Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information (11 pages)

Phototriggered release of amine from a cucurbituril macrocycle

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1. Materials and general procedures

o-Nitrobenzaldehyde (99%) was purchased from Sigma-Aldrich, Hoechst 33258 pentahydrate (Fluoropure grade) was supplied by Invitrogen, and 1,5-diaminopentane (cadaverine, 97%) was purchased from Fluka. Calf thymus DNA was purchased from Fluka. Cucurbit[7]uril was synthesized according to a published procedure.[1] Water was of Millipore quality.

The UV/vis absorption and fluorescence measurements were done at room temperature (24 ºC) using 1-cm quartz cuvettes. The absorption spectra were recorded with a Varian Cary 50 spectrophotometer and the fluorescence measurements were performed with a Varian Cary Eclipse fluorimeter. Induced circular dichroism (ICD) measurements were done on a Jasco J-810 circular dichrograph, using the buffer solution (10 mM sodium phosphate buffer; pH 7.0) for background correction.

The initial pH of the irradiation solution was adjusted by the addition of acid (HCl) or base (NaOH) and controlled with a pH meter (model HI221, HANNA Instruments). Where applicable, the pD value was calculated by pD = pH + 0.4.[2]

^{1}H NMR experiments were performed on an 400 MHz spectrometer (Agilent 400 MR). D_2O was used as solvent, and deuterium chloride or sodium deuteroxide were added to adjust the pH of the solutions. The chemical shifts (δ in ppm) were referenced to the residual HOD solvent peak at 4.79 ppm.

The binding constants of dye **1** at the different pH values were determined by adding aliquots of CB7 (direct titration) or competitor (competitive titration). The complexation was followed by fluorescence measurements. The resulting titration curves were fitted according to previously published procedures.[3, 4]

For the pH jump experiments the solutions were irradiated at 254 nm with a Vilber Loumat handheld UV lamp (model VL-4.LC).

The modelling of the multi-equilibrium system was done with MatLab.

The concentration of calf thymus DNA was determined by UV-absorption spectroscopy, using the molar absorption coefficient of 6600 $M^{-1}cm^{-1}$ at 260 nm.[5]

2. Binding constants of dye 1 at pH 8.0 and pH 5.0

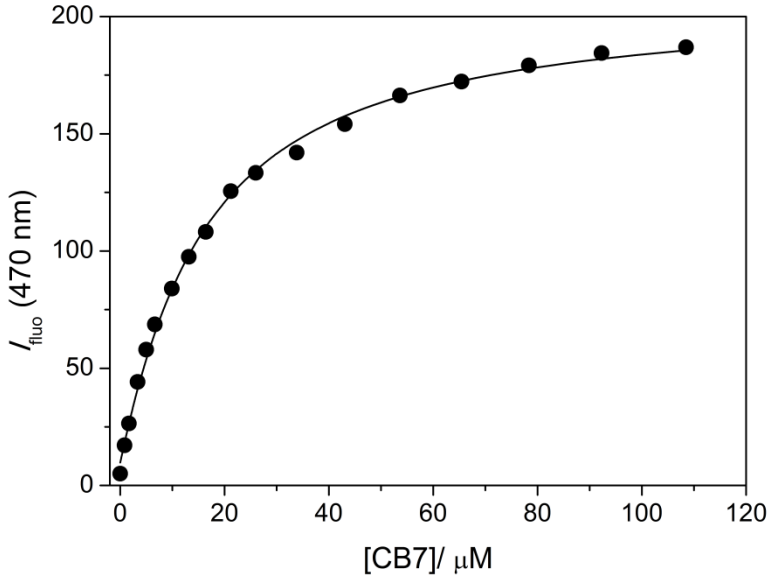

Figure S1. Fluorescence titration of **1** (5 μM) with CB7 at pH 8.0 (10 mM Tris buffer). The 1:1 binding constant was determined as $K = 7.4 \times 10^4$ M^{-1}.

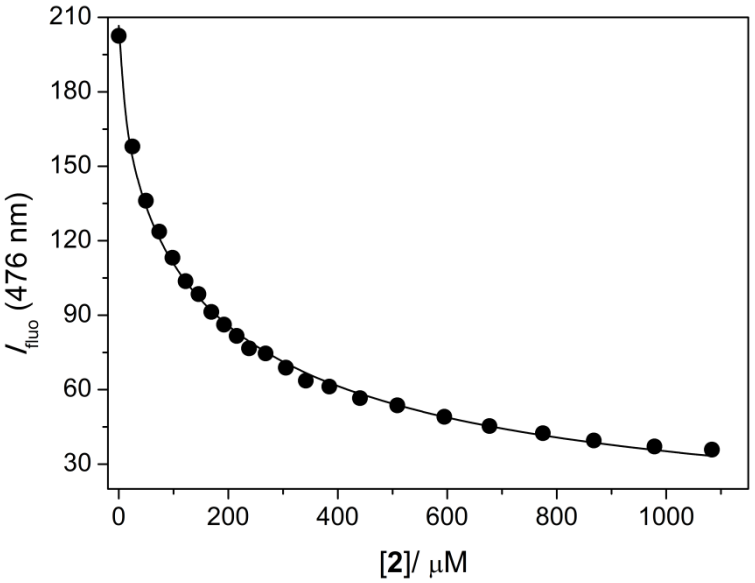

Figure S2. Competitive fluorescence titration of an equimolar mixture of **1** and CB7 (each 10 μM) with **2** at pH 5.0. The binding constant was determined as $K = 3.3 \times 10^8$ M^{-1}.

3. Control experiments and spectral data (see main text)

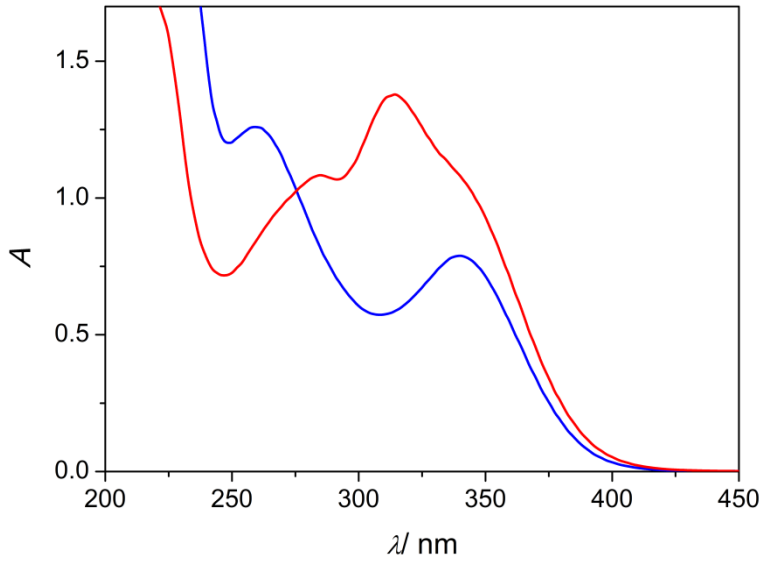

Figure S3. UV/vis absorption spectra of a mixture of 15 µM **1**, 15 µM CB7, and 150 µM **3** in buffered solution (Tris-HCl, 10 mM, pH 8.0) before (blue line) and after (red line) 5 minutes of irradiation at 254 nm. Irradiation of a non-buffered solution (initial pH 8.1) leads to the same spectral changes.

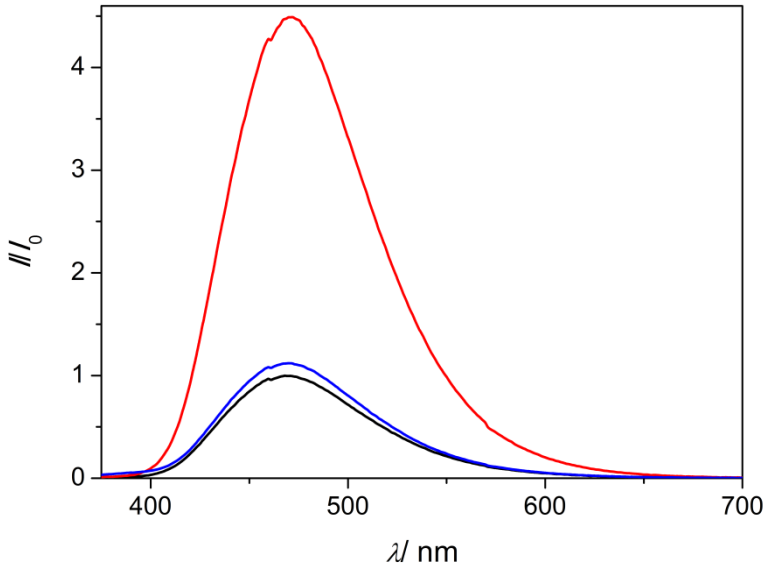

Figure S4. Normalized fluorescence spectra of a mixture of 15 µM **1**, 15 µM CB7, and 150 µM **3** before and after irradiation at 254 nm for 5 minutes. The black line corresponds to the initial spectrum (same spectrum for non-buffered and 10 mM Tris-buffered solutions at pH 8) before irradiation. The blue line is the spectrum after irradiation of the buffered solution and the red line corresponds to the spectrum after irradiation of the non-buffered solution (reaching pH 5.1).

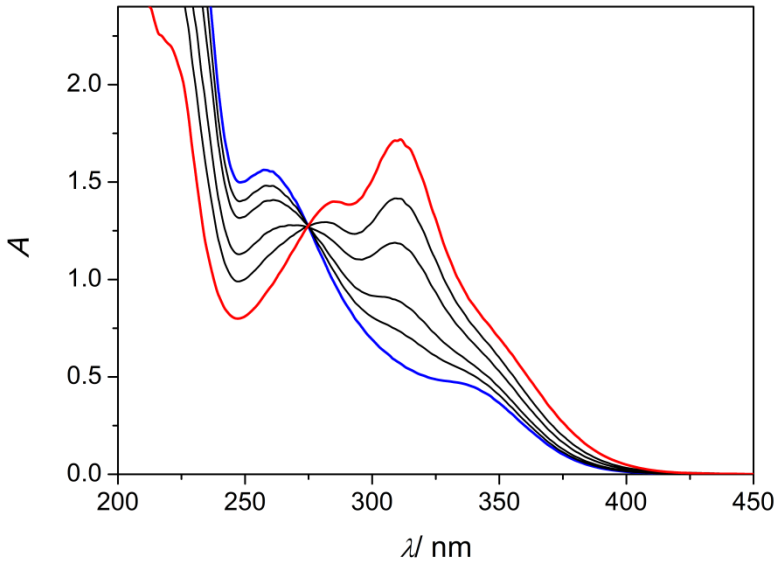

Figure S5. UV/vis absorption spectra of a mixture of 8.4 µM **1**, 10 µM CB7, 250 µM **3**, and 10 µM 1-aminoadamantane during the irradiation at 254 nm. The blue line corresponds to the initial spectrum at pH 8.0 and the red line to the spectrum after 5 minutes of irradiation (pH 4.9). The black lines correspond to spectra taken at irradiation intervals of 30 s, 60 s, 120 s, and 180 s.

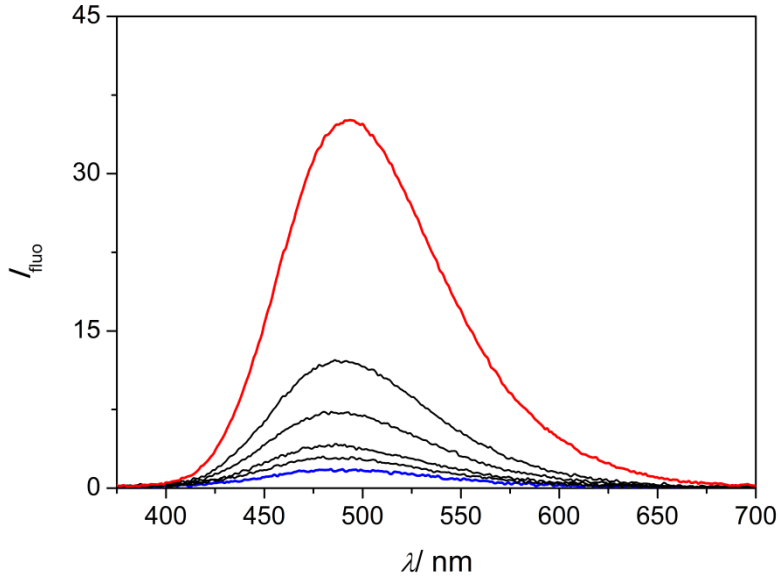

Figure S6. Fluorescence spectra of a mixture of 8.4 µM **1**, 10 µM CB7, 250 µM **3**, and 10 µM 1-aminoadamantane during the irradiation at 254 nm. The blue line corresponds to the initial spectrum at pH 8.0 and the red line to the spectrum after 5 minutes of irradiation (pH 4.9). The black lines correspond to spectra taken at irradiation intervals of 30 s, 60 s, 120 s, and 180 s.

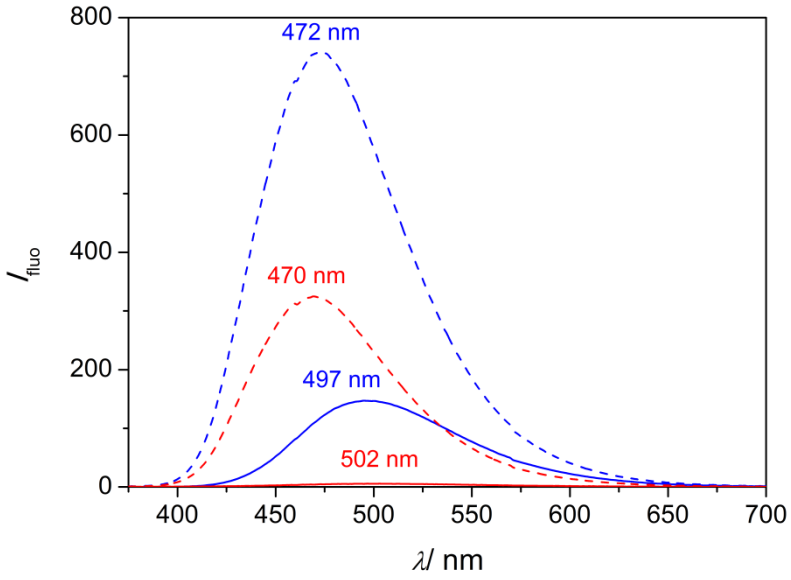

Figure S7. Fluorescence spectra of **1** (15 μM, full lines) and **1•CB7** (15/15 μM, dashed lines) at pH 5.0 (blue) and pH 8.0 (red); λ_{exc} = 275 nm.

4. ^{1}H NMR spectra

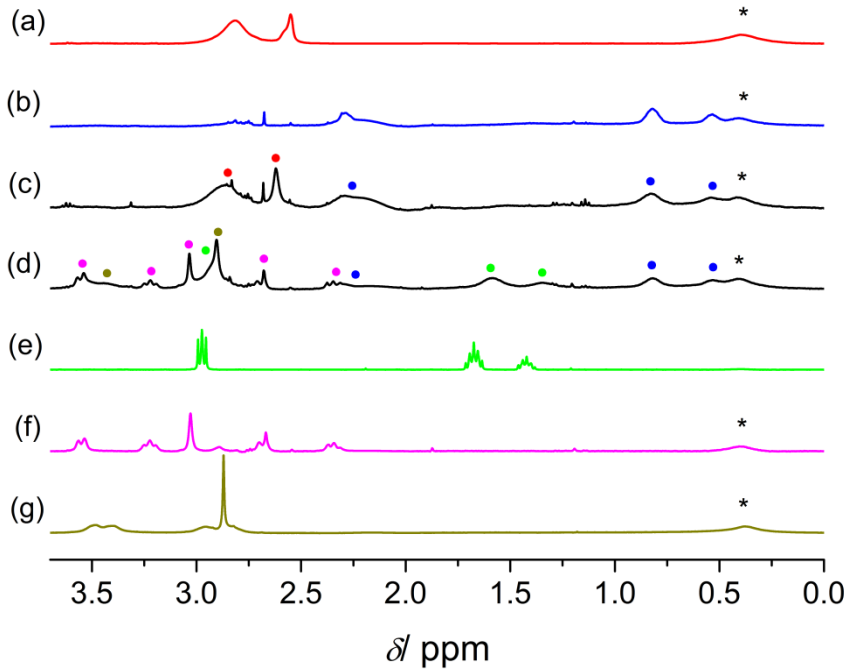

Figure S8. Partial ^{1}H NMR spectra of (a) dye **1** at pD 8.0, (b) amine **2** in presence of CB7 at pD 8.0, (c) dye **1** in presence of CB7 and amine **2** at pD 8.0, (d) dye **1** in presence of CB7 and amine **2** at pD 5.4, (e) free amine **2** at pD 7.4, (f) dye **1** in presence of CB7 at pD 5.4, and (g) dye **1** at pD 5.4. Concentrations: [**1**] = 0.93 mM, [**2**] = [CB7] = 1.0 mM. The coloured dots in (c) and (d) show the assignments of the signals to free and complexed dye at the different pH values, based on the colour-matching spectra in (a), (b), (e), (f), and (g). The asterisks mark an unidentified impurity signal.

5. Competition between calf thymus DNA and CB7 for Hoechst 33258 binding

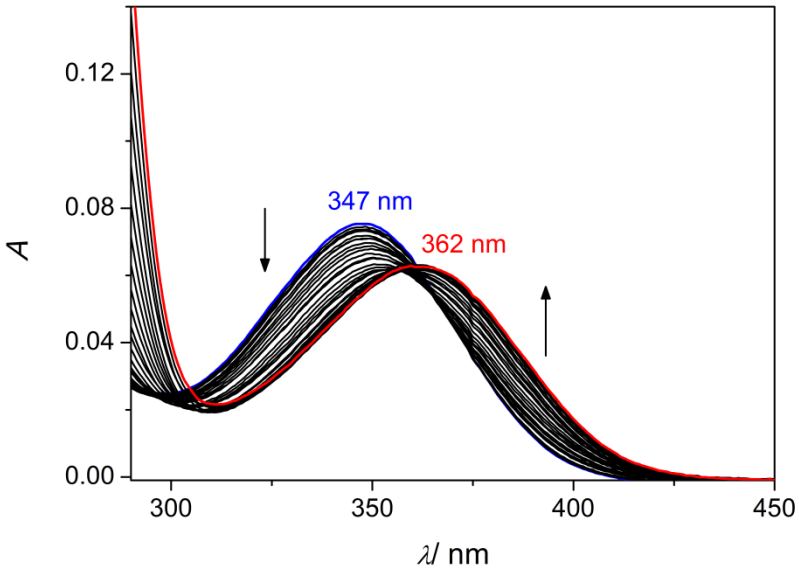

Figure S9. UV/vis-absorption titration of dye **1** (2 μM) in presence of CB7 (6 μM) with calf thymus DNA (up to *ca*. 60 μM) in 1 mM phosphate buffer solution at pH 7.2. The blue spectrum corresponds to the initial solution and the red spectrum marks the endpoint of the titration.

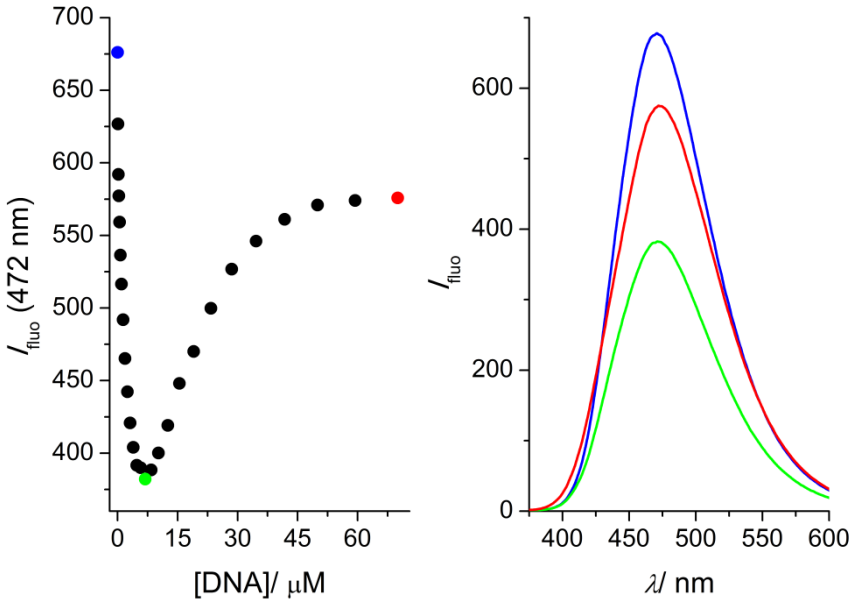

Figure S10. Fluorescence titration curve (left) for the addition of calf thymus DNA to a solution of dye **1** (2 μM) in presence of CB7 (6 μM) in 1 mM phosphate buffer solution at pH 7.2. On the right the emission spectra corresponding to the color-coded titration points are shown; excitation at 358 nm.

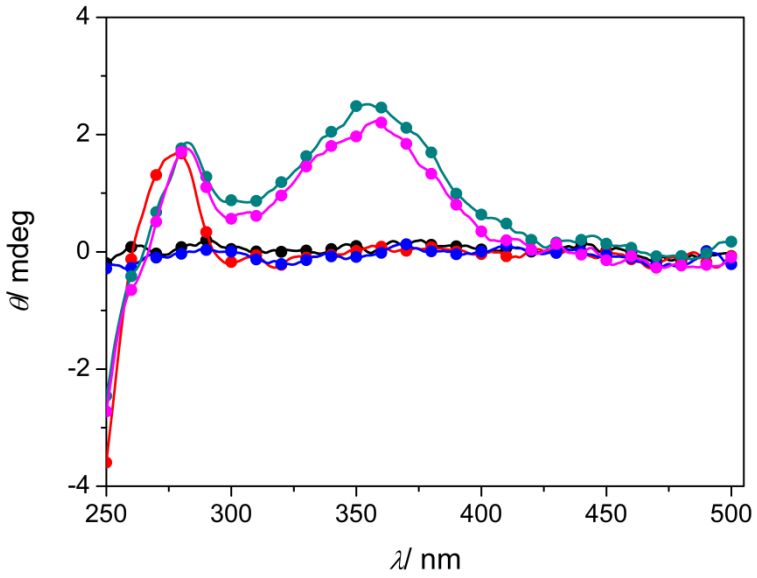

Figure S11. ICD spectra of dye **1** alone (black), calf thymus DNA alone (red), dye **1** in the presence of CB7 (blue), dye **1** in the presence of calf thymus DNA (dark cyan), dye **1** in the simultaneous presence of C7 and calf thymus DNA (magenta). [**1**] = 2 μM, [CB7] = 6 μM, [DNA] = 59 μM.

Note: Under the chosen concentration conditions *ca*. 90% of dye **1** are initially complexed by CB7 (K = 1.7 × 10^6 M^{-1} at pH 7.2).[6] On addition of calf thymus DNA a typical bathochromic shift of the UV/vis-absorption spectrum is observed (Figure S9). The final spectrum (red in Figure S9) with a maximum at 362 nm corresponds to the DNA-bound dye (determined in a separate titration of the dye alone, not shown). The corresponding fluorescence titration (Figure S10) shows two phases, first a quenching (at high dye/DNA ratio) and then a recovery of the emission (at low dye/DNA ratio). These observations are tentatively assigned to (a) the quenching of intercalated dye by unspecifically bound dye (at high dye/DNA ratio)[7] and (b) to the exclusive intercalation at low dye/DNA ratio with the corresponding light-up behaviour. Very compelling evidence for the dye release from the CB7 macrocycle on addition of calf thymus DNA comes from the induced circular dichroism (ICD) measurements (Figure S11). The ICD

spectrum that is obtained on addition of calf thymus DNA to the CB7-complex of dye **1** practically coincides with the spectrum of the dye-DNA complex. The combined experimental evidence supports the competitive binding of dye **1** by calf thymus DNA.

6. References

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