Supporting Information

A hybrid composite of gold and graphene oxide as PCR enhancer

Ha Young Jeong\textsuperscript{a}, Seung Hun Baek\textsuperscript{a}, Sung-Jin Chang\textsuperscript{a}, MinHo Yang\textsuperscript{b}, Seok Jae Lee\textsuperscript{b}, Kyoung G. Lee\textsuperscript{b,*} and Tae Jung Park\textsuperscript{a,*}

\textsuperscript{a}Department of Chemistry, Chung-Ang University, 84 Heukseok-ro, Dongjak-gu, Seoul 06974, Republic of Korea

\textsuperscript{b}Department of Nanobio Research, 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea
**Experimental Section**

**Reagents and apparatus**

Graphite powder was obtained from Kanto Chemical (Kanto, Japan). Potassium permanganate (KMnO₄), phosphorus pentoxide (P₂O₅), concentrated sulfuric acid (H₂SO₄), potassium persulfate (K₂S₂O₈), Gold (III) chloride trihydrate (HAuCl₄·3H₂O), trisodium citrate (Na₃C₆H₅O₇), and phosphate-buffered saline (PBS, 20 mM, pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). Hydrazine solution (NH₂NH₂), hydrochloric acid (HCl), and hydrogen peroxide (H₂O₂) were obtained from Samchun (Seoul, Korea). Trypsin-EDTA solution was purchased from Welgene (Gyeongsan, Korea). Filter papers were purchased from Whatman (Dassel, Germany). Ultrapure deionized (DI) water obtained from a Millipore water purification system (Milli-Q, Billerica, MA) was used in all assays. The FAM-modified ssDNA sequence (5′-FAM-GAG ATT CAT CAC GCG CAT AGT CCC AAG GCC TGC AAG GGA ACC AAG GAC ACA GCG ACT ATG CGA TGA TGT CTT C-3′) was synthesized by MACROGEN (Seoul, Korea). The genomic DNA were purified by G-DEX™ IIC genomic DNA extraction kit and PCR reaction mixture were performed using MAXIME PCR premix Kit from Intron (Seoul, Korea). The Taq polymerase was purchased from BioLabs (Lpswich, MA).

**Synthesis of graphene oxide**

Graphene oxide (GO) was synthesized from graphite powder adopting a modified Hummers and Offeman method. For the details, the solution of 50 wt% H₂SO₄ (27 mL), K₂S₂O₈ (2 g), and P₂O₅ (2 g) were mixed in the flask and heated at 85 °C. Then,
2 g of graphite powder was added the solution under magnetic stirring for 5 h. After that, 500 mL of deionized (DI) water was added slowly into the solution and mixture was stirred for 14 h. The mixture was filter and dried at room temperature. As the prepared before were put into a mixture cool solution of 50 wt% H₂SO₄ (270 mL) and added slowly 10 g of KMnO₄ in a stirred system with an ice bath for 1 h. The solution was cooled to room temperature (25 °C) under vigorous magnetic stirring for 24 h. Then, 500 mL of DI water and 20 mL of 30% H₂O₂ were then added respectively, the color of the solution was change from dark to yellow. After, the mixture was washed and filtered with 10 wt% HCl (1 L) and DI water (1 L) aqueous solution to remove ions and was dry at 60 °C in air.

**Synthesis of Au/GO hybrid composite**

The synthesis of Au/GO hybrid composite was based on the reduction of Au (III) complex by sodium. Generally, 1.5 mg mL⁻¹ GO (5 mL) was sonicated for 5 min to form dispersed GO. Then, 100 mL of 38 mM HAuCl₄ were added to the GO solution with magnetic stirring for 0.5 h. After, the reaction solution was heated until 80 °C, after a solution of sodium citrate (0.85 M, 1.92 mL) was added drop wise. The reaction was continued at these maintain during 1 h. The result solution was washed with filtration (0.1 μm, Whatman) to remove the free Au NPs in aqueous solution.

**Characterization**

UV/vis spectrophotometer and transmission electron microscope (TEM) analysis were conducted with OPTIZEN POP spectroscopy (Mecasys, Daejeon, Korea) and Tecnai
G2 F30 S-Twin microscope (FEI, Hillsboro, OR). Atomic force microscope (AFM) characterizations were conducted with non-contact mode on an XE-100 instruments at a scanning rate of 1 Hz and operated by XEP software (Park Systems, Suwon, Korea).

**Conventional PCR**

All experiments were performed using a PCR machine (T100™ Thermal cycler, Bio-rad, Hercules, CA) with premix solution. Specific primers for amplification from genomic DNA of aquatic fish (*Scomber japonicas*) and *Listeria monocytogenes* 19115 were used as shown in Table S1. The conventional PCR reaction was performed with a final concentration of 20 μL: which commonly contained 0.1 μM each primer, 20 μg L⁻¹ of genomic DNA, 1×10⁻² μg μL⁻¹ of each nanomaterials and PCR reaction mixture. The PCR reaction mixture contained the following final concentrations: i-Taq™ DNA polymerase 2.5 U μL⁻¹ in the reaction buffer, dNTP 2.5 mM each, Reaction buffer 1X, Gel loading buffer 1X. For PCR protocol followed initial denaturation step at 94 °C for 5 min, which consisted of 30 cycles of amplification: 30 s at 94 °C denaturation step, 30 s at 50 °C annealing step, and 50 s at 72 °C elongation step. Then after an extension step of 72 °C for 5 min, PCR tubes were maintained at 4 °C. The PCR products were analyzed by 1.0% (w/v) agarose gel electrophoresis and Quantity one program.

**Real-time PCR amplification**

For each real-time PCR, 2X SYBR Green I (SYBR Premix Ex Taq™ II, Takara Bio, Shiga, Japan) was used with the components as follows: Takara Ex Taq HS, dNTP
Mixture, Mg\(^{2+}\), Tli RNaseH, and SYBR Green I. Like as conventional PCR method, genomic DNAs of aquatic fish and *L. monocyte* were used as a template, respectively, with forward and reverse primers of 10 pmol for the reaction and with or without nanomaterials in a total volume of 25 μL. The real-time PCR was performed with a 2-step using real-time PCR machine (CFX96, Bio-Rad). Initial denaturation at 94 °C for 5 min was followed by 50 cycles of 30 s at 95 °C (denaturation), 30 s at 55 °C (annealing). After the reaction, the qPCR results were confirmed by Bio-Rad CFX Manager 3.1 program.

**Interaction between ssDNA and Au/GO hybrid composite using fluorescence quenching effect**

The FAM-ssDNA was suspended in a PBS solution (20 mM, pH 7.4) and 1 μL of this 20 nM solution was mixed with 18 μL of a 1 mg mL\(^{-1}\) solution of Au NPs, GO, and Au/GO hybrid composite in a 1.5 mL tube, respectively. This mixture was allowed to react for 15 min. The fluorescence measurements were performed using Synergy H1 Hybrid Reader from BioTek (Winooski, VT). The excitation wavelength was 480 nm, and emission spectra were collected at 520 nm.

**Adhesion of PCR elements on Au/GO surface**

Forward primer of *L. monocyte* genomic DNA (1 μL of 100 pmol) and *Taq* polymerase (1 μL of 5,000 U mL\(^{-1}\)) were mixed with 100 μL DI water, respectively. Then, 18 μL of a 1 mg mL\(^{-1}\) solution of Au NPs, GO, and Au/GO hybrid composite was added to above solutions. These solutions were incubated for 15 min at room
temperature. After 15 min reaction, the solutions were centrifuged at 3,000 rpm (3,650 \times g) for 10 min using the Smart R17 Micro Refrigerated Centrifuge by Hanil (Incheon, Korea). Then, 50 µL of supernatant was measured by recording UV/vis spectrum data.
**Fig. S1.** UV-vis absorption spectra of (a) GO and (b) Au/GO hybrid composite. Inset: UV-vis absorption spectrum obtained for the Au NPs of synthesized Au/GO hybrid composite at 520 nm.
**Fig. S2.** The effect of the annealing temperature on the PCR using genomic DNA of aquatic fish and (a) Au NPs, (b) GO, and (c) Au/GO hybrid composite. Lane M: DNA marker; lane 1: 55 °C; lane 2: 50 °C; lane 3: 45 °C; lane 4: 40 °C.
**Fig. S3.** Schematic illustration of the differences between quenching of dye-labeled primers and the amplification of fluorescence signal through intercalation of dye into the amplified DNA during real-time PCR. (a) The FAM dye labeled primers immobilized on the surface of Au/GO hybrid composite, and the fluorescence was quenched efficiently. (b) SYBR green intercalated double-stranded DNA (dsDNA) during real-time PCR and resulted in fluorescence emission.
### Table S1. DNA oligonucleotides and primers used in this study

<table>
<thead>
<tr>
<th>Genomic DNA</th>
<th>Length (bp)</th>
<th>Primer (5’→3’)</th>
<th>GC %</th>
</tr>
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<tbody>
<tr>
<td><strong>Aquatic fish</strong> <em>(Scomber japonicus)</em></td>
<td>800</td>
<td>F: 5’-CAACCAACCAACAAAGACATTGGCAC-3’</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5’-ACTTCAGGGTGACCCGAAGAATCAGAA-3’</td>
<td>48</td>
</tr>
<tr>
<td><strong>Listeria monocytogenes 19115</strong></td>
<td>280</td>
<td>F: 5’-GCGCCACTACGGACGTTAACCAAG-3’</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R: 5’-ACAATCGCATCCGCAAGCCTGTAG-3’</td>
<td>56</td>
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