

## Supporting Information

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### 3 **Fluorescence Detection of Telomerase Activity in High Concentration** 4 **of Cell Lysates Based on Strand-displacement Mediated Recycling**

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**Table S1. The Sequences of the DNA in Scheme 1**

DNA	Sequence
Hairpin DNA	5'-NH <sub>2</sub> -TTT TTT TCT TGG ACA CAC TAA CCC TAA CCC TAA CCC TAA CTC TGC TCG ACG GAT TTG TGT CCA AGA-3'
Fluorescent DNA	5'-FAM-TTT TTT CCT AGC GAC-NH <sub>2</sub> -3'
Primer 1	5'-AAT CCG TCG AGC AGA GTT-3'
Primer 2	5'-NH <sub>2</sub> -TTT TCT TGG ACA CA-3'

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**3 2. Experimental section****4 2.1. Materials**

5 Carboxyl-coated MBs and Carboxyl-coated uniform polystyrene microsphere (PSM) were  
6 ordered from Tianjin Baseline ChromTech Research Centre. Telomerase was obtained from  
7 Shanghai Biotechnology Co., Ltd. Deoxynucleoside triphosphate (dNTP) and Klenow DNA  
8 polymerase were obtained from Dalian treasure biological engineering Co., Ltd. All the reagents  
9 were analytical grade and used without further purification. Phosphate buffer saline (PBS, pH 7.4)  
10 contained 0.03 M NaCl, 0.1 M NaH<sub>2</sub>PO<sub>4</sub>, 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, 0.1 M imidazole buffer (pH 6.0)  
11 were prepared by standard methods. Deionized and autoclaved water was employed to prepare all  
12 solutions. All aqueous solutions were of analytical grade. DNA sequences were purchased from  
13 Sangon Biological Engineering Technology & Co., Ltd. (Shanghai, China). Sequences of the  
14 oligos are listed in Table 1.

**15 2.2. Cells**

16 HeLa cell was cultured in DMEM (GIBCO) medium supplemented with 10% fetal bovine  
17 serum (FBS) and 100 IU mL<sup>-1</sup> penicillin-streptomycin. The cells were maintained at 37 °C in a  
18 humidified atmosphere (95% air and 5% CO<sub>2</sub>). The cancer cells densities were determined by  
19 using a hemocytometer, and this was performed prior to any experiments.

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### 1 2.3. Instrument

2 All fluorescence measurements were performed on an A Hitachi F-4600 fluorescence  
3 spectrophotometer (Tokyo, Japan) with excitation wavelength set at 490 nm. The slit width was 10  
4 nm for both excitation and emission. The transmission electron microscopic (TEM) images were  
5 obtained on a JEM-2100 transmission electron microscope (JEOL Ltd.,Japan). The pH of all  
6 solutions was measured by a Model pHs-25 digital acidometer (Rex Electric Chemical Products  
7 Department, Shanghai Precision Scientific Instrument Co., Ltd.). Adjustable pipettes were from  
8 Thermoelectric instruments co., LTD., Shanghai. TG18KR Centrifuge came from Dong Wang  
9 Instrument (Hu nan,China).All glassware used in the experiment are immersed in a chromic acid  
10 solution, washed twice with deionized water and after drying use.

### 11 2.4. Preparation of PSM-FL signal NPs.

12 Firstly, 10  $\mu\text{L}$  of carboxyl-coated PSM were transferred into a 1.5 mL Eppendorf tube and  
13 were washed three times with 100  $\mu\text{L}$  PBS (10 mM phosphate, 0.1 M NaCl, pH 7.4) and then  
14 activated in 150  $\mu\text{L}$  0.1 M imidazole buffer (pH 6.0) containing 0.2 M EDC with gentle shaking  
15 for 90 min. Secondly, the PSMs were resuspended in 50  $\mu\text{L}$  pH 7.4 PBS, then 25  $\mu\text{L}$  100 nM  
16 Primer 2 and 50  $\mu\text{L}$  100 nM of FL DNA were added. The mixture solution was incubated at 37 °C  
17 overnight with gentle shaking in dark. The formed PSM-FL signal NPs were separated from the  
18 incubation solution and washed three times with 100  $\mu\text{L}$  PBS, then kept in an ice bath at 4 °C.

### 19 2.5.Preparation of hairpin DNA magnetic nanoprobe.

20 The MBs, as well as the PSMs, were synthesized according to a previous work with a light  
21 modification.<sup>[1]</sup> Firstly, 50  $\mu\text{L}$  of carboxyl-coated MBs were transferred into a 1.5 mL Eppendorf  
22 tube and were washed three times with 100  $\mu\text{L}$  PBS (10 mM phosphate, 0.1 M NaCl, pH 7.4)  
23 while physically retaining the particles with a magnetic field and then activated in 150  $\mu\text{L}$  of 0.1  
24 M imidazole buffer (pH 6.0) containing 0.2 M EDC with gentle shaking for 90 min. Secondly, the  
25 MBs were resuspended again in 100  $\mu\text{L}$  pH 7.4 PBS , and then 100  $\mu\text{L}$  500 nM hairpin DNA was  
26 added. The mixture solution was incubated for 24 h at 37 °C with gentle shaking in dark. The  
27 formed hairpin DNA magnetic nanoprobes were separated from the incubation solution and  
28 washed three times with 100  $\mu\text{L}$  PBS, then kept in an ice bath at 4 °C.

1 *2.6. Telomerase Extracted from Cultured Cells*

2 Cells were collected in the exponential phase of growth, and  $5.8 \times 10^5$  cells were dispensed in  
3 a 1.5 mL EP tube (3500 rpm for 5 min) in culture medium, washed twice with ice-cold PBS (0.1 M,  
4 pH 7.4), and resuspended in 200  $\mu$ L of ice-cold CHAPS lysis buffer containing 0.5% CHAPS, 10  
5 mM Tris-HCl, pH 7.4, 1 mM  $MgCl_2$ , 1 mM EGTA, 5 mM  $\beta$ -mercaptoethanol, 0.1 mM PMSF,  
6 10% glycerol. The mixture was incubated on ice for 30 min and centrifuged at 16,000 rpm for 20  
7 min at 4 °C. The supernatant was collected carefully as cell extract for analysis or stored in liquid  
8 nitrogen at -80 °C.

9 *2.7. Telomerase kit detection.*

10 50  $\mu$ L sample solution or telomerase standard solution was added in the 96 wells of ELISA  
11 plate, and incubated at 37 °C for 30 min. After washing with wash solution, 50  $\mu$ L solution of  
12 labeling reagent (from the kit) was added to the each well, and the mixture was incubated at 37 °C  
13 for 30 min. Then the medium was removed, and 50  $\mu$ L of color development agent A and 50  $\mu$ L of  
14 color development agent B (from the kit) was added to the each well. After the plate was vibrated  
15 for 15 min at 37 °C, 50  $\mu$ L of stop buffer was added to each well to stop the color reaction. The  
16 absorbance of each well was measured at 450 nm using a BioTek ELx800 microplate  
17 reader (USA) [2]

18 *2.8. Determination of the telomerase activity.*

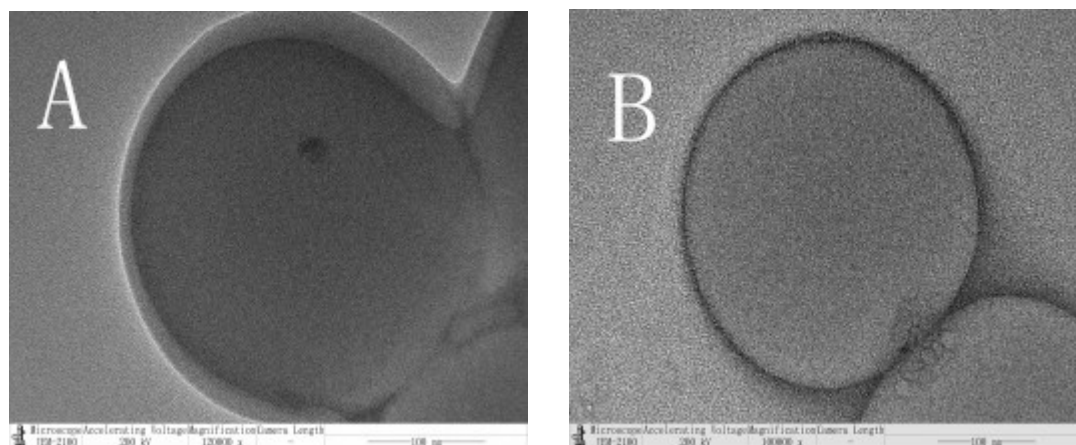
19 Hairpin DNA magnetic nanoprobe, Primer 1 (10  $\mu$ L, 100 nM), dNTPs (10  $\mu$ L, 10 mM), and  
20 different concentration of telomerase (10  $\mu$ L) were added in 200  $\mu$ L extension solution at 37 °C  
21 for 3 h with gentle shaking in dark. After the telomerization reaction, the formed PSM-DNA FL  
22 signal NPs were incubated to the preceding reaction system, and was incubated at 37 °C for 2 h  
23 with gentle shaking in dark. Then 4 U polymerase Klenow Fragment and dNTPs (10  $\mu$ L, 10 mM)  
24 were added to the mixture solutions, the mixture system was incubated at 37 °C for 2 h. The  
25 magnetic nanoprobe was separated from the incubation solution and washed three times with  
26 100  $\mu$ L PBS. The 10  $\mu$ L hydrochloric acid solution (hydrochloric acid: water=1:6) were added in  
27 the mixture solution at 37 °C for 30 min with gentle shaking in dark before fluorescence detection.

28

1 2.9. Characterization of PSM and PSM-FL signal NPs

2 The as-prepared PSM and PSM-FL signal NPs were characterized by transmission electron  
3 microscopy (TEM). A uniform spherical crystallite film formed with the carboxyl groups  
4 modified on the surface of PSM could be seen as shown in Figure S1A. The spherical crystallite  
5 film disappeared after the amide signal DNA was attached to the PSM with the formation of  
6 amido band between the PSM and FL signal DNA as shown in Figure S1B. The result showed that  
7 the PSM-FL signal NP was prepared as expected. The diameter of MB is 500 nm.

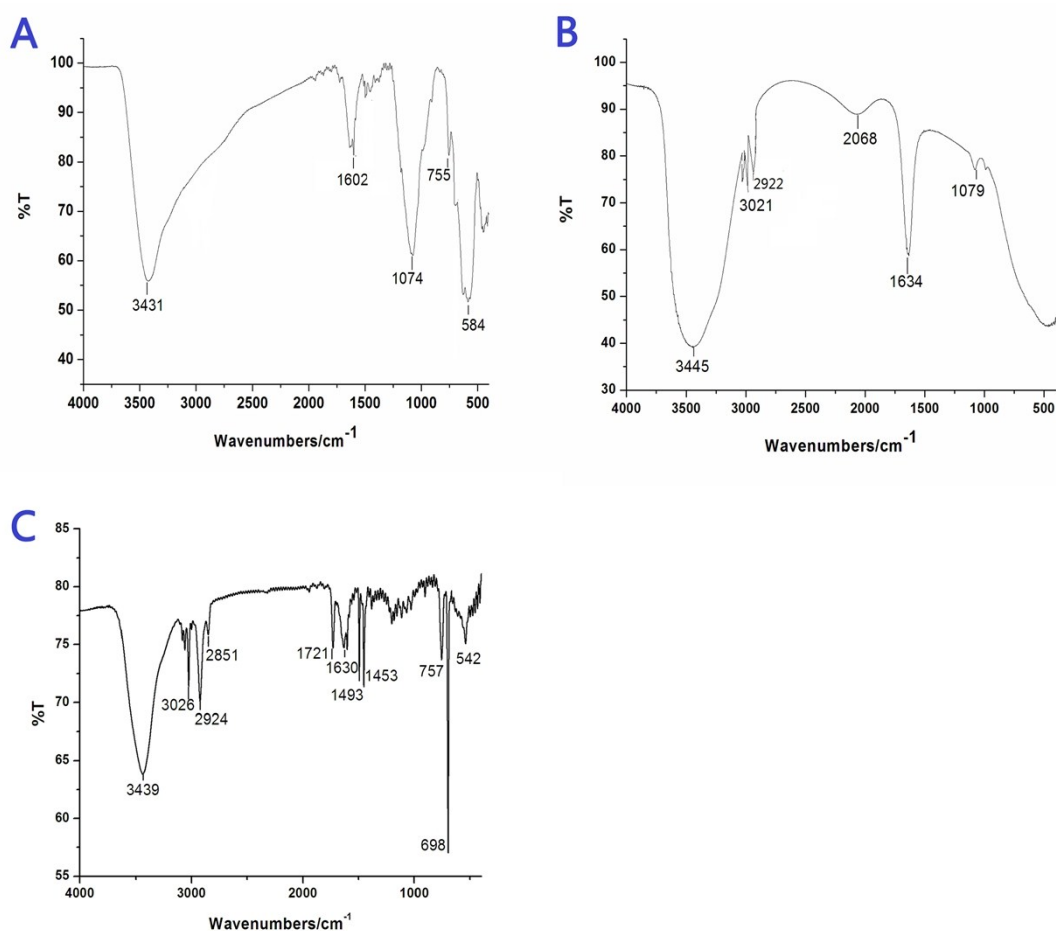
8 At the same time, the as-prepared PSM and PSM-FL signal NPs were characterized by  
9 fourier transform infrared spectroscopy (FTIR). The characteristic peak of DNA is nearby 3000  
10  $\text{cm}^{-1}$ (in Figure S2 B:  $3021 \text{ cm}^{-1}$  and  $2921 \text{ cm}^{-1}$ ). Relative to the as-prepared PSM (in Figure S2 A),  
11 the PSM- FL signal DNA (in Figure S2 C) have the characteristic peak of DNA. The characteristic  
12 peak of amido bond is nearby  $3439 \text{ cm}^{-1}$  and  $1630 \text{ cm}^{-1}$ (in Figure S2 C). The result showed that  
13 the PSM-FL signal NP was prepared as expected.



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**Figure S1.** TEM images of PSM (A) and PSM-FL signal NPs (B).



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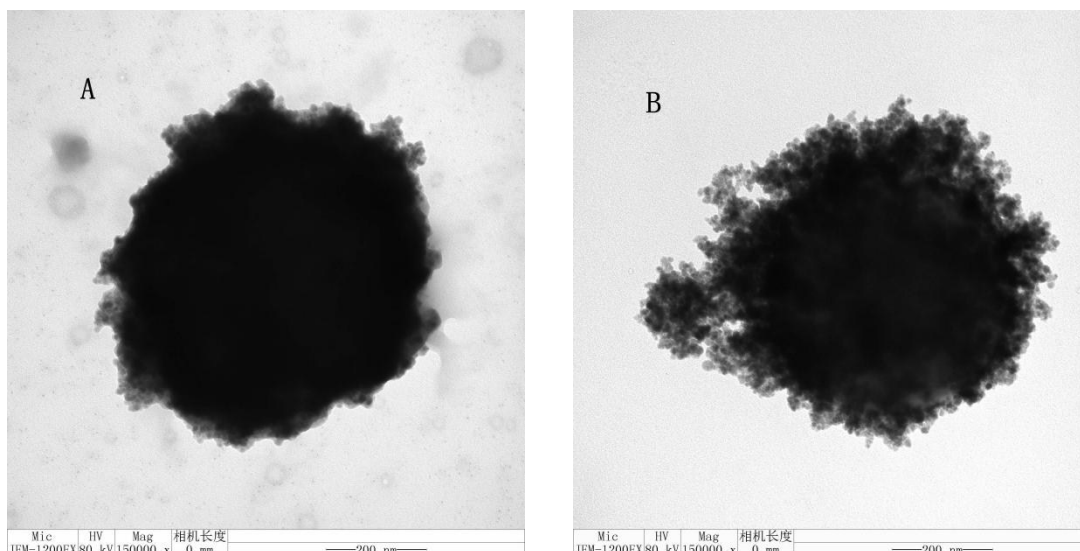
2

**Figure S2.** FTIR images of PSM (A), signal DNA (B) and PSM-FL signal NPs (C).

### 3 2.10. Characterization of MB and MB- Hairpin DNA

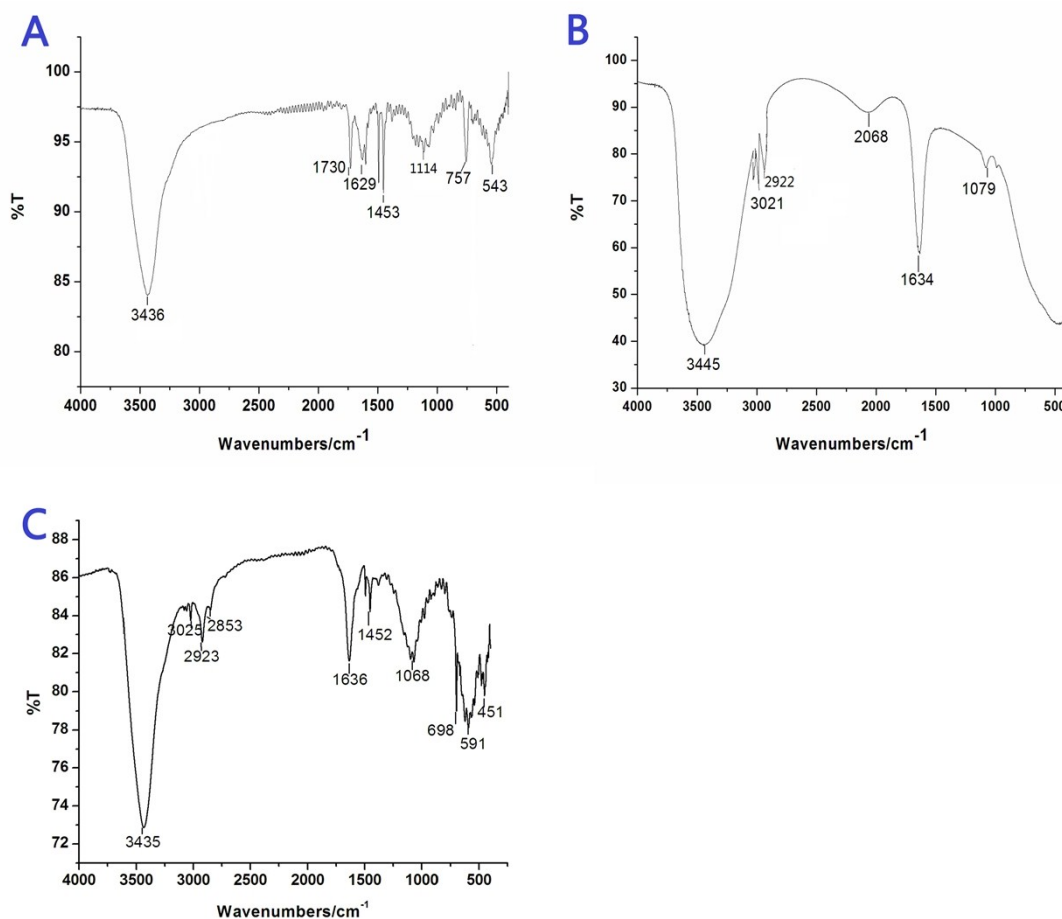
4 The as-prepared MB and MB-Hairpin DNA were characterized by transmission electron  
 5 microscopy (TEM). Relative to the as-prepared MB (in Figure S3 A), the MB-Hairpin DNA (in  
 6 Figure S3 B) have much rougher surface. The result showed that the hairpin DNA has connected  
 7 with the MB. The diameter of MB is 450 nm.

8 At the same time, the as-prepared MB and MB-Hairpin DNA were characterized by fourier  
 9 transform infrared spectroscopy (FTIR). The characteristic peak of DNA is nearby 3000 cm<sup>-1</sup>( in  
 10 Figure S4 B: 3021 cm<sup>-1</sup> and 2921 cm<sup>-1</sup>). Relative to the as-prepared MB (in Figure S4 A), the MB-  
 11 Hairpin DNA (in Figure S4 C) have the characteristic peak of DNA. The characteristic peak of  
 12 amido bond is nearby 3545 cm<sup>-1</sup> and 1636 cm<sup>-1</sup>(in Figure S4 C). The result showed that the MB-  
 13 Hairpin DNA was prepared as expected.



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**Figure S3.** TEM images of MB (A) and MB-Hairpin DNA (B).



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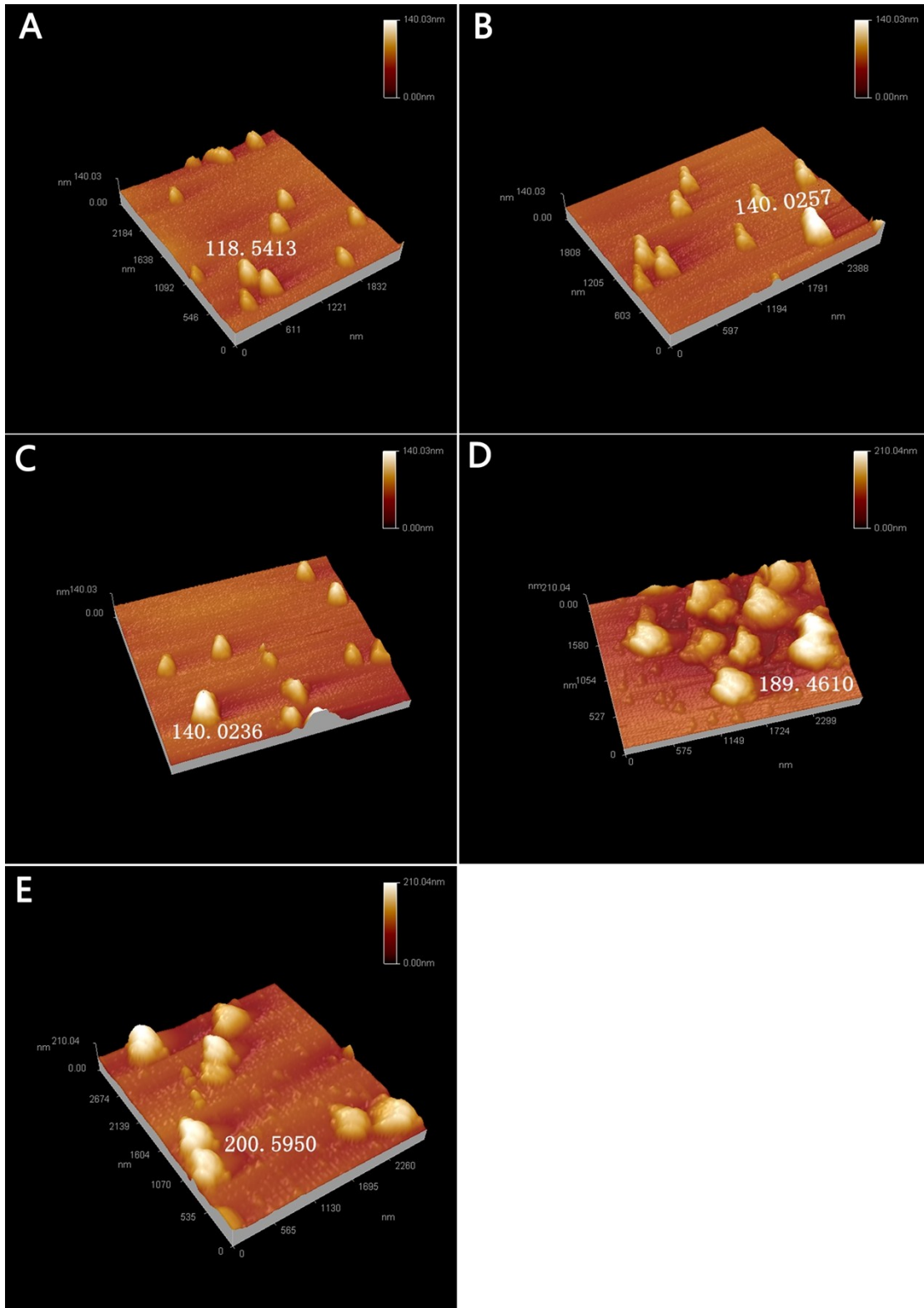
**Figure S4.** FTIR images of MB (A), Hairpin DNA (B) and MB-Hairpin DNA (C).

### 5 2.11. Characterization of PSM-FL signal NPs linked MB- Hairpin DNA

6 The as-prepared MB, MB-Hairpin DNA, PSM and PSM-FL signal NPs were characterized  
7 by atomic force microscope (AFM). Relative to the as-prepared MBs (in Figure S5 A), the MBs-

1 Hairpin DNA (in Figure S5 B) have much larger volume and higher height. The result showed that  
2 the hairpin DNA has connected with the MBs. In a similar way, relative to the as-prepared PSM  
3 (in Figure S5 C), the PSM- FL signal NPs (in Figure S5 D) have much larger volume and higher  
4 height. Due to the hybridization reaction of DNA was happen, PSM-FL signal NPs have been  
5 linked MB- Hairpin DNA (Figure S5 F).





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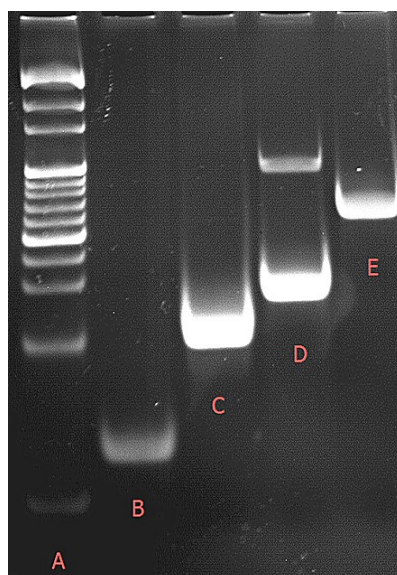
2 **Figure S5.** AFM images of MB (A), MB -Hairpin DNA (B), PSM(C), PSM-FL signal NPs (D), PSM-FL signal

3

NPs linked MB- Hairpin DNA (F)

4 *2.12. Characterization of polyacrylamide gel electrophoresis*

1 A nondenaturating polyacrylamide gel electrophoresis (PAGE) analysis was also employed  
2 to monitor the reaction product (Figure S6). From lane B to E, more and more strong bands were  
3 observed and run slowly with the numbers of base pairs increased by the addition of primer 1 and  
4 primer 2. Lane A and B are marker and hairpin DNA, respectively. The result indicated that the  
5 hairpin DNA, primer 1 and primer 2 could assembled to two-chain DNA and three-chain DNA  
6 structure as assumed. The DNA band in lane E was a little stronger and run slowly than lane D,  
7 indicated that telomerization reaction produced a long chain DNA structure and S1 was replaced.  
8 After the stepwise hydrolysis of probe DNA in duplex DNA in the presence of Klenow  
9 polymerase, the DNA band in lane d became as weak as lane C but run slower, showed the S1  
10 with more base sequence were generated, indicating the growth of primer 2 by Klenow  
11 polymerase.



12

13 **Figure S6.** The nondenaturating polyacrylamide gel electrophoresis (PAGE) analysis.

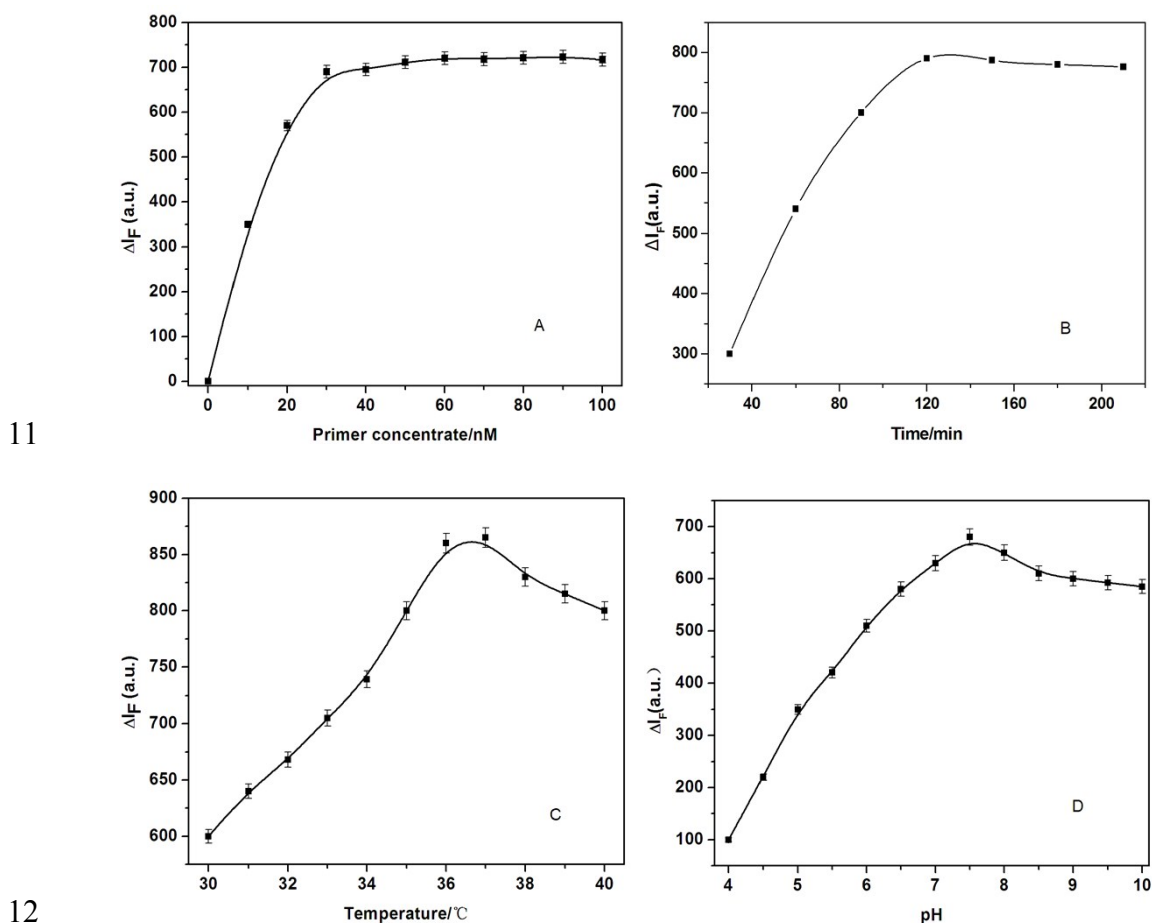
### 14 **3. Optimization of the Experimental Conditions.**

15 Several parameters were investigated systematically to establish optimal conditions for the  
16 ultrasensitive DNA detection, the experimental conditions were optimized including the  
17 concentration of primer 1, time, temperature and pH. [Figure S7A](#) showed that the intensities of  
18 fluorescence spectra increased rapidly upon raising the concentration of primer 1 from 0 to 30 nM,  
19 and then the fluorescence intensity increased slowly. To ensure the telomerization reaction  
20 proceeding completely, concentration of 60 nM was used all through the assays. In order to ensure

1 the circular strand-displacement polymerization reaction proceed completely, 100 nM of PSM-  
2 DNA FL signal NPs was used in the experiment.

3 The influence of telomerization reaction time of primer 2 was also investigated as shown in  
4 [Figure S7 B](#). At first, the response intensity of fluorescence was rapidly increasing and reached an  
5 equilibration step after 2 h. So the polymerization reaction time was controlled at 2 h for the  
6 subsequent assays.

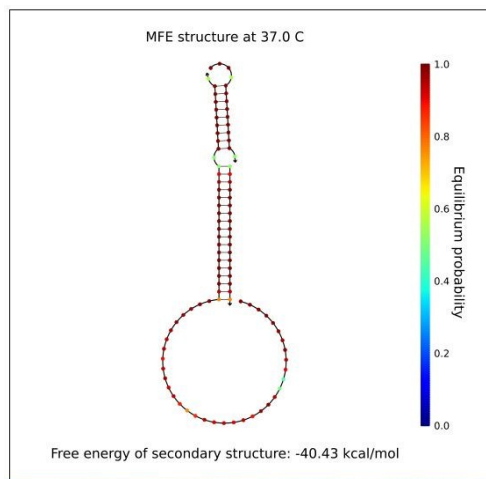
7 The influence of the polymerization reaction temperature and pH were investigated as shown  
8 in [Figure S7 C](#) and [Figure S7 D](#). The result in these figures showed that when the temperature was  
9 37 °C and the pH was 7.4, the fluorescence intensities were strongest. Thus, the subsequent  
10 experiment was all proceeded in 37 °C and experimental pH was 7.4.



11  
12  
13 **Figure S7.** Effect of concentrations of primer 1 (A), the polymerization reaction time (B), the polymerization  
14 reaction temperature (C), and the polymerization reactionon pH (D) on the fluorescence intensities.

#### 15 4. The feasibility of design

1 To obtain the best feasibility of the opened hairpin DNA-PSM, we used NUPACK [3] platform  
2 for aided design. As it shown in Figure S8, the Free Energy of bonding is -40.43 kcal/mol, it's  
3 easy to happen hybrid reaction, and it prove funtionalized polystyrene microspheres can be was  
4 attached to the opened hairpin DNA.

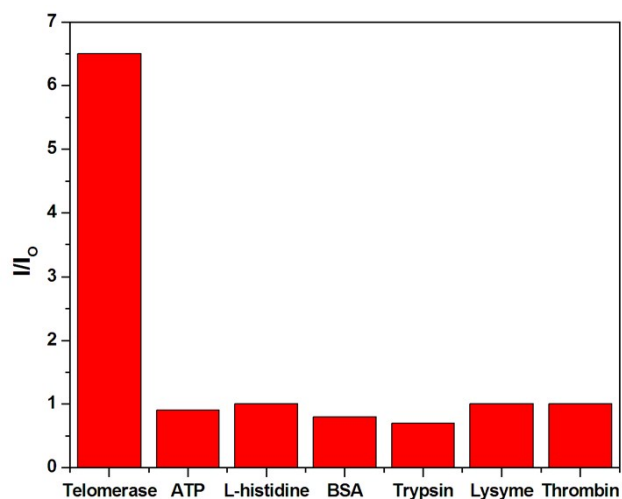


5

6 **Figure S8.** The free energy of second structure of hairpin DNA-primer 1-primer 2.

## 7 **5. Specificity**

8 In order to verify the selectivity of this design, the influence of interference of a variety of  
9 biorelevant analytes on monitoring telomerase were also tested for control experiments. The  
10 results in Fig. S9 indicate that various bioanalytes produced no or negligible interference with  
11 telomerase detection. The applications of the proposed method were performed using the  
12 telomerase, ATP, L-histidine, BSA, trypsin, lysyme, thrombin samples. The suitability of the  
13 developed method was carried out with respect to ICH guidelines [4].



1

2 **Figure S9.** The intensity of fluorescence to diverse bioanalytes in PBS.( the concentration of Telomerase, ATP, L-

3 histidine, BSA, Trypsin, Lysyme, Thrombin were  $1 \times 10^{-7}$ M,  $1 \times 10^{-3}$  M,  $1 \times 10^{-4}$  M, 2.5 g/L,  $4.3 \times 10^{-2}$  M,  $1 \times 10^{-}$

4  $^4$  M,  $1 \times 10^{-4}$  M)

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## 6 **6.Notes and references**

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