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Multi-Stimuli Responsive FRET Processes of Bifluorophoric AIEgens in an Amphiphilic Copolymer and Its Application to Cyanide Detection in Aqueous Media

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ASSOCIATED CONTENT

Supporting Information

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Experimental details and additional data including ^1H , ^{13}C NMR, HRMS, elemental analysis (EA), GPC, UV–vis, and fluorescence spectra (PDF)

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Abstract

A novel amphiphilic aggregation-induced emission (AIE) copolymer, that is, **poly(NIPAM-co-TPE-SP)**, consisting of N-isopropylacrylamide (NIPAM) as a hydrophilic unit and a tetraphenylethylene-spiropyran monomer (**TPE-SP**) as a bifluorophoric unit is reported. Upon UV exposure, the close form of non-emissive spiropyran (SP) in **poly(NIPAM-co-TPE-SP)** can be photo-switched to the open form of emissive merocyanine (MC) in **poly(NIPAM-co-TPE-MC)** in an aqueous solution, leading to ratiometric fluorescence of AIEgens between green TPE and red MC emissions at 517 and 627 nm, respectively, via Förster resonance energy transfer (FRET). Distinct FRET processes of **poly(NIPAM-co-TPE-MC)** can be observed under various UV and visible light irradiations, acid-base conditions, thermal treatments, and cyanide ion interactions, which are also confirmed by theoretical studies. The subtle perturbations of environmental factors, such as UV exposure, pH value, temperature, and cyanide ion, can be detected in aqueous media by distinct ratiometric fluorescence changes of the FRET behavior in the amphiphilic **poly(NIPAM-co-TPE-MC)**. Moreover, the first FRET sensor polymer **poly(NIPAM-co-TPE-MC)** based on dual AIEgens of TPE and MC units is developed to show a very high selectivity and sensitivity with a low detection limit (LOD = 0.26 μM) toward the cyanide ion in water, which only contain an approximately 1% molar ratio of the bifluorophoric content and can be utilized in cellular bioimaging applications for cyanide detections.

Graphical Abstract

Keywords

aggregation-induced emission (AIE); cyanide; Förster resonance energy transfer (FRET); spiropyran; tetraphenylethylene

1. INTRODUCTION

As one of the most prevailing anions, cyanide (CN^-) is a very dangerous and toxic anion, so the maximum allowable concentration of cyanide anion in drinking water is only 1.9 μM based on the conclusion of the World Health Organization (WHO).^{1–4} Therefore, the

developments of highly sensitive, selective, and effective sensor materials for the detection of CN^- have drawn extensive attention of our eco-environments. So far, various fluorescence sensors for CN^- recognition based on nucleophilic addition reaction into indolium moieties with poor aqueous solubilities^{5–10} and AIE properties^{11,12} have been presented. Besides, some water-soluble sensor polymers were also designed and reported to detect CN^- in aqueous media.^{13,14} Especially, a water-soluble N-isopropylacrylamide (NIPAM) based copolymer containing another conjugated coumarin--spiropyran monomer was synthesized to be used for CN^- detection in water.¹⁵

The concept of aggregation-induced emission (AIE) was firstly reported by Tang and co-workers in 2001 based on 1-methyl-1,2,3,4,5-pentaphenylsilole molecule.¹⁶ As a well-known AIE luminogen, tetraphenylethylene (TPE) possesses the interesting AIE properties in the aggregation and solid states,^{17–19} so TPE-based materials have been broadly applied to various fluorescent bioprobes^{20–24} and chemosensors^{25–28} in aqueous (or semi-aqueous) solutions. In addition, some stimuli-responsive luminescent materials with mechanochromism, thermochromism,²⁹ and photochromism³⁰ for various applications have been reported in recent years. As a photochromic molecule, the close form of spiropyran (SP) can be reversibly photo-isomerized to the open form of merocyanine (MC), attributed to the cleavage of the C-O bond in the SP ring upon external stimuli.³¹ The reversible structural conversion of SP unit has been broadly employed in a wide range of applications, including optical switches, photo-patterning, biological systems, and chemosensors,^{32–38} where most SP and MC structures were utilized via their photochromic behavior. So far, just a few fluorophoric properties of SP and MC derivatives have been used for applications,³⁹ pH sensing,⁴⁰ especially for the sensor applications in aqueous solutions.^{41,42} With the advantages of Förster resonance energy transfer (FRET) in photoluminescence (PL),^{43–45} various bifluorophoric combinations of FRET processes between two energy donor and acceptor chromophores within a molecule^{46,47} (or supramolecule)^{48–50} and a mixture⁵¹ were reported. For instance, some FRET fluorescence probes of TPE donors with doxorubicin and rhodamine B derivatives^{52,53} or MC acceptors with azocarbazole⁵⁴ and naphthalimide^{55,56} units have been presented. Moreover, the direct-coupling and conjugated connection of both TPE and SP chromophores without FRET behavior^{57,58} or with energy transfer^{59,60} have been designed and reported in recent years. To the best of our knowledge, no FRET behavior between TPE and SP (or MC) chromophores has been explored for the applications of sensor materials.

Though various bifluorophoric combinations of FRET donors and acceptors can be utilized as fluorescent bioprobes and chemosensors, the poor aqueous solubilities of bifluorophoric sensor materials need to be further improved to enhance their sensitivities in water. Hence, the mentioned FRET bifluorophoric monomer containing TPE and MC (or SP upon UV exposure) units is copolymerized with an NIPAM monomer to induce better water solubility for the detection of water-soluble analysts (for example, the cyanide detection in aqueous solutions). Besides the photo-responsive SP unit in the FRET bifluorophoric system, the introduction of poly(N-isopropyl-acrylamide) (PNIPAM) offers not only water solubility but also the temperature-responsive capability to this FRET luminescent copolymer, which also displays the upper critical solution temperature (UCST) or lower critical solution temperature (LCST) phase behavior in the aqueous solution. Herein, the dual TPE and MC

AI-Egens are integrated in the amphiphilic copolymer, that is, **poly(NIPAM-co-TPE-MC)**, and its multi-stimuli responsive FRET processes were investigated by sensitive ratiometric fluorescence between bifluorophoric TPE and MC units, which also can be utilized for cyanide detection in water and bioimaging in living cells.

2. EXPERIMENTAL SECTION

2.1. Materials

All chemical reagents were purchased from commercial sources (TCI Japan, Sigma-Aldrich, Fisher Scientific, and J. T. Baker) and used directly as received without further purification. The solvents used for reactions were purified/dried by the solvent purification system before using. For polymerization, the initiator AIBN (2,2'-azobis(2-methylpropionitrile)) and NIPAM monomer were purified by recrystallizing from methanol and *n*-hexane, respectively, prior to use. All reactions were carried out under nitrogen atmosphere and vacuum-line manipulations. Some reactants, catalysts, and organic solvents were used for reactions and purification of compounds, such as tetra-*n*-butylammonium bromide (TBAB), tosyl chloride (TsCl), disodium ethylenediaminetetraacetate dihydrate (Na₂EDTA.H₂O), triethylamine (TEA), acetonitrile (ACN), ethanol (EtOH), dichloromethane (DCM), dimethylformamide (DMF), tetrahydrofuran (THF), diethyl ether (Et₂O), ethyl acetate (EtOAc), hexane (Hex), and methanol (MeOH). The buffer solution (1 M MOPS, pH = 7.0) was prepared from 4-morpholinepropanesulfonic acid and sodium hydroxide solutions. All anion solutions (CN⁻, F⁻, Cl⁻, Br⁻, I⁻, OH⁻, NO₃⁻, PF₆⁻, HCO₃⁻, HSO₄⁻, H₂PO₄⁻, CH₃COO⁻, SO₄²⁻, HPO₄²⁻, and S²⁻) were prepared in distilled water from their sodium, potassium, and ammonium salts.

2.2. Characterizations and Measurements

Nuclear magnetic resonance (NMR) spectra were recorded at room temperature on a Bruker Avance 300 and 500 MHz instruments. High-resolution mass spectra were recorded on a Bruker-Impact HD mass spectrometer (ESI mode). Elemental analysis was performed using an Elementar Vario CUBE (CHN-OS Rapid, German). Polymer molecular weight and the polydispersity index were analyzed by gel permeation chromatography (Shodex GPC KF-805 L) with tetrahydrofuran as the eluent at a flow rate of 1.0 mL/min, 25 °C. Absorption spectra were recorded with an ultraviolet-visible near-infrared spectrophotometer (Lambda 950, PerkinElmer) between 200–800 nm. A fluorescence spectrophotometer (HITACHI F-4500) was used to measure the fluorescence properties of polymer.

2.3. Cell Culture and Confocal Fluorescence Imaging

The HeLa cells were grown in DMEM media supplemented with 10% (v/v) FBS (fetal bovine serum) and penicillin/streptomycin (100 µg/mL) at 37 °C in a 5% CO₂ incubator. The experiments to evaluate the sensing capability of polymers (close and open forms, i.e., **poly(NIPAM-co-TPE-SP)** and **poly(NIPAM-co-TPE-MC)**, respectively) for CN⁻ in living cells were performed in 0.1 M phosphate-buffered saline (PBS). The cells were treated with the close and open forms of polymers (10 µM based on SP and MC units) and incubated for 30 min at 37 °C. The culture medium was removed, and the treated cells were washed with

0.1 M PBS (2 ML $\times$ 3) before observation. Confocal fluorescence imaging of cells was performed with a Leica TCS SP5 X AOBS confocal fluorescence microscope (Germany), and a 63 $\times$ oil-immersion objective lens was used. The excitation wavelength of 365 nm was applied for this experiment. Green and red emissions were collected at 500–550 and 600–650 nm, respectively. Before and after the addition of CN^- (10 μM), the cells were incubated with the close and open forms of polymers (i.e., **poly(NIPAM-co-TPE-SP)** and **poly(NIPAMco-TPE-MC)**, respectively) treated cells and their confocal fluorescence imaging changes were compared by different fluorescence colors from RGB channels.

2.4. Cytotoxicity Assay

The cytotoxicity of the close and open forms of polymers (i.e., **poly(NIPAM-co-TPE-SP)** and **poly(NIPAMco-TPE-MC)**, respectively) were evaluated by MTT assay using HeLa cells. The cells were grown in a 96-well cell culture plate. Different amounts of polymers (0–25 μM based on SP and MC units) were added in the culture medium and then kept at 37 $^\circ\text{C}$ with 5% CO_2 for 24 h. The culture medium was added with 20 μL methyl thiazolyl tetrazolium (1 mg/mL) and then kept at 37 $^\circ\text{C}$ with 5% CO_2 for 4 h. Thereafter, the culture medium was removed, then 200 μL DMSO was added, and finally, 20 μL Sorenson's glycine buffer was also added to dissolve the yellow precipitates (formazan). Multiskan GO microplate reader was used to read the absorbance at 570 nm. The cell viability was evaluated by the equation shown below.

$$\text{cell viability (\%)} = (\text{mean absorbance of treatment group})/(\text{mean absorbance of control group})$$

2.5. Synthesis and Characterization

The detailed information on the synthesis and characterization of compounds **1–8** and **SP-N₃** are shown in the Supporting information (section 1). The synthetic procedures of monomer **TPE-SP** and copolymer **poly(NIPAM-coTPE-SP)** are illustrated in Scheme 1.

2.5.1. Synthesis of Monomer TPE-SP—Compound **8** (0.87 g, 1.50 mmol) and **SP-N₃** (0.63 g, 1.65 mmol) were dissolved in dry DCM (20 mL). Then, $[\text{Cu}(\text{CH}_3\text{CN})_4]\text{PF}_6$ (0.58 g, 1.50 mmol) was added. The reaction mixture was stirred for 24 h at room temperature under nitrogen gas. Then, 100 mL Na_2EDTA solution was added in the previous resulting mixture and extracted with CH_2Cl_2 (3 $\times$ 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was further purified on silica gel using column chromatography with a mixed solvent (DCM:MeOH = 250:1) as the eluent to gain the desired yellow solid product (bifluorophoric monomer **TPE-SP**). Yield: 1.24 g (87%). ^1H NMR (300 MHz, CDCl_3) δ 8.00 (dd, J = 2.7 Hz, J = 9 Hz, 1H), 7.93 (d, J = 2.7 Hz, 1H), 7.50 (s, 1H), 7.21–7.20 (m, 1H), 7.12–7.00 (m, 11H), 6.97–6.89 (m, 5H), 6.73–6.66 (m, 4H), 6.60–6.54 (m, 3H), 6.10 (s, 1H), 5.55 (t, J = 1.5 Hz, 1H), 5.11–5.10 (m, 2H), 5.00–4.97 (m, 1H), 4.60–4.57 (m, 2H), 4.15 (t, J = 6.6 Hz, 2H), 3.85 (t, J = 6.6 Hz, 2H), 3.75–3.52 (m, 2H), 1.95 (s, 3H), 1.75–1.68 (m, 4H), 1.47–1.43 (m, 4H), 1.24 (s, 3H), 1.05 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 167.5, 158.9, 157.6, 156.7, 145.8, 144.3, 144.2, 144.1, 141.2, 139.9, 139.5, 137.0, 136.5, 136.1, 136.0, 132.7, 132.5, 131.4, 131.3, 128.7, 127.9, 127.7, 126.1, 125.9, 125.2, 124.1, 122.8, 122.2, 120.6, 120.5, 118.3, 115.4, 113.6, 113.5,

106.4, 106.2, 67.6, 64.6, 61.7, 52.6, 48.8, 44.5, 30.3, 29.2, 28.5, 25.8, 25.7, 19.8, 18.3. HRMS (ESI⁺) [M + H]⁺: calcd for C₅₃H₅₅N₆O₄ 948.4331; found 948.4343. Elemental analysis: calcd for C₅₃H₅₅N₆O₄, C 75.74, H 6.06, N 7.39%, found C 75.07, H 6.35, N 7.42%.

2.5.2. Synthesis of Poly(NIPAM-co-TPE-SP)—This copolymer was synthesized by free radical polymerization in the presence of thermal initiator AIBN with the hydrophilic monomer (NIPAM) and bifluorophoric monomer TPE-SP. First, monomers NIPAM (307 mg, 2.72 mmol) and **TPE-SP** (25.8 mg, 0.0272 mmol) were mixed with initiator AIBN (7 mg) in dry THF. The mixture was degassed under the freeze-pump-thaw cycle three times and then heated up to 70 °C to react for 2 days. A few drops of CH₃OH were added to terminate the polymerization. The resultant mixture was poured into cool ether and precipitated with ethyl ether several times and then filtrated. The desired copolymer was dried under vacuum to acquire copolymer **poly(NIPAM-co-TPE-SP)** as a slight orange solid. Yield: 200 mg. The composition of **poly(NIPAM-co-TPE-SP)** was determined by comparing the integral values of the band centered at 7.50 ppm (one hydrogen of triazole group in monomer **TPE-SP**) and the band centered at 3.96 or 1.10 ppm (one hydrogen CH- or six hydrogens CH₃- of the isopropyl group in NIPAM, respectively) from ¹H NMR spectrum. According to the result of NMR spectrum (Figure S16), it indicates that the monomer ratio of NIPAM and TPE-SP (x:y) is 100:1. As shown in Figure S17a, the weight average molecular weight (M_w) and polydispersity index (PDI = M_w/M_n) of polymer obtained by gel permeation chromatography (GPC) were 12,344 Da and 1.80, respectively.

3. RESULTS AND DISCUSSION

3.1. Design and Synthesis of Monomer and Copolymer

The bifluorophoric monomer **TPE-SP** was synthesized via “click” reaction (CuAAC) of alkyne **8** and **SP-N₃** (see Scheme S1) in DCM with a [Cu(CH₃CN)₄]PF₆ catalyst under a mild condition. The detailed synthetic procedure of this monomer and **SP-N₃** are described in Scheme 1 and Schemes S1 and S2. The chemical structure of monomer **TPE-SP** is fully characterized by ¹H NMR, ¹³C NMR, HRMS spectra, and EA results, which are shown in the Supporting information. With two hydrophobic fluorophores (TPE and SP) in the monomer **TPE-SP**, it shows poor water solubility, leading to the reduction of the fluorescence property as well as its applications in aqueous solutions. Therefore, a novel amphiphilic copolymer **poly(NIPAM-co-TPE-SP)** was designed and synthesized by the incorporation of **TPE-SP** into NIPAM through free radical copolymerization with 2,2'-azobis(2-methylpropionitrile) (AIBN) as an initiator (Scheme 1), which has a molar ratio of NIPAM:TPE-SP = 100:1 with a molecular weight M_w = 12,344 Da and PDI = 1.80 (see Figures S16 and S17a). This polymer showed the typical properties of PNIPAM, including LCST and water solubility, as well as the AIE behavior of TPE units in the aggregation state and underwent the photo-isomerization of SP upon UV-vis irradiation. Hence, the FRET behavior between TPE and MC units in the aqueous solution of **poly(NIPAM-co-TPE-SP)** after UV exposure can be observed and utilized in the sensor applications. Most interestingly, this FRET process in water induced changeable dual emissions, which were used to detect cyanide ion and applied for bioimaging in living cells. Various FRET

phenomena were also confirmed by UV-vis and fluorescence spectra, as well as DFT computation in this report.

3.2. AIE Properties

In order to survey the AIE properties of this amphiphilic copolymer, the photoluminescence (PL) spectra of **poly(NIPAM-co-TPE-SP)** with the excitation wavelength of 365 nm are obtained in the THF/H₂O system with different water fractions (Figure 1a). We observed non-emissive PL behavior in pure and concentrated THF solutions of **poly(NIPAM-co-TPE-SP)** due to the intramolecular rotations of TPE units in low water content solutions, which led to the consummation of the excited state energy non-radiatively.⁶¹ However, the PL emission intensity of polymer increased gradually in THF/H₂O solutions with water fractions 80% and reached the maximum emission at 100% water (Figure 1b) where the inset revealed the PL photo-images of polymer solutions with non-emission and green emission in pure THF and water, respectively. It suggests that the aggregation state of **poly(NIPAM-co-TPE-SP)** arose at high water contents (> 80% H₂O) to turn on the AIE fluorescence due to the restriction of intramolecular motions of TPE units.¹⁸ Moreover, the average particle size of insoluble aggregates in the water solution is about 55.3 nm, which was determined by dynamic light scattering (DLS) measurements (Figure S17b). These results indicate that the AIE behavior of the **poly(NIPAM-co-TPE-SP)** solution occurred over 80% water content and reached the largest AIE effect in the aqueous solution (100% H₂O). Besides, the AIE effect of spiropyran and impact on FRET process in the aggregates of **poly(NIPAM-co-TPE-MC)** have been carried out and shown in Figure 1c,d (with the excitation wavelength of 365 nm) where the spiropyran unit is opened by UV exposure to become MC moiety. In addition, we also proceeded the PL measurements of **poly(NIPAM-co-TPE-MC)** with different H₂O fractions in THF/H₂O solutions with the excitation at 554 nm of the best absorption of MC moieties, but the results reveal that the MC moieties exhibited the similar AIE behaviors in both excitation wavelengths of 554 and 365 nm. Hence, the AIE effect on the open form of MC moiety in **poly(NIPAM-co-TPE-MC)** can be observed with stronger red emissions of MC at 627 nm at water contents higher than 50% (see Figure 1d), which occurred at an earlier AIE stage than the TPE emission at 517 nm at water contents larger than 70% (see Figure 1b). Moreover, the FRET contribution from the AIE donor of the TPE unit to the AIE acceptor of the MC unit can be observed in Figure 1c where the TPE emission of the AIE donor at 517 nm was reduced due to the FRET process to render its energy to the AIE acceptor of the MC unit in contrast to Figure 1a. Therefore, these amphiphilic copolymers, **poly(NIPAM-co-TPE-SP)** and **poly(NIPAM-co-TPE-MC)**, which exhibited the strongest AIE emissions in pure water, are further studied to demonstrate their novel applications.

3.3. PL Properties and FRET Behavior

The SP unit of copolymer could be gradually changed from the close form (SP) to the open form (MC) due to the cleavage of C(spiro)-O bond upon UV irradiation,³⁸ which lets **poly(NIPAM-co-TPE-SP)** convert to **poly(NIPAM-co-TPE-MC)** (Scheme 1). In order to investigate the rate of the ring-opening process for SP unit by UV light and thus to control the bifluorophoric FRET behavior between TPE and SP (or MC) units in copolymer, the

UV-vis and fluorescence spectra of the **poly(NIPAM-co-TPE-SP)** solution (1 g/L) in deionized water (DI water) are studied and displayed in Figure 2a,b.

The absorption spectra of **poly(NIPAM-co-TPE-SP)** in water are illustrated upon UV irradiation at 365 nm (by a hand-held ultraviolet lamp) in Figure 2a, measured at different UV exposure time intervals with a 10 s time span (from 0 to 90 s). We found that **poly(NIPAM-co-TPE-SP)** and **poly(NIPAM-co-TPE-MC)** could undergo reversible photo-isomerization between SP and MC isomers after UV and visible light irradiation, respectively, where a new absorption peak at 554 nm attributed to the MC appeared and saturated at approximately 80–90 s of UV exposure time, and the color of polymer solutions changed from yellow in the original close form (SP) to purple in the open form (MC) (inset photo-images of Figure 2a). Moreover, the fluorescence color of polymer solutions showed more significant changes upon UV exposure. The PL spectra ($\lambda_{\text{ex}} = 365 \text{ nm}$) of **poly(NIPAM-co-TPE-SP)** in water are demonstrated in Figure 2b, with similar UV exposure processes to induce the open form of **poly(NIPAM-co-TPEMC)** at different UV exposure time intervals. The fluorescence intensities of MC unit at 627 nm were gradually enhanced and saturated at approximately 80–90 s of UV exposure time. However, due to the FRET process, the fluorescence intensity of TPE unit at 517 nm in Figure 2b reduced gradually with increased UV exposure time and also saturated at approximately 80–90 s, which was matchable with the PL saturation of the open form (MC) at 627 nm in **poly(NIPAM-co-TPE-MC)**. Finally, the PL emission color of polymer solutions changed from green in the original close form (SP) to red in the open form (MC) (inset photo-images of Figure 2b) where the initial green emission color (before UV exposure) was attributed to the major TPE at 517 nm and the final red emission (after UV exposure) was attributed to both contributions of enhanced MC red emission (at 627 nm) and reduced TPE green emission (at 517 nm). The FRET process of **poly(NIPAM-co-TPE-MC)** can be explained by the partial energy transfer from the TPE unit to the MC unit and was shown in Figure 2c. Hence, the PL emission at 517 nm of the TPE unit in **poly(NIPAM-co-TPE-SP)** was gradually dropped after UV exposure, owing to the FRET process of TPE energy transferred to MC units in the open form of **poly(NIPAM-co-TPE-MC)**. Indeed, the MC absorption band of **poly(NIPAM-co-TPE-MC)** (absorption spectrum) is almost overlapped with the TPE emission band of **poly(NIPAM-co-TPE-SP)** (PL spectrum) in Figure 2d, leading to the energy absorption (energy acceptor) of the MC unit from the energy emission (energy donor) of the TPE unit, and thus to the enhancement of the MC emission at 627 nm and reduction of the TPE emission at 517 nm, simultaneously. In addition, the time-resolved photoluminescence (TRPL) measurements of **poly(NIPAM-co-TPE-SP)** have been done to further confirm the FRET process between green TPE and red MC units, which is shown in Figure S18f. Regarding the close form in **poly(NIPAM-co-TPE-SP)**, it shows a longer lifetime of 2.110 ns in the TPE emission (at 517 nm) without FRET than that (1.228 ns) of the open form in **poly(NIPAM-co-TPE-MC)** with the FRET process. These results indicated that the novel red emission of **poly(NIPAM-co-TPE-MC)** in water was not only produced by the red MC emission (at 627 nm) in its open form upon UV exposure but also further enhanced by the FRET process contributed from this specially designed bifluorophoric TPE and MC systems.

To characterize the optical reversibility of visible light on **poly(NIPAM-co-TPE-MC)**, the polymer solution was kept at room temperature under ambient light in a short time till no further intensity changes of UV-vis and PL spectra for the photo-isomerization process. We found that the naked-eye observation color of the solution changed from purple to pale yellow, and the green fluorescence was also turned back after 12 h (Figures S18a,b). Moreover, Figure S18c shows the disappearance of the absorption peak at 554 nm under the ambient light for 12 h to verify the regain of the close form (SP), and the disappearance of the PL emission peak at 627 nm and the increased PL emission peak at 517 nm to reveal the recovery of the close form (SP) (Figure S18d). To investigate the photo-fatigue resistant property of **poly(NIPAM-co-TPE-SP)** under UV and visible light, an aqueous solution of this polymer was exposed under UV (365 nm) and visible light for 2 and 7 min, respectively. We observed that **poly(NIPAM-co-TPE-SP)** underwent the photo-isomerization between SP and MC to enhance emission at 627 nm and to reduce emission at 517 nm where the ratios of PL intensities (I_{627}/I_{517}) were slightly changed for 10 cycles (see Figure S18e), indicating that **poly(NIPAM-co-TPE-SP)** possesses a good photo-fatigue resistant property. These results indicated that the reversible UV and visible exposure processes to produce **poly(NIPAM-co-TPE-MC)** and **poly(NIPAM-co-TPE-SP)**, respectively, can be easily carried out under a handheld ultraviolet lamp (with 2 min of UV exposure time) and ambient light or a common LED lamp with white light at room temperature, promising possible practical applications for photo-switchable materials and smart devices in the future.

3.4. pH Effects on FRET Behavior

Interestingly, **poly(NIPAM-co-TPE-SP)** in its close form (non-emissive SP) showed strong green fluorescence originated from the TPE unit in Figure S19a where the fluorescence of **poly(NIPAM-co-TPE-SP)** and the non-emissive SP unit could not be influenced by the acidic and basic conditions, so the PL intensities of **poly(NIPAM-co-TPE-SP)** with different pH values were not affected (see in Figure S19b). However, the emission properties of **poly(NIPAM-co-TPE-MC)** in its open form (MC) were obviously changed in different pH values (Figure 3a and inset). As shown in Figure 3b, the MC emission of **poly(NIPAM-co-TPE-MC)** at 627 nm decreased in the lower and higher ranges of pH values (i.e., pH = 1–3 and 11–13) and possessed the strongest red emissions in near-neutralized conditions (i.e., pH = 4–10), but the TPE emission of **poly(NIPAM-co-TPE-MC)** at 517 nm revealed the opposite way to have the lowest emission intensities in near-neutralized conditions (i.e., pH = 4–10) and increased in the lower and higher ranges of pH values (i.e., pH = 1–3 and 11–13). Therefore, the FRET process of TPE energy transferred to MC units in the open form of **poly(NIPAM-co-TPE-MC)** was strongly dependent on the pH environment, and the best FRET process to induce the highest MC emissions in **poly(NIPAM-co-TPE-MC)** occurred at near-neutralized conditions (i.e., pH = 4–10). However, the TPE emissions of **poly(NIPAM-co-TPE-SP)** at 517 nm without the FRET process will not be changed at all pH values.

3.5. Temperature Effects on FRET Behavior

PNIPAM is a well-known water-soluble and thermo-responsive polymer. The lower critical solution temperature (LCST) of this polymer is typically approximately 32 °C in aqueous

media.⁶¹ Herein, we determined LCST of **poly(NIPAM-co-TPE-SP)** in water by transmittance measurement. The LCST transition behavior of aqueous polymer solutions was studied through UV-vis measurements at 10 to 60 °C. We found that the transparent solution almost did not change at a low temperature (below 27 °C), which became turbid after increasing the temperature to 37 °C (Figure S20a). Moreover, the transmittance was dramatically fallen to 50% of the initial value at 32 °C, indicating that **poly(NIPAM-co-TPE-SP)** still had temperature-responsive characteristics of PNIPAM with the LCST at 32 °C. The reversible nature of **poly(NIPAM-co-TPE-SP)** solution was observed and shown in the inset of Figure S20a (top). Figure 4 and Figure S20b show the temperature effect on the fluorescence of **poly(NIPAM-co-TPE-MC)** in an aqueous solution by heating up to 60 °C and cooling to room temperature with UV exposure, respectively. The fluorescence intensities of **poly(NIPAM-co-TPE-MC)** in Figure 4a decreased at 627 nm and increased at 517 nm during the heating process (20–60 °C) under UV exposure due to the photo-isomerization from the MC to the SP form and turn to **poly(NIPAM-co-TPE-SP)** by heating, where Figure 4b reveals the relative changes of PL intensities at 627 and 517 nm for the MC and TPE units, respectively. Without UV exposure, **poly(NIPAM-co-TPE-SP)** maintained the temperature-independent close form and remained constant and strong green fluorescence from the TPE unit (see the bottom inset of Figure S20a), so the temperature changes could not affect the close form of **poly(NIPAM-co-TPE-SP)** at the range of 20–60 °C. However, with UV exposure, the thermally reversible red emission of **poly(NIPAM-co-TPE-MC)** gradually turned to green by heating over 40 °C and changed to green completely at approximately 55–60 °C due to the isomerization of the close form (SP) and vice versa by cooling back to 20 °C to recover the open form (MC) (Figure S20b) where the relative changes of PL intensities upon cooling is illustrated in the inset of Figure S20b. Hence, the temperature-dependent effect of thermally reversible fluorescence under UV exposure are also demonstrated in Figure S20c where the open form of **poly(NIPAM-co-TPE-MC)** under UV exposure could be transformed to the close form of **poly(NIPAM-co-TPE-SP)** by heating up to 55–60 °C so as to eliminate the FRET process. According to these results, the temperature-dependent FRET process of **poly(NIPAM-co-TPE-MC)** after UV exposure occurred due to the isomerization from the MC form to the SP form by heating, so the temperature effect was dominated over the UV exposure to induce the SP form of **poly(NIPAM-co-TPE-SP)** even with unfavorable UV exposure condition. However, the temperature-independent close form of **poly(NIPAM-co-TPE-SP)** indicates that the SP unit can only be opened by UV light rather than by heating.

3.6. Ratiometric Fluorescent Probe for Cyanide Sensing

Since **poly(NIPAM-co-TPE-SP)** was unable to react with CN^- in its close form (SP) to detect the cyanide anion, no changes in its absorption and PL spectra were observed upon the addition of CN^- (Figure S21). Herein, only **poly(NIPAM-co-TPE-MC)** as an amphiphilic FRET sensor material could react with CN^- to enlarge and magnify the ratiometric fluorescence changes at two respective wavelengths of 517 and 627 nm to detect the cyanide ion by the relative ratios of their fluorescence intensities (i.e., I_{627}/I_{517}), which were much more distinct than their absorption changes by naked eye observation (Figures S22 and S23). According to the previous PL results in Figure 2b, the ratiometric fluorescence detection of CN^- was carried out by continuous titrations with CN^- (0–110

μM) in a MOPS (3-(N-morpholino) propanesulfonic acid) buffer solution (10 mM, pH = 7.0), and the diminished FRET process was induced by the reduction of MC emission at 627 nm (owing to the reaction of MC with CN^-) and the recovery of TPE emission at 517 nm (Figure 5a). Besides, Figure S24c shows an excellent linear relationship between I_{627}/I_{517} of **poly(NIPAM-co-TPE-MC)** and CN^- concentration (0–80 μM) where the PL emission also illustrated a significant color change from red to green during this CN^- titration process (Figure S24a). Thus, the limit of detection (LOD) was calculated to be 0.26 μM for CN^- based on the $3\sigma/k$ method from the fluorescence titration profile (Figure S24b,c), and the LOD of this work is much lower than most of the previous reports^{10,13–15} due to this excellent ratiometric PL results via the efficient FRET process. Moreover, the LOD of **poly(NIPAMco-TPE-MC)** is seven times lower than the maximum allowable CN^- concentration in drinking water by WHO (1.9 μM),⁴ which provides a more sensitive FRET sensor material for the CN^- detection in the aqueous solution. Since the CN^- anion is a strong nucleophile group to attack the electron-deficient group easily, including the indolium ($-\text{C}=\text{N}^+$) group,^{5–10,15} CN^- could react with carbon atom ($-\text{C}=\text{N}^+$) in MC moieties of **poly(NIPAM-co-TPE-MC)** to form an MC-CN system via nucleophilic addition, resulting in the interruption of MC π conjugation¹⁰ to block the FRET process (see Figure 5b). Moreover, as shown in Figure S18f, the longer lifetime of **poly(NIPAM-co-TPE-MC)** + CN^- in the reacted open form with CN^- (2.109 ns) demonstrated the shutdown of FRET to regain the donor energy in the green TPE emission. Therefore, the FRET fluorescence of the MC unit was gradually quenched at 627 nm due to the reaction of CN^- with MC to recover TPE emission at 517 nm.

To examine the selectivity of this FRET sensor polymer toward CN^- over other anions, the PL spectra of **poly(NIPAMco-TPE-MC)** in the MOPS buffer solution were recorded in the presence of respective analytes (200 μM), including F^- , Cl^- , Br^- , I^- , OH^- , NO_3^- , PF_6^- , HCO_3^- , HSO_4^- , H_2PO_4^- , CH_3COO^- (AcO^-), SO_4^{2-} , HPO_4^{2-} , S^{2-} , GSH, and L-Cys. Among these analytes, CN^- revealed the best contrast in their PL spectra and the most obvious emission color change from red to green (see Figure 5c and inset in Figure 5d) due to the largest elimination of its FRET process. The largest reduction of I_{627}/I_{517} with an eight-fold smaller value for CN^- was also observed in Figure 5d. These results verify that **poly(NIPAM-co-TPE-MC)** as a highly sensitive and selective FRET sensor toward CN^- in water can be utilized for the detection applications in environmental and biological systems.

3.7. Theoretical Calculations

To better understand the FRET processes of **poly(NIPAM-co-TPE-MC)**, we carried out DFT calculations of **TPE-SP**, **TPE-MC**, and **TPE-MC-CN $^-$** using time-dependent density functional theory (TD-DFT) at IEF-PCM-B3LYP/6–31G(d) in water (see Table S1 and Figure 6). All computations were performed using the Gaussian 16 program. The optimized structures of **TPE-SP** and **TPE-MC** in their respective close and open forms revealed an approximately 1.3 nm central-to-central distance between the TPE to SP or MC centers (Figure 6a and Figure S25) and falls within the range of donor-acceptor distance (1–10 nm) required for the FRET process.⁴⁴ The absorption wavelengths for **TPE-SP**, **TPE-MC**, and **TPE-MC-CN $^-$** are within the range of 360–380 nm (Table S1), but an extra prominent peak is found for TPE-MC at 514 nm (close to TPE emission at 517 nm, based on fluorescence

spectra, see Figure 2b), suggesting a possible FRET process. The molecular orbital diagrams for **TPE-SP**, **TPE-MC**, and **TPEMC-CN⁻** are shown in Figure 6b–d. In **TPE-SP**, two absorptions occur at 359 nm (Figure 6b), a strong HOMO → LUMO+2 transition (oscillator strength $f = 0.51$) corresponds to excitation on the TPE fragment, and a weaker HOMO-2 → LUMO transition ($f = 0.15$) corresponds to excitation on the SP fragment. In **TPE-MC**, the same absorptions occur at 360 and 361 nm (Figure 6c), and a strong absorption at 514 nm, corresponding to HOMO-1 → LUMO transition in the MC motif ($f = 0.77$), indicates energy transfer from the TPE to the MC units. In **TPE-SP**, the SP unit adopts a close form and absorption for the HOMO-1 → LUMO transition is weak due to limited orbital overlap (absorption at 435 nm ($f = 0.01$)), leading to the absence of energy transfer (Figure 6b). In **TPEMC-CN⁻**, the MC unit reacted with CN⁻ raises the energy of the LUMO orbital (from -2.92 to -1.71 eV) (Figure 6c,d), resulting in a larger HOMO → LUMO gap of 0.7 eV for **TPEMC-CN⁻** (3.4 eV), a shorter absorption wavelength of 379 nm for HOMO → LUMO transition, and thus a shutdown of the FRET process.

The differences in the FRET behavior of **TPE-MC** at pH = 1 and pH = 13 (see the PL spectra in Figure 3a) can be explained by the changes in the orbital energies of the MC unit. The computational results in Figure 7a,b suggest that in the acidic media, a proton resides in between the oxygen atom attached to the TPE unit and the oxygen atom at the nitrophenolate in the MC unit (Figure 7a), and in the basic media, the alpha position neighboring the positively charged carbon is deprotonated (Figure 7b). The computed relative energies of different protonated/deprotonated forms are included in the Supporting Information (Figure S26 and Table S2). Both protonation and deprotonation widen the HOMO-LUMO gaps (to 3.2 and 3.4 eV, respectively, Figure 7c,d) and reduce the absorption wavelength (to 437 and 377 nm, respectively, Table S1) of TPE-MC to a range where the FRET process becomes inaccessible.

3.8. Cell Culture and Confocal Fluorescence Imaging of Polymers in Living Cells

With dual fluorescence emissions, **poly(NIPAM-co-TPE-SP)** and **poly(NIPAM-coTPE-MC)** could be utilized for cellular imaging on HeLa cells by a confocal fluorescence microscope. As shown in Figure 8, without CN⁻, we observed strong green fluorescence emissions at channels f-j and strong red emissions at channels p-t for close and open forms, respectively. Moreover, the overlay of TPE green or MC red emissions with the blue emission of DAPI revealed that **poly(NIPAM-co-TPE-SP)** and **poly(NIPAM-coTPE-MC)** were mostly localized in the cytoplasm over the nucleus of HeLa cells without and with CN⁻ treatments (Figures 8j,t and 8o,y, respectively). Upon addition of CN⁻ (10 μM) to the close form treated cells, no conspicuous changes were obtained in the green fluorescence channel (Figure 8h,m), as well as no obvious changes in the overlay images (Figure 8j,o). However, the cells incubated with the open form exhibited the guest selectivity with CN⁻ by enhanced green fluorescence (Figure 8r,w) and quenching of the red emission (Figure 8s,x) and thus induced significant changes in the overlay images (Figure 8t,y) after the treatment of CN⁻ (10 μM). These results indicate that **poly(NIPAM-co-TPE-MC)** could be particularly used for the bioimaging of the cyanide detection in living cells. In addition, the cytotoxicities of close and open forms were also evaluated by a standard MTT (methyl thiazolyl tetrazolium) assay using HeLa cells. The cells were grown in a 96-well cell culture plate, and different

amounts of polymers (0–25 μM based on SP and MC) were added in the medium and then kept at 37 °C with 5% CO_2 for 24 h. The medium was added 20 μL methyl thiazolyl tetrazolium (1 mg/mL) and then kept at 37 °C with 5% CO_2 for 4 h. As shown in Figure S27, the cell viabilities are higher than 85% when the cells were treated with 25 μM fluorophores for 24 h, which demonstrates that both close and open forms of respective **poly(NIPAM-co-TPE-SP)** and **poly(NIPAM-co-TPE-MC)** had low cytotoxicities.

4. CONCLUSIONS

In summary, we synthesized an amphiphilic polymer **poly(NIPAM-co-TPE-SP)** and developed a novel FRET sensor material, **poly(NIPAM-co-TPE-MC)**, based on the photoisomerization of SP and MC moieties under UV irradiation. Various FRET processes of this polymer were observed by UV–vis and PL spectra under UV and visible light, pH conditions, thermal treatments, and the interaction of CN^- , which were further verified by DFT calculations. The FRET process of **poly(NIPAM-co-TPE-MC)** is proven to be pH and temperature-dependent, whereas **poly(NIPAM-co-TPE-SP)** maintained the strong green emission of TPE in different pH values and temperatures by the lack of FRET phenomena. Owing to the useful FRET behavior, **poly(NIPAM-co-TPE-MC)** could be utilized for detecting CN^- by distinct ratiometric fluorescence changes with a very high selectivity and sensitivity (LOD = 0.26 μM) in water. In addition, due to the good biocompatibility of **poly(NIPAM-co-TPE-MC)**, this novel FRET sensor could be utilized for cellular imaging and CN^- detection in living cells. Finally, the amplification of detection signals by ratiometric PL designs through FRET processes between TPE and MC AIEgens in **poly(NIPAM-co-TPE-MC)** might facilitate many promising sensor applications for chemical, environmental, and biological detections in aqueous media.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. (a) PL spectra and (b) relative fluorescence intensity at 517 nm of **poly(NIPAM-co-TPE-SP)** and (c) PL spectra and (d) relative fluorescence intensity at 627 nm of **poly(NIPAM-co-TPE-MC)** with different H₂O fractions in THF/H₂O solutions. Inset: PL photo-images of panel (b) **poly(NIPAM-co-TPE-SP)** and panel (d) **poly(NIPAM-co-TPE-MC)** in THF (left) and water (right). Concentration: 1 g/L, λ_{ex} : 365 nm.

Figure 2. Time-dependent (a) absorption and (b) PL spectra ($\lambda_{\text{ex}} = 365 \text{ nm}$) of **poly(NIPAM-co-TPE-SP)** and **poly(NIPAM-co-TPE-MC)** (1 g/L) in water upon UV exposure (0–90 s). Insets: Photo-images of naked-eye observation and photoluminescence color changes. (c) Schematic illustration of the energy transfer from TPE to MC unit via FRET process after UV exposure and (d) spectral overlap of absorption spectra of **poly(NIPAM-co-TPE-MC)** and emission spectra of **poly(NIPAM-co-TPE-SP)**.

Figure 3. (a) PL spectra of **poly(NIPAM-co-TPE-MC)** and (b) relative fluorescence intensities of **poly(NIPAM-co-TPE-MC)** at 517 and 627 nm with different pH values. Insets: PL photos of polymer solutions at different pH values. Concentration: 1 g/L, λ_{ex} : 365 nm.

Figure 4.

(a) PL spectra of **poly(NIPAM-co-TPE-MC)** and (b) relative fluorescence intensities of **poly(NIPAM-co-TPE-MC)** at 517 and 627 nm under UV exposure upon heating (20–60 °C). Insets: PL photo-images of polymer solutions at different temperatures. Concentration: 1 g/L, λ_{ex} : 365 nm.

Figure 5.

(a) Titration profile change and hypsochromic shift of **poly(NIPAM-co-TPE-MC)** after addition of different CN⁻ concentrations, (b) proposed schematic illustration of the CN⁻ detection mechanism, (c) PL spectra (insets: PL photo-images of polymer solution with/without cyanide ion), and (d) fluorescence intensity ratios response of **poly(NIPAM-co-TPE-MC)** upon addition of various analytes (200 μM). Inset: PL photo-images of polymer solutions in the presence of various analytes. Concentration: 1 g/L, λ_{ex} : 365 nm, MOPS buffer solution, ***poly(NIPAM-co-TPE-MC)**.

Figure 6. (a) Optimized structures of **TPE-SP**, **TPE-MC**, and **TPE-MC-CN⁻** at B3LYP/6-31G(d). Only MC and MC-CN⁻ fragments of the latter two structures are shown, since the TPE fragment of all three compounds is similar (the optimized coordinates are included in the Supporting Information). Electronic transitions and corresponding molecular orbitals for (b) **TPE-SP**, (c) **TPE-MC**, and (d) **TPE-MC-CN⁻**. Orbital energies were computed at IEF-PCM-B3LYP/6-31G(d).

Figure 7. Optimized structures of (a) protonated and (b) deprotonated **TPE-MC** at B3LYP/6-31G(d). Protonated and deprotonated positions are circled in red. Molecular orbital diagrams of (c) protonated and (d) deprotonated **TPE-MC**, with orbital energies computed at IEF-PCM-B3LYP/631G(d).

Figure 8. Confocal fluorescence images of HeLa. (a–e) Control experiments of HeLa. (f–j) Cells were incubated with **poly(NIPAM-co-TPE-SP)** for 30 min. (k–o) Cells were treated with **poly(NIPAM-co-TPE-SP)** for 30 min followed by incubation with CN⁻ for 30 min. (p–t) Cells were incubated with **poly(NIPAM-co-TPE-MC)** for 30 min. (u–y) Cells were treated with **poly(NIPAM-co-TPE-MC)** followed by incubation with CN⁻ for 30 min. The polymeric fluorophores and cyanide concentration = 10 μ M. Green and red emissions were collected at 500–550 and 600–650 nm, respectively. Scale bars = 10 μ m, λ_{ex} : 365 nm.

Scheme 1.
Synthetic Routes of Monomer TPE-SP and Copolymers Poly(NIPAM-co-TPE-SP) and Poly(NIPAM-co-TPE-MC)