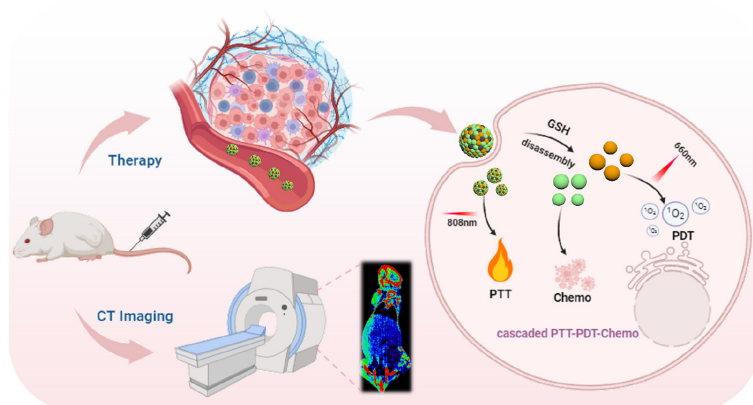


Research Article

Natural supramolecular photosensitizer with *in situ* switchable photothermal/photodynamic effects as an imaging platform for precise and controlled cancer synergistic therapy

Graphical abstract



Highlights

- Phototherapeutic nanoplatforms assembled with ET, Ce6, and disulfide bonds.
- ETSCe6@Bi NPs with PTT/PDT effects for tumor site response switching.
- ETSCe6@Bi NPs integrate imaging, chemotherapy, and PDT and PTT treatments.
- ETSCe6@Bi NPs offer controlled phototherapy and excellent anti-tumor properties.

Authors

Shiyao Fu, Jianjun Cheng, Hua Zhang, Haitian Zhao, Jiacheng Wang, Jing Wang and Xin Yang

Correspondence

yangxin@hit.edu.cn (X. Yang);
jcw@yzu.edu.cn (J. Wang)

In brief

A nanoplatform strategy of supramolecular photosensitizers (ETSCe6 NPs) and chelating metal ions is proposed to enable shifting TME responsiveness from PTT to PDT, and to enable precise diagnosis and treatment for tumor visualization.

Natural supramolecular photosensitizer with *in situ* switchable photothermal/photodynamic effects as an imaging platform for precise and controlled cancer synergistic therapy

Shiyao Fu^{a,b,c,1}, Jianjun Cheng^{c,1}, Hua Zhang^c, Haitian Zhao^b, Jiacheng Wang^{d,*}, Jing Wang^a and Xin Yang^{a,b,c,*}

^aInstitute of Plant Virology, Ningbo University, No. 818 Fenghua Road, Jiangbei District, Ningbo 315211, China

^bSchool of Medicine and Health, Harbin Institute of Technology, No. 92, West Dazhi Street, Nangang District, Harbin 150001, China

^cSchool of Chemistry and Chemical Engineering, Harbin Institute of Technology, No. 92, West Dazhi Street, Nangang District, Harbin 150001, China

^dMedicine School, Yangzhou University, No. 88, Daxue South Road, Yangzhou 225000, China

¹Shiyao Fu and Jianjun Cheng contributed equally to this work.

*Correspondence: yangxin@hit.edu.cn (X. Yang); jcw@yzu.edu.cn (J. Wang)

Received: 19 November 2024; Revised: 13 January 2025; Accepted: 31 January 2025

Published online: 26 February 2025

DOI 10.15212/AMM-2024-0081

ABSTRACT

Treatment of cancer can be challenging, because of the disease's intricate and varied nature. Consequently, developing nanomedicines with multimodal therapeutic capabilities for precise tumor therapy holds substantial promise in advancing cancer treatment. Herein, a nanoplatform strategy involving supramolecular photosensitizers (ETSCe6 NPs) and chelated metal ions (Au and Bi) was found to induce a shift in tumor microenvironment responsiveness from photothermal therapy to photodynamic therapy, thus facilitating tumor visualization for precise diagnosis and treatment. Self-assembly of supramolecular photosensitizers enhanced photothermal therapy through aggregation-caused quenching. Moreover, glutathione triggered disulfide bond cleavage, and consequently Ergosterol (ET) and Chlorin e6 release. Therefore, chemotherapy and photodynamic therapy achieved synergistic anticancer effects. Ce6 cavities were also used to chelate high valence Au and Bi metal ions for computed tomography. The ETSCe6@Au, Bi NPs demonstrated remarkable efficacy *in vitro* and *in vivo*, achieving complete tumor elimination after treatment. This integrated approach combining imaging, chemotherapy, photodynamic, and photothermal therapies has substantial promise for clinical applications, and may provide an innovative strategy for developing intelligent nanomedicines with promising prospects.

Keywords: Natural small molecules, Aggregation-caused quenching, Photodynamic therapy, Photothermal therapy, Synergistic cancer treatment

1. INTRODUCTION

Cancer's complexity and diversity pose substantial treatment challenges [1]. Currently, the exploration of single-molecule multifunctional nanosystems to simultaneously achieve various therapeutic effects has emerged as a prominent focus in anticancer research. Among them, photothermal therapy (PTT) and photodynamic therapy (PDT) have shown promise in clinical cancer treatment, because of their low toxicity, high

efficacy, and non-invasiveness [2-9]. These therapies offer further advantages such as high responsiveness and better selectivity than conventional treatments [10, 11]. Consequently, the development of nanomedicines with multimodal therapeutic capabilities for precision tumor therapy has substantial promise in advancing and optimizing cancer treatment [12-14].

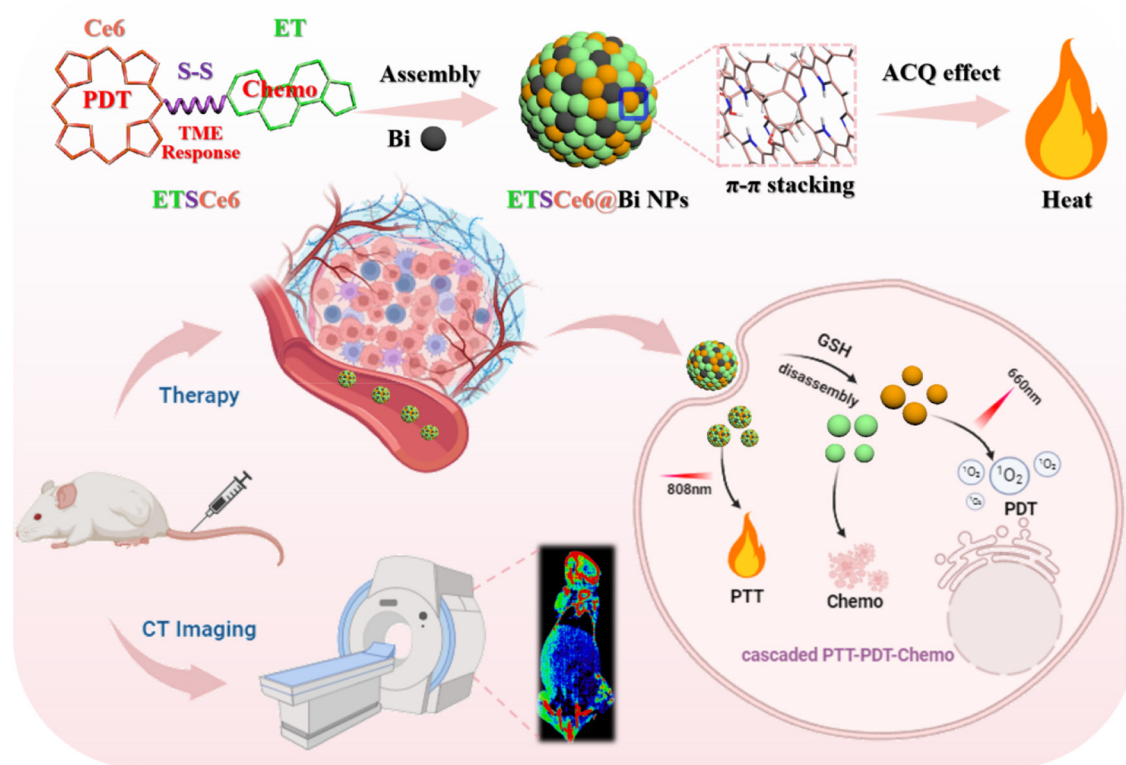
Compared with numerous photothermal reagents, organic photothermal molecules have potential advantages in the field of photothermal therapy, because

Research Article

of their unique biodegradability. Organic small molecule self-assembled materials based on porphyrins or porphyrins have been found to achieve favorable PTT performance. The self-assembly of photosensitizers induces the quenching of fluorescence and reactive oxygen species (ROS) of the photosensitized drug, owing to specific aggregation-caused quenching (ACQ) effects [15]. This phenomenon enhances non-radiative relaxation and amplifies the PTT effect [16–19]. Du et al. have developed self-assembled nanoparticles (NPs) for encapsulating cyanine molecules, exploiting intra- and intermolecular fluorescence bursts to enhance photothermal conversion efficiency [20]. Porphyrin-like molecules have emerged as optimal candidates for dual PDT and PTT platforms. Currently, an important challenge lies in inducing ordered self-assembly of photosensitizers to achieve PTT while preserving inherent PDT activity. Therefore, the selection of appropriate functional nanomaterials is paramount in this endeavor. However, these materials inevitably face the fundamental problems of nanomaterials, such as difficulties in large-scale fabrication with high reproducibility, or nanotoxicity due to their long-term metabolism *in vivo* [21, 22]. In recent years, nanocarriers derived from natural small molecules (NSMs) with self-assembly functions have become a promising approach to cancer treatment [23–26]. Because of their

ability to effectively interact with biological receptors, NSMs usually have a wide range of biological activities and are considered an important source in drug development [27]. Biologically active nanocarrier systems of natural small molecules, such as ursolic acid, betulinic acid, β -sitosterol, and dehydrofolic acid, have been constructed for synergistic chemo- and photodynamic therapy of cancer, on the basis of their self-assembling properties [28–34]. Assembly strategies can be used to create multifunctional nanocarrier platforms based on NSMs with synergistic anti-tumor effects. Ergosterol (ET) is an important steroidal compound with cholesterol-like functions, which is found primarily in the cell membranes of fungi, yeast, and some plants. In recent years, ET and its derivatives have been found to have favorable pharmacological effects, including anticancer, anti-inflammatory, and antioxidant properties. Introducing ET into nanocarrier systems enables the pharmacological effects of this molecule, such as anti-cancer and anti-inflammatory functions, to be fully exploited, while overcoming deficiencies in bioavailability, targeting, controlled release, and stability [35].

Drawing inspiration from the aforementioned investigations, we developed a supramolecular photosensitizer, referred to as ETSCe6 NPs, with *in situ* switchable PTT and PDT effects (Scheme 1). This novel approach



Scheme 1 | Schematic representation of the synthesis process and anti-tumor effects of ETSCe6@Bi NP nanocomposites with chemotherapy/PTT/PDT/CT imaging effects.

is aimed at providing precise and controllable phototherapy for the treatment of tumors [36]. ETSCe6 NPs not only reaches the tumor cells but also is affected by its ACQ properties, thus enhancing the efficiency of PTT against tumors. The ET molecule, with excellent biosafety and unique self-assembly function, was disulfide bonded to the photosensitizer Ce6, and ETSCe6 NPs were self-assembled through antisolvent precipitation. Ce6 cavities have also been used to chelate high valence Au and Bi metal ions for computed tomography (CT) [37-41]. Interestingly, the presence of large amounts of GSH under TME conditions leads to disulfide bond breakage, have been found to promote the release of ET and Ce6, and achieve synergistic anti-cancer effects of chemotherapy and PDT. This study is an example of an NP integrating the therapeutic functions of four-modality imaging, chemotherapy, and photodynamic and photothermal characteristics, thereby offering significant advantages for potential clinical applications.

2. MATERIALS AND METHODS

2.1 Preparation of ETSCe6 NPs

The nanocarriers were prepared through antisolvent precipitation. Briefly, ETSCe6 was dissolved in dimethyl sulfoxide (DMSO; 33.54 mM). The carrier solution was quickly added to 1 mL water. The mixture was then sonicated for 10 minutes (ultrasonic cleaner, Shanghai Hengqin Instrument Equipment Co., Shanghai, China). Centrifugation at 4°C and 12,000 rpm for 20 minutes yielded ETSCe6 NPs (high-speed freezing centrifuge, Changsha Xiangyi Centrifuge Instrument Co., Changsha, China). The precipitated NPs were washed twice with double-distilled water to remove excess DMSO. Other nano-assemblies with various initial additions (10, 20, 30, or 40 μ L), were prepared similarly.

2.2 Preparation of ETSCe6@Bi NPs

ETSCe6 and metal ions (Au and Bi) were dissolved in DMSO (33.54 mM). The ETSCe6@Au NP or ETSCe6@Bi NP preparations involved various constituent ratios (ETSCe6/Au = 10 μ L:5 μ L, 10 μ L:10 μ L, 10 μ L:20 μ L, 20 μ L:5 μ L, 20 μ L:20 μ L, 20 μ L:30 μ L, 20 μ L:40 μ L, and 20 μ L:60 μ L; ETSCe6/Bi = 10 μ L:5 μ L, 10 μ L:10 μ L, 10 μ L:20 μ L, 10 μ L:30 μ L, 20 μ L:5 μ L, 20 μ L:30 μ L, 20 μ L:40 μ L, and 20 μ L:60 μ L). The carrier solution was quickly added to 1 mL water. The mixture was then sonicated for 10 minutes. ETSCe6 NPs were obtained by centrifugation at 12,000 rpm for 20 min at 4°C. The precipitated NPs were washed twice with double-distilled water to remove excess DMSO.

2.3 Characterization of ETSCe6 NPs

The morphology of the prepared NPs was characterized with a Tecnai G2 20 TWIN transmission electron microscope (FEI USA, Inc., Hillsboro, USA) and a Quanta 200FEG scanning electron transmission microscope (FEI USA, Inc., Hillsboro, USA) at 20 kV. UV-visible spectra

were obtained with a TU-1900 PERSEE spectrometer (Beijing Pujin General Instrument Co., Ltd., Beijing, China). A full scan (200–800 nm) was performed on the ETSCe6 NPs. Infrared spectra in the range of 4,000 to 400 cm^{-1} were obtained through the KBr method with a Fourier transform infrared spectrometer (Nicolet iS10, Thermo, USA).

2.4 UV-visible spectroscopy

For UV determination, equivalent Ce6 concentrations of 0.4 $\mu\text{g/mL}$ were configured as a monomeric Ce6 DMSO solution, Ce6 aggregate DMSO- H_2O solution, ETSCe6 NPs, ETSCe6@Bi NPs, and ETSCe6@Au NPs. A full scan (200–800 nm) was performed on the ETSCe6 NPs, ETSCe6@Bi NPs, and ETSCe6@Au NPs.

For photothermal assays, ETSCe6 NPs, ETSCe6@Bi NPs, and ETSCe6@Au NPs were prepared with a Ce6 concentration of 50 $\mu\text{g/mL}$ and continuously irradiated at 808 nm (500 mW cm^{-2}) for 300 s (808 infrared laser, Beijing Nyubit Technology Co., Beijing, China). An infrared thermography camera (Fluke thermal imaging camera, Fluke Co., Washington, USA) was used to obtain temperature changes at various time points, and double-distilled water was used as a control.

The photothermal conversion efficiency (η) was measured according to the reported method and calculated with equation 1:

$$\eta = \frac{hs(T_{\text{max}} - T_{\text{surr}}) - Q_{\text{Dis}}}{I(1 - 10^A)} \quad (1)$$

where h is the heat transfer coefficient, s is the surface area of the container, Q_{Dis} is the amount of heat dissipated by the laser mediated by the solvent and container, and I is the laser power.

2.5 Extracorporeal phototherapy

In 96-well and six-well plates, 4T1 cells were seeded at a density of 10^4 cells/well for 24 h. The supernatants from the 96-well plates were removed, and 100 μL fresh DMEM at various concentrations was added to each well. Fresh medium containing ETSCe6 NPs at a concentration of 100 $\mu\text{g mL}^{-1}$ was added to each well of a six-well plate. Cells were irradiated at 808 nm with a power density of 0.5 W cm^{-2} for 5 min for PTT and PDT. Cell viability was also assessed with the MTT method after 24 h and with calcein-AM/propidium iodide (PI) co-staining and inverted fluorescence microscopy (Chongqing Opal Optoelectronic Technology Co., Chongqing, China) to visualize live/dead cells for 30 min.

2.6 In vivo biodistribution and CT imaging

Female BALB/c mice (6–8 weeks old, 18–22 g) from the Second Affiliated Hospital of Harbin Medical University (Harbin, Heilongjiang, China) were used for the experiments. All animal experiments were conducted according to protocols approved by the Harbin Medical University Animal Care and Use Committee, in accordance with the

Research Article

standards and norms of the Animal Ethics Society. When 4T1 cells mouse tumors reached 150–200 mm³, ETSCe6 NPs and ETSCe6@Au NPs (150 µL each) were injected *in vivo* through the tail vein. Subsequently, the mice received isoflurane anesthesia and were imaged with 661 nm excitation at various time points with a Maestro Mouse *In Vivo* Fluorescence Imaging System (Cambridge Research & Instrumentation, Inc., Massachusetts, USA). At 24 h after injection administration, the mice were euthanized through cervical dislocation, and major organs, including the heart, liver, spleen, lungs, and kidneys, as well as tumor tissue, were collected for *ex vivo* fluorescence imaging.

For CT imaging of ETSCe6 NPs, 150 µL ETSCe6@Bi NPs was injected into the tail vein in mice when the tumor volume (4T1) was approximately 150–200 mm³. The mice were then anesthetized with isoflurane. The imaging signals of the ETSCe6@Bi NPs were detected at various times through CT imaging.

2.7 *In vivo* anti-tumor therapy

When the mean tumor volume approached 75 mm³, 150 mm³, or 400 mm³, the mice were randomly divided into nine groups (n = 5) receiving the following treatments: (1) 5% glucose aqueous solution, (2) ETSCe6 NPs, (3) ETSCe6 NP-PDT, (4) ETSCe6 NP-PTT, (5) ETSCe6 NP-PDT-PTT, (6) ETSCe6@Bi NP-PDT, (7) ETSCe6@Bi NP-PDT-PTT, (8) ETSCe6@Au NP-PDT, or (9) ETSCe6@Au NP-PDT-PTT. The first administration was designated as day 0, and 200 µL of the corresponding drug was injected into the tail vein on days 0, 2, and 4. In the PTT group, mice were anesthetized with isoflurane and treated with light for 15 min at the tumor site 6 h after each administration. The PDT group was irradiated at the tumor site with a 600 nm laser at 150 mW cm⁻² for 15 min. Changes in tumor volume and body weight were obtained every 2 days. Tumor volume was calculated with equation 2:

$$V = a \times b^2 \times 0.5 \quad (2)$$

where *a* and *b* represent the long and short diameters of the tumor, respectively.

At the end of the experiment, the mice were euthanized, and the tumors were weighed. The average tumor inhibition rate (TIR) was estimated with equation 3:

$$\text{TIR}(\%) = (1 - W_{\text{sample}}/W_{\text{control}}) \times 100\% \quad (3)$$

where *W*_{sample} and *W*_{control} indicate the average tumor weight in the treatment and control groups, respectively.

2.8 *In vivo* safety evaluation

At the end of the PTT/PDT/chemotherapy treatment, major organs (heart, liver, spleen, lungs, and kidneys) and tumors from each group were collected, fixed in 10% neutral formalin buffer for 2 days, processed for paraffin embedding, sectioned, and stained with hematoxylin and eosin (H&E). The results were photographed under a light microscope and analyzed.

At the end of the treatment, blood was collected retro-orbitally (without anticoagulant) and allowed to stand for 2–4 h at 4°C, then centrifuged at 3,000 rpm for 10 min. The upper layer of yellowish transparent serum was collected for blood macrobiochemical analysis. The recorded blood data included liver and kidney function indexes, specifically albumin transaminase, aminotransferase, alkaline phosphatase, albumin, total bilirubin, creatinine, uric acid, and urea.

2.9 Statistical analysis

All data are expressed as mean ± standard deviation, and two-sided Student's *t* test (SPSS 19.0 software, Chicago, IL, USA) was used to compare the differences between groups. *P* < 0.05, **P* < 0.01, and ****P* < 0.001 indicated significant differences.

3. RESULTS AND DISCUSSION

3.1 Preparation and characterization of ETSCe6 NPs

The GSH-responsive ETSCe6 synthetic routes are shown in [Figure 1a](#). The chemical structure of ETSCe6 was confirmed by NMR carbon, hydrogen, and high-resolution mass spectrometry ([Figure S1–S3](#)). To prepare ETSCe6 NPs, we used an anti-solvent precipitation method [30]. The morphology of the prepared ETSCe6 NPs is shown in [Figures 1b and S4](#). As expected, the obtained NPs showed morphologically intact, uniformly sized spherical shapes under scanning microscopy. The hydrodynamic diameter, measured with dynamic light scattering, was ~129.5 ± 1.2 nm ([Table S1](#)). The zeta potentials of the ETSCe6 NPs ([Table S1](#)), showed a negatively charged distribution at -26.5 ± 1.5 mV. To verify the successful assembly of ETSCe6 NPs, we assessed the photochemical properties of aqueous dispersions of ETSCe6 NPs ([Figure 1c](#)). The nano-assembled ETSCe6 NPs showed the typical Soret band and Qy band of Ce6 at 409 and 669 nm, respectively. Notably, red-shifted Soret and Qy bands with respect to those for free ETSCe6 indicated successful assembly and possible intermolecular π-π stacking interactions between species. Meanwhile, the appearance and shift of the C=N characteristic vibration in the Fourier transform infrared spectra further confirmed the successful assembly of the ETSCe6 NPs ([Figure 1d](#)).

On the basis of the above findings, we further prepared ETSCe6@Au NPs and ETSCe6@Bi NPs by chelating ETSCe6 NPs with Au or Bi ions, respectively ([Figure S5 and Table S2](#)). Subsequently, the surface elemental composition of ETSCe6@Bi NPs and ETSCe6@Au NPs was verified with XPS ([Figure 1e, f](#)). The ETSCe6@Bi NP spectrum exhibited four characteristic peaks: C 1s (284.6 eV), N 1s (399.1 eV), O 1s (531.7 eV), and Bi 4f (157.9 eV). Similarly, the ETSCe6@Au NP spectrum exhibited four characteristic peaks: C 1s (284.6 eV), N 1s (399.1 eV), O 1s (531.3 eV), and Au 4f (87.5 eV). XPS results confirmed the presence of Bi and Au ions. The UV absorption spectrum clearly indicated that the Soret and Qy bands were redshifted, and the Au and Bi had therefore been complexed

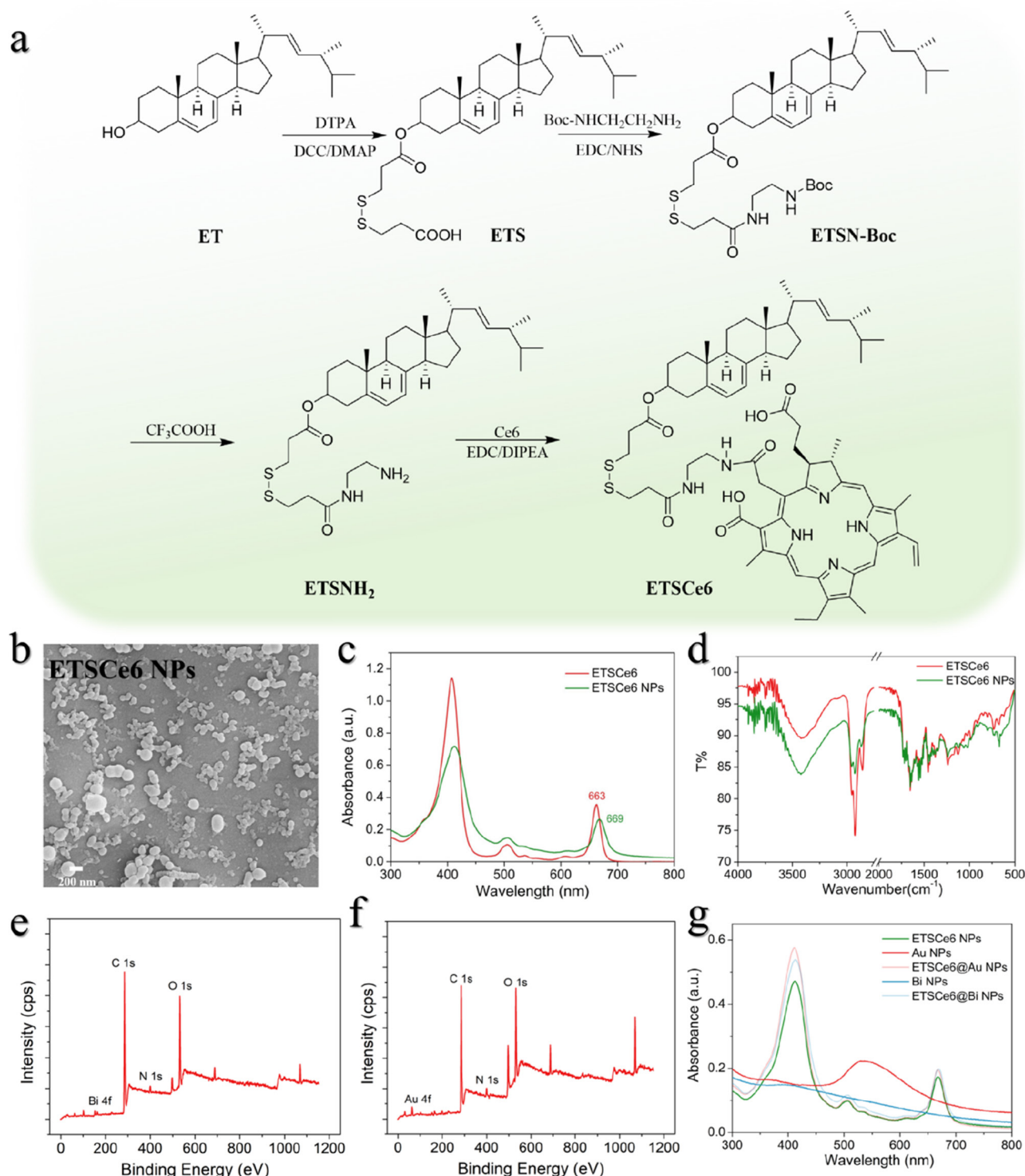


Figure 1 | Characterization of ETSCe6 NPs.

a) ETSCe6 synthesis route. b) Scanning electron micrograph of ETSCe6 NPs. c) UV spectra of ETSCe6 and ETSCe6 NPs. d) FT-IR spectra of ETSCe6 and ETSCe6 NPs. e, f) XPS of ETSCe6@Bi NPs and ETSCe6@Au NPs. g) UV spectra of ETSCe6@Bi NPs and ETSCe6@Au NPs.

Research Article

(Figure 1g). The encapsulation rates of ETSCe6 NPs, ETSCe6@Bi NPs, and ETSCe6@Au NPs were determined to be 66.0%, 79.0%, and 47.8%, respectively.

3.2 Assembly process of ETSCe6 NPs

Spectral absorption analysis of ETSCe6 NPs was used to study the driving forces for self-assembly. Ce6 tended to exist as H-type aggregates (dimers or multimers) in insoluble or highly polar media (e.g., aqueous solutions) in a coplanar arrangement, thus decreasing the absorbance and absorption wavelengths. With the addition of water, the absorbance of Ce6 in the Qy band decreased, and the absorption wavelength showed a significant blueshift (Figure 2a). ETSCe6 was added to water/DMSO solutions at various ratios to induce self-assembly of ETSCe6 NPs. Intermolecular π - π stacking interactions were facilitated within ETSCe6 NPs, thus resulting in a redshift of the Qy band (Figure 2b). According to the above results, ETSCe6 NPs consisted primarily of aggregates and not monomers of ETSCe6. To investigate the presence of monomeric forms in the NPs, we measured the supernatant of ETSCe6 NPs after centrifugation and the UV spectra of non-centrifuged NPs. ETSCe6 NPs and centrifuged supernatant (Figure 2c) both showed redshifts indicating the absence of ETSCe6 monomer in both samples. This observation supported the hypothesis that

ETSCe6 was present predominantly in ETSCe6 NPs in aggregated rather than monomeric form.

Subsequently, the interaction between ET and the aromatic ring of Ce6 was confirmed by measurement of UV-visible absorption spectra after incubation with surfactant sodium dodecyl sulfate (SDS; 0.2% w/v). Incubation of ETSCe6 NPs with SDS resulted in a prominent blueshifted UV absorption peak near 643 nm, thereby confirming the hydrophobic interactions between ET and the Ce6 aromatic ring (Figure 2d). The hydrophilicity of the ETSCe6 NPs was determined by contact angle measurements (Figure 2e). A significant enhancement in surface hydrophilicity of the NPs versus free ETSCe6 was observed, possibly because of the arrangement of hydrophilic groups on the outer surface. The aforementioned findings corroborated that π - π stacking and hydrophobic interactions were likely to be the primary driving forces underlying the formation of co-assembled ETSCe6 NPs.

3.3 Mechanism of PDT induced and photothermal conversion by ETSCe6 NPs

To evaluate the potential of ETSCe6 NPs for chemo/PDT/PTT therapy and determine the detailed PDT process, we assessed the drug's photochemical and photophysical properties, namely fluorescence and photothermal

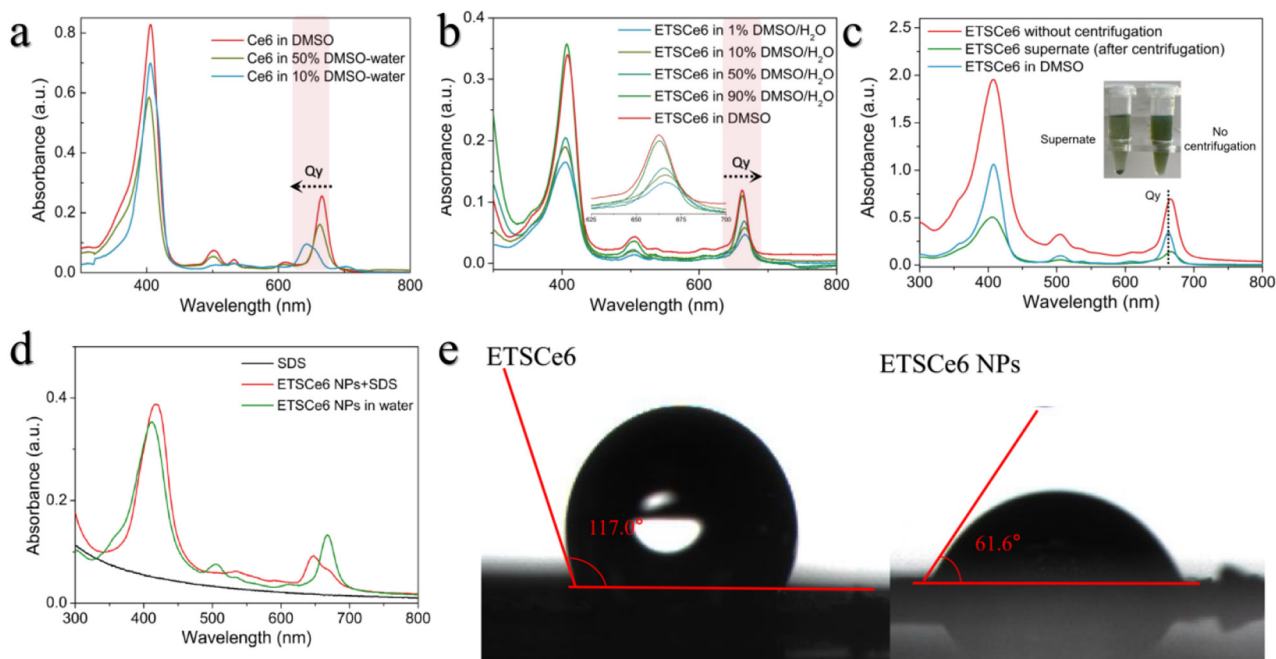


Figure 2 | Assembly mechanism of ETSCe6 NPs.

Different ratios of water/DMSO solution in a) Ce6 and b) ETSCe6 NPs. c) UV-visible absorption spectra ETSCe6 NPs before and after centrifugation. d) UV-visible absorption spectra of ETSCe6 NPs after incubation with 0.2% SDS and urea for 6 h. e) Contact angle tests of ETSCe6 and ETSCe6 NPs.

performance. The fluorescence spectra of ETSCe6 NPs were determined (Figure 3a). The fluorescence burst efficiency of ETSCe6 NPs in water was ~99.3% that of ETSCe6 in DMSO. The ETSCe6@Bi and ETSCe6@Au NPs fluorescence spectra are illustrated in Figure S6. The ACQ effect of ETSCe6 NPs was attributed primarily to the effective intramolecular PET effect, thus indicating that the excited state energy of ETSCe6 was easily attenuated through non-radiative pathways, thereby promoting photothermal conversion. This finding also confirmed the absence of ETSCe6 monomers in ETSCe6 NPs. The fluorescent probe 2,7-dichlorofluorescein diacetate (DCFH-DA) was used to directly measure ROS generation in ETSCe6 NPs with irradiation (Figures 3b and S7). Moreover, the fluorescence of ETSCe6 NPs gradually increased over time. After irradiation with ETSCe6 NPs and DCFH in a mixed aqueous solution for various times,

a significant increase in fluorescence emission near 520 nm was observed. Over time, ETSCe6 NPs showed increasing fluorescence. The levels of ROS with ETSCe6 NPs assembled in water were ~1.2 times higher than those of free ETSCe6 (Figure 3c, d). To further understand whether the synthesized ETSCe6 had photothermal conversion capability, we used density functional theory calculations with ETSCe6 to determine their optimized geometries, and to calculate the lowest unoccupied molecular orbital (LUMO) and the highest occupied molecular orbital (HOMO) (Figure 3e, f). Direct transitions from the higher excited state to the lower excited state can be facilitated by Ce6 and ETSCe6 NPs [42]. The energy gap (ΔE_{gap}) was calculated to be 1.8382 eV for Ce6 and 1.8604 eV for ETSCe6 NPs. A smaller ΔE_{gap} indicates a lower energy requirement for electron transition from the HOMO to the LUMO, thus necessitating less

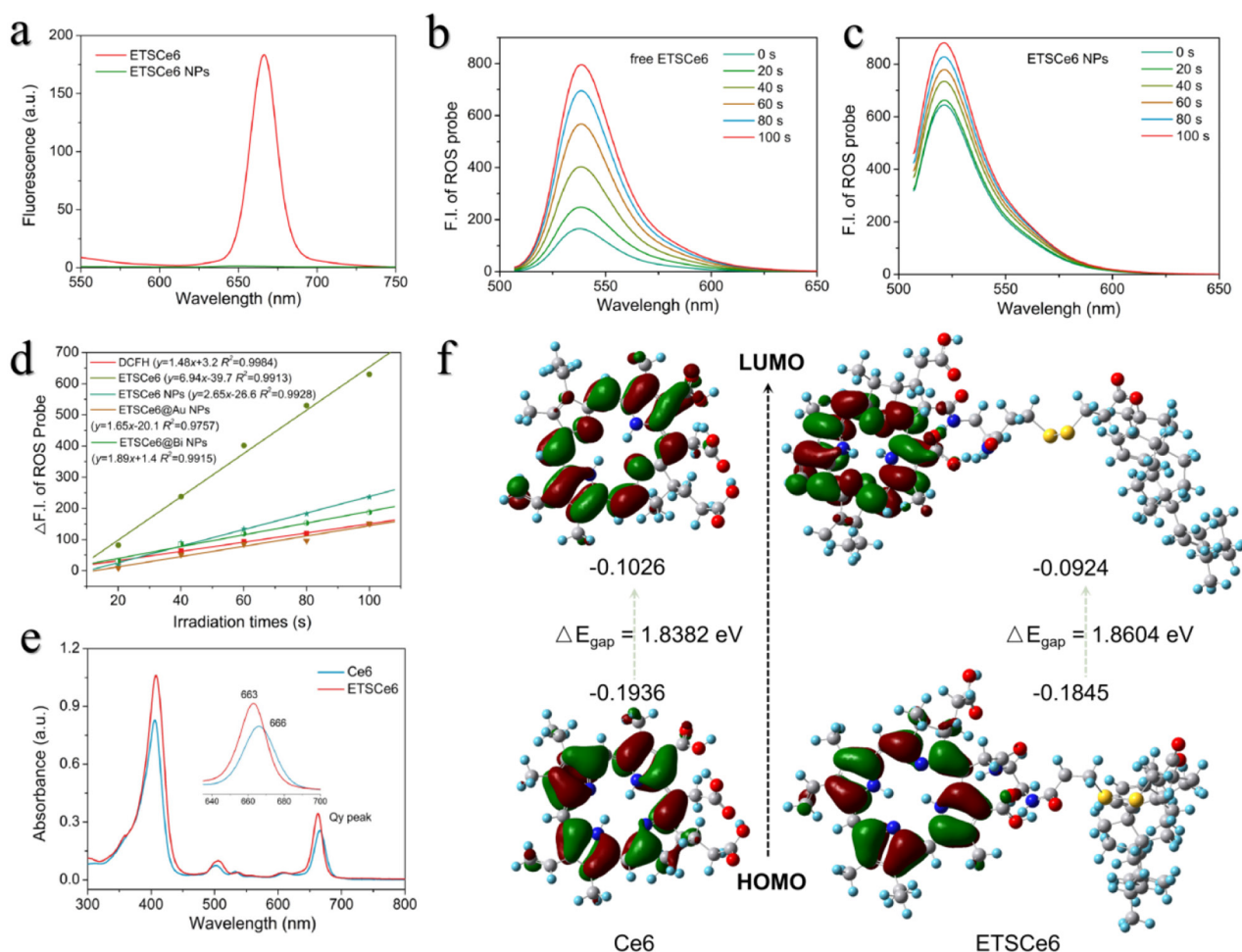


Figure 3 | Mechanism of photodynamic reaction triggered by ETSCe6 NP assembly.

a) Fluorescence emission spectra of ETSCe6 NPs assembled in water. b, c) ROS generation of ETSCe6 NPs and free ETSCe6, detected with the fluorescent probe 2,7-dichlorofluorescein diacetate (DCFH-DA). d) Changes in fluorescence intensity of the DCFH probe at 520 nm at various time points. e) UV-visible absorption spectra ETSCe6 and Ce6. f) Optimized geometry, HOMO, and LUMO of ETSCe6 NPs, calculated with density functional theory (DFT).

Research Article

energy for light absorption. This observation was consistent with the UV spectroscopy results, wherein Ce6 demonstrated a small Δ gap and UV absorption at 666 nm. In contrast, ETSCe6 exhibited a larger Δ gap, resulting in a blueshifted absorption wavelength at 663 nm. In conclusion, the ETSCe6 NPs have the optical properties necessary to achieve synergistic treatment of tumors.

For further study of the photothermal conversion with ETSCe6 NPs, we monitored the temperature change in the aqueous solution of ETSCe6 NPs with continuous irradiation from an 808 nm laser. After 600 seconds of irradiation at 500 mW cm^{-2} , pure water showed negligible temperature variation (Figure S8), whereas the temperature of the ETSCe6@Bi NPs solution showed a time-dependent increase (Figure 4a). On the basis of infrared thermogram recordings (Figure 4c), a peak temperature of 44°C was observed within 600 seconds at a concentration of 500 $\mu\text{g mL}^{-1}$. The photothermal conversion efficiency of the ETSCe6@Bi NPs was calculated to be 31.2%. Similarly, both ETSCe6 and ETSCe6@Au NPs showed similar behavior (Figures 4b, d and S9). These compelling findings regarding ETSCe6 NPs

suggested promise as a strong photothermal agent with a high photothermal conversion capacity suitable for photothermal therapy.

3.4 *In vitro* cellular response to TME stimulation

To elucidate the internalization and disintegration of ETSCe6 NPs at the cellular level, we incubated 4T1 cancer cells with ETSCe6 NPs (Figure 5a). ETSCe6 NPs efficiently targeted the tumor cells and used the ACQ effect, which significantly enhanced the efficiency of PTT. In addition, ET and Ce6 were released at tumor sites, because of the introduction of disulfide bonds in the presence of the tumor microenvironment. When ET and Ce6 were released, the PTT effect was lost because of weakening of the ACQ effect, thus restoring the chemotherapy and PDT effects. Free Ce6 showed intense fluorescence, thus indicating its presence in a monomeric form that was readily phagocytosed by the cells (Figure 5b). ETSCe6 did not exhibit strong fluorescence with cellular uptake. Over time, the gradual intensification of red fluorescence indicated that the intracellular reduction of GSH triggered the cleavage of disulfide bonds, and subsequently

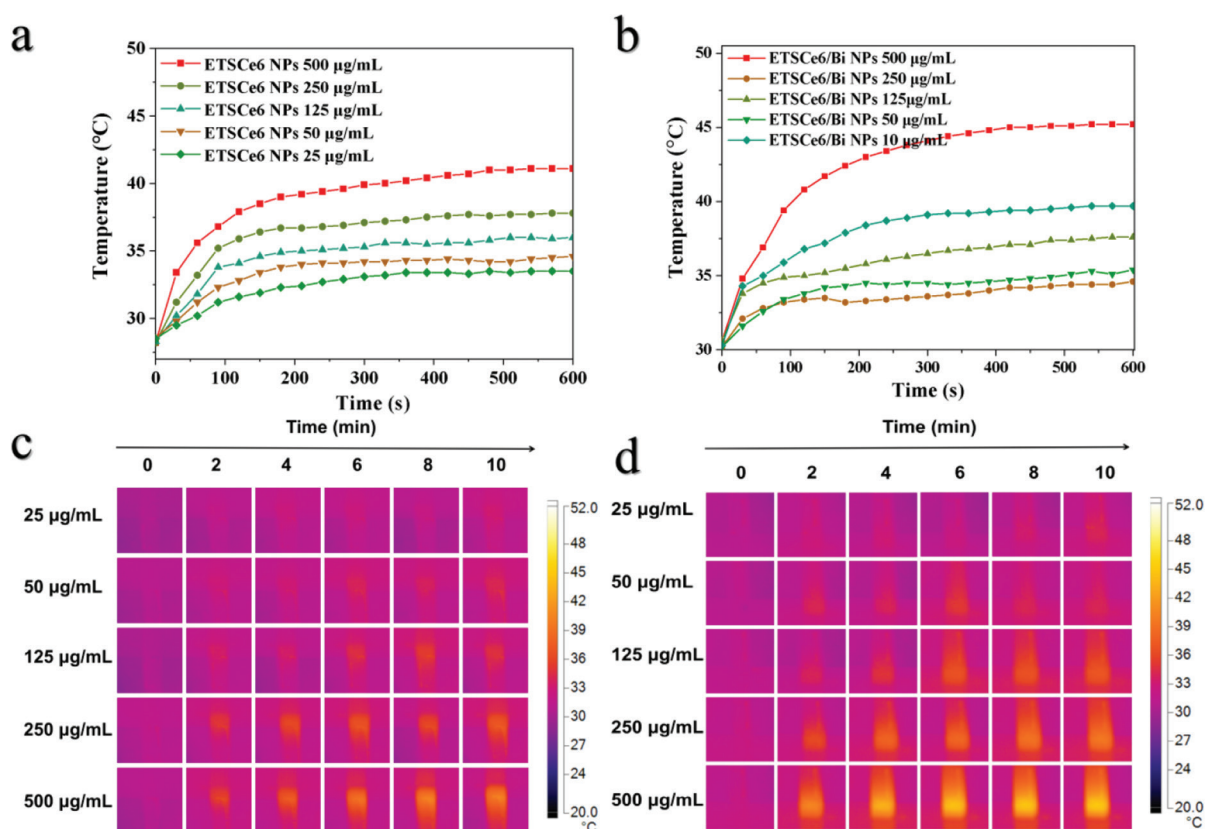


Figure 4 | Photothermal conversion of ETSCe6 NPs.

a, c) Photothermal heating curves of various concentrations of ETSCe6 NPs and aqueous solutions of ETSCe6@Bi NPs irradiated at 500 mW cm^{-2} with an 808 nm laser. b, d) Photothermal images of the ETSCe6 NPs and aqueous solutions of ETSCe6@Bi NPs, irradiated at 500 mW cm^{-2} with an 808 nm laser.

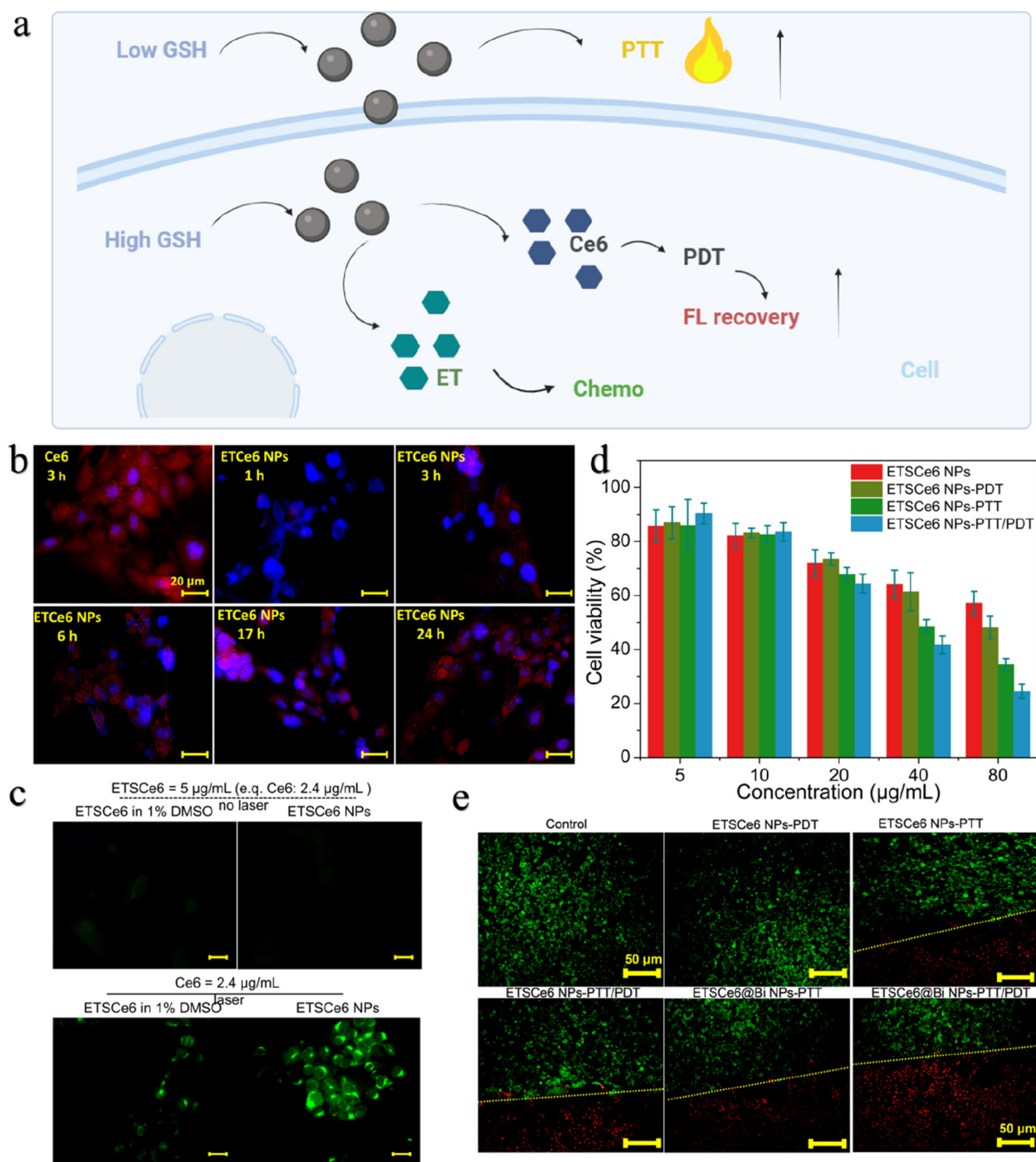


Figure 5 | In vitro cellular anticancer activity in response to TME stimulation.

a) Schematic illustration of intracellular treatment of ETSCe6 NPs (blue, DAPI; red, NPs). b) Fluorescence images of 4T1 after co-incubation with ETSCe6 NPs. Scale bar: 20 μm . c) Fluorescence images of intracellular ROS of various treatments. Scale bar: 20 μm . d) Cell survival after 24 h of treatment of 4T1 with various drugs. e) Staining analysis of live/dead cells of AM-PI after treatment of 4T1 with ETSCe6 NPs. Scale bar: 50 μm .

led to the dissociation and restoration of Ce6 molecule fluorescence. Therefore, GSH-activated Ce6 fluorescence served as a real-time indicator for monitoring NPs

breakdown *in vitro*. The DCFH-DA probe was co-incubated with Ce6 or ETSCe6 NPs (Ce6 concentration: 2.4 $\mu\text{g mL}^{-1}$) to assess intracellular ROS production. With

Research Article

illumination, cells exposed to ETSCe6 NPs showed notably more intense green fluorescence signals than those treated with Ce6 (**Figure 5c**). This finding suggested that the intracellular ROS levels induced by ETSCe6 NPs substantially increased in the presence of illumination. The ETSCe6 NPs therefore had significant phototoxicity in PDT applications.

To further illustrate and explore the photochemotherapeutic anticancer ability of ETSCe6 NPs, we measured the cytotoxicity of ETSCe6 NPs with MTT assays in 4T1 cells. The cell viability decrease was concentration dependent, and combined PTT and PDT treatment had the greatest cell killing effect, with ~76% of the cancer cells effectively killed with treatment with ETSCe6 NPs at a concentration of $80 \mu\text{g mL}^{-1}$ (**Figure 5d**). To illustrate the remarkable photochemotherapeutic efficacy of ETSCe6 NPs, we used calcein-AM and PI fluorescence double staining to distinguish live from dead cells, thereby detecting apoptosis and necrosis induced by the nanomedicine. Cells in the control group showed robust health, devoid of red fluorescence indicative of cell death (**Figure 5e**). Notably, ETSCe6 NPs showed significant induction of apoptosis and necrosis when subjected to PDT and PTT. This observation underscored the potential efficacy of combined treatment modes. **Figure S10** illustrates the outcomes of ETSCe6@Au NPs. ETSCe6 NPs therefore have favorable biocompatibility and *in vitro* phototherapeutic effects.

3.5 *In vivo* imaging

A small animal *in vivo* fluorescence imaging system was used to investigate the biodistribution of ETSCe6 NPs in tumor-bearing mice. The fluorescence emitted by free ETSCe6 in mice surpassed that in the other groups (**Figure 6a**). This finding indicated fluorescence quenching in NP-treated mice due to the ACQ effect. Simultaneously, at the tumor sites, the fluorescence intensity in all groups initially increased, peaked at 8 h, and subsequently decreased (**Figure 6b**). Additionally, images of the heart, liver, spleen, lungs, kidneys, and tumors were captured 24 h after *ex vivo* injection (**Figure 6c, d**). The weak fluorescence observed in the tumor tissue suggested gradual degradation of ETSCe6 NPs at tumor sites.

The CT capability of ETSCe6@Bi NPs was studied through both *in vitro* and *in vivo* experiments. Increasing brightness was observed with higher concentrations of ETSCe6@Bi NPs (**Figure 6e**). To further assess CT capability, we conducted *in vivo* studies in 4T1 tumor-bearing mice. In the initial experiments, mice bearing tumors were injected intratumorally, and significant enhancement of tumor signals was observed (**Figure 6f**), thereby supporting *in vivo* imaging applications of the NPs. After tail-vein injection in tumor-bearing mice, a sustained increased EPR effect was observed at tumor sites (**Figure 6g, h**), in agreement with the FL imaging data. These results indicated the accumulation of ETSCe6@Bi NPs at tumor sites, thus underscoring their utility as CT contrast

agents for guiding *in vivo* phototherapy. Encouraged by the promising results of *in vivo* CT imaging and the potential for GSH-triggered PTT/PDT switching, we studied the PTT efficacy of ETSCe6 NPs *in vivo* at various time intervals (0–10 min). With the injection of ETSCe6@Bi NPs, the temperature at the tumor site rose from 36.4 to 59.3°C within the same timeframe (**Figure 6i**). This finding indicated that metal ions confer CT imaging capability and synergistically enhance PTT.

3.6 Synergistic anti-tumor properties with *in vivo* PCT

The *in vivo* anticancer efficacy of nanomedicines was evaluated in 4T1 mice bearing tumor cells on the basis of the *in vitro* photochemical anticancer activity of ETSCe6@Bi NPs. **Figure 7a** shows the general schedule of animal experiments. Continuous tumor growth was observed in mice injected with PBS (**Figure 7b, c**), whereas all tumors in the group treated with PTT were significantly suppressed. However, solid tumors in both the free ETSCe6 and ETSCe6 NP-PDT groups could not be eliminated, and some recurred within 14 days. Solid tumors in several other groups of tumor-bearing mice were completely ablated and did not recur after 26 days, thereby achieving an early tumor suppression rate of 100% (**Figure 7d**). In view of the observed high anti-tumor efficacy, we extended the study to assess the therapeutic potential of ETSCe6 NPs in mid-tumor regions. After the tumor volume reached 150 mm^3 , the previously described treatment protocol was applied. The ETSCe6 NP-PDT-PTT group, along with the ETSCe6@Bi NP-PDT-PTT group, showed substantial tumor inhibitory effects after combined treatment (81.7% and 72.4% inhibition, respectively) (**Figures S11 and S12**). Representative images of mice undergoing treatment in various groups are displayed in **Figures S13 and S14**. During the subsequent monitoring period, rapid regeneration of tumor tissue was observed in both the ETSCe6 NP-PDT-PTT and ETSCe6@Au NP-PDT-PTT treatment groups. In contrast, the crusts observed in the ETSCe6@Bi NP-PDT-PTT group did not reappear within the tumor during the subsequent observation period after shedding 8 days later. Subsequently, a mouse model of advanced tumors was established to investigate ETSCe6 NPs' maximum therapeutic capacity. When the tumor volume reached 400 mm^3 , the treatment and determination of tumor volume were performed as previously described. The tumor inhibition rate in the ETSCe6 NP-PDT-PTT group reached nearly 70% (**Figures S15 and S16**). Together, the results indicated ETSCe6 NPs' excellent anticancer efficiency in chemo-PDT-PTT combination therapy. In addition, pathological analyses of tumor tissues from mice with medium-term tumors were performed. H&E staining indicated tissue deterioration and nuclear detachment in tumor sections of chemo-PDT-PTT combination-treated tumors (**Figure 7e**). The above results indicated that the successfully constructed ETSCe6 NPs achieved efficient anti-tumor efficacy.

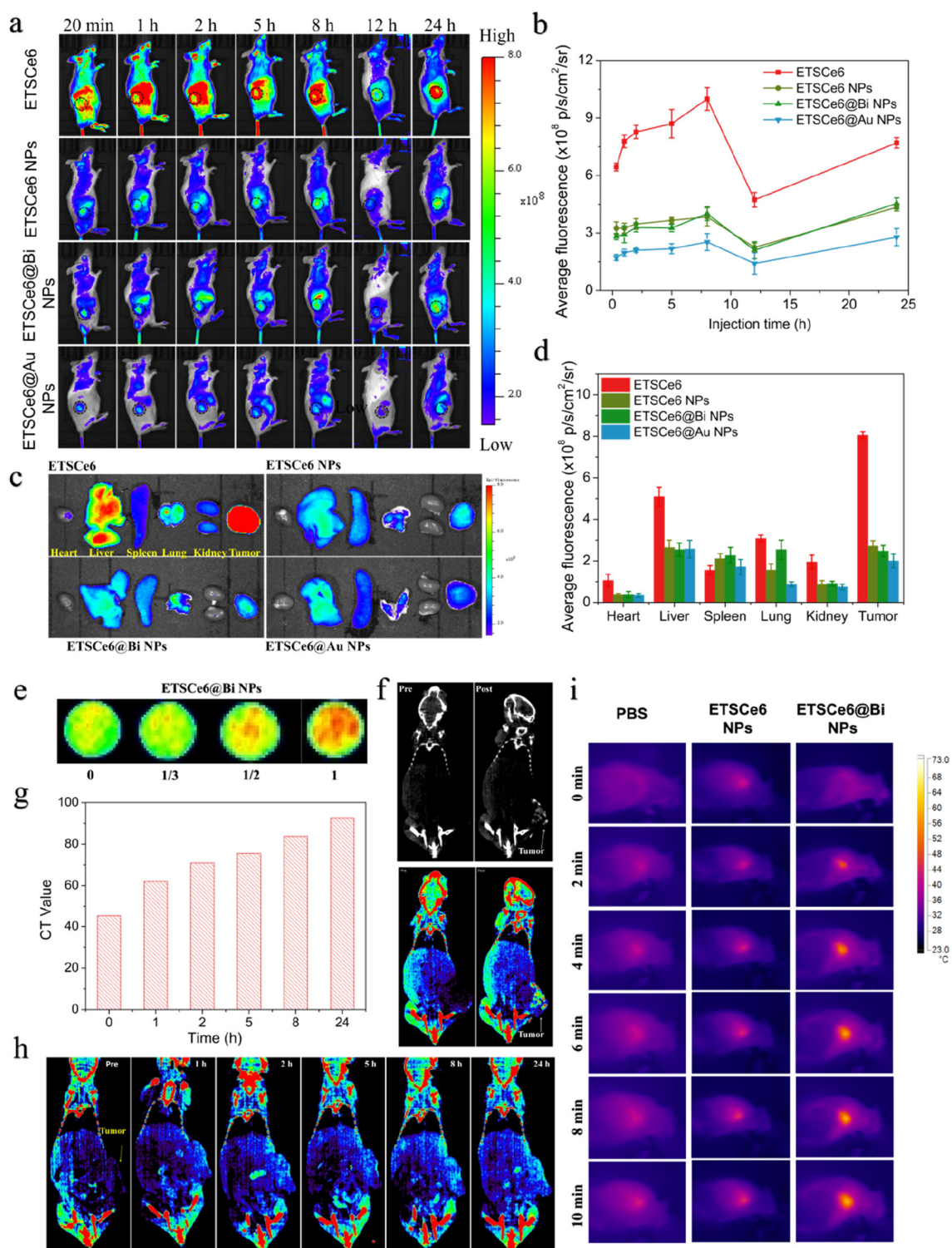


Figure 6 | In vivo biodistribution of ETSCe6 NPs.

a) *In vivo* real-time fluorescence imaging of each group (black circles mark the tumors). b) Real-time fluorescence intensity at tumor sites. c) Fluorescence imaging of isolated tumors and major organs at 24 h. d) Average fluorescence intensity in each isolated tissue. e) CT images of ETSCe6@Bi NPs. f) CT images of tumor-bearing mice injected intratumorally. g, h) CT signals of tumor-bearing mice treated with ETSCe6@Bi NPs at various time points and their quantification. i) Infrared thermal images of mice injected intravenously with ETSCe6@Bi NPs under continuous irradiation for 10 minutes.

Research Article

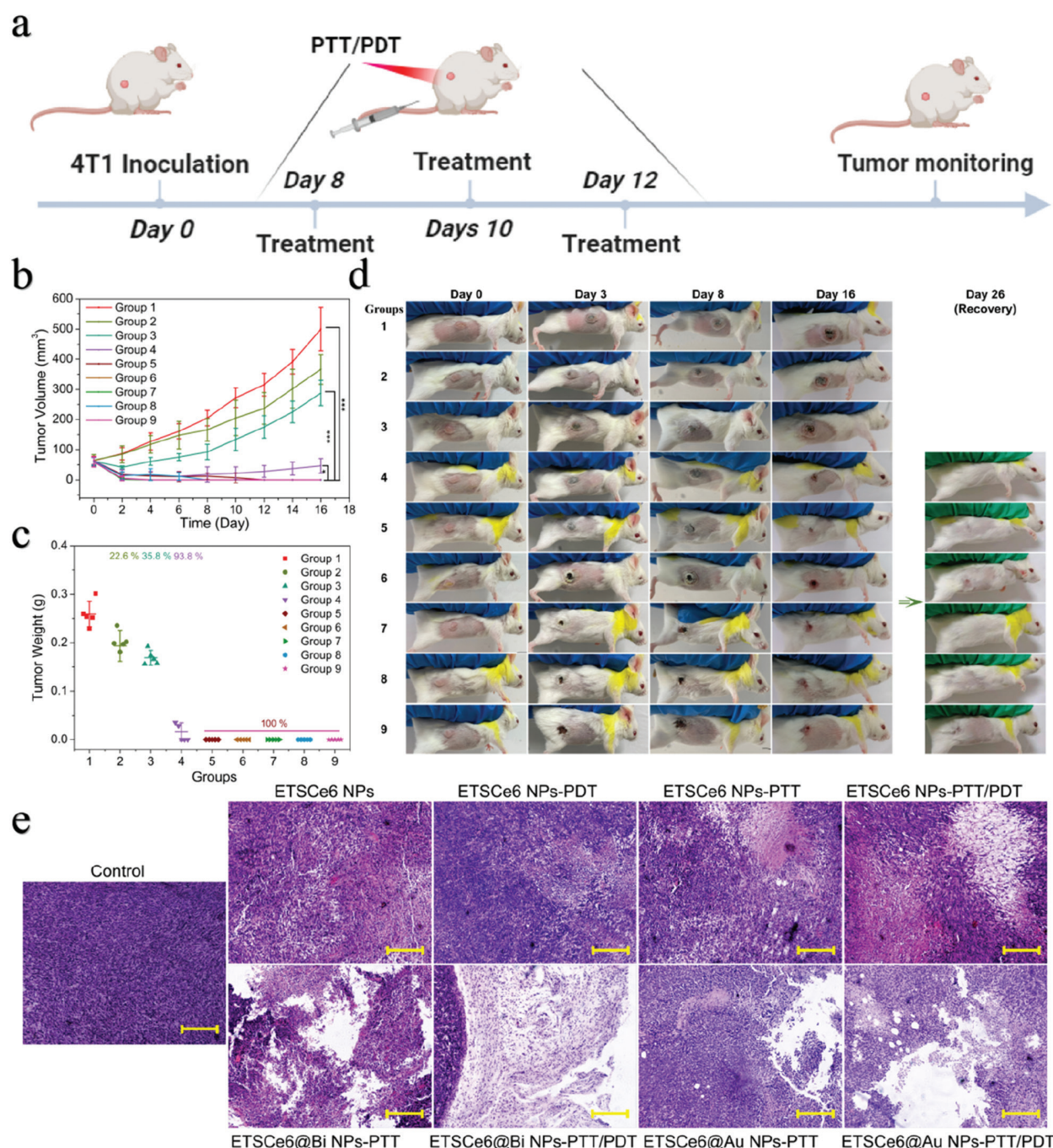


Figure 7 | In vivo assessment of anticancer activity in mice.

a) Overall timetable for animal experiments. b, c) Tumor volumes and inhibition rates in early stages of tumor growth. d) Images of representative mouse in various groups during treatment of early stage tumors. e) Tumor H&E staining results after various drug treatments. Scale bar: 50 μ m. Groups: 1) control; 2) ETSCe6 NPs; 3) ETSCe6 NP-PDT; 4) ETSCe6 NP-PTT; 5) ETSCe6 NP-PTT/PDT; 6) ETSCe6@Bi NP-PTT; 7) ETSCe6@Bi NP-PTT/PDT; 8) ETSCe6@Au NP-PTT; 9) ETSCe6@Au NP-PTT/PDT.

3.7 Biosafety

During the treatment period, the body weights of the mice were measured every other day (Figure S17). We

observed no significant changes in the body weights of tumor-bearing mice in the various experimental groups at various times. At the end of the treatment, the major

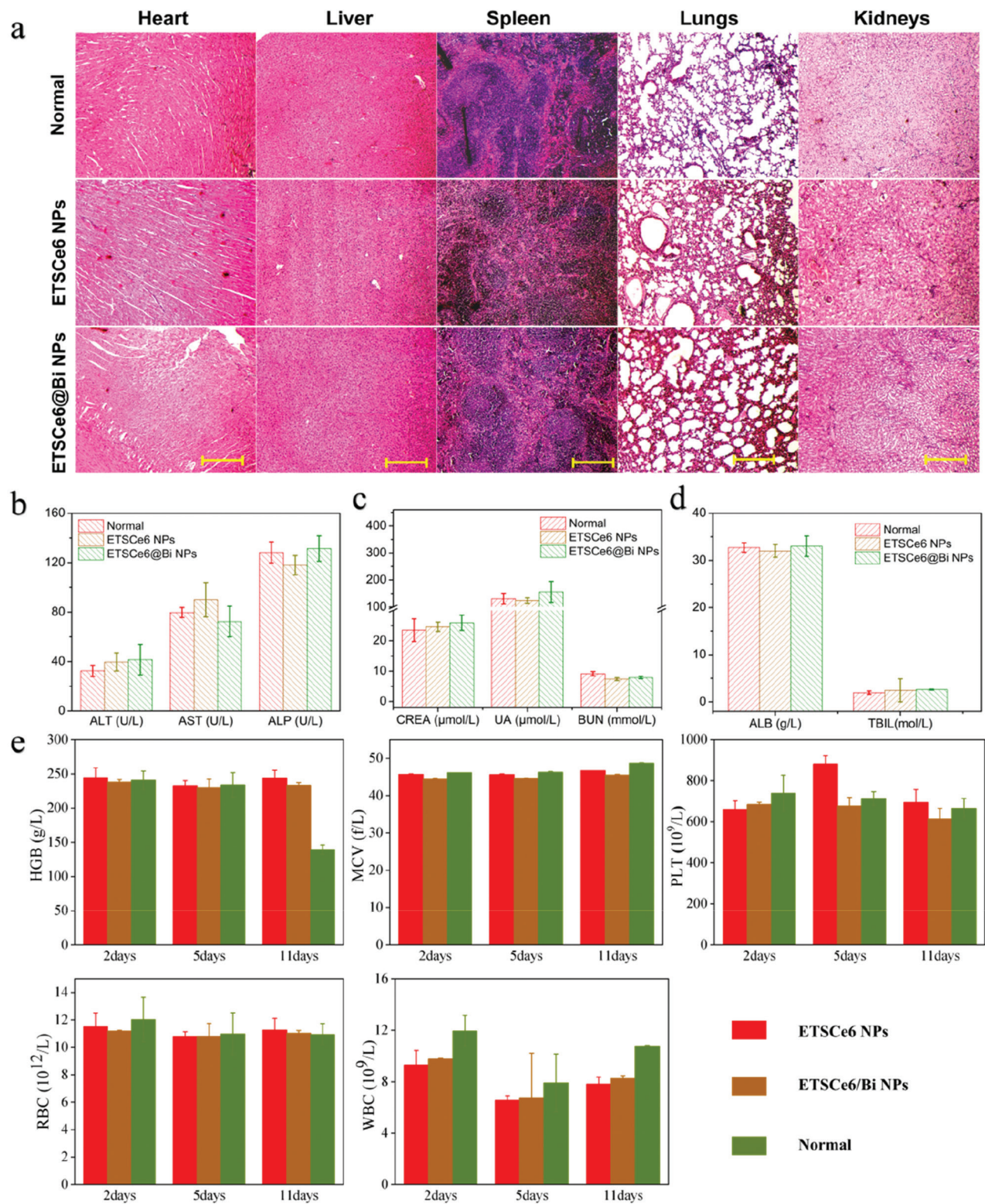


Figure 8 | Biosafety.

a) High-resolution organ histopathology assessment (H&E staining of heart, spleen, lungs, and kidneys). Scale bar: 50 μm . b–d) Analysis of biochemical indices in tumor-bearing mice. e) Analysis of blood routine indices in tumor-bearing mice.

Research Article

organs (heart, liver, spleen, lungs, and kidneys) of the mice with advanced tumors were collected for H&E staining analysis. No clear injuries or inflammatory lesions were observed in the major organs of mice in each group (Figure 8a and S18). The cellular morphology and structure were normal. In addition, we conducted blood macrobiotics analyses to further assess the biosafety and potential toxicity of ETSCe6 NPs (Figure 8b–d). No significant changes in major liver function indices (aminotransferase, alkaline phosphatase, and albumin transaminase), renal function indices (blood urea nitrogen, creatinine, and uric acid), and other blood biochemical indices were observed in ETSCe6 NPs-treated mice compared with normal healthy mice. Similarly, red blood cell count, hemoglobin, white blood cell count, mean corpuscular volume, and platelet count did not change significantly (Figure 8e). Therefore, the above results suggested that ETSCe6 NPs had favorable biocompatibility, biodegradability, low *in vivo* toxicity, and strong synergistic anti-tumor potential, and therefore may serve as a promising multiple synergistic chemotherapy/PDT/PTT drug carrier.

4. DISCUSSION

This study developed intelligent supramolecular NPs through the self-assembly of ETSCe6, thereby addressing the issue of low drug loading in supramolecular photosensitizers frequently arising with co-assembly strategies. The NPs chelated metal ions (Au and Bi) and consequently achieved TME-responsive, visualizable, and precise diagnosis and therapy at tumor sites. ETSCe6, a single-molecule photosensitizer based on Ce6, self-aggregates in NPs with strong π - π stacking. Because of the ACQ effect, the fluorescence quenching efficiency reached 99.34%, thus leading to quenching of both fluorescence and ROS production. This suppression instead enhanced the PTT effect and significantly increased PTT anti-tumor efficiency. ET and Ce6 are linked via disulfide bonds, thus allowing GSH-induced reduction to release free molecules and restore PDT capabilities. The morphology, PTT, and PDT performance of this material were comprehensively studied. On the basis of Gaussian theoretical calculations, the lower energy required for electron transitions from the HOMO to the LUMO in ETSCe6 indicated a diminished need for absorbing light energy. Given their excellent photophysical properties, ETSCe6 NPs achieved synergistic tumor therapy.

Extensive *in vitro* and *in vivo* experiments confirmed ETSCe6's photothermal and photodynamic capabilities, and strong ROS and photothermal performance. ETSCe6 NPs exhibited significantly enhanced, synergistic, and safe anticancer effects, achieving a 100% early tumor inhibition rate and partial tumor ablation. Additionally, ETSCe6@Bi NPs were demonstrated to serve as contrast agents for CT imaging to guide tumor therapy. The *in situ* activation of the PTT/PDT switch may provide an efficient strategy for 4T1 breast cancer treatment.

This study highlights the potential of NSMs in supramolecular photosensitizer self-assembly nano-systems, demonstrating the advantages and efficient assembly strategies of triterpenoid NSMs in constructing supramolecular photosensitizers. This approach offers significant benefits for potential clinical applications and provides an innovative pathway for developing intelligent nanomedicines. We believe that the designed ETSCe6@Bi NPs may provide new perspectives for stimulus-responsive intelligent phototherapy nanomedicines and, by integrating multiple bioactive molecules, hold promise for achieving precise, multifunctional therapeutic effects.

5. CONCLUSION

Smart supramolecular NPs were engineered to visualize the TME response at tumor sites by self-assembly of ETSCe6 with chelated metal ions, thus facilitating accurate diagnosis and treatment. ETSCe6-based photosensitizers self-aggregate into NPs with π - π stacking, and consequently increase the fluorescence efficiency to 99.3%. Furthermore, the efficient intramolecular PET effect enhances ETSCe6's photostability and boosts its photothermal capacity. The incorporation of ET and Ce6 with a disulfide bond was found to facilitate GSH-induced reductive dissociation into free molecules and to restore photodynamic capacity. Importantly, ETSCe6's PDT and PTT capabilities were confirmed through *in vitro* and *in vivo* experiments investigating ROS and photothermal properties. Remarkably, the ETSCe6 NPs showed significantly improved, synergistic, and safe anticancer efficacy, achieving 100% early tumor inhibition. In addition the ETSCe6@Bi NPs can serve as contrast agents for CT imaging-guided tumor therapy. The *in situ* activated PTT/PDT switch offers an efficient strategy for treating 4T1 breast cancer, and has potential for multifunctional precision therapy applications by integrating multiple pharmacologically active molecules.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from the National Natural Science Foundation of China (project No. 31972040). The manuscript was checked by a native English-speaking scientist, Prof. Joe M. Regenstein at Cornell University (USA). We express our sincere gratitude to Prof. Regenstein for his invaluable assistance and expertise in proofreading this manuscript. Schematic diagrams were generated with [BioRender.com](https://www.biorender.com).

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported herein.

SUPPLEMENTARY DATA

Supplementary data for this article can be found online at: https://amm-journal.org/wp-content/uploads/2025/02/amm20240081_Suppl.pdf.

REFERENCES

- [1] Miller KD, Nogueira L, Devasia T, Mariotto AB, Yabroff KR, Jemal A, et al.: Cancer Treatment and Survivorship Statistics. *CA: A Cancer Journal for Clinicians* 2019, 69:363–385.
- [2] Feng E, Jiao L, Tang S, Chen M, Lv S, Liu D, et al.: Anti-photobleaching Cyanine-based Nanoparticles with Simultaneous PET and ACQ Effects for Improved Tumor Photothermal Therapy. *Chemical Engineering Journal* 2022, 432:134355.
- [3] Yu Y, Tang D, Liu C, Zhang Q, Tang L, Lu Y, et al.: Biodegradable Polymer with Effective Near-Infrared-II Absorption as a Photothermal Agent for Deep Tumor Therapy. *Advanced Materials* 2022, 34:e2105976.
- [4] Wan Y, Fu LH, Li C, Lin J, Huang P: Conquering the Hypoxia Limitation for Photodynamic Therapy. *Advanced Materials* 2021, 33:2103978.
- [5] Li L, Shao C, Liu T, Chao Z, Chen H, Xiao F, et al.: An NIR-II-Emissive Photosensitizer for Hypoxia-tolerant Photodynamic Theranostics. *Advanced Materials* 2020, 32:e2003471.
- [6] Fu Z, Williams GR, Niu S, Wu J, Gao F, Zhang X, et al.: Functionalized Boron Nanosheets as an Intelligent Nanopatform for Synergistic Low-temperature Photothermal Therapy and Chemotherapy. *Nanoscale* 2020, 12:14739–14750.
- [7] Wang L, Gao C, Liu K, Liu Y, Ma L, Liu L, et al.: Cypate-conjugated Porous Upconversion Nanocomposites for Programmed Delivery of Heat Shock Protein 70 Small Interfering RNA for Gene Silencing and Photothermal Ablation. *Advanced Functional Materials* 2016, 26:3480–3489.
- [8] Feng S, Lu J, Wang K, Di D, Shi Z, Zhao Q, et al.: Advances in Smart Mesoporous Carbon Nanopatforms for Photothermal-enhanced Synergistic Cancer Therapy. *Chemical Engineering Journal* 2022, 435:134886.
- [9] Wang C, Cao F, Ruan Y, Jia X, Zhen W, Jiang X: Specific Generation of Singlet Oxygen Through the Russell Mechanism in Hypoxic Tumors and GSH Depletion by Cu-TCPP Nanosheets for Cancer Therapy. *Angewandte Chemie (International ed. in English)* 2019, 58:9846–9850.
- [10] Sun J, Du K, Diao J, Cai X, Feng F, Wang S: GSH and H₂O₂ Co-activatable Mitochondria-targeted Photodynamic Therapy under Normoxia and Hypoxia. *Angewandte Chemie (International ed. in English)* 2020, 59:12122–12128.
- [11] Lan JS, Liu L, Zeng RF, Qin YH, Hou JW, Xie SS, et al.: Tumor-specific Carrier-free Nanodrugs with GSH Depletion and Enhanced ROS Generation for Endogenous Synergistic Anti-tumor by a Chemotherapy-photodynamic Therapy. *Chemical Engineering Journal* 2021, 407:127212.
- [12] Li W, Wang S, Ren C, Liu P, Lu Q, Yang L, et al.: Exo/Endogenous Dual-augmented Chemodynamic Therapy Based on Bio-reducible and Bio-breakable Copper (II)-Based Truncated Octahedron. *Chemical Engineering Journal* 2020, 396:125280.
- [13] Hu K, Xie L, Zhang Y, Hanyu M, Yang Z, Nagatsu K, et al.: Marriage of Black Phosphorus and Cu²⁺ as Effective Photothermal Agents for PET-guided Combination Cancer Therapy. *Nature Communications* 2020, 11:2778.
- [14] He T, Jiang C, He J, Zhang Y, He G, Wu J, et al.: Manganese-Dioxide-Coating-Instructed Plasmonic Modulation of Gold Nanorods for Activatable Duplex-Imaging-Guided NIR-II Photothermal-chemodynamic Therapy. *Advanced Materials* 2021, 33:e2008540.
- [15] Zhou Y, Liang X, Dai Z: Porphyrin-loaded Nanoparticles for Cancer Theranostics. *Nanoscale* 2016, 8:12394.
- [16] Song F, Peng X, Lu E, Wang Y, Zhou W, Fan J: Tuning the Photoinduced Electron Transfer in Near-infrared Heptamethine Cyanine Dyes. *Tetrahedron Letters* 2005, 46:4817–4820.
- [17] Zou Q, Abbas M, Zhao L, Li S, Shen G, Yan X: Biological Photothermal Nanodots Based on Self-assembly of Peptide-porphyrin Conjugates for Antitumor Therapy. *Journal of the American Chemical Society* 2017, 139:1921–1927.
- [18] Gao S, Wei G, Zhang S, Zheng B, Xu J, Chen G, et al.: Albumin Tailoring Fluorescence and Photothermal Conversion Effect of Near-Infrared-II Fluorophore with Aggregation-induced Emission Characteristics. *Nature Communications* 2019, 10:2206.
- [19] Xing R, Zou Q, Yuan C, Zhao L, Chang R, Yan X: Self-assembling Endogenous Biliverdin as a Versatile Near-infrared Photothermal Nanoagent for Cancer Theranostics. *Advanced Materials* 2019, 31:e1900822.
- [20] Du W, Chong Y, Hu X, Wang Y, Zhu Y, Chen J, et al.: Increasing Photothermal Efficacy by Simultaneous Intra- and Intermolecular Fluorescence Quenching. *Advanced Functional Materials* 2020, 30:1908073.
- [21] Nel A, Xia T, Mädler L, Li N: Toxic Potential of Materials at the Nanolevel. *Science* 2006, 311:622–627.
- [22] Li J, Rao J, Pu K: Recent Progress on Semiconducting Polymer Nanoparticles for Molecular Imaging and Cancer Phototherapy. *Biomaterials* 2018, 155:217–235.
- [23] Yang X, Ma C, Chen Z, Liu J, Liu F, Xie R, et al.: Single Small Molecule-assembled Nanoparticles Mediate Efficient Oral Drug Delivery. *Nano Research* 2019, 12:2468–2476.
- [24] Bag BG, Majumdar R: Self-assembly of Renewable Nano-sized Triterpenoids. *Chemical Record* 2017, 17:841–873.
- [25] Paterson I, Anderson EA: The Renaissance of Natural Products as Drug Candidates. *Science* 2005, 310:451–453.
- [26] Rodrigues T, Reker D, Schneider P, Schneider G: Counting on Natural Products for Drug Design. *Nature Chemistry* 2018, 8:531–541.
- [27] Fu S, Yang X: Recent Advances in Natural Small Molecules as Drug Delivery Systems. *Journal of Materials Chemistry B* 2023, 11:4584–4599.
- [28] Wang J, Zhao H, Zhi K, Yang X: Exploration of the Natural Active Small-molecule Drug-loading Process and Highly Efficient Synergistic Antitumor Efficacy. *ACS Applied Materials & Interfaces* 2020, 12:6827–6839.
- [29] Wang J, Zhao H, Qiao W, Cheng J, Han Y, Yang X: Nanomedicine-Cum-Carrier by Co-assembly of Natural Small Products for Synergistic Enhanced Antitumor with Tissues Protective Actions. *ACS Applied Materials & Interfaces* 2020, 12:42537–42550.
- [30] Cheng J, Zhao H, Wang J, Han Y, Yang X: Bioactive Natural Small Molecule-tuned Coassembly of Photosensitive Drugs for Highly Efficient Synergistic and Enhanced Type I Photochemotherapy. *ACS Applied Materials & Interfaces* 2020, 12:43488–43500.
- [31] Cheng J, Li X, Wang S, Han Y, Zhao H, Yang X: Carrier-free Triterpene Prodrugs with Glutathione Response and Biosafety for Synergistically Enhanced Photochemotherapy. *ACS Applied Materials & Interfaces* 2021, 13:245–256.

Research Article

- [32] Cheng J, Zhao H, Yao L, Li Y, Qi B, Wang J, et al.: Simple and Multifunctional Natural Self-assembled Sterols with Anticancer Activity-mediated Supramolecular Photosensitizers for Enhanced Antitumor Photodynamic Therapy. *ACS Applied Materials & Interfaces* 2019, 11:29498–29511.
- [33] Cheng J, Fu S, Qin Z, Han Y, Yang X: Self-assembled Natural Small Molecule Diterpene Acids with Favorable Anticancer Activity and Biosafety for Synergistically Enhanced Antitumor Chemotherapy. *Journal of Materials Chemistry B* 2021, 9:2674–2687.
- [34] Lovell JF, Jin CS, Huynh E, Jin H, Kim C, Rubinstein JL, et al.: Porphysome Nanovesicles Generated by Porphyrin Bilayers for use as Multimodal Biophotonic Contrast Agents. *Nature Materials* 2011, 10:324–332.
- [35] Zhang HY, Firempong CK, Wang YW, Xu WQ, Wang MM, Cao X, et al.: Ergosterol-loaded Poly(lactide-co-glycolide) Nanoparticles with Enhanced in vitro Antitumor Activity and Oral Bioavailability. *Acta Pharmacologica Sinica* 2016, 37:834–844.
- [36] Liu Y, Wang H, Li S, Chen C, Xu L, Huang P, et al.: In Situ Supramolecular Polymerization-enhanced Self-assembly of Polymer Vesicles for Highly Efficient Photothermal Therapy. *Nature Communications* 2020, 11:1724.
- [37] Fu S, Wang M, Li B, Li X, Cheng J, Zhao H, et al.: Bionic Natural Small Molecule Co-assemblies Towards Targeted and Synergistic Chemo/PDT/CDT. *Biomaterials Research* 2023, 27:43.
- [38] Zhang L, Jiang C, Li B, Liu Z, Gu B, He S, et al.: A Core-shell $\text{Au@Cu}_2\text{S}_x\text{Se}$ Heterogeneous Metal Nanocomposite for Photoacoustic and Computed Tomography Dual-Imaging-Guided Photothermal Boosted Chemodynamic Therapy. *Journal of Nanobiotechnology* 2021, 19:410.
- [39] Li X, Li Y, Yu C, Bao H, Cheng S, Huang J, et al.: ROS-responsive Janus Au/Mesoporous Silica Core/Shell Nanoparticles for Drug Delivery and Long-term CT Imaging Tracking of MSCs in Pulmonary Fibrosis Treatment. *ACS Nano* 2023, 17:6387–6399.
- [40] Jin L, Jia G, Hao Q, Jun JS: Simultaneous Realization of Persistent Luminescence and CT Dual-mode Imaging by X-ray Recharged $\text{Bi}_2\text{Ga}_4\text{O}_9\text{:Cr}$ Nanoprobes in Depth-independent Tumors. *Chemical Engineering Journal* 2020, 406:126008.
- [41] Meng N, Xu P, Wen C, Liu H, Gao C, Shen XC, et al.: Near-Infrared-II-Activatable Sulfur-deficient Plasmonic $\text{Bi}_2\text{S}_3-x\text{Au}$ Heterostructures for Photoacoustic Imaging-guided Ultrasound Enhanced High Performance Phototherapy. *Journal of Colloid and Interface Science* 2023, 644:437–453.
- [42] Long R, Guo M, Liu L, Fang W: Nonradiative Relaxation of Photoexcited Black Phosphorus is Reduced by Stacking with MoS_2 : A Time Domain Ab Initio Study. *The Journal of Physical Chemistry Letters* 2016, 7:1830–1835.