Supporting information

Comparison Study of Two Near-Infrared Coumarin-BODIPY Dyes for Bioimaging and Photothermal Therapy of Cancer

Yuandong Zhang,[a] Nan Song,[a] Yuanyuan Li,[c] Zhiyu Yang,[a] Li Chen,*[a] Tingting Sun, [b] Zhigang Xie*[b]

[a]Department of Chemistry, Northeast Normal University, 5268 Renmin Street, Changchun 130024, P. R. China. E-mail: chenl686@nenu.edu.cn

[b]State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun 130022, P. R. China. E-mail: xiez@ciac.ac.cn

[c]The First Hospital of Jilin University, Xinmin Street, Changchun, Jilin 130021, P.R. China. E-mail: lyy2017@ciac.ac.cn

Instruments

Bruker 400 DRX NMR spectrometer was employed to record the ^{1}H NMR spectra. A Shimadzu UV-2450 PC UV/Vis spectrophotometer and a PerkinElmer LS-55 fluorescence spectrophotometer were hired to perform the measurements of UV/Vis absorption spectrum and fluorescence emission spectrum. A matrix-assisted laser desorption/ionization time of flight mass spectrometry was used to record MALDITOF/MS. Fluorescence quantum efficiencies were determined by using a Hamamatsu

Absolute PL Quantum Yield Measurement System C9920-02. Size, size distribution

(DLS). The morphology of the nanoparticles was measured by TEM with a JEOL JEM1011 electron microscope operating at an acceleration voltage of 100 kV. A Zeiss LSM

700 camera (Zurich, Switzerland) was taken to photo the confocal laser scanning microscopy (CLSM) images. Flow analysis of the cells was analyzed by Guava easyCyte 6-2L Base System (Merck Millipore, USA).

Materials and Reagents

Solvents and reagents were purchased commercially as reagent grade and used as received unless otherwise mentioned. 2, 4-Dimethyl-pyrrole was purchased from TCI reagents, and the purity of them was 98 %. Dichloromethane (CH_2Cl_2) was distilled over calcium hydride. The solvents used for spectroscopic measurements were of HPLC grade. Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were purchased from Sigma-Aldrich. 0.25 % Trypsin-EDTA was provided by Dalian Meilun Biotechnology Co., Ltd. MTT and Live-Dead Cell Staining Kit were purchased from KeyGen Biotech Co., Ltd.

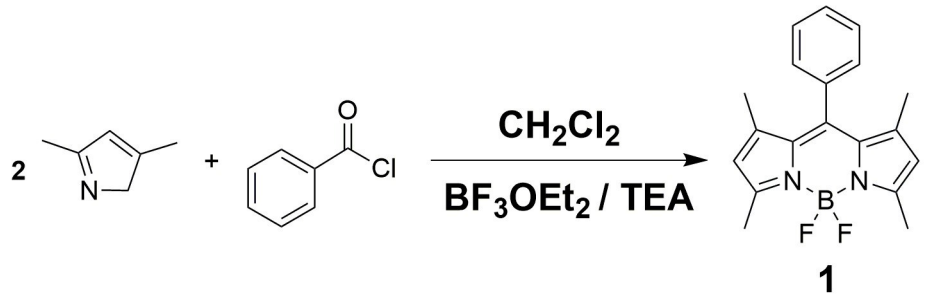
Synthesis

Synthesis of phenyl Bodipy

According to Scheme S1, we first prepared phenyl Bodipy. In detail, benzoyl chloride (1.0 g, 7.8 mM) and 2, 4-dimethyl pyrrole (2.0 ml, 19.6 mM) was dissolved in 120 mL of dry DCM with protection of Ar atmosphere and kept stirring overnight at room temperature. In an ice bath, 10 mL of triethylamine was added and 10 mL of boron trifluoride ether was instilled after reacted for 30 min. Then, the reaction temperature rose to the room temperature and the solution was stirred for 8 hours. After the reaction

completed, 3 × 30 mL of brine was used to wash the crude product. The crude product was dried and purified by silica gel with petroleum ether: dichloromethane (DCM) = 1:1 as eluent. An orange red solid was obtained with a yield of 36.1% (0.92 g 2.83 mM).

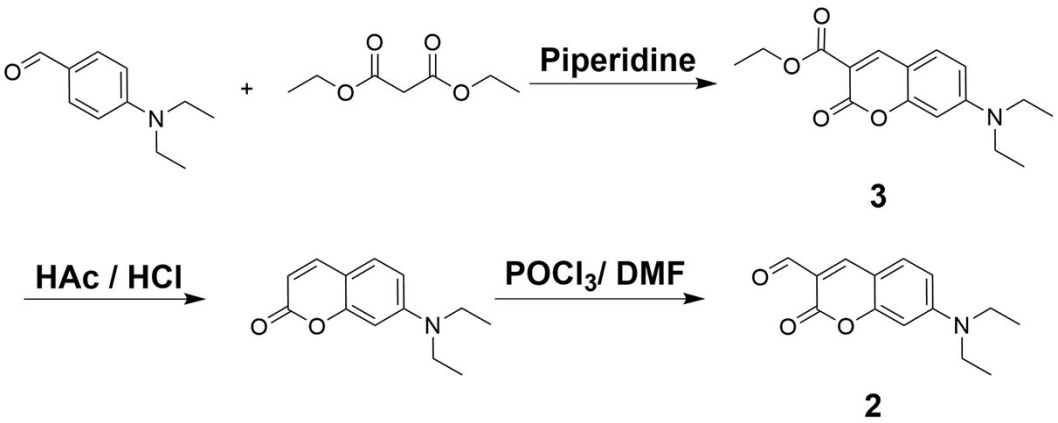
^{1}H NMR (400MZ, $CDCl_3$) 7.51-7.46 (m, 3H), 7.93-7.27 (m, 2H), 5.98 (s, 2H), 2.56 (s, 6H), 1.37 (s, 6H).



Scheme S1 Synthesis of phenyl Bodipy.

Synthesis of ethyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate (3)

4-*N,N*-Diethylaminosalicyaldehyde (1.93 g, 10.0 mM), diethyl malonate (3.2 g, 20.0 mM), and 0.3 mL of piperidine was dissolved in 50 mL of ethanol under an Ar atmosphere. The reaction was stopped and cooled to room temperature after refluxing for one night. Under the reduced pressure the solvent was moved, a dark brown oil liquid was got, then recrystallized from ethanol to afford a pure yellow crystal. Yield: 67 % (1.93 g, 6.7 mM). ^{1}H NMR (400 MHz, $CHCl_3$) δ 8.38 (s, 1H), 7.33-7.31 (d, J = 9.2 Hz, 1H), 6.59 – 6.56 (dd, J = 8.8, 2.4 Hz, 1H), 6.41-6.40 (d, J = 2.4 Hz, 1H), 4.364.31 (q, J = 7.2 Hz, 2H), 3.44-3.38 (q, J = 7.2 Hz, 4H), 1.37-1.34 (t, J = 7.2 Hz, 3H), 1.21-1.18 (t, J = 7.2 Hz, 6H).



Scheme S2 Synthesis of 7-diethylaminocoumarine-3-aldehyde.

Synthesis of 7-(diethylamino)-2-oxo-2H-chromene-3-carbaldehyde (2)

In a 50 mL stand-up flask, ethyl 7-(diethylamino)-2-oxo-2H-chromene-3carboxylate (**3**) (1.49 g, 5.0 mM), and 10 mL of HAC/HCl (1:1) was added and refluxed at 100 °C overnight. Then the reactions were cooled to room temperature and poured into 50 mL of ice water, 10 % sodium hydroxide solution as a neutralizing reagent was hired to adjust the mixture's pH value to 7. A large amount of precipitate was afforded. After being filtered, washed with water, collected the filter cake and dried by vacuum, a dark gray solid was obtained (0.85 g). The unpurified gray solids directly participated in the next reaction.

Under ice water bath and Ar atmosphere protection conditions, 2.0 mL of DMF was added in a 50 mL double-necked flask, and 2 mL of $POCl_3$ was dropwise under stirring. After dropping, the ice water bath was removed and the reaction kept for 1 h at room temperature. After a colorless Vilsmeier-Haack reagent was obtained, 10 mL of gray solid DMF solution (0.85 g) in a constant pressure dropping funnel was added dropwise. After the completion of the dropwise addition, the mixture was heated to 80 °C and reacted for 16 hours. Then the reaction was stopped and cooled to room temperature. The reactions were added dropwise to 50 mL of ice water and neutralized with a 10 %

aqueous NaOH solution to pH= 7. A large amount of orange precipitate was produced, suction filtered, washed with water, and solids were collected and recrystallized from anhydrous ethanol to give yellow needle crystals. Yield: 66 % (0.56 g, 2.4 mM). ^{1}H NMR (400MZ, $CDCl_3$) δ 10.12 (s, 1H), 8.24 (s, 1H), 7.41-7.39 (d, J = 8.8 Hz, 1H), 6.64-6.61 (dd, J = 8.8, 2.4 Hz, 1H), 6.48-6.47 (d, J = 2.4 Hz, 1H), 3.50-3.44 (m, 4H), 1.27-1.23 (t, J = 7.2 Hz, 6H).

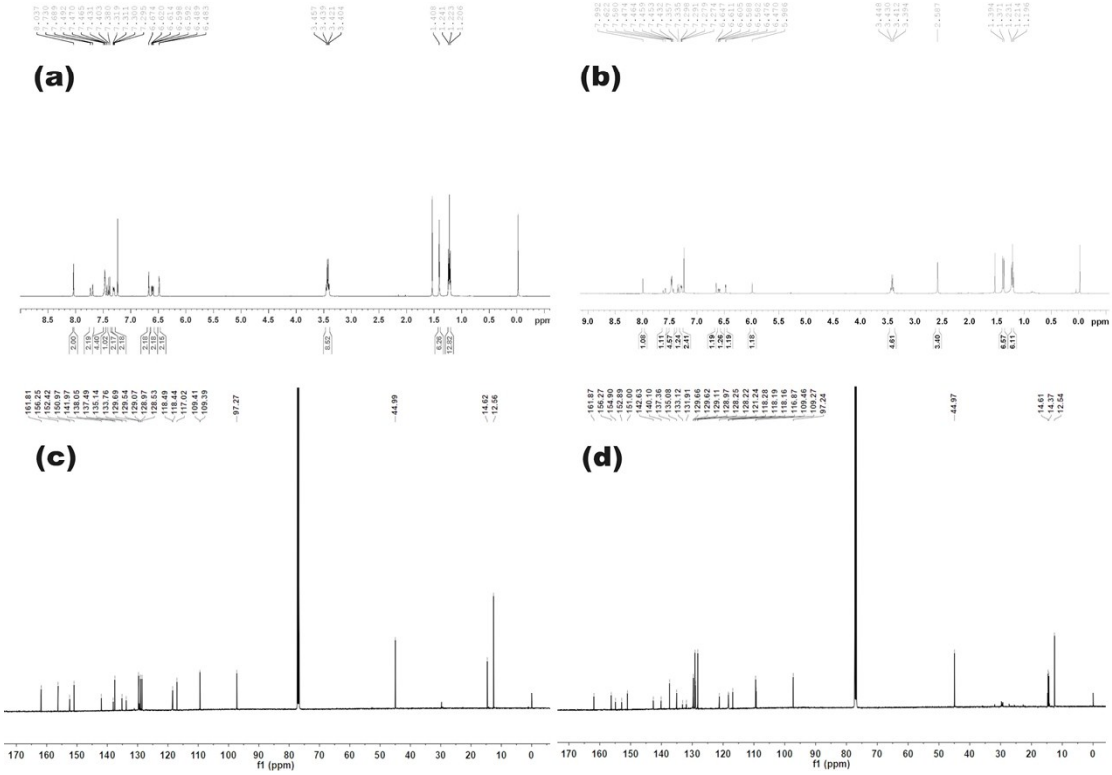


Fig. S1 ^{1}H NMR spectra of BDC (a) and BSC (b); ^{13}C NMR spectra of BDC (c) and BSC (d). Solvent: $CDCl_3$.

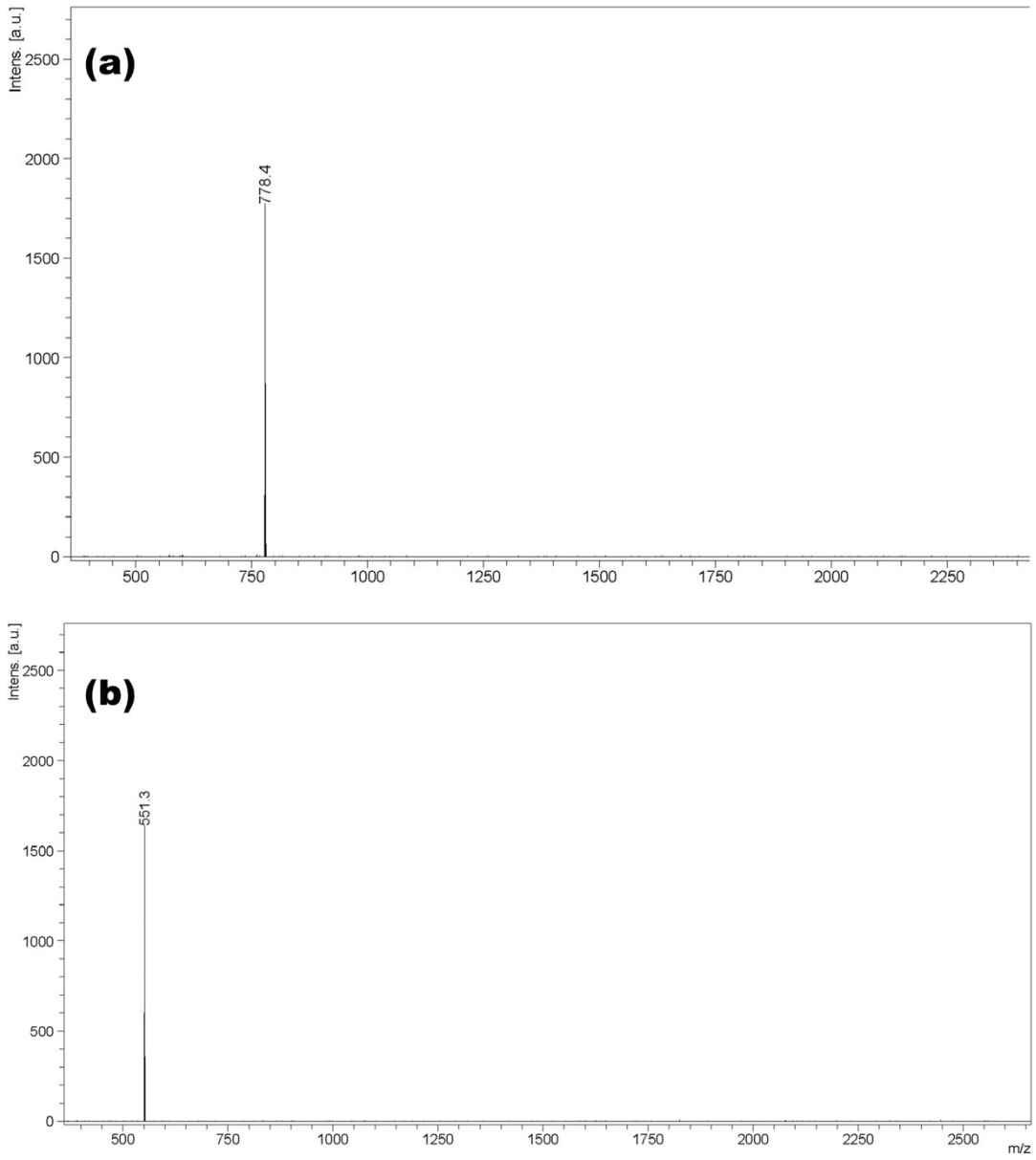


Fig. S2 MALDI-TOF spectra of (a) BDC and (b) BSC in CH_2Cl_2.

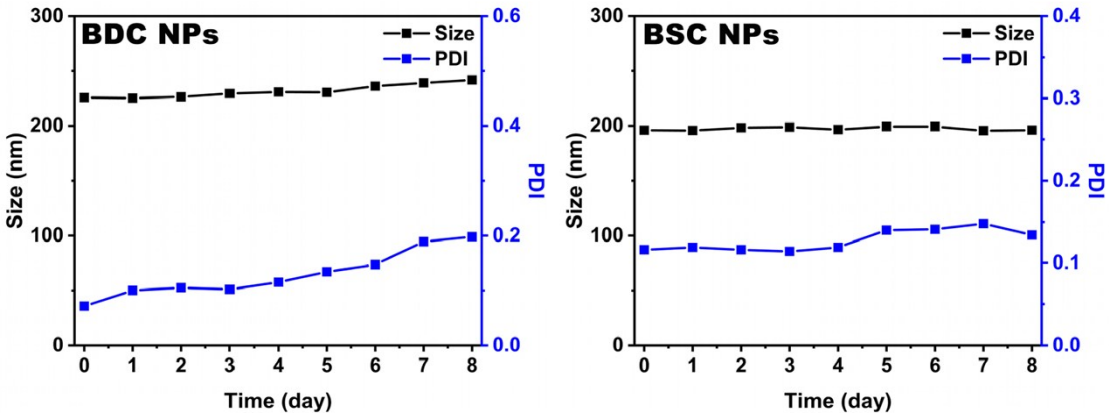


Fig. S3 Stability of BDC NPs and BSC NPs measured by DLS for 8 days.

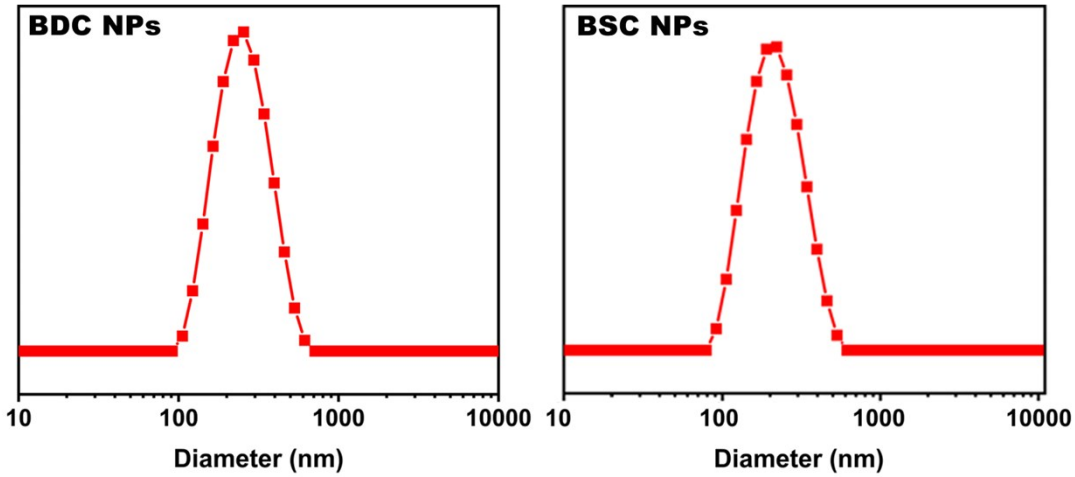


Fig. S4 DLS results for BDC NPs and BSC NPs.

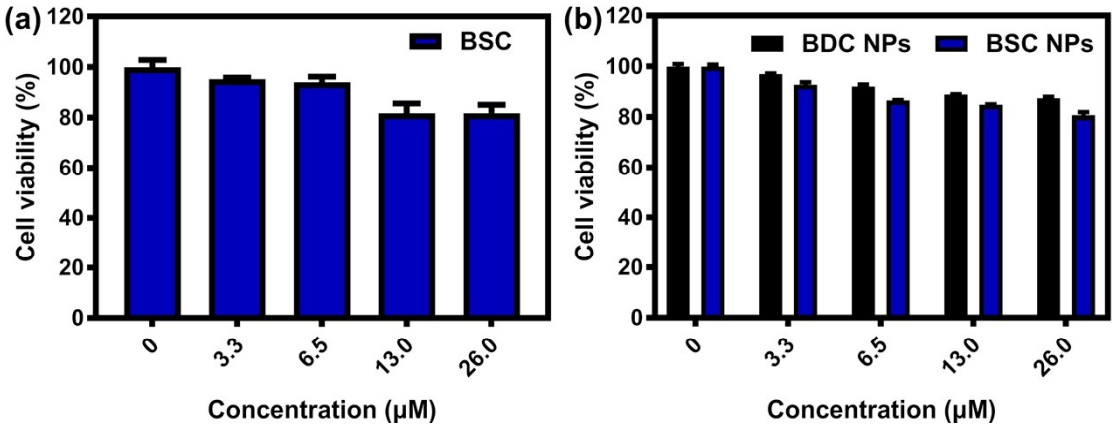


Fig. S5 (a) Viability of HeLa cells after a 24 h incubation with different doses of BSC NPs in dark. (b) Viability of L929 cells after a 24 h incubation with different doses of BDC NPs and BSC NPs in dark.