Rapid Generation of HNO Induced by Visible Light.

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Supporting Information:

All solvents were analytical grade or better. Commercially available reagents were used as received. cis-$[Ru(bpy)_2(PMe_3)(Glut)][PF_6]_2$[24] (Glut = Glutamate) and N-hydroxy-4-nitro benzene sulfonamide[25] (4-nitro Piloty´s acid) were prepared according to literature procedures. NMR spectra were obtained using a 500 MHz Bruker AM-500.

$[Ru(bpy)_2(PMe_3)(Glut)][PF_6]_2$: 50 mg of $[Ru(bpy)_2(PMe_3)Cl)]PF_6$[24] was dissolved in 2 ml of acetone. A suspension of 2 mL of water with 200 mg of a chloride-containing anionic exchange resin (DOWEX) was added, and stirred for 10 min. The resin was filtered to remove the resin, obtaining a $[Ru(bpy)_2(PMe_3)Cl]Cl$ water solution. Then a solution containing 250 mg of monosodium glutamate and 1.2 mL of 1 M NaOH was added, and the resulting mixture was heated for 3 h. Saturated KPF_6 (0.5 ml) was added, and the resulting precipitate was discarded. The solution was then cooled to 0°C and acidified with the addition of 5 M HCl until pH 2. The final compound, $[Ru(bpy)_2(PMe_3)(GluH_2)](PF_6)_2$, precipitated upon addition of excess of KPF_6. The solid was then washed three times with cold water and then dried. Yield: 43%. ^{1}H NMR (500 MHz, D_2O, d = doublet, m = multiplet, t = triplet): δ = 1.05 (d, 18H); 1.54 (m, 2H); 1.68 (m, 2H); 1.95 (m, 2H); 2.12 (m, 1H); 2.28 (m, 1H); 2.69 (m, 1H); 3.47 (t, 1H); 4.05 (d, 1H); 4.43 (d, 1H); 7.02 (t, 2H); 7.18 (t, 2H); 7.38 (d, 3H); 7.43 (d, 1H); 7.66 (m, 2H); 7.72 (t, 2H); 7.76 (m, 2H); 7.85 (t, 2H); 8.08 (t, 1H); 8.11 (d, 1H); 8.16 (m, 3H); 8.26 (d, 1H); 8.29 (d, 1H); 8.38 (d, 2H); 8.41 (t, 2H); 8.94 (d, 1H); 8.97 (d, 1H); 9.03 (d, 1H); 9.14 (d, 1H).

4-nitro Piloty´s acid: 360 mg of $NH_2OH{\cdot}HCl$ and 170 mg of MgO were added in a mixture of 1.5 ml of methanol and 1.25 of water (A). 470 mg of R-SO_2Cl (R = p-nitrophenyl) and 80 mg of MgO were added to 15 ml of THF (B). A and B were mixed and left reacting for 1 hour at room temperature. The reaction mixture was filtered, and MgO (solid) was discarded. The solvent was evaporated under reduced pressure and the obtained solid was dried under vacuum. ^{1}H NMR (500 MHz, d_6-DMSO, d = doublet, m = multiplet, t = triplet): d = 8.13 (d, 2H); 8.49 (d, 2H); 9.90 (d, 1H); 9.99 (d, 1H).

The UV-Vis spectra were acquired with an Ocean Optics CHEM2000 diode-array spectrometer. All samples were irradiated with a 405 nm laser diode.

Experimental Setup Description:

The experimental setup used for the photochemical reaction measurements is shown in Figure S1.a. The cell holder was made with an aluminum plate (coated with a black paint to avoid scattering) of 8 cm x 2.5 cm and 1 cm high that had two metallic sheet leaf that hold the cell. The spectrophotometer lamp was connected to the cell holder through a fiber-optic that fits in a hole made in the cell. The fiber-optic connected to the spectrophotometer detector was placed 3 cm far from the cell in order to allow the manipulation and irradiation of the cell. The light source used was a 405 nm diode laser. The light beam was expanded by a lens in order to irradiate the whole cell in a reproducible way.

The cell was made with two cover glasses (previously cleaned with sodium laurilsulphate solution, distilled water and ethanol) and a piece of Parafilm® was used as spacer, which was fixed between the two cover glasses heating at around 50 °C for three seconds. Each cell constructed in this way was used for only one experiment. Figure S1.b shows a scheme of the cell. The sample (10 μL) was introduced in the cell using a pipette with a thin tip.

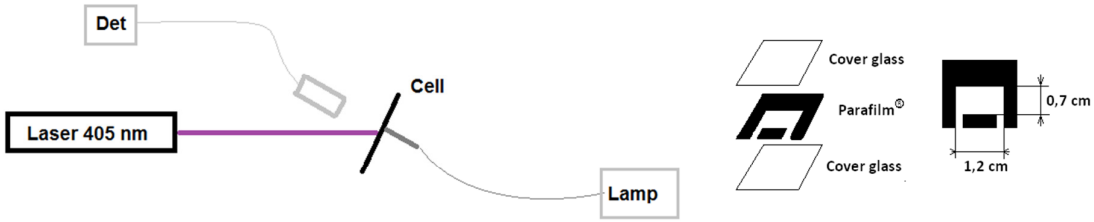

Figure S1. a) Scheme of the setup used to acquire the UV-Vis spectra in the photochemical reaction. b) Scheme of the cell used in the experiment.

Determination of pathlenght:

The UV-Vis spectra of a solution of Ru-Glut ($[Ru(bpy)_2(PMe_3)(Glut)]^{2+}$) in water was measured in the cell and compared with a 1:100 dilution of this solution measured in a 1 cm pathlenght cuvette (Abs = 0.284), in order to determine the pathlenght of the cell. The procedure was repeated for 3 different cells, the absorbance measured was 0.322, 0.345 and 0.333 respectively. The results show that the procedure of fabrication has a high reproducibility. The calculated pathlenght of the cells was 117.1 +/- 4.1 µm.

Light power measurement:

As some part of the light used to irradiate scatters on the cell, not all the laser light reaches the sample. In order to calculate the laser power that irradiates the sample an actinometric reaction (Ru-Glut photolysis, Figure 1.b) was done.

In order to calculate the light power that reaches the sample, 10 µl of a 5 mM aqueous solution of Ru-Glut was introduced in the cell and then irradiated (λ = 405 nm) until there were no changes on the UV-Vis spectra (complete photolysis). Under these conditions, after 12 sec of irradiation photolysis was completed. The calculated light power was (51.2 +/- 0.3) mW, and as the irradiated area in the sample was 0.85 cm^2 the density power was 60.2 mW/cm^2. For higher concentrations of Ru-Glut (15 mM), treated by the same procedure, after 20 sec of irradiation complete photolysis was done, which was expected according to the light power calculated before.

Calculation of $[Ru(bpy)_2(PMe)_3(H_2O)]^{2+}$ pKa and pH reached after pH jump:

Figure S2 shows the UV-Vis spectra of Ru-H_2O at pH = 5 (λ_{max} = 453 nm), at pH = 12 (λ_{max} = 466 nm) and a simulated spectra where [Ru-H_2O]/[Ru-OH] = 1 (where pH = pKa). The simulated spectra were compared with different buffer solutions of different pH values. The one measured at pH = 10.7 fitted with the simulated for pH = pKa. Then, pKa of Ru-H_2O = 10.7.

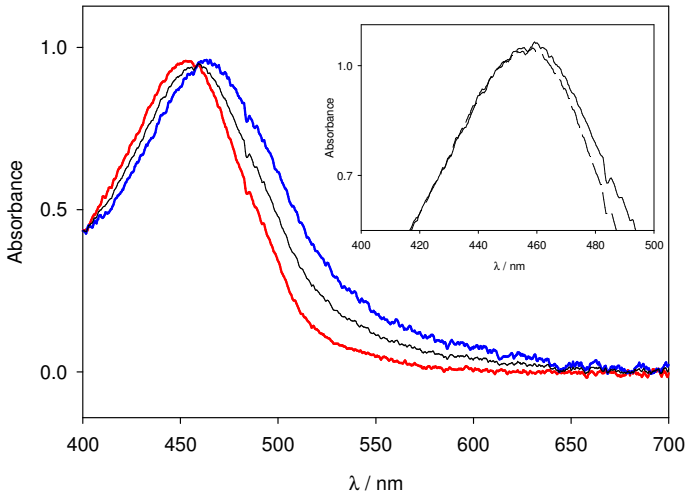

Figure S2. Normalized Absorption UV-Vis spectra of $[Ru(bpy)_2(PMe_3)(H_2O)]^{2+}$ (pH = 5, red; pH = 12 blue) in aqueous solution and simulated for pH = pKa (black). Inset: Amplified region of spectra of $[Ru(bpy)_2(PMe_3)(H_2O)]^{2+}$ in aqueous solution, simulated (dashed) and in buffer (line), at pH = 10.7.

In order to calculate the pH value reached after complete photolysis of Ru-Glut, spectra which fitted with the experimental final spectra after photolysis was simulated. Comparison of this spectrum with a $[Ru(bpy)_2(PMe)_3(H_2O)]^{2+}$ spectrum measured in buffer solution at the corresponding pH value shown concordance with the simulated results. This experiment was done for different concentrations (3 and 10 mM) of Ru-Glut in aqueous solution and (3 and 15 mM) of Ru-Glut in buffer (pH = 7.4; 2.5 mM), results are shown in Table S1. Figure S3 shows the experimental and the simulated for the different measurements.

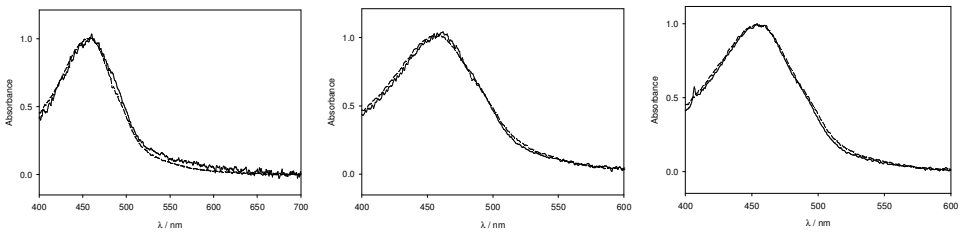

Figure S3. Normalized experimental (dotted) and simulated (line) UV-Vis spectra after complete photolysis of actuator. a) 10 mM, aqueous solution, final pH = 10.7. b) 3 mM, aqueous solution, final pH = 10.4. c) 3 mM, buffer pH = 7.4, final pH = 9.7.

Table S1. Absorption maximum of product after complete photolysis of the actuator.

pH	λ_{max}
5.0	453
9.7	455
10.4	458
10.7	460
12.0	466

Comparison of speed of HNO dimerization vs speed of HNO trapped by the probe:

HNO is generated by PA (Eq. S1), and then reacts with Mn-TSPP[23] (Eq. S2) or with itself in the dimerization reaction[5] (Eq. S3). Results are shown in Table 1 in main text.

Eq. S1 PA -> HNO

Eq. S2 HNO + MnTSPP -> MnTSPP-NO

Eq. S3 2 HNO -> N_2O + H_2O

Then,

$V_{PA} = k_{PA}*[PA]$
$V_{MnTSPP} = k_{MnTSPP} * [MnTSPP] * [HNO]$
$V_D = 2 * k_D * [HNO]^2$

Where V_{PA} = PA decomposition rate, V_{MnTSPP} = trapping rate of MnTSPP, V_D = HNO dimerization rate; k_{PA} = rate constant for Eq. S1 (see Table 1), k_{MnTSPP} = rate constant for Eq. S2[23], k_D = rate constant for Eq. S3[5].

In stationary state:

$d[HNO]/dt = 0 = k_{PA} *[PA] - k_{MnTSPP} * [MnTSPP] * [HNO] - 2 * k_D * [HNO]^2$

$k_{PA} *[PA] = k_{MnTSPP} * [Por] * [HNO] + 2 * k_D * [HNO]^2$

[HNO] is calculated for initial conditions, in each experiment, and then V_{PA}, V_{MnTSPP} and V_D are calculated.

In order to define if V_D is depreciable V_D / V_{MnTSPP} was calculated.

For reactions with rate constants equal (or lower) than 10^{-2} $M^{-1}s^{-1}$ then V_D / V_{MnTSPP} < 1%. And for reaction with higher rate constant values, V_D / V_{MnTSPP} = 5% aprox. For a qualitative determination of HNO, it is possible to assume that generated HNO is trapped by the MnTSPP and dimerization is negligible.

Experimental Conditions:

Photo-induced HNO generation was measured in duplicate for each condition. The solution used was prepared mixing stock solutions of the ruthenium complex, the PA derivative and the Mn(III) TSPP porphyrinate. Experimental conditions for each experiment are shown in Table S2. Rate constant calculation was done by standard fitting process between experimental data and simulated values. As some photobleaching was observed in MnTSPP that has reacted with HNO (λ_{max} = 425 nm), HNO determination was done by the decrease of the Soret band of MnTSPP (that has not reacted with HNO, λ_{max} = 465 nm).

Table S2. Experimental conditions for photo-induced HNO generation.

	Medium	Ru (10^{-3}M)	MnTSPP (10^{-4}M)	**PA (10^{-4}M)	*Irradiation time (s)
EXP1	Aqueous	3	5.46	1.5	26
EXP2	Aqueous	10	1.20	0.5	30
EXP3	Buffer, pH = 7.4	3	2.03	2.0	16
EXP4	Buffer, pH = 7.4	6	1.56	1.0	20

*As MnTPSS also absorbs at 405 nm, irradiation time was higher than in samples containing only Ru-Glut.

**For highest concentration of actuator in order to reach a higher pH value after irradiation, concentration of PA used for the experiment was lower (than in experiments with lowest concentration of actuator) in order to avoid HNO dimerization reaction.

Buffer solutions: pH 6 = Citrate, pH 7.4 = Phosphate, pH 8 = Tris, pH 9.7 = Borate, pH 10.4 = Carbonate, pH 10.7 = Carbonate.

<u>HNO generation in water:</u>

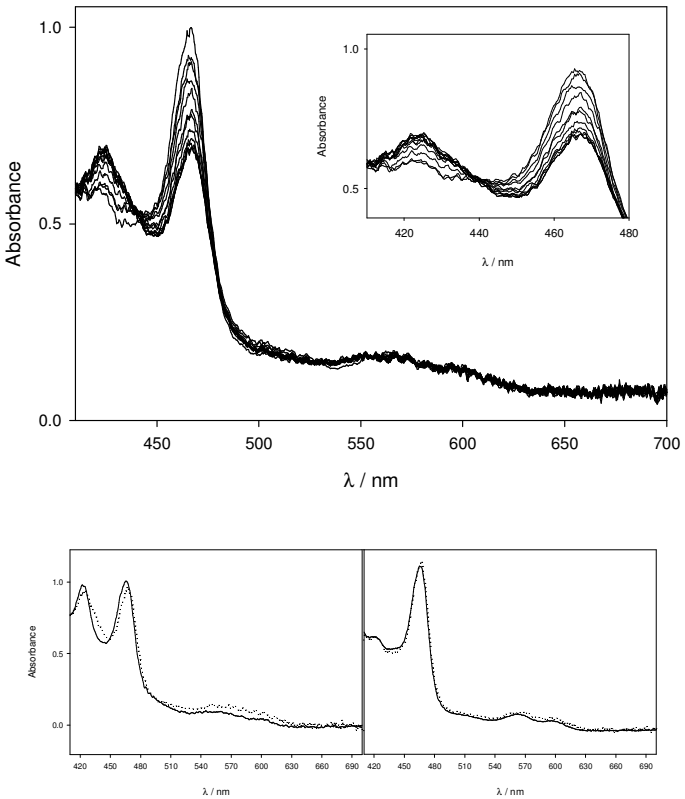

Figure S4. Top: Selected spectra during photo-induced generation of HNO, in EXP 1 conditions. Inset: Amplified region of the spectra, initial spectra was discarded, only spectra obtained after photolysis are shown (isosbestic point = 442 nm). Bottom: Initial (right) and final (left), experimental (doted) and simulated (line), normalized spectra. After photolysis is completed, the increase of the Mn porphyrin Soret band at 425 nm and the decrease of the band at 465 nm indicate that HNO is being generated.

Simulated spectra:

Concentration values of the initial species were calculated using the values of absorbance obtained by fitting, by standard procedures, the initial UV-Vis spectra measured with the simulated one (using UV-visible spectra of Ru-Glut, probe in absence of HNO).

The fraction of probe that has reacted with HNO was determined by the same fitting procedure than the once described above, using the experimental UV-Vis spectra measured after each reaction and the simulated one. In this case, UV-Vis spectra was simulated using spectra of MnTSPP, MnTSPP-HNO, Ru-H_2O, and PA byproduct.

Figure S5 shows the normalized spectra of each reactant and product. PA (λ_{max} = 261 nm) and its PA subproduct (λ_{max} = 269 nm) are not shown since their absorbance, on the studied region of the spectra, is negligible at the concentrations used.

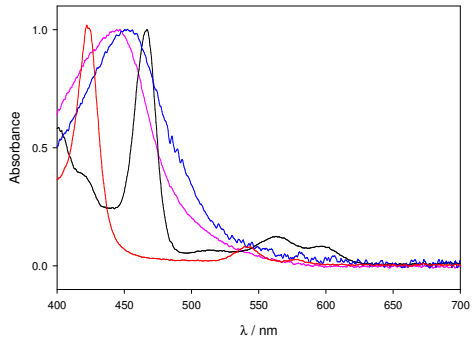

Figure S5.Normalized spectra of each reactant $[Ru(bpy)_2(PMe3)(Glut)]^{n+}$, λ_{max}=446 nm; MnTSPP, λ_{max}=465 nm and product $[Ru(bpy)_2(PMe3)(H_2O)]^{2+}$/$[Ru(bpy)_2(PMe3)(OH)]^{+}$ mixture, λ_{max} 450-460 nm, see S4; MnTSPP-NO, λ_{max}=425.

For initial spectra:

Eq S4. $Abs\,(\lambda) = a * Abs_{PA}\,(\lambda) + b * Abs_{Ru\text{-}Glut}\,(\lambda) + c * Abs_{MnTPPS}\,(\lambda) + e_1\,(\lambda)$

The parameters a, b, c, were optimized in order to minimize errors ($\Sigma\,|e_1|$) between experimental and simulated spectra.

For final spectra:

Eq S4. $Abs\,(\lambda) = a * Abs_{PAsubprod}\,(\lambda) + b * Abs_{Ru\text{-}H2O}\,(\lambda) + c_1 * Abs_{MnTPPS}\,(\lambda) + c_2 * Abs_{MnTPPS\text{-}HNO}\,(\lambda) + e_2\,(\lambda)$

In this case, parameters a, b, c_1, c_2 were optimized in order to minimize errors ($\Sigma\,|e_2|$) between experimental and simulated spectra.

Accumulated concentration of HNO was calculated by Ec S5.

Eq S5. $[HNO] = [Mn\text{-}TPPS]_o * c_2/(c_1+c_2)$.

HNO generation in buffer:

The initial (top) and final (bottom), experimental and simulated, normalized spectra of reaction under EXP 3 conditions are shown in Figure S6.

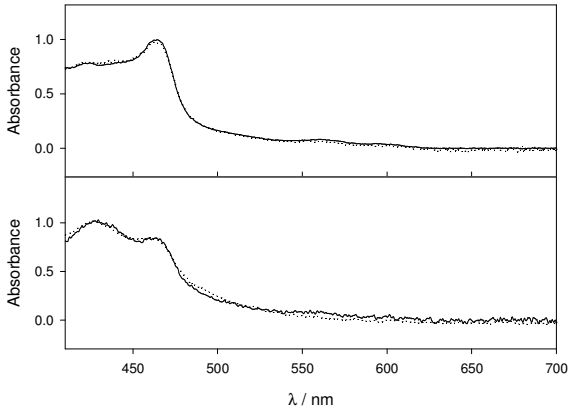

Figure S6. Initial (top) and final (bottom), experimental (dotted) and simulated (line), normalized spectra of reaction under EXP 3 conditions.

Selected spectra during photo-induced generation of HNO, in EXP 4 conditions, are shown in Figure S7.

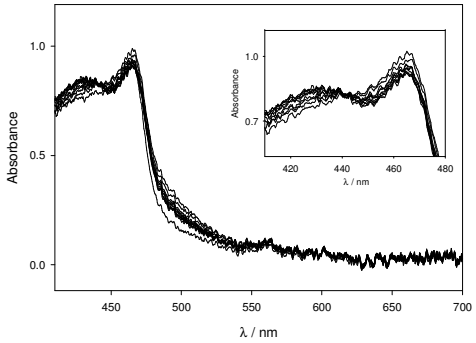

Figure S7. Selected normalized spectra during photo-induced generation of HNO, in EXP 4 conditions. Inset: Amplified region of the spectra, initial spectra was discarded, only spectra obtained after photolysis are shown (isosbestic point = 442 nm). After photolysis is completed, the increase of the Mn porphyrin Soret band at 425 nm and the decrease of the band at 465 nm indicate that HNO is being generated

The initial (top) and final (bottom), experimental and simulated, spectra of reaction under EXP 4 conditions are shown in Figure S8.

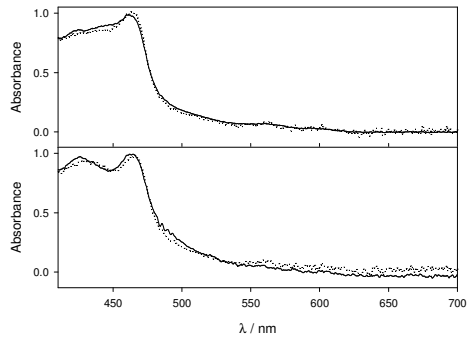

Figure S8. Initial (top) and final (bottom), experimental (dotted) and simulated (line), normalized spectra of reaction under EXP 4 conditions.

Rate constant comparison:

The rate constant of HNO generation induced by a macroscopic change of pH was calculated by the same procedure as the other experimental data. In this case, a solution of PA was added to a buffered solution of the MnTSPP contained on a 1 cm pathlenght glass cuvette. Both solutions were degassed in order to eliminate the oxygen that quenches the detection of HNO.

Table S3 shows the rate constant measured with photo-induced (k_{phot}); and macroscopic pH jump (k_{buffer}) adding PA solution to a buffer solution of MnTSPP. Values of k vs pH are represented in Figure S9.

Table S3. Rate constant measured at different final pH values.

pH	$k_{phot} * 10^2$ ($M^{-1} s^{-1}$)	$K_{buffer} * 10^2$ ($M^{-1} s^{-1}$)
6	--	~ 0*
7.4	--	~ 0*
8	--	~ 0*
9.7[b]	0.65**	0.71
10.2[b]	2.9**	--
10.4[c]	1.03*	1.52
10.7[c]	4.36*	~ 5

[a] after 20 min there was no HNO production

[b] pH calculated by pH = pKa - log ([ac]/[ba])

[c] pH calculated by UV-Vis spectra

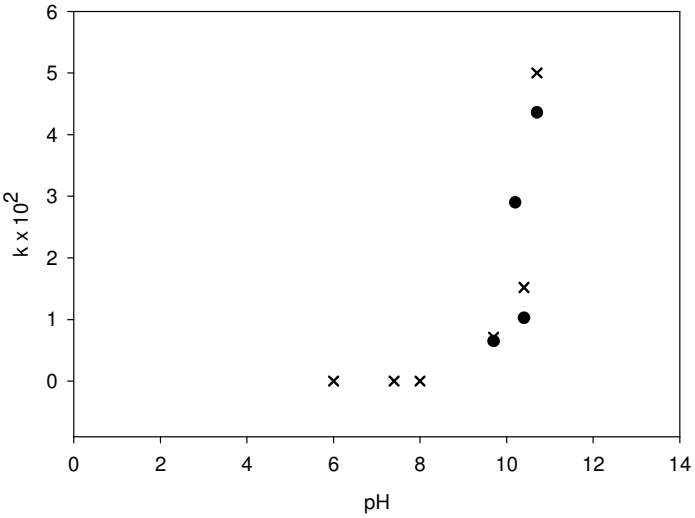

Figure S9. Rate constants measured with photo-induced (k_{phot}, dots), and macroscopic (k_{buffer}, cross), pH jump at different final pH values.