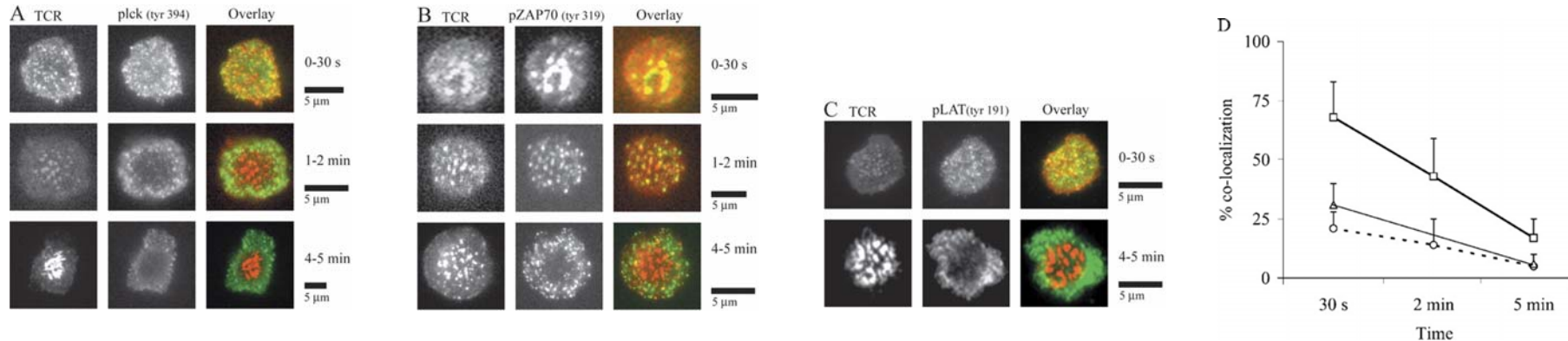




## Quantification of T cell receptors colocalizing with activated kinases



Dr Michael Dustin from the Program in Molecular Pathogenesis at the Skirball Institute of Biomolecular Medicine in New York studies T cell activation which plays a key role in Immune response to pathogens.

Antigen recognition induces T cell activation where molecular rearrangements lead to the formation of an organized immunologic synapse, characterized by a central cluster of T cell receptors (TCRs) surrounded by a ring of adhesion molecules and smaller TCR microclusters. It is known that TCR signalling recruits tyrosine kinases such as Lck and ZAP-70 and an adaptor molecule called LAT, but the kinetics of signalling and the role of TCR microcluster formation in signaling is unknown.

In order to find out more about microcluster formation, Dr Dustin and his colleagues studied the relationship of three specific proteins Lck, ZAP-70 and LAT, to TCR microclusters during immunologic synapse formation. Mouse T cells were incubated with a supported planer bilayers with adhesion molecules ICAM-1 and activating MHC-peptide complexes for 30 s, 2 min or 5 min and then fixed, permeabilized and stained with specific antibodies to phosphorylated forms of the proteins Lck, ZAP and LAT. Using wide field fluorescence microscopy to image the cells, Dr Dustin and colleagues analyzed colocalization of TCR microclusters and phosphoproteins using **Volocity Classification**.

Each image here shows the overlay of TCRs (red) and phosphoproteins Lck, ZAP or LAT (green) at 30 s, 2 min and 5 min after exposure of T cells to the activating surface. Images were imported into **Volocity** and the amount of colocalization of TCRs with phosphoproteins was measured. Dr Dustin used 2D images for his experiments, but Volocity allows colocalization to be analysed in 3D when using volumes of images. **Volocity Classification** will calculate measurement statistics including Colocalization coefficients and Pearson's correlation. Alternatively, a classifier can be used to identify objects then measure colocalization coefficients, which is the method chosen by Dr Dustin. **Volocity Classification** offers many charting options from bars and pies to interactive 3D charts. Here, the graph clearly reveals that at 30 s after antigen exposure, the degree of colocalization of phosphoproteins with TCR clusters is high but then decreases over time.

This result made a valuable contribution to demonstrating that early after antigen exposure TCR microclusters colocalize with activated forms of kinases Lck and ZAP-70 and the adapter protein LAT. Colocalization decreases over time as kinase activity is lost in the older central TCR cluster and sustained in younger peripheral TCR microclusters.

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