Amphiphilic porphyrin-based nanoparticles as a nuclei-approaching delivery platform for enhanced cancer photochemotherapy

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Abstract

Intelligent porphyrin photosensitizer integrating precise subcellular-targeting capability, enhanced solubility, and efficient phototheranostics is significant for cancer clinical treatment. Herein, we utilize a unilateral amphiphilic porphyrin (P) as nuclei-approaching drug carrier to interact with camptothecin (CPT), a potent DNA topoisomerase I inhibitor, to form supra-amphiphile-assembled nanoparticles (CPT@P). The nanoparticles are capable of directly entering and delivering CPT into nuclei to improve the therapeutic efficacy of photochemotherapy. Cancer cell membrane (M) is further used to camouflage CPT@P, endows the resulting CPT@P@M with excellent stability, homologous tumor targeting ability, and specific capability to co-deliver the chemotherapeutic drug and photosensitizer to tumor cells, even into the nuclei. This work takes advantage of unique structure and intrinsic performance of P to construct an integrative co-delivery system and modify by membrane-coating technology, which not only extends the application scopes of porphyrin photosensitizers but also provides a promising nanoplatform for cancer therapy.

Introduction

Photodynamic therapy (PDT) employs photosensitizers that upon absorbing a specific wavelength of light to release the cell damaging version of reactive oxygen species and provides a straightforward way to ablate tumor tissues with the advantages of minimally invasive, negligible systemic toxicity, fewer side effects and avoidance of drug resistance, which has been an effective alternative to traditional treatments^{1–5}. Porphyrin derivatives, intrinsically possess unique π -conjugated architectures and excellent imaging capacities, have been extensively used as the photosensitizers for PDT^{6–13}. However, previously reported works exhibit their respective and collective drawbacks including inferior singlet oxygen ($^1\text{O}_2$) quantum yields, poor solubility, the lack of subcellular-targeting capability and dissatisfactory biocompatibility^{14–19}. Evidently, the development of a novel porphyrin photosensitizer with intelligent structure to simultaneously solve these multiple problems is an appealing yet significantly challenging task.

Meanwhile, the distal end of the primary tumor and the infiltrating cancer cells cannot be effectively eliminated by a single treatment of PDT due to the limited light transmittance of tumor tissue^{20–22}. Combination of PDT and systemic chemotherapy in a synergistic therapeutic way has demonstrated superb efficacy in optimizing the outcome of cancer treatments^{23–26}. Chemotherapeutic drugs can spread to a wide range of cancer cells after the primary tumor is treated, thus inhibiting tumor recurrence^{27–31}. A large number of clinically employed chemotherapy drugs, such as platinum-based drugs (DNA cross-linker), camptothecin (topoisomerase I inhibitor) and doxorubicin (topoisomerase II inhibitor), are DNA-replication-associated toxins^{32–35}, however, direct delivery of these drugs into the nuclei is a fatal obstacle because of the lack of an ideal drug carrier, increasing the risks of drug pump and denaturation during intracellular transport^{36,37}. Moreover, nuclei are more sensitive for photodynamic damage as compared with other subcellular organelles^{38–40}. Therefore, the employment of nuclei-

approaching delivery platform combining chemotherapy and photodynamic therapy will provide good chance to significantly improve cancer theranostic effects.

To overcome these issues and to realize the goal of nuclei-approaching delivery, we design and synthesize an amphiphilic porphyrin photosensitizer (**P**) that consists of a hydrophobic urea-derived porphyrin and four hydrophilic PEG chains pointing to the same side of the porphyrin plane (Fig. 1). This unique structure can not only inhibit the $\pi - \pi$ stacking interactions and boost the PDT efficacy due to the nonplanar structure but also possess excellent solubility and prolonged circulation time. As our previous reports revealed^{41,42}, the introduction of four urea-groups into the porphyrin moieties endows the photosensitizer with high affinity towards various anions. We speculated that the strong binding affinity towards anions might lead to its entrance and accumulation in the cell nuclei with anionic properties. Camptothecin (**CPT**)^{43–45}, a chemotherapeutic agent exerting therapeutical effect in nuclei, is further employed as a guest to self-assemble with **P** to construct the supra-amphiphile-assembled nanoparticles (**CPT@P**) via $\pi - \pi$ stacking interaction, hydrogen bonds, hydrophilic and hydrophobic forces, and act as a dual functionalized system to achieve precise nuclei-approaching and synergistic anticancer efficacy.

Another fatal practical challenge hindering the clinical trials of the conventional nanosystems is their unstability in physiological environments and the inefficient accumulation in tumors. To further increase the stability and cancer-targeting capability, cancer cell membrane is used to wrap on the **CPT@P** as a camouflage body to endow the resulting **CPT@P@M** with excellent homologous targeting ability, precise and efficient phototheranostics, and favourable biocompatibility^{46–52}. In vivo anti-tumor studies demonstrate that **CPT@P@M** can completely eliminate the subcutaneous tumors without recurrence after three-dose injection through combining the chemotherapy and photodynamic therapy. This superior antitumor performance is attributed to the rational design of the novel photosensitizer drug carrier and the successful construction of supramolecular nuclei-approaching delivery system, providing a promising nanoplatfrom for cancer therapy.

Results

Synthesis of amphiphilic porphyrin photosensitizer. The synthetic route for **P** is illustrated in Supplementary Fig. 1 Polyethylene glycol substituted paracetaminobenzene **2** was synthesized from **1** that was prepared through the reaction of methoxypolyethylene glycols 350 and *p*-toluene sulfonyl chloride in dichloromethane at reflux for 24 h, by the introduction of paracetyl aminophenyl group. After treatment of **2** with 10% hydrochloric acid in aqueous solution, polyethylene glycol substituted aniline **3** was produced. In the presence of triphosgene, the amino groups on α,α,α -H₂TamPP converted into isocyanates, and then reacted with excess **3** in dichloromethane to generate the amphiphilic porphyrin photosensitizer **4** (**P**) in 96% yield. Their structures were well characterized by ¹H NMR, ¹³C NMR, electrospray ionisation mass spectrometry (ESI-MS), high-resolution mass spectroscopy (HRMS), infrared spectroscopy (IR) and UV-vis spectroscopy (Supplementary Fig. 2–14). The solubility of the amphiphilic porphyrin is greatly improved due to the introduction of four long PEG chains on the same side of the

porphyrin ring, with more than 450 mg of **P** dissolved in 1 mL of water to afford a transparent aqueous solution.

Host-guest assembly of P and CPT. The host–guest complexation between **P** and **CPT** was confirmed by the ^1H NMR spectrum, in which significant downfield shifts were observed after the addition of **CPT** into a solution of **P** (Fig. 2a). The existence of nuclear Overhauser effect (NOE) between the protons on **P** and the protons on **CPT** in NOESY NMR spectrum and the change of UV-vis spectrum provided further evidences for the host–guest complexation (Fig. 2b-c). A 1:1 binding stoichiometry for the complexation between **P** and **CPT** was determined by a mole ratio plot based on the UV-vis titration experiment, so it can be concluded that one porphyrin photosensitizer complexes with one equivalent camptothecin (Fig. 2d). ESI-MS further testified the stoichiometry of the self-assembly structure. In the ESI mass spectrum of **CPT@P**, all of the main peaks supported the 1:1 binding stoichiometry assignment, including four peaks at m/z 2851.3479, 2852.3733, 2874.3684 and 2897.4020 attributed to $[\text{P} + \text{CPT}]$, $[\text{P} + \text{CPT} + \text{H}]^+$, $[\text{P} + \text{CPT} + \text{Na}]^{2+}$ and $[\text{P} + \text{CPT} + 2\text{Na}]^{2+}$, respectively (Fig. 2f). The critical aggregation concentration (CAC) of **CPT@P** in aqueous solution was measured to be $\sim 7.0 \times 10^{-6}$ mol/L based on the UV-vis transmittance (Fig. 2e). All these results proved the host–guest complexation existed between **P** and **CPT** with a 1:1 stoichiometry.

Fabrication of CPT@P◀M. With the amphiphilic porphyrin photosensitizer (**P**) in hand, it was then used as a drug carrier to transport **CPT**, which can lead to the formation of nanoparticles (**CPT@P**) owing to various intermolecular forces. In order to reward **CPT@P** with better stability and tumor targeting ability, we coated **CPT@P** nanoparticles with B16F10 or 4T1 cancer cell membrane to form core – shell nanostructures (**CPT@P◀M_{B16F10}** and **CPT@P◀M_{4T1}**). The preparation process of **CPT@P◀M** was divided into three steps: preparation of nanoparticles **CPT@P**, cell membrane-derived vesicles from B16F10 or 4T1 cells and vesicle-nanoparticle fusion (Supplementary Figs. 16 and, 17). **CPT@P** was initially obtained with a particle size of 87.8 ± 0.8 nm and a zeta potential of -0.28 mV. After coating with cancer cell membrane, the resulting **CPT@P◀M** appeared with an average diameter of 99.8 nm and a zeta potential of -21.93 mV, suggesting the successful coating (Fig. 3a-b). TEM images also indicated the successful fusion, the **CPT@P** showed solid spheres in the PBS solution (Fig. 3c), while the final **CPT@P◀M** exhibited an obvious core – shell structure with smooth surfaces and with an increased diameter (Fig. 3d-e). TEM micrographs of **CPT@P◀M** upon negative staining with uranyl acetate also strongly proved that cell-membrane-derived vesicles evenly coated onto the **CPT@P** nanoparticles (Fig. 3f-g). Cryo-TEM imaging apparently showed that **CPT@P◀M** had a core-shell structure, where the **CPT@P** core was surrounded by the cell membrane shell whose thickness was measured to be ~ 8.7 nm (Fig. 3h). The CAC of **CPT@P◀M** in aqueous solution was estimated to be $\sim 9.0 \times 10^{-6}$ mol/L (Supplementary Fig. 18).

The singlet oxygen generation and stability study of CPT@P◀M. As we expected, **CPT@P◀M** exhibited higher stability than **CPT@P** in physiological environment (PBS with 10% serum). Negligible changes in diameter were detected by DLS over 3 days (Fig. 4d and Supplementary Fig. 19). More importantly, in the bio-TEM images of the B16F10 cells treated with **CPT@P◀M** for 3 h, the undamaged nanoparticles with a

diameter of 80–100 nm in the cytoplasm and the clustered nanoparticles in the nuclei could be clearly observed (Fig. 4a-c). These results implied that cell membrane coating could maintain the relative stability of **CPT@P◀M**, therefore, **CPT@P◀M** held the promise of delivering **CPT** to the nuclei target site for chemotherapy and was advantageous to obtain a better photodynamic effect. Inspired by these interesting phenomena, photosensitizing action was subsequently investigated using the singlet oxygen sensor green (SOSG). The SOSG fluorescence exhibited a significant enhancement after introduction of **P**, **CPT@P** and **CPT@P◀M** upon laser irradiation, indicative of a high photosensitizing efficacy (Fig. 4e and Supplementary Fig. 20). When sodium azide (NaN_3) was added as a $^1\text{O}_2$ scavenge, the fluorescence changes at 530 nm were inhibited, further confirming the production of $^1\text{O}_2$ during laser irradiation. Singlet oxygen quantum yield (QY) measurements were carried out in ethanol with 1,3-diphenylisobenzofuran (DPBF) as a detector molecule for trapping singlet oxygen and using methylene blue (MB) as a reference ($^1\text{O}_2$ generation QY = 0.52)^{53,54}. The $^1\text{O}_2$ generation QYs of **P**, **CPT@P** and **CPT@P◀M** were calculated to be 0.217, 0.283 and 0.243 (Fig. 4f), respectively, around 70-fold higher than that of *meso*-tetrakis(*p*-sulfonatophenyl) porphyrin tetrasodium salt (TPPS), demonstrating that the unique structure of the unilateral porphyrin and the formation of host-guest assembly played significant roles in improving the $^1\text{O}_2$ generation. Notably, the significant differences of SOSG fluorescence and $^1\text{O}_2$ generation yields between **P** and **CPT@P/CPT@P◀M** emphasized that the host-guest assembly behavior improved the photodynamic effect. More interestingly, the photostability of **P**, **CPT@P** and **CPT@P◀M** were excellent, with less than 5% reduction in absorption observed after thirteen cycles of irradiation at 15 mW/cm² (Fig. 4g). Comparatively, 73% of indocyanine green (ICG) dye was annihilated under the same laser density with severe photobleaching.

Homologous targeting ability of CPT@P◀M. To demonstrate the ability of **CPT@P◀M** homotypically target cancer cells for drug delivery applications, membranes from the mouse melanoma cell line B16F10 and the mouse breast cell line 4T1 were collected and coated onto **CPT@P**. As flow cytometric analysis was carried out to quantify the difference in uptake, it was manifested that **CPT@P◀M_{B16F10}** and **CPT@P◀M_{4T1}** had significantly increased uptake in comparison with the bare **CPT@P** cores (Fig. 5a and 5d). To further confirm that **CPT@P◀M** could target homologous cancer cells, 4T1, MCF7, MAD-MB-435 and B16F10 cell lines were selected for incubation with **CPT@P◀M_{B16F10}** or **CPT@P◀M_{4T1}**. Flow cytometric analysis indicated that **CPT@P◀M_{B16F10}** was more likely to be internalized by B16F10 cells with a high efficiency of 41.9%, while 4T1 cells showed the highest uptake to **CPT@P◀M_{4T1}** as compared to the other three cell lines (Fig. 5b-c; Fig. 5e-f). These results testified that **CPT@P◀M** can actively target the source cancer cells due to homologous homing because cancer cells express surface adhesion molecules with homologous adhesion domains^{55–58}.

Cellular uptake of CPT@P◀M. After camouflaged via cancer cell membrane, **CPT@P◀M** can enter cell through a variety of ways by comparison with **P** and **CPT@P**. Endocytic pathways of these three nanoparticles were evaluated using flow cytometry by applying different endocytosis inhibitors. As shown in Fig. 5g-i, the internalization of **P**, **CPT@P** and **CPT@P◀M** by the cells were significantly inhibited

at 4°C, suggesting the cellular uptake were mainly energy-dependent. Treatment of the cells with chlorpromazine (CPZ), amiloride-HCl (AMD), nystatin (NYS), methyl- β -cyclodextrin (CB) and genistein (Gen) indicated that **P** was internalized via macropinocytosis-, actin- and caveolae-mediated endocytosis; **CPT@P** can internalized via clathrin-mediated endocytic pathway with the assistance of macropinocytosis-, actin- and caveolae-mediated endocytosis. What is more, the internalization of **CPT@P◀M** decreased 69.0%, 38.2%, 22.7%, 32.2%, 41.6% and 84.2%, respectively, after treatment with different cellular inhibitors, indicating that **CPT@P◀M** can be easier to get into the cells due to the increased phagocytosis pathway. Flow cytometric quantification manifested that the uptake of **CPT@P◀M** by B16F10 cells was time-dependent (Supplementary Fig. 21). The intracellular porphyrin content increased from 49.2–83.3% when the incubation time extended from 1 to 10 h.

Nuclei-approaching ability of CPT@P◀M. To verify the nuclei-approaching ability, the cellular presence of **P**, **CPT@P** and **CPT@P◀M** were inspected by confocal laser scanning microscopy (CLSM). As shown in Fig. 6a, a red fluorescence of porphyrin was observed in nuclei and cytoplasm after the cells were incubated for 8 h with the three nanoparticles, respectively. In order to ascertain whether **CPT@P◀M** entered the cell nuclei and to investigate its distribution in the cell, bio-TEM images of B16F10 cells incubated with **CPT@P◀M** at different stages were performed (Fig. 6c). The **CPT@P◀M** nanoparticles with a diameter of 80–100 nm could be apparently observed in the cytoplasm, even in some of which the structure of **CPT@P** enveloped by the cancer membrane was displayed very clearly. When the **CPT@P◀M** entered the nuclei, the core-shell structure began to collapse and gather together more and more as time went on, which is probably due to the release of **CPT**. In this system, **CPT**, a potent DNA topoisomerase I inhibitor, can kill cancer cells more effectively since it can be directly delivered into the nuclei. It is interesting that the porphyrin photosensitizer, meanwhile, act as a drug carrier to deliver **CPT** into the nuclei. It is reasonable to infer that the unique structure of porphyrin plays a key role. The four urea-based groups on the porphyrin can not only provide strong intermolecular hydrogen bonds to facilitate self-assembly between adjacent porphyrins, but also contribute strong binding affinity towards various anionic species^{41,59,60}, which bestow **CPT@P◀M** to bind the anionic nucleic acids in the nuclei. Moreover, we observed different stages of B16F10 cells death after treatment with **CPT@P◀M** and laser (Fig. 6b-c). At the initial stage (Fig. 6c_A), **CPT@P◀M** dispersed in the cytoplasm and slowly entered the nuclei through the nuclear pore. The cells were intact and the organelles were healthy at this stage. With further increase of time, the drug slowly released and began to work as a chemotherapy agent. As shown in Fig. 6c_B, mitochondria in the cytoplasm swelled and became two or three times in size as compared with normal mitochondria. After laser irradiation, a cell damaging version of reactive oxygen species could be released. As the cell being destroyed, the cell membrane ruptured and the cytoplasm exposed, following with that the cell's nuclear membrane began to slowly break down (Fig. 6c_{C-D}). Eventually, the B16F10 cells died under the combined action of chemotherapy and photodynamic therapy.

Intracellular photodynamic study. Subsequently, the intracellular generation of $^1\text{O}_2$ by **P**, **CPT@P** and **CPT@P◀M** after cell uptake were measured upon light irradiation (645 nm, 15 mW/cm²) by using 2',7'-dichlorofluorescein diacetate (DCFDA) as the indicator before carrying out in vitro PDT. Strong green

fluorescence was detected in the cells that treated with **P**, **CPT@P** and **CPT@P◀M** after laser irradiation (Fig. 7a-b), which evidenced the generation of $^1\text{O}_2$ inside the cells. Flow cytometric analysis also showed strong signals from DCF after light irradiation, however, negligible green fluorescence was seen without light irradiation (Fig. 7c). Notably, the green signal was a little weaker for the cells cultured with **P** than for those treated with **CPT@P** or **CPT@P◀M** at the same concentration (Fig. 7d), illustrating that host-guest interaction can inhibit the π - π stacking among the porphyrins and generate more $^1\text{O}_2$. Moreover, unlike ordinary photosensitizers, it was found that **P**, **CPT@P** and **CPT@P◀M** could enter and stay in the nuclei to produce $^1\text{O}_2$, which would be greatly beneficial to the photodynamic therapy.

In vivo synergistic photochemotherapy. MTT assay indicated that the cytotoxicity of **CPT@P◀M** upon irradiation against 4T1 cell was greatly improved by combining PDT and chemotherapy as it was compared with other formulations (Fig. 8a). To further evaluate the relative contributions of chemotherapy and PDT towards the cytotoxicity of the photochemotherapy, 4T1 cells were treated with **CPT**, **P**, **P + L**, **CPT@P**, **CPT@P + L**, **CPT@P◀M_{4T1}** and **CPT@P◀M_{4T1} + L**, respectively, then labelled with propidium iodide (PI) and Annexin V (Fig. 8c-d). For the cells treated with **CPT**, the populations of the early apoptotic, late apoptotic, and necrotic cells were 6.1%, 9.6%, and 4.2%, respectively. For combining PDT and chemotherapy (**CPT@P + L**) at a light dose of 15 mW/cm² for 3 min, the percentage of the cells at early apoptotic and late apoptotic increased to 35.0% and 10.6%, respectively. Excitingly, **CPT@P◀M_{4T1}** increased percentage of the cells with early apoptotic 35.6% and late apoptotic 25.9% at the same laser conditions, confirming the promising application of the **CPT@P◀M_{4T1}** in synergistic treatment against cancers. Additionally, the in vivo safety of the components of **CPT@P◀M_{4T1}** was evaluated using the hemolysis assay. It was demonstrated that all of them were not hemolytic, as indicated by the negligible changes in hemolysis percentage in the tested concentration range (Fig. 8b and Supplementary Fig. 22). Hepatotoxicity and nephrotoxicity of each component of **CPT@P◀M_{4T1}** were tested by in vivo blood biochemistry (Supplementary Fig. 23). Routine blood analysis indicated that the levels of the biomarkers were in a normal range and a negligible difference was found between the healthy mice and the mice treated with each component of the **CPT@P◀M_{4T1}**.

Pharmacokinetic studies. Since porphyrin has fluorescence itself, a whole-body fluorescence imaging study was conducted to evaluate the targeting specificity ability and monitor the delivery of **CPT@P◀M_{4T1}** after i.v. injection. A significant amount of fluorescence signals accumulated in the tumor at 8 h post injection, indicating that **CPT@P◀M_{4T1}** possessed high tumor accumulation (Supplementary Fig. 24). Meanwhile, the time-dependent biodistribution of **CPT@P◀M_{4T1}** in tumor and liver was observed, which also suggested **CPT@P◀M_{4T1}** could improve the accumulation at tumor area via both EPR and active targeting effect (Fig. 8f-g). It was noted that almost no fluorescence signal could be detected in the kidneys. Therefore, employment of the amphiphilic porphyrin as a drug-delivery carrier camouflaged through the cell membrane can greatly avoid the renal toxicity.

In vivo targeting studies. The safe and effective delivery of porphyrin photosensitizer and **CPT** to the target in vivo is a key step for supramolecular nanomaterials to play as drug carriers. The above in vitro experiments proved that **CPT@P«M_{4T1}** has the ability to target homologous cancer cells and good stability in vivo environment. To evaluate the tumor targeting capability, the nude mice bearing 4T1 tumor were injected with **P**, **CPT@P** and **CPT@P«M_{4T1}**, respectively, and the porphyrin signals were monitored. The **CPT@P«M_{4T1}** showed enhanced targeting behavior due to the cancer cell membrane camouflage, which awards **CPT@P«M_{4T1}** with homologous targeting capability and higher stability (Fig. 8h).

In vitro synergistic anticancer treatments against 4T1 breast cancer. Inspired by the outstanding in vitro synergistic efficacy, high tumor accumulation, excellent biodistribution and precise nuclei-approaching capability, in vivo anti-tumor studies and systemic toxicity of the **CPT@P** were further evaluated. Nude mice bearing 4T1 tumor were randomly divided into eight groups when the tumor volume reached ~ 100 mm³, and the mice were then treated with PBS, Laser (**L**), **P**, **P + L**, **CPT@P**, **CPT@P + L**, **CPT@P«M_{4T1}** and **CPT@P«M_{4T1} + L** (n = 5/group), respectively. For photodynamic therapy, the mice received three laser irradiations (645 nm, 80 mW/cm², 10 min) on days 1, 5 and 9 after injection for another 8 h, respectively (Fig. 8e). As shown in Fig. 8i-j, the average tumor volumes of the mice treated with PBS or laser increased rapidly and limited anti-tumor effect was observed for the mice treated with **P**. For the mice treated with **CPT@P**, the growth of tumors was moderately suppressed. Unfortunately, severe recurrence occurred after the drug administration ended. An obvious tumor growth inhibition was achieved after the laser treatment for PDT alone (**P + L**), whereas a tumor recurrence appeared on the 11th day due to that the invasive cancer cells far away from the primary tumor can be hardly killed by photodynamic therapy. The **CPT@P«M_{4T1}** mediated chemotherapy inhibited around two thirds of the tumor growth, more efficient than **CPT@P**, which could be explained by the coating of 4T1 cell membranes that endows **CPT@P«M_{4T1}** the ability to target homologous cancer cell tumors and the long-term stability in the blood. The inhibition ratio was calculated to be 60.7% for the mice administrated with **CPT@P + L**, indicating that the combination of **CPT@P**-mediated chemotherapy and laser irradiation-activated PDT could play a better therapeutic effect than single treatment. Excitingly, the mice treated with **CPT@P«M_{4T1} + L** presented the highest antitumor efficiency among these groups with complete eradication of tumors without recurrence (Fig. 8k). The high antitumor efficiency could be attributed to the efficient synergic effects including the enhanced photodynamic action, reduced loss of camptothecin in the blood circulation, the tumor enrichment capacity and enhanced stability by 4T1 cancer cell membrane. In vivo safety of this nanosystem, as a crucial issue for practical applications, was simultaneously evaluated by survival rate and body weight (Fig. 8l-m). The survival study indicated that 80% of mice treated with **CPT@P«M_{4T1} + L** were still viable after 48 days from the first treatment, while the survival time of mice from the other treatments (**CPT@P**, **CPT@P + L** and **CPT@P«M_{4T1}**, etc) did not all exceed 46 days. These results demonstrated that the photochemotherapy camouflaged with cell membrane greatly prolonged mice survival. Negligible body weight reduction was observed during the therapy period for the mice treated with all treated groups. Moreover, there were no obvious signs of toxic effects including urination or nervous state.

In vitro and in vitro synergistic anticancer treatments against B16F10 melanoma cancer. To demonstrate the adaptability of **CPT@P◀M** for cancer therapy, we further evaluated the photochemotherapy effect of **CPT@P◀M** on melanomas. The photodynamic effect from porphyrins and chemotherapy from CPT, against B16F10 cells was firstly investigated by MTT assay. The half growth inhibition concentration (IC_{50}) was determined to be 1223 ± 3.1 , 542.9 ± 2.7 , 216.2 ± 2.6 , 147.4 ± 2.2 , 157.1 ± 2.2 , and 22.6 ± 1.4 nM, for **CPT**, **CPT@P**, **P + L**, **CPT@P + L**, **CPT@P◀M_{B16F10}**, and **CPT@P◀M_{B16F10} + L**, respectively (Fig. 9a). The IC_{50} of the photochemotherapy (**CPT@P + L**) was much lower than those of single chemotherapy (**CPT** and **CPT@P**) or single PDT (**P + L**), underlining that the host-guest interaction of **CPT@P** played a significant role in the synergistic photochemotherapy. Remarkably, the **CPT@P◀M_{B16F10}** coated with cancer cell membrane showed better photochemotherapy effects owing to the homologous targeting capacity. Calcitronin AM/PI co-staining demonstrated that negligible cell death was induced by light and the **CPT@P◀M_{B16F10}** could cause more cell death than **CPT** due to the increased solubility owing to the supramolecular nanoplatform (Fig. 9b). As compared with these control groups, significantly increased cell death was observed after incubating with **CPT@P◀M_{B16F10}** followed by light exposure. The percentage of the dead cells increased obviously along with the extension of laser exposure time and all cells could be killed within 300 s, proving that **CPT@P◀M_{B16F10}** possessed good drug delivery performance and excellent photodynamic therapeutic effect.

Subsequently, we evaluated the in vivo anti-tumor efficiency of **CPT@P◀M_{B16F10}** in B16F10 tumor bearing C57BL/6 mice (Fig. 9c). As shown in Fig. 9d and Supplementary Fig. 25, treatment with laser and **P** showed little inhibition on tumor progression similar with the PBS control. For the mice treated with single PDT or single chemotherapy (**P + L**, **CPT@P** and **CPT@P◀M_{B16F10}**), about 64.3%, 62.2%, and 63.0% tumor reduction was achieved as compared with the PBS group at 21 d post-treatment. In comparison, the mice treated with photochemotherapy (**CPT@P + L**) not only significantly inhibited the tumor growth, but also delayed the growth rate of malignant tumor (Fig. 9e and 9g). Notably, a superior antitumor outcome was achieved for the mice administrated with **CPT@P◀M_{B16F10} + L**, in which the tumor inhibition rate reached 98.7%, further attesting its excellent anti-tumor performance. Moreover, the enrichment of **P**, **CPT@P** and **CPT@P◀M_{B16F10}** in B16F10 melanoma tissues was observed by fluorescence microscope (Supplementary Fig. 26). Blue was the nuclei of tumor cells, and red was the porphyrin fluorescence of each material. The **CPT@P◀M_{B16F10}** coated by B16F10 cancer cell membrane enriched more than **P** and **CPT@P** in the tumor tissues, which also proved the tumor targeting specificity of **CPT@P◀M_{B16F10}**. Immunofluorescence of TUNEL from tumor tissues in treatment groups indicates that only PDT or chemotherapy (**P + L**, **CPT@P** or **CPT@P◀M_{B16F10}**) induced limited lethal effects on melanoma cells, showing insufficient capacity to eliminate the established tumor. When chemotherapy and PDT was combined, the lethal effects on melanoma cells further boosted after the treatment (**CPT@P + L** and **CPT@P◀M_{B16F10} + L**) (Fig. 9h). We speculated that the stronger antitumor activity of **CPT@P◀M_{B16F10} + L** was attributed to the synergistic photochemotherapy and the homologous tumor targeting ability. Furthermore, H&E staining confirmed the synergistic anticancer efficacy of **CPT@P◀M_{B16F10}**. TUNEL staining also showed that **CPT@P◀M_{B16F10} + L** caused the highest cell apoptosis in the tumor sites,

which was much higher than mono chemotherapy or PDT (Fig. 9i). The mice that received photochemotherapy exhibited longer survival by comparison with the mice treated with mono PDT or chemotherapy. Especially, the **CPT@P◀M_{B16F10} + L** treatment greatly prolonged mice survival over 50 days without any death (Fig. 9f). In the meantime, negligible body weight variation of the mice was observed during the treatment, implying a good safety profile (Supplementary Fig. 27). Moreover, H&E staining demonstrated that no pathological abnormality or inflamed cells existed in the lung, liver, kidney, and other tissues (Supplementary Fig. 28).

Discussion

In conclusion, we synthesized a unique amphiphilic porphyrin photosensitizer and used it as a nuclei-approaching drug carrier to self-assemble with **CPT** to construct a supramolecular nanoplatform. The participation of **CPT** boosted the photodynamic effect by inhibiting the $\pi - \pi$ stacking among adjacent porphyrins while the solubility of **CPT** was greatly enhanced and could be precisely delivered to cell nuclei to exert therapeutic effect, resulting in the construction of **CPT@P** with the capability to co-deliver a chemotherapeutic agent and a photosensitizer. Cancer cell membrane was further coated on the surface of **CPT@P**, endowing the formed **CPT@P◀M** with the ability to target homologous cancer cells and possess excellent stability in serum. In vivo anti-tumor studies demonstrated that **CPT@P◀M** completely eliminated the subcutaneous tumors without recurrence after three-dose injection by combining chemotherapy and photodynamic therapy. Benefiting from this co-therapy design, well construction of supramolecular nanoplatform and cell membrane nanotechnology, high tumor accumulation and excellent photochemotherapy effect were achieved. This work provides a smart porphyrin photosensitizer with multiple advantages and demonstrates a strategy for combining chemotherapy and photodynamic therapy through supramolecular strategy, which possess promising potential in cancer therapy.

Methods

Materials and general methods. Methoxypolyethylene glycols 350, 4-toluene sulfonyl chloride, 4-acetamidophenol, K_2CO_3 , NaI, triphosgene, $Zn(OAc)_2 \cdot H_2O$ and other reagents were purchased from Sigma-Aldrich or Aladdin and used as received. The compound meso-tetrakis($\alpha, \alpha, \alpha, \alpha$ -2-aminophenyl)porphyrin was synthesized according to the method reported in the literature.⁶¹ Solvents were either employed as purchased or dried according to procedures described in the literatures. 1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III-500 spectrometry and chemical shifts (δ) are referenced to the residual solvent or internal standard TMS peaks. UV-vis spectra were taken on a HITACHI U-2800 spectrometer. The fluorescence titration experiments were conducted on a Perkin Elmer LS55 at room temperature. Mass spectra were recorded on a Micromass Quattro II triplequadrupole mass spectrometer using electrospray ionization and analyzed with the MassLynx software suite or an AB TripleTOF 5600^{plus} System spectrometer with an ESI resource. Transmission electron microscopy (TEM) studies were conducted on a Hitachi HT7700 operating at an accelerating voltage of 120 kV. Cryo transmission electron microscopy (cryo-TEM) studies were conducted on a FEI Talos F200c operating at

an accelerating voltage from 20 kV to 200 kV. Zeta potential measurements were carried out using a Zetasizer Nano ZS90. Flow cytometry studies were taken on a Becton Coulter cytometer. Confocal laser microscopy was taken on a Zeiss LSM 880 with Airyscan.

Synthesis of compound 1. To a mixture of methoxypolyethylene glycols 350 (20.0 g, 57.0 mmol), triethylamine (16.0 mL, 114 mmol) and dichloromethane (30 mL), a dichloromethane solution of 4-toluene sulfonyl chloride (17.4 g, 91.0 mmol) was added dropwise in an ice-salt bath in 5 min. The mixture was stirred in the salt bath for 30 min and then stirred at room temperature for 24 h before filtering and concentrating. The crude product was purified by column chromatography (silica gel, with petroleum ether/ethyl acetate = 1:1 to petroleum ether/ethyl acetate = 3:1 as the eluent) to afford the product (12.5 g, 69% yield) as a white solid. ^1H NMR (500 MHz, CDCl_3) δ 7.80 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.17–4.14 (m, 2H), 3.70–3.67 (m, 2H), 3.67–3.59 (m, 18H), 3.58 (s, 4H), 3.54 (dd, J = 5.7, 3.7 Hz, 2H), 3.37 (s, 3H), 2.45 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 144.8, 133.1, 129.9, 128.0, 72.0, 71.4, 70.8, 70.6, 70.5, 69.3, 68.7, 60.4, 59.1, 21.7. ESI-MS: Calcd for $\text{C}_{22}\text{H}_{38}\text{NaO}_{10}\text{S}$ $[\text{M} + \text{Na}]^+$: 517.2078, Found: 517.2284.

Synthesis of compound 2. To a mixture of **1** (10.6 g, 24.5 mmol), K_2CO_3 (5.10 g, 36.8 mmol), NaI (5.50 g, 36.8 mmol) and acetonitrile (550 mL), 4-acetamidophenol (7.40 g, 49.0 mmol) was added. The mixture was stirred and refluxed for 24 h at 75°C. After cooling down to room temperature, the mixture was filtered and the filtrate was concentrated in vacuo. The crude product was dissolved in dichloromethane and washed with brine for three times, then dried over anhydrous Na_2SO_4 . The crude product was purified by column chromatography (silica gel, with dichloromethane/methanol = 200:1 to dichloromethane/methanol = 40:1 as the eluent) to afford the product (12.5 g, 69% yield) as a white solid. ^1H NMR (500 MHz, CDCl_3) δ 7.46 (s, 1H), 7.43–7.38 (m, 2H), 6.85 (d, J = 9.0 Hz, 2H), 4.12–4.07 (m, 2H), 3.85–3.79 (m, 2H), 3.70 (dd, J = 5.9, 3.4 Hz, 2H), 3.67–3.65 (m, 2H), 3.64–3.61 (m, 18H), 3.55–3.51 (m, 2H), 3.37–3.35 (m, 3H), 2.13 (s, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 168.4, 155.5, 131.7, 121.7, 115.0, 72.0, 70.9, 70.7, 70.7, 70.6, 69.9, 67.8, 59.1, 24.4. ESI-MS: Calcd for $\text{C}_{23}\text{H}_{39}\text{NNaO}_9$ $[\text{M} + \text{Na}]^+$: 496.2523, Found: 496.2658.

Synthesis of compound 3. Compound **2** (4.00 g, 8.45 mmol) was dissolved in 900 mL HCl (1 mol/L) aqueous solution and stirred for 24 h at 80°C. After cooling down to room temperature, NaOH (1 mol/L) aqueous solution was added dropwise to adjust the pH of solution to be ~ 10, the mixture was then concentrated in vacuo. The crude residue was re-dissolved in dichloromethane and filtered, then purified by column chromatography (silica gel, with dichloromethane/methanol = 1000:1 to dichloromethane/methanol = 100:1 as the eluent) to afford the product (12.5 g, 69% yield) as a white solid. ^1H NMR (500 MHz, CDCl_3) δ 6.76 (d, J = 8.7 Hz, 2H), 6.65–6.61 (m, 2H), 4.07–4.03 (m, 2H), 3.83–3.79 (m, 2H), 3.72–3.69 (m, 2H), 3.68–3.66 (m, 2H), 3.65–3.63 (m, 18H), 3.54 (dd, J = 5.7, 3.7 Hz, 2H), 3.37 (s, 3H). ^{13}C NMR (500 MHz, CDCl_3) δ 116.59, 116.55, 116.51, 116.0, 72.1, 70.9, 70.78, 70.76, 70.73, 70.72, 70.67, 70.1, 59.2. ESI-MS: Calcd for $\text{C}_{23}\text{H}_{41}\text{NO}_9$ $[\text{M} + \text{H}]^+$: 475.2781, Found: 475.2850.

Synthesis of compound 4. Under nitrogen atmosphere, 12 mL of triphosgene (78.0 mg, 0.260 mmol) solution in dichloromethane was added dropwise to a mixture of *a,a,a,a*-H₂TamPP (meso-tetrakis(*a,a,a,a*-2-aminophenyl)porphyrin (118 mg, 0.175 mmol), 0.3 mL of triethylamine and 30 mL of dichloromethane in an ice-salt bath. The mixture was stirred for 30 min in the ice-salt bath and 3 hours at room temperature. After **3** (1.00 g, 2.10 mmol) was added, the reaction mixture was stirred at room temperature for 48 h, then washed with brine for three times and dried over anhydrous Na₂SO₄. The crude product was purified by column chromatography (silica gel, with dichloromethane/methanol = 200:1 to dichloromethane/methanol = 20:1 as the eluent) to afford the product (539 mg, 68% yield) as a purple oil. ¹H NMR (500 MHz, CD₂Cl₂) δ 8.83 (br, 8H), 8.34 (br, 4H), 7.93 (br, 4H), 7.82 (br, 4H), 7.48 (br, 4H), 6.71 (br, 8H), 6.50 (br, 8H), 5.94 (br, 8H), 3.70–3.31 (m, 112H), 3.26 (s, 12H), –2.72 (s, 2H). ¹³C NMR (151 MHz, CD₂Cl₂) δ 154.7, 153.6, 139.8, 135.2, 133.1, 132.2, 131.4, 130.1, 129.7, 123.1, 122.2, 116.2, 114.6, 72.1, 70.6, 69.6, 67.5, 59.0, 30.1. ESI-MS: Calcd for C₁₃₂H₁₇₄N₁₂O₃₆ [M + 2H]²⁺: 2505.2299, Found: 2505.2236.

Preparation of P, CPT@P and CPT@P◀M. A mixture solution of **P** and **CPT** with a mole ratio of 1 : 1 at a concentration of 1 × 10^{−3} mol/L in THF was prepared by sonication for 5 min, then the solution was dropwise into ten times MilliQ water with sonication of the mixture for another 10 min. The obtained mixture solutions were stirred overnight until THF was volatilized completely. A solution of **CPT@P** dissolved in MilliQ water at a concentration of 1 × 10^{−4} mol/L was resulted. DLS and TEM were used to characterize these nanostructures.

Cancer cell membranes were collected by freezing and thawing B16F10 or 4T1 cancer cells. In order to coat **CPT@P** core with B16F10 or 4T1 cancer cell membranes, we mixed **CPT@P** with purified cell membranes. After sonication for 5 min, the mixture was placed at 4°C overnight and coextruded through a 200 nm porous polycarbonate membrane to form the **CPT@P◀M_{B16F10}** or **CPT@P◀M_{4T1}** nanoparticles. The nanoparticles were concentrated by centrifugation at 18,000 rpm for 30 min and then redispersed in PBS for their use.

DLS and TEM were used to characterize **P**, **CPT@P** and **CPT@P◀M**. Cryo-TEM was used to characterize **CPT@P◀M**. The protein contents in membrane-coated **CPT@P◀M** were determined by the BCA assay.

Preparation of CMCs solutions. Solutions of **P**, **CPT@P** and **CPT@P◀M** at a concentration of 1 × 10^{−3} mol/L in water were prepared, respectively. The solutions were then diluted to obtain the aqueous solutions with different concentrations at a range from 1 × 10^{−4} mol/L to 1 × 10^{−7} mol/L. The transmittances of the aqueous solutions were measured by UV-vis spectroscopy, and the correlation between the concentration and transmittance was made at the point where the ultraviolet absorption wavelength was 435 nm.

Detection of ¹O₂ generation in solution. The **P**, **CPT@P** and **CPT@P◀M** samples were respectively suspended in water containing singlet oxygen sensor green (SOSG, 2.50 μmol/L). The concentration of porphyrin photosensitizer in **P**, **CPT@P** or **CPT@P◀M** was 2.00 μmol/L. The mixture was then placed in a

cuvette and the solution was irradiated at 645 nm (15 mW/cm²) for different time. The fluorescence emission of SOSG (upon excitation at 470 nm) was then measured using a fluorescence spectrophotometer.

Measurement of singlet oxygen quantum yield (¹O₂ QY). The ¹O₂ QY of P, CPT@P or CPT@P◀M was calculated by using Methylene blue (MB) as a standard and 1,3-diphenylisobenzofuran (DPBF) as a singlet oxygen scavenger. Upon laser irradiation (645 nm, 15 mW/cm²) for different time, the absorption of the solution containing DPBF was detected using UV-vis spectroscopy. The slope of the absorbance at 414 nm versus irradiation time was determined. The ¹O₂ QY was calculated according to equation list as follows: $\Phi_{\Delta} = \Phi_{(TPPS)} \times k_{(MNPs)} \times F_{(TPPS)} / k_{(TPPS)} \times F_{(MNPs)}$, where k is the slope of the absorbance at 414 nm versus irradiation time, F is the absorption correction factor calculated by $F = 1 - 10^{OD}$ (OD at the irradiation wavelength).

Test of photostability. The P, CPT@P and CPT@P◀M samples were respectively suspended in water at a concentration of 20 μmol/L and indocyanine green (ICG) dye was used as a control photosensitizer with the same concentration. The mixture was then placed in a cuvette and the solution was irradiated at 645 nm (15 mW/cm²) for different time. The absorption of the aqueous solutions was then measured by UV-vis spectroscopy, and the correlation between the irradiated time and absorption was made at the point where the ultraviolet absorption wavelength was 435 nm.

Cell culture. B16F10, 4T1, MCF7 and MDA-MB-435 cell lines were purchased from Sigma. B16F10 and MDA-MB-435 cells were incubated in Dulbecco's Modified Eagle's Medium (DMEM) containing FBS (10%) and penicillin/streptomycin (1%). 4T1 and MCF7 cells were incubated in RPMI-1640 containing FBS (10%) and penicillin/streptomycin (1%). Cells grew as a monolayer and were detached upon confluence using trypsin (0.5% w/v in phosphate-buffered saline). The cells were harvested from cell culture medium by incubating in the trypsin solution for 5 min. The cells were centrifuged, and the supernatant was discarded. A 3.00 mL portion of serum-supplemented medium was added to neutralize any residual trypsin. The cells were re-suspended in serum-supplemented medium at a concentration of 1.00×10^4 cells/mL. Cells were cultured at 37°C and 5% CO₂.

Homologous targeting validation. B16F10 and 4T1 cells were incubated with CPT@P or CPT@P◀M_{B16F10}/CPT@P◀M_{4T1} at 37°C for 3 h. The cells were then washed three times with PBS and harvested with trypsin. The collected cells were analyzed by flow cytometry. The 4T1, B16F10, MDA-MB-435 and B16F10 cells were incubated with CPT@P◀M_{B16F10} or CPT@P◀M_{4T1} at 37°C for 30 min. The cells were then washed three times with PBS and harvested with trypsin. The collected cells were analyzed by flow cytometry. A total of 1×10^4 events were counted for analysis.

Determination of endocytosis pathways of P, CPT@P or CPT@P◀M determined by flow cytometry. B16F10 cells were pre-incubated with endocytosis inhibitors (inhibitor of clathrin-mediated endocytosis: chlorpromazine (CPZ); inhibitor of macropinocytosis: amiloride-HCl (AMD); inhibitor of caveolin-mediated

endocytosis: nystatin (NYS) and methyl- β -cyclodextrin (MCD); inhibitor of caveolin-mediated endocytosis: genistein (Gen)) at 37°C or at 4°C for 30 min. Then the **P**, **CPT@P** or **CPT@P«M** in serum-free medium with corresponding concentration of inhibitors were added to replace the medium at 37°C and the energy-dependent inhibition continue to incubate at 4°C for 3 h. The cells were analyzed by flow cytometry. A total of 1×10^4 events were counted for analysis.

Intracellular delivery and distribution of P, CPT@P or CPT@P«M determined by CLSM. B16F10 cells were incubated with **P**, **CPT@P** or **CPT@P«M**_{B16F10} at 37°C for 8 h. The cells were then washed three times with PBS and fixed with fresh 4.0% formaldehyde at room temperature for 15 min. After washing with PBS, the cells were stained with DAPI for 5 min and Actin-Tracter Green for 30 min. The images were taken using a LSM-880 confocal laser scanning microscope .

Cellular TEM imaging. B16F10 cells were cultured with **CPT@P«M** for 1 h, 3 h, or cultured for 3 h followed by laser irradiation (645 nm, 15 mW/cm²) for 2 min. The cells were then detached by trypsinization and collected by centrifugation. The cell pellets were fixed in 2.5% glutaraldehyde for 24 h and washed with PBS for three times (15 min $\times$ 3). Then the cell pellets were fixed in 1% osmic acid for 2 h and washed with PBS for three times (15 min $\times$ 3). The sample was dehydrated with gradient concentrations of ethanol (50%, 70%, 80%, 90% and 100%) for 15 minutes at each concentration, then treated with 100% ethanol and acetone for 20 min, respectively. Finally, the embedded sections were stained with ethanol solutions of lead citrate and uranium dioxy acetate for 15 minutes and observed under an electron microscope.

Intracellular ¹O₂ detection. The generation of ¹O₂ inside cells was detected immediately after the photosensitization experiments by using a singlet oxygen indictor. Briefly, the cells were cultured with **P**, **CPT@P** or **CPT@P«M** for 3 h, respectively. Then **P**, **CPT@P** or **CPT@P«M** were removed and DCF-DA (0.50 μ mol/L) was added, the cells were incubated for 30 min. Then the cells were subjected to photosensitization for 2 min by using laser irradiation at 645 nm with a density of 15 mW/cm². After irradiation, the medium was removed carefully and the cells were washed with PBS three times. The nuclei were stained with Hoechst 333248. The images were taken using a fluorecence microscope. Additionally, flow cytometry was used to monitor the formation of DCF by measuring the intracellular green fluorescence.

Cytotoxicity evaluation. The cytotoxicity of the nanostructures (**CPT**, **P**, **CPT@P** or **CPT@P«M**) against 4T1 and B16F10 cells were evaluated by a 3-(4',5'-dimethylthiazol-2'-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were seeded at a density of 1.00×10^4 cells/well, and incubated for 24 h for attachment. The cells were then cultured with fresh serum-supplemented medium without/with **CPT**, **P**, **CPT@P** or **CPT@P«M** at various concentrations. The cells were irradiated with light at 645 nm by controlling the laser density and irradiation time after 12 h incubation. After irradiation, the cells were further incubated for 12 h. For chemotherapy, the cells were cultured with camptothecin at various concentrations for 24 h. Then a MTT solution (200 μ L, 0.50 mg/mL) was added to each well. After incubating the cells at 37°C for 4 h, the MTT solution was removed and DMSO (100 μ L) was added to

dissolve the insoluble formazan crystals. The absorbance was measured by a spectrophotometer (570 nm). Untreated cells in media were used as a control. All experiments were carried out with five replicates.

Determination of the percentage of apoptotic cells by Annexin-V FITC/PI assay. The 4T1 cells were seeded in 6-well plates at a density of 5×10^5 cells/well and incubated in culture medium for 18 h at 37°C. After that, the culture medium containing **CPT**, **P**, **CPT@P**, or **CPT@P@M_{4T1}** were added. For chemotherapy, the cells were cultured for 2.5 h. For PDT, the cells were cultured for 2 h followed by laser irradiation (645 nm, 15 mW/cm²) for 2 min. Then the cells were further cultured for 30 min, after which the cells were thoroughly washed with PBS and harvested with trypsin. The collected cells were washed three times with PBS and resuspended in 0.5 mL of PBS. The cells were stained with Annexin V-FITC and PI according to the protocol. The cells were incubated for 10 min at room temperature in the dark and added with 500 μ L of 1 $\times$ binding buffer. The stained cells were analyzed by flow cytometry. A total of 1×10^4 events were counted for analysis.

Tumor model. The C57BL/6 mice and BALB/c nude mice were purchased from Zhejiang Academy of Medical Sciences and maintained in a pathogen-free environment under controlled temperature. Animal care and handling procedures were in agreement with the guidelines evaluated and approved by the ethics committee of Zhejiang University. Study protocols involving animals were approved by the Zhejiang University Animal Care and Use Committee. The C57BL/6 mice were subcutaneously injected with B16F10 cell suspension (100 μ L, 1×10^6) in the right flank. The BALB/c nude mice were injected with 4T1 cell suspension (100 μ L, 1×10^6) in the right flank. The tumors were allowed to grow to ~ 100 mm³ before experimentation. The tumor volume was calculated as (length) $\times$ (width)²/2.

Evaluation of hemolysis. Blood was collected from the BALB/c nude mice. The blood sample was diluted 10 times with PBS and centrifuged at 1500 rpm for 10 min. The centrifuged sample was washed with sterile PBS for three times by centrifugation and suction to isolate erythrocytes. The concentration of the resultant blood cells was adjusted to 2% (v/v). A 100 μ L sample solution (**P**, **M_{4T1}**, **CPT@P** or **CPT@P@M_{4T1}**) was added to 1 mL blood cells, respectively, and the mixtures were incubated for 1 h, 4 h, and 8 h at 37°C. The corresponding samples were centrifuged for 10 min at 1500 rpm. In order to assess the hemolytic activity, the released haemoglobin in supernatant was determined by measuring the absorbance at 541 nm. The percentage of hemolysis was calculated by $(A_{\text{sample}} - A_0)/(A_{100} - A_0) \times 100\%$, where A_{sample} , A_{100} , and A_0 is the absorbance of the sample, the completely lysed red blood cells in distilled water, and zero hemolysis in PBS, respectively. All hemolysis assays were conducted with five replicates.

In vivo anti-tumor activity. The mice were randomly divided into eight treatment groups (n = 5) when the mean tumor volume (B16F10 and 4T1 tumor models) reached around 100 mm³ and this day was set as the first day. Mice were received different treatments: PBS, Laser (L), **P**, **CPT@P**, **CPT@P@M**, **P + L**, **CPT@P + L**, and **CPT@P@M + L**. The dosage of **P**, **CPT@P** and **CPT@P@M** was 1.00 mg CPT·kg⁻¹ (2.87 μ mol/L P·kg⁻¹) in these formulations, respectively. For photodynamic therapy and photochemotherapy, the mice

only received three times laser irradiation (645 nm, 80 mW/cm², 10 min) at 8 h post the injection. Tumor volume and body weight were measured every 2 days. The tumors were excised at day 21 post treatment, and the weight of the tumor was assessed.

Tissue histopathology evaluation. In the histological assay, the heart, liver, spleen, lung, kidney, and tumor tissues were fixed in 4% paraformaldehyde for 24 h. The specimens were dehydrated in graded ethanol, embedded in paraffin, and cut into 5 mm thick sections. The fixed sections were deparaffinized and hydrated according to a standard protocol and stained with hematoxylin and eosin (H&E) for microscopic observation. Apoptosis of the tumor cells in the mice after treatments was determined by the TUNEL method according to the manufacturer's instructions.

Statistical analysis. Data are expressed as mean standard deviation (SD). Analysis of variance (ANOVA), followed by Student's *t*-test, was used to determine the significant differences among the groups (***P* < 0.01, and ****P* < 0.001).

Declarations

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information file.

Competing interests: The authors declare no competing interests.

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Figures

Figure 1

Nuclei-approaching delivery platform constructed from the host-guest nanoparticles between CPT and amphiphilic porphyrin photosensitizer and camouflaged with cancer cell membrane for enhanced cancer photochemotherapy.

Figure 2

a Partial ^1H NMR (500 MHz, CD_2Cl_2 , 25 $^\circ\text{C}$) spectra of **P**, **CPT@P** and **CPT**. **b** Partial NOESY spectrum (500 MHz, CD_2Cl_2 , 25 $^\circ\text{C}$) spectra of a mixture of **P** and **CPT**. **c** UV-vis spectra in aqueous solution of **P**

and **CPT@P**. **d** Mole ratio plot for the complexation between **P** and **CPT** in CHCl_3 at 25 °C, indicating a 1:1 stoichiometry. **e** The Tyndall effect of **CPT@P** and CAC of **CPT@P** in aqueous solution measured via the UV-vis transmittance to be about 7.0×10^{-6} mol/L. **f** ESI mass spectrum of **P** : **CPT** = 1 : 1. Calcd for $\text{C}_{152}\text{H}_{190}\text{N}_{14}\text{O}_{40}$ [**P** + **CPT**]: 2851.3264, Found: 2851.3479; Calcd for $\text{C}_{152}\text{H}_{191}\text{N}_{14}\text{O}_{40}$ [**P** + **CPT** + H^+]: 2852.3337, Found: 2852.3733; Calcd for $\text{C}_{152}\text{H}_{190}\text{NaN}_{14}\text{O}_{40}$ [**P** + **CPT** + Na^+]: 2874.3156, Found: 2874.3684; Calcd for $\text{C}_{152}\text{H}_{190}\text{Na}_2\text{N}_{14}\text{O}_{40}$ [**P** + **CPT** + 2Na^{2+}]: 2897.3048, Found: 2897.4020.

Figure 3

a DLS data of **CPT@P** and **CPT@P«M** in PBS. **b** Zeta potential of **CPT@P** and **CPT@P«M** in aqueous solution. **c** TEM image of **CPT@P** (1.00×10^{-4} mol/L) in PBS. **d** and **e** TEM images of **CPT@P«M** (1.00×10^{-4} mol/L) in PBS. **f** and **g** TEM images of **CPT@P«M** negatively stained with uranyl acetate. **h** Cryo-TEM images of **CPT@P«M** in aqueous solution. The thickness of the cell membrane was measured ~ 8.7 nm.

Figure 4

a, b and **c** Bio-TEM images of the cross section of B16F10 cell and its local view after being incubated with **CPT@P«M** for 4 h. Red circles show **CPT@P«M**. **d** The diameter changes of **CPT@P«M** determined by DLS tests in PBS containing 10% FBS after different time periods of incubation. **e** The fluorescence intensity changes at 530 nm of the solution containing SOSG in the presence of **CPT@P«M** or **CPT@P«M**/NaN₃ after irradiation for different time (645 nm, 15 mW/cm²). **f** Absorbance changes of DPBF at 414 nm vs irradiation time in ethanol in the presence of methylene blue (MB), **P**, **CPT@P**, or **CPT@P«M**. **g** The fluorescence changes of **P**, **CPT@P**, **CPT@P«M** and **ICG** after laser irradiation (645 nm, 15 mW/cm²) for different cycles. The irradiation time was 3 min for each irradiation cycle.

Figure 5

a and **d** B16F10 and 4T1 cells incubation with **CPT@P** and **CPT@P«M_{B16F10}/CPT@P«M_{4T1}** for 2 h and 4 h. Flow-cytometric analysis of 4T1, MCF7, MDA-MB-435, and B16F10 incubation with **b CPT@P«M_{B16F10}** and **e CPT@P«M_{4T1}**. The light grey peaks refer to the control cells without treatment. The statistical results of relative uptake difficiency of 4T1, MCF7, MDA-MB-435, and B16F10 treated with **c CPT@P«M_{B16F10}** or **f CPT@P«M_{4T1}** (mean $\pm$ SD, n = 3). Relative uptake efficiency of **g P**, **h CPT@P**, or **i CPT@P«M** on B16F10 cells in the presence of various endocytosis inhibitors (mean $\pm$ SD, n = 3).

Figure 6

a CLSM image of B16F10 cells treated with **P**, **CPT@P**, or **CPT@P«M** for 3 h. Blue fluorescence is from the nuclei stained with DAPI, green fluorescence is from the Actin-Tracker Green, and red fluorescence is from porphyrin. Scale bar is 20 mm. **b** Schematic representation of the nanoparticles **CPT@P«M** as a nuclei-approaching delivery platform for cancer photochemotherapy. **c** Bio-TEM images of the cross section of B16F10 cell at different stages of death treated with **CPT@P«M** at different stages. Red rectangles are the areas where have been enlarged. The broken nuclei are marked by red dotted circles. Blue circles show **CPT@P«M** nanoparticles. Green arrows indicate the damaged mitochondria. The yellow arrow indicates that the **CPT@P«M** nanoparticles are entering into the nuclei. Yellow dotted lines shows the **CPT@P«M** nanoparticles clustered in the nuclei. fluorescence is from the Actin-Tracker Green, and red fluorescence is from porphyrin.

Figure 7

a and **b** Fluorescence images, **c** Flow-cytometric analysis and **d** statistical result of B16F10 treated with **P**, **CPT@P**, or **CPT@P@M**_{B16F10} and DCFDA with/without irradiation for 2 min (645 nm, 15 mW/cm²). Scale bar is 50 mm.

Figure 8

a In vitro cytotoxicity of different formulations against 4T1 cells treated for 24 h. **b** Hemolysis rates of **CPT@P@M_{4T1}** at various concentrations after different time periods of incubation. **c** Statistical result and flow-cytometric analysis **d** of 4T1 cell apoptosis induced by different treatments. (645 nm, 15 mW/cm², 2 min). **e** Schematic illustration of **CPT@P@M_{4T1}** based photochemotherapy in 4T1 model. **f** Time-dependent ex vivo fluorescence imaging of dislodged organs of tumor-bearing 4T1 tumor-bearing mice i.v. injection of **CPT@P@M_{4T1}**. **g** Quantitative analysis of fluorescence intensity of dislodged organs

harvested at different time post i.v. injection of **CPT@P $\ll$ M_{4T1}** (mean $\pm$ SD, n = 3). **h** In vivo fluorescence imaging results of 4T1 tumor-bearing mice after the injection of **P**, **CPT@P** and **CPT@P $\ll$ M_{4T1}** and representative photos of 4T1 tumor-bearing mice in different treatments at 21 day of treatment. **i** Photo image of the tumors harvested from the mice after different treatments. **j** In vivo tumor growth inhibition curves, **k** tumor inhibition rate, **l** body weight changes and **m** survival curves of the mice bearing orthotopic 4T1 breast tumors after different treatments (n = 5). The irradiation density was 80 mW/cm² at 645nm and the irradiation time was 10 min.

Figure 9

a In vitro cytotoxicity of different formulations against B16F10 cells treated for 24 h. **b** Fluorescence images of B16F10 cells co-stained with Calcein AM/PI after different treatments. The drug concentration of CPT and CPT@P«M_{B16F10} was kept at 50 mmol/L. (Scale bars: 50 μm). **c** Schematic illustration of CPT@P«M_{B16F10} based photochemotherapy in B16F10 model. **d** Individual tumor growth kinetics of the mice treated with different formulations (n = 5). **e** Tumor weight of the mice treated with different

formulations. **f** Survival curves of the mice bearing B16F10 melanin tumors after different treatments (n = 5). **g** Photo image of the tumors harvested from the mice treated with different treatments. **h** TUNEL staining of the tumors in the therapeutic efficacy experiment. **i** H&E and TUNEL staining of the tumor tissues of the mice treated with different formulations (Scale bars: 50 μm). The irradiation density was 80 mW/cm^2 at 645nm and the irradiation time was 10 min.

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