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# Exploring the Origins of Enthalpy-Entropy Compensation by Calorimetric Studies of Cyclodextrin Complexes

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## Abstract

Cyclodextrin complexes were used as simple model systems to explore the enthalpy-entropy compensation phenomenon which is often observed in biomolecular processes, e.g. in protein-ligand binding. The complexation thermodynamics for the binding of a series of adamantane derivatives to several cyclodextrin hosts were determined by isothermal titration calorimetry in the temperature range 10-55 °C. As for other cyclodextrin complexes, the thermodynamic parameters depended systematically on the structural modifications of the cyclodextrins. Hydroxypropyl chains at the rims of the cyclodextrin hosts changed the thermodynamic fingerprint of binding to all guests by inducing significant increases in the complexation enthalpies and entropies. Similarly, the heat capacity changes upon complexation also showed a linear dependence on the number of hydroxypropyl chains. The altered complexation thermodynamics was ascribed to the increased dehydration of polar groups on the guest by the hydroxypropyl chains on the host. This unfavorable interaction destabilized the complexes as the enthalpic penalty was only partially compensated by the gain in entropy. The degree of enthalpy-entropy compensation depended on the guest molecule and seems to be related to the hydrophilicity/hydrophobicity of the desolvated molecular surface.

## Introduction

Molecular recognition depends on numerous noncovalent interactions between the associating molecules. When the association takes place in a solvent the interactions with the solvent molecules also contribute. Given the complexity of such systems it is no surprise that binding free energies ( $\Delta G^\circ$ ) are extremely difficult to predict, even for simple binary host-guest systems. It is often challenging to obtain even a qualitative understanding of the main driving forces for formation of host-guest complexes. Driving forces that may contribute to the formation of a complex include electrostatic/coulombic forces, van der Waals interactions, hydrogen bonding, etc. In aqueous solution, the hydrophobic effect also plays an important, and often dominant, role.

The binding free energy,  $\Delta G^\circ$ , can be divided into enthalpic and entropic contributions, which are often interpreted in terms of driving forces. Attractive electrostatic and van der Waals forces are assumed to result in an enthalpy-driven binding, while hydrophobic attraction supposedly is characterized by a large gain in entropy. This simplistic interpretation of binding thermodynamics has caused some confusion and seemingly contradictory observations. For example, there are many experimental indications that the hydrophobic effect is a crucial driving force for the formation of cyclodextrin (CD) inclusion complexes.<sup>1</sup> However, the thermodynamic fingerprint of these interactions does not conform to the conventional view that hydrophobic interactions are entropy-driven. On the contrary, the vast majority of CD complexes are enthalpy-driven.<sup>2</sup> This “nonclassical hydrophobic effect” is not only observed for CDs but may be a general characteristic of the solvent-driven association of molecules of moderate polarity,<sup>3</sup> keeping in mind that the CD cavity is considered semipolar.<sup>1</sup> Release of water molecules from the CD cavity constitutes another explanation of the enthalpy-driven complexation.<sup>4</sup> The latter

explanation is hypothesized to constitute a general driving force in systems where water molecules are confined in small cavities.<sup>5</sup>

The example above illustrates how the experimentally observed thermodynamic fingerprint plays a pivotal role in the arguments for the driving forces and their molecular interpretation.

Similarly, thermodynamic data play a central role in the recent arguments for the existence of a distinct chaotropic effect, orthogonal to the hydrophobic effect.<sup>6</sup> But is it really possible to conclude anything about the driving forces based on the enthalpic and entropic contributions? Possibly, but it requires a thorough understanding of the various contributing factors to these thermodynamic functions. Especially, the phenomenon called enthalpy-entropy compensation (EEC) needs to be understood as it can mask the thermodynamic fingerprint of the mechanisms that are responsible for the binding free energy. As the name indicates, EEC refers to processes where an enthalpic contribution is partially or wholly offset (compensated) by a similar but oppositely directed entropic contribution. Consequently, such processes do not contribute much to the free energy although they may dominate the total thermodynamic fingerprint.

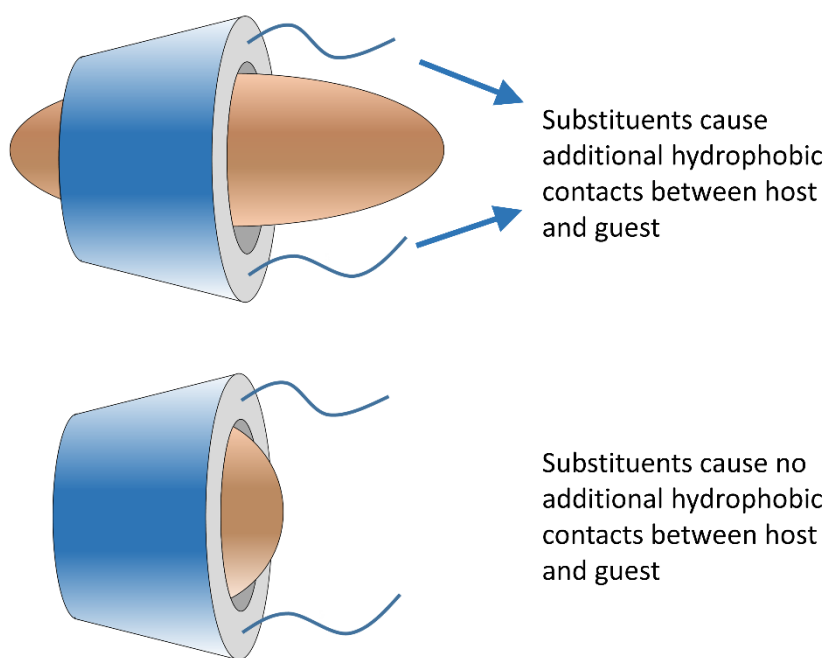
Reorganization of water molecules near solute surfaces have been suggested to be a source of EEC.<sup>7,8</sup>

EEC is a long known and much debated topic, but its underlying causes are not clear.<sup>9-12</sup> We have previously reported the complexation thermodynamics of numerous natural and modified CD host molecules with a number of bile salt guests.<sup>13-15</sup> For those complexes, a strong EEC effect was observed. Complexes with natural  $\beta$ CDs were driven by a large negative enthalpy ( $\Delta H^\circ \approx -25$  to  $-30$  kJ/mol) and slightly destabilized by entropy ( $T\Delta S^\circ \approx 0$  to  $-7$  kJ/mol). Chemical modification of the CD hosts resulted in a significant increase in  $\Delta H^\circ$  but also in a compensating increase in  $T\Delta S^\circ$ , so that the effect on the binding free energy was relatively small (see Eq. 3).

This effect scaled with the size and number of substituents at the rims of the CDs so that formation of complexes with some of the highly substituted CDs were mainly driven by entropy ( $T\Delta S^\circ \approx 10$  to  $15$  kJ/mol) and to a much lesser extent by enthalpy ( $\Delta H^\circ \approx -5$  kJ/mol). A similar picture was obtained for complexation with natural and modified  $\gamma$ CDs.<sup>16</sup> Although the presence of substituents at the rims of the CDs completely altered the thermodynamic fingerprint of the complexation process, this was not interpreted as a change in the driving force. Rather, the observed EEC was attributed to the increased hydrophobic contacts between the guest and the extended host molecule. This interpretation was supported by molecular dynamics (MD) simulations, measured heat capacity changes, and osmotic stress measurements.<sup>17–19</sup> On the other hand, a detailed analysis of the MD simulations revealed that altered energetics of the water in the cavity of the free CD constituted at least part of the explanation for the observed EEC.<sup>20</sup> Upon formation of the complex, release of the cavity water into the bulk will thus exhibit an altered thermodynamic fingerprint.

The present work aims to explore these hypotheses about the origins of the EEC observed for CD complexes. Is the EEC brought about by increased hydrophobic contacts between the guest and the CD substituents and the concomitant release of surface water? Or is there another explanation, such as altered energetics of the cavity water that is expelled upon complexation? The hypotheses and the experimental strategy are illustrated in Figure 1. Increased hydrophobic contacts occur when the guest molecule protrudes from the CD cavity and interacts with the substituents at the rims of the CD, but a sufficiently small guest molecule will not protrude from the CD cavity and will not form hydrophobic contacts with the substituents. Thus, if the hydrophobic contacts hypothesis is valid, no substituent-induced EEC is expected for the complexation with small guest molecules. Testing this hypothesis requires a suitable guest

molecule. Adamantane is a spherical hydrocarbon that is able to fit almost perfectly into the cavity of  $\beta$ CD without protruding from the cavity.<sup>21-23</sup> As the adamantane moiety also has a strong affinity for  $\beta$ CD this binding motif is an excellent model for testing the hydrophobic contacts hypothesis. The present work explores the complexation thermodynamics of several water-soluble adamantane derivatives (AD) upon binding to natural and modified  $\beta$ CDs.



*Figure 1 Small guest molecules are not expected to interact with the substituents at the CD rims. The small guest could be adamantane and the large guest could be a bile salt.*

## Experimental Section

### Chemicals

Natural  $\beta$ -cyclodextrin, randomly substituted (2-hydroxypropyl)- $\beta$ -cyclodextrin (HP093), and 1-Adamantylamine were purchased from Adamas-Beta, Shanghai, China. Randomly substituted (2-hydroxypropyl)- $\beta$ -cyclodextrin (HP063) was from Alfa Aesar, Shanghai, China.

Adamantanecarboxylic acid (AdCOOH) and 1-adamantaneacetic acid (AdCH<sub>2</sub>COOH) were from J&K Chemical, Shanghai, China. 1-Adamantanol (AdOH) and 1-Adamantanemethanol (AdCH<sub>2</sub>OH) were purchased from Sigma-Aldrich. The water content of the three CD samples were determined by Karl-Fischer titration to 11.2, 7.1, and 6.4 wt% for  $\beta$ CD, HP063 and HP093, respectively. The water content was taken into account when calculating the concentration of the cyclodextrins.

### Nuclear Magnetic Resonance Spectroscopy

Spectra were recorded at 25°C on a Bruker Avance-600 spectrometer, equipped with a cryoprobe. For structural characterization of HP $\beta$ CDs, 1D <sup>1</sup>H and <sup>13</sup>C and 2D HSQC, HMBC, H2BC spectra were recorded on samples containing around 10 mM CD in D<sub>2</sub>O. For structural characterization of the complexes, 2D ROESY spectra (16 scans, 400 ms mixing time) were recorded on samples with around 10 mM of CD and AD dissolved in D<sub>2</sub>O based phosphate buffer, pH 7.2.



### Isothermal titration calorimetry

Titration were made on a VP-ITC (Malvern Panalytical, Malvern, UK) with a cell volume of 1.4 ml. Aqueous solutions of CDs were titrated into solutions of ADs in aliquots of 10  $\mu$ L, in total 28 injections. The first injection of only 2  $\mu$ L was not included in the data analysis. All solutions were buffered with a 50 mM phosphate buffer, pH 7.20. At this pH the ADs with carboxylic acid or amino groups carry one negative or positive charge, respectively. AD concentrations were in the range 0.3-1 mM and CD concentrations were approximately 10 or 20 times higher, resulting in a molar ratio of 2 or 4 at the end of the titration. The lower the binding constant, the higher the concentrations. Titrations were conducted at 10, 25, 40 and 55 °C, except for the adamantols which caused some problems at elevated temperatures, possibly due to problems with solubility or chemical stability.

### Molecular dynamics simulations

Complexes of natural  $\beta$ CD and a HP $\beta$ CD with the two anionic guests were simulated, as well as the uncomplexed species. Each of the 4 complexes were simulated in two binding modes where the carboxylate group protruded from either the primary or the secondary CD rim. For the native CD structures, bonded and nonbonded parameters were from the CHARMM carbohydrate force field.<sup>24,25</sup> Parameters for the 2-hydroxypropyl substituents and the adamantane derivatives were generated by the CGenFF program version 1.0.0,<sup>26,27</sup> with the CHARMM General Force Field version 3.0.1.<sup>28</sup> See ref. 23 for details of the AD parameters. Molecules and complexes were solvated in cubic boxes of TIP3P water with a side length of 34-36 Å. If necessary, charge neutrality was obtained by adding a sodium ion.

Initial structures of the complexes were generated by placing the AD guest at the opening of the secondary CD rim with the charged group pointing away from the cavity. Simulations were run with 2 fs time steps in the NAMD software.<sup>29</sup> Complexes and free HP $\beta$ CDs were simulated with a 10 ns equilibration run and a 20 ns production run. Free ADs and free natural  $\beta$ CD have fewer structural degrees of freedom and required less simulation time. ASA was determined in the VMD software<sup>30</sup> using a spherical probe of radius 1.4 Å at a sampling frequency of 10 ps and averaged.

## Results

A total of 15 AD:CD complexes were characterized with respect to structure and complexation thermodynamics. Two neutral ADs, AdOH and AdCH<sub>2</sub>OH, two negatively charged ADs, AdCOO and AdCH<sub>2</sub>COO, and a single positively charged AD, AdNH<sub>3</sub>, were chosen as guest molecules. Natural  $\beta$ CD and two HP $\beta$ CDs were used as host molecules. Structures are shown in Figure 2.

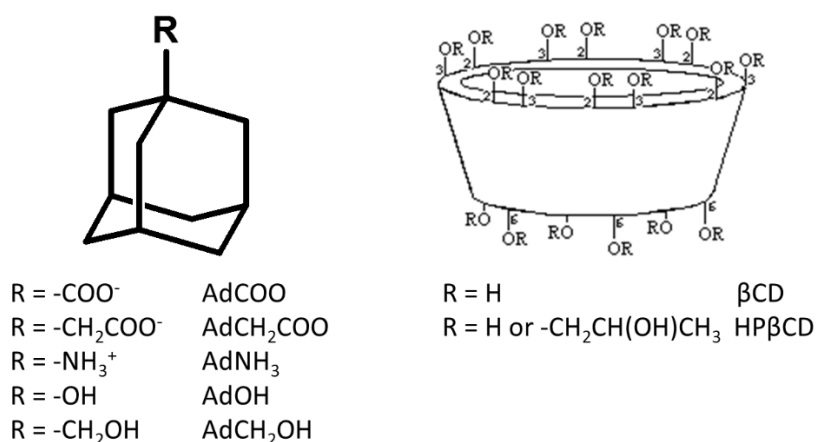


Figure 2 Structures of the investigated adamantane derivatives and cyclodextrins.

### Characterization of CDs and their complexes

The two HP $\beta$ CD samples were characterized by NMR according to a previously described method,<sup>13</sup> using the areas of selected peaks in the <sup>1</sup>H and <sup>13</sup>C spectra (Figures S1 and S2 in the Supporting Information). The average degree of substitution, expressed as the number of HP chains per glucose unit, was 0.63 and 0.93 for HP063 and HP093. Around 58% of the HP chains were located on O2 of the secondary CD rim, while around 10% were located on O6 of the primary rim. The remaining 30% were assumed to be attached to O3 on the secondary rim.

Previous analyses of HP $\beta$ CD samples by other analytical techniques yielded similar patterns of substitution.<sup>31,32</sup>

NMR ROESY spectra confirmed the inclusion of adamantane derivatives into the CD cavity, as evidenced by strong interactions between the interior CD protons, H3 and H5, and all of the protons on the adamantyl moiety (Figures S3 and S4). The complexity of the HP $\beta$ CD samples resulted in significantly broadened and overlapping signals, which prevented an unambiguous determination of the orientation of the adamantane derivatives. For complexes with natural  $\beta$ CD, it was recently established that the charged group of AdNH<sub>3</sub> protrudes from the secondary rim, while AdCOO and AdCH<sub>2</sub>COO are oriented in both directions.<sup>23</sup> Those orientations were also observed in methylated  $\beta$ CDs,<sup>23</sup> and it seems likely that HP $\beta$ CDs behave the same way. Very weak ROESY crosspeaks were observed between the protons on the AD guests and the methyl group on the HP chains of HP093, indicating limited interactions between these moieties (Figure S5)

### Molecular Dynamics Simulations

Molecular dynamics simulations of four of the investigated complexes were conducted to explore their structure and dynamics. In particular, the influence of the HP substituents was explored to test the validity of the hypothesis expressed in Figure 1. To this end, inclusion complexes with a hydroxypropylated  $\beta$ CD were compared to the corresponding inclusion complexes with natural  $\beta$ CD. The HP $\beta$ CD was named HP086 as its degree of substitution was 0.86 (6 HP chains distributed over 7 glucose units). To resemble the substitution pattern of the

experimental CD samples as closely as possible, three HP chains were attached to O2's on the glucose units, two to O3's, and a single HP chain to an O6.

Each combination of host and guest was simulated with two different guest orientations in which the carboxylate groups on the guest protruded from either the wider secondary rim of the CD or from the primary rim. Which one is the correct guest orientation is difficult to determine experimentally, and both secondary and primary complexes may coexist, as observed for natural  $\beta$ CD and methylated  $\beta$ CDs.<sup>23</sup>

During the 30 ns of simulation, none of the complexes dissociated. Some mobility of the guests was observed, but in all complexes the adamantyl moiety stayed inside the CD cavity. The HP chains on HP086 were very mobile and interacted with the guest and each other. To quantify their interaction with the guest molecule the water accessible surface area (ASA) was used. This parameter is also expected to be correlated with experimentally determined thermodynamic functions such as  $\Delta C_p$ .<sup>33</sup> Numerous interactions between the HP-chains and the guest would result in a large burial of surface area, that is, a large negative change in the water accessible surface area.

All ASA values for free and complexed species are presented in Table S1 in the Supporting Information. The change in ASA upon complexation was calculated as the difference in ASA between the complexes and the free species:

$$\Delta ASA = ASA_{\text{complex}} - (ASA_{\text{CD}} + ASA_{\text{AD}}) \quad \text{Eq. 1}$$

The buried surface area ( $\Delta ASA$ ) was grouped into polar ( $\Delta ASA_p$ ) and nonpolar surface area ( $\Delta ASA_{np}$ ). The resulting values appear in Table 1 for both secondary and primary complexes. It is clear that more nonpolar than polar surface area was buried upon complexation, which is not

surprising given the nonpolar nature of the adamantyl moiety and the CD cavity. The presence of HP chains on the CD hardly resulted in increased burial of surface area, i.e. there were few direct contacts between the HP chains and the guest. In the most extreme case, the secondary HP086:AdCH<sub>2</sub>COO complex, the presence of 6 HP chains only resulted in increased burial of 21 Å<sup>2</sup> of nonpolar surface when compared to the corresponding complex with natural βCD. For comparison, *each* HP chain in complexes with bile salt guests dehydrated an estimated 12-16 Å<sup>2</sup> of nonpolar surface due to numerous contacts between the HP chains and the protruding guest molecules.<sup>17</sup> Thus, the MD simulations essentially confirmed the picture in Figure 1, in which the HP chains interact with large protruding bile salt guests but not with small cavity-included adamantane guests.

*Table 1 Buried polar (ΔASA<sub>p</sub>) and nonpolar (ΔASA<sub>np</sub>) surface area of the simulated complexes. All values are in Å<sup>2</sup>. It is not clear whether the ADs are oriented with the charged carboxylate group protruding from the secondary or primary rim of the CDs, therefore both orientations were simulated.*

| Complex                     | Secondary complex |                    | Primary complex   |                    |
|-----------------------------|-------------------|--------------------|-------------------|--------------------|
|                             | ΔASA <sub>p</sub> | ΔASA <sub>np</sub> | ΔASA <sub>p</sub> | ΔASA <sub>np</sub> |
| βCD:AdCOO                   | -68               | -306               | -130              | -290               |
| HP086:AdCOO                 | -75               | -316               | -102              | -307               |
| βCD:AdCH <sub>2</sub> COO   | -76               | -311               | -130              | -311               |
| HP086:AdCH <sub>2</sub> COO | -67               | -332               | -121              | -322               |

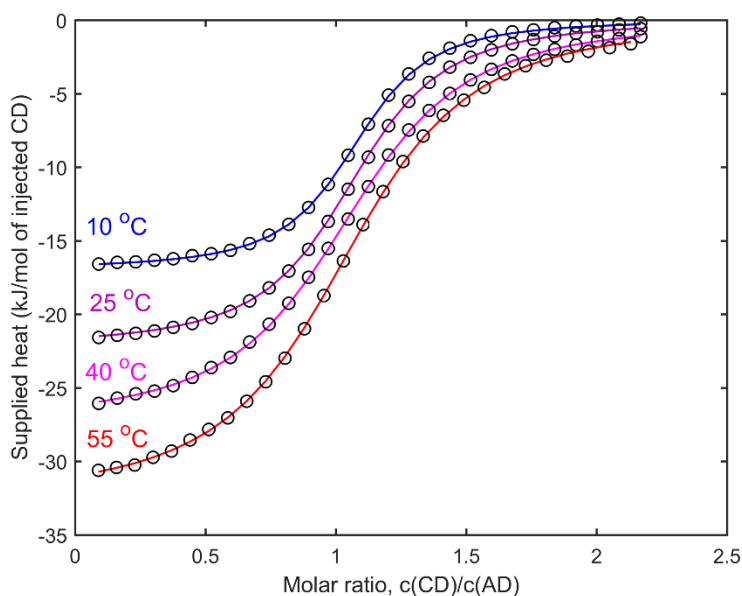
### Complexation Thermodynamics

Complexation thermodynamics was determined by isothermal titration calorimetry for all combinations of the three host molecules (βCD, HP063 and HP093) and the 5 guest molecules (AdCOO, AdCH<sub>2</sub>COO, AdNH<sub>3</sub>, AdOH and AdCH<sub>2</sub>OH), in total 15 complexes. Binding thermodynamics are usually obtained by fitting the ‘one set of sites’ model to each enthalpogram, yielding the binding constant,  $K$ , binding enthalpy,  $\Delta H^\circ$ , and stoichiometry,  $N$ , as best fit parameters. Conducting titrations at different temperatures allows for the change in heat

capacity,  $\Delta C_p$ , to be determined as the temperature derivative of  $\Delta H^\circ$ . By exploiting that the temperature dependence of the binding constant is governed by  $\Delta H^\circ$ , as expressed by the van't Hoff equation, more accurate thermodynamic data can be obtained by doing a global fit to multiple titrations at different temperatures.<sup>34</sup> In the global fit  $\Delta C_p$  is assumed to be temperature independent. This assumption is based on personal experience with CD complexes,<sup>15,18,34–36</sup> and there is no indication in the literature that  $\Delta C_p$  for CD complexes should exhibit any significant temperature dependence within the currently investigated temperature range.<sup>37–39</sup> Assuming a temperature independent  $\Delta C_p$  the binding constant can be expressed as:

$$\ln(K) = \frac{\Delta H_0 - T_0 \Delta C_p}{R} \left( \frac{1}{T_0} - \frac{1}{T} \right) + \frac{\Delta C_p}{R} \ln \left( \frac{T}{T_0} \right) + \ln(K_0) \quad (\text{Eq. 2})$$

where  $\Delta H_0$  and  $K_0$  are the complexation enthalpy and binding constant at a reference temperature,  $T_0$ , here chosen to be 25 °C. The global fitting procedure then yielded  $\Delta H_0$ ,  $K_0$  and a temperature independent  $\Delta C_p$  as global best fit parameters. Additionally, a set of local parameters,  $N$ , one for each titration, were varied to fit the data. An example of a global fit is shown in Figure 3 together with the obtained fitting parameters and their statistics. More examples are shown in the Supporting Information, and all obtained thermodynamic parameters are presented in Table S2. In most cases, excellent fits of the enthalpograms were obtained, and the errors on the obtained thermodynamic parameters were very small.



| Parameter                     | Best fit value | Error at 95% CL | Correlation Matrix |      |       |
|-------------------------------|----------------|-----------------|--------------------|------|-------|
| K at 25 °C (M <sup>-1</sup> ) | 109789         | 1173            | 1                  | 0.38 | 0.28  |
| ΔH at 25 °C (kJ/mol)          | -23.52         | 0.04            |                    | 1    | -0.31 |
| ΔC <sub>p</sub> (J/mol/K)     | -374           | 2               |                    |      | 1     |

Figure 3 Global fit of 4 calorimetric titrations of AdCH<sub>2</sub>COO with βCD, conducted at 10, 25, 40 and 55 °C. The fitting parameter, *N*, was local and assumed the values 0.942, 0.943, 0.938, and 0.936.

Once the binding constant and binding enthalpy was determined, the binding free energy and binding entropy was calculated:

$$\Delta G^\circ = -RT\ln(K) = \Delta H^\circ - T\Delta S^\circ \quad (\text{Eq. 3})$$

where *R* is the gas constant.

The obtained binding thermodynamics for the complexes of natural βCD with the three charged ADs are in good agreement with values previously reported by Cameron *et al.*, with the exception of Δ*H*<sup>°</sup> for the βCD:AdCH<sub>2</sub>COO complex where the previous value is too negative by around 5 kJ/mol.<sup>40</sup> The complexes of βCD with the two neutral ADs are less characterized in the



literature, and only the  $\beta$ CD:AdCH<sub>2</sub>OH complex has been studied by calorimetry at a single temperature (25 °C), at which slightly deviating values of  $K$  and  $\Delta H^\circ$  were reported.<sup>41</sup> None of the complexes with HP $\beta$ CDs have previously been described in the literature

The thermodynamic parameters,  $\Delta G^\circ$ ,  $\Delta H^\circ$ , and  $T\Delta S^\circ$ , for all 15 complexes are plotted in Figure 4, along with previously published data for complexes of HP $\beta$ CDs with three bile salt guests.<sup>13</sup> Although the most apparent feature is the large linear increase in  $\Delta H^\circ$  and  $T\Delta S^\circ$  with the number of HP chains on the host CD, it is also noteworthy that the HP chains severely destabilized the complexes. Going from  $\beta$ CD to HP091, the binding free energy increased 5-6 kJ/mol, meaning that the binding constants were reduced by a factor of 10 in the highly modified CD complexes (corresponding to an increase in  $\Delta G^\circ$  of 5.7 kJ/mol). Complexes with charged ADs were destabilized slightly more than complexes with neutral ADs.

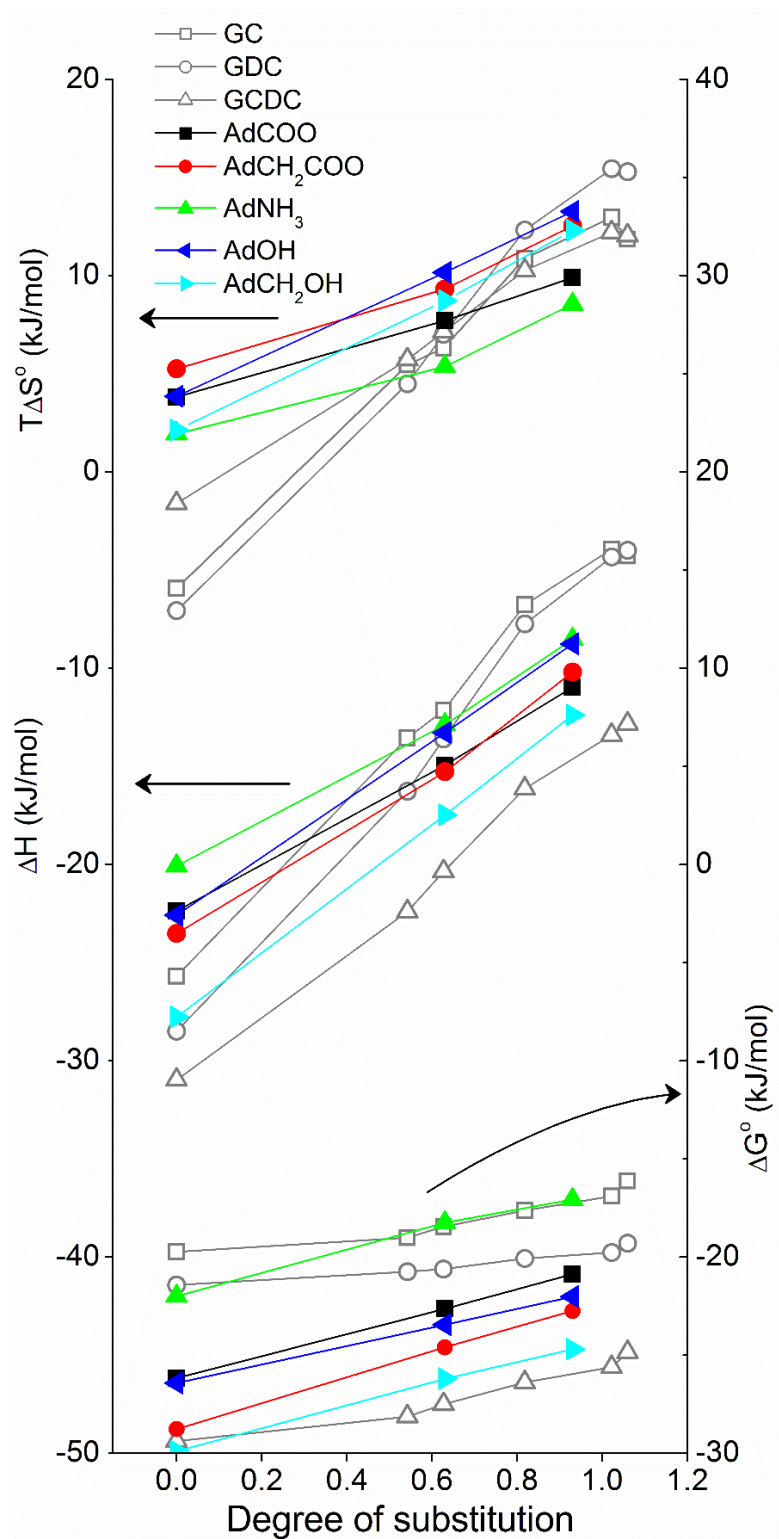


Figure 4  $\Delta H^\circ$ ,  $T\Delta S^\circ$  (left axis) and  $\Delta G^\circ$  (right axis) for the formation of complexes with  $\beta$ CD and HP $\beta$ CDs, plotted as a function of the number of HP chains per glucose unit of the CD hosts. Data for AD guests are shown with filled symbols while previously reported data<sup>13</sup> for three bile salt guests, glycocholate (GC), glycodeoxycholate (GDC) and glycochenodeoxycholate (GCDC), are shown with open symbols. All data are from Table S2.

## Discussion

### Disrupted solvent interactions with polar groups determine binding affinity

The measured binding constants can be easily rationalized. For the  $\beta$ CD complexes, the binding affinity correlates with the distance between the nonpolar adamantyl moiety and the attached polar group. The adamantyl moiety is located in the hydrophobic cavity of the CD with the polar group protruding from the cavity. The complexes are presumably destabilized when the polar group is too close to the CD host, resulting in reduced interactions between the polar group and the solvent. The charged  $-\text{NH}_3$  group is directly attached to the adamantyl moiety and is therefore strongly dehydrated upon complexation, thus  $\text{AdNH}_3$  had the lowest binding affinity. The charged carboxylate oxygens are separated from the adamantyl moiety by two and three bonds in  $\text{AdCOO}$  and  $\text{AdCH}_2\text{COO}$ , respectively, thus fewer interactions with the solvent are broken upon complexation, and the binding affinity increases in the order  $\text{AdNH}_3 < \text{AdCOO} < \text{AdCH}_2\text{COO}$ . Same pattern is seen for the two neutral guests. Although the hydroxyl groups in  $\text{AdOH}$  and  $\text{AdCH}_2\text{OH}$  are separated from the adamantyl moiety by only one and two bonds, respectively, the destabilizing effect is not as severe as for the charged guests due to the weaker interactions of the hydroxyl group with the solvent.

Similar reasoning can be used to rationalize the destabilizing effect of the HP chains. The chains presumably disrupt the attractive interactions between the polar guest groups and the solvent. This may explain why the HP chains caused a larger destabilization of complexes with charged ADs than neutral ADs. The partial dehydration of the neutral hydroxyl groups in  $\text{AdOH}$  and  $\text{AdCH}_2\text{OH}$  presumably cost less energy than dehydration of the charged carboxylate and amino groups. This interpretation is supported by the relatively small destabilizing effect of HP chains

on complexes with bile salts,<sup>13</sup> where solvent interactions with the polar hydroxyl groups on the guest probably are less disrupted by the HP chains.

#### Cyclodextrin substituents induced large increase in $\Delta H^\circ$ and $T\Delta S^\circ$

As seen on Figure 4, the HP chains significantly altered the thermodynamic fingerprint of the complexation process. Upon going from  $\beta$ CD to HP091,  $\Delta H^\circ$  increased with 11-15 kJ/mol, depending on the guest. The increase in  $T\Delta S^\circ$  was a little smaller: 6-7 kJ/mol for complexes with charged ADs and around 10 kJ/mol for neutral ADs. Whereas the complexes with natural  $\beta$ CD were almost exclusively driven by enthalpy, the binding free energy of complexes with HP091 was defined by almost equal contributions from enthalpy and entropy. The observed increase in  $\Delta H^\circ$ , and especially the increase in  $T\Delta S^\circ$ , were less pronounced than for previously studied complexes with bile salt guests, also shown in Figure 4.

Interestingly, it seems to be a general trend that substituents at the rims of the CD host increase the enthalpy and entropy of complexation. This has been observed for ibuprofen binding to HP $\beta$ CD,<sup>39</sup> adamantane-based drugs binding to sulfobutylether- $\beta$ CD,<sup>42</sup> rimantadine and amantadine ( $\text{AdNH}_3$ ) binding to sulfobutylether- $\beta$ CD (compare data for sulfobutylether- $\beta$ CD<sup>43</sup> to data for  $\beta$ CD<sup>41</sup>), numerous drugs binding to sulfobutylether- $\beta$ CD,<sup>44</sup> aminobenzoic acids binding to HP $\alpha$ CD and HP $\beta$ CD<sup>45</sup>, and the already mentioned binding of bile salts to methylated, hydroxypropylated and sulfobutylated  $\beta$ CDs and  $\gamma$ CDs. Despite the large experimental evidence of such effects on the complexation thermodynamics the underlying reasons are still somewhat speculative. As outlined in the introduction, the working hypothesis ascribes the substituent-induced increments in  $\Delta H^\circ$  and  $\Delta S^\circ$  to increased hydrophobic contacts between substituents and

guest. The current MD simulations indicated limited contacts between the HP chains and AD guests, so the observed increase in  $\Delta H^\circ$  and  $\Delta S^\circ$  seems to disprove this hypothesis. However, the measured heat capacities creates a more ambiguous picture, as discussed below.

#### Decrease in $\Delta C_p$ indicates hydrophobic contacts between HP-chains and guest

The change in isobaric heat capacity,  $\Delta C_p$ , is an often used measure of the molecular surface area that becomes exposed to water during a molecular process. Exposure of nonpolar surface is characterized by a large positive  $\Delta C_p$  while exposure of polar surface gives a negative  $\Delta C_p$ .<sup>33,46</sup> The measured negative values of  $\Delta C_p$ , plotted in Figure 5 are thus consistent with the large burial of hydrophobic surface upon insertion of the adamantyl moiety into  $\beta$ CD.

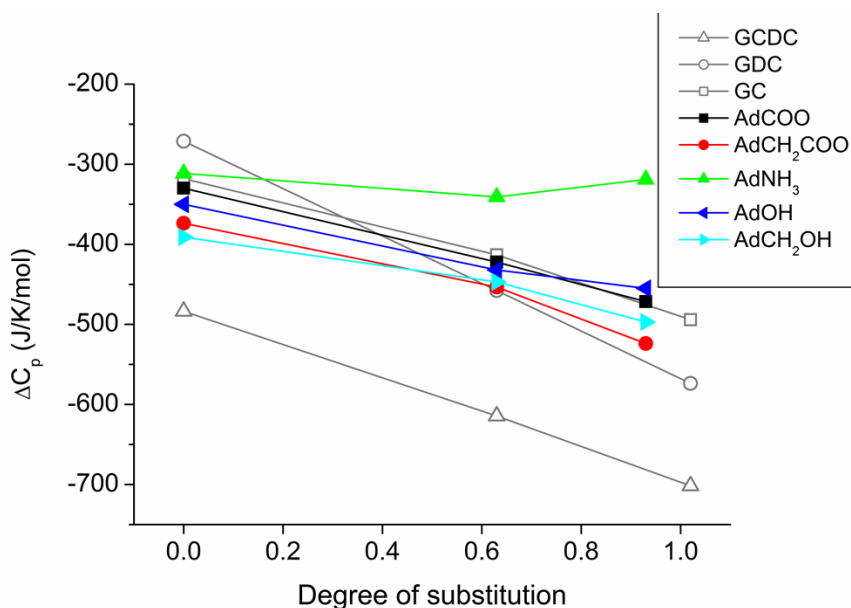


Figure 5  $\Delta C_p$  for the formation of complexes with  $\beta$ CD and HP $\beta$ CDs. Data for AD guests are shown with filled symbols while previously reported data<sup>17</sup> for three bile salt guests, glycocholate (GC), glycodeoxycholate (GDC) and glycochenodeoxycholate (GCDC), are shown with open symbols. All data are from Table S2.

What is really interesting is the effect of the hydroxypropyl substituents on  $\Delta C_p$ . With the exception of AdNH<sub>3</sub>, there was a substantial decrease in  $\Delta C_p$  with increasing degree of substitution, indicating formation of hydrophobic contacts between the HP chains and the AD guests. The literature does not present any universal quantitative relationship between  $\Delta C_p$  and  $\Delta ASA_{np}$  but previous studies suggest a proportionality constant of around 2 J/mol/K per Å<sup>2</sup> for extracavity interactions in cyclodextrins.<sup>17,47</sup>  $\Delta C_p$  for complexes with HP093 were more negative than for the corresponding complexes with natural  $\beta$ CD by around 100-150 J/mol/K. This decrease in  $\Delta C_p$  translates into an additional burial of 50-75 Å<sup>2</sup> in complexes with HP093 but analysis of the MD simulations, presented in Table 1, yields a much smaller number. Is something wrong with the MD simulations, or is the interpretation of  $\Delta C_p$  in terms of accessible surface area too simplistic? While it is worth noting that there are other contributions to  $\Delta C_p$ ,<sup>11</sup> it is also questionable whether  $\Delta ASA$  is a sufficient measure of the hydration changes. Without going into a deeper discussion of these issues, it is noted that both the MD simulations and the  $\Delta C_p$  values showed that the AD guests interacted less with the HP chains than did the previously studied BS guests. The HP chains also have a smaller impact on the enthalpies and entropies of binding to the AD guests, suggesting that the observed substituent-induced variations in complexation thermodynamics indeed are related to the interactions between the HP chains and the guest.

#### Disrupted polar interactions reduce the degree of enthalpy-entropy compensation

The studied AD:CD complexes were investigated to see whether the presence of HP chains at the CD rims would produce compensating changes to  $\Delta H^\circ$  and  $T\Delta S^\circ$ . The HP chains brought about

significant increases in  $\Delta H^\circ$  and  $T\Delta S^\circ$  but they also destabilized the complexes, i.e. the observed changes in  $\Delta H^\circ$  and  $T\Delta S^\circ$  were not fully compensating. The low degree of compensation is also apparent from the enthalpy-entropy compensation plot in Figure 6 where the slopes are much lower than unity. Only if the slope is unity there will be exact EEC and an unaltered  $\Delta G^\circ$ .

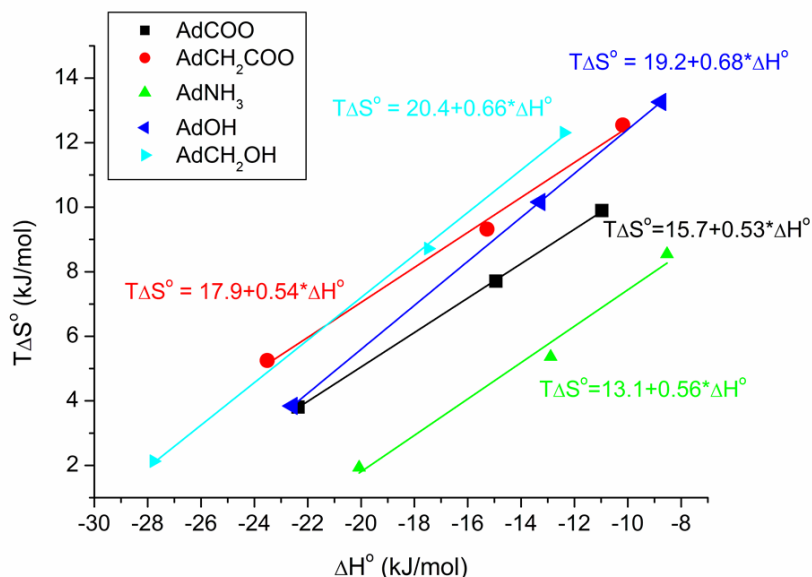


Figure 6 Enthalpy-entropy compensation plot for all AD:CD complexes. For each AD guest, the complexes with the three CD hosts lie very close to the linear regression lines.

It is noteworthy that the slopes in the EEC plot can be grouped in two. For the charged AD guests the slopes are in the range 0.53-0.56, meaning that only half of the destabilizing effect resulting from an increase in  $\Delta H^\circ$  is compensated by increased  $T\Delta S^\circ$ . For the neutral hydroxyl containing ADs, the slopes are significantly higher, around 0.67. For a large number of complexes of bile salts with various modified CDs, the slopes in the EEC plot were closer to unity, between 0.75 and 0.90,<sup>48</sup> and even closer, 0.93-0.94, when only methylated CDs were considered.<sup>18</sup> It is tempting to interpret the slopes in terms of the hydrophilicity of the molecular

surfaces that are dehydrated upon complexation. Partial dehydration of highly hydrophilic molecular moieties, like the charged carboxylate and amino groups, results in a low degree of EEC. Dehydration of nonpolar surface, as in the case of the bile salt complexes, yields an almost complete compensation with slopes close to unity. Dehydration of hydroxyl groups with intermediate hydrophilicity results in an intermediate degree of compensation. This interpretation is corroborated by a large study of the complexation thermodynamics of charged CDs with ionic guests.<sup>49</sup> No compensation was observed for complexes with significant electrostatic (coulombic) interactions between host and guest. Only the complexes with a neutral guest exhibited EEC, but with a slope of 0.5. According the interpretation above, this low degree of compensation is due to dehydration of the hydrophilic zwitterionic headgroup of the employed neutral guest.

The results presented in the present study add to the growing body of evidence that liberation of water molecules from the hydration layers of molecular surfaces is a major source of EEC.<sup>50–52</sup> Much of the evidence comes from protein-ligand interactions, but cyclodextrin complexes are in many ways better model systems due to their smaller and less complex structure which allows for a more detailed and precise structural and thermodynamical characterization.



## Conclusion

The small AD guest molecules were anticipated to be completely included in the cavity of  $\beta$ CD and should therefore not interact with the sidechains at the rims of modified  $\beta$ CDs. However, the results suggest that the sidechains disturb the hydration of the polar moieties of the guest molecules that protrude from the CD cavity. Thus, the studied systems were not as ideal as anticipated and could not provide an unambiguous confirmation of the hypothesis that dehydration of guest and sidechains is the cause of previously observed examples of EEC. Nevertheless, the study resulted in several important findings. As for other CD complexes, the presence of substituents at the CD rims resulted in increased enthalpies and entropies of complexation as well as more negative values of  $\Delta C_p$ , presumably due to the release of hydration waters from the guest and the substituents. Unlike other complexes, where the increase in enthalpy was almost completely compensated by increased entropy, a lower degree of enthalpy-entropy compensation was observed. Importantly, the degree of compensation seems to be related to the polarity of the dehydrated surface, with a low degree of compensation for hydrophilic surfaces and almost complete compensation for hydrophobic surfaces.

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**Supporting Information.** 1D and 2D NMR spectra. Water accessible surface areas. All thermodynamic data. Fitted enthalpograms.

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