Supporting Information

Enhancement of Electrocatalytic Activity of DNA-Conjugated Gold Nanoparticles and Its Application to DNA Detection

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Dependence of Electrocatalytic Activity on the Time. Figure S2 shows cyclic voltammograms obtained in a phosphate buffer solution containing 2 mM hydrazine at an ITO electrode modified with detection probe-conjugated AuNPs by changing the time of NaBH_4 treatment. The peak current of hydrazine electrooxidation increases with the time, and it becomes almost constant after 15 min. It indicates that the electrocatalytic activity plateaus at some incubation times.

Figure S3 shows cyclic voltammograms obtained at an ITO electrode modified with detection probe-conjugated AuNPs in a phosphate buffer solution containing 2 mM hydrazine by increasing the incubation time in air or phosphate buffer solution after NaBH_4 treatment of AuNPs.

Surface Density of DNA on DNA-Conjugated AuNPs. To measure total DNA amount on DNA-conjugated AuNPs, DNA was detached from AuNPs by using dithiothreitol (DTT). 100 μL of DNA-conjugated AuNPs was mixed with 100 μL of 0.1 M phosphate buffer solution (pH 8) containing 1 M DTT and the mixture was kept at 50 $^\circ\text{C}$ for 7 h. The mixture was then kept at room temperature for 2 h. The detached DNA was separated from the Au precipitate by centrifuging at 7,000 g for 15 min at room temperature using amicon ultra-4 (50,000 MW, Millipore Corporation, USA). The residue was washed with 100 μL TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) and again centrifuged at 7,000 g for 15 min at room temperature using the same centrifugal filter device. Both the filtrate was mixed together. This filtrate contains liberated DNA. The liberated DNA was measured using a commercially available single-stranded DNA quantification kit (Oligreen, Invitrogen) by following the manufacturer's protocol. 300 μL of oligreen solution in TE buffer (200-fold diluted original solution) was mixed well with liberated-DNA containing solution and incubated for 5 min in room temperature, protected from light. After incubation, fluorescence was measured using a spectrofluorometer (FP-750, Jasco), and quantified by comparing to a standard curve that was obtained by using same DNA. The excitation wavelength was 480 nm, and the

emission wavelength was 522 nm. The calculated surface coverage of DNA on AuNP was 14 pmol/cm^2 by considering the size of AuNP (8.5 nm) and 25% loss of AuNPs^{S1} in the time of centrifugation and workup. This surface coverage was comparable to published data.^{S2}

DNA Detachment during NaBH_4 Treatment. 100 μL of DNA-conjugated AuNPs was mixed with 100 μL of Tris buffer solution containing 20 mM NaBH_4 and the mixture was kept at room temperature for 15 min. The mixture was then centrifuged at 7,000 g for 15 min at room temperature using amicon ultra-4 (50,000 MW, Millipore Corporation, USA). The residue was washed with 100 μL TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) and again centrifuged at 7,000 g for 15 min at room temperature using the same centrifugal filter device. Both the filtrate was mixed together. This filtrate contains liberated DNA if any. The filtrate stored for 9 h at room temperature to fully decompose NaBH_4 . The liberated DNA was measured using a commercially available single-stranded DNA quantification kit (Oligreen, Invitrogen) by following the above mentioned protocol. The fluorescence intensity was similar to that of a solution containing no DNA-conjugated AuNPs and much smaller than that of the DNA detached from DNA-conjugated AuNPs (Figure S4). These results show that NaBH_4 does not remove the DNA layer on DNA-conjugated AuNPs.

SEM Images. The SEM images of ITO electrodes modified with detection probe-conjugated AuNPs were obtained before and after NaBH_4 treatment, and after NaBH_4 treatment followed by hydrazine electrooxidation (Figure S5). It shows that there is no significant change in the size and shape of AuNPs after NaBH_4 treatment.

Effect of NaBH_4 Treatment on Hydrazine Electrooxidation. Figure S6 shows cyclic voltammograms obtained in a phosphate buffer solution containing 2 mM hydrazine at an electrode modified with streptavidin-conjugated AuNPs before and after NaBH_4 treatment. The electrode was prepared by dropping and spreading streptavidin-conjugated AuNP onto biotin-modified ITO electrode for 30 min, followed by being washed with washing buffer. Streptavidin-conjugated AuNPs were synthesized by following the reported protocol.^{S3}

Figure S7 shows cyclic voltammograms obtained in a phosphate buffer solution containing 2 mM hydrazine at an electrode modified with IgG-conjugated AuNP before and after NaBH_4 treatment. The electrode was prepared by dropping and spreading biotinylated antimouse IgG-conjugated AuNP onto streptavidin-modified ITO electrode for 30 min, followed by being washed with washing buffer. Biotinylated antimouse IgG-conjugated AuNPs were synthesized by following the reported protocol.^{S3}

In all cases, the anodic current of hydrazine electrooxidation increased after NaBH₄ treatment. There is no change in the distance between AuNP and ITO electrode in all cases after NaBH₄ treatment. Accordingly, it indicates that the improved electrooxidation is related to faster electron-transfer kinetics rather than faster electron tunneling.

Surface Coverage of Dendrimer on an ITO Electrode. To measure the surface coverage of dendrimer on ITO electrode, ITO electrodes were modified with partially ferrocenyl-tethered dendrimer (6% Fc-D) instead of dendrimer. 6% represents the ratio of ferrocene groups to dendrimer amines. The surface concentration of 6% Fc-D on ITO electrode was calculated by dividing the surface concentration of ferrocene group (3.06×10^{-11} mol/cm²) by the number of ferrocene groups per dendrimer (3.84). The surface concentration of ferrocene group (Γ) was obtained by using the equation^{S4}

$$\Gamma = Q / nFA$$

where Q is the coulometric charge, n is the number of electrons transferred in the reaction, F is Faraday constant, and A is the electrode area (0.28 cm²). Q (8.23×10^{-7} C) was calculated by integrating the anodic peak area in the cyclic voltammogram obtained with a 6% Fc-D-modified ITO electrode in a Tris buffer solution (pH 9.0) at a scan rate of 50 mV/s (Figure S8). It is assumed that all of the ferrocene groups are electrochemically active.^{S3} Using this approach, we found that the surface coverage of 6% Fc-D on ITO electrode (i.e., the surface coverage of dendrimer on ITO electrode) is 7.97×10^{-12} mol/cm². It means that the number of dendrimer per cm² is 4.80×10^{12} . The circle area of a single dendrimer molecule is 1.26×10^{-13} cm², assuming that the diameter of dendrimer is 4 nm. Accordingly, the total area of dendrimer per cm² on ITO electrode is 0.60 cm², indicating that the surface coverage of dendrimer on ITO electrode is 60%.

Surface Density of Capture Probe on Dendrimer-Modified ITO electrodes.

Chronocoulometry (CC) was performed in 10 mM Tris buffer (pH 7.4) in the presence and absence of 50 μ M [Ru(NH₃)₆]³⁺ after purging it thoroughly with argon gas with a pulse period of 1s and a pulse with of 0.6 V (from + 0.15 V to – 0.45 V) to calculate surface density of capture probe DNA (Figure S9). The electrode was allowed to equilibrate with [Ru(NH₃)₆]³⁺ at 0.15 V for 10 s before starting CC. The surface density was derived from the charge of the surface confined [Ru(NH₃)₆]³⁺, which was obtained from the difference between the CC intercepts in the absence and presence of [Ru(NH₃)₆]³⁺. Electrode area of ITO was 0.28 cm². Following the published protocol,^{S6, S7} calculated surface density of capture probe DNA was $3.9 \pm 0.5 \times 10^{12}$ molecules/cm². This surface density is very efficient for hybridization of target DNA.^{S8}

References

- (S1) Storhoff, J. J.; Elghanian, R.; Mucic, R. C.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1998**, *120*, 1959–1964.
- (S2) Lee, J.-J.; Seferos, D. S.; Gijohann, D. A.; Mirkin, C. A. *J. Am. Chem. Soc.* **2008**, *130*, 5430–5431.
- (S3) Hermanson, G. T. *Bioconjugate Techniques*, 1st ed., Academic Press, San Diego, **1996**, pp. 593–604.
- (S4) Wang, J. *Analytical Electrochemistry*, 2nd ed., John Wiley & Sons, Inc., New York, **2000**, pp. 36–39.
- (S5) Kim, E.; Kim, K.; Yang, H.; Kim, Y. T.; Kwak, J. *Anal. Chem.* **2003**, *75*, 5665–5672.
- (S6) Steel, A. B.; Herne, T. M.; Tarlov, M. J. *Anal. Chem.* **1998**, *75*, 4670–4677.
- (S7) Ge, B.; Huang, Y.-C.; Sen, D.; Yu, H.-Z. *J. Electroanal. Chem.* **2007**, *602*, 156–162.
- (S8) Lee, C.-Y.; Nguyen, P.-C. T.; Grainger, D. W.; Gamble, L. J.; Castner, D. G. *Anal. Chem.* **2007**, *79*, 4390–4400.

Figure S1. Schematic representation of the preparation of a DNA-sensing electrode.

Figure S2. Cyclic voltammograms obtained at an ITO electrode modified with detection probe-conjugated AuNPs in a 0.1 M phosphate buffer solution containing 2 mM hydrazine at a scan rate of 50 mV/s by increasing the treatment time in a NaBH_4 -containing solution.

Figure S3. Cyclic voltammograms obtained at an ITO electrode modified with detection probe-conjugated AuNPs in a 0.1 M phosphate buffer solution containing 2 mM hydrazine at a scan rate of 50 mV/s by increasing the incubation time in (a) air or (b) phosphate buffer solution after activation of DNA-conjugated AuNPs. For the activation of DNA-conjugated AuNPs, the ITO electrode was dipped for 15 min in Tris buffer containing 10 mM NaBH_4 .

Figure S4. Fluorescence spectra of oligreen-containing solutions. The background corresponds to the spectrum obtained in a solution containing no DNA-conjugated AuNPs, the filtrate after NaBH₄ treatment corresponds to the spectrum obtained with the filtrate after NaBH₄ treatment of DNA-conjugated AuNPs, and the DNA from DNA-conjugated AuNPs corresponds to the spectrum obtained with the DNA detached from DNA-conjugated AuNPs by DTT treatment.

Figure S5. SEM images of DNA-conjugated AuNPs adsorbed on an dendrimer-modified ITO electrode, which were obtained (a) before and (b) after NaBH_4 treatment, and (c) after NaBH_4 treatment followed by hydrazine electrooxidation. To clearly see the images of particles, 20-nm AuNPs were used.

Figure S6. Cyclic voltammograms obtained at an electrode modified with streptavidin-conjugated AuNP before and after NaBH_4 treatment in a 0.1 M phosphate buffer solution containing 2 mM hydrazine at a scan rate of 50 mV/s.

Figure S7. Cyclic voltammograms obtained at an electrode modified with IgG-conjugated AuNP before and after NaBH_4 treatment in a 0.1 M phosphate buffer solution containing 2 mM hydrazine at a scan rate of 50 mV/s.

Figure S8. Cyclic voltammograms of a 6% Fc-D-modified ITO electrode in a Tris buffer solution (pH 9) containing 1 g/L MgCl_2 at a scan rate of 50 mV/s.

Figure S9. Chronocoulometry of capture probe-modified DNA-sensing electrode in a 10 mM Tris buffer solution (pH 7.4) in the presence and absence of 50 μM $[\text{Ru}(\text{NH}_3)_6]^{3+}$.