Electronic Supplementary Material (ESI) for ChemComm.


# Electronic Supplementary Information

## Multifunctional supramolecular vesicles for combined photothermal/photodynamic/hypoxia-activated chemotherapy

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## *1. General information and experimental procedure*

General information

All reactions were performed in atmosphere unless noted. The commercially available reagents and solvents were either employed as purchased or dried according to procedures described in the literature. Compounds **WP5**,[S1] and **1**,[S2] were prepared according to literature procedure. NMR spectra were recorded on a Bruker DPX 400 MHz spectrometer with internal standard tetramethylsilane (TMS) and solvent signals as internal references, and the chemical shifts (δ) were expressed in ppm and *J* values were given in Hz. Low-resolution electrospray ionization mass spectra were obtained on Finnigan MatTSQ 7000 instruments.

Experimental procedure

Fabrication of the TPZ-loaded vesicles: TPZ-loaded vesicles were prepared as follows: A certain amount of TPZ was added to an aqueous solution of **G**, then a certain amount of **WP5** solution was added quickly, and finally water was added until the volume of the solution reached 10 mL. The ultimate concentrations of TPZ, **G**, and **WP5** were 0.002, 0.01, and 0.0033 mM, respectively. After standing overnight, the prepared TPZ-loaded vesicles were purified by dialysis (molecular weight cutoff 10000) in distilled water for several times until the water outside the dialysis tube exhibited negligible TPZ fluorescence.

The TPZ encapsulation efficiency was calculated by the following equation:

Encapsulation efficiency (%) = $(m_{\text{TPZ-loaded}}/m_{\text{TPZ}}) \times 100$

where $m_{\text{TPZ-loaded}}$ and $m_{\text{TPZ}}$ are mass of TPZ encapsulated in vesicles and mass of TPZ added, respectively. The mass of TPZ was measured by the fluorescence emission intensity at 540 nm and calculated as relative to a standard calibration curve in the concentrations from 0.85 to 5 μM in water.

pH-responsive behavior of the TPZ-loaded vesicles: 0.01 M tris-HCl (pH = 7.4) and 0.01 M phosphate (pH = 6.0 and 4.5) buffer solutions were used as drug release media to simulate normal physiological conditions and the intracellular conditions of

tumor. In a typical release experiment, 6 mL of TPZ-loaded vesicles were added into 4 mL of appropriate release medium at 37 °C. At selected time intervals, 2 mL of the release media was taken out for measuring the released TPZ concentrations by the fluorescence emission technique and then was returned to the original release media. The concentration of TPZ was determined by measurement of fluorescence emission intensity at 540 nm using a standard intensity *vs* concentration curve constructed for TPZ in the corresponding release buffer. By presenting the vesicles to very low pH (the solution of HCl, pH = 3.0), a nearly 100% release of TPZ from TPZ-loaded vesicles could be obtained.

Cell culture: MCF-7 cells were cultured in the Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 50 U/mL$^{-1}$ penicillin and 50 U/mL$^{-1}$ streptomycin at 37 °C in a humidified atmosphere containing 5% $CO_2$.

*In vitro* cell cytotoxicity assay: The relative cytotoxicities of biomaterials against MCF-7 cells were evaluated in vitro by using the MTT assay. First, the cells were seeded in 96-well culture plates ($5\times10^3$ cells per well) with 150 μL DMEM medium containing 10% fetal bovine serum, supplemented with 50 U/mL$^{-1}$ penicillin and 50 U/mL$^{-1}$ streptomycin, and cultured under 5% $CO_2$ at 37 °C for 24 h. Second, fresh DMEM containing unloaded vesicles, TPZ-loaded vesicles were cultured with cells for 24 h with the concentration of **G** ranging from 5 to 30 μg/mL. For dark cytotoxicity, the cells were incubated for another 24 h. For combination therapy, the cells were irradiated by 660 nm laser (1.5 W/cm$^2$) for 10 min after 4 h of incubation, and then incubated in fresh DMEM for 24h. After that, 20 μL of MTT solution was added into each cell and incubated for another 4 h. Later, the medium containing MTT was removed and DMSO (100 μL) solution was added to each well to dissolve the MTT formazan crystals. Finally, the plates were shaken for 10 min, and the absorbance of formazan product was measured at 490 nm by a microplate reader (BioTek ELx808). Untreated cells in media were used as the blank control. All experiments were carried out with three replicates. The cytotoxicity was expressed as the percentage of the cell viability as compared with the blank control.

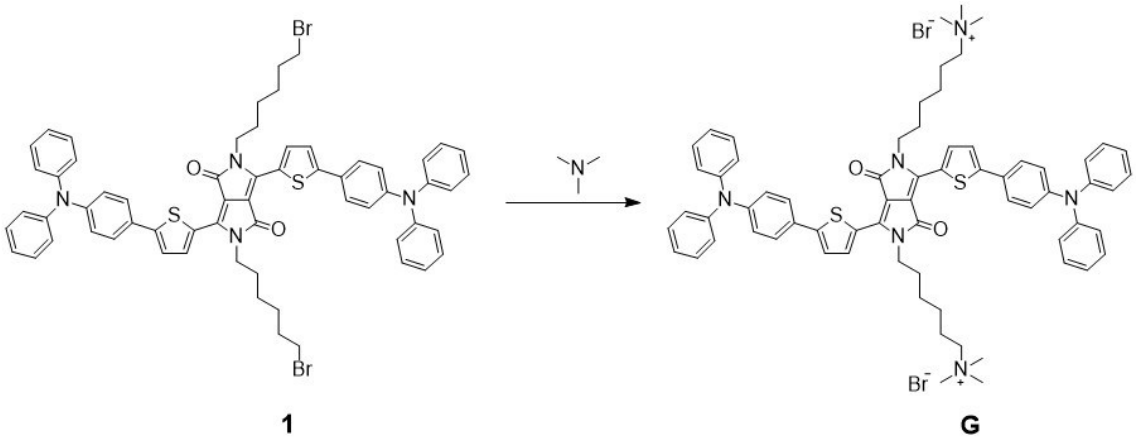
Cellular uptake and intracellular localization observed by CLSM: The cellular

uptake and intracellular localization of TPZ were examined in MCF-7 cancer cells. Briefly, the cells were seeded into the confocal dishes with 2 mL DMEM medium containing 10% fetal bovine serum, supplemented with 50 U/mL$^{-1}$ penicillin and 50 U/mL$^{-1}$ streptomycin, and cultured under 5% $CO_2$ at 37 °C for 24 h. The cells were incubated with TPZ-loaded vesicle (1 μg/mL TPZ) at 37 °C for 1 h and 5 h. Then, the culture medium was removed and cells were washed with PBS thrice. Thereafter, the Lyso-Tracker Red (Nanjing KeyGen Biotech. Co. Ltd.) was added to the medium to label lysosomes. Next, the cells were washed three times with PBS and the Hochest 33342 (Nanjing KeyGen Biotech. Co. Ltd.) was added to the medium to stain nuclei. Finally, all of the cell samples were observed using CLSM.

Flow cytometry: MCF-7 cells ($5 \times 10^4$ cells/well) were seeded into the dishes with 2 mL DMEM medium containing 10% fetal bovine serum, supplemented with 50 U/mL$^{-1}$ penicillin and 50 U/mL$^{-1}$ streptomycin and cultured at 37 °C in a 5% $CO_2$ humidified atmosphere for 24 h. Then, the cells were incubated with free TPZ and TPZ-loaded vesicles for 1 h and 5 h with the concentration of TPZ at 1μg/mL. After that, the cells were then rinsed three times with cold PBS. After trypsinizing, the cells were washed with cold PBS, centrifuged, and dispersed in cold PBS. And then, the cells were tested by using flow cytometry (Merck Millipore, Darmstadt, Germany).

Statistical analysis: All the results were expressed as means ± standard deviation. Significant differences of the mean values were evaluated by unpaired Student's t-test.

## 2. *Synthesis of guest* ***G***




**Scheme S1** Synthesis of guest **G**.

Compound **1** (0.4 g, 0.36 mmol) was dissolved in $CHCl_3$ (20 mL), and trimethylamine (33% in ethanol, 10 mL) was added. The resulting solution was refluxed for 24 h. Then, the mixture was concentrated under reduced pressure and the obtained residue was dissolved in a small amount of anhydrous MeOH (1 mL), which was then added dropwise to plenty of diethyl ether (150 mL), the precipitates was collected by filtration, washed by diethyl ether and dried in vacuum, and the target guest compound **G** was obtained as a black solid (0.3 g, 0.28 mmol, 78%). $^1$H NMR (400 MHz, DMSO-$d_6$, 298 K) $\delta$ (ppm): 8.91 (d, 2H), 7.74-7.70 (m, 6H), 7.39-7.35 (m, 8H), 7.16-7.09 (m, 12H), 7.00 (d, 4H), 4.08 (t, 4H), 3.26-3.22 (m, 4H), 3.00 (s, 18H), 1.73-1.64 (m, 8H), 1.47-1.39 (m, 4H), 1.34-1.28 (m, 4H). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 298 K) $\delta$ (ppm): 160.7, 150.0, 148.6, 146.9, 138.6, 136.9, 130.2, 127.5, 127.4, 126.0, 125.3, 124.6, 122.3, 107.1, 65.7, 52.6, 41.8, 29.9, 26.3, 26.0, 22.5. LR-ESI-MS: $m/z$ calcd. for $[M - 2Br]^{2+}$ = 535.27, found 535.55 (100%).

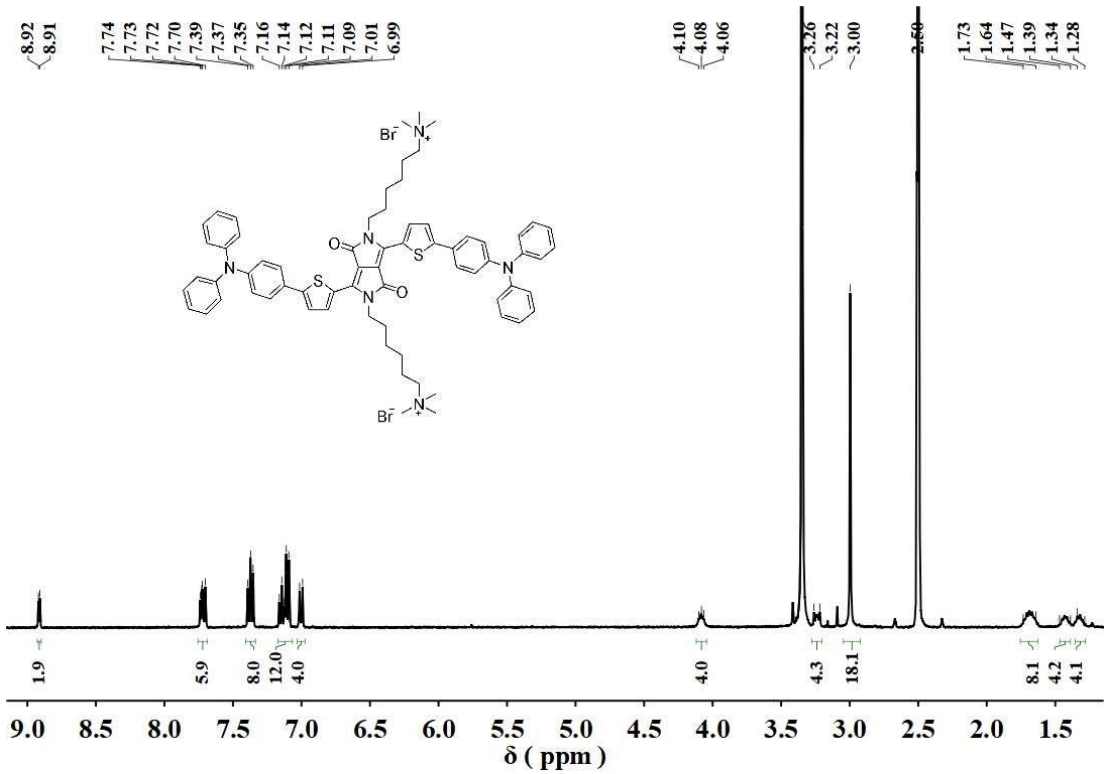



**Fig. S1** $^{1}$H NMR spectrum (400 MHz, DMSO-$d_6$, 298 K) of **G**.

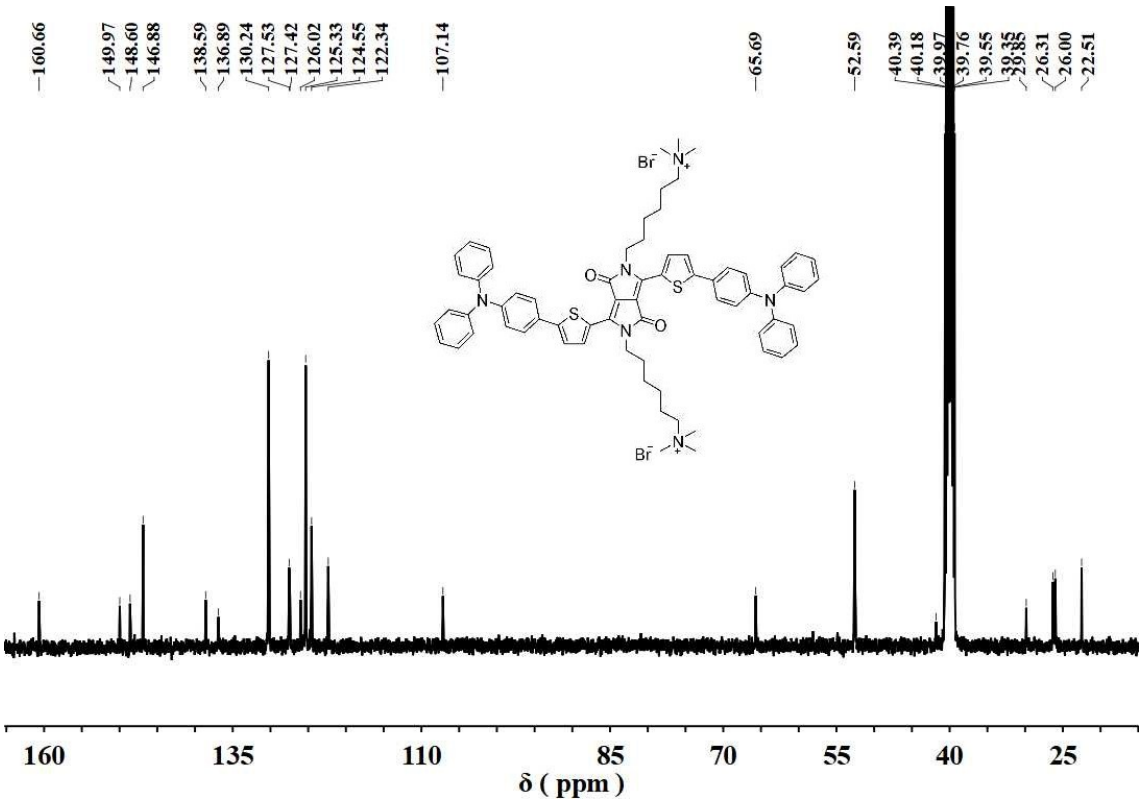



**Fig. S2** $^{13}$C NMR spectrum (100 MHz, DMSO-$d_6$, 298 K) of **G**.

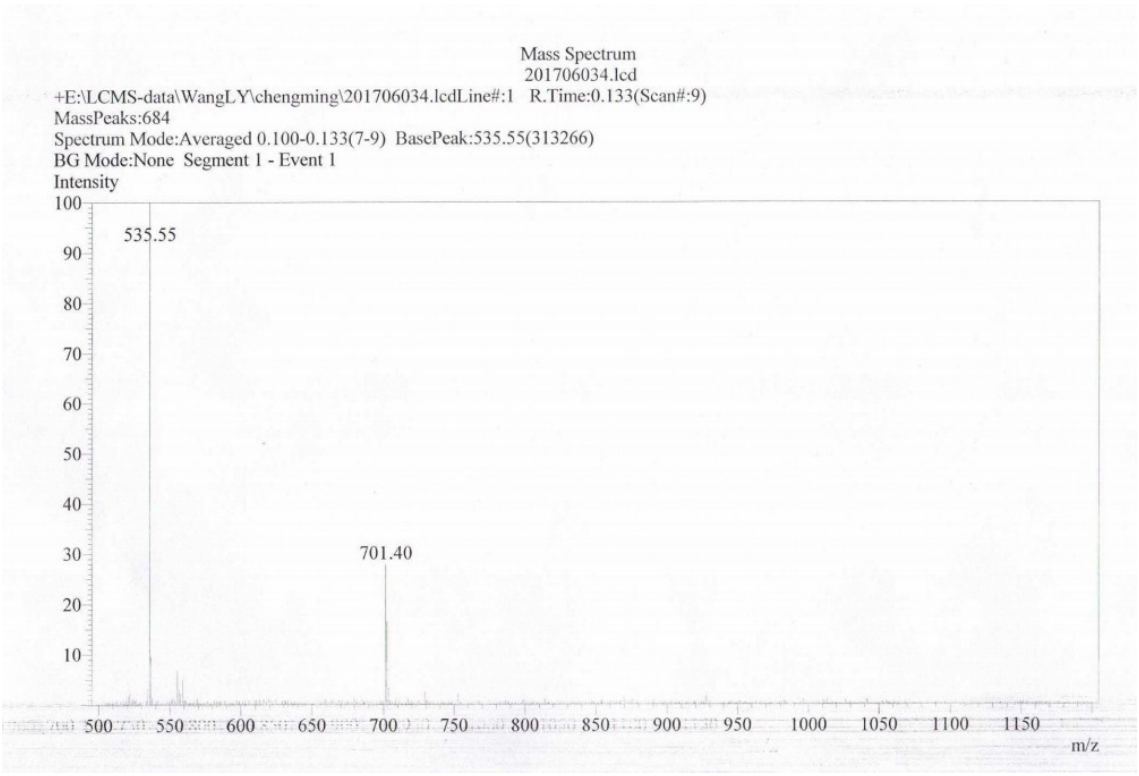



**Fig. S3** LR-ESI-MS spectrum of **G**.

## *3. Critical aggregation concentration determination of **G** and **(WP5)$_2$⊃G***

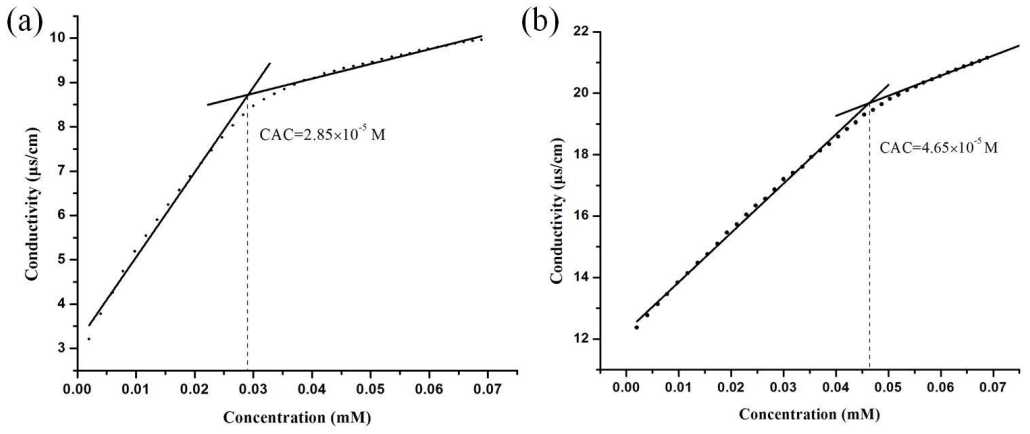



**Fig. S4** The concentration-dependent conductivity of **G** in the absence (a) and presence (b) of **WP5** ([**WP5**]/[**G**] = 1:8). The critical aggregation concentration (CAC) was determined to be 2.85 × $10^{-5}$ M for **G** and 4.65 × $10^{-5}$ M for **(WP5)$_2$⊃G**, respectively.

## *4. Determination of the best molar ratio of **WP5** and **G** leading to aggregation*

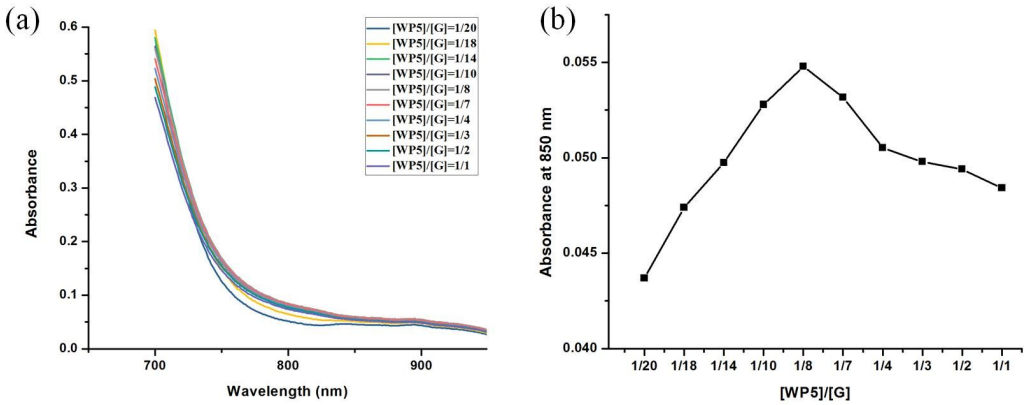



**Fig. S5** (a) UV-vis absorption of a mixture of **WP5** and **G** in water with constant **G** concentration (0.1 mM) upon increasing the concentration of **WP5** at 25 °C. (b) Dependence of the relative absorption intensity at 850 nm on the **WP5** concentration with a fixed concentration of **G** (0.1 mM) at 25 °C.

The best molar ratio between **WP5** and **G** for constructing supramolecular aggregates was investigated by UV-vis absorption spectroscopy. As shown in Fig. S5, upon gradually increasing the amount of **WP5**, the absorbance at 850 nm underwent a sharp increase and then an inverse decrease. The rapid increase of the absorbance implied that **WP5** and **G** formed a higher-order complex with a tendency toward amphiphilic aggregation, whereas it disassembled upon further addition of **WP5**, generating a simple 1:1 supramolecular inclusion complex. Thus, the best molar ratio of 1:8 ([**WP5**]/[**G**]) for the formation of supramolecular aggregates was observed at the inflection point.

## 5. *Models of* ***(WP5)$_2$⊃G*** *complex*

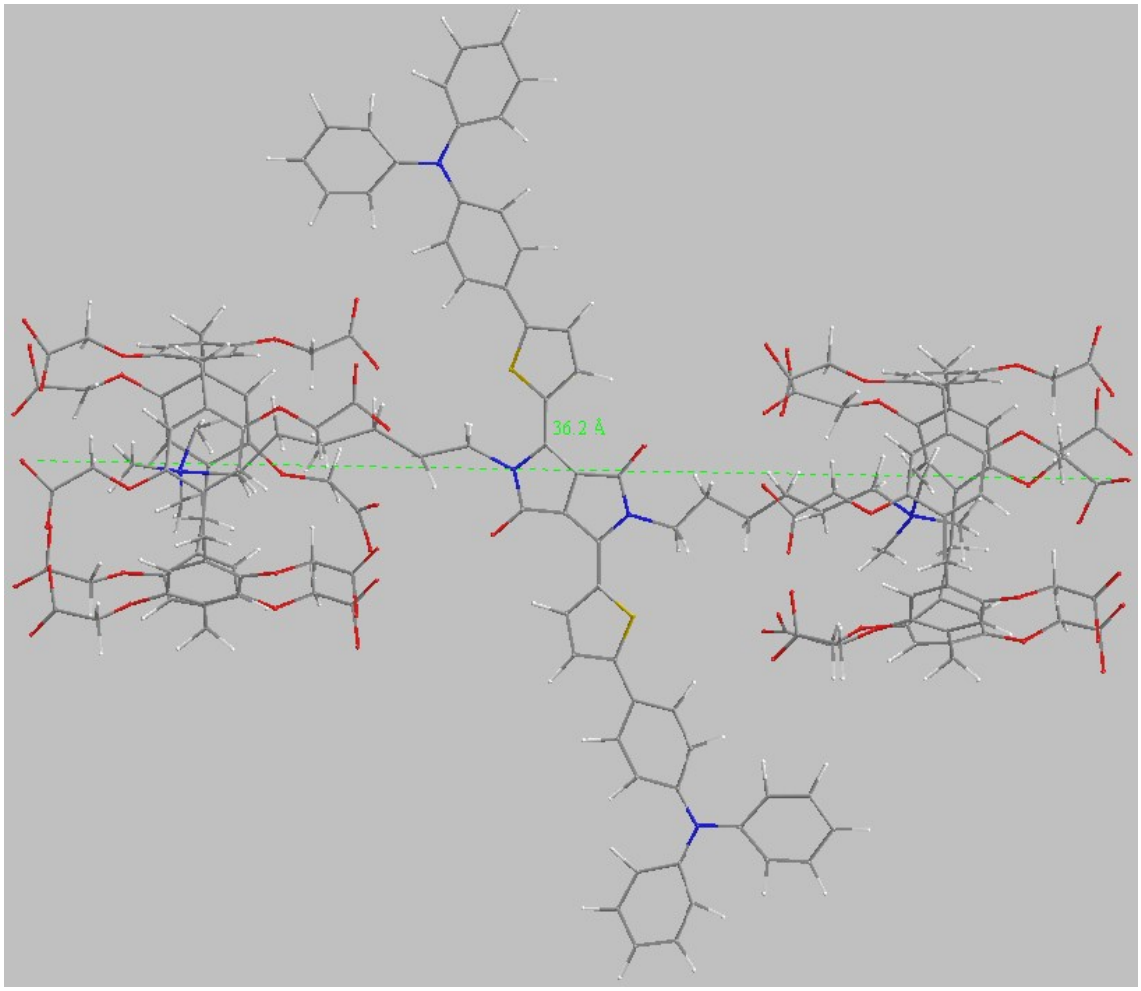



**Fig. S6** Models of the (**WP5**)$_2$⊃**G** complex (carbon atoms are gray, oxygen atoms are red, nitrogen atoms are blue, and hydrogen atoms are white). It was estimated by ChemBio3D Ultra 14.0.

## *6. ζ-potentials of the aggregates formed by* ***(WP5)$_2$⊃G***

ζ-potential experiment suggested that the formed supramolecular vesicles at the molar ratio of [**WP5**]/[**G**]=1/3 had a large negative ζ-potential (-30.99 mV), indicating the existence of repulsive forces among vesicles which can obviously enhance the stability of the obtained vesicles.[S3]

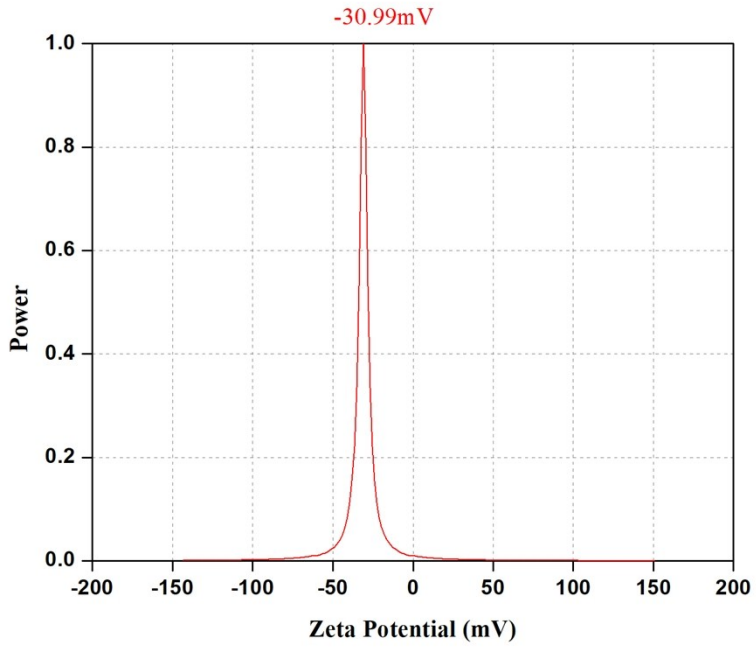



**Fig. S7** The ζ-potentials of (**WP5**)$_2$⊃**G** aggregates. [**WP5**]/[**G**]=1/3.

## 7. *UV-vis-NIR spectra of the vesicles*

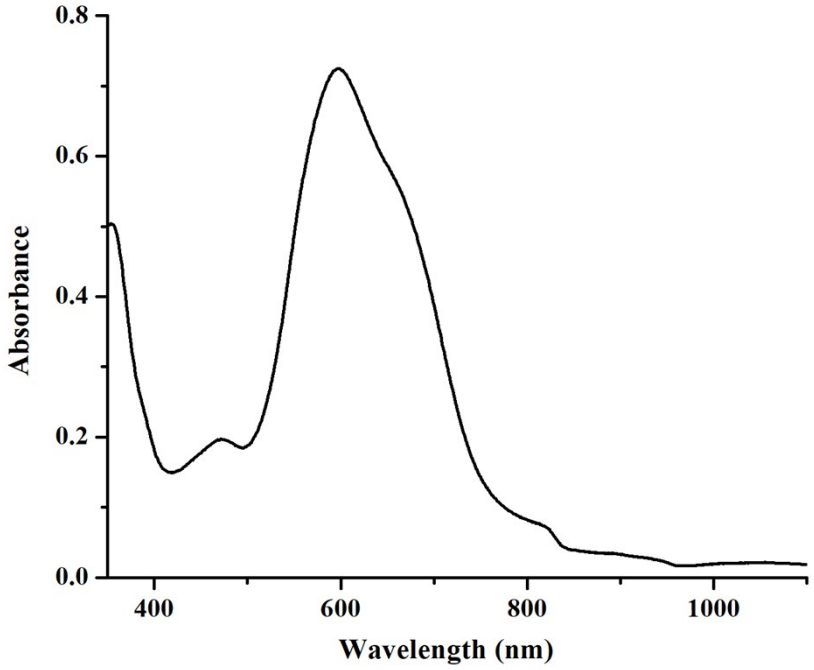



**Fig. S8** The UV-vis-NIR spectra of the vesicles ([**WP5**]/[**G**] = 1:3, [**G**]=0.05 mM).

## 8. *Infrared thermal images of the vesicles*

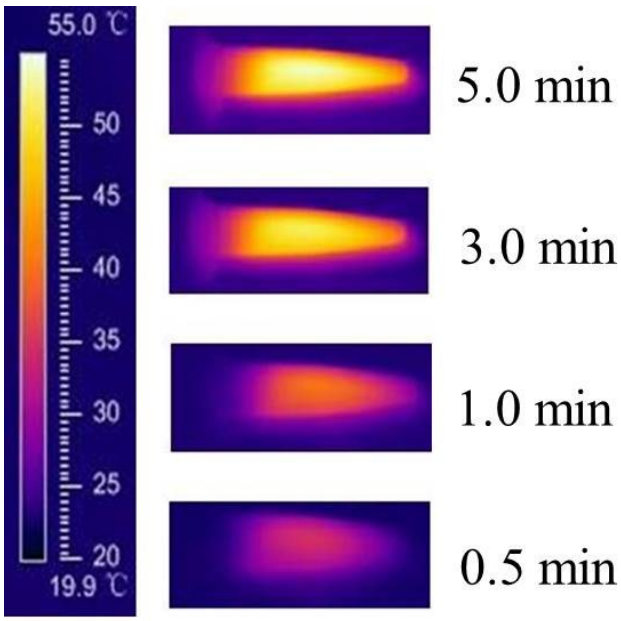



**Fig. S9** Infrared thermal images of the vesicles in water after laser irradiation for different times (660 nm, 1.5 W/cm$^2$).

## 9. *The biocompatibility of the vesicles against NIH3T3 normal cells*

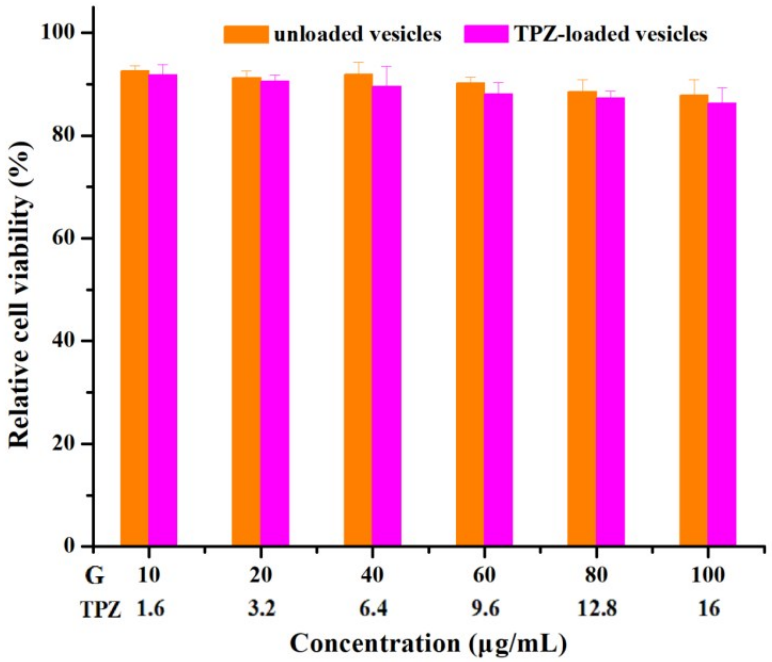



**Fig. S10** *In vitro* biocompatibility of unloaded vesicles and TPZ-loaded vesicles towards NIH3T3 normal cells after 24 h incubation.

## *10. The cytotoxicity of free TPZ against MCF-7 cancer cells under normoxia and hypoxia conditions*

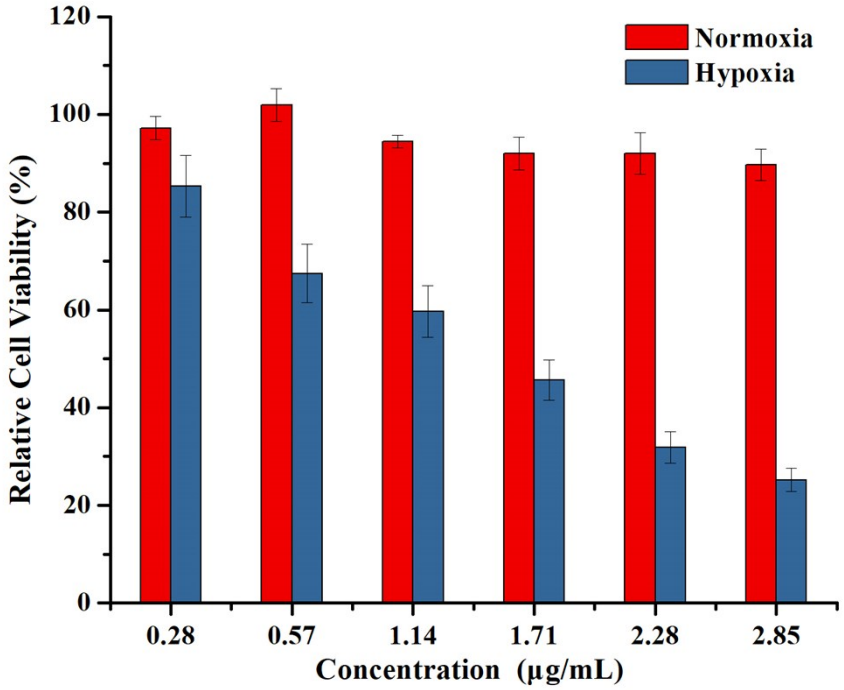



**Fig. S11** Cytotoxicity of free TPZ against MCF-7 cancer cells under normoxia and hypoxia conditions measured by MTT assay.

## *11. The cell apoptosis assay of MCF-7 cancer cells treated with as-prepared materials*

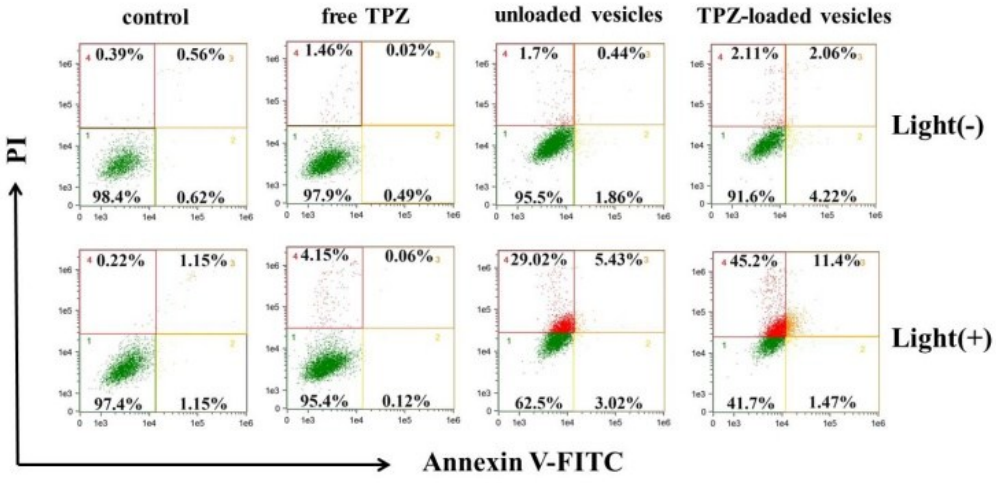



**Fig. S12** The cell apoptosis assay of MCF-7 cancer cells treated with as-prepared materials without or with 660 nm laser irradiation.

*12. The quantitative uptake activity of MCF-7 cells treated with as-prepared materials*

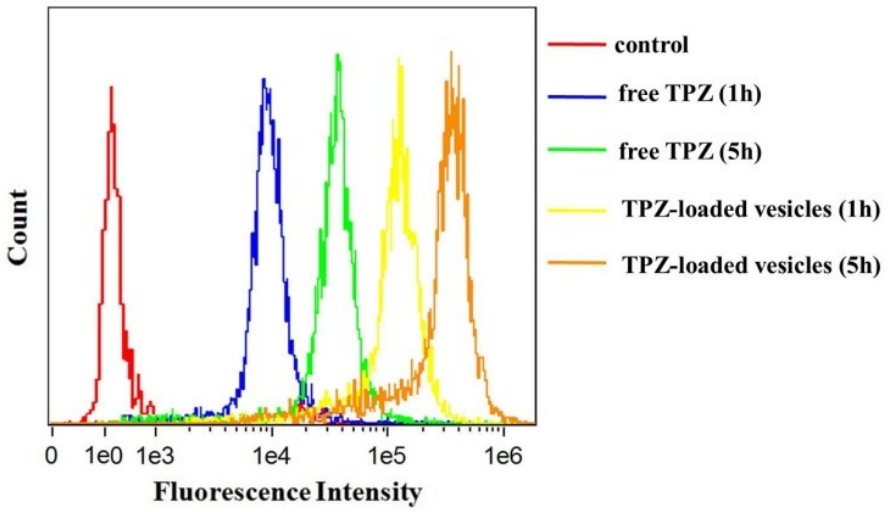



**Fig. S13** The quantitative uptake activity of MCF-7 cells treated with free TPZ or TPZ-loaded vesicles for 1 h and 5 h, respectively.

## *References:*

S1. H. Li, D.-X. Chen, Y.-L. Sun, Y. B. Zheng, L.-L. Tan, P. S. Weiss and Y.-W. Yang, *J. Am. Chem. Soc.*, 2013, **135**, 1570-1576.

S2. C. Yin, X. Zhen, H. Zhao, Y. Tang, Y. Ji, Y. Lyu, Q. Fan, W. Huang and K. Pu, *ACS Appl. Mater. Interfaces*, 2017, **9**, 12332-12339.

S3. L.-B. Meng, W. Zhang, D. Li, Y. Li, X.-Y. Hu, L. Wang, G. Li, *Chem. Commun.*, 2015, **51**, 14381-14384.