

A Water-soluble NIR-II Polyzwitterionic Aza Boron Dipyrromethene Probe with Prolonged Blood Circulation for High-contrast Vascular Imaging

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Electronic Supplementary Information

Abstract The development of NIR-II fluorescence imaging agents with high aqueous solubility and prolonged circulation time remains challenging, primarily because of the intrinsic hydrophobicity of the widely used small-molecule fluorophores. Herein, we rationally designed an aza-boron dipyrromethene (BODIPY)-based atom transfer radical polymerization (ATRP) initiator and employed it to polymerize the zwitterionic monomer, 2-methacryloyloxyethyl phosphorylcholine (MCP), yielding a water-soluble polyzwitterionic NIR-II probe, designated as **JNBDP-PM-CP₆₄**. The resulting **JNBDP-PMCP₆₄** exhibited excellent biocompatibility, remarkable chemical/photostability, and a high NIR-II fluorescence quantum yield ($\Phi_{\text{f-1000 nm}}=0.032\%$). Furthermore, its prolonged blood circulation half-life ($t_{1/2\beta}=11.8$ h) enables high-fidelity vascular imaging with a superior signal-to-background ratio (SBR). Collectively, this study provides a robust strategy to overcome the solubility and biocompatibility limitations of NIR-II fluorophores, paving the way for advanced bioimaging applications.

Keywords NIR-II probe; Polyzwitterionic; Long circulation; Bioimaging

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INTRODUCTION

The emergence of near-infrared II (NIR-II, 1000–1700 nm) fluorescence imaging has revolutionized the field of bioimaging by enabling deeper tissue penetration, reduced photon scattering, and lower autofluorescence compared with conventional visible and NIR-I (700–900 nm) windows.^[1–6] These intrinsic advantages render NIR-II imaging a powerful tool for real-time visualization of deep-seated anatomical structures and dynamic physiological processes with unprecedented spatial and temporal resolution. Among the various NIR-II fluorophores developed to date, organic small-molecule dyes have attracted significant attention owing to their well-defined chemical structures, tunable photophysical properties, and favorable biocompatibility.^[1,2,6] Representative classes, including donor–acceptor–donor (D–A–D)-type fluorophores,^[1,6] cyanines,^[7–10] and boron dipyrromethene (BODIPY)/aza-BODIPY derivatives,^[11–14] have been explored extensively. However, despite their remarkable brightness and spectral tunability, most conventional small-molecule NIR-II fluorophores suffer from inherent hy-

drophobicity, which necessitates the use of organic solvents or surfactant-assisted dispersions for biological applications. This poor aqueous solubility often leads to severe aggregation, quenched fluorescence, non-specific interactions with biomolecules, and rapid reticuloendothelial system (RES) clearance, thereby compromising imaging fidelity and *in vivo* performance.

Macromolecular fluorophores have emerged as attractive alternatives to address these limitations.^[15] Specifically, the conjugation of NIR-II emissive dyes to hydrophilic polymer scaffolds not only improves aqueous solubility and colloidal stability but also provides abundant sites for subsequent functionalization, thereby enabling tailored pharmacokinetic profiles.^[1,16] Moreover, the covalent attachment of fluorophores to polymer backbones prevents premature dye leakage and enhances photostability under physiological conditions. Nevertheless, the development of NIR-II fluorescent polymeric probes remains relatively limited^[16] largely because of the poor compatibility of many NIR-II dyes with radical polymerization conditions. These dyes often undergo degradation or undesirable reactions with radicals and metal catalysts, which can result in fluorescence loss or compromised structural integrity.

Aza-BODIPY dyes exhibit exceptional stability, high molar extinction coefficients, and tunable absorption and emission

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wavelengths.^[12,13,17,18] Importantly, the aromatic cores of such dyes display remarkable chemical stability under radical-mediated conditions, rendering them compatible with radical polymerization processes. This unique feature makes azabodipy an ideal building block for the rational construction of well-defined NIR-II fluorescent polymeric probes.

On the other hand, zwitterionic polymers, have attracted growing interest due to their superior resistance to non-specific protein adsorption, prolonged blood circulation, and excellent hydration capability.^[19–25] These polymers exhibit ultralow fouling characteristics and do not elicit immune responses.^[26] Moreover, their high water solubility and antifouling properties are derived from electrostatically induced hydration, enabling a stealth effect that significantly reduces uptake by the mononuclear phagocyte system. These attributes render zwitterionic polymers highly desirable for the construction of long-circulating water-soluble imaging probes.

Herein, we report an NIR-II emissive polyzwitterionic probe, designated as **JNBDP-PMCP₆₄**, which was synthesized via the polymerization of 2-(methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate using JNBDP-Br as the atom-transfer radical polymerization (ATRP) initiator. The obtained **JNBDP-PMCP₆₄** exhibited a molecular weight of approximately 20.6 kDa and an average hydrodynamic diameter of about 9 nm in an aqueous solution. Importantly, the resulting probe not only exhibits excellent aqueous stability and high fluorescence quantum yield ($\Phi_{\text{F-1000 nm}}=0.032\%$), but also demonstrates a prolonged blood circulation half-life ($t_{1/2\beta}=11.8$ h), thereby enabling visualization of vascular imaging with a high signal-to-background ratio.

EXPERIMENTAL

Materials

1-(4-(Methylamino)phenyl)ethan-1-one, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinoline-9-carbaldehyde, *N,N*-diisopropylethylamine (DIPEA), and boron trifluoride etherate (BF₃·Et₂O) were purchased from Sigma-Aldrich and used as received. Fetal bovine serum (FBS), penicillin, streptomycin, and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO and used as received. 3-Bromoprop-1-yne, nitromethane, potassium hydroxide (KOH), cuprous bromide (CuBr), 1,4-dioxane, ethyl acetate (EtOAc), acetonitrile (MeCN), dichloromethane (DCM), methanol, ethanol, diethyl ether, *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), *n*-hexane, and all other reagents were purchased from Adamas-beta Co., Ltd. and used as received. 2-(Methacryloyloxy)ethyl 2-(trimethylammonio)ethyl phosphate (MCP) was purchased from Sigma-Aldrich and stored at −20 °C prior to use. Tetrahydrofuran (THF) was dried over sodium and distilled before use. Water was deionized using a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.4 MΩ·cm. All other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd., and used as received.

Instruments

All nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV400 NMR spectrometer operated in the Fourier transform mode using CDCl₃, DMSO-*d*₆, and CD₃OD as solvents. High-resolution mass spectra were obtained on a Bruker Amazon ETD ion trap mass spectrometer (Bruker-Franzen Analytik

GmbH, Bremen, Germany) with positive electrospray ionization (ESI⁺), and the resulting data were expressed in mass/charge (*m/z*) units. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was conducted using an AB SCIEX 4800 Plus MALDI-TOF/TOFTM mass spectrometer. UV-Vis-NIR absorbance spectra were recorded on a PerkinElmer Lambda 750S spectrometer at a scan rate of 300 nm/min. Fluorescence spectra were recorded on an Edinburgh Fluorescence Spectrometer FLS980 instrument equipped with a 450 W Xe lamp, a visible photomultiplier tube (PMT) detector (200–870 nm), an NIR PMT detector (800–1700 nm), and an external 808 nm (MDL-III-2 W) semiconductor laser (Changchun New Industries Optoelectronics Tech. Co., Ltd.) as the excitation source. Quartz cuvettes (1 cm) were used for both the absorbance and emission measurements.

Synthesis of Compound C

Compound **A** (1.50 g, 10.68 mmol, 1.0 equiv.) and compound **B** (1.61 g, 10.68 mmol, 1.0 equiv.) were dissolved in ethanol (20–30 mL). Subsequently, KOH (1.19 g, 21.36 mmol, 2.0 equiv.) was added. The reaction mixture was then stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was re-dissolved in DCM (50 mL) and washed twice with saturated NaCl (50 mL). The organic phases were combined and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the residue was further purified by silica gel column chromatography, affording compound **C** as a yellow solid (2.10 g, 59.5% yield). ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 8.04–7.99 (m, 2H), 7.69 (d, *J*=15.3 Hz, 1H), 7.32 (d, *J*=15.3 Hz, 1H), 7.10 (s, 2H), 6.85–6.79 (m, 2H), 4.13 (d, *J*=2.5 Hz, 2H), 3.26–3.19 (m, 4H), 3.08 (d, *J*=1.9 Hz, 3H), 2.76 (t, *J*=6.4 Hz, 4H), 2.22 (d, *J*=4.7 Hz, 1H), 1.99–1.94 (m, 4H). ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 188.29, 151.67, 144.88, 144.47, 130.46, 128.57, 127.88, 122.10, 121.00, 115.77, 112.27, 78.72, 72.29, 49.96, 41.87, 38.40, 27.72, 21.65. ESI-MS (*m/z*): calculated. for [M + H]⁺, 371.4962, found, 371.4234.

Synthesis of Compound D

Compound **C** (0.8 g, 2.16 mmol, 1.0 equiv.) was dissolved in ethanol (20 mL), followed by addition of KOH (0.242 g, 4.32 mmol, 2.0 equiv.) and nitromethane (CH₃NO₂, 5.27 g, 86.4 mmol, 30.0 equiv.). The reaction mixture was heated at 70 °C overnight. After cooling the mixture to room temperature, the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography to obtain compound **D** as a faint yellow solid (560 mg, 60.0% yield). ¹H-NMR (400 MHz, CDCl₃, δ , ppm): 7.91–7.85 (m, 2H), 6.79–6.74 (m, 2H), 6.66 (s, 2H), 4.75 (dd, *J*=12.2, 6.4 Hz, 1H), 4.57–4.50 (m, 1H), 4.11 (d, *J*=2.6 Hz, 2H), 3.98 (tt, *J*=8.3, 6.1 Hz, 1H), 3.35–3.17 (m, 2H), 3.14–3.03 (m, 7H), 2.71 (t, *J*=6.6 Hz, 4H), 2.23 (t, *J*=2.4 Hz, 1H), 2.00–1.86 (m, 4H). ¹³C-NMR (101 MHz, CDCl₃, δ , ppm): 195.52, 152.31, 142.34, 130.47, 130.24, 126.80, 126.29, 121.78, 112.07, 80.13, 78.57, 72.45, 49.92, 49.76, 41.79, 41.30, 38.89, 38.39, 27.67, 21.98. ESI-MS (*m/z*): calculated. for [M + H]⁺, 432.5364, found, 432.5692.

Synthesis of the Alkyne-JNBDP

Ammonium acetate (1.875 g, 24.33 mmol, 30.0 equiv.) and compound **D** (350.0 mg, 0.811 mmol, 1.0 equiv.) were dissolved in *n*-BuOH (20 mL) and heated at 110 °C for 12 h under N₂ atmosphere. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was diluted with CH₂Cl₂ (100 mL) and washed twice with saturated NaCl (50 mL). The organic phase was then dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The resulting solid

was subsequently dissolved in dry CH_2Cl_2 (20 mL), cooled to -20°C under N_2 atmosphere, and treated with DIPEA (314.4 mg, 0.811 mmol, 3.0 equiv.) followed by slow addition of $\text{BF}_3\cdot\text{Et}_2\text{O}$ (575.5 mg, 4.055 mmol, 5.0 equiv.) and the mixture was stirred at room temperature for 3 h. The reaction was quenched with ice and the product was extracted with CH_2Cl_2 (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The residue was further purified by silica gel column chromatography (DCM:PE=1:1, V:V) to obtain the product as a blue solid (48.0 mg, 14.4% yield). ^1H -NMR (400 MHz, CDCl_3 , δ , ppm): 8.12–8.06 (m, 4H), 7.62 (s, 4H), 6.89 (d, $J=8.8$ Hz, 4H), 6.82 (s, 2H), 4.15 (d, $J=2.3$ Hz, 4H), 3.68 (q, $J=2.8$ Hz, 2H), 3.28 (t, $J=5.7$ Hz, 8H), 3.11 (s, 6H), 2.81 (t, $J=6.4$ Hz, 8H), 2.05–2.00 (m, 8H). MALDI-TOF-MS (m/z): calculated for $[\text{M} + \text{H}]^+$, 822.8282, found, 822.8963.

Synthesis of Compound JNBDP-Br

Alkyne-JNBDP (30.0 mg, 0.0365 mmol, 1.0 equiv.) and 2-azidoethyl 2-bromo-2-methylpropanoate (17.5 mg, 0.0804 mmol, 2.2 equiv.) were dissolved in DCM (2 mL), followed by addition of DIPEA (17.5 mg, 0.0730 mmol, 2.0 equiv.), CuI (9.3 mg, 0.0730 mmol, 2.0 equiv.), and CH_3COOH (4 μL , 0.0730 mmol, 2.0 equiv.). After stirring at room temperature for 24 h, the reaction mixture was concentrated and purified using silica gel column chromatography, affording JNBDP-Br as a blue solid (24.0 mg, 60.1% yield). ^1H -NMR (400 MHz, $\text{DMSO}-d_6$, δ , ppm): 8.10–7.54 (m, 10H), 7.10 (d, $J=22.0$ Hz, 2H), 6.89 (d, $J=8.6$ Hz, 4H), 5.74 (d, $J=4.1$ Hz, 4H), 4.69–4.64 (m, 4H), 4.51–4.44 (m, 4H), 3.35 (s, 8H), 3.07 (d, $J=3.9$ Hz, 8H), 2.85–2.55 (m, 6H), 1.89 (t, $J=6.2$ Hz, 8H), 1.73 (s, 12H). ^{13}C -NMR (101 MHz, $\text{DMSO}-d_6$, δ , ppm): 170.98, 162.54, 154.82, 150.59, 144.46, 144.02, 141.42, 131.41, 128.43, 123.81, 121.27, 120.64, 120.01, 114.36, 112.19, 64.48, 56.83, 49.92, 48.77, 47.25, 38.19, 28.07, 21.90. MALDI-TOF-MS (m/z): calculated for $[\text{M} + \text{H}]^+$, 1292.4179, found, 1292.4650.

Synthesis of JNBDP-PMCP_n

JNBDP-Br (5.0 mg, 0.00379 mmol, 1.0 equiv.), MPC (223.6 mg, 0.757 mmol, 200.0 equiv.) and PMDETA (1.3 mg, 0.00758 mmol, 2.0 equiv.) were charged into reaction tube. The reaction mixture was carefully degassed via three freeze-pump-thaw cycles, followed by the addition of CuBr (0.5 mg, 0.00379 mmol, 1.0 equiv.), which was then sealed under vacuum. After stirring at 70°C for 12 h, the reaction tube was opened and the mixture was precipitated with excess of diethyl ether. The resulting precipitate was dissolved in deionized water and dialyzed using a dialysis bag with a molecular weight cut-off of 5000 Da. The dialyzed water was replaced every 4 h. After 48 h of dialysis, the solution was lyophilized to obtain a freeze-dried powder of JNBDP-PMCP₆₄ (105 mg, 45.9% yield). The molecular weight and molecular weight distribution of JNBDP-PMCP_n were determined by gel permeation chromatography (GPC) using water as the eluent, which revealed an M_n of 20.6 kDa and an M_w/M_n of 1.32. The average degree of polymerization (DP) of the MCP block was determined to be 64, and the polymer was denoted as JNBDP-PMCP₆₄.

Determination of Fluorescence Quantum Yields (Φ_f)

Fluorescence quantum yields were determined using a standard dye as a reference from plots of integrated fluorescence intensity versus absorbance. The calculation equation is calculated as follows:

$$\Phi_{f,\text{sam}} = \Phi_{f,\text{ref}} \times (n_{\text{sam}}^2/n_{\text{ref}}^2)(K_{\text{sam}}/K_{\text{ref}}) \quad (1)$$

where $\Phi_{f,\text{sam}}$ is the fluorescence quantum yield of the test sample, and $\Phi_{f,\text{ref}}$ is the fluorescence quantum yield of the reference.

K is the slope of the integrated fluorescence intensity versus absorbance plot. IR 26 in 1,2-dichloroethane was used as a reference ($\Phi_f=0.05\%$). An 808 nm laser was used as the excitation source, and emission spectra were collected from 818 nm to 1300 nm. Measurements were performed with the absorbance of all dye solutions at 808 nm kept below 0.1, in order to maximize illumination homogeneity and optical transparency. n_{sam} and n_{ref} are the refractive indices of the respective solvents.

Evaluation of Chemical Stability

Preparation of various bio-reactive reagents solutions is presented as follows.

H_2O_2 : commercial H_2O_2 solution (30% in H_2O) was used directly. The concentration of the H_2O_2 stock solution was determined by measuring the absorbance at 240 nm, with a molar extinction coefficient of $43.6 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$;

ONOO $^-$: peroxyntirite stock (30 $\text{mmol}\cdot\text{L}^{-1}$ in 0.1 $\text{mol}\cdot\text{L}^{-1}$ NaOH) was prepared following the literature procedure, and the concentration was quantified with a spectrophotometer at $\epsilon_{302\text{nm}}=1670 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$;

ClO $^-$: a hypochlorite stock (ClO $^-$, 50 $\text{mmol}\cdot\text{L}^{-1}$ in H_2O , pH=12) was prepared by diluting a commercial NaClO solution in water (pH=12) and assayed with a spectrophotometer at $\epsilon_{293\text{nm}}=350 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$;

$\text{O}_2^{\cdot-}$: solid KO_2 was added into the solution directly.

JNBDP-PMCP₆₄ was suspended in PBS at a final concentration of $10 \mu\text{mol}\cdot\text{L}^{-1}$ upon treatment with H_2O_2 (1 $\text{mmol}\cdot\text{L}^{-1}$) and $\text{O}_2^{\cdot-}$ (1 $\text{mmol}\cdot\text{L}^{-1}$), ClO $^-$ (100 $\mu\text{mol}\cdot\text{L}^{-1}$), and ONOO $^-$ (100 $\mu\text{mol}\cdot\text{L}^{-1}$) for 12 h, and the absorption spectra were obtained.

In vivo Imaging

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Anhui Normal University. Female BALB/c mice (6–8 weeks, 18–22 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. All mice were randomly allocated and housed under standard environmental conditions ($23\pm1^\circ\text{C}$, $50\%\pm5\%$ humidity, and 12/12 h light/dark cycle) with free access to water and standard laboratory food. Before imaging, all mice were anesthetized via an abdominal injection of avertin. NIR-II fluorescence images were collected on a home-built small animal imaging system with a 640×512 pixel 2D InGaAs NIRvana CCD camera (Princeton Instrument) under 808 nm laser excitation with long-pass (LP) filters. All results are presented as the mean $\pm$ SD ($n=3$ mice), and images were processed using LightField imaging software and Image J.

Pharmacokinetics Studies

Healthy female BALB/c mice ($n=3$ per group) were intravenously injected with JNBDP-PMCP₆₄ (2.0 mg/mL, 200 μL) at predetermined time points. Blood samples were collected from the orbital venous plexus in heparin sodium tubes and stored at 4°C . After completing the full set of time points, 50 μL aliquots of blood were taken for NIR-II fluorescence imaging using 1000 nm long-pass filters under 808 nm laser excitation. A standard curve of fluorescence intensity versus concentration was generated for the blood, yielding a predicted R-squared value of 0.99. This standard curve was then used to determine fluorophore concentrations in the blood at different time points. Pharmacokinetic parameters were calculated using the Kinetica software (version 3.0) with a two-compartment model, following the calculation procedure described below.

$$C=A'e^{-\alpha t}+B'e^{-\beta t}-C'e^{-K_d t} \quad (2)$$

where C is the concentration at the specified time, t is the speci-

fied time, A' is the intercept for the fast distribution phase, B' is the intercept for the terminal elimination phase, C' is the coefficient for the absorption phase, α is the distribution rate constant, β is the elimination rate constant, K_a is the first-order absorption rate constant.

When the time is long enough, $K_a \gg \alpha$, $\alpha \gg \beta$, for $e^{-\beta t} \rightarrow 0$ and $e^{-\alpha t} \rightarrow 0$, the above equation is simplified as follows:

$$C = B'e^{-\beta t} \quad (3)$$

$$\text{So } \lg C = \lg B' - 0.4343\beta t \quad (4)$$

A semi-logarithmic graph was then drawn from the concentration-time data, and the elimination rate constant (β) was calculated based on the slope. For **JNBDP-PMCP**₆₄, $\beta = 0.0587$, so $t_{1/2} = 0.693/\beta = 11.8$ h.

RESULTS AND DISCUSSION

The synthetic routes employed for the preparation of the ATRP initiator, JNBDP-Br, and the polyzwitterionic NIR-II probe, **JNBDP-PMCP**₆₄, are depicted in Fig. 1. First, alkyne-JNBDP was synthesized following the established O'Shea procedure, which involves a sequential cascade including aldol-ketone condensation to form a chalcone, Michael addition with nitromethane to yield a β -nitro ketone, condensation cyclization with ammonium acetate to generate azadipyrromethene, and boron trifluoride diethyl etherate-mediated coordination-induced ring closure. Subsequently, alkyne-JNBDP was reacted with 2-azidoethyl 2-bromo-2-methylpropanoate *via* copper-catalyzed azide-alkyne cycloaddition (CuAAC) "click" chemistry to afford JNBDP-Br. Subsequently, ATRP polymerization of MCP was carried out using JNBDP-Br as the initiator in CH₃OH to obtain the

final polyzwitterionic probe, **JNBDP-PMCP**_n. Detailed synthetic routes and comprehensive characterization data for all intermediates are provided in the electronic supplementary information (ESI). All the synthesized compounds were fully characterized by nuclear magnetic resonance (¹H-NMR and ¹³C-NMR) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) (Fig. 2 and Figs. S1–S8 in ESI). The molecular weight and molecular weight distribution were determined using aqueous GPC, and the degree of polymerization was determined to be 64, denoted as **JNBDP-PMCP**₆₄.

The photophysical properties of JNBDP-Br were first investigated (Fig. 3 and Table 1). JNBDP-Br exhibited intense near-infrared (NIR) absorption in the range of 600–900 nm and emission beyond 950 nm in organic solvents. Notably, in polar solvents, such as dimethyl sulfoxide (DMSO), the absorption and emission peaks reached 845 and 1029 nm, respectively. Using IR26 in 1,2-dichloroethane as a reference (quantum yield, QY=0.05%), the NIR-II fluorescence quantum yield and brightness of JNBDP-Br in DMSO were determined to be 0.389% and 215.51 L·mol⁻¹·cm⁻¹, respectively.

The molecular weight of **JNBDP-PMCP**₆₄ was determined to be 20.6 kDa using aqueous gel permeation chromatography (GPC), with a polydispersity index of 1.32 (Fig. 4a). **JNBDP-PMCP**₆₄ is a freeze-dried solid powder at room temperature. It can be dissolved in PBS before use, dynamic light scattering (DLS) characterization revealed a hydrodynamic diameter of approximately 9.0 nm (Fig. 4b), while transmission electron microscopy (TEM) confirmed a spherical morphology (Fig. S9 in ESI). Furthermore, **JNBDP-PMCP**₆₄ exhibited excellent colloidal stability in PBS, DMEM, and fetal bovine serum (FBS) for 24 h, with no significant change in particle

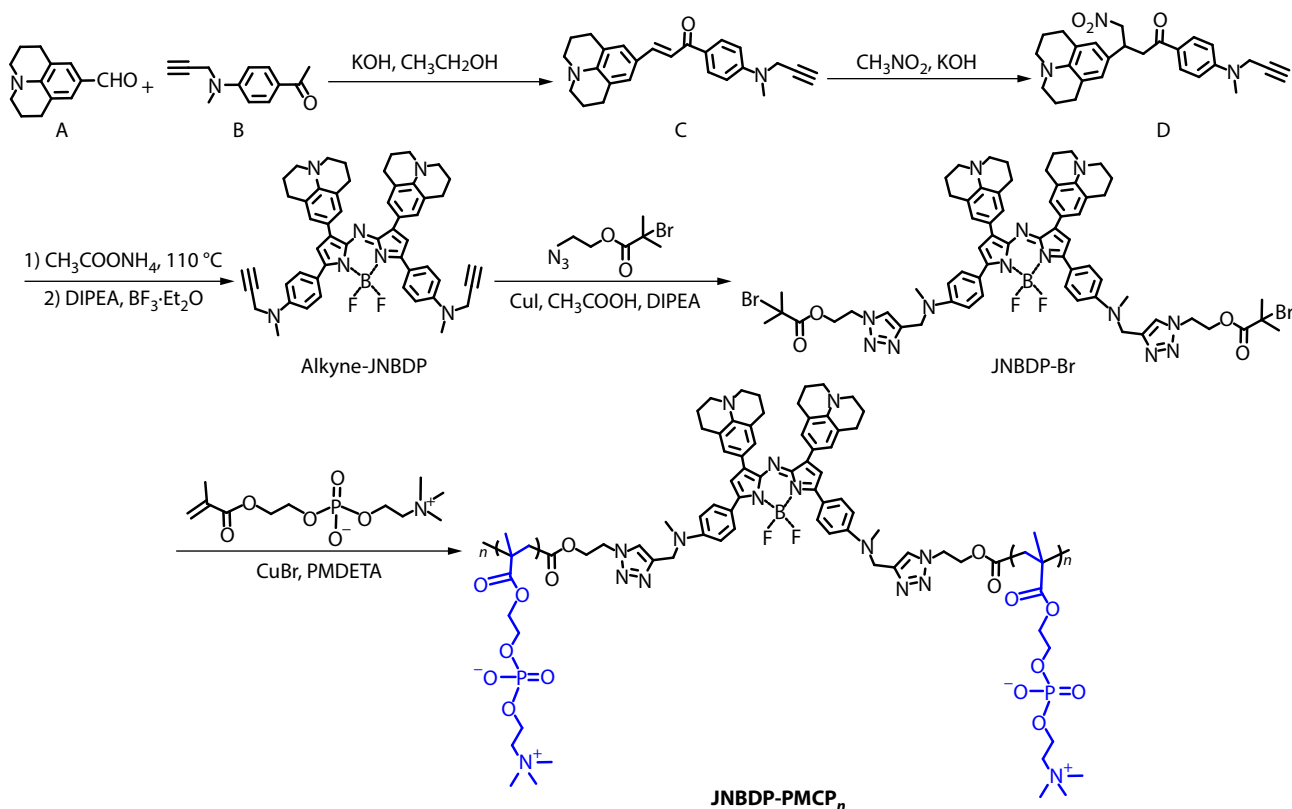


Fig. 1 Synthesis route employed for the preparation of NIR-II polyzwitterionic probes, **JNBDP-PMCP**_n.

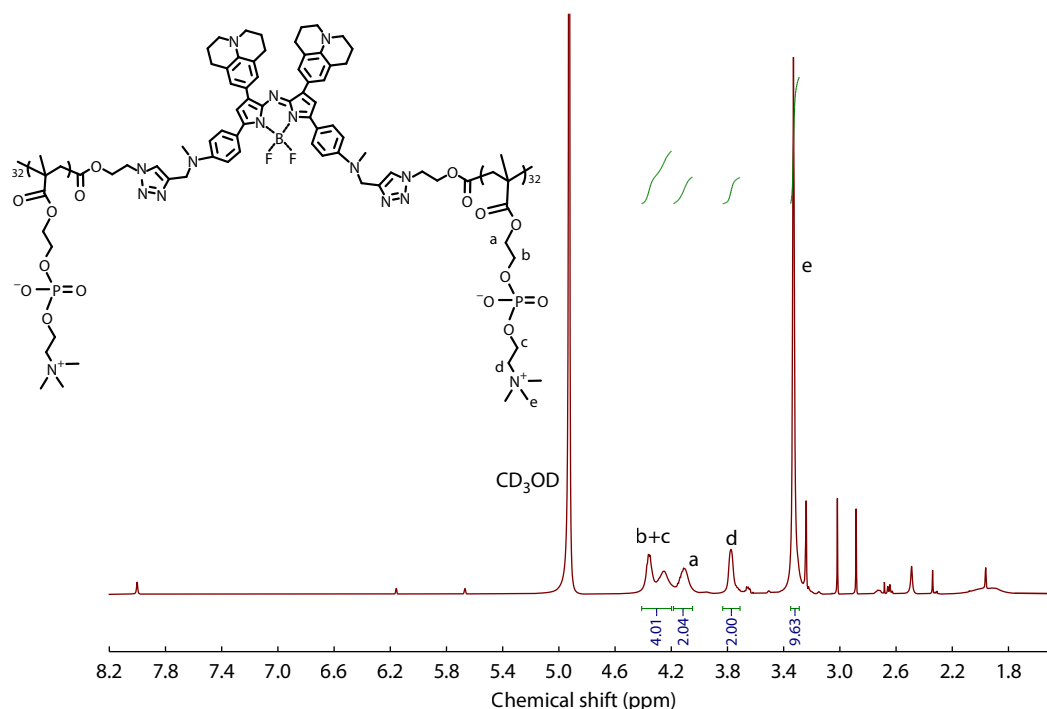


Fig. 2 ^1H -NMR spectra recorded for **JNBDP-PMCP**₆₄ in CD_3OD .

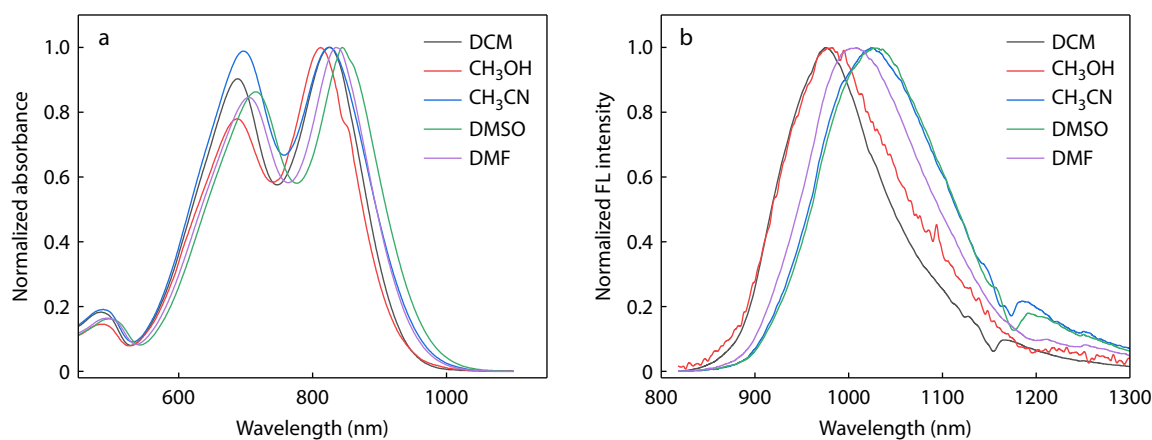


Fig. 3 (a) Normalized absorbance and (b) fluorescence intensity ($\lambda_{\text{ex}}=808\text{ nm}$) recorded for **JNBDP-Br** in various solvents as indicated.

Table 1 Photophysical properties of **JNBDP-Br** in various solvents.

Solvent	λ_{abs} (nm)	λ_{em} (nm)	Stokes shift (nm)	Molar extinction coefficients (ϵ_{max}) ($\times 10^3\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)	Quantum yields ($\Phi_{\text{f}} > 1000\text{ nm}$) (%)	$\epsilon\Phi_{\text{f}}$ ($\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$)
DCM	825	976	151	0.506	0.707	357.42
DMF	836	1005	169	0.567	0.359	203.55
DMSO	845	1029	184	0.554	0.389	215.51
CH_3OH	810	980	170	0.290	0.043	12.47
CH_3CN	825	1025	200	0.430	0.232	99.76

size (Fig. S10 in ESI). In aqueous solution, **JNBDP-PMCP**₆₄ exhibited a maximum absorption peak at 840 nm and an emission peak at 1030 nm (Fig. 4c), with a fluorescence quantum yield of 0.032% (Fig. 4d). Notably, the high-molecular-weight polyzwitterionic shell effectively suppressed aggregation-induced quenching of the central emissive dye. Experimentally, within a dye concentration range of 2.6–32.5 $\mu\text{mol}\cdot\text{L}^{-1}$, the fluorescence intensity shows a good linear correlation with

concentration, and no obvious aggregation-induced quenching is observed (Fig. S11 in ESI).

JNBDP-PMCP₆₄ showed excellent photostability upon continuous 808 nm laser irradiation (1.0 $\text{W}\cdot\text{cm}^{-2}$), whereas free ICG showed rapid fluorescence decay under identical irradiation conditions (Fig. 5a and Fig. S12 in ESI). Moreover, **JNBDP-PMCP**₆₄ exhibited significantly enhanced chemical stability in the presence of various reactive oxygen species (ROS), where-

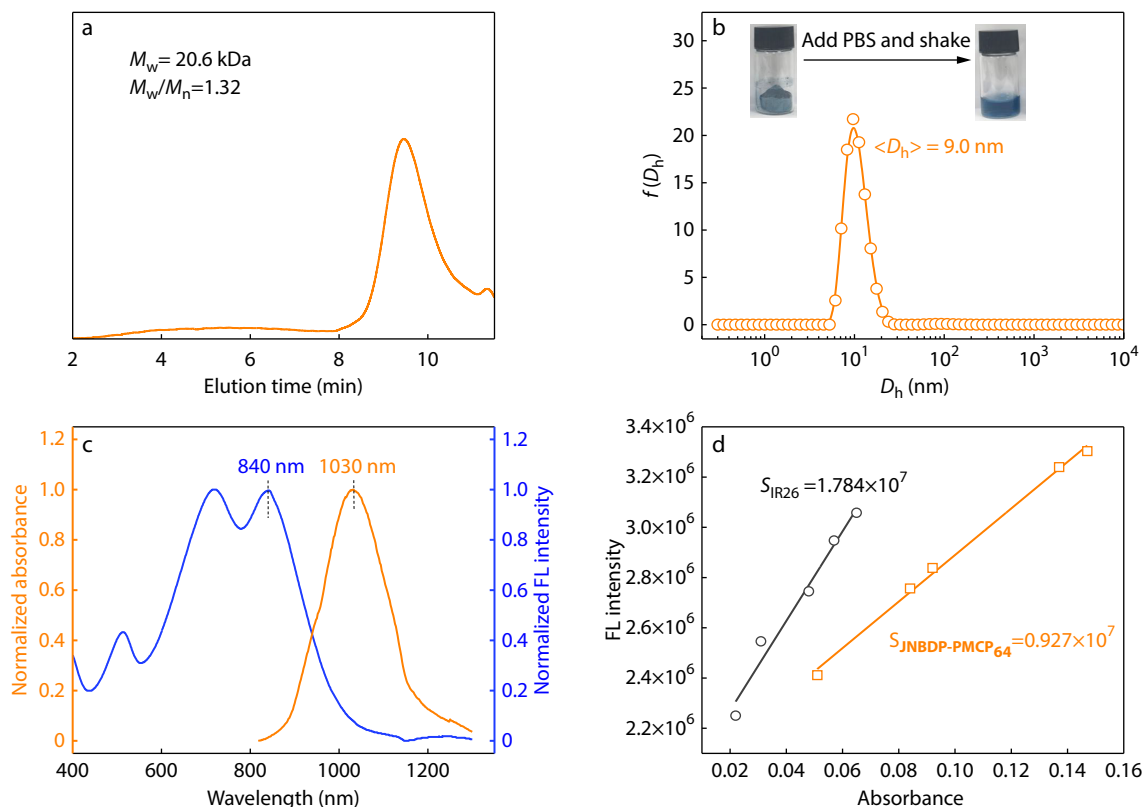


Fig. 4 (a) Aqueous-phase gel permeation chromatography (GPC) trace recorded for **JNBDP-PMCP₆₄**; (b) Dynamic light scattering (DLS) size distribution of **JNBDP-PMCP₆₄**, the inset figure shows the photographs of the corresponding lyophilized powder and aqueous solution samples of **JNBDP-PMCP₆₄**; (c) The normalized absorbance and fluorescence intensity recorded for **JNBDP-PMCP₆₄** in PBS at pH=7.4; (d) Calculating NIR-II quantum yield ($\Phi_{f>1000\text{ nm}}$) of **JNBDP-PMCP₆₄** in PBS (pH=7.4).

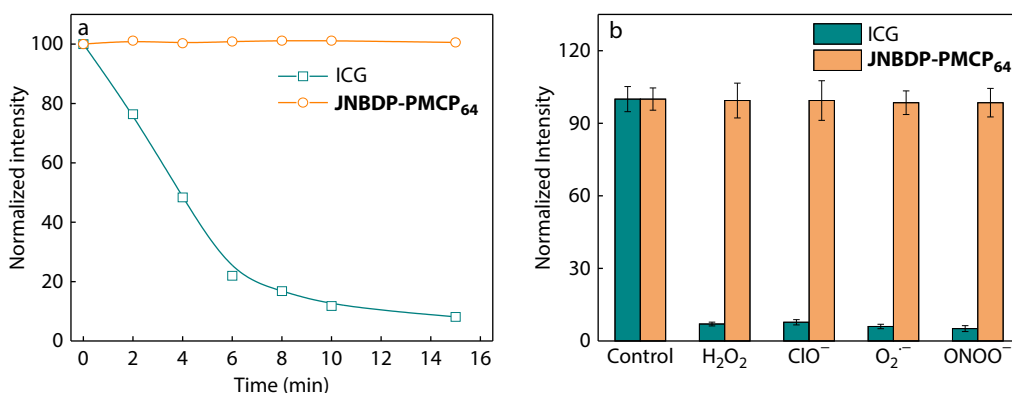


Fig. 5 (a) Photostability comparison of **JNBDP-PMCP₆₄** and free ICG in PBS under 808 nm laser irradiation at a constant power intensity of 1.0 W·cm⁻²; (b) Normalized fluorescence intensities of **JNBDP-PMCP₆₄** in PBS (pH=7.4, 10 mmol·L⁻¹) after 12 h incubation with various reactive oxygen species (ROS).

as free ICG underwent considerable bleaching under identical conditions (Fig. 5b and Fig. S13 in ESI).

The potential cytotoxicity of **JNBDP-PMCP₆₄** was evaluated in 4T1 cells and human umbilical vein endothelial cells (HUVEC), both cells exhibited over 95% viability after incubation with 1000 mg·L⁻¹ of **JNBDP-PMCP₆₄** for 24 h (Figs. 6a and 6b). Furthermore, no noticeable abnormalities were observed in major organs, including the heart, liver, spleen, lung, and kidneys, after intravenous administration of **JNBDP-PMCP₆₄** (1000 mg·L⁻¹, 200 μ L) into living mice for 48 h (Fig. 6c), indicating the high biocompatibility of **JNBDP-PMCP₆₄**.

In vivo NIR-II fluorescence imaging was performed following the intravenous injection of **JNBDP-PMCP₆₄** into BALB/c mice (Fig. 7a). A distinct fluorescence signal was observed in the liver as early as 1-min post-injection, indicating the rapid uptake of **JNBDP-PMCP₆₄** by hepatic macrophages. At 20 min post-injection, a detectable signal emerged in the bladder region, which markedly intensified at 60 min. The liver-to-kidney signal ratio peaked at approximately 40 min and gradually declined thereafter (Fig. 7b). This phenomenon is attributed to the polydispersity of **JNBDP-PMCP₆₄**; although the average hydrodynamic size is approximately 9 nm, a small frac-

tion of polymer single chains possesses a hydrodynamic diameter below the renal filtration threshold (5.5 nm), thereby enabling glomerular filtration into the bladder and subsequent excretion *via* urine. Although no significant signal was discernible in the kidney region *via* dorsal *in vivo* fluorescence imaging, *ex vivo* tissue imaging revealed predominant probe accumulation in the liver and kidneys 4 h post-injection (Figs. 7c and 7d). After 24 h, the renal signal notably diminished, which is consistent with the dynamic trends observed in real-time *in vivo* imaging.

After intravenous injection of **JNBDP-PMCP₆₄** (200 μ L, 2 g·L⁻¹) into the tail vein, blood samples were collected at various time points. The dye concentration in the blood at each time point was determined using a pre-established *in vitro* standard curve correlating fluorescence intensity with dye

concentration, allowing for the calculation of the blood circulation half-life of **JNBDP-PMCP₆₄** (Figs. 8a and 8b). The half-life was determined to be 11.8 h (Fig. 8c), which is significantly longer than that of most previously reported water-soluble near-infrared fluorescent probes. This prolonged circulation is likely attributable to the protein adsorption-resistant properties of the polyzwitterionic structure, which reduces capture by hepatic and splenic macrophages, thereby extending the probe's bloodstream residence time. This extended blood circulation half-life confers a distinct advantage to vascular imaging. Imaging of the hindlimb vasculature revealed that as the filter wavelength increased from 1200 nm to 1500 nm long-pass filter (LP), the signal-to-background ratio progressively improved, rising from 1.48 (1200 nm LP) and 1.59 (1300 nm LP) to 2.73 (1400 nm LP) and 2.99 (1500 nm LP). Concur-

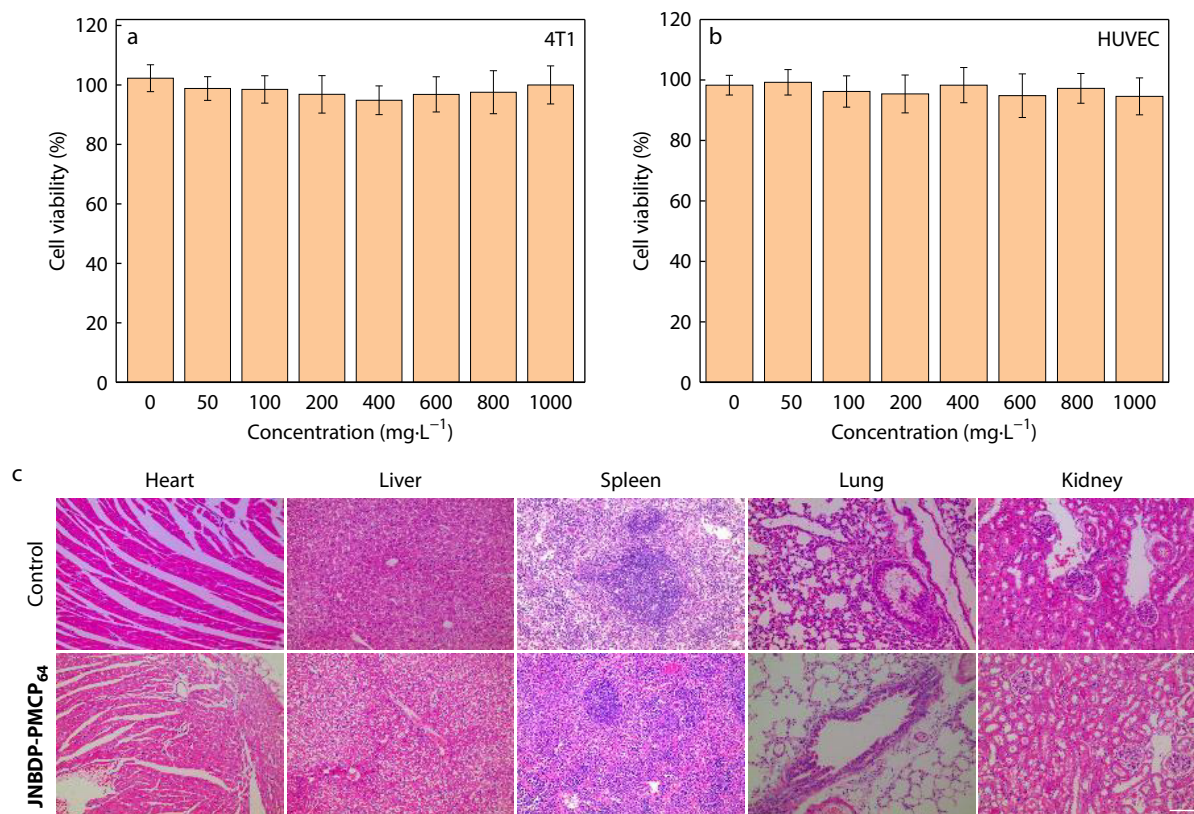
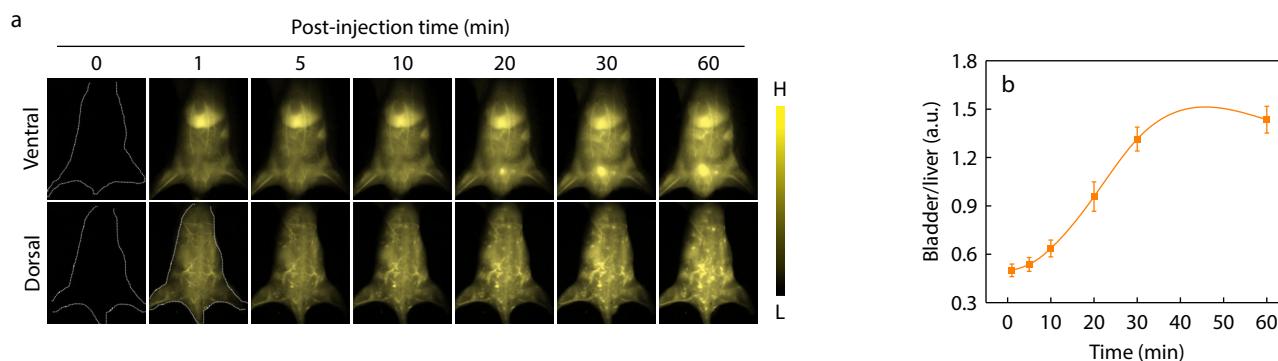


Fig. 6 *In vitro* cytotoxicity assessment of (a) 4T1 and (b) HUVEC cells after incubation with various concentration of **JNBDP-PMCP₆₄** for 24 h, the bars represent mean $\pm$ s. d., derived from $n=6$ biological independent measurements; (c) H&E staining of main organs (including heart, liver, spleen, lung and kidney) after intravenous administration of **JNBDP-PMCP₆₄** (1000 mg·L⁻¹, 200 μ L) into living mice for 48 h ($n=3$), scale bars: 100 μ m.



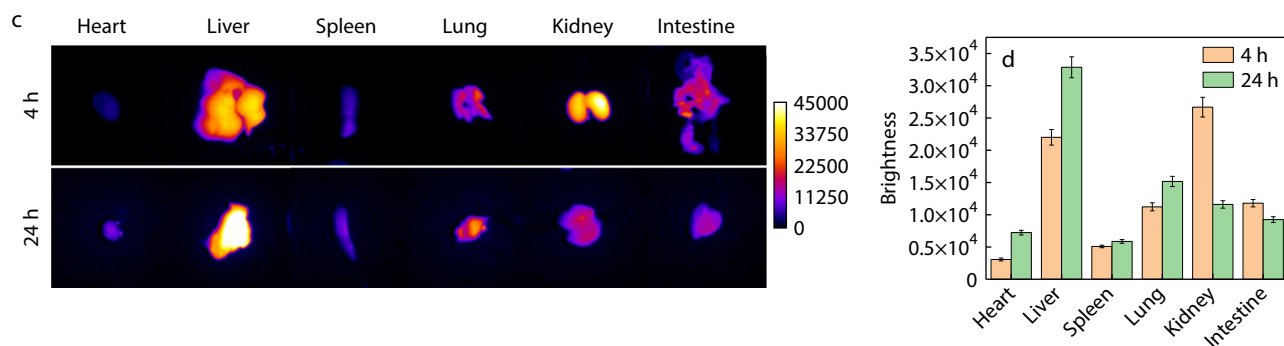


Fig. 7 (a) *In vivo* bioimaging of BALB/c mice from the dorsal or ventral position after intravenous administration of **JNBDP-PMCP₆₄** (1000 mg·L⁻¹, 200 μL) under 808 nm irradiation with a 1000 nm long-pass filter; (b) The normalized brightness ratio of bladder-to-liver intensity plotted as a function of time post-injection of **JNBDP-PMCP₆₄** in living mice; (c) *Ex vivo* NIR-II imaging of main organs at 4 and 24 h after intravenous administration of **JNBDP-PMCP₆₄** (1000 mg·L⁻¹, 200 μL); (d) Mean brightness of the main organs at 4 and 24 h. The data are presented as mean ± s.d., derived from *n*=3 biologically independent mice.

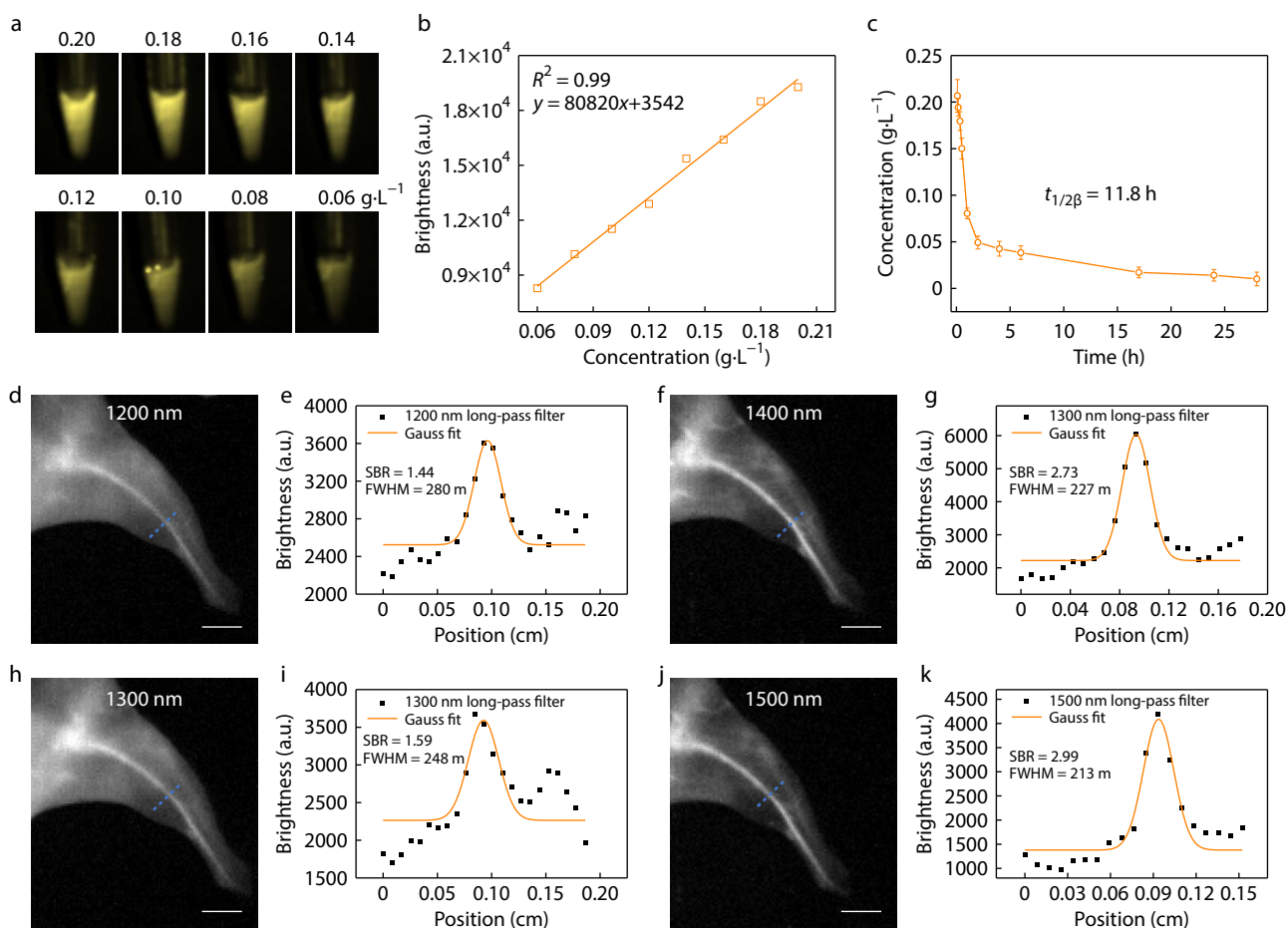


Fig. 8 (a) Concentration-dependent NIR-II brightness of **JNBDP-PMCP₆₄** under 808 nm laser excitation (1000 nm LP); (b) Linear relationship between the concentration of **JNBDP-PMCP₆₄** and the brightness; (c) Blood concentration (g·L⁻¹) decay of **JNBDP-PMCP₆₄** after intravenous injection into BALB/c mice (2 g·L⁻¹, 200 μL); (d, f, h, j) Wide-field whole-body NIR-II imaging of BALB/c mice with sequential long-pass filters after intravenous injection with **JNBDP-PMCP₆₄** (2 g·L⁻¹, 200 μL), scale bars: 2 mm; (e, g, i, k) The corresponding signal-to-background ratio and full width at half maximum (FWHM) of hindlimb vessels with various long-pass filters.

rently, the full width at half maximum (FWHM) decreased from 280 μm (1200 nm LP) to 248 μm (1300 nm LP) and 227 μm (1400 nm LP) to 213 μm (1500 nm LP) (Figs. 8d–8k). Collectively, these results demonstrated the substantial application potential of **JNBDP-PMCP₆₄** in terms of imaging perfor-

mance.

CONCLUSIONS

In conclusion, we designed and synthesized an NIR-II emissive

aza-BODIPY-based ATRP initiator and used it to polymerize zwitterionic monomers, thereby yielding water-soluble polyzwitterionic fluorescent probes. Such probes exhibit excellent colloidal stability and resistance to fluorescence quenching, as well as a high fluorescence quantum yield and prolonged blood circulation half-life, which together enable high-resolution imaging of vascular structures. We propose that the hydrophilic PMCP block in the **JNBDP-PMCP_n** probe warrants particular attention because it critically governs the aggregation behavior of the polymer, which in turn dictates its photophysical properties and subsequently influences both the biodistribution and pharmacokinetic profiles. For practical bioimaging or sensing applications, it is essential to select an appropriate PMCP block length that ensures complete molecular dispersion, thereby fully exploiting the high brightness potential of the JNBDP chromophore while mitigating aggregation-induced adverse effects. Ongoing investigations are focused on the precise modulation of the degree of polymerization to systematically elucidate the structure-property relationships among the molecular weight, self-assembled morphology, and fluorescence performance of the polymeric probes.

Conflict of Interests

The authors declare no interest conflict.



Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://doi.org/10.1007/s10118-026-3797-0>.

Data Availability Statement

The data that support the finding of this study are available from the corresponding author upon reasonable request. The authors' contact information: czhao@ahnu.edu.cn

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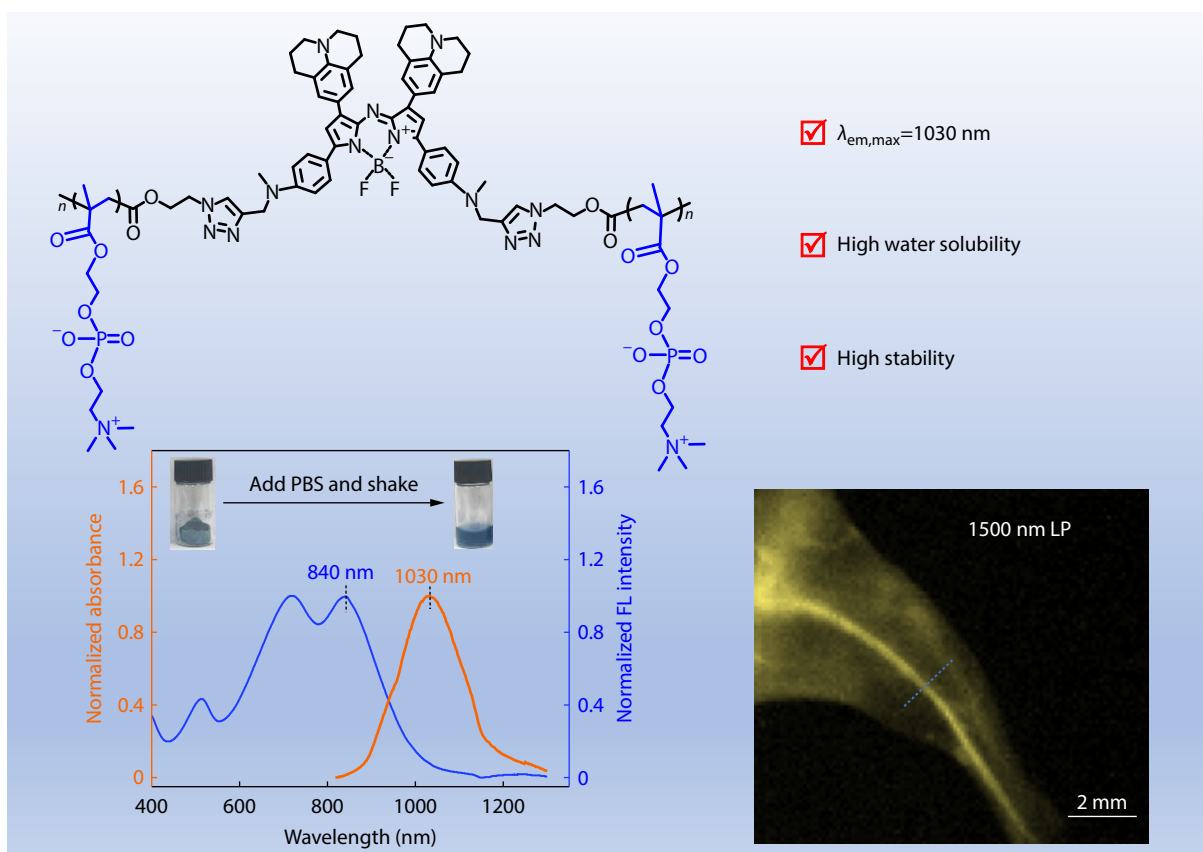
Graphical Abstract

A Water-soluble NIR-II Polyzwitterionic Aza Boron Dipyrromethene Probe with Prolonged Blood Circulation for High-contrast Vascular Imaging

Ju-Li Duan, Xue Shuai, Tian Chen, Sheng-Yu Shi, Yong Fan, Kuo Jia, and Chen-Zhi Yao

Anhui Normal University; Fudan University; Anhui Polytechnic University

A polyzwitterionic NIR-II probe (**JNBDP-PMCP₆₄**) with a high aqueous solubility was developed *via* atom transfer radical polymerization (ATRP), enabling prolonged circulation and high-fidelity vascular imaging.



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