# Light Control of Stoichiometry and Motion in Pseudorotaxanes Comprising a Cucurbit[7]uril Wheel and an Azobenzene–Bipyridinium Axle

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# Photochemistry

# Light Control of Stoichiometry and Motion in Pseudorotaxanes Comprising a Cucurbit[7]uril Wheel and an Azobenzene-Bipyridinium Axle

Massimo Baroncini,[a] Chao Gao,[a, b] Valentina Carboni,[a] Alberto Credi,[a] Elia Previtera,[a, c] Monica Semeraro,[a] Margherita Venturi,[a] and Serena Silvi*[a]

**Abstract:** Pseudorotaxanes are the simplest prototypes for the construction of molecular machines based on threaded species. Investigation on molecular motions in these model systems is a necessary action for an efficient design of working molecular machines and motors. Herein we report on photoactive pseudorotaxanes based on the interaction between bipyridinium and cucurbit[7]uril (CB7). The molecular axle is composed of a central bipyridinium unit and two azobenzene moieti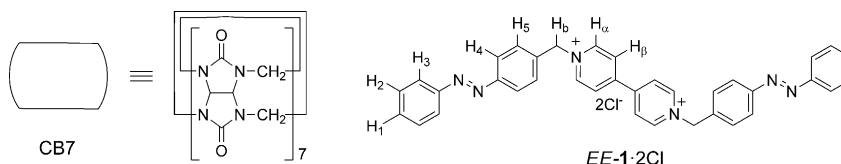es at the extremities. CB7 can form two different complexes with this molecule: a [2]pseudorotaxane, in which the macrocycle shuttles fast along the length of the axle, and a [3]pseudorotaxane, in which two CB7s are confined at the extremities of the axle. Upon *trans* to *cis* isomerization of the azobenzene moieties, the [3]pseudorotaxane is destabilized, and only one CB7 resides on the axle, surrounding the bipyridinium unit. The system was successfully inserted into the core of liposomes, and preliminary investigations confirmed that it maintains its switching ability.

## Introduction

Cucurbit[*n*]urils (CBs) are barrel-shaped macrocycles made of *n*-methylene-bridged glycoluril units (Scheme 1).[1] They have a rigid structure in which it is possible to recognize two regions with different properties: the interior of the cavity is hydrophobic, whereas the two portals are lined with carbonylic moieties, which confer a polar character to the entries of the cavity. These characteristics endow cucurbiturils with extraordinary recognition properties[2] that were exploited for versatile applications[3] in the fields of catalysis,[4] sensing,[5] drug delivery,[6] logic gates,[7] and molecular machines.[8] Because of the carbonylic units on the portals

of their cavity, CBs show a good affinity for cationic guests,[2, 9] giving host–guest complexes the stability of which can be controlled by means of pH changes,[6, 10] electrochemistry,[11] competitive reactions,[2, 7] and light.[6, 12]

The large association constants of CBs with various guests in aqueous solution and the possibility to control the stability of the adducts by means of different inputs make these macrocycles good candidates for the construction of molecular machines.[8] Pseudorotaxanes are simple prototypes of artificial molecular machines, wherein an axle-like molecule is surrounded by (at least) one macrocyle. These supramolecular systems can perform threading-dethreading motions, and are convenient precursors for the synthesis of more complex interlocked structures, such as rotaxanes and catenanes.[13] Bipyridinium derivatives are commonly used as recognition units in interlocked supramolecular systems, because they can be involved in charge-transfer interactions, which can be easily switched on and off by means of redox stimuli.[14] It is well known that 1,1′-disubstituted-4,4′-bipyridinium (also called viologen) derivatives can form very stable inclusion complexes with CB7, with two modes of interaction:[15] the macrocycle can either include



**Scheme 1.** Structures of the examined compounds.

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the aromatic nucleus of the bipyridinium inside its cavity, with the two quaternary nitrogen atoms positioned on the portals, or it can be placed on one side of the bipyridinium, with one portal interacting with one quaternary nitrogen, and the substituents of the bipyridinium included in the cavity. The control on the position of the macrocycle and on the stability of the adduct can be exerted by several methods, which depend also on the nature of the bipyridinium substituents.[8, 10, 16] In this paper, we investigate a new CB7-based pseudorotaxane in which the axle-like component is the bipyridinium derivative *EE*-**1**·2 Cl shown in Scheme 1. *EE*-**1**·2 Cl is a symmetric molecule in which the central bipyridinium unit is derivatized with two azobenzene moieties. Azobenzene is a well known photochromic compound,[17] which can be efficiently isomerized from the thermodynamic stable *E* form to the *Z* isomer by means of UV light, and can be switched back from *Z* to *E* either by visible light or thermally. In the present work, compound **1**$^{2+}$ and its host–guest complexes with CB7 have been fully characterized from the thermodynamic and kinetic point of view. The bipyridinium unit of **1**$^{2+}$ can be hosted inside the cavity of CB7,[15] and the two molecules can form adducts with 1:1 and 2:1 stoichiometry. In this regard, the isomerization state of the azobenzene moieties can be exploited to control the stability and the stoichiometry of the supramolecular complexes.

## Results and Discussion

The synthetic route and details for the preparation of the dumbbell compound **1**$^{2+}$ are presented in the Experimental Section.

NMR and UV/Vis spectroscopic experiments have been performed in NaCl water solutions: the salt is necessary on one side to favor the solubilization of CB7, and on the other side to control the concentration of Na$^+$ ions. It is known[18] that CB7 is an excellent host for Na$^+$ ions, which as a matter of fact cannot be completely removed from synthesized CB7. As Na$^+$ is a competitive guest for CB7, its presence affects the stability of any other host–guest complex and all the corresponding equilibria are conditioned by the association between CB7 and Na$^+$ ions.[18, 19] The presence of cations that bind to the CB7 portals decreases the concentration of free host available to bind **1**$^{2+}$. As a consequence when evaluating the equilibria between CB7 and **1**$^{2+}$, we measured overall binding constants,[18b] which are related to the equilibrium constants between CB7 and one Na$^+$ ion, two Na$^+$ ions and one **1**$^{2+}$ molecule. For this reason the concentration of NaCl was kept constant: a fixed concentration of Na$^+$ ion guarantees reproducibility of the experiments and enables the determination of the individual equilibrium constants between CB7 and **1**$^{2+}$ (vide infra).[18b]

The absorption spectrum of *EE*-**1**$^{2+}$ shows the typical features of its chromophores (Figure 1): an intense band at 322 nm and a broad and weak band at 428 nm, which can be ascribed to the π–π* and n–π* transitions of the azobenzene units, respectively;[17] the shoulder at 260 nm originates from the absorbance of the bipyridinium central moiety.[20]
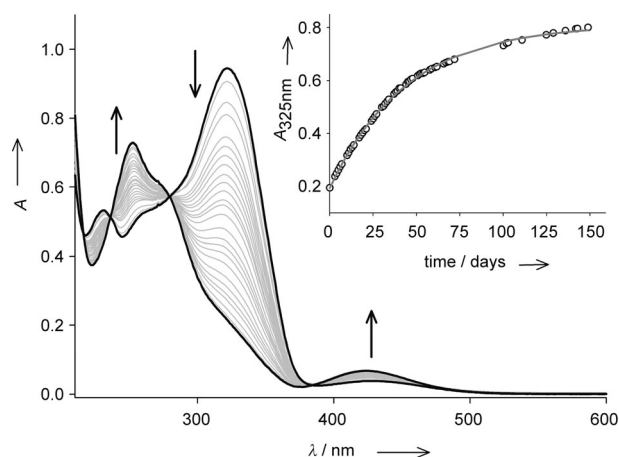


**Figure 1.** Absorption spectra (H$_2$O, 5 mM NaCl) of a 2.1×10$^{-5}$ M solution of *EE*-**1**$^{2+}$ upon irradiation with 365 nm light. The inset shows the absorption changes at 325 nm associated with the *Z*→*E* thermal reaction (○). —— represents the fitting of the data according to a first-order model.

Irradiation of *EE*-**1**$^{2+}$ with 365 nm light (Figure 1) affords *ZZ*-**1**$^{2+}$ with a quantum efficiency of 0.12[17] and a yield of 85% at the photostationary state (Table 1). *ZZ*-**1**$^{2+}$ is very stable and at room temperature isomerizes back to *EE*-**1**$^{2+}$ with $t_{1/2}=33$ days (Figure 1, inset). *Z*→*E* isomerization can also be carried out by irradiation with visible light, with a quantum yield of 0.46[17] (Table 1).

**Table 1.** Photoisomerization quantum yields.[a]

| Compound | $\Phi_{E \to Z}$[b] | $\Phi_{Z \to E}$[c] |
|---|---|---|
| *EE*-**1**$^{2+}$ | 0.12 | 0.46 |
| [*EE*-**1**⊂CB7]$^{2+}$ | 0.10 | 0.3 |
| [*EE*-**1**⊂2 CB7]$^{2+}$ | 0.08 | |

[a] The experimental error on the quantum yield values is 10%. [b] $\lambda_{irr}=$ 365 nm. [c] $\lambda_{irr}=$ 436 nm.

The persistence of the same isosbestic points throughout the irradiation experiments suggests that the two azobenzene moieties of **1**$^{2+}$ are spectroscopically independent. As a consequence, the *ZZ*, *EE*, and *ZE* (or *EZ*) isomers present throughout the irradiation cannot be distinguished with UV/Vis spectroscopy. It can also be reasonably assumed that the two azobenzene moieties are isomerized independently and are therefore statistically distributed among the **1**$^{2+}$ molecules. Considering that at the photostationary state in our conditions 85% of the *E* units have been isomerized to the *Z* form, the solution contains 72% *ZZ*-**1**$^{2+}$, 2% *EE*-**1**$^{2+}$, and 26% *EZ(ZE)*-**1**$^{2+}$. The thermodynamic and kinetic experiments were fitted with the simplest model, which considers the presence of only one species after exhaustive irradiation. As this model affords a very good data-fitting, it can be concluded that the behaviour of the *EZ* isomer with the CB7 host is very similar to that of the dominant *ZZ* species. It cannot be excluded, however, that the presence of more than one type of guest has an influence on the

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measured thermodynamic and kinetic constants. Nevertheless, as it will be discussed further below, the effect of photoisomerization on the stoichiometry of the pseudorotaxanes is unquestionable. This is an additional evidence of the similar behaviour of the EZ and ZZ isomers. Therefore, for the sake of simplicity, in the following discussion we will refer to the UV irradiated solution at the photostationary state as if it would contain only the ZZ species.

Upon addition of up to one equivalent of CB7 to a deuterated water solution of EE-1·2 Cl, the NMR spectrum shows a new set of resonances (Figure 2a–c), indicative of the formation of
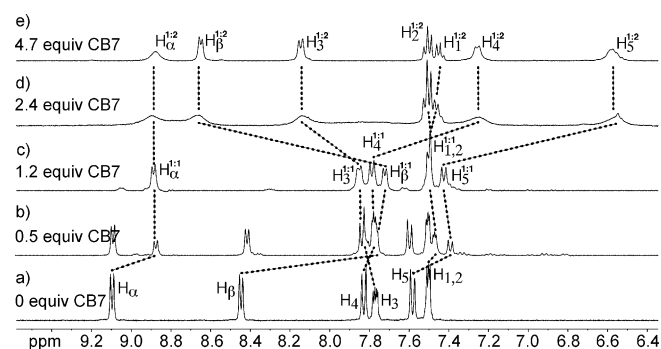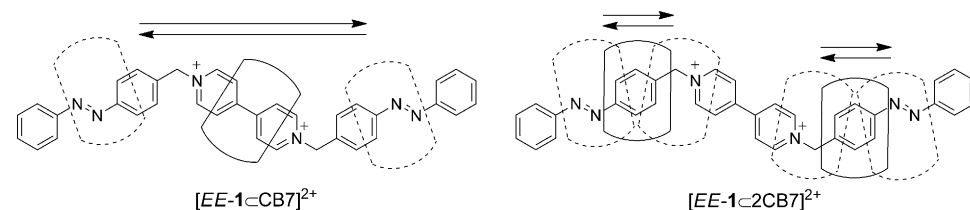


**Figure 2.** Partial $^1$H NMR spectra (D$_2$O, 0.1 M NaCl, 400 MHz, 298 K) of a solution of EE-$1^{2+}$ ($10^{-3}$ M) with an increasing amount of CB7: a) 0 equiv, b) 0.5 equiv, c) 1.2 equiv, d) 2.4 equiv, e) 4.7 equiv. Superscript 1:1 and 1:2 refers to resonances of the [EE-$1 \subset$CB7]$^{2+}$ and [EE-$1 \subset$2CB7]$^{2+}$ pseudorotaxanes, respectively.

a pseudorotaxane species with a 1:1 stoichiometry, namely [EE-$1 \subset$CB7]$^{2+}$, in slow equilibrium, on the NMR time scale, with the free components. It is well known that the proton resonances of a guest molecule exhibit upfield shifts when it is located inside the CB cavity, while the resonances undergo downfield shifts when the guest molecule is outside the cavity and near the rims of the CB macrocycle.[21] The resonances of protons H$_\alpha$ and H$_\beta$ of the bipyridinium as well as H$_4$ and H$_5$ of the azobenzene units are shifted upfield while H$_3$ protons show a significant downfield shift, and H$_1$ and H$_2$ protons resonances are almost unaffected. These observations indicate that the CB7 macrocycle shuttles fast around the bipyridinium unit within the range of the H$_3$ protons of the azobenzene units while hosting protons H$_\alpha$, H$_\beta$ of the bipyridinium unit and protons H$_4$ and H$_5$ of the azobenzene moieties inside its cavity (Scheme 2, left). Upon addition of more than one equivalent of CB7 (Figure 2d) the NMR spectrum shows the appearance of new broad resonances due to the formation of a [3]pseudorotaxane species, [EE-$1 \subset$2CB7]$^{2+}$, in fast equilibrium, on the NMR timescale, with the [2]pseudorotaxane [EE-$1 \subset$CB7]$^{2+}$.[22] Addition of an excess of CB7 (Figure 2e) pushes the equilibrium toward the 1:2 complex [EE-$1 \subset$2CB7]$^{2+}$ and reduces the broadening of the spectrum due to the fast exchange with the 1:1 adduct [EE-$1 \subset$CB7]$^{2+}$. Resonances of protons H$_4$ and H$_5$ in the [3]pseudorotaxane [EE-$1 \subset$2CB7]$^{2+}$ are strongly shifted upfield compared to the free dumbbell EE-$1$·2 Cl and to [EE-$1 \subset$CB7]$^{2+}$, and a large downfield shift of protons H$_\beta$ and H$_3$ is evident in the spectrum (Figure 2e). This is consistent with each CB7 macrocycle hosting inside its cavity protons H$_\alpha$, H$_4$, and H$_5$ while shuttling fast within the range of protons H$_\beta$ of the bipyridinium central unit and H$_3$ of the azobenzene moieties (Scheme 2, right).

The NMR spectrum of a sample of EE-$1$·2 Cl exhaustively irradiated at 365 nm displays the characteristic resonances of Z azobenzene moieties. From integration of the relevant peaks it is possible to estimate a conversion of around 85% of all the azobenzene units, in line with UV/Vis absorption experiments. The complexity of the spectra obtained upon irradiation of solutions of [EE-$1 \subset$CB7]$^{2+}$ and [EE-$1 \subset$2CB7]$^{2+}$ does not allow assignment of all the resonances. Despite this drawback, the spectra of the irradiated adducts do not show significant differences, regardless of the amount of CB7 present, pointing to a single stoichiometry for the ZZ complex. Insightful information in this regard can be obtained from UV/Vis titration experiments (vide infra).

Upon titration of a solution of EE-$1^{2+}$ with CB7, the absorption changes reported in Figure 3 are observed: the azobenzene $\pi$–$\pi^*$ band at 322 nm experiences a 5 nm hypsochromic shift, whereas in the region around 260 nm two trends are clearly discernible. The bipyridinium band decreases when up to one equivalent of CB7 is added, in line with what is observed for related host–guest systems;[15] conversely, on adding an excess of CB7, the absorption increases until reaching a plateau (Figure 3). These data can be fitted with a model that implies the formation of two adducts between CB7 and EE-$1^{2+}$: namely, a 1:1 host–guest complex in which the CB7 encircles the bipyridinium unit and a 2:1 complex, wherein two macrocycles interact with the axle. Unfortunately an accurate determination of the $K_{1E}$ is prevented by its large value and by the presence of the subsequent association process, which affects the shape of the titration curve. The values obtained by means of a global fit analysis of the spectra of the titration experiments are overall binding constants $\beta$,[18b] as they are determined in the presence of sodium ions, and they are related to the equilibrium constants between CB7 and one Na$^+$ ion, two Na$^+$ ions, and one guest molecule. The individual association constants $K$ for the formation of the pseudorotaxanes, purged from the dependence



**Scheme 2.** Representation of the CB7 shuttling motion in pseudorotaxanes [EE-$1 \subset$CB7]$^{2+}$ (left) and [EE-$1 \subset$2CB7]$^{2+}$ (right).

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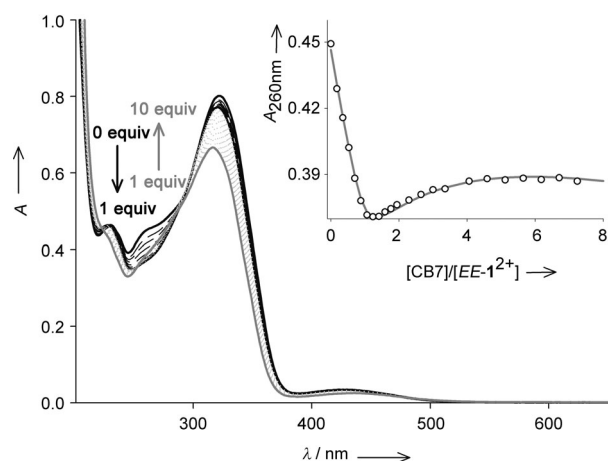
**Figure 3.** Absorption spectra of a $1.9 \times 10^{-5}$ M solution of $EE$-$\mathbf{1}^{2+}$ upon titration with CB7 ($H_2O$, 5 m M NaCl). The inset shows the plot of the absorption changes at 260 nm (—— represents the fitting of the experimental data).



**Figure 4.** AM1 minimized molecular models of a) $[EE$-$\mathbf{1} \subset CB7]^{2+}$ and b) $[EE$-$\mathbf{1} \subset 2CB7]^{2+}$.[25]

on metal cations concentration, can be calculated as follows:[18b]

$$K = \beta(1 + K_{Na1}[Na^+] + K_{Na1}K_{Na2}[Na^+]^2)$$

in which $K_{Na1}$ and $K_{Na2}$ are the equilibrium constants between CB7 and one and two $Na^+$ ions, respectively [Eqs. (4) and (5)]. The 1:1 binding constant $K_{1E}$ for the equilibrium reported in Equation (1) is $\sim 7 \times 10^6$ M$^{-1}$; the 2:1 association constant $K_{2E}$ [Eq. (2)] is $3 \times 10^4$ M$^{-1}$. Since the bipyridinium moiety cannot accommodate two CB7 macrocycles, it is likely that the second CB7 stays on the azobenzene end unit, causing the displacement of the first CB7 from the bipyridinium towards the other azobenzene. The final adduct is a [3]pseudorotaxane in which two macrocycles reside partially on the azobenzene units, with one portal lined with the quaternary nitrogen atom of the bipyridinium moiety (Figure 4).

It is known that the $E$ and $Z$ isomers of azobenzene have different electronic properties and, more importantly, significantly different shapes. It can, therefore, be expected, also on the basis of earlier observations on related systems,[23,24] that $E$–$Z$ isomerization of $\mathbf{1}^{2+}$ can have an effect on both the thermodynamic and kinetic behavior of its complexes with CB7. To investigate this aspect, a solution of $EE$-$\mathbf{1}^{2+}$ was irradiated at 365 nm till reaching a photostationary state and then titrated with CB7 (Figure 5). The absorption spectrum decreases in the region around 260 nm: the experimental data can be fitted with a model that implies the formation of just one supramolecular adduct with 1:1 stoichiometry ($K_{1Z} \sim 1 \times 10^6$ M$^{-1}$). Despite the good quality of the fitting, the determination of $K_{1Z}$ is complicated by the presence of 15% of $EE$-$\mathbf{1}^{2+}$, which, in its turn, can form the 1:1 and 2:1 complexes. Nevertheless the data suggest that isomerization of the azobenzene end groups causes a slight destabilization of the 1:1 complex. The association constant, however, remains large, and ensures more than 70% association under our experimental conditions.

Both threading and dethreading processes have also been investigated from the kinetic point of view by means of
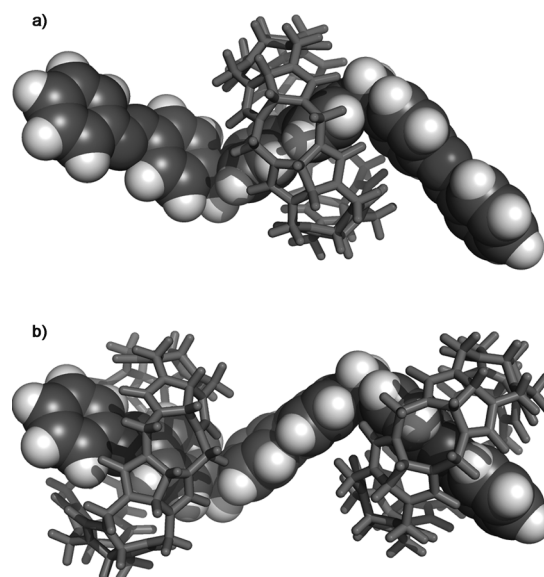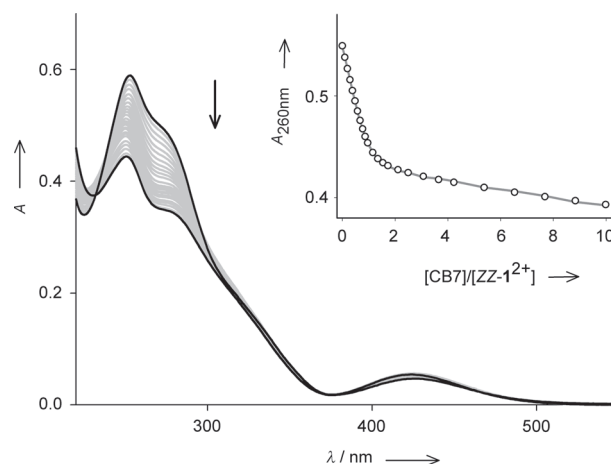


**Figure 5.** Absorption spectra of a $1.9 \times 10^{-5}$ M solution of $ZZ$-$\mathbf{1}^{2+}$ upon titration with CB7 ($H_2O$, 5 m M NaCl). The inset shows the plot of the absorption changes at 260 nm (—— represents the fitting of the experimental data).

stopped-flow spectrophotometry (Table 2). The three processes of Equations (1–3) were investigated in the following experiments: 1) two equimolar solutions of $EE$-$\mathbf{1}^{2+}$ and CB7 were mixed in a 1:1 ratio (Figure 6a); 2) a solution of $[EE$-$\mathbf{1} \subset CB7]^{2+}$ was mixed with a solution of CB7 in a 1:5 molar ratio; 3) a solution of $EE$-$\mathbf{1}^{2+}$ was irradiated at 365 nm till reaching a photostationary state and was then mixed with a solution of CB7 in a 1:5 ratio. The absorption changes at 260 nm were monitored, and the experimental traces were fitted with a mixed order model, that is second- (threading) and first-order (dethreading) opposing reactions [Eqs. (1–3)]. As a matter of fact, the association process is slowed down by the presence of $Na^+$ ions, which trap CB7 as CB7·$Na^+$ and $Na^+$·CB7·$Na^+$ complexes; therefore, the pre-equilibrium between CB7 and $Na^+$ ions is included in the fitting model, [Eqs. (4) and (5)] (see the Support-

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**Table 2.** Thermodynamic and kinetic constants for the threading and dethreading processes.

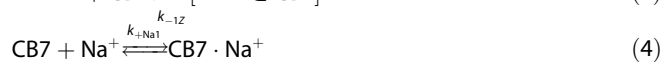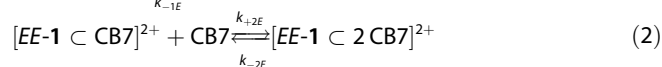| Compound | $K^{[a]}$ [M$^{-1}$] | $k_+^{[b]}$ [M$^{-1}$ s$^{-1}$] | $k_-^{[c]}$ [s$^{-1}$] |
|---|---|---|---|
| $[EE\text{-}1\subset CB7]^{2+}$ | $1.6\times10^7$ | $6.9\times10^6$ | 0.43 |
| $[EE\text{-}1\subset 2\,CB7]^{2+}$ | $3.8\times10^4$ | $3.5\times10^6$ | 92,$^{[d]}$ 0.48$^{[e]}$ |
| $[ZZ\text{-}1\subset CB7]^{2+}$ | $6.6\times10^6$ | $2.5\times10^6$ | 0.38 |

[a] Equilibrium constant determined from the ratio of the threading and dethreading rate constants. [b] Threading rate constant. [c] Dethreading rate constant. [d] $k_-$ for the dethreading of the first CB7 from $[EE\text{-}1\subset 2\,CB7]^{2+}$. [e] $k_-$ for the dethreading of the second CB7 from $[EE\text{-}1\subset 2\,CB7]^{2+}$.
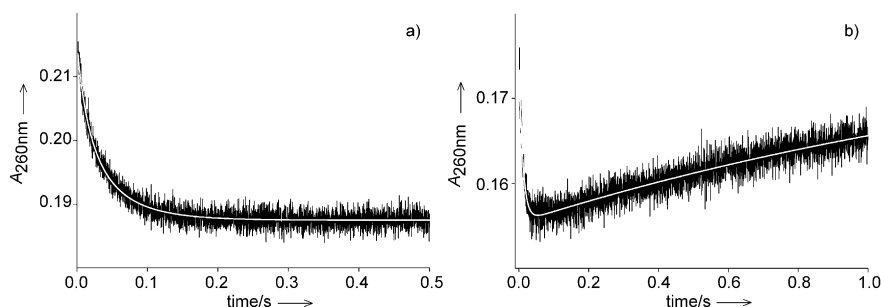


**Figure 6.** Stopped-flow kinetic traces recorded at 293 K for the absorbance change at 260 nm obtained upon mixing equal volumes of a) a solution of $EE\text{-}1^{2+}$ and a solution of CB7 (concentration after mixing $9\times10^{-6}$ M and $1.2\times10^{-5}$ M, respectively) and b) a solution of $[EE\text{-}1\subset 2\,CB7]^{2+}$ and a solution of AD (concentration after mixing $9\times10^{-6}$ M and $1.1\times10^{-4}$ M, respectively) (H$_2$O, 5 mM NaCl). —— represents the fitting of the experimental data (see text for details).

ing Information for details). This model would imply the simultaneous determination of six rate constants, but some of these values can be set as fixed parameters. First of all, the kinetic constants for the equilibria of Equations (5) and (6) can be fixed to reasonable values, knowing that the binding of Na$^+$ to CB7 is fast[18b] (see the Supporting Information for details). Moreover, the dethreading rate constants can be determined in independent experiments, by means of a competitive guest.

$$EE\text{-}1^{2+} + CB7 \underset{k_{-1E}}{\overset{k_{+1E}}{\rightleftharpoons}} [EE\text{-}1 \subset CB7]^{2+} \tag{1}$$

$$[EE\text{-}1 \subset CB7]^{2+} + CB7 \underset{k_{-2E}}{\overset{k_{+2E}}{\rightleftharpoons}} [EE\text{-}1 \subset 2\,CB7]^{2+} \tag{2}$$

$$ZZ\text{-}1^{2+} + CB7 \underset{k_{-1Z}}{\overset{k_{+1Z}}{\rightleftharpoons}} [ZZ\text{-}1 \subset CB7]^{2+} \tag{3}$$

$$CB7 + Na^+ \underset{k_{-Na1}}{\overset{k_{+Na1}}{\rightleftharpoons}} CB7 \cdot Na^+ \tag{4}$$

$$CB7 \cdot Na^+ + Na^+ \underset{k_{-Na2}}{\overset{k_{+Na2}}{\rightleftharpoons}} Na^+ \cdot CB7 \cdot Na^+ \tag{5}$$
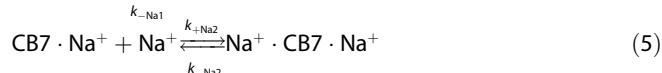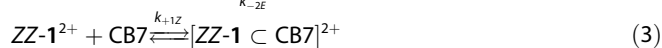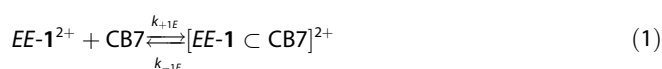
It is known[2] that adamantane derivatives like 1-adamantylamine (AD) are suitable guests for CB7, with association constants as large as $10^{12}$ M$^{-1}$. Therefore on addition of AD to a solution of $E$ or $Z$ pseudorotaxanes, fast and complete dissociation is achieved,[10] as confirmed by titration experiments of $[EE\text{-}1\subset 2\,CB7]^{2+}$ and $[ZZ\text{-}1\subset CB7]^{2+}$ with AD. Stopped-flow experiments have been performed under simplified conditions, namely by mixing a solution of each pseudorotaxane with a solution of AD in excess. The data were fitted with a first-order

mechanism (Figure 6b), considering that the rate-determining step is the dethreading of the supramolecular complex[10] (Table 2). The analysis of the data for the dethreading process is much more simple, as the dissociation reaction is not affected by the presence of ions. The values of $k_-$ determined from the fitting are introduced as a fixed parameter in the fitting model for the threading experiments (see the Supporting Information for details). The values of $k_+$ and $k_-$ are gathered in Table 2. The rate constant $k_{+1Z}$ is almost one third of $k_{+1E}$, which suggests a small but measurable kinetic effect of the isomerization state of the azobenzene units (Table 2).[23] The values of $k_+$ and $k_-$ could be used for a more accurate estimation of the thermodynamic constants for the $Z$ and $E$ complexes (Table 2). It is worth noting that the first dethreading process of $[EE\text{-}1\subset 2\,CB7]^{2+}$ is faster than the second one, which, on its turn, takes place in the same timescale of the dethreading from both $[EE\text{-}1\subset CB7]^{2+}$ and $[ZZ\text{-}1\subset CB7]^{2+}$. These results suggest that 1) as expected, after removal of one CB7 from the 2:1 complex, the other one shuttles back to the bipyridinium unit and 2) the rate of slippage of the macrocycle over the azobenzene unit when it dethreads from the 1:1 complexes is not affected by the isomerization state. It is likely, therefore, that the $Z$ axle cannot arrange two CB7 rings for electronic reasons, rather than for the steric hindrance exerted by the $Z$ isomer. It is actually recognized that the $Z$ isomer is more polar with respect to the $E$ one,[26] and this could be the reason for the slightly lower affinity of the CB7 for the $ZZ\text{-}1^{2+}$ axle.

The photochemical properties of $[EE\text{-}1\subset CB7]^{2+}$ and $[EE\text{-}1\subset 2\,CB7]^{2+}$ have also been investigated: irradiation of $[EE\text{-}1\subset CB7]^{2+}$ and $[EE\text{-}1\subset 2\,CB7]^{2+}$ with 365 nm light affords in both cases $[ZZ\text{-}1\subset CB7]^{2+}$ with quantum efficiencies of 0.10 and 0.08, respectively (Table 1). Therefore the encapsulation of the axle into the wheel does not influence the photochemical properties of the azobenzene units. It is worth noting that when the axle is in the $Z$ configuration it cannot host two CB7s; therefore upon irradiation of the [3]pseudorotaxane, the release of one CB7 is achieved (Scheme 3). Indeed the shape of the absorption spectra of an irradiated solution of $[EE\text{-}1\subset 2\,CB7]^{2+}$ and of a solution of $[ZZ\text{-}1\subset CB7]^{2+}$ (obtained either by titration of $ZZ\text{-}1^{2+}$ or by irradiation of a solution of $[EE\text{-}$
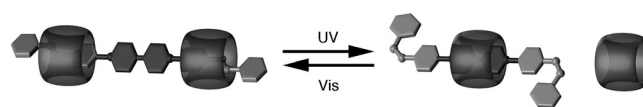


**Scheme 3.** Schematic representation of the light-controlled release and uptake of CB7.

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$1 \subset CB7]^{2+})$ match properly. The thermal stability of $[ZZ$-$1 \subset CB7]^{2+}$ could not be investigated because of precipitation problems over a timescale of hours; nevertheless, $Z \rightarrow E$ isomerization could be obtained by exposure to visible light (436 nm). Irradiation of $[ZZ$-$1 \subset CB7]^{2+}$ affords $[EE$-$1 \subset CB7]^{2+}$ with a quantum efficiency of 0.3; in presence of an excess of CB7, the 1:1 $E$ complex can uptake a second macrocycle, yielding the [3]pseudorotaxane $[EE$-$1 \subset 2CB7]^{2+}$ (Scheme 3). In summary, in presence of an excess of CB7, it is possible to switch the system from a [3]pseudorotaxane to a [2]pseudorotaxane, and back, by means of light inputs.

An interesting aspect of pseudorotaxanes, related to the controlled assembly/disassembly process, is the possibility to tune the concentration of solutes in a solution. For instance, the dethreading of a [2]pseudorotaxane increases the concentration of free species in solution, up to twice the starting value. Consequently, insertion of a pseudorotaxane into a compartmentalized environment and actuation of the disassembly process should cause an osmotic stress.[27] In this regard the use of liposomes[28] as scaffolds proves very convenient, as they are well known, easy to make containers, which provide the needed compartmentalization while maintaining the possibility to work in solution.

To investigate this possibility we inserted the [3]pseudorotaxane $[EE$-$1 \subset 2CB7]^{2+}$ in the aqueous core of 1-palmitoyl-2-oleoylphosphatydilcholine (POPC)-based liposomes. A dehydrated film of POPC was rehydrated with a solution of the [3]pseudorotaxane; after extrusion and filtration, the mean diameter of the loaded liposomes, evaluated by means of dynamic light scattering measurements, was 98 nm. The absorption spectrum of the suspension (Figure 7) shows a shoulder between 300 and 350 nm that is clearly assigned to the $E$ azobenzene moieties of $EE$-$1^{2+}$. The absorption spectral changes observed on irradiation with UV light are consistent with the $E \rightarrow Z$ isomerization of the azobenzene units. Unfortunately an estimation of the efficiency of the process is prevented by the scattering of the liposome suspension, but the spectroscopic

changes suggest a lower percentage of $E$ to $Z$ conversion with respect to the homogeneous solution.

As demonstrated above, the isomerization of the [3]pseudorotaxane should cause the release of one molecule of CB7, thus increasing the concentration of free species inside the core of the liposomes. The change in the osmotic pressure inside the liposomal core could possibly cause a swelling of the vesicles. As a matter of fact, the mean diameter of the liposomes is not changed after irradiation. This result is not surprising, if we consider that the [3]pseudorotaxane is obtained upon mixing $EE$-$1 \cdot 2Cl$ and CB7 in 1:5 molar ratio: this implies that, even in the most favorable conditions (i.e. quantitative 2:1 association before irradiation and quantitative dissociation upon irradiation) the change in concentration cannot exceed 25 %.

## Conclusion

We have designed and characterized a new pseudorotaxane based on a cucurbit[7]uril wheel and a photoactive axle component. In the latter compound, two azobenzene moieties are appended to a central bipyridinium unit, which is a well-known guest for CBs; the two azobenzenes can be switched between $E$ and $Z$ configurations by means of UV and visible light. The $E$ isomer of the axle can form two types of adducts with CB7: 1) a [2]pseudorotaxane with one CB7 wheel shuttling fast around the bipyridinium unit and 2) a [3]pseudorotaxane with two CB7 wheels, either one residing close to an azobenzene group and shuttling around on a much shorter distance. On the other hand, the $Z$ isomer can form only the 1:1 adduct. The systems were fully characterized from the thermodynamic and kinetic point of view. The photochemical properties of the azobenzene units are not affected by the presence of CB7; therefore, it is possible to control the stoichiometry of the pseudorotaxane by means of light. Pseudorotaxanes can be considered as simple prototypes of artificial molecular machines, and the possibility to control their motion by means of photochemical inputs is very convenient, especially for the design of more complex supramolecular systems. In this regard, a preliminary investigation on the insertion of the [3]pseudorotaxane into the aqueous core of POPC-based vesicles suggests that this system is compatible with the liposome environment, and keeps its ability to switch upon irradiation. These results are important for future applications of pseudorotaxanes as functioning devices, for example, to control the release of molecular species inside the core of a nanocontainer. Further developments in this direction are under investigation in our laboratory.

## Experimental Section

### General

$EE$-$1 \cdot 2Cl$ was synthesized with a slight modification of a previously published procedure.[29] 4-Chloromethyl azobenzene was synthesized according to a previously published procedure. Commercially available compounds, including cucurbit[7]uril (Aldrich), were reagent grade quality and were used without further purification.
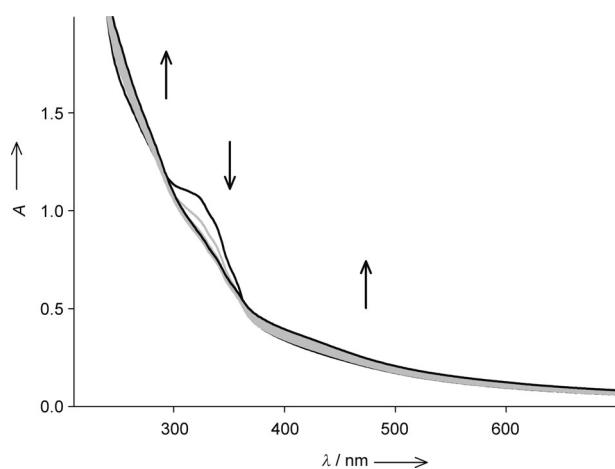


**Figure 7.** Absorption spectrum of a solution of $[EE$-$1 \subset 2CB7]^{2+}$ inside the aqueous core of POPC-based liposomes upon irradiation with 365 nm light.

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Solvents were dried according to literature procedures. The $^1H$ NMR spectra were recorded at 298 K in $D_2O$, 0.1 M NaCl, with a Varian Mercury 400 spectrometer with the deuterated solvent as the lock and the residual solvent as the internal standard. All chemical shifts are quoted by using the $\delta$ scale and all coupling constants (J) are expressed in Hertz (Hz). Melting points were determined using a Büchi 510 series apparatus and are uncorrected.

EE-**1**·2 Cl was prepared by refluxing a solution of 4-chloromethyl azobenzene (1.7 g, 7.4 mmol) and 4,4′-dipyridine (580 mg, 3.7 mmol) in acetonitrile (50 mL) under a nitrogen atmosphere for 24 h. After being cooled to room temperature, the yellow precipitate was collected by filtration and washed with n-hexane and ethyl ether. M.p. 230 °C decomposition; $^1H$ NMR (400 MHz; $D_2O$): $\delta = 9.24$ (d, $J = 6.3$ Hz, 4H), 8.59 (d, $J = 6.3$ Hz, 4H), 7.97 (d, $J = 8.4$ Hz, 4H), 7.92 (m, 4H), 7.73 (d, $J = 8.4$ Hz, 4H), 7.65 (m, 6H), 6.06 ppm (s, 4H); $^{13}C$ NMR (101 MHz; $D_2O$): $\delta = 139.44$, 126.08, 125.96, 124.30, 124.09, 123.88, 123.34, 122.91, 120.95, 117.04, 116.27, 45.83 ppm; ESI-MS: m/z: calcd: 617.57; found: 582.12 [$M^+$ −Cl].

Absorption spectra were recorded at room temperature on air-equilibrated solutions ($H_2O$, 5 mM NaCl) contained in 1 cm quartz cuvettes by using Perkin–Elmer Lambda 40 and Lambda 650 and Varian Cary 50 Bio spectrophotometers. The experimental error on the wavelength values was estimated to be ±1 nm. Photochemical reactions were performed in stirred air equilibrated aqueous solutions at RT by using a Hanau Q400 medium pressure Hg lamp (150 W). The selection of the desired irradiation wavelength ($\lambda = 365$ or 436 nm) was accomplished by the use of an appropriate interference filter. The number of incident photons was determined by ferrioxalate actinometry in its micro version.[30] The E→Z photoisomerization quantum yield ($\lambda_{irr} = 365$ or 436 nm) was determined from the changes of the $\pi$–$\pi^*$ absorption band of the azobenzene units of the reactant at low conversion percentages (<10%; extrapolation to t = 0 was made). The fraction of light transmitted at the irradiation wavelength was taken into account in the calculation of the yields. The experimental error on the quantum yield values was estimated to be 10%. Thermal Z→E isomerization was performed at 293 K in the dark; the experimental data were fitted by using the SPECFIT fitting program[31] with a first-order kinetic equation.

Reaction kinetic profiles were collected on air-equilibrated aqueous solutions at 298 K with an Applied Photophysics SX 18-MV equipment. The standard flow tube used had an observation path length of 1.0 cm, and the driving ram for the mixing system was operated at the recommended pressure of 8.5 bar. Under these conditions, the time required to fill the cell was 1.35 ms (based on a test reaction). A baseline correction was applied to the stopped-flow traces, to take into account the dependence of the instrument response on pressure. In all the experiments, the cell block and drive syringes were thermostatted by using a circulating constant-temperature bath maintained at the required temperature. In all cases the data were elaborated by means of the SPECFIT fitting program.

1-Palmitoyl-2-oleoylphosphatydilcholine (POPC) was purchased from Avanti Polar Lipids (Alabaster, AL). Sephadex G-25 was purchased from Sigma. POPC liposomes were prepared by rehydration of a homogeneous thin film, obtained by evaporation of a chloroform solution (100 μL) of POPC (25 mg mL$^{-1}$). The phospholipidic film was kept at 4 °C overnight before rehydration with a solution of EE-**1**·2 Cl $1.7 \times 10^{-4}$ M and CB7 $8.5 \times 10^{-4}$ M in 5 mM NaCl. The obtained liposomal suspension was stirred and extruded 20 times through a polycarbonate membrane with 100 nm pores on an Avanti Polar Lipids (Alabaster, AL, USA) miniextruder. The liposome suspension was filtered four times through a Sephadex G-25 column. Vesicle size was evaluated by dynamic light scattering measurements with a Malvern Nano ZS instrument equipped with a 633 nm laser diode.

## Acknowledgements

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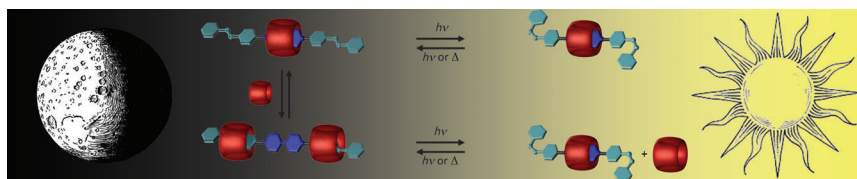
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