# Complexation Kinetics of Cyclodextrins with Bile Salt Anions

Energy Barriers for the Threading of Ionic Groups

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# Complexation Kinetics of Cyclodextrins with Bile Salt Anions: Energy Barriers for Threading of Ionic Groups

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# Abstract

Binding constants for thousands of cyclodextrin complexes have been reported in the literature but much less is known about the kinetics of these host-guest complexes. In the present study, inclusion complexes of bile salts with β-cyclodextrin, γ-cyclodextrin and a methylated β-cyclodextrin were studied by NMR lineshape analysis to explore the structural factors that govern the complexation kinetics. For complexes with β-cyclodextrin, the association rate constants ranged from $2 \times 10^6$ to $2 \times 10^7$ $M^{-1}s^{-1}$ while the dissociation rate constants ranged from 12 $s^{-1}$ to 6000 $s^{-1}$ at 25 °C. The kinetics were thus significantly slower than for any other β-cyclodextrin complex reported in the literature, due to the large energy barrier for threading the ionic sidechains of the bile salt anions. Bile salts with taurine and glycine sidechains had identical binding affinities but the kinetics differed by a factor of 10. Introduction of a single hydroxyl group at the binding site of the bile salts reduced the lifetimes and binding constants of the complexes more than 50 times. The strong temperature dependence of the rate constants revealed that the large activation energies were mainly enthalpic with a small contribution from entropy. The larger γ-cyclodextrin was threaded by the non-ionic end of the bile salts and the kinetics were too fast to be accurately determined. The study demonstrates that ionic groups on guest molecules constitute significant energy barriers for threading and dethreading of β-cyclodextrin hosts.

# Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides with a shape sometimes described as a hollow truncated cone. Due to their hydrophobic interior and hydrophilic exterior they readily form water soluble inclusion complexes with a large variety of guest molecules.[1] For this reason they have found applications within pharmacy, food and environmental technologies, advanced materials, etc.[2] They are also important building blocks for supramolecular structures and molecular machines, for example as wheels on molecular axles.[3] CD inclusion complexes also serve as simple model compounds for protein-ligand binding and molecular recognition, and they are used to develop and validate molecular modelling force fields.[4]

Most of the applications rely on the binding affinity of the guest moiety for the CD host, and binding constants for thousands of CD complexes with predominantly small guest molecules have been reported. However, the kinetics of the complexation and dissociation processes have only been determined for relatively few complexes, probably less than a hundred.[5–22] Of these, most are with the small αCD, fewer with the wider βCD and its derivatives, and rate constants have only been reported for a few complexes with γCD,[23–26] which has the largest diameter of the natural CDs. With timescales ranging from nanoseconds to seconds for the vast majority of complexes, the dynamics are sufficiently fast to be of negligible importance for many practical applications but may be crucial for understanding the properties of some supramolecular systems.[27] Also, for molecular machines that rely on the shuttling of a CD along an axle, or the threading/dethreading of an axle, it is of great importance to identify the energetic barriers that control the motion of the CD. Kinetic data may also add a new dimension to the understanding of the molecular recognition properties of CDs and advance the understanding of the relationships between structure and binding

affinity.[27] As shown in equation 1, the binding constant, K, is equal to the ratio of the complexation and dissociation rate constants.

$$G + CD \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CD{:}G \qquad\qquad K = \frac{k_1}{k_{-1}} \qquad\qquad \text{(Eq. 1)}$$

A strong binding can thus be due to fast complexation kinetics or slow dissociation kinetics. Conversely, a low binding constant can be due to a short-lived complex or a poor ability of the host and guest to structurally adapt to each other and form a complex. Thus, knowledge of the kinetics can assist the structural and mechanistic interpretation of the equilibrium complexation thermodynamics.

The present study reports the complexation and dissociation kinetics for complexes of several bile salts (BS) (Figure 1) with two βCDs and a γCD. In addition to being of importance for the intestinal absorption of CD-bound drugs,[28] BSs are suitable model compounds as many structural analogues are available, thereby making it feasible to probe the effects of systematic structural variations. Further, their complexes with various CDs have previously been thoroughly characterized in terms of structure and binding thermodynamics.[29–33] The present systematic study of structurally similar systems at a range of temperatures aims to contribute to the general understanding of the structural factors that control the complexation kinetics.
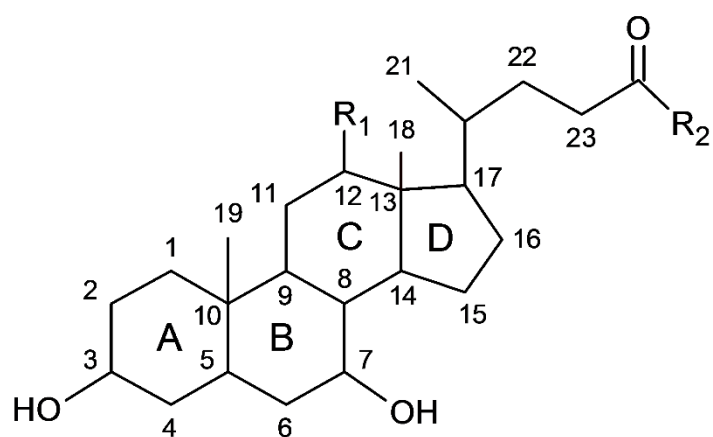
| Bile salt | $R_1$ (C12) | $R_2$ (C24) |
|-----------|-------------|-------------|
| GC | OH | $NHCH_2COO^-$ |
| TC | OH | $NHCH_2CH_2SO_3^-$ |
| GCDC | H | $NHCH_2COO^-$ |
| TCDC | H | $NHCH_2CH_2SO_3^-$ |
| C | OH | $O^-$ |

*Figure 1 Structures of the 5 bile salt anions employed in present study. Full names of the BSs are glycocholate (GC), taurocholate (TC), glycochenodeoxycholate (GCDC), taurochenodeoxycholate (TCDC), and cholate (C).*

# Experimental Section

## Chemicals

Sodium salts of the bile salt anions glycocholate (GC), taurocholate (TC), cholate (C), glycochenodeoxycholate (GCDC), and taurochenodeoxycholate (TCDC) were purchased from Sigma-Aldrich at a purity of at least 97%. βCD and γCD were from Sigma-Aldrich. Heptakis(2,6-di-O-methyl)-β-cyclodextrin (DIMEB) with an isomeric purity of 95% was purchased from Cyclolab (Budapest, Hungary). All chemicals were dried overnight in vacuum at 55 °C prior to weighing the amounts for the solutions.

## Isothermal Titration Calorimetry

Titrations were made on a VP-ITC (Malvern Panalytical, Malvern, UK) at several temperatures ranging from 10-70 °C. Solutions of CD in Milli-Q water were titrated into solutions of BS in Milli-Q water in aliquots of 10μL, except for the first injection which was only 2μL and neglected in the data analysis. Peaks were integrated in the Origin Microcal software. Cell concentrations and integrated heat signals were exported to Matlab where all titrations for a given host-guest combination were globally fitted with a 'One Set of Sites' binding model which requires ΔH to be linearly dependent on the temperature and K to follow the van't Hoff equation.[34]

## NMR

Titration series were prepared directly in the NMR tubes by mixing stock solutions of CD and BS in $D_2O$. $^1$H-NMR spectra were recorded at several temperatures in the interval 10-55 °C on a 400 MHz Bruker spectrometer. After insertion of the sample into the spectrometer, but prior to the locking

and shimming procedures, the sample was left to thermally equilibrate for 300 seconds. Each spectrum was acquired with 128 scans and a spectral resolution of 0.0488 Hz per point in the range -1 to 8 ppm. Spectra were phase and baseline corrected prior to analysis. The intensities of all spectra in a titration series were adjusted for differences in receiver gain.

Rate constants were obtained by fitting the Bloch-McConnell equations[35] for a two-site exchange system to the BS 18-CH$_3$ NMR singlet. The solution to the Bloch-McConnell equations in the form presented as equation 14 in the article by Římal *et al.*[36] was used to the fit the experimental NMR peaks using in-house Matlab scripts, inspired by the Asymexfit[36] and the LineShapeKin[37] Matlab packages. More details of the fitting procedure are provided in the Supporting Information.

# Results and Discussion

Complexation and dissociation rate constants were determined by NMR lineshape analysis. A proton in exchange between two different environments show up as two distinct peaks in the NMR spectrum if the exchange kinetics are sufficiently slow. If the exchange kinetics are fast, the proton only senses a single averaged environment and show up as a single peak. Between these two kinetic extremes, the exchange process results in a broadening of the NMR peak(s), and the exchange kinetics can be derived from the exact shape of the peak(s). This has previously been exploited to obtain complexation and dissociation kinetics for guest molecules that are in exchange between a nonpolar CD cavity and a polar aqueous solvent.[5]

The current study explores the kinetics for complexes of 5 bile salts with βCD at a range of temperatures, and discusses the relationship between the structure of the guest molecule and the complexation kinetics. Further, to elucidate the influence of the host structure, a few complexes with a partially methylated βCD, 2,6-dimethyl-βCD (DMβCD), and natural γCD were also studied.

**NMR lineshape analysis**

In all of the studied host-guest systems the complexation kinetics were determined from the shape of the 18-$CH_3$ peak. The 18-$CH_3$ group in the BSs is particularly suited for this purpose as its chemical shift is relatively sensitive to complexation and is well separated from the other peaks in the spectrum.[5] A titration series of NMR samples were prepared for each host-guest system. Within each series the concentration of BS was constant (around 1 mM) while the concentration of CD in most of the series ranged from 0 to 8 mM. Thus, the fraction of bound BS ranged from 0 to almost 100%. For the systems with the slowest complexation kinetics two separate peaks of 18-$CH_3$ were observed; one for the free BS and one for the bound BS. As the concentration of CD increased, the

bound peak grew at the expense of the free peak. Other systems were in fast exchange and produced only a single 18-CH$_3$ peak at a chemical shift that is a population-weighted average of the chemical shifts of the free and bound states. Some complexes went from the slow-exchange regime to the fast-exchange regime with increasing temperature, as shown in Figure 2.
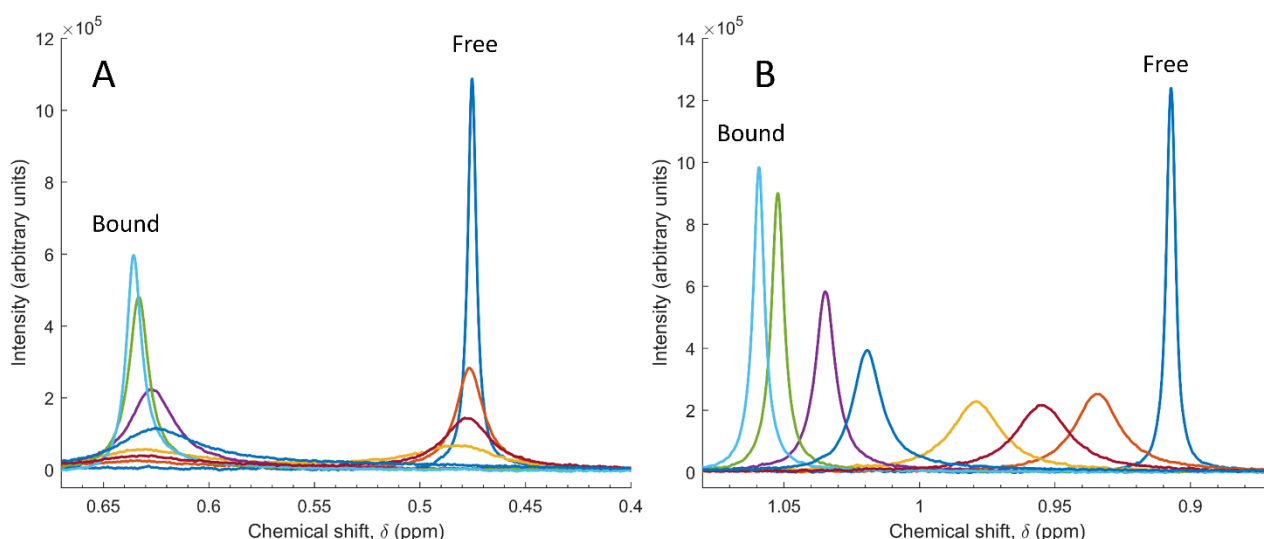


*Figure 2 The 18-CH$_3$ group in TCDC underwent slow exchange at 15 °C (A) and fast exchange at 55 °C (B) in the presence of βCD. In each of the 8 samples the concentration of TCDC was 1.04 mM while the concentration of βCD ranged from 0 to 4.03 mM.*

For each complex, the Bloch-McConnell equations[35] were fitted to the 18-CH$_3$ peak(s) in each spectrum of the titration series to yield the complexation and dissociation rate constants, $k_1$ and $k_{-1}$. In addition to these two rate constants the equations include the transverse relaxation rates for the free and bound states, $R_A$ and $R_B$, and the difference in resonance frequency between the two states, $\Delta\omega$. Further, the binding constant, K, must be known in order to calculate the populations of the free and bound BS from the known total concentrations of BS and CD. Due to the relation expressed in equation 1 knowledge of K eliminates one of the reaction rate constants. A detailed description of the fitting process is provided in the Supporting Information. In short, K for each complex were obtained from ITC experiments, $\Delta\omega$ was estimated from the spectra of free and bound BS, $R_A$ was

determined from the lineshape of the free 18-CH$_3$, and only k$_1$ and R$_B$ were varied to obtain the best

fit of the simulated peaks to the experimental peaks. Excellent fits to experimental data were

obtained in most cases, as illustrated in Figure 3 and in the Supporting Information.
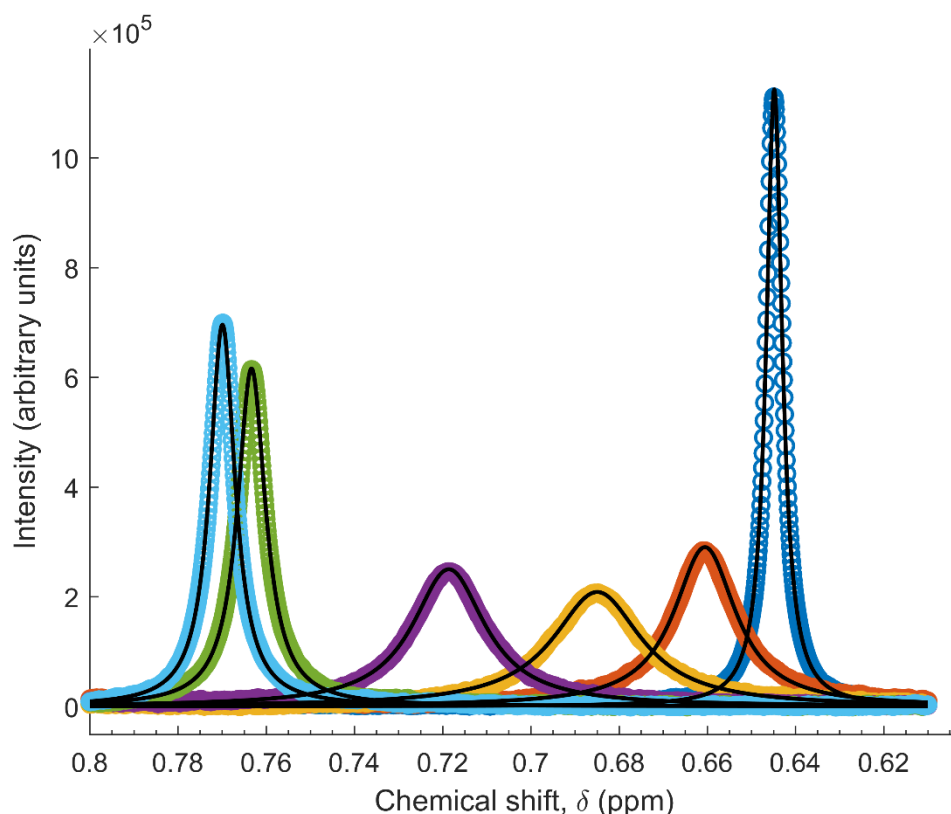


*Figure 3 The 18-CH$_3$ singlet of GC exhibited intermediate exchange at 25 °C in the presence of βCD. In all spectra, the concentration of GC was 1.04 mM , and the concentration of βCD incrementally increased from 0 mM (rightmost peak) to 8.06 mM (leftmost peak). Experimental data are shown with circles and the fit is shown with solid lines.*

**Kinetics of βCD complexes**

For the βCD complexes, the complexation reaction took place at a time scale that allowed for

precise determination of the kinetic parameters, except for the complex with TC which was so fast

at the highest temperatures that practically no broadening of the peaks was observed. All rate

constants are presented in Table 1 along with binding constants determined by ITC experiments and

reported in the literature. Only for a single complex, βCD:C, was it necessary to conduct new ITC experiments. The resulting enthalpograms are shown in Figure S1, and the analysis yielded a binding constant and binding enthalpy of 3974 $M^{-1}$ and -27.0 kJ/mol, both at 25 °C, and a differential heat capacity of 330 J/mol/K. The binding constant is very similar to a previously reported ITC value and confirms that the bile salt C binds to βCD with a slightly higher affinity than GC and TC.[38]

*Table 1 Rate constants for the complexation and dissociation reactions along with the binding constants that were used in the fitting procedure.*

| | Temperature (°C) | $k_1{}^a$ ($10^6$ $M^{-1}s^{-1}$) | $k_{-1}{}^a$ ($s^{-1}$) | K ($10^3$ $M^{-1}$) | Source of $K^b$ |
|---|---|---|---|---|---|
| βCD:GC | 15 | 1.2 | $2.9 \times 10^2$ | 4.14 | |
| | 25 | 2.0 | $6.9 \times 10^2$ | 2.96 | Ref. 34 |
| | 35 | 3.0 | $1.4 \times 10^3$ | 2.08 | |
| | 45 | 4.6 | $3.2 \times 10^3$ | 1.44 | |
| | 55 | 6.0 | $6.1 \times 10^3$ | 0.98 | |
| βCD:TC | 15 | 13 | $3.2 \times 10^3$ | 4.14 | |
| | 25 | 17 | $5.8 \times 10^3$ | 2.96 | Ref. 34$^c$ |
| | 35 | 23 | $1.1 \times 10^4$ | 2.08 | |
| βCD:C | 15 | 4.0 | $6.8 \times 10^2$ | 5.84 | |
| | 25 | 5.3 | $1.3 \times 10^3$ | 4.12 | This work |
| | 35 | 8.1 | $2.8 \times 10^3$ | 2.85 | |
| | 45 | 11 | $5.6 \times 10^3$ | 1.94 | |
| | 55 | 14 | $1.1 \times 10^4$ | 1.30 | |
| βCD:GCDC | 15 | 1.1 | 4.8 | 232 | |
| | 25 | 1.9 | 12 | 156 | Ref. 31 |
| | 35 | 3.3 | 32 | 102 | |
| | 45 | 3.5 | 55 | 64.6 | |
| | 55 | 4.4 | $1.1 \times 10^2$ | 39.8 | |
| βCD:TCDC | 15 | 13 | 56 | 232 | |
| | 25 | 17 | $1.1 \times 10^2$ | 156 | Ref. 31$^c$ |
| | 35 | 21 | $2.1 \times 10^2$ | 102 | |
| | 45 | 28 | $4.3 \times 10^2$ | 64.6 | |
| | 55 | 35 | $8.7 \times 10^2$ | 39.8 | |

*a*) Errors on the rate constants are estimated to be around 5% for complexes with C, GC and TC and up to 20% for complexes with GCDC and TCDC.

*b*) Binding constants at the exact temperatures were calculated from the binding constant and binding enthalpy at 25 °C and the differential heat capacity as explained in the Supporting Information.

*c*) The binding thermodynamics of tauroconjugated and glycoconjugated bile salts seem identical.[39,40] Therefore, the binding constants of TC and TCDC are assumed identical to their glycoconjugated counterparts at all temperatures.

Rate constants for the $\beta$CD:GCDC complex have previously been reported in the literature.[5] While the presently reported $k_{-1}$ does not differ remarkably from the previous value, $k_1$ differs by almost two orders of magnitude, most likely due to the previously reported binding constant being 40 times too small. The presently used binding constant was determined by ITC which is better suited for determination of large binding constants than the previously used NMR titration. Thus, the value of $k_1$ reported in the present work is undoubtedly more accurate.

The structures of complexes between $\beta$CDs and BSs have been thoroughly described in the literature. The D ring of the steroid body and part of the conjugation tail of the BSs are included in the $\beta$CD cavity, while the charged end of the conjugation tail protrudes from the narrow primary rim of the CD.[29,30,41] While the structures of the complexes are known, it is less certain how the bile salts *enter* the CD cavity. The kinetic results strongly indicate that the BSs thread the CD via the conjugation tail, as the identity of the conjugation tail seems to be the decisive structural parameter that determines the value of the association rate constant, $k_1$. The two glycoconjugated BSs, GC and GCDC, have more or less identical $k_1$'s at all temperatures. Similarly, the $k_1$'s of the two tauroconjugated BSs, TC and TCDC, are almost identical, albeit almost 10 times larger than their glycoconjugated analogues. The complexation rate constant for C is somewhere in between those of the tauro- and glycoconjugated bile salts.

Whereas the rate constant for the formation of the complexes is determined by the identity of the BS conjugation tail, the dissociation rate constants also depend on the steroid moiety of the guest. Comparing GC to GCDC and TC to TCDC, it is clear that the presence of a hydroxyl group on C12 in GC and TC increases $k_{-1}$ by a factor of around 50. It has long been established that the presence of the 12-OH leads to a significant decrease in binding constants, presumably because it prevents $\beta$CD from encroaching onto the C-ring of the BSs.[30,42] The kinetic parameters reveal that the 12-OH reduces the lifetime of the complex, i.e. it reduces the binding constant by destabilizing the

complexed state rather than preventing the formation of the complex. This is not surprising but it confirms the overall picture of a complexation/decomplexation process in which the conjugation tail penetrates the CD, and the CD resides on the C and D rings of the BS for an extended time if there is no 12-OH to disturb the interaction.

The energetics of the complexation process is illustrated in Figure 4. The primary energetic barrier for the formation and dissociation of the complexes is related to moving the charged conjugation tail through the hydrophobic cavity of the CD. This situation inevitably involves the dehydration of the hydrophilic charged group and may be regarded as the transition state. Glycoconjugation increases the transition state energy relative to tauroconjugation. The 12-OH on the steroid body of the BSs does not affect the energy of the transition state but decreases the binding constant by increasing the energy of the complexed state.
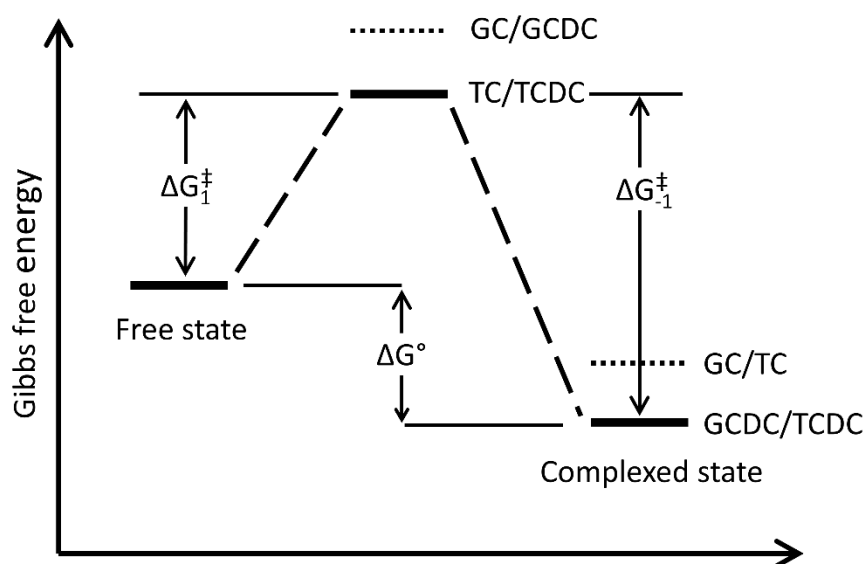


*Figure 4 Glycoconjugation (GC/GCDC) of the BSs increases the energy of the transition state relative to tauroconjugation (TC/TCDC), leading to slower kinetics. The presence of 12-OH (GC/TC) on the steroid body of the BSs increases the energy of the complexed state, leading to smaller binding constants.*

**Activation energy, enthalpy and entropy**

Activation energies, enthalpies and entropies ($\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$) were determined by linear regression to the Eyring plots in Figure 5 and are listed in Table 2. For all complexes, the negative slopes clearly indicate positive activation enthalpies for complexation and dissociation. Unfortunately, the errors on $\Delta H^{\ddagger}$ are $\Delta S^{\ddagger}$ are relatively large, especially when the data points are few, and consequently it is not possible to identify any differences in the enthalpic and entropic contributions among the complexes. Nevertheless, some overall conclusions can still be made. The activation energies are dominated by large enthalpies with only a minor entropic contribution. This is the case for complexation as well as for dissociation, but the entropic barrier seems slightly higher for the complexation reaction. This could be related to the flexible sidechain on the BSs which must adopt a proper conformation to penetrate the CD. In the complex, the motion of the sidechain is already somewhat restricted and therefore less entropy is lost when it moves through the CD during dissociation.
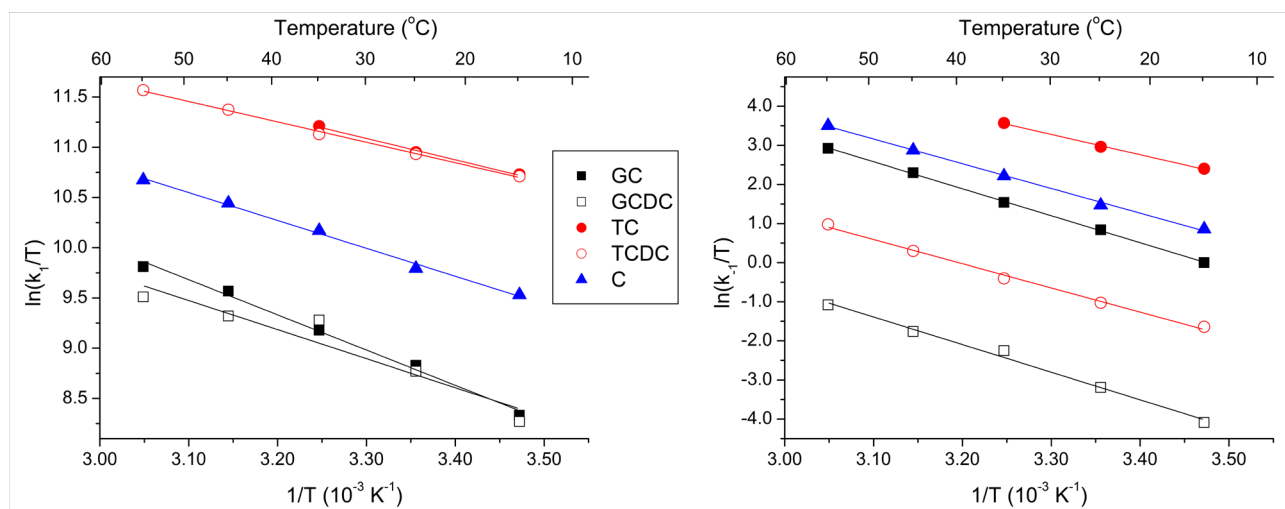


*Figure 5 Eyring plots for the complexation (left) and dissociation (right) reactions for complexes with natural βCD.*

Table 2 Activation enthalpies, entropies and energies for the complexation ond dissociation of BS complexes with natural βCD. The listed errors are at the 95% confidence level and determined from the linear regression to the Eyring plots.

| | Complexation | | | Dissociation | | |
|---|---|---|---|---|---|---|
| | $\Delta H^{\ddagger}$ (kJ/mol) | $T\Delta S^{\ddagger}$ at 298 K (kJ/mol) | $\Delta G^{\ddagger}$ at 298 K (kJ/mol) | $\Delta H^{\ddagger}$ (kJ/mol) | $T\Delta S^{\ddagger}$ at 298 K (kJ/mol) | $\Delta G^{\ddagger}$ at 298 K (kJ/mol) |
| GC | 29 ± 4 | -8 ± 4 | 37.1 ± 0.2 | 57 ± 2 | 1 ± 2 | 56.8 ± 0.1 |
| GCDC | 24 ± 13 | -13 ± 13 | 37.2 ± 0.7 | 59 ± 9 | -8 ± 9 | 66.8 ± 0.5 |
| TC | 18 ± 15 | -14 ± 15 | 31.7 ± 0.4 | 43 ± 24 | -8 ± 24 | 51.5 ± 0.7 |
| TCDC | 17 ± 1 | -15 ± 1 | 31.8 ± 0.1 | 51 ± 6 | -10 ± 6 | 61.3 ± 0.3 |
| C | 23 ± 3 | -11 ± 3 | 34.5 ± 0.2 | 53 ± 4 | -3 ± 4 | 55.1 ± 0.2 |

Due to the relatively large errors, the thermodynamic parameters do not bring us closer to understanding why taurine sidechains penetrate the CD cavity more easily than glycine sidechains. The taurine sidechain is one methylene group longer and ends in a negatively charged sulfonate group whereas the glycine sidechain ends in a negatively charged carboxylate group. The difference does not seem to be steric in nature as the sulfonate group is larger than carboxylate. It is more likely due to the weaker hydration of the sulfonate group in which the negative charge is more spread out than in the carboxylate group. However, the faster kinetics of C relative to GC, both anions containing a carboxylate group, reveals that more factors are at play.

**Kinetics of complexes with γCD and DMβCD**

To explore the effects of host structure, a few complexes with other CDs were studied. The larger γCD was employed to study the effect of ring size, and 2,6-dimethyl-βCD (DMβCD) was employed to study the effect of replacing two thirds of the hydroxyl groups at the rims of the CD with less polar methoxy groups.

The exchange dynamics of the studied γCD complex, γCD:GCDC, were too fast to determine the kinetic parameters. The 18-CH$_3$ peak gradually broadened as the fraction of bound GCDC increased (Figure S17), but this was merely due to the reduced tumbling rate of the complex. No exchange

broadening was observed. Simulating the peaks with various values of $k_{-1}$ revealed that $k_{-1}$ must be larger than $2 \times 10^8$ $M^{-1}s^{-1}$ to properly reproduce the experimental peaks. The much faster kinetics of $\gamma$CD relative to $\beta$CD is most likely due to the ability of $\gamma$CD to enter the steroid body of the BS via the A-ring. NMR and molecular modelling has shown that $\gamma$CD is not restricted to the C and D-rings of the guest but is large enough to encapsulate most of the steroid structure by shuttling back and forth on the BS.[33,43] It seems reasonable that the uncharged and relatively nonpolar A-ring has a much lower energy barrier for penetrating the nonpolar CD cavity than the ionic hydrophilic sidechain.

Rate constants for complexes with DM$\beta$CD are shown in Table 3. Chemical modification of the CD rims only had a slight effect on the complexation kinetics with GCDC as the parameters are very similar to the complex with natural $\beta$CD (Table 1). However, methylation of the CD had a significant impact on the complexation with GC where rate constants increased 3-4 times relative to the natural $\beta$CD. The binding constant was only slightly affected which means that methylation apparently lowers the energy of the transition state (see Figure 4). This raises the question: Why does host methylation lower the energy barrier for the GC guest but not for the GCDC guest? For both guests it is the same glycine sidechain that must penetrate the CD, so it makes no sense that methylation lowers the transition state energy for GC but not for GCDC. This puzzling observation must be left unanswered. The kinetic effects of host modification is a largely unexplored topic. Bohne *et al.* investigated the dynamics of triplet xanthone in complex with a few modified $\beta$CDs, including a partially methylated $\beta$CD, and the reported alterations of the rate constants were somewhat smaller than those presently observed for GC.[14] More kinetic studies of complexes with modified CDs are required to understand the effects of host modification. Such studies may also assist the molecular interpretation of why modifications of the CD sometimes increase binding constants and sometimes reduce binding constants.

*Table 3 Rate constants for complexation and dissociation reactions for complexes with γCD and DMβCD. Binding constants for the complexes with DMβCD were obtained by ITC. Global fits to ITC enthalpograms at several temperatures are shown in the Supporting Information along with the best fit parameters.*

| | Temperature (°C) | $k_1{}^a$ ($10^6$ M$^{-1}$s$^{-1}$) | $k_{-1}{}^a$ (s$^{-1}$) | K ($10^3$ M$^{-1}$) | Source of K$^b$ |
|---|---|---|---|---|---|
| DMβCD:GC | 15 | 4.3 | $1.3 \times 10^3$ | 3.18 | This work |
| | 25 | 5.3 | $2.0 \times 10^3$ | 2.68 | |
| DMβCD:GCDC | 25 | 2.3 | 15 | 150 | This work |
| | 35 | 2.8 | 27 | 103 | |
| | 55 | 4.5 | $1.0 \times 10^2$ | 42.9 | |
| γCD:GCDC | 25 | > 200 | > 2000 | 96 | Ref. 33 |

*a)* Errors on the rate constants are estimated to be around 5% for the complex with GC and up to 20% for the complex with GCDC.

*b)* Binding constants at the exact temperatures were calculated from the binding constant and binding enthalpy at 25 °C and the differential heat capacity as explained in the Supporting Information.

## General discussion of cyclodextrin kinetics

The association rate constants for the studied BS complexes with βCDs are significantly smaller than those previously reported for βCD complexes, which are often close to the diffusion controlled limit.[7,11–13,22,24,44] The pure diffusion-controlled rate constants for a bimolecular reaction between molecules of the relevant sizes is around $10^{10}$ M$^{-1}$s$^{-1}$ at 25 °C,[12,22] but due to geometric constraints it has been argued that the diffusion controlled limit for CD complexes is around $10^9$ M$^{-1}$s$^{-1}$.[44] The association rate constants for the studied BS complexes range from $2\times10^6$ to $2\times10^7$ at 25 °C and are thus at least one order of magnitude smaller than the reported association rate constants for other βCD complexes. (Even smaller rate constants were obtained for βCD complexes by a capillary electrophoresis method but these seem unrealistic)[45]. The small rate constants for the BS guests are most likely due to the large energy required to move the ionic groups on the sidechains through the CD cavity. The previously studied complexes with βCD can form without moving an ionic group through the CD and therefore have much lower activation energies.

It has often been noted that for a series of guests binding to βCD the association rate constants differed very little while the dissociation rate constants varied significantly more, meaning that the binding affinity was dictated by the dissociation rate constants.[12,22,44] This can be interpreted by referring to Figure 4. It seems that for many of these complexes structural modifications of the guest hardly altered the energy barrier for forming the complex. The energy required for those guests to penetrate the βCD is likely very small as no steric barriers or dehydration of ionic groups hinder complexation. The host and guest only need to meet and reorient to form a complex. Variations in guest structure mainly affected the energetic stabilization of the complexed state and thereby the lifetimes of the complexes. For the BSs, on the contrary, replacing a taurine sidechain with a glycine sidechain reduced the complexation rate constant by one order of magnitude but did not affect the binding affinity as the modified part was sufficiently separated from the binding site. While this feature seems unique among all studied βCD complexes it is widely observed for αCD complexes. Cramer *et al.* studied a series of azo dyes binding to αCD and found only slight variations in the binding constant while the rate constants spanned seven orders of magnitude.[46] The guest modifications only affected the energy barrier but hardly impacted on the energy difference between the free and bound state. Similar observations were made for other azo dyes binding to αCD.[47] In a study of the complexation dynamics of αCD with alkanes having bulky and ionic end groups, Lyon et al. provided a beautiful example of the structural factors that control the complexation rate and the binding affinity. Increasing the size of the end groups decreased the rate constants without affecting the binding affinity. Increasing the length of the alkyl chain increased the binding affinity without affecting the rate of complexation.[48] In the literature in general, the reported dynamics of αCD complexes are much slower than for βCD complexes; complexes between certain bolaform surfactants and αCDs even have lifetimes lasting thousands of seconds.[17,21] The generally slower kinetics of αCD complexes is undoubtedly related to the smaller

cavity of αCD and the larger abundance of structural motifs that act as barriers to the threading of αCD. The present work has identified functional groups which act as barriers to the threading of βCDs, not because of steric factors but due to hydrophilicity.

# Conclusions

The kinetics of the studied β-cyclodextrin complexes with bile salts were remarkably slow compared to all other complexes with β-cyclodextrins reported in the literature. The slow kinetics were due to the large energy required to move the hydrophilic ionic conjugation tail of the bile salt anions through the hydrophobic cavity of the cyclodextrin. The energy barrier was highest for glycine conjugated bile salts for which the kinetics were ten times slower than for their taurine conjugated analogues. The type of conjugation only affected the energy barrier and not the energy of the bound state, thus the two types of bile salts had the same binding affinity despite the large difference in kinetics. On the contrary, the molecular structure of the binding site affected the energy of the bound state without affecting the complexation rate. A hydroxyl group on C12 of the steroid body of the bile salt severely destabilized the complex and reduced the lifetime of the complex and the binding affinity by a factor of ~50.

The energy barrier for the complexation and dissociation processes was predominantly enthalpic with a slight contribution from entropy. Partial methylation of the hydroxyl groups at the rims of the β-cyclodextrin host reduced the energy barrier for binding to one bile salt guest while the kinetics were unaltered for another bile salt guest. This puzzling observation could not be explained. The larger γCD could be threaded by the other less hydrophilic end of the bile salts, resulting in a lower energy barrier and much faster kinetics.

**Supporting Information.** Procedure for fitting NMR peaks. Fitted ITC enthalpograms. Fitted NMR spectra.

# References

(1) Rekharsky, M. V.; Inoue, Y. Complexation Thermodynamics of Cyclodextrins. *Chem. Rev.* **1998**, *98*, 1875–1918.

(2) Crini, G. Review: A History of Cyclodextrins. *Chem. Rev.* **2014**, *114*, 10940–10975. See p 10964 for an overview of reviews.

(3) Hashidzume, A.; Yamaguchi, H.; Harada, A. Cyclodextrin-Based Molecular Machines. In *Molecular Machines and Motors: Recent Advances and Perspectives*; Credi, A., Silvi, S., Venturi, M., Eds.; Springer International Publishing: Cham, 2014; Vol. 310, pp 71–110.

(4) Henriksen, N. M.; Gilson, M. K. Evaluating Force Field Performance in Thermodynamic Calculations of Cyclodextrin Host-Guest Binding: Water Models, Partial Charges, and Host Force Field Parameters. *J. Chem. Theory Comput.* **2017**, *13*, 4253–4269.

(5) Yim, C. T.; Zhu, X. X.; Brown, G. R. Kinetics of Inclusion Reactions of Beta-Cyclodextrin with Several Dihydroxycholate Ions Studied by NMR Spectroscopy. *J. Phys. Chem. B* **1999**, *103*, 597–602 and references therein.

(6) Abou-Hamdan, A.; Bugnon, P.; Saudan, C.; Lye, P. G.; Merbach, A. E. High-Pressure Studies as a Novel Approach in Determining Inclusion Mechanisms: Thermodynamics and Kinetics of the Host-Guest Interactions for α-Cyclodextrin Complexes. *J. Am. Chem. Soc.* **2000**, *122*, 592–602.

(7) Fukahori, T.; Kondo, M.; Nishikawa, S. Dynamic Study of Interaction between β-Cyclodextrin and Aspirin by the Ultrasonic Relaxation Method. *J. Phys. Chem. B* **2006**, *110*, 4487–4491.

(8) Kano, K.; Nishiyabu, R.; Asada, T.; Kuroda, Y. Static and Dynamic Behavior of 2:1 Inclusion Complexes of Cyclodextrins and Charged Porphyrins in Aqueous Organic Media. *J. Am. Chem. Soc.* **2002**, *124*, 9937–9944.

(9) Nishikawa, S.; Fukahori, T.; Ishikawa, K. Ultrasonic Relaxations in Aqueous Solutions of Propionic Acid in the Presence and Absence of β-Cyclodextrin. *J. Phys. Chem. A* **2002**, *106*, 3029–3033.

(10) Park, S.; Bae, J. Ultrasonic Relaxation Associated with the Complex Formation of Benzoic Acid Derivative and β-Cyclodextrin. *J. Acoust. Soc. Korea* **2017**, *36*, 387–393.

(11) Zhang, X.; Gramlich, G.; Wang, X.; Nau, W. M. A Joint Structural, Kinetic, and Thermodynamic Investigation of Substituent Effects on Host-Guest Complexation of Bicyclic Azoalkanes by β-Cyclodextrin. *J. Am. Chem. Soc.* **2002**, *124*, 254–263.

(12) Al-Soufi, W.; Reija, B.; Novo, M.; Felekyan, S.; Kühnemuth, R.; Seidel, C. A. M. Fluorescence Correlation Spectroscopy, a Tool to Investigate Supramolecular Dynamics: Inclusion Complexes of Pyronines with Cyclodextrin. *J. Am. Chem. Soc.* **2005**, *127*, 8775–8784.

(13) Christoff, M.; Okano, L. T.; Bohne, C. Dynamics of Complexation of Flavone and Chromone to β-Cyclodextrin. *J. Photochem. Photobiol., A* **2000**, *134*, 169–176.

(14) Okano, L. T.; Barros, T. C.; Chou, D. T. H.; Bennet, A. J.; Bohne, C. Complexation

Dynamics of Xanthone and Thioxanthone to β-Cyclodextrin Derivatives. *J. Phys. Chem. B* **2001**, *105*, 2122–2128.

(15)   Baer, A. J.; Macartney, D. H. Orientational Isomers of α-Cyclodextrin [2]Semi-Rotaxanes with Asymmetric Dicationic Threads. *Org. Biomol. Chem.* **2005**, *3*, 1448–1452.

(16)   Park, J. W.; Song, H. J.; Cho, Y. J.; Park, K. K. Thermodynamics and Kinetics of Formation of Orientationally Isomeric [2]Pseudorotaxanes between α-Cyclodextrin and Aliphatic Chain-Linked Aromatic Donor-Viologen Acceptor Compounds. *J. Phys. Chem. C* **2007**, *111*, 18605–18614.

(17)   Quiroga, M.; Parajó, M.; Rodríguez-Dafonte, P.; García-Río, L. Kinetic Study of [2]Pseudorotaxane Formation with an Asymmetrical Thread. *Langmuir* **2016**, *32*, 6367–6375.

(18)   García-Martínez, F.; Quiroga, M.; Rodríguez-Dafonte, P.; Parajó, M.; Garcia-Rio, L. Displacement Assay Methodology for Pseudorotaxane Formation in the Millisecond Time-Scale. *Pure Appl. Chem.* **2017**, *89*, 821–827.

(19)   Wenz, G.; Gruber, C.; Keller, B.; Schilli, C.; Albuzat, T.; Müller, A. Kinetics of Threading α-Cyclodextrin onto Cationic and Zwitterionic Poly(Bola-Amphiphiles). *Macromolecules* **2006**, *39*, 8021–8026.

(20)   Xue, J.; Chen, L.; Zhou, L.; Jia, Z.; Wang, Y.; Zhu, X.; Yan, D. Effect of End Groups on Complexation Kinetics between Cyclodextrins and Guest Polymers. *J. Polym. Sci. Part B Polym. Phys.* **2006**, *44*, 2050–2057.

(21)   Nilsson, M.; Valente, A. J. M.; Olofsson, G.; Söderman, O.; Bonini, M. Thermodynamic and Kinetic Characterization of Host−Guest Association between Bolaform Surfactants and α- and β-Cyclodextrins. *J. Phys. Chem. B* **2008**, *112*, 11310–11316.

(22)   Nishikawa, S.; Kondo, M. Kinetic Study for the Inclusion Complex of Carboxylic Acids with Cyclodextrin by the Ultrasonic Relaxation Method. *J. Phys. Chem. B* **2006**, *110*, 26143–26147.

(23)   Dyck, A. S. M.; Kisiel, U.; Bohne, C. Dynamics for the Assembly of Pyrene-γ-Cyclodextrin Host-Guest Complexes. *J. Phys. Chem. B* **2003**, *107*, 11652–11659.

(24)   Franchi, P.; Casati, C.; Mezzina, E.; Lucarini, M. Kinetic Control of the Direction of Inclusion of Nitroxide Radicals into Cyclodextrins. *Org. Biomol. Chem.* **2011**, *9*, 6396–6401.

(25)   Turro, N. J.; Okubo, T.; Chung, C. J. Analysis of Static and Dynamic Host-Guest Associations of Detergents with Cyclodextrins via Photoluminescence Methods. *J. Am. Chem. Soc.* **1982**, *104*, 1789–1794.

(26)   Al-Soufi, W.; Reija, B.; Felekyan, S.; Seidel, C. A. M.; Novo, M. Dynamics of Supramolecular Association Monitored by Fluorescence Correlation Spectroscopy. *ChemPhysChem* **2008**, *9*, 1819–1827.

(27)   Bohne, C. Supramolecular Dynamics. *Chem. Soc. Rev.* **2014**, *43*, 4037–4050.

(28)   Holm, R.; Müllertz, A.; Mu, H. Bile Salts and Their Importance for Drug Absorption. *Int. J. Pharm.* **2013**, *453*, 44–55.

(29)  Cabrer, P. R.; Álvarez-Parrilla, E.; Al-Soufi, W.; Meijide, F.; Núñez, E. R.; Tato, J. V. Complexation of Bile Salts by Natural Cyclodextrins. *Supramol. Chem.* **2003**, *15*, 33–43.

(30)  Holm, R.; Shi, W.; Hartvig, R. A.; Askjær, S.; Christian Madsen, J.; Westh, P. Thermodynamics and Structure of Inclusion Compounds of Tauro- and Glyco-Conjugated Bile Salts and Beta-Cyclodextrin. *Phys. Chem. Chem. Phys.* **2009**, *11*, 5070–5078.

(31)  Schönbeck, C.; Holm, R.; Westh, P.; Peters, G. H. Extending the Hydrophobic Cavity of β-Cyclodextrin Results in More Negative Heat Capacity Changes but Reduced Binding Affinities. *J. Incl. Phenom. Macrocycl. Chem.* **2014**, *78*, 351–361.

(32)  Schönbeck, C.; Westh, P.; Holm, R. Complexation Thermodynamics of Modified Cyclodextrins: Extended Cavities and Distorted Structures. *J. Phys. Chem. B* **2014**, *118*, 10120–10129.

(33)  Holm, R.; Schönbeck, C.; Askjær, S.; Westh, P. Thermodynamics of the Interaction of γ-Cyclodextrin and Tauro- and Glyco-Conjugated Bile Salts. *J. Incl. Phenom. Macrocycl. Chem.* **2013**, *75*, 223–233.

(34)  Schönbeck, C.; Holm, R.; Westh, P. Higher Order Inclusion Complexes and Secondary Interactions Studied by Global Analysis of Calorimetric Titrations. *Anal. Chem.* **2012**, *84*, 2305–2312.

(35)  McConnell, H. M. Reaction Rates by Nuclear Magnetic Resonance. *J. Chem. Phys.* **1958**, *28*, 430–431.

(36)  Římal, V.; Štěpánková, H.; Štěpánek, J. Analysis of NMR Spectra in Case of Temperature-Dependent Chemical Exchange between Two Unequally Populated Sites. *Concepts Magn. Reson. Part A* **2011**, *38A*, 117–127.

(37)  Kovrigin, E. L. NMR Line Shapes and Multi-State Binding Equilibria. *J. Biomol. NMR* **2012**, *53*, 257–270.

(38)  Liu, Y.; Yang, Y. W.; Cao, R.; Song, S. H.; Zhang, H. Y.; Wang, L. H. Thermodynamic Origin of Molecular Selective Binding of Bile Salts by Aminated β-Cyclodextrins. *J. Phys. Chem. B* **2003**, *107*, 14130–14139.

(39)  Schönbeck, C.; Westh, P.; Madsen, J. C.; Larsen, K. L.; Städe, L. W.; Holm, R. Hydroxypropyl-Substituted β-Cyclodextrins: Influence of Degree of Substitution on the Thermodynamics of Complexation with Tauroconjugated and Glycoconjugated Bile Salts. *Langmuir* **2010**, *26*, 17949–17957.

(40)  Schönbeck, C.; Westh, P.; Madsen, J. C.; Larsen, K. L.; Städe, L. W.; Holm, R. Methylated β-Cyclodextrins: Influence of Degree and Pattern of Substitution on the Thermodynamics of Complexation with Tauro- and Glyco-Conjugated Bile Salts. *Langmuir* **2011**, *27*, 5832–5841.

(41)  Schönbeck, C. *Structure and Thermodynamics of Inclusion Complexes between Cyclodextrins and Bile Salts in Aqueous Solution*; Roskilde University: Roskilde, 2012.

(42)  Tan, X.; Lindenbaum, S. Studies on Complexation between β-Cyclodextrin and Bile Salts. *Int. J. Pharm.* **1991**, *74*, 127–135.

(43)  Køhler, J.; Schönbeck, C.; Westh, P.; Holm, R. Hydration Differences Explain the Large

Variations in the Complexation Thermodynamics of Modified γ-Cyclodextrins with Bile Salts. *J. Phys. Chem. B* **2016**, *120*, 396–405.

(44)  Fukahori, T.; Ugawa, T.; Nishikawa, S. Molecular Recognition Kinetics of Leucine and Glycyl-Leucine by β-Cyclodextrin in Aqueous Solution in Terms of Ultrasonic Relaxation. *J. Phys. Chem. A* **2002**, *106*, 9442–9445.

(45)  Mironov, G. G.; Okhonin, V.; Gorelsky, S. I.; Berezovski, M. V. Revealing Equilibrium and Rate Constants of Weak and Fast Noncovalent Interactions. *Anal. Chem.* **2011**, *83*, 2364–2370.

(46)  Cramer, F.; Saenger, W.; Spatz, H.-C. Inclusion Compounds. XIX. 1a The Formation of Inclusion Compounds of α-Cyclodextrin in Aqueous Solutions. Thermodynamics and Kinetics. *J. Am. Chem. Soc.* **1967**, *89*, 14–20.

(47)  Hersey, A.; Robinson, B. H. Thermodynamic and Kinetic Study of the Binding of Azo-Dyes to α-Cyclodextrin. *J. Chem. Soc. Faraday Trans. 1 Phys. Chem. Condens. Phases* **1984**, *80*, 2039-2052.

(48)  Lyon, A. P.; Banton, N. J.; Macartney, D. H. Kinetics of the Self-Assembly of Alpha-Cyclodextrin [2]Pseudorotaxanes with Polymethylene Threads Bearing Quartenary Ammonium and Phosphonium End Groups. *Can. J. Chem.* **1998**, *76*, 843–850.

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