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Visualizing the Molecular Dynamics of Adaptive and Innate Immune Signaling at the Cell Surface

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One of the great challenges in biology is to understand the emergence of spatial and temporal order in cellular processes. This challenge is particularly relevant to cell signaling, where the localized assembly and activation of protein complexes is used to control everything from cell fate to hormone release. While several decades of research have yielded many of the players in signaling pathways, most of the fundamental, conceptual questions of how signaling works at a molecular level remain mysterious. Immune cell activation represents an ideal system for understanding the spatial and temporal dynamics of cell signaling since the immune system faces an extreme version of many of the problems that all signaling systems must face: how to discriminate between closely related signals and activate the appropriate pathway even when the amount of signal is vanishingly small. In this paper, we have used high-resolution Total Internal Reflection Fluorescent (TIRF) imaging combined with functionalized supported lipid bilayers and engineered receptors and cell lines to visualize the molecular dynamics of immune signaling pathways. We will give details of our experiments that have revealed how the T cell receptor and IL1 receptor signaling networks activate an immune response.

A fundamental question in adaptive immunity is T cell antigen discrimination: How do T cells measure ligand affinity and respond to high-affinity ligands but ignore ligands of slightly lower affinity? This property allows T cells to activate towards pathogenic antigens while ignoring antigens derived from self. When this mechanism breaks down it can lead to self-reactive T cells and autoimmune disorders. A biochemical mechanism for this phenomena is lacking due to the inability to engineer the physical and kinetic properties of the TCR:peptide-MHC interaction. We overcame this hurdle by taking a synthetic biology approach and built a T cell receptor where the extracellular domains of the TCR and pMHC ligand were replaced by hybridizing strands of DNA (DNA – Chimeric Antigen Receptor (CAR), **Fig 1**) [1]. By varying the GC content and length of the complementary receptor-ligand oligonucleotides, we can precisely control and calibrate the binding energy of the receptor-ligand bond (**Fig. 1**). We then functionalized supported lipid bilayer with DNA-ligand and used two-color single molecule-imaging to directly visualize how T cell receptor ligand binding was converted to receptor activation. Receptor activation was imaged by visualizing the recruitment of ZAP70, a cytosolic kinase that binds to the phosphorylated T cell receptor, fused to a green fluorescent protein. These experiments reveal that receptor clustering is critical for receptor triggering, and that receptor clusters assemble more frequently with higher affinity ligands. This work uncovered the essential role of spatial organization in the mechanism of TCR ligand discrimination.

In the second part of this presentation, I will detail a system we have developed to image the spatial organization and protein dynamics of innate immune signaling. While the adaptive immune response is comparatively well characterized, the protein dynamics of innate immune signaling are poorly understood. The innate immune system is a network of cells and signaling pathways that are the first line of defense against infection. While genetics has revealed the molecular players of innate immune signaling networks, how these signaling systems self-organize to catalyze a powerful all-or-nothing immune response remains

a mystery. We have recently developed a system for understanding the spatial and temporal dynamics of IL1 receptor signaling that activates inflammation, an innate immune response. IL1 receptor activation recruits MyD88 and catalyzes the assembly of an intricate higher order protein complex referred to as the 'myddosome'. However, this dynamic process remains mechanistically unclear. To visualize the molecular dynamics of IL1 signaling, we have purified recombinant IL1beta with a poly-histidine tag to tether it to a supported lipid bilayer (**Fig. 2A**). We have labeled this recombinant IL1beta with mScarlet (a red fluorescent protein variant) or Halo tag (a self-labeling enzyme). These bright fluorescent probes allow IL1beta binding to IL1 receptor to be imaged at single molecule resolution. When EL4 cells are plated onto this functionalized surface, we find that IL1beta accumulates into clusters at the cell-bilayer interface. Using EL4 cells that have been engineered with CRISPR/Cas9 to express MyD88-GFP, we have observed the recruitment of MyD88 to the cell surface (**Fig. 2B**). We are currently using this system to understand the spatial and temporal organization of the IL1 receptor signaling pathway.

References:

[1] MJ Taylor et al., Cell **169** (2017), p. 108.

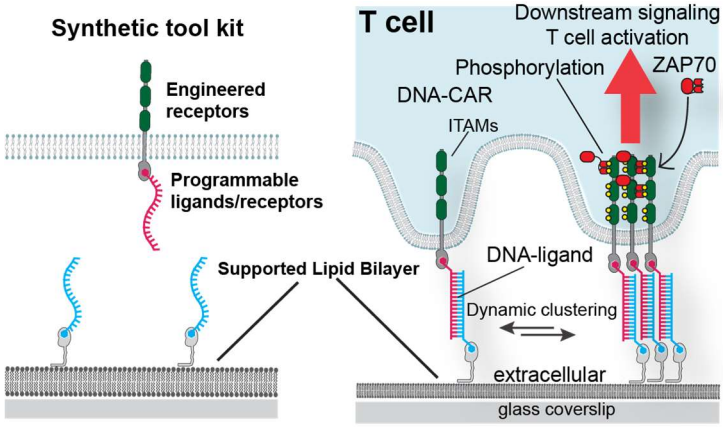

Figure 1. A schematic of the synthetic tool kit we have developed to interrogate how T cell signaling convert extracellular chemical signals into intracellular biochemical reactions.

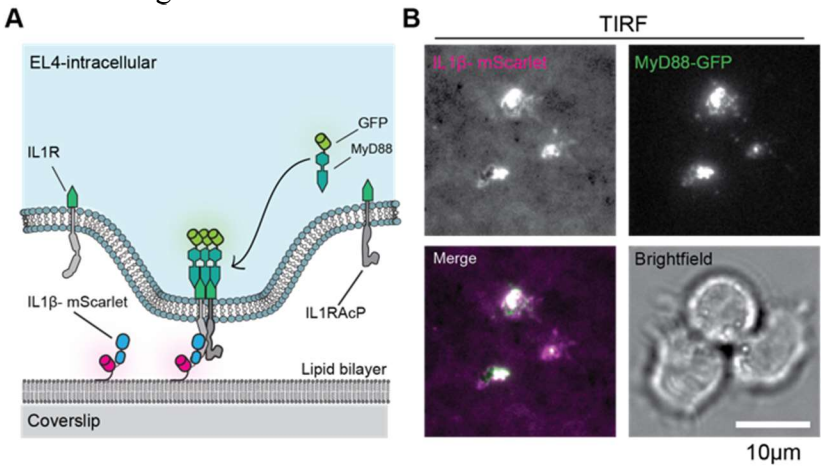

Figure 2. A. Schematic of a support lipid bilayer system to image the dynamics of IL1 receptor signaling at the cell surface. B. TIRF images showing EL4 cells plated on to IL1beta functionalized supported lipid bilayers.

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