

Ultrastable TPE-DNA nanoaggregates enable bright NIR-II fluorescence vascular imaging

Min Zhou^{1,§}, Ying Wang^{1,§}, Chu Zhang¹, Zichen Qin¹, Zicong Liang¹, Zhiguo Fang^{2,†}, Yeteng Zhong², Yu Xiang¹  , and Aijun Tong¹  

¹ Department of Chemistry, Tsinghua University, Beijing 100084, China

² National Center for Nanoscience and Technology, Beijing 100190, China

[†] Present address: College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, China

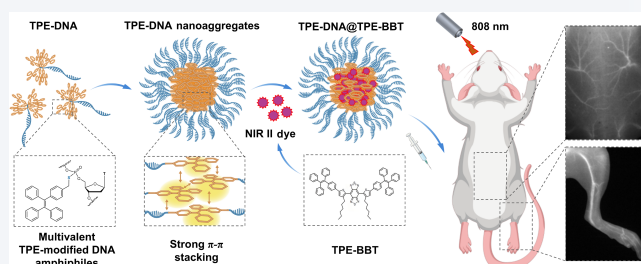
[§] Min Zhou and Ying Wang contributed equally to this work.



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ABSTRACT: The development of bright and stable near-infrared II (NIR-II) fluorescent probes is crucial for deep-tissue vascular imaging but remains challenging due to dye leakage and poor carrier stability. Here, we report a multivalent tetraphenylethylene (TPE) modification strategy to construct DNA amphiphiles that self-assemble into ultrastable nanoaggregates. Unlike conventional C18-DNA or cholesterol-DNA assemblies that readily dissociate, our 7-TPE-DNA nanoaggregates remain intact even under denaturing conditions, owing to cooperative π - π stacking among multiple TPE units. The same hydrophobic core efficiently loads the aggregation-induced emission (AIE)-active NIR-II dye TPE-benzobisthiadiazole (BBT), affording bright nanoprobes with outstanding resistance to dye leakage. Following intravenous injection into living mice, the probe enables high-resolution visualization of femoral arteries and subcutaneous capillary networks with markedly improved signal-to-background ratios compared to polyethylene glycol (PEG)- or F127-based controls. This work establishes a multivalent AIE engineering strategy for DNA amphiphiles, providing a robust and versatile nanoplatform for advanced NIR-II bioimaging applications.

KEYWORDS: DNA amphiphiles, multivalent aggregation-induced emission (AIE) engineering, near-infrared II (NIR-II) imaging, π - π stacking



1 Introduction

Fluorescence imaging in the second near-infrared window (near-infrared II (NIR-II), 1000–1700 nm) has emerged as a powerful technique for deep-tissue visualization because of reduced light scattering, negligible autofluorescence, and excellent penetration depth compared to the visible and NIR-I regions [1–3]. This optical advantage enables non-invasive angiography, tumor margin delineation, and image-guided surgery with high spatiotemporal resolution [4–6]. Among various NIR-II probes, organic fluorophores are particularly attractive owing to their well-defined chemical structures, good biocompatibility, and facile excretion

profiles [7, 8]. However, most organic NIR-II dyes are highly hydrophobic and suffer from aggregation-caused quenching (ACQ) when encapsulated in conventional nanocarriers [9, 10].

The emergence of aggregation-induced emission (AIE) has provided a paradigm shift. AIE luminogens (AIEgens) are weakly emissive in solution but become strongly fluorescent upon aggregation due to restriction of intramolecular motion (RIM) [11–13]. This unique property makes AIEgens ideal for constructing bright nanoprobes without the ACQ drawback. Representative AIEgens such as tetraphenylethylene (TPE) derivatives have been widely used in biosensing and bioimaging [14, 15]. In the NIR-II region, AIEgens with donor-acceptor-donor (D-A-D) structures, exemplified by TPE-BBT, exhibit high quantum yields and excellent photostability, enabling deep-tissue imaging in small animals and even non-human primates [16, 17].

Despite these advances, the delivery of hydrophobic AIE-active NIR-II dyes requires nanocarriers that can stably encapsulate the dye while maintaining good aqueous dispersibility. Conventional

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✉ Address correspondence to Aijun Tong, tongaj@mail.tsinghua.edu.cn; Yu Xiang, xiang-yu@mail.tsinghua.edu.cn



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strategies rely on amphiphilic polymers such as PEGylated or Pluronic F127 lipids to physically entrap the dye via nano-precipitation [18–20]. However, these carriers often suffer from limited structural stability: the hydrophobic cores are held together by relatively weak van der Waals forces, leading to premature dye leakage upon dilution, protein adsorption, or contact with biological lipids [21, 22]. Dye leakage not only reduces imaging contrast but also increases non-specific background signals, hampering high-resolution vascular imaging.

DNA amphiphiles, in which hydrophilic DNA strands are covalently linked to hydrophobic moieties, offer a promising alternative [23, 24]. They can self-assemble into micelle-like nanoaggregates with a hydrophobic core and a DNA corona, and the DNA shell provides programmable recognition sites for functionalization [25–27]. Recent studies have exploited nucleic-acid-based nanostructures for NIR-II bioimaging, including programmable assembly for cancer theranostics [28], deep-tissue imaging with fluorescent DNA frameworks [29], and *in vivo* microRNA detection using DNA-based NIR-II probes [30]. At the level of DNA-amphiphile carrier design, classic hydrophobic tails include cholesterol and C18 alkyl chains [31, 32]. Nevertheless, these single-tail modifications rely on isolated hydrophobic interactions and are prone to disassembly under physiological or denaturing conditions, which limits their *in vivo* stability [33]. Therefore, a DNA-based nanoplatform that integrates programmable DNA functionality, ultrastable dye encapsulation, high NIR-II brightness, and facile modular modification remains highly desirable.

Herein, we report a multivalent TPE modification strategy to construct ultrastable DNA nanoaggregates. By introducing multiple phosphorothioate (PS) sites on an oligonucleotide and coupling them with TPE-Br, we obtained TPE-DNA conjugates with precise stoichiometry. The resulting 7-TPE-DNA spontaneously formed ~17.5 nm spherical nanoaggregates driven by strong π - π stacking of TPE units (Scheme 1). Unlike conventional C18-DNA or cholesterol-DNA, 7-TPE-DNA nanoaggregates remained intact under denaturing gel electrophoresis conditions, including 15% polyacrylamide gel electrophoresis (PAGE) containing 8 M urea and sodium dodecyl sulfate-PAGE (SDS-PAGE), demonstrating exceptional structural stability. TPE-BBT, an AIE-active NIR-II dye, was then loaded to the nanoaggregates to afford 7-TPE-DNA@TPE-BBT nanoprobles, which exhibited bright NIR-II emission (quantum yield 2.45%) and superior resistance to dye leakage compared to PEG or F127 based polymer controls. In living mice,

tail-vein injection of 7-TPE-DNA@TPE-BBT enabled high-resolution visualization of the femoral artery and subcutaneous capillary networks with significantly improved signal-to-background ratio. This work establishes a highly stable and modular DNA nanoaggregate platform for advanced NIR-II bioimaging.

2 Experimental

2.1 Materials

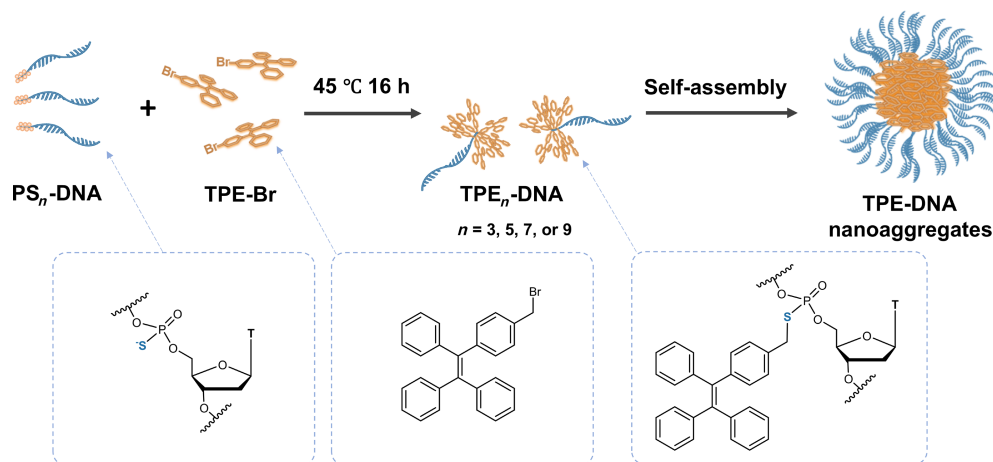
DNA oligonucleotides were purchased from Beijing Hema Biotechnology Co., Ltd. N,N-Dimethylformamide (DMF, $\geq 99.9\%$) and 1-[(4-bromomethyl)phenyl]-1,2,2-triphenylethylene (97%) were obtained from Hangzhou Aladdin and Energy Chemical, respectively. *n*-Butanol, ethyl acetate, acrylamide (30%), and urea were from Shanghai Meryer, Beijing Solarbio, and Beijing Lanyi. Cell counting kit-8 (CCK-8) was from Beyotime. PEG-2000, Pluronic F127, IR-1061, IR-1048, and tetrakis(triphenylphosphine) palladium(0) ($\text{Pd}(\text{PPh}_3)_4$) (99%) were from Sigma-Aldrich. FM-1210 and TTQ-F-NH₂ were from Beijing J&K and Xi'an Ruixi, respectively. All chemicals and materials were used as received without further purification.

2.2 Instruments

Polymerase chain reaction (PCR) was performed on a ProFlex System (Thermo Fisher). Ultraviolet-visible (UV-Vis) and fluorescence spectra were recorded on JASCO V-550 and FP-8600, respectively. Gel electrophoresis and imaging used Bio-Rad PowerPac and ChemiDoc. Electrospray ionization-mass spectrometry (ESI-MS) was on a Thermo Finnigan LCQ Deca XP. The NIR-II fluorescence intensity was measured on Edinburgh FLS900 and FLS920. Transmission electron microscopy (TEM) was on a Hitachi H-7650B. Dynamic light scattering (DLS) and zeta potential used a Horiba SZ-100. Absorbance and cell viability were read on a Multiskan SkyHigh microplate reader. Confocal imaging used an Olympus FV3000RS. *In vivo* NIR-II imaging used a custom system at the National Center for Nanoscience and Technology, China.

2.3 Synthesis and characterization of TPE-DNA nanoaggregates

We designed a DNA oligonucleotide sequence (Table S1 in the Electronic Supplementary Material (ESM)) with two functional



Scheme 1 Synthesis of TPE-DNA nanoaggregates ($n = 3, 5, 7$, or 9).

regions. The 5'-end contained a variable number of PS modification sites for multivalent TPE conjugation, whereas the 3'-terminal hydrophilic DNA segment was retained as a programmable anchoring sequence for subsequent multifunctional modification via Watson-Crick base pairing. This terminal sequence can, in principle, hybridize with complementary DNA strands bearing targeting ligands, aptamers, peptides, therapeutic modules, or additional imaging units. 1-[(4-Bromomethyl)phenyl]-1,2,2-triphenylethylene (TPE-Br, 20 mM) was dissolved in anhydrous DMF. PS-DNA (20 μM) was dissolved in nuclease-free water. The two solutions were mixed in 0.01 M phosphate buffer (pH 6.0) and incubated in a PCR thermal cycler at 45 °C for 16 h. The mixture was extracted with ethyl acetate and *n*-butanol (three times each) to remove excess TPE-Br. The aqueous phase was then purified by repeated centrifugation (100 kDa Amicon Ultra) and washed with ultrapure water. The resulting TPE-DNA nanoaggregates were stored at 4 °C.

The successful conjugation was confirmed by UV-Vis absorption (characteristic TPE peak at ~ 320 nm) and fluorescence spectroscopy. The number of TPE units per DNA strand was determined by ESI-MS. Self-assembly and stability were evaluated by 15% PAGE containing 8 M urea and 12% SDS-PAGE, which monitored migration shifts and nanoaggregate formation. The morphology of the resulting 7-TPE-DNA nanoaggregates was visualized by TEM (80 kV), and their hydrodynamic size and dispersity were measured by DLS after dilution in water.

2.4 Design and characterization of C18-DNA and cholesterol-DNA

To assess the self-assembly stability of 7-TPE-DNA nanoaggregates, we employed two well-established hydrophobic modification strategies commonly used in DNA nanotechnology as controls: octadecyl (C18) and cholesterol functionalization. Specifically, a DNA oligonucleotide identical in sequence to that of 7-TPE-DNA (Table S2 in the ESM) was synthesized and site-specifically conjugated at the 5'-terminus with either a C18 alkyl chain or a cholesterol group, yielding structurally matched C18-DNA and cholesterol-DNA nanoparticles. These controls were chosen to represent conventional single-tail hydrophobic-anchor strategies rather than to match the exact number of hydrophobic groups in multivalent TPE-DNA. This comparison allowed us to evaluate whether cooperative interactions among multiple aromatic TPE units could provide higher structural stability than classical C18 or cholesterol anchors. The structural stability of all three nano-assemblies was comparatively evaluated by 15% PAGE containing 8 M urea and 15% SDS-PAGE.

2.5 Synthesis and characterization of 7-TPE-DNA@NIR-II dye nanoprobes

7-TPE-DNA nanoaggregates (40 μM in 10 mM phosphate-buffered saline (PBS), pH 7.0) were mixed with a solution of NIR-II dye (TPE-BBT, FM-1210, TTQ-F-NH₂, IR-1061, or IR-1048) in tetrahydrofuran (THF) (1 mg·mL⁻¹). The mixture was annealed in a PCR thermocycler with a gradient cooling program from 95 to 25 °C at a stepwise rate of 5 °C per 2 min, following an initial hold at 95 °C for 5 min. Unbound dye and residual THF were removed by sequential extraction with ethyl acetate and *n*-butanol (three times each), followed by purification using 100 kDa Amicon Ultra filters. The final nanoprobe dispersions were stored at 4 °C.

To further optimize the loading conditions, the concentration of

7-TPE-DNA was fixed, while different concentrations of TPE-BBT (0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mg·mL⁻¹) were added for co-assembly. The optimal feeding ratio was determined by evaluating the fluorescence intensity. The morphology of the fluorescent nanoprobe was observed by TEM. The probe size distribution and hydrodynamic diameter were determined by DLS at room temperature. The NIR-II fluorescence intensity was measured using an Edinburgh FLS900 fluorescence spectrometer ($\lambda_{\text{ex}} = 808$ nm, $\lambda_{\text{em}} = 1000\text{--}1300$ nm). The absolute photoluminescence quantum yield (PLQY) of 7-TPE-DNA@TPE-BBT in aqueous solution was measured using an integrating sphere method on an Edinburgh Instruments FLS920 fluorescence spectrometer equipped with an NIR-sensitive photomultiplier tube. All measurements were performed in 1 cm quartz cuvettes at 808 nm. The absolute PLQY of 7-TPE-DNA@TPE-BBT in PBS (10 mM, pH 7.4) was determined to be 2.45%.

2.6 Synthesis of PEG@TPE-BBT and F127@TPE-BBT nanoprobes

To evaluate the potential of 7-TPE-DNA nanoaggregates as a functional carrier for *in vivo* NIR-II imaging, parallel comparisons were performed against two widely used commercial amphiphilic systems: methoxy-poly(ethylene glycol) (mPEG₂₀₀₀) and Pluronic F127. To ensure a fair and meaningful comparison among the three formulations, we have carefully controlled the experimental conditions to be as consistent as possible across all samples. Specifically, for the preparation of 7-TPE-DNA@TPE-BBT, PEG@TPE-BBT, and F127@TPE-BBT, the TPE-BBT feed concentration was fixed at 1.0 mg·mL⁻¹ in THF for all three formulations. The carrier concentrations were set as follows: 7-TPE-DNA at 40 μM , PEG and F127 at 10 mg·mL⁻¹, with the latter two being well above their critical micelle concentrations to ensure complete encapsulation. After assembly, residual THF was removed under a gentle nitrogen stream, followed by repeated extraction with ethyl acetate and *n*-butanol to eliminate unencapsulated dye. The resulting aqueous dispersions were further purified using 100 kDa Ultra centrifugal filters, with ultrapure water as the exchange buffer, to remove organic solvents and impurities. All three formulations were diluted to the same volume, and equal aliquots were taken for subsequent experiments. The optical properties of the purified nanoprobes were characterized by UV-Vis-NIR absorption spectroscopy and steady-state/time-resolved fluorescence spectroscopy (900–1700 nm). All comparative experiments were conducted under identical conditions to ensure a fair comparison.

2.7 Cytotoxicity assay

HeLa cells were seeded in 96-well plates (1 $\times 10^4$ cells per well) and incubated at 37 °C in 5% CO₂ for 24 h. Then 7-TPE-DNA nanoaggregates were added at various concentrations (0, 0.1, 0.2, 0.5, 1.0 μM). After 24 h, CCK-8 solution (10 μL per well) was added and incubated for 2 h. Absorbance at 450 nm was read using a microplate reader. Cell viability was normalized to the control group (without nanoaggregates).

2.8 *In vivo* vascular imaging

To further evaluate blood circulation dynamics and vascular imaging performance, *in vivo* experiments were conducted in live mice. Female BALB/c mice (7 weeks old, $n = 3$) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing,

China). Mice were anesthetized with isoflurane and intravenously injected via the tail vein with 150 μL of NIR-II probe solution ($1 \text{ mg}\cdot\text{mL}^{-1}$). The experimental group received the 7-TPE-DNA@TPE-BBT nanoprobe, while control groups were administered the PEG@TPE-BBT or F127@TPE-BBT nanoprobe. Immediately after injection, real-time NIR-II fluorescence imaging was performed using a small-animal imaging system equipped with a long-pass emission filter ($\lambda_p = 1000 \text{ nm}$) to capture blood-pool contrast. High-resolution visualization of the femoral artery in the hindlimb and the dermal capillary network in the dorsal region was specifically assessed for image clarity and signal-to-background ratio.

2.9 Ex vivo organ biodistribution

At 48 h post-injection, mice were euthanized, and major organs (liver, spleen, lung, heart, kidney, stomach, intestines) were harvested. *Ex vivo* NIR-II fluorescence imaging was performed to assess the biodistribution and clearance of the nanoprobes.

3 Results and discussion

To construct a programmable and robust DNA-based nanocarrier, we designed a series of DNA oligonucleotides bearing 3, 5, 7, or 9 PS modifications at the 5'-end, while the 3'-terminal hydrophilic segment was reserved as a universal anchoring sequence for subsequent functionalization through Watson-Crick base pairing (Table S1 in the ESM). These PS-DNAs were reacted with TPE-Br via nucleophilic substitution to afford TPE-DNA amphiphiles (Scheme 1). This design allows the TPE-rich hydrophobic core to

provide stable dye encapsulation, while leaving the external DNA shell available for future modular introduction of targeting, therapeutic, or additional imaging functions.

ESI-MS confirmed the successful synthesis of 3/5/7/9-TPE-DNA, with observed molecular weights matching the calculated values (Fig. 1(a)). Denaturing polyacrylamide gel electrophoresis was then employed to evaluate the assembly stability (Figs. 1(b) and 1(c)). Under 8 M urea, 7-TPE-DNA nanoaggregates remained fully trapped in the loading wells, whereas 3- and 5-TPE-DNA partially migrated, indicating that at least 7 TPE units were required to form stable aggregates without apparent monomer dissociation under the denaturing condition. SDS-PAGE further demonstrated that 7-TPE-DNA nanoaggregates possessed superior resistance to surfactant-induced disruption. Although 9-TPE-DNA nanoaggregates exhibited even higher stability, its synthesis suffered from low yield and batch-to-batch variation, likely due to excessive hydrophobicity and rapid aggregate formation. Considering the trade-off between structural robustness and synthetic practicality, 7-TPE-DNA was selected as the optimal carrier for subsequent NIR-II nanoprobe construction.

DLS and TEM characterizations revealed that 7-TPE-DNA self-assembled into uniform spherical nanoparticles with a hydrodynamic diameter of approximately 17.5 nm (Figs. 1(d) and 1(e)). The UV-Vis spectrum displayed a characteristic TPE absorption peak at $\sim 320 \text{ nm}$, and the aggregates exhibited strong AIE fluorescence at 463 nm (Fig. 1(f)). CCK-8 assays confirmed that 7-TPE-DNA nanoaggregates maintained $> 90\%$ cell viability even at concentrations up to $1 \mu\text{M}$ (Fig. S1 in the ESM), demonstrating good biocompatibility. These ultrastable

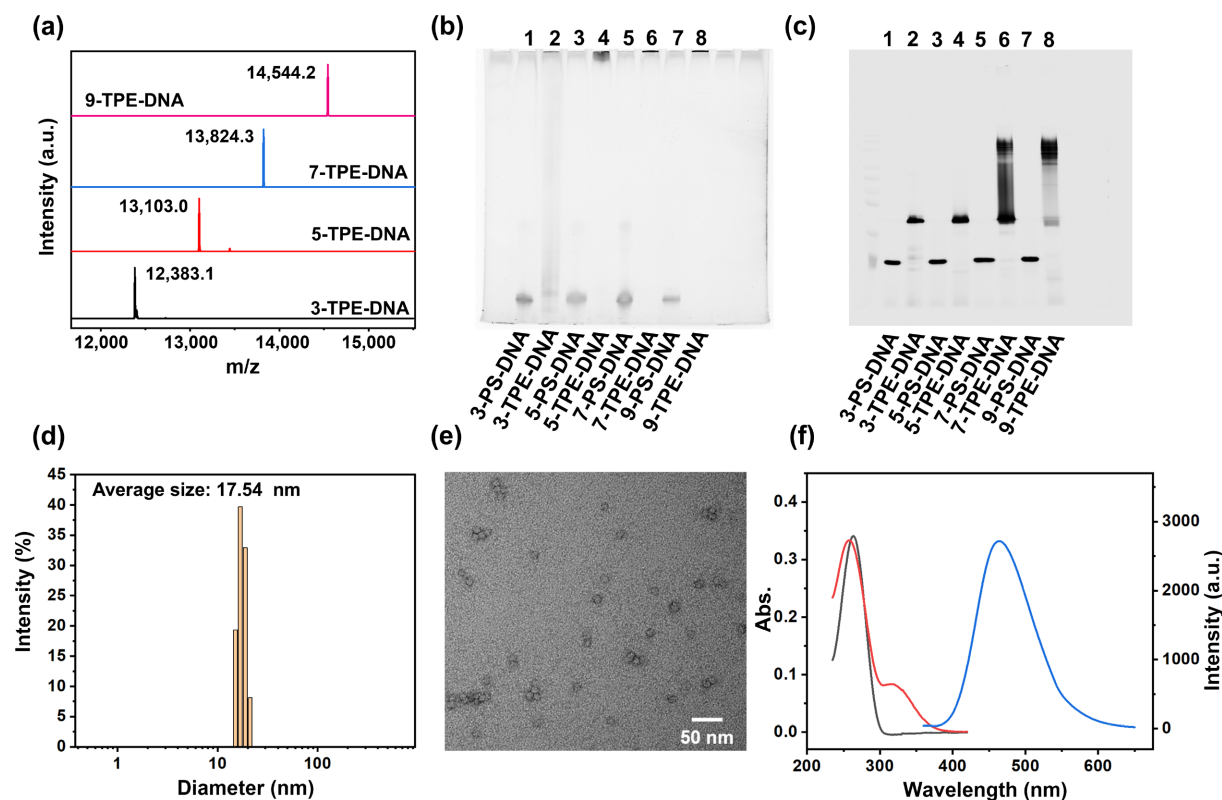


Figure 1 Characterization of TPE-DNA conjugates and nanoaggregates. (a) ESI-MS spectra of 3/5/7/9-TPE-DNA. (b) Stability evaluation by 15% PAGE containing 8 M urea. (c) Stability evaluation by 12% SDS-PAGE. (d) DLS size distribution of 7-TPE-DNA nanoaggregates. (e) TEM image of 7-TPE-DNA nanoaggregates (scale bar: 50 nm). (f) UV-Vis absorption (red) and fluorescence emission (blue) spectra of 7-TPE-DNA nanoaggregates ($\lambda_{\text{ex}} = 324 \text{ nm}$) in 0.01 M PBS; the UV-Vis spectrum of 7-PS-DNA (black) is shown for comparison.

nanoaggregates hold great promise as carriers capable of maintaining structural integrity even under harsh denaturing conditions.

The structural robustness of 7-TPE-DNA nanoaggregates was evaluated by denaturing gel electrophoresis in comparison with two classical DNA amphiphile controls, C18-DNA and cholesterol-DNA. The successful synthesis of these control constructs was confirmed by ESI-MS, and both formed spherical nanoaggregates as visualized by TEM (Fig. S2 in the ESM). Under 15% PAGE containing 8 M urea, 7-TPE-DNA aggregates remained trapped in the loading wells due to their large aggregate size, whereas both C18-DNA and cholesterol-DNA showed only migration bands of monomers, indicating complete disassembly (Fig. 2(a)). Under 15% SDS-PAGE conditions, 7-TPE-DNA nanoaggregates were still intact, while the controls were fully dissociated (Fig. 2(b)). In contrast, cholesterol and C18 relied mainly on weaker hydrophobic interactions, leading to loose and easily disrupted assemblies. Thus, multivalent TPE modification affords DNA nanoaggregates with ultra-high stability far beyond conventional single-tail anchors, highlighting their potential as a robust platform for biomedical applications (Fig. 2(c)).

We employed an annealing method to load organic NIR-II fluorophores into 7-TPE-DNA nanoaggregates, where the hydrophobic interior serves as an anchor for the hydrophobic dyes (Fig. 3(a)). This general encapsulation strategy enables the loading of diverse NIR-II fluorophores without requiring dye-specific chemical modification. To identify the most suitable NIR-II fluorophore for 7-TPE-DNA platform, we first synthesized TPE-BBT, an AIE-active dye with reported high quantum yield, and confirmed its structure by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), ^{13}C NMR, UV-Vis,

and fluorescence spectroscopy (Fig. S3 in the ESM). We then evaluated the generality of 7-TPE-DNA platform for loading different hydrophobic NIR-II fluorophores (Fig. 3(a)). Five representative dyes (TPE-BBT, TTQ-F-NH₂, FM-1210, IR-1061, and IR-1048) were separately assembled with 7-TPE-DNA under identical conditions. All formed stable nanoaggregates and exhibited NIR-II emission, as shown by the complete fluorescence spectra (Fig. S4 in the ESM). Among them, TPE-BBT gave the highest fluorescence intensity, likely due to the structural match and enhanced π - π stacking with the TPE core of the carrier. Therefore, TPE-BBT was selected for the subsequent experiments. It is worth noting that TPE-BBT was physically encapsulated into the TPE-rich hydrophobic domain rather than covalently conjugated to DNA. It enables the loading of structurally diverse hydrophobic NIR-II dyes using the same carrier without the need for dye-specific synthesis and purification, thus improving the generality and reproducibility of the platform.

The successful loading of TPE-BBT into 7-TPE-DNA nanoaggregates was confirmed by multiple characterizations. DLS showed a hydrodynamic diameter of approximately 135 nm for 7-TPE-DNA@TPE-BBT, and TEM revealed uniform spherical nanoparticles (Figs. 3(b) and 3(c)). The UV-Vis spectrum of 7-TPE-DNA@TPE-BBT exhibited a clear absorption peak at ~ 700 nm attributable to TPE-BBT, whereas no such peak was observed for the control 7-PS-DNA@TPE-BBT or for 7-TPE-DNA alone (Fig. 3(d)), confirming that TPE-driven assembly is required for stable dye loading. In contrast, the control sample 7-PS-DNA@TPE-BBT (without TPE modification) showed no significant TPE-BBT absorption, confirming that the TPE units are essential for stable dye encapsulation. The NIR-II fluorescence spectra (Fig. 3(e)) demonstrated that 7-TPE-DNA@TPE-BBT exhibited bright

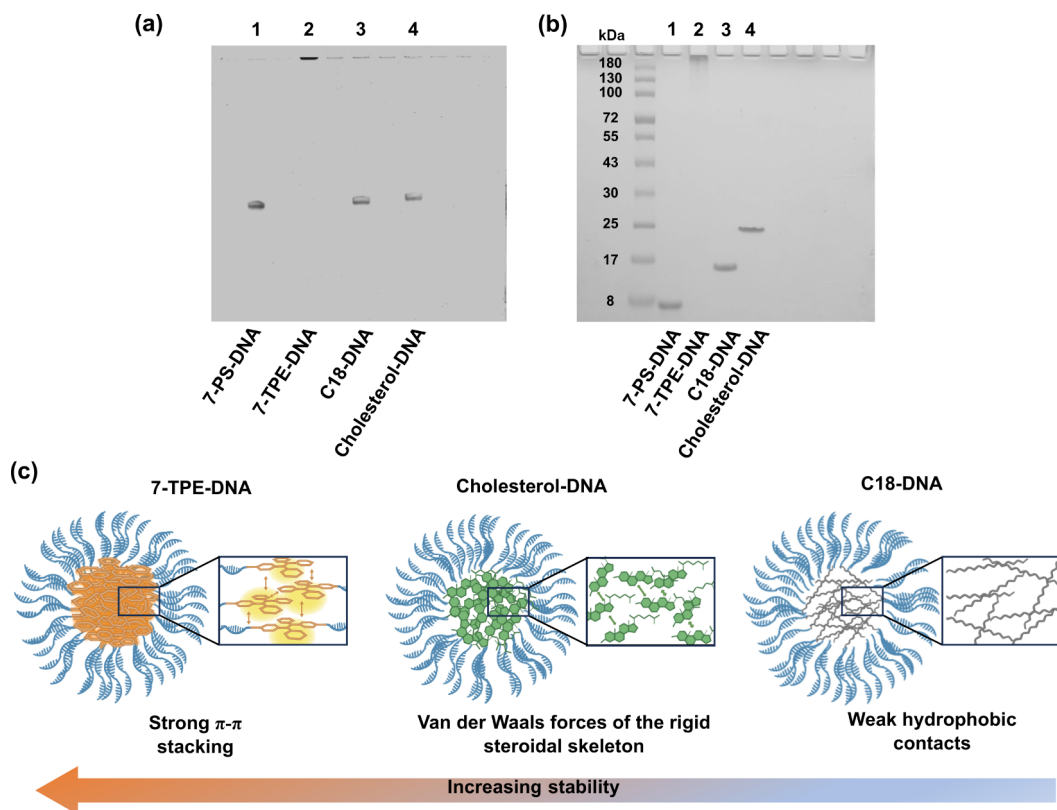


Figure 2 Stability evaluation of 7-PS-DNA, 7-TPE-DNA, C18-DNA, and cholesterol-DNA by (a) 15% PAGE with 8 M urea and (b) 15% SDS-PAGE. (c) Self-assembly driving forces and stability of 7-TPE-DNA, C18-DNA and cholesterol-DNA.

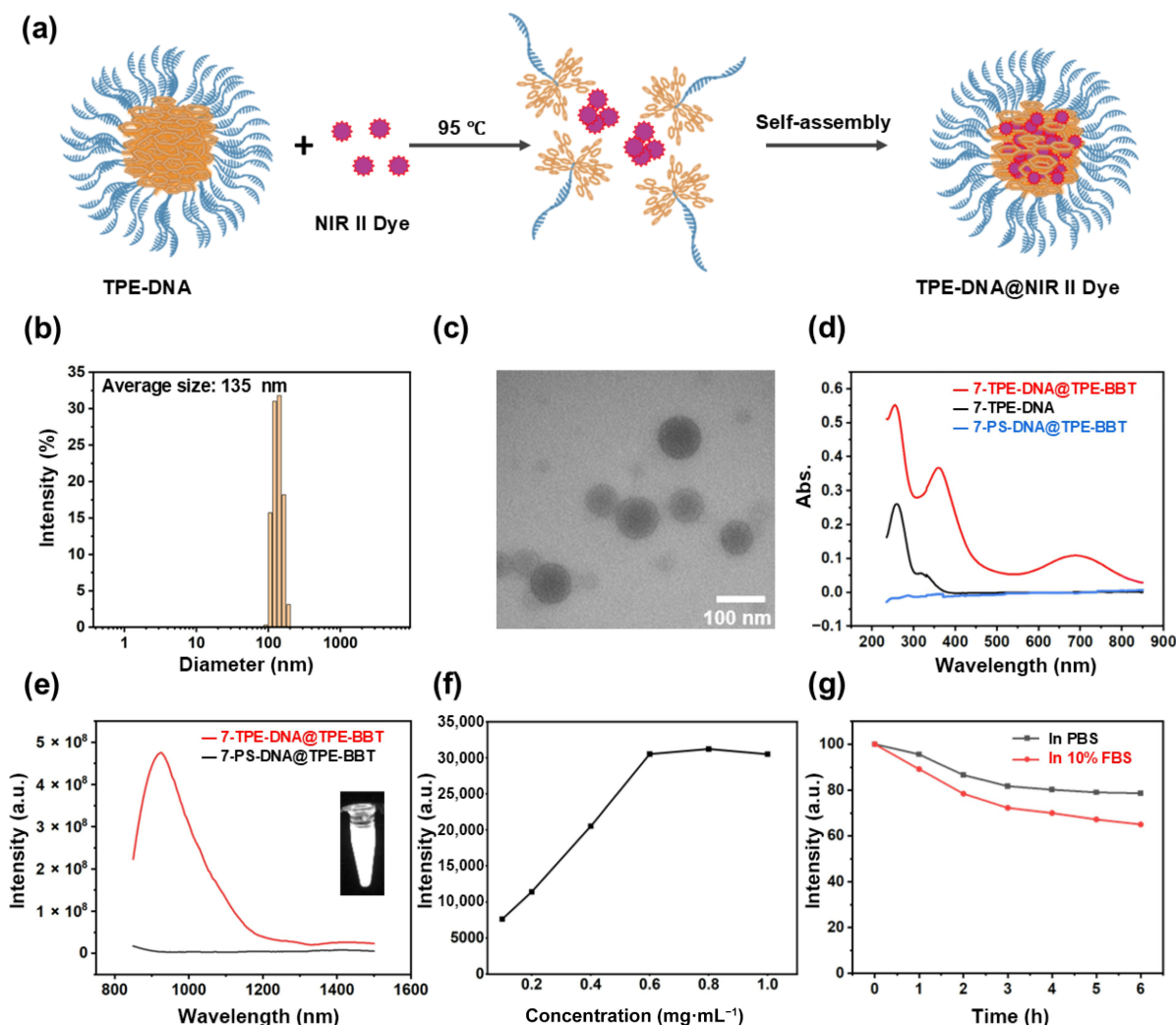


Figure 3 Characterization of 7-TPE-DNA nanoaggregates loaded with NIR-II dyes. (a) Schematic illustration of the self-assembly process. (b) DLS size distribution of 7-TPE-DNA@TPE-BBT. (c) TEM image of 7-TPE-DNA@TPE-BBT (scale bar: 100 nm). (d) UV-Vis absorption spectra of 7-TPE-DNA@TPE-BBT, 7-PS-DNA@TPE-BBT, and 7-TPE-DNA. (e) NIR-II fluorescence spectra of 7-TPE-DNA@TPE-BBT and 7-PS-DNA@TPE-BBT (λ_{ex} = 808 nm). (f) NIR-II fluorescence intensity at 1100 nm as a function of TPE-BBT feed concentration (0.1–1.0 mg·mL⁻¹) with fixed 7-TPE-DNA concentration (40 μ M). (g) Normalized NIR-II fluorescence intensity of 7-TPE-DNA@TPE-BBT over time in PBS and 10% FBS at 37 °C (λ_{ex} = 808 nm, λ_{em} = 1000 nm).

emission with a peak fluorescence intensity at 1100 nm increased up to 0.6 mg·mL⁻¹ and then plateaued, establishing 0.6 mg·mL⁻¹ as the optimal concentration for subsequent experiments (Fig. 3(f)). The colloidal stability of 7-TPE-DNA@TPE-BBT was assessed in PBS and 10% fetal bovine serum (FBS) at 37 °C (Fig. 3(g)). After 6 h, the nanoprobe retained 78% of initial fluorescence in PBS and 68% in 10% FBS, indicating satisfactory stability in serum-containing medium for *in vivo* imaging.

We next benchmarked the encapsulation performance of 7-TPE-DNA@TPE-BBT against two conventional carriers, PEG@TPE-BBT and F127@TPE-BBT. Under identical TPE-BBT loading conditions, the UV-Vis absorption spectra showed comparable dye loading for 7-TPE-DNA@TPE-BBT and PEG@TPE-BBT, whereas F127@TPE-BBT exhibited an absorbance approximately one order of magnitude lower (Fig. 4(a)). Consistently, the NIR-II fluorescence intensity of 7-TPE-DNA@TPE-BBT was similar to that of PEG@TPE-BBT, but about ten-fold higher than that of F127@TPE-BBT. The resistance to dye leakage was evaluated by solvent extraction with ethyl acetate and *n*-butanol. After vigorous

extraction, 7-TPE-DNA@TPE-BBT retained 80% and 60% of its initial fluorescence at 1000 nm, respectively, whereas PEG@TPE-BBT retained only 18% and 2%, and F127@TPE-BBT lost almost all signal (Fig. 4(b) and Fig. S5 in the ESM). Physiological stability was further assessed in 10% FBS at 37 °C (Fig. 4(c)). 7-TPE-DNA@TPE-BBT probe maintained > 50% of its initial fluorescence after 24 h, comparable to PEG@TPE-BBT and significantly better than F127@TPE-BBT, which rapidly lost signal. These results confirm that the multivalent TPE modification not only achieves high loading efficiency comparable to PEG but also provides superior protection against dye leakage and serum destabilization.

Encouraged by the good *in vitro* performance, we proceeded to evaluate the 7-TPE-DNA@TPE-BBT probe for *in vivo* NIR-II imaging. BALB/c mice were intravenously injected with the probe, using PEG@TPE-BBT and F127@TPE-BBT as controls (all at the same TPE-BBT dose of 1 mg·mL⁻¹, 150 μ L per mouse). For femoral artery imaging (Fig. 4(d)), the 7-TPE-DNA@TPE-BBT probe clearly delineated the main arterial trunk with high brightness and exceptionally low background signal, which we attribute to the ultrastable core that effectively prevents dye leakage. In contrast,

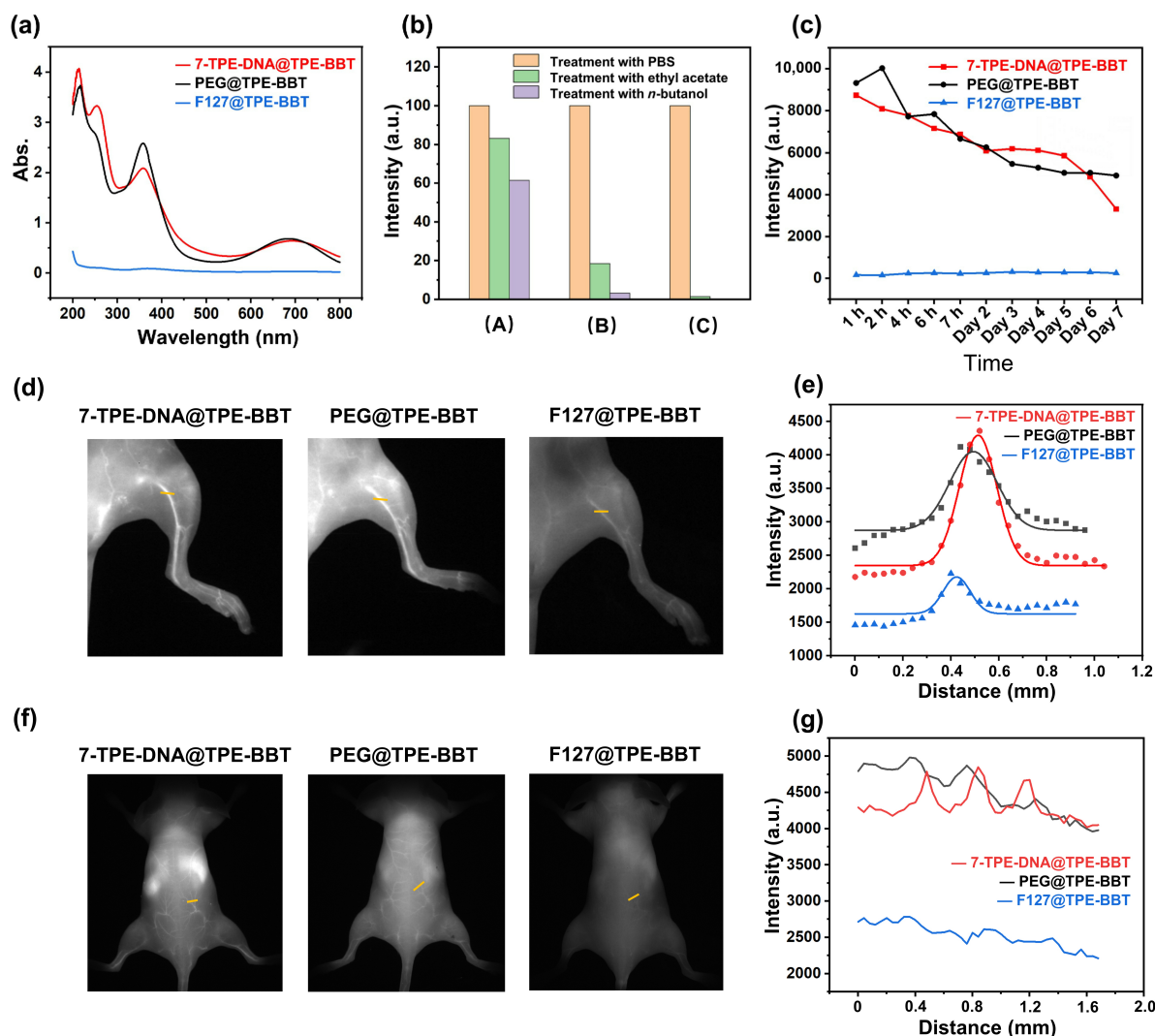


Figure 4 Comparison of the three TPE-BBT encapsulated nanoprobes. (a) UV-Vis absorption spectra. (b) Relative fluorescence intensity (at 1000 nm) before and after extraction with ethyl acetate and *n*-butanol ($\lambda_{ex} = 808$ nm) for: (A) 7-TPE-DNA@TPE-BBT, (B) PEG@TPE-BBT, and (C) F127@TPE-BBT. (c) Time-dependent fluorescence decay (at 1100 nm) in 10% FBS at 37 °C ($\lambda_{ex} = 808$ nm). (d) *In vivo* NIR-II fluorescence imaging of mice femoral artery after intravenous injection of the three nanoprobes. (e) Corresponding cross-sectional fluorescence intensity profiles along the yellow lines in panel (d). (f) *In vivo* NIR-II fluorescence imaging of mice subcutaneous capillary network on the mouse back. (g) Corresponding cross-sectional fluorescence intensity profiles along the yellow lines in panel (f) ($\lambda_{ex} = 808$ nm, $\lambda_{IP} = 1000$ nm, exposure time = 10 ms).

PEG@TPE-BBT produced a weak, diffuse signal with poor visualization of the artery. Quantitative analysis of the cross-sectional profiles along the yellow lines confirmed that the 7-TPE-DNA@TPE-BBT probe exhibited lower background signal and higher resolution, with a signal-to-background ratio approximately 1.3-fold higher than that of PEG@TPE-BBT, while F127@TPE-BBT barely visualized the vessels (Fig. 4(e)). In capillary imaging (Fig. 4(f)), only the 7-TPE-DNA@TPE-BBT probe resolved dense, well-defined networks, displaying three distinct peaks corresponding to parallel microvessels (Fig. 4(g)), further demonstrating its superior spatial resolution and contrast for fine vascular structures. *Ex vivo* organ imaging at 48 h post-injection (Fig. S6 in the ESM) showed predominant accumulation in the liver and spleen for all three probes. No obvious retention was observed in other organs, confirming the favourable biosafety profile of the TPE-DNA platform. These results demonstrate that the ultra-stable TPE-DNA core prevents dye leakage and background, enabling high-resolution NIR-II angiography.

4 Conclusions

In summary, we have developed a multivalent TPE modification strategy to construct DNA nanoaggregates with exceptional structural stability. Unlike conventional C18-DNA or cholesterol-DNA assemblies that readily dissociate under denaturing conditions, the TPE-DNA nanoaggregates remain intact in urea and SDS, owing to the dense hydrophobic core formed by cooperative π - π stacking and hydrophobic interactions of multiple TPE units. Leveraging this robust platform, we encapsulated the AIE-active NIR-II dye TPE-BBT to obtain 7-TPE-DNA@TPE-BBT probe. The probe exhibits bright NIR-II emission (quantum yield 2.45%) and outstanding resistance to dye leakage, outperforming PEG and F127 based counterparts. In living mice, it enables high-resolution visualization of femoral arteries and subcutaneous capillary networks with significantly improved signal-to-background ratio. This work establishes multivalent AIE engineering of DNA amphiphiles as a versatile and powerful

strategy for constructing ultra-stable NIR-II imaging platforms, opening new avenues for high-contrast vascular imaging and beyond.

Electronic Supplementary Material: Supplementary material (cytotoxicity assays, control DNA amphiphile characterization, TPE-BBT characterization, additional fluorescence spectra, dye-leakage analysis, *ex vivo* biodistribution imaging, and DNA sequences) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94909091>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

M. Z.: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing – original draft. Y. W.: Investigation, data curation, visualization, and writing – original draft. C. Z.: Investigation, methodology, and data analysis. Z. C. Q. and Z. C. L.: Validation and visualization. Z. G. F. and Y. T. Z.: *In vivo* imaging, resources, and validation. Y. X. and A. J. T.: Conceptualization, supervision, project administration, funding acquisition, and writing – review and editing. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

The animal experiments were carried out following the guidelines of the Tsinghua University Institutional Committee for the Care and Use of Laboratory Animals and were approved by the Committee on Ethical Use of Animals of Tsinghua University (Ethical code: THU-02-2026-0292A).

Use of AI statement

None.

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