A Tumor-targeting Ru/Polysaccharide/Protein Supramolecular Assembly with High Photodynamic Therapy Ability

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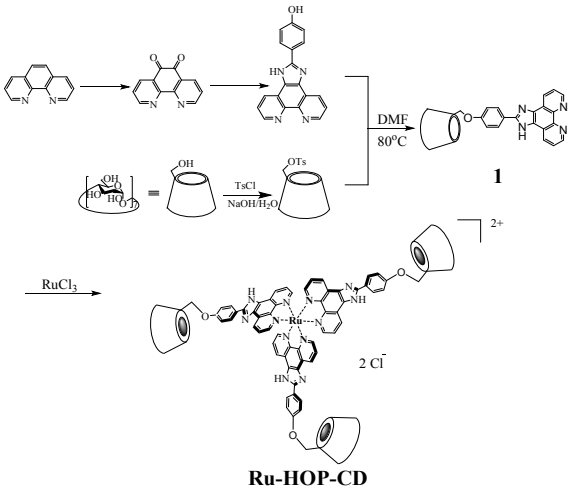
Experimental Procedures

All the reagents and solvents were commercially available and used as received unless otherwise specified purification. Compound **1** was prepared according to the literature procedure.[1]



Scheme S1. Synthetic pathway to Ru-HOP-CD.

Synthesis of Ru-HOP-CD. A two neck flask was charged with **1** (314.45 mg, 0.22 mmol), $RuCl_3 \cdot 3H_2O$ (12.86 mg, 0.062 mmol), C_2H_5OH (10 mL) and H_2O (50 mL), and the resulting solution was degassed via three freeze-pump-thaw cycles. The mixture was refluxed for about 36 h resulting in a turbid solution. After the removal of solvents under vacuum, the crude product was purified on a Sephadex G-25 column using distilled, deionized water as an eluent and dried in vacuo to give Ru-HOP-CD (110 mg, 40% yield).^{1}H NMR (DMSO-d_6, TMS, ppm): 3.4-3.8 (m, 126 H), 4.0-4.5(br m, 18 H), 4.8-5.0 (br m, 21 H), 5.8 (br s, 42 H), 7.2 (br m, 6 H), 7.8 (br s, 6 H), 8.0(br s, 6 H), 8.3 (br m, 6 H), 9.2 (br s, 6 H), 13.7(s, 3 H). IR (KBr): ν/cm^{-1} 3365, 2925, 1611, 1521, 1481, 1453, 1364, 1246, 1153, 1078, 1031, 944, 842, 810, 746, 722, 652, 580, 529, 479.

Synthesis of Ad-TRF. To a solution of 1-adamantane carboxylic acid (10 mg) in PBS (10 mL, pH ≈ 7.2 - 7.4, 0.05 M), a few drops of NaOH (10 M) was added. The mixture was stirred continuously, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 10 mg), N-hydroxysuccinimide (NHSS, 11 mg) were added. After 20 minutes, 50 mg of transferrin in 20 mL of PBS was added. The mixture was stirred at 4 °C for 12 h. The resulting solution was dialyzed against excess amount of deionized water for 5 days. After being freeze-dried, the product was obtained in 81% yield.

Measurements

NMR spectroscopy. NMR spectra were recorded on a Brucker AV400 spectrometer.

UV/Vis spectroscopy. UV/Vis spectra and the optical transmittance were recorded in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller.

TEM microscopy. High-resolution Transmission electron microscopy (TEM) images were acquired using a Tecnai 20 high-resolution transmission electron microscope operating at an accelerating voltage of 200 keV. The sample for high-resolution TEM measurements was prepared by dropping the solution onto a copper grid. The grid was then air-dried.

DLS spectroscopy. Solution samples were examined on a laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (TurboCorr) at 636 nm at a scattering angle of 90°. The hydrodynamic diameter (Dh) was determined by DLS experiments at 25°C.

Zeta potential. The zeta potential was recorded on NanoBrook 173 Plus (Brookhaven company) at 25 °C.

Light irradiation. The light irradiation was performed using a Xe lamp (CEL-HXF300 14V 50W) with an optical filter which provides visible light around at 450 nm. The light intensity was around 50 mW·cm-2 and the distance was kept at 10 cm.

Gel electrophoresis. Gel electrophoresis was run on a 1% (w/v) agarose gel at 60 V for 30 min and photographed by means of a UV transilluminator and WD-9415B gel documentation system (Beijing Liuyi Instrument Factory, P. R. China).

Cytotoxicity experiments. A549 cells were seeded in 96-well plates (5×10^4 cells mL^{-1}, 100 μL per well) for 24 h at 37 °C in 5% CO_2. Then, the cells were incubated with the complex at different concentrations ([Ad-TRF/Ru-HOP-CD] = 0, 20, 40, 80, and 160 mg/mL, respectively) for 12 h, respectively. After irradiation under the Xe lamp at 450 nm for 10 min, the cells were incubated for another 12 h. Then the cells were collected, washed, and resuspended in 500 μL of buffer containing 5 μL of annexin V-FITC and 5 μL of PI. The cells were incubated in the dark for 10 min at 25 °C and then analyzed by the flow cytometer (FCM) (FACSCalibur, BD Biosciences, San Jose, CA, USA). All data were presented as the mean ± standard deviation.

Measurement of intracellular reactive oxygen species (ROS). A549 cells were treated with the Ru-HOP-CD (20 mg/L) or Ad-TRF/Ru-HOP-CD (20 mg/L) for 24 h and then incubated with DCFH-DA (20 μg/mL) in serum-free DMEM for 15 min at 37 °C in dark. After irradiation under the Xe lamp at 450 nm for 10 min, the cells were incubated for another 12 h. The fluorescence intensity of the cells was measured immediately by flow cytometry with excitation at 480 nm and emission at 520 nm.

Apoptosis analysis. A549 cells were seeded in 96-well plates (5×10^4 cells mL^{-1}, 100 μL per well) for 24 h at 37 °C in 5% CO_2. Then, the cells were incubated with the complex (160 mg/L, respectively) for 12 h, respectively. After irradiation under the Xe lamp at 450 nm for 10 min, the cells were incubated for another 12 h. Then the cells were collected, washed, and resuspended in 500 μL of binding buffer containing 5 μL of annexin V-FITC. The cells were incubated in the dark for 10 min at 25 °C and then analyzed by the flow cytometer (FCM) (FACSCalibur, BD Biosciences, San Jose, CA, USA). All data were presented as the mean ± standard deviation.

Colocalization assay. A549 cells were seeded in 35 mm dishes for 24 h and then incubated with complex ([Ru] = 2 μM) at 37 °C for 24 h. Cells were washed three times with PBS and visualized by a confocal microscope (LSM 710, Carl Zeiss, Gottingen, Germany) immediately. The complex was excited at 450 nm, collected at 600 nm.

Figures

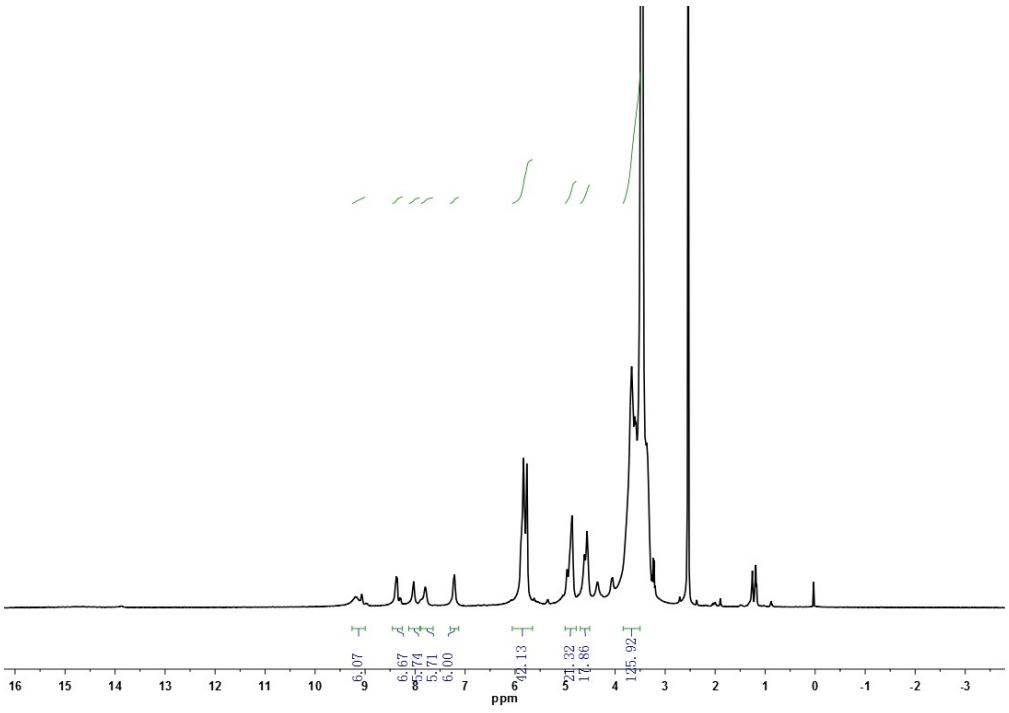


Figure S1. ^{1}H NMR (400 MHz) spectrum of **Ru-HOP-CD** in DMSO-d_6 at 25 °C.

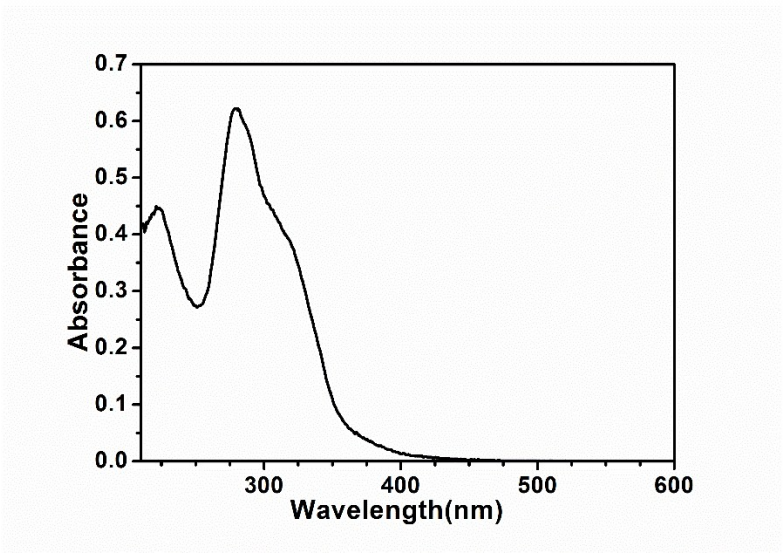


Figure S2. UV/Vis spectra of **1** in H_2O at 25°C.

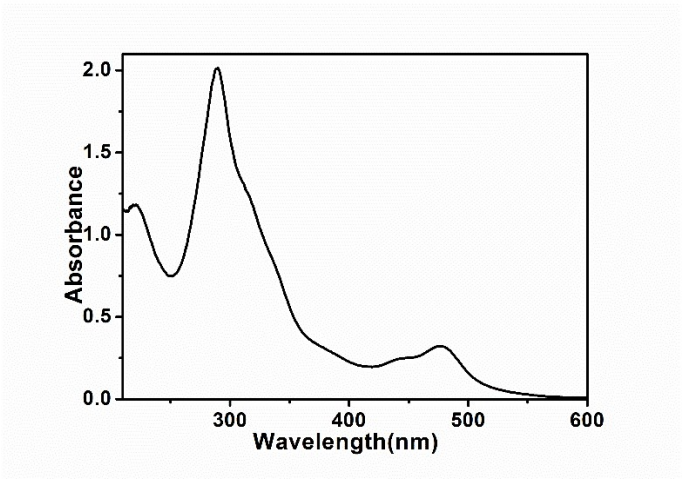


Figure S3. UV/Vis spectra of Ru-HOP-CD in H_2O at 25°C.

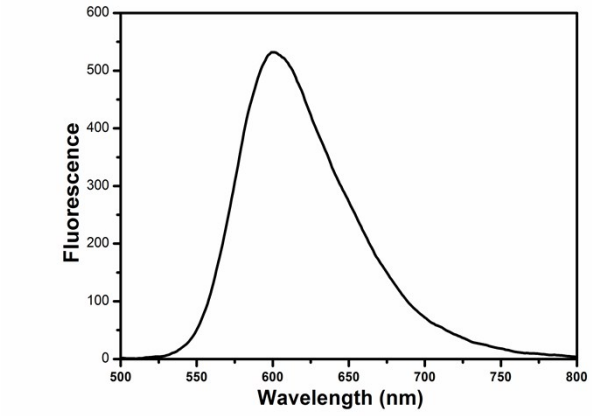


Figure S4. Fluorescence emission spectra of Ru-HOP-CD in H2O (λex = 450 nm).

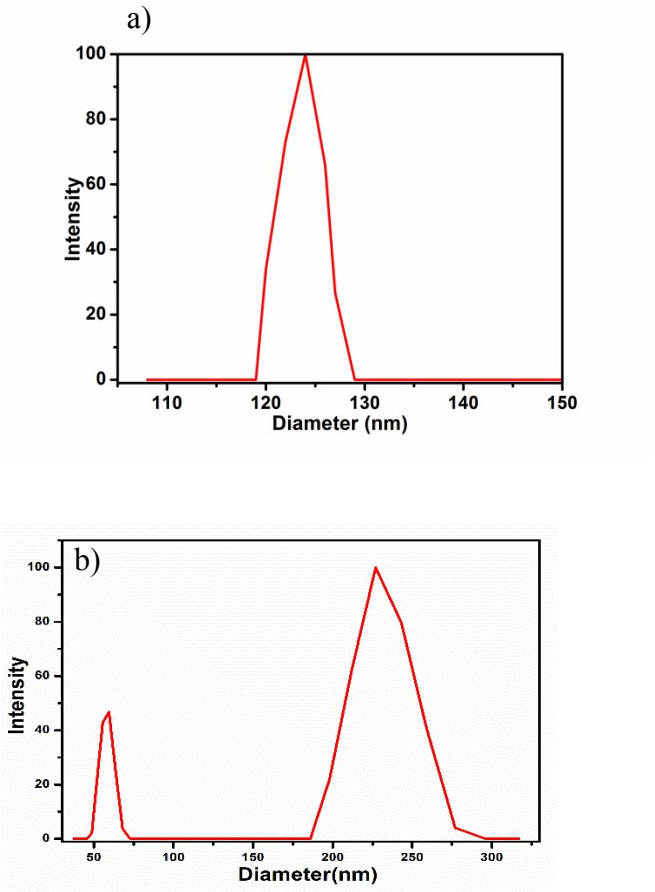


Figure S5. DLS of a) Ru-HOP-CD, b) the assembly.

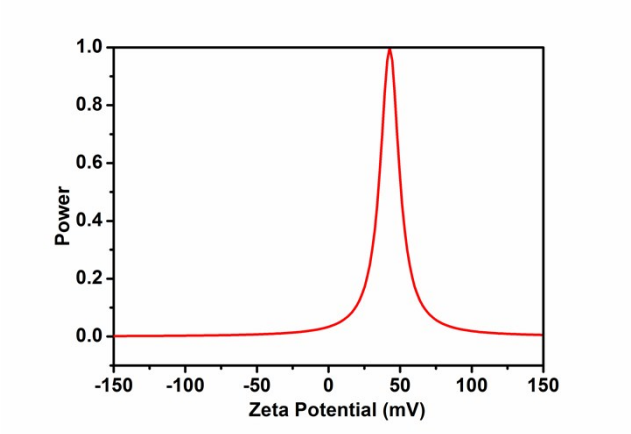


Figure S6. Zeta potential of Ru-HOP-CD.

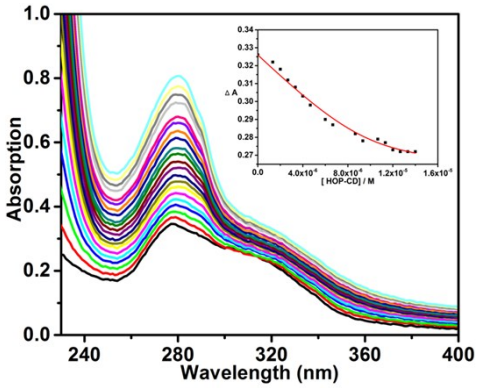


Figure S7. UV/vis titration spectra of HOP-CD (10 μM) upon addition of Ad-TRF (0 – 13 μM) Insert: Nonlinear least-squares analysis of the differential absorbance (ΔA, λ =280 nm) to calculate the complex binding constant.

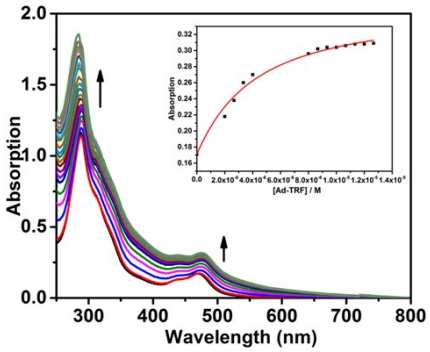


Figure S8. UV/vis titration spectra of Ru-HOP-CD (10 μM) upon addition of Ad-TRF (0 – 13 μM) Insert: Nonlinear least-squares analysis of the absorbance (A, λ = 470 nm) to calculate the complex binding constant.

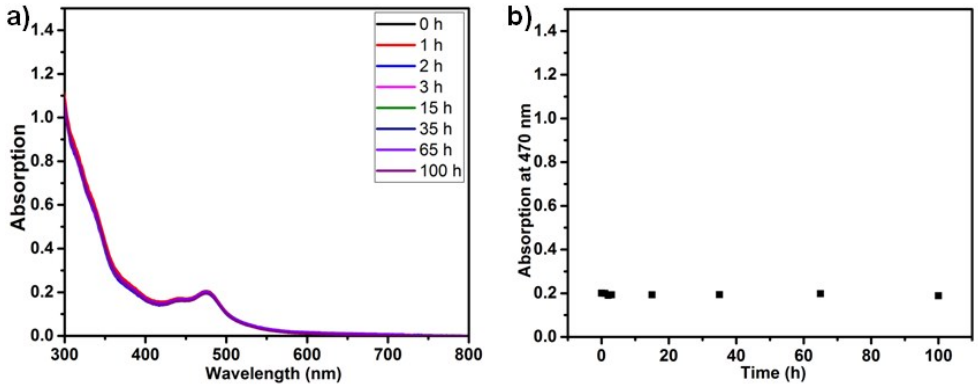


Figure S9. (a) UV/Vis spectra of Ad-TRF/Ru-HOP-CD assembly within 100 hours in serum. (b) Dependence of the UV absorption at 470 nm on time, [Ad-TRF] = 0.01 mM, [Ru-HOP-CD] = 0.01 mM.

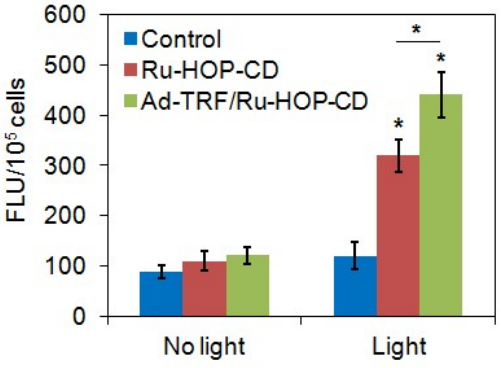


Figure S10. Effects of the complex on ROS generation: the fluorescence intensity of DCF in A549 cells after treatment with complex with or without light irradiation. Data are represented as means ± SD of three independent experiments.

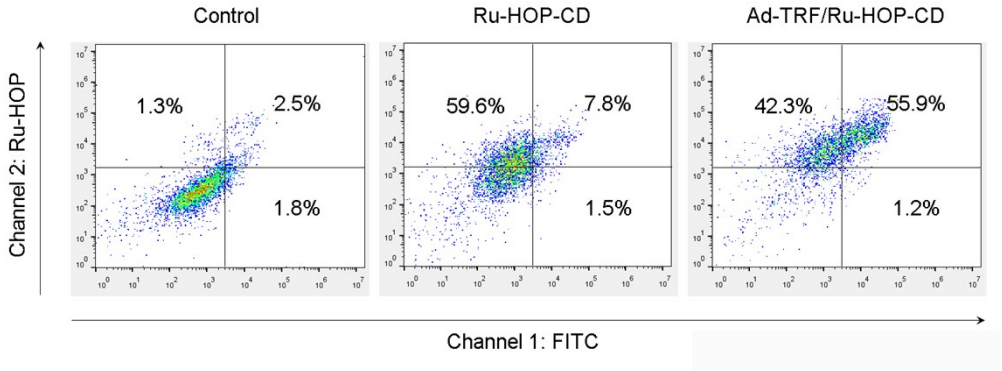


Fig. S11. Apoptosis analysis of Ru-HOP-CD (160 mg/L) and Ad-TRF/Ru-HOP-CD (160 mg/L) in A549 cells.

References

(1) Liu, Y.; Chen, Y.; Duan, Z.-Y.; Feng, X.-Z.; Hou, S.; Wang, C.; Wang, R. *ACS Nano* **2007**, *1*, 313-318.