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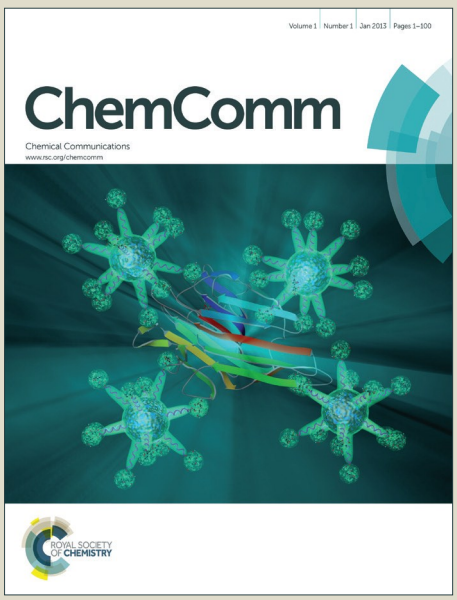

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Moving systems of polar dimeric capsules out of thermal equilibrium by light irradiation†

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Heterodimeric capsules self-assembled from tetraurea calix[4]pyrrole and tetraurea calix[4]arene provide unique molecular containers for the organised inclusion of small polar molecules. By inserting stimuli-responsive groups (azobenzene) in the heterocapsule structure, we are able to modify the equilibrium state of the system or the exchage between different host-guest assemblies in a reversible manner.

Encapsulation of key molecules in mono- and poly-dispersed molecular containers is an idea being explored in the last decades for several technological applications.[1] Many encapsulation approaches have been developed, but still some challenges and limitations need to be solved. Main issues are related to controlling the capture-release processes, the reversibility of the systems and the type of guests that can be captured.[2]

Self-assembled supramolecular capsules[3] are convenient systems to study and tackle these challenges, since they are well-defined, monodisperse assemblies that can be precisely designed and manipulated.[4] In this field, our group has pioneered the overcoming of one of the main deficiencies of molecular capsules (particularly regarding potential biological applications): assembling systems with polar interiors. Some of the first examples of capsules with internal H-bond donors were reported combining tetraurea α,α,α,α aryl-extended calix[4]pyrroles (tuC4P) and tetraurea calix[4]arenes (tuC4A).[5] The formation of capsules based on tuC4P and tuC4A scaffolds allowed tuning of their internal cavity properties, leading to bespoke systems able to encapsulate guests with different sizes, polarities and electronic nature.[6] But, to exploit all the potential of these capsules, it is necessary to control the capture and release of guest molecules (cargo), ideally in a reversible manner.

Light is one of the most appealing communication channels between molecules and humans because it is fast, clean and accurately managed in both worlds. Among the available photoresponsive chemical groups,[7] azobenzene has been the most extensively employed by scientists owing to its relatively simple synthesis and derivatisation and also its reliable photochemical and thermal responses.[8]

The introduction of azobenzene units into molecular capsules has been reported very recently by our group[9] and others,[10] showing an unprecedented potential for controlling the behaviour of the parent structures. The general action mechanism of these azobenzene-equipped capsules is based on the formation of well-defined capsular assemblies when all the azobenzene units are in the *trans*-form (thermodynamically stable state) and their isomerisation to the *cis*-form upon light irradiation. The irradiated assemblies become enriched in *cis*-azo groups and eventually a photostationary state (PSS) is reached in which the ratio between *trans* and *cis* azo-conformers remains constant. The increasing amount of azo-groups in the *cis*-form modifies the properties of the capsular aggregates (e.g. cavity volume), and may even lead to the formation of non-capsular aggregates. The *cis*-enriched assemblies show a reduced affinity for the cargo that may result in its partial release to the bulk solution.

In this work, we describe the light-triggered response of a series of dynamic systems based on dimeric capsules bearing photoresponsive azo-groups in only one of their components. Heterodimer **1•2** was assembled from the social self-sorting of all-*trans* tuC4A **1**,[9b] equipped with four terminal azobenzene units, and tuC4P **2** by encapsulating one molecule of trimethylphosphine oxide **3**, or trimethylamine oxide **4** (Fig. 1). Addition of bis-(*N*-oxide *N,N,N′,N′*-tetramethylamino)hexane **5**[6b] to the heterocapsule **3⊂1•2** produced a system of two capsules, **3⊂1•2** and **5⊂2•2**. Light irradiation switched the systems between two states and induced the formation of non-capsular assemblies. We also show preliminary results in the use of light-irradiation for controlling the selective assembly of a capsular aggregate in complex systems.

Azobenzene units were introduced exclusively in the tuC4A

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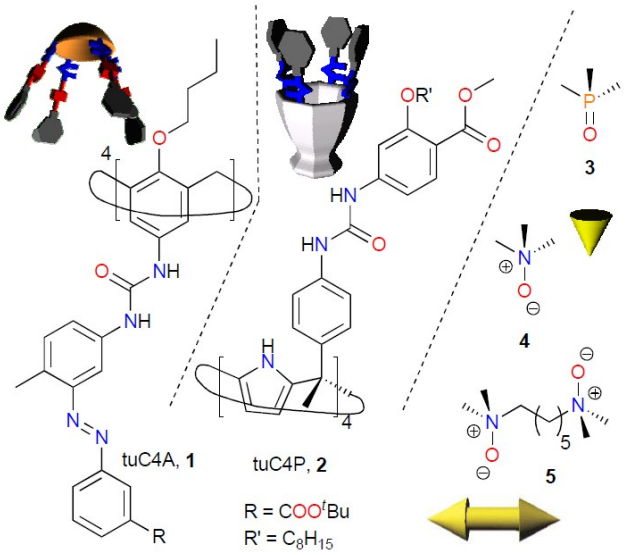

Fig. 1 Chemical structures and schematic representations of capsular components (left and centre) and polar guest molecules (right) studied in this work.

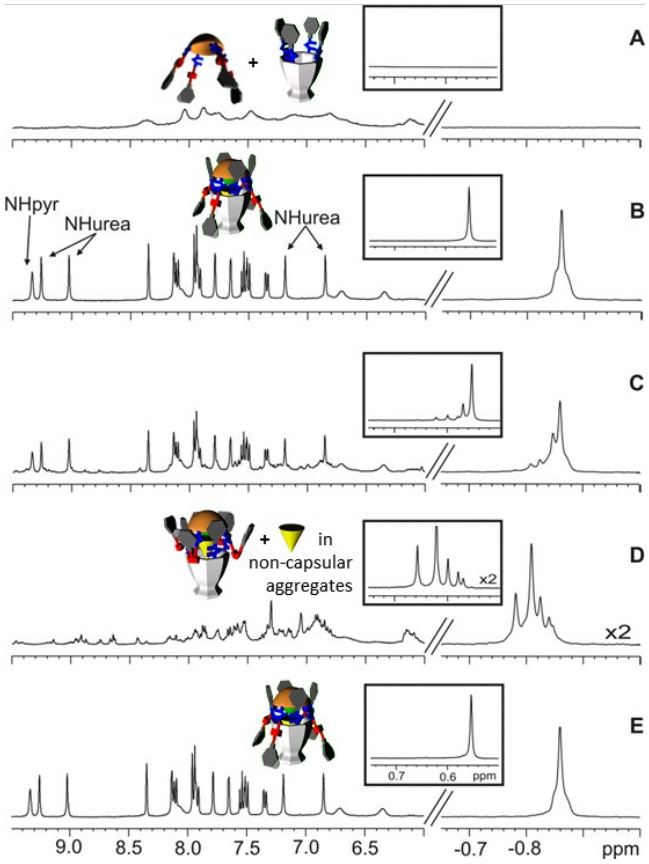

Fig. 2 Selected downfield and upfirld regions of the $^1H\{^{31}P\}$ NMR spectra of A) equimolar mixture of **1** and **2**; B) Addition of 1 molar equivalent of guest **3** to the previous mixture. The signals are diagnostic of **3⊂1·2**. C) Previous sample UV-irradiated for 1 min and D) for 10 min. E) Previous sample thermally equilibrated. The upfield regions for the analogous spectra using **4** as guest are also shown as insets in the different panels.

structure **1** for two reasons: i) to test if the presence of photoresponsive groups in just one half of the capsule was enough to trigger capsular destabilisation (previous reports on the subject were based on homocapsules having photoresponsive groups in all their components),[9,10c] and ii) to allow the self-assembly of tuC4P **2** (azobenzene-free half) into non-responsive homodimeric capsules **2•2** upon light irradiation of the system.

^1H NMR spectroscopy evidenced that tuC4A **1** did not assemble into the homodimeric capsule **1•1** when dissolved in CD_2Cl_2 or by addition of any of the polar guests **3**-**5** (Fig. S7, S13-S15). On the other hand, the tuC4P **2** counterpart dimerised in CD_2Cl_2 solution only by encapsulating bis-N-oxide **5** and yielded the capsular assembly **5⊂2•2** (Fig. S8, S16, S17, S20 and S23). In striking contrast to the observations made for **1**, the ^1H NMR spectra of the mixtures of **2** with **3** or **4** did not show the expected signals for the protons of the free guests. This finding together with the observation of broad signals for the protons of **2** suggested the formation of ill-defined non-capsular aggregates that trapped the polar guests, **3** and **4**, by hydrogen-bonding with the pyrrolic NHs.[11] Equimolar mixtures of all-*trans*-**1** and **2** in CD_2Cl_2 solution also formed ill-defined non-capsular aggregates (Fig. 2A and S12). However, the addition of one molar equivalent of the monodentate guests, **3** or **4**, induced their assembly in the corresponding heterocapsular aggregates **3⊂1•2** or **4⊂1•2** as the only species detected in solution by ^1H NMR spectroscopy (Fig. 2B, S18 and S19). None of the experiments discussed above produced the formation of detectable precipitates.

The assembly of the well-defined capsular hetero-aggregates **3⊂1•2** and **4⊂1•2** was evidenced by the observation of the diagnostic signals in the ^1H NMR spectra of the mixtures (Fig. 2B). DOSY experiments (Fig. S22) also supported the presence of a single supramolecular entity in solution with a calculated spherical diameter of ~1.43 nm. This value was in good agreement with the spherical volumes of the energy minimised structures of the capsules (Fig. S24), and with previously reported values for similar entities.[6,9,12] The downfield shift experienced by the pyrrole NHs of **2** in both aggregates was indicative of strong hydrogen-bonding interactions with the oxygen atoms of the polar guests.[12c] This produces a unique orientation of the guest inside the capsules.[5] In turn, the methyl protons of guests **3** and **4** resonated highly upfield shifted (Δδ = 2.5 ppm) owing to the magnetic shielding exerted by the four *meso*-phenyl groups of **2** (Fig. 2, S9, S10, S18, S19). Taken together, these results supported the single-orientation encapsulation of the polar guests in the tuC4P component **2** of the heterocapsules.

In separate experiments both heterocapsules (**3⊂1•2** and **4⊂1•2**) were irradiated with UV light (365 nm[‡]) for 0, 1, 5, 10 and 15 minutes. NMR spectra were recorded after each irradiation phase. Several sets of new proton signals appeared immediately and grew with irradiation time at the expenses of the signals of the original all-*trans* heterocapsules (Fig. 2C-D, S25 and S29). This was the expected result for the azobenzene groups photo-isomerisation process, leading to the formation of up to 6 different conformational isomers for the tuC4P **1** unit (i.e.: *cccc*, *tccc*, *tctc*, *ttcc*, *tttc*, *tttt*), which might be involved in the formation of capsular and non-capsular aggregates (*vide infra*). In the **3⊂**all-*trans*-**1•2** capsular

assembly, the broad singlet resonating higher upfield (phosphorous decoupled spectrum Fig. 2B, S25 and S29) was assigned to the methyl protons of the encapsulated *P*-oxide **3**. This signal splits in 6 different singlets during irradiation. All new singlets appeared downfield shifted with respect to the original signal and their relative intensity changed with irradiation time. In the PSS (~10-15 minutes) the original singlet had disappeared completely and the relative intensity of the remaining 5 singlets was maintained constant. We assigned these 6 singlets to the methyl protons of **3** encapsulated in each one of the 6 possible **1•2** capsular isomers. The observation of 6 different signals for bound **3** indicated that all the capsular isomers, even the all-*cis*-**1•2**, were able of encapsulating this guest, but possibly to different extents (*vide infra*). We assigned the most downfield shifted singlet to the protons of **3** in the **3**⊂all-*cis*-**1•2** complex (Fig. S31). The downfield shift experienced by the protons of encapsulated **3** in *cis*-enriched containers suggested an increase in the cavity volume that was modulated by the *trans*-to-*cis* azo-isomerisation. Interestingly, the sum of the integral values of the singlets in the PSS decreased 32% in the case of *P*-oxide **3** and 11% in the case of *N*-oxide **4**, with respect to the area of the original singlet. However, the expected signals for the free guests were not detected. Taken in concert, these results evidenced that in the PSS both polar guests must also be involved in the formation of homo/hetero non-capsular aggregates to different extents.

N-oxide **4** forms stronger hydrogen bonds than *P*-oxide **3** with α,α,α,α-aryl-extended calix[4]pyrroles.[13] For this reason, it's sensible to speculate that all isomers of the encapsulation complex **4**⊂**1•2** were thermodynamically more stable than their **3**⊂**1•2** analogues. This might be especially relevant for the case of all-*cis* capsular isomers, which most likely equilibrate with ill-structured non-capsular aggregates that do bind the guests. This hypothesis was substantiated by the larger intensity observed in the PSS for the singlet corresponding to the methyl protons of the polar encapsulated guest in the **4**⊂all-*cis*-**1•2** compared to the **3**⊂**1•2** counterpart (Fig. 2D and S31). Assuming that only the all-*cis* capsular isomers are in equilibrium with non-capsular aggregates, and taking into account the relative amounts of the other 4 isomeric encapsulation complexes, we calculated that, in the PSS, the *cis*:*trans* ratio of the azobenzene groups in **1** was 80:20 (Fig. S31). We obtained the same value independently of the guest, **3** or **4**, and this value matched nicely with the one previously reported for a model system of a single azobenzene unit.[9b] These coinciding results reinforced the assumption of an exclusive or preferential equilibrium between the all-*cis* capsular isomers and the non-capsular aggregates, and that each azo group in tuC4A **1** experienced an independent light-driven isomerisation process. Thermal equilibration (60 °C) in the dark for 12 h restored both systems to the initial state: exclusive and quantitative observation of the all-*trans*-**1•2** encapsulation complexes of **3** or **4** (Fig. 2E). The isomerisation processes were repeated over 5 consecutive cycles of irradiation and thermal equilibration in the dark without noticeable photodegradation of any of the molecular components (Fig. S27). The all-*trans* encapsulation complexes were also stable at room temperature in the dark for at least 2 weeks. The use of *P*-oxide **3** as guest allowed monitoring the isomerisation process using $^{31}P\{^{1}H\}$ NMR spectroscopy (Fig. S28). The phosphorus signal for the encapsulated *P*-oxide appeared downfield shifted (~54 ppm) respect to free **3** (~40 ppm). In the initial state, the *P*-oxide **3** was completely encapsulated and upon irradiation the integral of the phosphorous signal resonating at ~54 ppm decreased and split into multiple overlapping singlets. Concomitantly, a new and broad phosphorus signal grew in intensity at a chemical shift value close to that of free **3**. After reaching the PSS the relative integral of the downfield:upfield signals was 68:32, respectively. These observations were in complete agreement with the results obtained in the ^{1}H NMR studies. The broadening and chemical shift difference (Δδ = 1.15 ppm) observed for the upfield phosphorus signal appearing after UV-light irradiation provided additional support to the existence of interactions between the guests released form the capsular aggregates with non-capsular homo/hetero aggregates that were also present in solution.

We became interested in performing similar light-irradiation experiments in even more complex system of dimeric capsules. We added 0.5 molar equivalents of the ditopic bis-*N*-oxide **5** to a CD_2Cl_2 solution containing the thermally equilibrated heterocapsule **3**⊂all-*trans*-**1•2** (final molar ratio of **1**:**2**:**3**:**5** was 1:1:1:0.5). The ^{1}H NMR analysis of the mixture revealed the presence of a new set of proton signals that was assigned to the homocapsule **5**⊂$\mathbf{2_2}$. The protons of encapsulated **5** resonated between 1 and 0 ppm (protons for free **5** appeared between 3.2 and 1.5 ppm, Fig. S11). The heterocapsule **3**⊂all-*trans*-**1•2** remained the main component of the mixture.[§] Integration of separate proton

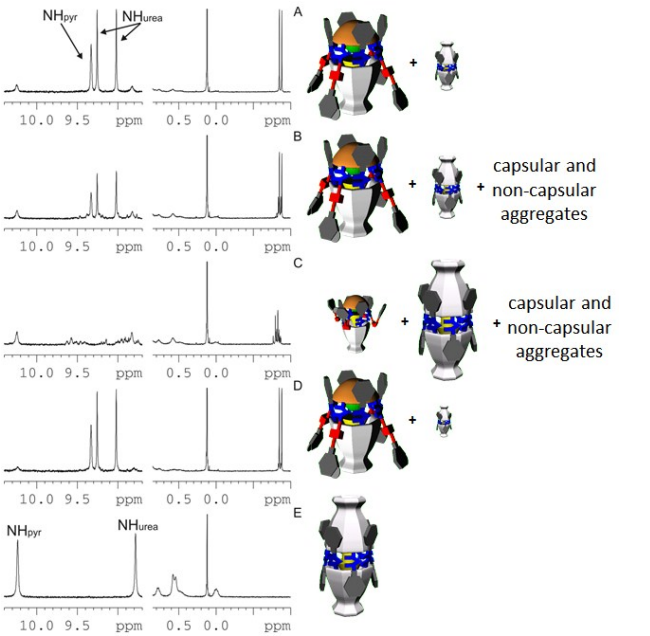

Fig. 3 (Left) Upfield and downfield regions of ^{1}H NMR spectra of A) 1:0.5 mixture of **3**⊂**1•2** and **5**; previous sample irradiated for B) 1 min or C) 7 min; D) previous sample warmed at 60 °C for 12 h; and E) **5**⊂$\mathbf{2_2}$. (Right) Schematic representation of the capsules present in each sample.

signals in the two assemblies allowed the calculation of the molar ratio [**3**⊂all-*trans*-**1●2**]/[**5**⊂$\mathbf{2_2}$] as 90:10 (Fig. 3A and S32). Using an internal standard (see ESI), we also realised that the addition of **5** had decreased the signal intensity of the **3**⊂all-*trans*-**1●2** assembly by half. This significant intensity diminution for the proton signals in **3**⊂all-*trans*-**1●2** was not accounted simply by the emergent proton signals related with the **5**⊂$\mathbf{2_2}$ assembly. This observation suggested the existence of non-capsular homo/hetero aggregates in equilibra with the capsular counterparts. Most likely, the protons of the non-capsular aggregates were not dectable in the NMR spectrum of the mixture because they produced broad and ill-defined signals.

The UV-light irradiation of the mixture induced the disappearance of the proton signals corresponding to the **3**⊂all-*trans*-**1●2** complex and the emergence of new broad and ill-defined signals. This result paralleled the observations made above for a solution exclusively containing the homocapsule. To our delight, after 7 minutes of irradiation, we observed that the proton signals in the **5**⊂$\mathbf{2_2}$ assembly doubled in intensity with respect to their initial values. We also detected that the amount of encapsulated **3** experienced a 25% decrease (Fig. 3C and S33). In this case, the proton signals corresponding to free **3** grew during irradiation (1.45 ppm in ^{1}H NMR). Collectively, these observations evidenced that the di-topic *N*-oxide **5** free in solution was able to recruit tuC4P **2** units involved in *cis*-enriched capsular and non-capsular aggregates to assemble larger amounts of **5**⊂$\mathbf{2_2}$. During this process, *N*-oxide **3** bound to **2** in capsular and non-capsular aggregates was released to the bulk solution. Unfortunately, the difference in thermodynamic stability that existed between the *cis*-enriched capsular, non-capsular aggregates and the homocapsule **5**⊂$\mathbf{2_2}$ was not enough to move the system towards the quantitative formation of the later by light irradiation. The system was only partially reversible by thermal equilibration in the dark. We discovered later that **5**⊂$\mathbf{2_2}$ was prone to photodecomposition (Fig. S35).

In summary, we presented here the exclusive assembly in solution of a dimeric heterocapsule all-*trans*-**1●2** from the social self-sorting process of a photoresponsive (azobenzene equipped) tetraurea calix[4]arene **1** and a tetraurea calix[4]pyrrole **2**. The capsule was assembled by encapsulating one polar guests **3** or **4** in its functionalised cavity. The *trans*-to-*cis* photoisomerisation of the azobenzene groups in all-*trans*-**1·2** induced the formation of *cis*-enriched capsular and non-capsular assemblies that were in equilibria. The process was fully reversible by thermal treatment of the samples. This approach also allowed us to switch the equilibrium state in a more complex system composed of two different capsules all-*trans*-**1●2** and **2●2** and two different guests *P*-oxide **3** and bis-*N*-oxide **5**. We demonstrated that the light-controlled selective encapsulation of the two different guests possible.

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Notes and references

‡ The absorption ratio between *trans* and *cis* isomers of azobenzene units is maximum in this region of UV-light, promoting the *trans* to *cis* isomerisation but minimising the reverse cis to trans conversion. REF: Photoisomerization of Azobenzenes, in *Photochemistry and Photophysics*, CRC Press, Boca Raton, **1990**.

§ If the initial heterocpasule contained the monodentate *N*-oxide **4** (**4**⊂**1●2**), the addition **5** did not affect the system and the homocpasule **5**⊂$\mathbf{2_2}$ was not formed even after UV irradiation. On the other hand, if *bis*-pyridine *bis*-*N*-oxide was added as bidentate competing guest, the corresponding homocapsule quickly became the main product in and the heterocapsule **3**⊂**1●2** could not be recovered.

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