PHOSPHORYLATION-BASED SIGNALING NETWORKS MEDIATE THE EFFECT OF LIGAND AFFINITY ON THE ACTIVATION OF NAIVE PRIMARY T-CELLS

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Systems biology studies the structure and dynamics of biological systems with the goal to generate dynamic models that can predict their complex behaviors. In this sense, quantitative analysis of protein phosphorylation dynamics by high-resolution MS-based phosphoproteomics is emerging as one of the essential methodology for understanding cellular signaling networks at a global scale.

In this work, we presented quantitative time-resolved phosphoproteomics data used to construct phosphorylation-based signaling networks for the study of TCR-activation in naive primary T-cells. How the TCR distinguishes between antigens of different quality is a mechanism that has fascinated the immunologist community for many years. Several studies have provided evidence that the affinity between the TCR and the peptide-MHC complexes plays an essential role for controlling T-cell responses upon ligand binding. Dysregulations in this mechanism could potentially cause autoimmune diseases.

To study how TCR affinity for antigens impacts T-cell activation, we chose a model in which naive primary $CD8^+$ T-cells purified from TCR transgenic OT-1 mice, were stimulated at different time points with low and high-affinity ligands (Q4H7 and Q4R7 tetramers). After trypsin digestion and purification by TiO_2 columns, phosphopeptides were fragmented by CID on a LTQ-Orbitrap. More than 1450 unique phosphopeptides were identified by Sequest (FDR<1%). Comparison with the Phospho.ELM database revealed >350 new phosphorylation sites. Changes in phosphopeptide abundance following stimulation were analyzed by a label-free method using the ion intensities from extracted ion chromatograms, which revealed differential responses upon low and high affinity ligand binding.

In summary, our study for the first time allows insights into the differential global phosphoproteome dynamics in naive primary T-cells following stimulation with high and low affinity ligand. The integration of this data with information on predicted upstream kinases and protein interaction information provides a new framework for the modeling of T-cell activation in response to different ligand affinities.