Electronic Supplementary Information

Tailorable Optical Properties of Polymer Nanodots for Triple-Mode Fluorescence Detection of Nucleic Acids

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Reagents

Poly(styrene)-graft-poly(ethylene oxide) (PSPEO, Mw/Mn = 1.02) was purchased from Polymer Source Inc. (Canada). Chromoionophore I (CHI) was purchased from Heowns (China). Sodium tetrakis[3,5-bis(trifluoromethyl)-phenyl]borate (NaTFPB), bis(2-ethylhexyl) sebacate (DOS), phosphate and phosphate-buffered saline (PBS) instant granules were purchased from Aladdin (China). Dimethyldioctadecylammonium chloride (R^+Cl^-) was from Bidepharm (China). Rhodamine B, 1-bromooctadecane, cesium carbonate (Cs_2CO_3), N,N-dimethylformamide (DMF) and tetrahydrofuran (THF) were provided by Macklin (China). The synthetic oligonucleotides (Table S1) were obtained from General Biosystems Co. Ltd. (China). The serum samples from healthy individuals were provided by Xuzhou Central Hospital. Deionized water was used after purification by a water purification system from the Thermo Scientific technology Co., Ltd. (Shanghai, China).

Synthesis of octadecyl rhodamine B

Octadecyl rhodamine B was synthesized according to the previously reported method by Viricel et al [1]. NMR characterization is shown in Figure S10. ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): (d, *J* = 7.7 Hz, 1H), 7.76 (dt, *J* = 24.8, 7.4 Hz, 2H), 7.30 (d, *J* = 7.3 Hz, 1H), 7.06 (d, *J* = 9.6 Hz, 2H), 6.81 (d, *J* = 7.3 Hz, 4H), 3.99 (t, *J* = 6.6 Hz, 2H), 3.59 (q, *J* = 7.1 Hz, 8H), 1.39 (q, *J* = 6.8 Hz, 2H), 1.31 (t, *J* = 6.9 Hz, 12H), 1.27 – 1.06 (m, 30H), 0.86 (t, *J* = 6.7 Hz, 3H).

Instrumentation and measurements

Fluorescence anisotropy measurements were carried out on a F4600 spectrophotometer equipped with polarization filters (Hitachi, Japan), while other fluorescence measurements were performed using a G9800A spectrophotometer (Agilent Technologies, USA). Absorption spectra were recorded on a UV-visible absorption spectrometer (UV-2600i, Shimadzu, Japan). The zeta-potentials of nanodots were estimated using a Nano Brook Omni instrument (Brookhaven instruments, USA). The size of nanodots were determined by Nicomp 380/ZLS Zeta Potential/Particle Sizer (PSS Nicomp, Santa Barbara, USA). The transmission electron microscope

(TEM) images were observed at 100 kV using a Tecnai G2 Spirit TWIN TEM (FEI, USA). For TEM sample preparation, a drop of the nanodot suspension was deposited onto carbon-coated copper grids and dried at room temperature, and then negatively stained with 1.5% uranyl acetate for 30 s. The 400 MHz ^{1}H NMR analysis was carried out on a Bruker (Bruker BioSpin GmbH, Rheinstetten, Germany).

FA measurements

The anisotropy value (*r*) was determined as the ratio between the difference in linearly polarized emission components and the total light intensity. The excitation wavelength was 470 nm, and the *r* was calculated at the emission wavelength of 525 nm using the following equations:

$$r = \frac{I_{VV} - G \times I_{VH}}{I_{VV} + 2G \times I_{VH}}$$

and

$$G = \frac{I_{HV}}{I_{HH}}$$

where the subscripts V and H are used to denote the orientation (vertical or horizontal) of the polarizer for the intensity measurements. The first subscript indicates the position of the excitation polarizer, while the second subscript represents the position of the emission polarizer. To account for variations in detection efficiencies of parallel and perpendicular emission pathways, an instrumental correction factor, *G*, was implemented.

The FRET efficiency (*E*) calculation

The FRET efficiencies were calculated from the equation given below:

$$E = 1 - F/F_0$$

where F is the fluorescence intensity of the donor in the presence of acceptor and F_0 is the fluorescence intensity of the donor in the absence of the acceptor.

The Förster distance (R_0) calculation

The Förster distance was calculated to be 5.2 nm for FRET-based quenching assay by the following equation:

$$R_0 = 0.02108(\kappa^2 \Phi_D n^{-4} J)^{1/6} \text{ nm}$$

The relative orientation κ^2 was taken as 2/3 because of random orientation of donor and acceptor. The refractive index (n) was taken as 1.5. The quantum yield (Φ_D) was 0.95 for FAM [2]. The overlap integral *J* was calculated by the following equation:

$$J = \int_{\lambda_1}^{\lambda_2} I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$$

where $I_D(\lambda)$ is the emission intensity from the normalized emission spectrum of donor and $\varepsilon_A(\lambda)$ is the acceptor extinction coefficient.

Estimating the number of CHI molecules in a single PSPEO nanodot

The average size of PSPEO nanodots was estimated to be approximately 23.8 nm by measuring from the TEM image using Nano Measure 1.2 software. Some assumptions were made: (1) The PSPEO nanodot was a perfect sphere. (2) It was assumed that CHI was evenly distributed in the core of the nanodots. The density of PSPEO nanodots was taken as 1.0 g/cm^3. The calculation showed that each PSPEO nanodot contains about 746 CHI molecules. The number of CHI molecules (*N*) encapsulated inside a single nanodot was calculated using the following equation:

$$N = \frac{\rho \times V \times m_C \times N_A}{m_P \times M_W \times 10^{21}}$$

where ρ is the density of the PSPEO nanodot (g/cm^3), V is the volume of the nanodot (nm^3), m_P and m_C is the total mass of PSPEO nanodots and CHI respectively, N_A is the Avogadro constant ($N_A = 6.02 \times 10^{23}$ mol^{-1}) and M_w is the molecular weight of CHI (M_w= 583.85 g/mol).

The quantum yield (Φ) calculation

The relative fluorescence quantum yields (Φ) of the nanodonors were calculated using the following formula:

$$\Phi_u = \Phi_s \times \frac{F_u}{F_s} \times \frac{A_s}{A_u} \times (\frac{n_u}{n_s})^2$$

where *F* is the fluorescence integral region, *A* is the absorbance, *n* is the refractive index, u denotes unknown samples, and s denotes the standard dye (rhodamine B, Φs = 0.31 in water) [3]. The absorbance at the excitation wavelength (530 nm) was kept between 0.02 and 0.05 to avoid inner filter effects and ensure a linear response in intensity.

Fabrication of the nanoprobes

For FA detection, a cocktail was prepared by adding 8.0 mg of PSPEO, 4.0 mg of DOS and 1.2 mg R^+ into 1 mL THF to form a homogeneous solution. Then 62.5 µL of the cocktail was quickly injected into 2.5 mL PBS (pH 7.4) containing 900 nM probe DNAs on a vertex spinning at 1000 rpm. The mixture was blown by compressed air to remove THF, forming a concentrated suspension of the nanoprobes. Based on the classic "sandwich" structure, 67 µL of suspended nanodots, 6 µL of signal DNA (5 µM) and different concentrations of target DNA were mixed to a final volume of 200 µL, and then hybridized at 37 °C in a water bath for 1 h.

For the nanoquencher-based FRET assays, an extra 1.2 mg of CHI was introduced to the cocktail as a quencher. The preparation of suspended nanoprobes and target nucleic acid detection followed the similar procedure as described above. Briefly, 100 µL of suspended nanodots, 9 µL of signal DNA (5 µM) and different concentrations of target DNA were mixed to a final volume of 300 µL,

and then hybridized at 37 °C in a water bath for 1 h. Human serum samples were obtained from healthy volunteers, which were centrifuged at 5000 rpm for 3 minutes and diluted with PBS prior to detection.

For the nanodonor-based FRET assays, a cocktail was prepared by dissolving 8.0 mg PSPEO, 4.0 mg DOS, 1.6 mg R18 and 0.8 mg NaTFPB in 1 mL THF to form a homogeneous solution. Then 50 µL of the cocktail was rapidly injected into 2 mL PBS (pH 7.4) containing 900 nM probe DNA21 while spinning at 1000 rpm on a vortex mixer. Subsequently, 756 µL of TCS-ATTO 647N (5 µM) was added to 1400 µL of suspended nanodots and hybridized at 25 °C for 1 h in a water bath. The solution was then purified by centrifugation using centrifugal filters (Amicon, 0.5 mL, 100 kD) at 5000 g for 10 min. The centrifugation procedure was repeated 3 times to remove free species. Prior to measurement, 77 µL of the above mixture, target DNA21/miR21 and PBS were mixed to a final volume of 300 µL and reacted at 25°C for 1.5 h in a water bath.

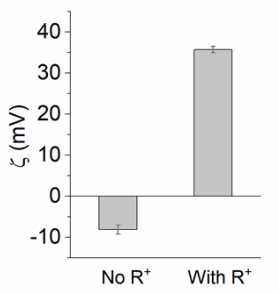

Figure S1. Zeta potentials of PSPEO nanodots in the absence and presence of R^+(weight ratio at 15% to PSPEO).

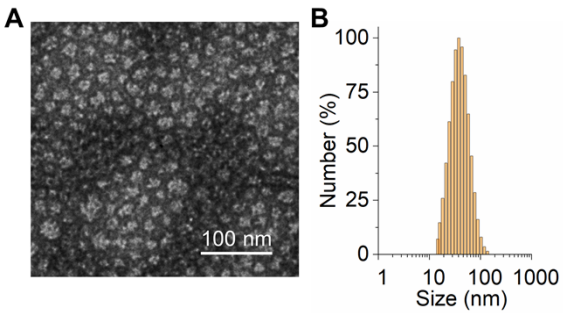

Figure S2. (A) A TEM image of the PSPEO nanoprobes. (B) The DLS distribution of the PSPEO nanoprobes.

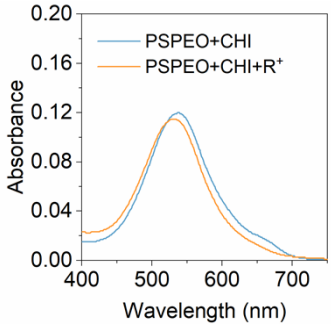

Figure S3. The absorbance spectra of PSPEO nanodots loading with CHI in the absence and presence of R^+.

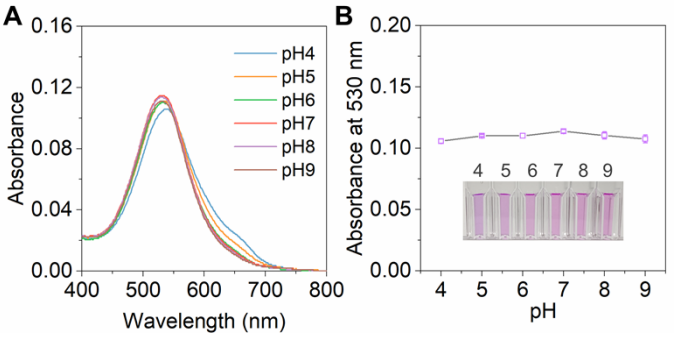

Figure S4. (A) The absorbance spectra of the nanodots containing CHI and R^+ in different pH solutions. (B)The absorbance variation with pH at 530 nm. The inset is a picture of the nanodots in 2.5 mM phosphate buffer (PB) solutions.

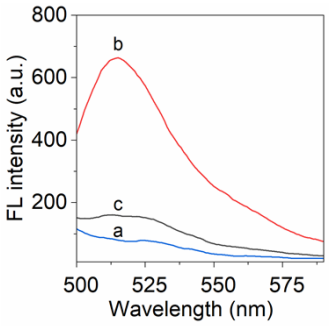

Figure S5. The fluorescence spectra were measured for three samples: (a) the nanoprobes alone (blue line), (b) the nanoprobes with the addition of 150 nM signal DNAs (red line), and (c) the nanoprobes with the addition of 200 nM target DNAs and 150 nM signal DNAs (gray line).

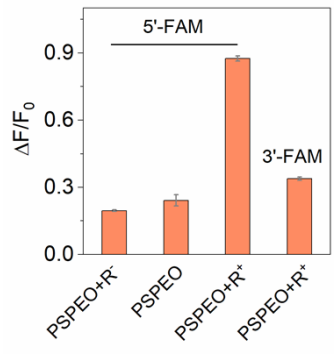

Figure S6. Influence of R^- ($TFPB^-$), R^+ and FAM-labelled DNA position on nanoprobes for FRET quenching assays.

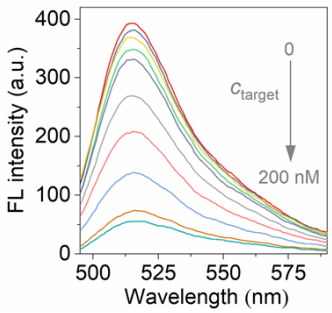

Figure S7. The fluorescence spectra of the nanoprobes with addition of different concentrations of target DNAs (0, 5, 10, 20, 40, 60, 90, 120, 150 and 200 nM) in presence of 150 nM signal DNAs.

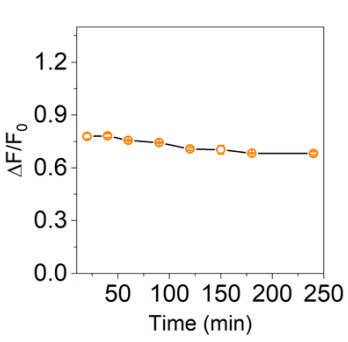

Figure S8. Time-dependent variation of nanoprobe response signal ($\Delta F/F_0$) at room temperature (ca. 25 °C). Target DNA concentration is 150 nM.

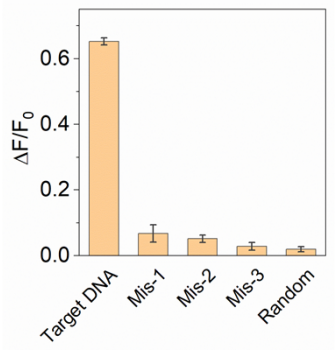

Figure S9. Selectivity of target DNA detection. Concentrations of target DNA, mis-1, mis-2, mis-3, and random DNA at 150 nM.

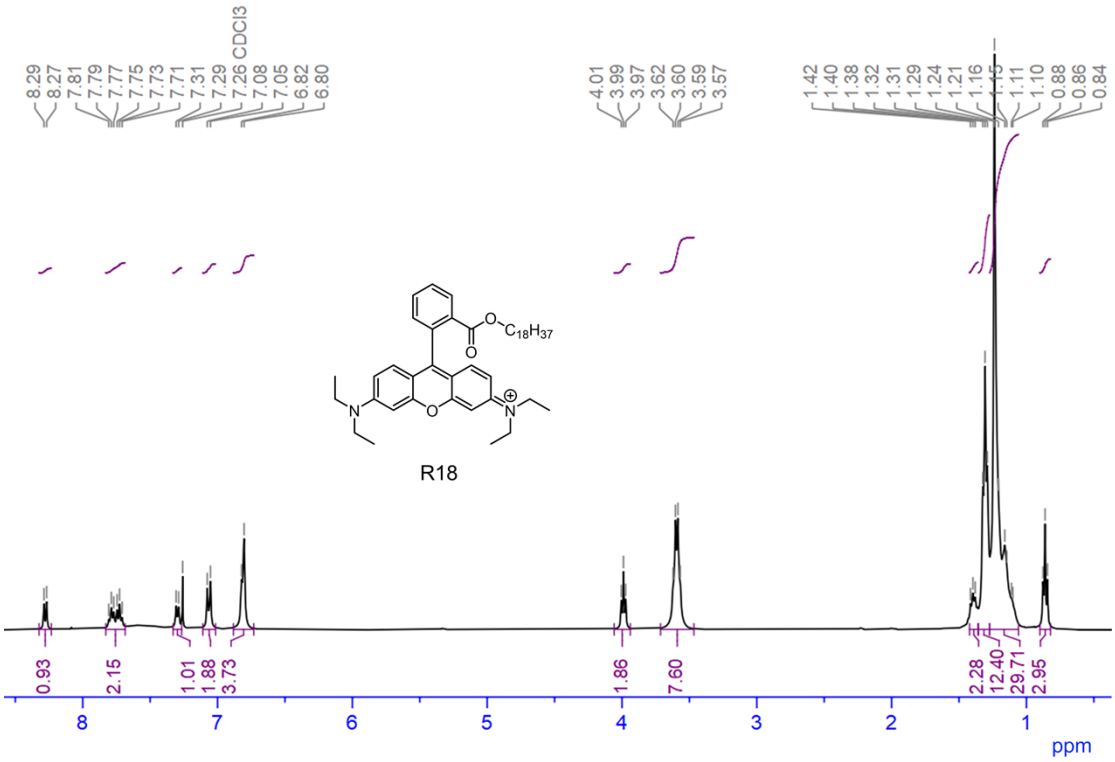

Figure S10. ^{1}H NMR spectrum of R18 in $CDCl_3$.

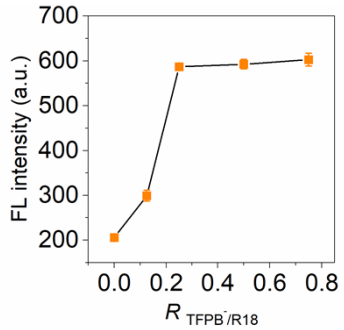

Figure S11. Effect of different $TFPB^-$/R18 ratios on the fluorescence intensity of PSPEO nanodots at a fixed R18 concentration of 20% (weight ratio to PSPEO).

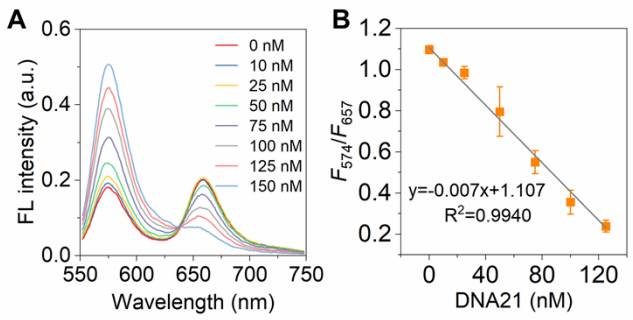

Figure S12. (A) Fluorescence spectra of PSPEO nanodonor-based FRET assay incubated with increasing concentrations of the target DNA21 sequence. (B) FRET ratio as a function of target DNA21 concentration.

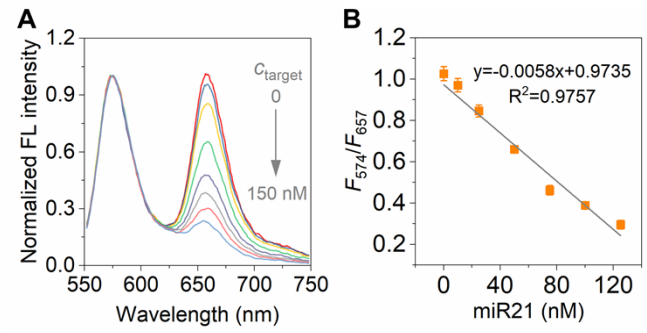

Figure S13. (A) Fluorescence spectra of PSPEO nanodonor-based FRET assay incubated with increasing concentrations (0, 10, 25, 50, 75, 100, 125 and 150 nM) of the target miR21 sequence. The spectra were normalized at the donor intensity. (B) FRET ratio as a function of target miR21 concentration.

Table S1. The oligonucleotides used in the experiments.

Name	Sequence
Probe DNA	5'-cholesterol-CCGTCTGCGGTAT -3'
Target DNA	5'-CCATAACCTTTCCACATACCGCAGACGG-3'
Signal DNA (5'FAM)	5'-FAM-GGAAAGGTTATGG-3'
Signal DNA (3'FAM)	5'-GGAAAGGTTATGG-FAM-3'
Probe DNA21	5'-TCAACATCAGTCTGATAAGCTA- cholesterol-3'
Target DNA21	5'-TAGCTTATCAGACTGATGTTGA-3'
miR21	5'-UAGCUUAUCAGACUGAUGUUGA-3'
TCS-ATTO 647N	5'-ATTO 647N-TAGCTTATCAGA-3'
Mis-1	5'-CCATAACCTTTCCACATACCGCAGATGG-3'
Mis-2	5'-CCATAACCTTTCCACATACCGCACATGG-3'
Mis-3	5'-CCATAACCTTTCCACATACCGGACAAGG-3'
Random	5'-GCTATATTGGTTGCATGTTGTATCATTC-3'

Table S2. Determination results of target DNA in human serum samples.

Added (nM)	Found (nM)	RSD (n=3, %)	Recovery (n=3, %)
30	29.2	9.9	97.5
60	57.9	5.5	96.5

References

1 W. Viricel, A. Mbarek and J. Leblond, *Angew. Chem. Int. Ed.*, 2015, **54**, 12743-12747.

2 M. V. Bharathi, S. De, T. Lavanya, S. Maiti, B. Sarkar, S. K. Ashok Kumar and P. Paira, *New J. Chem.*, 2018, **42**, 9116-9125.

3 D. Yang, H. Wang, C. Sun, H. Zhao, K. Hu, W. Qin, R. Ma, F. Yin, X. Qin, Q. Zhang, Y. Liang and Z. Li, *Chem. Sci.*, 2017, **8**, 6322-6326.