Supporting Information

Electrofocusing-Enhanced Localized Surface Plasmon Resonance Biosensors

Jinling Zhang [a] ‡, Yi Wang [a] ‡, Ten It Wong [b], Xiaohu Liu [a], Xiaodong Zhou [b], and Bo Liedberg*[a]

Experimental Section

1. Materials:

Cardiac troponin I (cTnI) full length protein (MW ca. 24kDa, 0.48mg/mL) was purchased from Abcam. Phosphate buffered saline (PBS), bovine serum albumin (BSA, ca. ~66kDa, ≥96%) and rabbit serum (R9133) were purchased from Sigma-Aldrich. CALNN-$(PEG)_4$-FYSHSFHENWPS and CALNN were purchased from GL Biochem(Shanghai). Absolute ethanol (99.9%) was purchased from Merck.

2. Instrument and method:

AuNH array substrate was fabrication by nano-imprinting lithography, a previously reported method. [1] Briefly, the Au-hole array were fabricated with an electroplated nickel mold (with nanoholes structure), which was used to nanoimprint the UV curable photoresist layer (mr-UVCur21-300nm from micro resist technology GmbH). The photoresist was then treated with reactive ion etching (RIE) to etch the indented photoresist down to the glass substrate. Afterwards, 5 nm of chromium and 45 nm thick of gold were deposited, and the photoresist was lifted-off by plasma etching and subsequent rinsing with acetone and isopropyl. The parameters of the AuNH array were characterized by Scanning Electron Microscopy (SEM) (JSM-7600F), Atomic force microscope (AFM) (Digital Instruments, DI-3100) and UV-vis spectrometer (Perkin Elmer, Lambda 35).

In order to modify the AuNH array, the AuNH array chip was treated with oxygen plasma for 30 s under 10 sccm oxygen at pressure of about 500 mtorr and power of 18 W (plasma cleaner PDC-32G). Then, it was incubated in an ethanol solution containing a mixture of CALNN and CALNN-PEG-FYSHSFHENWPS at molar ratio of 1:1 (total 50 μM). After one days' incubation, the chip was rinsed by ethanol and dried by nitrogen gas. The chip was fixed into a reaction chamber made off PDMS in which the Pt wire counter electrode and Ag/AgCl electrode was inserted in the chamber. The sensor chip was covered with a thin layer of PDMS with a hole (about 2 mm in diameter) to allow the AuNH array to be contacted with the solution in the chamber. The incident light was normally illuminated through the backside of the AuNH chip. The transmittance spectra were acquired every second by a spectrometer (Insplorion Xnano instrument, Sweden). The electrodes were connected with the Multi Autolab (Metrohm Autolab B.V., Netherlands) for electric bias application.

On the detection of cTnI, about 4 μL of cTnI solutions were sequentially injected into the chamber to achieve cTnI concentrations of 1ng/mL, 10ng/mL, 100ng/mL and 1μg/mL in the buffer solution. The *Extd* values (i.e. the extinction difference between the wavelength at about 700nm and the other wavelength at 730nm) were monitored in real time. The Δ*Extd* of each concentration was estimated as the *Extd* after 30 min incubation of cTnI subtracted to the background value. The electric bias was applied on the sensor chip through the multi Autolab based on the reference of the Ag/AgCl electrode.

Equation SI.1 Sigmoidal model.

$$y = START + (END - START) * \frac{x^n}{k^n + x^n} \qquad \text{Equation S1}$$

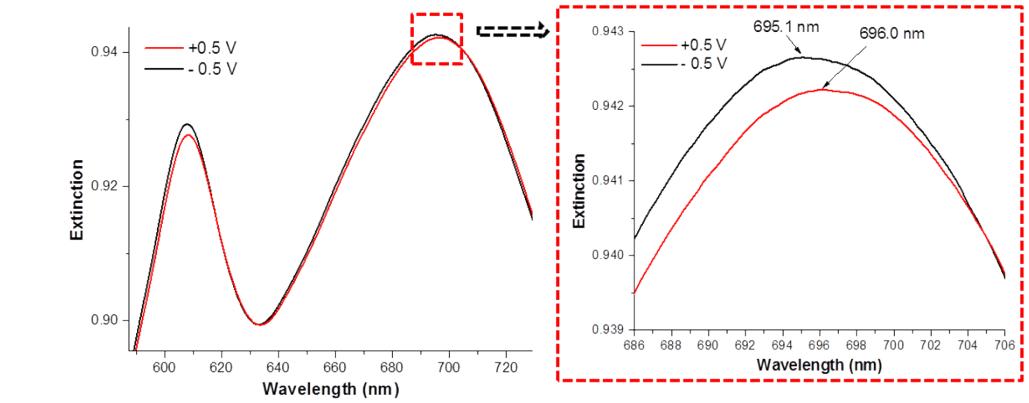
END stands for the y value of the end.
START stands for the y value of the start.
k and *n* are two parameters.
The bounds of this equation are: $k>0$, $x\geq 0$ and $n>0$.

3. Diffusion constant
The diffusion constant D is estimated as,

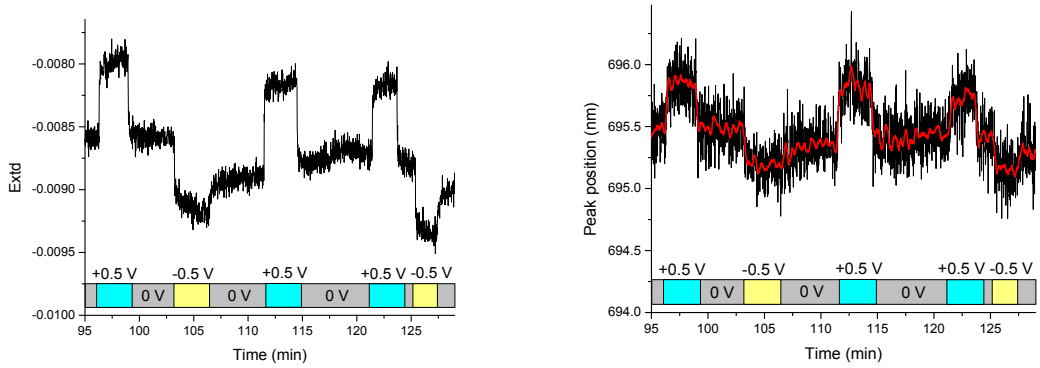
$$D = \frac{K_B T}{6\pi r \eta}, \qquad \text{Equation S2}$$

where K_B=1.38×10^{-23} m^2 kg s^{-2} K^{-1} is the Boltzmann constant, T is the absolute temperature, η=8.9×10^{-4} kg m^{-1} s^{-1} is the viscosity of the water and r is the radius of the sphere approximating the molecule size. Troponin I has the molecular weight of 23.8 KDa with radius about r=2 nm.

Fig. S1a shows the extinction spectra changes under applied biases on the AuNH sensor chip. Fig. S1a, S1b and S1c indicate the resonant peak red-shifted upon applied positive biases and blue-shifted with negative biases on the AuNH sensor chip, as measured in PBS buffer.



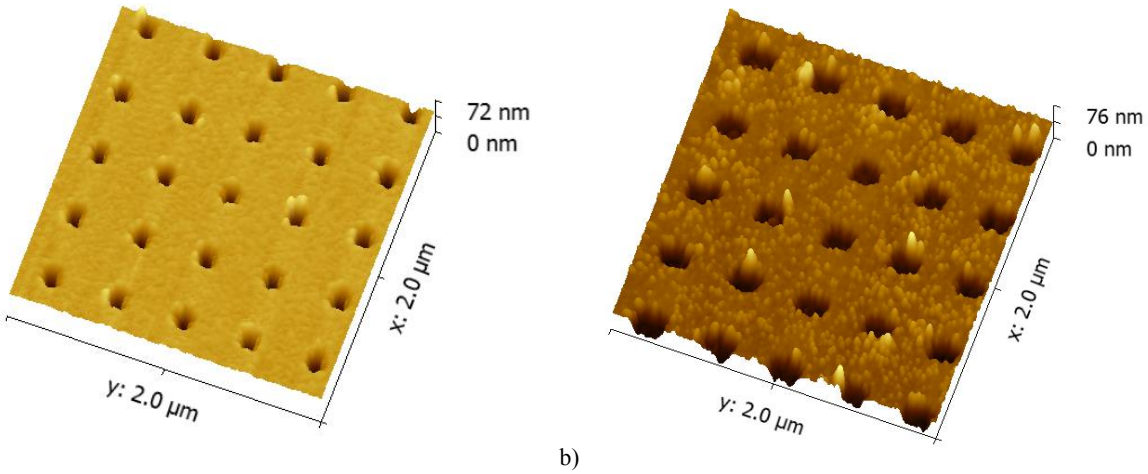
a)



b) c)

Fig. S1. a) Extinction spectra of the AuNH array applied with -0.5 V and +0.5 V biases. The time dependent b) extinction difference (Extd) and c) peak position changes upon applying different biases on the sensor surface.

Fig. S2 indicates the increase of surface roughness due to the binding of cTnI on the AuNH sensor chip.



a) b)

Fig. S2. AFM imging of the AuNH array a) before and b) after 30-min incubation of 3 µg/ml cTnI in PBS buffer under -0.2 V bias applied on the sensor chip.

Fig. S3 shows the low nonspecific adsorption of cTnI at concentration from 1 ng/ml to 1 µg/ml on the surface modified with CALNN peptides.

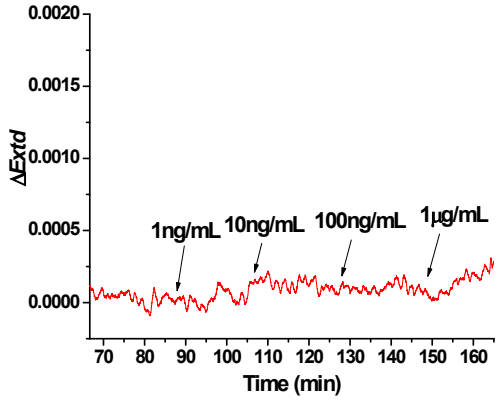


Fig. S3. LSPR response (*ΔExtd*) of the AuNH array modified by CALNN under no bias, after the injection of cTnI at the concentration of 1ng/mL, 10ng/mL 100ng/mL and 1µg/mL.

Fig. S4 shows about two-fold lower nonspecific adsorption of 3 mg/ml BSA on the peptide-modified AuNH array chip after appling negative bias (-0.2 V) as compared with the one without bias (0 V).

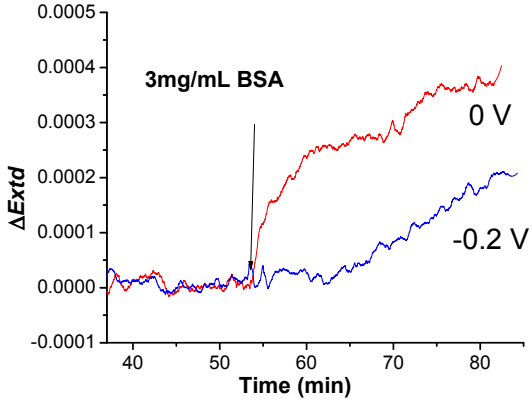


Fig. S4. Real-time monitored LSPR response (ΔExtd) to the incubation of 3 mg/ml BSA on the peptide-modified AuNH array under 0 V and -0.2V.

Fig. S5 shows the nonspecific adsorption of 10× diluted rabbit serum on the AuNH sensor surface is about 2 fold lower after applying -0.2 V bias.

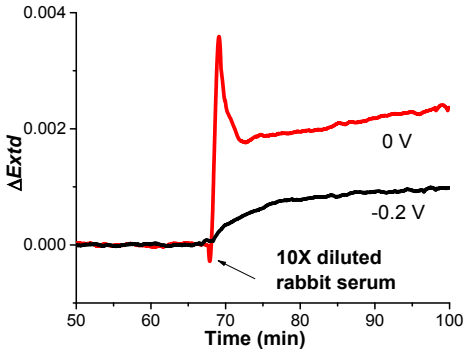


Fig. S5. Real-time monitored Δ*Extd* upon the injection of 10× diluted rabbit serum on the peptide-modified AuNH array under bias of 0 V and -0.2V.

Fig. S6 shows the calibration curve for the detection of cTnI in 10× diluted rabbit serum. The sensor response is corresponding to Δ*Extd* after 30 min incubation of cTnI subtracted to the Δ*Extd* due to serum adsorption. The limit of detection (LOD) is estimated to 25 ng/ml, based on the noise level 3σ~0.0001.

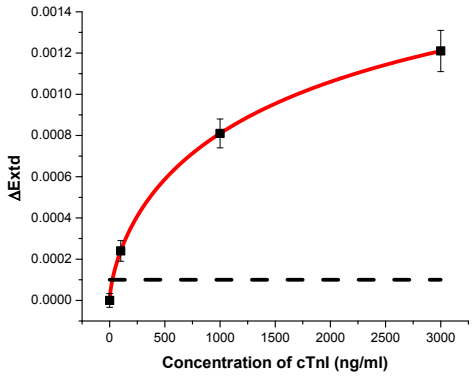


Fig. S6. Calibration curve for the detection of cTnI in 10× diluted rabbit serum. Dash line shows the noise level (3σ).

References:

[1] a) Y. Wang, L. Wu, X. D. Zhou, T. I. Wong, J. L. Zhang, P. Bai, E. P. Li, B. Liedberg, Sensors and Actuators B-Chemical 2013, 186, 205-211; b) T. I. Wong, S. Han, L. Wu, Y. Wang, J. Deng, C. Y. L. Tan, P. Bai, Y. C. Loke, X. D. Yang, M. S. Tse, S. H. Ng, X. Zhou, Lab. Chip 2013, 13, 2405-2413.