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Unveiling the Formation 1:2 Supramolecular Complexes Between Cucurbit[7]uril and a Cationic Calix[4]arene Derivative

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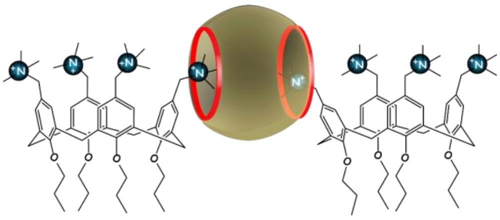
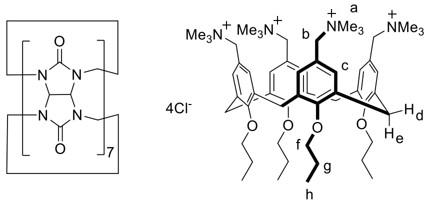
The formation of host-guest complexes between cucurbit[7]uril (CB7) and a tetracationic calix[4]arene derivative in the so-called cone conformation was investigated by ^{1}H NMR, DOSY NMR, isothermal titration calorimetry and ESI-MS. The results point out to the formation of 1:1, 1:2 and 2:1 CB7: calixarene complexes with binding constants of 3×10^6 M^{-1}, $\approx2\times10^2$ M^{-1},and 9×10^4 M^{-1} respectively. The study demonstrates, on one hand, that the despite of having four potential recognition sites, the calixarene only binds two CB7 molecules and, on the other, that for sterically crowded binding motifs that prevent CB7 inclusion with optimized hydrophobic and ion-dipole interactions, the formation of 1:2 complexes can be observed most likely due to formation of external binding of the cationic moieties to the CB7 carbonyl portals.



Macrocyclic receptors are ubiquitous components of host-guest systems owing to their ability to selectively recognize guest molecules of appropriate size, shape and complementary noncovalent interactions.[1-3] In addition to their fundamental relevance, macrocyclic host compounds are found in numerous applications such as catalysis, sensing, molecular machines, transport across membranes, etc.[4-11] Beyond of discrete complexes, the high affinity and selectivity demonstrated by selected host-guest binding pairs have been widely explored to design larger and more organized assemblies such supramolecular polymers, micelles, vesicles, 2D and 3D supramolecular frameworks, to give some examples.[12-16] These organized self-assembled materials require careful and subtle design of the host and guest building blocks which often need additional recognition points with high degree of orthogonality with respect to that of the host-guest binding motifs.



CB7

1

Scheme 1. Structures of cucurbit[7]uril (CB7) and tetracationic calix[4]arene **1**.

Cucurbit[n]urils (CBn) are pumpkin-shaped macrocyclic containers characterized by a rigid and symmetric hydrophobic cavity decorated by electronegative carbonyl groups at the portals.[17-18] This structural and electronic properties make them particularly good receptors for positively charged amphiphilic molecules, displaying ultrahigh affinity and selectivity for complementary guest molecules in aqueous solution.[19-22] While larger homologues such as CB8 and CB10 are widely known to include more than one guest in their cavities, for CB7 and smaller homologues the formation of higher order complexes is not well stablished.[17] In order to

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Electronic Supplementary Information (ESI) available: Experimental details, calixarene critical micelle concentration, NMR titrations and ESI-MS fragmentation spectrum. See DOI: 10.1039/x0xx00000x explore the potential of CB7 to simultaneously include two guest molecules an amphiphilic tetracationic calix[4]arene (**1**) in the cone conformation was used as model guest.[23]

Amphiphilic calixarenes in the cone conformation are known to self-assemble into globular micelles in aqueous solution.[24,25] In the case of ionic amphiphiles conductivity measurements allows straightforward determination of the critical micelle concentrations (CMC, i.e., defined as the onset of micelle formation). For compound **1** a CMC of 10 mM was found (see Supporting Information) and therefore all studies on interaction of monomeric **1** with CB7 were carried out at concentrations well below the CMC to avoid phenomena arising from the self-association of **1**.

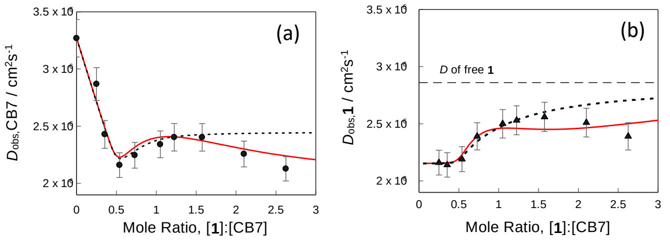


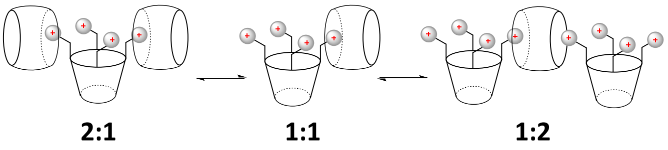
Figure 1. Observed diffusion coefficients for (a) CB7 and (b) **1** plotted against the concentration of **1** at fixed concentration of CB7 (2mM). All experiments were carried out in D_2O at 25ºC. Error bars correspond to 5% of the absolute D_{obs} values. The red line represents the fitting to a binding model that accounts for formation of 1:1, 1:2 and 2:1 complexes while the dashed line only considers the formation of 1:1 and 2:1 complexes.

Diffusion coefficients, obtained from DOSY NMR experiments, provide extremely useful information on the stoichiometric elucidation of supramolecular systems[26]. Figure 1 shows the observed diffusion coefficients (D_{obs}) obtained for CB7 and **1** as a function of the [**1**]:[CB7] mole ratio. As can be observed the D_{obs} of CB7 (Fig.1a) linearly decreases to reach a minimum for [**1**]:[CB7]=0.5. This behavior strongly suggests the efficient formation of 2:1 complexes (CB7:**1**). For [**1**]:[CB7]>0.5, the D_{obs} increases due to the consumption of 2:1 species to give 1:1 complexes (that due to lower hydrodynamic radius are expected to diffuse faster) reaching a maximum at [**1**]:[CB7]≈1.5, followed by a second decrease that can be assigned to the (unexpected) formation of 1:2 complexes (CB7:**1**) (see Scheme 2). The behavior of D_{obs} observed for **1** as function of [**1**]:[CB7] mole ratio (Fig. 1b) is also in agreement with the formation of complexes shown in Scheme 2. At [**1**]:[CB7]<0.5, the D_{obs} is low because 2:1 (CB7:**1**) complexes predominate, while above this value an increase can be observed due to the gradual formation of the 1:1 complexes. In the case of **1**, for molar ratio [**1**]:[CB7] > ≈1.5, the decrease in the D_{obs} owing to the proposed formation of the 1:2 (CB7:**1**) complex is not so evident because these effect is counterbalanced by the increase in the mole fraction of free **1**.

Quantitatively, D_{obs} is the average of the diffusion coefficients of free and complexed species, weighed by the correspondent mole fractions (equations 1 and 2).[26] Using the $K_{1:1}$ and $K_{2:1}$ values determined from ITC experiments (see below, Table 2), the data of Figure 1a and 1b can be fitted to equations 1 and 2, respectively, to obtain the diffusion coefficient of all species (Table 1) and a relatively small value for the $K_{1:2}$≈2x10^2 M^{-1}, as optimized parameters. D_{obs} show the expected trend i.e. slower diffusion for higher order complexes < 1:1 < free species. As can be observed, the experimental data are better described when 1:1, 2:1 and 1:2 complexes (CB7:**1**) are postulated (continuous red line) than when only 1:1 and 2:1 complexes are accounted (dotted black line) thus supporting the first model. Although relatively rare, the inclusion of two guests in the cavity or at the carbonyl portals of CB7 and CB6 has been previously reported, demonstrating that these small receptors can accommodate two molecules in their cavities in particular conditions[27-29].

$$D_{obs,CB7} = D_{CB7}\chi_{CB7,f} + D_{1:1}\chi_{1:1} + 2D_{2:1}\chi_{2:1} + D_{1:2}\chi_{1:2} \quad (1)$$

$$D_{obs,\mathbf{1}} = D_{\mathbf{1}}\chi_{\mathbf{1},f} + D_{1:1}\chi_{1:1} + D_{2:1}\chi_{2:1} + 2D_{1:2}\chi_{1:2} \quad (2)$$



Scheme 2. Cartoon representation of the different complexes formed between cucurbit[7]uril (CB7) and calix[4]arene **1**.

Table 1. Diffusion coefficients (cm^{2}s^{-1}) determined for **1**, CB7 and their respective complexes in D_2O at 25°C.

D_1	D_{CB7}	$D_{2:1}$	$D_{1:1}$	$D_{1:2}$
2.88x10^{-6}	3.26x10^{-6}	2.15x10^{-6}	2.56x10^{-6}	1.70x10^{-6}

Further evidence on the formation of complexes outlined in Scheme 2 were obtained by ESI-MS. This technique is recognized to be a powerful tool for the elucidation of cucurbituril-based host-guest systems.[30-33] Complexes 2:1 and 1:1 are readily seen in the gas phase after spraying an aqueous solution 100 μM of **1** and CB7 (1:1 stoichiometry). Complexes 1:1 can be seen at m/z 693.2 ([CB7+**1** +Cl]$^{3+}$) and 1057.6 ([CB7+**1** +2·Cl]$^{2+}$) while 2:1 were observed at m/z 801.6 ([2·CB7+**1**]$^{4+}$) and 1080.6 ([2·CB7+**1**+Cl]$^{3+}$) (Figure 2a). The observation of 1:2 complexes was achieved using very soft ionization conditions and a solution containing CB7 and **1** in 1:5 molar ratio (50 μM:250 μM). The signal of 1:2 complexes was observed at m/z 1034.6 ([CB7+2·**1**+5·Cl]$^{3+}$) (Figure 2b) with an isotope distribution consistent with the assignment which was further supported by fragmentation experiments (Figure S3, SI).

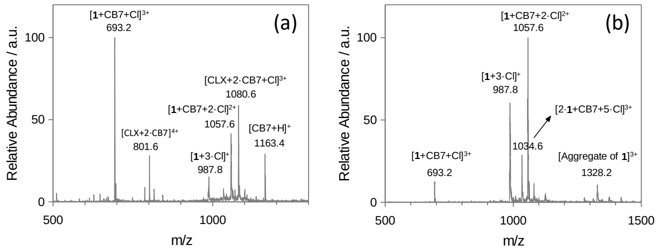


Figure 2. Electrospray ionization mass spectrum of **(a)** a mixture of CB7 (100 μM) and **1** (100 μM) and **(b)** a mixture of CB7 (50 μM) and **1** (250 μM) in neutral water. The [Aggregate of **1**]$^{3+}$ was also observed in 250 μM solutions containing only **1**.

Isothermal titration calorimetry (ITC) was used to investigate the stability of the complexes formed betweenCB7 and **1**. As can be observed from Figure 3a, when **1** is titrated with a CB7 solution, endothermal calorimetric peaks are observed thorough the titration process. Noteworthy, an inflexion point can be observed at CB7:**1** molar ratio near 2, confirming that two CB7 molecules bind to calixarene **1**. The same conclusion can be reached when **1** is added to the CB7 solution (Fig. 3b), now showing an inflexion point near 0.5 **1**:CB7 molar ratio. Interestingly, after a significant heat release at the beginning of titration, small endothermic peaks can observe. These peaks are due to dissociation of 2:1 complexes to give 1:1 (and potentially 1:2) complexes and account for the fact that formation of this last species is less endothermic than the former.

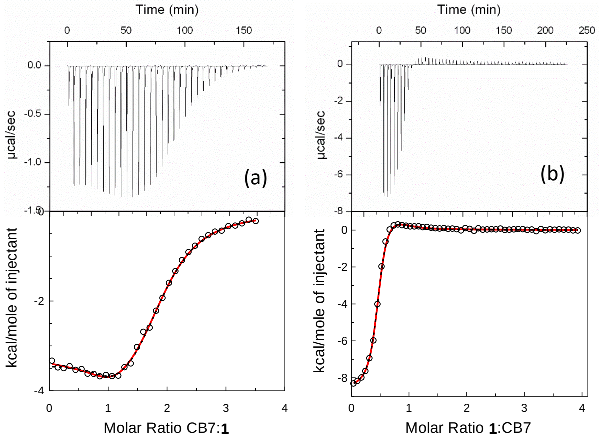


Figure 3. Isothermal titration calorimetry (ITC) data obtained upon (a) titration of 0.08 mM of **1** with 1.45 mM of CB7 and (b) titration of 0.25 mM of CB7 with 5 mM of **1**. All experiments were carried out in H_2O at 25°C. The data was fitted to a 2:1/1:1/1:2 binding model (continuous red line).

The ITC data was fitted to a model that accounts for the 2:1/1:1/1:2 complexes. As can be observed, the experimental data can be satisfactorily fitted with the proposed model. The obtained thermodynamic parameters are shown in table 2. The thermodynamic data for the 1:2 (CB7:**1**) complexes could not be accurately obtained owing to their low binding constant $K_{1:2} \approx 2 \times 10^2$ M^{-1} and therefore will not be discussed. The obtained binding constants for 2:1 and 1:1 complexes show values in the micromolar range with modest negative cooperativity ($K_{2:1} < K_{1:1}/4$). It is worth noting that the value obtained for $K_{1:1}$ compares with the one recently obtained in buffer free solution for tetramethylammonium (1.5×10^6 M^{-1})[34] suggesting a similar complexation driving force in both cases.The thermodynamic data demonstrates that the formation of both 2:1 and 1:1 complexes is enthalpically and entropically favorable. A closer inspection at these data shows that for the binary complex the entropy and enthalpy changes are relatively unexpected if compared with the data reported for more conventional trimethylalkyl ammonium cations (TMAC).[32,35] For the binary CB7:**1** complex the entropy has a higher contribution for the free energy while conventional CB-based host-guest systems are enthalpy driven with small favorable or unfavorable entropy changes. When the absolute values are compared, it can be verified that the enthalpic variation is ca. 3 times smaller for the CB7:**1** species while the entropic change is significantly more favorable. This suggests the existence of an important enthalpic penalty upon binding calixarene **1** to the first CB7 molecule that, on the other hand, is entropically overcome, resulting in an overall Gibbs free energy comparable with conventional systems. Noteworthy, the thermodynamic data for 2:1 complex is in line with reports for other TMAC ditopic guests, i. e; enthalpy changes of the order of 20 kJ.mol^{-1} associated with positive entropy changes.[36] Together, these observations suggest that the desolvation/disruption of highly stable and organized hydrogen bond network around the calixarene ammonium headgroups may have a major contribution for 1:1 complexation energetics. For 2:1 complex, the thermodynamics parameters are compatible with the major driving force being the release of high energy water from the CB7 cavity as generally accepted for these systems.[20]

Table 2. Thermodynamic parameters obtained from ITC experiments for the formation of host guest complexes between CB7 and calixarene **1** in H_2O at 25°C.

	1:1	**2:1**	**1:2**[a]
K (M^{-1})	$(3.0 \pm 0.2) \times 10^6$	$(8.80 \pm 0.7) \times 10^4$	$(2 \pm 1) \times 10^2$
ΔH (kJ/mo)	-14.6±0.2	-19.4±0.1	---
$T\Delta S$ (kJ/mol)	22.3±0.5	8.9±0.2	---

[a] Reliable thermodynamic parameters could not be obtained for this species owing to their comparatively low binding constants.

^1H NMR experiments provide direct evidences on the structure of inclusion complexes involving cucurbituril host. On one hand, protons deeply included in the cavity of the cucurbituril are magnetically shielded leading to upfield shifts on their ^1H NMR signals, while on the other hand, protons located lying outside the cavity and near the carbonyls portals are deshielded resulting in downfield complexation induced chemical shifts. As can be observed from figure 4, protons a and b of the calixarene are displaced to higher filed upon complexation while protons c and d undergo downfield displacements suggesting that the trimethylammonium group is included inside the cavity of the CB7 receptor. The NMR data can be simulated with the model that accounts for 2:1/1:1/1:2 species, using the binding constants of table 2 as fixed parameters, and the following limiting complexation induced chemical shifts: $\Delta\delta_{1:1}$=0.14 ppm, $\Delta\delta_{2:1}$=0.28 ppm and $\Delta\delta_{1:2}$=0.03 ppm for protons c and $\Delta\delta_{1:1}$=-0.235 ppm, $\Delta\delta_{2:1}$=-0.47 ppm and $\Delta\delta_{1:2}$=-0.1 ppm. These values are in agreement with the proposed binding model showing higher absolute values for the 2:1 complexes due to the inclusion of two recognition sites per calixarene molecule. On the other hand, the 1:2 complex shows lower absolute values (although with high uncertainty) which are in agreement with a structure where the calixarene binding sites are located more externally interacting predominantly with the carbonyl portals. Some structural features of these complexes deserve further discussion: (i) as for other calixarenes blocked in the cone conformation, **1** may adopt a C_{2v} "boat" conformation with CB7 binding to the opposite aromatic units showing larger distance. This conformational arrangement minimizes unfavorable repulsion and steric interaction. (ii) Despite of presenting four possible recognition points, only 1:1 and 2:1 complexes are observed. The access to the two remaining trimethylammonium headgroups is blocked by steric effects imposed by the two CB7 molecules.

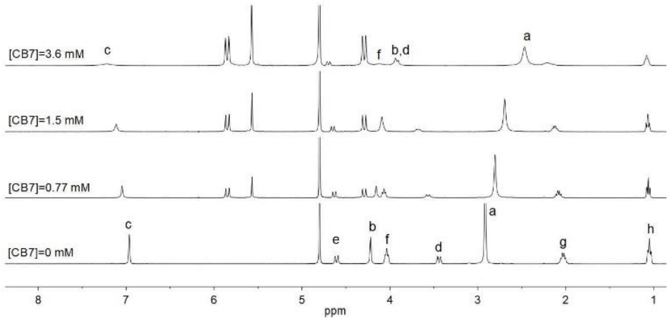


Figure 4. ^{1}H NMR spectra of calixarene **1** (1.5mM) in the presence of different concentrations of CB7. All experiments were carried out in D_2O at 25°C. Please see Scheme 1 for H labelling.

In conclusion, we showed that CB7 can bind a tetracationic calixarene derivative in the cone conformation to form 2:1, 1:1 and 1:2 CB7:calixarene complexes with high binding constants for the first two stoichiometries and much lower affinity for the last. The thermodynamic results show that formation of 1:1 and 2:1 complexes is both enthalpic and entropically favorable as result of a combination of factors such as the release of high-energy water from the cavity of CB7 and dehydration of the calixarene cationic groups and as well ion-dipole interactions. The proposed structure for the 2:1 complex in which the calixarene adopts a C_{2v} "boat", conformation seems to be adequate for complexation of the other smaller guests. On the other hand, the rather unusual 1:2 recognition motif may of interest to be explored in a context of self-assembled soft-materials such as supramolecular polymer and supra-amphiphiles.

Acknowledgements

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