

# Nanofabrication of curvature sensitive protein screening phospholipid membrane platform

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The curvature of lipid membranes plays a key role in many relevant biological processes such as membrane trafficking, vesicular budding or host-virus interactions.<sup>1,2</sup> In-vitro studies on biomimetic models in the nanoscale are challenging, due to the resolution and high cost of the available techniques.

In this work, we have developed a simple and low-cost platform for curvature sensitive protein screening, prepared through scanning probe lithography (SPL) methods, where lipid bilayer patches of different compositions can be multiplexed onto substrate areas with tailored local curvature<sup>3</sup> (Figure 1). The desired curvature is imposed by anchoring nanoparticles of the desired size to the substrate prior to lithography. We have optimized the nanolithography process (Figure 2) and demonstrated, as a proof of principle, that a biosensor for positive membrane deformations derived from the BAR domain<sup>4</sup> of Nadrin2 binds selectively to lipid patches patterned on substrates areas coated with 100 nm nanoparticles. The platform opens up a path to screening applications for protein / curved membrane interaction studies by providing a flexible and easy to prepare substrate with control over lipid composition and membrane curvature.

<sup>1</sup> Vanni, S.; Hirose, H.; Barelli, H.; Antony B.; Gautier, R. A sub-nanometre view of how membrane curvature and composition modulate lipid packing and protein recruitment. 2014, *Nat. Commun.*, 5, 4916.

<sup>2</sup> Galic, M.; Ebrahimkutty, M.P. Receptor-Free Signaling at Curved Cellular Membranes. 2019, *BioEssays* 41, 1900068.

<sup>3</sup> Berganza, E.; Ebrahimkutty, M.P.; Vasantham S.K.; Zhong, C.; Wunsch, A.; Navarrete, A.; Galic M.; Hirtz, M. Multiplexed phospholipid membrane platform for curvature sensitive protein screening. 2021, *Nanoscale* 13, 12642-12650.

<sup>4</sup> Peter, B. J.; Kent, H. M.; Mills, I. G.; Vallis, Y.; Butler, P. J. G.; Evans P. R.; McMahon, H. T. BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. 2004, *Science* (80-. ), , 303, 495–499.

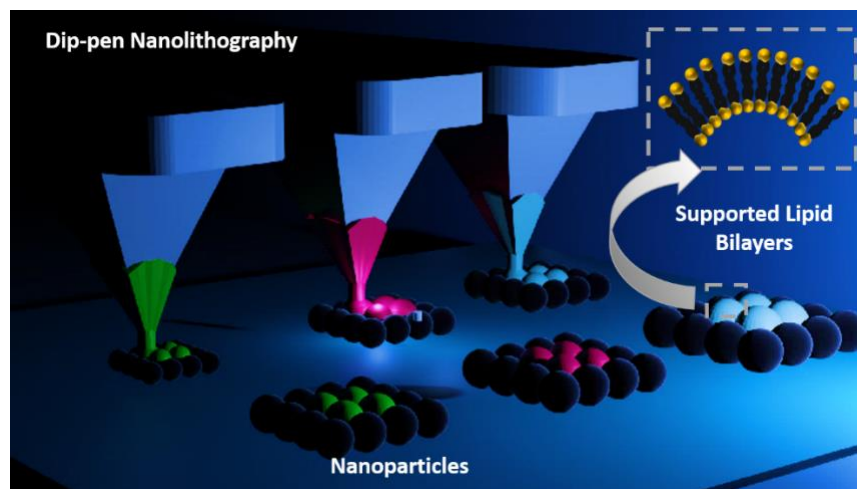


Figure 1: Schematic representation of a platform for the multiplexing of lipid patches via Dip-Pen Nanolithography onto locally curved surfaces.

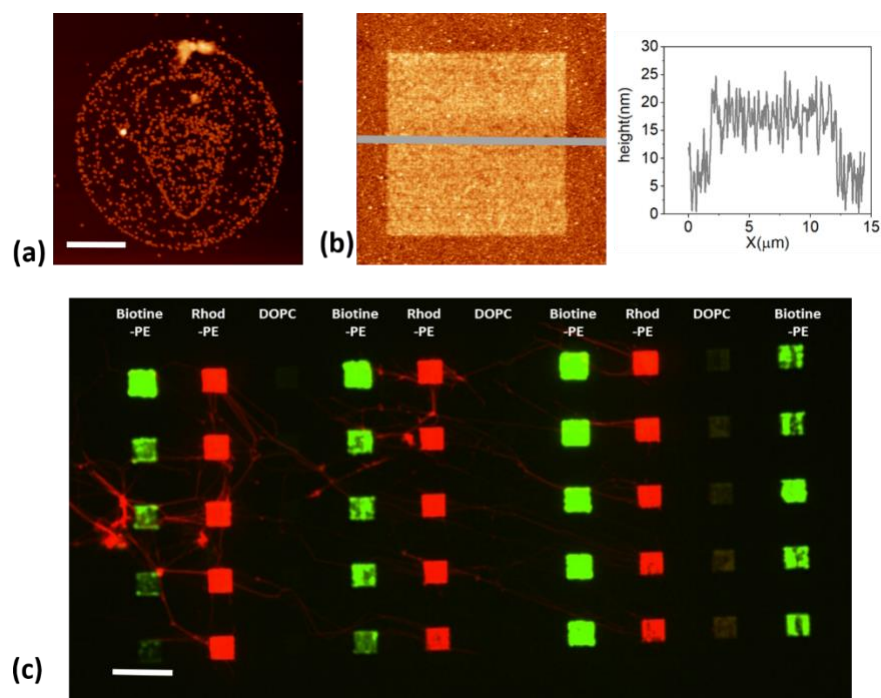


Figure 2: Different perspectives of the nanofabrication process. (a) AFM image of spotted nanoparticles on a gold coated surface. (b) Squared shaped lipid patch patterned on a surface fully coated with nanoparticles and corresponding profile. Scale bar equals 5 μm. (c) Fluorescence microscopy image of different phospholipid mixtures patterned in close proximity to each other. Scale bar

*equals 50  $\mu\text{m}$ .*