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Supplementary information

Novel indole-BODIPY photosensitizers based on iodine promoted ISC enhancement for lysosome-targeted imaging and photodynamic therapy

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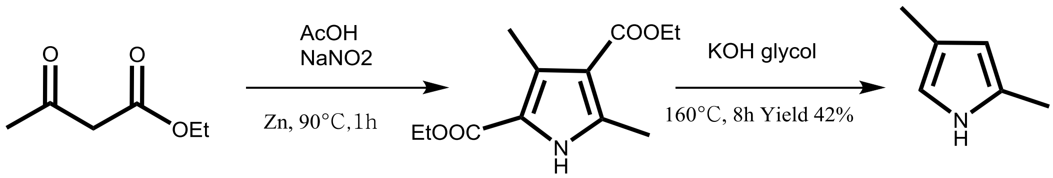
Experimental details

Synthesis of 2, 4-Dimethyl-1H-pyrrole

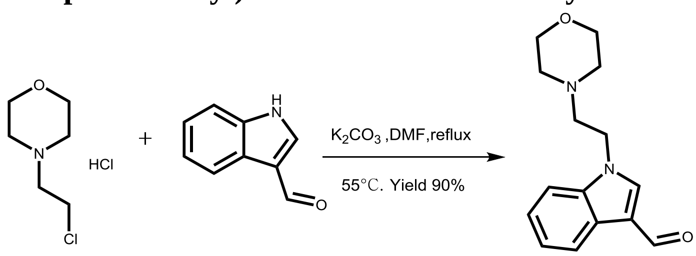


Scheme S1. Synthesis route of 2, 4-dimethylpyrrole.

A suspension of ethyl acetoacetate 50.4 mL (52.0 g, 400.0 mmol) in AcOH (100.0 mL) was treated with $NaNO_2$ (13.7 g, 200.0 mmol) in water added dropwise slowly. The mixture was stirred at room temperature for 2.5 h. Next, zinc powder (26 g, 400.0 mm) was slowly added while the reaction mixture was kept below 25 ℃, and the mixture was stirred at room temperature until the zinc powder reacted completely, then the reaction was stirred at 95 ℃ for 1 h, and poured into iced water and suction filtration to white solid and directly put into the next reaction.

Under the protection of oil seal, The KOH (22.4 g, 400.0 mmol) in ethylene glycol (80.0 mL) was treated with the newly prepared white powder, and stirred evenly. After stirring at 110 ℃ for 8 h, the mixture was cooled to room temperature by pour into 100 mL saturated salt solution. Extract with 100 mL dichloromethane three times. The combined organic phases are dried over anhydrous sodium sulfate. The solvent is pumped dry by a rotary evaporator to obtain the crude product as a as dark brown oil (8.0 g, yield: 42%).

Synthesis of 1-(2-morpholinoethyl)-1H-indole-3-carbaldehyde



Scheme S2. Synthesis route of 1-[2-(4-Morpholinyl) ethyl]-1H-indole

N-(2- chloroethyl) morpholine hydrochloride (2.5 g, 16.5 mmol), indole -3-formaldehyde (1.2 g, 8.3 mmol) and potassium carbonate (2.3 g, 16.5 mmol) were dissolved in 50.0 mL of a solution of N,N- dimethylformamide and stirred at 55 ℃ for 48 h. the reaction progress was detected by TLC washed three times with dichloromethane and water, the organic phases were combined and dried over Na_2SO_4, the product was purified by silica gel chromatography (EA: PE = 2: 1) to get yellow solid (2.1 g, Yield: 90%).

Methods

Fluorescence quantum yield: fluorescein (0.01M NaOH, $\Phi_F = 0.95$) was selected as reference for BDP-Lys dye, rhodamine B(EtOH, $\Phi_F = 0.50$) was selected as reference for IBDP-Lys and I_2BDP-Lys photosensitizer according to the wavelength of the target compound, and The fluorescence quantum yield was calculated on the basis of the equation [1]

$$\emptyset_{F(X)} = \emptyset_{F(s)} \frac{A_S}{A_X} \frac{F_X}{F_S} \left(\frac{n_x}{n_s}\right)^2 \qquad \text{Eq.1}$$

Φ_F is the fluorescence quantum yield, A is the absorption value, F is the area of fluorescence spectrum, n is the refractive index of solvent, s represents the reference substance, and x represents the analyte.

Singlet oxygen yield: In the determination of singlet oxygen quantum yield, 1, 4-Diphenyl-2, 3-benzofuran (DPBF) as singlet oxygen capture agent and (Rose Bengal) as reference, (Φ_Δ= 0.68 in EtOH) [2]. In the testing process, it is necessary to regulate the ethanol solution of photosensitizer so that its maximum absorption value is between 0.2 and 0.3, and the absorption value of DPBF is about 1 at 414 nm. Calculate the singlet oxygen yield according to the following formula.

$$\emptyset_{\Delta(s)} = \emptyset_{\Delta(f)} \frac{K_S}{K_f} \frac{F_f}{F_S} \qquad \text{Eq.2}$$

In which, Φ_Δ represents the singlet oxygen efficiency, k represents the slope of the absorption value of DPBF at 414 nm decreasing with illumination time, F is absorption correction factor and $F=1-10^{-OD}$ and OD means the absorbance value at its main absorption, f represents the reference substance.

A549 cell culture: A549 cells were obtained from American Type Culture Collection (ATCC). The cells were cultured in 1640 incomplete medium 10% fetal bovine serum at 37 ℃ in a humid atmosphere containing 5% CO_2. All cell procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Southeast University and approved by the Animal Ethics Committee of Southeast University

Cytotoxicity assay (MTT): A549 cells were inoculated into 96-well plate and culture medium containing IBDP-Lys photosensitizer with different concentration (0 μM - 4 μM), incubated for another 24 h. For photo toxicity, cells incubate with the IBDP-Lys photosensitizer with green light of 525 nm (0.8 mW/cm^2) for 15 min. For dark toxicity, cells without light, incubated for another 24 h. Cells containing photosensitizer were washed three times with PBS buffer solution, and fresh culture medium was added. Then continue to incubate with MTT (15 μL, 5 mg/mL) solution added in each well for 4 h, terminate the culture and carefully discard the supernatant add 100 μL DMSO solution to each well. The absorption at 490 nm was recorded by enzyme-labeled instrument. The cell activity test is based on the following formula. Cell viability (%) = A_1/A_2, here A_1 represents the experimental absorbance value at 490 nm, A_2 represents the control absorbance value at 490 nm.

Intracellular (ROS) production: 2,7-dichlorofluorescin diacetate (DCFH-DA) was used as the intracellular $^1O_2$ indicator, which can be converted to DCF and emit bright green fluorescence in the presence of $^1O_2$, A 549 cells cultured in 1640 incomplete medium were inoculated in a 20 mm confocal dish. The cells containing photosensitizer (1 μM)) were incubated for 1 h in the dark and treated with DCFH-DA, and incubated for another 30 minutes. The medium was discarded and washed three times with PBS buffer. Here, a controlled experiment was performed to exclude the effects of the presence of light and DCFH-DA alone. Then use a confocal laser scanning microscope (CLSM, DCFH-DA: Ex: 488 nm, Em: 488-520 nm) to image cells in the green and red channels.

AO/EB staining: A549 cells cultured were inoculated into 20 mm confocal dishes, and fresh 1640 medium containing photosensitizer IBDP-Lys (1 μM) was added and incubated for 1 h. The washed with 2 mL PBS solution and fresh medium was added again, The solution were incubated with light of 525 nm (20 mW/cm^2) for 0 min, 5 min and 15 min, then acridine orange /ethidium bromide were added in the dark. Tests involved were all in dark conditions to avoid interference from outside light.

Lysosome co-localization experiment: Cells were evenly seed on the confocal culture dish (3×105 cells/dish). Cells with photosensitizer (1 μM) were incubated for another 1 h in the dark environment. Then old medium was discarded and fresh medium with Lyso-Tracker Green (1 μM) was added. Lysosome co-localization imaging was performed on confocal laser scanning microscopy. (Lyso-Tracker Green: λ_{ex}: 488 nm, λ_{em}: 515-545 nm).

Zebrafish imaging: Zebrafish purchased from commercial supply. The zebrafish were incubated in the aqueous solution at 28 ℃ for around 3 day, and zebrafish were evenly divided into each confocal dish (4-5 fish / dish). Before performing fluorescence imaging, proper anesthetic was added to each dish to prevent the movement of the fish from affecting the imaging. Then fluorescence imaging was conducted through the confocal laser scanning microscopy.

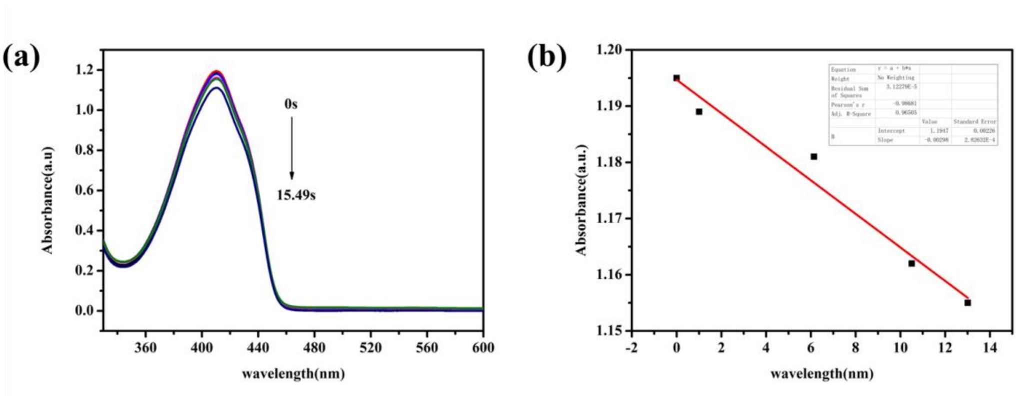


Fig.S1 (a) Time-dependent absorbance decrease of DPBF under light illumination (525 nm, 0.8 mW/cm^2); (b) The linear fitting curve of the absorbance at 410 nm of DPBF versus irradiation time and its corresponding linear regression equation.

^{1}HNMR and HRMS spectra of compounds

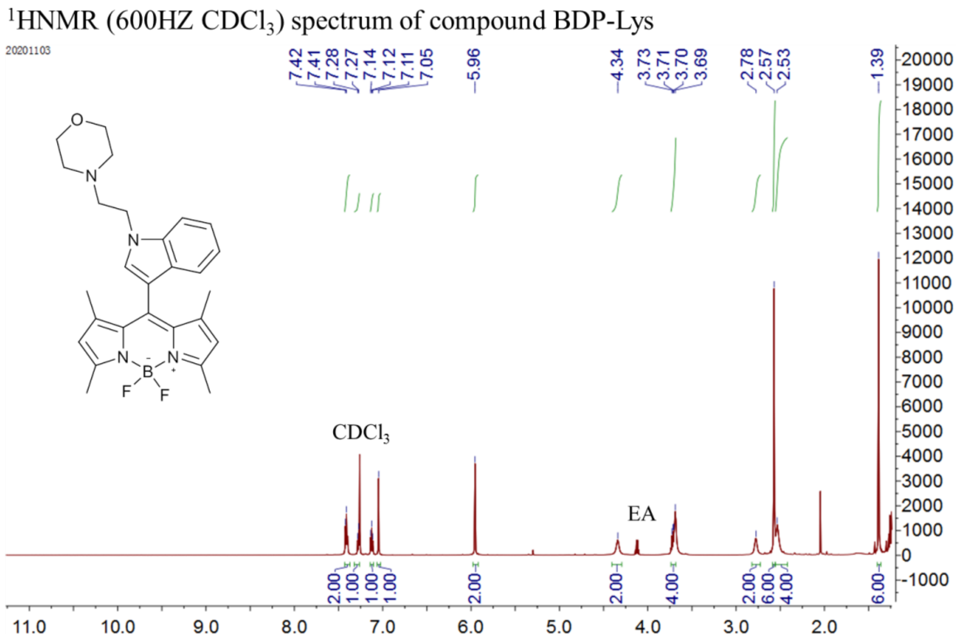


Fig. S2. ^{1}HNMR spectra of BDP-Lys in CDCl3

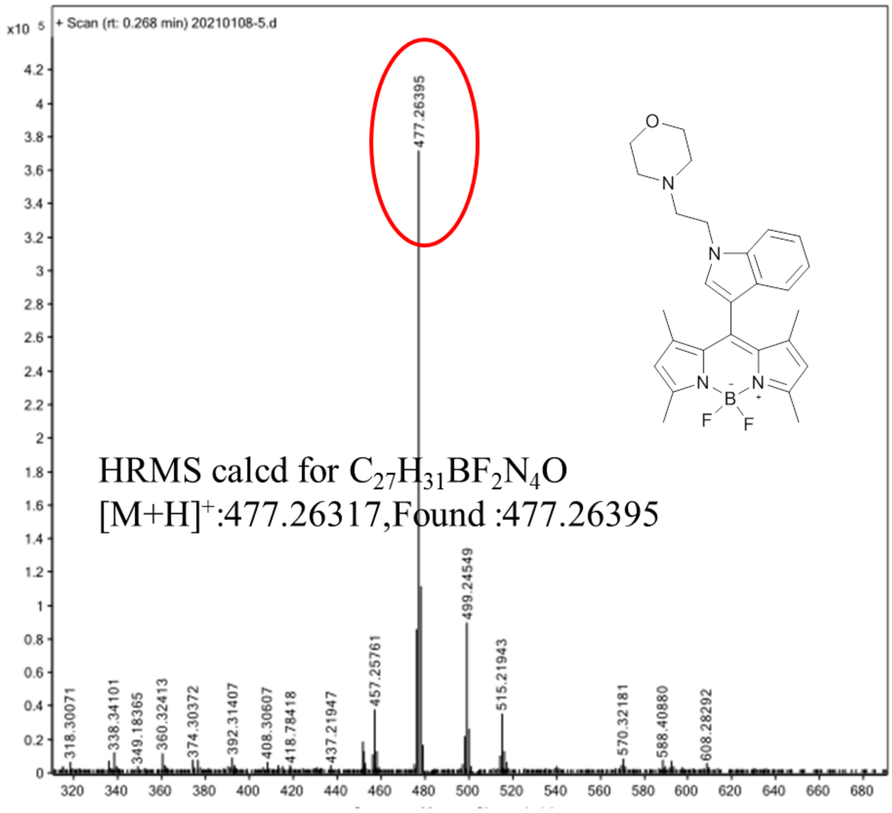


Fig. S3 HRMS spectra of dye BDP-Lys

^{1}HNMR (600HZ $CDCl_3$) spectrum of compound IBDP-Lys

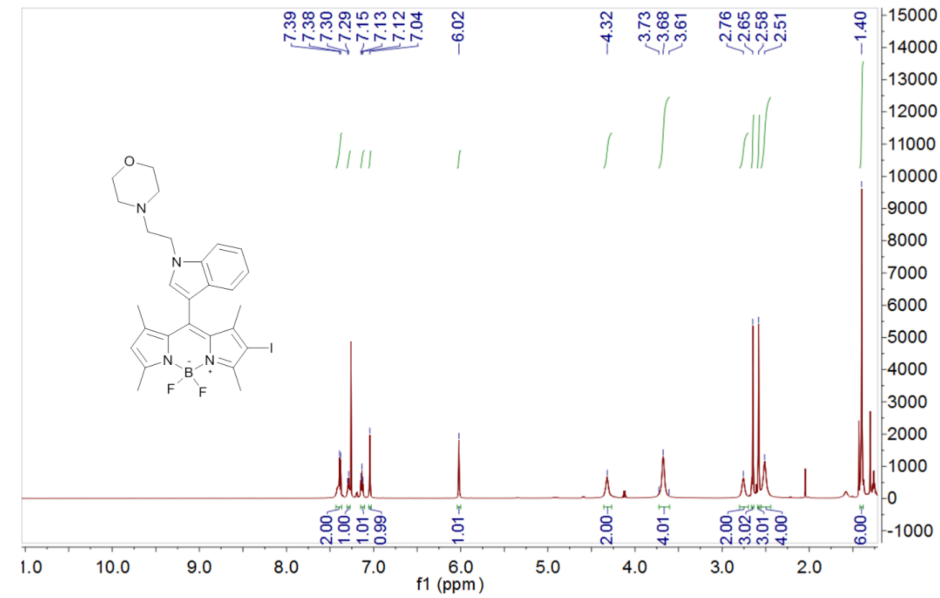


Fig. S4. ^{1}H NMR spectra of photosensitizer IBDP-Lys in $CDCl_3$

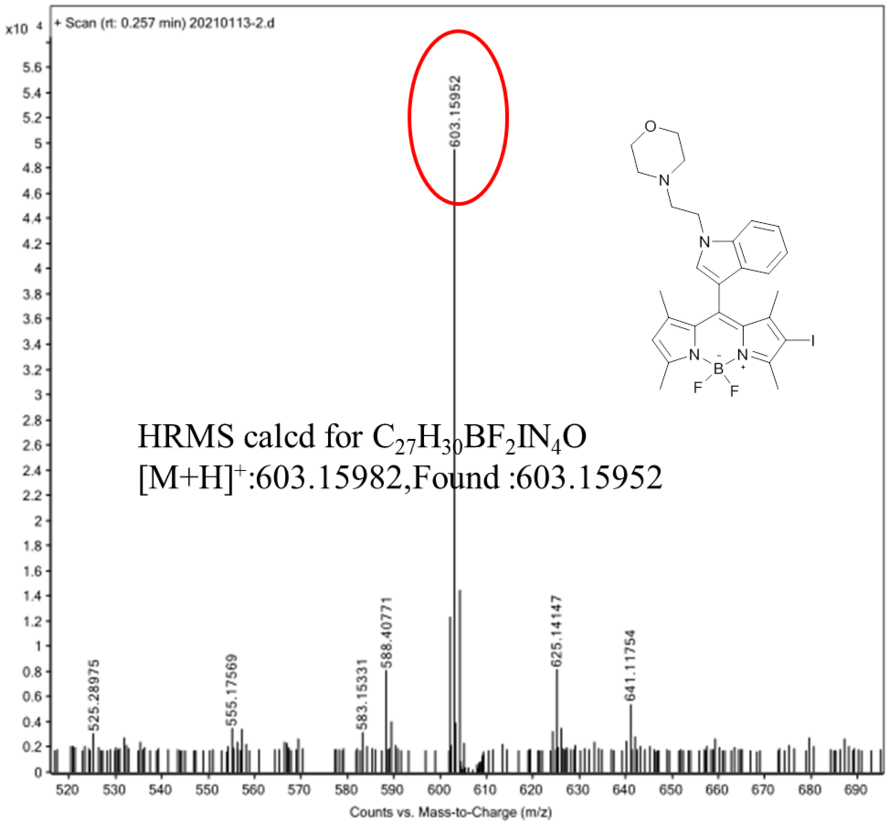


Fig. S5. HRMS spectra of photosensitizer IBDP-Lys

[1]HNMR (600Hz $CDCl_3$） spectrum of compound I_2BDP-Lys

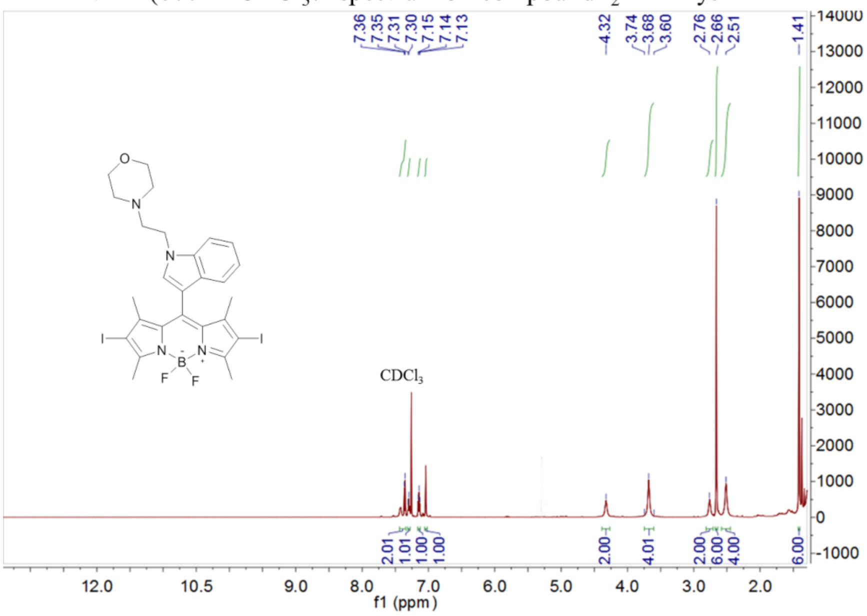


Fig. S6. [1]HNMR spectra of photosensitizer I_2BDP-Lys in $CDCl_3$

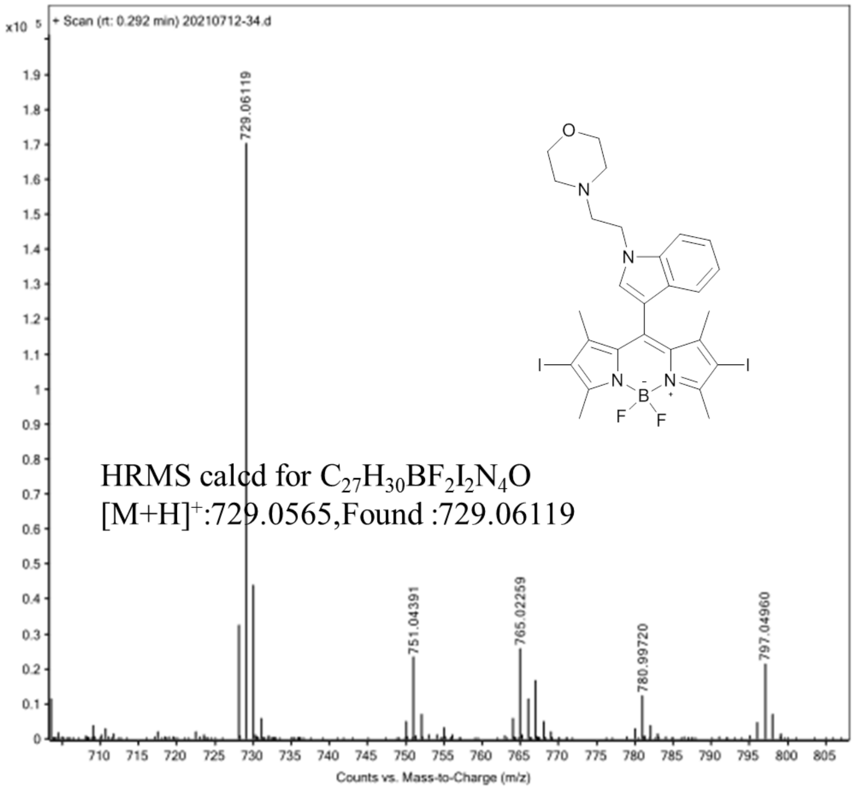


Fig. S7. HRMS spectra of photosensitizer I_2BDP-Lys

Theoretical computation to the rationalized intersystem crossing in indole-BODIPY dyes

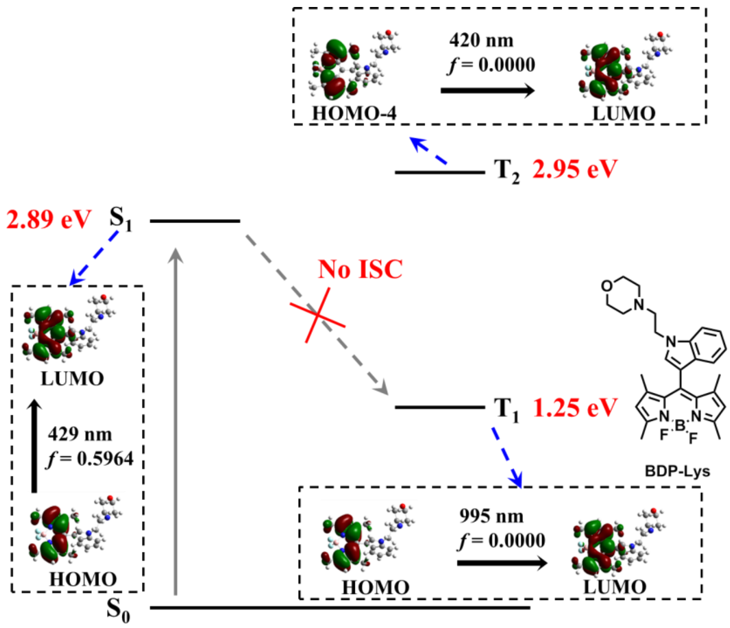


Fig.S8. The energy and frontier molecular orbital diagrams of BDP-Lys dyes obtained with EtOH-TDDFT/cam-B3LYP/6-31G(d) level based on the optimized ground state geometries at DFT/B3LYP/6-31G (d) basis set, isovalue was 0.02.

Table S1. Selected parameters for the vertical excitations of BDP-Lys obtained with EtOH-TDDFT/cam-B3LYP/6-31G(d) level based on the optimized ground state geometries at DFT/B3LYP/6-31G (d) basis set and IBDP-Lys and I_2BDP-Lys with EtOH-TDDFT/cam-B3LYP/TD-DFT//B3LYP/6-31G(d)/LANL2DZ level based on the optimized ground state geometries at DFT/B3LYP/6-31G(d)/LANL2DZ level basis ground state geometries at DFT/B3LYP/6-31G(d)/LANL2DZ level basis set.

Compounds	Excited states	Electronic transition	Energy, eV/λ nm	f^a	Composition[b]	CI[c]
BDP-Lys	Singlet	$S_0 \rightarrow S_1$	2.89/429	0.5964	H→L	0.69943
		$S_0 \rightarrow S_2$	3.11/398	0.0199	H-1→L	0.61098
	Triplet	$S_0 \rightarrow T_1$	1.25/995	0.0000	H→L	0.72846
		$S_0 \rightarrow T_2$	2.95/420	0.0000	H-4→L	0.62300
IBDP-Lys	Singlet	$S_0 \rightarrow S_1$	3.22/385	0.7033	H-1→L	0.69314
		$S_0 \rightarrow S_2$	3.38/367	0.0509	H-2→L	0.66412
	Triplet	$S_0 \rightarrow T_1$	1.43/867	0.0000	H-1→L	0.68753
		$S_0 \rightarrow T_2$	2.75/450	0.0000	H-3→L	0.56767
		$S_0 \rightarrow T_3$	2.80/443	0.0000	H-5→L	0.59486
		$S_0 \rightarrow T_4$	3.07/404	0.0000	H-2→L+1	0.48543
I_2BDP-Lys	Singlet	$S_0 \rightarrow S_1$	3.13/395	0.7505	H-1→L	0.69413
		$S_0 \rightarrow S_2$	3.28/378	0.0380	H-2→L	0.66501
	Triplet	$S_0 \rightarrow T_1$	1.44/860	0.0000	H-1→L	0.66950
		$S_0 \rightarrow T_2$	2.67/465	0.0000	H-4→L	0.47812
		$S_0 \rightarrow T_3$	2.72/456	0.0000	H-3→L	0.56704
		$S_0 \rightarrow T_4$	3.06/405	0.0000	H-2→L+1	0.44971

[a]Oscillator strength. [b]H, HOMO (highest occupied molecular orbital) and L, LUMO (lowest unoccupied molecular orbital). [c]Coefficient of the wavefunction for each excitation.

Supporting references

[1] T. Karstens, K. Kobs, J Phys. Chem., 1980, 84, 1871.

[2]R.W. Redmond, J.N. Gamlin, Photochem. Photobiol., 1999, 70, 391.