Guest-induced capsule formation based on concerted interactions in water at neutral pH⁺

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The interaction of a tetracationic calixarene (TAC4) with dianionic guest (BS) in water was studied with NMR, nano-ITC and ESI-MS experiments; the convergent evidence provided by these different techniques shows that the addition of a suitable guest molecule triggers the formation of a homodimeric capsule in aqueous neutral solution.

One of the vital current tasks in supramolecular chemistry is to develop simple yet comprehensive and reliable methods to effectively hold together separate parts of complex architectures, exclusively through cooperative non-covalent interactions. Only in the past couple of decades have researchers learned how to program well-designed building blocks which self-assemble into defined aggregates.¹ In this context, assembling molecular structures that are able to encapsulate guest molecules in solution has turned out to be a very interesting yet challenging area that has attracted the interest of many research groups.² Gibb and Simin have elegantly shown that these structures can be used to perform reactions that are normally difficult to carry out in aqueous solution.²

Hence, water solubility is a key issue for these structures. However, working in water implies disadvantages not involved when dealing with organic solvents; these include solubility, the use of a buffer and the drop of the binding constant values. Water soluble non-covalent assemblies have been obtained mainly by metal-ligand interactions.³ For example, in order to explain the rapid capture and crystallisation of (di)cationic cyclic (aza)crown ethers in molecular capsules based on two p-sulfonatocalix[4]arenes, Raston et al. carried out a diffusion study in water indicating that more than one species seems to be present in solution, at least one of which displays diffusion behaviour that would be consistent with a 2:1 host-guest complex.³ Only few papers, though, deal with structures that are not based on metal-ligand interactions; however the systems reported are not entirely soluble in water and need mixtures of water/organic solvent.^{2,4} To the best of our knowledge there are only two examples of self-assembling capsules that are not based on metal-ligand interactions and are soluble in water;^{2,5} both these assemblies are not soluble in neutral region and reach full solubility in water at pH 9.

We have previously investigated the inclusion properties of the tetracationic calix[4]arene (TAC4) depicted in Scheme 1 and have shown its power to promote the inclusion of organic anions in water in neutral solution.⁶ If anionic guests can be included in the cavity of TAC4, can a gemini-like guest trigger the formation of a capsule? To answer this question we designed and synthesized a dianionic guest (BS) (Scheme 1)⁷ and studied its interaction with TAC4 by ¹H NMR titrations, DOSY, ROESY, nano-ITC and ESI-MS measurements, to show that they may form a capsule in neutral aqueous solution, that would rely on concerted hydrophobic and electrostatic interactions.

In ¹H NMR titrations⁸ the signals for the guest shift upfield from where they occur in free solution as the host concentration increases: the complexation induced shifts (CIS) follow the order Me > H_2 > H_1 > H_3 > H_{α} > H_{β} > H_{γ} (Fig. S3, ESI[†]). This shows that the guest is included via its aromatic moiety, most likely to maximize the burial of lipophilic surfaces from water, with CH₃ groups pointing deeply into the cavity. The large CIS (up to 2 ppm for the methyl group) reveals that the aromatic moieties of BS are included and experience the magnetic shielding provided by the four aromatic rings that shape the cavity. Guest signals are detected as single resonance due to the fast exchange between the free and the complexed species on the NMR timescale. Complexation of **BS** could not be described using a 1 : 1 or a 2 : 1 host-guest binding model; a good fit of the data was instead obtained with the 1:1 and 2:1 association model (Fig. 1 and Fig. S4, ESI[†]), and this yields log K values of 4.34 \pm 0.01 and 3.06 ± 0.01 , respectively (Table 1). The overall binding constant for the formation of the capsule (log $\beta_{21} = 7.4$)



Scheme 1 Host and guest investigated.

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Fig. 1 Schematic representation of the assemblies formed.

Table 1 Log K values and thermodynamic parameters for the host-guest comp
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Equilibrium	NMR $\log K^a/\mathrm{dm^3} \mathrm{mol^{-1}}$	ITC			
		$\log K^a/\mathrm{dm^3} \mathrm{mol^{-1}}$	$\Delta G^a/{ m kJ}~{ m mol}^{-1}$	$\Delta H^a/\mathrm{kJ}~\mathrm{mol}^{-1}$	ΔS^a /J K ⁻¹ mol ⁻¹
$H + G \leftrightarrows HG \\ HG + H \leftrightarrows H_2G$	4.34 (1) 3.06 (1)	3.6 (2) 2.9 (3)	-20.5(1) -16.5(2)	-11.9(1) -9.1(2)	30 (4) 25 (5)
^{<i>a</i>} σ in parentheses.					

nicely compares with the value reported for a deep cavitand-based capsule.²

The ESI-MS analysis of an aqueous solution of TAC4/BS (4/1) showed signals at 1861.8 and 1111.2 corresponding to $[2(TAC4) \cdot BS \cdot H_2PO_4 \cdot 2H]^+$ and $[TAC4 \cdot BS \cdot H]^+$, respectively, confirming the co-existence of a 2 : 1 (capsule) and a 1 : 1 host–guest complex (Fig. S5, ESI†).

This model was checked by nano-ITC. A satisfactory fit of the nano-ITC data to the 1 : 1 and a 2 : 1 host–guest association model was again obtained, yielding log *K* values equal to 3.6 ± 0.3 and 2.9 ± 0.3 for the two species, respectively, which is consistent with the ¹H NMR experiments.^{6,9}

The enthalpic and entropic values show that both steps are favored by enthalpy and entropy. This indicates that the entropic penalties associated with organizing the guest and the host for complex formation have been paid for by the desolvation of the guest and the host. The burying of the aromatic moiety from water, which is also facilitated by the ionic interactions upon self-assembly of the complexes, contributes to the free energy of formation; the enthalpically favorable contribution overrides the cost in energy needed for the desolvation of all the interacting particles prior to the self-assembly process.

To prove that the ionic interaction between the host(s) and the guest is crucial for the formation of the supramolecular assemblies we run the same experiments in 0.1 mol dm⁻³ NaNO₃. The introduction of high salt concentrations determined a dramatic drop in the heat developed and the binding constant (Fig. 2); in any case the model obtained in the absence of sodium nitrate both *via* NMR and ITC could no longer be reproduced. This clearly shows that the driving forces for the complex formation include both electrostatic as well as CH– π and π – π interactions.¹⁰

The formation of a "capsule" in solution was further confirmed using diffusion NMR spectroscopy (DOSY). This method has proved to be useful for the detection of aggregates which were difficult to characterize due to their high symmetry and non-covalent character.¹¹

The increase of TAC4 concentration markedly affects the motion of **BS**, while it has a smaller effect on the TAC4 diffusion coefficients (Fig. 3); the guest diffuses more slowly



Fig. 2 Thermograms obtained in the absence (red) and in the presence (blue) of NaNO₃.



Fig. 3 BS self-diffusion coefficients. Red full circles, experimental data points; broken line, HG model; solid line, HG + H_2G model.

than in its free form, which is consistent with an increase of its effective size due to complex formation.¹²

Two models have been used to fit the experimental data points, *i.e.* a model that takes into account the existence of HG only and a second one that considers the formation of HG + H₂G (see ESI[†], DOSY experiments). Noteworthy, the mole fraction values needed to generate the theoretical curves were computed by using the log *K* values obtained *via* ITC (Fig. S7, ESI[†]).¹³ The use of data from NMR and ITC, that probe different physical observables, provides a way to cross-check the results. Fig. 3 indisputably shows that whilst the first model (HG only) fails to reproduce the experimental



Fig. 4 Top: 2D ROESY (phosphate buffer, D_2O , pD 6.8, TAC4/BS = 6); off-diagonal peaks of interest are encircled. Bottom: optimized model (see ESI[†]); arrows indicate the spatially correlated groups.

1.00

H

M

E

680

E

0

H

-4.0

5.0

7.0

2.0

3.0

4.0

5.0

6.0

-7.0

620

CH

C

ō

data, the second model (HG + H₂G) nicely fits the experimental self-diffusion coefficients, thus pointing out that the two species coexist in neutral solution. The self-diffusion coefficient of **BS**, "extrapolated" to larger H/G ratio $(2.4 \times 10^{-10} \text{ m}^2 \text{ s}^{-1})$, is significantly smaller than that of free **TAC4** $(3.1 \times 10^{-10} \text{ m}^2 \text{ s}^{-1})$ and free **BS** $(3.8 \times 10^{-10} \text{ m}^2 \text{ s}^{-1})$, and confirms the "weighed average" larger size of the species in solution; the "extrapolated" value nicely matches the theoretically predicted value $(2.4 \times 10^{-10} \text{ m}^2 \text{ s}^{-1})$ for the guest-containing homodimeric capsule.¹²

2D ROESY experiments were performed to map out the capsule geometry in details. The cross peaks corresponding to intermolecular NOE contacts confirmed the spatial proximity of the different components of the complex. The results are consistent with the observed changes in the chemical shift and in the diffusion coefficients, confirming that the **TAC4** units are wrapped around the guest.

The H₁ and H₂ protons correlate with all the calix[4]arene signals, while the H₃ proton interacts with the upper rim groups (Fig. 4). The Me protons show a NOE effect both with the calix[4]arene cavity (Hc) and the ammonium methyl groups (Ha). The off-diagonal peaks between the β and γ methylene protons and the ammonium methyl groups confirm

their spatial proximity; the signals of the Hb groups overlap with those of the α methylene groups and cannot be used as reporters for the interaction. Noteworthy, all these spatial correlations are no longer observed when HG is the main species (H/G < 4) (Fig. S10, ESI†). Taken together, these observations prove unambiguously the existence of a self-assembled homodimeric capsule.

This work shows that a suitable choice of the anchoring groups of the upper rim of the hydrophobic cavity can be a powerful approach to high definition self-assembly in water, provided that the upper rim substituents match the electrostatic and steric requirements of the guest. NMR, MS and ITC, that probe different physical observables, cross-validate one another. We have demonstrated that (i) a simple host like **TAC4** may form a homodimeric capsule; (ii) the capsule forms in neutral aqueous solution; (iii) significant amounts of the capsule (50%) form even with relatively small (H/G) ratios.

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