

Supporting Information

Maximal Emission beyond 1200 nm Dicyanovinyl-Functionalized Squaraine for in vivo Vascular Imaging

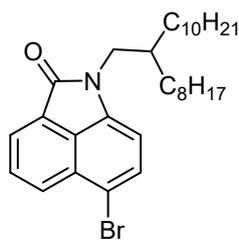
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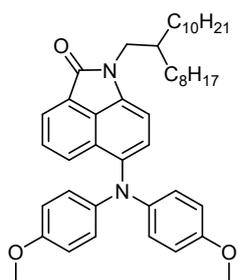
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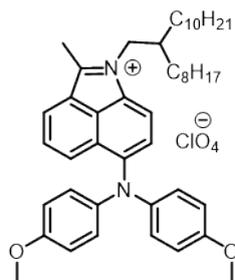
Y. W., and M. W. contributed equally to this work.



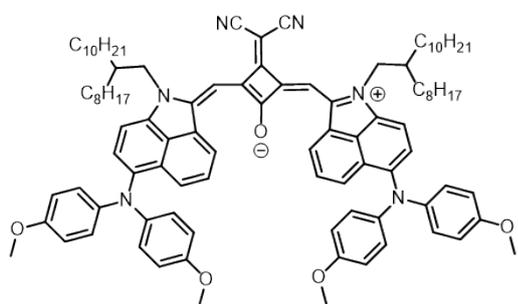
Synthesis of compound **3**. compound **2** (2.48 g, 10 mmol), 1-Bromo-2-octyldodecane (10.8 g, 30 mmol), and K_2CO_3 (8.29 g, 60 mmol) were dissolved in acetonitrile (50 ml). After the mixture was refluxed for 72 h, the solvent was evaporated under reduced pressure, and the mixture was extracted with ethyl acetate (2×50 ml) and water. The organic layer was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure to obtain the crude product and further purified via solid silica gel column chromatography (Petroleum ether / Ethyl acetate = 50/1) to afford compound **3** as a yellow oil (4.03 g, 76.3% yield). 1H NMR (400 MHz, Chloroform-*d*): δ 8.10 (d, J = 8.3 Hz, 1H), 8.05 (d, J = 7.0 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.61 (d, J = 7.5 Hz, 1H), 6.70 (d, J = 7.5 Hz, 1H), 3.75 (d, J = 7.4 Hz, 2H), 1.93 (p, J = 6.3 Hz, 1H), 1.32 (q, J = 5.9 Hz, 8H), 1.23 (d, J = 16.7 Hz, 23H), 0.86 (q, J = 6.5 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*): δ 167.61, 139.69, 131.10, 130.11, 129.57, 128.82, 126.87, 126.09, 124.99, 113.76, 106.10, 44.76, 37.24, 31.89, 31.84, 31.58, 29.92, 29.59, 29.55, 29.50, 29.31, 29.25, 26.49, 22.67, 22.63, 14.10, 14.09. TOF-MS m/z : calculated for compound **6**; $[M+H]^+$ $C_{31}H_{47}BrNO^+$: 528.2836, found: 528.2842.



Synthesis of compound **4**. 4,4'-dimethoxy-4-biphenylamin (1.374 g, 6 mmol), compound **3** (2.64 g, 5 mmol), sodium tert-butoxide (1.44 g, 15 mmol), and tri-tert-butylphosphine tetrafluoroborate (4.35 g, 15 mmol) was added to toluene (50 mL). The mixture was then purged with argon for 30 min. $Pd_2(dba)_3$ (458 mg, 0.50 mmol) was added to the mixture. The reaction was stirred at 110 °C for 16 h under Ar. After cooling to room temperature, the solvent was evaporated under reduced pressure, and the resulting mixture was extracted with ethyl acetate (2×100 ml) and water. The organic layer was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure to obtain the crude product and further purified by solid silica gel column chromatography (Petroleum ether / Ethyl acetate = 50/1) to afford compound **4** as a clear red oil (2.31 g, 68.3 % yield). 1H NMR (400 MHz, Chloroform-*d*) δ 7.99 (d, J = 6.9 Hz, 1H), 7.77 (d, J = 8.3 Hz, 1H), 7.52 – 7.45 (m, 1H), 7.03 (d, J = 7.5 Hz, 1H), 6.97 (d, J = 8.6 Hz, 4H), 6.81 – 6.73 (m, 5H), 3.76 (m, J = 7.4 Hz, 8H), 1.95 (s, 1H), 1.36 (d, J = 5.1 Hz, 6H), 1.23 (s, 25H), 0.86 (d, J = 4.0 Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*): δ 168.37, 154.85, 142.79, 140.07, 136.50, 128.88, 128.08, 127.18, 126.85, 126.48, 125.62, 124.22, 124.09, 114.50, 106.26, 55.48, 44.66, 37.44, 31.92, 31.89, 31.58, 30.01, 29.64, 29.59, 29.36, 29.31, 26.55, 22.71, 22.68, 14.16, 131.52, 131.49, 131.47, 129.71, 129.66, 129.49, 128.37, 125.58, 121.56, 109.99, 106.67, 91.77, 38.59, 13.94. TOF-MS m/z : calculated for compound **4**, $[M+H]^+$ $C_{45}H_{61}N_2O_3^+$: 677.4667, found: 677.4585.



Synthesis of compound 5. Methylmagnesium chloride (2.0 mL, 3 M solutions in THF, six mmol) was added to a solution of compound 4 (1.35 mg, two mmol) in anhydrous THF (10 mL) at 0 °C. The mixture was stirred at 60 °C for two hours. After cooling to 0 °C, water (0.5 mL) and then perchloric acid (70% solution in water, 2 mL) were added to the reaction mixture. The orange-red solution obtained was extracted with dichloromethane (2×50 ml) and water. The organic layer was dried over sodium sulfate and filtered. The solvent was removed under reduced pressure to obtain the crude product as an orange-red oil. The resulting crude product was used directly in the next step without purification.



Synthesis of NSQ1270. A solution of compound 5 (2 mmol) and compound 6 (290 mg, one mmol) in toluene (10 mL) and n-butanol (10 mL) was heated to reflux in a Dean-Stark apparatus for one h. After the completion of the reaction, the mixture was concentrated in a vacuum. The residue was purified

by solid silica gel column chromatography (Petroleum ether / Ethyl acetate = 15/1) to afford **NSQ1270** as a black solid (477 mg, 46.7% yield). ¹H NMR (400 MHz, Tetrahydrofuran-*d*₈): δ 8.51 (d, J = 7.2 Hz, 2H), 7.58 (d, J = 8.1 Hz, 2H), 7.41 (t, J = 7.7 Hz, 2H), 7.03 (d, J = 7.8 Hz, 2H), 6.94 – 6.83 (m, 10H), 6.69 (d, J = 8.5 Hz, 10H), 4.01 (d, J = 6.2 Hz, 4H), 3.61 (s, 12H), 2.11 (s, 2H), 1.34 (s, 12H), 1.15 (d, J = 10.6 Hz, 50H), 0.75 (t, J = 6.7 Hz, 12H). ¹³C NMR (101 MHz, Tetrahydrofuran-*d*₈) δ 173.04, 166.60, 161.29, 155.62, 149.93, 142.44, 142.15, 137.19, 131.08, 129.82, 127.74, 127.61, 126.12, 125.82, 124.60, 123.69, 117.07, 114.17, 113.83, 109.72, 93.25, 54.26, 42.44, 37.46, 31.53, 31.51, 31.01, 29.62, 29.26, 29.17, 29.12, 29.03, 28.97, 28.92, 25.93, 24.38, 23.97, 23.78, 23.58, 22.22, 13.17, 13.16. TOF-MS *m/z*: calculated for NSQ1270, [M+H]⁺ C₉₉H₁₂₃N₆O₅⁺: 1475.9550, found: 1475.9437.

4. Determination of fluorescence quantum yield (QY) of the dyes

The fluorescence quantum yield (ϕ) was measured following the previous method. [2] Briefly, ϕ was calculated by using the following equation:

$$\phi_X = \phi_S \frac{R_X \left(\frac{\eta_X}{\eta_S} \right)^2}{R_S}$$

where the subscripts *S* and *X* mean the standard and test samples, respectively. *R* is the slope from the integrated emission intensity versus absorbance. η is the refractive indices of solvents. NSQ1270 were measured by using IR-26 ($\phi = 0.05\%$ in dichloroethane) as a standard. [3]

NSQ1270 and IR-26 were excited at 1064 nm. The absorbance of each dye was below 0.05 at the excited wavelength.

5. DFT Calculations

Density functional theory (DFT) calculations were performed on Gaussian 09 program. [4] The single molecular geometries and the frontier orbital energy levels were obtained by the Becke three-parameter hybrid functional combined with Becke-Lee-Yang-Parr correlation functional (B3LYP) with 6-31G(d) basis sets. Theoretical predictions on all long alkyl substituents were replaced with methyl groups in the calculations.

6. Fabrication of NSQ1270@BSA NCs.

BSA (100 mg) was dissolved in 5 mL of water, and then one equivalent amount of NSQ1270 methanol solution (1.0 mL) was added to the BSA solution dropwise with vigorous ultrasonic (19 W) at room temperature; consequently, NSQ1270@BSA NCs were obtained. Glutaraldehyde solution (25%, 5 μ L) was added to cross-link NCs for 12 h at room temperature. An optically clear aqueous dispersion containing NSQ1270@BSA NCs was prepared, which was then purified with a 0.22 μ m PES membrane syringe-driven filter. The final aqueous dispersions were obtained by ultrafiltration centrifuge filtration (100 kDa filter, 6000 rpm for 20 min) tube for further experiments.

7. Cell culture

B16F10 tumor cells were incubated in Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in humidified atmosphere of 5% CO₂, and then the cells were collected for the following cell experiments.

8. Animal Experiments.

The use of mice in this study was in accordance with the Regulations for the Administration of Laboratory Animals in China and obtained the ethical approval of the Animal Protection and Utilization Committee of Nanchang University (NCU-ACC-202210134681). C57BL/6 mice (18–20 g, male) were obtained from the Hunan Laboratory Animal Co., Ltd. (Changsha, China). The mice were housed under specific-pathogen-free (SPF) conditions at 24 °C with a 12 h light–dark cycle and fed with standard laboratory water and chow.

9. NIR-II Fluorescence Vascular Imaging of Mice

NSQ1270@BSA NCs (100 μ L, 0.5 mg/mL) were intravenously injected. NIR-II fluorescence imaging was explored at whole-body vasculature of living mice upon exposed to 1064 nm laser (1 W/cm²) irradiation, and signals were collected by a 1200 nm LP filter. And then, the mice were sacrificed.

10. Supplementary Figures and tables.

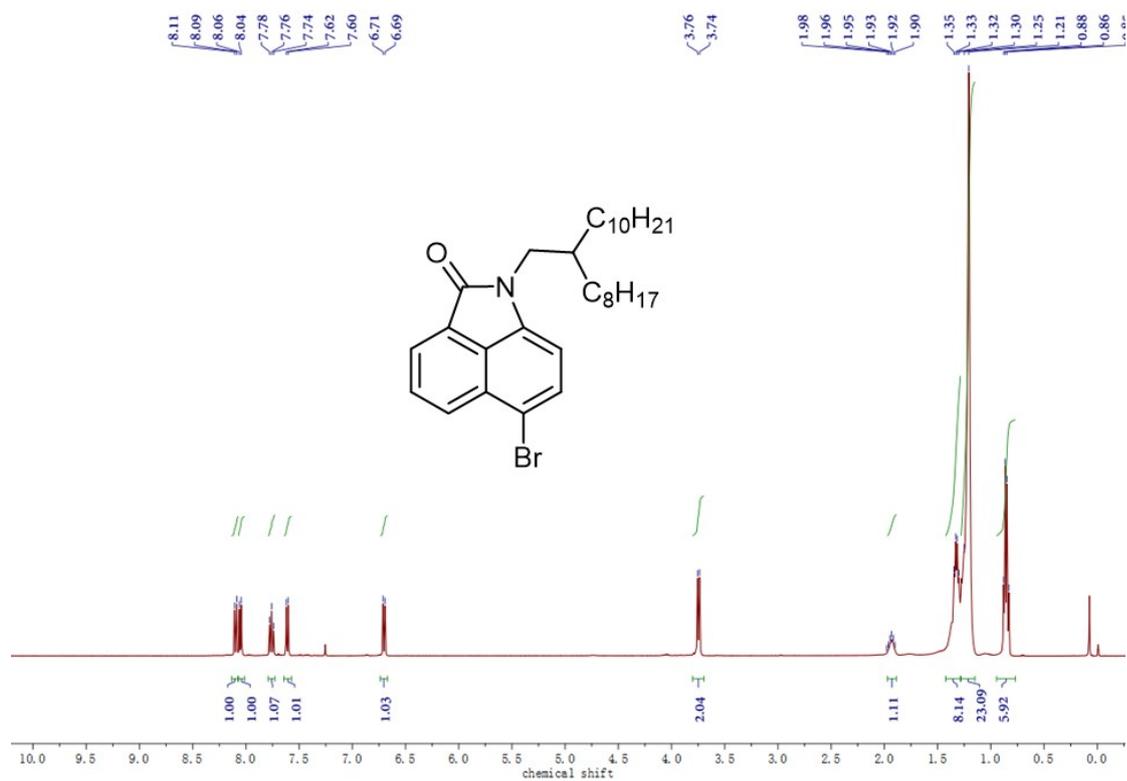


Figure S1. ¹H-NMR spectrum of Compound 3 in Chloroform-*d*.

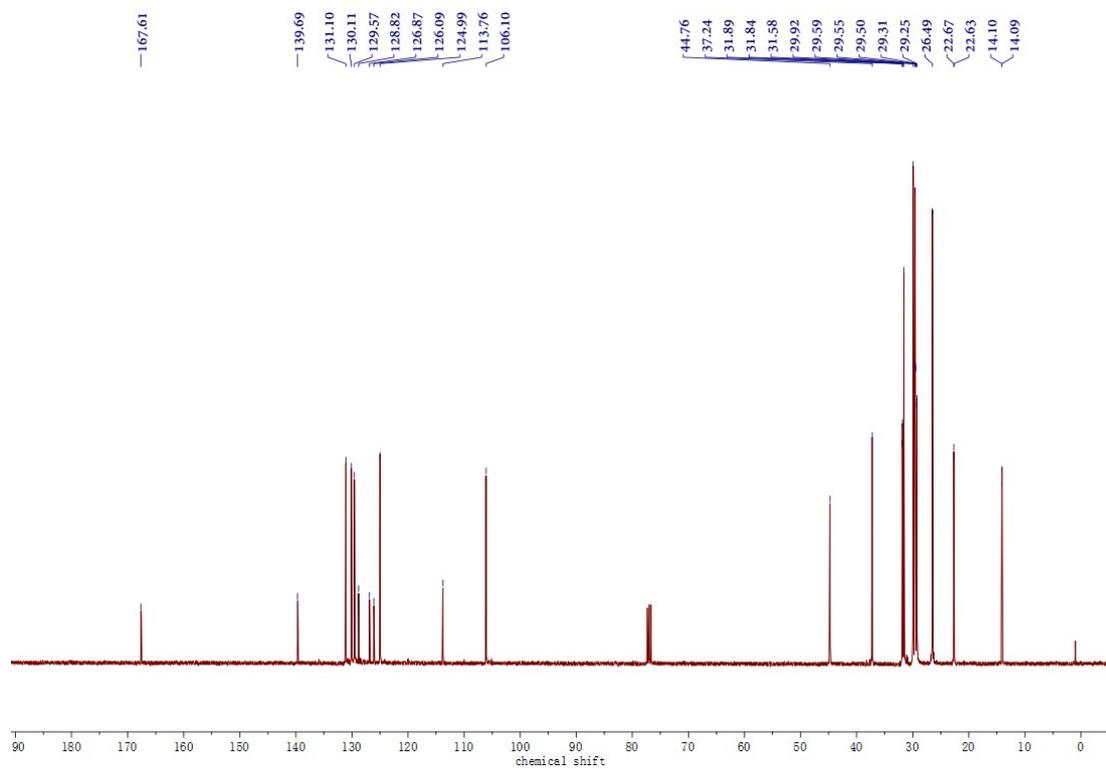


Figure S2. ¹³C-NMR spectrum of Compound 3 in Chloroform-*d*.

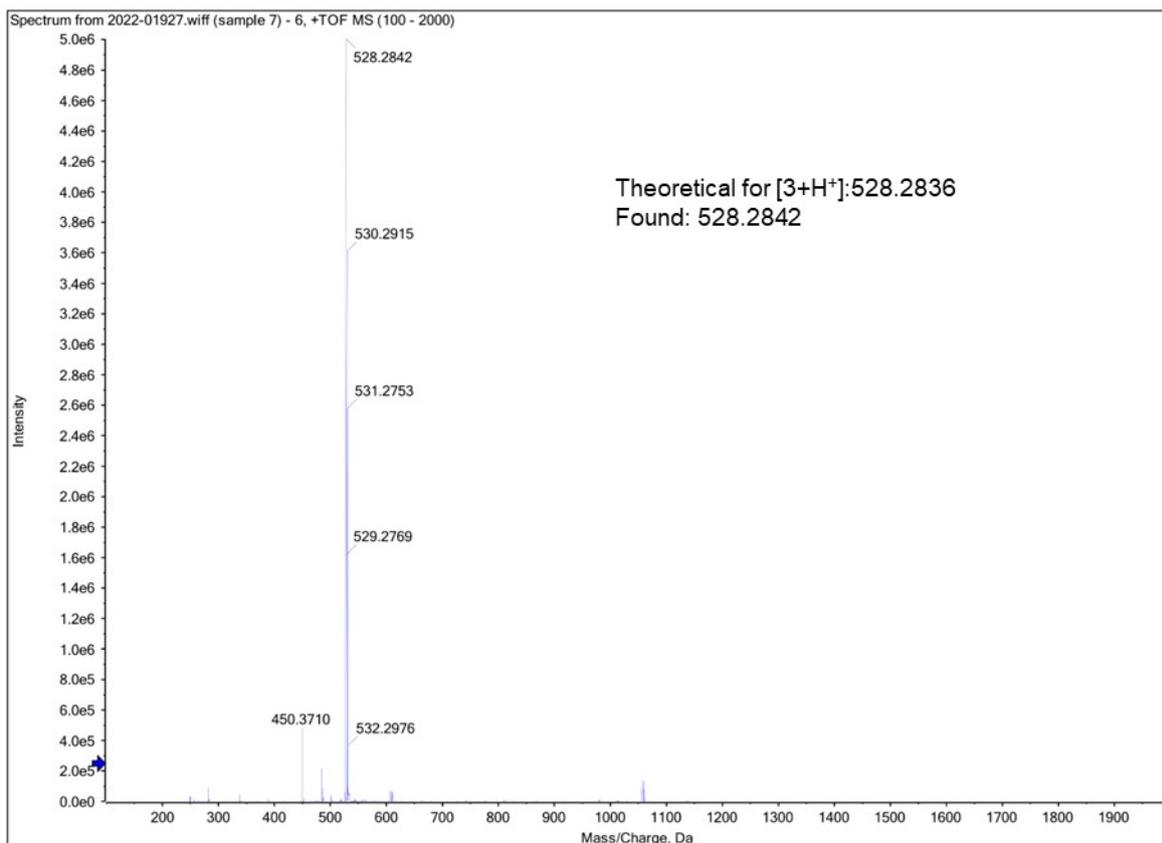


Figure S3. TOF-MS mass spectrum of Compound 3.

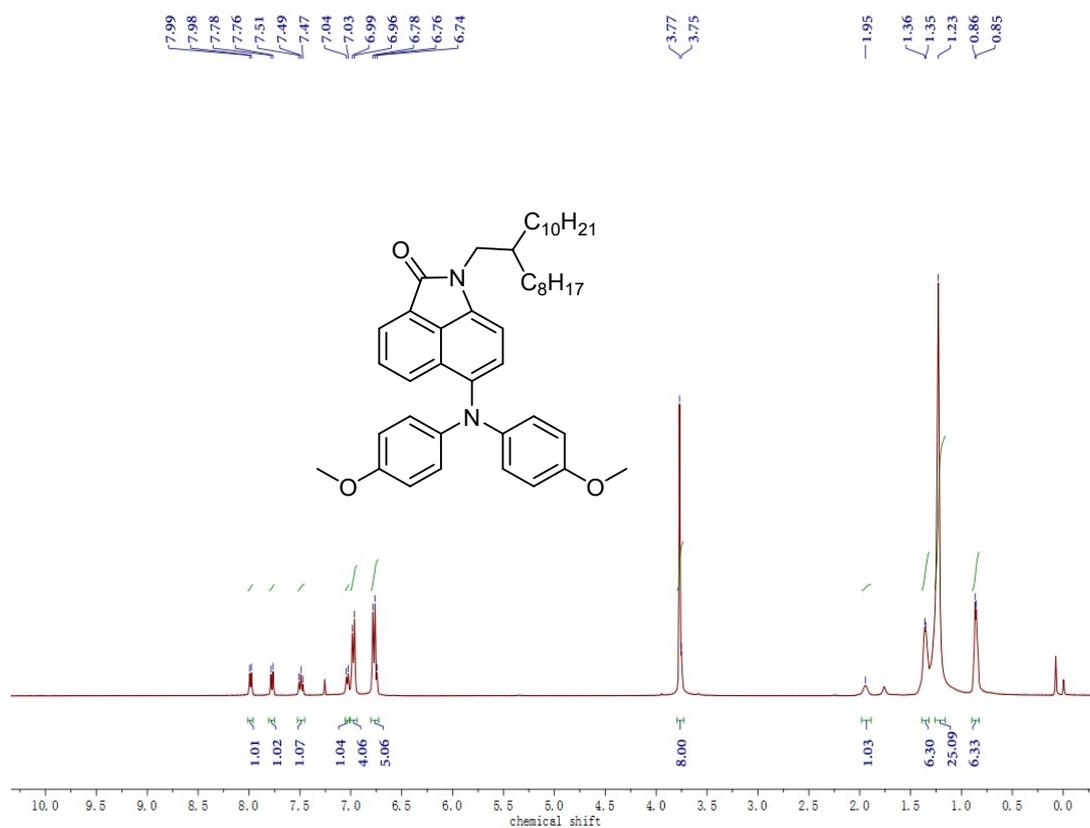


Figure S4. $^1\text{H-NMR}$ spectrum of Compound 4 in Chloroform-*d*.

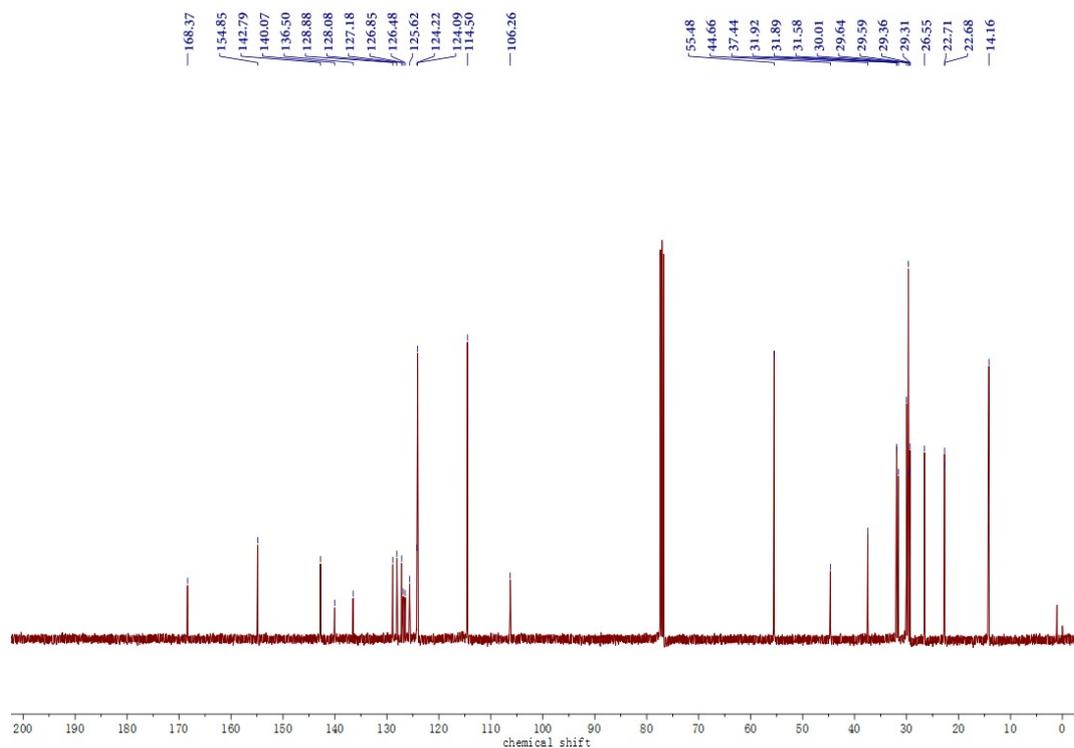


Figure S5. ^{13}C -NMR spectrum of Compound 4 in Chloroform-*d*.

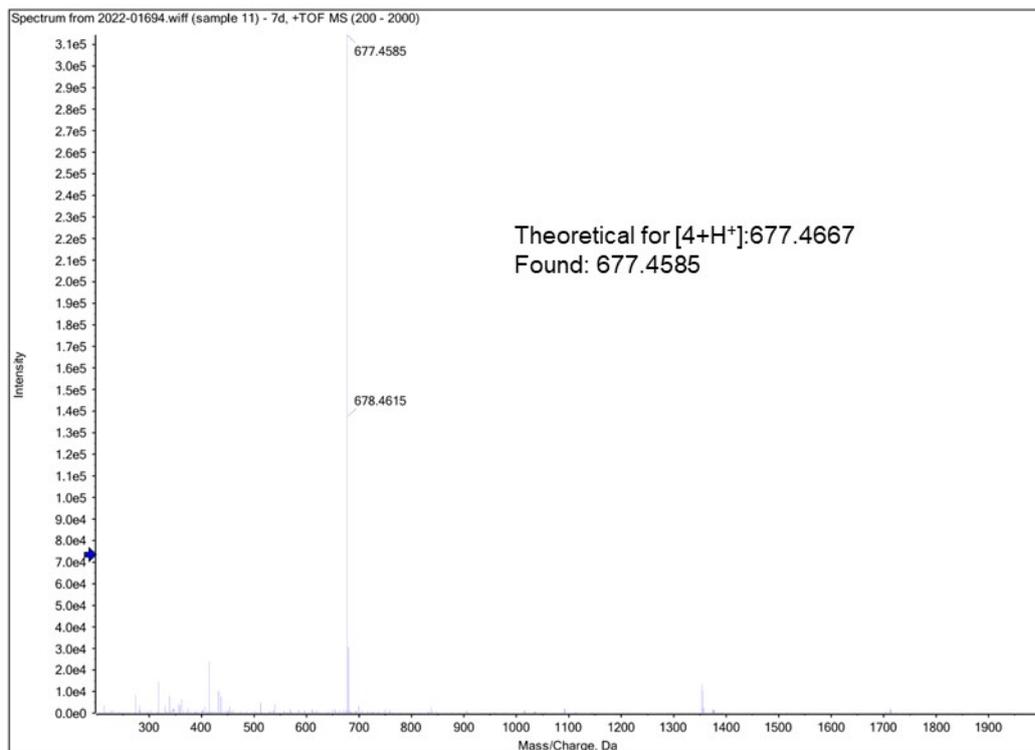


Figure S6. TOF-MS mass spectrum of Compound 4.

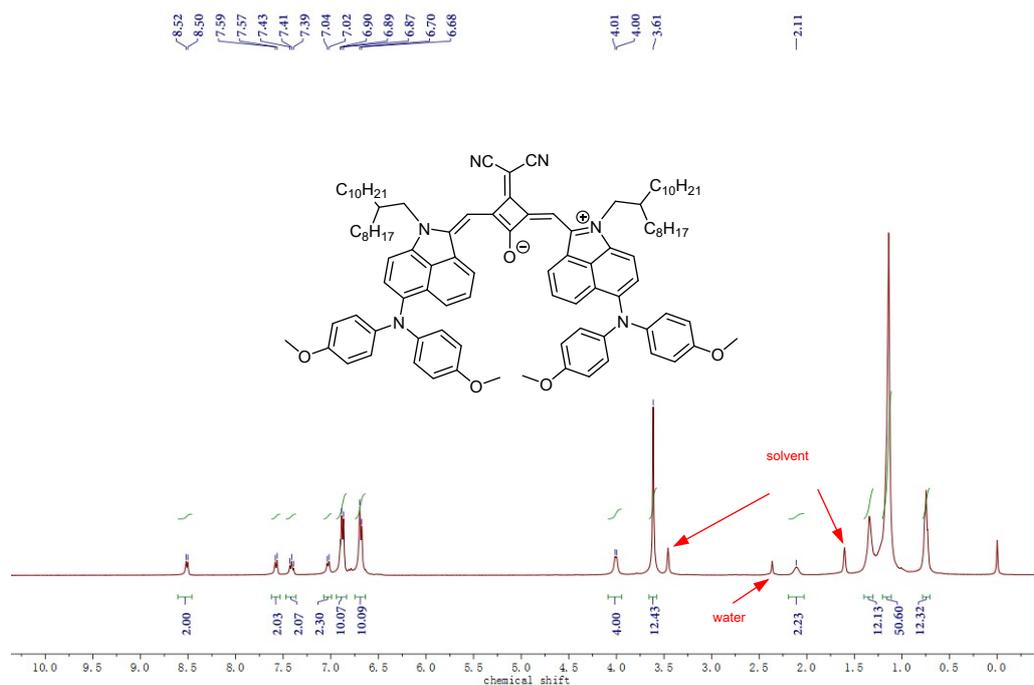


Figure S7. ¹H-NMR spectrum of NSQ1270 in THF-*d*₈.

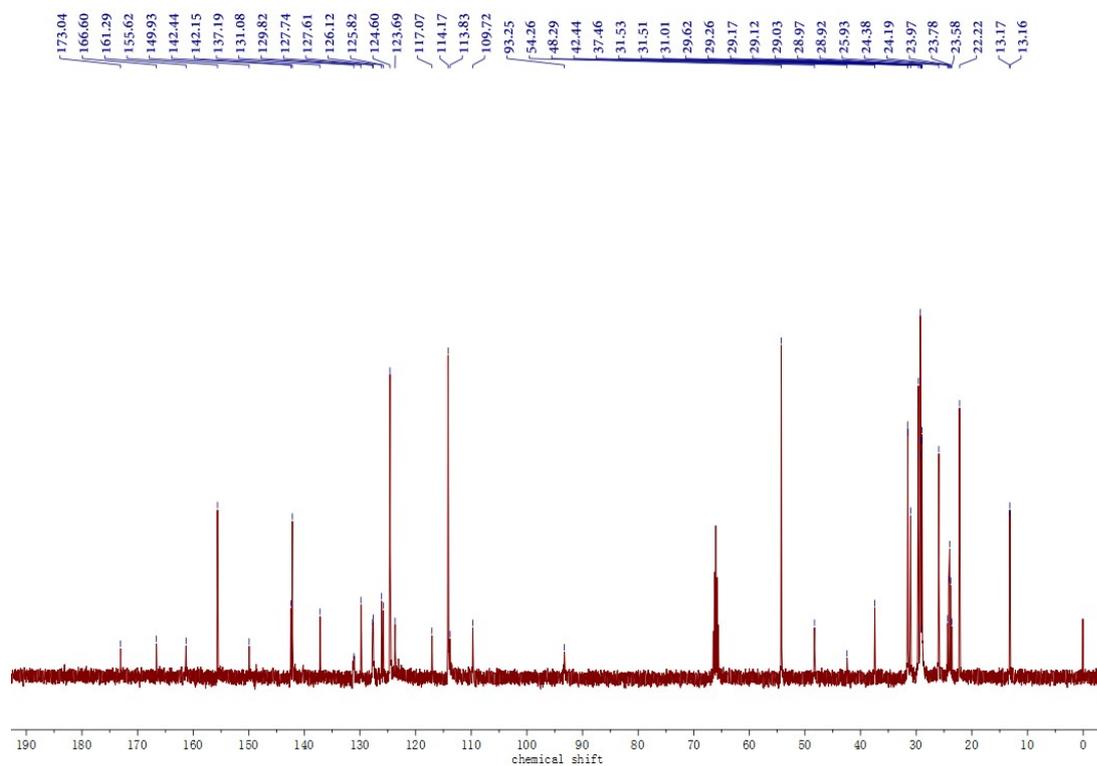


Figure S8. ¹³C-NMR spectrum of NSQ1270 in THF-*d*₈.

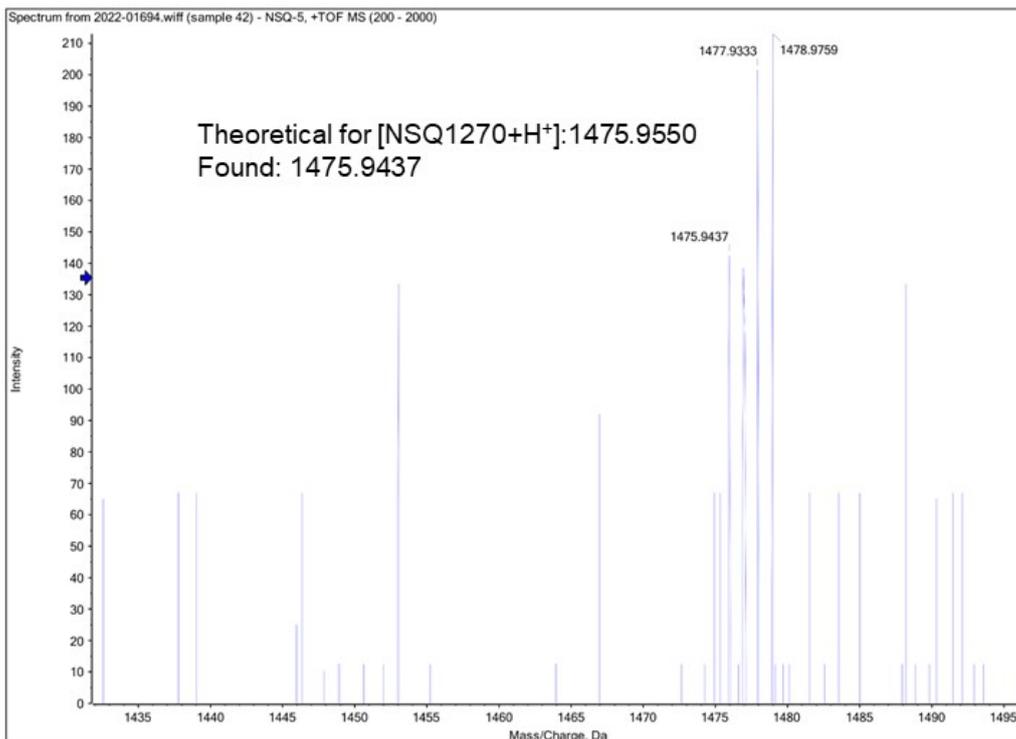


Figure S9. TOF-MS mass spectrum of NSQ1270.

Table S1. The photophysical data for NSQ1270 at 298K.

condition	λ_{ab} [nm]	ϵ [M ⁻¹ cm ⁻¹]	$\lambda_{em}^{a)}$ [nm]	$\phi_{PL}^{b)}$ [%]
n-Hexane	1091	143,000	1153	0.88
DCM	1074	102,000	1138	0.42
MeOH	1052	101,000	1149	0.12
H ₂ O	1124	29000	1270	-

a) λ_{ex} = 1064 nm; b) calculated by indirect method (with IR-26).

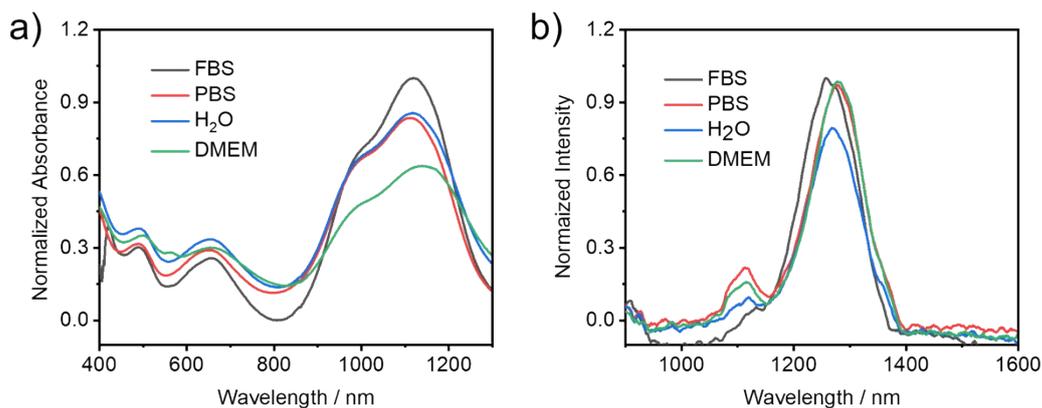


Figure S10. a) Normalized absorption, and b) normalized fluorescence spectra of NSQ1270 in FBS, PBS, H₂O, and DMEM.

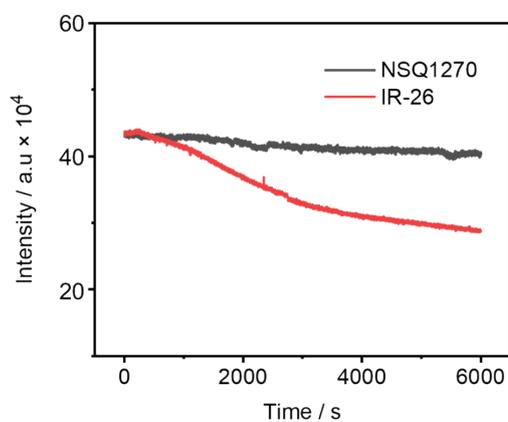


Figure S11. Fluorescence intensity of NSQ1270 and IR-26 in PBS under 6000s successive irradiation.

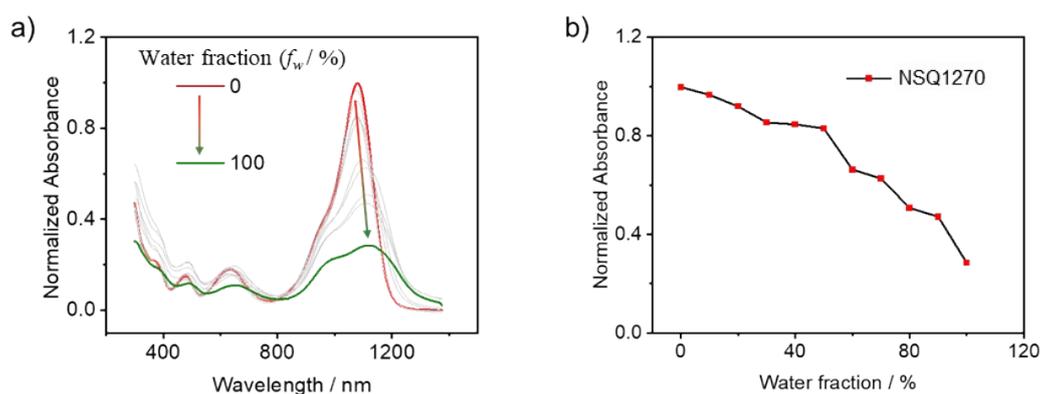


Figure S12. a) Normalized absorption spectral of NSQ1270 with variation of f_w , b) the absorbance trend diagram with different f_w .

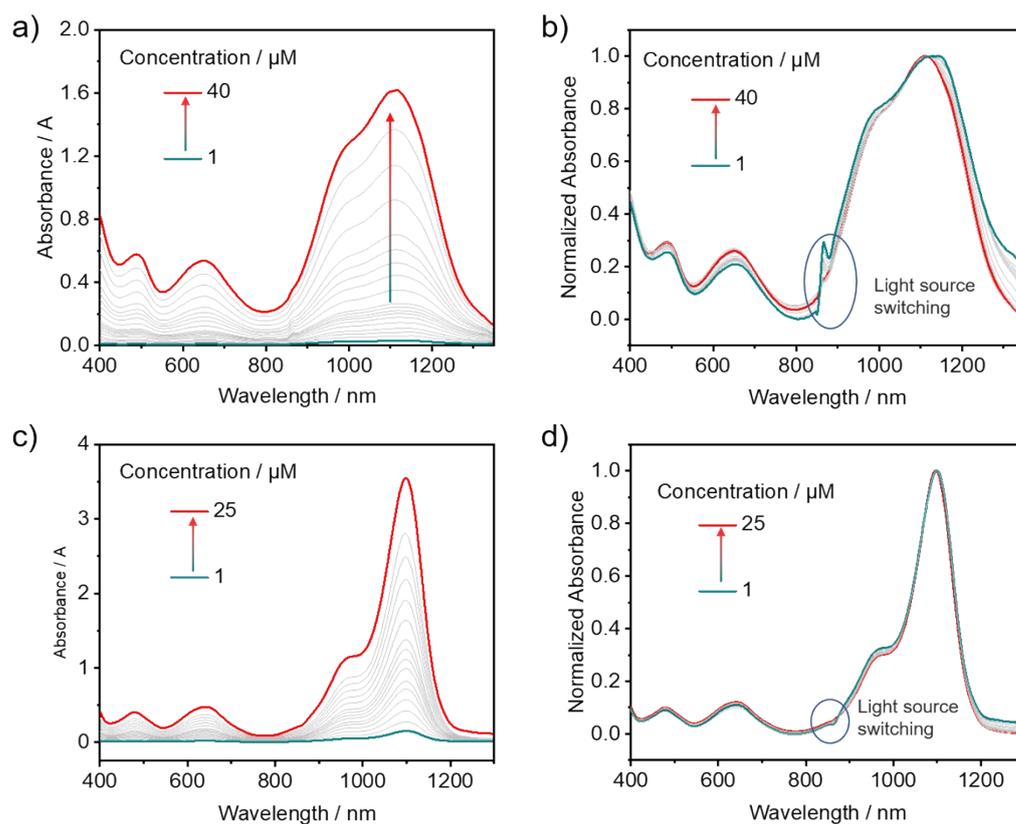


Figure S13. Concentration-dependent absorption spectra of NSQ1270 in a): water and c): methyl cyclohexane, and its normalized absorption spectra in b): water and d): methyl cyclohexane.

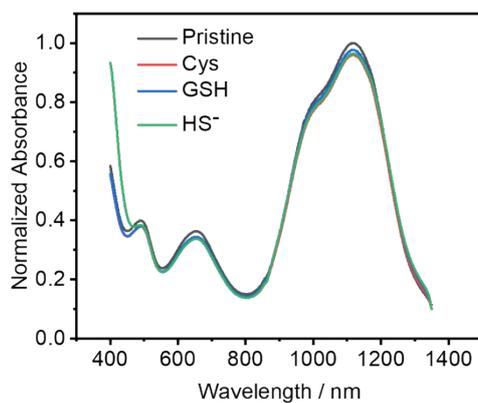


Figure S14. Absorption spectra NSQ1270 and in the presence of different nucleophiles.

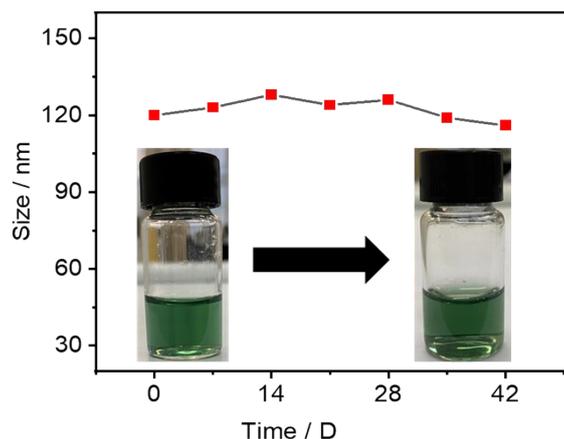


Figure S15. Particle size of NSQ1270@BSA NCs after 6-week storage under 4 °C. (insert: before and after photographs of NSQ1270@BSA NCs dispersion in water)

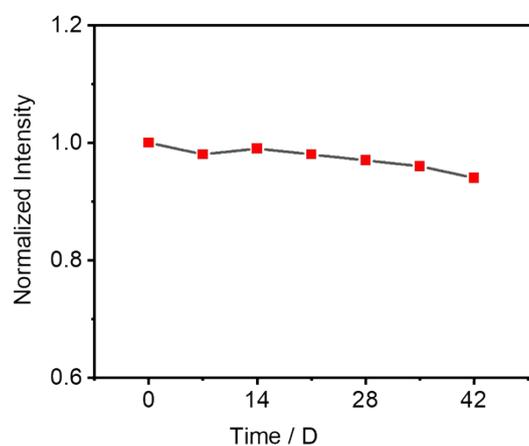


Figure S16. Normalized absorption of NSQ1270@BSA NCs after 6-week storage under 4 °C.

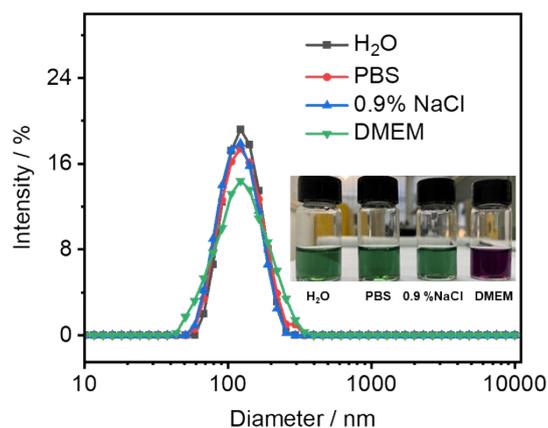


Figure S17. Particle size of NSQ1270@BSA NCs in water, PBS, 0.9% NaCl, and DMEM (insert: from left to right, photographs of NSQ1270@BSA NCs incubated with water, PBS, 0.9% NaCl, and DMEM).

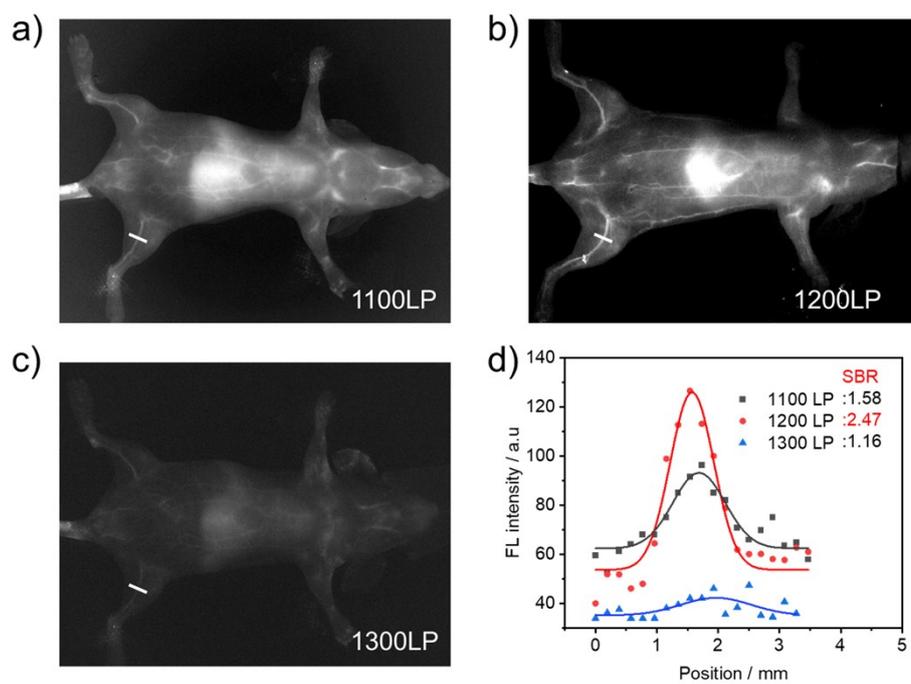


Figure S18. NIR-II fluorescence images of whole body treated with NSQ1270@BSA NCs at 5 min post-injection under a) 1100 long-pass filter, b) 1200 long-pass filter, and c) 1300 long-pass filter; d) Cross-sectional intensity profiles (dots) and Gaussian fit (lines) along the white line (hindlimb vasculature) of different long-pass filter.

References

1. a) Y. Haishima, Y. Kubota, K. Manseki, J. Jin, Y. Sawada, T. Inuzuka, K. Funabiki, and M. Matsui, *J. Org. Chem.* 2018, **83**, 4389–4401; b) U. Mayerhöffer, M. Gsänger, M. Stolte, B. Fimmel, and F. Würthner, *Chem. Eur. J.* 2013, **19**, 218 – 232.
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