Supplementary Information

Near infrared mediated photoactivation of cytotoxic Re(I) complex by lanthanide-doped upconversion nanoparticles

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Contents.

Section1:

Materials:

$Y(CH_3CO_2)_3$, $Yb(CH_3CO_2)_3$, $Tm(CH_3CO_2)_3$, oleic acid, 1-octadecene, NaF, polyacrylic acid (PAA, Mw 1800), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), Poly(D-glucosamine) (chitosan, medium molecular weight), N,N-Dimethyl-4-nitrosoaniline (RNO) and imidazole were purchased from Sigma-Aldrich. RPMI1640 Medium, fetal bovine serum (FBS), penicillin-streptomycin and trypsin-EDTA were obtained from Invitrogen (Carlsbad, CA, USA). All the commercially reagents were used as received unless otherwise noted.

Cytotoxicity Assay upon UV irradiation:

Generally, cells were seeded in 96-wells plates with a density of 10^4 cells per well. After 24 hours of incubation at 5% CO_2 and 37°C, the medium was replaced with fresh medium containing Re(I) complex. After another 24-hour incubation at 37°C, culture media were removed, cells were washed and then incubated with 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) containing culture media. After 5 hours incubation, media were removed and 100μL of DMSO was added. The absorbance at 570 nm was measured by a Tecan's Infinite M200 microplate reader. In a typical experiment with UV irradiation cells were treated with Re(I) complex for 2 hours. Subsequently, the drug-containing medium was replaced with fresh medium and cells were irradiated with UV light (365nm, 8.9mW/cm^2). After 24 hours of incubation cell viability was evaluated by standard MTT assays. In phototoxicity tests, cells were exposed to UV irradiation for different period of time followed by 24 hours incubation and MTT assays to investigate cell viability.

Light irradiation:

In a typical NIR irradiation experiment, a 980 nm NIR laser (EINST Technology Pte Ltd, Singapore) was used for irradiation of solution or cells in the dark. The power density of the NIR laser was adjusted by fixing the power of the laser beam and the distance between the laser beam and the

cells. The power density of the laser beam was determined by a power meter (Newport, Israel). UV lamp (Blak-Ray, B-100AP/R, 100 w/365nm) was used in a similar way. The power density was adjusted by the distance between the UV lamp and samples. A power meter was used to determine the power density of UV light.

Investigation on mechanism of photoactivated cytotoxicity.

N,N-dimethyl-4-nitrosoaniline (RNO) imidazole assay was performed to investigate the mechanism of photoactivated cytotoxicity. In general, PBS (10mM, pH 7.4) solution containing RNO (50μM), imidazole (8mM) and the Re(I) complex (50μM) or Re-UPC (500μM) were irradiated (UV 8.9mW/cm^2 30min, NIR 1.5W/cm^2 1h) in fluorescence quartz cuvettes. Absorbance of N,N-dimethyl-4-nitrosoaniline at 440 nm was monitored by UV-VIS spectrometer.

Myoglobin assay

Myoglobin (Mb) assay was carried out to study the CO release from the Re(I) complex or Re-UPC upon light irradiation. Generally, PBS (10mM, pH 7.4) solution containing Mb (50μM), dithionite (10mM) and the Re(I) complex (15μM) or Re-UPC (15μM) were irradiated with UV or NIR light respectively (UV 8.9mW/cm^2 30min, NIR 1.5w/cm^2) in fluorescence quartz cuvettes. Absorbance of Mb at 557 nm was monitored by UV-VIS spectrometer.

Section 2:

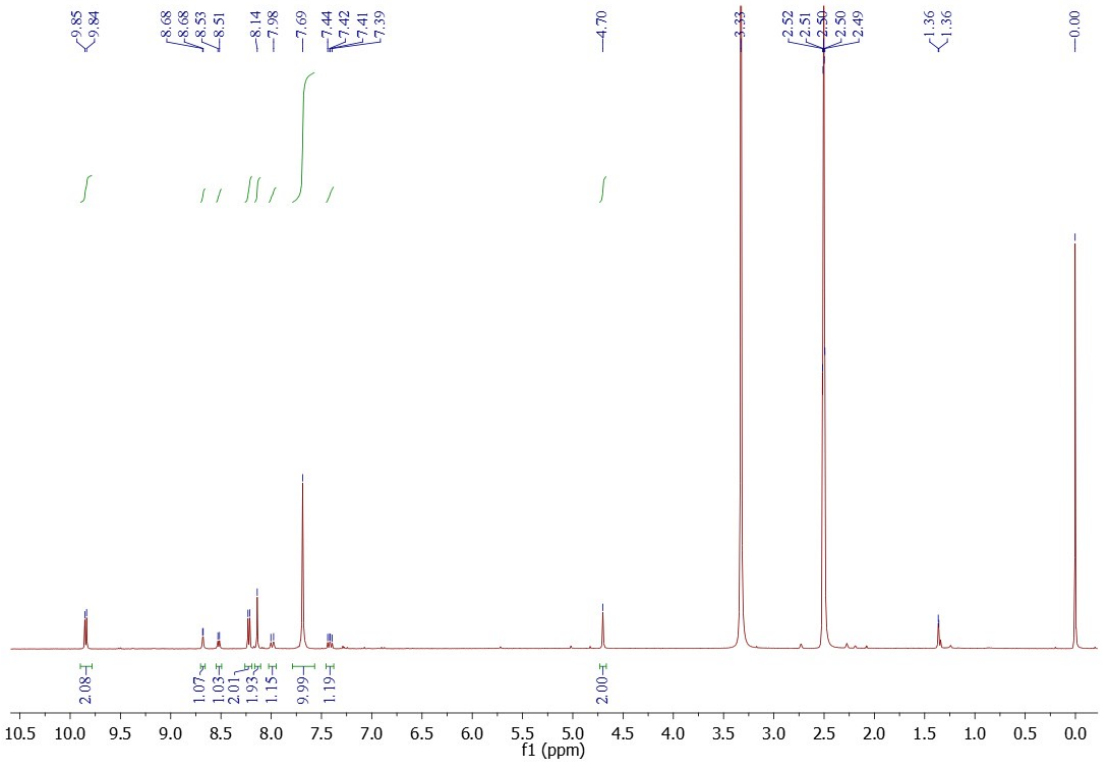

Fig.S1[1]HNMR spectrum of [Re(DIP)$(CO)_3$(cpy)](PF_6)

[1]H NMR (300MHz, DMSO) δ 9.85 (d, *J* = 5.4 Hz, 2H; H of Ph_2-phen), 8.68 (s, 1H; H of pyridine), 8.52 (d, *J* = 4.9 Hz, 1H; H of pyridine), 8.22 (d, *J* = 5.4 Hz, 2H; H of Ph_2-phen), 8.14 (s, 2H; H of Ph_2-phen), 7.99 (d, *J* = 8.1 Hz, 1H;H of pyridine), 7.69 (s, 10H; C_6H_5 at Ph_2-phen), 7.42 (t, *J* = 7.9, 5.7 Hz, 1H;H of pyridine), 4.70 (s, 2H; CH_2).

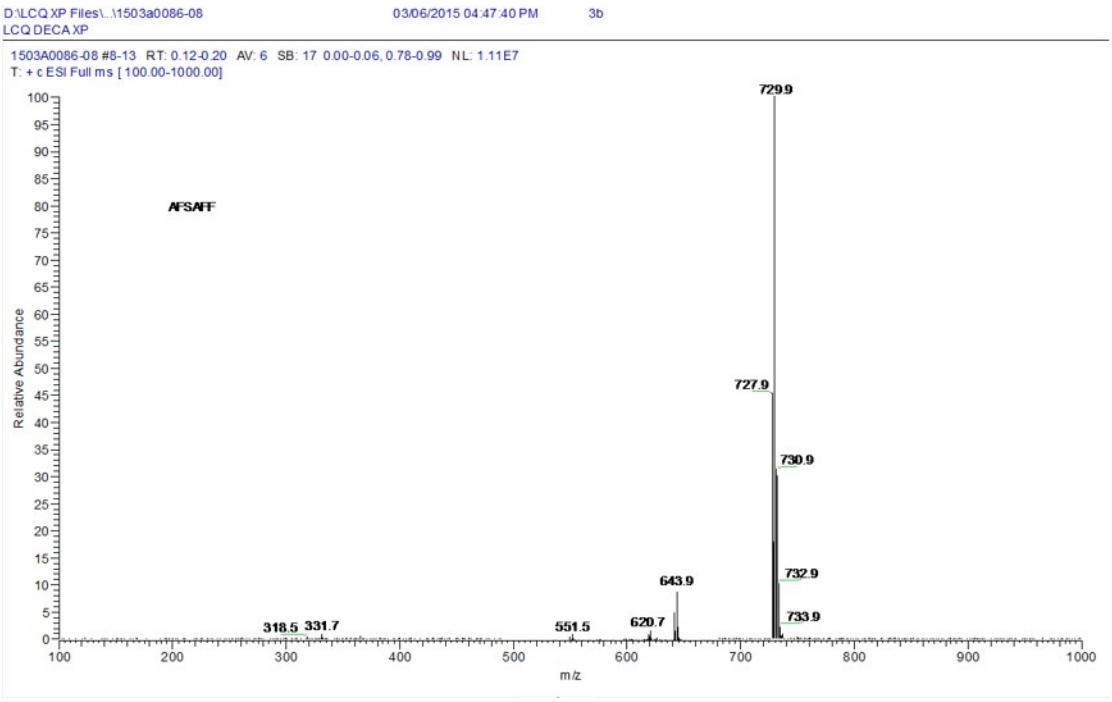

Fig. S2 ESI-MS spectrum of $[Re(DIP)(CO)_3(cpy)](PF_6)$, ESI-MS: m/z 729.9 $[M\text{-}PF_6^-]^+$.

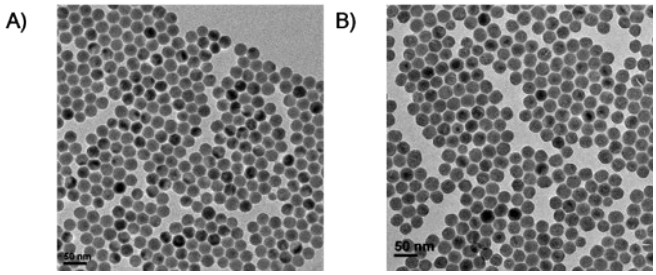

Fig. S3 TEM images of A) NaYF4:Yb^{3+}/Tm^{3+} core nanoparticles and B)NaYF4:Yb^{3+}/Tm^{3+}@NaYF4 core-shell nanoparticles

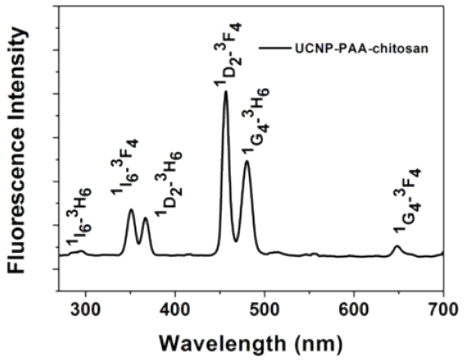

Fig. S4 Emission spectra of UCNP-PAA-Chitosan (1mg/mL) in PBS buffer (10mM, pH7.4), λ_{ex}=980nm.

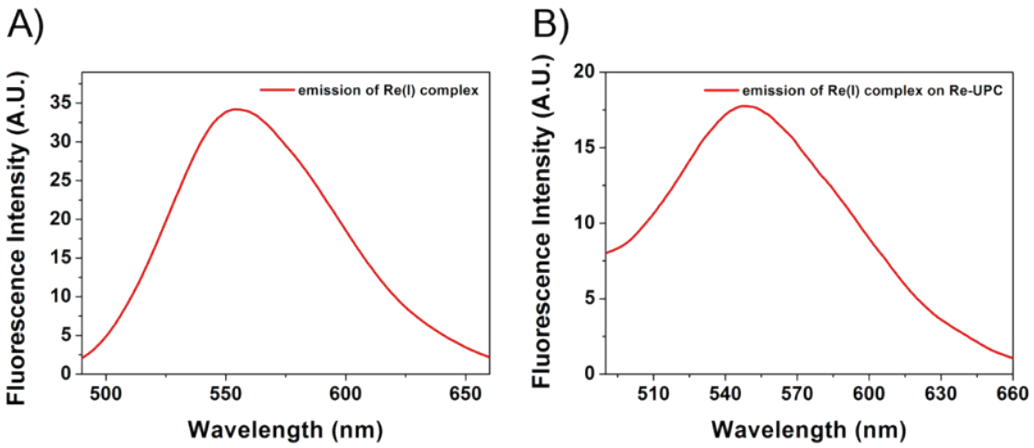

Fig. S5 Fluorescence emission spectra of A) free Re(I) complex (10μM) and B) the Re(I) complex loaded on Re-UPC (5μM) in PBS (10mM, pH7.4), λ_{ex}=290nm.

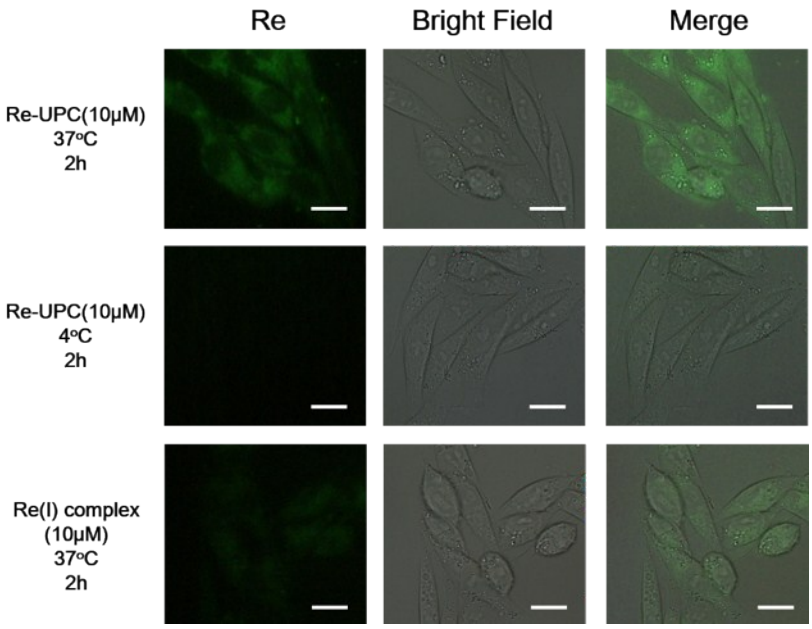

Fig.S6 Fluorescence microscope images of A2780cis cells incubated with Re-UPC (10μM) at 37°C, Re-UPC (10μM) at 4°C and Re(I) complex (10μM) at 37°C for 2h, λ_{ex}=364nm. All scale bars are 10μm.

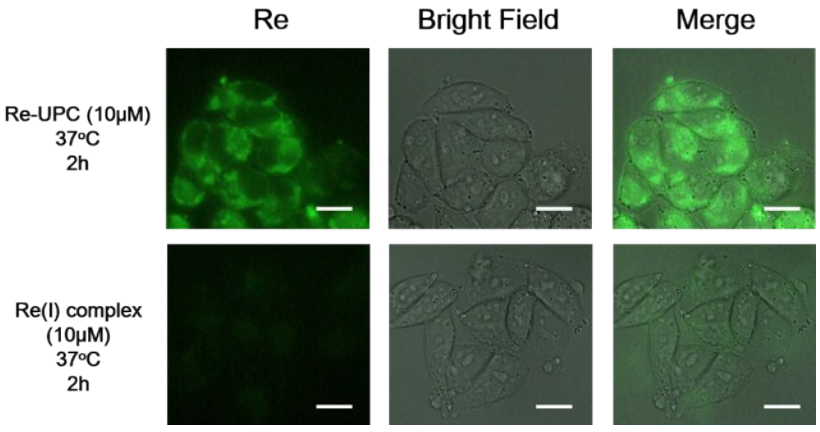

Fig.S7 Fluorescence microscope images of A2780 cells incubated with Re-UPC (10μM) at 37°C and Re(I) complex (10μM) at 37°C for 2h, λ_{ex}=364nm. All scale bars are 10μm.

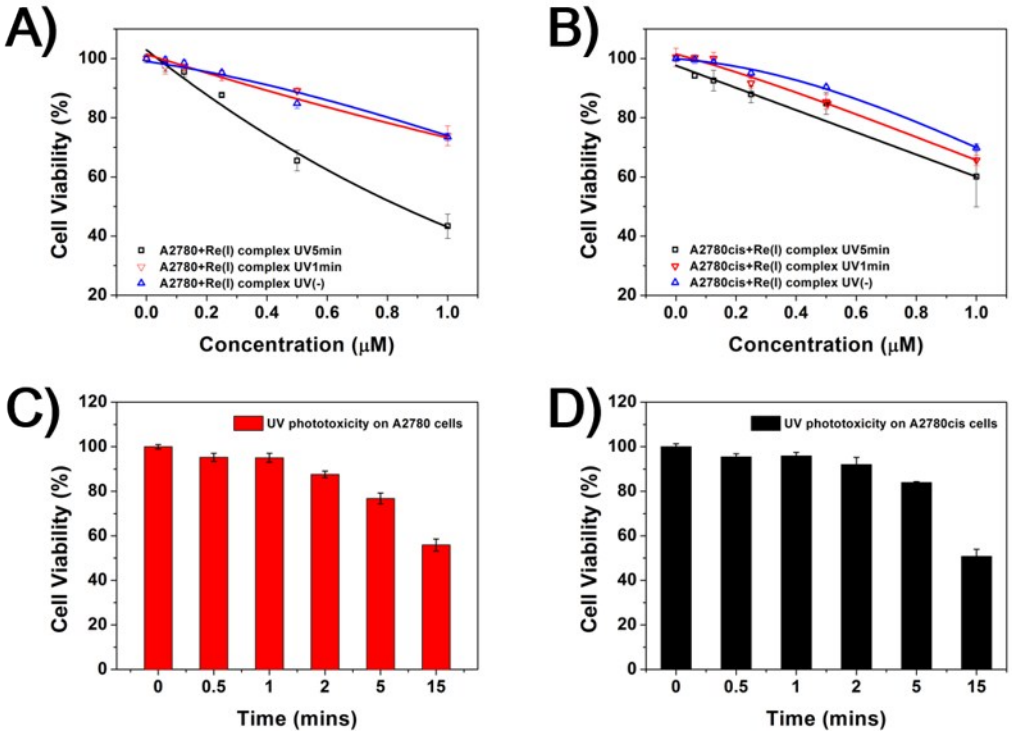

Fig.S8 The cytotoxicity assays of free Re (I) complex in A2780 cells (A) and A2780cis cells (B) treated with UV light for different periods of time (0 minute (no, UV irradiation, blue), 1 minute (pink) and 5 minutes (black). The phototoxicity of UV light over increasing time on C) A2780 cells (red) and D) A2780cis cells (black) respectively.

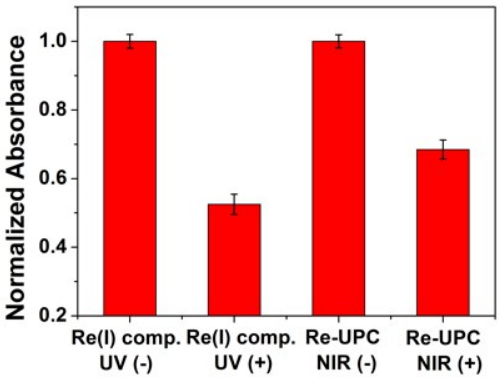

Fig.S9 RNO/imidazole assay for investigation on mechanism of photoactivated cytotoxicity: Normalized absorbance (440nm) of RNO (50μM) in the presence of Re(I) complex (50μM) or Re-UPC (500μM) with or without light irradiation.

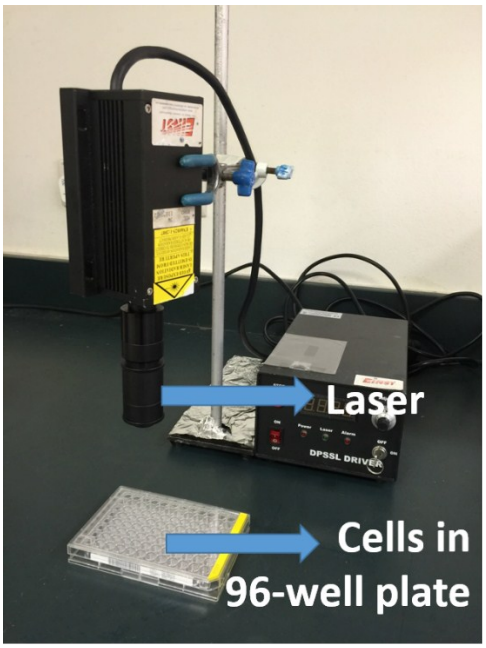

Fig.S10 Photograph showing the experimental setup for NIR irradiation of cell experiments.

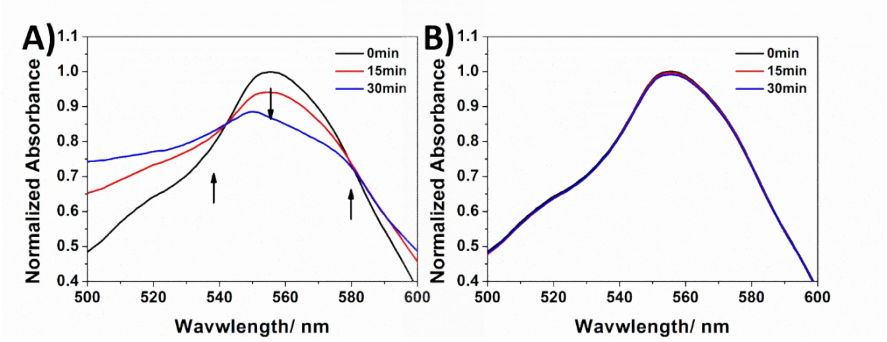

Fig.S11 Time course of absorption spectra change of Myoglobin (50μM) in PBS solution (pH 7.4) containing the rhenium (I) complex 15 μM A) with UV irradiation (365nm) and B) without light irradiation

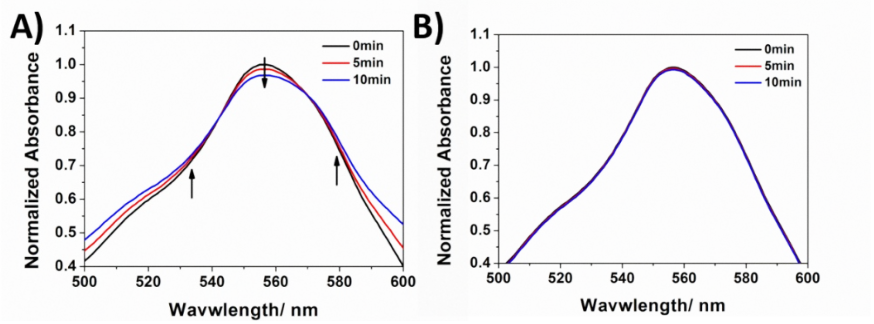

Fig.S12 Time course of absorption spectra change of Myoglobin (50μM) in PBS solution (pH 7.4) containing Re-UPC 15 μM A) with NIR irradiation (980nm) and B) without irradiation.

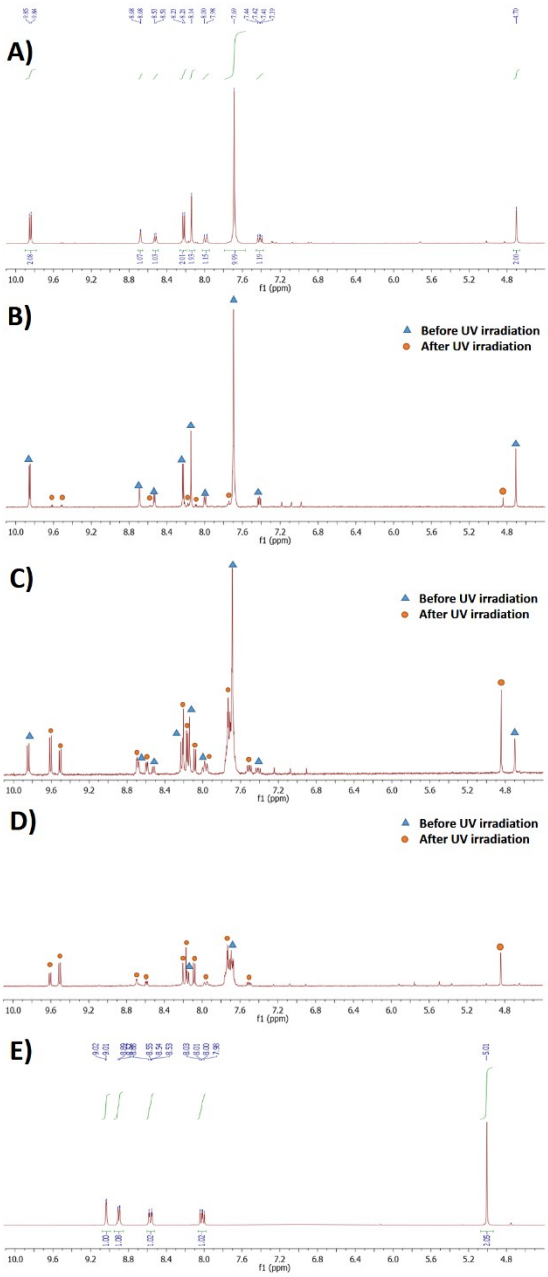

Fig.S13 A) 1H NMR spectrum of the Re(I) complex before UV irradiation (in DMSO). B), C) and D) 1H NMR spectrum of the Re(I) complex (in DMSO) after UV irradiation (UV 8.9mW/cm^2) for B) 30min, C) 6h and D) >12h. E) 1H NMR spectrum of the pyridyl ligand 3-(chloromethyl) pyridine in DMSO.