Biocompatible fluorescent polymers from PEGylation of an aggregation-induced emission dye

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Abstract

Novel aggregation-induced emission (AIE) polymers (**NS1** and **NS2**) were prepared through reversible addition fragmentation chain transfer polymerization of poly(ethylene glycol) monomethyl ether methacrylate and a new AIE monomer with a vinyl group. The **NS1** and **NS2** copolymers were characterized by gel permeation chromatography, ^1H NMR spectroscopy, FT-IR spectroscopy, X-ray photoelectron spectroscopy, which proved their successful syntheses. Such amphiphilic copolymers could self-assemble into fluorescent polymeric nanoparticles (FPNs) in water with good dispersibility, which were spherical in shape. The prepared FPNs of **NS1** had a size range of 50–150 nm, strong green fluorescence in aqueous solution with fluorescence quantum yield of 31%, and low critical micelle concentration (CMC) of 0.030 mg mL^{-1} . The **NS2** FPNs exhibited size range of 200–300 nm, intense green fluorescence with quantum yield of 38%, and CMC of 0.037 mg mL^{-1} . These FPNs were demonstrated highly biocompatible for cell imaging. By virtue of excellent biocompatibility via PEG modification, good particle stability with low CMC, and AIE fluorescent polymers with high photostability, the FPNs constructed in this work showed obvious advantages. This strategy should inspire new approaches to prepare novel biocompatible AIE fluorescent polymers for biomedical application.

Keywords: Aggregation-induced emission; Fluorescent polymeric nanoparticles; Particle stability; Photostability; Biocompatibility; Cell imaging

1. Introduction

The development of fluorescent polymeric nanoparticles (FPNs) for biomedical applications has recently attracted great attention due to their remarkable advantages as compared with small

organic dyes and fluorescent inorganic nanomaterials [1-3]. Among these FPNs, aggregation-induced emission (AIE) dyes generating FPNs have aroused great research interest owing to their own advantages including high water dispersibility, high fluorescence quantum yield, excellent biocompatibility, and easy functionalization [4-7]. A series of AIE dyes comprising tetraphenylethylene [8-10], siloles [11-13], triphenylethylene [14-18], cyano-substituted diarylethylene [19, 20], and distyrylanthracene [21-23] have been utilized to construct AIE-based FPNs. Various strategies for the fabrication of AIE-based FPNs have been developed for bioimaging. The first kind of strategy is non-covalent amphiphilic polymer encapsulation, which provides an easy preparation approach. Self-assembly of Pluronic F127 and an AIE dye has been reported to construct water soluble and biocompatible FPNs [24]. Biocompatible bovine serum albumin has also been introduced as the matrix to encapsulate AIE dye and fluorescent polymer, and self-assembled into FPNs with unique fluorescence-amplified far-red/near-infrared fluorescent feature for cellular imaging and *in vivo* tumor diagnosis [25]. Folate-functionalized 1,2-distearoylsn-glycero-3-phosphoethanolamine-*N*-poly(ethylene glycol) has been employed as encapsulation matrix to load AIE dye, and formed FPNs for two-photon excited fluorescence imaging, which was advantageous to minimize autofluorescence from biological tissues [26]. The second kind of strategy is covalent linkage of AIE dyes with amphiphilic polymers, which improves the chemical and colloidal stability of the FPNs. An AIE-conjugated block copolymer containing an AIE fluorophore, hydrophobic poly(3-caprolactone) segments, hydrophilic poly(ethylene glycol) segments and folate groups, has been presented and self-assembled into biocompatible FPNs, which could avoid dye leakage phenomenon due to the stable chemical bond among AIE fluorophore, poly(3-caprolactone) and

poly(ethylene glycol) segments [27]. Schiff base condensation of 4-(tetraphenylethene)benzaldehyde and **polylysine**, and subsequent reduction by sodium borohydride, has been demonstrated to form an AIE fluorescent polymer, which showed amphiphilic **properties** and formed FPNs for cell imaging [28]. Tetraphenylethene labeled to a chitosan chain to prepare biocompatible polymers with AIE behavior has been reported, and aggregates of AIE polymers could be readily internalized by HeLa cells for long-term fluorescent cellular tracing [29]. The third kind of strategy is copolymerization of AIE dye with biocompatible amphiphilic polymer, which offers easy functionalization of the FPNs. **Reversible addition fragmentation chain transfer (RAFT) polymerization** and enzymatic transesterification were utilized to fabricate AIE-based FPNs for cell imaging [30]. AIE amphiphilic block copolymers were developed based on the living ring-opening metathesis polymerization, and self-assembled into biocompatible FPNs [31]. Anhydride ring-opening polymerization with an amino-ended AIE dye at room temperature and under an air atmosphere was reported to synthesize copolymers and self-assembly of the copolymers afforded FPNs, which were suitable for cell imaging [32]. Emulsion polymerization was also reported to prepare AIE dye-based FPNs for cell imaging [33, 34]. In addition to the fabrication strategies of AIE-based FPNs, the familiar biocompatible polymers to construct AIE dye-based copolymers included poly(ethylene glycol) [35, 36], chitosan [37], **poly(lysine)** [38], and **phospholipids** [39, 40]. With great advances of the preparation methodology, many AIE-based FPNs with high-performance have been fabricated and exploited for bioimaging application [41-43]. Despite the significant development in this field, new strategy to fabricate biocompatible FPNs possessing good particle stability with low CMC

and AIE fluorescent polymers with high photostability is highly demanded to expand their biomedical applications.

In this work, novel AIE polymers (**NS1** and **NS2**) were prepared through RAFT polymerization of poly(ethylene glycol) monomethyl ether methacrylate and a new AIE monomer (NSP2E) with a vinyl group (scheme 1). When **NS1** and **NS2** were dispersed in water, the AIE fluorogen aggregated into fluorescent cores and the PEG segments covered the surfaces, leading to excellent water solubility of the FPNs. The chemical structure and optical property of the prepared FPNs were systematically characterized by a series of methods including ¹H NMR spectroscopy, gel permeation chromatography, X-ray photoelectron spectroscopy, FT-IR spectroscopy, transmission electron microscopy, dynamic light scattering, fluorescence and UV-visible absorption spectra. Finally, the biocompatibility and cell stain behaviour of the **NS1** and **NS2** FPNs were evaluated for cell imaging application.

<Scheme 1>

2. Experimental procedure

2.1 Materials and characterization

4-Bromobenzaldehyde (99%, Sigma-Aldrich), 4-vinylphenylboronic acid (97%, TCI), benzothiazole-2-yl-acetonitrile (98%, Sigma-Aldrich), potassium bicarbonate (99.5%, J&K), palladium(II) chloride (99.9%, J&K), azobisisobutyronitrile (99%, J&K), 1,4-dioxane (99.5%, J&K), isopropanol (99.7%, J&K), tetrabutylammonium hydroxide (40% in water, J&K), and poly(ethylene glycol) monomethyl ether methacrylate ($M_n = 480$, Sigma-Aldrich) were used as received. All other agents and solvents were purchased from Sigma-Aldrich and used as received without further purification. Ultra-pure water used in the experiments was obtained from a Milli-Q

ElementQ water system.

Gel permeation chromatography (GPC) measurements were determined on Shimadzu LC-20AD pump system, and DMF was used as the eluent at a flow rate of 1.0 mL min⁻¹. The system was calibrated with narrow molecular weight distribution polystyrene standards ranging from 200 to 10⁶ g mol⁻¹. ¹H NMR and ¹³C NMR spectra were recorded with a Mercury-Plus 400 MHz spectrometer using CDCl₃ as the solvent and tetramethylsilane as the internal standard. High resolution mass spectra (HRMS) were measured using a Bruker micrOTOF-Q II high resolution mass spectrometry. Fourier transform infrared spectroscopy (FT-IR) was achieved by Shimadzu Spectrum 8400 spectrophotometer (Japan) at a resolution of 1 cm⁻¹ and the range of 4000–400 cm⁻¹. The X-ray photoelectron spectra (XPS) of the prepared FPNs were detected using a VGESCALAB 220-IXL spectrometer with an Al K α X-ray source (1486.6 eV). The particle sizes of the prepared FPNs were measured by ZetaPlus Zeta Potential Analyzer (Brookhaven Instruments) at 25 °C with a scattering angle of 90°. Transmission electron microscopy (TEM) images were obtained on a HT7700 microscope (Hitachi, Japan) operated at 100 kV, and the TEM samples were made by dropping a droplet of the FPNs in water on a carbon-coated copper grid. UV-vis spectra of the prepared FPNs were recorded using a UV/Vis/NIR 2600 spectrophotometer (Shimadzu, Japan). Fluorescent spectra were measured on a Hitachi F4600 spectrofluorimeter with both slit width of excitation and emission are 3 nm.

2.2 Synthesis and characterization of NSP2E

As shown in scheme 1, NSP2E was synthesized from 4-(4-vinylphenyl)benzaldehyde (intermediate **1**) and benzothiazole-2-yl-acetonitrile. The intermediate **1** was synthesized according to the previous literature [44].

To synthesize the compound of NSP2E, A solution of **1** (0.75 g, 3.60 mmol) and benzothiazole-2-yl-acetonitrile (0.63 g, 3.60 mmol) in ethanol (40.0 mL) was stirred at room temperature. Then tetrabutylammonium hydroxide solution (40% in water, 5 drops) was added and the mixture was heated to reflux for 2 h. The reaction mixture was cooled to room temperature and filtered, washed with ethanol for three times to afford NSP2E (1.11 g, yield 84.7%). **Melting point:** 192–193 °C. **FT-IR (cm⁻¹):** 2227, 1625, 1587, 1494, 1476, 1400, 1313, 1192, 820, 766, 732. **¹H NMR (400 MHz, CDCl₃) δ (ppm):** 5.31 (d, 1H, J = 10.8 Hz), 5.83 (d, 1H, J = 17.6 Hz), 6.76 (q, 2H, J = 6.8 Hz), 7.39–7.46 (m, 1H), 7.47–7.54 (m, 3H), 7.56–7.66 (m, 3H), 7.69–7.78 (m, 2H), 7.90 (d, 1H, J = 8.0 Hz), 8.05–8.13 (m, 3H), 8.25 (s, 1H). **¹³C NMR (100 MHz, CDCl₃) δ (ppm):** 163.04, 153.73, 149.28, 146.35, 144.37, 138.85, 137.66, 136.25, 134.95, 131.37, 131.12, 130.90, 127.60, 127.35, 127.06, 126.98, 126.92, 126.08, 124.13, 123.67, 121.59, 116.78, 114.65, 104.99. **HRMS calcd. for C₂₄H₁₆N₂S:** 364.1034, **found** 364.1004.

2.3 Preparation of NS1 and NS2 FPNs

As shown in scheme 1, in a typical synthesis of **NS1**, NSP2E (0.025 g, 0.068 mmol), PEGMA-480 (0.324 g, 0.67 mmol), **chain transfer agent** (CTA, 19.5 mg, 0.074 mmol), and AIBN (3.28 mg, 0.02 mmol) were dissolved in **dioxane (2 mL)**. The solution was degassed three times through a standard freeze–thaw process. **The polymerization of NSP2E and PEGMA-480 was through RAFT polymerization, and was initiated by AIBN at 70 °C. The above mixture was put into an oil bath maintained at 70 °C for 12h.** Then stop the reaction of polymerization, the purified **NS1** was obtained via precipitation from dioxane to petroleum ether for three times, and then dried under vacuum for further characterization. **FT-IR (cm⁻¹):** 2884, 1726, 1660, 1465, 1392, 1344, 1104, 961, 841, 766. **GPC (Da):** 28104 for *M_n*, 31829 for *M_w*, 1.13 for polydispersity index. **XPS:**

C : N : O : S = 72.1 : 0.5 : 27.1 : 0.3.

The synthetic procedure of **NS2** was similar to that of **NS1**, whereas the amount of CTA was changed to 9.7 mg. FT-IR (cm^{-1}): 2884, 1726, 1660, 1465, 1392, 1344, 1104, 961, 841, 766. GPC (Da): 38439 for M_n , 61209 for M_w , 1.59 for polydispersity index. XPS: C : N : O : S = 73.6 : 1.2 : 24.4 : 0.8.

2.4 Cytotoxicity and confocal microscopic cell imaging of NS1 and NS2 FPNs

The cell viability was evaluated by cell counting kit-8 (CCK-8) assay with A549 cells. Confocal microscopic imaging of A549 cells incubated with the prepared FPNs was measured with a confocal laser scanning microscope (CLSM) Zeiss 710 3-channel (Zeiss, Germany), as the excitation wavelength was set as 405 nm. The specific details were adopted according to our previous literature [45].

3. Results and discussion

A new AIE fluorogen of NSP2E was prepared *via* the Knoevenagel condensation reaction of 4-(4-vinylphenyl)benzaldehyde with benzothiazole-2-yl-acetonitrile in high yield as described in Scheme 1. The structure of the NSP2E was characterized and confirmed by NMR spectroscopy and mass spectrometry, which proved that NSP2E was successfully synthesized. The AIE performance of NSP2E was investigated and is shown in Fig. 1. Fig. 1A represented the molecular structure of NSP2E. THF/water mixed solvent was used and increasing the water fraction would cause NSP2E to aggregate in the solution. The UV-vis absorption spectra of NSP2E in THF/water mixtures were conducted to reveal the emergence of aggregates at high water fraction. When the water fraction was lower than 80%, there were no significant changes for the absorption spectra (Fig. 1B). When the water fraction reached and

exceeded 80%, an obvious levelling-off and red-shift of the absorption peak in the spectra appeared, indicating the formation of aggregates at high water fraction and causing light scattering effect. In the case of PL spectra, when the water fraction was lower than 80%, the PL intensity of NSP2E only had a small increment as the water fraction increased (Fig. 1C). When it reached and exceeded 80%, the emission intensity increased rapidly, showing distinct AIE effect of NSP2E. Fig. 1D shows the changes of fluorescent intensity and images of NSP2E at different water fraction under excitation with a 365 nm UV lamp. The fluorescent quantum yields of the new prepared NSP2E in THF and the mixture of THF/water with different water fraction have been shown in Fig. S1, which indicated a similar trend as that of fluorescent intensity in Fig. 1D.

<Fig. 1>

To prepare NS1 and NS2 FPNs, AIE dye, NSP2E, was copolymerized with an amphiphilic monomer of PEGMA through RAFT polymerization (Scheme 1). The feed ratio of monomer PEGMA to NSP2E (y/x) is 10 : 1, and the designed degrees of polymerization are 10 (for NS1) and 20 (for NS2). The number average molecular weights and weight average molecular weights of NS1 and NS2 were determined by gel permeation chromatography, showing 28104 and 31829 Da with a narrow polydispersity index of 1.13 for NS1, and 38439 and 61209 Da with a polydispersity index of 1.59 for NS2 (Fig. S2). Due to the amphiphilic property of the synthesized polymers, when NS1 and NS2 were dispersed in water, FPNs were formed through self-assembly.

The successful formation of NS1 and NS2 FPNs could be qualitatively confirmed by FT-IR spectroscopy. As shown in Fig. 2A, after RAFT polymerization of NSP2E with PEGMA,

strong stretching vibration band C–H stretching vibration band at 2884 cm^{-1} and C=O stretching vibration band at 1726 cm^{-1} derived from PEGMA was found in **NS1** and **NS2**. C=C stretching vibration band of vinyl group from PEGMA located at 1634 cm^{-1} disappeared in the **NS1 and NS2 samples**, meanwhile a new C=C stretching vibration band of an aromatic ring located at 1660 cm^{-1} was observed in the prepared polymers. Furthermore, C–O stretching vibration at 1104 cm^{-1} from PEGMA and =C–H out-of-plane bending vibration located at 766 cm^{-1} from NSP2E were observed in the prepared samples of **NS1** and **NS2**. These FT-IR results demonstrated the successful syntheses of **NS1** and **NS2**.

The successful syntheses of **NS1** and **NS2** copolymers were also quantitatively confirmed by ^1H NMR spectroscopy. Fig. 2B represented the ^1H NMR spectra of NSP2E, **NS1** and **NS2**. After RAFT polymerization of NSP2E with PEGMA, the chemical shifts of 5.3, 5.8 and 6.8 ppm derived from the vinyl group of NSP2E disappeared in those of **NS1** and **NS2**, and the peaks of C-H from the benzene ring around 8.0 ppm in NSP2E broadened in **NS1** and **NS2**, the chemical shifts of methyl and methylene groups at 2.9 and 3.6 ppm from PEGMA were observed in **NS1** and **NS2**, proving the successful syntheses of **NS1** and **NS2**. According to the hydrogen integral ratio of methylene group from PEGMA to the phenyl group derived from NSP2E, the values of y/x were calculated as 7.2 for **NS1** and 4.7 for **NS2**, while the degrees of polymerization were obtained as 7.5 for **NS1** and 15 for **NS2**, respectively.

<Fig. 2>

XPS experiments were detected to analyse the chemical composition of **NS1** and **NS2**. As shown in Fig. 2C and 2D, the XPS results indicated the presence of carbon and oxygen as major components along with the minor components of nitrogen and sulfur. The overall wt %

of four elements (C : N : O : S) in **NS1** was $\sim 72.1 : 0.5 : 27.1 : 0.3$ and in **NS2** was $\sim 73.6 : 1.2 : 24.4 : 0.8$. The high-resolution C 1s XPS spectrum of **NS1** could be deconvoluted into 2 components (Fig. 3A), which located at ~ 285 eV and ~ 286 eV, corresponding to carbon with sp^2 and sp^3 carbon atoms, respectively. The high-resolution N 1s spectrum of **NS1** showed a peak at ~ 399 eV (Fig. 2B), and the high-resolution O 1s and S 2p spectra of **NS1** obtained peaks at ~ 532 eV and ~ 164 eV (Fig. 3C and 3D). The high-resolution XPS spectra of these elements in **NS2** were shown in Fig. S3, and indicate comparable results.

<Fig. 3>

The size distribution and morphology of the FPNs have been investigated by dynamic light scattering (DLS) and transmission electron microscopy (TEM) study. The size distribution of **NS1** FPNs in phosphate buffer solution (PBS) determined by DLS provided the size range as 109 ± 10 nm, with a polydispersity index of 0.268 (Fig. 4A). The size distribution observed by TEM image indicated the size range from 50 nm to 150 nm (Fig. 4B). **NS1** FPNs in TEM images exhibited spherical nanoparticle structure. The size of **NS2** FPNs were found much larger than that of **NS1**, as indicated from the DLS result, the size value was 276 ± 1 nm with a much smaller polydispersity index of 0.005 (Fig. 4C). TEM image of **NS2** FPNs revealed the size range located between 200 nm and 300 nm with spherical nanoparticle morphology (Fig. 4D). With regard to the controllability of the FPNs sizes, the previous references have proved that regulation of the polymeric molecular weight could control the particle size [46, 47].

<Fig. 4>

PEGylation has been widely utilized to improve the water dispersibility of hydrophobic

materials. After RAFT polymerization of the hydrophobic AIE dye with PEGMA, amphiphilic polymers were formed and self-assembled into nanoparticles in aqueous solution with high water dispersibility. The inset of Fig. 5A and Fig. S4 showed the high dispersibility of the NS1 and NS2 FPNs in water. The absorption peaks of both NS1 and NS2 FPNs were quantitatively mensurated by UV-vis absorption spectroscopy and demonstrate a peak at 378 nm, which referred to conjugated system of NSP2E. The UV-vis spectra did not appear with Mie effect (light scattering due to extrinsic electrodynamic effects) as reported in the previous literature,[32] which might be due to the high water solubility of NS1 and NS2. The fluorescent properties of NS1 and NS2 FPNs in water were studied, and the maximum fluorescence excitation wavelength and emission wavelength of the FPNs were determined as 394 nm and 500 nm, respectively, for both NS1 and NS2 (Fig. 5B). The fluorescence quantum yields of the NS1 and NS2 FPNs were calculated as 31% and 38%, using quinine sulfate as the reference dye. Due to the introduction of the AIE fluorogen, the NS1 and NS2 FPNs showed strong fluorescence in water (right insets in Fig. 5C and 5D). The PL spectra of NS1 and NS2 FPNs in ethanol and water were conducted and shown in Fig. 5C and 5D. As compared with the FPNs in ethanol, the emission wavelength of the FPNs in water red-shifted from 480 to 500 nm, which was probably caused by the solvent effect. More importantly, the PL intensities of the FPNs in water increased greater than those in ethanol, proving both prepared NS1 and NS2 were AIE polymers.

<Fig. 5>

Fluorescence stability of the FPNs are important for long-term imaging application. Fig. 6A represented the PL intensity of the NS1 FPNs in water under continuous UV exposure of 365

nm for 60 min and 120 min, the result indicated that fluorescence after UV exposure remained almost unchanged as compared to the initial PL intensity. The long-term PL stability of the NS1 FPNs might be attributed to the introduction of AIE component, leading to a more efficient anti-quenching effect by aggregation. The fluorescence stability study of the NS2 FPNs also showed excellent photostability (Fig. S5). Moreover, we have conducted the emission images of NS1 FPNs and NS2 FPNs in water with a 365 nm UV lamp after storing at room temperature for one month (Fig. S6), and determined the dynamic light scattering of NS1 FPNs and NS2 FPNs in PBS after storing at room temperature for one month (Fig. S7). These results showed almost unchanged fluorescent emission and particle size distribution, indicating excellent colloidal stability of the FPNs. In a previous work, low CMC of the nanoparticles has been demonstrated highly desirable to broaden their application scope in extremely dilute solution [45]. Thus, PL spectra of the FPNs in water at different concentrations were measured to obtain the CMC values (Fig. 6B and Fig. S8). Along with the increased concentration of the FPNs, the fluorescence intensity enhanced. After the concentration exceeded 0.01 mg mL^{-1} , the fluorescence intensity increased rapidly, indicating an inflection point emerged. Therefore, the CMC values of NS1 and NS2 were quantitatively determined according to the inflection point of the curves between the intensity of the fluorescent emission vs the logarithm of the concentration of NS1 and NS2. The result showed that NS1 and NS2 FPNs had very low CMC values, which were 0.030 mg mL^{-1} for NS1 and 0.037 mg mL^{-1} for NS2 (Figure 6C, 6D), suggesting those were even lower than the previous reported AIE-based FPNs [39, 47].

<Fig. 6>

The performance study of the prepared FPNs revealed they had high water dispersibility, suitable size, strong fluorescent intensity, and excellent fluorescent stability. To apply them for potential cell imaging, the biocompatibility of the NS1 and NS2 FPNs was determined with A549 cell lines. CCK-8 assays were used to investigate the cytotoxicity of NS1 and NS2 FPNs to A549 cells after incubation for 8 and 24 h. Fig. 7A revealed cell viability remained unchanged after the cells incubated NS1 FPNs with different concentrations for either 8 h or 24 h. 95% of cell viability was achieved even at very high concentration of $120 \mu\text{g mL}^{-1}$ after 24 h. As shown in Fig S5A, the NS2 FPNs were also proved with high cell viability, which made them suitable for fluorescent cell labels. Finally, to explore the FPNs for bioimaging application, A549 cells were incubated with NS1 and NS2 FPNs for 3 h, imaged under fluorescence mode, and observed by Confocal Laser Scanning Microscope (CLSM). After incubating with $10 \mu\text{g mL}^{-1}$ of NS1 and NS2 FPNs, strong green fluorescence could be found in the cytoplasm area of the cells (Fig. 7B and Fig. S9B). Within the cytoplasm area appeared relative weak fluorescent intensity, which indicated the location of the cell nucleus. This result demonstrated NS1 and NS2 FPNs were uptaken by endocytosis of the cells and stained the cytoplasm. The results also indicated that both of NS1 and NS2 could be applied to the cell images without big difference, even with different sizes. In fact, many previous references about PEGylation of AIE dye to form FPNs have demonstrated that the FPNs with different molecular weights or different sizes have similar cell viability and CLSM images [46-49].

<Fig. 7>

4. Conclusions

In this work, we reported RAFT polymerization of PEGMA with a new AIE monomer to

obtain the amphiphilic polymers of **NS1** and **NS2**, which self-assembled into FPNs in aqueous solution. ^1H NMR and FT-IR results confirmed the successful syntheses of **NS1** and **NS2**. The prepared FPNs of **NS1** showed high water dispersibility with size range of 50–150 nm, strong green fluorescence in aqueous solution with fluorescence quantum yield of 31%, and low CMC of 0.030 mg mL^{-1} . And the **NS2** FPNs exhibited size range of 200–300 nm, intense green fluorescence with quantum yield of 38%, and CMC of 0.037 mg mL^{-1} . Such FPNs were proved highly biocompatible for cell imaging. As compared to the previous work about fluorescent polymer based on AIE dye [45, 48], this work has some obvious advantages, such as excellent biocompatibility *via* PEG modification, good particle stability with low CMC, and AIE fluorescent polymers with high photostability. More importantly, this work would inspire new approach to prepare novel biocompatible AIE fluorescent polymers for biomedical application.

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Figure Captions

Scheme 1 Synthetic route of **NS1** and **NS2**: RAFT polymerization of PEGMA with a new AIE monomer (NSP2E) to obtain the amphiphilic polymer of **NS1** and **NS2**, the ratio of monomer PEGMA to NSP2E is 10 : 1, the designed degrees of polymerization for **NS1** and **NS2** are 10 and 20, respectively.

Fig. 1 (A) The molecular structure of NSP2E; (B) UV-vis absorption spectra of NSP2E at 10 mM in THF/water solution with different water fractions; (C) PL spectra of NSP2E at 10 mM in THF/water solution with different water fractions; (D) The curve of fluorescent intensity vs. water fraction, the insets are the emission images of the compounds in THF/water solution with a 365 nm UV lamp.

Fig. 2 (A) FT-IR spectra of NSP2E, PEGMA, **NS1** and **NS2**. Strong stretching vibration band C-H stretching vibration band at 2884 cm^{-1} , C=O stretching vibration band at 1726 cm^{-1} , C=C stretching vibration band of aromatic ring located at 1660 cm^{-1} , C-O stretching vibration at 1104 cm^{-1} and =C-H out-of-plane bending vibration located at 766 cm^{-1} were observed in the prepared **NS1** and **NS2**, suggesting their successful syntheses; (B) ^1H NMR spectra of NSP2E, **NS1** and **NS2**. The chemical shifts of 5.3, 5.8 and 6.8 ppm derived from the vinyl group of NSP2E were disappeared in **NS1** and **NS2**, and the peaks of C-H from the benzene ring around 8.0 ppm in NSP2E broadened in **NS1** and **NS2**, and the chemical shifts of methyl and methylene group at 2.9 and 3.6 ppm were observed in **NS1** and **NS2**, proving the successful syntheses of **NS1** and **NS2**; XPS spectra of (C) **NS1** and (D) **NS2** FPNs showing the presence of carbon, nitrogen, oxygen, and sulfur.

Fig. 3 High-resolution (A) C 1s spectrum, (B) N 1s spectrum, (C) O 1s spectrum, and (D) S 2p spectrum of **NS1** FPNs.

Fig. 4 (A) Dynamic light scattering result of **NS1** FPNs in PBS; (B) TEM image of **NS1** FPNs dispersed in water; (A) Dynamic light scattering result of **NS2** FPNs in PBS; (B) TEM image of **NS2** FPNs dispersed in water.

Fig. 5 (A) UV-vis spectra of **NS1** FPNs in water at different concentrations (0.1, 0.25, 0.50, 1.00 mg mL⁻¹), inset is the visible image of **NS1** FPNs in water; (B) Fluorescent excitation (Ex) and emission (Em) spectra of **NS1** and **NS2** FPNs in water; (C) PL spectra of **NS1** FPNs in ethanol and water, the insets are the emission images of **NS1** FPNs in ethanol and water with a 365 nm UV lamp, respectively; (D) PL spectra of **NS2** FPNs in ethanol and water, the insets are the emission images of **NS2** FPNs in ethanol and water with a 365 nm UV lamp, respectively.

Fig. 6 (A) Fluorescence stability of the **NS1** FPNs under continuous UV light exposure of 365 nm for 60 min and 120 min; (B) PL spectra of **NS1** FPNs in water at different concentrations excited by 405 nm; (C) Intensity of the fluorescent emission vs the logarithm of the concentration of **NS1** ($\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 500 \text{ nm}$), and the CMC value is calculated as 0.030 mg mL⁻¹; (D) Intensity of the fluorescent emission vs the logarithm of the concentration of **NS2** ($\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 500 \text{ nm}$), and the CMC value is calculated as 0.037 mg mL⁻¹.

Fig. 7 (A) Cell viability of A549 cells incubated with different concentrations of **NS1** FPNs for 8 h and 24 h, indicating good biocompatibility of the FPNs. CLSM images of A549 cells incubated with 10 $\mu\text{g mL}^{-1}$ of **NS1** FPNs for 3 h: (B) excited with 405 nm laser, (C) bright field, (D) merged image of B and C. Scale bar = 20 μm .

Scheme 1 Synthetic route of **NS1** and **NS2**: RAFT polymerization of PEGMA with a new AIE monomer (**NSP2E**) to obtain the amphiphilic polymer of **NS1** and **NS2**, the ratio of monomer PEGMA to **NSP2E** is 10 : 1, the designed degrees of polymerization for **NS1** and **NS2** are 10 and 20, respectively.

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