DNA ORIGAMI AS A NANOSCALE PLATFORM FOR T-CELL ACTIVATION

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INTRODUCTION

Every cell of our body is surrounded by a plasma membrane that separates the inside of a cell from the outside. This is not just a static barrier but the stage of an intricate interplay of invisibly small structures, proteins and even smaller lipids, that move and assemble themselves in complex ways to mediate membrane function. A special type of cells, T-cells, are major players in our immune system. One protein in their plasma membrane, the T-cell receptor (TCR), recognizes a small part of a pathogen (the antigen), which is presented by the protein mayjor histocompatibility complex (MHC) on an antigen presenting cell (APC). Upon this recognition process, the T-cell becomes activated and a series of events is initiated ultimately leading to an immune response. Deregulation of this process can lead to inadequate immune responses such as autoimmunity or immunodeficiency.

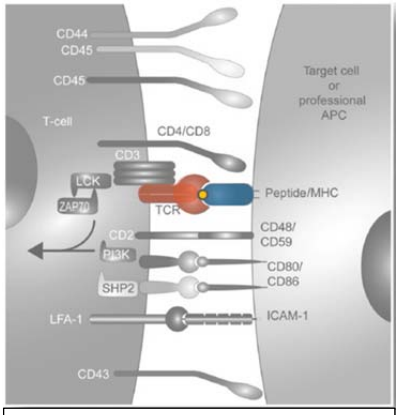


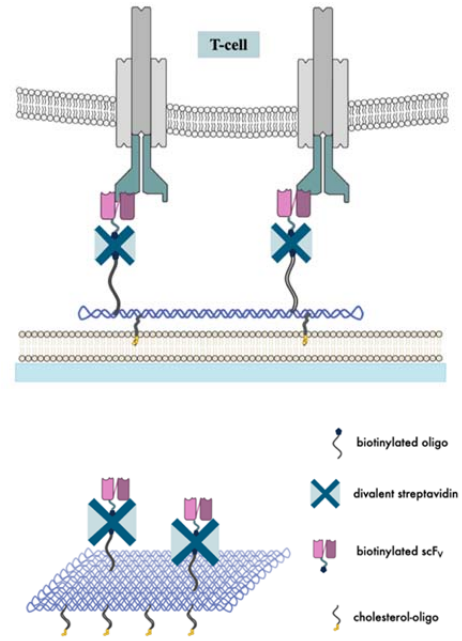
Figure 1: *Schematic overview of an immunological synapse formed by a T-cell and an APC [Huppa et al., 2004].*

T cells recognize peptide-antigens presented by MHCs (pMHCs) with remarkable specificity and sensitivity. While the molecular players in the activation cascade, as well as the sequence of events, are rather well characterized, we still do not understand how and where the decision for activation is made. It is thought that the nanoscale spatial distribution of ligand and TCR plays an essential role in the initiation of an immune response but events at this time and length scale are intrinsically hard to study in a living system.

A great asset of being part of the TU DK "Biointerface" is working with colleagues from various natural scientific fields and thus being able to combine all the expertise and benefits to view a task from every angle and perspective.

EXPERIMENTS / FUNDAMENTAL OF THE PROBLEM / EXAMINATIONS

Several studies have already tried to assess the importance of the nanoscale distribution of TCR ligands by mimicking the surface of an APC with nanostructured surfaces. So far, only static systems that do not allow for rearrangement (e.g. accumulation, segregation) of molecules within the immunological synapse during T cell activation were extensively used. Since the physiological scenario is not static at all, we were aiming for a novel nanostructured surface, which does not only allow positioning of single ligands with nanoscale precision but also permits reorganization of molecules during T-cell activation. Therefore, we use DNA origami decorated with TCR ligands anchored to a planar glass-supported lipid bilayer containing adhesion (ICAM-1) and costimulatory



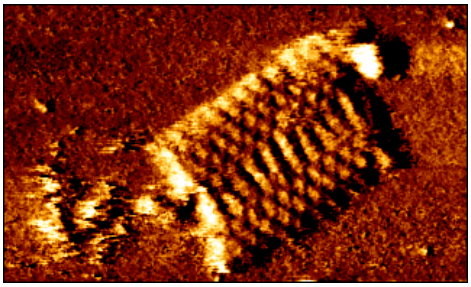


Figure 2: *Schematic overview of a T-cell interfaced with a DNA origami bilayer platform decorated with an antibody scF$_V$*

(B7-1) proteins to assess the effects of nanoscale ligand arrangement on T-cell activation. In order to achieve that, we used a sandwich structure consisting of biotinylated oligos, divalent streptavidin and biotinylated ligand labelled with a fluorophore. Intriguingly, T-cells cannot only be activated by binding to a cognate ligand (pMHC) but also by binding to antibodies. Thus, we used an antibody single chain fragment (scF$_V$) to the TCR and assessed its spatial requirements for T-cell activation. Additionally, our DNA origami platform creates an exclusion zone that prohibits the approach of ligands below a minimum distance set by the platform size. In order to observe potential differences in the T-cell response DNA origami platforms of different size and ligand occupancy were created. Actual ligand occupancies of DNA origami platforms and overall densities were determined by relating the platform brightness to the brightness of a single fluorophore.

We used intracellular calcium levels as an indicator for T cell activation. Thus, we labeled the T cells with a calcium sensitive dye to see differences in the release of calcium. Besides that, phosphorylation of the ζ-chain of the TCR, the earliest indicator of T cell activation, and subsequent recruitment of the kinase ZAP70 was determined, to be able to understand the molecular events in early t cell activation in more detail. To quantify the data, we used a positive control containing B7-1, ICAM and peptide MHC and a negative control which contained solely B7-1 and ICAM.

RESULTS AND DISCUSSION

We found a pronounced effect of ligand spacing on its potency to activate T cells. At a ligand spacing of 50nm, T cell activation was almost completely abolished. This is in line with a recent study, that varied anti-TCR antibody fragment spacings in a slightly larger size range (distances 40 – 120 nm) found an optimum spacing at 40 nm (Cai et al., 2018), albeit at much higher overall densities of immobilized Fab. In our experiments, a separation of 10 nm between ligands was 9-fold more efficient than 30 nm. Interestingly, a distance of 10nm corresponds to the spacing of two epitope-binding sites in a divalently bound antibody, a potent activator of T cell signaling... Taken together, our results suggest that single phosphorylated TCRs at distances of more than 30 nm are not signalling-competent. For efficient T cell activation, ligand and TCR need to be freely rearrangeable to from small clusters or ligand needs to be pre-arranged in dimers with a spacing of ~10 nm.

CONCLUSION

We find a pronounced influence of nanoscale ligand spacing on T cell activation. This not only sheds new light on the mechanisms of early T cell signalling but also will be important in the design of nanostructured surfaces that can be used to activate T cells in T cell immunotherapy.