

Supporting information for Basic-to-acidic reversible pH switching with a merocyanine photoacid

Laura Wimberger, Joakim Andréasson, Jonathon E. Beves
School of Chemistry, UNSW Sydney, Sydney, Australia

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S1. General information

Reagents and solvents were purchased from Sigma-Aldrich, Chem Supply, BDH Chemicals or Combi-Blocks and used without further purification unless stated otherwise.

Qualitative TLC was performed on silica-coated aluminium plates (Merck, silica 60 F254) with detection by UV light ($\lambda = 254$ nm). Flash column chromatography (FCC) was carried out with SilicaFlash® P60 (Velocity Scientific Solutions, 63 μm) with the indicated eluent.

Nuclear magnetic resonance spectra were recorded on a Bruker Avance III 300, Bruker Avance III 400 with a Prodigy CryoProbe, a Bruker Avance III 500 or a Bruker Avance III 600. Signals are reported relative to the residual ^1H NMR signal of DMSO- d_5 ($\delta = 2.50$ ppm) or D $_2$ O ($\delta = 4.79$ ppm). ^{13}C NMR spectra were referenced to the ^{13}C -D septet of DMSO- d_6 ($\delta = 39.5$). Coincidentally equal coupling constants of magnetically non-equivalent protons are marked as virtual (virt.). The signal multiplicities are indicated by the following abbreviations: s-singlet, d-doublet, t-triplet, q-quartet, quin-quintet, sex-sextet, sept-septet, m-multiplet, br-broad. The coupling constants J are reported in Hertz (Hz) as an average of the experimentally determined ones. Structural assignments are based on COSY, HMBC, and HSQC experiments.

UV-vis absorption spectra were measured in 41FLUV10 cells from fireflysci made of quartz (pathway 10 mm) on an Agilent Cary 60 UV-vis spectrometer equipped with a customized Cary Single Cell Peltier Accessory for temperature control ($T = 22$ °C) and stirring of the samples. A LED irradiation setup was employed as described previously by our group.^[1] A Luxeon Rebel LED mounted ca. 4 cm away from the sample holder and focused with a Carelo 20.0 Fibre coupling lens served as irradiation source. Photoswitching cycles were controlled with a timer relay module (FRM01).

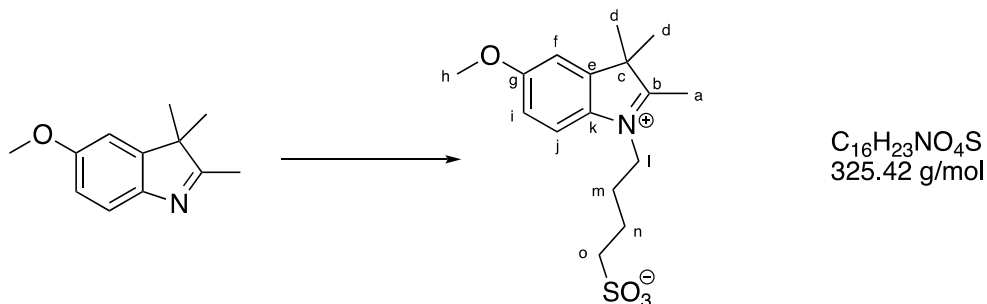
High resolution mass spectrometry (HRMS) was measured on a hybrid linear quadrupole ion trap mass spectrometer (Thermo LTQ Orbitrap XL) with an external nanospray ionisation (NSI) source.

pH values were measured with a *Metrohm* 913 pH meter in conjunction with the *Metrohm* LL Biotrode 3mm WOC. The Pt1000 Temp Sensor Steel was used to monitor the temperature. Solutions in D $_2$ O were also measured with this pH meter and are reported as pD values.

S2. Experimental procedures and analytical data

Precursor synthesis

4-(5-methoxy-2,3,3-trimethyl-3*H*-indol-1-ium-1-yl)butane-1-sulfonate



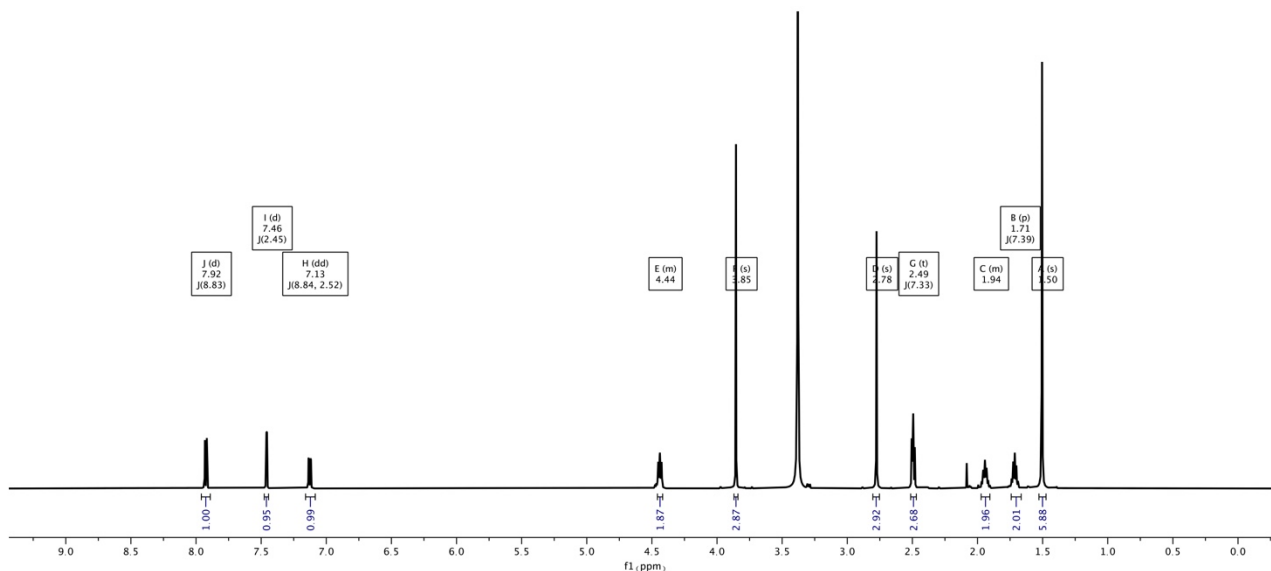
5-Methoxy-2,3,3-trimethyl-3*H*-indole was prepared according to a literature procedure.^[2] To a solution of propane 1,4-butane sulfone (1.14 mL, 1.51 mg, 1.05 eq.) in toluene anhydrous (8.6 mL), 5-methoxy-2,3,3-trimethyl-3*H*-indole (1.92 mL, 2.00 g, 1.00 eq.) was added under inert atmosphere and the solution was heated to 125 °C for 2.5 d. After letting it cool to r.t. the resulting solid was suspended in 8 mL of toluene. The dark purple solid was collected by vacuum filtration and washed with toluene (100 mL) and acetone (10 mL). The solid was dried *in vacuo* and resulted in 2.93 g of 4-(5-methoxy-2,3,3-trimethyl-3*H*-indol-1-ium-1-yl)butane-1-sulfonate (9.00 mmol, 85%) as dark purple solid and used without further purification in the consecutive reaction.

¹H NMR (600 MHz, DMSO-*d*₆): δ 7.92 (d, *J* = 8.8 Hz, 1H, H^j), 7.46 (d, *J* = 2.5 Hz, 1H, H^f), 7.13 (dd, *J* = 8.8, 2.5 Hz, 1H, Hⁱ), 4.46 – 4.41 (m, 2H, H^l), 3.85 (s, 3H, H^a), 2.78 (s, 3H, H^o), 2.49 (t, *J* = 7.3 Hz, 2H, H^o), 1.98 – 1.90 (m, 2H, H^m), 1.71 (p, *J* = 7.4 Hz, 2H, Hⁿ), 1.50 (s, 6H, H^d).

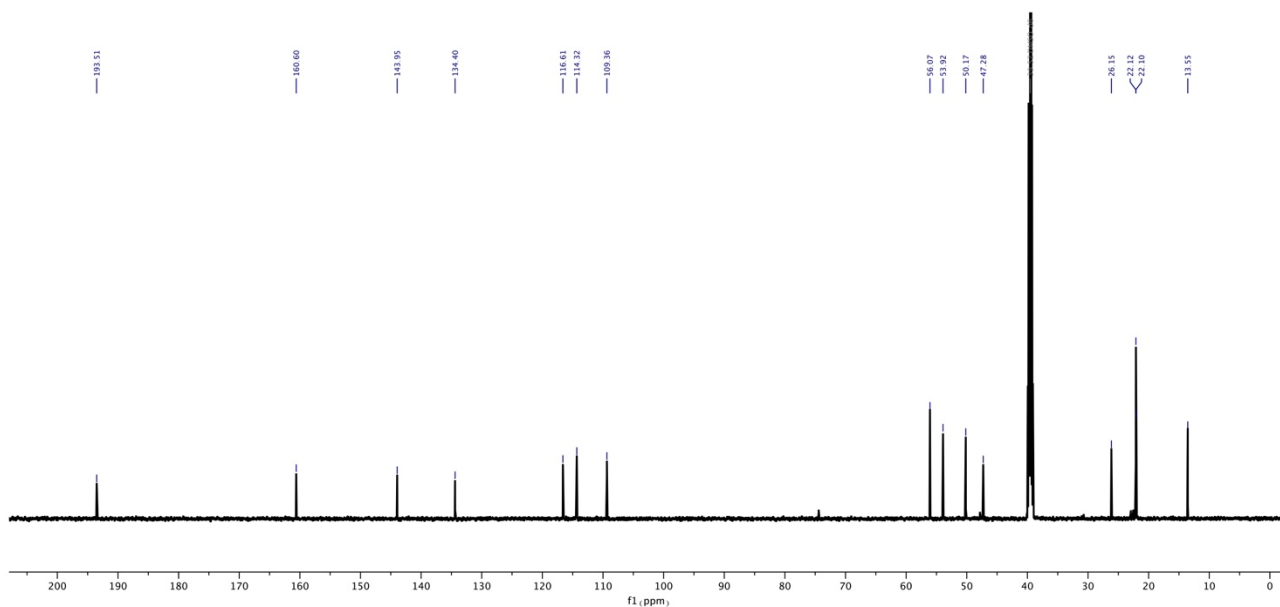
¹³C NMR (151 MHz, DMSO-*d*₆): δ 193.5 (C^b), 160.6 (C^g), 144.0 (C^e), 134.4 (C^k), 116.6 (C^j), 114.3 (Cⁱ), 109.4 (C^f), 56.1 (C^h), 53.9 (C^c), 50.2 (C^o), 47.3 (C^l), 26.2 (C^m), 22.1 (Cⁿ), 22.1 (C^d), 13.6 (C^a).

HRMS (ESI): calc. for C₁₆H₂₃NO₄S: [M–H]⁺: 326.1421 [M+Na]⁺: 348.1240
found: [M–H]⁺: 326.1423 [M+Na]⁺: 348.1240.

¹H NMR (600 MHz, DMSO-*d*₆):

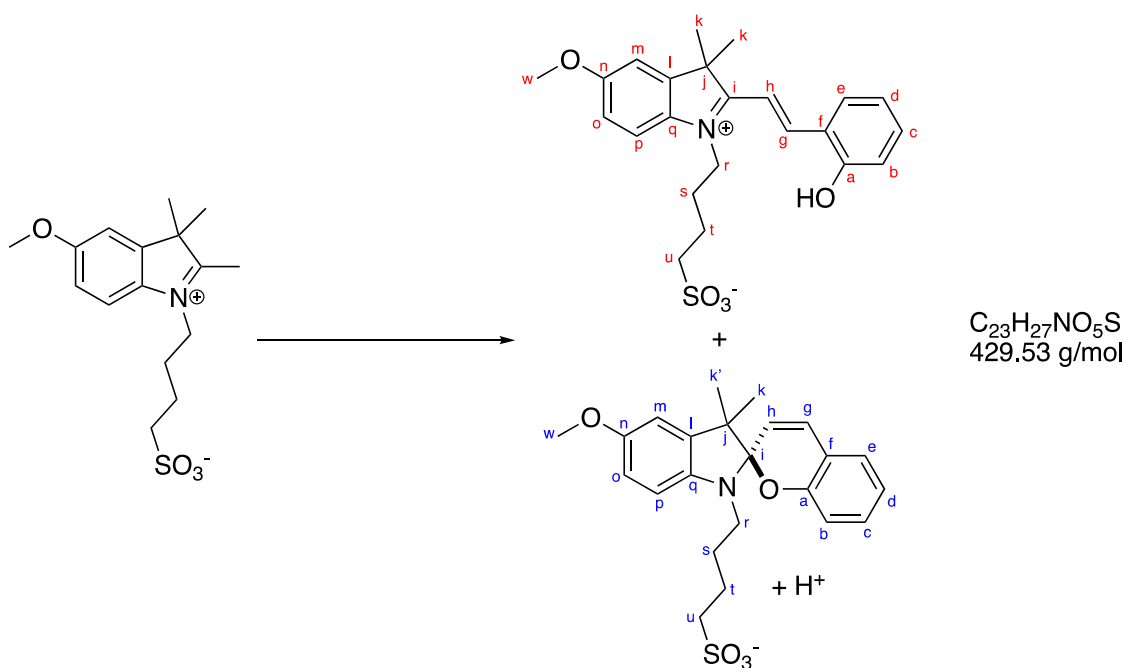


^{13}C NMR (151 MHz, $\text{DMSO-}d_6$):



Synthesis of SP-1/MCH-1

(*E*)-4-(2-(2-hydroxystyryl)-5-methoxy-3,3-dimethyl-3*H*-indol-1-ium-1-yl)butane-1-sulfonate (**1-MCH**)/ (*R/S*)-4-(5'-methoxy-3',3'-dimethylspiro[chromene-2,2'-indolin]-1'-yl)butane-1-sulfonate (**1-SP**)



Salicylaldehyde (278 μL , 289 mg, 2.37 mmol, 1.10 eq., *BDH Chemicals*) was added to a solution of the substituted indolinium (700 mg, 2.15 mmol, 1.00 eq.) in anhydrous ethanol (0.27 M, 8.00 mL). The reaction mixture was heated to 100 $^\circ\text{C}$ for 19 h and cooled down to r.t. The reaction mixture was added dropwise to ice-cold diethyl ether (200 mL) resulting in an orange precipitate. The orange precipitate was collected by vacuum filtration and washed with ice cold diethyl ether (ca. 10 mL). The solid was purified by column chromatography (4.5 \times 26 cm, $\text{CH}_2\text{Cl}_2/\text{MeOH} = 95/5 \rightarrow 93/7 \rightarrow 90/10$) resulting in an orange powder as the product (454 mg, 1.06 mmol, 49%). In solution ($\text{DMSO-}d_6$) **1** is present as a mixture of **MCH-1** and **SP-1**

TLC: $R_f = 0.38$ ($\text{CH}_2\text{Cl}_2/\text{MeOH} = 95/5$) [UV].

Merocyanine form

¹H NMR (600 MHz, DMSO-*d*₆) δ 11.05 (s, 1H, OH), 8.46 (d, *J* = 16.4 Hz, 1H, H^g), 8.15 (dd, *J* = 8.0, 1.7 Hz, 1H, H^e), 7.91 (d, *J* = 8.9 Hz, 1H, H^p), 7.74 (d, *J* = 16.4 Hz, 1H, H^h), 7.51 (d, *J* = 2.5 Hz, 1H, H^m), 7.44 (ddd, *J* = 8.5, 7.1, 1.7 Hz, 1H, H^s), 7.15 (dd, *J* = 8.9, 2.5 Hz, 1H, H^o), 7.03 (d, *J* = 8.3 Hz, 1H, H^{d/b}), 7.00 – 6.97 (m, 1H, H^{d/b}), 4.59 (t, *J* = 7.8 Hz, 2H, H^r), 3.89 (s, 3H, H^w), 2.54 – 2.51 (m, 2H, H^u), 1.96 (tt, *J* = 8.2, 6.4 Hz, 2H, H^s), 1.82 – 1.77 (m, 2H, H^t), 1.76 (s, 6H, H^k).

¹³C{¹H} NMR (151 MHz, DMSO-*d*₆) δ 179.5 (Cⁱ), 160.8 (Cⁿ), 158.8 (C^a), 147.2 (C^g), 145.7 (C^l), 135.1 (C^c), 134.2 (C^q), 130.2 (C^e), 121.4 (C^f), 119.9 (C^{d/b}), 116.6 (C^{d/b}), 116.4 (C^p), 114.9 (C^o), 111.8 (C^h), 108.8 (C^m), 56.2 (C^w), 51.9 (C^j), 50.3 (C^u), 46.6 (C^r), 27.1 (C^s), 26.4 (C^k), 22.3 (C^t).

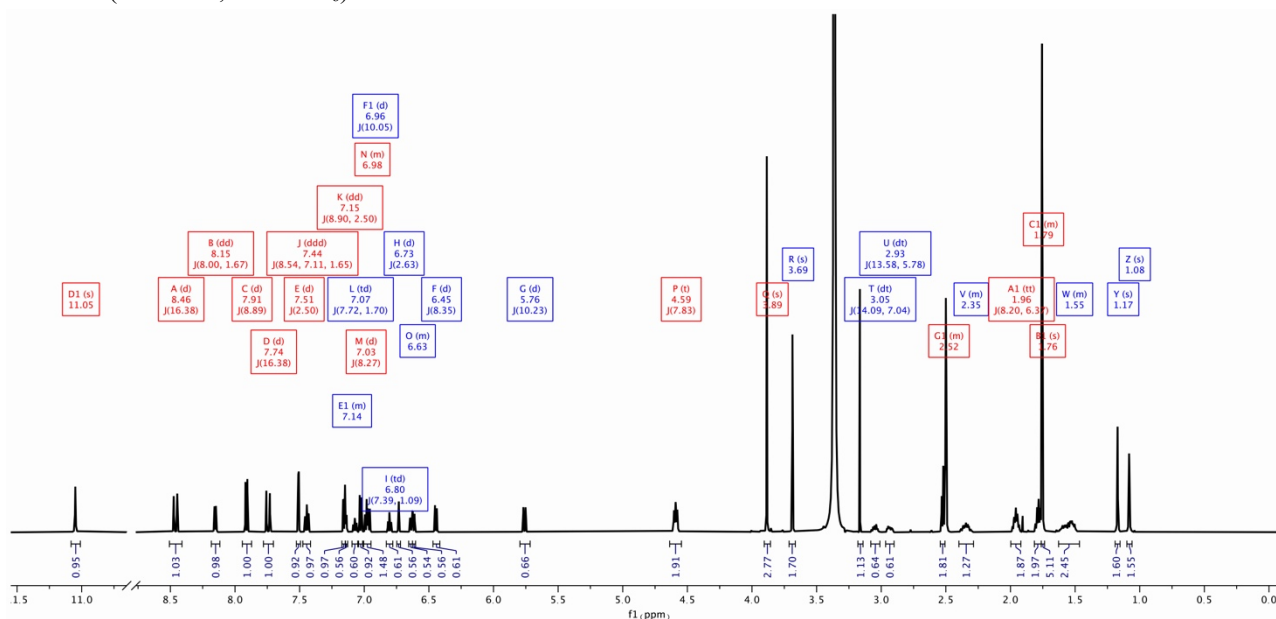
Spiropyran form

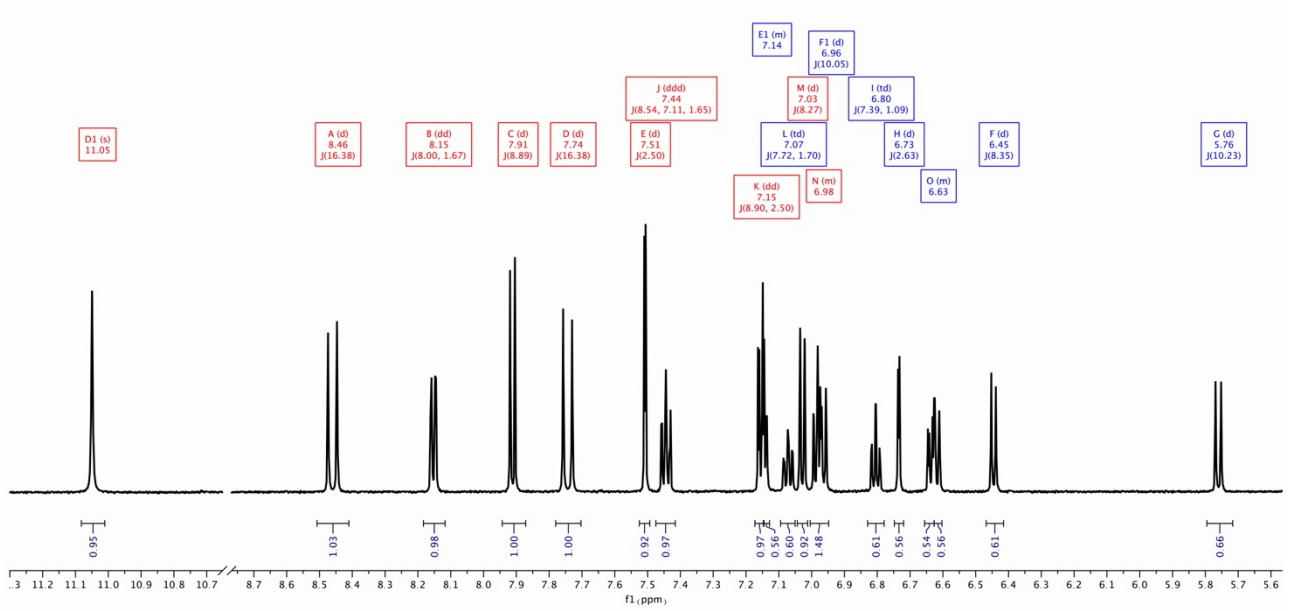
¹H NMR (600 MHz, DMSO-*d*₆) δ 7.15 – 7.13 (m, 1H, H^{e/c}), 7.07 (td, *J* = 7.7, 1.7 Hz, 1H, H^{e/c}), 6.96 (d, *J* = 10.0 Hz, 1H, H^s), 6.80 (td, *J* = 7.4, 1.1 Hz, 1H, H^{d/b}), 6.73 (d, *J* = 2.6 Hz, 1H, H^m), 6.65 – 6.60 (m, 2H, H^o, H^{d/b}), 6.45 (d, *J* = 8.4 Hz, 1H, H^p), 5.76 (d, *J* = 10.2 Hz, 1H, H^h), 3.69 (s, 3H, H^w), 3.05 (dt, *J* = 14.1, 7.0 Hz, 1H, H^r), 2.93 (dt, *J* = 13.6, 5.8 Hz, 1H, H^r), 2.41 – 2.27 (m, 2H, H^u), 1.64 – 1.45 (m, 4H, H^{s/t}), 1.17 (s, 3H, H^{k/k'}), 1.08 (s, 3H, H^{k/k'}).

¹³C{¹H} NMR (151 MHz, DMSO-*d*₆) δ 153.9 (C^a), 153.1 (Cⁿ), 141.5 (C^q), 137.7 (C^l), 129.7 (C^{e/c}), 129.0 (C^g), 126.9 (C^{e/c}), 120.0 (C^{d/b}), 119.8 (C^h), 118.5 (C^f), 114.4 (C^{d/b}), 111.3 (C^o), 109.5 (C^m), 106.3 (C^p), 104.8 (Cⁱ), 55.6 (C^w), 51.9 (C^j), 51.3 (C^u), 43.3 (C^r), 27.9 (C^{s/t}), 25.7 (C^{k/k'}), 22.8 (C^{s/t}), 19.7 (C^{k/k'}).

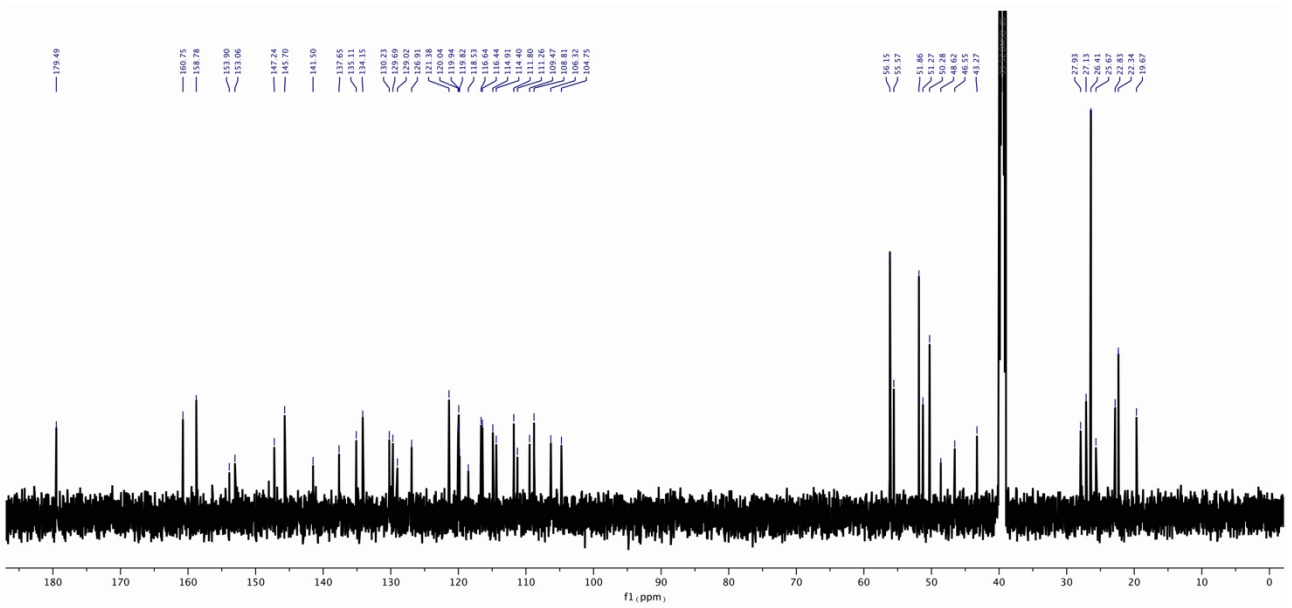
HRMS (ESI): calc. for C₂₃H₂₇NO₅S: [M–Na]⁺: 452.1502
found: [M–Na]⁺: 452.1502.

¹H NMR (600 MHz, DMSO-*d*₆):





¹³C NMR (151 MHz, DMSO-d₆):



S3. NMR studies

S3.1. NMR titration of 1

Aqueous samples of **1** (1 mM) containing 10% D₂O were prepared at different pH values with respective phosphate buffers (final phosphate concentration = 40 mM). At pH ~ 2 a mixture of the MCH form and *cis*-MCH form can be observed. The coupling constants of the alkene protons are characteristic for the individual species especially for the *cis*-MCH form which has previously been reported in the literature ($^3J_{cis} = 13.0$ Hz)^[3] and is in agreement with observed coupling constant of $^3J = 12.9$ Hz. At pH ~ 5 predominantly the MCH form can be observed. At pH ~ 7 the concentration of MCH is lower, and small amounts of MC and SP are present. At the highest pH value (~ 9) the merocyanine form is predominantly deprotonated resulting in mostly the MC and SP forms.

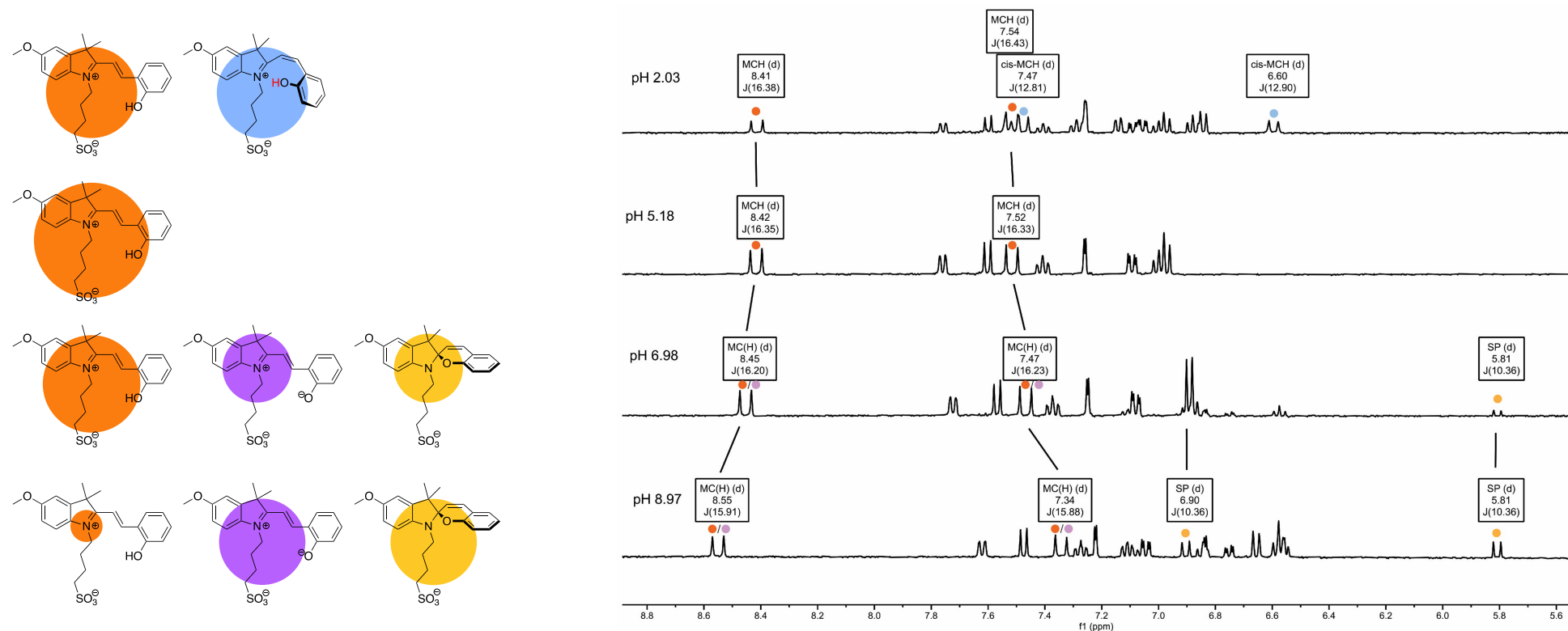


Figure S1. Representative distribution of species of **1** (left) for respective ¹H NMR (400 MHz, 10% D₂O in water, reduced power water suppression - zgpgpgpr) spectra at varying pH values (right). The alkene doublets and coupling constants are characteristic for the respective species and are highlighted with respective colours (*cis*-MCH, MCH, MC, SP).

Ratios of the individual species were determined by NMR by calculating the integral ratio of signals characteristic for the individual species: 6.60 ppm for *cis*-MCH, 8.41–8.55 for MC(H) and 5.81 ppm for SP. Additionally, ratios at respective pH values were calculated from pK_a and K_c values (determined by UV-vis absorbance titrations) following a reported method (see Eq. S1-3, ratios under light irradiation are calculated from Eq. S4-5).^[2] The ratios calculated from UV-vis and NMR measurements at different pH values (see Table S1) are in good agreement which suggests that the parameters determined at micromolar concentrations from UV-vis absorbance studies are also applicable at higher concentrations (1 mM).

$$\chi_{MCH} = \frac{[MCH]}{C} = \frac{1}{(K_c + 1) \cdot 10^{pH-pK_a} + 1} \quad (\text{Eq. S1})$$

$$\chi_{MC} = \frac{[MC]}{C} = \frac{1}{(K_c + 1) + \frac{1}{10^{pH-pK_a}}} \quad (\text{Eq. S2})$$

$$\chi_{SP} = \frac{[SP]}{C} = \frac{K_c}{(K_c + 1) + \frac{1}{10^{pH-pK_a}}} \quad (\text{Eq. S3})$$

$$\chi_{cis-MCH} = \frac{[cis-MCH]}{C} = \frac{1}{10^{pH-pK_a} + 1} \quad (\text{Eq. S4})$$

$$\chi_{SP_{hv}} = 1 - \chi_{cis-MCH} \quad (\text{Eq. S5})$$

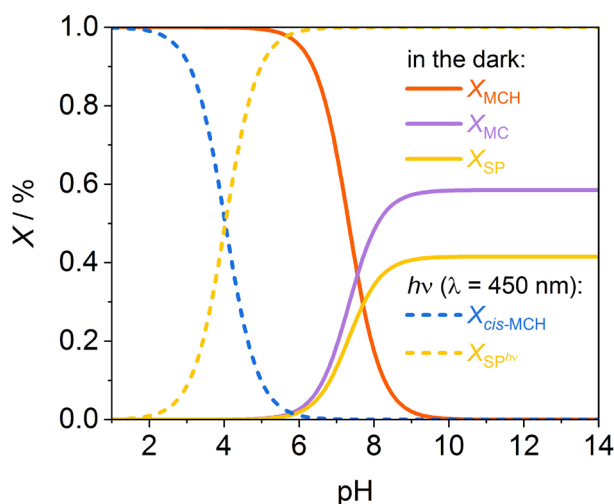


Figure S2. Overview of pH dependent mole fractions of MCH, MC, SP and *cis*-MCH calculated from Eq. S1-5.

Table S1. Comparison of ratios of individual species *cis*-MCH, MCH, MC and SP determined by NMR with ratios calculated from pK_a and K_c determined by UV-vis absorption.

| pH | Determined by NMR | | | | Calculated from parameters determined from UV-vis | | | |
|-----|-------------------|-----|----|----|---|-----|----|----|
| | <i>cis</i> -MCH | MCH | MC | SP | <i>cis</i> -MCH | MCH | MC | SP |
| 2.0 | 56 | 44 | – | – | – | – | – | – |
| 5.2 | – | 100 | – | – | – | 99 | 0 | 0 |
| 7.0 | – | 89 | – | 11 | – | 68 | 19 | 13 |
| | | | | | | 87 | | |
| 9.0 | – | 64 | – | 36 | – | 2 | 57 | 41 |
| | | | | | | 58 | | |

S3.2. Concentration dependent NMR of 1

Samples of increasing concentrations (0.8–3 mM) of **1** were prepared in D₂O. Increasing the concentration of **1** to approximately its solubility limit (3 mM) did not appear to result in aggregation of the MC(H) form. Since the equilibrium position of **1** is not significantly shifted towards the SP form we did not observe aggregation of the SP form as we did in an earlier study for concentrations above 1 mM.^[2]

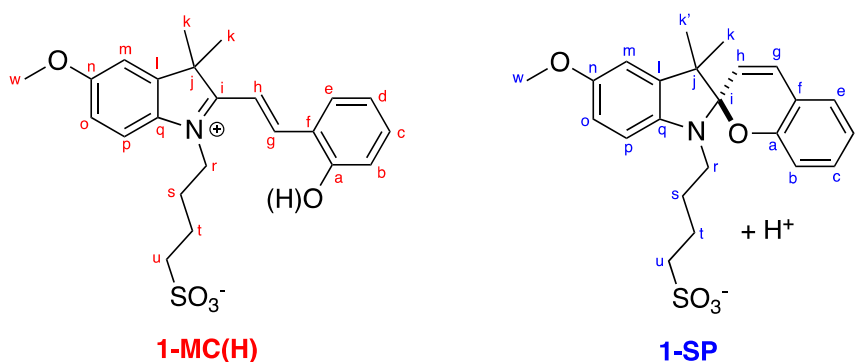


Figure S3. Structures of **1-MC(H)** and **1-SP** present at varying concentrations in the ¹H NMR spectra shown below.

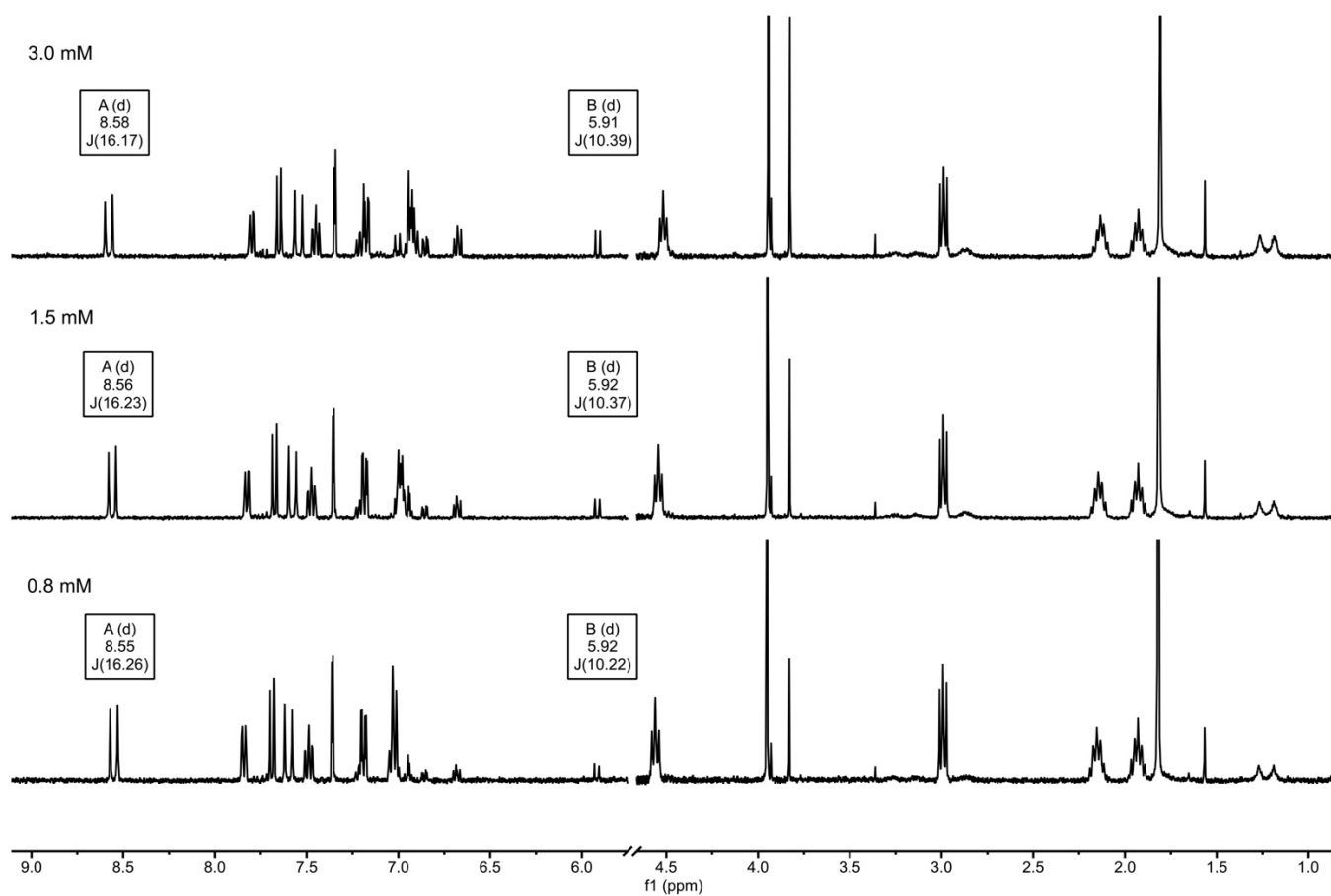


Figure S4. ¹H NMR (400 MHz, D₂O) spectra of different concentrations of **1**. The doublet labelled as A corresponds to proton **g** of **1-MC(H)** and the doublet labelled as B corresponds to proton **h** of **1-SP**.

S3.3. *In situ* irradiation ¹H NMR spectra at increasing pD values

Solutions of **1** (1 mM) in D₂O at increasing pD values were irradiated and monitored by ¹H NMR spectroscopy. The NMR *in situ* irradiation setup^[4] uses a fiber optic cable (Thorlabs, FT1500UMT) attached to a LUXEON Rebel color line LED light source (LXML-PR02-A900, Royal Blue) mounted to a heatsink. The fiber optic was fed into a screw cap NMR tube and inserted into the NMR spectrometer. The end of the fiber optic cable (ca. 3–4 cm) was roughened with sandpaper to ensure light emission across the entire sample volume. This setup allowed light irradiation during NMR acquisition.

The initial ¹H NMR spectra in the dark at increasing pD values are shown below. The ratio of the MC(H) to SP form is calculated based on the characteristic alkene integrals (A: proton **g** of **1-MC(H)**, B: proton **h** of **1-SP**) which are highlighted in the NMR stack plot. As expected, the SP ratio increased with increasing pD values.

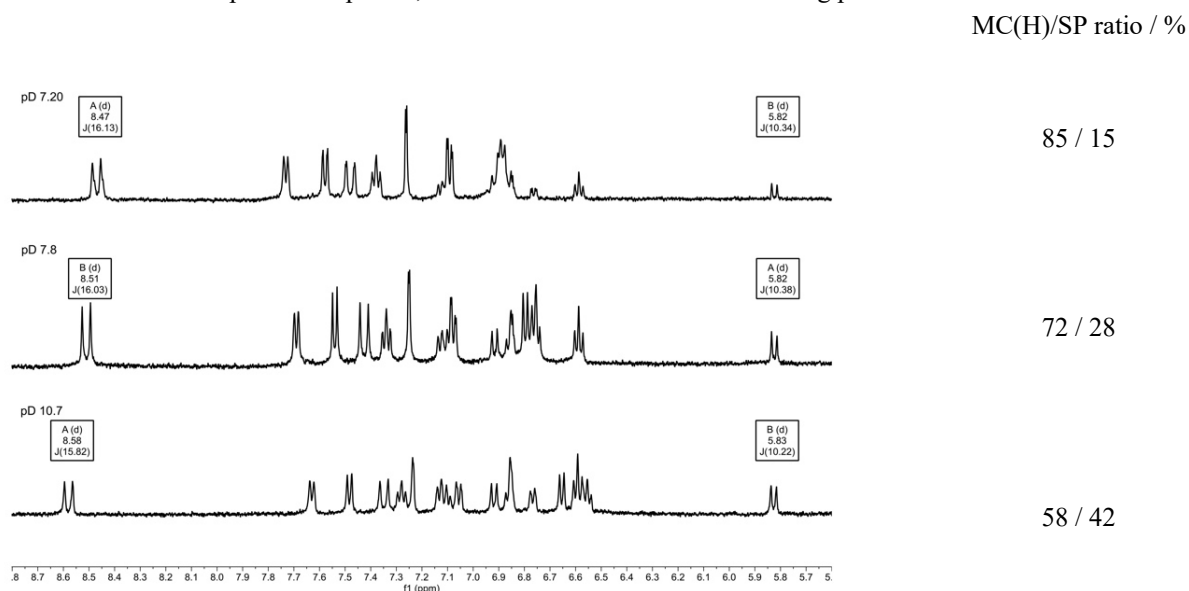


Figure S5. ¹H NMR (500 MHz, D₂O) spectra in the dark at respective pD values with corresponding MC(H)/SP ratio determined from highlighted integrals (A: proton **g** of **1-MC(H)**, B: proton **h** of **1-SP**).

When irradiated with 455 nm light the doublet at 8.47–8.58 ppm characteristic for the MC(H) form disappears for samples below pD 8 which indicates a complete bleach. For the sample above pD 10 the integral of the doublet at 8.59 ppm corresponding to the MC(H) form is slightly decreased. This resulted in a larger SP ratio (51%) compared to in the dark (42%) which indicates that the sample is partly bleached.

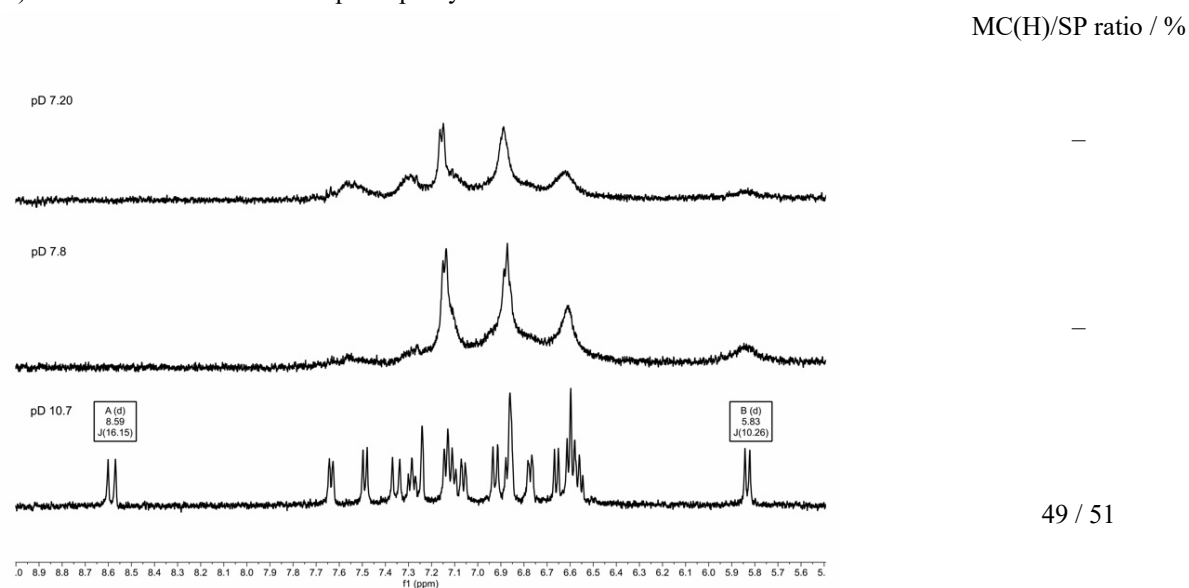


Figure S6. ¹H NMR (500 MHz, D₂O) spectra under *hν* ($\lambda = 455$ nm) labelled according to initial pD values. The MC(H)/SP ratio was determined from highlighted integrals (A: proton **g** of **1-MC(H)**, B: proton **h** of **1-SP**).

After irradiation with 455 nm light all samples recovered back to their initial distribution in the dark (see below).

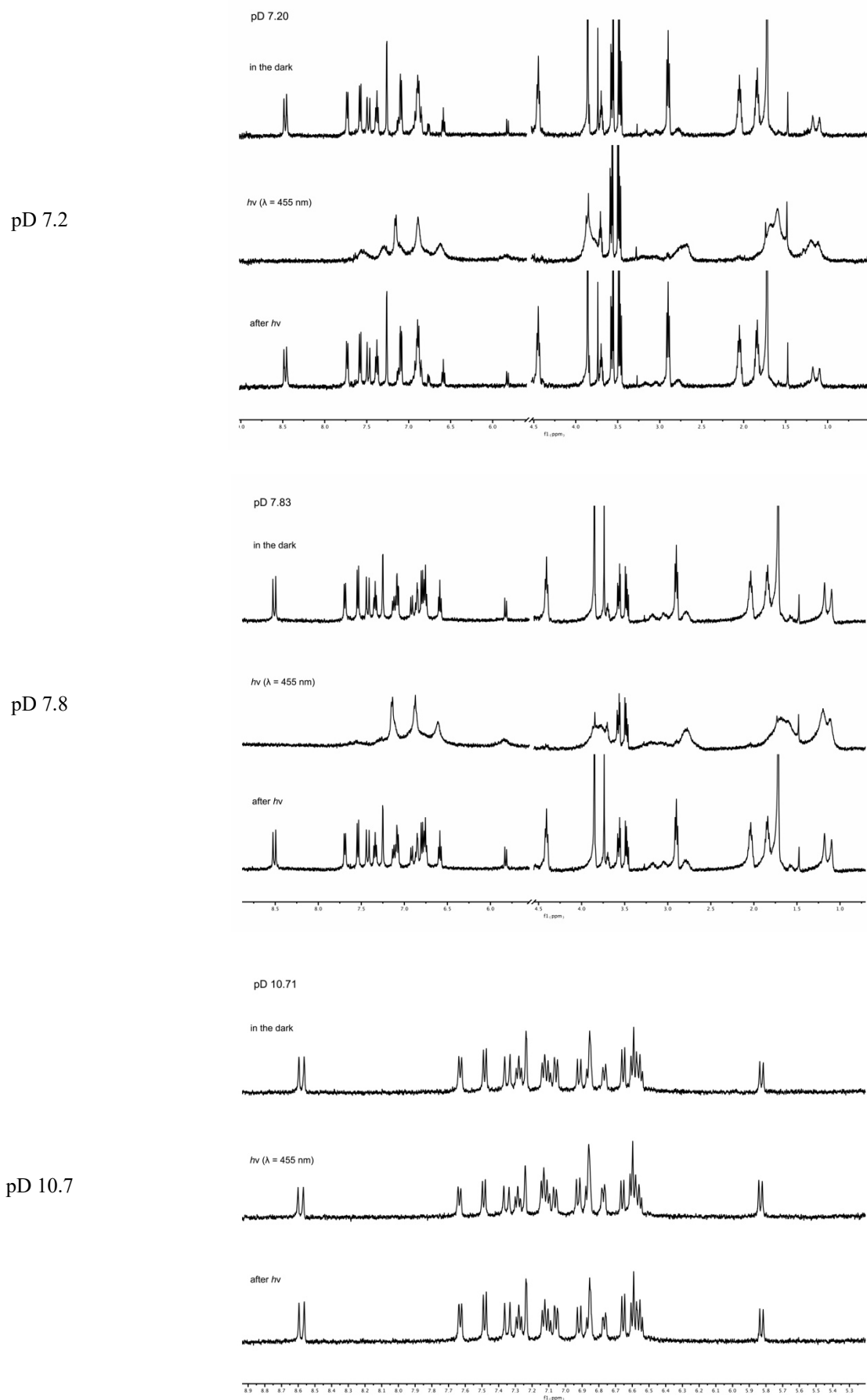


Figure S7. ^1H NMR (500 MHz, D_2O) spectra of **1** at increasing pD values (7.2-10.7). The respective top spectra were collected in the dark before irradiation, the middle spectra show the photo-stationary state under irradiation with 455 nm light, the bottom spectra show the fully recovered spectrum in the dark after irradiation.

The *in situ* irradiation in the NMR was monitored over time by collecting ^1H NMR (500 MHz, D_2O) spectra every 31 seconds. Initially spectra were collected in the dark (2 min) followed by 5.2 min of irradiation at 455 nm and recovery in the dark after irradiation. Normalized integrals characteristic for the MC(H) (8.47–8.64 ppm) and SP (5.88–5.89 ppm) form were chosen to follow the bleaching process over time and are shown in Figure S8. For pD values 7.20 and 7.83 the samples appear to be fully bleached whereas at pD 10.7 no significant change is observed under light irradiation as indicated earlier (51% SP in the dark vs 42% SP under $h\nu$).

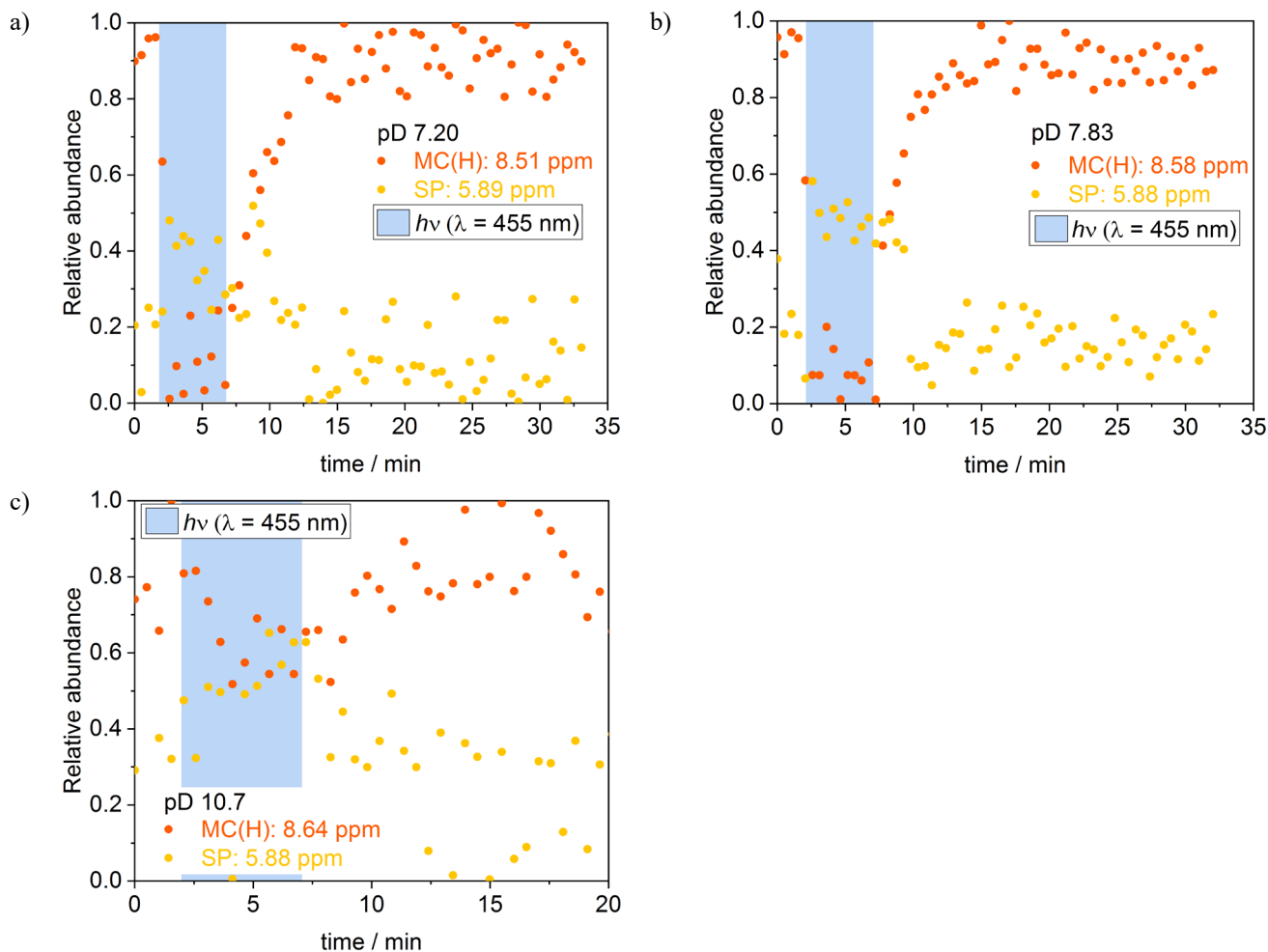


Figure S8. Kinetic traces following the *in situ* irradiation ($\lambda = 455$ nm, indicated by blue box) in the NMR at a) pD 7.20, b) pD 7.83 and c) pD 10.7. The respective normalized integrals characteristic for the MC(H) form at 8.5-8.6 ppm (orange) and the SP form at 5.9 ppm (yellow) are shown over time and were extracted from the respective ^1H NMR spectra (500 MHz, D_2O).

S4. UV-Vis studies

S4.1. General procedure

Buffer solutions ranging from pH 2.5–10 were prepared by adding KOH (10 M) to H₃PO₃ (100 mM) solutions and pH 2.0–2.5 by adding standard HCl. The pH of the prepared solutions was measured with a *SPEER* benchtop meter with a *ThermoFisher Scientific* Orion™ PerpHecT™ ROSS™ combination pH microelectrode.

A stock solution of **1** (*c* = 5.05 mM) was prepared with anhydrous methanol filtered through syringe filters (0.45 μm) and stored in a 10 mL volumetric flask wrapped in aluminum foil at 4 °C.

The stock solution was diluted with milliQ water to give 10% MeOH v/v (*c* ~500 μM).

UV-vis samples preparation. In a 1 cm cuvette 600 μL of a phosphate buffer of respective pH was mixed with 1800 μL of milliQ water, put in the spectrometer and let equilibrate thermally for 5 min. The acquisition was started and 450 μL of a premixed solution of milliQ water with 10% MeOH v/v dilution was added *in situ*, mixed by gentle pipetting and continuous stirring of the solution. Scans were collected until no further noticeable change in the spectrum occurred (20 min to ~3 h, pH dependent). The last spectrum of the scanning collection was used as the equilibrated spectrum in the dark.

For measurements of the *cis*-MCH and spiropyran forms, spectra were recorded under continuous irradiation (λ = 455 nm). For most pH values (except pH > 9) the equilibration was almost immediate and no change in the spectrum was observed over time. The last spectrum of the scanning collection under irradiation was used as the equilibrated spectrum in the light.

S4.2. Setup for visible irradiation experiments (λ = 455 nm)

UV-Vis absorbance spectroscopy experiments were performed on an Agilent Cary 60 Bio UV-visible spectrophotometer equipped with a customized Cary Single Cell Peltier Accessory, maintaining the samples at a set temperature. The cell holder was modified to allow for irradiation perpendicular to the direction of measurement, as previously described.^[4] A Luxeon Rebel LED was mounted on a heat sink positioned 4 cm away from the cell and driven using a 1000 mA LuxDrive PowerPuck. A Carclo 20.0 mm Fibre Coupling Lens was used to focus the beam. Samples were stirred to ensure homogeneity and a timer relay module (FRM01) was used to control the irradiation cycles.

Table S2. Technical information for LUXEON Rebel color line LED light source (<https://www.lumileds.com/wp-content/uploads/files/DS68.pdf>).

| LED part number | Descriptor maximum emission wavelength / nm | Dominant wavelength / nm | | Radiometric power / mW | | Test current / mA |
|-----------------------------|---|--------------------------|---------|------------------------|---------|-------------------|
| | | Minimum | Maximum | Minimum | Typical | |
| LXML-PR02-A900 (Royal Blue) | 455 | 440 | 460 | 900 | 1030 | 700 |

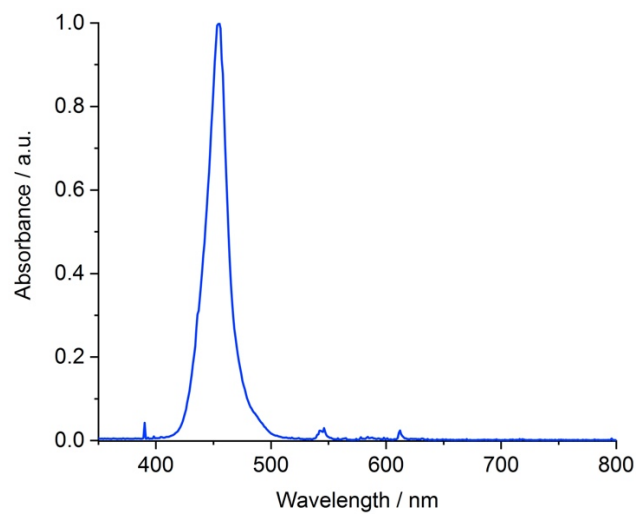


Figure S9. Emission spectrum of blue LED light source ($\lambda_{\text{max}} = 455 \text{ nm}$) for UV-vis irradiation experiments.

S4.3. pK_a^{dark} and $pK_a^{h\nu}$ determination

The observed pK_a values in the dark (pK_a^{dark}) and under irradiation ($pK_a^{h\nu}$) were determined using UV-vis spectroscopy based on previously reported methods.^[2-3] Absorption spectra of equilibrated samples were recorded over the pH range in the dark and under irradiation with 455 nm light and are shown in Figure S10 a) and b). The characteristic absorbance maxima of the respective species were plotted over the pH range (Figure S10 c, d) and fitted to a sigmoidal function using origin:

$$A_{\text{eq}} = A_{\text{OH}} + \frac{A_{\text{H}} - A_{\text{OH}}}{1 + \exp\left(\frac{\text{pH} - pK_a^{\text{dark}/h\nu}}{P}\right)} \quad (\text{Eq. S6})$$

A_{eq} : absorbance at equilibrium

A_{OH} : absorbance at lowest recorded pH

A_{H} : absorbance at highest recorded pH

$pK_a^{\text{dark}/h\nu}$ approximate inflection point from plot

P = fitting factor

The reported pK_a^{dark} and $pK_a^{h\nu}$ values are averaged and obtained from duplicate experiments. The reported errors are from standard deviation.

$$pK_a^{\text{dark}} = 7.33 \pm 0.01$$

$$pK_a^{h\nu} = 4.03 \pm 0.03$$

The previously defined photoacidity Π parameter was then calculated as:

$$\Pi = pK_a^{\text{dark}} - pK_a^{h\nu} = 3.29 \pm 0.03 \quad (\text{Eq. S7})$$

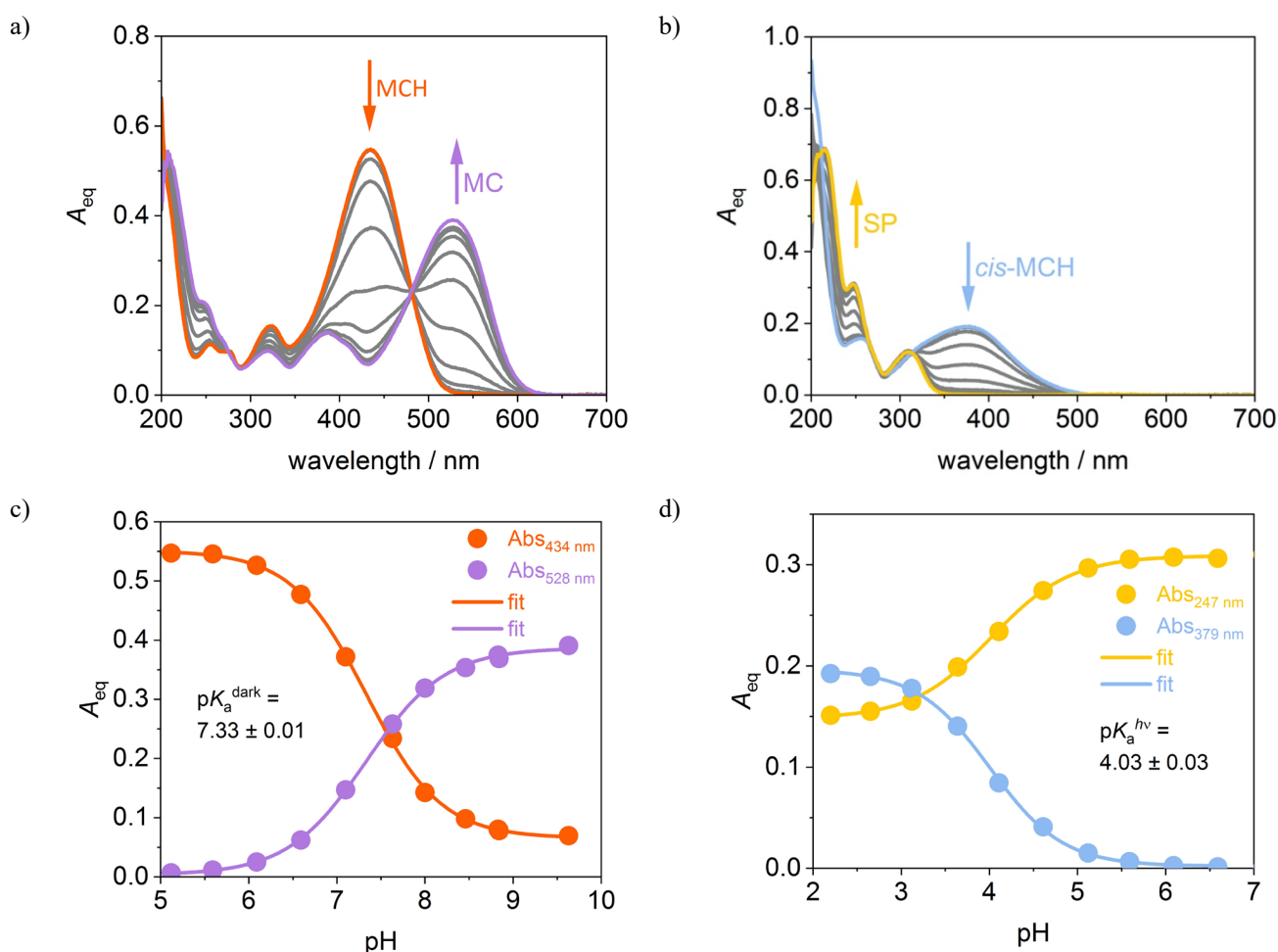


Figure S10. UV-vis absorption titrations of photoacid **1**. a) Equilibrated absorbance spectra at different pH values in the dark (orange: pH = 5.1, purple: pH = 9.6). b) Change in absorbance maxima of **1-MCH** (orange, $\lambda = 434$ nm) and **1-MC** (purple, $\lambda = 528$ nm) over the pH range with corresponding fits to Eq. S6 shown as solid lines. Experimental conditions: $[1] = 20.0 \mu\text{M}$, [phosphate buffer] = 20 mM, pH 5.1-9.6, $T = 22$ °C. c) Equilibrated absorbance spectra at different pH values (blue: pH = 2.2, yellow: pH = 8.8) under continuous blue light irradiation ($\lambda = 455$ nm). d) Change in absorbance maxima of **1-SP** (yellow, $\lambda = 247$ nm) and **1-cis-MCH** (blue, $\lambda = 379$ nm) over the pH range with corresponding fits to Eq. S6 shown as solid lines. Experimental conditions: $h\nu$ ($\lambda = 455$ nm), $[1] = 20.0 \mu\text{M}$, [phosphate buffer] = 20 mM, pH 2.2-8.8, $T = 22$ °C.

S4.4. Determination of kinetic parameters and pK_a

Kinetic parameters were determined by monitoring the equilibration process at varying pH values using UV-vis spectroscopy. The change of the maximum absorbance of the MCH form and MC form were monitored over time (Figure S11 a, b). These kinetic traces were fit to a single exponential function:

$$A_t = A_{eq} + (A_0 - A_{eq})e^{-k_{obs,eq}t} \quad (\text{Eq. S8})$$

A_t : time dependent absorbance

A_{eq} : absorbance at equilibrium

A_0 : absorbance at time = 0

The observed rate constants k_{obs} are obtained from fitting the data to Eq. S8 and are averaged over duplicate experiments. Errors given are from standard deviation. The change of k_{obs} over the pH range is shown in Figure S11 c). As reported previously^[2-3] k_{obs} is equal to the rate of ring opening k_{open} where the pH value is $< pK_a^{dark}$ and $> pK_a^{hv}$. At $pH \gg pK_a^{dark}$, the observed rate constant is equal to the sum of ring opening and ring closing rate constants ($k_{open} + k_{close}$). The equilibrium constant K_c can then be calculated as:

$$K_c = \frac{k_{close}}{k_{open}} \quad (\text{Eq. S9})$$

Determined rate constants:

$$k_{open} = 0.33 \pm 0.07 \times 10^{-2} \text{ s}^{-1} \quad (\text{pH 5.1})$$

$$k_{close} + k_{open} = 0.57 \pm 0.11 \times 10^{-2} \text{ s}^{-1} \quad (\text{pH 8.9})$$

$$k_{close} = 0.24 \pm 0.14 \times 10^{-2} \text{ s}^{-1}$$

$$K_c = 0.71 \pm 0.44$$

The pK_a can then be calculated using Eq. S10:^[2]

$$pK_a = pK_a^{dark} + \log(1 + K_c) \quad (\text{Eq. S10})$$

$$pK_a = 7.56 \pm 0.11$$

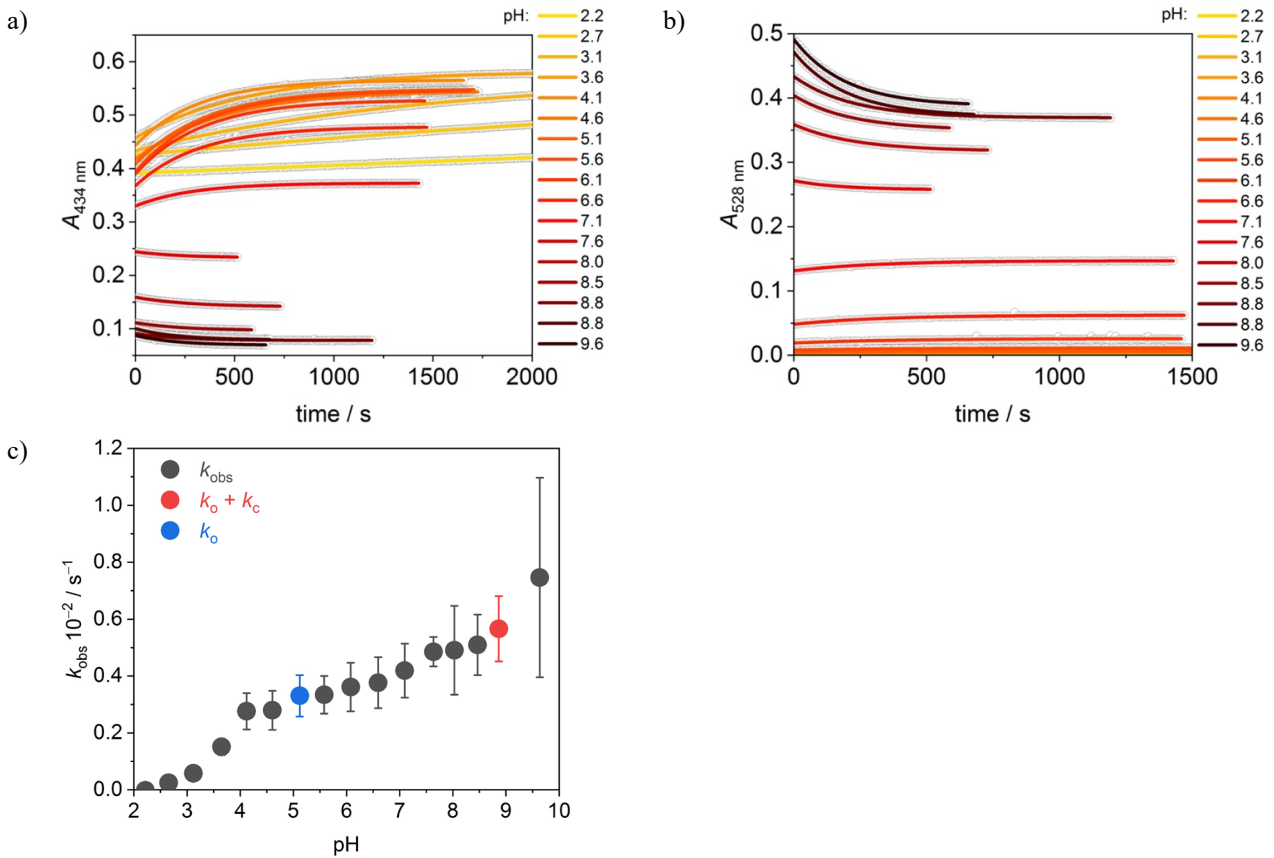


Figure S11. Kinetic parameters of photoacid **1**. An aqueous sample of the photoacid **1** was added to respective buffer solution *in situ* to monitor equilibration processes. a) Kinetic profiles for the equilibration of MCH ($\lambda = 434 \text{ nm}$) at different pH (yellow = low pH, dark red = high pH) with corresponding exponential fits (Eq. S8) depicted as solid lines. Experimental conditions: $[1] = 20.0 \mu\text{M}$, [phosphate buffer] = 20 mM, pH 2.2-9.6, $T = 22 \text{ }^\circ\text{C}$. b) Kinetic profiles for the equilibration of MC ($\lambda = 528 \text{ nm}$) at different pH (yellow = low pH, dark red = high pH) with corresponding exponential fits (Eq. S8) depicted as solid lines. Experimental conditions: $[1] = 20.0 \mu\text{M}$, [phosphate buffer] = 20 mM, pH 2.2-9.6, $T = 22 \text{ }^\circ\text{C}$. c) Averaged observed rate constants k_{obs} from duplicate experiments obtained from the exponential fit to Eq. S8 in a) (error bars from standard deviation) with $k_o + k_c$ highlighted in red and k_o highlighted in blue.

Table S3. k_{obs} values from Figure S11 c) with respective half-lives ($t_{1/2} = \ln 2/k_{\text{obs}}$).

| pH | $k_{\text{obs}} 10^{-2} / \text{s}^{-1}$ | $t_{1/2} / \text{min}$ |
|------|--|------------------------|
| 2.22 | 0.00 ± 0.00 | – |
| 2.66 | 0.02 ± 0.01 | 46.3 |
| 3.12 | 0.06 ± 0.01 | 19.9 |
| 3.65 | 0.15 ± 0.03 | 7.7 |
| 4.12 | 0.28 ± 0.06 | 4.2 |
| 4.61 | 0.28 ± 0.07 | 4.1 |
| 5.12 | 0.33 ± 0.07 | 3.5 |
| 5.58 | 0.33 ± 0.07 | 3.5 |
| 6.08 | 0.36 ± 0.09 | 3.2 |
| 6.60 | 0.38 ± 0.09 | 3.1 |
| 7.10 | 0.42 ± 0.09 | 2.8 |
| 7.64 | 0.49 ± 0.05 | 2.4 |
| 8.03 | 0.49 ± 0.16 | 2.4 |
| 8.47 | 0.51 ± 0.11 | 2.3 |
| 8.87 | 0.57 ± 0.11 | 2.0 |
| 9.64 | 0.75 ± 0.35 | 1.5 |

S4.5. Extinction coefficients

By choosing specific experimental conditions pure absorbance spectra of the SP and MCH form could be obtained. Experimental conditions are given below Figure S12. At high pH an absorbance spectrum of a mixture of the SP and MC was obtained and used to calculate the extinction coefficients of the MC form ($\varepsilon_{MC,\lambda}$) using Eq. S11:^[3]

$$\varepsilon_{MC,\lambda} = \frac{A_{\text{eq}} - \varepsilon_{SP,\lambda} \chi_{SP} C}{\chi_{MC} C} \quad (\text{Eq. S11})$$

A_{eq} : absorbance at equilibrium

$\varepsilon_{SP,\lambda}$: extinction coefficient of the SP form

χ_{SP} : mole fraction of the SP form

χ_{MC} : mole fraction of the MC form

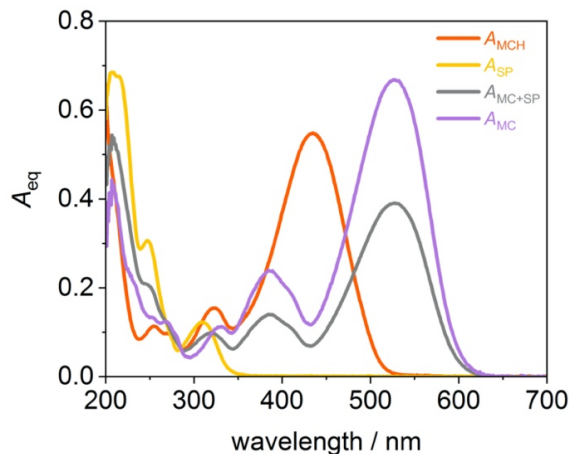


Figure S12. Absorbance spectra of SP (yellow), MCH (orange), an equilibrated absorbance spectrum at high pH containing SP and MC (grey) used to calculate spectrum of MC (purple) with Eq. S12. Experimental conditions: $[I] = 20.0 \mu\text{M}$, [phosphate buffer] = 20 mM, $T = 22 \text{ }^\circ\text{C}$.

| A_{SP} under $h\nu$ ($\lambda = 455 \text{ nm}$) | A_{MCH} (dark) | A_{SP+MC} (dark) | $A_{MC}^{[3]}$ |
|--|------------------|--------------------|---|
| pH 6.6 | pH 5.1 | pH 9.6 | $A_{MC}^\lambda = \frac{A_{\text{eq}}^\lambda - A_{SP}^\lambda \chi_{SP}}{\chi_{MC}}$ where $A_{SP}^\lambda = \varepsilon_{SP}^\lambda C$ (Eq. S12) |

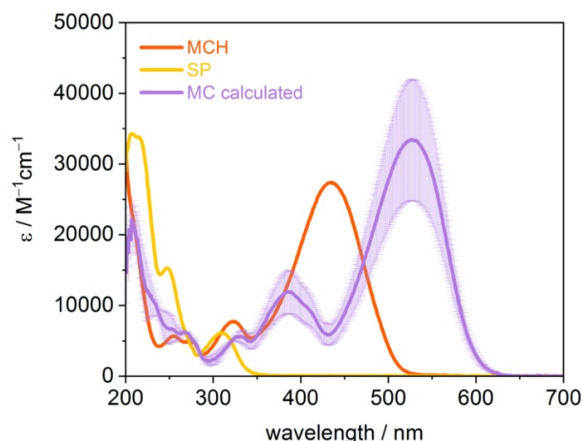


Figure S13. Extinction coefficients of the respective species showing error bars ($\delta\varepsilon_{MC,\lambda_{max}}$) for the calculated MC extinction coefficients (Eq. S11) which were determined by error propagation: Errors of A_{eq} , $\varepsilon_{SP,\lambda_{max}}$, C assumed to be negligible compared to the error from K_c :

$$\delta\varepsilon_{MC,\lambda_{max}} = \sqrt{\left(\frac{\partial\varepsilon_{MC,\lambda_{max}}}{\partial K_c}\right)^2} (\delta K_c)^2 = \sqrt{\left(\frac{A_{eq}}{C} - \varepsilon_{SP,\lambda_{max}}\right)^2} (\delta K_c)^2$$

Table S4. Overview of extinction coefficients of the SP, MCH and MC form at selected absorbance maxima characteristic for the individual species

| λ / nm | $\varepsilon_{SP} / 10^3 \times \text{L mol}^{-1}\text{cm}^{-1}$ | $\varepsilon_{MCH} / 10^3 \times \text{L mol}^{-1}\text{cm}^{-1}$ | $\varepsilon_{MC} / 10^3 \times \text{L mol}^{-1}\text{cm}^{-1}$ |
|----------------|--|---|--|
| 247 | 15.3 | 5.0 | 6.8 ± 2.2 |
| 434 | – | 27.4 | 5.9 ± 1.5 |
| 528 | – | – | 33.4 ± 8.6 |

S5. pH switching

Concentrated solutions of **1** (2-3 mM) were prepared in 20 mM aqueous KCl solution. Less concentrated samples were prepared by diluting the concentrated solution by a factor of 10.

S5.1. Penn PhD photoreactor

The sample was irradiated in a *Penn PhD* photoreactor m2 with 10 s of pre-stirring, 30–60 s of irradiation with 450 nm light at full power followed by stirring in the dark. The cooling fan was set to its maximum capacity. Using the internal temperature control of the photoreactor in combination with a photocoupler resulted in larger temperature spikes under irradiation compared to setting the fan to full speed.

pH switching at different initial pH values

The pH value was adjusted by adding 0.7–2 μL of aqueous KOH (1 M) to 3.2 mL of a solution of **1** and letting the sample equilibrate for a minimum of five half-lives (changes depending on the pH). The corresponding amounts of base added resulting in $\text{pH}_{\text{initial}}$ values and the pH values reached under irradiation are shown in Table S5-6.

Table S5. pH adjustments of a concentrated solution of **1** (2.4 mM) and respective light induced pH changes.

| # | V of 1 M KOH added / μL | Sum of equivalents of KOH added | $\text{pH}_{\text{initial}}$ | pH_{hv} | ΔpH |
|---|------------------------------------|---------------------------------|------------------------------|-------------------------|-------------------|
| 1 | – | – | 7.24 | 3.74 | 3.50 |
| 2 | 0.7 | 0.0002 | 7.33 | 3.85 | 3.48 |
| 3 | 1.5 | 0.0007 | 7.48 | 4.04 | 3.44 |
| 4 | 1.5 | 0.0012 | 7.55 | 4.20 | 3.35 |
| 5 | 2.0 | 0.0019 | 7.82 | 4.59 | 3.23 |
| 6 | 2.0 | 0.0026 | 8.31 | 5.74 | 2.57 |
| 7 | 1.0 | 0.0029 | 8.67 | 7.78 | 0.89 |

Table S6. pH adjustments of a diluted solution of **1** (0.21 mM) and respective light induced pH changes.

| # | V of 0.1 M KOH added / μL | Sum of equivalents of KOH added | $\text{pH}_{\text{initial}}$ | $\text{pH}_{h\nu}$ | ΔpH |
|---|--------------------------------------|---------------------------------|------------------------------|--------------------|-------------------|
| 1 | – | – | 7.00 | 4.29 | 2.71 |
| 2 | 0.7 | 0.0002 | 7.12 | 4.41 | 2.71 |
| 3 | 1.5 | 0.0007 | 7.27 | 4.67 | 2.60 |
| 4 | 1.5 | 0.0012 | 7.41 | 5.01 | 2.40 |
| 5 | 2.0 | 0.0019 | 7.56 | 5.74 | 1.82 |
| 6 | 2.0 | 0.0026 | 7.75 | 6.37 | 1.38 |
| 7 | 1.0 | 0.0029 | 7.78 | 6.56 | 1.22 |
| 8 | 2.0 | 0.0036 | 7.92 | 6.98 | 0.94 |
| 9 | 2.0 | 0.0042 | 8.14 | 7.62 | 0.52 |

The change in pH and temperature over time of experiments listed in Tables S5-6 are shown in Figure S14.

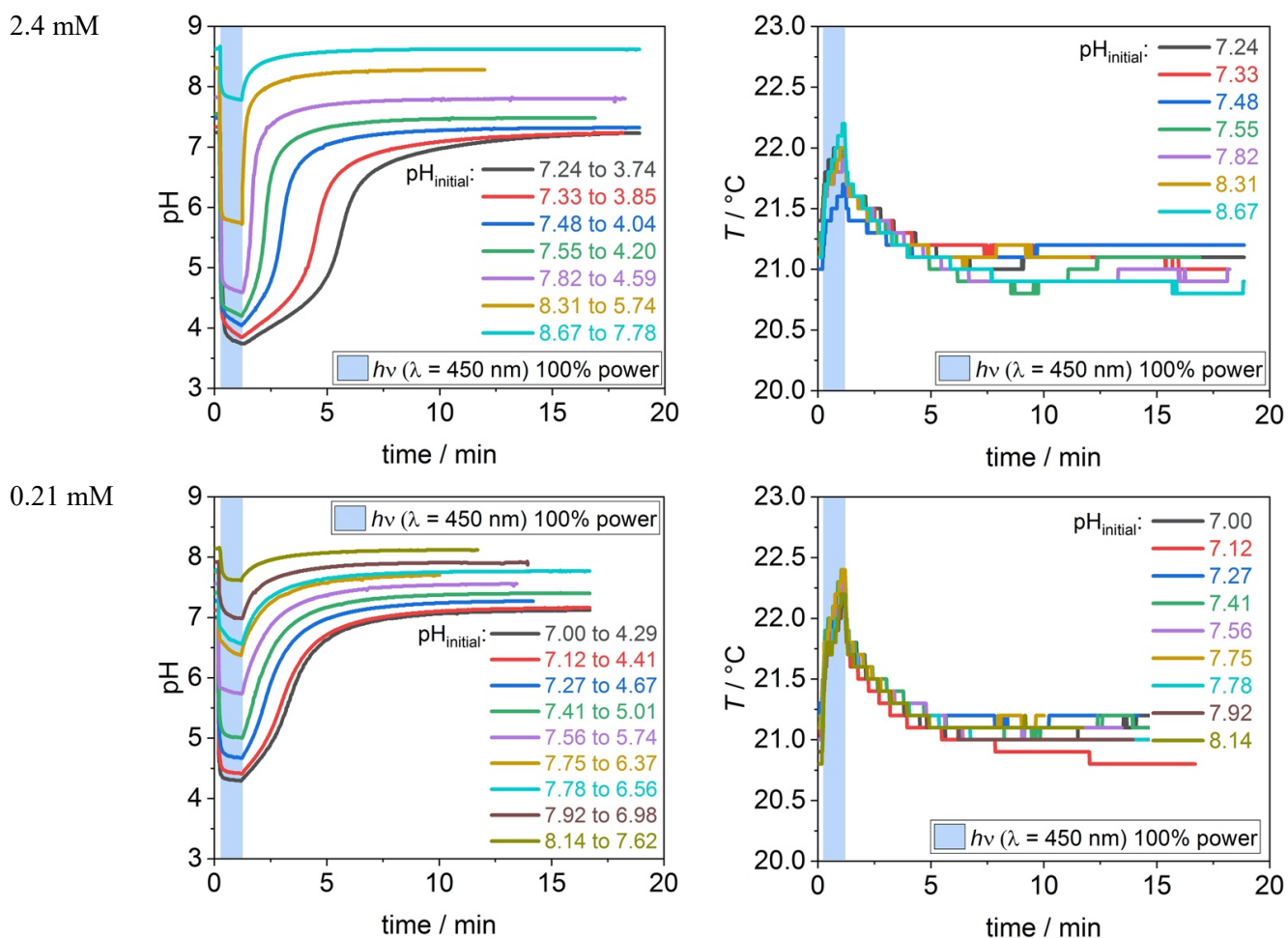
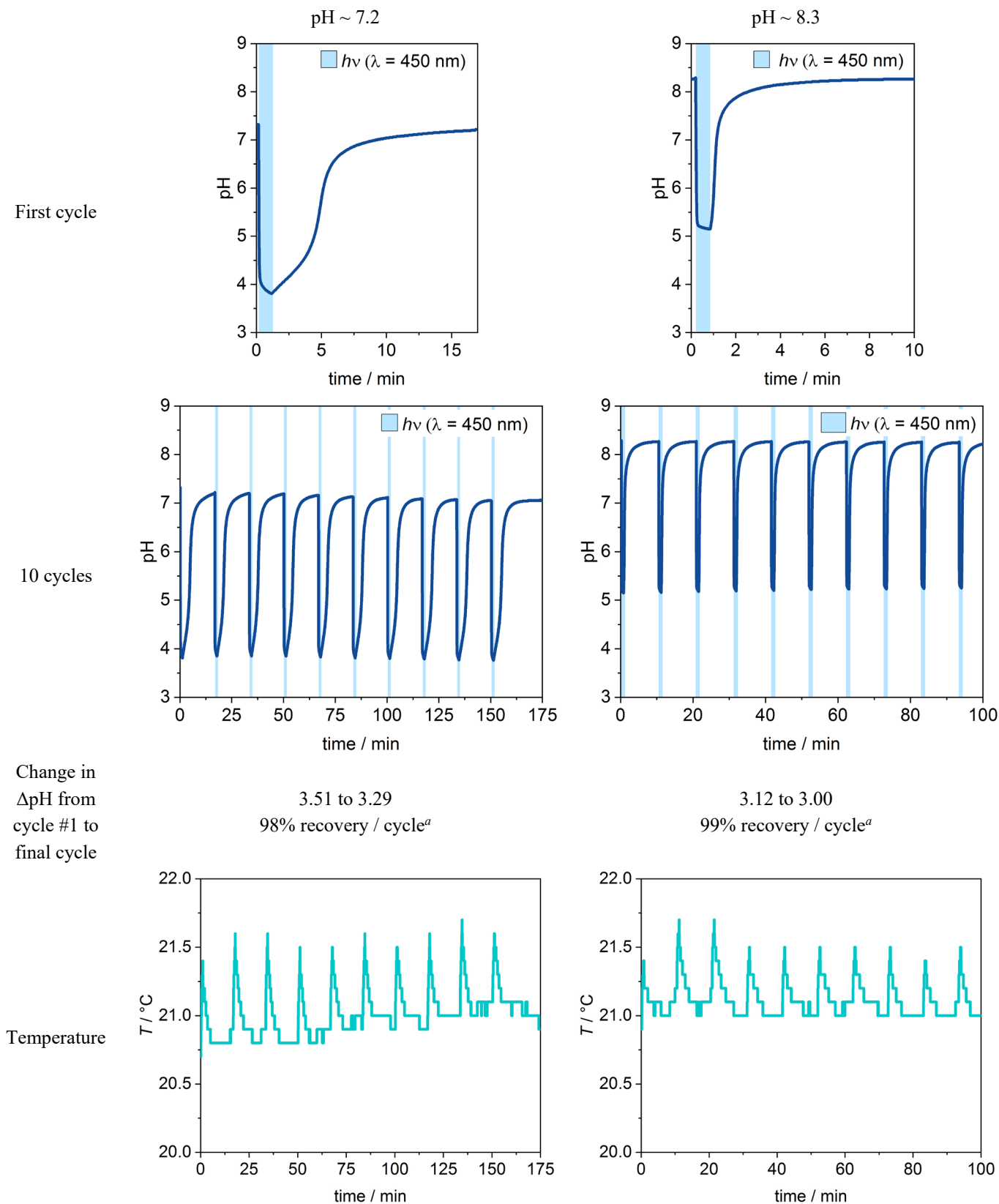


Figure S14. pH switching of a solution of **1** (2.4 mM, top and 0.21 mM bottom): pH trace over time at different initial pH values (left) and respective temperature profiles of measurements (right).

Repeated pH switching cycles

To measure the fatigue resistance of **1**: the initial pH with the maximum pH jump (pH ~ 7.2) and the highest initial pH with significant switching (pH ~ 8.3). We measure ten consecutive cycles by restarting the program of the photoreactor immediately after finishing. The first irradiation cycle followed by recovery of the pH is shown in Table S7 (top) as well as consecutive switching cycles (bottom). At both pH values we observed a high percentage of recovery per switching cycle (based on the pH).

Table S7. pH switching experiments of photoacid **1** in Penn PhD photoreactor. Experimental conditions: [1] = 3.0 mM in aqueous 20 mM KCl with addition of 0.7 μ L (for pH ~ 7.2) or 7 μ L (for pH ~ 8.3) of 1.0 M KOH respectively.



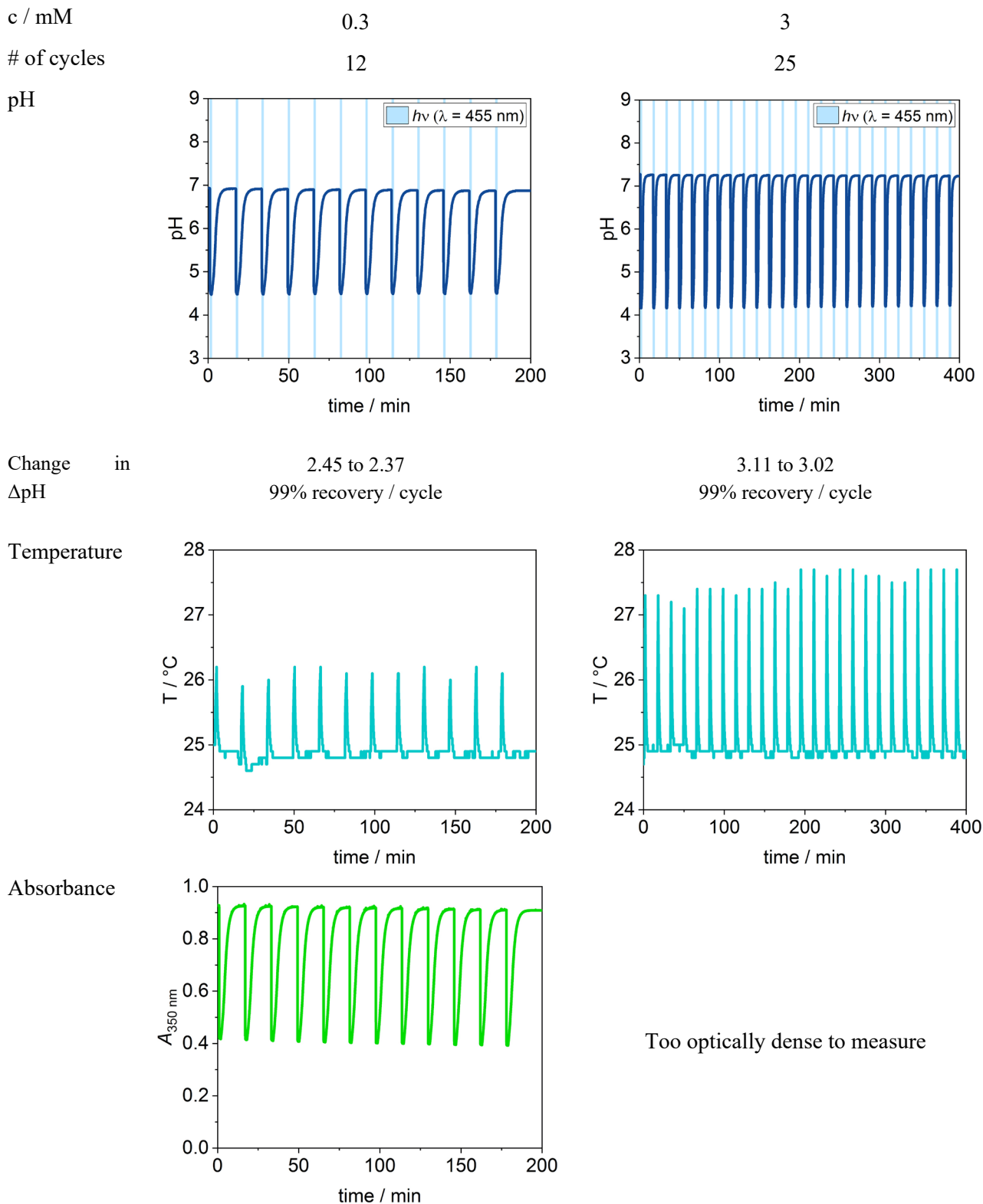
$$a) \%recovery/cycle = \left(\frac{\Delta pH_{final\ cycle}}{\Delta pH_{first\ cycle}} \right)^{\frac{1}{\#\ of\ cycles}}$$

S5.2. Cary 60 LED setup

The light irradiation setup in the Cary60 UV-vis spectrometer (as described in section S4.2) was used to increase the number of pH switching cycles by automation. In addition to pH and temperature the change in absorbance of a dilute sample (0.3 mM) was monitored. Despite using the temperature control spikes in temperature during the irradiation time could not be avoided.

Repeated pH switching cycles

Table S8. Light induced ($\lambda = 455$ nm) pH switching of **1** in a 1 cm cuvette at 25°C: changes in pH, temperature, and absorbance of a dilute (0.3 mM) and concentrated (3 mM) sample monitored simultaneously. The pH and temperature were constant after the irradiation cycles.



Variable temperature

The effect of temperature on the pH change and equilibrium position was investigated at 22°C, 25°C and 28°C (Figure S15) of a 0.3 mM solution of **1**. As to be expected the lowest temperature resulted in the slowest pH recovery after irradiation and the highest temperature resulted in the fastest pH recovery (18 vs 7 min). The equilibrium positions appear to be slightly shifted with the highest temperature having a lower equilibrium pH and the lowest temperature having the highest equilibrium pH. However, the equilibrium of the lowest and the highest temperature only differ by 0.05 pH units.

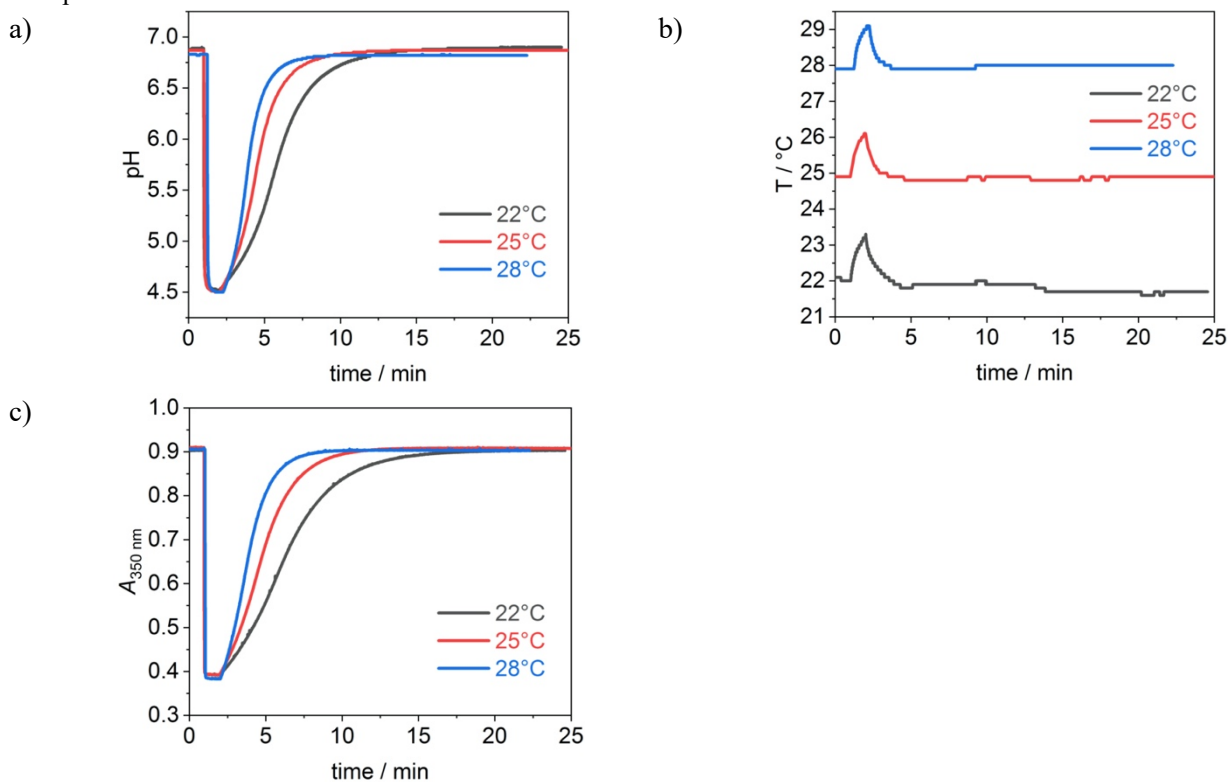


Figure S15. pH switching of a dilute sample of **1** (0.3 mM) at different temperatures in the Cary 60. a) pH change over time at 22, 25 and 28°C, b) Temperature change over time at initial temperatures of 22, 25 and 28°C, c) Change in absorbance at 350 nm over time at 22, 25 and 28°C.

S6. Bibliography

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