

Synthesis and Characterization of Novel Reversible Photoswitchable Fluorescent Polymeric Nanoparticles via One-Step Miniemulsion Polymerization

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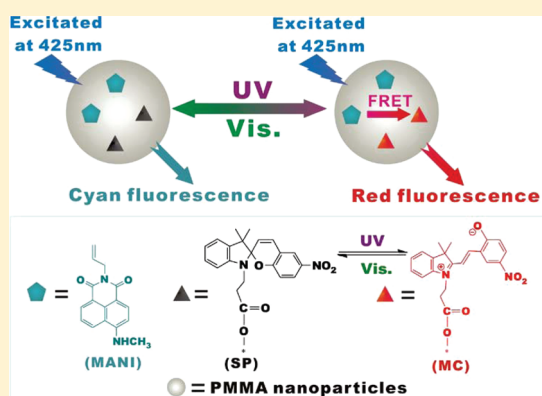
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S Supporting Information

ABSTRACT: In the present study, novel polymeric nanoparticles of ca. 55 nm in diameter with reversibly photoswitchable fluorescence properties were synthesized using a facile one-step miniemulsion polymerization, in which the donor of fluorescence resonance energy transfer (FRET), 4-methamino-9-allyl-1,8-naphthalimide (MANI), and the acceptor, spiropyran-linked methacrylate (SPMA), were covalently incorporated into a polymeric matrix during the polymerization process. The fluorescence emission of MANI dye in nanoparticles can be reversibly switched using the alternating irradiation of UV and visible light, which can induce the structural interconversion between the SP form and MC form of spiropyran moieties inside nanoparticles and thus reversibly switch on and switch off the FRET process. The prepared photoswitchable fluorescent polymer nanoparticles not only show a high load capacity of dyes, controllable amount and ratio of the two dyes, and tunable FRET efficiency but also exhibit higher spectral stability because of covalent linkage between dye molecules and the particle, relatively fast photoresponsibility, and better photoreversibility compared to some previously reported systems.



INTRODUCTION

In recent years, novel fluorescent materials, especially those that can be reversibly switched, are of high interest in selectively highlighting biological substances (proteins, cells, or organelles) and individually light-addressable nanoscale devices.^{1–8} Photochromic compounds such as spiropyran, which can be reversibly converted upon the irradiation of UV or visible light between the open-ring state and the close-ring state with different spectroscopic properties, have been used to achieve a reversible photoswitchable fluorescent system through constructing a dyad or triad with another energy level matched fluorescent dye and inducing the occurrence of fluorescence resonance energy transfer (FRET) from the fluorescent dye to the open-ring state of spiropyran moieties.^{9,10} However, the low solubility in aqueous media as well as pH dependence of the fluorescence restricted the use of these photoswitchable fluorescent compounds in biological labeling and imaging. In addition, their cytotoxicity represents additional drawbacks for *in vivo* applications.² To address these issues, the photoswitchable fluorescent nanoparticles containing fluorescent dye and photochromic compounds have been developed to serve as an alternative approach for biological

applications.^{3,8,11–13} These photoswitchable fluorescent nanoparticles exhibit several advantages over traditional organic dyes in that they not only exhibit extraordinarily high brightness, excellent stability, and biocompatibility, but also feature versatility in the design and synthesis of photoswitchable fluorescent system.^{3,8}

Conventional photoswitchable fluorescent nanoparticles generally incorporated the dye molecules into the particle matrix through the modification of nanoparticles, or by physical doping or adsorption during the synthesis process, which usually involve complicated synthetic pathways or have the problem of dye leakage. For example, Medintz et al.¹¹ labeled maltose binding protein (MBP) with a photochromic dye, then coordinated multiple copies of this labeled MBP to individual QDs. They demonstrated that the photochromic dye attached to MBP can be used in the reversible modulation of QD emission. Our group also fabricated two kinds of amphiphilic fluorescent polymer

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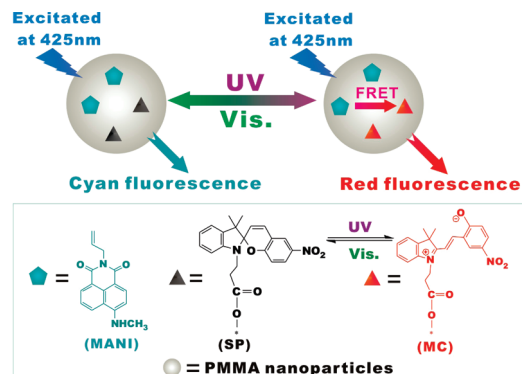
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nanoparticles with reversibly light-induced fluorescence modulation properties.^{14,15} However, synthesis of these photoswitchable fluorescent nanoparticles requires a relatively complicated process, and we could not control the quantity of the dyes within the nanoparticles, especially in the core/shell interface. In addition, the leakage of unbound dyes is also a disadvantage for potential biological application. Later on, we employed a one-step miniemulsion polymerization to noncovalently incorporate two chromophores into individual nanoparticles and formed the nanoparticle-based fluorescence modulation systems.¹⁶ This one-step approach can produce fluorescent nanoparticles with a higher dye loading, improved dye stability, and a controllable amount and ratio of the two dyes. However, the slow release of fluorescent dyes during a synthesis or preservation process might be disadvantageous in terms of long-term stability. One promising strategy to address this issue, we suppose, is to covalently incorporate dye molecules into polymer nanoparticles, which may avoid the dyes' leakage and significantly improve the structural stability of the whole FRET system.

In recent years, various methods, such as microemulsion polymerization, sol–gel synthesis, and atom transfer radical polymerization (ATRP), have been developed to covalently incorporate both photochromic components and fluorescent dyes into either uniform or composite nanostructures to fabricate photoswitchable fluorescent nanoparticles. For example, Li et al.¹⁷ covalently incorporated a FRET donor (perylene derivative) and acceptor (spiropyran derivative) into polymer nanoparticles via a modified microemulsion polymerization method to generate optically addressable dual-color fluorescent systems, which exhibit high photoluminescence and superior resistance to photobleaching.¹⁷ Hell and co-workers covalently incorporated a rhodamine fluorescent dye and a photochromic diarylethene into a silica network via a sol–gel process, fabricating FRET-based photoswitchable systems.¹⁸ With the photoactive units covalently linked to the silica network, it could avoid any dye leakage in biological labeling. Recently, Liu et al.¹⁹ combined photoswitchable FRET systems with organic/inorganic hybrid nanoparticles coated with responsive polymer brushes via ATRP method, and this greatly enhanced the structural stability of the system in aqueous solution. Moreover, the aqueous dispersion of this type of hybrid silica nanoparticle is capable of emitting multicolor fluorescence, which can be facily tuned by UV irradiation, visible light, and temperatures or a proper combination of these factors. However, it is worthy to note that for all of the above examples, the use of ATRP method to covalently incorporate multiple dyes into nanoparticles still needs relatively complicated routes, while the use of microemulsion polymerization or a sol–gel approach usually is not able to effectively control the quantity or ratio of dyes in nanoparticles due to the different diffusion coefficients in water (or another liquid phase) between the dyes and monomer during the preparation process.

On the basis of past experiences with fabrication of photoswitchable fluorescent nanoparticles, we anticipate that the combination of covalent incorporation of dyes with a miniemulsion polymerization approach^{16,20,21} should be able to provide many advantages for constructing a particle-based FRET system, such as a facile and flexible synthesis route, high dye loading, enhanced dye stability, controllable quantity, and the ratio of dyes. In the present work, we employed a modified one-step miniemulsion polymerization to prepare reversible photoswitchable fluorescent polymeric nanoparticles by covalently

Scheme 1. Schematic Illustration of Novel Reversible Photoswitchable Fluorescent Nanoparticles Covalently Incorporating Fluorescent Dye (MANI) and Photochromic Spiropyran (SPMA)



incorporating a fluorescent dye (4-methamino-9-allyl-1,8-naphthalimide, MANI, donor) and a photochromic spiropyran derivative (acceptor) into individual polymeric nanoparticles. The donor MANI is one of the 1,8-naphthalimide derivatives, which are well-known fluorescent probes for biosensors and chemosensors,^{22,23} useful *n*-type materials in organic light-emitting diodes,^{24,25} and laser dyes.²⁶ Despite the large amount of research on the 1,8-naphthalimides, little is known about their application in photoswitchable fluorescent materials as a fluorescent donor. For this approach, the two chromophores, with their amount and ratio being easily controllable, can be covalently linked to the polymeric matrix during the polymerization process, which can greatly increase the stability of photoswitchable fluorescent nanoparticles. The fluorescence emission of MANI dye in nanoparticles can be reversibly switched “on” and switched “off” as a result of the structural interconversion between two states of spiropyran moieties upon UV and visible light irradiation (Scheme 1). Moreover, these photoswitchable fluorescent polymeric nanoparticles also exhibit other advantages, such as the tunable FRET efficiency, faster photoresponsibility, and better photoreversibility compared to our previously reported systems.^{14–16}

EXPERIMENTAL SECTION

Materials. The surfactant sodium dodecyl sulfate (SDS, 99%, Aldrich), *n*-hexadecane (HD, 99%, Aldrich), 4-bromo-1,8-naphthalic anhydride (99%, TCI), methylamine water solution (MA, 40%, Aladdin), allylamine (99%, Aladdin), *N,N'*-dicyclohexylcarbodiimide (DCC, 99%, Alfa), and 4-dimethylamino-pyridine (DMAP, 99%, Alfa) were used as received. Dichloromethane (A. R.) was washed with sulfuric acid and then distilled from CaH₂. 2-Hydroxyethyl methacrylate (HEMA, 97%, Aldrich) was dissolved in water (25 vol %) and washed four times with an equal volume of hexane, then dried over MgSO₄ and distilled under a vacuum prior to use. Potassium persulfate (KPS, 99.99%, Aldrich) was recrystallized from water and dried under a vacuum. The water used in this work is the double-distilled water, which was further purified with a Milli-Q system. Tetrahydrofuran (THF, A.R.) and triethylamine (A.R.) were distilled over CaH₂. Petroleum ether, benzene, and other reagents were analytical reagents and used without further purification.

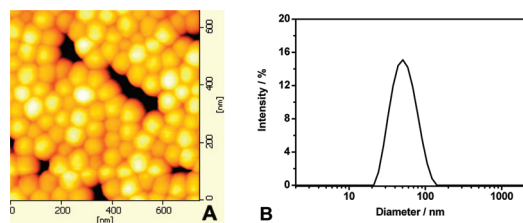


Figure 1. (A) AFM image of photoswitchable fluorescent nanoparticles (sample NP-N4). (B) Size distribution for the nanoparticle sample NP-N4 determined by DLS.

Synthesis of 4-Methamino-9-allyl-1,8-naphthalimide (MANI) Monomer. MANI was synthesized according to modified literature procedures.²⁷ First, 4-bromo-*n*-substituted-1,8-naphthalimides was prepared according to literature procedures.²⁸ Typical procedures employed for the preparation of MANI are as follows. A mixture of 0.01 mol of 4-bromo-*n*-substituted-1,8-naphthalimides, 0.015 mol of methylamine, 0.01 mol of triethylamine, and 0.1 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was dissolved in 100 mL of 2-methoxyethanol. The mixture was heated at 90 °C for 5 h, and after cooling to room temperature, the liquor was poured into 400 mL of water. The solid residue was filtered, washed with water, dried, and then purified by silica gel column chromatography using ethylacetate/petroleum ether (1:3 v/v) as an eluent. The product was yellow powder in 65% yield. The ^1H NMR spectrum for the product is shown in Figure S1 (Supporting Information; 500 MHz, CDCl_3 , 25 °C; ppm): 3.2 (CH_3 , 3H), 4.8 ($-\text{CH}_2-$, 2H), 5.2 (olefinic protons, CH_2 , 2H), 6.0 (olefinic protons, CH, 1H), 6.7–8.6 (aromatic protons).

Synthesis of the Spiropyran-Linked Methacrylate (SPMA) Monomer. SPMA was synthesized with a slightly modified procedure reported elsewhere.¹⁶ During the synthesis process, all of the reaction vessels were wrapped with aluminum foil, so as to ensure the reaction was performed in the dark. For the synthesis of SPMA, first, 1-(β -carboxyethyl)-3',3'-dimethyl-6-nitrospiro(indoline-2',2-chromane) (SPCOOH) was synthesized according to a literature procedure.²⁹ Subsequently, SPCOOH (0.38 g, 1 mmol), HEMA (0.39 g, 3 mmol), and DMAP (0.027 g, 0.2 mmol) were added to a 50 mL round-bottomed flask equipped with a pressure-equalized dropping funnel, magnetic stirrer, and nitrogen inlet. Cold, dry THF (10 mL) was added to the flask, and the solution was cooled to 0 °C. A red brown solution resulted. A DCC solution (0.206 g, 1 mmol in THF) was added to the resulting solution via the pressure-equalized dropping funnel over 45 min. The flask was maintained at 0 °C for 2 h; then the temperature was raised gradually to room temperature for about 24 h. The product was filtrated with cold dry THF (10 mL) three times, giving a red filtrate. After removal of most of the solvent under vacuum distillation, the residue was washed with a larger amount of distilled water to remove the unreacted HEMA. The precipitate was dissolved in benzene and filtrated again to remove the unreacted SPCOOH; afterward, most of the solvent was evaporated under a vacuum and below 45 °C for avoiding SPMA polymerization. The solution was precipitated in a large amount of petroleum ether; finally, a fine red purple precipitate of purified 2-(3-(3',3'-dimethyl-6-nitrospiro(indoline-2',2-chromane)-1'-yl)propanoyloxy)-ethyl-methacrylate (SPMA) was obtained. The target product was dried in a vacuum oven overnight at room temperature. The product was a red purple powder in 85% yield. The ^1H NMR spectrum for the

product is shown in Figure S2 (Supporting Information; 500 MHz, CDCl_3 , 25 °C, ppm): 1.0–1.3 (CH_3 of spiropyran, 6H), 1.8–1.9 (CH_3 of HEMA, connected to olefinic carbon, 3H), 2.6–2.7 ($-\text{CH}_2\text{COO}-$ of spiropyran, 2H), 3.5–3.6 ($-\text{CH}_2\text{N}-$ of spiropyran, 2H), 4.2 ($-\text{CH}_2\text{O}-$ of HEMA, 4H), 5.5–6.0 (olefinic protons, CH_2 and two CH, 4H), 6.6–8.1 (aromatic protons).

Preparation of Novel Photoswitchable Fluorescent Polymeric Nanoparticles. A mixture containing the monomers and hydrophobes (MMA, MANI, SPMA, HD) was added to a water solution with emulsifier (SDS) and stirred (1000 r/min) for 15 min; then, the mixture was ultrasonicated for 30 min (KQ2000DE) to obtain a miniemulsion. The mixture was cooled in an ice-bath during ultrasonication to avoid being heated. The resulting miniemulsion was put into a 50 mL flask equipped with a condenser, which was immersed in an oil bath with a thermostat. The polymerization was started by adding an aqueous solution of KPS and preceded at 60 °C for 210 min. After the polymerization, the as-prepared nanoparticle dispersions were percolated, and finally the novel nanoparticle dispersions were obtained.

Characterization. ^1H NMR spectra were recorded on a Bruker Avance 500 MHz NMR spectrometer. The nanoparticle diameters were determined by a Malvern Nano-ZS90 instrument, and their morphology was observed with an atomic force microscope (AFM, Seiko SII 400) in the tapping mode. UV–vis spectra were recorded on a Shimadzu UV-2501PC spectrophotometer at room temperature. Fluorescence spectra were recorded on a Shimadzu RF-5301PC fluorescence spectrophotometer at room temperature. The cyclic voltammetric measurements were carried out with a CH Instruments 631A apparatus. Ag/Ag^+ was used as the reference electrode, a glassy carbon as the working electrode, and a Pt wire as the auxiliary electrode. The redox potentials were measured in Ar bubbled acetonitrile using 0.1 M tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) as the supporting electrolyte. The scanning speed was maintained at 0.1 V/s.

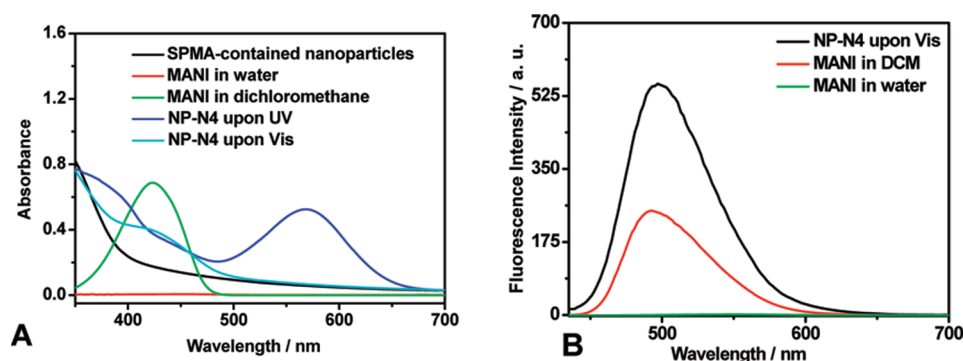
RESULTS AND DISCUSSION

Preparation of Novel Photoswitchable Fluorescent Polymeric Nanoparticles. In this study, a modified one-step miniemulsion polymerization is employed for the preparation of novel photoswitchable fluorescent polymeric nanoparticles, in which the fluorescent dye (MANI) was covalently bound into a polymeric matrix, as the presence of an unsaturated polymerizable double bond in MANI dye enables them to be copolymerized with vinyl monomers to form a covalent bond in the polymer matrix.^{27,28} Importantly, this can effectively avoid the dye leakage, which is an undesired effect observed with nanoparticles, as the dyes were noncovalently incorporated into the polymeric nanoparticles.^{14–16} In order to prepare the photoswitchable fluorescent polymeric nanoparticles dispersion, we first synthesized a cyan fluorescent dye (MANI) and a photochromic spiropyran derivative (SPMA), as described in the Experimental Section. As for the preparation of the particle-based FRET system, first a mixture containing the monomers and cosurfactant (MMA, HD, MANI, SPMA) were dispersed into water with the surfactant (SDS) under ultrasonic shear force; then the water-soluble initiator (KPS) was used to start the miniemulsion polymerization. Finally, nanoparticle dispersion with covalently incorporating two dyes was produced. The prepared fluorescent

Table 1. List of Some Data and Parameters of Two Dye-Incorporated Nanoparticle Samples

sample ^a	MANI [mg] ($[\times 10^{-4} \text{ M}]$)		SPMA [mg] ($[\times 10^{-3} \text{ M}]$)		diameter ^d [nm]
	feed	determined ^b	feed	determined ^c	
NP-N1	6(9.00)	5.4(8.10)	10(0.81)	8.6(0.70)	54.9
NP-N2	6(9.00)	5.5(8.25)	20(1.63)	17.8(1.45)	57.5
NP-N3	6(9.00)	5.5(8.25)	30(2.44)	26.4(2.15)	52.8
NP-N4	6(9.00)	5.5(8.25)	40(3.25)	33.8(2.75)	55.4

^a The MMA/HD/SDS/KPS feed is 2.5/0.15/0.10/0.085 g, respectively. ^b Calculated by using the absorbance of MANI at 424 nm in a nanoparticle dispersion (eliminate the effect of scattering light) and the molar extinction coefficient of MANI in dichloromethane ($\epsilon = 14000 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$). ^c Calculated by using the absorbance of SPMA at 340 nm in a nanoparticle dispersion (eliminate the effect of scattering light) and the molar extinction coefficient of SPMA in dichloromethane ($\epsilon = 8780 \text{ mol}^{-1} \cdot \text{L} \cdot \text{cm}^{-1}$). ^d Average nanoparticle diameter, determined from DLS data.

**Figure 2.** (A) Absorption spectra of MANI dye in pure water, in dichloromethane solution, and in a photoswitchable fluorescent nanoparticle dispersion sample (NP-N4), as well as an SPMA-containing nanoparticle dispersion. (B) Fluorescence emission spectra of MANI dye in pure water, in dichloromethane solution, and in sample NP-N4.

nanoparticles show typical reversible photoswitchable fluorescence upon irradiation with UV or visible light in aqueous media, as illustrated in Scheme 1.

By typical one-step miniemulsion polymerization, nanosized to submicrometer-sized polymer particles can be prepared by varying some experimental parameters, such as the amount of surfactant and hydrophobe, the concentration of the monomer in aqueous solution, and the ultrasonic time.^{20,21} It has been well-documented that the spectral properties of the fluorescent nanoparticles can be affected by the particle size due to the fact that the scattering light by a particle is at a maximum when the diameter of the particle is equivalent to one-half of the wavelength of incident light.³⁰ Thus, nanoparticles with a smaller size should be synthesized to reduce the scattering effect. In this paper, by optimizing some experimental parameters, we obtained several nanosized particle samples with diameters of around 55 nm (Table 1). Table 1 also indicates that the presence of dye has little effect on the particle size.

Figure 1A shows the atomic force microscopy (AFM) of a photoswitchable fluorescent nanoparticle sample (NP-N4). It is clearly shown that most of the nanoparticles were discrete, smooth, and regular, with their diameters ranging from 50 to 70 nm. And NP-N4's stable dispersion with an average diameter of 55.0 nm is determined by DLS (Figure 1B). The above results prove that uniform polymer nanoparticles with defined size can be prepared by miniemulsion polymerization.

Spectroscopic Properties of Photoswitchable Fluorescent Nanoparticles. Figure 2 shows the absorption and fluorescence emission spectra for MANI dye in pure water, in dichloromethane solution, and in a nanoparticle dispersion sample

(NP-N4). The MANI dye, which is a typical hydrophobic fluorescent dye, is hardly soluble in water and exhibits very low absorption and weak fluorescence in pure water (Figure 2). However, upon being incorporated into the nanoparticles, the fluorescent dye (MANI) exhibits prominent absorption at around 424 nm, which is close to that for MANI dye in a low-polarity solvent like dichloromethane (Figure 2A). We determined the percentage of the covalently bound MANI molecules in nanoparticles during the copolymerization according to a previously reported procedure.²⁸ The collected dried nanoparticles containing only MANI dye were repeatedly dissolved in dichloromethane and precipitated in methanol several times to remove free dye molecules, and then the absorption spectra for the dichloromethane solution of the polymer were measured. We found that the absorption spectra for the dichloromethane solution of the purified polymers showed an identical λ_{max} to that of the pure MANI dyes in dichloromethane, indicating that the basic spectral property for the MANI dye did not change during the polymerization (Figure S3, Supporting Information). By comparing the absorbance value,²⁸ it can be found that over 90% of the initially introduced dyes were covalently bound to the polymer chains. Moreover, the fluorescence intensity of the MANI/nanoparticles dispersion increased greatly compared to the saturated MANI aqueous solution, and its maximum fluorescence emission peak is close to that for MANI dye in a dichloromethane solution, as shown in Figure 2B. It is well-known that the photochromic spiropyran derivatives can assume one of two stable states: the close-ring state, known as the spiro (SP) form, and the open-ring state, known as the merocyanine (MC) form. Upon irradiation with UV light, the spiropyran

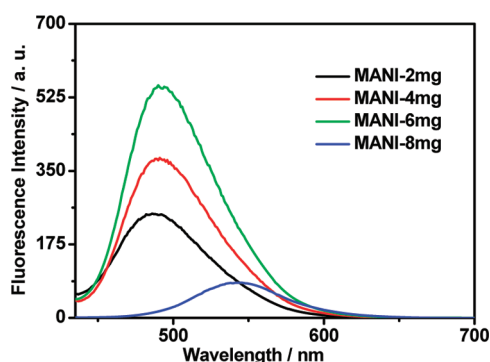


Figure 3. Fluorescence emission spectra for MANI dye in nanoparticle samples with different MANI feed contents (for samples MANI-2mg to MANI-8mg, the MANI feed content increases by a certain value).

molecules adopt the MC form, while they adopt the SP form under visible light.^{14–16,31} As can be seen from Figure 2A, upon visible light irradiation, spiropyran moieties in polymer nanoparticles exhibit no absorption band from 500 to 700 nm. However, after UV irradiation, a new absorbance band in the range of 500 to 700 nm can be observed, which can be ascribed to the isomerization of the SP form to the MC form. The UV–vis absorption spectrum property displayed by the nanoparticles is also indicative of the successfully covalent incorporation of spiropyran moieties in the nanoparticles.

It has been previously established that the fluorescence intensity of the nanoparticles dispersion can be varied by changing the content (concentration) of fluorescent dye in nanoparticles.¹⁵ In this study, we used four different nanoparticle samples for investigating the relationship between the content of fluorophores and the fluorescence intensity of the nanoparticle dispersions, as shown in Figure 3.

As can be seen in Figure 3, with increasing MANI content in nanoparticles, the fluorescent intensity first increases and then decreases. For the nanoparticle dispersion (MANI-8mg) with the highest MANI concentration, its fluorescence intensity is much lower than that of MANI-6mg, and its emission maximum exhibits a remarkable red shift (~ 51 nm). The decreased fluorescence intensity may be caused by self-quenching of fluorescence caused by the interaction of the fluorophores, and the significant red shift of the maximum emission wavelength may be due to the formation of an excited-state complex of two identical fluorophores, which takes place as the concentration of dye molecules inside the nanoparticles reaches a certain value.³² Therefore, to ensure high fluorescence intensity, an appropriate amount of fluorophore should be incorporated into the nanoparticles.

The amount of chromophores covalently incorporated into the nanoparticles can be deduced by using the absorption values, assuming that the molar extinction coefficient of the chromophore in nanoparticles is the same as that in some organic solutions.³³ In the present study, the amounts of the two dyes covalently incorporated within the nanoparticles were deduced by using the extinction coefficient of the MANI or SPMA (SP form) monomer in dichloromethane, and the actual absorbance values for MANI (at 424 nm) and the SP form of spiropyran moieties (at 340 nm, upon visible light irradiation) can be obtained by eliminating the light scattering effect (Figure S4, Supporting Information). The determined amounts of MANI and SPMA in nanoparticle samples from the absorbance values

are listed in Table 1. The amounts of MANI and SPMA calculated through the absorption measurement for the nanoparticle samples are about 85–91% of the feed amount, and we think the difference may arise from the systematic error of this determination method during polymerization. On the other hand, as shown in Figure S4, with the gradual increase in SPMA feed, the absorbance value also increases by a certain proportion, and the determined weight ratio of SPMA is close to the feed ratios (Table 1). These results indicate that the miniemulsion polymerization is suitable for the incorporation of two chromophores (MANI and SPMA), with their ratios close to pre-designed values, into polymer particles.

Photoreversible Modulation (Switching) of Fluorescence of Nanoparticle Dispersion. Figure 4 shows fluorescence emission spectra of MANI dye covalently incorporated into four different spiropyran-containing nanoparticle dispersions upon UV and visible light irradiation. After these fluorescent nanoparticle dispersions were irradiated upon UV light (300 nm, 15 W) for 15 min, they were then immediately moved into the fluorescence spectrometer for the emission measurement (at the excitation wavelength of 425 nm). The characteristic fluorescence intensity at 497 nm of MANI decreased remarkably, and a new emission band at 650 nm appeared, which can be attributed to the MC form of spiropyran moieties. After irradiation with visible light (525 nm, 15W LED lamp) for 20 min, the fluorescence intensity at 497 nm recovered, as shown in Figure 4. In addition, the appearance of an aqueous dispersion of fluorescent nanoparticles also reversibly modulates with the irradiation of UV or visible light, as shown in Figure 5. The color of the nanoparticle dispersion (NP-N4) turns red after UV irradiation and reverts to cyan after visible light illumination. The red color corresponds to the MC form of spiropyran moieties in the nanoparticles, while the cyan corresponds to the MANI dye.

As for the photoreversible fluorescence modulation systems, the emissive behavior of the donor–acceptor (fluorophore–spiropyran or other photochromes) dyads can be varied through the photoinduced electron transfer (PET), FRET, or both of them.^{34–41} The measured reduction potentials and the spectral data allow a quantitative estimation of the thermodynamic driving force for the PET process in terms of the free energy changes (ΔG). The ΔG values have been calculated using $\Delta G = E_{\text{ox}}(\text{D/D}^+) - E_{\text{red}}(\text{A}^-/\text{A}) - E_{0,0} - 0.06$,^{35,38,41} where $E_{\text{ox}}(\text{D/D}^+)$ represents the oxidation potential for the MANI, $E_{\text{red}}(\text{A}^-/\text{A})$ represents the reduction potential of the MC state of SPMA, and $E_{0,0}$ denotes the energy of the first-excited singlet state of the fluorophore. The $E_{0,0}$ values used in the calculation of ΔG have been estimated from the location of the first peak position in the fluorescence spectrum.³⁸ The measured ΔG values in this system have been shown in Table S1 (see the Supporting Information). As can be seen, the ΔG value (0.04) for PET for this system is positive, showing that PET is thermodynamically impossible in this system. Presumably, the FRET from the excited MANI to the MC state of the spiropyran moiety is mainly responsible for the decrease in the emission intensity of this system.

According to the FRET principle,⁴¹ as long as the emission band of the fluorophore (donor) and the absorption band of the photochrome (acceptor) can overlap well, and the distance between the fluorophore to the spiropyran moiety is within the Förster radius (generally, 1 to 10 nm), the FRET from the excited fluorophore to the colored state of the photochrome is efficient. In this work, the emission band of the fluorophore MANI (450–620 nm) generally matches the absorption band of the

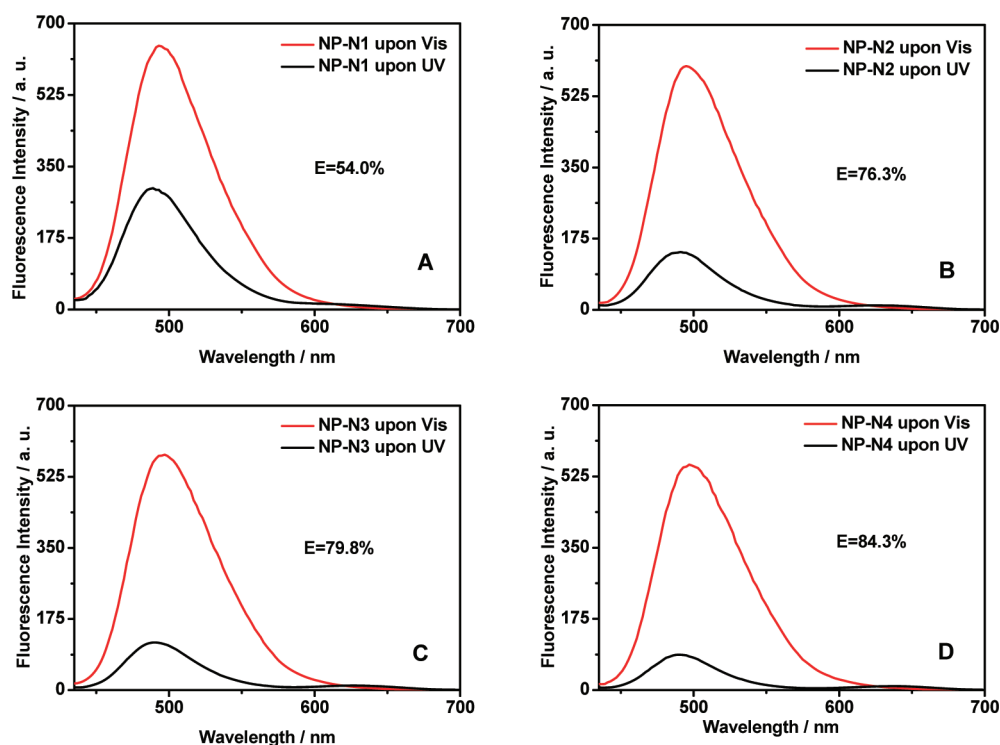


Figure 4. Fluorescence emission spectrum (excited at 425 nm, 25 °C) of photoswitchable fluorescent nanoparticle dispersions modulated by UV irradiation (300 nm) or by visible light (525 nm) irradiation. The concentration for MANI is 8.25×10^{-5} M in an aqueous dispersion, and that for the spiropyran moiety is 0.70×10^{-4} M (sample NP-N1, spiropyran feed: 0.010 g; A), 1.45×10^{-4} M (sample NP-N2, spiropyran feed: 0.020 g; B), 2.15×10^{-4} M (sample NP-N3, spiropyran feed: 0.030 g; C), and 2.75×10^{-4} M (sample NP-N3, spiropyran feed: 0.040 g; D).



Figure 5. Photograph for a nanoparticles dispersion (NP-N4) after visible light irradiation (I) and UV irradiation (II) under a dark environment.

MC state of spiropyran (470–650 nm) in nanoparticles (Figure 6). However, the SP state does not exhibit any absorption band in the emission band of MANI (450–620 nm), as shown in Figure 2A. Thus, energy transfer from the excited state of MANI to the SP form of spiropyran is impossible, whereas energy transfer to the MC form is possible. On the other hand, from the perspective of an energy-level match, the energy of the first-excited singlet state of MANI was estimated to be 2.50 eV, as described above.³⁸ Similar calculations based upon absorption and emission maxima gave a first-excited singlet-state energy of 3.65 eV for the SP form of the spiropyran moieties in nanoparticles, and 2.05 eV for the MC form of the spiropyran moieties.^{14,38} These data also indicate that energy transfer from MANI to the SP form of spiropyran is impossible, whereas that from MANI to the MC form is possible.

On the basis of the results in Figure 4, the fluorescence intensity of MANI can be remarkably quenched by the MC form of SPMA moieties through an intraparticle FRET process.

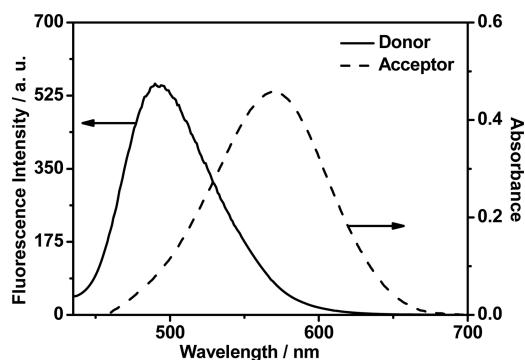


Figure 6. Fluorescence emission spectrum of MANI (solid curve) and absorption spectrum of the MC form of spiropyran moieties (dashed curve) in nanoparticles.

According to the Förster nonradiative energy transfer theory,⁴¹ the Förster's critical distance R_0 between the MANI (donor) and the acceptor (MC) in nanoparticles has been calculated to be 37.6 Å (see the Supporting Information). The FRET efficiency E is expressed as

$$E = \frac{R_0^6}{R_0^6 + r^6} \quad (1)$$

where r is the distance between the donor (MANI) and the acceptor (MC form of spiropyran). The energy transfer is effective over distances in the $R_0 \pm 50\% R_0$ range,^{33,41} namely, $18.8 \text{ Å} \leq r \leq 56.4 \text{ Å}$. However, unlike the dyad systems, the MANI dye molecules and spiropyran moieties are dispersed in

Table 2. Characteristics of Four MANI and Spiropyran-Containing Nanoparticle Samples

sample ^a	D_{NP} ^b [nm]	C_{MANI} ^c [$\times 10^{-4}$ M]	N_{MANI}/NP	C_{SP} ^c [$\times 10^{-3}$ M]	N_{SP}/NP	N_{SP}/N_{MANI}	N_A ^d	$E[\%]$ ^e	r^f [nm]
NP-N1	54.9	8.10	500	0.70	458	0.9	4.0	54.0	4.80
NP-N2	57.5	8.25	571	1.45	1048	1.8	7.9	76.3	1.75
NP-N3	52.8	8.25	444	2.15	1185	2.7	11.6	79.8	1.43
NP-N4	55.4	8.25	500	2.75	1708	3.4	14.4	84.3	1.05

^a The MMA/HD/SDS/KPS feed is 2.5/0.15/0.10/0.085 g, respectively. ^b D_{NP} : average nanoparticle diameter, determined from DLS data. ^c C_{MANI} and $C_{spiropyran}$: mole concentrations of MANI and spiropyran in dispersion, respectively, determined from the absorbance values. ^d The number of spiropyrans residing around one donor (MANI) within the effective energy transfer distance (see the Supporting Information). ^e The experimental energy transfer efficiency (see the Supporting Information). ^f The estimated average MANI–spiropyran distance (see the Supporting Information).

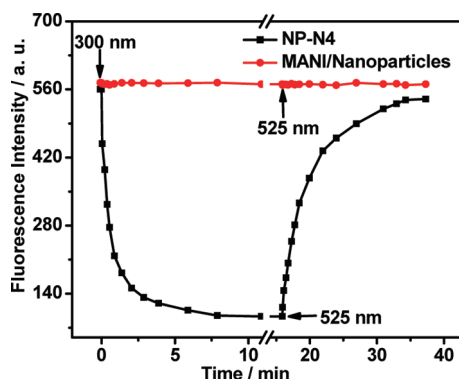


Figure 7. Fluorescence intensity (ca. 497 nm) changes of MANI-containing fluorescent nanoparticles dispersion and photoswitchable fluorescent nanoparticles dispersion (sample NP-N4) upon irradiation with 300 nm UV and 525 nm visible light (15 W). The concentration of MANI in dispersion is 8.25×10^{-5} M.

the particles and separated by the polymer matrix, and the distance between the donor and acceptor is governed by the amount of donors and acceptors incorporated in the individual nanoparticles, assuming the fluorophores are uniformly dispersed. In this study, we calculated the average number of MANI (N_{MANI}/NP) and spiropyran (N_{SP}/NP) in a nanoparticle, the average ratio of the spiropyran number to the MANI number in one nanoparticle (N_{SP}/N_{MANI}), the experimental energy transfer efficiency (E) for the nanoparticle samples, and the estimated average distance between MANI and spiropyran in nanoparticles (r) for the samples, which are listed in Table 2. In addition, it should be considered that the energy can be transferred from a donor to an acceptor if their separation distance is less than the upper limit of efficient energy transfer (56.4 Å). We also estimated the number of the spiropyran (N_A) residing around one donor within the effective energy transfer distance, assuming that the dispersion of spiropyran molecules or MANI molecules in the whole nanoparticle is homogeneous, and the existence of the donor (MANI) does not affect the dispersion of the acceptor (spiropyran) and vice versa. The data (N_A) are given in Table 2.

The data in Table 2 indicate that for the four nanoparticle samples from NP-N1 to NP-N4, with the irradiation of UV light, in most of the samples there are multiple acceptors (MC) around a donor within the effective energy transfer distance of 56.4 Å; this ensures that the intraparticle FRET process takes place from donor to acceptor as well as the light-induced fluorescent modulation. Moreover, for the nanoparticle samples with a higher ratio of spiropyran to MANI (NP-N3 and NP-N4), there are more MC molecules around a MANI molecule (donor), and

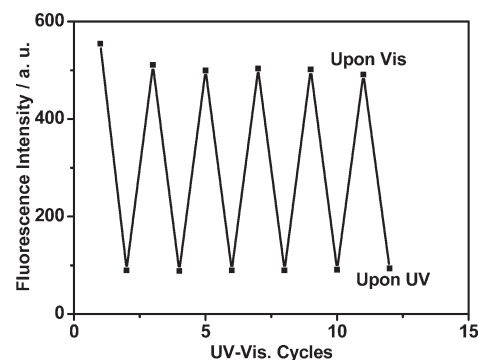


Figure 8. Fluorescence intensity of a photoswitchable fluorescent nanoparticle dispersion (NP-N4; $\lambda_{ex} = 425$ nm, 25 °C) recorded at 497 nm upon alternate UV and visible-light irradiation cycles. The concentration of MANI in dispersion is 8.25×10^{-5} M.

higher experimental energy transfer efficiency can be achieved. However, for the sample with the lowest ratio of spiropyran to MANI (NP-N1), on average, there are only 4.0 effective acceptors (MC) around a MANI within 56.4 Å, and the experimental energy transfer efficiency is 54.0%. Also, the fluorescence emission of MANI cannot be effectively quenched under UV-light irradiation, as illustrated in Figure 4A. Under the current experimental conditions, the critical $N_{spiropyran}/N_{MANI}$ value, which ensures effective fluorescence quenching ($E = 84.3\%$), is estimated to be 3.4. Moreover, the control of the acceptor to donor ratio during the polymerization is important for obtaining nanoparticle dispersion with well-defined energy transfer efficiency as well as fluorescence emission property.

The typical photoresponsive behaviors of the aqueous dispersion of fluorescent nanoparticles containing only the MANI dye and the photoswitchable fluorescent nanoparticles (NP-N4) upon irradiation with UV (15 W) and visible light (15 W) are illustrated in Figure 7. It is clearly evident that there are no significant fluorescence intensity changes in the dispersion of nanoparticles containing only MANI, indicating that the photo-degradation of the donor (MANI) is negligible with such a short-time irradiation with low energy (15 W) UV or visible light. As for the sample NP-N4, upon irradiation with UV at 300 nm, the fluorescence intensity of MANI moieties rapidly decreases over 10 min and then tends toward stability, indicating that the fluorescent quenching of MANI moieties occurs due to the transformation of spiropyran moieties from the SP form to the MC form. While it gradually recovers its fluorescence intensity upon irradiation with 525 nm visible light within 20 min, which can be ascribed to the photoinduced conversion from the MC form to the SP form of spiropyran moieties, for some water-

soluble spiropyran derivatives, this process is much slower in water because the water tends to stabilize the zwitterionic MC form.⁴² In the present study, the spiropyran groups reside in the hydrophobic core of the nanoparticles, in which the zwitterionic MC form cannot be stabilized by water, and the fluorescence intensity of MANI can recover much more quickly.

Figure 8 shows the reversible nature of the fluorescence modulation of the photoswitchable fluorescent nanoparticles dispersion via cycled irradiation with UV (300 nm) and visible light (525 nm). As can be seen, the fluorescence of MANI can be reversibly switched “on” and “off” upon irradiation with UV and visible light. On the other hand, the “fatigue” effects caused by photodegradation of the chromophores under UV irradiation have been commonly observed and discussed extensively elsewhere.⁴³ However, from Figure 8, one can see that the covalent incorporation of MANI dyes and spiropyran moieties into nanoparticles can make the fluorescent nanoparticles show quite good photoreversibility. The optical switching of fluorescence can be repeated for six cycles with a slight “fatigue” effect. Obviously, the novel polymeric nanoparticles prepared by employing a covalent bonding strategy possess better resistance to fatigue when compared to our previous photoswitchable systems, in which dye molecules are incorporated by doping or adsorbing to the polymeric matrix.^{14–16}

CONCLUSION

In summary, novel photoswitchable fluorescent polymeric nanoparticles have been synthesized using a facile one-step miniemulsion polymerization, in which the FRET donor, 4-methamino-9-allyl-1,8-naphthalimide (MANI), and photoswitchable acceptor, spiropyran derivative, were successfully covalently incorporated into a polymeric matrix. They possess good performances, including no dye leakage, intense fluorescence, a controllable amount and ratio of the two dyes, and tunable FRET efficiency. The fluorescence emission of MANI dye in nanoparticles can be reversibly modulated by the transformation of spiropyran moieties’ structures upon UV and visible light irradiation. Overall, the as-prepared novel photoswitchable fluorescent polymeric nanoparticles not only show higher stability and relatively fast photoresponsivity but also possess better photoreversibility compared to some previous reports. This class of photoresponsive nanoparticles may find applications in biological labeling and imaging, as well as in optical fields like individually light-addressable nanoscale devices.

ASSOCIATED CONTENT

S Supporting Information. ¹H NMR spectrum of MANI and SPMA monomers. Detailed description of calculation of the Förster radii (R_0), experimental energy transfer efficiency and estimation of donor–acceptor distance, and estimation of N_A (number of NRs residing around one donor within the effective energy transfer distance). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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