated force probe. This directly reveals the single molecule interaction process (Fig. 3C). On-rate information is provided by the waiting-time for bond formation, which is the period from the instant of dissociation of an existing bond to the instant of formation of the next bond. The bond lifetime, which is the period from the instant of bond formation to the instant of bond dissociation, provides information about the off-rate. The on-rate or off-rate is obtained by fitting the distribution of waiting-times or lifetimes pooled from many bond formation or dissociation events using a single-step association or dissociation model, respectively (Chen et al., 2008a; Chen et al., 2008b). These mechanical assays showed that a TCR binds to its antigenic pMHC with high 2D affinities and fast kinetics, and the 2D

affinities and on-rates of a panel of pMHC ligands possess broad dynamic ranges that match their corresponding potencies to trigger T cell responses (Huang et al., 2010).

It is relatively easy to adapt single-molecule mechanical assays to measure other cell surface molecular interactions with simple biochemical modifications. Indeed, these assays have been used to measure 2D kinetics of ligand binding of TCRs, CD8, Fc receptors, selectins, integrins and cadherin (Chen et al., 2008a; Chen et al., 2010; Chien et al., 2008; Huang et al., 2004; Huang et al., 2007; Huang et al., 2010; Jiang et al., 2011; Sabatino et al., 2011; Zhang et al., 2005). Another advantage is that they can be further extended to measure mechanical regulation of TCR-pMHC interactions, e.g., force-dependent bond lifetimes and unbinding forces. However, these are low throughput assays because measurements are made for one pair of cells at a time. The only readout for these assays is the binding events, making it nontrivial to simultaneously determine interactions between more than one receptor-ligand pair on the cell surface.

3.2.3 The role of co-receptors—The co-receptor CD4 or CD8 alone binds to pMHC with low *in vitro* 3D affinities (K_d of 100–200 μM for human co-receptors and 10–100 μM for mouse co-receptors) in solution (Gao et al., 2002). 3D *in vitro* SPR measurements showed that TCR and co-receptor CD4 or CD8 bind to pMHC independently and with distinct kinetics (Wyer et al., 1999; Xiong et al., 2001).

To date there are no quantitative *in situ* 2D kinetic measurements for CD4-pMHC interactions, which might be due to their weak interactions. The 2D interaction between CD8 and pMHC is readily measurable and we have quantified the *in situ* binding kinetics of this interaction using the adhesion frequency assay. We found that the CD8-pMHC interaction has a very low 2D affinity that depends on the MHC alleles and membrane lipid rafts, but not on the bound peptide or the composition of CD8 α and β chains (Huang et al., 2007). Importantly, the presence of CD4 and CD8 affects the *in situ* TCR binding distinctly. CD4 does not appear to affect the kinetics of *in situ* TCR-pMHC interactions (Huppa et al., 2010), while CD8 greatly promotes the *in situ* cooperative binding of the trimolecular interactions among TCR, pMHC, and CD8 (Jiang et al., 2011). CD4 and CD8 also play different roles in meditating the nonstimulatory/endogenous pMHCs for helping TCR antigen recognition (Gascoigne, 2008; Krogsgaard et al., 2005; Yachi et al., 2005). It is not clear what causes this difference between CD4 and CD8. A possible reconciliation might be that the main purpose of these two co-receptors is to direct Lck to the TCR, regardless of TCR stabilization (Artyomov et al., 2010).

3.3 The gap and possible bridge between 2D and 3D measurements

3D kinetic assays of TCR-pMHC interactions measure the *in vitro* molecular binding properties at the ensemble level while 2D kinetic assays quantify the physiological *in situ* kinetics at the single molecule level. A TCR can recognize a specific agonist and a series of altered peptides identified as co-agonist, weak agonist, antagonist and null according to their potencies to activate T cells (Alam et al., 1996; Davis et al., 1998; Gascoigne et al., 2001; Kersh et al., 1998). 3D binding assays have been used to analyze the kinetics of many such peptide panels with little consensus and discrepant correlation with peptide biological functions, requiring complex models to explain the mechanism of T cell recognition and discrimination (Dustin and Depoil, 2011; Fahmy et al., 2002; Gascoigne et al., 2001; Laugel et al., 2007; van der Merwe and Dushek, 2010). In sharp contrast, recent 2D *in situ* measurements show a broad dynamic range of kinetics parameters for a panel of ligands that correlate well with the peptide biological functions (Huang et al., 2010; Huppa et al., 2010). For agonist peptides, 3D measurements suggest that TCR-pMHC interactions have low binding affinities, slow off-rates and on-rates, while 2D measurements observe high binding

affinities, fast off-rates and on-rates. Such substantial differences between 3D and 2D results are not seen in other receptor-ligand interactions such as selectins and integrins (Huang et al., 2004; Mehta et al., 1998; Shimaoka et al., 2003; Zhang et al., 2005). The key might lie in the special cellular microenvironment of TCRs. For 3D measurements, both TCRs and pMHCs are truncated, recombinant purified soluble proteins isolated from this microenvironment with a 3D diffusion freedom in solution. On the cell surface under physiological conditions, TCRs may interact laterally with each other or other molecules to form membrane lipid-dependent nanostructures and associate with the cytoskeleton to restrict their 2D diffusion. It has been shown that both the cytoskeleton and membrane lipid nanostructures play critical roles in TCR antigen recognition, signaling and triggering (Brdickova et al., 2003; Fuller et al., 2003; Harder, 2004; Harder and Engelhardt, 2004; He et al., 2005; Huppa and Davis, 2003; Magee et al., 2002). Recent total internal reflection fluorescence microscopy experiments have shown that TCRs form microclusters during antigen recognition, which mediate early T cell signaling in a cholesterol and actin cytoskeleton dependent fashion (Campi et al., 2005; Yokosuka et al., 2005). Further studies using super resolution electron microscopy and high-speed photoactivated localization microscopy suggest that membrane-associated TCRs form 10–200 nm multivalent TCRs (Kumar et al., 2011; Schamel et al., 2005) or protein islands (Lillemeier et al., 2010). Indeed, inhibition of actin polymerization or depletion of cholesterol dramatically changed the *in situ* TCR-pMHC binding kinetics in both single-molecule FRET and single-molecule mechanical assays. In the FRET measurements, actin depolymerization produced an almost identical 2D off-rate to that measured by the 3D SPR method; cholesterol depletion also severely impaired the TCR-pMHC interactions (Huppa et al., 2010). Similarly, actin polymerization inhibition and cholesterol depletion significantly affected the 2D on-rates and dramatically reduced the effective 2D affinities in the mechanical assays (Huang et al., 2010). The importance of the TCR cellular microenvironment has also been demonstrated with cell-free 2D assays. By coating purified TCR and pMHC molecules onto 2D surfaces, Robert et al. measured cell-free 2D binding kinetics using a flow chamber with single-bond resolution. They found that cell-free 2D off-rates were comparable to 3D parameters obtained with the same purified molecules. Additionally, there is no significant correlation between cell-free 2D on-rates and activating potency of pMHCs (Robert et al., 2012). Thus, the specific cellular microenvironment of the T cell may impose unique regulations on TCR organization, orientation, and conformation, which may greatly increase TCR availability. This may in turn promote a higher binding frequency of TCR-pMHC interactions to enable T cells effectively scan and recognize rare antigens presented on APC surface. Therefore, the *in situ* 2D measurements may represent an integration of molecular interaction, TCR structure, membrane organization, cytoskeleton regulation, cell signaling and feedback. Future studies are needed to further address the discrepancies between 3D and 2D measurements.

4. Issues and models of T cell antigen recognition

The interaction of TCR-pMHC determines the fate and response of a T cell, yet much is still unknown for this process. Mature T cells have undergone both positive and negative selection. It has been proposed that positive selection requires TCR recognition of self-pMHCs with low affinities to provide signals for T cell survival, while negative selection eliminates T cells with high affinities to self-pMHCs. In physiological conditions, T cell activation is trigged by specific TCR recognition of a very low dose of foreign pMHCs that are randomly distributed on the APC surface and surrounded by a large excess of self-pMHCs. The relatively small TCRs (7 nm) co-exist with other large molecules such as CD45 (40–50 nm) on the T cell surface. However, we still do not fully understand how TCRs overcome steric hindrance in such a dense and crowded cell surface and recognize extremely low-density antigens from the sea of self-pMHCs with high sensitivity and

specificity (Fahmy et al., 2002; Springer, 1990). In addition, although many studies have highlighted the critical role of co-receptors to augment TCR signaling, the mechanism of co-receptor communication with TCR remains elusive. Recent 2D assays suggest that CD4 and CD8 distinctly assist TCR in antigen recognition: CD4 has a negligible role in affecting TCR-pMHC binding (Huppa et al., 2010) while CD8 significantly promotes antigen recognition by cooperation with the TCR in pMHC binding in a signaling-dependent fashion (Jiang et al., 2011). Furthermore, the association of CD3δε, γε, and ζζ subunits to the TCR is necessary for T cell intracelluar signal transduction (Call et al., 2002; Call and Wucherpfennig, 2005; Kuhns and Davis, 2007; Kuhns et al., 2006). However, it is not fully understood how the TCR αβ domains associate with the CD3 signaling subunits and how the TCR-pMHC interaction affects the association, conformation and signaling of the CD3 subunits with the TCR αβ domains. Finally, T cell antigen recognition is a dynamic and complex process that involves the cell membrane environment, cytoskeleton, molecular orientation and organization in the cell surface nanostructure, and adhesion and co-stimulatory/inhibitory molecular interactions (Davis et al., 2003). These fascinating phenomena have motivated numerous studies over the past twenty years. Many models have been proposed to explain the mechanism of T cell antigen recognition. A complete model should be able to explain how the recognition signal is initiated, controlled, and transmitted. Here we will review some selected models.

4.1 Kinetic proofreading and related models

The kinetic proofreading model was proposed to explain how T cells discriminate ligands based on small differences of off-rates (McKeithan, 1995; Rabinowitz et al., 1996). It proposes that the full activation of a T cell requires the TCR-pMHC interaction to be sustained long enough to complete a series of necessary modification steps. Without this long-lasting interaction, the molecules will completely revert to their unmodified forms (McKeithan, 1995; Rabinowitz et al., 1996). These sequential modification steps exponentially amplify minor or moderate differences in kinetics, explaining the profoundly different biological outcomes. Some 3D measurements found positive correlations between reciprocal off-rates and peptide potencies (Davis et al., 1998; Gascoigne et al., 2001; Kersh et al., 1998). However, many exceptions have reported that slow off-rates do not necessarily result in better activations (Hlavacek et al., 2001; Kalergis et al., 2001; Laugel et al., 2007; Rosette et al., 2001). This model has been improved over the years into several modified forms, e.g., the kinetic-segregation model (Davis and van der Merwe, 2006). Recently, it has been further revised to an integrated TCR triggering model by summarizing and incorporating recent data and findings (van der Merwe and Dushek, 2010).

4.2 Dimer models

The co-receptor plays a critical role in T cell activation. Based on how the co-receptor binds to pMHC with the TCR, two dimer models have been proposed. The classic heterodimer model postulates that the co-receptor binds a pMHC that is concurrently engaged with a TCR. Several studies have shown that the TCR and co-receptor must bind to same pMHC in order to fully initiate the T cell activation (Block et al., 2001; Gao et al., 2002; Gascoigne, 2008; Janeway et al., 1999; Rudolph et al., 2006). Using the 2D *in situ* micropipette adhesion frequency assay, we have shown that CD8 binds to same pMHC with TCR to further amplify the T cell response (Jiang et al., 2011). TCR and co-receptor binding to the same pMHC can promote the signaling between the co-receptor associated Lck and the TCR intracellular CD3 signaling module (Jiang et al., 2011; Xu and Littman, 1993). The heterodimer model is challenged by the finding that soluble dimeric or oligomeric pMHCs, but not monomeric pMHCs, can efficiently trigger T cell activation and signaling (Boniface et al., 1998; Stone and Stern, 2006). Also, 2D *in situ* FRET measurements found that CD4 does not stabilize TCR-pMHC interactions (Huppa et al., 2010).

Davis et al. proposed an alternative pseudodimer model by highlighting the role of endogenous pMHC in a $CD4^+$ T cell system. Based on the soluble dimeric pMHC data, this model suggests that the interaction of one TCR with an agonist pMHC can recruit a second TCR through an associated CD4 molecule. This recruited TCR can bind an endogenous pMHC, thereby forming a stable pseudodimer to initiate activation using the tyrosine kinase Lck (Davis et al., 2007; Krogsgaard et al., 2005). However, Ma et al. claimed that endogenous pMHCs have a negligible role in triggering T cell activation (Ma et al., 2008). Nevertheless, the pseudodimer model is attractive due to its emphasis on the role of excess endogenous pMHCs on the cell surface (Davis et al., 2007; Gascoigne, 2008; van der Merwe and Dushek, 2010).

Future physiological studies with detailed spatial and temporal resolution are needed to further elucidate these models and to understand the interactions between TCRs and co-receptors during antigen recognition. This is necessary since TCRs, co-receptors and pMHCs are expressed on the cell surface and associated with the lipid rafts and the cytoskeleton in physiological conditions, and are not expressed as soluble monomers or oligomers.

4.3 Conformational model

The conformational model proposes that TCR conformational changes are necessary for signal transmission across the cell membrane. The conformational model suggests that upon encountering an agonist pMHC, a TCR changes its conformation to induce the T cell activation signal. Alarcón's group has shown that the engagement of TCR with pMHC can cause the exposure of an epitope on CD3 revealed by a reporting antibody (Gil et al., 2002; Gil et al., 2005; Risueno et al., 2005; Schamel et al., 2006). However, except for the LC13 TCR crystal structure, most TCR crystal structures do not show any direct evidence for conformational changes of the TCR following binding to pMHC (Levin and Weiss, 2005; Rudolph and Wilson, 2002). There is still a lack of direct structural evidence, partially due to the technical challenges of understanding and solving the complex multi-unit structures of TCR-CD3 complexes in physiological conditions.

4.4 Serial triggering model

A TCR serial triggering model was proposed based on the observation that a small number of agonist pMHCs can achieve a high level of TCR internalization (Valitutti et al., 1995). The serial triggering model postulates that a single pMHC can serially engage and trigger multiple TCRs, leading to TCR down-regulation so that a T cell can sensitively detect a small number of antigenic pMHCs through high TCR occupancy. Serial engagement was originally proposed to explain TCR recognition for low affinity and fast off-rate ligands, and suggested that the high affinity and slow off-rate of the TCR-pMHC interaction will prevent T cell antigen recognition by reducing or preventing TCR usage. A drawback of this serial triggering model is that it is based on the assumption that TCR internalization is triggered by TCR-pMHC engagements and correlated with T cell activation. However, simple CD3 surface staining experiments after hours of T cell/APC binding could not provide any direct information of initial dynamic TCR-pMHC engagements (Valitutti et al., 1995). It has been shown that TCRs can be down-regulated either by other ligand engagements or cell signaling (Niedergang et al., 1997; San Jose et al., 2000). On the other hand, Evavold et al. found that a 90% reduction in TCR level did not affect the ability for the T cell to be stimulated by agonist pMHCs (McNeil and Evavold, 2002; McNeil and Evavold, 2003). In other words, the number of internalized TCRs may not correlate with the number of engaged receptors and the T cell activation.

Recent 2D *in situ* kinetic measurements provide new appealing features and important information for TCR antigen recognition, and we have accordingly proposed a fast kinetics based serial triggering model (Fig. 4). Both single-molecule FRET and single-molecule mechanical based 2D measurements found that the TCR-pMHC interaction has a high affinity, fast on-rate and off-rate for antigen recognition. Although the off-rate of the TCR-pMHC interaction is very fast, the extremely fast on-rate promotes bond re-formation with high frequency and generates high binding affinity. Furthermore, the formation of TCR clusters/protein islands provides a high local TCR concentration to facilitate TCR antigen recognition and amplify the T cell antigen recognition signal (Campi et al., 2005; Kumar et al., 2011; Lillemeier et al., 2010; Schamel et al., 2005; Yokosuka et al., 2005). Fast kinetics and serial TCR triggering enables a T cell to efficiently scan, detect and engage rare antigens presented on the APC surfaces. This fast kinetics based serial triggering model can also explain how TCRs discriminate pMHC ligands with very different kinetic rates that will lead to distinct TCR occupancy rates. This model reconciles the paradox of high sensitivity and specificity of T cell antigen recognition and allows both the quality and quantity of pMHCs to be measured by the frequency of bond formation.

5. Conclusion and Future directions

Recent advances in the study of T cell antigen recognition have provided new excitement to T cell biology. Along with the discoveries of TCR clusters and/or TCR-enriched protein islands, the *in situ* 2D kinetics measurements suggest a fast kinetics based serial triggering model (Fig. 4). This model can explain how T cells efficiently scan and sensitively recognize a very low number of antigens on APCs and maximize the T cell response. However, direct verification of TCR serial engagement is technically challenging. Current studies are unable to resolve whether the TCR clusters are formed before or after TCR engagement, and cannot reveal the dynamic TCR nanostructures on live cell membrane during TCR triggering. The roles of the cytoskeleton and lipid rafts in dynamic TCR antigen recognition remain unclear, and there is a lack of direct information about aspects of T cell surface nanostructures such as their composition, size, heterogeneity, and dynamics in physiological condition. Developing a new imaging technique that can reliably and continuously detect serial interactions of a single antigen with an array of TCRs would provide direct evidence to prove or disprove this revised serial triggering model. It is still unclear whether a T cell is triggered by the accumulated high frequency of serial engagements, a high quality single ligand binding, or a combination of both. Future advances and breakthroughs will probably rely on the development and combination of super resolution fluorescent microcopy, novel biochemistry and single-molecule kinetic measurements in the coming years.

Acknowledgments

The authors are grateful to Dr. Mark M. Davis for his generous support and critical comments to this paper. We acknowledge the contributions of our colleagues whose original works are reviewed and the support of NIH grant (R01 GM096187).

Abbreviations

TCR	T cell receptor
pMHC	peptide bound major histocompatibility complex
APC	antigen presenting cell
SPR	surface plasmon resonance

FRET	förster resonance energy transfer
ITAM	immunoreceptor tyrosine-based activation motif
3D	three-dimensional
2D	two-dimensional
RBC	red blood cell

References

Alam SM, Travers PJ, Wung JL, Nasholds W, Redpath S, Jameson SC, Gascoigne NR. T-cell-receptor affinity and thymocyte positive selection. Nature. 1996; 381:616–620. [PubMed: 8637599]

Alegre ML, Frauwirth KA, Thompson CB. T-cell regulation by CD28 and CTLA-4. Nat Rev Immunol. 2001; 1:220–228. [PubMed: 11905831]

Aleksic M, Dushek O, Zhang H, Shenderov E, Chen JL, Cerundolo V, Coombs D, van der Merwe PA. Dependence of T cell antigen recognition on T cell receptor-peptide MHC confinement time. Immunity. 2011; 32:163–174. [PubMed: 20137987]

Altschuh D, Dubs MC, Weiss E, Zeder-Lutz G, Van Regenmortel MH. Determination of kinetic constants for the interaction between a monoclonal antibody and peptides using surface plasmon resonance. Biochemistry. 1992; 31:6298–6304. [PubMed: 1627568]

Artyomov MN, Lis M, Devadas S, Davis MM, Chakraborty AK. CD4 and CD8 binding to MHC molecules primarily acts to enhance Lck delivery. Proc Natl Acad Sci U S A. 2010; 107:16916–16921. [PubMed: 20837541]

Billadeau DD, Nolz JC, Gomez TS. Regulation of T-cell activation by the cytoskeleton. Nat Rev Immunol. 2007; 7:131–143. [PubMed: 17259969]

Block MS, Johnson AJ, Mendez-Fernandez Y, Pease LR. Monomeric class I molecules mediate TCR/CD3 epsilon/CD8 interaction on the surface of T cells. J Immunol. 2001; 167:821–826. [PubMed: 11441088]

Boniface JJ, Rabinowitz JD, Wulfing C, Hampl J, Reich Z, Altman JD, Kantor RM, Beeson C, McConnell HM, Davis MM. Initiation of signal transduction through the T cell receptor requires the multivalent engagement of peptide/MHC ligands [corrected]. Immunity. 1998; 9:459–466. [PubMed: 9806632]

Brdickova N, Brdicka T, Angelisova P, Horvath O, Spicka J, Hilgert I, Paces J, Simeoni L, Kliche S, Merten C, Schraven B, Horejsi V. LIME: a new membrane Raft-associated adaptor protein involved in CD4 and CD8 coreceptor signaling. J. Exp. Med. 2003; 198:1453–1462. [PubMed: 14610046]

Call ME, Pyrdol J, Wiedmann M, Wucherpfennig KW. The organizing principle in the formation of the T cell receptor-CD3 complex. Cell. 2002; 111:967–979. [PubMed: 12507424]

Call ME, Wucherpfennig KW. The T cell receptor: critical role of the membrane environment in receptor assembly and function. Annu. Rev. Immunol. 2005; 23:101–125. [PubMed: 15771567]

Campi G, Varma R, Dustin ML. Actin and agonist MHC-peptide complex-dependent T cell receptor microclusters as scaffolds for signaling. J Exp Med. 2005; 202:1031–1036. [PubMed: 16216891]

Catimel B, Nerrie M, Lee FT, Scott AM, Ritter G, Welt S, Old LJ, Burgess AW, Nice EC. Kinetic analysis of the interaction between the monoclonal antibody A33 and its colonic epithelial antigen by the use of an optical biosensor. A comparison of immobilisation strategies. J Chromatogr A. 1997; 776:15–30. [PubMed: 9286074]

Chen W, Evans EA, McEver RP, Zhu C. Monitoring receptor-ligand interactions between surfaces by thermal fluctuations. Biophys J. 2008a; 94:694–701. [PubMed: 17890399]

Chen W, Lou J, Zhu C. Forcing switch from short- to intermediate- and long-lived states of the alphaA domain generates LFA-1/ICAM-1 catch bonds. J Biol Chem. 2010; 285:35967–35978. [PubMed: 20819952]

Chen W, Zarnitsyna VI, Sarangapani KK, Huang J, Zhu C. Measuring Receptor-Ligand Binding Kinetics on Cell Surfaces: From Adhesion Frequency to Thermal Fluctuation Methods. Cell Mol Bioeng. 2008b; 1:276–288. [PubMed: 19890486]

Chesla SE, Selvaraj P, Zhu C. Measuring two-dimensional receptor-ligand binding kinetics by micropipette. Biophys. J. 1998; 75:1553–1572. [PubMed: 9726957]

Chien YH, Jiang N, Li F, Zhang F, Zhu C, Leckband D. Two stage cadherin kinetics require multiple extracellular domains but not the cytoplasmic region. J Biol Chem. 2008; 283:1848–1856. [PubMed: 17999960]

Cullen DC, Brown RG, Lowe CR. Detection of immuno-complex formation via surface plasmon resonance on gold-coated diffraction gratings. Biosensors. 1987; 3:211–225. [PubMed: 3504151]

Davis MM, Boniface JJ, Reich Z, Lyons D, Hampl J, Arden B, Chien Y. Ligand recognition by alpha beta T cell receptors. Annu Rev Immunol. 1998; 16:523–544. [PubMed: 9597140]

Davis MM, Krogsgaard M, Huppa JB, Sumen C, Purbhoo MA, Irvine DJ, Wu LC, Ehrlich L. Dynamics of cell surface molecules during T cell recognition. Annu Rev Biochem. 2003; 72:717–742. [PubMed: 14527326]

Davis MM, Krogsgaard M, Huse M, Huppa J, Lillemeier BF, Li QJ. T cells as a self-referential, sensory organ. Annu Rev Immunol. 2007; 25:681–695. [PubMed: 17291190]

Davis SJ, van der Merwe PA. The kinetic-segregation model: TCR triggering and beyond. Nat Immunol. 2006; 7:803–809. [PubMed: 16855606]

Demotz S, Grey HM, Sette A. The minimal number of class II MHC-antigen complexes needed for T cell activation. Science. 1990; 249:1028–1030. [PubMed: 2118680]

Dustin ML. Cell adhesion molecules and actin cytoskeleton at immune synapses and kinapses. Curr Opin Cell Biol. 2007; 19:529–533. [PubMed: 17923403]

Dustin ML, Depoil D. New insights into the T cell synapse from single molecule techniques. Nat Rev Immunol. 2011; 11:672–684. [PubMed: 21904389]

Ebert PJ, Ehrlich LI, Davis MM. Low ligand requirement for deletion and lack of synapses in positive selection enforce the gauntlet of thymic T cell maturation. Immunity. 2008; 29:734–745. [PubMed: 18993085]

Evans E, Berk D, Leung A. Detachment of agglutinin-bonded red blood cells. I. Forces to rupture molecular-point attachments. Biophys J. 1991; 59:838–848. [PubMed: 2065188]

Evans E, Ritchie K, Merkel R. Sensitive force technique to probe molecular adhesion and structural linkages at biological interfaces. Biophys J. 1995; 68:2580–2587. [PubMed: 7647261]

Evavold BD, Allen PM. Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. Science. 1991; 252:1308–1310. [PubMed: 1833816]

Fahmy TM, Bieler JG, Schneck JP. Probing T cell membrane organization using dimeric MHC-Ig complexes. J. Immunol. Methods. 2002; 268:93–106. [PubMed: 12213346]

Fuller CL, Braciale VL, Samelson LE. All roads lead to actin: the intimate relationship between TCR signaling and the cytoskeleton. Immunol Rev. 2003; 191:220–236. [PubMed: 12614363]

Gao GF, Rao Z, Bell JI. Molecular coordination of alphabeta T-cell receptors and coreceptors CD8 and CD4 in their recognition of peptide-MHC ligands. Trends Immunol. 2002; 23:408–413. [PubMed: 12133804]

Gascoigne NR. Do T cells need endogenous peptides for activation? Nat Rev Immunol. 2008; 8:895–900. [PubMed: 18846098]

Gascoigne NR, Zal T, Alam SM. T-cell receptor binding kinetics in T-cell development and activation. Expert Rev Mol Med. 2001; 2001:1–17. [PubMed: 14987373]

Gil D, Schamel WW, Montoya M, Sanchez-Madrid F, Alarcon B. Recruitment of Nck by CD3 epsilon reveals a ligand-induced conformational change essential for T cell receptor signaling and synapse formation. Cell. 2002; 109:901–912. [PubMed: 12110186]

Gil D, Schrum AG, Alarcon B, Palmer E. T cell receptor engagement by peptide-MHC ligands induces a conformational change in the CD3 complex of thymocytes. J. Exp. Med. 2005; 201:517–522. [PubMed: 15728235]

Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. The immunological synapse: a molecular machine controlling T cell activation. Science. 1999; 285:221–227. [PubMed: 10398592]

Ha T, Enderle T, Ogletree DF, Chemla DS, Selvin PR, Weiss S. Probing the interaction between two single molecules: fluorescence resonance energy transfer between a single donor and a single acceptor. Proc Natl Acad Sci U S A. 1996; 93:6264–6268. [PubMed: 8692803]

Harder T. Lipid raft domains and protein networks in T-cell receptor signal transduction. Curr Opin Immunol. 2004; 16:353–359. [PubMed: 15134785]

Harder T, Engelhardt KR. Membrane domains in lymphocytes - from lipid rafts to protein scaffolds. Traffic. 2004; 5:265–275. [PubMed: 15030568]

Harding CV, Unanue ER. Quantitation of antigen-presenting cell MHC class II/peptide complexes necessary for T-cell stimulation. Nature. 1990; 346:574–576. [PubMed: 2115981]

He HT, Lellouch A, Marguet D. Lipid rafts and the initiation of T cell receptor signaling. Semin Immunol. 2005; 17:23–33. [PubMed: 15582486]

Hlavacek WS, Redondo A, Metzger H, Wofsy C, Goldstein B. Kinetic proofreading models for cell signaling predict ways to escape kinetic proofreading. Proc Natl Acad Sci U S A. 2001; 98:7295–7300. [PubMed: 11390967]

Huang J, Chen J, Chesla SE, Yago T, Mehta P, McEver RP, Zhu C, Long M. Quantifying the effects of molecular orientation and length on two-dimensional receptor-ligand binding kinetics. J. Biol. Chem. 2004; 279:44915–44923. [PubMed: 15299021]

Huang J, Edwards LJ, Evavold BD, Zhu C. Kinetics of MHC-CD8 interaction at the T cell membrane. J Immunol. 2007; 179:7653–7662. [PubMed: 18025211]

Huang J, Zarnitsyna VI, Liu B, Edwards LJ, Jiang N, Evavold BD, Zhu C. The kinetics of two-dimensional TCR and pMHC interactions determine T-cell responsiveness. Nature. 2010; 464:932–936. [PubMed: 20357766]

Huppa JB, Axmann M, Mortelmaier MA, Lillemeier BF, Newell EW, Brameshuber M, Klein LO, Schutz GJ, Davis MM. TCR-peptide-MHC interactions in situ show accelerated kinetics and increased affinity. Nature. 2010; 463:963–967. [PubMed: 20164930]

Huppa JB, Davis MM. T-cell-antigen recognition and the immunological synapse. Nat Rev Immunol. 2003; 3:973–983. [PubMed: 14647479]

Huse M, Klein LO, Girvin AT, Faraj JM, Li QJ, Kuhns MS, Davis MM. Spatial and temporal dynamics of T cell receptor signaling with a photoactivatable agonist. Immunity. 2007; 27:76–88. [PubMed: 17629516]

Irvine DJ, Purbhoo MA, Krogsgaard M, Davis MM. Direct observation of ligand recognition by T cells. Nature. 2002; 419:845–849. [PubMed: 12397360]

Janeway CA Jr. The T cell receptor as a multicomponent signalling machine: CD4/CD8 coreceptors and CD45 in T cell activation. Annu Rev Immunol. 1992; 10:645–674. [PubMed: 1534242]

Janeway, CA.; Travers, P.; Walport, M.; Capra, JD. Immunobiology: The Immune System in Health and Disease. New York, NY: Elsevier Science Ltd/Garland publishing; 1999.

Jiang N, Huang J, Edwards LJ, Liu B, Zhang Y, Beal CD, Evavold BD, Zhu C. Two-stage cooperative T cell receptor-peptide major histocompatibility complex-CD8 trimolecular interactions amplify antigen discrimination. Immunity. 2011; 34:13–23. [PubMed: 21256056]

Kalergis AM, Boucheron N, Doucey MA, Palmieri E, Goyarts EC, Vegh Z, Luescher IF, Nathenson SG. Efficient T cell activation requires an optimal dwell-time of interaction between the TCR and the pMHC complex. Nat Immunol. 2001; 2:229–234. [PubMed: 11224522]

Kersh GJ, Allen PM. Essential flexibility in the T-cell recognition of antigen. Nature. 1996; 380:495–498. [PubMed: 8606766]

Kersh GJ, Kersh EN, Fremont DH, Allen PM. High- and low-potency ligands with similar affinities for the TCR: the importance of kinetics in TCR signaling. Immunity. 1998; 9:817–826. [PubMed: 9881972]

Klonisch T, Panayotou G, Edwards P, Jackson AM, Berger P, Delves PJ, Lund T, Roitt IM. Enhancement in antigen binding by a combination of synergy and antibody capture. Immunology. 1996; 89:165–171. [PubMed: 8943709]

Krogsgaard M, Li QJ, Sumen C, Huppa JB, Huse M, Davis MM. Agonist/endogenous peptide-MHC heterodimers drive T cell activation and sensitivity. Nature. 2005; 434:238–243. [PubMed: 15724150]

Kuhns MS, Davis MM. Disruption of extracellular interactions impairs T cell receptor-CD3 complex stability and signaling. Immunity. 2007; 26:357–369. [PubMed: 17368054]

Kuhns MS, Davis MM, Garcia KC. Deconstructing the form and function of the TCR/CD3 complex. Immunity. 2006; 24:133–139. [PubMed: 16473826]

Kumar R, Ferez M, Swamy M, Arechaga I, Rejas MT, Valpuesta JM, Schamel WW, Alarcon B, van Santen HM. Increased sensitivity of antigen-experienced T cells through the enrichment of oligomeric T cell receptor complexes. Immunity. 2011; 35:375–387. [PubMed: 21903423]

Laugel B, van den Berg HA, Gostick E, Cole DK, Wooldridge L, Boulter J, Milicic A, Price DA, Sewell AK. Different T cell receptor affinity thresholds and CD8 coreceptor dependence govern cytotoxic T lymphocyte activation and tetramer binding properties. J Biol Chem. 2007; 282:23799–23810. [PubMed: 17540778]

Levin SE, Weiss A. Twisting tails exposed: the evidence for TCR conformational change. J Exp Med. 2005; 201:489–492. [PubMed: 15728230]

Lillemeier BF, Mortelmaier MA, Forstner MB, Huppa JB, Groves JT, Davis MM. TCR and Lat are expressed on separate protein islands on T cell membranes and concatenate during activation. Nat Immunol. 2010; 11:90–96. [PubMed: 20010844]

Lofas S, Johnsson B. A Novel Hydrogel Matrix on Gold Surfaces in Surface-Plasmon Resonance Sensors for Fast and Efficient Covalent Immobilization of Ligands. Journal of the Chemical Society-Chemical Communications. 1990:1526–1528.

Lyons DS, Lieberman SA, Hampl J, Boniface JJ, Chien Y, Berg LJ, Davis MM. A TCR binds to antagonist ligands with lower affinities and faster dissociation rates than to agonists. Immunity. 1996; 5:53–61. [PubMed: 8758894]

Magee T, Pirinen N, Adler J, Pagakis SN, Parmryd I. Lipid rafts: cell surface platforms for T cell signaling. Biol Res. 2002; 35:127–131. [PubMed: 12415729]

McKeithan TW. Kinetic proofreading in T-cell receptor signal transduction. Proc Natl Acad Sci U S A. 1995; 92:5042–5046. [PubMed: 7761445]

McNeil LK, Evavold BD. Dissociation of peripheral T cell responses from thymocyte negative selection by weak agonists supports a spare receptor model of T cell activation. Proc Natl Acad Sci U S A. 2002; 99:4520–4525. [PubMed: 11904393]

McNeil LK, Evavold BD. TCR reserve: a novel principle of CD4 T cell activation by weak ligands. J Immunol. 2003; 170:1224–1230. [PubMed: 12538680]

Mehta P, Cummings RD, McEver RP. Affinity and kinetic analysis of P-selectin binding to P-selectin glycoprotein ligand-1. J Biol Chem. 1998; 273:32506–32513. [PubMed: 9829984]

Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. Three-dimensional segregation of supramolecular activation clusters in T cells. Nature. 1998; 395:82–86. [PubMed: 9738502]

Niedergang F, Dautry-Varsat A, Alcover A. Peptide antigen or superantigen-induced down-regulation of TCRs involves both stimulated and unstimulated receptors. J Immunol. 1997; 159:1703–1710. [PubMed: 9257831]

Purbhoo MA, Irvine DJ, Huppa JB, Davis MM. T cell killing does not require the formation of a stable mature immunological synapse. Nat Immunol. 2004; 5:524–530. [PubMed: 15048111]

Rabinowitz JD, Beeson C, Lyons DS, Davis MM, McConnell HM. Kinetic discrimination in T-cell activation. Proc Natl Acad Sci U S A. 1996; 93:1401–1405. [PubMed: 8643643]

Risueno RM, Gil D, Fernandez E, Sanchez-Madrid F, Alarcon B. Ligand-induced conformational change in the T-cell receptor associated with productive immune synapses. Blood. 2005; 106:601–608. [PubMed: 15790785]

Robert P, Aleksic M, Dushek O, Cerundolo V, Bongrand P, van der Merwe PA. Kinetics and mechanics of two-dimensional interactions between T cell receptors and different activating ligands. Biophys J. 2012; 102:248–257. [PubMed: 22339861]

Rosette C, Werlen G, Daniels MA, Holman PO, Alam SM, Travers PJ, Gascoigne NR, Palmer E, Jameson SC. The impact of duration versus extent of TCR occupancy on T cell activation: a revision of the kinetic proofreading model. Immunity. 2001; 15:59–70. [PubMed: 11485738]

Roy R, Hohng S, Ha T. A practical guide to single-molecule FRET. Nat Methods. 2008; 5:507–516. [PubMed: 18511918]

Rudolph MG, Stanfield RL, Wilson IA. How TCRs bind MHCs, peptides, and coreceptors. Annu. Rev. Immunol. 2006; 24:419–466. [PubMed: 16551255]

Rudolph MG, Wilson IA. The specificity of TCR/pMHC interaction. Curr Opin Immunol. 2002; 14:52–65. [PubMed: 11790533]

Sabatino JJ Jr, Huang J, Zhu C, Evavold BD. High prevalence of low affinity peptide-MHC II tetramer-negative effectors during polyclonal CD4+ T cell responses. J Exp Med. 2011; 208:81–90. [PubMed: 21220453]

San Jose E, Borroto A, Niedergang F, Alcover A, Alarcon B. Triggering the TCR complex causes the downregulation of nonengaged receptors by a signal transduction-dependent mechanism. Immunity. 2000; 12:161–170. [PubMed: 10714682]

Schamel WW, Arechaga I, Risueno RM, van Santen HM, Cabezas P, Risco C, Valpuesta JM, Alarcon B. Coexistence of multivalent and monovalent TCRs explains high sensitivity and wide range of response. J Exp Med. 2005; 202:493–503. [PubMed: 16087711]

Schamel WW, Risueno RM, Minguet S, Ortiz AR, Alarcon B. A conformation- and avidity-based proofreading mechanism for the TCR-CD3 complex. Trends Immunol. 2006; 27:176–182. [PubMed: 16527543]

Shimaoka M, Xiao T, Liu JH, Yang Y, Dong Y, Jun CD, McCormack A, Zhang R, Joachimiak A, Takagi J, Wang JH, Springer TA. Structures of the alpha L I domain and its complex with ICAM-1 reveal a shape-shifting pathway for integrin regulation. Cell. 2003; 112:99–111. [PubMed: 12526797]

Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. Annu. Rev. Immunol. 1996; 14:1–27. [PubMed: 8717505]

Sloan-Lancaster J, Evavold BD, Allen PM. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. Nature. 1993; 363:156–159. [PubMed: 8483498]

Springer TA. Adhesion receptors of the immune system. Nature. 1990; 346:425–434. [PubMed: 1974032]

Stenberg E, Persson B, Roos H, Urbaniczky C. Quantitative-Determination of Surface Concentration of Protein with Surface-Plasmon Resonance Using Radiolabeled Proteins. Journal of Colloid and Interface Science. 1991; 143:513–526.

Stone JD, Stern LJ. CD8 T cells, like CD4 T cells, are triggered by multivalent engagement of TCRs by MHC-peptide ligands but not by monovalent engagement. J. Immunol. 2006; 176:1498–1505. [PubMed: 16424178]

Sykulev Y, Joo M, Vturina I, Tsomides TJ, Eisen HN. Evidence that a single peptide-MHC complex on a target cell can elicit a cytolytic T cell response. Immunity. 1996; 4:565–571. [PubMed: 8673703]

Turner JM, Brodsky MH, Irving BA, Levin SD, Perlmutter RM, Littman DR. Interaction of the unique N-terminal region of tyrosine kinase p56lck with cytoplasmic domains of CD4 and CD8 is mediated by cysteine motifs. Cell. 1990; 60:755–765. [PubMed: 2107025]

Valitutti S, Muller S, Cella M, Padovan E, Lanzavecchia A. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. Nature. 1995; 375:148–151. [PubMed: 7753171]

van der Merwe PA, Dushek O. Mechanisms for T cell receptor triggering. Nat Rev Immunol. 2010; 11:47–55. [PubMed: 21127503]

Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. Cell. 1994; 76:263–274. [PubMed: 8293463]

Wyer JR, Willcox BE, Gao GF, Gerth UC, Davis SJ, Bell JI, van der Merwe PA, Jakobsen BK. T cell receptor and coreceptor CD8 alphaalpha bind peptide-MHC independently and with distinct kinetics. Immunity. 1999; 10:219–225. [PubMed: 10072074]

Xiong Y, Kern P, Chang H, Reinherz E. T Cell Receptor Binding to a pMHCII Ligand Is Kinetically Distinct from and Independent of CD4. J Biol Chem. 2001; 276:5659–5667. [PubMed: 11106664]

Xu H, Littman DR. A kinase-independent function of Lck in potentiating antigen-specific T cell activation. Cell. 1993; 74:633–643. [PubMed: 8358792]

Yachi PP, Ampudia J, Gascoigne NR, Zal T. Nonstimulatory peptides contribute to antigen-induced CD8-T cell receptor interaction at the immunological synapse. Nat. Immunol. 2005; 6:785–792. [PubMed: 15980863]

Yokosuka T, Sakata-Sogawa K, Kobayashi W, Hiroshima M, Hashimoto-Tane A, Tokunaga M, Dustin ML, Saito T. Newly generated T cell receptor microclusters initiate and sustain T cell activation by recruitment of Zap70 and SLP-76. Nat Immunol. 2005; 6:1253–1262. [PubMed: 16273097]

Zhang F, Marcus WD, Goyal NH, Selvaraj P, Springer TA, Zhu C. Two-dimensional kinetics regulation of alphaLbeta2-ICAM-1 interaction by conformational changes of the alphaL-inserted domain. J. Biol. Chem. 2005; 280:42207–42218. [PubMed: 16234238]

Highlights

- The TCR-pMHC interaction determines T cell fate and responsiveness.
- Single molecule assays measure the *in situ* binding kinetics of TCR-pMHC interactions.
- We propose a fast kinetics based TCR serial triggering model for T cell activation

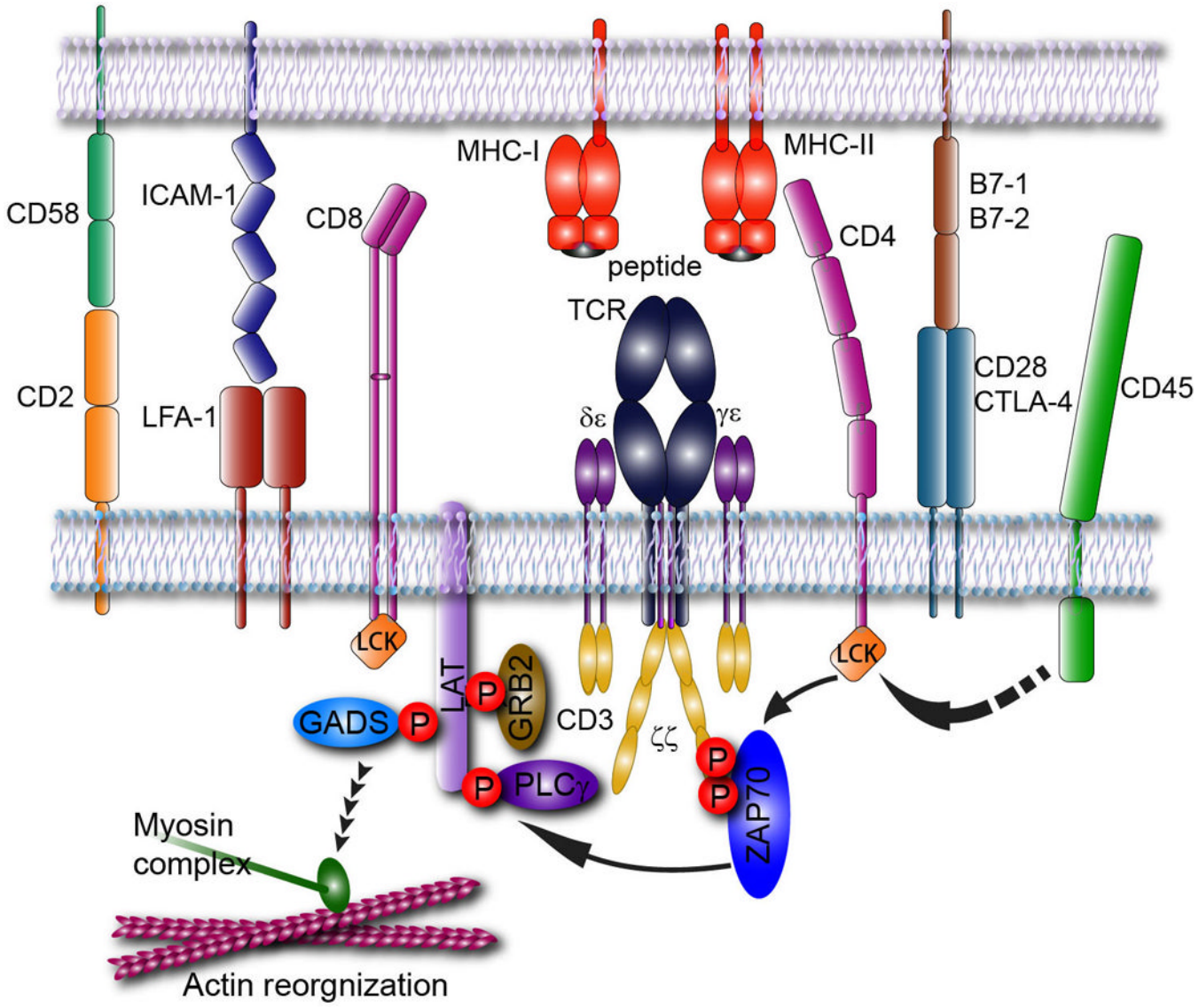

Figure 1. Important molecules on and underneath either the T cell or APC surface

TCRs recognize antigen pMHCs on the cell membrane with the help of co-receptors and accessory molecules. These molecules play critical roles in the T cell recognition and the function of each of which is discussed in the text. Also depicted are possible regulatory mechanisms of TCR-pMHC interaction by the T cell membrane and intracellular structures.

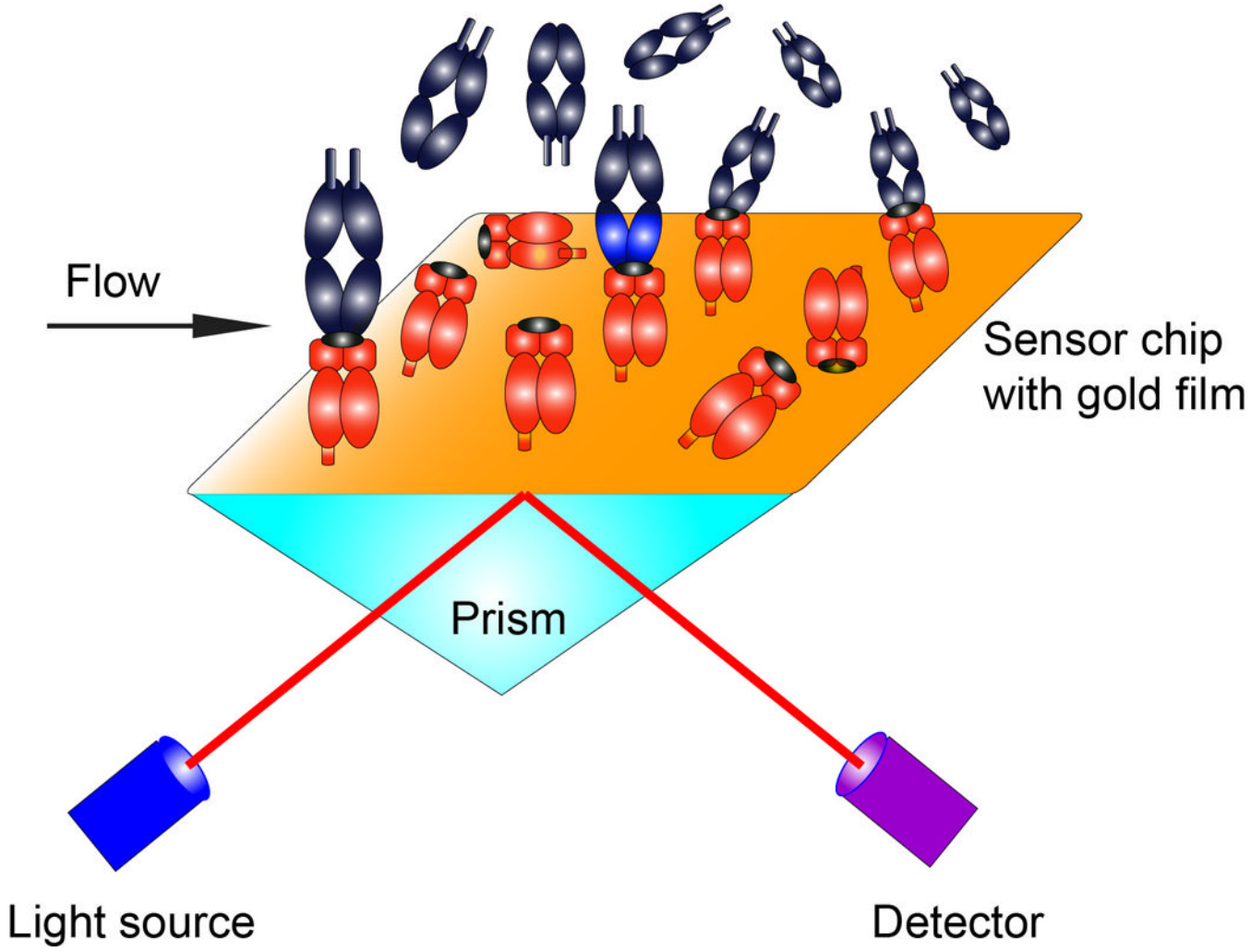

Figure 2. Schematic of SPR for measuring the *in vitro* 3D kinetics of TCR-pMHC interactions

Interaction between purified soluble TCRs in solution and immobilized pMHCs on a sensor chip is measured by SPR angle shifts when the mass of the surface layer changes.

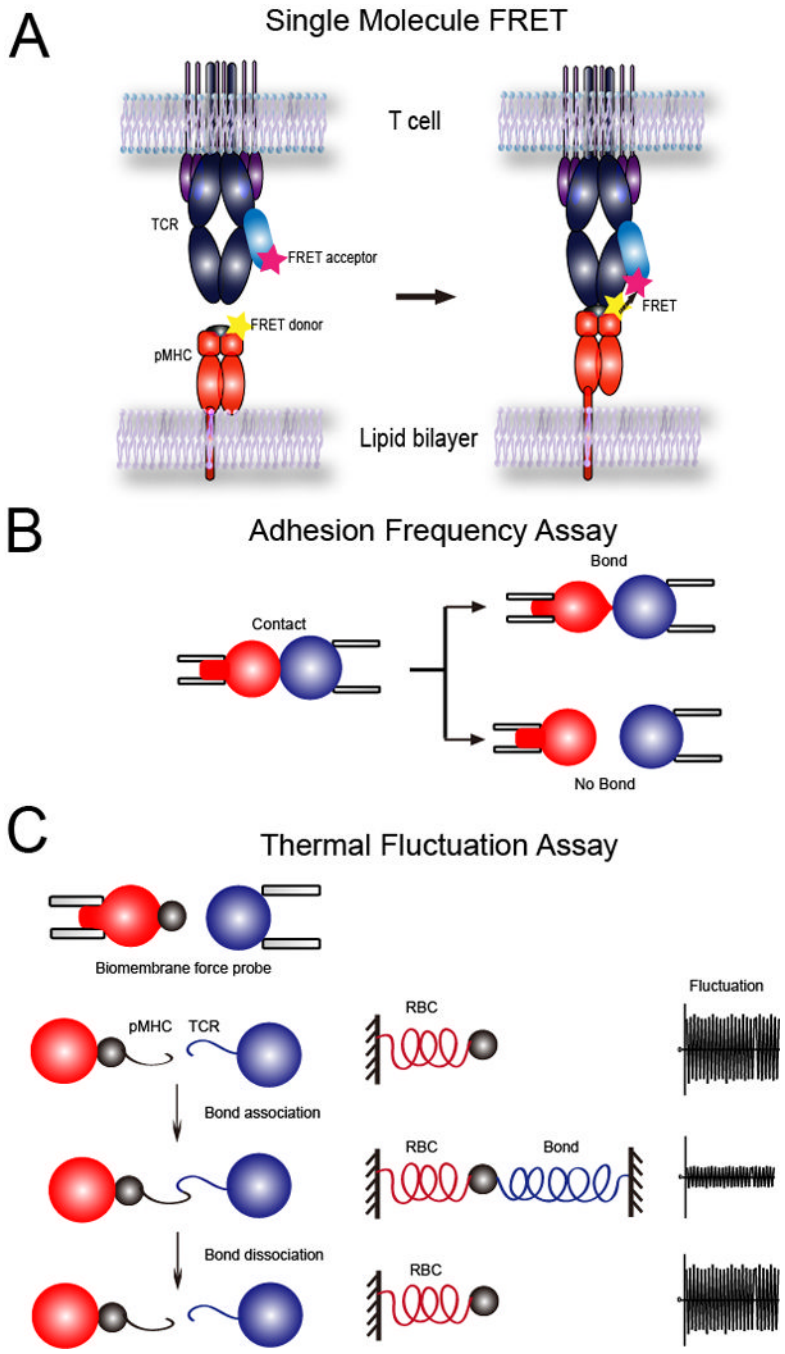

Figure 3. 2D methods for measuring *in situ* TCR-pMHC interactions

(A) Single-molecule FRET. A TCR on the T cell membrane is labeled with a single chain antibody fragment conjugated with a FRET acceptor, and the peptide on a pMHC anchored to the lipid bilayer is labeled with a FRET donor. The TCR-pMHC interaction brings the donor and acceptor into close distance to trigger FRET. (B) Adhesion frequency assay. A T cell (right) is brought in and out of contact with a pMHC coated RBC (left) to estimate an adhesion frequency using micropipette manipulation. The presence or absence of RBC deformation signifies whether a TCR-pMHC bond is present on T cell retraction. (C) Thermal fluctuation assay. A T cell (right) is brought into close proximity to a pMHC coated bead attached to a RBC (left). Single bond association and dissociation events are detected

by monitoring the thermal fluctuation amplitude of the bead that is either restrained by only the RBC or the combination of the RBC and the single TCR-pMHC bond.

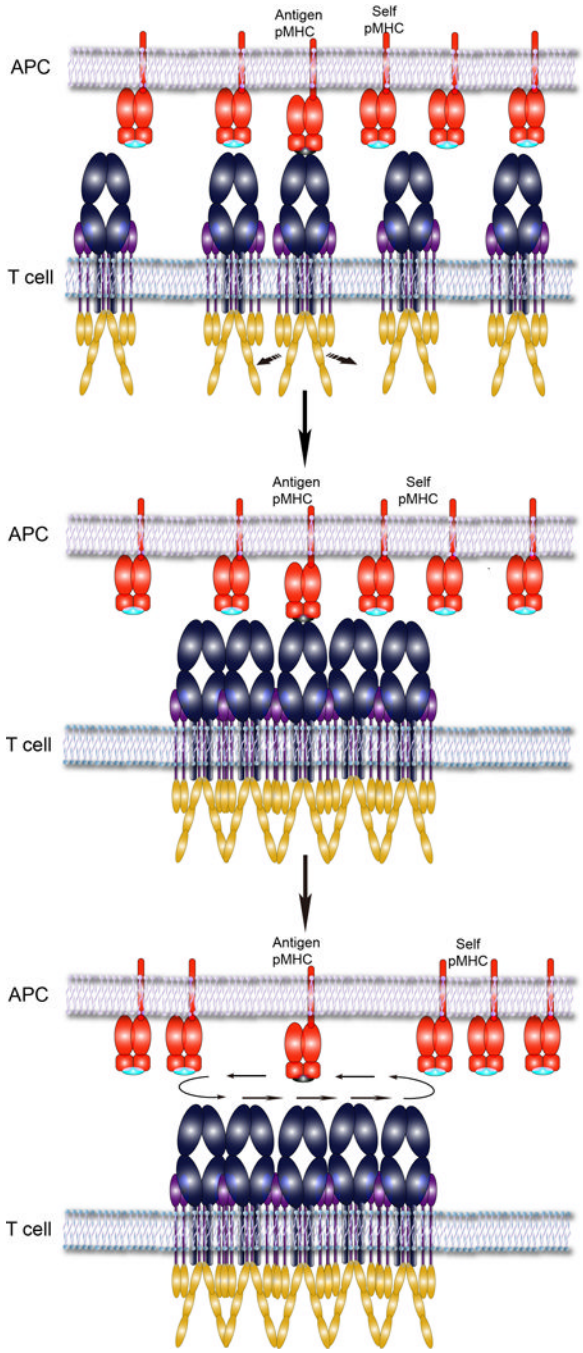

Figure 4. A fast kinetics based serial triggering model

TCR recognition of an antigenic pMHC leads to CD3 ITAM phosphorylation and signaling. The TCR ligation signal promotes the formation of TCR protein islands/clusters on the T cell membrane. The fast kinetics of TCR-pMHC interaction allows the TCRs in the cluster to serially engage with a small number of antigens on the APC surface and maximize T cell signaling and activation.