Electronic Supporting Information

Quartz crystal microbalance for telomerase sensing based on gold nanoparticles induced signal amplification

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1. Chemicals and Materials. Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) was purchased from Sigma-Aldrich Co. Ltd. (St. Louis, MO) Tris(2-carboxyethyl)phosphine (TCEP) were ordered from Sangon Biotech Co., Ltd. (Shanghai, China). 6-mercaptohexanol (MCH) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). The oligonucleotides were synthesized and modified by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd (Table S1).

In this study, the buffer solutions were employed as follows: telomerase extension reaction buffer (20 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.63 mM KCl, 0.005% (v/v) Tween 20). dNTPs was prepared by dissolving in extension reaction buffer. Telomerase solution consisted of telomerase originating from the specified number of cancer cells was prepared by dissolving in extension reaction buffer containing 1 mM dNTPs. The TS primer and GNPs-cDNA were prepared by dissolving in Tris-NaCl (10 mM Tris-HCl, pH 7.4, 0.1 M NaCl).

2. Apparatus. TEM images were collected on a transmission electron microscope (JEM-2010, Hitachi, Japan). Before TEM examination, 10μL of each sample was first dispersed onto a copper grid coated with the carbon film and was then dried in air. Piezoelectric measurements were performed with AT-cut gold coated quartz crystals with a resonance frequency of 5 MHz in flow through mode with a Q-Sense explorer QCM-D instrument (Biolin Scientific AB, Sweden). The maximum mass sensitivity of the instrument we use in liquid is 0.5 ng/cm^2 (5 pg/mm^2). The oscillating frequency of the piezoelectric crystal decreases with the adsorption of a foreign substance on the surface. To calculate the mass uptakes (Δm) the simplified relation between the shift in frequency (Δf) and the mass of the adsorbed layer described by the Sauerbrey equation was used. $\Delta m = -(C \cdot \Delta f)/n$. Where C = 17.7 ng Hz^{-1} cm^{-2} for a 5 MHz quartz crystal. n = 1, 3, 5, 7 is the overtone number.

3. Cell Culture and Telomerase Extraction. Telomerase was extracted using the CHAPS method.[1, 2] Before telomerase extraction, A549 cells, HeLa cells, and MCF-7 cells were seeded in DMEM supplemented with 10% FBS, penicillin (100 μg/mL), and streptomycin (100 μg/mL) in 5% CO2, 37 °C incubator. Three kinds of cells were collected in the exponential phase of growth, and the number of cells was calculated by flow cytometry. Then 1 million cells were dispensed in a 1.5 mL eppendorf tube, washed twice with ice-cold PBS solution by centrifugation at 1800 rpm for 5 min, after discarding the supernatant carefully, the cells were redispersed in 200 μL of ice-cold CHAPS lysis buffer. The cells were incubated for 30 min on ice and then centrifuged for 20 min (12 000 rpm, 4 °C). Finally, the cleared lysate which contained cell extracts corresponding to one million cells, was carefully transferred to a fresh tube, flash frozen, and stored at -80 °C before use.

For extraction of telomerase from urine samples, fresh urine (4 mL) was collected, centrifuged and washed with PBS solution. After 1800 rpm centrifugation for 5 min at 4 °C, the precipitate was redispersed in 40 uL of ice-cold lysis buffer and then incubated on ice. After 12000 rpm centrifugation for 20 min at 4 °C, the supernatant was transferred and stored at -80 °C before analysis.

4. Preparation of GNPs-cDNA. Citrate-stabilized GNPs with diameters of 13 nm were synthesized according to the classic method.[3] First, all glasswares used in experiment were thoroughly washed with aqua regia (volume ratio 3:1, HCl/HNO_3), rinsed in DI water, and oven-dried prior to use. Second, an aqueous solution of $HAuCl_4 \cdot 4H_2O$ (1 mM, 50 mL) was brought to reflux with continuous stirring. Third, sodium citrate

solution (0.057 g sodium citrate + 5 ml H_2O, freshly prepared) was quickly added, stirred, and kept boiling for another 15 min. The solution will turn from yellow to clear, to black, to purple to deep red during this period. After 15 min, turn heat off and allow the reaction to cool to room temperature. Finally, the AuNPs solution was stored in deep color bottles at 4 °C for further use. Concentration of the as-prepared AuNPs was determined using UV-vis spectroscopy.[4]

GNPs-cDNA was prepared following a literature procedure.[5] In order to increase the negative surface charge of the particle and consequently ensure that the GNPs modified with DNA were well-dispersed at high ionic strength. BSPP-protected GNPs were firstly prepared. Then the BSPP-protected GNPs were mixed with cDNA at a molar ratio of 1:5 for GNPs-cDNA, followed by rocking gently at room temperature for 24 hours. Finally, the prepared GNPs-cDNA was further incubated with thiol-PEG-methyl for 2 hous to passivate the GNPs surface. The sample was then centrifuged to remove uncoupled oligonucleotides.

5. QCM Measured Procedure. Prior to use, the gold-coated crystal chip (5 MHz, AT-cut) was immersed in a mixture of milliQ water, ammonia (25%) and hydrogen peroxide (30%) with a volume ratio of 5:1:1 for 5 min at 75°C, then the chips were rinsed with deionized water and dried by nitrogen gas before loading to measuring cells. In a typical experiment, after the frequency stabilization with Tris-NaCl as mobile phase, mixture of the TS primer (40 nM) and reducer TCEP (100 nM) was injected for 60 min, then the chip was rinsed for 30 min to remove weakly bound TS primer. MCH (100 nM) flowed through the channels for 30 min to block the gold surface. Then the

chip was rinsed with Tris-NaCl to remove weakly bound MCH to frequency stabilization. Next, different concentrations of A549 cells were injected for 60min. After rinsing with Tris-NaCl for 50 min, the amplified response was obtained from the final injection of the gold nanoparticles immobilized cDNA (GNPs-cDNA). The running rate was 20 μL/min set by an ismatec IPC tubing pump (Glattbrugg, Switzerland). To reduce the effect of system error, the frequency shift of the normalized ninth overtone which has the smallest noise was used to quantify.

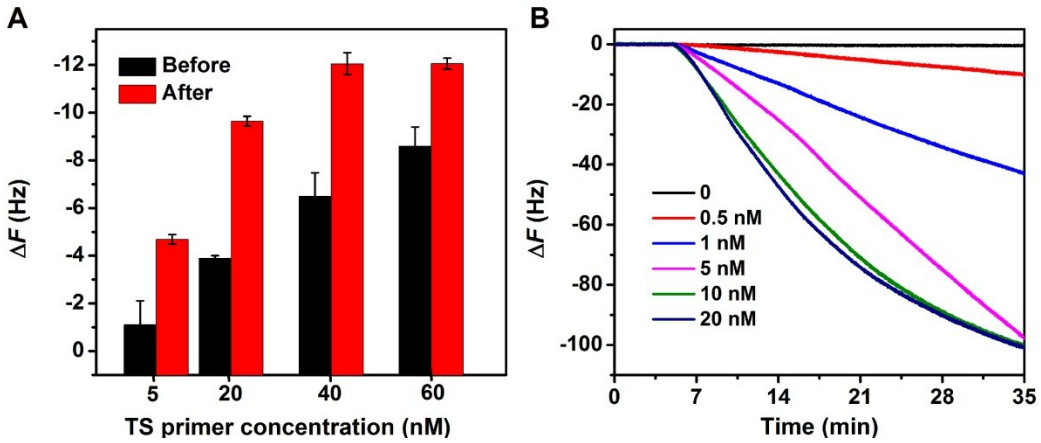
For telomerase inhibitor evaluation, the experiments were conducted using the similar procedure except that telomerase was replaced with a mixture of different concentrations of BIBR 1532 or curcumin with a fixed concentration of 3000 A549 cells/mL. The inhibition efficiency was calculated based on the change of telomerase activity.

6. Atomic Force Microscope (AFM) Characterization. Freshly exfoliated mica was immersed in acetone (95%) containing 1% APTES for 5 min, washed with acetone and dried in the air. Then, it was immersed in 5% glutaraldehyde for 30 min, washed with water and then added with 1 μM of NH_2-TS. 2.6% ice dissolved sodium borohydride was used for blocking. After washing with water, 80 μL of extension reaction buffer containing 1 mM dNTPs and 7800 A549 cells/mL was added and incubated for 1.5 h. After washing, GNPs-cDNA was added and incubated for 30 min. Afterwards, the final prepared mica was rinsed with deionized water and dried for AFM characterization. The surface morphology was observed using a tapping mode.

7. Calculation of the limit of detection. The detection limit was calculated according

123 to the IUPAC recommendations. The equation was $X_D = kS_b/m$. Where S_b is the the
124 standard deviation of ten blank solution; m is the slope of the linear equation of ΔF to
125 the concentration of A549 cells: $\Delta F = -29.86 - 0.024C$, k is 3 when confidence level
126 reached 99.86%.
127

128 **Fig. S1**



129

130 **Fig. S1** The optimization of the concentrations of TS primer (A) and GNPs-cDNA (B).

131 Black and red bars represent before and after telomerase extension reactions,

132 respectively. 3000 A549 cells/mL were used.

133

Fig.S2

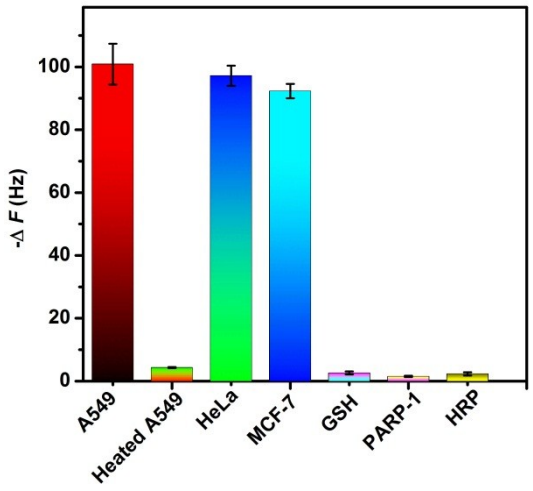


Fig. S2 Telomerase activity detection extracts from various cell lines and selectivity of the biosensor. Heated inactive A549 cells were used as negative control. Error bars show the standard deviation of three experiments.

Fig. S3

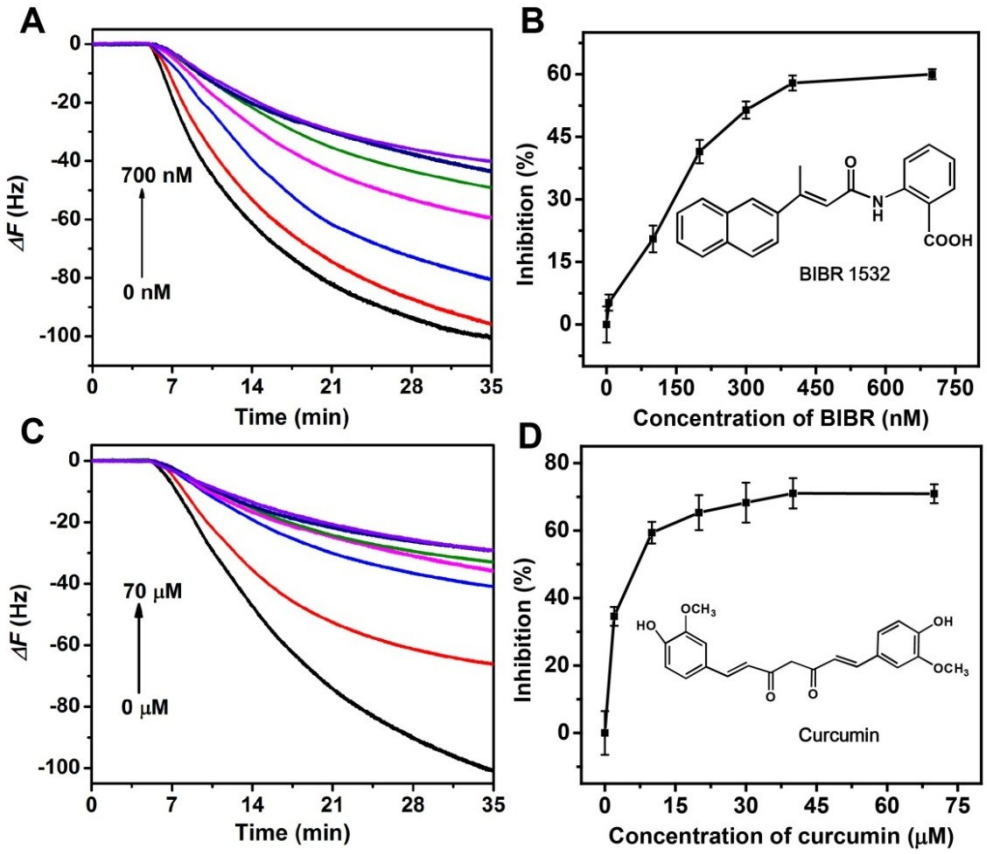


Fig. S3 Frequency responses of the amplified QCM biosensor with the increasing concentrations of BIBR (A) and curcumin (C). The inhibition effect of BIBR 1532 (B) and curcumin (D) on telomerase activity. 3000 A549 cells were used. Error bars showed the standard deviation of three experiments.

147 **Table S1** Sequences of oligonucleotides used in this study.

Name	Sequences (5' to 3')
TS	$SH(CH_2)_6$-TTTTTTTTTTAATCCGTCGAGCAGAGTT
NH_2-TS	$NH_2(CH_2)_6$-TTTTTTTTTTAATCCGTCGAGCAGAGTT
cDNA	SH-$(CH_2)_6$-CCCTAACCCTAACCCTAACCCTAACCCTAAAACT

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Table S2 A comparison in sensitivity between the proposed method and other reported assays for the detection of telomerase activity.

Method	System	Detection range	Detection limit	Reference
UV-vis	Hemin-graphene conjugates	100 - 2300 cells/mL	60 cells/mL	6
UV-Vis	Etching of GNRs	200-15000 cells/mL	90 cells/mL	7
Fluorescence	DNA tetrahedron-hairpin probe	0-82000 cells/mL	90 cells/mL	8
Fluorescence	Molecular beacons	50-2000 cells/mL	50 cells/mL	9
EIS	$Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$	10^3-10^5 cells/mL	1000 cells/mL	10
DPV	Structure-switching DNA	10^2 - 6×10^4 cells/mL	100 cells/mL	11
LSV	Pt NPs encapsulated MOFs	5×10^2-10^7 cells/mL	100 cells/mL	12
Glucometer	DNA-capped MSNs	100-5000 cells/mL	80 cells/mL	13
SERS	Nanogap-rich Au nanowire	0-10^4 cells/mL	0.2 cells/mL	14
SERS	TEC-SERS	1-10000 cells/mL	1 cells/mL	15
QCM	GNPs	100-7800 cells/mL	37 cells/mL	This work

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