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Supramolecular Chemistry of Cucurbiturils: Tuning Cooperativity with Multiple Non-Covalent Interactions from Positive to Negative

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Abstract

Rational control over cooperativity of multiple non-covalent interactions often plays an important role in the design and construction of supramolecular self-assemblies and materials, especially in precision supramolecular engineering. However, it still remains a challenge to control the cooperativity of multiple non-covalent interactions through tuning the hydrophobic effect. In this work, we demonstrate that the binding cooperativity of cucurbit[8]uril(CB[8])-mediated homoternary complexes is strongly influenced by the amphiphilicity of guest molecule side groups on account of an interplay between both classical (entropy-driven) and non-classical (enthalpy-driven) hydrophobic effect. To this end, we rationally designed and prepared a series of guest molecules bearing a benzyl

group as the CB[8] homoternary binding motif with various hydrophilic and hydrophobic side groups for cooperative control. By gradually tuning side groups of the guest molecules from hydrophilic to hydrophobic, we are able to control binding from positive to negative cooperativity. Advanced molecular recognition process and self-assembling system can be developed by adjusting positive and negative cooperativity. The ability to regulate and control binding cooperativity will enrich the field of supramolecular chemistry, and employing cooperativity-controlled multiple non-covalent interactions in precision supramolecular engineering is highly anticipated.

Introduction

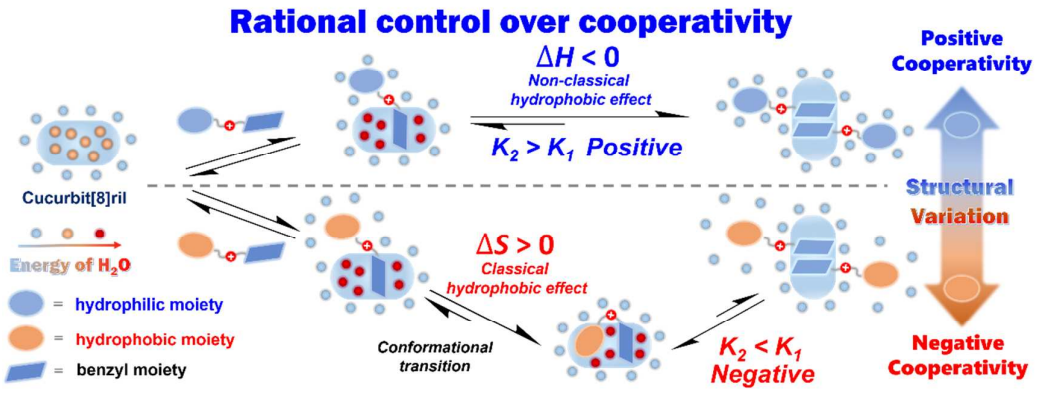
Precision supramolecular engineering is of fundamental importance in both chemistry and biology, as structure determines function. In recent years, lots of strategies, such as self-assembled supramolecular coordination complexes,[1] template-directed supramolecular synthesis,[2] controllable supramolecular polymerization,[3-4] and selective self-assemblies,[5] have been developed in this field to precisely construct supramolecular architectures by utilizing non-covalent interactions. A wide variety of non-covalent interactions, including multiple hydrogen bonding, metal-coordination, host-guest and ·π-π· interaction, have been successfully utilized as driving forces in supramolecular engineering. More importantly, achieving rational control over multiple and directional non-covalent interactions is of critical importance in realizing precision supramolecular engineering. As stoichiometry and complexity of non-covalent interactions increase,

cooperative effects can play an important role in multiple non-covalent interactions. We believe that controlling cooperativity represents an efficient strategy to rationally modulate non-covalent interactions which can be used for supramolecular engineering with precisely-defined structure and function.

Cooperativity can be classified as a) positive cooperativity, when one interaction favors that of the others, b) negative cooperativity, when one interaction suppresses other interactions, and c) non-cooperativity, when multiple interactions occur independently from one another.[6] The synergistic binding of oxygen to hemoglobin is an example from nature, which has encouraged scientists to study cooperative effects in biological systems.[7-8] Cooperativity of artificial supramolecular systems has attracted lots of interest,[9-15] nevertheless, chemists are not simply satisfied to understand its origins, but aim to control it. Limited success has been achieved designing cooperative processes in supramolecular multiple binding systems. Thus, it remains a challenge to rationally control the cooperativity of multiple non-covalent interactions in water through modulating the hydrophobic effect.

Cucurbit[n]urils (CB[n]s) are a family of water-soluble macrocyclic hosts, which have a hydrophobic cavity capable of the binding one or two guest molecules.[16-22] Three points contribute to binding between CB[n] host and guest molecules: i) attraction between the host and guest, ii) desolvation of the host cavity, and iii) desolvation of the guest.[23-24] The attraction between host and guest includes size/shape complementarity as well as ion-dipole and dipole-dipole interactions arising from the electron-rich carbonyl rims of CB[n]s. The desolvation of CB[n] host cavity releases high-energy water trapped in the

cavity which is regarded as a non-classical hydrophobic effect as it has a favorable enthalpic signature. In contrast, the desolvation of the guest molecules is regarded as a classical hydrophobic effect, containing a favorable entropic component as a result of release of surface-bound solvent molecules on the guest. On account of their high affinities, CB[n]-mediated host-guest interactions are of great interest in the field of supramolecular chemistry and chemical biology.[25-32] While the ability to exhibit multiple non-covalent interactions, CB[8]-mediated heteroternary and homoternary complexes have been exploited in the construction of supramolecular polymers,[33-36] supramolecular hydrogels,[37-38] supra-amphiphiles[39-40] and other supramolecular functional self-assembled systems[41-46].



Scheme 1 Schematic diagram of design principles on rationally controlling cooperativity of cucurbit[8]uril-mediated π-π interactions by tuning classical and non-classical hydrophobic effect (blue balls represent bulk water, while orange and red balls represent high-energy water and the energy of the red one is higher than the orange one's).

Herein we aim to control binding cooperativity of CB[8]-mediated homoternary

interactions by tuning the classical and non-classical hydrophobic effects involved in their formation. A large number of suitable guests for CB[8] generally contain two components: an aromatic moiety representing a cavity binding motif to release high-energy water molecules trapped inside the CB[8] cavity, and a positively-charged side group to form ion-dipole interactions with the carbonyl rims. As shown in Scheme 1 (top), we posited that the amphiphilicity of the positively-charged side group substantially influences high-energy water release. One can envision that a guest molecule with a hydrophilic side group would result in only partial release of the trapped water and serve to increase the energy of any residual confined water molecules. This would occur as formation of the 1:1 complex creates an even more confined geometry and hydrophobic cavity. Subsequently, the secondary binding to the 2:1 complex becomes more favorable exhibiting positive cooperativity, which is dominated by non-classical hydrophobic effect.

In contrast, if the side group of the guest molecules is hydrophobic, simultaneous incorporation of both the aromatic moiety and the side group into the same CB[8] can occur as illustrated in Scheme 1 (bottom). This 1:1 complex results in the release of fewer high-energy water molecules compared to 2:1 homoternary complex; nevertheless, a higher entropy contribution towards binding compensates arising from desolvation of water molecules on the surface of the hydrophobic group, leading to an overall stabilization of the 1:1 complex. Suppressing the association of the second guest molecule results in negative cooperativity, which is mainly influenced by the classical hydrophobic effect. Moreover, if the hydrophobic side group is large enough, the non-classic hydrophobic effect plays an even greater role, completely suppressing the

formation of the 2:1 homoternary complex. In addition, by modulating the hydrophilicity and hydrophobicity of the guest side groups to balance the classical and non-classical hydrophobic effect, one can also achieve non-cooperativity. Eventually, we may be able to control cooperative bindings of cucurbit[8]uril-mediated homoternary interactions by simply varying the amphiphilicity of the guest side groups.

Results and discussion

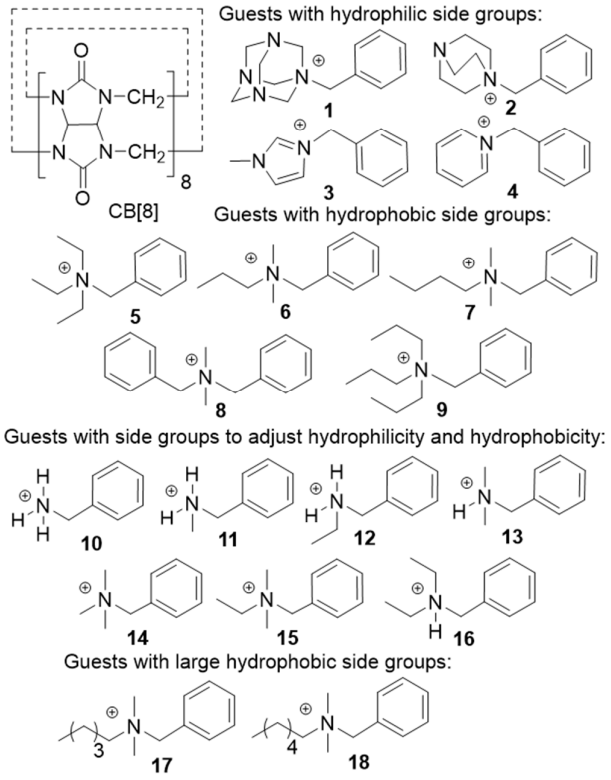
To this end, we designed and prepared a series of benzyl-based guest molecules bearing different positively-charged substituents varying from hydrophilic to hydrophobic. The cooperativity of their homoternary interactions with CB[8] was investigated by ^{1}H NMR spectroscopy, rotating-frame overhauser effect spectroscopy (ROESY), electrospray ionization mass spectrometry (ESI-MS) and isothermal titration calorimetry (ITC). Interestingly, through structural variation of the guests, we achieved three different types of cooperative binding by tuning the classical and non-classical hydrophobic effect, which exhibit positive cooperativity, negative cooperativity and non-cooperativity.

Molecular design of a library of guest molecules.

A series of guest molecules (Chart 1) were designed and prepared such that each guest molecule has a benzyl group as the π-π stacking motif to form homoternary π-π interactions. For tuning the amphiphilicity of the guest molecules, different positively-charged side groups have been introduced. Guests **1-4** contain hydrophilic side groups while guests **5-9** contain hydrophobic groups. In order to achieve the transition from positive to negative cooperativity and seek non-cooperative cases, we systematically

adjusted the hydrophilicity and hydrophobicity of guest molecules **10-16**. Finally, two guests modified with long alkyl chains, **17-18** were prepared to investigate entropy compensation. In order to compare all bindings of guests, the counterions are bromides in all cases.

Chart 1 CB[8] host and guest molecules used in this study and the counterions are bromides in all cases.



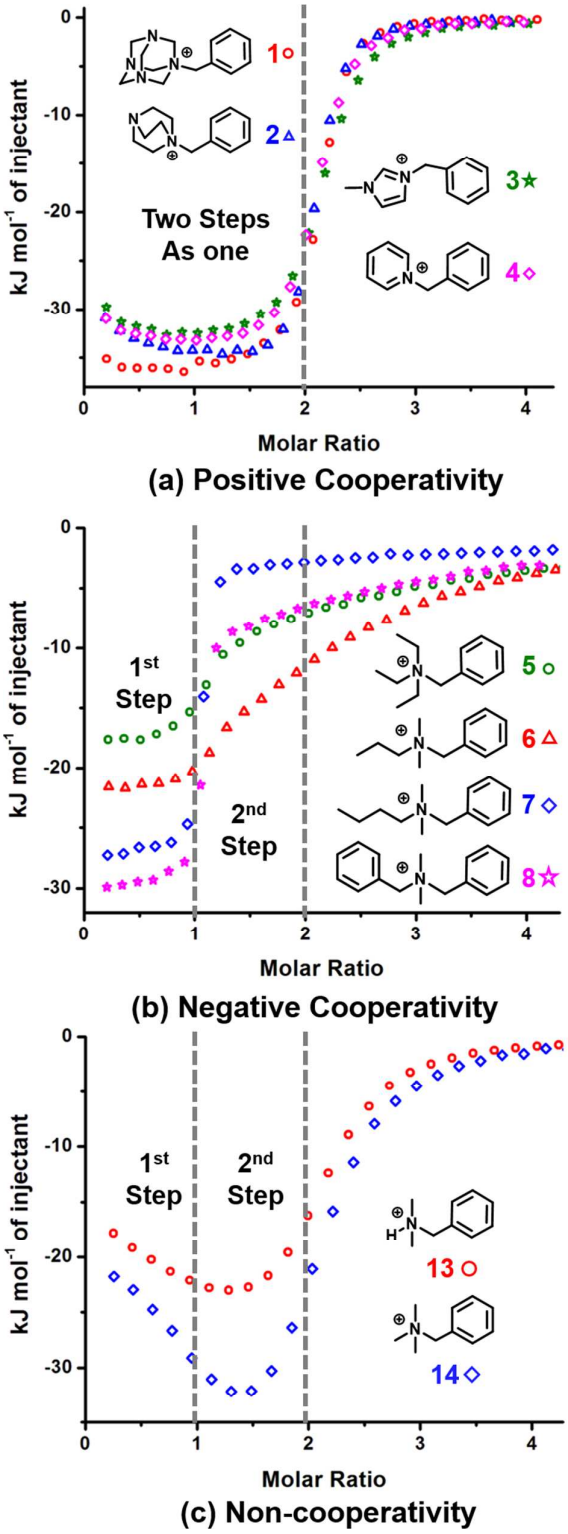


Figure 1 ITC titration plots in the same experimental conditions representing three kinds of cooperative bindings: (a) positive cooperativity; (b) negative cooperativity and (c) non-cooperativity.

Realization of three types of cooperative bindings.

Isothermal titration calorimetry (ITC) was employed to observe the cooperativity of CB[8]-mediated homoternary complexation for all of the different guest molecules (**1**-**19**) bearing a variety of hydrophilic and hydrophobic side groups. Three types of titration profiles were observed as illustrated in Figure 1a-c. Qualitatively, sharper transitions in an ITC plot suggest stronger binding. In Figure 1a, the transition at the molar ratio of 2:1 is indeed very sharp while any transition at a ratio of 1:1 is hard to observe. This means that the binding of a second guest molecule is much stronger than the first, indicative of positive cooperativity. On the contrary, when the first transition is clearly sharper than the second, as is the case for guest **5-8** shown in Figure 1b, the first binding event is substantially stronger than the binding of the second guest molecule, representing a system of negative cooperativity. In Figure 1c, two titration curves for **13** and **14** are similar to each other. On account of their stepwise enthalpy changes ($\Delta H_2 > \Delta H_1$), they display a distinctive sinusoidal shape. Both of them has two mild transitions at the molar ratio of 1:1 and 2:1, representing non-cooperativity. All of these 2:1 or 1:1 complexations can be clearly observed in ^{1}H NMR (Figure S1-42), ROESY (Figure S43-46), ESI-MS (Figure S47-87) spectra. Eventually, we have realized a control over cooperativity by simply varying the structures of guest molecules.

In order to quantitatively evaluate the cooperativity for the guest **1**-**18** with CB[8], we utilized sedphat[47-48] and NIPIC[49-50] to fit the data and obtained macroscopic stepwise association constants (K_n). Sedphat is a powerful mathematics tool which can take into consideration the concentrations of the host and the guest during fitting, which can help us

jump out of the fitting traps and provide us better parameters with less error. By importing the fitted thermodynamic parameters and concentrations from sedphat as initial setting, we fitted the raw data again by Origin to produce ITC final figures. As shown in Figure S94, the macroscopic stepwise association constants should be transformed into microscopic stepwise association constants (k_n) due to statistical considerations. An interaction parameter (α) was then calculated based on equation ($\alpha = k_2/k_1 = 4K_2/K_1$) to describe the cooperativity in a quantitative manner.[6] When the value of log α is larger than 0.5, the system displays positive cooperativity, when log α is smaller than -0.5, the system displays negative cooperativity, and when log α is in between -0.5 to 0.5, the system is non-cooperative.

Table 1 Thermodynamic parameters of all the host-guest complexations and interaction parameters of all guest molecules.

No.	log α[a]	K_1[b] (10^5 M^{-1})	K_2[b] (10^5 M^{-1})	ΔH_1[b] (kJ mol^{-1})	$-T\Delta S_1$[b] (kJ mol^{-1})	ΔH_2[b] (kJ mol^{-1})	$-T\Delta S_2$[b] (kJ mol^{-1})
1	**2.32±0.02**	1.24±0.04	64.60±1.6	-36.4±1.1	7.3±1.2	-37.8±1.1	-1.1±1.2
2	**0.91±0.02**	11.70±0.29	24.00±0.32	-28.2±0.2	-6.4±0.2	-42.6±0.2	6.1±0.2
3	**1.28±0.02**	2.64±0.05	12.60±0.21	-26.6±0.3	-4.3±0.3	-42.8±0.3	8.0±0.3
4	**0.87±0.01**	7.76±0.12	14.40±0.24	-30.0±0.1	-3.6±0.2	-39.1±0.2	3.9±0.2
5	**-1.89±0.02**	26.00±0.81	0.083±0.001	-17.9±0.1	-18.7±0.1	-47.7±0.5	25.3±0.5
6	**-1.11±0.03**	7.17±0.30	0.139±0.004	-22.5±0.1	-11.0±0.2	-46.3±0.6	22.6±0.6
7	**-3.30±0.05**	193±10	0.024±0.001	-26.9±0.1	-14.6±0.2	-46.6±2.3	27.2±2.5
8	**-3.02±0.08**	377±50	0.089±0.005	-29.4±0.1	-13.9±0.4	-42.7±1.3	20.1±1.5
9	-[c]	363±43	-[c]	-26.0±0.1	-17.2±0.4	-[c]	-[c]
10	**1.53±0.02**	1.29±0.03	10.90±0.20	-19.5±0.3	-9.6±0.4	-35.4±0.3	0.9±0.4
11	**1.00±0.02**	6.66±0.20	16.50±0.38	-16.4±0.3	-16.9±0.4	-42.3±0.3	6.8±0.3
12	**0.59±0.11**	10.9±1.9	10.50±0.82	-23.5±0.7	-11.0±1.1	-41.2±0.7	6.8±0.9
13	**0.06±0.01**	6.86±0.09	1.99±0.02	-16.9±0.1	-16.5±0.7	-34.1±0.1	3.8±0.1
14	**-0.38±0.02**	24.50±0.63	2.58±0.03	-20.9±0.1	-15.6±0.1	-47.6±0.1	16.7±0.1
15	**-0.71±0.01**	35.30±0.47	1.74±0.02	-20.6±0.1	-16.7±0.1	-44.1±0.1	14.1±0.1
16	**-1.89±0.02**	31.00±0.98	0.101±0.004	-21.3±0.1	-15.9±0.1	-47.3±0.4	24.5±0.5
17	-[c]	415±56	-[c]	-30.0±0.1	-13.5±0.4	-[c]	-[c]
18	-[c]	542±56	-[c]	-33.0±0.1	-11.1±0.4	-[c]	-[c]

Note: [a] interactions parameters (log α) are calculated by microscopic stepwise binding constants. [b] Mean value measured from ITC experiments at 25.0 °C in 50 mM sodium acetate buffer (pH 4.75), which are fitted by Sedphat and NIPIC; K_1 and K_2 are macroscopic stepwise binding constants; ΔH_1 and ΔS_1 are the binding enthalpy and binding entropy for the first step; ΔH_2 and ΔS_2 are the binding enthalpy and binding entropy for the second step. [c] not determined. All the detailed data are shown in ESI.

Positively cooperative guest molecules.

In order to achieve positive cooperativity, the second association of the guest molecules must be more favorable than the first. As Biedermann and co-workers reported[23-24], the release of "high-energy" water can provide an enthalpic driving force which is regarded as a non-classical hydrophobic effect. After the incorporation of the first guest, the conformational space of the residual "high-energy" water is further restricted by geometric confinement and the hydrophobic nature of the first guest. This may serve to further enhance the energy of the residual water in the cavity and may lead to a more favorable second association. Therefore, by introducing hydrophilic side groups on the guest molecules, the first binding event may release relatively few of the confined water molecules leaving more high-energy water in the CB[8] cavity for a more favorable second association. This leads to positive cooperativity as shown in Figure 1a, Figure S88 and Table 1 for four guest molecules **1**-**4** modified with hydrophilic side groups, which exhibited similar titration curves. Moreover, the mechanism behind their positive binding is corroborated by the stepwise enthalpy changes in Table 1. For guests **1**-**4**, all the second binding steps provides a greater change in enthalpy than the first binding step ($\Delta H_2 > \Delta H_1$).

The homoternary complexation between CB[8] and hexamethylenetetramine mono benzyl ammonium (**1**) exhibits the largest log α value (2.32) of all 18 guests displaying a high level of positive cooperativity. As hexamethylenetetramine group has significant steric bulk and four tertiary nitrogen atoms capable of forming multiple hydrogen bonds with bulk water, a considerable entropy penalty may exist for the first binding step,

resulting in a much weaker binding event than the second. Guests **2**-**4** contain moieties that are also readily hydrated by bulk water and thus do not enter the CB[8] cavity, which contributes to their positively cooperative complexation with log α values of 0.91, 1.28 and 0.87, respectively. In addition, Urbach et. al. revealed that the tripeptide Phe-Gly-Gly can form CB[8]-mediated homoternary interactions with positive cooperativity, and the single crystal structures of 2Phe-Gly-Gly-CB[8] was also obtained.[51] The positive cooperativity behind its binding can be ascribed to the abundant hydration of Gly. Therefore, based on non-classical hydrophobic effect, guest molecules with hydrophilic side groups can exhibit positive cooperativity.

Negatively cooperative guest molecules.

In contrast to positive cooperativity, negative cooperativity is realized by ensuring that the first binding with the macrocycle is more favorable than the second. Our molecular design enables straightforward introduction of hydrophobic groups to the benzyl unit in the form of alkyl chains or additional aromatic rings. We envisioned that for certain structures, simultaneous incorporation of both the aromatic benzyl moiety as well as a second hydrophobic group into the CB[8] cavity could be achieved. While complexation from such a structure may only release a portion of the high energy water molecules, it would also give rise to considerable entropy compensation due to the reduction of a water-alkyl chain interface,[52] which represents a typical classical hydrophobic driving force to stabilize the 1:1 complex. In effect, the 1:1 complex with two cavity-bound hydrophobic components would suppress a second molecule from associating to the CB[8], resulting in negative

cooperativity. As shown in Figure 1b and Figure S89, a different type of titration plot was obtained for four guest molecules (**5**-**8**) with hydrophobic side groups, which exhibited two binding steps and the first transition was sharper than the second one representing strong negative cooperativity. Quantitatively, the log α value for these four guest molecules were all less than -0.5 (Table 1), which indicates the first binding affinity is considerably larger than the second.

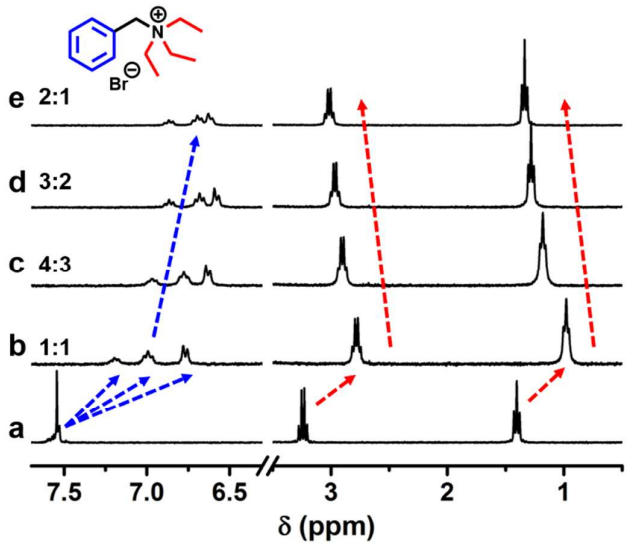


Figure 2 Partial ^{1}H NMR (300 MHz, D_2O, 25.0 °C) spectra of a) **5**; b) **5**·CB[8]; c) **5**·0.75CB[8]; d) **5**·0.667CB[8] and e) **5**·0.5CB[8]. (The concentration of CB[8] = 1.0 mM in all cases)

As a very stable 1:1 complex should form when negative cooperativity is observed, the association of a second guest should be suppressed. In order to confirm this assumption, ^{1}H NMR titration experiments were carried out with benzyl triethylammonium (**5**) and CB[8]. As shown in Figure 2, the protons of the three ethyl groups in **5**·CB[8] showed a significant upfield shift compared with **5** itself, which indicated that upon formation of the 1:1 complex, both the benzyl unit as well as the ethyl groups can be encapsulated into the CB[8] cavity. On account of fast exchange,[53-54] only one set of

peaks for the ethyl groups can be observed. When an additional quantity of guest **5** was added to the 1:1 **5**·CB[8] complex, we observed that the proton shifts of the ethyl group gradually moved back downfield suggesting that complexation of the (benzyl group of the) second guest molecule forces the ethyl group out of the CB[8] cavity. Titration experiments carried out with *N*, *N*-dimethyl-*N*-propylammonium (**6**), *N*, *N*-dimethyl-*N*-butylammonium (**7**) and *N*, *N*-dimethyl-*N*-benzylammonuim (**8**) and CB[8] all showed similar trends in their ^{1}H NMR spectra (Figure S12-20).

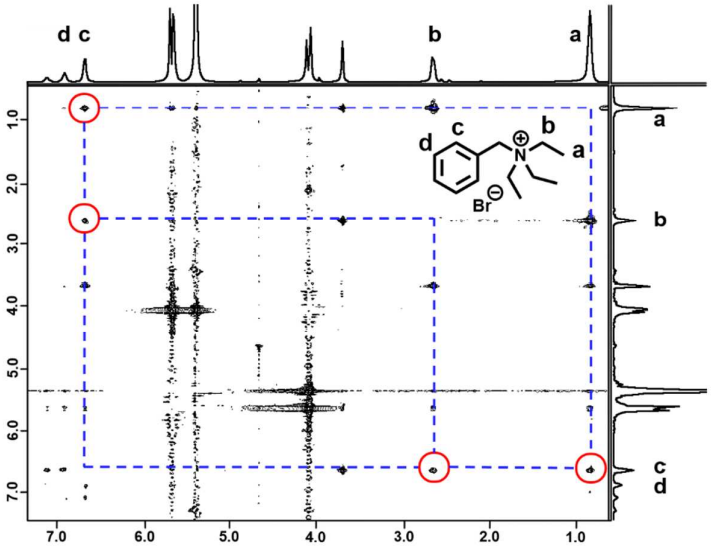


Figure 3 ROESY ^{1}H-^{1}H spectrum (400 MHz, D_2O, 25.0 °C) of **5**·CB[8] (4.0 mM).

A ROESY NMR experiment was carried out in order to confirm simultaneous encapsulation of both the benzyl and ethyl groups inside the CB[8] cavity. As shown in Figure 3, protons labelled (a, b) of ethyl groups show a strong ROESY ^{1}H-^{1}H correlation with the (c) protons on the benzyl group. Importantly, no clear correlation between the (a, b) protons and the (d) protons exists as the ethyl groups do not extend enough into the CB[8] cavity to interact with the (d) protons on the benzyl group. A ROESY spectrum was

also obtained for guest **5** itself in which no correlation between the (a, b) protons and the (d) protons was observed (Figure S44).

According to the enthalpy and entropy changes recorded in Table 1, a mechanism for negative cooperativity can be illustrated. In a similar manner to guests **1**-**4**, the second step (molecule) binding for guests **5**-**8** all provide a greater enthalpy change than the binding of the first molecule, strongly suggesting that the non-classical hydrophobic effect plays an important role in the final 2:1 homoternary complex formed with guests **5**-**8**. However, for guests **5**-**8**, which carry hydrophobic side groups, their simultaneous incorporations of the benzyl group and another hydrophobic moiety in the initial binding step offers much greater entropic compensation ($-T\Delta S_1 < -10$ kJ mol^{-1}) than for guests **1**-**4**, which represents a classical hydrophobic driving force. Second guest molecule complexation with CB[8] to form the 2:1 homoternary complex yields a large entropy penalty ($-T\Delta S_2 > 20$ kJ mol^{-1}) suppressing the second association and also decreases the overall binding affinity compared with guests carrying a hydrophilic side group such as **1**-**4**. These two entropic effects can outweigh the non-classical hydrophobic effect making the first binding step substantially stronger than the second. It appears, therefore, that the stronger classical hydrophobic effect drives the occurrence of negatively cooperative events; thus negative cooperativity can be readily achieved in this system by selecting guests with hydrophobic side groups.

Guest molecules with well-tuned cooperativity from positive to negative.

In an effort to achieve systems that displayed non-cooperativity, we attempted to find

the right balance between hydrophilicity and hydrophobicity of the guest molecules. Thus we designed and prepared a series of guest molecules with increasing hydrophobicity of the side groups by systematically increasing the alkyl content one methylene group at a time. As shown in Table 1 and Figure S90b-90d, three hydrophilic guests, ammonium (**10**), *N*-methylammonium (**11**) and *N*-ethylammonium (**12**) still exhibited considerable positive cooperativity on account of their abundant hydrogen-bonding with bulk water. However, by gradually increasing the hydrophobic alkyl chains of guest molecules to *N*, *N*-dimethylammonium (**13**) and trimethylammonium (**14**), they displayed smaller and similar interaction parameter (α) as shown in Table 1 and Figure S91a-91b. Their log α values are in the range of -0.5 and 0.5, which is close to 0, indicating non-cooperativity. This data confirms that non-cooperativity can be readily achieved by balancing the hydrophilicity and hydrophobicity of the guest molecules. It should be pointed out that since CB[8] only have one hydrophobic cavity, the second guest can always feel the presence of the first one, thus it is not a strict non-cooperative case and the balance of positive and negative cooperativity is responsible for this non-cooperativity.

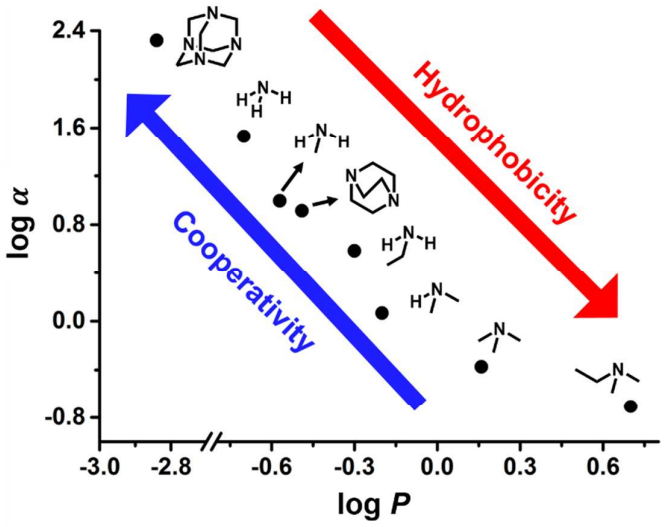


Figure 4 Plots of octanol-water partition coefficients (log *P*) and interaction parameters (α).

In order to evaluate the amphiphilicity in a semi-quantitative manner, we utilized partition coefficients (log *P*, between octanol and water, as shown in Table S1) of the side groups in the series and obtained a structure-activity relationship as shown in Figure 4.[55] We also included hexamethylenetetramine and 1, 4-diazabicyclooctanetriethylenediamine into consideration as their molecular structure and amount of the positive charge are the same as those of the above series. As is evident in Figure 4, a negative correlation between the octanol-water partition coefficients of the side groups and their interaction parameters exists. This indicates that guests bearing side groups with lower hydrophobicity show higher positive cooperativity as higher hydrophilicity leads to situations where the non-classical hydrophobic effect outweighs the classical hydrophobic effect. Thus, by increasing the hydrophobicity of the side groups, we can tune systems that display positive cooperativity into systems that do not display cooperative.

We wondered if further increasing the hydrophobicity of the guest molecules would

lead to systems displaying strong negative cooperativity. To prove this assumption, we continued to increase the alkyl chain preparing *N*, *N*-dimethyl-N-ethylammonium (**15**); however, this guest only exhibited weak negative cooperativity as seen in Table 1 and Figure S91c. When the rearranged variant *N*, *N*-diethylammonium (**16**) was prepared, as indicated by the data in Table 1 and Figure S91d, guest **16** exhibited a log α value of -1.89, typical for negative cooperative complexation with CB[8] and similar to guests **5**-**8**. This minor structural change results in a large difference in how the guests interact with CB[8] as the two ethyl moieties on guest **16** can play the same role as the triethyl group of guest **5** leading to negative cooperativity. Thus, a stepwise progression from positive cooperativity to negative cooperativity can be achieved by simply increasing hydrophobicity of the guest molecules. Side groups with weak hydrophilicity or hydrophobicity have little influence on release of high-energy water or the entropy compensation during the first binding step, resulting in a “balance” of the binding affinity between the two independent complexation steps and leading to the observation of non-cooperative binding.

Non-classical hydrophobic effect can hinder 2:1 complexation and favor 1:1 complexation.

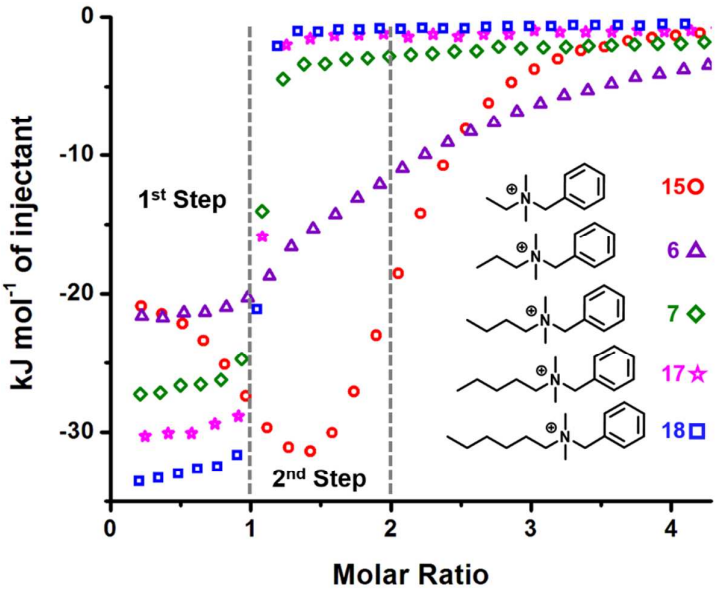


Figure 5 ITC titration plots using guest molecules modified with different length of the alkyl side chains obtained in acetate buffer of pH = 4.75, 25.0 °C.

We observed that the guest molecule **9**, bearing three propyl chains, forms an extremely stable 1:1 complex with CB[8] similar to guests **5**-**8** confirmed by ^{1}H NMR, ROESY and ITC (as shown in Figure S21-22, S46 and S90a, respectively); the binding of a second molecule was surprisingly inhibited. This led us to believe that a distinct boundary may exist between 2:1 and 1:1 complexation. In order to observe the transition from 2:1 to 1:1 complexation, we designed and prepared several guest molecules (**17**-**18**) bearing longer, more hydrophobic alkyl chains to further stabilize the formation of the 1:1 complex. As shown in Figure 5 and Figure S92, when the guest molecule contained a pentyl or hexyl chain, the second binding step could hardly be observed in the titration plots. Based on this finding, 2:1 homoternary interactions can be prevented by design, which may be of interest in newly developed supramolecular systems.

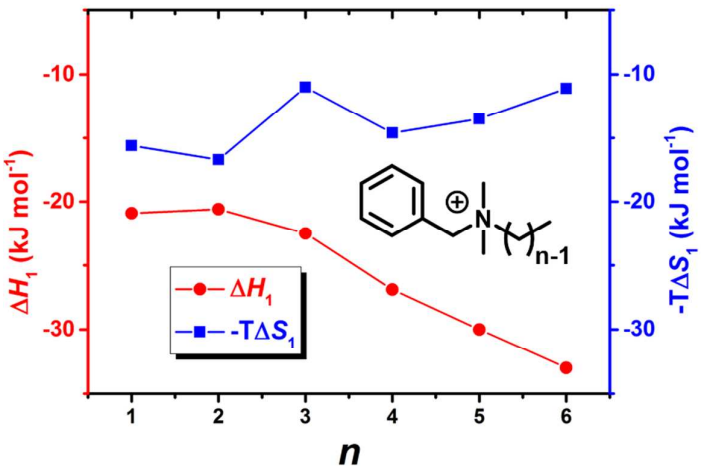


Figure 6 Plots of thermodynamic parameters (ΔH_1 and $-T\Delta S_1$) in the first binding step of guests 14-18 where n = 1-6, respectively.

Analysis of all the thermodynamic parameters for the guests shown in Figure 6 yields mechanistic insight into the complexation of each guest with CB[8]. As depicted by the blue data set in Figure 6, the entropy compensation arising from reduction of the water-alkyl chain interface in the first binding step for each guest (**14**-**18**) is roughly the same (-10 to -15 kJ mol^{-1}), which strongly suggests that the classical hydrophobic effect plays the same role. However, as the length of the alkyl chain increases, a larger enthalpic contribution (red plot) from -20 to -35 kJ mol^{-1} is evident in the first binding step. This corresponds to a larger number of high-energy water molecules being released from the CB[8] cavity upon 1:1 complexation. In other words, while the classical hydrophobic effect (entropy compensation) is essential, the non-classical hydrophobic effect (enthalpy-driven complexation) plays an increasingly important role in the first binding step. When the enthalpy change becomes large enough to suppress the second binding step, 2:1 complexation can be inhibited resulting in 1:1 complexation.

Advanced self-sorting system can be developed by adjusting negative and positive cooperativity.

Self-sorting is a self-assembly process in which molecules are endowed with the ability to form complexes selectively and specifically with their own recognition units within a mixture.[56-59] We wondered if it would be possible to design an advanced self-sorting system by adjusting the cooperativity in complexation for the benzylamine guests with CB[8]. Based on the thermodynamic stability of 1:1 complexes arising from negative cooperativity, and 2:1 complexes stemming from positive cooperativity, we envisioned that a mixture of guests that distinctly resulted in either 1:1 or 2:1 complexation with CB[8] may exhibit self-sorting. To test our hypothesis, guests **1** and **5** were selected representing systems exhibiting positive cooperativity and negative cooperativity, respectively.

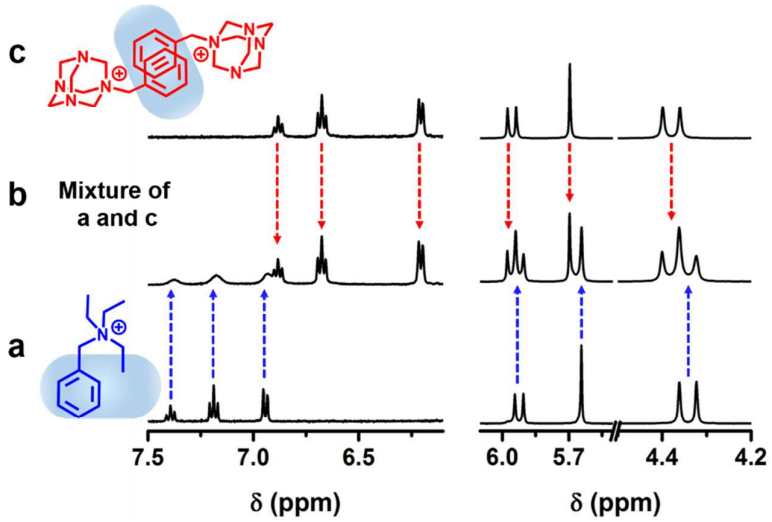


Figure 7 Partial ^{1}H NMR (400 MHz, D_2O, 25.0 °C) spectra of a) **5**·CB[8]; b) mixture of **1**$_2$·CB[8] and **5**·CB[8] and c) **1**$_2$·CB[8]. (The concentration of CB[8] = 1.0 mM in all cases)

To investigate the self-sorting process between guests **1** and **5** and CB[8], a series of ^{1}H NMR experiments were performed. As shown in Figure 7, the identical peaks of protons on the aromatic rings (left) and CB[8] (right) of the 1:1 complex of **5**·CB[8] (Figure 7a) and the 2:1 complex of $\mathbf{1}_2$·CB[8] (Figure 7c) can be clearly observed. Interestingly, when we prepared a mixture containing 2.0 equiv. of **1**, 1.0 equiv. of **5** and 2.0 equiv. of CB[8], we observed the identical proton peaks arising from the 1:1 complex of **5**·CB[8] and the 2:1 complex of $\mathbf{1}_2$·CB[8] as shown in Figure 7b. This mixture gives clear indication that these three components exhibit self-sorting, resulting in the simple combination of **5**·CB[8] and $\mathbf{1}_2$·CB[8]. After mixing, the exchange time of host-guest complex **5**·CB[8] could be close to the detection time of ^{1}H NMR, leading to broadening of the peaks related to the protons of guest **5**. Thus, negatively cooperative events and positively cooperative events do not appear to disturb each other, and such a coexistence suggests that self-sorting can indeed be tuned by adjusting positive and negative cooperativity for structurally similar molecular species. We anticipate that this new self-sorting system can be easily utilized in advanced self-assembling system to fabricate multifunctional supramolecular polymers, supra-amphiphiles or other complex and precise supramolecular architectures.

Conclusions

In this work, we have successfully achieved rational control over cooperativity of multiple non-covalent interactions in water by tuning the interplay between the classical and non-classical hydrophobic effects. Specifically, positive cooperativity, non-cooperativity

and negative cooperativity can all be achieved for structurally similar guest molecules with the same host by varying the amphiphilicity of side groups present on the guest molecules from hydrophilic to hydrophobic. Similar design methodology may also be applicable in other multiple supramolecular complexations driven by enthalpic high-energy water release or classical entropy compensation. Advanced molecular recognition process and advanced self-assembling system can be developed by adjusting the positive and negative cooperativity. We anticipate that this line of research representing an in-depth study of modulating classical and non-classical hydrophobic effects will enrich the field of supramolecular chemistry leading to important advances and the realization of controlling cooperativity of non-covalent interactions, which can be employed in precision supramolecular engineering.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, ^{1}H NMR spectra, ROESY, COSY spectra and ESI MS spectra of all guest molecules and their 2:1 or 1:1 complexes with CB[8], all binding isotherms, fitted curves and experimental results, along with supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.

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