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A photoinduced pH jump applied to drug release from cucurbit[7]uril†

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A proof-of-principle for the application of a photoinduced pH jump for delivery of the Hoechst 33258 drug by disassembly of its host–guest complex with cucurbit[7]uril is described.

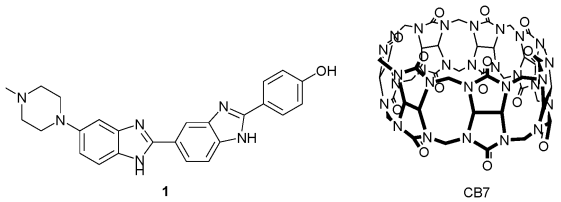
The manipulation of supramolecular assemblies with light (“photoswitching”) allows spatiotemporal control in a remote and non-invasive fashion. This is desirable, for example, for targeted drug delivery with macrocycles. Traditionally, the *cis–trans* isomerisation of azobenzenes,[1] the ring opening/closing of diarylethene switches,[2] or the reversible intramolecular [4 + 4] photocycloaddition of anthracenes[3,4] have been used to trigger a photochemical response of host macrocycles. In other case studies, it has been the photochromic guest itself which is complexed only in one isomeric form.[5–7] Except for the extremely unlikely event that the drug itself would be photochromic, this greatly complicates the design of such systems, because both the drug and one isomeric form of the photoactive guest would need to show a high affinity to the macrocycle (“competitive approach”). Since numerous drug complexes of different macrocycles have already been characterised and are in fact partially being used,[8–13] a “stimulus approach” would be complementary, in which the photoactive component does not need to compete for the macrocycle, but rather causes an effect on the macrocycle–drug equilibrium through a relay mechanism or chemical output. In the working principle established herein, this is a photoinduced pH jump.

Interestingly, light-induced pH changes have been rarely explored for supramolecular assembly and disassembly; in fact, our awareness is limited to a single study.[14] And there exists no precedent for a photoinduced assembly or disassembly of a macrocycle–drug complex.

Macrocycles with cation- or anion-receptor properties, whose complexation is driven by electrostatic interactions, are expected to display variations in binding constants when either the host or the guest undergoes protonation or deprotonation in a particular pH range; they could serve as prototypes for the present investigation. For example, pH-dependent binding of guests is well known for calixarenes[15–17] and, in particular, for cucurbiturils (CBs).[18–20] CBs have received much attention in recent years,[21] which has led to versatile applications, including the stabilisation of dyes and drugs,[8,13,22] chemosensing and monitoring,[5,23–25] the implementation of logic gates,[20] and the use as nanoreactors.[26,27] As a consequence of the increased affinity of CBs for cationic species,[28] they display a pronounced preference for binding the protonated forms of guests over their neutral forms.[8,29,30] Accordingly, host–guest binding of CBs is strongly pH-dependent and the differential binding manifests itself in complexation-induced pK_a shifts.[18,30,31] Invariably, when basic guests are investigated, their affinity increases at low pH and decreases at high pH. When coupled with a photoinduced pH jump, it should be possible to exploit this known dependence to affect either the selective binding or release of a guest, for example a drug, which defines exactly the idea of our present study.

We have selected the Hoechst 33258 dye as a model guest (**1**, Scheme 1), which finds wide application as a sequence-specific DNA stain[32] and an antihelmintic drug.[33] **1** is also known to inhibit the enzymatic activity of helicase and topoisomerases, which has implications for its antitumor activity.[34,35] The dye exists in various protonation states and shows a pH-dependent fluorescence response, which should report directly on the pH jump. Moreover, the fluorescence changes also markedly upon macrocyclic complexation by CB7,[36] such that we could follow its uptake by and release from the host by the same method.

In a first step, the formation of the supramolecular host–guest complex between **1** and CB7 was investigated.‡ The photophysical properties of **1** have been subject of previous works[37,38] and it is well known that the free dye is only very weakly fluorescent at pH 7 (Φ_f = 0.01). Upon addition of



Scheme 1 Chemical structures of the guest dye Hoechst 33258 (**1**) and of the cucurbit[7]uril (CB7) receptor macrocycle.

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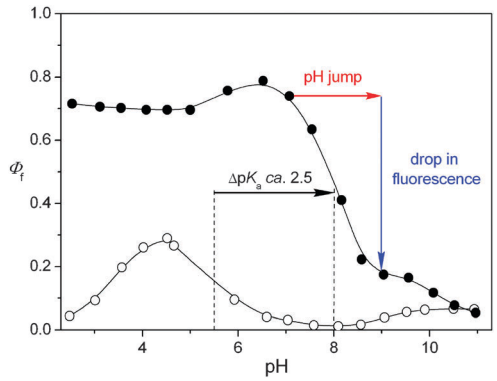
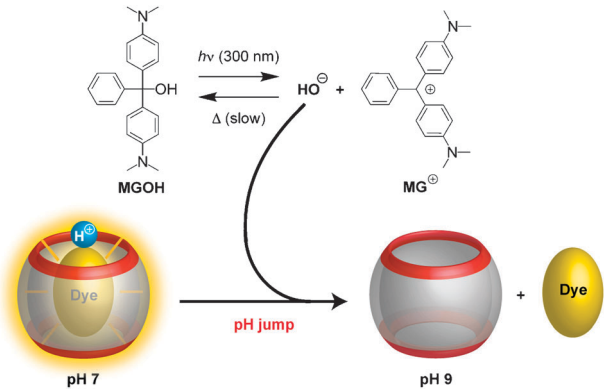


Fig. 1 pH titration for the fluorescence quantum yield (Φ_f) of **1** (1 μM) in the absence (empty circles) and presence (filled circles) of CB7 (30 μM).



Scheme 2 Switching of the CB7·**1** complex by a photoinduced pH jump. Note that only one benzimidazole residue is actually immersed inside the cavity, and not the entire dye, see text.

CB7 a remarkable fluorescence enhancement was observed (Φ_f = 0.74 in the presence of 30 μM CB7, Fig. 1), which can be assigned to dye confinement in the nonpolar CB7 cavity and the concomitant host-assisted protonation of **1** (see below); both are indicative for inclusion of the dye.[18]

A pH titration of free **1** revealed a maximum emission of the dye near pH 4.5 (Φ_f = 0.29, Fig. 1), where the doubly protonated form, $[\mathbf{1}H_2]^{2+}$, is known to be most abundant (pK_a *ca.* 5.5; this work).[39] The same titration in the presence of 30 μM CB7 (98% dye complexation at pH 7.2, Fig. 1) shifted the maximum of fluorescence close to pH 7, and from the titration curve a pK_a value of *ca.* 8.0 for $[\mathbf{1}H_2]^{2+}$ was estimated. The observed large pK_a shift of *ca.* 2.5 units is characteristic for CB7 complexes.[8,19,20,29,30] The variation of the dye fluorescence upon CB7 addition afforded the effective binding constant of the 1 : 1 CB7·**1** complex as K_b = (1.7 ± 0.4) × 10^6 M^{-1} at pH 7.2 (ESI†), verified by UV/vis titration, which is a factor of 10 larger than the one reported in an independent study.[36] The 1 : 1 stoichiometry was verified by Job's plot analysis, mass spectrometry, and ^{1}H NMR spectroscopy (ESI†).‡ Selective ^{1}H NMR upfield shifts further established the preferential immersion of the piperazinyl-benzimidazole residue of **1** into the CB7 cavity (ESI†). At pH 8.7, the effective binding constant dropped by two orders of magnitude to K_b = (2.8 ± 0.2) × 10^4 M^{-1}, as expected from the more than 2 units large pK_a shift and the known preference for binding of more highly charged cations. This drop in affinity should result in a significant increase in the fraction of uncomplexed guest (from 2% to 55% with 1 μM **1** and 30 μM CB7) as the pH is suddenly increased from 7.2 to 8.7.

With the fluorescence variation of the dye in dependence on pH and the complexation by CB7 being established,‡ we returned to the ultimate objective of switching this assembly by a photoinduced pH jump. As sketched in Scheme 2, we expected that a pH change from neutral to basic (from pH 7 to 9, red arrow in Fig. 1) triggers the release of the dye from the complex, which could in turn be monitored through a decrease in fluorescence (blue arrow in Fig. 1). Among the available photobases we selected malachite green leucohydroxide (MGOH, see Scheme 2, top).[40] Its irradiation with UV light (300 nm, Fig. 2) produces with high efficiency MG^+, the malachite green cation and conjugate Lewis acid, as well as

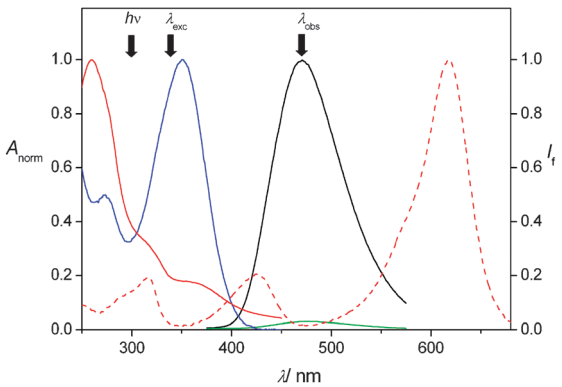


Fig. 2 Normalised absorption spectra of CB7·**1** (blue line), MGOH (red line), and MG^+ (red dashed line) as well as relative fluorescence spectra of **1** (green line) *versus* CB7·**1** (black line), all at pH 7. Note the selective dye excitation and the absence of competitive absorption at the observation wavelength (arrows). The irradiation wavelength to induce the photoinduced pH jump is indicated with *hν*; see also ESI.†

hydroxide ions. MG^+ has an absorption window near 340 nm to allow selective excitation of **1**, and another one near 470 nm to exclude inner filter effects when monitoring the fluorescence of **1** (Fig. 2). While the photophysical prerequisites for this multi-component system presented a challenge, they are actually not required from the viewpoint of drug release, which could be effected independent of the spectral properties of the drug itself.

The actual pH jump was induced by irradiation for 10 minutes after thermal equilibration of the reaction mixture for 90 minutes (Fig. 3). Indeed, the fluorescence of the dye dropped dramatically as the pH jumped to 8.7. Control experiments demonstrated that the fluorescence decrease was a genuine consequence of the pH jump. Most importantly, an experiment under equal conditions, but in a *buffered* solution (pH 7.2), showed no significant pH or fluorescence response upon irradiation or prolonged standing (inset in Fig. 3).

Within *ca.* 60 minutes after the irradiation, the fluorescence recovered again to its initial levels, due to the slow recombination of MG^+ and hydroxide ions, which was accompanied by a similarly slow restoration of the initial neutral pH, see ESI.† This (thermal) reversibility of the photoinduced host–guest complex dissociation establishes a special example of a photochromic effect. In contrast to the very robust azobenzene and

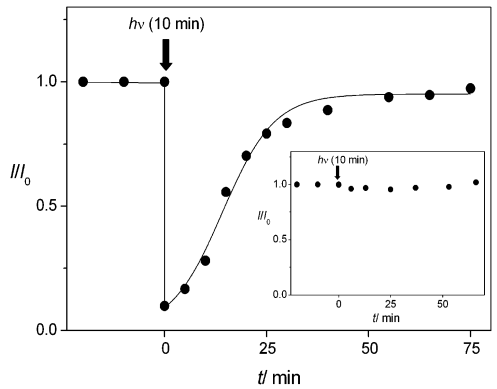


Fig. 3 Fluorescence response of the CB7·**1** complex (1 μM **1**, 30 μM CB7) in the presence of 100 μM MGOH and irradiation at 300 nm for 10 minutes with an initial pH of 7. The inset shows the negative control experiment in buffered solution (pH 7.2, 10 mM phosphate buffer).

diarylethene switches, MGOH undergoes secondary irreversible photoprocesses,[41] which prevented our system to "cycle" through repetitive dissociation–association cycles. In fact, the fluorescence response was much reduced already in the second cycle (to *ca.* 20% of the initial value). Of course, the use of this particular photobase demonstrates only a proof-of-principle. In fact, a potential application in a targeted photoinduced drug delivery system would neither require a reversible uptake of the drug nor a multiple release. For example, photocaged compounds, which are used for the triggering of biochemical processes, are invariably based on irreversible photoreactions.[42]

In summary, we have realised the dissociation of the host–guest complex between cucurbit[7]uril and Hoechst 33258 by a phototriggered pH jump. The sudden increase in pH from 7 to 9 lowers the binding constant by two orders of magnitude. This results in an immediate (on the time scale of the photolysis) release of Hoechst 33258, which was employed as a model for a potential drug. The release event is conveniently signalled by fluorescence modulations. Our approach opens a conceptually novel and unconventional pathway for photo-controlled drug release. Although the transfer of the method to actual biological systems may not be straightforward, especially in view of their natural buffering capacity, pH jumps have been realised in living cells.[43]

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Notes and references

‡ During the preparation of this manuscript we became aware of an independent study on the host–guest complexation of dye **1** by CB7 (ref. 36). The formation of a higher-order (2 : 1) CB7·**1** complex at acidic pH has been observed in this study, which does not need to be invoked for the neutral and basic pH studied herein (see ESI†).

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