A Label-Free Strategy for *In-situ* Analysis of Protein Binding Interaction Based on Attenuated Total Reflection Surface Enhanced Infrared Absorption Spectroscopy

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Supporting Information (SI)

Preparation of an IR enhanced Film and Calculation the SEIRA Enhancement Factor.

A layer of gold film was chemically deposited on a hemisphere Si prism following the steps: The hemispherical silicon prism was rinsed by sonication in anhydrous ethanol and ultrapure water respectively after polishing with alumina power (0.3 μm). Native silicon oxide layer was removed by immersing the surface of the silicon prism in 10% HF for 5 min, and then in 40% NH_4F for 3 min, followed by rinsing in ultrapure water. This process is known to result in Si-H surfaces. Prior to the deposition, a plating solution contained 0.015M $HAuCl_4$+0.15M Na_2SO_3+0.05M $Na_2S_2O_3$+0.05M NH_4Cl was prepared. Deposition of Au nanoparticles film was performed at 60 ^oC simply by dropping a mixture of 1.0 mL plating solution + 0.5 mL 2% HF onto the hydrogen-terminated Si surface and maintained in the dark for 3 min. Ultrapure water was added to end the reaction finally. This chemical deposition process resulted in a nanoparticulated gold film with the size distributions of 50 nm on the surface of a silicon optical window as indicated by an atomic force microscopic image. The close proximity of the small islands was observed in Figure S1. The effect extent of signal enhancement can be seen in Figure S2. The apparent enhancement factor (EF) can be calculated as 30 showing one to two orders of amplification of Infrared signal for analytical protein on the presented IR enhanced substrate

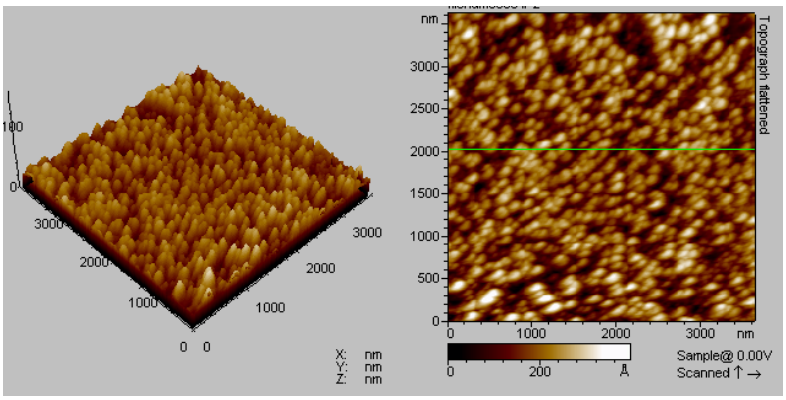

Figure S1. AFM image of nanoparticulated gold film deposited on a surface of silicon optical prism.

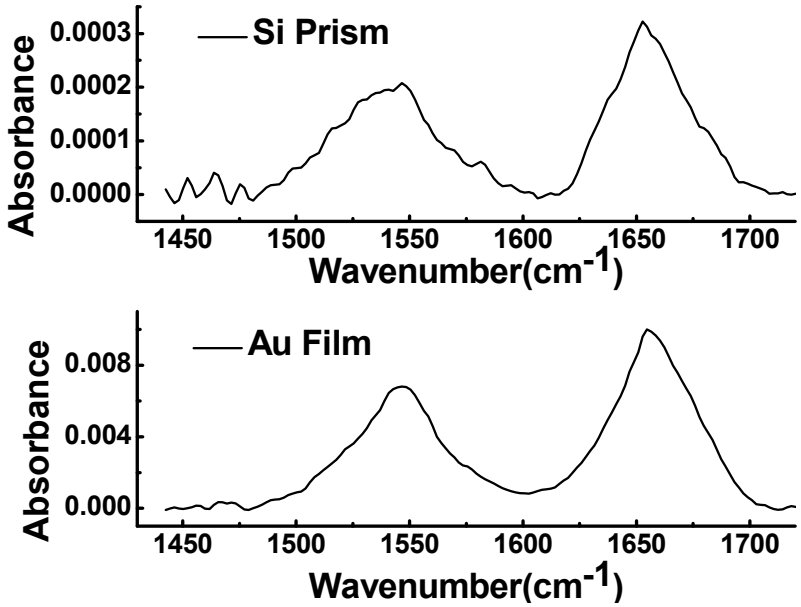

Figure S2. The surface enhanced (black) and nonenhanced (red) IR spectra of bovine serum albumin (BSA) solution (1mg/ml).

ATR-SEIRAS Characterization of the Interference from the Environment.

It is known that interfacial water molecule shows the specific bending vibration bands around 1650 cm-1 region, which may overlap with the amide I and amide II signal of target protein. In order to demonstrate the negligible effect of water in the spectrum, a background spectrum is first directly measured by ATR-SEIRAS after the immobilization of the secondary antibody on the intermediate layer formed on IR enhanced substrate, and then continuous spectra as a function of incubation time are collected after introducing the same pure PBS buffer solution (pH 7.4). The resulting IR spectra are shown in Figure S3. The IR spectra in the region of characteristic absorption bands for proteins remain almost constant within 30min. The result suggested that the environmental interferences (such as water, spectators and bulk species) are almost negligible.

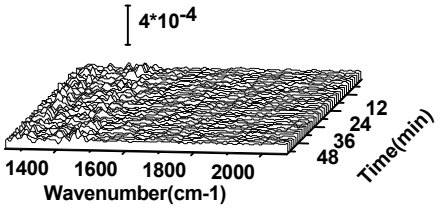

Figure S3. The continuous spectra as a function of incubation time are collected after introducing the same pure PBS buffer solution (pH 7.4) after **b**ackground spectrum is first directly measured by ATR-SEIRAS.

EIS Characterization of the Stepwise Assembly Processes and Protein Binding

Electrochemical impedance spectroscopy (EIS) measurements in PBS buffer (10 mM, pH 7.4) contains 5 mM $Fe(CN)_6^{3-/4-}$ at 210 mV reveal the successful functionalization of the chemically deposited nanoparticulated gold film, the assembling process of goat anti-rIgG and the formation of immunocomplex on the substrate. The semicircle portion at higher frequencies is attributed to the electron-transfer-limited process and the diameter of the semicircle reflects the interfacial electron transfer resistance.

It clearly shows that with the subsequently assembly of MUA, goat anti-rIgG, the electron transfer resistance (the diameter of the semicircle part at high frequencies) increases due to the blockage of the assembled layer. In addition, the electron transfer resistance further increases after the sensing surface immunobingding with rIgG (0.2 μg/mL) for 30 min. The detail functionalization process of sensing surface is specified in “Preparation of an Immunosensing Layer”. These results clearly demonstrate that the immobilization process of MUA, goat anti-rIgG on gold film is successful and the immunoreaction exactly occurs.

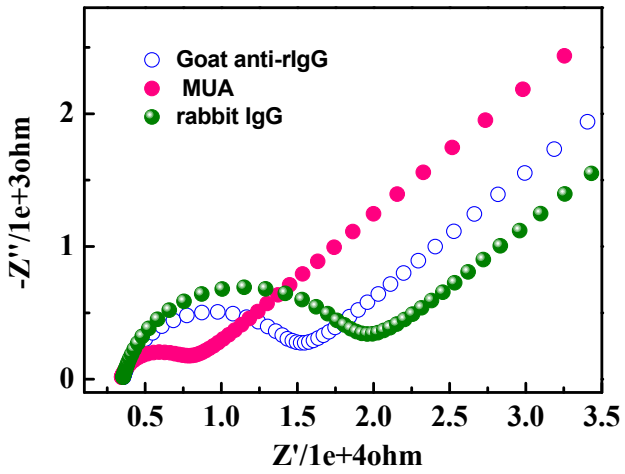

Figure S4. Nyquist plot of impedance measurement for Au substrates with stepwise assembly processes.