Electronic Supplementary Information

Parameters influencing the photo-induced electron transfer from tryptophan containing peptides to a Ru^{II} complex: a systematic study

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Synthesis of the peptide sequences

Peptides were assembled on a Syro II peptides synthesizer (Biotage) following Fmoc strategy. The following side chain protected amino acids were used: Glu(tBu), Trp(boc), Lys(boc) and Tyr(tBu). Rink amide resin, loaded to 0.79 mmol/g, was used. Syntheses were performed on a 0.1 mmol scale using 0.5 M solution of amino acids dissolved in NMP (4 equiv.), 3.9 equivalents HBTU and 8 equivalents DIEA. Deprotection was performed by a NMP/piperidine (3:2) solution.
Acetylation was performed by shaking resin in a Ac_2O/Pyridine/NMP (1:25:25) for 5 minutes at RT (x2). Final deprotection was achieved by shaking resin in 10 mL TFA/H_2O/TIS (95:2.5:2.5) for 2 h.
Deprotection solution was filtrated and evaporated to dryness. The resulting oily residue was precipitated in cold Et_2O/pentane (2:1) (20 mL) and centrifuged. White precipitate was washed by a cold Et_2O/pentane (2:1) solution (3×30 mL) and purified by RPHPLC on a Gilson GX281 apparatus. Eluent A is a 0.1% TFA solution and eluent B is 0.1% TFA solution in MeCN/H_2O (9:1). Elution was monitored at 214 nm and 300 nm. Fractions were gathered and lyophilized to afford white powder.

Experimental measurements of k_q

The luminescence lifetimes were measured by Time-Correlated Single Photon Counting (TC-SPC) in aqueous solution ($[Ru(TAP)_2phen]^{2+}$ (10^{-5} M) and Tris-HCl buffer (10^{-3} M)) as a function of the concentration of the selected peptide. From these experiments the rate constant (k_q) for the quenching of the excited state was then calculated.
TC-SPC is an FL-900 CDT Edinburgh Instruments spectrometer equipped with a PDL445 laser diode and a Peltier-cooled photomultiplier (R955, Hamamatsu). The samples are thermostated at 20 °C with a Haake F3 temperature controller. A multichannel analyzer collected the data with a minimum number of counts in the first channel equal to 10^4. The resulting decays were deconvoluted for the instrumental response and fitted to single exponential functions using the original manufacturer software package (Fig. 1).

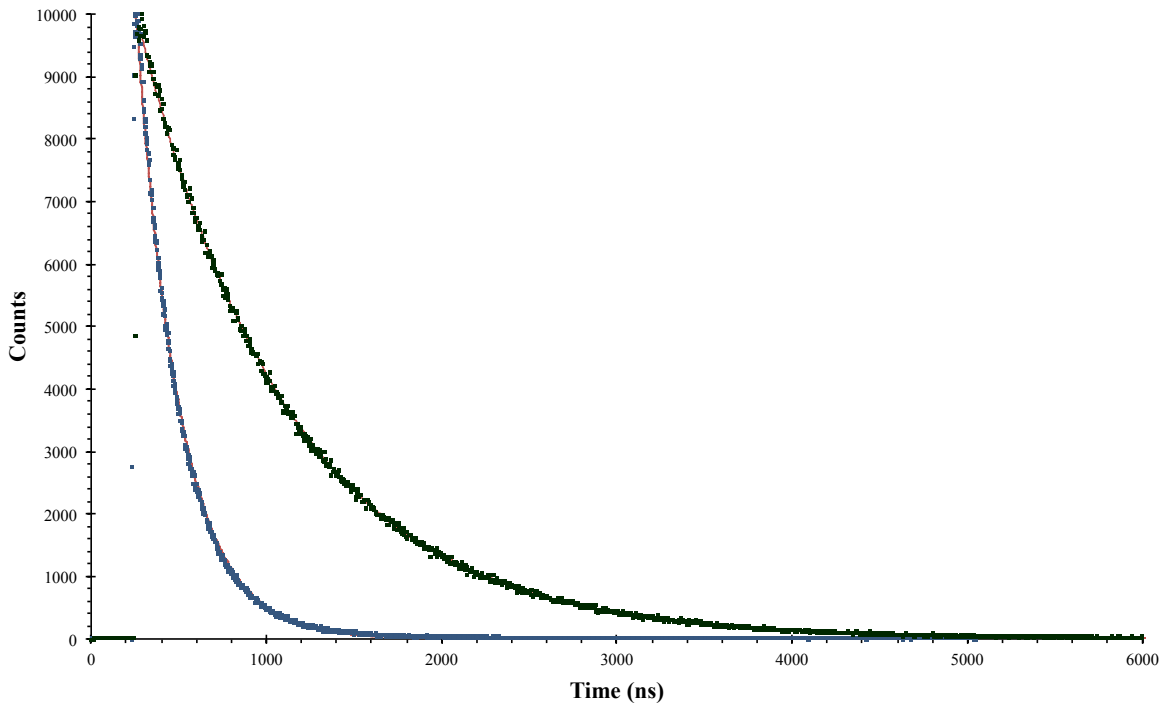

Fig. 1. Luminescence decay for $[Ru(TAP)_2phen]^{2+}$ (10^{-5}M) in aqueous solution in presence of pentapeptides EWGWG and single exponential fitted curve (red line). Blue: 1mM EWGWG, τ=248ns. Green: 21µM EWGWG, τ=863ns.

Stern-Volmer plots

The luminescence lifetime of the ^{3}MLCT state of $[Ru(TAP)_2phen]^{2+}$ in water at pH 7 (100mM Tris HCl buffer solution) and under inert atmosphere was measured by Time-Correlated Single Photon Counting (TC-SPC) as a function of the concentration of the selected peptide. From these experiments the rate constants (k_q) for the quenching of the excited state were then calculated.

Tripeptides

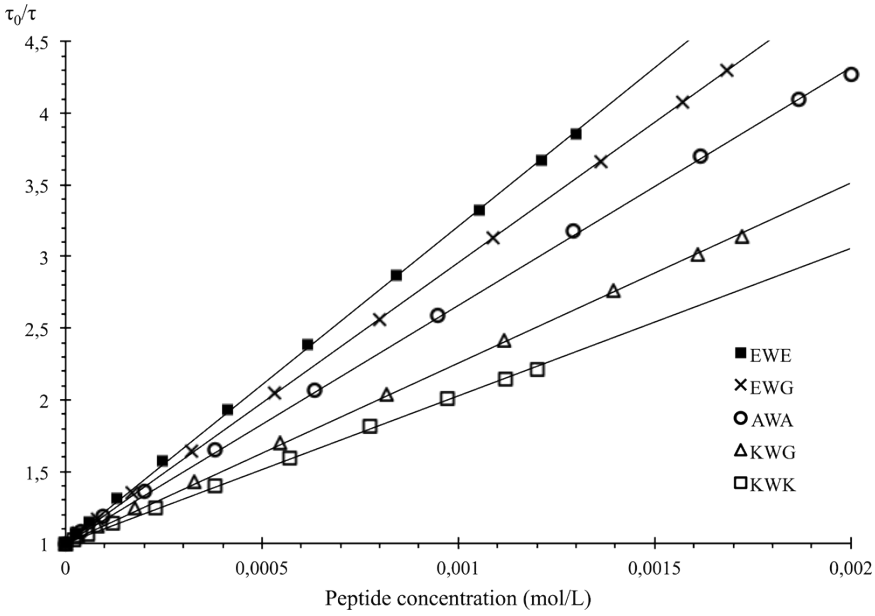

Fig. 2. Stern-Volmer plots for the luminescence quenching of $[Ru(TAP)_2phen]^{2+}$ (10^{-5} M) in presence of tripeptides in water at pH 7 under argon.

Tetrapeptides

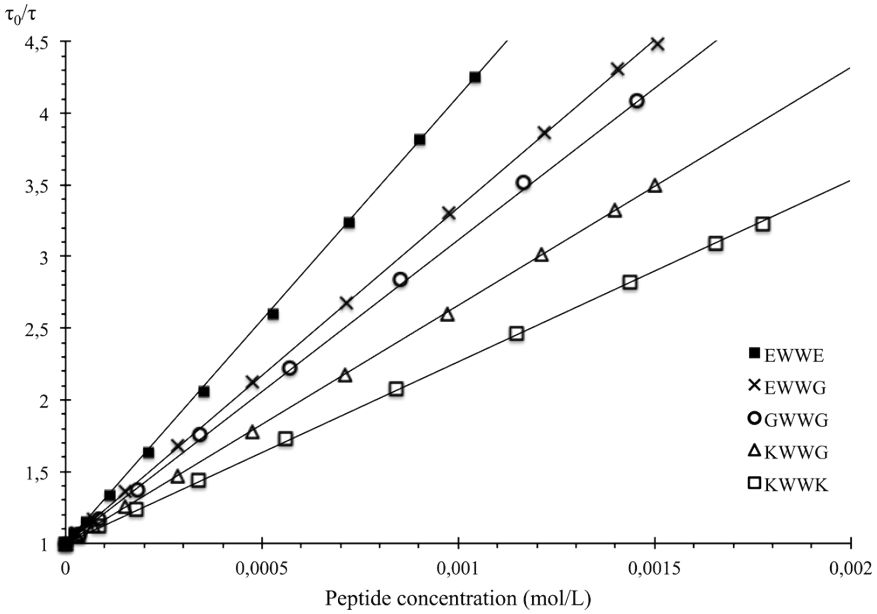

Fig. 3. Stern-Volmer plots for the luminescence quenching of $[Ru(TAP)_2phen]^{2+}$ (10^{-5} M) in presence of tetrapeptides in water at pH 7 under argon.

Pentapeptides

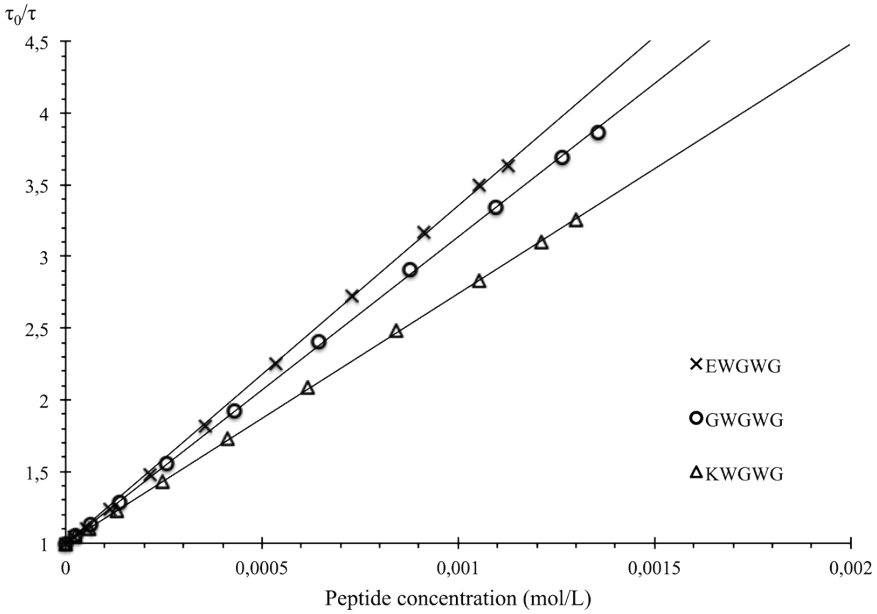

Fig. 4. Stern-Volmer plots for the luminescence quenching of $[Ru(TAP)_2phen]^{2+}$ (10^{-5} M) in presence of pentapeptides in water at pH 7 under argon.

Photoadducts formation

Continuous irradiation in the presence of Glu-Trp-Trp-Gly or Glu-Trp-Gly-Trp-Gly was performed with a 250W xenon lamp (Thermo Oriel), cooled by a system of water circulation. IR (water) and UV (KNO2) cut-off filters were inserted between the irradiation cell and the excitation source. A solution of $[Ru(TAP)_2phen]^{2+}$ ($2\ 10^{-5}$ M) and the selected peptide ($1.65\ 10^{-3}$ M) was illuminated for 6 hours in a quartz cell (1 cm pathlength) under argon and stirred continuously. The resulting crude solution was analyzed by MALDI-ToF mass spectrometry.

Mass spectrometry

All MS experiments were performed on a Waters QToF Premier mass spectrometer in the positive ion mode by using the MALDI source. The MALDI source consisted of a nitrogen laser, operating at 337 nm with a maximum output of 500 mW delivered to the sample in 4 ns pulses at 20 Hz repeating rate.
All samples were prepared by dilution of the reaction mixture in methanol and analyzed by using a solution (20 mg/mL) of trans-2-[3-(4-tbutyl-phenyl)-2-methyl-2-propenylidene]- malononitrile (DCTB) in chloroform as the matrix. The matrix solution (1 mL) was spotted onto a stainless steel target and air dried. Then, the analyte methanol solution (1 mL) was applied onto the spots of matrix crystals, and air dried. For the recording of the single-stage MALDI-MS spectra, the quadrupole (rf-only mode) was set to pass ions between m/z 100 and 2500, and all ions were transmitted into the pusher region of the time-of-flight analyzer where they were mass-analyzed with a 1 s integration time.

Mass spectra after 6 hours illumination

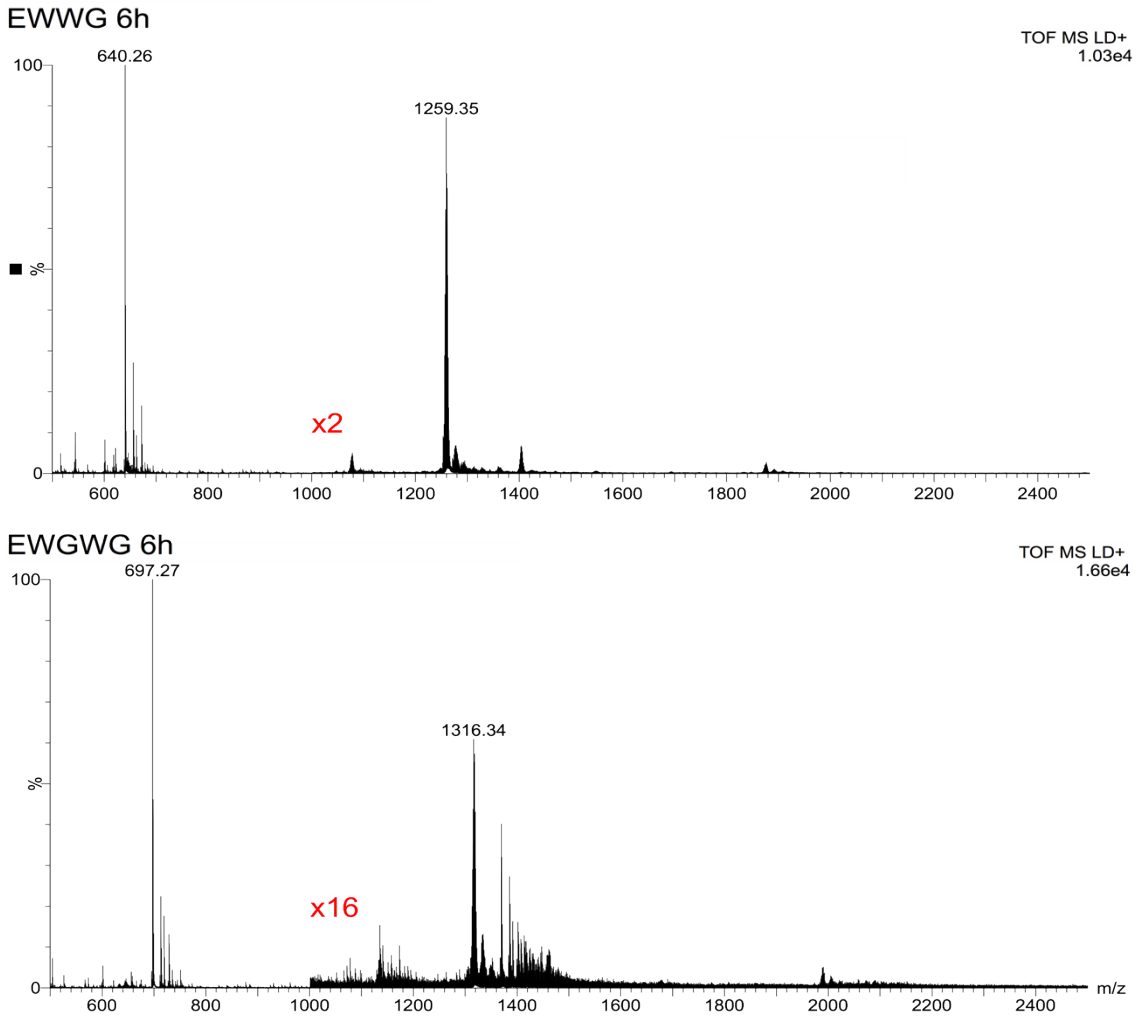

Fig. 5. MALDI-ToF analyzes of the crude reaction medium after 6 hours irradiation. Top : $[Ru(TAP)_2phen]^{2+}$ in presence of EWWG. Bottom: $[Ru(TAP)_2phen]^{2+}$ in presence of EWGWG