Supporting Information

Label-free photoelectrochemical aptasensor for facile and ultrasensitive mercury ion assay based on solution-phase photoactive probe and exonuclease III-assisted amplification

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Table of Contents

Experimental section

1. Reagents and Materials

Exonuclease III (Exo III), 10× Reaction buffer (660 mM Tris-HCl, 6.6 mM $MgCl_2$, pH 8.0) and the synthetic HPLC-purified DNA oligonucleoties were obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequence of the hairpin DNA probe (HP) is shown below:

5′-C**TTT**AGGGTGGGGAGGGTGGGGCCCCACCCT**TTT**G -3′

in which, the underlined letters represent the bases in the stem of the hairpin, and the bold letters stand for the thymines capable of forming T-Hg^{2+}-T bonds. Tris(hydroxymethyl) aminomethane (Tris), methylene blue (MB), hydrochloric acid (HCl), NaOH, NaCl, KCl, and $MgCl_2$ were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Mercuric chloride ($HgCl_2$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid (AA) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). GelRed was from Solebo Biotechnology Co., Ltd. (Beijing, China). All chemicals were of analytical grade without further purification. Indium tin oxide (ITO)-coated glass (sheet resistance ＜ 10 Ω/square) was purchased from Shenzhen Nanbo Display Technology Co. Ltd. (Shenzhen, China). Ultrapure water (resistivity of 18.2 MΩ cm at 25 °C) produced by Milli-Q ultrapure water system (Millipore, Bedford, MA, USA) was used throughout the experiments. All oligonucleotides were diluted in 10 mM Tris-HCl buffer (pH 7.4) to make stock solutions, which, prior to use, were heated up to 90 °C and maintained at this temperature for 10 min, and then slowly cooled down to room temperature.

2. ITO Electrode Pretreatment

ITO-coated glass was cut into 1.0 cm × 4.0 cm slices, which were then pretreated as follows: firstly, the ITO electrodes were cleaned by sequential ultrasonic treatment in 1 M NaOH of

water/ethanol mixture (1:1, v/v), acetone, and ultrapure water for 30 min, respectively; next, the ITO electrodes were immersed in 1 mM NaOH solution for 5 h at room temperature, followed by sonication in ultrapure water for 10 min, and then blown dry with nitrogen gas. Upon these treatments, negatively charged ITO electrode surface was obtained. Furthermore, for each cleaned 1.0 cm × 4.0 cm ITO electrode, a strip of Scotch tape was stuck on the conducting side of the ITO slice, positioning at 1.0 cm from one end. As a result, a conducting surface of 1.0 cm × 1.0 cm was confined by the tape, with an active area of 1.0 cm^2, and such ITO electrodes were ready for use in PEC measurements.

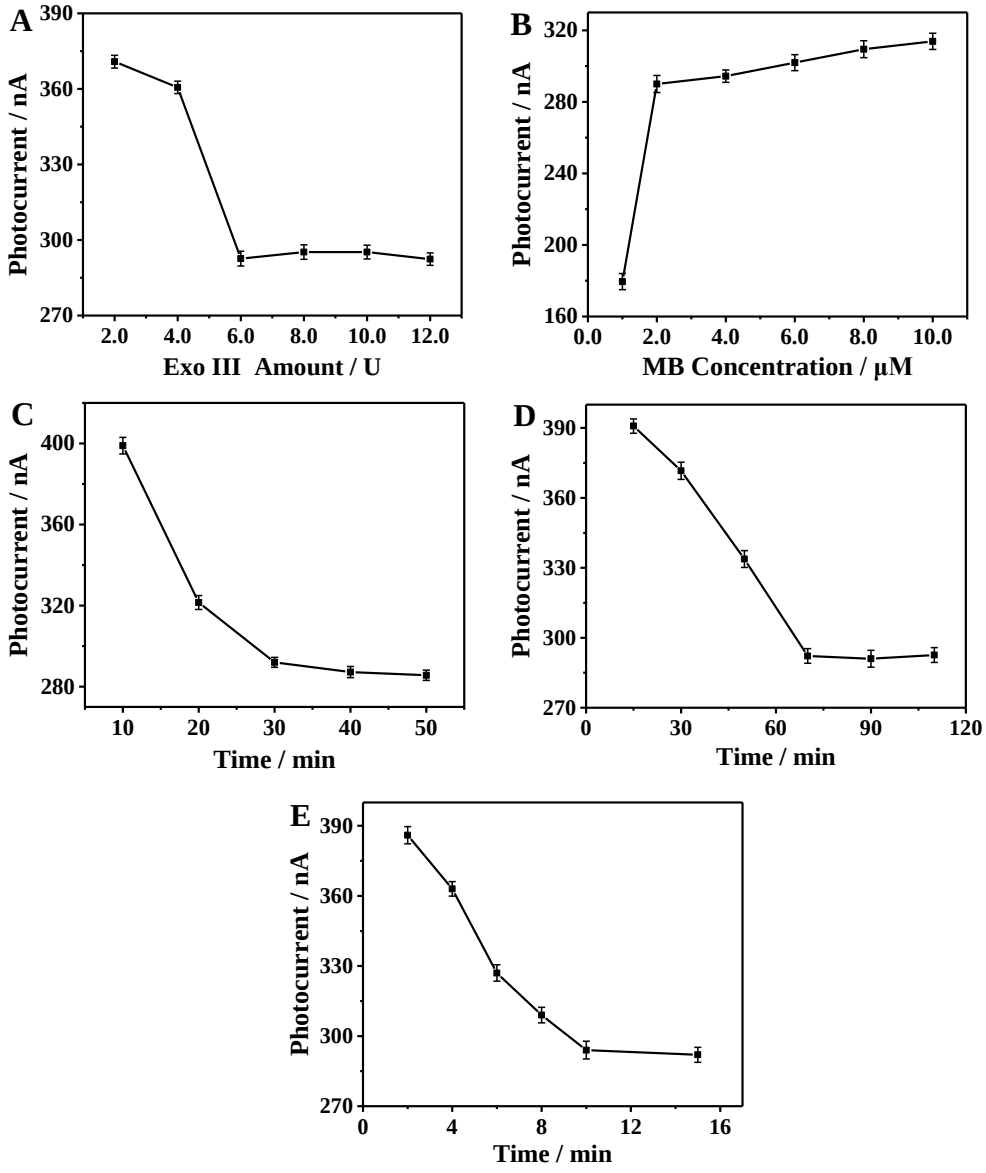

Figure S1. Photocurrent versus (A) Exo III amount (2, 4, 6, 8, 10 and 12 U), (B) MB concentration (1, 2, 4, 6, 8 and 10 μM), (C) interaction time between HP and Hg^{2+} (10, 20, 30 ,40, 50 min), (D) Exo III-assisted digestion time (15, 30, 50, 70, 90, 110 min), and (E) MB intercalation time (2, 4, 6, 8, 10 and 15 min). The concentration of Hg^{2+} is 4 nM in all cases, and the other experimental conditions are the same as those mentioned in Experimental Section, if not stated otherwise. The error bars stand for the standard deviation of five repetitive measurements.

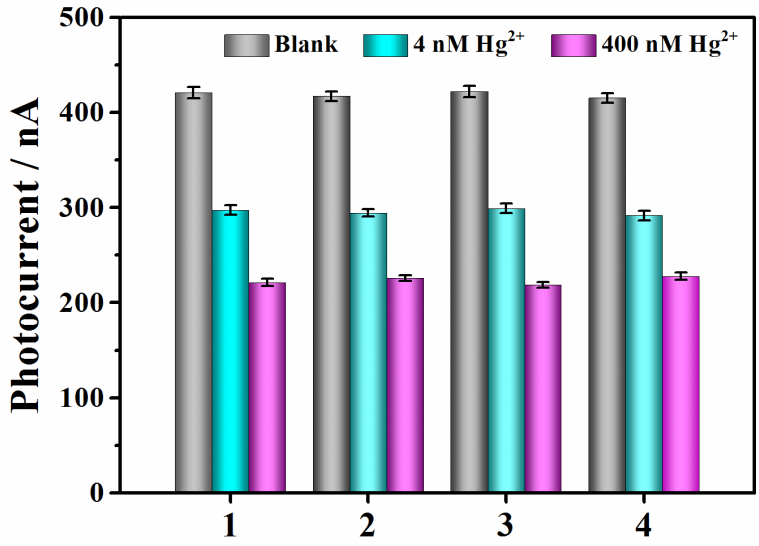

Figure S2. Photocurrents obtained on four different bare ITO electrodes in the absence and presence of 4 nM and 400 nM Hg^{2+}. The other experimental conditions are the same as those mentioned in Experimental Section, and the error bars represent the standard deviation of five repetitive measurements.

Table S1. Comparison of analytical performance of Hg^{2+} detection by our method and those reported in literature.

Detection Method	Linear Detection Range	Limit of Detection	Reference
Label-free & Immobilization-free PEC	4 pM ~ 500 nM	1.2 pM	This work
Immobilization-based PEC	0.01 nM ~ 10 nM	2.5 pM	1
Immobilization-based PEC	5 pM ~ 500 pM	1 pM	2
Electrochemistry	50 pM ~ 100 nM & 100 nM ~ 10 μM	10 pM	3
Electrochemistry	0.5 nM ~ 80 nM	0.2 nM	4
Fluorescence	5 nM ~ 100 nM	3.49 nM	5
Fluorescence	10 pM ~ 100 nM	4.5 pM	6
Fluorescence	10.0 nM ~ 8.0 μM	2.6 nM	7
Colorimetry	1 nM ~ 28 nM	32 pM	8
Colorimetry	10 nM ~ 500 nM	3.3 nM	9
Colorimetry	0.1 nM ~ 25 nM	0.39 nM	10
Chemiluminescence	0.01 ng/mL ~ 1000 ng/mL (0.05 nM ~ 5000 nM)	50 pM	11
Electrochemiluminescence	0.2 ng/mL ~ 1000 ng/mL (1 nM ~ 5000 nM)	0.06 ng/mL (0.3 nM)	12

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