

# Fluorescent Nanoparticles Comprising Amphiphilic Rod–Coil Graft Copolymers

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**ABSTRACT:** Three fluorescent amphiphilic rod–coil grafted copolymers comprising fluorene-based backbones and PEO side chains have been synthesized through the Suzuki coupling reaction. Stable and uniform fluorescent micelles were formed from the fluorescent amphiphiles in aqueous solution. The micelle size was tuned from 85 to 178 nm through controlling the hydrophilic/hydrophobic ratio and molecular weight of the amphiphiles. Preliminary biocompatibility and bioimaging investigation with BV-2 cells indicates that the fluorescent micelles are noncytotoxic, and BV-2 cells can be uniformly labeled by the micelles. Because of the intense fluorescence, biocompatibility, and noncytotoxicity of the amphiphiles, the fluorescent micelles may find potential applications in visualization of microscopic structures and drug delivery tracing.

## Introduction

Self-assembly is a powerful and versatile method to construct nanostructural materials. The unique self-assembly properties of amphiphilic block copolymers result from the inherent immiscibility between different building blocks and the competing thermodynamic effects.<sup>1–5</sup> Polymeric micelles formed from amphiphilic block copolymers have found a rich variety of applications in nanotechnology as solubilizers and surface modifiers as well as gene and drug delivery vehicles.<sup>6–11</sup> For drug delivery tracing, it makes strategic sense to combine the properties of drug delivery with optical labeling.<sup>12</sup>

Luminescent nanoparticles, such as quantum dots (QDs)<sup>13,14</sup> and dye-encapsulated silicon nanoparticles (SNs),<sup>15,16</sup> represent new classes of fluorescent probes, which demonstrate high efficiency and long lifetime. However, protective coating and functionalization of the particle surface are indispensable for these nanoparticles. In addition, their toxicity and environmental impact need to be assessed. Thus, biocompatible and environmentally friendly luminescent micelles make things simple.

In this paper, we report a simple, flexible, and effective approach to fluorescent micelles with intense fluorescence, excellent biocompatibility, and nontoxicity. The amphiphilic molecules are based on rod–coil graft copolymers containing oligofluorene (OF)/polyfluorene (PF) backbones and poly(ethylene oxide) (PEO) side chains. PEOs have been widely used in cosmetics and pharmaceutical applications due to their excellent hydrophilicity, biocompatibility, and noncytotoxicity.<sup>17–20</sup> OF/PF and their derivatives are a class of well-known conjugated molecules that are widely used in organic electronics due to their intense luminescent properties<sup>21,22</sup> and convenient color tunability.<sup>23</sup> Three fluorescent amphiphiles with different molecular weights and hydrophilic/hydrophobic ratios were synthesized. The chemical structures of the amphiphiles are shown

in Figure 1. In these amphiphiles, PEO with molecular weight of 2000 Da was selected as hydrophilic segments and linked to OF/PF side chains. Hexyl-substituted fluorene formed the backbone of conjugated oligomers and polymers. The backbones are composed of 3 and 5 fluorene repeat units for **3FP** and **5FP** and 18 units for **PFP**. Their structural effects on particle size, size distribution, and micelle morphology were investigated.

## Experimental Section

**Materials.** 2-Bromofluorene, 2,7-dibromofluorene, 1-bromohexane, and 1,6-dibromohexane were purchased from Lancaster Co. *n*-Butyllithium (1.6 M in hexane), anhydrous sodium hydride, 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, and monomethoxyl-capped poly(ethylene oxide) ( $M_n = 2000$  g/mol) were purchased from Sigma-Aldrich Chemical Co. PEOs were dried at 40 °C under vacuum overnight prior to use. Catalyst tetrakis(triphenylphosphine)palladium(0) [Pd(PPh<sub>3</sub>)<sub>4</sub>] was from Strem Co. All the above chemicals were used as received without further purification. Tetrahydrofuran (THF) was distilled from sodium benzophenone prior to use.

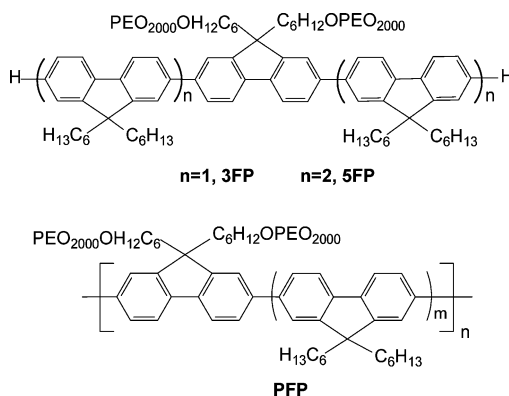
**Synthesis.** 2,7-Dibromo-9,9-bis(6'-polyethylene oxide hexyl)-fluorene. In a three-necked 150 mL flask under argon atmosphere was placed sodium hydride (0.96 g, 40 mmol) and anhydrous THF (30 mL), and then PEO (8 g, 4 mmol,  $M_n = 2000$  g/mol) in THF (50 mL) was dropped in at room temperature. After the solution was stirred for 4 h, 2,7-dibromo-9,9-bis(6'-bromohexyl)fluorene (0.65 g, 1 mmol) was added and stirred at room temperature. TLC showed that the reaction completed in 1 week. Water was added dropwise to terminate the reaction. The mixture was evaporated off to remove THF, and the residue was dissolved in 20 mL of dichloromethane and 4 mL of methanol. Then the solution was precipitated in 500 mL of ether with stirring for 1 h. After the solvent was centrifuged off, the solid was dissolved in dichloromethane/methanol again and precipitated in ether. The procedure of dissolution/precipitation/centrifugation was repeated three times, and the final solid was dissolved in dichloromethane and loaded into dialysis tube to remove the salts and unreacted PEOs. After dialysis for 1 week, the aqueous solution was freeze-dried and 1 g (50%) of white powders was obtained. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz, ppm): 7.54–7.49 (m, from fluorenyl group), 7.47–7.40 (m, from fluorenyl group), 3.64 (s, from PEO), 2.10–1.90 (m, broad), 1.43–

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**Figure 1.** Chemical structures of fluorescent graft copolymers.

1.39 (m, broad), 1.30–1.19 (m, broad), 1.10–1.00 (m, broad), 0.90–0.78 (m, broad). GPC (254 nm, THF),  $M_n = 4100$ ,  $M_w = 4200$ , PDI = 1.02. Anal. Calcd: C, 55.04, H, 8.84. Found: C, 54.80, H, 8.96.

**3FP.** **3FP** was synthesized by following the standard Suzuki coupling reaction. A mixture of 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dihexylfluorene (0.46 g, 1 mmol), 2,7-dibromo-9,9-bis(6'-polyethylene oxide hexyl)fluorene (1.12 g, 0.25 mmol),  $\text{Pd}(\text{PPh}_3)_4$  (50 mg, 0.04 mmol), aqueous sodium carbonate (2 M, 1.24 mL), and toluene (10 mL) was deoxygenated and then heated to reflux under nitrogen. The mixture was stirred for 3 days and then cooled to room temperature. The organic solvent in the mixture was allowed to evaporate. The residue was dissolved in 20 mL of dichloromethane and precipitated in 800 mL of ether. The solvents were removed by centrifuge. The precipitation process was repeated three times. The crude product was dissolved in dichloromethane and subject to dialysis using dialysis tube. The solution was freeze-dried to afford **3FP** as pale powders with a yield of 50%.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, ppm): 7.82–7.70 (m, from fluorenyl group), 7.68–7.57 (m, from fluorenyl group), 7.35–7.31 (m, from fluorenyl group), 3.64 (s, from PEO), 2.10–1.96 (m, broad), 1.43–1.33 (m, broad), 1.17–1.00 (m, broad), 0.83–0.65 (m, broad). GPC (254 nm, THF),  $M_n = 4000$ ,  $M_w = 4100$ , PDI = 1.03. Anal. Calcd: C, 61.44, H, 9.27. Found: C, 59.56, H, 9.38.

**5FP.** **5FP** was synthesized by following the same procedure as the preparation of **3FP**. After purification by precipitation, dialysis, and ultrafiltration, **5FP** was obtained as pale powder with a yield of 37%.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, ppm): 7.86–7.72 (m, from fluorenyl group), 7.72–7.58 (m, from fluorenyl group), 7.40–7.30 (m, from fluorenyl group), 3.64 (s, from PEO), 2.15–1.97 (m, broad), 1.45–1.33 (m, broad), 1.28–1.18 (m, broad), 1.18–1.02 (m, broad), 0.90–0.70 (m, broad). GPC (254 nm, THF),  $M_n = 3900$ ,  $M_w = 4400$ , PDI = 1.13. Anal. Calcd: C, 64.81, H, 9.32. Found: C, 61.86, H, 9.45.

**PFP.** **PFP** was synthesized by following the same procedure as preparation of **3FP**. After purification by precipitation, dialysis, and ultrafiltration, **PFP** was obtained as light yellow powder with a yield of 14%.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz, ppm): 7.85 (s, from fluorenyl group), 7.67 (s, broad, from fluorenyl group), 3.64 (s, from PEO), 2.13 (s), 1.14 (s, broad), 0.80 (s, broad). GPC (254 nm, THF),  $M_n = 9900$ ,  $M_w = 19\,600$ , PDI = 1.98. Anal. Calcd: C, 75.82, H, 9.48. Found: C, 73.55, H, 9.66.

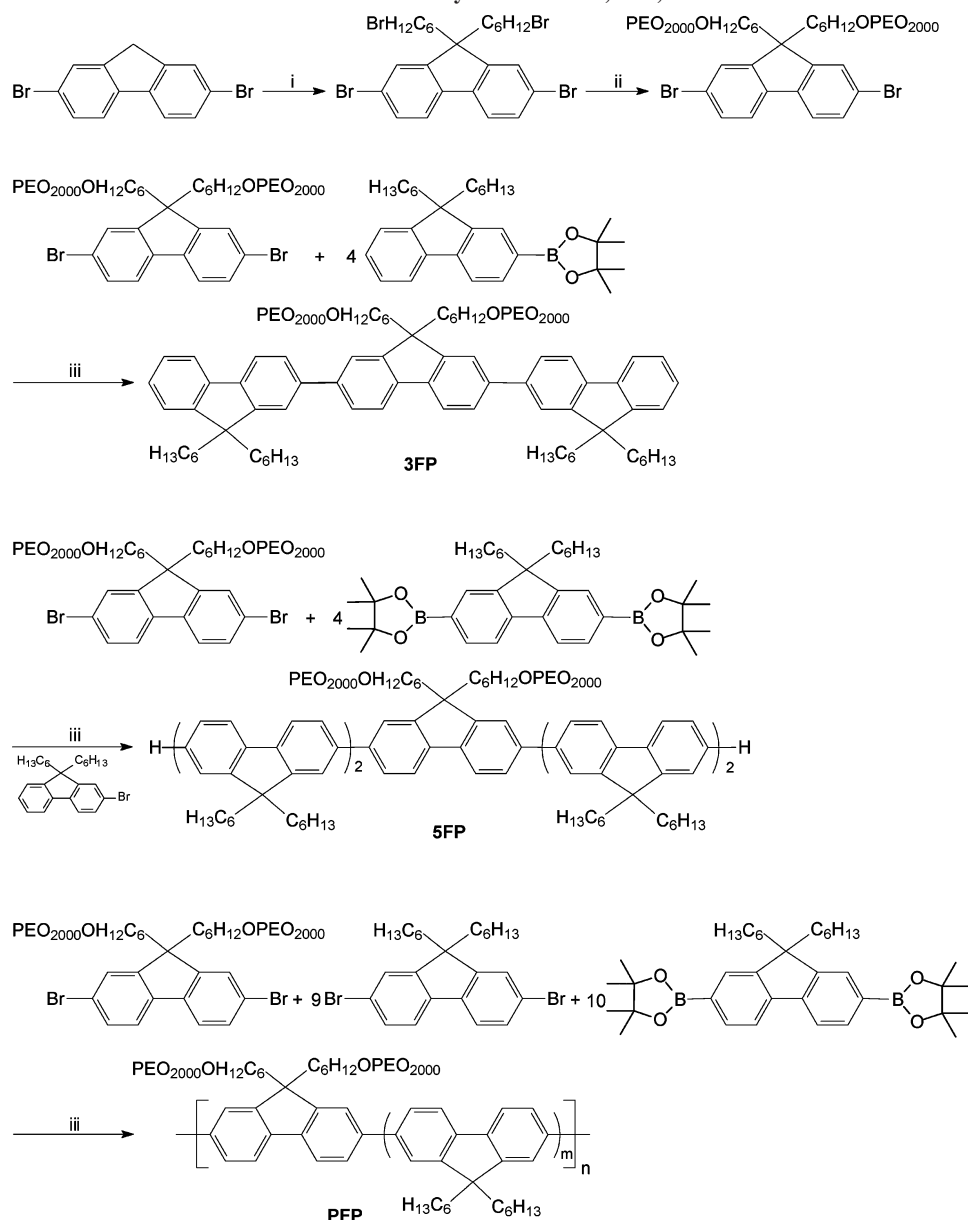
**Preparation of Aqueous Solutions of Micelles.** The graft copolymers were dissolved in a good solvent (THF), in which all blocks of the polymer are soluble. Water was added slowly in order to change the solvent system gradually. Then THF was completely evaporated off to leave water in the system, and micellization was started.

**Characterization.** The  $^1\text{H}$  NMR spectra were recorded in solution of  $\text{CDCl}_3$  on a Bruker DPX (400 MHz) NMR spectrometer with tetramethylsilane (TMS) as the internal standard. GPC analysis was conducted on a Shimadzu SCL-10A and LC-8A system equipped with two Phenogel 5  $\mu\text{m}$ , 50 and 1000 Å columns (size  $300 \times 4.6$  mm) in series and a Shimadzu RID-10A refractive index

detector. Tetrahydrofuran (THF) was used as eluent at a flow rate of 0.20 mL/min at 45 °C. Monodispersed poly(ethylene glycol) standards ( $M_n = 400\text{--}70\,000$  g/mol) were used to obtain a calibration curve. UV–vis–NIR absorption spectroscopy was measured by a Shimadzu UV-3101 PC spectrometer at room temperature. Fluorescence spectra were measured by a Shimadzu RF-5301 PC spectrophotometer at room temperature. Fourier transform infrared spectrophotometry (FTIR) was recorded on a Perkin-Elmer 2000 and measured in KBr discs. Dynamic and static light scattering (DLS and SLS) measurement was conducted with a Brookhaven light scattering instrument that applied vertically polarized laser light with wavelength of 632.8 nm. The spectrometer was calibrated by using polystyrene standard solution of  $97 \pm 3.2$  nm in size. Prior to light scattering measurement, all the sample solutions were filtrated through 0.2  $\mu\text{m}$  Millipore membrane filter to remove dust particles. Atomic force microscopy (AFM) micrographs were performed on DI multimode scanning probe microscope with a Nanoscope IV controller. All measurements were carried out with tapping mode in air at room temperature. Sample solutions were dropped onto freshly cleaved mica surface and dried overnight. The concentration is 0.5 mg/mL for **3FP** and 0.6 mg/mL for **PFP**. Transmission electron microscopy (TEM) images were obtained on a JEOL JEM 2010F transmission electron microscope operating at 300 kV accelerating voltage. Phosphotungstic acid (PTA) was used as staining agent for these PEO-containing micellar samples. A 90  $\mu\text{L}$  sample solution was mixed with 10  $\mu\text{L}$  1% PTA aqueous solution by vortex. A drop of solution mixture was then put onto a 400-mesh carbon-coated copper grid and dried overnight.

## Results and Discussion

The synthetic routes to **3FP**, **5FP**, and **PFP** are illustrated in Scheme 1. The starting materials of 2-bromo-(9,9-dihexyl)fluorene, 2,7-dibromo-(9,9-dihexyl)fluorene, and 2,7-dibromo-9,9-bis(6'-bromohexyl)fluorene were synthesized from fluorene through alkylation reaction in the presence of phase transfer catalyst tetrabutylammonium bromide in 50 wt % KOH aqueous solution with high yields.<sup>24,25</sup> 2-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dihexylfluorene and 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dihexylfluorene were obtained by standard *n*-butyllithium reaction at  $-78$  °C in anhydrous THF solution, followed by adding 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane with around 60–70% yields.<sup>26</sup> 2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene was prepared by introduction of 6-bromohexyl groups into 2,7-dibromofluorene at the position of C-9 with a yield of 75%. Then PEOs with one –OH end group were coupled with 6-bromohexyl groups on fluorene through etherification reaction in the presence of NaH in anhydrous THF to offer the key intermediate 2,7-dibromo-9,9-bis(6'-polyethylene oxide hexyl)fluorene. The crude product of 2,7-dibromo-9,9-bis(6'-polyethylene oxide hexyl)fluorene was purified by dissolving the product in dichloromethane/methanol and precipitating in ether three times. The final solid was dissolved in dichloromethane and loaded into dialysis tube to further remove the salts and unreacted residual PEOs. After dialysis, the aqueous solution was freeze-dried to offer white powders with a yield of 50%. 2,7-Dibromo-9,9-bis(6'-polyethylene oxide hexyl)fluorene was then coupled with 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dihexylfluorene, or 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dihexylfluorene and 2-bromo-(9,9-dihexyl)fluorene, or 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dihexylfluorene and 2,7-dibromo-(9,9-dihexyl)fluorene following the standard Suzuki coupling reaction to afford the amphiphilic graft copolymers of **3FP**, **5FP**, and **PFP**, respectively. The three copolymers were purified by precipitation in ether three times, followed by ultrafiltration and dialysis in water sequentially. In order to enhance the yields of final products

Scheme 1. Routes for Synthesis of **3FP**, **5FP**, and **PFP**<sup>a</sup>

<sup>a</sup> Reagents and conditions: i =  $\text{BrC}_6\text{H}_{12}\text{Br}/50\%$  KOH; ii =  $\text{NaH}/\text{THF}/\text{PEO}_{2000}$ ; iii =  $\text{Pd}(\text{PPh}_3)_4/2\text{M Na}_2\text{CO}_3/\text{toluene}$ .

and simplify the purification, some of the reagents were added in excess. For example, the actual amount of fluorene boronic ester added for synthesis of **3FP** was 4 equiv. instead of the 2 equiv. based on theoretical amount. The same principle was also applied to the synthesis of **5FP**: 4 equiv. of diboronic ester and 6 equiv. of end-capping reagent 2-bromofluorene were used to guarantee that the majority of product is **5FP**.

The chemical structures of the synthesized copolymers were verified by  $^1\text{H}$  NMR spectra. As an example, the  $^1\text{H}$  NMR spectrum of **PFP** is shown in Figure 2. Clear signals can be found at 7.9 and 7.7 ppm for aromatic protons on fluorenyl groups, 3.6 ppm for  $-(\text{CH}_2\text{CH}_2\text{O})-$  of PEO, 2.1 ppm for  $-\text{CH}_2-$  linked at C9 position of fluorenyl groups, 1.1 ppm for  $-\text{CH}_2(\text{CH}_2)_3\text{CH}_3-$  of the alkyl chains attached to fluorenyl groups, and 0.8 ppm for  $-\text{CH}_2\text{CH}_3$  of the alkyl chains. The integration of the signals indicated that the ratios of protons on the aromatic rings, PEO chains, and  $-\text{CH}_2-$  linked at the C9 position of fluorenyl group are 3:10:2, which implied that the ratio of fluorenyl group with PEO side chains and fluorenyl

group with hexyl side chains is 1:17. This ratio matched well with the feed ratio of 1:19 for the polymer preparation.

GPC analysis reveals that the weight-average molecular weight ( $M_w$ ) and number-average molecular weight ( $M_n$ ) of the copolymer are 4100/4000, 4400/3900, and 19 600/9900 for **3FP**, **5FP**, and **PFP**, respectively. GPC results indicated that no free PEOs remain in the synthesized copolymers, and each copolymer chain contains only one unit of fluorenyl group attached with two PEO side chains. For **PFP**, the molecular weight measured by GPC is in good agreement with that obtained from NMR measurement. As discussed in NMR measurement, each **PFP** polymer chain contains 18 fluorenyl units (1 unit attached with two PEO side chains and 17 units substituted with two hexyl side chains), and the calculated  $M_n$  is 9976.

All the amphiphiles formed core-shell or core-corona micellar structure in aqueous solution with the compact fluorescent hydrophobic core and swollen PEO shell. The luminescent hydrophobic core is well stabilized by the hydrophilic shell. All the micelles showed good stability over 6 months storage.

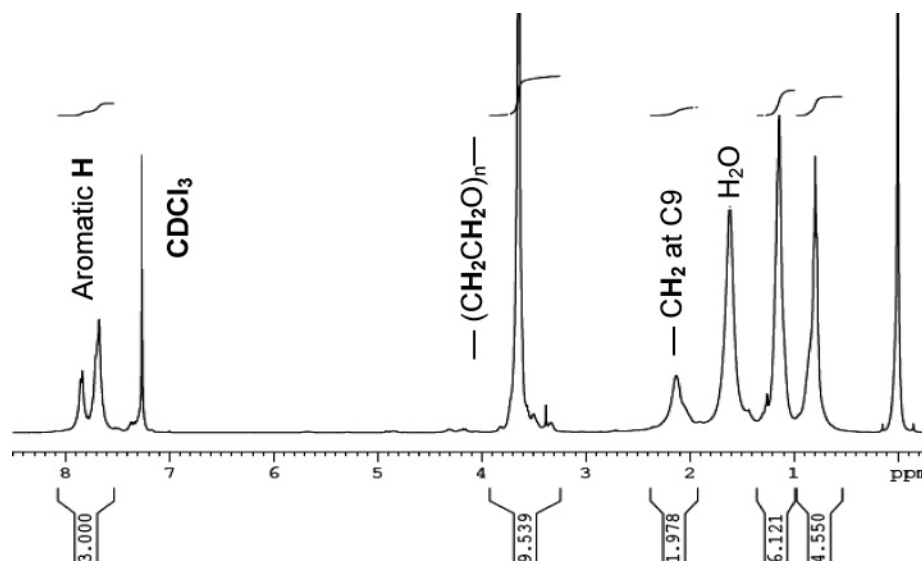


Figure 2.  $^1\text{H}$  NMR spectrum of PFP.

Table 1. Physical Properties of 3FP, 5FP, and PFP

sample	$M_w$	$M_n$	PDI	CAC (mg/mL)	UV $\lambda_{\text{max}}$ (nm)		PL $\lambda_{\text{max}}$ (nm)	
					in DCM	in H <sub>2</sub> O	in DCM	in H <sub>2</sub> O
3FP	4100	4000	1.03	0.1	349	343	395, 416, <sup>a</sup> 443 <sup>b</sup>	400, 421, <sup>a</sup> 446 <sup>b</sup>
5FP	4400	3900	1.13	0.08	365	363	413, 434, <sup>a</sup> 463 <sup>b</sup>	422, 445, <sup>a</sup> 511 <sup>b</sup>
PFP	19600	9900	1.98	0.008	383	378	422, 442, <sup>a</sup> 471 <sup>b</sup>	427, 446, <sup>a</sup> 518 <sup>a</sup>

<sup>a</sup> Side peak. <sup>b</sup> Tail emission band.

The critical aggregation concentration (CAC) of the amphiphilic copolymers has been measured. CAC can be determined by fluorescence, conductance, surface tension, or light scattering measurements.<sup>27–30</sup> Because of the fluorescent properties and nonconductivity of these micelles, the traditional fluorescent probe method and conductance measurement cannot be used for CAC determination for our copolymers. Since the transition from single molecules to micelles involves a considerable

change in scattered light, light scattering measurements have been extensively used to measure CACs for several micellar systems. Here, the CACs were determined by static light scattering measurement and found that the CACs of **3FP**, **5FP**, and **PFP** were 0.1, 0.08, and 0.008 mg/mL, respectively. The micelle size was tuned in a wide range; **5FP** and **PFP** showed monodistribution. The average diameters of micelle in aqueous solution were 15 and 130 nm for **3FP**, 178 nm for **5FP**, and 85

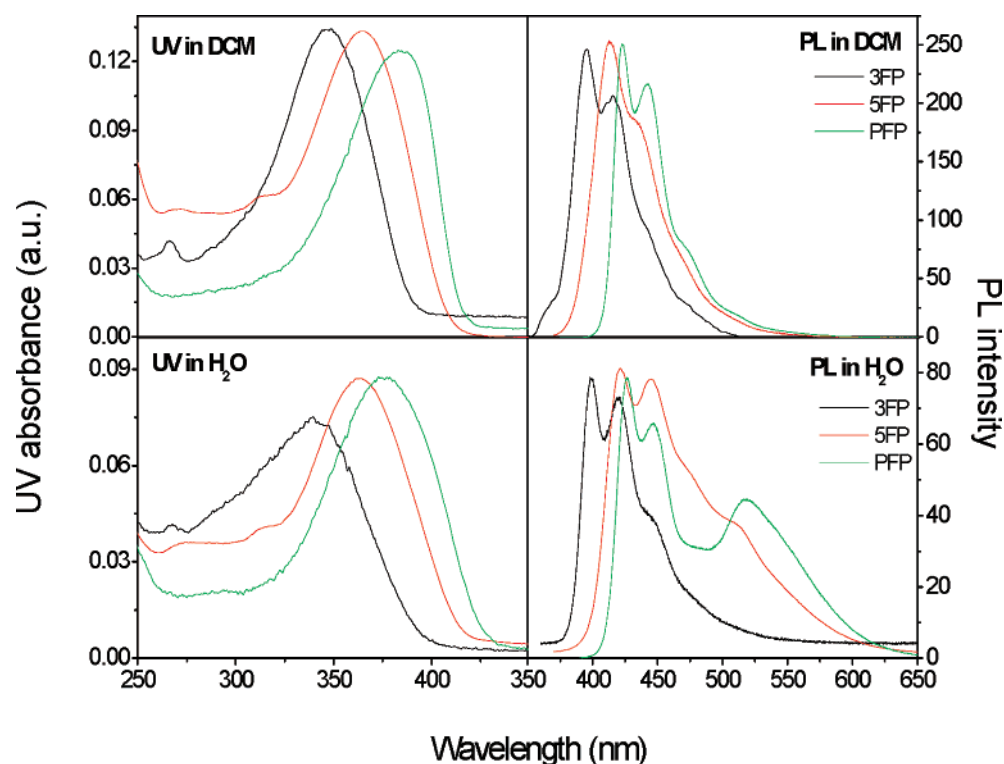


Figure 3. UV and PL spectra of **3FP**, **5FP**, and **PFP** in DCM and in H<sub>2</sub>O.



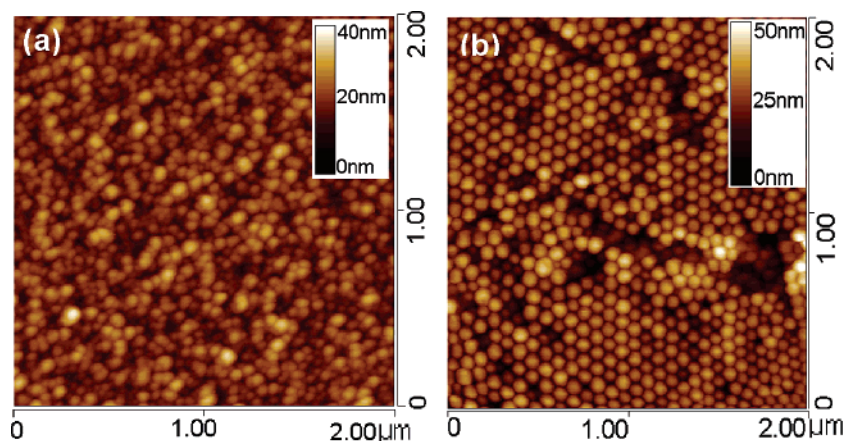


Figure 4. AFM height images (tapping mode) on mica: (a) **3FP** (concentration 0.5 mg/mL) and (b) **PFP** (concentration 0.6 mg/mL).

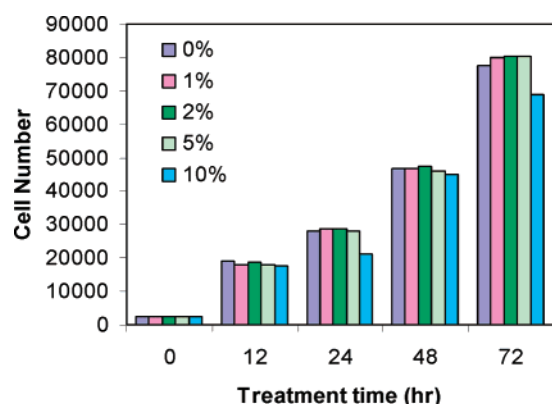


Figure 5. Effect of culture time and concentration of fluorescent micelles on the growth of BV-2 cells.

nm for **PFP**. It can be seen that there are two types of particles in **3FP** micellar solution. The smaller ones are either unimolecules or aggregates comprising a few molecules. The presence of small but stable particles of **3FP** is ascribed to the relatively good solubility of the amphiphile in water, which is of the highest hydrophilic/hydrophobic ratio in the molecules among the three amphiphilic copolymers. The CAC measurement results are summarized in Table 1.

In the fluorescent core of the micelles, molecular aggregation and stronger intermolecular interaction occur, which can be reflected in their optical properties.<sup>31</sup> The UV-vis absorption and photoluminescence (PL) spectra of the three amphiphiles in dichloromethane (DCM) (at a concentration of 0.001–0.01 mg/mL) and aqueous solution (at a concentration of 0.01–0.1 mg/mL) were measured at room temperature (shown in Figure 3). DCM is a good solvent for the amphiphiles, but selective

solvent, H<sub>2</sub>O, will induce micellization. The UV-vis absorption spectra of **3FP**, **5FP**, and **PFP** in DCM are peaked at 349, 365, and 383 nm, respectively. Their absorption spectra in aqueous solution are quite similar to those in DCM. However, their PL spectra in DCM and in H<sub>2</sub>O showed remarkable difference. The PL spectra of **3FP**, **5FP**, and **PFP** in DCM resemble the spectra of oleophilic oligofluorenes and polyfluorenes in other organic solvent, which show the maximum emission at 395, 413, and 422 nm, respectively.<sup>32,33</sup> In comparison with the spectra of DCM samples, all the emission peaks of the amphiphiles in H<sub>2</sub>O are red-shifted by 5–10 nm. In addition, the very weak emission tails of the amphiphiles in DCM were enhanced remarkably in H<sub>2</sub>O, especially for **5FP** and **PFP**. A clearly broad new peak appeared at 518 nm for **PFP**. The enhanced tail emission at the longer wavelength region is due to excimer emission resulting from the  $\pi$ - $\pi$  stacking of the oligomer/polymer backbones.<sup>34</sup> Another possibility of the origin of the emission band at 518 nm is keto defects in polyfluorene backbone, which may exist in the polymer backbone caused by oxidation. In order to affirm the origin of the longer wavelength band emission, FTIR spectra of the copolymers dispersed in KBr pellets were measured. As an example, the FTIR spectrum of **3FP** showed clear vibration bands at 2905 and 2871 cm<sup>-1</sup> for CH<sub>2</sub>  $\nu_{as}$  and  $\nu_s$  stretching, 1635 cm<sup>-1</sup> for benzene ring stretching, 1460 cm<sup>-1</sup> for CH<sub>2</sub>  $\delta_s$  bending, 1453 and 1350 cm<sup>-1</sup> for CH<sub>3</sub>  $\delta_{as}$  and  $\delta_s$  bending, 1250 and 1098 cm<sup>-1</sup> for C-O-C  $\nu_{as}$  and  $\nu_s$  stretching, and 949 cm<sup>-1</sup> for aromatic C-H out-of-plane bending. No signal was observed in the region from 1650 to 1800 cm<sup>-1</sup>, indicating that no detectable keto defects exist in the polyfluorene backbone.<sup>35,36</sup> Thus, the possibility of longer wavelength emission originated from keto defects was excluded. As the chain length of the

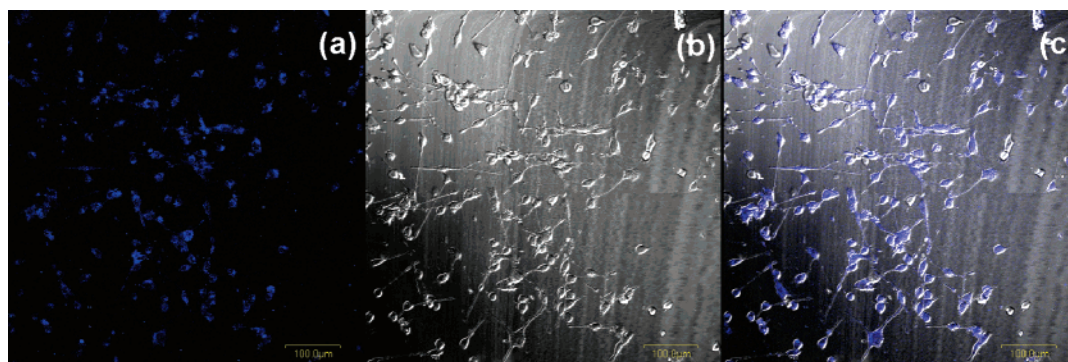


Figure 6. Confocal images of BV-2 cells cultured for 2 h in the presence of **PFP** fluorescent micelles solution (0.003 mg/g): (a) fluorescence view; (b) phase-contrast view, and (c) picture overlapped from (a) and (b).

backbones increases from **3FP** to **5FP**, and then to **PFP**, the excimer emission becomes more intense and obvious, indicating that the polymer chains in **PFP** micelles are more close-packed than the other two amphiphiles. It is rational because (1) **PFP** with longer chain length and higher percentage of hydrophobic segment in the polymer chain will have stronger hydrophobic–hydrophobic interactions between polymer chains and (2) fluorenyl units with hexyl side chains are much easier to form  $\pi$ – $\pi$  stacking than fluorenyl units with PEO side chains. This observation was further confirmed by the AFM images of the micelle samples. The variable intensity of excimer emission of the micelles indicates that the molecular packing density of the micelles could be facily tuned by controlling the molecular weight and the ratio of hydrophilic/hydrophobic components in the polymers.

The solid-state morphologies of micelles were investigated by AFM and TEM. It can be seen from the AFM images (Figure 4) that **3FP** formed soft elliptical micelles. TEM images (figures are not shown) illustrated that the average diameters of the micelles are 10 and 105 nm, which is a little bit smaller than the diameters measured by DLS due to shell shrinkage after dried in air. In comparison with **3FP** and **5FP**, the balanced hydrophilic/hydrophobic ratio and higher molecular weight of polymer **PFP** force the amphiphiles to form uniform and densely packed micelles with thin shell layer in large area. The AFM images of **PFP** showed clear particle boundaries and revealed uniformly dispersed spherical objects possessing an average diameter of 60 nm.

The preliminary investigation of the fluorescent micelles for bioimaging application has been performed with BV-2 microglial cells, the brain macrophages. **PFP** fluorescent micelle aqueous solution (0.3 mg/mL) was added to the culture media with the concentration of 1%, 2%, 5%, and 10% (v/v). After being cultured for 12, 24, 48, and 72 h, the viability of BV-2 cells was measured (Figure 5). The number of the cells cultured with the fluorescent micelle solutions increased synchronously with the number of cells in pure culture media, indicating that the fluorescent micelles are noncytotoxic. The uptake of the fluorescent micelles by BV-2 cells was recorded by confocal laser scanning microscope. Figure 6 shows the confocal images of BV-2 cells cultured for 2 h in a culture media containing 0.003 mg/mL of fluorescent micelles solution. Parts a and b of Figure 6 are recorded in the fluorescent view and phase-contrast view, respectively, and (c) is the overlapped picture from (a) and (b). The confocal images illustrated that almost all the cells can be uniformly labeled by the micelles.

## Conclusion

Three amphiphilic graft copolymers comprising OF/PF backbone and PEO side chains have been developed. Their micellization in aqueous solution has been investigated by light scattering and optical spectroscopy. Among the three amphiphiles, **PFP** with polyfluorene backbone demonstrated the best molecular packing and formed the most stable, uniform, and dense nanoscale micelles. The micelle size can be tuned through modifying the structure and adjusting the hydrophilic/hydrophobic ratio of the amphiphilic copolymers. The fluores-

cent property, good biocompatibility, and excellent long-term stability of the micelles allow the stable fluorescent micelles wide applications in biolabeling and drug delivery tracing. Further functionalization of the hydrophilic corona will allow them to perform as molecular recognition or anchors for specific surface. The color tunability of polyfluorene backbone allows synthesis of nanoparticles with various emissive colors by minor modification of the polymer backbones.

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