Electronic Supplementary Information

A facile method of achieving low surface coverage of Au nanoparticles on an indium tin oxide electrode and its application to sensitive protein detection

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Chemicals and reagents. ITO-coated glasses were obtained from Geomatec (www.geomatec.co.jp). AP, APTES, $HAuCl_4 \cdot 3H_2O$, H_2O_2, NH_4OH, $H_2NOH \cdot HCl$, HNO_3, and H_2SO_4 were purchased from Sigma-Aldrich. Ascorbic acid, Avidin, biotinylated goat antimouse IgG, mouse IgG from serum, and ALP-conjugated goat antimouse IgG, were supplied by Sigma. APP was received from LKT Laboratories, Inc. Absolute ethanol was obtained from Fisher Scientific. All buffer reagents and other inorganic chemicals were supplied by Sigma, Aldrich, or Fluka, unless otherwise stated. All chemicals were used as received. All aqueous solutions were prepared in doubly distilled water.

The phosphate buffered saline (PBS) consisted of 0.01 M phosphate, 0.138 M NaCl, and 0.0027 M KCl (pH 7.4). The PBSB buffer solution contained all the ingredients of PBS with additional 1% (w/v) albumin-bovine serum (pH 7.4). The rinsing buffer (RB) was prepared by using 50 mM tromethamine, 40 mM HCl, 0.5 M NaCl, and 0.05% (w/v) albumin-bovine serum (pH 7.6). The Tris buffer solution for the electrochemical experiment consisted of 50 mM tromethamine, 10 mM KCl, 2.13 g/L $MgCl_2$, and 7 mM HCl (pH 9.0).

Formation of Au NPs on ITO electrodes and their growth. ITO electrodes were successively cleaned with trichloroethylene, ethanol, and water with 15-min sonication, followed by drying at 50°C. The cleaned substrates were pretreated in a mixture of 5:1:1 H_2O/H_2O_2 (30%)/NH_4OH (30%) (v/v/v) at 70 °C for 1.5 h to ensure the presence of hydroxyl groups on the surface. The substrates were then washed with copious amount of water and dried at 50°C for 20 min. The pretreated ITO electrodes were immersed in an ethanolic solution containing 2% APTES (v/v) for 12 h to form silane monolayer. It was then washed thoroughly with ethanol (three times), and dried at room temperature. The amine-functionalized ITO electrodes were dipped in 1 mM $HAuCl_4$ (pH 3.13) for 30 min. The $AuCl_4^-$ ions are electrostatically bound to protonated amine groups. After thorough rinsing with water, the electrostatically bound $AuCl_4^-$ ions were reduced in an aqueous solution containing 0.5 mM ascorbic acid for 2 h. The Au NP-

and APTES-modified ITO electrodes were washed with water and dried at 50 °C for 20 min.

To increase the size of Au NPs, the electrodes were treated in an aqueous solution containing 0.3 mM $HAuCl_4$ and 0.3 mM $NH_2OH{\cdot}HCl$.

Construction of immunosensing layer. The Au NP- and APTES-modified ITO electrodes were immersed for 6 h in PBS (pH 7.4) solution containing 100 μg/mL avidin and then washed twice with PBS. Subsequently, the avidin-coated electrodes were immersed in a PBSB solution containing 0.05% (v/v) Tween-20 (pH 7.4) for 30 min to prevent nonspecific adsorption of proteins. After washed with RB, the electrodes were incubated for 30 min in PBSB containing 10 μg/mL biotinylated goat antimouse IgG and washed with RB. The electrodes were then treated in a PBSB solution containing different concentrations of mouse IgG for 30 min. After rinsed with RB, the resulting assemblies were dipped in PBSB containing 10 μg/mL ALP-conjugated goat antimouse IgG for 30 min, followed by washing with RB.

Electrochemistry. The electrochemical experiment was performed using a CHI 708C (CH instruments). The electrochemical cell consisted of the modified ITO working electrode, a Pt counter electrode, and an Ag/AgCl reference electrode.

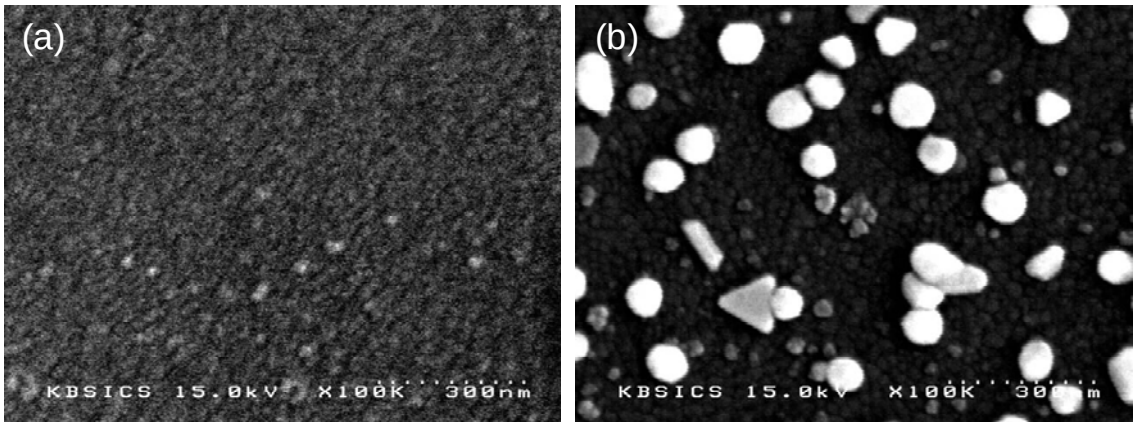

Fig. S1 SEM images of (a) APTES-modified ITO electrode and (b) Au NP- and APTES-modified ITO electrode obtained after immersing the electrodes in an aqueous solution containing 0.3 mM $HAuCl_4$ and 0.3 mM $H_2NOH{\cdot}HCl$ for 20 min.

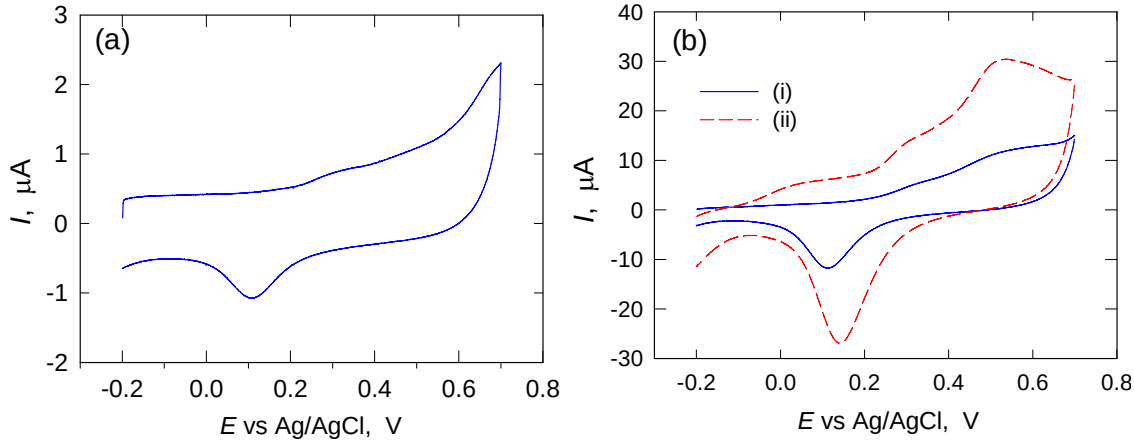

Fig. S2 Cyclic voltammograms obtained (in 0.1 M NaOH at a scan rate of 100 mV/s) at Au NP- and APTES-modified ITO electrodes (a) before and (b) after immersing the electrodes in an aqueous solution containing 0.3 mM $HAuCl_4$ and 0.3 mM $H_2NOH{\cdot}HCl$ for (i) 5 and (ii) 20 min.

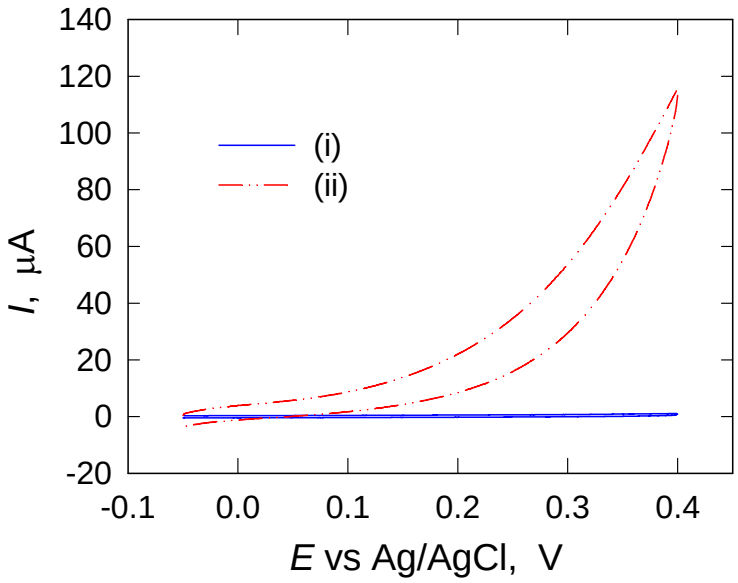

Fig. S3 Cyclic voltammograms were obtained at Au NP- and APTES-modified ITO electrode (i) and Au disc electrode (ii) in a Tris buffer solution (pH 9.0) at scan rate of 20 mV/s.

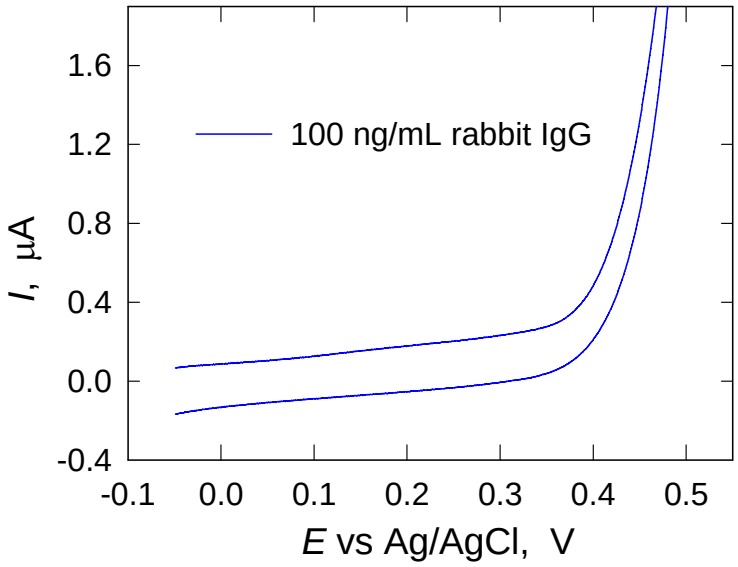

Fig. S4 Cyclic voltammogram obtained at a scan rate of 20 mV/s after incubation for 10 min in a Tris buffer containing 1.0 mM APP. The sensing electrode was treated with a solution containing 100 ng/mL rabbit IgG.