SUPPORTING INFORMATION

Light-induced cargo release from a cucurbit[8]uril host by means of a sequential logic operation

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1. Materials and methods

All solvents and chemicals employed for synthesis and for preparation of samples were of reagent or spectrophotometric grade and used as received. Water was Millipore grade. Cucurbit[8]uril was purchased from Aldrich. UV/vis titration experiments at pH 1 were done by addition of CB8 stock solution (compensated with the same flavylium cation concentration as in the cuvette) to flavylium cation **2** (18 μ M) in 1-cm quartz cuvettes. Fluorescence titration were performed in the same way but with a lower concentration of flavylium cation (4 μ M).

The pH of the citrate-buffered solutions was adjusted with HCl or NaOH and monitored with a pH meter (model HI221, Hanna Instruments). NMR experiments were performed at 25 °C on a Bruker AMX 400 spectrometer operating at 400 MHz (¹H) and 101 MHz (¹³C). The irradiation experiments were done at 365 nm with a TLC 24W lamp (typically $I_{abs} = 4.4 \times 10^{-8}$ mol photons s⁻¹). The UV/vis-absorption spectra were recorded with a Shimadzu UV-1603 or a Varian Cary 50 spectrophotometer and the fluorescence was detected with a Varian Cary Eclipse fluorimeter. Photoreaction quantum yields were determined with the aid of the ferrioxalate actinometer.¹

Flash photolysis experiments were performed on a Varian Cary 5000 spectrophotometer equipped with a Harrick FiberMate interface/fiber optic probe coupled to an Ocean Optics 4-way cuvette holder. The compartment was isolated from external light and a flash from a commercially available Achiever 630AF camera (Hong Kong, China) was used as a pulsed white light source (placed in close contact with the 10×2 mm quartz cuvette). The optical density was monitored along the 10 mm optical path and pulsed excitation through the 2 mm optical path to reduce diffusion effects.²

2. Synthesis of chalcone 1



4'-(1-Sulfo-4-butyloxy)-acetophenone sodium salt³ (0.48 g, 1.7 mmol) and 2-hydroxy-4-methoxybenzaldehyde (0.25 g, 1.7 mmol) were dissolved in 5 mL 40% NaOH. The mixture was refluxed under nitrogen atmosphere for 35 minutes with vigorous stirring. After cooling in an ice bath, the solution was neutralized with concentrated HCl and the solvent removed by evaporation. The crude product was dissolved in distilled water and any insoluble material was removed by filtration. The aqueous phase was concentrated by evaporation the product was isolated by reversed-phase (C₁₈) column flash chromatography with gradient elution from 100% H₂O to 70% H₂O/30% CH₃CN. The final product **1** was obtained as an orange amorphous solid (0.13 g, 20% yield).

¹H-NMR (400 MHz, D₂O) δ 7.71 (m, 3H), 7.35 (d, *J* = 8.5 Hz, 1H), 7.19 (d, *J* = 15.4 Hz, 1H), 6.79 (d, *J* = 8.6 Hz, 2H), 6.40 (d, *J* = 8.5 Hz, 1H), 6.35 (s, 1H), 3.87 (t, *J* = 5.9 Hz, 2H), 3.73 (s, 3H), 2.94 (t, *J* = 7.2 Hz, 2H), 1.89–1.74 ppm (m, 4H).

¹³C-NMR (101 MHz, CD₃OD) δ 191.96, 164.83, 164.36, 163.44, 143.13, 132.70, 131.92, 131.79, 118.75, 117.12, 115.36, 106.83, 102.69, 68.94, 55.68, 52.27, 29.32, 22.92 ppm.

HRMS (negative mode) [M–Na]⁻: Calcd for C₂₀H₂₁O₇S 405.1003; Found 405.1017.







Figure S2. ¹³C-NMR spectrum of **1**.

3. Characterization of the chalcone/flavylium photoswitch

3.1. p*K*a Determination of the light-induced transformation of the chalcone 1 into flavylium cation 2



Figure S3. UV/vis-absorption spectra upon irradiation of a solution of 1 in water (18 μ M, 10 mM citrate buffer) at 365 nm until reaching the photostationary state at different pH.



Figure S4. pH Titration curve (monitored at 458 nm, data from Fig. S3) of a solution of 1 in water (18 μ M, 10 mM citrate buffer), irradiated at 365 nm until reaching the photostationary state.



Figure S5. UV/vis-absorption spectra after 24 hours of thermally activated back reaction (298 K) of a solution of **1** in water (18 μ M, 10 mM citrate buffer), previously irradiated at 365 nm until reaching to the photostationary state at different pH values.



Figure S6. pH Titration curve (monitored at 458 nm, data from Fig. S5) after 24 hours of thermally activated back reaction (298 K) of a solution of **1** in water (18 μ M, 10 mM citrate buffer), previously irradiated at 365 nm until reaching the photostationary state.

3.2. p*K*a Determination of the light-induced transformation of the chalcone 1 into flavylium cation 2 in presence of CB8



Figure S7. UV/vis-absorption spectra upon irradiation of a solution of **1** and CB8 in water (18 μ M **1**, 9 μ M CB8, 10 mM citrate buffer) at 365 nm until reaching the photostationary state at different pH.



Figure S8. pH Titration curve (monitored at 458 nm, data from Fig. S7) of a solution of **1** and CB8 in water (18 μ M **1**, 9 μ M CB8, 10 mM citrate buffer), irradiated at 365 nm until reaching the photostationary state.



Figure S9. UV/vis-absorption spectra after 24 hours of thermally activated back reaction (298 K) of a solution of **1** and CB8 in water (18 μ M **1**, 9 μ M CB8, 10 mM citrate buffer), previously irradiated at 365 nm until reaching to the photostationary state at different pH values.



Figure S10. pH Titration curve (monitored at 458 nm, data from Fig. S9) after 24 hours of thermally activated back reaction (298 K) of a solution of **1** and CB8 in water (18 μ M **1**, 9 μ M CB8, 10 mM citrate buffer), previously irradiated at 365 nm until reaching the photostationary state.

3.3. Reversibility of the photoswitch



Figure S11. UV/vis absorbance at 458 nm of a solution of **1** in water (5 μ M, 10 mM citrate buffer, pH 4.5) upon several cycles of irradiation (3 minutes at 365 nm) and thermally activated back reaction (50 minutes, 298 K).

4. Supramolecular characterization

4.1. Determination of the stoichiometry of the flavylium-CB8 complex



Figure S12. Job's plot monitored at 458 nm upon changing the molar fraction of flavylium cation **2**. The total molar concentration was kept constant at 10 μ M; pH 1.

4.2. Determination of the 2:1 binding constant of flavylium cation 2 by CB8



Figure S13. UV/vis-absorption titration curve of flavylium cation **2** (18 μ M) with CB8 at pH 1. $K_{CB8}(2) = 1.3 \times 10^{12} \text{ M}^{-2}$. Global fitting^{4, 5} of the complete spectral data yielded the same value for the binding constant. The red line shows the corresponding fit according to 2:1 binding.



Figure S14. Fluorescence titration curve of flavylium cation **2** (4 μ M) with CB8 at pH 1. $K_{CB8}(2) = 5.0 \times 10^{12} \text{ M}^{-2}$. Global fitting^{4, 5} of the complete spectral data yielded a similar value for the binding constant (1.5 $\times 10^{12} \text{ M}^{-2}$). The red line shows the corresponding fit according to 2:1 binding.

5. Photorelease of tripeptide 3



Figure S15. Normalized UV/vis-absorption spectra of irradiated solutions (365 nm, 60 s) in water at pH 4.5 (10 mM citrate buffer): blue - 1 (5 μ M), tripeptide 3 (5 μ M), and CB8 (2.5 μ M); black - 1 (5 μ M); red - 1 (5 μ M) and CB8 (2.5 μ M).

6. Sequential logic

6.1. Continuous irradiation



Figure S16. UV/vis-absorption spectra of **1** (18 μ M) in water: at pH 7.4 (black); at pH 4.5 (red); at pH 7.4 and then irradiated with 365-nm light (blue); at pH 4.5 and then irradiated at 365 nm (green); at pH 7.4 irradiated at 365 nm first and then set to pH 4.5 (cyan).

6.2. Flash photolysis experiments

Millisecond-second time scale flash photolysis experiments (see materials and methods section above for experimental details) are particularly useful for the characterization of the photochromic properties of chalcone/flavylium systems. In general, these experiments allow access to the kinetics of the photochemically generated *cis*-chalcone (Cc) from the *trans*-chalcone (Ct) in *the ground state*. In the present work these experiments were conducted to support the formation of the flavylium cation through the sequential application of stimuli (pH and light).



Scheme S1. Cc+B, formed from Ct after light excitation, can relax through two competing pathways: backward to the Ct or forward to the colored AH^+ species.

Scheme S1 illustrates the chalcone/flavylium multistate system generated after excitation of the Ct with pulsed UV light. At neutral and slightly acidic conditions the Ct is the main species in dark equilibrated solutions. Upon excitation with UV light, Ct is photo-isomerized to Cc that equilibrates fast with the hemiketal (B) in a ring-closing tautomerization process. Then, in a slower process, photogenerated B+Cc can relax back to the Ct or forward to AH⁺ through two parallel kinetic pathways. From basic kinetics, it is well known that the relative yield of the two products is given by:

$$\chi_{Ct} = \frac{k_{backward}}{k_{backward} + k_{forward}} \quad (S1); \qquad \chi_{AH+} = \frac{k_{k_{forward}}}{k_{backward} + k_{forward}} \quad (S2)$$

and that for pseudo-first order conditions, both products are formed at the same rate with:

$$k_{obs} = k_{backward} + k_{forward} \tag{S3}$$

In the case of chalcone/flavylium systems, the backward reaction rate ($k_{backward}$) is given by the *cis* to *trans* isomerization rate constant (k_i) multiplied by mole fraction of Cc (owing to the fast equilibrium between B and Cc).

$$k_{backward} = \frac{K_t k_i}{1 + K_t} \tag{S4}$$

The forward reaction rate is controlled by the de-hydration reaction of B that is catalyzed by H^+ and must be multiplied by mole fraction of B.

$$k_{forward} = \frac{k_{-h}[H^+]}{1+K_t} \tag{S5}$$

Thus, the relative yield of AH^+ formed after light excitation is $[H^+]$ -dependent and given by:

$$\chi_{AH+} = \frac{[H^+]}{\frac{K_t k_i}{k_{-h}} + [H^+]}$$
(S6)

These kinetic features of chalcone/flavylium systems lead to their well-known pHdependent photochromic properties.^{6, 7} Devising sequence dependent two-input systems requires not only efficient formation of AH^+ at acidic pH values but also fast recovering of Ct at neutral pH. At this point it must be stressed that for chalcone/flavylium systems bearing high isomerization barrier (very low k_i) Cc and B accumulates in the photostationary state upon light irradiation at neutral pH and are converted into AH^+ after acidification. In such cases the formation of colored AH+ is fully controlled by the apparent pKa of the photostationary state and does not depend on the input sequence.

On the other hand, candidates with high k_i and/or low k_{-h} may lead to inefficient formation of AH⁺ in the reversible acidic region making these compounds poor candidates for supramolecular applications.

After some screening, we found that compound **1** met the requirements to enable phototriggered formation AH^+ via sequence-dependent application of pH and light stimuli which are supported by flash photolysis experiments. As can be observed from Figure S17a, at pH = 7.4 absorption of the light pulse leads to a bleaching at 360 nm due to the disappearance of Ct to give a mixture B+Cc which has lower extinction coefficient. After the bleaching, Ct is fully recovered in *ca*. 1 minute. On the other hand, when the process is monitored at the flavylium cation maximum absorption wavelength (460 nm) no positive ΔA can be observed confirming that this species is not formed under these pH conditions. Instead, a small bleaching is also observed probably due a low absorption arising from a small fraction of ionized Ct. In contrast, at pH = 4.0 (Figure S17b) the observed bleaching at 360 nm is not recovered while at 460 nm a positive ΔA of *ca*. 0.3 units was registered. Together, these results show that under these conditions the formation of the flavylium cation is very efficient (i.e. $k_{\text{forward}} \gg k_{\text{backward}}$)

while at neutral conditions the recovery of the Ct controls the process (i.e. $k_{backward} >> k_{forward}$). It should also be stressed that at pH = 4.0 the system is in the reversible pH range as the apparent p K_a is ≤ 2.2 .



Figure S17. Flash photolysis traces of **1** in aqueous citrate buffer at (a) pH = 7.4 and (b) pH = 4.0. All solutions were monitored at 360 nm (*trans*-chalcone) and at 460 nm (flavylium cation) in two independent experiments.

Similar experiments to that described above were conducted in the presence of 0.5 equivalents of CB8 (Figure S18).



Figure S18. Flash photolysis traces of **1** in the presence of 0.5 equiv. of CB8 (10mM of citrate buffer) at (a) pH = 7.4 and (b) pH = 4.4. All solutions were monitored at 360 nm (*trans*-chalcone) and at 460 nm (flavylium cation) in two independent experiments.

7. NMR spectra



Figure S19. ¹H-NMR spectra of **1** (0.7 mM) in D_2O at pD 7 (bottom); the same at pD 2.0 and after irradiation at 365 nm (middle); and photogenerated flavylium cation **2** (at 0.4 mM) after addition of 0.5 equiv. (0.2 mM) of CB8 (top).

8. References

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