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Supporting Information

For

A Potential Water-Soluble Ytterbium-Based Porphyrin-Cyclen Dual Bio-Probe for Golgi apparatus Imaging and Photodynamic Therapy

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Experimental

Preparation of Ligand L^1 and L^2

The starting materials, 1-[4-[5,10,15-Tris(3,4,5-trimethoxylphenyl)-20-porphyrinyl]-phenoxy]-5-(p-tolylsu-lfonyloxy)-ethoxyethane (**Por-OTs**), 1,4,7-tri(tertbutoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**Cyclen-1**) and 1,4,7-triacetamide-1,4,7,10-tetraazacyclododecane (**Cyclen-2**) were prepared by the methods described in these references.[1-3]

L^1: Por-OTs (57.2 mg, 0.05mmol), **Cyclen-1** (25.7mg, 0.05mmol) and potassium carbonate (6.9mg, 0.05mmol) were added in a dry reaction flask, and then 15ml dry acetonitrile were injected in the flask. The reaction was heated to 60°C and continuously stirred for overnight. The reaction was monitored by TLC plate and stopped after 20h. The organic solvent was removed and the residue was dissolved in CH_2Cl_2, and filter out of the solid. The organic phase was collected and purified on silica gel (mixed solvent CH_2Cl_2/MeOH = 20:1 as eluent). Yield a purple solid 55.7 mg, 75.0%. The intermediate product was identified by ^{1}H NMR and MALDI-TOF HRMS. ^{1}HNMR ($CDCl_3$) δ -2.77 (s, 2H), 1.49 (s, 9H, tert-butyl), 1.55 (s, 18H, tert-butyl), 1.88 (s, 6H), 2.26-3.34 (m, 18H), 3.91 (d, 2H, *J* = 4.81Hz), 3.97 (s, 18H, methoxyl on porphyrin), 4.01 (d, 2H, *J* = 4.67Hz), 4.18 (s, 9H, methoxyl on porphyrin), 4.44 (s, 2H), 7.30 (d, 2H, *J* = 8.49Hz), 7.47 (s, 6H), 8.11 (d, 2H, *J* = 8.50 Hz), 8.85 (d, 2H, *J* = 4.65Hz), 8.94 (m, 6H). (MALDI-TOF HRMS) ($[M]^+$, m/z): Calcd for $C_{83}H_{104}N_8O_{17}$, 1484.7519; found for $[M+H]^+$, 1485.7279; $[M+Na]^+$, 1507.7408. Then, complete removal of tert-butyl groups from this intermediate product by a mixture of trifluoroacetic acid (1.5 mL) and CH_2Cl_2 (0.5 mL) afforded ligand **L^1** quantitatively. The solvent was removed and the product **L^1** in a little $CHCl_3$ solution was precipitated after the addition of large scales of hexane. λ_{abs}/nm(logε) 423(5.49), 516(4.31), 553(3.90), 591(3.83), 648(3.75). ^{1}HNMR ($CDCl_3$) δ -2.83 (s, 2H), 1.48-2.33 (s, br., 16H), 2.47-3.14 (m, 10H), 3.81 (s, 18H, methoxyl on porphyrin), 4.01 (s, 9H, methoxyl on porphyrin), 4.08 (s, 2H), 4.25 (s, 2H), 7.25 (overlap with $CDCl_3$ peak, 2H), 7.39 (s, 6H), 8.05 (d, 2H, *J* = 7.50 Hz), 8.87 (d, 8H, *J* = 9.50 Hz). (MALDI-TOF HRMS) ($[M]^+$, m/z): Calcd for $C_{71}H_{80}N_8O_{17}$, 1316.5641; found for $[M+H]^+$, 1317.5708; $[M+Na]^+$, 1340.5750; $[M+2Na-H]^+$, 1362.6210.

L^2: Por-OTs (57.2 mg, 0.05mmol), **Cyclen-2** (25.6mg, 0.05mmol) and potassium carbonate (6.9mg, 0.05mmol) were added in a dry flask, and then 15ml dry acetonitrile were injected in the flask. After continuously stirred at 60°C for 20h, the organic solvent was removed and the residue was purified on silica gel using CH_2Cl_2/MeOH = 20:1 as eluent. Purple solid **L^2** 53.4mg (yield 72.1%) was collected. λ_{abs}/nm(logε) 423(5.50), 516(4.34), 553(3.91), 591(3.84), 648(3.75). ^{1}H NMR ($CDCl_3$) δ -2.78 (s, 2H), 1.00-1.10 (m, 9H, $3CH_3$), 1.20-1.25 (m, 9H, $3CH_3$), 1.72-2.31 (br., 9H), 2.39-2.91 (m, br., 12H), 3.16-3.48 (m, 16H), 3.86(s, 2H), 3.97 (s, 18H, methoxyl on porphyrin), 4.18 (s, 9H, methoxyl on porphyrin) 4.46 (m, 4H), 7.29 (d, 2H, *J* = 8.62Hz), 7.47 (d, 6H, *J* = 1.51 Hz), 8.10 (d, 2H, *J* = 8.43 Hz), 8.84 (d, 2H, *J* = 4.74Hz), 8.94 (m,

6H). (MALDI-TOF HRMS) ([M]$^+$, m/z): Calcd for $C_{83}H_{107}N_{11}O_{14}$, 1481.7999; found for [M+H]$^+$, 1482.8112; [M+Na]$^+$, 1504.8332.

Preparation of Yb-L^1 and Yb-L^2

Ligand **L^1** (25mg, 19.0μmol) in MeOH (3ml) was added respectively in H_2O (0.5ml) solution of $YbCl_3$•$6H_2O$ (1.1 eq., 8.07mg) and the mixture was stirred at room temperature for 72h. Then, the solvent was removed under reduced pressure and the remaining residue was dissolved in a little MeOH. The complex **Yb-L^1** was precipitated from the solution after the slow addition of diethyl ether, and then the product dissolved again in MeOH and was precipitated by the slow addition of diethyl ether. The procedure was repeated 4 times and pure purple solid (20mg, 13.3μmol) was obtained. λ_{abs}/nm(logε) 423(5.55), 516(4.36), 553(3.94), 591(3.85), 648(3.77). (MALDI-TOF HRMS) ([M]$^+$, m/z): Calcd for $C_{71}H_{77}N_8O_{17}Yb$, 1505.4901; found for [M-H_2O(Coordinated)+H]$^+$, 1488.4843.

Water-soluble green solid, **Yb-L^2** (18.9mg, 12.6μmol) was prepared with the ligand **L^2** (25mg, 16.8μmol) and $YbCl_3$•$6H_2O$ (1.1 eq., 8.07mg) as the same procedure described above. λ_{abs}/nm(logε) 423(5.52), 516(4.34), 553(3.92), 591(3.84), 648(3.76). (ESI HRMS$^+$) ([M]$^+$, m/z): Calcd for $C_{83}H_{109}N_{11}O_{15}Yb$, 1673.7493; found for [M-H_2O+F^-]$^{2+}$, 837.3589; [M-H_2O+CH_3O^-]$^{2+}$, 843.3681; [M-H_2O+$CH_3CH_2O^-$]$^{2+}$, 850.3562; [M-H_2O+CH_3COO^-]$^{2+}$, 857.3619.

Photo-physical measurement of Yb-L^1 and Yb-L^2

UV-Visible absorption spectra in the spectral range 200 to 1100 nm were recorded by an HP UV-8453 spectrophotometer. Single-photon luminescence spectra were recorded using an Edinburgh Instrument FLS920 Combined Fluorescence Lifetime and Steady state spectrophotometer that was equipped with a red sensitive single photon counting photomultiplier by Peltier Cooled Housing. The spectra were corrected for detector response and stray background light phosphorescence. The quantum yields of the compounds were measured by Demountable 142mm (inner) diameter barium sulphide-coated integrating sphere supplied with two access ports. The NIR emission spectra of Yb-**L^1** and Yb-**L^2** were obtained by InGaAs detector on a PTI QM4 luminescence spectrometer.

Singlet Oxygen Quantum Yield Measurement

Singlet oxygen was detected directly by its phosphorescence emission at 1270 nm using an InGaAs detector on a PTI QM4 luminescence spectrometer. The singlet oxygen quantum yields (Φ_Δ) of the test compounds were determined in $CHCl_3$ by comparing the singlet oxygen emission intensity of the sample solution to that of a reference compound (H_2TPP, Φ_Δ = 0.55 in $CHCl_3$) according to below equation,

$$\Phi_\Delta^S = \Phi_\Delta^{REF} \times \left(\frac{n_S}{n_{REF}}\right)^2 \frac{G_\Delta^S}{G_\Delta^{REF}} \times \frac{A_{REF}}{A_S}$$

where Φ_Δ is the singlet oxygen quantum yield, G_Δ is the integrated emission intensity, *A* is the absorbance at the excitation wavelength, *n* is the refractive index of the solvent. Superscripts *REF* and *S* correspond to the reference and the sample, respectively. In all measurements, the $^1O_2$ emission spectra were obtained using an excitation with the absorbance set at 0.05 in order to minimize re-absorption of the emitted light. [4]

Cell culture

Human cervical carcinoma (HeLa) cells were maintained in an RMPI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin in 5% CO_2. Culture medium in each dish was changed prior to exposure to the test compounds. Stock solutions of the test compounds (1 mM) were prepared in aqueous solution and stored in dark at room temperature. These compounds, when used in the imaging and bioassay experiments involving cultured cells, were diluted with the corresponding culture media to appropriate concentrations. Human lung carcinoma A549 cells were purchased from the American type Culture Collection (ATCC) (#CCL-185, ATCC, Manassas, VA, USA). Cells were cultured in Ham's F12K medium with L-glutamine and phenol red (N3520, Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum at 37º C and 5% CO_2. Cells were passaged every 3–5 days.The cell lines were incubated at 37 ^oC in a humidified incubator with 5% CO_2. Human cervical carcinoma (HeLa) cells were maintained in an RMPI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin in 5% CO_2.

Confocal Microscopic imaging of compounds Yb-L^1 and Yb-L^2

HeLa/A549 cells (1 x 10^5) were seeded onto coverslip in 35-mm culture dishes for overnight. The cells were initially incubated with compounds Yb-$\mathbf{L^1}$ and Yb-$\mathbf{L^2}$ (1 μM) for 30 min in dark. For co-localization experiments, the cells were then washed and stained with 100 nM Golgi marker Alexa Fluor 647 conjugates of HPA, Invitrogen L32452 or mitochondria-specific probe Mito Tracker Green FM dye M7514 or lysosomes-specific probe Lyso Tracker Green DND-26 L7526, for 30 min. The emitted fluorescent signals of tested compounds and the organelle-specific probes were examined using the Leica SP5 (upright configuration) confocal microscope equipped with argon laser, HeCd laser and a femtosecond-pulsed Ti:Sapphire laser (Libra II, Coherent) inside the tissue culture chamber (5% CO_2, 37 ^oC) A 40x oil immersion objective and pinhole size of 110 μm was used for image capturing. For two-photon-induced confocal microscopic imaging of Yb-$\mathbf{L^1}$ and Yb-$\mathbf{L^2}$ were captured using the Leica SP5 (upright configuration) confocal microscope equipped with a femtosecond-pulsed Ti:Sapphire laser (Libra II, Coherent) inside the tissue culture chamber (5% CO_2, 37 ^oC). The excitation beam produced by the femtosecond laser, which was tunable from 680-1050 nm, was focused on the adherent cells through a 40x oil immersion objective. For the evaluation of effectiveness of photodynamic therapy, the time-lapse images (0 - 30 min, one laser shot per 1 min) were obtained with femtosecond laser excitation at 850 nm (laser power ~ 8 mW).

Fluorometric analysis of cellular uptake

HeLa cells (2×10^5 cell/well) were incubated separately with 1 μM of Yb-$\mathbf{L^1}$ and Yb-$\mathbf{L^2}$ in the culture medium in dark for 6 hours. Afterward, the cells were washed with phosphate buffered

saline (PBS) twice and then trypsinized. The cell-suspended solutions were centrifuged and then washed with PBS for several times. The cell-suspended solutions were then diluted to a final cell concentration of 4×10^5 cell/mL in PBS. Fluorescence spectra (λ_{ex} = 430 nm) of the cell suspensions were measured fluorometrically. The amounts of conjugates taken up by these cells were estimated semi-quantitatively based on the calibration curves obtained by plotting the fluorescence intensities versus the concentrations of the standard solutions of Yb-**L**^{1} and Yb-**L**^{2} in PBS.

Dark-cytotoxicity Assay

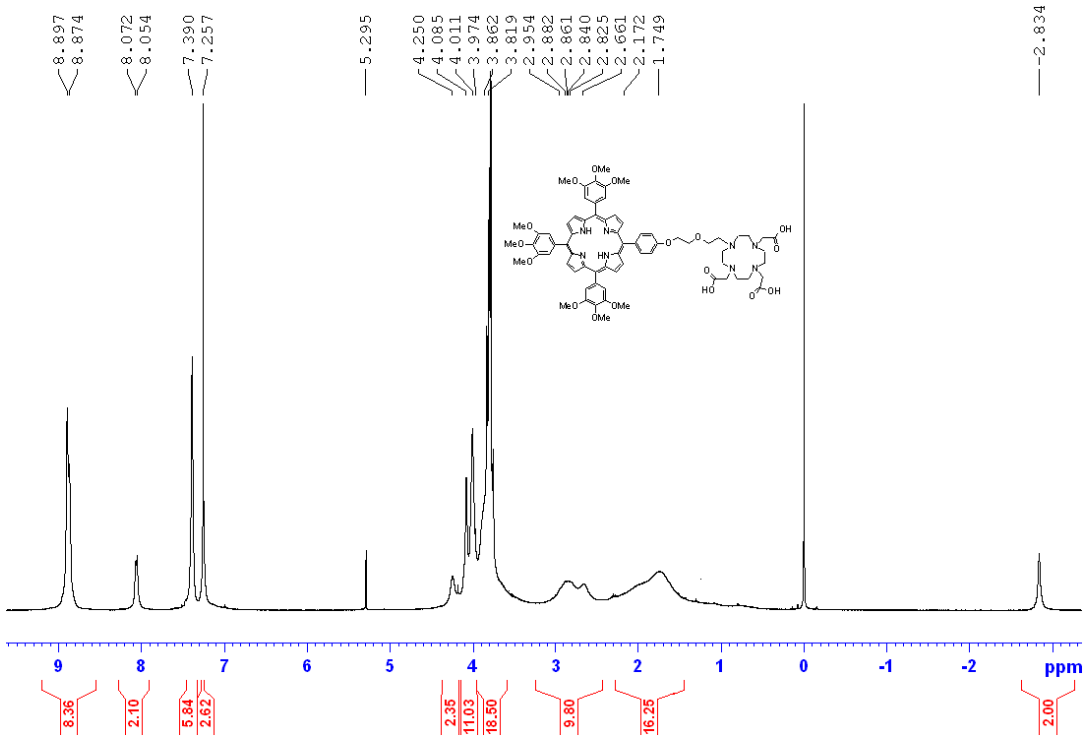
MTT viability assay was performed as reported in previous literature. Briefly, three thousand HeLa cells were seeded in 96-well plates 24 hr prior to exposure to the europium complex or DMSO as control. After various exposure time points, 20 µl MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (5 mg/ml) was added to the culture medium in each well and incubated for 5 hours at 37°C. The media was removed, 200 µl DMSO solubilizing reagent was added and incubation was carried out for another hour to dissolve the formazan crystals. The absorbance was measured at 570 nm on a Labsystem Multiskan microplate reader (Merck Eurolab, Switzerland). MTT assays were conducted in triplicate wells, and repeated twice. Each data point represents the ratio of mean values between the europium versus the DMSO control. [5]

Photocytotoxicity Assay

HeLa cells (2 x 10^4/well) were incubated in wells of 96-well plate for overnight. The cells were treated Yb-**L**^{1} and Yb-**L**^{2} for 6 h in dark. The culture medium was then replaced with fresh medium and the cells were exposed to yellow light (1-8 J/cm^2) produced from a 400 W tungsten lamp fitted with a heat-isolation filter and a 500 nm long-pass filter. The fluence rate was 4 mW/cm^2. Cells viability was determined by the MTT reduction assay at 24 h post-PDT. [6] The cell monolayers were rinsed twice with phosphate-buffered saline (PBS) and then incubated with 250 µg/mL MTT solution at 37 °C for 3 h. The formazan crystal formed was dissolved in DMSO and the absorbance of dissolved formazan crystal at 540 and 690 nm was measured using a 96-well plate reader (ELx800 Absorbance Microplate Reader).

$$\frac{\text{(Absorbance of cell control-Absorbance of blank) - (Absorbance of treatment-Absorbance of}}{\text{(Absorbance of cell control-Absorbance of}} \times 100\%$$

(a)



(b)

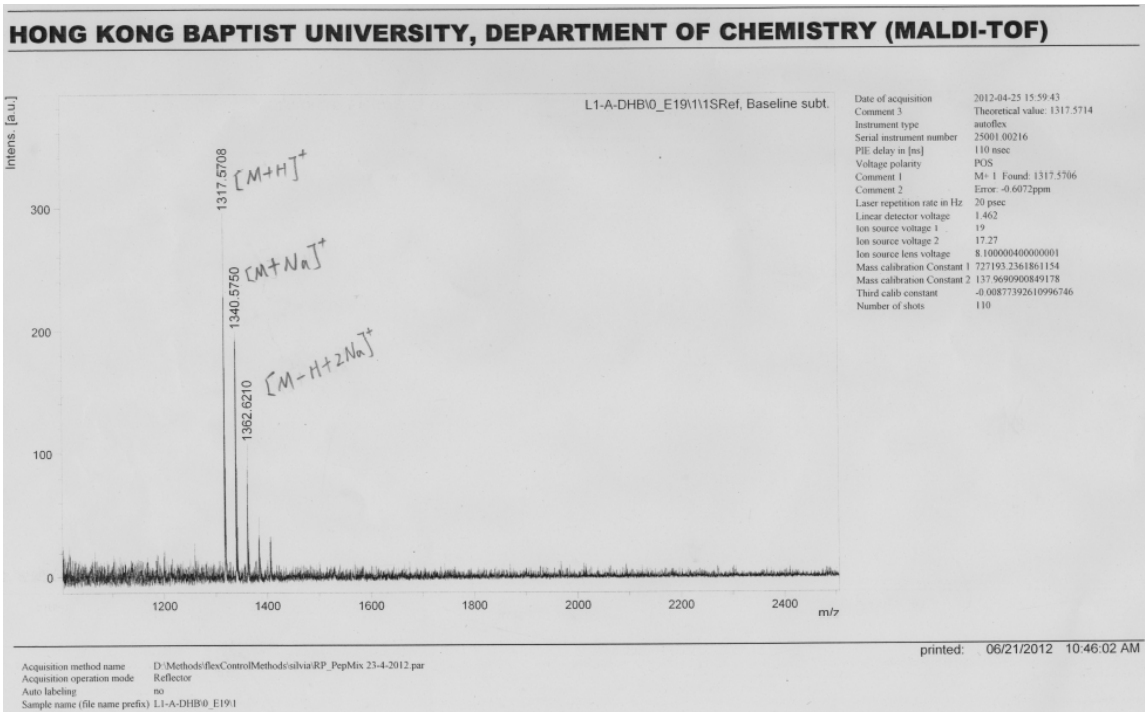
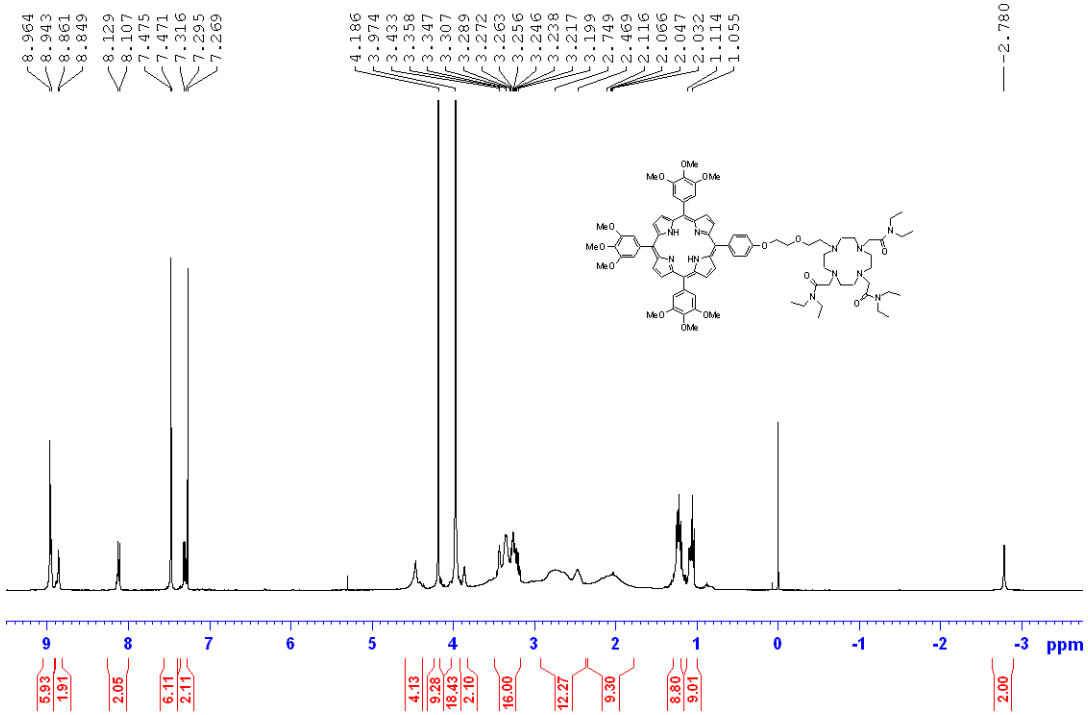


Figure S1. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum (a) and MALDI-TOF spectrum (b) of **L**^{1}

(a)



(b)

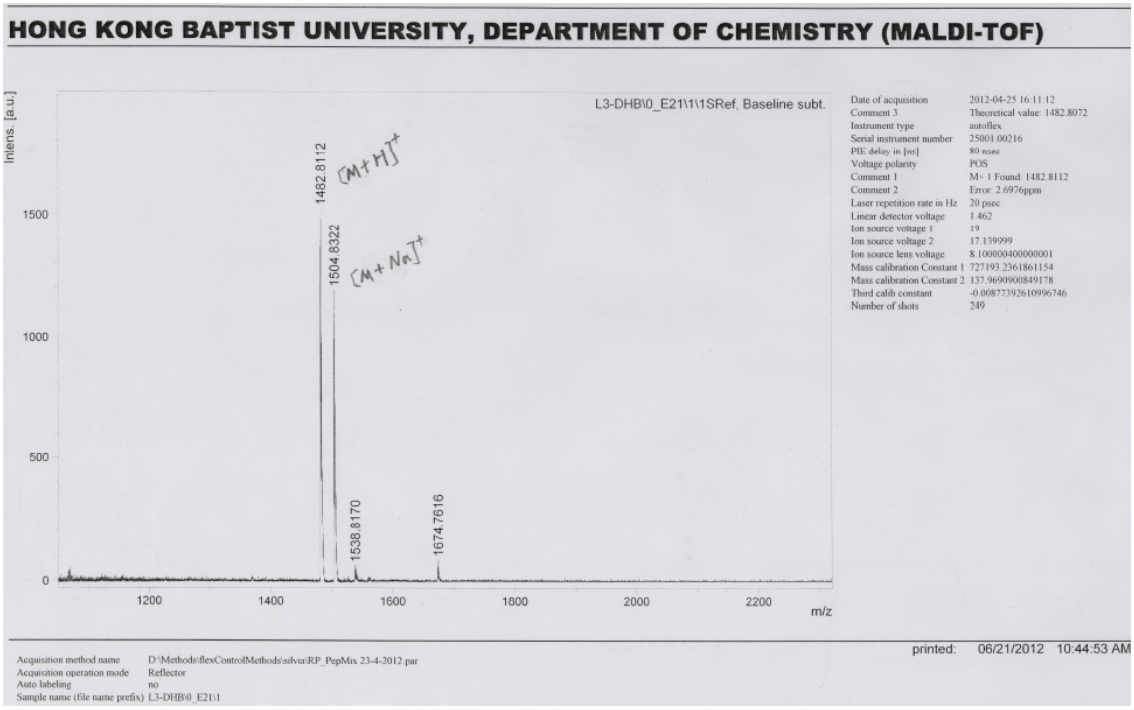
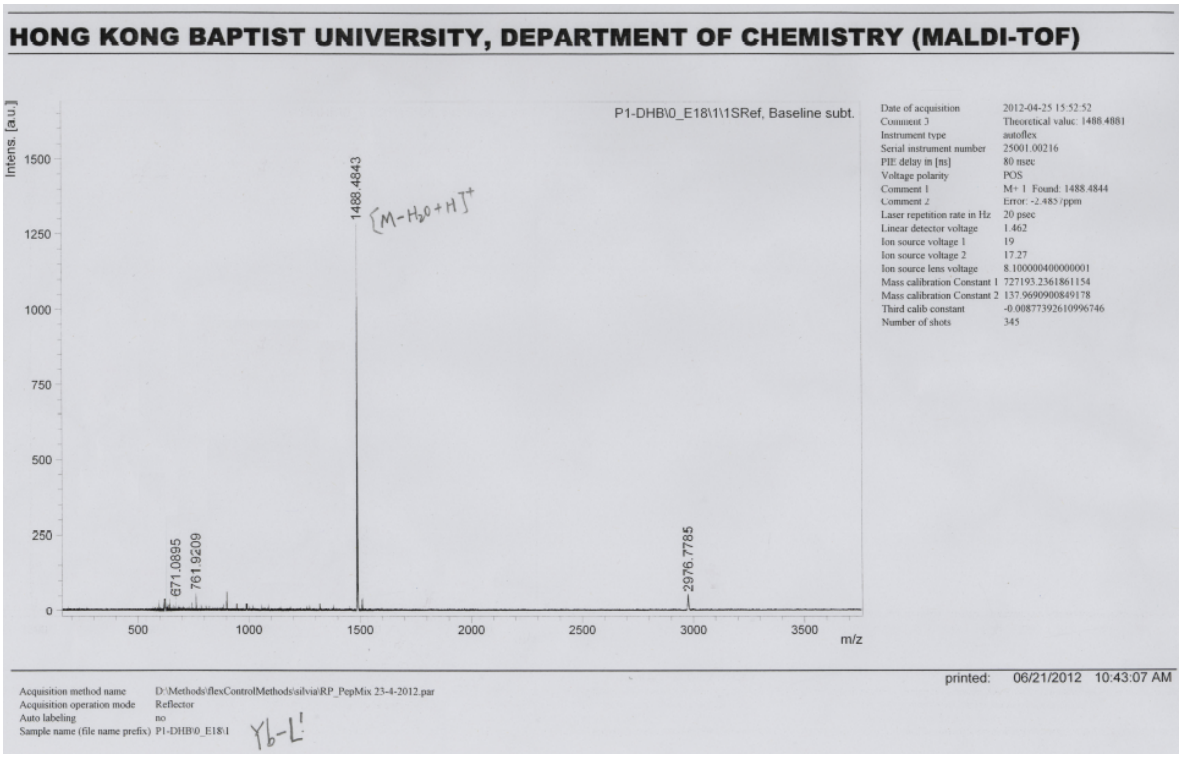


Figure S2. 400 MHz-^{1}H-NMR ($CDCl_3$) spectrum (a) and MALDI-TOF spectrum of **L**^{2}

(a)



(b)

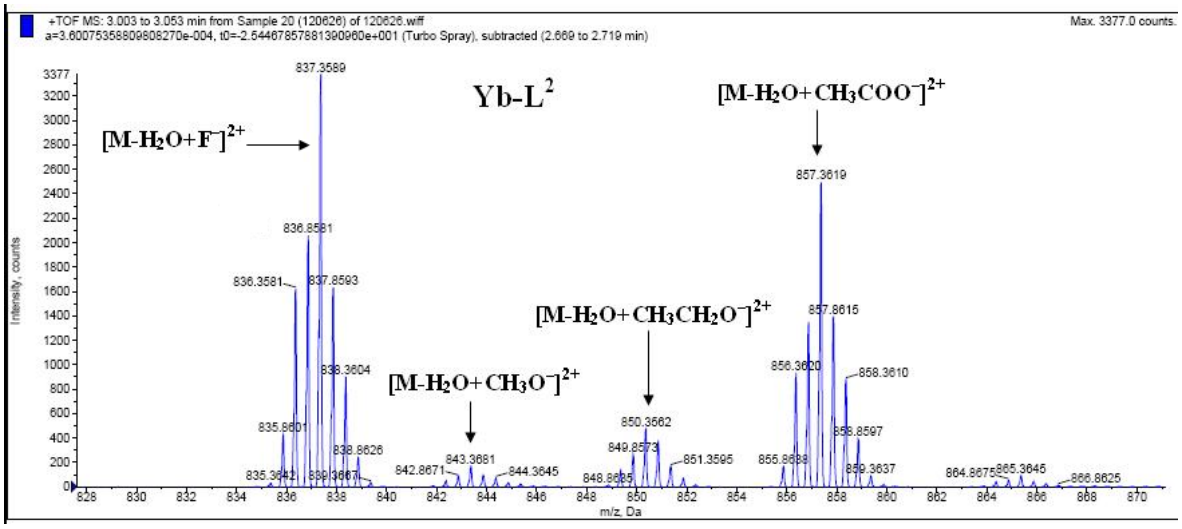


Figure S3. MALDI-TOF HRMS of Yb-**L**^{1} (a) and ESI HRMS of Yb-**L**^{2} (b)

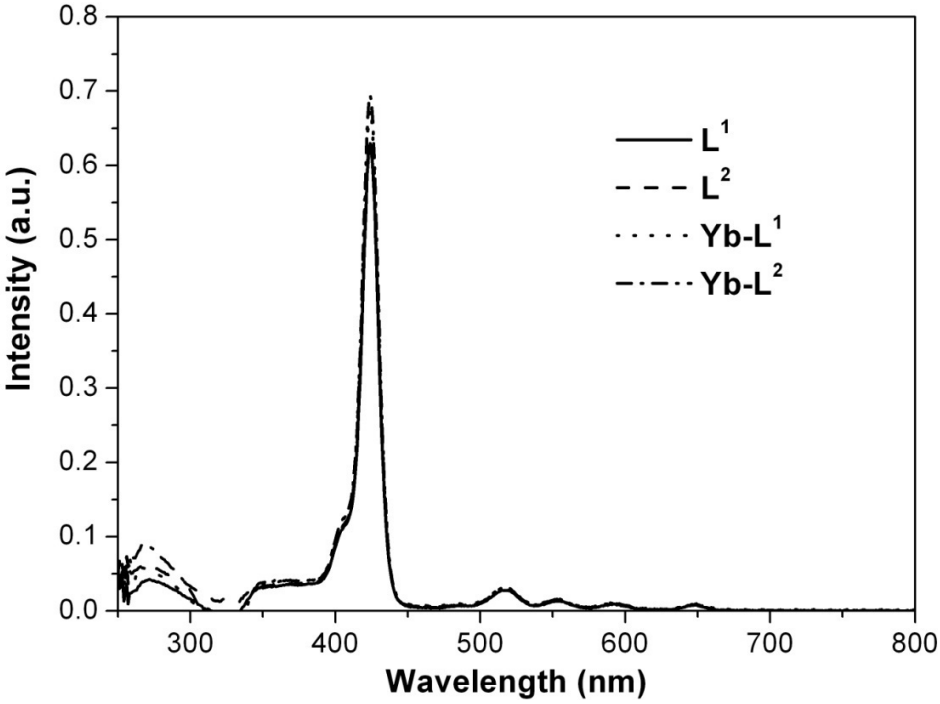
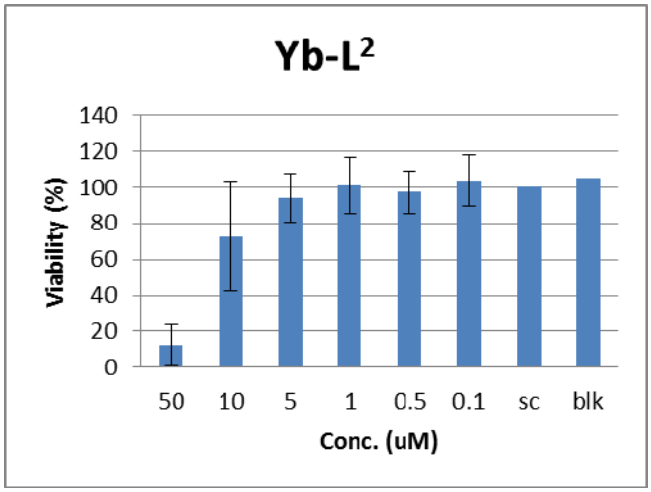


Figure S4. UV-absorption spectra of 2μM **L**^{1}, **L**^{2}, Yb-**L**^{1} and Yb-**L**^{2} in DMSO.

a)



b)

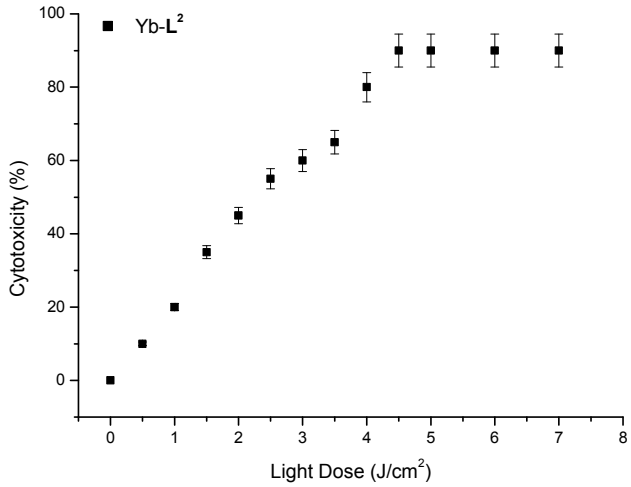


Figure S5. Dose-response curves of the dark cytotoxicity (left) and photo-cytotoxicity (right) of conjugates Yb-**L**^{2}. Dark cytotoxicity curves were obtained using dosed concentration from 1 to 250 μM. Photo-cytotoxicity curves were obtained using 1 μM of conjugates and various light doses from 0 to 8 J/cm^2. MTT assay was carried out after incubation for 24 h. The results were expressed as the mean ± S.D. of three separate trials. [7]

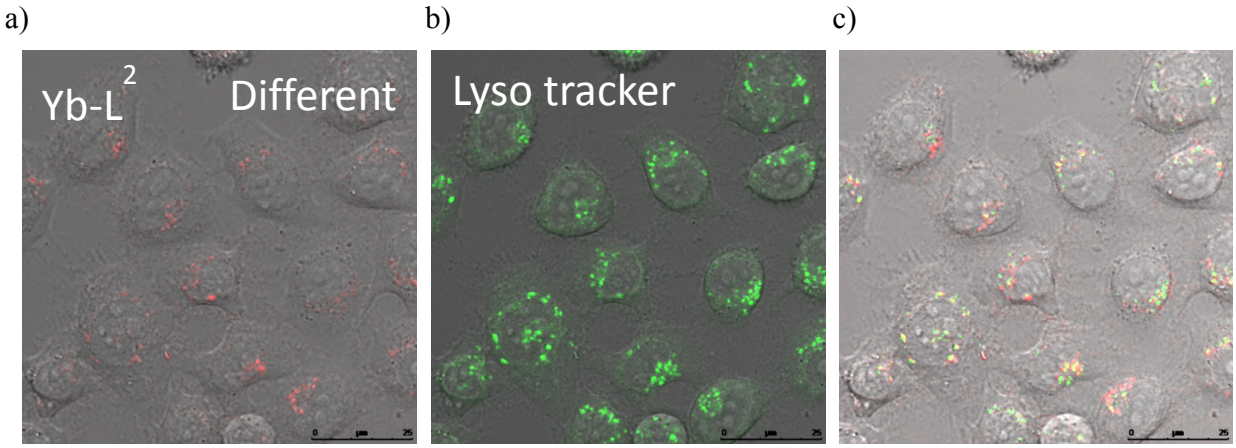


Figure S6. Confocal microscopic analysis of subcellular localization of Yb-**L**^{2} with lysosome (negative control) specific marker. Negative control: (a) Linear confocal microscopy images of the red in-vitro emission from Yb-**L**^{2} (10 μM, λ_{ex} = 430 nm), 30 min exposure in HeLa cells; (b) Green lysosome-specific probe Lyso-Tracker Green DND-26L7526 (negative control) (c) Merged image.

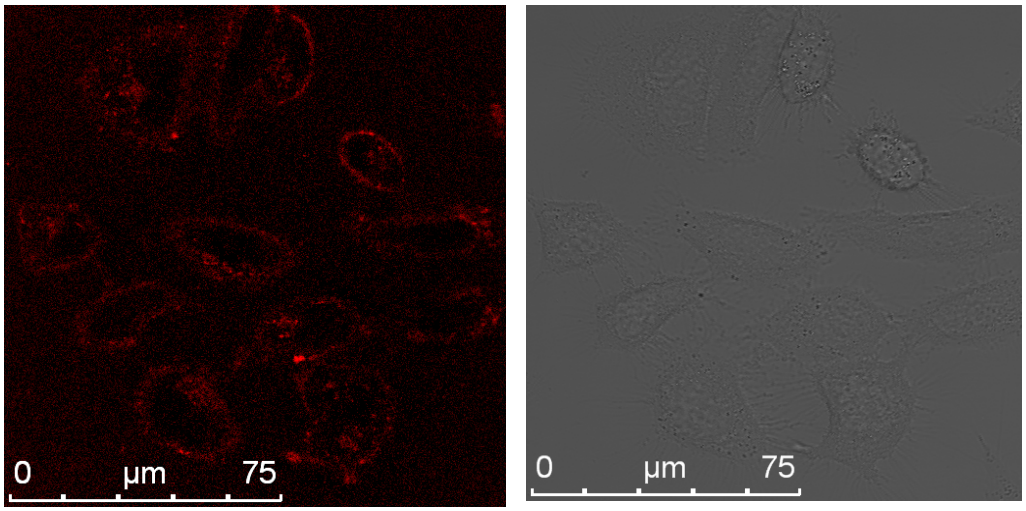


Figure S7. Confocal fluorescent images of Yb**L**^{1} (10 μM, λ_{ex} = 430 nm) with 180 min incubation time in HeLa cells and only localized in cytoplasm (served as negative control for Yb-**L**^{2})

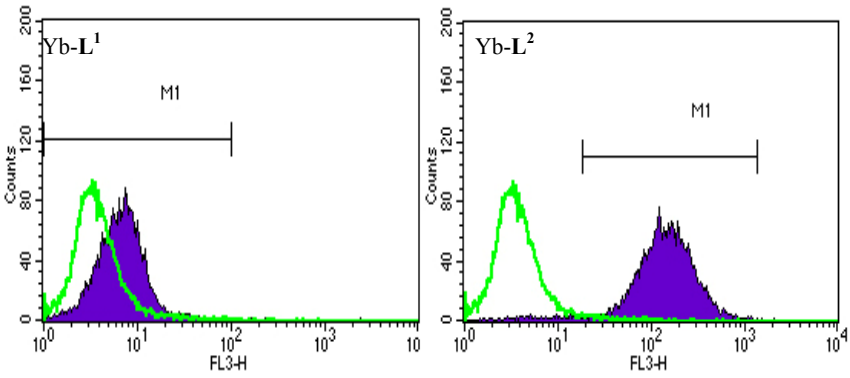


Figure S8. Flow cytometric analysis of the cellular uptake of Yb-L1 and Yb-L1 in the HeLa cells. The cells were incubated with 1 μM of Yb-L1 and Yb-L2 for 0 hr (control, green line), 6 hr (purple) in dark.

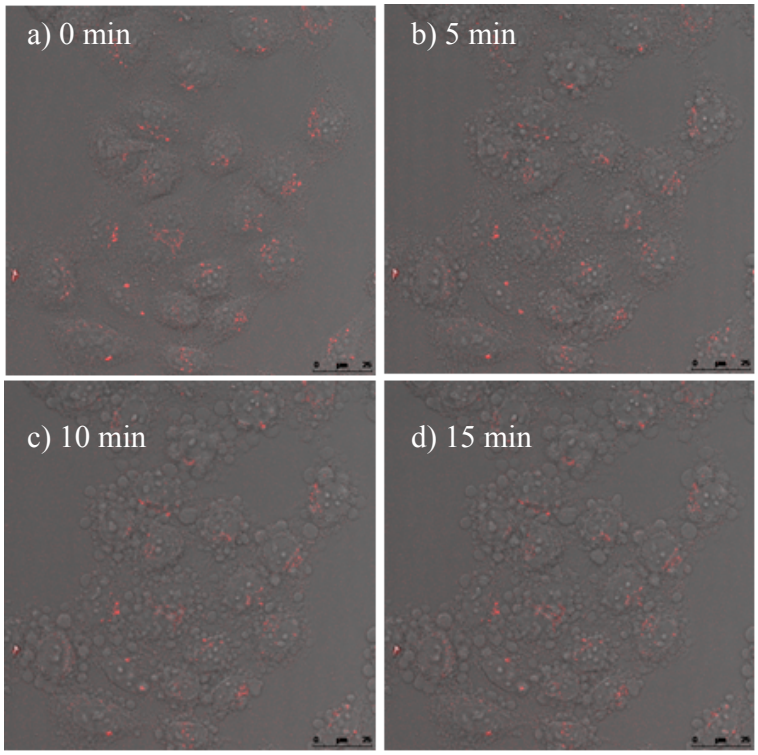


Figure S9. Effectiveness of Yb-**L**^{2} as PDT agents. Confocal microscopic images of HeLa cells treated with 5 μM of Yb-**L**^{2}. Images were obtained after different time of laser irradiation.

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