Supplementary Information

A water soluble carbazolyl-BODIPY photosensitizer with orthogonal D-A structure for photodynamic therapy in living cells and zebrafish

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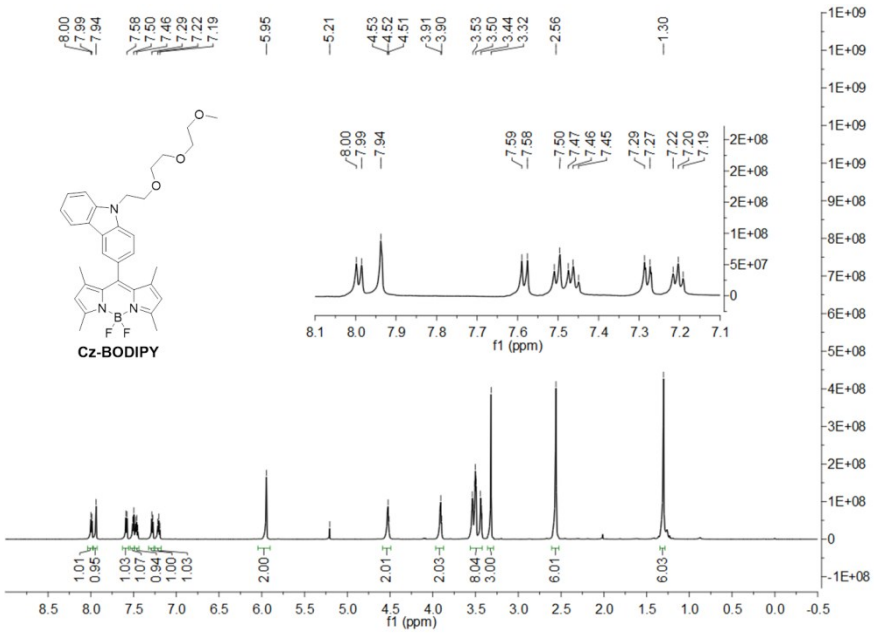

Figure S1. ^{1}H NMR of Cz-BODIPY.

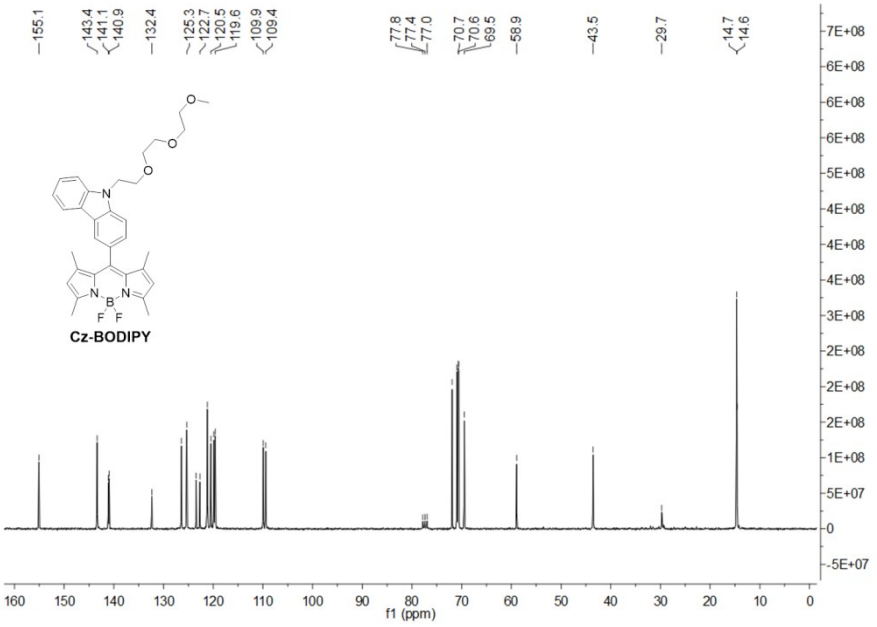

Figure S2. ^{13}C NMR of Cz-BODIPY.

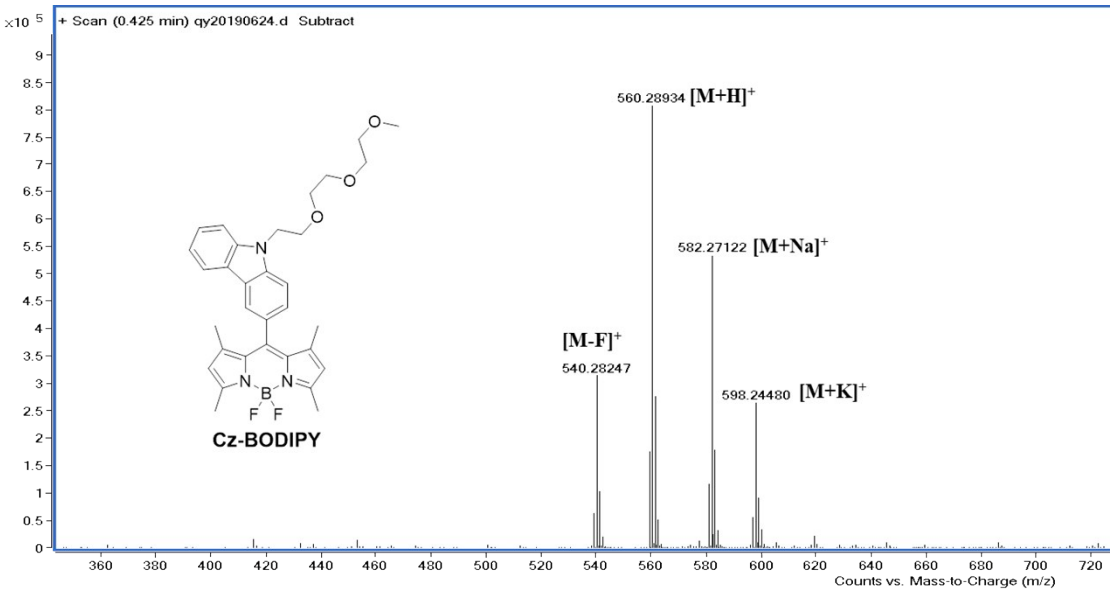

Figure S3. HRMS of Cz-BODIPY.

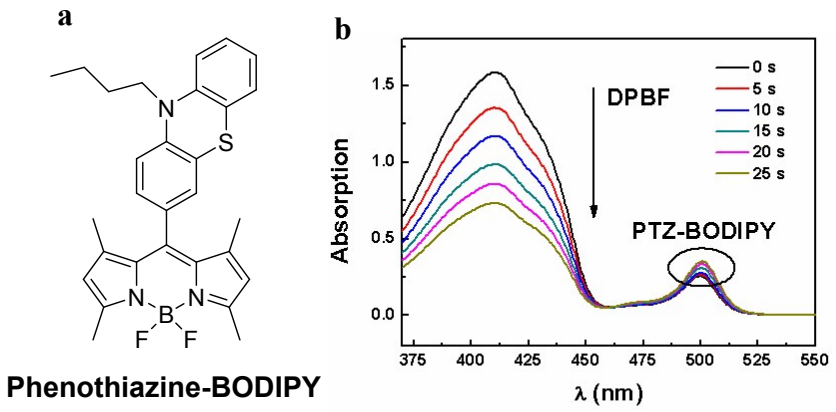

Figure S4. (a) The structure of Phenothiazine-BODIPY; (b) The absorption change of DPBF and Phenothiazine-BODIPY in MeOH upon light irradiation (0.8 mW/cm^2) for various time.

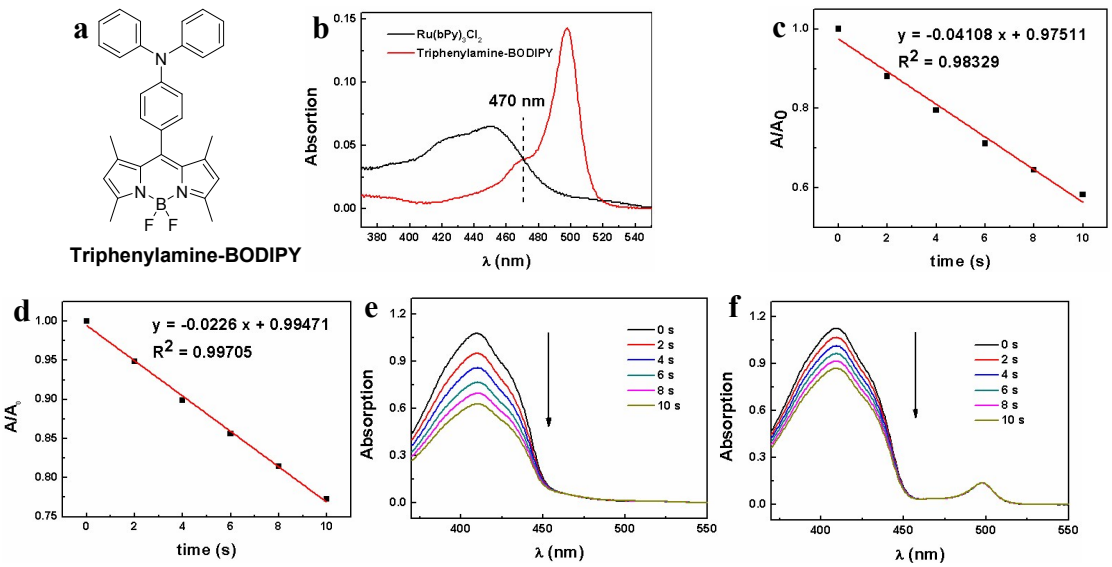

Figure S5. (a) The structure of Triphenylamine-BODIPY; (b) The absorption spectra of Triphenylamine-BODIPY in the MeOH and $Ru(bPy)_3Cl_2$ in MeOH; (c) Plot of normalized DPBF absorption (A/A_0) at 410 nm in MeOH against various light (0.8 mW/cm^2, 470 nm) illumination time in the presence of $Ru(bpy)_3Cl_2$; (d) Plot of normalized DPBF absorption (A/A_0) at 410 nm in MeOH against various light (0.8 mW/cm^2, 470 nm) illumination time in the presence of Triphenylamine-BODIPY; (e) The degradative absorption of DPBF at 410 nm in the presence of $Ru(bpy)_3Cl_2$ in MeOH upon light irradiation (0.8 mW/cm^2) for various time; (f) The degradative absorption of DPBF at 410 nm in the presence of Triphenylamine-BODIPY in MeOH upon light irradiation (0.8 mW/cm^2) for various time.

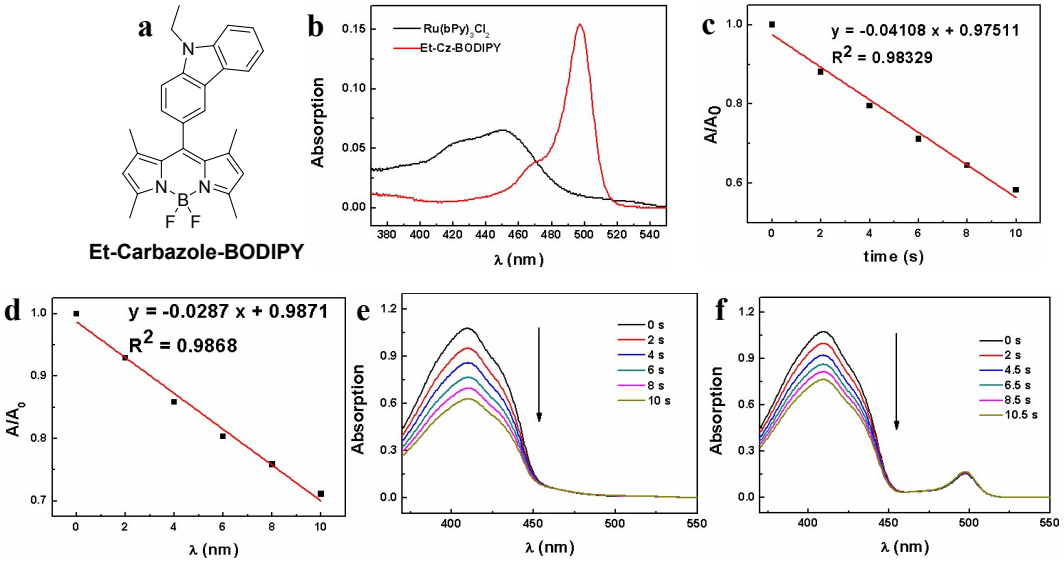

Figure S6. (a) The structure of Et-Carbazole-BODIPY; (b) The absorption spectra of Et-Carbazole-BODIPY and $Ru(bPy)_3Cl_2$ in MeOH; (c) Plot of normalized DPBF absorption (A/A_0) at 410 nm in MeOH against various light (0.8 mW/cm^2, 470 nm) illumination time in the presence of $Ru(bpy)_3Cl_2$; (d) Plot of normalized DPBF absorption (A/A_0) at 410 nm in MeOH against various light (0.8 mW/cm^2, 470 nm) illumination time in the presence of Et-Carbazole-BODIPY; (e) The degradative absorption of DPBF at 410 nm in the presence of $Ru(bpy)_3Cl_2$ in MeOH and light irradiation (0.8 mW/cm^2); (f) The degradative absorption of DPBF in the presence of Et-Carbazole-BODIPY in MeOH and light illumination (0.8 mW/cm^2).

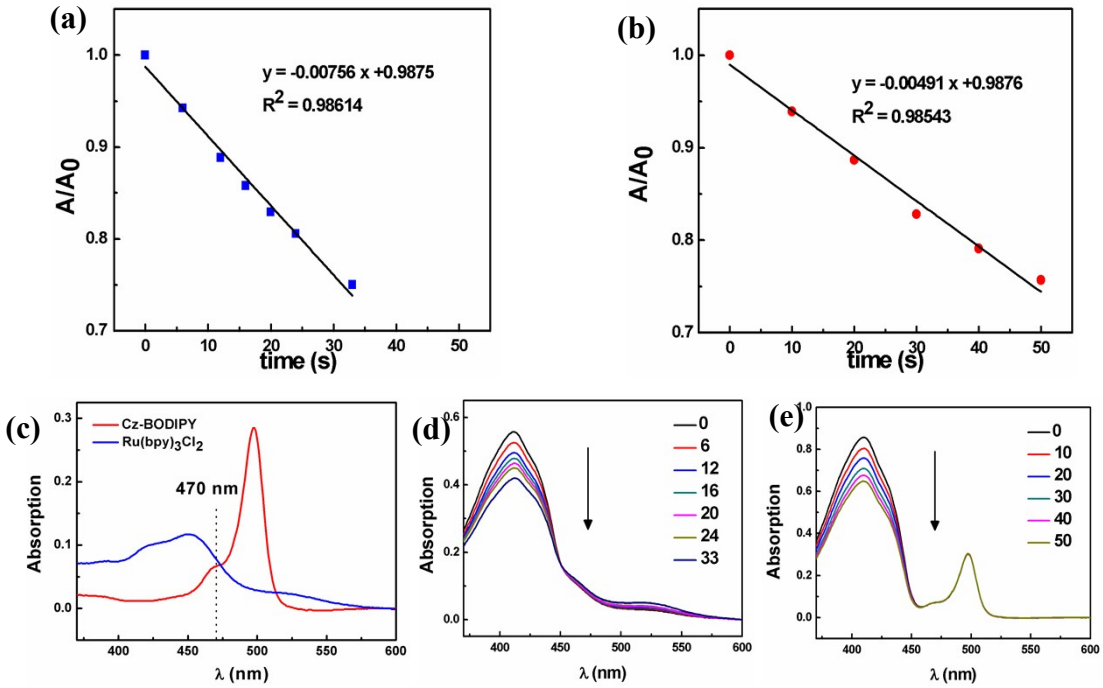

Figure S7. (a) linear fitting of normalized DPBF absorption (A/A_0) at 410 nm in MeOH against various light (0.8 mW/cm^2, 470 nm) illumination time in the presence of $Ru(bpy)_3Cl_2$; (b) linear fitting of normalized DPBF absorption (A/A_0) at 410 nm in MeOH against various light (0.8 mW/cm^2, 470 nm) illumination time in the presence of Cz-BODIPY; (c) the absorption of Cz-BODIPY and $Ru(bpy)_3Cl_2$ in MeOH before addition of DPBF; (d) The degradative absorption of DPBF in the presence of $Ru(bpy)_3Cl_2$ in MeOH and light illumination (0.8 mW/cm^2, 470 nm); (e) The degradative absorption of DPBF in the presence of Cz-BODIPY in MeOH and light illumination (0.8 mW/cm^2, 470 nm).

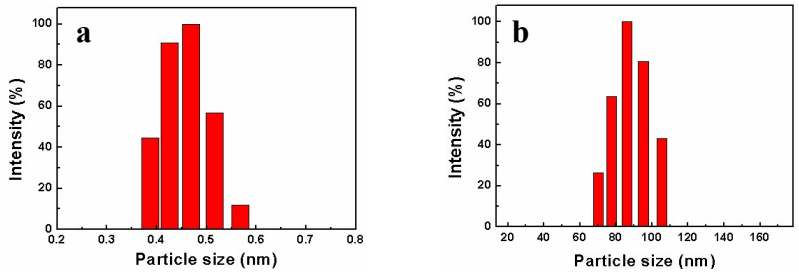

Figure S8. Average sizes of Cz-BODIPY in water solution with the concentrations of 0.1 μM (a) and 1 μM (b).

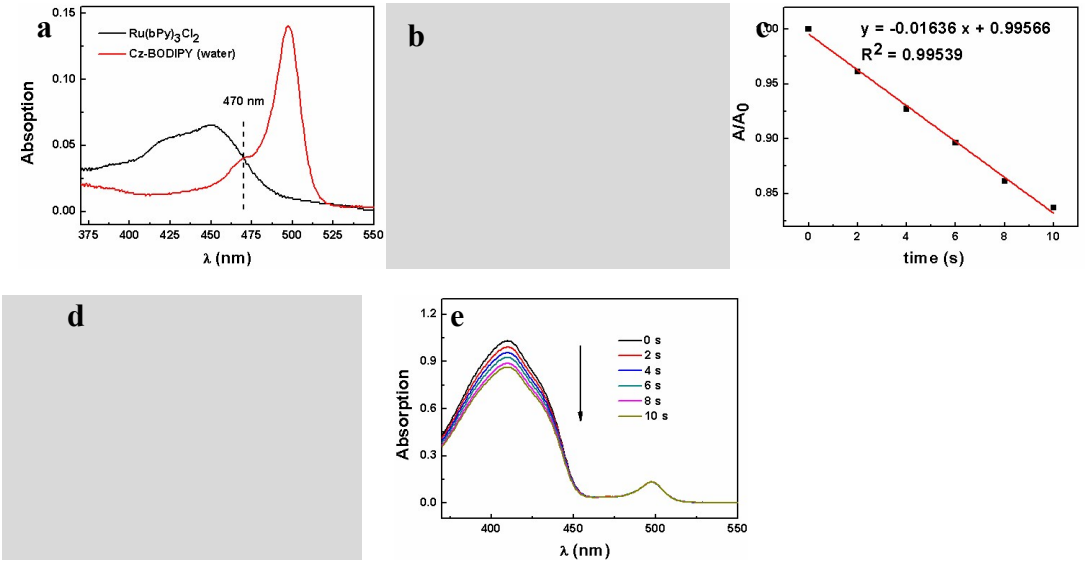

Figure S9. (a) The absorption spectra of Cz-BODIPY in the solution of MeOH/H_2O 1:1 and $Ru(bPy)_3Cl_2$ in MeOH; (b) Plot of normalized DPBF absorption (A/A_0) at 410 nm in MeOH against various light (0.8 mW/cm^2, 470 nm) illumination time in the presence of $Ru(bpy)_3Cl_2$; (c) Plot of normalized DPBF absorption (A/A_0) at 410 nm in MeOH/H_2O 1:1 against various light (0.8 mW/cm^2, 470 nm) illumination time in the presence of Cz-BODIPY ; (d) The degradative absorption of DPBF in the presence of $Ru(bpy)_3Cl_2$ in MeOH and light irradiation (0.8 mW/cm^2); (e) The degradative absorption of DPBF in the presence of Cz-BODIPY in MeOH/H_2O 1:1 and light illumination (0.8 mW/cm^2).

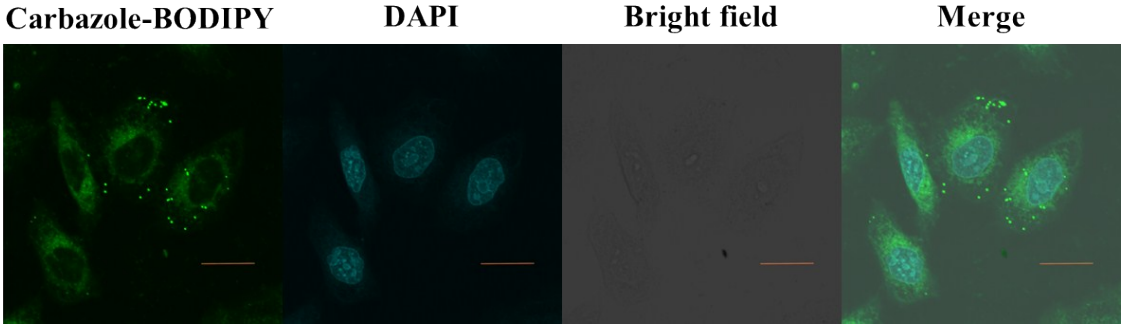

Figure S10. The CLSM image of cells with incubation of Cz-BODIPY (1 μM) and DAPI (0.5 μg/mL).

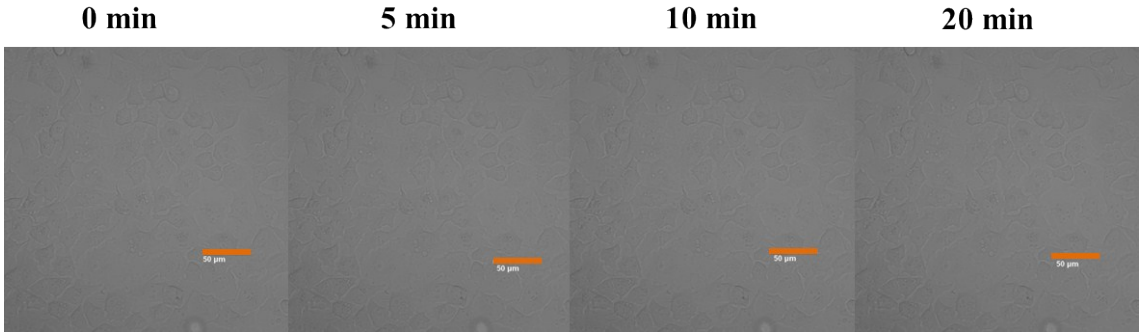

Figure S11. The CLSM image of cells without incubation of Cz-BODIPY after illumination of light (460 nm, 4 mW/cm^2) for various times.

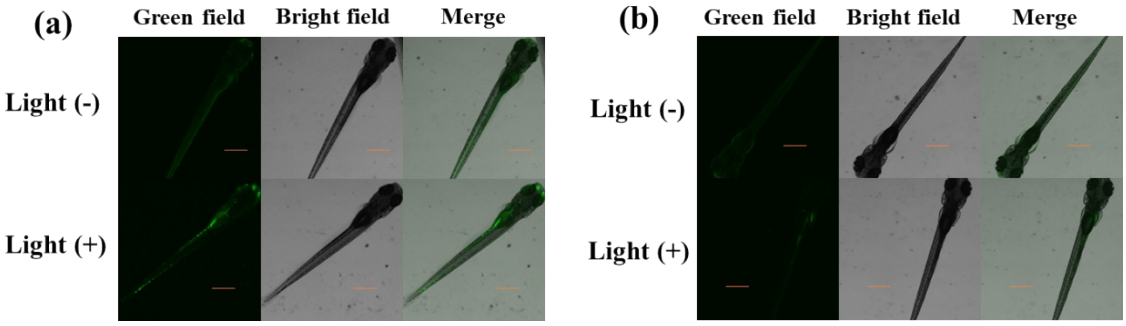

Figure S12. (a) The CLSM image of zebrafish with incubation of Acridine Orange (10 μg/mL) before and after illumination of light (460 nm, 4 mW/cm^2) for 5 min, Scale bar = 500 μm; (b) The CLSM image of zebrafish with incubation of Cz-BODIPY (1 μM) before and after illumination of light (460 nm, 4 mW/cm^2) for 5 min, Scale bar = 500 μm.

Methods

1. Measurement of fluorescence quantum yield of Cz-BODIPY

Fluorescence quantum yield of Cz-BODIPY in MeOH solution was determined using Fluorescein as standard (Φ_r = 0.95 in 0.1 M NaOH), [1] within an absorbance around 0.05. The quantum yields were calculated based on Eq. 1:

$$\Phi_s = \Phi_r \times \frac{A_r}{A_s} \times \frac{F_s}{F_r} \times \frac{{n_s}^2}{{n_r}^2} \qquad \text{Eq. 1}$$

Where Φ is the fluorescence quantum yield, A is the absorbance at the excitation wavelength (A<0.05), F is the area under the corrected emission curve, and n is the refractive index of the solvent used. Subscripts s and r refer to the unknown and to the standard, respectively.

2. Measurement of singlet oxygen quantum yield of Cz-BODIPY

Singlet oxygen quantum yield (Φ_Δ) of Cz-BODIPY in MeOH solution was determined using DPBF as singlet oxygen trapping agent and $Ru(bpy)_3Cl_2$ as standard (Φ_Δ = 0.87 in MeOH).[2] The quantum yields were calculated based on Eq. 2:

$$\Phi_{\Delta,\,s} = \Phi_{\Delta,\,RB} \times \frac{k_s}{k_r} \times \frac{F_r}{F_s} \qquad \text{Eq. 2}$$

Where $\Phi_{\Delta,s}$ is the singlet oxygen quantum yield of Cz-BODIPY, $\Phi_{\Delta,ref}$ is the singlet oxygen quantum yield of the reference compound $Ru(bPy)_3Cl_2$, *k* is the bleach rate of DPBF absorbance (410 nm) against illumination time, F is the absorption correction factor at the excitation wavelength (470 nm).

3. Cell viability by MTT assay

Preparation of the Cz-BODIPY pretreated Bel-7402 cells: Bel-7402 tumor cells were seeded in 96-well plates at 100 μL/well (density of 5000 cells/well) and incubated for 24 h. After removal of medium, various concentrations of Cz-BODIPY (0, 0.25, 0.5, 1, 2, 4 μM) in fresh medium were added into each well and incubated for 12 h.

Dark cytoctoxity: After the removal of Cz-BODIPY-containing medium, the cells were rinsed twice with PBS and the fresh medium containing MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) was added and

incubated for 4 h. After removal of the MTT-containing medium, DMSO (100 μL/well) was added and the absorbance at 490 nm was recorded. The cell viability was calculated utilizing the following equation:

$$\text{Cell Viability} = OD_{490}\ (\text{sample})/OD_{490}\ (\text{control})$$

Phototoxity: After the removal of Cz-BODIPY-containing medium, the cells were rinsed twice with PBS and the fresh medium was added. Then the cells were illuminated by the LED lamp (460 nm) at a power density of 4 mW/cm^2 for 15 min and 40 min with an ice bag disposed below the 96-well plates to eliminate the influence of temperature. Then the cells were subject to continuous incubation for 12 h. After removal of the medium and the fresh medium containing MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, 0.5 mg/mL) was added and incubated for 4 h. After removal of the MTT-containing medium, DMSO (100 μL/well) was added and the absorbance at 490 nm was recorded.

4. Confocal Fluorescence Microscopy

Cell images: Bel-7402 cells were seeded in glass-bottomed plates (Φ = 24 mm) at a density of 1×10^5 cells per well. After incubation for 24 h, the medium was removed and washed with PBS twice. Fresh medium containing Cz-BODIPY (1 μM) was added and incubated for 30 min. The medium was removed and fresh medium was added. Light irradiation (460 nm, 4 mW/cm^2) or near infrared (808 nm) laser (0.72 W) irradiation were conducted and visualized in green channel using the cofocal laser scanning microscopy (CLSM).

Reference

1 J.H. Brannon, D. Magde, *J. Phys. Chem.*, 1978, **82**, 705–709.

2 C. Tanielian, C. Wolff, M. Esch, *J. Phys. Chem.*, 1996, **100**, 6555–6560