Electronic Supplementary Information

Redox-Active Gold Nanoclusters Immobilized ZnO Nanorod Electrodes for Electrochemical Sensing Applications

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Materials and Measurements.

Alkaline phosphatase enzyme (EC 3.1.3.1, from Bovine intestinal mucosa 7500 U/mg), hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$, reagent grade), sodium borohydride ($NaBH_4$, 99%), and L-glutathione reduced (GSH, >99%), Zinc acetate dihydrate ($Zn(OAc)_2 \cdot 2H_2O$, 99.5%), Zinc nitrate hexahydrate ($ZnNO_3.6H_2O$, 99%) and hexamethylenetetramine ($C_6H_{12}N_4$, 99%) were purchased from Sigma-Aldrich and used as received. ITO electrodes were purchased from Samsung Fine Chemicals. Water was purified using a Millipore Milli-Q system (18.2 MΩ·cm). Voltammetric measurements of ITO, ZnO/ITO, and Au_{25}/ZnO/ITO composite electrodes were performed with an electrochemical workstation (CHI 660B, CH Instruments) in a three electrode cell. Platinum wire and Ag/AgCl (3 M NaCl) were used as a counter and a reference electrode, respectively. All electrochemical experiments were carried out in a deoxygenated solution purged with argon. UV-vis absorption spectrum of aqueous solution of Au_{25} clusters was obtained using a Shimadzu UV-Vis-NIR spectrophotometer (UV 3600). Electrospray ionization (ESI) mass spectrometry was carried out on a Waters Micromass Q-TOF mass spectrometer. TEM images of ZnO nanorods and Au_{25}/ZnO were obtained using a JEOL JEM-2010 microscope. SEM images and Energy-dispersive X-ray spectroscopy (EDS) spectra were obtained using a JEOL JSM-6701F or a JEOL JSM-7001F microscope at 200 kV.

Synthesis of Glutathione-Stabilized Au_{25} clusters.

Glutathione-stabilized Au_{25} clusters (Au_{25}) were synthesized according to a procedure described elsewhere.[1] In a typical procedure, 1.23 g of glutathione (4 mmol) was dissolved and stirred in 40 mL of water. To this solution, 0.394 g of $HAuCl_4 \cdot 3H_2O$ (1 mmol) dissolved in 80 mL methanol was added, which generated white, cloudy suspension of Au(I)SG polymer. Addition of 0.3784 g of $NaBH_4$ (10 mmol) in 10 mL of water to the above suspension resulted in an immediate colour change to dark brown, indicating formation of glutathione stabilized clusters. Stirring was continued for another 1.5 h and the solution was evaporated to near dryness. The product was then dissolved in minimal amount of water and methanol was added to induce precipitation of the larger clusters. After the precipitate was separated by centrifugation, the supernatant liquid was treated with more methanol and centrifuged again. This recrystallization procedure was repeated until a clear supernatant was obtained. Highly pure, glutathione stabilized Au_{25} nanoclusters were obtained in the final stage of the recrystallization.

Preparation of ZnO/ITO Electrodes.

ZnO/ITO Electrodes were prepared according to a literature procedure with some modifications.[2a] In a typical procedure, an ITO electrode (2.5×1.0 cm^2) was first cleaned by immersing it in KOH-saturated isopropanol solution for 2 h and subsequently washed with ethanol, water and then blown dry with a stream of argon. The cleaned ITO surface was wet with a droplet of 0.005 M $Zn(OAc)_2\cdot2H_2O$ in ethanol, rinsed with ethanol after 30 s, and then dried with a stream of argon. This coating process was repeated five times. The ITO surface coated with a film of zinc acetate crystallites was heated at 350 °C in air for 20 min to form aligned ZnO seeds on the ITO surface. ZnO nanorods were grown on the aligned ZnO seeds in 5 mL of aqueous solution containing zinc nitrate (0.025M) and hexamethylenetetramine (0.025 M) at 80° C for 3 h. The resulting ZnO/ITO electrodes were rinsed thoroughly with water and dried in air. This procedure typically produced highly oriented ZnO nanorods with average diameter of 150 nm and length of 1.5 μm.

Fabrication of Au_{25}/ZnO/ITO Electrodes.

The prepared ZnO/ITO electrode was partially covered with insulating epoxy resin to expose a constant surface area (1.0×1.0 cm^2). The Au_{25}/ZnO/ITO electrodes were fabricated by dropcasting an aqueous Au_{25} solution on the ZnO/ITO electrode and heating at 50°C in air for 10 min. The Au_{25} loading was controlled by varying the concentration of the Au_{25} solution (0.1-1 mg/mL) and by the number of the repeating dropcasting process. The fabricated Au_{25}/ZnO/ITO electrodes were rinsed thoroughly with water and dried in air.

Determination of ALP activity

Cyclic voltammograms of Au_{25}/ZnO/ITO electrodes were recorded at 37 °C at an incubation time of 5 min after the addition of ALP in the range of 50 – 300 U/L in 7mL of 50 mM of Tris buffer solution (pH = 8.0) containing 0.5 mM of APP along with 0.1M KCl and 1mM

$MgCl_2$. The current response was averaged out of three independent measurements. Amperometric responses of Au_{25}/ZnO/ITO electrodes were also recorded in the same electrolyte solution at 37 °C. A predetermined amount of ALP in range of 5 to 300 U/L was added after the current response was stabilized for 120 s and the corresponding amperometric responses were recorded for 280 s at a fixed potential of 0.25V (vs. Ag/AgCl).

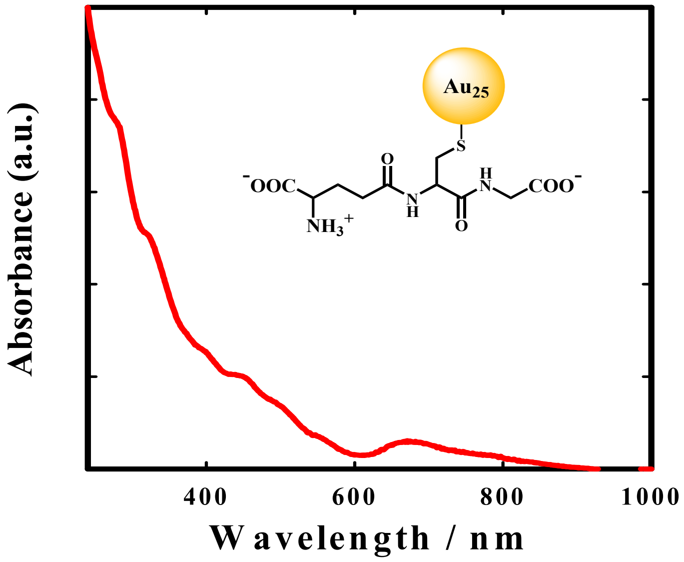

Fig. S1 UV-vis absorption spectrum of aqueous solution of Au_{25} clusters. Inset shows the structure of glutathione stabilizing Au_{25} nanocluster.

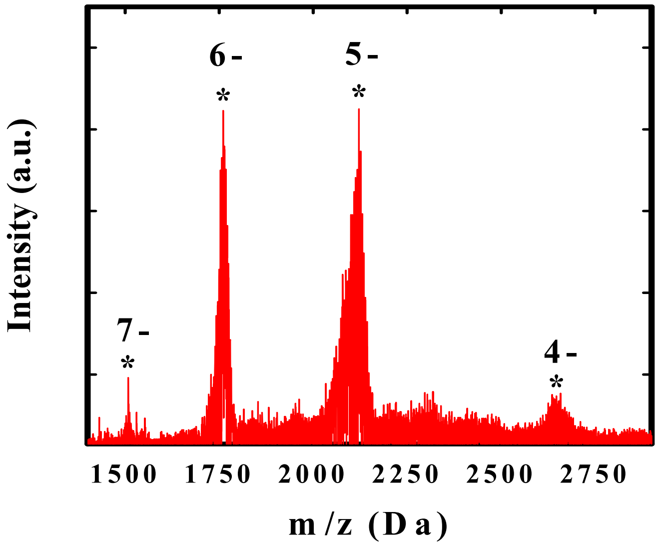

Fig. S2 Negative-ion ESI mass spectrum of glutathione-stabilized Au_{25} clusters.

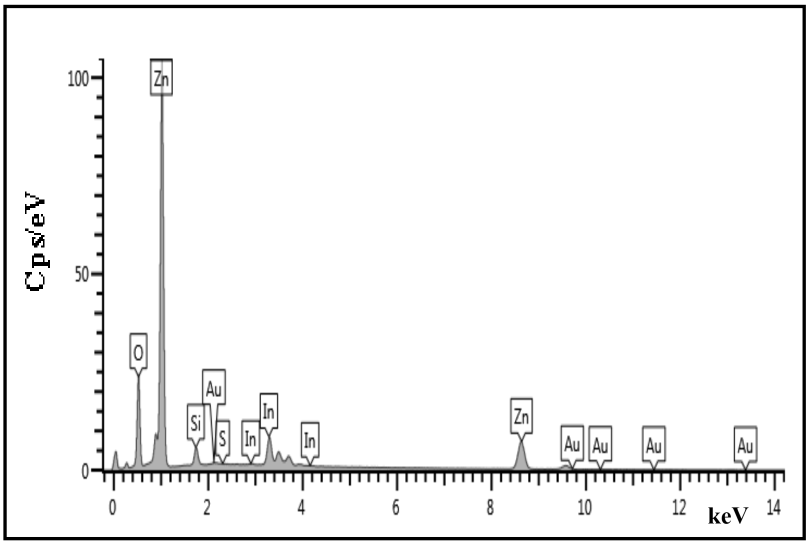

Fig. S3 SEM energy dispersive spectrum of Au_{25}/ZnO/ITO electrode surface.

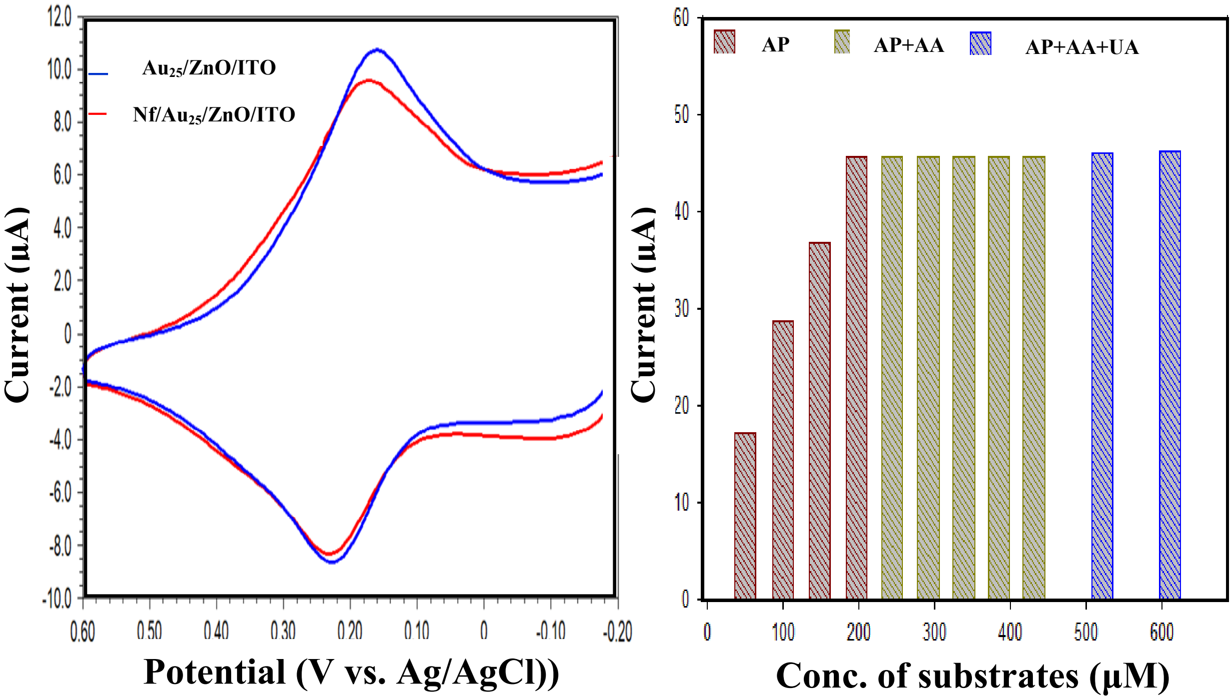

Fig. S4 (A) CVs of Au_{25}/ZnO/ITO electrode and Au_{25}/ZnO/ITO electrode coated with Nafion (Nf) in 0.1 M KCl (pH = 7.0) at 50 mV/s. (B) Interference effect of ascorbic acid (AA) and uric acid (UA) towards the determination of AP at the Au_{25}/ZnO/ITO electrode obtained from amperometric response conducted at fixed potential of 0.25V.

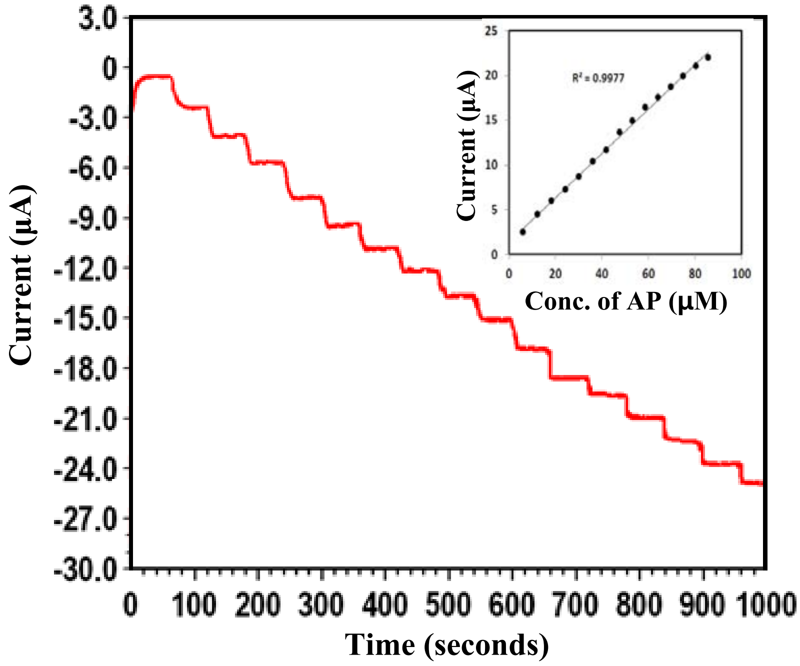

Fig. S5 Amperograms of Au_{25}/ZnO/ITO electrode for the successive additions of 0.05mL of 1mM AP in 8mL of 0.1M KCl under stirring with 60 s interval. Inset shows the calibration plot for the determination of AP.

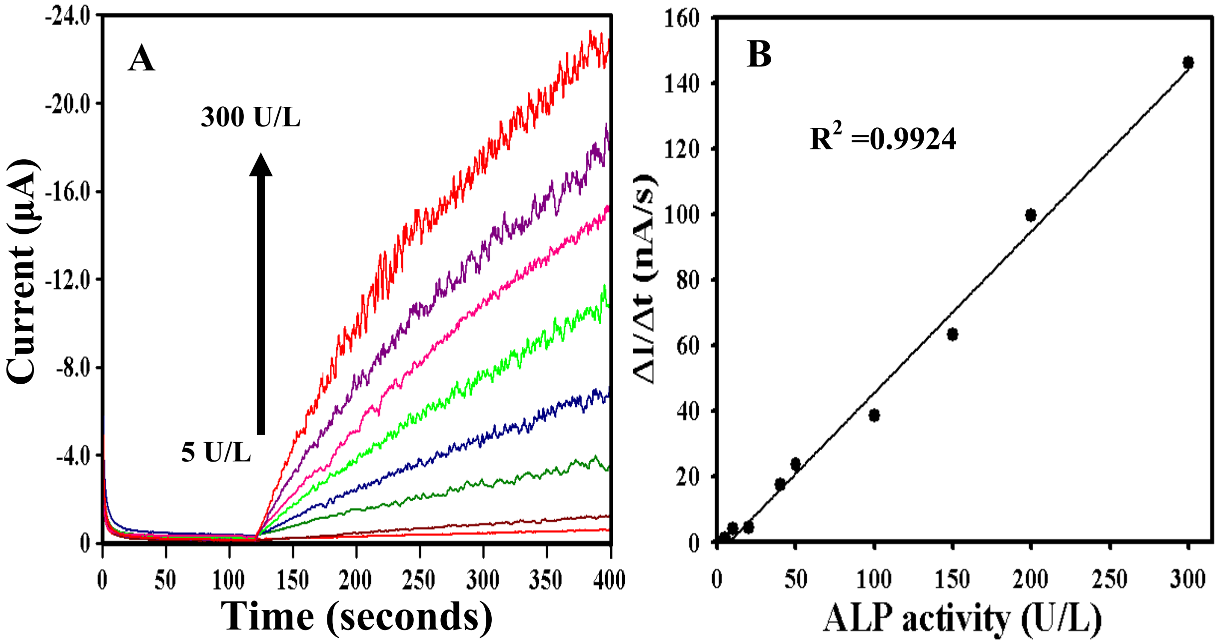

Fig. S6 (A) Amperometric response of Au_{25}/ZnO/ITO electrode for the addition of ALP (5-300 U/L) recorded at 0.25 V (vs. Ag/AgCl) in presence of 0.5 mM of APP, Tris buffer (pH = 8.0) containing 0.1 M KCl and 1mM $MgCl_2$ under stirring at 37^{o}C. (B) Corresponding calibration plot for ALP. The initial current-time slopes (for 30 s) from the amperometric responses of Au_{25}/ZnO/ITO electrode are plotted as a function of ALP activity.

References:

1. (a) K. Kwak, S. S. Kumar and D. Lee, *Nanoscale*, 2012, **4**, 4240; (b) S. S. Kumar, K. Kwak and D. Lee, *Electroanalysis*, 2011, **23**, 2116.

2. (a) L. E. Greene, M. Law, D. H. Tan, M. Montano, J. Goldberger, G. Somorjai and P. Yang, *Nano Lett.*, 2005, **5**, 1231; (b) L. E. Greene, B. D. Yuhas, M. Law, D. Zitoun and P. Yang, *Inorg. Chem.*, 2006, **45**, 7535.