Supporting Information

Distance Determined Sensitivity in Attenuated Total

Reflection-Surface Enhanced Infrared Absorption Spectroscopy:

Aptamer-Antigen Compared to Antibody-Antigen

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SI 1. Experimental Section.

SI 1.1 Material and apparatus.

The hemispherical Si (111) ATR prism (36 mm in diameter) was purchased from Alkor Technologies (Saint-Petersburg, Russia). Mercapto-hexanoic acid (MHA), 6-mercaptohexanol (MCH), N-Hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) were obtained from Sigma-Aldrich. L-selectin was purchased from Sino Biological Inc. (Beijing, China) and used without further purification. The L-selectin aptamer, 5'-SH C6 GCC AAG GTA ACC AGT ACA AGG TGC TAA ACG TAA TGG CTT-3', was custom synthesized from Sangon Biotechnology Co., Ltd. (Shanghai, China). Rabbit anti-L-selectin was obtained from Boster Biological Technology., Ltd (Wuhan, China). Other reagents were of analytical grade and used as received. All aqueous solutions were prepared using ultrapure water (Millipore, USA).

Infrared spectra were measured with a Bruker Tensor27 Fourier transform spectrometer equipped with a liquid-nitrogen-cooled MCT detector. Atomic force microscopy (AFM) imaging was performed on an Agilent 5500 AFM/SPM system using tapping mode under ambient conditions. Scanning electron microscopy (FE-SEM, S-4800, Hitachi) at an accelerating voltage of 5 kV was used to characterize the morphology of the deposited gold films. Circular dichroism (CD) spectra of L-selectin and the aptamer were obtained from a J-810 circular dichroism spectrometer (JASCO, Japan). Electrochemical impedance spectroscopy (EIS) measurement was performed with a CHI electrochemical workstation (CH Instruments, U.S.A.) with a bias potential of +0.22 V and the frequency ranging from 0.01 Hz to 100 kHz in a solution containing 1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) and 0.1 M KCl.

SI 1.2 Preparation of aptamer-coated gold nanoparticle film.

A gold nanoparticle film with efficient SEIRAS-activity was first deposited on a hemispherical silicon prism via galvanic displacement reaction. In detail, the hemispherical silicon prism was rinsed by sonication in anhydrous ethanol and ultrapure water respectively after polishing with alumina power (1 μ m). Native silicon oxide layer was removed by immersing the surface of the silicon prism in 40% NH₄F for 3 min, followed by rinsing with ultrapure water. Deposition of an Au nanoparticle film was performed at 60 °C simply by dropping a mixture of 1.0 mL of plating solution containing 0.01 M HAuCl₄ + 0.1 M Na₂SO₃ + 0.033 M Na₂S₂O₃ + 0.033 M NH₄Cl + 0.5M HF onto the hydrogen-terminated Si surface and maintaining in the dark for 30-60s. Ultrapure water was added to end the reaction finally. The morphology and structure of Au nanoparticle film was characterized by SEM and AFM (Figure S1). The SEIRAS substrate was then immersed in 400 μ L 0.25 μ M L-selectin aptamer overnight to obtain a homogeneous DNA layer. In order to eliminate the electrostatic repulsion interaction among DNA strands, the solution of aptamer was prepared in 1 M NaCl. The modified Au NP film was subsequently dipped in 325 μ L 0.3 mM MCH for 30 min to remove aptamers physically adsorbed on the surface and acted as passivation layer. Afterwards, the surface was rinsed with 50 mM PBS (pH=7.4) solution.

SI 1.3 Preparation of antibody-coated gold nanoparticle film.

Similarly, an Au NP film was first chemically deposited on the silicon optical prism. Afterwards, the SEIRA substrate was immersed in 400 μ L 5 mM 6-Mercaptohexanoic acid (MHA) overnight to form a stable self-assembled layer terminated with carboxyl groups. 400 μ L mixed solution of

25 mM EDC/NHS (1:1, in 10 mM PBS, pH=5.8) was added to the surface at room temperature for 30 min and then the activated surface was immediately incubated in 500 μ L 10 μ g/mL anti-L-selectin (in 50 mM PBS, pH=7.4) for 1 h after rinsing with 50 mM PBS. To minimize the non-specific interactions, the surface was soaked in 300 μ L 1% BSA for 10 min and carefully rinsed by PBS buffer.

SI 1.4 ATR-SEIRAS detection of aptamer/antibody-protein binding.

Detection was processed on a FTIR spectrometer with a homemade ATR accessory. The diameter of the detection cell is 10 mm. Unpolarized IR radiation was totally reflected at the silicon prism/solution interface with an incident angle $\theta = 70^{\circ}$ and was detected with a liquid-nitrogen-cooled MCT detector. All the spectra were plotted in absorbance unit relative to a baseline which was recorded by immersing the as-prepared surface in phosphate buffer solution for 30 min before further measurement. The O-H stretching of water was automatically subtracted by the OPUS IR software. The spectral range was 4000-1000 cm⁻¹ at a 4 cm⁻¹ resolution.

SI 2. Characterization of the Au nanoparticle film.

A shiny, golden thin film was formed on the surface of Si prism within tens of seconds. The morphology of the SEIRAS-active Au NP film was investigated with SEM and AFM. As shown in Figure S1, the chemically deposited gold film has an island structure with an average particle size of about 50 nm. The gold nanoparticles are closely packed to form large numbers of nanogaps, which have been reported to produce large infrared signal enhancement. In addition, it was investigated by AFM and XPS that the preformed Si-H bond after treatment by NH₄F facilitated the formation of silicide between Si and the Au deposits,^[1,2] so the chemically deposited Au NP film behaved strong adhesion to the silicon substrate and long-term stability during the detection. This property is particularly important for biochemical analysis and biomimetic devices.



Figure S1. (a) SEM and (b) AFM images of the gold NP film deposited on the surface of silicon semispherical optical prism at 60 °C for 30 s.

SI 3. EIS characterization of the aptamer assembly processes and protein binding.

We applied electrochemical impedance spectroscopy to characterize the entire assembly and binding process (Figure S2). The electron transfer resistance (the diameter of the semicircle part at high frequencies) of ferricyanide/ferrocynide electrochemical probes gains due to the increasing steric hindrance of the assembled layer with the subsequently assembly of aptamer, MCH and capture of L-selectin to the Au NP surface and the increased Ohmic resistance, which demonstrates the exact and successful binding of L-selectin to the stepwise assembled anti-L-selectin aptamer layer at the Au NP surface.



Figure S2. Nyquist plots of impedance measurements obtained from aptamer (black squares), aptamer + MCH (red circles) and aptamer + MCH + L-selectin (blue triangles) modified Au NP film. The buffer solution consists of 1 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1) + 0.1 M KCl, and the frequency range from 0.01 Hz to 100 kHz.

SI 4. The secondary structure analysis of L-selectin.

The secondary structure of proteins, i.e. helices, turns, sheets and unordered structures, can be analyzed by deconvolution of the amide I region.^[3,4] Here, we compared the fraction of the secondary structure of L-selectin in three different states: (1) L-selectin adsorbed on Au NP film exhibited natural secondary structures, which could be concluded from our previous work; (2) L-selectin that bound to its aptamer and (3) its antibody at the interface showed almost the same secondary structure with the adsorbed L-selectin, suggesting there was no obvious structural changes for proteins upon binding to the corresponding aptamers and antibodies.



Figure S3. Curve-fitting analysis of the secondary structure of L-selectin (a) adsorbed on the Au NP film; (b) captured by L-selectin aptamer and (c) captured by antibody of L-selectin on the Au NP film. The raw amide I bands, from Figure 1a, are presented as black lines, and the red ones are fitting lines composed of five secondary structures (green lines) shared by proteins; (d) Fractions of the secondary structures of L-selectin adsorbed on the Au NP film (black bars) and L-selectin recognized by L-selectin aptamer (dark grey bars) and anti-L-selectin (light grey bars) modified Au NP film.

| | | | - | - | - | | |
|-----|---|-----------|-------------|-----------|-------------|-----------|-------------------------------------|
| | Adsorbed L-selectin (cm ⁻¹) | | L-selectin | | L-selectin | | |
| | | Band area | binded to | Band area | binded to | Band area | A ani num out ^[5] |
| | | (a.u) | antibody | (a.u) | aptamer | (a.u) | Assignment |
| | | | (cm^{-1}) | | (cm^{-1}) | | |
| | 1621 | 5.7 | 1620 | 10.9 | 1620 | 9.4 | Antiparallel β-sheet |
| | 1634 | 28.5 | 1634 | 30.4 | 1634 | 24.7 | Parallel β-sheet |
| | 1645 | 9.2 | 1644 | 7.5 | 1645 | 8.2 | Unordered |
| | 1656 | 49.1 | 1657 | 43.4 | 1654 | 48.6 | α-helix |
| | 1678 | 7.5 | 1678 | 7.8 | 1677 | 9.1 | Turn |
| 1.1 | | | | | | | |

Table S1. Positions (cm⁻¹) and fractional areas (%) of the amide I bands of L-selectin adsorbed on Au NP film and L-selectin recognized by L-selectin aptamer modified Au NP film.

SI 5. Circular dichroism characterization of the secondary structure of aptamer and protein.

Circular dichroism is a useful technique to study the interaction between DNA and protein, and herein it was applied to provide supplementary structural information in the specific recognition of L-selectin to its aptamer. L-selectin aptamer exhibits as typical B-type DNA: a positive band appears in the vicinity of 280 nm, while a negative one at around 245 nm, and the ellipticity turns into zero at about 260 nm.^[6] The CD spectrum of L-selectin shows a double-negative peak located at 208 nm and 220 nm separately, which is a representative character shared by α -helix-rich proteins.^[7] The spectrum of aptamer-protein complex comprises of the sum signal from L-selectin aptamer and L-selectin in the region above 240 nm (the CD spectra of DNA below 220 nm attract less attention because slight secondary structural changes usually give rise to large signal variations in this spectral range^[8,9]), implying that only minor changes in the secondary structure of aptamer take place upon binding to the target protein.



Figure S4. Circular dichroism spectra of 1.0 μ M L-selectin aptamer (black line), 0.30 μ M L-selectin (red line) and their complex (blue line).

SI 6. Numerical simulation of the near-field intensity distribution around the surface of Au nanoparticle film.

The simulation was carried out using the COMSOL Multiphysics. The Au NP film made of numerous nanoparticles was simulated as an array of hexagonal close-packed gold ellipsoid (Figure S5). The lengths of the semi-principal axes of each ellipsoid were a=b=25nm and c=5nm with periodic distance P=47nm, which was obtained from the results of SEM and AFM. A Si substrate with a refractive index of 3.4 was located at the bottom of Au ellipsoid array and the medium refractive index was set as 1.33. The incident planar wave, with the wave vector and electric field vector along the z-axis and y-axis respectively, excited the surface plasmon of Au NP film.



Figure S5. Illustration of the structure unit of Au NP film as the model for numerical simulation.

SI 7. Quantitative determination of protein at the interface via the intensity of amide II bands.

The area of amide II band in ATR mode has been widely applied for quantitative analysis of proteins.^[10] Jeon et al.^[11] integrated the spectral area of amide II and calculated the adsorption density of BSA on a polyurethane-coated ZnSe prism. The peak intensity of amide II is also used to analyze the amount of protein in a few reports.^[12] In order to verify the feasibility of the latter method, the association rate constant was calculated based on the same set of data respectively by fitting the binding time-dependent intensity and area of amide II bands. The experiment was repeated three times and similar rate constants were obtained with an average relative standard deviation (RSD) of 2.4%. Therefore, the peak intensity of amide II band was used for quantitative analysis of L-selectin.



Figure S6. (a) Series of the SEIRA spectra as a function of binding time in a solution of 50 nM L-selectin + 50 mM PBS (pH=7.4) on the surface of aptamer-modified Au NP film; (b) The peak intensity (black squares) and area (red circles, divided by 50) of amide II bands obtained from spectra in (a) and fitting curve (red and blue lines for intensity and area, respectively) as functions of binding time.

SI 8. Modified two-compartment model.

The binding kinetics of proteins in the solution to captured aptamers at an interface is typically modeled as a two-compartment reaction. In this model, both reaction and transport kinetics are incorporated. In our measurement based on ATR-SEIRAS, proteins are unlabeled, leading to rapid rates of diffusion, so the concentration of proteins in the reaction compartment can be quickly replenished by diffusion of the bulk proteins.

The binding equilibrium of aptamer-protein interaction is described as:

A (aptamer) + P (protein)
$$\underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} M$$
 (aptamer · protein)

the net reaction rate and dissociation constant can be defined by the following set of equations:

$$\frac{d[M]}{dt} = k_1([A] - [M])([P] - [M]) - k_{-1}[M]$$

$$K_d = \frac{[A]_e[P]_e}{[M]_e} = \frac{([A] - [M])([P] - [M])}{[M]}$$
(1)
(2)

(2)

where k_1 and k_{-1} are the association and dissociation reaction rate constants respectively; K_d is the dissociation equilibrium constant; [A], [P], and [M] are the concentrations of aptamer, protein and their complex in the reaction compartment, respectively; while $[A]_e$, $[P]_e$, and $[M]_e$ are the equilibrium concentrations of aptamer, protein and their complex in the reaction compartment, respectively.

Since aptamers recognize their target proteins with nanomolar affinities, the dissociation of surface bound complexes can be ignored in the measurements of binding kinetics. As a result, the two equations mentioned above are transformed to:

$$[M] = [A][P] \frac{1 - \exp[-k_1([P] - [A]) t]}{[P] - [A] \exp[-k_1([P] - [A]) t]}$$
(3)

$$[M] = \frac{[P] + ([A] + K_d) - \sqrt{[P]^2 + 2(K_d - [A])[P] + ([A] + K_d)^2}}{2}$$
(4)

therefore the association rate constant and association equilibrium constant could been obtained by fitting our experimental data to the above two equations.

SI 9. SEIRAS characterization of the binding kinetics of the protein at a low concentration to its aptamer.

It could take a longer period of time to attain a nearly saturated binding for a lower concentration of the protein solution. To ensure the attainment of equilibrium state, the binding kinetics of 1 nM target protein to its aptamer was investigated. We found that a nearly saturated binding event could been obtained within 30 min for 1 nM target protein in solution (Figure S7), so we believed that it was reasonable to choose 30 min as the equilibrium binding time for quantitative analysis.



Figure S7. The intensity of amide II bands for 1 nM of L-selectin on the aptamer-modified Au NP film surface as a function of binding time.

SI 10. SEIRAS characterization of the binding kinetics and thermodynamics of protein to its antibody.

The kinetics and thermodynamics of immune interaction between L-selectin and its antibody were investigated in comparison with that from binding of L-selectin to its aptamer. The association rate constant is calculated to be 2.3×10^4 M⁻¹·s⁻¹, slightly smaller than the aptamer-protein's. As shown in Figure S5, it takes a longer time for L-selectin to achieve the equilibrium state on the anti-L-selectin decorated Au NP film surface, quite probably because of low flexibility and motional degree of freedom (DOF) for the antibody layer compared with aptamer. The association equilibrium constant is 6.2×10^7 M⁻¹, indicating that anti-L-selectin and L-selectin aptamer have the similar high affinity towards their target.



Figure S8. (a) Series of the SEIRA spectra as a function of binding time in a solution of 50 nM L-selectin + 50 mM PBS (pH=7.4) on the surface of anti-L-selectin modified Au NP film; (b) The intensity of amide II bands (black squares) of spectra in (a) and fitting curve (red line) as a function of binding time; (c) ATR-SEIRA spectra of L-selectin recognized by immobilized anti-L-selectin from different concentrations of L-selectin in the bulk solution. The concentrations from top to bottom are as follows: 92.4, 73.6, 51.6, 34.8, 15.9, 5.5 nM. All the spectra were collected after 30 minutes' binding so as to represent the equilibrium state; (d) The intensity of amide II bands (black square) of spectra in (c) and fitting curve (red line) as a function of the concentrations of L-Selectin in solution.

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