Molecular Scale Spatio-Chemical Control of the Activating-Inhibitory Signal Integration in NK Cells

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Innate immune system is based on natural killer (NK) cells – lymphocytes that distinguish between healthy and diseased cells, and attack tumor. The activity of NK cells regulate through a delicate balance between activating and inhibitory signals delivered by a multitude of activating and inhibitory receptors. The role of juxtaposition of activating and inhibitory receptors in signal inhibition of cytotoxic lymphocytes remains strongly debated. The challenge lies in the lack of tools that allow simultaneous spatial manipulation of signaling molecules. Recently, biomimetic devices that control spatial organization of receptors within the cell membrane have been extensively used to study how the receptor spatial order regulates cell function, including that of immune lymphocytes. Yet, these devices have been limited to control only receptor of one type, and thus could not be used to study signal integration between different receptors.

To circumvent this, we produced a nanoengineered multifunctional platform with molecular scale spatial control of ligands. This platform was fabricated by an "out-of-the-box" fabrication approach, in which were included nanoimprint lithography with double angle evaporation. This approach was allowed to produce bimetallic nanoarrays registered with nanoscale accuracy using only one lithographic step, and with no need for alignment between different nanodots. We first patterned a thermal nanoimprint resist on a Si substrate and transferred the imprinted pattern into a resist by angle deposition of a metallic protection mask, resist over-etch by plasma, two sequential metal evaporations, Au and Ti/Cr in, and liftoff . This platform was conceived by bimetallic nanodot patterning with molecular-scale registry, followed by a ternary functionalization with distinct moieties. We found that a 40-nm gap between activating and inhibitory ligands provided optimal inhibitory conditions. Supported by theoretical modelling, we interpret these findings because of the size mismatch and conformational flexibility of ligands in their spatial interaction. This highly versatile approach provides an important insight into the spatial mechanism of inhibitory immune checkpoints, which is essential for the rational design of future immunotherapies.

Reference:

Toledo, E.; Le Saux, G.; Edri, A.; Li, L.; Rosenberg, M.; Keidar, Y.; Bhingardive, V.; Radinsky, O.; Hadad, U.; Di Primo, C.; Buffeteau, T.; Smith, A.-S.; Porgador, A.; Schvartzman, M. Molecular-Scale Spatio-Chemical Control of the Activating-Inhibitory Signal Integration in NK Cells. *Sci. Adv.* **2021**, *7* (24), eabc1640. https://doi.org/10.1126/sciadv.abc1640.

Figures:

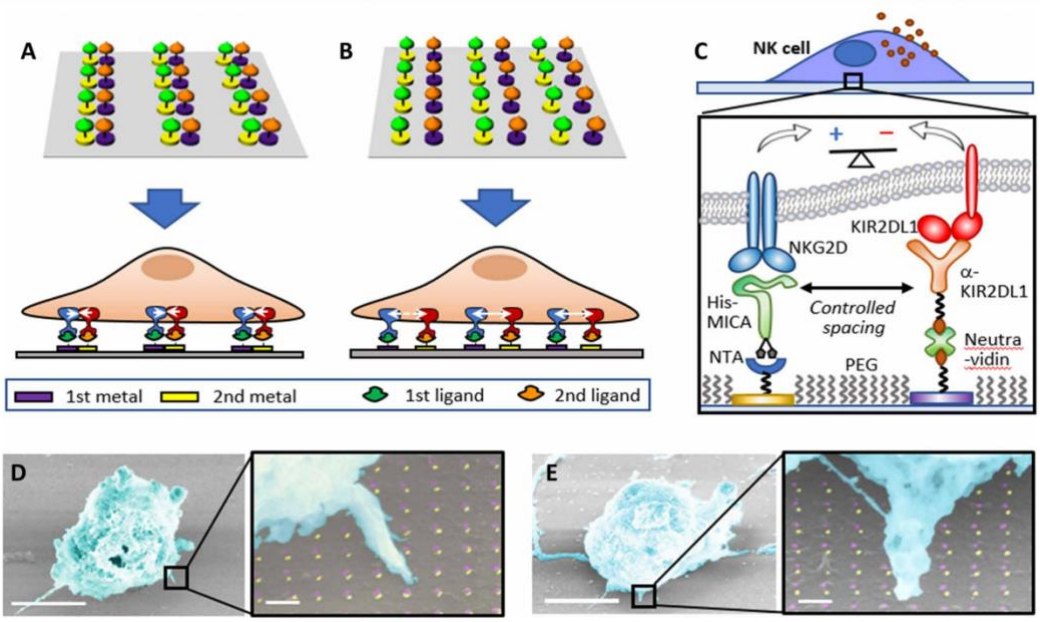

Figure 1: *Platform for the molecular-scale spatial control of two extracellular ligands. (A and B) Schematic representation of ligand patterns in which colocalized or spatially segregated ligands, respectively, control the spatial arrangement of integrating receptors (not to scale). (C) Scheme of a ligand pattern that controls activating inhibitory balance in NK cells. PEG, polyethylene glycol. (D and E) False-colored scanning electron micrograph of NK cells stimulated in activating-inhibitory ligand array with different spacings between the ligands. Scale bars, 5 μm; scale bars in high-magnification images, 200 nm.*

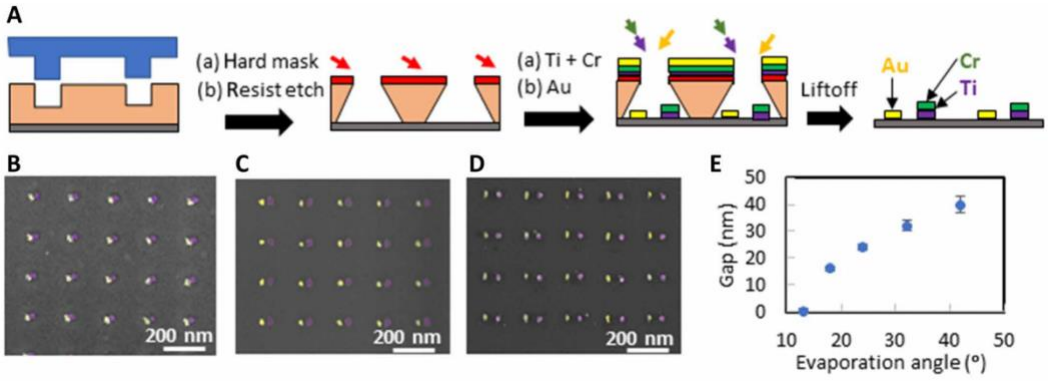

Figure 2: *Fig. 2. Flowchart of the fabrication of bimetallic nanopatterns functionalized with two different biomolecules. (A) Fabrication of heterogeneous metallic arrays. False-colored scanning electron micrograph of (B) colocalized nanodots, (C) nanodots separated by ~20-nm gap, and (D) nanodots separated by ~40-nm gap. (E) Segregation control via evaporation angle.*

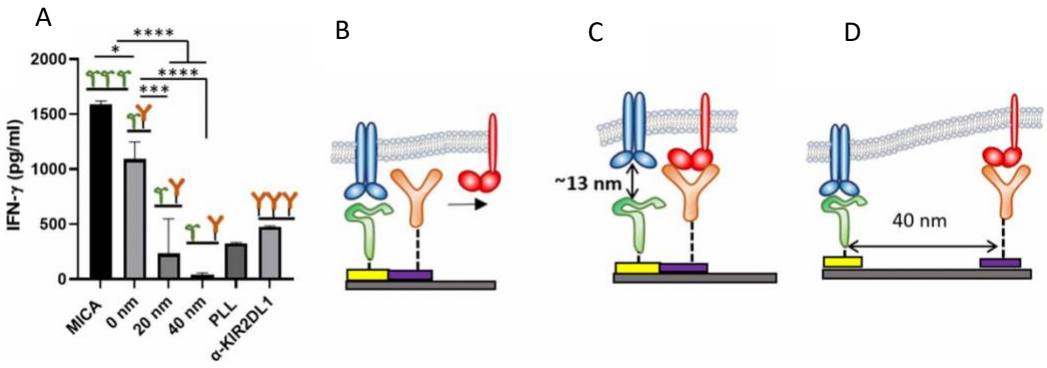

Figure 3: *Impact of molecular-scale spatial control of two extracellular ligands on NK cell immune response. (A) Interferon-γ (IFN-γ) release intensity. (B to D) Schemes of the possible mechanisms explaining the observed effect of the gap between the ligands in NK cell activation: (B) long ligand binds first, precluding binding to the colocalized shorter ligand; (C) short ligand binds first, precluding binding to the colocalized longer ligands; and (D) both ligands can bind their receptors owing to the spatial segregation*