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Hydrophobic Effect

The Hydrophobic Effect Revisited—Studies with Supramolecular Complexes Imply High-Energy Water as a Noncovalent Driving Force

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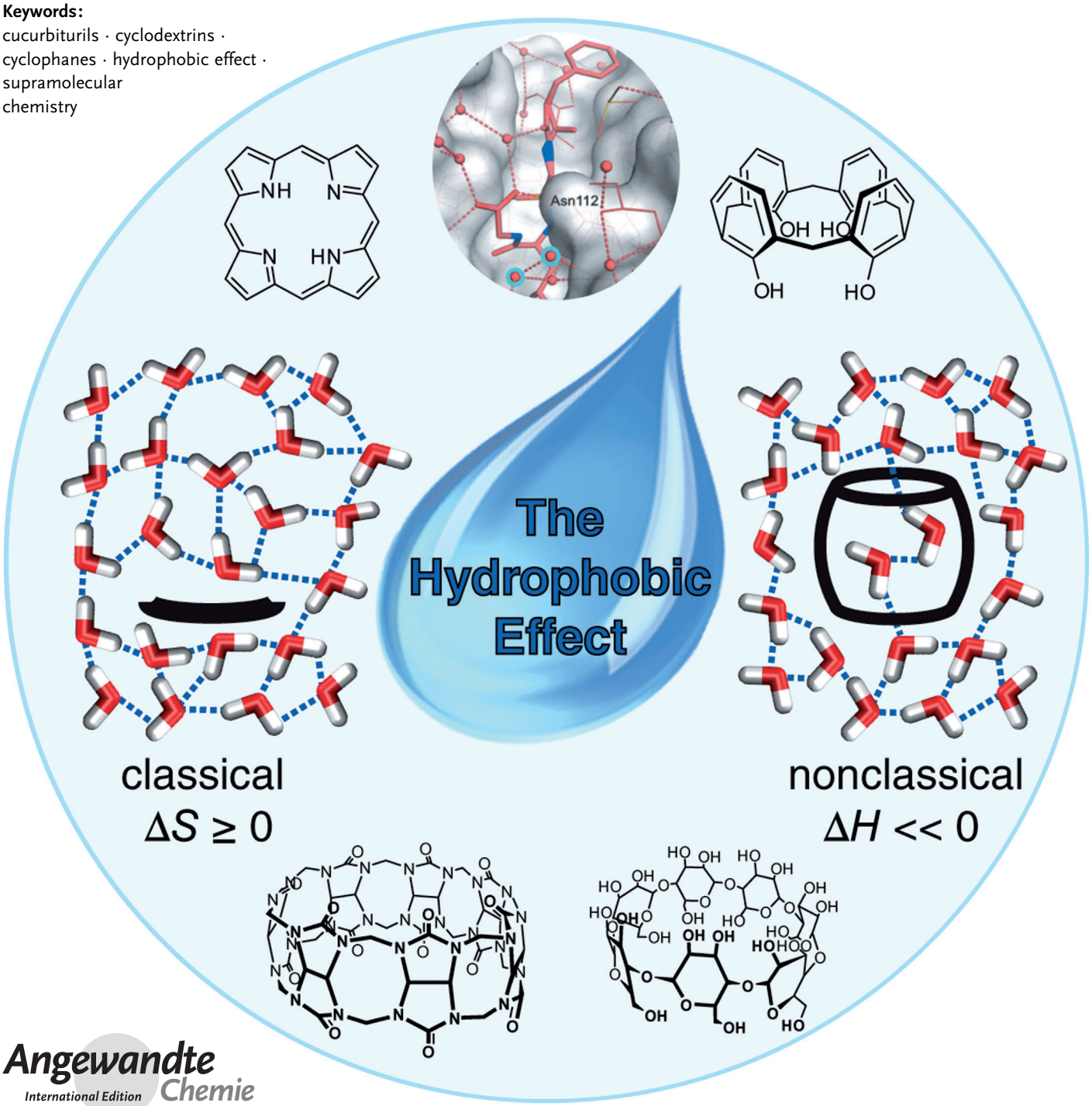
Keywords:
cucurbiturils · cyclodextrins · cyclophanes · hydrophobic effect · supramolecular chemistry



Traditional descriptions of the hydrophobic effect on the basis of entropic arguments or the calculation of solvent-occupied surfaces must be questioned in view of new results obtained with supramolecular complexes. In these studies, it was possible to separate hydrophobic from dispersive interactions, which are strongest in aqueous systems. Even very hydrophobic alkanes associate significantly only in cavities containing water molecules with an insufficient number of possible hydrogen bonds. The replacement of high-energy water in cavities by guest molecules is the essential enthalpic driving force for complexation, as borne out by data for complexes of cyclodextrins, cyclophanes, and cucurbiturils, for which complexation enthalpies of up to $-100\ kJ\,mol^{-1}$ were reached for encapsulated alkyl residues. Water-box simulations were used to characterize the different contributions from high-energy water and enabled the calculation of the association free enthalpies for selected cucurbituril complexes to within a 10% deviation from experimental values. Cavities in artificial receptors are more apt to show the enthalpic effect of high-energy water than those in proteins or nucleic acids, because they bear fewer or no functional groups in the inner cavity to stabilize interior water molecules.

From the Contents

1. Introduction

Of all factors driving intermolecular interactions, the hydrophobic effect is the most often cited.[1] At the same time, it is the least understood. The traditional explanation of hydrophobic forces rests on the formation of a large cavity around two lipophilic particles, for which a smaller number of solvating water molecules is required than for complexation in two smaller, separate cavities; in the Frank–Evans description, the liberation of solvating water molecules in this way can lead to an entropic advantage.[2] In a complementary model, the liberated water enables more cohesive water–water interactions, which would lead to an enthalpy gain.[3] Studies with cyclodextrin and cyclophane complexes (Scheme 1; see also Sections 5.1 and 5.2) have shown that, contrary to conventional interpretations (based on the entropy gain of liberated water), enthalpic contributions can indeed become dominant,[3a,4] and that hydrophobic solvent parameters are best suited to describe the influence of the medium on the complex stability.[5] The enthalpic, so-called "nonclassical" hydrophobic effect[6] correlates with the surface tension, γ, of water, which reflects the strength of hydrogen bonds between water molecules, as described by Equation (1a) (derived by Sinanoglu), in which ΔA is the

$$\Delta G_{hp} = \text{const.}\,\gamma\,\Delta A \qquad \Delta G_{hp} = \text{const.}\,\Delta A \qquad (1a, b)$$

difference in the solvent-accessible surface area of the interacting particles before and after association.[7] In particular for protein complexes, the hydrophobic contributions are usually quantified by a similar equation [Eq. (1b)] by the use of empirically derived constants instead of γ; however, these constants vary strongly, from 200 to $500\ J\,(mol\,Å^{-2})^{-1}$.[1d]

Investigations with supramolecular complexes show that established concepts for these fundamentally important interactions require reconsideration. Early on, it was suggested that the expulsion of water from cyclodextrin cavities by guest molecules is an important factor for complexation, as the expelled water can form more hydrogen bonds in the bulk medium.[8] New studies on the complexation between cucurbiturils and electroneutral ligands have furnished compelling evidence for the importance of such "high-energy water". The exceedingly high binding constants of up to $K_a = 7 \times 10^{17}\ M^{-1}$,[9] which even exceed the strongest binding constants known for biological complexes, correlate with the number and energetic frustration of high-energy water molecules displaced from the receptor cavity upon ligand binding,[3c] as established by calorimetric measurements and molecular-dynamics (MD) simulations. The thermodynamic fingerprint for the formation of cucurbituril complexes is consistent with the nonclassical hydrophobic effect (see Section 5.5).

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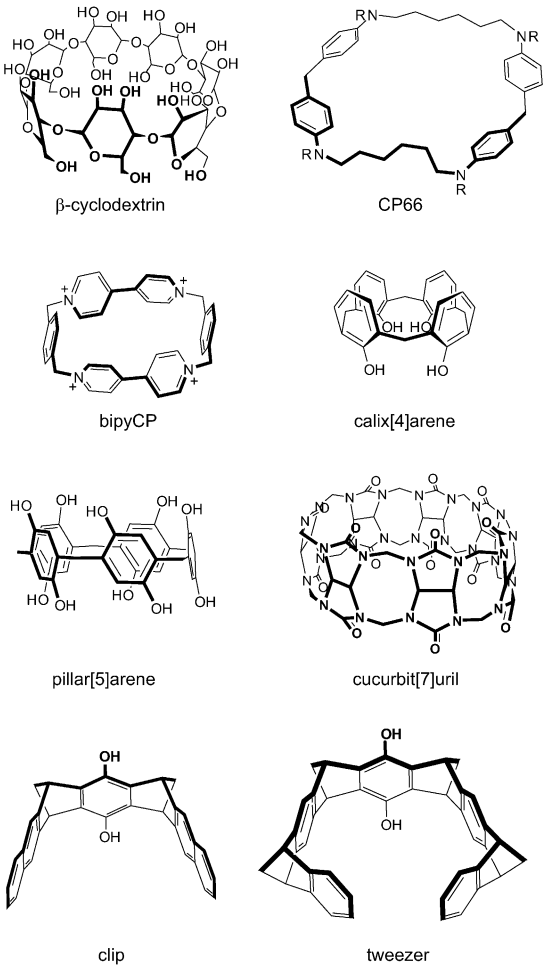

Scheme 1. Structures of host scaffolds discussed in this Review.

2. Theoretical Considerations

Computational methods have underlined the importance of enthalpy gain due to the liberation of water from cavities.[3b,10] In this context, the number of hydrogen bonds that water molecules form in bulk water is a key parameter. Most recent ab initio molecular-dynamics calculations on the energetics and symmetry of local interactions, and comparison with X-ray diffraction data, reconcile earlier controversies[11] and indicate that bulk water consists of intertwined rings of tetracoordinated water;[12] each water molecule is involved (on average) simultaneously in two donor and two acceptor bonds in a virtually symmetrical tetrahedral conformation.

Generally, favorable free-energy changes characterize the transfer of water from the bulk to a confined volume or surface. Both favorable and unfavorable entropic changes have been predicted to accompany water binding to hydrophobic cavities or protein crystals.[13] Recent solvent MD simulations and potential of mean force (PMF) calculations indicate that hydrophobic enthalpy-driven binding is mainly favored by cavity dehydration, with sizable, favorable energetic changes of up to $\Delta G_W = -10.5\ kJ\,mol^{-1}$ resulting from the formation of new hydrogen bonds upon the expulsion of water molecules from a hydrophobic cavity.[3b,10] Water entropy seems to be more favorable for water inside the cavity than for highly correlated water–water interactions in the bulk; however, exceptions exist, such as the streptavidin binding pocket.[13c] Compensation by a favorable enthalpy of bulk water–water interactions upon cavity-water displacement by the ligand then leads to a large gain in free energy.

3. Overview of Hydrophobic Contributions and General Design Principles for High-Affinity Receptors and Hosts

For the maximization of host–guest affinity through the hydrophobic effect, the optimal release of high-energy water is a critical design criterion. Three considerations have to be taken into account: First, some host geometries impede hydrogen-bond formation of the cavity water molecules, whereas others do not. Most notably, concave, deep macrocyclic hosts, such as molecular barrels, cups, and some molecular tweezers, can efficiently screen the cavity water from contact with the bulk (Figure 1a). Cyclodextrins, some cyclophanes, cucurbit[*n*]urils, and cryptophanes belong to this class. On the other hand, flat structures or those with a wide opening, such as porphyrins or simple calixarenes, allow the surface/cavity water to form hydrogen bonds with adjacent bulk-water molecules, thereby lowering hydrophobic contributions to binding. Second, within the preferred class of concave hosts, there is a critical range for the cavity diameter/

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Werner M. Nau obtained his MSc in 1991 from St. Francis Xavier University (Canada) and his PhD in 1994 from the University of Würzburg with Waldemar Adam. Following postdoctoral studies at the University of Ottawa with J. C. "Tito" Scaiano, he completed his habilitation with J. Wirz at the University of Basel, where he became Assistant Professor in 2000. Since 2002, he has been Professor of Chemistry at Jacobs University Bremen. His research ranges from fundamental studies on intermolecular interactions to the development of (bio)analytical methods based on macrocyclic recognition.

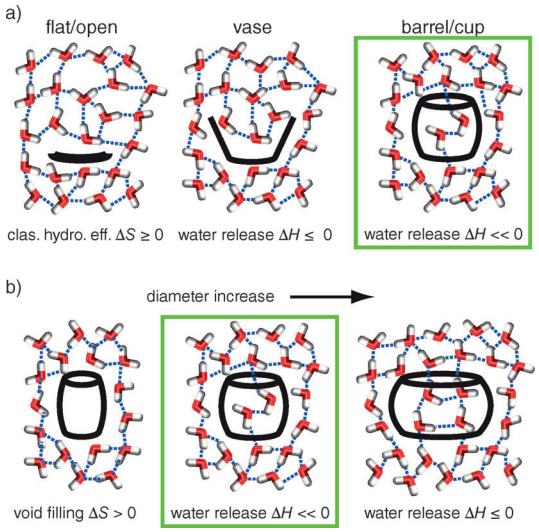


Figure 1. Different host geometries and associated effects on the dehydration thermodynamics accompanying host–guest binding as interpreted in terms of the classical ($\Delta S > 0$) or nonclassical ($\Delta H < 0$) hydrophobic effect. a) Concave hosts shield water molecules better from hydrogen bonding with bulk solvent than open/flat structures. b) An optimal cavity diameter exists for which the host cavity is not empty but contains an intermediary number of energetically highly frustrated water molecules. Hydrogen bonds of water molecules near/inside the host are indicated by dashed lines.

volume in which the maximal hydrophobic gain can be expected (Figure 1 b): Hosts with a very small cavity diameter are not filled by water molecules, as the entropic gain of filling a small void would not compensate for the required breaking of intersolvent hydrogen bonds. The cavity of such small-diameter hosts remains unfilled or "dry",[14] as was also observed computationally for small protein pockets.[13b] Binding of a very small hydrophobic guest that fills the "vacuum" is in this case expected to be entropically favorable (Figure 1 b, left).

When the host diameter is increased, some water molecules need to reside in the cavity to avoid creating a vacuum. Such cavity water molecules cannot form a stable hydrogen-bonded network with their few neighbors in a medium-sized hydrophobic cavity (Figure 1 b, middle). Thus, their individual energetic frustration will be substantial ("high-energy" water) and a strong enthalpic driving force for the binding of guest molecules is expected, because the release of cavity water molecules to the bulk restores their hydrogen-bonding ability.

A further increase in the cavity diameter increases the number of cavity water molecules that can be released but also lowers their individual energetic frustration, as more partners for hydrogen bonding become available (Figure 1 b, right). Simulations of water clusters in both fullerenes and cucurbit[*n*]urils have indicated that beyond a cavity diameter of approximately 1 nm, stable hydrogen-bonded water clusters can be formed inside the cavity.[1c,3c,14] In essence, the water inside such large cavities behaves as a nanoscale droplet of condensed water (or "molecular ice", to adopt a formulation used for the water decamer cluster observed experimentally inside a self-assembled coordination cage),[15] whereas the physical state of high-energy water inside medium-sized cavities is probably better characterized as supercritical.[16] Novel high-affinity hosts may be obtained if the number of water molecules to be released is increased, thus preserving their individual energetic frustration. One promising strategy towards this goal is the enlargement of the cavity height as opposed to the cavity diameter of barrel- or cup-shaped hosts or almost-closed molecular tweezers. Nanotubes are examples of such long containers; they usually contain single chains of water molecules linked by only few hydrogen bonds (Figure 2 a).[17] The replacement of such high-energy water

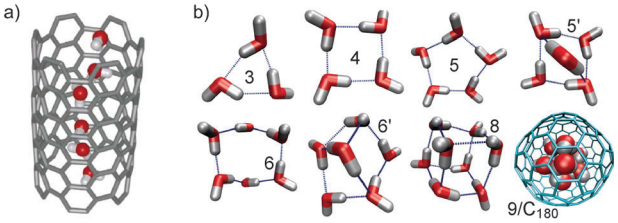


Figure 2. a) MD-simulation snapshot of a 1D hydrogen-bonded water wire in the pore of a short (6,6) nanotube.[17c] Reprinted with permission from Ref. [14b]. Copyright 2001 Macmillan Magazines Ltd. b) Structures of clusters of 3–8 water molecules inside spherical cavities, and of 9 water molecules inside a C_{180} fullerene.[14] Reprinted from Ref. [14a] with permission. Copyright 2004 The National Academy of Sciences.

with ligands will lead to significantly increased affinity even in the absence of direct interactions of the ligands with the nanotube walls. This important aspect for the use of such nanomaterials as sensors and in other applications, for example, for drug release and water purification, has often been overlooked. Similarly frustrated water molecules are also expected inside spherical pores and cavities,[18] such as those of fullerenes (Figure 2 b).[14]

A third important design aspect, besides the shape of the host and the size of its cavity, is the type and arrangement of functional groups on the host. The host should not stabilize the cavity water molecules; that is, any hydrogen-bond-donor or -acceptor moieties on the host should not be oriented towards the cavity water molecules. This limitation is particularly important for cyclodextrins, but also for many cyclophanes and calixarenes, which can engage in O–H···π interactions.

Hans-Jörg Schneider studied in Munich, Berlin, and Tübingen, where he obtained his PhD in organic chemistry in 1967 with Michael Hanack. After postdoctoral research at the University of California, San Diego with R. C. Fahey, he completed his habilitation in the laboratory of Walter Hückel in Tübingen and became Professor at the Universität des Saarlandes in Saarbrücken in 1972. His research has focused on conformational analysis, reaction mechanisms, NMR spectroscopy, supramolecular chemistry, and, more recently, smart materials based on chemomechanical polymers.

To make the thread of thought in this Review easier to follow, and to base comparative interpretations on a consistent data set, we investigated the number of cavity water molecules and their degree of hydrogen bonding for some of the most popular host molecules by MD simulations (Table 1, Scheme 1). Our exploratory MD simulations were performed on constrained host geometries derived from experimental crystal structures or DFT-optimized structures in a vacuum. For rigid hosts, such as cucurbit[*n*]uril (CB*n*), and clefts, this simplification may be a good approximation for their solution-phase structures; however, substantial distortion can be expected for the more flexible cyclodextrins and cyclophanes. Indeed, MD calculations showed that flexible hosts, such as γ-cyclodextrin,[19] may be distorted/collapse in aqueous solution to bypass the filling of the cavity with water molecules. This "hydrophobic collapse" of large flexible hosts is a further indication of the high energy required for cavity filling, which, indeed, is the last resort of a host to avoid a vacuum. The actual hydrophobic contributions to binding are probably much smaller for the flexible and large hosts than implied by the data in Table 1. To scale the possible effect of high-energy water for the different host molecules, we multiplied the number, N, of water molecules in a cavity (Table 1) by the average deficiency of water hydrogen bonds of these molecules, m. The result is assumed to reflect the difference between the hydrogen-bond number in bulk water ($m = 3.62$) and that of cavity water. Thus, the total effect of high-energy water is given as $Z = N(3.62 - m)$. For consistency, we used $m = 3.62$ and not $m = 4$ as the maximum coordination number for bulk water, as this value was the highest reached in TIP5P simulations with rather liberal assumptions regarding distance and H–O···H angle limits. The calculated values depend not only on these limits but to some degree also on the arbitrarily chosen separation between cavity and bulk water molecules.[20] For example, it has been shown that lipophilic parts of large guest molecules that extend beyond the host cavity can significantly enhance the affinity.[21]

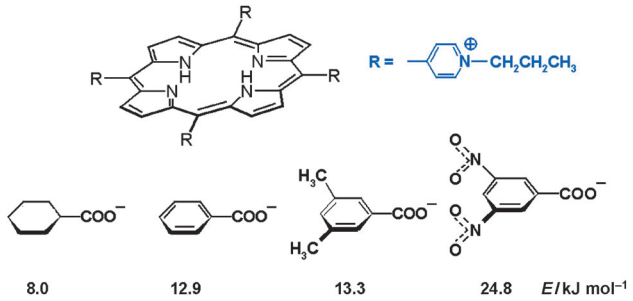
Table 1: Results from water-box simulations for water molecules inside cavities

System[a]	N[b]	m[c]	Z[d]
bulk water		3.62	
CB6	3.3	1.71	6.3
CB7	7.9	2.52	8.7
CB8	13.1	3.06	7.3
calix[4]arene	0.8	2.15	1.2
clip	3.8	2.67	3.6
tweezer	0.7	1.00	1.8
CP66	4.9	2.92	3.4
bipyCP	3.2	2.73	2.9
α-CD	3.6	2.86[e]	3.1
β-CD	4.4	2.96[e]	3.1
pillar[5]arene	0.5	1.24	1.2

[a] See Scheme 1. For geometric cutoff boundaries, see Scheme S1 in the Supporting Information. [b] Number of inner-phase water molecules. [c] Average number of hydrogen bonds of the inner-phase water molecules (with limits of the H-O-H angle and the H–OH distance of 130° and 3.5 Å, respectively). Note that the actual number of water molecules shown in the MD snapshots in Figures 3–5, 7, and 9 may differ from the tabulated average values. [d] Relative measure of the anticipated effect of high-energy water; see text. [e] Hydrogen bonds between the O1 and O4 CD oxygen atoms and cavity water molecules are included.

4. Association with Flat Molecular Surfaces—Absence of a Sizeable Hydrophobic Effect

From studies with open flat molecular surfaces, it has become clear that the association of even the most hydrophobic alkanes in water is weak unless confined water molecules are involved.[22] Thus, binding contributions of alkyl groups in complexes with water-soluble porphyrins (Scheme 2), despite their large molecular surface, are barely measurable, at less than 1 kJ mol^{-1} per CH_2 group. In contrast, more hydrophilic heteroatoms undergo sizeable interactions as a function of their polarizability.[22] Micelle formation is also characterized by only small free energies. Even for long-chain surfactants, such as hexadecyltrimethylammonium bromide (CTAB), the total free energy amounts only to approximately 17 kJ mol^{-1} and is thus consistent with a "classical" hydrophobic effect dominated by entropic contributions, as also observed for complex formation between surfactants and amphiphilic calixarenes.[23]



Scheme 2. Porphyrin complexes in water show no measurable hydrophobic, but large dispersive binding contributions (experimental ΔG values in kJ mol^{-1}).[22]

5. Representative Concave Hosts with Hydrophobic Binding Contributions

5.1. Cyclodextrin Complexes

The analysis of the different binding mechanisms in cyclodextrin (CD) complexes[24] is hampered by the multitude of simultaneously occurring interactions, which include, besides polar interactions with polar ligands, dispersive interactions and the hydrophobic effect. Since cyclodextrins are known to also bind some ligands outside the cavity, even the interpretation of the data in terms of the formation of inclusion or exclusion complexes is not always straightforward.[1d] Recent MD simulations, coupled with parameter-

dependent force-field calculations, have led to unrealistically large $T\Delta S$ and ΔH terms for cyclodextrin complexes (up to 100 kJ mol^{-1}) with associated ΔG values surprisingly close to experimental data.[25] Until now, computational approaches have not explicitly taken into account the presence of high-energy water inside the cyclodextrin cavity. Calorimetric measurements most often show the signature of predominant enthalpic contributions, not only for associations with polarizable guest molecules, which provide sizable dispersive contributions to binding, but also for alkane derivatives.[24] Only for wider CD cavities, which involve less precise binding of the guest in a particular position, sizeable entropic contributions may result.[26] Table 2 shows thermodynamic data for some representative examples of CD complexes for which the inclusion mode was confirmed by NMR spectroscopy, including NOE measurements.[8e] These data were interpreted as evidence for the presence of high-energy water inside the cavities of hydrated CDs.[8e]

Contrary to earlier claims,[24] ionic forms of the same ligand do not always bind less strongly, as demonstrated for the nitrophenol/phenolate and iodophenol/phenolate pairs (Table 2). This observation and the strong increase in binding enthalpy with the charged phenolates indicates that factors other than the classical hydrophobic effect dominate, most likely dispersive interactions between the aryl moiety and the cyclodextrin C–H bonds, in particular with the narrower α-CD. In contrast, the behavior of the aliphatic adamantyl derivatives has the typical characteristics of a nonclassical hydrophobic effect: a lower affinity for the more hydrophilic ionic form (for β-CD), but a dominant enthalpic driving force.

Our water-box simulations indicate for α-CD the presence of $N = 3.6$ water molecules with on average only 2.9 hydrogen bonds and a corresponding Z value of 3.1; for β-CD, the values found were $N = 4.4$ with 3.0 hydrogen bonds and $Z = 3.1$ (Figure 3a,b). The effect of high-energy water in cyclodextrins is thus much lower than that observed for cucurbiturils, but close to that of cyclophanes. That neither the experimentally determined free energies nor the enthalpies correlate directly with the Z values is understandable in view of the dramatically different entropic contributions (see Table 2), the formation of specific hydrogen bonds between water and the oxygen atoms of the host, and other complementary host–guest binding contributions.

Neutron diffraction of crystalline β-CD dodecahydrate showed on average 6.1 disordered water molecules inside the cavity[27,28] (Figure 3c), in agreement with MD and Monte Carlo (MC) simulations.[28,29] The MD results further suggested that the number of water molecules inside the cavity roughly doubles per glucose unit, and that the residence time of an encapsulated water molecule is inversely proportional to the cavity size.[30] Earlier simulations showed for α-CD only 2–3 water molecules, and for γ-CD more than for β-CD, namely, 8.8 water molecules.[29] Almost all intracavity water molecules in the β-CD structure (Figure 3c) can only form less than the theoretically possible four hydrogen bonds, and only some are involved in stabilizing hydrogen-bonding interactions with the CD skeleton (W8 and W12 in Figure 3c).

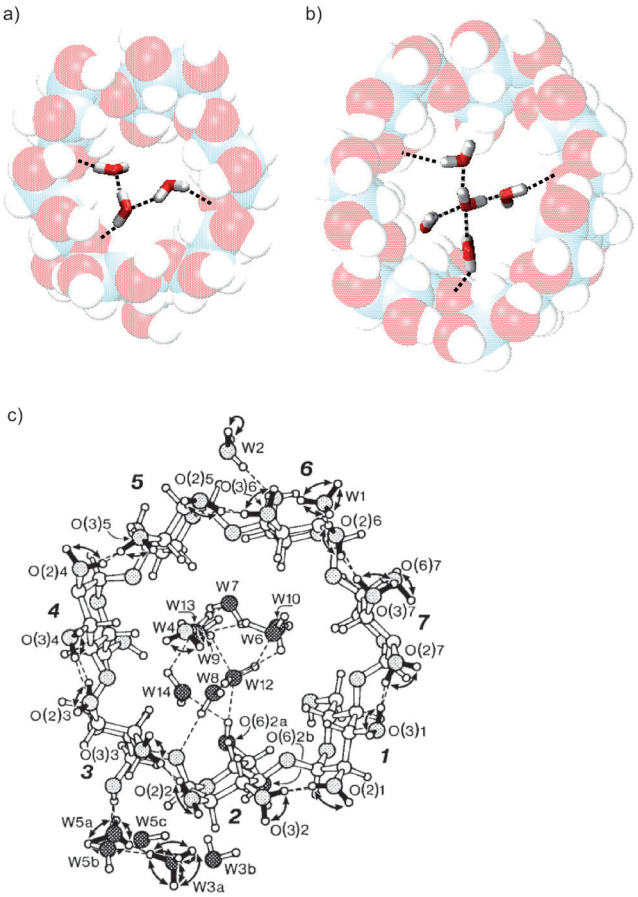


Figure 3. Snapshot from MD simulations for a) α-CD and b) β-CD with 3 and 5 cavity water molecules, respectively. Hydrogen bonds are represented by a dashed line. c) Structure of β-CD dodecahydrate, as derived from neutron diffraction.[27] Reprinted with permission from Ref. [27]. Copyright 1984 American Chemical Society.

Table 2: Representative experimental thermodynamic data (in kJ mol^{-1}) for the formation of inclusion complexes with α-CD and β-CD.[8e]

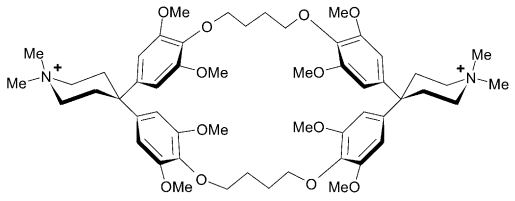
	α-CD			β-CD		
	ΔG	ΔH	$-T\Delta S$	ΔG	ΔH	$-T\Delta S$
p-$NO_2C_6H_4OH$	−11.5	−23.0	11.5	−14.2	−10.2	−3.9
p-$NO_2C_6H_4O^-$	−18.7	−42.8	24.1	−15.0	−16.1	1.1
p-IC_6H_4OH	−16.8	−26.3	9.5	−17.0	−16.1	−0.9
p-$IC_6H_4O^-$	−19.1	−36.5	17.4	−17.1	−12.7	−4.5
1-adamantyl-COOH	–	–	–	−32.4	−42.1	9.7
1-adamantyl-COO^-[a]	−11.6	−14.3	2.7	−24.5	−21.6	−2.9

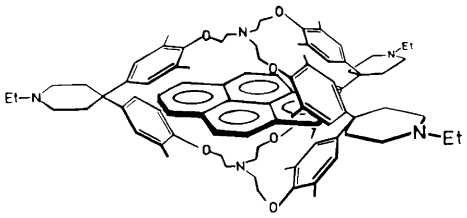
[a] Partial inclusion according to NMR spectroscopic data and molecular modeling.

5.2. Cyclophanes

Most water-soluble cyclophanes possess a lipophilic cavity, however, often in combination with polar donor or acceptor sites, which, in a similar way to the oxygen atoms in CDs, moderate the effects of high-energy water. Diederich and co-

workers showed that cyclophane hosts CP1 and CP2 (Scheme 3) form essentially enthalpy driven complexes with aromatic hydrocarbons not only in water, but also in other media, such as methanol.[3a,31] The maximal binding strength was observed in water (Table 3). The results were discussed in terms of solvent cohesiveness, which increases upon solvent liberation as outlined above for entropically driven associations.





Scheme 3. Chemical structures of water-soluble cyclophanes CP1 and CP2 (the latter as its host–guest complex with pyrene).[3a,31a]

Table 3: Complexation thermodynamics (in kJ mol^{-1}) of cyclophanes CP1 and CP2.[3a,31a]

Host	Guest	Solvent	ΔG	ΔH	$-T\Delta S$
CP1	*p*-MeC_6H_4Me	water	−22.3	−30.1	7.8
CP1	*p*-$MeOC_6H_4OMe$	water	−22.5	−41.8	19.3
CP1	*p*-$MeOC_6H_4OMe$	MeOH	−5.0	−15.5	10.5
CP2	pyrene	water (1% DMSO)	−39.3	–	–
CP2	pyrene	MeOH	−26.7	−50.1	23.4
CP2	pyrene	$CHCl_3$	−9.6	−13.0	3.4

The more confined cavity of the three-dimensional trimeric host CP2 shields the intracavity water from hydrogen bonding with the bulk more effectively than that of the two-dimensional dimeric host CP1. Thus, the energy gain upon the release of high-energy water is expected to be larger for host CP2 than for CP1. Indeed, the complex of CP2 with pyrene showed the highest affinity, in particular in water (Table 3). Furthermore, the stability of the pyrene complex with CP2 decreased sharply in less polar solvents on account of a drop in the enthalpic driving force for binding that is not compensated by an increase in entropy. All these findings point to the increasing importance of the hydrophobic effect in closed cavities with fewer water-binding elements. The solvent dependence of complexes with CP2 was investigated by free-energy calculations in water and in chloroform on the basis of the deletion of the pyrene guest in the pure solvent and inside the cyclophane cavity by the double-annihilation technique.[32] The calculations led to ΔG values that were an order of magnitude higher than the experimental values, but confirmed that the displacement of cavity water by pyrene is the essential reason for the strong effect of the medium.

The cyclophanes CP3 and CP4 (Figure 4) have geometrically similar cavities, but differ by the presence in CP3 of a charged nitrogen atom, which leads, on account of the

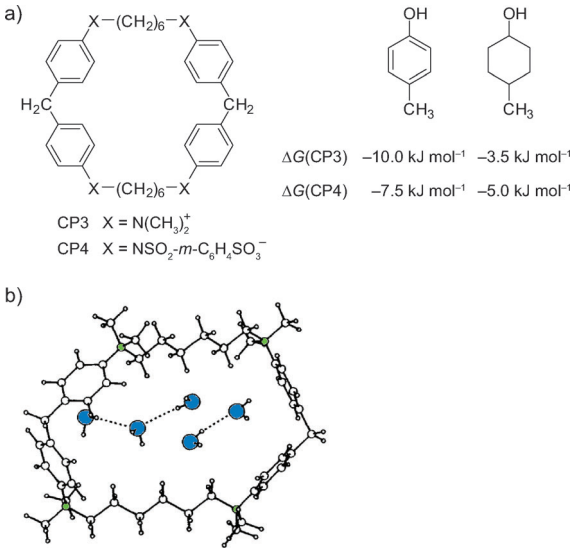


Figure 4. a) Structures of cyclophanes CP3 and CP4 along with representative guest molecules and their complexation free energies.[8d,e,33] b) Snapshot of an early TIP3P water-box simulation for CP3.[8e] Reprinted with permission from Ref. [8e]. Copyright 1991 Wiley-VCH.

cation–π effect, to the preferential binding of aromatic guest molecules.[33] In the absence of cation–π interactions, that is, for cyclophane CP4, the preferential binding of saturated frameworks was observed. Early water-box simulations of host CP3 (Figure 4b) showed the presence of five water molecules inside the cavity; these water molecules could form much fewer water–water hydrogen bonds than they would in solution.[33] Our new simulations with an electroneutral analogous host functionalized with $NHSO_2Me$ instead of the corner $-NMe_2^+-$ groups (see Scheme S1b in the Supporting Information) also indicate the presence of high-energy water, with 4.9 water molecules inside the cavity. These molecules are on average involved in only 2.9 hydrogen bonds, as compared to 3.6 bonds in bulk water. The calculated Z value of 3.4, which assumes a nondistorted host geometry, indicates a high-energy-water effect similar to that of α- or β-cyclodextrin and significantly stronger than that of calix[4]-arenes.

The electron-poor tetracationic viologen-based cyclophane bipyCP[34] (see Scheme 1) binds electron-rich guest molecules particularly well by electrostatic attraction, but it also forms complexes with electroneutral compounds.[35] For example, indole is bound in water with $K_a = 7100\,M^{-1}$.[35a,36] The water-box simulations showed the presence of 3.2 water molecules inside the cavity. These water molecules are involved in 2.7 hydrogen bonds on average,[36] thus resulting

in a Z value of 2.9. This value is smaller than that for cucurbiturils but larger than that for calixarenes (see Section 4.3).

5.3. Calixarenes, Resorcarenes, and Related Hosts

The shallow bowl of the calix[4]arene cone is less suited for shielding cavity water from hydrogen bonding with the bulk (Figure 5a and Table 1). Indeed, host–guest complexes with small lipophilic guests have almost exclusively been

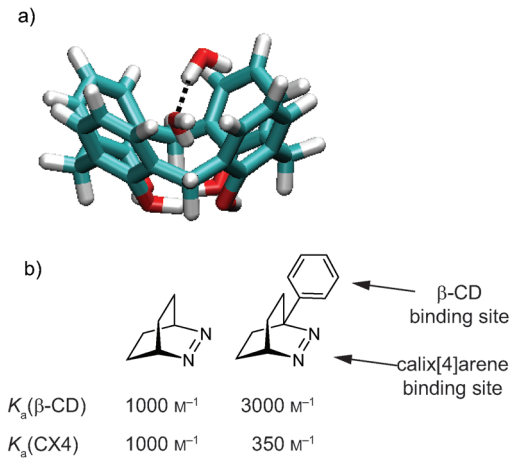


Figure 5. a) TIP5P water-box simulation for calix[4]arene. b) The phenyl-substituted azoalkane shows different binding-site selectivity with β-CD and *p*-sulfonatocalix[4]arene (CX4) because of the dominance of high-energy-water release (β-CD) or C–H···π interactions (CX4), respectively.[38]

reported for solid calixarene derivatives, which bind gaseous small molecules, such as CH_4, CO_2, CF_3Br, C_2F_6 and CF_4,[37] through dispersive forces. *p*-Sulfonatocalix[4]arene (CX4) is the prototype of a water-soluble calixarene. Its flexibility allows this macrocycle to bind to similarly large guests, such as β-CD and CB7. However, the largest binding constants observed for neutral guest molecules, that is, in the absence of auxiliary ionic interactions, did not exceed $10^3\,M^{-1}$.[38] For the binding of small neutral molecules, such as alcohols, by this host, much lower K_a values of less than $100\,M^{-1}$ were obtained, even after correction for competitive binding by added inorganic cations.[38,39]

Recent structural evidence in both the solid state[40] and the gas phase[41] revealed the presence of only a single water molecule, bound through two O–H···π hydrogen-bonding interactions with two aryl rings, inside the bowl-shaped cavity of CX4. These two hydrogen bonds contribute up to 15 kJ mol^{-1} to the complex stability, or about one half of the total binding energy of the single water molecule (37 kJ mol^{-1}).[40] The remainder can be attributed to dispersive interactions with the electron-rich cavity. As the cavity is occupied by a single water molecule, CX4 has been termed "the smallest cup of water".[41] The structural studies are in good agreement with our MD calculations and, in particular, our conjecture that high-energy water constitutes only a minor driving force for complex formation with open, small host cavities (Figure 5a). Indeed, guest binding of calixarenes in aqueous solution is dominated by direct host–guest interactions. Specifically, the strong tendency of CX4 to bind aliphatic moieties through C–H···π interactions rather than aromatic residues through stacking interactions was borne out in studies of 1-phenyl-2,3-diazabocyclo[2.2.2]oct-2-ene (Figure 5b).[38] In this case, ROESY NMR spectroscopy demonstrated preferential binding of the less hydrophobic azoalkane residue over the more hydrophobic phenyl group; in fact, the presence of a bridgehead phenyl group on the azoalkane reduced the binding constant to $350\,M^{-1}$ as compared to that for the unsubstituted guest ($1000\,M^{-1}$). These results are in stark contrast to the affinity observed for β-CD, in which case the expected increase in the binding constant was observed upon the introduction of the hydrophobic anchor (from 1000 to $3000\,M^{-1}$), and preferential binding of the phenyl group was corroborated by ROESY (Figure 5b).

Calixarene-based capsules are accessible either by hydrogen bonding (Caps1)[42] or by ion pairing (Caps2)[43] between the monomeric partners (Figure 6). As shown with water-soluble ion-paired capsules, such as Caps2, they hold more promise for hydrophobic binding effects than simple calixarenes,[44] because any intracavity water is better shielded from hydrogen bonding with the bulk. Notably, such cavities effectively complex alkanes, also in usually unstable conformations and even in aqueous solution (Figure 6a).[42a,45] Resorcarenes, which are structurally closely related to calixarenes and which become water-soluble under alkaline conditions, have an even flatter cavity, with an associated smaller driving force for the hydrophobically driven complexation of guests.[46]

5.4. Other Aromatic Macrocycles

The water-soluble aromatic host "octaacid" (Octa) resembles calixarenes and resorcarenes in that its inner cavity walls incorporate highly polarizable, electron-rich aromatic building blocks (Scheme 4).[47] A computational study revealed ordered (entropically unfavorable) encapsulated water molecules for this macrocyclic host.[47b] The hydrophobic effect was further probed by the binding of adamantanecarboxylate, a guest molecule with a highly hydrophobic core, in the presence of different sodium salts. The anions of the latter were chosen to fall on extreme sides of the Hofmeister scale, with either strongly kosmotropic (fluoride, sulfate) or strongly chaotropic behavior (iodide, thiocyanate, perchlorate).[47a] Kosmotropic anions are known to "increase water structure" and accordingly emphasize the hydrophobic effect, which led to an increase in binding of the hydrophobic guest and a more favorable complexation enthalpy by about −2 kJ mol^{-1}.[47a] Chaotropic anions are known to "decrease water structure" and accordingly weaken the hydrophobic driving force for complexation, which accordingly led to a strong decrease in binding of the hydrophobic guest and a less favorable complexation enthalpy by up to approximately 36 kJ mol^{-1}, but a more favorable complexation

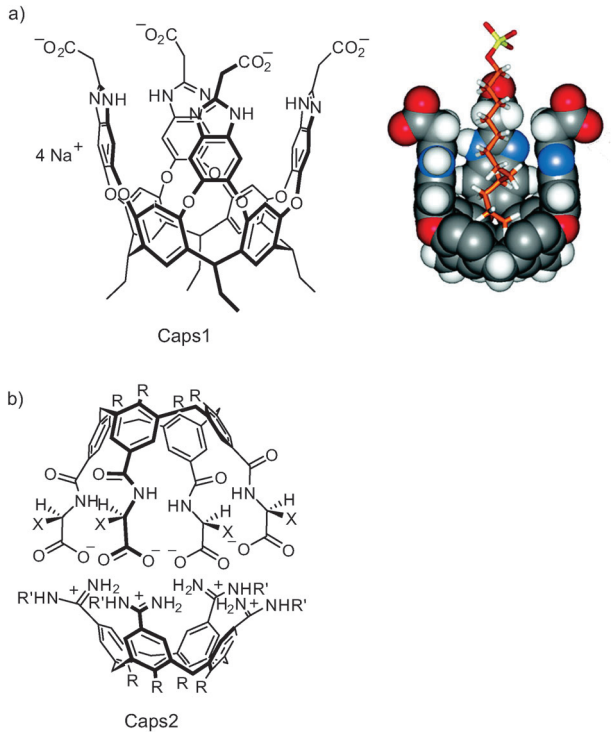
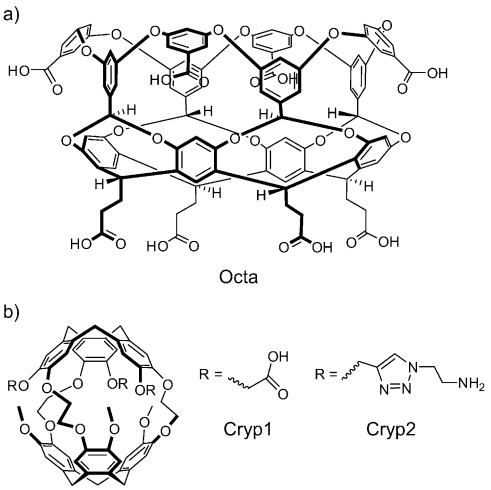


Figure 6. a) Chemical structure of capsule Caps1 and 3D rendering of its host–guest complex with coiled sodium dodecyl sulfate (SDS). Reprinted with permission from Ref. [42a]. Copyright 2003 American Association for the Advancement of Science.[42a] b) Chemical structure of capsule Caps2.[43]



Scheme 4. a) Chemical structure of "octaacid" (Octa).[47] b) Cryptophanes for noble-gas complexation.[50]

entropy term by about 32 kJ mol^{-1}.[47a] The marked change in the complexation thermodynamics of the adamantane guest in the presence of chaotropes, and specifically perchlorate, was not only attributed to a Hofmeister-type solvent effect but also to direct host–guest interactions between perchlorate and the octaacid ($K_a = 95\,\text{M}^{-1}$). Interestingly, the first observation of anion binding to a hydrophobic cavity—in fact, of the perchlorate ion itself, with a binding constant of 29 M^{-1}—was documented for α-CD.[48] An earlier, qualitative observation of the binding of another chaotropic anion, iodide, to α-CD dates back even more than 50 years.[49] These old and new studies deserve attention, because they demonstrate that the release of high-energy water can drive not only the encapsulation of electroneutral, but even of anionic guests.

Pillararenes are macrocyclic hosts (Scheme 1) that have just recently been discovered.[51] Our computational predictions (Table 1) for pillar[5]arene suggest that the cavity contains no or very little water; it is therefore very unlikely that the complexation of nonpolar guests is significantly driven by the release of high-energy water. Therefore, the potential of pillar[5]arene as a high-affinity binder for nonfunctional guests in aqueous solution is limited.

The water-soluble cryptophane Cryp1 (Scheme 4) complexes xenon strongly with $K = 33\,000\,\text{M}^{-1}$ ($\Delta G = -25.3$ kJ mol^{-1}, $\Delta H = -18$ kJ mol^{-1}, and $-T\Delta S = -7$ kJ mol^{-1}).[50] Furthermore, Cryp2 was found to bind xenon and radon (radioactive ^{222}Rn) in water with record binding constants of 42 000 and 49 000 M^{-1}, respectively.[52] By isothermal calorimetry (ITC), the binding of xenon was found to be enthalpically driven ($\Delta H = -15.0$ kJ mol^{-1}) with a sizable entropic contribution ($-T\Delta S = -11.0$ kJ mol^{-1}), which was attributed to "the release of one or more water molecules from the cryptophane cavity" and to dispersive interactions, particularly with radon, among other factors. The significant contribution of dispersive interactions is presumably also responsible for the fact that these cryptophanes bind xenon even more strongly than CB5 ($K \approx 4000\,\text{M}^{-1}$)[53] and CB6 ($K \approx 1300\,\text{M}^{-1}$).[54]

5.5. Cucurbiturils

The most compelling evidence for the action of high-energy water stems from recent studies of cucurbituril complexes, which for the first time also enabled the quantification of these contributions. The host cavity of the barrel-shaped, glycoluril-based CB*n* macrocycles is hydrophobic,[55] as no hydrogen-bond-donor or -acceptor moiety is pointing inwards.[20] Recent MD simulations confirmed early suggestions that the release of cavity water contributes to CB*n*·guest complex formation.[3c,56] Indeed, the cavity water molecules are structurally and energetically frustrated, for example, they show a lower hydrogen-bond count than bulk water, in particular for the smaller CB*n* homologues.[3c] The agreement between the simulated hydration structure of CB6 and a crystal structure of an isomer of cucurbit[6]uril, in which one glycoluril unit is pointing inwards (*i*CB6),[57] is very close (Figure 7).

The affinity of CB*n*, in particular CB7, for organic guests in water is very high (K_a up to $10^{17}\,\text{M}^{-1}$),[9] and in most cases stronger than that of any other synthetic host for the same guests.[55] Generally, there is a strong exothermic driving force for binding, coupled with a smaller entropic contribution that can be favorable or unfavorable. In fact, ΔH values of up to -90 kJ mol^{-1} were observed for CB7 complex formation, regardless of whether the ferrocene guest was noncharged,

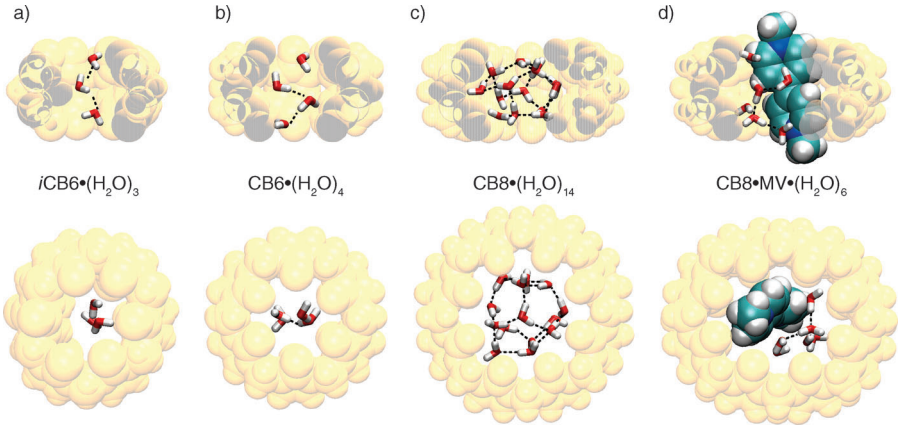


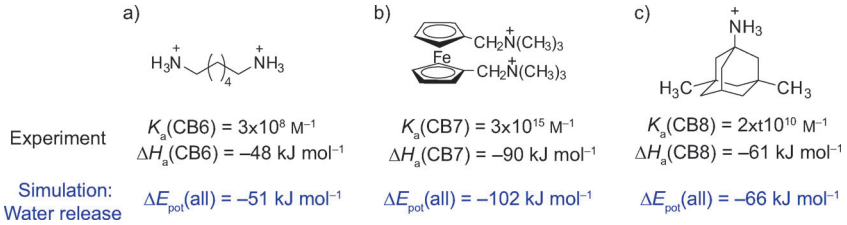
Figure 7. a) Crystal structure (NEBDII)[57] of inverted CB6 with 3 intracavity water molecules. b–d) Snapshot from MD simulations for CB6 (b), CB8 (c), and CB8·viologen (d) complexes with 4, 14, and 6 cavity water molecules, respectively. Top: Complexes viewed from the side (CB*n* atoms in the front have been removed for clarity). Bottom: Complexes viewed from the top.

monocationic, or dicationic.[58] Similarly, several adamantane (K_a up to $10^{14}\,M^{-1}$, ΔH up to $-80\,kJ\,mol^{-1}$) and bicyclo-[2.2.2]octane derivatives (K_a up to $10^{15}\,M^{-1}$, ΔH up to $-60\,kJ\,mol^{-1}$) were also reported as rigid, spherical, high-affinity guests for CB7.[59]

The large differences observed in the binding strength between the charged and noncharged analogues (K(monocationic)/K(neutral) $\approx 10^3$) almost exclusively resulted from entropic differences,[58,59] which is typical for ionic interactions. Specifically, desolvation of the charged ammonium groups of the guests upon binding to the CB*n* carbonyl-fringed portals liberates a number of solvating water molecules, which is entropically favorable.[60] Indeed, the additional release of portal water molecules by an elongated diamantane guest was utilized to reach a record binding affinity of $7\times10^{17}\,M^{-1}$.[9] Conversely, polar interactions between the charged functional groups of the guest and water (prior to binding) or CB7 (after binding) were computed to be similar in magnitude, and thus do not affect the overall ΔH value.[59]

Simulations show that cavity water in CB6 exhibits a much lower hydrogen-bond count than in CB8, but more water molecules can be released from the larger-cavity host CB8 (Table 1 and Figure 7). From the MD analysis, it was predicted that the energetic contribution from the release of all high-energy water molecules should be maximal for the medium-sized CB7 host,[3c] which agrees with the trends in the Z value (see Section 3). In strikingly close agreement, the exothermic driving force for complex formation is significantly smaller for CB6 and CB8 than for CB7, even for the respective highest-affinity guests known (e.g. CB6·1,6-diaminohexane: $\Delta H = -48\,kJ\,mol^{-1}$;[61] CB8·1-amino-3,5-dimethyladamantane: $\Delta H = -61\,kJ\,mol^{-1}$; see Scheme 5).

If the hydrophobic effect is the main determinant for CB*n* binding, then guests that are too small to displace all cavity water molecules cannot achieve the entire energetic contribution of the release of high-energy water, in particular, since residual cavity water molecules can engage even less in hydrogen bonding.[3c] Indeed, size-selective binding is well-documented with CB*n* complexes.[20,55] An instructive example is the formation of binary complexes of CB8 with quaternary ammonium salts.[62] It was observed that 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 twice as high ($-39\,kJ\,mol^{-1}$). NMR spectroscopic experiments showed a back-folded, tightly packed structure of the C_{12} guest in the CB8 cavity. However, the C_6 guest is too small to fill the CB8 cavity,[62] so that residual high-energy water molecules are probably present.[36]



Scheme 5. Ultra-high-affinity guests for a) CB6, b) CB7, and c) CB8 and their enthalpic contribution to binding.[58,61] The estimated energy gain through cavity-water release is denoted as ΔE_{pot}(all).[3c]

The large host CB8 typically accommodates two aromatic guests simultaneously to ensure the complete release of cavity water, thus resulting in 2:1 homoternary or 1:1:1 heteroternary complexes (Figure 8).[36] Specifically, inclusion of the first guest, such as the dicationic auxiliary guest viologen (paraquat), in the cavity of CB8 restricts the hydrogen-bonding opportunities of the residual water molecules (Figure 8a). Therefore, subsequent binding of an additional aromatic guest is strongly exothermic (ΔH up to $-60\,kJ\,mol^{-1}$), in particular for guests that are large enough to displace all cavity water molecules (naphthalenes, indoles, but not benzenes): another example of size-selective binding.[36] In contrast, charge-transfer (CT) or π–π-stacking interactions are of little energetic significance in these systems.[63] Pronounced solvent and solvent-isotope effects provide further experimental verification for the release of high-energy water:[36] 1) The binding affinity for the second aromatic guest drops by a factor of more than 1000 when the solvent is changed from water to acetonitrile,[64] in analogy with the findings for the well-shielded cyclophane CP2 (Table 3),[3a,31a] whereas binding in the open or small-cavity systems was much less affected by the solvent (factor of 2–100).[65] 2) Auxiliary-guest binding to CB8 is stronger and more exothermic in H_2O than in D_2O, whereas the opposite

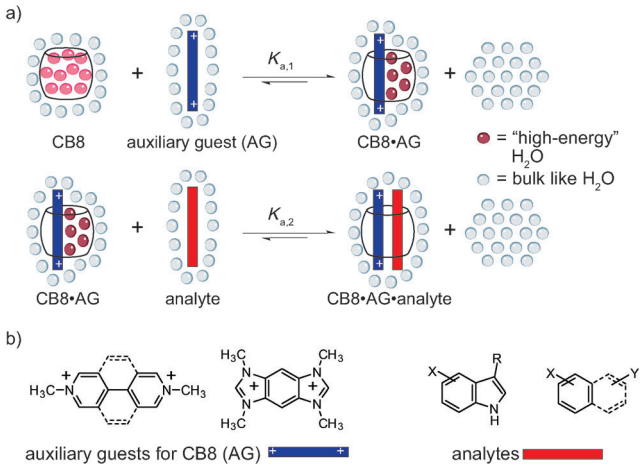
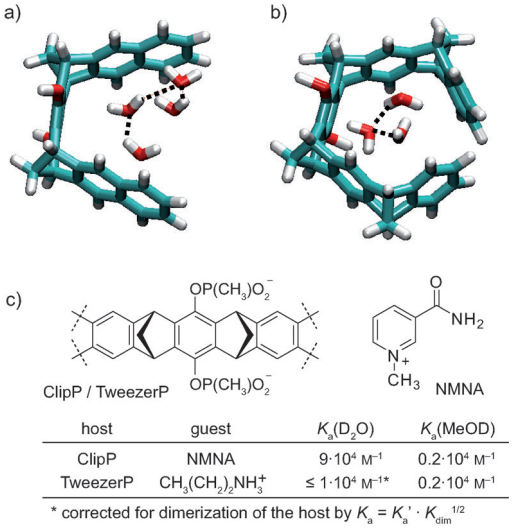


Figure 8. a) Stepwise formation of 1:1:1 ternary complexes of CB8 in aqueous solution. The first binding event increases the energetic frustration of the residual cavity water, so that the subsequent binding of an aromatic analyte is energetically favored. b) Typical auxiliary guests are dicationic heteroaromatic compounds, and typical analytes are phenyl, naphthyl, and indoyl derivatives, including the natural amino acids Phe, Tyr, and Trp.[36]



host	guest	$K_a(D_2O)$	K_a(MeOD)
ClipP	NMNA	$9\cdot10^4\ M^{-1}$	$0.2\cdot10^4\ M^{-1}$
TweezerP	$CH_3(CH_2)_2NH_3^+$	$\le 1\cdot10^4\ M^{-1}$*	$0.2\cdot10^4\ M^{-1}$

* corrected for dimerization of the host by $K_a = K_a' \cdot K_{dim}^{1/2}$

Figure 9. TIP5P water-box simulation for the core structure of a) a clip and b) a tweezer. Note that the average occurrence of cavity water in the tweezer is low (Table 1). c) Experimental affinities for the methanephosphonate-substituted hosts ClipP and TweezerP with cationic guests in D_2O and MeOD.[67a,c]

was observed for the subsequent binding step to form a ternary complex.[36] This effect is in line with the presence of residual high-energy cavity water in binary CB8–auxiliary-guest complexes because hydrogen bonding in D_2O is stronger than in H_2O owing to the lower zero-point vibrational energy of O–D versus O–H bonds.[36,66]

5.6. Acyclic Concave Hosts: Clips and Tweezers

The methanephosphonate-substituted molecular hosts ClipP and TweezerP (Figure 9) serve as impressive examples of large binding contributions by nonclassical hydrophobic contributions in the absence of a closed cavity.[67] The affinity of ClipP for cationic aromatic guest molecules is high; it reaches, for example, $9\cdot10^4\ M^{-1}$ in D_2O and $2\cdot10^4\ M^{-1}$ in MeOD for *N*-methyl nicotinamide (NMNA; Figure 9c).[67a] Polyglycerol-tagged clips exhibit lower affinities for organic cations by 1–2 orders of magnitude as compared to anion-bearing clips.[67e] Remarkably, the affinity is higher in water than in methanol, although the electrostatic attraction between the electron-rich inside of the concave clips and the electron-poor guest, and the ion pairing between the methanephosphonate groups and the positively charged NMNA guest are weaker in the more polar medium. Solvent effects are significantly lower for the smaller host TweezerP, which preferentially binds alkylammonium cations (Figure 9).[67c]

Water-box simulations showed for the parent clip the presence of about four water molecules inside the cavity, with 2.7 hydrogen bonds per water molecule (Figure 9a), whereas the average number of cavity water molecules in the parent tweezer is low ($N<1$), as was also observed for the similarly shaped pillar[5]arene (Table 1). The corresponding Z numbers are 3.6 for the clip and 1.8 for the tweezer, and indeed the medium effects (clip: $K_a(D_2O) > K_a$(MeOD); tweezer: $K_a(D_2O) \approx K_a$(MeOD)) suggest that the release of high-energy water is more important for the clip than for the small tweezer. Interestingly, a slightly enlarged version of TweezerP, with one naphthalene panel instead of a phenyl panel (see Figure S2 in the Supporting Information), showed a high tendency to self-aggregate in water ($K_{dim} = 2\times10^6\ M^{-1}$), but not in methanol.[67a] This observation and the very large favorable enthalpy of dimerization ($\Delta H_{dim} = -87\ kJ\,mol^{-1}$), as determined by a van 't Hoff plot, are another indication of a powerful nonclassical hydrophobic effect originating from the release of high-energy water.

Acyclic cucurbituril derivatives, which have recently been introduced,[68] adopt an almost closed tweezer-type structure. They also display very high binding affinities, generally only 1–2 orders of magnitude lower than those for the related cyclic CB*n* hosts.

6. Studies with Biopolymers

Many studies have highlighted the importance of ordered water in protein pockets; its liberation into the bulk can enhance ligand binding. Various computational methods have been applied[69] and have revealed classical entropic as well as compensating enthalpic binding contributions.[70] In a recent study, several thermolysin–inhibitor complexes were crystallographically characterized, and it was shown that the hydrogen-bonding network of the residual cavity water molecules strongly depends on the ligand structure (Figure 10a). The corresponding calorimetric measurements showed that variable enthalpy and entropy contributions are involved as a function of the inhibitor structure, with significant enthalpy–entropy compensation and therefore an overall smaller variation in the free energy. A similar investigation with thrombin complexes indicated that an increase in the hydro-

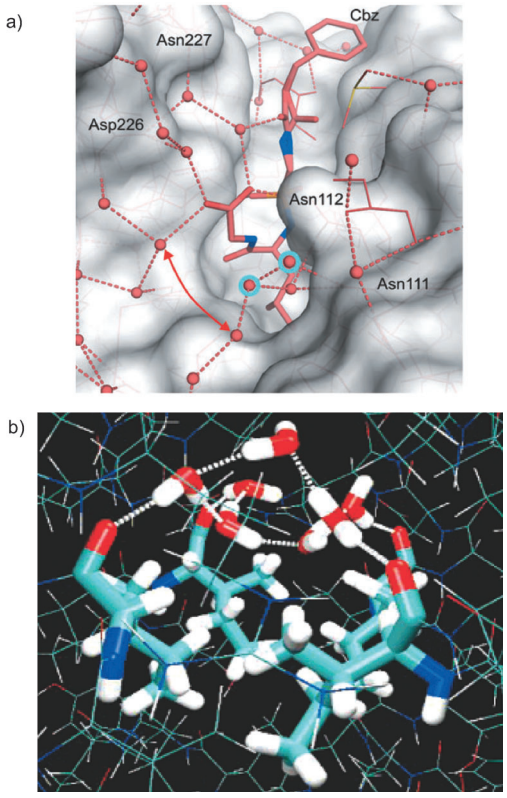


Figure 10. a) Water network in a thermolysin–inhibitor complex.[70a] Reprinted with permission from Ref. [70a]. Copyright 2013 Wiley-VCH. b) Tetrabrachion cavity with water molecules, from TIP3P MD water-box simulations.[71] Reprinted with permission from Ref. [71]. Copyright 2007 American Chemical Society.

phobicity of substituents results in greater affinity, which is associated with an enhanced entropic term resulting from the release of conformationally restricted water molecules from a well-hydrated binding pocket.[70] Some proteins lacking polar groups in the cavity walls, such as tetrabrachion, contain cavities filled with up to nine water molecules, each of which can form less than four hydrogen bonds (Figure 10b).[71] For several carbonic anhydrase complexes, calorimetric measurements in combination with crystal-structure analysis demonstrated that it is not the atomic composition or structure of the ligand that determines binding affinity, but rather the number of water molecules that are displaced from the binding pocket into the bulk.[72] In nanotubes of apolar peptides, the water molecules were oriented in a linear, wirelike arrangement.[73] Water in proteins can also serve as a bonding unit between peptide chains and/or peptides and ligands, thus serving as a kind of hydrophilic "glue".[74] In complexes of double-stranded DNA with antibiotic minor-groove binders, the presence of water molecules as bridging units leads to a sizeable affinity increase.[75] Complexes of proteins with DNA are also stabilized by water release from the interface between the partners.[76]

The decisive difference between water effects in biopolymer pockets and cavities in artificial receptors is that the latter usually contain fewer or no functional groups that can interact with water and at the same time can be sufficiently large to accommodate an intermediary number of water molecules. Consequently, the theoretically predicted enthalpic advantage of water liberation is rarely seen in biopolymers, but becomes important, if not dominant, in many synthetic host–guest complexes.

7. Summary and Outlook

The enormous differences in the calculated hydrophobic contributions from high-energy water in different cavities can be compared in Figure 11 with typical experimental host–guest affinities ($\lg K_a$) observed with electroneutral guest molecules, as far as these data are available (see Table S2 in the Supporting Information). Although the correlation between host Z values and the observed affinities is crude, the strongest complexes are generally those with the largest Z values, as expected. Deviations from the correlation of Z with $\lg K_a$ could be due to adverse entropic contributions as a result of additional host–guest interactions or due to water molecules that are directly hydrogen bonded to host components. Nevertheless, water-box simulations can provide a useful lead with respect to the large variation of high-energy-water effects, and therefore for the design of high-affinity hosts and of drugs.

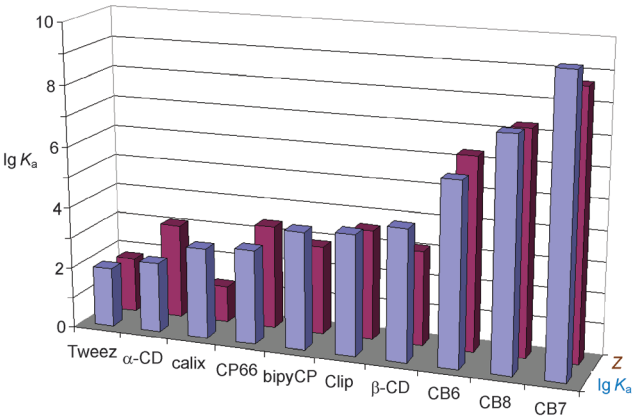


Figure 11. Front, light purple row: Typical experimental $\lg K_a$ values observed for different complexes of synthetic hosts with electroneutral guest molecules, with corrections for ion pairing (see Table S2 in the Supporting Information). Back, dark purple row: Hydrophobic effect Z of the high-energy water in different hosts, as obtained by multiplying the number N of water molecules in the cavity (Table 1) by the average deficiency of hydrogen bonds per water molecule [$Z = N\,(3.62 - m)$].

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