

Fluorescent Nanomicelles for Selective Detection of Sudan Dye in Pluronic F127 Aqueous Media

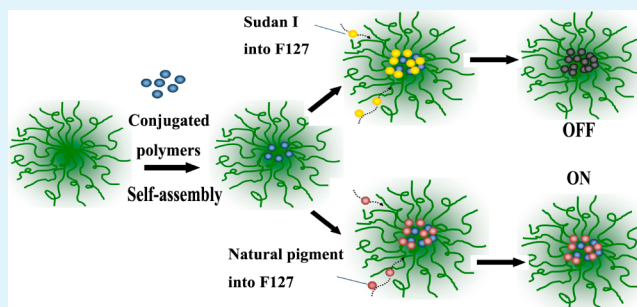
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S Supporting Information

ABSTRACT: Novel self-assembled water-soluble nanomicelles that contain fluorescent conjugated polymers (poly(9,9-dioctylfluorene) (PFO) or poly[2,7-(9,9-dihexylfluorene)-alt-4,4'-phenylether] (PF-PE)) have been obtained and used as the highly sensitive/selective platform for Sudan dye detection. The fluorescent nanomicelles exhibited a highly selective fluorescence quenching by the prohibited food additive Sudan I, while not for the natural pigments: Capsanthin and Beta-carotene, due to the more suitable matching of the LUMOs (lowest unoccupied molecular orbital) of the conjugated polymers with that of Sudan I molecules. The Stern–Volmer constants (K_{SV}) of PF-PE/F127 and PFO/F127 for Sudan I were 1 040 480 and 665 000 M^{-1} , respectively, which were more than 100 times higher than those of the same conjugated polymers in the organic solvents. The significantly enhanced sensitivity was due to the collective effect of the F127 micelles to both chromophore and analyte, through which the fluorophore–analyte binding interaction is significantly strengthened and efficient photoinduced charge transfer occurs. The as-proposed materials and approach may be potentially applied in the real-time food safety screening.

KEYWORDS: conjugated polymers nanomicelles, self-assembly, amphiphilic polymer carriers, hydrophobic synthetic dyes, biological sensing



1. INTRODUCTION

Conjugated polymers (CPs) with unique photophysical properties and electrical conductivity are considered as promising candidates for the development of fluorescent sensors, biological imaging probes, etc.^{1–5} However, the wide spread application of conjugated polymers to bioimaging and sensing in aqueous environment is hindered by their poor water solubilities. In recent years, great efforts have been devoted to developing colloidal stable conjugated polymer nanoparticles.^{6–8} Conjugated polymer dots prepared by rapid precipitation conjugated polymer's tetrahydrofuran (THF) solution in water have received attention because of their small size and well-controlled fluorescence properties.^{9–11} In another approach, highly fluorescent conjugated polymer nanoparticles were prepared via polymerization in aqueous mini-emulsion.^{12,13} The fluorescent nanoparticles possess unique functional and structural properties such as small size, high fluorescence brightness/volume ratios, and good photochemical stability. These properties indicate an enormous potential for fluorescent bioimaging and sensing.^{14–19}

Synthetic dyes have been widely used as coloring agents in the food industry for many years. Many synthetic dyes, including Sudan dyes, disperse dyes, Rhodamine B, direct black dyes, and Carmosine, contain azo functional groups and

aromatic rings that may have adverse effects on health and are prohibited to be applied as additives in food products worldwide.²⁰ Sudan dyes (Sudan I, II, III, and IV) are a kind of phenyl-azoic dyes that are commonly used in chemical industry for coloring hydrocarbon solvents, oils and waxes. Sudans have been documented as human carcinogen and mutagen, and are not allowed to be used as additives in foods according to both the Food Standards Agency and the European Union.²¹ Analytical methods for the determination of Sudan dyes in food are commonly based on chromatography and mass spectrum.^{22–24} Recently, enzyme-linked immune method (ELISA)^{25,26} and molecular imprinting method have also been developed.^{27,28} However, most of these methods require comprehensive instruments and time-consuming pretreatment procedures, and are not suitable for real-time and on-site measurement. In contrast, the fluorescence analytical technique is a rapid, highly sensitive, and low-cost method for the detection and quantification of trace analyte, offering the possibility for development of portable point-of-care instruments.

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In this contribution, we report a simple and straightforward strategy which allows the formation of CPs-based nanomicelles in water by using Pluronic F127 as an encapsulating material. Pluronics are amphiphilic triblock copolymers composed of poly(oxyethylene)-block-poly(oxypropylene)-block-poly(oxyethylene)(PEO_x-PPO_y-PEO_z) that are commonly used as nonionic macromolecular surfactants.^{29,30} Pluronic F127 (PEO100-PPO65-PEO100) is one of the most widely adapted biocompatible polymers for preparing polymeric nanomicelles for drug and gene delivery, which was reported to facilitate the particles–cell interaction and enhance the cellular uptake efficiency.^{31,32} This strategy provides an alternative approach for preparing highly fluorescent and ultrasensitive CPs colloidal nanoparticles in water by employing F127 nanomicelles as carriers. The CPs-containing nanoparticles only require commercially available materials, and based on which the fast and ultrasensitive detection of hydrophobic Sudan dyes in aqueous solution could be easily reached. To the best of our knowledge, this is the first report on fluorescent conjugated polymer nanomicelles for selective detection of Sudan dyes in water.

2. EXPERIMENTAL SECTION

Chemicals. Conjugated polymer poly(9,9-dioctylfluorene) (PFO, M_w 38 131, polydispersity 2.9)³³ and poly[2,7-(9,9-dihexylfluorene)-alt-4,4'-phenylether](PF-PE, M_w 66 317, polydispersity 1.8)³⁴ were synthesized following literature methods, respectively. Pluronic nonionic surfactant Pluronic F127 (M_w 12 600) and two natural pigment Capsanthin and Beta-carotene were purchased from Sigma-Aldrich. All other chemicals were purchased from Aldrich or Alfa Aesar and used without further purification.

Preparation of Conjugated Polymers/F-127 Nanomicelles. The aqueous dispersion of CPs/F127 fluorescent nanomicelles was prepared by a micellization method. In a typical procedure, conjugated polymer was dissolved in THF by stirring overnight under inert atmosphere. The solution was then filtered through a 0.44 μm filter and furtherly diluted to a concentration of 0.05 g/L. F127 was also dissolved in THF to make a 0.1 g/L solution. The conjugated polymer solution was mixed with F127 solution to produce solution mixtures with a constant host concentration of 10 g/L and dopant fractions of 5 wt %. The mixtures were stirred to form homogeneous solutions. A 2 ml quantity of the mixture solution was added rapidly to 8 mL of deionized water while sonicating the mixture. The THF was removed by rotary evaporation followed by filtration through a 0.25 μm microfilter. The concentration of conjugated polymers for the fluorescence quenching measurements was kept constant at 0.005 g/L. The resulting micelle dispersions are clear and stable for months with no signs of aggregation.

Characterization Methods. Morphology and size distribution of the polymer blend nanoparticles were characterized by transmission electron microscopy (TEM). TEM observations were performed on a Tecnai G2 F20 electron microscope at an acceleration voltage of 200 KV. For all TEM observations, a drop of micelles water solution was cast on the carbon-coated copper grids and stained with phosphotungstic acid (PTA, 2%, w/w) for 3 min before drying in a desiccator for 24 h. The average hydrodynamic diameter and size distribution of the as prepared nanomicelles were determined using a Malvern 90 Plus particle size analyzer equipped with a 30 mW semiconductor laser diode (659 nm) with an output at a scattering angle of 90°. All measurements were made at 25 °C. The UV–vis absorption spectra were recorded with a Shimadzu UV-3600 scanning spectrophotometer, using 1 cm quartz cuvettes. Fluorescence spectra were collected with a commercial fluorometer (Jobin Yvon, FL-3), using a 1 cm quartz cuvette. Cyclic voltam-mograms (CV) were recorded on a CHI 660A electrochemical workstation. The CV was performed in a solution of tetrabutylammonium hexafluorophosphate (Bu₄NPF₆) (0.1 M) in methylene dichloride at a scan rate of 50 mV/s

at room temperature, using saturated calomel electrode (SCE) and a platinum wire as reference and counter electrode, respectively, A platinum electrode was used as the working electrode. The highest occupied molecular orbital (HOMO) level values of the polymers were calculated according to the following empirical formulas³⁵

$$E_{\text{HOMO}} = -e(E_{\text{ox}} + 4.40) \text{ (eV)}$$

The E_{ox} is the onset oxidation potential vs SCE. The lowest unoccupied molecular orbital (LUMO) level values of the copolymers were estimated from the HOMO energy levels and optical band gap ($E_{\text{g}}^{\text{opt}}$) using the equation

$$E_{\text{LUMO}} = E_{\text{HOMO}} + E_{\text{g}}^{\text{opt}}$$

Fluorescence Quenching Experiments with Analytes. For the Stern–Volmer measurements, 2 mL of conjugated polymers/F127 nanomicelles solution were placed in a covered quartz cuvette. Fluorescence quenching of CPs micelles was obtained by successive addition of aliquots of analyte solution at room temperature. Fluorescence spectra were recorded immediately after each addition. The concentration of conjugated polymers for the fluorescence quenching measurements was kept constant at 0.005 g/L.

3. RESULTS AND DISCUSSION

Self-Assembly of CPs/F127 Nanomicelles. The schematic illustration of the formation of CPs/F127 nanomicelles and chemical structures of PFO, PF-PE, Sudan I, Capsanthin and β -carotene employed in this study are shown in Scheme 1. The commercially available conjugated polymer PF-PE and PFO were used for the preparation of CPs/F127 nanomicelles. The conjugated polymer loaded F127 nanomicelles were prepared by mixing the tetrahydrofuran dispersions of F127 and conjugated polymer with deionized water. After rapid addition of a mixing solution of the polymers to deionized water, the conjugated polymer-F127 complexes spontaneously self-aggregated into micelles with hydrophobic cores and hydrophilic shells in dilute aqueous solution driven by hydrophobic interaction.^{36,37} Conjugated polymer deposited on the interior PPO segments of the PF127 micelle, leaving the exterior PEO segments stretching out in aqueous media, which yielded clear and transparent nanoparticle dispersions.³⁸

The morphology of the F127 and conjugated polymers/F127 micelles were observed with TEM (Figure 1). As both the PPO and PEO chains are highly transparent to an electron beam, the F127 micelles were stained by PTA to give well-defined sphere particles with average diameter of 25–30 nm, which is consistent with the literature.^{39,40} The conjugated polymers entrapped in the hydrophobic core of F127 micelles appearing as dark dots in panels c and d in Figure 1 are surrounded by a white disk of unstained corona of micelles composed by PEO segments that stands out against the stained background. The sizes of the dark dots are around 10 nm, which are consistent with the size of the PPO core of pure F127 micelles determined in previous cryogenic temperature transmission electron microscopy (cryo-TEM) and small-angle neutron scattering (SANS) studies.^{41–43} Moreover, the size range of the white disks surrounding the dark dots and some empty white disks are similar to that of pure F127 micelles positively stained by PTA. This suggests the conjugated polymers are located at the core of F127 micelles, and the size and shape of F127 micelles are not obviously changed by conjugated polymers deposition.

The hydrodynamic size distributions of CPs loaded F127 micelles and empty F127 micelles were determined by the dynamic light scattering (DLS) technique. The size distribution histograms of F127, PF-PE/F127, and PFO/F127 micelles are

Scheme 1. Formation of Fluorescent CPs-F127 Nanomicelles and Chemical Structures of Conjugated Polymer PF-PE, PFO, Sudan I Capsanthin, and Beta-Carotene in This Study

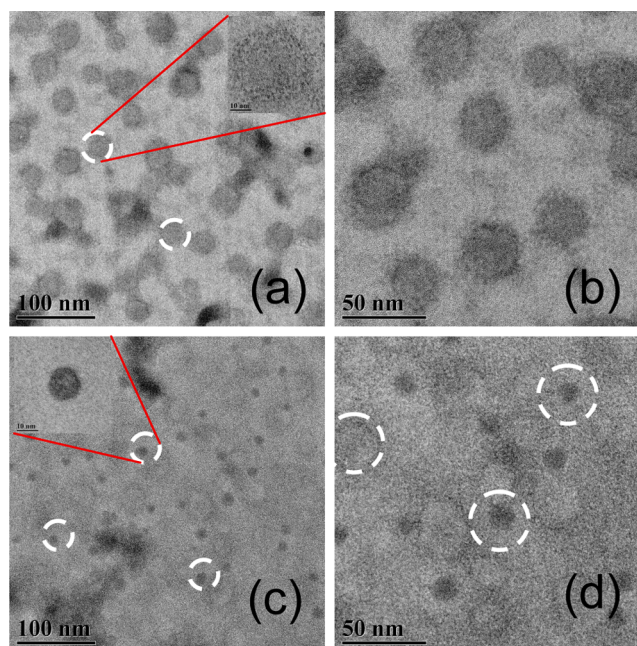
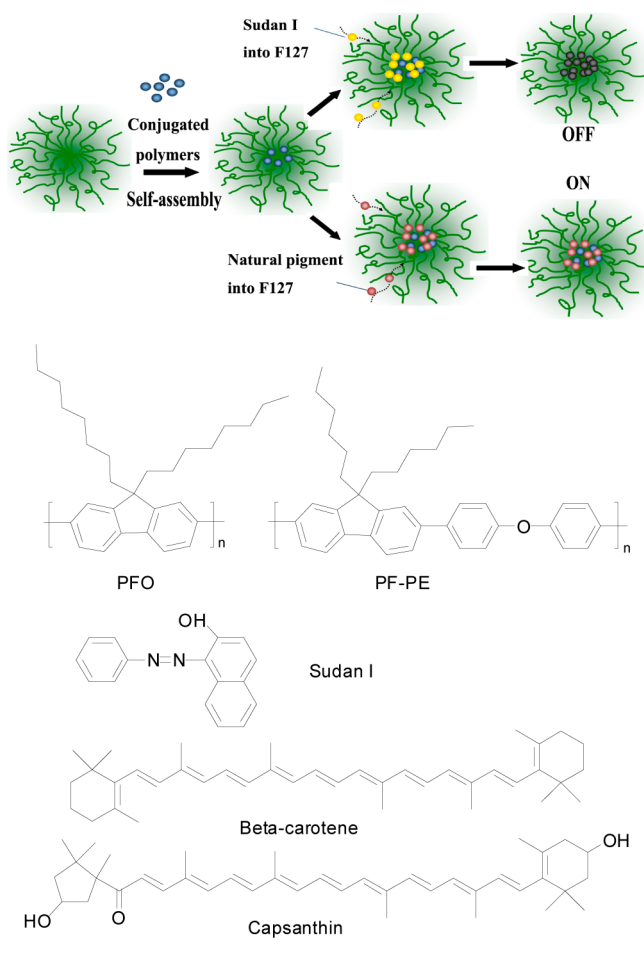


Figure 1. TEM image for (a, b) the micelles of F127 formed in DI water obtained by positive staining with 2% PTA; and (c, d) the PF-PE/F127 micelles in DI water obtained by negative staining with 2% PTA.

similar, which is consistent with the results of TEM. The mean sizes of F127, PF-PE/F127, and PFO/F127 micelles determined by the software were 36.4 nm, 38.6 nm and 39.2 nm, respectively, which are a little larger than those observed by TEM. This may be due to the difference in sample preparation process. The apparent hydrodynamic radii analyzed by DLS include the hydrophilic PEO blocks stretching out in aqueous media, whereas the samples observed by TEM are at contracted dry state, which do not contain the similar phenomenon as commonly found in the previous reports.

It was found that the self-assembly of CPs/F127 nanomicelles occurs immediately when adding a THF solution of PFO and F127 into an aqueous solution. The conjugated polymers loaded F127 in water is quite stable and no precipitate and aggregation is observed several weeks after preparation. In the current method, common commercially available light emitting CPs and surfactants are able to self-assemble into nanomicelles in aqueous solution at low concentrations through a facile method.

Photophysical Properties. The optical properties of the fluorescent nanomicelles were investigated by Ultraviolet-visible (UV-vis) absorption and fluorescence spectroscopy. Figure 2a shows the absorption spectra of PF-PE and PFO in THF solution and in F127 aqueous solutions. In the THF solutions

of PFO and PF-PE, the maximal absorptions are observed at 390 and 340 nm, respectively. The PF-PE in F127 aqueous solution shows a similar absorbance main peak at around 340 nm and retains its structure as in the THF solution. On the other hand, the PFO's F127 aqueous solutions undergo a slight blue-shifted absorption as compared to that of the PFO in THF solution, which is attributable to the bending or kinking of the polymer backbone. Moreover, there is a small shoulder peak appearing at around 436 nm in the UV-vis spectrum of PFO's F127 aqueous solutions. This implies the existence of aggregation that results from interchain interactions or β -phase formation of PFO.^{44,45} The corresponding fluorescence spectra of above samples are displayed in Figure 2b. When excited at 340 nm, PF-PE in THF solution has PL peaks at 360 and 390 nm, and PFO in THF solution exhibits PL peaks at 420 and 450 nm. Both of the two samples exhibited obvious red-shifted PL emission when they were transferred to the F127 aqueous solution. It can be found from Figure 2b that PF-PE's PL peaks shift to 400 and 420 nm, whereas PFO's PL peaks shift to 450 and 475 nm with a more pronounced side peak at 500 nm. The red shift of PFO's PL peaks should be caused by the aggregation or crystalline phase changes induced by the F127 encapsulation and dispersion in water. Similarly, the red-shifted PL peaks and broadened absorption signal of PF-PE in F127 aqueous solution also indicate the existence of aggregation in some degree.

Accordingly, the UV-vis absorption and photoluminescence (PL) spectra of Sudan I, capsanthin, and β -carotene in the ethanol solution are illustrated in Figure 3. The absorption spectra of Sudan I, capsanthin, and β -carotene peak at 450, 475, and 480 nm, respectively. As shown in Figure 3, Sudan I, capsanthin, and β -carotene exhibit PL peaks at 566, 569 and 510, 569 nm in the ethanol solution, but show weak fluorescent intensity.

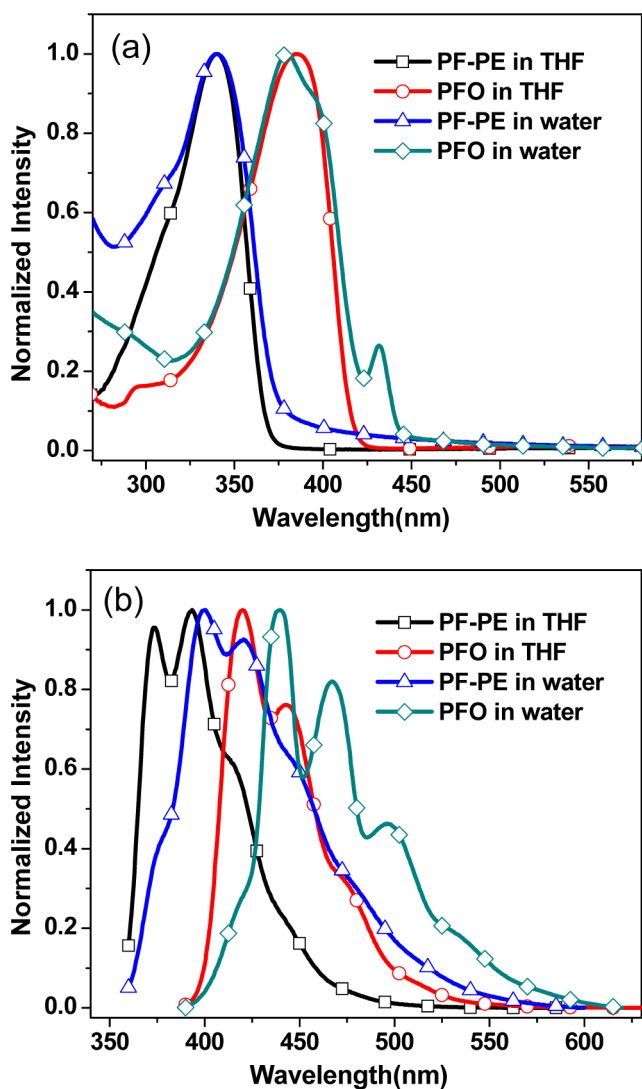


Figure 2. (a) UV-vis and (b) PL spectra of conjugated polymers in solution and in F127 aqueous solution.

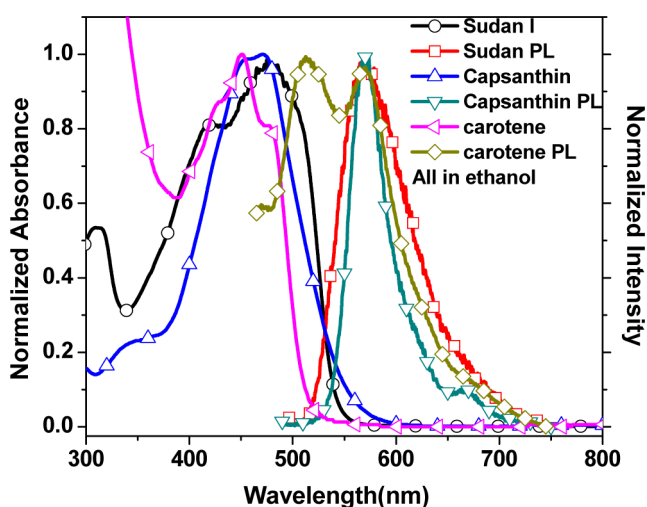


Figure 3. UV-vis and PL spectra of Sudan dye and natural pigments in solution.

Fluorescence Quenching Studies with Sudan Dye in Solution and Aqueous Medium. The response of the two

fluorescent conjugated polymers nanomicelles to Sudan I and two natural pigments (capsanthin, β -carotene) in F127 aqueous solutions were studied firstly. Capsanthin and β -carotene were chosen as the control to examine whether the quenching effect of natural pigments could be observed, which may affect the selectivity of the designed sensing system. For each measurement, the steady-state fluorescence intensity changes of the conjugated polymer samples in response to the addition of increasing amount of dyes: Sudan I, Capsanthin, and β -carotene were examined to evaluate their quenching effect. Figure 4a

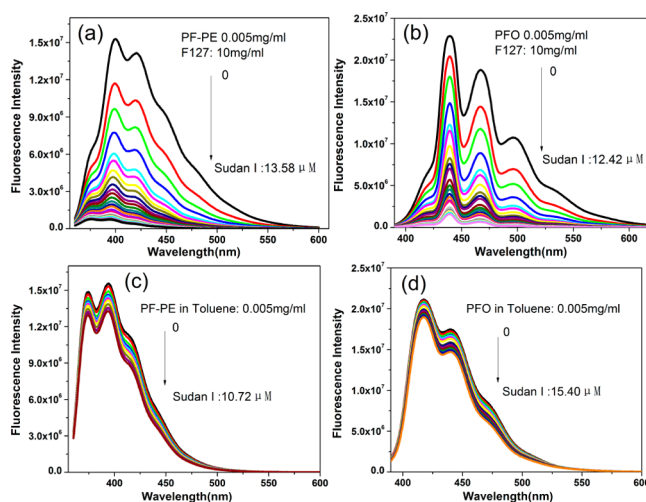


Figure 4. Fluorescence emission spectra of conjugated polymers in the presence of different concentrations of Sudan I: (a) PF-PE in F127 water solution; (b) PFO in F127 water solution; (c) PF-PE in toluene solution; (d) PFO in toluene solution.

shows the fluorescence quenching of PF-PE nanomicelles as a function of added Sudan I. According to Figure 4a, significant quenching could be obtained when 5 μ M of Sudan I is added and the PL of nanomicelles could be completely quenched with the addition of 13.58 μ M Sudan I. The fluorescence emission profiles of PFO/F127 were monitored before and after the addition of the Sudan I. A similar quenching effect with the fluorescence quenching properties of PF-PE in response to Sudan I was observed. As shown in Figure 4b, the fluorescence of the PFO nanomicelles is almost completely quenched by 12.24 μ M Sudan I, indicating that the conjugated polymers in F127 aqueous solution are fully accessible to Sudan I. The quenching effect of capsanthin and β -carotene to the conjugated polymers/F127 nanomicelles were shown in Figure 5a, b and Figure 6a, b. As illustrated in Figure 5a and Figure 6a, addition of capsanthin and β -carotene can only slightly quench the fluorescence of PF-PE/F127 aqueous solutions, and a maximum fluorescence loss of 10% is measured even with the addition of 12.5 μ M capsanthin and β -carotene. Their quenching effect against PFO/F127 aqueous solutions, as shown in Figures 5b and 6b, were similar to that of PF-PE/F127 aqueous solutions. This means that the conjugated polymers/F127 nanomicelles can efficiently discriminate Sudan I against other natural dyes interferences.

The fluorescence behaviors of free PF-PE and PFO in the presence of analytes in the toluene solution were also investigated, as shown in Figures 4c, d, 5c, d, and 6c, d. Without the encapsulation of F127 nanomicelles, there is no significant quenching on the signals of PF-PE and PFO with the

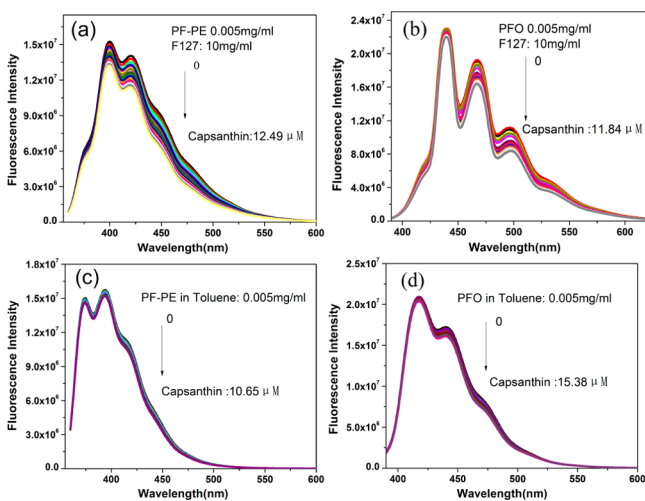


Figure 5. Fluorescence emission spectra of conjugated polymers in the presence of different concentrations of capsanthin: (a) PF-PE in F127 water solution; (b) PFO in F127 water solution; (c) PF-PE in toluene solution; (d) PFO in toluene solution.

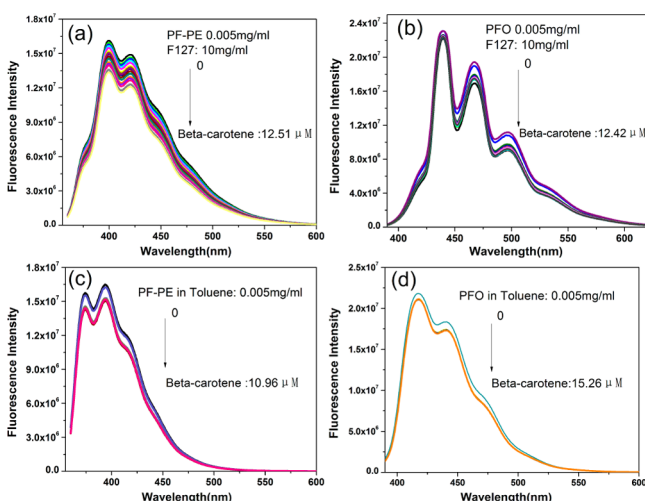


Figure 6. Fluorescence emission spectra of conjugated polymers in the presence of different concentrations of β -carotene: (a) PF-PE in F127 water solution; (b) PFO in F127 water solution; (c) PF-PE in toluene solution; (d) PFO in toluene solution.

addition of each analyte. For Sudan I, only small changes in fluorescence intensity could be observed for the PF-PE and PFO toluene solution. On the other hand, Capsanthin and β -carotene have almost no effect on the emission spectra of the conjugated polymers' toluene solutions. These results suggest that the entrapment effect of F127 nanomicelles plays an important role in enhancing the quenching efficiencies of Sudan I with conjugated polymers. The reason is because most of analytes become entrapped after added into F127 nanomicelles aqueous solution,⁴⁶ either within the micelles or in the interior of the micelles, where they can effectively interact with the imbed conjugated polymers.

The quenching behaviors can be simply described by the Stern–Volmer equation as following:

$$I_0/I - 1 = K_{SV}[Q]$$

In this equation, I_0 is the initial fluorescence intensity of conjugated polymers without analyte, I is the fluorescence

intensity with added analyte of concentration $[Q]$, and K_{SV} is the Stern–Volmer constant. The Stern–Volmer plots were made in order to access a quantitative measurement of fluorescence quenching. Figure 7 shows the Stern–Volmer

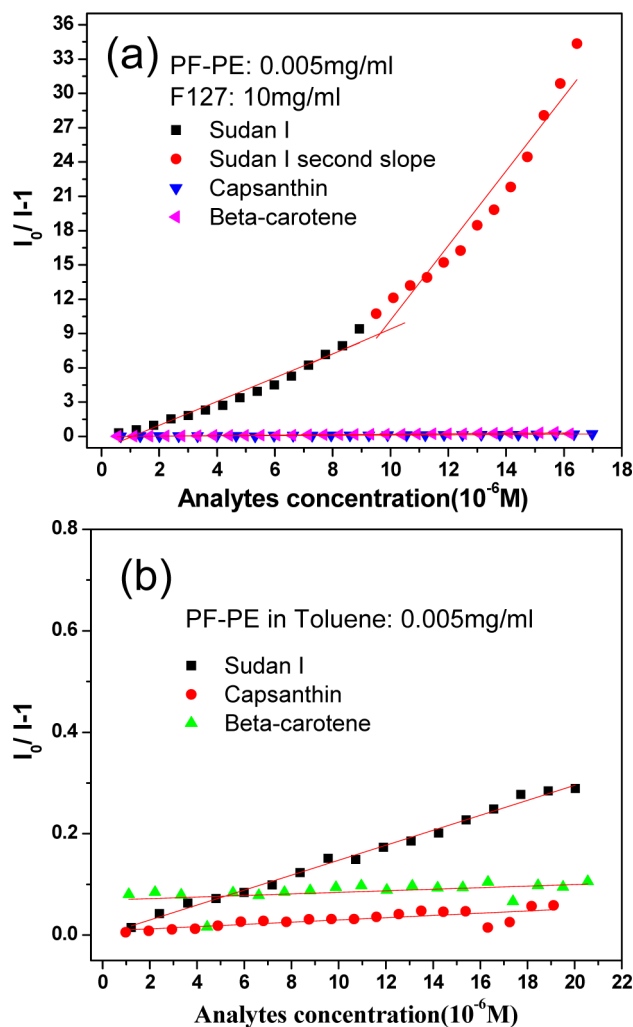


Figure 7. Stern–Volmer plots for PF-PE (a) in F127 water solution and (b) in toluene solution with the analytes (Sudan I, capsanthin, and β -carotene, quenching versus analytes concentration).

plots of PF-PE and PFO for each analytes in F127 aqueous solution and in toluene solution. A linear Stern–Volmer relationship is observed in almost all cases, except for the Stern–Volmer plot of PF-PE in F127 solution for Sudan I. In that case, the Stern–Volmer plot becomes nonlinear when the concentration of Sudan I is higher than $9 \mu\text{M}$. A linear Stern–Volmer relationship may be observed if either a static or dynamic quenching process is dominant. Thus, in the case of with higher concentrations of Sudan I, the two processes may be competitive, which results in a nonlinear Stern–Volmer relationship. As the concentration increases, the Stern–Volmer plots for the Sudan I become superlinear, consistent with “sphere-of-action” quenching.^{47,48} Significant fluorescence quenching was also observed with the PFO/F127 solution upon the addition of Sudan I. At a higher concentration of Sudan I, the plot bent upward (Figure 8a) indicating superamplified quenching effect. On the contrary, the Stern–Volmer plots of PF-PE and PFO in toluene solution with

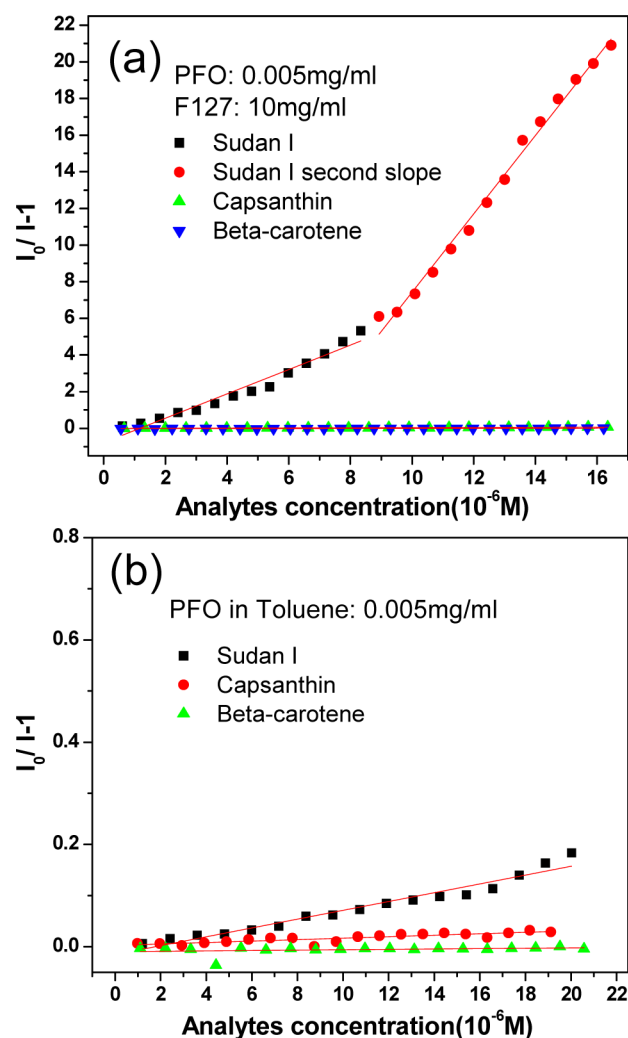


Figure 8. Stern–Volmer plots for PFO in F127 water solution (a) and in toluene solution (b) with the analytes (Sudan, Capsanthin, β -carotene, quenching versus analytes concentration).

Sudan I and two natural pigments all show linear relationship. It is very prominent that the application of F127 could significantly increase the quenching sensitivity of conjugated polymers toward Sudan I, but their quenching response toward the natural pigments are kept low. This indicates the application of F127 can increase conjugated polymers selectivity toward Sudan I.

The sharply increased quenching sensitivity may be assigned to the entrapment of the hydrophobic analyte into F127 micelles that leads to contact quenching as well as the collisional quenching happening between CPs nanoparticles with the quencher.^{49,50} On the other hand, nonionic surfactant was also reported to be able to enhance the photoluminescence of the water-soluble conjugated polymers by breaking up polymer aggregates and by incorporating the polymer chain into micelles at high concentration.⁵¹ Thus, the increased fluorescence of CPs in micelles may also contribute to the improved sensitivity.

The PL quenching efficiencies, as indicated by the values of K_{SV} of each samples, were determined from the slopes of steady-state Stern–Volmer plots and listed in Table 1. The Stern–Volmer constant (K_{SV}) of PF-PE/F127 aqueous solution for Sudan I was $1.04 \times 10^6 \text{ M}^{-1}$, whereas it was only $14\,740$

Table 1. Summary of the Stern–Volmer plots Constants obtained from Fluorescence Quenching Data for Each Material with Analytes

sample	$K_{SV}(\text{M}^{-1})$			
	PF-PE in toluene	PF-PE in water	PFO in toluene	PFO in water
Sudan I	14 740	1 040 480	8610	665 000
capsanthin	2210	11 980	1430	4000
β -carotene	1540	19 800	375	1000

M^{-1} for PF-PE in the toluene solution. The PFO/F127 aqueous solution also show a significantly enhanced K_{SV} of around $6.65 \times 10^5 \text{ M}^{-1}$ with Sudan I in compare with the value of 8610 M^{-1} in toluene solution. It is noteworthy that the K_{SV} constants for Sudan I in CPs/F127 solution are almost 2 orders higher than that of Sudan I in CP toluene solution. However, there is no obvious fluorescence quenching for the two natural pigments in the CP/F127 solution with the K_{SV} values about 2 orders of magnitudes lower than that for Sudan I, showing a high selectivity of CPs/F127 to Sudan I. The K_{SV} values for PF-PE and PFO in toluene solution with Capsanthin and β -carotene are calculated to be mostly in the same order of magnitude with a negligible sensing response. The conjugated polymers in toluene solution also show a higher sensitivity toward Sudan I than the natural pigments although their K_{SV} values are much lower than that in the F127 system. These results indicate the sensitivity and selectivity of CPs toward Sudan I are both enhanced by the encapsulation of F127 micelles, and not influenced by capsanthin and β -carotene. This phenomenon may arise from the contact of Sudan I with chromophore in the core of F127 micelles, which is driven by a collective effect of the F127 micelles. In the fluorescence emission spectra, a linear response of fluorescence intensity by conjugated polymers nanomicelles vs. Sudan I was observed in the range of $0\text{--}2.5 \mu\text{M}$ with detection limits of $0.33 \mu\text{M}$ and $0.28 \mu\text{M}$ for PF-PE and PFO, respectively (see Figures S2 and S3 in the Supporting Information). Under the same conditions, CPs nanomicelles exhibited strong fluorescence responses toward other “azo” dyes, such as Sudan II, Sudan III, and Sudan IV (see Figures S4–S6 in the Supporting Information). Moreover, the effects of interference of the above-mentioned analytes on monitoring “azo” dyes were studied. It is was found that the sensing systems only exhibited significant fluorescence intensity changes upon the addition of Sudan dyes, whereas there is only a very little fluorescence change observed in the presence of natural pigments (see Figure S3 in the Supporting Information). It allowed the selectively determination of Sudan dye by a fluorescence method.

Investigation of the Photochemical Mechanisms for Micelle-Induced Fluorescence Quenching. In principle, the fluorescence quenching process could be considered as energy transfer or photo induced electron transfer between donor and acceptor. The different fluorescence quenching responses of PFO and PF-PE in F127 aqueous solution and in toluene solution may be explained by the energy levels of dye receptors compared to the HOMO and LUMO of conjugated polymers. Efficient energy transfer usually requires good spectra overlap between donor emission and acceptor absorption spectra. The selective fluorescence quenching of PF-PE and PFO by Sudan dyes could be explained by the lower LUMO energy level of Sudan dyes.

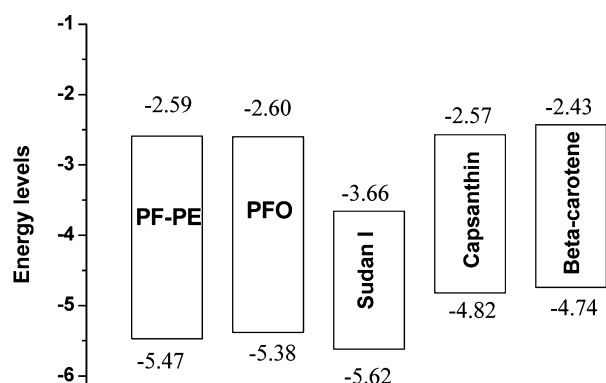
The HOMO and LUMO energy levels of the different compounds were estimated using cyclic voltammetry, and the corresponding bandgap from absorption measurements are listed in Table 2. The HOMO level values of PF-PE and PFO were calculated to be -5.47 and -5.38 eV, respectively. As shown in Table 2, the LUMO levels of PF-PE and PFO are -2.59 and -2.60 eV.

Table 2. Photophysical Data for Conjugated Polymers (Including Sudan Dye and Capsanthin)

sample	λ_{abs} (nm) solution	λ_{abs} (nm) film	$E_{\text{g}}^{\text{opt}}$ (eV)	E_{ox} (eV)	E_{HOMO} (eV)	E_{LUMO} (eV)		
PF-PE	340	346	2.88	1.07	-5.47	-2.59		
PFO	385	390	4.30	0.98	-5.38	-2.60		
Sudan I	417	470	493	538	1.96	1.22	-5.62	-3.66
capsanthin	453	470	460	479	2.25	0.42	-4.82	-2.57
β -carotene	450	478	461	495	2.31	0.34	-4.74	-2.43

The HOMO energy level of Sudan I (-5.62 eV) is lower than those of the conjugated polymers: PFO (-5.38 eV) and PF-PE (-5.47 eV). The LUMO energy of Sudan I (-3.66 eV) is contained within the HOMO-LUMO gap of the two conjugated polymers (Scheme 2). Photoinduced charge

Scheme 2. Energy Level Diagram of the Component Materials Used in the Sensor



transfer (PCT) occurs when lowest unoccupied molecular orbital (LUMO) of the electron acceptor is between highest occupied molecular orbital (HOMO) and LUMO energy levels of electron donors and when they are in close spatial proximity.⁵² In fact, the HOMO-LUMO levels of Sudan I are well contained between the levels of PF-PE and PFO, which should favor PCT. Therefore, it is reasonable to expect that PCT to the LUMO of Sudan I is taking place.⁵³ This order of energy levels leads to an energetically favored situation for excited-state quenching via PCT upon excitation of either the donor or the acceptor, thereby their probabilities vary to different extents with the donor-acceptor distance. In CPs toluene solution, much less quenching is observed, despite the similar optical properties of PF-PE and PFO. This result indicates that the mechanism of photoluminescence quenching is attributable to electron transfer from the excited PF-PE and PFO polymers to the LUMO of the Sudan I. When Sudan I is close enough to CPs, e.g., co-entrapped in the nanosized micelles, electron-transfer quenching occurs from the conjugated polymers to the LUMO of the analyte. PCT is essentially a contact process characterized by an exponential distance dependence and functions effectively at donor-

acceptor distances considerably shorter than those probed by FRET processes. Control experiments using toluene solutions of PF-PE and PFO displayed little change in the photoluminescence spectrum, which could be explained by the difference in polarity or hydrophobicity between water and micelle core environment.

It should be noted that the HOMO energy levels of the pigments (capsanthin and β -carotene) are higher than those of the conjugated polymers (PFO and PF-PE), and thus the charge transfer may also occur between them and consequently leads to fluorescence quenching as well. However, there is only a little fluorescence quenching observed in the presence of high concentrations of capsanthin and β -carotene (see Figure S3 in the Supporting Information). It is well-known that the energy and charge transfer are highly sensitive to the distance between the host and the guest complexes and the structure of the complex matrixes.^{54,55} Moreover, it has been reported that the shape and the orientation of the fluorophore molecules also play important roles on the charge/energy transfer efficiencies between different fluorophores.^{56,57} Thereby, in addition to the energy level difference, the very different molecule shapes between Sudan dyes and natural pigments (capsanthin, and β -carotene), which will lead to different interactions with CPs/F127 nanomicelles, may also contribute to their dramatically different quenching efficiencies for the CPs/F127 nanomicelles. It is believed that the efficient interaction between the CPs and analyte in the F127 micelles plays an important role in the signal amplification.^{58,59} It is also possible that the trace amount of hydrophobic dye in the aqueous solution can be collected and enriched in the hydrophobic inner part of F127 nanomicelles by the hydrophobic-hydrophobic interaction, resulting in the enhancement of the quenching effect.⁶⁰

4. CONCLUSIONS

In summary, we present an effective approach to design and prepare conjugated polymers nanoparticles based on a nanomicelles system. In this method, light-emitting CPs are easily dispersed in water by encapsulating them in the common commercially available block copolymer F127. Significantly enhanced sensitivity of the fluorescent nanomicelles aqueous solution towards trace amounts of Sudan I is established. The Stern-Volmer constant of CPs/F127 aqueous solution for Sudan I was 2-fold higher than that of the CPs in organic solvent. The detailed photochemical mechanism for amplified fluorescence quenching was revealed to be due to the charge transfer between CPs and analytes and the enriching and collecting effect micelles hydrophobic core. Potentially, the fluorescent nanomicelles in aqueous media can work as efficient and selective fluorescent sensor for the fast contamination monitor, food safety assurance, or imaging biological cells in water. Moreover, this strategy provides a general approach for extending the applications of conjugated polymers.

■ ASSOCIATED CONTENT

Supporting Information

Hydrodynamic sizes and size distributions of F127 micelles and conjugated polymers/F127 micelles. Detection limit measurement and PL spectra of the resulting samples (Sudan II, Sudan III, Sudan IV). Fluorescence quantum yields of the nanomicelles in the absence and presence of analytes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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REFERENCES

- (1) Thomas, S. W.; Joly, G. D.; Swager, T. M. Chemical Sensors Based on Amplifying Fluorescent Conjugated Polymers. *Chem. Rev.* **2007**, *107*, 1339–1386.
- (2) Zhu, C.; Liu, L.; Yang, Q.; Lv, F.; Wang, S. Water-Soluble Conjugated Polymers for Imaging, Diagnosis, and Therapy. *Chem. Rev.* **2012**, *112*, 4687–4735.
- (3) Feng, X. L.; Yang, G. M.; Liu, L. B.; Lv, F. T.; Yang, Q.; Wang, S.; Zhu, D. B. A Convenient Preparation of Multi-Spectral Microparticles by Bacteria-Mediated Assemblies of Conjugated Polymer Nanoparticles for Cell Imaging and Barcoding. *Adv. Mater.* **2012**, *24*, 637–641.
- (4) Feng, X. L.; Liu, L. B.; Tang, H. W.; Xing, C. F.; Yang, Q.; Wang, S. Conjugated Polymer Nanoparticles for Drug Delivery and Imaging. *ACS Appl. Mater. Interfaces.* **2010**, *2*, 2429–2435.
- (5) Kumaraswamy, S.; Bergstedt, T.; Shi, X.B.; Rininsland, F.; Kushon, S.; Xia, W. S.; Ley, K.; Achyuthan, K.; McBranch, D.; Whitten, D. Fluorescent-Conjugated Polymer Superquenching Facilitates Highly Sensitive Detection of Proteases. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7511–7515.
- (6) Kaeser, A.; Schenning, A. Fluorescent Nanoparticles Based on Self-Assembled pi-Conjugated Systems. *Adv. Mater.* **2010**, *22*, 2985–2997.
- (7) Tuncel, D.; Demir, H. V. Conjugated Polymer Nanoparticles. *Nanoscale.* **2010**, *2*, 484–487.
- (8) Tian, Y. Q.; Chen, C. Y.; Cheng, Y. J.; Young, A. C.; Tucker, N. M.; Jen, A. K.-Y. Hydrophobic Chromophores in Aqueous Micellar Solution Showing Large Two-Photon Absorption Cross Sections. *Adv. Funct. Mater.* **2007**, *17*, 1691–1697.
- (9) Rong, Y.; Wu, C.; Yu, J.; Zhang, X.; Ye, F.; Zeigler, M.; Gallina, M. E.; Wu, I. C.; Zhang, Y.; Chan, Y.-H.; Sun, W.; Uvdal, K.; Chiu, D. T. Multicolor Fluorescent Semiconducting Polymer Dots with Narrow Emissions and High Brightness. *ACS Nano* **2013**, *7*, 376–384.
- (10) Wu, C.; Bull, B.; Szymanski, C.; Christensen, K.; McNeill, J. Multicolor Conjugated Polymer Dots for Biological Fluorescence Imaging. *ACS Nano* **2008**, *2*, 2415–2423.
- (11) Tian, Z.; Yu, J.; Wu, C.; Szymanski, C.; McNeill, J. Amplified Energy Transfer in Conjugated Polymer Nanoparticle Tags and Sensors. *Nanoscale.* **2010**, *2*, 1999–2011.
- (12) Landfester, K. Miniemulsion Polymerization and the Structure of Polymer and Hybrid Nanoparticles. *Angew. Chem., Int. Ed.* **2009**, *48*, 4488–4507.
- (13) Baier, M. C.; Huber, J.; Mecking, S. Fluorescent Conjugated Polymer Nanoparticles by Polymerization in Miniemulsion. *J. Am. Chem. Soc.* **2009**, *131*, 14267–14273.
- (14) Wang, X. H.; Guo, Y. Z.; Li, D.; Chen, H.; Sun, R. C. Fluorescent Amphiphilic Cellulose Nanoaggregates for Sensing Trace Explosives in Aqueous Solution. *Chem. Commun.* **2012**, *48*, 5569–5571.
- (15) Li, K.; Pan, J.; Feng, S. S.; Wu, A. W.; Pu, K.-Y.; Liu, Y. T.; Liu, B. Generic Strategy of Preparing Fluorescent Conjugated-Polymer-Loaded Poly(DL-lactide-co-Glycolide) Nanoparticles for Targeted Cell Imaging. *Adv. Funct. Mater.* **2009**, *19*, 3535–3542.
- (16) He, G.; Yan, N.; Yang, J. Y.; Wang, H. Y.; Ding, L. P.; Yin, S. W.; Fang, Y. Pyrene-Containing Conjugated Polymer-Based Fluorescent Films for Highly Sensitive and Selective Sensing of TNT in Aqueous Medium. *Macromolecules.* **2011**, *44*, 4759–4766.
- (17) Tian, Y. Q.; Shumway, B. R.; Gao, W. M.; Youngbull, C.; Holl, M. R.; Johnson, R. H.; Meldrum, D. R. Influence of Matrices on Oxygen Sensing of Three Sensing Films with Chemically Conjugated Platinum Porphyrin Probes and Preliminary Application for Monitoring of Oxygen Consumption of *Escherichia coli* (*E. coli*). *Sens. Actuators, B.* **2010**, *150*, 579–587.
- (18) Lee, S. Y.; Lee, K. M.; Lee, M. J.; Yoon, J. Y. Polydiacetylenes Bearing Boronic Acid Groups as Colorimetric and Fluorescence Sensors for Cationic Surfactants. *ACS Appl. Mater. Interfaces.* **2013**, *5*, 4521–4526.
- (19) Cai, X. H.; Shi, L.; Liu, X. F.; Huang, Y. Q.; Fan, Q. L.; Huang, W. Functionalized Conjugated Polymers and Their Application in the Biological and/or Chemical Analysis. *PROG CHEM.* **2013**, *25*, 1375–1382.
- (20) Martinez-Huitle, C. A.; Brillas, E. Decontamination of Wastewaters Containing Synthetic Organic Dyes by Electrochemical Methods: A General Review. *Appl. Catal., B.* **2009**, *87*, 105–145.
- (21) Stiborova, M.; Martinek, V.; Rydlova, H.; Hodek, P.; Frei, E. Sudan I is a Potential Carcinogen for Humans: Evidence for its Metabolic Activation and Detoxication by Human Recombinant Cytochrome P450 1A1 and Liver Microsomes. *Cancer Res.* **2002**, *62*, 5678–5684.
- (22) Tateo, F.; Bononi, M. Fast Determination of Sudan I by HPLC/APCI-MS in Hot Chilli, Spices, and Oven-Baked Foods. *J. Agric. Food Chem.* **2004**, *52*, 655–658.
- (23) Donna, L. Di.; Maiuolo, L.; Mazzotti, F.; De Luca, D.; Sindona, G. Assay of Sudan I Contamination of Foodstuff by Atmospheric Pressure Chemical Ionization Tandem Mass Spectrometry and Isotope Dilution. *Anal. Chem.* **2004**, *76*, 5104–5108.
- (24) Ma, M.; Luo, X. B.; Chen, B.; Sub, S. P.; Yao, S. Z. Simultaneous Determination of Water-Soluble and Fat-Soluble Synthetic Colorants in Foodstuff by High-Performance Liquid Chromatography-Diode Array Detection-Electrospray Mass Spectrometry. *J. Chromatogr. A.* **2006**, *1103*, 170–176.
- (25) Wang, J.; Wei, K.-Y.; Li, H.; Li, Q. X.; Li, J.; Xu, T. A Sensitive and Selective Enzyme-Linked Immunosorbent Assay for the Analysis of Para Red in Foods. *Analyst* **2012**, *137*, 2136–2146.
- (26) Han, D.; Yu, M.; Knopp, D.; Niessner, R.; Wu, M.; Deng, A. P. Development of a Highly Sensitive and Specific Enzyme-Linked Immunosorbent Assay for Detection of Sudan I in Food Samples. *J. Agric. Food Chem.* **2007**, *55*, 6424–6430.
- (27) Yan, H.; Qiao, J.; Pei, Y.; Long, T.; Ding, W.; Xie, K. Molecularly Imprinted Solid-Phase Extraction Coupled to Liquid Chromatography for Determination of Sudan Dyes in Preserved Beancurds. *Food Chem.* **2012**, *132*, 649–654.
- (28) Zheng, M. M.; Wu, J. H.; Feng, Y. Q.; Huang, F. H. Rapid and Sensitive Determination of Sudan Dyes in Hot Chilli Products by Solid-Phase Extraction Directly Combined with Time-of-Flight Mass Spectrometry. *Anal. Methods* **2011**, *3*, 1851–1858.
- (29) Nguyen, P. M.; Zacharia, N. S.; Verploegen, E.; Hammond, P. T. Extended Release Antibacterial Layer-by-Layer Films Incorporating Linear-Dendritic Block Copolymer Micelles. *Chem. Mater.* **2007**, *19*, 5524–5530.
- (30) Alexander, S.; de Vos, W. M.; Castle, T. C.; Cosgrove, T.; Prescott, S. W. Growth and Shrinkage of Pluronic Micelles by Uptake and Release of Flurbiprofen: Variation of pH. *Langmuir.* **2012**, *28*, 6539–6545.
- (31) Hartono, S. B.; Gu, W.; Kleitz, F.; Liu, J.; He, L.; Middelberg, A. P. J.; Yu, C.; Lu, G. Q.; Qiao, S. Z. Poly-L-lysine Functionalized Large Pore Cubic Mesostructured Silica Nanoparticles as Biocompatible Carriers for Gene Delivery. *ACS Nano* **2012**, *6*, 2104–2117.
- (32) Kong, W.; Bae, K. H.; Hong, C. A.; Lee, Y.; Hahn, S. K.; Park, T. G. Oil-Encapsulating PEO-PPO-PEO/PEG Shell Cross-Linked Nanocapsules for Target-Specific Delivery of Paclitaxel. *Biomacromolecules.* **2007**, *8*, 650–656.

- (33) Hou, Q.; Xu, Y. S.; Yang, W.; Yuan, M.; Peng, J. B.; Cao, Y. Novel Red-Emitting Fluorene-Based Copolymers. *J. Mater. Chem.* **2002**, *12*, 2887–2892.
- (34) Huang, F.; Niu, Y. H.; Liu, M. S.; Zhou, X. H.; Tian, Y. Q.; Jen, A. K.-Y. Efficient Ultraviolet-Blue Polymer Light-Emitting Diodes Based on a Novel Fluorene-Based Non-Conjugated Polymer. *Appl. Phys. Lett.* **2006**, *89*, 081104.
- (35) Li, Y. F.; Cao, Y.; Gao, J.; Wang, D. L.; G. Yu, G.; Heeger, A. J. Electrochemical Properties of Luminescent Polymers and Polymer Light-Emitting Electrochemical Cells. *Synth. Met.* **1999**, *99*, 243–248.
- (36) Li, Y.; Xu, R.; Couderc, S.; Bloor, D. M.; Wyn-Jones, E.; Holzwarth, J. F. Binding of Sodium Dodecyl Sulfate (SDS) to the ABA Block Copolymer Pluronic F127 (EO₉₇PO₆₉EO₉₇): F127 Aggregation Induced by SDS. *Langmuir.* **2000**, *17*, 183–188.
- (37) Zhang, W.; Gilstrap, K.; Wu, L.; RB, K C; Moss, M. A.; Wang, Q.; Lu, X.; He, X. Synthesis and Characterization of Thermally Responsive Pluronic F127–Chitosan Nanocapsules for Controlled Release and Intracellular Delivery of Small Molecules. *ACS Nano* **2010**, *4*, 6747–6759.
- (38) Tian, Z. Y.; Yu, J. B.; Wang, X.; Groff, L. C.; Grimland, J. L.; McNeill, J. D. Conjugated Polymer Nanoparticles Incorporating Antifade Additives for Improved Brightness and Photostability. *J. Phys. Chem. B.* **2013**, *117*, 4517–4520.
- (39) Gai, F.; Zhou, T.; Zhang, L.; Li, X.; Hou, W.; Yang, W. X.; Li, Y.; Zhao, X.; Xu, D.; Liu, Y.; Huo, Q. Silica Cross-Linked Nanoparticles Encapsulating Fluorescent Conjugated Dyes for Energy Transfer-Based White Light Emission and Porphyrin Sensing. *Nanoscale* **2012**, *4*, 6041–6049.
- (40) Szymanski, C.; Wu, C.; Hooper, J.; Salazar, M. A.; Perdomo, A.; Dukes, A.; McNeill, J. Single Molecule Nanoparticles of the Conjugated Polymer MEH-PPV, Preparation and Characterization by Near-Field Scanning Optical Microscopy. *J. Phys. Chem. B* **2005**, *109*, 8543–8546.
- (41) Lam, Y. M.; Grigorieff, N.; Goldbeck-Wood, G. Direct Visualisation of Micelles of Pluronic Block Copolymers in Aqueous Solution by Cryo-TEM. *PCCP Phys. Chem. Chem. Phys.* **1999**, *1*, 3331–3334.
- (42) Mortensen, K.; Talmon, Y. Cryo-TEM and SANS Microstructural Study of Pluronic Polymer Solutions. *Macromolecules* **1995**, *28*, 8829–8834.
- (43) Tan, H.; Liu, N. S.; He, B.; Wong, S. Y.; Chen, Z. K.; Li, X.; Wang, J. Facile Synthesis of Hybrid Silica Nanocapsules by Interfacial Templating Condensation and Their Application in Fluorescence Imaging. *Chem. Commun.* **2009**, 6240–6242.
- (44) Chen, X.; Tseng, H.-E.; Liao, J. L.; Chen, S. A. Green Emission from End-Group-Enhanced Aggregation in Polydiocetylfluorene. *J. Phys. Chem. B.* **2005**, *109*, 17496–17502.
- (45) Wu, L.; Sato, T.; Tang, H. Z.; Fujiki, M. Conformation of a Polyfluorene Derivative in Solution. *Macromolecules.* **2004**, *37*, 6183–6188.
- (46) Yu, H.; Qi, L. Polymer-Assisted Crystallization and Optical Properties of Uniform Microrods of Organic Dye Sudan II. *Langmuir* **2009**, *25*, 6781–6786.
- (47) Chen, J. S.; Dong, W. F.; Mohwald, H.; Krastev, R. Amplified Fluorescence Quenching of Self-Assembled Polyelectrolyte-Dye Nanoparticles in Aqueous Solution. *Chem. Mater.* **2008**, *20*, 1664–1666.
- (48) Wang, J.; Wang, D.; Miller, E. K.; Moses, D.; Bazan, G. C.; Heeger, A. J. Photoluminescence of Water-Soluble Conjugated Polymers: Origin of Enhanced Quenching by Charge Transfer. *Macromolecules.* **2000**, *33*, 5153–5158.
- (49) Knaapila, M.; Almásy, L.; Garamus, V. M.; Pearson, V. M. C.; Pradhan, S.; Petty, M. C.; Scherf, U.; Burrows, H. D.; Monkman, A. P. Solubilization of Polyelectrolytic Hairy-Rod Polyfluorene in Aqueous Solutions of Nonionic Surfactant. *J. Phys. Chem. B* **2006**, *110*, 10248–10257.
- (50) Olley, D. A.; Wren, E. J.; Vamvounis, G.; Fernee, M. J.; Wang, X.; Burn, P. L.; P. Meredith, P.; Shaw, P. E. Explosive Sensing with Fluorescent Dendrimers: The Role of Collisional Quenching. *Chem. Mater.* **2010**, *23*, 789–794.
- (51) Attar, H. A. Al.; Monkman, A. P. Effect of Surfactant on Water-Soluble Conjugated Polymer Used in Biosensor. *J. Phys. Chem. B* **2007**, *111*, 12418–12426.
- (52) Wasielewski, M. R. Photoinduced Electron Transfer in Supramolecular Systems for Artificial Photosynthesis. *Chem. Rev.* **1992**, *92*, 435–461.
- (53) Liu, B.; Bazan, G. C. Optimization of the Molecular Orbital Energies of Conjugated Polymers for Optical Amplification of Fluorescent Sensors. *J. Am. Chem. Soc.* **2006**, *128*, 1188–1196.
- (54) Mailänder, V.; Landfester, K. Interaction of Nanoparticles with Cells. *Biomacromolecules.* **2009**, *10*, 2379–2400.
- (55) Zhang, L. B.; Zhu, J. B.; Guo, S. J.; Li, T.; Li, J.; Wang, E. G. Photoinduced Electron Transfer of DNA/Ag Nanoclusters Modulated by G-Quadruplex/Hemin Complex for the Construction of Versatile Biosensors. *J. Am. Chem. Soc.* **2013**, *135*, 2403–2406.
- (56) Wang, S.; Gaylord, B. S.; Bazan, G. C. Fluorescein Provides a Resonance Gate for FRET from Conjugated Polymers to DNA Intercalated Dyes. *J. Am. Chem. Soc.* **2004**, *126*, 5446–5451.
- (57) Liu, B.; Wang, S.; Guillermo, C.; Bazan, G. C.; Mikhailovsky, A. Shape-Adaptable Water-Soluble Conjugated Polymers. *J. Am. Chem. Soc.* **2003**, *125*, 13306–13307.
- (58) Zhang, X.; Wu, Y. B.; Ji, S. M.; Guo, H. M.; Song, P.; Han, K. L.; Wu, W. T.; Wu, W. H.; James, T. D.; Zhao, J. Z. Effect of the Electron Donor/Acceptor Orientation on the Fluorescence Transduction Efficiency of the d-PET Effect of Carbazole-Based Fluorescent Boronic Acid Sensors. *J. Org. Chem.* **2010**, *75*, 2578–2588.
- (59) Jia, L.; Guo, C.; Xiang, J.; Wang, N.; Yang, L.; Tang, Y.; Liu, H. Interaction between PEO-PPO-PEO Copolymers and a Hexapeptide in Aqueous Solutions. *Langmuir* **2011**, *28*, 1725–1732.
- (60) Niamnont, N.; Siripornnoppakhun, W.; Rashatasakhon, P.; Sukwattanasinitt, M. A Polyanionic Dendritic Fluorophore for Selective Detection of Hg²⁺ in Triton X-100 Aqueous Media. *Org. Lett.* **2009**, *11*, 2768–2771.