Supporting information

Single-molecule detection assisted by the target-triggered signal amplification strategy for ultrasensitive quantitative analysis of intracellular telomerase activity

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Contents

1. Materials and reagent

The oligonucleotides used in this work were synthesized and purified with HPLC by Sangon Biotech Co., Ltd. (Shanghai, China). The QDs were purchased from Wuhan Jiayuan Quantum Dot Technology Development Co., LTD. The streptavidin (SA) was purchased from Shanghai Macklin Biochemistry. The poly(L-lysine)-poly(ethylene glycol)-biotin (PLL-PEG-Biotin) and poly(L-lysine)-poly(ethylene glycol) (PLL-PEG) were purchased from Xi'an Ruixi Biotechnology Co., LTD. The telomerase activity detection kit was purchased from Shanghai Keshun Biotechnology Co., LTD. and the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) provided the rest chemical reagents of analytical grade. The trizol reagent used for the gel-electrophoresis analysis including acrylamide, ammonium persulfate (APS), 1,2-bis(dimethylamino)-ethane (TEMED), and DNA ladder were obtained from Sangon Biotech Co., Ltd. All sample solution was prepared by the purified water from an OKP purification system. For the cell experiment, the cell culture solution, pancreatin and PBS buffer were obtained from Sangon Biotech Co., Ltd.

2. Apparatus

The general morphology of the products was characterized by transmission electron microscopy (TEM, JEOL JEM-2100). Ultraviolet-visible (UV-vis) absorption spectra were recorded on a spectrophotometer (Agilent). The Bio-Rad imaging system was used to record the polyacrylamide gel-electrophoresis result. The Zeta-potential (ζ) values measurements were performed on a Zetasizer Nano ZS90 (Malvern) with 90^{o} scattering angle and a He-Ne laser.

3. Coverslip Pretreatment

Coverslips (25 mm×25 mm) were cleaned via the below procedure. In detail, the coverslips were firstly cleaned with 1.0 M of KOH aqueous solution under the ultrasonication for 10 min to dislodge the surface grease. Then, these treated coverslips were cleaned in ultrapure water and ethanol for 10 min, respectively. For further use, the cleaned coverslips were dried by N_2 stream.

4. Preparation and Surface Modification of Sample Cells

A hole with the diameter of 5 mm was made in the middle of the glass slide (30 mm×30 mm, 5 mm). Then, the glass slide was glued to the coverslip to form

the reaction cell which was further functionally modified based on the previously reported methods with some modification. In detail, the vacuum plasma (PDC-32G-2, Harrick Plasma Inc., U.S.) was used to etch the obtained sample cell for 10 min. Then, the etched cell was incubated with a mixture of PLL-PEG and PLL-PEG-Biotin with the volume ratio of 10:1 for 1 h. After that, the treated cell was washed with PBS buffer three times to dislodge the excessive polymer. Then, 0.2 mg/mL of SA (25 μL) was carefully added into the sample cell and modified onto the surface of the cells based on the interaction with the biotin. Then, the excess SA was removed via the repetitive wash with PBS buffer. Finally, the SA modified sample cell was stored in 25 μL of PBS buffer at 4^{o}C for the subsequent use.

5. Preparation of the Catalytic Probe H2 Modified QDs (QDs-H2)

800 nM of biotin-labeled H2 was added into 3.0 nM of SA modified of QDs (QDs-SA) and the mixture reacted at room temperature for 4 h to obtain the QDs-H2. After the removal of the excess H2, the obtained QDs-H2 was stored in PBS at 4^{o}C for further use.

6. Cell Culture and Extraction of the Intracellular Telomerase

Cancerous cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cell culture condition chose 37ºC in a humidified atmosphere containing 5% CO_2. To extract the intracellular telomerase, cancer cells were collected with the trypsinization, washed twice with ice-cold PBS buffer and then treated with 200 μL of the cold cracking buffer containing 0.5% (3-[(3-cholamidopropyl)dimethylammonio]1-propanesulfonate), CHAPS), 1 mM of magnesium chloride ($MgCl_2$), 1 mM of ethylenebis (oxyethylenenitrilo) tetraacetic acid (EGTA), 0.1 mM of phenylmethanesulfonyl fluoride (PMSF), 5 mM of pyrine-based ethanol, 10% glycerin and 10 mM Tris-HCl (pH=7.5) for 30 min. Then, the above mixing solution was centrifuged under 12,000 rpm for 20 min at 4^{o}C. The extracted intracellular telomerase in the upper extraction solution was carefully collected which was used immediately or be frozen at -80^{o}C for further use. As a control experiment, the telomerase extraction solution was heated at 95^{o}C for 10 min to make the telomerase inactive. For the

single-molecule detection, the extracted telomerase in living cells was diluted by the commercial dilution solution for different folds to obtain different telomerase activity.

7. Telomerase Extension Reaction

The reaction solution was comprised with 30 mM Tris-HCl (pH=8.3), 0.3 mM of $MgCl_2$, 70 mM of KCl, 1 mM of EGTA and 0.05% Tween 20. Then, 1 μL of the telomerase extension reacted in 10 μL of the reaction solution containing 2 μL of telomerase extracts, 3 μM of telomerase substrate primer and 1 mM of dNTPs to obtain the TEP.

8. Detection of TEP

The sample cell modified with SA was first treated with 50 nM of the biotin labeled capture probe H1 (50 μL) for 15 min, then washed with the Tris-HCl buffer three times to remove the unbounded H1. Subsequently, 1 μL of TEP solution and 25 μL of QDs-H2 were added into the above sample cell in sequence to let the designed CHA assay occur. After 12 h, the Tris-HCl buffer was used to remove the unbounded DNA in the sample cell and another 25 μL was used to immerse the sample cell for the single fluorescence imaging.

9. Single-Molecule Detection and Data Analysis

The images of single molecules were acquired by the TIRF microscopy (Nikon, Ti-E, Japan). The 562 nm laser was used to excite the fixed QDs and the excited QDs photons were observed under an 100×oil immersion objective. The fluorescence image was obtained at a high-frequency with an exposure time of 100 ms and then collected by the camera (Photometrics, Evolve 512). For the data analysis, regions of interest of 320×320 pixels were selected for the counting of the fluorescent QDs and the amount of the imaging spots was calculated with the Image J software. To guarantee the accuracy, the average amount of the imaging spots was obtained via 10 frames.

10. Detection of Telomerase Activity

The telomerase activity was quantified with the telomerase activity detection kit according to the standard procedure provided by the manufacturer (Human the elisa kit, Shanghai Keshun Biology).

11. Inhibition of Telomerase Activity

In this experiment, MST-312 was chosen as the inhibitor and studied its inhibition effect on the telomerase activity. Different concentrations of MST-132 (0.25 μM, 0.5 μM, 1.0 μM, 2.0 μM, and 5.0 μM) were used to treated 1×10^6 HeLa cell for 72 h, then the telomerase in these treated HeLa cells was extracted and detected based on the above developed procedure. The inhibition efficiency (%) was calculated according to the below equation.[19] Inhibition efficiency (%)=$(N_2-N_3)/(N_2-N_1)\times100\%$ where N_1 was the average counts of imaging spots in the control group which did not contain cancer cells; N_2 was the average counts of imaging spots when no MST-132 was used to treat cancer cells; N_3 was the average counts of imaging spots when different concentrations of MST-312 were used to treat cancer cells.

12. QDs characterization

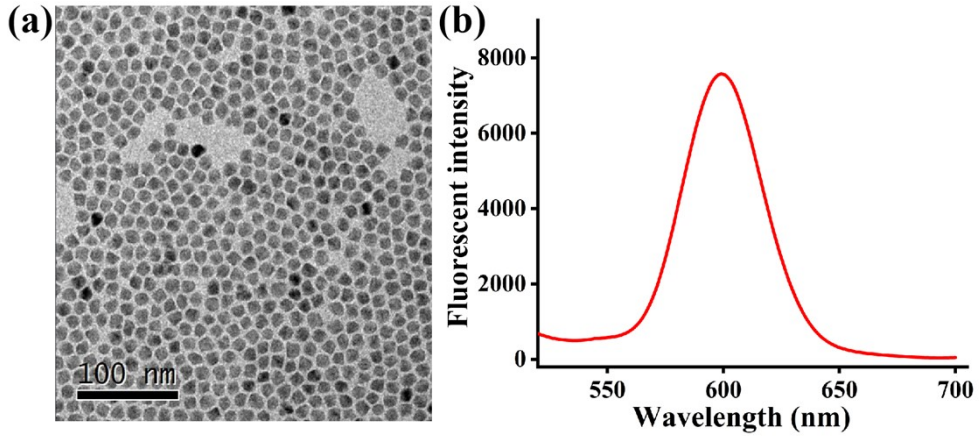

Figure S1: (a) TEM image of the QDs; (b) the fluorescence image of the QDs. The excitation wavelength was set at 562 nm.

13. QDs-H2 characterization

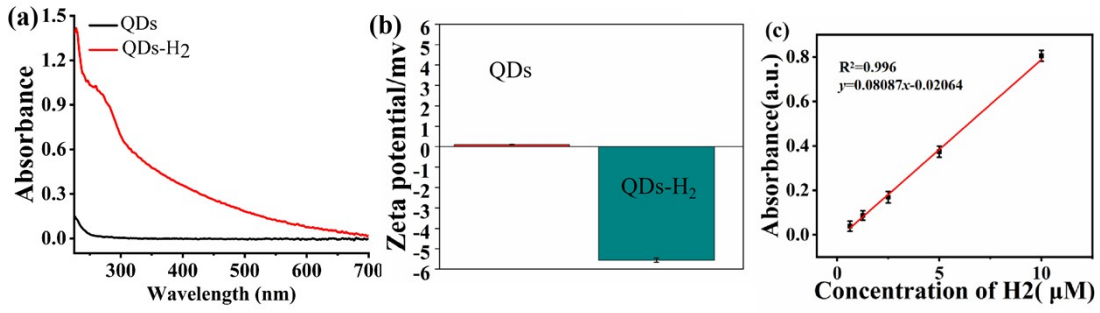

Figure S2: (a) Uv-Vis spectra; (b) Zeta-potential (ζ) values of QDs and QDs-H2 in Tris-HCl buffer; (c) the UV-Vis calibration curve of the H2.

14. The designed DNA sequence

Table S1: Sequences of the Oligonucleotides

Name	Sequence (5′-3′)
Primer	AATCCGTCGAGCAGAGTT
Synthesized TEP	AATCCGTCGAGCAGAGTTAGGGTTAGGGTTAGGG
H1	CCCTAACCCTAACCCGCATGTGTAGAGGGTTAGGGAGTTGTC AGAGCGA-biotin
H2	TAACCCTCTACACATGCGGGTTAGGGTTCCCGCATGTGTAGA-biotin

15. The single-step photobleaching and photoblinking

In this experiment, the nature of the single molecule was validated by the single-step photobleaching and photoblinking. The fluorescence dye Cy5 was used instead of the QDs.

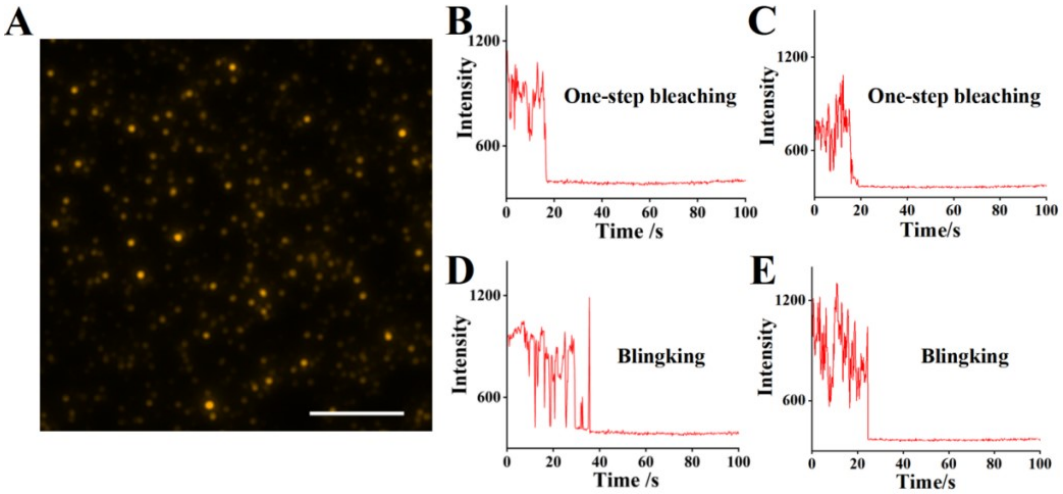

Figure S3: The single-step photobleaching and photoblinking.

16. Comparison of the reported sensors for the detection of telomerase activity

Table S2: Comparison of the reported sensors for the detection of telomerase activity.

Detection method	Nanoprobes	Indicators	DNA Amplification	LOD	Ref.
	DNA	Cy5	with	0.1 aM	1
	DNA	SYBR Gold	with	3 cells	2
	DNA	ThT	with	1 cell	3
	DNA	Cy5	with	1 cells	4
	DNA tweezer	FAM, TAMRA	with	141 cells/μL	5
	DNA nanotetrads	Cy5	with	90	6

				cells/mL	
	Self-assembled DNA polymer	Cy5	with	30 cells	7
	DNAzyme motor modified AuNPs	FAM	with	46 cells/mL	8
	DNA modified MBs, DNA modified UCNPs	UCNPs	with	23 cells	9
	DNA	$[Fe(CN)_6]^{4-/3-}$, Methylene blue	without	20 cells/ml	10
	DNAzyme spiders	Ru-PEI@ZIF-8	with	11 cells	11
	Au nanorods	3,3',5,5'-tetramethylbenzidine (TMB)	without	90 cells/mL	12
	Au nanorods	glucose	without	5 cells/mL	13
	DNA modified AuNPs	$[Fe(CN)_6]^{4-/3-}$	without	7 cells	14
	DNA modified MOFs	acetaminophen	without	2×10^{-11} IU	15
Chemilum-inescence detection	DNA	hemin	with	1 cell	16
Electroche-milumines cence	DNA modified AuNPs	DNA modified magnetic beads	with	500 cells/mL	17
Photoelect-rochemical detection	AuNPs/Cu^{2+}-BNNS, $Ag_2S/Ag/ZnIn_2S_4/C_3N_4$	Insoluble BCP	without	35 cells/mL	18
SERS	Tetrahedron Probes	Cy5, TAMRA	without	7.6×10^{-16} IU	19
	DNA modified MBs, DNA modified AuNPs	AuNPs	with	1 cell	20
Dynamic light scattering method	DNA modified AuNPs	AuNPs aggregation	with	1-8 cells	21
POC assay	Chemiluminescence-based POC assay	DNA modified magnetic beads	Oxidized luminol	20 cells	22
	Pressure readings-based POC assay	DNA modified magnetic beads, DNA-modified PtNP	Oxidized TMN	1 cells	23
	Colorimetry-based POC assay	DNA modified magnetic beads	Oxidized TMN	5 cells	24

FAM: 5-Carboxy Fluorescein; ThT: ThioflavinT; MBs: magnetic beads; UCNPs: upconversion nanoparticles; AuNPs: gold nanoparticles; MOF: metal-organic framework; QDs: quantum dots; AgNCs: silver nanoclusters; NAA: nanoporous anodic alumina; BCP: biocatalytic precipitation; Cu^{2+}-BNNS: Cu^{2+}-modified boron nitride nanosheets; SERS: surface-enhanced Raman scattering.

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