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A single quantum dot-based biosensor for telomerase assay†

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Telomerase is a promising biomarker and a therapeutic target due to its extensive expression in human tumors such as lung cancer and breast cancer. Here, we develop a single quantum dot (QD)-based biosensor for the sensitive detection of telomerase activity. This single QD-based biosensor has significant advantages of simplicity and high sensitivity, and it can be applied for the discrimination of tumor cells from normal cells as well as the screening of anticancer drugs.

Human telomerase is a reverse transcriptase with a special complex structure including a functional protein component and an endogenous RNA template, and it can specifically add several repeat units to the ends of a telomere.[1] In normal cells, the expression of telomerase is usually repressed, and the telomeres shorten progressively with each cell division, resulting in cell senescence and death.[2] However, in human cancer cells, the length of telomeres is maintained due to either the up-regulation or the reactivation of telomerase activity, making cancer cells divide indefinitely.[3] Especially, a majority of cancers demonstrate positive telomerase activity.[4] Therefore, the measurement of telomerase activity is essential for both clinical diagnosis and anticancer-drug discovery.

Since the discovery of telomerase in 1985,[5] a variety of methods have been developed for the telomerase assay. Among them, the radioactive approach enables the direct measurement of telomerase activity, but it suffers from poor sensitivity and radiation risk.[6] To improve the detection sensitivity, a polymerase chain reaction (PCR)-based telomere repeat amplification protocol (TRAP) is introduced[7] and it can measure telomerase at the single-cell level,[8] but it involves the precise control of temperature cycling and cannot detect very short telomerase products.[9] So far, the reported alternative methods for the telomerase assay include immunoassay,[10] the molecular beacon-based assay,[11] the isothermal amplification reaction-based method,[12] the electrochemical assay,[13] and the gold nanoparticle-based method.[14] However, the requirement of expensive antibodies[10] and doubly labeled fluorescent probes,[11] the involvement of various enzymes[12] and the immobilization/separation steps,[13] and the time-consuming synthesis of gold nanoparticles[14] might hinder their practical applications. Thus the development of a simple and sensitive method for the telomerase assay still remains a great challenge.

Semiconductor quantum dots (QDs) exhibit unique optical properties including high quantum yield, good stability against photobleaching, narrow luminescence bands and size-tunable luminescence spectra, and have been widely applied in fluorescence imaging, drug delivery and biomedical research.[15] Especially, the QDs can be employed for the development of fluorescence resonance energy transfer (FRET)-based sensors for the detection of nucleic acids, proteins, and even small molecules.[16] Here, we develop a single QD-based biosensor for sensitive detection of telomerase activity. In the presence of telomerase, Cy5 molecules can be assembled in an orderly manner into the ends of primer with the assembly direction towards the QD, enabling the enhanced FRET efficiency between the QD and Cy5. However, in the absence of telomerase, no Cy5 molecule can be assembled onto the surface of QD and consequently no FRET occurs between the QD and Cy5, resulting in a near zero-background signal. This single QD-based biosensor has the significant advantages of simplicity and high sensitivity, and it can be applied for the discrimination of tumor cells from normal cells as well as the screening of anticancer drugs.

The principle of the telomerase assay is illustrated in Scheme 1. We designed a specific primer with 18 nucleotides, and incubated the primers with cancer cell extracts in the presence of the nucleotides of dGTP, dTTP and Cy5-dATP at 37 °C for 1 h. The telomerase-induced extension reaction adds the repeat units of AGGGTT onto the 3′-end of the primer and simultaneously assembles the Cy5 molecules into them, generating a Cy5-labeled primer. Subsequently, the Cy5-labeled primer can hybridize with the biotinylated capture probe to form a Cy5-labeled biotinylated double-stranded DNA (dsDNA).

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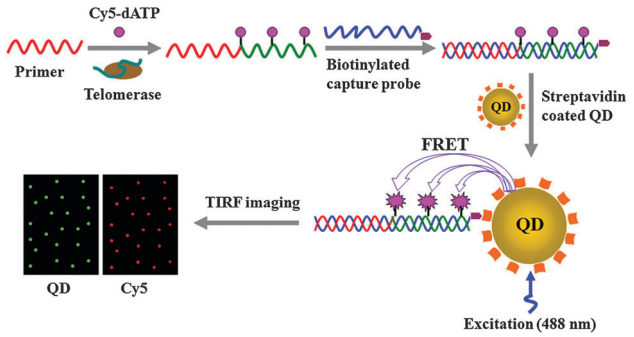

Scheme 1 Schematic illustration of single QD-based biosensor for telomerase assay.

Since the numbers of repeat units added at the ends of primer are usually 1, 2 and 3, which accounts for about 90% in all cases,[17] we design the capture probe with 36 nucleotides which consist of two regions: one is the complementary region of primer, and the other is the complementary region of the added repeat units. The obtained Cy5-labeled biotinylated dsDNAs can attach onto the surface of streptavidin-coated QDs through the biotin–streptavidin interaction to form a Cy5-dsDNA–QD assembly (Scheme 1). Theoretically, the length between the adjacent bases in dsDNA is about 0.34 nm.[18] When the numbers of repeat units added onto the ends of primer are 3, the distances between Cy5 molecules in the extended primer and the surface of streptavidin-coated QD is estimated to be 2.04 nm, 4.08 nm and 6.12 nm, respectively. Taking into account the radius of a streptavidin-coated QD (~7.5 nm),[16a] the corresponding QD-Cy5 separation distances are calculated to be 9.54 nm, 11.98 nm and 13.62 nm, within the efficient range of FRET ($2R_0$ = 138.8 Å),[16a] suggesting that efficient fluorescence resonance energy transfer (FRET) may occur between the QD donor and the Cy5 receptor. When the Cy5-dsDNA-QD assembly is excited using a 488 nm argon laser, the fluorescence signals of QD and Cy5 can be observed simultaneously (Scheme 1). Due to the availability of multiple streptavidins on the surface of a single QD, a number of Cy5 molecules can be assembled onto a single QD, thus significantly improving the FRET efficiency. In addition, Cy5 molecules are assembled in an orderly manner onto the ends of primer with the assembling direction towards the QD, which may greatly enhance the FRET efficiency. The telomerase activity can be quantitatively evaluated through the measurement of Cy5 counts by total internal reflection fluorescence (TIRF) microscopy. However, in the absence of telomerase, the specific primer cannot be elongated and no Cy5-dATP is assembled into the primer. Consequently, no Cy5-dsDNA-QD assembly can be obtained and no Cy5 signal is observed.

To demonstrate the feasibility of a single QD-based biosensor for the telomerase assay, we measured the fluorescence images in the presence of cell extracts and in the control group with heat-inactivated extracts, respectively. In the presence of cell extracts, the fluorescence signals of QDs (Fig. 1A) and Cy5 (Fig. 1B) are observed simultaneously due to the efficient FRET between the QD donor and the Cy5 acceptor in the Cy5-dsDNA-QD assembly induced by the telomerase. In contrast, in the

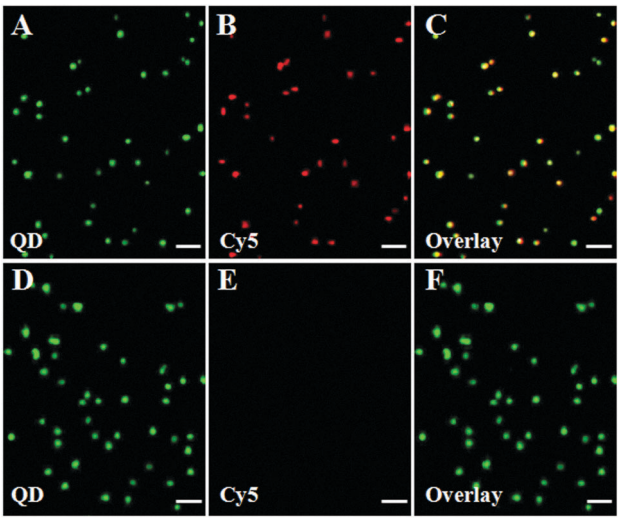

Fig. 1 Fluorescence images of single QD-based biosensors in the presence of cell extracts (A–C) and in the control group with heat-inactivated extracts (D–F) at the excitation wavelength of 488 nm. The fluorescence images of the QDs (A and D) are shown in green, and the fluorescence images of Cy5 (B and E) are shown in red. The fluorescence images with the blended color in yellow and orange (C) indicate the co-localization of QD and Cy5. The telomerase is extracted from 15 000 HeLa cells. The scale bar is 2 μm.

control group, only the QD fluorescence signal (Fig. 1D) is observed, but no Cy5 fluorescence signal is detected (Fig. 1E), because no Cy5 acceptor can be assembled onto the surface of QD due to the inactivation of telomerase. Notably, the QD fluorescence intensities in the presence of cell extracts (Fig. 1A) are much less than those in the control group (Fig. 1D) due to the quenching of QD fluorescence as a result of energy transfer from the QD donor to the Cy5 acceptor in the Cy5-dsDNA-QD assembly. Even though there are some free Cy5-dATP molecules in solution, they are far away from the QD in the single-particle/molecule imaging and no Cy5 fluorescence signal is observed in the control group (Fig. 1E).

We obtained the cell extracts from different numbers of HeLa cells and investigated the variance of fluorescence emission spectra with the number of cells under the optimally experimental conditions (see ESI,† Fig. S1). As shown in Fig. 2A, with the increase of cell number from 1000 to 20 000, the QD fluorescence intensity decreases, accompanied by the increases of the Cy5 fluorescence intensity (inset of Fig. 2A). Notably, the Cy5 fluorescence intensity shows a linear correlation with the cell number over a range from 1000 to 15 000 (see ESI,† Fig. S2). The regression equation is F = 172.88 + 0.00565 N with a correlation coefficient of 0.979, where F is the Cy5 fluorescence intensity and N is the number of cells, respectively. The limit of detection is 1667 cells (67 cells per μL) based on the calculation of the average signal of the blank plus three times the standard deviation.

We further employed the single QD-based biosensor to measure the Cy5 counts. As shown in Fig. 2B, the Cy5 counts increase with the increase of cell number from 500 to 20 000. Notably, the Cy5 counts exhibit a linear correlation with the cell number over a range from 500 to 10 000 (Fig. 2B). The regression

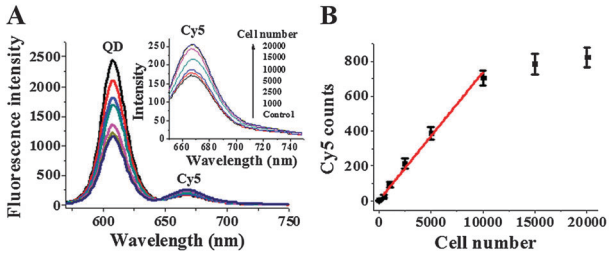

Fig. 2 (A) Variance of the fluorescence emission spectra as a function of HeLa cell number. The inset shows the variance of Cy5 fluorescence intensity with cell number 0, 1000, 2500, 5000, 10 000, 15 000, and 20 000 from bottom to top, respectively. (B) Variance of the Cy5 count as a function of HeLa cell number, with a linear correlation between the Cy5 count and the cell number in the range from 500 to 10 000. Error bars show the standard deviations of three experiments.

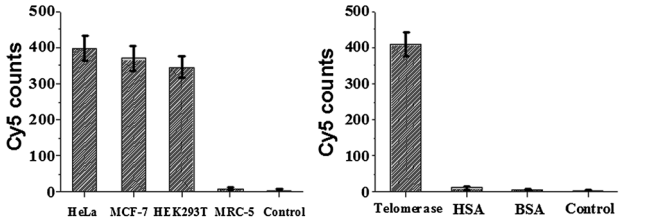

Fig. 3 (A) Measurement of Cy5 counts in response to the cell extracts (equivalent to 5000 cells) obtained from HeLa cells, MCF-7 cells, HEK293T cells, MRC-5 cells, and the control group with heat-inactivated cell extracts. (B) Selectivity of single QD-based biosensor for the telomerase assay. Error bars show the standard deviations of three experiments.

equation is $Y = 0.349 + 0.0738\ N$ with a correlation coefficient of 0.984, where Y is the Cy5 count and N is the cell number, respectively. The limit of detection is 185 cells (7 cells per μL) based on the calculation of the average signal of blank plus three times standard deviation, which has improved by as much as 9-fold as compared with that of fluorescence spectrum measurement. Moreover, the sensitivity of single QD-based biosensor has improved by as much as 38-fold as compared with that of QD-based electron-transfer assays using either hemin[19] (270 cells per μL) or doxorubicin[20] (269 cells per μL) as the electron acceptors, by 5.7-fold as compared with that of electrochemical method (40 cells per μL),[21] and by 3.8-fold as compared with that of the Au nanoparticle-based colorimetric assay (27 cells per μL).[22] The improved sensitivity may be attributed to the enhanced FRET efficiency resulting from (1) the telomerase-induced orderly assembly of Cy5 molecules onto the ends of primer with the direction towards the QD, and (2) the availability of multiple streptavidins on the surface of single QD to catch a number of Cy5-labeled biotinylated dsDNAs as well as the near-zero background signal resulting from the single-particle/molecule imaging.

The broad-spectrum expression of telomerase in most malignancies makes it a promising target for cancer diagnosis. To evaluate the feasibility of a single QD-based biosensor for clinical diagnosis, we further measured the telomerase activity in different cell lines including the human cervical cancer cell line of HeLa, breast cancer cell line of MCF-7, embryonic kidney cell line of HEK-293T, and normal cell line of MRC-5. As shown in Fig. 3A, high Cy5 counts are detected in the presence of HeLa cells and MCF-7 cells, consistent with the over-expression of telomerase in human cancer cells.[3] Moreover, high Cy5 counts are detected in the presence of HEK-293T cells, consistent with the unlimited cell division capability and the over-expression of telomerase in HEK-293T cells.[12c] However, no distinct Cy5 counts are detected in the presence of MRC-5 cells due to the lack of telomerase in normal cells.[2] Similar results are observed in the control group with heat-inactivated cell extracts due to the loss of telomerase activity. These results demonstrate the feasibility of single QD-based biosensor for the detection of telomerase activity in different kinds of cells.

To evaluate the selectivity of a single QD-based biosensor, we employed the cell extracts from 5000 HeLa cells, human serum albumin (HSA), and bovine serum albumin (BSA) as the model. As shown in Fig. 3B, high Cy5 counts are detected only in the presence of HeLa cell extracts. In contrast, no distinct Cy5 counts are observed in the presence of either HSA or BSA, suggesting the high selectivity of the single QD-based biosensor for the telomerase assay.

We further investigated the feasibility of single QD-based biosensor for the screening of telomerase inhibitor using *N*,*N*′-1,3-phenylenebis-[2,3-dihydroxy-benzamide] (MST-312) as the model. MST-312 is a synthetic small molecule which can efficiently inhibit the telomerase activity.[23] After the incubation with various concentrations of MST-312 for 72 h, the HeLa cells were collected for the measurement of telomerase activity. The relative enzyme activity (R_A) is determined as $R_A = N_i/N_0$, where N_i is the Cy5 count in the presence of inhibitors, and N_0 is the Cy5 count in the control sample without the inhibitor treatment. As shown in Fig. 4, the relative activity of telomerase decreases with the increase of MST-312 concentration, and the half-maximal inhibitory concentration (IC_{50}) value of MST-312 is calculated to be 0.72 μM. These results indicate that single QD-based biosensor can be applied for the screening of telomerase inhibitors.

In summary, we have developed a single QD-based biosensor for the sensitive detection of telomerase activity. In comparison with the conventional PCR-based TRAP assay,[7–9] this method is very simple without the requirement of a sophisticated double-primer design and precise thermal cycling. This single QD-based

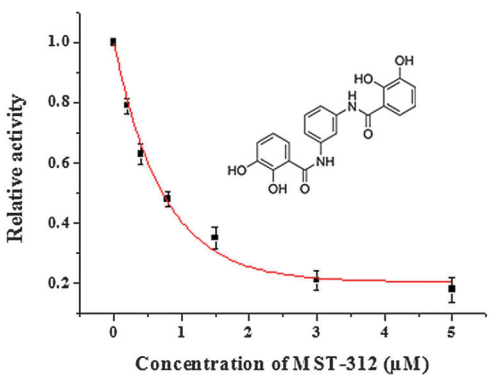

Fig. 4 Dose-dependent inhibition of telomerase activity in HeLa cells induced by MST-312. Inset shows the chemical structure of MST-312. Error bars show the standard deviations of three experiments.

biosensor can be used for the discrimination of tumor cells from normal cells as well as the screening of anticancer drugs, and it might be further applied for the detection of various targets including microRNAs, metal ions, and small molecules in combination using different probes/aptamers.

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Notes and references

1 (*a*) D. Shippenlentz and E. H. Blackburn, *Science*, 1990, **247**, 546; (*b*) E. H. Blackburn, *Nature*, 1991, **350**, 569.
2 K. Masutomi, E. Y. Yu, S. Khurts, I. Ben-Porath, J. L. Currier, G. B. Metz, M. W. Brooks, S. Kaneko, S. Murakami, J. A. DeCaprio, R. A. Weinberg, S. A. Stewart and W. C. Hahn, *Cell*, 2003, **114**, 241.
3 J. W. Shay, Y. Zou, E. Hiyama and W. E. Wright, *Hum. Mol. Genet.*, 2001, **10**, 677.
4 (*a*) J. L. Mergny, J. F. Riou, P. Mailliet, M. P. Teulade-Fichou and E. Gilson, *Nucleic Acids Res.*, 2002, **30**, 839; (*b*) M. Meyerson, *J. Clin. Oncol.*, 2000, **18**, 2626; (*c*) E. H. Blackburn, *Mol. Cancer Res.*, 2005, **3**, 477.
5 C. W. Greider and E. H. Blackburn, *Cell*, 1985, **43**, 405.
6 G. B. Morin, *Cell*, 1989, **59**, 521.
7 (*a*) N. W. Kim, M. A. Piatyszek, K. R. Prowse, C. B. Harley, M. D. West, P. L. Ho, G. M. Coviello, W. E. Wright, S. L. Weinrich and J. W. Shay, *Science*, 1994, **266**, 2011; (*b*) N. W. Kim and F. Wu, *Nucleic Acids Res.*, 1997, **25**, 2595.
8 H. Wege, M. S. Chui, H. T. Le, J. M. Tran and M. A. Zern, *Nucleic Acids Res.*, 2003, **31**, e3.
9 S. Huard, T. J. Moriarty and C. Autexier, *Nucleic Acids Res.*, 2003, **31**, 4059.
10 S. B. Cohen and R. R. Reddel, *Nat. Methods*, 2008, **5**, 355.
11 C. Ding, X. Li, Y. Ge and S. Zhang, *Anal. Chem.*, 2010, **82**, 2850.
12 (*a*) L. Tian and Y. Weizmann, *J. Am. Chem. Soc.*, 2013, **135**, 1661; (*b*) L. Wang, Y. Zhang and C. Y. Zhang, *Anal. Chem.*, 2013, **85**, 11509; (*c*) Y. Zhang, L. Wang and C. Y. Zhang, *Chem. Commun.*, 2014, **50**, 1909.
13 X. Zhou, D. Xing, D. Zhu and L. Jia, *Anal. Chem.*, 2009, **81**, 255.
14 (*a*) R. Qian, L. Ding, L. Yan, M. Lin and H. Ju, *J. Am. Chem. Soc.*, 2014, **136**, 8205; (*b*) R. Duan, B. Wang, T. Zhang, Z. Zhang, S. Xu, Z. Chen, X. Lou and F. Xia, *Anal. Chem.*, 2014, **86**, 9781.
15 (*a*) I. L. Medintz, H. T. Uyeda, E. Goldman and H. Mattoussi, *Nat. Mater.*, 2005, **4**, 435; (*b*) R. Gill, M. Zayats and I. Willner, *Angew. Chem., Int. Ed.*, 2008, **47**, 7602; (*c*) R. C. Somers, M. G. Bawendi and D. G. Nocera, *Chem. Soc. Rev.*, 2007, **36**, 579; (*d*) I. L. Medintz, M. H. Stewart, S. A. Trammell, K. Susumu, J. B. Delehanty, B. C. Mei, J. S. Melinger, J. B. Blanco-Canosa, P. E. Dawson and H. Mattoussi, *Nat. Mater.*, 2010, **9**, 676; (*e*) J. B. Delehanty, C. E. Bradburne, K. Susumu, K. Boeneman, B. C. Mei, D. Farrell, J. B. Blanco-Canosa, P. E. Dawson, H. Mattoussi and I. L. Medintz, *J. Am. Chem. Soc.*, 2011, **133**, 10482; (*f*) Q. Wang, Y. Liu, Y. Ke and H. Yan, *Angew. Chem., Int. Ed.*, 2008, **47**, 316; (*g*) A. Samanta, Y. Zhou, S. Zou, H. Yan and Y. Liu, *Nano Lett.*, 2014, **14**, 5052–5057.
16 (*a*) C. Y. Zhang, H. C. Yeh, M. T. Kuroki and T. H. Wang, *Nat. Mater.*, 2005, **4**, 826; (*b*) K. Boeneman, B. C. Mei, A. M. Dennis, G. Bao, J. R. Deschamps, H. Mattoussi and I. L. Medintz, *J. Am. Chem. Soc.*, 2009, **131**, 3828; (*c*) C. Y. Zhang and L. W. Johnson, *Anal. Chem.*, 2009, **81**, 3051.
17 (*a*) J. L. Chen and C. W. Greider, *EMBO J.*, 2003, **22**, 304; (*b*) X. Ren, H. Li, R. W. Clarke, D. A. Alves, L. Ying, D. Klenerman and S. Balasubramanian, *J. Am. Chem. Soc.*, 2006, **128**, 4992.
18 S. B. Smith, Y. J. Cui and C. Bustamante, *Science*, 1996, **271**, 795.
19 E. Sharon, R. Freeman, M. Riskin, N. Gil, Y. Tzfati and I. Willner, *Anal. Chem.*, 2010, **82**, 8390.
20 S. Raichlin, E. Sharon, R. Freeman, Y. Tzfati and I. Willner, *Biosens. Bioelectron.*, 2011, **26**, 4681.
21 S. B. Sato, H. Kondo, T. Nojima and S. Takenaka, *Anal. Chem.*, 2005, **77**, 7304.
22 E. Sharon, E. Golub, A. Niazov-Elkan, D. Balogh and I. Willner, *Anal. Chem.*, 2014, **86**, 3153.
23 H. Seimiya, T. Oh-hara, T. Suzuki, I. Naasani, T. Shimazaki, K. Tsuchiya and T. Tsuruo, *Mol. Cancer Ther.*, 2002, **1**, 657.

