Electronic Supplementary Information

Single Quantum Dot-Based Biosensor for Telomerase Assay

Guichi Zhu, Kun Yang, and Chun-yang Zhang*

Single-Molecule Detection and Imaging Laboratory, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Guangdong 518055, China

* Tel.: +86 755 86392211; fax: +86 755 86392299. E-mail address: zhangcy@siat.ac.cn

EXPERIMENTAL SECTION

Materials. The primer (5'-AAT CCG TCG AGC AGA GTT-3'), the capture probe (5'-biotin-AAC CCT AAC CCT AAC CCT AAC TCT GCT CGA CGG ATT-3'), dGTP, dTTP, recombinant RNase inhibitor, and DEPC-treated water (RNase free) were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Cyanine 5-dATP (Cy5-dATP) was obtained from Perkin-Elmer. The streptavidin-coated CdSe/ZnS QDs with the maximum emission at 605 nm (Qdot 605 ITK) were obtained from Invitrogen Corporation (CA, USA). TRAPeze 1× CHAPS lysis buffer was obtained from Millipore. Telomerase inhibitor of MST-312 (N,N'-1,3-Phenylenebis-[2,3-dihydroxy -benzamide]) was purchased from Calbiochem. Other chemical reagents were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cell Culture and Telomerase Extract Preparation. HeLa cells, MCF-7 cells, and HEK-293T cells were cultured with 10% fetal bovine serum in Dulbecco's modified Eagle's medium. MRC-5 cells were cultured with 15% fetal bovine serum in Dulbecco's modified Eagle's medium. The cells were collected in the exponential phase of growth, and 2×10^6 cells were dispensed in an EP tube, washed twice with ice-cold PBS, and re-suspended in 200 μL of ice-cold CHAPS lysis

buffer (10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM PMSF, 5 mM β-mercaptoethanol, 0.5% CHAPS, 10% glycerol). The lysate was incubated for 30 min on ice and centrifuged at 12000 rpm for 30 min at 4 °C. After the centrifugation, the supernatant was carefully transferred into a fresh tube and stored at −80 °C. For the control experiments, the telomerase extracts were pre-treated at 95 °C for 10 min prior to the measurement.

Telomerase Assay and Data Analysis. First, the primer extension reaction was carried out in 25 μL of volume in a buffer containing 1 μM primer, 10 μM Cy5-dATP, 1 mM dGTP, 1 mM dTTP, 20 U RNase inhibitor, 1× PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1 mM $MgCl_2$), 1 mM EGTA, DEPC-treated water and the telomerase extracts, and incubated at 37 ºC for 60 min. Then 25 μL of extension products was incubated with a solution containing 20 μL of 1.25 μM capture probe at 95 °C for 5 min, followed by cooling to the room temperature. Finally, 5 μL of 0.2 μM QDs was added to the reaction solution and incubated at room temperature for 15 min.

Prior to the imaging, the glass slides were pretreated according to the method developed by Chan et al.[1] The 50 μL of reaction solution was diluted 1000-fold with the buffer containing 67 mM glycine-KOH (pH 9.4), 50 μg/mL BSA, 2.5 mM $MgCl_2$, 1 mg/mL Trolox. The QDs were excited by a Jive 488-nm DPSS laser (Cobolt) via total internal reflection. Both the QD and Cy5 fluorescence signals were collected by an oil immersion objective (NA 1.45, 100×, Olympus), and imaged onto the two halves of an Andor Ixon DU897 EMCCD with a time resolution of 50 ms. For data analysis, the number of Cy5 from ten frames with an imaging region of 200×400 pixels in each frame are counted.

Optimization of Experimental Condition. The concentration of Cy5-dATP is essential to the primer extension reaction and the subsequent FRET efficiency. To optimize the Cy5-dATP

concentration, we measured the FRET efficiency at various concentrations of Cy5-dATP. The FRET efficiency can be calculated according to the equation $E = 1 - (F / F_0)$, where F and F_0 are the fluorescence intensity of QDs in the presence of cell extracts and in the control group with heat-inactivated extracts, respectively. As shown in Fig. S1A, the FRET efficiency enhances with the increase of Cy5-dATP concentration from 2.5 μM to 10 μM, and levels off beyond the concentration of 10 μM. The high-concentration Cy5-dATP may result in high primer extension efficiency and the assembling of more Cy5 molecule onto the surface of QD and consequently high FRET efficiency. However, when the Cy5-dATP concentration is saturated for the primer extension reaction, there is no significant improvement in FRET efficiency. Therefore, we selected 10 μM as the optimal Cy5-dATP concentration in the subsequent research. We further optimized the incubation time of primer extension reaction. As shown in Fig. S1B, the FRET efficiency improves with the increase of incubation time from 0 to 60 min, and levels off beyond the incubation time of 60 min. Therefore, we used 60 min as the optimal incubation time for primer extension reaction.

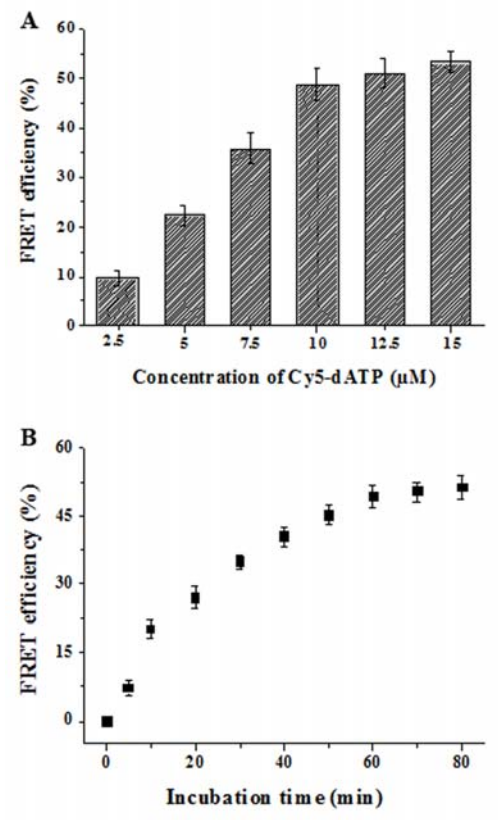

Fig. S1 (A) Variance of FRET efficiency with the concentration of Cy5-dATP. (B) Variance of FRET efficiency with the incubation time of primer extension reaction. The telomerase is extracted from 15000 HeLa cells. Error bars show the standard deviation of three experiments.

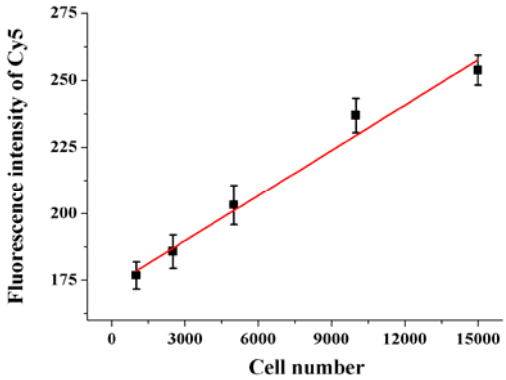

Fig. S2 The linear correlation between Cy5 fluorescence intensity and the cell number in the range from 1000 to 15000. Error bars show the standard deviation of three experiments.

REFERENCES

(1) H. M. Chan, L. S. Chan, R. N. S. Wong, H. W. Li, *Anal. Chem.*, 2010, **82**, 6911.