Supporting information for

Mediator-free NADH photochemical regeneration with the aid of the amino acid L-Cysteine

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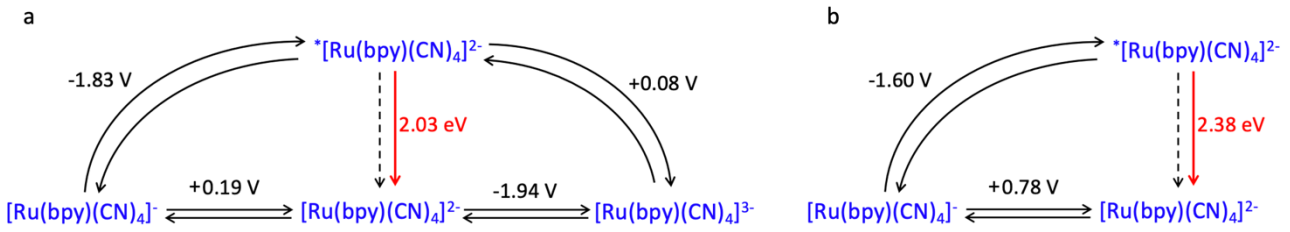
Chemicals and materials

β-Nicotinamide adenine dinucleotide hydrate (NAD^+, grade I, free acid, product code 10127965001), reduced β-Nicotinamide adenine dinucleotide hydrate (1,4-NADH, grade I, disodium salt, product code 10107735001) were purchased from Roche Diagnostic GmbH and used as received. High-purity triethanolamine (TEOA), L-Cysteine, tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl) and acetaldehyde were purchased from Merck and used with no further purification. N_2 used for purging (filtered on Drierite™, 99.999% purity) was supplied by SIAD. Type 1 ultrapure water was obtained with an Elga PURELAB® Classic UV apparatus. $[Ru(bpy)(CN)_4]^{2-}$ (as sodium salt) was available from previous work.[1]

Photophysical characterization

UV/Vis absorption spectra were recorded on a Perkin Elmer λ45 or Agilent Cary 300 double beam spectrophotometers, using quartz gas-tight cuvette with 1 cm path length; emission spectra were recorded on a Perkin Elmer LS55 spectrofluorometer equipped with a Hamamatsu R928 photomultiplier tube or Edinburgh Instruments FS5 spectrofluorometer equipped with a Hamamatsu R13456 photomultiplier tube; all the spectra were corrected for detector' sensitivity.

Emission lifetime decays were recorded on an Edinburgh Instruments FSL920 spectrofluorometer equipped with a TCC2 electronic module for time-correlated single photon counting data acquisition (305 fs resolution), a PicoQuant LDH-P-C-405 pulsed diode laser and a Hamamatsu R928 photomultiplier tube. All the decays were fitted with mono-exponential functions.



Scheme S1. Ground and excited state potentials (vs SCE) of mono(bipyridyl)-tetracyanoruthenate in DMF (panel a) and in water (panel b).[2]

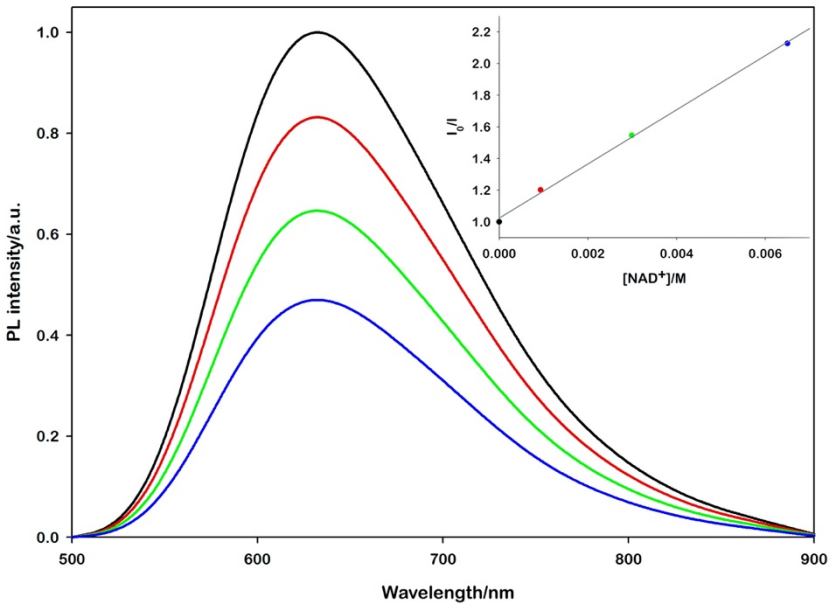


Figure S1. Emission spectra obtained upon 440 nm excitation of $[Ru(bpy)(CN)_4]^{2-}$ in air-equilibrated aqueous solution (black), $[NAD^+]$ = 0.93 mM (red), $[NAD^+]$ = 2.99 mM (green), $[NAD^+]$ = 6.5 mM (blue). Inset shows the Stern-Volmer plot with linear fitting (R^2 = 0.9985).

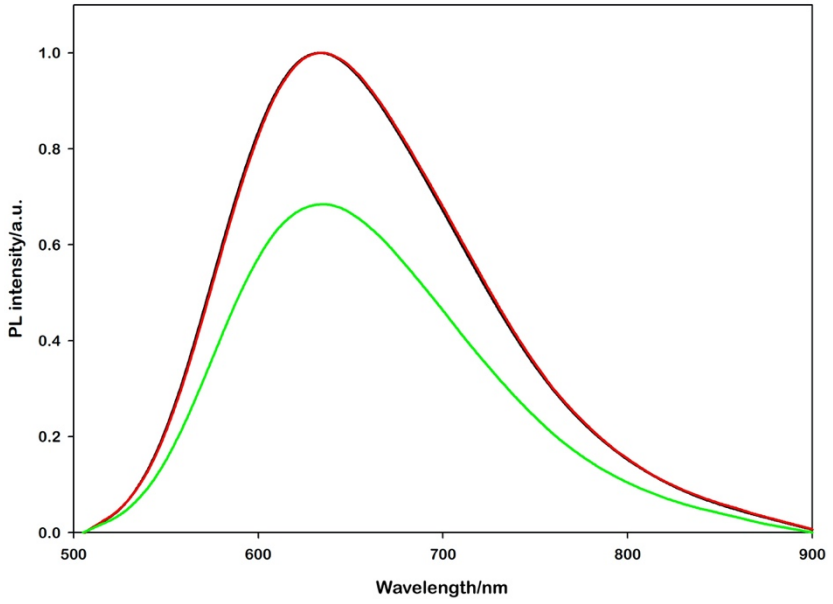


Figure S2. Emission spectra obtained upon 440 nm excitation of $[Ru(bpy)(CN)_4]^{2-}$ in water (black line), upon 0.1 M L-Cysteine addition (red line) and 2 mM NAD^+ addition (green line). At excitation wavelength there were no absorbance variations.

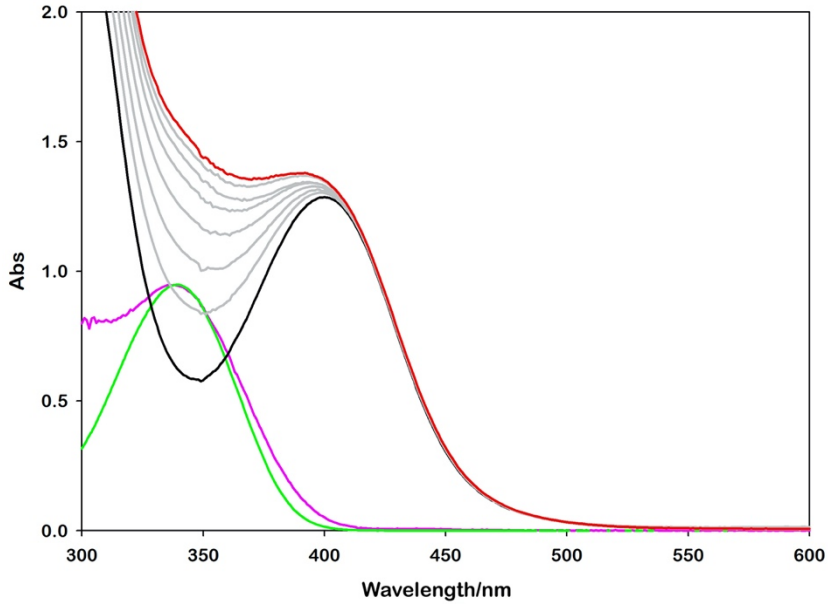


Figure S3. Absorption spectra before (black line), during (grey lines) and after irradiation (red line). Pink line represents the difference absorption spectra. For comparison commercial 1,4-NADH spectrum (green line) is also reported.

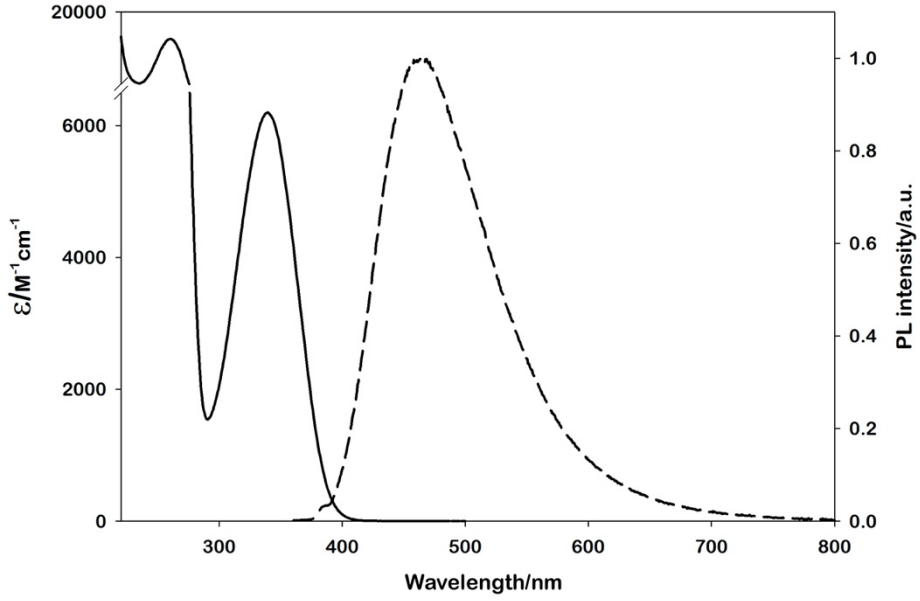


Figure S4. Absorption (solid line) and emission (dashed line) spectra of commercial 1,4-NADH in water. Emission spectrum is obtained upon excitation at 340 nm.

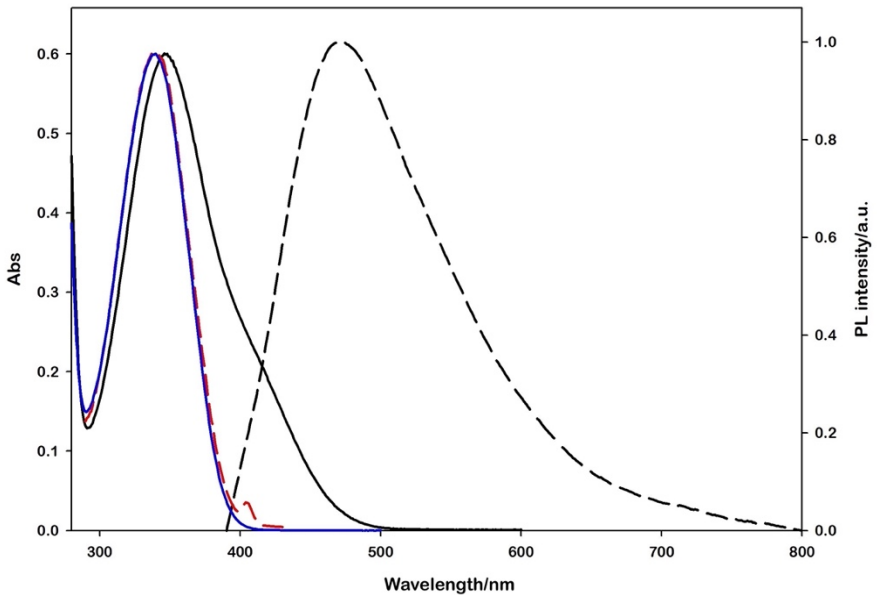


Figure S5. Absorption (solid black line) and emission (dashed black line) spectra of NAD^+ chemical reduction with $NaBH_4$, emission obtained upon non-selective 260 nm excitation. Excitation spectrum (dashed red line) obtained recording emission signal at 470 nm. Commercial 1,4-NADH absorption spectrum (solid blue line) is reported for comparison.

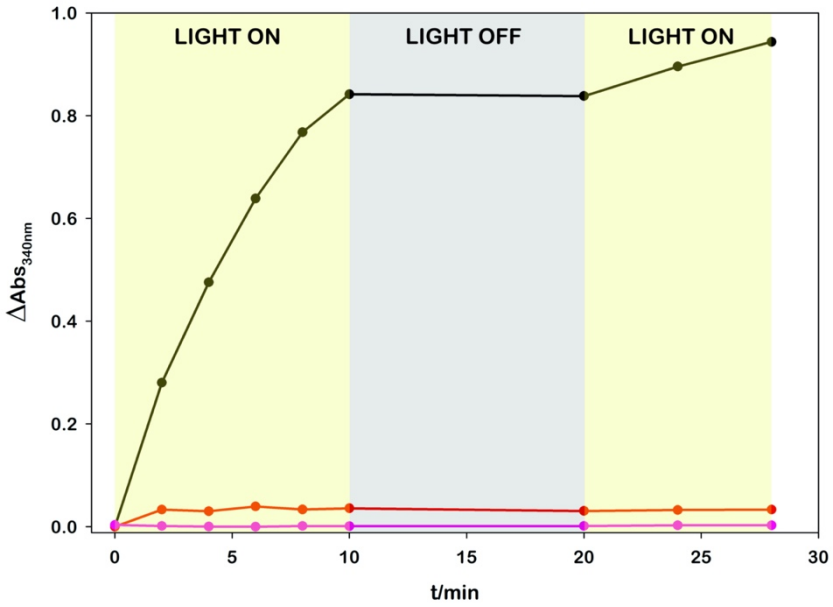


Figure S6. Comparison between N_2-purged (black line) and air-equilibrated (red line) reaction mixtures irradiation; irradiation of N_2-purged reaction mixture without L-Cysteine is also reported (pink line).

HPLC analysis

The reaction mixture was analysed, before and after irradiation and subsequent ADH1 enzymatic assay, using an HPLC Agilent 1260 Infinity II system equipped with a Luna Phenomenex C18(2) column (4.6 x 250 mm, 5µm particle size), a flexible pump, an autosampler, a multicolumn

thermostat and a WR diode array detector. Mixed solutions of methanol/100mM potassium phosphate buffer pH 7.1 (1:9 v/v) were used as eluent.

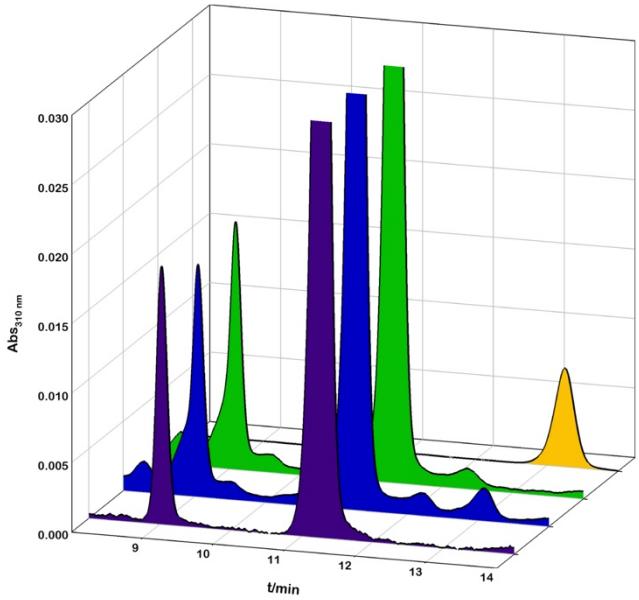


Figure S7. HPLC-chromatograms of reaction mixture before irradiation (purple), after irradiation (blue) and after enzyme assay (green). A chromatogram of commercial 1,4-NADH standard for signals attributions is also reported (orange).

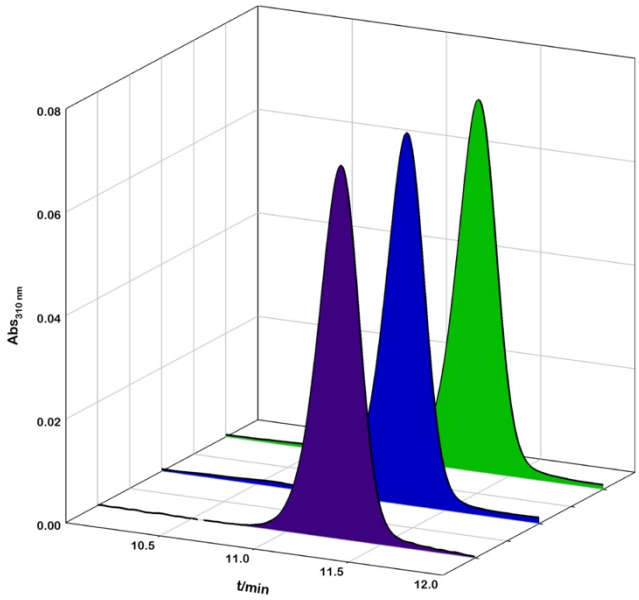


Figure S8. HPLC-chromatograms of reaction mixture before irradiation (purple), after irradiation (blue) and after enzyme assay (green). The peak at 11.30 min is attributed to Ru complex.

Calculation of absorbed light fraction

While quantification of photon flux through chemical actinometry is very easy and accurate in case of monochromatic light sources, this type of measurements became very complex in case of polychromatic ones.

The most straightforward measurement in this instance is absolute spectral irradiance $E_{e,\lambda}$, which is the radiant flux received by a surface per unit area per wavelength (u.m. $\frac{W}{m^2 \cdot nm}$), that can be recorded in the same geometry of photoreaction setup thanks to a calibrated diode array detector (Avantes AvaSpec 2048) equipped with an optical fiber and a cosine corrector.

Once obtained the spectral irradiance of the source at the same geometry of the experiment, it is possible to multiply this for the irradiated surface S, obtaining the spectral flux $\Phi_{e,\lambda}$, which is the radiant energy received per unit time per wavelength (u.m. $\frac{W}{nm}$):

$$\Phi_{e,\lambda} = E_{e,\lambda} \cdot S$$

Moreover, if not all photons are absorbed by the sample ($Abs < 2$), the fraction of light absorbed at each wavelength $\chi_{abs,\lambda}$ must also be considered. The latter quantity is determined by the Beer-Lambert-Bouguer law for liquid solutions:

$$\chi_{abs,\lambda} = 1 - 10^{-Abs_\lambda}$$

At this point it is possible to obtain the radiant energy absorbed per unit time per wavelength:

$$\Phi_{e,abs,\lambda} = \Phi_{e,\lambda} \cdot \chi_{abs,\lambda} = E_{e,\lambda} \cdot S \cdot \left(1 - 10^{-Abs_\lambda}\right)$$

Therefore, the total radiant energy absorbed per unit time is given by the following formula:

$$\Phi_{e,abs} = S \cdot \int_{\lambda_1}^{\lambda_2} E_{e,\lambda} \cdot \left(1 - 10^{-Abs_\lambda}\right) d\lambda$$

The integral is evaluated in a spectral range specified by the experimental conditions, typically the overlapping area between the source's spectral profile and the absorption spectrum of the reaction mixture.

This approach does not consider any possible sample absorption variation during irradiation.

$\Phi_{e,abs}$ (u.m. W) can be simplified as 'Absorbed power'.

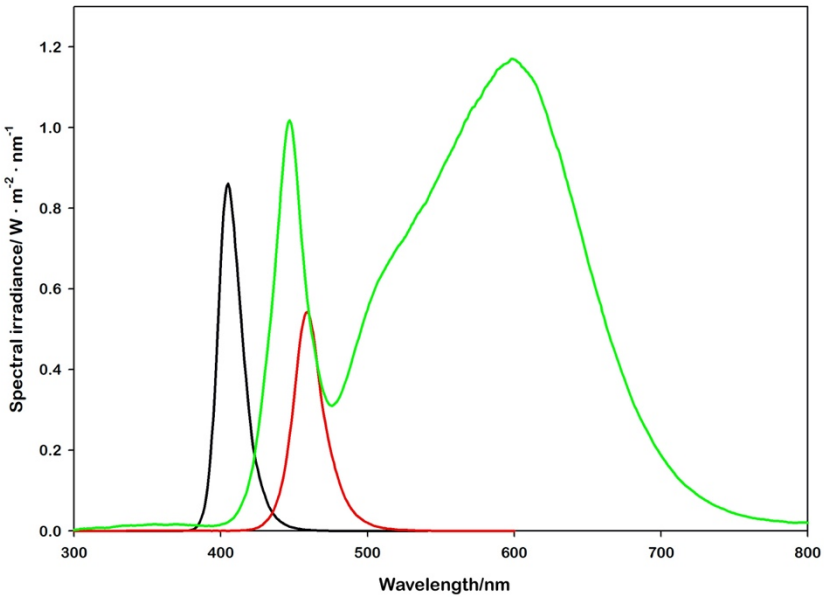


Figure S9. Sources' spectral profiles at 20 cm distance. Black line represents LED Engin LuxiGen™ LZ1-10UB0RM1U8 operating at 600mA (scaled down by a factor of 20 for comparison), red line represents LED Engin LuxiGen™ LZ1-10B202-0000 operating at 600mA and green line represent a commercial 35W white LED lightbulb.

Quenching constant calculation

Quenching constant can be obtained thanks to Stern-Volmer equation:

$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + k_q \tau_0 [Q]$$

where τ_0 is the lifetime of the excited state without the quencher Q (I_0 represents emission intensity without Q), τ is its lifetime in presence of Q (I is the emission intensity with Q), k_q is the dynamic quenching constant and $[Q]$ is the concentration of the quencher.

References

1 G. Bergamini, C. Saudan, P. Ceroni, M. Maestri, V. Balzani, M. Gorka, S.-K. Lee, J. van Heyst and F. Vögtle, *J. Am. Chem. Soc.*, 2004, **126**, 16466–16471.

2 C. A. Bignozzi, C. Chiorboli, M. T. Indelli, M. A. Rampi Scandola, G. Varani and F. Scandola, *J.*

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