

Electronic supplementary information (ESI) for
**Towards a high-throughput label-free detection system
combining localized-surface plasmon resonance and
microfluidics**

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† Electronic supplementary information (ESI) available.

Reagents

(3-Aminopropyl) trimethoxysilane (APTMS) (#281778, 97%) and ethanol (#34923, SPECTRANAL, $\geq 99.8\%$) were from Sigma-Aldrich. Acetone (#0701001, A.R.) was from Beijing Chemical Works. Four biotinylation linkers: EZ-Link Sulfo-NHS-Biotin (#21217, MW: 443.43, spacer arm: 13.5 Å), EZ-Link Sulfo-NHS-LC-Biotin (#21335, MW: 556.59, spacer arm: 22.4 Å), EZ-Link Sulfo-NHS-SS-Biotin (#21331, MW: 606.69, spacer arm: 24.3 Å) and EZ-Link NHS-PEG₁₂-Biotin (#21312, MW: 941.09, spacer arm: 56.0 Å), and dithiothreitol (DTT) (#20290) were from Thermo Scientific (Fig. 4a). Streptavidin (#bs-0437P) and Human IgG (#bs-0297P) were from Beijing Biosynthesis Biotechnology Co., Ltd. Ethylene glycol-thiol (PEG-thiol) (#TH 001-m11.n6-1), HS-(CH₂)₁₁-EG₆-OH, was from ProChimia. Glutaraldehyde (GA) (#1131189, electron microscopy grade) was from Aladdin. Recombinant HIV-1 envelope gp41 antigen (#HIV-112, which is fused to β -galactosidase and has a total molecular mass of 146 kDa) and HIV-1 gp41 antibody (#ANT-160, which could be considered as IgG antibodies with molecular mass of 150 kDa) were from ProSpec-Tany TechnoGene Ltd. Phosphate-buffered saline (PBS) (#ST476, 135 mM NaCl, 4.7 mM KCl, 10 mM Na₂HPO₄, 2 mM NaH₂PO₄, pH 7.4) was from Beyotime Institute of Biotechnology.

Fabrication of Au-LSPR substrate

We newly prepared plasmonic gold nanostructure on the glass surface with the optimized protocol, aiming to a uniform localization (i.e. diameter and interparticle gap) of the AuNPs (Fig. S3†). The partial encrusting of the AuNPs within glass enables the tight encasing of AuNPs that survives organic solvent (e.g., ethanol) and the mechanical peeling of the adhesive tape.

Initially, glass slides (10.0 mm × 10.0 mm, Corning Eagle 2000) were soaked in 20% H₂O₂ solution for 30 min, rinsed with ultrapure water and dried under a stream of nitrogen gas. Au film with a thickness of approximately 2 nm was deposited onto a glass substrate to form a piece of Au-sputtered glass. This coating technology was accomplished using a magnetic sputter instrument (SC3000, Quorum Tech). The thickness was controlled and monitored by a film thickness monitor controller. 20 samples of 2 nm-thick Au film glass were subsequently put into a microwave oven (1800 W; 2.45 GHz) equipped with a quartz chamber. When the pressure of the quartz chamber reached 0.3 Torr (≈ 40.0 Pa), the microwave generator would turn on to ignite Ar plasma ions for 45 s. On the basis of analysis of AFM and FE-SEM images, the 2 nm-thick Au film was converted into a monolayer of AuNPs, and size distribution of particles was in the range of 8 nm ~ 11 nm. Importantly, such as-fabricated nanoparticles were embedded into the surface of glass to form a very stable Au-LSPR substrate. Furthermore, UV-VIS spectra showed that the LSPR peaks of Au-LSPR substrates were of 525 nm ~ 530 nm.

The microwave plasma treatment could produce stable gold nanostructures partially encrusted by the glass in a short time, and a thin (2 nm) Au film could produce uniform nanoparticles. Both features will guarantee the spectral reproducibility of the Au-LSPR substrate.

Fabrication of microfluidic channels

Briefly, we designed the photomask by CAD software and printed it into a film at a local company (Shenzhen Microcad Photo-mask Ltd). We made the master by contact photolithography (MJB4, SUSS MicroTec) using SU-8 photoresist (SU-8 2100, MicroChem). The microfluidic channels were replica-molded using poly(dimethylsiloxane) (PDMS) (Sylgard 184, Dow Corning) elastomer. We cured and cut the elastomer from the master, and punched holes at the inlets and outlets.

Surface modification

For this work, however, we must obtain a highly uniform surface because different targets compared with each other located in different areas (i.e. channels). We must eliminate the bias resulting from the surface modification instead of the targets.

We newly prepared plasmonic gold nanostructure on the glass surface with the optimized protocol, aiming to a uniform localization (i.e. diameter and interparticle gap) of the gold nanoparticles (Fig. S3[†]). We treated the surface with oxygen plasma at low RF power (7.16 W) for 5 min (Harrick Plasma Cleaner) before further functionalization, to achieve a uniform hydroxyl moiety on the glass surface. Each washing and drying steps should be careful enough to avoid the scratch of the surface.

In this study, we discussed the "length effect" and "concentration effect" by using biotin-streptavidin model system, and carried out HIV antibody detection by using another glutaraldehyde-mediated chemical crosslinking. Details are described below.

(1) Biotinylated surface:

The hydroxylated surface was incubated in 10 mM APTMS solution (acetone 3 mL + 7 μ L APTMS) for 3 h, rinsed thoroughly with 100% ethanol and deionized water in turn for 5 min 3 times, and dried under N₂ gas carefully. APTMS-treated substrate was treated for 2 h at 80 °C in a vacuum oven, providing stable cross-linking siloxane bonding between APTMS and silica substrates.^{1,2}

By amine coupling chemistry, the surface of APTMS/Au-LSPR substrate was incubated in Sulfo-NHS-Biotin, Sulfo-NHS-LC-Biotin, Sulfo-NHS-SS-Biotin or NHS-PEG₁₂-Biotin (Fig. 4a) at a given concentration (9.0 mM, 0.45 mM, 0.090 mM, 0.022 mM or 0.0090 mM) in PBS for 1 h at 20 °C, rinsed with PBS and deionized water on a shaker in turn for 5 min 3 times, respectively, and dried under N₂ gas. We call this treatment "biotinylation", and the resulting surface is "biotinylated".

(2) Protein-immobilized surface:

The Au-LSPR substrate was functionalized with APTMS as above. The silanized surface was then incubated in PEG-thiol solution (2 mM in ethanol) for 2 h (Fig. 3a), providing sulfur-gold bond.³ The substrate was rinsed thoroughly with 100% ethanol and deionized water in turn for 5 min 3 times, respectively, and dried under N₂ gas

carefully. We call this treatment "PEGylation", and the resulted surface is "PEGylated".

The immobilization of glutaraldehyde on the PEG-APTMS/ Au-LSPR substrate was achieved by standard imine coupling chemistry. Briefly, the surface of PEG-APTMS/Au-LSPR substrate was incubated in 10% GA solution (diluted in deionized water) at 20 °C for 3 h to form new imine bonding between the amino group of APTMS and the aldehyde group of GA molecules,¹ rinsed with deionized water for 5 min 3 times, and dried with N₂ gas.

This GA/PEG-APTMS/Au-LSPR substrate was incubated in 2.5 µg mL⁻¹ HIV-1 gp41 antigen (diluted in PBS) at 4 °C for 12 h to form new imine bonding between the aldehyde group of GA molecules and the amino group of the antigen,⁴ rinsed with PBS and deionized water, in turn, and dried under N₂ gas.

Label-free detection

(1) Biotin-streptavidin system:

Streptavidin solutions at a given concentration were incubated onto the biotinylated Au-LSPR substrate for 20 min, followed by thorough rinsing with PBS and deionized water, and dried under N₂ gas carefully.

(2) HIV detection system

The maximal dilution times can be used to determine the sensitivity when the concentration of the sample is unknown.⁵ 10, 100, 1000, 10000, and 100000 times diluted (in PBS) rabbit antiserum against gp41 were introduced into microfluidic channels. The last channel was introduced by 1 µg mL⁻¹ human IgG (diluted in PBS) as the negative control. The antibodies in the antiserum could specifically bind to gp41 antigens which were covalently linked to the glass surface by glutaraldehyde. After 20 min incubation (Fig. S5), the solutions were removed. The microfluidic chip was peeled off from the substrate. The substrate was rinsed thoroughly with PBS and deionized water by gentle pipetting, in turn, and dried under N₂ gas.

When we measure the extinction spectra after the target is captured by the substrate, we should peel off PDMS channels from the substrate, because the optical absorption of PDMS is not negligible. The ability to reversibly seal PDMS against glass substrates with gold nanoparticles is thus required to the success of this assay.

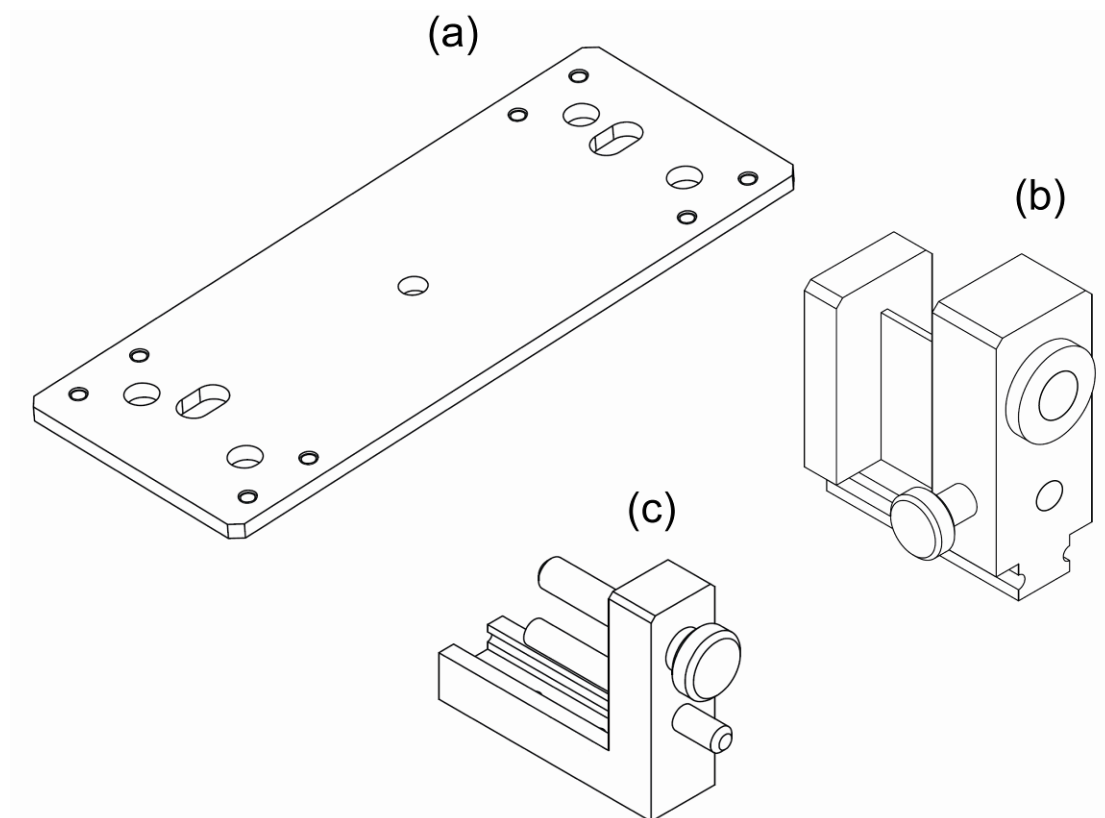


Fig. S1: Some separate parts of the optical bench. (a) The base punched holes fitting to the upper elements and the lower central hole; (b) the movable holder; (c) the adjustment with the clamping bar.

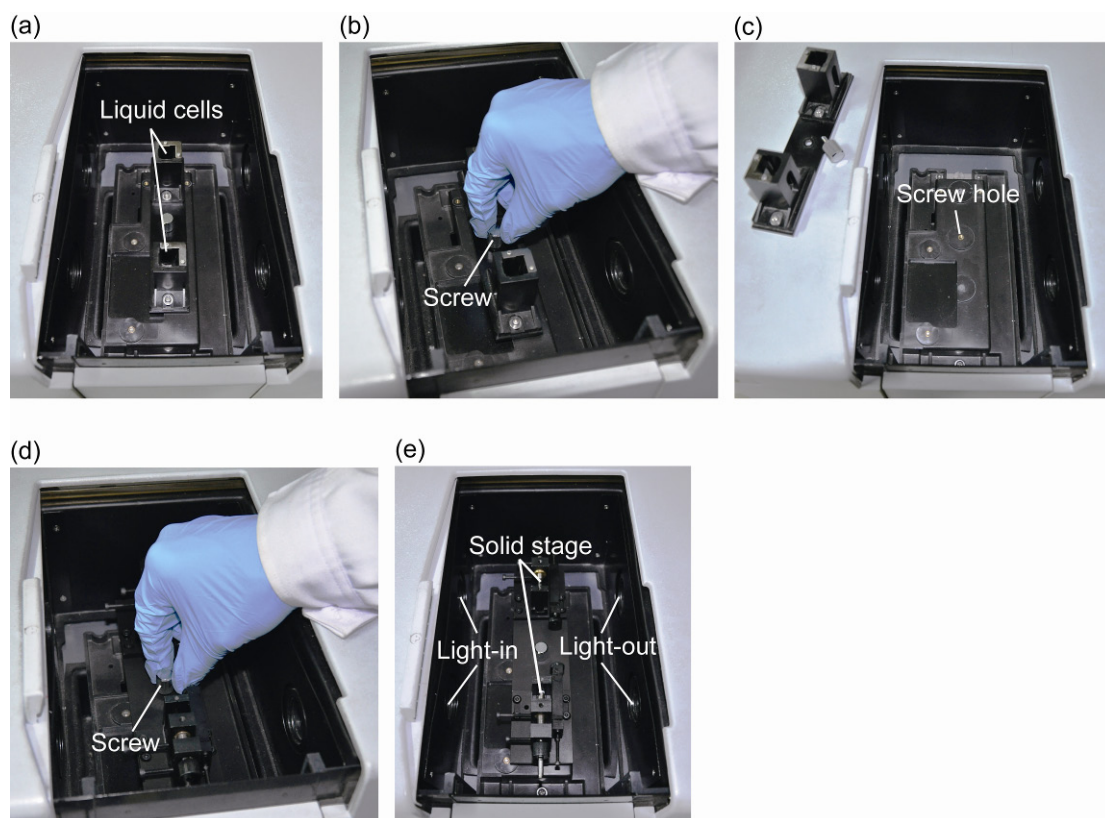


Fig. S2: The displacement of standard liquid cells with our newly designed optical bench in which the solid samples can be loaded. (a) The original assembly with standard liquid cells. (b) We disassemble the liquid cells by removing the screw out of the hole. (c) The empty cavity. (d) We assemble the new optical bench by putting the screw in the hole. (e) The final assembly of the solid stage fitting to the dual optical path system.

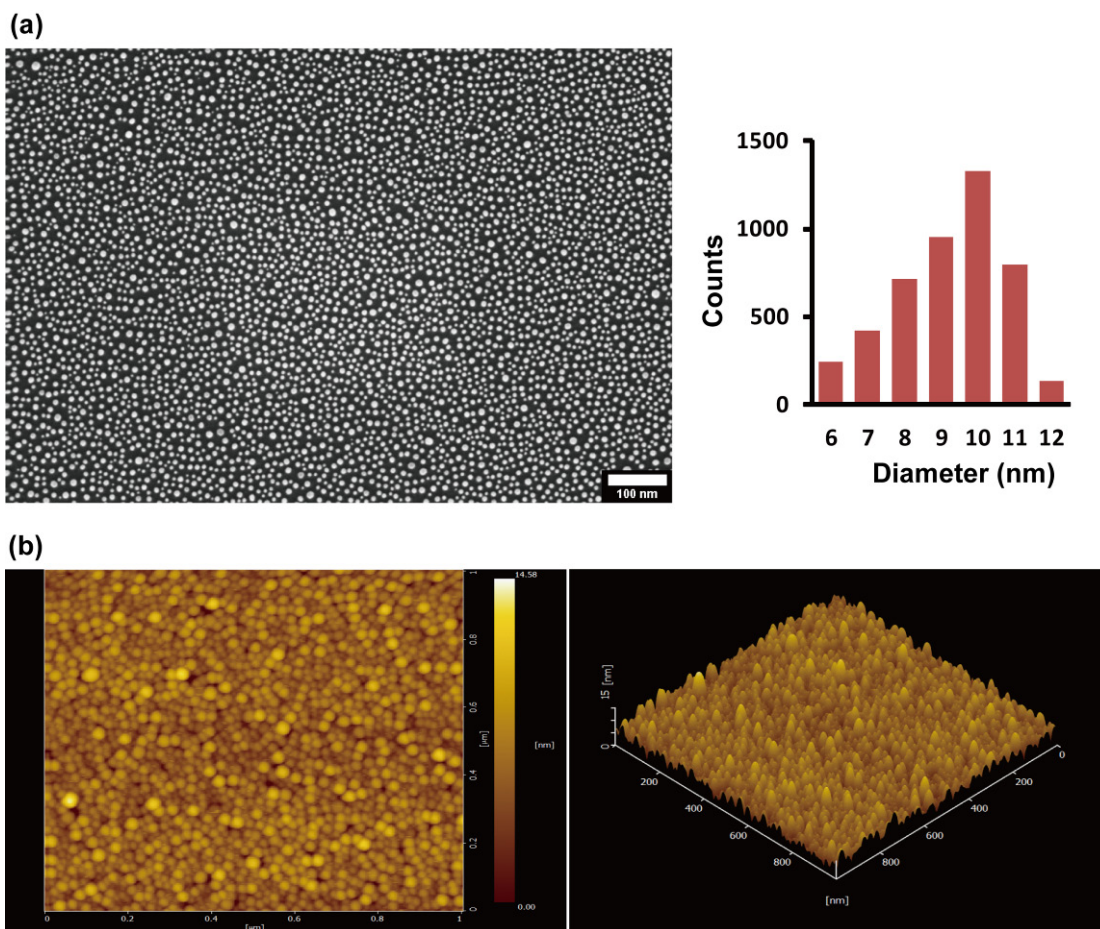


Fig. S3: The characterization of the surface of bare Au-LSPR substrate. (a) FE-SEM image of the surface (Zeiss ULTRA plus) and the diameter distribution. (b) AFM image of the surface (the top view and the 45° angle view of the same area) (SPI 4000, Seiko Instruments Inc.). The statistical analysis show that the diameter of the gold nanoparticles is $8.7 \text{ nm} \pm 1.5 \text{ nm}$ (the mode is $\sim 10 \text{ nm}$) and the depth of the interstices is $\sim 10 \text{ nm}$.

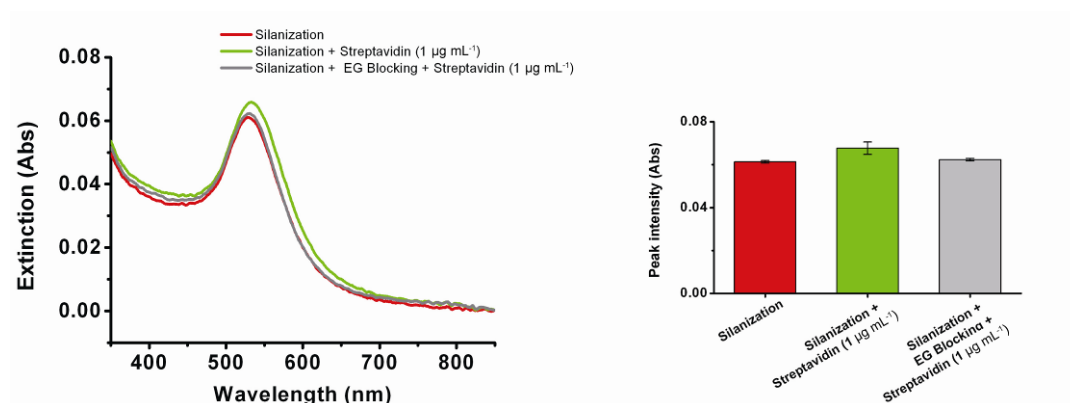


Fig. S4: The nonspecific binding of streptavidin to the silanized substrate can be repressed by poly(ethylene glycol). The red and green curves represent the extinction spectra of the silanized substrate before and after immobilization of streptavidin, respectively. The gray curve represents the extinction spectrum of another substrate which is silanized and PEGylated, followed by immobilization of streptavidin. These experiments were repeated three times and the statistical analysis for the peak intensity of each curve (mean value \pm 1 standard deviation) is showed in the column diagram. The effect of nonspecific binding has set the demand for blocking, and the results suggest PEG-thiol is an effective blocking reagent.

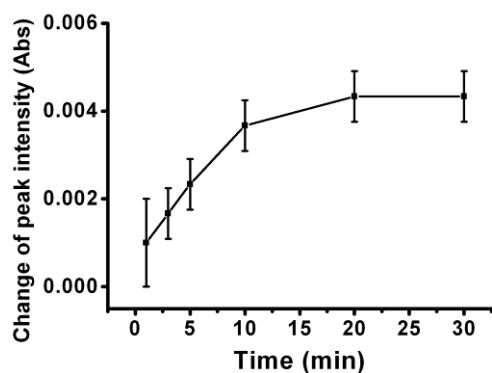


Fig. S5: Time-dependent measurement. The antiserum against gp41 was diluted 1000 times, and was incubated with the Au-LSPR substrate covalently immobilized with the gp41 antigen (all in microchannels). We stopped the incubation at different time points and measured corresponding extinction spectra. The peak intensity resulted from the antibody-antigen interaction increased along with the incubation time of antibody in a gradually slow-down manner. The result showed that the peak intensity could reach saturation in 20 min.

Anti DEV-1 serum end point ELISA

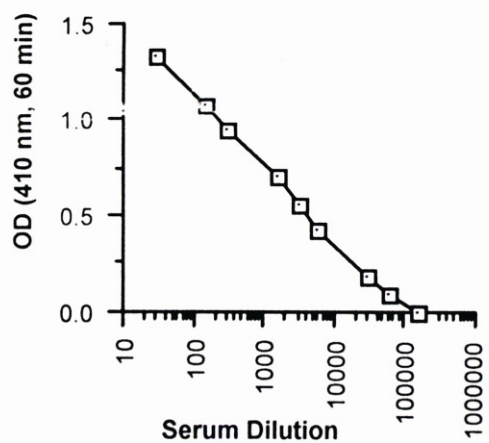


Fig. S6: The titration curve measured by the manufacturer (ProSpec-Tany) of the antibody. This picture is from the specification sheet of the product.

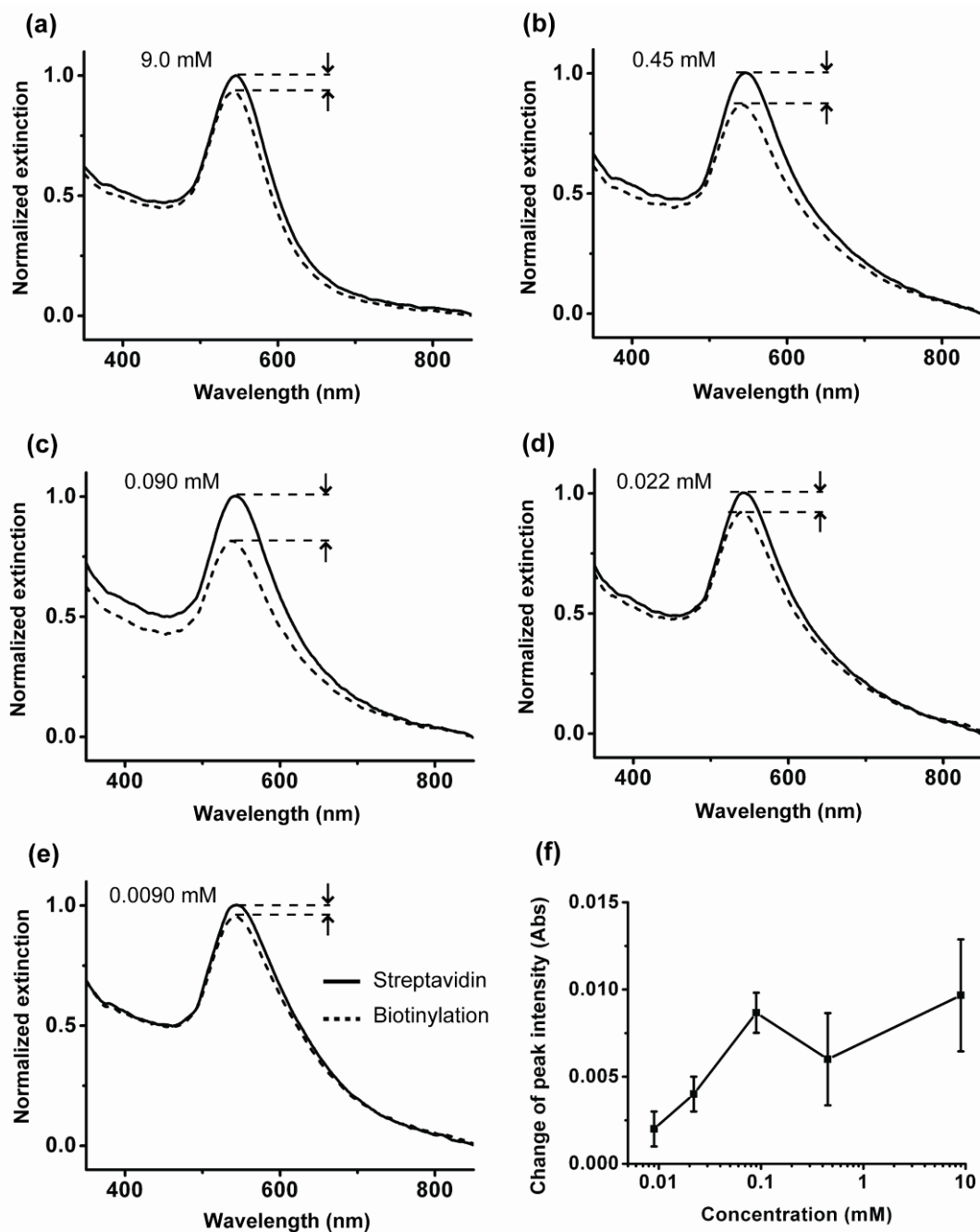


Fig. S7: The optimization for the concentration of the biotinylation linkers. The biotinylation linker is Sulfo-NHS-SS-Biotin. The concentration of streptavidin detected was also $1 \mu\text{g mL}^{-1}$. The double-headed arrow indicates the change of peak intensity between biotinylation and streptavidin binding, representing the response of the biotinylated chip. The concentrations from (a) to (e) are 9.0 mM, 0.45 mM, 0.090 mM, 0.022 mM and 0.0090 mM, respectively. The results show that the optimal concentration of biotinylation linker is 0.090 mM (i.e. (c)) due to the relative best response. Higher and lower concentration showed the worse response. Too high

concentration of linker molecule gave a large variation of the response (i.e. (f)). Error bar in (f): standard deviation.

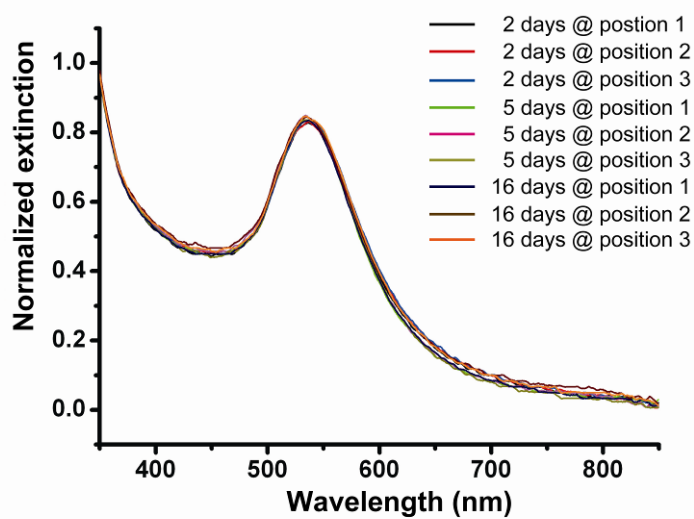


Fig. S8: The stability test for the modified surface which is stored at room temperature in air. This surface was modified by biotinylation linker. We measured the substrate from 2 days to 16 days at three different positions within the same chip. The result showed that the modified surface was highly stable.

Movie S1: This video showed the procedure of assembly of the optical bench with the UV-VIS spectrophotometer. We took down the standard liquid cell from the instrument before we assembled the newly designed optical bench into the cavity. This video was recorded by a digital camera (D90, Nikon).

References for Electronic Supplementary Information (ESI)

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