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Electronic Supplementary Information

Three-dimensional DNA walker amplified FRET sensor for detection of telomerase activity based on MnO_2 nanosheets-upconversion nanoparticles sensing platform

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EXPERIMENTAL SECTION

Reagents and Chemicals.

$YbCl_3 \cdot 6H_2O$ (99.9%), $YCl_3 \cdot 6H_2O$ (99.99%), $TmCl_3 \cdot 6H_2O$ (99.99%), glycerol phosphate disodium salt hydrate, 1-octadecene (ODE) (90%), oleic acid (OA) (90%) and $MnCl_2 \cdot 4H_2O$ were purchased from Sigma-Aldrich. Tetramethylammonium hydroxide (TMA OH), 30 wt% H_2O_2, ethylene diamine tetraacetic acid (EDTA), ammonium fluoride (NH_4F), methanol and cyclohexane were obtained from Aladdin-E.com (Shanghai, China). Streptavidin-coated magnetic beads (2.8 μm in diameter) were purchased from Dynal Biotech (Lake Success, NY). All other chemicals used in this work were of analytical grade and used without further purification. Ultrapure water used in all runs was obtained from a Millipore water purification system (18.2 $M\Omega\ cm^{-1}$, Milli-Q). All oligonucleotides were synthesized by Sangon Biotech Inc. (Shanghai, China), and the sequences were listed in Table S1.

Table S1 Oligonucleotide sequences used in this work.

Oligonucleotides	Sequences (5' → 3')
TS primer	AATCCGTCGAGCAGAGTT
Walker strand	Biotin-$(T)_{45}$AGGGTTAGGGTTATCTCTTCTCCGAGCCGGTCGAAATAGT
Locking strand	AAGAGATAACCCTAACCCTAACCCTAAAACTC
Substrate strand	Biotin-$(T)_{14}$ACTATrAGGAAGAGATAGCTAACCTGGCCAATCGAT
Substrate strand-FAM	Biotin-$(T)_{14}$ACTATrAGGAAGAGATAGCTAACCTGGCCAATCGAT-FAM
Complementary strand	TTTTTTTTATCGATTGGCCAGGTTAGCT
Random DNA	GACGAATCGATGCTTAACGCGGTCACG
Trigger strand	GGAAGAGATAGCTAACCTGGCCAATCGAT
One-mismatched trigger	GGAAGAGATAGCT**C**ACCTGGCCAATCGAT
Three-mismatched trigger	GGAAGA**C**ATAGCT**C**ACCTGGC**G**AATCGAT
Four-mismatched trigger	GGAAGA**C**ATAGCT**C**ACCTGGC**G**A**T**TCGAT

Synthesis of $NaYF_4$:Yb,Tm Upconversion Nanoparticles.

The oleic acid (OA) stabilized $NaYF_4$:Yb,Tm UCNPs was prepared according to previous method[1]. In detail, the salts YCl_3 $6H_2O$ (0.78 mmol), $YbCl_3$ $6H_2O$ (0.20 mmol), and $TmCl_3$ $6H_2O$ (0.02 mmol) were mixed with 7 mL oleic acid and 15 mL 1-octadecene in a 100 mL flask. The mixture solution was heated to 150 ℃ under a gentle nitrogen flow and maintained at 150 ℃ for 40 min, resulting in a transparent colorless solution. After cooling down to 50 ℃, 10 mL methanol containing 4.0 mmol NH_4F and 2.5 mmol NaOH was dropwise added into the mixture solution and stirred at 50 ℃ for 40 min. After evaporating the methanol at 100 ℃, the mixture was heated to 290 ℃ for 1.5 h and then cooled down to room temperature. The resulting OA-UCNPs were precipitated with ethanol and redispersed in 5 mL cyclohexane.

Preparation of DNA-conjugated UCNPs.

To conjugate the complementary (CP) DNA strand with UCNPs, ligand-free UCNPs were firstly prepared according to previously reported method[2]. In detail, 1.0 mg of the OA-UCNPs were dispersed in 1.0 mL of 0.1 mM HCl and ultrasonicated for 1 h to remove the OA. The resulting ligand-free UCNPs were then collected by centrifugation at 12000 rpm for 0.5 h, followed by washing with deionized water for three times. Ligand-free UCNPs were re-dispersed in 1.0 mL of water for further use. CP strand were conjugated with ligand-free UCNPs by mixing 100 μL of CP strand (5.0 μM) with 0.5 mL of the above ligand-free UCNPs solution, followed by stirring for 24 h. Subsequently, glycerol phosphate disodium salt hydrate (GDSH) as the blocking reagent was added to the mixture solution to give a final concentration of 100 μM. After stirring for another 24 h, the resulting CP@UCNPs were collected by centrifugation and thoroughly washed with deionized water for three times. Finally, the CP@UCNPs were redispersed in 10 mM Tris-HCl buffer (pH 7.4, containing 50 mM NaCl) and stored at 4 ℃ for further use. The successful conjugation of CP strand with UCNPs was confirmed by testing the characteristic absorption peak of DNA at 260 nm (Fig. S1).

Preparation of MnO_2 Nanosheets.

The MnO_2 nanosheets were synthesized according to previous literature[3]. Briefly, the 20 mL of aqueous solution containing 0.6 M tetramethylammonium hydroxide (TMA•OH) and H_2O_2 (3.0 wt %) was rapidly added to 10 mL of $MnCl_2$•$4H_2O$ (0.3 M) within 15 s. A dark brown solution generated immediately, and was agitated intensely overnight at RT to ensure the sufficient oxidation in air. The resulting bulk MnO_2 was collected by centrifuging at 2000 rpm for 15 min, followed by washing with ultrapure water and methanol alternately for several times. Subsequently, 25 mg bulk MnO_2 was redispersed in 20 mL of ultrapure water and sonicated for 10 h. The residual unexfoliated bulk MnO_2 was removed by centrifugation at 2000 rpm for 30 min, and the supernatant containing MnO_2 nanosheet was collected for further use. The concentration of MnO_2 nanosheets determined by ICP-OES was 5 mg L^{-1}. The MnO_2-UCNPs complexes suspension was prepared by directly mixing the CP@UCNPs with MnO_2 nanosheets under the aid of vortex.

Preparation of DNA Walker-Magnetic Beads (MBs).

Firstly, the double-stranded DNA walker scaffold was prepared by annealing the walking strand (1 μM) with the locking strand (1 μM) at 95 ℃ for 3 min in 5 mM Tris-HCl buffer (pH 7.4, containing 10 mM $MgCl_2$ and 0.1 M NaCl), followed by cooling slowly to room temperature over a 10-min period. Before conjugation, streptavidin (SA)-coated MBs were thoroughly washed using 5 mM Tris-HCl buffer (pH 7.4, containing 0.5 mM EDTA and 1 M NaCl). The SA-MBs were then resuspended in 500 μL of above-mentioned Tris-HCl buffer, followed by injecting 500 μL of 5 nmol DNA walker scaffold and substrate strand. After incubation at room temperature for 15 min, the unbound walker scaffold and substrate strand were removed via magnetic separation. The resulting DNA walker-MBs were thoroughly washed with buffer solution for 3 times. Finally, the DNA walker-MBs were resuspended in 500 μL of buffer solution and stored at 4 ℃ for further use.

Preparation of Telomerase Extract.

HeLa cells were cultured in DMEM medium supplemented with 10% fetal calf serum at 37 ℃ in a humidified atmosphere (95% air and 5% CO_2). Cells were collected in the exponential phase of growth and counted with a hemocytometer. About 1 × 106 cells were collected and dispensed in a 1.5mL EP tube. After washing twice with ice-cold PBS (0.1 M, pH 7.4), these cells were resuspended in 200 μL of ice-cold CHAPS lysis buffer (10mM Tris-HCl, pH 7.5, 1mM $MgCl_2$, 1mM EGTA, 0.1mM PMSF, 0.5% CHAPS, 10% glycerol). The lysate was incubated for 30 min on ice and then centrifuged at 16,000 rpm for 30 min. The supernatant was collected and transferred into a 1.5mL EP tube, and used immediately for telomerase assay or frozen at -80 ℃.

Detection of Telomerase Activity.

20 μL of telomerase extract was added into 30 μL of extension reaction buffer containing 20 mM Tris-HCl buffer (pH 8.3, 4 mM $MgCl_2$, 1 mM EGTA, 63 mM KCl, 0.05% Tween 20 and 2.5 mM dNTPs), and 20 nM TS primer. After incubation at 37 ℃ for 1 h, the elongation reaction was terminated by heat denaturing at 95 ℃ for 15 min. For the negative control experiments, the telomerase extracts were first heated at 95 ℃ for 10 min. Subsequently, the reaction solution was mixed with 50 μL of DNA walker-MBs and 50 μL of $MnCl_2$ solution with certain concentration. After incubation at 37 ℃ for 30 min, the MBs were removed by magnetic separation and the supernatant was transferred into a cuvette containing 50 μL of MnO_2-UCNPs complexes suspension. After incubation at 37 ℃ for another 25 min, the luminescence of the nanosystem was measured using the F-4600 fluorescence spectrophotometer (Hitachi Co., Japan) equipping with a 980 nm laser (200 mW) as the excitation source.

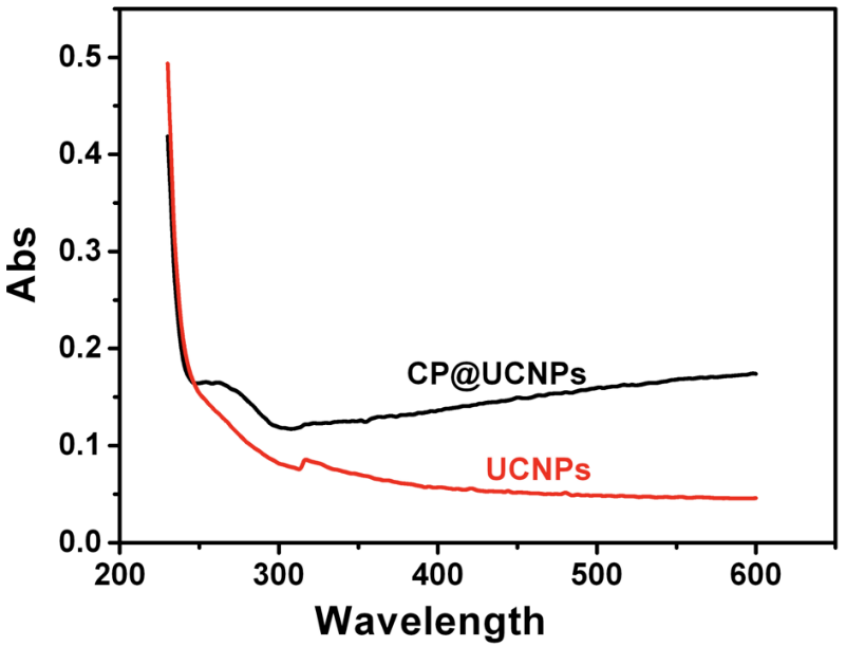

Fig. S1. UV-vis absorption spectra of UCNPs (red line) and CP@UCNPs (black line).

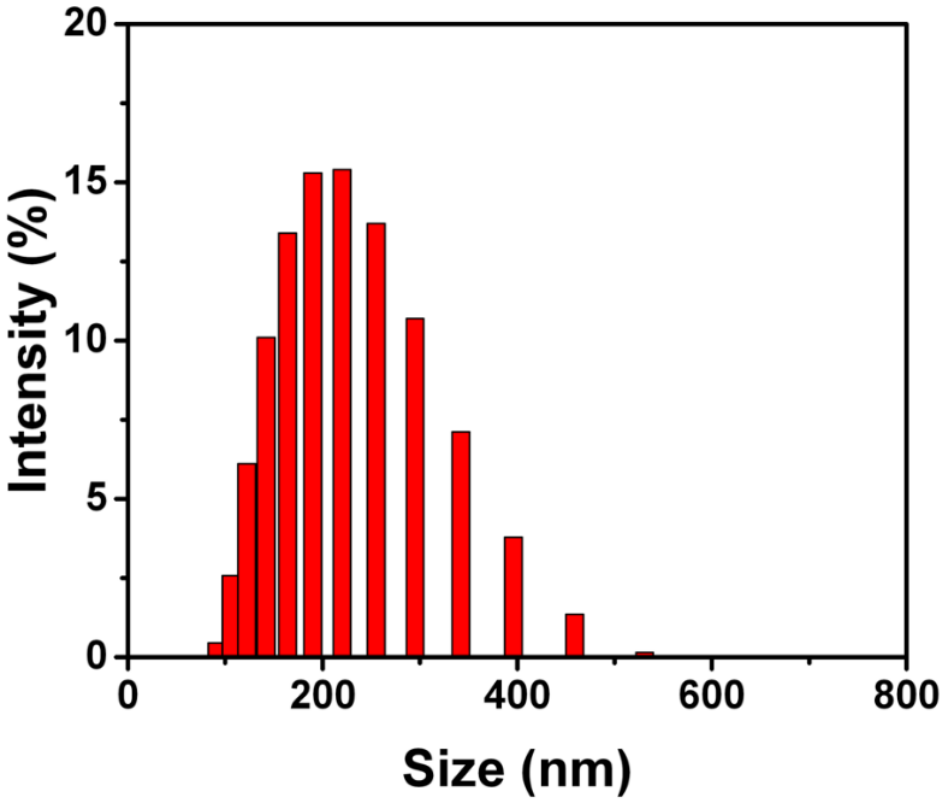

Fig. S2. DLS data of MnO_2 nanosheets.

Characterization of the MnO_2-UCNPs FRET system

The assembly of CP@UCNPs with MnO_2 nanosheets after mixing was characterized by TEM. As show in Fig. S3A, the CP@UCNPs were well anchored on the surface of MnO_2 nanosheets. The element distribution on the MnO_2-UCNPs complexes was also confirmed by EDS mapping (Fig. S3B). These results indicated that the CP@UCNPs could uniformly assemble on the MnO_2 nanosheets. This assembly reaction is mainly ascribed to van der Waals forces between MnO_2 and DNA nucleobases. Fig. S3C displays the UV-vis absorption spectrum of MnO_2 nanosheets (red line). It was observed that the MnO_2 nanosheets possessed a broad absorption band from 250 to 500 nm, matching well with previously reported results[3b]. Significantly, the absorbance spectrum of MnO_2 nanosheets overlapped well with the fluorescence emission of $NaYF_4$:Yb,Tm UCNPs at 450 nm and 479 nm (blue line in Fig. S3C), which may lead to the FRET from UCNPs to the MnO_2 nanosheets. As shown in Fig. S3D, the fluorescence emission of $NaYF_4$:Yb,Tm UCNPs decreased with the increasing concentrations of MnO_2 nanosheets (in the range from 0 mg mL^{-1} to 1.2 mg mL^{-1}), indicating that the MnO_2 nanosheets could quench the fluorescence of UCNPs due to the FRET effect. Remarkably, A 90% reduction in fluorescent intensity could be readily achieved by adding 1.0 mg mL^{-1} MnO_2 nanosheets. These results revealed that the MnO_2-UCNPs FRET system was successfully established.

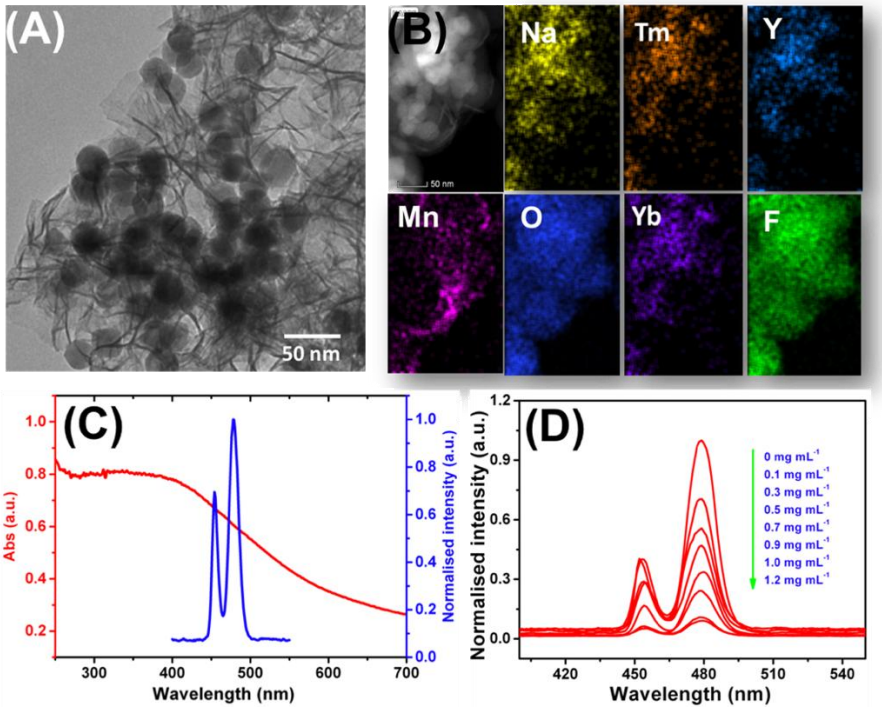

Fig. S3. TEM (A) and EDS mapping (B) of MnO_2-UCNPs complexes; (C) UV-vis absorption (red line) and fluorescence spectra (blue line) of $NaYF_4$:Yb,Tm UCNPs; (D) Fluorescence quenching of the CP@UCNPs (0.03 mg mL^{-1}) by varying amounts of the MnO_2 nanosheet.

Specificity of the Trigger Strand to the Disintegration of MnO_2-UCNPs System.

To illustrate the fact that the disintegration of MnO_2-UCNPs system and the following recovery of fluorescence signal were dependent on the hybridization of trigger strand with CP strand, we challenged the MnO_2-UCNPs system with mismatched trigger strand, proteins, anionic species and random DNA. As shown in Fig. S4, the respective incubation of Cl^- (200 mM), BSA (100 mg mL^{-1}) and random DNA with the MnO_2-UCNPs system gave rise to no significant fluorescence signal compared with the background signal. In addition, the trigger strands with base mismatch aroused relatively weaker fluorescence signals compared with that caused by perfectly matched trigger strand. Importantly, more mismatched bases in the trigger strand resulted in weaker fluorescence signals. These results indicated that the response signal generated by the MnO_2-UCNPs system was highly dependent on the trigger strand and not interfered by other extraneous components.

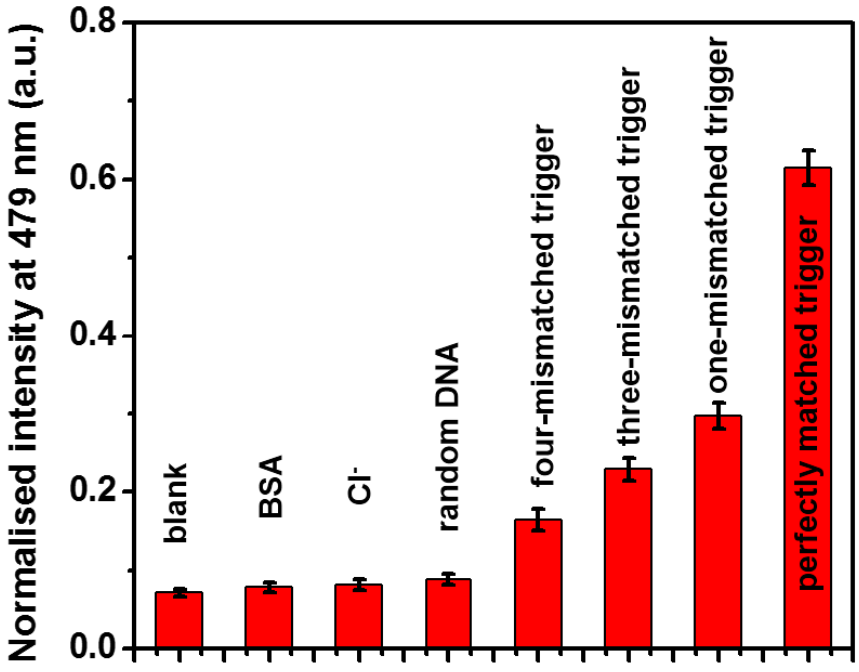

Fig. S4. Specificity of the trigger strand to the diintegration of MnO_2-UCNPs system.

Optimization of Experimental Conditions.

To achieve an optimal analytical performance of the 3D DNA walker-based FRET sensing method, several experiment conditions including the reaction time for telomerase-catalyzed extension reaction, the incubation time of extension strand with DNA walker-MBs, the concentration of Mn^{2+} as cofactor and the incubation time of trigger strand with MnO_2-UCNPs complexes were investigated (350 Hela cells used as an example). To comprehensively consider the effect of these parameters on both response signal and background signal, the signal-to-background (S/B) ratio was chosen as the test criterion. As shown in Fig. S5A, the S/B ratio increased with the increasing extension reaction time, and a plateau was reached after 60 min. Thus, 1 h was used as the elongation reaction time. Fig. S5B shows the effect of the concentration of Mn^{2+} on the fluorescence signal from 0.2 mM to 1.8 mM. It was observed that the S/B ratio values increased with the increscent concentrations of Mn^{2+} and tended to level off after 1.2 mM. Therefore, 1.2 mM Mn^{2+} was employed as the cofactor for DNAzyme. Next, the optimal incubation time of extension strand with DNA walker-MBs was studied. As seen from Fig. S5C, the S/B ratio values increased with the increasing incubation time and a plateau was reached after 30 min. Hence, 30 min was used as the incubation time of extension strand with DNA walker-MBs. In a similar way, the optimal incubation time of trigger strand with MnO_2-UCNPs complexes was determined to be 25 min (Fig. S5D).

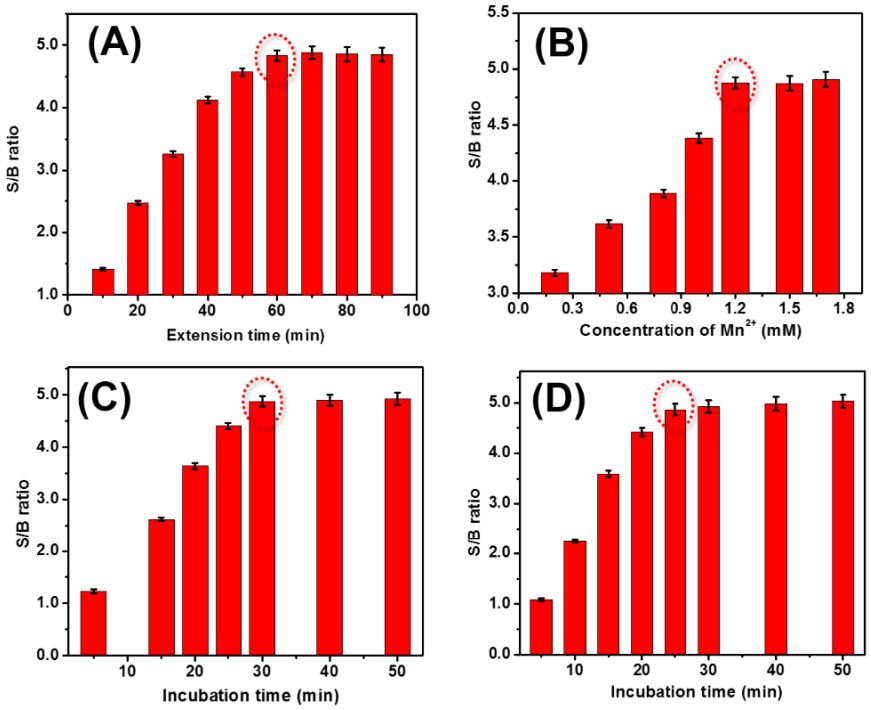

Fig. S5. Effects of (A) extension time, (B) concentration of Mn^{2+}, (C) incubation time of extension strand with DNA walker-MBs and (D) incubation time of trigger strand with MnO_2-UCNPs complexes on the S/B ratio of the developed sensing platform. Telomerase extracts from 350 HeLa cells were used in these cases.

Analytical Performance of the Sensing Platform Using FAM as the Signal Tag.

To demonstrate the signal amplification ability of the MnO_2-UCNPs system, the analytical performance of the sensing platform toward telomerase activity using FAM as the signal tag was investigated. Fig. S6 displayed the linear relationship between the response signals and the logarithm of HeLa cells numbers in the range from 250 to 1200. The regression equation could be expressed as y = 0.4373 lgx – 0.835 (y: the fluorescence intensity, x: the number of HeLa cell, $R^2 = 0.9916$), and the detection limit was calculated to be 187 Hela cells. Significantly, the sensing platform using MnO_2-UCNP as the signal tag was of more excellent assay performance than that using FAM as tag.

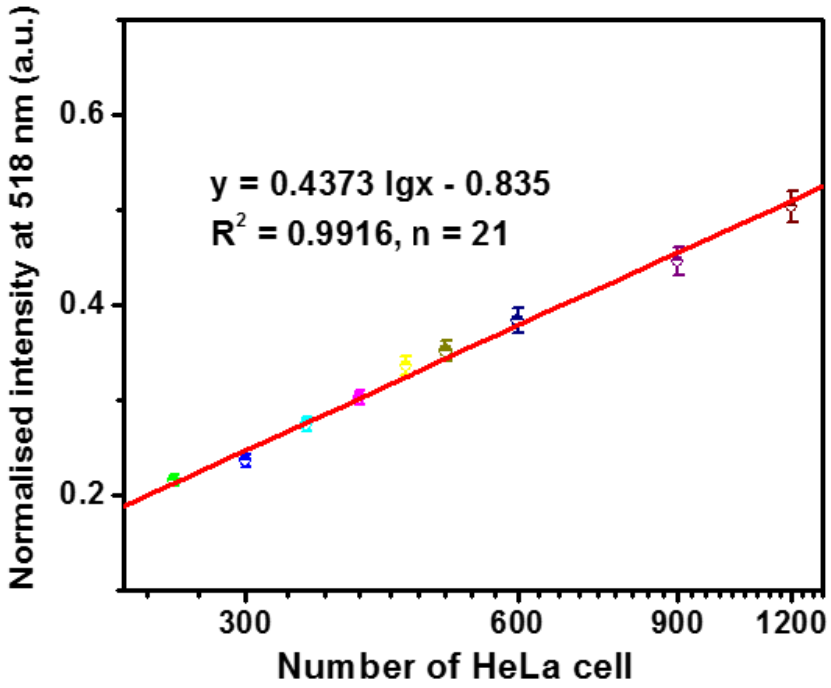

Fig. S6. The corresponding calibration curve of the sensing platform toward telomerase using FAM as the signal tag.

Detection of Telomerase Activity in Urine Sample.

Urinary telomerase has been proved to be a promising biomarker for the prognosis and diagnosis of bladder cancer[4]. Therefore, we further investigated the feasibility of developed sensing platform in complicated biological matrixes by conducting the recovery experiment in spiked human urine samples. Firstly, five cell extracts samples achieved from different number of HeLa cells were spiked in 10% human urine collected from normal individuals. The artificially positive urine samples were then test by using the developed sensing platform. As shown in Table S2, the recoveries varied from 92.3% to 105.7%, while the relative standard deviation (RSD) varied from 1.5% to 3.6%. These results clearly demonstrated the capability of this sensing platform to accurately assay the telomerase activity in biological samples.

- **Table S2** Determination of telomerase activity in artificially positive urine samples by using the developed sensing platform method.

Samples	Spiked (number of HeLa cells)	Found (number of HeLa cells)	Recovery (%)	RSD (%, n = 3)
1	50	46.2	92.3	3.3
2	100	94.7	94.7	2.9
3	500	528.5	105.7	3.6
4	800	776.8	97.1	2.5
5	1500	1537.5	102.5	1.5

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