Supporting Information

3,4-Ethylenedithio Thiophene Donor for NIR-II Fluorophores with Improved Quantum Yields

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General information and methods

Materials. Unless otherwise noted, all reagents were obtained commercially and used without further purification. Tetrahydrofuran (THF), toluene, and dimethyl formamide (DMF) used for reactions were purified by a solvent purification system (Innovative Technology, Inc.) before using. All air and moisture sensitive reactions were carried out in flame-dried glass-wares under a nitrogen atmosphere.

Measurements. ^{1}H and ^{13}C NMR spectra were performed on 500 MHz NMR spectrometers (Bruker AVANCE) using $CDCl_3$. Mass spectra were in general recorded on QSTAR Elite (ABI). Ultraviolet-visible-near infrared (UV-VIS-NIR) absorption spectra were recorded on Shimadzu UV-3600Plus. All UV-Vis-NIR measurements were conducted using quartz cuvettes with 1 cm light path and the sample volume was 3 mL. Background measurement was made by using bank solvents without any sample.

Quantum yield test. The fluorescence quantum yields of the fluorophores were measured in a similar way to a previous method.[1] The fluorescence spectra in the region of 900-1500 nm were measured by a spectrometer with a thermoelectrically cooled InGaAs detector (HORIBA Ihr320) under an 808 nm diode laser excitation (Thorlabs lasers, 180 mW). During emission measurements, one 850-nm short pass filter (Thorlabs) was used as the emission filter. The obtained emission spectra were further corrected by the detector sensitivity profile and the absorbance features of the filter. The fluorescence quantum yield was determined against the reference fluorophore **IR-nFE** with a known quantum yield of 3.1% (Φ_{st}) in toluene, which was previously determined with **IR-26** of 0.050% as reference in dichloroethane. All

samples were measured at 25 °C with optical density (*OD*) below 0.1 at 808 nm. The intensity read out from the InGaAs camera was a spectrally integrated total emission intensity in the 900-1400 nm region. Using the measured optical density (*OD*) at 808 nm and spectrally integrated fluorescence intensity (*F*), the quantum yield of the test sample can be calculated according to the following equation:

$$\Phi_x = \Phi_{st} \times \frac{F_x}{F_{st}} \times \frac{A_{st}}{A_x} \times \frac{\eta_x{}^2}{\eta_{st}{}^2}$$
$$= \Phi_{st} \times \frac{F_x}{F_{st}} \times \frac{1 - 10^{-OD_{st}}}{1 - 10^{-OD_x}} \times \frac{\eta_x{}^2}{\eta_{st}{}^2}$$

Φ_{st} and F_{st} are data of the IR-nFE standard, Φ_x and F_x are data of the studied sample. η is the refractive index of solvent.

Encapsulation procedures for NFs. For IR-nFES NFs, 0.5 ml of IR-nFES (2mg/ml in THF) and 0.5ml of DSPE-PEG2000 (4mg/ml in THF) were mixed and then sonicated to obtain a clear solution (1ml). The mixture was quickly injected into 10 mL of deionized water. Sonication was applied to disperse organic components vigorously into water for 2 minutes. The mixture was then stirred for 24 h at 37 °C to remove THF. Finally, the suspension was filtered through a membrane filter (diameter = 220 nm) and subsequently concentrated to 1 mg/mL with a filtration concentrator (Corning, Mw = 3K 3000r/5min). The IR-nFE NFs were prepared by the exactly same procedure. The obtained NPs were stored at 4 °C for further usage.

NIR-II fluorescence imaging. For dynamic NIR-II imaging, IR-nFE NFs, IR-nFES NFs and indocynine green (ICG) in deionized water with the same mass concentration of 20 μg/ml) were utilized for the measurement. NIR-II fluorescence images were

collected using a liquid-nitrogen-cooled, two-dimensional InGaAs array (Princeton Instruments, 640 × 512 pixels). The excitation light was provided by a fiber-coupled 808-nm diode laser (RMPC) with an in-plane excitation power density of 180 mW/cm^2. The light was collimated and filtered through a 4.5 mm collimator and an 850-nm and a 1000-nm short pass filter (Thorlabs). The emission light was filtered using a 900 or 1000 nm long pass filter (Thorlabs), and focused onto the detector to be collected as images.

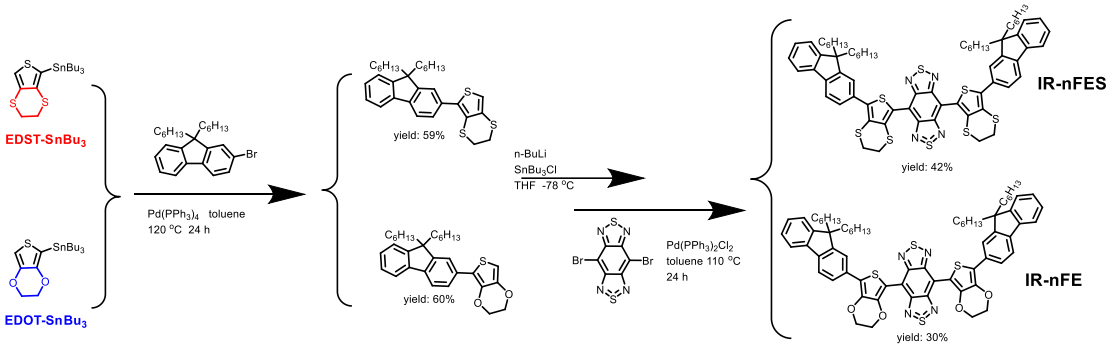
Female BALB/c mice (6-8 weeks) were bought from Beijing Vital River Laboratory Animal Technology Co., Ltd. (China). All procedures were sanctified by Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences Animal Care and Use Committee. Female BALB/c mice were randomly divided into 2 groups (n = 6): (1) Blank control (treated with 0.9% Nacl); (2) IV injection of nanoparticles (NPs dosage = 5 mg kg−1). At 14 days post injection of NPs, the treated mice were euthanized, and the main organs of mice in the two groups were collected for the H&E staining (CM1950, Lecia.).

Blood circulation studies: mice (5, 45, 90, 30, 180, 360, 720, 1440, 2880 min; n = 3 for each group) were intravenous administration with IR-nFES NFs (1 mg/mL, 100 μL) and then placed in metabolic cages. Blood was collected through orbital sinus method at various time points and then imaging by InGaAs NIRvana CCD camera under 808 nm laser excitation with 1200 nm long-pass filter. The concentrations of IR-nFES NFs at various time point were determined by fluorescence intensity.

Density functional theory calculations. All the calculations were performed using the

Gaussian 09 software.[2] To reduce the computational cost, alkyl substituent groups on fluorene units were replaced by methyl groups. The ground-state (S_0) geometries of the simplified structures IR-nFE and IR-nFES were firstly optimized using B3LYP/6-31G(d) method and re-optimized at the tuned-ωB97XD*/6-31G(d) level. The corresponding range-separation parameter (ω, in $Bohr^{-1}$) for each molecule was optimally tuned according to the GAP-tuning method. The excited-state (S_1) geometries of these molecules were optimized using time dependent (TD)-ωB97XD*/6-31G(d) method. The HOMOs and LUMOs of two molecules were obtained at the ωB97XD*/6-31G(d) level based on their optimized S_0 geometries.

Synthetic procedures and characterization data for IR-nFE and IR-nFES



Scheme S1. Synthetic route of molecular fluorophores IR-nFES and IR-nFE.

Synthesis of IR-nFES.

Synthesis of monomer 1. 2-Bomo-9,9-dihexyl-9H-fluorene (1.477 g, 3.5 mmol) and tributyl(2,3-dihydrothieno[3,4-b][1,4]dithiin-5-yl)stannane (1.62 g, 3.5 mmol) were dissolved in toluene (40 mL) under protective gas atmosphere, then $Pd(PPh_3)_4$ (150 mg) was added. After refluxing for 20 h and then cooling to room temperature, the mixture was poured into water and extracted twice with ethyl acetate. The organic phase was

dried with $MgSO_4$ and evaporated *in vacuo*. The crude product was subjected to column chromatography on silica gel with PE/DCM 20:1 as eluent to afford **1** as a light white oil (1.05 mg, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (t, *J* = 8.0 Hz, 2H), 7.54 (s, 1H), 7.50 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.37 – 7.29 (m, 3H), 7.01 (s, 1H), 3.29 – 3.25 (m, 2H), 3.24 – 3.19 (m, 2H), 2.03 – 1.92 (m, 4H), 1.15 – 1.02 (m, 12H), 0.76 (t, *J* = 6.8 Hz, 6H), 0.72 – 0.61 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.11, 151.01, 140.82, 140.48, 136.69, 131.76, 128.66, 128.60, 127.39, 127.22, 126.78, 123.20, 122.86, 119.81, 119.75, 116.95, 55.13, 40.24, 31.46, 29.68, 28.37, 27.67, 23.73, 22.55, 14.01. HRMS (ESI) calcd for C31H39S3+, ([M+H+]) 507.2199, Found 507.2208.

Synthesis of IR-nFES. To a solution of compound **1**(760 mg, 1.5 mmol) in THF (20 mL) at -78 ºC under protection gas atmosphere, *n*-BuLi (1.6 M in hexane, 1.4 mL, 2.2 mmol) was added dropwise. After stirring at this temperature for another 1.5 h, tributyltinchloride (0.76 g, 2.5 mmol) was added to the solution. The reaction mixture was then slowly warmed to room temperature and stirred for 8 h. After that the mixture was poured into water and extracted twice with ethyl acetate. The combined organic phase was dried with MgSO4 and evaporated *in vacuo* without further purification.

To a solution of the crude product (1.19 g, 1.5 mmol) and dibromo-benzobisthiadiazole (DBr-BBTD) (175 mg, 0.5 mmol) in toluene (20 mL) under protection gas atmosphere, $Pd(PPh_3)_2Cl_2$ (60 mg) was added. The mixture was stirred at 110 ºC for 24 h. After cooling to room temperature, the mixture was poured into water and extracted twice with ethyl acetate. The organic phase was dried with $MgSO_4$ and evaporated *in vacuo*. The crude product was subjected to column chromatography on silica gel with PE/DCM

2:1 to afford **IR-nFES** as a dark green solid (252 mg, 42%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.0 Hz, 2H), 7.74 (d, *J* = 5.5 Hz, 4H), 7.70 (d, *J* = 8.0 Hz, 2H), 7.39 – 7.31 (m, 6H), 3.41 – 3.32 (m, 4H), 3.13 (m, 4H), 2.11 – 1.94 (m, 8H), 1.17 – 1.04 (m, 24H), 0.81 – 0.64 (m, 11H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.63, 151.22, 151.08, 141.38, 140.54, 140.44, 131.50, 131.12, 127.88, 127.82, 127.37, 126.83, 124.32, 123.67, 122.89, 119.93, 119.81, 114.73, 55.23, 40.30, 31.51, 29.71, 29.05, 28.46, 23.80, 22.58, 14.04. HRMS (ESI) calcd for C68H74N4S8+, ([M+H+]) 1203.3727, Found 1203.3751.

Synthesis of IR-nFE. The synthesis procedures for **IR-nFE** are same with that for **IR-nFES**.

Synthesis of 2. (996 mg, 60%).^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.62 (m, 4H), 7.40 – 7.27 (m, 3H), 6.31 (s, 1H), 4.32 (m, 4H), 2.06 – 1.90 (m, 4H), 1.18 – 0.99 (m, 12H), 0.76 (t, *J* = 6.8 Hz, 6H), 0.72 – 0.58 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.45, 150.83, 145.20, 140.66, 140.58, 133.11, 128.00, 127.06, 126.77, 124.82, 124.41, 122.81, 122.79, 120.12, 119.99, 119.65, 55.11, 40.39, 31.45, 29.67, 23.69, 22.55, 13.99. HRMS(ESI) calcd for C31H39O2S+, ([M+H+]) 475.2657, Found 475.2665.

Synthesis of IR-nFE. (170 mg, 30%).^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, *J* = 9.5 Hz, 2H), 7.77 (s, 2H), 7.75 – 7.69 (m, 4H), 7.37 – 7.28 (m, 6H), 4.56 – 4.48 (m, 4H), 4.43 – 4.32 (m, 4H), 2.08 – 1.93 (m, 8H), 1.18 – 0.99 (m, 24H), 0.77 (t, *J* = 7.0 Hz, 12H), 0.74 – 0.60 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.57, 151.12, 151.00, 141.89, 140.76, 140.33, 138.24, 131.57, 127.02, 126.74, 125.44, 122.84, 122.80, 120.77, 119.77, 119.66, 113.11, 108.66, 64.68, 64.56, 55.17, 40.40, 31.48, 29.68, 23.71,

22.56, 14.02. HRMS (ESI) calcd for C68H74O4N4S4+, ([M+H+]) 1139.4670, Found 1139.4666.

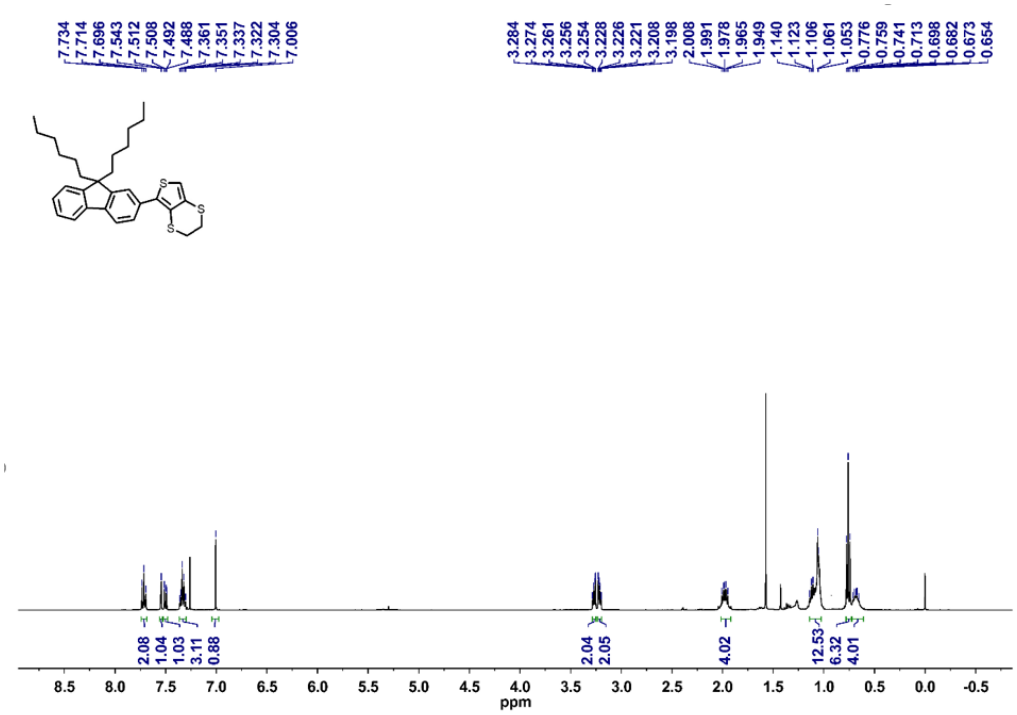


Figure S1. ^{1}H NMR of monomer 1.

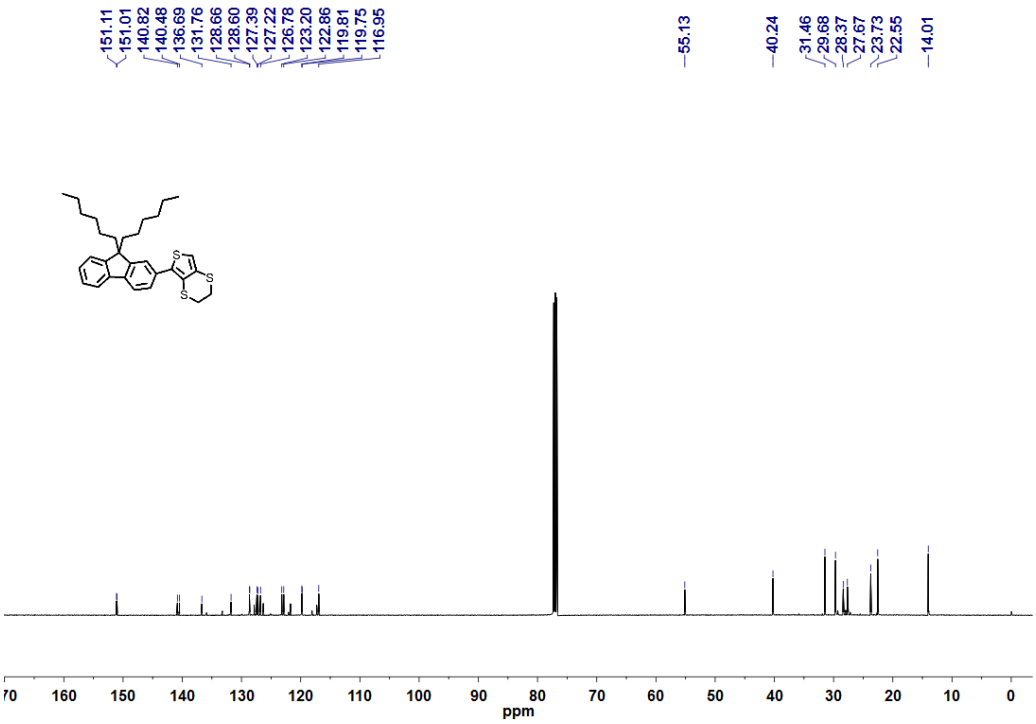


Figure S2. ^{13}NMR of monomer 1.

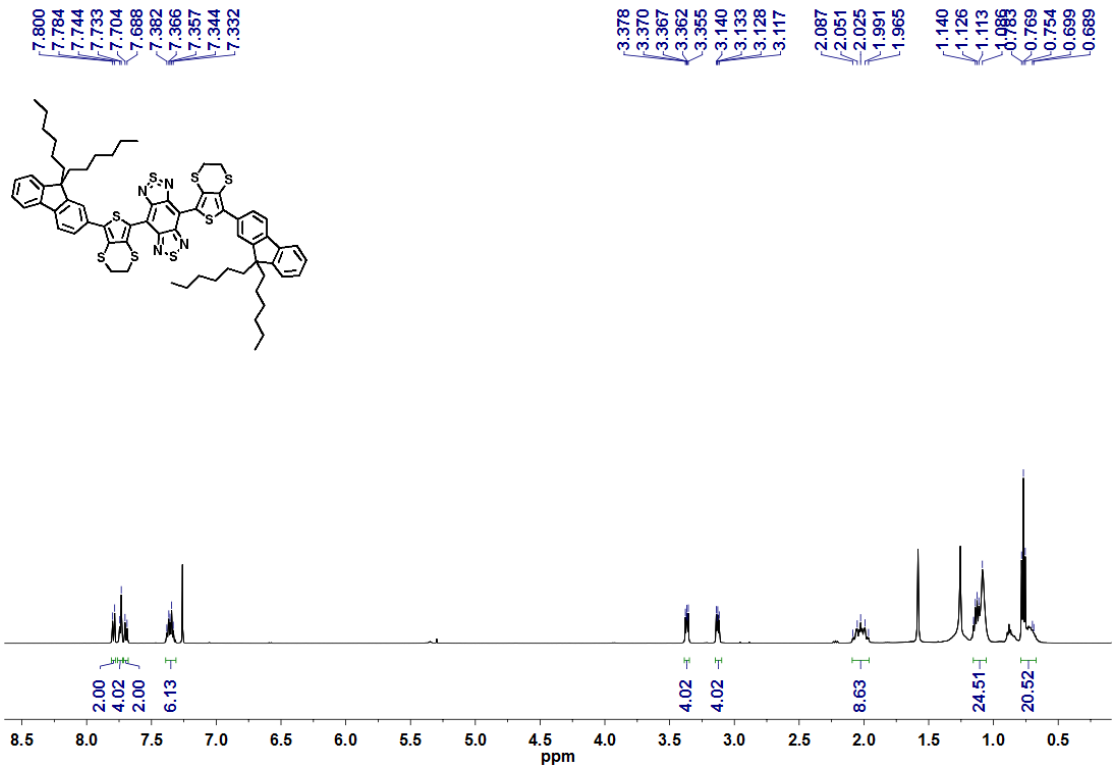


Figure S3. ^{1}H NMR of IR-nFES.

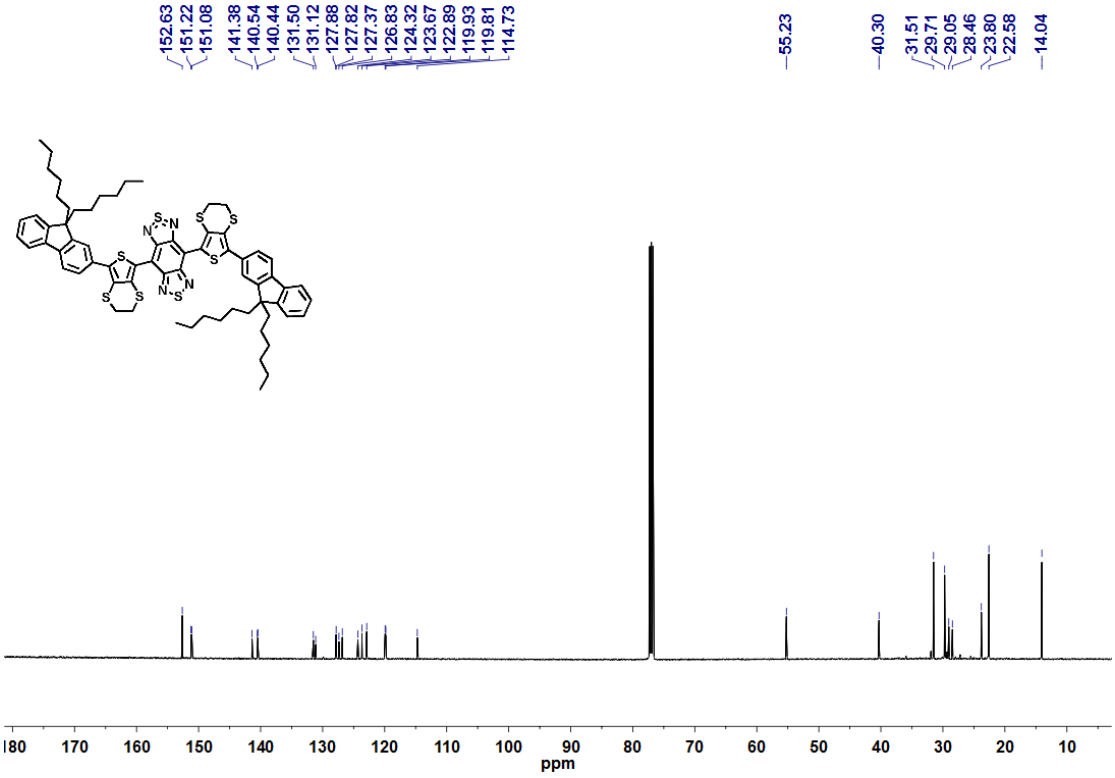


Figure S4. ^{13}NMR of IR-nFES.

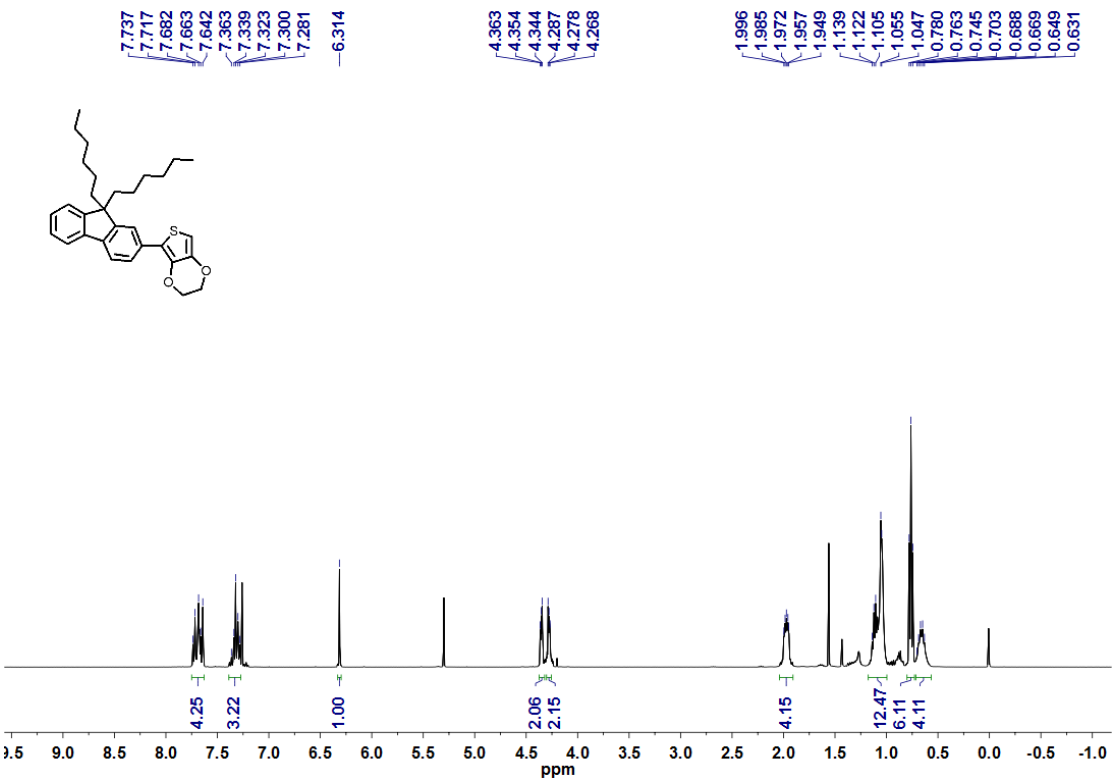


Figure S5. ^{1}H NMR of monomer 2.

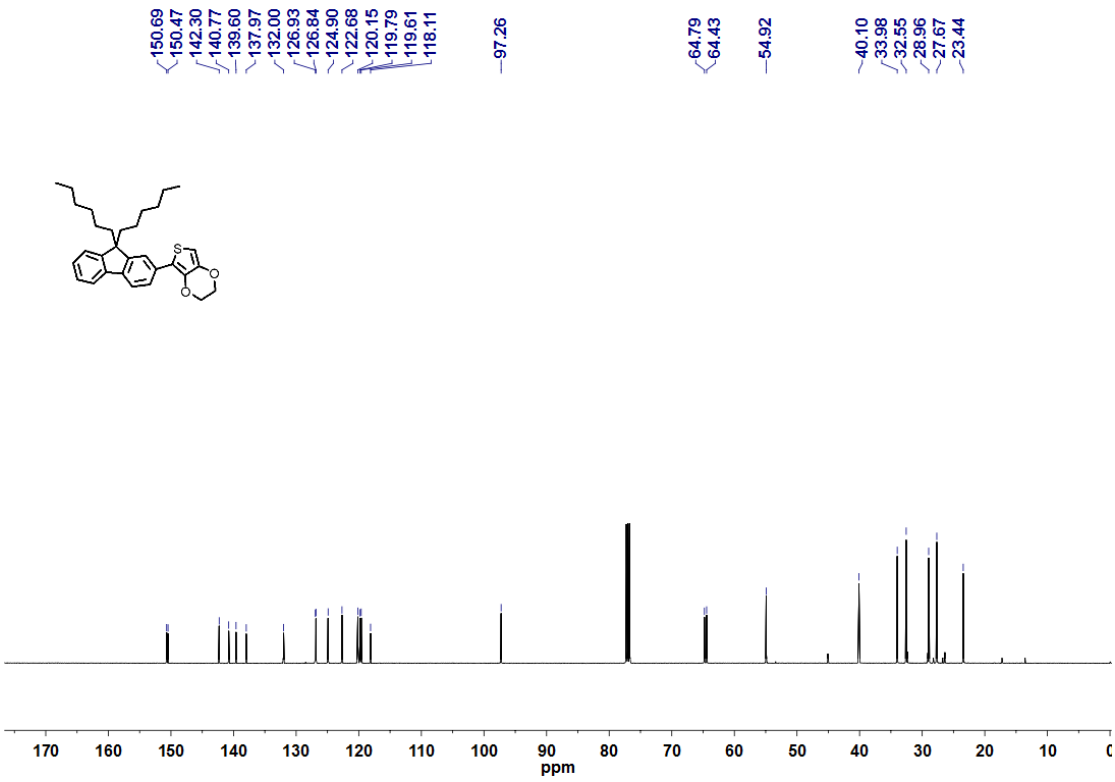


Figure S6. ^{13}C NMR of monomer 2.

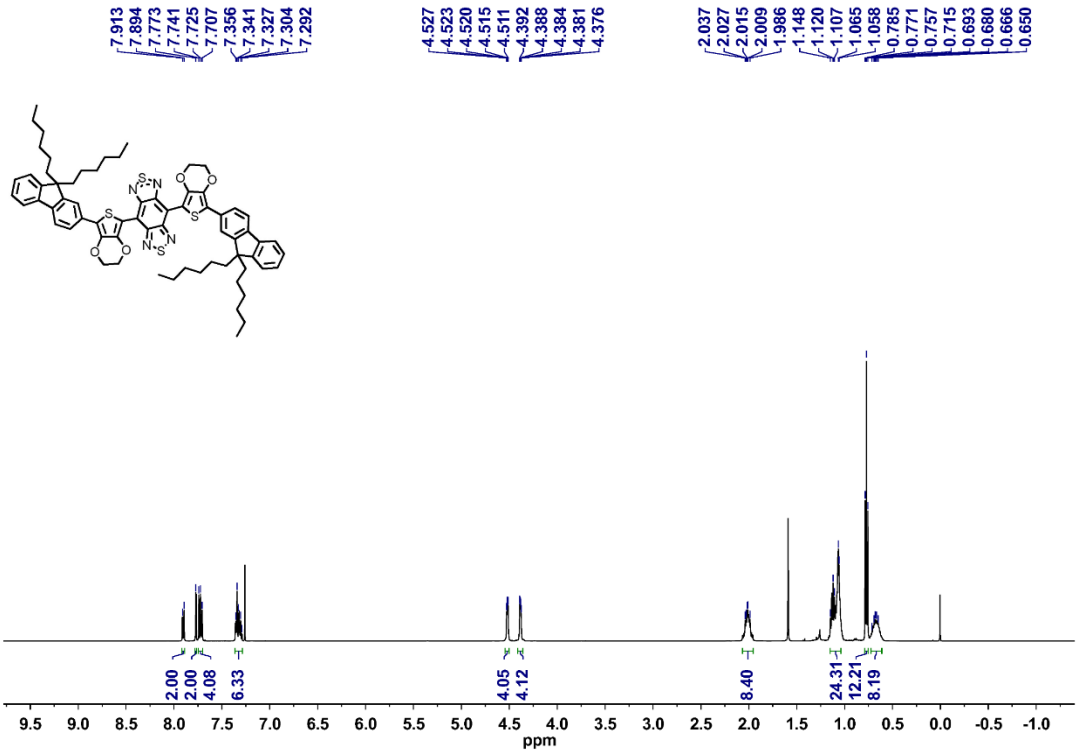


Figure S7. ^{1}H NMR of IR-nFE.

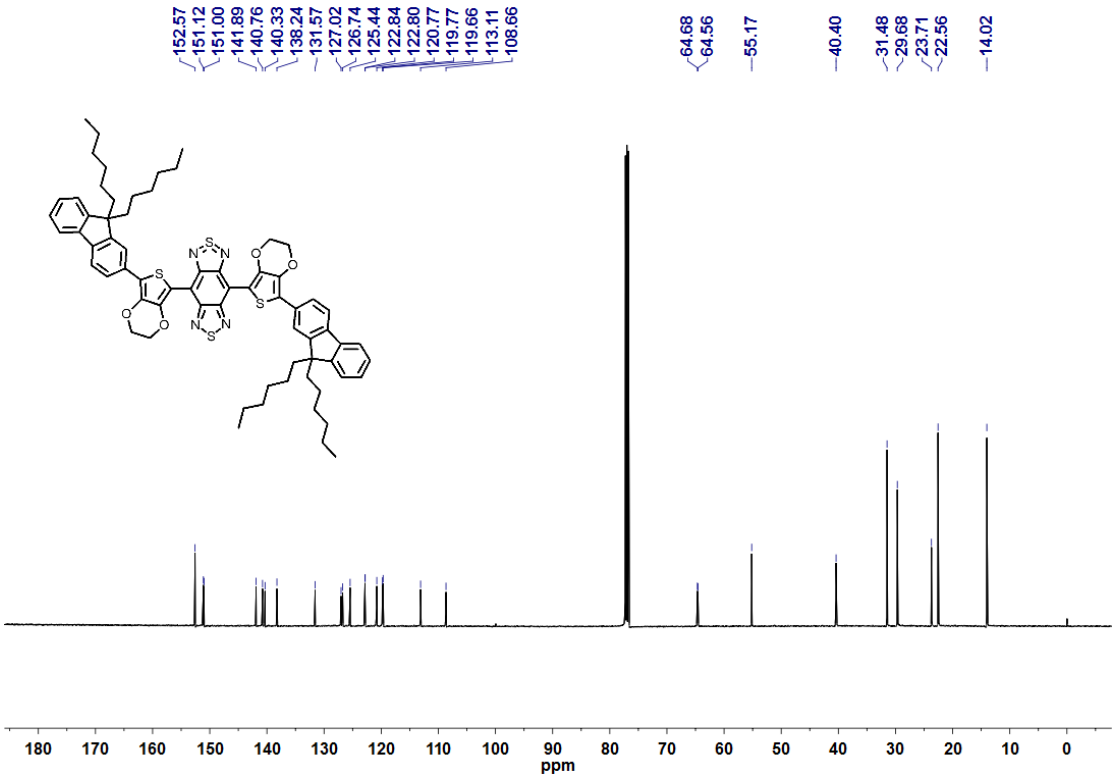


Figure S8. ^{13}C NMR of IR-nFE.

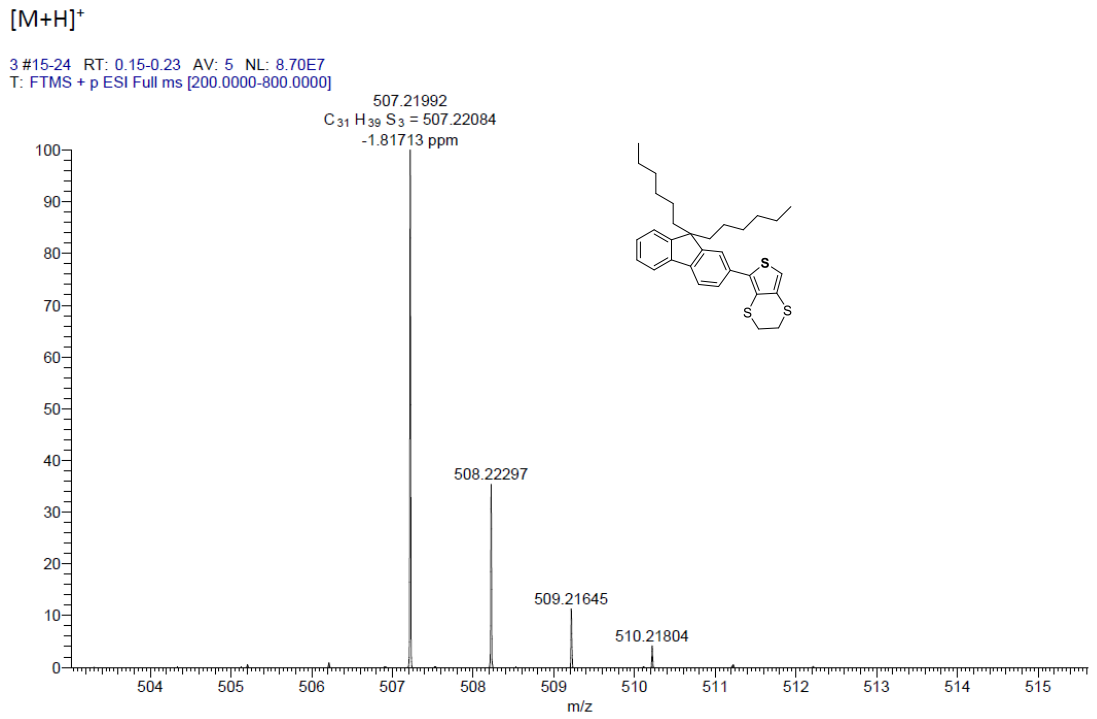


Figure S9. HR-Mass of monomer 1.

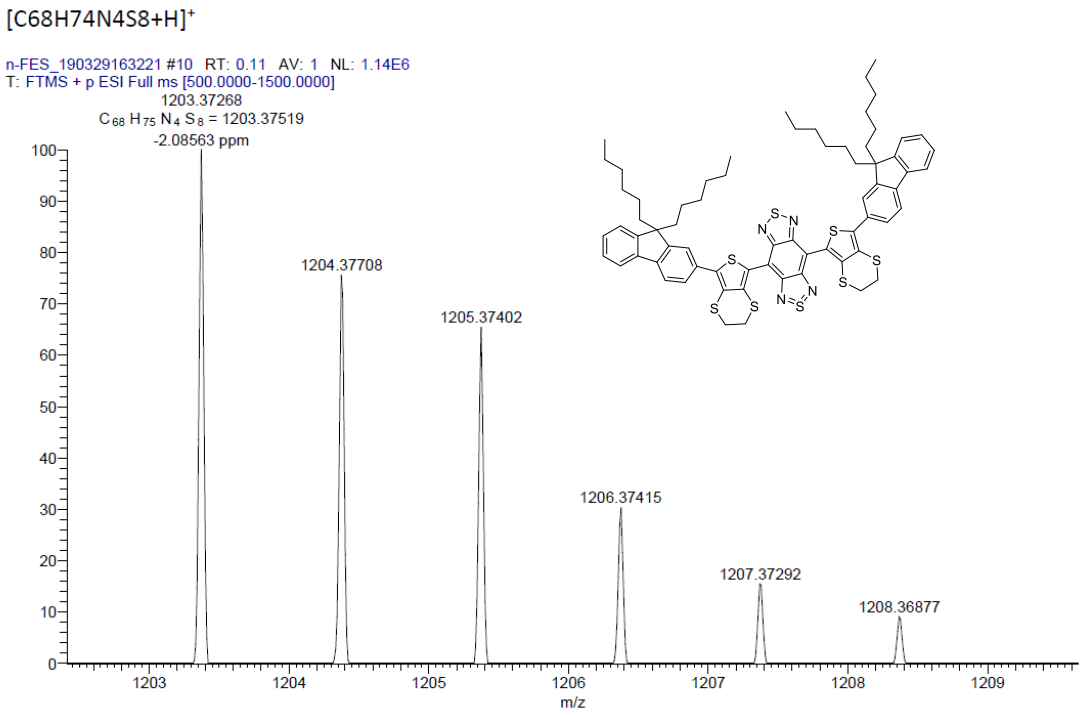


Figure S10. HR-Mass of IR-nFES.

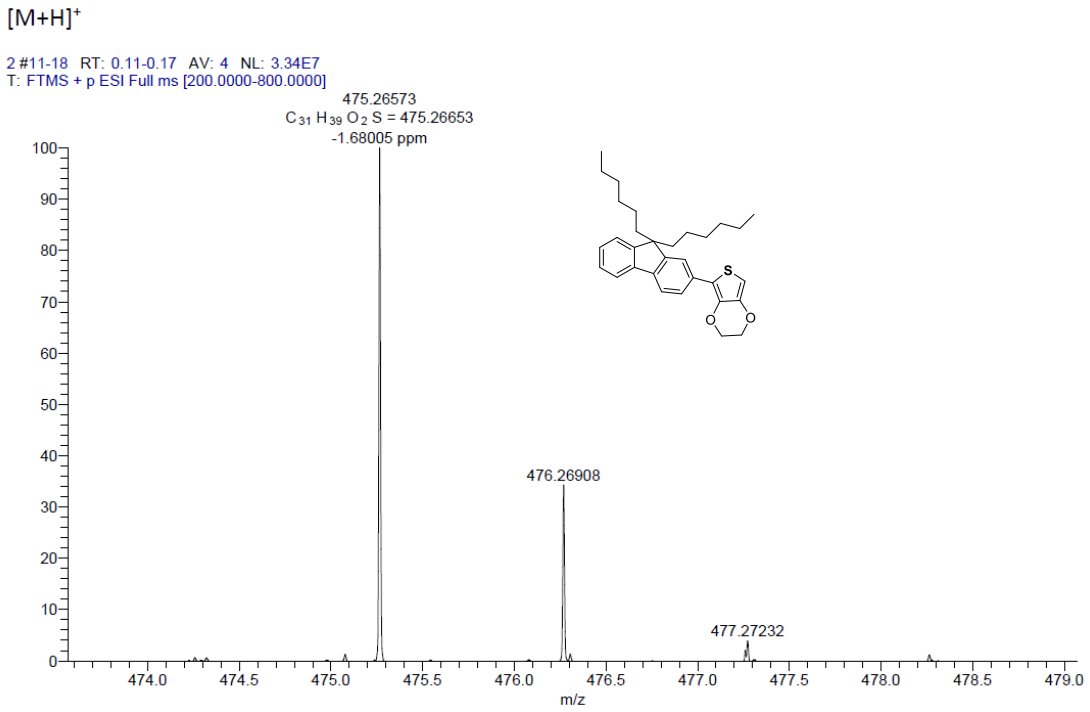


Figure S11. HR-Mass of monomer 2.

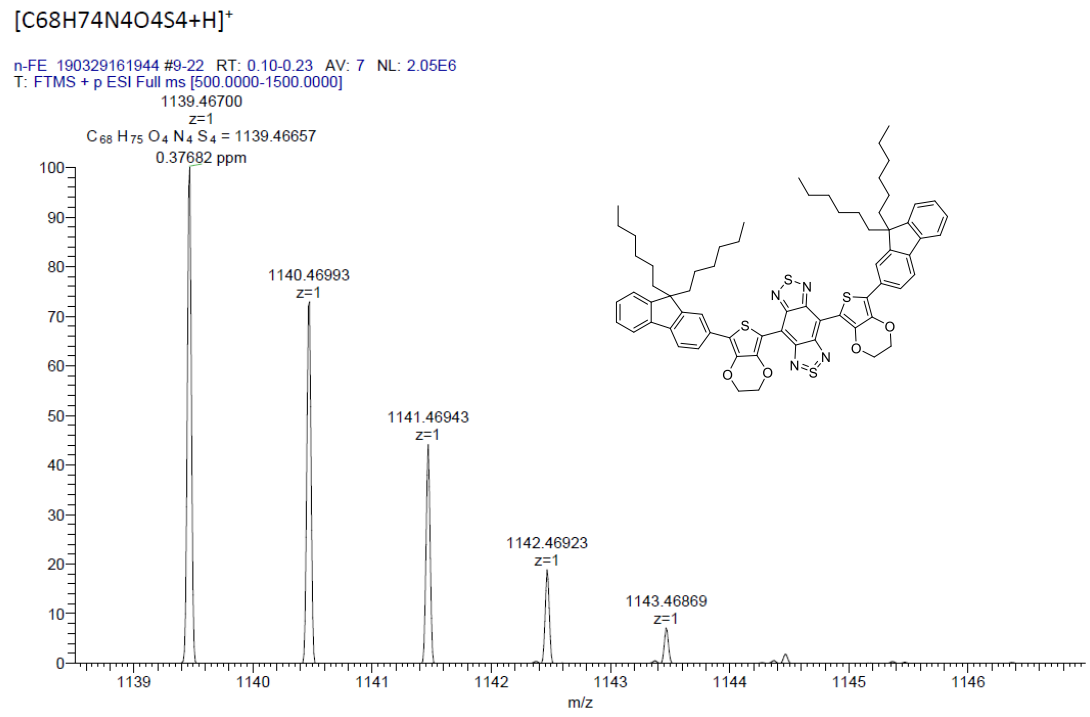


Figure S12. HR-Mass of IR-nFE.

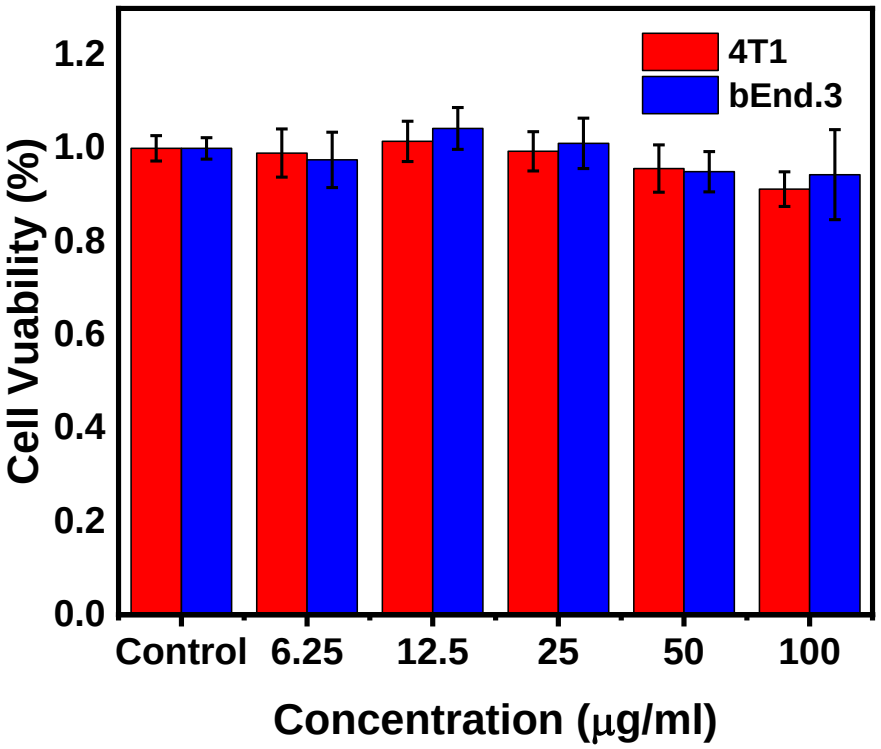


Figure S13. The viability of IR-nFES NFs treated 4T1 and bEnd.3 cells in the dark.

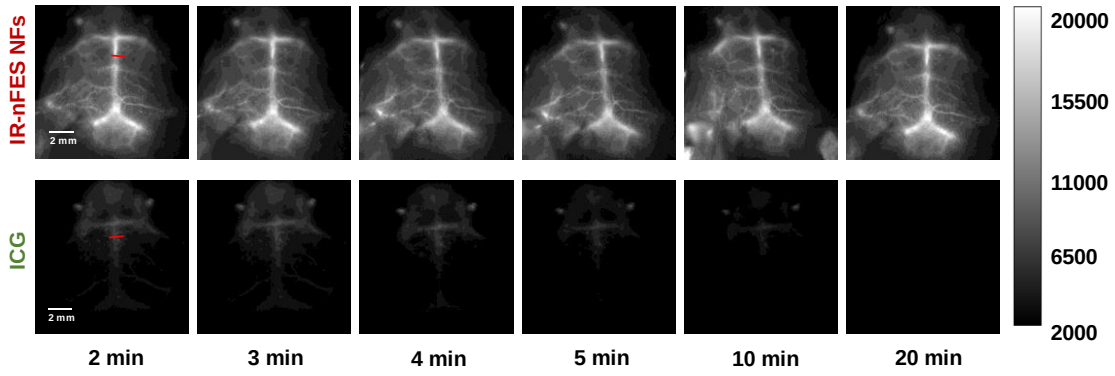


Figure S14. Non-invasive *in vivo* NIR-II imaging of cerebral vessels of IR-nFES NFs and ICG with same concentration (150 μL, 1 mg/mL) in PBS at 1300 nm long-pass (LP) filter. The red line represents the cross-section of the vessels. An 808 nm laser was used for excitation, providing a power density of 150 $mW \cdot cm^{-2}$ and exposure time of 100 ms.

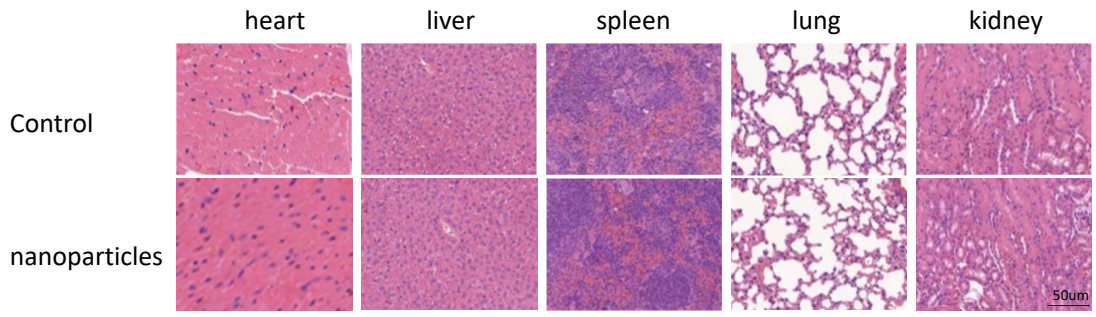


Figure S15. The hematoxylin and eosin (H&E) stain of organs in BALB/c bearing mice after injection of IR-nFES NFs.

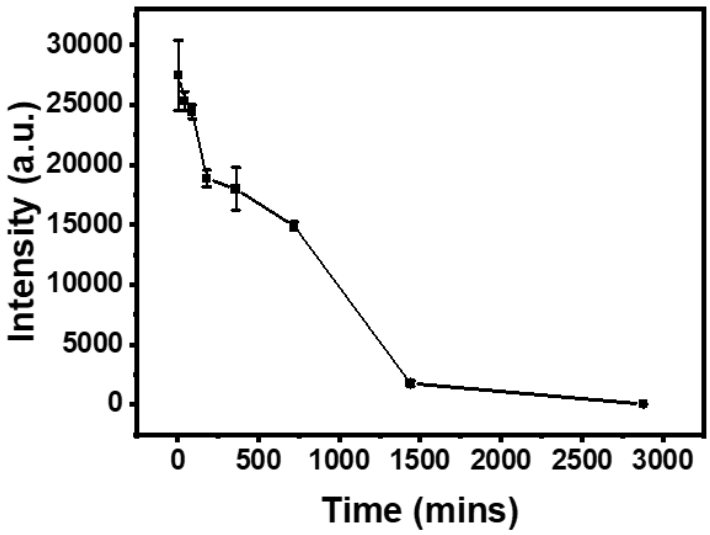


Figure S16. Blood circulation of IR-nFES NFs within 48 hours after intravenous injection

References

[1] Q. Yang, Z. Ma, H. Wang, B. Zhou, S. Zhu, Y. Zhong, J. Wang, H. Wan, A. Antaris, R. Ma, X. Zhang, J. Yang, X. Zhang, H. Sun, W. Liu, Y. Liang and H. Dai, *Adv. Mater.*, 2017, **29**, 1605497.

[2] M. Frisch, G. Trucks, H. Schlegel, G. Scuseria, M. Robb, J. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. Petersson, Inc., Wallingford, CT 2009, 4.