Supporting Information

Real-time monitoring of electrochemical controlled protein adsorption by a plasmonic nanowire based sensor

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Methods

Synthesis of Au NW arrays

Before any modification, glass coverslips (24 x 24 mm N°1, Carl Roth, Germany) were cleaned in piranha solution (H_2SO_4 (c): H_2O_2 30 % v/v 3:1) for one hour, then exhaustively rinsed with MilliQ water and dried under a stream of nitrogen. Glass substrates were then coated with a 5 nm titanium adhesion layer and 20 nm of gold at room temperature by physical vapor deposition (PVD). Ion-track polycarbonate (PC) membranes (it4ip, Seneffe, Belgium) were PVD coated with a 20-nm layer of gold. This layer provided good adherence to the gold-coated glass substrates reducing defects due to the presence of bubbles between both surfaces. PC membranes with 25-µm thickness and 100 nm pore diameter with different pore densities ($\rho_1 = 10^6$ cm^{-2}, $\rho_2 = 6x10^8$ cm^{-2}) were used. Prior to the electrodeposition procedure, PC membranes were placed in gold electrolyte solution (Conrad Electronics, Germany) for 20 min at room temperature to promote pore filling by the electrolyte. Subsequently the wet PC membrane was placed and fixed onto the gold-coated glass substrate within a Teflon cell with a platinum mesh as counter electrode and the gold-coated glass as working electrode. Growth of Au NWs was achieved by applying a voltage of -1.5 V on an area of ~1.75 cm^2 under smooth stirring (~4 Hz) with an Autolab PGSTAT302N potentiostat (Echo Chemie, Germany). Under these conditions a growth rate of 4.7 nm/s was achieved. Deposition time was optimized to render Au NWs with intense and sharp plasmon extinction band. A deposition time of 400 s (1.9 µm length) was found to fulfill these requirements, as can be seen from Figure S1. After that, the membrane was dissolved

by dichloromethane and the Au NW array was gently rinsed with DMF and MilliQ water. Finally, the Au NW array was subjected to O_2 plasma treatment (RIE Oxford Plasmalab, Oxford Instruments, t = 5 min, f(O_2) = 40 sccm, P_{RF} = 50 W, P_{ICP} = 50 W, p = 80 mTorr, T = 20 °C) to remove any remaining organic matter.

Electron microscopy characterization

SEM imaging was achieved with a Zeiss Gemini Ultra 55 (4kV) to investigate the morphology and size of the deposited Au NWs. HRTEM images were taken with a JEOL ARM 1250kV operated at 1000 kV. For a detailed description of the sample preparation please refer to Schneckenburger et al.[1]

Optical measurements

All optical measurements were carried out in transmission mode with a UV-Vis spectrophotometer Cary 4000 (Varian Inc., USA) at room temperature. Measurements in air were performed with a standard sample holder. Bulk RI sensitivity and biosensing measurements with or without electrochemical control were performed with a flow cell under normal illumination (θ = 0°). In the case of bulk RI sensitivity measurements, Au NW arrays were placed into the cell and glycerol-water solutions of different RIs were injected at 250 µL/min. Transmission spectra were acquired in the 560-640 nm range at 1 min intervals until the optical response was stable. The reproducibility and stability of the optical response was verified by restoration of the initial resonance wavelength after rinsing the cell with MilliQ water. A Gaussian function was used to detect the resonance wavelength and the resonance peak shift ($\Delta\lambda$) was plotted as a function of the RI of the solutions to calculate the corresponding bulk RI sensitivity. Based on 6 different Au NW arrays, an average value of 246 ± 12 nm/RIU was obtained. This value was taken as quality standard for further biosensing measurements. Biosensing measurements were performed in PBS buffer at a flow rate of 250 µL/min. Typically, after establishing the baseline with PBS buffer, a solution of the protein of interest, dissolved in the identical buffer, was introduced to the flow cell. Transmission spectra were acquired in the 572-598 nm range at 1 min intervals. Under electrochemical stimulation, potentials were applied between the Au NW array (working electrode) and a Ag/AgCl reference electrode. A loop made of platinum mesh was used as counter electrode. The baseline was obtained at a defined applied bias in PBS buffer. $\Delta\lambda$ was determined by fitting the extinction band to a Gaussian function. BSA (fraction V, Serva, Germany) and LZM (from chicken egg white, Jena Bioscience, Germany) solutions were prepared in PBS buffer the same day of the experiments.

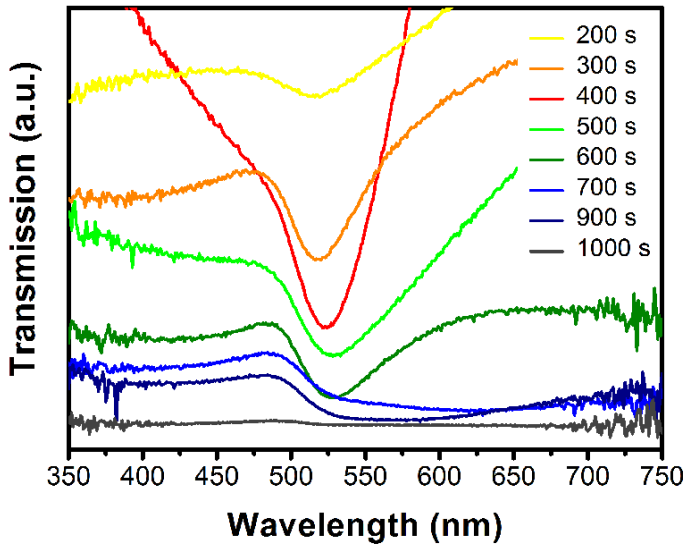

Figure S1. Transmission spectra of Au NW arrays of different length. Au NWs obtained after different deposition time were evaluated in terms of their optical response in air at room temperature. The synthesis rate achieved during the deposition procedure corresponds to 4.7 nm/s. The sharpest and most intense transmission band was obtained for Au NWs grown for 400 s, which render an average nanowire length of ~1.9 μm

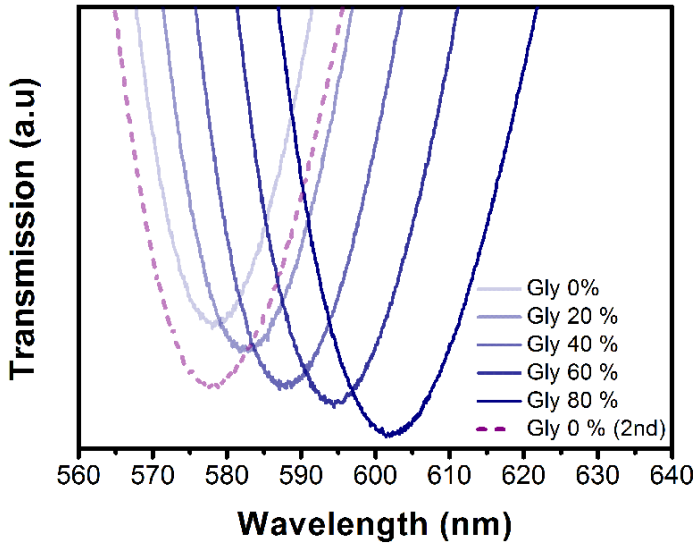

Figure S2. Bulk RI sensitivity measurements. Transmission spectra of a Au NW array with an average nanowire length of 1.9 μm in glycerol-water solutions with different RIs. The measurements correspond to Gly 0 % (n = 1.333), Gly 20 % (n = 1.358), Gly 40 % (n =1.384), Gly 60 % (n = 1.412) and Gly 80 % (n = 1.443). Measurements were performed in a flow cell in increasing concentrations of glycerol under normal illumination (θ = 0°) at room temperature. The optical response (resonance peak shift (Δλ)) of the nanoarray was restored to its initial value after rinsing the whole device with MilliQ water, which evidence the remarkably reproducible and stable optical response of the Au NW array. Under these conditions a bulk RI sensitivity of 246 ± 12 nm/RIU was obtained (based on 6 different Au NW arrays).

Electrochemical characterization

Electrochemical experiments aiming to estimate the surface roughness factor f_R of the Au NW arrays were performed with an Autolab PGSTAT302N potentiostat (Echo Chemie, Germany) in a three-electrode electrochemical cell equipped with an Ag/AgCl (3 M KCl) reference electrode and a platinum mesh counter electrode. Cyclic voltammetry experiments were carried out by scanning the potential from -0.6 to 1.4 V at 25 mV/s at room temperature in PBS buffer in a Teflon cell (exposed area to solution: 0.16 cm^2 of the electrode). The real area of the gold surface was estimated by measuring the charge related to the AuO $\rightarrow$ Au phase change, i.e., the electroreduction charge. Average data presented in this work correspond to measurements of at least three low- and high-density nanoarrays.

The potential of zero charge (PZC) of the AuNW array in PBS solution was estimated by electrochemical impedance spectroscopy (EIS). The PZC was deducted from measurements of the double layer capacitance (C_{dl}) as a function of the applied potential (E), where it manifests itself by a minimum on the C_{dl}(E) plot. EIS measurements were recorded with 10 mV AC perturbation in the frequency range of 10 kHz to 0.05 Hz between -0.5 and +0.5V in a three-electrode electrochemical cell equipped with an Ag/AgCl (3 M KCl) reference electrode and a platinum mesh counter electrode, using an Autolab PGSTAT302N potentiostat (Echo Chemie, Germany) equipped with a frequency analyzer module (FRA2). Capacitance values were obtained by applying a circle fit to the complex capacitance plot[2] recorded at each potential. Under these conditions, a value of PZC = 78 ± 4 mV was obtained. According to this value, when the electrode is biased at -0.5 V (vs. Ag/AgCl), it carries an excess of negative charges. Similarly, at 0.5 V (vs. Ag/AgCl) the electrode has an excess of positive charges.

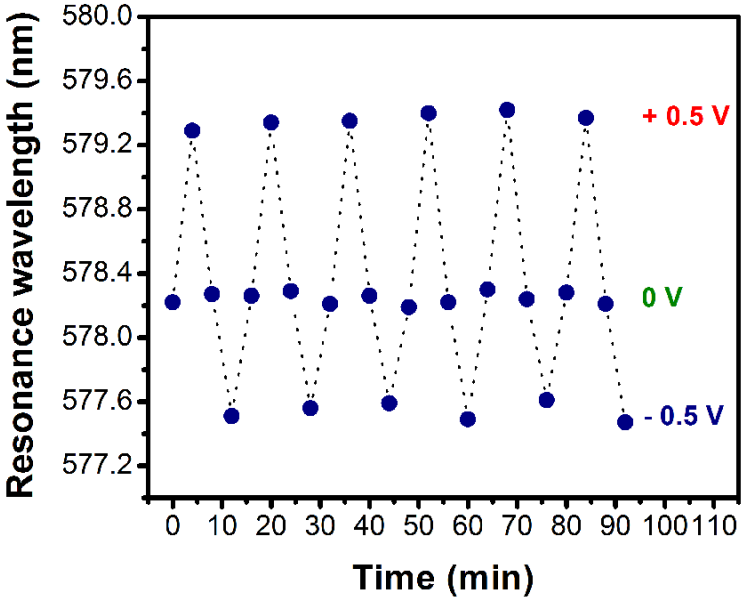

Figure S3. LSPR response in the presence of applied bias. Resonance wavelength as a function of time of the Au NW array in PBS buffer under applied bias and normal illumination (θ = 0°) at room temperature.

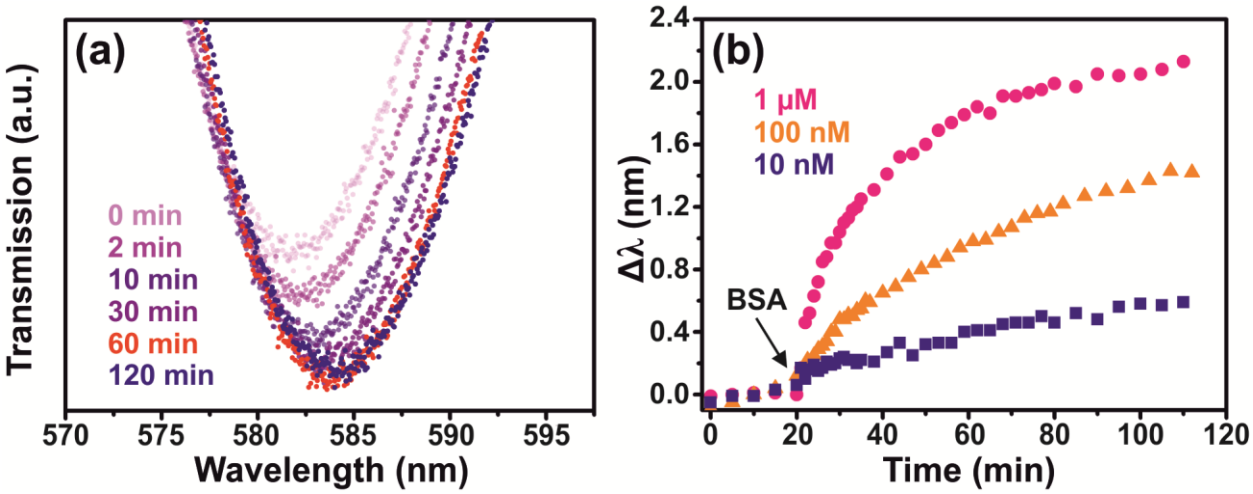

Figure S4. BSA adsorption kinetics in the absence of applied bias. (a) Transmission spectra for the adsorption process of BSA 1 μM on the nanostructures at different time values in PBS buffer. (b) Resonance peak shift (Δλ) response as a function of time for the adsorption process of BSA at different concentrations in PBS buffer. The values of Δλ for the different time points are the result of fitting the transmission spectra (as in Figure 3) to a Gaussian function. The arrow indicates the addition of the BSA solution into the flow cell.

Figure S4a illustrates the optical behavior of the nanoarray in the presence of 1 μM solution of BSA in PBS buffer (PBS formulation: NaCl 0.1369 M; KCl 0.0027 M; Na_2HPO_4 0.0081 M;

KH_2PO_4 0.0019 M; pH 7.4; ionic strength 0.163 M) at different time values in the absence of applied bias. The corresponding transmission spectra evidenced a well-defined red shift in the LSPR peak wavelength, which originates from local RI changes as water is replaced by the protein. Δλ was determined by fitting the extinction bands to a Gaussian function and plotted as a function of time in Figure S4b. Typically, after establishing the baseline with PBS solution during 20 min BSA was injected into the flow chamber. A rapid increase in resonance peak wavelength (red shift) was observed due to the adsorption of the protein onto the nanostructured surface. For 1 μM BSA solution, half of the peak shift was reached ~9 min after protein injection, and it took ~60 min for the signal to stabilize to a value of ~2.1 nm. Rinsing with buffer did not evidence major changes in the LSPR response (data not shown), as typically observed for irreversible adsorption processes. The array behavior in 100 and 10 nM BSA solutions is also included in Figure S4b. In the latter cases, Δλ monotonically increased during the whole time interval evaluated, which correlates with the strong influence that bulk concentration have on the kinetics of protein adsorption and therefore the resulting surface density. [3,4] The signal response of the nanoarray, considering the Δλ after an adsorption time of 60 min, exhibited a semilogarithmic dependence with the concentration resembling Langmuir behavior. Further dose-response assessment provided a limit of detection of 1.5 nM, as determined as the concentration of BSA that corresponds to three times the standard deviation (3σ) of the baseline noise. These results clearly confirm the concordance between the bulk sensitivity and the localized biosensing capability of the Au NW array.

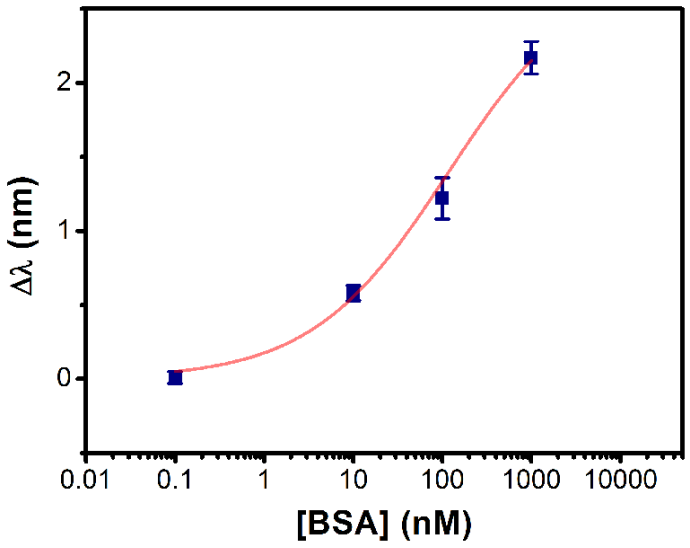

Figure S5. Dose-response assessment for the adsorption of BSA onto the Au NW array. Resonance peak shift (Δλ) response as a function of BSA concentration for the adsorption of BSA on the sensor surface.

References

1. Schneckenburger, M.; Kelsch, M.; van Aken, P. A.; Richter, G.; Spatz, J. P.; Rustom, A. *Small* **2012,** 8, (22), 3396-3399.
2. Bard, A. J.; Faulkner, L. R., Electrochemical Methods: Fundamentals and Applications, chapter 10. In 2nd ed.; 2001.
3. Ramsden, J. J. *Chemical Society Reviews* **1995,** 24, (1), 73-78.
4. Rabe, M.; Verdes, D.; Seeger, S. *Advances in Colloid and Interface Science* **2011,** 162, (1-2), 87-106.