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Conjugated Polyelectrolyte/DNA Complexes for Multi-Color and One-Tube SNP Genotyping Assays

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The Design of Multi-step FRET system

To evaluate the multi-step FRET property of CCP/DNA complex, we designed a complex of water-soluble cationic poly [(9,9-bis (6'-N, N,N- trimethylammonium)hexyl)fluorenylene phenylene][4] (PFP) with two separate single-stranded DNAs respectively labeled at 5'-terminus with fluorescein and Cy3 (PFP/ssDNA-Fl/ssDNA-Cy3) (see Figure 1a for chemical structures) where PFP acts as the donor for fluorescein and Cy3, fluorescein acts as the acceptor for PFP and the donor for Cy3 to satisfy the overlap integral requirement for FRET (Figure 1b)[14]. As shown in Figure 1c, the emission maximum of PFP itself in buffer solution appeared at around 425 nm. For PFP/ssDNA-Fl complex, the efficient FRET from PFP to fluorescein (FRET-1 with efficiency of

75%) led to a significant quenching of PFP emission at 425 nm and the appearance of the fluorescein emission at 530 nm upon exciting PFP at 380 nm. For PFP/ssDNA-Cy3 complex, FRET-2 occurred with an efficiency of 69% and the Cy3 emission at 574 nm was observed. For PFP/ssDNA-Fl/ssDNA-Cy3 complex, irradiation at 380 nm exclusively excites PFP, and multi-step FRET occurs: from the PFP to the fluorescein (FRET-1), followed by FRET from the fluorescein to the Cy3 (FRET-3 with efficiency of 85%). The more efficient quenching of PFP than those in PFP/ssDNA-Fl and PFP/ssDNA-Cy3 indicates that direct FRET from PFP to Cy3 (FRET-2) also occurs. The energy transfer process regulates the fluorescence intensity of the PFP, fluorescein and Cy3 components, which provide a chance for multicolor bioassays.

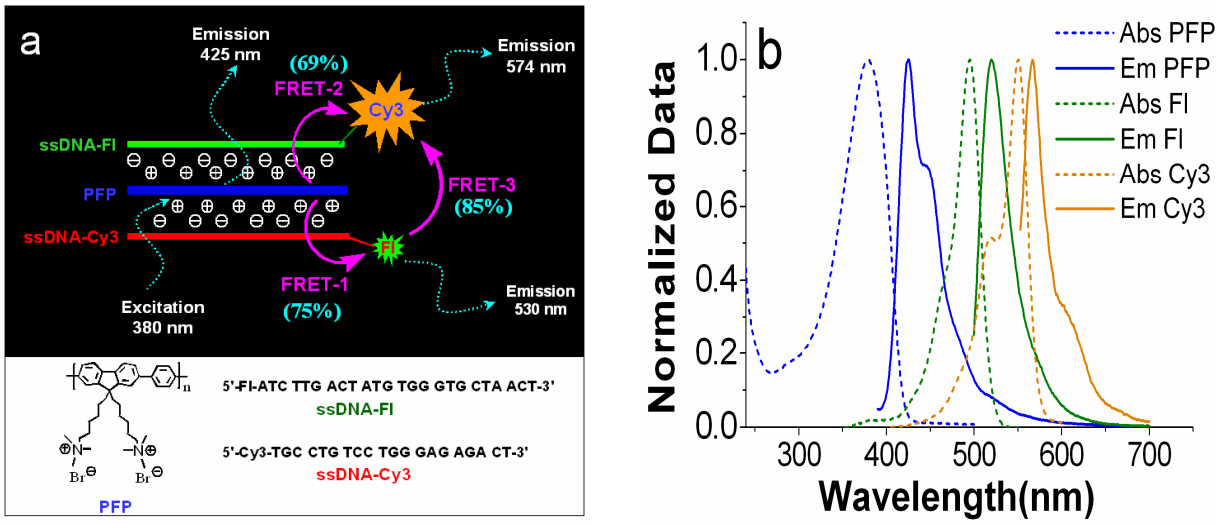

Fig S1. (a) Schematic representation of the PFP/ssDNA-Fl/ssDNA-Cy3 complex with multi-step FRET processes. (b) Normalized absorption and emission spectra of PFP, ssDNA-Fl and ssDNA-Cy3. The excitation wavelength is 380 nm.

Experimental Section

PAGE-purified oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd or SBS Genetech Co., Ltd. The concentrations of oligonucleotides were determined by measuring the absorbance at 260 nm in a 160 μL quartz cuvette. Taq DNA polymerase and shrimp alkaline phosphatase were obtained from TaKaRa Biotechnology Co., Ltd (Dalian, China). The dCTP-Cy3 was purchased from Perkin Elmer. The dUTP-Fl was obtained from Fermentas. Mineral oil was purchased from Sigma. The PFP was prepared according to the procedure in the literature.[1] All base extension reactions were carried out in a Bioer Little Genius TC-25/H Thermal Cycler (Hangzhou, China). UV-vis absorption spectra were taken on a JASCO V-550 spectrophotometer. The fluorescence spectra were measured with a Hitachi F-4500 fluorimeter equipped with a Xenon lamp. The slit width and PMT voltage of the measurements were 5 nm and 700 V, respectively. The heterozygous G/A target is prepared by mixing mutant target and wild target at molar ratio of 1:1.

The base extension reactions were carried out in a total volume of 10 μL containing 1 pmol synthetic DNA target (except for dynamic range experiments), 10 pmol probe, 20 pmol dUTP-Fl, 20 pmol dCTP-Cy3 and 2 unit Taq DNA polymerase in TaKaRa Taq reaction buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3). To prevent the evaporation of water, 20 μL mineral oil was added. The extension solutions were incubated for 30 cycles of 80 ℃ for 15s and 57 ℃ for 1 min. After base extension reaction, 2 μL shrimp alkaline phosphatase (SAP, 0.5 unit/μL) was added to extension solution and incubated at 37 ℃ for 10 min to degrade unreacted dNTPs, and then the reaction solutions were held at 4 ℃. For fluorescence measurements, 5.0 μL extension solution were taken out and diluted with 595 μL HEPES buffer (25 mM, pH 8.0) containing PFP ([PFP] = 2.5×10^{-7} M in RUs), then the fluorescence spectra were measured in 3 mL cuvettes with

an excitation wavelength of 380 nm. The SAP treatment only degrades the negative charge of unreacted dNTPs. After SAP treatment, the electrostatic interaction between PFP and unreacted dNTPs-dye become negligible and the FRET from PFP to unreacted dNTPs-dye doesn't occur. In image experiments, the solutions containing 1 μL extension products and 0.6 μL PFP (5.0×10^{-5} M in RUs) were dotted on a slide with an eppendorf pipette. The image was taken with a Canon IXUS 750 digital camera in a WD-9403F UV Viewing Cabinet (Beijing Liuyi instrument factory, Bejing) equipped with a 550-650 nm band-pass optical filter under 300 nm transmission light.

References

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