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Supporting Information

Kelvin probe force microscopy on patterned large-area biofunctionalized surfaces: a reliable ultrasensitive platform for biomarker detection

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Supplementary Information

Content:

- **<u>Supporting Note 1:</u> Patterned biofunctionalized surfaces.**

Figure S1 shows the biofunctionalization flowchart. It encompasses four steps: a polimeric mask (7 mm x 7 mm), purchased from Shin-Etsu Chemical, is placed on a portion of the gold coated substrate. Anti-human immunoglobulin M (anti-IgM) or Anti-human immunoglobulin G produced in goat polyclonal antibodies (Sigma–Aldrich) were used with no further purification as bio-recognition receptors via physisorption. The gold surface was submerged in a phosphate buffer saline (PBS) solution of antibody 0,1 mg/ml for 150 min, at 25 °C. After the physisorption, the mask was removed, leaving a dense thin layer of capturing sites in the unprotected sample area. The surfaces were extensively rinsed in PBS, then in HPLC water and dried by spinning at 3000 rpm for 60 s.

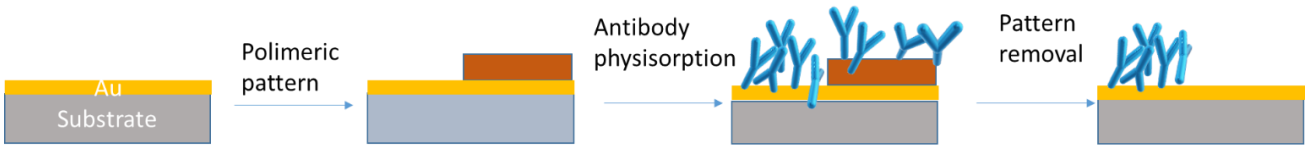

Figure S1: Flowchart of the biofunctionalization protocol.

<u>Supporting Note 2:</u> Surface potential shifts induced by affinity binding events – anti-IgM/IgM

Figure S2 shows the KPFM images and the distributions of SPD, defined as the surface potential difference calculated between the peaks of the SP distributions corresponding to the anti-IgM and the Au areas, respectively, following the incubation of Au/anti-IgM in IgM PBS standard solutions at nominal concentrations of 100 zM, 10 aM, 100 aM. and 1 fM.

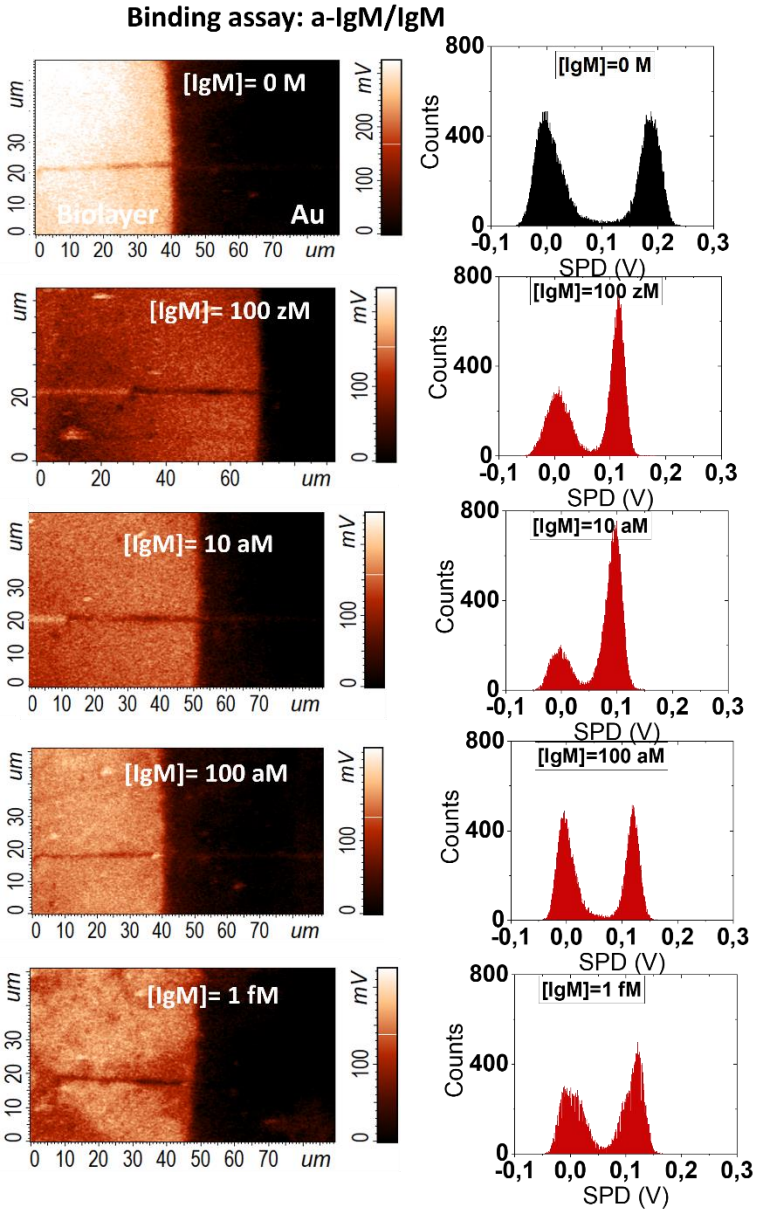

Figure S2: KPFM images and SPD histograms across the interface between the Au region and the anti-IgM one after the incubation with IgM standard solutions at different nominal concentrations, ranging from 0 zM to 10^6 zM.

In order to demonstrate the high selectivity and specificity of the KPFM-based sensing platform, a different anti-IgM patterned sample was exposed to increasing concentrations of the immunoglobulin

IgG. As IgG does not bind to anti-IgM, this suitably serves as the negative control experiment. The KPFM images and the corresponding distributions of SPD are shown in Figure S3. No significative change in biolayer SPD values has been measured. These data are included in Figure 5a.

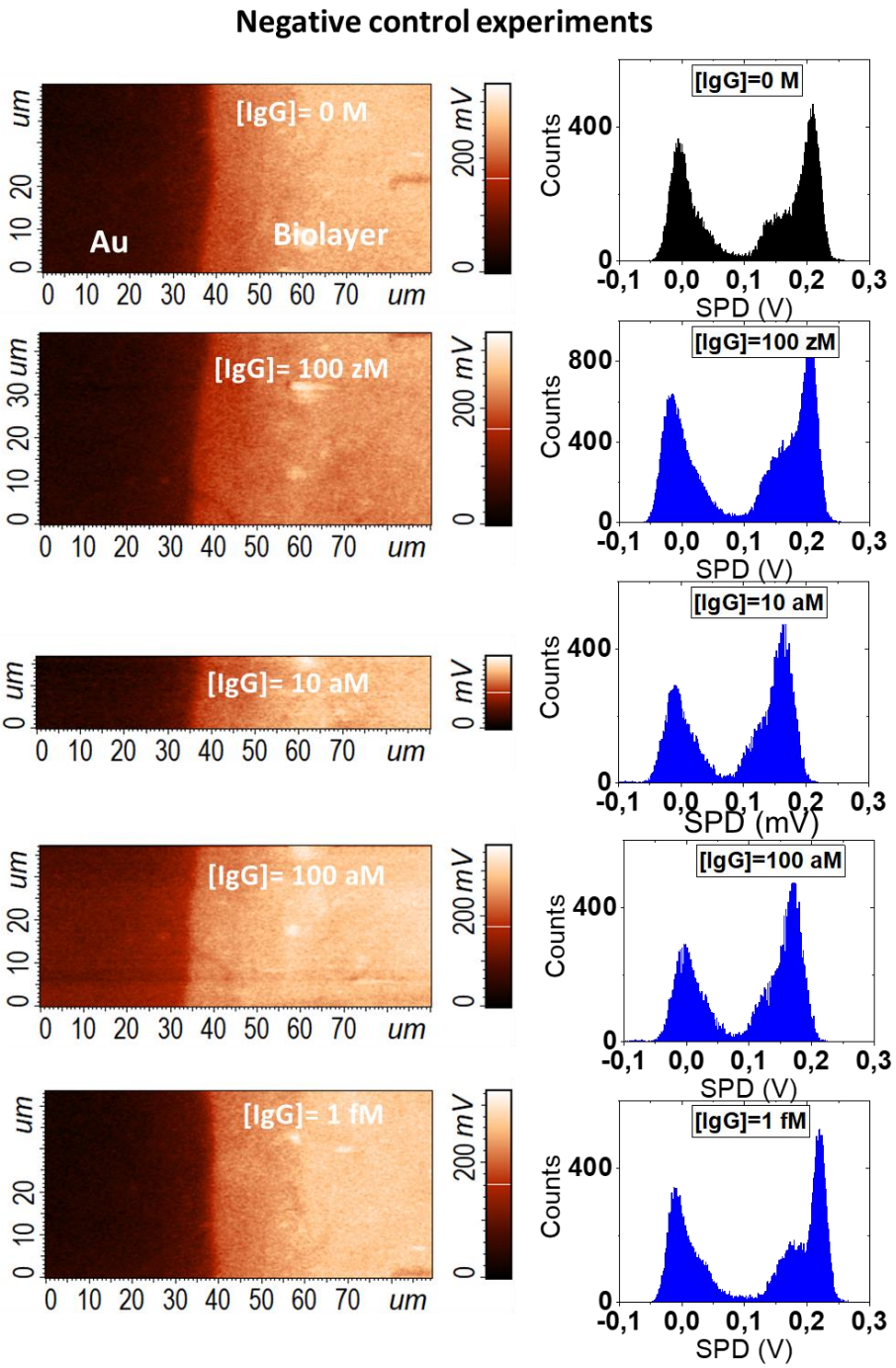

Figure S3: KPFM images and corresponding SPD histograms at the edge between the gold region and the anti-IgM one following the incubation with the unbinding IgG standard solutions at different nominal concentrations, ranging from 100 zM to 10^6 zM, taken as negative control.

<u>Supporting Note 3:</u> Surface potential shifts induced by affinity binding events – anti-IgG/IgG

Patterned Anti-IgG/Au samples were produced following the protocol described in the text. The Anti-IgG/Au morphology was evaluated by atomic force microscopy, operated in the semi-contact mode. The micrographs have been recorded in air (Si probes, mod. NGS01, NanoTips, resonant frequency of 150 kHz, force constant of 5 N/m, and a nominal tip radius of 10 nm). Figures S4a and S4b show the 3D morphology of a representative 2 μm x 2 μm area of a patterned Anti-IgG sample and the corresponding average height profile. A step is detected between the leftmost side Anti-IgG area and gold rightmost one, corresponding to the physisorption of a dense and compact layer of capturing antibodies with a thickness of about 3 nm.

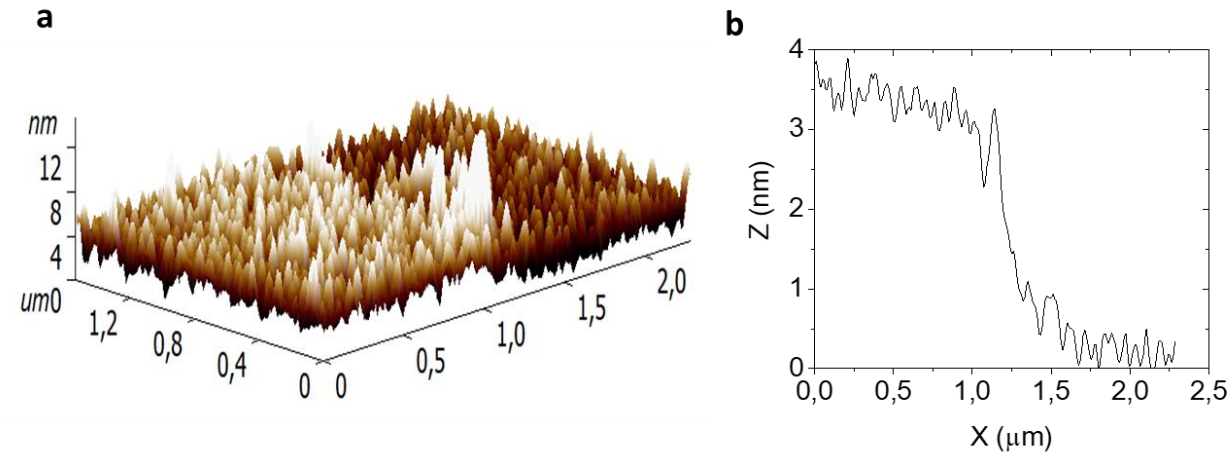

Figure S4 (a) Representative semi-contact mode 3D morphology of a patterned Anti-IgG/Au sample and (b) corresponding average height profile, measured by AFM.

The IgG/Anti-IgG affinity-binding was studied after the incubation in IgG standard PBS solutions at concentrations of 100 zM, 10 aM, 100 aM. The negative control experiment consisted of incubating the same Anti-IgG/Au sample in 500 μL of a 1 fM IgM solution in PBS. The KPFM images and the corresponding SPD distributions are shown in Fig. S5. The average SPD (260 ± 20 mV) is comparable to the SPD baseline (240 ± 20 mV), within error bars. The KPFM images change significantly after incubation in 100 zM, and 10 aM IgGs, and the $mean\ \Delta SPD$ is 94 $\pm$ 10 mV, compared to the baseline. These data are included in Figure 5b.

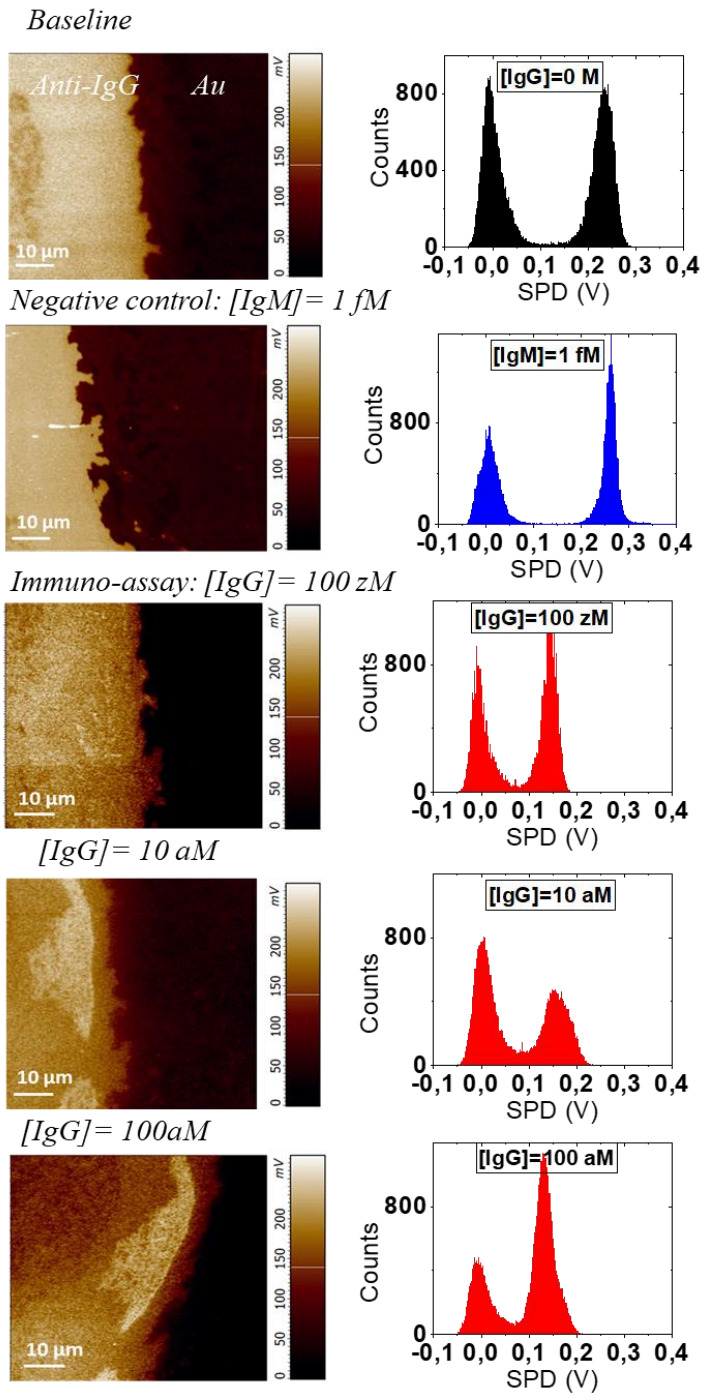

Figure S5: KPFM images and corresponding SPD histograms at the edge between the Au region and the anti-IgM one following the incubation with the negative control (IgM) and different nominal concentrations of ligands (IgG), in the range 100 zM – 100 aM.