Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Multi-cycle signal amplification-mediated single quantum dot nanosensor for PIWI-interacting RNA detection

Qi-qin Ge,[a] Qian Han,[a] Yun Han,[a] Fei Ma, [a,*] Chen-zhong Li,[b,*] and Chun-yang Zhang [a,*]

[a] School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China.

[b] Biomedical Engineering, School of Medicine, The Chinese University of Hong Kong, Shenzhen, 518172, China.

* Corresponding author. E-mail: fei@seu.edu.cn (F. Ma); lichenzhong@cuhk.edu.cn (C.-z, Li); zhangcy@seu.edu.cn (C.-y. Zhang).

EXPERIMENTAL SECTION

Chemicals and Materials. All oligonucleotides (Table S1) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Ampligase® thermostable DNA ligase and its 10× reaction buffer (200 mM Tris-HCl, 250 mM KCl, 100 mM $MgCl_2$, 5 mM NAD, 0.1% Triton® X-100, pH 8.3) were purchased from Epicentre Biotechnologies (Madison, WI, USA). T4 RNA Ligase 2 and 10× reaction buffer (500 mM Tris-HCl, 20 mM $MgCl_2$, 10 mM DTT, 4 mM ATP, pH 7.5) were obtained from New England Biolabs (Ipswich, MA, USA). The streptavidin-conjugated CdSe/ZnS QDs with a maximum emission of 605 nm (Qdot 605 ITK) were obtained from Invitrogen Corporation (California, USA). Human breast cancer cells (MCF-7), human nonsmall cell lung cancer cells (A549), human cervical carcinoma cell line (HeLa cells), human hepatocellular carcinoma cells (HepG2) and human colon cancer cells (HCT 116) were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). RNA 1st Strand cDNA Synthesis Kit and SYBR Green Premix Pro Taq HS qPCR KitIIwere obtained from Accurate Biotechnology Co. Ltd. (Hunan, China). Other chemicals were of analytical grade and used without further purification. The ultrapure water was prepared by a Millipore filtration system (Millipore, Milford, MA, U.S.A.). The human tissues were obtained from the Affiliated Hospital of Weifang Medical University (Weifang, Shandong, China), and the research was approved by the ethics committee of the Affiliated Hospital of Weifang Medical University.

Table S1. Sequences of the oligonucleotides

Name	Sequence (5'-3')
piR-36026	GGC CCC AUG GUG UAA UGG UCA GCA CUC
Sensing probe A	GTG CTG ACC AT/rU/rA
Sensing probe B	P-CAC CAT GGG GC
Reporter probe A	Cy5-GGC CCC ATG GTG
Reporter probe B	P-TAA TGG TCA GCA CTC-Biotin
Reporter probe AB	Cy5-GGC CCC ATG GTG TAA TGG TCA GCA CTC-Biotin
mis-1	GGC CCC AUG GUC UAA UGG UCA GCA CUC
mis-2	GGC CCG AUG GUC UAA UGG UCA GCA CUC
PCR primer	GGC CCC ATG GTG TAA TGG TCA GCA CTC

Gel electrophoresis. The products of ligase amplification reaction were analyzed by 12% desaturating polyacrylamide gel electrophoresis (PAGE) in 1× TBE buffer (9 mM Tris-HCl, pH 7.9, 9 mM boric acid, 0.2 mM EDTA) at a 120 V constant voltage for 50 min at room temperature. The gel was analyzed by a Bio-Rad ChemiDoc MP Imaging System (Hercules, CA, USA).

Fluorescence measurements. Fluorescence emission spectra were recorded by using a FLS1000 photoluminescence spectrometer (Edinburgh Instruments Ltd., Livingston, UK) with both the emission and excitation slits of 5 nm. The excitation wavelength was 488 nm. Data analysis was performed by using the 605QD fluorescence intensity at 605 nm and the Cy5 fluorescence intensity at 670 nm, respectively. The time-correlated single photon counting was employed to measure the fluorescence lifetime of 605QDs.

Single-molecule detection

Total internal reflection fluorescence (TIRF) microscopy (Nikon, Ti-E, Japan) was used for single-molecule imaging, with 10 μL of reaction product being directly dropped onto a glass cover slide for imaging. A sapphire 488nm laser (50 mW, Coherent, USA) was used as the excitation light source to excite the 605QD. The oil immersed objective (CFI Apochromat TIRF 100 ×) was used to collect the emitted photons, and the dichroic mirror was used to divide the fluorescence signal into a Cy5 channel and a 605QD channel. The photos were collected by an EMCCD camera (Hamamatsu Photos K.K., Japan) with an exposure time of 500 ms. ImageJ software was used to count the Cy5 fluorescence spots in the region of interest of 600 × 600 pixels, with Cy5 counts being the average of ten frames for data analysis. Statistical significance was assessed by using two-tailed Student's t-test.

Cell culturing and piRNA extraction

MCF-7 cells, A549 cells, HepG2 cells, Hela cells and HCT-116 cells were cultured in Dulbecco's modified Eagle medium (Invitrogen, USA) containing 1% penicillin-streptomycin (Invitrogen, USA) and 10% fetal bovine serum (FBS, Gibco, USA). All cells were cultured at 37 °C in a humidified chamber with 5% CO_2. The numbers of cells were counted by using a Countstar cell Counter. Cells were collected with trypsinization and washed twice with the ice-cold PBS (pH 7.4, Gibco, USA), followed by centrifugation at 1,000 rpm for 5 min. The collected cells were suspended in 50 μL of lysis buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% NP-40, 0.25 mM sodium deoxycholate, 1.0% glycerol, and 0.1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride), incubated on ice for 30 s for 5 min, and then centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was transferred into a fresh tube and stored at −80 °C. The RNA

concentration was measured by using a NanoDrop 2000c Spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA).

SUPPLEMENTARY RESULTS

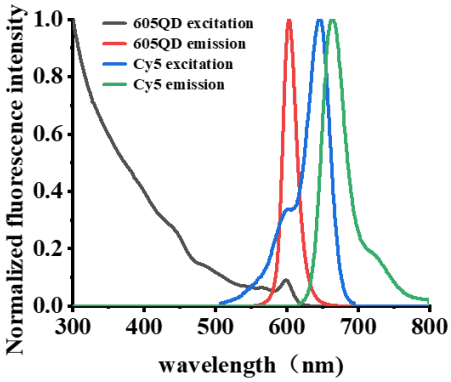

Fig. S1. Normalized excitation and emission spectra of the 605 nm-emitting QDs and Cy5. Black line, excitation spectrum of the 605 nm-emitting QD; red line, emission spectrum of the 605 nm-emitting QD; blue line, excitation spectrum of Cy5; green line, emission spectrum of Cy5.

As shown in Fig. S1, there is no overlap between the 605QD emission and the Cy5 emission, but there is large spectral overlap between the 605QD emission and the Cy5 absorption, indicating that the 605QD and Cy5 can function as the energy donor and the energy acceptor, respectively, to build an efficient FRET system.

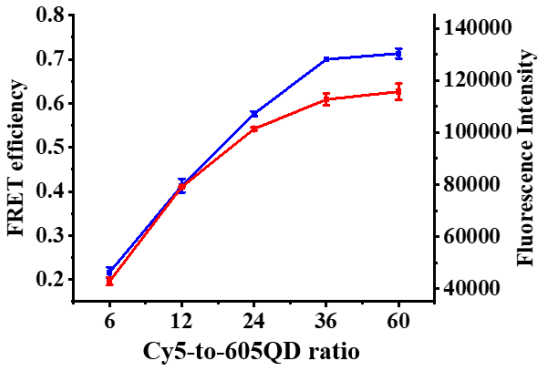

Fig. S2. Variance of FRET efficiency (blue curve) and Cy5 fluorescence intensity (red curve) with the Cy5-to-605QD ratio. The concentration of 605QD is 1.39 nM. Error bars show the standard deviation of three experiments.

To investigate the influence of the Cy5-to-605QD ratio upon the assay performance, varied concentrations of Cy5- and biotin-labeled reporter probe AB were mixed with a fixed concentration of 605QDs (1.39 nM) to obtain the 605QD/reporter probe AB/Cy5 nanostructures with different Cy5-to-605QD ratio, and the fluorescence intensities of both 605QDs and Cy5 at the excitation wavelength of 488 nm were measured. The FRET efficiency (E) is calculated according to equation S1.

$$E\,(\%) = (1 - F/F_0) \times 100 \qquad \text{(S1)}$$

where F is the fluorescence intensity of 605QD in the presence of piRNA, and F_0 is fluorescence intensity of 605QD in the absence of piRNA. The obtained FRET efficiency and Cy5 fluorescence intensity are plotted versus the Cy5-to-605QD ratio. As shown in Fig. S2, the FRET efficiency enhances with the increasing Cy5-to-605QD ratio from 6 to 36, and reaches a plateau beyond the ratio of 36. Moreover, the Cy5 fluorescence enhances with the increasing Cy5-to-605QD ratio from 6 to 36, and reach a plateau beyond the ratio of 36. Therefore, reporter probe A/B-to-605QD ratio of 36 is used in the subsequent researches.

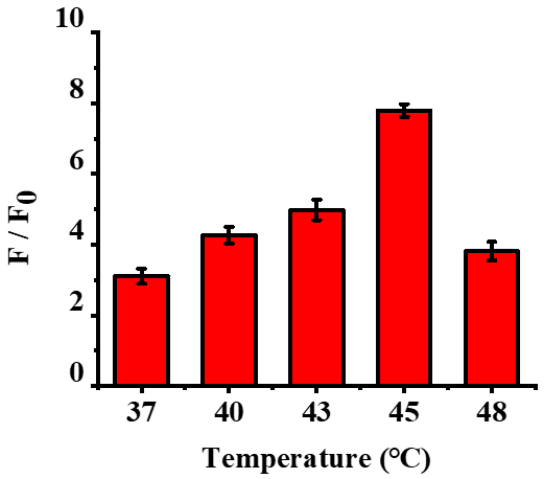

Fig. S3. Variance of F/F_0 value with the ligation temperature. Error bars show the standard deviations of three independent experiments.

We optimized the ligation temperature of ligase chain reaction. As shown in Fig. S3, the F/F_0 value enhances with the reaction temperature from 37 to 45 °C, follow by a decrease beyond 45 °C. Thus, the ligation temperature of 45 °C is used in the subsequent researches.

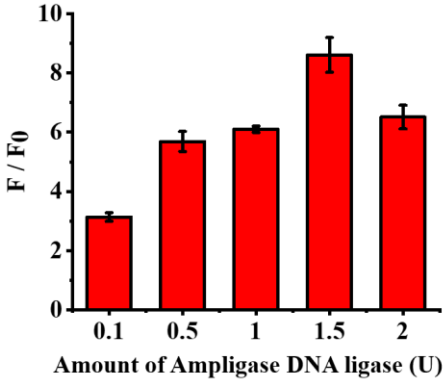

Fig. S4. Variance of F/F_0 value in response to different amounts of Ampligase DNA ligase. Error bars show the standard deviations of three independent experiments.

We further optimized the concentration of Ampligase DNA ligase. As shown in Fig. S4, the F/F_0

value enhances with the increasing concentration of Ampligase from 0.1 to 1.5 U/μL, and levels off at the concentration of 1.5 U/μL. Thus, 1.5 U/μL of Ampligase is used in the reaction system.

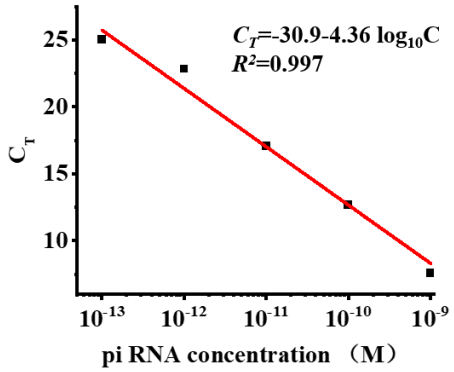

Fig. S5. Linear relationship between the C_T value and the logarithm of piR-36026 concentration. Error bars show the standard deviation of three independent experiments

The standard qRT-PCR was employed to quantify piR-36026 concentration (Fig. S5). After the reverse transcription of target piRNA, the reaction products were subjected to quantitative PCR analysis. The real-time fluorescence measurements in response to different concentrations of piR-36026 were performed with SYBR Green I as the fluorescent indicator. When the piR-36026 concentration increases from 0 to 1 nM, the real-time fluorescence intensity enhances in a sigmoidal fashion. The threshold cycle (C_T) value (i.e., the fractional cycle number at which amount of amplified target reaches a fixed threshold) exhibits a linear correlation with the logarithm of the piR-36026 concentration in the range from 100 fM to 1 nM, and the regression equation is C_T = -30.90 – 4.36 lg $C_{piR\text{-}36026}$ with a correlation coefficient (R^2) of 0.997, where C is the concentration of piR-36026 (M).