# Title: T cell adhesion on engineered substrates: influence of ligand nano-clustering


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## Abstract

The interface between an Antigen Presenting Cell (APC) and a T-lymphocyte (T cell), sometimes called a synapse, plays a key role in sensitivity and precision of antigen recognition by T-cells. The importance of clustering of T cell receptors (TCR) is well established. In addition, it has recently been shown that the antigens, in the form of peptide Major Histocompatibility Complex (pMHC) recognized by TCR, are present on the membrane of APCs as defined submicronic clusters. The aim of this work is to study how such clustering of ligands influences T-cell membrane and actin organization.

To achieve this, we developed a novel hybrid system, where a synthetic substrate mimics the APC-membrane. The substrate consists of an array of sub-micrometric protein dots (diameter: 800±100 nm; spacing: 2μm), surrounded by a fluid supported lipid bilayer (SLB), which is optionally functionalized. The dots and the SLB are alternatively functionalized with molecules of anti-CD3 (targeting the TCR-complex), or ICAM-1 (ligand for the T-cell integrin LFA-1).

In T cells adhered to these substrates, local organization of TCR, the kinase ZAP-70 (one of the first molecules to be recruited to the TCR complex on activation), the actin distribution and membrane topography (measured using Reflection Interface Contrast Microscopy - RICM and Total Internal Reflection Fluorescence Microscopy - TIRFM) are impacted by ligand clustering. Colocalization of microclusters of both TCR and ZAP-70 with anti-CD3 dots is seen. The presence of ICAM-1 on the SLB does not appreciably perturb this organization, whereas B7 may have a slight impact. On ICAM-1 dots the TCR is not organized in micro-clusters.

The membrane of the adhering T cells exhibits a characteristic topography, when adhesive ligands (either ICAM-1 or anti-CD3) are present only in the dots but not on the SLB. Interestingly, the presence of an artificial polymer like polyethylene glycol on the SLB enhances the membrane topographical patterning, pointing to the repulsive role of membrane polymers in defining the topography of the synaptic interface.

Global parameters like cell area depend on the nature (ICAM-1 or anti-CD3) of the ligands present. On substrates with anti-CD3 (no ICAM), the cell area is the same on clustered and homogeneously distributed ligands as long as the average ligand density remains the same. However, in presence of ligands of LFA1, the global parameters are influenced by clustering or not of the TCR ligands. Specifically, the cell area is significantly increased when the same amount of anti-CD3 is clustered, rather than homogeneous.

Classically, the actin distribution in T cells adhering to a SLB functionalized with both ICAM-1 and anti-CD3, is in the form of a peripheral ring. However, on substrates patterned with dots of either ICAM-1 or anti-CD3 (no SLB functionalization), the actin is in form of colocalized dots. When in addition, the SLB is functionalized with the complementary ligand, the actin distribution becomes either homogeneous or peripheral. Dynamic imaging hints that TCR organizes the actin at early time and LFA-1 at late time, thus pointing to the crucial but different role of both in adhesion of T cells.