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The HYDROPHOBE Challenge: A Joint Experimental and Computational Study on the Host-Guest Binding of Hydrocarbons to Cucurbiturils Allowing Explicit Evaluation of Guest Hydration Free Energy Contributions

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Abstract

The host-guest complexation of hydrocarbons (22 guest molecules) with cucurbit[7]uril (CB7) was investigated in aqueous solution. Association constants were determined by using the indicator displacement strategy, which allows binding constant determinations also for poorly water-soluble (hydrophobic) guests. The binding constants (10^3 – 10^9 M⁻¹) increased with the size of the hydrocarbon, pointing to the hydrophobic effect and dispersion interactions as driving forces. Besides potential applications for the sensing and separation of hydrocarbons, the measured affinities provide unique benchmark data for the binding of neutral guest molecules. Consequently, a computational blind challenge, the HYDROPHOBE challenge, was conducted in order to allow a comparison with state-of-the-art computational methods for predicting host-guest affinity constants. In total, 5 computational data sets were submitted, which allowed the comparison of experimental binding constants with those predicted by coupled-cluster theory (DLPNO-CCSD(T)), dispersion-corrected density functional theory (DFT), and explicit solvent molecular dynamics (MD) simulations parameterized with two different force field combinations from the AMBER simulation package. All submissions were capable of predicting the general binding trend, with a slightly better correlation for the MD compared to the quantum-chemical (QM) data sets ($R^2_{\text{MD}} = 0.80$ vs $R^2_{\text{QM}} = 0.66$, average values for the submitted data sets). On the other hand, QM calculations showed better predictions for the absolute values of the binding

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Notes

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affinities as reflected by the mean signed errors (4.3 kcal mol⁻¹ for MD vs 1.8 kcal mol⁻¹ for QM). When searching for sources of uncertainty in predicting the host-guest affinities, the experimentally known hydration energies of the investigated hydrocarbons could be employed, which provided a distinct advantage of the HYDROPHOBE challenge. The comparison with the employed solvation models (explicit solvent for MD and COSMO-RS for QM) confirmed a good correlation for both methods, but revealed a rather constant offset of the COSMO data, by ca. +2 kcal mol⁻¹, which was traced back to a required reference-state correction in the QM submissions (2.38 kcal mol⁻¹). Introduction of the reference-state correction improved the predictive power of the QM methods, particularly for small hydrocarbons up to C₅. The correlations of both QM and MD submissions also exposed specific outliers, which could be due to peculiarities of the investigated guests, for example, different degrees of conformational changes upon complexation, such as helical structures of the longer *n*-alkyl chains within the cavity. The latter was confirmed by 2D NMR experiments and both the MD as well as QM calculations.

TOC Graphic

INTRODUCTION

The chemistry of host-guest systems describes the complexation of two or more molecules through non-covalent interactions.¹⁻³ Supramolecular hosts with defined shape, cavity size, and chemical environment offer different types of non-covalent interactions,⁴⁻⁶ the understanding of which dates back to the lock-and-key principle introduced by Fischer in the 1890s.⁷ Hydrogen and halogen bonding, electrostatics, London dispersion interactions, and solvophobic effects are common attractive forces involved in the formation of stable host-guest complexes. In aqueous solution, the classical hydrophobic effect is frequently thought to be the dominant contributor to the inclusion of a nonpolar guest molecule into the cavity of concave host molecules.^{8,9} Both the desolvation of the hydrophobic guest and the release of so-called high-energy water molecules from the host cavity may contribute to the hydrophobic effect.¹⁰⁻¹⁵ In addition, dispersion interactions present an important modulator in host-guest chemistry;^{16,17} however, dissecting the contribution of dispersion interactions from that of the hydrophobic effect has proven difficult, since both increase with the size of the guest.¹⁸⁻²¹ Computational methods are therefore particularly valuable in order to rationalize experimental trends and to understand the interplay between the two main contributors.²²⁻²⁷

Studying the host-guest complexation of simple hydrocarbons as guest molecules in water is a promising approach to understand the interplay between hydrophobic and dispersion

interactions, because electrostatic (ion-ion, ion-dipole, dipole-dipole, and hydrogen-bonding) interactions between the host and guest are largely eliminated. Their propensity to bind within the hydrophobic cavity of macrocyclic hosts is well known.^{21,28–37} The encapsulation of hydrocarbons in self-assembled molecular capsules has been intensively experimentally investigated by Rebek and coworkers.^{38–42} Cucurbit[*n*]urils (CB*n*, Figure 1) are water-soluble macrocyclic host molecules with a nonpolar cavity that consist of *n* glycoluril units bridged by methylene groups, and whose shape resembles that of a pumpkin.^{5,43–47} CB*n* macrocycles show record affinities for guest binding^{44,48–52} with well-defined application potential.^{53–57} The parent homologue CB6 is able to accommodate small hydrocarbons (gases) in its cavity with high affinities (up to micromolar),²¹ while the larger homologue CB7 also complexes large hydrocarbons or steroids strongly.^{53,58,59}

Here we report experimental affinities of 22 hydrocarbons with CB7, which formed the basis for a blinded prediction challenge, the HYDROPHOBE challenge.⁶⁰ Such challenges are of great use to developers of computational prediction methods, because they allow an unbiased test of their accuracy. Indeed, methods tuned via retrospective studies on existing datasets do not always show the same level of accuracy in prospective studies. Here, measured host-guest binding free energies are compared with predictions made by two very different computational approaches: 1) a quantum-mechanical electronic structure approach which considered between one and seven low-energy bound conformers in a continuum solvent; and 2) classical all-atom molecular dynamics simulations in explicit solvent. Published results for some of the same guests with CB6²¹ were available as a calibration set.

Previous host-guest blinded prediction challenges involving CB*n* contained predominantly cationic guests,^{52,61–65} which introduced strong electrostatic interactions with the carbonyl rim of the CB*n* that caused a separate challenge for some computational approaches. Moreover, the solubility of the cationic guests was unknown, which precluded a comparison of calculated *versus* experimental hydration thermodynamics. In addition, the experimental measurements were done in the presence of significant (up to 50 mM)^{48,62} cation concentrations due to added buffer/salt, conditions which were invariably expected to lead to lower affinities than in the absence of metal ions (due to metal ion binding to the carbonyl portals of these hosts)^{21,66,67} – and conditions which could not be consistently incorporated in all computational methods. Moreover, the guest molecules studied did not span a wide range of sizes.^{61–63} In contrast, the dataset generated for the HYDROPHOBE challenge was measured in the absence of added metal ions (solely 3 mM HCl to maintain constant pH, conditions which do not affect binding of neutral guests to cucurbiturils).⁶⁸ The employed hydrocarbons also covered a homologous series with a wide range of guest size and hydrophobicity, and the hydration free energies of these common guests are known. As will be seen, the cross-check of the hydration thermodynamics proved essential to introduce a reference-state correction.

In the Results and Discussion sections below, we address as separate subsections the experimental data, the quantum-mechanical methods and results, and the molecular dynamics methods and results. We then consider the relationship between the two different computational approaches taken in this challenge, QM and MD. In addition to the HYDROPHOBE challenge results, we consider the binding conformations of the guests,

decomposition of the binding free energy calculations, the comparison of guest hydration free energies, and possible sources of error. An extensive Supporting Information (SI) section is attached for expert details.

EXPERIMENTAL and COMPUTATIONAL METHODS

Experimental Details.

Fluorescence measurements were done with a Varian Eclipse fluorimeter. ^1H NMR spectra were recorded on a JEOL ECX 400 MHz NMR spectrometer. Displacement experiments with the CB7•4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) reporter pair were done by using a rubber-sealed long-neck quartz cuvette. The different gases were introduced to the aqueous mixture containing the CB7•dye complex by slowly purging (ca. 1 bubble per second) with a long needle. For the online monitoring of the complexation of volatile liquid hydrocarbons, we employed N_2 as carrier gas to transfer them through the gas phase into the aqueous solution containing the reporter pair until the solubility limit was reached. All binding constants (K_a values in Table 1) were calculated by assuming a 1:1 complexation stoichiometry and obtained through repetitive single-point measurements of the fluorescence intensity after dye displacement in the respective hydrocarbon-saturated solution, which was indicated through a plateau value of the fluorescence intensity.²¹ Further details are given in SI.

QM Calculations.

The binding free energy, G , was computed as the sum of three contributions, the electronic gas phase binding energy E , the thermal free energy ΔG_{RRHO}^T , and the solvation free energy $\Delta\delta G_{\text{solv}}^T(X)$. The temperature was set to 298 K and water was used as solvent, which matches the experimental conditions.

$$\Delta G = \Delta E + \Delta G_{RRHO}^T + \Delta\delta G_{\text{solv}}^T(\text{water})$$

All DFT calculations were performed with the TURBOMOLE 7.0.2 program package.^{69,70} The geometries were fully optimized at the composite PBEh-3c⁷¹ level using the resolution of identity (RI) approximation for the Coulomb integrals.⁷² Vibrational frequencies were subsequently calculated to confirm the optimized as local minima, 2) to obtain ΔG_{RRHO}^T (using frequencies scaled by a factor of 0.95), and to generate the quantum-mechanically derived force field (QMDFF) for each complex.⁷³ The QMDFF was then used in conformational searching using the simulated annealing approach for low-lying structures (see SI), followed by further refinement at the PBEh-3c level to find one to seven low-lying structures per guest molecule to be used for the evaluation of the final G on higher level of theory given as the Boltzmann-weighted average over all distinct conformers including conformational entropy. For the submission QM3, to include also host-guest structures potentially favored in aqueous solution, the PBEh-3c level was also used in combination with the COSMO solvation model for water (with a dielectric constant of 78.39 and a

solvent radius of 1.93 Å);⁷⁴ this eventually led to improved structures for nine CB7 complexes (with guests **1**, **7**, **8**, **9**, **11**, **13**, **15**, **19**, **20**).

For the submissions QM1, QM2, and QM3, better binding energies E were computed at the dispersion corrected^{75,76–78,79,80} meta-GGA TPSS-D3^{ATM}/def2-QZVP^{81,82,83}, and meta-hybrid PW6B95-D3^{ATM}/def2-QZVP⁸⁴ (with discarded g- and f-functions on non-hydrogen and hydrogen atoms, respectively) and the DLPNO-CCSD(T)/CBS*⁸⁵ levels, respectively. For each molecule, the thermostatical free energy correction G_{RRHO}^T was computed at the PBEh-3c level (or PBEh-3c + COSMO level when COSMO was included for geometry optimization) at normal conditions (298 K and 1 atm) according to the modified rigid-rotor-harmonic-oscillator (RRHO) scheme,⁸⁶ using a scaling factor of 0.95⁷¹ for computed harmonic vibrational frequencies. The solvation free energy $\delta G_{\text{solv}}^T(\text{water})$ was computed by the COSMO-RS solvation model^{87,88} in water using the COSMO-RS 12fine (for QM1) and COSMO-RS 13 (for QM2 and QM3) parametrizations and the 1 atm gas/mole fraction reference state.

To account more efficiently for the solvation effects on the optimized complex structures in water, a revised QM3 procedure (i.e., QM4) was also developed. The conformational searching was now done with the semi-empirical XTB Hamiltonian⁸⁹ together with the GBSA solvation model for water, followed by harmonic frequency calculations also at this level to provide the thermostatical free-energy corrections (G_{RRHO}^T). The geometry refinement was performed at the PBEh-3c level together with the DCOSMO-RS solvation model⁹⁰ and DLPNO-CCSD(T)/CBS* single-point and COSMO-RS(13) calculations. The final binding free energy, G , was calculated either as the Boltzmann average of an ensemble of individual conformers including the conformational entropy (QM1, QM2, and QM4) or by using the lowest-lying conformer (QM3, due to high computational demands for DLPNO-CCSD(T)/CBS* calculations and small effects of Boltzmann averaging on final binding free energies G).

MD Simulations.

The MD1 and MD2 submissions were absolute binding free energy calculations generated using molecular dynamics simulations and referenced to the standard concentration of 1 M. The free energy difference between the bound and unbound state was computed using the attach-pull-release (APR) method described in detail by Henriksen et al.²⁷ Briefly, this approach involves attaching a set of restraints to control the position and orientation of the guest relative to the host, followed by removal of the guest from the host into bulk solvent. The work of moving the guest along this free energy path can be computed from the mean restraint coordinate values using thermodynamic integration (or other similar approaches, such as multi-state Bennet acceptance ratio (MBAR)). An analytic correction to place the unbound guest at standard concentration allows the results to be compared with experiment. In order to avoid complications from hysteresis, the APR steps are split into a series of independent simulation windows, which has the added benefit of enabling simultaneous calculation of the entire free energy pathway on a computing cluster. In addition to the binding free energy, we also compute the binding enthalpy by taking the difference of the mean potential energy between the bound and unbound state simulation windows. In order

to achieve meaningful uncertainties for these values, these simulations must be run long enough to converge the large energy fluctuations from solvent. All simulations were performed with the Amber14 molecular dynamics package.⁹¹ The specific force field parameters and simulation settings are described in the following sections.

The MD1 and MD2 calculations differed in the choice of force fields for both the solute and solvent. MD1 combined the OPC water model,⁹² RESP partial charges via the R.E.D. server,⁹³ and the new GAFF2 force field for bonded and Lennard-Jones parameters. The MD2 calculations followed a traditional, widely used approach: the TIP3P water model,⁹⁴ AM1-BCC partial charges,^{95,96} and the GAFF (v1.7) force field for bonded and Lennard-Jones parameters.⁹⁷

The initial configuration of the bound host-guest pair was generated via manual placement. The guest was subsequently translated via automated script to the appropriate location along the APR pathway for each simulation window. Three non-interacting anchor particles were placed in the box and restrained in the lab frame in order to align the pulling axis and thereby reduce the total solvent required for the simulation box. In particular, to carry out the “pull” phase, the guest was moved ~ 18 Å from the host cavity by stepwise increasing the target value of a distance restraint between an anchor particle and the guest, to define a set of 45 simulation windows, in each of which the guest was restrained to the target distance from the host. The resulting host-guest setups were independently set up and solvated in an orthorhombic box with 2210 water molecules and dimensions of approximately $36 \times 36 \times 53$ Å (Figure S21). The orientation of the CB7 host portal openings was aligned with the long box axis via six restraints to three non-interacting anchor particles at fixed positions in the laboratory frame. These restraints (one distance, two angle, and three dihedral) were present in all phases of the APR approach and were chosen in such a way as to not perturb the internal degrees of freedom of the host (see Henriksen et al.²⁷ for complete description). In contrast, restraints between the anchor particles and the guest molecule, as well as conformational restraints which forced apart the CB7 portal openings, were attached by increasing the force constant over a series of 15 simulations. The CB7 conformational restraints are released via an additional 15 simulation windows, leaving only the restraints maintaining separation between the host and guest.

All production simulations were performed with the GPU accelerated pmemd.cuda program from Amber14. Following a brief energy minimization and NVT thermalization at 298.15 K, the density was equilibrated for 2.0 ns under NPT conditions. The endpoint simulation windows (the bound and unbound states) were extended to 250 ns of simulation time for the purpose of computing binding enthalpies. The rest of the simulation windows were run to between 5.0 and 50 ns, terminating when they reached a specified error threshold in the mean restraint coordinate uncertainties. A longer-than-usual time step of 4 fs was employed in conjunction with hydrogen mass repartitioning of the solute. This combination was found previously to have no adverse effect on binding thermodynamics.²⁷ Temperature regulation of the system was controlled via the Langevin thermostat⁹⁸ and pressure regulation via the Monte Carlo barostat.⁹⁹ A cutoff value of 9.0 Å was used for Lennard-Jones interactions as well as limiting the direct space sum for PME electrostatics. Default values for all other PME parameters in Amber14 were employed.

RESULTS AND DISCUSSION

1 Experimental Results

1.1 Binding Affinities—The hydrocarbons studied here include saturated and unsaturated, linear, cyclic and bicyclic, as well as branched and unbranched molecules (Figure 1). The binding constants (Table 1) were measured by using fluorescent indicator displacement,^{21,100,101} which has the distinct advantage that even highly hydrophobic guests, including large hydrocarbons with very low solubilities (μM), can be studied at low host concentrations.^{21,101} A 1:1 binding stoichiometry was assumed, based on ^1H NMR data for the small hydrocarbons, and based on the fact that the CB7 cavity can only fit one molecule of the large hydrocarbons. 4',6-diamidino-2-phenylindole, DAPI (Figure 1),^{101,102} was employed as indicator dye, which, upon complexation with CB7 in aqueous solution, undergoes a large fluorescence enhancement ($\lambda_{\text{ex}} = 361 \text{ nm}$, $\lambda_{\text{em}} = 468 \text{ nm}$, $I_{\text{final}}/I_{\text{initial}} = 15$). The binding constant of DAPI was previously determined, and was taken as $1.1 \times 10^7 \text{ M}^{-1}$, see Figure S1.^{101,102} Transferring gaseous (**1–12**, and **15**), volatile liquid (**13**, **14**, **16–21**), and solid (**22**) hydrocarbons into the aqueous CB7•DAPI solution ($2.4 \mu\text{M}$ CB7 and $1.0 \mu\text{M}$ DAPI) resulted in a decrease in the fluorescence signal, indicating the displacement of the dye by the competitive hydrocarbon binder (see Methods and Figure S2). The binding constants were determined analogously to the thoroughly tested procedure for CB6,²¹ where the concentration of the hydrocarbon corresponds to its known aqueous solubility; solubility data are given in Table S2 in the SI. The data were fitted according to a competitive binding model in which the binding constant of the dye was fixed (see ref.²¹ for details). Uncertainties in the experimental binding constants depend on reported solubilities of the hydrocarbons in water. For small hydrocarbons, the solubilities are accurately known.¹⁰³ A predicted solubility was used for norbornene (see Table S2), where no experimental data were available.

All hydrocarbon guests **1–24** showed binding to CB7 (Table 1). The compounds that bind to CB7 have up to 14 carbons (e.g., diamantane, **24**). The smaller hydrocarbons, which are gases at ambient temperature and pressure and which were known to bind to CB6 (**1–14**, and **16**),²¹ were complexed by CB7 as well, but with relatively low affinities (millimolar K_{a}); acetylene exhibited the lowest affinity. The binding affinity tends to increase for the larger hydrocarbons, as expected from their higher hydrophobicity and better space filling inside the cavity (see packing coefficients, PC , in Table 1). That said, a similar or slightly higher affinity was found for adamantane ($2.2 \times 10^9 \text{ M}^{-1}$) than for the larger diamantane ($1.6 \times 10^9 \text{ M}^{-1}$), which points to a packing limitation.^{43,53}

While the HYDROPHOBE challenge was ongoing, binding affinities of alkanes (n -hexane to n -dodecane) with CB7 were reported by using the solubilization method, showing similar trends but large variations in the absolute values.⁵⁸ This variation is attributed to the differences in the methodology used to determine the binding affinities. Contrary to the dye displacement technique, the solubilization method requires high mM concentrations of CB n and excess of alkanes, which can lead to underestimated binding constants. For example, high CB n concentrations result in the formation of higher aggregates.¹⁰⁴ It should be noted that the computational methods used herein are invariably in better absolute agreement with

the experimental affinities obtained by the displacement methodology, which operates under more ideal, since more dilute, conditions.

1.2. Structure of the Host-Guest Complexes—The complexation of hydrocarbons with CB7 was examined by using ^1H NMR spectroscopy (Figure 2 and SI: Figures S3–12). When CB7 and the hydrocarbons were mixed, up-field shifts of the guest protons were observed. These up-field shifts are diagnostic for the formation of inclusion complexes.^{58,105} For example, the *n*-pentane (**13**) protons experience considerable up-field shifts upon encapsulation by CB7 ($\delta = 0.60\text{--}0.70$ ppm). The longer *n*-alkanes ($n > 5$) showed upfield shifts of the central CH_2 and the CH_3 groups as well. The NMR data suggest that the *n*-alkanes adopt a folded shape inside the CB7 cavity (see Table S1 in SI). A dimension comparison between the CB7 cavity height (6.20 Å, from the oxygen centers of the glycoluril unit) and *n*-alkane lengths (distance between the two terminal methyl carbon atoms, for *n*-hexane = 6.43 Å up to *n*-nonane = 10.21 Å) and the fact that all protons showed up-field shifts suggest a compressed conformation of *n*-alkanes inside the hydrophobic cavity of CB7 (see below). Moreover, the coupling constant of the terminal CH_3 -protons in the complexed form was found to be different from that of the uncomplexed *n*-alkanes in neat form or dissolved in D_2O . For example, the methyl protons in *n*-heptane (**21**) have a coupling constant of 6.87 Hz in the neat liquid and in D_2O , which increased to 7.33 Hz upon inclusion complex formation with CB7. The increase in coupling constant reveals that *n*-heptane (**21**) adopts a folded skeleton rather than an extended zig-zag one.¹⁰⁶ This folding can maximize favorable dispersion interactions and hydrophobic desolvation.^{38,39,107}

The conformation of the free and encapsulated *n*-alkanes was further analyzed by 2D-NOESY experiments (Figures S13–16 in SI), which have been previously used to determine their conformations.^{108,109} NOE cross-peaks between C_1 and C_3 as well as C_4 were observed in the case of *n*-heptane (**21**) inside the CB7 cavity, pointing to an onset of helical distortion. With one more methylene unit (*n*-octane) the guest actually assumes a more pronounced helical conformation, as clearly demonstrated by the presence of the diagnostic $\text{C}_i\text{--C}_{i+2}$ and $\text{C}_i\text{--C}_{i+3}$ NOE cross-peaks (SI, Figure S16). This “induced hydrophobic fit” was an important structural detail, which was correctly reflected by the calculations (see below).

2 Comparison of Calculations with Experiment: The HYDROPHOBE Challenge

Table 1 and Figures 3 and 4 compare the experimental binding free energies with blinded predictions by QM electronic structure methods (three submissions: QM1, QM2, and QM3) and classical MD simulations (two submissions: MD1 and MD2). Although guests **23–26** are not regarded as part of the challenge, because their affinities had been published or estimated previously,^{53,101} they are included in the table, but not the summary statistics, as instructive extra cases. Overall, both classes of computational methods yield positive correlations with the experimental results. The MD1 method yields the highest correlation, but the QM results agree better with experiment in absolute terms (Table 1).

Before, however, entering a more in-depth analysis of quantitative data, it is important to define the quantities relevant for comparison between theory and experiment. In particular, the reference state, which the thermodynamic data refer to, remains a recurring source of

formal discrepancy. Experimental affinity data that describe supramolecular, macromolecular, and biomolecular processes, and particularly those referring to association or host-guest complexation, are conventionally reported in units of M^{-1} (affinity constants K_a) or M (dissociation constants K_d), that is, the derived binding free energies (ΔG) refer to a reference state of 1 mole per liter solution, which we indicate here as $\Delta G(1\text{ M})$. In the QM approaches used in the present challenge, binding free energies include contributions attributable to direct host-guest interactions of the isolated guests in the gas phase (ΔG_{gas}) and related to solvation (ΔG_{solv}), which need then to be provided in the same molar reference framework. For consistency, and to ensure comparable entropic components to the driving force for bimolecular and higher-order reactions, the molar reference state framework needs to be also conserved in the gas phase, that is, 1 mole per liter gas, which corresponds to an ideal-gas pressure of 24.5 atm at standard temperature (298.15 K). These standard conditions, namely 1 M or a pressure of 24.5 atm in the gas phase and 1 M in solution, have been advocated by Ben-Naim and named accordingly.^{110,111} In contrast, many quantum chemistry programs use the 1-atm standard state in reporting thermochemical quantities, which is the reference state typically employed for gas-phase binding phenomena.

When the components to the binding free energy are calculated at different standard pressures or concentrations (indicated in parentheses in eqs 1–7), correction terms need to be considered. If ΔG_{gas} or ΔG_{solv} are computed with a reference pressure of 1 atm, then correction terms of $R T \ln(24.5) = 1.90\text{ kcal mol}^{-1}$ need to be added (eq. 2) or subtracted (eq. 3); and when solvation free energies are obtained with a mole fraction concentration reference state (1 mole mole⁻¹), a solvent-dependent correction term applies (eqs. 4 and 5), which amounts to $R T \ln(55.5) = 2.38\text{ kcal mol}^{-1}$ in water, with 55.5 being the molarity of water in 1 liter of water. In some mixed cases both corrections terms need to be summed up, which leads to different correction terms (eqs. 6 and 7). As can be seen, the required corrections, which range from 0.48–4.28 kcal mol^{-1} , are significant and cannot be ignored when comparing absolute thermodynamic data. This is particularly true because predictions frequently aim at or claim accuracies better than $\pm 1\text{ kcal mol}^{-1}$, and because correlations or absolute agreement between experiment and theory are used for methodological adjustments or parameterizations.

$$\Delta G(1\text{ M}) = \Delta G_{\text{gas}}(24.5\text{ atm}) + \Delta G_{\text{solv}}(24.5\text{ atm}/1\text{ M}) \quad (1)$$

$$\Delta G(1\text{ M}) = \Delta G_{\text{gas}}(24.5\text{ atm}) + \Delta G_{\text{solv}}(1\text{ atm}/1\text{ M}) \quad + 1.90\text{ kcal mol}^{-1} \quad (2)$$

$$\Delta G(1\text{ M}) = \Delta G_{\text{gas}}(1\text{ atm}) + \Delta G_{\text{solv}}(24.5\text{ atm}/1\text{ M}) \quad - 1.90\text{ kcal mol}^{-1} \quad (3)$$

$$\Delta G(1 \text{ M}) = \Delta G_{\text{gas}}(24.5 \text{ atm}) + \Delta G_{\text{solv}}(24.5 \text{ atm}/1 \text{ mole mole}^{-1}) + 2.38 \text{ kcal mol}^{-1} \quad (4)$$

$$\Delta G(1 \text{ M}) = \Delta G_{\text{gas}}(1 \text{ atm}) + \Delta G_{\text{solv}}(1 \text{ atm}/1 \text{ mole mole}^{-1}) + 2.38 \text{ kcal mol}^{-1} \quad (5)$$

$$\Delta G(1 \text{ M}) = \Delta G_{\text{gas}}(24.5 \text{ atm}) + \Delta G_{\text{solv}}(1 \text{ atm}/1 \text{ mole mole}^{-1}) + 4.28 \text{ kcal mol}^{-1} \quad (6)$$

$$\Delta G(1 \text{ M}) = \Delta G_{\text{gas}}(1 \text{ atm}) + \Delta G_{\text{solv}}(24.5 \text{ atm}/1 \text{ mole mole}^{-1}) + 0.48 \text{ kcal mol}^{-1} \quad (7)$$

The MM-calculated binding free energies from submissions MD1 and MD2 as well as the associated hydration free energies refer directly to $G(1 \text{ M})$ and, implicitly, to $G_{\text{solv}}(24.5 \text{ atm}/1 \text{ M})$ as required in eq. 1 such that no corrections need to be considered in the Results or Discussion sections. The original QM submissions (see SI, Table S3 and Figure S20), referred to $G_{\text{gas}}(1 \text{ atm})$ and $G_{\text{solv}}(1 \text{ atm}/1 \text{ mole mole}^{-1})$ data, which required an *ex post* reference-state correction by $+2.38 \text{ kcal mol}^{-1}$ according to eq. 5 for direct comparison with experimental binding free energies. The following Results section analyzes the QM1–QM3 results after this correction; the Discussion part illustrates the improvements related to the correction in the context of the original submitted data as well as the newly added QM4 submission.

2.2. Quantum-Chemical Calculations—Electronic structure calculations were used to compute the binding free energies (G). Each host-guest complex is represented by a set of discrete structures that model the thermodynamically favored binding geometries that the guest adopts inside the host. These are determined as potential energy minima by geometry optimization following a conformational search. The structure with minimal (free) energy was taken as representative of the free (unbound) flexible guest. For the linear alkanes (7, 13, 17 and 21), this is the fully extended conformation. The minimal energy structures of the other guests (8, 14, 16, 18 and 19) were identified by manual scans of their rotatable bonds. The present computational approach was introduced in ref.⁸⁶ (see Methods and SI) and since then applied several times.^{24,61,113} A recent overview also of work by other groups is given in ref.¹¹⁴.

2.2.1 Calculated Structures: Figure 5 shows the optimized structures that give the largest contribution to G for the CB7 complexes of the guest molecules 1–22 of the HYDROPHOBE challenge. It appears that all guest molecules can be accommodated inside the host cavity. The linear alkanes (*n*-pentane, *n*-hexane, and *n*-heptane) adopt a folded (g+g+, g+g+g–, and g+g+g–g–, respectively) conformation inside CB7 (Figure 5), in contrast to the fully extended structure preferred in solution and inside the smaller homologue CB6 (see

S17). The CB7 cavity is sufficiently large to allow both axial and equatorial binding modes. For benzene (**20**) the equatorial binding mode is not much disfavored over the axial orientation shown in Figure 5. No direct interaction with the carbonyl groups at the entrance of the host occurs. In four cases the host-guest complex is represented by a single structure (guests **1**, **3**, **4**, and **15**), since no other binding modes were found by the search procedure. The other complexes are modeled by ensembles of up to seven conformers (guests **13** and **21**), such that in total 70 structures are considered. In some cases, only one structure has a considerable contribution (e.g., guest **19**), because it is much more stable than all others, while for the complex with guest **22**, the individual G values of four structures span a range of less than 1 kcal mol⁻¹. The Cartesian coordinates of the structures with the largest contribution to G are provided in the SI.

2.2.2. Comparison to Experiment: Figure 3 compares the QM-calculated and experimental binding free energies of the host-guest complexes. Three QM method combinations have been contributed, denoted as QM1 (TPSS⁸¹-D3^{ATM}^{75,78}/def2-QZVP^{82,83} // PBEh-3c⁷¹ // COSMO-RS^{87,88} (12-fine)), QM2 (PW6B95⁸⁴-D3^{ATM}/def2-QZVP' // PBEh-3c // COSMO-RS (13)), and QM3 (DLPNO-CCSD(T)^{85,115}/CBS* // PBEh-3c (PBEh-3c +COSMO for guests **1**, **7**, **8**, **9**, **11**, **13**, **15**, **19**, **20**) // COSMO-RS (13)). For QM1 and QM2, the G data were obtained for both, as single values for the lowest-lying conformer and as Boltzmann average of the individual G values for an ensemble of low-energy conformers, and the latter are entered in Figure 3. However, since the averaging over several conformers led only to minor variations, the computationally most demanding QM3 values were only obtained for the lowest-lying conformer.

For the three submissions, good agreement with experiment was obtained. While the experimental G values vary between -2.9 and -8.9 kcal mol⁻¹ for the guests **1–22**, the computational results fall into the range from 1.2 to -13.4 kcal mol⁻¹ (Table 1). In general, QM1 tends to systematically underestimate the G values (MSE of 2.29 kcal mol⁻¹, Table 1) but this can be evidently improved at higher QM2 and QM3 levels. For the set of the smallest guests **1–16**, with up to five carbon atoms, good absolute accuracy (MUE and RMSE of 1.1 and 1.3 kcal mol⁻¹) and correlations (with $R^2 \sim 0.9$ and linear regression slope ~ 1.7) with respect to experimental data were observed at both QM2 and QM3 levels. In all cases, the use of PBEh-3c/COSMO-optimized geometries in QM3 led to more negative G values than in QM2, likely due to better binding structures. The largest outliers are cyclohexane (guest **19**) and *n*-heptane (guest **21**) that are consistently over- and under-bound at all QM levels (especially for QM2 and QM3, by ~ 5 kcal mol⁻¹), respectively, which evidently reduce the overall correlation between experiment and QM results (with $R^2 \sim 0.6$ and slope ~ 1.4 , Table 1). Nevertheless, good correlations (with $R^2 \sim 0.9$) were observed for all QM results, and the overall *MSE*, *MUE*, and *RMSE* for the most robust QM2 results are -0.01, 1.48, and 1.94 kcal mol⁻¹, indicating strong prediction power of this theoretical approach.

2.2.3. Contributions to G : Figure 6 shows the contributions to the calculated free energy of association for the 22 CB7•hydrocarbon complexes adopting the structures shown in Figure 5 according to the QM2 submission: the electronic binding energy E , thermal

corrections from energy to free energy $G^{\text{T}}_{\text{RRHO}}$, and the solvation free energy $\delta G^{\text{T}}_{\text{solv}}$ of the gas phase species. For analysis purposes the electronic binding energy is furthermore divided into the dispersion correction and the part from the pure DFT calculation itself; and the dispersion part is, finally, subdivided into the two- and three-body terms. Although the dispersion correction is not the dispersion energy, e.g., since its value depends on the choice of the density functional, its size is a good measure for the importance of dispersion interactions in the association reaction under consideration, in particular, when comparisons between similar systems are done. While this estimate of the dispersion energy contribution is readily accessible in case of the submissions QM1 and QM2 which are based on dispersion-corrected DFT, submission QM3 relies on wave function theory and quantifying the dispersion contribution requires an energy decomposition analysis¹¹⁶ which, however, was not performed. The resulting free energy of binding is displayed as a thin line. It is composed of mutually compensating terms with opposite signs. The ro-vibrational term, including a translational component, is always repulsive, due to the loss of three translational and three rotational degrees of freedom upon host-guest complex formation (i.e., two molecules forming a one single complex); it varies between 6.0 kcal mol⁻¹ for guest **1** (methane) and 15.4 kcal mol⁻¹ for guest **21** (*n*-heptane). In many cases, it effectively compensates the electronic binding energy. The binding energy is dominated by the DFT dispersion correction term except for acetylene (guest **4**), where the DFT contribution exceeds E_{disp} . The three-body dispersion term, the smallest contribution to G , is always positive and varies from 0.6 to 2.9 kcal mol⁻¹. The dispersion interaction of the host and the guests with the solvent, which is included in $\delta G^{\text{T}}_{\text{solv}}$, is not separately displayed.

The binding energies E of the QM1 submission (SI, Table S10) are consistently less negative than those of the QM2 submission except for those of guests **9** (*cis*-butene) and **19** (cyclohexane), which differ by only 0.05 and 0.03 kcal mol⁻¹, respectively. The MUE of the two sets of binding energies is 0.4 kcal mol⁻¹ with the maximal deviation of 1.1 kcal mol⁻¹ for the complex of guest **18** (2,3-dimethylbutane). The E estimates of QM3 (SI, Table S14) are generally more negative than those of QM2. The mean average deviation is 0.9 kcal mol⁻¹ and therefore larger than that between the QM1 and QM2 E values. The difference ranges from 0.8 kcal mol⁻¹ for guest **20** (benzene) to -2.6 kcal mol⁻¹ for guest **8** (1-butene). It should be noted, however, that a part of this deviation originates from the different choice of structures in QM3 than in QM1 and QM2. This, together with the fact that in nine cases the harmonic frequencies have been calculated in the presence of the COSMO continuum solvation model for water, is the reason for the differences of the $G^{\text{T}}_{\text{RRHO}}$ contributions of submissions QM2 and QM3, which is exactly the same in QM1 and QM2. The difference ranges from -1.2 kcal mol⁻¹ for the complex with guest **18** (2,3-dimethylbutane) to 2.4 kcal mol⁻¹ for guest **16** (cyclopentane) with a MUE of 0.9 kcal mol⁻¹. The largest deviation occurs between the solvation contributions $\delta G^{\text{T}}_{\text{solv}}$ to the free energy of binding, which is by 0.4 to 4.0 kcal mol⁻¹ more negative for QM2 than for QM1, on average by 2.3 kcal mol⁻¹. Even between QM2 and QM3, deviations of the solvation term from -0.9 to 4.3 kcal mol⁻¹ with a MUE of 1.1 kcal mol⁻¹ occur due to differences in structure selection. The variability of the solvation contribution is in some part related to the fact that the COSMO-RS parameterizations have been done against databases of experimental reference values for smaller molecules than the CB7 host and the COSMO-RS error increases with the size of the

molecular surface area. All contributions for the submissions QM1 to QM3 are listed in the SI.

2.3. Molecular Dynamics Simulations—The free energy calculations using molecular dynamics simulations were performed via the attach-pull-release (APR) approach.²⁷ The two submissions, MD1 and MD2, differ only in the potential functions used, among which MD1 can be considered more “cutting-edge” (see Methods section for details). Although experimental data for several of the guests with CB6 were available in the literature,²¹ these were not used to select or calibrate the present MD models or methods.

2.3.1. Comparison with Experiment: Overall, the MD1 and MD2 submissions performed similarly. Both significantly overestimated the CB7 binding affinity for most guests, particularly those with high affinity. This led to a linear regression slope greater than 2.0, RMSE values greater than 5.5 kcal mol⁻¹, and MSE values ranging from -3.8 to -4.7 kcal mol⁻¹ (see Figure 4 and Table 1). Nonetheless, the calculated values correlate well with experiment, with R^2 values of 0.84 and 0.75 for the MD1 and MD2 submissions, respectively (Table 1). If the four additional guests which were not part of the formal challenge are included (22–26), the error metrics worsen slightly (Table S6). Although the MD1 and MD2 submissions had similar error statistics with respect to experiment, there were some predictions, particularly for the unsaturated guests, which differed significantly. For example, the deviation in MUE between the MD1 and MD2 predictions for the (saturated) alkane guests, excluding diamantane which we discuss below, amounted to 0.4 kcal mol⁻¹. In contrast, the deviation in MUE between the two submissions for the unsaturated and perfluorinated guests was 1.8 kcal mol⁻¹. However, the overall correlation between the two submissions is high ($R^2 = 0.93$) and the relative offset between the submissions is modest (MSE = +0.62 kcal mol⁻¹, MD1 relative to MD2).

2.3.2. Thermodynamic Contributions to Binding Affinity: To gain deeper insight into the thermodynamic determinants of host-guest binding, we routinely compute the binding enthalpy, which is straightforward in the APR framework. When this quantity is subtracted from the binding free energy, the entropic contribution to binding is obtained, thus providing a full thermodynamic profile of binding. Although the experimental binding enthalpy is not available for this challenge, a number of interesting observations may be made when comparing the MD1 and MD2 submissions. For example, binding is favored both enthalpically and entropically for all guests, except for the lowest affinity binders in submission MD1, which are enthalpically disfavored (Figure 7 and Table S4), and for the adamantyl/diamantyl and perfluorinated guests in the MD2 submission, which are entropically disfavored (Figure S22 and Table S5). Interestingly, the binding enthalpy values for the MD2 predictions are significantly more negative than the MD1 predictions by an average MSE of 4.5 kcal mol⁻¹, while the correlation between the two remains high ($R^2 = 0.91$). This difference is largely compensated via more unfavorable entropic components for MD2 relative to MD1, by an average of 3.9 kcal mol⁻¹.

The binding enthalpy may itself be decomposed into contributions from the valence energy (bonds, angles, dihedrals, and 1–4 interactions), van der Waals (VDW) energy, and electrostatic energy. We find that the valence and VDW contributions are the primary source

of the difference in binding enthalpy between MD1 and MD2 (Table S4 and S5). Although electrostatics do not contribute to this difference, it should be noted that they contribute a significant and variable energy to the overall binding enthalpy, up to $-25 \text{ kcal mol}^{-1}$ for the diamantane prediction in MD2. This might result in part from the ejection of “unhappy” water from the CB7 cavity by the guests.^{10–12,15,43,117} In addition, the partial charges themselves are quite different between the MD1 and MD2 submissions, a consequence of using different charge assignment schemes (see SI, Figure S23 and Table S7). Although the largest deviation from experiment was observed for adamantane (**23**), which also happens to bind with the highest affinity, the greatest deviation from the linear regression line was observed for diamantane (**24**). In contrast to adamantane (**23**), for which binding affinity is dominated by enormous VDW and electrostatic components and essentially neutral valence energy in both MD1 and MD2, diamantane (**24**) binding has an enormous valence energy penalty of 8.2 and 17.0 kcal mol^{-1} for MD1 and MD2 respectively, in addition to favorable VDW and electrostatic energies. This valence penalty effectively opposes the tendency of MD1 and MD2 to overestimate the binding affinity and places the diamantane predictions closer to experiment than any of the other high-affinity guests.

2.3.3. Unusual Binding Conformations: A surprising deviation between MD1 and MD2 occurs for binding of the benzene (**20**) guest. The binding affinity predictions differ by 4 kcal mol^{-1} , the largest difference among all guests, and the binding enthalpy difference is even larger, at 9.6 kcal mol^{-1} . Most striking is that the binding affinity of benzene (**20**) is driven mainly by the entropic contribution for MD1 ($H = -3.0 \text{ kcal mol}^{-1}$, $-T S = -6.7 \text{ kcal mol}^{-1}$), whereas the reverse is true for MD2 ($H = -12.7 \text{ kcal mol}^{-1}$, $-T S = -1.1 \text{ kcal mol}^{-1}$). We speculate that this difference results from a difference in the binding poses of benzene (**20**), and indeed, inspection of the bound-state simulation trajectories reveals that benzene is much less constrained in the MD1 simulation than for MD2 (see Figure S24). It is likely that subtle decreases in the Lennard-Jones radius parameters of both the host and guest in MD1, relative to MD2, allow more room in the host cavity which permits the dramatic change in entropic contribution (see atom types “ca”, “c”, and “n” in Table S8).

As noted earlier, for larger *n*-alkanes, a helical conformation is adopted to maximize dispersion and minimize solvent exposure. This is also observed for the *n*-alkanes in the MD1 and MD2 submissions (see Figure 8). A histogram of the end-to-end distance for *n*-heptane in the bound and unbound states shows a large shift to a more compact structure (Figure S25), which matches the NMR results.

Structural ensembles for the bound state of each CB7-guest complex, taken from the MD1 and MD2 submissions, are provided in the SI. For each CB7-guest complex, a PDB file is provided with 50 evenly-spaced snapshots from a 250-ns bound state trajectory.

3. Discussion

3.1. Experimental Trends—The newly measured affinities of several hydrocarbons with CB7 exceed their previously measured values for the smaller homologue CB6²¹ and for acyclic CB*n*⁵⁸, as well as those of other macrocyclic hosts in aqueous solution.^{118–121} The selectivity in binding is strongly modulated by the size and the shape of the hydrocarbons.

For example, the affinity of linear alkanes increases monotonically with the number of carbons (Figure 9), while a preferential binding of CB7 for compact and branched hydrocarbons (e.g., isobutane, neopentane, and 2,3-dimethylbutane) over their linear isomers (e.g., *n*-butane, *n*-pentane, and *n*-hexane) was also observed. On the other hand, cyclic homologues were found to bind more weakly or with the same affinities as the noncyclic ones. Moreover, saturated hydrocarbons showed higher binding constants (by a factor of ca. 6) compared to unsaturated or aromatic ones with the same numbers of carbon atoms. The drop in the packing coefficients (*PC*%), and the fact that unsaturated hydrocarbons have 4–5 times higher water solubilities than saturated ones, are the main intuitive reasons for the reduced binding affinities. The same argument (in terms of *PC*% and solubility) may apply for cyclic and noncyclic alkanes (e.g., cyclopentane *versus* *n*-pentane). Good linear correlations between the experimental binding affinities with the octanol-water partition coefficient ($\log K_{ow}$, see Table S2 in SI) and the static electric dipole polarizability (α , see Table S2 in SI) of the guest molecules were observed (Figure 10), confirming that the hydrophobic effect, dispersion interactions, or both dominate the binding. In fact, the correlation coefficients of these empirical correlations ($R^2 = 0.79$ – 0.85) are better than those of the QM submissions ($R^2 = 0.59$ – 0.77) and similar as those obtained from the best MD submission, MD2 ($R^2 = 0.84$), see Table 1.

3.2. Discussion of QM Results—The quantum-mechanically determined binding free energies G of the CB7•hydrocarbon complexes are obtained as the sum of three terms, which are computed separately, however, for the same geometry, thus allowing independent choices for the applied levels of theory to determine E , G^T_{RRHO} , and δG^T_{solv} . Three different approaches for calculating the electronic structure are employed. The binding energy of two approaches is based on dispersion-corrected density functional theory (QM1 and QM2), and the binding energy of the other on a wave-function based approach (QM3). The thermal corrections from energy to free energy are the same in submissions QM1 and QM2 and the difference for QM3 originates from the different choice of structures which in nine cases were obtained in the presence of the COSMO continuum model for water. Finally, two different parameterizations of the COSMO-RS model to determine the solvation free energy of the gas-phase species were chosen by minimizing the mean average deviation of the calculated and published experimental binding free energy of the CB6•hydrocarbon complexes. These parametrizations differ amongst others in the level of the underlying DFT calculation and are denoted here according to the year of their release as COSMO-RS 12-fine (QM1) and COSMO-RS 13 (QM2 and QM3). The choice of the optimal continuum solvation model depends on the selected density functional or wave-function based method.

This indicates that, although being computed separately, the three components of the QM computed G values cannot be converged independently to the exact result, but have an influence on each other. While being quite similar in general, looking closely at the three QM-based submissions their performance slightly deteriorates from QM2 to QM3 (see Table 1). This behavior is opposite to the refinement of the quantum-mechanical level of theory applied which is highest for QM3. Thus, it appears that the interference between the three components of the computed G originates from a different degree of error compensation.

Error discussion.: Figure 11, top, shows the binding energies E computed at the two DFT levels of theory *versus* the results obtained for the same structures with the wave-function method. The higher computational effort for coupled-cluster calculations required the smaller number of complexes compared to those considered by DFT. In the estimate of the free energy of association, only the contribution of one structure per guest was used for QM3, as opposed to submissions QM1 and QM2, where a Boltzmann averaging was made. The mean absolute deviation between the PW6B95-D3^{ATM}/def2-QZVP' and DLPNO-CCSD(T)/CBS* results amounts to 0.5 kcal mol⁻¹ and for TPSS-D3^{ATM}/def2-QZVP to 0.7 kcal mol⁻¹. The maximal deviation is 1.9 and 1.7 kcal mol⁻¹, respectively. Most DFT values are within the 5% error range of the DLPNO-CCSD(T) reference values. In contrast, the contributions of the solvation free energy of the gas-phase species to the free energy of association compared between the two COSMO-RS parameterizations (Figure 11, bottom) have almost no correlation. It is obvious that at least one model for the solvation contribution differs from the true value considerably.

Another source of error might be defects of the optimized structures, due to the omission of solvent effects in structure determination. In submission QM3, this has been realized already for nine out of 22 host-guest complexes by including the COSMO solvation model for water into the geometry optimization (see SI, Section 2. Computational Part 2.1. Quantum-Chemical Calculations. QM technical details). In order to address this issue completely, we repeated the entire study from scratch after release of the experimental reference data set, now using the direct COSMO-RS (DCOSMO-RS)⁹⁰ model for geometry optimization and including the GBSA approach into the conformation search, based on a semi-empirical tight-binding Hamiltonian⁸⁹ instead of a force field. For more details please refer to the SI, Section 2. Computational Part, 2.1. Quantum-Chemical Calculations, New procedure (QM4). Furthermore, we included adamantane (**23**), diamantane (**24**), perfluorohexane (**25**), and perfluorocyclohexane (**26**) into the study for which experimental reference data are published.^{53,101} We denote this fourth set of quantum-chemically calculated results in the following as QM4 (DLPNO-CCSD(T)/CBS* // XTB + GBSA // COSMO-RS(13) on PBEh-3c + DCOSMO-RS optimized structures). The correlation of the QM4-calculated binding free energies with experimental values is shown in Figure 12. Finally, one could consider the selection of not optimally binding conformers as being a source of error. Aside from the observation that only underestimation of the experimental result can be explained that way, it is striking that cyclohexane (**19**), adamantane (**23**), and diamantane (**24**), which have no conformational issue (variability) at all in their complex with CB7, nonetheless show a large mismatch. It thus appears that the limit of the accuracy a continuum solvation model can deliver for the host-guest complexes investigated is reached.

Since the hydration free energies of the various hydrocarbons are known experimentally (in contrast to the salts investigated in previous host-guest affinity prediction challenges, see Table 2),^{52,61–65} it was possible to directly cross-check the COSMO-RS calculated values with experimental data. This comparison, which was only made after submission of the original QM1–3 data sets, revealed a rather uniform offset by 2 kcal mol⁻¹ along the series and indicated a systematic source of deviation. This led us to notice that the selected COSMO-RS reference state referred to a guest to water molar-fraction of 1.0 (the mole-

fraction reference state), which differs by a factor of 55.5 (the value for the molar concentration of water in water) from the 1.0 M concentration reference state relevant for the experimental hydration free energies. To account for the different reference state used in the COSMO-RS calculations, a correction by $-2.38 \text{ kcal mol}^{-1}$ ($-R \ln(55.5)$) is required which nicely accounted for the observed offset. However, due to the required correction in the COSMO-RS term for each species, the original QM data sets (QM1–QM3 in SI) as well as the expanded QM4 submission were adjusted by the same term, namely by $+2.38 \text{ kcal mol}^{-1}$ since a bimolecular associative reaction is involved according to eq. 5 (see Table 2 and SI, Table S3 and Figure S20). The reason that the correction term is negative for the hydration free energies but positive for the binding free energies is because the quantity that enters in eq. 5 is the *difference* of three hydration energies, one for the product and two for the reactants. The QM1–QM4 data shown in Tables 1 and 2 and discussed in the main text and all Figures are corrected for the reference-state.

After adjusting the COSMO-RS-calculated values to the Ben-Naim reference state (Table 2) an excellent correlation was obtained with experimental hydration free energies ($G_{\text{solv}}(\text{calc}) = -0.31 + 0.96 G_{\text{solv}}(\text{exp})$, in kcal mol^{-1} , $R^2 = 0.92$, Figure 13). Moreover, the COSMO-RS(12fine) data agree very well with the COSMO-RS(13) data (with the respective MSE and RMSE of only -0.13 and $0.16 \text{ kcal mol}^{-1}$) for all guests **1–22**. It is thus not the hydration free energies of the guests but more likely the hydration free energies of the CB7•guest complexes (Figure 11) that present the main source of error for the deviation between experimental and theoretical G values.

As can be seen from Table 2 (DEV values for the reference state-corrected QM4 data), the agreement across the entire set of guests of the HYDROPHOBE challenge (**1–22**) is much improved (MSE = $0.01 \text{ kcal mol}^{-1}$, MUE = $1.47 \text{ kcal mol}^{-1}$, and RMSE = $2.03 \text{ kcal mol}^{-1}$) in comparison to the QM1–3 submissions (Table 1) as well as the uncorrected QM4 submission (see SI), even if specific outliers slightly reduce the correlation ($R^2 = 0.45$). Excellent agreement is observed for hydrocarbons up to C5 (**1–16**), with the MUE value being as small as $0.79 \text{ kcal mol}^{-1}$, the smallest value obtained across all data sets. These smaller hydrocarbons **1–16** have in common that they can be completely immersed in the cavity of CB7 without geometrical deformations of the guests. For the longest included linear alkane, *n*-heptane (**21**), the binding is much underestimated by the QM4 method (by $+5.37 \text{ kcal mol}^{-1}$), a trend which already emerges for *n*-hexane (**17**, by $+2.45 \text{ kcal mol}^{-1}$). Indeed, helical deformations upon encapsulation have been experimentally detected for these longer *n*-alkanes (see above), which may account for this deviation. For most other large hydrocarbons (**19,20,22,23**), the QM4 data afford a significant overbinding by up to -4 kcal mol^{-1} . Since conformational fluctuations cannot be major contributors for these cyclic and bicyclic guests, and since their hydration free energies are also well reproduced (see Table 2), these deviations must be due to other reasons, e.g., the hydration free energies of the unbound host and of the host-guest complex.

Strikingly, even though perfluorinated hydrocarbons were not part of the original HYDROPHOBE challenge, the largest deviations in predicted binding free energies were observed for exactly these molecules (underbinding by $+4.5$ – $11.5 \text{ kcal mol}^{-1}$). While in the case of the extended-chain perfluorohexane (**25**) conformational sampling effects may again

contribute to the predicted far too positive free energies (similar to *n*-heptane), the underestimated binding for perfluorocyclohexane (**26**) indicates also a systematic variation related to guest perfluorination. Again, this large deviation is not due to an ill-predicted hydration free energy of the perfluorinated guests (see Table 2 and Figure 13). Strikingly, while the experimental binding free energies of the two perfluoroalkanes (**25** and **26**) and their alkane analogues (**17** and **19**) are essentially the same, within 1 kcal mol⁻¹, their calculated values differ largely, by up to 8 kcal mol⁻¹, both between their aliphatic and alicyclic forms and also between their substitution patterns. Such structure-activity relationships between homologous compounds are, of course, of great experimental and also practical interest, for example, for separation applications (see Figure S12 in the SI for an experimental demonstration), and pose an ongoing challenge for computational approaches.

3.3. Discussion of MD Results—The affinity predictions based on MD simulations correlate well with the *hitherto* blinded experimental data. However, they systematically overestimate the measured affinities, increasingly so for the higher affinity guests. Also, despite the overall correlation with experiment, for both MD1 and MD2, some of the experimental trends in binding affinity as a function of the size and character of the guests are not well replicated. For example, although experiment showed that there is a steady increase in binding affinity going from methane (**1**) up to *n*-heptane (**21**) (and beyond), the binding affinity of *n*-heptane is noticeably decreased for MD1 and MD2 relative to *n*-hexane by about 3 kcal mol⁻¹ (Tables S4 and S5). Similarly, while the branched-chain molecules isobutane (**11**), neopentane (**15**), and 2,3-dimethylbutane (**18**) bind with greater affinity experimentally than their *n*-alkyl isomers, both MD1 and MD2 predict that *n*-butane binds with greater affinity than isobutane (by 0.1 and 1.3 kcal mol⁻¹, respectively). Finally, whereas *n*-alkyl guests bind with greater affinity experimentally than their cyclic isomers, the reverse is true for *n*-hexane/cyclohexane in MD1 and both *n*-pentane/cyclopentane and *n*-hexane/cyclohexane in MD2.

We considered whether the systematic overestimation of binding affinities by the MD simulation methods could result from systematic underestimation of the hydration free energies of the guest molecules: less favorable interactions with solvent would tend to favor binding. To evaluate this, we compared the difference between calculated and experimental hydration free energies for 18 of the 22 hydrophobic guests (Table 2, obtained from the Mobley lab FreeSolv database¹²⁴) with the differences between the calculated and experimental binding free energies to CB7 (Figure 14), using the MD2 method. Crucially, the FreeSolv database provides computed hydration free energies obtained with the same force field as used in the present MD2 calculations: AM1-BCC charges and GAFF parameters for the solute, in TIP3P water. There is indeed a very good correlation ($G_{\text{solv}}(\text{calc}) = 0.73 + 0.90 G_{\text{solv}}(\text{exp})$, in kcal mol⁻¹, $R^2 = 0.83$) between calculated and experimental hydration free energies (Figure 13, blue squares). In terms of the absolute error shown in Figure 14, which is modest, constant, and typically less than 1 kcal mol⁻¹, it is clear that the observed deviations between calculated and experimental binding free energy are not related to incorrect predictions of the hydration free energies of the guest molecules, a conclusion also reached for the QM data (see Section 3.2). This suggests that the errors in

the binding calculations may trace, instead, to errors in the direct interactions between the host and its guests or between the host and structured water within the cavity.

Some of the errors in the MD data may result from subtle problems with the host force field. Because the binding cavity of CB7 is restrictive and rigid, even a slightly wrong bond length could result in the inability of the host to properly discriminate among guests by size. It is likely that improvements over the GAFF parameterization could be obtained with careful QM calculations. Along these lines, it is tempting to inspect the binding affinity decompositions for the QM (Figure 6) and MD results (Figure 7) for clues as to why the predictions are different. However, given the lack of explicit solvent in the QM calculations, along with a different overall approach, a meaningful comparison is difficult to achieve. For instance, the $G_{\text{RRHO}}^{\text{T}}$ term from the QM calculations can be considered as a primarily entropic term, but it is difficult to compare this to the $-T \Delta S$ values in Figure 7 as the former value does not contain the water contribution, which is folded into the $\delta G_{\text{solv}}^{\text{T}}$ term, which also has an entropic contribution as well. Likewise, the pairwise dispersion term in Figure 6, $E_{\text{disp}}^{(2)}$, does not include solvent dispersion contribution, whereas H_{VDW} in Figure 7 contains both solvent and solute contributions. Further decomposition of the H_{VDW} term is possible, into solute, solvent, and solute-solvent components, which reveals that the solute contribution to H_{VDW} is about the same magnitude as $E_{\text{disp}}^{(2)}$ (results not shown).

The performance of the MD1 and MD2 approaches observed here is rather consistent with that seen in prior studies of similar methods. In particular, previous host-guest calculations with more polar host and/or guests than those studied here also yielded significant overestimation of both the binding free energy and binding enthalpy,²⁷ as well as a tendency for TIP3P to overestimate the binding enthalpy relative to OPC.¹²⁵ There are currently several approaches which the Gilson lab is pursuing for improving these and other host-guest calculations, with the ultimate goal of improving force fields for computer-aided drug design. These include altering solute parameters on an *ad hoc* basis,¹²⁶ designing purpose-built water models, and incorporating binding calculations into a broader force field optimization scheme which also includes predicting pure liquid properties, mixing properties, and crystal lattice values.

3.4. Relationship of the QM and MD Calculations—Both QM and MD calculations yield good correlations with the blinded experimental binding free energies; the correlation is somewhat larger for the MD methods, but the absolute errors are significantly smaller for the QM methods, particularly after applying the standard corrections identified after the close of the challenge. When considering these differences, it is worth noting the two participating computational research groups adopted different, yet equally valid, philosophies regarding the use of existing experimental data for the cucurbiturils. For the QM calculations, the DFT parameterizations used in the COSMO-RS solvent models was selected based on comparisons with prior experimental data for CB6, as detailed in the SI. This approach hopefully led to increased accuracy, relative to what would have been obtained without the benefit of prior results. In contrast, the MD calculations were not adjusted based on previously available binding data. This approach may have led to somewhat larger errors, but was chosen so that the results would be informative regarding the performance of generally applicable MD potential functions and methods. Overall, the

difference in how prior data were or were not used arguably makes it difficult to draw a clear conclusion that either method is fundamentally more accurate than the other.

The strength of the present challenge compared to previous challenges proved to be the availability of experimental hydration free energies at least for one component (the hydrocarbon guests). In this manner, the calculated hydration energies of the guests could not only be excluded as a common or unknown source of error, but the correlation also revealed a systematic offset in the employed QM data. Thus, it became evident in the course of the comparison of calculated and experimental data that the QM data required a reference-state adjustment. This *ex post* correction limits a more direct comparison of the performance of the different approaches, because the solvation model chosen for the QM submissions had been selected by comparison of calculated and experimental affinity data that referred to different reference states. Nevertheless, it could be convincingly demonstrated that QM methods can perform very well in predicting the absolute binding free energies of CB7 complexes with small alkanes (up to C5) in water, while for large hydrocarbons, elongated alkanes, and perfluorinated derivatives large errors may result.

Finally, it is instructive to consider the relation between the main strengths and weaknesses of the QM and MD approaches. The QM approach is expected to provide relatively reliable solute-solute interaction energies, via electronic structure calculations, but is based on only a few thermodynamically dominant structures of the solutes, and it uses an implicit solvent model, which may introduce error, particularly for water molecules in the concave interior of the CB7 host. In contrast, the MD approach uses a relatively crude potential function, but treats solvent molecules explicitly and incorporates extensive conformational sampling. The MD method, accordingly, appears to yield good relative binding free energies, suggesting that its absolute errors (overbinding) derive primarily from errors in the solute-solute interactions, which the QM method captures relatively well. As a particular advantage of the HYDROPHOBE challenge, the predictive power of both methods, MM and COSMO-RS, could be tested for the hydration free energies of the guest molecules, and both methods were found to display good accuracy, within 1 kcal mol⁻¹. Nevertheless, their performance for predicting the hydration free energies of the large concave host and the host-guest complexes cannot be evaluated (because the experimental values are inaccessible) which remains a potential source of error in both approaches. Thus, a method combining the strengths of the two approaches might be especially predictive. Especially the correct prediction of absolute binding free energies must remain the major goal, because the QM submissions still could be much improved by *ex post* comparison with experimental data (which revealed a required reference-state correction term of 2.38 kcal mol⁻¹) and although the MM submission provided better correlations and, therefore, predictive power, these correlations did not prove superior to much simpler empirical correlations with polarizability or octanol-water partition coefficients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ASSOCIATED CONTENT

Supporting Information: Materials and Methods; fluorescence and NMR experiments; physical parameters of the investigated guest molecules; computational details (all as PDF file). Structural ensembles for the bound state of each CB7-guest complex, taken from the MD1 and MD2 submissions (Provided as ZIP).

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Figure 1. Chemical structures of CB_n , the indicator dye (DAPI), and the guest molecules (1-26) studied in this work. Guests 23-26 are included as instructive additional cases but were not part of the original HYDROPHOBE challenge.

Figure 2. ^1H NMR spectra of selected hydrocarbons complexed with CB7: a) n -pentane (**13**), b) isopentane (**14**), c) cyclopentane (**16**), d) benzene (**20**), e) n -heptane (**21**), and f) norbornene (**22**).

Figure 3.

Experimental *versus* calculated binding free energies (kcal mol^{-1} , 1 M reference state) for 22 CB7•hydrocarbon complexes. Top: QM1 with conformational-entropy and reference-state correction; middle: QM2 with conformational-entropy and reference-state correction; bottom: QM3 with reference-state correction. Solid red lines: linear regression. Dashed black lines: identity.

Figure 4.

Experimental *versus* calculated binding free energies (kcal mol⁻¹, 1 M reference state) for 22 CB7•hydrocarbon complexes. Top: MD1; bottom: MD2. Solid red lines: linear regression. Dashed black lines: identity.

Figure 5. QM-calculated structures for CB7•hydrocarbon complexes. The structures shown have the largest contribution to the calculated binding free energy at the QM2 level.

Figure 6. Contributions to calculated G values (in kcal mol^{-1} , 1 M reference state) for those structures of CB7•hydrocarbon complexes having the largest contribution to the calculated binding free energy. E : PW6B95-D3^{ATM}/def2-QZVP'. $G^{\text{T}}_{\text{RRHO}}$: PBEh-3c. $\delta G^{\text{T}}_{\text{solv}}$: COSMO-RS (13) (QM2 level). The depicted $G^{\text{T}}_{\text{RRHO}}$ and $G^{\text{T}}_{\text{solv}}$ data correspond to $G_{\text{gas}}(24.5 \text{ atm})$ and $G_{\text{solv}}(24.5 \text{ atm}/1 \text{ M})$ values.

Figure 7.

Thermodynamic contributions to the binding free energy for all guests (**1–26**) using the MD1 submission protocol. Three components of the binding enthalpy are provided (the valence energy which consists of bonds, angles, dihedrals, and 1–4 energies; the VDW energy; and the electrostatic energy), as well as the entropic component of the free energy. It should be noted that the depicted $T\ S$ and G data refer to the 1 M reference state and contribute or correspond directly to the $G(1\text{ M})$ values relevant for direct comparison with experiment (see Results).

Figure 8.

A snapshot from a CB7•*n*-heptane simulation showing a helical conformation developing in the hydrocarbon guest.

Figure 9. Dimensional comparison between CB7 and the longer *n*-alkanes (left). Experimental binding free energy *versus* number of carbon atoms in *n*-alkanes (right).¹²²

Figure 10.

a) Experimental binding free energy *versus* a) the octanol-water partition coefficient¹²³ and
b) the polarizability¹⁰³ of the guest molecules.

Figure 11.

Top: Comparison of the electronic binding energies afforded by dispersion-corrected density-functional theory (QM1 and QM2) *versus* those obtained by a wave-function based approach (QM3). The two solid lines refer to a $\pm 5\%$ error region above/below the identity. Bottom: Comparison of the solvation free energies of the gas-phase species to the binding free energies obtained by the COSMO-RS 12-fine solvation mode (QM1) *versus* those obtained by COSMO-RS 13 (QM2 and QM3). Data shown refer to the 49 CB7 host-guest complexes involved in the preselection for the QM3 submission (see SI, Section 2).

Computational Part, 2.1. Quantum-Chemical Calculations, QM-technical details). Data in the bottom graph refer to the Ben-Naim reference state (24.5 atm/1 M).

Figure 12. Experimental versus *a posteriori* calculated G values by QM4 (in kcal mol⁻¹, 1 M reference state) for CB7•hydrocarbon complexes.

Figure 13. Correlation of COSMO-RS 13 (red circles, dashed line) and MD (blue squares, dotted line) calculated *versus* experimental hydration free energies. Identity line is solid. All data from Table 2.

Figure 14.

Differences in hydration free energy (blue, in kcal mol^{-1} from Table 2) and binding free energy (orange, in kcal mol^{-1} from Table 1) between MD2-calculated and experimental values. The calculated values used the same force field approach: AM1-BCC/GAFF solute force field in TIP3P water.

Table 1.

Experimental association constants (K_a , M^{-1} , 10% error) and experimental as well as computed binding free energies (G , kcal mol^{-1} , 1 M reference state) for hydrocarbons (1–24) and perfluorinated hydrocarbons (25 and 26) as guest molecules with CB7 as host in water. Packing coefficients (PC , %) are also provided. Italic font indicates previously published data not included in the summary statistics in the last four rows.

	Experimental			Calculated <i>G</i>					
	<i>PC</i>	<i>K_a</i> (10 ³)	<i>G</i>	QM1	QM2	QM3	MD1	MD2	
1	methane	12	3.0	-4.74	0.46	-2.62	-2.43	-0.76	-1.78
2	ethane	19	3.4	-4.82	0.05	-2.55	-3.63	-3.80	-4.91
3	ethene	17	1.1	-4.15	0.71	-2.05	-2.58	-2.88	-3.39
4	acetylene	14	0.13	-2.87	1.20	-0.88	-0.98	-3.45	-5.12
5	propane	26	6.0	-5.15	-2.14	-4.78	-5.92	-8.34	-8.60
6	propene	24	1.2	-4.19	-0.72	-3.10	-3.45	-4.92	-6.16
7	<i>n</i> -butane	33	170	-7.14	-3.74	-7.00	-7.97	-12.18	-12.60
8	1-butene	31	26	-6.02	-2.31	-5.86	-6.95	-8.45	-9.25
9	<i>cis</i> -butene	31	35	-6.20	-3.89	-6.26	-6.57	-8.92	-10.84
10	<i>trans</i> -butene	31	14	-5.66	-2.80	-5.62	-6.90	-10.17	-11.69
11	isobutane	33	270	-7.40	-5.00	-9.23	-9.67	-12.04	-11.33
12	isobutene	31	43	-6.32	-3.35	-6.52	-6.27	-8.79	-10.54
13	<i>n</i> -pentane	40	480	-7.75	-5.11	-8.14	-8.14	-14.12	-14.42
14	isopentane	40	630	-7.92	-5.72	-8.57	-8.62	-13.79	-13.95
15	neopentane	40	1000	-8.19	-7.48	-8.57	-9.11	-14.91	-14.86
16	cyclopentane	36	200	-7.22	-7.05	-9.12	-9.29	-13.66	-14.61
17	<i>n</i> -hexane	47	1500	-8.43	-5.98	-6.48	-6.98	-15.81	-15.68
18	2,3-dimethylbutane	47	1800	-8.53	-7.80	-10.68	-9.60	-15.80	-15.84
19	Cyclohexane	42	1500	-8.43	-9.98	-13.25	-13.41	-17.32	-18.03
20	Benzene	37	17	-5.77	-7.64	-7.93	-9.53	-9.75	-13.77
21	<i>n</i> -heptane	54	3600	-8.94	-6.85	-4.94	-5.21	-12.44	-12.54
22	Norbornene	43	570	-7.85	-8.17	-9.69	-12.32	-16.15	-17.03
23	Adamantane	61	2.2×10 ^{6a}	-12.74	<i>c</i>	<i>c</i>	<i>c</i>	-24.85	-24.63
24	Diamantane	79	1.6×10 ^{6a}	-12.56	<i>c</i>	<i>c</i>	<i>c</i>	-14.84	-18.30

		Experimental				Calculated			
		PC	K_a (10^3)	G	QM1	QM2	QM3	MD1	MD2
25	Perfluorohexane	54	1.2×10^4 ^b	-8.38	c	c	c	-18.90	-14.92
26	perfluorocyclohexane	61	1400 ^b	-9.66	c	c	c	-16.15	-16.15
MSE ^d					2.29 (3.05) ^e	-0.01 (0.30) ^e	-0.54 (-0.17) ^e	-3.85 (-2.84) ^e	-4.69 (-3.64) ^e
MUE ^d					2.63 (3.05) ^e	1.48 (0.98) ^e	1.71 (1.14) ^e	4.42 (3.62) ^e	5.03 (4.11) ^e
RMSE ^d					2.97 (3.33) ^e	1.94 (1.30) ^e	2.18 (1.32) ^e	5.05 (4.17) ^e	5.64 (4.62) ^e
R ² ^d					0.77 (0.88) ^e	0.66 (0.93) ^e	0.59 (0.90) ^e	0.84 (0.84) ^e	0.75 (0.79) ^e

PC: Packing coefficient, (volume of guest)/(cavity volume of CB7, 242 Å³); QM1: TPSS-D3-ATM_{def2-QZVP} // PBEh-3c // COSMO-RS (12-fine); QM2: PW6B95-D3-ATM_{def2-QZVP} // PBEh-3c // COSMO-RS (13); QM3: DLPNO-CCSD(T)/CBS* // PBEh-3c // COSMO-RS (13); MD1: OPC water model, RESP point charges and GAFF2 bonded and Lennard-Jones parameters on CB7 and guests; MD2: TIP3P water model, AM1-BCC point charges and GAFF bonded and Lennard-Jones parameters on CB7 and guests. The uncertainties (standard errors of the mean) for the MD1 and MD2 values do not exceed 0.38 kcal mol⁻¹ (see Tables S4 and S5).

^aValues reported here represent a correction of the previously published data in ref. 53.

^bFrom ref. 101.

^cNo data given. In Figure 12 and Table 2, results obtained by a fourth submission (QM4), which were prepared after publication of the experimental data, are presented.

^dMSE: mean signed error in computed free energies. MUE: mean unsigned error in computed free energies. RMSE: root mean squared error of computed free energies. R²: correlation coefficient of determination for computation vs. experiment.

^eThe error-statistical data in parentheses refer to the set of small guest molecules only (1–16). Molecules 23–26 are not included in the error statistics since they were provided as optional additions following the close of the HYDROPHOB challenge.

Table 2.

Experimental and COSMO-RS-calculated hydration free energies (G_{solv}) as well as experimental and QM-calculated (QM4 method) binding free energies in aqueous solution (G , from Table 1) and their deviation (Dev). All data are in kcal mol⁻¹ and refer to the Ben-Naim reference state, that is 24.5 atm/1 M for G_{solv} and 1 M for G .

Guest	G_{solv}			G		Dev^a
	exp. ^b	COSMO-RS(13)	MD ^c	exp. ^d	QM4	
1	1.99	1.63	2.54	-4.74	-2.40	2.34
2	1.82	1.79	2.58	-4.82	-3.24	1.58
3	1.27	1.03	2.36	-4.15	-2.56	1.59
4	-0.01	-0.95	---	-2.87	-1.77	1.10
5	1.94	1.87	2.56	-5.15	-4.91	0.24
6	1.27	1.06	2.44	-4.19	-4.62	-0.43
7	2.06	1.97	2.54	-7.14	-7.62	-0.48
8	1.38	1.2	2.48	-6.02	-6.98	-0.96
9	1.37	1.19	---	-6.20	-6.78	-0.58
10	1.36	1.32	---	-5.66	-5.91	-0.25
11	2.24	1.89	2.74	-7.40	-7.22	0.18
12	1.28	1.09	2.34	-6.32	-6.26	0.06
13	2.29	2.07	2.67	-7.75	-7.62	0.13
14	2.37	1.96	2.52	-7.92	-7.43	0.49
15	2.65	1.85	2.53	-8.19	-7.48	0.71
16	1.2	0.97	1.53	-7.22	-8.81	-1.59
17	2.45	2.17	3.05	-8.43	-5.98	2.45
18	2.39	1.95	2.69	-8.53	-10.16	-1.63
19	1.19	0.83	1.67	-8.43	-12.49	-4.06
20	-0.89	-0.82	-0.7	-5.77	-9.60	-3.83
21	2.85	2.27	3.2	-8.94	-3.57	5.37
22	0.98	-0.06	---	-7.85	-10.06	-2.21
23	1.32	0.07	---	-12.74	-16.65	-3.91
24	0.12	-0.65	---	-12.56	-12.99	-0.43
25	6.32	5.27	---	-8.38	3.07	11.45
26	3.52	3.6	---	-9.66	-5.16	4.50

^a $Dev = G_{\text{exp}} - G_{\text{QM4}}$.

^bFrom Table S2 in SI.

^cTaken from the FreeSolv database in ref. ¹²⁴, which uses the same force field as used in the present MD2 calculations.

^dFrom Table 1.