A versatile single-molecule nanoarray platform for T-cell activation

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In the immune recognition process, in which a T-cell engages with an antigen presenting cell (APC), an elaborate collage of adhesion, costimulatory, and signaling molecules is formed into a stereotypic geometric structure known as the immunological synapse (IS). Key roles in the IS are played by the T-cell receptor (TCR) and leukocyte function associated antigen-1 (LFA-1) on the T-cell side, and antigenic peptide bound to major histocompatibility complex (pMHC) and intercellular adhesion molecule-1 (ICAM-1) on the APC. The specific geometric structure of the IS has inspired major research efforts, as understanding the role of geometric organization at the molecular level that underlies the formation of the IS can lead to potential applications in immunotherapy. Recent studies using arrays of immobilized TCR agonists (pMHC and anti-CD3 antibodies) formed by micellar diblock copolymer lithography identified a threshold for TCR stimulation in the range of 90-140 molecules per μm^2[1] or a spacing of 69nm.[2] This is in contrast to stimulation by an APC in solution, which requires only a single agonist and only ten agonists to initiate IS formation.[3]

In order to clearly identify the respective roles of the key IS components in the immune recognition process, we have developed a versatileÍ engineered platform that allows us to control the precise arrangement of TCR binding sites in terms of density and stoichiometry, and to sort out the role of the costimulatory molecule, ICAM-1. Arrays of TCR binding site, comprising AuPd nanodots patterned by electron beam or nanoimprint lithography and self-aligned pattern transfer were functionalized with a biotinylated UCHT1 Fab antibody (which binds to a single TCR) via a streptavidin linker (Fig. 1a). The surrounding surface was passivated by a PEG-silane brush to prevent non-specific binding. On extended hexagonal arrays, the average intensity of phosphorylated tyrosine (pY), an indicator of immune response signaling strength, decreased with increasing spacing to a threshold at a spacing ~100-120 nm, corresponding to a density of ~80-115/μm^2, similar to previously reported observations.[1,2] T-cells plated on heptamer arrays with the same inter-dot spacing but a constant low global density of 50/μm^2, displayed a low stimulatory response. This suggests that, in the absence of ICAM-1, which binds to LFA-1 and enhances cell adhesion, a greater number of TCR agonists are needed to play that role. His-tag ICAM-1 can be introduced by adding a nickel-NTA bridge to the PEG-silane brush (Fig. 2a).[4] This may provide sufficient adhesion to allow TCR stimulation at a lower threshold. However, there is evidence that T-cell triggering requires mechanical pulling on the TCR,[5] and thus it is desirable for the ICAM-1 to be mobile. This can be accomplished by replacing the PEG passivation with a fluid lipid bilayer to which ICAM-1 can be bound while remaining mobile (Fig. 2b). Figure 3a shows clusters of AuPd nanodots with diameters ~7nm. A supported lipid bilayer with 200/μm^2 ICAM-1 was formed by vesicle fusion from a solution of single unilamellar vesicles (SUVs) containing 12.5% Ni-DOPGS and 87.5 % DOPC. Fig. 3b shows a fluorescence image of Fab-568 on a 200x200μm^2 square of heptamer arrays with 200 nm inter-cluster spacing. When this spacing is as large as 1μm, individual clusters can be clearly resolved. Field-stop aperture fluorescence recovery after photobleaching (FRAP) of ICAM-1-405 in a region with embedded nanoarrays is shown in Fig. 3c, which confirms the membrane continuity and mobility. Comparison of cell assays among these platforms will provide insights into both T-cell activation threshold of TCR arrangement and the respective role of different molecules in this process.

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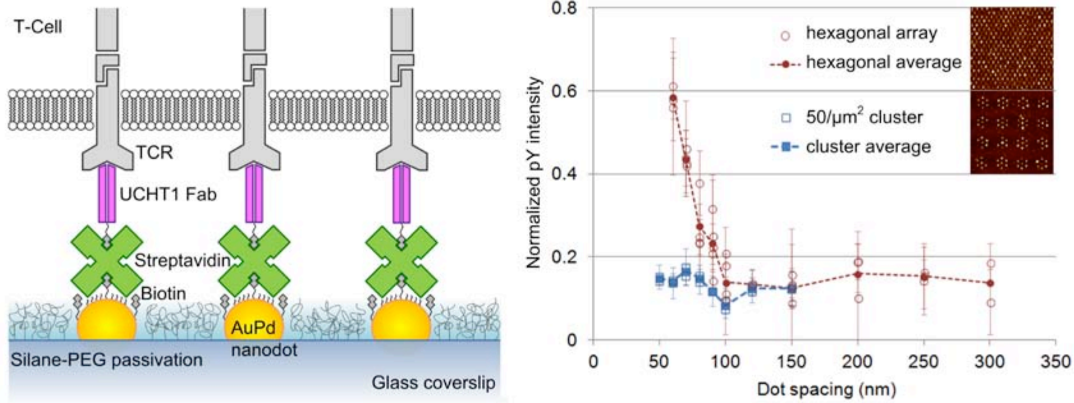

Figure 1. (a) Nanoarray with PEG passivation; (b) T-cell assays results: the dependence of pY intensity on dot spacing.

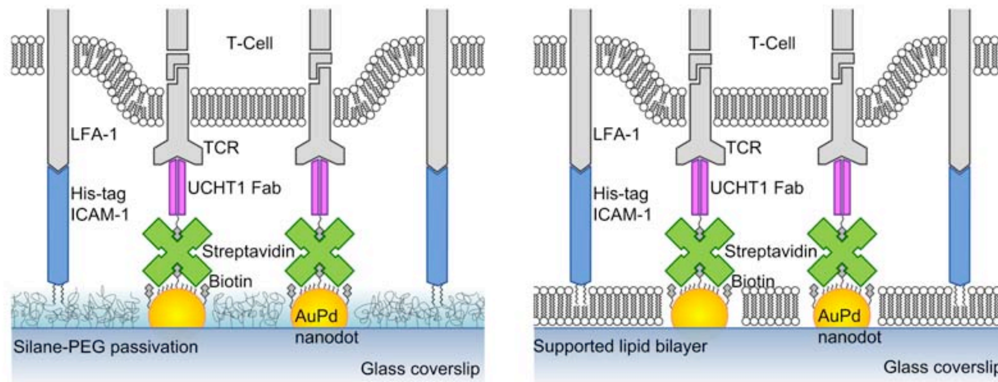

Figure 2. (a) Nanoarray with Silane-PEG-NTA passivation; (b) Nanoarray with fluid lipid bilayer.

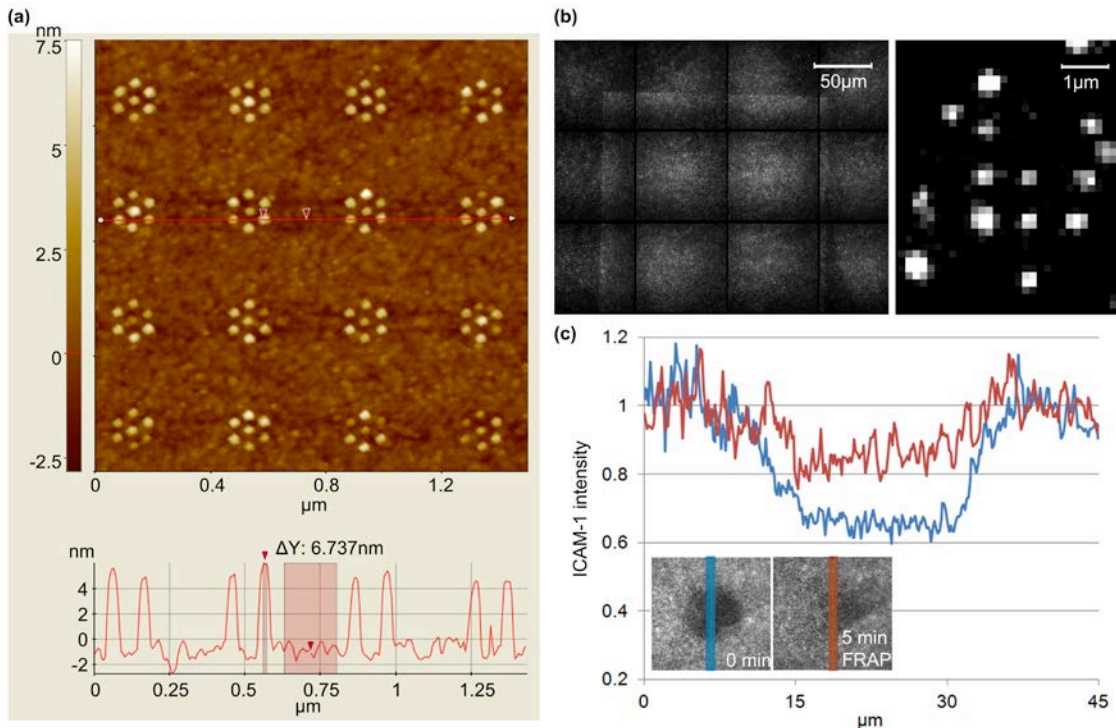

Figure 3. (a) AFM image of a heptamer array with 60nm inter-dot spacing, 200nm inter-cluster spacing, and ~7nm dot size;
(b) Fluorescence images of Fab-568 on heptamer arrays with 200nm and 1μm inter-cluster spacing;
(c) Fluorescence recovery of ICAM-1-405 in the lipid bilayer with embedded nanoarray.