Enzyme-mimicking accelerated signal enhancement for visually
multiplexed quantitation of telomerase activity at single-cell level

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Experimental Methods

Materials and chemicals. All the oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China), and the oligonucleotides sequences were as following: TS (5’-NH$_2$C$_6$AATCCGTCGAGCAGAGTT-3’), ACX(5’-GC GCCGCTACCCTACCCTACCCTACC-3’). The mixture nucleotides (dNTPs) were purchased from Sangon Biotech (Shanghai, China). Bovine serum albumin (BSA) was obtained from Sigma-Aldrich, Inc. (St. Louis, MO). Tetraethyl orthosilicate (TEOS), FeCl$_3$·6H$_2$O, 1H,1H,2H,2H-Perfluoroctyltrichlorosilane were obtained from Shanghai Macklin Biochemical Co., Ltd. 4-mer-captophenylboronic acid was fabricated by Energy Chemical. N-Hydroxysuccinimide, Dopamine hydrochloride were obtained from Aladdin. N-(3-Dimethylaminopropyl)-N’–ethylcarbodiimide hydrochloride was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Glass slides (75 × 50 × 1.5 mm), amorphous diamond-coated drill bits (1.0 mm in diameter), CNC 4303 engraving and milling machine were purchased from Jing Yan Instruments Technology Co., Ltd. (Shenzhen, China). All graphics were designed using Auto CAD software and then printed out as transparency photomasks by Ji Xian Guang Dian Co., Ltd. (Shenzhen, China). SPR220-7 photoresist and AZ400K developer were purchased from Suzhou Research Materials Microtechnology Co., Ltd (Suzhou, China, MicroChem Corp).

TV- Chip fabrication. The devices were fabricated according to our previously reported methods. Firstly, TV-Chip was designed by Auto CAD software and then fabricated with the standard protocols of photolithography and wet etching. The structure of the glass plates with a depth of approximately 50 μm by maintaining the etching temperature at 40 °C in 40 min. Access holes were fabricated a diamond drill of 1 mm diameter. The glass plates were cleaned by using acetone, isopropanol solution and oxygen plasma thoroughly. Then, to make the surface of chips hydrophobic, the chips were modified with 1H,1H,2H,2H-perfluoroctyltrichlorosilane. Finally, we added 2.5 μL 2-methyl silicon oil fully dispersed between the two glass plates to assist the device assembly. The ultrathin oil layer served as a lubricant and prevents O$_2$ leakage during operation.

Preparation of MSNPs. Specifically, FeCl$_3$·6H$_2$O (2.73 g) and sodium acetate (7.22 g) were added into 80 mL ethylene glycol with magnetic stirring, and PEG (1.00 g) was added to the solution. After fully dissolved, the mixture was decanted into a Teflon-lined stainless-steel autoclave (120 mL). It was heated for 12 h at 200 °C in the oven. After the autoclave was cooled, the obtained precipitate was washed three times with ethanol, followed by washed three times with water. To prepare MSNPs, 120 mg Fe$_3$O$_4$ nanoparticles were suspended in 100 mL ethanol and 24 mL water by sonication for 10 min at room temperature. Then, 1.6 mL of ammonia aqueous solution (NH$_3$·H$_2$O) and 1.6 mL of tetraethyl orthosilicate (TEOS) were added to the above solution continuously stirred 12 h. The solution was washed three times with ethanol and three times with water. To modify the amino group (NH$_2$) to the surface of MSNPs, 50 μL
of (3-aminopropyl) triethoxysilane (APTES) was loaded into the solution containing 1 mL of methanol and 25 mg of MSNPs, followed by continuously stirred 12 h, and washed three times with ethanol. TS was conjugated to the surface of MSNPs through the glutaraldehyde crosslinking method. 500 μL glutaraldehyde was added to the solution of MNPs for 1 h, the MNPs were separated by magnet and washed five times with PBS, then, 250 μL TS solution (10 μM) was added. The solution was incubated 8 h, followed by the addition of BSA (0.5 %). After the reaction for another 1 h, and washed several times with PBS.

**Preparation of BA-PtNPs.** The PtNPs were synthesized according to the previously reported strategy. Briefly, 2 mL H₂PtCl₆ (100 mM) was added into water (198 mL) and the solution heated to boiling. Then, 20 mL of sodium citrate aqueous (38.8 mM) was added quickly. The mixture was boiled for 40 min. To modify 4-mercaptophenylboronic acid to the surface of PtNPs (BA-PtNPs), 100 μM 4-mercaptophenylboronic acid was added to PtNPs in alkaline solution (pH=11) with mechanical stirring for 12 h. The concentration (m/v) ratio between 4-mercaptophenylboronic acid and platinum nanoparticles was 8:35. Finally, the solution was thoroughly washed five times.

**Telomerase extension reaction.** The telomerase extracts (5 μL) added to MSNPs-TS (200 μL) with extension solution containing 20 mM Tris-HCl (pH=8.3), 2 mM dNTP, 63 mM KCl, 0.005 % Tween-20, 1.5 mM MgCl₂, 1 mM EGTA. For inhibition assay (TMPyP4), TMPyP4 was mixed with telomerase extracts (the number of 10000 cancer cells) for 1 h before the primer extension process. The solution was incubated for 1.5 h at 37 °C. Then washed the buffer away to stop the reaction, all washing steps were done by using a magnet with the corresponding buffer. Subsequently, 20 μL of hemin (0.5 μM) was added into the solution (10 mM pH=7.3 Tris-HCl, 10 mM KCl, 100 mM NaCl, 0.002 % TritonX-100) for 1 h, washed three times. 0.0025 g dopamine dissolved in an alkaline solution and 60 μL of H₂O₂ (1 M) was added to the suspension reacted for 20 min and washed five times. After the step of PDA development, 50 μL of 4-MPBA-PtNPs (3.5 mg mL⁻¹) was added to the above solution for 1 h and washed five times immediately. The sample was dispersed in 40 μL water.

**Detection of telomerase activity.** 10 M H₂O₂ and the sample were loaded into their respective fluidic channel, twenty microliters of pink ink loaded into “Z”-shaped fluidic paths of TV-chip. After reagent loading, the TV-Chip was slid manually to make the sample well overlaps the H₂O₂ well. Meanwhile, the PtNPs which attached to the surface of PDA were reacted to H₂O₂ to generate O₂ to push the ink bars movement. The results were recorded at 5 min by reading ink bar advancements relies on the scales beside the wells.

**Cell Culture and telomerase extraction.** The different cancer cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 10 % fetal bovine serum (FBS)
maintained at 37 °C in a humidified atmosphere (95 % air and 5 % CO₂). The cells were cultured in T25 flask, the volume of cell culture is 6 mL. These cells were harvested with trypsin, counted and collected in a fresh EP tube, and washed twice by using ice-cold phosphate buffered saline (PBS), and dispensed in 200 μL ice-cold CHAPS lysis buffer, and immediately incubated on ice 30 min. For inhibition assay (AZT), the breast cancer cells were treated with different concentration of inhibitors for 48 h at 37 °C before extraction. For control assay, the extract production was heat-treated at 98 °C for 10 min. Then, the solution was centrifuged at 12000 rpm for 20 min at 4 °C to remove insoluble components. The supernatant was transferred to a fresh EP tube and stored at -80 °C.

Conventional TRAP assays and polyacrylamide gel electrophoresis (PAGE) analysis. First of all, 10 μL of telomerization products were added to 10 μL the primer(TS), 10 μL anchor reverse primer (ACX) and reaction solution containing Taq DNA polymerase, dNTP. Then, PCR was carried out with the following program: 94 °C for 4 min; 30 cycles at 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 30 s; 72 °C for 5 min; 4 °C hold. Finally, the products were analyzed by polyacrylamide gel (PAGE). Different DNA solution mixed with loading buffer added into polyacrylamide gel in TBE buffer. Electrophoresis was performed under 200 V voltage for 1 h on electrophoresis analyzer. Then the gel was visualized on ultraviolet illumination gel imaging analysis system. The conventional TRAP assay was employed to prove the activity of telomerase in different numbers MDA-MB-231 cells. As shown in Figure S3b, the increased the number of MDA-MB-231 cells result in the higher intensity of the band. Meanwhile, there is no obvious elongated band observed from the heated-inactive control (lane 5). The results illustrated that the primer TS successfully extended the repeats (TTAGGG)n by telomerase.

For telomerase quantification. Telomerase extracts were diluted in CHAPS lysis buffer step by step with the specific number of cells. The specific steps are as follows: the cells (1 × 10⁶) were suspended in 200 μL ice-cold CHAPS lysis buffer, and immediately incubated on ice for 30 min. Then 10 μL of the telomerase extracts (from 1 × 10⁶ cells) were diluted with 15 μL of CHAPS lysis buffer. 5 μL of the diluted telomerase extracts (corresponding to the cells number of 1 × 10⁴) were reacted with MSNPs-TS to execute the EMASE process, followed by incubation with BA-PtNPs to fabricate MGHPP. MGHPP were analyzed on TV-chip for detecting the telomerase activity, and the result represents the telomerase activity of 1 × 10⁴ cells. The above steps are used to quantitatively detect the telomerase activity of different cell numbers.

The mechanism of PDA deposition on the surface BSA. A previous study involving a surface-tethered catechol revealed that the catechol and quinone states are in equilibrium in aqueous media, with the equilibrium shifted toward the quinone at alkaline pH. The quinone have the latent reactivity toward amine groups of proteins.

(Figure S5a) Therefore, in our strategy, the scheme of PDA deposition on the surface
of BSA as shown in Figure R5b. PDA can reacted with amine groups of BSA in an alkaline solution.

**The mechanism of PDA reacted to BA-PtNPs.** Certain diols and phenylboronic acid (PBA) react together to form boronate esters, to an extent dependent upon the pH of the solution.\(^6\)\(^-\)\(^8\) These properties extend to the boronate ester formed by reacting phenylboronic acid (PBA) with dopamine.\(^9\)\(^-\)\(^11\) (Figure S6a) In our experiment, Pt-nanoparticle was modified with 4-mercaptophenylboronic acids (4-MPBA). Due to PDA containing ortho-quinols moieties can covalently react with cis-diol moieties-containing 4-MPBA to form boronate esters, 4-MPBA modified PtNPs (BA-PtNPs) can covalent bind to PDA. (Figure S6b)

**Optimization of experimental conditions.** To achieve excellent sensitivity for the detection of telomerase activity, it was necessary to optimize the experimental conditions. BSA has been shown to not only reduce the non-specific binding, but also provide sufficient binding sites for PDA deposition. So we first evaluated the amount of BSA to use. As illustrated in Fig. S4a, the optimal BSA concentration was determined to be 0.5% (w/v). Concentrations of BSA higher than 0.5% may have provided shielding from charge repulsion and induced MGH aggregation, leading to less PDA deposition on the surface of MGH and resulting in low sensitivity. The concentration of the TS was also found to affect the sensitivity. As shown in Fig. S4b, we found that the displacement of the ink bar increased markedly as the concentration of the primer TS was increased from 0.3 to 10 mM. The ink bar advanced the farthest when the concentration of TS was 10 mM. Therefore, 10 mM TS was adopted in this system. The extension time was also optimized. The signal reached a plateau when the telomerase extension time was 1.5 h (Fig.S4c). Therefore, 1.5 h was chosen in the subsequent experiment.
Supporting Figures

Figure S1. (a) Particle size distribution of MSNPs. (b) The Zeta potentials of modified-MSNPs varies with the preparation process. (c) Particle size distribution of PtNPs. (d) Zeta potentials of PtNPs, 4-MPBA, BA-PtNPs.
Figure S2. Photomasks used for the preparation of TV-Chip (a) Top plate and (b) bottom plate; Images of TV-Chip (c) top plate and (d) bottom plate.
Figure S3. (a) TEM image of PtNPs. (b) Conventional PAGE image analysis of extension products: DNA ladder marker (M), telomerase extracts from MDA-MB-231 of 6000 cells (lane 1), 4000 cells (lane 2), 1000 cells (lane 3), 100 cells(lane 4), heated-inactive control (lane 5). (c) UV–vis spectra of MGH catalyzing H$_2$O$_2$-mediated oxidation TMB in pH 5.5 buffer (H$_2$O$_2$ : 50 mM, TMB : 800 mM, Reaction time: 15 min) from different numbers of MDA-MB-231 cell.
Figure S4. Optimization of the experimental conditions: the concentrations of (a) BSA and (b) TS, (c) the telomerase extension time.
Figure S5. (a) Aqueous chemical equilibrium between catechol (left) and quinone (middle). The equilibrium shifts toward quinone under alkaline conditions, conferring latent reactivity toward amine groups of proteins (right). The figure originated from: Adv. Mater. 2009, 21, 431. (b) Schematic of PDA reaction with BSA.
Figure S6. (a) Schematic of certain diols and boronic acids react together to form boronate esters. The figure originated from: Chem. Commun., 2000, 2393. (b) Schematic of the reaction of PDA and BA-PtNPs.
Supplementary References