

Photosensitive MRI Biosensor for BCRP-Targeted Uptake and Light-Induced Inhibition of Tumor Cells

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1. Materials and Instruments

In the article, except Elacridar purchased from Sigma-Aldrich and Cryptophanol-A from Sinopharm Chemical Reagent Co. Ltd, all other the chemicals we used were purchased from Sinopharm Reagent Co., Ltd., Shanghai Aladdin Bio-Chem Technology Co., LTD. and J&K Scientific Ltd. without further purification. All reagents were of analytical grade, and besides DMSO we purchased Chemical Reagent Co. Ltd is a spectral grade and the other reagents are analytical grade.

^{13}C NMR and ^1H NMR and spectra were acquisition on a Bruker AVANCE 500 Hz spectrometer and TMS as an internal standard. ^{129}Xe NMR spectra and MRI results were acquired on a 400 MHz Bruker AV400 wide bore spectrometer.

The high resolution mass spectrometry (HRMS) was obtained on an Agilent 6530 accurate mass Q-TOF spectrometer. The pH meter was obtained by Mettler Toledo SevenEasy pH meter. UV-Vis spectra were collected on a Thermo Scientific evolution 220 UV-Vis spectrometer. Fluorescence spectra was recorded with an Edinburgh FS5 fluorescence spectrophotometer. Thin layer chromatography (TLC) ultraviolet light of 254 nm was used for detection of the silica gel plate.

2. Experimental section

2.1 Cellular fluorescence imaging

The culture of human lung cancer cells A549 cells and breast cancer cells MCF-7 cells were in RPMI 1640 medium (Boster, China)^[1]. A549 cells, $\sim 10^5/\text{mL}$, were seeded in a 6-well chamber slide at the bottom of each chamber with a cover glass and then incubated with +inhibitor and -inhibitor for 12 h. Next, cells were washed three times with PBS and incubated with CrA-2 for 3 h. And cells were incubated with DAPI for 30 minutes (staining of nuclei). To fix cell morphology, A549 cells were immersed in 4% paraformaldehyde for 15 minutes. Finally, the cells were fixed on the glass slides to obtain a fluorescence image microscope (A1R/A1, Nikon, Japan) by confocal laser scanning microscope. For MCF-7 cells imaging, the procedure was the same as mentioned above. Human lung cancer cells A549 and breast cancer cells MCF-7 were purchased from the Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

2.2 Cell viability assay

A549 cells (MCF-7 cells) were seeded to 96-well plate density of 0.5×10^5 cells/well and incubated overnight. CrA-2 or photoproducts (CrA-2 after applying four-hours radiation, expressed as the concentrations of CrA initial concentration were diluted to a specific concentration with a diluent and added to the medium, and incubated for 3 h at 37 °C under 5% CO₂ . After the end, cells were washed three times with PBS and further continue to be cultured 44 hours in fresh medium. Thereafter, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium added bromide (MTT) to the medium keeping the concentration at 0.5 mg/mL, and Incubate for another 4 hours at 37 °C. After removal of the medium, 200 μL of DMSO was added to per well and sand shaken for 30 min to fully dissolve the colored formazan, which was produced by cellular reduction of MTT. In the per well, the solution was measured at the 490 nm absorbance by an ELISA plate reader (Spectra MAX 190, Molecular Devices, USA).

3. Synthesis of the ^{129}Xe biosensor: CrA-1/CrA-2

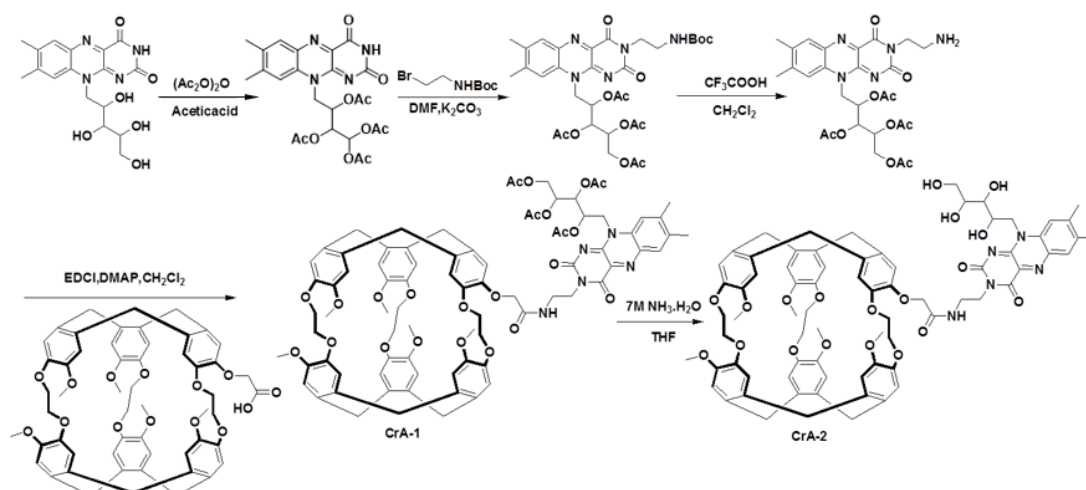


Figure S1. The synthetic route of CrA-1/CrA-2.

3.1 Tetra-O-acetyl-riboflavin

In a two-necked round-bottomed flask, riboflavin (2.0 g, 5.3 mmol), acetic anhydride (40 mL), and 4-dimethylaminopyridine (1.3 g, 10.2 mmol) were mixed and stirred under argon for 24 h at 40 °C. After this period, the solution was left to cool to room temperature, diluted in dichloromethane (60 mL) and followed by addition of saturated aqueous NH_4Cl solution (40 mL). After phase separation, the aqueous layer was extracted with dichloromethane (2×60 mL). The combined organic layers were dried over Na_2SO_4 , filtered off and the solvent removed under vacuum. The residue was purified by column chromatography on silica gel, using dichloromethane and methanol (10:1 v/v) as eluent to afford tetra-O-acetyl riboflavin (**R1**). ^1H (NMR 500 MHz, CDCl_3) δ : 8.90 (s, 1H), 8.01 (s, 1H), 7.57 (s, 1H), 5.67 (s, 1H), 5.47-5.42 (m, 2H), 5.13-4.91 (m, 2H), 4.45 (d, $J=12$ Hz, 1H), 4.26 (d, $J=12$ Hz, 1H), 2.57 (s, 3H), 2.45 (s, 3H), 2.29 (s, 3H), 2.22 (s, 3H), 2.08 (s, 3H), 1.76 (s, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ : 170.7, 170.3, 169.9, 169.8, 159.4, 154.6, 150.7, 148.1, 137.0, 136.1, 134.6, 132.9, 131.2, 115.6, 70.5, 69.4, 69.0, 61.9, 21.5, 21.1, 20.8, 20.7, 20.4, 19.5. HRMS [ESI]: $[\text{M}+\text{H}]^+$ Calculated for ($\text{C}_{25}\text{H}_{29}\text{N}_4\text{O}_{10}$) 545.1878; measured: 545.1888.

3.2 Tetra-O-acetyl-N(3)-2-(Boc-amino) ethyl-riboflavin

In a two-necked round-bottomed flask, a mixture of tetra-O-acetyl-riboflavin (544 mg, 1.0 mmol), K_2CO_3 (346 mg, 2.5 mmol), a catalytic amount of potassium iodide, and dry DMF (20 mL) was stirred under argon at room temperature for 30 min. Then, a solution of tert-Butyl N-(2-Bromoethyl) carbamate (1.12 g, 5.0 mmol) was added slowly and the stirring was continued for 20 h at 40 °C. The reaction mixture was diluted with dichloromethane (60 mL) and the organic phase was washed with saturated aqueous solution of NaHCO_3 (60 mL), then water (120×3 mL), and then brine (30 mL). The organic extract was dried over Na_2SO_4 , filtered off and the solvent was removed under vacuum. The residue was purified by column chromatography on

silica gel using a mixture of dichloromethane and methanol (10:1 v/v) as eluent to afford product (**R2**). ¹H NMR (500 MHz, CDCl₃): 8.06 (s, 1H), 7.57 (s, 1H), 7.27 (m, 1H), 5.67 (d, J=9 Hz, 1H), 5.48 (s, 1H), 5.45-5.42 (bs, 1H), 4.93-4.77 (m, 2H), 4.47 (dd, J₁= 12 Hz, J₂=2 Hz, 1H), 4.29-4.25 (m, 3H), 3.58-3.45 (m, 2 H), 2.58 (s, 3H), 2.46 (s, 3H), 2.30 (s, 3H), 2.23 (s, 3H), 2.10 (s, 3H), 1.79 (s, 3H), 1.39 (s, 9H). ¹³CNMR (125 MHz, CDCl₃) δ: 170.7, 170.3, 169.9, 169.7, 160.2, 156.1, 155.2, 149.2, 147.8, 136.7, 135.6, 134.5, 132.9, 131.2, 115.4, 79.1, 70.4, 69.1, 61.9, 61.8, 44.5, 41.5, 40.0, 28.4, 21.5, 21.1, 20.8, 20.7, 20.4, 19.5. HRMS [ESI]: [M+H]⁺ Calculated for (C₃₂H₄₂N₅O₁₂) 688.2825; measured: 688.2631.

3.3 Tetra-O-acetyl-N(3)-2-(amino)ethyl-riboflavin

In a two necked round-bottomed flask containing a solution of **R2** (137 mg, 0.2 mmol) in dichloromethane (20 mL), then, trifluoroacetic acid (5 mL) was added slowly at 0 °C. The solution was stirred for 12 h at room temperature. The reaction mixture was then poured into ice-water mixture, and a saturated aqueous solution of NaHCO₃ was added until pH=5. After the phase separation, the water layer was washed with dichloromethane (30×3mL) and the combined organic extracts were washed with brine (30 mL) and then with water mL). After drying over Na₂SO₄, the desiccant was filtered off and the solvent was removed under vacuum. The residue was purified by column chromatography on silica gel using a mixture of dichloromethane and methanol (5:1 v/v) as eluent to afford Tetra-O-acetyl-N(3)-2-(amino) ethyl-riboflavin (**R3**). ¹H NMR (500 MHz, CDCl₃): 7.96 (s, 1H), 7.50 (s, 1H), 5.59 (s, 1H), 5.47-5.40 (m, 2H), 5.16-4.93 (m, 2H), 4.43 (dd, J₁= 12 Hz, J₂=2 Hz, 1H), 4.28 (dd, J₁= 12 Hz, J₂=5 Hz, 1H) 3.55-3.49 (m, 2H), 3.22 (br, 2 H), 2.85-2.69 (m, 2H), 2.52 (s, 3H), 2.42 (s, 3H), 2.24 (s, 3H), 2.21 (s, 3H), 2.09 (s, 3H), 1.68 (s, 3H). ¹³CNMR (125 MHz, CDCl₃) δ: 171.6, 170.8, 169.6, 169.5, 160.7, 155.8, 149.1, 147.4, 136.8, 135.7, 134.8, 132.5, 131.5, 115.6, 70.0, 69.5, 69.0, 61.6, 44.0, 39.6, 38.9, 21.5, 21.2, 20.74, 20.70, 20.3, 19.3. HRMS [ESI]: [M+H]⁺ Calculated for (C₂₇H₃₄N₅O₁₀) 588.2300, measured: 588.2300.

3.4 CrA-1

In a two-necked round-bottomed flask, a mixture of Cryptophane-A (20 mg, 0.02 mM), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI, 20mg, 0.1 mM), 4-dimethylaminopyridine (DMAP, 12 mg, 0.1 mM) and dichloromethane (10 mL), stirred at room temperature for 30 min. Then, Tetra-O-acetyl-N(3)-2-(Boc-amino)ethyl-riboflavin was added slowly at room temperature for 8 h. After this period, the solution was left to cool to room temperature, diluted in water (20 mL), the aqueous layer was extracted with dichloromethane (3×30 mL). The combined organic layers were dried over Na₂SO₄, filtered off and the solvent removed under vacuum. The residue was purified by column chromatography on silica gel, using dichloromethane and methanol (10:1 v/v) as eluent to afford CrA-1. ¹H NMR (500 MHz, CDCl₃): 7.96 (s, 1H), 7.55 (s, 1H), 7.40 (br, 1H), 6.94-6.67 (m, 12H), 5.68 (br, 1H) 5.44-5.40 (m, 1H), 5.37 (s, 1H), 5.10-4.86 (m, 2H), 4.65-4.55 (m, 6H), 4.44-4.38 (m, 6H), 4.25-4.13 (m, 12H), 3.89-3.76 (m, 15H), 3.52-3.38 (m, 8H), 2.56 (s, 3H), 2.41 (s, 3H), 2.28 (s, 3H), 2.23 (s, 3H), 2.09 (s, 3H), 1.79 (s, 3H) ¹³CNMR (125 MHz, CDCl₃) δ: 171.5, 170.7, 170.6,

169.6, 169.3, 160.4, 155.6, 149.7, 149.65, 149.60, 149.5, 149.2, 148.6, 147.8, 146.3, 136.7, 136.4, 135.8, 135.7, 134.7, 134.6 132.4, 134.1, 133.8, 133.7, 133.5, 133.1, 132.5, 131.9, 131.8, 131.6, 131.3, 131.25, 122.6, 122.4, 121.4, 120.8, 120.7, 120.5, 120.0, 115.4, 114.3, 113.8, 113.7, 113.5, 70.4, 70.2, 69.8, 69.5, 69.3, 69.2, 69.05, 69.01, 61.8, 55.8, 55.7, 55.4, 55.0, 53.6, 44.3, 39.8, 38.4, 36.5, 36.2, 21.5, 21.3, 20.8, 20.7, 20.3, 19.9. HRMS [ESI]: $[M+H]^+$ Calculated for $C_{82}H_{86}N_5O_{23}$ $[M + H]^+$ =1508.5708; found, 1508.5730.

3.5 CrA-2

7 N ammonia in THF (20 mL) was added to a solution of CrA-1 (15 mg; 0.01 mmol). The solution was stirred at room temperature for 24 h^[3]. After completion of reaction the solvent was removed under high vacuum and dissolved the residue in dichloromethane: methanol (1:20), workup with 5% NH_3 in water, removed the solvent under high vacuum and the residue was purified by column chromatography (CH_2Cl_2 :MeOH; 10:1–5:1) to get CrA-2. 1H NMR (500 MHz, DMSO) δ 8.01 (t, J = 5.7 Hz, 1H), 7.96 (s, 1H), 7.87 (s, 1H), 6.85 (t, J = 7.9 Hz, 6H), 6.81 – 6.75 (m, 6H), 5.14 (d, J = 2.3 Hz, 1H), 5.01 (s, 1H), 4.91 (s, 1H), 4.81 (d, J = 5.6 Hz, 1H), 4.49 (dd, J = 24.2, 12.6 Hz, 7H), 4.36 (dd, J = 24.7, 14.9 Hz, 3H), 4.24 (dd, J = 19.0, 10.3 Hz, 3H), 4.09 (dddd, J = 24.2, 21.0, 18.2, 8.5 Hz, 15H), 3.72 (s, 12H), 3.65 (t, J = 7.3 Hz, 6H), 3.60 – 3.39 (m, 6H), 2.49 (s, 3H), 2.38 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d₆) 171.2, 170.4, 160.1, 155.8, 149.1, 147.4, 136.8, 135.7, 134.8, 132.5, 131.5, 115.6, 70.0, 69.5, 69.0, 61.6, 44.0, 39.6, 38.9, 21.5, 21.2, 20.74, 20.70, 20.3, 19.3. ^{13}C NMR (125 MHz, $CDCl_3$) δ : 170.7, 170.6, 169.7, 169.2, 160.8, 155.1, 149.9, 149.8, 149.6, 149.5, 149.0, 148.5, 147.6, 146.1, 136.8, 136.1, 135.6, 135.6, 134.5, 134.7 132.4, 134.1, 133.8, 133.9, 133.5, 133.5, 132.0, 131.95, 131.8, 131.7, 131.5, 131.4, 131.25, 122.4, 122.5, 121.6, 120.7, 120.6, 120.5, 120.1, 115.9, 114.7, 113.8, 113.7, 113.2, 70.6, 70.0, 69.9, 69.7, 69.3, 69.2, 69.1, 69.0, 61.8, 55.8, 55.6, 55.2, 55.0, 53.4, 44.3, 39.6, 38.8, 36.3, 36.2, 22.1, 20.3. HRMS [ESI]: $[M+H]^+$ $C_{74}H_{78}N_5O_{19}$ $[M + H]^+$ =1340.5286; found, 1340.5268.

4. Photolysis results of CrA-1/CrA-2 for UV-vis spectra

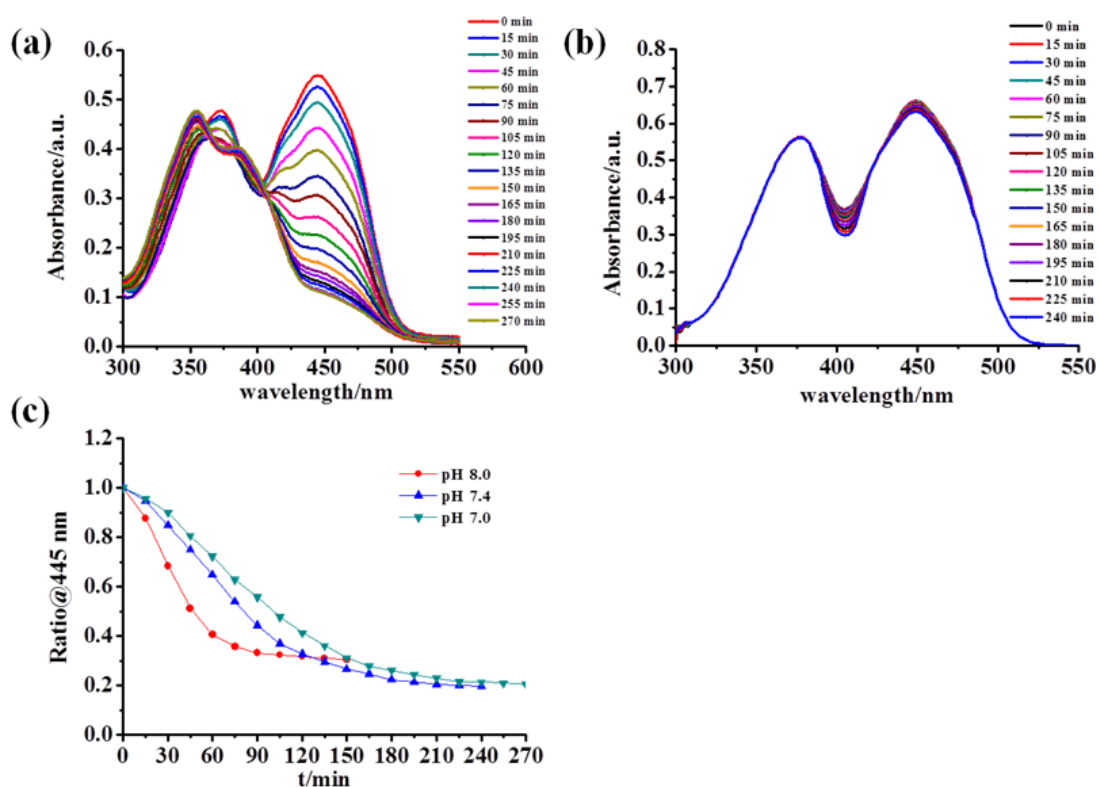


Figure S2. Photolysis of 50 μM (a) CrA-2 and (b) CrA-1 UV-vis absorbance spectra in pH 7.4; (c) The curves of 50 μM CrA-2 in pH 7.0, 7.4, 8.0 UV-vis absorbance at 445 nm with applying irradiation time; 20 mM phosphate buffer, 20% DMSO, 250 W high-voltage mercury lamp with emission at 350~450nm.

5. Photolysis results of CrA-1/CrA-2 for fluorescence spectra

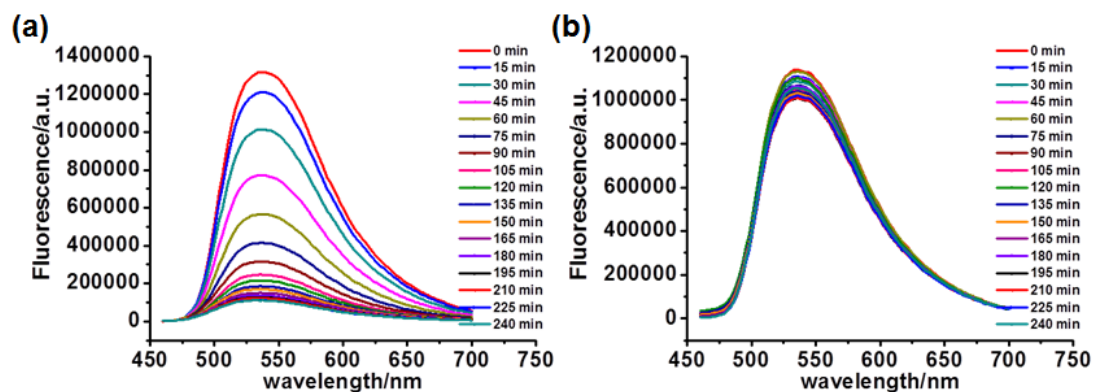


Figure S3. Photolysis of 50 μ M (a) CrA-2 and (b) CrA-1 fluorescence spectra are excitation at 446/450 nm; 20 mM phosphate buffer; pH=7.4; 20% DMSO, 250 W high-voltage mercury lamp with emission at 350~450nm, t=0~ 240 min.

6. Hyper-CEST NMR spectrum of CrA-2

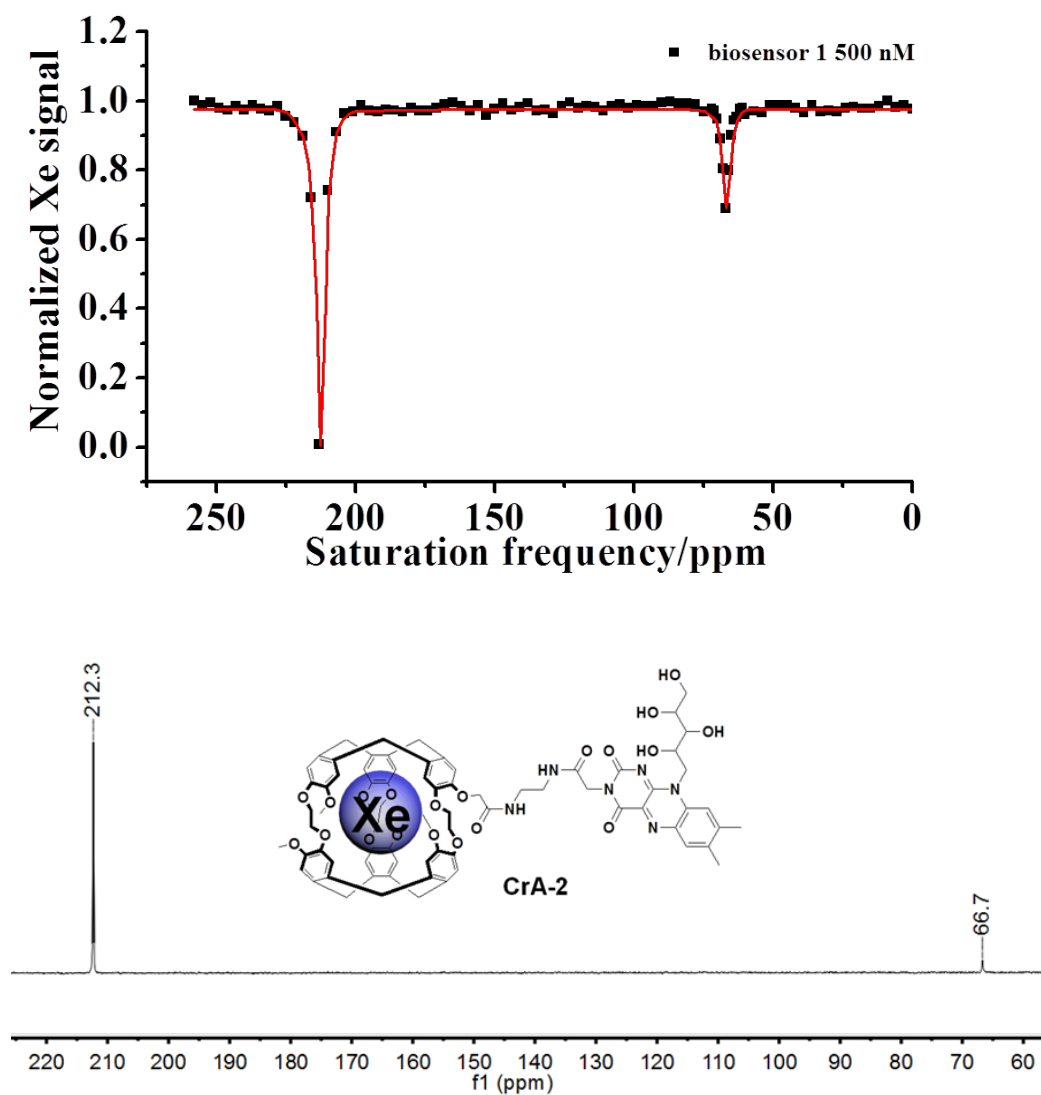


Figure S4. above: hyper-CEST spectrum of CrA-2 (500 nM); below: ^{129}Xe NMR spectrum of CrA-2 (50 μM , 16 scans, LB=4 Hz). The spectrum was measured with a 10 s saturation pulse with of amplitude 13 μT , and the frequency interval from 54 ppm to 78 ppm is 1 ppm, and the frequency interval of the rest spectrum (0-54, 78-254 ppm) is 3 ppm. Temperature was set to 298 K, CrA-2 in PBS buffer (pH=7.4, 20 mM), containing 20% DMSO (v/v).

7. Hyper-CEST NMR spectra of biosensors and their photoproducts

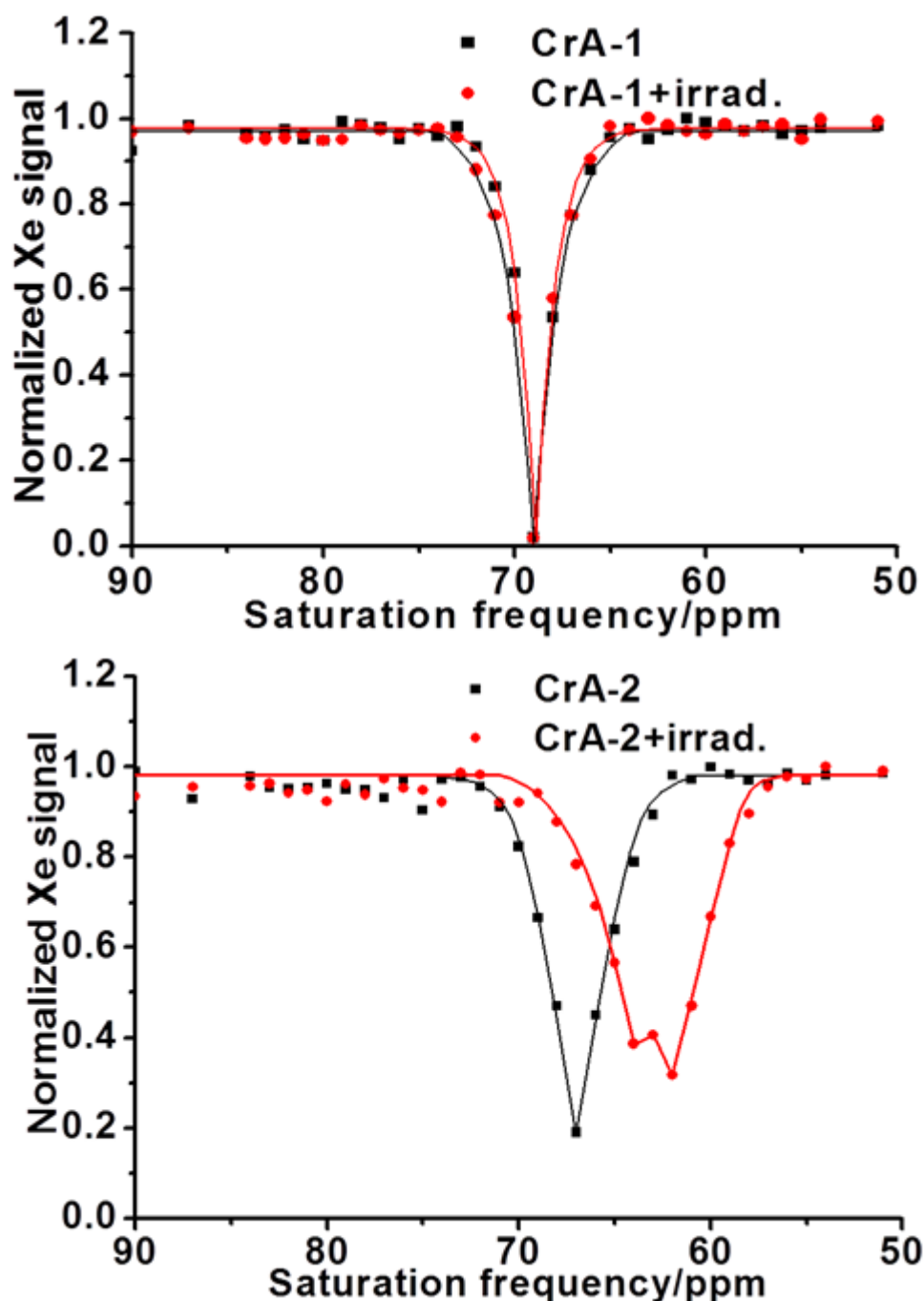


Figure S5. Hyper-CEST spectra of (above) CrA-1 and (below) CrA-2 (12.5 μM). The spectrum was measured with a 10 s saturation pulse and amplitude of 3.0 μT . The frequency interval from 54 ppm to 84 ppm is 1 ppm, and while that of the remaining spectrum (21-54, 84-108 ppm) is 3 ppm. The irrads. stands for samples with applying 3-h radiation. Temperature was set to 298 K, CrA-1/CrA-2 in PBS buffer (pH=7.4, 20 mM), containing 20% DMSO (v/v).

8. TOF-MS spectrum of photoproducts of the CrA-1

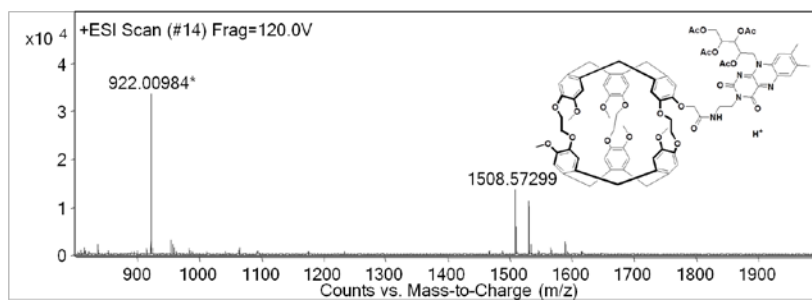


Figure S6. ESI-HRMS spectra of photoproducts of the CrA-1 with applying three-hour radiation.

9. TOF-MS spectrum of photoproducts of the CrA-2

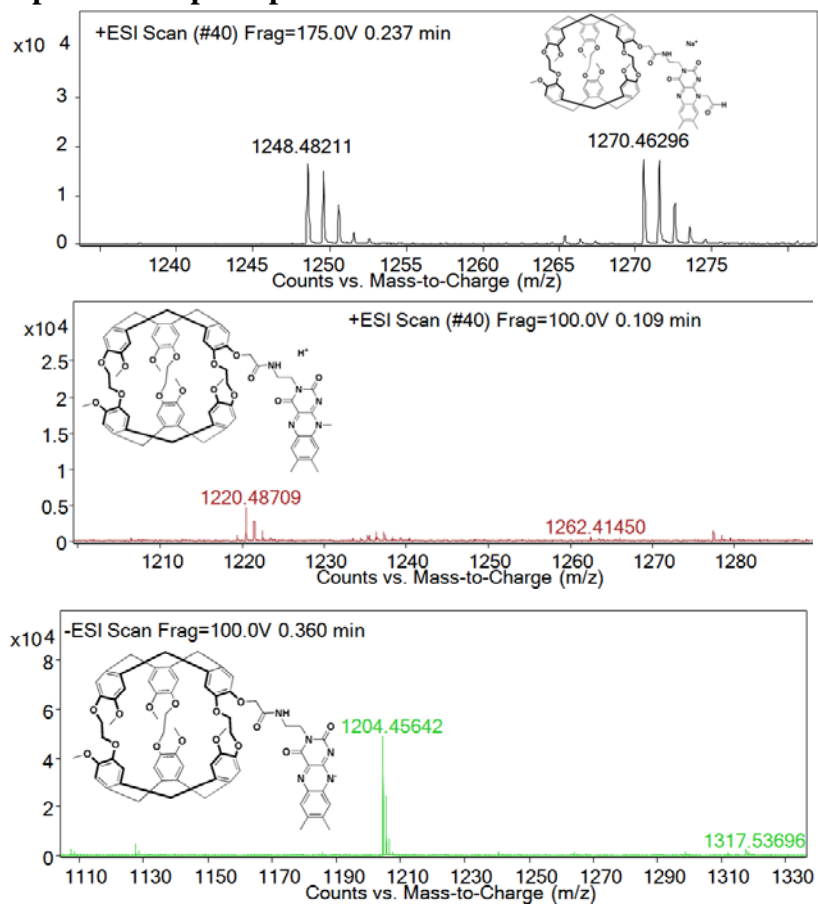


Figure S7. ESI-HRMS spectra of photoproducts of the CrA-2 with applying three-hour radiation. It shows three signals: 1270.4630, 1220.4871, 1204.4564, representing three kinds of photoproducts in samples.

10. Additional cellular fluorescence imaging with A549 and MCF-7 cells

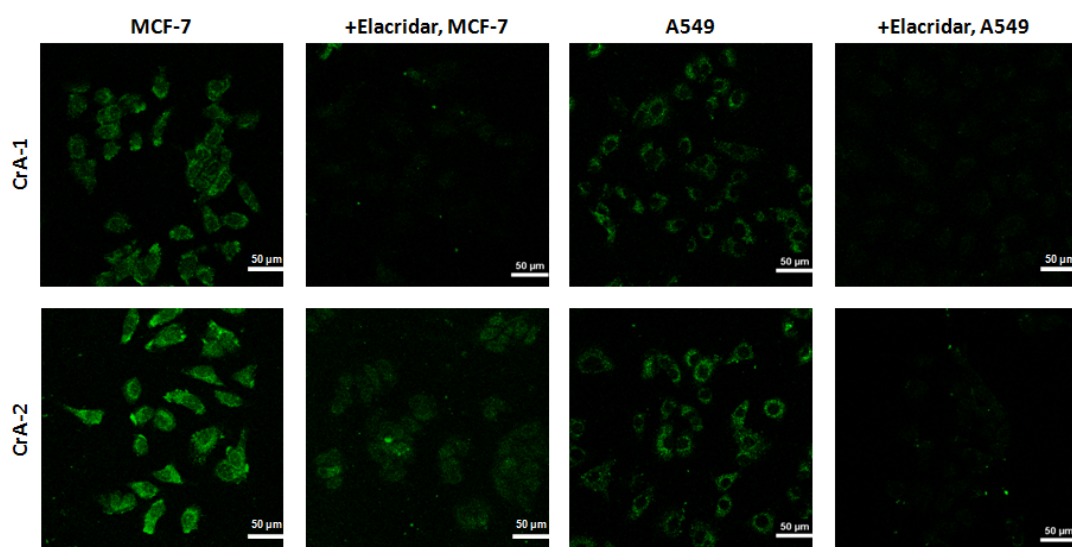


Figure S8. Fluorescence images of MCF-7 cells and A549 cells treated by Elacridar and CrA-1/CrA-2. Cells were incubated with Elacridar (2.5 μM) or PBS at 37 $^{\circ}\text{C}$ for 12 h. Then, the medium was replaced by fresh medium containing CrA-1/CrA-2 (40 μM) and the cells were incubated for another 3 h at 37 $^{\circ}\text{C}$, $\lambda_{\text{ex}} = 488 \text{ nm}$.

11. Additional cellular hyper-CEST NMR spectra of the CrA-1

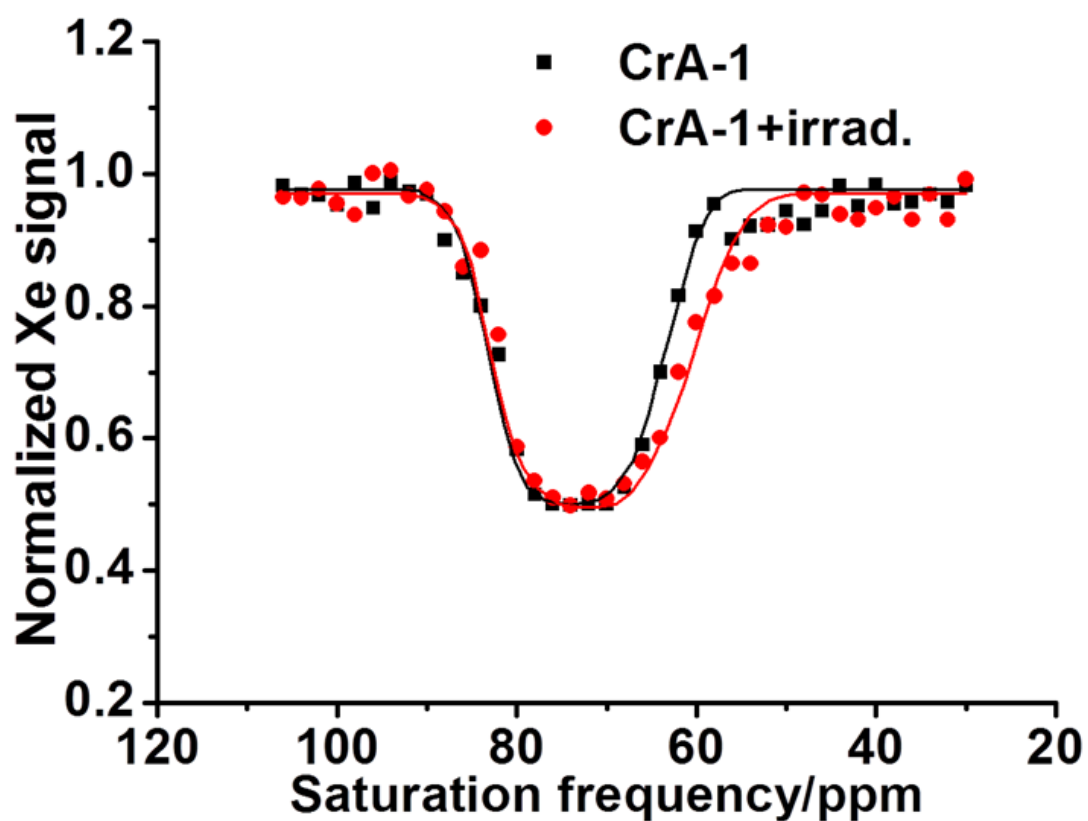


Figure S9. Hyper-CEST spectra of 50 μM CrA-1 (black line) and irradiated CrA-1 (black line) in A549 cells. The spectrum was measured with a 10 s saturation pulse and amplitude of 3.0 μT , and the frequency interval from 30 to 106 ppm is 2 ppm. The irradiated stands for samples with applying 4-h radiation.

12. Additional MTT assay of the CrA-1

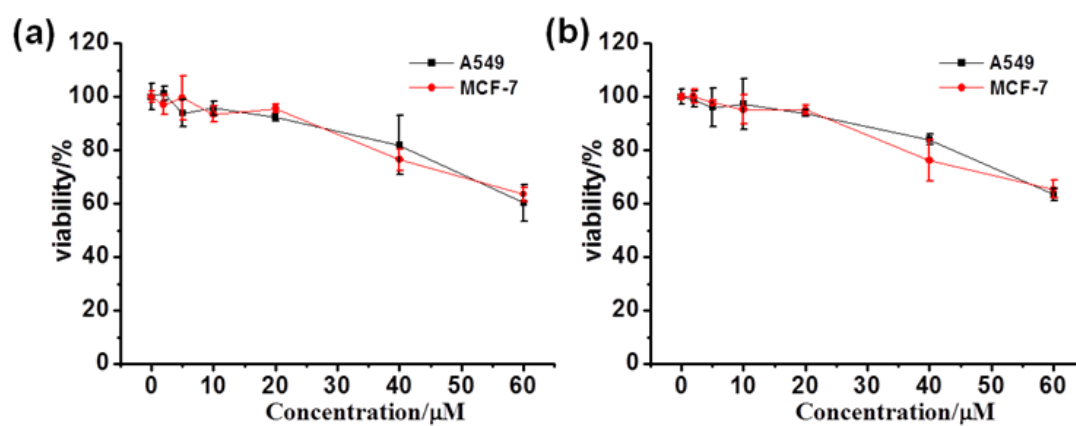


Figure S10. CrA-1 and its photoproducts were incubated with cells for MTT assay. A549 (black line) and MCF-7 (red line) cells after treatment with increasing concentrations of (a) CrA-1 and (b) its photoproducts. Viability was measured using MTT assay after 24 h of incubation.

13. ¹H NMR of the CrA-2

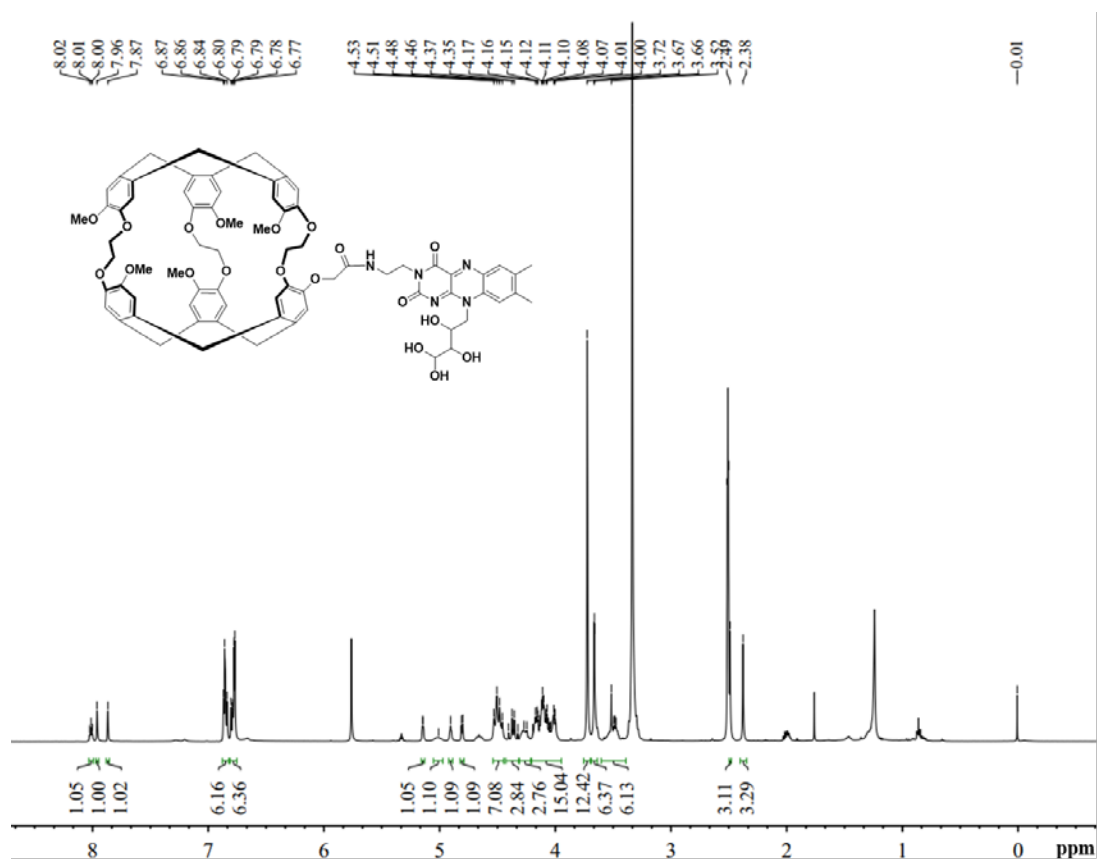


Figure S11. The ¹H NMR spectrum of CrA-2.

References

- [1] Li, S.; Yuan, Y. P.; Yang, Y. Q.; Li, C. G.; McMahon, M. T.; Liu, M. L.; Chen, S. Z.; Zhou, X. *J. Mater. Chem. B*, **2018**, *26* (6): 4293-4300.
- [2] Patil, S. P.; Jeong, H. S.; Kim, B. H. *Chem. Commun.*, **2012**, 48(9), 8901-8903.