Electronic Supplementary Information

Quantitatively analyzing the dissociation and release of disulfide-containing organic Nanoparticles

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Experimental

Materials. Glutathione and 1-ethyl-3-(3-dimethylaminopro pyl)carbodiimide hydrochloride (EDC·HCl) were purchased from Shanghai Yuanye Biological Technology Co., Ltd. 3,3-Dithiodipropionic acid and 4-dimethylaminopyridine (DMAP) were purchased from Shanghai Sun Chemical Technology Co., Ltd. Suberic acid and 4-dimethylaminobenzaldehyde was purchased from 9ding Chemical Technology Co., Ltd. 3-Mercaptopropionic acid was purchased from Aldrich. G-OHBDP was synthesized according to the previous literature.[1] All reagents were purchased from commercial sources and used without further treatment, unless indicated otherwise.

Characterizations. The details of proton nuclear magnetic resonance spectra (^{1}H NMR), dynamic light scattering (DLS) and transmission electron microscopy (TEM) were shown in our previous work.[2-4] The Shimadzu HPLC coupled with a Qtrap 5500 mass spectrometer (Sciex, Ontario, Canada) equipped with a TurboIonSprapy source (HPLC-ESI-MS) was used for quantification. Data acquisition and integration were controlled by Analyst 1.61 Software. The chromatography was performed on a ZORBA×300SB-C8 column (150 × 4.6 mm, 5 μm, Sciex) maintained at room temperature. Gradient elution was 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B). Statistical significance analysis was assessed by SPSS via one-way ANOVA test. N.s. meant no difference ($P > 0.05$) but *$P < 0.05$ was considered statistically highly significant.

Synthesis of G-BDPSS and G-BDPSCOOH. G-OHBDP (82 mg, 0.231 mmol),

3,3-dithiodipropionic acid (24 mg, 0.116 mmol), DMAP (2.8 mg, 0.023 mmol) and EDC·HCl (88.9 mg, 0.462 mmol) were dissolved in dichloromethane (DCM, 10 mL) (Fig. 1A in the manuscript). The mixture was stirred for an hour at room temperature. Then DMAP (2.8 mg, 0.023 mmol) and EDC·HCl (44.4 mg, 0.231 mmol) was added to the mixture followed by stirring for 24 h. The mixture was chromatographed on a silica gel column (gradient elution: hexane: DCM = 1: 2). MS: $[M+H]^+$ =863.5.

The synthesis steps of G-BDPSCOOH were similar to the synthesis steps of G-BDPSS. The amount of all compounds was the same except 3,3-dithiodipropionic acid (240 mg, 1.16 mmol). The gradient elution was ethyl acetate. MS: $[M-H]^-$ =545.3.

Synthesis of G-SHBDP. G-OHBDP (74 mg, 0.21 mmol), 3-mercaptopropionic acid (91.5 μL, 1.05 mmol), DMAP (2.5 mg, 0.021 mmol) and EDC·HCl (76.3 mg, 0.42 mmol) were dissolved in DCM (10 mL) (Fig. 1A). The mixture was stirred for an hour at room temperature. Then DMAP (2.5 mg, 0.021 mmol) and EDC·HCl (38 mg, 0.21 mmol) was added to the mixture followed by stirring for 24 h. The mixture was chromatographed on a silica gel column (gradient elution: hexane: DCM = 1: 2). MS: $[M+H]^+$ =443.2

Synthesis of G-BDPCC and G-BDPCCOOH. G-OHBDP (47 mg, 0.134 mmol), suberic acid (11.6 mg, 0.067 mmol), DMAP (1.6 mg, 0.013 mmol) and EDC·HCl (50 mg, 0.268 mmol) were dissolved in DCM (10 mL) (Fig. 1A). The mixture was stirred for an hour at room temperature. Then DMAP (1.6 mg, 0.013 mmol) and EDC·HCl (25 mg, 0.134 mmol) was added to the mixture followed by stirring for 24 h. The mixture was chromatographed on a silica gel column (gradient elution: hexane: DCM

= 1: 2). MS: $[M+H]^+$ =847.5

The synthesis steps of G-BDPCCOOH were similar to the synthesis steps of G-BDPCC. The amount of all compounds was the same except suberic acid (116 mg, 0.67 mmol). The gradient elution was ethyl acetate. MS: $[M-H]^-$ =509.3

Synthesis of OHBDP. G-OHBDP (216 mg, 0.61 mmol) and 4-dimethylaminobenzaldehyde (363 mg, 2.44 mmol) in 25 mL toluene were stirred followed by added with piperidine (2.6 mL) and acetic acid (0.6 mL). The mixture was refluxed (120 ^oC) for 2 h in presence of Dean-Stark apparatus (Scheme S2). The solvent was removed and the crude product was purified by silica gel column chromatograph (gradient elution: DCM: ethyl acetate = 20:1). At last, OHBDP was obtained by settling in n-hexane and filtration.

Synthesis of BDPSS and BDPSCOOH. OHBDP (38.8 mg, 0.0629 mmol), 3,3-dithiodipropionic acid (6.6 mg, 0.0314 mmol), DMAP (0.7 mg, 0.0063 mmol) and EDC·HCl (24 mg, 0.126 mmol) were dissolved in dichloromethane (DCM, 10 mL) (Scheme S2). The mixture was stirred for an hour at room temperature. Then DMAP (0.7 mg, 0.0063 mmol) and EDC·HCl (12 mg, 0.063 mmol) was added to the mixture followed by stirring for 24 h. The mixture was chromatographed on a silica gel column (gradient elution: DCM).

The synthesis steps of BDPSCOOH were similar to the synthesis steps of BDPSS. The amount of all compounds was the same except 3,3-dithiodipropionic acid (66 mg, 0.314 mmol). The gradient elution was ethyl acetate.

Synthesis of BDPCC and BDPCCOOH. OHBDP (33 mg, 0.0536 mmol), suberic

acid (4.6 mg. 0.0268 mmol), DMAP (0.6 mg, 0.0054 mmol) and EDC·HCl (21 mg, 0.107 mmol) were dissolved in DCM (10 mL) (Scheme S2). The mixture was stirred for an hour at room temperature. Then DMAP (0.6 mg, 0.0054 mmol) and EDC·HCl (10 mg, 0.054 mmol) was added to the mixture followed by stirring for 24 h. The mixture was chromatographed on a silica gel column (gradient elution: DCM).

The synthesis steps of BDPCCOOH were similar to the synthesis steps of BDPSS. The amount of all compounds was the same except suberic acid (46 mg, 0.268 mmol). The gradient elution was ethyl acetate.

Preparation of G-BDPCC NPs and G-BDPSS NPs. G-BDPCC (2 mg) was dissolved in acetone (5 mL), and then the solution was added dropwise to deionized water (10 mL) and stirred for 10 h to evaporate the organic solvent followed by dialysis for 24 h.

The preparation steps of G-BDPSS NPs were similar to those of G-BDPCC NPs.

Preparation of BDPCC@PEG-PLA NPs and BDPSS@PEG-PLA NPs. BDPCC (1 mg) and PEG-PLA (10 mg) were dissolved in acetone (5 mL), and then the solution was added dropwise to deionized water (10 mL) and stirred for 10 h to evaporate the organic solvent followed by dialysis for 24 h.

The preparation steps of BDPSS@PEG-PLA NPs were similar to those of BDPCC@PEG-PLA NPs

Cellular uptake studies. Similar experimental methods and instruments were shown in our previous work.[3] The cell nuclei were stained with Hoechst 33258. The incubation time was 0.5 h, 2 h, 6 h and 24 h, respectively. 24 h indicated that cells

were incubated with nanoparticles for 6 h, washed with PBS and incubated with Dulbecco Modified Eagle Medium (DMEM, GIBCO) containing 10% fetal bovine serum for additional 18 h. CLSM images of cells were obtained by Carl Zeiss LSM 710 (Zurich, Switzerland). The blue fluorescence from Hoechst 33258 was obtained through the DAPI channel. The green fluorescence from G-BDP was obtained through the FITC channel. To regulate intracellular GSH, cells were first treated with GSH or N-ethylmaleimide (NEM) for 2 h.

Animal model. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. Female Kunming mice with naked-eye-visible U14 tumor nodules in the armpit of the left anterior limb were applied.

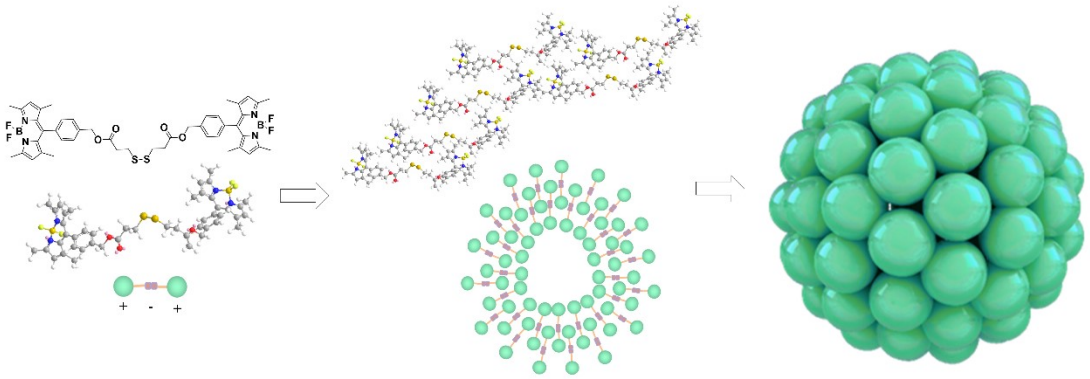
Reference：

1 C. Li, W. Lin, S. Liu, W. Zhang and Z. Xie, *J. Mater. Chem. B*, 2019, **7**, 4655-4660.

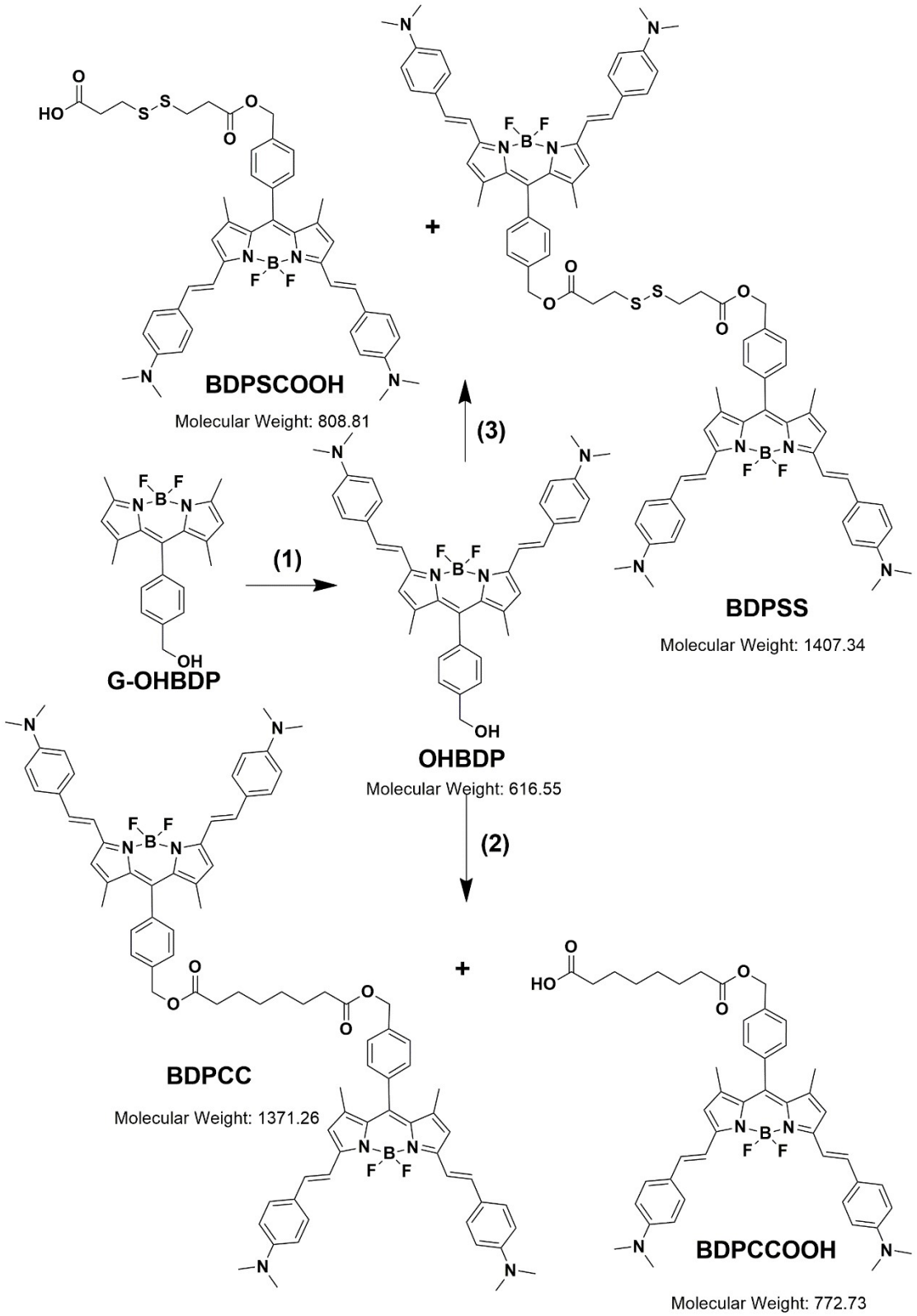
2 W. Lin, W. Zhang, S. Liu, Z. Li, X. Hu, Z. Xie, C. Duan and G. Han, *ACS Appl. Mater. Interfaces*, 2019, **11**, 43928-43935.

3 W. Lin, T. Sun, Z. Xie, J. Gu and X. Jing, *Chem. Sci.*, 2016, **7**, 1846-1852.

4 Y. Zhu, W. Lin, W. Zhang, Y. Feng, Z. Wu, L. Chen and Z. Xie, *Chin. Chem. Lett.*, 2017, **28**, 1875-1877.



Scheme S1 Illustration of the formation of supramolecular nanoparticles according to ground-state geometrical structure and calculated electron density distribution of G-BDPSS.



Scheme S2 Synthesis of OHBDP, BDPCC, BDPCCOOH, BDPSS and BDPSCOOH. (1) pyridine, CH_3COOH, dimethylaminobenzaldehyde, 120 ^{o}C. (2) suberic acid, EDC·HCl, DMAP, room temperature. (3) 3,3'-dithiodipropionic acid, EDC·HCl,

DMAP, room temperature.

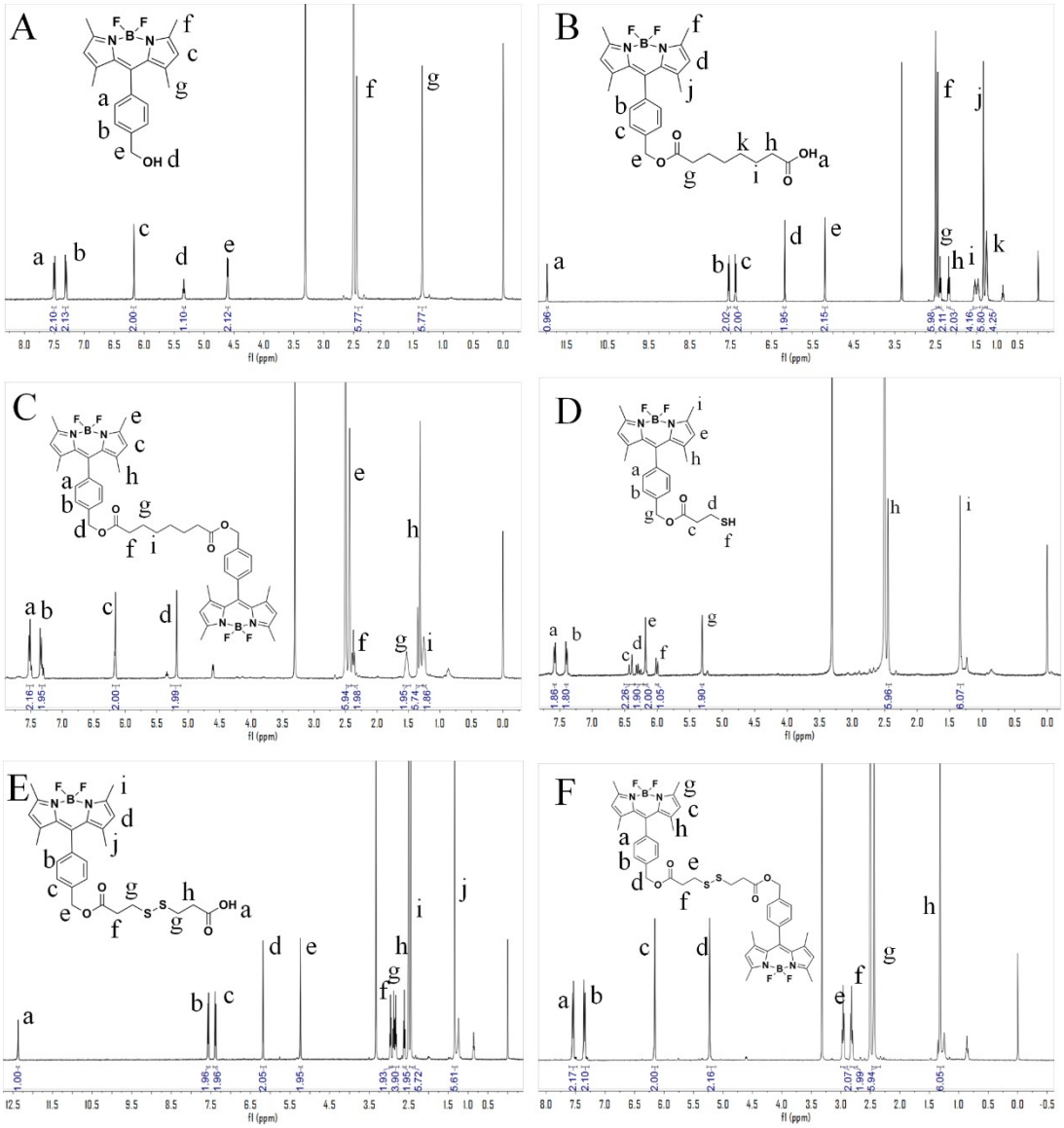


Fig. S1 ^{1}H NMR of (A) G-OHBDP, (B) G-BDPCCOOH, (C) G-BDPCC, (D) G-SHBDP, (E) G-BDPSCOOH and (F) G-BDPSS.

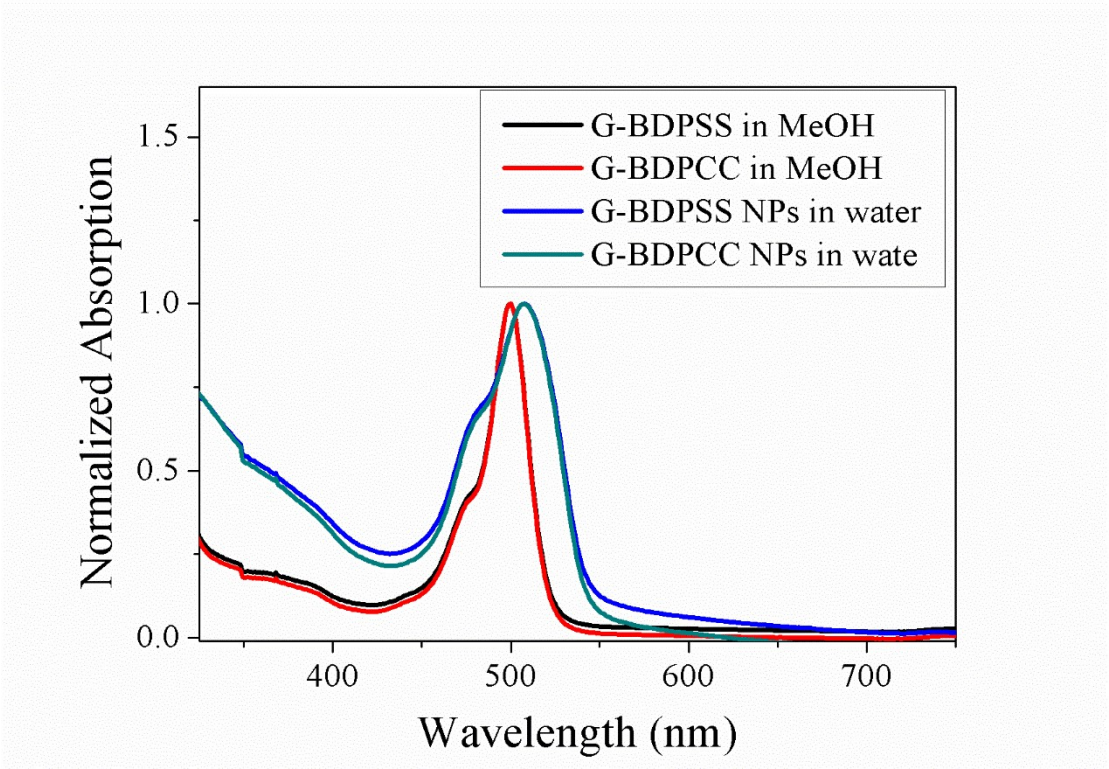


Fig. S2 The absorption of G-BDPSS and G-BDPCC in MeOH and their nanoparticles in water.

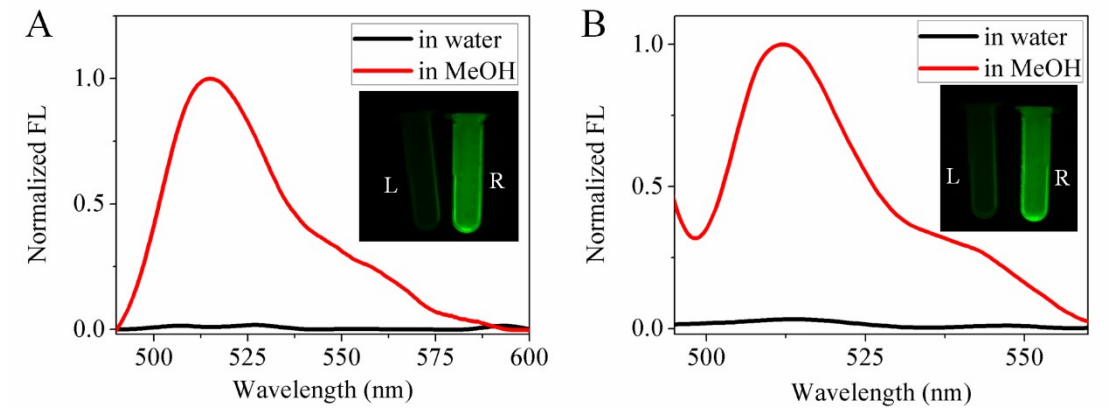


Fig. S3 The FL spectra of (A) G-BDPCC NPs in water and water+MeOH (v/v, 1:20) at the same concentration, and (B) G-BDPSS NPs in water and water+MeOH (v/v, 1:20) at the same concentration. In the inserted photos, NPs in water are on the left (L), and G-BDPSS in water+MeOH are on the right (R).

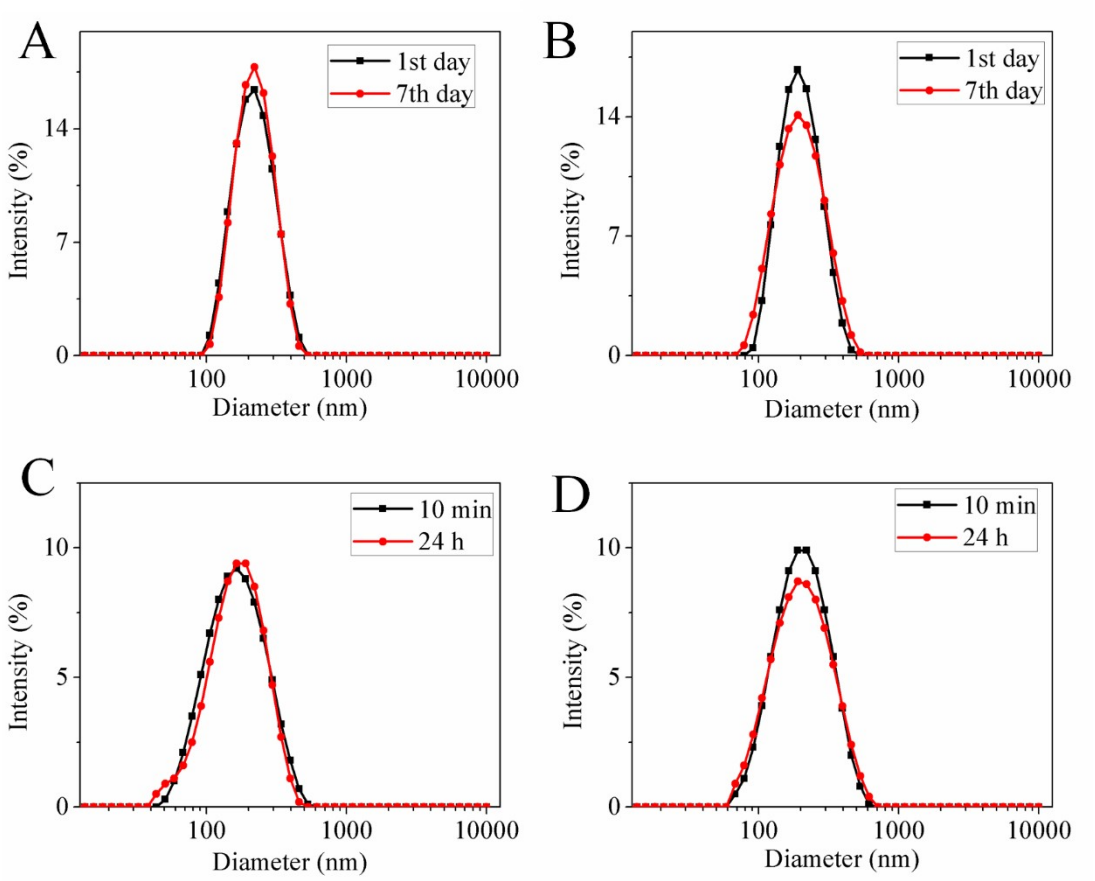


Fig. S4 The DLS results of G-BDPCC NPs and G-BDPSS NPs (A and B) at room temperature for 7 days and (C and D) in DMEM with 10 % FBS for 24 h.

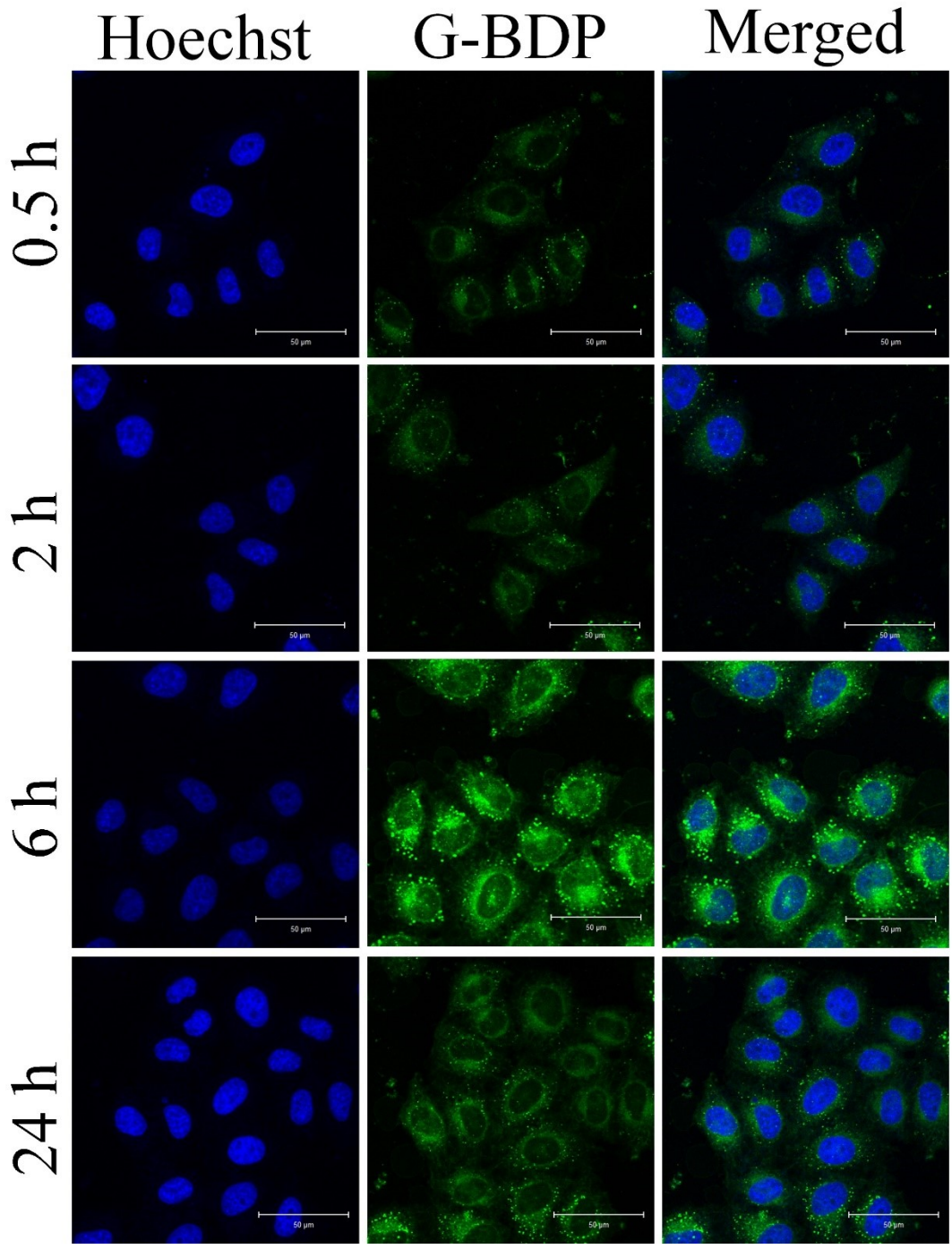


Fig. S5 Representative CLSM images of HeLa cells incubated with G-BDPSS NPs for 0.5, 1, 6 and 24 h. 24 hours contain 6 hours incubated with G-BDPSS NPs and following 18 hours incubated with DMEM (10 % FBS). For each panel, the images from left to right show cell nuclei stained by Hoechst 33258 (blue), G-BDP fluorescence in cells (green), and overlays of both images. Scale bar, 50 μm.

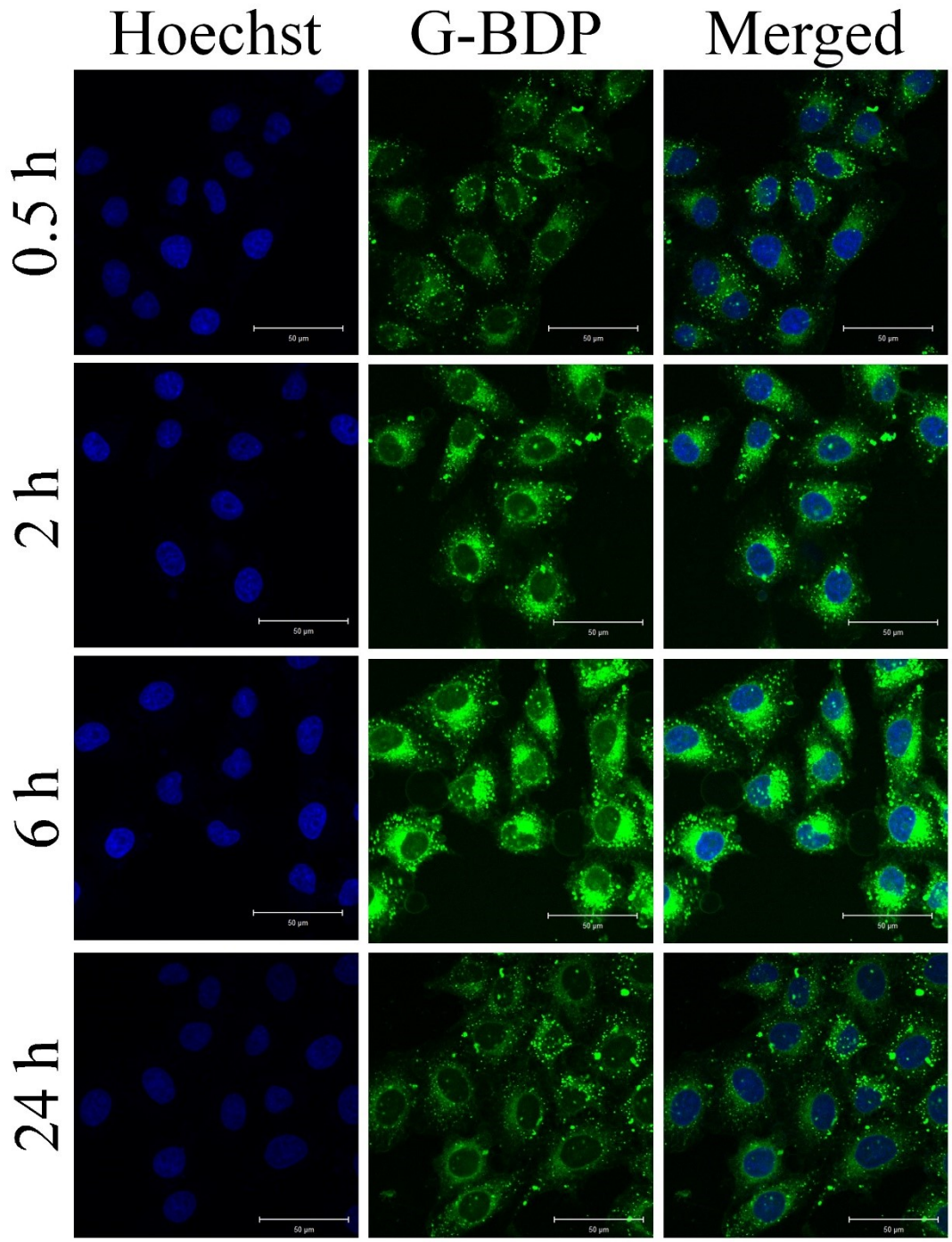


Fig. S6 Representative CLSM images of HeLa cells incubated with G-BDPCC NPs for 0.5, 1, 6 and 24 h. 24 hours contain 6 hours incubated with G-BDPCC NPs and following 18 hours incubated with DMEM (10 % FBS). For each panel, the images from left to right show cell nuclei stained by Hoechst 33258 (blue), G-BDP fluorescence in cells (green), and overlays of both images. Scale bar, 50 μm.

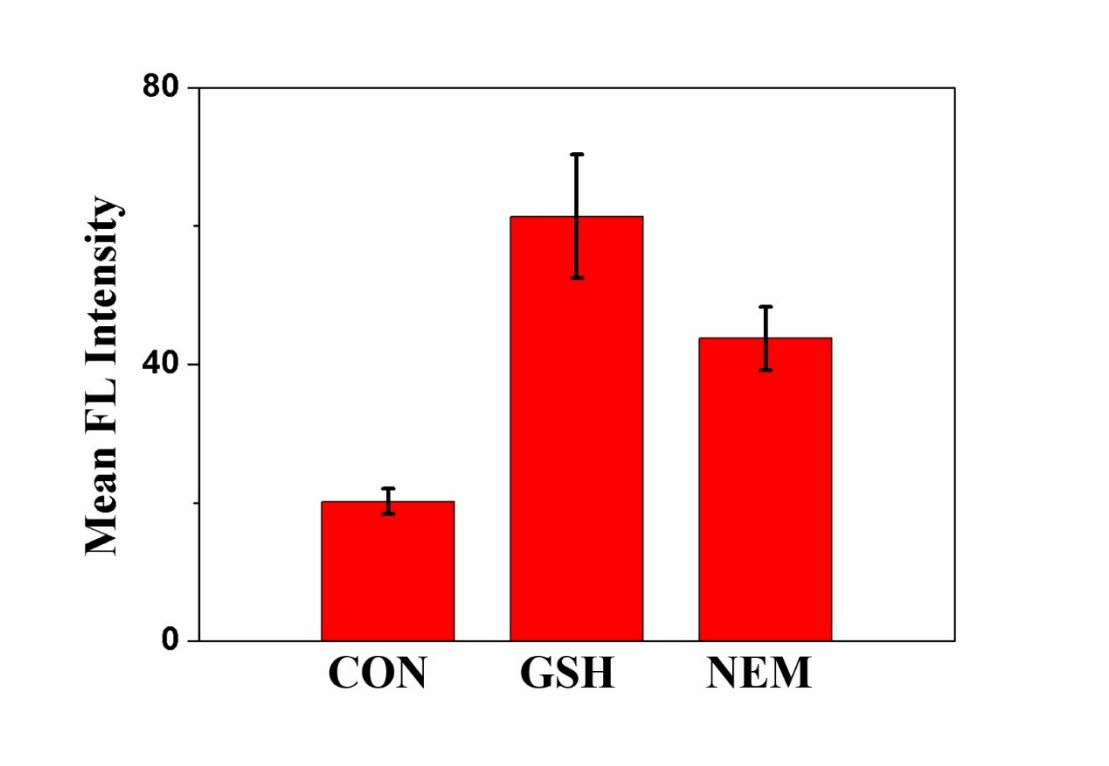


Fig S7. The mean fluorescence intensity of G-BDP in cells according to the CLSM images (Fig 2).

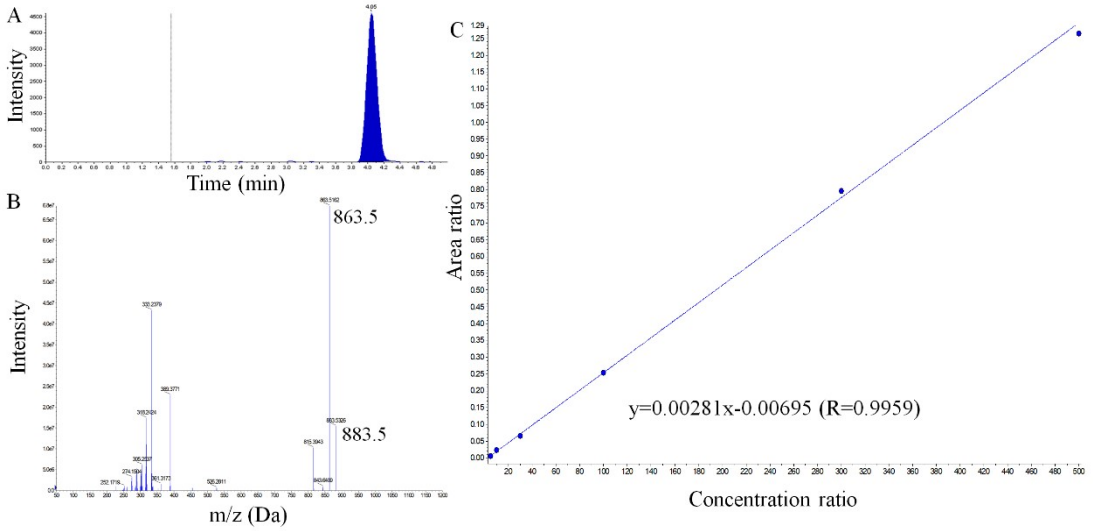


Fig. S8 (A and B) LC-MS/MS of G-BDPSS. (C) The quantitative standard curve of GBDPSS.

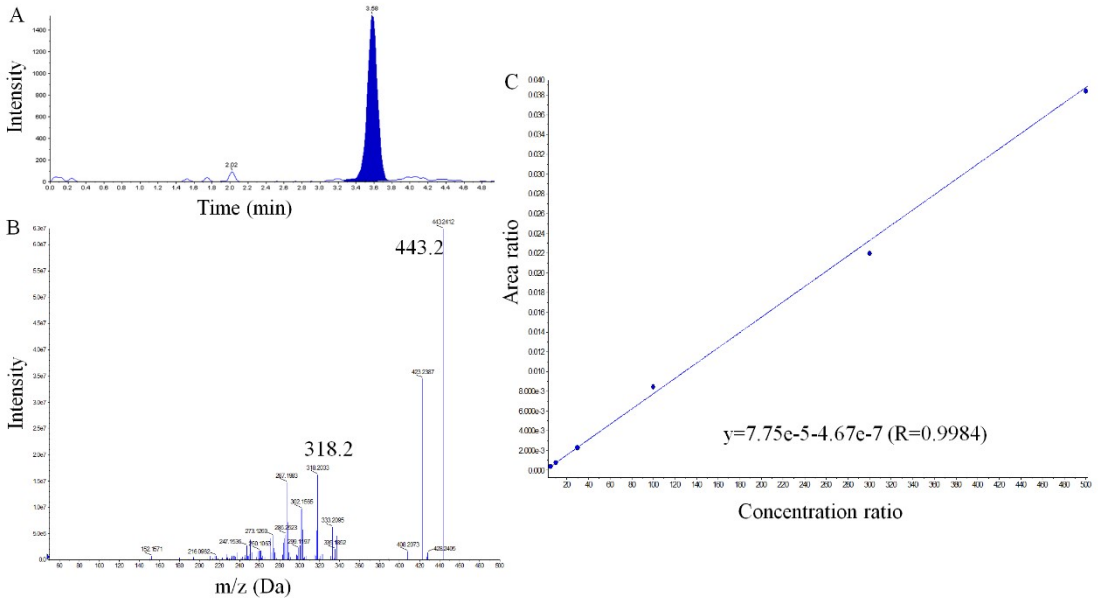


Fig. S9 (A and B) LC-MS/MS of G-SHBDP. (C) The quantitative standard curve of GSHBDP.

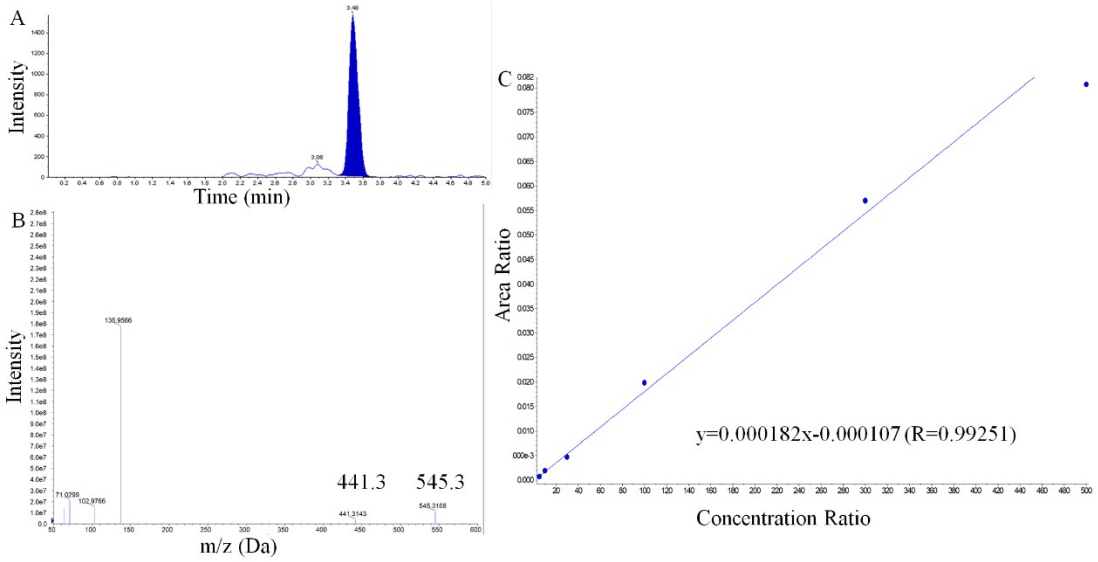


Fig. S10 (A and B) LC-MS/MS of G-BDPSCOOH. (C) The quantitative standard curve of GBDPSCOOH.

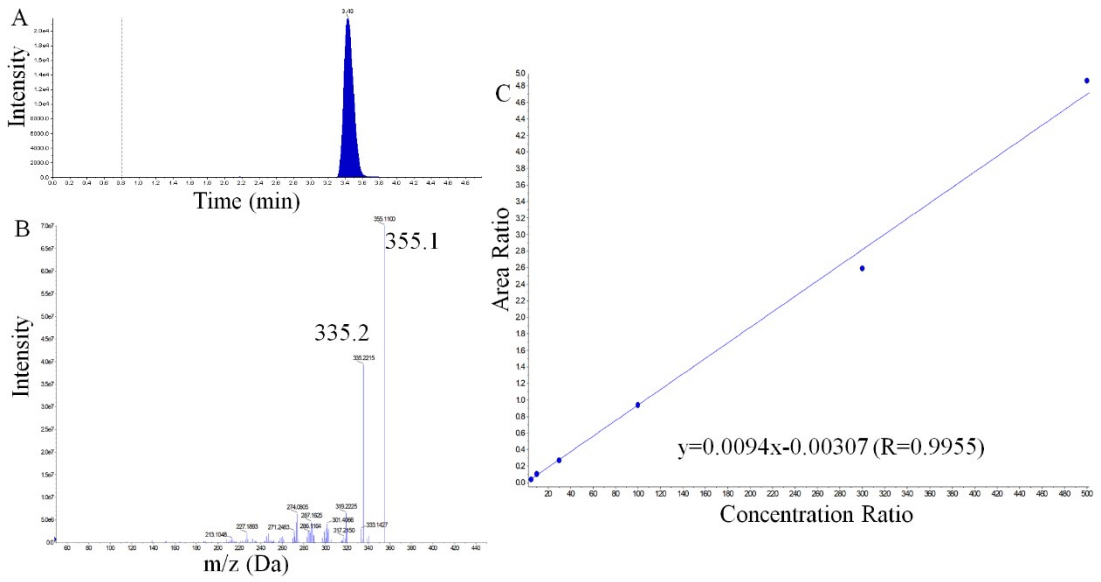


Fig. S11 (A and B) LC-MS/MS of G-OHBDP. (C) The quantitative standard curve of G-OHBDP.

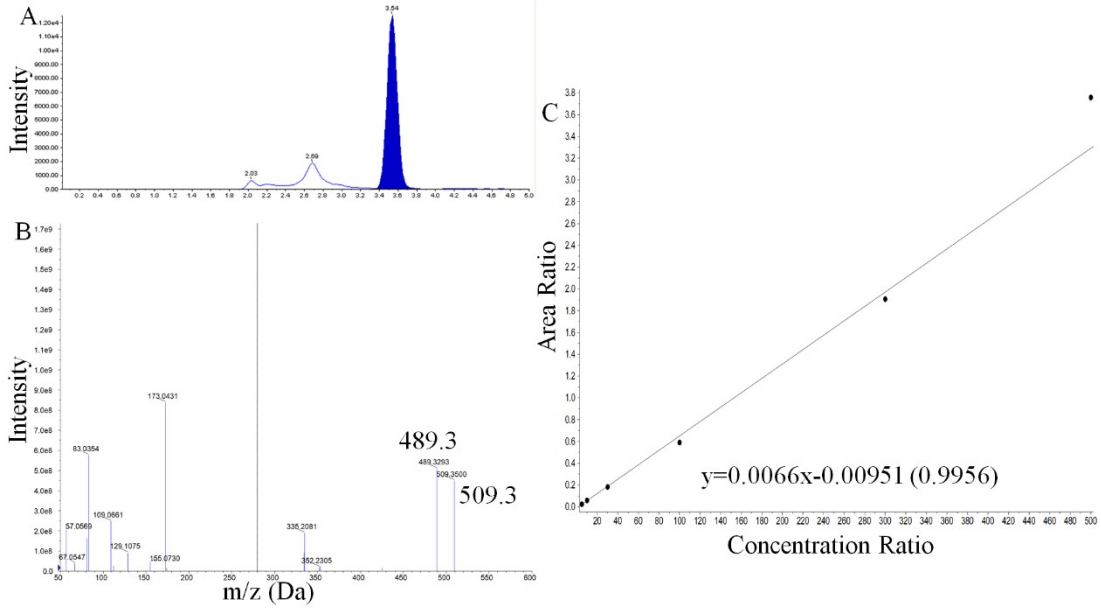


Fig. S12 (A and B) LC-MS/MS of G-BDPCCOOH. (C) The quantitative standard curve of G-BDPCCOOH.

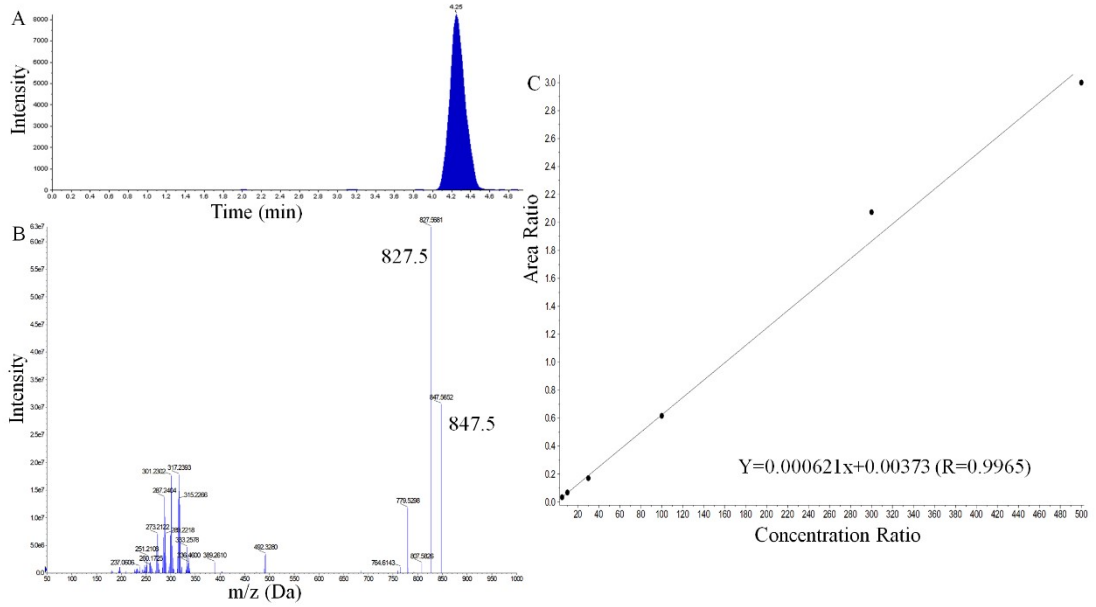


Fig. S13 (A and B) LC-MS/MS of G-BDPCC. (C) The quantitative standard curve of G-BDPCC.

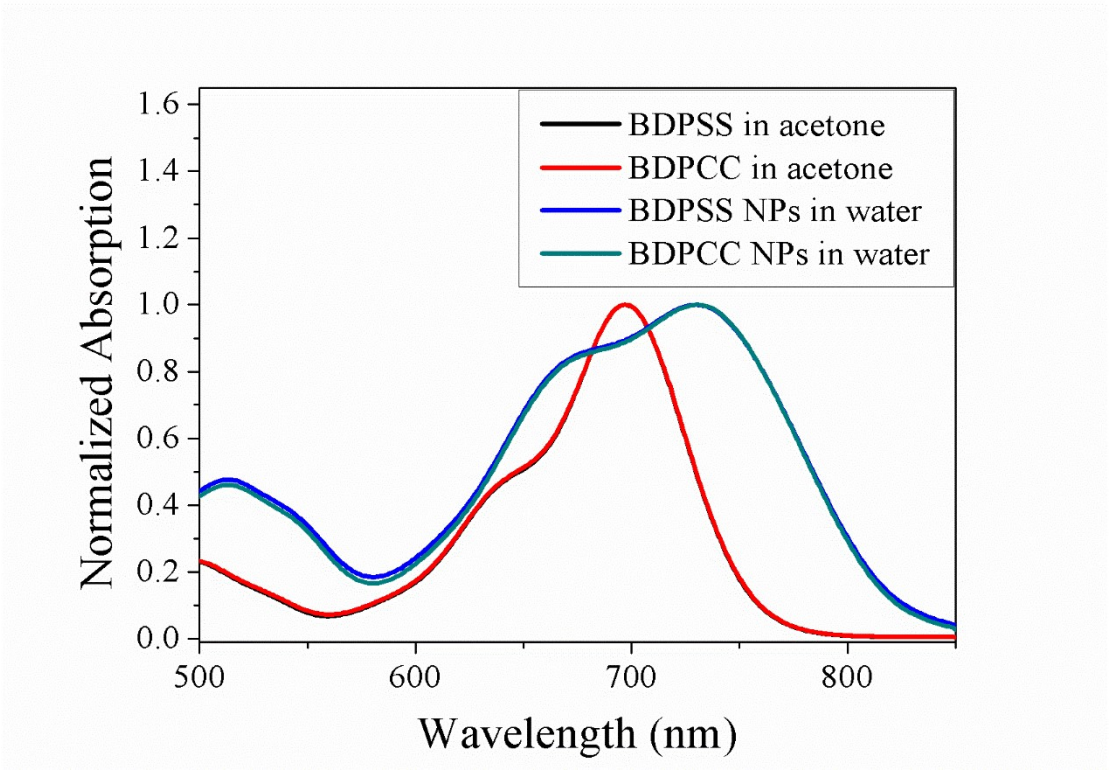


Fig. S14 The absorption of BDPSS and BDPCC in acetone and their nanoparticles in water.

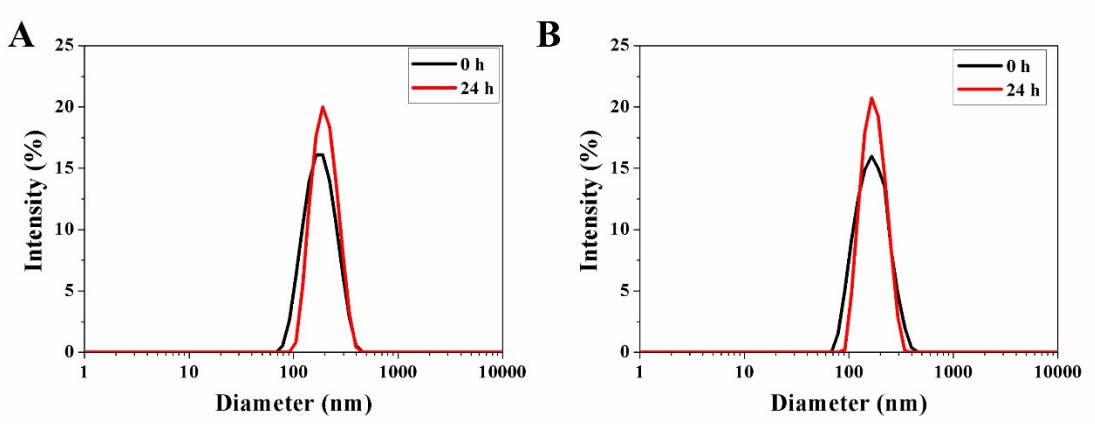


Fig. S15 The DLS result of (A) BDPCC@PEG-PLA NPs and (B) BDPSS@PEG-PLA NPs in DMEM with 10 % FBS for 24 h.

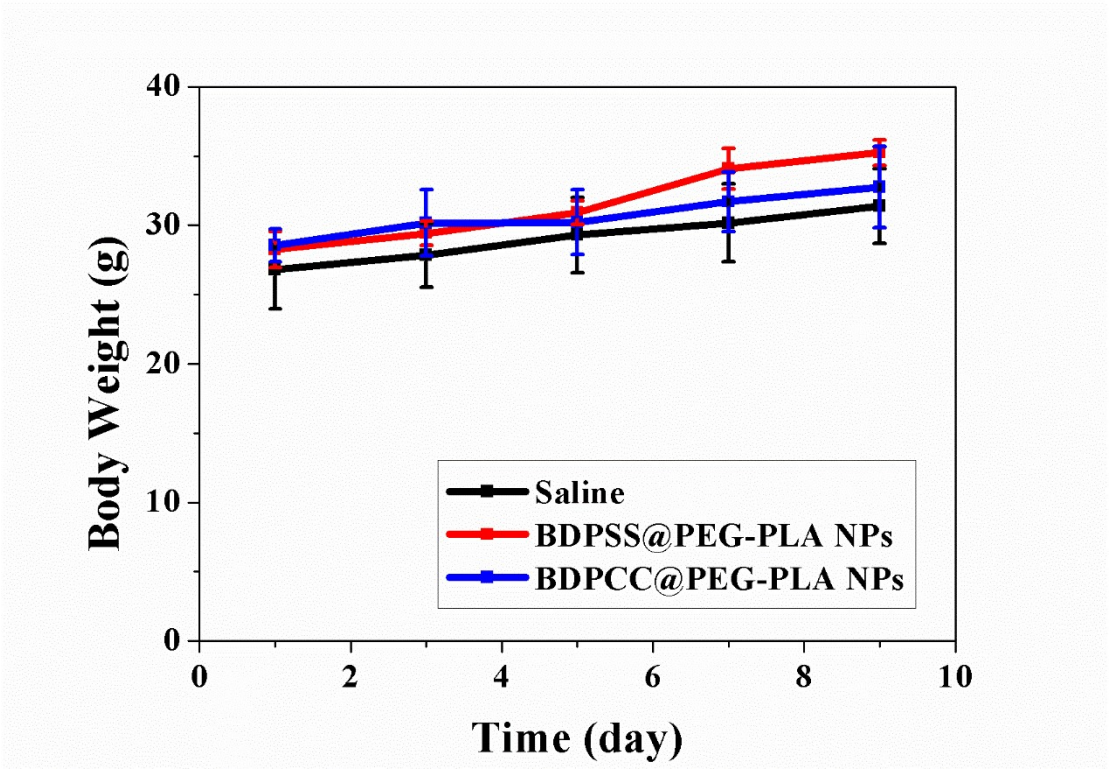


Fig. S16 The body wights of mice (n +) treated with saline, BDPSS@PEG-PLA NPs (500 μg/mL) and BDPCC@PEG-PLA NPs (500 μg/mL), respectively.

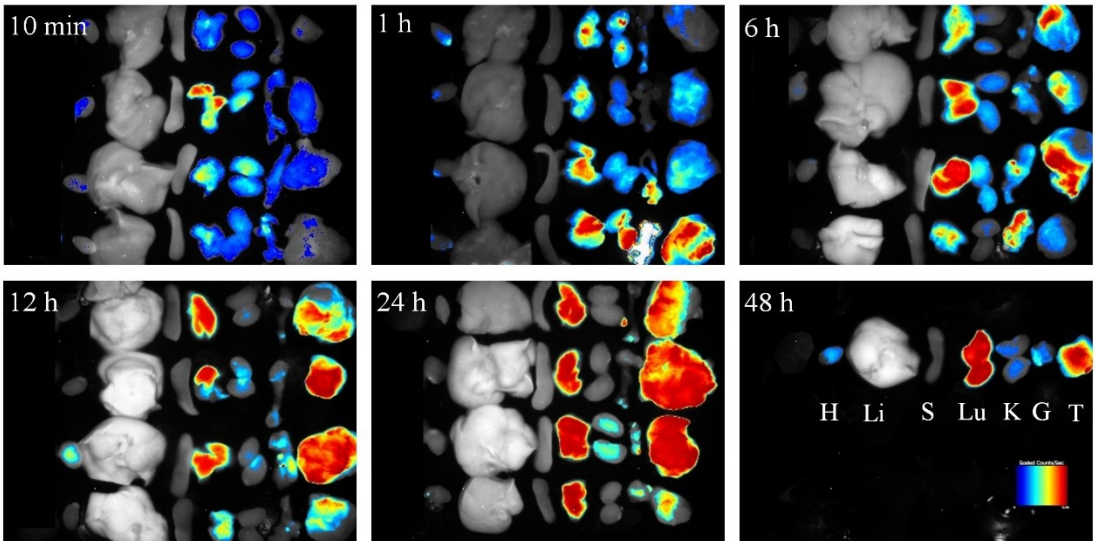


Fig. S17 NIRF imaging of main organs and tumors after the mice were injected with BDPSS@PEG-PLA NPs. Heart (H), liver (Li), spleen (S), lung (Lu), kidney (K), genitals (G) and tumor (T).

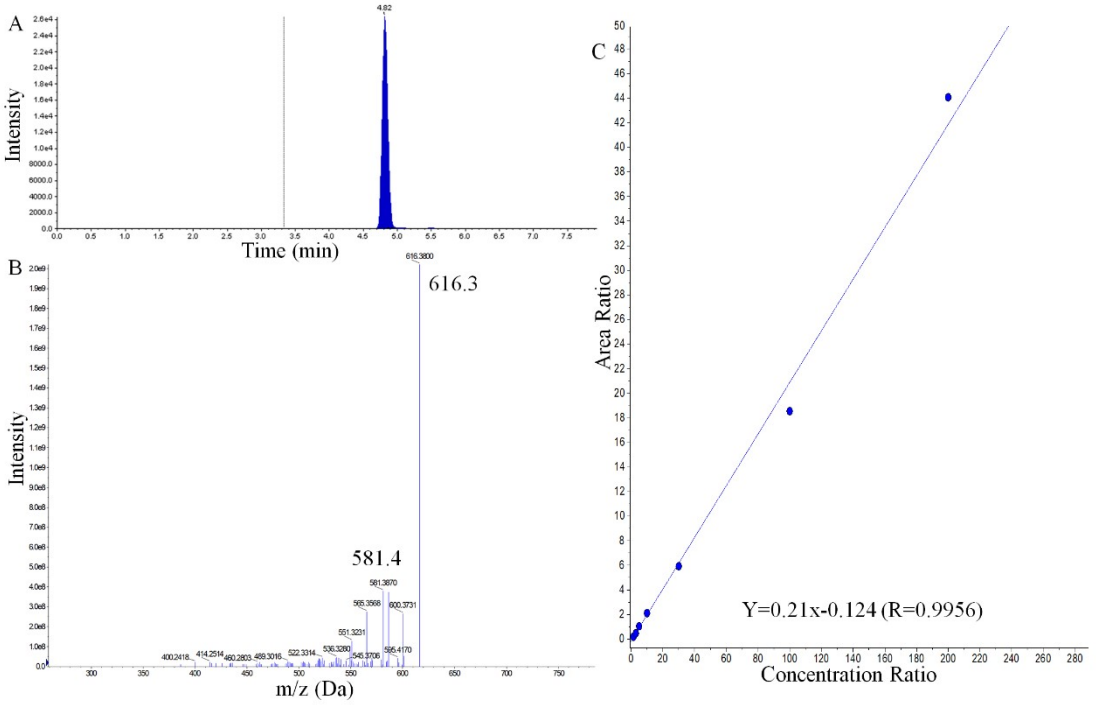


Fig. S18 (A and B) LC-MS/MS of OHBDP. (C) The quantitative standard curve of OHBDP.

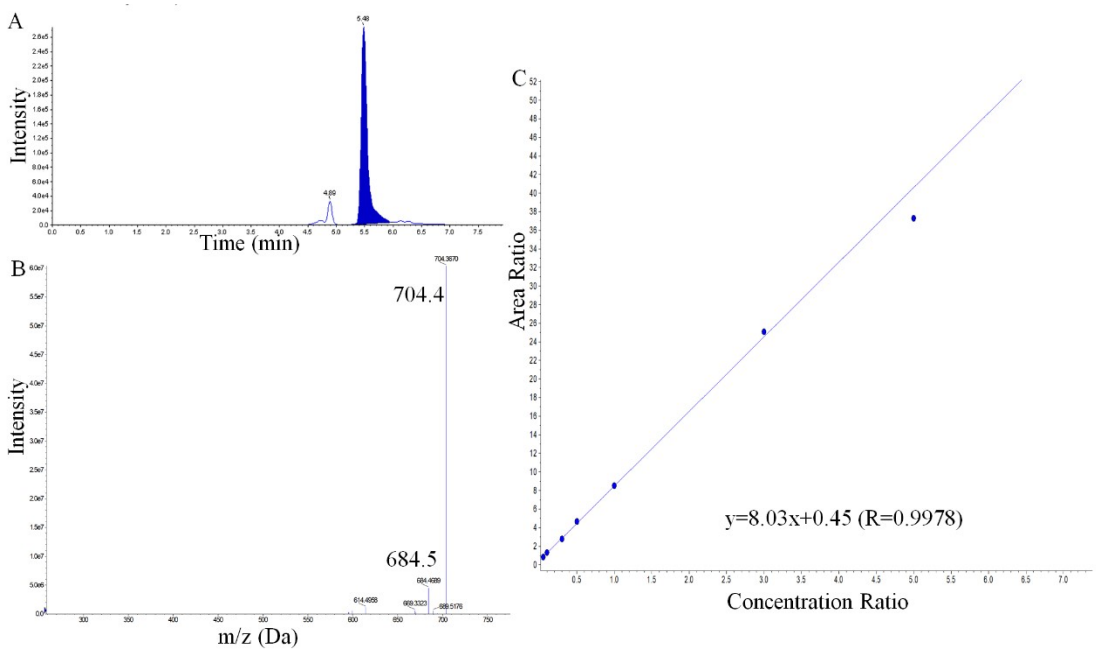


Fig. S19 (A and B) LC-MS/MS of BDPSS. (C) The quantitative standard curve of BDPSS.

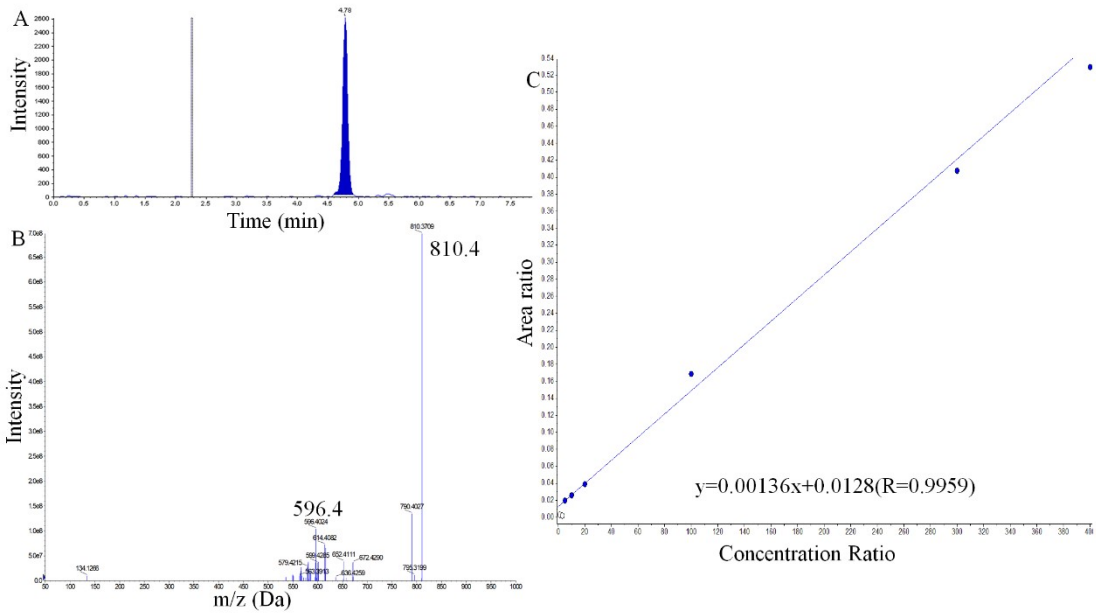


Fig. S20 (A and B) LC-MS/MS of BDPSCOOH. (C) The quantitative standard curve of BDPSCOOH.

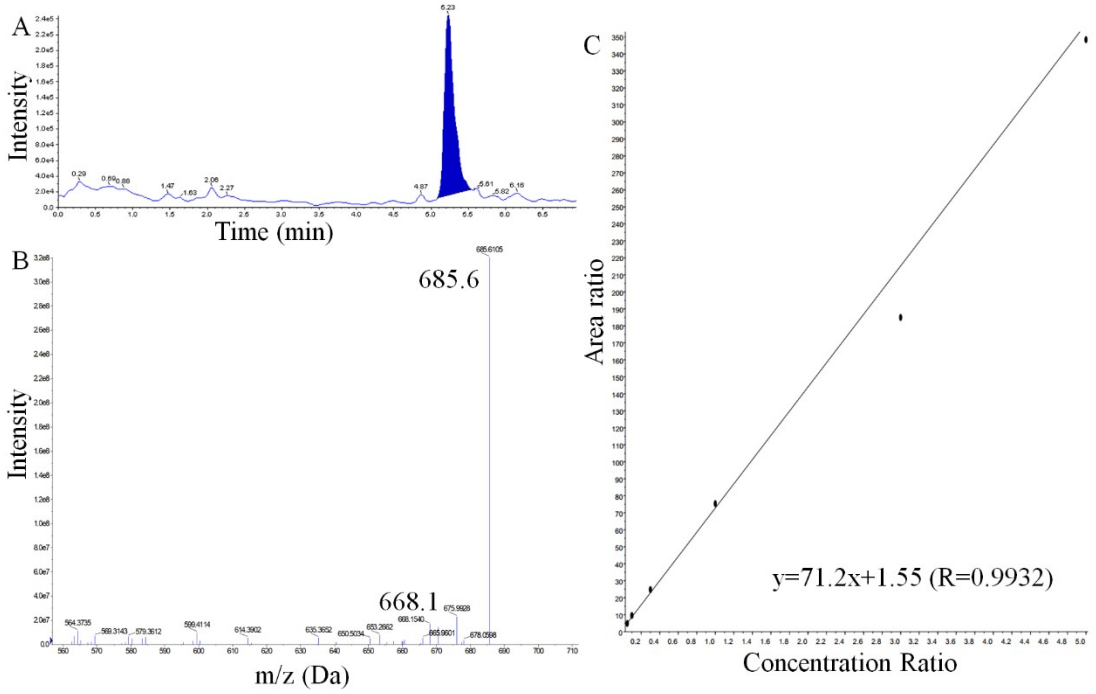


Fig. S21 (A and B) LC-MS/MS of BDPCC. (C) The quantitative standard curve of BDPCC.

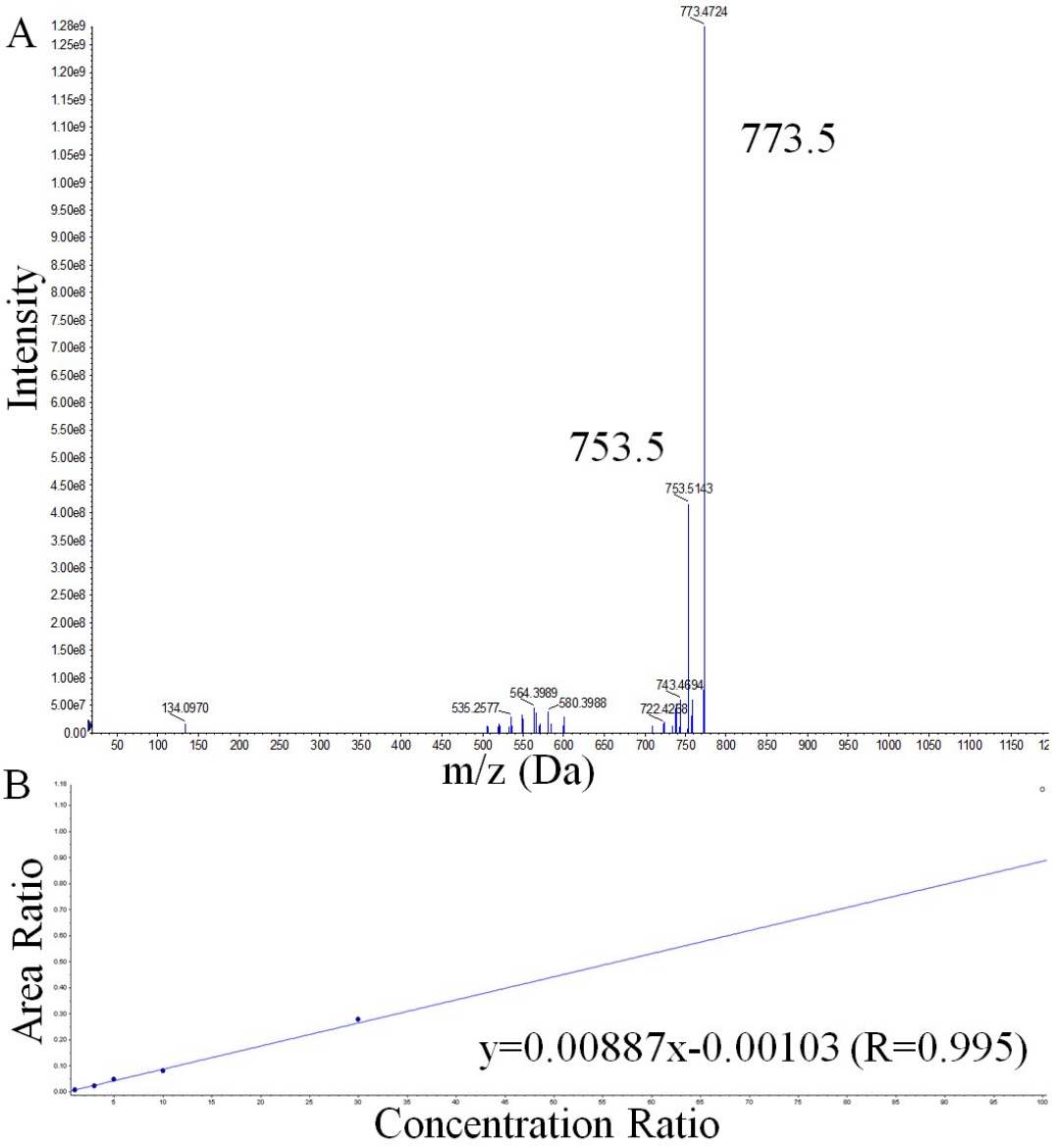


Fig. S22 (A) LC-MS/MS of BDPCCOOH. (B) The quantitative standard curve of BDPCCOOH.