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Upconversion Fluorescence Resonance Energy Transfer Biosensor with Aromatic Polymer Nanospheres as the Lable-Free Energy Acceptor

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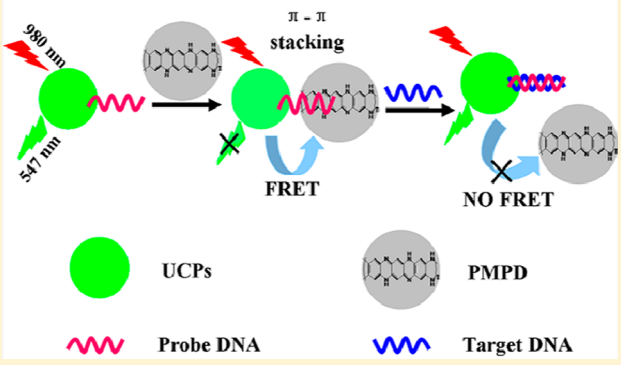
ABSTRACT: We report a new upconversion fluorescence resonance energy transfer (UC-FRET) biosensor using poly-*m*-phenylenediamine (PMPD) nanospheres as the energy acceptor in this paper. A single-stranded DNA (ssDNA) tagged with a sulfydryl group at the 5′-terminus was covalently linked to poly(ethylenimine) (PEI) functionalized upconversion phosphors (UCPs, the energy donor). Because of the π-rich electronic structure of PMPD, self-assembly of the donor and the acceptor was achieved through the $\pi-\pi$ stacking interaction between ssDNA and PMPD. The fluorescence of the donor was quenched by the acceptor in a PMPD-concentration-dependent manner. A maximum quenching degree of 90% was acquired, which was among the highest levels of all previous reports. Upon the formation of double-stranded DNA (dsDNA) between the target DNA and the probe DNA, the energy acceptor was separated from the donor due to the weakened interaction between dsDNA and PMPD. The fluorescence of UCPs was accordingly restored, and a linear response was obtained with the target concentration ranging from 0.1 to 6.0 nM. The limit of detection was calculated as 0.036 nM, which was a highly competitive sensitivity. The sensor also showed high precision, pronounced specificity, and the applicability to complicated sample matrix (human serum). The UCPs−PMPD FRET sensing platform takes advantages of both the optical merits of the upconversion donors and the superquenching ability and good water-solubility of the aromatic polymer nanoparticles. This study will open the opportunity to develop a new class of UC-FRET biosensors.



Upconversion phosphors (UCPs) are kinds of fluorophores codoped with rare-earth ions that with near-infrared (NIR) excitation and anti-Stokes emission (in visible region) hold high photoluminescence intensity, narrow emission band, good chemical stability, and photostability.[1,2] Under the excitation of low-energy NIR photons (normally 980 nm), the autofluorescence from biological macromolecules and scattered excitation light are eliminated, providing enlarged signal-to-background ratio and hence increased sensitivities.[3,4] More importantly, the coexcitation of energy donors and acceptors in fluorescence resonance energy transfer (FRET)-based assays, which is always seen in down-conversion fluorescence applications due to the overlap of their optical spectra, can be avoided because of the very large anti-Stokes shift of UCPs (>300 nm).[5,6] Therefore, UCPs are promising energy donors for FRET assays with the ability to overcome the inherent drawbacks of down-conversion fluorescence, as was first documented by Li and Kuningas in 2005.[4,6] In the past few years, the upconversion (UC)-FRET technique has gained increasing attention and found wide applications in biosensing.[7−12] Especially, the NIR-excitation nature makes UCPs-based analytical methods applicable directly in complicated biological matrixes (such as urine, serum, or whole blood samples).[7,13]

However, a major concern of the UC-FRET technique could be the energy transfer efficiency from the upconversion donors to the energy acceptors. Due to the fact that the upconversion materials currently used are normally particles with diameters of tens of nanometers and that FRET is a highly distance-dependent process, only the emitters (doped rare-earth ions) near the surface of particles can be efficiently quenched. Therefore, the energy transfer efficiency between UCPs and the acceptors, which mainly are organic dyes, is rather low, and accordingly, the fluorescence quenching degree of the donor is limited, which impairs the assay sensitivity.[14,15] To overcome this shortcoming, some kinds of inorganic nanomaterials, like gold nanoparticles (nano-Au),[5,6,8] carbon nanoparticles (CNPs),[16,17] and graphene (or graphene oxide),[18−20] have been introduced into the UC-FRET technique. They were used as the energy acceptors because of their enhanced fluorescence quenching abilities as compared to organic dyes, which may be attributed to the surface energy transfer mechanism that is a longer-distance process following a $1/R^4$ distance dependence (R is the separation distance between the energy donor and the

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energy acceptor).[21,22] Particularly, the newly emerging carbon materials, including the two-dimensional graphene and zero-dimensional carbon particles, also possess the label-free merit in ssDNA or peptide involved assays, which originates from the $\pi-\pi$ stacking interaction between these biomolecules and the carbon atoms with sp^2 orbital hybridization. Owing to the higher florescence quenching power and the label-free feature of these carbon materials, improved analytical performances have been achieved with simplified operations in UC-FRET biosensing.[16−20] Nonetheless, the lack of water-solubility of graphene and CNPs has somewhat restricted the analytical application of these materials. Solubilizers like surfactants are always required to assist dispersion of the nanocolloid in aqueous solutions.

An efficient strategy to acquire water-solubility is to partially oxidize these carbon nanomaterials, so that graphene oxides or CNPs oxides with oxygen-containing groups are generated, exhibiting improved colloidal stability and better dispersibility in aqueous solutions. Unfortunately, such oxidation will inevitably result in the decrease of the percentage of sp^2 carbon atoms and hence will reduce the fluorescence quenching ability. It can be rather tricky to control the oxidation extent and to balance between the two aspects, i.e., the water-solubility and the quenching ability of the carbon materials. Most recently, aromatic polymers (APs) such as poly-*m*-phenylenediamine (PMPD) have been reported to quench the emission of organic fluorophores.[23−25] Since APs possess a large conjugate plane similar to the aforementioned nanosized graphitic carbon materials, it is reasonable to expect their good fluorescence quenching capability toward upconversion donors. Besides, the large conjugate system with a delocalized π-electron structure also enables label-free assembly of ssDNAs and peptides on the surface of APs through $\pi-\pi$ stacking. More attractively, it is very convenient to fuse hydrophilic motifs like carboxyl or amine groups onto the aromatic rings, and thus good water-solubility can be easily obtained with minimum influences on the conjugate structure. Inspired by these prospects, we hereby constructed the FRET-based sensing platform using UCPs and PMPD as the energy donor−acceptor pair and ssDNA as the model analyte. It is revealed that the aromatic polymer nanospheres exhibit strong power to quench the emission of UCPs and allow label-free assembly of the single-stranded DNA chain on the surface, and the abundant amino groups ensure good thermodynamic stability of the nanocolloid. These merits in combination endow the new UC-FRET platform with satisfying analytical performances.

EXPERIMENTAL SECTION

Materials and Reagents. Poly(ethylenimine) (PEI, $M_{average}$ = 25 000) and sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC) were from Sigma-Aldrich. IgG antibody and bovine serum albumin (BSA) were obtained from Zhongshan Golden Bridge Biotechnology Co., Ltd. (Beijing, China). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was from Alfa Aesar. All oligonucleotides were supplied by Sangon Biotechnology Co. Ltd. (Shanghai, China). Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were of analytical grade and were used without further purification.

The sequences of oligonucleotides used in this work are as follows:

Probe DNA: 5′-SH-GAGTTACCGCATAGTAAGCA-CATT-3′
Target DNA: 5′-AATGTGCTTACTATGCGG-TAACTC-3′
Single-base mismatched chain: 5′-AATGTGCT-TACGTGCGGTAACTC-3′ (mismatch underlined)
Noncomplementary chain: 5′-TTTTTTTTTTTTTTT-TTTTTTTTTT-3′

Instrumentations. The crystal phase of UCPs was characterized by an X-ray diffractometer (XRD, Bruker D8 Discover) with a 2θ range from 10° to 70° at a scanning rate of 4°/min, with Cu Kα irradiation (k = 1.5406 Å). The size and morphology of PEI-coated UCPs were identified by a JEM-2010 transmission electron microscope (TEM) with an accelerating voltage of 200 kV. FT-IR spectra of PEI−UCPs were measured on a Magan-IR spectrometer 500 (Nicolet) with the KBr pellet technique. Scanning electron microscopy (SEM) (FEI Quanta 200) was utilized to identify the size and morphology of PMPD. The absorption spectrum of PMPD nanospheres was recorded with a UV-2550 UV/vis spectrometer (Shimadzu). A 980 nm diode laser (Beijing Hi-Tech Optoelectronic Co., Ltd.) was used as the excitation source, with the power being set at 800 mW. The upconversion fluorescence was measured with a DCS200PC single-photon counter by an Omni-λ300 monochromator (Beijing Zolix Instruments Co., Ltd.).

Synthesis of PEI-Coated $NaYF_4$:Yb,Er Upconversion Phosphors. PEI-modified Yb,Er-codoped $NaYF_4$ water-soluble UCPs were synthesized using a one-pot hydrothermal method as described in our previous works.[17] Briefly, 0.25 mmol of lanthanide oxides Ln_2O_3 (Y:Yb:Er = 0.78:0.2:0.02 in mol) was dissolved in hot nitric acid (65 °C) to acquire $Ln(NO_3)_3$, and the solvent was evaporated after a 6 h reaction. The prepared nitrate salts were added to an aqueous solution containing 340 mg of PEI. Thereafter, another aqueous solution containing 0.126 g of NaF was added to the above solution under vigorous stirring. The whole mixture (36 mL, $V_{ethanol}/V_{water}$ = 1.0) was transferred into a 50 mL Teflon autoclave and heated to 200 °C for a 10 h hydrothermal treatment. After the autoclave cooled down to room temperature naturally, a precipitate was harvested by centrifuging and washed several times with ultrapure water and absolute ethanol. The product was dried under vacuum before use.

Preparation of PMPD Nanospheres. PMPD nanospheres were synthesized by room-temperature chemical oxidation polymerization of *m*-phenylenediamine (MPD) monomers with ammonium persulfate (APS).[24] Typically, 0.72 mL of APS solution (0.5 M) was mixed with 10 mL of *N*-methylpyrrolidone, and then 1.2 mL of 0.1 M MPD aqueous solution was quickly injected to the above mixture under vigorous stirring. After a 24 h reaction at room temperature, a black precipitate was obtained by centrifuging. The precipitate was washed three times with ethanol and water, respectively. The product was dried under vacuum and weighed. Finally, the precipitate was diluted in 1 mL of water (ca. 0.5 mg/mL).

Attachment of the Probe DNA to UCPs. Sulfydryl-tagged probe ssDNA was covalently conjugated with UCPs using Sulfo-SMCC as the cross-linking agent. In brief, 2 mg of PEI−UCPs was mixed with 5 mL of PB buffer solution (pH 7.4, 10 mM), followed by adding 0.2 mg of Sulfo-SMCC to the mixture. After 1 h of reaction, the mixture was centrifuged and washed three times with PB buffer solution to remove excess

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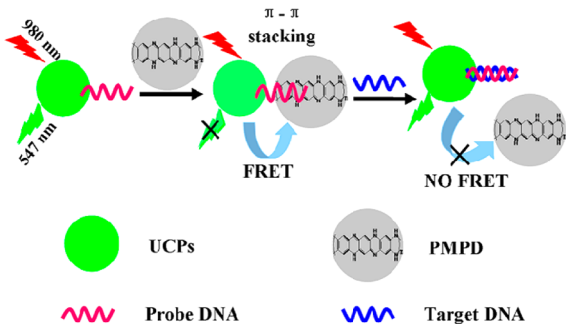
Sulfo-SMCC. The maleimide-activated UCPs were diluted in 2 mL of HEPES buffer solution and incubated with SH-DNA (10 μM) under gentle shaking overnight at room temperature. The UCPs-ssDNA conjugate was harvested with centrifugation and washing. Finally, the product was diluted with 2 mL of HEPES buffer solution and stored at 4 °C for further use.

Upconversion Fluorescence Experiments. To investigate the fluorescence quenching degree, a fixed amount of UCPs–probe DNA (0.005 mg/mL) was incubated with an increasing amount of PMPD at room temperature for 2 h, and the mixtures were then subject to fluorescence measurements. The same experiment was also done with a bare UCPs sample (without linking the probe DNA) to test nonspecific quenching. In a typical UC-FRET assay procedure, UCPs–probe DNA (0.005 mg/mL) and various concentrations of target DNA were mixed in tubes. After adjusting the total volume to 740 μL with HEPES buffer solution, the mixtures were heated at 90 °C for 3 min and cooled down to room temperature naturally. Thereafter, 60 μL of PMPD was individually added to the above mixtures. After 2 h of incubation at room temperature, the upconversion fluorescence was recorded. To examine the specificity of the UCPs–PMPD sensor, a single-base mismatched DNA chain, a noncomplementary chain, and some other biomolecules were added in place of the target DNA following the identical procedure. To verify the ability of the method to resist background interference, the target DNA detection was also performed in 100-fold diluted serum samples. In the upconversion fluorescence measurements, the samples were excited with a 980 nm laser, and the emission intensity at 547 nm was taken for quantification.

RESULTS AND DISCUSSION

Principle of the UC-FRET Sensor and Characterizations of the Materials. Taking the advantage of the large conjugate structure of PMPD, the sensor is set up by coating the probe ssDNA on the surface of UCPs, followed by simply mixing the UCPs–probe DNA complex with PMPD (Scheme 1). That is, only a one-step label is involved and no more chemical assembling or packing is needed for the sensor construction. The donor–acceptor assembly is mediated by the π–π stacking between the ssDNA chain and PMPD, which initiates the nonradiative energy transfer from UCPs to PMPD and leads to the quenching of the donor fluorescence. Upon the hybridization between the target DNA and the probe DNA, dsDNA forms with sharply reduced π–π stacking interaction on PMPD surface. The fluorescence of UCPs is therefore restored in response to the release of the quencher, which makes the foundation of the hybridization assay. In addition to the quantification of the target DNA with the sequence complementary to the probe DNA, the sensor is also designed to discriminate noncomplementary or mismatched chains. Moreover, because of the very flexible configuration of the FRET system, further extensions to other sensing targets can be achieved by coupling specific probes (such as aptamers and peptide substrates) with UCPs.

Scheme 1. Schematic Illustration (not to real scale) of the Upconversion FRET Sensor with UCPs and PMPD Nanospheres as the Energy Donor–Acceptor Pair



To realize the above design, water-soluble upconversion phosphors (Yb,Er-codoped $NaYF_4$) coated with PEI and PMPD nanospheres were first synthesized. The UCPs nanoparticles exhibited approximately spherical morphology and good dispersibility in aqueous solution, with the diameter distribution ranging from 20 to 40 nm (Figure 1A). The XRD pattern (Figure 1B) showed that the crystal phase of the phosphors was mainly hexagonal phase, which guaranteed the high luminescence efficiency of the prepared UCPs.[26,27] Although the absolute quantum yield (QY) of UCPs in aqueous solutions is usually low and the QY value tends to decrease rapidly with reducing the size of material, the overall photoluminescence intensity of UCPs is nevertheless high enough. In combination with their pronounced photostability and the ability to eliminate background signal, UCPs are capable of providing satisfying assay sensitivities in biological sample matrixes. Despite the better water-solubility of carboxyl, amine was selected as the functional group of the phosphors considering the fact that the acceptor (poly-*m*-phenylenediamine) also carries amine, so that the possible electrostatic-effect-induced nonspecific donor–acceptor adsorption is precluded. The FT-IR spectrum of the PEI-coated UCPs (Figure 1C) confirmed the existence of $-NH_2$ groups on the surface, which exhibited characteristic absorption peaks of PEI molecules, including the methylene asymmetric and symmetric C–H stretching (2960, 2850 cm^{-1}), amine N–H bending (1640 cm^{-1}), and amide bonds internal vibration (1380 cm^{-1}). These amine groups were also utilized to covalently link the sulfydryl-tagged probe DNA to the surface of the donor. As to the energy acceptor, the as-prepared PMPD nanoparticles were in uniform spherical shape with an average diameter of ca. 40 nm (Figure 1D), with favorable solubility in water. Compared to the previous several reports where PMPD nanorods[24] or nanobelts[23] were used in sensing, the nanospheres with symmetrical morphology and relatively smaller size should have better colloidal stability, which can be beneficial to homogeneous bioassays. The UV–vis spectrum (Figure 1E) showed strong wide-band absorption (from 200 to 700 nm) of the aromatic polymer nanospheres, making it a potential dark quencher to a wide variety of fluorescence donors, including the upconversion phosphors used in this study.

Quenching of the UCPs Fluorescence by PMPD. Upon incubating the UCPs–probe DNA conjugate (with a fixed amount, 0.005 mg/mL) with the PMPD solution, the upconversion fluorescence of UCPs was quenched in a PMPD-concentration-dependent manner (Figure 2A). The quenching efficiency was calculated using the equation $(F_0 - F)/F_0$, where F_0 and F represent the upconversion fluorescence intensity at 547 nm in the absence and presence of PMPD, respectively. The maximum quenching degree reached to ca. 90% with 0.038 mg/mL PMPD, and a platform was observed with further increasing the concentration of PMPD (inset in Figure 2A). Such a quenching degree is significantly higher than

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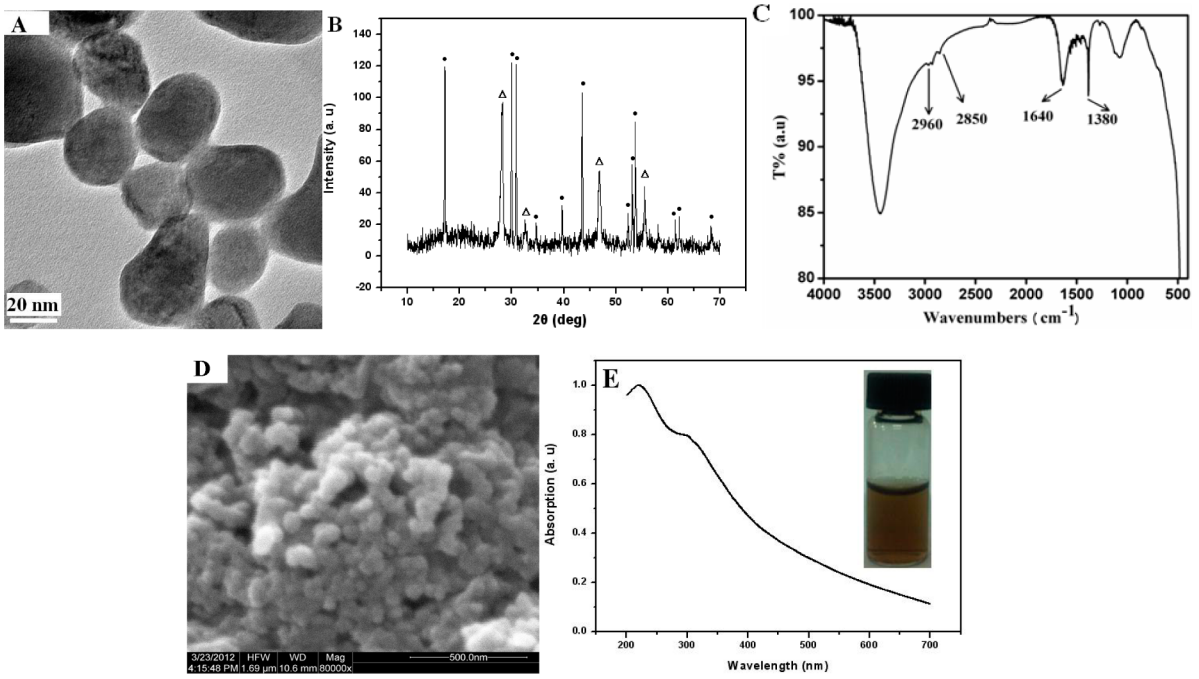

Figure 1. (A) TEM image of PEI-coated $NaYF_4$:Yb,Er upconversion phosphors. (B) XRD pattern of $NaYF_4$:Yb,Er nanocrystals: Δ, cubic phase (JCPDS file no. 77-2042); ●, hexagonal phase (JCPDS file no. 28-1192). (C) FT-IR spectrum of PEI–UCPs. (D) SEM image of the as-prepared PMPD nanospheres. (E) UV–vis absorption spectrum of PMPD nanospheres with a concentration of 0.025 mg/mL. Inset: photograph of 0.05 mg/mL PMPD aqueous solution.

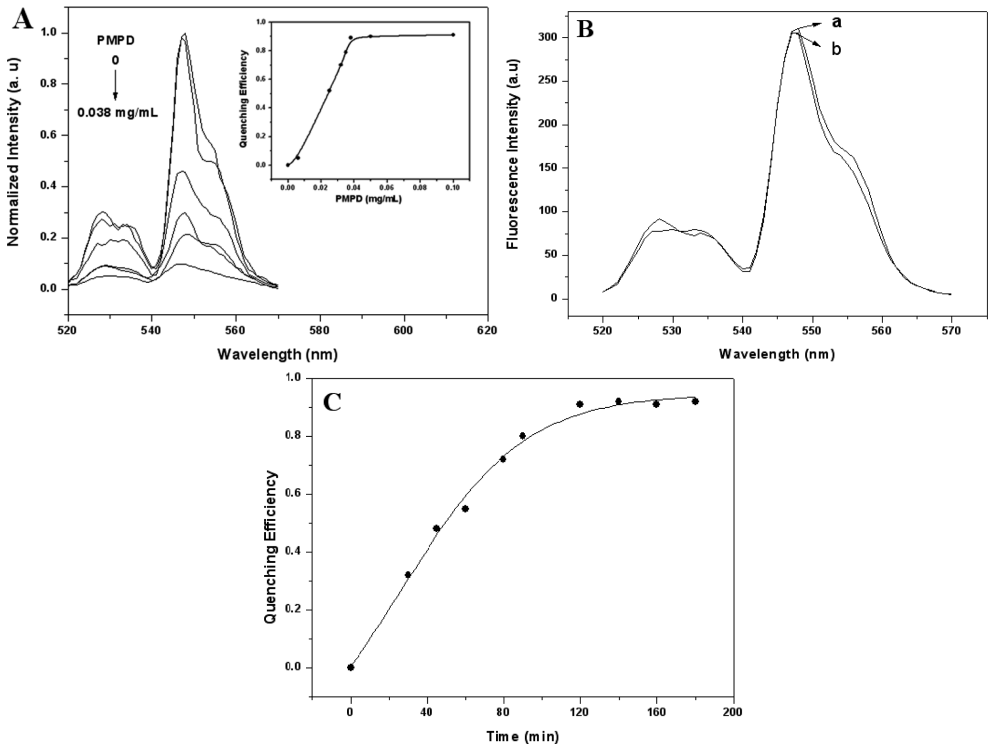

Figure 2. (A) Fluorescence quenching of UCPs–probe DNA (0.005 mg/mL) with various concentrations of PMPD. Inset: fluorescence quenching degree versus PMPD concentration (0, 0.006, 0.025, 0.032, 0.035, 0.038, 0.05, and 0.10 mg/mL). (B) Upconversion fluorescence spectra of 0.005 mg/mL PEI-coated UCPs in the absence (curve a) and presence (curve b) of 0.038 mg/mL PMPD. (C) Time dependence of the fluorescence quenching degree with 0.005 mg/mL UCPs–probe DNA and 0.038 mg/mL PMPD. All experiments were performed in HEPES buffer (10 mM, 50 mM NaCl, pH 7.0) under excitation at 980 nm.

all the reported upconversion FRET pairs with organic dyes as the energy acceptors, which were generally lower than 50%.[28] It is also comparable to or better than most cases using gold nanoparticles or carbon nanomaterials as the acceptor of UCPs. Taking into account the avoidance of the acceptor labeling (which is necessary for gold nanoparticles) and the exemption

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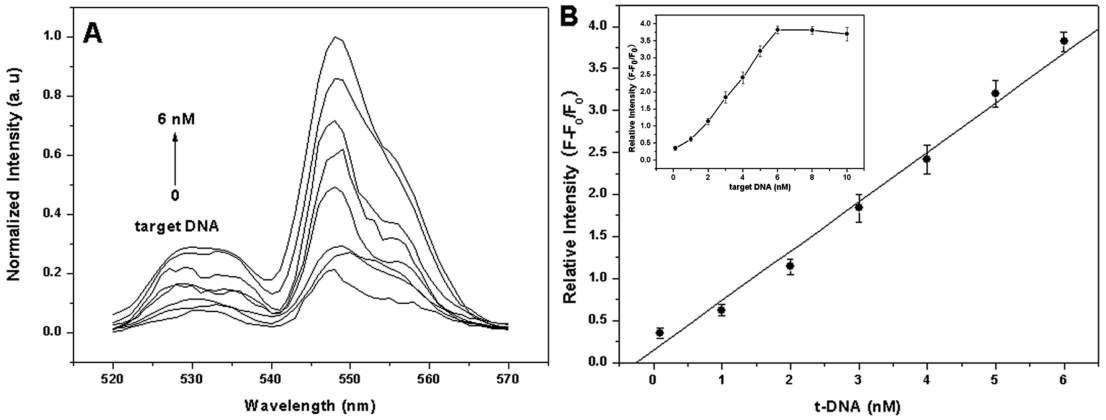

Figure 3. (A) The upconversion fluorescence restoration of the UCPs–PMPD sensor with various concentrations of target DNA. (B) The linear relationship between the fluorescence recovery (at 547 nm) and the concentration of target-DNA within the range of 0.1–6.0 nM, Inset: The fluorescence recovery of the sensor in the presence of target DNA ranging from 0.1 to 10.0 nM; data are presented as average ± SD from three independent measurements. All experiments were performed in HEPES buffer (10 mM, 50 mM NaCl, pH 7.0) under excitation at 980 nm, in the presence of 0.005 mg/mL UCPs–probe DNA conjugate and 0.038 mg/mL PMPD. Fluorescence was given in normalized form.

of solubilization with surfactants (which is always required for carbon nanomaterials), the aromatic polymer nanosphere can be a promising alternative as the energy acceptor to construct UC-FRET sensors. The fluorescence quenching is attributed to the π-rich electronic structure of PMPD and the $\pi-\pi$ stacking between ssDNA and PMPD,[29] which took the energy donor and acceptor in close proximity. To further confirm this and exclude other possibilities, a control experiment was conducted where bare UCPs (without linking the probe DNA) at 0.005 mg/mL were incubated with 0.038 mg/mL PMPD. In this case, the emission of UCPs remained nearly unchanged (Figure 2B), suggesting that the quenching was exclusively induced by the specific interaction between the probe DNA and the acceptor. The time course of the quenching experiment (Figure 2C) revealed that a 2 h reaction was needed to achieve the quenching equilibrium, which is similar to the cases with carbon nanomaterials as the acceptor.[16,19]

Target DNA Sensing with the UCPs–PMPD FRET Sensor. For a FRET-based analytical method when the fluorescence quenching-and-restoration protocol is adopted for quantitative assay, a higher quenching degree generally foreshows a higher sensitivity due to the lowered background. With the 90% of quenching extent in hand, we then tested the as-built UC-FRET sensor for its analytical performances. With the introduction of an increasing amount of the target ssDNA, which has a sequence complementary to the probe DNA, the emission intensity of UCPs was restored gradually as expected (Figure 3A). This is explained by the separation of the acceptor from the donor, resulting from the very weak interaction between the dsDNA (formed by the hybridization between the target and the probe ssDNAs) and the PMPD particles, which is similar to the weakened $\pi-\pi$ stacking interaction between double-stranded oligonucleotides and graphitic carbon materials that has been well-documented. The fluorescence restoration was linearly related to the concentration of the target in the range from 0.1 to 6.0 nM. No further fluorescence recovery was observed at concentrations higher than 6.0 nM, which was probably a result from the saturation of the hybridization (inset in Figure 3B). The calibration curve also shows quite low standard deviation levels, demonstrating good precision of the detection. This may also benefit from the favorable water-solubility of the nanomaterials used, which ensures the thermodynamic stability of the assay solutions. The limit of detection (LOD) of the target DNA calculated according to the $3s_b/m$ criterion, where m is the slope for the range of the linearity used and s_b is the standard deviation of the blank (n = 7), was 0.036 nM. Such a LOD level was at least 1 order of magnitude lower than that of the reported UC-FRET-based hybridization assays.[9,10,30,31]

To assess the specificity of this UC-FRET sensor for target ssDNA, the responses of the sensor toward some other biomolecules (proteins) and noncomplementary ssDNA chains were investigated. As shown in Figure 4, the single-base

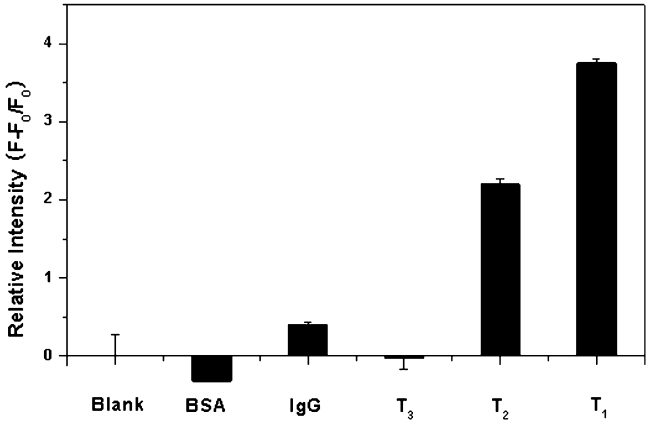

Figure 4. Relative fluorescence intensity $(F - F_0/F_0)$ of the UC-FRET biosensor in the presence of different substances. The blank was the UCPs–PMPD biosensor and F_0 is the intensity of the blank. The concentration of all tested species was 6.0 nM. Experiments were performed in HEPES buffer (10 mM, 50 mM NaCl, pH 7.0) under excitation at 980 nm. T_1: target DNA, T_2: single-base mismatched DNA, T_3: noncomplementary target.

mismatched DNA chain caused significantly lower (by about 40%) restoration of the donor emission than the target DNA, while the totally noncomplementary chain was unable to recover the donor fluorescence. The other protein molecules (BSA and IgG) did not cause an obvious signal change either. The results have shown a pronounced specificity of the UC-FRET sensor for the target DNA, together with a promising ability to discriminate even single-base mismatched oligonu-

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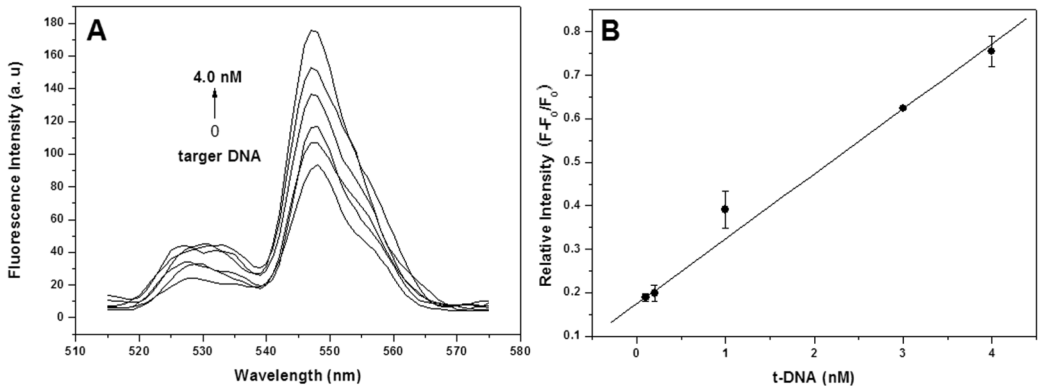

Figure 5. (A) The upconversion fluorescence emission of the UCPs−PMPD sensor with various concentrations of target DNA in 100-fold diluted human serum. (B) The linear relationship between the fluorescence recovery (at 547 nm) and the concentration of target DNA within the range of 0.1−4.0 nM; data are presented as average ± SD from three independent measurements. Fluorescence was excited at 980 nm, and 0.005 mg/mL UCPs−probe DNA conjugate and 0.038 mg/mL PMPD were used.

cleotides. Since upconversion fluorescence has been best known for its competence in homogeneous assay in a complicated sample matrix, we applied the developed sensor to spiked serum samples to show the sensitivity and specificity of this system. As shown in Figure 5, a linear calibration was also obtained for the target DNA in the concentration range of 0.1−4.0 nM. The limit of detection was calculated as 0.07 nM. It is seen that the linear range was relatively narrower and the sensitivity was lower than that in aqueous buffer. Although the designed target ssDNA does not have much clinical significance in this case (since the hybridization assay was just selected as a model for evaluating the new UC-FRET sensor), the results indicate that the UCPs−PMPD FRET sensor is applicable in such complicated matrix, which lays the foundation of biological and clinical applications of the UC-FRET sensing platform with PMPD as the energy acceptor.

■ CONCLUSIONS

We have developed a new upconversion FRET sensing platform using aromatic polymer nanospheres (poly-*m*-phenylenediamine, PMPD) as the energy acceptor. The π-rich electronic structure of PMPD enables the label-free assembly of the acceptor through $\pi-\pi$ stacking interaction. PMPD quenched the fluorescence of the donor by up to 90% without any nonspecific quenching, which effectively resolved the problem of the low energy-transfer efficiency of upconversion donors. Moreover, the good water-solubility of PMPD ensured the thermodynamic stability of the assay solutions. Benefiting from these merits of PMPD, high sensitivity and precision were achieved in quantitative detection of target DNA. The sensor also showed good specificity and the applicability to complicated sample matrix. Because of the very simple and flexible configuration of the sensor, it can be readily extended to other targets by linking specific ligands or substrates on the surface of the donor. This study may open the way for a new class of UC-FRET biosensors with wide applications.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Zhou, J.; Liu, Z.; Li, F. *Chem. Soc. Rev.* **2012**, *41*, 1323−1349.
(2) Haase, M.; Schäfer, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 5808−5829.
(3) Liu, L.; Dong, X.; Lian, W.; Peng, X.; Liu, Z.; He, Z.; Wang, Q. *Anal. Chem.* **2010**, *82*, 1381−1388.
(4) Kuningas, K.; Rantanen, T.; Ukonaho, T.; Lövgren, T.; Soukka, T. *Anal. Chem.* **2005**, *77*, 7348−7355.
(5) Peng, J.; Wang, Y.; Wang, J.; Zhou, X.; Liu, Z. *Biosens. Bioelectron.* **2011**, *28*, 414−420.
(6) Wang, L.; Yan, R.; Huo, Z.; Wang, L.; Zeng, J.; Bao, J.; Wang, X.; Peng, Q.; Li, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 6054−6057.
(7) Kuningas, K.; Ukonaho, T.; Päkkilä, H.; Rantanen, T.; Rosenberg, J.; Lövgren, T.; Soukka, T. *Anal. Chem.* **2006**, *78*, 4690−4696.
(8) Wang, M.; Hou, W.; Mi, C. C.; Wang, W. X.; Xu, Z. R.; Teng, H. H.; Mao, C. B.; Xu, S. K. *Anal. Chem.* **2009**, *81*, 8783−8789.
(9) Zhang, P.; Rogelj, S.; Nguyen, K.; Wheeler, D. *J. Am. Chem. Soc.* **2006**, *128*, 12410−12411.
(10) Chen, Z.; Chen, H.; Hu, H.; Yu, M.; Li, F.; Zhang, Q.; Zhou, Z.; Yi, T.; Huang, C. *J. Am. Chem. Soc.* **2008**, *130*, 3023−3029.
(11) Xie, L.; Qin, Y.; Chen, H. Y. *Anal. Chem.* **2012**, *84*, 1969−1974.
(12) Deng, R.; Xie, X.; Vendrell, M.; Chang, Y. T.; Liu, X. *J. Am. Chem. Soc.* **2011**, *133*, 20168−20171.
(13) Kuningas, K.; Päkkilä, H.; Ukonaho, T.; Rantanen, T.; Lövgren, T.; Soukka, T. *Clin. Chem.* **2007**, *53*, 145−146.
(14) Rantanen, T.; Järvenpää, M. L.; Vuojola, J.; Kuningas, K.; Soukka, T. *Angrew. Chem. Int. Ed.* **2008**, *47*, 3811−3813.
(15) Soukka, T.; Rantanen, T.; Kuningas, K. *Ann. N.Y. Acad. Sci.* **2008**, *1130*, 188−200.
(16) Wang, Y.; Bao, L.; Liu, Z.; Pang, D. *Anal. Chem.* **2011**, *83*, 8130−8137.
(17) Wang, Y.; Shen, P.; Li, C.; Wang, Y.; Liu, Z. *Anal. Chem.* **2012**, *84*, 1466−1473.

(18) Liu, C.; Wang, Z.; Jia, H.; Li, Z. *Chem. Commun.* **2011**, *47*, 4661−4663.
(19) Zhang, C.; Yuan, Y.; Zhang, S.; Wang, Y.; Liu, Z. *Angew. Chem., Int. Ed.* **2011**, *50*, 6851−6854.
(20) Wu, S.; Duan, N.; Ma, X.; Xia, Y.; Wang, H.; Wang, Z.; Zhang, Q. *Anal. Chem.* **2012**, *84*, 6263−6270.
(21) Yun, C. S.; Javier, A.; Jennings, T.; Fisher, M.; Hira, S.; Peterson, S.; Hopkins, B.; Reich, N. O.; Strouse, G. F. *J. Am. Chem. Soc.* **2005**, *127*, 3115−3119.
(22) Swathi, R. S.; Sebastian, K. L. *J. Chem. Phys.* **2009**, *130*, 086101.
(23) Wang, L.; Zhang, Y.; Tian, J.; Li, H.; Sun, X. *Nucleic. Acids. Res.* **2011**, *39*, e37−e42.
(24) Zhang, Y.; Sun, X. *Chem. Commun.* **2011**, *47*, 3927−3929.
(25) Li, H.; Wang, L.; Zhai, J.; Zhang, Y.; Tian, J.; Sun, X. *Macromol. Rapid Commun.* **2011**, *32*, 899−904.
(26) Kramer, K. W.; Biner, D.; Frei, G.; Güdel, H. U.; Hehlen, M. P.; Lüthi, S. R. *Chem. Mater.* **2004**, *16*, 1244−1251.
(27) Boyer, J. C.; van Veggel, F. C. J. M. *Nanoscale* **2010**, *2*, 1417−1419.
(28) Yuan, Y.; Liu, Z. *Chem. Commun.* **2012**, *48*, 7510−7512.
(29) Varghese, N.; Mogera, U.; Govindaraj, A.; Das, A.; Maiti, P. K.; Sood, A. K; Rao, C. N. R. *ChemPhysChem* **2009**, *10*, 206−210.
(30) Zhang, P.; Rogelj, S.; Nguyen, K.; Wheeler, D. *Langmuir* **2006**, *25*, 6024−6027.
(31) Rantanen, T.; Järvenpää, M. L.; Vuojola, J.; Arppe, R.; Kuningas, K.; Soukka, T. *Analyst* **2009**, *134*, 1713−1716.