More Is Better: Acceptor Engineering for Constructing NIR-II AIEgens to Boost Multimodal Phototheranostics

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Abstract: The manipulation of electron donor/acceptor (D/A) shows endless impetus for innovating optoelectronic materials. Because of the diversity of electron-donating species, the study on donor engineering has explored systematically, whereas the research on electron acceptor engineering received a snub by contrast. Inspired by the philosophical idea of “more is different”, two systems with D’−D−A−D−D’ (1A system) and D’−D−A−A−D−D’ (2A system) structures based on the acceptor engineering are ingeniously designed and studied. It is demonstrated that the 1A system presents a weak aggregation-induced emission (AIE) to aggregation-caused quenching (ACQ) phenomenon along with the increased acceptor electrophilicity. Interestingly, the 2A system exhibits an opposite ACQ-to-AIE transformation, manifesting the dual-acceptor tactic could facilitate AIE activity. Thanks to the highest molar absorptivity, near-infrared-II (NIR-II, 1000–1700 nm) emission, superior AIE effect, favorable reactive oxygen species generation and high photothermal conversion efficiency, a representative member of 2A system handily perform in fluorescence-photoacoustic-photothermal multimodal imaging-guided photodynamic-photothermal synergetic therapy for efficient tumor elimination. Meanwhile, NIR-II fluorescence imaging of blood vessels and lymph nodes in living mice are also accomplished. This work provides a fascinating molecular design philosophy for developing versatile phototheranostic agent with a higher molar absorptivity, superb aggregation-intensified NIR-II fluorescent emission, and improved heat generation capacity.

1. Introduction

Nature is a complex and multifaceted system, and each constituent performs its own function. That is to say, when many individual components are assembled or admixed into a collective group/aggregate, there could produce superior outputs and simultaneously execute multiple tasks (beyond independent components).\cite{1,2} Aristotle, a Greek philosopher in the Classical period, has already pioneered the emergentism view that “The whole is greater than the sum of its parts”. This heuristic epistemology is consistent with the philosophical perspectives of “quantity breeds quality” and “more is different”.\cite{3} Unambiguously, the field of materials science has achieved flourishing developments under the guidance of such research philosophies, especially for luminescent materials.\cite{4–6}

Promoting the advancement of luminescent materials would undoubtedly facilitate the high-tech innovation and benefit societal progress.\cite{7–10} There are mainly two general tactics for manipulating the luminescence color, absorption/emission wavelength, molar absorptivity (\(\varepsilon\)), and luminescence efficiency of luminescent materials.\cite{11} One feasible way is to subtly regulate
the conjugation extent of a molecular skeleton. However, it should be pointed out that the red-shift in the absorption/emission wavelength caused by extending the π-conjugation could lead to the risk of strengthening the detrimental π–π stacking interaction.\cite{5,12,13} Alternatively, electron donor–acceptor (D–A) engineering provides a sophisticated and profound combination protocol.\cite{9,14–16} Due to the variety (e.g., carbazole, diphenylamine, triphenylamine) and accessible modification of electron donors, feasibly customized optical properties, and promising functionalities, the construction of organic small-molecule fluorophores with D–A–D-type scaffolds have attracted immense attention.\cite{11,17,18} As a rule, the highest occupied molecular orbital (HOMO) is broadly delocalized along the whole conjugated molecular backbone, but the lowest unoccupied molecular orbital (LUMO) is almost distributed on the acceptor moiety in those fluorophores. Thus, the manipulation of electron acceptor could exert a greater impact on the structure-property relationships of D–A–D fluorophores. Unfortunately, although enthusiastic research endeavors have been injected in the past few decades, especially in organic photovoltaic field,\cite{19–22} the number of practical electron acceptors with distinct electron-withdrawing ability for pursuing tunable and high-performance fluorophores are still limited. Inevitably, employing the acceptor engineering to develop novel and multifunctional fluorophores remain as a bottleneck and receive a stagnancy up to now.\cite{23,24} In this circumstance, how to make full use of the existing and typical electron acceptors to excavate the undisclosed realm of multi-acceptors (“more”) in a D–A–D skeleton, is anticipated to be an exotic and exciting wonderland with limitless treasures, which could breed a conspicuous molecular design philosophy.

On the other hand, fluorophores are practically frequently used as a whole in the aggregate state, rather than in their single-molecule state, such as in the areas of optoelectronic devices and bioimaging.\cite{7,10} However, traditional luminescent molecules generally suffer from the problem of aggregation-caused quenching (ACQ), which gives rise to the result that a separated element is more brilliant than the whole, meaning more is worse.\cite{12,13,25} Fortunately, this common belief of ACQ is subverted by the phenomenon of aggregation-induced emission (AIE), which is exactly opposite to the ACQ effect and the collaboration and synergism from many isolated molecules in the aggregate state (“more”) promote it to perform better than its elementary compositions.\cite{26,27} For AIE phenomenon, non-emissive luminogens in the monodispersed state are induced to emit intensely upon the formation of aggregates, which could be understood by the mechanism of restriction of intramolecular motions (RIM).\cite{5} Furthermore, aggregation generally induces the divergence (the multipath distribution of excitons), in which the equilibrium between radiative and nonradiative decays could be subtly
regulated to generate a multifunctional system.\textsuperscript{[28]} The distinct behaviors and qualitative jumps after forming aggregates from a simple molecule could be ascribed to the change of morphologies, quantities, or diverse interactions that render manifold properties in the aggregate state.\textsuperscript{[27]} Overall, AIE effect is a representative example exhibiting a non-linear addition of properties from simple molecules to versatile aggregates.\textsuperscript{[29,30]} In this regard, the AIE technology offers a new platform and territory for researchers to explore and access the philosophy of “more is better”.

As a proof-of-concept, in this work, acceptor engineering was systematically studied by using two approaches. Firstly, as shown in Scheme 1a, four small molecules with a structural type of D’–D–A–D–D’ were designed and classified as 1A system. These four molecules possess the same electron donor but different electron acceptors with an orderly increased electrophilic capability from benzo[2,1,3]thiadiazole to benzo[1,2-c:4,5-c’]bis([1,2,5]thiadiazole). Furthermore, to explore the property changes that resulted from introducing one more acceptor at the molecular level, the construction of 2A system with the molecular structure of D’–D–A–A–D–D’ was conducted. More importantly, the essence or disparity of the optical behaviors of these two systems entices us to unravel the veil in their aggregate states. The results revealed that the 1A system encountered a change of photophysical properties from weak AIE to ACQ upon enhancing the electron-withdrawing capacity of the acceptors. In contrast, the 2A system demonstrated an opposite ACQ-to-AIE transformation (Scheme 1a). Strikingly, the members of 2A system possessed a higher ε than their counterparts in the 1A system, no matter which acceptor was utilized. In addition, among all the molecules of 2A system, 2TT-2BBTD with the strongest electron acceptor exhibited the highest ε, best AIE activity, and maximal emission wavelength located in the NIR-II region. These advantageous properties, together with appreciable reactive oxygen species (ROS) and prominent photothermal generation, jointly enabled 2TT-2BBTD nanoparticles (NPs) to serve as an ideal NIR-II fluorescence imaging (FLI)-featured multimodal phototheranostic system for breast cancer elimination in live mice (Scheme 1b,c). This work definitely exemplifies the molecular design philosophy of “more is better” and provides a new avenue for developing multifunctional phototheranostic systems.
Scheme 1. Schematic illustration of the investigation on acceptor engineering, nanofabrication and multifunctional phototheranostic application. a) Molecule types and the structures of diverse acceptors employed in the 1A or 2A system. b) Molecular structure of 2TT-2BBTD and the preparation of 2TT-2BBTD NPs by a nanoprecipitation method. c) Application of 2TT-2BBTD NPs in NIR-II fluorescence-photoacoustic-photothermal trimodal imaging-guided photodynamic-photothermal synergetic therapy of cancer.

2. Results and Discussion
2.1. Design, Synthesis and Characterization

To systematically study the effect of acceptor engineering in modulating the photophysical properties of luminogens at the molecular and aggregate levels, four frequently used electron acceptors were employed to construct luminogens with one or two acceptor units, respectively (Figure 1a,e). To be specific, in the 1A system, benzo[2,1,3]thiadiazole (BTD), pyridal[2,1,3]thiadiazole (PTD), 6,7-diphenyl-[1,2,5]thiadiazolo[3,4-g]quinoxaline (DPTDQ) and benzo[1,2-c:4,5-c']bis([1,2,5]thiadiazole) (BBTD) with the progressively increased electron-withdrawing ability were selected as the electron acceptors. Besides, alkyl thiophene serving as both π-conjugation bridge and electron donor, and triphenylamine (TPA) unit acting as the second donor and molecular rotor were integrated to produce D′−D−A−D−D′-type molecules. The long alkyl chains were introduced as shielding units to provide steric hindrance, which could weaken the intermolecular interactions and facilitate the intramolecular motions even within NPs.\textsuperscript{[31]} Particularly, the alkyl group on the thiophene ring close to the TPA unit
could enable a longer absorption wavelength. In addition, the molecules in the 2A system with \(D'-D-A-D-D'\) structure were established through grafting one more electron acceptor in comparison with their counterparts in the 1A system. The synthetic routes of molecules in the 1A (including 2TT-BTD, 2TT-PTD, 2TT-DPTDQ and 2TT-BBTD) and 2A (including 2TT-2BTD, 2TT-2PTD, 2TT-2DPTDQ and 2TT-2BBTD) systems were provided in Schemes S1−S6 in the Supporting Information, and their structures were satisfactorily characterized by NMR and mass spectroscopy (Figures S1−S59, Supporting Information).

Subsequently, the photophysical properties of the molecules in the 1A system were investigated by measuring their UV-Vis-NIR absorption and photoluminescence (PL) spectra. It was noticeable that the absorption and emission maxima of these four luminogens showed obvious red shifts in THF solution by orderly changing the electron acceptor unit from BTD to BBTD (Figure 1b,c). 2TT-BBTD exhibited the strongest intramolecular charge transfer (ICT) effect than the other three compounds, which could be reflected by their low-energy charge transfer (CT) absorption bands that steadily increased in the sequence of 511, 541, 743, and 824 nm for 2TT-BTD, 2TT-PTD, 2TT-DPTDQ and 2TT-BBTD, respectively (Figure 1b). Additionally, their corresponding emission maxima were also gradually bathochromic shift in the line of 663, 716, 952 and 1104 nm, demonstrating that the enhanced ICT effect was derived from the elaborate acceptor engineering (Figure 1c).

The PL intensity fluctuations of these four molecules in THF/H\(_2\)O mixtures with different water fractions \((f_w)\) as poor solvent were further evaluated. As illustrated in Figure 1d and Figure S60 (Supporting Information), for 2TT-BTD and 2TT-PTD, upon gradually increasing the \(f_w\) to 40%, their emission intensities decreased significantly due to the twisted intramolecular charge transfer (TICT) effect,\(^{[33,34]}\) which then turned to slightly enhance along with further elevating the \(f_w\). As a result, 2TT-BTD and 2TT-PTD analogously displayed the features of TICT plus weak AIE. However, 2TT-DPTDQ and 2TT-BBTD exhibited a typical ACQ phenomenon with the characteristics of obviously decreased fluorescence intensities as aggregates in comparison to the isolated molecules. Definitely, at \(f_w = 90\%\), the \(I/I_0\) values \((I_0\) is the PL intensity of molecules in pure THF solution) of 2TT-DPTDQ and 2TT-BBTD were determined to be as low as 0.14 and 0.08, respectively. In addition, the solvatochromic effects of the molecules in the 1A system were carried out to verify the TICT property. With the increase of solvent polarity, the red-shifted emission maxima and weakened PL intensities were monitored along with the negligible variations in the corresponding absorption spectra (Figure S61, Supporting Information). These results fully signified the TICT behavior of these four luminogens. As a whole, the \(D'-D-A-D-D'\)-type molecules in the 1A system exhibited the
conversion of AIE activity to ACQ behavior in the wake of employing acceptor with enhanced electron deficiency.

**Figure 1.** The designed compounds and photophysical properties. a), e) Chemical structures of 2TT-BTD, 2TT-PTD, 2TT-DPTDQ, 2TT-BBTD, 2TT-2BTD, 2TT-2PTD, 2TT-2DPTDQ and 2TT-2BBTD, respectively. b), f) Normalized absorption spectra in THF solution. c), g) Normalized emission spectra in THF solution. d), h) Plots of the relative emission intensity \(\frac{I}{I_0}\) versus the composition of the THF/H\(_2\)O mixture, where \(I_0 =\) PL intensity in pure THF solution. Inset: partially enlarged \(\frac{I}{I_0}\) plots of 2TT-2BTD, 2TT-2PTD, 2TT-2DPTDQ and 2TT-2BBTD, respectively. Concentration = 10 \(\mu\)m.

To probe the photophysical properties of the 2A system, their absorption and emission spectra in THF solution were measured. At the molecular level, these four compounds affirmed the concrete bathochromic shifts both in absorption maxima (509, 546, 688, and 773 nm) and emission maxima (668, 716, 886, and 1004 nm) from 2TT-BTD to 2TT-2BBTD (Figure 1f,g), respectively, attributed to the orderly enhanced electron-withdrawing ability of the electron acceptors. Compared with the 1A system, interestingly, the corresponding luminogens with one more acceptor in the 2A system possessed a definitely higher \(\epsilon\), which furnished an attractive and robust strategy to improve the molecular light-harvesting ability (Figure 1b,f; Figure S62,
Supporting Information). It is worth noting that 2TT-2BBTD was resplendently standing out from the others owing to its highest ε (4.29 × 10^4 M^{-1} cm^{-1}) and maximum emission peak in the NIR-II region, which laid a solid foundation for the multimodal phototheranostics application (Figure 1f).[35,36] Moreover, since an 808 nm laser is frequently used in phototheranostics due to its minimal biological absorption and superior penetration depth in biological tissue, the ε of a phototheranostic agent at 808 nm will definitely determine the phototheranostic outcome. Gladly, the ε of 2TT-2BBTD at 808 nm was still as high as 3.76 × 10^4 M^{-1} cm^{-1} (Figure 1f), which was higher than that of a majority of previously developed NIR-II AIE luminogens (AIEgens).[16,37,38] Therewith, the PL of molecules in the 2A system was studied in THF/H_2O mixtures with different f_w. As displayed in Figure 1h and Figure S63a (Supporting Information), the PL intensities of 2TT-2BTD decreased prominently from the molecularly dispersed state in THF solution (f_w = 0) to the aggregate state, where an ACQ appearance was monitored. In contrast, benefitting from the elaborate acceptor engineering, 2TT-2PTD, 2TT-2DPTDQ and 2TT-2BBTD revealed the sequential promotion of the AIE effect, as evidenced by their gradually increased I/I_0 values of 0.57, 1.19 and 33.65 at f_w = 90%, respectively (Figure 1h; Figure S63b–d, Supporting Information). Meanwhile, the intrinsic TICT characteristic of compounds in the 2A system was also proved by their AIE curves and solvatochromic effects (Figure 1h; Figure S64, Supporting Information). To sum up, by virtue of the acceptor engineering strategy, the compounds in the 2A system with the molecular structure of D’−D−A−A−D−D’ demonstrated the transformation of ACQ-to-AIE, which nourished the desired AIE property of 2TT-2BBTD ultimately.

2.2. Theoretical Calculation

To obtain more in-depth insights into the opposite photophysical properties of the 1A and 2A systems, the geometries of all molecules were optimized using the density functional theory (DFT) method. As illustrated in Figure 2a–h, the compounds in 1A and 2A systems showed the dihedral angles of 40–48° between the twisted TPA and alkyl thiophene units. Clearly, the 1A system demonstrated gradually decreased dihedral angles of the thiophene-acceptor-thiophene core (from 6.7° in 2TT-BTD to 1.1° in 2TT-BBTD), which stemmed from the orderly attenuated repulsion effect between the hexyl chain in the thiophene ring and the adjacent acceptor unit. In this context, together with the enhanced D−A and TICT effects, the more coplanar backbone would strengthen the intermolecular interactions and nonradiative decay to produce the ACQ effect.[32,34] In the case of 2A system, interestingly, the elevated dihedral angles of two acceptors (from 32° in 2TT-2BTD to 55° in 2TT-2BBTD) were triggered by well-
designed acceptor engineering. Thus, it would be reasonable that the distorted backbone of the thiophene-acceptor-acceptor-thiophene moiety played a key role in realizing the transformation of ACQ to AIE in the 2A system. The ACQ effect could be suppressed effectively by employing more electrophilic and twisted dual-acceptor. Particularly, a more twisted conformation could provide a more flexible environment and higher degree of freedom for intramolecular motions, thus leading to an extremely low PL intensity \(I_0\) of 2TT-2BBTD in the solution state. Upon the aggregate formation, the intramolecular motions of 2TT-2BBTD were restricted to some extent, and gradually enhanced emission intensities were monitored due to the RIM mechanism, to give rise to the increased \(I/I_0\) values. Taken together, the prominent AIE activity of 2TT-2BBTD prevailed over the dark TICT effect plus the weak intermolecular interactions in the aggregate state.

Then, the frontier molecular orbitals of all compounds were calculated based on the optimized ground-state geometries. As demonstrated in Figure S65 (Supporting Information), the HOMO orbitals delocalized across the whole molecular skeleton, while the LUMO was mainly distributed on the central core of the acceptor and thiophene units. The energy gaps between HOMO and LUMO were determined to be 2.28, 2.05, 1.53, and 1.26 eV for compounds in the 1A system, respectively (Figure 2i). Similarly, a decreased trend of 2.18, 1.98, 1.64, and 1.29 eV was observed for compounds in the 2A system (Figure 2j). These orderly narrow energy gaps were consistent with the red-shifted absorption and emission wavelengths of the compounds in 1A and 2A systems along with the increased electron-withdrawing ability of acceptors. Notably, the energy levels of LUMO in the 1A or 2A system exhibited an obviously downward trajectory along with the slightly upraised energy levels of HOMO. This confirmed that the untraversed acceptor engineering could be a credible tactic to construct luminescent materials with desirable absorption and emission wavelengths. In addition, the reorganization energies \(\lambda\) of these compounds were calculated, which could quantitatively describe the inherent geometry changes and intramolecular motions upon photoexcitation.\[^{39}\] Moreover, the contribution from the dihedral angle (at low-frequency region with wavenumbers \(<500\) cm\(^{-1}\)) to the total \(\lambda\) was prone to reflect the intramolecular twisting motions to nonradiative decay.\[^{40}\] Interestingly, the introduction of one more acceptor in the 2A system endowed a higher contribution of dihedral angle to the total \(\lambda\) than that of the counterparts in the 1A system (Figure 2k; Figure S66, Supporting Information). These outcomes indicated that the compounds in 2A system exhibited a higher proportion of nonradiative decay and potential for photothermal conversion than those in the 1A system.
Figure 2. Theoretical calculation analysis. a)–h) Optimized ground-state geometries of all compounds in the 1A and 2A systems. i), j) Calculated energy levels of all compounds in the i) 1A system and j) 2A system. k) Plots of the reorganization energy contributed by the dihedral angle change between the ground-state and excited-state geometries. l) The schematic definition of dominant dihedral angle (see the red arrow) between the two BBTD groups of 2TT-2BBTD studied by molecular dynamics simulation. m) Molecular dynamics simulation snapshot of 2TT-2BBTD aggregates in THF/water mixture (10/90, v/v). The innermost molecule was given in space-filling mode and the other molecules were shown in licorice style. n) Dihedral angle (between the BBTD and BBTD group) distribution of a single molecule (THF solution) and the innermost molecule of the aggregate.

To further analyze the superiorities of the twisted dual-acceptor strategy, molecular dynamics (MD) simulations were conducted by selecting 2TT-BBTD and 2TT-2BBTD in the 1A and 2A systems as representative members. Besides, we mainly focused on the dihedral angle changes
of the thiophene-BBTD unit in 2TT-BBTD and BBTD-BBTD core in 2TT-2BBTD under different states, respectively. Thus, the mentioned dihedral angle distributions of thiophene-BBTD and BBTD-BBTD units were studied. Consistent with the results of DFT calculation, the distorted acceptor-acceptor moiety acted a key role in realizing the transformation of ACQ-to-AIE from 2TT-BBTD to 2TT-2BBTD, again indicating the reliable tactic of dual-acceptor engineering for constructing NIR-II AIEgens (Figure 2l,m; Figures S67–68, Supporting Information). In addition, the intramolecular motions of 2TT-2BBTD in the solution and aggregate states were investigated. As illustrated in Figure 2n, compared with the widely distributed dihedral angle of 2TT-2BBTD in the isolated state, a narrower distribution of BBTD-BBTD moiety was observed for the innermost molecule in the aggregate, suggesting the effect of RIM.[41] Interestingly, the BBTD-BBTD unit adopted a obviously distorted conformation in the single-molecule state and even in the aggregate state. In this regard, the intramolecular motions of 2TT-2BBTD in the aggregates were partially restricted but still active, thus providing a promising multifunctional system for phototheranostics.

2.3. Preparation and Characterization of Nanoparticles

Considering the eminent AIE feature and emission wavelength located in the NIR-II region, the hydrophobic 2TT-2BBTD was encapsulated into NPs using an amphiphilic co-polymer called DSPE-mPEG2000 (Scheme 1b). Hence, the prepared NPs were endowed with improved biocompatibility and water dispersity. Dynamic light scattering (DLS) analysis revealed that the average hydrodynamic diameter of 2TT-2BBTD NPs was about 66.76 nm with a desired polydispersity index of 0.165 (Figure 3a). Furthermore, transmission electron microscope (TEM) measurement clarified that these NPs hold a spherically shaped morphology with a particle size of approximately 40 nm. Noteworthy, 2TT-2BBTD NPs displayed excellent storage stability both in deionized water and PBS buffer under ambient condition for at least four weeks (Figure S69a, Supporting Information). The particle size of 2TT-2BBTD NPs also maintained a good colloidal stability in PBS + FBS (10%) solution over 4 days (Figure S69b, Supporting Information). In view of the fact that 2TT-DPTDQ, 2TT-2DPTDQ and 2TT-BBTD also hold the potential for intravital applications due to their NIR emission, their corresponding NPs were prepared via the same nanoprecipitation method with average sizes of 64.17, 72.38 and 119.20 nm, respectively (Figure S70, Supporting Information). To investigate their ability for NIR FLI, the relative quantum yields (QYs) of 2TT-DPTDQ, 2TT-2DPTDQ, 2TT-BBTD and 2TT-2BBTD in both single-molecule state (THF solution) and NPs state were determined. By using indocyanine green (ICG) as a reference, the QYs of 2TT-DPTDQ and 2TT-DPTDQ
NPs were determined to be 1.18% and 0.31%, respectively (Figure S71a–i, Supporting Information). The QY of 2TT-2DPTDQ NPs (1.59%) was slightly higher than that of 2TT-2DPTDQ (1.23%) in THF solution (Figure S71j–o, Supporting Information). Meanwhile, the QYs of 2TT-BBTD, 2TT-2BBTD and their corresponding NPs in the NIR-II region were also measured by adopting IR-26 as a reference (Figure S72, Supporting Information). Obviously, the QY of 2TT-BBTD (3.14%) in THF solution sharply declined to 0.19% in NPs. As expected, the QY of 2TT-2BBTD NPs enhanced by more than thrice to 0.25%, compared with the QY (0.08%) in the solution state. These results again suggested the inherent AIE nature of 2TT-2DPTDQ and 2TT-2BBTD, but both 2TT-DPTDQ and 2TT-BBTD clearly showcased the ACQ characteristics, respectively.

**Figure 3.** Characterization of 2TT-2BBTD NPs. a) DLS analysis of 2TT-2BBTD NPs. Inset: TEM image of 2TT-2BBTD NPs. b) The absorption and emission spectra of 2TT-2BBTD NPs in aqueous solution. c) ROS generation of 2TT-2BBTD NPs (1 μm 2TT-2BBTD) upon 808 nm laser irradiation. d), e) Temperature elevation of 2TT-2BBTD NPs in aqueous solution with different concentrations and at different power densities under 808 nm laser irradiation, respectively. f) Photothermal stability of 2TT-2BBTD NPs (50 μm 2TT-2BBTD) and ICG (50 μm) in aqueous solution during five heating-cooling cycles under 808 nm laser irradiation (0.8 W cm\(^{-2}\)). g) The linear fitting of time from the cooling period versus the negative natural logarithm of driving force temperature for 2TT-2BBTD NPs. h) Temperature profile of 2TT-BBTD NPs (50 μm 2TT-BBTD), 2TT-2BBTD NPs (50 μm 2TT-2BBTD) and pure water irradiated by 808 nm laser (0.8 W cm\(^{-2}\)) for 8 min.
Due to the excellent properties, 2TT-2BBTD NPs were selected for further photophysical study and bioapplications. The UV-Vis-NIR absorption and PL spectra disclosed that 2TT-2BBTD NPs showed absorption and emission maxima at 799 and 1065 nm, respectively (Figure 3b). Notably, the spectral tail of 2TT-2BBTD NPs was observed to extend to 1400 nm, providing a superiority for in vivo applications due to the deep tissue penetration of NIR-II emission. Furthermore, the ROS generation and photothermal conversion capacities of 2TT-2BBTD NPs were evaluated. As illustrated in Figure 3c and Figure S73 (Supporting Information), along with the continuous laser irradiation, the PL signal of dichlorofluorescein (DCFH) at 525 nm was strengthened rapidly in the presence of 2TT-2BBTD NPs, while negligible elevation in fluorescence intensity was monitored for the solution of DCFH alone. This nearly 10-fold higher fluorescence intensity of DCFH than the initial value after 9 min laser exposure manifested the favorable ROS generation ability of 2TT-2BBTD NPs. To further distinguished the type of ROS, 9,10-anthracenediylbis(methylene)-dimalonic acid (ABDA), hydroxyphenyl fluorescein (HPF) and dihydrorhodamine123 (DHR123) as indicators were utilized. As displayed in Figure S74 (Supporting Information), 2TT-2BBTD NPs were prone to generate O$_2^{-}$ through the type I pathway. Additionally, it was also proved that 2TT-2BBTD NPs possessed the characteristics of NPs concentration- and laser power density-dependent temperature elevation (Figure 3d,e). These results suggested that the heat generation from 2TT-2BBTD NPs in aqueous solution reached to the plateau at 66 °C at a low concentration of 50 μm under 808 nm laser irradiation (0.8 W cm$^{-2}$) for 8 min, demonstrating the considerable photothermal conversation performance. After five consecutive cycles of the heating-cooling process, 2TT-2BBTD NPs also retained splendid and unaltered photothermal generation ability (Figure 3f). In sharp contrast, the photothermal stability of ICG showed a poor performance and decreased by nearly 50% under the same conditions. Besides, the photostability of 2TT-2BBTD NPs is also far superior to ICG under continuous 808 nm laser illumination (Figure S75, Supporting Information). Thereupon, the photothermal conversion efficiency (PCE) of 2TT-2BBTD NPs was determined to be 41.7% (Figure 3g). As expected, 2TT-2BBTD NPs revealed superior heat generation ability to 2TT-BBTD NPs under the same circumstances (Figure 3h; Figure S76, Supporting Information). It could be ascribed to the introduction of one more acceptor into the 1A system, which could result in intensive intramolecular twisting motions on account of the increased contribution of dihedral angle change to the total $\lambda$ (Figure 2k).
2.4. In Vitro Experiments

Encouraged by the great potential of 2TT-2BBTD NPs in multimodal phototheranostics, the synergistic phototherapeutic efficacy was firstly assessed at the cellular level. For easy observation, the fluorescein isothiocyanate (FITC)-labeled 2TT-2BBTD NPs, abbreviated as FITC-2TT-2BBTD NPs were prepared. Imaging using confocal laser scanning microscopy (CLSM) was then carried out to investigate the intracellular uptake behavior. As shown in Figure 4a, an obvious co-localization of fluorescence signal between FITC-2TT-2BBTD NPs and LysoTracker Red was monitored, suggesting that 2TT-2BBTD NPs entered the 4T1 cells possibly through a lysosome-mediated endocytosis pathway. Next, CCK-8 assay was performed to evaluate the tumoricidal effect of 2TT-2BBTD NPs in vitro (Figure 4b). Obviously, negligible toxicity to 4T1 cancer cells was detected even at a high concentration of 2TT-2BBTD NPs under dark condition, revealing its desired biocompatibility. After NIR laser irradiation at 808 nm and 0.6 W cm\(^{-2}\), more than 70% cell viability was still detected at a dye concentration was lower than 10 μm, while almost all the 4T1 cells were deactivated by increasing the dye concentration to 50 μm. Besides, the half-maximal inhibitory concentration (IC\(_{50}\)) of 2TT-2BBTD NPs to 4T1 cells was determined to be 13.6 μm based on 2TT-2BBTD, demonstrating its excellent cytotoxic activity upon laser irradiation. The phototherapeutic effect of 2TT-2BBTD NPs was further verified by the co-stained approach using fluorescein diacetate (FDA) and propidium iodide (PI), where the green and red fluorescence were responsible for live and dead cells, respectively. The staining results displayed that a strong green fluorescence signal was obtained in the groups of PBS, PBS + laser (L), and 2TT-2BBTD NPs (dark condition), whereas nearly all of the 2TT-2BBTD NPs-loaded 4T1 cells entered the death state upon laser irradiation (Figure 4c). Furthermore, flow cytometric analysis was also conducted by employing Annexin V-FITC detection assay to elucidate the cell death mechanism of cells. As illustrated in Figure 4d, 56.0% of 4T1 cells were involved in the apoptosis pathway after treatment with 2TT-2BBTD NPs plus laser irradiation (2TT-2BBTD NPs + L), and 20.4% of cancer cells were related to necrosis. However, the unobtrusive apoptosis or necrosis was surveyed in other control groups. Taken together, the inappreciable side effect of the operational laser power density (808 nm, 0.6 W cm\(^{-2}\)), outstanding biocompatibility, and photoinduced cytotoxic ability of 2TT-2BBTD NPs are incontrovertible.
Figure 4. In vitro phototherapy application. a) CLSM images of 4T1 cells after being stained with Hoechst 33342, FITC-2TT-2BBTD NPs, and LysoTracker Red. b) Cell viability of 4T1 cells after being treated with different concentrations of 2TT-2BBTD NPs under dark or 808 nm laser irradiation (0.6 W cm\(^{-2}\)). c) Live/dead cell staining of 4T1 cells after various treatments. The green and red fluorescence mean live and dead cells, respectively. d) Flow cytometric analysis of 4T1 cells treated with PBS and 2TT-2BBTD NPs (50 μM 2TT-2BBTD) with or without laser irradiation (808 nm, 0.6 W cm\(^{-2}\)).

2.5. In vivo NIR-II Fluorescence Imaging and Phototheranostics

Considering the excellent photophysical properties of 2TT-2BBTD NPs, the performance of the NPs for NIR-II angiography in vivo was evaluated firstly. Clearly, as shown in Figure 5a, the vascular structures of the whole body of the mouse were visualized under a 1300 nm long-pass (LP) filter after intravenous injection of 2TT-2BBTD NPs (laser: 808 nm, 30 mW cm\(^{-2}\); exposure time: 500 ms). To further investigate and optimize the imaging quality of 2TT-2BBTD NPs, the blood vessels in the hindlimb of the mouse was imaged under different wavelengths of LP filters. As expected, the imaging clarity was gradually improved along with the range of LP filter extended to 1350 nm (laser: 808 nm, 30 mW cm\(^{-2}\); exposure time: 750 ms), thus providing the highest signal-to-background ratio (SBR) of 3.74 and the smallest full width at half-maxima (FWHM) of 0.29 mm (Figure 5b,c; Figure S77, Supporting Information). Subsequently, the dorsal body of the mouse was also outlined clearly under a 1350 nm LP filter after administration of 2TT-2BBTD NPs into the bloodstream (Figure 5d. laser: 808 nm, 10
mW cm\(^{-2}\); exposure time: 200 ms). In addition, as displayed in Figure 5e and Figure S78 (Supporting Information), bright NIR-II fluorescence signals could also be detected in the lymph nodes of the mouse at 30 min post-treatment under a 1150 nm LP filter (laser: 808 nm, 20 mW cm\(^{-2}\); exposure time: 500 ms).

**Figure 5.** In vivo fluorescence imaging performance of 2TT-2BBTD NPs (\(\lambda_{\text{ex}} = 808 \text{ nm}\)). a) NIR-II fluorescence imaging of whole body (supine position), b) hindlimb and d) dorsal vessels of BALB/c nude mice after the intravenous injection of 300 µL 2TT-2BBTD NPs. The fluorescence images were taken after the injection for 10 min. c) Cross-sectional fluorescence intensity profile in blood vessel along the red line shown in b) under a 1350 nm LP filter. e) NIR-II fluorescence imaging of lymph nodes after administration of 30 µL 2TT-2BBTD NPs from footpad of the mouse. The fluorescence image was taken after the injection for 30 min.

Then, inspired by the excellent in vitro cytocidal efficacy and in vivo NIR-II imaging ability, the multimodal imaging-guided phototherapy performance of 2TT-2BBTD NPs was investigated based on an orthotopic 4T1 breast tumor mouse model. Preliminarily, the NIR-II FLI and accumulation ability of 2TT-2BBTD NPs in the tumor site were estimated. Before injection of 2TT-2BBTD NPs, the mice exhibited extremely feeble autofluorescence interference upon using a LP filter of 1000 nm, which could provide a minimal background signal. After intravenous injection of 2TT-2BBTD NPs, the fluorescence signal gradually arose and increased in the tumor region over time, suggesting a desirable tumor-site accumulation ability (**Figure 6a**). Particularly, the fluorescence intensity reached its maximum at 12h post-injection and subsequently became weaker due to the metabolism (**Figure 6a; Figure S79a**,
Supporting Information). In order to quantitatively analyze the bio-distribution of 2TT-2BBTD NPs after 24 h injection, the tumor tissue and major organs were isolated. As illustrated in Figure S79b–c (Supporting Information), it was affirmed that a distinct fluorescent signal was found in the tumor region, while the fluorescent emission in liver and spleen was also visualized. Consistent with the results of FLI, the photoacoustic signal also presented a maximum level at 12 h and maintained for 24 h after tail vein injection (Figure 6b; Figure S80, Supporting Information). There is no doubt that the synergism of FLI and photoacoustic imaging (PAI) would be a powerful weapon to visualize and diagnose tumor. Considering that 2TT-2BBTD NPs possessed a substantial heat generation capacity, in vivo photothermal imaging (PTI) was conducted by using 808 nm laser (0.6 W cm$^{-2}$) as an illumination source after 12 h intravenous administration of the NPs. According to the infrared thermal images shown in Figure 6c, a rapid temperature increment from 32.8 to 62.3 °C in the tumor site only within 2 min irradiation was recorded, and a temperature plateau of about 65.5 °C was observed after 4 min irradiation. In contrast, the saline-treated mouse indicated negligible temperature elevation under the same circumstance, again demonstrating the reliable photothermal conversion performance of 2TT-2BBTD NPs (Figure S81, Supporting Information). Collectively, these results strongly manifested that 2TT-2BBTD NPs possessing NIR-II FLI, PAI, and PTI functions would be an impressive diagnostic agent for precise cancer therapy.

**Figure 6.** The investigation of in vivo multimodal phototheranostics and therapeutic mechanism. a) NIR-II fluorescence and b) photoacoustic imaging of orthotopic 4T1 breast tumor-bearing BALB/c mice at different time after intravenous injection of 2TT-2BBTD NPs.
(λ_{ex} = 808 \text{ nm}, 200 \mu\text{L}), \text{ respectively. c)} \text{ Photothermal imaging of 4T1 tumor-bearing mice treated with 2TT-2BBTD NPs through intravenous injection, followed (12 h later) by 808 nm laser irradiation (0.6 W cm}^{-2} \text{) for 2, 4, 6, and 8 min, respectively. d)} \text{ Time-dependent tumor growth curves of 4T1 tumor-bearing mice (n = 5) after various treatments, respectively. e)} \text{ H&E, TUNEL, CD31, and Ki67 staining analyses of tumor tissues under various treatments. AIE NPs = 2TT-2BBTD NPs. f)} \text{ Body weight changes of 4T1 tumor-bearing mice (n = 5) during the treatments.}

Later on, the in vivo antitumor efficacy of 2TT-2BBTD NPs was assessed by randomly dividing the orthotopic 4T1 breast tumor (~100 mm³)-bearing mice into four groups. Upon merely a single intravenous injection of the NPs for 12 h and followed once laser exposure (808 nm, 0.6 W cm}^{-2}, 10 \text{ min}), the growth of the solid neoplasms was thoroughly suppressed and almost eliminated at day 3 in the phototherapeutic group of 2TT-2BBTD NPs plus laser irradiation (Figure 6d). After 15-day treatment, full tumor eradication and undetected recurrence were realized, where the treated mice were only left behind with a scar (Figure 6d; Figure S82, Supporting Information). The tumor volumes in other three groups (saline, saline plus laser irradiation, and 2TT-2BBTD NPs alone) grew rapidly and delivered no inhibition effect on the tumor growth (Figure 6d). On the other side, the histological and immunohistochemical analyses of the tumor slices were also adopted to verify the in vivo therapeutic mechanism of 2TT-2BBTD NPs. As depicted in Figure 6e, the hematoxylin and eosin (H&E) assay exhibited that extensive destruction of tumor tissues was confirmed in the therapeutic group of 2TT-2BBTD NPs plus laser irradiation. In contrast, the tumor cells still remained a vibrant and dense arrangement in the control groups. The denouement of severe apoptosis or necrosis of the treated 4T1 cells (underwent PDT plus PTT) was proved by the TUNEL staining. Meanwhile, after being treated with 2TT-2BBTD NPs plus laser exposure, the inhibited vessel formation and suppressed cell proliferation of the tumor region were further convinced according to the CD31 and Ki67 staining (Figure 6e). These outcomes solidly manifested that the synergistic PDT and PTT could afford the exciting therapeutic efficiency of 2TT-2BBTD NPs in vivo.

In addition, in vivo biosafety of 2TT-2BBTD NPs was also systematically evaluated. During the whole therapeutic course, negligible mouse body weight losses were observed along with similar growth tendencies in these four groups (Figure 6f). After accomplishing the various treatments at day 15, the mice were sacrificed and analyzed. Results from H&E staining of major organs revealed that the heart, liver, spleen, lung, and kidney had no pathological
anomalies or inflammatory lesions (Figure S83, Supporting Information). In contrast to the seriously abnormal routine blood indexes in the other control groups, all the measured parameters of the mice in the group of 2TT-2BBTD NPs plus laser irradiation belonged to normal ranges (Table S1, Supporting Information). Moreover, the blood biochemistry test of hepatic and renal function indicators displayed the comparable results in these four groups, respectively, and no significant statistical difference was observed (Figure S84, Supporting Information). Overall, the above-mentioned results exemplified that the acceptor engineering and “more” strategy could provide the versatile phototheranostics system for cancer elimination accompanying with desirable biocompatibility and biosecurity.

3. Conclusion

In this work, we tactfully designed and synthesized four pairs of luminogens with one electron acceptor (1A system) and dual-connected electron acceptors (2A system) based on the strategy of acceptor engineering. Among them, the red-shifted absorption and emission wavelengths were accomplished through modulating the acceptor with incremental electron-withdrawing capability. Because of the gradually enhanced TICT effect and planar π-conjugated structure, the features of these compounds in 1A system showed a change from AIE to ACQ. Excitingly, the 2A system exhibited the transformation of ACQ to AIE behavior, where more distorted molecular conformation (especially for the twist between two acceptor units) and RIM mechanism played critical roles. This clear distinction suggested the effectiveness of the acceptor engineering and “more” strategy in guaranteeing AIE property. Eminently, among all the compounds, 2TT-2BBTD exhibited the best AIE feature, the highest $\varepsilon$, and the satisfactory NIR-II emission. In addition, 2TT-2BBTD NPs were proven to show favorable ROS generation and appreciable photothermal conversion capacities. Accordingly, the trimodal NIR-II FLI/PAI/PTI-guided PDT and PTT for eradicating the orthotopic 4T1 mouse breast tumor was successfully realized by employing 2TT-2BBTD NPs as a phototheranostic agent. Beyond all doubt, this strategy not only offers a spiffy and robust route to improve $\varepsilon$, absorption/emission maximum, AIE activity and photo-driven heat generation ability, but also highlights the success of the proposed molecular design concept of “more is better”. By employing this distinctive paradigm of acceptor engineering, it is anticipated that a series of high-performance and novel all-round phototheranostic agents would be boosted for potent bioimaging and cancer therapy.
Supporting Information
Supporting Information is available from the corresponding authors upon reasonable request.

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Conflict of Interest
The authors declare no conflict of interest.

References