Supporting information

Visual Electrochemiluminescence Detection of Telomerase Activity Based on Multifunctional Au Nanoparticles modified with G-quadruplexes Deoxyribozymes and Luminol

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SI 1. Experimental Section

SI 1.1. Reagents

3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonic acid (CHAPS), phenylmethylsulfonyl fluoride (PMSF), ethylene glycol bis (-aminoethyl ether)-N, N, N, N tetraacetic acid (EGTA), glycerol, and Tween 20 were purchased from Biosharp Biotechnology. Hemin was purchased from Aladdin (Shanghai, China) and used without further purification. Hemin solution was prepared in DMSO and stored in the dark at -20 ˚C. ITO-coated (thickness ~100 nm, resistance ~10 Ω/square) aluminosilicate glass slides were purchased from CSG (Shenzhen, China). SG-2506 borosilicate glass (with 145 nm thick chrome and 570 nm thick anodic S-1805 type photoresist) was purchased from Shaoguang Chrome Blank Co. Ltd. Sylgard 184 (including poly (dimethylsiloxane) (PDMS) monomer and curing agent) was from Dow Corning (Midland, MI). Oligonucleotides of telomerase primer, complementary DNA, and G-quadruplexes DNA were purchased from Sangon Biotech Co. Ltd (Shanghai, China), and the sequences were listed in Table 1. Deoxynucleotide solution mixture (dNTPs), bovine serum albumin (BSA), tri (2-carboxyethyl) phosphine hydrochloride (TCEP) and luminol were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. 0.1 M PBS (pH 7.4) buffer containing K_2HPO_4 and KH_2PO_4 was used to wash cancer cells. 0.1 M luminol solution was first dissolved in 0.1 M KOH solution and then diluted into required concentrations by PBS buffer (pH 7.4). H_2O_2 solution was also diluted by PBS buffer (pH 7.4). 0.1 M, pH 7.4 PBS buffer containing 0.1 M NaCl and 5 mM $MgCl_2$ was

employed for preparation of DNA stock solutions. All other reagents were of analytical grade and used as received. All the water used in the work was RNAase-free.

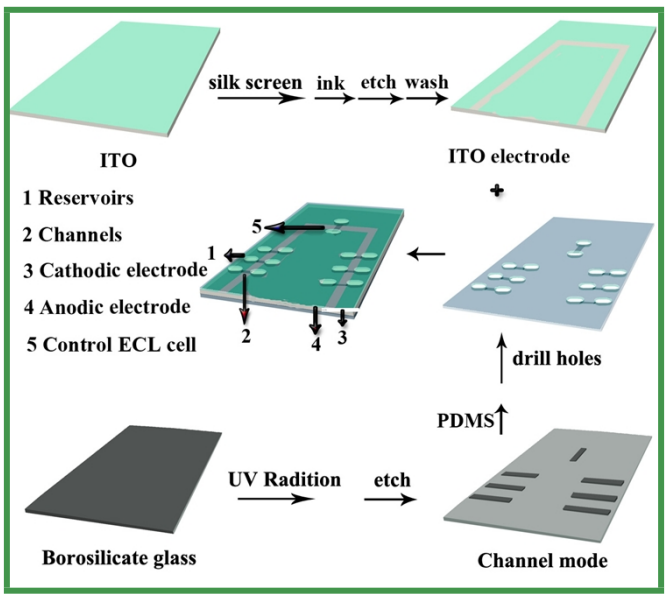
Table 1. The DNA Sequences used in this work.

Name	Sequences (5' to 3')
Telomerase primer	5'-SH-$(CH_2)_6$-TTTTTTAATCCGTCGAGCAGAGTT-3'
Complementary DNA	5'- AACTCTGCTCGACGGATTAAAAAA -3'
G-quadruplex DNA	5'-CTAACCCTAACCCTAACCTTTTTTTTGGGTAGGGCGGGTTGGGTTTTTTTTTT-$(CH_2)_6$-SH-3'

SI 1.2. Instrument:

CHI-660 A was used to apply a voltage of 1.6 V on the two ITO electrodes to induce the ECL of luminol- H_2O_2 system. An Olympus DP71 cooled CCD camera was applied for chip imaging, results of which were analyzed by Image-Pro Plus (IPP) 6.0 software. Transmission electron microscopy (TEM) images were performed with a JEOL model 2000 instrument operating at 200 kV accelerating voltage to characterize the sensing surfaces. The UV–vis absorption spectra were recorded on a Shimadzu UV-3600 UV–vis–NIR photospectrometer (Shimadzu Co.).

SI 1.3. ITO Electrode Fabrication and Design.



Scheme S1. The process of fabricate and design ITO electrode.

Firstly, a piece of ITO was first cut into small slices (6 cm * 6.5 cm). Then as can be seen in scheme S1, those ITO slices were fabricated on a silk-screen. For screen-printing, oil ink (essential ingredient of acrylic resin) was transmitted through a U shape silk-screen mode onto the ITO layer by brush. Once the oil ink on the ITO was dry, a wet chemical etching procedure was carried out with chemical solution (HF: NH_4F: HNO_3=1: 0.5: 0.5), which produced the desired shape of ITO electrodes. Those ITO electrode slices were cleaned by immersion into a boiling solution of 2 M KOH in 2-propanol for 20 min, followed by washing with water and milli-Q water. To obtain the PDMS molds with one or more predesigned channels, SG-2506 borosilicate glass were fabricated by traditional photolithography and wet chemical etching techniques. SG-2506 borosilicate glass plates were exposed to UV radiation under a mask with the designed patterns, followed by developing with a 0.5% NaOH solution, the Cr layer was then removed by a 0.2 M ammonium cerium (IV) nitrate solution. Etching of these glass plates was carried out in 1 M HF-NH_4F solution (40 ˚C) with a water bath for 28 min. After that, designed channel structures mold were obtained. Then degassed PDMS was cast on these glass masks for 1 h in 80 ˚C. After cooling at room temperature, the PDMS was stripped from the masks, producing the PDMS molds. The PDMS molds were been drilled with 4 mm holes as reservoirs and attached to the ITO-coated glass slice.

SI 1.4. Preparation of Telomerase Primer Modified ITO Electrode.

Au NPs was synthesized according to previous work and the TEM image in Fig. S1 shows the diameter of Au NPs is about 25nm. Au NPs solution was stored in fridge (4 ˚C) for further use after being synthesized. After ITO electrode was covered with reservoirs decorated polydimethylsiloxane (PDMS) slice without channels, 40 μL Au NPs solution mixed with 4 μL chitosan was added into each reservoir on the anodic ITO electrode and then air-dried at room temperature to form seven Au NPs modified circles. The solution of 1×10^{-5} M (20 μL) telomerase primer was pretreated by 2 μL TCEP and then it added into the reservoirs and kept for 24 h at 4 °C in order to assure the fastness of Au-S bond. The obtained surface was rinsed with 20 μL of 0.1 M PBS buffer to remove the nonspecific binding telomerase primer.

SI 1.5. Hybridization of the G-quadruplexes - L-Au NPs DNAzyme Labels

L-Au NPs (20 nm) were synthesized via the direct reduction of chloroauric acid ($HAuCl_4$) by luminol. A number of luminol molecules as stabilizers were coated on the Au NPs through such a simple one-pot method. The L-Au NPs solution was stored

in fridge (4˚C) for further use after synthesized. 100 mM (200 μL) G-quadruplex DNA was added into L-Au NPs solution and kept for 24 h to form G-4 DNA-Au NPs bioconjugates. Then the solution of bioconjugates was incubated in a buffer solution (pH 7.4, composed of 25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100 and 1% DMSO), with 0.2 mM hemin for 1 h to form DNAzyme-Au NPs biomarkers.

SI 1.6. Telomerase Extension Experiment and Hybridization of L-Au NPs.

HL-60 cancer cells were cultured in DMEM medium supplemented with 10 % fetal calf serum, and maintained at 37 °C in a humidified atmosphere (95% air and 5% CO_2). HL-60 cancer cells were collected in the exponential phase of growth and washed twice with ice-cold sterile PBS, then 10^5 HL-60 cancer cells was resuspended in 200 μL of ice-cold CHAPS lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM mercaptoethanol, 0.5% CHAPS, 10% glycerol). The lysate was incubated for 30 min on ice and centrifuged for 15 min at 16000 rpm in 4 °C to pellet insoluble material. Then the cleaned lysate was carefully transferred to a 1.5 mL EP tube. The lysate was used immediately for telomerase assay or frozen at -80 °C. 60 μL telomerase reaction solution which contained 20 μL telomerase extracts from different number of HL-60 cancer cells, and 40 μL telomerase reaction buffer (20 mM Tris-HCl buffer, pH 8.3, 1.5 mM $MgCl_2$, 0.63 mM KCl, 0.05% Tween 20, 1 mM EGTA, 0.1 mM dNTPs) were added into the reservoirs on the anodic electrode, respectively, at 37 °C for 1 h. For control experiments, telomerase extracts were pretreated at 95 °C for 20 min and added into control ECL cell. Then the electrode was incubated with 40 μL telomerase reaction buffer which was mentioned above. After that, the electrode was washed thoroughly with 0.1 M PBS buffer for further use. Then, 20 μL complementary DNA and 50 μL DNAzyme modified L-Au NPs solution were added into the reservoirs on the anodic ITO electrode, at 37 °C for 1 h. Finally the reservoirs were washed thoroughly with 0.1 M PBS buffer for further detection.

SI 1.7. Visual Luminescence Measurement.

After telomerase primer DNA was modified in the reservoirs on the anodic ITO electrode, 20 μLof 50 mM H_2O_2 and 20 μL of 5.0 mM luminol mixed solution was added into the reservoirs on the ITO electrodes. A voltage of 1.6 V was applied on the two ITO electrodes. The ECL intensity was measured using Olympus DP71 cooled CCD camera as original signals. The data of the luminescence intensity for each spot were obtained by Image-Pro Plus (IPP) 6.0 software analysis. After all the modified

precedures completed, telomerase from different number of HL-60 cancer cells was detected using designed method and the enhanced luminescence emission was recorded as finally signals in the same way.

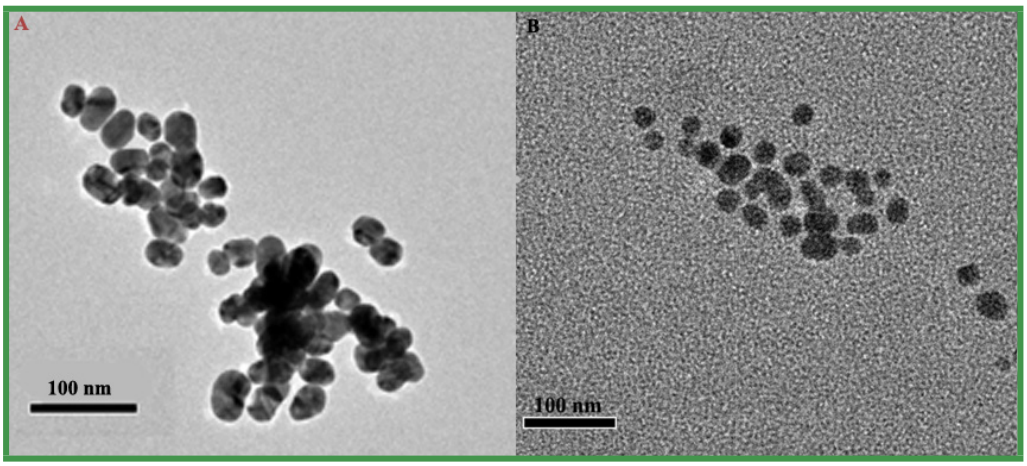


Figure S1.（A）TEM image of Au NPs.（B）TEM image of L-Au NPs.

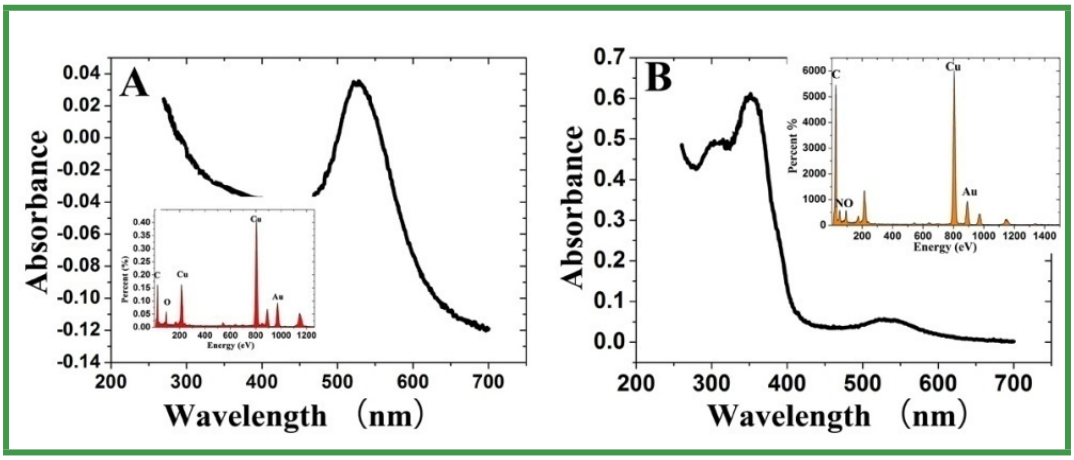


Figure S2. (A) UV-visual spectrum of Au NPs, inset: EDX of Au NPs. (B) UV-visual spectrum of L-Au NPs; inset: EDX of L-Au NPs.