Cucurbit[8]uril•Guest Complexes: Blinded Dataset for the SAMPL6 Challenge

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Abstract: We investigated the formation of host guest complexes between cucurbit[8]uril (CB[8]) and 14 cationic guest compounds (**2** – **15**, including seven drugs Lexapro, detrol, palonosetron, quinine, gallamine, aricept, oxaliplatin) by a combination of ^{1}H NMR spectroscopy and isothermal titration calorimetry (ITC). Although these two component systems generally form 1:1 host:guest complexes, other stoichiometries are also observed (e.g. 1:2 host:guest and 3:1 host:guest) in situations where one guest fills roughly half of the cavity of CB[8] or where the guest contains multiple binding epitopes. We used the changes in chemical shift observed in the ^{1}H NMR spectrum of the guest upon binding to glean information about the geometry of the host-guest complexes and in cases of slow exchange to confirm the host:guest stoichiometry. The complexes form with binding constants that range from 5.34×10^{4} to 8.26×10^{9} M^{-1} in 25 mM sodium phosphate buffered water at pH 7.4 at 298 ˚K. All of the complexes are driven in part by negative ΔH° values (-2.00 to -14.4 kcal mol^{-1}). The data from these experiments were used as a blinded dataset for the SAMPL6 computational chemistry challenge held in February 2018 in La Jolla, California.

Keywords: Cucurbituril; SAMPL challenge; host-guest chemistry; binding constant; drugs

Introduction.

The condensation of glycoluril with formaldehyde under strongly acidic conditions delivers a homologous series of macrocycles known as cucurbit[n]urils (CB[n], n = 5, 6, 7, 8, 10, 13-15, Figure 1)[1] whose cavity volumes range from 82 to 870 Å^3.[2] CB[n] molecular containers are highly symmetrical structures (D_{nh}) and feature two equivalent ureidyl C=O portals that are regions of highly negative electrostatic potential that guard entry to a hydrophobic central cavity.[2a,3] Due to these structural features, CB[n] compounds bind to alkali and alkaline earth cations at their portals[4] but are also able to bind to neutral guests within their hydrophobic central cavity.[2d,5] Guest compounds that feature both cationic and hydrophobic regions (e.g. alkane (di)ammonium ions) bind to CB[n] both with remarkable affinity (K_a commonly 10^6, often 10^9, sometimes 10^{12}, and up to 10^{17} M^{-1} in special cases) and selectivity.[6] The extraordinarily high binding affinities of CB[n]•guest complexes arise from the presence of high-energy intracavity H_2O molecules that lack a full complement of H-bonds that deliver a substantial enthalpic driving force upon CB[n]•guest binding.[2d,5b,7] Because of the high K_a values and high selectivity, CB[n]•guest derived systems are inherently stimuli responsive (e.g. pH, photochemical, electrochemical, chemical) and thereby function as high fidelity switching elements.[6d,7b,8] Accordingly, CB[n]-type molecular containers enable the creation of supramolecular polymers and materials, supramolecular organic frameworks, supramolecular catch and release separations systems, sensing ensembles, solubilizing agents for insoluble drugs, *in vitro* and *in vivo* sequestration agents, and theranostic systems.[8d,9] Beyond CB[6] and CB[7] which typically form host•guest complexes of 1:1 stoichiometry lies CB[8] whose cavity is large enough to simultaneously host two guests.[8a,9n,9o,10] Most commonly, CB[8] binds to an electron

poor aromatic (e.g. methyl viologen) as a first guest followed by an electron rich aromatic (e.g. tryptophan) as a second guest to form a CB[8]•guest1•guest2 complex. Accordingly, investigations of CB[8] complexation requires an elucidation of both stoichiometry and K_a values.

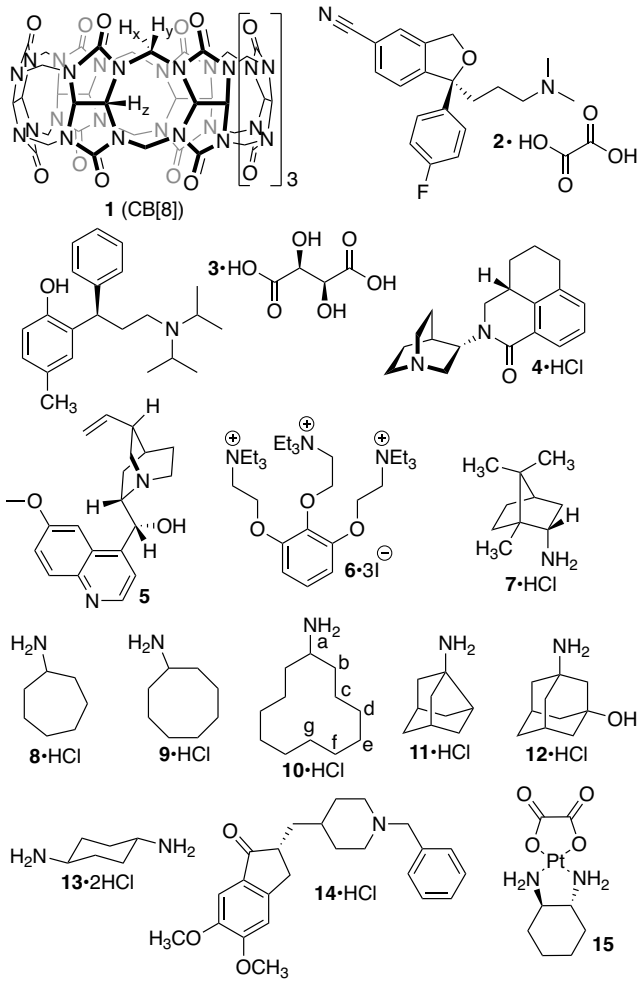

Figure 1. Chemical structures of CB[8] and the guest compounds used in this study.

The features that render CB[n]-type containers attractive to supramolecular chemists[6b,6d,7b,11] – high K_a, high selectivity – make them attractive to computational chemists who value them for their small size (relative to proteins) which makes CB[n] a tractable model system to test and improve new computational approaches toward protein•ligand binding free energies.[5d,12] The SAMPL challenges – originally organized

by OpenEye Scientific Software and now organized by the Drug Design Data Resource[13] – bring together an experimental group that prepares a blinded unpublished dataset (e.g. K_a for host•guest or protein•ligand, solvation free energy, tautomer ratios, vacuum water transfer energies, pK_a prediction) with computational chemists who are given the structures of the complexes of interest and seek to compute the parameters of interest as a test of new computational methodologies. The experimental and computational results are discussed at the SAMPL conference to promote synergy between the communities and assess the current state-of-the-art in computational predictions. CB[n] type receptors have been featured prominently in previous SAMPL challenges. For example, SAMPL3 featured one of our acyclic CB[n]-type receptors and CB[8],[12a,14] SAMPL4 featured CB[7] and Gibb's octaacid,[12b,15] SAMPL5 featured our methylene bridged glycoluril dimer and Gibb's methylated octaacid.[12c,16] For our experimental contribution to the SAMPL6 we created a dataset comprising the thermodynamic binding parameters of CB[8] toward 14 guests with several guests being pharmaceuticals to enhance the relevance for the computationalists.

Results and Discussion. This results and discussion section is organized as follows. First, we discuss the criteria used to select the guests used in this study and the buffer system used to conduct the binding studies. Subsequently, we present the results of our measurement of the CB[8]:guest binding stoichiometries and K_a values of guests **2** – **15** toward CB[8] by isothermal titration calorimetry and their CB[8]•guest geometries by ^{1}H NMR spectroscopy. Finally, we discuss selected trends seen in the binding data.

Selection of Guest Compounds. Figure 1 shows the chemical structures of 14 guest compounds (**2** – **15**) that were used in this study. Initially, we selected guests **2** – **6**, **14**,

and **15** which are well known pharmaceutical agents (**2**: Lexapro; **3**: Detrol; **4**: Palonosetron; **5**: Quinine; **6**: Gallamine; **14**, Aricept; **15**, Oxaliplatin) in the hope that this would provide a stimulating challenge for the computationalists and K_a values that would cover several orders of magnitude to make observed correlations more statistically meaningful. Compounds **2** – **5** and **14** contain a single basic N-atom which is expected to be protonated at pH 7.4 which renders each of these guests monocationic. In consultation with the organizers, **15** was only included as a bonus challenge due to the presence of the Pt atom which presents significant challenges to computational predictions. After initial measurements found that the K_a values for these pharmaceutical agents cluster at the lower end of affinity, we added in a series of hydrophobic cycloalkyl (di)ammonium ions (**7** – **13**) which were expected to bind more strongly.

Selection of a Buffer System. Amongst the unfunctionalized CB[n], CB[5] and CB[7] possess good aqueous solubility (> 5 mM) whereas CB[6], CB[8], and CB[10] are poorly soluble (< 100 μM) in pure water.[1a,1c] Of course, CB[n] are known to bind metal ions (e.g. Na^+) or even H^+ at their portals which can improve their aqueous solubility.[4,6a] In our previous work, we have conducted binding studies between CB[6], CB[7], and CB[8] toward guests in a common medium comprising 50 mM NaOAc buffered D_2O at pH 4.74 to facilitate comparisons between CB[n]•guest complexes.[6c,17] In SAMPL4 we conducted our binding studies of CB[7] towards its guests in 100 mM sodium phosphate buffered D_2O at pH 7.4 to provide conditions that would better reflect those typically encountered in the protein•ligand interaction systems of interest to the computationalists.[15a] In this paper we decided to use 25 mM NaH_2PO_4 buffered H_2O because it provided sufficient levels of solubility of CB[8] (100 μM) and also provided

a biologically relevant buffer. The modest ionic strength of the buffer provides an additional challenge to the computationalists since metal ions are known to reduce the observed binding constants of CB[n] toward its guests.[2b,18]

^{1}H NMR Investigations of the Host-Guest Complexes. Before proceeding to the measurement of the binding constants by ITC, we decided to investigate the CB[8]•guest binding by ^{1}H NMR spectroscopy at different CB[8]:guest ratios to glean information about host:guest complex stoichiometry and geometry. For example, Figure 2 shows the ^{1}H NMR spectra recorded for cyclododecaneammonium ion (**10**) alone, as its CB[8]•**10** complex, and as a mixture of CB[8]•**10** with excess free **10**. Figure 2b shows that all of the ^{1}H NMR resonances (H_a – H_g) of **10** undergo substantial upfield shifts ($\Delta\delta \approx 0.6$ ppm) upon formation of complex CB[8]•**10**. It is well known that the central cavity of CB[n] constitute a magnetically shielding region whereas the region just outside the C=O portals constitute a deshielding region.[2b,6a] Accordingly the consistent upfield shifts observed for CB[8]•**10** establishes that the cycloalkyl ring is fully buried within the cavity of CB[8]. In Figure 2c recorded at a 1:2 CB[8]:**10** molar ratio we observe the presence of two sets of resonances; one set appears at the chemical shifts observed for the CB[8]•**10** complex and one set appears at the chemical shifts observed for uncomplexed **10**. This means that the dynamics of chemical exchange between uncomplexed **10** and CB[8]•**10** is slow on the chemical shift timescale which is typically observed for complexes of high thermodynamic stability. Since exchange is slow on the chemical shift timescale, we can use ^{1}H NMR spectral integration of the resonances for CB[8] and **10** in the CB[8]•**10** complex to confirm the 1:1 stoichiometry of this complex. Interestingly, even though the CB[8]•**10** complex is unsymmetrical (e.g. 2 different C=O portals), the H_x and H_y resonances each appear as a single doublet.

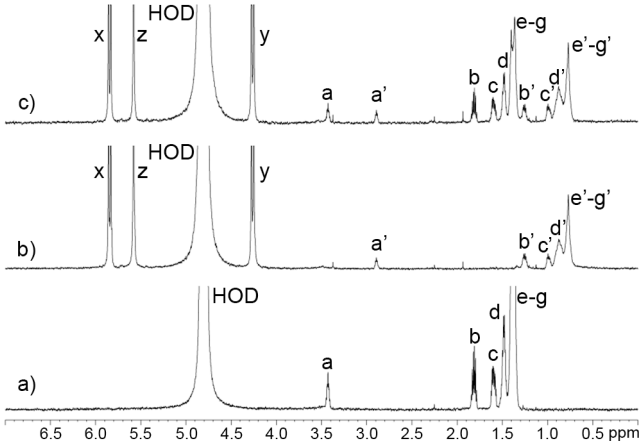

Figure 2. ^{1}H NMR spectra recorded (600 MHz, RT, D_2O) for a) **10** (0.25 mM), b) an equimolar mixture of **10** and CB[8] (0.125 mM), and c) a mixture of **10** (0.250 mM) and CB[8] (0.125 mM).

Analogous ^{1}H NMR investigations were performed for the remainder of the guests with CB[8] at several different stoichiometries as presented in the Supporting Information. The remaining (cyclo)alkaneammonium ions behaved similarly to CB[8]•**10**. For example, the complexes of CB[8] with guests **7** – **9** and **11** – **13** display sizable upfield shifts for all guest resonances upon encapsulation which indicates that the entire guest is bound within the magnetic shielding region inside the CB[8] host. The maximum upfield shift is seen at a 1:1 CB[8]:guest stoichiometry. Amongst these CB[8]•guest complexes, the CB[8]•**7** and CB[8]•**12** show intermediate exchange on the chemical shift timescale whereas CB[8]•**8**, CB[8]•**9**, and CB[8]•**11** display fast exchange kinetics of the chemical shift timescale. When CB[8] is mixed with 2 equivalents of 1,4-cyclohexanediammonium ion (**13**) we observe two sets of broadened resonances which correspond to CB[8]•**13** and uncomplexed **13** indicating some chemical exchange between free and bound **13**.

Next, we consider the ^{1}H NMR characteristics of the drugs **2** – **6**, **14**, and **15**. For guest **2** (Lexapro), complexation with CB[8] results in a broadening of the aromatic and aliphatic resonances into the baseline of the spectrum which is indicative of cavity binding but does not provide evidence for a single well-defined geometry for the CB[8]•**2** complex. For guest **3** (detrol), we observe ≈ 0.5 ppm upfield shifts for the isopropyl and NCH_2CH_2 groups upon binding whereas the chemical shifts of the Ar-H atoms remain nearly constant indicating that the isopropyl groups are the dominant binding region upon complexation with CB[8]. At stoichiometries of >2:1 CB[8]:**3** the upfield shifted isopropyl resonances are sharp whereas at a 1:1 stoichiometry the resonances are broadened which suggests this complex deviates from 1:1 binding stoichiometry (*vide infra)*. Complexes between CB[8] and guests **4** (palonosetron) and **5** (quinine) which contain quinuclidine moieties behave similarly in that the quinuclidine protons undergo upfield shifts with some broadening indicating inclusion of this moiety in the CB[8] cavity. Whereas the Ar-H resonances for **5** do not shift upon binding with CB[8], the resonances for **4** undergo upfield shifts which indicates that the Ar ring of **5** may constitute a secondary binding site for CB[8]. Guest **6** (the neuromuscular blocker gallamine) contains three quaternary triethylammonium ion moieties. At a 3:1 CB[8]:**6** ratio we observe maximal changes in ^{1}H NMR chemical shift and relatively sharp resonances which strongly suggests a $CB[8]_3$•**6** complex. Interestingly, the Ar-H resonances display a downfield change in chemical shift indicating that these protons reside outside the cavity of CB[8] but near the C=O portals. Conversely, upfield shifts are seen for the NEt_3 resonances and the OCH_2CH_2 resonances which suggests these are the dominant binding regions for CB[8]. Guest **14** (Aricept) was specifically included to challenge the computationalists regarding host:guest stoichiometry. Guest **14** contains the benzylammonium binding epitope

which is well known to bind to cucurbiturils;[9p,10b,10e,10f,19] in the case of CB[8] we expected it to form a head-to-tail 2:1 complex with CB[8] and were interested to look for cooperativity (positive or negative). Experimentally, we find that the Ph resonances undergo approx. 1 ppm upfield shifts upon complexation whereas the resonances for protons on the dimethoxy substituted aromatic ring, the OMe groups, and the piperidine rings remain nearly constant suggesting the benzyl ammonium groups are complexed inside CB[8]. Figure 3 shows an MMFF minimized model of the CB[8]•**14**$_2$ complex that illustrates the NH•••O=C H-bonding and π–π stacking that occurs inside the ternary complex. Finally, guest **15** (oxaliplatin) displays upfield shifted resonances for all of the cyclohexyl protons up to a 1:1 CB[8]:**15** ratio, whereas at higher CB[8]:guest ratios, the resonances broaden and shift back toward the chemical shift for the free guest **15** which establishes intermediate exchange kinetics on the NMR timescale and suggests a 1:1 binding stoichiometry for CB[8]•**15**. After having gleaned information about the complexation stoichiometry and preferred binding regions for the complexes between CB[8] and guests **2** – **15** by ^{1}H NMR we proceeded to conduct isothermal titration calorimetry experiments to measure the host•guest binding constants and provide further evidence for host•guest stoichiometry.

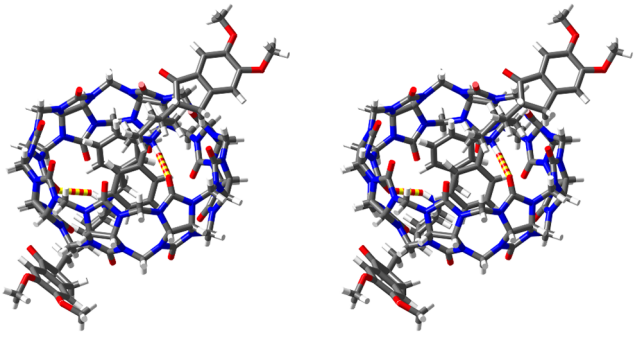

Figure 3. Cross-eyed stereoview of the MMFF minimized geometry of the head-to-tail geometry of the CB[8]•**14**$_2$ ternary complex. Color code: C, grey; H, white; N, blue; O, red; H-bonds, red-yellow stripped.

Determination of the Thermodynamics of CB[8]•Guest Complexes by Isothermal Titration Calorimetry. To determine the binding constants for the interaction between the CB[8] host and guests **2** – **15** we used ITC. For most of the guests (**2** – **8**, **11**, **12**, **13**, **15**), we performed direct titrations involving ≤100 μM CB[8] in the ITC cell with more concentrated solutions of guest (often 1 mM) in the syringe. Figure 4a shows a plot of DP versus time during the titration of CB[8] with **11** which was integrated to transform the data into a plot of DH versus molar ratio given in Figure 4b. The solid line in Figure 2b is the best fit of the data to the single set of sites binding model in the PEAQ-ITC data analysis software which allowed us to determine the values of the thermodynamic parameters K_a, ΔH°, and ΔG° as well as the binding stoichiometry (n) for these complexes (Table 1). As suspected based on the ^{1}H NMR experiments described above, the complexes between CB[8] and guests **3** and **6** deviate from 1:1 and are best formulated as CB[8]•$\mathbf{3}_2$ and CB[8]•$\mathbf{6}_3$ as determined by the n values of 0.5 and 0.33, respectively, from the ITC data fitting. Interestingly, the data for CB[8]•$\mathbf{3}_2$ and CB[8]•$\mathbf{6}_3$ fit well to a single set of sites model which suggests that the binding events are independent. Figure 4c,d shows the data recorded during the titration of CB[8] with **14** (Aricept). In this case, we observe two sequential inflection points in the data corresponding to the formation of CB[8]•**14** and CB[8]•$\mathbf{14}_2$ complexes. We analyzed the data using the sequential binding model in the PEAQ ITC data analysis software and found that the K_a value for the formation of CB[8]•**14** is approximately 100-fold stronger than CB[8]•$\mathbf{14}_2$ which reflects a substantial negative cooperativity in the formation of the ternary complex. For the more tightly binding complexes ($K_a > 10^7$ M^{-1}) between CB[8] and guests **9** and **10** we turned to competitive ITC titrations. In competitive ITC titrations, a solution of CB[8] and an excess of a weak binding guest whose K_a and ΔH

values had been previously measured is titrated with a stronger binding guest and the data is fitted to a competitive binding model to extract the thermodynamic parameters for the stronger binding complex. Figure 4e,f shows the ITC thermograms recorded for the titration of a mixture of CB[8] (0.129 mM) and **11** (2.5 mM) with a solution of **10** (1.0 mM) which allowed us to determine $K_a = 8.26 \pm 0.31 \times 10^9$ M^{-1} for the CB[8]•**10** complex.

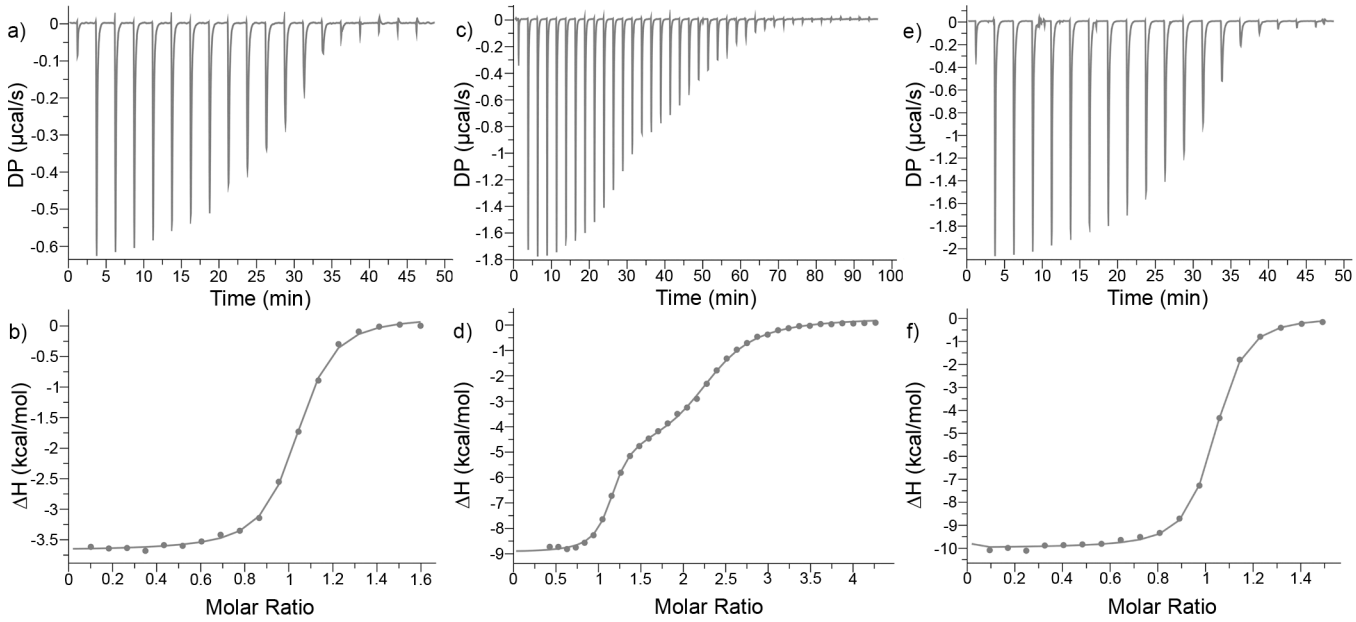

Figure 4. Plots of change in DP vs time from the titration of: a) CB[8] (100 µM) in the cell with guest **11** (0.829 mM) in the syringe, c) CB[8] (100 µM) in the cell with guest **14** (1.0 mM) in the syringe, and e) CB[8] (129 µM) and **11** (2.5 mM) in the cell with guest **10** (1.0 mM) in the syringe. All experiments were performed in 25 mM NaH_2PO_4 buffered water (pH = 7.4) at 298 K. Plots of ΔH as a function of the molar ratio of CB[8] toward guests fitted (solid line) to an appropriate binding model: b) guest **11** (single set of sites binding model with $K_a = 2.29 \pm 0.32 \times 10^6$ M^{-1}), d) guest **14** (sequential binding model with $K_a = 1.67 \pm 0.04 \times 10^7$ and $1.46 \pm 0.02 \times 10^5$ M^{-1}, f) guest **10** (competition binding model with $8.26 \pm 0.31 \times 10^9$ M^{-1}).

Table 1. Thermodynamic parameters (K_a (M^{-1}), ΔH° and ΔG° (kcal mol^{-1}) and stoichiometry determined for the CB[8]-guest complexes by ITC. [a] Direct ITC titration, [b] competition ITC titration.

Guest	K_a (**1**•guest) (M^{-1})	ΔH^o (kcal/mol)	ΔG^o (kcal/mol)	$-T\Delta S^o$ (kcal/mol)	*n*
2	$8.06 \pm 0.38 \times 10^{4}$[a]	-4.22 ± 0.079	-6.69	-2.48	1
3	$4.03 \pm 0.16 \times 10^{5}$[a]	-5.05 ± 0.039	-7.65	-2.60	0.5
4	$4.08 \pm 0.24 \times 10^{5}$[a]	-6.50 ± 0.084	-7.66	-1.16	1
5	$5.34 \pm 0.36 \times 10^{4}$[a]	-2.46 ± 0.071	-6.45	-3.99	1
6	$5.13 \pm 0.19 \times 10^{5}$[a]	-9.83 ± 0.073	-7.8	2.03	0.33
7	$9.90 \pm 0.60 \times 10^{5}$[a]	-3.18 ± 0.027	-8.18	-5.00	1
8	$1.3 \pm 0.07 \times 10^{6}$[a]	-5.69 ± 0.040	-8.34	-2.65	1
9	$2.08 \pm 0.27 \times 10^{7}$[b]	-6.48 ± 0.064	-9.99	-3.50	1
10	$8.26 \pm 0.31 \times 10^{9}$[b]	-14.4 ± 0.060	-13.5	0.880	1
11	$2.29 \pm 0.32 \times 10^{6}$[a]	-4.63 ± 0.043	-8.68	-4.05	1
12	$1.05 \pm 0.09 \times 10^{6}$[a]	-2.00 ± 0.022	-8.22	-6.22	1
13	$4.98 \pm 0.29 \times 10^{5}$[a]	-2.11 ± 0.021	-7.77	-5.67	1
14	$1.67 \pm 0.04 \times 10^{7}$[a] $1.46 \pm 0.02 \times$	-9.16 ± 0.034 -4.83 ± 0.041	-9.86 -7.05	-0.696 -2.23	2

	10^{5}[a]			
	1.61 ± 0.04 ×		-0.310	
15	10^{5}[a]	-6.80 ± 0.038	-7.11	1

Discussion of the Binding Affinity Data. In the selection of guests to use to create a blinded dataset for the SAMPL6 challenge, one of the goals was to achieve as wide a dynamic range of binding constants as possible. In this study, the K_a values ranged from a low of 5.34 ± 0.36 × 10^4 to a high of 8.26 ± 0.31 × 10^9 M^{-1} which corresponds to more than five orders of magnitude and a span of ΔG of more than 7 kcal mol^{-1} between weakest and tightest. Drug molecules **2** – **6** and **14** contain aromatic rings along with hydrophilic alkylated tertiary and quaternary ammonium ions; they bind more weakly than the more hydrophobic primary cycloalkylammonium ions **7** – **12**. The relatively weak binding of CB[8] toward its drug-like guests could be advantageous toward its use to create new drug formulations that exhibit passive release.[20] As expected, all of the complexes are driven by favorable ΔH˚ values presumably due to the expulsion of high energy water molecules from inside the cavity of CB[8] upon host•guest complexation.[2d,5b,7,21] The complexes with the smallest enthalpic driving force (CB[8]•**5**, CB[8]•**12**, and CB[8]•**13**) are those that position hydrophilic OH functional groups (**5** and **12**) within the CB[8] cavity which results in an enthalpic desolvation penalty upon binding and **13** that does not fill the CB[8] cavity completely whereby energetically frustrated waters remain inside CB[8] within the CB[8]•**13** complex. The trends in binding strength for the alkyl (di)ammonium ions **7** – **13** is noteworthy. For example, as the number of methylene units in cycloalkanes is increased (**8** – **10**) from 7 to 8 to 12, the binding constant increases by 16-fold and 397-fold, respectively which is

probably due to the enhanced hydrophobicity of the hydrocarbon skeleton as the number of C-atoms increases.[17b] Previously, we have measured the K_a value for CB[8]•1-aminoadamantane as $K_a = 8.2 \times 10^8$ M^{-1} in 50 mM NaOAc at pH 4.74.[17b] In this work, we determined a 358-fold lower Ka value for the CB[8]•noradamantaneammonium ion complex ($K_a = 2.29 \times 10^6$ M^{-1}), which we believe reflects the decrease of the number of C-atoms in the hydrophobic binding domain. Similarly, guest **12** with its hydroxyl substituent forms a 780-fold weaker complex with CB[8] ($K_a = 1.05 \pm 0.09 \times 10^6$ M^{-1}) than 1-aminoadamantane does. As for guest **7**, we have previously found that it binds 4-fold weaker to CB[7] than **11** does;[15a] in this work we observe a 2.3-fold difference in K_a between the CB[8]•**7** and CB[8]•**11** complexes.

Conclusion. In summary, we have investigated the formation of complexes between CB[8] and guests **2** – **15** by a combination of ^{1}H NMR spectroscopy and ITC measurements to serve as a blinded dataset for the SAMPL6 challenge. We find that the drug-like guests **2** – **6** and **15** form relatively weak complexes with CB[8] with K_a values in the 10^4 – 10^6 M^{-1} range. Interestingly, however, some drugs contain multiple binding domains and form complexes that deviate from 1:1 host:guest binding stoichiometry. For example, **3** forms the $CB[8]_2$•**3** complex whereas **6** with its 3 quaternary ammonium ion binding domains prefers to form the $CB[8]_3$•**6** complex. In contrast, guest **14** (Aricept) forms a CB[8]•$\mathbf{14}_2$ complex whereby the benzylammonium ion binding epitopes from two molecules of **14** combine to fill the cavity of CB[8] in a head-to-tail binding mode. All of the complexes are driven by favorable enthalpic contribution to ΔG° probably due to the release of high energy H_2O molecules from the cavity of CB[8] upon complex

formation. The influence of the number of C-atoms in the guest across a homologous series (e.g. **8** – **10**) correlates with increases in both K_a and enthalpic driving force. Overall, the work provides a blinded dataset of Ka values and CB[8]:guest stoichiometries that promotes synergistic interactions between the supramolecular chemistry and computational chemistry communities who both seek to advance the state of the art of understanding and predicting of host•guest complexation phenomena in aqueous solution.

Experimental Details. *General.* Drugs **2** – **6**, **14**, and **15** were purchased from commercial suppliers and used without further purification. Compounds **7** – **13** were purchased as their free base and converted to their hydrochloric salts by adding conc. HCl, concentrating by rotary evaporation, and then drying at high vacuum overnight. ^{1}H NMR spectra were measured on commercial NMR spectrometers operating at 600 MHz. ITC data was collected on a Malvern Microcal PEAQ-ITC instrument with a 200 µl cell volume. The ITC method used consisted of 19 injections at a 2 µl injection volume for all titrations except **14** which was two successive 19 injection titrations concatenated with the ConCat32 software. For direct titrations with binding constants exceeding $K_a = 1 \times 10^7$ M^{-1} such as guests **9** and **10**, a competition titration was completed with an appropriate weak guest, **11**. Data was fit with either the single set of sites model, the competitive binding model (for **9** and **10**), or in the case of **14** sequential binding model on the MicroCal PEAQ-ITC analysis software.

Disclosure statement. No potential conflict of interest was reported by the authors.

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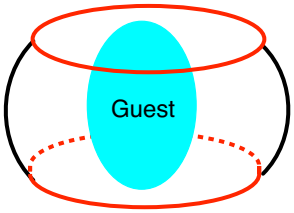

14 CB[8]•guest complexes
Blinded Dataset for
SAMPL6 Challenge

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