Out-of-plane spatial control at single-molecule resolution on biomimetic surfaces

Haogang Cai[1], James Muller[2], David Depoil[3], Michael Sheetz[4], Michael Dustin[3] and Shalom J. Wind[5]
[1]Dept. of Mechanical Engineering, [4]Dept. of Biological Sciences,
[5]Dept. of Applied Physics and Applied Mathematics, Columbia University, New York
[2]Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York
[3]The Kennedy Institute of Rheumatology, University of Oxford, Oxford, UK

Metallic nanoarrays, fabricated by either conventional electron-beam lithography or self-assembled block-copolymer micelle nanolithography, have been widely used to create biomimetic surfaces to explore the geometric effect of key biomolecules involved in critical cellular functions. For example, nanoarrays functionalized with RGD (a ligand of integrin) have been used to mimic the extracellular matrix (ECM) for cell adhesion and spreading[1-4]. Nanoarrays functionalized with ligands of T cell receptor (TCR) form an artificial antigen presenting cell (APC) surface for immune recognition[5-8]. While these experiments have provided insights of geometric effect in biological processes where only a single type of biomolecule is crucial, more often, multiple molecules could be involved in complicated cellular functions. In the latter case, the relative positions of the multiple molecules, both in-plane and out-of-plane, would affect the cellular responses. For example, in the immune recognition, besides the TCR which triggers tyrosine phosphorylation (pY, an indicator of immune response signaling strength), other molecules also play important roles, such as CD45, a receptor-linked protein which constrains pY in resting cells, and the integrin leukocyte function associated antigen-1 (LFA-1) for adhesion. Due to the smaller size of TCR-ligand pair (~ 15 nm), the larger integrin-ligand pair (~ 40 nm), i.e. LFA-1 and intercellular adhesion molecule-1 (ICAM-1), and CD45 (~ 20 nm) are excluded by the diffusion barrier formed by TCR clustering. The molecule segregation or TCR mechanical pulling by membrane bending, have been suggested to be important in immune response[9, 10]. Therefore, the out-of-plane spatial control of these molecules could be used to adjust the membrane bending and molecule segregation, which is essential for further investigation of the mechanism.

In our previous work, a hybrid artificial APC surface was developed, with TCR binding ligands (anti-CD3 antibody, UCHT1 Fab) immobilized on AuPd nanodots and mobile ICAM-1 on the supported lipid bilayer (Fig. 1a). Now we introduce the out-of-plane spatial control to this platform by etching the glass substrate and forming nanopillar arrays. The glass pillar raises the TCR-ligand pair, which reduces the membrane bending and makes more room for the diffusion of LFA-1 and CD45 (Fig. 1b). This is more accurate and reliable than the conventional way using elongated molecule, which is usually not fully extended and flexible[9]. After dry etching with CHF_3, the nanodots profile measured by atomic force microscopy (AFM) shows an increment of ~ 10 nm (Fig. 2). 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. Field-stop aperture fluorescence recovery after photobleaching (FRAP) of ICAM-1-405 confirmed the bilayer mobility is similar when penetrated by either nanodot or nanopillar arrays. Fresh human T cells were plated and fixed after 5 min on both the nanodot and nanopillar surfaces. Then pY and CD45 were stained respectively (Fig. 3). The pY-488 is found to be colocalized with UCHT1 Fab-568 (not shown for clarity) immobilized on nanodot/pillar arrays (the inset of Fig. 3a shows the AFM image of a nanodot cluster). The intensity profiles of pY-488 and CD45-647 are opposite on nanodots, while no obvious trend is observed on nanopillars. This implies that the CD45 exclusion is weaker on nanopillars than nanodots, because the diffusion barrier by membrane bending is reduced. More data analysis is underway to fully understand the mechanism of immune recognition at single-molecule resolution, which can lead to potential applications in immunotherapy. This technique also serves as a general platform for cellular studies involving multiple biomolecules or membrane deformation.

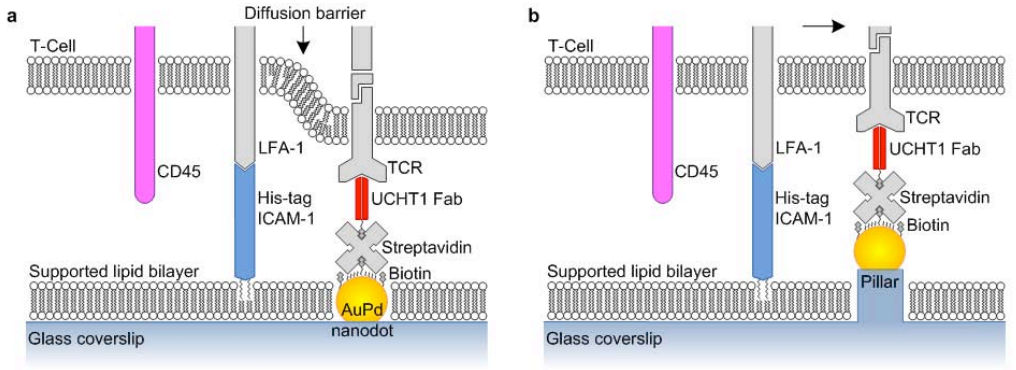

Figure 1. Schematic diagram of a live T cell on (a) nanodot arrays, (b) nanopillar arrays. The pillars introduce out-of-plane spatial control between TCR-ligand pair and integrin-ligand pairs, adjusting the membrane bending and molecule segregation.

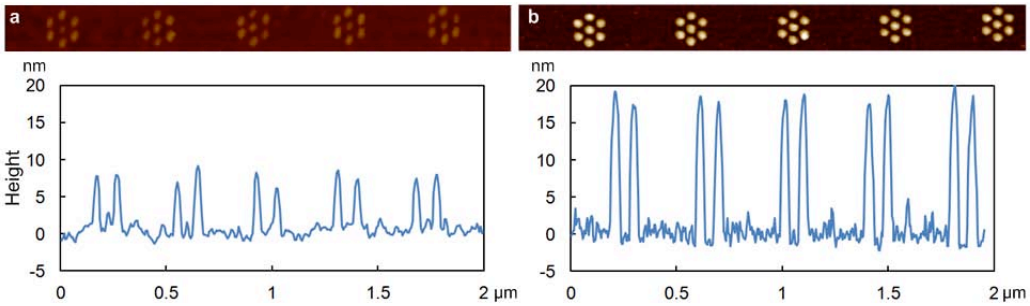

Figure 2. AFM image with height profile of (a) nanodot arrays, (b) nanopillar arrays. The dry etching of glass substrate has raised the nanodots by ~ 10nm.

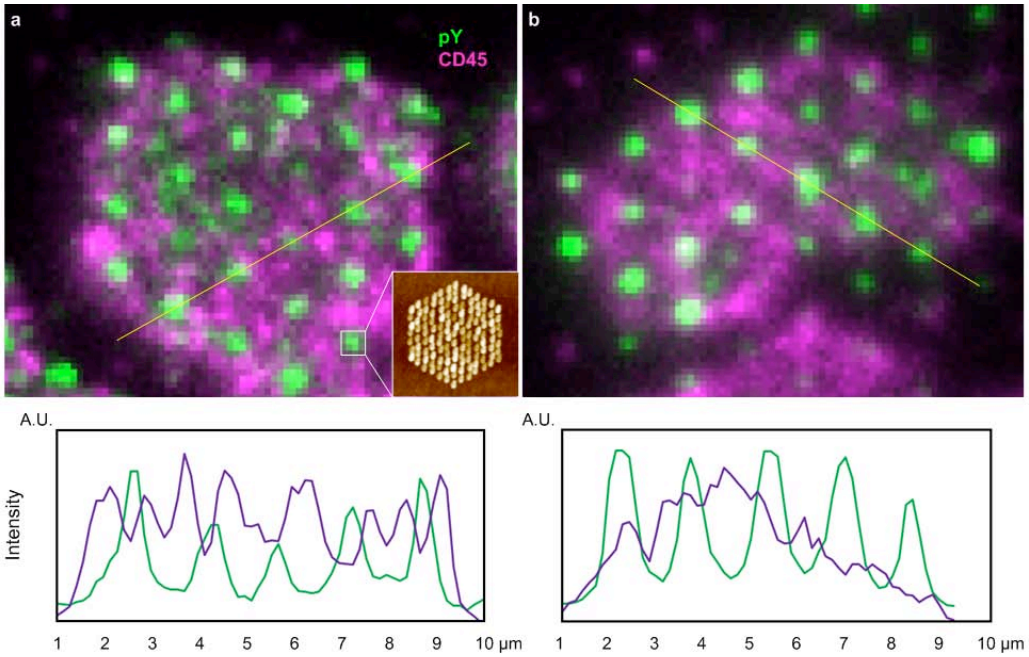

Figure 3. T cell fluorescence image with intensity profiles of pY and CD45: (a) nanodot arrays (the inset shows the AFM image of a nanodot cluster), (b) nanopillar arrays. The CD45 exclusion is weaker on nanopillars than nanodots.

1. M. Schvartzman, M. Palma, J. Sable, J. Abramson, X. Hu, M. P. Sheetz and S. J. Wind, Nano Lett **11** (3), 1306-1312 (2011).
2. M. Arnold, E. A. Cavalcanti-Adam, R. Glass, J. Blummel, W. Eck, M. Kantlehner, H. Kessler and J. P. Spatz, Chemphyschem **5** (3), 383-388 (2004).
3. E. A. Cavalcanti-Adam, T. Volberg, A. Micoulet, H. Kessler, B. Geiger and J. P. Spatz, Biophys. J. **92** (8), 2964-2974 (2007).
4. J. H. Huang, S. V. Grater, F. Corbellinl, S. Rinck, E. Bock, R. Kemkemer, H. Kessler, J. D. Ding and J. P. Spatz, Nano Lett. **9** (3), 1111-1116 (2009).
5. H. Cai, D. Depoil, M. Palma, M. P. Sheetz, M. L. Dustin and S. J. Wind, Journal of vacuum science and technology. B, Nanotechnology & microelectronics : materials, processing, measurement, & phenomena : JVST B **31** (6), 6F902 (2013).
6. J. Deeg, M. Axmann, J. Matic, A. Liapis, D. Depoil, J. Afrose, S. Curado, M. L. Dustin and J. P. Spatz, Nano Lett **13** (11), 5619-5626 (2013).
7. J. Matic, J. Deeg, A. Scheffold, I. Goldstein and J. P. Spatz, Nano Lett. **13** (11), 5090-5097 (2013).
8. D. Delcassian, D. Depoil, D. Rudnicka, M. L. Liu, D. M. Davis, M. L. Dustin and I. E. Dunlop, Nano Lett. **13** (11), 5608-5614 (2013).
9. K. Choudhuri, M. Parker, A. Milicic, D. K. Cole, M. K. Shaw, A. K. Sewell, G. Stewart-Jones, T. Dong, K. G. Gould and P. A. van der Merwe, J. Biol. Chem. **284** (38), 26096-26105 (2009).
10. J. M. Alakoskela, A. L. Koner, D. Rudnicka, K. Kohler, M. Howarth and D. M. Davis, Biophys. J. **100** (12), 2865-2874 (2011).