Electronic Supplementary Information

A Fullerene-Rhodamine B Photosensitizer with pH-Activated Visible-

light Absorbance/Fluorescence/Photodynamic therapy

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1. Materials and Reagents

Fullerene was obtained from Nanjing XFNANO Materials Tech Co. Ltd. (Nanjing, China). Rhodamine B and dichloro[1,2-bis(diphenylphosphino)ethane]cobalt (II) (CoCl₂dppe) were purchased from Alfa Aesar. Cupric bromide, 1,3-diphenylisobenzofuran (DPBF) were obtained from Aladdin (Shanghai, China). Hydrochloric acid, sodium hydroxide, hydrazine hydrate and 1,2-dichlorobenzene were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Manganese, methyl 3-(bromomethyl)benzoate and N,N-dimethylformamide was purchased from Adamas Reagent Co. Ltd. (Shanghai, China). DSPE-mPEG₂₀₀₀ was purchased from Shanghai ZZBIO. Co. Ltd. (Shanghai, China). All the chemical agents are analytical pure and are used as received.

2. Characterization

Nuclear magnetic resonance (NMR) spectra were measured by using a BrukerAV-300, BrukerAV-400 or BrukerAV-500 spectrometer. MALDI-TOF-MASS (Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry) was performed on a Bruker Autoflex speed MALDI-TOF for data acquisition. The UV-visible absorption and fluorescence spectra were recorded by using an UV-3600 UV-Vis spectrophotometer (Shimadzu, Japan) and an F-4600 Spectro-fluorophotometer (Hitachi, Japan), respectively. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-2100 transmission electron microscope. A xenon lamp was used to carry out the PDT and PTT study. Dynamic light scattering was measured on a 90 Plus particle size analyzer (Brookhaven Instruments, USA). Confocal images were obtained by confocal laser scanning microscopy (Olympus IX 70 inverted microscope).

3. Experimental section

3.1 Synthesis and Characterization of C₆₀-RB

3.1.1 Synthesis of Methyl 3-[([60]Fulleren-1(2H)-yl)methyl]benzoate (1)

Compound 1 was synthesized according to the reported method with slight modification.^{S1} CoCl₂dppe (14.4 mg, 0.027 mmol), Manganese (45.0 mg, 0.83 mmol), fullerene (64.8 mg, 0.090 mmol) and methyl 3-(bromomethyl)benzoate (31.0 mg, 0.14 mmol) in 1,2-dichlorobenzene (12 mL) were stirred under nitrogen atmosphere for 1 h. H₂O (30 μ L, 1.6 mmol) and N,N-dimethylformamide (100 μ L) were added subsequently and the mixture was stirred at room temperature for 24 h. The mixture was filtered and washed with 1,2-dichlorobenzene. The filtrate was purified by silica gel chromatography with toluene/hexane (v:v = 1: 2) to afford the product in 70% yield (54.8 mg). ¹H NMR (300 MHz, CDCl₃) δ 8.51 (s, 1H), 8.12 (d, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 7.6 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 6.63 (s, 1H), 4.83 (s, 2H), 3.98 (s, 3H).

3.1.2 Synthesis of Rhodamine B hydrazide (2)

Rhodamine B hydrazide was synthesized according to the reported method.^{S2} ¹H NMR (300 MHz, CDCl₃) δ 7.99 - 7.88 (m, 1H), 7.50 - 7.38 (m, 2H), 7.17 - 7.04 (m, 1H), 6.59 - 6.37 (m, 4H), 6.29 (d, *J* = 8.4 Hz, 2H), 3.62 (s, 2H), 3.34 (d, *J* = 7.0 Hz, 8H), 1.17 (t, *J* = 6.9 Hz, 12H).

3.1.3 Synthesis of C₆₀-RB

Compound 1 (87.0 mg, 0.1 mmol), compound 2 (91.3 mg, 0.2 mmol), cupric bromide (2.2 mg, 0.01 mmol), N, N-dimethylformamide (1.4 mL) and 1,2-dichlorobenzene (14 mL) were stirred

for 18 h under oxygen atmosphere at 70 °C. The mixture was cooled to room temperature and purified through silica gel column chromatography with the eluent of CS₂/ethyl acetate (v:v = 30:1) to give the brown solid of C₆₀-RB (110.0 mg, yield: 83%). ¹H NMR (500 MHz, CDCl₃) δ 8.33 (s, 1H), 7.97 (d, *J* = 7.1 Hz, 1H), 7.89 (t, *J* = 7.3 Hz, 2H), 7.59 (m, 2H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.24 (m, 1H), 6.69-7.13 (m, 6H), 4.42 (s, 2H), 3.93 (s, 3H), 3.49 – 3.13 (m, 8H), 1.18 (m, 12H). ¹³C NMR (126 MHz, CDCl₃) δ 169.71, 167.25, 154.97, 150.74, 148.76, 147.07, 146.98, 146.67, 145.03, 144.59, 144.36, 144.27, 144.19, 143.97, 143.93, 143.19, 142.82, 142.75, 142.68, 142.46, 133.43, 131.16, 130.43, 128.81, 128.75, 125.66, 125.00, 123.27, 107.73, 70.69, 68.12, 67.92, 59.75, 52.35, 44.48, 12.96. MALDI-TOF MS [M+H]⁺: calcd for C₉₇H₄₁N₄O₄, 1326.3161; found, 1326.4206.

3.2 Fluorescence quantum yield detection

Fluorescence quantum yields of C_{60} -RB were determined in the presence or absence of trifluoroacetic acid in ethanol. Rhodamine 6G (Φ_{FL} = 0.94 in ethanol) was adopted as a reference. Fluorescence quantum yields (Φ_{FL}) was calculated according to the following equation:

$$\Phi_{FL(X)} = \Phi_{FL(R)} \times \frac{I_R}{I_X} \times \frac{A_R}{A_X} \times \frac{n_X^2}{n_R^2} \times \frac{D_X}{D_R}$$
(1)

Where, 'X' and 'R' designate C_{60} -RB and Rhodamine 6G, respectively. I is the relative power of the light source of the spectrofluorimeter at the excitation wavelength. A is the optical density at the excitation wavelength, n is the refractive index of the solvent and D is the integrated emission signal.

3.3 Singlet oxygen detection

The singlet oxygen generation of C₆₀-RB was measured by monitoring oxidation of DPBF in the presence or absence of trifluoroacetic acid in ethanol. Rose bengal (Φ_{Δ} = 0.79 in ethanol) was used as the standard. The mixed solution was irradiated by xenon lamp (550 nm) for 25 s. The decrease of the absorption of DPBF was monitored by UV-VIS-NIR spectrophotometer. Singlet oxygen quantum yield (Φ_{Δ}) of C₆₀-RB was calculated according to the following equation:

$$\Phi_{\Delta(sam)} = \Phi_{\Delta(std)} \times \frac{S_{sam}}{S_{std}} \times \frac{F_{std}}{F_{sam}}$$
(2)

Where, 'sam' and 'std' designate C_{60} -RB and Rose bengal, respectively. S represents the slope of the plots of the absorbance of DPBF (at 410 nm) vs. the irradiation time, F represents the absorption correction factor, which is given by F =1-10^{-O.D.} (O. D. is the optical density of the samples at 660 nm).

The singlet oxygen generation of C₆₀-RB NPs was detected with SOSG (2.5 μ M) as ¹O₂ probe in PBS solution (pH 7.4 or 5.0). The SOSG solution in PBS 7.4 was also irradiated as the control group. Different samples were irradiated by xenon lamp (> 510 nm) at power density of 20 mW cm⁻². The recovered SOSG fluorescence was determined under 494 nm excitation.

3.3 Cellular uptake

Cells of different cell lines were seeded into the glass bottom confocal dishes and incubated in 2 mL culture media for 24 h and 2 mL media containing C_{60} -RB NPs (IC₅₀ values for each cells) for additional 24 h. Washed with PBS solution, these cells were incubated with paraformaldehyde (4%) for 20 min and treated with DAPI for 3 min in darkness. After rinsing thrice with PBS solution (at pH 7.4), these cells were refilled with 2 mL PBS solution. These Page S5

cells were imaged by a confocal laser scanning microscopy. C_{60} -RB NPs was excited with a 559-nm laser, and the fluorescence from 570 nm to 670 nm was collected. A 405-nm laser was used for the excitation of DAPI, and the fluorescence from 420 nm to 500 nm was collected.

3.4 ROS detection in vitro

Cells of four different cell lines seeded in the glass bottom confocal dishes were incubated in 2 mL media containing C_{60} -RB NPs (IC₅₀ values for each cell) for 24 h. Washed with PBS solution, these cells were incubated with DCFH-DA for 30 min, paraformaldehyde (4%) for 20 min and DAPI for 3 min at 37 °C in darkness, respectively. After rinsing and refilling with PBS solution (pH 7.4), these dishes were illuminated with a xenon lamp (> 510nm, 20 mW cm⁻²) for 10 min and imaged using a confocal laser scanning microscopy. A 488-nm laser was used for excitation of DCF, and the fluorescence from 500 nm to 550 nm was collected. A 405-nm laser was used for excitation of DAPI, and the fluorescence from 420 nm to 500 nm was collected.

4. Supplemental Figures



Fig. S1. The MALDI-TOF mass spectrum of C₆₀-RB.



Fig. S2. The resonance structures of C_{60} -RB-H1 according to theoretical calculation.



Fig. S3. (a) Absorbance spectra of DPBF incubated with C_{60} -RB in ethanol under irradiation of xenon lamp (550 nm) for different time. (b) Absorption spectra of DPBF incubated with C_{60} -RB and TFA in ethanol upon irradiation with xenon lamp (550 nm) for different time. (c) Absorbance of DPBF incubated with Rose bengal in ethanol under irradiation of xenon lamp (550 nm) for different time.



Fig. S4. Possible Mechanism for enhanced singlet oxygen generation induced by pH.



Fig. S5. Size distribution of C₆₀-RB NPs.



Fig. S6. (a) TEM image of C_{60} -RB NPs after 30 days. (b) Size distribution of C_{60} -RB NPs after Page S9





Fig. S7. MTT assay of HCT-116 cells treated with C_{60} -RB NPs at different light dose under xenon lamp.

5. Supplemental References

(S1) Lu, S.; Jin, T.; Bao, M.; Yamamoto, Y. (2011) Cobalt-Catalyzed Hydroalkylation of
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(S2) Xiang, Y., Tong, A., Jin, P., Ju, Y. (2006) New Fluorescent Rhodamine Hydrazone

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