

## Supporting Information

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### **Electrooxidative Grafting of Amine-terminated Dendrimers Encapsulating Nanoparticles for Spatially Controlled Surface Functionalization of Indium Tin Oxide**

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## Experimental Section

**Chemicals and materials** Amine-terminated sixth-generation polyamidoamine dendrimers (G6-NH<sub>2</sub> PAMAM dendrimers), H<sub>2</sub>AuCl<sub>4</sub>•3H<sub>2</sub>O, K<sub>2</sub>PtCl<sub>4</sub>, NaBH<sub>4</sub>, LiClO<sub>4</sub>, K<sub>3</sub>Fe(CN)<sub>6</sub>, H<sub>2</sub>O<sub>2</sub>, phosphate buffered saline (PBS), silver enhancement kit, 4-chloro-1-naphthol (4-CN), and cellulose dialysis sacks (MW cutoff of 12,000) were purchased from Sigma-Aldrich, Inc. (USA). Indium tin oxide (ITO)-coated glass slides were obtained from Delta Technologies (USA). *N*-hydroxysuccinimide (NHS) esters of biotin (EZ-Link® Sulfo-NHS-LC-LC-Biotin) and streptavidin-conjugated horseradish peroxidase (Streptavidin-HRP) were used as received from Pierce Biotechnology (USA). 18 MΩ•cm deionized (DI) water was used in the preparation of aqueous solutions (Ultra370, Younglin Co., Korea).

**Synthesis of dendrimer-encapsulated nanoparticles (DENs)** Pt and Au DENs were synthesized as previously reported with some modification.<sup>1-2</sup> Briefly, 147 mol equivalent of an aqueous 0.1 M K<sub>2</sub>PtCl<sub>4</sub> or 10 mM H<sub>2</sub>AuCl<sub>4</sub> was added to an aqueous 10 μM G6-NH<sub>2</sub> PAMAM dendrimer solution for complexation of the metal ions with the interior amines of the dendrimers. Especially, the mixture solution (pH 5) of Pt ions and dendrimers was stirred for 76 h to ensure the binding of the Pt ions to the intradendrimer tertiary amines. A stoichiometric excess of aqueous NaBH<sub>4</sub> was then added slowly to the mixture under vigorous stirring. Specifically, a 20-fold excess of an aqueous NaBH<sub>4</sub> solution was added to the Pt ion-dendrimer complex solution. The mixture solution (pH 8) was kept in a closed vial for 24 h to ensure complete reduction of the Pt. Similarly, a 5-fold excess of NaBH<sub>4</sub> in 0.3 M NaOH was added to the Au ion-dendrimer complex solution, which resulted in color change of the solution from pale yellow to brown. This color change indicates reduction of the complexed Au ions to zerovalent Au nanoparticles inside

the dendrimers. Finally, the prepared DEN solutions were dialyzed using cellulose dialysis sacks to remove impurities.

**Fabrication of functionalized ITO** The electrochemical immobilization of DENs on ITO electrodes was carried out, similarly as we reported for electrooxidative grafting of DENs onto glassy carbon electrodes.<sup>1,2</sup> Briefly, an ITO electrode was ultrasonically cleaned with acetone, ethanol, and water subsequently and dried under a stream of N<sub>2</sub>. Then, the ITO electrode was cleaned further in a plasma cleaner/sterilizer (PDC-32G, Harrick Scientific, Ossining, NY) at medium power for 2 min. Immediately after the plasma treatment, the ITO electrode (area: 0.096 cm<sup>2</sup>) was exposed to an aqueous 10 μM DEN solution containing 0.1 M LiClO<sub>4</sub> and the potential of the electrode was cycled three times between 1.20 and 1.75 V (vs. Ag/AgCl) using a Model 440 potentiostat (CH Instruments, USA). A Pt wire and a Ag/AgCl electrode were used as a counter and a reference electrode, respectively. After immobilization, the modified ITO was rinsed with DI water, ultrasonicated in DI water thoroughly for 10 min, and then blown dry.

Streptavidin-conjugated horseradish peroxidase (HRP) enzymes were attached onto the DEN-grafted ITOs through biotin-streptavidin chemistry. Briefly, the DEN-grafted ITO was exposed to a 10 mM NHS esters of biotin solution for biotinylation of the terminal amine groups of immobilized dendrimers. Then, a 0.1 mg/mL streptavidin-HRP conjugate solution was introduced onto the biotinylated ITO surface, which resulted in binding of the HRP enzymes onto the surface. After rinsing with PBS buffer (pH 7.4), the electrode was blown dry by N<sub>2</sub> stream.

For electrochemical grafting of DENs on interdigitated array (IDA) ITO electrodes, we fabricated IDA microelectrodes (~50  $\mu\text{m}$  wide with a spacing of ~50  $\mu\text{m}$ ) using a standard photolithography. Briefly, cleaned ITO substrates were spin coated with photoresist (AZ P4330-RS, AZ Electronic Materials) at 6000 rpm for 30 s, baked at 115  $^{\circ}\text{C}$  for 5 min, and exposed to UV light for 90 s by use of a photolithography system (LABSYS-2, Nextron, Korea) and a photographic film mask. The resulting image was then developed with AZ 400K developer solution (AZ Electronic Materials) for 150 s to form a photoresist pattern, rinsed with DI water, and baked at 90  $^{\circ}\text{C}$  for 5 min. Next, the photoresist-patterned ITO was etched with an etchant solution (TE-100, Transene Company, Inc., USA) at 60  $^{\circ}\text{C}$  for 15 min, which resulted in formation of IDA ITO microelectrodes. All photoresist residues were removed by rinsing the ITO microelectrodes with acetone.

**Characterization** TEM images were collected using a Tecnai G2 F30 instrument (FEI Co., USA) operating at 200 kV. TEM samples were prepared by evaporating a drop of aqueous sample solution on a 200 mesh carbon-coated copper grid (Ted Pella Inc., USA). XPS measurements were performed with a PHI 5000 spectrometer (Physical Electronics Inc., USA) using an Al  $K\alpha$  radiation ( $h\nu = 1486.6$  eV). XPS samples were prepared by dropping the sample solutions on Si wafers and allowing them to dry. A fluorescence microscope (Nikon ECLIPSE Ti-U, Nikon Co., Japan) equipped with a CCD camera (CoolSNAP EZ, Photometrics Ltd., USA) was used to acquire optical micrographs.

The DEN-grafted ITO surfaces were electrochemically characterized to estimate the surfaces areas of grafted DENs. For measurement of surface areas of grafted Pt DENs, we used two electrochemical methods: CO stripping and hydrogen adsorption/desorption. Briefly, the Pt

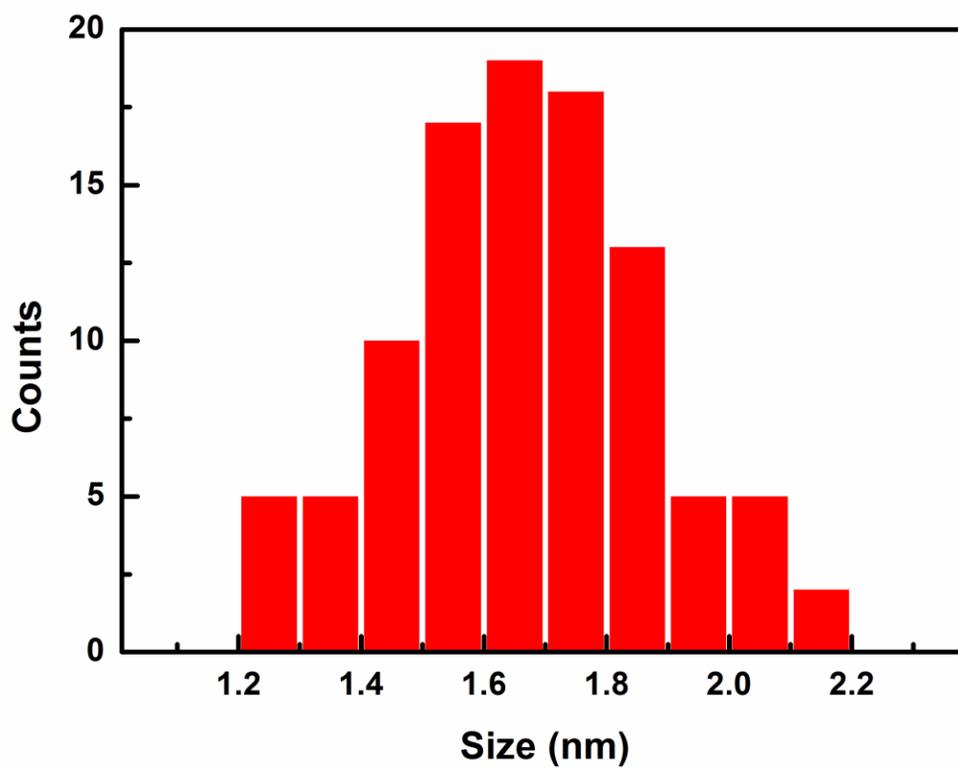
DEN-grafted electrodes (area: 0.096 cm<sup>2</sup>) were placed in a CO-saturated 0.5 M H<sub>2</sub>SO<sub>4</sub> solution and the electrode potential was held at 0.1 V (vs. Ag/AgCl) for 3 min, which resulted in adsorption of CO on the Pt surface.<sup>3</sup> Then, the electrolyte solution was purged with N<sub>2</sub> gas for 10 min to remove dissolved CO gas in the solution while maintaining the electrode potential. Finally, the potential of the electrode was scanned to strip the adsorbed CO on the Pt surface. After stripping the adsorbed CO, the potential scan was repeated to make sure absence of the CO oxidation peak, which confirms that the oxidation of CO arises from adsorbed CO and not from dissolved CO. Integration of the resulting CO oxidation peaks and hydrogen-desorption peaks allowed to estimate the total Pt surface area using the reported values about the amount of charges required to oxidize CO (420 μC/cm<sup>2</sup>) or hydrogen (210 μC/cm<sup>2</sup>) per unit area of Pt.<sup>3-5</sup> Similarly, we estimated the surface areas of Au DENs grafted on ITO using the reduction peaks of Au oxide in cyclic voltammograms of the Au DEN-grafted ITOs obtained in 0.5 M H<sub>2</sub>SO<sub>4</sub> solution. The calculation was also based on a reported value (400 μC/cm<sup>2</sup>) about the amount of charge required to reduce Au oxide per unit area of Au.<sup>6-8</sup>

## References

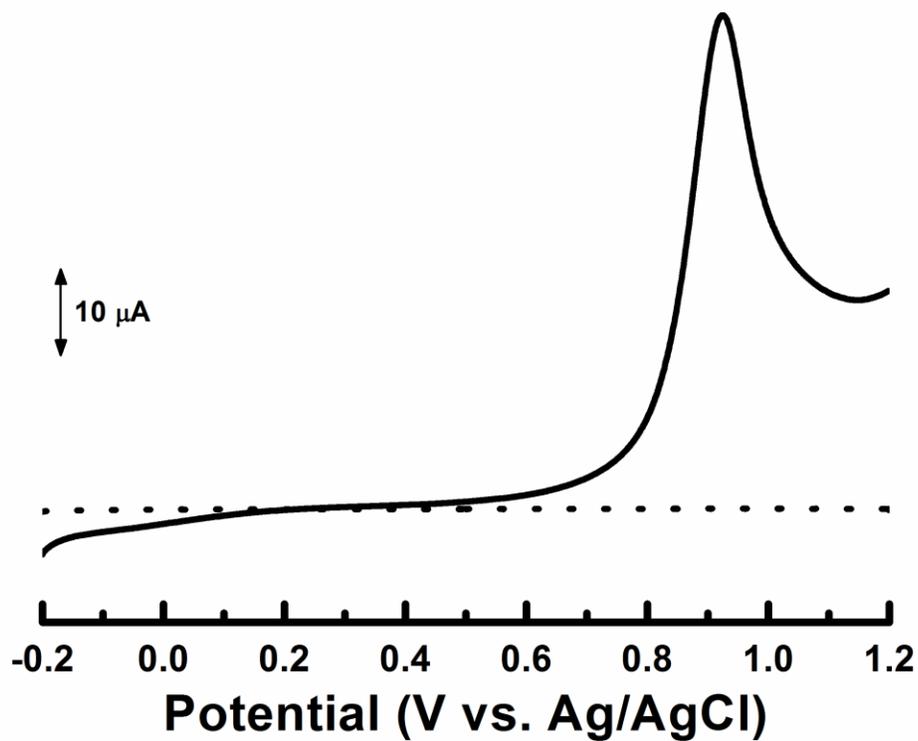
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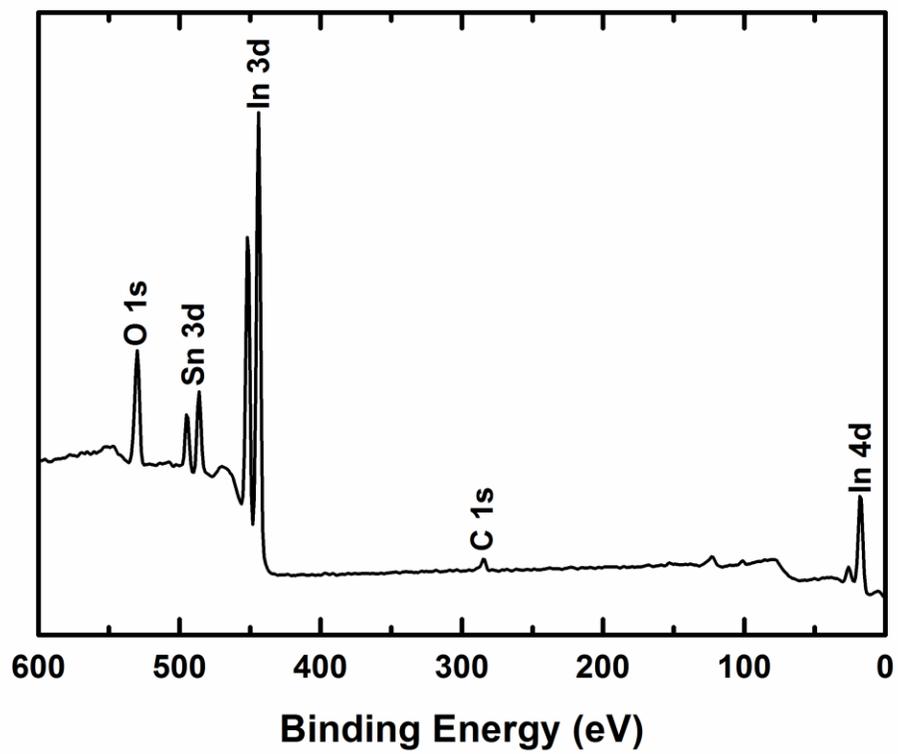
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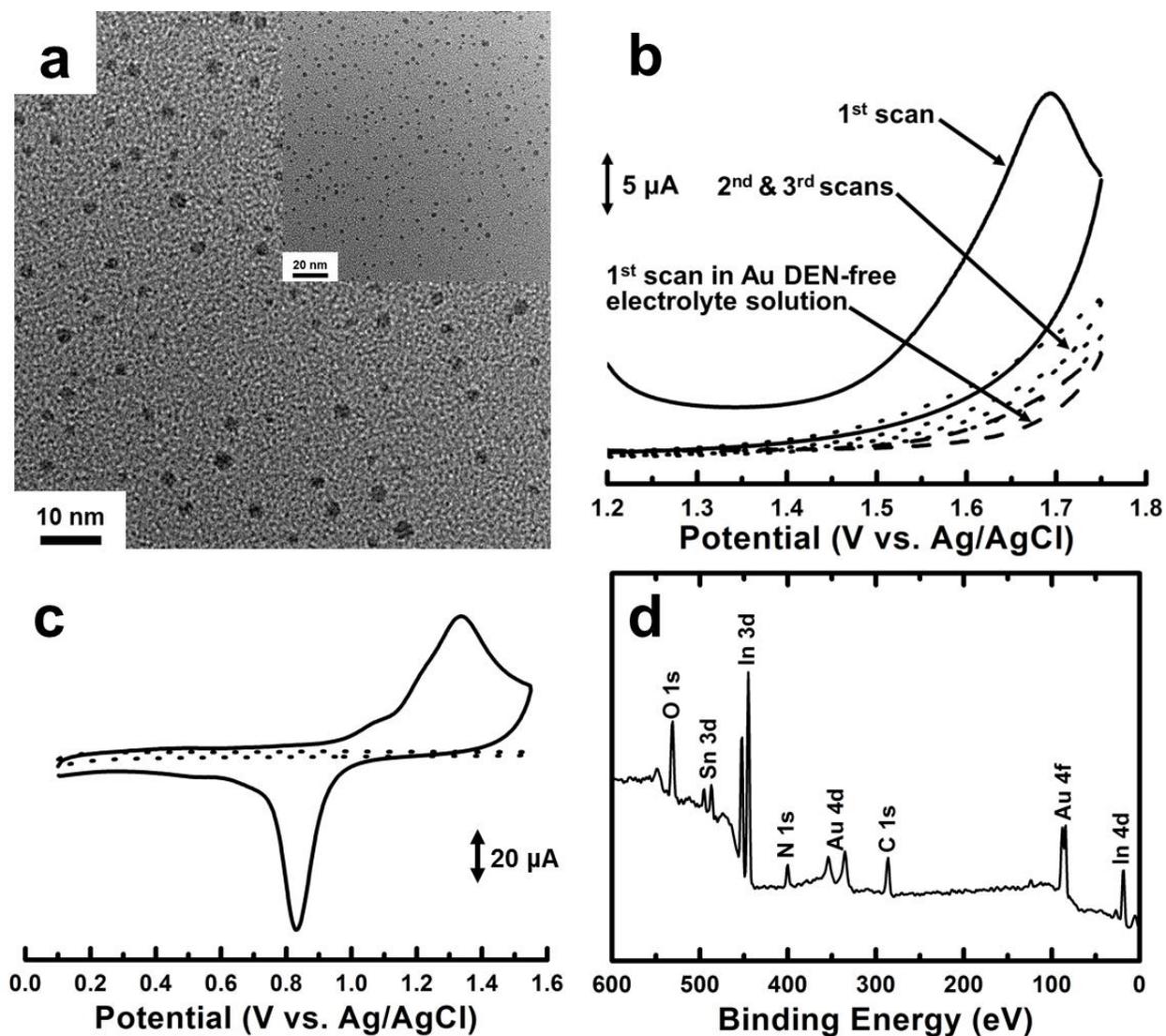
**Fig. S1.** Particle size distribution of as-prepared Pt DENs, *i.e.* G6-NH<sub>2</sub>(Pt<sub>147</sub>) DENs.



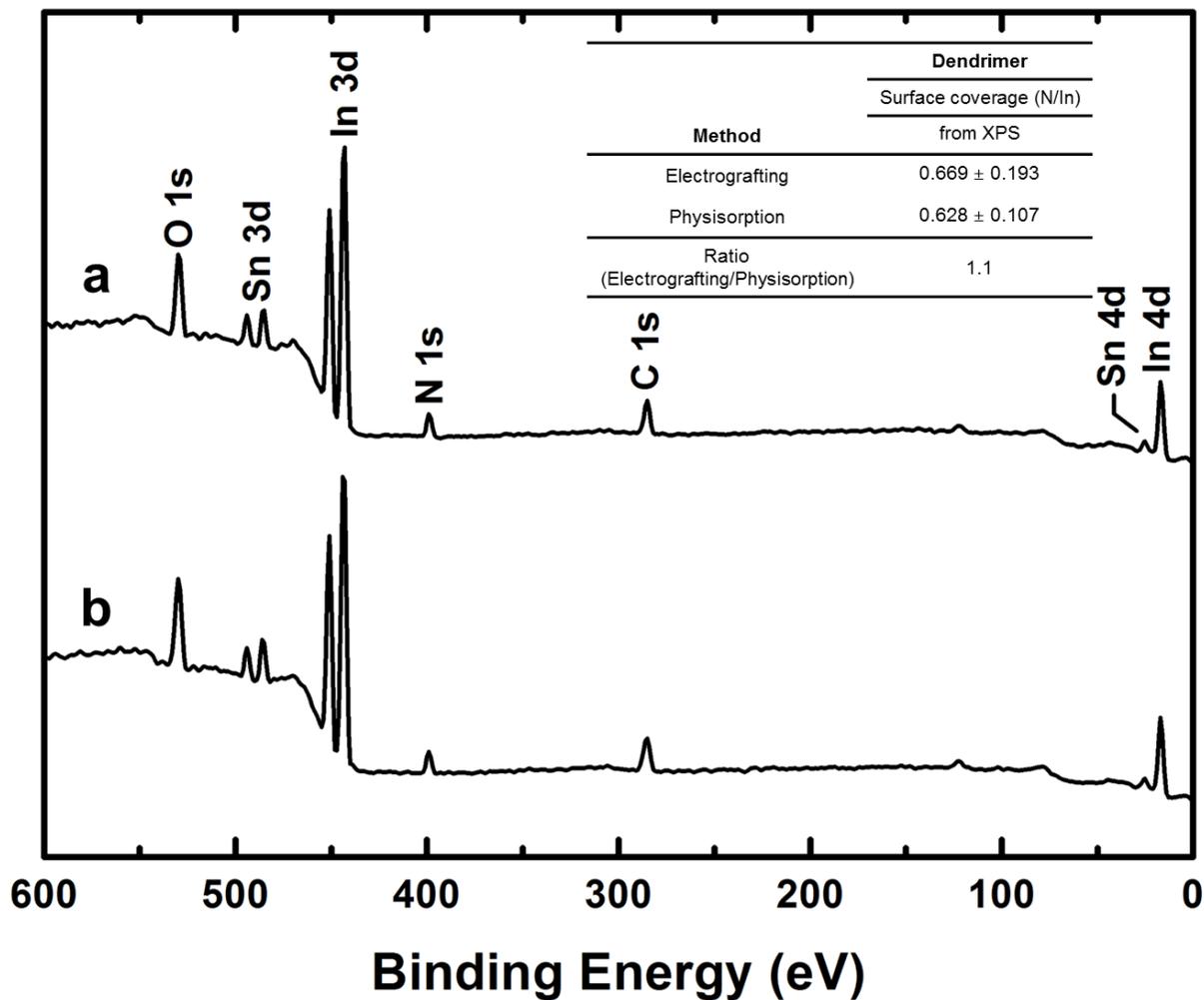
**Fig. S2.** CO stripping voltammograms obtained from (solid line) a Pt DEN-grafted and (dotted line) a bare ITO electrode after baseline correction.



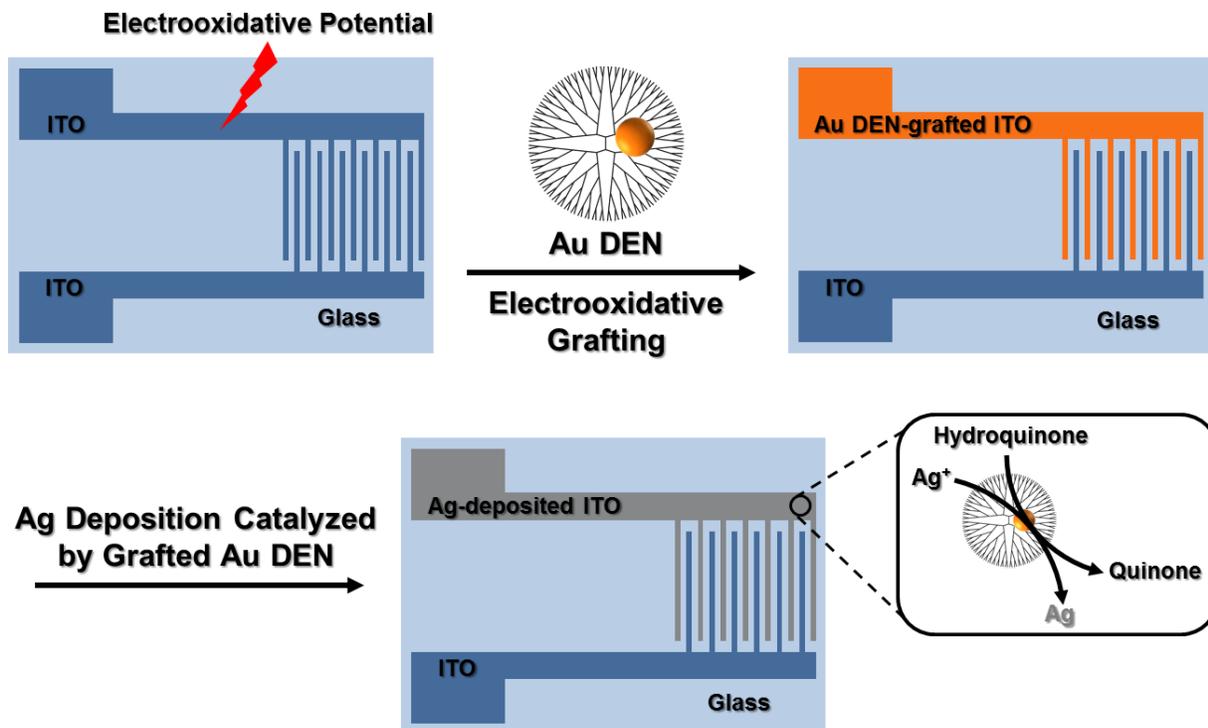
**Fig. S3.** XPS spectrum of a bare ITO electrode.



**Fig. S4.** (a) TEM images of as-prepared Au DENs, *i.e.* G6-NH<sub>2</sub>(Au<sub>147</sub>) DENs. (b) CVs obtained on ITOs in an aqueous 10  $\mu$ M Au DEN solution containing 0.1 M LiClO<sub>4</sub> and in a Au DEN-free 0.1 M LiClO<sub>4</sub> solution. Scan rate: 10 mV s<sup>-1</sup>. (c) CVs of a Au DEN-grafted and a bare ITO electrode in 0.5 M H<sub>2</sub>SO<sub>4</sub>. Scan rate: 500 mV s<sup>-1</sup>. (d) XPS spectrum of a Au DEN-grafted ITO electrode.

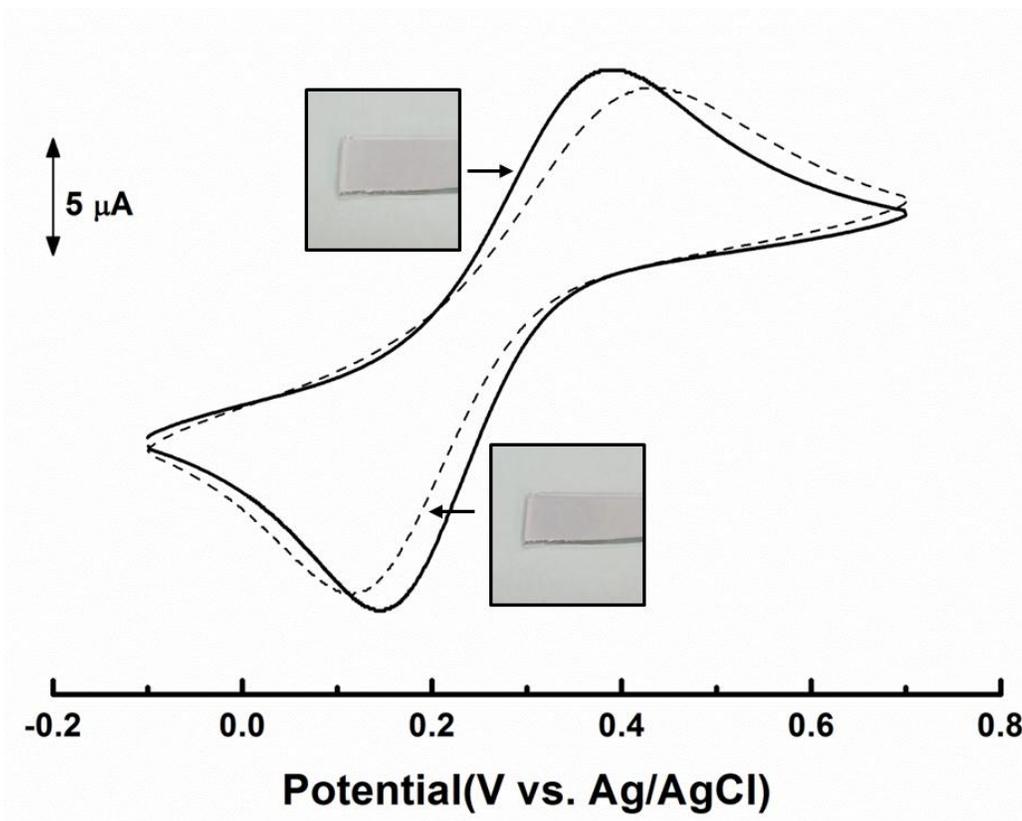


**Fig. S5.** XPS spectra of (a) a dendrimer-grafted ITO, which was modified identically to the DEN-grafted ITO but in the absence of nanoparticles encapsulated in the dendrimers, and (b) a dendrimer-physisorbed ITO, which was treated identically to the DEN-physisorbed ITO. 10  $\mu\text{M}$  G6-NH<sub>2</sub> dendrimer solution containing 0.1 M LiClO<sub>4</sub>. Inset: Comparison of surface coverage of dendrimers on dendrimer-grafted and dendrimer-physisorbed ITOs.



**Fig. S6.** Schematic illustration of the experimental approach used to demonstrate spatially controlled surface functionalization of ITO with catalytic Au DENs.

To confirm that the HRP-catalyzed deposition of the insulating film is due to HRP linked to the terminal amine groups of grafted dendrimers through biotin-streptavidin chemistry, we carried out a control experiment in which Au DEN-grafted ITO was incubated in a streptavidin-HRP conjugate solution but with no biotinylation on the DEN-grafted ITO. The ITO electrode prepared in this control experiment did not show any dramatic blocking of the redox reaction of  $\text{Fe}(\text{CN})_6^{3-}$  (Fig. S7), thus verifying that the biocatalytic precipitation of 4-CN was due to HRP enzymes conjugated onto the Au DEN-grafted ITO and not due to the physisorbed ones.



**Fig. S7.** CVs of 1.0 mM  $\text{Fe}(\text{CN})_6^{3-}$  in 0.1 M PBS buffer (pH 7.0) on a HRP-physisorbed ITO, which was treated identically to the HRP/Au DEN-functionalized ITO but with no biotinylation on the Au DEN-grafted ITO, before (solid line) and after (dotted line) its exposure to a 2.5 mM of 4-chloro-1-naphthol (4-CN) solution (10 mM PBS, pH 7.4) containing 0.5 M  $\text{H}_2\text{O}_2$  for 10 min. Scan rate:  $100 \text{ mV s}^{-1}$ .