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Phototriggered release of amine from a cucurbituril macrocycle

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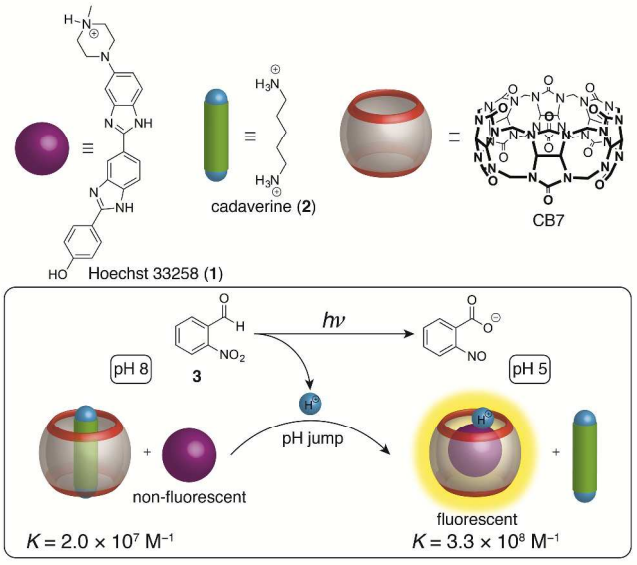
A method for the phototriggered release of a biogenic amine from the host-guest complex with the cucurbit[7]uril macrocycle in aqueous solution was devised. The approach exploits a photoinduced pH jump from 8 to 5, combined with the pH-dependent switching of the competitive capacity of a guest dye. The fluorescence fingerprint of the competitor can be used to monitor the amine release in the micromolar concentration regime.

The control of the binding characteristics of supramolecular assemblies by means of light irradiation is an instructive example for the combination of chemical complexity and functional design.[1-8] The spatiotemporal nature of photostimulation makes such procedures especially interesting for delivery applications,[9-11] not only in biological contexts. Among other macrocyclic host structures, cucurbiturils (CBn) have received increased attention with a special focus on biological, pharmacological, and nanotechnological applications.[12-24] The observation of high binding constants with molecularly very diverse guests in water has contributed to these developments.[23, 25-30] In this context the direct photostimulation of cucurbituril host-guest complexes by means of photoreactive/photochromic guests has been employed for the design of supramolecular switches[7, 31, 32] and valves[33] as well as for supramolecular photocatalysis.[22, 34]

Recently we have shown a different approach by harnessing the well-known pH-dependence of guest binding to cucurbiturils in combination with a photoinduced pH jump as relay mechanism.[35] The binding constants of most guests with CBs are higher at acidic pH, being a consequence of the phenomenon of host-assisted guest protonation.[36-38] Hence, a jump from acidic to basic pH can trigger the release of the guest as demonstrated for the CB7-complex of the Hoechst 33258 dye (**1**),[35, 39] also known as DNA minor groove binder (see also ESI†).[40] In the present work we envisioned to combine the pH jump (from basic to acidic pH) with the switchable binding of a competitor (dye **1**) in order to remove an amine guest (cadaverine, **2**) from the CB7 cavity; see Scheme 1. The binding of the latter is largely independent on the pH due to its high basicity (pK_a 9–10). **2** was chosen as model for biogenic polyamines, which for example are recognized for their role in cell proliferation.[41]



Scheme 1. Working principle of phototriggered amine release from CB7 with pH jump as relay mechanism.

The pH jump was produced by the known and very efficient proton-releasing photoreaction of 2-nitrobenzaldehye (**3**) upon UV irradiation, yielding a decrease of the pH from 8 to 5; see Scheme 1.[42] Recently, compound **3** has been used for pH-jump experiments to achieve control of biological processes[43-45] and materials' functions.[46, 47]

At basic pH the **2**•CB7 complex prevails (91% amine complexation at pH 8 for [**1**] = 8.4 μM and [**2**] = [CB7] = 10 μM). This is rationalized with the three orders of magnitude higher binding constant of **2**[24] than observed for dye **1** at this pH ($K_{\mathbf{2}\bullet\mathrm{CB7}}$ = 2.0 × 10^7 M^{-1} *versus* $K_{\mathbf{1}\bullet\mathrm{CB7}}$ = 7.4 × 10^4 M^{-1}); see ESI†. This situation was dramatically inverted on changing to pH 5. Now the binding constant of dye **1** is *ca*. 20 times higher than that of **2** ($K_{\mathbf{1}\bullet\mathrm{CB7}}$ = 3.3 × 10^8 M^{-1} *versus* $K_{\mathbf{2}\bullet\mathrm{CB7}}$ = 2.0 × 10^7 M^{-1}); see ESI†. This led to a decrease of the amount of complexed amine **2** (27% complexation degree) on pH jump (same concentrations of the components as above), corresponding to a release of 70% of the cargo. A simulation of the multi-equilibrium system, based on the measured binding constants, indicates that this already very efficient release could be further improved (see Figure 1). However, in an effort to keep the amount of competitor as low as possible, while assuring efficient release, a sub-stoichiometric concentration of **1** was chosen for our experiments.

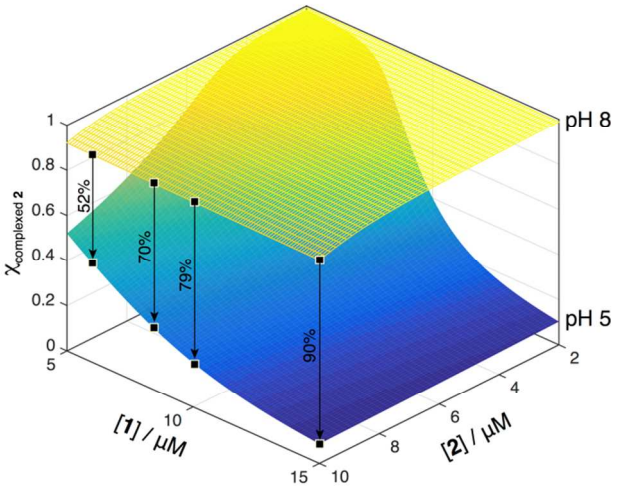


Figure 1. Modelling of the mole fraction of CB7-complexed amine **2** in dependence on the concentration of **2** and dye **1** at pH 8 and pH 5. The CB7 concentration was fixed at 10 μM. The arrows show the expected changes on pH jump for 10 μM **2** and varying dye concentrations (from left to right: 6 , 8.4, 10, 15 μM). The arrow labels indicate the release efficiency, calculated as ($[\mathbf{2}]_{\text{released}}$/$[\mathbf{2}\bullet\text{CB7}]_{\text{pH8}}$)×100%.

It is convenient, but no precondition, that dye **1** is practically non-fluorescent at pH ≥ 7 (Φ_{fluo} *ca*. 0.01 at pH 7), while it turns strongly fluorescent in the CB7 complex (Φ_{fluo} = 0.74 at pH 7).[35] This fluorescence light-up behaviour can be used to monitor the complexation of the dye on pH jump from basic to acidic pH. In Figure 2 the corresponding experiment is shown. A solution with the same concentrations of **1**, **2**, and CB7 as stated above, but containing additionally 125 μM of the acid generator **3**, was irradiated at 254 nm. This wavelength, although incompatible with biological contexts, was chosen for enabling a fast conversion even with a low-intensity light source, such as the used 4 W UV handheld lamp. Noteworthy, compound **3** was shown earlier to perform also as efficient photoacid when irradiated with a pulsed laser at 388 nm or under two-photon excitation conditions with a near-infrared fs-laser system.[45, 48] Under our experimental conditions the expected fast and clean photoreaction with an isosbestic point at 275 nm in the UV/vis absorption spectra was produced. This wavelength was used for exciting the sample in the fluorescence measurements. As predicted, the fluorescence of the sample was rather low at pH 8, but increased by a factor of *ca*. 40 after 4 minutes of irradiation, leading to pH 5. The fluorescence emission has its maximum at about 470 nm, as expected for the CB7-complexed dye; see ESI†. Notably, the free dye would also increase its fluorescence at pH 5, albeit less pronounced (Φ_{fluo} = 0.29 at pH 4.5)[35] and with a emission maximum that is red-shifted to *ca*. 500 nm; see ESI†. These characteristic spectral features lead to the safe confirmation that the dye was complexed and therefore the amine guest was liberated. Importantly, in the applied pH range, being below the pK_a of the amine, no release would have been observed in the absence of competitor **1**.[49]

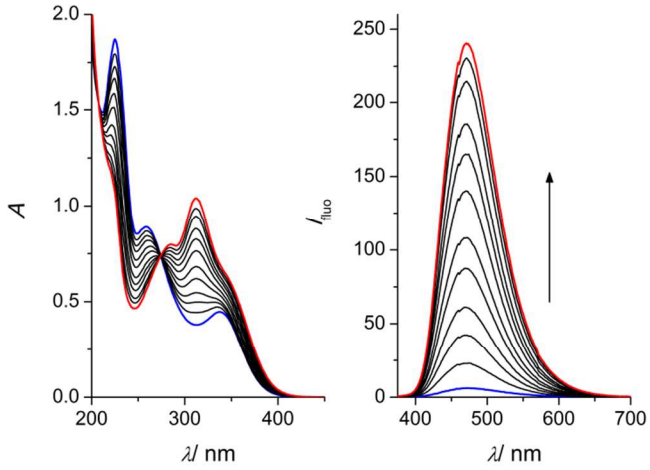


Figure 2. UV/vis absorption (left) and fluorescence spectra (right) on irradiation of an aqueous solution containing **1** (8.4 μM), **2** (10 μM), CB7 (10 μM), and **3** (125 μM) at 254 nm. The initial pH was 8.0 (blue spectra) and the final pH after 4 minutes of irradiation was 4.8 (red spectra). The fluorescence spectra were obtained by excitation at 275 nm.

Two control experiments (see also ESI†) were performed to confirm the determining role of **1** and its pH-switchable capability to compete with amine **2**. First, a Tris-buffered solution (pH 8) containing equimolar amounts (15 μM) of **1** and CB7 was irradiated in the presence of **3**. However, only a very minor fluorescence increase by 10% was seen for identical irradiation conditions as applied above. This contrasts the result in non-buffered solution, where a fluorescence increase by 350% was observed. Hence, it is safe to conclude that it is the pH jump which triggers the complexation of **1**.

The second control experiment consisted in the attempt of a phototriggered displacement of 1-aminoadamantane. However, this amine binds to CB7 with such high efficiency that even at pH 5 its binding constant ($K = 4.2 \times 10^{12}$ M^{-1}) is four orders of magnitude higher than that of dye **1**.[25] Hence, *de facto* there should be no displacement after the pH jump. This was indeed verified in the fluorescence spectra. There is some minor increase of the fluorescence of dye **1**, but the emission maximum (502 nm) coincides with that of the unbound dye (see above). This experiment shows unequivocally that amine release will only take place if **1** turns into an efficient competitor at acidic pH.

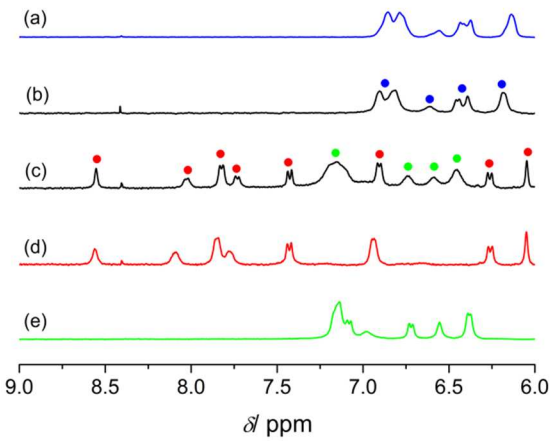


Figure 3. Partial ^{1}H NMR spectra of (a) dye **1** at pD 8.0, (b) dye **1** in presence of CB7 and amine **2** at pD 8.0, (c) dye **1** in presence of CB7 and amine **2** at pD 5.4, (d) dye **1** in presence of CB7 at pD 5.4, and (e) dye **1** at pD 5.4. Concentrations: [**1**] = 0.93 mM, [**2**] = [CB7] = 1.0 mM. The coloured dots in (b) and (c) show the assignments of the signals to free and complexed dye at the different pH values, based on the comparison with the colour-matching spectra in (a), (d), and (e).

To gain additional insight into the complexation of **1** and consequently the release of **2** on pH jump we performed ^{1}H NMR experiments, changing the pD by manual addition of acid. This procedure gave a realistic insight into the complexation/release situation without extra addition of **3** which would complicate the spectra (see Figure 3). We focussed on the region between 6 and 9 ppm, where exclusively the aromatic protons of dye **1** appear. At pD 8.0 and 5.4 the free dye is aggregated in the applied millimolar concentration regime (*ca*. 1 mM)[50] and groups of rather broad signals between 6.14 and 6.85 ppm were seen (Figure 3a and 3e). This changed when CB7 was added. The 1:1 complexation favours de-aggregation,[35, 51, 52] accompanied by the known upfield shifts of protons that are immersed in the CB cavity.[35] A well resolved spectrum in the expanded window between 6.0 and 8.6 ppm resulted (see Figure 3d for pD 5.4). Addition of stoichiometric amounts of amine **2** to a solution of **1** and CB7 at pD 8.0 showed exclusively the signals of the aggregated free dye (Figure 3b). On acidification to pD 5.4 (simulating the photoinduced pH jump) the signals of the CB7-complexed dye appeared, alongside with residual signals of free guest **1** (see colour-coded assignments in Figure 3c). Taking advantage of the slow exchange on the NMR time scale, signal integration yielded a dye complexation degree of *ca*. 50%, corresponding to about 50% release of the amine guest **2**. The amine release was confirmed by the appearance of signals ascribed to the free species in the aliphatic region of the ^{1}H NMR spectrum (0.5–3.0 ppm); see ESI†. The efficiency of cargo liberation is somewhat lower than estimated based on the binding constants for the micromolar concentration regime (see above). This is reasoned with the competing aggregation of dye **1** in the millimolar range, leading to less efficient displacement at pD 5.4.

In conclusion, with the described novel approach it is possible to photo-release amines from the CB7 macrocycle by producing a jump from basic to acidic pH. Noteworthy, the amine could not be released just by acidification, which in the herein covered pH window would have simply no effect on the stability of the **2**•CB7 complex.[49] This obstacle was overcome by introducing a pH-dependent guest dye that competes with the amine at acidic pH, but not at basic pH. Conveniently, the fluorescence fingerprint of the competitor provided a handle to monitor the amine release in the micromolar concentration range where NMR spectroscopy is not applicable. Although herein demonstrated in a proof-of-principle approach for 254-nm UV irradiation, the excitation conditions could be adapted to the near-UV region or even to the two-photon regime (*ca*. 700 mm) that are more compatible with bio-relevant applications.[45, 48] The approach can be easily extended to other guests and competitors, as long as the boundary conditions for the binding constants are fulfilled.

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Notes and references

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† Electronic Supplementary Information (ESI) available: experimental details, binding titrations of **1**, control experiments, additional spectral data, NMR spectra, competition with DNA binding. See DOI: 10.1039/c000000x/

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