

Supporting Information: A Quenched Binuclear Ruthenium (II) Dimer Photosensitizer Activated by Another Photosensitizer

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Materials and Methods

All reagents, including 5-amino-1,10-phenanthroline (5phen, Sigma # 631507), cis-dichlorobis(2,2'-bipyridine)ruthenium(II) (Sigma # 733202) and ammonium hexafluorophosphate (NH_4PF_6 , Sigma # 216593) were purchased from Sigma-Aldrich. ^1H NMR spectra were acquired on Varian Inova-500 and mass spectroscopy analysis was done on ThermoFinnigan MAT95XL spectrometer. HPLC analysis was done on a Waters 2790 HPLC.

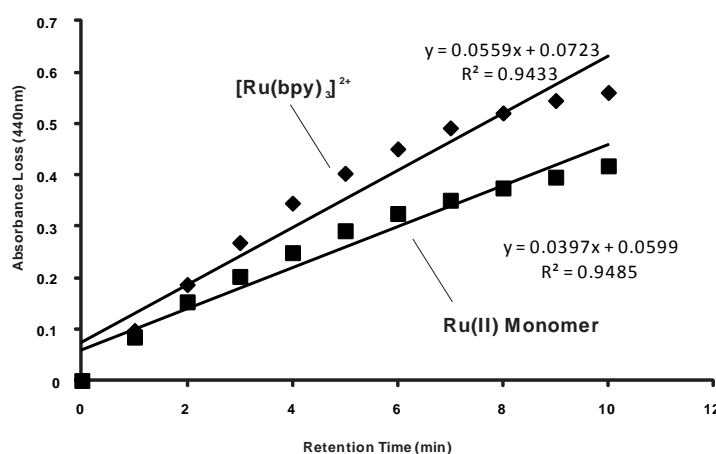
Optical Detection Protocols

Absorbance and fluorescence spectra was recorded on a StellarNet fiber Black-Comet spectrometer and a TECAN Safire 96-well microplate reader. Light irradiation treatment was carried out with 658 nm and 450 nm laser diodes in a 96-well microplate. D_2O was used as solvent unless otherwise mentioned and samples were irradiated as indicated. The blue laser used was a 450 nm focusable laser diode and the red laser used was a 658 nm diode laser system (LaserGlow). Laser power was monitored by a LaserBee power meter and 300 mW output was used unless otherwise mentioned. 15 minutes of irradiation in a single well of a 96-well plate yields $\sim 800 \text{ J/cm}^2$ fluence for either laser. For all irradiation experiments, 200 μL of as-prepared samples were added to a 96-well plate to be irradiated. Following light treatment, desired parameters (fluorescence/absorbance) were recorded in a TECAN Safire 96-well microplate reader. BiRD and Ru(II) monomer fluorescence was measured with 450 nm excitation and 602

nm emission. RNO absorbance was monitored at 440 nm. For the two step amplification shown in Figure 3, Route IIB, the RNO indicator was directly added to the activated BiRD from Route IIA to dilute the BiRD concentration to 25 μM from 100 μM without affecting the total number of mols in the well.

Singlet Oxygen Quantum Yield of Ru(II) Monomer (in D₂O)

The singlet oxygen quantum yield (Φ_{Δ}) of Ru (II) Monomer was determined by an experimental comparison method (Ogunsipe et al., J. Photochem. Photobiol. A: Chem., 173, 211, 2005) based on monitoring loss of RNO. $[\text{Ru}(\text{bpy})_3]^{2+}$, ($\Phi_{\Delta}=0.22$, DeRosa et al., Coord. Chem. Rev. 233–234 351–371, 2002) was selected as the standard reference. Assessment of RNO (100 μM) bleached by two photosensitizers (30 μM) aided by histidine (25 mM) was carried out by using the same method above. And a 0.16 singlet oxygen quantum yield was got for Ru (II) monomer.



Bleaching kinetics of RNO by $[\text{Ru}(\text{bpy})_3](\text{PF}_6)_2$ and Ru (II) monomer. Concentration used: RNO:100 μM , Photosensitizers:30 μM , histidine:25 mM. Irradiation protocol was same as the method mentioned above.

The following equation was employed to calculate the singlet oxygen quantum yield of Ru (II) monomer (Tuncel et al., Dalton Trans. 40(16):4067-79, 2011):

$$\Phi_{\Delta} = \frac{\Phi_{\Delta}^{\text{Std}} \cdot R \cdot I_{\text{abs}}^{\text{Std}}}{R^{\text{Std}} \cdot I_{\text{abs}}}$$

Where $\Phi_{\Delta}^{\text{Std}}$ refers to the singlet oxygen quantum yield of standard $[\text{Ru}(\text{bpy})_3]^{2+}$; R and R^{Std} denote RNO bleaching rate by Ru (II) and $[\text{Ru}(\text{bpy})_3]^{2+}$, respectively. $I_{\text{abs}}^{\text{Std}}$ and I_{abs} are absorbance of two compounds which were adjusted to be the same.

Synthesis of the Monomeric Ruthenium (II) Complex

Synthesis of the Ru (II) monomer was based on a previous method (Ellis et al., Inorg. Chem. 22-1283, 1983). A 10% excess of 5phen (0.205g, 1.08mmol) was dissolved in methanol. To this solution, 0.5g (0.96 mmol) cis-dichlorobis (2,2'-bipyridine)ruthenium(II) was added. The mixture was heated at reflux overnight for 12 h under argon. Following the reaction, insoluble materials were removed by filtration. A 5-fold molar excess of NH_4PF_6 dissolved in water was then added to the resulting orange solution. After incubation for 10 minutes, the red/orange precipitate was collected from suction filtration and washed with diethyl ether. Finally, the product was dried under vacuum. Yield: 80%. ^1H NMR (500 MHz, $\text{dms}\text{-d}_6$) δ 8.93 – 8.8 (m, 4H), 8.36–8.29 (d, 2H), 8.22-8.08 (m, 4H), 7.84 (dd, 2H), 7.68 – 7.52 (m, 8H), 7.4 (dd, 2H), 7.08 (s, 2H), 6.94 (s, 1H). MS: Observed: M^{2+} : 304.944, M^+ : 754.1. Expected: M^{2+} : 304.574, M^+ : 753.62.

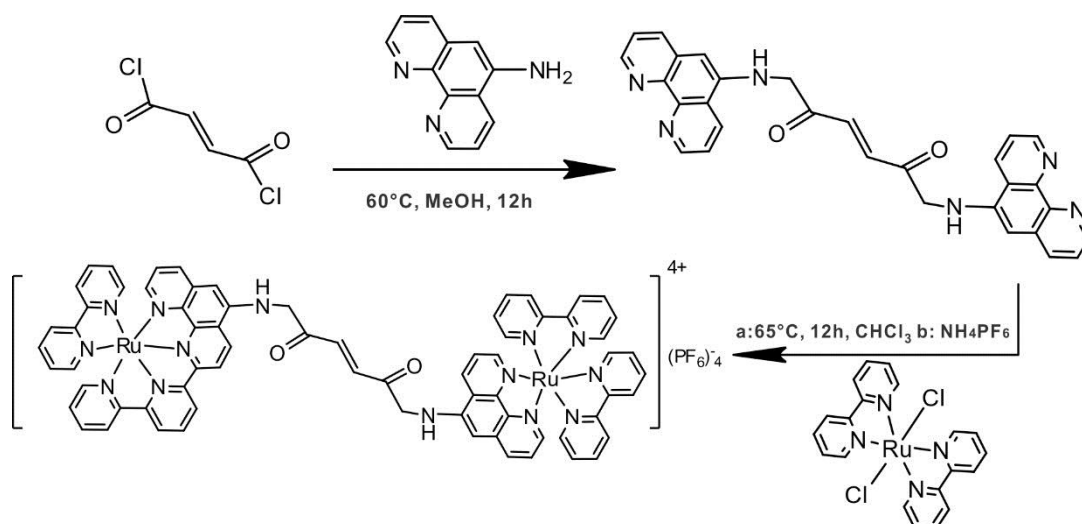
Synthesis of the 1, 6-fumaryl-amido-bis(1,10-phenanthroline-5-yl) ligand

100 mg (0.512 mmol) 5phen was dissolved in chloroform. 20% molar excess of fumaryl chloride (33.2 μL , 0.307 mmol) was dissolved in a small amount of chloroform and dropped into 5phen in chloroform under argon. The solution turned from yellow to orange and was allowed to reflux for 24 hours. A yellow/orange precipitate was collected following the reaction by suction filtration. The residue was rinsed with enough chloroform to wash off unreacted 5phen. The product was then

dried under vacuum and dropped into 20 mL chloroform and sonicated for 10 minutes. Suction filtration and chloroform washing were performed, yielding a light orange powder. The chloroform washing step was repeated for 2~3 times until there was no trace of free 5phen on thin layer chromatography ($R_f=0.2\text{--}0.3$, 10% methanol in dichloromethane). The product was then dried thoroughly by rotary evaporation; yield: 49%. $^1\text{H NMR}$ (500 MHz, $\text{dms}\text{-d}_6$) δ 10.79 (s, 2H), 9.17 (s, 2H), 9.07(s, 2H), 8.73 (d, 2H), 8.51 (d, 2H), 8.39 (s, 2H), 7.87 (m, 2H), 7.79 (m, 2H), 7.61 (s, 2H) MS: Observed: 471.327. Expected: 470.48.

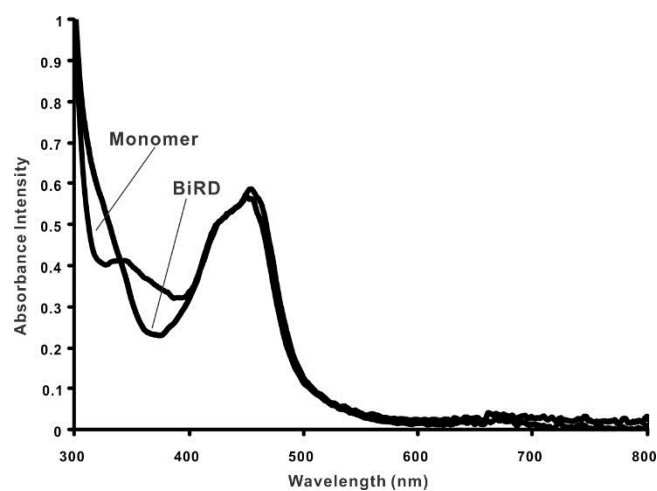
Synthesis of the Binuclear Ruthenium (II) Dimer

The overall synthetic scheme is shown in Scheme 1. Synthesis of the binuclear Ruthenium compound was similar to monomer Ruthenium compound as described above: 50mg (0.106mmol) of synthesized 1, 6-fumaryl-amido-bis(1,10-phenanthroline-5-yl) ligand was dissolved in methanol. 100mg (0.192mmol) cis-dichlorobis (2,2'-bipyridine)ruthenium(II) was added to the solution. The mixture was refluxed overnight for 12 h under argon. Unreacted starting material was filtered out. A 5-fold molar excess of NH_4PF_6 dissolved in water was then added to the resulting orange solution. red/orange precipitate was collected from suction filtration after incubation for 10 minutes, and washed with diethyl ether. Product was dried under vacuum ; yield: 60%. $^1\text{H NMR}$ (500 MHz, $\text{dms}\text{-d}_6$) δ 11.02 (s, 2H), 9.06 (d, 4H), 8.97 – 8.85 (m, 8H), 8.22 – 8.18 (m, 8H), 7.97 (d, 4H), 7.91 – 7.8 (m, 8H), 7.7 (d, 4H), 7.57 (m, 8H), 7.38 (m, 2H), 7.25(s, 2H). MS: Observed: M^{4+} : 324.374, M^{3+} : 480.753 Expected: M^{4+} : 324.558, M^{3+} : 481.067.



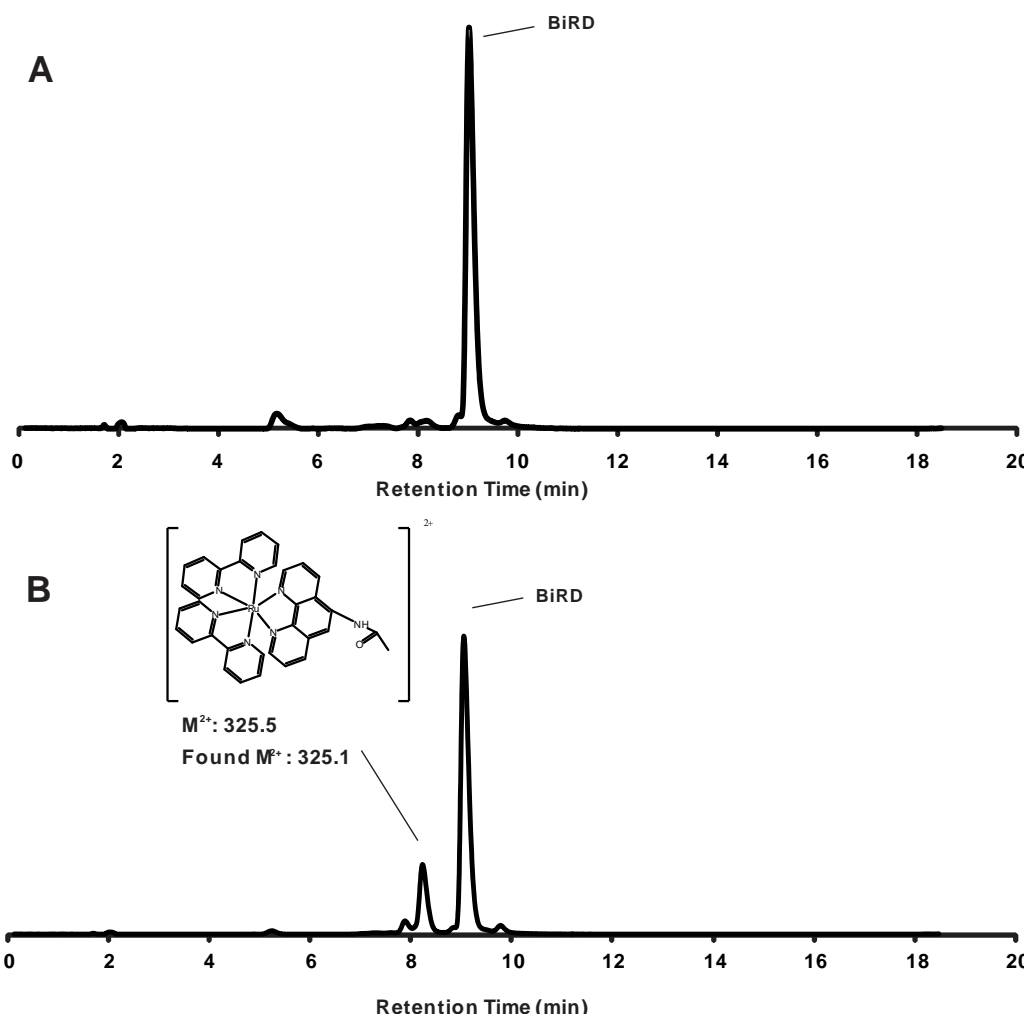
Scheme 1: BiRD synthesis

Supporting Figures

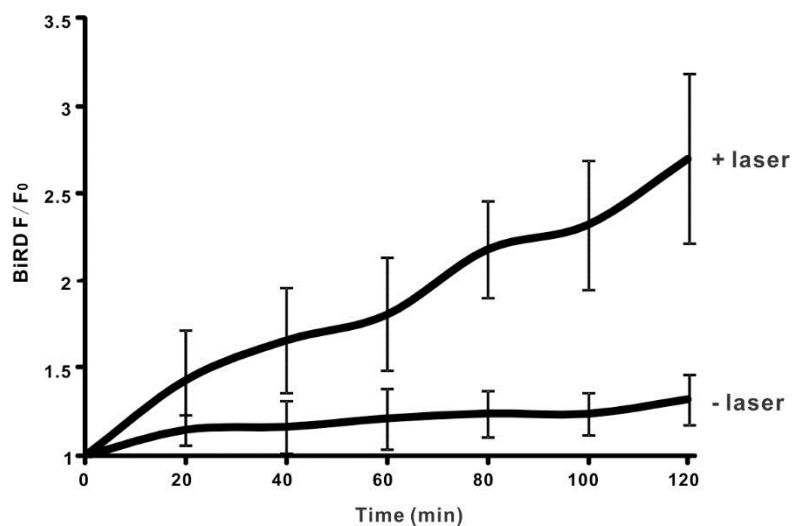


Supporting Figure 1: Absorption spectra of monomeric Ru(II) complex and the BiRD.

Absorption spectra recorded in methanol. Ru (II) absorption peak at 450nm is attributed to Ru(II) ($d\pi$) - bpy (*) charge transfer (MLCT) while monomer exhibits a shoulder at 350nm. This might be the neighbor Ru (II) shifts the reduction potential of the bridging ligand to a less negative value which leads to the elimination of Ru(II)-bridging phen charge transfer (Ohno T, Nozaki K, Haga M., *Inorg Chem.*31(21):4256–61,1992).



Supporting Figure 2: Observation of photoproduct before and after 15 min BiRD activation with methylene blue and red laser. HPLC trace monitored at 450 nm. a) Before irradiation. b) After irradiation. A new peak appeared at earlier retention time with mass detected. A potential photocleavage structure is shown at the inset based on the observed mass, however more work is required to elucidate the exact nature of the cleavage and photocleavage products. The HPLC gradient used was 0 to 30 % acetonitrile in 0.1% TFA.



Supporting Figure 3: Activation of BiRD by subnanomolar levels off methylene blue. Laser

power density: 200 mW/cm^2 ; BiRD concentration: $1 \mu\text{M}$; histidine concentration: 0.2 M ; Excitation:

450 nm , Emission: 600 nm