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Fluorescence Detection of DNA Hybridization Based on the Aggregation-Induced Emission of a Perylene-Functionalized Polymer

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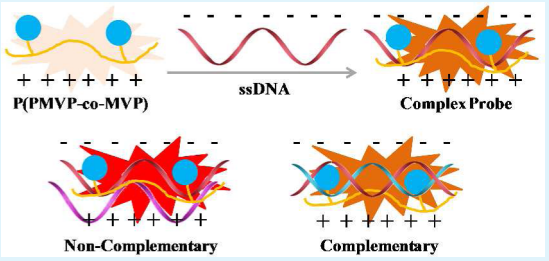
Supporting Information

ABSTRACT: A perylene-functionalized polycation was synthesized by quaternization of poly(4-vinylpyridine) with bromomethyl-perylene and methyl iodide, which exhibited a unique aggregation-induced emission (AIE) effect. The synthesized polycation and polyanion ssDNA could form a complex probe to detect DNA hybridization. Upon adding noncomplementary ssDNA, the fluorescence of the complex probe increased due to the AIE effect; upon adding complementary ssDNA, the fluorescence intensity changed little due to the combined effects of AIE and duplex-quenching resulting from the intercalation of perylene into the duplex.



KEYWORDS: *aggregation-induced emission, fluorescence, hybridization, polyelectrolyte, probe*

INTRODUCTION

The development of fluorescence probes for ultrasensitive, rapid, and cost-effective DNA detection have attracted great attention since they play important roles in medical diagnostics, identification of genetic mutations, and monitoring of gene delivery.[1−3] Among them, fluorophore-labeled probes, especially the representative stem-loop structured hairpin-like oligonucleotide sensors named molecular beacons, gained great recognition because of their high sensitivity.[4,5] Besides, some complex probes consisting of conjugated polyelectrolytes and fluorophore-labeled oligonucleotides exhibit excellent detection properties via the mechanism of fluorescence resonance energy transfer (FRET).[6−8] However, the preparation of such labeled oligonucleotides often needs a complex process and high cost, motivating researchers to develop label-free probes by cost-effective methods for the detection of DNA. The fluorescent polycations binding to the negatively charged phosphate backbone of DNA can be used to detect the biomacromolecules by employing their unique fluorescent properties.[9−11] For example, we have reported several label-free complex probes consisting of pyrene-functionalized polycations and single-stranded DNA (ssDNA) for the detection of DNA hybridization: the fluorescence intensity could be decreased further upon adding the complementary ssDNA due to the intercalation of pyrene into the duplex.[12−14]

Many fluorescent organics and polymers are highly emissive in their dilute solutions but become weakly luminescent with the increase of their concentration due to the formation of aggregates. Tang et al. first discovered an uncommon phenomenon that silole derivatives were weakly fluorescent in a good solvent (in which the substance has a good solubility), but the fluorescence could be enhanced efficiently in poor solvents (in which the substance has a poor solubility) due to the aggregation, as was called aggregation-induced emission (AIE).[15] Since then, researchers have synthesized a series of AIE molecules such as silole derivatives and tetraphenylethylene derivatives, which have made a great contribution to the detection of enzyme, protein, ions, etc.[16−19] However, the detection of DNA hybridization based on the AIE effect was rarely reported. Wang et al. have reported a fluorescence turn-on and label-free detection of DNA by using the AIE feature of the silole compound.[20] Liu et al. have also demonstrated a general light-up probe design strategy for specific DNA hybridization detection by labeling an AIE fluorogen, tetraphenylethene, to an oligonucleotide.[21] In addition, Lu et al. have developed a fluorescence turn-on biosensor for DNA detection and quantification by taking advantage of complexation-induced aggregation of a conjugated polyelectrolyte with AIE characteristics.[22] Herein, we report a simple label-free method for detection of DNA hybridization based on the AIE property of a nonconjugated fluorescent polycation, poly(*N*-perylenemethyl 4-vinylpyridine bromide-*co*-*N*-methyl 4-vinylpyridine iodide) P(PMVP-*co*-MVP), the chemical structure of which is shown in Scheme 1. This novel strategy to detect DNA hybridization based on the AIE effect is different from that only based on the intercalation effect we reported before.[12−14]

EXPERIMENTAL SECTION

Materials. 3-(Bromomethyl) perylene was purchased from Suna Tech Inc. Poly(4-vinylpyridine) (P4VP, weight-average molecular weight, M_w = 60 000 Da) was purchased from Sigma-Aldrich Chemical Co. and used without further purification. The organic reagents used

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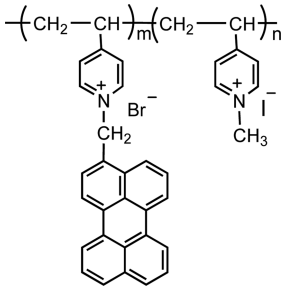
Scheme 1. Chemical Structure of the Synthesized Perylene-Functionalized Polymer P(PMVP-*co*-MVP)



in this work were commercial products. The HPLC-purified DNA oligonucleotides ssDNA1 (5′-CAA GTA GAA TGT ATG TGC-3′), ssDNA2 (5′-GCA CAT ACA TTC TAC TTG-3′), ssDNAt (5′-TTT TTT TTT TTT TTT TTT-3′), and ssDNA2-*n* (5′-AGT CTA GGT TAC GGC GTG-3′) (ssDNA2 is complementary with ssDNA1; ssDNAt and ssDNA2-*n* are noncomplementary with ssDNA1) and PBS buffer used in this work were purchased from the Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China).

Methods. ^{1}H NMR spectra were recorded with a Bruker DMX-400 spectrometer in deuterated solvent at 298 K. UV−visible absorption spectra were recorded with a Shimadzu UV-3100 UV−vis spectrophotometer. Elemental analysis was measured through the Heratus CHN-Rapid method. A Fourier transform infrared spectrometer (FTIR, PerkinElmer, USA) was used to obtain the evidence of the quaternization. The fluorescence spectra were measured on a Hitachi F-4500 spectrofluoremeter. CD spectra were obtained on a JASCO CD J-805 spectrophotometer, and all the data were collected at a scan rate of 50 nm/min and presented as an average of three successive scans.

Synthesis of the Perylene-Functionalized Polymer P(PMVP-*co*-MVP). The synthetic route of P(PMVP-*co*-MVP) is shown in the Supporting Information (Scheme S1). First, the intermediate poly(6*N*-perylenemethyl 4-vinylpyridine bromide-*co*-4-vinylpyridine) P(PMVP-*co*-VP) was synthesized from P4VP and Pe-MeBr by quaternization. P4VP (5 mmol of 4-vinylpyridine) and Pe-MeBr (0.2 mmol) (molar ratio, 25:1) were dissolved in 15 mL of chloroform. The solution was stirred and refluxed for 24 h. Then, THF was added into the mixture dropwise to precipitate the faint yellow intermediate. After filtering, abstersion, and vacuum-drying, the intermediate was obtained. Second, the intermediate P(PMVP-*co*-VP) was quaternized with methyl iodide to afford a water-soluble and positively charged product. To a solution of P(PMVP-*co*-VP) (5 mmol) in ethanol (25 mL), excess methyl iodide was added dropwise and then stirred at 40 °C for 24 h. Finally, the solvent and unreacted methyl iodide were evaporated under reduced pressure, and thus, the final product P(PMVP-*co*-MVP) was obtained. The structure of the polymer was determined by elemental analysis, NMR, FTIR, UV−vis absorption, and fluorescence spectra.

Degree of Functionalization. It is well-known that the absorbance of a substance is in proportion to its concentration in the same solvent, according to which we measured the absorbance of Pe-MeBr and the polymer P(PMVP-*co*-VP) in $CHCl_3$, respectively, with low concentrations to get the perylene functionalization. The degree of perylene functionalization was measured to be 2.5%.

Molecular Weight of P(PMVP-*co*-MVP). According to the molecular weight of P4VP (M_w = 60 000 Da) and the degree of perylene functionalization (2.5%), the molecular weight of the synthesized P(PMVP-*co*-MVP) was calculated to be 1.44 × 10^5 Da.

RESULTS AND DISCUSSION

The fluorescent polycation P(PMVP-*co*-MVP) was synthesized from poly(4-vinylpyridine) (P4VP) by two steps of protonation according to the method we reported earlier[12] (see the Supporting Information, Scheme S1): first, 3-(bromomethyl)-perylenes were grafted into P4VP partially by quaternization to get an intermediate product P(PMVP-*co*-VP); second, P(PMVP-*co*-VP) was quaternized further by methyl iodide to get the cationic and water-soluble polyelectrolyte P(PMVP-*co*-MVP) which could combine with anionic ssDNA by electrostatic interactions. The ^{1}H NMR spectra (see the Supporting Information, Figure S1) indicated that P4VP was quaternized by 3-(bromomethyl)perylene and methyl iodide completely.[12] The FTIR spectra of P(PMVP-*co*-MVP), P(PMVP-*co*-VP), and P4VP are shown in Figure 1. The disappearance of the band

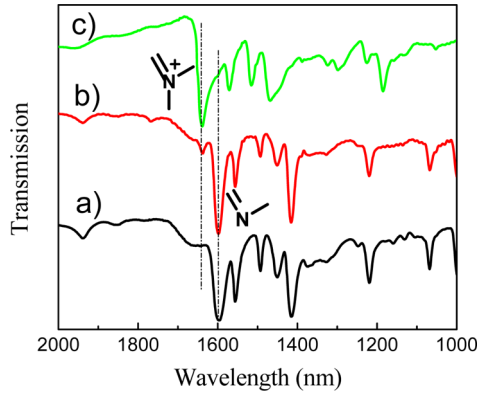


Figure 1. FTIR spectra of P4VP (a), P(PMVP-*co*-VP) (b), and P(PMVP-*co*-MVP) (c).

centered at 1600 cm^{-1} assigned to the deprotonated pyridine ring and the appearance of the band centered at 1640 cm^{-1} assigned to the protonated pyridine ring indicated the quaternization of P4VP. The degree of perylene functionalization was calculated to be 2.5% by measuring absorption of perylene, and the weight-average molecular weight of P(PMVP-*co*-MVP) was determined to be 1.44 × 10^5 Da. The fluorescence quantum yield (Φ) of P(PMVP-*co*-MVP) in water was measured to be 0.014 by using quinine sulfate in sulfuric acid as standard with a known Φ of 0.55. The UV−vis absorption and fluorescence spectra in PBS buffer (10 mM, pH = 7.4) are shown in the Supporting Information (Figure S2).

First, the AIE property of the polycation P(PMVP-*co*-MVP) was investigated in water−THF mixtures with different THF fractions. The polymer P(PMVP-*co*-MVP) was soluble in water while insoluble in THF, so we gradually increased the THF fraction in the mixed solvents to induce the aggregation of P(PMVP-*co*-MVP). Figure 2a shows the fluorescence change of P(PMVP-*co*-MVP) in water−THF mixtures with different THF fractions. The fluorescence intensity was very low in pure water, while it was enhanced obviously in the mixed solvents, especially when the THF fraction was more than 20%. The fluorescence quantum yield (Φ) of P(PMVP-*co*-MVP) increased from 0.014 in water to 0.20 in the water−THF mixture (THF volume fraction, 50%). The excimer band of perylene in the range between 500 and 600 nm demonstrated the aggregation of P(PMVP-*co*-MVP). Figure 2b shows that the corresponding integrated fluorescence intensities of P(PMVP-*co*-MVP) (from 420 to 700 nm) increased with the increase of THF volume fraction. The fluorescence intensity in the mixed solvent with 90% of THF fraction was approximately 600 times higher than that in pure water. Bright fluorescence could be observed in the mixed solvent, while the fluorescence was negligible in pure water under illumination of UV light (inset of Figure 2b). The increase in fluorescence due to the aggregation

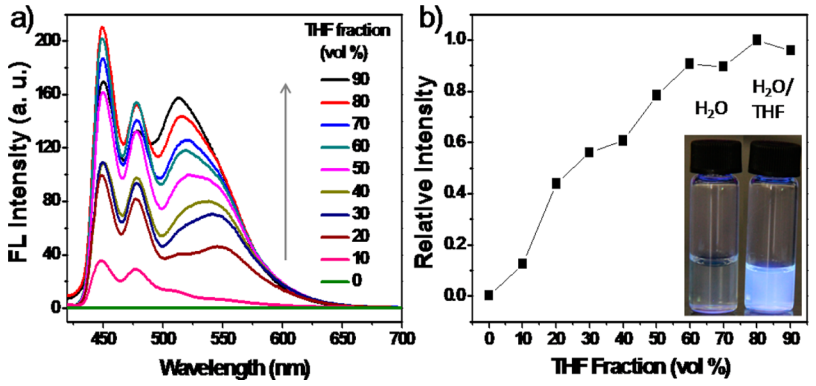


Figure 2. (a) Fluorescence spectra of P(PMVP-*co*-MVP) ([P(PMVP-*co*-MVP)] = 5×10^{-7} M) in water−THF mixtures with different THF volume fractions, λ_{ex} = 415 nm. (b) Corresponding relative integrated fluorescence intensity of P(PMVP-*co*-MVP) (from 420 to 700 nm) in the mixtures with different THF volume fractions. The inset shows photos of P(PMVP-*co*-MVP) in pure water and the water−THF mixture with 90% of THF, taken under the illumination of a 365 nm UV light.

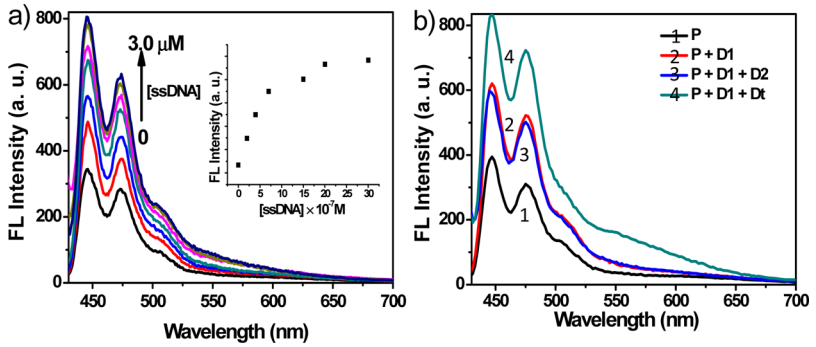


Figure 3. (a) Fluorescence spectra of P(PMVP-*co*-MVP) ([P(PMVP-*co*-MVP)] = 2×10^{-7} M) in the presence of different concentrations of ssDNA1 (from 0 to 3.0×10^{-6} M) in PBS buffer (10 mM, pH = 7.4), λ_{ex} = 415 nm. The inset depicts corresponding fluorescence intensities at 444 nm with different concentrations of ssDNA1. (b) Fluorescence spectra of the polymer P(PMVP-*co*-MVP), P(PMVP-*co*-MVP) + ssDNA1, P(PMVP-*co*-MVP) + ssDNA1 + ssDNA2, and P(PMVP-*co*-MVP) + ssDNA1 + ssDNAt in PBS buffer. [P(PMVP-*co*-MVP)] = 2×10^{-7} M, [ssDNA] = 3×10^{-7} M, λ_{ex} = 415 nm. Here P, D1, D2, and Dt stand for the polymer P(PMVP-*co*-MVP), ssDNA1, ssDNA2, and ssDNAt, respectively.

of the polymer P(PMVP-*co*-MVP) in the mixed solvents demonstrated that P(PMVP-*co*-MVP) could exhibit a strong AIE characteristic. Tang and co-workers have identified that the restriction of intramolecular rotation in the aggregates is the main cause for the AIE effect.[23−25] The AIE effect has been proved to be a general property for propeller-like molecules consisting of π-conjugated rotors and stators. In the solution state, such rotation is active, which serves as a relaxation channel for the excited state to decay, whereas, in the aggregated state, this rotation is restricted due to physical constraints on the molecular packing, blocking the nonradiative path, and activates the radiative decay. For the perylene-functionalized poly(4-vinylpyridine) (P(PMVP-*co*-MVP)), the pyridines of the polymer could be the π-conjugated stators and the perylenes could be the π-conjugated rotors. In the aggregated state, the rotation of perylene could be restricted and the emission was enhanced. It is noted that the excimer peaks of aqueous polymer solution blue-shifted upon addition of THF, which could be ascribed to the restriction of intramolecular rotation upon aggregation.[26,27] From Figure 2b, it can also be found that the fluorescence intensity increased with a zigzag pattern when increasing the THF fraction over 60%, where the large amount of THF induced the aggregation seriously and some of the polymers were precipitated, as was often observed in the determination of the AIE effect. On one hand, the aggregation could decrease the fluorescence intensity since only the molecules on the surface of the nanoparticles emitted light and contributed to the fluorescent intensity upon excitation; on the other hand, the restriction of intramolecular rotations of the aromatic rings in the aggregation state could enhance light emission.[28,29] The net output of these two antagonistic processes induced the zigzag pattern with further increasing THF fraction. The fluorescent polyelectrolyte P(PMVP-*co*-MVP) also exhibited the AIE phenomenon in water−EtOH mixtures, shown in the Supporting Information (Figure S3). (The polymer P(PMVP-*co*-MVP) can not dissolve in EtOH.)

Then, we investigated the interaction between P(PMVP-*co*-MVP) and DNA. When the perylene-functionalized polymer P(PMVP-*co*-MVP) was mixed with single-stranded DNA (ssDNA1: 5′-CAA GTA GAA TGT ATG TGC-3′) in PBS buffer (10 mM, pH = 7.4), the fluorescence intensity could be enhanced greatly, while the fluorescence of pyrene-functionalized P4VP decreased when mixed with ssDNA due to the electron transfer between excited pyrene and nucleotide bases, as we reported earlier.[12] The pyrene-functionalized P4VP did not show the AIE characteristic, while the polymer P(PMVP-*co*-MVP) functionalized with the large chromophore perylene did, where the restriction of intramolecular rotation could occur in the system with the large chromophore when aggregated. Figure 3a shows that the fluorescence of P(PMVP-*co*-MVP) increased quickly first and then slowly with the increase of the concentration of ssDNA1. In fact, when the concentration of added ssDNA was ten times higher than that of P(PMVP-*co*-

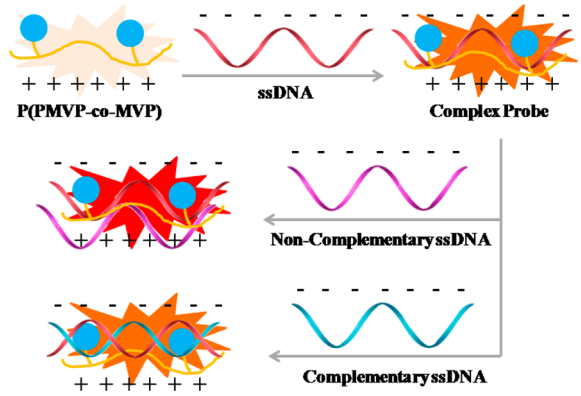
MVP), the polymer P(PMVP-*co*-MVP) was precipitated. The results above demonstrated that the polyanion DNA could induce aggregation of the polycation P(PMVP-*co*-MVP) and the fluorescence increased with the AIE characteristics. It is noted that the excimer band of perylene in the range between 500 and 600 nm shown in the spectra with the mixed solvents of water and THF (Figure 2a) did not appear here when the polymer P(PMVP-*co*-MVP) was mixed with ssDNA, where the perylene chromophores could be isolated by the polyanion ssDNA and thus no excimer emission could be observed.

Next, we detected DNA hybridization using the AIE characteristics of P(PMVP-*co*-MVP). In this assay, the positively charged P(PMVP-*co*-MVP) (2×10^{-7} M) and negatively charged ssDNA1 (5′-CAA GTA GAA TGT ATG TGC-3′, 3×10^{-7} M) were mixed in PBS buffer (10 mM, pH = 7.4) to form a complex probe by electrostatic interactions. Complementary DNA (ssDNA2, 5′-GCA CAT ACA TTC TAC TTG-3′) and noncomplementary DNA (ssDNAt, 5′-TTT TTT TTT TTT TTT TTT-3′) were used to investigate the detection of DNA hybridization by the complex probe. As shown in Figure 3b, the fluorescence of P(PMVP-*co*-MVP) increased when mixed with ssDNA1 to form the complex probe because of the AIE effect. When the complementary ssDNA2 (3×10^{-7} M) was added into the complex solution, the fluorescence changed little, while the fluorescence increased significantly upon addition of noncomplementary ssDNAt (3×10^{-7} M). Thus, we can distinguish the complementary/noncomplementary DNA based on the fluorescence change of the complex probe. When ssDNAt was replaced by an arbitrary noncomplementary DNA, ssDNA2-*n* (5′-AGT CTA GGT TAC GGC GTG-3′), the increased fluorescence could also be observed (see the Supporting Information, Figure S4).

The increase in fluorescence of the complex probe upon addition of noncomplementary ssDNA such as ssDNAt and ssDNA2-*n* could be ascribed to the further aggregation of the polymer when adding more negative ssDNA, where the aggregation enhanced its fluorescence.[20] However, the addition of complementary ssDNA2 did not induce the fluorescence increase as observed when adding noncomplementary ssDNAs, which could be ascribed to the intercalation of the chromophore perylene into the duplex. On one hand, the addition of the negative ssDNA2 could increase the fluorescence due to the AIE effect; on the other hand, the fluorescence could be quenched by the duplex base pairs due to the electron transfer from the intercalated chromophore to nucleotide bases.[12−14,30] The combined effects of AIE and duplex-quenching from intercalation could result in little change in the fluorescence intensity. Scheme 2 illustrates the formation of the complex probe and detection of DNA hybridization based on the AIE and intercalation effects. The intercalation of the chromophore perylene into the duplex was confirmed by the circular dichroism (CD) spectra and the fluorescence quenching by iodide.

CD is one of the most sensitive techniques for the detection of interactions and changes in nucleic-acid conformations, which has been widely used to probe the intercalation of guest molecules in DNA.[31] Therefore, to investigate the intercalation of perylene into the DNA duplex, we analyzed the differences in CD signals of DNA duplex in the absence and presence of P(PMVP-*co*-MVP). As shown in Figure 4a, the spectrum of the duplex of DNA1/DNA2 exhibited a positive band at 276 nm due to the base stacking and a negative band at 249 nm due to the right-handed helicity.[32] With the addition of P(PMVP-*co*-

Scheme 2. Conceptual Illustration of the Formation of the Complex Probe and Label-Free DNA Detection Based on the AIE and Intercalation Effects



MVP) to the duplex solution, the positive band red-shifted to 283 nm and the negative band red-shifted to 251 nm. Moreover, the intensities of both positive and negative bands decreased significantly. Groove binding, electrostatic interactions, and intercalation are three possible interactions between optical probes and DNA duplexes.[33] Groove binding and electrostatic interactions can show less or no perturbation on the base stacking, whereas intercalation can change the position and intensity of both bands, which could be ascribed to the local unwinding in the helical backbone as the result of the intercalation of perylene and the induced disruption of base stacking that perturbed the right-handed conformation of DNA duplex.[34] By this token, the CD spectra demonstrated that the perylene moieties intercalated into the DNA duplex.

Besides CD, the iodide fluorescence quenching measurements have also been widely used to probe the intercalation of chromophores in DNA duplex.[35,36] The negatively charged phosphate backbone of DNA duplex would repel the negatively charged quencher I^-. Therefore, if the binding mode of the perylene moieties with DNA duplex is intercalation, the chromophore perylene should be protected from being quenched by anionic quencher. Different from intercalation, groove binding and electrostatic interactions provide much less protection for the bound chromophores. Here, the fluorescence quenching of perylene by iodide further confirmed the intercalation of perylene into the duplex. As shown in Figure 4b, with the increase of the amount of iodide, the ratio between the fluorescence intensities in the absence and presence of iodide increased because of the quenching of iodide. It should be noted that the quenching effect in the duplex system (P(PMVP-*co*-MVP)/DNA1/DNA2) was less than that in the noncomplementary system (P(PMVP-*co*-MVP)/DNA1/DNAt), as demonstrated that perylene chromophores could be intercalated into the duplex and protected from the attack of iodide.

CONCLUSION

In summary, we synthesized a novel perylene-functionalized polycation P(PMVP-*co*-MVP) by quaternization of P4VP with bromomethyl-perylene and methyl iodide, which possessed a unique AIE property. With the addition of poor solvents such as THF and EtOH into the aqueous solution of the polycation, the fluorescence increased significantly due to the aggregation of the polymer. Besides, DNA polyanions could also induce the aggregation of P(PMVP-*co*-MVP) and enhance its fluorescence

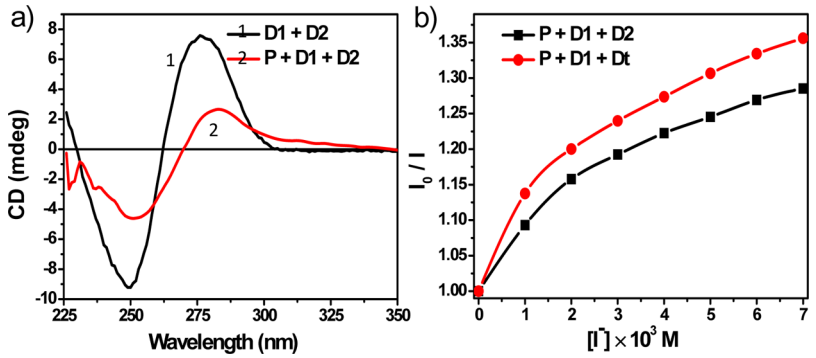


Figure 4. (a) CD spectra of DNA1/DNA2 duplex in the absence and presence of P(PMVP-*co*-MVP) in PBS buffer (10 mM, pH = 7.4). [P(PMVP-*co*-MVP)] = 1.0 × 10^{-5} M, [ssDNA] = 5.0 × 10^{-6} M. (b) Iodide quenching curves of complex probe with complementary ssDNA2 and noncomplementary ssDNAt. [P(PMVP-*co*-MVP)] = 2 × 10^{-7} M, [ssDNA] = 3 × 10^{-7} M, λ_{ex} = 415 nm. Here P, D1, D2, and Dt stand for the polymer P(PMVP-*co*-MVP), ssDNA1, ssDNA2, and ssDNAt, respectively. I_0 and I stand for fluorescence intensity of the polymer at 444 nm in the absence and presence of iodide.

in buffer. The polycation P(PMVP-*co*-MVP) and the polyanion ssDNA could form a complex probe to detect DNA hybridization. Upon adding noncomplementary ssDNA, the fluorescence of the complex probe increased due to the AIE effect; upon adding complementary ssDNA, the fluorescence intensity changed little due to the combined effects of AIE and duplex-quenching resulted from the intercalation of perylene into the duplex. This reported perylene-involved polyelectrolyte may provide a new direction to the development of AIE polymers and find broad applications in bioassays as a novel optical biosensor for the detection of DNA hybridization.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental information, ^{1}H NMR spectra, elemental analysis, absorption and fluorescence spectra of P(PMVP-*co*-MVP), AIE property of P(PMVP-*co*-MVP) in the water−EtOH mixture, and fluorescence spectrum of the complex probe upon adding noncomplementary ssDNA2-n. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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