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Cucurbit[8]uril and Blue-Box: High-Energy Water Release Overwhelms Electrostatic Interactions

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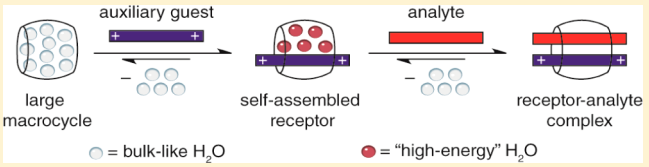
Supporting Information

ABSTRACT: The design of high-affinity and analyte-selective receptors operating in aqueous solutions is an outstanding problem in supramolecular chemistry. Directing the focus toward the unique properties of water, we present here a new strategy toward this goal and support it by molecular dynamics simulations and calorimetric measurements. We illustrate the procedure in the case of self-assembled 1:1 complexes of the rigid macrocycle cucurbit[8]uril (CB8) and dicationic auxiliary guests (AG). These CB8•AG complexes contain residual water molecules whose conformational space and hydrogen-bond formation ability is restricted by the geometrically confined and hydrophobic cavity of the receptor. We show that upon inclusion of an analyte to form a 1:1:1 CB8•AG•analyte complex, these "high-energy" cavity water molecules are released to the aqueous bulk, providing a strong enthalpic driving force to the association, and resulting in binding constants of up to 10^6 M^{-1} for aromatic analytes. This binding model is supported by the measurements of large solvent and solvent isotope effects. The selectivity of the CB8•AG receptor can be modified or even switched toward small aliphatic analytes by a rational choice of the auxiliary guest, demonstrating the tunable recognition features of such self-assembled receptors. Furthermore, by comparison of the results to those for the extensively studied macrocyclic host cyclobis(paraquat-*p*-phenylene)—the so-called "blue-box"—it is shown that in aqueous solution the release of "high-energy" water molecules from the CB8•AG cavity can be more favorable than the use of direct host−guest interactions



INTRODUCTION

The rational design of synthetic receptors that can complex analytes with high affinity and selectivity represents a key challenge of supramolecular chemistry, in particular for systems operating in aqueous solutions.[1−3] Many high-affinity receptors are based on the use of directional, noncovalent interactions between functional groups of the host and the guest such as hydrogen bonds or Coulombic interactions.[1] Extremely high binding constants, mostly in organic solvents, were obtained with rationally designed systems,[4,5] especially when multivalent interactions were utilized.[1,2,6−13] However, in aqueous solution, a dramatic drop in host−guest affinity is frequently observed as water competes strongly for hydrogen bonds and efficiently solvates charged species.[1−3]

The challenge to achieve high-affinity binding in water is surprising if one considers that the hydrophobic effect provides an intrinsic, omnipresent driving force for the association of nonpolar residues in aqueous solution.[1,14−24] An improved design of high-affinity receptors in water must therefore exploit the unique properties of water, in particular its very high cohesiveness, caused by strong hydrogen bonds, and its low polarizability. By drawing inspiration from biological receptors, it has been recognized that this hydrophobic driving force can be increased by designing concave receptors; prominent examples are macrocyclic hosts such as cyclodextrins,[25] cyclophanes,[26] resorcinarenes,[27] calixarenes[28] and cucurbit[*n*]urils.[29] Besides the diminished entropic penalty of analyte binding to the preorganized cavities, which is independent of the solvent environment, the distinct advantage of macrocycles as aqueous receptors is that their inner cavity has unusual hydrophobic properties in the sense that the encapsulated water molecules cannot readily form hydrogen-bonded networks. Consequently, these cavity water molecules are—in comparison to that in the aqueous bulk—high in energy, such that their release upon analyte entry is more favorable than the release of water associated with flexible and in particular acyclic receptors. Note that for entropic reasons ("nature abhors a vacuum") all cavities larger than a critical size are occupied by water solvent molecules, even if breaking of hydrogen bonds is required.[30]

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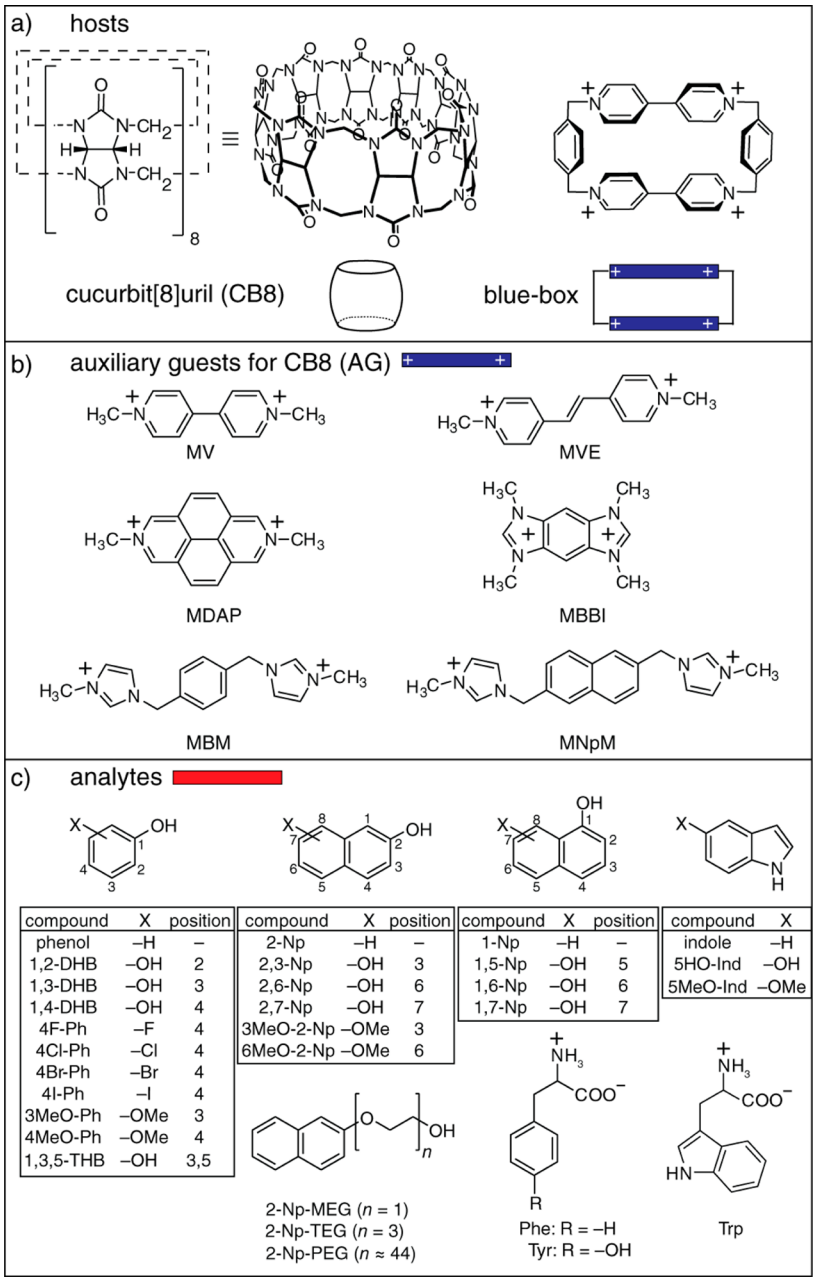

Figure 1. Chemical structures and cartoon representations of (a) the macrocyclic hosts CB8 and blue-box, (b) dicationic auxiliary guests for CB8 (halide counterions not shown) and (c) electron-rich aromatic analytes.

While such energetically frustrated water molecules have been identified as providing important driving forces in biological processes[31−33] such as protein−ligand interactions,[34−39] they have only rarely been taken into account for synthetic receptors such as for cyclophanes,[1,23,40,41] cyclodextrins[1,24,42] and cavitands.[1,25,43] Recently, we have computationally and experimentally quantified the effects of the release of such high-energy water molecules from the inner cavity of the rigid, hydrophobic, barrel-shaped cucurbit[*n*]uril (CB*n*) macrocycles (Figure 1). This analysis has provided evidence that solvent effects are the main determinant for the exceptionally high binding strength (K_a up to 10^{15} M^{-1})[44−48] of CB*n* with a wide range of guests in aqueous solutions.[49] In particular, the computational analysis predicted that the release of these cavity water molecules manifests itself enthalpically, in agreement with calorimetric observations. Additionally, a delicate balance applies because the enlargement of the cavity increases the absolute number of high-energy water molecules, but lowers the average energies of the individual water molecules, because larger cavities allow the formation of structurally optimized hydrogen-bonding networks. From this delicate balance, the cavity size of CB7 emerged as the optimal compromise between the number of inner cavity water molecules to be released and their individual energetic frustration.[49]

In this contribution, we demonstrate that receptors combining the release of high-energy water molecules with specific and tunable receptor-analyte interactions can be self-assembled from (i) a large macrocyclic host (CB8), providing a hydrophobic cavity, and (ii) a structurally modifiable, medium-sized auxiliary guest (AG) that is encapsulated in a 1:1 stoichiometry by CB8. Residual water molecules observed in

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Table 1. Calculated Properties for the Hydrated CB8•AG Complexes in Comparison to Bulk Water and the Selected Hosts, Using the tip5p Water Model[a]

system	cavity vol[b] (Å^3)	$N_{cavity}(H_2O)$[c]	PC[d] (%)	t_{res}[e] (ps)	H-bond count[f]	ΔE_{pot}(all)[g] (kJ mol^{-1})
bulk H_2O[49]			55	21	2.54	*i*
blue-box	96	3.2	57	90	1.94	−40.8
CB7[49]	214	7.9	64	529	2.01	−102.4
CB8[49]	356	13.1	63	449	2.55	−66.2
CB8•MV[h]	155	5.5	60	399	1.85	−113.1
CB8•MVE[h]	193	6.9	61	356	2.04	−112.5
CB8•MDAP[h]	179	2.9	27	504	1.01	−117.7
CB8•MBBI[h]	261	7.1	46	326	1.90	−125.4
CB8•MBM	199	5.2	44	382	1.56	−147.4
CB8•MNpM	202	4.4	37	577	1.34	−131.1

[a]See Supporting information for computational details. [b]Cavity volume calculated via Monte Carlo particle insertion method; see Supporting information for details. [c]Average number of cavity water molecules. [d]Packing coefficient (PC) = [$N(H_2O)$ × (water van der Waals volume)]/(cavity vol). [e]Residence times of water molecules determined by a single-exponential fit to an autocorrelation function. [f]Average number of hydrogen bonds between adjacent water molecules; hydrogen bonds were considered if O···O distance ≤ 3.5 Å and O−H−O angle ≥ 150° between neighboring water molecules; see Supporting Information for details. [g]Difference in potential energy for the removal of all cavity water and transfer of those to a spherical cavity in the aqueous bulk; see also Supporting Information. [h]An elliptically distorted CB8 was used, which was found to be adapted in DFT geometry optimizations of the CB8•AG complex. See Table S1 in the Supporting Information for values with a circular CB8. [i]Reference.

the CB8•AG complex are characterized by an unfavorable hydrogen bond count, and their release upon analyte binding is highly favorable. In this manner, noncharged analytes that do not bind strongly to the uncomplexed, well-solvated host CB8 can be tightly bound to the CB8•AG complex. In addition, direct noncovalent interactions between the analyte and the auxiliary guest can be exploited to define and tune the selectivity of the CB8•AG receptor for certain analyte classes.

RESULTS

Molecular Dynamics Simulations. The CB8•AG complexes and the role of high-energy cavity water were computationally investigated by molecular dynamics (MD) simulations with the GROMACS package[50] in a cubic box of >1000 explicit water molecules. Additional computational details, including the force-field parameters for CB8, are found Materials and Methods and in the Supporting Information. The MD simulations and analysis of the hydration patterns of the cavity water molecules were conducted with three different water models (tip3p, tip4pEW and tip5p) as well as with different complex geometries (circular vs elliptically distorted CB8; planar methyl viologen (MV) vs MV tilted by 45°) and in the absence and presence of chloride counterions. Additional sodium and chloride ions were also added to test the influence of salts on the computational results. All of the simulations showed similar trends, i.e., the conclusions discussed here based on these simulations are rather insensitive to the actual settings/parameters used (see Table S1 in the Supporting Information). Most importantly, all simulations revealed the existence of water molecules inside the CB8•AG complexes whose average hydrogen-bonding number is largely reduced as compared to the results in the bulk and in the CB8 cavity prior to the auxiliary guest binding event (Table 1).

Moreover, the cavity water molecules show a longer residence time and an increased relaxation time for reorientation of their dipoles, as compared to the bulk (Table 1 and Figure S1 in the Supporting Information), indicating the presence of correlated motions between water molecules, as expected from the known behavior of water molecules in the proximity of hydrophobic surfaces,[51] in the interior of reverse micelles,[52] and our previous observations for smaller cucurbiturils.[49] For comparison, the residence time in the more flexible and shallower macrocyclic host cyclobis(paraquat-*p*-phenylene)[53] (the "blue-box") is much shorter (ca. 90 ps) than in both CB8 (ca. 450 ps)[49] and the CB8•MV complex (ca. 400 ps, Table 1); note that the egression of water molecules from CBs is constricted by the carbonyl portals.[54] A representative snapshot of MD simulations with CB8•MV is shown in Figure 3, highlighting the impaired hydrogen-bond network of the cavity water molecules. The corresponding snapshot for blue-box can be found in Figure S8 in the Supporting Information.

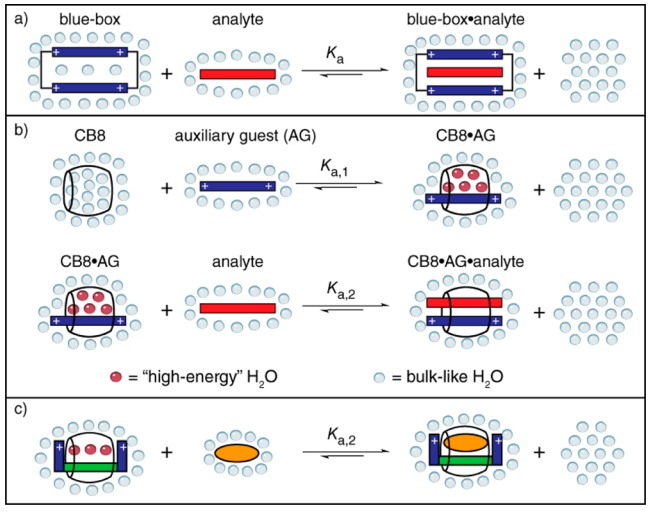

Figure 2. Schematic representation of (a) 1:1 binary complex formation with blue-box and (b) 1:1:1 ternary complex formation with CB8 in aqueous solution. (c) Changes in receptor selectivity for analytes through auxiliary guest modification.

In order to identify the energetic contribution of the high-energy water release to the host−guest binding event, one needs to consider the sum of the energetic terms of all encapsulated water molecules. Specifically, ΔE_{pot}(all) was defined as the potential energy difference upon removing all water molecules from the host's cavity in comparison to removing the same number of water molecules from bulk water

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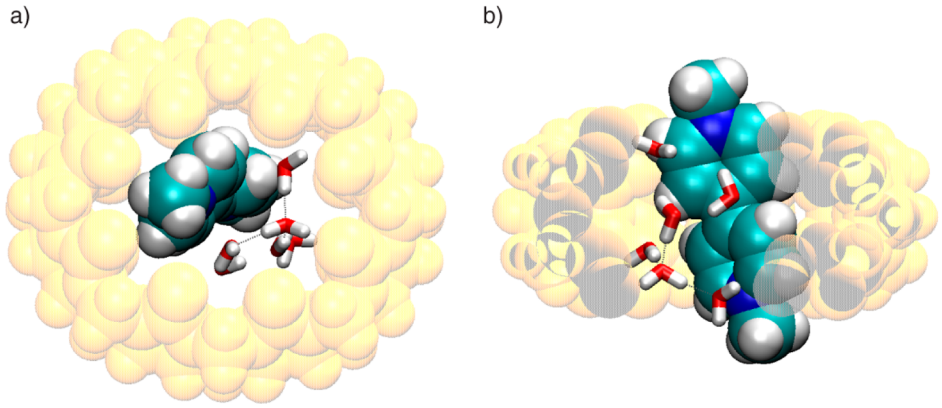

Figure 3. Representative MD snapshot of the CB8•MV complex shown in (a) top view and (b) side view. All water molecules outside the CB8 cavity and the front CB8 atoms in panel b have been removed for clarity. Hydrogen bonds between the cavity water molecules are shown as dashed lines.

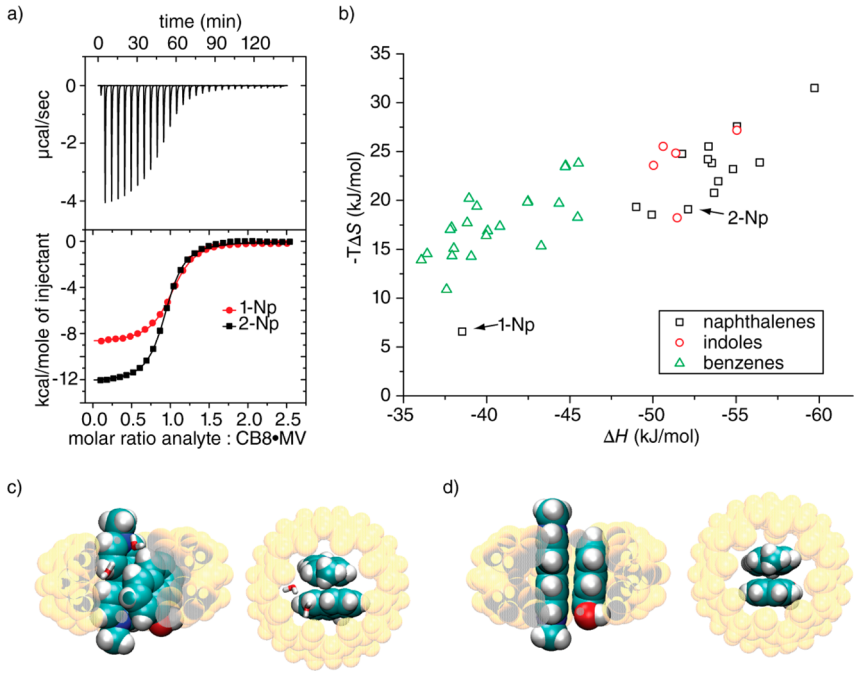

Figure 4. (a) ITC isotherm for titration of CB8•MV with 2-Np (top) and integrated heats for ternary complex formation of 1-Np (red) or 2-Np (black) with CB8•MV (bottom). (b) Plot of ΔH and $-T\Delta S$ values for the complexation of noncharged aromatic species with CB8•MV as determined by ITC experiments in buffered aqueous solution at 298 K. (c, d) Representative MD snapshots of the CB8•MV•1-Np and CB8•MV•2-Np complexes, respectively. All water molecules outside the CB8 cavity and the front CB8 atoms in the side view have been removed for clarity.

(see Figure S2 in the Supporting Information for the definition). The negative sign of the ΔE_{pot}(all) values that we found (Table 1) indicates that the transfer of the water molecules from the cavity to the bulk is energetically favorable. Strikingly, the ΔE_{pot}(all) values for the CB8•AG complexes are virtually twice as large as that for uncomplexed CB8, even though only a fraction of water molecules (3−7 compared to 13) are residing in the cavity alongside the auxiliary guest. In other words, binding of an auxiliary guest converts the relatively well-solvated CB8 cavity into a poorly solvated environment that reassembles the host cavity of CB7 in terms of high-energy water content (Table 1). For comparison, the ΔE_{pot}(all) value for the blue-box is much smaller than for the CB8•AG receptors and even smaller than for CB8 itself, which is mainly attributable to the lower number of cavity water molecules in this smaller-cavity host. In addition, the deep cavity of the CB8•AG complexes (depth = 6.2 Å) is more confined and thus shields the cavity water molecules from contact with bulk water more effectively than that of blue-box (depth = 4.3 Å). Table 1 also suggests that the CB8•MDAP complex contains a similar number of water molecules as blue-box (3), but nevertheless, its ΔE_{pot}(all) value is nearly three times that of blue-box. This observation, in turn, can be traced back to the extremely low hydrogen bond count in CB8•MDAP, which is an additional important parameter for determining the individual energy of the encapsulated water molecules.

The auxiliary guests MBM and MNpM differ from the other dicationic auxiliary guests MV, MBBI, MDAP and MVE, as they are not fully conjugated and nonplanar. In their 1:1 complex with CB8, a hydrophobic phenyl/naphthyl moiety is positioned

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Table 2. Thermodynamic Data for Ternary Complex Formation of the CB8•AG Receptor with Suitable Analytes, as Determined by ITC Experiments in Buffered Aqueous Solutions at 298 K

receptor	analyte	$K_{a,2}$ (10^3 M^{-1})	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	$-T\Delta S$ (kJ mol^{-1})
CB8•MVE	2,6-Np	2700 ± 100	−36.7 ± 0.3	−56.8 ± 1.0	20.1 ± 1.3
CB8•MV[60]	2,6-Np	590 ± 20	−32.9 ± 0.3	−53.7 ± 1.0	20.7 ± 1.3
CB8•MDAP	2,6-Np	390 ± 20	−31.9 ± 0.3	−65.4 ± 1.2	33.4 ± 1.5
CB8•MBBI[70]	2,6-Np	71 ± 9	−27.7 ± 0.3	−56.9 ± 1.0	29.2 ± 1.3
CB8•MNpM[62]	2,6-Np	1.2 ± 0.2	−17.5 ± 0.4	<0	na
CB8•MBM[62]	2,6-Np	*a*	*a*	*a*	*a*
CB8•MV	THF	*a*	*a*	*a*	*a*
CB8•MBBI	THF	*a*	*a*	*a*	*a*
CB8•MNpM[62]	THF	11 ± 4	−23.1 ± 0.7	<0	na
CB8•MBM[62]	THF	13 ± 1	−23.4 ± 0.2	<0	na
CB8•MV	acetone	*a*	*a*	*a*	*a*
CB8•MBBI	acetone	*a*	*a*	*a*	*a*
CB8•MNpM[62]	acetone	1.1 ± 0.1	−17.4 ± 0.2	<0	na
CB8•MBM[62]	acetone	3.9 ± 0.2	−20.5 ± 0.3	<0	na

[a]No binding detectable by ^{1}H NMR/ITC.

Table 3. Thermodynamic Data for CB8•MV and Blue-Box Host−Guest Complex Formation with Aromatic Analytes at 298 K

receptor	analyte	solvent	K_a (10^3 M^{-1})	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	$-T\Delta S$ (kJ mol^{-1})
CB8•MV[60]	1,5-Np	H_2O[a]	130 ± 20	−29.1 ± 0.3	−53.3 ± 1.0	24.2 ± 1.3
blue-box[88]	1,5-Np	H_2O	900 ± 90	−34.0 ± 0.3	−23.8 ± 3.0	−10.2 ± 2.0
CB8•MV	indole	H_2O	713 ± 50	−33.4 ± 0.2	−54.3 ± 1.0	20.9 ± 1.2
CB8•MV	indole	D_2O	1120 ± 50	−34.5 ± 0.2	−58.8 ± 1.0	24.3 ± 1.2
blue-box[89]	indole	H_2O	7.1 ± 0.9	−22.0 ± 0.3	na	na
CB8•MV[61]	Trp	H_2O[a,b]	43 ± 3	−26.5 ± 0.2	−44.4 ± 1.0	17.9 ± 1.2
blue-box[90]	Trp	H_2O	1.0 ± 0.2	−17.1 ± 0.4	na	na
CB8•MV[91]	Phe	H_2O[a,b]	5.3 ± 0.7	−21.3 ± 0.2	−37.2 ± 2.0[c]	15.9 ± 2.2[c]
blue-box[90]	Phe	H_2O	0.03 ± 0.02	−8.7 ± 1.2	na	na
CB8•MV[91]	Tyr	H_2O[a,b]	2.2 ± 0.1	−19.1 ± 0.2	−22.6 ± 0.6[c]	3.5 ± 0.8[c]
blue-box[90]	Tyr	H_2O	0.6 ± 0.1	−15.9 ± 0.4	na	na
CB8•MV[60]	1,2-DHB	H_2O[a]	10 ± 3	−22.9 ± 0.6	−38.0 ± 1.5	15.1 ± 2.1
blue-box[89]	1,2-DHB	H_2O	3.9 ± 0.3	−20.5 ± 0.3	na	na
CB8•MVrotax[84]	2,6-Np	H_2O[a]	130 ± 10	−29.2 ± 0.3	na	na
CB8•MVrotax[84]	2,6-Np	CH_3CN	0.09 ± 0.02	−11.2 ± 0.3	na	na
blue-box[86]	1,5-Np-DEG[d]	H_2O	1400 ± 140	−34.3 ± 1.0	−44.0 ± 5.0	9.7 ± 5.0
blue-box[86]	1,5-Np-TEG[e]	CH_3CN	43.5 ± 2.7	−26.5 ± 0.2	−57.0 ± 0.5	30.5 ± 0.5

[a]In 10 mM sodium phosphate buffer (pH 7); essentially identical values were obtained in pure water as tested for 2,6-Np as the guest. [b]Values determined at 300 K. [c]We thank Prof. Adam Urbach for providing us the raw data of ref 91. [d]1,5-Bis[(2-hydroxyethoxy)ethoxy]naphthalene. [e]1,5-Bis[((2-hydroxyethoxy)ethoxy)ethoxy]naphthalene.

inside the CB8 cavity while the portals are capped with the positively charged imidazolium units, as is schematically depicted in Figure 2c. Moreover, the positive charges of MBM and MNpM are rather localized at the imidazolium units of the auxiliary guests (Figures S3 and S4 in the Supporting Information) and are efficiently stabilized by the CB8 carbonyl-fringed portals. A representative snapshot from the MD simulations for the hydrated CB8•MBM complex can be found in Figure S3 in the Supporting Information.

A strong enthalpic driving force for binding should result when the high-energy water molecules are released from the CB8•AG cavity to the bulk by an incoming analyte, as those water molecules can then participate in an energetically optimized hydrogen bond network. In addition, the analyte should also be electron-donating in order to stabilize the positive charges on the neighboring dicationic AG moiety in the CB8•AG•analyte ternary complex with fully conjugated, dicationic auxiliary guests (MV, MBBI, MDAP and MVE).[55−57] This additional condition for the analyte structure leads to the strong preference of the CB8•MV receptor for electron-rich aromatic species whereas for the 1:1 CB7−guest complexes the main selectivity criterion for noncharged analytes is their size, i.e., their ability to replace all cavity water molecules.[49,58] From this model, it also becomes evident that if the size of the analyte is too small to displace all of the cavity water molecules from the CB8•MV cavity, a lower binding affinity is expected, in particular, since any residual cavity water molecules would have even more unfavorable potential energies.

To test this hypothesis, several 1:1:1 ternary CB8•MV•analyte complexes with noncharged aromatic species were investigated by MD simulations. For most of the indole/naphthalene type analytes, all cavity water molecules were replaced from the CB8•MV cavity in the simulated ternary complex structures. For the smaller phenol species and for 1-naphthol (1-Np), the CB8•MV•analyte complexes typically contained one or two water molecules. Not surprisingly, these residual water molecules do not participate in stable hydrogen-bond networks and are high in energy (Table S2 in the

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Supporting Information). Thus, the formation of ternary complexes with suboptimally sized analytes is expected to be accompanied by a reduced enthalpic driving force. Indeed, as described below, this is experimentally observed. Finally, restructuring of the solvent upon complex formation must also be accounted for.[59,60] Generally, better solvated guests have to pay a larger desolvation penalty, which reduces their overall binding affinity.[60] A detailed analysis of analyte desolvation on the binding affinity in aqueous solution is presented in a separate section in the Supporting Information.

Isothermal Titration Calorimetry (ITC). To experimentally evaluate the importance of high-energy water displacement as a driving force in the binding of analytes to the preformed CB8•AG complexes, ITC measurements were carried out (see Figure 4a and Figure S5 in the Supporting Information for representative examples). The binding constants $K_{a,1}$ of the selected auxiliary guests with CB8 are very high (6×10^5 M^{-1} to 4×10^6 M^{-1}),[56,61,62] see also Table S3 in the Supporting Information. Therefore, the experimental conditions can be adjusted such that the $K_{a,2}$ values for the CB8•AG•analyte complex formation can be directly determined. Table 2 and Table 3 show the thermodynamic data for the binding of a small selection of different analytes to preformed 1:1 CB8•AG complexes. The thermodynamic data sets for additional auxiliary guests and analytes are contained in the Supporting Information. In agreement with expectations based on the high-energy water release, strongly exothermic complex formation was observed with both aromatic and aliphatic analytes and various auxiliary guests (Table 2, Table 3 and Table S4 in the Supporting Information). The entropic contributions to ternary complex formation are generally unfavorable, which can be rationalized by the largely reduced degrees of freedom of the auxiliary guest and analyte in the tightly packed ternary complex. It is thus experimentally clear that the classical hydrophobic effect, which would have implied $-T\Delta S < 0$,[63] is not the driving force for binding.

The stepwise CB8•AG•analyte ternary complex formation was investigated in H_2O and D_2O as the involvement of cavity water in host–guest binding should manifest itself in a significant solvent isotope effect. It is well-known that D_2O forms stronger hydrogen bonds than H_2O, attributable to the lower zero-point vibrational energy of O–D versus O–H bonds.[64−66] Associative binding events driven by the classic hydrophobic effect are characterized by a reduction in enthalpic binding force in D_2O as compared to H_2O, i.e., $\Delta\Delta H_{D/H} = \Delta H(D_2O) - \Delta H(H_2O) > 0$.[66] Conversely, the effects on the $K(D_2O)/K(H_2O)$ values are usually much smaller because of enthalpy–entropy compensation.[66] For instance, $K(D_2O)/K(H_2O)$ and $\Delta\Delta H_{D/H}$ values reported for various 1:1 host–guest complexes with cyclodextrin are rather small, $K(D_2O)/K(H_2O) \leq 1.25$, with a maximum $\Delta\Delta H_{D/H}$ value of 1 kJ mol^{-1}.[67] On the other hand, a value of $\Delta\Delta H_{D/H} = 5.2$ kJ mol^{-1} was reported for the 1:2 complex formation of camphor with α-cyclodextrin, where the solvent was postulated to have a structural role in the host–guest complex.[68] Strikingly, the binding affinity of MV for CB8 was observed to weaken by a factor of 2 when changing the solvent from H_2O to D_2O, i.e. $K(D_2O)/K(H_2O) = 0.50$, which is primarily on account of a reduction in the enthalpic driving force for binding in D_2O ($\Delta\Delta H_{D/H} = (6.7 \pm 2.0)$ kJ mol^{-1}). This large solvent isotope effects can be attributed to a net loss of hydrogen bonds upon 1:1 CB8•MV complex formation. Conversely, the inclusion of indole into the CB8•MV complex is by a factor of $K(D_2O)/K(H_2O) = 1.5$ stronger and by $\Delta\Delta H_{D/H} = (-4.5 \pm 2.0)$ kJ mol^{-1} more exothermic in D_2O than in H_2O (Table 3 and Figure S5 in the Supporting Information). Similar trends were observed with 2-Np ($K(D_2O)/K(H_2O) = 1.7$, $\Delta\Delta H_{D/H} = (-2.3 \pm 2.0)$ kJ mol^{-1}) and 2,7-Np ($K(D_2O)/K(H_2O) = 1.6$, $\Delta\Delta H_{D/H} = (-3.0 \pm 2.0)$ kJ mol^{-1}) as the analytes. All these findings indicate that a substantial net increase in the number of hydrogen bonds occurs upon ternary complex formation, which supports the high-energy water release model.[69]

Based on the MD simulation results (Table S2 in the Supporting Information), the indole and naphthalene-type guests were expected to display a much more favorable complex formation enthalpy with CB8•MV than any of the phenol species on account of incomplete water release from the ternary complexes of the latter. Indeed, the experimentally determined ΔH values are in good agreement with this expectation.

As is depicted in Figure 4b, there is a clear separation between the ΔH values for analytes that can displace all the cavity water molecules, that is, indole and naphthalene type guests, and those that cannot, benzene-type guests. Notably, the ternary complex formation of 1-Np with CB8•MV shows a much less favorable ΔH value of −38.5 kJ mol^{-1} as compared to $\Delta H < -48$ kJ mol^{-1} for all other naphthol species, e.g. $\Delta H = -52.1$ kJ mol^{-1} for 2-Np. This finding cannot be explained by electron-donation or charge transfer arguments since 1-Np is even more electron-rich than 2-Np.[49] Interestingly, 1-Np is the only naphthol-species for which MD simulations resulted in an incomplete cavity water release upon ternary complex formation (Table S2 in the Supporting Information), which explains the uniquely different ΔH value of 1-Np. Representative snapshots from MD simulations for the CB8•MV•1-Np ternary complex as compared to CB8•MV•2-Np are shown in Figures 4c and 4d, respectively, displaying the presence (CB8•MV•1-Np) and absence (CB8•MV•2-Np) of residual cavity water molecules.

Similar ΔH and $-T\Delta S$ values for the ternary complex formation were observed when the auxiliary guest MV was replaced by the dicationic, fully conjugated molecules MVE, MDAP and MBBI, as can been seen from a comparison of the thermodynamic values for 2,6-Np as a representative aromatic analyte (Table 2). An enthalpy–entropy correlation plot for the binding of a wide range of aromatic analytes, including tryptophan-containing peptides and naphthyl/anthryl/fluorenyl or pyrenyl labeled PEG-polymers, to CB8•AG complexes is depicted in Figure S6 in the Supporting Information (see also Table S4 in the Supporting Information for numerical values). The small but systematic differences seen between the auxiliary guests can be rationalized by their structural properties. For instance, the viologen analogue MVE is preorganized in a planar conformation as opposed to the twisted ground state of MV. Thus, MVE generally displays a more exothermic driving force for analyte binding in comparison to MV, which needs to pay a planarization penalty upon analyte binding. Nevertheless, the similarity of the enthalpic and entropic characteristics for CB8•AG•analyte ternary complex formation further supports that the release of high-energy water is a unifying theme among these systems.

The 1:1 complexes of CB8•MBM and CB8•MNpM show a preference for small aliphatic analytes such as tetrahydrofuran, acetone and diethyl ether (Table 2).[62] These aliphatic molecules do not significantly bind to the 1:1 CB8 complexes with the dicationic, fully conjugated auxiliary guests MV, MVE, MDAP and MBBI. The inability of these aliphatic molecules to

function as analytes for CB8•MV and CB8•MBBI is in agreement with the aforementioned necessity of the analyte to provide some charge solvation for fully conjugated auxiliary guests. Conversely, the charges on MBM and MNpM are localized and can be efficiently stabilized by the CB8 portals, thus electron donation of the analyte is not required in their ternary complexes. In addition, through rational choice of the geometry of the auxiliary guest, size-selective binding can be achieved. For instance, the large analyte 2,6-Np is only a weak guest for CB8•MNpM and not complexed at all by CB8•MBM since the imidazolium units of MBM and MNpM act as lids for the CB8 barrel, restricting the available space for analyte binding (Table 2). On the other hand, the smaller analyte phenol is strongly complexed by both CB8•MBM and CB8•MNpM.[62]

■ DISCUSSION

Some macrocycles such as γ-cyclodextrin and cucurbit[8]uril are sufficiently large to encapsulate two organic guest molecules, which can result in the formation of a 1:1:1 heteroternary complex. We reasoned that cavity confinement and partial removal of water molecules upon auxiliary guest binding would increase the free energy of the residual cavity water molecules, whose release would thus provide a large favorable contribution to the binding affinity of the analyte (Figure 2b). CB8 was therefore selected as the large macrocyclic host, as it is more rigid than γ-CD and since it ensures, through ion−dipole interactions with the carbonyl portals, the tight binding of symmetric dicationic auxiliary guests such as those depicted in Figure 1b.[29,47]

If a sufficiently small auxiliary guest is complexed by a rigid macrocyclic host, then additional water molecules have to reside in the cavity in order to fill the remaining space.[30] For confined, hydrophobic cavities, such water molecules will be high in energy as their hydrogen-bonded network is disrupted.[71] Consequently, a subsequent binding of an analyte can be driven by the displacement of the cavity water molecules (see Figure 2b). According to this design principle, high-energy water release ensures a large driving force and, thus, a high affinity, while the shape of the auxiliary guest in combination with additional noncovalent interactions between the auxiliary guest and the analyte can be used to tune the receptor's selectivity.

In fact, CB8 has become a widely studied host to induce a face-to-face arrangement of pairs of one electron-poor, dicationic aromatic guest such as methyl viologen (MV) and another electron-rich, neutral aromatic guest such as naphthol (Figure 2) with high binding constants ($K_{a,ternary}$ up to 10^{13} M^{-2}) in aqueous solutions.[29,55,57,60,61] However, the driving force for binding remained puzzling, in particular as charge transfer (CT) interactions are likely not of *energetic* importance in this system.[57] CB8 mediated ternary complexes have found many applications ranging from reversible bioconjugation[72−75] and hydrogelation[76] to the formation of complex supramolecular architectures,[77−81] all of which rely on the high binding affinities of CB8 ternary complexes in aqueous solutions. A deeper understanding of the binding forces in CB8 ternary complexes is therefore not only of fundamental interest but also of practical relevance.

In order to evaluate the energetic importance of the release of cavity water molecules in comparison to direct host−guest interactions, the binding strengths of the self-assembled CB8•MV receptor and Stoddard's macrocyclic host cyclobis-(paraquat-*p*-phenylene) ("blue-box",[53] Figure 1a) with aromatic analytes were compared. Both for blue-box and for CB8•MV, there are confined inner cavities that are characterized by sizable positive electrostatic potentials (Figure S7 in the Supporting Information), which originate from the presence of one (CB8•MV) or two (blue-box) dicationic 4,4-bipyridinium units. The larger positive charge built up for blue-box is intuitively expected to result in stronger electrostatic attraction of the host to electron-rich analytes. In addition, polarization effects and dispersion interactions between the host and the guest are likely stronger in blue-box than in CB8•MV.[58,82,83] In contrast, the release of cavity water from the host cavity, which is associated with favorable enthalpic effects, is much more important for the deep-cavity receptor CB8•MV than for the shallower macrocycle blue-box, see Results and Table 1. In aqueous solutions, it was found that CB8•MV shows higher binding affinity than blue-box for most aromatic analytes (Table 3), demonstrating that the release of high-energy water molecules from the host cavity can be energetically more powerful than direct host−guest interactions. It is also worth mentioning that the enthalpic driving force for ternary complex formation with CB8•MV is significantly larger than that of blue-box with 1,5-Np box (Table 3), despite the stronger electrostatic and dispersive contributions to the host−guest binding energies in the blue-box system. Moreover, while the affinity of blue-box is evidently correlated to the electron-donating ability of the analyte (1,5-Np ≫ indole; Tyr > Phe, and dialkyl-1,5-Np > 1,5-Np), the trend in $K_{a,2}$ is reversed for the CB8•MV•analyte complexes (Table 3 and Table S4 in the Supporting Information). The interplay of two solvation effects, the desolvation of the analyte[60] (see also Supporting Information) and the release of high-energy water from the CB8•MV cavity can readily account for the observations. The largest enthalpic driving force for binding and thus the highest $K_{a,2}$ values are observed for analytes that are sufficiently large to displace all the water molecules from the CB8•MV cavity *and* that have a low desolvation cost.

If the release of cavity water molecules, as opposed to direct receptor−analyte interactions, is a major component of the driving force for binding, then large solvent effects on the binding affinities are expected. For a CB8•viologen-rotaxane receptor, binding of 2,6-Np was largely reduced upon changing the solvent from water to acetonitrile (decrease in K_a by a factor of 1400, Table 3).[84] The dipolar but aprotic acetonitrile molecules can efficiently solvate the CB8•viologen-rotaxane receptor such that the complexation of 2,6-Np is less favorable in acetonitrile than in aqueous solutions. Interestingly, the group of Diederich reported that their hydrophobic cyclophane-host pyrene-guest system displayed an approximately 1000-fold decrease of binding strength when the strongly cohesive solvent water was changed to solvents with a similar Hildebrand−Scott δ value as acetonitrile. By reference to empirical solvent scales, those effects were attributed to differences in the solvation structure of the host's cavity,[23,85] in close analogy to our binding model for CB8 ternary complexes. In contrast, the binding strength of blue-box with an alkylated 1,5-Np species and indole is only 30 times and 100 times, respectively, smaller in acetonitrile than in water (Table 3),[86,87] demonstrating that cavity (de)solvation effects are energetically less pronounced for this smaller and more flexible host.

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Most CB8 ternary reported consist of an electron-poor auxiliary guest and an electron-rich analyte. Therefore, it was commonly assumed that CT is an important driving force for binding. However, not only does our recent experimental evidence suggest otherwise,[57] there are also high-affinity 2:1 homoternary complexes with CB8 for which CT can be excluded.[92−94] Interestingly, stepwise inclusion of Trp-$(Gly)_2$ tripeptides by CB8 showed a more favorable binding enthalpy for the second binding step (ΔH_1 = −43.9 kJ/mol, ΔH_2 = −51.5 kJ/mol), in spite of charge repulsion between the positively charged ammonium groups of the *N*-terminal Trp-units in the 2:1 complex with CB8.[92] This difference in enthalpy is certainly too large to only originate from π−π-stacking effects.[14,15] Besides, the higher affinity of Phe-$(Gly)_2$ ($K_{a,ternary}$ = 2 × 10^{11} M^{-2}) in comparison to that of Trp-$(Gly)_2$ ($K_{a,ternary}$ = 3.6 × 10^9 M^{-2}) for CB8 also indicates that π−π-stacking interactions are not the main driving force for binding.[92] However, release of high-energy water from the 1:1 CB8•Trp-$(Gly)_2$ complex upon binding of the second peptide equivalent can rationalize the peculiar trends in ΔH_1 and ΔH_2.

It is plausible that CB8 ternary complexes with purely aliphatic components exist. From ^{1}H NMR experiments displayed in Figure S9 in the Supporting Information, it was confirmed that the fully protonated 1,5-diaminobutane (cadaverine) forms a binary complex with CB8, in analogy to the literature findings for CB6 and CB7,[29,47] increasing the aqueous solubility of CB8 by at least a factor of 5. Saturating the aqueous solution of CB8•cadaverine with *n*-butane resulted in a broadening and upfield shift of the *n*-butane protons, which is indicative for binding (Figure S10 in the Supporting Information). Please note that the poor solubility of CB8 in the absence of an auxiliary guest prevented significant complexation of *n*-butane (Figure S10b in the Supporting Information). Literature reports for the binary complex formation of quarternary ammonium salts with CB8 also support the high-energy water concept.[95] In fact, the shorter $Me_3N^+C_6H_{13}$ guest displayed only a small binding enthalpy (−15 kJ mol^{-1}) whereas the ΔH value for the $Me_3N^+C_{12}H_{25}$ guest was more than double (−39 kJ mol^{-1}).[95] NMR experiments secured a backfolded, tightly packed structure of the C_{12}-guest while the C_6-guest is too small to fill the CB8 cavity,[95] such that residual high-energy water molecules are likely present.

The release of high-energy water molecules represents a type of hydrophobic effect that can be considered as a general motif for the design of high-affinity receptors in aqueous solutions. It has to be mentioned, however, that the selectivity of such receptors for structurally different analytes will be rather low, in contrast to systems employing directional noncovalent interactions. For example, the host CB7 binds strongly to both aromatic and aliphatic analytes, where the selectivity is largely determined by the size of the analyte.[49,58] Thus, it is desirable to combine the release of cavity water—a guarantor for high complex affinities—with the use of more specific noncovalent interactions to control the receptor's selectivity for an analyte. The decoration of hydrophobic, rigid and concave macrocycles with specific noncovalent recognition units is the conventional way to reach this goal. The use of self-assembled receptors consisting of a large, common macrocycle and structurally variable auxiliary guests provides an attractive alternative, which also serves to bypass synthetic challenges, e.g. in the synthesis of cucurbituril derivatives.[46,96−98]

The selectivity of self-assembled CB8•AG receptors discussed here can indeed be readily tuned with rationally designed auxiliary guests. For instance, the dicationic, planar, and fully conjugated auxiliary guests such MVE and MBBI show a strong preference for aromatic analytes while the auxiliary guests MBM and MNpM that possess positively charged imidazolium panels acting as "lids" are characterized by a size-selective binding, where both aromatic and aliphatic analytes are strongly complexed. Furthermore, utilization of spectroscopic reporter molecules, such as the fluorescent MBBI[70] and MDAP,[56] as auxiliary guests in combination with the optoelectronically inactive CB8 provides the opportunity to follow the binding event of analytes by monitoring the absorption or emission spectra of the auxiliary guest in real time and in low concentrations, which opens up opportunities for real-life applications of self-assembled CB8•AG complexes as chemical sensors.

■ CONCLUSIONS

We have presented a strategy for the design of high-affinity and analyte-selective receptors operating in aqueous solutions. This procedure is based on the observation that large, well-solvated host cavities, such as that of CB8, can be rendered hydrophobic by restricting the cavity space through binding of an auxiliary guest (AG). Residual cavity water molecules cannot form stable hydrogen-bonded networks in the confined cavity of the 1:1 CB8•AG complex, i.e. they are high in energy. Subsequent binding of an analyte to these self-assembled receptors liberates the cavity water molecules to the aqueous bulk and restores their full hydrogen-bonding potential, providing a favorable enthalpic contribution for host−guest binding (K_a up to 10^6 M^{-1}, ΔH up to −70 kJ mol^{-1}). Consequently, even analytes that show only weak noncovalent interactions with the receptor are tightly complexed, in stark contrast to findings with macrocyclic host blue-box. Furthermore, facile structural modification of the auxiliary guests provides a convenient handle for tuning the receptor's analyte-selectivity without the need for changing the macrocyclic host. The binding model, which was developed though the use of molecular dynamics simulations, is experimentally supported by a wide range of calorimetric experiments and can also rationalize the uniquely large solvent isotope and solvent effects observed.

■ MATERIALS AND METHODS

Simulations Setup. Ab initio/DFT calculations were performed using the Spartan08 software package from Wave function. MD simulations were carried out with the Gromacs package[50] by using the all-atom amber99sb forcefield.[99,100] Modification of the CB8 force field parameters was needed to account for the low polarizability of the CB8 cavity, as we have described previously.[49] The explicit water models tip3p, tip4pEW and tip5p were employed.[101] The simulations were carried out in NPT ensemble with periodic boundary conditions at a constant temperature of 300 K. An initially cubic box (3.5 nm edge-length) was used for accommodating the CB*n*, guest molecules and water molecules. A time step of 1.0 ps was employed. All simulations were carried out for a time of 20 ns after the system was equilibrated for 10 ns. As the hydration of small molecules equilibrates in the time scale of picoseconds, the simulated time was enough to provide a robust statistics for the CB8•AG solvation. The V-rescale algorithm was applied for the temperature, an isotropic Berendsen-coupling for the pressure. The bonds were constrained by the Lincs algorithm. The particle-mesh Ewald (PME) method was used to account for the electrostatic contribution to nonbonded interactions (grid spacing of 0.12 nm). The equilibrated box-size and number of explicit water molecules used can be found in Table S5 in the Supporting

Information for each system studied. The calculation of ΔE_{pot}(all) values is explained schematically in Figure S2 in the Supporting Information.

Isothermal Titration Calorimetry. All starting materials were purchased from Alfa Aesar and Sigma Aldrich and used as received unless stated otherwise. MV and the small molecule analytes were purchased and used as received. CB8,[102] MDAP,[103] MBBI,[70] MNpM,[62] and MBM[62] and aryl-functional-PEG[104] polymers were synthesized according to literature methods. Isothermal titration experiments were carried out on a VIP-ITC from Microcal, Inc., at 25 °C. The ternary complex formation binding equilibria were studied using a cellular CB8•AG concentration of typically of 0.1 mM, to which the 10× higher concentrated analyte solution was titrated. The titrations were carried out in 10 mM sodium phosphate buffer (pH 7); essentially identical $K_{a,2}$ values were obtained in neat deionized water for noncharged analytes. Typically 20−30 consecutive injections of 10 μL each were used. All solutions were degassed prior to titration. Heats of dilution were determined by titration of the guest/analyte solution into water. The first data point was removed from the data set prior to curve fitting. The data was analyzed with Origin 7.0 software with the one-set-of-sites model. The knowledge of the complex stability constant (K_a) and molar reaction enthalpy ($\Delta H°$) enabled the calculation of the standard free energy ($\Delta G°$) and entropy changes ($\Delta S°$) according to $\Delta G° = -RT \ln K_a = \Delta H° - T\Delta S°$. For each system, 1−2 repetition experiments were conducted in order to estimate the error in the thermodynamic values.

■ ASSOCIATED CONTENT

Supporting Information

Detailed procedures for the force field parametrization of the CB[n] and guest molecules and for the analysis of the MD trajectories. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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