Supporting Information

for

Facile Synthesis of Fluorescent Tungsten Oxide Quantum Dots for Telomerase Detection Based on the Inner Filter Effect

Li Zhang,[a] Jia-Qing Chen,[a] Ming-Fang Hong,[a] Ru-Ping Liang,[a] and Jian-Ding Qiu [a,b*]

[a]*College of Chemistry, Nanchang University, Nanchang 330031, China*

[b]*Environmental Protection Materials and Equipment Engineering Technology Center of Jiangxi, Department of Materials and Chemical Engineering, Pingxiang University, 211 Pingan North Road, Pingxiang 337055, Jiangxi, China*

*Corresponding author. (J.-D.Qiu) Tel/Fax: +86-791-83969518. E-mail: jdqiu@ncu.edu.cn.

1. Experimental Section

1.1. Materials and reagents

The deoxynucleotide solution mixture (dNTP), DEPC-treated water, gel electrophoresis loading buffer, 5× Tris-borate-EDTA (TBE) and ladder DNA were purchased from TaKaRa Bio. Inc. (Dalian, China). HeLa, A549, and L929 cell lines were purchased from ATCC (Manassas, VA). Cells cultured chemicals: penicillin, streptomycin and fetal bovine serum were purchased from Thermo Scientific HyClone (USA). N,N,N',N'-tetramethylethylene diamine (TEMED), N,N′-1,3-Phenylenebis-[2,3-dihydroxy-benzamide] (MST), epigallocatechin gallate (EGCG) and thrombin were purchased from Sigma-Aldrich (Shanghai, China). [(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonic acid (CHAPS), ethylene glycol-bis (β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), trypsin and phenylmethylsulfonyl fluoride (PMSF) were purchased from Solarbio Co. Ltd. (Beijing, China). Bull serum albumin (BSA) was purchased from BoAo. Co. Ltd (Shanghai, China). Phosphate buffer saline (PBS, pH 7.4) containing 136.7 mM NaCl, 8.72 mM Na_2HPO_4, 2.7 mM KCl and 1.41 mM KH_2PO_4 was obtained from ZhongShan. Co. Ltd (Beijing, China). All other chemicals were of analytical grade and were used without further purification. Ultrapure water obtained from a Millipore filtration system was used in all experiments. The DNA sequences were purchased from Sangon Biological Engineering Technology Co. Ltd (Shanghai, China) with the following sequences: telomerase primer (primer-DNA): 5’-AAT CCG TCG AGC AGA GTT-3’.

1.2. Apparatus

The fluorescence analysis was conducted on a Hitachi F-7000 spectrometer (Tokyo, Japan) furnished with a Xenon lamp. The UV–vis absorption spectra were obtained by an UV-2450 spectrophotometer (Shimadzu, Japan). The gel electrophoresis image was obtained by using a Tanon-3500 digital gel imaging system (Tanon Science & Technology, China). The atomic force microscopy (AFM) image was mapped with the ScanAsyst mode of Bruker MultiMode-8 (Bruker, USA). The fluorescence images were obtained on a Zesis LSM 710 (Germany). The transmission electron microscopy (TEM) image was taken by a JEOL2010 microscope (Japan) operated at 200 kV. The sample for TEM measurement was suspended in water and dropped onto holey carbon films supported on Cu grids. The crystalline structure of the sample was investigated by X-ray diffraction (XRD) with Cu Kα radiation (Bruker AXS D8). Diffraction data (2θ) was collected between 15-75 º. The chemical state was analyzed using X-ray photoelectron spectroscopy (XPS, VG Multilab 2000X instrument, Thermal Electron, USA). The Fourier transform infrared (FT-IR) spectrum was acquired on a Thermo Nicolet 5700 spectrometer at resolution of 2 cm^{-1} in the range of 400−4000 cm^{-1}.

1.3. Cell culture and telomerase extraction

HeLa (human cervical cancer) cells, L929 (Mouse fibroblast) cells and A549 (human lung cancer) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin (100 $\mu g \cdot mL^{-1}$), and streptomycin (100 $\mu g \cdot mL^{-1}$) in a flask at 37 °C under a humidified atmosphere

containing 5% CO_2. All kinds of cells were collected by trypsinization in the exponential phase of growth, washed twice with ice-cold PBS (pH 7.4), and pelleted at 2000 rpm at 4 °C for 3 min. About 2×10^6 cells were then suspended in 200 μL of ice-cold 1× CHAPS lysis buffer (10 mM Tris–HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 0.5% CHAPS, 10% glycerol), and incubated on ice for 30 min. Then the mixture was centrifuged at 16000 rpm for 20 min at 4 °C, and the supernatant was transferred and stored at −80 °C. For the control experiments, the telomerase extracts (10000 cells equivalence) were pretreated at 95 °C for 15 min prior to detection.

1.4. Gel electrophoresis analysis

The steps for conducting gel electrophoresis were as follows: the samples were injected into polyacrylamide hydrogel in TBE buffer, and the lanes are listed as marker DNA (10 μM) (Lane M), primer-DNA (10 μM) (Lane 1), telomerase extract and primer-DNA incubation at 37 °C for 1 hour (Lane 2), telomerase extract with primer-DNA and hemin incubation for 1 hour at 37 °C (Lane 3), telomerase extract with inhibitor (MST) treatment and primer-DNA incubation at 37 °C for 1 hour (Lane 4), and heat-inactivated telomerase extract and primer-DNA incubation at 37 °C for 1 hour (Lane 5). Electrophoresis was carried out at 110 V in 1× TBE buffer for 2.5 h, and ethidium bromide was used for staining. The gel was observed under UV irradiation. The gel was prepared by using 6.6 mL of polyacrylamide (20%), 6.6 μL of TEMED, 2 mL of 5× TBE, and 73 μL of ammonium persulfate (10%).

Table S1 Fluorescence lifetimes obtained with two-exponential fit of the fluorescence decay curves of WO_x QDs, WO_x QDs/hemin, and WO_x QDs/hemin in the presence of telomerase, respectively.

Samples	τ_1 /ns	τ_2 /ns	τ /ns
WO_x QDs	5.14 (25.92%)	17.59 (74.08%)	14.36
WO_x QDs/hemin	3.92 (26.73%)	16.67 (73.27%)	13.26
WO_x QDs/hemin-telomerase	4.21 (30.48%)	17.25 (69.52%)	13.27

Inner filter effect (IFE) of hemin on WO_x QDs

Based on the cuvette geometry and the absorption characteristics of the aqueous solution of hemin and WO_x QDs, the IFE was corrected with the following equation.[1,2]

$$CF = \frac{F_{cor}}{F_{obsd}} = \frac{2.3dA_{ex}}{1-10^{-dA_{ex}}} 10^{gA_{em}} \frac{2.3sA_{em}}{1-10^{-sA_{em}}}$$

Where, CF is the corrected factor; F_{obsd} is the observed fluorescence intensity of WO_x QDs and F_{cor} is the corrected fluorescence intensity by removing IFE from F_{obsd}; A_{ex} and A_{em} represent the absorbance per centimeter at the excitation wavelength and the emission wavelength of WO_x QDs, respectively; s is the thickness of excitation beam (0.10 cm), g is the distance between the edge of the excitation beam and the edge of the cuvette (0.40 cm) and d is the width of the cuvette (1.00 cm). The maximum value of the correction factor could not exceed 3; otherwise the correction was not convincing.

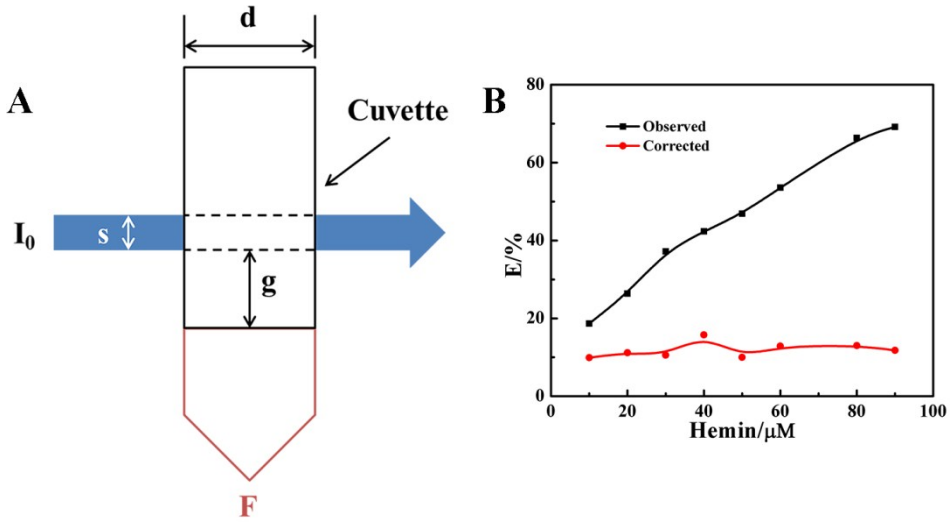

Figure S1. (A) Parameters used in the equation to correct for the inner filter effect. I_0 represents the excitation beam, and F represents the observed fluorescence beam. (B) Observed (black curve, E_{obsd}) and corrected (red curve, E_{cor}) quenching efficiency of hemin towards WO_x QDs. E = 1 - F/F_0, F_0 and F are the fluorescence intensities of WO_x QDs in the absence and presence of hemin, respectively.

Table S2 IFE of hemin on the fluorescence of WO_x QDs.

Hemin (μM)	A_{ex}[a]	A_{em}[b]	CF[c]	F_{obsd}[d]	F_{cor}[e]	$F_{cor,0}/F_{cor}$[f]	E_{obsd}[g]	E_{cor}[h]
0	0.022	0.005	1.029	5349	5501.745	1	0	0
10	0.065	0.057	1.140	4350	4957.414	1.110	18.68%	9.89%
20	0.104	0.097	1.241	3939	4886.682	1.126	26.36%	11.18%
30	0.183	0.174	1.464	3360	4919.56	1.118	37.18%	10.58%
40	0.195	0.187	1.503	3084	4635.676	1.187	42.34%	15.74%
50	0.271	0.253	1.743	2840.7	4951.251	1.111	46.89%	10.01%
60	0.323	0.300	1.930	2483.7	4792.624	1.148	53.57%	12.89%
80	0.505	0.438	2.656	1801.8	4785.743	1.150	66.32%	13.01%
90	0.549	0.498	2.943	1648.8	4853.033	1.134	69.18%	11.79%

A_{ex} is the absorbance of WO_x QDs with the addition of hemin at the excitation wavelength. A_{em} is the absorbance of WO_x QDs with the addition of hemin at the emission wavelength. Corrected factor (CF) was calculated as F_{cor}/F_{obsd}. F_{obsd} is the measured fluorescence intensity of WO_x QDs with the addition of hemin. F_{cor} is the corrected fluorescence intensity by removing IFE from the measured fluorescence intensity. $F_{cor,0}$ and F_{cor} are the corrected fluorescence intensities of WO_x QDs in the absence and presence of hemin, respectively. $E_{obsd} = 1 - F_{obsd}/F_{obsd,0}$, $F_{obsd,0}$ and F_{obsd} are the observed fluorescence intensities of WO_x QDs in the absence and presence of hemin, respectively. $E_{cor} = 1 - F_{cor}/F_{cor,0}$, $F_{cor,0}$ and F_{cor} are the corrected fluorescence intensities of WO_x QDs in the absence and presence of hemin, respectively.

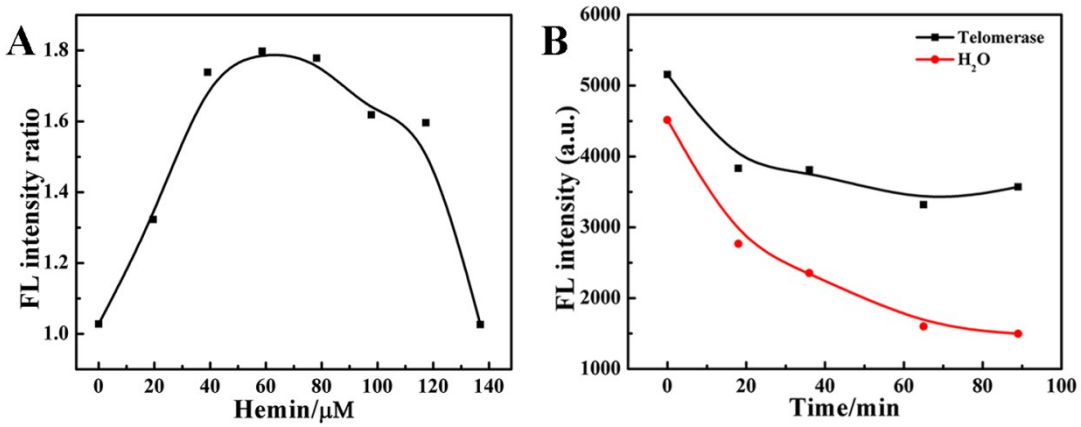

Figure S2. (A) The fluorescence responses toward telomerase of 10000 HeLa cells in the presence of different concentrations of hemin. (B) Fluorescence intensity of WO_x QDs with telomerase of 10000 HeLa cells or without telomerase versus the reaction time.

Table S3 Comparison of the sensing performance between the present method and other methods for telomerase detection.

Method	System	Detection limit (cells)	Detection range (cells)	Time	Ref.
Electrochemistry	Au NPs/biotin-streptavidin magnetic beads	500	5×10^2~2×10^4	2.5 h	3
	Bi@NOC NHs/Th-T	60	5×10^2~10^6	1.5 h	4
	p-$CuBi_2O_4$ nanorod	53	10^2~2×10^3	2 h 10 min	5
	CS/Ru-PEI@ZIF-8/PtNPs	11	50~10^6	4 h	6
Chemiluminescence	MB-AuNPs/hemin	100	10^2~10^3	2 h 10 min	7
	Au/G-quadruplex/hemin/luminol	1000	10^3~10^4	12 h	8
	MB-TS/HRP-cDNA/luminol/H_2O_2/PIP	9	10~10^3	4 h 25 min	9
	Catalytic beacons/hemin	500	5×10^2~10^4	/	10
Colorimetry	TC- AuNPs	500	5×10^2~10^4	1 h 20 min	11
	TS-PMPs/cDNA/urse	20	20~10^4	7 h	12
	Au NRs/CHA/hemin/TMB	15	20~5×10^2	2 h 20 min	13
Fluorescence	Exo III/GO/signaling DNA probe	250	250~10^4	2 h	14
	Hairpin-DNA@Cy5.5-Au NBPs	23	40~1.2×10^3	1.5 h	15
	Au NP/GO/mimic-HCR	480	2×10^3~4×10^4	2 h	16
	RecA-GFP fusion protein	8	50~10^3	2 h 40 min	17
	DNAzyme-CHA cascade	20	10^3~10^4	2 h 40 min	18
	RNA-aptamer/DFHBI/TRPs/RNaseH	100	10^2~5×10^4	3 h 10 min	19
	WO_x QDs/hemin	17	50~3×10^4	1 h	This work

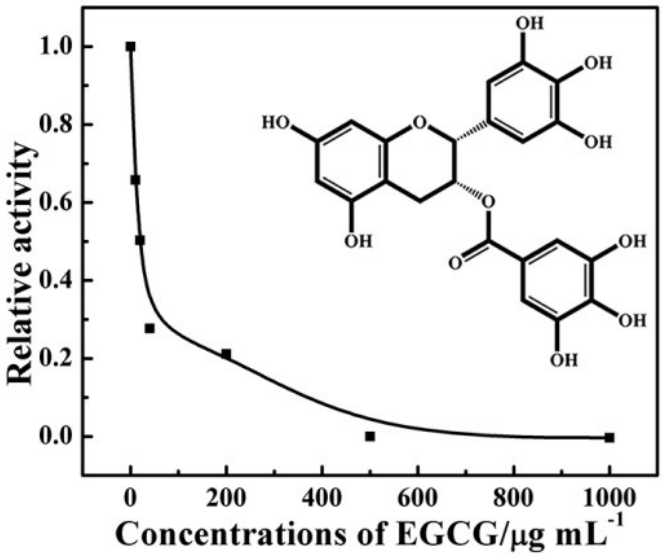

Figure S3. Inhibition effect of different concentrations of EGCG on telomerase activity.

References

1. T. D. Gauthier, E. C. Shane, W. F. Guerin, W. R. Seitz and C. L. Grant, *Environ. Sci. Technol.*, 1986, **20**, 1162-1166.
2. L. Zhang, D. Peng, R.-P. Liang and J.-D. Qiu, *Anal. Chem.*, 2015, **87**, 10894-10901.
3. X. Zhou, D. Xing, D. Zhu and L. Jia, *Anal. Chem.*, 2009, **81**, 255-261.
4. S. Liu, S. Zhao, W. Tu, X. Wang, X. Wang, J. Bao, Y. Wang, M. Han and Z. Dai, *Chem. Eur. J.*, 2018, **24**, 3677-3682.
5. J. Lei, B. Han, S. Lv, Y. Li, J. Tang, Y. Mao and J. Zhuang, *Electrochem. Commun.*, 2018, **92**, 43-47.
6. C. Xiong, W. Liang, Y. Zheng, Y. Zhuo, Y. Chai and R. Yuan, *Anal. Chem.*, 2017, **89**, 3222-3227.
7. Y. Li, X. Li, X. Ji and X. Li, *Biosens. Bioelectron.*, 2011, **26**, 4095-4098.
8. V. Pavlov, Y. Xiao, R. Gill, A. Dishon, M. Kotler and I. Willner, *Anal. Chem.*, 2004, **76**, 2152-2156.
9. J. Zhu, Y. Wang, B. Li and Y. Jin, *Sensor Actuat. B-Chem*, 2019, **301**, 127109.
10. Y. Xiao, V. Pavlov, T. Niazov, A. Dishon, M. Kotler and I. Willner, *J. Am. Chem. Soc.*, 2004, **126**, 7430-7431.
11. J. Wang, L. Wu, J. Ren and X. Qu, *Nanoscale*, 2014, **6**, 1661-1666.
12. L. Wang, C. Chen, H. Huang, D. Huang, F. Luo, B. Qiu, L. Guo, Z. Lin and H. Yang, *Biosens. Bioelectron.*, 2018, **121**, 153-158.
13. D. Wang, R. Guo, Y. Wei, Y. Zhang, X. Zhao and Z. Xu, *Biosens. Bioelectron.*, 2018, **122**, 247-253.
14. L. Peng, Z. Zhu, Y. Chen, D. Han and W. Tan, *Biosens. Bioelectron.*, 2012, **35**, 475-478.
15. S. Xu, L. Jiang, Y. Nie, J. Wang, H. Li, Y. Liu, W. Wang, G. Xu and X. Luo, *ACS Appl. Mater. Interfaces*, 2018, **10**, 26851-26858.
16. M. Hong, L. Xu, Q. Xue, L. Li and B. Tang, *Anal. Chem.*, 2016, **88**, 12177-12182.
17. T. Wu, Y. Zhang, T. Hou, Y. Zhang and S. Wang, *Anal. Chim. Acta*, 2018, **1038**, 120-125.
18. H. Fan, H. Bai, Q. Liu, H. Xing, X.-B. Zhang and W. Tan, *Anal. Chem.*, 2019, **91**, 13143-13151.
19. Y. Zhou, S. Shen, C. Lau and J. Lu, *Talanta*, 2019, **199**, 21-26.