

Supporting Information

Experimental Section

Reagents and Materials:

$\text{Y}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, $\text{Yb}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$, $\text{Er}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$, ethylene glycol (EG), Poly (acrylic acid) (PAA, $\text{mw} \approx 1800$), sodium cyanoborohydride (NaCNBH_3), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), sodium dodecyl sulfate (SDS), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC), and N-hydroxysuccinimide (NHS) were from Sigma Aldrich company. Sodium chloride (NaCl), ammonium fluoride (NH_4F), sodium periodate (NaIO_4), Lithium chloride (LiCl) were from Aladdin. All buffer solutions were prepared using a deionized water purification system (Milli-Q, $18 \text{ M}\Omega\text{cm}^{-1}$) and were autoclaved prior to use. The buffer solutions included: borate buffer (BB buffer) (50 mM, $\text{pH}=9.25$), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer (100 mM, $\text{pH}=6.0, 8.0$), Tris-HCl buffer (20 mM, $\text{pH}=8.0$) containing MgCl_2 (20 mM). The oligonucleotide used in this context was synthesized by Sangon Biotechnology Inc. (Shanghai, P.R. China). The sequences were as follows. $\text{NH}_2\text{-TS}$: $5'\text{-NH}_2(\text{CH}_2)_6\text{TTTTTTAATCCGTCGAGCAGAGTT-3'}$, $\text{NH}_2\text{-TC}$: $5'\text{-NH}_2(\text{CH}_2)_6\text{AAAAACCCTAACCCTAACCCTAACCCT-3'}$. The oligonucleotide sequences were dissolved in sterile Milli-Q water and stored at -20°C . All DNA binders and other chemicals were purchased from Sigma-Aldrich and used without further purification.

Apparatus and characterization:

UV-Vis absorbance measurement was carried out on a JASCO V-550 UV-vis spectrophotometer with a Peltier temperature control accessory. FT-IR spectra were carried out on a BRUKE Vertex 70 FT-IR spectrometer. The Zeta-potential was measured in a Zetasizer 3000HS analyzer and each value of zeta-potential was acquired by averaging three independent measurements. Scanning electron microscopic (SEM) images were recorded using a Hitachi S-4800 Instrument (Japan). Transmission electron microscopic (TEM) images of cells were captured with a FEI TECNAI G2 20 high-resolution transmission electron microscope operating at 200 kV. The UCL spectra were recorded by ACTON SpectraPro 2750 with equipped with diode 980 laser at RT.

Synthesis of Carboxylated UCNPs: The PAA-UCNPs was synthesized by the “one pot” method. Briefly, 0.21 g NaCl was dissolved in about 20 mL EG, then the solution was mixed with $\text{Y}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$, $\text{Yb}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$, $\text{Er}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$ (1.56 mmol, 0.4 mmol, 0.4 mmol respectively). After that the mixture was agitated at least 20 min to get a transparent and homogeneous solution. Subsequently, 18 mL of EG containing a stoichiometric amount of NH_4F was added to the above solution. The resulting solution was stirred for another 20 min, then transferred to a 40 mL of Teflon-lined autoclave, and heated at 200°C for 12 h. The product was centrifuged at

10000 rpm and washed with ethanol and deionized water 3 times. The obtained nanoparticles were dried in an oven at 37 °C.

Preparation of TC Modified UCNPs: The TC were conjugated to PAA-UCNPs via EDC-NHS mediated amide bond formation. 1 mL UCNPs solution (1 mg/mL) was mixed with 4mg EDC and 2 mg NHS and oscillated vigorously in MES buffer (pH=6.0). The mixture was incubated at room temperature for 18 hours and for another 18 hours after added TC. The products were separated by centrifugation at 10000 rpm. To remove excess unreacted DNA , EDC and NHS, The conjugates were redissolved in Tris-HCl buffer and centrifuged as above twice. The obtained TC-UCNPs conjugates was washed by deionized water 2 times and dissolved in 1 mL buffer. The solution was stored at 4°C prior to use.

Preparation of TS Modified Paper: Firstly, prepare the oxide solution: 0.3 g lithium chloride (LiCl) and 0.84 g sodium periodate (NaIO₄) were dissolved in 150 mL water. Filter paper samples (20 mm × 60 mm) were immersed in the oxide solution (15 mL) and the temperature of the solution was adjusted to 60 °C for 60 min. The paper was then dried in an oven at 37 °C after rinsed twice with deionized water, then the treated paper samples were sterilized. After that, the paper samples were cut into small pieces (5mm × 5mm). A low-volume HEPES buffer solution (2 μL, 50 mM, pH=8.0) containing NH₂-TS (3 μM) and NaCNBH₃ (50 mM), was pipetted on the small piece paper. The paper was incubated for 1 h at 37 °C kept under humidity and then washed

with BB buffer (containing 0.1% sodium dodecyl sulfate) and water for several minutes. Then, the 5 μ L 1 \times TRAP buffer, (20 mM Tris-HCl pH 8.3, 1.5 mM MgCl₂, 63 mM KCl, 0.005% Tween 20, 1 mM EGTA, BSA 0.1 mg/mL) and 1 mM dNTP mixtures were dropped on the paper and lyophilized.

Cell Culture and Telomerase Extract Preparation: Briefly, HEK-293T cell were cultured in Dulbecco's modified Eagle's medium (DMEM) medium supplemented with 10% fetal calf serum and the cells were maintained at 37 °C in a humidified atmosphere (95% air and 5% CO₂). Cells were collected in the exponential phase of growth and 1 \times 10⁶ cells were dispensed in a 1.5 mL Eppendorf (EP) tube, washed twice with ice-cold phosphate buffered saline (PBS), and resuspended in 100 μ L of ice-cold lysis buffer (10 mM Tris-HCl, pH=7.5, 1 mM MgCl₂, 1 mM ethylene glycol tetraacetic acid (EGTA), 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS), 5 mM β -mercaptoethanol, 10% glycerol). The CHAPS lysis buffer was pretreated with RNA secure according to the manufacturer's instructions. The lysate was incubated for 30 min on ice and centrifuged 20 min at 12000 rpm, 4°C, to pellet insoluble material. Without disturbing the pellet, the cleared lysate was carefully transferred to a fresh 1.5 mL EP tube. The lysate was used immediately for telomerase assay or frozen at -80 °C.

Telomerase Extension Reaction: The telomerase extracts were diluted in lysis buffer with respective number of cells; the extracts (5 μ L) were diluted to 50 μ L with RNA secure pretreated extension solution. Then a low-volume reaction solution prepared above (2 μ L) was placed on the TS primer modified paper. The paper was incubated for 60 min at 37 °C kept under humidity for the telomerase extension reaction. After extension reaction, the paper should be washed with Tris-HCl buffer (20 mM, pH=8.0) 5 times, then with water 5 times. For negative controls, telomerase extracts were heat-treated at 95 °C for 5 min. For inhibition of telomerase by the G-quadruplex ligand, indicated TMPyP₄ and [Ni₂L₃]Cl₄-P were mixed with telomerase extract equivalent to 250 HEK-293T cells/ μ L.

Detection of telomerase activity: After telomerase extension reaction, 4 μ L the prepared TC-UCNPs solution (containing 1% PEG used to suppress the physical absorption of UCNPs to the surface of paper) was pipetted on the small piece paper smoothly. After that the paper was kept at 37 °C for 15 min in a humidified atmosphere to hybridization, followed by washing with fresh hybridization buffer 5 times. It maybe not easy for UCNPs to quickly and completely pass through the pores of the paper substrate, the paper was first inverted and the wash buffer was then introduced. A soft tissue paper was placed under the paper strip to promote fluid flow through the paper due to wicking. After dried, the fluorescence of the substrate was recorded by a camera with the help of a portable NIR laser.

Figure S1

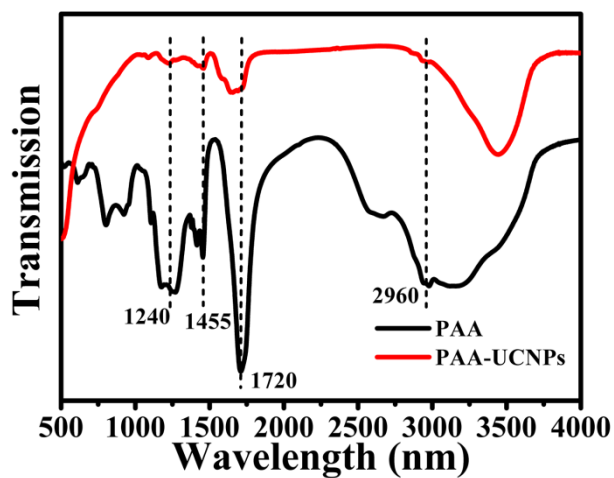


Figure S1. FT-IR spectra of PAA and PAA-UCNPs. The C-H stretching (2960 cm^{-1}), C=O stretching (1720 cm^{-1}), C-H bending (1455 cm^{-1}), C-C skeletal vibration (1240 cm^{-1}) indicated the PAA has been successfully introduced on the surface of UCNPs.

Figure S2

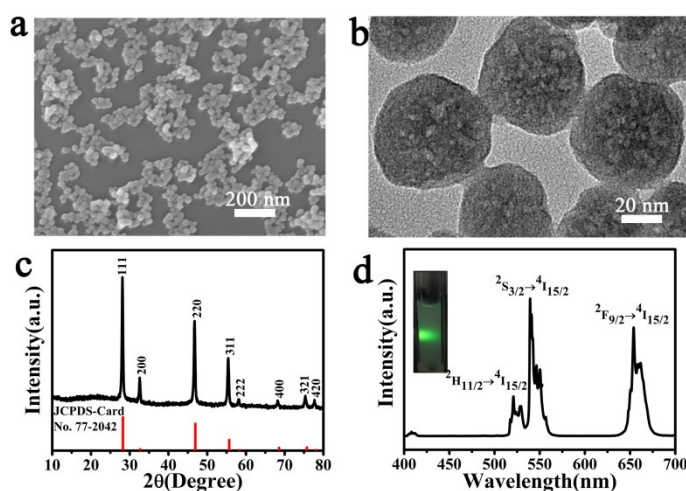


Figure S2. a, b) As shown in the scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images, the PAA-UCNPs nanoparticles were uniform and their average diameter was about 60 nm. c) The diffraction peaks in the X-ray diffraction (XRD) pattern could be clearly assigned to the NaYF₄ of cubic phase (JCPDS, Card No. 77-2042). d) Upon irradiated with 980 nm light, the UCNPs emitted photons around at 524, 544, and 655 nm, which was the characteristic emission peaks of Er³⁺. When the NIR laser traveled through the UCNPs colloidal solutions, the yellow-green photoluminescence was also readily observed (Figure S2d inset). This light is sensitive to our eyes and suitable for visual detection.

Figure S3

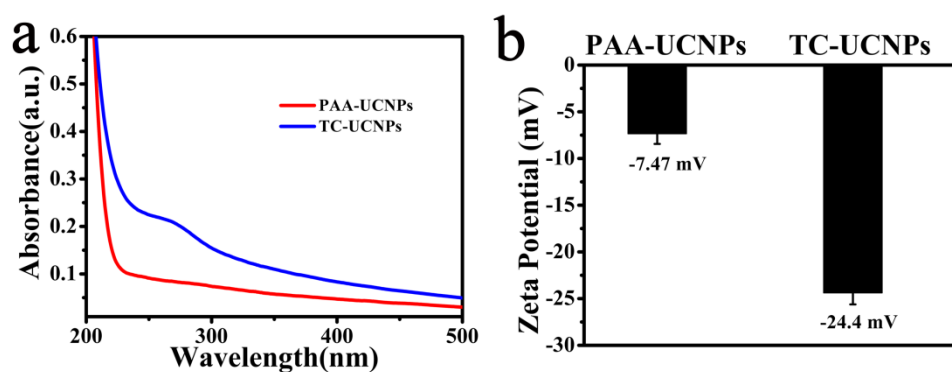
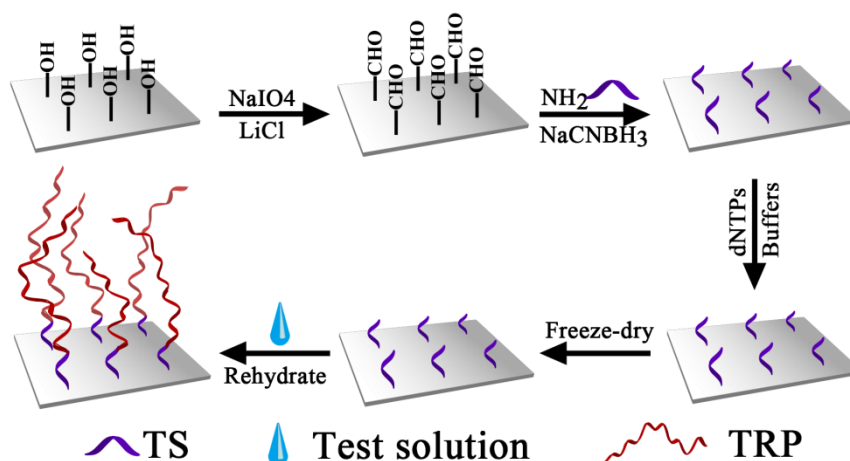


Figure S3. a) The UV-vis spectrum of the PAA-UCNPs and TC-UCNPs. The absorption band centered at 260 nm in DNA-UCNPs (blue line) shows the successful linkage of DNA with UCNPs. b) Zeta Potential of the PAA-UCNPs and TC-UCNPs. The decrease of Zeta Potential of UCNPs-DNA shows the successful linkage of DNA with UCNPs.

Scheme S2



Scheme S2. Synthetic steps of surface modification of the paper with TS-NH₂ and treatment of the paper platform.

Figure S4

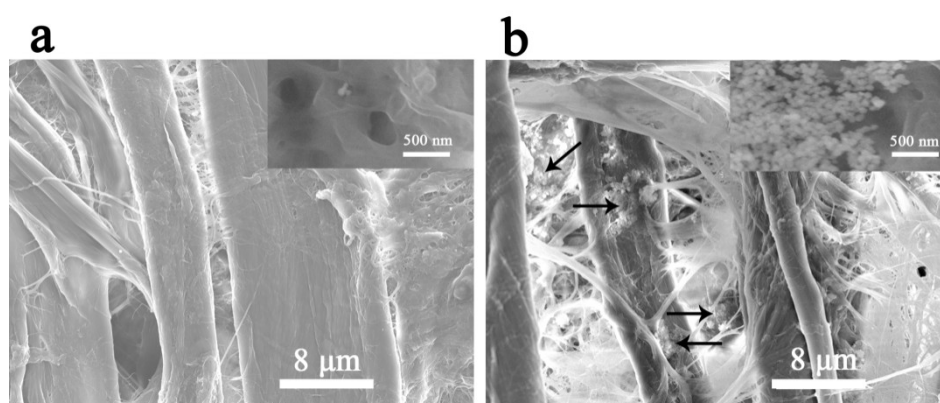


Figure S4. SEM image of the paper substrates dealt with blank solution (a) and test solution (b). Inserts were the magnified SEM images of the paper surface

Figure S5

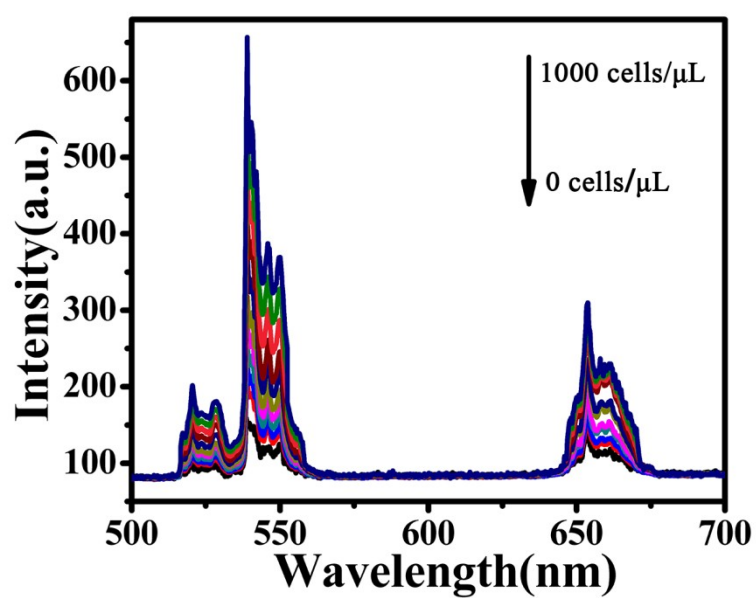


Figure S5. The upconversion luminescence spectra of the substrates that treated with different concentration of telomerase.

Figure S6

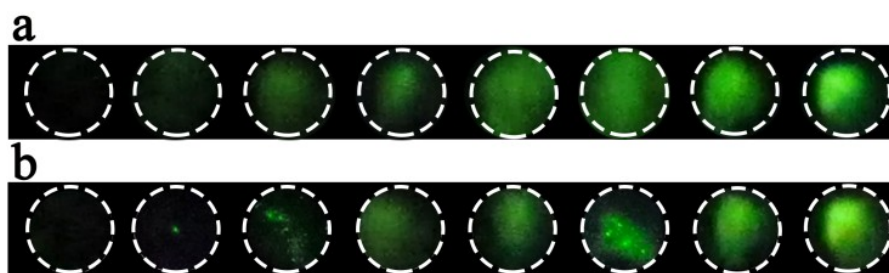


Figure S6. Visual photo images of the paper substrate got before (a) and after (b) one month respectively incubated with telomerase extract from different concentrations of HEK-293T cells: 0, 1, 10, 50, 100, 250, 500, 1000 cells/ μ L.

Figure S7

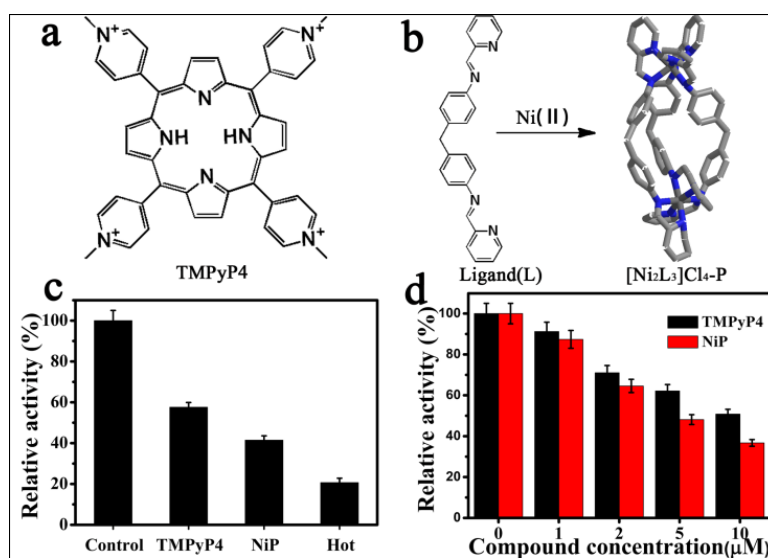


Figure S7 a, b) The chemical structures of TMPyP4 and [Ni₂L₃]Cl₄-P. c) Assess the inhibition of telomerase activity by G-quadruplex ligand TMPyP4, [Ni₂L₃]Cl₄-P and heat with our assay. d) The inhibition effect of TMPyP4 and [Ni₂L₃]Cl₄-P at different concentrations.

Table S1. The IC₅₀ of [Ni₂L₃]Cl₄-P and TMPyP4 measured with our paper-based method and the TRAP assay.

Compounds	IC ₅₀ (nM)	
	In This Work	TRAP ^[1]
[Ni ₂ L ₃]Cl ₄ -P	4000	89
TMPyP4	10000	658

[1] J. Wang, L. Wu, J. Ren, X. Qu, *Small* **2012**, 8, 259-264.