Supporting Information

Amplified fluorescence determination of MicroRNAs in Homogeneous solution with cationic conjugated Polymers

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Experimental Section

Materials and Reagents

The PFP was synthesized according to the procedure in the literature.[1] The chemical structure of PFP is shown in Figure S1. Klenow Fragment (3'→5' exo$^-$) was purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Fluorescein-12-dATP was purchased from PerkinElmer Life and Analytical Sciences, Ribonuclease Inhibitor, PAGE-purified DNA, and HPLC-purified miRNA oligonucleotides were purchased from TakaRa Biotechnology Co. Ltd. (Dalian, China). The concentration of the oligonucleotides was determined by measuring the absorbance at 260 nm in a 100 μL quartz cuvette by using a TU 1901 UV-Vis spectrophotometer (Purkinje General Instrument Co., Ltd., Beijing, China).

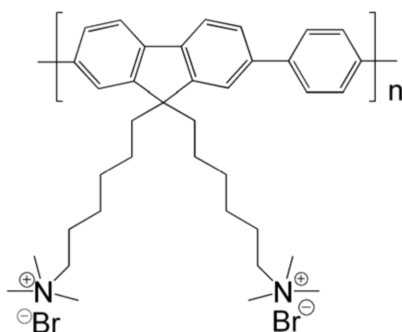

Figure S1. Chemical Structures of PFP

The miRNA-primed extension reactions were carried out in 200 μL PCR microtubes and the reaction temperature was controlled by a T1 Thermal Cycler (Biomera, Germany). All solutions were prepared with DEPC-treated water except 0.2 M PBS buffer (10 mM sodium phosphate buffer containing 0.2 M NaCl, pH 7.4), which was prepared with Millipore filtration

system-purified (18.2 MΩ) and sterilized water.

miRNA assay. In a 200 μL PCR microtube, 4.0 μL DNA probe (1.0×10^{-6} M) and 2.0 μL each miRNA were added into 10 μL buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9). The mixture solution was heated at 70 °C for 3 min, 55 °C for 1.5 h. After cooling to room temperature, 0.5 μL Ribonuclease inhibitor, 1.0 unit (or 2.5 unit) Klenow Fragement (exo^-) and 1.2 μL dATP-Fl (5.0×10^{-6} M) were added in and incubated at 37 °C for 1 h to perform the extension reaction, After cooling to room temperature again, the extension solution was diluted with 0.2 M PBS buffer into 200 μL and 8.0 μL PFP (1.0×10^{-5} M in RUs) was transferred in. Then the fluorescence spectra were measured in a 1 mL quartz cuvette with a Hitachi F-4500 spectrofluorimeter with a xenon lamp excitation source (Tokyo, Japan). The excitation wavelength is 380 nm.

Absorption spectrum of dATP-Fl and emission spectrum of PFP and

Fig. S2 shows the normalized absorption spectrum of dATP-Fl and the emission spectrum of PFP. As illustrated in Fig. S2, the absorption spectrum of dATP-Fl and the emission spectrum of PFP can well satisfy the overlap integral requirement for FRET, indicating that PFP and dATP-Fl can used as an ideal donor-acceptor pair for FRET. Therefore, the efficient FRET from PFP to dATP-Fl can be observed when the dATP-Fl is close to PFP by miRNA extension reaction.

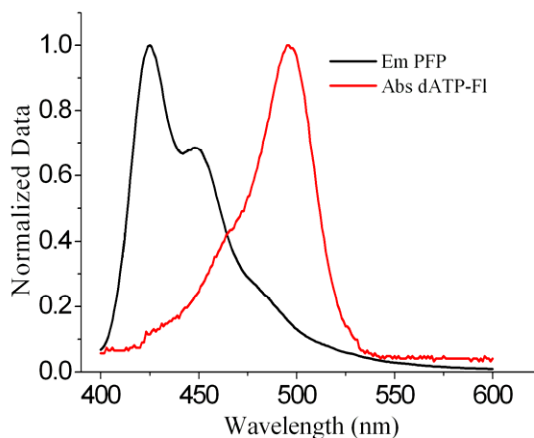

Figure S2. Normalized emission spectrum of PFP(Em PFP) and absorption spectrum of dATP-Fl (Abs dATP-Fl). The excitation wavelength of PFP is 380nm

1 M. Stork, B. S. Gaylord, A. J. Heeger and G. C.Bazan, *Adv. Mater.*， 2002, **14**, 361-366.