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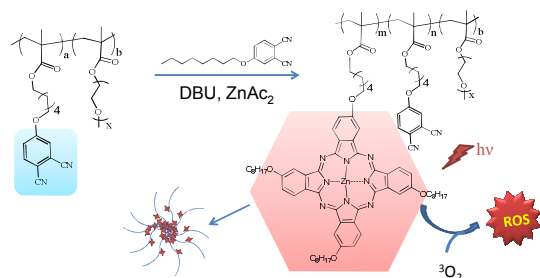
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## Graphical Abstract

### Novel Approach to Synthesize Polymers for Potential Photodynamic Therapy: from Benzendinitrile to Phthalocyanine

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RAFT synthesized polymers with benzendinitrile side chains were converted to phthalocyanine conveniently, providing a novel method of making PDT polymers.



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## ARTICLE TYPE

## Novel Approach to Synthesize Polymers for Potential Photodynamic Therapy: from Benzenedinitrile to Phthalocyanine

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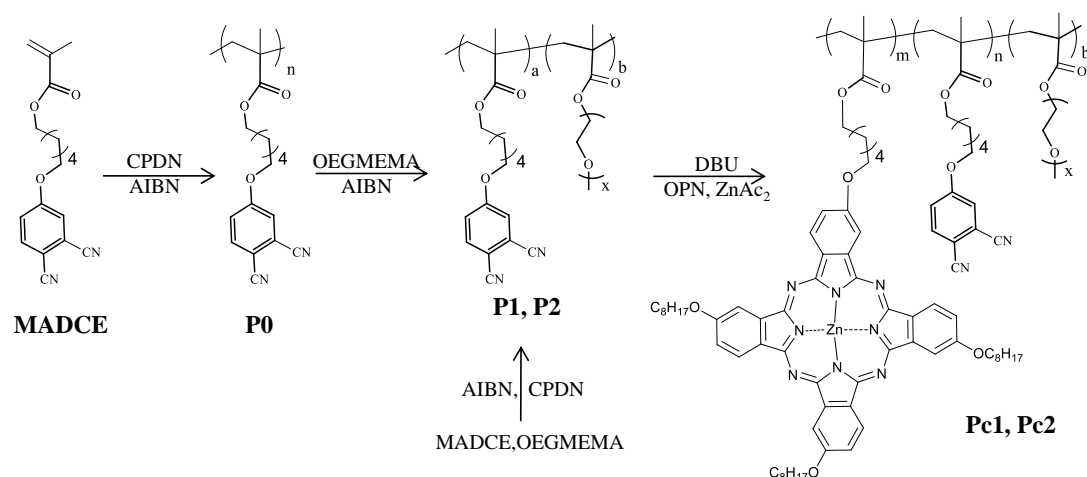
Benzenedinitrile, precursor for the synthesis of phthalocyanine, was incorporated into methacrylate monomer, 2-methyl-acrylic acid 6-(3,4-dicyano-phenoxy)-hexyl ester (MADCE). The benzenedinitrile monomer was homo-polymerized, chain-extended and copolymerized with oligo(ethylene glycol) methyl methacrylate (OEGMEMA) via the reversible addition-fragmentation chain transfer (RAFT) method. These homopolymer, block and random copolymers obtained were used to synthesize polymers with Zn(II) phthalocyanine (ZnPc) side-chains by reacting with excess benzenedinitrile derivatives, 4-(octyloxy) phthalonitrile (OPN). GPC, FTIR, UV-vis, NMR, fluorescence, atomic absorption spectroscopy were used to characterize the Zn(II) phthalocyanine-functionalized polymers. The ZnPc-functionalized polymers show good singlet oxygen quantum yields, which is believed to be the major cytotoxic reactive oxygen species (ROS) for photodynamic therapy. Among them, copolymers present enhanced singlet oxygen quantum yields ( $\Phi_{\Delta} = 0.49$  and  $0.40$  in DMF,  $\Phi_{\Delta} = 0.68$  and  $0.73$  in aqueous media) compared to the homopolymer ( $\Phi_{\Delta} = 0.28$ ). The ZnPc-functionalized copolymers self-assembled into nanoparticles in aqueous solutions, which were confirmed by DLS and TEM, and the nanoparticles show good stability in the presence of salt and protein. This novel and efficient method to synthesize water-soluble ZnPc-functionalized polymers with good singlet oxygen quantum yields provides simple approach to fabricate polymeric materials for photodynamic therapy.

## Introduction

Photosensitizing drugs have been known and applied in medicine for thousands of years. With the scientific progress in photobiology and cancer biology, as well as the development of modern photonic devices, such as lasers and (Light Emitting Diode) LEDs, photodynamic therapy (PDT) receives greater interests recently. PDT uses photosensitizer and light irradiation at certain wavelength onto target tissues.<sup>1-6</sup> Singlet oxygen generated through the photosensitization process is believed to be the major cytotoxic reactive oxygen species (ROS) responsible for the damage. However, these photosensitizers are far from ideal because of non-specific skin phototoxicity, poor water solubility, and inefficient delivery to target tumor tissues in cancer treatment. Incorporation of photosensitizers with hydrophilic polymers will be one good approach. Although physically loading photosensitizers into polymeric carriers is widely used, to link photosensitizers onto water-soluble polymers using chemical bond has seldom been reported.<sup>7-11</sup> The latter approach provides well-defined polymeric materials with precise photosensitizer content, and most importantly, avoids the unwanted drug release that is often happened in the case of physically loading. To increase its stability for *in vivo* applications, chemically attaching the photosensitizers to hydrophilic polymer backbone will be a good solution. Poly(ethylene glycol) (PEG) is an inexpensive, water-soluble and

biocompatible polymer and has been approved by Food and Drug Administration. Polymers with short oligo(ethylene glycol) side-chains have gained rapid success in fundamental polymer science and applied materials research.<sup>12-16</sup> PEGylated photosensitizers will not only increase the solubility but also the stability of the photosensitizers in the blood stream during cancer treatments.

Phthalocyanine (Pc)<sup>17-22</sup> is one of the most promising classes of second-generation photosensitizer, which has an absorption peak in the visible-near infrared region of 650-700 nm and allows the use of light with a high tissue penetration power. However the poor solubility and slippery  $\pi$ - $\pi$  stacks of Pcs limit their applications. Polymeric Pcs, either main-chain or side-chain type, which offers a unique combination of good properties of polymers and Pcs, have been paid much attention recently.<sup>23</sup> Among them, the side-chain polymeric Pcs/MtPcs were mainly prepared via polymerization of Pc/MtPc-containing monomers<sup>24-26</sup> or "grafting to" reactions.<sup>23, 27, 28</sup> However, the synthesis of Pc/MtPc containing monomers or asymmetric Pcs is relatively difficult due to the relatively low yield. Post-polymerization modification will be an attractive approach, allowing incorporation of functionality incompatible with the polymerization and greatly facilitates the establishment of structure-property relationships. Due to the difficulty in the synthesis and purification of Pc molecules and their poor solubility, instead of grafting Pc molecules onto preformed polymer, it will offer more advantages to synthesize Pc polymers



**Scheme 1** RAFT homo or block polymerization of MADCE and OEGMEMA, and formation of Zn(II) phthalocyanine on the side chain.

from well-defined polymers whose side-chain are intermediate molecules, which are starting reactants for the synthesis of Pc and are easier to synthesize and process.

As one of the most promising living radical polymerisation techniques, reversible addition-fragmentation chain transfer (RAFT) polymerization (RAFT) method has been successfully employed to prepare polymers with various functional groups due to its higher tolerance to many functional groups like allyl, amino, epoxy, hydroxy and vinyl groups present in the monomers.<sup>29-33</sup> Benzenedinitriles are starting molecules for the synthesis of phthalocyanines and easily dissolve in common organic solvents. We here synthesized novel copolymers with benzenedinitrile and oligo(ethylene glycol) side chains via RAFT polymerization. Excess benzenedinitrile derivatives were added to react with benzenedinitrile groups in the polymer chain for the synthesis of Zn(II) phthalocyanine-functionalized polymers. RAFT technique provides the possibility to tune the composition of the polymers, ZnPc groups are formed in-situ, which brings PDT effect and promotes the formation of micelles structure in aqueous solution for the polymers obtained. This provides a convenient approach for synthesizing PDT polymers.

## Experimental section

### Materials

All reagents were bought from Sigma-Aldrich, except *N,N*-dimethylacetamide (DMAc), acetonitrile, potassium carbonate, zinc acetate dihydrate and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (Sinopharm Chemical Reagent Co. Ltd., China). 2,2'-Azobis(isobutyronitrile) (AIBN) (Shanghai Chemical Reagent Co. Ltd., China, 99%) was recrystallized three times from ethanol. 2-Cyanoprop-2-yl- $\alpha$ -dithionaphthalate (CPDN) was prepared as reported previously.<sup>34</sup> Anthracene-9,10-bis-methylmalonate (ADMA, acid form) and Zn(II) Phthalocyanine tetrasulfonic acid (ZnPcS<sub>4</sub>) were obtained from Aldrich. All were used as purchased unless otherwise specified.

### Instruments and analysis

The molecular weights and molecular weight distributions of the polymers were measured on an Agilent PL-GPC 50 gel permeation chromatography (GPC) equipped with a refractive index detector, using a 5  $\mu$ m Guard, 5  $\mu$ m MIXED-D column with PMMA standard samples, and 0.05 mol·L<sup>-1</sup> lithium bromide solution in DMF was used as the eluent at a flow rate of 1 mL·min<sup>-1</sup> operated at 50 °C. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of the polymers were recorded on an INOVA 400 MHz nuclear magnetic resonance (NMR) instrument, using CDCl<sub>3</sub>, DMSO-*d*<sub>6</sub> or THF-*d*<sub>8</sub> as solvent. The content of N, H and O in the compounds was characterized by EA1110CHNO-S (Erba, Carlo). FT-IR spectra were recorded on a Nicolette-6700 FT-IR spectrometer. The fluorescence spectra of polymers in THF solution were recorded by HITACHI F-2500 fluorescence spectrophotometer. Atomic absorption spectrometry was detected by Varian Spectra 220FS atomic absorption spectrometer. Ultraviolet-visible (UV-Vis) absorption spectra of solutions in DMF were performed on a Shimadzu (Kyoto, Japan) UV-3600. The singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) of ZnPc-functionalized polymers in DMF were measured by the method of the rate of chemical quenching of DPBF using ZnPc as the reference ( $\Phi_{\Delta}$  = 0.56).<sup>35</sup> The light source consisted of a 75 W halogen lamp and a color glass filter cut-on 610 nm. The concentration of DPBF was lowered to  $\sim 8 \times 10^{-5}$  mol·L<sup>-1</sup>. The DPBF absorption decay was followed at 411 nm. The singlet oxygen quantum yields in aqueous media were measured using ADMA as quencher and ZnPcS<sub>4</sub> as reference. The size and intensity evolution analysis of polymers in aqueous solutions were performed using dynamic light scattering (DLS) measurements (Zetasizer Nano-ZS90; Malvern Instrument Ltd. UK) at room temperature. Transmission electron microscopy (TEM) was recorded on a FEI CM120 TEM at a 200 kV accelerating voltage.

### Synthesis

#### Synthesis of 4-(6-hydroxyhexyloxy) phthalonitrile (HHPN)

A typical synthetic procedure of HHPN was as follows: 3,4-dicyanophenol (0.29 g, 2.0 mmol) and potassium carbonate (0.36

g, 2.6 mmol) were dissolved in 15 mL dry *N,N*-dimethylacetamide, and stirred at 80 °C for 3 hours, then 6-bromo-1-hexanol (0.29 g, 1.6 mmol) and a small amount of potassium iodide were added into the above mixture, after that, the mixture was refluxed for 12 h. When the mixture was allowed to room temperature, 100 mL ethyl acetate was added, then the resulted mixture was washed with 100 mL deionized water for 3 times. After being dried over anhydrous  $\text{MgSO}_4$  overnight, ethyl acetate was evaporated and the crude product purified by column chromatography (silica gel, ethyl acetate/petroleum ether = 1/8) to yield a white solid (0.33 g, 60%). Elemental analysis calculated for  $\text{C}_{14}\text{H}_{16}\text{N}_2\text{O}_2$ : C, 68.83%; H, 6.60%; N, 11.47%. Found: C, 68.55%; H, 6.82%; N, 11.52%.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6/\text{TMS}$ , ppm) (Fig.S1): 7.99-8.05(d, 1H, CHCHCCN), 7.70-7.78 (s, 1H, CCHCCN), 7.40-7.46 (d, 1H, CHCHCCN), 4.32-4.38 (t, 1H, OH), 4.08-4.16 (t, 2H,  $\text{OCH}_2$ ), 3.35-3.42 (m, 2H,  $\text{CH}_2\text{OH}$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm): 162.48, 135.49, 119.93, 119.61, 117.30, 116.08, 115.60, 69.43, 62.65, 32.66, 28.87, 25.81, 25.61.

#### 20 Synthesis of 2-methyl-acrylic acid 6-(3,4-dicyano-phenoxy)-hexyl ester (MADCE).

HHPN (0.24 g, 1.00 mmol) and triethylamine (1.0 mL) were dissolved in 10 mL THF, then 10 mL Methacryloyl chloride (1.04 g, 0.97 mmol) THF solution was added in into the mixture at -10 °C. After the solution was stirred at room temperature overnight, ethyl acetate was added, and it was then washed with water and dried with anhydrous  $\text{MgSO}_4$ . After removing the solvent, column chromatography (silica gel, ethyl acetate/petroleum ether = 1/10) was undertaken to obtain the product. Yield: 85%. Elemental analysis calculated for  $\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_3$ : C: 69.21%; H: 6.45%; N: 8.97%. Found: C: 69.07%; H: 6.20%; N: 8.79%.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm)(Fig.S2): 7.65-7.76(d, 1H, CHCHCCN), 7.22- 7.30 (s, 1H, CCHCCN), 7.12- 7.21 (d, 1H, CHCHCCN), 6.05–6.15 (s, 1H,  $\text{C}=\text{CH}_2$ ), 6.52-6.60 (s, 1H,  $\text{C}=\text{CH}_2$ ), 4.11-4.22 (t, 2H,  $\text{OCH}_2$ ), 3.99-4.10 (t, 2H,  $\text{CH}_2\text{OCOC}$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm): 167.69, 162.43, 136.65, 135.47, 125.54, 119.84, 119.59, 117.54, 116.04, 115.59, 107.15, 69.38, 64.70, 28.87, 28.70, 25.89, 25.74, 18.56.

#### 40 Synthesis of homopolymer(P0) of MADCE via RAFT polymerisation.

A typical procedure of RAFT polymerization for the synthesis of PMADCE (P0) is as follow: MADCE (0.5 g, 1.6 mmol), CPDN (17.37 mg, 0.064 mmol) and AIBN (5.26 mg, 0.032 mmol) were dissolved in 1 mL of DMF. The above solution was purged with argon for 15 min to eliminate the oxygen. Then the ampule was flame-sealed and placed in an oil bath held by a thermostat at 70 °C to carry out the polymerization. After 5 h, the ampule was cooled with ice water and opened. The reaction mixture was diluted with 3 mL THF and precipitated into an excess of methanol three times. The polymer was dried at room temperature in vacuum to a constant weight.

#### 55 Synthesis of block copolymer (P1) of MADCE and oligo(ethylene glycol) methyl methacrylate (OEGMEMA).

The block polymerization for the synthesis of P1 is as follow: OEGMEMA (0.5 mL, 1.14 mmol), PMADCE (0.2852 g, 0.023 mmol,  $M_{n,\text{GPC}} = 12400 \text{ g}\cdot\text{mol}^{-1}$ ,  $M_w/M_n = 1.12$ ), and AIBN (1.9 mg, 0.0115 mmol) and DMAc (3 mL) were added into 5 mL

ampoule by the desired proportion, after purged with argon for 15 min, the ampule was flame-sealed and placed in an oil bath held by a thermostat at 70 °C for 24 h to carry out the polymerization. The reaction mixture was diluted with THF and precipitated with ether. The polymer was dried at room temperature in vacuum to a constant weight.

#### Synthesis of random copolymer (P2) of MADCE and OEGMEMA.

Typical procedure of RAFT polymerization for the synthesis of P2 is as follow: MADCE (0.1773 g, 0.568 mmol), OEGMEMA (1 mL, 2.27 mmol), CPDN (6.2 mg, 0.0227 mmol) and AIBN (1.9 mg, 0.0114 mmol) were dissolved in 3 mL of DMAc. The above solution was purged with argon for 15 min to eliminate the oxygen. After flame-sealed, the ampule was placed in an oil bath at 70 °C for 24 h to carry out the polymerization. Then, the reaction mixture was diluted with 8 mL THF and precipitated into an excess of ether three times. The polymer was dried at room temperature in vacuum to a constant weight.

#### Synthesis of polymers (Pc0, Pc1, Pc2) with Zn(II) phthalocyanine on the side chain.

A typical procedure of conjugating phthalocyanine to the prepolymer: PMADCE, OPN and zinc acetate dihydrate were mixed in 15 mL DMAc by the desired ratio and heated at 80 °C. After adding a few drops of DBU, the mixture was kept at 130 °C overnight. The contents were then diluted with 10 mL THF and precipitated into an excess of methanol. After filtered and extracted in a Soxhlet Extractor with ether, a dark green solid product was dried at room temperature in vacuum to yield Pc0. In the case of copolymers with OEGMEMA, reaction mixtures were precipitated into an excess of diethyl ether three times for purification to obtain Pc1 and Pc2.

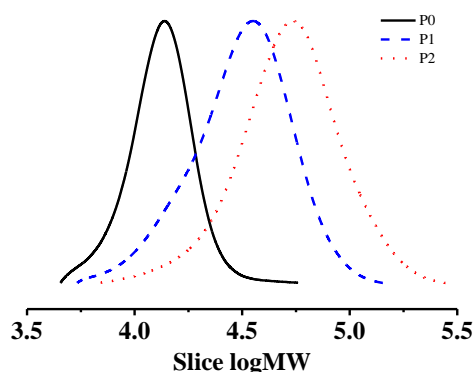
4-(octyloxy) phthalonitrile (OPN) used above was prepared with the similar procedure to that of HHPN. The product was recrystallized from methanol to yield a white solid (0.42 g, 78%). Elemental analysis calculated for  $\text{C}_{16}\text{H}_{20}\text{N}_2\text{O}$ : C, 74.97%; H, 7.86%; N, 10.93%. Found: C, 74.82%; H, 7.92%; N, 11.02%.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6/\text{TMS}$ , ppm) (Fig.S3): 8.02-8.07 (d, 1H, CHCHCCN), 7.73-7.77 (s, 1H, CCHCCN), 7.41-7.46 (d, 1H, CHCHCCN), 4.08-4.17 (t, 2H,  $\text{OCH}_2$ ).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3/\text{TMS}$ , ppm): 162.49, 135.43, 119.84, 119.55, 117.47, 116.05, 115.59, 106.99, 69.57, 31.95, 29.39, 28.93, 26.00, 22.83.

## Results and discussion

Well defined block and random copolymers containing oligo(ethylene glycol) methyl methacrylate (OEGMEMA) and benzenedinitrile were synthesized and used for the following synthesis of Zn(II) phthalocyanine (ZnPc) on the side-chain. Benzenedinitrile-containing homopolymers were firstly synthesized via RAFT polymerization of 2-methyl-acrylic acid 6-(3,4-dicyano-phenoxy)-hexyl ester (MADCE), which was carried out in DMF at 70 °C using CPDN as the RAFT agent and AIBN as the initiator. Block copolymers of MADCE and OEGMEMA were synthesized by chain-extension of the homopolymer of MADCE synthesized above with OEGMEMA as the second monomer. (Scheme 1) As presented in Fig.1, an apparent peak shift from the macro-CTA (P0,  $M_{n,\text{GPC}} = 12400 \text{ g}\cdot\text{mol}^{-1}$ ,  $M_w/M_n = 1.12$ ) to the chain extended PMADCE-*b*-POEGMEMA



(P1,  $M_{n(GPC)} = 27100 \text{ g}\cdot\text{mol}^{-1}$ ,  $M_w/M_n = 1.31$ ) was found.



**Fig. 1** GPC chromatograms of polymers with benzenedinitrile groups on the side chain.

Similarly, random copolymer was synthesized by RAFT polymerization of MADCE and OEGMEMA in DMAc at 70 °C using CPDN as the RAFT agent and AIBN as the initiator. The benzenedinitrile-containing homopolymer (P0), block (P1) and random polymer (P2) were summarized in Fig. 1 and Table 1.

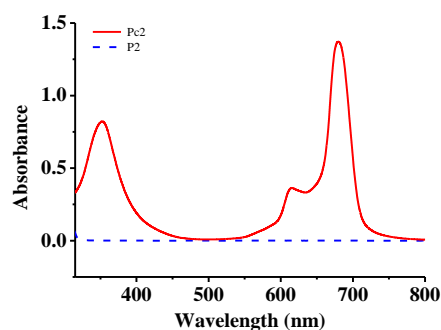
**Table 1** The benzenedinitrile polymers obtained by RAFT polymerization.

Name	Samples	[OEGMEMA] <sub>0</sub> / [MADCE] <sub>0</sub>	$M_{n,GPC}$ ( $\text{g}\cdot\text{mol}^{-1}$ )	$M_w/M_n$
P0	PMADCE	0/25	12400	1.12
P1	PMADCE- <i>b</i> - POEGMEMA	50/25	27100	1.31
P2	PMADCE- <i>co</i> - POEGMEMA	100/25	44800	1.35

The synthesis of Pc derivatives is relatively difficult with poor solubility, which limited the preparation of side chain Pc-containing polymers. Here we described the preparation of side chain Pc-containing polymers via post-polymerization modification technique. Zinc 2-hexyloxy-9,16,23-tri-octyloxy-29H,31H-phthalocyanine (ZnHOPc) was formed via chemical reaction between benzenedinitrile groups in PMADCE and those in added reagent (M in Scheme 1). Either the homopolymer P0, block copolymer P1 or random copolymer P2 is used for the synthesis of polymers with ZnPc units on the side chains, namely Pc0, Pc1 and Pc2. UV-Vis, FT-IR, GPC, NMR and atomic absorption spectra (AAS) were used to characterize the polymers before and after the formation of ZnPc unit in the polymeric chain.

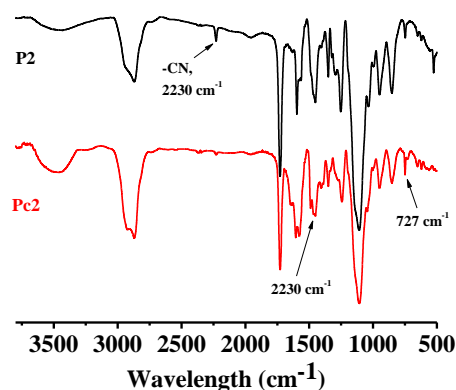
Phthalocyanines consisting of four isoindole units presenting an 18 $\pi$ -electron heterocyclic aromatic system, their intrinsic absorption spectra indicate intense Q-bands in the visible-near infrared optical region, approximately from 600 nm to 700 nm.<sup>36,37</sup> Fig.2, Fig. S4 and Fig. S5 shows the typical UV-vis spectra of the polymer precursors (P1, P2) and the Zn(II) phthalocyanine-functionalized polymers (Pc1, Pc2) in DMF, using Zinc Phthalocyanine (ZnPc) as the reference. As presented in Fig.2 and Fig. S4, the intense  $\pi$ - $\pi$  bands in the visible (from 660 nm to 690 nm,  $\lambda_{\text{max}} = 682 \text{ nm}$ ) and UV (from 330 nm to 360 nm,  $\lambda_{\text{max}} = 350 \text{ nm}$ ) spectral regions ascribed to the typical Q-

band and B-band of Pc ring was observed after post-polymerization modification, which indicates the successful formation of ZnPc units on the side chain. The UV-Vis spectra of the ZnPc-containing polymers show a slightly red shift for the Q-bands of the ZnPc moieties (from 669 nm to ~680 nm), due to the electron-donating effects of alkoxy groups. For the different polymers (Pc0, Pc1 and Pc2), the UV-Vis spectra are almost identical, indicating the polymer chain has little effect on the absorption property of ZnPc.



**Fig. 2** Typical UV-vis spectra of polymers before (P2) and after modification (Pc2) in DMF.

Further confirmation of the phthalocyanine-forming reaction can be taken from IR spectra. Fig.3 and Fig. S6 shows the typical IR spectra of the benzenedinitrile-containing polymers (P1, P2) and ZnHOPc-functionalized polymers (Pc1, Pc2). The signal at 2230  $\text{cm}^{-1}$  assigned to the cyano groups existed in P1, P2 almost disappeared after post-polymerization modification reaction, which proved the reaction leading to the covalently bound polymeric phthalocyanine while the absorptions at 2940 and 1725  $\text{cm}^{-1}$  remain. Moreover, the typical absorptions of Pc ring in PAMDCE-ZnPc at 1490 and 727  $\text{cm}^{-1}$  were observed respectively.<sup>38</sup>



**Fig. 3** Typical FT-IR spectra of polymers before (P2) and after modification (Pc2).

GPC has also been used to characterize the polymers before and after modification. We found that GPC traces of polymers before and after modification changed greatly, indicating the successful modification. However,  $M_{n,GPC}$  of the polymers after modification changed little or even decreased, as given in Fig. S7.

The molecular weight obtained by GPC may not represent the actual molecular weight of polymers after modification, due to the difference of polymers with GPC standards. We found that the  $M_w/M_n$  was slight ascent, indicating that there may be small amount of cross-linking reactions among polymer chains during the modification.

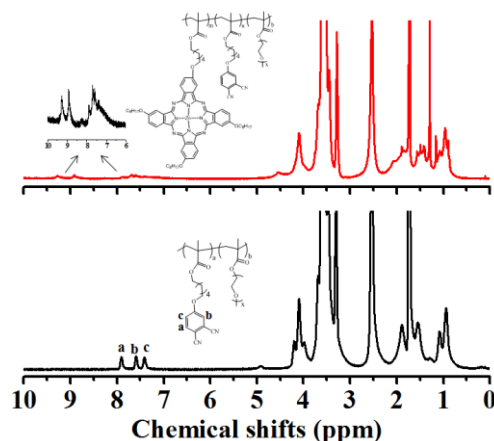


Fig. 4  $^1\text{H}$ -NMR spectra of benzenedinitrile-containing polymer (P2) and ZnPc-functionalized polymer (Pc2) in  $\text{THF-}d_4$ .

NMR could be used to further characterize Pc molecules with the Pc macrocycle protons in the downfield region at  $> 9$  ppm and the pyridyl protons between 8 and 9 ppm.<sup>8,39</sup> NMR characterisation of benzenedinitrile-containing and ZnHOPc-functionalized copolymers confirmed the successful formation of Pc units on the polymer chain with the observation of signals at 9.30 and 8.90 ppm as presented in Fig. 4. The chemical shifts of some protons in the Pc units locate in the range between 6.50 and 8.50 ppm, overlapping the signals of benzenedinitrile groups (7.90, 7.60 and 7.40 ppm), making it difficult to calculate accurately the conversion of benzenedinitrile on the side chain to ZnPc from NMR. To get a clear understanding of the content of ZnPc groups in the different polymeric chains, atomic absorption spectroscopy was employed to study the zinc content of the functionalized polymer Pc0, Pc1 and Pc2. The polymer samples were placed on the combustion boat in a horizontal quartz tube furnace, and then heated to 850  $^\circ\text{C}$  for 12 h with a constant oxygen gas flow. After being cooling naturally, the white power was found on the combustion boat. The ashed sample was dissolved in dilute hydrochloric acid and zinc content was analysed by atomic absorption spectroscopy. The zinc content in the polymers and loading of ZnPc were calculated and summarised in Table 2. The zinc content in the Pc0, Pc1 and Pc2 was calculated to be 2.1%, 0.96% and 0.56%, respectively. Based on the above results, the conversion of benzenedinitrile to phthalocyanine (Y) could further be calculated using the equation,  $Y = M_{\text{ZnPc}}/M_{\text{MADCE}}$ , where  $M_{\text{ZnPc}}$  is the molar content of ZnPc per gram in the modified polymer and  $M_{\text{MADCE}}$  is the molar content of MADCE per gram in the starting precursor polymer. For polymers used here, about 35% molar ratio of cyano groups in Pc0 and 30% in Pc1 and Pc2 took part in the chemical reaction with 4-(octyloxy) phthalonitrile in the presence of zinc acetate dehydrate.

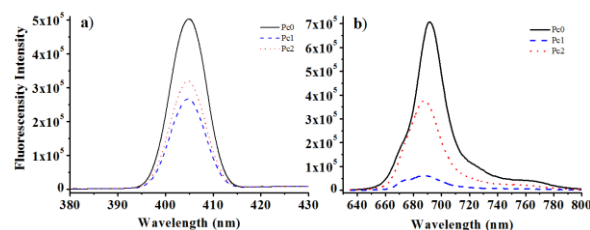


Fig. 5 Fluorescence spectra of polymers in THF at the same concentration ( $0.01 \text{ mg} \cdot \text{mL}^{-1}$ ), a,  $\lambda_{\text{ex}} = 350 \text{ nm}$ ; b,  $\lambda_{\text{ex}} = 620 \text{ nm}$

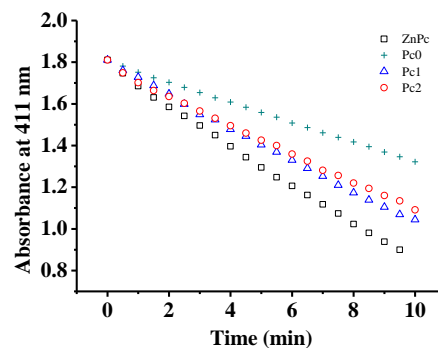


Fig. 6 Comparison of the rates of decay of DPBF in DMF using polymers and ZnPc as the photosensitizers.

The inherent fluorescent characteristic of the Pc moiety may be used for imaging applications in no need of further labeling. To evaluate the fluorescent property of the polymers, fluorescence emission spectra of Pc0, Pc1 and Pc2 were recorded in THF at the  $0.01 \text{ mg} \cdot \text{mL}^{-1}$  (Fig. 5). The emission bands appeared at 405 nm for all the polymers when excited by light with 350 nm. The bands were at  $\sim 690$  nm excited by 620 nm light. The fluorescent property of the Pc-functionalized polymers is similar to other typical Pc molecules,<sup>10</sup> and the emission at 690 nm may be suitable for bio-imaging and monitoring applications. To compare the fluorescence property of the different polymers obtained, the quantum yields of fluorescence in DMF were determined by comparative method<sup>40</sup> using the following equation:

$$\Phi_s = \Phi_r (A_r / A_s) (F_s / F_r) (n_s^2 / n_r^2)$$

where  $\Phi_s$  and  $\Phi_r$  are quantum efficiencies of the sample and reference compound;  $A_r$  and  $A_s$  are the respective absorbance of the sample and reference solutions at the excitation wavelength;  $F_s$  and  $F_r$  are the corresponding emission integration areas; and  $n_s$  and  $n_r$  are refractive indices of the sample and reference solutions, respectively. ZnPc in DMF was employed as the reference ( $\Phi_r = 0.3$ )<sup>41,42</sup>. The absorbance of the solutions at the excitation wavelength ranged between 0.02–0.08. As shown in Table 2, the functionalized polymer Pc0, Pc1 and Pc2 show relatively low fluorescence quantum yields. The relatively low fluorescence quantum yields are attributed to polymer coil overlap, which hindered the fluorescence emission. The emission intensity and fluorescence quantum yield of random copolymer Pc2 are higher than those of block copolymer Pc1, possibly due to the reduced self-aggregation of Pc units in the random copolymer.

**Table 2** Fluorescence quantum yields of the functionalized polymer Pc0, Pc1 and Pc2. (Excitation at 620 nm).

	Pc0	Pc1	Pc2
$Abs_{\lambda_{max}}(nm)$	679	680	680
$Ems_{\lambda_{max}}(nm)$	693	690	692
$n$	1.4321	1.4313	1.4325
$A^a$	0.051	0.029	0.019
$\Phi_s^b$	0.03	0.004	0.028

<sup>a</sup>The absorbances of the sample solutions at excitation wavelength and the concentration of all functionalized polymer is 0.002 mg·mL<sup>-1</sup> in DMF.

<sup>b</sup> $\Phi_s(\text{ZnPc}) = 0.30$ (value from Refs.<sup>41,42</sup>)

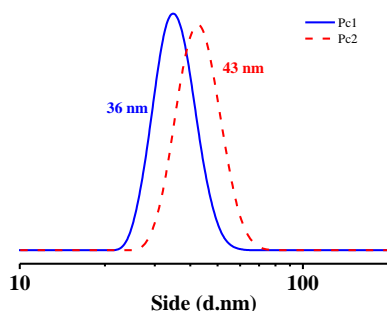
Singlet oxygen has been implicated as an intermediary species leading to cell death following the excitation of photosensitizers in photodynamic therapy. Measurements of singlet oxygen quantum yields are thus important in assessing the potential effectiveness of the new synthesized polymeric pre-drug for PDT. The singlet oxygen quantum yields ( $\Phi_{\Delta}$ ) of ZnPc-functionalized polymers were measured by the method of the rate of decay of DPBF in DMF, using ZnPc as the reference (Fig.6),<sup>35</sup> and the value obtained were summarize in Table 3. We found that copolymers Pc1 and Pc2 presented better singlet oxygen quantum yields compared to the homopolymer Pc0, possibly due to improved solubility and the reduced self-aggregation of Pc units in the polymer chain, which is similar to our previous findings,<sup>28</sup> where Pc-functionalized OEGMEMA copolymers show enhanced singlet oxygen quantum yields. In addition, the copolymers have presented good singlet oxygen quantum yield values in aqueous media, measured using ZnPcS<sub>4</sub> as reference.<sup>43</sup> This is promising for the polymers obtained to be used as potential PDT agents.

**Table 3** Content of ZnPc and characterization of singlet oxygen quantum yields of the polymers obtained.

	Pc0	Pc1	Pc2
Zn (wt %) <sup>a</sup>	2.1	0.96	0.56
ZnPc (mmol·g <sup>-1</sup> )	0.32	0.15	0.086
$\Phi_{\Delta}$ , DMF	0.28	0.49	0.40
$\Phi_{\Delta}$ , aqueous	--- <sup>b</sup>	0.68	0.73

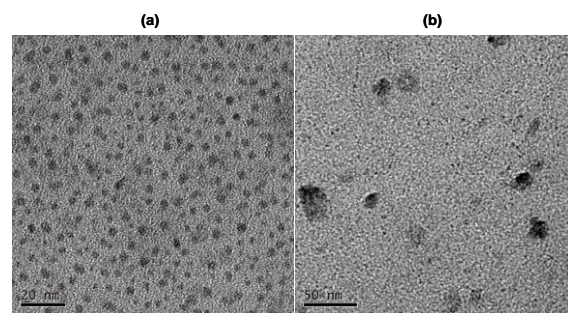
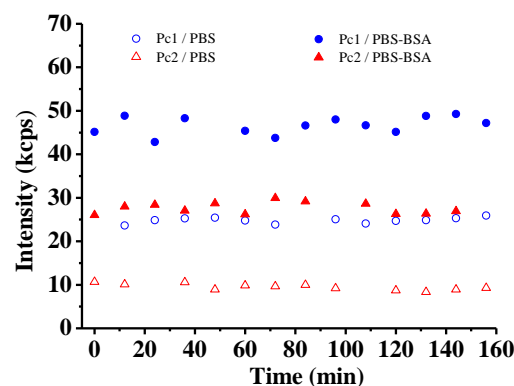
<sup>a</sup>Obtained from atomic absorption spectroscopy.

<sup>b</sup> Pc0 is insoluble in aqueous media

**Fig. 7** Dynamic light scattering of Zn-Pc containing micelles self-assembled in aqueous solution.

Knowing the morphology of polymers in aqueous solutions is important for bio-related potential applications. Homopolymer

Pc0 is not water soluble, and the amphiphilic copolymers Pc1 and Pc2 could self-assemble into micelles in aqueous solutions. The formed micelles of amphiphilic copolymers Pc1 and Pc2 were characterized by the dynamic light scattering (DLS) measurements as shown in Fig. 7. The micelle size of polymer Pc1 and Pc2 is 36 nm and 43 nm, respectively. The difference between micelle sizes of polymers Pc1 and Pc2 may be attributed to their different molecular weights. This micellar structure was further confirmed by TEM. As shown in Fig. 8, it is evident that the self-assembled micelles formed have a spherical shape and are well dispersed. From the TEM image, the sizes of micelles are about 9 nm for Pc1 and about 20 nm for Pc2. As TEM samples are taken without staining, therefore ZnPc groups mainly contribute the dark spheres seen in TEM, which will be the core of micelles. For block copolymer Pc1, ZnPc are packed more densely in the core, leading to smaller cores compared to random copolymers.

**Fig. 8** TEM image of Pc1 (a) and Pc2 (b) micelles self-assembled in aqueous solution.**Fig. 9** Evolution of count rates of Pc1 and Pc2 in PBS buffer (pH7.4) and PBS with BSA dissolved (BSA 0.5 mg·mL<sup>-1</sup>).

The effects of salt and protein on the stability of the ZnPc-functionalized polymer are important information for potential bio-applications. The polymers were mixed with PBS 7.4 buffer and PBS buffer with bovine serum albumin (BSA) dissolved (PBS-BSA, BSA concentration 0.5 mg·mL<sup>-1</sup>). DLS were used to monitor the evolution of intensity (count rates) of the mixtures, which is often used to monitor the change (caused by size or concentration) of the same sample over a period of time. As shown in Fig. 9, the intensity of both the block and random copolymer (Pc1 and Pc2) solutions keep constant, indicating a good stability in PBS and PBS-BSA over the period we tested. The good solubility and stability of Zn-Pc polymers in aqueous



solutions in the presence of salt and protein guarantee their further bio-related applications.

## Conclusions

In summary, homo-, block and random polymers with benzendinitrile on the side chain have been successfully synthesized via RAFT polymerization. Phthalocyanine functionalities were introduced by reactions of dinitrile groups with 4-(octyloxy) phthalonitrile in the presence of zinc acetate dihydrate. The Zn(II) phthalocyanine-functionalized polymers have an absorption peak in the far-red region with a sharp Q band absorption at 682 nm. Strong fluorescence emission ( $\lambda_{\text{ex}} = 620$  nm) was observed for the polymers except the block copolymer. Enhanced singlet oxygen quantum yields ( $\Phi_{\Delta} = 0.49$  and  $0.40$  in DMF,  $\Phi_{\Delta} = 0.68$  and  $0.73$  in aqueous media) are obtained for Zn-Pc functionalized copolymers compared to the homopolymer ( $\Phi_{\Delta} = 0.28$  in DMF). DLS measurements and TEM observation of the copolymers confirmed the formation of nanoparticles by the self-assembly of conjugates in aqueous solution. These Zn-Pc functionalized nanoparticles show good stability in the presence of salt and protein. This provides a simple method to fabricate novel Zn-Pc functionalized polymers as candidate for photodynamic therapy.

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## Notes and references

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- <sup>†</sup>Electronic Supplementary Information (ESI) available: <sup>1</sup>H NMR spectra of HHPN, MADCE and OPN, UV-Vis spectra of polymers before (P1) and after modification (Pc1) in DMF, FT-IR spectra of polymers before (P1) and after modification (Pc1), GPC traces of polymers before and after modification and fluorescence spectra of polymers (Pc0, Pc1 and Pc2) in DMF, The absorption spectra for the determination of singlet oxygen quantum yields of Pc1 and Pc2 in aqueous media. See DOI: 10.1039/b000000x/.
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