ACTIVATION OF T CELLS ON MICROPRINTED COVER GLASS SURFACES

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Recent advances in microscopy have made an enormous impact on cellular biology research in general and the study of the immune system in particular. T cells are crucial players in an immune response against infections and tumours. When a T cell encounters an antigen presenting cell (APC), they form a specialized cell-cell interface known as the immunological synapse. The engaged T cell receptor (TCR) then undergoes a series of phosphorylation events, known as the immediate TCR signalling pathway. Recently, it has been shown that subsynaptic vesicles carry signalling molecules that interact directly with the TCR [1].

There is still much debate on the importance of membrane versus vesicle bound signalling proteins in driving T cell activation and downstream TCR signalling, which is why our study focuses on vesicle movement during T cell activation. By using micro-contact printing, we can create cover glasses with patterned fluorescent activating dots to mimic interactions with an APC. The dots in the pattern are smaller than an average T cell, enabling us to do a comparison of vesicle dynamics in activated and non-activated areas within the same cell. By imaging cells as soon as they land on the surface and become engaged by the APC-mimicking dot, we can use the fast image acquisition ability of total internal reflection fluorescence (TIRF) microscopy to capture early T cell triggering events, which are rapid and potentially short-lived. We are able to monitor the movement of vesicles as they interact with key TCR signalling molecules such as Lck, TCRζ, LAT and membrane scaffolding molecules such as GADS and SLP76. With this approach, we aim to show that vesicle bound signalling and scaffolding molecules have different interactions with the APC during T cell triggering and during T cell activation.