Supporting Information

Photo-Responsive Hydrogels Based on Ruthenium Complex: Synthesis and Degradation

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Contents

1. General Experimental Section

1.1. Chemicals and Reagents

All chemicals were purchased from Merck KGaA, Acros Organics, ABCR, and its affiliates which they used without further purification. The solvents used herein, i.e., diethyl ethe (100%), *N,N*-dimethylformamide (99.8%), acetonitrile were bought from VWR chemicals and Acros Organics, respectively, while DCM (99%) and ethyl acetate were both obtained from Fischer Scientific. Triphenylphosphine (99%) and acryloyl was purchased from Alfa Aesar. 4-arm-PEG OH of ≈10 kDa average molecular weight was obtain from Jenkem Technology. For purification carried out with dialysis, Spectra Por dialysis tubing (MWCO = 2000 g mol^{-1}).

1.2.Characterization Methods

The synthesis of the Ru(II) complex were performed under an atmosphere of Argon and in the dark. The a Joel ECX 400 (400 MHz), Jeol Eclipse 500 MHz (Tokyo, Japan) or a Bruker AVANCE III 700 MHz spectrometer (Billerica, MA, USA) instruments were used to measure all the NMR spectra of all the compounds (^{1}H and ^{13}C) reported here were recorded at 300 K in the deuterated solvents. Chemical shifts δ were reported in ppm and the deuterated solvent peak was used as a standard. UV/Vis measurements were conducted on an Agilent Cary 300 Bio spectrophotometer using half-micro quartz cuvettes. A high-resolution mass spectra (HRMS) were obtained from an AGILENT 6210 ESI-TOF spectrometer. The samples were irradiated with a LED light (529 nm), high power LED-Chip. All the Rheology data reported here was measured and characterized by the Kinexus rheometer (NETZSCH GmbH, Selb, Germany). A parallel plate, 8 mm in diameter was used for all the measurements, with the average normal force maintained at ≈ 0.1 N at 25 °C. The data were analyzed by an oscillatory frequency sweep strain controlled test with 1% strain (which is obtained from a linear viscoelastic range of an amplitude sweep test) and the reported storage modulus (G´) of a rigid hydrogel were picked at 0.3 Hz. Cell absorbance was measured with Spark 20 M plate reader. Micrographs were recorded using the microscopes low-dose protocol at a primary magnification of 28000x and an acceleration voltage of 200 kV. Images were recorded by a Falcon 3CE direct electron detector (48 aligned frames) at full size (4k). In all cases, the defocus was chosen to be 5 µm to create sufficient phase contrast.

2. General Experimental Procedure

2.1. Synthesis of N-(pyridin-4-ylmethyl) acrylamide (4AMAM)

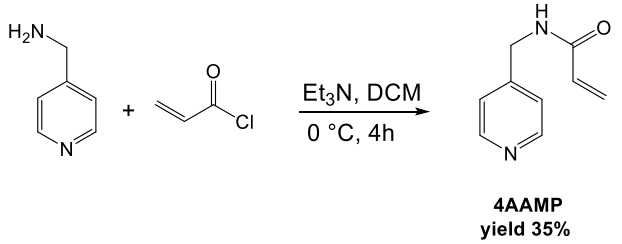


Figure S1. Synthesis of mono dentate ligand 4AAMP

According to the previous report[1] acryloyl chloride (505.97 mg, 5 mmol, 2 eq.) was placed in Schlenk flask and DCM (25 ml) was added. The reaction mixture

was cooled to -5 °C. After that a mixture of 4-aminomethylpyridine (505.97 mg, 5 mmol, 1 eq), trimethylamine (996.6 mg, 10 mmol, 2 eq.) and DCM (20 ml) was added dropwise over a period of 15 minutes. The reaction mixture was stirred at 25 °C for 4 h. Afterwards, the solvent was evaporated under reduced pressure. To purity the product, the mixture of reaction was extracted with saturated $NaHCO_3$ (100 ml) and DCM (3 × 40 ml). The combined organic phases were dried over Na_2SO_4, filtered and evaporated under reduce pressure at room temperature. To achieve a pure monomer, an organic solution was extracted again with EA (3 x 20 ml). In the end the pure product was obtained by evaporation of EA under reduced pressure. The product (285.3 mg, 1.75 mmol, 35%) was obtained as a brown oil.
^{1}H-NMR (500 MHz, MeOD-d_4): δ 8.51 (d, *J* = 5.92 Hz, 2H), 7.18 (d, *J* = 5.95 Hz, 2H), 6.33 (d, *J* = 1.21, 16.96 Hz, 1H), 6.18 (m, 1H), 5.76 (dd, *J* = 1.26, 10.31 Hz, 1H), 4.57 (d, *J* = 6.1Hz, 2H)

2.2. Synthesis of $[Ru(bpy)_2(4AAMP)_2](PF_6)_2$

2.2.1 Synthesis of $[Ru(bpy)_2(Cl)_2]$

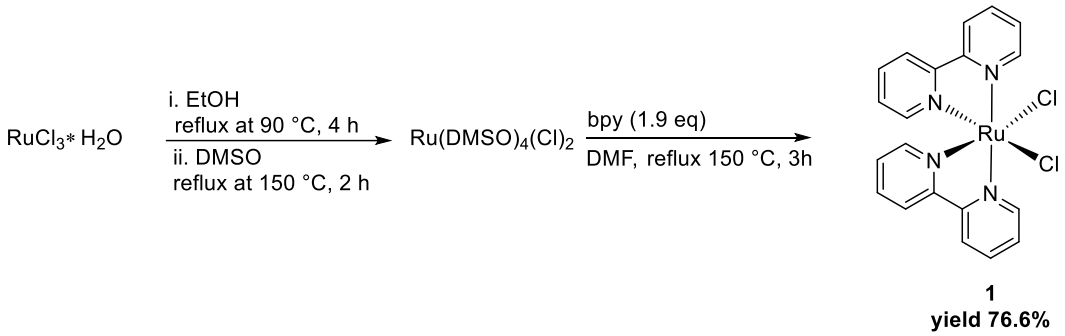


Figure S2. The procedure to synthesis of $[Ru(bpy)_2(Cl)_2]$

Product $[Ru(bpy)_2(Cl)_2]$ was synthesized from modification of previous report[2] The synthesis and workup of this reaction was performed under reduced light in order to avoid possible cis-trans isomerization. Ru $(dmso)_4Cl_2$ (145.34 mg, 0.3 mmol was dissolved in DMF (1.5 ml) to which the bpy ligand (1.90 eq, 0.57 mmol, 89.03 mg) was dissolved in 1.5 ml of DMF and added into the mixture of reaction in two portion during two hours. The reaction flask was wrapped with aluminum foil and refluxed under an Ar atmosphere for 3 h. The reaction mixture was cooled to 25 °C and filtered. A violate-black precipitate was washed with cold acetone three times (3x5 ml) and the excess solvents were removed under reduced pressure. (Rf) = 0.75 (DCM, MeOH (6%). (Yield: 76.6% 111.09 mg, 0.23 mmol).

^{1}H-NMR (400 MHz, CD_2Cl_2-d_2): δ 10.17 (d, *J* = 5.80 Hz, 2 H), 8.19 (d, *J* = 8.41 Hz, 2 H), 8.03 (d, *J* = 8.31 Hz, 2H), 7.9 (td, *J* = 7.90 Hz, 2 H), 7.62 (dd, *J* = 5.60 Hz, 4H), 7.51 (td, *J* = 7.83 Hz, 2H), 6.91 (dd, *J* = 6.53 Hz, 2H).

2.2.2. Synthesis of $[Ru(bpy)_2(4AAMP)_2](PF_6)_2$

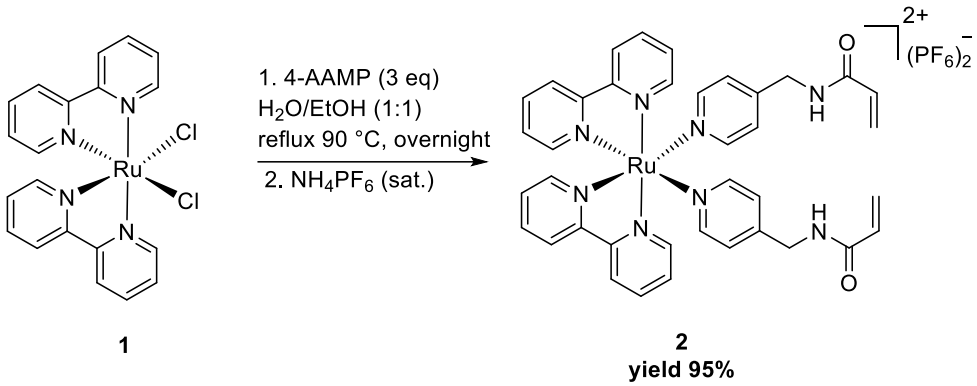


Figure S3. The procedure to synthesis of $[Ru(bpy)_2(4AAMP)_2](PF_6)_2$

According the previous procedure[3] product $Ru(bpy)_2Cl_2$ **1** (48.43 mg, 0.1 mmol) was suspended in H_2O (1.5 mL). The suspension was stirred at 90 °C under Ar while flask was wrapped with aluminum foil and after dissolution, *N*-(pyridin-4-ylmethyl) acrylamide pyridine (4AAMP) (3 eq, 48.62 mg, 0.3 mmol) was dissolved in 1 ml EtOH and dissolved by US then added to the mixture of reaction. The reaction mixture was kept at 90 °C for 24 h. The orange solution was cooled to RT and EtOH was evaporated. 5 mL water was added to the aqueous solution and extracted two times with DCM (20 mL). Then, an aqueous solution of NH_4PF_6 solution (~ 5 mL) was added. The precipitate was filtered, washed with H_2O, and dried. Ru complex $[Ru(bpy)_2(4AAMP)_2](PF_6)_2$ **3** was obtained an orange solid, (97.66 mg, 0.095 mmol, 95%). TLC: (Rf) = 0.2 (SiO_2, DCM: MeOH: sat. NH_4PF_6 (10%)).

^{1}H-NMR (400 MHz, acetone, d_6): δ 9.26 (d, *J* = 5.56 Hz, 2H), 8.62 (d, *J* = 8.14, 2H), 8.54 (m, 6H and NH), 8.24 (td, *J* = 7.89 Hz, 2H), 8.19 (dd, *J* = 5.63 Hz, 2H), 8.02 (td, *J* = 1.32, 7.87 Hz, 2H), 7.88 (td, *J* = 13.27 Hz, 2H), 7.82 (t, 2H), 7.48 (td, *J* = 6.26, 13.25 Hz, 2H), 7.28 (d, *J* = 6.15 Hz, 4H), 6.28 (m, 2H), 6.17 (dd, *J* = 6.41 Hz, 4H), 5.61 (dd, *J* = 4.06 Hz, 2H), 4.46 (d, *J* = 6.13 Hz, 4H)).
^{13}C {H} (125 MHz, Acetone): 165.33, 157.89, 157.70, 153.40, 152.88, 151.71, 138.13, 137.78, 131.07, 128.27, 127.89, 125.81, 124.52, 124.09, 123.84, 41.27.
MS (ESI): m/z = 369.150 $[Ru(bpy)_2(4\text{-acryloyl-AMP})_2]^{2+}$ and 883.1743 $[Ru(bpy)_2(4\text{-acryloyl-4AMP})_2](PF_6)$.

2.3. Synthesis and characterization of 4-arm-PEG thiol

2.3.1. Synthesis and characterization of 4-arm-PEG Oms

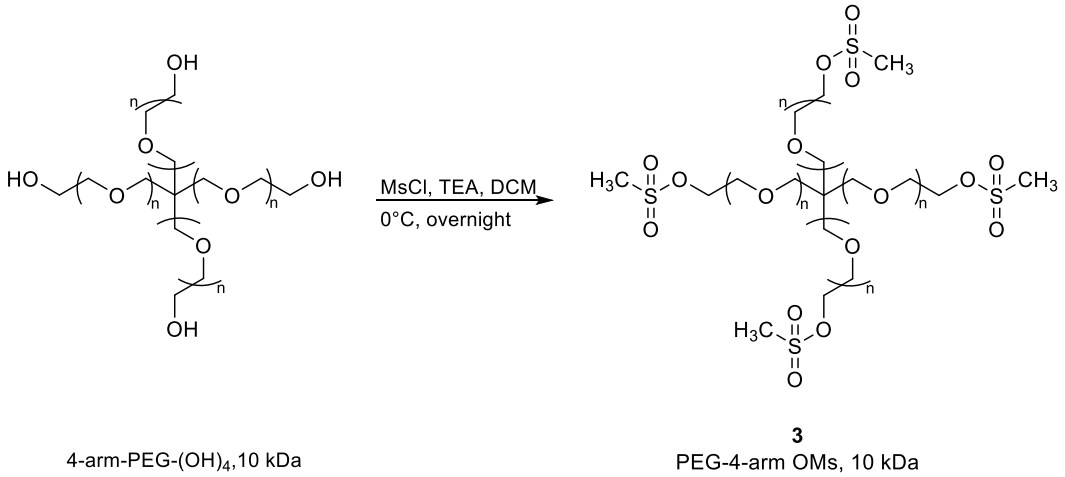


Figure S4. The procedure to synthesis 4-arm-PEG Oms

10 kDa 4-arm-PEG OH (7 g, 0.7 mmol, 1 eq.) was subjected to dry at 70°C under vacuum overnight and was later dissolved in anhydrous DCM (50 mL). TEA (0.97 mL, 7 mmol, 10 eq.) was added to the solution and the flask was then cooled on ice bath. Methane sulfonyl chloride (0.43 mL, 5.6 mmol, 8 eq.) was added dropwise to the mixture and it was carried overnight from 0°C till room temperature relating to the ice state. Afterwards the crude product was washed thrice with brine and the DCM layer was then dried with Na_2SO_4 and concentrated on the rotary evaporator. The concentrated mixture was then precipitated in cooled diethyl ether. After dried overnight under vacuum, the precipitate product results in a white powder with 85% isolated yield.

^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm)): 3.08 (3H, s), 3.40 - 3.78 (m), 4.37-4.38 (2H, t).

2.3.2. Synthesis and characterization of 4-arm-PEG thiol

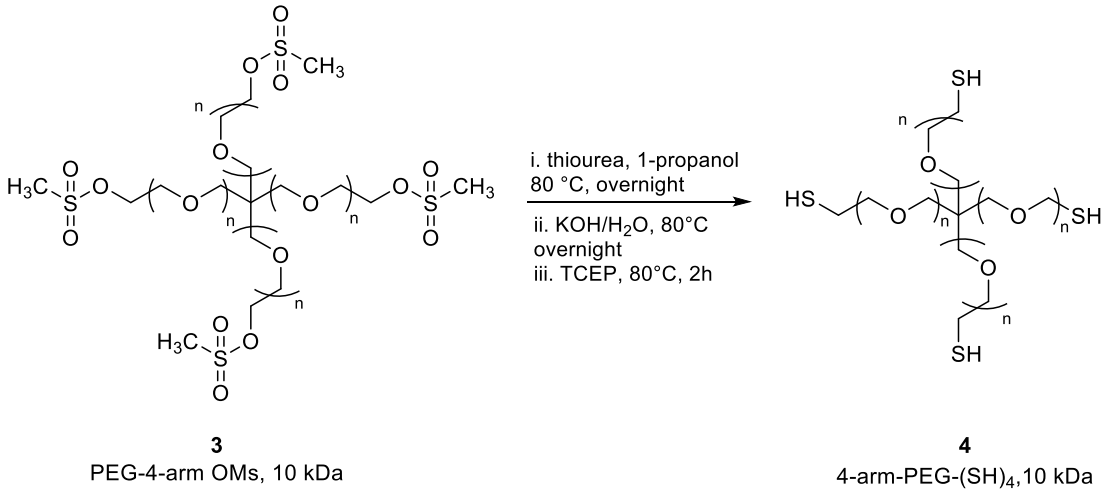


Figure S5. The procedure to synthesis 4-arm-PEG thiol

To the 1-propanol solution (10 mL) of 4-arm-PEG Oms (3) (4.33 g, 0.43 mmol, 1 eq.) was added thiourea (0.66 g, 8.7 mmol, 20 eq.) and the mixture was heated up to 80 °C overnight to obtain 4-arm-PEG isothiouronium intermediate.

After 1-propanol was removed from the mixture, KOH (0.024 g, 0.43 mmol, 4 eq.) and water (40 mL) were added and the solution was then set to 80 °C overnight. Afterwards, TCEP (0.5 g, 1.7 mmol, 4 eq.) was added to the crude mixture and it was stirred for 2 h. The product was later purified by first adding NaCl to the crude until reaching the saturated point, then extracting the product with DCM thrice and drying it with Na_2SO_4, concentrating the DCM layer and finally precipitating it in cooled diethyl ether. The precipitate were then dried under vacuum overnight to later obtain 4-arm-PEG thiol product as a pale yellowish powder with 90% yield.

^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm)): 1.598 – 1.622 (t, *J* = 6 Hz, 1H), 2.698-2.728 (quat, *J* = 4.5, 6 Hz, 2H).

2.4. Synthesis of metallo hydrogel

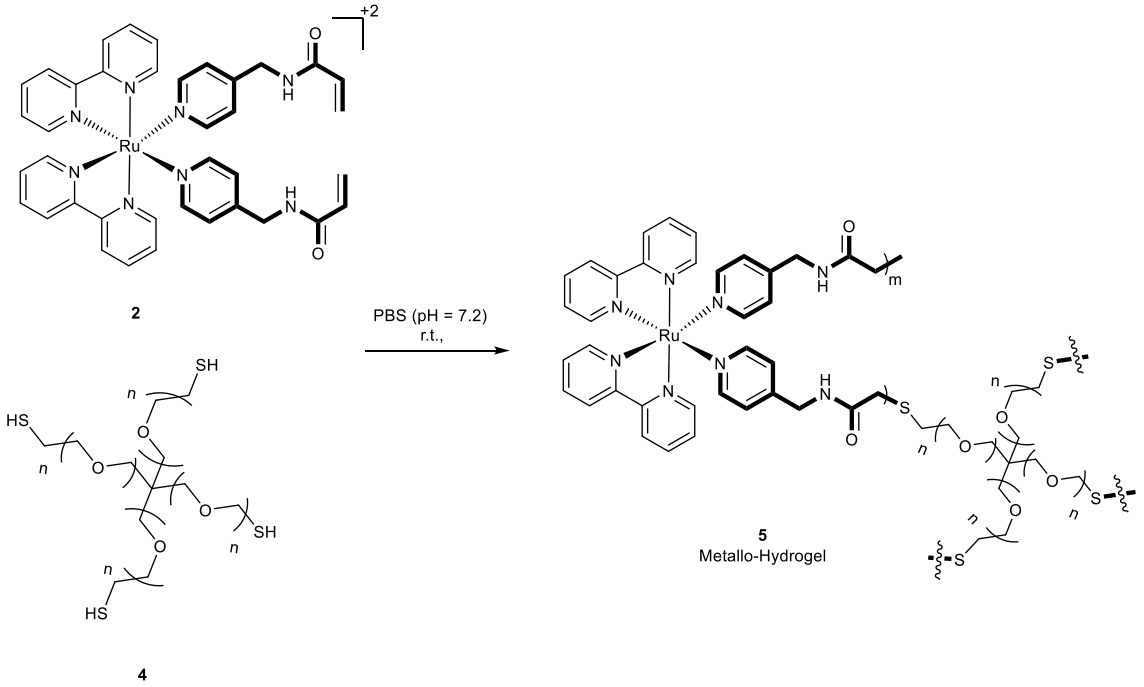


Figure S6. The procedure to synthesis metallo-hydrogel **5**

Metallo-hydrogel 5 was prepared from dissolved $[Ru(bpy)_2(4AAMP)_2](PF_6)_2$ **2** in the solution of DMSO and PBS (ratio 3:2 (10% w/v)) and 4 arm PEG-SH (10 kDa) 4 as a gel component in PBS (20% w/v) at room temperature. Also, an additional PBS was added to the mixture of reaction together to get the amount to a total of 100 µL gel volume. Solutions were combined and stirred at room temperature for 6-24 hours, depends on the gel percentages, until an orange gel is obtained. Subsequently hydrogel was washed with MeOH and water to remove an uncoordinated Ru complex.

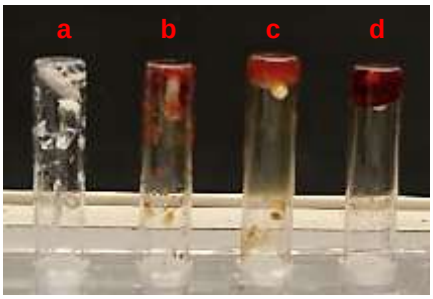


Figure S7. A photograph taken after gelation reaction which have been placed in PBS at r.t, control reaction 10% (a), 2% (b), 5% (c), 7% (d) of gel concentration respectively.

Table S1. Optimizing gel construction. The gelation reaction have been placed with PBS at 25 °C in the volume of 100 µL; (a) the control gelation reaction by 4arm-PEG10k-SH and PBS without Ru(II) as a crosslinker, (b) a solution of 10% of $[Ru(bpy)_2(4AAMP)_2]^{2+}$ in the mixture of PBS and DMSO (3:2) and (c) a solution of 20% w/v of 4arm-PEG10k-SH in PBS.

Gel entry	Ru:PEG 1.5mol ratio		Ru:PEG 2mol ratio		Gel conc. (%w/v)	Total Gel volume [μL]	Time (hour)
	[Ru][b] volume [μL]	PEG[c]	[Ru][b] volume [μL]	PEG[c]			
a[a]	-	-	0	41.50	10	56.78	5 days
b	1.35	4.35	3.42	8.30	2	100	48 h
c	6.71	21.7	8.6	20.70	5	100	24 h
d	9.36	30.33	11.95	29.03	7	100	12 h
e	13.55	43.22	17	41.50	10	100	6 h

3. Characterization of $[Ru(bpy)_2(4AAMP)_2]^{2+}$ and hydrogel

3.1. ^{1}H NMR Spectrum

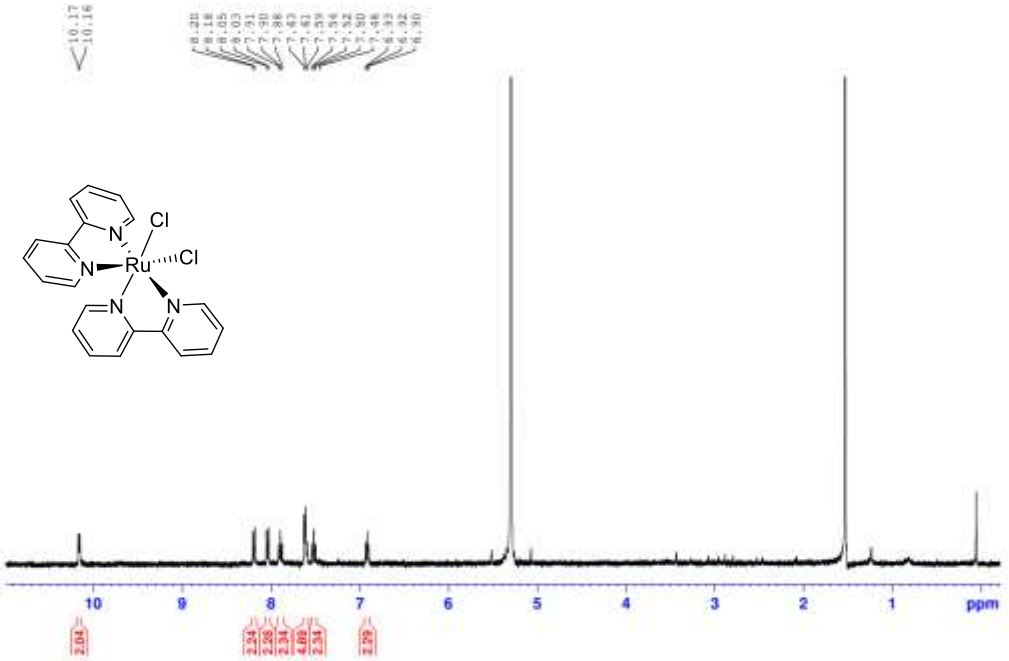


Figure S8. ^{1}HNMR spectra of $[Ru(bpy)_2(Cl)_2]^{2+}$[1] in methylene chloride-d_2

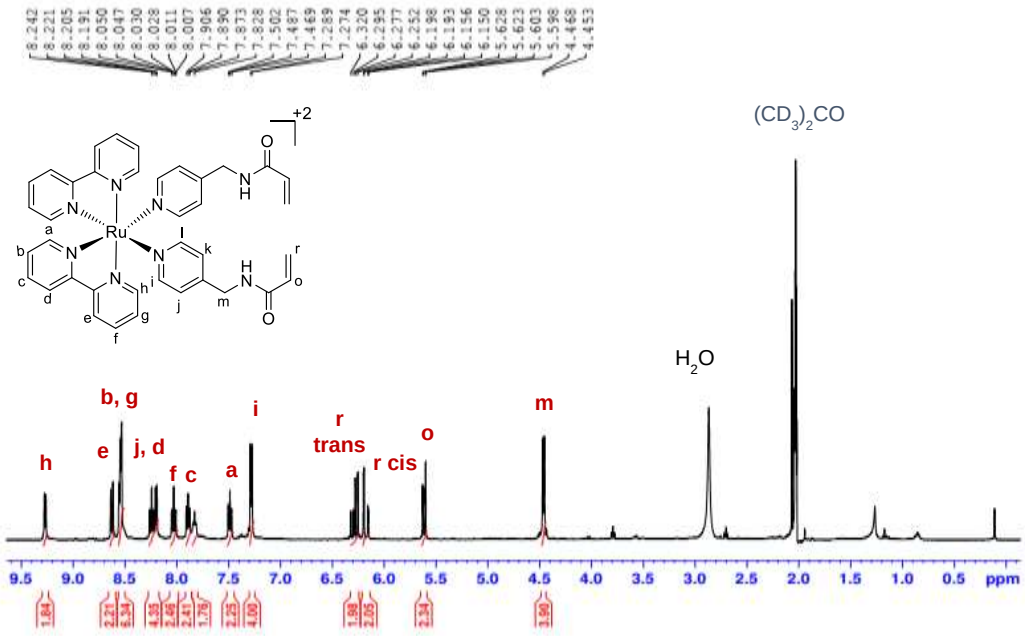


Figure S9. ^{1}HNMR spectra of $[Ru(bpy)_2(4AAMP)_2]^{2+}$ **2** in acetone-d_6.

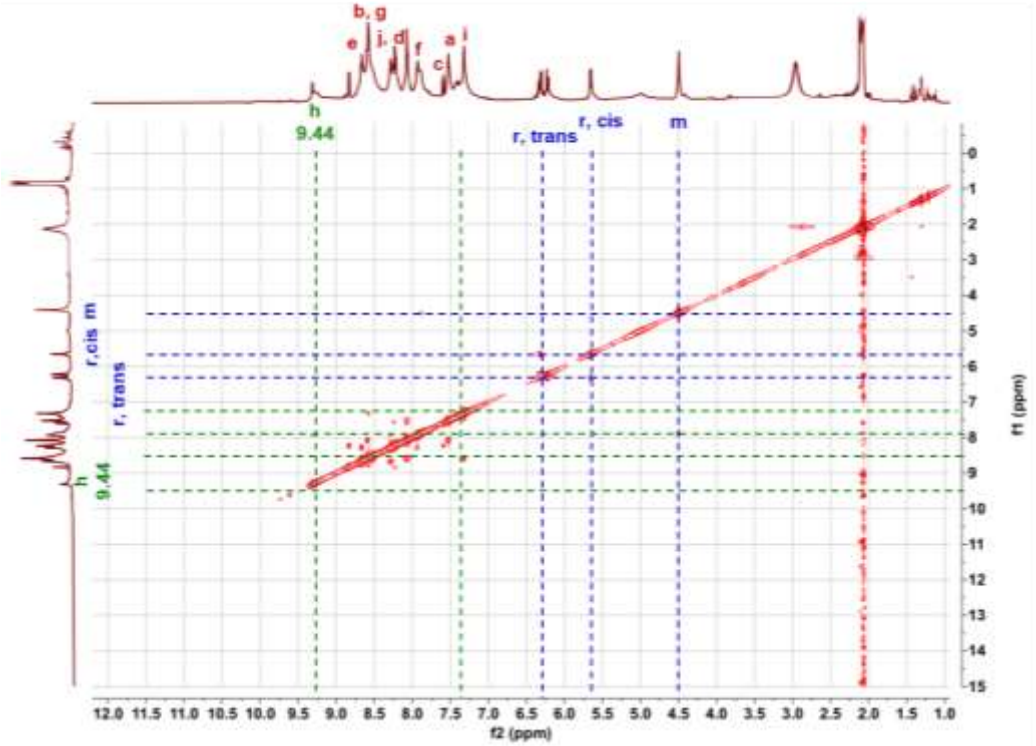


Figure S10. Partial COSY NMR 1H-H COSY HNMR (700 MHz, CD_3COCD3) spectrum of $[Ru(bpy)_2(4AAMP)_2]^{2+}$ **2** in acetone-d_6.

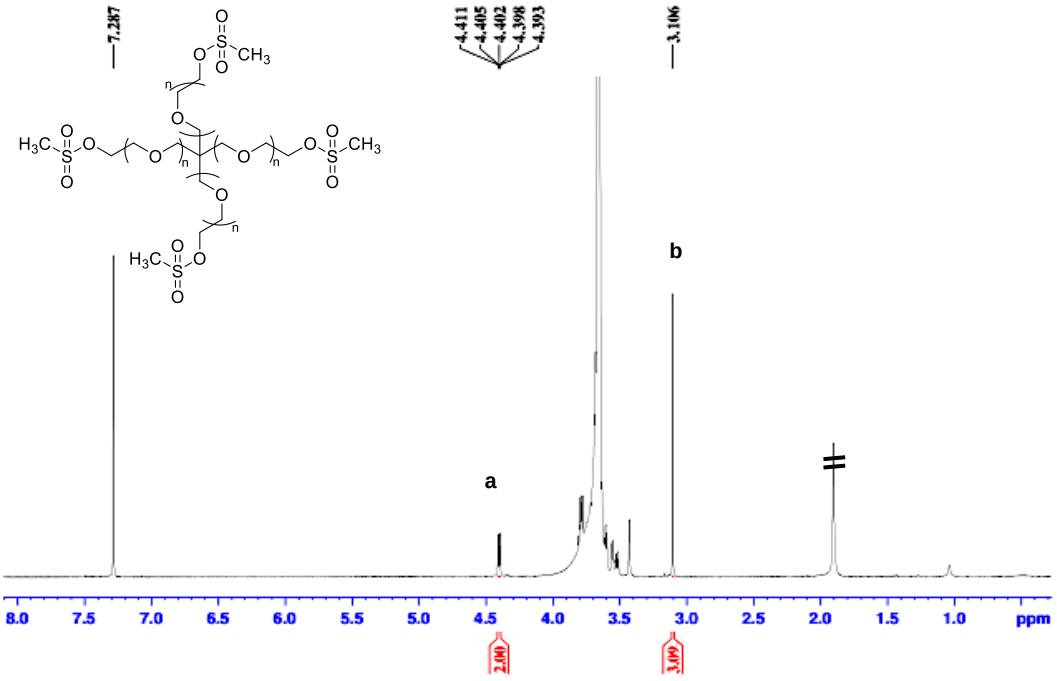


Figure S11. ^1H NMR (500 MHz, $CDCl_3$, δ (ppm)) of PEG 4 arm Oms **3**.

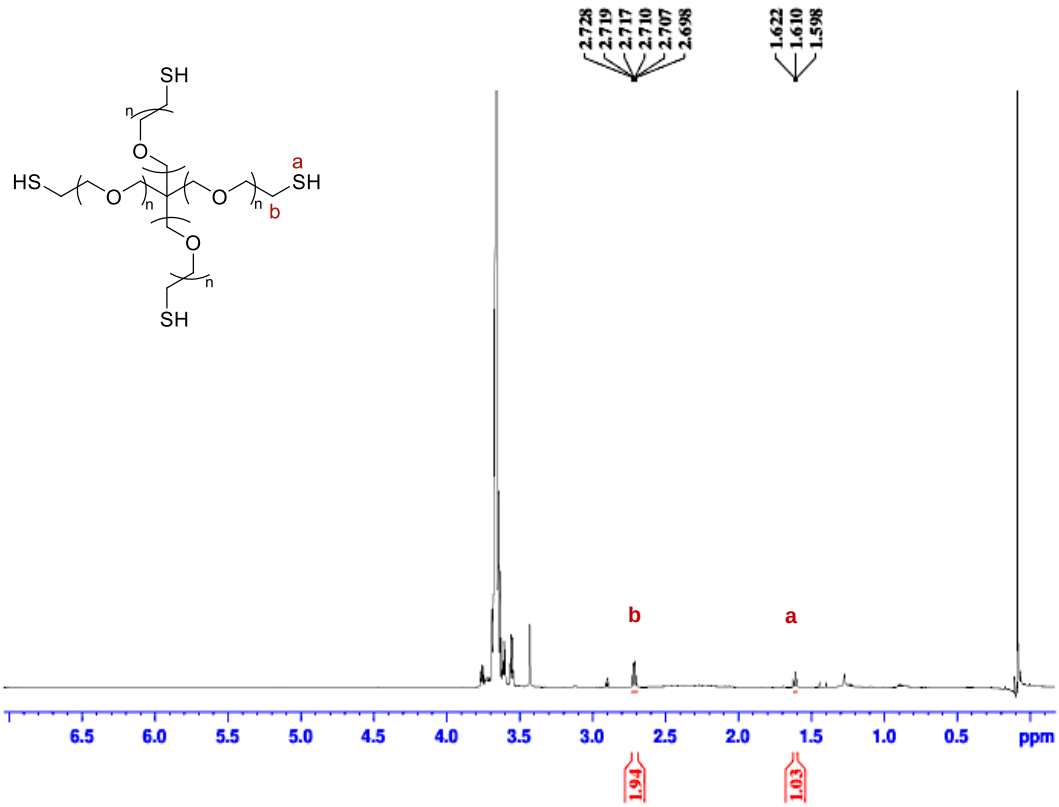


Figure S12. ^{1}H NMR (500 MHz, $CDCl_3$, δ (ppm)) of PEG 4 arm thiol **4**.

3.2. ^{13}CNMR Spectrum $[Ru(bpy)_2(4AAMP)_2]^{2+}$

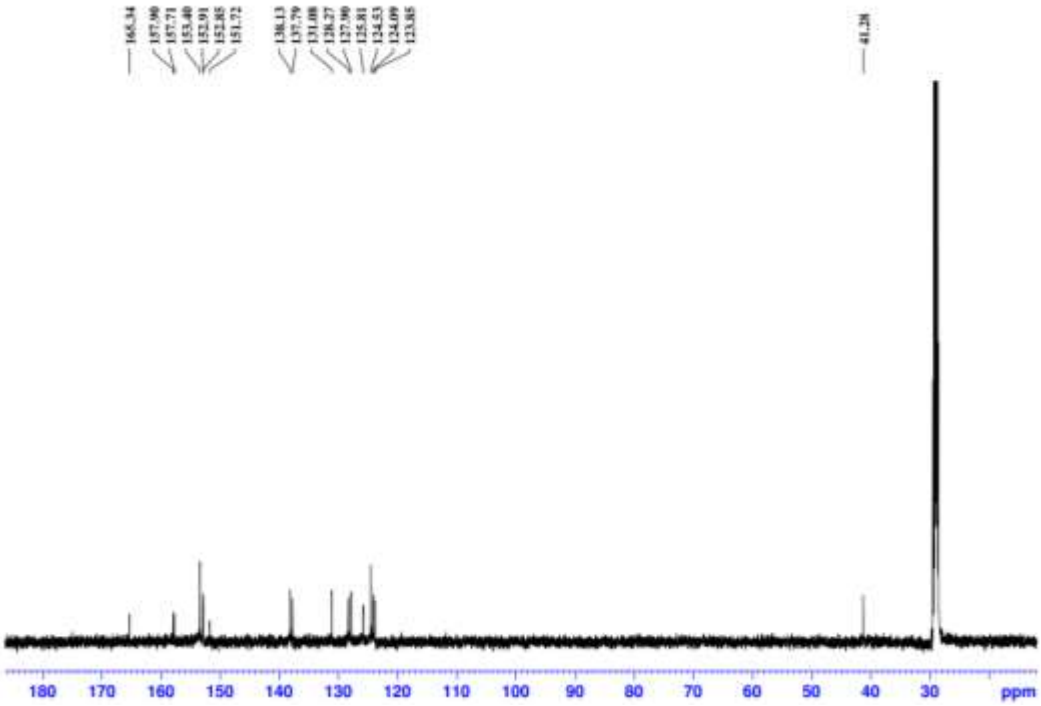
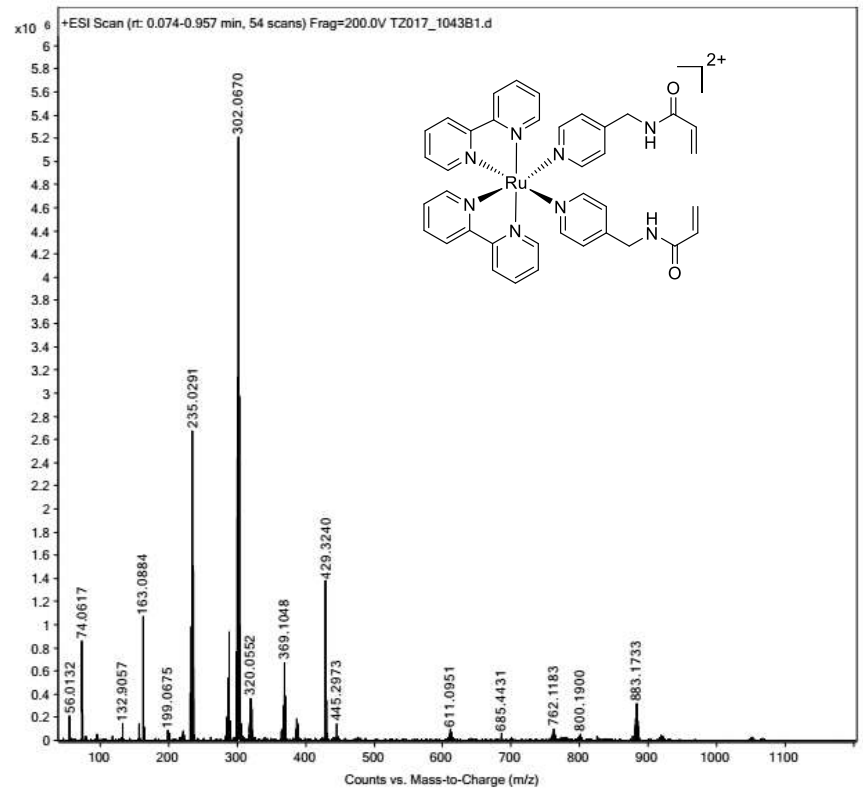
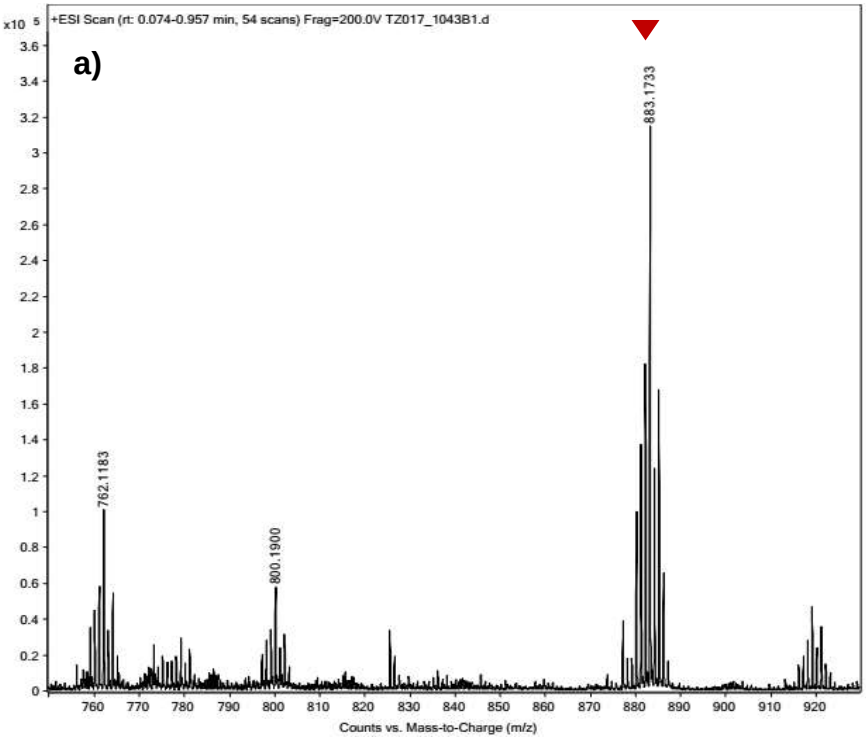


Figure S13. ^{13}CNMR spectrum of $[Ru(bpy)_2(4AAMP)_2]^{2+}$ **2** in acetone-d_6.

3.3. HR MS Spectrum





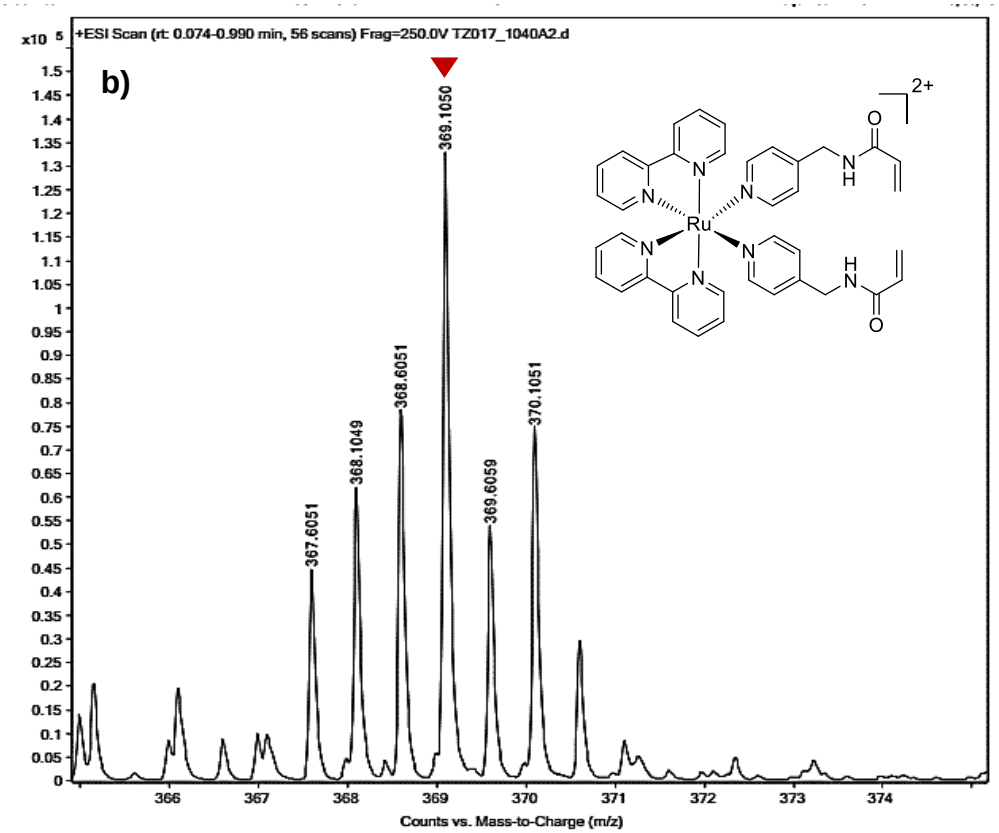


Figure S14. a) HR MS spectrum of $[Ru(bpy)_2(4AAMP)_2](PF_6)_2$ **2** in acetonitrile (m/z = 369.0999 represents $[Ru(bpy)_2(4\text{-}AAMP)_2]^{2+}$, and b) m/z = 883.1743 represents $\{[Ru(bpy)_2(4\text{-}AAMP)_2](PF_6)\}^+$

3.4. FTIR Spectrum

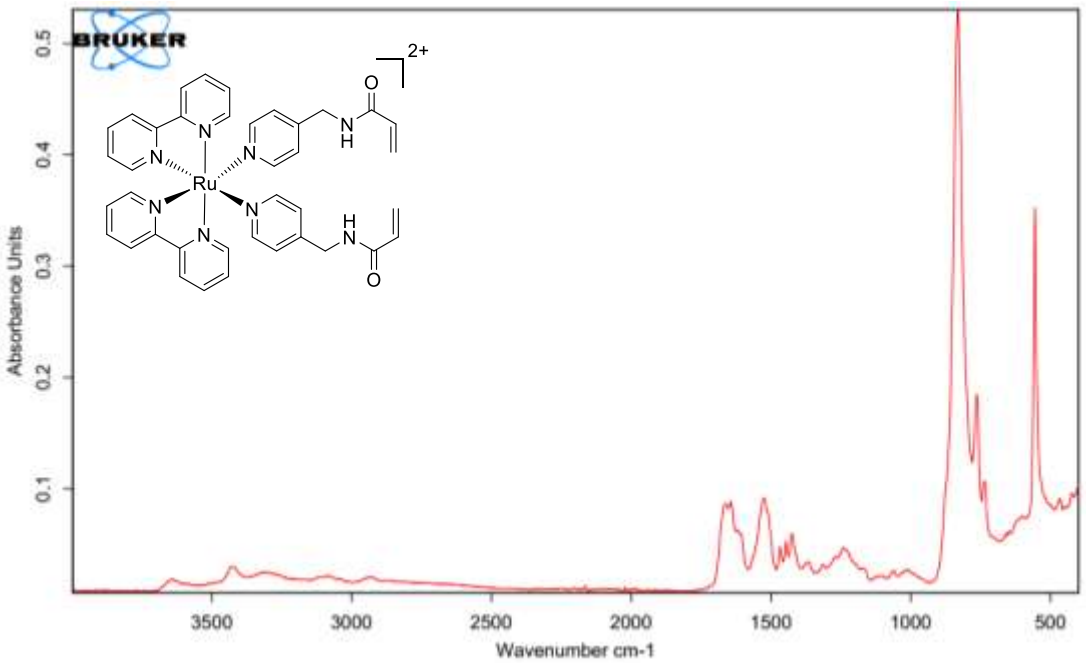


Figure S15. FT-IR Spectrum of $[Ru(bpy)_2(4\text{-}AAMP)_2]^{2+}(PF_6)_2^-$

3.5. Irradiation experiments of the complex in acetonitrile

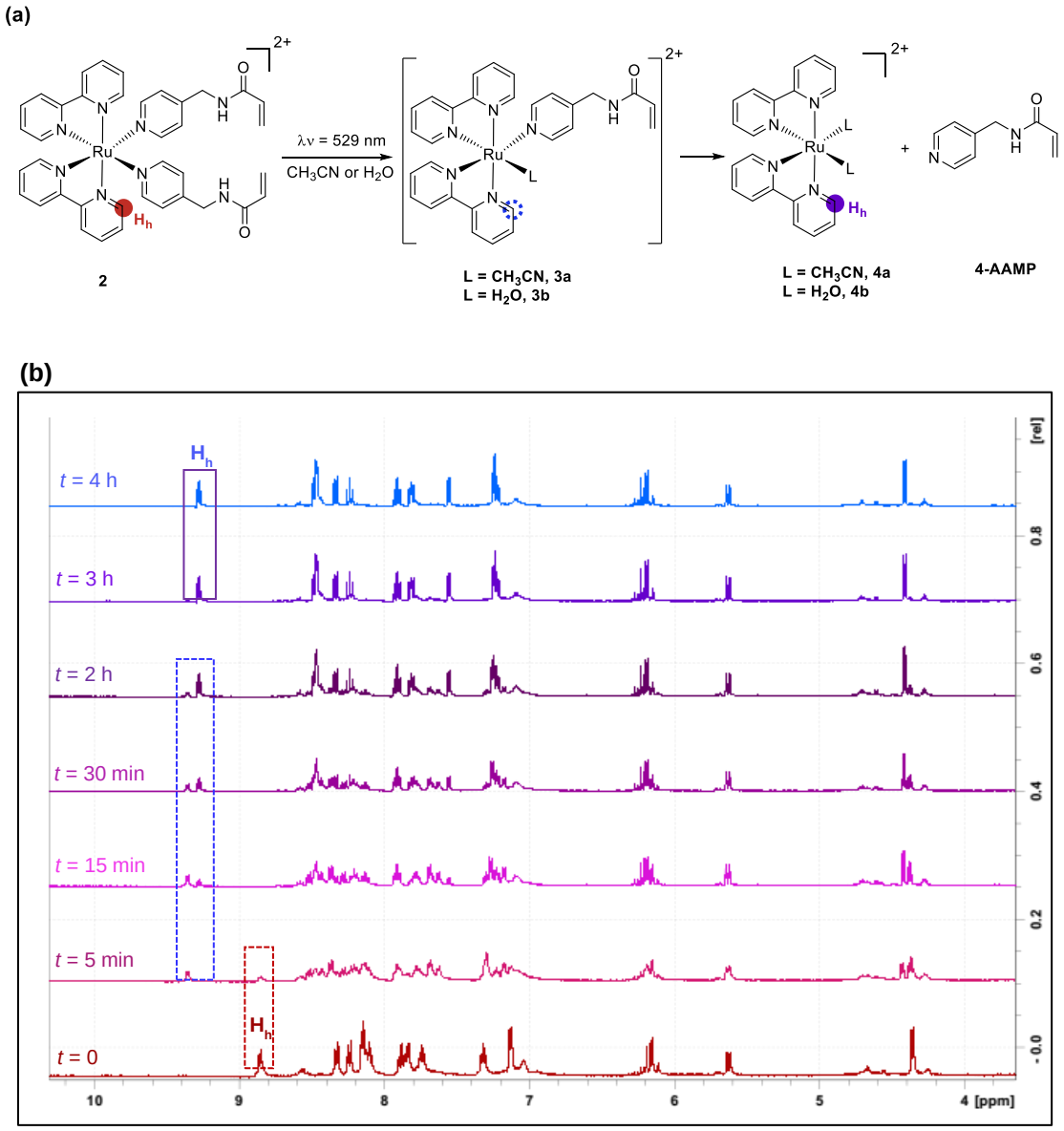


Figure S16. (a) photochemical reaction of Ru(II) complex in acetonitrile and water, (b) photochemical reaction was monitored as a function of the irradiation time in 4 h by ^{1}H NMR spectroscopy in CD_3CN (conc ≈ 1×10^{-2} mol.L^{-1}) at green light, λ = at 529 nm, intensity 28 mW.cm^{-2}, 4 h at 25 °C.

3.6. Irradiation experiments of the complex in water

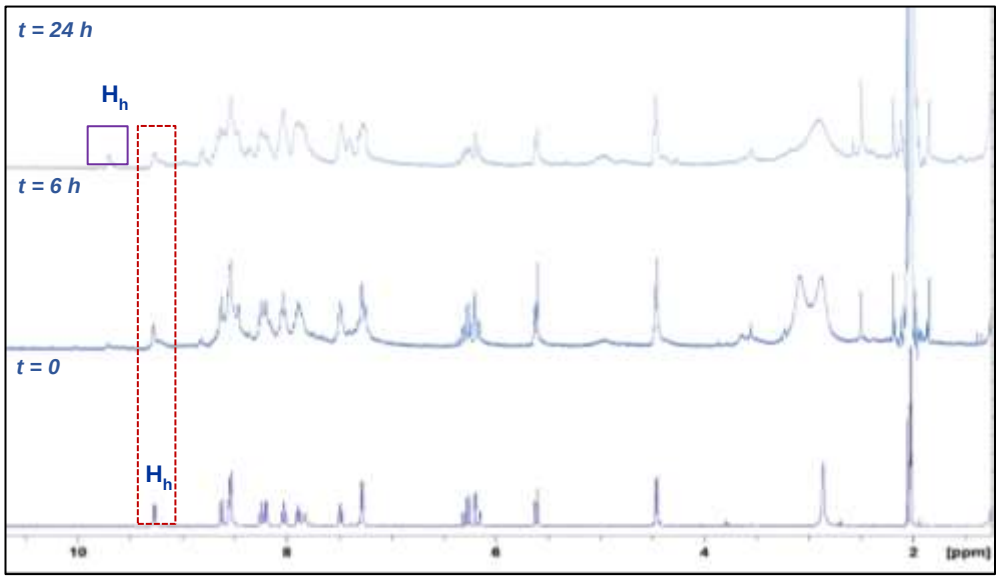


Figure S17. ^{1}HNMR (400 MHz, $(CD_3)CO$) of $[Ru(bpy)_2(4\text{-}AAMP)_2]^{2+}$ dissolved in two drops of acetone-d_6 and measured in D_2O within irradiation at 529 nm.

3.7. UV-Visible measurement of the complex $Ru(bpy)_2(4AAMP)_2]^{2+}$ in water

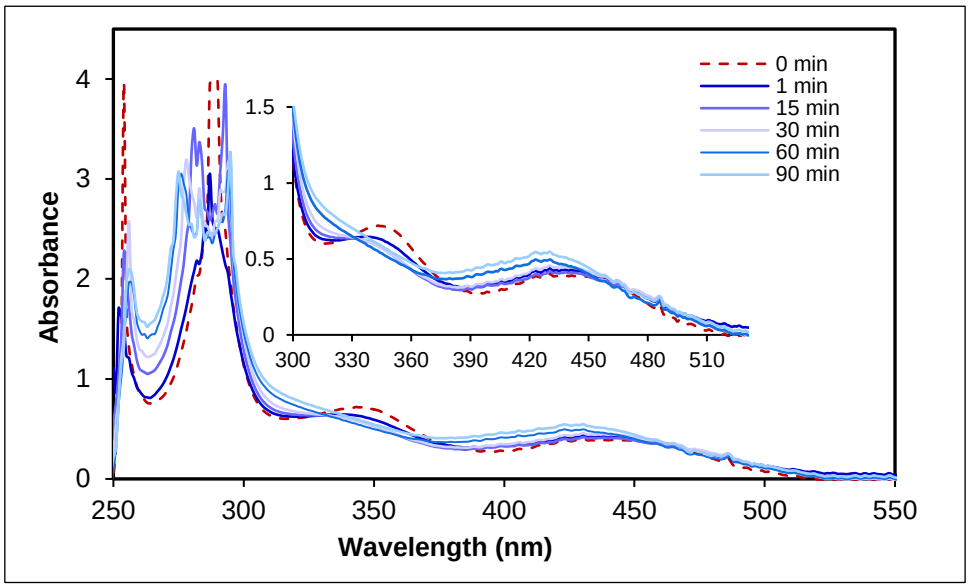


Figure S18. Change in the absorption spectra of $[Ru(bpy)_2(4AAMP)_2]^{2+}$ in H_2O solution, (conc. ≈ 7×10^{-5} mol.L^{-1}) with and without irradiation at 529 nm within 90 min.

3.8. Fluorescence measurement of the complex $Ru(bpy)_2(4AAMP)_2]^{2+}$ in water

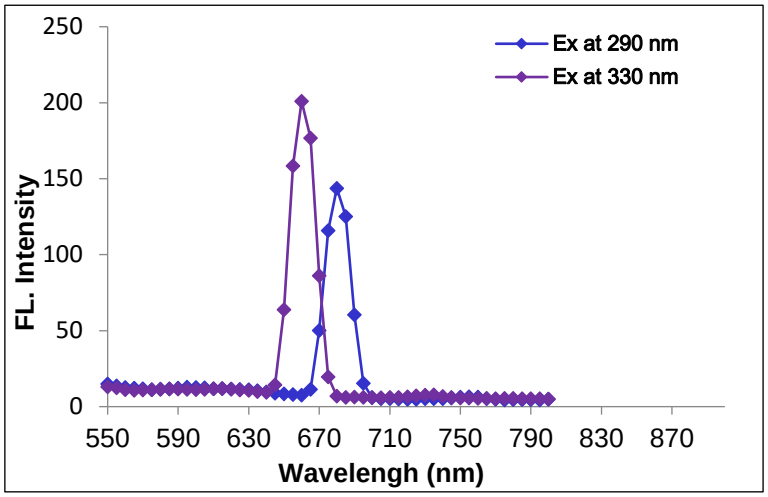


Figure S19. Fluorescence intensity of $Ru(bpy)_2(4AAMP)_2]^{2+}$ in H_2O solution (conc. ≈ 7×10^{-5} $molL^{-1}$) (down) FL. Intensity measured at 330 and 290 nm excitation to get an estimate of the emission spectra.

3.9. Rheological Characterization

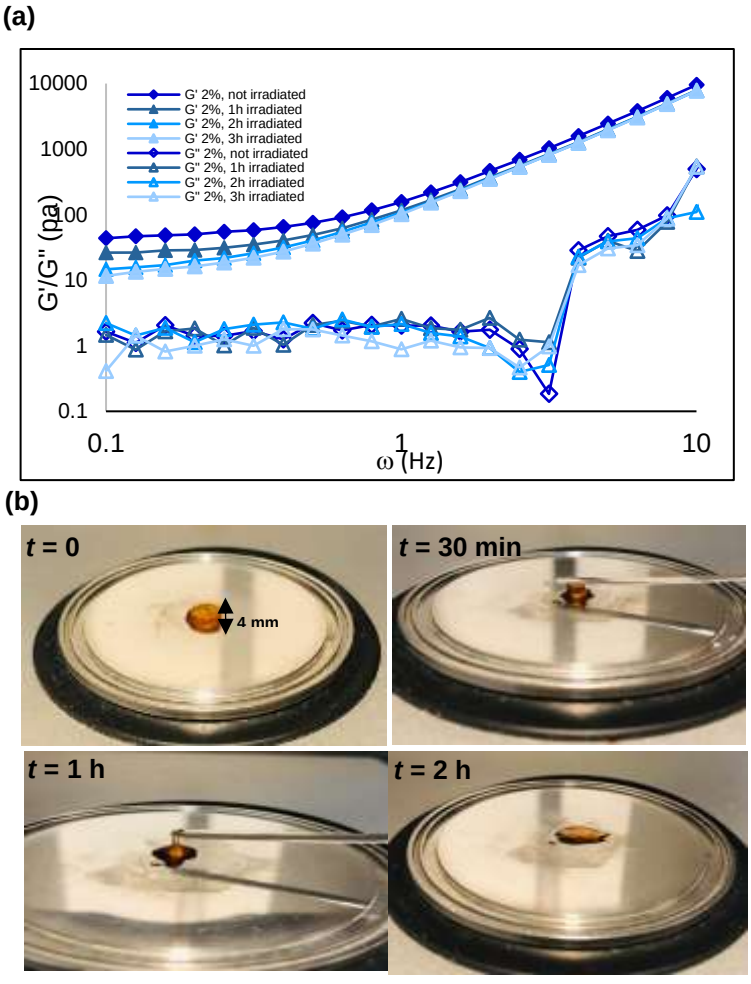
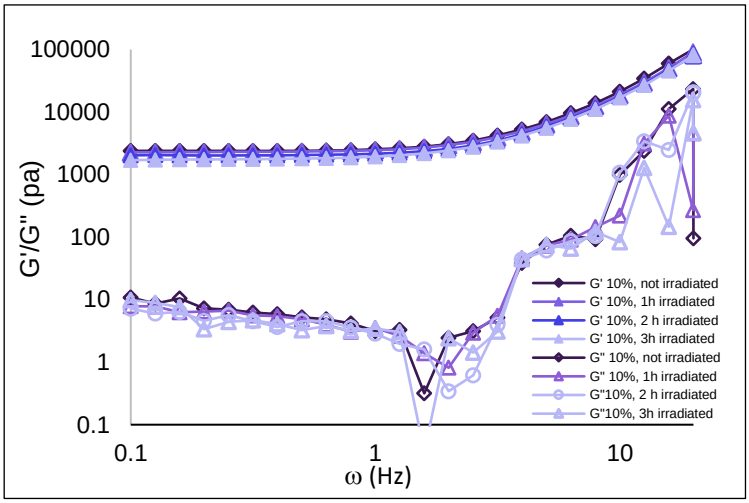


Figure S20. a) Light-induced de-gelation of the metallo hydrogel measured by rheology (light starts after 1-3 hours at 529 nm, intensity 28 mW cm^{-2}; with gel concentration (2% (w/v)), the ratio of 1.5 to 1 of [Ru] complex/PEG 4 arm thiol. Storage (G') and loss (G") module as a function of radial frequency (ω); Shear strain 0.1-20.000%, frequency: 0.1- 20 Hz, sample per decade: 10 at 25 °C, in this experiment; b) Photograph taken after irradiation of 2% gel with ratio of 1.5 of ruthenium complex within 2 hours under exposure of green light.



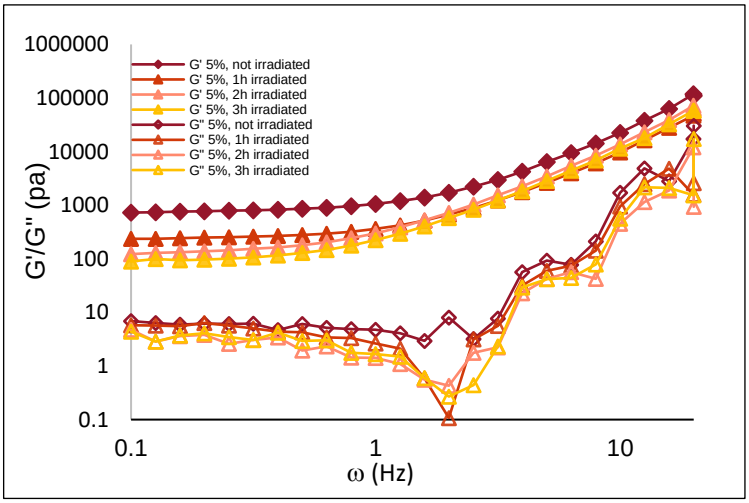


Figure S21. Light-induced de-gelation of the metallo hydrogel measured by rheology at 529 nm, intensity 28 mW cm^{-2}; with gel concentration (5% (w/v)) and 1.5 mmol ratio of [Ru] complex/PEG 4 arm thiol. Storage (G') and loss (G") module as a function of radial frequency (ω); Shear strain 0.1-20.000%, frequency: 0.1- 20 Hz, sample per decade: 10 at 25 °C, in this experiment.

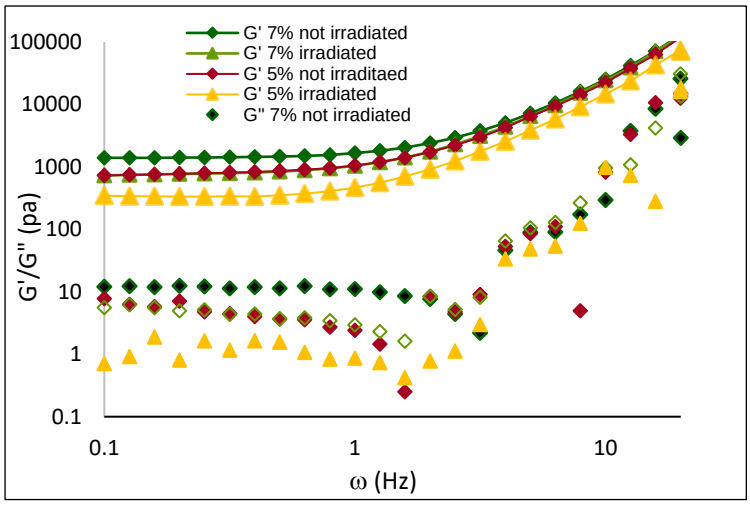


Figure S22. Light-induced de-gelation of the metallo hydrogel measured by rheology (light starts after 1-3 hours at 529 nm, intensity 28 mW cm^{-2}; with gel concentration (10%) and the 1.5 mmol ratio of [Ru] complex/PEG 4 arm thiol. Storage (G') and loss (G") module as a function of radial frequency (ω); Shear strain 0.1-20.000%, frequency: 0.1- 20 Hz, sample per decade: 10 at 25 °C, in this experiment.

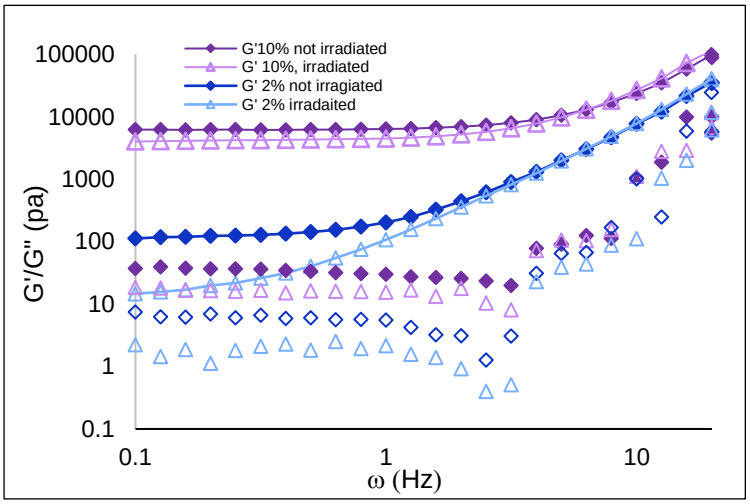


Figure S23. Light-induced degradation of the metallo-hydrogel measured by rheology before and after light irradiation at 529 nm within 3 hours, 5 and 7% (w/v) (up) and 2 and 10% (w/v) (down), and gel concentration with 2 mmol ratio of [Ru] complex/PEG 4 arm thiol.

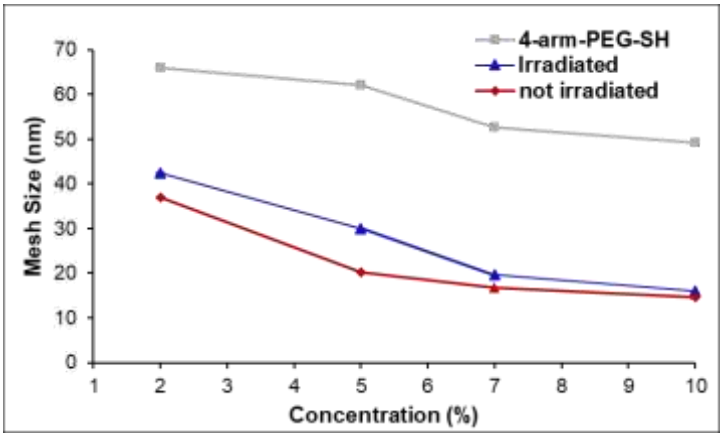


Figure S24. Mesh size, which is calculated by the storage modulus at 1 Hz from frequency sweep test at 25 °C, of different concentration of hydrogels before and after degradation for 2, 5, 7 and 10% (w/v) hydrogels. Where r is mesh size (nm), R is the universal gas constant (8.314 $J \cdot K^{-1} \cdot mol^{-1}$), T is temperature (K), π is Archimede's constant (3.141…), N_{AV} is Avogadro's number ($6.022 \cdot 10^{23}$ mol^{-1}), and G is the storage shear modulus (Pa).

4. Biological experiments

4.1 Cytotoxicity assay for Ru(II) complex

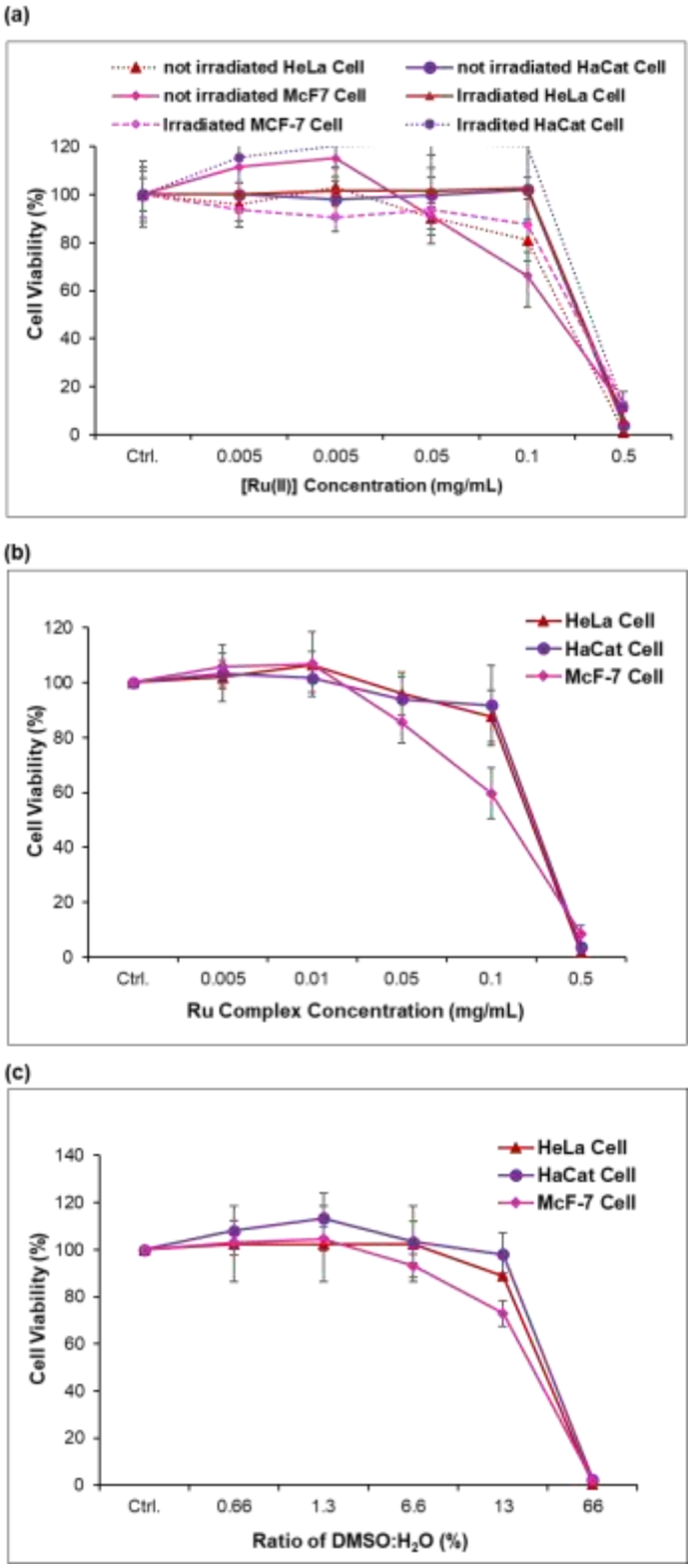


Figure S25. (a) Cytotoxicity assay of $[Ru(bpy)_2(4AAMP)_2]^{2+}$ for Hela, MCF-7 and HaCat cell line after 24 h incubator then, irradiated under green light (529 nm, 28 $mWcm^{-2}$) for 1 h and incubator for the next 24 h; (b) MTT cell viability assay of photoproducts after photo dissociation of $[Ru(bpy)_2(4AAMP)_2]^{2+}$ in water under green light (529 nm, 28 $mW \cdot cm^{-2}$) for 1 h was performed for HeLa, McF-7 and HaCat cell lines after 48 h incubation time; (c) MTT cell viability assay a solution of DMSO : H_2O for HeLa, McF-7 and HaCat cell lines after 48 h incubation time.

4.2. *In vitro* degradation assay

4.2.1. Cell lines were cultured in standard protocol to explore cells' growing capability and viability with metallo hydrogel. For this investigation, the final concentration of 2% (w/v) of Metallo hydrogel (1.5 and 2 mmol ratio of Ru(II) complex) were prepared in the 96-cell well plate under a sterilized situation overnight in an incubator (37 °C, maximum 80 rpm). In this experiment Ru complex 1mg/ml in PBS was dissolved to avoid using dmso. After washing hydrogel three times with PBS buffer to remove residual gel components. Afterwards, 200 µl DMEM medium were added to the all hydrogel samples. The next day upon irradiation with LED green light for approximately 1 hour, degradation of hydrogel was occurred. We separately seeded also HeLa cells simultaneous on the another 96 well plate incubated them for 24 h. Then we discarded the medium on top of the cells and add 100 µl DMEM medium of each gel and incubated it over night at 37 °C. the next day washed the cells with PBS, add new Medium and CCK8. After 3h incubation absorbance was measured in order to evaluate the toxicity of hydrogel supernatant before and after irradiation (Figure S26).

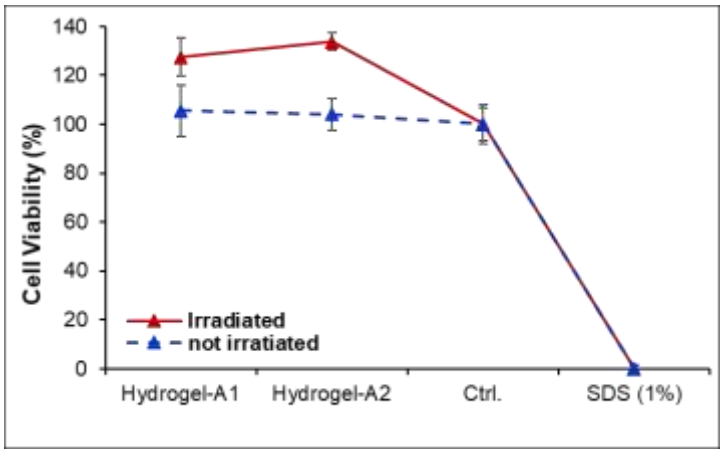


Figure S26. MTT assay of $[Ru(bpy)_2(4AAMP)_2]^2$ metallo hydrogel A1 and A2 (1.5 and 2 mmol ratio of Ru(II) complex, respectively) were prepared in the 96-cell well plate under a sterilized situation overnight in an incubator (37 °C). After irradiation of hydroge (529 nm, 28 $mW \cdot cm^{-2}$) for 1 h then the cytotoxicity of possible photoproduct has been addressed for HeLa cell line.

4.2.2. For this investigation, 10 µl of the Metallo hydrogel with final concentration of 2% (w/v) with 1.5 and 2 mmol ratio of [Ru]:4armPEG-SH were prepared in µ-slide 15 well 3D- ibidi for 3D cell culture under a sterilized condition overnight in an incubator (37 °C, maximum 80 rpm). In this experiment the concentration of Ru complex is 1mg/ml in PBS to avoid using dmso. Afterwards, 48 µl of GFP-Hella cells were added, then cells were incubated for 24 hours. After irradiating step for 1 hour, the GFP-Hella cells was stained with DAPI (1µg/ml, 2 µL) to observed dead cell line, while the waiting time is 10 minutes for DAPI, we were taken image for live cell by a confocal microscopy for irradiate and non irradiated samples. Additionally, dmso (10%, 5 µL) was added to form dead control (Figure S26, b).

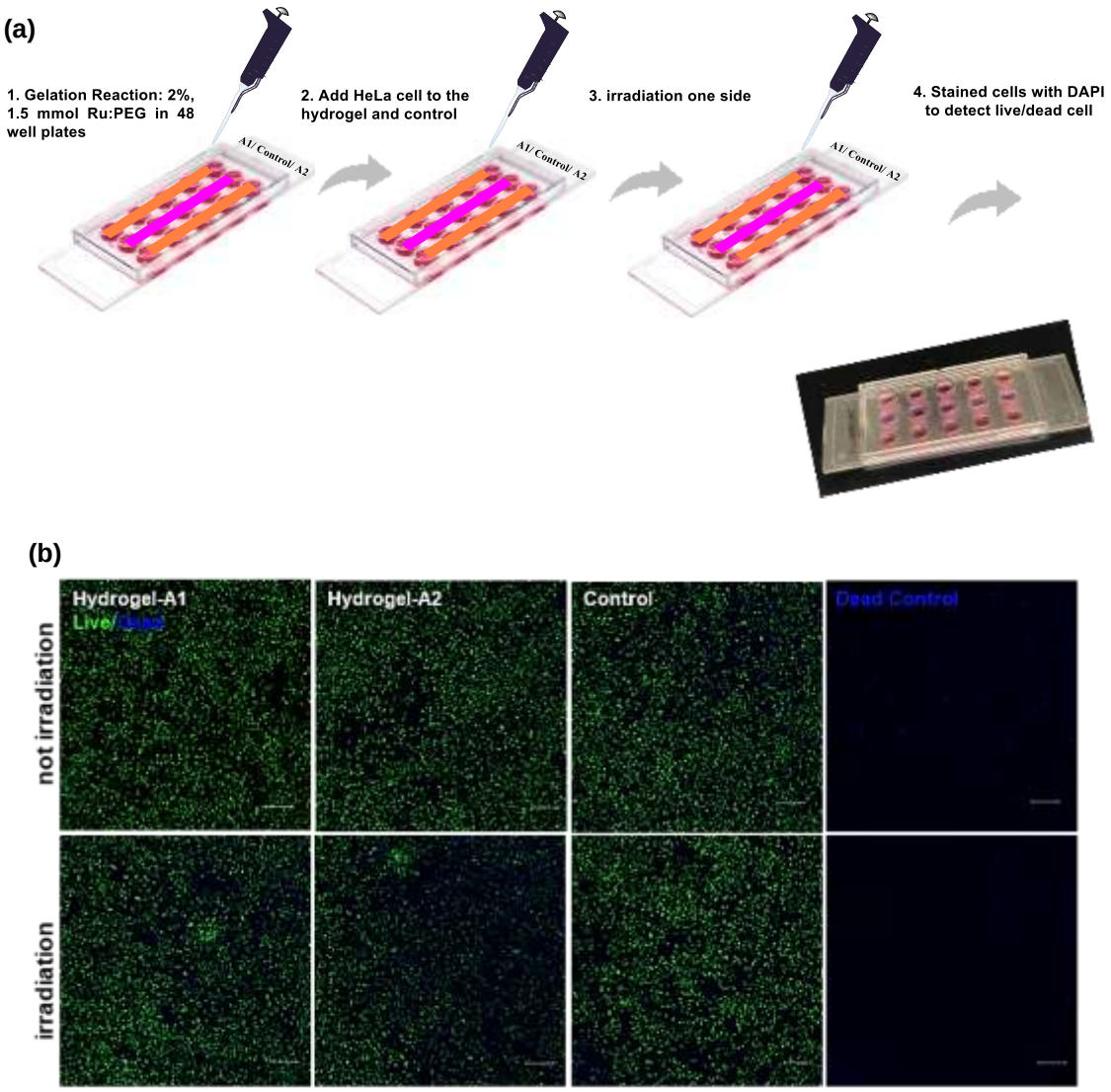


Figure S27. (a) Organization of the Standard irradiation *in vitro* degradation Assay under light irradiation by LED green light in µ-slide 15 well 3D- ibidi for (529 nm, intensity 28 mW cm^{-2}); (b) Confocal microscopy images of live HeLa cultured on metalo hydrogel 2% concentration (1.5 and 2 mmol ratio of [Ru]:4armPEG-SH in medium buffer) followed by counter staining with DAPI (0.5 µM for 10 min, stain nucleus). DAPI λem = 461 nm, λex = 353 nm, GFP λem= 509, λex = 395 nm, nm, Object: 5x, Inset scale bars: 250µm, irradiation time is 1h after 24 h incubation under light irradiation with 529 nm.

5. Reference

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2. S. M. Zakeeruddin, Md. K. Nazeeruddin,R. Humphry-Baker, M. Grätzel, *Inorg. Chem.* **1998,** *37,* 5251-5259.
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