Supporting Information

DNA-Fueled molecular machine for label-free and non-enzymatic

ultrasensitive detection of telomerase activity

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Experimental Section

Reagents and Materials: All oligonucleotides listed in Table S1 were obtained from Shanghai Sangon Biological Engineering Technology and Services (Shanghai, China). N-methyl mesoporphyrin IX (NMM) was purchased from Porphyrin Products Inc (Logan, UT), and its concentration was measured by absorbance at 379 nm assuming an extinction coefficient of 1.45 $\times 10^5 M^{-1} cm^{-1}$. All the reagents were of analytical grade and used without further purification, and solutions were prepared with ultrapure water (specific resistance of 18.3 MΩ•cm) during the experimental process.

Cell Culture and Telomerase Extract Preparation: The telomerase extracts were prepared according to the previously reported protocol.[1] Briefly, various cell lines used in the experiment were cultured in Dulbecco's modified Eagle's medium (DMEM) medium supplemented with 10% fetal calf serum and maintained the cells at 37 °C in a humidified atmosphere (containing 5% CO_2). Cells were collected in the exponential phase of growth and 1×10^6 cells were dispensed in a 1.5 mL EP tube, washed twice with ice-cold PBS, and resuspended in 100 μL of ice-cold lysis buffer (10 mM Tris HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM ethylene glycol tetraacetic acid (EGTA), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 5 mM β-mercaptoethanol, 0.5% 3-[(3-cholamido-propyl)-dimethylammonio]-1-pro-panesulfonic acid (CHAPS), 10% glycerol). Pretreated the CHAPS lysis buffer with RNA secure according to the manufacturer's instructions. The lysate was incubated for 30 min on ice and centrifuged at 12000 rpm for 20 min at 4 °C, to discard insoluble material. Finally, the cleared lysate was carefully transferred to a fresh 1.5 mL EP tube and stored at -80 °C.

Telomerase Extension Reaction: Telomerase extracts were diluted in lysis buffer with respective number of cells; the extracts (1 μL) were diluted to 10 μL with RNA secure pretreated extension solution containing 1× TRAP buffer (20 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 63 mM KCl, 0.005% Tween 20, 1 mM EGTA, bovine serum albumin (BSA) 0.1 mg mL^{-1}), 1 mM dATP, dTTP, dCTP, and dGTP, and 10μM TS primer. Then the mixtures were incubated at 30 °C for 60 min. For negative control experiments, telomerase extracts were heat-treated (95 °C for 10 min). In the inhibition experiments, different volumes of curcumin (50 μM) solution were added into the telomerase extension reaction buffer to achieve the desired final concentrations.

Detection of Telomerase Activity: After telomerase extension reaction, heated the reaction solution at 95°C for 5 min. Then cooled down quickly and added to 120 μL reaction system containing 10mM Tris-HCl (PH7.5), 10μM H1 and 10μM H2. The mixtures were incubated at 37 °C for 40 min and then the fluorescence intensities were recorded immediately at room temperature on a FP-6500 spectrofluorometer (Jasco International Co., Japan). The excitation wavelength was 399 nm and the emission wavelengths were in the range from 575 to 650nm with the slit widths of both excitation and emission of 5 nm. The fluorescent emission at 608 nm was used as the indicator of the performance of the telomerase detection approach. There were two important roles of heating before mix: for one thing, the telomerization reaction was stopped by heat denaturing of telomerase; for another, heating could disrupt the G-quadruplex conformation formed by the TRP and eliminate its interference for the reaction between TRP and H1. The TRP used here was from 0.05 μM TS primers which incubated with 100 HeLa cell extracts.
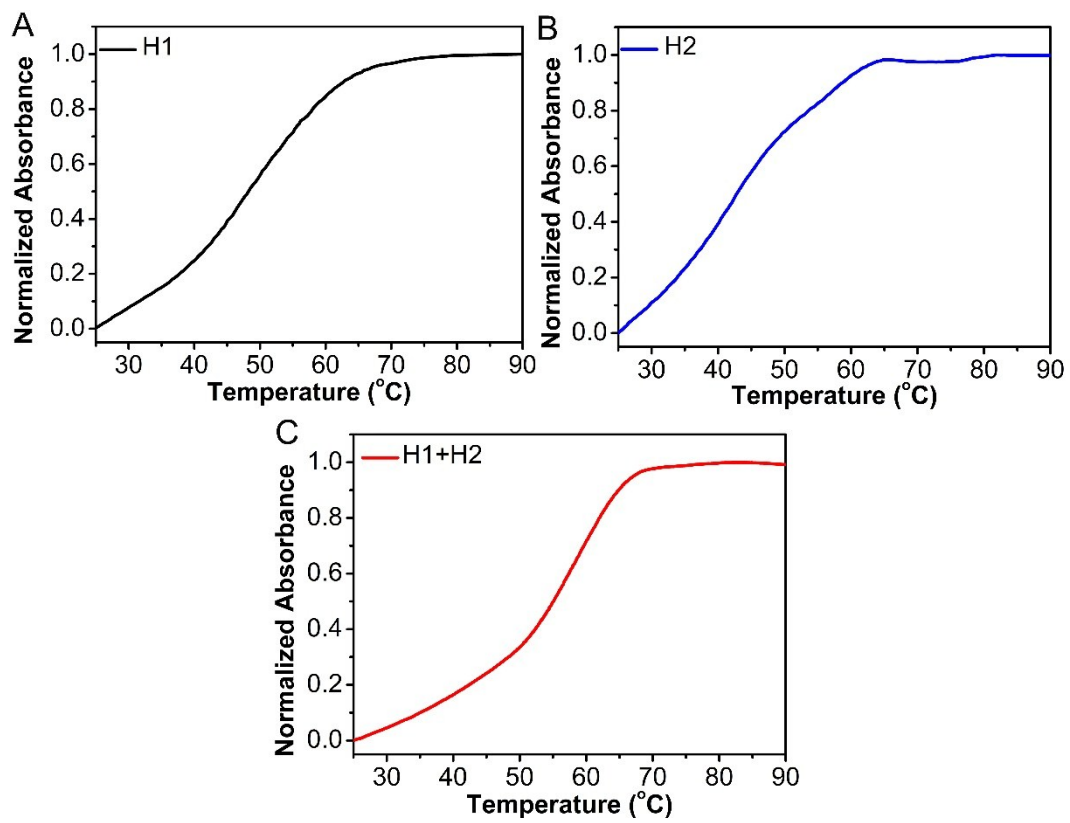
Figures:

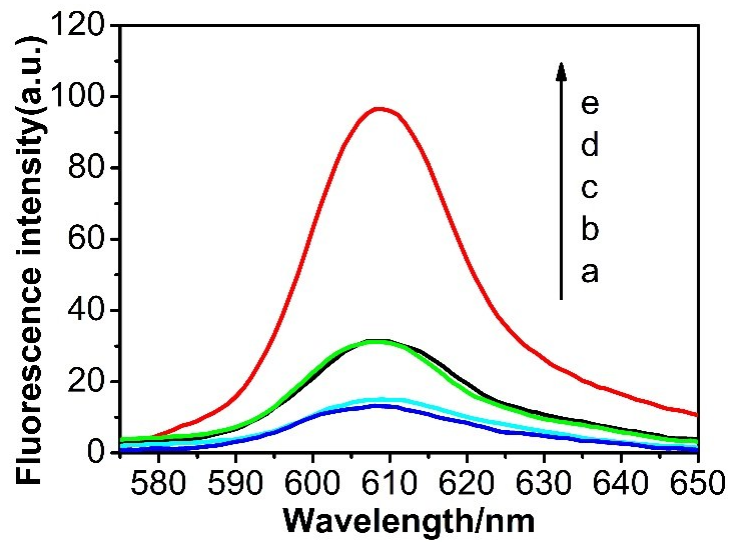Fig. S1. The melting curves of H1 (A), H2 (B) and H1+H2 complex (C).

Fig. S2.Fluorescence spectra of different mixtures: (a) NMM alone. (b) H2/NMM. (c) H1/H2/NMM. (d) H1/H2/TS primer. (e) H1/H2/NMM/ synthetic TRP.The concentrations of the synthetic TRP(with two TTAGGG repeats), H1, H2 and NMM were 0.05μM, 0.5μM,0.5μM and 2μM respectively.
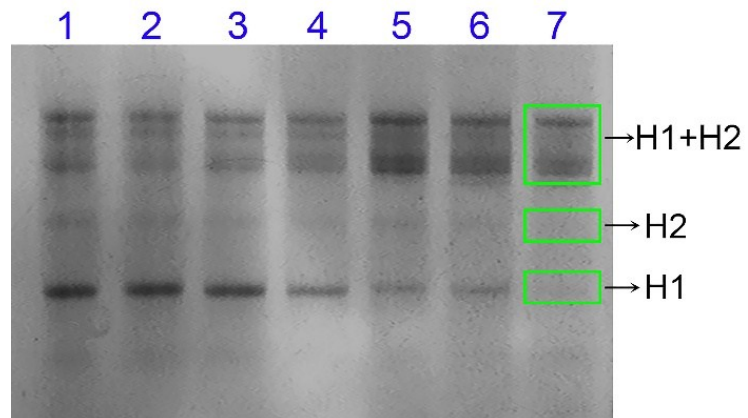
Fig. S3. Nondenaturing polyacrylamide gel (12%) analysis for the catalytic effect of different concentrations of synthetic TRP. (1) 0.2μM; (2) 0.4μM; (3) 1μM; (4) 2μM; (5) 4μM; (6) 6μM; (7) 10μM. [H1] = [H2] = 10μM.
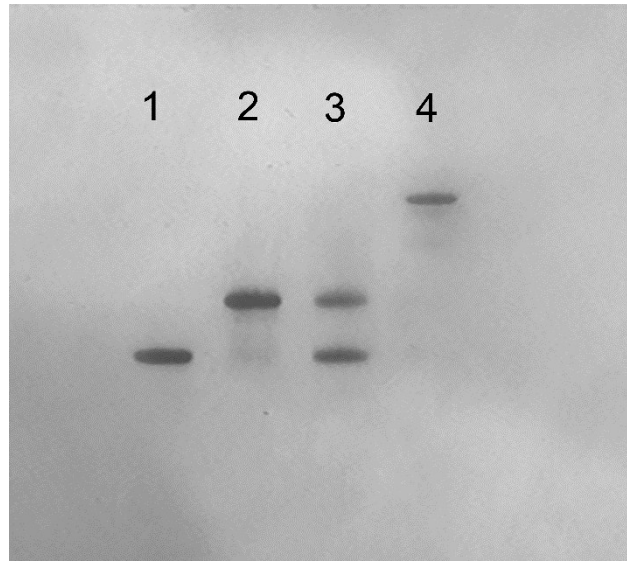
Fig. S4. Non-denaturing PAGE experiments to analyze of the formation of H1+H2 complex: (1) H1 alone; (2) H2 alone; (3) H1/H2; (4) H1/H2/TRP.
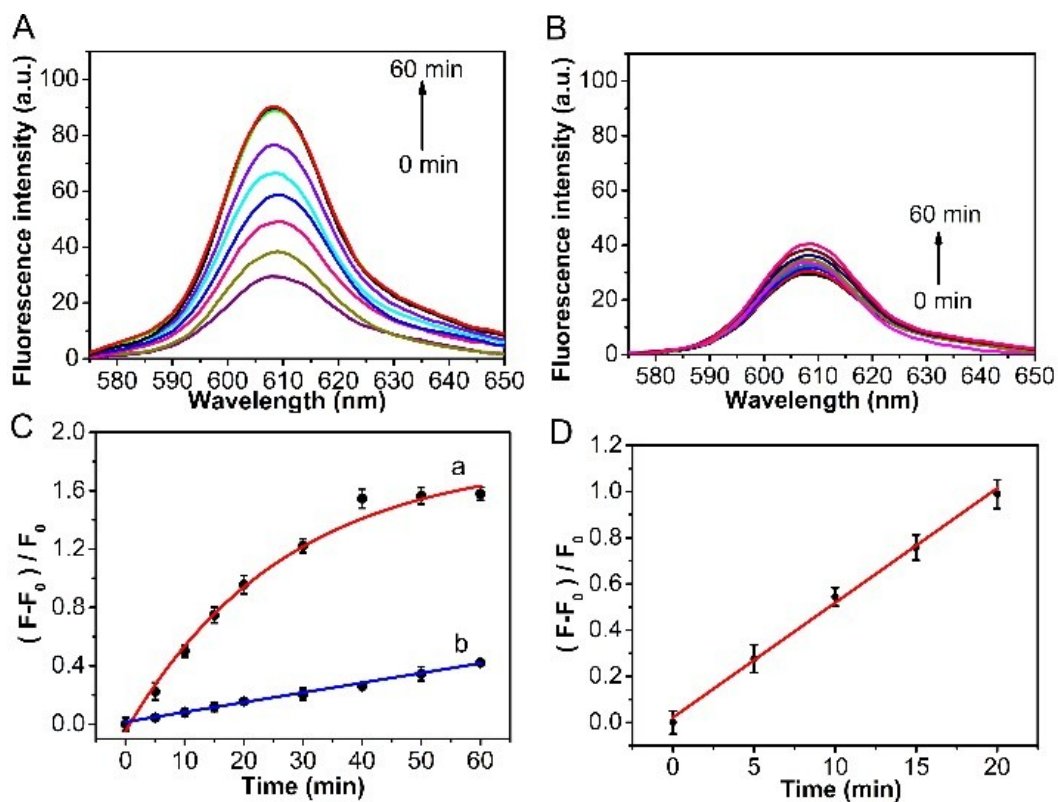
Fig. S5. The time-dependent fluorescence changes of the sensors (A) in the presence and (B) absence of TRP (0.05 μM TS primer extended in 100 HeLa cells). (C) Relationship between fluorescence intensity (at 608 nm) and incubation time with (a) and without(b) TRP. (D) magnification of the plot from 0 to 20 min.The concentration of H1 and H2 were all 0.5 μM.
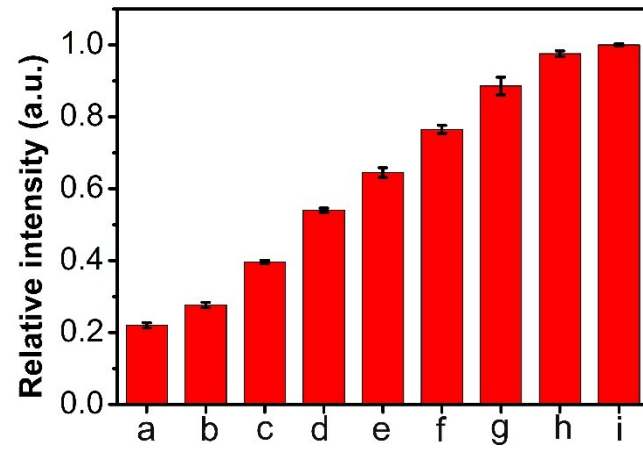
Fig. S6. Relative fluorescence intensity of the H1-H2 (H1 completely hybridized with H2) (i) and different concentrations of telomerase: a h represent the telomerase samples extracted from 0, 25, 50, 100, 200, 400, 1000, 2000 HeLa cells.
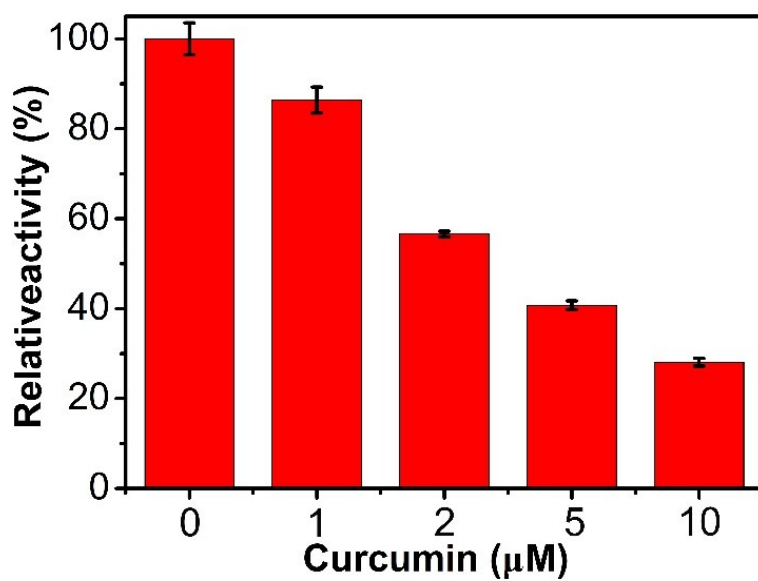
Fig. S7. The inhibition effect for telomerase activities by using different concentrations of curcumin.[H1] = [H2] = 0.5 μM.

Tables:

Table S1. The sequences of the oligonucleotides used in the experiments.

Name	Sequence
TS primer	5'-AATCCG TCGAGC AGAGTT-3'
H1	5'- CCCTAACCCTAAAACTCTACGCAGGGTAGGGCAGAGTTTTAGGG-3'
Fuel H2	5'- AACTCTGCCCTACCCTGCGTAGAGTTTTAGGGTTAGGGATAGGG TAGGGCGGGTTGGG-3'
Synthetic TRP	5'-AGAGTTTTAGGGTTAGGG-3'

Table S2. Comparison between our telomerase assay and other previously reported telomerase activity detection methods.

Method	System	Decetion limit	Time	Ref
PCR based assay	telomeric repeat amplification protocol (TRAP)	10-100 cells	1 day	2,3
Nanoparticle based assay	TS-primer modifiedAuNPs	1 HeLa cell/μL	1.5 h	1
	TS-primer modified UCNP	1 HeLa cell/μL		4
Magnetic and Fluorescence Assay	Polyvalentoligonucleotide nanoparticle conjugates	10 HeLa cells	6.5-7.5 h	5
	Zinc(II)-Protoporphyrin IX/G-Quadruplex labels	380 ± 20 293T cells	3.5h	6
	T7 Exonuclease/taqman probe	5 HeLa cells	3 h	7
Electrochemical Assay	ECL-nanoprobe / TS-primer modified magnetic bead	500 HeLa cells	3 h	8
	Porphyrin-Functionalized Graphene/Ru(bpy)$_3^{2+}$	10 HeLa cells/mL	4 h	9
HCR Assay	Hairpin fluorescence probe	4 HeLa cells	3h	10
	Arch-structure DNA probe	5HeLa cells	~2h	11
	DNA-Fueled molecular machine based assay	1.76 HeLa cells	<2h	**This assay**

Table S3. The turnover number for the target catalytic process in different concentrations of telomerase extracted from HeLa cells. (the fluorescence intensity when treated with the H1-H2 was regarded as 1.)

HeLa cells	0	25	50	100	200	400	1000	2000
Turnover number	0	0.05644	0.17625	0.31982	0.4248	0.54483	0.66551	0.75552

Reference

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