Supramolecular Chemistry

Deutsche Ausgabe: DOI: 10.1002/ange.201606038
Internationale Ausgabe: DOI: 10.1002/anie.201606038

Cooperative Self-Assembly of a Quaternary Complex Formed by Two Cucurbit[7]uril Hosts, Cyclobis(paraquat-*p*-phenylene), and a "Designer" Guest

*Mohammad Hossein Tootoonchi, Gaurav Sharma, Jonathan Calles, Rajeev Prabhakar, and Angel E. Kaifer**

Abstract: *The self-assembly in aqueous solution of the well-known cyclophane, cyclobis(paraquat-p-phenylene) (BB^{4+}), and two cucurbit[7]uril (CB7) hosts around a simple hydroquinol-based, diamine guest (GH_2^{2+}) was investigated by ^1H NMR and electronic absorption spectroscopies, electrospray mass spectrometry and DFT computations. The formation of a quaternary supramolecular assembly [GH_2^{2+}·BB^{4+}·$(CB7)_2$] was shown to be a very efficient process, which takes place not only because of the attractive forces between each of the hosts and the guest, but also because of the lateral interactions between the hosts in the final assembly. This complementary set of attractive interactions results in clear cooperative binding effects that help overcome the entropic barriers for multiple component assembly.*

The formation of noncovalent complexes usually takes place in spite of the entropic penalty associated with the assembly of several molecular components into a larger supramolecular entity. Although entropic penalties can be diminished by solvophobic interactions or by the judicious choice of rigid structural components for assembly, careful design of the components favors synergistic interactions in the final assembly that may overcome the entropic effects.[1] In this regard, the interactions between multiple hosts in large supramolecular assemblies have been relatively overlooked. Harada and co-workers prepared cyclodextrin-based pseudo-polyrotaxanes that resembled supramolecular necklaces,[2] in which hydrogen bonding interactions between neighboring cyclodextrins (the beads in the necklaces) seem to play an important role in the overall stabilization of the assemblies. Liu and co-workers have explored the formation of mixed supramolecular polymers of cucurbit[*n*]uril/bis(*p*-sulfonatocalix[4]arenes) and cucurbit[*n*]uril/cyclodextrins.[3] A more recent report by Stoddart and co-workers shows that hydrogen bonding interactions between the portals of cucurbit[6]uril and β-cyclodextrin favor the formation of complexes in which these two hosts occupy alternating binding stations along a molecular thread.[4] A similar host combination has been used by Francis and co-workers.[5] Here, we report the preparation of a structurally simple diamine guest, GH_2^{2+} (Figure 1), which can simultaneously interact with one molecule of the well-known tetracationic cyclophane cyclobis(paraquat-*p*-phenylene), BB^{4+}, and two molecules of cucurbit[7]uril, CB7, to form a quaternary supramolecular complex in which all components exhibit attractive interactions with their molecular neighbors that facilitate the formation of the assembly.

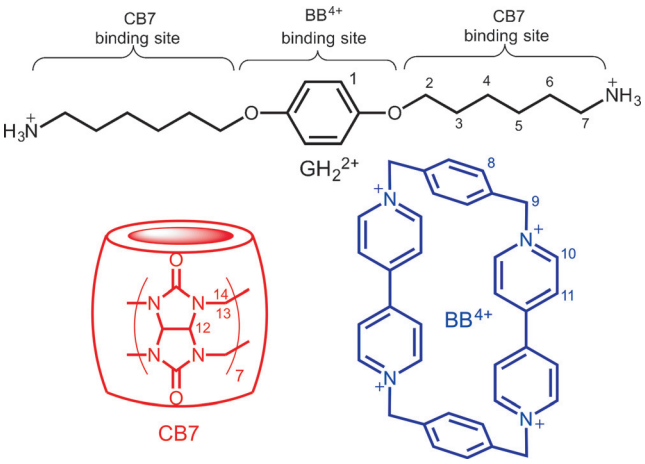


Figure 1. Structures of the diamine guest (GH_2^{2+}) and the hosts CB7 and BB^{4+} used in this work. The numbers around the structures are proton labels.

Binding interactions between the GH_2^{2+} guest and the BB^{4+} host. Titration of a 0.5 mM solution of GH_2^{2+} with the tetracationic cyclophane host BB^{4+} leads to the appearance of a new set of proton resonances for the complex, which coexist with the resonances of the free guest (Figure 2, G/BB and Figure S2 in the Supporting Information). The fact that both GH_2^{2+} and BB^{4+} proton signals preserved their symmetry within their complex is consistent with the host engulfing the central dioxybenzene residue of the guest. At any point in the titration with BB^{4+}, the relative integrals of the signals corresponding to the same set of protons in the free guest and the complex allow the straightforward calculation of the equilibrium association constant, which was found to be $6.4(\pm 1.0) \times 10^2\ M^{-1}$.

Binding interactions between the GH_2^{2+} guest and the CB7 host. When a 0.27 mM solution of GH_2^{2+} was titrated with CB7

[*] Dr. M. H. Tootoonchi, G. Sharma, J. Calles, Prof. Dr. R. Prabhakar, Prof. Dr. A. E. Kaifer
Department of Chemistry and Center for Supramolecular Science, University of Miami
Coral Gables, FL 33124-0431 (USA)
E-mail: akaifer@miami.edu
Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/anie.201606038.

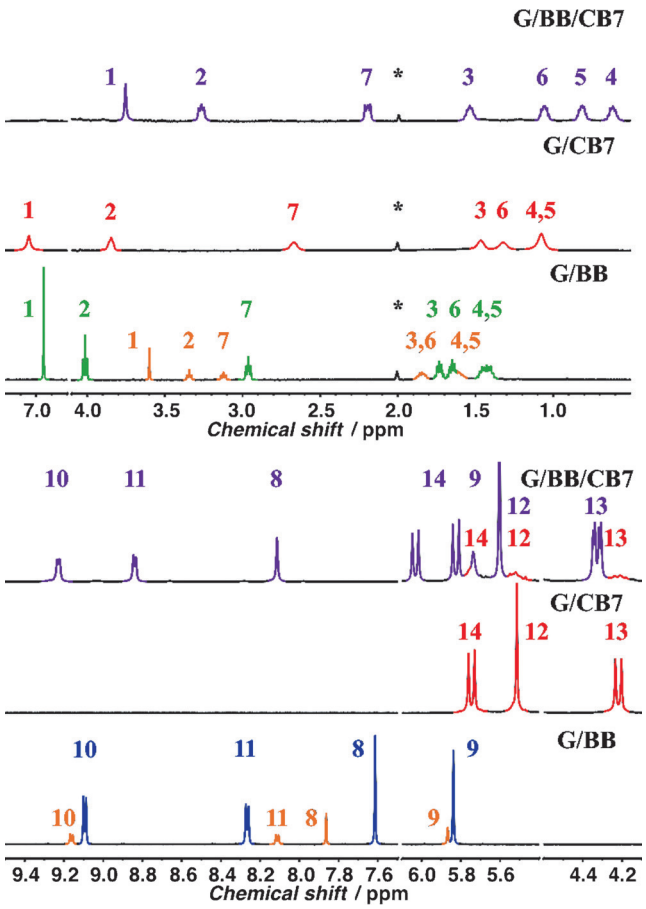


Figure 2. Partial ^{1}H NMR spectra: G/BB corresponds to $[GH_2^{2+}]=0.50$ mM, $[BB^{4+}]=0.41$ mM; G/CB7 is $[GH_2^{2+}]=0.27$ mM, [CB7] = 0.5 M; and G/BB/CB7 is $[GH_2^{2+}]=0.42$ mM, $[BB^{4+}]=0.42$ mM, [CB7] = 1.0 mM. All solutions contain 50 mM NaAc (pD = 4). ^{1}H NMR signals are labeled based on Figure 1 and color coded as follows: free GH_2^{2+} (green), free BB^{4+} (blue), $GH_2^{2+}\cdot BB^{4+}$ complex (orange), $GH_2^{2+}\cdot$CB7 complex (red), $GH_2^{2+}\cdot BB^{4+}\cdot(CB7)_2$ complex (purple). The asterisk marks the residual peak of NaAc.

(Figures 2, G/CB7 and S1) two distinct effects were observed in the ^{1}H NMR spectra: 1) Broadening of all proton resonances and 2) upfield shifts for the aliphatic protons on the guest and downfield shifts for its aromatic protons resonances (proton 1). It is now well established that CB7 inclusion[6] leads to substantial upfield shifts for the protons in the included unit and downfield shifts for those protons located outside the CB7 cavity, in the proximity of the carbonyl oxygens lining the cavity entrances. Therefore, the CB7-induced chemical shift displacements observed on the guest protons are consistent with the inclusion of the hexyl chain methylenes in the cavity, while the dioxybenzene aromatic ring remains close to one of the CB7 portals. The observed signal broadening can be attributed to fast exchange in the NMR time scale between the complex and the free guest. In the presence of less than 1.0 equiv of CB7 (Figure S1), the exchange between free and CB7-bound guest is fast in the NMR time scale, indicating that fast association/dissociation of CB7 to and from GH_2^{2+} happens in solution and $GH_2^{2+}\cdot$CB7 is the dominant complex species, for which we determined a binding constant of $3.2(\pm 0.7)\times 10^3\,M^{-1}$. We did not observe any evidence for the $GH_2^{2+}\cdot(CB7)_2$ ternary complex.

Binding interactions between the hosts BB^{4+} and CB7. A white precipitate was formed when solutions of these macrocycles at mM concentration levels were mixed in different proportions. The ^{1}H NMR spectra of these samples showed broadening and upfield shifts of the BB^{4+} resonances (Figure S7). The precipitate, after isolation and ^{1}H NMR spectroscopy analysis, was shown to be an equimolar mixture of both hosts. No change in the NMR spectra was detected when the overall concentration was kept under 0.3 mM to avoid precipitation. These observations suggest the existence of weak interactions between BB^{4+} and CB7 in aqueous solution.

In summary, the results of the binary interaction experiments, in which the guest is exposed to a single host, clearly indicate that the BB^{4+} host selectively binds to the central dioxybenzene ring, while CB7 prefers to bind to one of the terminal aliphatic chains of the guest. This is in excellent agreement with the known binding preferences for each host. In addition, there is evidence for interactions between the two hosts. The question is whether these complementary binding modes are maintained when the guest is simultaneously exposed to both hosts.

Binding interactions between the GH_2^{2+} guest and the two hosts. The ^{1}H NMR spectrum of a solution containing 0.42 mM GH_2^{2+}, 0.42 mM BB^{4+} and 1.0 mM CB7 provides information on this issue. Compared to their binary complexes, chemical shift changes are apparent for all protons, which suggests that the three-component solution cannot be treated as a simple superposition of the corresponding binary mixtures. In the presence of CB7, all the hexyl chain proton signals of the guest shift upfield, while the guest's aromatic peak protons shift downfield (Figure 2, top), when compared to those of the $GH_2^{2+}\cdot BB^{4+}$ complex. As mentioned before, this is in agreement with the CB7 hosts engulfing the hexyl chains and the CB7 portals affecting the adjacent phenyl ring. The methylene proton resonances of CB7 are split into two peaks of the same intensity, while the equatorial methyne peak stays as a singlet (Figure 2, bottom). This suggests that the symmetry of CB7 is broken along its longitudinal axis, which is in agreement with one CB7 portal being close to BB^{4+} and the other facing the terminal ammonium group. Finally, there is only one chemical shift for each proton on the guest and on BB^{4+}, with no apparent peak broadening (Figure 2). Therefore, both the guest and BB^{4+} preserve their symmetry within the complex, which means that the complex contains two CB7 hosts, one on each hexyl chain. Overall, the observations mentioned above, all consistently point to the formation of a quaternary complex in which BB^{4+} engulfs the central dioxybenzene moiety of the guest and two CB7 hosts are symmetrically placed on the guest's terminal hexyl arms (Figure 1).

We also carried out one-dimensional selective NOE experiments to investigate the spatial relations among the protons in the quaternary complex. For instance, irradiation of the β protons (labeled 11 in Figure 1) on the BB^{4+} host results in the detection of positive NOEs for the signals of

methylene protons on CB7 and the aromatic protons on the guest, as expected from the proposed structure of the quaternary complex.

Diffusion coefficient measurements. The diffusion coefficients of the various species where measured using the bipolar longitudinal eddy-current delay (BPLED) pulse sequence (Table S1). As expected, the diffusion coefficients of GH_2^{2+}, BB^{4+} and CB7, when present in the quaternary complex, are all identical (ca. $2\times10^{-10}\,m^2s^{-1}$) within experimental error. Also, the diffusion coefficient of the larger four-component complex is lower than that of either of the binary complexes ($2.8\times10^{-10}\,m^2s^{-1}$ for $GH_2^{2+}\cdot BB^{4+}$ and $2.6\times10^{-10}\,m^2s^{-1}$ for $GH_2^{2+}\cdot$CB7). These diffusion data are perfectly consistent with our previous observations.

The intermediate ternary complex. The first step toward formation of the quaternary complex is an intermediate three-component complex, in which BB^{4+} is positioned on the dioxyphenyl moiety of GH_2^{2+} while only one CB7 molecule is placed on one of the hexyl chains. To create conditions favorable to the formation of this intermediate, we titrated a solution of GH_2^{2+} and BB^{4+} with CB7, deliberately keeping the concentration of CB7 similar to that of the guest and the tetracationic host. The resulting ^{1}H NMR spectrum had new signals (Figure S5), suggesting the presence of a new species which can be assigned as the ternary $GH_2^{2+}\cdot BB^{4+}\cdot$CB7 complex (see the Supporting Information for more detailed discussion).

Electrospray mass spectrometric experiments. Further experimental evidence supporting the formation of a complex between GH_2^{2+}, BB^{4+} and CB7 was obtained using electrospray ionization mass spectrometry, ESI-MS. The major peaks in the spectrum (Figure S10) of a solution containing GH_2^{2+}, BB^{4+} and CB7 appear at m/z ratios of 526.0378 [calcd 526.0351] and 631.0420 [calcd 631.0408], which can be assigned to the quaternary complex containing one molecule of the guest, one molecule of BB^{4+} and two molecules of CB7 with +6 and +5 total charges, respectively. Both the proximity of the experimentally observed m/z ratios to the calculated values and the isotopic distribution patterns constitute good evidence for the existence of the quaternary complex, which appear to be stable enough to survive into the gas phase under our mass spectrometric conditions. Neither $GH_2^{2+}\cdot BB^{4+}$ nor $GH_2^{2+}\cdot BB^{4+}\cdot$CB7 were observed under these conditions.

Electronic absorption spectroscopic experiments. Formation of the $GH_2^{2+}\cdot BB^{4+}$ complex is accompanied by easily observable color changes in the solution. Mixing GH_2^{2+} and BB^{4+} solutions, both colorless, yields an orange solution owing to the development of a charge transfer band around 450 nm (Figure 3). This is due to the well-known ability[7] of BB^{4+} to form charge transfer complexes with electron rich, π-donor moieties like the central aromatic residue of GH_2^{2+}, and further confirms the placement of BB^{4+} around the central ring of the guest. Addition of CB7 to the above solution causes a red shift in the charge transfer band to 525 nm (Figure 3) and a substantial increase in its intensity. These findings indicate that the presence of CB7 not only preserves the position of BB^{4+} on the guest, but also results in a considerable increase in the concentration of the charge

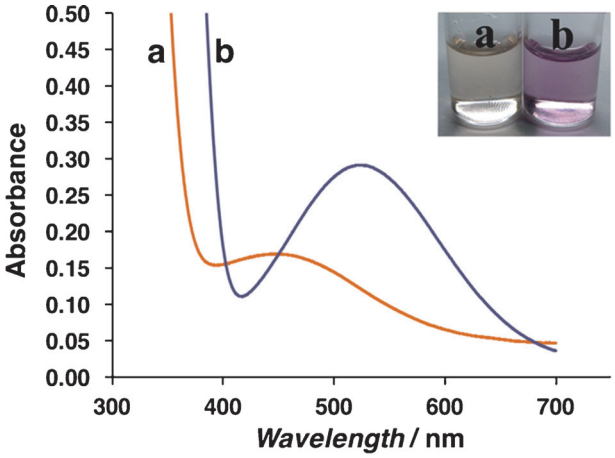


Figure 3. UV/Vis absorption spectra of a) $[GH_2^{2+}]=1.08$ mM, $[BB^{4+}]=0.84$ mM and b) $[GH_2^{2+}]=1.08$ mM, $[BB^{4+}]=0.84$ mM, [CB7] 2.5 mM. All solutions were prepared in water containing 50 mM NaAc (pH 4). Inset: Pictures of the same solutions. A small amount of $GH_2^{2+}\cdot BB^{4+}\cdot(CB7)_2$ precipitate was removed from solution b.

transfer complex. The red shift observed on the λ_{max} of the spectroscopic band is consistent with the stabilization of the acceptor (BB^{4+}) resulting from its interactions with the flanking CB7 hosts.

Computational studies. While all our experimental results are consistent with the formation of a quaternary complex between a molecule of the GH_2^{2+} guest, a molecule of the tetracationic host BB^{4+} and two molecules of CB7, the complexity of this molecular assembly led us to carry out a series of DFT computations at the B3LYP/6-31G(d) and M06-2X/6-31G(d) levels to provide additional support for the proposed complex structures.[8] We anticipated that the computational work may afford a better understanding of the interactions among all the molecular components in the quaternary complex. The resulting DFT-optimized structures are shown in Figure 4. It is particularly important to note that extensive hydrogen bonding[9] was observed in the optimized structure of the quaternary complex $GH_2^{2+}\cdot BB^{4+}\cdot(CB7)_2$ with a total of 20 hydrogen bonds formed between CB7 and BB^{4+} (Figure S13). This has a strong effect on the energy released during the formation of this complex, making it highly stable. In addition, four hydrogen bonds were observed between GH_2^{2+} and CB7 as shown in Figure S13. However, optimization of the ternary complex $GH_2^{2+}\cdot BB^{4+}\cdot$CB7 results in an optimized structure with 11 hydrogen bonds between $BB^{4+}$and CB7 and only two hydrogen bonds were observed in the structure of the complex formed between GH_2^{2+} and CB7 (Figure S12).

Table 1 shows the formation energies of the complexes in vacuum and water. The use of either density functional, B3LYP or M06-2X, leads to similar results with pronounced energy releases accompanying the formation of the complexes. Clearly, the formation of the quaternary complex $GH_2^{2+}\cdot BB^{4+}\cdot(CB7)_2$ releases the largest amount of energy, supporting the considerable stability of this structure, and the presence of multiple interactions between the various molecular components. Interestingly, the only complex which was

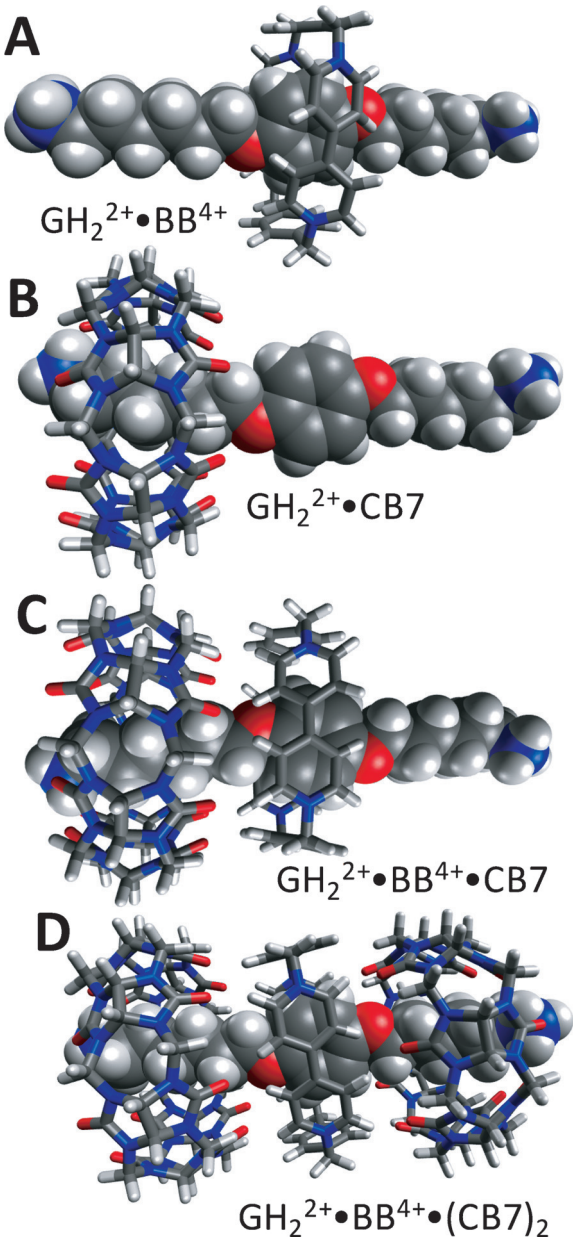


Figure 4. Energy minimized structures for the complexes formed by the guest GH_2^{2+} and (A) BB^{4+}, (B) CB7, (C) CB7 and BB^{4+} and (D) 2 *x* CB7 and BB^{4+}.

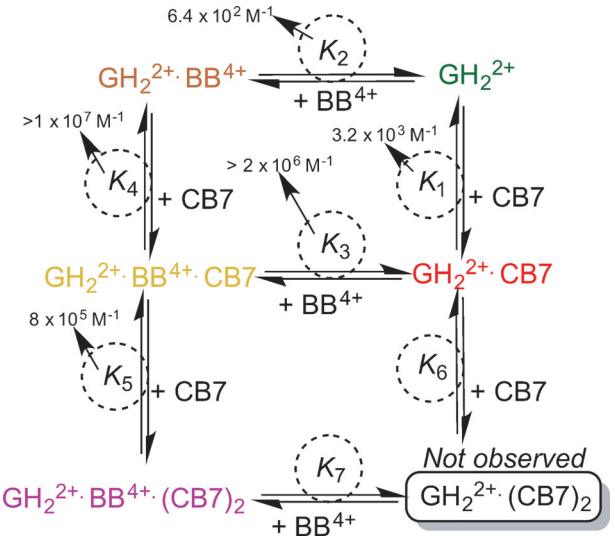
Table 1: Computed formation energies (in kcal mol^{-1}) for the various complexes obtained using the B3LYP density functional. Values in parenthesis were obtained with the M06-2X density functional.

Complex \ Medium	Gas Phase	H_2O soln.	Et_2O soln.
GH_2^{2+}·BB^{4+}	216.4 (198.3)	4.9 (6.4)	48.6 (50.6)
GH_2^{2+}·CB7	−74.3 (−89.9)	−17.9 (−6.9)	−38.0 (−27.9)
GH_2^{2+}·BB^{4+}·CB7	−92.0 (−64.9)	−40.2 (−31.9)	−49.7 (−41.3)
GH_2^{2+}·BB^{4+}·$(CB7)_2$	−165.3 (−191.5)	−82.1 (−64.5)	−134.7 (−119.2)

found to be relative unstable in our calculations was GH_2^{2+}·BB^{4+}, probably as a result of electrostatic repulsions between the two components. However, the presence of BB^{4+} around the central guest binding station results in stronger binding of CB7 than in the absence of the tetracationic host. For instance, the difference of formation energies in water (B3LYP functional) between the complexes GH_2^{2+}·BB^{4+} and GH_2^{2+}·BB^{4+}·CB7 is −45.1 kcal mol^{-1}, while the formation energy for GH_2^{2+}·CB7 from its two molecular components is −17.9 kcal mol^{-1}. This finding shows that the presence of BB^{4+} on the guest considerably increases its binding affinity for CB7, showing strong binding cooperativity between the two hosts. Similar calculations show that the presence of CB7 on one of the hexylamine residues also favors the binding of BB^{4+} on the dioxybenzene site. In general terms, it is clear that the computed structures (Figure 4 and S11-S13) provide additional support to the proposed complex structures.

The spatial relationships between the molecular assembly components, especially in the quaternary complex, give rise to chemical shift patterns that are strongly influenced by the relative positions of the components. Therefore, we used DFT calculations to compute the proton NMR chemical shifts on the complexes of Table 1 and investigate their correlation to the experimentally observed ^{1}H NMR chemical shifts. The corresponding set of data is compiled in the supporting information. For each set of protons in a particular complex we plotted calculated versus experimentally measured NMR chemical shifts and obtained straight lines in all cases with reasonably high correlation coefficients. These results provide further support to the proposed complex structures.

Modeling of the relevant equilibria. The binding interactions between the guest and the two surveyed hosts are shown in Scheme 1. Following up on the arguments derived from the computational data on Table 1, it is of maximum interest to us to compare some key association equilibrium constant values. For instance, what are the relative values of K_2 and K_3? If the K_3 value is substantially larger than K_2, we can conclude that the presence of CB7 in the complex favors the binding interaction between the guest and BB^{4+}. Similarly, what are the values of K_4 and K_5 compared to K_1? This comparison will



Scheme 1. Equilibrium processes between the guest GH_2^{2+} and the hosts BB^{4+} and CB7.

tell us about the effect of BB^{4+} on the guest's thermodynamic affinity for CB7. At the root of these comparisons lies the answer to the key question underlying this work: Is the binding of BB^{4+} and CB7 to the central guest cooperative as the quaternary complex is formed?

First, we must point out that the values of K_1 and K_2, characterizing the binary association equilibria between our guest and each of the hosts, CB7 and BB^{4+}, respectively, were measured in ^{1}H NMR spectroscopic experiments already discussed. Also, we can establish some mathematical relations between equilibrium constants, such as:

$$K_1 \cdot K_3 = K_2 \cdot K_4 \quad (1)$$

$$K' = K_5/K_4 \quad (2)$$

The first equation results from the fact that the corresponding chemical processes form a closed thermodynamic cycle. K' is the equilibrium constant for the process

$$2\,[GH_2^{2+} \cdot BB^{4+} \cdot CB7] \rightleftharpoons GH_2^{2+} \cdot BB^{4+} + GH_2^{2+} \cdot BB^{4+} \cdot (CB7)_2 \quad (3)$$

which results from the subtraction of process 4 from process 5 in Scheme 1. K' can be obtained from NMR experiments (Figure S5) in which the three relevant species, $GH_2^{2+}\cdot BB^{4+}$, $GH_2^{2+}\cdot BB^{4+}\cdot CB7$ and $GH_2^{2+}\cdot BB^{4+}\cdot (CB7)_2$, are present and their concentrations can be evaluated from integration of relevant peaks. From these experiments, we obtained a K' value of 0.077.

Because of the complexity of the equilibrium scheme, we used COPASI,[10] a software tool intended for the simulation and analysis of biochemical networks and their dynamics, which can in fact be readily applied to our system to obtain a minimum value for K_4 (see Figures S18–S20). From the fitting of the simulated concentration data to our experimentally measured values we obtained a minimum value $K_4 = 1 \times 10^7\,M^{-1}$. From the experimentally measured values of K_1 and K_2 previously obtained and Equation (1), we concluded that K_3 has a minimum value of $2 \times 10^6\,M^{-1}$. Finally using equation 2 and the known values of K' and K_4, we obtained a value of $8 \times 10^5\,M^{-1}$ for K_5. These values show that the presence of the BB^{4+} macrocycle bound to the guest substantially increases the binding affinity towards CB7 and vice versa, confirming our hypothesis on the cooperative character of the binding processes.

To conclude, we present here experimental evidence for the formation of a quaternary supramolecular complex resulting from the self-assembly in aqueous solution of two CB7 hosts and one BB^{4+} tetracationic cyclophane around a structurally simple diamine guest with a central *p*-dioxybenzene group (GH_2^{2+}). The molecular design of the guest targets the binding of the three hosts on specific sites, while leading to the development of strong attractive lateral (host-to-host) interactions among them. Binding cooperativity is evidenced by the computed complex formation energies and the K values obtained using COPASI simulations to fit the NMR experimental data. In a way, we can also conclude that the quaternary complex is equivalent to a [2]rotaxane with the peripherally threaded CB7 components playing the role of stoppers.

Acknowledgements

The authors are grateful to the National Science Foundation for the generous support of this research (to AEK, CHE-1412455).

Keywords: cooperativity · cucurbiturils · molecular recognition · self-assembly

How to cite: *Angew. Chem. Int. Ed.* **2016**, *55*, 11507–11511
Angew. Chem. **2016**, *128*, 11679–11683

[1] G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **1991**, *254*, 1312–1319.
[2] A. Harada, J. Li, M. Kamachi, *Nature* **1992**, *356*, 325–327.
[3] a) H. Qian, D. S. Guo, Y. Liu, *Chem. Eur. J.* **2012**, *18*, 5087–5095; b) Y. Chen, Y. M. Zhang, Y. Liu, *Isr. J. Chem.* **2011**, *51*, 515–524.
[4] C. F. Ke, R. A. Smaldone, T. Kikuchi, H. Li, A. P. Davis, J. F. Stoddart, *Angew. Chem. Int. Ed.* **2013**, *52*, 381–387; *Angew. Chem.* **2013**, *125*, 399–405.
[5] J. A. Finbloom, C. C. Slack, C. J. Bruns, K. Jeong, D. E. Wemmer, A. Pines, M. B. Francis, *Chem. Commun.* **2016**, *52*, 3119–3122.
[6] a) J. W. Lee, S. Samal, N. Selvapalam, H.-J. Kim, K. Kim, *Acc. Chem. Res.* **2003**, *36*, 621–630; b) J. Lagona, P. Mukhopadhyay, S. Chakrabarti, L. Isaacs, *Angew. Chem. Int. Ed.* **2005**, *44*, 4844–4870; *Angew. Chem.* **2005**, *117*, 4922–4949; c) E. Masson, X. Ling, R. Joseph, L. Kyeremeh-Mensah, X. Lu, *RSC Adv.* **2012**, *2*, 1213–1247; d) S. J. Barrow, S. Kasera, M. J. Rowland, J. del Barrio, O. A. Scherman, *Chem. Rev.* **2015**, *115*, 12320–12406.
[7] a) P. L. Anelli, P. R. Ashton, R. Ballardini, V. Balzani, M. Delgado, M. T. Gandolfi, T. T. Goodnow, A. E. Kaifer, D. Philp, M. Pietraszkiewicz, L. Prodi, M. V. Reddington, A. M. Z. Slawin, N. Spencer, J. F. Stoddart, C. Vicent, D. J. Williams, *J. Am. Chem. Soc.* **1992**, *114*, 193–218; b) A. Mirzoian, A. E. Kaifer, *J. Org. Chem.* **1995**, *60*, 8093–8095.
[8] T.-C. Lee, E. Kalenius, A. I. Lazar, K. I. Assaf, N. Kuhnert, C. H. Grun, J. Janis, O. A. Scherman, W. M. Nau, *Nat. Chem.* **2013**, *5*, 376–382.
[9] R. S. Rowland, R. Taylor, *J. Phys. Chem.* **1996**, *100*, 7384–7391.
[10] S. Hoops, S. Sahle, R. Gauges, C. Lee, J. Pahle, N. Simus, M. Singhal, L. Xu, P. Mendes, U. Kummer, *Bioinformatics* **2006**, *22*, 3067–3074.

Received: June 21, 2016
Published online: August 17, 2016

