Electronic Supplementary Information (ESI) for Analyst
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A novel DNA tetrahedron-hairpin probe for in situ "off-on" fluorescent imaging of intracellular telomerase activity†

Qiu-Mei Feng, Meng-Jiao Zhu, Ting-Ting Zhang, Jing-Juan Xu* and Hong-Yuan Chen

State Key Laboratory of Analytical Chemistry for Life Science and Collaborative Innovation Center of Chemistry for Life Sciences, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, P.R. China.
E-mail: xujj@nju.edu.cn (J.J. Xu); Fax: +86 25 89687294; Tel: +86 25 89687294

Table S1. DNA sequences employed in this work.

name	Sequences (5′ to 3′)
S1	SH-AGGGTT$(AAA)_7$AACCCTACATTCGTATGTGTGAAACATTACAGCTTGCTACACGAATCCGTCGAGCAGAGTT
Cy5-S2	Cy5-TATCACCAGTCAATTGACAGTGTAGCAAGCTGTAATAGATGCTAGTGTCTA ATAG
Cy5-S3	Cy5-TCAATTGACTGGTGATAAAACGACACTACGTGAGTATGAACTCTGCTCGAC GGAT
Cy5-S4	Cy5-TTCACACATACGAATGTGCTACTCACGTAGTGTCGTTTCTATTAG

	ACACTAG CAT
S5	SH- AGGGTT(AAA)$_7$AACCCTACATTCGTATGTGTGAAACATTACAGCT TGCTACACGAGAGCCGCCATAGTAGCT
bbcDNA	SH-GCGCGAACCGTATA

The telomerase strand primer (TSP) is highlighted in the box. The same color portion is complementary each other.

Telomerase activity in different media

The activity of telomerase activity was tested in different media (PBS, DMEM and RPMI-1640) based on the changes of fluorescence signal of T-H probe (Fig. S1). In these solutions, the fluorescence intensity of the T-H probe and solution containing dNTPs in the presence or absence of telomerase (extracted from HeLa cells, 30000 cells/mL) was recorded after incubation with different times (Fig. S1A-C). In the presence of telomerase, the fluorescence intensity increased with incubation time similarly in PBS, DMEM and RPMI-1640 (Fig. S1A-C, curve b), while no obvious fluorescence change was obtained in the absence of telomerase (Fig. S1A-C, curve a), suggesting good activity of the T-H probe in different media.

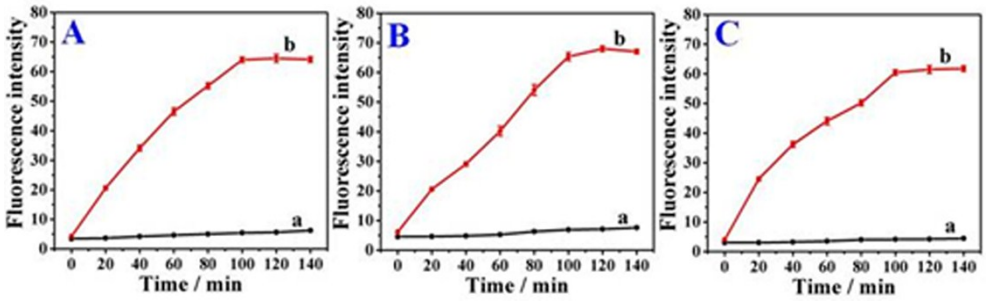

Fig. S1 Plots of fluorescence intensity of the T-H probe solution containing dNTPs *vs* incubation time in the (a) absence and (b) presence of telomerase (extracted from HeLa cells, 30000 cells/mL) in (A) PBS, (B) DMEM and (C) RPMI-1640.

Optimal molar ratio between S1 and Au NPs

The influence of molar ratio between S1 and Au NPs (1/1, 2/1, 3/1 and 4/1) on the fluorescence intensity of the corresponding T-H probe has been investigated. The concentration of S1 kept unchange and the Au NPs concentration altered with the different molar ratio. The molar amounts of Cy5-S2, Cy5-S3, Cy5-S4 were the equivalent with that of S1. According to Fig. S2, the fluorescence intensity decreased with the increase of molar ratio for S1/Au NPs. Due to the three-dimensional scaffold structure of DNA tetrahedron, the amounts of DNA tetrahedron on one Au NP would be restrained because of steric hindrance. The surplus and unreacted Cy5-S2 (Cy5-S3, Cy5-S4) would be removed by ultrafiltration, resulting in the reducing of Cy5 amount. The optimal molar ratio between S1 and Au NPs for constructing the DNA tetrahedron for sensing telomerase activity is 1/1.

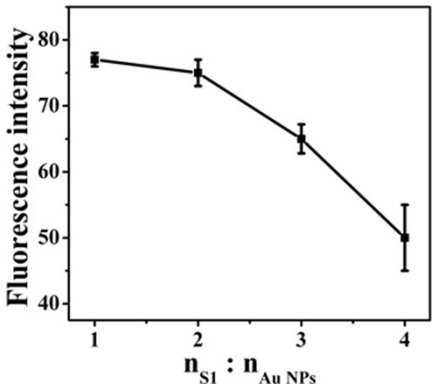

Fig. S2 Plots of fluorescence intensity of the T-H probe solution with different molar ratio between S1 and Au NPs (1/1, 2/1, 3/1 and 4/1) in the presence of telomerase (extracted from HeLa cells, 30000 cells/mL).

Nuclease stability assay

Nuclease stability is a key property of the T-H probe for imaging and therapeutic applications in living cells. After DNase I was added to the T-H probe and dNTPs solution, it showed negligible fluorescence recovery along with incubation time in the absence of telomerase. However, the fluorescence intensities of the solutions in the absence and presence of DNase I were both increased greatly after incubation with the telomerase extract from HeLa cells (Fig. S3, inset), indicating the good stability of the T-H probe and high resistance to the nuclease.

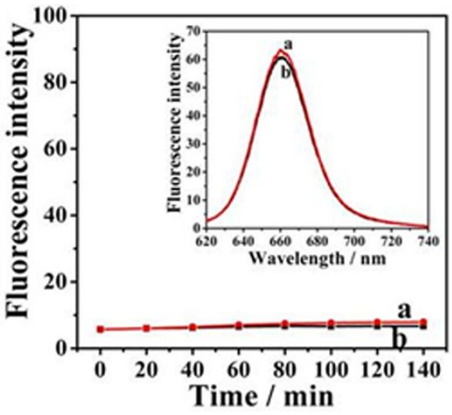

Fig. S3 Plots of fluorescence intensity of the T-H probe in (a) absence or (b) presence of DNsae I vs. incubation time. Inset: Fluorescence spectra of the T-H probe without (a) and with (b) 2 μg DNase I after incubation with dNTPs and telomerase (extracted from HeLa cells, 30000 cells/mL).

Optimization of T-H probe amount for incubation with HeLa cells

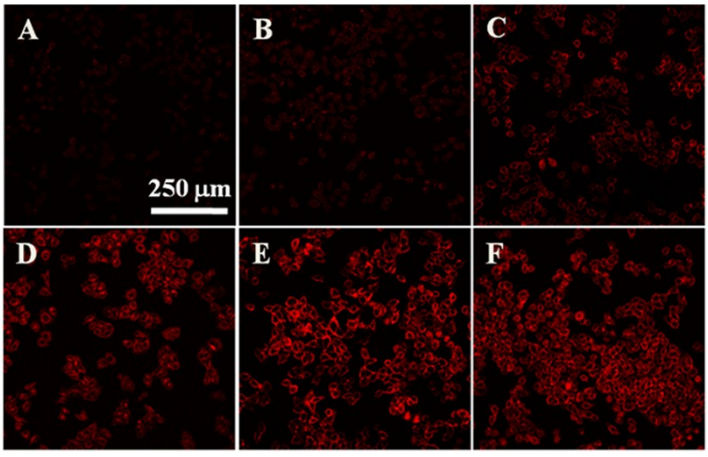

Fig. S4 Confocal microscopy images of HeLa cells (200 μL, 1×10^6 mL^{-1}) after incubation with 5, 10, 15, 20, 25 and 30 μL T-H probe (from A to F) at 37 °C for 2 h.

Cell viability assays

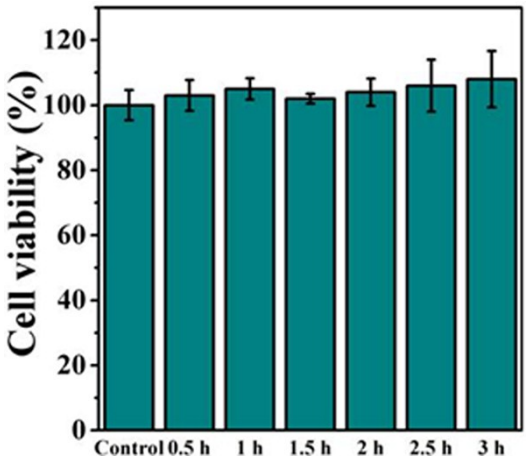

Fig. S5 Bar graph of viability of HeLa cells after incubation with 25 μL T-H probe for different times. Error bars represent relative standard deviation (RSD) based on five independent measurements.