Electronic Supplementary Information

Single quantum dot-based fluorescence resonance energy transfer biosensor for antibody-free detection of ten-eleven translocation 1

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Materials and methods

Materials

Ten-eleven translocation 1 protein (TET1) was purchased from Epigentek Group Inc (New York, NY, USA). The detection probe 5'-biotin - TTT TTC ACT C^{m}CG GTC ACG TTT TCG TGA CCG GAG TG - Cy5-3' was synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The 605 nm-emitting streptavidin-coated CdSe/ZnS QDs (605QDs) were purchased from Invitrogen Corporation (Carlsbad, CA, USA). T4 phage β-glucosyltransferase (T4-βGT), uridine diphosphate glucose (UDP-glucose), 10× NEBuffer 4 (500 mM potassium acetate, 200 mM Tris-acetate, 100 mM magnesium acetate, 10 mM DTT, pH 7.9), MspI, and 10× CutSmart buffer (100 mM magnesium acetate, 200 mM Tris-acetate, 500 mM potassium acetate, 1 mg/mL BSA, pH 7.9) were obtained from New England Biolabs (Ipswich, MA, USA). Ammonium iron (II) sulfate hexahydrate ($(NH_4)_2Fe(SO_4)_2$), α-ketoglutaric acid disodium salt dehydrate (α-KG), DL-dithiothreitol (DTT), ascorbic acid, HEPES, $(NH_4)_2SO_4$, $MgCl_2$, and NaCl were obtained from Sigma-Aldrich Company (St. Louis, MO, USA). Human neuroblastoma cell line (SK-N-BE(2) cells) and cervical carcinoma cell line (HeLa cells) were bought from Cell Bank of Chinese Academy of Sciences (Shanghai, China).

Detection of TET1

For TET1 assay, the detection probe was incubated with the annealing buffer (5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.0) at 95 °C for 5 min, followed by slowly cooling to room temperature. Then 144 nM pre-annealed detection probe and various concentrations of TET1 were added into 10 μL of reaction mixture containing 50 mM HEPES, 50 mM NaCl, 75 μM $Fe(NH_4)_2(SO4)_2$, 2 mM ascorbic acid, 2.5 mM DTT, and 1 mM α-KG and incubated at 37 °C for 30 min. The obtained product was incubated with 40 μM UDP-glucose and 3 U of T4-βGT in 1× NEBuffer 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM DTT, pH 7.9) at 37 °C for 2 h. Subsequently, the glucosylated product was digested by 30 U of MspI in 1× CutSmart buffer (10 mM magnesium acetate, 20 mM Tris-acetate, 50 mM potassium acetate, 0.1 mg/mL BSA, pH 7.9) at 37 °C for 2 h. Then 0.5 nM 605QDs was added into the digested product in 60 μL of incubation buffer (100 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, and 3 mM $MgCl_2$, pH 8.0) and incubated for 20 min at room temperature to obtain the 605QD-DNA-Cy5 nanostructure.

Gel electrophoresis and fluorescence measurement

The digested products were stained with SYBR Gold and analyzed with 12% nondenaturating polyacrylamide gel electrophoresis (PAGE) in 1× TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) at a 110 V constant voltage for 40 min. The multichannel imaging was performed by a ChemiDoc MP imaging system (Hercules, CA,

USA). The SYBR Gold signal was visualized using an illumination source of Epi-blue (460-490 nm excitation) and a 518-546 nm filter, and the Cy5 signal was visualized using an illumination source of Epi-red (625-650 nm excitation) and a 675-725 nm filter. The fluorescence spectra were detected by a FLS1000 fluorescence spectrophotometer (Edinburgh Instruments, UK) at an excitation wavelengths of 488 nm.

Single-molecule detection

After 25-fold dilution of the reaction products with the buffer (100 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, and 3 mM $MgCl_2$, pH 8.0), the 10 μL of sample was dropped onto the coverslip for total internal reflection fluorescence microscopy (TIRF, Nikon, Ti-E, Japan) imaging. The 605QDs were excited by a 488 nm laser. The photons emitted by Cy5 and 605QD were collected by 100× oil immersion lens. The Cy5 fluorescent molecules were counted in the image area of 600 × 600 pixels by Image J software.

Cell culture and cell extraction.

SK-N-BE(2) cells were cultured in 45% MEM cell culture medium, 45% F-12 cell culture medium, 10% fetal bovine serum (FBS) and penicillin streptomycin in humidification chamber containing 5% CO_2 at 37 °C. HeLa cells were cultured in 90% Dulbecco's modified Eagle's medium (DMEM), 10% FBS and penicillin streptomycin in humidification chamber containing 5% CO_2 at 37 °C. The cells were washed with PBS and then collected with cell scraper. After cell counting, ~1.5×10^6 SK-N-BE(2) cells and ~2.0×10^6 HeLa cells were used for extraction. The cell lysates were obtained by using the nuclear extract kit (Active Motif, Carlsbad, CA, USA) according to the manufacturer's protocol. The resultant supernatant was subjected to TET1 assay, with different dilutions of cell lysates being used for cellular TET1 assay and linear relationship analysis.

Supplementary results

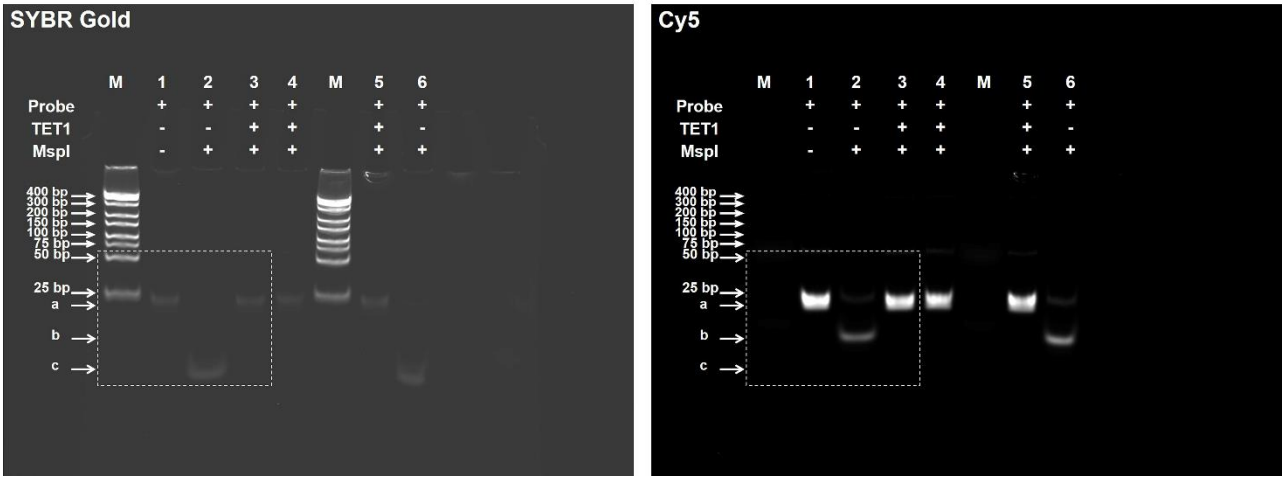

Fig. S1 Full gel image for the PAGE data shown in Fig. 1A. Images of the same field in the SYBR Gold channel (left part) and the Cy5 channel (right part) were obtained by a ChemiDoc MP imaging system (Hercules, CA, USA), followed by being merged into a superimposed image (Fig. 1A). Dashed regions containing lanes M and 1−3 are shown in Fig. 1A. Lanes 4-6 represent the products from another tubes and DNA marker.

Fluorescence spectra analysis

The normalized absorption and fluorescence emission spectra of 605QD and Cy5 are shown in Fig. S2. Because of significant spectral overlap between the emission spectra of 605QD and the absorption spectra of Cy5, efficient FRET from the 605QD to Cy5 can occur in the presence of TET1. We performed fluorescence spectra measurement (Fig. 1B). In the absence of TET1, no Cy5 fluorescence signal is detected at the excitation wavelength of 488 nm due to no occurrence of FRET between 605QD and Cy5 (Fig. 1B, blue line). In contrast, distinct Cy5 fluorescence signal is observed in the presence of TET1 (Fig. 1B, red line). The fluorescence intensity of 605QD in the presence of TET1 is significantly lower than that in the absence of TET1, indicating that the FRET from the 605QD to Cy5 occurs. The FRET efficiency is caudated based on equation 1.

$$E = \left(1 - \frac{F_{DA}}{F_D}\right) \times 100\% \qquad (1)$$

where F_{DA} is the 605QD fluorescence intensity in the presence of TET1, and F_{D} is the 605QD fluorescence intensity in the absence of TET1. The FRET efficiency is calculated to be 77%.

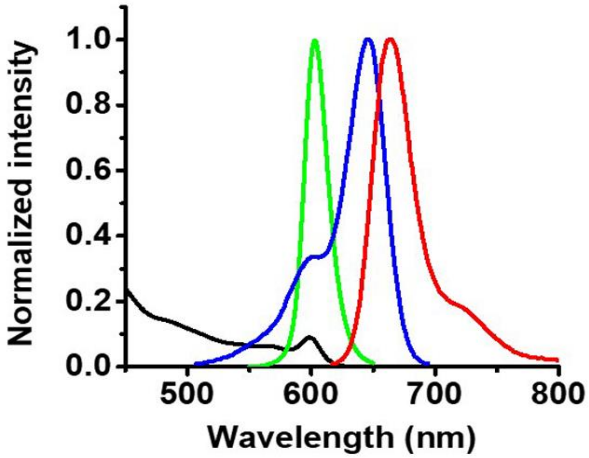

Fig. S2 Normalized absorption of 605QD (black line) and Cy5 (blue line), and normalized emission spectra of 605QD (green line) and Cy5 (red line).

Fluorescence lifetime analysis

To verify the FRET between 605QD and Cy5, we measured the fluorescence lifetime curves of 605QD in the absence and presence of TET1, respectively (Fig. 1C). The average lifetime of the 605QD in the absence of TET1 is measured to be 28 ns, whereas the average lifetime of the 605QD is reduced to 6.8 ns in the presence of TET1. The FRET efficiency can be calculated based on equation 2.

$$E = (1 - \frac{\tau_{DA}}{\tau_D}) \times 100\% \qquad (2)$$

where τ_{DA} is the 605QD fluorescence lifetime in the presence of TET1, and τ_D is the 605QD fluorescence lifetime in the absence of TET1. The FRET efficiency is calculated to be 76%, consistent with that obtained by fluorescence spectra measurement (77%).

Optimization of the amount of MspI

To ensure the best assay performance of single QD-based FRET biosensor, we optimized the amount of MspI and the ratio of detection probe to 605QD. The sensitive detection of TET1 in the assay relies on the MspI-assisted cleavage of methylated detection probe and the subsequent assembly of multiple glycosylated detection probes onto the surface of single 605QD. Therefore, the amount of MspI should be carefully optimized. As shown in Fig. S3, the Cy5 counts decrease with the increasing amount of MspI from 5 to 30 U and reach a plateau beyond the amount of 30 U due to the complete cleavage of the methylated detection probe by MspI. Thus, 30 U of MspI is used in the reaction system.

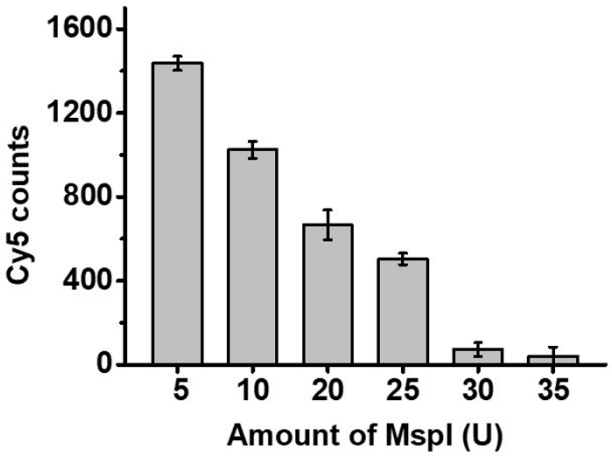

Fig. S3 Variance of Cy5 counts in response to different amounts of MspI. Error bars represent standard deviations of three experiments.

Optimization of the ratio of detection probe to 605QD

We further optimized the ratio of detection probe to 605QD. In this single QD-based FRET biosensor, multiple glucosylated detection probes can be assembled onto a single 605QD to form a single-donor/multiple-acceptor nanostructure, resulting in improved FRET efficiency. The FRET efficiency in single-molecule detection can be calculated according to equation 3.

$$E = \left(1 - \frac{\sum I_{DA}}{\sum I_D}\right) \times 100\% \qquad (3)$$

where ΣI_{DA} is the sum of 605QD fluorescence intensities in the presence of TET1, and ΣI_D is the sum of 605QD fluorescence intensities in the absence of TET1. As shown in Fig. S4, the FRET efficiency enhances gradually when the detection probe-to-605QD ratio increases from 12 to 48, and reaches a plateau beyond the ratio of 48 (Fig. S4, blue line). FRET efficiency is calculated to be 80% when the detection probe-to-605QD ratio is 48, consistent with the values obtained by fluorescence spectra measurement (77%) and fluorescence lifetime measurement (76%). Moreover, the Cy5 counts exhibit a linear relationship with the detection probe-to-605QD ratio in the range from 12 to 48 (Fig. S4, red line). Thus, the detection probe-to-605QD ratio of 48 is used in the experiments.

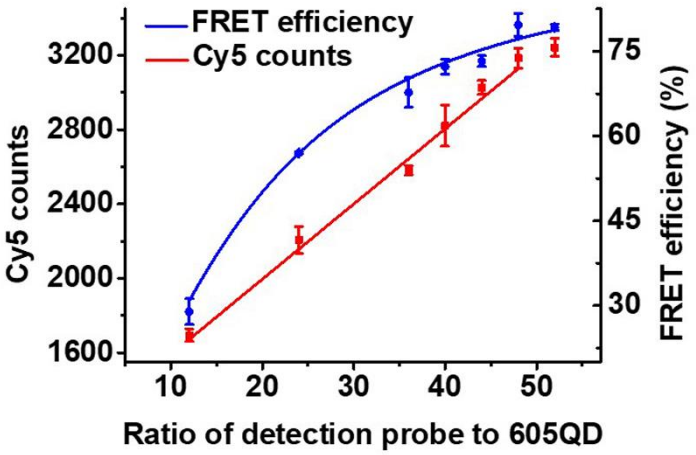

Fig. S4 Variance of Cy5 counts (red line) and FRET efficiency (blue line) as a function of detection probe-to-605QD ratio. Error bars represent standard deviations of three experiments.

Inhibition assay

We used nickel (Ni(II)) ion as the TET1 model inhibitor. To evaluate the effect of Ni(II) ion upon the TET1 activity, different concentrations of Ni(II) ions were incubated with the detection probe and 230 nM TET1 at 37 °C for 10 min. Subsequently, Fe(II) ion was added into the mixture and incubated at 37 °C for 30 min. The relative activity (*RA*) of TET1 is calculated based on equation 4.

$$RA = \frac{N_i - N_0}{N_t - N_0} \times 100\% \qquad (4)$$

where N_0 is the Cy5 counts in the absence of TET1, N_t is the Cy5 counts in the presence of TET1, and N_i is the Cy5 counts in the presence of both TET1 and Ni(II) ion. The IC_{50} value was calculated from the curve of relative activity versus the Ni(II) ion concentration.

Kinetic analysis

To evaluate the kinetic parameters of TET1, we performed the enzyme kinetics study (i.e., Michaelis-Menten plots of the steady-state kinetics). We measured the velocity in the presence of 230 nM TET1 and variable-concentration detection probe substrate at 37 °C for 3 min. As shown in Fig. S5, the initial rate of TET1 enhances with the increasing concentration of detection probe substrate. K_m and V_{max} are calculated according to equation 5.

$$V = \frac{V_{max}[S]}{K_m + [S]} \qquad (5)$$

where V_{max} is the maximum initial velocity, $[S]$ is the concentration of detection probe substrate, and K_m is the

Michaelis-Menten constant corresponding to the semi maximum velocity concentration. The V_{max} is estimated to be 2.2 ± 0.11 nM/min, and K_m is estimated to be 0.46 ± 0.054 μM. The K_{cat} value is calculated based on equation 6.

$$K_{cat} = \frac{V_{max}}{[C]} \qquad (6)$$

where $[C]$ is the concentration of TET1. The K_{cat} value is calculated to be (1.6 ± 0.080) × 10^{-4} s^{-1}.

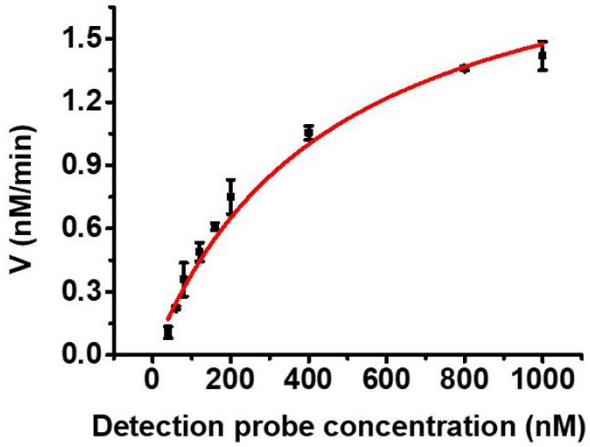

Fig. S5 Measured initial rate in response to different concentrations of the detection probe substrate for enzyme kinetic analysis. The TET1 concentration is 230 nM. Error bars represent standard deviations of three experiments.