Electronic Supplementary Information for

Synthesis of lipid-black phosphorus quantum dot bilayer vesicles for near-infrared-controlled drug release

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Experimental details

Materials. The BP crystals were purchased from a commercial supplier (Mophos, China) and stored in a dark Ar glove box. N-methyl-2-pyrrolidone (NMP) (99.5%, anhydrous) was purchased from Aladdin Reagents, $Ti(O^iPr)_4$ and *p*-toluenesulfonic acid were obtained from Alfa-Aesar. L-α-phosphatidylcholine (from dried egg yolk, 99%) and cholesterol were acquired from Sigma-Aldrich, and DOX was bought from Melone Pharmaceutical Co., Ltd (Dalian, China). All the reagents were analytical grade and used without further purification.

Synthesis and surface coordination of BPQDs. The BPQDs was synthesized by simple liquid exfoliation described previously.[1] Briefly, 30 mg of the BP powder were added to 30 mL NMP followed by sonication with a sonic tip of 19-25 KHz for 3 h (2 s with the interval of 4 s) at the power of 1200 W. The dispersion was sonicated in an ice bath for another 12 h at 300 W. The resulting dispersion was centrifuged at 9,000 rpm for 15 min and the supernatant containing BPQDs were decanted gently. Synthesis of TiL_4 and surface coordination of BPQDs proceeded as described in the literature.[2]

Preparation of BPQDs@Lipo. The BPQDs@Lipo was prepared by a modified thin lipid film hydration method. L-α-phosphatidylcholine (20 μmol), cholesterol (20 μmol), and BPQDs (5 μmol) were dissolved in $CHCl_3$ and dried by a rotary evaporator at reduced pressure at 37 °C to produce a thin film. The film was further dried under vacuum for 1 h to ensure complete solvent removal. The lipid film was hydrated with PBS (pH 7.4) and sonicated by a VCX 130 probe sonicator (Sonics and Materials, Inc.) for 20 min (50 s on and 10 s off in each cycle). The temperature was controlled with

the aid of an ice water bath and centrifugation at 14,000 rpm for 10 min was performed to remove the excess BPQDs not incorporated into the lipid bilayers.

Characterization. TEM and HR-TEM were performed on the JEM-3200FS field emission transmission electron microscope (JEOL, Japan) at 200 kV and HR-XPS was conducted on the Thermo Fisher ESCALAB 250Xi XPS. The size distribution and zeta potential of the liposomes before and after the incorporation of the BPQDs were determined by dynamic light scattering at room temperature following 1:20 dilution with PBS (Zetasizer 3000 HAS, Malvern Instruments Ltd., UK).

Loading of DOX into BPQDs@Lipo. DOX was loaded into liposomes by the transmembrane pH gradient-driven encapsulation technique (inside acidic) as described in the literature.[3] A Sephadex G-50 column eluted with PBS (pH 7.4) was used to separate the unencapsulated drug. The loading efficiency was calculated by fluorescence spectrophotometry (λ_{ex} = 505 nm, λ_{em} = 559 nm, Hitachi F-4600, Japan) according to the following equation: Encapsulation efficiency (%) = 100 × (I_{max} I_o)/I_{max}, where I_o is the fluorescence intensity of the liposome suspension at the initial time and I_{max} is the fluorescence intensity after the addition of 0.5% Triton X-100.

NIR-light-induced heat conversion. One milliliter of BPQDs@Lipo was placed in a quartz cuvette with a 1 cm path length and irradiated with a fiber-coupled continuous semiconductor diode laser (808 nm, KS-810F-8000, Kai Site Electronic Technology Co., Ltd. Shanxi, China) at a power density of 0.5 W/cm^2 for 10 min. The laser spot was adjusted to cover the entire surface of the sample and an infrared thermal imaging camera (Fluke Ti27, USA) was used to record the temperature change. The samples were irradiated with 1.0 and 1.5 W/cm^2 808 nm laser using the same method, respectively.

NIR-light-controlled drug release. One milliliter of DOX-loaded BPQDs@Lipo was placed in a quartz cuvette with a 1 cm path length and each sample was irradiated with the 0.5, 1.0, and 1.5 W/cm^2 808 nm laser, respectively. The DOX release behavior was measured by fluorescence spectrophotometry (Hitachi F-4600, Japan) at different time points. The amount of DOX released (%) from liposomes was calculated by the following equation: DOX released (%) = 100 × (I_t – I_o)/(I_{max} I_o), where I_o is the fluorescence intensity of the sample at the initial time, I_{max} is the fluorescence intensity after the addition of 0.5% Triton X-100, and I_t is the measured fluorescence intensity at various time intervals[3].

Cell cultures. The cell lines were purchased from China type culture collection (CTCC) through the American Type Culture Collection (ATCC). A549, MCF-7, and L929 were cultured in DMEM medium supplemented with 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin under humidified conditions of 5% CO_2 at 37 °C and QSG-7701 was cultured in the normal RPMI-1640 medium under the same conditions.

***In vitro* toxicity assay.** The cells were seeded on 96-well plates at a density of 5 × 10^4/mL and cultured for 24 h. The culture medium was replaced with a medium containing serial dilutions of BPQDs@Lipo and the final concentrations of the lipids were adjusted to 0.1, 0.2, 0.5, 1.0, and 2.0 μmol/mL. After incubation for 24 h, the medium was removed and a medium containing the 10% (v/v) CCK-8 solution was added to each well followed by incubation for 2 h. A microplate reader (FilterMax F5, Molecular Devices, USA) was used to measure the absorbance at 450 nm. The untreated cells were represented as control group.

Intracellular controlled drug release. The MCF-7 cells were seeded on confocal dishes at a density of 5 × 10^4/mL and cultured for 24 h. The culture medium was

removed and a medium containing DOX-loaded BPQDs@Lipo with DOX concentration of 20 μg/mL was added to each dish. After incubation for 2 h, the cells were irradiated with the 808 nm laser (0.5 W/cm^2 for 5 min, 1.0 W/cm^2 for 5 min, 1.0 W/cm^2 for 10 min, and 1.0 W/cm^2 for 15 min). The cells treated with the same sample but without laser irradiation served as the control. After culturing for another 1 h, the cells were washed with PBS twice. The cell nucleus were stained by DAPI (4',6-diamidino-2-phenylindole) with the concentration of 100 ng/mL for 10 min. Fluorescent images were taken on a confocal microscope (Leica TCS SP5). The cell viability in the different treatments was assessed by the CCK-8 kit.

***In vitro* antitumor efficiency.** The MCF-7 cells were seeded on a 96-well plate at a density of 5 × 10^4/mL and cultured for 24 h. The culture medium was replaced with blank BPQDs@Lipo or DOX-loaded BPQDs@Lipo in the medium and the lipid concentrations in different groups were 0.5 μmol/mL to keep the same BPQD concentration. After incubation for 4 h, the cells in the photothermal therapy group and chemo-photothermal therapy group were illuminated with the 808 nm laser (1.0 W/cm^2) for 10 min. The cells without sample treatment were also irradiated with the 808 nm laser (1.0 W/cm^2) for 10 min to determine the cytotoxicity of NIR light. The cells were incubated for another 24 h and the cell viability of each group was determined by the CCK-8 kit. In the live/dead assay, the medium was removed and Calcein-AM (5 μg/mL) and PI (5 μg/mL) were added. After incubation for 15 min, the cells were washed with PBS and fluorescent images were captured by a fluorescence microscope (IX71, Olympus, Japan).

Supplementary table and figures

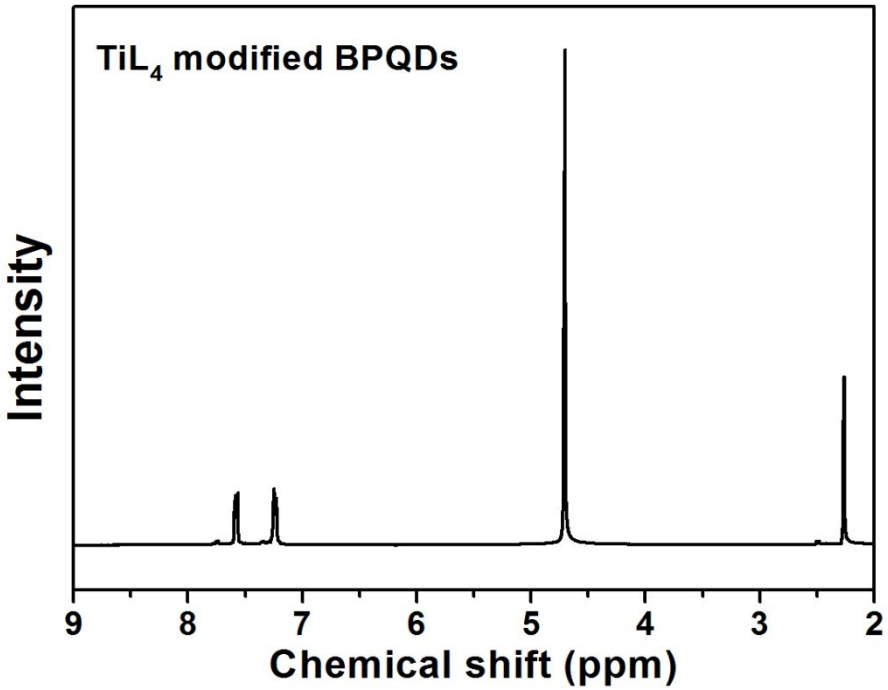

Fig. S1 ^{1}H NMR spectra of the BPQDs modified by TiL_4.

Table S1. Liposome characterization by DLS (n = 3).

Sample	Size (nm)	PdI	Zeta potential (mV)
Liposome	107.2 ± 4.9	0.19 ± 0.02	−5.6 ± 0.3
BPQDs	---	---	22.4 ± 0.3
BPQDs@Lipo	105.6 ± 6.8	0.20 ± 0.03	0.5 ± 0.1

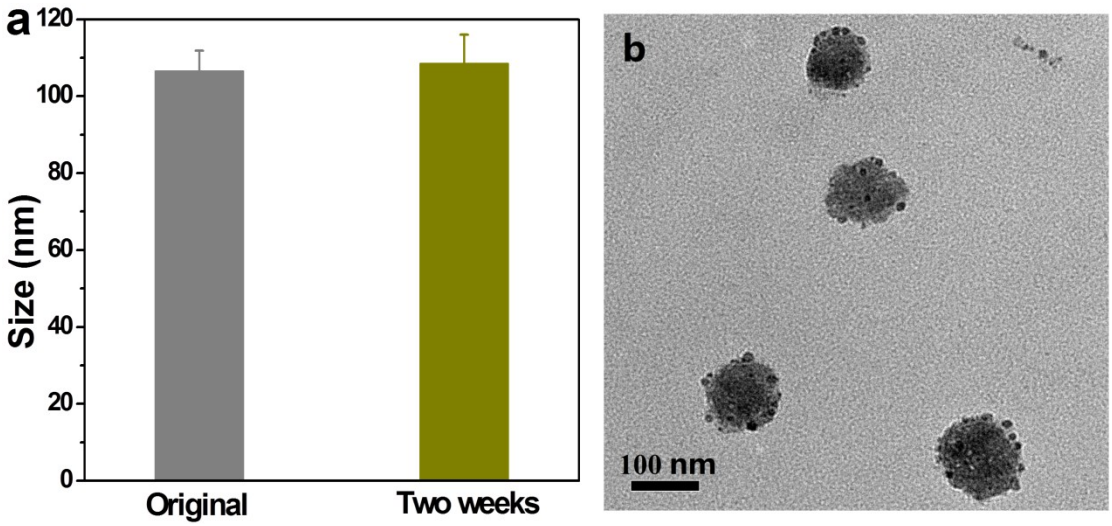

Fig. S2 (a) The size change of BPQDs@Lipo measured by DLS (n = 3); (b) TEM image after storing in 4 °C for two weeks.

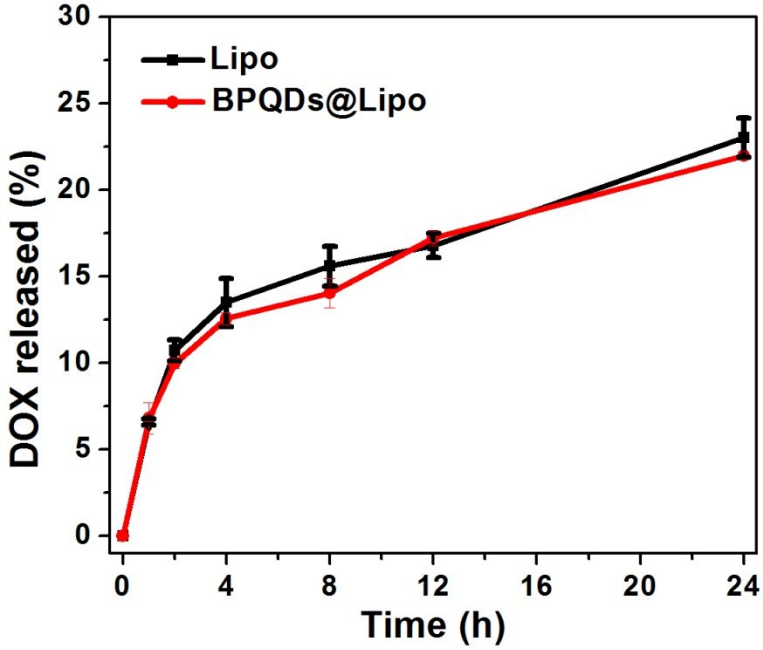

Fig. S3 Drug release behavior of liposomes before and after BPQDs incorporation in PBS at 37 °C (n = 3).

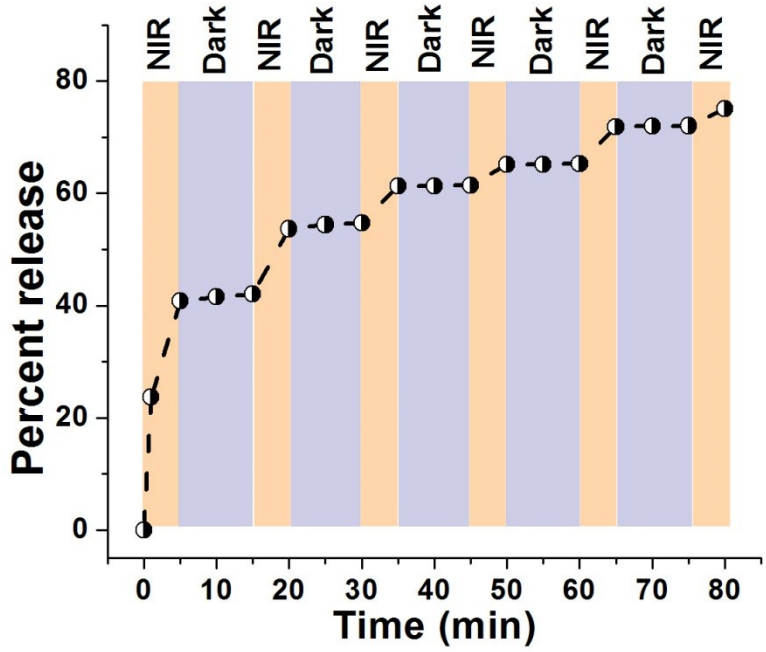

Fig. S4 Percent release profile of DOX from BPQDs@Lipo in the alternating presence of NIR light and dark. The power density of 808 nm laser is 1.0 W/cm^2.

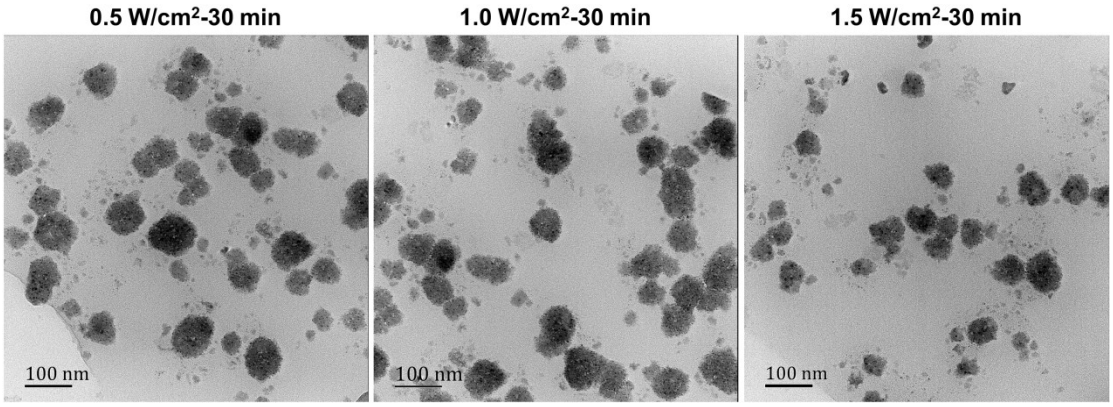

Fig. S5 TEM images of the BPQDs@Lipo after the NIR-controlled drug release experiments.

References

1 Z. B. Sun, H. H. Xie, S. Y. Tang, X. F. Yu, Z. N. Guo, J. D. Shao, H. Zhang, H. Huang, H. Y. Wang and P. K. Chu, *Angew. Chem. Int. Edit.*, 2015, **54**, 11526-11530.

2 Y. T. Zhao, H. Y. Wang, H. Huang, Q. L. Xiao, Y. H. Xu, Z. N. Guo, H. H. Xie, J. D. Shao, Z. B. Sun, W. J. Han, X. F. Yu, P. H. Li and P. K. Chu, *Angew. Chem. Int. Edit.*, 2016, **55**, 5003-5007.

3 S. Y. Geng, B. Yang, G. W. Wang, G. Qin, S. Wada and J. Y. Wang, *Nanotechnology*, 2014, **25**.