

## Self-assembled Dendrimer/Polyelectrolyte Layers on Indium Tin Oxide Electrodes as a Matrix for Immobilization of Gold Nanoparticles and Fluorophores

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Indium tin oxide (ITO) electrodes have been used in a wide variety of applications that require high optical transparency and low electrical resistance, such as liquid crystal displays, organic light-emitting diodes, and solar cells.<sup>1-4</sup> However, the applications of the ITO electrode often involve the modification of the surface of ITO due to its limited catalytic activity and intrinsic surface heterogeneity. For example, modification of ITO surface with gold nanoparticles was proposed to enhance the activity of ITO toward the oxidation of tri-*n*-propylamine (TPRA), which is the most common cofactor for Ru(bpy)<sub>3</sub><sup>2+</sup> electrogenerated chemiluminescence.<sup>5</sup> In addition, formation of self-assembled monolayers (SAM) on ITO was proven to be useful to improve homogeneity of the ITO surface despite the intrinsic surface roughness of the ITO electrode.<sup>6</sup>

Gold nanoparticles (Au NPs) have gained intense interests due to their tunable optical, electrical, and catalytic properties depending on the size and shape of Au NPs. For instance, the surface plasmon resonance bands of Au NPs, which result from collective oscillations of conduction electrons of Au NPs with visible light, show strong dependence in their size and shape.<sup>7,8</sup> Au NPs also show enhanced catalytic activity for CO oxidation depending on the size of Au NPs.<sup>9</sup> Based on those tunable properties of Au NPs, various strategies for the modification of ITO surface with Au NPs have been suggested.<sup>10-14</sup> However, assembling Au NPs in nanostructured thin films with precise controls in size and shape of Au NPs still remains a challenge.

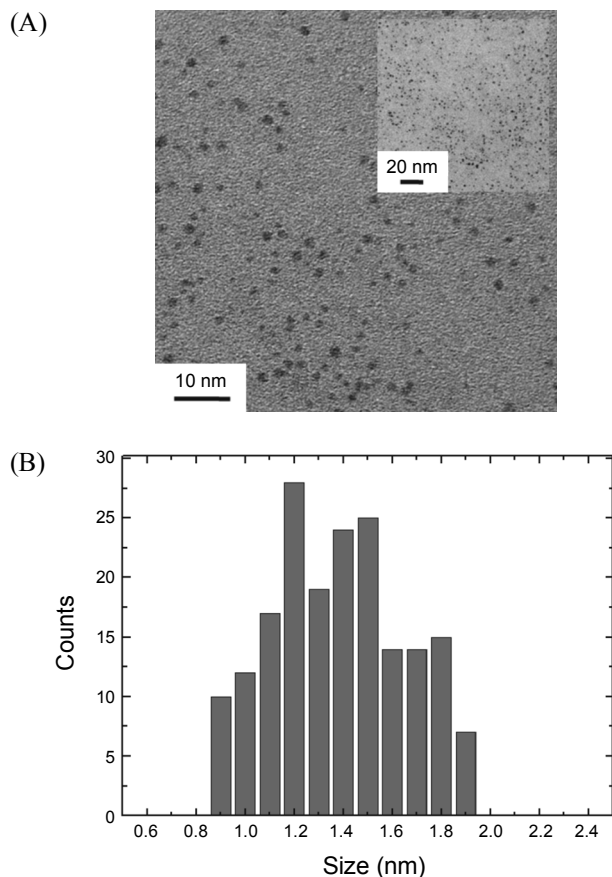
Here, we report self-assembly of amino-terminated poly(amidoamine) (PAMAM) dendrimers onto polyelectrolyte-films on ITO electrodes and subsequent modification of the assembled dendrimers with amine-reactive fluorophores. Since Au NPs were encapsulated inside the cores of the PAMAM dendrimers, the self-assembly of dendrimers resulted in modification of ITO electrodes with Au NPs. The Au dendrimer-encapsulated nanoparticles (Au DENs) were stable and nearly monodisperse in size. Therefore, the assembling of the dendrimers (thus, Au DENs) on ITO provides a convenient way to immobilize Au NPs on ITO surfaces with precise control in size of particles. In addition, the terminal amine groups of the PAMAM dendrimer were used to attach fluorescent organic substances, which demonstrated the possibility of subsequent modifications of the Au DENs-assembled ITO electrodes for later applications.

Au DENs were synthesized within sixth-generation PAMAM dendrimers having peripheral amine groups (G6-NH<sub>2</sub> den-

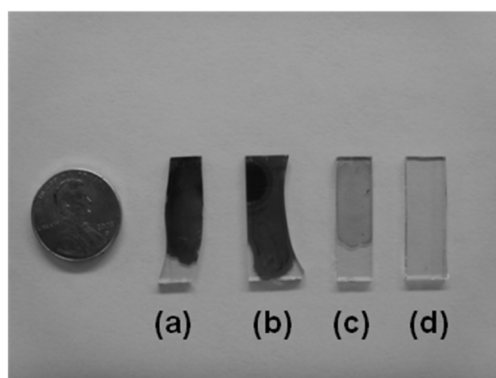
drimers) as previously reported with some modification.<sup>15</sup> First, AuCl<sub>4</sub><sup>-</sup> ions were coordinated inside the amino-terminated PAMAM dendrimers. Second, the coordinated AuCl<sub>4</sub><sup>-</sup> ions within the dendrimers were reduced to form dendrimer-encapsulated Au nanoparticles. Specifically, 147 mol equivalent of an aqueous 10 mM HAuCl<sub>4</sub> was mixed with an aqueous 10 μM G6-NH<sub>2</sub> PAMAM dendrimer solution ([AuCl<sub>4</sub><sup>-</sup>] : [G6-NH<sub>2</sub>] = 147 : 1). The mixture solution was vigorously stirred for 40 min, which provides enough time for AuCl<sub>4</sub><sup>-</sup> to complex with the interior amines of the dendrimers. Then, excess NaBH<sub>4</sub> in 0.3 M NaOH was added in drops to the pale yellow mixture with vigorous stirring. This resulted in change of the initial pale yellow mixture to the brown color solution, which indicates reduction of the Au complex to zerovalent Au NPs inside the dendrimers. Finally, the solution was dialyzed using a cellulose dialysis sack for overnight to remove impurities. Figure 1 shows transmission electron microscopy (TEM) images and a corresponding histogram of particle size distributions for the Au DENs (G6-NH<sub>2</sub>(Au147) DENs). The TEM images reveal little aggregation of the Au nanoparticles (Figure 1A), which suggests stabilization of the nanoparticles *via* their encapsulation inside dendrimers. The average size of the nanoparticles is 1.4 ± 0.3 nm (Figure 1B). This measured diameter of the G6-NH<sub>2</sub>(Au147) DEN is very close to the calculated value (1.6 nm) for nanoparticles containing 147 Au atoms. The value of 1.6 nm was calculated by assuming that Au nanoparticles are spherical in shape.<sup>15</sup> Those results reveal that the dendrimer templating approach enables the preparation of stable and nearly size-monodisperse Au nanoparticles inside dendrimers. Note that the size of the prepared Au DENs was controlled simply by adjusting the [AuCl<sub>4</sub><sup>-</sup>] : [G6-NH<sub>2</sub>] ratio, which is another beauty of the dendrimer templating approach for nanoparticle synthesis.<sup>16</sup>

The prepared Au DENs were assembled onto polyelectrolyte films-modified ITO surfaces as described in detail in Experimental Section. One unique characteristic of Au NPs is their capability for catalytic silver deposition, usually known as silver enhancement.<sup>17</sup> Therefore, the silver enhancement experiment was performed to verify the presence of assembled Au DENs on ITO surfaces. In a typical experiment, a polyelectrolyte films-modified ITO was assembled with Au DENs and then exposed to silver enhancement mixture solution (Silver enhancer kit, SE100, Sigma-Aldrich) for 3 min. The presence of Au DENs on ITO was visualized using the silver enhancement solution

in which silver ions are reduced by hydroquinone to silver at the surfaces of Au NPs. Figure 2a shows silver deposition (darkened area) on an Au DENs-assembled ITO electrode after exposure to silver enhancement solution, which confirms the



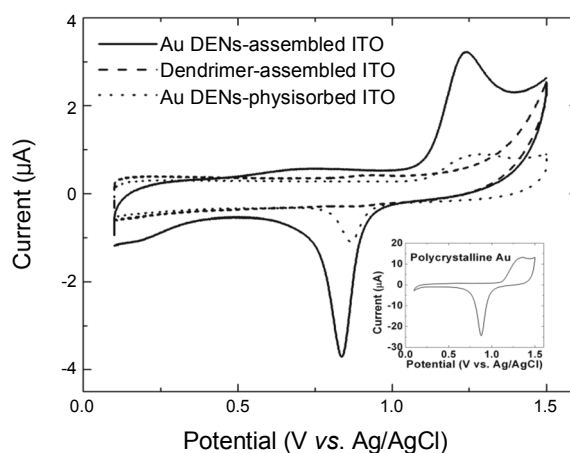
**Figure 1.** (A) Transmission electron microscopy (TEM) images of G6-NH<sub>2</sub>(Au147) DENs. The inset TEM image shows G6-NH<sub>2</sub>(Au147) DENs on a larger area. (B) A corresponding particle size distribution of the G6-NH<sub>2</sub>(Au147) DENs. The average G6-NH<sub>2</sub>(Au147) DENs diameter is  $1.4 \pm 0.3$  nm.



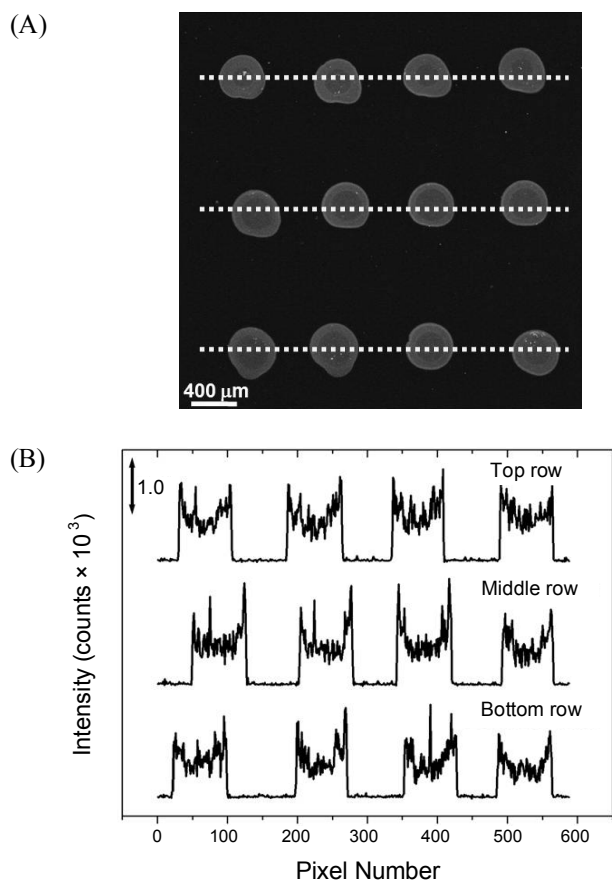
**Figure 2.** An image showing ITO electrodes after silver enhancement treatment. ITO pieces a-d correspond to one assembled with G6-NH<sub>2</sub>(Au147) on its polyelectrolyte film, one assembled with G6-NH<sub>2</sub>(Au147) on its polyelectrolyte film and ultrasonicated for 20 min in ethanol, one dipped in G6-NH<sub>2</sub>(Au147) solution for overnight, and one assembled with G6-NH<sub>2</sub> on its polyelectrolyte film, respectively.

presence of Au NPs on ITO. Note that we observed the silver deposition on the ITO only where the silver enhancement solution was dropped, which indicates Au NP-specific silver formation. In addition, we observed no detectable silver formation on PAMAM dendrimer-assembled ITO electrodes which were treated identically to the Au DENs-assembled ITO but in the absence of Au NPs, which also confirms silver formation specific to Au NPs (Figure 2d). The stability of the assembled Au DENs on ITO surface was tested by ultrasonating the Au DENs-assembled ITO for 20 min in ethanol which is a good solvent for amino-terminated PAMAM dendrimers. No significant difference in the amount of deposited silver was observed, which indicates the Au DENs assembled on ITO surface was stable even after the ultrasonication in ethanol for 20 min (Figure 2b). To confirm that Au DENs are not just physisorbed but self-assembled specifically on the polyelectrolyte layers-modified ITO *via* electrostatic attraction, we performed a control experiment in which a bare ITO electrode modified with no the polyelectrolyte films was immersed in a 10  $\mu$ M Au DEN solution for overnight and the soaked ITO electrode was exposed to silver enhancement solution. The ITO electrode prepared in this control experiment also showed silver deposition on the surface, but the extent of deposition was substantially smaller than one obtained using the ITO electrode modified with polyelectrolyte films and Au DENs (Figure 2c). The smaller extent of silver formation (*i.e.* smaller presence of Au DENs) on the Au DENs-physisorbed ITO indicates that the polyelectrolyte layers are essential for significant assembling of Au DENs on ITO surfaces and Au DENs are assembled mainly *via* electrostatic attraction.

Cyclic voltammetry was also employed to examine the Au DENs assembled on ITO electrodes. Figure 3 shows cyclic voltammograms (CVs) of Au DENs-assembled ITO, PAMAM dendrimer-assembled ITO, and Au DENs-physisorbed ITO electrodes obtained in 0.1 M H<sub>2</sub>SO<sub>4</sub> at a potential scan rate of 0.1 V/s. The Au DENs-assembled ITO electrode showed characteristic redox waves similar to ones of a polycrystalline Au



**Figure 3.** Cyclic voltammograms of Au DENs-assembled ITO, dendrimer-assembled ITO, and Au DENs-physisorbed ITO electrodes. For comparison, a cyclic voltammogram of a polycrystalline Au disk electrode is given in the inset. The voltammograms were obtained in 0.1 M H<sub>2</sub>SO<sub>4</sub> at a potential scan rate of 0.1 V/s.



**Figure 4.** (A) A fluorescence micrograph obtained by scanning a  $4 \times 3$  fluorescein-arrayed ITO surface on which Au DENs were assembled. (B) Fluorescence intensity profiles obtained along the dotted lines shown in (A).

electrode (Inset in Figure 3), *i.e.* an oxidation peak at  $\sim 1.3$  V in the positive scan and a reduction peak at  $\sim 0.9$  V in the reverse scan (Solid line in Figure 3). The Au DENs-physisorbed ITO also showed the Au characteristic redox waves, but much smaller waves than ones of Au DENs-assembled ITO electrode (Dotted line in Figure 3). The surface areas of the Au NPs assembled on the ITO electrodes were calculated based on the amount of the charge consumed during the reduction of the Au surface oxide monolayer. The surface area calculation used a reported value of  $400 \mu\text{C}/\text{cm}^2$ .<sup>12,18</sup> The calculated values of the Au surface areas on the Au DENs-assembled and Au DENs-physisorbed ITO electrodes were  $9.76 \times 10^{-3} \text{ cm}^2$  and  $1.85 \times 10^{-3} \text{ cm}^2$ , respectively. The larger value (about 5 times) of the Au surface area on the Au DENs-assembled ITO compared to one of the Au DENs-physisorbed ITO electrode is consistent with the results of the above silver enhancement experiments. In contrast, the characteristic redox waves of Au were not observed in the cyclic voltammogram of PAMAM dendrimer-assembled ITO electrode which was treated identically to the Au DENs-assembled ITO but in the absence of Au NPs encapsulated in the dendrimers (Dashed line in Figure 3). Those results indicate that Au DENs were significantly assembled on the polyelectrolyte films-modified ITO electrode and the assembled Au DENs were electrochemically active.

Thus far, we have demonstrated the use of PAMAM dendrimers for assembling Au NPs, which were encapsulated inside the core of the dendrimers, onto ITO electrodes. Next, we demonstrate the possibility of subsequent modification of the Au DENs-assembled ITO electrodes, which might be required for later applications. The PAMAM dendrimers have numerous peripheral amine groups which can be functionalized further. To demonstrate the possible subsequent modification, amine-reactive organic fluorophores, *i.e.* 5-carboxyfluorescein *N*-succinimidyl ester (10 mM in DMSO), were spotted onto the Au DENs-assembled ITO surface using a manual microarrayer (Xenopore Corp., NJ, USA). After incubation, the spotted slide was washed to remove unbound fluoresceins and blown dry by a  $\text{N}_2$  stream. Figure 4A is a fluorescence micrograph obtained by scanning the fluorescein-arrayed ( $4 \times 3$  array) ITO surface. All of the arrayed spots are fluorescent, which indicates that the amine-reactive fluorescein fluorophores were conjugated to the peripheral amine groups of PAMAM dendrimers assembled on the ITO surface. The fluorescence line profiles in Figure 4B show the relatively homogeneous intensities along the spots, representing that the PAMAM dendrimers encapsulating Au NPs were assembled uniformly on the ITO surface. We believe that the weaker fluorescence on the center of each spot is simply due to the well-known “coffee ring effect”, which was caused by the flux taking fluoresceins to the periphery of each spot during the incubation.<sup>19</sup> Those results indicate that the Au DENs-assembled ITO electrode can be subsequently modified via the reactive periphery amine groups of the dendrimers uniformly assembled on ITO surfaces.

In conclusion, we reported self-assembly of Au DENs onto polyelectrolyte-films on ITO electrodes and subsequent modification of the Au DENs-assembled ITO with amine-reactive fluorophores. This is a significant report because the approach described in this note provides a convenient method for immobilization of Au NPs having highly uniform small size (less than 2 nm) on ITO surfaces and subsequent functionalization of the Au NPs-modified ITO, which is challenging in conventional electrode modification methods. It is also easily envisioned to develop a variety of analytical devices using this facile electrode modification technique.

## Experimental Section

**Chemicals and materials.** Amino-terminated sixth-generation poly(amidoamine) dendrimers (G6-NH<sub>2</sub> PAMAM dendrimers, 5 wt % in methanol), HAuCl<sub>4</sub>, NaBH<sub>4</sub>, poly(diallyldimethylammonium) chloride (PDDA, 20 wt % in water, MW 400,000 - 500,000), poly(sodium-*p*-styrenesulfonate) (PSS, 30 wt % in water, MW 70,000), and cellulose dialysis sacks (MW cutoff of 12,000) were purchased from Sigma-Aldrich (MO, USA). 5-carboxyfluorescein *N*-succinimidyl ester was obtained from Invitrogen (CA, USA). ITO coated glass slides were purchased from Delta Technologies (MN, USA). 18 MΩ/cm deionized water was used to prepare aqueous solutions.

**Assembling of G6-NH<sub>2</sub>(Au147) on ITO.** An ITO electrode was ultrasonically cleaned with acetone, ethanol, and water subsequently and dried under a stream of  $\text{N}_2$ . 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 was immersed into 4% PDDA (containing 0.15 M NaCl) solution for 30 min, and then it was washed with water and dried. Next, the ITO electrode was dipped in 4% PSS for 30 min. After being washed and dried, the polyelectrolyte films-modified ITO was immersed into 10  $\mu$ M G6-NH<sub>2</sub>(Au147) DEN solution for overnight to assemble the Au nanoparticles on the surface. This assembling procedure was adopted from a similar one reported previously to obtain a multilayer PAMAM dendrimer films-modified ITO surface.<sup>13</sup>

**Instruments.** Transmission electron microscopy (TEM) images were obtained using a JEOL-2010 TEM. Samples were loaded on a carbon-coated grid and the solvent was evaporated in air. Electrochemical measurements were carried out in a small-volume electrochemical cell using a standard three-electrode configuration with a Model 440 electrochemical workstation (CH Instruments, USA). A Pt wire and a Ag/AgCl electrode were used as a counter and a reference electrode, respectively. Fluorescein-spotted arrays on Au DENs-assembled ITO electrodes were scanned using a microarray scanner (GenePix 4000B, Molecular Devices Corp., CA, USA).

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## References

1. Jhun, C. G.; Chen, C. P.; Lee, U. J.; Lee, S. R.; Yoon, T.-H.; Kim, J. C. *Appl. Phys. Lett.* **2006**, *89*, 123507/1.
2. Huang, Z. H.; Zeng, X. T.; Kang, E. T.; Fuh, J. Y. H.; Lu, L.; Sun, X. Y. *Solid-State Lett.* **2006**, *9*, H39.
3. Tak, Y.-H.; Kim, K.-B.; Park, H.-G.; Lee, K.-H.; Lee, J.-R. *Thin Solid Films* **2002**, *411*, 12.
4. Kim, H.-K.; Jeong, J.-A.; Choi, K.-H.; Jeong, S.-W.; Kang, J.-W. *Electrochem. Solid-State Lett.* **2009**, *12*, H169.
5. Chen, Z.; Zu, Y. *Langmuir* **2007**, *23*, 11387.
6. Hillebrandt, H.; Tanaka, M. *J. Phys. Chem. B* **2001**, *105*, 4270.
7. Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. *J. Phys. Chem. B* **2003**, *107*, 668.
8. Kim, J.; Lema, K.; Ukaigwe, M.; Lee, D. *Langmuir* **2007**, *23*, 7853.
9. Valden, M.; Pak, S.; Lai, X.; Goodman, D. W. *Catal. Lett.* **1998**, *56*, 7.
10. Kim, J.; Lee, S. W.; Hammond, P. T.; Shao-Horn, Y. *Chem. Mater.* **2009**, *21*, 2993.
11. Aziz, M. A.; Patra, S.; Yang, H. *Chem. Commun.* **2008**, 4607.
12. Dai, X.; Compton, R. G. *Anal. Sci.* **2006**, *22*, 567.
13. Yang, X.; Qian, L. *J. Phys. Chem. B* **2006**, *110*, 16672.
14. Zhang, J.; Oyama, M. *Anal. Chim. Acta* **2005**, *540*, 299.
15. Kim, Y.-G.; Oh, S.-K.; Crooks, R. M. *Chem. Mater.* **2004**, *16*, 167.
16. Crooks, R. M.; Zhao, M.; Sun, L.; Chechik, V.; Yeung, L. K. *Acc. Chem. Res.* **2001**, *34*, 181.
17. Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757.
18. Angerstein-Kozłowska, H.; Conway, B. E.; Hamelin, A.; Stoicoviciu, L. *J. Electroanal. Chem.* **1987**, *228*, 429.
19. Deegan, R. D.; Bakajin, O.; Dupont, T. F.; Huer, G.; Nagel, S. R.; Witten, T. A. *Nature* **1997**, *389*, 827.