Supplementary Material (ESI) for Chemical Communications

Supporting information

High Sensitivity and Selectivity of Human Antibody Attachment at the Interstices between Substrate-Bound Gold Nanoparticles

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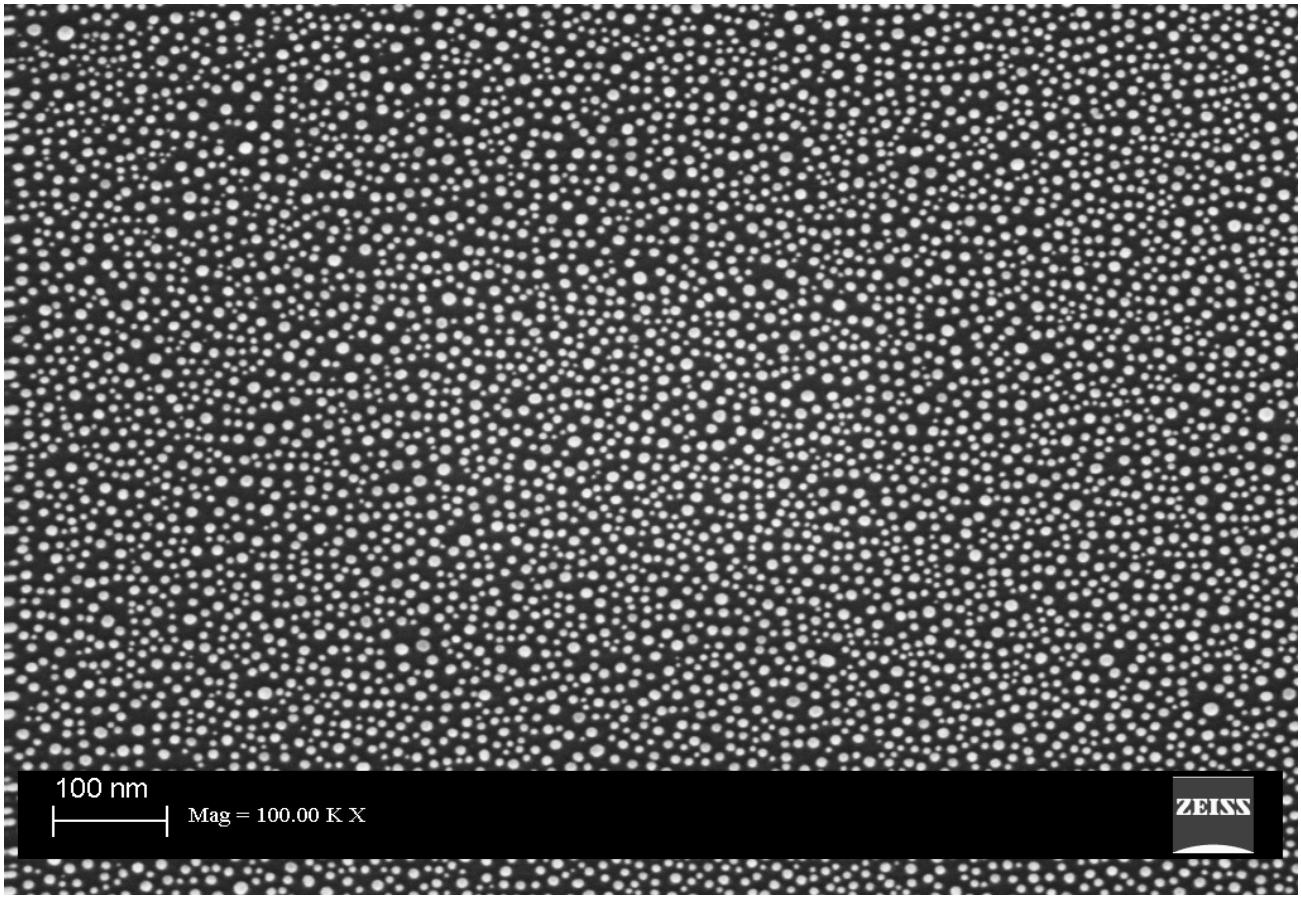

Fig S1. The original field-emission scanning electron microscopy image of gold nanopartlces on the substrate.

1. Reproducibility of Au-LSPR biochips:

The LSPR nano-biosensor will full bright for development of fast, real time, low-cost, label-free and miniaturized device in the future, especially in the field of point-of-care clinical diagnostics. However, it always suffers a huge hindrance to the precise control over the noble metal nanostructures with reproducible optical properties of LSPR. Thus far, it still lacks of effective methods for overcoming such critical hurdle. Here, we provide a proposal that gold nanostructures directly fabricate onto glass with unique slightly embedding properties via microwave plasma heating. As shown in Figure S1, every chip has realized the reproducibility of Au-LSPR biochips with UV/Vis record, demonstrating future potential in disposable LSPR chips.

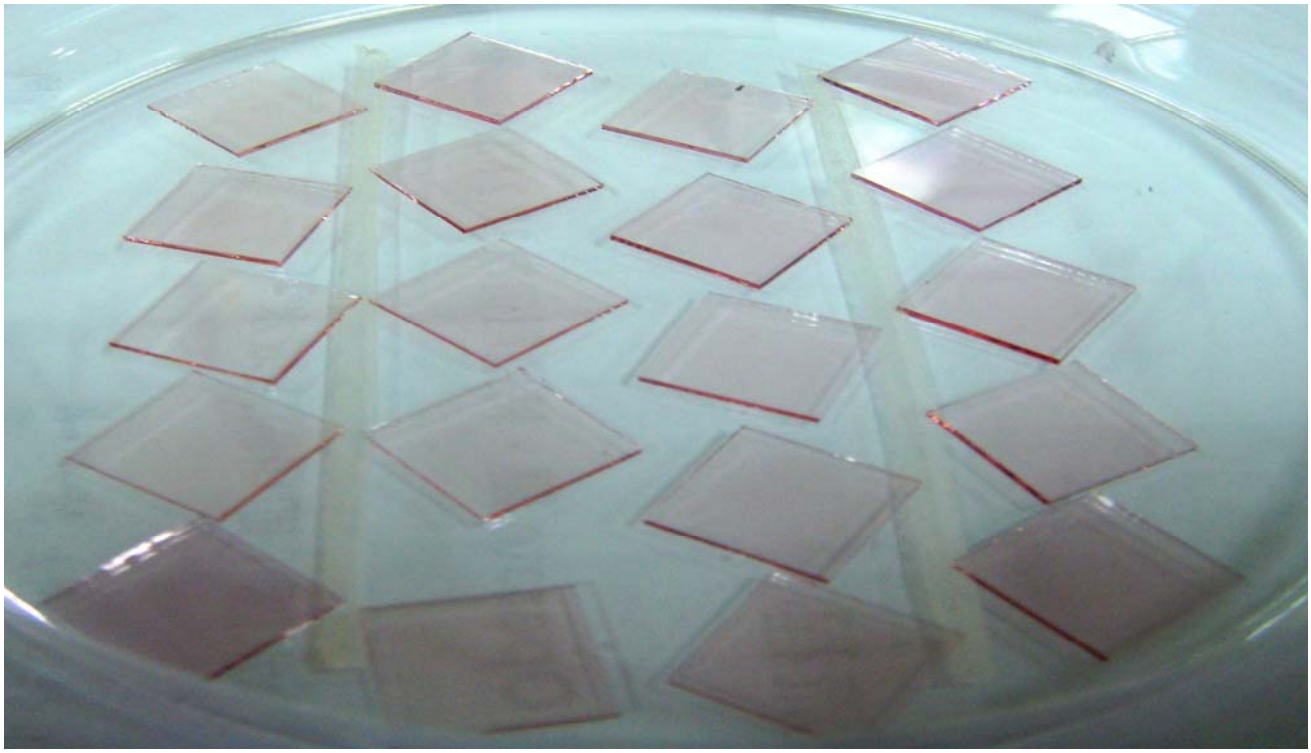

***Figure S2*.** Twenty Au-LSPR chips with the precise control over size, shape, and surface density of gold nanoparticles , showing high reproducibility.

2. Stability test:

It is important for plasmonic chips to be able to pass stability testing without causing signal changes. As such, we operate our Au-LSPR biochips in a series of solvents including DI-water, PBS buffer, and ethanol to prove substrate stability. As shown in Figure S2, when the as-prepared gold-bound plasmonic substrate was exposed to solvents, the optical extinction showed insignificant changes, indicating that the surface of gold nanostructures on the substrate is highly stable and has no detachment or deformation. It also illustrates that the signal transduction of the optical extinction of LSPR indeed comes from the binding of analytes of interest.

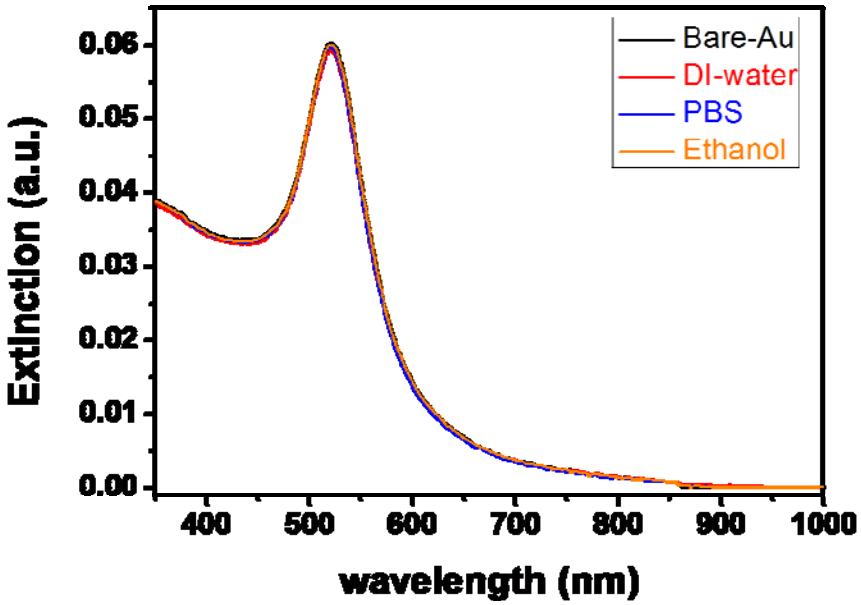

Figure S3. Optical extinction of Au-LSPR in solvents

3. Oxidation effect:

Metal nanostructures are extra active and thus oxidize in air. Hence, the oxidation effect is also an important parameter in the establishment of trustworthy LSPR readout signal. Figure S3 firmly indicates that the optical extinction of LSPR caused small changes due to the thin oxidation layer at the surface of gold nanostructures in air after one day. It seems that the as-prepared gold nanostructures on the glass substrate were initially prone to oxidation. Our Au-LSPR biochips can be stored at room temperature for several days without any optical changes.

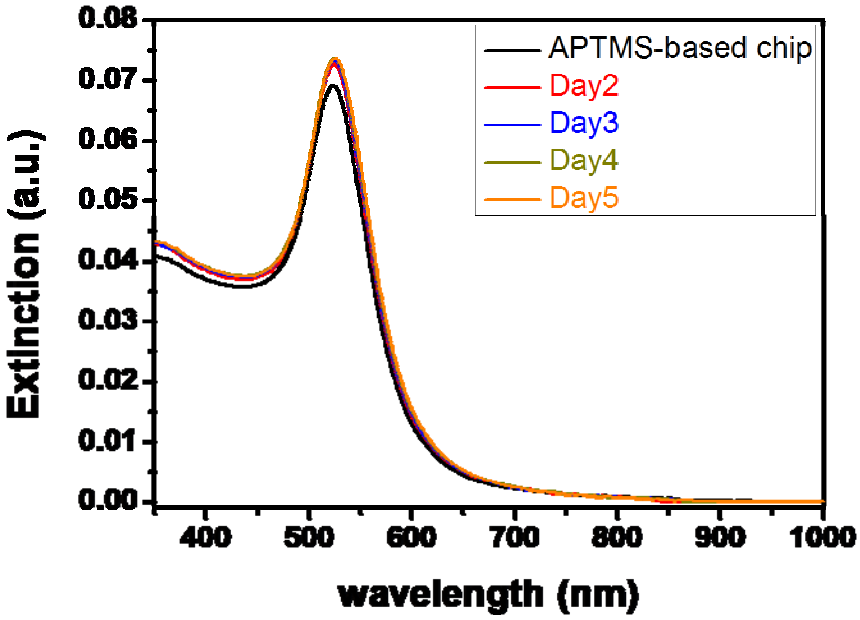

Figure S4. Probing oxidation effect of Au-LSPR biochips in air.

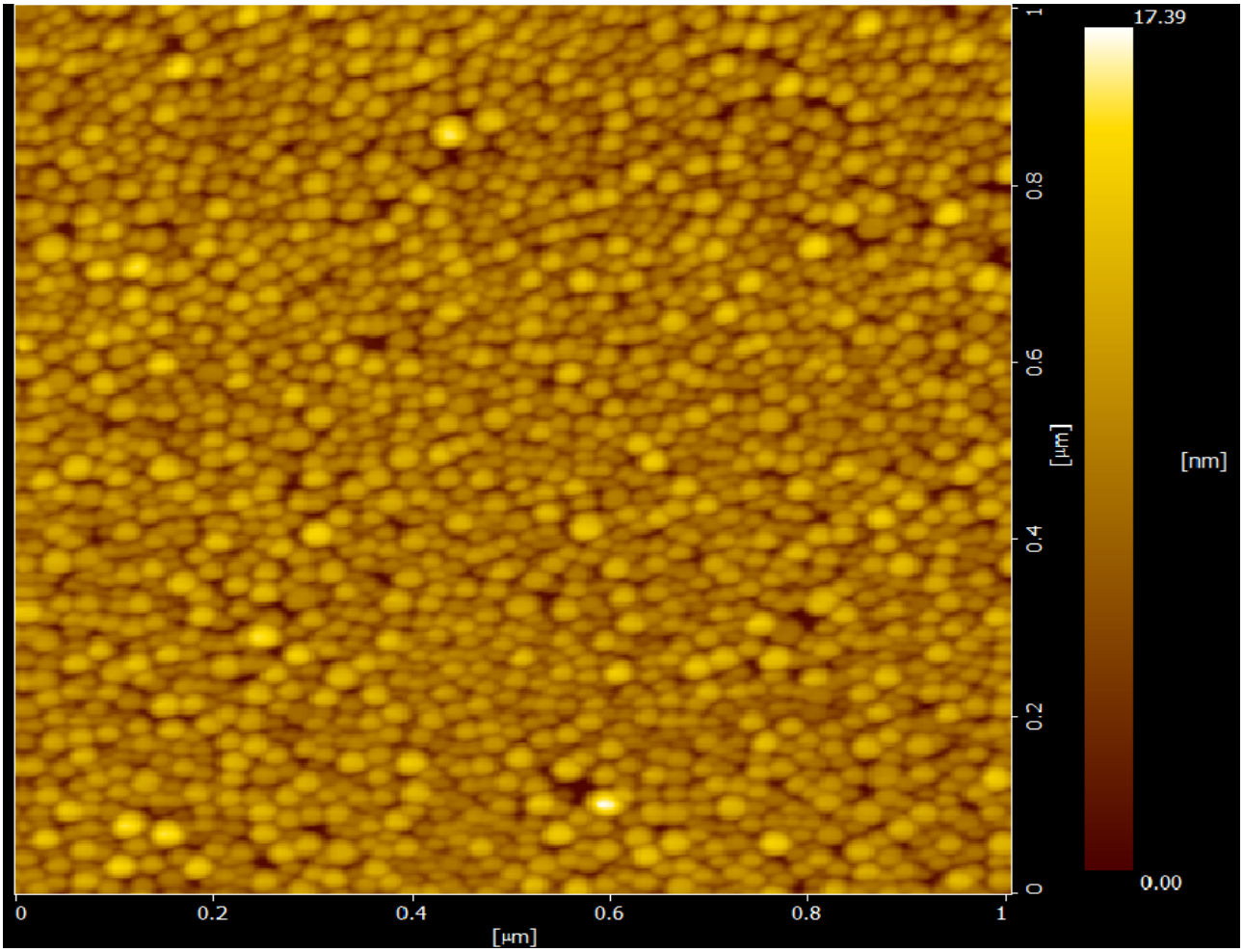

Figure S5. AFM image of Au-LSPR chip.

4. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra:

In the Figure S4, two significant features at 1641 and 1527 cm^{-1} are assigned to the amide I and amide II bonds of IgG.[1] The amide I band is attributed to the C=O stretching vibrations of the peptide moieties and the amide II band arises from C–N stretching modes of the polypeptide chains. Upon anti-IgG attachment, the two amide bands shift to higher frequencies of 1647 and 1531 cm^{-1}, respectively. In addition, there is a significant enhancement of the absorbance peaks at 1727 and 1445 cm^{-1}, which may be assigned to carboxylic acid C=O stretching and symmetric COO^- stretching, respectively. These results are interpreted as an increase in the symmetry of the α-helix structure of the polypeptide during the specific IgG and anti-IgG recognitions.

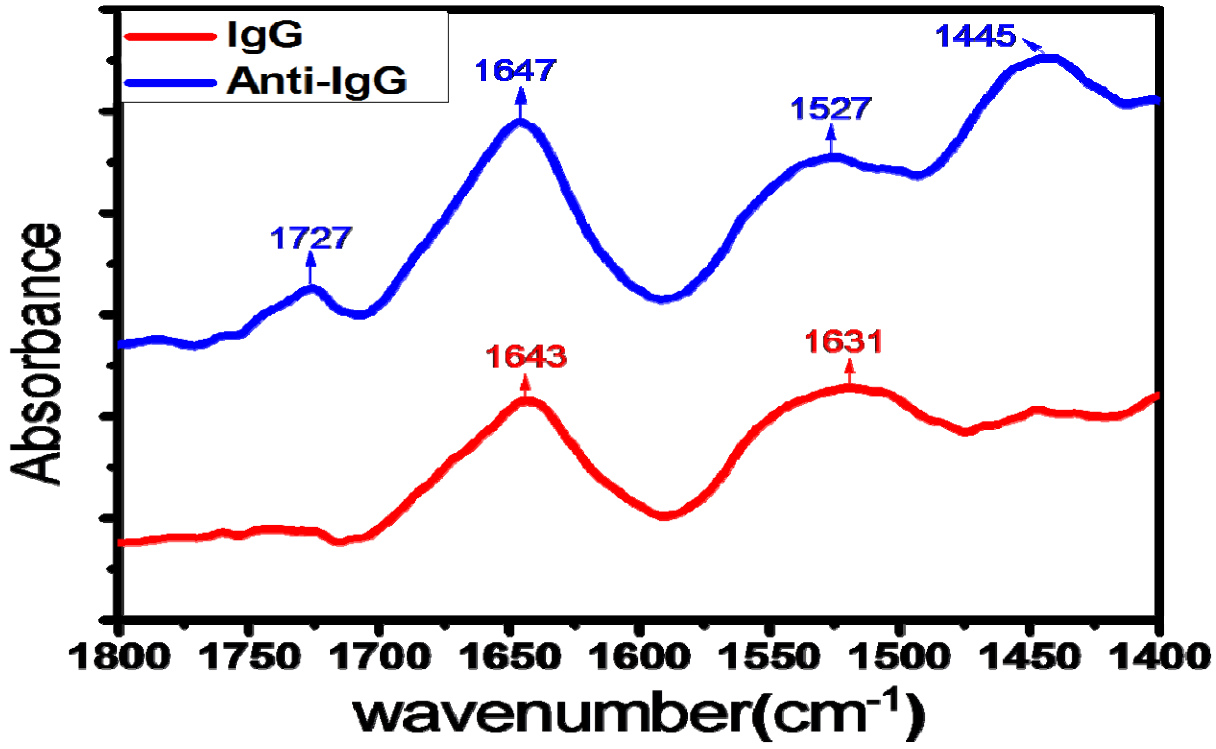

Figure S6. ATR-FTIR spectra of IgG-immobilized biochips before (red line) and after (blue line) anti-IgG attachment.

5. Monitor optical changes in real time for exposed to sensor chips:

We were able to observe biomolecular binding in real time. The extinction increase upon anti-IgG attachment was dependent on the anti-IgG concentration. Fig S6 shows the observation of the response of LSPR via stepwise-increasing concentrations from 2 ng/mL (13 pM) to 2000 ng/mL (13 nM) in the same chip, where the anti-IgG molecule weight is about 150 KDa. The saturated concentration of the Au-LSPR chip was determined to be about 1000 ng/mL (6.6 nM) from the lack of change in the optical extinction maximum after being exposed to 2000 ng/mL. Thus, our Au-LSPR biochips are adapted to slight analyte detection within a narrow low-concentration range.

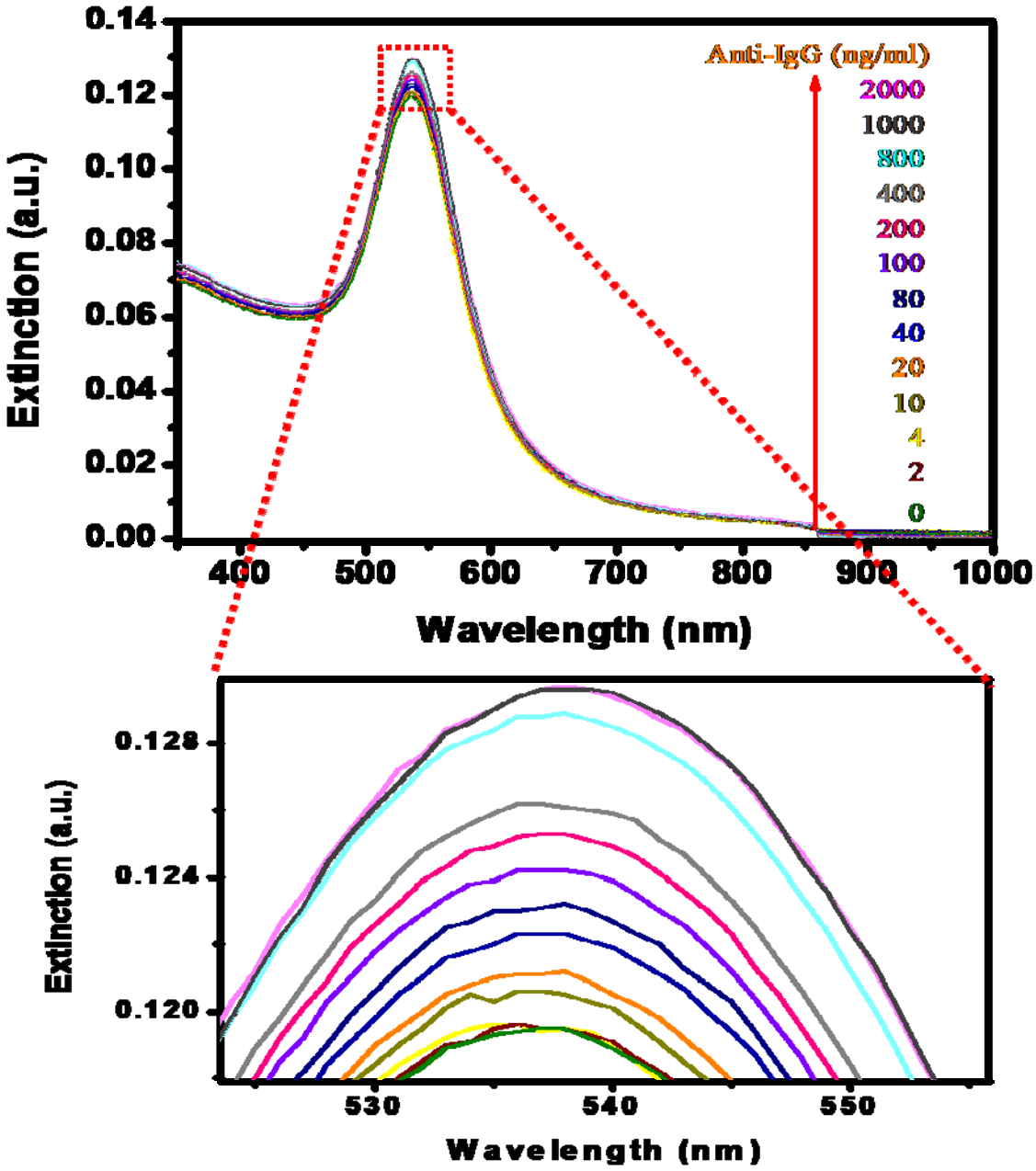

Figure S7. Optical extinction of IgG-based Au-LSPTR chips as a function of the anti-IgG concentration.

6. Materials and methods:

Materials*:* Aminopropyltrimethoxysilane (APTMS) was purchased from Sigma-Aldrich. Glutaraldehyde 50 % (GA) was purchased from Fluka. Immunoglobulin G and Anti Immunoglobulin G were purchased from Sigma-Aldrich. Phosphate-buffered saline (PBS) solutions of 10 mM, pH 7.4, were purchased from Uni Region. Ultrapure water (18.2 MΩ·cm) from Millipore was used for the preparation of all reagents, unless otherwise stated.

Characterizations: All UV/Vis transmission extinction spectra collected are macroscopic measurements performed in air using a PerkinElmer Lambda 990. Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra collected are

performed in air using a PerkinElmer Specstrum 100. Field-emission scanning electron microscopic (FE-SEM) images were obtained using a Zeiss ULTRA plus.

Fabrication of Au-LSPR glass substrates: Glass slides were cleaned thoroughly by ultrasonication for 30 min in ethanol, soaked in piranha solutions (3:1 concentrated H_2SO_4: 30% H_2O_2) for 30 min, rinsed thoroughly with ultrapure water, and then dried under a stream of nitrogen gas. (***Warning:*** *Piranha solution is hazardous and highly reactive. It may explode on contact with organic solvents. Extreme care and precaution must be taken at all times.)* The specimens processed through the aforementioned cleaning steps were placed inside a Quorum K575X Turbo sputter coater to deposit the Au films thereon. A film thickness measurement instrument was used to control the thicknesses of the films deposited on the specimens to 2 nm. Next, the specimens were exposed to home microwave plasma within 40 s in accordance with the protocol of the commercial home microwave oven that previously demonstrated that plasma ions can be generated as a novel heating source.

Self-assembled monolayer (SAM) of APTMS on the hot spot regions of Au-LSPR substrate: First, the LSPR substrates were treated with Harrick Plasma Cleaner for 5 min to achieve a hydrophilic surface; they were then incubated in the 10 mM APTMS solution for 3 h, rinsed with ethanol, PBS buffer solution, and deionized water, and dried with N_2 gas. Next, APTMS-treated substrates were treated for 2 h at 80 °C in the vacuum oven, providing stable cross-linking siloxane bonding between APTMS and silica substrates.

Immobilization of GA on APTMS/Au-LSPR substrate: The immobilization of GA on the APTMS/Au-LSPR substrates was achieved by standard imine coupling chemistry. Briefly, the surface of APTMS/Au-LSPR substrates was incubated in a 10 w/w % GA solution at 20 ^oC for 1 h to form new imine bonding between APTMS and GA molecules, rinsed with PBS buffer solution and deionized water, and dried with N_2 gas.

Immobilization of IgG on GA/APTMS/Au-LSPR substrate: The obtained GA/APTMS/Au-LSPR substrate was directly incubated in 100 ppm IgG PBS (pH 7.4) solution at 20 ^oC for 12 h, rinsed with PBS buffer solution and deionized water, and dried by N_2 gas.

Label-free detection of Anti-IgG: IgG molecules with precise control of concentration were stepwise introduced onto the IgG-immobilized Au-LSPR chips for periods of 15 min for every concentration, followed by thorough rinsing with PBS buffered solution to remove the nonspecific binding events.

Selectively binding of different anti-IgG to human IgG-modified Au-LSPR chips: Rabbit, mouse and human anti-IgG with the same concentrations of 2 μg/ml were introduced onto the human IgG-modified Au-LSPR chip in order at 20 ^oC for 1 h.

7. Reference

1. F. Caruso, D. N. Furlong, K. Ariga, I. Ichinose and T. Kunitake, *Langmuir*, 1998, **14**, 4559.