a highly specific pathogen-derived peptide binds to the T cell receptor (TCR). However, the concomitant signaling events are not specific to the TCR raising the question of how T cells recognize specific signals for activation. The organization of signaling proteins in time and space may establish hierarchies and, ultimately, control signaling outcomes that determine cell function in health and disease.

By manipulating lipid packing densities (Rentero et al PLoS One, 2008) and quantify membrane order microscopically (Gaus et al, J Cell Biol, 2005), our previous data revealed that membrane lipids and proteins co-operate to form stable membrane domains and protein clusters that are necessary for full T cell activation. To understand the underlying principles of the organization of signaling proteins in the T cell membrane, we established super-resolution microscopy approaches based on photo-activation localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM). These are a single molecule imaging technique that allows us to quantify the number of proteins participating in signaling clusters, the number of clusters and the ratio of proteins within clusters (Owen et al. J Biophotonics, 2010). In other words, we are able to quantify signaling efficiency and thus determine how lipids influence the organization and regulation of signaling process.

979-Plat
Competing Negative and Positive Feedback Generate Specific T Cell Responses by Tuning Duration and Amplitude of Itk Activation
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T cells, key orchestrators of adaptive immunity, sense pathogen-derived antigen through T cell receptors (TCRs). Developing T cells express TCRs of random antigen specificity that interact with self-peptides with a wide range of affinity. A strict selection process warrants generation of a functional, protective but self-tolerant T cell repertoire by removing T cell precursors failing to interact or stimulated strongly by self-peptides, and inducing survival and maturation for low-affinity/mild TCR signals. How different TCR signals can have such vastly different outcomes is ill understood. Among crucial TCR effectors, the oligomeric enzyme Interleukin-2 inducible T cell kinase (Itk) controls early (min scale) TCR signaling. Transient Itk activation is controlled by a positive feedback feeding into a negative feedback. Both are mediated by the soluble small messenger molecule inositol(1,3,4,5)tetrakisphosphate (IP4) generated via signal-dependent metabolism of membrane lipids (Huang et al, Science 2007). We combine computational modeling and biochemical experiments to elucidate the role of antigen affinity and Itk oligomerization in regulating duration and amplitude of Itk and T cell activation. Our results suggest that high affinity peptides cause strong but short-lived Itk activation necessary to induce downstream Ras and MAPK activation. Low affinity antigens cause prolonged Itk activation with smaller amplitudes. This is activation necessary to induce downstream Ras and MAPK activation. Low affinity antigens cause prolonged Itk activation with smaller amplitudes. This is sufficient to activate Erk, an essential mediator for survival in developing T cells. Our findings also suggest that certain modes of Itk oligomerization can inhibit signaling by low-affinity peptides. Regulation of transitive Itk activation by IP4 may point to a novel mechanism used by different cell signaling networks to generate specific functional decisions. In developing T cells, it may contribute to an enigmatic TCR signal splitter that determines whether TCR engagement causes death or survival and maturation.

980-Plat
High-Throughput Measurement of GPCR Stability At Femtomole Scale
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The inherent instability of heptahedral G protein-coupled receptors (GPCRs) during purification and reconstitution is a primary impediment to biophysical studies and to obtaining high-resolution crystal structures. New approaches to stabilize receptors during purification and to screen reconstitution procedures are needed. Here we report the development of a novel homogeneous time-resolved fluorescence assay (HTRF) to quantify properly folded CC-chemokine receptor 5 (CCR5). The assay permits high-throughput thermal stability measurements of femtomole quantities of CCR5 in detergent and in engineered nanoscale apoprotein bound bilayer (NABB) particles. We show that recombinant expressed CCR5 can be incorporated into NABB particles in high yield, resulting in greater thermal stability compared with CCR5 in detergent solution. We also demonstrate that CCR5 binding to the HIV-1 cellular entry inhibitors maraviroc, AD101, CMPD 167, and vicriviroc dramatically increases receptor stability. The HTFR assay technology reported here is applicable to other membrane proteins and could greatly facilitate structural studies of GPCRs.