Supporting Information for

570 nm-/770 nm Light-Excited Deep-Red Fluorescence Switch Based on Dithienylethenes Derived from BF_2-Curcuminoid

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1. General information

1.1 Materials

All manipulations were carried out under a nitrogen atmosphere by using standard Schlenk techniques unless otherwise stated. THF and toluene was distilled under nitrogen from sodium-benzophenone. DMF was dried by $MgSO_4$ and distilled under reduced pressure. The intermediates **1**,[1] **3**,[2] **4**[2] and **5**[3] were prepared by reported literature method, respectively. B16 cell lines were supplied by Procell Life Science and Technology Co., Ltd. (China). All other starting materials were obtained commercially as analytical-grade from Energy Chemical Reagent Co., Ltd (Shanghai, China) and used without further purification.

1.2 Instruments

^1H and ^{13}C NMR spectra were collected on German BRUKER AVANCE III 400 MHz (all the chemical shifts are relative to TMS). High resolution mass spectra were obtained on SCIEX X-500R QTOF (ESI mode). All the absorption spectra were collected on a SHIMADZU UV-2600 UV-Vis spectrophotometer, and the fluorescence spectra were obtained on a Hitachi Model F-4500 fluorescent spectrophotometer. The fluorescence lifetimes were determined by a fluorescent spectrophotometer (FLS1000). In the photochromic experiments, the visible light irradiation experiment was carried out using a 30 W yellow-green lamp (570-575 nm), 30 W green lamp (520-530 nm) and 30 W Near infrared lamp (760-770 nm), respectively. All these LED lamps were purchased from Shenzhen Boya Technology Co., Ltd (China).

1.3 Determination of the cyclization and cycloreversion quantum yields

The cyclization and cycloreversion quantum yields of **NBDC** were determined according to the standard procedure reported in previous literatures.[4,5] For the cyclization quantum yields, potassium ferrioxalate ($K_3[Fe(C_2O_4)_3]$) was used as actinometer.[6] The light intensity at 520 nm was determined by following steps: i) 3.0 mL of 0.15 M $K_3[Fe(C_2O_4)_3]$ solution in 0.05 M H_2SO_4 was irradiated for 180 s; ii) 0.5 mL phenanthroline (0.1 wt % in 0.5 M H_2SO_4 /1.6 M NaOAc) were added subsequently; iii) measuring the absorpance at 510 nm before and after irradiation. The light intensity could be calculated via Eq. (1).

$$I_0 = \frac{\Delta A_{510nm}}{\Delta t * \varepsilon_{510nm} * \emptyset_{irr} * 1000} * \frac{3.5mL}{3.0mL} \qquad (1)$$

In which ΔA_{510nm} is the difference of the absorption at 510 nm for an irradiated versus a nonirradiated solution, Δt is the irradiation time, ε_{510nm} is 11100 M^{-1} cm^{-1} and Φ_{irr} is the quantum yield at the irradiation wavelength (0.65 for 520 nm).

Because the wavelength of the irradiation light that was needed for the ring-opening was out of the range for ferrioxalate actinometry (220-550 nm). Therefore, for the cycloreversion quantum yields of **NBDC**, aberchrome 670 was used as actinometer.[6] The light intensity at 600 nm was determined by following steps: i) 3.0 mL aberchrome 670 solution (1.0×10^{-4} M in toluene) was irradiated with 365 nm light; ii) the formed isomer was irradiated back with 600 nm light, iii) measuring the absorpance at 519 nm before and after irradiation. The light intensity at 600 nm could be calculated via Eq. (2).

$$I_0 = \frac{\Delta A_{519nm}}{\Delta t * \varepsilon_{519nm} * \emptyset_{irr} * 1000 * (1 - 10^{-A'})} \qquad (2)$$

In which ΔA_{519nm} is the difference of the absorption at 519 nm before and after irradiation, Δt is the irradiation time, ε_{519nm} is 7760 M^{-1} cm^{-1}, Φ_{irr} is the quantum yield at the irradiation wavelength (0.27 for 600 nm), $1\text{-}10^{-A'}$ is the percentage of absorbance photons by the solution at irradiation wavelength, and A' is the initial absorpance at the irradiation wavelength.

Then, the solutions of ring-open isomer **NBDC(o)** (3.0 mL, 2.0×10^{-5} M) were irradiated with the same investigated light source, and the changes at corresponding maximum absorption wavelength were measured immediately. The cyclization quantum yields could be calculated via Eq. (3). Moreover, the similar operations were performed on ring-closed isomer **NBDC(c)**, and the cycloreversion quantum yields could also be calculated via Eq. (3).

$$\emptyset = \frac{\Delta A / \Delta t}{(1 - 10^{-A'}) * \varepsilon * I_0 * 1000} \qquad (3)$$

In which $\Delta A/\Delta t$ is the change rate of absorbance upon irradiating at excitation wavelength, $1\text{-}10^{-A'}$ is the percentage of absorbance photons by the solution at irradiation wavelength, A' is the absorbance of open-form at excitation wavelength, ε is extinction coefficient at detection wavelength, and I_0 is the light intensity calculated above.

1.4 Determination of the fluorescence quantum yields

The fluorescence quantum yields (Φ_f) of **NBDC(o)** in the various solvents were approximatively determined according to the following equation:

$$\emptyset_s = \emptyset_{ref} \times \frac{F_s}{F_{ref}} \times \frac{A_{ref}}{A_s} \times \frac{n_s^2}{n_{ref}^2}$$

in which Φ_{ref} is the fluorescence quantum yield of reference, F is the area under the emission spectra, A is the absorbance at the excitation wavelength, n is the refractive index of solvent. s and ref stand for sample and reference, respectively. We chose rhodamine 6G in water (Φ_{ref} = 0.75, λ_{ex} = 488 nm) as the reference. Absorbance of the unknown samples and the standard should be similar and small.[7]

1.5 Nanoparticle preparation

NBDC-loaded nanoparticles (**NBDC** NPs) were facilely prepared according to a modified literature method.[8] A mixture of **NBDC** (1.0 mg), Pluronic F127 (10.0 mg) and THF (1.0 mL) was added dropwise to distilled-deionized water (10.0 mL) under continuous sonication with a microtip-equipped probe sonicator for 3 min. After sonication, the mixture solution was stirred overnight at room temperature to ensure evaporation of the THF. The resulting **NBDC** NPs were obtained by filtration through a 0.22 μm syringe filter for further use.

1.6 Cytotoxicity evaluation

Cells were cultured in B16-F1 cell-specific medium supplemented with 10% heated-inactivated FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mmol/L glutamine. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2. Cell viability was determined to assess the in vitro cytotoxicity of **NBDC** NPs to B16 cells with MTT assay. The B16 cells were seeded onto 96-well plates at a density of 3 × 10^4 cells/well and cultivated in the incubator to adhere. The culture medium was substituted by medium containing **NBDC** NPs with different DS at the final concentrations of 50-1000 μg/mL. After incubation for 24 h, MTT solution（0.5 mg/mL) was added to each well and incubated for 4 h at 37 °C. Subsequently, the medium was removed and any formazan crystals formed were solubilized with dimethyl sulfoxide under gentle shaking for 10 min.The absorbance of each well was measured at 490 nm with a Bio-Rad Microplate Reader. Each concentration of the samples had six replicates. Every test includes a blank containing culture medium only. The viability was presented as the percent of sample well to the control well.

1.7 Cell culture and fluorescence imaging

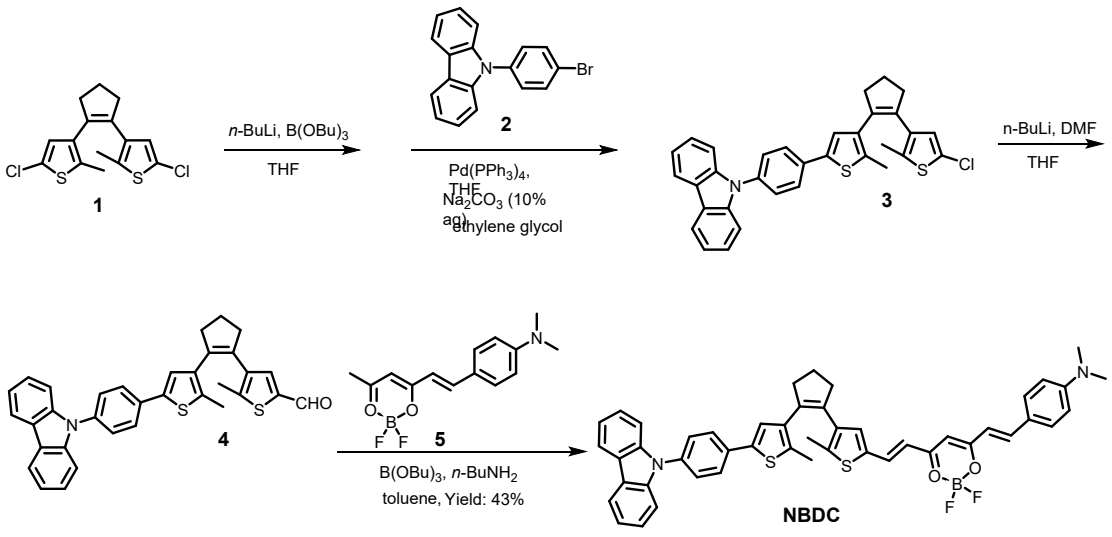
The cell experiments are conducted as follows: The B16 cell line is seeded into a six-well plate at a density of approximately 3×10^5 cells per well and cultured in B16-F1 cell-specific medium at 37°C in a 5%

CO_2 incubator for 24 hours. Subsequently, the fluorescent dye (**NBDC** NPs) is added to each well at a volume of 1.0 mL, followed by the addition of 1.0 mL of fresh medium per well. The medium and fluorescent dye mixture is gently mixed using a Pasteur pipette, and then the cells are placed back in the incubator for 30 minutes. Finally, the cells are washed three times with PBS (pH 7.4) to completely remove the uninternalized dye. A fluorescence inverted microscope is used to observe the cells. Fluorescence signals are collected at 530-750 nm upon excitation at 510 nm. The switchable irradiation experiments are conducted for 10 minutes under yellow-green light at 570 nm and NIR light at 770 nm, respectively.

1.8 Photoswitching experiments on living zebrafish

Zebrafish larvae were maintained in E3 medium (15 mM NaCl, 0.5 mM KCl, 1.0 mM $MgSO_4$, 1.0 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10^{-5}% methylene blue; pH 7.5) at a constant temperature of 25 °C. Three-day-old zebrafish were placed in a six-well plate, with 2.0 mL of E3 medium added to each well. 100 μL of fluorescent dye (**NBDC** NPs) was added to each well. The zebrafish were stained with fluorescent dyes at room temperature for 15 minutes, after which the E3 medium mixed with the fluorescent dyes was aspirated out, and the zebrafish were washed three times with clean E3 medium. Finally, a drop of clean E3 medium was placed in the center of each well, and the zebrafish were positioned in the middle of the droplet for observation under a fluorescence microscope. The six-well plate was subjected to photoswitching experiments with 570 nm yellow-green light and 770 nm NIR light, and fluorescence images were obtained immediately after each light exposure.

2. Experimental section



Scheme S1. Synthetic route of **NBDC**.

To a solution of intermediate **4** (265 mg, 0.5 mmol) and **5** (140 mg, 0.5 mmol) in toluene (3.0 mL) was added $B(OBu)_3$ (0.21 mL, 0.75 mmol) and *n*-Butylamine (13 μL, 0.125 mmol), and the resulting solution was stirred at 65 °C for 16 h. After cooling to room temperature, the solution was concentrated under reduced pressure and the residue was purified by column chromatography (silica gel: 200-300, PE : DCM = 1 : 2) to obtain the target **NBDC** as a brown solid (166 mg, Yield: 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (d, *J* = 7.7 Hz, 2H), 7.97 (dd, *J* = 23.2, 15.1 Hz, 2H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.54 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.9 Hz, 2H), 7.45 - 7.39 (m, 4H), 7.29 (t, *J* = 6.6 Hz, 2H), 7.09 (s, 2H), 6.67 (d, *J* = 8.8 Hz, 2H), 6.43 (d, *J* = 15.2 Hz, 1H), 6.27 (d, *J* = 15.1 Hz, 1H), 5.87 (s, 1H), 3.08 (s, 6H), 2.94 - 2.76 (m, 4H), 2.15 - 2.05 (m, 2H), 2.09 (s, 3H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.32, 176.43, 153.01, 148.49, 141.85, 140.75, 139.16, 138.05, 137.66, 136.45, 136.41, 136.24, 136.00, 135.09, 135.02, 133.66, 133.43, 131.86, 127.37, 126.59, 125.95, 124.14, 123.37, 122.09, 120.27, 119.95, 118.49, 114.36, 111.90, 109.81, 101.38, 40.10, 38.51, 38.29, 22.98, 15.26, 14.44. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{48}H_{42}BF_2N_2O_2S_2$ 791.2749; Found 791.2763; $[M + NH_4]^+$ Calcd for $C_{48}H_{45}BF_2N_3O_2S_2$ 808.3014; Found 808.3032; $[M + Na]^+$ Calcd for $C_{48}H_{41}BF_2N_2NaO_2S_2$ 813.2568; Found 813.2587; $[M + K]^+$ Calcd for $C_{48}H_{41}BF_2KN_2O_2S_2$ 829.2308; Found 829.2338.

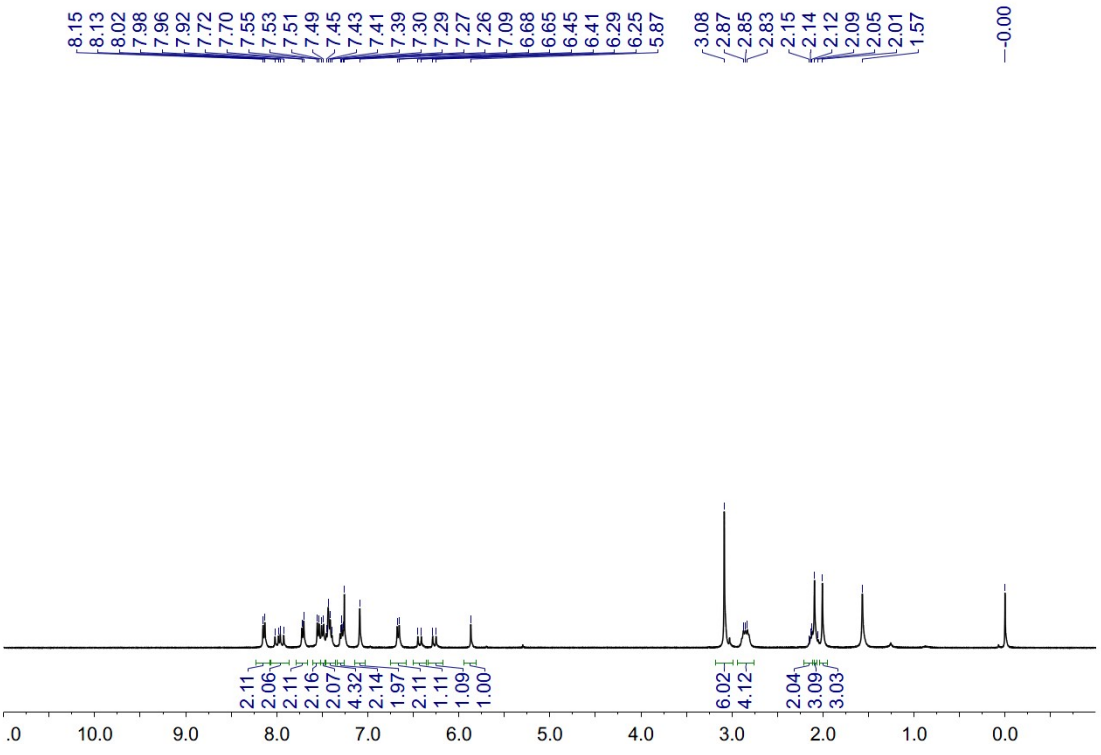


Figure S1. 400 MHz ^{1}H NMR spectrum of **NBDC** in $CDCl_3$ at room temperature.

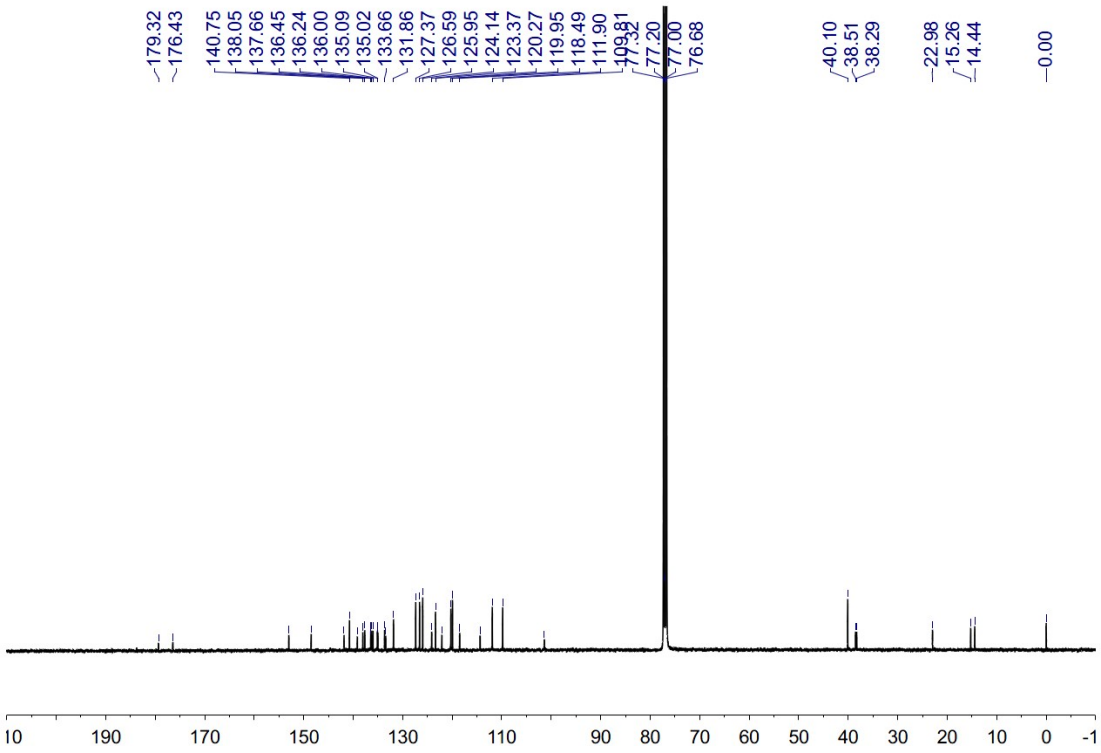


Figure S2. 100 MHz ^{13}C NMR spectrum of **NBDC** in $CDCl_3$ at room temperature.

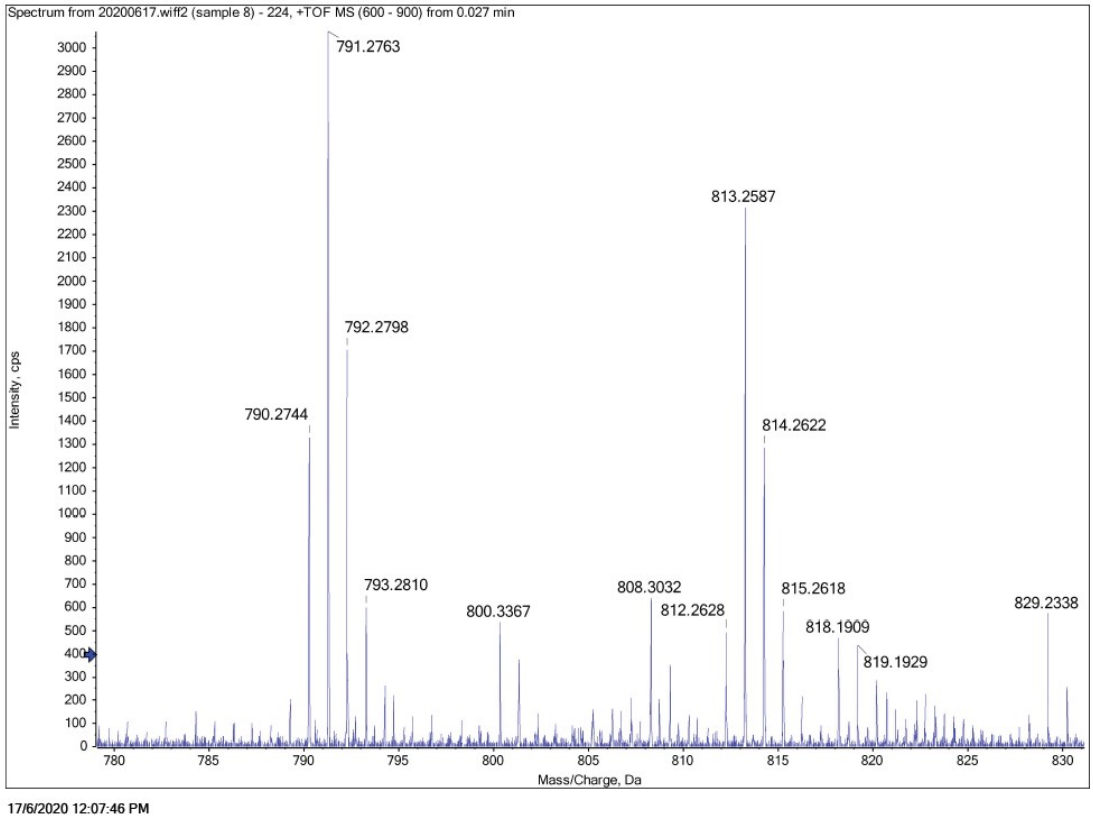
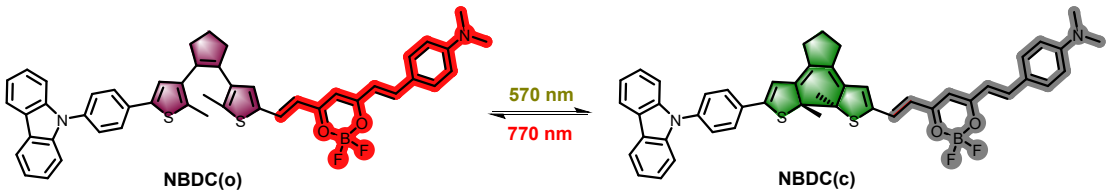


Figure S3. HRMS of **NBDC**.



Scheme S2. The photochromic reaction of **NBDC**.

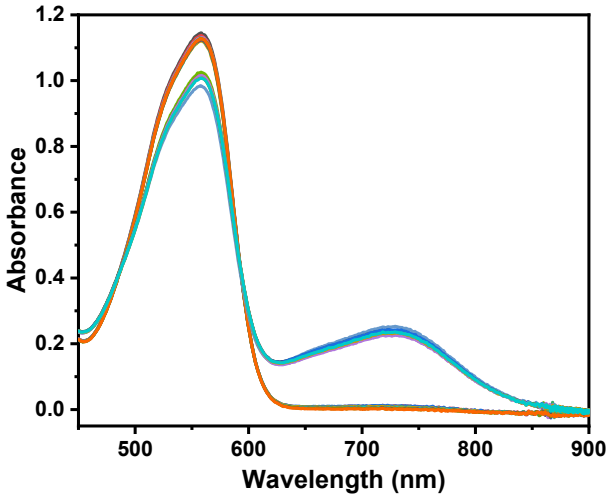


Figure S4. The absorption spectra changes of **NBDC** in toluene (2.0×10^{-5} mol/L) upon alternating irradiation with yellow-green light at 570 nm (8.9 mW/cm^2) and NIR light at 770 nm (8.9 mW/cm^2) for ten times.

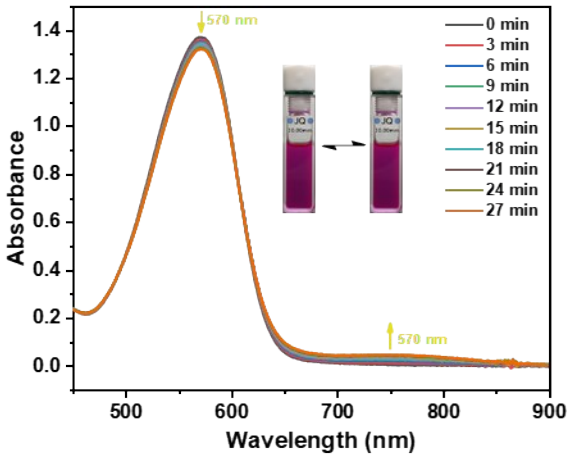


Figure S5. The absorption spectra changes of **NBDC** in $CHCl_3$ (2.0×10^{-5} mol/L) upon irradiation with yellow-green light at 570 nm (8.9 mW/cm^2). The insets show the corresponding color changes upon photoirradiation.

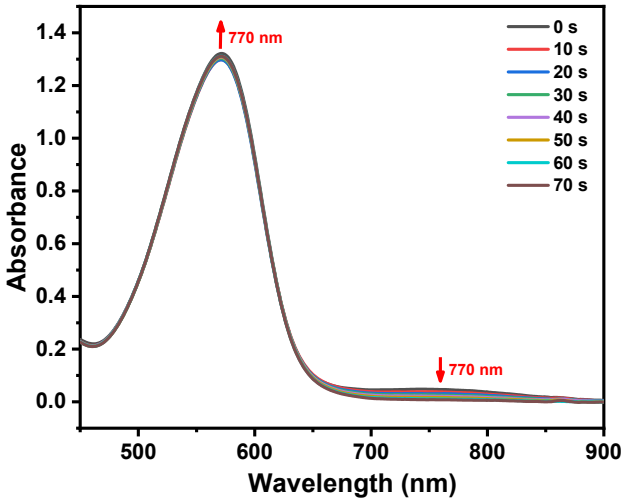


Figure S6. The absorption spectra changes of **NBDC** in $CHCl_3$ (2.0×10^{-5} mol/L) upon irradiation with NIR light at 770 nm (8.9 mW/cm^2).

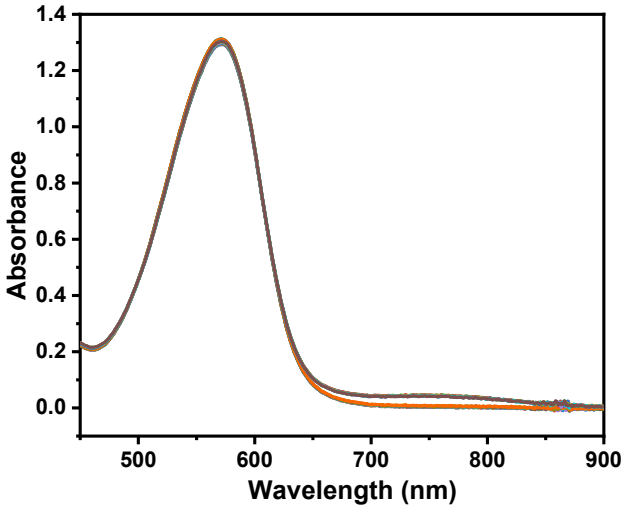


Figure S7. The absorption spectra changes of **NBDC** in $CHCl_3$ (2.0×10^{-5} mol/L) upon alternating irradiation with yellow-green light at 570 nm (8.9 mW/cm^2) and NIR light at 770 nm (8.9 mW/cm^2) for ten times.

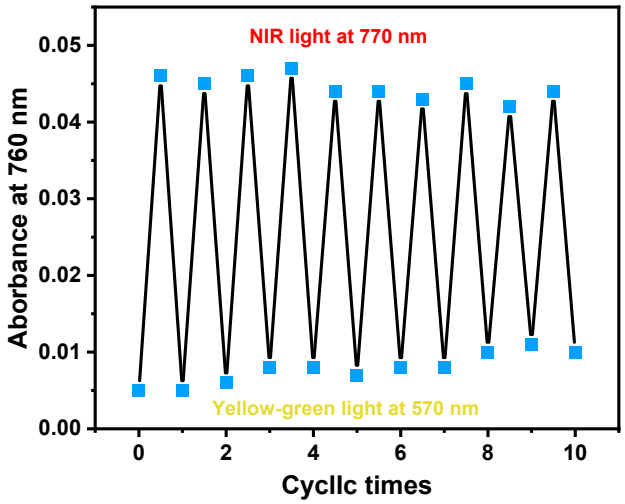


Figure S8. The absorption spectra changes of **NBDC** in $CHCl_3$ (2.0×10^{-5} mol/L) upon alternating irradiation with yellow-green light at 570 nm (8.9 mW/cm^2) and NIR light at 770 nm (8.9 mW/cm^2) for ten times.

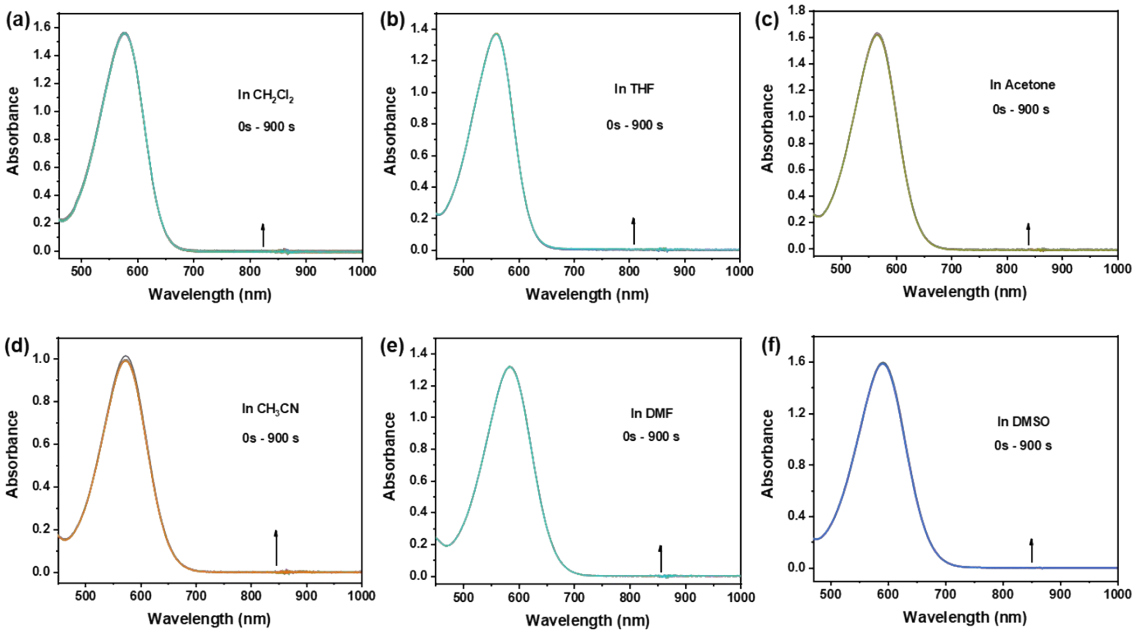


Figure S9. The absorption spectra changes of **NBDC** in CH_2Cl_2 (2.0×10^{-5} mol/L), THF (2.0×10^{-5} mol/L), Acetone (2.0×10^{-5} mol/L), CH_3CN (2.0×10^{-5} mol/L), DMF (2.0×10^{-5} mol/L), and DMSO (2.0×10^{-5} mol/L) upon irradiation with yellow-green light at 570 nm (8.9 mW/cm^2).

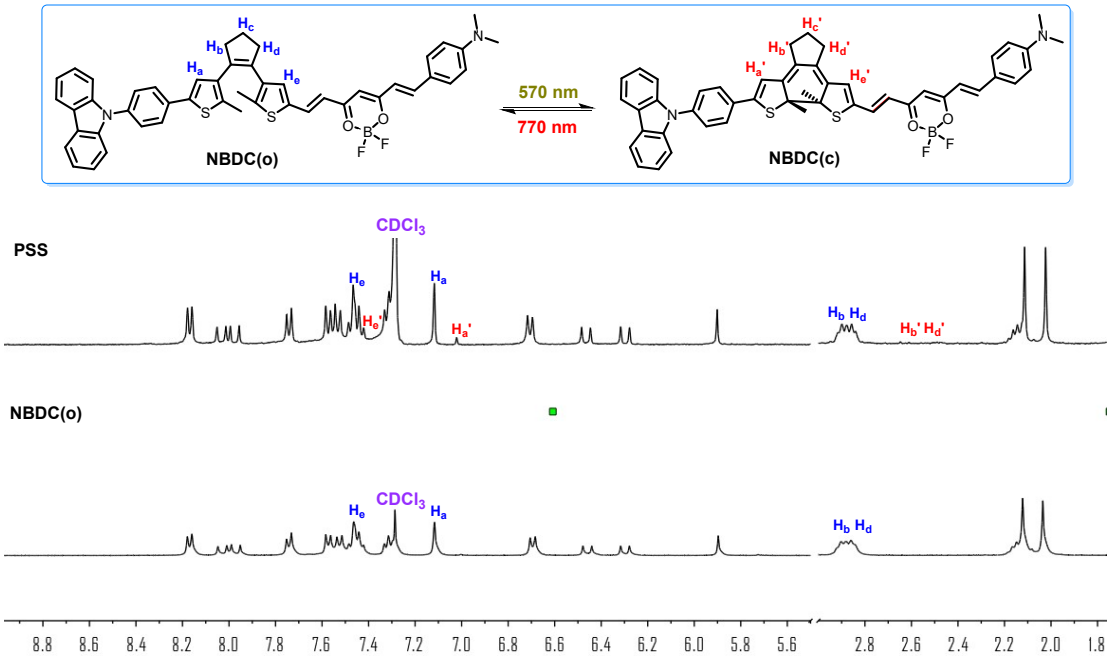


Figure S10. The partial ^{1}H NMR spectral variations of **NBDC** upon irradiation with yellow-green light in $CDCl_3$.

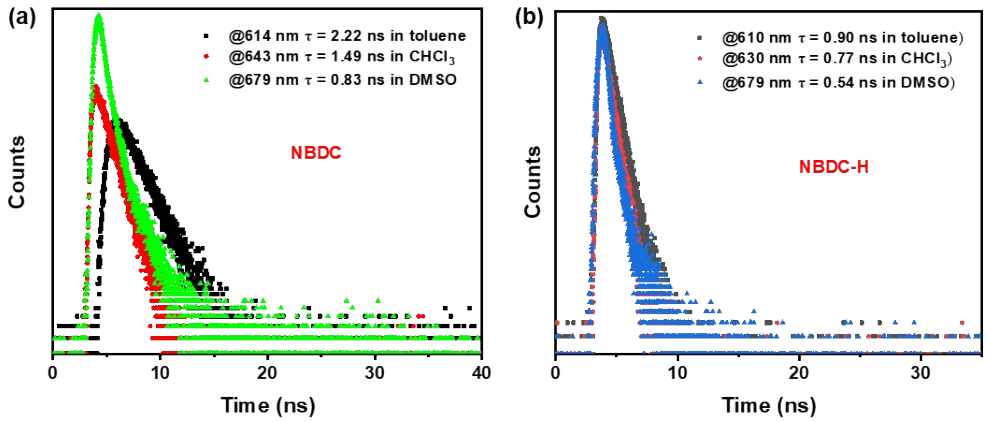


Figure S11. The prompt fluorescence decay curves of **NBDC** (a) and **NBDC-H** (b) in toluene, $CHCl_3$ and DMSO.

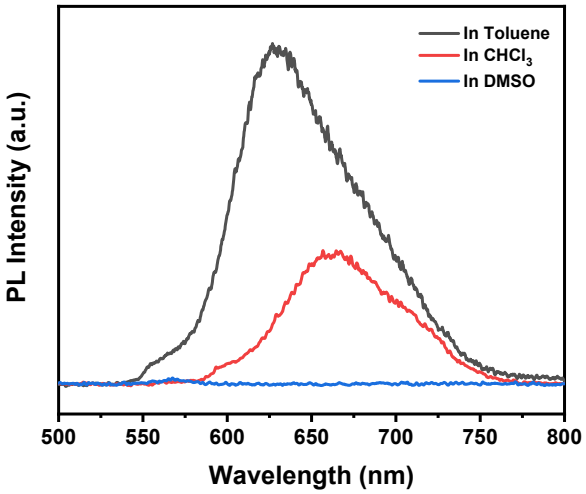


Figure S12. PL spectra of **NBDC** under delayed mode at room temperature in different solvents (λ_{ex} = 570 nm).

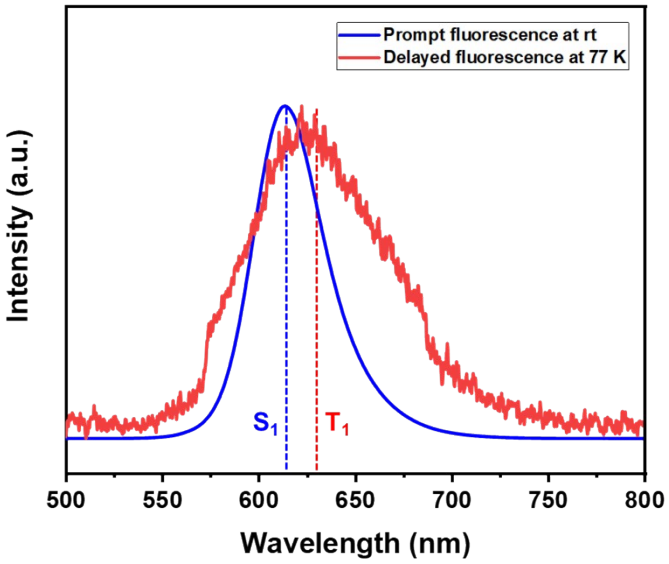


Figure S13. The prompt fluorescence of **NBDC** at room temperature and its phosphorescence spectra at 77 K.

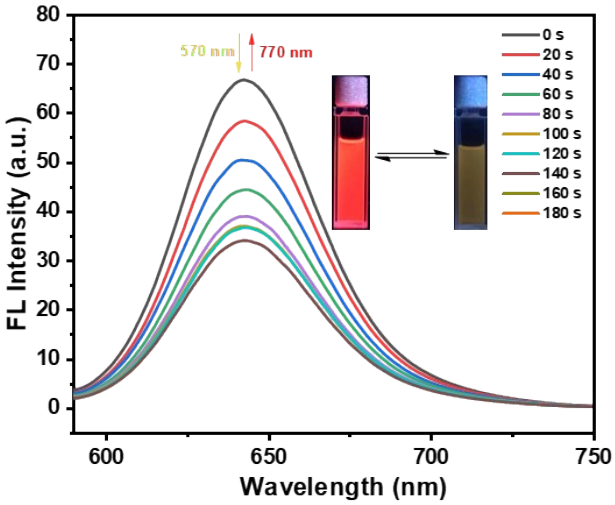


Figure S14. The prompt fluorescence spectra changes of **NBDC** in $CHCl_3$ (2.0×10^{-5} mol/L) upon alternating irradiation with 570 nm light (8.9 mW/cm^2) and NIR light at 770 nm (λ_{ex} = 570 nm) (8.9 mW/cm^2). (Inset) Corresponding fluorescent color changes upon photoirradiation.

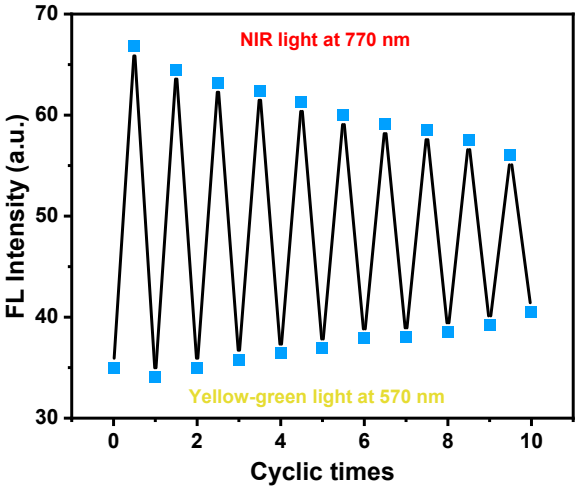


Figure S15. The fluorescence spectra changes of **NBDC** in $CHCl_3$ (2.0×10^{-5} mol/L) upon alternating irradiation with yellow-green light at 570 nm (8.9 mW/cm^2) and NIR light at 770 nm (8.9 mW/cm^2) for ten times.

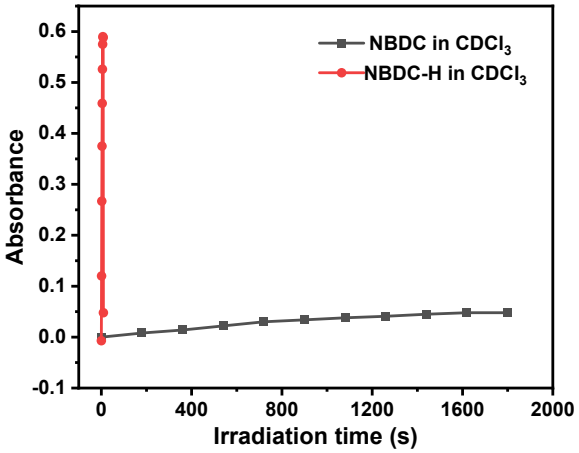


Figure S16. The optical response rate of **NBDC** and **NBDC-H** upon irradiation with 570 nm (8.9 mW/cm^2) and 520 nm (8.9 mW/cm^2) in $CHCl_3$ (2.0×10^{-5} mol/L) by monitoring changes in the maximum absorption wavelength (λ_{max}) of **NBDC(c)** and **NBDC-H(c)**, respectively.

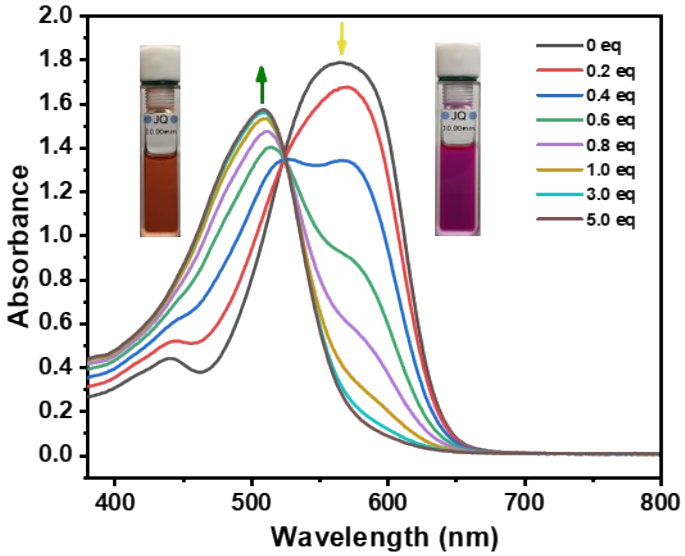


Figure S17. The absorption spectra changes of **NBDC(o)** in the presence of TFA (0-5.0 eq.) in toluene (2.0×10^{-5} mol/L). The insets show the corresponding color and fluorescence changes before and after addition of 5.0 eq. TFA.

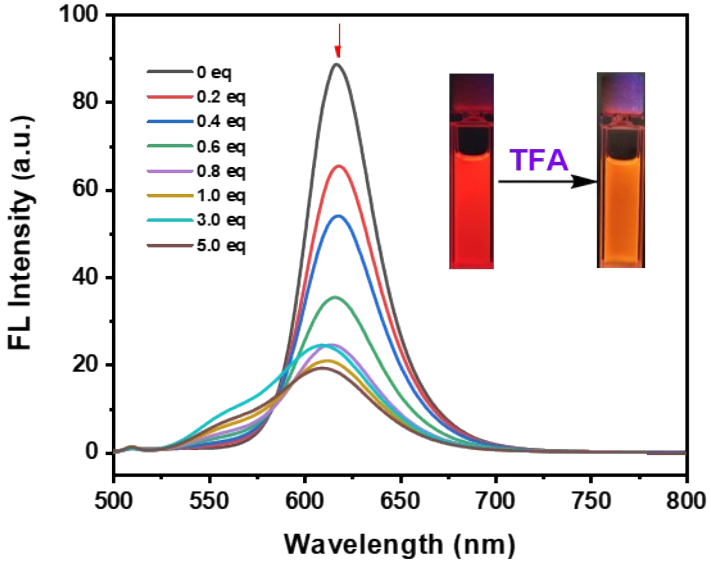


Figure S18. The fluorescence spectra changes of **NBDC(o)** in the presence of TFA (0-5.0 eq.) in toluene (2.0×10^{-5} mol/L). The insets show the corresponding color and fluorescence changes before and after addition of 5.0 eq. TFA.

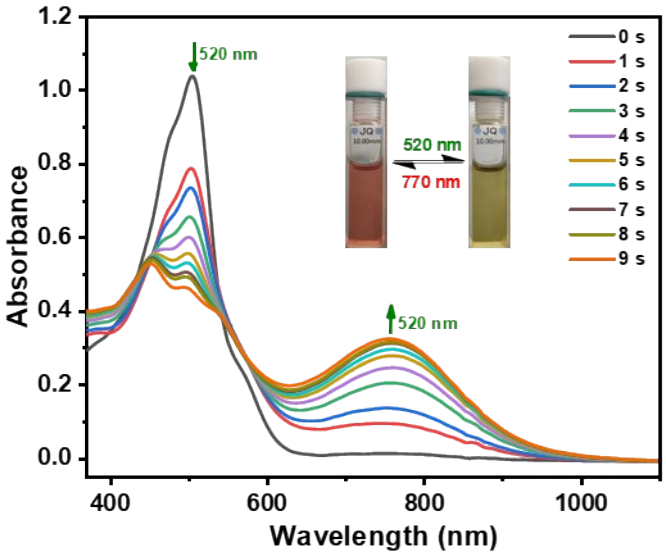


Figure S19. The absorption spectra changes of as-protonated DTE **NBDC-H** in toluene (2.0×10^{-5} mol/L) upon irradiation with green light at 520 nm (8.9 mW/cm^2). The insets show the corresponding color changes upon photoirradiation.

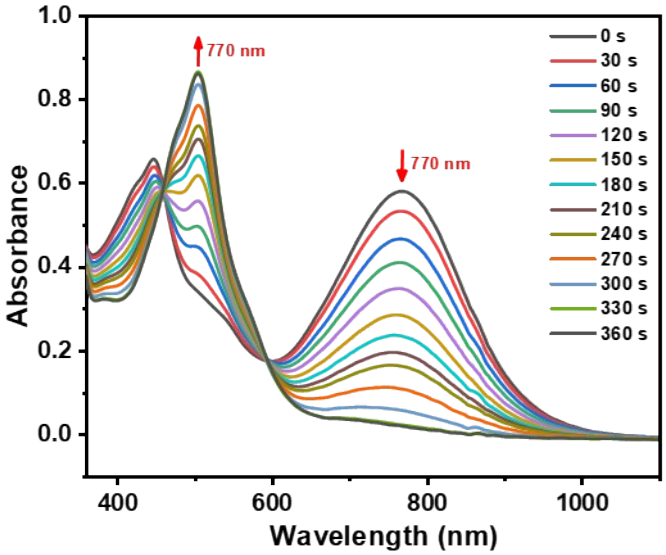


Figure S20. The absorption spectra changes of **NBDC-H(c)** in toluene (2.0×10^{-5} mol/L) upon irradiation with NIR light at 770 nm (8.9 mW/cm^2).

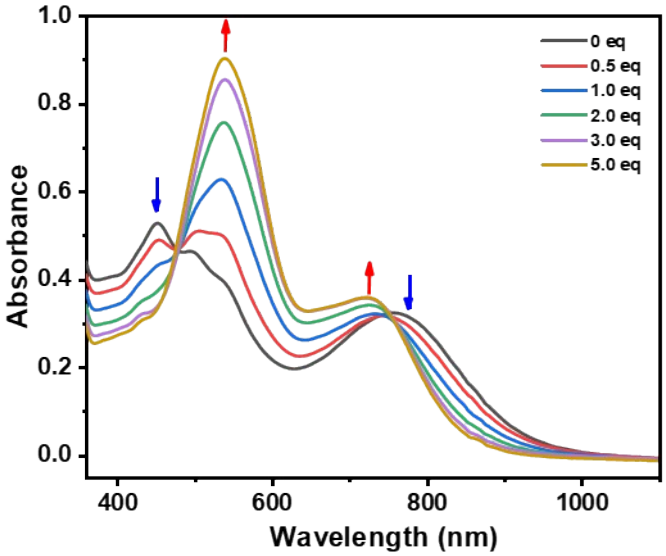
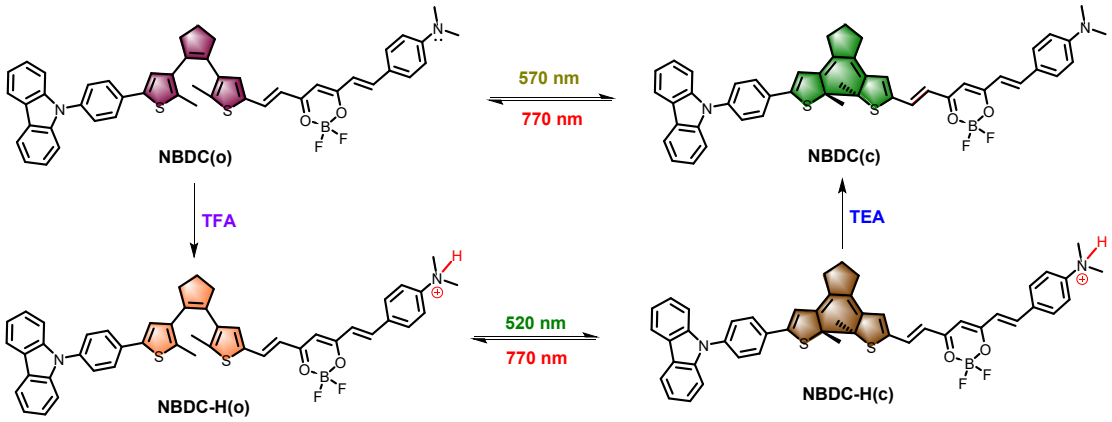


Figure S21. The absorption spectra changes of **NBDC-H(c)** in the presence of TEA (0-5.0 eq.) in toluene (2.0×10^{-5} mol/L).



Scheme S3. Schematic representation of protonation of **NBDC(o)** to generate **NBDC-H(o)** upon addition of TFA (5.0 eq) in toluene, the photoisomerization of **NBDC-H** upon irradiation with 520 nm/770 nm light (8.9 mW/cm^2), and deprotonation of **NBDC-H(c)** to generate **NBDC(c)** upon addition of TEA (5.0 eq) in toluene.

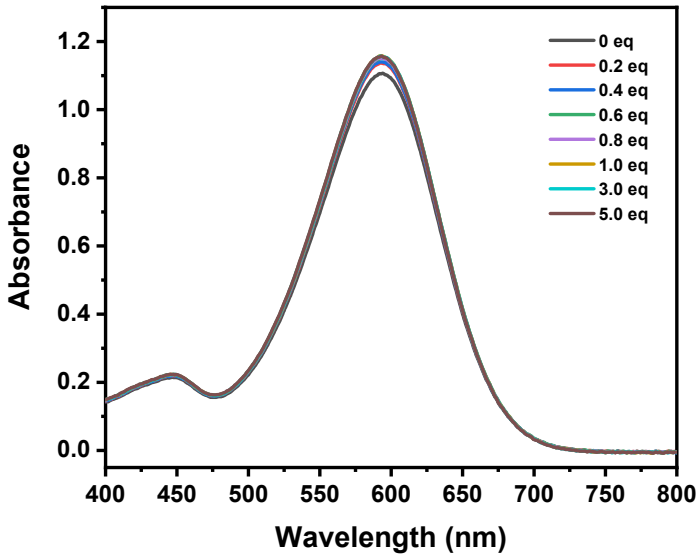


Figure S22. The absorption spectra changes of **NBDC(o)** in the presence of TFA (0-5.0 eq.) in DMSO (2.0×10^{-5} mol/L).

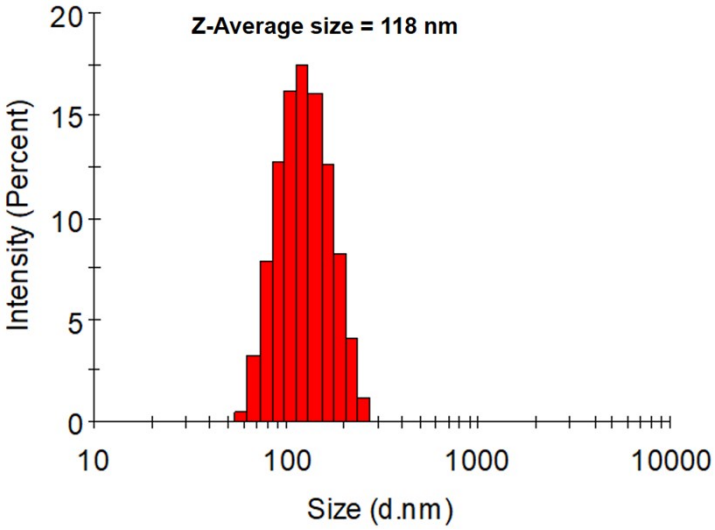


Figure S23. The dynamic light scattering (DLS) profiles of **NBDC** NPs.

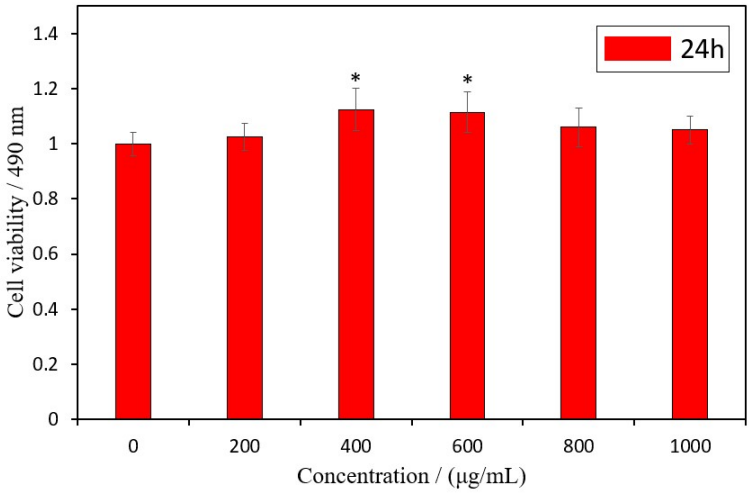


Figure S24. Cell viability value (%) by MTT method.

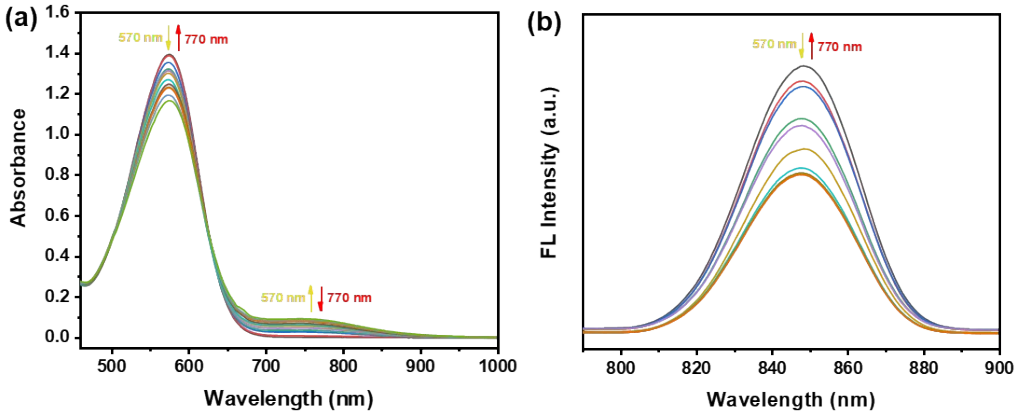


Figure S25. The absorption spectra (a) and the fluorescence spectra (b) changes and of **NBDC** NPs in water (2.0×10^{-5} mol/L) upon irradiation with yellow-green light at 570 nm (8.9 mW/cm^2) and NIR light at 770 nm (8.9 mW/cm^2).

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