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Star-shaped Poly(L-lactide)-b- poly(ethylene glycol) with Porphyrin Core: Synthesis, Self-Assembly, Drug-Release Behavior, and singlet oxygen research

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Abstract：

Star-shaped Poly(L-lactide)-b-poly(ethylene glycol) （ SPPLA-b-PEG ） block copolymers with porphyrin core were successfully synthesized from ring-opening polymerization (ROP) of L-Lactide initiated with porphyrin core, followed by coupling reaction with a hydrophilic polymer PEG shell. The structure of this novel copolymer was thoroughly studied by Nuclear magnetic resonance spectroscopy (NMR), Gel Permeation Chromatography (GPC), Fourier transform infrared (FT-IR), X-raydiffraction (XRD). Such SPPLA-b-PEG copolymer exhibits efficient singlet oxygen generation and indicates high fluorescence quantum yields. Notably, the as-prepared porphyrin-cored star-shaped copolymer self-assembly into micelle-like structures provides the great potential of using this well-defined porphyrin core --material for drug delivery system. Particularly, the doxorubicin-loaded SPPLA-b-PEG nanosphere exhibits property of pH-induced drug release.

Keywords: photodynamic therapy; star-shaped; self-assembly; singlet oxygen

1 Introduction

Porphyrin compounds for photodynamic therapy (PDT) is a burgeoning technology because of their unique characteristics, such as eradicating premalignant, controlling the early-stage cancer, and reducing the tumor size in end-stage cancers by photosensitizers (PSs). [1-4] When exposed to visible light, the PSs will produce highly reactive oxygen species (ROS) that are the responsible components for invoking cell death and destructing diseased tissues without damage to the surrounding

healthy tissues.[5-7] However, many drawbacks, such as molecular complexity, self-quenching, photo-toxicity to the skin induced by existing agents' hydrophobicity and hydrophobicity, greatly limit its in vivo application.[8-10] In aqueous media, most photosensitizers (PSs) are inclined to aggregate due to the tendency of the hydrophobic skeleton to resist connecting with water molecules. This inhibits the photo activity of monomeric species and result in remarkable inhibition of their photosensitizing efficacy because only monomeric species are appreciably photoactive. [11, 12]Therefore, great efforts have been made in the delivery systems to address the problem of aggregation of porphyrin derivatives such as liposomes,[13] polymeric particles,[14] and hydrophilic polymer.[10]

Among the various vehicle used for delivery systems, star-shaped amphiphilic copolymers may be the best one since this material always possess well-defined and flexible structure, controllable surface functionality, providing many advantages such as high surface reactivity and low hydrodynamic radius.[15, 16] . Poly-(lactide) (PLA), a US Food and Drug Administration (FDA) approved biomedical materials, has been increasingly studied as a biomedical and biocompatible material.[17, 18] However, problems like poor hydrophilicity, lacking bioactivity and slow drug-release rates greatly limit its in vivo applications.[19, 20] Fortunately, these defects could be settled through the adjustment of polymer hydrophilicity-hydrophobicity balance by precisely control of branched macromolecular architecture. Presently, poly(ethylene glycol) (PEG) has been widely investigated as an hydrophilic material. Using PEG with molecular weight less than 10000, it is able to efficiently modulate the

biodegradation and drug-release rates of the aforementioned PLA polymers.[15, 21-23] As an extension, Chen *et al.* investigated the synthesis of poly(L-lactide)-b-poly(ethylene oxide) (PLLA- b-PEG) copolymers with different branch arms.[15] Sun *et al.* synthesis triblock and pentablock copolymers composed of poly(ε-caprolactone) (PCL), poly(lactide) (PLA), and poly(ethylene glycol) (PEG) and investigated their drug-release behavior.[21] You Han Bae *et al*. synthesis flower-like micelles of PLLA-b-PEG-b-polyHis and studied its pH-dependent cell cytotoxicity.[24] Dijkstra and Feijen using ring-opening polymerization method synthesized star-shaped block copolymers PEG-$(PLLA)_8$ initiated with 8-arm PEG.[25, 26] However, the study on the biodegradable polymers with porphyrin-cored is still limited since their propensity are to complex and the synthesis process is difficult. Frechet[27], Lai[28] and Our group[29, 30] successfully synthesized a series of porphyrin-cored PLA/PCL polymeric shell based on ROP. But these porphyrin-cored PLA/PCL polymers lack water solubility which greatly limited in further application. Peng *et al*. used chlorin coupled with diblock copolymer PEG-PCL for dual chemo-photodynamic therapies.[10] REN *et al*. investigated thermo sensitive porphyrin-cored amphiphilic PCL-b-POEGMA polymer.[12] However, there is no report on the star-shaped biodegradable micelles prepared by ROP of L-LA initiated with porphyrin core with a hydrophilic polymer shell. This work focusing on the prophyrin-cored star-shaped SPPLA-b-PEG copolymer provides a high singlet oxygen production material and could be used for chemotherapy drug delivery system.

In this study, a novel and well-defined 4-armed porphyrin-cored star-shaped poly(L-lactide)-b-poly(ethylene glycol) (SPPLA-b-PEG) copolymer was synthesized. Specifically, tetrahydroxyethyl terminated porphyrin was used as an initiator for the ROP of L-lactide to prepare porphyrin-cored star-shaped poly(L-lactide) (SPPLA). Then, SPPLA-b-PEG was synthesized by the reaction of SPPLA with monomethoxy ethylene glycol (CMPEG), as shown in Scheme 1. The molecular structures, fluorescence quantum yields, self-assembly behavioring and singlet oxygen detection of these prophyrin-cored star-shaped SPPLA-b-PEG copolymers were thoroughly characterized by means of ^{1}H NMR, GPC, FT-IR, UV-vis, Fluorescent spectroscopy and TEM. In addition, its potential as a drug delivery carrier was also evaluated. Hydrophobic chemotherapeutic agent doxorubicin (DOX) as a kind of widely used anticancer drug can then be encapsulated into the core region of this micelle.

2 Experimental Sections

2.1 Materials. 4-dimethylamiopryidine(DMAP), dicyclohexylcarbodiimide (DCC), 1,6-diphenyl-1,3,5-hexatriene(DPH) and Doxorubicin (DOX) were purchased from Aldrich used as received. L-Lactide(L-LA) was purchased from Aldrich and It was recrystallized from toluene and dried in vacuo overnight. 5,10,15,20-Tetraphenylporphyrin ($TPPH_2$) were purchased from Shanghai Dibai Chemical Technology Co., Ltd. Tetrahydroxyethyl terminated porphyrin was synthesized from tetrakis(4-hydroxylphenyl)-21H,23H-porphine and bromoethanol according to our previous publication(50 % yield).[29] ^{1}H NMR (DMSO, ppm): 3.89 (t, 8H, -CH_2-OH), 4.28 (t, 8H, -O-CH_2-CH_2-), 5.03(s, 4H, -OH), 7.38 (d, 8H, o-Ar-H),

8.12 (d, 8H, m-Ar-H), and 8.87(s, 8H, â-pyrrole-H). Carboxyl-Terminated Poly(ethylene oxide) (CMPEG) was synthesized from poly(ethylene glycol) methyl ether and succinic anhydride according to the literature procedure (90.0 % yield).[15] Poly(ethylene glycol) methyl ether purchased from Aldrich (Mn~2000, M_w/M_n = 1.06 ; Mn ~5000, M_w/M_n=1.04) was dried at 50 ^oC in vacuo overnight, and its purity was 100 % within the error of 1H NMR measurement.^{1}H NMR ($CDCl_3$, ppm): 3.56 – 3.75 (-O-CH_2-CH_2-O-), 3.40 (CH_3-O-), 4.28 (-CH_2-OC-O-), 2.67 (-O-OC-CH_2-CH_2-CO-O-). Tetrahydrofuran (THF) were dried and distilled from Na and stored under a dry nitrogen atmosphere. N,N-Dimethylformamide (DMF) and dichloromethane (CH_2Cl_2) were dried and distilled from CaH_2 and stored under a dry nitrogen atmosphere. All other reagents and solvents were used without further purification.

2.2 Synthesis of Star-shaped Porphyrin-Cored Poly(L-LA) (SPPLA). According to our previous publication,[3031] the polymerization tubes were kept at 50 ℃ for 24 h. and a dry stirring bar were put into the warm tube quickly. A certain amount of porphyrin core initiator, DMAP, L-LA was added to a tube followed by connected to a Schlenk line, where an exhausting-refilling process was made then degassed THF was added. The tube was quickly put into an oil bath at 50 ℃ and vigorous stirred for about 24h. The obtained product was dissolved in dichloromethane and poured dropwise into an excess of methanol under vigorous stirring, and then the precipitate was filtered. The purified SPPLA polymer was dried in vacuo until a constant weight was obtained, and the polymer yields were determined gravimetrically. A typical procedure is as follow: the porphyrin core

initiator (8.5 mg，0.01 mmol) , L-LA monomer (116 mg，0.8 mmol) and the catalyst DMAP (9.8 mg 0.08 mmol) was added to a tube. Polymerization was carried out in THF at 50 ℃ for 24 h. Then, the resulting product was dissolved in 5 mL of dichloromethane and poured dropwise into 50 mL of cold methanol under vigorous stirring at room temperature. The precipitate was filtered and dried in vacuo at 40 ℃ to give 95.0 mg of the SPPLA sample (81.9 % yield; 94.1% monomer conversion).

2.3 Synthesis of Star-shaped Porphyrin-Cored poly(L-lactide)-b- poly(ethylene glycol)

A typical example is given below: SPPLA（117.00 mg，0.01 mmol）, CMPEG (M_w=5000, 217.6 mg，0.0435 mmol)，DCC（16.51 mg，0.08 mmol）, DMAP（4.89 mg，0.04 mmol）and 3 mL CH_2Cl_2 was added to a tube and quickly put into an oil bath at 25℃ with vigorous stirring for about 24 h. After cooling, 1 mL acetone was added into the crude product and the 1,3-dicyclohexylurea (DCU) precipitate was removed by filtration. The solvent was removed under vacuum, then 3 mL CH_2Cl_2 was added in order to extract the cooled polymer from the flask. Under stirring, the solution was added dropwise into 30 mL diethyl ether anhydrous. The obtained SPPLA-b-PEG was purified by solvent extraction with diethyl ether/benzene (2:1 v/v) as a cosolvent, and then methanol (50.0 mL) was used to completely extract unreacted CMPEG. The powder was dried in vacuo at 40 ℃ to give 110.5 mg of SPPLA-b-PEG (86.4% yield).

^{1}H NMR spectra were recorded at room temperature on a Varian Mercury-400 spectrometer. $CDCl_3$, DMSO-d_6, and D_2O were used as the deuterated solvents for the

SPPLA precursors and SPPLA-b-PEG block copolymers.

The molecular weight and molecular weight distribution of polymer were determined on GPC (Perkin-Elmer Series 200) and a refractive index detector at 30 ℃, The elution phase was DMF (elution rate: 1.0 mL/min), and polystyrene used as the calibration standard.

Powder X-ray diffraction (XRD) patterns were obtained on a D/MAX-2500 diffract meter (Rigaku, Japan) using Cu Ka radiation source (k = 1.54056 Å) at a scan rate of 10^{o} / min to determine then crystal phase of the obtained samples. The accelerating voltage and the applied current were 50 kV and 300 mA, respectively.

Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet FT-IR spectrophotometer (Nexus 470, Thermo Electron Corporation) at frequencies ranging from 400 to 4000 cm^{-1}. Samples were thoroughly mixed with KBr and pressed into pellet form.

2.4 Preparation of Aggregates in Water. SPPLA-b-PEG copolymer was dissolved in DMF (1 mg / mL), followed by added distilled water dropwise using a microsyringe. After stirring for 24 h dialyzing against distilled water was used for 3 days to guarantee DMF was completely removed. The morphology of aggregates was determined by TEM.

TEM micrographs were taken with a JEOL-JEM-2010 (JEOL, Japan) operated at 200 Kv. One drop of aggregates solution was deposited onto the surface of 300 mesh Formvar-carbon film-coated copper grids. Excess solution was quickly wicked away with a filter paper. The image contrast was enhanced by negative staining with phosphotungstic acid (0.5 wt. %)

2.5 Measurement of the Critical Aggregation Concentration of SPPLA-b-PEG.

Critical aggregation concentration (cac) of amphiphilic SPPLA-b-PEG copolymers was measured taking the hydrophobic dye solubilization method using DPH as a probe molecule.[32, 33] DPH was dissolved in methanol to produce 0.05 mM DPH methanol solution then 50 μL of DPH solutions were shift to containers (5mL), and the methanol was evaporated. Each aqueous sample solutions with various polymer concentrations from 10^{-5} mg/mL to 0.5 mg/mL contained the same concentration of excess DPH residue were obtained. At room temperature, UV-vis spectra of samples were recorded at 313 nm range.

2.6 Determination of the fluorescence quantum yields

[12, 34] Fluorescence quantum yields of SPPLA-b-PEG_{2000} and SPPLA-b-PEG_{5000} were obtained from the formula (1) and formula (2) and $TPPH_2$ was used as the standard (fluorescence quantum yield Φ= 0.11).[12, 34] The concentration of $TPPH_2$, SPPLA-b-PEG_{2000} and SPPLA-b-PEG_{5000} were 1.63 μmol • L^{-1}.

$$\Phi = 0.11 \frac{A_{TPPH2}}{S_{TPPH2}} \frac{S_{SPPLA-b-PEG}}{A_{SPPLA-b-PEG}} \quad (1) \qquad S = \int_{\lambda_{600nm}}^{\lambda_{800nm}} I_{f\,\lambda}\, d\lambda \quad (2)$$

A_{TPPH2}: $TPPH_2$ in absorbance at 425 nm; S_{TPPH2}: reference sample $TPPH_2$ integral area of the fluorescence emission spectra from 600 nm to 800 nm (E_X = 425 nm) ; $A_{SPPLA\text{-}b\text{-}PEG}$: SPPLA-b-PEG in absorbance at 425 nm absorbance; $S_{SPPLA-b-PEG}$: fluorescence emission spectra of integral area from 600nm to 800nm (E_X = 425 nm) . $I_{f\,\lambda}$: fluorescence emission curve of $TPPH_2$ or SPPLA-b-PEG .

Fluorescence spectra were performed at room temperature using a luminescence spectrometer (CaryEclipse, AUS). Fluorescence quantum yields test were performed at room temperature in the range of 600–800 nm using increment of 5 nm, and an

excitation wavelength of 425 nm, while singlet oxygen detection was performed at room temperature in the range of 425–600 nm using increment of 5 nm, and an excitation wavelength of 403 nm.

2.7 Detecting Singlet Oxygen ($^1O_2$) production.

In our study, 1,3-diphenylisobenzofuran (DPBF) as a trapping agent was proposed for detecting $^1O_2$ generated by SPPLA-b-PEG_{5000} photosensitizers ($TPPH_2$ as a standard regent) [10, 35, 36] DPBF reacts irreversibly with $^1O_2$ that lead to a decrease in the intensity of the DPBF absorption band at 456 nm. In a typical experiment, 150 µL $TPPH_2$ (0.147mM) or SPPLA-b-PEG (0.147mM) was mixed with 3mL of DPBF (2.5µM) in DMF respectively. Then, the samples were illuminated with a 650 nm laser source (5mw) and their absorbencies at 456 nm were recorded every 1 min in a luminescence spectrometer. The singlet oxygen quantum yield *(η)* of SPPLA-b-PEG_{5000} in DMF was calculated using following equation:[37, 38]

$$\eta = \Phi_{TPPH2} \frac{t_{SPPLA-b-PEG}}{t_{TPPH_2}} \quad (3)$$

t_{TPPH2} is the time for decrease in absorption of DPBF in the presence of $TPPH_2$ free in DMF solution adjusted to a first-order exponetial decay; $t_{SPPLA\text{-}b\text{-}PEG}$ is the time for decrease in absorption of DPBF in the presence of SPPLA-b-PEG in DMF adjusted to a first-order exponetial decay. The $^1O2$ yield of $TPPH_2$ free in DMF solution is 0.67 ± 0.09.

2.8 Preparation of DOX-Loaded Nanoparticles in Aqueous Solution

By means of a dialysis technique,[21] SPPLA-b-PEG_{5000} (5 mg) and DOX

hydrochloride (1mg, 1.7μmoL) were dissolved in 6.5 mL of DMF, in which 2.6μmoL triethyl amine (Et_3N) (2.6μmoL) was added to neutralize HCl in solution. 5mL distilled water was injected by a microsyringe to form nanoparticles. The obtained nanoparticles solution was then added into a dialyzing membrane and subjected to dialysis against distilled water for 24h. Then, the DOX-loaded nanoparticles solution was lyophilized and stored at 4℃. The DOX-loaded nanoparticles (1mg) was dissolved in 5mL of DMF and The loading efficiency of DOX in SPPLA-b-PEG was determined at an absorbance of 500 nm by UV-vis. Loading efficiency (L.E.) (%) = (weight of DOX in the SPPLA-b-PEG /weight of the feeding DOX) × 100%. Drug loading capacity (L.C.) (%) = (weight of DOX in the SPPLA-b-PEG /weight of the Nanoparticles) × 100%.The calibration curve of DOX in aqueous solution is y (abs) = 0.00116 + 19.5888x (C: mg/mL).

The (L.E.) (%) was found to be 27.7% and the (L.C.) (%) was 5.54%.

2.9 In Vitro DOX Release Studies of DOX-Loaded Nanoparticles

The lyophilized DOX-loaded nanoparticles (1.5 mg) were added into 1 mL of buffer solution (pH = 7.4 or 5.5) followed by put into a dialyzing membrane. The dialyzing membrane was put into 30 mL of buffer solution container at 37 ℃. The DOX-released solution was extracted 3mLperiodically (3, 6, 9, 21, 33, 45, 57, 81, 105 h) followed by added 3mL buffer solution, and by using UV–vis at 500 nm, the quantity of DOX released from nanoparticles was measured at room temperature.

3 Results and Discussion

3.1 Synthesis of SPPLA-b-PEG

In our study, firstly, initiated with tetrahydroxyethyl terminated porphyrin and used DMAP as the catalyst to prepare SPPLA polymers by the ROP of L-LA monomer in THF at 50 ℃. Then, using this SPPLA polymers reacted with CMPEG and two amphiphilic porphrin-cored SPPLA-b-PEG_{2000} and SPPLA-b-PEG_{5000} were obtained which have been summarized (Table 1). Notably, the unimodal elution peak of the purified block copolymer is apparently shifted toward a higher molecular weight region coupled with a narrow polydispersity in comparison with that of the original SPPLA. In addition, the number-average molecular weight of the as synthesized polymers SPPLA-b-PEG determined by GPC ($M_{n,GPC}$) were in well control when reacted with different molecular weight of CMPEG (Figure 1). Moreover, the polymer molecular weight determined by ^{1}H NMR ($M_{n,NMR}$) is reasonably consistent with the theoretical molecular weight of polymer ($M_{n,th}$), where $M_{n,th}$= [M] / [I] × $M_{monomer}$ × yield + $M_{initiator}$. Furthermore, both ^{1}H NMR and ^{13}C NMR of the purified block copolymers confirmed that each arm of the SPPLA precursor was successfully coupled with CMPEG.

The ^{1}H NMR spectrum of SPPLA-b-PEG_{5000} polymer is shown (Figure 2). 8.75-8.88 ppm （δH^8），8.15-8.25 ppm（δH^9）and 7.55-7.60 ppm（δH^{10}） are assigned to porphyrin core initiator benzene ring or pyrrole ring. Signals at 1.30 – 1.77（δH^1）and 5.07– 5.37 ppm（δH^2）are belong to poly(L-lactide) and there are typical proton signals of poly(ethylene glycol) at 3.50 – 4.00（δH^4）. Moreover, ^{13}C NMR spectra represented that the end-group peaks of the SPPLA precursor at 21.2ppm（$\delta H^{1'}$）, 67.3ppm （$\delta H^{2'}$）and 174.8ppm （$\delta H^{3'}$）completely disappeared in the related

SPPLA-b-PEG copolymer, and this phenomenon confirmed that all the hydroxyl groups in SPPLA were completely reacted with CMPEG within the limit of ^{13}C NMR measurements (Figure 3). In all, these results showed that porphyrin-cored SPPLA-b-PEG copolymers can be easily obtained by a two-step synthetic strategy (Scheme 1).

FT-IR is another characterization to prove the presence of both poly(L-lactide) and poly(ethylene glycol) components in purified block copolymers (Figure S1). The purified SPPLA-b-PEG block copolymers show the distinct carbonyl stretching bands at 1750 cm^{-1} for the SPPLA block, the intense at 2870 cm^{-1} was attributed to bands of methylene for the PEG block. Besides, SPPLA-b-PEG with a relatively short PEG_{2000} arm units show weak bands at 2870 cm^{-1} for the PEG block in addition to the strong bands for the SPPLA block.

XRD is also a very effective method to demonstrate the structure of the block copolymer SPPLA-b-PEG in the solid state. Purified poly(L-lactide) and poly(ethylene glycol) showed markedly diffraction peaks at about 16.5°, 19.0° and 23.2°, which are characteristic of poly(L-lactide) and poly(ethylene glycol) crystals, respectively.[15, 39] The diffraction peaks of both SPPLA and PEG blocks were approximately presented for SPPLA-b-PEG copolymers (Figure 4). Additionally, the relative signal intensity of the PEG block with in the copolymer progressively enhanced with an increasing molecular weight from Mn =2000 to Mn =5000 induced by the relatively increasing arm length ratio of poly(ethylene glycol) to poly(L-lactide). Notably, when physically mixed PLA with PEG, the signal of PLA

maintain stable compared with pure PLA. However, the relative signal intensity of PLA block within star-shaped SPPLA-b-PEG copolymer was dramatically decreased resulting in weak crystallinity. Therefore, the synthetic SPPLA with hydrophilic PEG will give a decreased crystallinity PLA-based biomaterials which could be used for drug release.facile method for improving the physical, biodegradation, biocompatibility, and drug release characteristics of PLA-based biomaterials.[40]

3.2 UV-vis Analyses.

The obtained SPPLA-b-PEG copolymers were further characterized using UV-vis spectroscopy (Figure 5). As the same as porphyrin, in the UV-vis, the Soret (435 nm) and Q bands (500-700 nm) SPPLA-b-PEG_{5000} were showed that are belonging to porphyrin. This proves that porphyrin moiety still retained the luminescent property within SPPLA-b-PEG. Thus, this will potentially enable SPPLA-b-PEG for the biological probe and PDT applications.[41, 42]

3.3 Self-Assembly Properties of Star-shaped Porphyrin-Cored SPPLA-b-PEG Block Copolymers

Then, the dye solubilizing method was used to examine SPPLA-b-PEG copolymers' critical aggregation concentration (cac),[33] The cac was an important parameter for the thermodynamic stability of self-assembled aggregates in aqueous solution. In our experiment, DPH was employed as a probe regentand the relationship of the absorbance intensity of DPH as a function of copolymer concentration at room temperature is shown. The absorbance intensity values of DPH keep consistent below a certain concentration (Figure 6). Above that concentration, the absorbance intensity

increased dramatically indicating that the DPH was incorporated into the hydrophobic region of aggregates. When the block length of PEG increased from MW = 2000 to MW = 5000, the cac value of SPPLA-b-PEG copolymers slightly increased from 0.0234 mg/mL to 0.0365 mg/mL which has a similar conclusion to other reports about amphiphilic copolymers.[43]

TEM was employed to study the morphology of the self-assembled aggregates from these two SPPLA-b-PEG copolymers (Figure 7). For investigating influence of PEG block length and / or the weight fraction (f) of PEG on the morphology of aggregates, the hydrophobic PLA block was kept at 20 repeating unit. In Figure 7A, with a relatively long hydrophilic PEG (e.g., SPPLA-b-$PEG_{5000,}$ f_{PEG} = 62.0 %), it can be seen that the normally spherical micelles were observed and the average diameter is around 22 nm. In case of the short PEG block bilayer forming copolymer aggregates, smaller spherical micelles about 18nm were obtained for SPPLA-b-PEG_{2000} block copolymer [f_{PEG} = 38.6 %] (Figure 7B). It can conclude that different aggregates morphologies can be formed through adjusting PEG weight fraction with in the copolymers, which has a very similar conclusion on the polymeric aggregates morphological transformation, as reported for biodegradable poly(L-LA) – and / or PCL-b-poly(ethylene oxide) copolymers.[44, 45] Significantly, it could provded a method to fabricate targeted drug delivery systems.

3.4 Fluorescence Quantum Yields

Because of π –π interactions and lipophilic nature, conventional Photosensitizer (PSs) tend to aggregate resulting in inferior bioavailability and decreased capacity to absorb

light which greatly affects their efficacy in vivo, that is, low tendency to aggregation is substantial for the photodynamic agents.[45] To overcome this drawback, method used to deliver porphyrin derivatives such as amphiphilic polymer-PS conjugates, liposomes, and hydrophilic polymer have been reported.[3, 41, 46]Herein, the fluorescence quantum yield of SPPLA-b-PEG was investigated. Based on formula (1) and formula (2), the fluorescence quantum yields of SPPLA-b-PEG_{5000} was 0.24 and SPPLA-b-PEG_{2000} was 0.20 ， which prove that this porphyrin core star-shaped block copolymers possessing high fluorescence quantum yields. Porphyrin derivatives have been demonstrated to influence the chemistry and photophysics of molecules by altering the microenvironment in which the molecules reside, so the reason could be attributed to the fact that porphyrin as the core being surrounded by the armed copolymer resulting in high steric hindrance which can prevent self-aggregation and self-quenching of the central porphyrin.[34, 47]

3.5 Singlet oxygen ($^1O_2$) production of SPPLA -b-PEG plus irradiation

$^1O_2$ is the prerequisite molecule in the PDT. DPBF, which was used as a chemically photosensitizer in this study to detect the production of singlet oxygen, reacts irreversibly with $^1O_2$ resulting into a decreased intensity of the DPBF fluorescence absorption band at ～400nm. The decrease in fluorescence intensity at 456 nm as a function of irradiation time was shown (Figure 8). According to the reported singlet oxygen generation estimation method, the singlet oxygen release delivery (η) by a suspension of porphyrin-cored SPPLA-b-PEG_{5000} is estimated to be 0.239,[38] while the photofrin with singlet oxygen quantum yield less than 0.3 as a sensitizer has been

used as a PDT agent in clinical trial.[48] This result suggests that the SPPLA-b-PEG_{5000} presented here is an interesting candidate as PDT agents that need much more clinical study to prop up this standpoint. Moreover, in the case of free porphyrin $TPPH_2$, the fluorescence intensity of DPBF caused by $TPPH_2$ dropped sharply to 20 percent in two minutes which proves a large number of singlet oxygen produced during this period, however, the fluorescence intensity of DPBF declined slowly when mixed with SPPLA-b-PEG, In other words, the singlet oxygen production ability of SPPLA-b-PEG can be well controlled by irradiation time, which is an interesting feature for PDT use.[49, 50]

3.6 In Vitro Drug Release

Doxorubicin, which has been widely used As a most common anticancer drug in anticancer clinical application,[51] could be used as a hydrophobic model drug since hydrophobic interactions with prophyrin-SPPLA segment could physically entrap and stabilize the DOX in the hydrophobic inner core of the micelle. To mimic the physiological and endosome/lysosome microenvironments, the experiment was made at aqueous pH 7.5 and 5.5 respectively to investigate the DOX-loaded nanoparticles release beavering (Figure 9). We can see from this figure that it shows a very different drug release result between the pH 7.5 and pH 5.5. The DOX release from the micelle at pH 5.5 was much faster and the reason could be attributed to protonation at lower pH. Therefore, it is particular interesting to apply this technique for tumor-targeted drug delivery since the polymeric micelles possess pH-dependent releasing behavior. In this study, it could be anticipated that DOX release will happen after micelles are

internalized in cancer tissues via the endocytosis pathway and entrapped in the acidic endosome/lysosome compartments. Consequently, this porphyrin-cored SPPLA-b-PEG copolymers improve targeting to pH 5.5 tumor tissues for drug delivery.

4 Conclusions

In this study, star-shaped porphyrin-core SPPLA-b-PEG amphiphilic copolymer were well synthesized and thoroughly characterized. Moreover, these amphiphilic copolymers could self-assemble to form micelles used for photosensitizing agents and further encapsulate hydrophobic drugs such as DOX. Furthermore, Such SPPLA-b-PEG copolymer exhibits efficient singlet oxygen generation and indicates high fluorescence quantum yields. In addition, our results proved that the drug release showed pH induced profile. This work focusing on the prophyrin-cored star-shaped SPPLA-b-PEG copolymer provides a high singlet oxygen production material and could be used for chemotherapy drug delivery system.

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1. W. M. Sharman, C. M. Allen and J. E. van Lier, *Drug discovery today*, 1999, **4**, 507-517.
2. Z. Huang, H. Xu, A. D. Meyers, A. I. Musani, L. Wang, R. Tagg, A. B. Barqawi and Y. K. Chen, *Technology in cancer research & treatment*, 2008, **7**, 309.
3. X. Liang, X. Li, X. Yue and Z. Dai, *Angewandte Chemie*, 2011, **123**, 11826-11831.
4. J. Hollingsworth, Louisiana State University2012.
5. J. F. Lovell, T. W. Liu, J. Chen and G. Zheng, *Chemical reviews*, 2010, **110**, 2839-2857.
6. B. C. Wilson and M. S. Patterson, *Physics in medicine and biology*, 2008, **53**, R61.
7. R. Dosselli, C. Tampieri, R. n. Ruiz-González, S. De Munari, X. Ragàs, D. Sánchez-García, M. Agut, S. Nonell, E. Reddi and M. Gobbo, *Journal of medicinal chemistry*, 2013, **56**, 1052-1063.
8. S.-T. Lin, K. Fuchise, Y. Chen, R. Sakai, T. Satoh, T. Kakuchi and W.-C. Chen, *Soft Matter*, 2009, **5**, 3761-3770.
9. J. Choi, C. R. Ruiz and E. E. Nesterov, *Macromolecules*, 2010, **43**, 1964-1974.
10. C.-L. Peng, M.-J. Shieh, M.-H. Tsai, C.-C. Chang and P.-S. Lai, *Biomaterials*, 2008, **29**, 3599-3608.
11. R. Teixeira, S. M. Andrade, V. Vaz Serra, P. M. Paulo, A. Sánchez-Coronilla, M. G. Neves, J. A. Cavaleiro and S. M. Costa, *The Journal of Physical Chemistry B*, 2012, **116**, 2396-2404.
12. T. Ren, A. Wang, W. Yuan, L. Li and Y. Feng, *Journal of Polymer Science Part A: Polymer Chemistry*, 2011, **49**, 2303-2313.
13. Y. Takeuchi, K. Ichikawa, S. Yonezawa, K. Kurohane, T. Koishi, M. Nango, Y. Namba and N. Oku, *Journal of controlled release*, 2004, **97**, 231-240.
14. D. Bechet, P. Couleaud, C. Frochot, M.-L. Viriot, F. Guillemin and M. Barberi-Heyob, *Trends in biotechnology*, 2008, **26**, 612-621.
15. C. Cai, L. Wang and C. M. Dong, *Journal of Polymer Science Part A: Polymer Chemistry*, 2006, **44**, 2034-2044.
16. X.-H. Dai, C.-M. Dong and D. Yan, *The Journal of Physical Chemistry B*, 2008, **112**, 3644-3652.
17. A. Bertin, *Macromolecular Chemistry and Physics*, 2012, **213**, 2329-2352.
18. L. Xiang, L. J. Shen, F. Long, K. Yang, J. B. Fan, Y. J. Li, J. Xiang and M. Q. Zhu, *Macromolecular Chemistry and Physics*, 2011, **212**, 563-573.
19. A. J. Lasprilla, G. A. Martinez, B. H. Lunelli and A. L. Jardini, *Biotechnology advances*, 2012, **30**, 321-328.
20. J. Lee, Y. Kim and Y. Do, *Inorganic chemistry*, 2007, **46**, 7701-7703.
21. L. Sun, L. J. Shen, M. Q. Zhu, C. M. Dong and Y. Wei, *Journal of Polymer Science Part A: Polymer Chemistry*, 2010, **48**, 4583-4593.
22. G. N. Tew and S. R. Bhatia, in *Engineering Biomaterials for Regenerative Medicine*, Springer2012, pp. 127-140.
23. I. V. Dimitrov, I. V. Berlinova and V. I. Michailova, *Polymer Journal*, 2012,45, 457-461
24. E. S. Lee, K. T. Oh, D. Kim, Y. S. Youn and Y. H. Bae, *Journal of Controlled Release*, 2007, **123**, 19-26.
25. C. Hiemstra, Z. Zhong, L. Li, P. J. Dijkstra and J. Feijen, *Biomacromolecules*, 2006, **7**, 2790-2795.
26. L. Yu, Z. Zhang and J. Ding, *Biomacromolecules*, 2011, **12**, 1290-1297.
27. S. Hecht, N. Vladimirov and J. M. Frechet, *Journal of the American Chemical Society*, 2001,

123, 18-25.
28. C.-Y. Hsu, M.-P. Nieh and P.-S. Lai, *Chemical Communications*, 2012, **48**, 9343-9345.
29. X.-H. Dai, C.-M. Dong, H.-B. Fa, D. Yan and Y. Wei, *Biomacromolecules*, 2006, **7**, 3527-3533.
30. X. H. Dai, W. Liu, Y. F. Huang and C. M. Dong, *Advanced Materials Research*, 2011, **239**, 1703-1706.
31. Dai, X.-H.; Dong, C.-M.; Fa, H.-B.; Yan, D.; Wei, Y., Biomacromolecules, 2006, 7, (12), 3527-3533.
32. X. H. Dai and C. M. Dong, *Journal of Polymer Science Part A: Polymer Chemistry*, 2008, **46**, 817-829.
33. S. Y. Park, B. R. Han, K. M. Na, D. K. Han and S. C. Kim, *Macromolecules*, 2003, **36**, 4115-4124.
34. Z. Y. Li, H. Y. Wang, C. Li, X. L. Zhang, X. J. Wu, S. Y. Qin, X. Z. Zhang and R. X. Zhuo, *Journal of Polymer Science Part A: Polymer Chemistry*, 2011, **49**, 286-292.
35. S. Ye, M. Czuba, A. Romiszewska, J. Karolczak and A. Graczyk, *Optica Applicata*, 2002, **33**, 489-504.
36. W. Spiller, H. Kliesch, D. Woehrle, S. Hackbarth, B. Roeder and G. Schnurpfeil, *Journal of Porphyrins and Phthalocyanines*, 1998, **2**, 145-158.
37. D. B. Tada, L. L. Vono, E. L. Duarte, R. Itri, P. K. Kiyohara, M. S. Baptista and L. M. Rossi, *Langmuir*, 2007, **23**, 8194-8199.
38. S. A. Gerhardt, J. W. Lewis, J. Z. Zhang, R. Bonnett and K. A. McManus, *Photochemical & Photobiological Sciences*, 2003, **2**, 934-938.
39. K. S. Kim, S. Chung, I. J. Chin, M. N. Kim and J. S. Yoon, *Journal of applied polymer science*, 1999, **72**, 341-348.
40. C. Hua and C. M. Dong, *Journal of Biomedical Materials Research Part A*, 2007, **82**, 689-700.
41. R. Ideta, F. Tasaka, W.-D. Jang, N. Nishiyama, G.-D. Zhang, A. Harada, Y. Yanagi, Y. Tamaki, T. Aida and K. Kataoka, *Nano letters*, 2005, **5**, 2426-2431.
42. H. Gu, K. Xu, Z. Yang, C. K. Chang and B. Xu, *Chemical communications*, 2005, 4270-4272.
43. D. E. Discher and F. Ahmed, *Annu. Rev. Biomed. Eng.*, 2006, **8**, 323-341.
44. H. Feng and C.-M. Dong, *Biomacromolecules*, 2006, **7**, 3069-3075.
45. R. Haag, *Angewandte Chemie International Edition*, 2004, **43**, 278-282.
46. A. S. Derycke and P. A. de Witte, *Advanced drug delivery reviews*, 2004, **56**, 17-30.
47. P. Sharma, S. Gandhi, A. Chopra, N. Sekar and C. Raman Suri, *Analytica chimica acta*, 2010, **676**, 87-92.
48. J. Moan, *Photochemistry and photobiology*, 1984, **39**, 445-449.
49. K.-H. Choi, K.-K. Wang, E. P. Shin, S.-L. Oh, J.-S. Jung, H.-K. Kim and Y.-R. Kim, *The Journal of Physical Chemistry C*, 2011, **115**, 3212-3219.
50. C. Ringot, V. Sol, M. Barrière, N. m. Saad, P. Bressollier, R. Granet, P. Couleaud, C. l. Frochot and P. Krausz, *Biomacromolecules*, 2011, **12**, 1716-1723.
51. E. R. Gillies and J. M. Fréchet, *Bioconjugate chemistry*, 2005, **16**, 361-368.

Legends

Scheme1. Synthesis of star-shaped porphyrin-cored SPPLA-b-PEG block copolymers.

Figure1. GPC traces of the SPPLA, SPPLA-b-PEG_{2000} and SPPLA-b-PEG_{5000} samples

Figure2. ^{1}H NMR spectra of SPPLA-b-PEG block copolymers in $CDCl_3$

Figure3. ^{13}C NMR spectra of SPPLA block copolymers in $CDCl_3$ (A) and ^{13}C NMR spectra of SPPLA-b-PEG_{5000} block copolymers in $CDCl_3$ (B)

Figure4. X-ray diffraction patterns of pure SPPLA, PEG, and the SPPLA-b-PEG copolymers

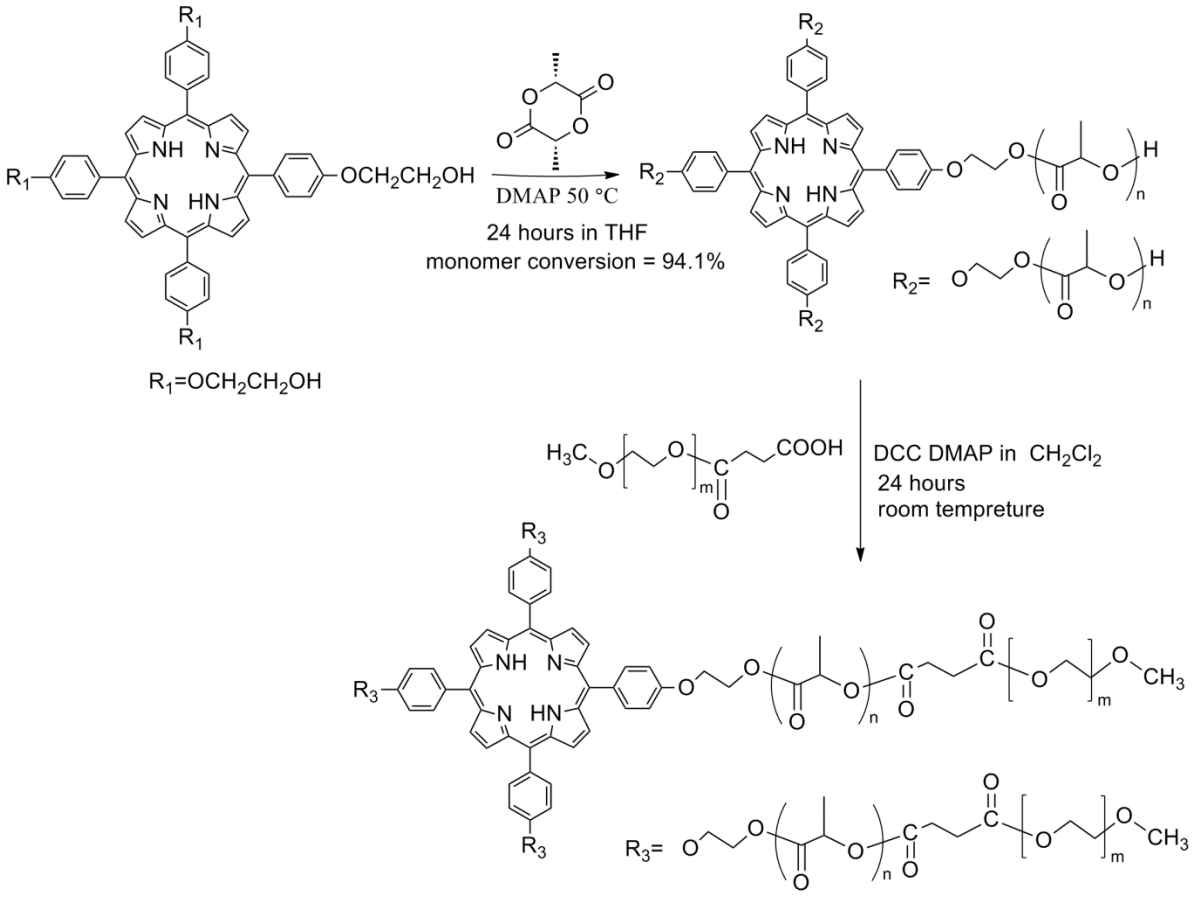
Figrue5. UV-vis spectra of $TPPH_2$ and SPPLA-b-PEG_{5000}

Figure6. The relationship of the absorbance intensity of DPH as a function of SPPLA-b-PEG block copolymer concentration at room temperature.

Figure7. TEM photographs of SPPLA-b-PEG block copolymers: SPPLA-b-PEG_{5000} (A), SPPLA-b-PEG_{2000} (B)： SPPLA-b-PEG_{5000} (A), SPPLA-b-PEG_{2000} (B)

Figure8. Fluorescence intensity (at 456nm) decay curves of DPBF with SPPLA-b-PEG_{5000} or $TPPH_2$ as function of time with a laser source (650 ±10 nm, 5 Mw)

Figure9. Drug release of DOX encapsulated micelle self-assembled from SPPLA-b-PEG_{5000} copolymer



Scheme1

Table1. Synthesis of star-shaped porphyrin-cored poly(L-lactide)-b- poly(ethylene glycol)

Entry	$M_{n,GPC}$[a]	$M_{n,th}$[b]	$M_{n,NMR}$[c]	M_w/M_n[a]	f_{PLA}/f_{PEG}[d] (%/%)	Yield (%)
SPPLA	13950	10270	11700	1.25	100 / 0	81.9
SPPLA-PEO_{2000}	21054	18070	20732	1.58	61.4 / 38.6	96.3
SPPLA-PEO_{5000}	33446	30070	32239	1.38	38.0 / 62.0	86.4

a:M_w/M_n denotes the molecular weight distribution of polymer, where weight-average molecular weight (M_w) and number-average molecular weight (M_n) are determined by GPC in DMF.

b:$M_{n,th}$ denotes the theoretical number-average molecular weight.

c:$M_{n,NMR}$ was determined from the integral ratio of the signal from the ^{1}H NMR spectra.

d:f_{PLA}/f_{PEO} denotes the weight fraction of PLA and/or PEO with in SPPLA-b-PEO copolymers, which was obtained from ^{1}H NMR;

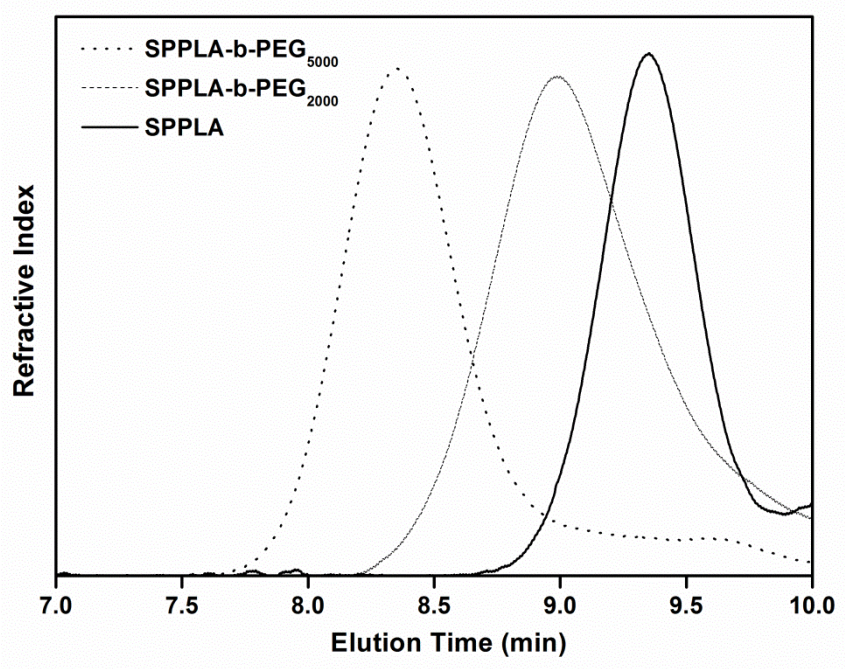


Figure1

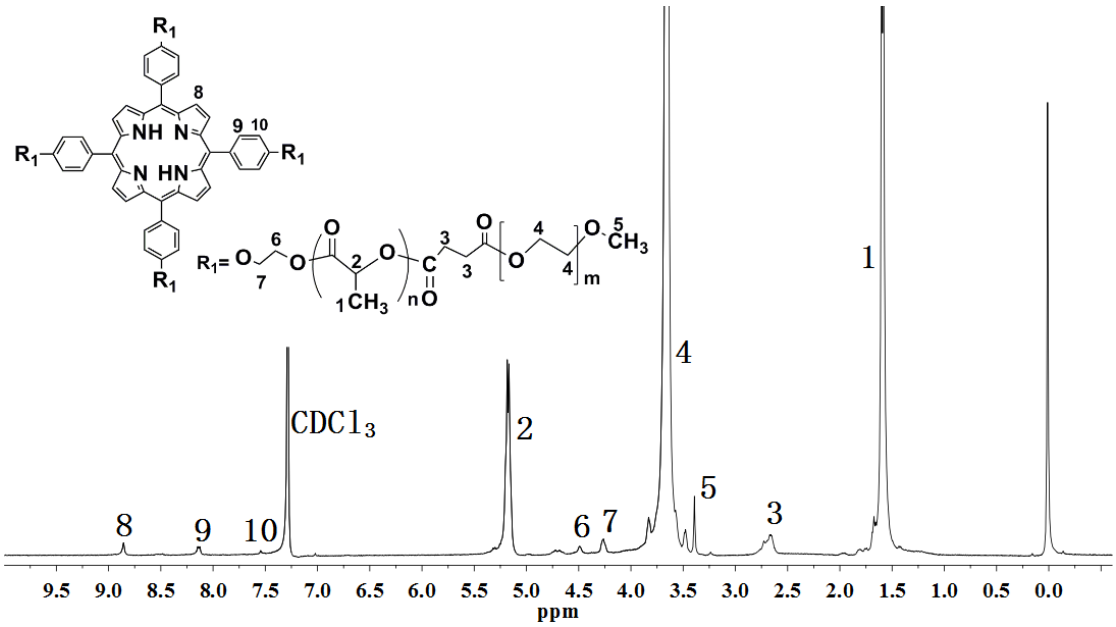


Figure2

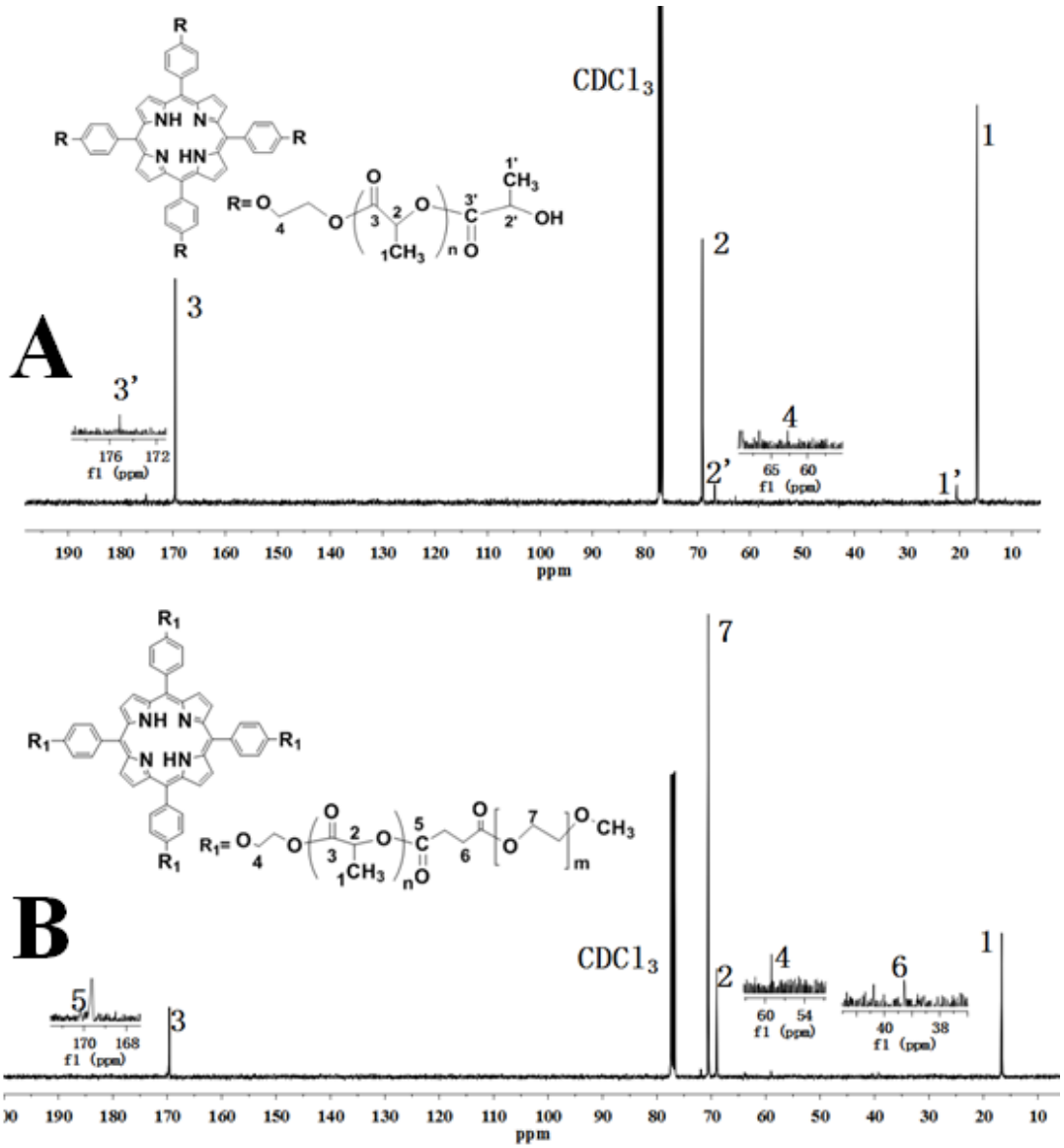


Figure3

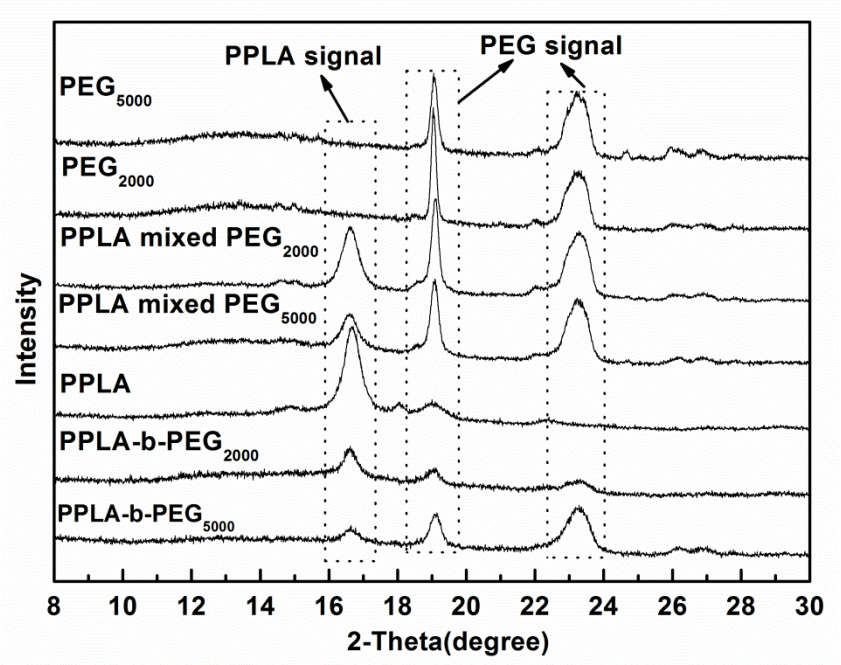


Figure 4

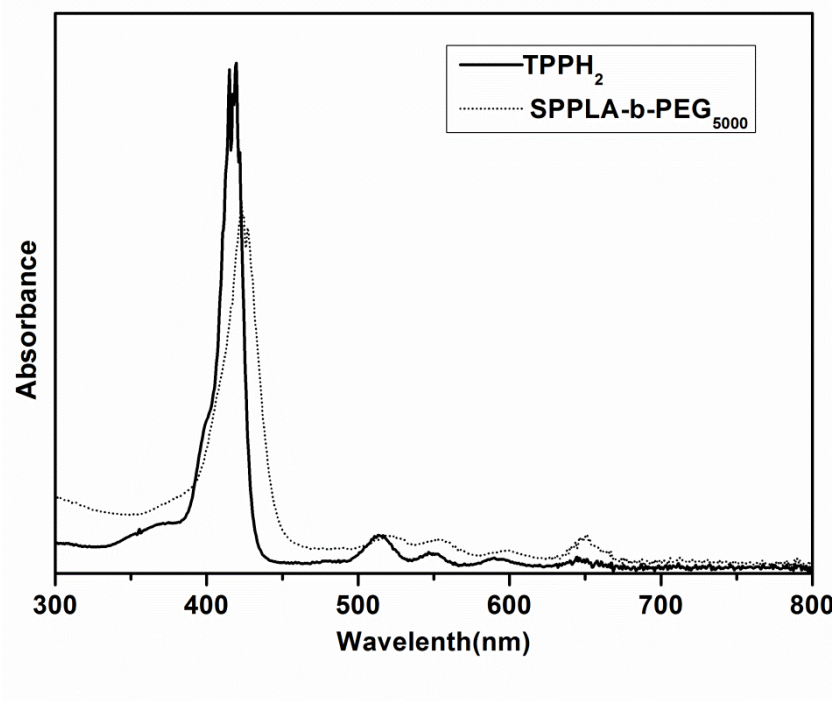


Figure 5

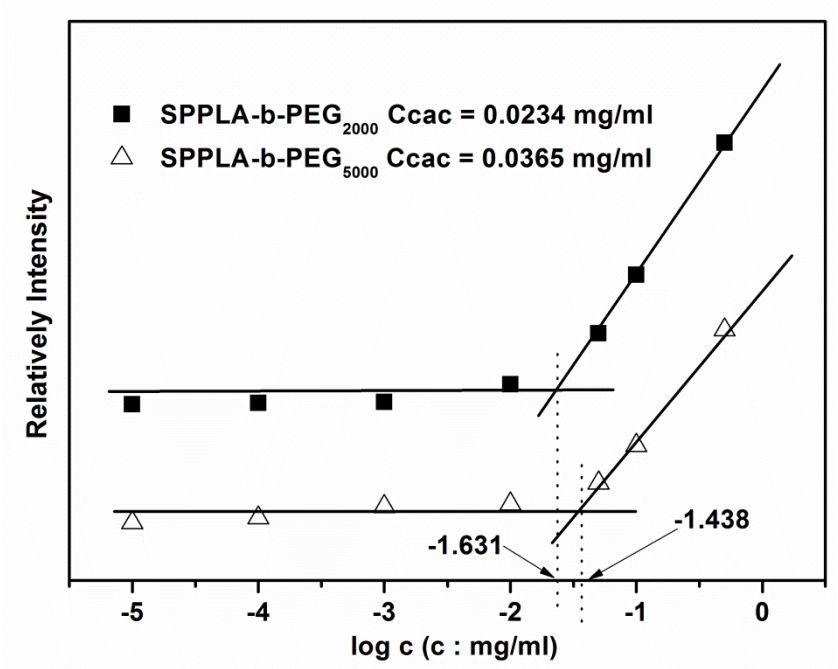


Figure 6

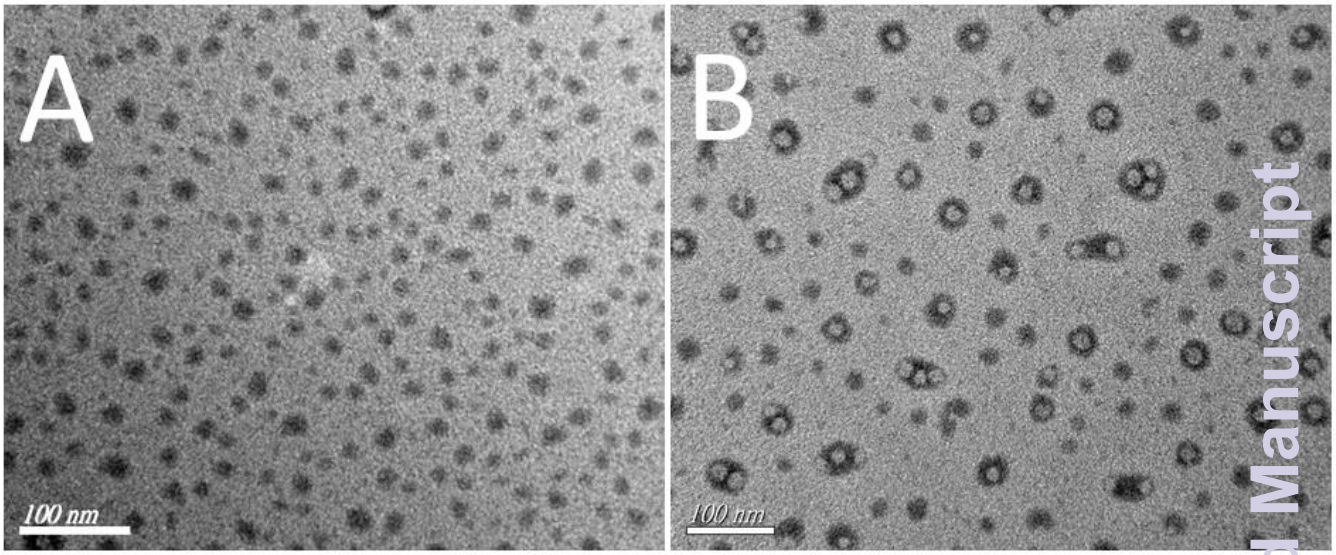


Figure 7

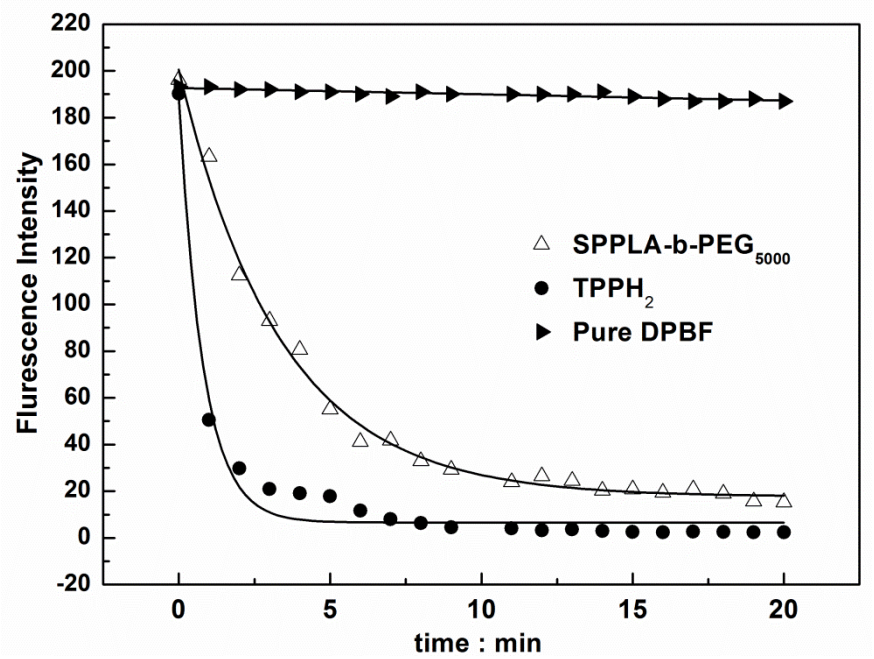


Figure 8

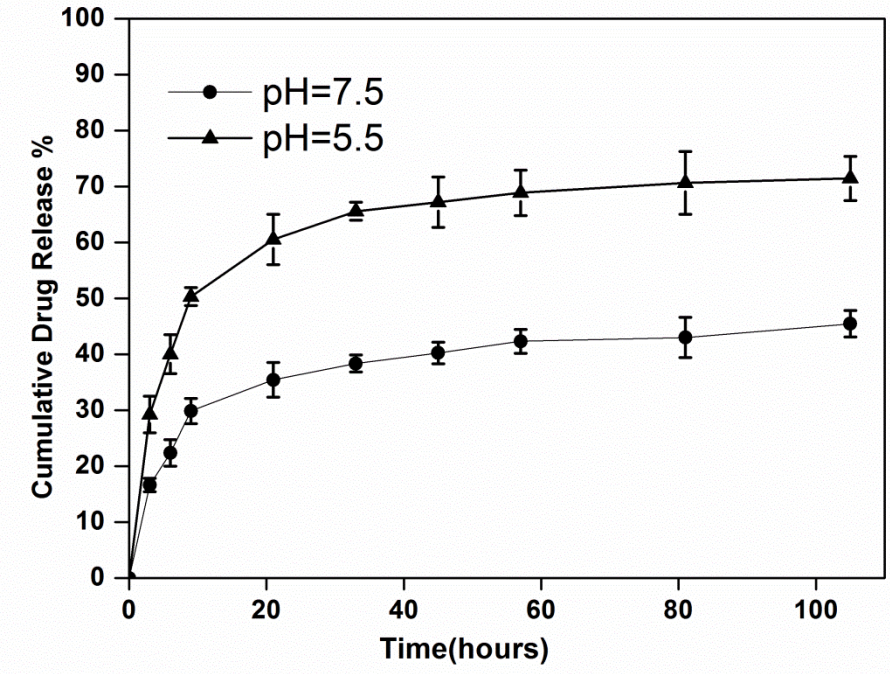
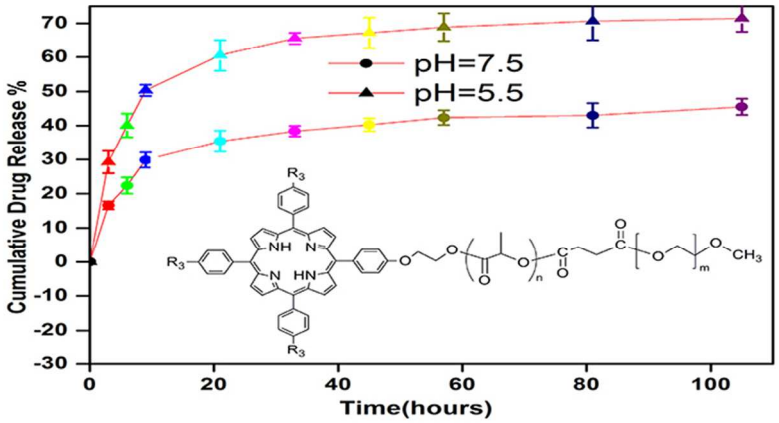


Figure 9



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