Supporting Information

Transforming *Escherichia coli* Proteomembranes into Artificial Chloroplasts Using Molecular Photocatalysis

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1. General Information

**Chemicals and Materials:** All reactions were performed under air if not otherwise stated. All solvents utilized for synthetic purposes were of technical grade and were redistilled prior to use. Oxygen-free solvents were obtained by bubbling argon through these liquids (2 min per 1 mL of solvent) using a common cannula piercing a septum. Sephadex™ LH-20 was obtained from GE Healthcare. Size exclusion chromatography was performed on Sephadex™ LH-20 using methanol as mobile phase. Inversion of cell membranes were performed with the French-Press Pressure Cell SLM Amic (SLM Instruments), membrane separation was performed by ultracentrifugation using an Optima XPN-100 ultracentrifuge (Beckman Coulter).

The nicotinamide adenine dinucleotides NAD⁺, NADH (ultra-pure, ≥ 98 %), nicotinamide adenine dinucleotide phosphate (NADP⁺), adenosine triphosphate sodium salt (ATP), adenosine diphosphate potassium salt (ADP) and glucose-6-phosphate disodium-salt (G6P) were purchased from Roche Diagnostics. Choline chloride and acridine orange (AO, AO-HCl:ZnCl₂) was purchased from Alfa Aesar (Thermo Fisher Scientific), 1,3-bis(tris(hydroxymethyl)methylamino)propane (BTP) from PanReac AppliChem. Manganese chloride hexahydrate (MgCl₂·6 H₂O), ammonium chloride (NH₄Cl), dithiothreitol, glycerol and D(+)-glucose were used from Carl Roth GmbH and tri-sodium phosphate-dodecahydrate (Na₃PO₄·12 H₂O), triethanolamine-hydrochloride (TEOA) was used from Merck Millipore. The compound (3-(4-iodophenyl)-2-(4-nitrophenyl)-5-phenyl-2H-tetrazol-3-ium chloride (INT) was used from Sigma-Aldrich (Merck). The enzymes hexokinase (HK, from yeast) and glucose-6-phosphate-dehydrogenase (G6P-DH, from Leuconostoc mesenteroides) were purchased from Roche Diagnostics and the enzyme diaphorase (from Clostridium kluyveri) was purchased from Sigma-Aldrich (Merck). Tris-HCl and NaCl were obtained from AppliChem GmbH, Tryptone (Bacto™ Tryptone) and yeast extract (Bacto™ Yeast extract) were purchased from Otto Nordwald GmbH.

[Rh(Cp*)Cl₂], ruthenium chloride (RuCl₃·xH₂O), trimethyl bromide (TMSBr), sodium aluminium hydride (NaAlH₄), triethylphosphine (PEt₃), dimethyl sulfoxide (DMSO), acetonitrile (MeCN) and [Ru(bpy)₃]Cl₂ (4) were all purchased from Sigma-Aldrich (Merck). 1,10-Phenanthroline and 4,4´-dimethyl-2,2´-bipyridine were obtained from abcr GmbH. Acetic acid and methanol (MeOH) were purchased from VWR GmbH. Sodium formate (NaHCO₃) was obtained from TCI GmbH.

([(bpy)₂Ru(tpphz)]Cl₂ [3], [(bpy₂h)₂Ru[phenO₂]][PF₆]₂ [2] and 1,10-phenanthroline-5,6-diamine [3] were synthesized according to literature procedures.

**UV-vis absorption spectroscopy** was performed on a JASCO V-670 UV-vis-NIR Spectrophotometer using gas-tight quartz glass cuvettes (d = 10.0 mm, Hellma). Enzyme kinetics were monitored at the Ultrospec 2100 pro (Amersham Biosciences) spectrophotometer using polystyrene cuvettes (d = 10.0 mm, Sarstedt). Microtiter plate based enzymatic read out was performed with the Tecan Infinite® M200 multimode plate reader (Tecan, Crailsheim, Germany) using transparent 96x well microtiter plates (Sarstedt).

**Emission spectroscopy** was performed on a JASCO FP-8500 Fluorescence Spectrometer with gas-tight quartz glass cuvettes (d = 10.0 mm, Hellma).

**Fluorescence measurements** (using AO as pH gradient sensor) were performed in an Amicon Bowman series 2 fluorescence spectrometer (spectronic industries) with single use fluorescence polystyrene makro cuvettes (ratiolab GmbH).
High resolution mass spectrometry (HRMS) was performed using a Fourier Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer solariX (Bruker Daltonics) equipped with a 7.0 T superconducting magnet and interfaced to an Apollo II Dual ESI/MALDI source. For all measurements trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) was used as matrix. Spectra were analyzed with the DataAnalysisViewer 4.2 from Bruker and transferred to Origin 9.0. Spectra simulation was performed with mMass Version 5.5.0 and transferred to Origin 9.0 as well.

$^1$H (400 MHz) and $^{31}$P-NMR (162 MHz) spectra were recorded on a Bruker Avance 400 MHz at room temperature and processed with MestReNova software (Version 12.0.0). The shift values are given in ppm and are referenced to the corresponding solvent residual peaks (1.94 ppm (quintet) for CD$_3$CN and 3.31 ppm (quintet) for d$_4$-MeOD).

Irradiation setup: For all catalysis runs and irradiation experiments analyzed in-situ via UV-vis or emission spectroscopy the irradiation setup consisted of a custom-made reactor equipped with four ventilators to exclude heating of the samples and one blue light emitting LED-stick ($\lambda_{\text{max}} = 465$nm, 45-50 mW/cm$^2$) in the center of this reactor.[4] The cuvettes were placed on top of the LED-stick. For the photobiocatalytic ATP formation the GC vials containing the photoactive components were irradiated with one blue light emitting LED-stick ($\lambda_{\text{max}} = 465$nm, 45-50 mW/cm$^2$) inside an argon filled glovebox (see chapter 2 for more details) at a distance of ca. 1cm.
2. Experimental details

The photobiocatalytic ATP generation was performed inside an argon filled glovebox using an aqueous buffer solution as solvent (50 mM BTP, 140 mM choline chloride, 5 mM MgCl$_2$, 5 mM Na$_3$PO$_4$, 1 mM NAD$^+$, 150 µM ADP and 0.2 M triethanolamine (TEOA) at pH 8.0). The amount of the vesicle solution used for the experiments was adjusted to 650 µg/mL total protein concentration. To the vesicle-buffer mixture 500 µM α-D-glucose and Hexokinase (HK, 1 U) as coupling enzyme were added. This mixture was kept on ice until degassing of the buffer with argon (2 min per mL) in an argon filled Schlenk flask. In detail, the experimental approach was as follows: First, glass vials (VWR Screw Vial, 4 mL, 45 x 14.15 mm) were charged with the respective amount of Ru complexes (5 and 25 µM solutions were evaluated) via MeOH or MeOH/H$_2$O stock solutions using Hamilton micro syringes. After evaporation using pressured air, the glass vials were introduced into an argon filled glovebox. Here, the complexes were dissolved in 2.0 mL degassed buffer solution (see above) and then the GC-vials were sealed with a gas-tight lid. After an incubation period of 5 min, the samples were irradiated with one LED-stick ($\lambda_{\text{max}} = 465$ nm, 45-50 mW/cm$^2$) from the bottom of the glass vials. Typically, every 15 min (if the photobiocatalysis experiments were performed for a duration of 1.5 h) or every 30 min (if the photobiocatalysis experiments were performed for a duration of 4 h) a 100 µL aliquot of the solution was taken and stored on ice outside of the glovebox until further use. The enzymatic readout was performed as follows: After irradiation of the reaction mixture all collected samples were boiled for 15 min at 95 °C to inactivate all enzymes avoiding reactions disturbing the following enzymatic readout (see Scheme 1). The denatured proteins were removed by centrifugation (20 min 20’800 g, 4 °C) and 50 µL of the supernatants were taken for the enzymatic quantification of the generated glucose-6-phosphate (G6P). The readout was performed in two steps in a 96x well microtiter plate (assay volume 100 µl). First, the enzyme diaphorase was added (0.8 U) to the reaction mixture containing 0.5 mM INT (3-(4-Iodophenyl)-2-(4-nitrophenyl)-5-phenyl-2H-tetrazol-3-ium chloride), 2.5 mM NADP$^+$ and 25 mM Tris-HCl (pH 7.5). After incubation for 30 min at room temperature, the absorbance at 492 nm (E1) was determined in a Tecan microplate reader. Afterwards, the enzyme G6P-DH (1 U) was added and incubated for 1 h at room temperature for conversion of the present G6P to 6-phospho-δ-lactone and generation of NADPH from NADP$^+$. The hereby formed NADPH is then used by diaphorase together with INT to yield additional formazan and NADP$^+$ (see Scheme 1). Subsequently, the absorbance at 492 nm is measured (E2). The difference of the absorbance values (Δ(E2-E1)) is used for the calculation of the G6P amount (stoichiometrically equivalent to produced ATP with the reaction equilibrium being strongly on the product site$^5$) based on a parallel performed calibration based on a serial dilution of G6P.
Scheme S1: Graphical representation of the enzymatic G6P readout using the diaphorase catalyzed reductive ring opening reaction of the INT tetrazolium ring yielding the strongly absorbing formazan product ($\lambda = 492$ nm). This process allows to quantify the amount of reduced nicotinamides (NADH and NADPH) by comparing the obtained values with those from an additional G6P calibration. The G6P itself, produced in the photobiocatalytic experiments, originates from the Hexokinase catalyzed phosphorylation of glucose.

The NAD$^+$ independent photoacidification of inverted E.coli vesicles using acridine orange (AO) as sensor for a proton gradient formation was performed as follows: To assess the capacity of the Ru complexes 1, 3 and 5 to induce proton gradient formation, a slightly varied photocatalysis buffer (50 mM BTP; 140 mM choline chloride; 5 mM MgCl$_2$; 5 mM Na$_3$PO$_4$; 0.2 M TEOA, pH 8.0) was used without added ADP, NAD$^+$ and Hexokinase enzyme. The same amount of inverted E. coli vesicles (total protein concentration 650 µg/mL) was added to the buffer as used for the photobiocatalysis experiments. The buffer containing the vesicles was first poured into a one-necked round bottom schlenk flask, which was already set under an argon atmosphere. After bubbling argon through the suspension (2 min per mL), the schlenk flask was introduced into a glovebox. Here, glass vials equipped with the corresponding amount of Ru complex (20 µM; via MeOH stock solutions using Hamilton micro syringes and pressured air to remove all volatiles) were filled with 2.49 mL of the degassed buffer and charged with 5 µM AO by adding 10 µL of 1.25 mM degassed aqueous AO stock solution. The final yellow suspension was subsequently transferred into a sealable gas-tight quartz glass cuvette ($d = 10.0$ mm, Hellma) equipped with a septum on the top. Then the sample was irradiated with one LED-stick ($\lambda = 465$ nm, 45-50 mW/cm$^2$) at room temperature from the bottom of the cuvette for the corresponding time in a home-made reactor as described in chapter 2. Afterwards the cuvette was placed into an Amicon fluorescence spectrometer ($\lambda_{exc} = 495$ nm; $\lambda_{det} = 530$ nm). After detecting a stable fluorescence signal, the proton gradient was
abolished by addition of an argon degassed aqueous NH$_4$Cl stock-solution (to reach a final concentration of 2.5 mM NH$_4$Cl) using a needle and puncturing the septum of the cuvette.

**Preparation of the inverted vesicles from Escherichia coli (E. coli) cells.**

Inverted vesicles were prepared as described previously.[6] In brief, *E. coli* TK2309 or *Pseudomonas putida* KT2440 cells were cultivated overnight in 500 mL LB medium (10 g tryptone/L; 5 g yeast extract/L; 10 g NaCl/L), harvested by centrifugation, resuspended in 50 mL of TCDG buffer (10 mM Tris-HCl, pH 7.5; 140 mM choline chloride; 0.5 mM dithiothreitol; 10% (v/v) glycerol), and washed three times. Afterwards, the cells were suspended in a French Press buffer containing additionally to the TCDG buffer protease inhibitor cocktail tablets (Roche Diagnostics) and a trace element of DNase (Roche Diagnostics). Inversion of cell membranes was done by a single passage through a French Press at 1 100 psi. Cell debris was removed by centrifugation and membranes were collected by ultracentrifugation (200 000 g; 90 min; 4 °C). Membrane pellets were resuspended in fresh TCDG buffer and stored at -80 °C at a concentration of 22 µg/µL before use (*E. coli*). The total protein content was quantified by the Qubit 4 Fluorometer (Thermo Fisher Scientific) according to the manufacturer’s instructions.

**Luminol-luminescence based evaluation of ATP-synthesis with inverted *E. coli* membrane vesicles.**

The qualitative evaluation of the ATP synthesis was done as described elsewhere.[6] In brief, 96x well microtiter plates (assay volume 200 µL) containing a buffered vesicle solution (50 mM BTP; 140 mM choline chloride; 5 mM MgCl$_2$; 150 µM ADP; 5 mM inorganic phosphate (P$_i$)) were mixed with a luciferase/luminol solution (*ATP-determination kit*, Thermo Fisher Scientific) in a ratio 10:1. ATP-synthesis was initiated by the injection of NADH to a final concentration of 500 µM. Luminescence was monitored in a kinetic cycle by Tecan infiniteM200 plate reader. As a control, the experiment was also performed in the absence of inverted vesicles.

To identify optimal conditions for an *in vitro* ATP synthesis, different concentrations of NADH (250-1000 µM, final concentration) were injected to the buffered vesicle solution (175 µg/mL) and resulting ATP levels were quantified after 2 min based on an ATP standard calibration. In an additional setup, an increasing amount of vesicles (0-550 µg/mL total protein concentration) were added to the reaction mixture and ATP levels were quantified after injection of NADH to a final concentration of 500 µM.

**Acridine orange assay to examine proton gradient formation across vesicular membrane.**

In every acridine orange (AO) assay a volume of 1 mL (in polystyrene makro-cuvettes) was used. To the assay buffer (50 mM BTP; 140 mM choline chloride; 5 mM MgCl$_2$), 1 µM AO and 650 µg/mL (total protein concentration) of inverted vesicles were added and the reaction mixture was incubated at room temperature for 30 min. Afterwards, the AO fluorescence was constantly monitored in an Amicon fluorescence spectrometer ($\lambda_{\text{exc}} = 495$ nm; $\lambda_{\text{det}} = 530$ nm). Proton gradient formation was initiated by addition of NADH (final concentration 500 µM), ATP (1 mM final concentration) or buffer as control to the cuvette. Proton gradient was abolished by addition of NH$_4$Cl to a final concentration of 2.5 mM.
The photostability of the various Ru complexes utilized for the photobiocatalytic experiments (1-5) was determined in H₂O:DMSO = 9:1 (v:v) under air. The samples were prepared as follows: starting from MeOH or MeOH/H₂O stock solutions of the different Ru complexes, an appropriate volume was transferred into a glass vial (VWR Screw Vial, 4 mL, 45 x 14.15 mm) using Hamilton micro-syringes. After evaporation of the solvent, the Ru complex was dissolved in 3 mL of H₂O:DMSO = 9:1 (v/v, 2700 µL:300 µL) and then transferred into a cuvette. Irradiation was performed in a custom-made reactor as described in chapter 1.[4]

The stepwise photobiocatalysis experiments used to verify the biological activity of nicotinamide reduction products was performed as follows: First, 2.5 mL of buffered solutions (50 mM BTP, 140 mM choline chloride, 5 mM MgCl₂, 5 mM Na₃PO₄, 0.2 M TEOA, pH 8.0, 1 mM NAD⁺) containing 25 µM Ru complex 1, 2, 3 or 4 were prepared inside of an argon filled glovebox in glass vials (VWR Screw Vial, 4 mL, 45 x 14.15 mm). Prior to use, the buffer solution was hence degassed for 2 min per mL. The solution was then transferred into sealable cuvettes. Irradiation for 2 h was performed using the setup described in chapter 1 and followed by UV-vis absorption and emission spectroscopy to verify NADH formation.

After these 2 h of irradiation, 1 mL of the samples was transferred into polystyrene makro-cuvettes equipped with a small stirring bar followed by the addition of AO (1 µM, final concentration). After stirring the sample for ca. 2 min to achieve a homogeneous distribution of AO, AO luminescence was detected as described above. Afterwards, 100 µL of a buffered solution (50 mM BTP, 140 mM choline chloride, 5 mM MgCl₂, 5 mM Na₃PO₄, 0.2 M TEOA, pH 8.0) containing 440 µg/mL (total protein concentration) inverted E. coli vesicles was added to the stirred solution. This resulted in a strong luminescence increase for the blank experiment as well as for Ru complexes generating no or only less NADH, whereas only low luminescence increase was observed for the samples containing much NADH (assessed by emission spectroscopy).

The photostability of acidine orange (AO) was assessed using UV-vis absorption as well as emission spectroscopy. Therefore, a suitable amount of AO-HCl-ZnCl₂ (M = 438.10 g/mol) was dissolved in deionized water and transferred into a 10 mm x 10 mm cuvette. Irradiation was performed with one LED-stick (λₘₐₓ = 465 nm, 45-50 mW/cm²) from the bottom of the cuvettes inside the custom-made reactor described in chapter 1. The emission spectra were recorded after excitation of AO with λₑₓ = 495 nm.

The photostability of the vesicles was examined by the analysis of proton gradient formation using an AO-assay. Inverted E. coli vesicles (650 µg/mL total protein concentration) were added to the AO-assay buffer (50 mM BTP; 140 mM choline chloride; 5 mM MgCl₂) and irradiated with one blue light emitting LED-stick (λₘₐₓ = 465 nm, 45-50 mW/cm²) for 3 days. After this time, the proton gradient formation was tested by the AO assay (1 µM acridine orange final concentration) as described above.

The emission quenching of acidine orange (AO) by Ru complex 1 was determined in air-equilibrated deionized water. Therefore, two solutions were prepared. The first solution only contained 1 µM AO, the second solution contained 1 µM AO and 100 µM 1. The addition of suitable volumes of the second
solution to the first one allowed to determine the emission quenching of AO by various equivalents of 1. The emission spectra were recorded after excitation of AO with $\lambda_{\text{exc}} = 495$ nm.

The formate driven NAD$^+$ reduction was determined via UV-vis absorption spectroscopy. Therefore, a 5 µM solution of the Ru complexes (1, 2, 3 and 7) in H$_2$O:DMSO =9:1 (v/v, $V_{\text{total}} = 3$ mL) containing 100 mM NaHCO$_3$ and 200 µM NAD$^+$ was prepared under argon atmosphere. Solvents were degassed as discussed in chapter 1. After recording a starting spectrum, the cuvettes, serving as the reaction vessels, were placed into a pre-warmed water bath (40 °C). Catalytic turnover was measured by recording an UV-vis spectrum every 10 min and determined using the extinction coefficient of NADH at 340 nm ($\epsilon = 6230$ L/mol*cm).\cite{7} In detail, the absorbance at 340 nm of the starting spectrum was subtracted from all absorbances at 340 nm of the spectra recorded upon heating the sample. The increase in absorbance at this wavelength was used to determine the turnover numbers (TONs) by dividing the concentration of NADH by the concentration of the utilized catalyst (5 µM).

The emission spectroscopic detection of the light switch effect for compounds 3 and 5 in the presence of inverted E.coli vesicles was performed as follows: To a 10 µM solution ($V = 2.5$ mL) of the respective complexes dissolved either in deionized water (condition i) or in the typical buffer (50 mM BTP, 140 mM choline chloride, 5 mM MgCl$_2$, 5 mM Na$_3$PO$_4$, condition ii), a buffered suspension of E.coli vesicles (total protein concentration 650 µg/mL; 50 mM BTP, 140 mM choline chloride, 5 mM MgCl$_2$, 5 mM Na$_3$PO$_4$) was added. For condition i, vesicle addition was performed in steps of 20 µL until $V_{\text{added, total}} = 180$ µL was reached. For condition ii, vesicle addition was performed first in smaller steps ($V_{\text{added, total}} = 5$ µL, 10 µL, 20 µL) then in steps of 20 µL until $V_{\text{added, total}} = 100$ µL was reached and then in larger steps of 100 µL until $V_{\text{added, total}} = 400$ µL was reached.

In all cases the excitation was performed at the maximum of the MLCT band ($\lambda_{\text{exc}} = 447$ nm for 3 and $\lambda_{\text{exc}} = 449$ nm for 5, respectively) and emission was recorded from 500 – 900 nm. After addition of the last vesicle aliquot and an incubation period of 1 h at room temperature, the emission spectrum was recorded again to test for a kinetically impeded complex-vesicle interaction (which was not observed, see Figure S12).

The UV-vis spectroscopic detection of supramolecular interactions between the Ru complexes 1, 2, 3, 4 and 5 with the inverted E.coli vesicles was performed as follows: First, a 10 µM aqueous buffered solution ($V = 1.6$ mL; buffer: 50 mM BTP, 140 mM choline chloride, 5 mM MgCl$_2$, 0.2 M TEOA, pH 8.0) of the respective complexes was prepared and then an UV-vis absorption spectrum was recorded. After that, 55 µL of the inverted E.coli vesicles (22 µg/µL total protein concentration, i.e. 730 µg/mL final total protein concentration) were added and then incubated for 1 h at room temperature. After centrifugation (21 000 g, 75 min, 4 °C), the supernatant was analyzed by UV-vis spectroscopy again. The loss of the Ru complex by interaction with the inverted E.coli vesicles was determined by the absorbance loss at the MLCT maximum of the Ru complexes.
Calculation of the energy efficiency of the photobiocatalytic process:

Based on the utilized volume of the photobiocatalytic solution \( V = 2.0 \text{ mL} \) and the concentration of RuRh complex 1 (5 \( \mu \text{M} \)) as well as the inner diameter of the GV vial (1.2 cm, \( r = 0.6 \text{ cm} \)) and the absorbance of 5 \( \mu \text{M} \) 1 at 465 nm if \( d = 1 \text{ cm} \), i.e. 0.09, the following initial parameters can be calculated:

The height of the solution inside the GC vial is \( 2 \text{ cm}^3 / (0.6 \text{ cm}^2 \times \pi) = 1.8 \text{ cm} \).

Consequently the absorbance of light in the system is \( E = 0.09 \times 1.8 = 0.16 \).

Application of \( E = \log(\text{l}_0/\text{l}) \) reveals that with \( E = 0.16 \) ca 30% of all incoming photons of \( \lambda = 465 \text{ nm} \) are absorbed by complex 1 in the utilized setup for photobiocatalysis.

Assuming that the distance of 1 cm between the GC vial and the LED stick as well as scattering of LED light by the round shaped geometry of the GC vial reduces the total photonic power input from 45 mW/cm\(^2\) (measured directly on top of the LED stick) to 20 mW/cm\(^2\) gives a total amount of photonic power input into the GC vial of ca. 22 mW considering the bottom area of the GC vial of \((0.6 \text{ cm})^2 \times \pi = 1.1 \text{ cm}^2\). 1.5 h (5400 s) of irradiation thus yields a photonic energy input of 22 mW \times 5400 s = 119 J from which only 30% is absorbed, i.e. ca 36 J.

As over the course of 1.5 h ca. 20 \( \mu \text{M} \) ATP were formed in 2.0 mL solution, ca \( 4.0 \times 10^{-8} \) mol ATP are present when the experiment is finished. Considering the energy of the ATP \( \rightarrow \text{ADP} + \text{P}_i \) conversion with -30.5 kJ/mol,\(^8\) \( 4.0 \times 10^{-8} \) mol \times 30.5 \times 10^3 J/mol = 1.2 \times 10^{-3} \text{ J} \) of photonic energy were stored.

Dividing this value by 36 J of photonic energy that was absorbed by complex 1, an absorbance-considered energy efficiency of ca. \( 3.3 \times 10^{-3} \% \) is obtained.
3. Utilized Ru complexes and their acronyms

In the course of the experiments, five different Ru complexes (1-5) were analyzed with respect to their photobiocatalytic response in the presence of inverted *E.coli* vesicles. The ester protected derivatives 6 and 7 served as the molecular precursors for compounds 2 and 5, which were overall negatively charged under the utilized catalysis conditions (pH 8.0).

![Scheme S2: Molecular structures and corresponding acronyms of the Ru complexes utilized for photobiocatalytic experiments (1-5) as well as synthetic precursors (6 and 7) towards the free phosphonic acid derivatives 2 and 5. The acidic moieties are highlighted by blue circles.](image)

[9]
4. Supplementary Figures S1-S19

Figure S1: Photostability of the Ru complexes utilized for photobiocatalytic experiments (1-5) determined via UV-vis spectroscopy in H2O:DMSO = 9:1. The measurements were performed as described in chapter 2. Detailed spectra of 1 (panel A), 2 (panel B), 3 (panel C), 4 (panel D) and 5 (panel E) are converted into a kinetic comparison (panel F) by plotting the relative absorbance of the MLCT-maximum with respect to the values obtained prior to the irradiation against the irradiation time.
Figure S2: Photocatalytic NADH formation by 1 (panel A), 2 (panel B), 3 (panel C) and 4 (panel D) in a TEOA containing (0.2 M, pH 8.0) buffer solution (50 mM BTP, 140 mM choline chloride, 5 mM Na₃PO₄, 0.4 mM NAD⁺). Additionally, a control experiment using 1 under TEOA-free conditions (panel E) and the difference spectra after 90 min of irradiation (panel F) are shown. The sample irradiation was performed for 90 min and is described in chapter 2; spectra were recorded in intervals of 10 min, the catalyst concentration was 20 µM.
**Figure S3:** Evaluation of the ATP-formation capacity of the inverted *E. coli* vesicles under varying conditions using a quantitative luminol-luciferase assay. The specific values of the x-axis are given on top of each bar. A: Calculated ATP levels in the presence of an increasing amount of the inverted *E. coli* vesicles utilizing a constant NADH concentration of 500 μM (in the presence of 0, 45 and 90 μg/mL inverted *E. coli* vesicles, no ATP was detected). B: Calculated ATP levels after addition of different concentrations of NADH to the reaction mixture containing 175 μg/mL (total protein concentration) of inverted *E. coli* vesicles.

**Figure S4:** A: Course of AO emission utilizing the irradiated solutions analyzed in Figures S5-S6. After the subsequent addition of AO (1 μM) to these solutions, the emission intensity was recorded at 530 nm. The black arrow indicates addition of vesicles (see chapter 2 for details) to these solutions which contained the respective complexes. B: Correlation of the NADH-based emission increase after 2 h of photocatalysis with the AO emission increase upon vesicle addition relative to the initially (at 0 s) recorded emission intensity.
**Figure S5:** Kinetic analysis of formazan absorbance (black) due to not consumed NADH remaining in the photobiocatalysis solution (E1-value, see Scheme S1) and ATP formation using 5 µM 1 under the typically utilized conditions (N = 2; if no error bars are visible, they are small and overlayed by the individual data points).

**Figure S6:** UV-vis absorption (A) and emission spectra (B) of a solution containing 1 mM NAD⁺ in the presence of 50 mM BTP, 140 mM choline chloride and 0.2 M TEOA at pH 8.0 prior to (black curves) and after 2 h of irradiation (red curves) using one LED-stick (λ_{max} = 465 nm, 45-50 mW/cm²). The irradiation was performed as described in chapters 1 and 2.
Figure S7: UV-vis absorption (left column) and emission spectra (right column) of buffered solutions (50 mM BTP, 140 mM choline chloride, 0.2 M TEOA at pH 8.0) containing 25 µM Ru complex and 1 mM NAD⁺ prior to (black curves) and after 2 h of irradiation (red curves) using one LED-stick (λ_{max} = 465 nm, 45-50 mW/cm²).

A and B: 25 µM 1; C and D: 25 µM 2; E and F: 25 µM 3, G and H: 25 µM 4. See Figure S4 for the corresponding blank experiment, i.e. in the absence of any Ru complex.
Figure S8: Formate driven NAD\(^+\) reduction in H\(_2\)O:DMSO = 9:1 (v:v) using 1 (panel A), 2 (panel B), 3 (panel C) and 7 (panel D). The reaction was performed as described in chapter 2. Panel E compares the kinetics of the obtained TONs (for calculation details see chapter 2, N = 2), panel F displays the general reaction mechanism according to Pitman et al.\(^{[10]}\)
Figure S9: Photocatalytic ATP formation using [(bpy)₂Ru(tpphz)]Cl₂ (3) in the presence (gray bars) as well as in the absence (hatched bars) of NAD⁺. No ATP was detected if 0 µM of the catalyst was used (NAD⁺ was present), whereas significant amounts of ATP were observed if 25 or 5 µM of 3 were utilized. The bars represent the obtained ATP amount after 1.5 h of irradiation (N = 2 for all experiments except 5 µM without NAD⁺).

Figure S10: Kinetic analysis of the photogenerated NADH by formazan absorbance at 492 nm after workup of the aliquots taken from the photobiocatalysis at the given irradiation time using 5 µM 1 as catalyst. A/B: Photobiocatalysis was performed for a total time of 4 h / 1.5 h, samples were taken at intervals of 30 min / 10 min, respectively.
**Figure S11:** Emission spectra of aqueous solutions containing 3 (panel A) or 5 (panel B) upon addition of a buffered vesicle solution (in 20 µL steps, 0.65 µg/µL total protein concentration). Panels C and D show plots of the relative emission intensity increase for 3 and 5 upon vesicle addition versus the added vesicle-solution volume (panel C, N = 2) or the total protein concentration (panel D, N = 2), respectively. Only a small increase in the emission intensity was observed if 3 (panel E) or 5 (panel F) were incubated for another 1 h after the last vesicle addition. The bottom row shows the change of the emission maximum with increasing vesicle amount for 3 (panel G) and 5 (panel H).
Figure S12: Kinetic analysis of formazan absorbance (black) due to not consumed NADH remaining in the photobiocatalysis solution (E1-value, see Scheme S1) and ATP formation using 5 µM 2 under the typically utilized conditions (N = 2).

Figure S13: UV-vis spectra of complex 1 (panel A, 10 µM), complex 2 (panel B, 10 µM), complex 3 (panel C, 10 µM), complex 4 (panel D, 10 µM) and complex 5 (panel E, 10 µM) prior (black curves) and after (red curves) an 1 h long incubation with inverted E.coli vesicles (730 µg/mL final total protein concentration). The red spectra were obtained after centrifugation of the mixture removing the Ru-loaded vesicles; the experiment was performed as described in chapter 2. The relative loss of Ru complexes is depicted in panel F (N = 2).
Figure S14: Dependence of the proton-gradient reporting ability of AO on the concentration of AO itself. The course of AO luminescence of 1 µM AO (A), 5 µM AO (B) and 10 µM AO (C) is detected at 530 nm in the presence of 650 µg/mL (total protein concentration) of inverted *E. coli* vesicles. Addition of NADH (final concentration: 500 µM) and NH₄Cl (final concentration: 2.5 mM) is indicated by the corresponding black arrows.

Figure S15: Emission spectra (panel A) of acridine orange (AO, 1 µM) upon addition of increasing amounts of Ru complex 1 and the corresponding plot of maximum emission intensity (\(\lambda = 529\) nm) versus equivalents of 1 (panel B).
Figure S16: UV-vis absorption (solid lines) and emission spectra (dotted lines) of 1 µM acridine orange (AO) in deionized water under air (panel A) or under argon (panel B). The different colors indicate the spectra recorded prior to (black curves), after 10 min (red curves) and after 20 min (blue curves) of irradiation using one LED-stick ($\lambda_{\text{max}} = 465$ nm, 45-50 mW/cm$^2$). No effect of the gaseous atmosphere on the photolysis of AO was observed.

Figure S17: Emission spectra of acridine orange (AO, 5 µM) in the absence (panel A) and the presence (panel B) of 20 µM 3 upon irradiation with one LED-stick ($\lambda_{\text{max}} = 465$ nm, 45-50 mW/cm$^2$). Panel C displays the emission spectra of 1 µM AO under the same conditions, i.e. in the presence of 20 µM 3. The plot of maximum emission intensity versus irradiation time (panel D) indicates that the presence of Ru complex 3 as additional absorber decreases the rate of photolysis of AO. In all cases air-equilibrated deionized water was used as solvent.
Figure S18: Evaluation of the AO luminescence, detected at 530 nm. In all cases, a solution containing 650 µg/mL (total protein concentration) of the inverted E. coli vesicles, 5 µM AO, 50 mM BTP, 140 mM choline chloride, 5 mM MgCl₂, 5 mM PO₄³⁻ and 0.2 M TEOA with pH 8.0 was used. The irradiation was performed using one LED-stick (λ_max = 465 nm, 45-50 mW/cm²). The addition of NH₄Cl is indicated by the black arrow.
A: The above described solution was irradiated for 15 min, then analyzed by luminescence spectroscopy. B: The solution, additionally containing 20 µM Ru complex 3, was analyzed after keeping it 15 min in the dark. C: The solution, additionally containing 20 µM Ru complex 3, was irradiated for 5 min and then analyzed by luminescence spectroscopy. D: The solution, additionally containing 20 µM Ru complex 3, was irradiated for 15 min and then analyzed by luminescence spectroscopy. E: The diagram compares the relative luminescence intensities (normalized to the emission intensity prior to NH₄Cl addition) prior to (gray bars) and after addition of NH₄Cl (hatched bars).
Figure S19: Evaluation of the AO luminescence, detected at 530 nm. In all cases, a solution containing 650 µg/mL (total protein concentration) of the inverted *E. coli* vesicles, 5 µM AO, 50 mM BTP, 140 mM choline chloride, 5 mM MgCl$_2$, 5 mM PO$_4^{3-}$ and 0.2 M TEOA with pH 8.0 was used. The irradiation was performed using one LED-stick ($\lambda_{\text{max}}$ = 465 nm, 45-50 mW/cm$^2$). The addition of NH$_4$Cl is indicated by the black arrow.

A: The above described buffer solution additionally containing 20 µM of Ru complex 5 was irradiated for 10 min, then analyzed by luminescence spectroscopy. B: The above described buffer solution, additionally containing 20 µM of Ru complex 1, was irradiated for 10 min, then analyzed by luminescence spectroscopy. C: The above described buffer solution, additionally containing 20 µM of Ru complex 3, was irradiated for 10 min, then analyzed by luminescence spectroscopy. D: The diagram compares the relative luminescence intensities (normalized to the emission intensity prior to NH$_4$Cl addition) prior to (gray bars) and after addition of NH$_4$Cl (hatched bars) for the given Ru complexes.
5. **Synthesis of the Ru complexes**

**Synthesis of [(bpy)$_2$Ru(tpphz)Rh(Cp*)Cl]Cl$_2$ (1)**

In a 50 mL one-necked round-bottom flask 38.2 mg [(bpy)$_2$Ru(tpphz)]Cl$_2$ (0.0440 mmol, 1.94 eq.) and 14.0 mg [Rh(Cp*)Cl]$_2$ (0.0227 mmol, 1.00 eq.) were suspended in 8.0 mL MeOH and stirred for 16 h at room temperature. After evaporation of the solvent, the crude mixture was purified using size-exclusion chromatography (Sephadex™ LH-20 with MeOH as solvent). The first fraction (which appeared reddish) was collected and separated from the following eluents (grayish and brownish compounds). Subsequent evaporation of the solvent followed by drying the red powder at 50 °C resulted in 44.5 mg of the desired compound (0.0378 mmol, 86 %).

$^1$H-NMR (400 MHz, $d_4$-MeOD, c = 1 mM) δ 10.28 (dd, $J = 8.2, 1.3$ Hz, 2H$_a$), 10.12 (dd, $J = 8.2, 1.2$ Hz, 2H$_b$), 9.59 (dd, $J = 5.3, 1.3$ Hz, 2H$_c$), 8.79 (d, $J = 8.3$ Hz, 2H$_d$), 8.76 (dd, $J = 8.0, 3.0$ Hz, 2H$_e$), 8.49 (dd, $J = 8.2, 5.3$ Hz, 2H$_f$), 8.42 (dd, $J = 5.3, 1.2$ Hz, 2H$_g$), 8.22 (td, $J = 8.0, 1.4$ Hz, 2H$_h$), 8.13 (dd, $J = 8.2, 5.3$ Hz, 2H$_i$), 8.11–8.08 (m, 2H$_j$), 7.97 (dd, $J = 5.6, 0.7$ Hz, 2H$_k$), 7.86 (dd, $J = 7.0, 6.0, 0.6$ Hz, 2H$_l$), 7.59 (dd, $J = 7.5, 5.6, 1.2$ Hz, 2H$_m$), 7.40–7.33 (m, 2H$_n$), 1.88 (s, 15H$_o$).

HRMS (MALDI-FT-ICR, MeOH ; [M] = C$_{65}$H$_{62}$Cl$_4$N$_{10}$RuRh; $M =$ 1177.78 g/mol): calcd. for $[M – Cl + MeO + CO]$ $^+ 1202.0965$; found 1202.1910; calcd. for $[M – 2Cl + 2MeO]$ $^+ 1168.1525$; found 1168.1036; calcd. for $[M – Cl]$$^+ 1143.0831$; found 1143.0825; calcd. for $[M – 2Cl + 2MeO]$$^+ 1133.1840$; found 1133.1322; calcd. for $[M – 2Cl]$$^+ 1106.1156$; found 1106.1106; calcd. for $[M – 3Cl]$$^+ 1071.1471$; found 1071.1459; calcd. for $[M – 4Cl]$$^+ 1036.1786$; found 1036.1758; calcd. for $[M – bpy – 2Cl + 2MeO]$$^+ 1012.0834$; found 1012.0335; calcd. for $[M – bpy – Cl]$$^+ 987.0139$; found 987.0135; calcd. for $[M – bpy – 2Cl]$$^+ 950.0466$; found 950.0451; calcd. for $[M – bpy – 4Cl + 3CH$_3$ + 2H]$$^+ 927.1957$; found 927.1999; calcd. for $[M – 3Cl – RhCp*]$$^+ 833.1239$; found 833.1220; calcd. for $[M – 4Cl – RhCp*]$$^+ 798.1554$; found 798.1531; calcd. for $[M – 4Cl – bpy – RhCp* + 2CH$_3$]$$^+ 699.1205$; found: 699.1572; calcd. for $[M – 3Cl – bpy – RhCp*]$$^+ 677.0548$; found 677.0536.

**Synthesis of [(bpy)$_2$Ru(tpphz)Rh(Cp*)Br]Br$_3$ (2)**

7 (28 mg, 14 µmol) was dissolved in dichloromethane and degassed with an argon-flow. To the solution TMSBr (46 µL, 350 µmol, 25 eq.) was added under argon and the mixture was stirred for 12 h at room temperature. After that time methanol was added and the mixture was further stirred for 6 h at room temperature. Diethyl ether was added, and the product was filtered off to yield a dark red powder (22 mg, 95 %, calculated for the bromide salt).

$^1$H NMR (400 MHz, CD$_2$CN:D$_2$O = 9:1, v:v) δ 10.14 (s, 2H$_a$), 9.98 (s, 2H$_b$), 9.44 (d, $J = 4.7$ Hz, 2H$_c$), 8.62 (s, 4H$_d$), 8.40 (s, 4H$_e$), 8.03 (s, 2H$_f$), 7.78 (s, 2H$_g$), 7.65 (s, 2H$_h$), 7.38 (s, 2H$_i$), 7.23 (s, 2H$_j$), 3.36 (t, $J = 26.3$ Hz, 4H$_k$), 1.85 (s, 15H$_l$).

$^{31}$P NMR (162 MHz, D$_2$O) δ 17.99, 17.49.
Synthesis of [(bpy\textsuperscript{P}Et\textsubscript{2})\textsubscript{2}Ru(tpphz)]Br\textsubscript{2} (5)

7.5 mg 6 (0.0044 mmol) were dissolved in 5 mL dry CH\textsubscript{3}CN under argon in a 25 mL one-necked round-bottom flask. After addition of 100 µL freshly distilled TMSBr, the mixture was stirred for 16 h at room temperature. Then the mixture was heated to 40 °C for 0.5 h. Afterwards the reaction vessel was opened under air and 1 mL of a 1:1 (v:v) mixture of acetone:H\textsubscript{2}O was added to the solution leading to the gradual formation of a precipitate. Next, the mixture was stirred for 1 h at room temperature and then all volatiles were evaporated. The quantitative formation of 5 was determined by using \textsuperscript{1}H-NMR spectroscopy showing that all protecting ethyl ester groups were cleaved.

\textsuperscript{1}H NMR (400 MHz, d\textsubscript{4}-MeOD) \(\delta\) 10.46 (dd, \(J = 8.3, 1.5\) Hz, 2H), 10.13 (dd, \(J = 8.3, 1.2\) Hz, 2H), 9.48 (dd, \(J = 5.0, 1.5\) Hz, 2H), 8.71 (s, 2H), 8.68 (s, 2H), 8.51 (dd, \(J = 8.3, 5.0\) Hz, 2H), 8.43 (dd, \(J = 5.3, 1.2\) Hz, 2H), 8.13 (dd, \(J = 8.2, 5.4\) Hz, 2H), 7.88 (d, \(J = 6.1\) Hz, 2H), 7.80 (d, \(J = 6.0\) Hz, 2H), 7.57 – 7.51 (m, 2H), 7.36 – 7.31 (m, 2H), 3.48 (d, \(J = 22.6\) Hz, 4H), 3.37 (d, \(J = 22.4\) Hz, 4H).

Synthesis of [(bpy\textsuperscript{P}Et\textsubscript{2})\textsubscript{2}Ru(tpphz)][PF\textsubscript{6}]\textsubscript{2} (6)

[(bpy\textsuperscript{P}Et\textsubscript{2})\textsubscript{2}Ru(phenO\textsubscript{2})\textsubscript{2}][PF\textsubscript{6}]\textsubscript{2} (50 mg, 33 µmol) was dissolved in 10 mL acetonitrile, acetic acid was added (2 mL) and the solution was degassed with argon. The mixture was heated to 80 °C and one equivalent of 1,10-phenantroline-5,6-diamin (7.0 mg, 33 µmol) was added. After 6 and 18 h two more equivalents of the diamine were added to the reaction mixture, respectively. After cooling to room temperature, the tpphz formed by self-condensation of the diamine was filtered off and the solvent was removed under reduced pressure. The crude oil was taken up in a minimum amount of methanol and upon addition of an aqueous solution of ammonium hexafluorophosphate the product could be filtered off. Further purification was done by size exclusion chromatography (Spehadex LH-20, methanol) to yield an orange-red powder (25 mg, 45 %).

\textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}CN) \(\delta\) 10.14 (dd, \(J = 7.5, 2.7\) Hz, 2H), 9.96 (d, \(J = 8.3\) Hz, 2H), 9.45 (dt, \(J = 5.1, 1.2\) Hz, 2H), 8.55 (dt, \(J = 3.7, 1.8\) Hz, 2H), 8.53 – 8.49 (m, 2H), 8.45 – 8.36 (m, 2H), 8.28 (dt, \(J = 5.4, 1.1\) Hz, 2H), 8.02 (ddd, \(J = 8.0, 5.4, 2.6\) Hz, 2H), 7.77 (dd, \(J = 5.5, 2.5\) Hz, 2H), 7.65 (d, \(J = 5.6\) Hz, 2H), 7.48 – 7.41 (m, 2H), 7.25 – 7.16 (m, 2H), 4.14 – 3.86 (m, 16H), 3.53 – 3.27 (m, 8H), 1.21 (td, \(J = 7.1, 1.8\) Hz, 12H), 1.14 – 1.04 (m, 12H).

\textsuperscript{31}P NMR (162 MHz, CD\textsubscript{3}CN) \(\delta\) 22.91, 22.89.

HRMS (MALDI-FT-ICR, CH\textsubscript{3}CN; \([M] = C\textsubscript{64}H\textsubscript{72}F\textsubscript{13}N\textsubscript{10}O\textsubscript{12}P\textsubscript{6}Ru; \(M = 1688.23\) g/mol): calcd. for \([M – PF\textsubscript{6}]^+\) 1543.2987; found: 1543.2971; calcd. for \([M – PF\textsubscript{6} – C\textsubscript{2}H\textsubscript{5} + H]^+\) 1515.2673; found: 1515.2691; calcd. for \([M – 2PF\textsubscript{6} – 2C\textsubscript{2}H\textsubscript{5}]^+\) 1485.2203; found: 1485.3373; calcd. for \([M – 2PF\textsubscript{6}]^2+\) 1398.3345; found: 1398.3331; calcd. for \([M – 2PF\textsubscript{6} – 2C\textsubscript{2}H\textsubscript{5}]^2+\) 1369.2953; found: 1369.2937; calcd. for \([M – 2PF\textsubscript{6}]^2+\) 699.1673; found: 699.1695.
Synthesis of [(bpyP^2)Ru(tpphz)Rh(Cp*)Cl]Cl(PF_6)₂ (7)

6 (25 mg, 15 µmol, 2 eq.) was dissolved in dichloromethane and [Rh(Cp*)Cl]_2 (4.6 mg, 7.5 µmol, 1 eq.) was added to the solution. The mixture was stirred for 6 h at room temperature, after that time diethyl ether was added and the product was filtered off to yield a dark red powder (28 mg, 95%).

^1H NMR (400 MHz, CD₃CN) δ 10.14 (dd, J = 7.4, 3.4 Hz, 2H), 9.97 (d, J = 8.5 Hz, 2H), 9.46 (d, J = 5.3 Hz, 2H), 8.59 – 8.54 (m, 4H), 8.43 – 8.39 (m, 2H), 8.29 – 8.27 (m, 2H), 8.04 – 8.00 (m, 2H), 7.78 – 7.76 (m, 2H), 7.65 (d, J = 6.9 Hz, 2H), 7.45 – 7.44 (m, 2H), 7.22 – 7.20 (m, 2H), 4.10 – 3.90 (m, 16H), 3.49 – 3.33 (m, 8H), 1.82 (s, 15H), 1.22 – 1.19 (m, 12H), 1.13 – 1.06 (m, 12H).

^31P NMR (162 MHz, CD₃CN) δ 22.82, 22.80.

HRMS (MALDI-FT-ICR, CH₃CN; [M] = C₇₆H₇₀Cl₃F₁₁₂N₁₀O₁₂P₆RhRu; M = 1997.27 g/mol): calcd. for [M – Cl]^+ 1961.2545; found: 1961.2578; calcd. for [M – 2Cl + CN]^+ 1952.2892; found: 1952.2901; calcd. for [M – 2Cl]^+ 1926.2861; found: 1926.2839; calcd. for [M – PF₆ – Cl]^+ 1816.2903; found: 1816.2890; calcd. for [M – PF₆ – 2Cl + CN]^+ 1807.3250; found: 1807.3256; calcd. for [M – PF₆ – Cl – C₂H₅]^+ 1787.2511; found: 1787.2554; calcd. for [M – PF₆ – 2Cl]^+ 1781.3229; found: 1781.3252; calcd. for [M – PF₆ – 2Cl – C₂H₅]^+ 1754.2827; found: 1754.2824; calcd. for [M – 2PF₆ – HCl]^+ 1670.3183; found: 1670.3185; calcd. for [M – 2PF₆ – 2Cl + CN]^+ 1662.3608; found: 1662.3576; calcd. for [M – 2PF₆ – Cl – C₂H₅]^+ 1642.2870; found: 1642.2882; calcd. for [M – 2PF₆ – Cl – HCl]^+ 1635.3499; found: 1635.3543; calcd. for [M – PF₆ – 2Cl – C₂H₅]^+ 1607.3185; found: 1607.3176; calcd. for [M – PF₆ – 2Cl – RhCp*]^+ 1543.2987; found: 1543.2980; calcd. for [M – PF₆ – 2Cl – RhCp* – C₂H₅ + H]^+ 1515.2673; found: 1515.2687; calcd. for [M – 2PF₆ – Cl – HCl – RhCp*]^+ 1397.3267; found: 1397.3280; calcd. for [M – 2PF₆ – 2Cl – RhCp* – C₂H₅]^+ 1369.2953; found: 1369.2939; calcd. for [M – PF₆ – Cl]^2+ 908.1442; found: 908.1447.
6. Characterization of the Ru complexes (Figures S20-S37)

Figure S20: $^1$H-NMR spectrum of 1 (c = 3.4 mM) in $d_4$-MeOD. For better visualization of the peaks corresponding to 1, the solvent residual signals of $d_3$-MeOD and H$_2$O were removed from the spectrum. Signal assignment was based on H,H-COSY spectra (Figures S19-20) and previous literature reports.[11,12]

Figure S21: $^1$H-NMR spectra of 1 in $d_4$-MeOD (spectra 1-3 show a dilution series with c(1) = 3.4 (bottom), 1.7 (center) and 1.0 mM (top), respectively). No signals were omitted by cutting the spectrum between 8.9 and 9.4 ppm (see Figure S17).
**Figure S22:** H,H-COSY spectrum of 1 (c = 3.4 mM) in d₄-MeOD covering the whole aromatic region (the signal numbering is based on the molecular structure of 1 depicted in Figure S17).

**Figure S23:** H,H-COSY spectrum of 1 (c = 3.4 mM) in d₄-MeOD covering the aromatic region in which the bpy based signals occur (the signal numbering is based on the molecular structure of 1 depicted in Figure S17).
Figure S24: $^1$H-NMR spectrum of 2 in CD$_3$CN/D$_2$O = 9:1 (v:v).

Figure S25: $^{31}$P-NMR spectrum of 2 in D$_2$O.
Figure S26: $^1$H-NMR spectrum of 5 in d$_4$-MeOD. For better visualization the large H$_2$O peak between 4.9 and 5.1 ppm was removed (no other signals are detected in this area). The signals marked with an asterisk are assigned to residual DMF impurities.

Figure S27: $^1$H-NMR spectrum of 6 in CD$_3$CN.
Figure S28: $^{31}$P-NMR spectrum of 6 in CD$_3$CN.

Figure S29: $^1$H-NMR spectrum of 7 in CD$_3$CN.
Figure S30: $^{31}$P-NMR spectrum of 7 in CD$_3$CN.

Figure S31: MALDI-TOF mass spectrum of 1.
Figure S32: Comparison of experimentally obtained mass spectrum (top) and simulated mass spectrum (bottom) of 1 depicting the fragments \([M-2Cl]^+\), \([M-3Cl]^+\) and \([M-4Cl]^+\).

Figure S33: MALDI-TOF mass spectrum of 6.
Figure S34: MALDI-TOF mass spectrum of 7 showing the m/z range from 1600 to 2000.

Figure S35: MALDI mass spectrum of 7 showing the m/z range from 900 to 1600.
Figure S36: UV-vis absorption (panel A; solvent: H$_2$O:DMSO = 9:1, v:v) and emission spectra (panel B; solvent: DMSO:H$_2$O = 19:1, v:v) of 2 (black curves) and 5 (red curves). For the UV-vis spectra 10 µM of the complexes were used, for the emission spectra the concentration was set to 5 µM. Although 5 was much better soluble in water compared to DMSO, no higher water content could be used in order to not fully quench the emission of 5 due to the well-known light-switch effect.[13]

Figure S37: UV-vis (panel A) and emission spectra (panel B) of 6 (red curves) and 7 (black curves) in DMSO (c = 5 µM).
7. References

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