EXPERIMENTAL DETAILS

**Preparation of the buffers.** The PBS buffer was made of 0.01 M KH$_2$PO$_4$ and 0.15 M NaCl and its pH was adjusted to 7.4 with 1M NaOH. The phosphate buffer used for the AFM-SECM experiments and for the preparation of the Fc-PEG-labeled IgG was made of 35 mM KH$_2$PO$_4$ and its pH was adjusted to 8 with 1M NaOH (final ionic strength 0.1 M). The buffers were filtered immediately before use on a 0.22 µm nylon VWR filter.

**Preparation of the Fc-PEG-labeled IgG.** Attachment of Fc-PEG chains to Goat anti-mouse IgG’s was carried out by reacting the NHS activated ester of home synthesized NHS-PEG$_{3400}$-Fc chains with the amino groups of the IgG species, as described previously. Briefly ~ 1 mg of NHS-PEG-Fc was added to a filtered 20 µM anti-mouse Goat IgG solution in phosphate buffer pH 8 (NHS-PEG-Fc was in a ~ 135 fold excess with respect to the IgG). The IgG’s were separated from unattached Fc-PEG chains by ultrafiltration through a Centricon 30 YM cellulose membrane (Millipore). The membrane is permeable to the PEG chains, while it retains the IgG molecules which are thus concentrated in the residual solution. Elution of unreacted NHS-PEG-Fc chains and liberated NHS product was monitored by UV/vis spectroscopy at 260 nm, a wavelength corresponding to the maximum absorbance of NHS. After 5 concentration/dilution cycles corresponding to a total dilution factor of $10^6$, no trace of NHS could be detected in the eluate. PBS pH 7.4 buffer containing 0.01% sodium azide was then added to the Fc-PEG-labeled IgG solution so that the final solution contained ~ 0.6 mg/mL labeled IgG. The IgG-PEG-Fc solution was stored in a refrigerator and used within 6 months.

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Measuring the coverage in Fc heads of HOPG surfaces bearing saturated antigen/IgG-PEG-Fc layers from cyclic voltammograms. Integration of the cathodic (or anodic) peak of the background corrected voltammograms recorded at HOPG surfaces bearing saturated antigen/IgG-PEG-Fc layers yielded the Faradaic charge $Q$ required for the full oxidation (or full reduction) of the IgG-PEG-Fc layer. The total amount of IgG-bound-Fc on the electrode surface $A \Gamma_{\text{Fc}}$, was then be quantitatively derived from the integrated charge: $Q = F A \Gamma_{\text{Fc}}$, $F$ being the Faraday constant, and $A$ the effective surface area (cm$^2$). Taking for $A$, the geometric area of the smooth HOPG surface, the saturating surface coverage in Fc heads $\Gamma_{\text{Fc}}$ ensued.

Fabrication of the antigen/IgG-PEG-Fc immunocomplex dot arrays on gold surfaces using particle lithography. The particle lithography technique used to form a regular array of Mouse IgG (antigen) dots on a gold surface was adapted from the procedure described by Schmidtke et al. to form protein dots on glass.$^{ii,iii}$ Polystyrene (PS) latex beads (either ~ 2 μm or ~ 0.5 μm in diameter) were washed twice by centrifugation before use. The gold substrate was placed in a Teflon cell which was designed so that only a circular area of 6 mm diameter was exposed to the PS bead solution. The cell was filled with 24 μL of the bead solution (1% wt). The concentration of beads in the solution was optimized to obtain at least one complete monolayer (typically multilayers were formed). Complete evaporation of the solution was performed at 30°C in a thermostatic bath and was achieved in ~ 2 hours. The substrate was then placed in a Petri dish onto a heating plate at about 80°C for 1 hour. Afterwards the surface was washed with Milli-Q water. A 1 mM PEG$_{2000}$ disulfide solution in Milli-Q water was then placed on the gold substrate overnight under an argon atmosphere. The beads were then removed by ultrasonication in Milli-Q water for 15 seconds. A 100 μL solution of 50 μg/mL Mouse antigen IgG in PBS buffer was deposited on the surface for 2 hours under an argon atmosphere. After desorption, molecular recognition of the adsorbed Mouse IgG’s by the IgG-PEG-Fc was carried out as described in the manuscript in the case of the HOPG


substrate, except that the gold surface was kept under an argon atmosphere to protect the PEG layer from air oxidation.

**Combined AFM-SECM experiments.** The AFM-SECM experiments were carried out with a Molecular Imaging PICOSPM I AFM microscope (Agilent), which was modified as described in previous contributions.\textsuperscript{iv,v} The electrochemical liquid cell contained 600 µL of filtered pH 8 phosphate buffer. A platinum wire was used as a counter-electrode, and a quasi-reference electrode (Pt/PPy) was freshly prepared by electropolymerization of polypyrrole on a platinum wire, as previously described.\textsuperscript{vi} All the potential values are given here versus the KCl saturated calomel electrode reference (SCE). A home-made bipotentiostat was used to control independently the potential applied to the substrate and to the electrochemical tip. Cyclic voltammograms could be independently recorded at the tip or at the substrate while maintaining a constant bias at the substrate or at the tip, respectively. For the tip and substrate currents acquisitions, high (10 pA/V) and low (20 µA/V) gain current measuring circuits were used. The tip-current signal was passed through a 10 Hz low-pass analogue filter. The substrate potential was generated by a PAR 175 programmer. The tip-current data were corrected, in all experiments (approach curves and images), from the nonspecific leakage current resulting from the imperfect insulation of the connecting wires. This current is non specific and independent of the tip-to-substrate distance and of the tip and substrate potential.

**Differential pulse voltammetry (DPV).** The patterned gold surfaces, bearing antigen/IgG-PEG-Fc immunocomplex dots, were characterized using CV and differential pulse voltammetry (DPV). The higher analytical sensitivity displayed by DPV, as compared to CV, was required to detect the low amount of Fc heads present on these surfaces (see Figure S2). A CHI600 Electrochemical Workstation (CH instrument, Austin, Texas) was used for recording DPV signals with the following parameters: init E (V) = 0, final E (V) = 0.30, incr E (V) = 0.005, amplitude (V) = 0.05, pulse width (sec) = 0.002, sample width (sec) = 0.001, pulse period (sec) = 0.1.

\textsuperscript{iv} Anne, A.; Demaille, C.; Goyer, C. *ACS Nano* 2009, 3, 819-827.
\textsuperscript{vi} Anne, A.; Cambril, E.; Chovin, A.; Demaille, C.; Goyer, C. *ACS Nano* 2009, 3, 2927-2940
**ADDITIONAL FIGURES**

**Figure S1.** In situ tapping mode AFM topography images of a gold surface bearing: (A) A layer of PEG$_{2000}$-disulfide. The flexible PEG chains escape compression by the sharp AFM tip and cannot be imaged. (B) Mouse IgG (antigen) dots formed by particle lithography using 2 μm diameter polystyrene beads. (C) Mouse IgG (antigen) dots formed by particle lithography using 0.5 μm diameter polystyrene beads. Cross-section of the images along the white line are shown. A commercial FM probe was used. Frequency = 17.2 kHz, image rate 1 Hz. 10% damping. Phosphate buffer pH 8, 0.1 M ionic strength.

**Figure S2.** (a) Cyclic voltammogram and (b) Differential pulse voltammogram (DPV) of a gold surface bearing a Mouse IgG dot array, before (green dotted trace), and after (blue solid trace) redox immunomarking with a Fc-PEGylated antimouse IgG. The Mouse IgG pattern was formed by particle lithography using 2 μm diameter polystyrene beads. Phosphate buffer pH 8, 0.1 M ionic strength. CV scan rate = 2V/s. DPV parameters: init E (V) = 0, final E (V) = 0.3, incr E (V) = 0.005, amplitude (V) = 0.05, pulse width (sec) = 0.002, sample width (sec) = 0.001, pulse period (sec) = 0.1
Figure S3. Mt/AFM-SECM tapping mode imaging of a gold surface bearing an ordered array of antigen/IgG-PEG-Fc immunocomplex. Illustration of the possibility of recording images with the substrate unbiased. (a) Topography image. (b) Simultaneously acquired tip current image. The image was scanned from top to bottom. In the upper part of the image $E_{\text{sub}} = -0.05$ V/SCE. In the lower part of the image the substrate was disconnected from the bipotentiostat. Aqueous phosphate buffer pH 8, 0.1 M ionic strength. Tip potential: $E_{\text{tip}} = +0.30$ V/SCE, The probe is oscillated at its fundamental flexural frequency $= 2.82$ kHz. ~ 25% damping. Imaging rate 0.3 Hz.