

Polymerization-Amplified Photoacoustic Signal by Enhancing Near-Infrared Light-Harvesting Capacity and Thermal-to-Acoustic Conversion

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Electronic Supplementary Information

Abstract As a frontier imaging technique for biomedical applications, photoacoustic (PA) imaging has been developed rapidly. The development of new design strategies and excellent PA imaging reagents to boost PA conversion is eagerly desirable for high quality PA imaging but complicated to realize. Herein, we develop a new strategy in which PA imaging reagents with better properties can be easily optimized by polymerization. A series of new PA imaging reagents were designed and synthesized. The polymerization strategy can effectively promote the PA signal by specifically increasing the thermal-to-acoustic conversion efficiency. As these materials shared the same building units, the optimized effectiveness of polymerization strategy in terms of near-infrared light-harvesting capacity and thermal-to-acoustic conversion efficiency are discussed, rationally. The polymers with intense intramolecular motion exhibit an amplified PA signal by elevating thermal-to-acoustic conversion and its higher light-harvesting capability at redshifted region. The simultaneously strong PA signal and photothermal conversion efficiency of p-TTmB NPs enable precise PA imaging and effective photothermal therapy. This work highlights a simple and available design guideline of polymerization for amplifying the PA effect and optimizing existing materials.

Keywords Intramolecular motion; Thermal-to-acoustic conversion; Photoacoustic imaging; Photothermal conversion efficiency; Polymerization

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INTRODUCTION

As a kind of precise and effective imaging approach for biomedical applications,^[1–3] photoacoustic (PA) imaging has been widely studied in disease diagnosis because it can offer high spatial resolution, deep penetration and high contrast disease information.^[4–11] However, it is too difficult to reach an ideal PA imaging quality under endogenous signal interference from hemoglobin and melanin *in vivo*.^[12,13] One of the most challenging issues is how to develop high-contrast PA imaging agents that are able to efficiently detect diseased sites. Recently, among various photoacoustic materials,^[14–18] organic small molecular chromophores/semiconducting conjugated polymers have been widely researched for PA imaging because of

their facile synthesis and tunable material properties.^[19–27] However, how to promote PA signal output and improve the imaging quality is still a major challenge in urgent demand.^[28]

As depicted in the Jablonski diagram, PA signal generation mainly depends on the thermal deactivation process of the absorbed photoexcitation energy.^[29–34] To obtain PA imaging agents with better performance, the inhibition of the other two main dissipation pathways (photoluminescence and intersystem crossing) is required. In addition, the PA effect is generated from two consecutive processes: photothermal conversion and the subsequent PA effect generated from the thermoelastic expansion.^[16] Usually, these two processes can be described by the formula $p = \Gamma\eta H$, where p is the PA amplitude, Γ (Grüneisen parameter) represents the heat-to-acoustic conversion efficiency, η represents the energy of the laser irradiation, and H refers to the photothermal conversion efficiency (PCE).^[35–37] Based on the above understanding, a lot of contributions have been made to promote the photoacoustic/photothermal performance in both small

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molecules and polymers.^[36] Several groups developed a strategy in which a high light-harvesting unit was introduced to increase the molar extinction coefficient.^[38,39] The utilization of twisted intramolecular charge transfer (TICT) to improve the nonradiative decay process has been demonstrated.^[40] A method of photoinduced electron transfer to quench the emission of semiconducting conjugated polymer nanoparticles was designed by Pu and coworkers.^[41,42] The aggregate-state intramolecular motion was proposed by our group in molecular design to promote heat-to-acoustic or heat conversion from the excited state.^[14,43] Aoki *et al.* reported an effective strategy for photoacoustic intensity enhancement by utilizing polymers with large Γ to fabricate nanoprobe with sizes >100 nm.^[44] Although these intensive investigations have focused on elevating PCE to improve the PA effect by rational design of small molecules or polymers, the thermal-to-acoustic conversion efficiency (Grüneisen parameter) or the conversion of small molecules to polymers to optimize the photoacoustic signal is still seldom considered.^[45]

As the strategy of boosting the photoacoustic effect by simultaneously introducing the dark TICT state and intramolecular motion was successfully demonstrated in our previous work,^[46–48] we rationally designed three materials based on the above consideration (namely s-TToB, p-TToB and p-TTmB). Herein, we reveal for the first time that the polymerization strategy could promote the PA effect by improving thermal-to-acoustic conversion and near-infrared (NIR) light-harvesting capacity, simultaneously. We confirmed that the PA signal enhancement originated from the polymerization strategy because these three materials shared the same building units. By comparing the photothermal and PA properties of these three materials in the nanoparticle state, we found that both PA intensity and PCE were enhanced *via* our polymerization strategy. Further study on the photothermal and PA transitions indicates that the polymerization strategy is capable of elevating the photothermal conversion and thermal-to-acoustic conversion. Encouraged by the excellent PA and photothermal performance of the optimal polymers, the *in vivo* PA imaging and photothermal therapy in orthotopic 4T1 breast tumor-bearing mice were further performed. The results display that the PA signal accurately outlined the tumor contour and the tumor in mice can be effectively inhibited thanks to intensive thermal inactivation process of the optimal polymers. This work presents a general and effective polymerization strategy, which is beneficial for boosting the thermal-to-acoustic transition and promoting the NIR light-harvesting capacity, thus promotes PA imaging quality in biomedical applications.

EXPERIMENTAL

Synthesis of s-TToB, p-TToB and p-TTmB, and Preparation the Corresponding Nanoparticles

The detailed synthetic procedures and characterization of s-TToB, p-TToB and p-TTmB are described in electronic supplementary information (ESI). The ultrasonic nanocoprecipitation method was selected to prepare the corresponding s-TToB, p-TToB and p-TTmB nanoparticles (NPs). The s-TToB, p-TToB or p-TTmB (1.0 mg) and amphiphilic doped

matrix (MPEG₂₀₀₀-DSPE, 4.0 mg) were dissolved in THF (1.0 mL), respectively. The corresponding THF solution was dropwise added in deionized water (9.0 mL) accompanied with sonication (40%, 60 W output) for 3 min. After that the THF in the obtained mixtures was purged and removed by the air pump for 4 h. Then the NPs suspensions were dialyzed overnight at room temperature. The obtained s-TToB, p-TToB and p-TTmB NPs were subsequently concentrated to 1.0 mg·mL⁻¹ by ultrafiltration before 4 °C storage. The s-TToB, p-TToB and p-TTmB NPs were filtered through a 0.2 μm syringe driven-filter before use.

Photophysical Properties Evaluation

UV-Vis absorption spectra of s-TToB, p-TToB and p-TTmB (in THF, 3.0 mL) as well as their corresponding nanoparticles (in water, 3.0 mL) of various concentrations were recorded by Thermo Scientific GENESYS 50. The photoluminescence spectra of s-TToB, p-TToB and p-TTmB (in THF/ water mixtures with various water fractions, 3.0 mL) as well as their corresponding s-TToB, p-TToB and p-TTmB NPs (in water, 3.0 mL) were measured by FLS1000 spectrofluorometer (excited wavelength 808 nm).

Photothermal Performance

The corresponding s-TToB, p-TToB and p-TTmB NPs (in water, 0.2 mL) with different concentrations were irradiated by 808 nm laser irradiation (0.46 W·cm⁻²) for 180 s, and subsequently cooled down to ambient temperature (300 s). The temperature changes of s-TToB, p-TToB and p-TTmB nanoparticles solutions were monitored by FLUKE Ti200 and digital thermometer every 10 s. The photothermal performance of commercial photothermal agent ICG was also evaluated under the same condition.

In vitro and *In vivo* PA Imaging

To study the relationship between PA intensity and UV absorption, the corresponding s-TToB, p-TToB and p-TTmB NPs with various concentrations (6.25, 12.5, 25, 50 and 100 μg/mL) were loaded into a tube in the phantom and subsequently recorded the PA signals under different excitation wavelength (680–900 nm, every 5 nm) on a MOST imaging system. After the tumor volumes reached approximately 50–100 mm³, three of these 4T1 tumors bearing models were selected, randomly. To evaluate the photoacoustic imaging potential of p-TTmB NPs, the p-TTmB NPs (200 μL; 1 mg·mL⁻¹ based on p-TTmB) were intravenously injected into these mice. Then the PA data were subsequently acquired at 0, 2, 4, 6, 8, 10 and 24 h post-injection of p-TTmB NPs. After obtaining the PA imaging data, the images were reconstructed using the model-based algorithm supplied within the View MSOT software suite (V3.6, iThera Medical).

In vivo PTT Experiments

The 4T1 tumor-bearing mice were randomly separated into 4 groups (3 mice per group) when the tumor volumes reached about 50–100 mm³: (1) "Saline + laser" group, (2) "p-TTmB NPs + laser" group, (3) "Saline" group, (4) "p-TTmB NPs" group. The p-TTmB NPs (200 μL; 1 mg·mL⁻¹ based on p-TTmB) or Saline (200 μL) were intravenously injected into the tumor-bearing mice, respectively. For the first two groups, the tumor areas of mice were irradiated by an 808 nm laser at 1 W·cm⁻² for 5 min at 8 h post-injection. After treatments, the mice weights and tumor volumes were measured every three days for 21 days. The

tumor volume was calculated using the following formula: length $\times$ width²/2 (length and width were the longest and shortest diameters of tumors, respectively).

RESULTS AND DISCUSSION

Design, Synthesis, and Characterization of s-TToB, p-TToB and p-TTmB

In order to investigate the influence of polymerization on the PA property, the "D- π -A- π -D" typed small molecule and polymers based on the same building blocks were constructed. The classic low-band-gap electronic acceptor benzo[1,2-*c*:4,5-*c'*]bis([1,2,5]thiadiazole) (BBTD) was employed as a strong electron-deficient core to redshift the absorption to near-infrared (NIR) region and introduce the strong intramolecular charge transfer (ICT) characteristics.^[49,50] The twisted triphenylamine (TPA) building block was selected as the molecular rotors to ensure the intramolecular rotation, which act as the electronic donor at the same time. As the intramolecular motions has been demonstrated as an effective approach to enhance both photoacoustic and phototherapy.^[14,48] The 3-(2-hexyldecyl)thiophene ring (T) was introduced as the π -bridge and the second electronic donor, which can facilitate the ICT progress from TPA to BBTD.

Additionally, the outwardly extended long alkyl chain can not only improve the solubility of polymers, but also provide screen effects for inhibition of intermolecular interaction, which is beneficial for the active molecular motions in aggregates.^[51] We thus synthesized the small molecule (s-TToB)

and the polymer (p-TToB), accordingly. In addition, we synthesized the p-TTmB by manipulating the substitution position of alkyl chain on thiophene (from *ortho*- to *meta*-positioned) to further promote the nonradiative decay and in turn obtain the better photoacoustic and phototherapy properties.^[40,51] The synthetic route to the s-TToB, p-TToB and p-TTmB are depicted in Fig. 1. All intermediates and target compounds were synthesized *via* a few steps of organic name reactions (Suzuki-Miyaura coupling, Stille coupling *et al.*) and confirmed by NMR and gel permeation chromatography (GPC) methods. The detailed synthetic procedures and characterization are shown in Figs. S1–S11 (in the electronic supplementary information, ESI). Number-average molecular weight (M_n) and polydispersity index ($\mathcal{D}$) are 17.9 kDa and 1.06 for p-TToB, 13.8 kDa and 1.45 for p-TTmB.

Photophysical Properties and Theoretical Calculation

Firstly, basic photophysical properties of these three materials were investigated. As shown in Figs. 2(a) and 2(b), the maxima absorption peaks in tetrahydrofuran (THF) of their UV-Vis spectra for s-TToB, p-TToB and p-TTmB were located at 696, 704 and 814 nm, respectively. And the emission peaks (in THF) for s-TToB, p-TToB and p-TTmB were located at 1020, 1050, and 1095 nm, respectively. The gradual red-shift results might be originated from the contribution of polymerization and regulation of alkyl chain position to the molecular conformation.^[51] Then the molar absorption coefficients (ϵ) were investigated (Fig. S12, in ESI) and the ϵ values were measured to be 1.04×10^4 L·mol⁻¹·cm⁻¹ at 696 nm, 1.19×10^4 L·mol⁻¹·cm⁻¹ at 700 nm and 1.26×10^4 L·mol⁻¹·cm⁻¹ at 814 nm

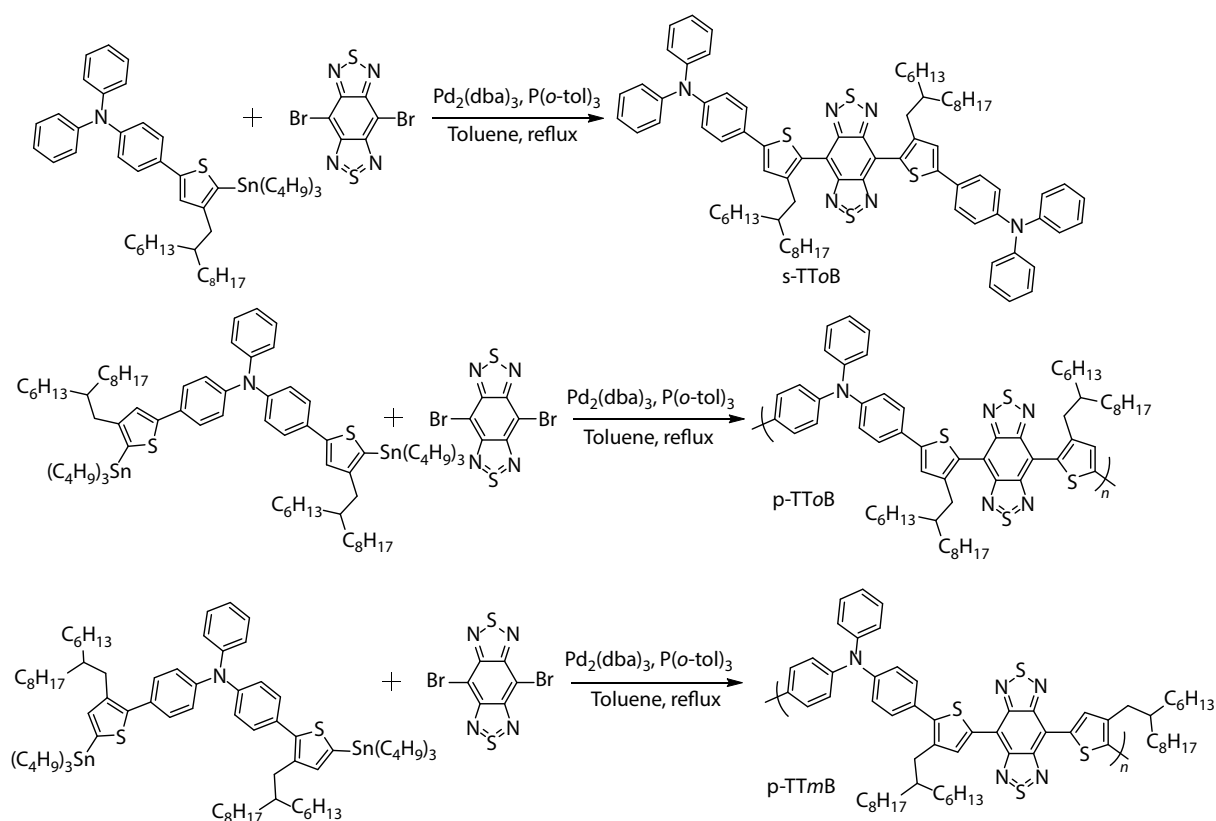


Fig. 1 Synthetic route to the s-TToB, p-TToB and p-TTmB.

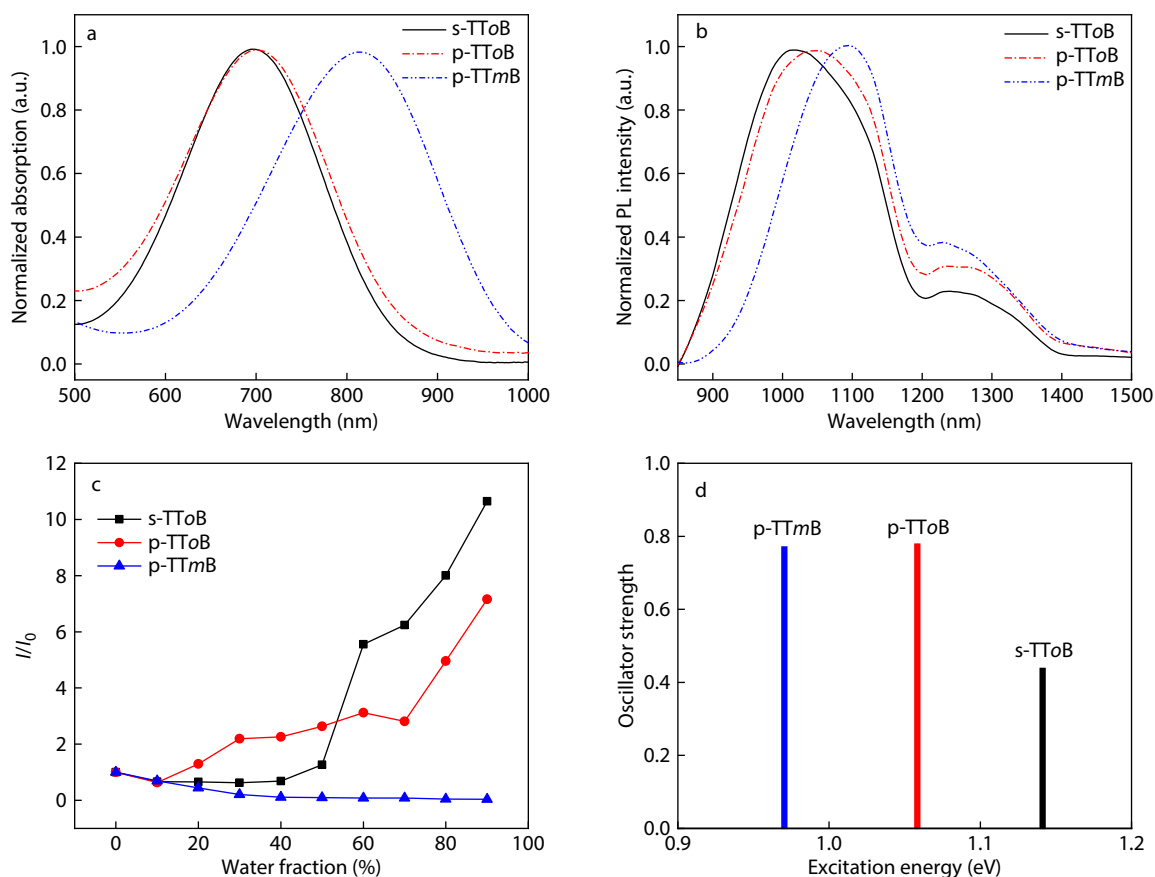


Fig. 2 Photophysical properties and theoretical calculation. (a) Normalized absorption spectra of s-TToB, p-TToB and p-TTmB in THF; (b) Normalized photoluminescence spectra of s-TToB, p-TToB and p-TTmB in THF; (c) Variation of PL intensity (I/I_0) with water fraction in THF/water mixtures, where I and I_0 are the maximum PL intensities in THF/water mixtures and pure THF, respectively; (d) Oscillator strength of S_0 to S_1 transitions for polymer trimers calculated by TD-DFT.

for s-TToB, p-TToB and p-TTmB, respectively. The increased ϵ values might be ascribed to the larger transition dipole moments originating from the extended effective conjugation length of polymers.^[52]

As THF is the good solvent for these three compounds while water is the poor solvent, the photoluminescence (PL) spectra in different aggregate states were investigated in THF/water mixtures (Fig. S13, in ESI). With the increase of water fractions, both s-TToB and p-TToB PL intensities exhibit a process of decreased first and then increased, while the p-TTmB PL intensities exhibit a process of continuous decline. The PL intensities decrease process of these three materials might be attributed to the TICT state or the dark TICT state formation, which has been demonstrated in our previous work.^[48] α (PL intensity at water fraction=90% versus water fraction=0%) is a factor which can represent the emission efficiency in the aggregated state. And it is easy to observe in Fig. 2(c) that the α value of p-TToB (7.16) and p-TTmB (0.04) are lower than s-TToB (10.64), suggesting the excited state energies of p-TToB and p-TTmB prefer to return to the ground state through the nonradiative decay.^[20]

Next, density functional theory (DFT) and time-dependent density functional theory (TD-DFT) calculations based on s-TToB and trimers of polymers (p-TToB, p-TTmB) were performed to further confirm our design influence of molecular

conformation. And the optimized ground-state (S_0) geometry was displayed in Fig. S14 (in ESI). Both s-TToB and p-TToB exhibit large dihedral angle ($\sim 50^\circ$) between T and BBTD units, while the T and BBTD units of p-TTmB are almost in the same plane ($< 5^\circ$). The planar architecture of the core moieties might be one of the reasons for the p-TTmB possessing the most red-shifted maximum wavelength (814 nm) and the largest ϵ value among these three compounds. But compared with s-TToB and p-TToB, the p-TTmB possesses a more twisted structural geometry between T and TPA units. These results confirmed the effectiveness of alkyl chains in determining the distortion of molecular structure at adjacent positions. Further, such twisted structural geometry can also reduce the stacking of molecular structures at adjacent units, leaving rooms for intramolecular motions. Fig. S15 (in ESI) revealed the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) of s-TToB, p-TToB and p-TTmB. All these HOMOs of three compounds were delocalized along the conjugated backbones and the LUMOs were almost all located on the BBTD moieties. And this kind of separated HOMO-LUMO distribution is instrumental in TICT state formation.^[30] Furthermore, the HOMO/ LUMO values (Fig. S16, in ESI) of s-TToB, p-TToB and p-TTmB are -3.31 eV/ -4.72 eV, -3.40 eV/ -4.72 eV and -3.42 eV/ -4.56 eV, respectively. Notably, compared with the HOMO-LUMO gap of

s-TToB (−1.41 eV), the p-TToB (−1.32 eV) and p-TTmB (−1.14 eV) possess lower gap values. These lower energy gaps were beneficial for theoretically generate more redshifted absorption in the NIR region, which in accordance with the absorption spectra of s-TToB, p-TToB and p-TTmB in THF (Fig. 2a). The excited state information of s-TToB, p-TToB and p-TTmB were also predicated by TD-DFT calculations (Table S1, in ESI). As shown in Fig. 2(d), the p-TToB (1.06 eV) and p-TTmB (0.97 eV) display lower S1-S0 bandgaps as compared to s-TToB (1.14 eV). In addition, a significant oscillator strength improvement of p-TToB and p-TTmB were carried out, which is match well with the increasing ϵ values of the polymers (Fig. S12, in ESI). Triplet states of dyes can usually dissipate energy via sensitizing the formation of reactive oxygen species. We thus calculated the energy gaps between S_1 and T_1 states (ΔE_{ST}). All the ΔE_{ST} values of s-TToB, p-TToB and p-TTmB were too high to generate effective intersystem crossing (ISC) process, which were beneficial for the nonradiative dissipation from excited states.^[16] Collectively, the calculated data confirmed the effectiveness of our strategy, demonstrating the potential of promoting the properties (such as the redshifted light-harvesting capacity) by polymerization and manipulation of alkyl chain position.

Fabrication and Characterization of Nanoparticles

To further investigate the properties of small molecule (s-TToB) and polymers (p-TToB and p-TTmB) in aggregate, a general nanoprecipitation method was employed to form the water-soluble nanoparticles, with amphiphilic MPEG₂₀₀₀-DSPE as the doped matrix (Fig. 3a). The corresponding nanoparticles (named as s-TToB NPs, p-TToB NPs, and p-TTmB NPs) exhibit almost the same hydrodynamic diameters of approximately 120 nm and share similar spherical shapes (Figs. 3b–3d). All the three absorption profiles of s-TToB NPs, p-TToB NPs, and p-TTmB NPs show red-shift maximum absorption peaks (Fig. 3e), which located at 724, 734 and 834 nm, respectively. As is known to all, both the absorption wavelength and ϵ play important roles in promoting maximum permissible exposure (MPE) laser power and penetration depth.^[38] Thus, the ϵ values at 808 nm were also measured because of the widespread use of 808 nm laser in biological applications (Fig. S17, in ESI). Both the ϵ of p-TToB ($6.8 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$) and p-TTmB ($7.7 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$) NPs at 808 nm are significantly higher than that of s-TToB ($5.3 \times 10^3 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$). The red-shifted absorption peaks in response to a large polar solvent (in water) indicated that a strong TICT effect existed in these three materials. And the TICT state usually returns to the ground state *via* the nonradiative decay. The s-TToB NPs, p-TToB NPs and p-TTmB NPs possess PL profiles in the wide range of 850–1400 nm with maximum emission wavelengths of approximately 970, 1030 and 1065 nm, respectively (Fig. 3f). Notably, s-TToB NPs exhibit over 3-fold and 49-fold higher PL intensity than p-TToB NPs, and p-TTmB NPs, respectively (Fig. S18 in ESI and Fig. 3g). Compared to s-TToB NPs, the weakened PL intensities of p-TToB NPs, and p-TTmB NPs suggest a largely increased nonradiative decay process. Furthermore, these three kinds of NPs could not generate any reactive oxygen species (ROS) under 808 nm laser irradiation as evidenced by the negligible decomposition at 400 nm of 9,10-anthracenediyl-bis(methylene)dimaronic acid (ABDA, a classic ROS indicator), demonstrating that the effect from ISC on

excited-state deactivation could be excluded (Fig. 3h). And this result was in accordance with our calculated data. Besides, s-TToB NPs, p-TToB NPs, and p-TTmB NPs show good anti-photobleaching capabilities. As shown in Fig. 3(i), the absorbance of these three nanoparticles remain over 90% during 4 min laser irradiation, whereas the maximal absorbance of indocyanine green (ICG, a widely used dyes in clinical research) dropped to nearly 10% (Fig. S19, in ESI). In addition, these three nanoparticles remain stable in blood serum solution after 7 days storage, suggesting that the good colloidal stability in biological matrices (Fig. 3j).

Photothermal Properties

As is known to all, photoluminescence, ISC, and internal conversion for heat deactivation are the three main dissipation pathways of absorbed photoexcitation according to the Jablonski diagram. The PL intensity decrease and almost no ROS generation of p-TToB and p-TTmB, revealed that these polymers were expected to become excellent candidates for photoacoustic and photothermal application. To investigate the photothermal properties, 808 nm laser was selected to irradiate s-TToB, p-TToB and p-TTmB NPs with various concentrations. As depicted in Figs. 4(a)–4(c), the platform temperature gradually increases as the concentrations of these three nanoparticles increase from $12.5 \mu\text{g} \cdot \text{mL}^{-1}$ to $200 \mu\text{g} \cdot \text{mL}^{-1}$. Compared with s-TToB NPs, p-TToB and p-TTmB NPs can reach higher elevated temperatures with different concentrations, indicative of the better photothermal conversion abilities can be obtained by the polymerization strategy. For instance, the p-TToB and p-TTmB NPs can reach plateau temperatures of 80 and 90 °C, respectively, while the s-TToB NPs can only increase to 73 °C under the same concentration ($200 \mu\text{g} \cdot \text{mL}^{-1}$). By contrast, the pure water just shows a slight temperature increase of ≈ 3 °C under the same conditions, suggesting that any difference in temperature increase shall be solely caused by s-TToB, p-TToB and p-TTmB NPs. To obtain the photothermal conversion efficiency (PCE, η) based on the reported calculation method,^[53] ICG was also measured at the same time (Fig. 4d). The PCE of these corresponding nanoparticles at 808 nm were calculated as 58.6%, 62.0% and 64.6% for s-TToB, p-TToB and p-TTmB NPs, respectively (Fig. 4e and Figs. S20 and S21 in ESI), which are higher than that of ICG (33.1%). As the photothermal stabilities of the photothermal agents are essential for clinical treatments, the photothermal stabilities of s-TToB, p-TToB and p-TTmB NPs were further evaluated by means of five heating-cooling cycles (Fig. 4f). Compared with ICG and s-TToB NPs, both p-TToB and p-TTmB NPs not only exhibit a better photothermal conversion but also remain excellent anti-photobleaching capabilities. Notably, the p-TTmB NPs can achieve a plateau temperature of 90 °C, which is approximately 10 °C higher than p-TToB NPs under the same concentration ($200 \mu\text{g} \cdot \text{mL}^{-1}$). The p-TTmB NPs possess better photothermal performance and higher PCE value than those of p-TToB NPs. The difference between p-TToB and p-TTmB NPs might be attributed to the enhanced dark TICT state formation and the planar core structure resulted from the alkyl modified position changes.^[29,48]

In vitro and In vivo PA Properties

According to the Jablonski diagram, PA signal generation is often along with the thermal deactivation process. To

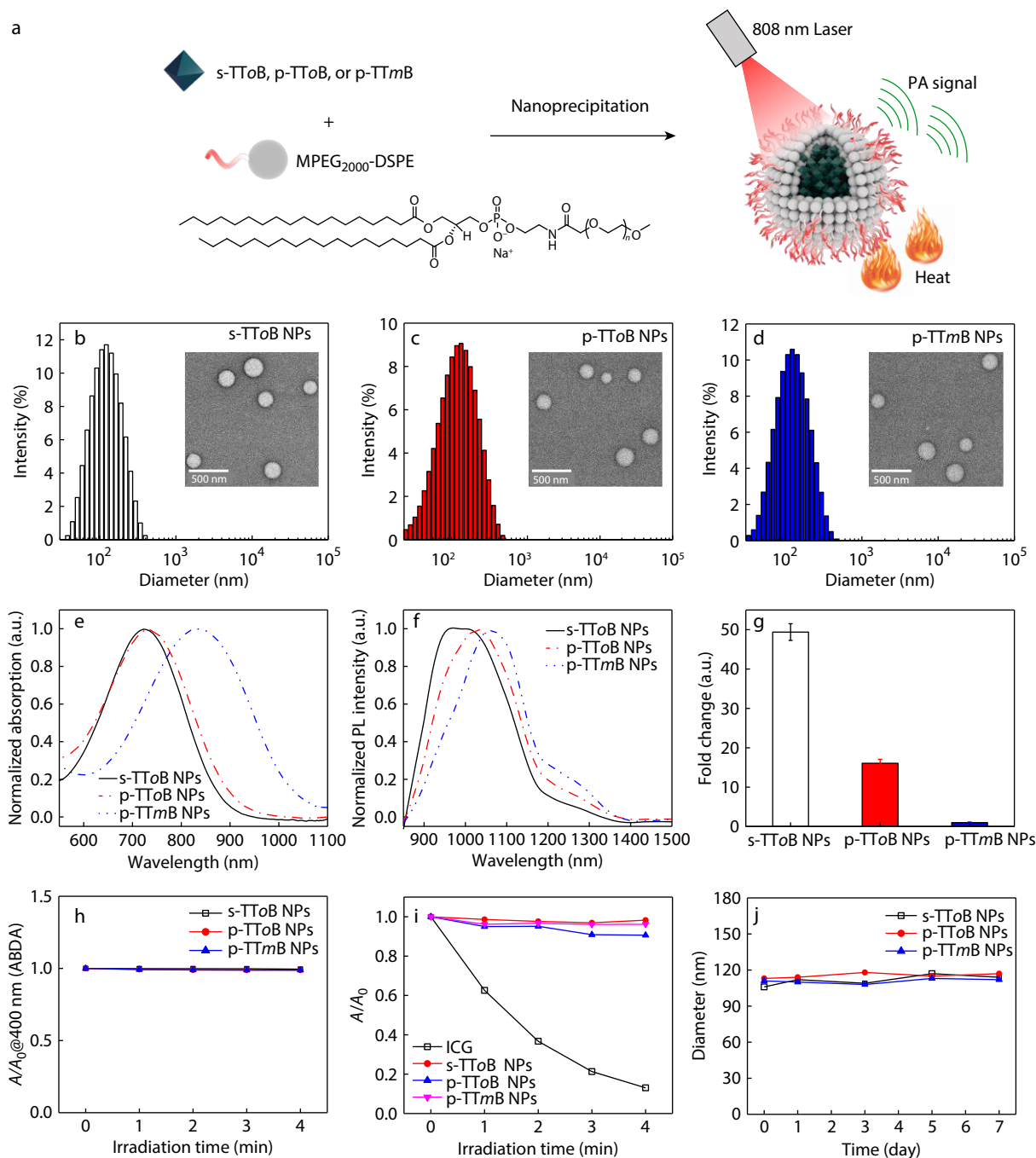


Fig. 3 Preparation, characterization and stability evaluation of these corresponding NPs. (a) Schematic of the preparation of s-TToB NPs, p-TToB NPs, and p-TTmB NPs via a nanoprecipitation method; Representative dynamic light scattering and transmission electron microscope results of s-TToB NPs (b), p-TToB NPs (c), and p-TTmB NPs (d); Absorption (e) and PL spectra (f) of s-TToB NPs, p-TToB NPs, and p-TTmB NPs, respectively; (g) Fold change plot of PL intensities for s-TToB NPs, p-TToB NPs and p-TTmB NPs under the same conditions, compared with the PL intensity of p-TTmB NPs. Error bars: mean±standard deviation ($n=3$); (h) Absorbance of ABDA at 400 nm as a function of 808 nm laser irradiation time of s-TToB NPs, p-TToB NPs, and p-TTmB NPs; (i) Plot of A/A_0 versus various irradiation time. A and A_0 are the maximal absorbance of s-TToB NPs, p-TToB NPs, p-TTmB NPs and ICG in PBS solutions after and before laser irradiation, respectively; (j) The diameter of s-TToB NPs, p-TToB NPs, and p-TTmB NPs after storage for 1, 3, 5 and 7 days, respectively.

investigate the PA properties of these three nanoparticles, the *in vitro* PA signals were acquired on a commercial small-animal opt-acoustic tomography system (MOST, iTheraMedical, Germany). The s-TToB, p-TToB and p-TTmB NPs were irradiated from 680 nm to 900 nm for recording the PA spectra. As shown

in Fig. 5(a), the PA spectra match well with the absorption profile, indicating the marked correlation between the absorption and photoacoustic signals. It can be intuitively observed that the PA signals of p-TToB and p-TTmB NPs were significantly stronger than s-TToB NPs at the same

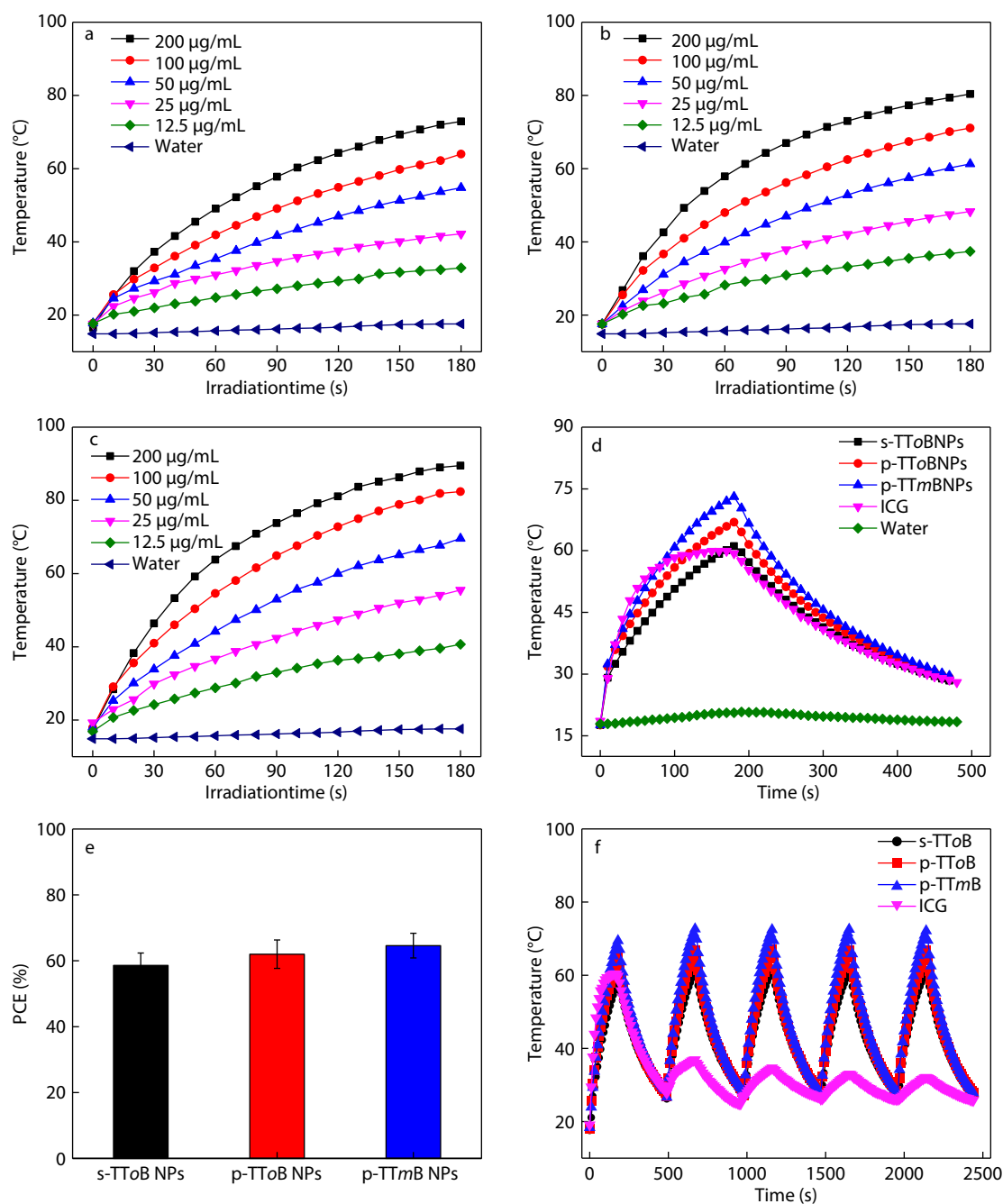


Fig. 4 Photothermal properties of these corresponding nanoparticles. Photothermal conversion behaviors of s-TToB (a), p-TToB (b) and p-TTmB (c) NPs at different concentrations (12.5–200 μg·mL⁻¹) under 808 nm light (460 mW·cm⁻²) irradiation; (d) Comparison of the photothermal conversion behaviors of s-TToB (50 μmol·L⁻¹, 65 μg·mL⁻¹), p-TToB (65 μg·mL⁻¹), p-TTmB NPs (65 μg·mL⁻¹), ICG (50 μmol·L⁻¹) and the blank in the PBS solution under 808 nm light (460 mW·cm⁻²) irradiation; (e) The calculated PCE values of s-TToB, p-TToB and p-TTmB NPs under 808 nm light (460 mW·cm⁻²) irradiation; (f) Photothermal stabilities of s-TToB, p-TToB and p-TTmB NPs (65 μg·mL⁻¹) and ICG (50 μmol·L⁻¹) during heating-cooling process for 5 cycles.

concentration (Fig. 5b). As displayed in Fig. 5(c), all these three NPs exhibited good linear correlation with the concentration, suggesting the great potential for quantitative analysis. The quantitative results revealed that, at the concentration of 100 μg·mL⁻¹, the p-TToB and p-TTmB NPs show about 1.3 times and 2 times PA amplitude intensities in comparison to s-TToB NPs, respectively. And this result directly proved feasibility of polymerization induced photoacoustic enhancement. In order

to obtain a deeper understanding of the reasons for PA signal enhancement, the relative Grüneisen parameter was estimated out according to the PA equation,^[43] since the same excitation pulsed laser was used. As depicted in Fig. S22 (in ESI), the relative Grüneisen parameters of p-TToB NPs and p-TTmB NPs are approximately 1.2- and 1.7-fold, respectively higher than that of s-TToB NPs. Moreover, Grüneisen parameter is the main factor responsible for the thermoelastic efficiency of PA

reagents, which closely related to the molecular structure. As the p-TTmB, and p-TTmB shared the same composition units with s-TTmB, we can confirm again that the photoacoustic enhancement comes from the polymerization.

The *in vitro* photoacoustic and photothermal properties revealed that p-TTmB NPs are the best photoacoustic and photothermal candidates for serving in the biomedical applications among these three materials. Thus, the *in vitro* and *in vivo* side toxicities were further evaluated. The result of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay demonstrated that p-TTmB NPs at a gradient of concentrations from $6.125 \mu\text{g}\cdot\text{mL}^{-1}$ to $100 \mu\text{g}\cdot\text{mL}^{-1}$ displayed negligible toxicity (Fig. S23, in ESI). The *in vivo* side toxicity was further evaluated by intravenous administration of the p-TTmB NPs ($200 \mu\text{L}$, $1 \text{ mg}\cdot\text{mL}^{-1}$) into a group of healthy mice. After 7 days, the blood samples of p-TTmB NPs-injected mice was collected for a series of biochemical index analyses, which were also compared with those of saline-injected healthy mice (Fig. S24 in ESI). The main organs of these mice were also collected and stained with H&E (Fig. S25 in ESI). Negligible differences of these results suggested that the p-TTmB NPs did not cause damage to the healthy mice, verifying the good biocompatibility of the p-TTmB NPs.

The PA properties *in vitro* illustrated p-TTmB NPs were a highly potent PA imaging contrast agent. To identify the therapeutic performance *in vivo*, the time-dependent PA imaging was performed on 4T1 tumor-bearing mice. The PA signals were monitored by MOST system after injected p-TTmB NPs ($200 \mu\text{L}$, $1 \text{ mg}\cdot\text{mL}^{-1}$) into the mice *via* tail vein, immediately. The PA images were recorded at 0, 2, 4, 6, 8, 10 and 24 h. As displayed in Figs. 5(d) and 5(e), the PA signals of tumor gradually increase during the 8 h post-injection. The maxim-

um PA intensity was detected at 8 h post-injection. From 8 h to 24 h, PA signal decreases slowly. The tissues with strong PA signals were collected and stained with H&E after the end of imaging, which suggested that the tumor was verified (Fig. S26, in ESI). These results manifested that the p-TTmB NPs could enrich at the tumor site and serve as an effective PA imaging agent to visualize the *in vivo* tumors.

In vivo Photothermal Therapy

Encouraged by pTTmB NPs exhibited a quite good photothermal conversion efficiency, the *in vivo* photothermal therapy (PTT) performance of pTTmB NPs was also investigated on 4T1 tumor-bearing mice. Mice with tumor volumes of 50 mm^3 were randomly divided into four groups. "Saline", "Saline + Laser", "pTTmB NPs" and "pTTmB NPs + Laser". Accordingly, the pTTmB NPs ($200 \mu\text{L}$, $1 \text{ mg}\cdot\text{mL}^{-1}$) and saline ($200 \mu\text{L}$) were injected into the corresponding group. As for these two group with laser treatment, the PTT was carried out at 8 h post-injection of pTTmB NPs *via* tail vein according to the PA imaging results (Fig. 5e). During the 5 min 808 nm laser irradiation ($1.0 \text{ W}\cdot\text{cm}^{-2}$), tumor temperatures for pTTmB NPs-treated mice gradually increase to 80°C , which is 45°C higher than the "Saline + Laser" treated mice (Figs. 6a and 6b). Tumor volumes was monitored after each treatment, the tumor volumes of "Saline", "Saline + Laser" and "pTTmB NPs" grew up rapidly without any hindrance. By contrast, the tumor growth of "pTTmB NPs + Laser" group was totally inhibited by PTT, which should be attributed to the excellent photo-thermal conversion of the pTTmB NPs upon laser irradiation (Fig. 6c). Meanwhile, the body weights of all groups were recorded, no significant body weight change can be found in four groups (Fig. S27 in ESI), indicating that the "pTTmB NPs + Laser" treatment did not cause *in vivo* side toxicity.

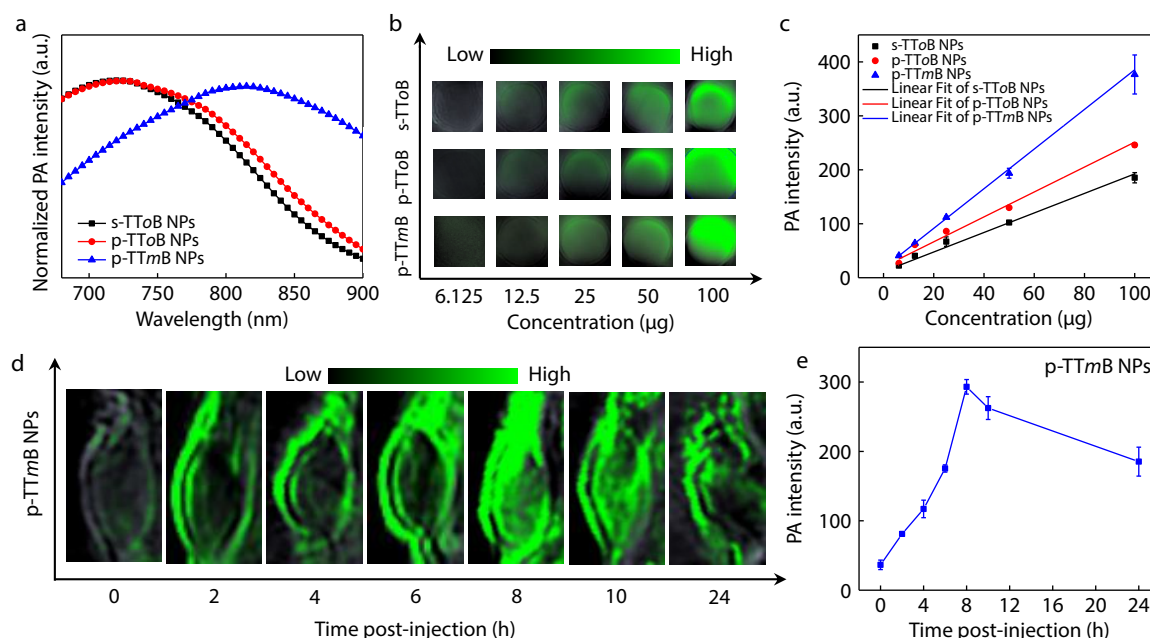


Fig. 5 *In vitro* and *in vivo* PA data of these corresponding nanoparticles. (a) PA spectra of s-TTmB, p-TTmB and p-TTmB NPs; (b) PA images and (c) corresponding PA intensities versus the concentration of s-TTmB, p-TTmB and p-TTmB NPs; (d) *In vivo* PA images of tumor sites at different times after injected p-TTmB NPs ($200 \mu\text{L}$, $1 \text{ mg}\cdot\text{mL}^{-1}$); (e) Quantitative analysis based on the typical images in (d). Error bars, mean $\pm$ s.d. ($n=3$ mice per group).

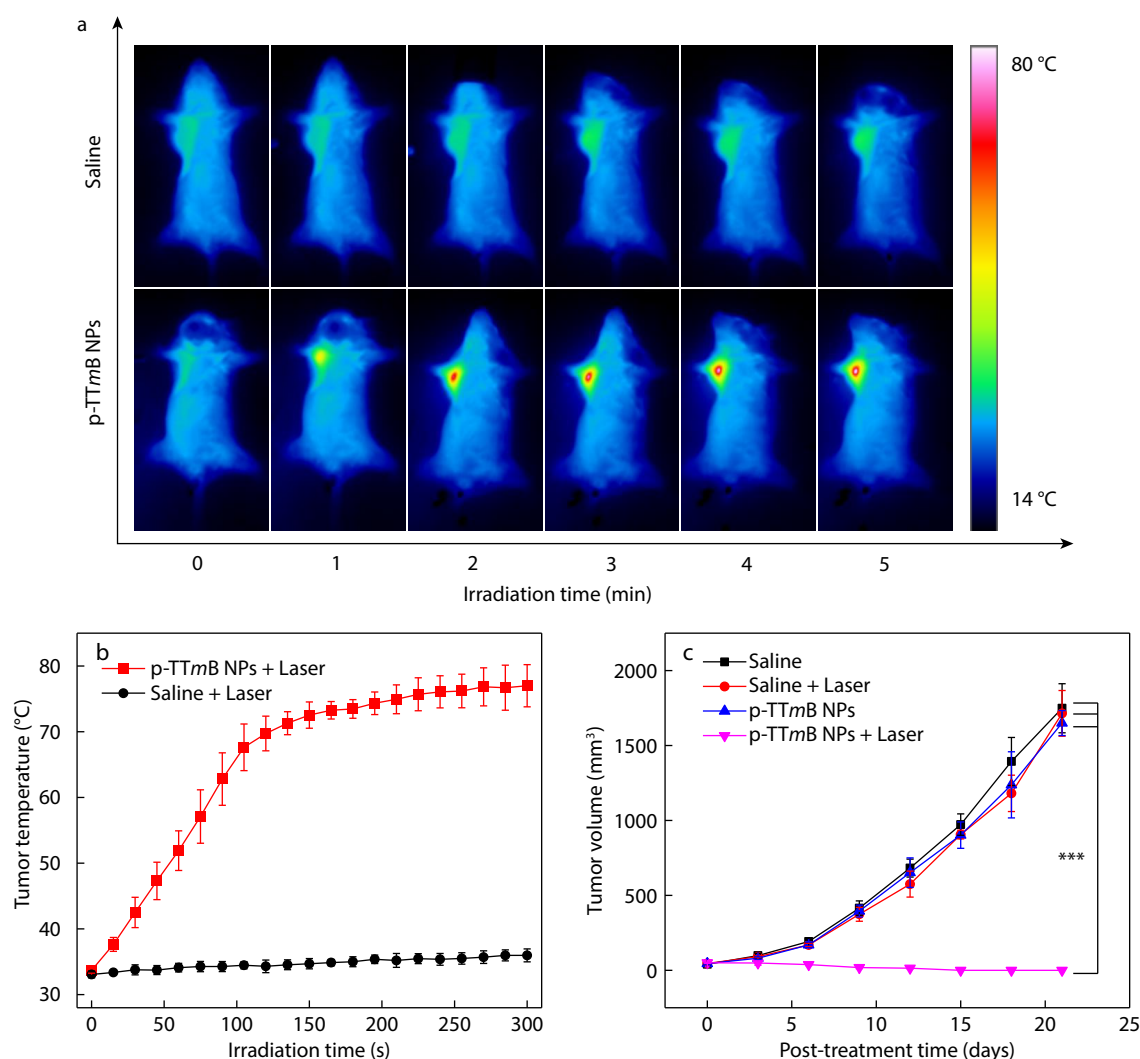


Fig. 6 *In vivo* photothermal therapy effect. (a) Photothermal images of 4T1 tumor-bearing mice under 808 nm laser irradiation with the laser power of 1.0 W·cm² for 5 min (saline=200 μ L, p-TTmB NPs=200 μ L, 1 mg·mL⁻¹); (b) Quantitative analysis based on the typical images in (a); (c) Tumor growth curves of 4T1 tumor-bearing mice after systemic administration of saline and pTTmB NPs with or without 808 nm laser irradiation for 5 min. Error bars, mean $\pm$ s.d. ($n=4$ mice). *** $p<0.001$, one-way ANOVA.

CONCLUSIONS

In summary, we successfully demonstrated that polymerization represented an efficient strategy to amplify the PA signal, especially in promoting thermal-to-acoustic conversion process and enhancing NIR light-harvesting capability. A series of functional materials were designed and synthesized, based on the same building units (namely s-TTmB, p-TTmB, and p-TTmB). Both p-TTmB and p-TTmB displayed higher NIR absorption because of the extended conjugation and obvious band-gap decrease of polymerization. Not only PL intensity decrease of p-TTmB and p-TTmB was observed in the aggregated, but also no ROS generation can be detected for p-TTmB and p-TTmB, suggesting that the absorbed excitation energy prefers to return to the ground state through nonradiative decay. Compared with s-TTmB NPs, the corresponding p-TTmB and p-TTmB NPs exhibited better photothermal performance and PA properties. However, the better performances of polymers are not entirely due to its high NIR light-harvesting capability.

The increased Grüneisen parameters of p-TTmB and p-TTmB NPs were obtained, indicating that the polymerization strategy also can effectively magnify the photoacoustic intensity *via* enlarging the thermal-to-acoustic conversion efficiency. Furthermore, the p-TTmB NPs exhibited negligible *in vitro* and *in vivo* toxicity, demonstrating that p-TTmB NPs possess good biosafety. The *in vivo* PA imaging performance and PTT effect of the optimized agent (p-TTmB NPs) were evaluated. The obtained results indicated that the p-TTmB NPs possess great potential for bioapplications. Collectively, this work provides a convenient and effective approach for developing better PA imaging materials. We hope this work that highlights the importance of polymerization strategy in guiding the design and preparation of advanced PA imaging agents.

NOTES

The authors declare no competing financial interest.

Electronic Supplementary Information

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