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Enhanced Plasmon-Induced Resonance Energy Transfer (PIRET)-mediated Photothermal and Photodynamic Therapy Guided by Photoacoustic and Magnetic Resonance Imaging

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Abstract: Phototherapy, containing photothermal and photodynamic therapy, has attracted extensive attention due to its noninvasive nature, low toxicity, and high anticancer efficiency. Charge-separation mechanism of plasmon-induced resonance energy transfer (PIRET), has been increasingly employed to design nanotheranotic agents. Herein, we developed a novel and smart PIRET-mediated nanoplatform for enhanced, imaging-guided phototherapy. Prussian blue (PB) was incorporated into Au@Cu2O nanostructure, which was then assembled with poly(allylamine) (PAH) modified black phosphorus quantum dots (Au@PB@Cu2O@BPQDs/PAH nanocomposites). The hybrid nanosystem exhibited great absorption in NIR region, as well as the ability to self-supply O2 by catalyzing hydrogen peroxide and convert O2 into singlet oxygen (1O2) under 650 nm laser light (0.5 W/cm2) irradiation. In vitro and in vivo assay showed that the generated heat and toxic 1O2 from Au@PB@Cu2O@BPQDs/PAH nanocomposites could effectively kill the cancer cells and suppress tumor growth. Moreover, the unique properties of PB modified nanosystem allowed for synergistic therapy with the aid of T1-weighed magnetic resonance imaging (T1-weighted MRI) and photoacoustic imaging (PAI). This study presented a suitable way to fabricate smart PIRET-based nanosystem with enhanced PTT/PDT efficacy and dual-modal imaging functionality. The great biocompatibility and low toxicity ensured their high potential for use in cancer therapy.

KEYWORDS: phototherapy, plasmon-induced resonance energy transfer, prussian blue, magnetic resonance imaging, photoacoustic imaging, biocompatibility
Introduction

In the past few years, phototherapy such as photothermal therapy (PTT) and photodynamic therapy (PDT) has attracted extensively attention in cancer treatment due to the advantages of specific tumor localization, remote control, and low system toxicity.\textsuperscript{1-4} PTT uses near-Infrared light (NIR) absorbing agents to produce heat from optical energy, leading to the ablation of tumor.\textsuperscript{5,6} Recently, many photothermal agents, such as gold nanoparticles (NPs),\textsuperscript{7} copper sulfide NPs,\textsuperscript{8} black phosphorus nanosheets,\textsuperscript{9} MoS\textsubscript{2} nanaosheets,\textsuperscript{10} boron nanosheets,\textsuperscript{11} have been extensively explored. For PDT, the treatment is based on the photosensitizer (PBs) triggered by specific NIR light, then producing reactive oxygen species (ROS) to kill the cancer cells.\textsuperscript{12,13} In general, the traditional organic PBs show poor water solubility, low stability and quantum yield, which limits their further application in PDT.\textsuperscript{14} Recently, the semiconductor NPs including TiO\textsubscript{2},\textsuperscript{15} ZnO,\textsuperscript{16} Cu\textsubscript{2}O,\textsuperscript{17} provide a new thought to design PBs. Nevertheless, some of these inorganic PBs can only be activated by narrow UV-vis light, which has a prominent limitation in tissue penetration.

According to the aforementioned limitation of PDT, localized surface plasmon resonance (LSPR) has emerged as a new strategy to fabricate efficient PBs with great NIR absorption by incorporating plasmonic metal nanostructures (e.g. Au, Ag and Cu) in semiconductor for enhanced PDT.\textsuperscript{18,19} Recently, Wu’s group fabricated the Au@SiO\textsubscript{2}@Cu\textsubscript{2}O nanosystem, which showed much higher photocatalytic activity than both Cu\textsubscript{2}O and Au@Cu\textsubscript{2}O.\textsuperscript{17} Introducing gold NPs into Cu\textsubscript{2}O can expand the light absorption and improve its depth of penetration into tissues. Most importantly, according to the unique charge-separation mechanism of PIRET, the SiO\textsubscript{2} layer in Au@SiO\textsubscript{2}@Cu\textsubscript{2}O nanosystem can serve as a separating layer and prominently enhances the generation of electron–hole pairs in the Cu\textsubscript{2}O shell, and consequently improve the generation of 1\textsuperscript{O}\textsubscript{2} for enhanced PDT.\textsuperscript{20-22} Similar to Au@SiO\textsubscript{2}@Cu\textsubscript{2}O nanosystem, a gold-copper sulfide yolk-shell (Au@CuS) nanoparticle has been synthesized. It enhanced PDT performance using PIRET effects.\textsuperscript{23} Therefore, the PIRET charge-separation mechanism based nanostructures seem to be promising phototherapy agents.

Although integrating Cu\textsubscript{2}O nanomaterials with plasmonic noble metal nanomaterials improved PDT, the use of such hybrid nanomaterials for cancer treatment is greatly limited by the hypoxia microenvironment, because the essential element of oxygen is insufficient in tumor. Another disadvantage of these nanosystems was that the photothermal property was lacking.\textsuperscript{17,24} In addition,
it remains a great challenge to develop multi-functional nanomaterials that can simultaneously provide multi-modality imaging and efficient PIRET-mediated PTT and PDT for tumor treatment.

Prussian Blue (PB) with excellent biocompatibility has been approved by American Food and Drug Administration (FDA) for clinical application.\textsuperscript{25-27} In addition to its excellent photothermal performance, it can be further used as strong contrast agent for both photoacoustic imaging (PAI) and magnetic resonance imaging (MRI) in early cancer diagnosing.\textsuperscript{28-30} Moreover, Prussian blue NPs possess a catalysis-like activity that can catalyze hydrogen peroxide, which is promising to overcome the problems of hypoxia tumor microenvironment that limits PDT efficacy.\textsuperscript{31} In addition, Black phosphorus quantum dots (BPQDs), as a new form of black phosphorus nanostructures, has emerged as functional nanomaterials in 2015.\textsuperscript{32,33} The nanostructures of BPQDs are obviously different from two-dimensional (2D) BP nanosheets and one-dimensional (1D) BP nanoribbons. BPQDs can enhance photodynamic performance by generating $^1$O$_2$ in tumor microenvironment under 650 nm laser light.\textsuperscript{34-36} Importantly, BPQDs might be effectively discharged from the liver and kidneys because its particle size is smaller than 10 nm.\textsuperscript{37}

Inspired by the above research results, we have successfully built a PIRET based, multimodal imaging-guided PTT and PDT nanosystem (Au@PB@Cu$_2$O@BPQDs/PAH nanocomposites) in this study. Firstly, gold nanorods were synthesized, and then a PB layer with the thickness of 10 ± 2 nm was coated onto the surface of gold nanorods (Au NRs). Next, Cu$_2$O shell was grafted on PB. Finally, PAH modified BPQDs were coupled on Cu$_2$O via electrostatic interaction. Here, PAH served as modified layer to increase water stability and biocompatibility of Au@PB@Cu$_2$O@BPQDs/PAH nanocomposites (Au@PB@Cu$_2$O@BPQDs/PAH NCs), as well as the prevention the oxidation of BPQDs. By taking advantage of the PIRET process, the Au@PB@Cu$_2$O NPs showed high generation of $^1$O$_2$ than Au@Cu$_2$O NPs under 650 nm laser light (0.5 W/cm$^2$), and verified great production of $^1$O$_2$ after combining with PAH modified BPQDs. Importantly, Au@PB@Cu$_2$O@BPQDs/PAH NCs displayed an excellent PTT performance at 650 nm laser light (1.5 W/cm$^2$) due to the presence of PB, while the property was lacking in other PIRET-mediated Cu$_2$O core-shell nanostructures. Moreover, the PB endowed the sample with simultaneous PA and $T_1$-weighted MR imaging properties. As a result, an enhanced PIRET-mediated phototherapy has been fabricated, which exhibited synergetic PAI, MRI-guided PTT/PDT efficacy in the treatment of cancer therapy.
**Experimental section**

**Chemicals.** All of the aforementioned chemicals were used in this article without further purification. All agents were obtained from Sigma-Aldrich except bulk BP and potassium ferrocyanide trihydrate, which were purchased from Smart Elements and Thermo Fisher. All procedures for animal experiments were handled under the guidelines approved and supervised by the ethics committee of Nanjing University.

**Apparatus.** The infrared spectrum was performed on a (FT-IR) Nexus 670 FTIR type (Nicolet). UV-Vis spectra were recorded using a Shimadzu UV-2600 spectrometer. The surface composition and element analysis of the samples were recorded using X-ray photoelectron spectroscopy (XPS, EscaLab-250, Thermo, USA). Transmission electron microscopy (TEM), high-resolution TEM (HRTEM), and energy-dispersive spectroscopy spectrum (EDS) analyses of samples were carried out on a Tecnai G20 operating. Raman spectrometer (LabRam HR800) with 514 nm laser excitation was used to measure the Raman spectra of BPQDs. ESR spectrum of sample was measured by using Bruker EMXplus Spectrometer System. $\zeta$ potential was conducted using Malvern DLS Zetasizer. The fluorescent images of cells were acquired by Confocal laser scanning microscopy (CLSM, TI-E-AIR, Nikon, Japan). The obtained infrared thermal images at tumor site exposing to 650 laser light irradiation were recorded with a PTT monitoring system MG33 (Shanghai Magnity Electronics Co. Ltd.). The process of synthesizing material are shown in the Supporting Information.

**Results and discussion**

**Synthesis and Characterization.** In this study, multifunctional Au@PB@Cu$_2$O@BPQDs/PAH NCs were fabricated and its multimodal-imaging guided therapeutic effect was shown in scheme 1. The NC contains two main composites: Au@PB@Cu$_2$O NPs and BPQDs/PAH NPs, respectively. In Au@PB@Cu$_2$O nanosystem, PB modified gold nanorods can equip Au@Cu$_2$O NPs with PTT effect, while the traditional Au@Cu$_2$O NPs were unable to exhibit the property due to their relative low absorption throughout the NIR region. In addition, we utilized PB to separate gold nanorod and Cu$_2$O to take full advantage of plasmonic gold nanorods and consequently improved the photocatalytic activity on the surface of Cu$_2$O, generating more $^{1}$O$_2$ to kill the cancer cells. Furthermore, It has been verified that Prussian Blue displays a catalase-like activity to catalyze hydrogen peroxide ($\text{H}_2\text{O}_2$) into oxygen ($\text{O}_2$) molecules
under the neutral pH condition (pH = 7.4). Due to the excellent absorption through the NIR region of PB, PB modified Au@Cu$_2$O nanostructures can also be used as photo-absorbing agent for PAI and $T_1$-MRI because of its Fe(II) and Fe(III) constituent. On the other hand, combining Au@PB@Cu$_2$O NPs with BPQDs/PAH NPs for further enhanced PDT, which has been demonstrated by zeta potential results (Figure S1). Thus, through integration of these two main composites, the Au@PB@Cu$_2$O@BPQDs/PAH NCs could be excited at the 650 nm laser light for PAI and $T_1$-MRI guided PTT/PDT.

Scheme 1. Schematic of (a) synthetic process and (b) Multimodal-Imaging guided therapeutic effect of Au@PB@Cu$_2$O@BPQDs/PAH NCs.

TEM images of obtained Au@PB NPs showed that the PB shell was well coated on the surface of Au NRs (about 110 nm) with a 10 nm thick layer (Figure 1a), and its elemental mapping images (Figure 1b) displayed the good distributions of the Au, Fe, and N elements. The TEM, interfacial high-resolution TEM (HR-TEM) and elemental mapping images of Au@PB@Cu$_2$O core-shell NPs with well size (250 ± 20 nm) and elements (Au, Fe, Cu) dispersion were shown in Figure 1c,
1d. The clearly exposed (111) plane and a lattice fringe spacing of 0.24 nm (Figure 1c), which belongs to Cu$_2$O small crystalline, verified that Cu$_2$O shell has been successfully coated on the surface of Au@PB NPs. While the PB layer could not be observed clearly due to the contrast between AuNR and Cu$_2$O shell. Besides, the formation of Au@PB@Cu$_2$O core-shell NPs were also confirmed by the FT-IR spectrum. Figure S2 shows the FT-IR absorption spectrum of the Au@PB@Cu$_2$O powder. The absorption band at 610 cm$^{-1}$ showed the typical characteristics of Cu$_2$O, which belongs to the stretching vibration of Cu-O bond.$^{39}$ The absorption peak at 2070 cm$^{-1}$ showed the common characteristics of PB, corresponding to the stretching vibration of the CN group.$^{40}$

The BPQDs were synthesized based on the solvothermal method (Figure S3). Its morphology and size (obtained via statistical TEM analysis) were shown in Figure S4. The average size of BPQDs were about 2.8 ± 0.8 nm (Figure S5). Three typical Raman peaks of BP (Figure S6) corresponded to the out-of-plane vibration mode $A_{1g}$ at 363.5 cm$^{-1}$, the in-plane vibration modes $B_{2g}$ at 440.8 cm$^{-1}$ and $A_{2g}$ at 467.0 cm$^{-1}$. Figure S7 shows the UV-vis spectrum of BPQDs dissolved in 200 mL NMP solution, indicating that the solvothermal method could achieve a large scale production of BPQDs. HR-TEM image in Figure 1e showed PAH-modified BPQDs. The lattice fringes were 0.34, corresponding to the (021) plane of the BP crystal. As shown in Figure 1e, the morphology and nanostructure of BPQDs/PAH coated Au@PB@Cu$_2$O did not change and the ultimate size of Au@PB@Cu$_2$O@BPQDs/PAH NCs were about 275 ± 25 nm (Figure 1g), which was suitable for biomedical uses.$^{41-43}$ According to the elemental mapping images (Figure 1f) Au, Fe (different valence state with Fe$^{2+}$ and Fe$^{3+}$ originated from Prussian Blue), K, Cu, and P elements were uniformly distributed. The energy-dispersive spectroscopy (EDS) spectrum of Au@PB@Cu$_2$O@BPQDs/PAH NCs further demonstrated the coexistence of Au, Fe, K, Cu, O and P elements (Figure S8). In addition to these characterizations, we also employed X-ray photoeleetroscopy (XPS) to investigate the components of the core-shell nanostructure. From the Figure 1h, the obtained XPS survey images of Au@PB@Cu$_2$O@BPQDs/PAH NCs proved that the chemical composition corresponded well to the previous research.$^{17,44}$ Two typical peaks located at 720.28eV (Fe 2p1/2) and 714.18 eV (Fe 2p3/2) in the high-resolution Fe 2p spectrum were attributed to the FeN$_x$ unit, while the peak at 707.08 eV (Fe 2p3/2) could be attributed to the [Fe$^{2+}$(CN)$_6$] units of PB shell (Figure 1i).$^{40}$ In XPS spectrum of Cu 2p, a typical peak at 931.68 eV (Cu$^+$ 2p3/2) and an obvious peak at 951.58 eV (Cu$^+$ 2p1/2) was displayed in Figure 1j, which was
due to the existence of Cu$_2$O, whereas the weak peak at about 943.18 eV was due to the existence of Cu$^{2+}$. The inconspicuous peak belongs to Au 4f in Figure S9, which might be shielded by the Cu$_2$O shell. Most importantly, we could still see two peaks at around 130.67 eV (P 2p1/2) and 129.27 eV (P 2p3/2) in Figure S10. A peak at high energy region (around 132.98 eV) indicated the oxidation of BPQDs. All TEM, EDS, XPS, and FT-IR results illustrated above demonstrated the successful fabrication of Au@PB@Cu$_2$O@BPQDs/PAH NCs.

Figure 1. Characterization of Au@PB@Cu$_2$O@BPQDs/PAH NCs. (a) TEM image and HR-TEM image of Au@PB NPs. (b) HAADF-STEM image and elemental mapping of Au@PB NPs. (c) TEM image and HR-TEM image of Au@PB@Cu$_2$O NPs. (d) HAADF-STEM image and elemental mapping of Au@PB@Cu$_2$O NPs. (e) TEM image and HR-TEM image of Au@PB@Cu$_2$O@BPQDs/PAH NCs. Inset: HR-TEM images of BPQDs. (f) HAADF-STEM image and elemental mapping of Au@PB@Cu$_2$O@BPQDs/PAH NCs. (g) Statistical analysis of the lateral sizes Au@PB@Cu$_2$O@BPQDs/PAH NCs determined by TEM. (h) XPS survey. (i) XPS spectrum of Fe 2p. (j) XPS image of Cu 2p of Au@PB@Cu$_2$O@BPQDs/PAH NCs.
Stability Assay of Au@PB@Cu$_2$O@BPQDs/PAH NCs. When the nanocomposites are applied as potential anticancer agents, it is essential to test its biostability in mimetic human body system. After cultivating with H$_2$O, 10% fetal bovine serum, and phosphate buffered saline (PBS) with different time interval (6h, 12h, 24h, 48h), respectively, the biostability of the nanocomposite was evaluated by observing the UV-vis absorption changes when dissolving them in above selected solutions. From the supporting information (Figure S11a, 11b, 11c), there was no obvious change of absorption curves in H$_2$O, 10% fetal bovine serum, and PBS. The above biostability analysis results suggested that Au@PB@Cu$_2$O@BPQDs/PAH NCs possessed excellent stability in simulative liquid system.

Catalytic performance of Au@PB@Cu$_2$O@BPQDs/PAH NCs for Generating O$_2$. When Au@PB@Cu$_2$O@BPQDs/PAH NCs were added to 20% H$_2$O$_2$ at 20 °C, plenty of bubbles were observed. By contrast, no bubbles were observed in the tube when 20% H$_2$O$_2$ solution was added into the PBS solution without Au@PB@Cu$_2$O@BPQDs/PAH NCs, which could be evaluated by the amount of dissolved O$_2$ in two different solution (Figure 2a). Besides, the size of the bubbles became bigger and bigger with increasing of reaction time to 5 min (Figure S12). It verified the ability of Au@PB@Cu$_2$O@BPQDs/PAH NCs to catalyze the decomposition of H$_2$O$_2$ into O$_2$ and directly demonstrated that Au@PB@Cu$_2$O@BPQDs/PAH NCs played a catalytic role in the decomposition of H$_2$O$_2$.

Photodynamic Effect of Au@PB@Cu$_2$O@BPQDs/PAH NCs. From the UV-vis absorbance spectrum of Au nanorods, Au@PB NPs, Au@PB@Cu$_2$O NPs, Au@Cu$_2$O NPs, and Au@PB@Cu$_2$O@BPQDs/PAH NCs (Figure 2b), we can see that the Au@Cu$_2$O NPs exhibited a low absorption through the absorbance range (300-900 nm), while after the modification by the PB and BPQDs, the Au@PB@Cu$_2$O@BPQDs/PAH NCs showed a significant absorption in the NIR I window (650-950nm). In order to evaluate the PDT efficiency of Au@PB@Cu$_2$O@BPQDs/PAH NCs, its $^1$O$_2$ production capacity was tested by using 1,3-diphenylisobenzofuran (DPBF), a common standard regent for the determination of $^1$O$_2$. Firstly, as presented in Figure 2c, the absorption spectrum of DPBF decreased with the increasing irradiation with 650 nm laser (0.5 W/cm$^2$). The $^1$O$_2$ generation from H$_2$O$_2$, Au@PB, Au@Cu$_2$O, Au@PB@Cu$_2$O, Au@PB@Cu$_2$O@BPQDs/PAH NCs, and Au@PB@Cu$_2$O@BPQDs/PAH NCs plus H$_2$O$_2$ when exposed to the 650 nm laser were assessed via monitoring the time-dependent photodegradation of DPBF, as presented in Figure 2d. The absorbance intensity at 410 nm of
DPBF (as control group) and Au@PB showed a negligible fluctuation. Comparing to the above two groups, the Au@Cu₂O NPs showed a slightly increment of \(^1\)O₂ production within 5 min according to the absorption at 410 nm. While the steep downtrend were obtained for Au@PB@Cu₂O and Au@PB@Cu₂O@BPQDs/PAH NCs, and Au@PB@Cu₂O@BPQDs/PAH NCs plus \(\text{H}_2\text{O}_2\) group. The latter generated much more \(^1\)O₂ generation than that of Au@PB@Cu₂O and Au@PB@Cu₂O@BPQDs/PAH groups within 5 min under laser light, since in the presence of \(\text{H}_2\text{O}_2\), a large amount of \(\text{O}_2\) generating from PB catalyzing \(\text{H}_2\text{O}_2\) then reacted with Cu₂O and BPQDs to generate efficient \(^1\)O₂. The proposed mechanism of enhanced photodynamic effect of Au@PB@Cu₂O@BPQDs/PAH NCs are schematically illustrated in Figure 2e. Generally, Cu₂O NPs can not be excited to generate \(^1\)O₂, since its band gap is less than the energy of 650 nm laser.

In Au@PB@Cu₂O@BPQDs/PAH and Au@Cu₂O NPs, the Au nanorods can convert the energy absorbed from laser light into LSPR oscillations. The generated energy from Au nanorods, which was then directly delivered to the Cu₂O shell, can induce the generation of electron–hole pairs in Cu₂O.\(^{20,21}\) The generated electrons in Cu₂O can then react with \(\text{O}_2\) to generate \(^1\)O₂.\(^{22}\) The existence of PB layer in Au@Cu₂O nanostructures can restrain the interfacial damping and the dephasing of the plasmon from hot-electron transfer as well as the back charge transfer, ensuring the nonradiative transfer of plasmonic energy from the Au NRs core to Cu₂O shell and enhanced the \(^1\)O₂ production through the PDT. In addition, the BPQDs also enhanced the generation of \(^1\)O₂. Attributed to the catalytic property of PB, the resulting NCs successfully achieved \(\text{O}_2\) self-supply via catalyzing \(\text{H}_2\text{O}_2\), which overcame the problem of the hypoxic microenvironment in tumor site, and improved PDT efficacy.

Electron spin resonance (ESR) spectrum was further employed to prove the above mechanism of the \(^1\)O₂ generation of Au@PB@Cu₂O@BPQDs/PAH NCs by using the TEMP to trap \(^1\)O₂. The Au@Cu₂O NPs, Au@PB NPs, Au@PB@Cu₂O NPs, Au@PB@Cu₂O@BPQDs/PAH NCs, and Au@PB@Cu₂O@BPQDs/PAH NCs plus \(\text{H}_2\text{O}_2\) were exposed to 650 nm laser light, and Au@PB@Cu₂O@BPQDs/PAH NCs without 650 nm laser light were set as control groups, respectively. From ESR spectrum, there was an obvious signal peak which belongs to \(^1\)O₂ species, was detected from the Au@PB NPs, Au@Cu₂O NPs, Au@PB@Cu₂O NPs, Au@PB@Cu₂O@BPQDs/PAH NCs, and Au@PB@Cu₂O@BPQDs/PAH NCs plus \(\text{H}_2\text{O}_2\) after irradiation with 650 nm laser light, and Au@PB@Cu₂O@BPQDs/PAH NCs plus \(\text{H}_2\text{O}_2\) group showed great intensity of signal peak compared to other 4 groups. This was because a large amount
of O$_2$ catalyzed by PB finally converted to $^1$O$_2$ when reacted with Cu$_2$O shell and BPQDs, whereas no observable signals could be detected from Au@PB@Cu$_2$O@BPQDs/PAH NCs without 650 nm laser light irradiation. These results sufficiently confirmed the proposed mechanism of O$_2$ generation and $^1$O$_2$ production ability of Au@PB@Cu$_2$O@BPQDs/PAH NCs (Figure 2f).

The photodynamic properties of prepared samples were evaluated in intracellular experiments, as shown in Figure 2g. Herein, 2,7-dichlorofluorescin diacetate (DCFH-DA) has been extensively applied in detecting the generation of $^1$O$_2$ inside cells. Briefly, DCFH-DA, a type of ROS responsive probe with no fluorescence, can be converted to DCF with the oxidization by $^1$O$_2$ and then presents green fluorescence under 488 nm stimulation. From Figure 2g, the CLSM images of HeLa cells dealt with Au@PB@Cu$_2$O@BPQDs/PAH NCs plus H$_2$O$_2$ under 650 nm laser light irradiation exhibited prominent bright green fluorescence with higher fluorescence intensity than the Au@PB@Cu$_2$O@BPQDs/PAH NCs and Au@PB@Cu$_2$O NPs groups. In contrast, the Hela cells cultivated with normal saline (control group), H$_2$O$_2$ as well as with Au@Cu$_2$O NPs exhibited almost invisible or weak green fluorescence, which further indicates that Au@PB@Cu$_2$O@BPQDs/PAH NCs can serve as efficient photodynamic agent.

**Photothermal Effect of Au@PB@Cu$_2$O@BPQDs/PAH NCs.** The photothermal performance of the nanocomposite was studied using a 650 nm laser (1.5 W/cm$^2$) as light source through the whole experiments. As presented in Figure 3a, the temperature of Au@PB@Cu$_2$O@BPQDs/PAH NCs at different concentrations increased quickly after 5 min of laser (1.5 W/cm$^2$) irradiation, showing a concentration-dependent photothermal effect. Especially for the concentration of 200 μg/mL, the temperature increased to 52.5 °C. However, there was no apparent temperature increase was observed in the deionized water. Infrared thermal images were taken to further detect temperature changes and evaluate the photothermal effect, as shown in Figure 3b. Obviously, the temperature of Au@PB@Cu$_2$O@BPQDs/PAH NCs after irradiation increased by 27.5 °C. The photothermal conversion efficiency of Au@PB@Cu$_2$O@BPQDs NCs was studied. Figure 3c showed the temperature curve with an exposure time of 5 min irradiation, followed by another 10 min without irradiation. The linear time data versus ln (θ) obtained during the cooling time (Figure 3d) was used to assess its heat transfer effect. Comparing to other PTT agents, Au@PB@Cu$_2$O@BPQDs/PAH NCs possessed a higher photothermal conversion efficiency ($\eta$) of 25.73%, while $\eta$ was 13% for Au nanoshells, and 22% for Cu$_{2-x}$Se.
Figure 2. (a) The concentration changes of dissolved O$_2$ with the increase of reaction time. Inset: the photo of the mixture of Au@PB@Cu$_2$O@BPQDs/PAH NCs and H$_2$O$_2$ solution in the PBS, the H$_2$O$_2$ solution in the PBS without the NCs. (b) UV-vis absorption spectra of Au NRs, Au@PB NPs, Au@PB@Cu$_2$O NPs, Au@Cu$_2$O NPs, and Au@PB@Cu$_2$O@BPQDs/PAH NCs aqueous solution. (c) Degradation of DPBF in Au@PB@Cu$_2$O@BPQDs/PAH NCs solution under 650 nm laser (0.5 W/cm$^2$) irradiation at each point time. (d) Time-dependent degradation of DPBF at 410 nm caused by $^1$O$_2$ generated by H$_2$O$_2$, Au@Cu$_2$O NPs, Au@PB@Cu$_2$O NPs, Au@PB@Cu$_2$O@BPQDs/PAH NCs, and Au@PB@Cu$_2$O@BPQDs/PAH NCs plus H$_2$O$_2$ under 650 nm laser irradiation. (e) Proposed mechanism of enhanced photodynamic effect of Au@PB@Cu$_2$O@BPQDs/PAH NCs. (f) ESR spectra of Au@PB NPs, Au@Cu$_2$O NPs, Au@PB@Cu$_2$O NPs, Au@PB@Cu$_2$O@BPQDs/PAH NCs, and Au@PB@Cu$_2$O@BPQDs/PAH NCs plus H$_2$O$_2$ in the presence of TEMP with or treated with Au@PB@Cu$_2$O@BPQDs/PAH without irradiation for 5 min (650 nm, 0.5 W/cm$^2$). (g) CLSM images of HeLa cells after treat with different groups and then stained with DCFH-DA (ROS fluorescence probe), the scale bar was 50 μm.

The hemocompatibility of Au@PB@Cu$_2$O@BPQDs NCs. The toxicity of the nanomaterials is a big concern when applied in biomedical field. Therefore, it is necessary to evaluate hemocompatibility of Au@PB@Cu$_2$O@BPQDs/PAH NCs. In this work, red blood cells (RBCs) were selected to test the toxicity. Generally, monitoring the morphology change of RBCs is a standard method to evaluate the hemocompatibility. As presented in Figure 3e, RBCs incubated
with phosphate buffered saline (PBS, as negative control group) and Au@PB@Cu$_2$O@BPQDs/PAH NCs solutions with different concentrations, in deionized water (Figure S13, positive control) suffered destruction. There was no obvious morphology changes, destruction or aggregation, implying the samples possessed no side effect on RBCs. After RBCs experiments, hemolysis rate, as another important parameter, was further calculated. In normal conditions, hemolysis rate below 5% is allowable. It can be seen clearly from Figure 3f that the highest hemolysis rate corresponding to the highest concentrations of samples (1000 µg/mL) was up to 0.82%, which was far less than the standard value (5%), indicating Au@PB@Cu$_2$O@BPQDs/PAH NCs caused negligible hemolysis effect.

![Figure 3](image-url)

**Figure 3.** (a) Photothermal heating curves of PBS and Au@PB@Cu$_2$O@BPQDs/PAH NCs in MiliQ water at different concentrations under 650 nm laser light (1.5 W/cm$^2$). (b) Infrared photothermal images of Au@PB@Cu$_2$O@BPQDs/PAH NCs at different irradiation time. (c) Photothermal response of Au@PB@Cu$_2$O@BPQDs/PAH NCs aqueous solution under irradiation for 5 min with an NIR laser (650 nm, 1.5 W/cm$^2$) and then the laser was shut off. d) Obtained time constant for heat transfer of this nanosystem ($\tau_s = 238.095$ s) by applying $-\ln(\theta)$ vs linear time data from the cooling stage. (e) The morphology of red blood cells treated with Au@PB@Cu$_2$O@BPQDs/PAH NCs at different concentrations, the scale bar was 50 µm. (f) Hemolytic assay of Au@PB@Cu$_2$O@BPQDs/PAH NCs by red blood cells.

**Evaluation Cytotoxicity and Ablation Performance of Au@PB@Cu$_2$O@BPQDs/PAH NCs.** We evaluated the *in vitro* anticancer effect of Au@PB@Cu$_2$O@BPQDs/PAH NCs. In order to investigate the cell uptake of resulting nanocomposites, we used CLSM to observe the red fluorescence intensity originated from Cyanine5.5-decorated Au@PB@Cu$_2$O@BPQDs/PAH NCs...
(Cy5.5@NCs). As shown in Figure 4a, strong red fluorescence could be observed inside cells after 3 h incubation, and homologous fluorescence signal distribution was marked by red cycle, demonstrating the high cellular uptake efficiency of Cy5.5@NCs. The in vitro cytotoxicity of Au@PB@Cu2O@BPQDs/PAH NCs was studied by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. We selected two common types of cells (NIH3T3 cells and Hela cells) as experimental subjects to evaluate its cell viability under dark and laser light. As shown in Figure 4b, there was no apparent cytotoxicity against NIH3T3 cells and Hela cells after 24 h incubation with the resulting NCs without laser irradiation. In contrast, two different power laser light (650 nm, 0.5 W/cm², 1.5 W/cm²) were used to calculate the cells viability under irradiation. Hela cells were treated with either NCs only or NCs plus H2O2, so that four combinations of conditions were tested. As shown in Figure 4c, the four groups showed concentration-dependent dramatic decrease in the percentage of cell viability. It is worth noting, that the group with higher laser power and treated with NCs plus H2O2 gave the most rapid downtrend in cell viability. Almost 100% of the Hela cells were killed as a result of combined heat and the large amount of toxic ¹O2 generated with 650 nm laser light irradiation. In order to get more facts of the synergistic anticancer therapy effect, HeLa cells in there were stained with calcein-AM (live cells, green), and propidium iodide (dead cells, red). As shown in Figure 4d, HeLa cells incubated with Au@PB@Cu2O@BPQDs/PAH NCs irradiated with 650 nm laser light (1.5 W/cm²) exhibited apparent killing effect in contrast to other control groups. In particular, the NCs at low concentration (100 µg/mL, 650 nm, 1.5 W/cm²) still possessed severe apoptosis compared with the group of NCs at 250 µg/mL under 650 nm laser light (1.5 W/cm²), which further demonstrated the catalytic performance of Au@PB@Cu2O@BPQDs/PAH NCs to catalyze H2O2 into O2 then generate a large amount of high toxic ¹O2. All the aforementioned results efficiently demonstrated the great biocompatibility and NIR-induced phototoxicity of Au@PB@Cu2O@BPQDs/PAH NCs.

**In vivo Fluorescence, MR, and PA imaging performance.** Before in vivo imaging experiments, the in vivo behaviors of Au@PB@Cu2O@BPQDs/PAH NCs by measuring Au element using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The blood was collected from mice at each time point and its levels of Au@PB@Cu2O@BPQDs/PAH NCs showed a gradual decrease over time, while still maintained a high level of ~4.3 % ID/g even after 24 h injection (Figure S14). To monitor the biodistribution of the Au@PB@Cu2O@BPQDs/PAH
NCs in vivo, the Cy5.5-decorated NCs were injected into mice by tail veins, and its biodistribution was directly observed by fluorescence imaging. From Figure 5a, we can clearly see the fluorescence signal from the tumor at 6 h post-injection, while the subcutaneous tumor could be definitely delineated from the other tissues. Besides, the fluorescence intensity located at tumor site exhibited a stable level, which was detected at 6 h, 12 h, and 24 h post-injection (Figure 5b). Moreover, no obvious signal was detected in other tissues site, suggesting the Au@PB@Cu$_2$O@BPQDs/PAH NCs could continuously accumulate at the tumor site due to the enhanced permeability and retention (EPR) effect of cancerous tumors.$^{51}$

Figure 4. (a) CLSM images of HeLa cells incubated with Cyanine5.5-decorated Au@PB@Cu$_2$O@BPQDs/PAH NCs (Cy5.5@NCs) and fluorescence signal distribution of Cy5.5@NCs marked by red cycle. The scale bar is 10 μm. (b) Cytotoxicity of the Au@PB@Cu$_2$O@BPQDs/PAH NCs at different concentrations (0–250 μg/mL) after 24 h of incubation with NIH3T3 cells and Hela cells. (c) Cell viability of HeLa cells treated with Au@PB@Cu$_2$O@BPQDs/PAH NCs at different concentrations (0-250 μg/mL) irradiated with different power (0.5 W/cm$^2$, 1.5 W/cm$^2$) of 650 nm laser light with H$_2$O$_2$ or without it. (d) Live/dead assay of HeLa cells after incubation with different groups. The scale bar is 50 μm.

Recently, $T_1$-weighted MRI that has great sensitivity to soft tissues, has been extensively used in bioimaging and cancer diagnosis application due to its excellent spatial resolution. It is well
known that Fe$^{3+}$ can be used as $T_1$-weighted MRI contrast agent by interacting the protons of water molecules. Since our Au@PB@Cu$_2$O@BPQDs/PAH NCs were equipped with the PB modified shell, they were able to be used for MR imaging.$^{28}$

To study the potential application of Au@PB@Cu$_2$O@BPQDs/PAH NCs as $T_1$-MRI contrast agents, the $T_1$ relaxation times of Au@PB@Cu$_2$O@BPQDs/PAH NCs were tested. Consequently, the longitudinal relaxivity values ($r_1$), were calculated from the slope of the plot of $T_1$ vs sample molar concentration of [Fe], respectively. The longitudinal relaxation rate $r_1$ was 5.578 mM$^{-1}$S$^{-1}$, (Figure S15). Next, after injection with Au@PB@Cu$_2$O@BPQDs/PAH NCs in different time interval (0 h, 6 h, 12 h, 24 h), it was observed that $T_1$-weighted MR signals at tumor site enhanced obviously after 6 h post-injection (Figure 5c). $T_1$-weighted MR intensity was scanned by a 3.0 T clinical MRI system. As shown in Figure S16, MR intensity located at tumor site increased to the maximum value (14676.7 ± 1174.136), and decreased a bit (13115.2 ± 849.216) after 24 h post-injection, which demonstrated the potential of Au@PB@Cu$_2$O@BPQDs/PAH NCs NCs as a contrast material for $T_1$-weighted MR imaging.

Photoacoustic imaging as an emerging noninvasive imaging technique, has attracted extensive attentions. Suitable photoacoustic contrast agents should possess great photothermal effect as to minimize the light scattering effect and improve the signal-to-noise ratio (SNR).$^{52}$ According to the previous work, PB NPs exhibited a high absorption in the NIR region, which tended to be a strong contrast agent for PA imaging.$^{26,30,53}$ In this study, the obtained photoacoustic images from tumor site was monitored before and after the injection of Au@PB@Cu$_2$O@BPQDs/PAH NCs (250 µg/mL) into hela tumor bearing mice via tail veins. As shown in Figure 5d, the photoacoustic signals in tumor increased obviously after injection of Au@PB@Cu$_2$O@BPQDs/PAH NCs due to the circulation of NPs in the blood. Besides, prominet photoacoustic signals emerged and scattered throughout the whole tumor, in which the total signals displayed a durable time-dependent increase, indicating the efficient tumor retention of Au@PB@Cu$_2$O@BPQDs/PAH NCs. Therefore, introducing the PB layer into the traditional Au@Cu$_2$O system not only enhanced PTT and PDT effect, but also achieved a $T_1$-weighted MRI, PAI-guided photodynamic therapy, which has never been reported in previous research. After evaluation of imaging performance in vivo, we further investigated the biodistribution of Au@PB@Cu$_2$O@BPQDs/PAH NCs by ICP-AES measurements of Au element in different organs. It can be seen from Figure S17, the tumor uptake of NCs was found to be relatively high, at a level of $\sim$14.9 % ID/g at 24 h post injection, which
could be attributed to the EPR effect of tumor tissues. As for other NCs’ distribution in different organs, such NCs also showed high accumulation in the liver because of the macrophage clearance of NCs. What is more, the high Au level in the kidney indicated that these NCs could be excreted by renal excretion.

**In vivo Tumor Ablation.** The antitumor performance was further evaluated *in vivo*. Firstly, we selected infrared thermal imager to evaluate the *in vivo* PTT effects of our Au@PB@Cu$_2$O@BPQDs/PAH NCs in Hela-bearing tumor mice model (Figure 6a). After the tumor volume has reached ca. 100 mm$^3$, 12 mice were randomly divided into two groups with tail veins of our NCs, and PBS, respectively. Animal NIR images and temperature changes ($\Delta T$) were monitored during 5 min laser light (650 nm, 1.5 W/cm$^2$) irradiation. Image J software was used to detect the tissue penetration of 650 nm laser light. As presented in Figure 6b, $\Delta T$ of injected PBS group was only 0.61, while that of NCs were 16.9 throughout the whole irradiation period, which was attribute to the great absorption in at 650 nm and high photothermal conversion efficiency, indicating that our samples maintained an excellent photothermal effect when injected into mice model. The antitumor effect was evaluated based on the relative change of tumor volumes.

![Figure 5](image_url)
MRI in Hela tumor-bearing mice before and after tail veins of Au@PB@Cu₂O@BPQDs/PAH NCs. (d) PA images of Hela tumor-bearing mice in vivo before and after the tail veins of Au@PB@Cu₂O@BPQDs/PAH NCs.

As displayed in Figure 6c, the Au@PB@Cu₂O@BPQDs/PAH NCs injected hela-bearing tumor mice indicated showed an prominent degradation on the fourth day after irradiation with 650 nm laser light (1.5 W/cm²) for 5 min each day, and the tumor was almost ablated after 14 days treatment. In contrast, PBS treated hela-bearing tumor (control group), exhibited rapid growth of tumor, while group of Au@PB@Cu₂O@BPQDs/PAH NCs with 650 nm laser light (0.5 W/cm²) illumination displayed a slow growth trend. The body weight of hela-bearing tumor mice was kept at a stable level during the treatment, demonstrating that our NCs has good biocompatibility and almost negligible toxic effect (Figure 6d). In Figure 6e, the tumors were excised after 14 days treatment, and the associated tumor weight are provided in Figure S18. The hematoxylin and eosin (H&E) staining was used to detect the apoptosis and shape of the cancer cells. It can be seen clearly that the cells from the control group was mainly intact, while the cancer cells began to be damaged in group of Au@PB@Cu₂O@BPQDs/PAH NCs with 650 nm light (0.5 W/cm²) irradiation and largely destructed in Au@PB@Cu₂O@BPQDs/PAH NCs with 650 nm light (1.5 W/cm²) irradiation (Figure 6e, bottom). Besides, as shown in Figure S19, images of organs slices stemming from different treatment groups after H&E staining indicated that the antitumor agents caused ignorable toxic effects and no remarkable histopathological abnormalities to mice. Based on these preliminary results, the Au@PB@Cu₂O@BPQDs/PAH NCs hold great potential as PAI, T₁-weighted MRI guided PTT/PDT anticancer agents for future clinical applications.

The complete blood count assessment are discussed to further reveal any potential toxicology of Au@PB@Cu₂O@BPQDs/PAH NCs. To our best knowledge, liver function, and kidney function markers including alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea level (BUN), creatinine were measured. As shown in Figure S20, all above test results are in normal reference value range in the blood of the Au@PB@Cu₂O@BPQDs/PAH NCs injected mice, suggesting that no obvious toxicity or kidney disorder is induced by Au@PB@Cu₂O@BPQDs/PAH NCs.
Figure 6. (a) Thermal imaging of Hela tumor-bearing mice treated with PBS (top) and Au@PB@Cu$_2$O@BPQDs/PAH NCs (bottom) before and after tail veins under 650 nm laser irradiation (1.5 W/cm$^2$, 5 min). (b) Temperature change curves at tumor sites. (c) Changes of tumor volume and (d) body weight of the mice treated with various groups after therapy (e) Photographs of excised tumors on day 14 (top) and H&E-stained images of the tumors from different groups (bottom).

Conclusions

In summary, we have successfully fabricated Au@PB@Cu$_2$O@BPQDs/PAH NCs, a multifunctional therapeutic nanoplatform with multilayer core-shell structures for enhanced synergistic phototherapy. By introducing PB as a modified layer and BPQDs/PAH into Au@Cu$_2$O NPs, we sufficiently utilized the mechanism of PIRET and catalase-like property of PB to achieve an dual enhanced photodynamic performance, which generated more $^1$O$_2$ (from photosensitizer Cu$_2$O shell and BPQDs). The as-prepared NCs also exhibited great absorption in NIR region, and
its photothermal conversion was as high as 25.73% upon 650 nm laser light irradiation (1.5 W/cm²).
Attributed to PB the NCs achieved a visualized synergistic therapy with the aid of MRI and PA imaging. Comparing to the Au@Cu₂O NPs, our Au@PB@Cu₂O@BPQDs/PAH NCs exhibited drastic improvement in bioimaging functionality and phototherapy efficiency, which offers a way to design of PIRET based nanosystems. The NCs also possessed excellent biocompatibility and photostability, which have favorable development prospect for nanomedical therapeutic applications.

Supporting Information
The Supporting Information is available free of charge on the ACS Publications. Detailed description of experimental methods and Figures S1–S16 (PDF)

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Conflicts of interest
The authors have no existing conflicts to declare.

Reference


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