Regioselective fabrication of gold nanowire using open-space laminar flow for attomolar protein detection

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Chemicals

Unless otherwise specified, all reagents were dissolved in water purified by a Milli-Q system (Sitaba Scientific Technology, Itd, Japan). Gold(III) chloride acid ($HAuCl_4$, Kishida Chemical Co., Ltd., Osaka, Japan), sodium borohydride ($NaBH_4$, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), tin(II) chloride ($SnCl_2$, Kanto Chemical Co., Ltd., Tokyo, Japan), hydroiodic acid (*HI*, Fujifilm Wako Pure Chemical Industries Co. Osaka, Japan), sodium fluorescein (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan), sodium chloride (*NaCl*, Fujifilm Wako Pure Chemical Industries, Osaka, Japan), disodium hydrogen phosphate (12-water) ($Na_2HPO_4 \cdot 12H_2O$, Kanto Chemical Company, Tokyo, Japan), potassium chloride (*KCl*, Kanto Chemical Company, Tokyo, Japan), potassium dihydrogen phosphate (KH_2PO_4, Nakalai Tesque Co. Ltd., Kyoto, Japan), potassium hexacyanoferrate(III) ($K_3[Fe(CN)_6]$, Kanto Chemical Co., Ltd., Tokyo, Japan) were dissolved in water. 3-mercaptopropionic acid ($C_3H_6O_2S$, Tokyo Chemical Industry Co., Ltd. Tokyo, Japan), carbodiimide (EDC) ($C_{13}H_{22}N_2$, Tokyo Chemical Industry Co., Ltd. Tokyo, Japan), N-hydroxychosuccinic acid imide (NHS) ($C_4H_5NO_3$, Tokyo Chemical Industry Co., Ltd. Tokyo, Japan), bovine serum albumin (BSA) (Fujifilm Wako Pure Chemicals Co., Ltd. Osaka, Japan), Human IgA antibody (Bethyl laboratories, Inc. Texas, USA), Human IgA (Bethyl laboratories, Inc. Texas, USA) were dissolved in PBS (potassium chloride, sodium chloride, disodium hydrogen phosphate (12-water) and potassium dihydrogen phosphate dissolved in water. pH: 7.4)

Materials

Fused silica capillary tubes (ID: 100 μm, OD: 200 μm) were purchased from GL Science Corporation (Tokyo, Japan). Glass capillaries (ID: 0.6 mm, OD: 1.0 mm) were purchased from Nihon Rikagaku Kikai K.K. (Tokyo, Japan), PEEK tubes (ID: 350 μm, OD: 500 μm) and PEEK connector and PEEK lualock were purchased from IDEX Health & Science K.K. (Saitama, Japan). Glass slides were purchased from Toshin Rikoh Corporation (Tokyo, Japan), and glass with ITO film was purchased from Geomatek Corporation (Kanagawa, Japan). Platinum wire (0.1 mm) was purchased from Nilaco Co. Silver chloride electrode (RE-1B) was purchased from BAS Inc. 10 mL gas tight syringe (1010TLL) and 1 mL gas tight syringe (1001TLL) were purchased from HAMILTON. The motorized X,Y-stage (SOM-C25E) was purchased from Sigma Koki Co. The motorized X,Y-stage controller was purchased from Sigma Koki Co. The X,Y,Z-stage was purchased from Sigma Koki Co. Petri dish (FS-70) was purchased from Flatt Corporation. Conductors were purchased from MOGAMI. The current amplifier (5725A) was purchased from FLUKE.

Fabrication of the chemical pen

Chemical pen was fabricated using the heat stretching technique. First, three glass capillaries were bundled together using heat-shrinkable tubing to make a template. Three fused silica capillaries were passed through each of the three glass capillaries. The silica capillaries coming out of the other side were bundled using a micropipette tip (Fig. S1a), and the three bundled silica capillaries were passed through a single glass capillary. (Fig. S1b) Then, the center of the glass capillary was heated to 1200°C using a micropipette preparation equipment (PB-07, NARISHIGE, Tokyo, Japan) and stretched. (Fig. S1c) The tip of the capillary was aligned by polished the cut portion with water-resistant paper, and the capillary was fixed on a glass slide with adhesive. (Fig. S1d) Finally, each of the three silica capillaries was connected to a PEEK tube and fitted with a PEEK connector and PEEK luer lock.

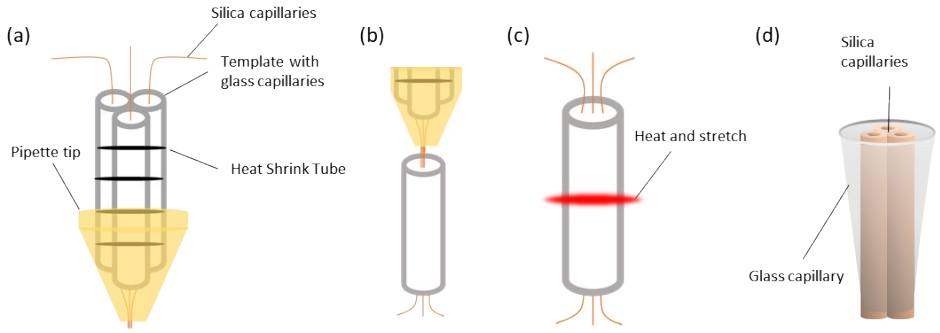

Fig. S1 Brief fabrication method of the chemical pen. (a) Three silica capillaries were bundled using a template. (b) The bundled three silica capillaries were put through one glass capillary. (c) The glass capillary was heated and stretched to fold the silica capillaries. (d) Schematic diagram of the fabricated the chemical pen.

Determine the solutions concentration by simulation (Comsol Multiphysics Software)

Because the microreaction field was formed underneath the chemical pen, the original solution was diluted in the microreaction field. Therefore, it is necessary to adjust the concentration for the suitable chemical reaction to form gold nanowire.

In this study, the concentrations of gold chloride and sodium borohydride in the microreaction field were calculated by using the simulation (Comsol Multiphysics Software) to determine how much the concentrations are diluted compared to the original concentrations (Fig.S2). Here, the gap was 30 um, the injection and the aspiration flow rates were 50 uL/h and 2000 uL/h respectively.

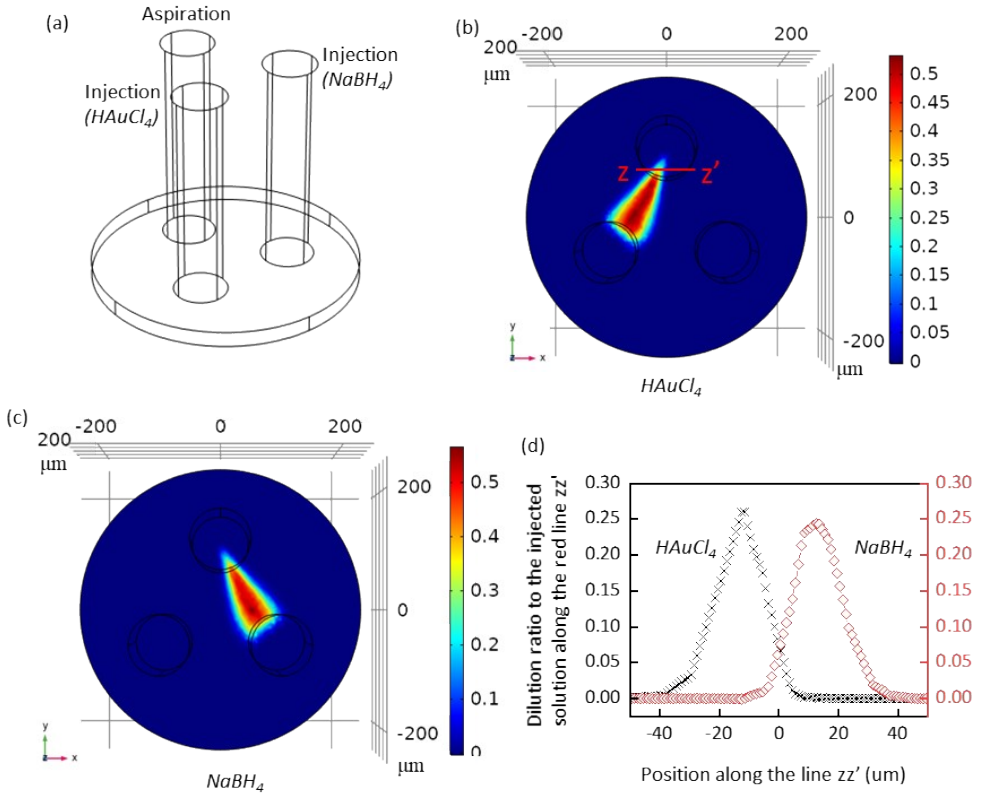

Fig. S2 Caluculated solution concentration at middle position between the substrate and the bottome surface of the chemical pen. (a) 3D model of chemical pen. (b)Concentration distribution of gold chloride. (c) Concentration distribution of sodium borohydride. (d) Dilution ratio to the injected solution along the red line zz' shown in Fig. S2b.

Formation of solution reaction field using chemical pen and investigation of gold nanowire fabrication conditions

When using chemical pen, chemical pen was fixed so that the tip of the pen was placed in a petri dish filled with distilled water (Fig. S3a). The two solutions were injected from two of the three capillaries using two 1 mL gas tight syringes and syringe pumps and injected the solutions was aspirated by the remaining capillary using a 10 mL gas tight syringe and syringe pump. At this time, the two injected solutions mixed underneath the aspiration capillary of chemical pen and a chemical reaction occurred. This area is called the solution reaction field. By moving the chemical pen to a desired location using the XY stage, the solution reaction field could also be moved to the desired location. To create nanowires, the ratio Q_A/Q_I (where Q_I represents the injection flow rate and Q_A represents the aspiration flow rate), the Gap (the distance between the tip of chemical pen and the substrate), and the moving speed of the substrate by an automatically controlled XY stage can be optimized to reduce the size of the solution reaction field, resulting in nanometer-sized wires. Q_A and Q_I can be changed with the syringe pumps, Gap with a Z-stage, and drawing speed with a computer program. In this study, we optimized each condition (Q_A/Q_I, gap, drawing speed) to achieve gold nanowires (Table S1). The width of the fabricated gold wire was measured using Inverted microscope (LX-71, Olympus Corporation, Tokyo, Japan), CCD camera (DP72, Olympus Corporation, Tokyo, Japan) and imaging software (cellSens standard, Olympus Corporation, Tokyo, Japan) (Fig.S3b-d).

Table. S1 Experimental conditions.

Q_A / Q_I	Gap / μm	Drawing speed / μm・s^{-1}
10	20	0.2
20	20	0.2
40	20	0.2
60	20	0.2
80	20	0.2
100	20	0.2
120	20	0.2
60	**5**	0.2
60	**10**	0.2
60	**20**	0.2
60	**30**	0.2
60	**40**	0.2
60	**50**	0.2
60	20	**0.5**
60	20	**0.33**
60	20	**0.25**
60	20	**0.2**

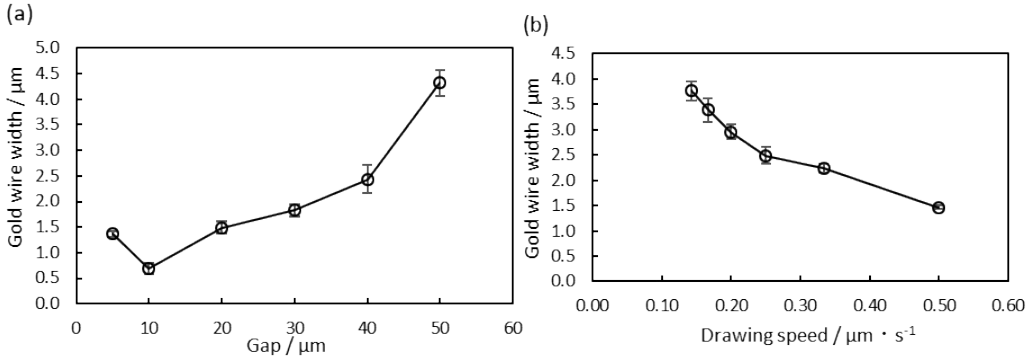

Fig. S3 Width of gold wire in each condition as optimization results. (a) Gold wire width when gap was changed. (b) Gold wire width with various drawing speeds.

Fabrication and Examination of gold nanowire

From Fig. S3, we inferred the conditions under which gold nanowires can be stably fabricated, and gold nanowires were drawn on the glass substrate under the conditions shown in Table S2. The fabricated gold nanowire was also observed using the tabletop SEM (Thermo Scientific Phenom ProX G6, Thermo Fisher SCIENTIFIC) and AFM (AFM5100N, Hitachi High-Tech Corporation, Tokyo, Japan). Elemental analysis was also performed by the EDS system installed in the tabletop SEM.

Table.S2 Conditions of gold nanowire fabrication.

Q_A / Q_I	Gap / μm	Drawing speed / μm・s^{-1}
180	10	0.2

Electrochemical measurements

Gold nanowires with a width of 300 nm were drawn over a 3 mm area using a chemical pen near the boundary between the ITO area and the area where the ITO was removed under the conditions shown in Table S3. Then, enamel coating was applied for 2 cm from the boundary between the ITO and the ITO-removed area to prevent electrochemical reaction at the ITO area. (Fig.S4a) Gold nanowires were drawn from the boundary between the ITO part and the part where the ITO was removed using chemical pen under the conditions shown in Table S3. Cyclic voltammetry measurements were performed using this as the working electrode. In addition, an enamel coating was applied to the ITO glass part to avoid an extra electrochemical reaction (Fig. S4a). A silver/silver chloride electrode was used as the reference electrode, and a commercially available platinum wire with a diameter of 100 μm was used as the counter electrode. The Petri dish was filled with 1 mM of potassium ferricyanide ($K_3[Fe(CN)_6]$) solution in PBS (pH7.4). The working electrode, reference electrode, and counter electrode were then immersed in the solution, and cyclic voltammetry measurements were performed by varying the scan rate between 0.005 V/s and 0.2 V/s using the Electrochemical Analyzer (ALS model 802D, BAS Inc, Tokyo, Japan) (Fig.S4b).

Table. S3 Conditions of gold nanowire fabricated by chemical pen for CV measurement.

Conditions	
Nanowire length / μm	3000
Q_A/Q_I	120
Gap /μm	10
Drawing speed / μm・s^{-1}	0.2
wire width / nm	300

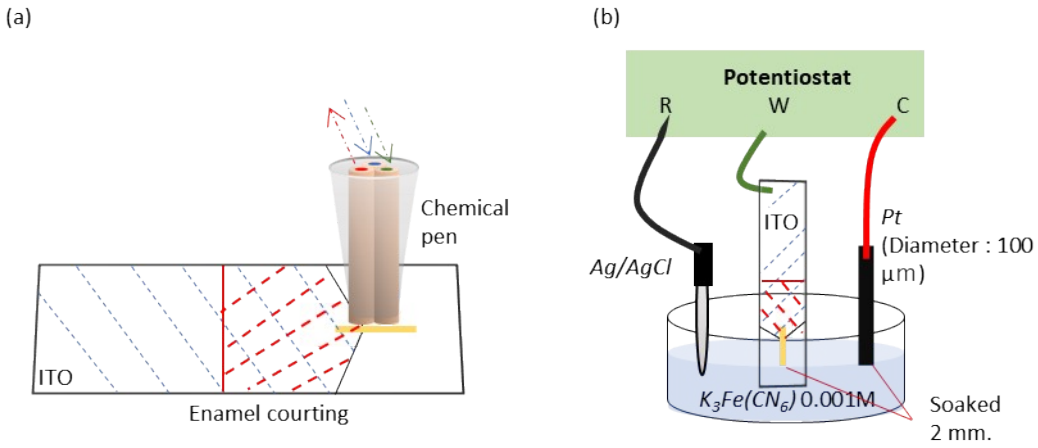

Fig. S4 Scheme of CV measurement. (a) Fabrication of working electrode with chemical pen. (b) Scheme of CV measurement.

Setup of the detection system

Detection system is shown in Fig. S5a. In the detection system, the voltage is amplified and measured using a computer. A resistances of known size were connected to the electrical circuit, and the voltage value at this time was measured. The relationship between resistance and voltage was then determined (Fig. 4a). As a good linear relationship was obtained from Fig. 4a, we can say that this detection system is reliable.

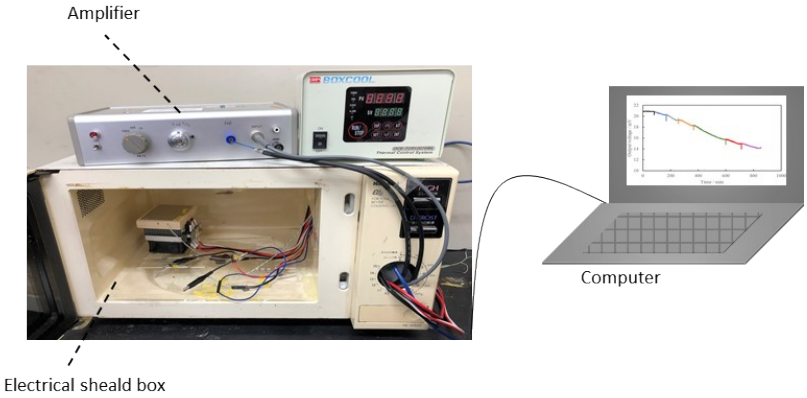

Fig. S5 Detection system configuration.

Fabrication of baiosensor

The ITO glass was covered with a mask and chemically etched by immersed in hydroiodic acid (HI) for 30 min (Fig. S6b). Then, gold nanowires were fabricated using a chemical pen under the conditions shown in Table S4 (Fig. S6c), and conductive wires were connected to both ends of the ITO using conductive adhesive. Finally, masking (FEP adhesive sheet film.) was applied to expose only the gold nanowires, and the sample slot (Cut off the tip of a 1 mL pipette tip.) was placed (Fig. S6d).

Table.S4 Conditions of gold nanowire fabricated by chemical pen for biosensor.

Conditions	
Nanowire length / μm	2000
Q_A/Q_I	120
Gap / μm	10
Drawing speed / μm・s^{-1}	0.2
wire width / nm	300

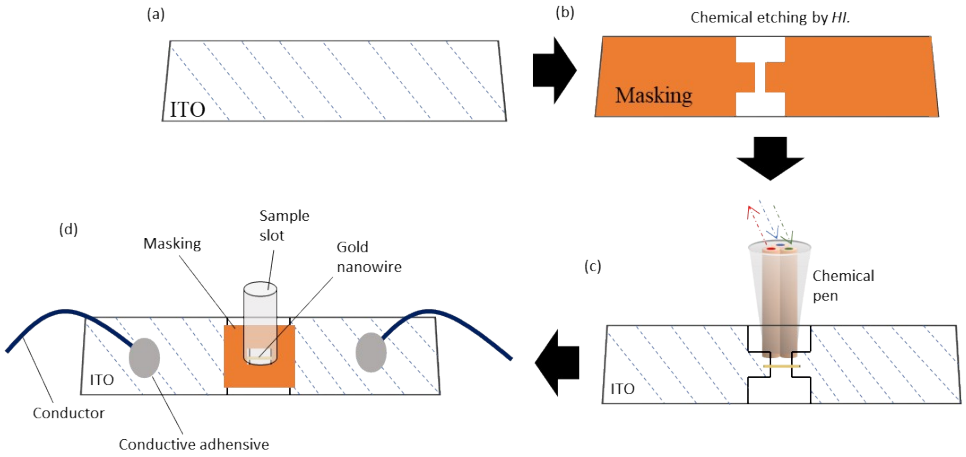

Fig. S6 Scheme of fabricating biosensor. (a) ITO glass. (b) Masking of chemical etching. (c) Fabrication of the gold nanowire between ITO electrodes. (d) Installation of conductors and sample slot and protection of the gold nanowire.

Functionalization of the gold nanowire

All the experimentss were performed with the fabricated biosensor connected to the circuit and the voltage was measured in real time (Fig. S8a). First, 10 mM 3-mercaptopropionic acid and 50 mM EDC were mixed for 15 minutes. Then 125 mM NHS was added to the mixture and mixed for 15 min. The gold nanowire were then functionalized by immersing them in 3-mercaptopropionic acid modified EDC/NHS for 1 hour. After the voltage value was sufficiently stabilized, 10 μM of anti-IgA was added. Finally, BSA (1% w/v) was added for blocking to prevent non-specific adsorption (Fig. S7). These voltage values were then converted to resistances based on Fig. S5b (Fig. 8b).

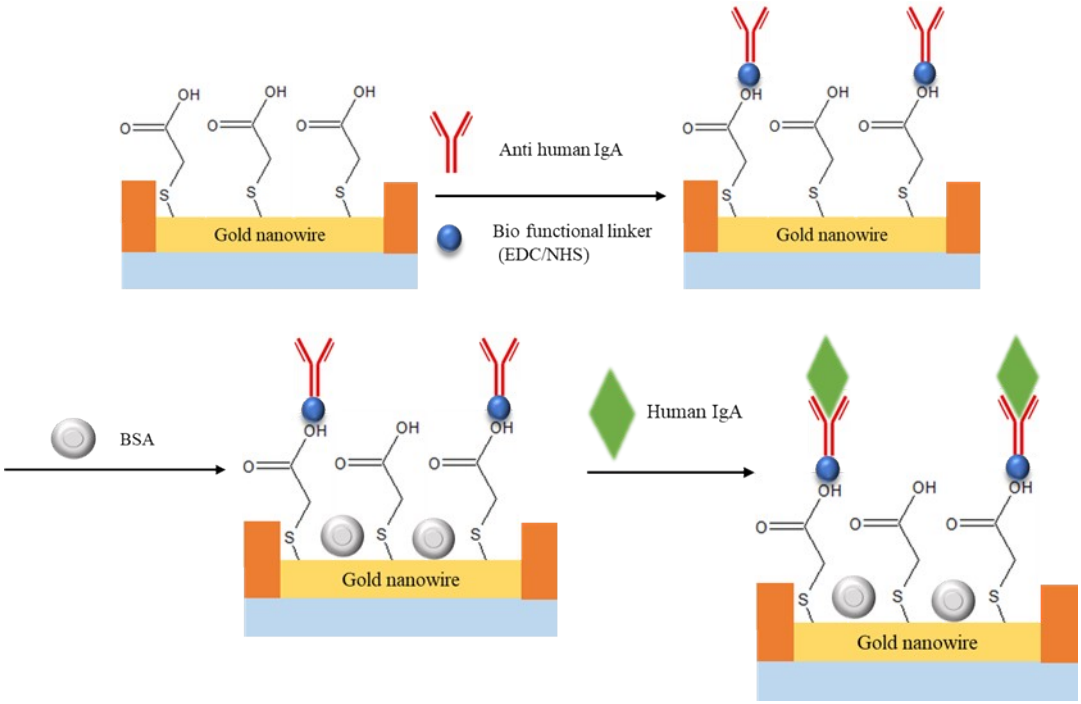

Fig. S7 The scheme of the gold nanowire functionalization.

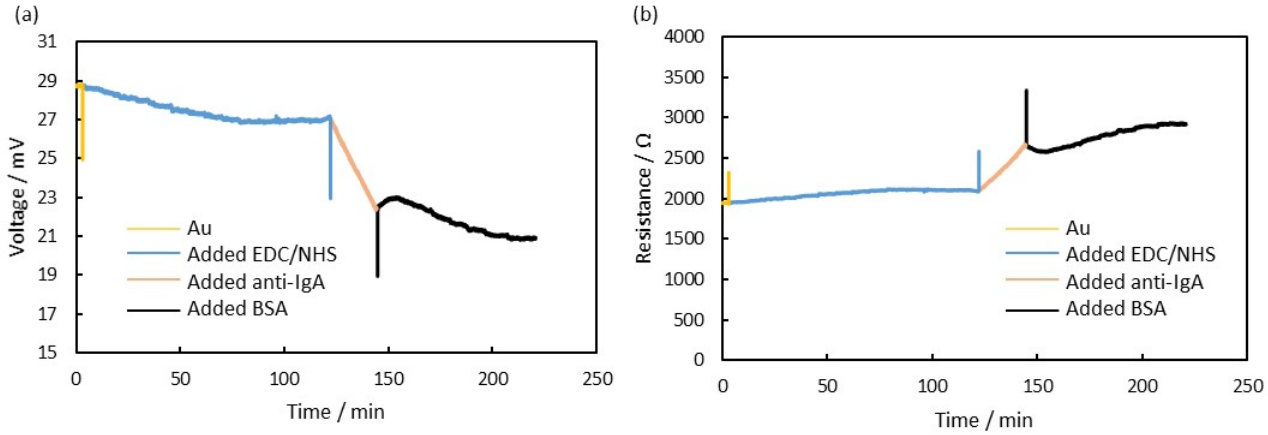

Fig. S8 Real time measurement when functionalizing the gold nanowire. (a) Real time measurement of voltage. (b) Calculated resistance.

Detection of human IgA

After blocking, various concentrations of IgA were added, and the culresistance at this time was measured (Fig. S9). In case the immobilized IgA was not removed, IgA was added in the order of 1 aM, 100 aM, 10 fM, 1 pM, 100 pM, and 10 nM to avoid the influence of the previous sample. The voltage values obtained were then converted to resistance based on Fig. 4a.

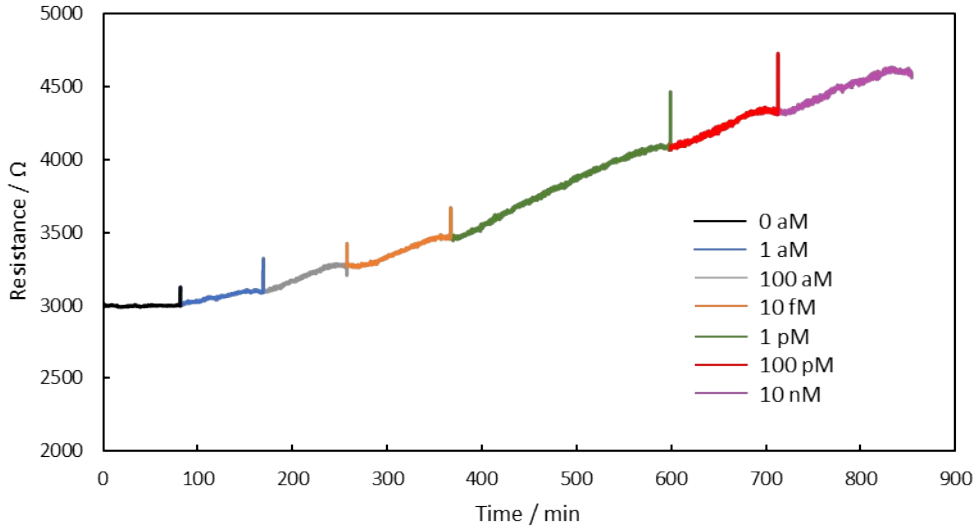

Fig. S9 Real time measurement when antigen measurement.