Electronic supplementary information

An Exonuclease III-aided “‘Turn-on’” Fluorescence Assay for Mercury Ions Based on Graphene Oxide and Metal-Mediated “Molecular Beacon”

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Optimization of the Concentration of GO

To optimize the concentration of GO, different concentrations of GO were added to 10 µL of the probe stock solution (1.0 µM) and diluted with 20 mM Tris-HCl (pH 7.5) buffer to 250 µL and incubated 30 min with gentle shake at room temperature. Finally, the fluorescence intensity was measured at 520 nm with excitation at 480 nm.

Optimization of the Concentration of Exo III

10 µL of the probe stock solution (1.0 µM) in the absence and presence of 20 µL Hg²⁺ (45.75 µM) was incubated for 1 h at 25 ºC. Then different concentrations of Exo III solution were added into this mixture, and allowed to incubate for 15 min at 25 ºC. Finally, 10 µL of GO (250 µg/mL) was added to the above mixture and diluted with 20 mM Tris-HCl buffer to 250 µL and incubated 30 min with gentle shake at room temperature. After that, the fluorescence intensity was measured at 520 nm with excitation at 480 nm.

Optimization of the Reaction Time between MB and Exo III

To optimize the reaction time between MB and Exo III, 10 µL of the probe stock solution (1.0 µM), 20 µL of Hg²⁺ (45.75 µM) were mixed, and was incubated for 1 h at 25 ºC. Then 0.5 U of Exo III solution was added into this mixture, and allowed to incubate for different
time (0, 10, 20, 30, 40, 50 min) at 25 ºC. Finally, 10 µL of GO (250 µg/mL) was added to the above mixture and diluted with 20 mM Tris-HCl buffer to 250 µL and incubated 30 min with gentle shake at room temperature. After that, the fluorescence intensity was measured at 520 nm with excitation at 480 nm.

Optimization of the Reaction Temperature between MB and Exo III

To optimize the reaction temperature between the probe and Exo III, 10 µL of the probe stock solution (1.0 µM), 20 µL of Hg^{2+} (45.75 µM) were mixed, and incubated for 1 h at 25 ºC. Then 0.5 U of Exo III solution was added into this mixture, and allowed to incubate for 15 min at different temperatures. Finally, 10 µL of GO (250 µg/mL) was added to the above mixture and and diluted with 20 mM Tris-HCl buffer to 250 µL and incubated 30 min with gentle shake at room temperature. After that, the fluorescence intensity was measured at 520 nm with excitation at 480 nm.
**Fig. S1** Image of the gel electrophoresis to verify the Amplified Mechanism of Exo III:

Lane 1: 3.3 µM MB; Lane 2: 3.3 µM MB + 6 U Exo III, enzyme reaction time: 10 min;
Lane 3: 3.3 µM MB + 15.25 µM Hg²⁺ + 6 U Exo III, enzyme reaction time: 10 min; Lane 4: 3.3 µM MB + 0.61 µM Hg²⁺ + 6 U Exo III, enzyme reaction time: 10 min; Lane 5: 3.3 µM MB + 0.61 µM Hg²⁺ + 6 U Exo III, enzyme reaction time: 70 min
**Fig. S2** Exo III Activity on DNA Duplex with T–Hg$^{2+}$–T Base Pairs: Lane 1: 3.33 μM DNA 2 + 7.625 μM Hg$^{2+}$; Lane 2: 1.67 μM DNA 1 + 1.67 μM DNA 2 + 7.625 μM Hg$^{2+}$; Lane 3: 1.67 μM DNA 1 + 1.67 μM DNA 2 + 6 U Exo III + 7.625 μM Hg$^{2+}$; Lane 4: 1.67 μM DNA 3 + 1.67 μM DNA 1 + 6 U Exo III + 7.625 μM Hg$^{2+}$; Lane 5: 1.67 μM DNA 4 + 1.67 μM DNA 1 + 6 U Exo III + 7.625 μM Hg$^{2+}$; Lane 6: 1.67 μM DNA 5 + 1.67 μM DNA 1 + 6 U Exo III + 7.625 μM Hg$^{2+}$; Lane 7: 1.67 μM DNA 6 + 1.67 μM DNA 1 + 6 U Exo III + 7.625 μM Hg$^{2+}$. 
Fig. S3 Fluorescence spectra of MB under different concentrations of GO
**Fig. S4** Fluorescence intensity of MB in the absence and presence of Hg$^{2+}$ upon the addition of different concentrations of Exo III. Inset: Relative fluorescence intensity versus concentrations of Exo III, where $F_0$ and $F$ are the fluorescence intensities in the absence and presence of Hg$^{2+}$, respectively.
**Fig. S5** Fluorescence intensity in the absence and presence of Hg$^{2+}$ upon different reaction time between MB and Exo III. Inset: Relative fluorescence intensity versus reaction time between MB and Exo III, where $F_0$ and $F$ are the fluorescence intensities in the absence and presence of Hg$^{2+}$, respectively.
**Fig. S6** The change of fluorescence intensity of MB in the absence and presence of Hg$^{2+}$ under different reaction temperatures between MB and Exo III. Inset: Relative fluorescence intensities of MB under different reaction temperatures between MB and Exo III, where $F_0$ and $F$ are the fluorescence intensities in the absence and presence of Hg$^{2+}$ under different reaction
<table>
<thead>
<tr>
<th>Designation</th>
<th>Sequence (5' to 3')</th>
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</tr>
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<tr>
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