Electronic Supporting Information

Label-free ultrasensitive detection of telomerase activity *via* multiple telomeric hemin/G-quadruplexes triggered polyaniline deposition and DNA tetrahedron-structure regulated signal

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List of Contents:

1. Chemicals and Materials

All oligonucleotides synthesized and modified by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). The sequences are shown in Table S1. RuHex, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 3-[(3-Cholamidopropyl)dimethylammonio]propanesulfonate (CHAPS), ethylene glycol tetraacetic acid (EGTA), ethylene diamine tetraacetic acid (EDTA), phenylmethanesulfonyl fluoride (PMSF), 6-Mercapto-1-hexanol (MCH), and aniline (99.5%) were obtained from Sigma-Aldrich Co. Ltd. (St. Louis, MO). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Life Technologies (California, USA). Hemin was purchased from Porphyrin Products (Logan, UT, USA) and used without further purification. 2-[(E)-3-naphthalen-2-ylbut-2-enoylamino] benzoic acid (BIBR 1532) was received from Selleck Chemicals (Houston, USA). Curcumin, genistein, and 2,6-diaminoanthraquinone were obtained from J & K Chemical Ltd. (Shanghai, China).

The buffer solutions employed in this study were as follows: 1×PBS (pH 7.2 ~ 7.4, 136.89 mM NaCl, 2.67 mM KCl, 8.24 mM Na_2HPO_4, 1.76 mM NaH_2PO_4); Lysis buffer (0.5% (w/v) CHAPS, 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 10% (v/v) glycerol); Telomerase extension reaction buffer (20 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 1 mM EGTA, 63 mM KCl, 0.005% (v/v) Tween 20, 1 mM dNTPs); TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA); TM buffer (20 mM Tris, pH 8.0, 50 mM $MgCl_2$); TK buffer (20 mM Tris-HCl, pH 8.0, 50 mM KCl, 2 μM hemin); Deposition buffer (100 mM acetic acid-sodium acetate (HAc-NaAc), pH 4.3, 150 mM aniline, 100 mM H_2O_2, 50 mM KCl, prepared daily); Electrolyte (I) (100 mM HAc-NaAc buffer, pH 4.3); Electrolyte (II) (10 mM Tris, pH 7.4, 50 μM RuHex); Electrochemical impedance spectroscopy (EIS) buffer (1×PBS, 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1), 100 mM KCl). All other reagents of analytical grade were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used without any purification. Milli-Q water (18.2 MΩ cm at 25 °C, Barnstead, Thermo Scientific, USA) was used throughout the experiments.

2. Cell Culture and Telomerase Extraction

The telomerase extracts were prepared according to the CHAPS method.[S1] Briefly, various cell lines (HeLa cervical cancer, A549 lung cancer, MCF-7 breast cancer, and MDA-MB-231 breast cancer) were cultured in DMEM medium

supplemented with 15% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL) in 5% CO_2, 37 °C incubator. All kinds of cells were collected in the exponential phase of growth, and 1 × 10^6 cells were transferred into an RNase-free 1.5 ml eppendorf tube, washed twice with ice-cold 1×PBS by centrifugation at 1 800 rpm for 5 mins. After discarding the supernatant carefully, the cells were resuspended in 200 μL of ice-cold lysis buffer. The cells were incubated for 30 mins on ice and then centrifuged for 20 mins (12 000 rpm, 4 °C). Without disturbing the pellet, the cleared lysate was collected carefully and transferred to a fresh RNase-free eppendorf tube, flash frozen, and stored at -80 °C before analysis.

For extraction of telomerase from urine samples, fresh urine (200 mL) was collected and centrifuged at 1 000 rpm for 10 mins at 4 °C and washed with 1×PBS. The above samples were centrifuged at 1 800 rpm for 5 mins at 4 °C. The precipitate was resuspended in 2 mL of ice-cold lysis buffer and then incubated on ice for 30 mins. The mixture was centrifuged at 12 000 rpm for 20 mins at 4 °C. The supernatant was transferred, aliquoted, and stored at -80 °C before analysis.

3. Fabrication of Sensors

DNA tetrahedron-structured primer (DTS-primer) was formed as reported previously.[S2−S5] In brief, equimolar quantities of four strands (Table S1) for the formation of the DTS-primer were dissolved in TE buffer, yielding a final concentration of 50 μM. 1 μL of each strand was mixed with 1 μL of TCEP (500 mM) and 45 μL of TM buffer. The resulting mixture was heated to 95 °C for 10 mins, then cooled to 4 °C for 30 mins using a thermal cycler S1000TM (MJ Research Inc., SA). In this way, DTS-primer with primer at one vertex and three thiol groups at the other three vertices was constructed. Then, 3 μL of DTS-primer (1 μM) were added to the cleaned gold electrode for 12 hours at room temperature for immobilization. The ss-TS was immobilized according to the same procedures. Subsequently, the resulting electrode was incubated in 1 mM MCH for 1 hour to eliminate nonspecific binding. The resulting modified electrodes were rinsed with 1×PBS and dried gently with nitrogen before telomerase extension.

4. Formation of Telomeric Hemin/G-quadruplexes and Deposition of PANI

Telomerase extracts were firstly diluted with lysis buffer, and then 4 μL of the resulted solution was mixed with 6 μL of telomerase extension reaction buffer. The

mixture was pipetted to the surface of modified gold electrode and incubated at 37 °C for a certain period of time. For inhibition of telomerase, BIBR 1532, curcumin, genistein, or 2,6-diaminoanthraquinone was performed in the presence of 1 000 HeLa cells. Then the solution was incubated at 30 °C for 60 mins. For negative control experiments, telomerase extracts were heat-treated at 95 °C for 10 mins.

After telomerization, the above modified electrodes were immersed in TK buffer at room temperature for 40 mins. For the deposition of PANI, the electrode was incubated in deposition buffer for 90 mins at room temperature.

Table S1. Sequences of Oligonucleotides Employed in This Work

name	Sequence and modifications (from 5′-3′)
TS	AATCCGTCGAGCAGAGTT
ss-TS	SH-$(CH_2)_6$-TTTTTTTTTTAATCCGTCGAGCAGAGTT
tetra-A7	CGCCTAAACAAGTGGAGACTGTGTTTTTTTTTTAATCCGTCGAGCAGAGTT
tetra-B7	SH-$(CH_2)_6$-GAGCGTTAGCCACACACACAGTC
tetra-C7	SH-$(CH_2)_6$-TTAGGCGAGTGTGGCAGAGGTGT
tetra-D7	SH-$(CH_2)_6$-AACGCTCACCACTTGAACACCTC
tetra-A13	ACACTACGTCAGAACAGCTTGCATCACTGGTCACCAGAGTATTTTTTTTTTTAATCCGTCGAGCAGAGTT
tetra-B13	SH-$(CH_2)_6$-ACGAGCGAGTTGATGTGATGCAAGCTGAATGCGAGGGTCCT
tetra-C13	SH-$(CH_2)_6$-TCAACTCGCTCGTAACTACACTGTGCAATACTCTGGTGACC
tetra-D13	SH-$(CH_2)_6$-TCTGACGTAGTGTATGCACAGTGTAGTAAGGACCCTCGCAT
tetra-A17	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTATTTTTTTTTTAATCC GTCGAGCAGAGTT
tetra-B17	SH-$(CH_2)_6$-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
tetra-C17	SH-$(CH_2)_6$-TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
tetra-D17	SH-$(CH_2)_6$-TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
tetra-A26	GCCTGGAGATACATGCACATTACGGCTTTCCCTATTAGAAGGTCTCAGGTGCGCGTTTCGGTAAGTAGAC GGGACCAGTTCGCC TTTTTTTTTTAATCCGTCGAGCAGAGTT
tetra-B26	SH-$(CH_2)_6$- CGCGCACCTGAGACCTTCTAATAGGGTTTGCGACAGTCGTTCAACTAGAATGCCCTTTGGGCTG TTCCGGGTGTGGCTCGTCGG
tetra-C26	SH-$(CH_2)_6$-GGCCGAGGACTCCTGCTCCGCTGCGGTTTGGCGAACTGGTCCCGTCTACTTACCGTTTCCGAC GAGCCACACCCGGAACAGCCC
tetra-D26	SH-$(CH_2)_6$-GCCGTAATGTGCATGTATCTCCAGGCTTTCCGCAGCGGAGCAGGAGTCCTCGGCCTTTGGGCA TTCTAGTTGAACGACTGTCGC
tetra-A37	GCCCAGATTAAAACGTGAATTCCTAGCCAGTACAGGGTTTCCGGACTGACGTAAATCGGTTTTTTTTTTA ATCCGTCGAGCAGAGTT
tetra-a37	CGGTATTATTCTCATGGGTTTGGCACCACCTGAGTCTCGCCCGGCTCTTGTAAGTCGG
tetra-B37	SH-$(CH_2)_6$-CCCTGTACTGGCTAGGAATTCACGTTTTAATCTGGGCTTTGGGTTAAGAAACTCCCCG
tetra-b37	CGCTGGAGGCGCATCACCGTTTGCGTATGTGTTCTGTGCGGCCTGCCGTCCCGTGTGGG
tetra-C37	SH-$(CH_2)_6$-CGGTGATGCGCCTCCAGCGCGGGGAGTTTCTTAACCCTTTCCGACTTACAAGAGCCGG
tetra-c37	GCGAGACTCAGGTGGTGCCTTTGGCATTCGACCAGGAGATATCGCGTTCAGCTATGCCC
tetra-D37	SH-$(CH_2)_6$-CCCATGAGAATAATACCGCCGATTTACGTCAGTCCGGTTTCCCACACGGGACGGCAGGC
tetra-d37	CGCACAGAACACATACGCTTTGGGCATAGCTGAACGCGATATCTCCTGGTCGAATGCC

5. Characterization and Measurements

Surface plasmon resonance (SPR, DyneChem HiTech Ltd., China) was used to investigate the binding performance of DNA and PANI deposition on SPR chip. The morphology of PANI was analyzed using scanning electron microscopy (SEM) system (JEM-2100, JEOL, Japan). EIS was performed on a VersaSTAT 3 workstation (Princeton Applied Research, USA). Chronocoulometry (CC), cyclic voltammetry (CV), and differential pulse voltammetry (DPV) were performed with a CHI 660D electrochemical analyzer (Chenhua Instruments, Shanghai, China). A three-electrode system was employed with platinum wire as the auxiliary electrode, saturated calomel electrode (SCE) as the reference electrode, and gold electrode or modified gold electrode as the working electrode, respectively. The electrochemical signal was measured with CV and DPV by scanning from -0.4 V to 0.2 V in electrolyte (I), which was degassed with nitrogen for 15 mins. Perform CC in electrolyte (II) with the following parameters: potential step from 0.2 to -0.5 V, step = 2, pulse width = 0.5 s, sample interval = 5 ms, quiet time = 2.0 s, sensitivity = 1e-5 A/V. EIS was recorded with a frequency range of 0.01 to 10^5 Hz.

6. Quantification of the Nanospacing between TS Primers

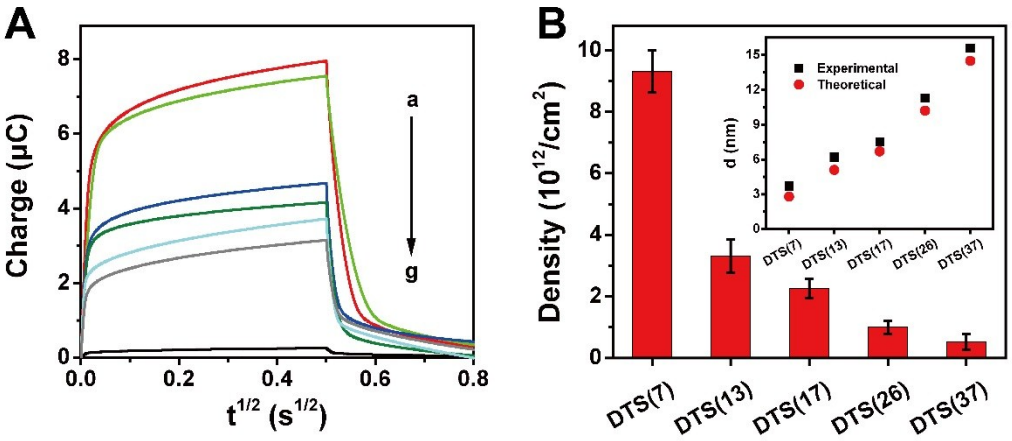

Fig. S1 (A) Chronocoulometry for different size of DTS immobilized electrode in the presence of RuHex. From a to g: ss-TS, DTS(7), DTS(13), DTS(17), DTS(26), DTS(37), and bare gold electrode without RuHex. (B) The densities of the primer decreased with increasing DTS size. Inset: The nanospacing between primers were dependent on the DTS size. The experimental data correlated highly with the theoretical data. Error bars showed the standard deviation of three samples.

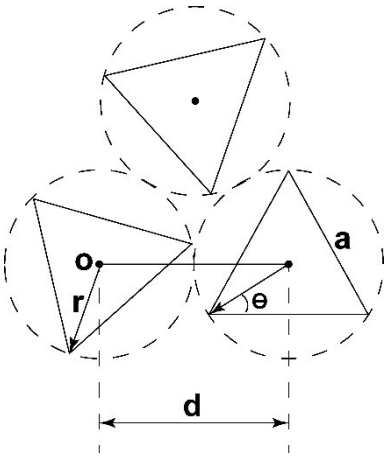

Fig. S2 The local planar graph of DTS-primers distributed in the gold electrode surface.

In this paper, each DTS carries a TS primer at one vertex and three thiol groups at the other three vertices, which can be firmly anchored at the gold electrode surface using well-established Au-S bonds (see Scheme 1). We hypothesize DTS-primers were evenly distributed in the gold electrode surface. Considering the space steric hindrance effect resulted by electrostatic repulsion, each DTS-primer is anchored in a circular area (Fig. S2). This circle is the excircle of the triangle. Obviously, the triangle with three thiol groups in regular tetrahedron is a regular triangle. In this way, 1) the center of regular triangle is also the center of its excircle; 2) the nanospacing between TS primers is equivalent to the distance between centers of the two triangles. Detailed deduced processes were presented in the following:

$$\theta = 30° \quad (1)$$

$$r = \frac{a}{2cos\theta} \quad (2)$$

$$d = 2r \quad (3)$$

The parameters used are as follows: a, length of regular triangle side; d, distance between centers of two triangles; r, radius of the circle.

Because each base pair was separated by 0.34 nm in a double helix, we were able to precisely calculate the edge length of DTSs (length of regular triangle side) and to quantification of the nanospacing between TS primers theoretically.

7. Quantification of Surface Density of DTS-primers and Multiplicity of Extension (x in $(TTAGGG)_x$) on Gold Electrode by Chronocoulometry

Herein, we attempt to quantify the surface density of the DTS-primers and the

multiplicity of extension (x in $(TTAGGG)_x$) by telomerase immobilized on gold electrode surface by chronocoulometry (CC) with hexaammineruthenium(III) chloride (RuHex).[S6,S7] RuHex binds electrostatically to the phosphate backbone of DNA immobilized on the electrode to give rise to an electrochemical signal whose intensity is proportional to the amount of DNA.[S8] Detailed deduced processes were presented in the following:

$$Q = \frac{2nFAD_0^{1/2}C_0^{+}}{\pi^{1/2}}t^{1/2} + Q_{dl} + nFA\Gamma_0 \quad (1)$$

$$\Gamma_0 = \frac{(Q_{before} - Q_{dl})}{nFA} \quad (2)$$

$$\Gamma_{DNA} = \Gamma_0\left(\frac{z}{m}\right)N_A \quad (3)$$

$$\Delta\Gamma = \frac{Q_{after} - Q_{before}}{nFA} \quad (4)$$

$$(TTAGGG)_x = \left(\frac{m}{6}\right)\left(\frac{Q_{after} - Q_{before}}{Q_{after} - Q_{dl}}\right) \quad (5)$$

The parameters used are as follows: n, number of electronsper molecule for reduction (n = 3); F, Faraday constant (C/equiv); A, electrode area (cm^2); Q_{dl}, capacitive charge (C); Γ_0, amount of redox marker, RuHex (mol/cm^2); Γ_{DNA}, probe surface density ($molecules/cm^2$); m, the number of bases in the DTS-primer; $\Delta\Gamma$, elongated products per electrodearea (mol/cm^2); z, charge of the redox molecule (z = 3); N_A, Avogadro's number (molecules/mol); Q_{before}, charge before elongation reaction; Q_{after}, charge after elongation reaction, and $(TTAGGG)_x$, average elongation time per hexanucleotide per primer molecule.

In the Cottrell equation, eq 1, for chronocoulograms (plot of Q vs $t^{1/2}$) in the absence and presence of RuHex, the y-intercept and Γ_{DNA} is derived from eqs 2 and 3 using the obtained Q_{dl} and Q_{before}. After telomerase reaction of this electrode, the y-intercept at time zero in the chronocoulogram in the presence of RuHex gives Q_{after} (charge after elongation reaction, curve c in Fig. 2A of manuscript). Accordingly, the elongation time x in $(TTAGGG)_x$ is given by eqs 4 and 5 using the obtained Q_{before} and Q_{after}.

8. Optimization of Experiment Conditions

To improve the analytical performances of the sensor, some detection conditions such as telomerase elongation time, H_2O_2 concentration, and aniline concentration were optimized. The current increased with increasing telomerase elongation time, H_2O_2 concentration, and aniline concentration in the range of 0 ~ 180 mins (Fig. S3A), 20 ~ 100 mM (Fig. S3B), and 5 ~ 150 mM (Fig. S3C), respectively, then reached a plateau. Thus, 120 mins telomerase elongation time, 100 mM H_2O_2, and 150 mM aniline were used in the subsequent research.

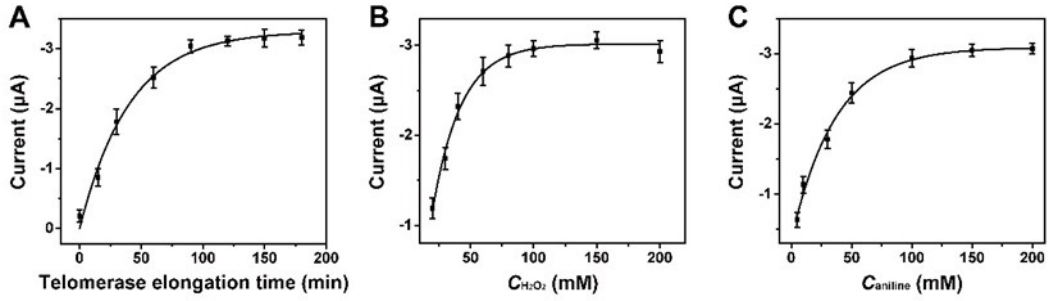

Fig. S3 The influence of different (A) H_2O_2 concentration: 20, 30, 40, 60, 80, 100, 150, and 200 mM; (B) aniline concentration: 5, 10, 30, 50, 100, 150, and 200 mM; (C) telomerase elongation time: 0, 15, 30, 60, 90, 120, 150, and 180 mins on the response of the sensor. 1 000 HeLa cells were used. Error bars showed the standard deviation of three experiments.

9. Evaluation of Telomerase Inhibition

Telomerase activity is usually reactivated in most cancer cells but not in neighboring normal cells. Activation of telomerase enzyme and telomere stabilization are important necessary steps in tumorogenesis.[S9] Four kinds of compounds such as BIBR 1532, curcumin, genistein, and 2,6-diaminoanthraquinone were selected as model inhibitors to verify the proposed method. For all the tested inhibitors, the DPV current decreased gradually with its increasing concentration and began to level off when the inhibitor concentrations increased to a certain level (Fig. S4), indicating all the tested inhibitors were in a dose-dependent manner. The inhibition efficiency (%) was evaluated as follows:

$$\text{Inhibition (\%)} = \frac{I_3 - I_2}{I_3 - I_1} \times 100\%$$

where I_1 was the peak current of PANI obtained from DTS(37)/gold electrode

system without telomerase. I_2 was the inhibited current of PANI obtained from DTS (37)/gold electrode system with telomerase and inhibitors. I_3 was the peak current of PANI obtained from DTS (37)/gold electrode system with telomerase. The derived IC_{50} value for BIBR 1532, curcumin, genistein, and 2, 6-diaminoanthraquinone were 113 ± 12 nM, 3.3 ± 0.4 μM, 25 ± 2 μM, and 52 ± 3 μM, respectively, which was in accord with the previously reported result.[S10−S13] Hence, the assay can be used for rapid evaluation and screening of the telomerase inhibitors, which has a potential application in discovery of new anticancer drugs.

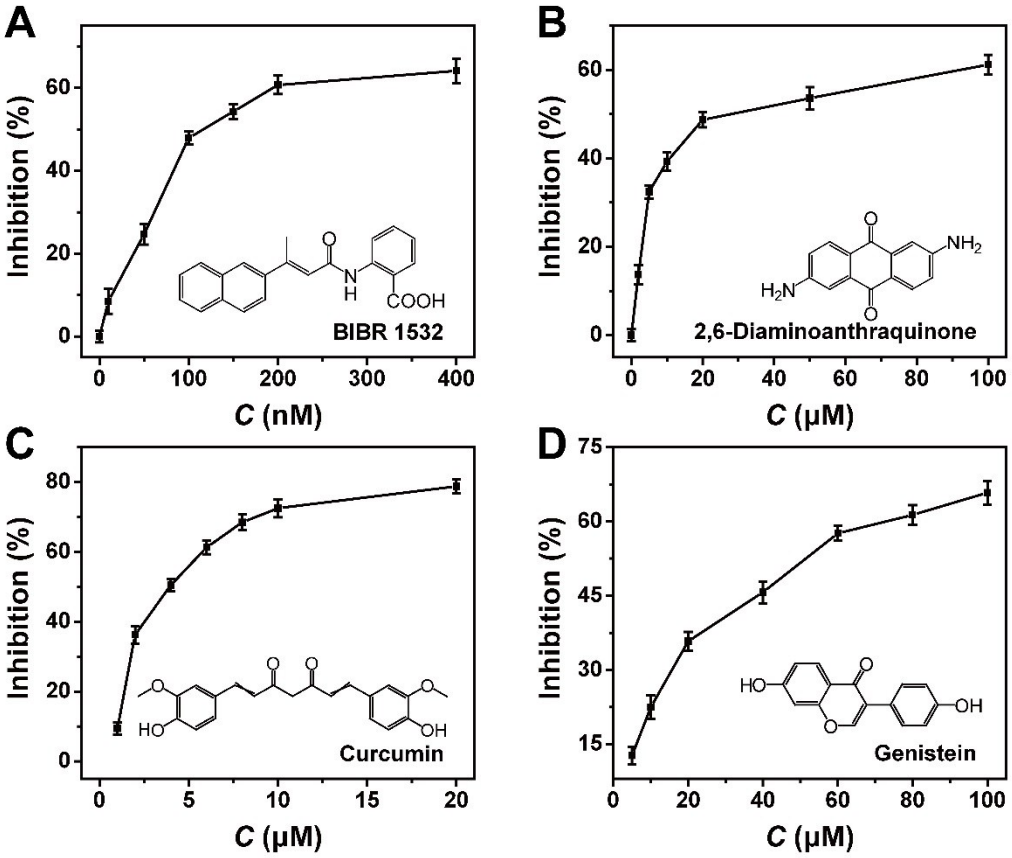

Fig. S4 The inhibition effect of BIBR 1532 (A), 2,6-diaminoanthraquinone (2,6-dia) (B), curcumin (C), and genistein (D) on telomerase activity. 1 000 HeLa cells were used. Error bars showed the standard deviation of three samples.

10. Clinical Applicability

The DPV peak was obvious in the positive sample (ID: 1004473251), while no obvious DPV peaks were found in normal individual or inflammation patient (ID: 1003729557) (Fig. S5). Typical DPV spectra were shown in the inset of Fig. S5.

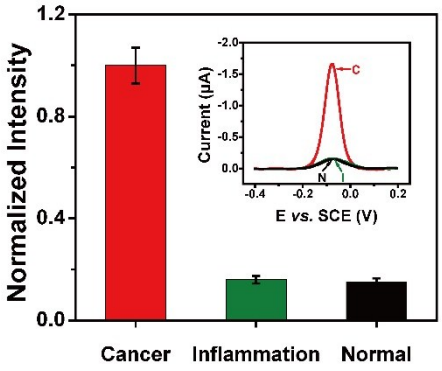

Fig. S5 (A) Histogram for DPV intensities of representative urine specimens (C, bladder cancer patient; I, inflammation patient; N, normal individual). Inset: DPV corresponding to the representative urine specimens. Clinical diagnosis provided by Nanjing General Hospital of Nanjing Military Command, Nanjing, China. Error bars showed the standard deviation of three samples.

14. References

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