Electronic Supplementary Information for

Portable Dual-Mode Sensor Based on TiO$_2$ Nanotube Membrane for the Evaluation of Telomerase Activity

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Part 1: Experimental details

1. Materials

Titanium foil (0.1 mm thickness, 99.6% purity) was purchased from Bao Tai Metal Co. (Baoji, China). The recombinant RNase inhibitor, deoxynucleotide solution (dNTPs) mixture, and RNase-free water were purchased from Sangon Biotech Co. Ltd. (Shanghai, China). The 1X CHAPS lysis buffer was purchased from Millipore (Bedford, MA). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was purchased from Sigma-Aldrich. Chloroauric acid (HAuCl₄), hydrochloric acid (HCl), silver nitrate (AgNO₃), trisodium citrate dihydrate, citric acid monohydrate, and hydroquinone were purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received without further purification. All aqueous solutions were prepared with deionized (DI) water (>18 MΩ). Cervical cancer cells (HeLa cell line) and human breast cancer cells (MCF-7) were obtained from Sangon Biotech Co. Ltd. (Shanghai, China).

The template strand (TS) primer (5′p - TTTTTTTTTT AAT CCG TGG AGC AGA GTT 3′) and the reporter DNA (5′ SH - CCC TAA CCC TAA CCC 3′) were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China).

This research was approved by the Institutional Committee of Northeastern University and complies with all applicable laws and institutional guidelines, together with individual written informed consent.

2. Instrumentation

UV-vis absorbance was recorded on a Lambda 365 spectrophotometer (PerkinElmer, USA). The current-voltage (I-V) curves were measured using an electrochemical workstation (CHI660E, Chenhua, China). The particle size was measured on a Zetasizer Nano ZS90 analyzer (Malvern, USA). The morphology of TiNM was characterized on a scanning electron microscope (SEM, S-4800, Japan). The XRD patterns were acquired on an X’Pert X-ray diffraction spectrometer (Philips, USA) using a CuKα X-ray source. X-ray photoelectron spectra (XPS) were recorded on a Perkin-Elmer Physical Electronics 5600 spectrometer using AlKα radiation at 13 kV as excitation source. The takeoff angle of the emitted photoelectrons was 45°, and the binding energy of the target elements was determined at a pass energy of 23.5 eV with a resolution of 0.1 eV. The binding energy of C 1s signal (284.6 eV) was used as the reference.

3. Preparation of TiO₂ nanotube membrane (TiNM)

Ti foils were first cleaned as described elsewhere.¹ The TiNMs were synthesized by electrochemical anodization in an ethylene glycol-based electrolyte containing 0.1 M NH₄F and 2.7 vol% H₂O. The preparation of TiNM was according to Ref [2 and 3]. Briefly, the conversion of the nanotubular layers to membranes was achieved by growing a second amorphous short-length nanotube layer underneath, then the upper membrane was removed from the substrate by immersion in H₂O₂.²,³ Ti and platinum foils served as the working electrode and the counter electrode, respectively. The prepared samples were annealed at 450 °C for 2 h in air with a heating rate of 3 °C/min.

4. Modification of TiNM with template strand (TS) primer

The blank TiNMs were protected by two poly(ethyleneterephthalate) (PET) films (70 μm thick, DELI group Limited Company, Zhejiang, China). The PET films were prepunched with 2 mm well aligned holes, and then laminated together with a heating laminator. The prepared TiNMs were immersed in a TS primer solution (2.5 μM). After the reaction with DNA for 12 h, the unreacted DNA was removed by washing with distilled water. The reactions were performed at room temperature.
5. Preparation of gold nanoparticle (AuNPs)

Citrate-stabilized AuNPs were prepared through the thermal reduction of HAuCl₄ by sodium citrate.¹ For the synthesis of ~10, ~17, ~30, and ~50 nm AuNPs, 50 mL of 0.01 wt.% HAuCl₄ was heated to boiling and then 2.5, 1.3, 0.7, and 0.35 mL of 1 wt.% sodium citrate was immediately added to the boiling solution under vigorous stirring, respectively. The reactants were boiled for 15 min. During this time, the solution color changed from colorless to deep red. After turning off heating, the system was naturally cooled to room temperature under stirring. The suspension of AuNP was stored at 4 °C and centrifuged at 12,000 rpm for 20 min before use. The diameters of the prepared nanoparticles determined by dynamic light scattering (DLS) are ~10±3, ~17±3, ~30±4, and ~50±6 nm, respectively. And their extinction value of the plasmon peak in 520-531 nm region are ~1.9, ~1.8, ~2.0, and ~1.7, respectively.

6. Preparation of AuNPs-reporter DNA

The AuNPs labeled reporter DNA was prepared according to the previous report with a few of revision.⁵ Typically, twenty microliters (20 µL) of 0.76 mM DNA solution was pipetted into a microcentrifuge tube. Then, 4 µL of 500 mM acetate buffer (pH 5.2) and 4 µL of 10 mM TCEP were added to the tube to activate the thiol-modified DNA. The mixture was incubated at room temperature for 1 h. Afterwards, 5 mL of the obtained gold nanoparticles (~10 or ~17 nm) suspension were introduced in a NaOH-treated glass vial, followed by the addition of TCEP-treated thiol DNA with gentle shaking. The vial was closed and stored in a drawer at room temperature for at least 16 h, and magnetic stirring was also applied to facilitate the reaction. In the next step, 50 µL of 500 mM Tris acetate (pH 8.2) buffer was slowly dropped into the vial with gentle shaking by hand. Finally, 2.4 mL of 1 M NaCl solution was added dropwise to the vial with gentle shaking. The vial was stored in a drawer for at least a day before use. The mixture was centrifuged at 12,000 rpm at room temperature for 20 min in a bench-top centrifuge. At the end, the free DNA was removed by taken off as much supernatant as possible. The resulted nanoparticles were redispersed in 200 µL of buffer containing 100 mM NaCl and 25 mM Tris acetate (pH = 8.2). It should be noted that, for ~30 or ~50 nm AuNPs, the concentration of reporter DNA solution added was 3 mM.

7. Cell culture and telomerase extraction

Telomerase was extracted from cancer cells according to the previous report.⁶ Typically, cervical cancer cells (HeLa cell line) and human breast cancer cells (MCF-7) were cultured in a culture flask containing 1640 (GIBCO) medium with 10% fetal calf serum (FBS) and 1% penicillin streptomycin (PS, 10 000 IU penicillin and 10 000 µg/mL streptomycin, Multicell) at 37 °C in a humidified atmosphere containing 5% CO₂. HeLa and MCF-7 cancer cells were first suspended in 1X CHAPS lysis buffer to obtain a concentration of 5000 cells per µL and then incubated on ice for 30 min. Then, the mixture was centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was transferred, aliquoted, and stored at -80 °C.

8. Telomerase extraction from urine samples

Telomerase was extracted from urine samples according to the previous report.⁶ Briefly, fresh urine samples were collected and centrifuged at 850 g for 10 min at 4 °C and washed once with Tris-HCl buffer (10 mM, pH = 7.4). The samples were centrifuged again at 2,300 g for 5 min at 4 °C. The precipitate was resuspended in 200 µL of ice-cold 1X CHAPS lysis buffer and then incubated on ice for 30 min. In the next step, the mixture was centrifuged at 10,000 g for 20 min at 4 °C. The supernatant was transferred, aliquoted, and stored at -80 °C.

9. Telomerase extension reaction
Telomerase extracts were diluted in distilled water with a known number of cells. The extracts (10 μL) were added to 140 μL of extension solution containing 129 μL NEBuffer (12 mM Tris-HCl, pH = 7.4, 6 mM MgCl₂, 60 mM NaCl), 10 μL of 10 mM dNTPs, and 1 μL of RNase inhibitor. Then, TiNM grafted with TS primer was submerged in the mixture and the sample was incubated at 37 °C for 2 h. Once the extension reaction completed, the sample was washed with Tris-HCl buffer (10 mM, pH = 7.4) three times. Finally, the sample was immersed into a solution containing the reporter DNA modified AuNPs (150 μL) and incubated at 37 °C for 2 h to obtain the assembly between the reporter DNA and elongated TS primer. Next, the product was washed with Tris-HCl buffer (10 mM, pH = 7.4) three times.

10. Silver amplification

Silver amplification solutions (A and B) were prepared according to the previous report freshly prior to use. For the solution A, 200 mg of silver nitrate were dissolved in 25 mL of deionized water (silver nitrate can be dissolved by continuous stirring within about 15 min). For the solution B, 410 mg of hydroquinone were dissolved in 50 mL of citrate buffer (pH = 3.5). The citrate buffer was prepared by dissolving 1.382 g of trisodium citrate dihydrate and 1.5 g of citric acid monohydrate in 50 mL of deionized water. Solutions A and B were immediately mixed with an equal volume of 1 mL : 1 mL before use. To increase the size of AuNPs attached on DNA, a suspension containing the AuNPs-reporter DNA was mixed with 2 mL of A and B mixed solution. The resulted mixture was kept for 15 min at room temperature to allow silver deposition on AuNPs. Then, the unreacted solutions A and B were removed by washing with distilled water. Because the silver nitrate is light sensitive, the amplification procedure has to be carried out in dark conditions.

11. Current-voltage (I-V) curves measurement

The I-V curve of TiNM is measured using a home-made electrochemical cell (Figure 3B). The effective exposed area of TiNM was 0.20 mm². The membrane was clamped between two PDMS films and then placed between two Teflon cells. Due to their advantages, such as non-polarizability and high stability, two Ag/AgCl electrodes were used to apply the trans-membrane potential and measure the ionic current. I-V curves were measured using a CHI660E electrochemical workstation. The electrochemical detection was performed in a 1 mM NaCl solution. The transport of ionic current through the nanotube array was measured by scanning the voltage from -1 to +1 V at a scan rate of 100 mV/s. The membranes were immersed in a 1 mM NaCl solution (pH 7.0) before use.
Part 2: Additional figures and tables

12. Characterization of as-prepared TiNM

![Fig. S1](image)

**Fig. S1** Images of self-standing TiNMs: (A) before and (B) after annealing at 450 °C for 2 h in air.

These membranes are defect-free and able to withstand annealing treatments at 450 °C, without curling or cracking (Fig. S1B).
**Fig. S2** Bottom-view SEM images of TiNMs (A and B). Top-view SEM images of TiNMs before (C) and after (D) hybridization with AuNPs-reporter DNA. Top-view (E) and bottom-view (F) SEM images of TiNMs with AuNPs amplified with silver.
13. The high affinity of phosphate/phosphonic acid groups for TiNM

**Fig. S3** UV-vis absorption spectra of TS primer solution after incubation treatment with TiNM/TS primer in (A) 10 mM Tris-HCl buffer (pH 7.4) and (B) 10 mM PBS buffer (pH 7.4). (C) Bidentate interaction of the TS primer with TiNM.

As shown in Fig. S3A and Fig. S3B, instead of Tris-HCl buffer (pH 7.4), the adsorbed DNA was displaced by adding free phosphate from PBS buffer (pH 7.4). And UV-vis absorption spectra of TS primer solution after incubation treatment with TiNM/TS primer in 10 mM PBS buffer (pH 7.4) is enhanced. Therefore, the TS primer is attached on the internal surface of TiNM via a bidentate interaction between PO$_4^{3-}$ on TS primer and Ti(IV)O$_6$ (Fig. S3C).
14. Optimization of TS primer absorption conditions

**Fig. S4** TS primer absorption capacity of TiNM as a function of incubation time (0-36 h) and TS primer concentrations (1-5 μM) at room temperature. The absorbance value of the TS primer solution was recorded at 260 nm.

To evaluate the optimal DNA adsorption capacity over time, the samples were incubated for well-established time intervals (0-36 h) followed by measurement of the absorbance of TS primer in the UV-vis range (Fig. S4). The optimal DNA concentration is 2.5 μM and the optimal DNA incubation time is 12 h.
Fig. S5 High-resolution (A) N 1s and (B) P 2p spectra. High-resolution O 1s spectra of TiNM before (C) and (D) after adsorption of TS primer. High-resolution C 1s spectra of TiNM before (E) and (F) after modification of TS primer. CPS = counts per second.

The high resolution O 1s spectra depicted in Fig. S5C and S5D display peaks at (i) ~529.5 eV, which is attributed to oxygen atoms on TiO$_2$ surface, (ii) ~531.2 eV, which is assigned to oxygen atoms bound to phosphorus atoms as P=O or P-O$_2$,$^9$ and (iii) ~532.8 eV, which is due to oxygen atoms bound to carbon atoms, such as C-O or C=O.$^9$ The high resolution C 1s spectra depicted in Fig. S5E and S5F display peaks at (i) ~285 eV, which is due to the carbon in hydrocarbons (C-C, C-H), (ii) ~286 eV, which is assigned to carbon bound to nitrogen (C-N, C-O), (iii) ~288 eV, which originates from carbon in urea [N-C(=O)-N], and (iv) ~287 eV, which is assigned to carbon in amides [N-C(=O)-C, N=C-N, N=C-O].$^{10}$
16. Optimization of experimental conditions

The UV-vis spectra (Fig. S6A) show that the absorbance peak of AuNPs is centered at ~520 nm. After DNA modification, this peak shifted to ~524 nm. In addition, the dynamic light scattering data (Fig. S6B) show that the hydrodynamic size of AuNPs is ~17±3 nm. And the dynamic light scattering data also show that the hydrodynamic size of AuNPs is increased from ~17.0 to ~20.6 nm (Fig. S6B). The size changed as well as the red shift in UV-vis spectra demonstrate the successful grafting of DNA on AuNPs. Figure S6C proves that the diameter of AuNP is ~17 nm.

**Fig. S6** UV-vis absorption spectra (A) and size (B) of ~17 nm AuNPs determined by dynamic light scattering before and after modification with the reporter DNA. (C) SEM image of ~17 nm AuNPs.
To obtain a highly sensitive telomerase activity assay, the time of the reaction of elongated primer hybridized with the reporter probe DNA was optimized based on the color change of TiNM (Fig. S7). It can be seen that the pink color of membrane becomes more intense with the incubation time. It should be point out that the control sample (in the absence of telomerase) keeps the original yellow color in the first 2 h (Fig. S7). After that, the control sample turns to pink, which can be attributed the nonspecific binding (physical absorption) of the reporter DNA. Therefore, an incubation time of 2 h was selected for the further experiments.
**Fig. S8** $I$-$V$ curves of TiNMs modified with AuNPs in the presence of different concentrations of telomerase.
Fig. S9 Diameters of ~10 nm (A), ~30 nm (B), and ~50 nm (C) AuNPs determined by dynamic light scattering before and after modification with the reporter DNA. SEM image of ~10 nm (D), ~30 nm (E), and ~50 nm (F) AuNPs. UV-vis absorption spectra of ~10 nm (G), ~30 nm (H), and ~50 nm (I) AuNPs before and after modification with the reporter DNA. (J) I-V curves of TiNMs modified with AuNPs in the presence of telomerase from 50,000 HeLa cells. (K) I-V curves of TiNMs after silver amplification of AuNPs in the presence of telomerase from 50,000 HeLa cells. (L) Photos showing the colorimetric response of the proposed detection system to different sizes of AuNPs observed by naked eye (i): AuNPs; (ii): silver amplified).

The dynamic light scattering data (Fig. S9A-C) show that the hydrodynamic sizes of AuNPs are ~10±3, ~30±4, and ~50±6 nm, respectively. Figure S9D-E prove that the diameters of AuNP are ~10, ~30, and ~50 nm, respectively. UV-vis spectra (Fig. S9F) show that the absorbance peak of ~10, ~30, and ~50 nm AuNPs are centered at ~520, ~529, and ~531 nm, respectively. After DNA modification, this peak shifted to ~522, ~533, and ~538 nm, respectively. The size changed as well as the red shift in UV-vis spectra demonstrate the successful grafting of DNA on AuNPs. Moreover, the ~10, ~30, and ~50 nm AuNPs were attached on TiNM internal surface via the hybridization of reporter DNA with elongated TS primer. However, the change of the ionic current of TiNM with ~10 nm (~6.6%), ~30 nm (~8.8%) or ~50 nm (~4%) AuNPs is insufficient (Fig. S9J). After silver amplification, the reduction of the ionic current of TiNM with ~10 nm (~23.4%), ~30 nm (~26.9%) or ~50 nm (~4%) AuNPs also is less than that of ~17 nm AuNPs (~50.3%) (Fig. S9K). The size of ~10 nm AuNPs is so small that it is not enough
to induce a considerable reduction of the ionic current of TiNM. In addition, the color of TiNM proves that only fewer ~30 nm or ~50 nm AuNPs are attached on TiNM (Fig. S9L), because of difficulty in reporter DNA grafting on the surface of ~30 or ~50 nm AuNPs.\textsuperscript{12} Thus, the optimal diameter of AuNPs is ~17 nm.
17. Clinical applicability

**Table S1** Comparison of the results by clinical diagnosis and the proposed method

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<th>III</th>
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Clinical outcomes provided by Shengjing Hospital of Shenyang.
**Fig. S10** Top-view and bottom-view SEM images of TiNMs with AuNPs amplified with silver after the contact with urine samples from the healthy individuals (sample II - A and B) and patients with bladder cancer (sample III - C and D, sample IV - E and F).
18. Comparison of various methods for telomerase activity assay

**Table S2** Comparison of various methods for telomerase activity assay

<table>
<thead>
<tr>
<th>Method</th>
<th>Mechanisms</th>
<th>Sensitivity data</th>
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<tr>
<td>DNAzyme-based colorimetric strategy</td>
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<td>Modification of the TRAP assay</td>
<td>Primer-modified AuNPs reduce polymerase chain reaction (PCR) artifacts and improve sensitivity and specificity</td>
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<td>Primer-modified AuNPs-based Colorimetric assay</td>
<td>Telomerization induced the formation of G-quadruplex to protect the AuNPs from the aggregation</td>
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<td>DNA-metallization-based electrochemical method</td>
<td>Telomerization increased the deposition of silver nanoparticles along DNA, serving as electroactive labels</td>
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<tr>
<td>Electrochemical impedance Spectroscopy (EIS)</td>
<td>Telomerization blocks the transfer of electrons on electrode surface, increasing in impedance measured by EIS</td>
<td>1000 HeLa cells</td>
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<tr>
<td>Nanochannel-based electrochemical assay</td>
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<td>Surface-enhanced Raman scattering (SERS)</td>
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<td>This work</td>
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19. References

(15) J. Wang, L. Wu, J. Ren and X. Qu, Small, 2012, 8, 259-264.