
by

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Supervisory Committee


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Abstract

Supramolecular systems, which are formed by the noncovalent intermolecular interactions between molecules, are highly dynamic. The high reversibility of supramolecular systems leads to some functional features that cannot be achieved by the single chemical component. The kinetic information for the supramolecular systems cannot be inferred from thermodynamic studies or structural studies. Furthermore, the information provided by the dynamic study can be employed to infer or explain the results from the thermodynamic study and the structural study.

The first objective of this work was to study the dynamics and the binding mechanism of cucurbit[7]uril with a charged guest molecule (2-naphthyl-1-ethylammonium cation, NpAmH⁺). In general, the binding affinity of cucurbit[7]uril to the positively charged guests are very high compared with other host systems such as cyclodextrins and bile salt aggregates. In this work, the complexation of cucurbit[7]uril and NpAmH⁺ was studied from a kinetic point of view. Results showed that the high binding affinity of cucurbit[7]uril to NpAmH⁺ was due to the high association rate constant and the low dissociation rate constant for the complexation of cucurbit[7]uril and NpAmH⁺. Moreover, the competition between co-cations and NpAmH⁺ for the binding sites of cucurbituril molecules retarded the complexation process for cucurbit[7]uril binding to

The second objective of this work was to study the chiral recognition observed for the formation of 2:2 complexes between β-cyclodextrin and 2-naphthyl-1-ethanol (NpOH). The binding of β-cyclodextrin and NpOH leads to the formation of two 1:1 complexes and three 2:2 complexes. The binding dynamics of NpOH with β-cyclodextrin in the 1:1 complex is fast and occurs within microseconds. A much slower dynamics was observed for the formation of the 2:2 complex. Results showed that more 2:2 complex were formed for (R)-NpOH than for (S)-NpOH, which is due to the difference of the dissociation rate constant of the 2:2 complex for both NpOH enantiomers. The dissociation rate constant of the 2:2 complex for (R)-NpOH is 46.8% lower than that for (S)-NpOH while the association rate constant of the 2:2 complex are similar for both NpOH enantiomers.

The third objective of this work was to study the dynamics and the binding mechanism of octa acid with pyrene. As known from the work of other researchers, the accessibility of small molecules (e.g. I⁻ or O₂) to pyrene bound to octa acid is largely limited by the octa acid capsule. In this study, a two-step successive process was observed for the complexation of octa acid and pyrene. The first step, which was related to the formation of octa acid-pyrene 1:1 complex, was sufficiently fast to be viewed as a pre-equilibrium process. The second step, which was related to the formation of octa acid-pyrene 2:1 complex, was slow on the millisecond – second time scale. The high binding affinity of octa acid to pyrene was observed, which is due to the low dissociation rate constant for the octa acid-pyrene 2:1 complex.
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1 General introduction

1.1 Host-guest system in supramolecular chemistry

Supramolecular chemistry, which is defined as “chemistry beyond the molecule” by Nobel Prize laureate Jean-Marie Lehn, refers to the association of two or more chemical components by the noncovalent, intermolecular forces. Supramolecular systems, which are formed by the interaction between molecules, can achieve functions that cannot achieve by the single chemical component. One may say that the relationships between supramolecular systems, the individual chemical components and the intermolecular forces are analogous to those between houses, bricks and concretes. The noncovalent, intermolecular forces include electrostatic interactions, hydrogen bonding, van der Waals forces, \( \pi-\pi \) stackings and hydrophobic effects. These forces are relatively weak when compared to the covalent bonds within molecules, which lead to the high reversibility in the supramolecular systems.

In the last forty years, supramolecular chemistry has been developed into three areas: the area of molecular recognition, the area of self-processes and constitutional dynamic chemistry. Molecular recognition, which is defined as “binding with a purpose” by Jean-Marie Lehn, refers to the selective binding of one chemical component (guest) by another chemical component (host) to build up a well-defined structure (host-guest complex) with intermolecular interactions. The host is defined as a molecule, an ion or aggregates with its binding site converging in the complex while the guest is defined as any molecule or ion with its binding site diverging in the complex. The self-processes, including self-assembly (which is driven by free energy minimization) and self-organisation (which is driven by an external energy source), refers to the process of the
formation of well-defined and organized architectures by the spontaneous interaction between the components. Constitutional dynamic chemistry, i.e. self-organisation with selection, refers to the formation of the supramolecular entity through the self-processes where the specific components of the supramolecular entity were selected from a collection of components according to the experimental conditions (e.g. pH, the presence of metal ions, the temperature). The molecular recognition is the most fundamental part among these three areas of supramolecular chemistry since it covers the principles of the molecular complementarity and the intermolecular interactions, which are crucial information for designing and studying the complex systems of the other two areas.

In the following sections, several popular supramolecular host molecules are described to provide a perspective on the area of the molecular recognition.

1.1.1 The cucurbit[n]uril host-guest system

Cucurbituril is a macropolycyclic compound formed by linking the glycoluril units with the methylene units (CB[n], Scheme 1.1). The trivial name “cucurbituril” was named after the pumpkin (from the Cucurbitaceae family) because of the similarity of the shape between the molecule and the pumpkin. Cucurbituril was first synthesized by the condensation of glycoluril and formaldehyde in a concentrated HCl solution in 1904 and the structure was recognized as cucurbit[6]uril (CB[6]) by Mock’s group in 1981. CB[6] was found to be a rigid molecule with a hydrophobic cavity and two identical portals lined with carbonyl groups. Therefore, CB[6] can bind with metal cations, the hydronium cation and ammonium cations at the portals of CB[6] by an ion-dipole interaction or with the neutral guest molecules inside the cavities of CB[6] by the hydrophobic effect. Moreover, CB[6] can bind with charged guest molecules to form an inclusion complex.
where the hydrophobic moieties of the guest molecules stay inside the cavity of CB[6] and the charged moieties bind to the portals of CB[6].\textsuperscript{10,13,21-27} The equilibrium binding constants for CB[6] binding to charged guest molecules are relatively high, indicating that CB[6] could be a powerful host molecule for potential applications.\textsuperscript{18,28} However, the capacity of CB[6] being used as a host molecules is limited by two factors: (i) The solubility of CB[6] in water is very low, e.g. 13 $\mu$M reported by Jekel’s group\textsuperscript{18} or 20 $\mu$M reported by Buschmann’s group\textsuperscript{15}. (ii) The size of CB[6] is relatively small with a 5.5-Å diameter cavity (measured at the equator of the molecule) and 4-Å diameter portals. Consequently, only a small ranges of the guest molecules with the proper sizes, such as alkylammonium cations, can fit into the cavity of CB[6].

Scheme 1.1. Structure for the CB[n] ($n = 5 – 8, 10$) homologues.

Other cucurbit[n]uril homologues, i.e. cucurbit[n]uril ($n = 5, 7$ and 8), were first synthesized and characterized by Kim’s group in 2000\textsuperscript{29}. Moreover, cucurbit[10]uril was synthesized by Day’s group\textsuperscript{30} and isolated by Isaacs’s group\textsuperscript{31}. The first cucurbit[n]uril derivative, i.e. decamethylcucurbit[5]uril (Scheme 1.2), was synthesized by Stoddart’s group in 1992.\textsuperscript{32} However, it was after the discovery of CB[n] homologues that more cucurbit[n]uril derivatives were synthesized\textsuperscript{33-38} These CB[n] homologues and derivatives have new physical properties such as different sizes of the molecular cavities.
and a large range of solubilities in water, e.g. up to 10 µM for CB[8] \( ^{39} \) – 0.2 M for cyclohexanocucurbit[6]uril (Scheme 1.2) \(^{36} \). Consequently, the research area for the supramolecular chemistry of the cucurbit[n]uril family has dramatically expanded. In the next sections, the synthesis and physical properties of cucurbit[n]uril compounds will be introduced and the corresponding supramolecular chemistry will be discussed.

![Scheme 1.2. Structures for some cucurbit[n]uril derivatives. a, the cyclohexanocucurbit[n]uril (n = 5 or 6) homologues; b, decamethylcucurbit[5]uril; c, (±)-bis-ns-CB[6]; d, a hexamethylated cucurbit[6]uril compound; e, the perhydroxycucurbit[n]uril (n = 5 – 8) homologues.](image-url)
1.1.1.1 The synthesis and physical properties of cucurbit[n]uril compounds

CB[6] was first synthesized via a two-step process by Behrend et al and was then repeated by Mock et al.9,10 (i) The precipitates were formed from the condensation of glycoluril and formaldehyde in HCl solution. (ii) The precipitates were then dissolved in concentrated H2SO4 at 110 °C to form CB[6]. There was no evidence for the formation of other cucurbit[n]uril compounds by using this synthetic protocol.

The synthetic procedures for CB[n] (n = 5, 7 8 and 10) homologues were developed by Kim’s group29 and Day’s group30 and were modified by several research groups.41-43 The synthetic protocols for these CB[n] homologues were similar to that for CB[6] except that the milder reaction conditions, e.g. lower reaction temperature and lower concentration of acid, were employed for the synthesis of these CB[n] homologues. Furthermore, the product distribution, i.e. the ratio of the amount of the CB[n] homologues, was affected by the reaction conditions e.g. the reaction temperature, the ratio of reactants, the type of the acid involved, the concentration of the acid and the presence of metal cations or guest molecules.40,44,45

Some CB[n] derivatives were synthesized as the reactants i.e. glycoluril and formaldehyde were replaced by the corresponding derivatives. For example, decamethylcucurbit[5]uril32 was synthesized by the condensation of dimethylglycoluril and formaldehyde; cyclohexanocucurbit[6]uril36 was synthesized by the condensation of cyclohexanoglycoluril and formaldehyde; and a chiral nor-seco-cucurbituril compound (±)-bis-ns-CB[6] (Scheme 1.2)35 was synthesized by the condensation of glycoluril and paraformaldehyde.
The cavity sizes of the cucurbit[n]uril homologues increase with the increase of the number of the glycoluril units in the CB[n] molecules. The molecular dimension parameters for CB[n] homologues are summarized in Table 1.1. The portal diameter increases from 2.4 Å for CB[5] to ca. 10 Å for CB[10] while the cavity diameter determined at the equator of the molecule increases from 4.4 Å for CB[5] to ca. 11.7 Å for CB[10]. The cavity volume increase dramatically from 82 Å³ for CB[5] to ca. 870 Å³ for CB[10]. As a result, the cavity of CB[5] can only hold small guest molecules such as propene or xenon, while the cavity of CB[10] can hold large guest molecules such as CB[5], cationic calix[4]arene derivatives or tetra(N-methylpyridyl)porphyrins.

Table 1.1. The molecular dimension parameters and the solubilities for CB[n] homologues

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<td>portal diameter / Å</td>
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<td>cavity volume / Å³</td>
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The values were determined by Kim’s group. The values were determined by Kim’s group. The cavity diameter was measured at the equator of the molecule. The values were determined by Isaacs’s group and the solubility was determined by using D₂O as solvent. The values were determined by Kim’s group. The values were reported by Jekel’s group and Buschmann’s group.

The cavity sizes of CB[n] derivatives are affected not only by the number of the glycoluril units of the CB[n] derivatives, but also by the way how the CB[n] derivatives...
are modified from their parent CB[n] molecules. In general, as the molecular modification is conducted on the outer surface of the corresponding parent CB[n], the cavity size of the CB[n] derivative is similar to that of the corresponding parent CB[n]. This observation is the case for some CB[n] derivatives, such as cyclohexanocucurbit[n]uril (n = 5 or 6)\textsuperscript{36,49}, hexamethylated cucurbit[6]uril (Scheme 1.2)\textsuperscript{50,51}, decamethylcucurbit[5]uril\textsuperscript{32,52}, perhydroxycucurbit[6]uril\textsuperscript{37}. As the molecular modification is conducted on the inner surface of the corresponding parent CB[n], the cavity size of the CB[n] derivative could be different from that of the corresponding parent CB[n]. For example, the inverted CB[n] derivatives (n = 6 or 7)\textsuperscript{34,53}, which contain a single inverted glycoluril unit, have molecular cavities smaller than the corresponding parent CB[n]. On the contrary, the nor-seco-cucurbituril compound (±)-bis-ns-CB[6] (Scheme 1.2)\textsuperscript{35}, has a molecular cavity larger than CB[6].

The solubilities of CB[n] homologues are low (i.e. 10 µM level) except for the solubilities of CB[5] and CB[7] which are about 20 mM – 30 mM. Therefore, the supramolecular system for the CB[n] homologues are always studied in the presence of co-cations, e.g. the hydronium, ammonium ions or metal cations since the co-cations can solubilize the CB[n] homologues.

The solubilities of some CB[n] derivatives are relatively high compared to their parent CB[n] molecules, e.g. the solubilities of the cyclohexanocucurbit[n]uril (n = 5 or 6) compounds are both ca. 0.2 M, which are 10 times higher than that of CB[5] or $10^4$ times higher than that of CB[6]. Although this thesis focuses on the supramolecular system in water, it is worth noting that some CB[n] derivatives are much more soluble in organic solvents than the corresponding parent cucurbit[n]urils. This observation is crucial since
these organic-soluble CB[n] derivatives can be employed in the functionalization reactions that can be only performed in organic solvents. For example, perhydroxycucurbit[6]uril, which is soluble in dimethyl sulfoxide and dimethylformamide, can be functionalized by the alkylation reactions or the carboxylation reactions.\textsuperscript{36}

The electronic structures of the CB[n] homologues were studied by density functional theory calculations. These results show that the carbonyl oxygens at the portals of all cucurbit[n]uril homologues are partially negatively charged and the charge densities on the carbonyl oxygen increase with the increase of the sizes of the CB[n] homologues.\textsuperscript{54} Moreover, the charge densities is delocalized to envelop the entire portal for CB[5] but become more localized on the carbonyl oxygen with the increase of the sizes of the CB[n] homologues.\textsuperscript{55,56} As the portals of CB[n] homologues become more hydrophilic with the increase of sizes of the CB[n] homologues, the cavities of the CB[n] homologues become, on the contrary, more hydrophobic.\textsuperscript{57} Although CB[n] homologues molecules have an overall neutral charge, the electrons are asymmetrically distributed. The outer surfaces of all CB[n] homologues are slightly positive,\textsuperscript{39,52} while the inner surfaces of all CB[n] homologues are weakly negative.\textsuperscript{39,57}

1.1.1.2 The cucurbit[5]uril host-guest system

The cavity size of CB[5] is relatively small as aforementioned. Therefore, the CB[5] cavity can only hold small molecules such as N\textsubscript{2}, O\textsubscript{2}, Ar, Ne, Xe, CO, NO, H\textsubscript{2}S, methane, ethylene, acetylene, dioxane, propene and hexamethylene diammonium ions.\textsuperscript{25,46,47} However, a wide range of guest molecules with different sizes can bind to the portals of CB[5] to form exclusion complexes in which the guest is not located in the cavity of
CB[5]. The guest molecules can be the hydronium ion, metal cations (e.g. Na\(^+\), K\(^+\), Ca\(^{2+}\), Ba\(^{2+}\), Pb\(^{2+}\), Hg\(^{2+}\)), charged organic guest molecules (e.g. protonated lysine\(^{59}\) and thioflavin T\(^{60}\)), or even large particles (e.g. the gold nanoparticles with 3.5 nm – 7.0 nm diameter\(^{61}\)).

The equilibrium binding constants (logK) of the 1:1 host:guest complex formed by the metal cations (M\(^+\)) and CB[5] were determined as 1.85, 1.31, 1.01 and 0.9 for Na\(^+\), K\(^+\), Rb\(^+\) and Cs\(^+\), respectively.\(^{17}\) The values of logK for the CB[5]-M\(^+\) complexes decrease with the increase of the sizes of the metal cations, which is consistent with the observation that the 2.4-Å diameter portals of CB[5] match the metal cations better in the order of the sizes of metal cations, i.e. Na\(^+\) (2.04-Å diameter), K\(^+\) (2.76-Å diameter), Rb\(^+\) (2.98-Å diameter) and Cs\(^+\) (3.4-Å diameter).\(^{62}\)

An interesting study shows that CB[5] can encapsulate anions (e.g. Cl\(^-\) or nitrate ion) in the presence of the metal cations, e.g. K\(^+\), Ba\(^{2+}\), La\(^{3+}\).\(^{39,57,63,64}\) The metal cations, which bind to the portals of CB[5], act as lids to keep the anion inside of the CB[5] cavity.\(^{63,64}\) Furthermore, the interaction between the metal cations and the encapsulated anions would be a crucial force to stabilize the CB[5]-anion complexes, otherwise the CB[5]-anion complexes could not be formed due to the repulsion between the anion and the inner surface of CB[5] which is partially negatively charged.\(^{39,57}\)

1.1.1.3 The cucurbit[6]uril host-guest system

The CB[6] host-guest system were first studied in 1981. In the same paper where the structure of CB[6] was reported, Mock’s group briefly discussed the remarkable feature for CB[6] host-guest system that the binding affinities of CB[6] for the charged guest molecules are very high, e.g. the equilibrium constant for CB[6] binding with
cyclopentanemethylammonium ion was ca. $10^6$ M$^{-1}$ at 40 °C in the 1:1 formic acid:water solution.\textsuperscript{10} In Mock’s following work, the binding of CB[6] to a range of the substituted ammonium ions, which contain different aliphatic chain lengths and different numbers of ammonium groups, were systematically studied.\textsuperscript{22-24,65} The results showed that the CB[6] cavity can hold the substituted ammonium ions with sizes smaller than a para-disubstituted benzene ring. The $\alpha,\omega$-alkanedi ammonium ions bind to CB[6] much more tightly than the alkylammonium ions with the same length of alkyl chain but only one ammonium group, indicating that the ion-dipole interactions between the charged guest and CB[6] stabilize the CB[6]-guest complexes. For example, the equilibrium constant for 1,5-pentanedi ammonium ion binding with CB[6] ($2.4 \times 10^6$ M$^{-1}$) is ca. 100 times higher than that for $n$-pentyl ammonium ion binding with CB[6] and the equilibrium constant for 1,4-butanedi ammonium ion binding with CB[6] ($1.5 \times 10^5$ M$^{-1}$) is ca. 1.5 times higher than that for $n$-butylammonium ion binding with CB[6]. Moreover, the 1,6-hexanedi ammonium ion binds to CB[6] more tightly than its $\alpha,\omega$-alkyl homologues. This observation can be explained by the fact that the distance between two ammonium ions in the 1,6-hexanedi ammonium ion perfectly matches the height of the CB[6] molecule. Consequently, both ammonium ions of the 1,6-hexanedi ammonium ion can tightly bind to the portals of CB[6]. It is worth noticing that the $n$-butyl ammonium ion binds to CB[6] more tightly than its $n$-alkyl homologues. This observation was not explained until the role of co-cations in the CB[6] host-guest system were thoroughly discussed by Kim’s and Inoue’s groups.\textsuperscript{21,66} These two research groups studied the binding affinity of CB[6] to a series of aliphatic alcohols, ammonium ions and diamonium ions in the presence of co-cations (e.g. hydronium ion, Li$^+$, Na$^+$, K$^+$ and Cs$^+$). The results show that the binding
affinities of CB[6] to n-alkyl alcohols are much weaker than those of CB[6] to n-alkylammonium ions with the same length of the alkyl chains. For example, the equilibrium binding constants for CB[6] with n-alkyl alcohols in the presence of 0.05 M NaCl are 90 M$^{-1}$, 710 M$^{-1}$, 1220 M$^{-1}$ and 410 M$^{-1}$ for ethanol, propanol, butanol and pentanol, respectively. The equilibrium binding constants for CB[6] with n-alkylammonium ions in the presence of 0.05 M NaCl are 900 M$^{-1}$, $1.55 \times 10^5$ M$^{-1}$, $3.1 \times 10^6$ M$^{-1}$ and $2.2 \times 10^6$ M$^{-1}$ for ethylammonium ion, 1-propylammonium ion, 1-butylammonium ion and 1-pentylammonium ion, respectively. This observation indicates that the ion-dipole interaction between the host and guest molecules plays a key role for the CB[6]-guest complexation. The maximum binding affinity of CB[6] to n-alkyl alcohols was observed for butanol while the maximum binding affinity of CB[6] to n-alkylammonium ions was observed for 1-butylammonium ion. These observations were explained by the following reasons. (i) The guests, i.e. n-alkyl alcohols and n-alkylammonium ions, become more hydrophobic with the elongation of alkyl chains, leading to the stronger hydrophobic interaction between the alkyl chains of the guests and the CB[6] cavities. As a result, the binding affinities of CB[6] to the guests increase with the elongation of the alkyl chains from ethyl to butyl. (ii) The other portal of CB[6] is capped with Na$^+$ as the guest binds to one portal of CB[6] to form the inclusion complex. Consequently, the steric effects between the alkyl chains of the bound guests and Na$^+$ reduce the binding affinities of CB[6] to the guests, as the alkyl chains of the guests are longer than butyl. The formation of the n-alkylammonium@CB[6]\textbullet Na$^+$ complexes were confirmed by the electrospray ionization mass spectrometry.$^{67}$ Moreover, the maximum binding affinity of CB[6] to n-alkylammonium ions was observed for 1-butylammonium
ion, which is consistent with Kim’s and Inoue’s aforementioned study. These results illustrate not only the role of ion-dipole interactions and hydrophobic effect between the host and the guest molecules for the formation of the CB[6]-guest inclusion complex, but also the effect of co-cations on the binding of CB[6] to the guests.

The effect of co-cations on the binding of CB[6] to the guests were illustrated by the observation that the equilibrium binding constants for CB[6] with the same guest could be very different when determined by different research groups. For example, the equilibrium constants for CB[6] binding to tricationic spermine and tricationic spermidine are respectively $4.1 \times 10^5 \text{ M}^{-1}$ and $6.6 \times 10^5 \text{ M}^{-1}$ determined in pure water by Buschmann’s group, which are respectively 3.3 times and 20 times lower than those determined in 50% formic acid by Mock’s group. The equilibrium constant for CB[6] binding with 1,6-hexane-diammonium determined in 50 mM acetate buffer in D$_2$O by Isaacs’s group was $4.5 \times 10^8 \text{ M}^{-1}$, which is ca. 150 times larger than that determined in 50% formic acid by Mock’s group.

The binding of hydronium ion, ammonium ion and metal cations to the portals of CB[6] was studied by Buschmann’s and Knoche’s groups. The equilibrium binding constants (logK) for CB[6] with the univalent and the divalent cations (Table 1.2) were determined under the assumption that only the 1:1 CB[6]-cation complex was formed. The value of logK for CB[6] binding to the same cation varies according to the methodology used. For example, the values of logK listed in the third and the fifth rows of Table 1.2 were both determined in water at 25 °C while the values of logK listed in the forth rows of Table 1.2 were determined in HCOOH/H$_2$O (1:1) solution at 25 °C. Moreover, the concentration ranges of cations for logK listed in the third and fifth rows of
Table 1.2 were 2 mM – 20 mM and 0.01 mM – 0.5 mM, respectively. The high concentration of cations favours the formation of the 1:2 CB[6]-cations complexes, which may lead to errors in the determination of log\(K\) for the 1:1 CB[6]-cation complex since the assumption that only the 1:1 CB[6]-cation complex is formed in the CB[6]-cation system cannot hold any more. The equilibrium binding constant (log\(K\)) for CB[6] with the trivalent lanthanide cations (Ln\(^{3+}\)) in water at 25 °C were determined. As the diameters of the trivalent lanthanide cations decrease from 2.04 Å for La\(^{3+}\) to 1.70 for Lu\(^{3+}\), the values of log\(K\) for the CB[6]-Ln\(^{3+}\) complexes gradually increase from 2.50 to 3.36, indicating that the smaller size of Ln\(^{3+}\) match the portals of CB[6] better.

Table 1.2. The diameter of cations and the equilibrium binding constants (log\(K\)) for cations with CB[6] at 25 °C

<table>
<thead>
<tr>
<th></th>
<th>H(_2)O(^+)</th>
<th>NH(_4)(^+)</th>
<th>Li(^+)</th>
<th>Na(^+)</th>
<th>K(^+)</th>
<th>Rb(^+)</th>
<th>Cs(^+)</th>
<th>Ca(^{2+})</th>
<th>Sr(^{2+})</th>
<th>Ba(^{2+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>diameter(^a) / Å</td>
<td>2.76</td>
<td>3.40</td>
<td>1.52</td>
<td>2.04</td>
<td>2.76</td>
<td>3.04</td>
<td>3.40</td>
<td>2.00</td>
<td>2.32</td>
<td>2.72</td>
</tr>
<tr>
<td>log(K(^b)</td>
<td>-</td>
<td>2.84</td>
<td>-</td>
<td>3.49</td>
<td>2.85</td>
<td>2.98</td>
<td>2.52</td>
<td>3.61</td>
<td>2.90</td>
<td>-</td>
</tr>
<tr>
<td>log(K(^c)</td>
<td>-</td>
<td>2.69</td>
<td>2.38</td>
<td>3.23</td>
<td>2.79</td>
<td>2.68</td>
<td>-</td>
<td>2.80</td>
<td>3.18</td>
<td>2.83</td>
</tr>
<tr>
<td>log(K(^d)</td>
<td>3.02</td>
<td>3.97</td>
<td>-</td>
<td>3.69</td>
<td>3.96</td>
<td>4.41</td>
<td>4.82</td>
<td>4.57</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\)The values were calculated from the corresponding crystal structures.\(^62\)\(^-\)\(^73\) \(^b\)The values were measured in water.\(^17\) \(^c\)The values were determined in HCOOH/H\(_2\)O (1:1) solution.\(^15\)\(^25\) \(^d\)The values were measured in water.\(^12\) The errors for the diameter of cations are less than 0.05 Å. The errors for log\(K\) are less than 0.05 except that no errors were reported for those in the last row of the table.

1.1.1.4 The cucurbit[7]uril host-guest system

The CB[7] host-guest system has attracted a lot of attention during the last ten years due to the following reasons. (i) CB[7], as other CB[n] homologues, can bind tightly with
charged guests by the ion-dipole interaction and the hydrophobic effect between the host and guest molecules. (ii) CB[7], with a 5.4 Å diameter portal and a 279 Å³ cavity, is much larger than CB[5] and CB[6]. (iii) The solubilities of CB[7] and CB[5] are ca. $1 \times 10^3$ times higher than those of other CB[n] homologues.

Some of the guest molecules, which cannot bind to CB[5] and CB[6] but can bind to CB[7], are listed as follows with the equilibrium binding constants for the CB[7]-guest 1:1 complexes in brackets: ferrocene and its derivatives (ca. $10^9$ M⁻¹ – $10^{15}$ M⁻¹ determined in water), adamantaneammonium and its derivatives (ca. $10^{12}$ M⁻¹ at pD 4.74 in D₂O buffered by 50 mM NaO₂CCD₃); cationic form of dyes, e.g. the Rhodamine derivatives, cresyl violet, pyronin Y, coumarin 102, neutral red and brilliant green (ca. $10^4$ M⁻¹ – $10^5$ M⁻¹ determined in water); dipeptides (ca. $10^4$ M⁻¹ – $10^7$ M⁻¹ determined in 0.1 M NaCl aqueous solutions); viologen and its derivatives (ca. $10^5$ M⁻¹ in 0.1 M NaCl aqueous solutions); 2-aminoanthracene (ca. $8 \times 10^5$ M⁻¹ determined at pH 1.5 in water); acridizinium derivatives (ca. $4 \times 10^8$ – $7 \times 10^8$ M⁻¹ determined in D₂O); N-benzyl-1-(1-naphthyl)ethylamine (ca. $1 \times 10^8$ M⁻¹ determined in D₂O); 2,3-diazabicyclo[2.2.2]oct-2-ene i.e. DBO (ca. $4 \times 10^5$ M⁻¹ determined in D₂O); anthraquinone derivatives (ca. $10^3$ M⁻¹ determined in D₂O); and dendrimer derivatives (ca. $10^4$ M⁻¹ at pH 3.2 in 0.2 M formic acid buffer and ca. $10^4$ M⁻¹ at pH 7.3 in 0.03 M Tris buffer).

The studies on the binding of CB[7] to dyes show that CB[7] can enhance the fluorescence of the dyes, stabilize the dyes against photobleaching and against the unspecific adsorption onto the surface of the equipment. For example, the fluorescence quantum yield of brilliant green bound to CB[7] increases 300 fold as compared with that
of Brilliant Green free in water.\textsuperscript{91} The rate of photobleaching for Rhodamine 6G bound to CB[7] is 30 times lower than that for Rhodamine 6G free in water. Moreover, the rate of the unspecific adsorption of Rhodamine 6G-CB[7] complexes onto the glass and plastic surfaces is at least 100 times lower than that of Rhodamine 6G free in water.\textsuperscript{76} The high binding affinity of dyes to CB[7] is not only due to the high hydrophobic effect between CB[7] and the dyes but also due to the high ion-dipole interaction between the charged moieties of the dyes and the portals of CB[7]. For example, the equilibrium constant for neutral red binding with CB[7] was $6.5 \times 10^3 \text{ M}^{-1}$ at pH 11, which is 100 times lower than that for the protonated form of neutral red with CB[7] at pH 2.\textsuperscript{91,92} It is worth noticing that the negatively charged dyes, e.g. the deprotonated form of fluorescein and deprotonated form of the arylsulfonated Cyanine 5 derivative, do not bind to CB[7].\textsuperscript{77}

The role of the ion-dipole interaction for the CB[7]-guest complexation can be represented by the study on the binding of CB[7] to the ferrocene derivatives.\textsuperscript{74,93} The neutral ferrocene derivatives, e.g. hydroxymethylferrocene, (ferrocenylmethyl)dimethylamine and ferrocenecarboxylic acid, bind to CB[7] with equilibrium binding constants in the range of $10^9 - 10^{10} \text{ M}^{-1}$. The cationic ferrocene derivatives, e.g. (ferrocenylmethyl)dimethylammonium ion and (ferrocenylmethyl)trimethylammonium ion, bind to CB[7] with equilibrium binding constants in the range of $10^{12} - 10^{13} \text{ M}^{-1}$. Furthermore, the equilibrium binding constant of 1,1-bis(trimethylammoniomethyl)ferrocene cation with CB[7], ca. $3 \times 10^{15} \text{ M}^{-1}$, is the highest binding affinity reported for the CB[n] host-guest system. However, the anionic derivatives, e.g. ferrocenecarboxylate, do not bind to CB[7] due to the repulsion between the negative charged inner surface of the CB[7] cavity and the negative charged guest.\textsuperscript{99,57}
The binding affinities of CB[7] to different methylviologen species were studied.\textsuperscript{94,95} Results show that CB[7] binds to methylviologen (MV\textsuperscript{2+}) tightly with the equilibrium binding constant of $2 \times 10^5 \text{ M}^{-1}$. MV\textsuperscript{••}, which is a cation radical formed by the reduction of MV\textsuperscript{2+}, binds to CB[7] with a slightly smaller equilibrium binding constant of $8.5 \times 10^4 \text{ M}^{-1}$. Moreover, MV\textsuperscript{0}, which is a neutral species formed by the reduction of MV\textsuperscript{+•}, bind to CB[7] with a much smaller equilibrium binding constant of $2.5 \times 10^2 \text{ M}^{-1}$. In other words, the binding affinity of the methylviologen species to CB[7] is in the order of MV\textsuperscript{2+} > MV\textsuperscript{••} >> MV\textsuperscript{0}. These results were determined in 0.1 M phosphate buffer solutions (pH 7.0) by Kim’s group.\textsuperscript{94} The same trend was observed by Kaifer’s group as well, i.e. the equilibrium binding constants of CB[7] with MV\textsuperscript{2+} ($1 \times 10^5 \text{ M}^{-1}$ determined in 0.2 M NaCl aqueous solutions) are higher than that of CB[7] with MV\textsuperscript{••} ($0.5 \times 10^5 \text{ M}^{-1}$ determined in 0.2 M NaCl aqueous solutions).\textsuperscript{95}

CB[7] binds to the hydronium ion, ammonium ions and metal cations. The effect of these cations in the CB[7]-guest system, which was observed and discussed by a few groups, will be introduced in Chapter 2.

1.1.1.5 The cucurbit[8]uril host-guest system

Cucurbit[8]uril (CB[8]) can bind to large guest molecules, e.g. other macrocycles (cyclen, cyclam and their metal complexes).\textsuperscript{96} Moreover, CB[8] can bind to two identical guest molecules (e.g. 2,6-bis(4,5-dihydro-1H-imidazol-2-yl)naphthalene, MV\textsuperscript{••}, phenylphosphonic acid, acridinium and its derivatives, coumarin) to form the 1:2 CB[8]-guests complexes,\textsuperscript{29,39,85,97-99} or two different guest molecules (e.g. 2,6-dihydroxynaphthalene together with methylviologen, 1,4-dihydroxybenzene together with methylviologen) to form the 1:1:1 ternary complexes.\textsuperscript{100} The formation of the 1:2 or
the 1:1:1 ternary CB[8]-guests complexes is not only driven by the ion-dipole interaction and the hydrophobic effect between host and guest molecules as aforementioned for other CB[n]-guest systems (n = 5, 6 and 7), but also affected by the intermolecular interactions between guest molecules. For example, 2,6-dihydroxynaphthalene itself does not bind to CB[8] in the absence of MV$^{2+}$ but binds to CB[8] in the presence of MV$^{2+}$. Therefore, the formation of CB[8]-2,6-dihydroxynaphthalene-methylviologen 1:1:1 ternary complex is driven by the intermolecular interaction between 2,6-dihydroxynaphthalene and MV$^{2+}$. Another example is that MV$^{2+}$ cannot bind to CB[8] to form a 1:2 CB[8]-MV$^{2+}$ complex, which could be due to the electrostatic repulsion between two MV$^{2+}$. However, the 1:2 CB[8]-MV$^{2+}$ inclusion complex can be quickly formed as the 1:1 CB[8]-MV$^{2+}$ complex is reduced to the 1:1 CB[8]-MV$^{2+}$ complex. The equilibrium dimerization constant of MV$^{2+}$ in the presence of CB[8] is ca. $2 \times 10^7$ M$^{-1}$, which is 10$^5$ times higher than that of MV$^{2+}$ in the absence of CB[8]. As CB[8] binds to 2 guest molecules, CB[8] can be employed as a template to induce the photodimerization of guests (e.g. dipyridyl ethylenes, stilbazoles, stilbazolium salts).  

1.1.1.6 The cucurbit[10]uril host-guest system

Cucurbit[10]uril (CB[10]) was first discovered as the CB[5]•CB[10] inclusion complex by Day’s group in 2002 and isolated by Isaacs’s group in 2005. As a host molecule, CB[10] has a high binding affinity to CB[5] (> $10^6$ M$^{-1}$). The method to isolate CB[10] from the CB[5]•CB[10] complex is using another guest molecule (e.g. melamine diamine) to displace CB[5] from the cavity of CB[10] and then washing out the guest molecule inside of the cavity of CB[10]. CB[10] binds with other macrocyclic molecules (e.g. the cationic calix[4]arene derivatives, porphine) or complexes (e.g.
K⁺•CB[5]•K⁺, porphyrins with metals in their cavity, the dinuclear platinum(II) and ruthenium(II) complexes. Moreover, CB[10] can bind with two guests to form ternary complexes, e.g. a porphyrin•CB[10]•pyridine complex. The intermolecular interactions between two guests in some specific cases, e.g. the π-π stacking between porphyrin and pyridine, can be the main driving force for the formation of the ternary complexes.

1.1.1.7 The host-guest system of cucurbit[n]uril derivatives

Although this thesis focuses on the supramolecular chemistry of CB[7] in water, it is worth noting that the host-guest systems for the cucurbit[n]uril derivatives have been developed extensively in recent years. The CB[n] derivatives, which carry functional groups (e.g. the hydroxyl groups on the outer surface of perhydroxycucurbit[n]uril) or have different shape (e.g. (±)-bis-ns-CB[6] shown in Scheme 1.2), can build up interesting supramolecular structures. For example, the long-chain guest, which contains two adamantylammonium ions separated by the xylylene spacing groups, can bind to bis-ns-CB[10] to form supramolecular polymers.

1.1.2 The cyclodextrin host-guest system

Cyclodextrins (CD) are macropolycyclic compounds formed by linking the α-D-glucose units through α-1,4 bonds (Scheme 1.3). The most common cyclodextrin homologues, which contain 6, 7 and 8 glucosidic units, are named as α-cyclodextrin (α-CD), β-cyclodextrin (β-CD) and γ-cyclodextrin (γ-CD), respectively. CD were first isolated by Villiers in 1891 and identified as α-CD, β-CD and γ-CD by Schardinger and Freudenberg. Each CD homologue has the shape of a truncated cone. The cavity of
CD is hydrophobic due to the protons located at C-3 and C-5 and due to the lone pairs of glucosidic oxygen on the inner surface of the CD. The portals of the CD, lined with the free hydroxyl groups of the glucose moieties, are hydrophilic. The primary hydroxyl groups, i.e. the hydroxyl groups located at C-6, can freely rotate at the portal of CD and define the narrower portal of the CD. The secondary hydroxyl groups, i.e. the hydroxyl groups located at C-2 and C-3, are situated quite still and define the wider portal of CD.

Scheme 1.3. Structure for the CD (n = 6 – 8) homologues (left) and the α-D-glucose unit (right).

The molecular dimension parameters for CD homologues are summarized in Table 1.3. The cavity sizes of CD homologues increase with the increase of the number of glucose units. The solubility of β-CD in water is quite low, which is probably due to the fact that all secondary hydroxyl groups in one β-CD molecule can associate with each other to form a complete belt by the intramolecular hydrogen bonds rather than associate with water molecules by intermolecular hydrogen bonds. Comparatively, the hydrogen-bond belts formed by the secondary hydroxyl groups are not complete for α-CD and γ-CD, as the positions of some secondary hydroxyl groups in α-CD and γ-CD are distorted. Consequently, the secondary hydroxyl groups in α-CD and γ-CD have a higher chance to bind to water molecules, leading to high solubilities for α-CD and γ-CD.
Table 1.3. The molecular dimension parameters and the solubilities for CD homologues\textsuperscript{111,112}

<table>
<thead>
<tr>
<th>CD</th>
<th>portal diameter / Å</th>
<th>height / Å</th>
<th>cavity volume / Å\textsuperscript{3}</th>
<th>solubility / mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CD</td>
<td>4.7</td>
<td>5.3</td>
<td>7.9</td>
<td>174</td>
</tr>
<tr>
<td>β-CD</td>
<td>6.0</td>
<td>6.5</td>
<td>7.9</td>
<td>262</td>
</tr>
<tr>
<td>γ-CD</td>
<td>7.5</td>
<td>8.3</td>
<td>7.9</td>
<td>427</td>
</tr>
</tbody>
</table>

According to Szejtli’s analysis, the CD chemistry can be related to three stages: the discovery period (1891 – 1930s), the period of the systematic studies on the CD host-guest system (1930s – 1970s) and the period of the application of CD (from 1970s onward).\textsuperscript{111} There have been ca. 15,000 publications (i.e. papers, patents and conference abstracts) related to CD until the end of 1997 and the number of the publications has more than tripled until the end of 2010.

The binding affinities of CD to guest molecules were extensively studied over one century. The most important feature of CD in supramolecular chemistry is that CD can bind to a wide range of guest molecules, e.g. alcohols, aldehydes, organic acids, amines, aliphatic and aromatic hydrocarbons, organometallic complexes, inorganic anions and noble gases.\textsuperscript{112-118} Two factors play key roles in the CD-guest complexation: (i) the steric effect, i.e. the cavity of CD should be large enough to accommodate the guest molecules or the specific functional groups of the guest molecules; (ii) the intermolecular interaction as the driving force, which mainly is the hydrophobic effect between the cavity of CD and the hydrophobic moieties of the guest molecules, but could also be hydrogen bondings and van der Waals forces.\textsuperscript{119-122} The papers concerning CDs as the hosts for the guest molecules and as the building blocks for the supramolecular structures have been
Therefore, only two topics, i.e. (i) the binding of β-CD to naphthalene (Np, Scheme 1.4) and to Np derivatives and (ii) the chiral recognition of guest with β-CD, will be introduced hereafter to provide a necessary background for the β-CD host-guest system reported in this thesis.

Scheme 1.4. Structure for naphthalene (Np).

1.1.2.1 The binding of β-CD to naphthalene and its derivatives

The complexation between naphthalene and β-CD were firstly studied by Hamai. The fluorescence emission of Np at 330 nm, i.e. the monomer emission, increased with the addition of β-CD, which was due to the formation of β-CD-Np 1:1 complex. Moreover, a new emission peak at 410 nm, i.e. the excimer emission, was observed and increased with the further addition of β-CD, which was due to the formation of a β-CD-Np 2:2 complex. Three lifetimes of singlet-excited states of Np species, i.e. 40 ns, 48 ns and 68 ns, were determined and respectively assigned to Np free in water, Np in the 1:1 complexes and Np in the 2:2 complexes. Furthermore, the fluorescence emissions of all Np species were quenched by I⁻ or IO⁻; and the fluorescence quenching rate constant for each Np species was determined. The longest singlet-excited states and the smallest fluorescence quenching rate constant are observed for Np species in the 2:2 complexes, indicating that Np in the 2:2 complexes is more isolated from the aqueous phase than other Np species. Moreover, Np species in the 1:1 complexes are associated with the longer lived singlet-excited states and the smaller fluorescence quenching rate constant.
when compared to the photophysics of the singlet excited state of Np free in water, indicating that Np in the 1:1 complexes is more isolated from aqueous phase than Np free in water. The equilibrium constant for the formation of 1:1 complex and 2:2 complex were determined as 685 M\(^{-1}\) and 4000 M\(^{-1}\) at 25 °C, respectively.\(^{134}\)

Hamai’s study on the complexation of Np with β-CD was followed by Grabner’s study by using structural model calculations (i.e. a dynamic Monte Carlo technique) and spectroscopic techniques.\(^{135}\) The formation of the β-CD-Np 1:1 complexes leads to the increase of the lifetime of the triplet-excited state for the Np species. However, no long-lived component can be assigned to the triplet-excited state of the 2:2 complexes, although the presence of the 2:2 complexes in the system is indicated by the excimer emission. This observation may be due to the rapid self-quenching of the triplet-excited state of the excimer or the inefficient intersystem crossing of the singlet-excited state of the excimer. The formations of β-CD-Np 1:1 and 2:2 complexes were observed in the results of the structural model calculations. Moreover, the results of the structural model calculations indicate that the hydrogen bonding between the secondary hydroxyl groups of two β-CDs in the 2:2 complexes play a key role for stabilization of the 2:2 complexes and the guests are flexible within the rather stable β-CD complex.

The complexations between β-CD and the Np derivatives are affected by the position of the substituted functional group on the Np molecule. One example is the study conducted by Harata et al using the circular dichroism spectroscopy.\(^{136}\) The naphthalene derivatives employed in the study include 1-naphthylacetic acid, 2-naphthylacetic acid, 1-naphthoic acid, 2-naphthoic acid, 1-naphthylamine, 2-naphthylamine, 1,8-diaminonaphthalene and 2,3-diaminonaphthalene. Results indicate that the position of the
2-substituted Np derivatives with respect to the β-CD cavity are different from that of the 1-substituted Np derivatives. The longitudinal axis of the 2-substituted Np derivative is parallel to the symmetry axis of β-CD. Comparatively, two kinds of the β-CD-guest complexes were observed for each 1-substituted Np derivative, where the longitudinal axis of the 1-subsituted Np derivative was either parallel or perpendicular to the symmetry axis of β-CD. The study conducted by Warner’s group shows that the lifetime of the singlet-excited state of the 2-substituted Np derivative is longer than that of the 1-substituted Np derivative as these Np derivatives are bound to β-CD.\textsuperscript{137} This observation indicates that the 2-substituted Np derivative is more isolated from the aqueous phase than the 1-substituted Np derivative when these Np derivatives are bound to β-CD.

Another binding study of β-CD with Np derivatives was conducted by using 2-naphthyloxyacetic acid and 1-naphthylacetic acid as the model guest molecules.\textsuperscript{138} Results show that the binding affinity of β-CD to the 2-substituted Np derivative (560 M\textsuperscript{-1} at 20 °C) is much higher than that to 1-substituted Np derivative (100 M\textsuperscript{-1} at 20 °C). Moreover, the values of pKa for each guest molecule does not change in the absence and presence of β-CD, indicating that the substitution group of each guest molecule protrudes from the cavity of β-CD and is in the aqueous phase. These studies suggest that the naphthyl moieties in the 2-substituted Np derivatives are inserted into the β-CD cavity deeply to achieve the higher binding affinity of β-CD to the 2-substituted Np derivatives while only the unsubstituted benzene rings of 1-substituted Np derivatives can be included into the β-CD due to the steric hindrance, leading to the lower binding affinity of β-CD to the 1-substituted Np derivatives.
The effect of the charge state of the Np derivatives on the binding affinity of β-CD to the Np derivatives was studied by using 1-α-naphthyl-3-(dimethylamino)propane as the guest molecule.\textsuperscript{139} The binding affinity of β-CD to the unprotonated form of the guest (630 M\textsuperscript{-1}) is much higher than that to the protonated form of the guest (250 M\textsuperscript{-1}). This observation may be due to the fact that the protonated form of the guest is less hydrophobic than the unprotonated form of the guest. Moreover, two lifetimes of the singlet-excited states of the unprotonated form of the guest in the β-CD-guest complex were observed, indicating the formation of two types of the β-CD-guest complexes with different geometries.

The binding process of 2-naphthol with β-CD was studied by a statistical approach (i.e. a molecular mechanics method).\textsuperscript{140} The low energy structures of β-CD free in water and the β-CD-guest complex were calculated and the corresponding conformational variabilities for each β-CD species were studied. The calculated results for β-CD free in water indicate that the local, rigid hydrogen-bonding domains are generated at the wider portal of β-CD, as the secondary hydroxyl groups in two or more glucose units are associated with each other. The structure of β-CD becomes less flexible and more asymmetric when β-CD is bound to 2-naphthol. Moreover, van der Waals forces between the 2-naphthol molecule and the localized hydrogen-bonding domains of β-CD is the main driving force to stabilize the β-CD-guest complex. Two different types of complex structure were characterized, suggesting the presence of two states of complexation: the docking state (i.e. the stage for the binding site of 2-naphthol encountering that of β-CD).
and the final state (i.e. the state for the formation of the complex with the lowest conformational energy).

The complexation of 2-methylnaphthalene with β-CD as the 1:1 and 2:2 complexes was studied by fluorescence spectroscopic methods. The formation of 1:1 and 2:2 complexes with the addition of β-CD were respectively indicated by the fluorescence enhancement at 330 nm and the formation of a new fluorescence emission band at 410 nm (the excimer emission). The equilibrium binding constants for the 1:1 and 2:2 complexes at 25 °C were 1190 M⁻¹ and 1400 M⁻¹, respectively. The excimer emission was not observed for the β-CD-guest system once the pH was adjusted to above 12.2, indicating that the 2:2 complexes were dissociated into two 1:1 complexes in such a pH range (i.e. > 12.2). The pH value of 12.2 for the dissociation of 2:2 complexes coincides with the pKa value of the secondary hydroxyl groups of β-CD, indicating that the electrostatic repulsion between the deprotonated secondary hydroxyl groups of two β-CDs plays a key role for the dissociation of 2:2 complexes at high pH. The circular dichroism results indicate that the longitudinal axis of the guest molecule is parallel to the symmetry axis of β-CD. Moreover, the positions of the guest molecules with respect to the β-CD cavity are identical with each other for the 1:1 and 2:2 complexes, indicating that the guest molecules in the 2:2 complexes may not interact with each other when in the ground state.

1.1.2.2 The chiral recognition of guest with β-CD

Cyclodextrins, which are chiral, can exhibit different binding affinity to the enantiomers of the guest molecules. Therefore, one of the most important application for
CD and its derivatives is their use as the chiral selectors in separation science.\textsuperscript{111,142,143} $\beta$-CD is the most common choice for the chiral recognition among all parent CDs, which may be due to the proper size of $\beta$-CD cavity for guests (e.g. phenyl or naphthyl compounds) and the cheap price of $\beta$-CD.\textsuperscript{144} The mechanisms for the chiral recognition of the enantiomeric guests by $\beta$-CD were summarized by Kano et al.\textsuperscript{122,145} The main mechanism was the lock-and-key mechanism, i.e. one guest molecule could be complementary to the $\beta$-CD cavity in size, shape and charge distribution to a better extent than its enantiomer, which leads to the higher binding affinity of $\beta$-CD to the former guest than to the latter guest. It is worth noticing that the lock-and-key mechanism is valid not only for the total inclusion of the guest molecule in the $\beta$-CD cavity but also for the partial inclusion of the guest molecule.\textsuperscript{146} The point interactions between guest and $\beta$-CD through some interaction forces (e.g. hydrogen bonding, Coulomb forces or coordinative bonding) could also lead to chiral recognition. For example, the hydrophobic moieties of the guest could cover the portal of $\beta$-CD, leading to the desolvation of the secondary hydroxyl groups of $\beta$-CD. In this case, the hydrogen bonding between the guest and $\beta$-CD can be the main driving force for the $\beta$-CD-guest complexation. Moreover, the chiral recognition can be observed if one of the enantiomeric guests could cover the portal of $\beta$-CD more efficiently than the other.

$\beta$-CD has a low ability to chirally recognize the guests with a central chirality but has quite strong ability to chirally recognize the guests with axial chirality.\textsuperscript{142,146-150} This observation is probably due to the fact that $\beta$-CD is quite flexible and can change its shape according to the shape of guest molecule inside the $\beta$-CD cavity.\textsuperscript{144} Consequently,
the \( \beta \)-CD-guest complementarity for one guest may not be very different from that for its enantiomer. On the other hand, the flexibility of \( \beta \)-CD, as well as many different chiral recognition sites in each \( \beta \)-CD molecule, lead to the broad chiral recognition capability of \( \beta \)-CD, i.e. \( \beta \)-CD can chirally recognize a wide range of enantiomeric guests.\(^{142,145,151,152}\)

### 1.1.3 The octa acid host-guest system

In the early 2000’s, Gibb’s group synthesized a series of deep-cavity cavitands by using calix[4]resorcinarenes as templates.\(^{153-156}\) These cavitands represent hydrophobic cavities which can associate with guest molecules by noncovalent interactions (e.g. the hydrophobic effect and the hydrogen bonding).\(^{153,157-159}\) However, the insolubility of these cavitands in water limits the capacity of these cavitands as a host system in supramolecular chemistry. In 2004, Gibb’s group reported a new water-soluble cavitand,\(^{160}\) which has become a very popular cavitand from then on.

Scheme 1.5. Structure for octa acid
The water-soluble cavitand was named as octa acid (or octaacid, OA, Scheme 1.5) since it contains eight carboxylic acid groups.\textsuperscript{161,162} The cavity of OA is 1 nm wide and 1 nm long. The hydrodynamic volume of the OA molecule is ca. 7200 Å\textsuperscript{3}.\textsuperscript{163} The wall of the cavity of OA consists twelve aromatic rings, leading to the strong hydrophobicity of the cavity of OA.

The solubility of OA in neutral water is very low (ca. tens of micromolar). However, in basic solution, the carboxylic acid groups on the outer surface of OA can be deprotonated, leading to the enhancement of the solubility of OA. Consequently, most of the binding studies on the OA system were conducted in 10 mM sodium tetraborate buffer at pH 8.9.\textsuperscript{160} It is worth noticing that OA exist as monomers at millimolar concentrations but starts to form aggregates at the higher concentrations (> 5 mM). Moreover, the aggregation process of OA can be tuned by the addition of the inorganic salts and the guest molecules. For example, the presence of guest deaggregates the OA aggregates while the addition of excess sodium tetraborate (50 mM) led to the aggregation of OA at the concentration of 1 mM.\textsuperscript{164}

OA can include a variety of hydrophobic molecules e.g. hydrocarbon guests from butane to octadecane,\textsuperscript{163,165} steroids,\textsuperscript{161} methyl viologen,\textsuperscript{166} adamantane\textsuperscript{164} and the polyaromatic compounds.\textsuperscript{167,168} Furthermore, two OAs can dimerize with each other to form a supramolecular capsule. The dimerization of OA is induced by the presence of the guest molecules that can be included in the OA cavity.\textsuperscript{163} The driving force for the dimerization of OA is the hydrophobic effect between the aromatic rings at the entrance of each OA cavity. The OA capsule is ca. 1 nm wide and 2 nm long, with a hydrodynamic volume of 1.49 × 10\textsuperscript{4} Å\textsuperscript{3}.\textsuperscript{163} The cavity size of the OA capsule is quite
Therefore, the OA capsule can bind to a wide range of guest molecules to form OA-guest 1:1, 2:1 or 2:2 complexes. The type of complexes is mainly dependent on the sizes, the hydrophobicities or the polarities of the guests.\textsuperscript{164,165,167,169,170} For example, the smaller straight-chain alkanes (i.e. butane – heptane) can associate with OA to form OA-alkane 2:2 complexes, while the larger straight-chain alkanes (i.e. octane – heptadecane) can associate with OA to form OA-alkane 2:1 complexes. Furthermore, octadecane is too large to be included into OA to form a stable complex.\textsuperscript{165} Another example is that the cyclic and acyclic carboxylic acids, i.e. the amphiphilic guest molecules, associate with OA to form the 1:1 complex rather than the 2:2 complex.\textsuperscript{169}

Gibb’s group studied the association between steroids and OA by NMR spectroscopy.\textsuperscript{161} The NOESY results for the OA-estradiol complex indicated that two OA molecules formed a dimer by the intermolecular interaction between the wide hydrophobic rims of the OA cavity, and the guest molecules were located in the cavity. The overall equilibrium binding constant for the OA-estradiol complex is higher than $1 \times 10^8$ M$^{-1}$. Moreover, the overall equilibrium binding constants of OA binding to other steroids were determined. Results show that the binding affinities of OA to the steroids vary according to the shape and polarity of the steroids.

Kaifer’s group\textsuperscript{166} studied the interaction between OA and a neutral (ferrocene) or a charged guest molecule (butyl viologen). The NMR results suggest that ferrocene moves into the OA cavity to form a OA-ferrocene 2:1 complex. Moreover, the diffusion coefficients for 1 mM OA in 10 mM sodium tetraborate solution are very different in the absence and presence of ferrocene, indicating that (i) OA (1 mM) does not dimerize in the absence of guest molecules and (ii) the guest bound inside the OA cavity induces the
dimerization of OA. Moreover, the results indicate that the addition of NaCl (40 mM) can induce the dimerization of OA in the absence of guest. The NMR proton signals of OA do not change in the absence and presence of butyl viologen, while the diffusion coefficients for OA are very different in the absence and presence of butyl viologen. These observations indicate that butyl viologen does not move into the OA cavity but associates with the carboxylate moieties on the outer surface of OA.

A few equilibrium binding constants for the OA-guest complex were reported up to the present. Gibb’s group determined the overall equilibrium binding constant for the formation of the 2:2 OA-butane complex. The value of the equilibrium binding constant of the 2:2 OA-butane complex, e.g. $1.4 \times 10^3 \text{ M}^{-1}$ in the presence of 1 mM Na$^+$, $3 \times 10^4 \text{ M}^{-1}$ in the presence of 5 mM NaCl and $1 \times 10^5 \text{ M}^{-1}$ in the presence of 14 mM NaCl, increases dramatically with the addition of NaCl. Gibb’s group studied the binding between OA and a series of acyclic and cyclic aliphatic carboxylic acids. The formation of the OA-guest 1:1 complexes is indicated from the results. Moreover, the standard molar heat-capacity changes for the OA-guest complex are proportional to the accessible surface areas of the guests, suggesting that the complexation process between OA and these carboxylic acids are mainly driven by the hydrophobic effect.

One of the most interesting features for OA as a host system is that the OA capsule can be employed as a nano-scale reaction container. Moreover, the local environment in the OA capsule, which is hydrophobic, dry (i.e. with only 4 or 5 water molecules in each OA capsule on average), leak-proof and confined, is very different from the aqueous phase. Therefore, the reaction pathway for the guest inside the OA capsule can be modified by the OA capsule. For example, anthracene in water can dimerize with each other
easily after irradiation. Comparatively, anthracene in the OA capsule associates with each other to form a excimer instead of a dimer even after irradiation for long time (> 10 hours).\textsuperscript{172}

1.1.4 Other host systems

The cucurbit[n]urils, cyclodextrins and cavitand octa acid are well-defined oligomolecular host systems,\textsuperscript{1} which associate with guest molecules to generate the small host-guest units, such as the host-guest complexes with the 1:1, 2:1, 1:2 and 2:2 host-guest stoichiometries. Moreover, there are other types of host systems, e.g. the polymolecular host systems,\textsuperscript{1} formed by the aggregation of numerous molecular assemblies. The polymolecular host systems, e.g. the detergent micelles and the bile salt aggregates, can associate with the guest molecules with the undefined stoichiometries.

The ordinary detergents (e.g. sodium lauryl sulfate) contain the well-defined hydrophobic tails and the well-defined hydrophilic heads. The micelle sizes, the aggregation numbers of detergents and the critical micelle concentrations are well-defined for the formation of the detergent micelles. For example, the micelles of sodium lauryl sulfate with the aggregation number of 50 are spontaneously formed in water at 25 $^\circ$C, as the concentration of the sodium lauryl sulfate is higher than 8.32 mM.\textsuperscript{177,178}

Bile salts are 24-carbon-5β-cholanates, consisting of a rigid moiety (the steroid moiety) and a flexible moiety (the side chain). The hydrophilic moiety and the hydrophobic moiety of the bile salt molecules are not as well-defined. The hydroxyl groups on the steroid moiety and the carboxyl group on the side chain define the hydrophilic parts of the bile salts, while the steroid moiety on the face opposite to the one containing the
hydroxyl groups defines the hydrophobic parts of the bile salts. The number, the location and the orientation of the hydroxyl groups are essential to the properties of the bile salt aggregates, such as the aggregation number, aggregate size and the way how the bile salt monomers pack in the aggregates. According to the number of the hydroxyl groups on the steroid moiety, the bile salts can be grouped as the monohydroxy bile salts, the dihydroxy bile salts and the trihydroxy bile salts. The carboxylate group on the side chain of the bile salt molecule can be conjugated with amino acids such as taurine and glycine.

The structure of the aggregates of bile salts is different from the detergent micelles. Bile salts can form small aggregates (e.g. dimers, trimers and tetramers) and large aggregates (e.g. helical structures and liquid crystals). The aggregation number for bile salt aggregates are not well-defined and vary depending on the experimental condition used (e.g. pH, the ionic strength and the temperature) and the molecular structure of bile salts. Three models have been employed to describe the bile salt aggregates: (i) the primary-secondary aggregate model,\textsuperscript{179} (ii) the disk-like aggregate model\textsuperscript{180} and (iii) the helical aggregate model\textsuperscript{181}. The primary-secondary aggregation model illustrates that the hydrophobic moieties of the bile salt monomers can bind with each other through the hydrophobic interactions to form the primary aggregates. Furthermore, the primary aggregates can interact with each other by hydrogen bonding to build a loose and less hydrophobic structure (i.e. the secondary aggregates), as the concentration of bile salt increases. The disk-like aggregate model represents that bile salt aggregates are generated by retaining the hydrophobic parts of the bile salt monomers inside the aggregates and exposing the hydrophilic parts on the outer surface of the aggregates. In another words, the carboxylate groups in the alkyl chains of bile salts, which associate with the co-
cations in the solution, are oriented toward the top or the bottom of the aggregates. In the helical aggregate model, the hydrophilic parts of the bile salt molecules are oriented toward the axis of helix, and hydrophobic parts point toward the outside of the aggregates. All of these models, i.e. the primary-secondary aggregates model\textsuperscript{181-193} the disk-like aggregate model\textsuperscript{180} and the helical aggregate model\textsuperscript{194-196}, are supported by the experimental results. The difference in the structures of the bile salts aggregates may be due to different intermolecular interactions predominant in the bile salt systems. In the primary-secondary aggregate model, the hydrophobic interaction between the bile salts induces the formation of the primary aggregates while the hydrogen bonding between the primary aggregates induces the formation of the secondary aggregates. In the disk-like aggregate model, the hydrophobic interaction between the bile salts as well as the ion-dipole interaction between bile salts and co-cations induces the formation of bile salt aggregation. In the helical aggregate model, the hydrogen bonding between the hydroxyl groups of bile salts as well as the ion-dipole interaction between bile salts and co-cations induces the formation of the bile salt aggregates.

1.2 Dynamic study in supramolecular chemistry

One of the most important features of supramolecular systems is the dynamic feature of the supramolecules.\textsuperscript{1} The intermolecular interactions between the chemical components in the supramolecular systems are noncovalent and relatively weak compared to the covalent bond in the molecular systems. Therefore, the chemical components in supramolecular systems associate and dissociate with each other easily, leading to the high reversibility of supramolecular systems. As written by Jean-Marie Lehn, “indeed, supramolecular chemistry is intrinsically a dynamic chemistry in view of
the lability of the non-covalent interactions connecting the molecular components of a supramolecular entity.\(^2\) The information provided by dynamic studies cannot be inferred from thermodynamic studies or structural studies. Furthermore, the information provided by the dynamic study can be employed to infer or explain the results from the thermodynamic study and the structural study.

The dynamic study in the supramolecular chemistry has not been developed to the same extent as the thermodynamic study or the structural study. This situation may be due to fact that the complexity and the diversity of the supramolecular systems limit the equipment and the technique that can be employed. For example, the dissociation of the supramolecules can proceed with a very high rate constant (e.g. close to \(10^7\) s\(^{-1}\) for the β-CD-xanthone complex\(^{197}\)) or with a very low rate constant (e.g. lower than \(10^{-5}\) s\(^{-1}\) for the CB[7]-1,1′-bis(trimethylammoniomethyl)ferrocene complex\(^{93}\)).

In 1950’s, Manfred Eigen developed a method (i.e. “kinetic relaxation”, or called “chemical relaxation”) to study very fast reactions, e.g. the neutralization process of hydronium ion and hydroxide ion.\(^{198,199}\) In brief, the methodology of chemical relaxation studies includes perturbing a system at equilibrium, recording the relaxation process (i.e. the process of the system re-achieving the equilibrium) as the kinetic trace and analyzing the kinetic trace. Consequently, the association and dissociation rate constants for the relevant reactions in the system can be determined. The perturbation process, which must be faster than the relaxation process, can be achieved by changing temperature (i.e. “temperature jump”), changing pressure (i.e. “pressure jump”), changing the reagent concentration (i.e. “stopped flow”) or changing the energy state of the chemical species in the solutions in flash photolysis experiments.\(^{200}\)
In general, the binding process between the guest and the host molecules proceeds by two steps: (i) the formation of intermediates driven by the host-guest recognition according to the surface properties of the guest and the host molecules. (ii) the formation of the final host-guest complexes whose geometry and structural distribution are dependent on the dynamic properties of the complex.\(^{201}\) In the following sections, the dynamic studies for three host-guest systems (i.e. the CB\([n]\)-guest system, the \(\beta\)-CD-guest system and the OA-guest system) are presented. It is worth noticing that the number of papers related to the dynamic study is limited for the CB\([n]\)-guest system and the OA-guest system. This situation may be due to the fact that these two systems have been developed only for a few years.

1.2.1 The dynamic study for the cucurbit\([n]\)uril host-guest system

The dynamics for the formation of the CB\([6]\)-guests complexes were studied by Mock’s group using the alkylammonium ions and the arylammonium ions as the guest molecules.\(^{23}\) The experiments were conducted by displacing the guest bound inside of the CB\([6]\) cavity (i.e. the displaced agent) with another guest (i.e. the displacing agent) and the rate constants for the displacement process were determined. As the concentration of the displacing agent increased ten-fold, the rate constants for the displacement process were only slightly changed, suggesting that the replacement process proceeds by two steps instead of one step. The first step, i.e. the rate limiting step, is that the displaced guest dissociates from the CB cavity. The second step, which should be fast, is that the displacing guest moves into the CB cavity.

The binding of CB\([6]\) to the 4-methylbenzylammonium ion in the presence of cations were studied by Hoffmann \textit{et al.}\(^ {13}\) Their results show that the charged guest
molecule associates with CB[6] by two steps: (i) The charged moiety of the guest molecule associate with the portal of CB[6] while the hydrophobic moiety of the guest molecule stay in the aqueous phase. (ii) The hydrophobic moiety of the guest molecule moves into the CB[6] cavity. Moreover, the two-step model was developed by Nau’s group as the flip-flop mechanism, which will be discussed in Chapter 2.

The association and dissociation rate constants for the CB[6]-guest complexes were determined by using 4-amino-4′-nitroazobenzene as the guest molecule. Results showed that the association rate constants for the CB[6]-guest complexes decreased dramatically (i.e. from $10^4$ M$^{-1}$s$^{-1}$ to $10^2$ M$^{-1}$s$^{-1}$) with the addition of HCl (i.e. from 0.05 M to 2 M) while the dissociation rate constants for the CB[6]-guest complexes (ca. $1.6 \times 10^{-3}$ s$^{-1}$) did not change, suggesting that the association process of the CB[6]-guest complex is affected by the presence of co-cations while the dissociation process of the CB[6]-guest complex is not affected.

Kim’s group estimated the dissociation rate constant for the CB[7]-MV$^{2+}$ complex as $1.9 \times 10^{-2}$ s$^{-1}$. The dissociation rate constant is very low compared with that for the complexation of β-CD and reduced viologen derivatives (ca. $10^4$ s$^{-1}$), which may be attributed to the strong ion-dipole interaction between CB[7] and MV$^{2+}$ bound inside of the CB[7] cavity.

### 1.2.2 The dynamic study for the cyclodextrin host-guest system

The recognition of β-CD to the guest molecules is not specific and mainly due to the hydrophobic effect. The dynamics between β-CD and guest depend on the guest’s topology, electronic state, charge state, and polarity. Higher order complexes, i.e. with more than one β-CD molecule or guest molecule, have slower
dynamics. For example, the entry and exit rate constants for the 1:1 complex between 2-naphthyl-1-ethanol (NpOH) and β-CD were respectively $3 \times 10^8$ M$^{-1}$s$^{-1}$ and $1.8 \times 10^5$ s$^{-1}$. Comparatively, the dynamics for the β-CD-NpOH 2:2 complex were estimated to be much slower (ca. $1 \times 10^3$ s$^{-1}$).

The association and dissociation rate constants for the β-CD-guest complexes were determined by using a series of cationic phosphorescence detergents as the guest molecules. The association rate constants for the β-CD-guest complexes are very similar for all guests with the alkyl chains of different lengths, suggesting that the rate-limiting step for the association between β-CD and guest is the dehydration of the β-CD cavity and the guest molecules. The dissociation rate constants for the β-CD-guest complexes decrease with the increase of the lengths of the alkyl chains of the guests, suggesting that the increase of the hydrophobicity of the guest slows down the dissociation process for the β-CD-guest complexes.

The binding dynamics of xanthone with β-CD was studied in laser flash photolysis experiments. Results show that the binding of β-CD to the triplet-excited state of xanthone (i.e. the polarized xanthone) is weak (ca. 48 M$^{-1}$) compared to that to the ground state of xanthone (ca. $1.1 \times 10^3$ M$^{-1}$). Moreover, the dissociation rate constant for the complexation between the triplet-excited state of xanthone and β-CD, which is very high (ca. $8.4 \times 10^6$ s$^{-1}$), is similar with that for the complexation between the triplet-excited state of xanthone and other CD molecules (e.g. γ-CD and hydroxypropyl-β-CD). This observation indicates that the dissociation process for the complexes between the triplet-excited state of xanthone and different CD molecules is not affected by the structure of
CD molecules. Furthermore, the decrease of the polarity of the aqueous phase, which can be adjusted by the addition of alcohol, leads to the decrease of the dissociation rate constants for the complex between β-CD and the triplet-excited state of xanthone.\textsuperscript{213} These observations indicate that the interaction between the triplet-excited state of xanthone and the aqueous phase (e.g. the dipole-dipole interactions) may play a role in the dissociation process for the complexes between the triplet-excited state of xanthone and CD molecules.

The binding between β-CD and guest molecules with the same functional group but different molecular sizes were studied.\textsuperscript{202} All these guest molecules (i.e. flavone, chromone and xanthone) contain the same 4H-pyran-4-one moiety and have different molecular sizes. Results show that the smallest guest molecule, i.e. chromone, binds with β-CD with the highest association (ca. $3 \times 10^9$ M$^{-1}$s$^{-1}$) and the highest dissociation (ca. $2 \times 10^7$ s$^{-1}$) rate constants. While the largest guest molecule, i.e flavone, binds with β-CD with the smallest dissociation rate constant ($4.4 \times 10^6$ s$^{-1}$), which is probably due to the fact that the phenyl ring of flavone can be included deeply into the cavity of β-CD.

The oxidation of the anion form of ferrocenecarboxylic acid was studied in the presence of β-CD by the cyclic voltammetry and the uv-vis absorption spectroscopy.\textsuperscript{214} Results indicate that the oxidation of the guest located inside of the β-CD cavity proceeds by two steps, i.e. the guest is dissociated from the β-CD cavity firstly and is then oxidized in the aqueous phase. The dissociation rate constant and the equilibrium binding constant for the β-CD-guest complex was determined as $2.1 \times 10^4$ s$^{-1}$ and $2.2 \times 10^3$ M$^{-1}$ at 20 °C, respectively.
It is worth noticing that the studies on the chiral recognition of guests with β-CD are mainly to optimize the experimental conditions (e.g. temperature, solvent compositions, pH) or modifying the molecular structure of β-CD. As a result, a high recognition ability of the host molecule to the guest enantiomers can be achieved. There is no kinetic study reported for the chiral recognition of guest enantiomers with β-CD up to the present. However, the kinetic aspect of the chiral recognition process is very important for two reasons. Firstly, the efficient chiral recognition relies on the fast association and dissociation between CD and guest. Secondly, the kinetic study could provide useful information to help understand the mechanism of the chiral recognition process and to help design the functionalized CD.

1.2.3 The dynamic study for the cavitand octa acid host-guest system

The only two works related to the dynamic study on the OA-guest system were reported by Ramamurthy’s group. The triplet-excited state of dimethyl benzil located in the OA capsule was quenched by the singlet oxygen and the rate constants for the quenching process were determined. The value of the rate constants for the quenching process, which is close to $10^8 \text{ M}^{-1}\text{s}^{-1}$, is ten times smaller than the diffusion rate constant. This observation suggests that the process of the opening and closing the OA capsule may be the rate-limiting step in the whole dynamic quenching process of the triplet-excited state of dimethyl benzil by the singlet oxygen. The oxygen-quenching experiments were conducted for nine guest molecules (e.g. pyrene, xanthione and adamantanethione) in the absence and presence of OA. The excited guests with the lifetime of excited state shorter than 5 µs were not quenched by oxygen in the presence of OA. The excited guests with the lifetime of excited state longer than 5 µs were quenched
by oxygen and the oxygen-quenching rate constants for the excited guests bound to the OA capsule are all much smaller than those for the excited guests free in water. The authors proposed that the opening-closing process of the OA capsule, which did not occur within 5 µs, limited the oxygen-quenching process for the excited guest bound to the OA capsule. Moreover, the opening-closing process of the OA capsule is affected by the guest located inside the OA capsule.

### 1.3 Objectives

The objectives of this work is to study the dynamics of the host-guest complexation in three different host systems i.e CB[7]-guest system (Chapter 2), β-CD-guest system (Chapter 3) and OA-guest system (Chapter 4). Furthermore, the information provided by the dynamic study together with the information provided by the equilibrium binding study for each host-guest system were analyzed in association with the molecular structures of the host and the guest molecules and with the intermolecular interactions between the host and the guest molecules. For example, the dynamic study in the CB[7]-guest system is to explain the high binding affinity of CB[7] to the positive charged guest and to study the effect of co-cations on the complexation of CB[7] and the positive charged guest. The dynamic study in the β-CD-guest system is to explain the chiral recognition observed for the complexation of β-CD and guest enantiomers as 2:2 host-guest complex. The dynamic study in the OA-guest system is to explain the efficient protection provided by the OA capsule to the excited guest against being quenched.
2 The Complexation Mechanism For A Cucurbit[7]uril Host-Guest System

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2.1 Introduction

2.1.1 The role of co-cations in cucurbit[7]uril host-guest systems

The intriguing feature of cucurbiturils as host molecules is that cucurbituril molecules bind with guest molecules with high equilibrium binding constants\textsuperscript{21,33,69,79,220-223} For example, the binding affinities of adamantane derivatives (> 10\textsuperscript{12} M\textsuperscript{-1})\textsuperscript{69} or charged ferrocene derivatives (10\textsuperscript{9} M\textsuperscript{-1} – 10\textsuperscript{13} M\textsuperscript{-1})\textsuperscript{74} to cucurbit[7]uril (CB[7]) are so high that these binding affinities are comparable to the binding affinities of avidin to biotin (10\textsuperscript{15} M\textsuperscript{-1})\textsuperscript{224} However, cucurbituril molecules are not very soluble in aqueous solution,\textsuperscript{223} e.g. the solubility of CB[7] in water is ca. 20 mM – 30 mM\textsuperscript{69} Therefore, cucurbituril molecules were solubilised with the addition of co-cations, e.g. hydronium ion or metal cations, to ensure that the complexation processes in the cucurbituril/guest systems could be studied. Several scientists have noticed that the equilibrium constants for the cucurbituril-guest complex were underestimated in the presence of co-cations due to the competition between guest molecules and co-cations for the binding sites of cucurbituril molecules.\textsuperscript{21,27,83,89,220,222,225,226} For example, the equilibrium binding constant of the CB[7]-acetophenone complex decreased from 9600 M\textsuperscript{-1} in the absence of co-cations to 1350 M\textsuperscript{-1} and 350 M\textsuperscript{-1} in the presence of 0.2 M Na\textsuperscript{+} and 0.2 M K\textsuperscript{+}, respectively;\textsuperscript{222} the equilibrium
constant for the complexation of the protonated acridine orange and CB[7] decreased from $2.0 \times 10^5 \text{ M}^{-1}$ in the absence of Na$^+$ to $6.7 \times 10^3 \text{ M}^{-1}$ in the presence of 1 M Na$^+$.\textsuperscript{225} The equilibrium binding constant of the CB[7]-methyl viologen complex decreased from $2.24 \times 10^5 \text{ M}^{-1}$ in the presence of 0.03 M Tris buffer to $1.67 \times 10^5 \text{ M}^{-1}$ in the presence of 0.01 M Na$^+$, and kept decreasing to $2.49 \times 10^4 \text{ M}^{-1}$ with the addition of Na$^+$ up to 0.2 M.\textsuperscript{83}

The equilibrium constants for cucurbiturils binding with co-cations are required to be known to recover the equilibrium binding constants of the cucurbituril-guest complexes in the presence of co-cation. The binding between cucurbiturils and co-cations have been studied by a few research groups and results show that the cucurbituril molecules can bind with one or two co-cations.\textsuperscript{12,13,17,226-228} Nau’s group studied the binding behaviour of the protonated neutral red with CB[7] in the presence of Na$^+$.\textsuperscript{225} The equilibrium binding constant for the CB[7]-Na$^+$ complex was estimated as 80 M$^{-1}$ although the stoichiometries of the CB[7]-Na$^+$ complex were not determined. Biczok’s group studied the binding behaviour of protonated berberine with CB[7] in the presence of Na$^+$.\textsuperscript{226} The equilibrium binding constant for the CB[7]•Na$^+$ complex was determined as 120 M$^{-1}$ while the equilibrium constant for Na$^+$ binding to CB[7]•Na$^+$ was determined as 11 M$^{-1}$. Kim’s group determined the pK$a$ value of CB[7] as 2.2.\textsuperscript{227} However, this pK$a$ value was not assigned to the association of the first or the second hydronium cation with CB[7]. Mezzina’s group determined the equilibrium binding constants for the CB[7]•K$^+$ complex by employing nitroxide radicals (e.g. 2,2,6,6-tetramethyl piperidine-N-oxyl) as probes in EPR experiments.\textsuperscript{228} The equilibrium binding constant for the CB[7]•K$^+$ complex was determined as 600 M$^{-1}$ while the equilibrium constant for K$^+$ binding to CB[7]•K$^+$ was determined as 53 M$^{-1}$. 
2.1.2 The complexation dynamics of the cucurbituril-guest system

Most studies with cucurbiturils focused on thermodynamic aspects, such as the determination of the equilibrium binding constants of cucurbiturils to guest molecules, how the properties of the guests (e.g. polarity, charge, size) affect the equilibrium constants and how the properties of guests are modified when incorporated into the host. Few research groups studied the binding dynamics for the cucurbituril-guest system. These authors observed that the binding of guests to CB[6] were affected by the charge states, the sizes and the functional groups of guests.

The guests with different charge states but with same sizes bind to CB[6] by following different mechanisms. Large charged guests associate with CB[6] by following a flip-flop mechanism. In the first step, the charged group of the guest molecule binds to ureido-carbonyl rim of CB[6] while the organic moiety of the guest molecule still stays in the solvent, i.e. a exclusion complex (CB[6]•guest) is formed. In the second step, the charged group of the guest molecule binds to ureido-carbonyl rim of CB[6] while the organic moiety of the guest molecule is desolvated and moves into the cavity of CB[6], i.e. the inclusion complex (guest@CB[6]) is formed. In contrast, neutral guests with similar size directly move into the cavity of CB[6] to form a inclusion complex with a much larger association rate constant. For example, the association rate constants for the cyclohexylmethylammonium cation binding to CB[6] were ca. $4 \times 10^{-4} - 10 \times 10^{-4} \text{ M}^{-1}\text{s}^{-1}$ while those for the unprotonated state of cyclohexylmethylamine binding to CB[6] were ca. $50 \times 10^{-4} \text{ M}^{-1}\text{s}^{-1} - 150 \times 10^{-4} \text{ M}^{-1}\text{s}^{-1}$. Moreover, the dissociation rate constants for the cyclohexylmethylammonium cation binding to CB[6] ($3 \times 10^{-6} \text{ s}^{-1} - 6 \times 10^{-6} \text{ s}^{-1}$) were
much lower than those for the unprotonated state of cyclohexylmethylamine binding to CB[6] (e.g. $1100 \times 10^{-6}$ s$^{-1}$ at pH 12.2). These observations indicate that the ion-dipole interactions between the charged guest and CB[6] decelerate not only the association process but also the dissociation process for the guest-CB[6] complexation. The equilibrium binding constants of the cyclohexylmethylammonium cation to CB[6] (ca. 170 M$^{-1}$) were higher than those of the unprotonated state of cyclohexylmethylamine to CB[6] (ca. 10 M$^{-1}$).

The dynamic process between CB[6] and guests with the same functional group but with different molecular sizes were studied. The results indicate that the association and dissociation rate constants for the guest-CB[6] complexes decreased with the increase of the guest size. For example, the association and dissociation rate constants for the guest-CB[6] complexes were respectively listed as follows: $> 1 \times 10^6$ M$^{-1}$s$^{-1}$ and $> 1 \times 10^2$ s$^{-1}$ for cyclopropylmethylammonium,$^{65}$ 5.9 $\times$ 10$^5$ M$^{-1}$s$^{-1}$ and 1.6 $\times$ 10$^2$ s$^{-1}$ for cyclobutylmethylammonium,$^{65}$ 5.5 M$^{-1}$s$^{-1}$ and 1.6 $\times$ 10$^5$ s$^{-1}$ for cyclopentylmethylammonium,$^{65}$ and 8.8 $\times$ 10$^4$ M$^{-1}$s$^{-1}$ and 1.1 $\times$ 10$^5$ s$^{-1}$ for cyclohexylmethylammonium.$^{26}$ Moreover, the results show that the equilibrium binding constants of the guest-CB[6] complexes for cyclobutylmethylammonium ($3.7 \times 10^5$ M$^{-1}$)$^{65}$ and the cyclopentylmethylammonium ($3.3 \times 10^5$ M$^{-1}$)$^{65}$ were much higher than those for cyclopropylmethylammonium ($1.5 \times 10^4$ M$^{-1}$)$^{65}$ and for cyclohexylmethylammonium (80 M$^{-1}$)$^{26}$. This observation is probably due to the fact that cyclobutylmethylammonium and cyclopentylmethylammonium fit into the cavity of CB[6] well while the other two guest cations are either too small (cyclopropylmethylammonium) or too large (cyclohexylmethylammonium) for the cavity of CB[6]. It is worth noticing that only the
cyclohexylmethylammonium bind to CB[6] by following the flip-flop mechanism. This observation may be due to the size mismatch between the cyclohexyl moiety of guest and the portal of CB[6]. The studies on the guest-CB[7] binding dynamics have not been reported yet.

2.1.3 Objectives

The first objective of this work was to study the binding dynamics of a charged guest with CB[7] to provide mechanistic insights into the high binding affinities of charged guests to CB[7]. In this work, the charged guest employed is 2-naphthyl-1-ethylammonium cation (NpAmH⁺). The inclusion complex (NpAmH⁺@CB[7]), where the naphthyl moiety of NpAmH⁺ is located inside the cavity of CB[7] and the ethyl ammonium moiety associates with the ureido-carbonyl rim of CB[7], was expected to be formed in the CB[7]-guest system.

![Scheme 2.1. Structure for the 2-naphthyl-1-ethylammonium cation](image)

The second objective of this work was to study the competitive binding with CB[7] of NpAmH⁺ and of co-cations (M⁺, e.g. H₃O⁺ or Na⁺), which could lead to the better understanding on the role of co-cations in the cucurbiturils-guest system. The role of co-cations in the cucurbituril-guest system is an essential topic since co-cations, employed as solubilizers for cucurbituril molecules, are always present in the cucurbituril-guest systems. The kinetic scheme with all relevant reactions for the NpAmH⁺/CB[7]/co-cations system is shown in Scheme 2.2.
Scheme 2.2. Reactions for the binding of NpAmH$^+$ with CB[7] in the presence of co-cation (M$^+$, which represents H$_3$O$^+$ or Na$^+$ in this study). The symbol “•” signifies the binding of a cation to the portal of the CB[7], while the symbol “@” signifies the formation of an inclusion complex.
2.2 Experimental Section

2.2.1 Instrumentation

UV-vis absorption spectra were recorded by using a Varian Cary 1 spectrophotometer at room temperature. A PTI QM-2 fluorimeter was employed to measure steady-state fluorescence spectra. The excitation wavelength was set to 280 nm. The emission light was recorded in the wavelength range of 300 to 550 nm. The excitation and emission slit bandwidths on the monochromators were set to 2 nm. Control experiments were performed by collecting the emission spectra of solutions containing all chemicals with the exception of NpAmH+. This baseline spectrum contains the Raman peak for water and a very small emission from CB[7] (≤ 15% of the Raman peak for the solution at a concentration of CB[7] at 25 µM). All fluorescence spectra were corrected by subtracting the baseline spectrum.

Time-resolved fluorescence decays were measured with an Edinburgh OB 920 single photon counting (SPC) system. The excitation source was a 277 nm pulsed light emitting diode (EPLED280 – Edinburgh instruments Ltd.). The bandpass for the emission monochromator was ca. 16 nm (2 mm slits). The stop counts were set to 10,000, and the accumulation of data stopped when the highest counts at a channel reached 10,000. The instrument response function (IRF) was collected with a Ludox (Aldrich) solution by scattering light at the wavelength used for the collection of the emission of NpAmH+. The IRF was used to deconvolute the lamp profile from the fluorescence decays. Quartz cells with dimensions of 10 mm × 10 mm or 10 mm × 2 mm were employed for steady-state and time-resolved fluorescence experiments. The samples for both experiments were
thermostated before data collection at 20.0 ± 0.1 °C for at least 15 min using a circulating water bath.

A SX20 stopped flow system (Applied Photophysics) was employed to measure the kinetics for the formation of the NpAmH⁺-CB[7] complex using the fluorescence detection mode. The excitation wavelength was 280 nm with a monochromator slit bandwidth of 4.65 nm (1 mm slits). The emission was collected at 90 degrees with respect to the excitation beam via two kinds of channels. One channel is the monochromator channel, i.e. the fluorescence signal was collected at 330 nm using a monochromator with a slit bandwidth of 37.2 nm (8 mm slits). The other channel is the cut-off filter channel, i.e. the fluorescence signal was collected with a 310 nm cut-off filter to increase the sensitivity. The samples contained in the two injection syringes were thermostated for at least 15 min at 20 °C. A mixing ratio of 1:1 for the NpAmH⁺ and CB[7] solutions was employed. Therefore, the final concentrations of NpAmH⁺ and CB[7] were half of the concentrations prepared for the solutions contained in the syringes. The concentrations of chemicals mentioned below are the final concentrations of chemicals unless otherwise stated. The detection photomultiplier (PMT) voltage was adjusted so that the highest expected emission intensity was at 6 V or lower. The same photomultiplier voltage was used throughout one experiment to ensure that intensities at different concentrations of NpAmH⁺ or CB[7] can be compared. At least 20 kinetic traces were averaged to increase the signal-to-noise ratio. The following control experiments were performed: (i) Injection of water against water to determine the zero intensity value for the system to be subtracted from intensity values for solutions containing NpAmH⁺ but not CB[7]. (ii) Injection of CB[7] against water to determine the baseline value to be
subtracted from all intensities measured for solutions containing CB[7]. The values for control experiments (i) and (ii) are very similar and are less than 0.5% of the intensities measured for the 1 µM NpAmH⁺ emission. (iii) Injection of NpAmH⁺ against water to determine the emission intensity of NpAmH⁺ in the absence of CB[7]. For these control experiments all other experimental conditions, such as the concentrations of H₃O⁺ and Na⁺, were kept constant in both syringes.

2.2.2 Materials

CB[7] samples (\((\text{C}_{42}\text{H}_{42}\text{N}_{28}\text{O}_{14})\cdot (2\text{HCl})\cdot (18\text{H}_2\text{O})\), lots Y121-CB[7], 73% and (\((\text{C}_{42}\text{H}_{42}\text{N}_{28}\text{O}_{14})\cdot (9.4\text{H}_2\text{O})\), lots Q469GA/C, > 99%) were synthesized by Kim’s group as previously described.²⁹,⁹³ The purities of CB[7] samples were checked by steady-state fluorescence experiments (see Section 2.2.6 below for more details). CB[7] samples, R- (+)-2-naphthyl-1-ethylamine (NpAm, Fluka, ≥ 99%), NaCl (BDH, > 99%), standardized volumetric solution of hydrochloric acid (Anachemia, A.C.S. reagent grade), standardized volumetric solution of sodium hydroxide (Anachemia, A.C.S. reagent grade), CB[6] (\((\text{C}_{36}\text{H}_{36}\text{N}_{24}\text{O}_{12})\cdot (7\text{H}_2\text{O})\), Kim group, lots NS-II-66 CB[6]), ferrocene (\((\text{C}_{10}\text{H}_{10}\text{Fe})\), Fluka, ≥ 98%) and methanol (Caledon Laboratories, spectrgrade, > 99.8%) were used without further purification. NpAmH⁺ was checked for the presence of fluorescent impurities by measuring the fluorescence decay of a 10 µM aqueous solution of NpAmH⁺ at pH 3. The decay was mono-exponential indicating that no other emissive species were present in solution. Deionized water (Barnstead NANOpure deionizing systems ≥ 17.8 MΩ cm⁻¹) was employed for all samples.
2.2.3 Solution preparation

HCl (1.000 M) and NaOH (1.000 M) stock solutions were prepared by diluting the standardized volumetric solutions of hydrochloric acid and the standardized volumetric solutions of sodium hydroxide, respectively. NaCl (2.000 M), NpAm (625 μM) and CB[7] (800 μM) stock solutions were prepared by dissolving the appropriate amount of solid NaCl, NpAm and CB[7] in water, respectively. The solutions with lower concentrations of species (e.g. NpAm, CB[7], HCl, NaOH and NaCl) were prepared by injecting the corresponding stock solutions into water. The ferrocene stock solution (1mM) was prepared by dissolving the appropriate amount of solid ferrocene into methanol. Aerated samples were used for all experiments because the consistent deaeration for the stopped flow experiment is difficult to achieve leading to a larger uncertainty in the fluorophore’s emission intensity.

2.2.4 Time-resolved fluorescence

The fluorescence decays were fitted to a sum of exponentials (Equation 2.6) using the Edinburgh software (version 3, Edinburgh Instruments Ltd.) to determine the lifetimes ($\tau_i$) and the pre-exponential factors ($A_i$). The quality of each fit was judged by the residuals plot and the value of the reduced chi-squared parameter ($\chi^2$). The number of exponentials for each fit was increased until the residuals were random and the $\chi^2$ values were between 0.9 and 1.2.\textsuperscript{241}

\[
I_t = I_0 \times \sum_{i=1}^{n} \left[ A_i \times e^{-\frac{t}{\tau_i}} \right]
\]  
(2.6)
2.2.5 Data treatment for steady-state fluorescence experiments

Spectra were determined for solutions of NpAmH⁺ in the absence and presence of CB[7]. Spectra were corrected by subtracting a baseline which corresponds to a spectrum for a solution containing all chemicals with the exception of the fluorophore (NpAmH⁺). The correction was to separate the fluorescence emission of NpAmH⁺ from Raman emission and the fluorescence emission of impurities present in CB[7]. The corrected spectra were integrated between 315 nm and 350 nm and the integrated intensity for each spectrum ($I_{\text{flu}}^*$) was related to the instrumental settings, such as the excitation light intensity and the bandwidth. Therefore, the values of $I_{\text{flu}}^*$ can vary between experiments performed on different days. The values of $I_{\text{flu}}^*$ were divided by the integrated intensity for NpAmH⁺ in the absence of CB[7] ($I_{\text{flu},0}^*$) to determine the normalized intensity for each spectrum ($I_{\text{flu}}$). The values of $I_{\text{flu}}$, the normalized $I_{\text{flu}}^*$, are independent of the instrumental settings mentioned above.

2.2.6 Purity check for CB[7] samples

The purities of CB[7] samples were checked by studying the dependence of the fluorescence intensity ($I_{\text{flu}}^*$) determined for the NpAmH⁺/CB[7] system with the addition of ferrocene (Figure 2.1). The binding affinity of ferrocene with CB[7] is very high ($> 4 \times 10^5 \text{ M}^{-1}$). Moreover, the fluorescence emission efficiency for NpAmH⁺ free in water is lower than that for NpAmH⁺ in the complex (see Section 2.4.1 below). Therefore, as ferrocene is added into the NpAmH⁺/CB[7] solution, the competition between ferrocene and NpAmH⁺ for the cavity of CB[7] leads to the dissociation of NpAmH⁺/CB[7] complexes and the decrease of the fluorescence intensity is observed for the NpAmH⁺/CB[7]/ferrocene system. The fluorescence intensity keeps decreasing with the
addition of ferrocene until the breaking point is reached where all of NpAmH$^+$ in the CB[7] cavity is replaced by ferrocene. The concentration of ferrocene at the breaking point is equal to the actual concentration of CB[7] ([CB[7]]$_{bp}$). The purity of CB[7] can then be calculated as the ratio of [CB[7]]$_{bp}$ to the concentration of CB[7] prepared ([CB[7]]$_{pr}$).

![Graph](image)

Figure 2.1. The dependence of the fluorescence intensity (●) for the NpAmH$^+$/CB[7] solution with the addition of ferrocene. [NpAmH$^+$] = 10 µM, [CB[7]] = 10 µM and [H$_3$O$^+$] = 1 mM. The two solid lines correspond to the fits of the data to a linear function for each concentration range of ferrocene. [CB[7]]$_{bp}$ (9.55 µM) is equal to the concentration of ferrocene at the intersection of these two solid lines.

The purity for each CB[7] sample with a specific lot number was recovered from three independent experiments. The impurities could be water, HCl or other cucurbituril homologues such as cucurbit[5]uril or cucurbit[8]uril. However, there was no evidence that the impurities affected the binding process of guest-CB[7] complexation during the study.

2.2.7 Data treatment for stopped flow experiments

The stopped-flow traces were determined as the intensity change of the fluorescence emission over time. The stopped-flow traces provided the kinetic information of the
complexation between NpAmH\(^+\) and CB[7] as well as the information to determine the overall equilibrium binding constant of NpAmH\(^+\) to CB[7].

The stopped-flow traces were treated by two methods to obtain the kinetic information: (i) the exponential curve fitting method\(^{108}\) and (ii) the second-order global analysis method\(^{242}\). When the stopped-flow traces were studied by the exponential curve fitting method, the traces obtained for different concentrations of CB[7] were fitted with a mono-exponential function (n = 1) or with a function equal to a sum of exponentials (Equation 2.7) if necessary, where \(I_0\) is the initial intensity, \(I_{\infty,i}\) and \(k_{obs,i}\) are respectively the pre-exponential factor and the relaxation rate constant (i.e. inverse of the relaxation time of the system) for each kinetic process \(i\). The quality of each fit was judged by the residuals plot. The number of exponentials for each fit was increased until the residuals were random. The dependence of \(k_{obs}\) on the concentration of CB[7] was obtained and fitted with the linearized rate equations. The linearized rate equations were derived from the reaction scheme proposed by the chemical relaxation method (see the section 2.3.2).

\[
I_i = I_0 + \sum_{i=1}^{\infty} I_{\infty,i} \left(1 - e^{-k_{obs,i}t}\right)
\]  

(2.7)

When the stopped-flow traces were studied by the second-order global analysis method, the traces determined for different concentrations of CB[7] and different concentrations of the co-cations were simultaneously fitted with the non-linear least-squares regression method using Pro-Kineticist II (Applied Photophysics Ltd). The fitting models were derived according to the reaction scheme proposed (see the section 2.3.1). The equilibrium and rate constants for the complexation process were the global parameters in the common fitting models. These global parameters were shared by all
traces during the fitting and were varied to minimize the sum of the chi-squared values for all traces.

The stopped-flow traces were treated by the following method to ensure that the traces determined on different days can be compared. The intensity of the fluorescence emission determined in the stopped flow experiments corresponds to the output voltage of PMT, which is related to the instrumental settings, such as the excitation light intensity, the voltage applied to the PMT and the bandwidth of the monochromators. Therefore, the kinetic traces determined on different days cannot be compared directly. The kinetic traces were corrected by subtracting a baseline which corresponds to the fluorescence intensity for a mixture containing all chemicals with the exception of the fluorophore (NpAmH+). The corrected kinetic traces were then divided by the corrected fluorescence intensity for NpAmH+ in the absence of CB[7], i.e. the corrected kinetic traces were normalized. The fluorescence intensities for each time point \( t \) in the normalized traces \( I_{\text{SF}}^t \) are independent of the instrumental settings and only depend on the emission efficiencies \( C_i \) of each species \( i \) and the concentration of the corresponding species \( ([i]) \).

Another data treatment for the normalized stopped-flow traces was employed to determine the overall equilibrium binding constant for the NpAmH+/CB[7] complex. The normalized fluorescence intensities for the solutions at the equilibrium state \( I_{\text{SF}}^{\text{eq}} \) were determined by fitting the levelling regions of the normalized traces into a horizontal line. The values of \( I_{\text{SF}}^{\text{eq}} \) are independent of the instrumental settings and only depend on the emission efficiency \( C_i \) of each species \( i \) and the concentration of the corresponding species \( ([i]) \).
2.2.8 Binding isotherms for 1:1 complexes

The dependence of $I_{\text{SF}}^{\text{eq}}$ or $I_{\text{Flu}}$ on the CB[7] concentration was fitted numerically using Scientist 3 from Micromath (see Section 2.4.3). Briefly, an equation is defined for the measured variable, i.e. the normalized fluorescence intensity ($I_{\text{SF}}^{\text{eq}}$ or $I_{\text{Flu}}$), as a function of emission efficiencies ($C_{\text{Np}}$ and $C_{11}$) and concentration of NpAmH$^+$ in the aqueous phase ([G]) or bound to CB[7] ([HG]). The emission efficiencies ($C_{i}^{0}$) are related to the fluorescence quantum yields for each species and instrumental settings, such as the excitation light intensity and bandwidth of the monochromators. The magnitude for the $C_{i}^{0}$ values can vary between experiments performed on different days. However, relative $C_{i}$ values, the ratios of $C_{i}^{0}$ to $C_{\text{Np}}^{0}$, are constant for each NpAmH$^+$ species. The emission efficiencies discussed below are the relative emission efficiencies unless otherwise stated.

The relationship between the concentrations of various NpAmH$^+$ and CB[7] species is defined by the equilibrium binding constant for the 1:1 complex and the mass balance equations. The equilibrium binding constant for the 1:1 complex determined by this procedure ($\beta_{11}$) corresponds to the overall equilibrium binding constant for the binding of NpAmH$^+$ to CB[7] in the presence of co-cations. (See Appendix a.1).

The model employed in Scientist 3 (Model 1) is listed as follows:

The total concentration of CB[7] ([H]) is defined as the independent variable. The equilibrium concentration of free host ([H]), free guest ([G]), 1:1 complex ([HG]) and the normalized fluorescence emission intensity ($I_{\text{mon}}$, which represents $I_{\text{SF}}^{\text{eq}}$ or $I_{\text{Flu}}$) are defined as the dependent variables. The emission efficiency for the 1:1 complex ($C_{11}$) and the overall equilibrium binding constant for the 1:1 complex ($\beta_{11}$) are defined as the parameters in the model. $R$, the ratio of the normalized fluorescence emission intensity
\( (I_{\text{mon,0}}) \) of NpAmH\(^+\) in the absence of CB[7] to the total concentration of NpAmH\(^+\) ([G]_T), is a constant for each independent experiment.

Equations for Model 1 are listed as follows:

\[
\begin{align*}
[HG] &= \beta_{11} \times [G] \times [H] \\
[H] &= [H]_T - [HG] \\
I_{\text{Mon}} &= R \times ([G] + C_{11} \times [HG])
\end{align*}
\]

(2.8) \hspace{1cm} (2.9) \hspace{1cm} (2.10) \hspace{1cm} (2.11)

where \( R = \frac{I_{\text{mon,0}}}{[G]_T} \) \hspace{1cm} (2.12)

Ranges for the dependent variables are listed as follows:

\[
\begin{align*}
0 < [H] < [H]_T \\
0 < [G] < [G]_T \\
0 < [HG] < [G]_T
\end{align*}
\]

(2.13) \hspace{1cm} (2.14) \hspace{1cm} (2.15)

2.3 Data analysis

2.3.1 The simplified reaction model for the kinetic study in the NpAmH\(^+\)/CB[7]/M\(^+\) system

The model shown in Scheme 2.2 contains all possible reactions for the NpAmH\(^+\)/CB[7]/M\(^+\) system. This general model can be simplified for the kinetic study in the specific case where the binding of NpAmH\(^+\) (at the \( \mu \)M level) with CB[7] (at the \( \mu \)M level) is studied in the presence of the high concentration of M\(^+\) (from the mM level to the M level). Briefly, the presence of co-cations affects the concentration distribution of CB[7] species and lowers the concentration of CB[7] species which is available to bind with NpAmH\(^+\). Consequently, the reactions relating to the binding of CB[7] species to NpAmH\(^+\) are slow enough to be studied on the stopped flow time scale.
In this study, several models (Model 2 – 5) were proposed on the basis of the model shown in Scheme 2.2 and were employed in the kinetic study. Model 2 (Scheme 2.3) was experimentally proved to be the right model for the kinetic study in the NpAmH⁺/CB[7]/M⁺ system (see Section 2.4.6.1).

\[
\begin{align*}
\text{CB[7]} + \text{M}^+ & \rightleftharpoons_{K_{ij}}^{\text{CB[7]} \cdot \text{M}^+} \\
\text{CB[7]} \cdot \text{M}^+ + \text{M}^+ & \rightleftharpoons_{K_{ij}}^{\text{M}^+ \cdot \text{CB[7]} \cdot \text{M}^+} \\
\text{CB[7]} + \text{NpAmH}^+ & \rightleftharpoons_{\frac{k_{001}^+}{k_{001}}}^{\text{NpAmH}^+ \cdot \text{CB[7]}}
\end{align*}
\]  

(2.16)  
(2.17)  
(2.18)

Scheme 2.3. Mechanism for the binding of NpAmH⁺ with CB[7] in the presence of co-cations (Model 2, M = H₃O⁺ or Na⁺). The numbered subscripts correspond to the stoichiometries of the co-cation or guest bound to the CB[7] shown for the species on the right side of the equilibrium. From the left to the right the subscripts correspond to H₃O⁺ (i = 1 or 2; j = 0), Na⁺ (i = 0; j = 1 or 2) and NpAmH⁺. Any “zeros” not followed by an integer are not shown.

Three other models (Model 3 shown in Scheme 2.4, Model 4 shown in Scheme 2.5 and Model 5 shown in Scheme 2.6) were employed for the kinetic study in the NpAmH⁺/CB[7]/M⁺ system. However, these three models cannot explain some of the experimental data, indicating that these three models are not suitable to describe the complexation of CB[7] with co-cations and with guest molecules (see Sections 2.4.5.2 and 2.4.6.2).
Scheme 2.4. Mechanism for the binding of NpAmH$^+$ with CB[7] in the presence of co-cations (Model 3, M = H$_3$O$^+$ or Na$^+$). The numbered subscripts correspond to the stoichiometries of the co-cation or guest bound to the CB[7] shown for the species on the right side of the equilibrium. From the left to the right the subscripts correspond to H$_3$O$^+$ (i = 1 or 2; j = 0), Na$^+$ (i = 0; j = 1 or 2) and NpAmH$^+$. Any “zeros” not followed by an integer are not shown.

Scheme 2.5. Mechanism for the binding of NpAmH$^+$ with CB[7] in the presence of co-cations (Model 4, M = H$_3$O$^+$ or Na$^+$).

Scheme 2.6. Mechanism for the binding of NpAmH$^+$ with CB[7] in the presence of co-cations (Model 5, M = H$_3$O$^+$ or Na$^+$).
2.3.2 The relationship between the observed rate constants determined for the NpAmH⁺/CB[7]/M⁺ system by stopped flow experiments and [CB[7]]

The stopped flow experiments were conducted under the specific condition where one reaction was slow while the others were sufficiently fast to be viewed as pre-equilibrium processes (see Section 2.4.4 below for more details). Theoretically, two different trends can be predicted for the dependence of the observed rate constants ($k_{\text{obs}}$) with the concentrations of CB[7], as the slow reaction is assigned to different reactions in the proposed mechanism. Which assignment is correct can be checked by comparing the experimental data with the theoretical trends predicted for the dependence of $k_{\text{obs}}$ with the concentrations of CB[7].

(i). If the reaction for the binding between the CB[7] species and co-cations (e.g. Reaction 2.16 or 2.17 in Scheme 2.3) is slow on the stopped flow time scale while the reaction for the binding between the CB[7] species and NpAmH⁺ (e.g. Reaction 2.18 in Scheme 2.3) is sufficiently fast to be viewed as a pre-equilibrium process, $k_{\text{obs}}$ for the relaxation kinetics should be related to the formation of the CB[7]•M⁺ complex or the M⁺•CB[7]•M⁺ complex. In other words, $k_{\text{obs}}$ should be related to the association process, which is defined by the rate constant and the concentration of the reagents (M⁺ and the CB[7] species), and the dissociation process. Since the concentration of M⁺ (from the millimolar level to the molar level) is much higher than the concentration of CB[7] species (at the µM level), the reaction for the binding between the CB[7] species and co-cations can be treated as a pseudo first order reaction (e.g. Reaction 2.27). Therefore, the values of $k_{\text{obs}}$ should be independent of the concentrations of CB[7].

$$\text{H}_3\text{O}^+\cdot\text{CB}[7] \xrightleftharpoons[k_2 \cdot [\text{H}_3\text{O}^+]]{k_2} \text{H}_3\text{O}^+\cdot\text{CB}[7] \cdot \text{H}_3\text{O}^+$$

(2.27)
(ii). If the reactions for the complexation of the CB[7] species and co-cations are sufficiently fast to be viewed as pre-equilibrium processes while the reaction for the binding between the CB[7] species and NpAmH⁺ is slow on the stopped flow time scale, \( k_{\text{obs}} \) for the relaxation kinetics should be related to the complexation process between NpAmH⁺ and the CB[7] species. In other words, \( k_{\text{obs}} \) should be related to the association process, which is defined by the rate constant and the concentration of the reagents (NpAmH⁺ and the CB[7] species), and the dissociation process. Since the concentration of NpAmH⁺ (at the µM level) is lower than the concentration of CB[7] species (at the µM level), the values of \( k_{\text{obs}} \) should be dependent on the concentrations of CB[7].

### 2.4 Results

#### 2.4.1 Steady-state and time-resolved fluorescence

The pKₐ of NpAm is 7.6.²⁴³ The absorption spectrum of NpAm does not change when the concentration of the hydronium cation in the solution was varied from \( 10^{-11} \) M to \( 10^{-4} \) M (Figure 2.2). This result is important for the fluorescence experiments because the assumption can be made that the excitation efficiency for unprotonated NpAm and protonated NpAmH⁺ is the same. The fluorescence efficiency for NpAmH⁺ is much higher than for NpAm (a factors of ca. 7 at 330 nm) which can be explained by a well-established theory.²⁴⁴ The singlet-excited state fluorophore in the unprotonated NpAm can be quenched by the nitrogen lone pair, i.e. the electron donor, through a photoinduced electron transfer mechanism. Upon the protonation of the amine, the photoinduced electron transfer quenching is inhibited. In addition, the shape of the emission spectrum for NpAmH⁺ and NpAm are slightly different (Figure 2.3).
The fluorescence lifetime of NpAmH$^+$ (10 µM) was measured in the presence of 1 mM H$_3$O$^+$, where only the protonated NpAmH$^+$ was present. The fluorescence decay was mono-exponential and a lifetime of 35.8 ± 0.1 ns was determined according to three independent experiments. In aqueous solutions with no addition of HCl the decay for the fluorescence of NpAm did not follow a mono-exponential decay and was fitted to the sum of two exponentials, where the recovered lifetimes were 2.7 ns and 35.6 ns. Both NpAmH$^+$ and NpAm should be present in the solution at ca. neutral pH. Therefore, the lifetimes of 2.7 ns and 35.6 ns were assigned to the emission from NpAm and NpAmH$^+$, respectively. This result is consistent with the decrease in the steady-state emission intensity observed when NaOH was added to the aqueous solution of NpAmH$^+$ at pH 3.
Figure 2.3. Fluorescence spectra normalized at 331 nm for the aqueous solutions of 150 µM NpAmH⁺ (a, black, 100 µM HCl) and of 150 µM NpAm (b, red, 1 mM NaOH).

The fluorescence of NpAmH⁺ was enhanced in the presence of CB[7] (Figure 2.4). An artifact was observed when the intensity of the excitation beam was high and oxygen was present, which led to the slow formation over minutes to hours of an emitting species at longer wavelengths than observed for the emission of NpAmH⁺ (see Section 2.4.2 for details). All fluorescence and stopped flow experiments were performed at excitation intensities that did not lead to the red-shifted emission. In the presence of CB[7] the maximum close to 300 nm for the NpAmH⁺ fluorescence shifted by 1 to 2 nm to longer wavelengths and a change in shape was observed for the peak at the shorter wavelengths (inset Figure 2.4). The increase in the emission intensity and the change in the shape of the emission spectra support the formation of a complex between NpAmH⁺ and CB7 and indicate that the fluorescence emission efficiency for NpAmH⁺ in the complex is higher than that for NpAmH⁺ free in water. The fluorescence decay for 10 µM NpAmH⁺ in the presence of 18.3 µM CB[7] and 2 mM H₃O⁺ did follow a mono-exponential decay with a lifetime of 62.3 ± 0.4 ns according to two independent experiments. The lengthening of the lifetime is consistent with the increase in the emission intensity when NpAmH⁺ is
complexed to CB[7]. The mono-exponential decay suggests that at these concentrations of NpAmH\(^+\) and CB[7] all the NpAmH\(^+\) was bound to CB[7]. The fluorescence decay was also measured at a lower concentration of CB[7] (3.7 \(\mu\)M) and the decay did not follow a mono-exponential function. Fitting of the data to the sum of two exponentials recovered lifetimes of 36 ns and 63 ns, consistent with the presence of NpAmH\(^+\) in the aqueous phase and bound to CB[7].

![Fluorescence spectra](image)

Figure 2.4. Fluorescence spectra for NpAmH\(^+\) (50 \(\mu\)M) in the absence (a) or presence of CB[7] (b, 9.1 \(\mu\)M; c, 27.4 \(\mu\)M; d, 45.6 \(\mu\)M and e, 100.7 \(\mu\)M). [HCl] = 0.2 M. The inset shows an expanded region for the normalized spectra of NpAmH\(^+\) in the absence of CB[7] and presence of 100.7 \(\mu\)M of CB[7].

2.4.2 Artifacts observed in steady-state fluorescence experiments

The formation of complexes of NpAmH' with CB[7] led to the emission enhancement as shown in Figure 2.4. This emission enhancement was observed right after the injection of CB[7] into aerated NpAmH\(^+\) solutions. However, slow kinetics, which led to the decrease of the emission at 333 nm and the emission enhancement at 450 nm, were observed on the minute time-scale when the excitation slit bandwidths on the monochromators of the PTI QM-2 fluorimeter were set to 2 nm (Figure 2.5). The
emission enhancement at 450 nm indicated the formation of a product. No product formation was observed in the absence of CB[7] indicating that CB[7] was involved in the process of the formation of this product. For the short irradiation times the product formation was reversible while for the long irradiation times the formation of the product was permanent. The rate of the formation of this species depended on the photon flux and was absent (for at least 15 min) when the bandwidth for the excitation monochromator was narrowed to 0.5 nm.

Figure 2.5. Formation of a product which emits at 450 nm when the NpAmH⁺ (5 μM)/CB[7] (18.3 μM)/HCl (2 mM) solutions were continuously irradiated at 280 nm with a excitation monochromator bandwidth of 2 nm for: (a) 2 min, (b) 62 min, (c) 240 min, (d) 453 min, (e) 555 min.

The formation of this product was absent when oxygen was removed from the solution by bubbling the solution for 30 min with argon. This statement was supported by the observation that fluorescence spectra for the deaerated sample did not change before and after 30 min irradiation (the red and blue spectra in Figure 2.6) while fluorescence spectra for the aerated sample changed dramatically under the same experimental condition (the black and green spectra in Figure 2.6).
Figure 2.6. Fluorescence spectra for the NpAmH\(^{+}\) (60 µM)/CB[7] (219 µM)/HCl (1 mM) solution in the deaerated (red and blue) and aerated states (black and green) determined before (red and black) and after the irradiation of 30 minutes (blue and green).

2.4.3 Determination of the binding isotherms for the NpAmH\(^{+}@CB[7]\) complex at low concentration of Na\(^{+}\)

The emission spectra of 1 µM NpAmH\(^{+}\) in the absence and presence of CB[7] were determined in steady-state fluorescence experiments. The concentration of hydronium ion was maintained at 0.01 mM to keep the NpAm species protonated. The concentration of sodium cation was maintained at different concentrations (2, 4, 6 and 8 mM) and the corresponding binding isotherm was determined for each concentration of Na\(^{+}\) chosen.

The overall equilibrium binding constants (\(\beta_{11}\)) and the relative emission efficiencies (\(C_{11}\)) for the NpAmH\(^{+}@CB[7]\) complex were determined by the numerical fitting (see Section 2.2.8). The residuals between the fitting curve and the experimental data (e.g. the dots in bottom panels of Figure 2.7 and Figure 2.8) are randomly scattered around 0, indicating a good fit between the model and the data.
Figure 2.7. Binding isotherm for the complexation of NpAmH\(^+\) (1.0 µM) with CB[7] in the presence of 2 mM Na\(^+\) and 0.01 mM H\(_3\)O\(^+\). The fit of the 1:1 binding isotherm was obtained using Scientist and the residuals between the fitting curve (black curve) and the experimental data (red dots in the top panel) are shown in the bottom panel. The recovered \(\beta_{11}\) value is \((8.3 \pm 0.7) \times 10^6\) M\(^{-1}\).

Figure 2.8. Binding isotherm for the complexation of NpAmH\(^+\) (1.0 µM) with CB[7] in the presence of 8 mM Na\(^+\). The fit of the 1:1 binding isotherm was obtained using Scientist and the residuals between the fitting curve (black curve) and the experimental data (red dots in the top panel) are shown in the bottom panel. The recovered \(\beta_{11}\) value is \((5.1 \pm 0.5) \times 10^6\) M\(^{-1}\).

The values of \(C_{11}\) for the NpAmH\(^+\)@CB[7] complex in the presence of different concentrations of Na\(^+\) are similar (Table 2.1). The average value of \(C_{11}\) from six experiments is 1.362 ± 0.006. The values of \(\beta_{11}\) for the NpAmH\(^+\)@CB[7] complex (Table
2.1) decrease with the increase of concentration of Na\(^+\), which coincides with the trend predicted by Equation 2.28 (See Appendix a.1 for the mathematical transformation processes for Equation 2.28).

<table>
<thead>
<tr>
<th>[Na(^+)] / mM</th>
<th>(\beta_{11} / 10^6) M(^{-1})</th>
<th>(C_{11})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>8.3 ± 0.6</td>
<td>1.363</td>
</tr>
<tr>
<td>2</td>
<td>8.3 ± 0.7</td>
<td>1.363</td>
</tr>
<tr>
<td>4</td>
<td>7.2 ± 0.7</td>
<td>1.361</td>
</tr>
<tr>
<td>6</td>
<td>6.2 ± 0.6</td>
<td>1.362</td>
</tr>
<tr>
<td>8</td>
<td>5.1 ± 0.5</td>
<td>1.369</td>
</tr>
<tr>
<td>8</td>
<td>5.3 ± 0.6</td>
<td>1.351</td>
</tr>
</tbody>
</table>

\(^a\)values and errors of \(\beta_{11}\) and \(C_{11}\) were recovered by fitting the dependence of fluorescence intensity with the concentration of CB[7] into Model 1 in Scientist 3; the errors of \(C_{11}\) were less than 0.005; [H\(_3\)O\(^+\)] = 0.01 mM

\[ \beta_{11} = \frac{K_{001}}{1 + K_{01}[\text{Na}^+] + K_{01}K_{02}[\text{Na}^+]^2} \]  

(2.28)

The high-order term \(K_{01}K_{02}[\text{Na}^+]^2\) of the term of \(1 + K_{01}[\text{Na}^+] + K_{01}K_{02}[\text{Na}^+]^2\) can be ignored when the concentration of Na\(^+\) is very low, leading to a linear relationship between the inverse of \(\beta_{11}\) and the concentration of Na\(^+\) (Equation 2.29). This linear relationship predicted by Equation 2.29 coincides with the observation for the plot of the experimental results (Figure 2.9), indicating that the concentration of Na\(^+\) at the mM level is sufficiently low to keep Equation 2.29 valid. Consequently, the values of \(K_{01}\) (130 ± 10
M\(^{-1}\)) and \(K_{001} \times 10^7\) M\(^{-1}\) were recovered from the fit of the experimental results to Equation 2.29.

\[
\frac{1}{\beta_{11}} = \frac{1 + K_{01}[\text{Na}^+]}{K_{001}}
\]  

(2.29)

Figure 2.9. Dependence of the inverse of the overall binding constant for the formation of NpAmH\(^+\)@CB[7] complex with the Na\(^+\) concentration.

### 2.4.4 Optimization of the experimental conditions for the stopped flow experiments on the NpAmH\(^+\)/CB[7]/M\(^+\) system

The emission change over time was measured in stopped flow experiments and the collected spectral region, 292.8 nm – 367.2 nm, relates to the emission from the NpAmH\(^+\) free in water and bound to CB[7]. The timescale for the stopped flow experiments was set to 100 ms. The following experiment was conducted to check if the complex formation is complete within 100 ms. Figure 2.10 shows the kinetic traces determined for the mixing of a 50 \(\mu\)M NpAmH\(^+\) solution with a CB[7] (0 – 73 \(\mu\)M) solution in the presence of 0.2 M H\(_2\)O\(^+\). The fluorescence intensities increased with time and reached a limiting value (i.e. the final fluorescence intensity) within a very short time period (ca. 5 ms). The traces for the control experiments \(i\) and \(ii\) (i.e. mixing water against water and mixing CB[7]
against water as aforementioned in Section 2.2.1) are horizontal lines and the intensities are ca. 0.02, which is about 0.5% of the lowest offset, i.e. the offset of the trace \(a\) observed in Figure 2.10.

The trace \(a\) was recorded for the injection of NpAmH\(^{+}\) against water and was fitted well into a horizontal line. The intercept of the trace \(a\) relates to the emission intensity of NpAmH\(^{+}\) in the absence of CB[7]. The final fluorescence intensities for the traces \(i\) (\(i = b\) to \(h\)) increased with the concentration of CB[7]. This observation indicated that more NpAmH\(^{+}\) was bound to CB[7] as the concentration of CB[7] increased. The corrected final fluorescence intensity for each trace was determined by averaging the intensities between 50 – 100 ms and then normalized as \(I_{SF}^{eq}\) (see Section 2.2.7). Consequently, the values of \(I_{SF}^{eq}\) can be compared with the values of \(I_{flu}\) determined from the steady-state fluorescence experiments (see section 2.2.5). The comparison (Figure 2.11) shows that the changes in fluorescence intensity measured by the steady-state fluorescence experiments and the stopped flow experiments follow exactly the same trend, confirming

![Figure 2.10. Stopped-flow traces for NpAmH\(^{+}\) (50 µM) mixing with CB[7] in the presence of 0.2 M HCl. [CB[7]] = 0 µM (a), 7.3 µM (b), 14.6 µM (c), 21.9 µM (d), 29.2 µM (e), 36.5 µM (f), 43.8 µM (g) and 73 µM (h).]
that at 100 ms the complex formation is complete. If a slow process would happen on
time scales longer than 100 ms, the amplitudes derived from the kinetic and equilibrium
experiments should be different.

Figure 2.11. Dependence of the changes in the fluorescence intensity measured by the
steady-state fluorescence (●, red, $I_{SF}^{eq}$) and stopped flow (●, black, $I_{flu}$) experiments for
the addition of CB[7] to the 50 µM NpAmH$^+$ solution in the presence of 0.2 M H$_3$O$^+$.

The kinetic traces $i$ ($i = b$ to $h$) did not start at the intercept of the kinetic trace $a$ and a
large offset was observed. This observation indicates that most of the dynamics for the
formation of the NpAmH$^+$/CB[7] complexes occurred within the dead time of the stopped
flow equipment (1 ms). As a result, the kinetic traces determined for the system with
[NpAmH$^+$] at the 10 µM level and [H$_3$O$^+$] at the 0.1 M level contain limited kinetic
information and are not suitable for the kinetic study.

The experimental conditions for the stopped flow experiments, e.g. the concentrations
of NpAmH$^+$ and co-cations, need to be optimized to recover the kinetic information from
the NpAmH$^+$/CB[7] system. Moreover, it is worth noting that two co-cations, Na$^+$ and
H$_3$O$^+$, were both present in the NpAmH$^+$/CB[7]/Na$^+$ system since the addition of H$_3$O$^+$
is required to maintain the 2-naphthyl-1-ethylamine molecule in its protonated state.
Therefore, the effect of the co-presence of H$_3$O$^+$ on the binding dynamics in the NpAmH$^+/\text{CB}[7]/\text{Na}^+$ system needed to be investigated.

The kinetic traces were determined for the NpAmH$^+$ (10 µM)/Na$^+$ solutions mixing with the CB[7] (25 µM)/Na$^+$ solutions in the presence of different concentrations of Na$^+$ and H$_3$O$^+$ (Figure 2.12). The kinetic traces were normalized by the method aforementioned in Section 2.2.7 to ensure that the traces determined in different days can be compared directly. Consequently, the value “1” for the normalized fluorescence intensity relates to the normalized intensity for the control experiment where the NpAmH$^+$ solution was mixed with a solution containing all co-cations, but not containing CB[7]. When the concentration of Na$^+$ was 25 mM, the normalized kinetic traces did not start at the value “1”, indicating that the kinetics observed for the guest-host binding were fast and occur within the dead time of the stopped flow equipment. When the concentration of Na$^+$ was 200 mM, the normalized kinetic traces started at the value “1” indicating that the kinetics observed for the guest-host binding did not occur within the dead time of the stopped flow equipment. Moreover, the normalized kinetic traces determined for the NpAmH$^+/\text{CB}[7]/\text{Na}^+$ (200 mM) system coincide with each other as the concentration of H$_3$O$^+$ changed from 0.05 mM to 0.4 mM. This observation indicates that the presence of H$_3$O$^+$ up to the 0.4 mM level in the NpAmH$^+/\text{CB}[7]/\text{Na}^+$ system does not affect the binding kinetics observed in the stopped flow experiments.
Figure 2.12. The normalized stopped-flow traces for NpAmH\(^+\) (10 \(\mu\)M) mixing with CB[7] (25 \(\mu\)M) in the presence of Na\(^+\) and H\(_3\)O\(^+\). The values of [Na\(^+\)] and [H\(_3\)O\(^+\)] were respectively 25 mM and 0.4 mM (black), 200 mM and 0.05 mM (blue) and 200 mM and 0.4 mM (red).

Another method to treat the stopped flow data was employed to check if the presence of H\(_3\)O\(^+\) up to the 0.1 mM level in the NpAmH\(^+\)/CB[7]/Na\(^+\) system affects the binding affinity of NpAmH\(^+\) to CB[7]. The normalized fluorescence intensities for the mixing solutions at the equilibrium state (\(I_{SF}^{eq}\)) were determined for each kinetic trace as aforementioned in Section 2.2.7. The dependence of \(I_{SF}^{eq}\) with the concentration of CB[7] were plotted for the NpAmH\(^+\)/CB[7]/Na\(^+\) systems with different concentrations of Na\(^+\) and H\(_3\)O\(^+\) (Figure 2.13). As the concentration of Na\(^+\) increased, more CB[7] was required to saturate the fluorescence of a fixed amount of NpAmH\(^+\), i.e. the overall binding affinity of NpAmH\(^+\) to CB[7] decreased. When the concentration of Na\(^+\) was 200 mM, the dependence of \(I_{SF}^{eq}\) with the concentration of [CB[7]] fall on the same curve for the NpAmH\(^+\)/CB[7]/Na\(^+\) systems with different concentrations of H\(_3\)O\(^+\) (0.05 mM – 0.4 mM). This observation indicates that the co-presence of H\(_3\)O\(^+\) up to the 0.4 mM level in the NpAmH\(^+\)/CB[7]/Na\(^+\) system does not affect the binding affinity of NpAmH\(^+\) to CB[7] observed in the stopped flow experiments.
Figure 2.13. Dependence of the fluorescence intensity ($I_{SF}^{eq}$) with the concentration of CB[7] for the NpAmH$^+$ (10 $\mu$M)/CB[7]/Na$^+$ system. [Na$^+$] and [H$_3$O$^+$] for each system were 25 mM and 0.4 mM (●), 100 mM and 0.4 mM (●), 200 mM and 0.05 mM (●), 200 mM and 0.1 mM (●) and 200 mM and 0.4 mM (●), respectively.

Since the presence of H$_3$O$^+$ up to the 0.1 mM level in the NpAmH$^+$/CB[7]/Na$^+$ system does not affect either the binding dynamics observed or the binding affinities of NpAmH$^+$ to CB[7] in the NpAmH$^+$/CB[7]/Na$^+$ systems, the only co-cation that needs to be considered for the kinetic and equilibrium binding studies in the NpAmH$^+$/CB[7]/Na$^+$ systems was Na$^+$ when the simplified model for the NpAmH$^+$/CB[7]/Na$^+$ system (Scheme 2.3) was adapted.

2.4.5 The chemical relaxation approach to determine the dissociation rate constant for the complexation between NpAmH$^+$ and the CB[7] species

The stopped flow experiments were conducted by mixing the NpAmH$^+$/Na$^+$ solutions with the CB[7]/Na$^+$ solutions under the experimental condition (sodium system I) where the concentrations of NpAmH$^+$ (e.g. 0.5 $\mu$M or 1 $\mu$M), H$_3$O$^+$ (e.g. 0.1 mM or 1 mM) and Na$^+$ ($\geq$ 75 mM) were constant while the concentrations of CB[7] ($\leq$ 30 $\mu$M) were varied. The dynamics for the formation of the NpAmH$^+$/CB[7] complexes were recorded (Figure 2.14).
Figure 2.14. Kinetics for the formation of NpAmH⁺@CB[7] by mixing a NpAmH⁺ (0.5 µM)/Na⁺ solution with a CB[7]/Na⁺ solution at various CB[7] concentration. [Na⁺] (100 mM) and [H₃O⁺] (0.1 mM) were constant during the stopped flow experiments. [CB[7]] = 0 µM (a, black), 2.5 µM (b, green), 5.0 µM (c, red), 7.5 µM (d, blue), 10.0 µM (e, green), 12.5 µM (f, red) and 15 µM (g, blue).

The kinetic trace a, which was determined for NpAmH⁺ mixing with water, is a horizontal line. The intercept of this line is related to the fluorescence intensity for NpAmH⁺ in the absence of CB[7]. The kinetic traces i (i = b to f) start at the intercept of the kinetic trace a and reach a limiting value within 100 ms. This observation indicates that the dynamics for the formation of the NpAmH⁺/CB[7] complexes did not occur within the dead time of the stopped flow equipments and reached the equilibrium states on the stopped flow time scale. As a result, the kinetic traces determined for the system with both [NpAmH⁺] and [CB[7]] at the µM level and [Na⁺] at 100 mM level are suitable for the kinetic study.

Each kinetic trace i (i = b to f) was studied by the exponential curve fitting method (see section 2.2.7) to check how many kinetic relaxation processes occurred on the stopped flow time scale. Each kinetic trace i (i = b to f) is fitted well with a mono-exponential function (Equation 2.30, which was derived from Equation 2.7) and the residuals between the experimental data and the fitting curve are random scattered around 0 (Figure 2.15).
This observation indicates that there is only one kinetic relaxation process occurring on the 100 ms time scale, i.e. only one reaction is slow on the stopped flow time scale. The relaxation rate constant \(k_{\text{obs}}\) was determined for each kinetic trace.

\[ I_t = I_0 + I_\infty \left(1 - e^{-k_{\text{obs}}t}\right) \] (2.30)

Figure 2.15. Stopped-flow traces fitted with a mono-exponential function. Top: Experimental trace for the 0.5 µM NpAmH\(^+\) solution mixed with the 15 µM (red), 2.5 µM (blue) or 0 µM (green) CB[7] solutions and the corresponding fitting curves (black). [Na\(^+\)] (100 mM) and [H\(_3\)O\(^+\)] (0.1 mM) were constant during the stopped flow experiments. Middle: The residuals between the fitting curve and the experimental data for the NpAmH\(^+\) solution mixed with the 15 µM CB[7] solution (red). Bottom: The residuals between the fitting curve and the experimental data for the NpAmH\(^+\) solution mixed with the 2.5 µM CB[7] solution (red).

The dependence of \(k_{\text{obs}}\) on the concentration of CB[7], which was plotted in Figure 2.16, follows a non-horizontal linear trend. This observation coincides with the prediction \(ii\) as aforementioned in Section 2.3.2, indicating that \(k_{\text{obs}}\) obtained from the relaxation kinetics corresponds to the binding process between NpAmH\(^+\) and the CB[7] species
under the specific experimental condition (i.e. \([\text{NpAmH}^+]\) is at \(\mu\)M level, \([\text{CB}[7]]\) is at 10 \(\mu\)M level, \([\text{H}_3\text{O}^+]\) is at mM level and \([\text{Na}^+]\) is higher than 75 mM).

Figure 2.16. Dependence of the observed rate constant with the total concentration of \([\text{CB}[7]]\) for the kinetic relaxation process studied by mixing the solution of \([\text{NpAmH}^+]\) \((0.5\ \mu\text{M})/\text{Na}^+\) with the solutions of \([\text{CB}[7]]/\text{Na}^+. [\text{Na}^+]\) \((0.1\ \text{M})\) and \([\text{H}_3\text{O}^+]\) \((0.1\ \text{mM})\) were constant during the stopped flow experiments.

A proposed mechanism is required to recover the kinetic information from the stopped flow experiments. Model 2, 3, 4 and 5 were employed to fit the dependence of \(k_{\text{obs}}\) with the concentration of \([\text{CB}[7]]\) and the results are listed as follows.

2.4.5.1 Determination of the dissociation rate constant for \(\text{NpAmH}^+@\text{CB}[7]\) complex by using Model 2

As Model 2 (Scheme 2.3) is employed, three reactions, i.e. the reactions for \(\text{Na}^+\) binding to the \([\text{CB}[7]]\) species (Reactions 2.16 and 2.17) and the reaction for \(\text{NpAmH}^+\) binding to \([\text{CB}[7]]\) (Reaction 2.18) are predominated in \(\text{NpAmH}^+/@\text{CB}[7]/\text{Na}^+\) system. Moreover, according to the prediction \(\text{ii}\), Reactions 2.16 and 2.17 are sufficiently fast to be viewed as pre-equilibrium processes and Reaction 2.18 is slow on the stopped flow time scale. Consequently, the dependence of \(k_{\text{obs}}\) with \([\text{CB}[7]]\) (Figure 2.16) should
follow Equation 2.31 (see Appendix a.2 for the mathematical transformation processes for Equation 2.31).

\[
k_{\text{obs}} = k_{001}^{*} \frac{1}{1 + K_{01}\left[\text{Na}^{+}\right]_{T} + K_{02}\left[\text{Na}^{+}\right]_{T}^{2}} \left[\text{CB}[7]\right]_{T} + k_{001}^{-}
\]  \hspace{1cm} (2.31)

where \([\text{CB}[7]]_{T}\) and \([\text{Na}^{+}]_{T}\) correspond to the total concentration of the CB[7] species and the total concentration of the sodium cation, respectively.

The dependence of \(k_{\text{obs}}\) with the concentration of CB[7] was fitted well with Equation 2.31, and the value of \(k_{001}^{-}\) for Reaction 2.18 was determined as the intercept of the fitting line. The values of \(k_{001}^{-}\) were determined for the sodium system 1 where different concentrations of reagents (i.e. [NpAmH\(^+\)], [CB[7]], [Na\(^+\)] and [H\(_3\)O\(^+\)]) were employed (Table 1.1).

Table 2.2. The dissociation rate constants for the NpAmH\(^+\)@CB[7] complex determined by fitting the dependence of \(k_{\text{obs}}\) on [CB[7]] with Equation 2.31\(^a\)

<table>
<thead>
<tr>
<th>[NpAmH(^+)] / (\mu)M</th>
<th>[CB[7]] / (\mu)M</th>
<th>[Na(^+)] / mM</th>
<th>[H(_3)O(^+)] / mM</th>
<th>(k_{001}^{-}) / s(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 – 25</td>
<td>75</td>
<td>0.1</td>
<td>45 ± 7</td>
</tr>
<tr>
<td>1</td>
<td>0 – 25</td>
<td>75</td>
<td>1</td>
<td>59 ± 1</td>
</tr>
<tr>
<td>0.5</td>
<td>0 – 15</td>
<td>100</td>
<td>0.1</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>1</td>
<td>0 – 25</td>
<td>100</td>
<td>0.1</td>
<td>53 ± 1</td>
</tr>
<tr>
<td>1</td>
<td>0 – 20</td>
<td>125</td>
<td>0.1</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>1</td>
<td>0 – 25</td>
<td>125</td>
<td>1</td>
<td>49 ± 2</td>
</tr>
<tr>
<td>1</td>
<td>0 – 25</td>
<td>200</td>
<td>1</td>
<td>61 ± 1</td>
</tr>
</tbody>
</table>

\(^a\)Values and errors were recovered from the fittings of the data with Equation 2.31 for each independent experiment.
According to Equation 2.31, the value of \( k_{001} \) is independent of the concentrations of reagents (i.e. \([\text{NpAmH}^+], [\text{CB}[7]], [\text{Na}^+] \) and \([\text{H}_3\text{O}^+]\)) used in the sodium system 1. This statement is consistent with the observation that the values of \( k_{001} \), which were determined by using different concentrations of reagents, show no trend. The value of \( k_{001} \) was determined as \( 55 \pm 7 \text{ s}^{-1} \) according to seven independent experiments.

### 2.4.5.2 Determination of the dissociation rate constant for NpAmH\(^+\) binding to the CB[7] species by using other models (Model 3, 4 or 5)

The equations, which represent the relationship between \( k_{\text{obs}} \) and the concentration of \( \text{Na}^+ \), can be derived for NpAmH\(^+\)/CB[7]/\( \text{Na}^+ \) system according to the model employed (i.e. Model 3, 4 or 5). The mathematical transformation to derive the equation for each model is similar as the one listed in Appendix a.2. As a result, Equations 2.32, 2.33 and 2.34 were derived for Model 3, 4 and 5, respectively.

\[
\begin{align*}
  k_{\text{obs}} &= k_{001}^* \frac{1}{1 + K_{01}[\text{Na}^+]_T}[\text{CB}[7]]_T + k_{001}^- \\
  \text{where } [\text{CB}[7]]_T \text{ and } [\text{Na}^+]_T &\text{ correspond to the total concentration of the CB[7] species and the total concentration of the sodium cation, respectively.}
\end{align*}
\] (2.32)

\[
\begin{align*}
  k_{\text{obs}} &= k_{011}^* \frac{K_{01}[\text{Na}^+]_T}{1 + K_{01}[\text{Na}^+]_T + K_{01}K_{02}[\text{Na}^+]_T^2}[\text{CB}[7]]_T + k_{001}^- \\
  \text{As Model 3 (Scheme 2.4) was employed, the dependence of } k_{\text{obs}} \text{ with } [\text{CB}[7]] \text{ (Figure 2.16) was fitted well with Equations 2.32 and the value of } k_{001}^- \text{ was determined as the}
\end{align*}
\] (2.33)

\[
\begin{align*}
  k_{\text{obs}} &= k_{001}^* \frac{K_{01}[\text{Na}^+]_T}{1 + K_{01}[\text{Na}^+]_T + K_{01}K_{02}[\text{Na}^+]_T^2}[\text{CB}[7]]_T + k_{001}^-[\text{Na}^+]_T \\
  \text{value of } k_{001}^- \text{ was determined as the}
\end{align*}
\] (2.34)
intercept of the fitting line. The determination process for the value of $k_{001}$ in Reaction 2.20 is similar as the analysis stated in Section 2.4.5.1. The average of $k_{001}$ is $55 \pm 7 \text{ s}^{-1}$.

As Model 4 (Scheme 2.5) was employed, the dependence of $k_{\text{obs}}$ with [CB[7]] (Figure 2.16) was fitted well with Equations 2.33 and the value of $k_{011}$ was determined as the intercept of the fitting line. The determination process for the value of $k_{011}$ in Reaction 2.23 is similar as the analysis stated in Section 2.4.5.1. The average of $k_{011}$ is $55 \pm 7 \text{ s}^{-1}$.

As Model 5 (Scheme 2.6) was employed, the dependence of $k_{\text{obs}}$ with [CB[7]] (Figure 2.16) was fitted well with Equations 2.33. The product of $k_{011}$ and [Na$^+$] was determined as the intercept of the fitting line. The determination process for the value of $k_{001}[\text{Na}^+]$ is similar as the analysis stated in Section 2.4.5.1. In principle, the value of $k_{001}[\text{Na}^+]$ should increase with the concentration of sodium cation. This prediction is in conflict with the observation that the values of the intercept of the fitting line, which were determined by using different concentrations of reagents, show no trend with the concentration of Na$^+$. Therefore, Model 5 is not the right model to describe the reactions in the sodium system 1.

In summary, results in Sections 2.4.5.1 and 2.4.5.2 indicate that Model 2, 3 and 4 fit the data determined for the sodium system 1 equally well. However, Model 5 cannot be right model due to the confliction between the experimental data and the model. The dissociation rate constant for NpAmH$^+$ bound to the CB[7] species was determined and assigned to different reactions (i.e. Reactions 2.18, 2.20, 2.23) according to different models (i.e. Model 2, 3 and 4) employed.
2.4.6 The chemical relaxation approach to determine the association rate constant for NpAmH$^+$ binding to the CB[7] species and the equilibrium constant for Na$^+$ binding to the CB[7] species

The stopped flow experiments were conducted by mixing a NpAmH$^+$/Na$^+$ solution with a CB[7]/Na$^+$ solution under the experimental condition (sodium system 2) where the concentrations of NpAmH$^+$ (1 µM), CB[7] (10 µM) and H$_3$O$^+$ (0.1 mM) were constant while the concentration of Na$^+$ ($\geq$ 50 mM) was varied. The dynamics for the formation of the CB[7]/NpAmH$^+$ complexes were recorded (Figure 2.17).

![Figure 2.17. Kinetics for the formation of the NpAmH$^+$@CB[7] complex by mixing the NpAmH$^+$ (1 µM)/Na$^+$ solution with the CB[7] (10 µM)/Na$^+$ solution at various Na$^+$ concentrations. [H$_3$O$^+$] (0.1 mM) was constant during the stopped flow experiments. [Na$^+$] = 50 mM (a, red), 75 mM (b, blue), 100 mM (c, green), 125 mM (d, black), 150 mM (e, red) and 200 mM (f, blue). Each trace was determined by averaging 75 traces for good signal-to-noise quality.](image)

Each kinetic trace reached a limiting value within 50 ms. Moreover, it took more time for the CB[7]/NpAmH$^+$ system to reach a limiting value in the presence of higher concentration of Na$^+$, indicating that the binding dynamics for the binding between NpAmH$^+$ and the CB[7] species was retarded. The limiting value for each kinetic trace decreased with the increase of the concentration of Na$^+$, indicating that the overall
binding affinity of NpAmH with CB[7] was decreased. Each trace was fitted well with a mono-exponential function (Equation 2.30) and the residuals between the experimental data and the fitting curve were random scattered around 0 (Figure 2.18), indicating that only one kinetic relaxation process was observed for each trace. The relaxation rate constant ($k_{obs}$) was determined for each kinetic trace.

Figure 2.18. Stopped-flow traces fitted with a mono-exponential function. Top: Experimental trace for the 1 μM NpAmH$^+$ solution mixed with the 10 μM (red and blue) CB[7] solutions and the corresponding fitting curves (black). [Na$^+$] (red – 50 mM and blue – 200 mM) and [H$_3$O$^+$] (0.1 mM) were constant during the stopped flow experiments. Middle: The residuals between the fitting curve and the experimental data for the NpAmH$^+$ solution mixed with the CB[7] solution in the presence of 50 mM Na$^+$ and 0.1 mM H$_3$O$^+$ (red). Bottom: The residuals between the fitting curve and the experimental data for the NpAmH$^+$ solution mixed with the CB[7] solution in the presence of 200 mM Na$^+$ and 0.1 mM H$_3$O$^+$ (red).
2.4.6.1 Determination of the association rate constant for NpAmH$^+@$CB[7] complex and the equilibrium binding constant for Na$^+$•CB[7]•Na$^+$ complex by using Model 2

Model 2 was employed to fit the data determined for the sodium system 2. Equation 2.35, which was obtained by rearranging Equation 2.31, was employed to fit the dependence of a function of $k_{\text{obs}}$ (i.e. $\frac{k_{\text{obs}} - k_{\text{001}}^-}{[\text{CB}[7]]_T}$) with the concentration of Na$^+$ (Figure 2.19). The value of $k_{\text{001}}^-$ was fixed to 55 s$^{-1}$ (determined as aforementioned in Section 2.4.5.1). When the data in Figure 2.19 were fitted with Equation 2.35 and three parameters ($K_{01}$, $K_{02}$ and $k_{\text{001}}^+$) were treated as free parameters, the fitting curve (i.e. the red curve in Figure 2.19) fitted data well. However, the errors for $K_{01}$, $K_{02}$ and $k_{\text{001}}^+$ are large, which may be due to the fact that these parameters are correlated with each other. When the value of $K_{01}$ was fixed to 130 M$^{-1}$ (determined as aforementioned in Section 2.4.3) and two parameter ($K_{02}$ and $k_{\text{001}}^+$) were treated as free parameters, the fitting curve (i.e. the red curve in Figure 2.19) fitted data equally well with small errors for $K_{02}$ and $k_{\text{001}}^+$ (Table 2.3).

$$\frac{k_{\text{obs}} - k_{\text{001}}^-}{[\text{CB}[7]]_T} = \frac{k_{\text{001}}^+}{1 + K_{01}[\text{Na}^+]_T + K_{01}K_{02}[\text{Na}^+]_T^2}$$  \hspace{1cm} (2.35)
Figure 2.19. Dependence of a function of the observed rate constant for the formation of the NpAmH⁺@CB[7] complex with the concentration of sodium. The solid red curve corresponds to the fit of the data to Equation 2.35 (Model 2). The dashed line corresponds to the fit of the data when Model 3 or 4 is considered. The data from three independent experiments were simultaneously employed in the fit.

Table 2.3. Equilibrium and rate constants determined for NpAmH⁺/CB[7]/Na⁺ system in stopped flow experiments

<table>
<thead>
<tr>
<th>[Na⁺] / M</th>
<th>K₀₂ / M⁻¹</th>
<th>k⁺₀₀₁ / 10⁸ M⁻¹s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 – 0.2</td>
<td>21 ± 2</td>
<td>6.3 ± 0.3</td>
</tr>
</tbody>
</table>

The value of $k_{001}^-$ was fixed as 55 ± 7 s⁻¹. The value of $K_{01}$ was fixed as 130 ± 10 M⁻¹. Values and errors for $K_{02}$ and $k_{001}^+$ were recovered by simultaneously fitting the data from three independent experiments with Equation 2.35.

2.4.6.2 Fitting the data determined for the sodium system 2 by using other models (Model 3 and 4)

Model 3 was employed to fit the data determined for the sodium system 2. Equation 2.36, which was obtained by rearranging Equation 2.32, was employed to fit the dependence of a function of $k_{\text{obs}}$ (i.e. $k_{\text{obs}} - k_{001}^-$) with the concentration of Na⁺ (Figure 2.19). The value of $k_{001}^-$ was fixed to 55 s⁻¹ (determined as aforementioned in Section
2.4.5.1). When the data in Figure 2.19 were fitted with Equation 2.36, and two parameters ($K_{01}$ and $k_{001}^-$) were treated as free parameters, the fitting curve (i.e. the dashed line in Figure 2.19) did not fit data well.

$$\frac{k_{\text{obs}} - k_{001}^-}{[\text{CB}[7]]_T} = \frac{k_{001}^+}{1 + K_{01}[\text{Na}^+]_T}$$

(2.36)

Model 4 was employed to fit the data determined for the sodium system 2. Equation 2.37, which was obtained by rearranging Equation 2.33, was employed to fit the dependence of a function of $k_{\text{obs}}$ (i.e. $\frac{k_{\text{obs}} - k_{011}^-}{[\text{CB}[7]]_T}$) with the concentration of Na$^+$ (Figure 2.19). The value of $k_{001}^-$ was fixed to 55 s$^{-1}$ (determined as aforementioned in Section 2.4.5.1). When the data in Figure 2.19 were fitted with Equation 2.37, and three parameters ($K_{01}$, $K_{02}$ and $k_{001}^+$) were treated as free parameters, the fitting curve (i.e. the dashed line in Figure 2.19) did not fit data well.

$$\frac{k_{\text{obs}} - k_{011}^-}{[\text{CB}[7]]_T} = \frac{k_{001}^+}{\frac{1}{K_{01}[\text{Na}^+]_T} + 1 + K_{02}[\text{Na}^+]_T}$$

(2.37)

In summary, results in Section 2.4.6.1 and 2.4.6.2 indicate that Model 2 can fit the data determined for the sodium system 2 well while Model 3 and 4 cannot. Therefore, Model 2 is the right model to describe the dynamic processes in the NpAmH$^+$/CB[7]/Na$^+$ system.

2.4.7 The second-order global analysis for the binding kinetics between NpAmH$^+$ and CB[7]

Another approach to study the dynamics in NpAmH$^+$/CB[7]/Na$^+$ system, rather than the exponential curve fitting performed to the individual kinetic trace, was the second-
order global analysis. Each proposed mechanism was employed to build up a global analysis model in Pro-Kineticist II. The rate constants for the pre-equilibrium processes cannot be recovered from stopped flow experiments although the corresponding equilibrium binding constants can be determined. The association and dissociation rate constants for the slow reaction can be recovered.

The validity of the model employed can be checked by the residuals between the fitting curve and the experimental curve. The fact that the residuals are randomly scattered around 0 indicates the model fit the data well. On the contrary, the systematic deviations between the fitting curve and the experimental curve indicates the model is not suitable to fit the data.

The kinetic traces determined for the sodium system 2 were employed in the second-order global analysis. Model 2 was employed in the second-order global analysis. The value of $k_{001}$ was fixed to 55 s$^{-1}$ (determined as aforementioned in Section 2.4.5.1), the value of $K_{01}$ was fixed to 130 M$^{-1}$ (determined as aforementioned in Section 2.4.3) and other parameters (i.e. $K_{02}$ and $k_{001}$) were treated as free parameters. The residuals between the experimental data and the fit to the second-order global fitting model are randomly scattered around 0 for the whole data set analyzed (Figure 2.20), indicating that Model 2 is suitable to fit the data determined for the sodium system 2. The values of $K_{02}$ and $k_{001}$ recovered are closed to the values recovered in Section 2.4.6.1. The errors for $K_{02}$ and $k_{001}$ are large and are higher than the corresponding values recovered in Section 2.4.6.1.
Figure 2.20. Residuals between the experimental data and the fit to the second-order global fitting model (Model 2). The stopped-flow traces for the NpAmH$^+$ (1 μM)/Na$^+$ solution mixing with the CB[7] (10 μM)/Na$^+$ solution were shown in Figure 2.17. [H$_3$O$^+$] (0.1 mM) was constant during the stopped flow experiments. [Na$^+$] from top to bottom are: 0.05 M, 0.075 M, 0.100 M, 0.125 M, 0.150 M and 0.200 M.

When Model 3 or 4 was employed in the second-order global analysis, the value of the dissociation rate constant for NpAmH$^+$ binding to CB[7] was fixed to 55 s$^{-1}$ (determined as aforementioned in Section 2.4.5.2), the value of $K_{01}$ was fixed to 130 M$^{-1}$ (determined as aforementioned in Section 2.4.3) and other parameters were treated as free parameters. The residuals between the experimental data and the fit to the second-order global fitting model are not randomly scattered around 0 for the whole data set analyzed (Figure 2.21), indicating that neither Model 3 nor Model 4 is suitable to fit the data determined for the sodium system 2.
Figure 2.21. Residuals between the experimental data and the fit to the second-order global fitting model (Model 3 or 4). The stopped-flow traces for the NpAmH⁺ (1 μM)/Na⁺ solution mixing with the CB[7] (10 μM)/Na⁺ solution were shown in Figure 2.17. [H₃O⁺] (0.1 mM) was constant during the stopped flow experiments. [Na⁺] from top to bottom are: 0.05 M, 0.075 M, 0.100 M, 0.125 M, 0.150 M and 0.200 M.

Results in Section 2.4.7 indicate that Model 2 can fit the data determined for the sodium system 2 well while Model 3 and 4 cannot. This statement is consistent with the statement made in Section 2.4.6. Therefore, Model 2 is the right model to represent the dynamic processes in the NpAmH⁺/CB[7]/Na⁺ system.

2.4.8 The dynamic study for the NpAmH⁺/CB[7]/H₃O⁺ system

The stopped flow experiments (hydronium system 1) were conducted by mixing NpAmH⁺ (1 μM)/H₃O⁺ (1 M) solutions with the CB[7] (0 – 25 μM)/H₃O⁺ (1 M) solutions, and the dynamics for the formation of the NpAmH⁺/CB[7] complexes were recorded (Figure 2.22). The fluorescence intensities increased with time and reached a
limiting value within 50 ms. The fluorescence intensities reached the limiting values in shorter time for the NpAmH⁺/CB[7]/H₂O⁺ system with the higher concentration of CB[7], indicating that the dynamics for the formation of NpAmH⁺/CB[7] complexes were accelerated with the addition of CB[7]. Moreover, the final fluorescence intensities increased with the addition of CB[7], indicating that more NpAmH⁺ were bound to CB[7] at higher concentrations of CB[7].

![Image of fluorescence intensity vs time](image)

Figure 2.22. Kinetics for the formation of the NpAmH⁺@CB[7] complex by mixing the NpAmH⁺ (1 µM)/H₂O⁺ (1 M) solution with the CB[7]/H₂O⁺ (1 M) solution at various CB[7] concentrations. [CB[7]] = 0 µM (a, red), 5.00 µM (b, black), 10.0 µM (c, green), 15.0 µM (d, blue), 20.0 µM (e, red) and 25.0 µM (f, black).

The kinetic trace a, which was determined for the NpAmH⁺ solution mixing with water, was a horizontal line. The intercept of this line is related to the fluorescence intensity for NpAmH⁺ in the absence of CB[7]. The kinetic trace i (i = b to f) started at the intercept of the kinetic trace a and reached a limiting value within 50 ms. This observation indicates that dynamics for the formation of the NpAmH⁺/CB[7] complexes did not occur within the dead time of the stopped flow equipment and reached the equilibrium on the stopped flow time scale. As a result, the kinetic traces determined for the system with 1 µM NpAmH⁺ and 1 M H₂O⁺ are suitable for the kinetic study.
Each trace can be fitted well with a mono-exponential function (Equation 2.30), indicating that only one kinetic relaxation process can be observed on the stopped flow time scale (Figure 2.23). The relaxation rate constant \( k_{\text{obs}} \) was determined for each kinetic trace. The dependence of \( k_{\text{obs}} \) with the concentration of CB[7], which was plotted in Figure 2.24, follows a non-horizontal linear trend. According to the argument made in Section 2.3.2, this observation indicates that the binding dynamics of H$_3$O$^+$ binding to the CB[7] species are pre-equilibrium processes on the stopped flow time scale and the binding dynamics of NpAmH$^+$ to the CB[7] species was slow on the stopped flow time scale.

![Stopped-flow trace fitted with a mono-exponential function](image)

**Figure 2.23.** Stopped-flow trace fitted with a mono-exponential function. Top panel: Experimental trace for the NpAmH$^+$ (1 μM)/H$_3$O$^+$ (1 M) solution mixed with the CB[7] (5 μM)/H$_3$O$^+$ (1 M) solution (black) and the fit with a mono-exponential function (red). Bottom panel: The residuals between the fitting curve and the experimental data (green).
Figure 2.24. Dependence of the observed rate constant with the total concentration of CB[7] for the kinetic relaxation process studied by mixing the solution of NpAmH⁺ (1 µM)/H₃O⁺ (1 M) with the solutions of CB[7]/H₃O⁺ (1 M).

Therefore, the dissociation rate constant for the binding process between NpAmH⁺ and the CB[7] species (k) was determined by fitting the dependence of $k_{\text{obs}}$ with the concentration of CB[7] to a linear line. The value of k was determined as $36 \pm 4$ s⁻¹ according to two individual experiments. k was assigned to the specific parameter according to the model employed. For example, k was assigned to $k_{001}^{++}$, $k_{001}^{−−}$ and $k_{010}^{−−}$ when Equations 2.38, 2.39 and 2.40 were employed, respectively. Equations 2.38, 2.39 and 2.40 were derived according to the corresponding model (e.g. Model 2, Model 3 and Model 4) employed for the NpAmH⁺/CB[7]/H₃O⁺ system. The mathematical transformation processes for these equations are similar as those shown in Appendix a.2.

$$k_{\text{obs}} = k_{001}^{+} \frac{1}{1 + K_1[H_3O^+]_T + K_1K_2[H_3O^+]_T^2}[\text{CB}[7]]_T + k_{001}^{−−}$$  \hspace{1cm} (2.38)

where $[\text{CB}[7]]_T$ and $[H_3O^+]_T$ correspond to the total concentration of the CB[7] species and the total concentration of the hydronium cations, respectively.

$$k_{\text{obs}} = k_{001}^{+} \frac{1}{1 + K_1[H_3O^+]_T}[\text{CB}[7]]_T + k_{001}^{−−}$$  \hspace{1cm} (2.39)
The dynamics for the formation of the NpAmH⁺/CB[7] complexes were recorded by mixing NpAmH⁺ (1 μM)/H₃O⁺ solutions with CB[7] (10 μM)/H₃O⁺ solutions in the presence of H₃O⁺ (0.5 – 2 M) (hydronium system 2, Figure 2.25).

![Figure 2.25](image)

**Figure 2.25.** Kinetics for the formation of NpAmH⁺@CB[7] (a – e) by mixing NpAmH⁺ (1 μM)/H₃O⁺ solutions with CB[7] (10 μM)/H₃O⁺ solutions at various H₃O⁺ concentrations. The traces at the bottom of figure (f – j) were determined by mixing NpAmH⁺ (1 μM)/H₃O⁺ solutions with H₂O⁺ solutions. [H₂O⁺] = 0.5 M (a and f, black), 0.75 M (b and g, blue), 1.0 M (c and h, red), 1.5 M (d and i, green) and 2.0 M (e and j, purple).

The fluorescence intensities for traces a – e increased over time and reached the equilibrium state within 40 ms. No step-function was observed for the NpAmH⁺-CB[7] binding kinetics in the presence of different concentrations of H₂O⁺, i.e. each kinetic trace started at the fluorescence intensity for the corresponding control experiment where the NpAmH⁺ solution was mixed with a solution containing the co-cation, but not containing CB[7]. Moreover, all of the kinetic traces follow a mono-exponential growth. These observations indicate that one kinetic relaxation process was observed for each kinetic
trace and this process did not occur within the dead time of the stopped flow equipment. Therefore, the NpAmH⁺-CB[7] binding kinetics can be employed to check the validity of the model (i.e. Model 2, 3 and 4) stated in Section 2.3.1.

Model 2 was employed to fit the data determined for the hydronium system 2. Equation 2.41, which was obtained by rearranging Equation 2.38, was employed to fit the dependence of a function of $k_{obs}$ (i.e. $\frac{k_{obs} - k_{001}^-}{[CB[7]]_T}$) with the concentration of H$_3$O$^+$ (Figure 2.26). The value of $k_{001}^-$ was fixed to 36 s$^{-1}$. When the data in Figure 2.25 were fitted with Equation 2.41 and three parameters ($K_1$, $K_2$ and $k_{001}^+$) were treated as free parameters, the fitting curve (i.e. the red curve in Figure 2.26) fitted data well. However, the errors for $K_1$, $K_2$ and $k_{001}^+$ are large, which may be due to the fact that these parameters are correlated with each other. An assumption was then made for the fitting process that the ratio of $K_1$ to $K_2$ was equal to the ratio of $K_{01}$ to $K_{02}$. The fitting curve (i.e. the red curve in Figure 2.26) fitted data equally well with small errors for $K_1$, $K_2$ and $k_{001}^+$ (Table 2.4).

$$\frac{k_{obs} - k_{001}^-}{[CB[7]]_T} = \frac{k_{001}^+}{1 + K_1[H_3O^+] + K_2[H_3O^+]^2}$$  (2.41)
Figure 2.26. Dependence of a function of the observed rate constant for the formation of the NpAmH⁺@CB[7] complex with the concentration of H₃O⁺. The solid red curve corresponds to the fit of the data to Equation 2.41. The dashed line corresponds to the fit of the data when the binding of only one H₃O⁺ to CB[7] is considered. The data from three independent experiments were simultaneously employed in the fit.

Table 2.4. Equilibrium and rate constants estimated for NpAmH⁺/CB[7]/H₃O⁺ system

<table>
<thead>
<tr>
<th>[H₃O⁺] / M</th>
<th>K₁ / M⁻¹</th>
<th>K₂ / M⁻¹</th>
<th>k⁺₀₀₁ / 10⁸ M⁻¹s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 – 2</td>
<td>~11</td>
<td>~2</td>
<td>~8</td>
</tr>
</tbody>
</table>

The value of k⁻₀₀₁ was fixed as 36 s⁻¹. The ratio of K₁ to K₂ was fixed as 6.2, which was determined as the ratio of K₀₁ to K₀₂. Values of K₁, K₂ and k⁺₀₀₁ were recovered by simultaneously fitting the data from two independent experiments with Equation 2.41.

The data determined for the hydronium system 2 was fitted using Model 3 or 4. The fitting curve (i.e. the dashed line in Figure 2.26) did not fit data well and deviated from the data, indicating that Model 3 or 4 was not suitable for the dynamic study in the NpAmH⁺/CB[7]/H₃O⁺ system.

In summary, the equilibrium and rate constants determined for the NpAmH⁺/CB[7]/M⁺ systems are listed in Table 2.5.
Table 2.5. Equilibrium and rate constants determined for the NpAmH⁺/CB[7]/M⁺ system in stopped flow experiments

<table>
<thead>
<tr>
<th>M⁺</th>
<th>$K_i$ / M⁻¹</th>
<th>$K_j$ / M⁻¹</th>
<th>$k_{001}^+$ / $10^8$ M⁻¹s⁻¹</th>
<th>$k_{001}^-$ / s⁻¹</th>
<th>$K_{01}$ / $10^7$ M⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>130 ± 10</td>
<td>21 ± 2</td>
<td>6.3 ± 0.3</td>
<td>55 ± 7 (7)</td>
<td>1.2 ± 2</td>
</tr>
<tr>
<td>H₃O⁺</td>
<td>~ 11</td>
<td>~ 2</td>
<td>~ 8</td>
<td>36 ± 4 (2)</td>
<td>~ 2</td>
</tr>
</tbody>
</table>

*a*the results were recovered from one independent fitting unless otherwise stated as numbers in parentheses; the values of $K_{001}$ were determined as the ratio of $k_{001}^+$ to $k_{001}^-$. 

*b*i and *j* refer to 01 and 02 for NpAmH⁺/CB[7]/Na⁺ system, respectively; the value and error for $K_{01}$ were recovered from the fit of the experimental results to Equation 2.29; the value and error for $k_{001}^-$ were recovered from the fit of the experimental results to Equation 2.31; values and errors of $K_{02}$ and $k_{001}^+$ were recovered from the fit of the experimental results to Equation 2.35. 

*c*i and *j* refer to 1 and 2 for NpAmH⁺/CB[7]/H₃O⁺ system, respectively; the value and error for $K_{01}$ were recovered from the fit of the experimental results to Equation 2.38; values and errors of $K_1$, $K_2$ and $k_{001}^+$ were estimated from the fit of the experimental results to Equation 2.41 as the ratio of $K_1$ and $K_2$ was fixed as 6.2

2.5 Discussion and conclusions

2.5.1 The binding dynamics of NpAmH⁺ with CB[7]

The binding kinetic study, from the dynamic point of view, provided further mechanistic information on the reasons for the high binding affinity of guests to cucurbit[n]urils. The association rate constant of NpAmH⁺ binding with CB[7] in the presence of H₃O⁺ ($k_{001}^+$), within the experimental errors, is not apparently different from that in the presence of Na⁺ ($k_{001}^+$). The values of $k_{001}^+$ are close to association rate constants ($2.9 \times 10^8$ M⁻¹s⁻¹) reported for the binding of NpOH to β-CD as 1:1 complexes. This observation is not surprising since the sizes of NpAmH⁺ and NpOH as well as the sizes of the host cavities for CB[7] and β-CD are similar in these two guest-host systems. The values of $k_{001}^-$ are lower than 100 s⁻¹, which is much lower than the dissociation rate constants ($1.8 \times 10^5$ s⁻¹) reported for the binding of NpOH to β-CD.
This observation is consistent with the mechanism that CB[7] can bind to the guest cation through the ion-dipole interaction and the hydrophobic effect while β-CD can bind to the guest only through the hydrophobic effect. Consequently, the large difference between the dissociation rate constants and the small difference between the association rate constants in these two guest-host systems leads to the fact that the binding efficiency of NpAmH$^+$ with CB[7] ($10^7$ M$^{-1}$) is much higher than the binding efficiency of NpOH with β-CD ($10^3$ M$^{-1}$).

The width of naphthyl moiety is ca. 5.6 Å, while the portal diameters for CB[6] and CB[7] are 3.9 and 5.4 Å, respectively. As a result, NpAmH$^+$ can move into the CB[7] cavity but not into the CB[6] cavity. No fluorescence intensity change or singlet-excited state lifetime change was observed for NpAmH$^+$ (10 µM) with the addition of CB[6] (1 mM) in the presence of 0.02 M NaCl at pH 3, indicating that NpAmH$^+$ does not associate with CB[6]. These observations indicate that a stable NpAmH$^+$•CB[n] complex cannot be formed if the hydrophobic moieties of NpAmH$^+$ cannot move into the cavity of CB[n].

The binding kinetic study provides the mechanistic information on how the size of the guest molecules and the host molecules affect the binding dynamics. There is no intermediate observed for the formation of the NpAmH$^+$-CB[7] complex, indicating that the naphthyl moieties of the NpAmH$^+$ molecule moves into the CB[7] cavity directly. Moreover, no intermediate was observed for the binding of small charged guests (e.g. cyclopropylmethylamine) with CB[6] and the association rate constant for small charged guests binding to CB[6] is high (e.g. $>10^6$ M$^{-1}$s$^{-1}$ for cyclopropylmethylamine binding to CB[6]). In contrast, the binding of the large charged guests (e.g. cyclohexylmethylamine
and 4-methylbenzylammonium ion) with CB[6] follows a flip-flop mechanism. In other words, an intermediate is formed as an exclusion complex firstly. Sequentially, the large hydrophobic moiety of the guest moves into the CB[6] cavity with a very small overall association rate constant (e.g. $8.8 \times 10^{-4} \text{ M}^{-1} \text{s}^{-1}$ for cyclohexylmethylamine binding to CB[6]). These observations show that the steric hindrance for the ingression of guest into host has a significant effect on the binding dynamics observed for the cucurbituril-guests complex.

2.5.2 The binding affinity of co-cations to cucurbit[7]uril

The values of equilibrium constants for Na$^+$ binding to CB[7] ($K_{01}$, $130 \text{ M}^{-1}$) and to CB[7]$\cdot$Na$^+$ ($K_{02}$, $21 \text{ M}^{-1}$) are similar as those determined in other groups, e.g. $120 \text{ M}^{-1}$ for $K_{01}$ and $11 \text{ M}^{-1}$ for $K_{02}$. The values of equilibrium constants for H$_3$O$^+$ binding to CB[7] ($K_1$, $11 \text{ M}^{-1}$) to CB[7]$\cdot$H$_3$O$^+$ ($K_2$, $2 \text{ M}^{-1}$) are repetitively smaller than $K_{01}$ and $K_{02}$, although the size of H$_3$O$^+$ (1.38 Å in radius) matches the portal size of CB[7] (2.7 Å in radius) better than that of Na$^+$ (1.02 Å in radius) does. The trend for the binding affinity of co-cations is also observed for the crown ether systems, e.g. the equilibrium constant for Na$^+$ binding to 18-crown-6 (6 M$^{-1}$) is higher than for H$_3$O$^+$ binding (0.4 M$^{-1}$); the equilibrium constant for Na$^+$ binding to 15-crown-5 (5 M$^{-1}$) is higher than that for H$_3$O$^+$ binding (0.3 M$^{-1}$). The higher binding affinity of Na$^+$ to these hosts may be due to the fact that the interaction (ion-dipole interaction) between Na$^+$ and host is stronger than the interaction (hydrogen bond) between H$_3$O$^+$ and host.

The co-cations bind to CB[7] with high binding affinities (Table 2.5) while they do not bind to β-CD at non-basic pH, although the portal size of β-CD (3 Å – 3.2 Å in radii)
is similar to that of CB[7] (2.7 Å in radii). This example shows that ion-dipole interaction plays a key role for the binding of co-cation to CB[7].

The binding affinity of Na\(^+\) to CB[7] (130 M\(^{-1}\)) is much higher than that of Na\(^+\) to crown ethers (e.g. 6 M\(^{-1}\) for 18-crown-6 and 5 M\(^{-1}\) for 15-crown-5) although the size of Na\(^+\) (1.02 Å in radius) fits the cavity of crown ethers (0.9 Å and 1.4 Å in cavity radii for 15-crown-5 and 16-crown-6, respectively\(^{11,249}\) better than the portal of CB[7] (2.7 Å in portal radii). This observation may be due to two reasons: (i) The interaction between co-cations and the oxygen of ureido-carbonyl rim of CB[7] is stronger than that between co-cations and oxygen of crown ethers.\(^{250}\) (ii) The crown ethers are flexible compounds while the cucurbituril molecules are rigid. As a result, the complexation of co-cations with crown ethers is more disfavoured in terms of entropy than that with cucurbiturils.\(^{71}\)

### 2.5.3 The effect of co-cations in cucurbit[7]uril host-guest systems

The presence of Na\(^+\) weakens the binding affinity of NpAmH\(^+\) to CB[7], which has been observed in Figure 2.13 and discussed qualitatively in Section 2.4.4. The quantitative analysis can be conducted by comparing the overall equilibrium constants (\(\beta_{11}\)) determined for the NpAmH\(^+\)-CB[7] complex in the presence of different concentration of co-cations (Table 2.1). The equilibrium constants for the binding of NpAmH\(^+\) to CB[7] determined by the binding study ((1.06 ± 0.05) \times 10^7 M\(^{-1}\) as aforementioned in Section 2.4.3) were the same as those determined by the kinetic study ((1.2 ± 2) \times 10^7 M\(^{-1}\) as aforementioned in Section 2.4.8). This observation indicates that the results from the kinetic study and the equilibrium binding study are self-consistent. Moreover, the results in Table 2.1 show that the overall equilibrium constant (\(\beta_{11}\)) for the binding of NpAmH\(^+\) to CB[7] decreased with the increase of the concentration of Na\(^+\).
Therefore, the equilibrium constant for the binding of guest to CB[7] can be recovered only when the competition between co-cations and the guest for CB[7] is taken into account.
3 Chiral Recognition for the Complexation Dynamics of β-Cyclodextrin with the Enantiomers of 2-Naphthyl-1-ethanol

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3.1 Introduction

3.1.1 2-Naphthyl-1-ethanol / β-CD system

The enantiomers of 2-naphthyl-1-ethanol (Scheme 3.1, NpOH) were previously shown to form 1:1 and 2:2 complexes with β-CD. Complexation of NpOH as a 1:1 complex led to an increase in its fluorescence intensity, while the NpOH excimer emission was observed for the guest’s dimer in the 2:2 complex. The quenching efficiency for the triplet-excited state of NpOH by Mn$^{2+}$ ions was shown to be much lower for the 2:2 complex when compared to the 1:1 complex showing that the guest in the 2:2 complex is much more isolated from species in the aqueous phase than the guest in the 1:1 complex. The binding dynamics of NpOH with β-CD in the 1:1 complex is fast and occurs within microseconds. A much slower dynamics was observed for the 2:2 complex where the relaxation process was estimated to be slower than 0.4 ms. The effect of chirality was apparent from the higher intensity for the excimer emission observed in the case of (R)-NpOH when compared to its enantiomer. However, this chiral discrimination was not apparent, within experimental errors, for the equilibrium constants for the formation of the 1:1 and 2:2 complexes.
Scheme 3.1. Structure for (R)-2-Naphthyl-1-ethanol

NMR experiments\textsuperscript{203} and theoretical studies\textsuperscript{252} showed that NpOH is axially included in the β-CD cavity. Two 1:1 complexes were formed, which differ in the positioning of the naphthyl (N) or the ethanol (E) groups with respect to the CD cavity. The N structure has the naphthyl group positioned close to the narrower entrance of the CD containing the primary hydroxyl groups (Figure 3.1, red CD rim), while in the E structure the ethanol moiety is positioned close to the narrower entrance and the naphthyl moiety is located at the wider entrance. Despite a lower energy calculated for N a comparison of the calculated and experimental induced circular dichroism spectra showed that both N and E were present in solution.\textsuperscript{252} For this reason, three types of 2:2 complexes can be formed which have different relative orientations of the naphthyl and ethyl groups within the β-CD complex (NN, EE (Figure 3.2) and EN). The calculated energies for these structures were similar and the comparison of the calculated and experimental circular dichroism spectra supported the presence of multiple 2:2 complexes. Therefore, different excimer intensities observed for (R)- and (S)-NpOH in the presence of β-CD could be due to different binding dynamics for 2:2 complexes or due to a different distribution of the three 2:2 complexes (NN, EE, EN).

The formation of multiple 2:2 complexes for the NpOH/β-CD system was supported by the simultaneous observation of the excimer emission from a NpOH dimer and the presence of long-lived triplet-excited states. In previous work where 2:2 complexes were
formed with CDs for naphthalene/β-CD or α-terthiopene/γ-CD, the observation of excimer emission was accompanied by the lack of detection of any triplet-excited states of the guest probably due to the efficient formation of the excimer. These guests were symmetrical and sufficient mobility existed in their 2:2 complexes for the formation of the dimer responsible for the excimer emission. In the case of NpOH bound to β-CD, the ethanol substituent can “lock” the naphthyl moieties in the $N$ complex eliminating the possibility that the naphthyl moieties can overlap in the $NN$ complex, while in the $EE$ complex the naphthyl moieties are in close proximity. Excimer emission from the $EN$ complex is unlikely because the two naphthyl moieties are also far apart. Therefore, the excimer emission in the NpOH/β-CD system is due to the presence of the $EE$ complex, while the long-lived triplet states occur in the $EN$ and $NN$ complexes.

Figure 3.1. Formation of 1:1 complexes of (R)-NpOH with β-CD where the naphthyl (N) or ethanol (E) moieties of the guest enter the CD cavity from the wider rim. The secondary hydroxyl groups on the wider rim are shown in blue while the primary hydroxyl groups on the narrower rim are shown in red. The structures were calculated previously in our group.²⁵²
Figure 3.2. The structures of two types of 2:2 (R)-NpOH-β-CD complex. The structures were calculated previously in our group.\textsuperscript{252}

3.1.2 Objectives

The first objective of this work was to study how chirality affects the binding dynamics of chiral guests to β-CD. In this work the kinetics for the formation of 2:2 complexes was investigated using stopped-flow on the millisecond time-scale. These studies were performed at 10 °C in order to further differentiate the kinetics from the time-resolution of the stopped-flow system. The complexation dynamics for the $E$ complex and the $N$ complex is fast on the millisecond time-scale and reactions 3.1 and 3.2 (Scheme 1.1) are complete within the mixing time of the stopped-flow experiment (~1 ms).
The complexation process for the NpOH-β-CD system

\[
\begin{align*}
\text{Np} + \text{CD} & \xrightleftharpoons{K_N} \text{N} \quad \text{(3.1)} \\
\text{Np} + \text{CD} & \xrightleftharpoons{K_E} \text{E} \quad \text{(3.2)} \\
\text{N} + \text{N} & \xrightleftharpoons{K_{NN}} \text{NN} \quad \text{(3.3)} \\
\text{E} + \text{N} & \xrightleftharpoons{K_{EN}} \text{EN} \quad \text{(3.4)} \\
\text{E} + \text{E} & \xrightleftharpoons{\frac{k_{EE}^+}{k_{EE}^-}} \text{EE} \quad \text{(3.5)} \\
K_{EE} &= \frac{k_{EE}^+}{k_{EE}^-} \quad \text{(3.6)}
\end{align*}
\]

Scheme 3.2. The complexation process for the NpOH-β-CD system

The second objective of this work was to study the chiral discrimination for the formation of 1:1 and 2:2 complexes using a kinetic technique. Chiral discrimination was not apparent, within experimental errors, for the equilibrium constants for the formation of the 1:1 and 2:2 complexes in the previous work.\textsuperscript{203} In other words, the binding study did not provide sufficient information to explain the higher intensity for the excimer emission observed in the case of (R)-NpOH when compared to its enantiomer.\textsuperscript{252} The kinetic technique employed in this work provided information unavailable from binding studies in order to understand the chiral recognition observed from steady-state fluorescence experiments.

3.2 Experimental Section

3.2.1 Instrumentation

UV-vis absorption spectra were recorded by using a Varian Cary 1 spectrophotometer with samples kept at room temperature.
A PTI QM-2 fluorimeter was employed to measure steady-state fluorescence spectra. The excitation wavelength was set to 280 nm, and the emission spectra were recorded between 300 nm and 500 nm. The bandwidths for the excitation and emission monochromators were 2 nm. The samples were contained in 10 mm × 10 mm quartz cells which were thermostated using a circulating water bath for at least 15 min at (10.0 ± 0.1) °C before experiments were performed.

Fluorescence decays and time-resolved emission spectra (TRES) were measured with an Edinburgh OB 920 single photon counter. The excitation source was a 277 nm pulsed light emitting diode (EPLED280 – Edinburgh Instruments Ltd.). Decays were collected at 330 nm or 380 nm (monochromator bandwidth of 16 nm) and a Ludox (Aldrich) solution was used to collect the instrument response function (IRF) by scattering the EPLED light. The IRF was used to deconvolute the lamp profile from the fluorescence decays. The solutions containing NpOH or the mixture of NpOH and β-CD were held in 10 mm × 10 mm quartz cells which were thermostated to (10.0 ± 0.1) °C for at least 15 min using a circulating water bath. The decays were collected until at least 2,000 counts were reached in the channel with maximum intensity.

TRES were collected at every 10 nm between 300 nm and 500 nm by keeping the collection time constant at 5 min, which corresponded to a collection of ca. 20,000 counts in the channel of maximum intensity for the maximum emission wavelength of 330 nm. Each decay trace was integrated between specific time delays to construct the emission spectra.

A SX20 stopped flow system from Applied Photophysics Ltd was employed to measure the kinetics for the formation of the 2:2 host-guest complexes. The dead time for
the mixing of two solutions was 1 ms. The samples were excited at 280 nm (9.3 nm bandwidth) and fluorescence was collected by selecting the emission wavelengths by means of an interference filter with the center wavelength of 405 nm (FWHM of 38 nm, Melles Griot, lot 13637 (ANDV7248)). The NpOH solution was contained in one syringe while the β-CD solution was contained in the second syringe and the two solutions were mixed in a 1:1 ratio. Therefore, the final concentrations of NpOH and β-CD were half of the concentrations prepared for the solutions contained in the syringes. Samples were thermostated at (10.0 ± 0.1) °C using a circulating water bath for at least 20 min before experiments were performed. The following control experiments were performed: (i) injection of 0.2 M NaCl solution against 0.2 M NaCl solution to determine the zero intensity value for the system. (ii) injection of β-CD solution against 0.2 M NaCl solution to determine the baseline value for the system. The values for control experiments (i) and (ii) are very similar and are less than 1.8% of the lowest offset for the system containing the lowest concentration of NpOH (50 µM) and β-CD (50 µM).

Laser flash photolysis (LFP) was employed to measure the kinetics for the triplet-excited state of NpOH. Samples were excited with a Quanta-Ray Lab 130-4 pulsed Nd:YAG laser at 266 nm (≤ 20 mJ/pulse, Spectra Physics). The transient absorption changes at 420 nm were simultaneously detected on two oscilloscopes. This procedure was required to determine the kinetics at short and long times. The samples contained in 10 mm × 10 mm quartz cells were maintained at (10.0 ± 0.1) °C for at least 15 min using a modified cryostat (Unisoku USP-203 cryostat) with 4 windows. The laser irradiation exits the sample compartment through a back window. The monitoring beam was perpendicular to the laser beam and a pinhole was placed on the entrance for the
monitoring beam into the cryostat to ensure that the width of the monitoring beam was smaller than the width of the laser excitation beam.

### 3.2.2 Materials.

β–CD was a generous gift from Cerestar (lots C6034-13 and F6080-191)), (R)- and (S)-2-naphthyl-1-ethanol (NpOH, Fluka), sodium chloride (BDH) were used without further purification. (R)- and (S)-NpOH were checked for the presence of fluorescent impurities by measuring the fluorescence decay of 10 µM aqueous solutions of NpOH. The decay was mono-exponential for each enantiomer indicating that no other emissive species were present in solution. NaNO$_2$ (Aldrich) was recrystallized from water. Deionized water (Barnstead NANOpure, 17.8 MΩ cm$^{-1}$) was employed for all samples.

### 3.2.3 Solution preparation

All aqueous solutions contained 0.2 M NaCl. NpOH (1 mM) and β–CD stock solutions (10 mM) were prepared by dissolving the appropriate amount of solids in 0.2 M NaCl aqueous solutions. The NpOH/β-CD solution with the most concentrated β-CD (10 mM) was prepared by dissolving solid β-CD into the NpOH/NaCl aqueous solution. Solutions at lower concentrations were prepared by dilution. All solutions were shaken overnight at room temperature before experiments were conducted. Samples for laser flash photolysis experiments were purged with N$_2$O for 20 min in order to trap solvated electrons formed from the photoionization of aromatic guests in aqueous host-guest solutions.$^{254,256}$ Oxygen, which is a triplet-excited state quencher,$^{204,257,258}$ was also removed by the purging process. Samples for other experiments, such as steady-state fluorescence, stopped-flow, SPC and TRES experiments, were performed using aerated solutions.
3.2.4 Quenching procedures

For the triplet quenching experiments, NaNO$_2$ solutions were employed as the quencher solutions. NaNO$_2$ solutions were prepared daily by dissolving the appropriate amount of solids in aqueous solutions. NaNO$_2$ solutions were deaerated by purging N$_2$O for 20 min prior to use. The appropriate amount of quencher solution was injected into the 2.5 mL samples by using a gas-tight syringe.

3.2.5 Time-resolved fluorescence

The fluorescence decays were fitted to a sum of exponentials (Equation 3.7) using the Fast software (version 3, Edinburgh Instruments Ltd.) so that the lifetimes ($\tau_i$) and the pre-exponential factors ($A_i$) were determined. The quality of each fit was judged by the residuals plot and the value of the reduced chi-squared parameter ($\chi^2$). The number of exponentials for each fit was increased until the residuals were random and the $\chi^2$ values were between 0.9 and 1.2.$^{241}$

$$I_t = I_0 \times \sum_{i=1}^{n} A_i \times e^{-\frac{t}{\tau_i}}$$

(3.7)

At high NpOH concentrations multicomponent mixtures of fluorophores with different emission spectra are present in the sample. The lifetime for each fluorophore is common to the fluorescence decays collected at different wavelengths for the same solution. For this reason, the global analysis technique$^{241,259}$ was employed to determine the lifetime for each fluorophore. The fluorescence decays collected at two wavelengths (330 nm and 380 nm) for the same solutions were simultaneously fitted using the Fast software.
3.2.6 Binding isotherms for 1:1 complexes

Binding isotherms for 1:1 complexes were determined using solutions with NpOH at low concentrations (5 µM). Spectra of NpOH in the absence or presence of β-CD were corrected by subtracting a baseline, which corresponded to a spectrum for a solution containing all chemicals with the exception of the fluorophore (NpOH). Such a correction was required because of the Raman emission from the solvent and impurities in the CD sample which showed a low, continuously decreasing emission between 300 nm and 375 nm. The corrected spectra were integrated between 315 nm and 500 nm and the dependence of the integrated intensity on the β-CD concentration was fitted numerically using Scientist 3 from Micromath (see below). Briefly, an equation is defined for the measured variable, i.e. the fluorescence intensity, as a function of emission efficiencies (\(C_N\) and \(C_{11}\)) and concentration of NpOH in the aqueous phase ([\(G\)]) or bound to CD ([\(HG\)]). The emission efficiencies (\(C_i^0\)) are related to the fluorescence quantum yields for each species and instrumental settings, such as the excitation light intensity and bandwidth. The magnitude for the \(C_i^0\) values can vary between experiments performed on different days. However, relative \(C_i\) values, i.e. the ratios of \(C_i^0\) to \(C_N^0\), are constant for each NpOH enantiomer. The emission efficiencies discussed below are the relative emission efficiencies unless otherwise stated. The relationship between the concentrations of the various NpOH and CD species is defined by the equilibrium constant for the 1:1 complex and the mass balance equations. The equilibrium constant determined by this procedure (\(K_{11}\)) corresponds to the sum of \(K_N\) and \(K_E\) (see Section 3.3.2).

The model employed in Scientist 3 (model 1) is listed as follows:
The total concentration of β-CD ([H$_0$]) is defined as the independent variable. The equilibrium concentration of free host ([H]), free guest ([G]), 1:1 complex ([HG]) and the fluorescence emission intensity ($I_{\text{mon}}$) are defined as dependent variables. The emission efficiency for the 1:1 complex ($C_{11}$) and the equilibrium binding constant for the 1:1 complex ($K_{11}$) are defined as parameters in the model. $R$, the ratio of the fluorescence emission intensity ($I_{\text{mon,0}}$) of NpOH in the absence of β-CD to the total concentration of NpOH ([G$_0$], 5 µM), is a constant for each independent experiment.

Equations for model 1 are listed as follows:

\[ [HG] = K_{11} \times [G] \times [H] \] \hspace{1cm} (3.8)
\[ [H] = [H_0] - [HG] \] \hspace{1cm} (3.9)
\[ [G] = 5 \times 10^{-6} - [HG] \] \hspace{1cm} (3.10)
\[ I_{\text{Mon}} = R \times ([G] + C_{11} \times [HG]) \] \hspace{1cm} (3.11)

where $R = \frac{I_{\text{mon,0}}}{[G_0]}$ \hspace{1cm} (3.12)

Ranges for the dependent variables are listed as follows:

\[ 0 < [H] < 0.01 \text{ M} \] \hspace{1cm} (3.13)
\[ 0 < [G] < 5 \text{ µM} \] \hspace{1cm} (3.14)
\[ 0 < [HG] < 5 \text{ µM} \] \hspace{1cm} (3.15)

### 3.2.7 Determination of monomer and excimer emission intensities

At high NpOH concentrations 1:1 and 2:2 complexes are present. Concentrations of $EN$ and $NN$ are incorporated into the intensity for the monomer emission, which was related to the integration of the spectrum between 322 nm and 338 nm ($I_{330}$). The concentration of $EE$ is related to the excimer intensity ($I_{390}$) integrated between 382 nm
and 398 nm. However, the monomer and excimer spectra overlap and each one of the intensities was corrected for the contribution from the other species, where $f_i$ was related to the monomer intensity ratio between 390 nm and 330 nm and $f_2$ was related to the excimer emission intensity ratio between 330 nm and 390 nm.

$$I_{330} = I_{330}^{cor} + I_{390}^{cor} \times f_2$$  \hspace{1cm} (3.16)

$$I_{390} = I_{330}^{cor} \times f_1 + I_{390}^{cor}$$  \hspace{1cm} (3.17)

A value of $0.036 \pm 0.002$ was determined for $f_1$ from NpOH emission spectra in water, while the value of $0.565 \pm 0.007$ for $f_2$ was determined from the TRES spectra of NpOH/β-CD solutions at long delays (see Section 3.4.3). The corrected intensities for the monomer (Equation 3.18) and excimer (Equation 3.19) emissions are given by:

$$I_{330}^{cor} = 1.02 \times I_{330} - 0.58 \times I_{390}$$  \hspace{1cm} (3.18)

$$I_{390}^{cor} = 1.02 \times I_{390} - 0.037 \times I_{330}$$  \hspace{1cm} (3.19)

The values for the emission efficiencies were different for experiments performed on different days as aforementioned. For this reason, the corrected intensities for the monomer ($I_{330}^{cor}$) were normalized to unity for experiments performed with the same concentrations of NpOH in the absence of β-CD.

### 3.2.8 Binding isotherms for the formation of 2:2 complexes

Binding isotherms for $EE$ complexes were determined by numerically fitting the dependence of the corrected intensities for the excimer on the β-CD concentration. The Scientist 3 program was employed for the data fitting (see below). Briefly, an equation is defined for the measured variable, i.e. the fluorescence intensity for the excimer ($I_{390}^{cor}$), as a function of emission efficiencies ($C_{EE}$) and concentration of NpOH bound to CD as the $EE$ complex. The emission efficiency $C_{EE}^0$ is related to the fluorescence quantum yields
for each species and instrumental settings, such as the excitation light intensity and bandwidth. The magnitude for the absolute \( C_{EE}^0 \) values can vary between experiments performed on different days. However, the relative \( C_{EE} \) values, i.e. the ratio of \( C_{EE}^0 \) values to \( C_{Np}^0 \) values, are constant. The relationship between the concentrations of the various NpOH and CD species is defined by \( K_N, K_E \), the equilibrium constant for the \( EE \) complex \( (K_{EE}) \) and the mass balance equations. The fittings were conducted under the assumption that the values of the equilibrium constants for all 2:2 complexes are the same, i.e. the equilibrium constants for the \( EN \) complex \( (K_{EN}) \) and the \( NN \) complex \( (K_{NN}) \) are the same as \( K_{EE} \) (see Section 3.3.1).

The model employed in Scientist 3 (model 2) is listed as follows:

The total concentration of \( \beta-CD \) species \( ([H_0]) \) was defined as the independent variable. The equilibrium concentration of \( \beta-CD \) free in water \( ([H]) \), NpOH free in water \( ([G]) \), \( E \) complex \( ([E]) \), \( N \) complex \( ([N]) \), \( EE \) complex \( ([EE]) \), \( EN \) complex \( ([EN]) \), \( NN \) complex \( ([NN]) \) and the fluorescence emission intensity for the \( EE \) complex \( (I_{EE}) \) were defined as dependent variables. The relative emission efficiency for the \( EE \) complex \( (C_{EE}) \), the equilibrium constant for the \( E \) complex \( (K_E) \) and the equilibrium constant for the \( EE \) complex \( (K_{EE}) \) were defined as parameters in the model. The total concentration of NpOH species was 150 \( \mu \)M.

Equations for model 2 are listed as follows:

\[
[E] = K_E \times [G] \times [H] \tag{3.20}
\]
\[
[N] = (K_{11} - K_E) \times [G] \times [H] \tag{3.21}
\]
\[
[EE] = K_{EE} \times [E]^2 \tag{3.22}
\]
\[
[EN] = K_{EN} \times [E] \times [N] \tag{3.23}
\]
\[ [\text{NN}] = K_{\text{NN}} \times [\text{N}]^2 \quad (3.24) \]
\[ [\text{H}_0] = [\text{H}] + [\text{E}] + [\text{N}] + 2 \times ([\text{EE}] + [\text{EN}] + [\text{NN}]) \quad (3.25) \]
\[ 1.50 \times 10^{-4} = [\text{G}] + [\text{E}] + [\text{N}] + 2 \times ([\text{EE}] + [\text{EN}] + [\text{NN}]) \quad (3.26) \]
\[ I_{\text{EE}} = C_{\text{EE}} \times [\text{EE}] \quad (3.27) \]

Ranges for the dependent variables are listed as follows:

\[
0 < [\text{H}] < 0.01 \text{ M} \quad (3.28)
\]
\[
0 < [\text{E}] < 150 \mu\text{M} \quad (3.29)
\]
\[
0 < [\text{N}] < 150 \mu\text{M} \quad (3.30)
\]
\[
0 < [\text{EE}] < 75 \mu\text{M} \quad (3.31)
\]
\[
0 < [\text{EN}] < 75 \mu\text{M} \quad (3.32)
\]
\[
0 < [\text{NN}] < 75 \mu\text{M} \quad (3.33)
\]

### 3.2.9 Triplet quenching methodology

In the absence of \(\beta\)-CD the decay for the triplet-excited state of NpOH follows a mono-exponential function, while in the presence of \(\beta\)-CD the kinetics follow the sum of two exponential functions (Equation 3.34) instead of a mono-exponential function.

\[
\Delta A = a_0 + a_1 e^{-k_{\text{obs}_1}^\text{fp} t} + a_2 e^{-k_{\text{obs}_2}^\text{fp} t} \quad (3.34)
\]

where \(a_1\) and \(a_2\) are pre-exponential factors for the fast \((k_{\text{obs}_1}^\text{fp})\) and slow \((k_{\text{obs}_2}^\text{fp})\) decays.

The rate constants \((k_{\text{obs}_i}^\text{fp})\) are the reciprocals of the lifetimes of the triplet-excited states of the species \(i\). The values of \(k_{\text{obs}_i}^\text{fp}\) recovered from the fit to equation 3.34 are very dependent on the value of \(a_0\). Therefore, \(a_0\) was determined from a kinetic trace collected at a sufficiently long collection time to determine a constant absorbance value after the decay of all triplets. The kinetic traces at two different collection times were determined simultaneously. The kinetic trace at a long collection time contains a fast decay followed by a slow decay. The slow decay corresponds to the time window when the fast
component had mostly decayed (e.g. when the starting time of the time window is 10 times longer than the shorter lifetime of the triplet-excited NpOH species, only 0.0045% of this triplet-excited NpOH species still remains in the system). As a result, the value of \( k_{\text{obs2}}^{\text{lfp}} \) was obtained by fitting the slow decay to a mono-exponential decay with a fixed value of \( a_0 \) (Figure 3.3). The kinetic trace at a short collection time corresponds to the decay of two triplet-excited NpOH species with different lifetimes. Therefore, the kinetic trace at a short collection time was fit with Equation 3.34. The values of \( k_{\text{obs2}}^{\text{lfp}} \) and \( a_0 \) were fixed and the parameters \( a_1 \), \( a_2 \) and \( k_{\text{obs1}}^{\text{lfp}} \) were determined (Figure 3.4). The values of \( a_i \) and \( k_{\text{obs1}}^{\text{lfp}} \) determined by the aforementioned method (multi-step fitting) are more accurate than those determined by one-step fitting (i.e. simply fitting the kinetics to equation 2.30 without any fixed parameters) since the standard deviation for each parameter recovered from the multi-step fitting is much lower than that from the one-step fitting. The multi-step fitting and the one-step fitting were both conducted with Kaleidagraph 4.1 (Synergy Software).

Figure 3.3. Data fitting with the mono-exponential function model for a LFP trace collected in a long collection time. The value of \( a_0 \) was fixed and the value of \( k_{\text{obs2}}^{\text{lfp}} \) was recovered. (red: the experimental curve; black: the calculated curve; \([\text{S)}-\text{NpOH}] = 150 \mu\text{M}; [\beta\text{-CD}] = 10 \text{ mM}; [\text{NaNO}_2] = 0.35 \text{ mM}; [\text{NaCl}] = 0.2 \text{ M}; 10 ^\circ\text{C})
Figure 3.4. Data fitting with the sum of two exponential functions model for a LFP trace collected in a short collection time. The values of \( a_0 \) and \( k_{\text{obs}1}^{\text{lip}} \) were fixed and the values of \( a_1, a_2 \) and \( k_{\text{obs}2}^{\text{lip}} \) were recovered. (red: the experimental curve; black: the calculated curve; \( [(S)\text{-NpOH}] = 150 \text{ } \mu\text{M}; \beta\text{-CD} = 10 \text{ mM}; [\text{NaNO}_2] = 0.35 \text{ mM}; [\text{NaCl}] = 0.2 \text{ M}; 10^\circ \text{C} \))

Parameter \( k_{\text{obs}1}^{\text{lip}} \) is related to the decay of triplet NpOH in water and in the \( N \) and \( E \) complexes for which quenching is relatively efficient and the interconversion between NpOH in water and in the \( E \) and \( N \) complexes is fast. Parameter \( k_{\text{obs}2}^{\text{lip}} \) is related to the decay of the \( NN \) and \( EN \) complexes (for \( EE \) no triplet is formed) for which access of an ionic quencher is significantly inhibited. Therefore the addition of nitrite anions as quenchers leads to a more pronounced increase in \( k_{\text{obs}1}^{\text{lip}} \) than in \( k_{\text{obs}2}^{\text{lip}} \) achieving a larger differentiation between these two rate constants.

In principle, the values of \( a_2 \) can be related to the concentrations of NpOH in the \( NN \) and \( EN \) complexes (see Appendix a.5). The decay traces for both NpOH enantiomers were collected under the same experimental condition (i.e. the same energy of the excited laser pulses, the same concentration of both NpOH enantiomers and the same concentration of \( \beta\text{-CD} \)). Therefore, the comparison between the values of \( a_2 \) for (R)- and
(S)-NpOH could be employed to study in which NpOH enantiomer/β-CD system more NN and EN complexes are formed compared to the EE complex.

3.2.10 Fitting procedures for stopped-flow experiments

The kinetics for the formation of the EE complex was studied by stopped-flow. All reactions shown in Scheme 3.2 are coupled and therefore need to be considered when analyzing the kinetics for EE formation. Several approaches were employed: (i) fit of the data to a mono-exponential function or a function of the sum of exponentials using the Pro-Data viewer from Applied Photophysics; (ii) the second-order global analysis. The exponential fitting methods were employed to check how many kinetic relaxation processes could be observed for the kinetic traces. When the stopped-flow traces were studied by the second-order global analysis, the traces determined under different experimental conditions (e.g. different initial concentrations of NpOH and β-CD) were simultaneously fitted with non-linear least-squares regression using Pro-Kineticist II from Applied Photophysics Ltd. The fitting models were based on a kinetic scheme where a fast equilibrium for the formation of 1:1 complexes is followed by a slow process for the formation of 2:2 complexes. The equilibrium constant for the formation of 1:1 complexes ($K_{11}$), the association ($k_{+2}^{22}$) and dissociation rate constants ($k_{-2}^{22}$) for the formation of 2:2 complexes were the global parameters in the common fitting models. These global parameters were shared by all traces during the fitting and were varied to minimize the sum of the chi-squared values for all traces. The equilibrium constant ($K_{22}$) for the formation of 2:2 complexes was then calculated from the ratio of rate constants. The equilibrium and rate constants for the formation of 2:2 complexes were employed to determine the equilibrium and rate constants for the formation of EE complex under the
assumption that the values of the equilibrium constants for all 2:2 complexes are the same, i.e. the equilibrium constants for the EN complex ($K_{EN}$) and the NN complex ($K_{NN}$) are the same as $K_{EE}$ (see Section 3.3.1).

3.2.11 The simulations for stopped-flow experiments

Simulations of the kinetics were conducted to check to what extent a difference between the rate constants for the formation of different 2:2 complex will lead to two kinetic relaxation processes differentiable in the kinetic measurements. The model for the simulations, which includes all reactions shown in Scheme 1.1, was employed in Pro-Kineticist II. The simulated kinetic traces were generated by two methods: (i) varying the association rate constants for the NN ($k_{NN}^+$) and EN complex ($k_{EN}^+$) while other parameters ($K_E$, $K_N$, $k_{EE}^+$ and the dissociation rate constants for all 2:2 complexes) were fixed. The dissociation rate constants for all 2:2 complexes were set to the same value. (ii) varying the dissociation rate constants for the NN ($k_{NN}^-$) and EN complex ($k_{EN}^-$) while other parameters ($K_E$, $K_N$, $k_{EE}^+$ and the association rate constants for all 2:2 complexes) were fixed. The association rate constants for all 2:2 complexes were set to the same value. Moreover, the simulations were conducted under three different conditions to check how the ratio of $K_E$ to $K_N$ affected the differentiation of two kinetic relaxation processes: (i) the value of $K_E$ was equal to that of $K_N$, (ii) the value of $K_E$ was higher than that of $K_N$, e.g. the value of $K_E$ was 5 times higher than that of $K_N$, (iii) the value of $K_E$ was lower than that of $K_N$, e.g. the value of $K_E$ was 5 times lower than that of $K_N$.

The differentiation of two kinetic relaxation processes was indicated by the fact that the simulated kinetic traces cannot be fitted well with the analysis model which was
employed to fit the experimental data (shown in Section 3.4.8 below) and the residuals plot showed nonrandom scatter around 0 (shown in Figure 3.5).

Figure 3.5. Residuals between the simulated data and the fit to the second-order fitting model. The simulated data were generated under the condition listed as follows: [NpOH] = 300 µM, [β-CD] = 100 µM, $K_N = 500 \ M^{-1}$, $K_N = 500 \ M^{-1}$, $k_{EE}^{EE} = 9.28 \times 10^5 \ M^{-1}s^{-1}$, the dissociation rate constants for all 2:2 complex were set to 115 s$^{-1}$. The association rate constants for the EN and NN complexes were set to different percentage of $k_{EE}^{EE}$: (a) 90%, (b) 95%, (c) 100%, (d) 105%, (e) 110%. The traces b, c and d were recognized as random patterns while the traces a and e were not.

### 3.3 Data analysis

#### 3.3.1 Prerequisites for the determination of the equilibrium constants for 2:2 complexes

The emission spectra of 150 µM NpOH in the absence and presence of β-CD were determined in steady-state fluorescence experiments. The numerical curve fitting method
was employed to fit the data using model 2 in Scientist 3 (as aforementioned in Section 3.2.8).

The fitting was conducted under the assumption that the values of the equilibrium constants for all 2:2 complexes (e.g. $K_{EE}$, $K_{EN}$ and $K_{NN}$) are the same. This assumption is reasonable for the following reasons. Only one kinetic relaxation process can be observed for every stopped-flow trace indicating that the rate constants for all 2:2 complexes are similar (see Section 3.5.2). Therefore, the equilibrium constants for all 2:2 complexes, calculated from the ratio of rate constants, would be similar. The assumption of equal equilibrium constants is also supported by the simulation for the kinetics (see Section 3.5.2), which led to an upper limit of 25% for the difference between the equilibrium constants for the formation of the 2:2 complexes. Another supporting evidence is that the total energy calculated for the three 2:2 complexes were similar, i.e. no stereoselectivity is apparent for different 2:2 complexes from the energetic point of view.\(^\text{252}\)

The equilibria for the formation of 1:1 and 2:2 complexes are coupled, so the value of $K_{EE}$ can be determined only when the value of $K_E$ is known and is fixed in the numerical analysis. The value of $K_E$ cannot be determined from steady-state fluorescence experiments. However, a range of $K_E$ values can be determined (see Section 3.3.2), which was then employed to determine a range of $K_{EE}$ values.

### 3.3.2 Determination of $K_{11}$

The value of $K_E$ was required to determine the equilibrium and rate constants for the formation of the 2:2 complexes as aforementioned. However, the value of $K_E$ cannot be determined by numerically fitting the dependence of the fluorescence intensity on the $\beta$-CD concentration while the value of $K_{11}$ can. This phenomenon happens because there is
no spectroscopic signature differentiating the E complex from the N complex, as detailed below.

The key equation 3.35 was defined for the measured variable, the fluorescence intensity \( I_{\text{Mon}} \), as a function of emission efficiencies \( C_{\text{Np}}, C_{\text{E}} \) and \( C_{\text{N}} \) and the concentration of NpOH in the aqueous phase \([\text{Np}]\) or bound to CD \([\text{E}]\) and \([\text{N}]\). Equation 3.36 was derived from equation 3.35 and was then employed in the fitting model for the determination of the values of \( K_{\text{E}} \) and \( K_{\text{N}} \). However, the values of \( K_{\text{E}} \) and \( K_{\text{N}} \) cannot be determined using this methodology because of the following reason. The ratio of \( C_{i} \) can be determined as the ratio of excited state lifetimes \( \tau_{i} \) for the E and N complex (see Appendix a.3). The value of \( C_{\text{N}} \) and \( C_{\text{E}} \) should be very similar since the lifetimes for the E and N complex were very similar. Therefore, equation 3.36 can be derived as equation 3.37 where \( C_{11} \) was defined as the emission efficiencies for both E and N complexes. Equation 3.37 indicates that only the sum of \( K_{\text{E}} \) and \( K_{\text{N}} \) can be determined no matter how \( K_{\text{E}} \) and \( K_{\text{N}} \) are different. The value for the sum of \( K_{\text{E}} \) and \( K_{\text{N}} \) \((K_{11} \text{ in equation 3.38})\) constitutes the theoretical upper limit for the values of \( K_{\text{E}} \).

\[
I_{\text{Mon}} = C_{\text{Np}} \times [\text{Np}] + C_{\text{N}} \times [\text{N}] + C_{\text{E}} \times [\text{E}] \quad (3.35)
\]
\[
I_{\text{Mon}} = C_{\text{Np}} \times [\text{Np}] + (C_{\text{N}} \times K_{\text{N}} + C_{\text{E}} \times K_{\text{E}}) \times [\text{Np}] \times [\text{CD}] \quad (3.36)
\]
\[
I_{\text{Mon}} = C_{\text{Np}} \times [\text{Np}] + C_{11} (K_{\text{N}} + K_{\text{E}}) \times [\text{Np}] \times [\text{CD}] \quad (3.37)
\]
\[
I_{\text{Mon}} = C_{\text{Np}} \times [\text{Np}] + C_{11} \times (K_{11} [\text{Np}] \times [\text{CD}]) \quad (3.38)
\]
3.4 Results

3.4.1 Steady-state fluorescence

The binding behavior for the enantiomers of NpOH with β-CD was investigated at 10 °C. This temperature was lower than for previous experiments (20 °C)\textsuperscript{203,252} in order to lengthen the kinetics and to differentiate it from the dead time of the stopped flow system. The fluorescence intensity of NpOH increases with the addition of β-CD and at high NpOH concentrations an excimer emission was observed as a shoulder around 380 nm (Figure 3.6). In the absence of β-CD or for low concentrations of NpOH (5 µM) in the presence of β-CD, only the structured monomer emission with a maximum at 330 nm was observed (inset Figure 3.6). It is important to note that for 5µM NpOH the enhancement for the monomer emission was the same for both enantiomers of NpOH. At a NpOH concentration of 150 µM the excimer emission is higher for (R)-NpOH than (S)-NpOH, while the relative intensity for the monomer is higher for (S)-NpOH than (R)-NpOH (Figure 3.6).

Figure 3.6. Fluorescence spectra for (R)- and (S)-NpOH (150 µM) in the absence (black) and in the presence of 10 mM β-CD (red: (S)-NpOH, blue: (R)-NpOH). The inset shows the fluorescence spectra for (R)- and (S)-NpOH (5 µM) in the absence (black) and in the
presence of 10 mM β-CD (red: (S)-NpOH, blue: (R)-NpOH; both spectra are superimposed).

3.4.2 Time-resolved fluorescence

Fluorescence lifetime measurements are instrumental to differentiate between the various NpOH species present, because the lifetime of fluorophores in general lengthen when the fluorophore is included in a constraint microenvironment. In water, the decays for the (R) and (S)-NpOH fluorescence measured at 330 nm were mono-exponential with a lifetime of $25.6 \pm 0.2$ ns. This value was only slightly longer than the lifetime determined for NpOH at 20 °C (25 ns), suggesting that the photophysics of NpOH is not very temperature dependent.

The fluorescence decays for 5 µM NpOH in the presence of β-CD were measured at 330 nm because at this NpOH concentration only the monomer emission was observed due to NpOH in water and in the N and E complexes. The impurity emission from β-CD contributes to the decay because of the low concentration ratio between NpOH and β-CD and the lifetime for this impurity was $4.6 \pm 0.7$ ns. The fluorescence decay could not be adequately fitted to a mono-exponential function or to the sum of two exponentials, but fit well to the sum of three exponentials, where one of the lifetimes was close to that observed for the CD impurity emission and the second lifetime was close to 25 ns. Therefore, the lifetimes for the CD impurity emission and for NpOH in water were fixed and a lifetime of 38.1 ns was recovered for NpOH in the 1:1 complex with β-CD. This longer lifetime is consistent with the increase in the steady-state emission intensity observed when NpOH was complexed to β-CD, and is close to the 34 ns lifetime observed at 20 °C. The fact that the same lifetime for the 1:1 complex was observed for
(S)- and (R)-NpOH, despite the fact that a different ratio of $E$ and $N$ complexes exists for the two enantiomers (see discussion section below) showed that the lifetime for the $E$ and $N$ complexes were very similar. The pre-exponential $A$ factors can be related to relative concentrations of different fluorophores when the lifetime of these fluorophores are sufficiently short so that no interconversion between the species occurs while the fluorophore is excited.$^{252,262}$ The lifetimes for the singlet-excited state of NpOH in the $E$ and $N$ complexes are much shorter (< 50 ns) than the relaxation time, i.e. the time for the association and dissociation process to occur, for 1:1 complexes at the highest $\beta$-CD concentration employed (310 ns).$^{203}$ For this reason, $A_1$ and $A_2$ can be related to the fraction of NpOH free in water and bound as 1:1 complexes, respectively.

Table 3.1. Lifetimes ($\tau$, ns) and pre-exponential factors ($A$) for the emission of NpOH at different concentrations in the presence of 10 mM $\beta$-CD.

<table>
<thead>
<tr>
<th>NpOH</th>
<th>$\lambda_{em}$ / nm</th>
<th>[NpOH] / $\mu$M</th>
<th>$\tau_1$ ($A_1$)</th>
<th>$\tau_2$ ($A_2$)</th>
<th>$\tau_3$ ($A_3$)</th>
<th>$\chi^2$ (global $\chi^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)</td>
<td>330</td>
<td>5</td>
<td>25.4$^{b,c}$ (0.14)</td>
<td>37.6$^c$ (0.86)</td>
<td>-</td>
<td>1.064</td>
</tr>
<tr>
<td>(S)</td>
<td>330</td>
<td>5</td>
<td>25.4$^{b,c}$ (0.13)</td>
<td>38.1$^c$ (0.87)</td>
<td>-</td>
<td>1.007</td>
</tr>
<tr>
<td>(R)</td>
<td>380</td>
<td>150</td>
<td>25.4$^{b,d}$ (0.17)</td>
<td>38.0$^{b,d}$ (0.11)</td>
<td>72.7$^d$ (0.72)</td>
<td>0.967 (1.056)</td>
</tr>
<tr>
<td>(R)</td>
<td>330</td>
<td>150</td>
<td>25.4$^{b,d}$ (0.22)</td>
<td>38.0$^{b,d}$ (0.70)</td>
<td>72.7$^d$ (0.08)</td>
<td>1.146 (1.056)</td>
</tr>
<tr>
<td>(S)</td>
<td>380</td>
<td>150</td>
<td>25.4$^{b,d}$ (0.21)</td>
<td>38.0$^{b,d}$ (0.38)</td>
<td>72.0$^d$ (0.42)</td>
<td>1.070 (1.120)</td>
</tr>
<tr>
<td>(S)</td>
<td>330</td>
<td>150</td>
<td>25.4$^{b,d}$ (0.09)</td>
<td>38.0$^{b,d}$ (0.86)</td>
<td>72.0$^d$ (0.05)</td>
<td>1.170 (1.120)</td>
</tr>
</tbody>
</table>

$^a$Errors for $A$ were 0.03 and the errors for the lifetimes were 0.3 ns unless otherwise stated. $^b$Lifetime value was fixed. $^c$The contribution for the emission from the $\beta$-CD impurity was not taken into account in the calculation of the $A$ values. $^d$The global analysis was employed. One independent experiment was performed.
The decay for the excimer emission was measured at 330 nm and 380 nm at a high NpOH concentration (150 µM) when the 1:1 and 2:2 complexes were present. No growth was observed for the emission at 380 nm, which indicates that the excimer was formed within the excitation pulse (1 ns) because the naphthyl moieties of the NpOHs were in close proximity. This assignment is consistent with the absence of triplets when excimer emission is observed in 2:2 complexes of CDs, because intersystem crossing cannot compete with the formation of excimer. It is worth noting that the 2:2 complex has an excimer-like emission because the EE complex has the two naphthyl moieties in close proximity so that excimer emission can occur. However, the 2:2 complex is not an excimer since the 2:2 complex is not a short-lived dimer while the excimer is. The intensity at 380 nm had a significant contribution from the monomer emission and the fluorescence decay also reflects the kinetics of these species. There is no contribution of the CD impurity emission at 380 nm. The decay at 380 nm was adequately fitted to the sum of three exponentials. A longer lifetime was recovered when two lifetimes were fixed as the lifetimes for NpOH in water and in 1:1 complex. The values of the longer lifetime, 73.5 ns and 71.7 ns for (R)- and (S)-NpOH, respectively, were close to the 70 ns lifetime observed at 20 °C. These lifetimes of ca. 70 ns were assigned to the excimer emission.

There is no contribution of the CD impurity emission at 330 nm for an NpOH concentration of 150 µM, which is due to the high concentration ratio between NpOH and β-CD. The decay at 330 nm was adequately fitted to the sum of three exponentials. A longer lifetime was recovered when two lifetimes were fixed as the lifetimes for NpOH in water and in 1:1 complexes. The values of the longer lifetime, 74.5 ns and 59 ns for (R)-
and (S)-NpOH, respectively correlate with those recovered from the decay at 380 nm. It is worth noting that the value of the longer lifetime for (S)-NpOH, 59 ns, is quite shorter than the one recovered from the decay at 380 nm. This observation is due to the fact that the pre-exponential for the component with longer lifetime is small (0.05) and the precision is low. To increase the precision of the lifetimes recovered, a global analysis was employed to simultaneously fit the fluorescence decays collected at 330 nm and 380 nm using the Fast software. The results are shown in Table 3.1.

The $A$ values are related to the concentration of each emissive species because the decays of the singlet-excited state NpOH in the 2:2 complexes are much faster than the 2:2 complex binding dynamics (< 0.4 ms). The lower $A_3$ value observed for (S)-NpOH at 380 nm when compared to (R)-NpOH is consistent with the lower steady-state intensity of excimer emission observed for the (S)-enantiomer at 380 nm (Figure 3.6) and indicates that the concentration of the EE complex for (S)-NpOH is lower than the concentration for the EE complex formed with (R)-NpOH.

### 3.4.3 TRES

The emission spectra for the $EE$ complex were required to determine the monomer and excimer intensities (see Section 3.2.7). However, the emission spectra of $EE$ cannot be obtained from the steady-state fluorescence experiments since the monomer and the excimer spectra overlap. As a result, the normalized TRES spectra of NpOH/$\beta$-CD solutions at long delays were measured to determine the emission spectra of $EE$.

The TRES were constructed by integrating all decay traces (Figure 3.7) between specific time delays. The TRES collected at different time delays were normalized and are shown in Figure 3.8. The normalized TRES obtained for short delays have the bands
at 330 nm. The intensity for the bands at 330 nm decreased while the intensity for bands at 390 nm increased in the normalized TRES obtained for longer time delays. The TRES obtained at short delays contain contributions from all fluorophores with different singlet-excited state lifetimes. The TRES obtained at long delays correspond to the fluorophores with long singlet-excited state lifetimes since the fluorophores with short singlet-excited state lifetimes decayed before long delays. Therefore, the TRES obtained at long delays can be regarded as the emission spectra of the species with long lifetime. In case of NpOH / β-CD system, 2:2 complexes are NpOH species with the longest singlet-excited state lifetime. Therefore, the spectra obtained in long delays can be regarded as the emission spectra of 2:2 complexes. The spectra obtained in long delays have the same shape for both NpOH enantiomers.

Figure 3.7. Decay traces employed to determine the TRES for (R)-NpOH in the presence of β-CD. For all traces, the excitation wavelength was 277 nm. The emission wavelength for each trace was 310 nm (red), 330 nm (black), 390 nm (green), 450 nm (purple) and 500 nm (blue). ([R]-NpOH] = 150 µM, [β-CD] = 10 mM, [NaCl] = 0.2 M at 10 °C.)
Figure 3.8. Normalized TRES for (R)-NpOH in the presence of β-CD. The time window for each spectrum was 0 – 50 ns (red), 100 – 150 ns (green), 200 – 250 ns (blue), 300 – 350 (purple) and 400 – 450 (black). ([ (R)-NpOH] = 150 µM, [β-CD] = 10 mM, [NaCl] = 0.2 M at 10 °C) One independent experiment was conducted.

### 3.4.4 Transient absorption spectroscopy

The decays for the triplet-excited states were measured for NpOH in the absence of β-CD by LFP and the triplet-excited states were quenched by NO$_2^-$ at 10 °C. The triplet decays for NpOH in the presence of NO$_2^-$ could be fitted with a mono-exponential function and the observed rate constants ($k_{\text{obs}}^{\text{lfp}}$) were determined. The values of $k_{\text{obs}}^{\text{lfp}}$ increased with the concentration of NO$_2^-$. The quenching rate constants in aqueous solution ($k_q$) were determined from the quenching plots (Equation 3.39) as shown in Table 3.2.

$$k_{\text{obs}}^{\text{lfp}} = k_{h,i} + k_q \times [\text{NO}_2^-]$$  \hspace{1cm} (3.39)

where $k_{h,i}$ is the rate constant of the triplet decays for NpOH species $i$ in the absence of NO$_2^-$. The triplet decays were measured for NpOH in the presence of β-CD by LFP and the triplet-excited states were quenched by NO$_2^-$ at 10 °C. The triplet decays for NpOH in the presence of β-CD and NO$_2^-$ were fitted with a mono-exponential function and the
residuals between the experimental data and the fit were non-random. This observation indicated that there was more than one type of triplet-excited state for \( \text{NpOH} \). Therefore, the triplet decays were fitted with the sum of two exponentials and the observed rate constants (\( k_{\text{obs}}^{\text{lip}} \) and \( k_{\text{obs}}^{\text{lip}} \)) were determined as aforementioned in Section 3.2.9. The value of \( k_{\text{obs}}^{\text{lip}} \) increased with the addition of \( \text{NO}_2^- \) more dramatically than the value of \( k_{\text{obs}}^{\text{lip}} \) did (Figure 3.9). The quenching rate constants (\( k_q \)) were determined from the quenching plots (Equation 3.39)\(^{261} \) as shown in Table 3.2.

![Graph](image)

Figure 3.9. Quenching plots for the triplet-excited states of \( \text{NpOH} \) species quenched by \( \text{NO}_2^- \) at 10 °C ([\((\text{S})-\text{NpOH}\)] = 150 \( \mu \)M, [\( \beta \)-CD] = 10 mM, [NaCl] = 0.2 M). The data shown in blue correspond to the quenching of \( \text{NpOH} \) in the 1:1 complexes whereas the data in red are for the quenching of \( \text{NpOH} \) in the 2:2 complexes.

Table 3.2. Quenching rate constants for triplet-excited states of \( \text{NpOH} \) species by \( \text{NO}_2^- \)

<table>
<thead>
<tr>
<th>( \text{NpOH} ) species</th>
<th>( k_q / 10^7 \text{ M}^{-1} \text{ s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{NpOH} ) free in water (( k_{\text{obs}}^{\text{lip}} ))</td>
<td>251 ( \pm ) 3</td>
</tr>
<tr>
<td>1:1 complex (( k_{\text{obs}}^{\text{lip}} ))</td>
<td>88 ( \pm ) 2</td>
</tr>
<tr>
<td>2:2 complex (( k_{\text{obs}}^{\text{lip}} ))</td>
<td>2.9 ( \pm ) 0.1</td>
</tr>
</tbody>
</table>

*The errors correspond to the standard deviation obtained from two independent experiments. The experiment was conducted at 10 °C.*
The quenching rate constants for the triplet-excited states of NpOH free in water determined at 10 °C were similar to the value of (3.1 ± 0.1)×10⁹ M⁻¹ s⁻¹ determined at 20 °C in a previous study.¹⁸⁹ The quenching rate constants for the triplet-excited state of NpOH in the absence of β-CD were much higher than those for the triplet-excited state of NpOH in the presence of β-CD. Moreover, the quenching rate constants for the fast decay component \( k_{\text{obs}1}^{\text{fip}} \) were much higher than those for the slow decay component \( k_{\text{obs}2}^{\text{fip}} \).

The quenching rate constants indicate how efficiently the quencher accesses different NpOH species in the β-CD complexes. The results in Table 3.2 show that the NpOH species bound with β-CD are accessed by NO₃⁻ less efficiently than NpOH free in water, i.e. the β-CD cavity protects the chromophore from quenchers in solution. Two different quenching rate constants were determined for 150 µM NpOHs in the presence of β-CD, as described in the previous paper where Mn²⁺ was employed as quencher.²⁰³ The NpOH species related to the fast decay were accessed by NO₃⁻ much more efficiently than the NpOH species related to the slow decay were accessed. These results indicated that there are at least two different complexes between the triplet-excited state of NpOH and β-CD in the solution and the complexes related to the slow decays offer a better protection from quenching than the complexes related to the fast decay. Since NpOHs in 2:2 complexes are better protected by β-CD than NpOHs in 1:1 complex, the NpOH species related to the fast decay and the slow decay are assigned to the 1:1 and 2:2 complexes, respectively. Moreover, since the triplet-excited state of NpOH in the EE complex can be quenched efficiently by the interaction with another NpOH close-by, as was previously seen for naphthalene as a guest,¹³⁵ 2:2 complexes related to the slow triplet decays correspond to the EN and NN complex instead of the EE complex.
The values of \( a_2 \), i.e. the pre-exponential factor for the slow decay, are 0.023 ± 0.004 and 0.020 ± 0.001 for (S)- and (R)-NpOH, respectively. According to Equation A.64, the values of \( a_2 \) for (R)-NpOH, within the experimental errors, is the same as that for (S)-NpOH, indicating that the concentrations of \( NN \) and \( EN \) complexes in the NpOH/\( \beta \)-CD system are not different for both NpOH enantiomers.

### 3.4.5 Determination of the equilibrium constants for 1:1 complexes

The emission spectra of 5 \( \mu \)M NpOH in the absence and presence of \( \beta \)-CD were determined in steady-state fluorescence experiments. Since there was no 2:2 complex formed when the concentration of NpOH was low, the dependences of the fluorescence intensities for (S)- and (R)-NpOH with the concentration of \( \beta \)-CD were employed to determine the equilibrium constants for the 1:1 complexes. The apparent equilibrium constants of the 1:1 complexes (\( K_{11} \)) and the relative emission efficiencies for the 1:1 complexes (\( C_{11} \)) were determined by numerical fitting (Table 3.3). The residuals plot in Figure 3.10 shows random scatter around 0 indicating that the model employed fits the data well. The apparent equilibrium constants for the 1:1 complexes are the same for the (S)- and (R)-NpOH enantiomers, although the \( K_E \) and \( K_N \) values for each NpOH enantiomer could be different. The values of \( C_{11} \) and the ratios of \( C_{11}^0 \) to \( C_{Np}^0 \), are constant for each NpOH enantiomer and are dependent on the photophysics of each NpOH enantiomer.

The equilibrium constants for the 1:1 complex (\( K_{11} \)) were determined to be ca. 1.1 × 10^3 M\(^{-1}\) at 10 °C. The values of \( K_{11} \) were higher than the value of 8 × 10^2 M\(^{-1}\) determined at 20 °C\(^{203}\) suggesting that low temperature favors the formation of the 1:1 complex.
Table 3.3. Apparent equilibrium constants $K_{11}$ determined from changes in the steady-state fluorescence intensity in the range of 315 – 350 nm at 10 °C. \([\text{NpOH}] = 5 \, \mu\text{M}, [\beta-\text{CD}] = 0 – 10 \, \text{mM}, [\text{NaCl}] = 0.2 \, \text{M}.$

<table>
<thead>
<tr>
<th>enantiomer</th>
<th>$K_{11} / 10^3 , \text{M}^{-1}$</th>
<th>$C_{11}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-NpOH</td>
<td>$1.10 \pm 0.03$</td>
<td>$1.74 \pm 0.01$</td>
</tr>
<tr>
<td>(S)-NpOH</td>
<td>$1.08 \pm 0.03$</td>
<td>$1.78 \pm 0.02$</td>
</tr>
</tbody>
</table>

*Values and errors correspond to averages from three independent experiments.*

Figure 3.10. Numerical fitting for the dependence of the steady-state fluorescence intensity in the range of 315 – 350 nm on [\(\beta\)-CD] (0 – 10 mM) to recover the value of $K_{11}$ of (R)-NpOH-\(\beta\)-CD 1:1 complex ([\(\text{NpOH}\)] = 5 \(\mu\)M and [\(\text{NaCl}\)] = 0.2 M. 10 °C)

3.4.6 Determination of the equilibrium constants for the 2:2 complexes

The emission spectra of 150 \(\mu\)M NpOH in the absence and presence of \(\beta\)-CD were determined in steady-state fluorescence experiments. The numerical curve fitting method was employed to fit the data with model 2 (as aforementioned in 3.2.8) under the assumption that the equilibrium constants for all of three 2:2 complexes are the same. The recovered relative emission efficiencies ($C_{EE}$) and the equilibrium constants for the $EE$ complex ($K_{EE,\text{flu}}$) changed when different values for $K_E$ were used in the fits. However, the fits (e.g. Figure 3.11) based on the distribution of the residuals were equally good for
$K_E$ values between 0 M$^{-1}$ and the value of $K_{11}$, the minimum and the maximum theoretical value possible for this parameter.

The values for $K_{EE,flu}$ which varied between limiting conditions are shown in Figure 3.12. The minimum value for $K_{EE,flu}$ was reached when only one kind of 1:1 complex ($E$ or $N$) predominated in the system, i.e. $K_E$ was equal to $K_{11}$ (condition 1) or $K_N$ was equal to $K_{11}$ (condition 2). The maximum value of $K_{EE,flu}$ was reached when there was no stereoselectivity for different 1:1 complexes, i.e. $K_E$ was equal to $K_N$ (condition 3). The limiting values for $K_{EE,flu}$ are shown in Table 3.4.

The range of the equilibrium constants for the $EE$ complex ($K_{EE}$) at 10 °C were determined as 5800 – 7800 M$^{-1}$ and 4500 – 6000 M$^{-1}$ for (R)- and (S)-NpOH, respectively. The values of $K_{EE}$ are higher than 3000 M$^{-1}$ determined at 20 °C$^{203}$ suggesting that low temperature favors the formation of the 2:2 complex. Moreover, the $K_{EE}$ value for (R)-NpOH is always higher than for (S)-NpOH no matter which $K_E$ value was assumed.

$C_{EE}$ corresponds to the ratio between the fluorescence quantum yield of the $EE$ complex at 380 nm and the quantum yield of NpOH free in water. The values of $C_{EE}$ decreased with the increase of $K_E$ as shown in Figure 3.13. As the value of $K_E$ increased and the value of $K_{11}$ stayed constant, more $E$ complexes were formed which then led to more $EE$ complex generated in the system. As a result, the increase of [EE] (e.g. 0 – 33.6 μM for the solution containing 150 μM (R)-NpOH and 10 mM β-CD) led to the decrease of $C_{EE}$ when the dependence of $I_{EE}$ on [β-CD] was fitted with Equation 3.27. It is worth noting that [E] as well as [EE] is close to 0 when $K_E$ is close to 0 under condition 2. In this case, the value of $C_{EE}$ is close to infinity. This extreme of $C_{EE}$ has no physical
meaning. However, the trend of $C_{EE}$ to approach infinity indicates that there is no upper limit for $C_{EE}$ recovered from changes in the steady-state fluorescence intensity. The limiting values for $C_{EE}$ are shown in Table 3.4.

![Graph showing fluorescence intensity vs. [β-CD] mM](image1)

**Figure 3.11.** Numerical fitting for the dependence of the steady-state fluorescence intensity of the $EE$ complex on [β-CD] to recover the value of $K_{EE}$ of $EE$ complex ([R]-NpOH) = 150 μM, [NaCl] = 0.2 M, and $K_E = 0 – 1100 \text{ M}^{-1}$, 10 °C) using the data from three independent experiments.

![Graph showing $K_{EE,flu}$ vs. $K_E$](image2)

**Figure 3.12.** Dependence of $K_{EE,flu}$ recovered from changes in the steady-state fluorescence intensity on the values of $K_E$ used in the fit. (red: (S)-NpOH, blue: (R)-NpOH.) The lines were included to guide the eye.
Figure 3.13. Dependence of $C_{EE}$ values recovered from changes in the steady-state fluorescence intensity on the values of $K_E$ used. (red: (S)-NpOH, blue: (R)-NpOH.) The lines were included to guide the eye.

Table 3.4. Limiting values of $K_{EE}$ and $C_{EE}$ determined for the three different limiting conditions for $K_E$.$^a$

<table>
<thead>
<tr>
<th>condition</th>
<th>enantiomer</th>
<th>$K_{EE,rej} / 10^3 \text{ M}^{-1}$</th>
<th>$C_{EE} / 10^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ($K_E = K_{II}$)</td>
<td>(R)-NpOH</td>
<td>5.8 ± 0.2</td>
<td>1.43 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(S)-NpOH</td>
<td>4.5 ± 0.2</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>2 ($K_N = K_{II}$)</td>
<td>(R)-NpOH</td>
<td>5.8 ± 0.2</td>
<td>$\infty$</td>
</tr>
<tr>
<td></td>
<td>(S)-NpOH</td>
<td>4.5 ± 0.2</td>
<td>$\infty$</td>
</tr>
<tr>
<td>3 ($K_E = K_N$)</td>
<td>(R)-NpOH</td>
<td>7.8 ± 0.3</td>
<td>4.30 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>(S)-NpOH</td>
<td>6.0 ± 0.3</td>
<td>2.16 ± 0.04</td>
</tr>
</tbody>
</table>

$^a$Values and errors were recovered from the nonlinear fit of the data determined from three independent experiments.

3.4.7 Stopped flow experiments

The emission change over time was measured in stopped flow experiments and the collected spectral region, 385 nm – 423 nm, mainly relates the emission from the $EE$ complex. Figure 3.14 shows that the fluorescence intensities increased with time and
reached a limiting value within 100 ms, i.e. the system reached an equilibrium state within 100 ms. The traces for control experiments are horizontal lines and the intensities are ca. 0.008, which is about 1.8% of the lowest offset, i.e. the offset of the trace \(j\) observed in Figure 3.14.

The magnitude of the fluorescence enhancement increased with the concentration of \(\beta\)-CD in the presence of the same concentration of NpOH, e.g. the magnitude in Figure 3.14 for trace \(a\) for a \(\beta\)-CD concentration of 100 \(\mu\)M is higher than that for \(b\) with a \(\beta\)-CD concentration of 50 \(\mu\)M. Moreover, the magnitude of the fluorescence enhancement increased with the concentration of NpOH in the presence of same concentration of \(\beta\)-CD, e.g. the magnitude increases in order of the traces listed as follows: \(i, g, e, c\) and \(a\) (Figure 3.14). Since the dynamics observed in the stopped flow experiments relates to the formation of 2:2 complexes (see Section 3.5.2), the fact that the intensity amplitudes increased with the addition of NpOH and \(\beta\)-CD indicated that more 2:2 complexes were formed as the concentration of either NpOH or \(\beta\)-CD was enhanced. This inference was consistent with the observation in the steady-state fluorescence experiment.

The kinetic traces did not start at zero intensity and an offset was observed. The offsets were much higher than the values determined in the control experiments (see Section 3.2.1 for the description concerning the control experiments), e.g. the values determined in the control experiments are less than 1.8% of the lowest offsets. The offsets for the same concentration of NpOH increased with the concentration of \(\beta\)-CD, e.g. the offset for trace \(a\) was higher than that for trace \(b\). Since the dynamics for the formation of 1:1 complexes occur during the dead time of the stopped flow equipment, the offset is related to the fluorescence intensity increase because of the formation of 1:1 complexes. The
higher offsets were observed at higher concentration of β-CD. This observation indicates that more 1:1 complexes were formed with the addition of β-CD. This inference is also consistent with the observation in the steady-state fluorescence experiment, where an increase in intensity was observed for the formation of 1:1 complexes.

The kinetic traces were fitted with a mono-exponential function and the residual plot shows random scatter around 0 (e.g. Figure 3.15 and Figure 3.16). This observation indicated that there was only one kinetic relaxation process occurring on the 100 ms time scale. The observed rate constants recovered from the fitting were higher for (S)-NpOH than for (R)-NpOH for the same experimental conditions. This observation is due to different association/dissociation rate constants for the 2:2 complexes for each enantiomer.

![Figure 3.14](image)

Figure 3.14 The stopped-flow traces for (R)-NpOH mixing with β-CD. [(R)-NpOH] = 300 µM (a, b), 200 µM (c, d), 150 µM (e, f), 100 µM (g, h) and 50 µM (i, j); [β-CD] = 100 µM (a, c, e, g, i) and 50 µM (b, d, f, h, j).
3.4.8 Determination of the rate constants for the association and dissociation of the 2:2 complexes

The dynamics observed in the stopped flow experiments is related to the formation of the 2:2 complexes since the kinetics for the formation of 1:1 complex occurs on a time scale faster than milliseconds. The observation of only one relaxation process indicated that the dynamics for the formation of three possible 2:2 complexes (EN, NN, and EE) occur on similar time scales. As a result, the simplified reaction scheme (Scheme 3.3)
was employed to build up a global analysis model in Pro-Kineticist II to fit the kinetic data. The values of $K_{11}$ were fixed to the values determined in the steady-state fluorescence experiments (as aforementioned in Section 3.4.5). The association ($k_{+}^{22}$) and dissociation rate constants ($k_{-}^{22}$) for the formation of the 2:2 complexes were recovered from the global analysis. The residuals pattern shows random scatter around 0 for the complete data set analyzed (Figure 3.17). The fitting results are shown in Table 3.5.

\[
\begin{align*}
\text{Np} + \text{CD} & \xrightleftharpoons{K_{11}} \text{Np-CD} \\
2 \text{Np-CD} & \xrightleftharpoons{k_{+}^{22} / k_{-}^{22}} (\text{Np-CD})_2 \\
K_{22} &= \frac{k_{+}^{22}}{k_{-}^{22}}
\end{align*}
\] (3.40)

Scheme 3.3 A simplified reaction scheme for the β-CD–NpOH system

<table>
<thead>
<tr>
<th>Enantiomer</th>
<th>$k_{+}^{22} / 10^5 \text{ M}^{-1}\text{s}^{-1}$</th>
<th>$k_{-}^{22} / 10^2 \text{ s}^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)-NpOH</td>
<td>6.9 ± 0.3</td>
<td>1.11 ± 0.05</td>
</tr>
<tr>
<td>(S)-NpOH</td>
<td>7.2 ± 0.4</td>
<td>1.63 ± 0.02</td>
</tr>
</tbody>
</table>

*Values and errors were recovered from two independent experiments where the second-order global fittings of the data were conducted independently.*
Figure 3.17. Residuals between the experimental data and the fit to the second-order global fitting model. The stopped-flow traces for (R)-NpOH mixing with β-CD were shown in Figure 3.14. [(R)-NpOH] = 300 µM (a, b), 200 µM (c, d), 150 µM (e, f), 100 µM (g, h) and 50 µM (i, j); [β-CD] = 100 µM (a, c, e, g, i) and 50 µM (b, d, f, h, j).

Within experimental errors the $k_{+22}$ values are the same while a higher $k_{-22}$ value was observed for (S)-NpOH than for (R)-NpOH, explaining the faster growth of the excimer emission observed for the (S)-enantiomer. The kinetics for the formation of the 2:2 complexes follows equation 3.43 when the simplified reaction scheme (Scheme 3.3) was employed. This analysis is under the assumption that only one type of 1:1 complex and
one type of 2:2 complex are formed in the system. However, there are two types of 1:1 complexes and three types of 2:2 complexes in the system. The formation of each 2:2 complex should be described separately (Scheme 3.2) so that the kinetics for the formation of EE complex can be studied (equation 3.44).

\[
\frac{d[(Np \cdot CD)]_2}{dt} = k_{22}^{+22}[(Np \cdot CD)_2] - k_{-22}^{22}[(Np \cdot CD)_2] \tag{3.43}
\]

\[
\frac{d[EE]}{dt} = k_{+EE}^{EE}[E]^2 - k_{-EE}^{EE}[EE] \tag{3.44}
\]

Equation 3.46 can be derived from equation 3.45 under the assumption that the values of the equilibrium constants for all 2:2 complexes are the same. This assumption is based on the fact that the dynamics for the formation of the 2:2 complexes occur on similar time scales. Equation 3.47 was then derived from equation 3.46.

\[
[(Np \cdot CD)_2] = K_{EE}[E]^2 + K_{EN}[E][N] + K_{NN}[N]^2 \tag{3.45}
\]

\[
[(Np \cdot CD)_2] = K_{EE}(E)^2 + [E][N] + [N]^2 \tag{3.46}
\]

\[
[(Np \cdot CD)_2] = (1 + c + c^2)K_{EE}[E]^2 \tag{3.47}
\]

where \( c = \frac{K_{NN}}{K_{EE}} \tag{3.48} \)

Equation 3.49 can be derived from equation 3.43 and equation 3.47. The rate constants for the formation of the EE complex were then related to those for the formation of 2:2 complexes by equation 3.50 and 3.51.

\[
\frac{d[EE]}{dt} = \frac{(1 + c)^2}{(1 + c + c^2)} k_{+}^{22} [E]^2 - k_{-}^{22} [EE] \tag{3.49}
\]

\[
k_{+}^{EE} = \frac{(1 + c)^2}{(1 + c + c^2)} k_{+}^{22} \tag{3.50}
\]

\[
k_{-}^{EE} = k_{-}^{22} \tag{3.51}
\]
The values of \( k^{\text{EE}}_+ \) were varied between two extremes. The minimum value of \( k^{\text{EE}}_+ \) was reached when only one kind of 1:1 complex (\( E \) or \( N \)) predominated in the system, i.e. \( K_E \) was equal to \( K_{11} \) (condition 1) or \( K_N \) was equal to \( K_{11} \) (condition 2). The maximum value of \( k^{\text{EE}}_+ \) was reached when there was no stereoselectivity for different 1:1 complexes, i.e. \( K_E \) was equal to \( K_N \) (condition 3). The values of \( k^{\text{EE}}_- \) were the same as those of \( k^{22}_- \). The equilibrium constants for the \( EE \) complex (\( K_{\text{EE, sf}} \)) were determined as the ratio between \( k^{\text{EE}}_+ \) and \( k^{\text{EE}}_- \). The results are shown in Table 3.6.

### Table 3.6. Limiting values for the association and dissociation rate constants and equilibrium constants for the \( EE \) complexes derived from the fitting results of the stopped flow experiments under limiting conditions for the \( K_E \) values.

<table>
<thead>
<tr>
<th>Condition</th>
<th>enantiomer</th>
<th>( k^{\text{EE}}_+ ) / ( 10^5 ) M(^{-1})s(^{-1})</th>
<th>( k^{\text{EE}}_- ) / ( 10^2 ) s(^{-1})</th>
<th>( K_{\text{EE, sf}} ) / ( 10^3 ) M(^{-1})</th>
<th>( K_{\text{EE, flu}} ) / ( 10^3 ) M(^{-1})</th>
<th>( C_{\text{EE}} ) / ( 10^4 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (( K_E = K_{11} ))</td>
<td>(R)-NpOH</td>
<td>6.9 ± 0.3</td>
<td>1.11 ± 0.05</td>
<td>6.2 ± 0.4</td>
<td>5.8 ± 0.2</td>
<td>1.43 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(S)-NpOH</td>
<td>7.2 ± 0.4</td>
<td>1.63 ± 0.02</td>
<td>4.4 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>0.72 ± 0.01</td>
</tr>
<tr>
<td>2 (( K_N = K_{11} ))</td>
<td>(R)-NpOH</td>
<td>6.9 ± 0.3</td>
<td>1.11 ± 0.05</td>
<td>6.2 ± 0.4</td>
<td>5.8 ± 0.2</td>
<td>( \infty )</td>
</tr>
<tr>
<td></td>
<td>(S)-NpOH</td>
<td>7.2 ± 0.4</td>
<td>1.63 ± 0.02</td>
<td>4.4 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>( \infty )</td>
</tr>
<tr>
<td>3 (( K_E = K_S ))</td>
<td>(R)-NpOH</td>
<td>9.2 ± 0.4</td>
<td>1.11 ± 0.05</td>
<td>8.3 ± 0.5</td>
<td>7.8 ± 0.3</td>
<td>4.30 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>(S)-NpOH</td>
<td>9.6 ± 0.5</td>
<td>1.63 ± 0.02</td>
<td>5.9 ± 0.3</td>
<td>6.0 ± 0.3</td>
<td>2.16 ± 0.04</td>
</tr>
</tbody>
</table>

The equilibrium constants for the \( EE \) complex (\( K_{\text{EE, sf}} \)) can be determined as the ratio of \( k^{\text{EE}}_+ \) to \( k^{\text{EE}}_- \). The \( K_{\text{EE, sf}} \) values recovered from the kinetic study are the same as the \( K_{\text{EE, flu}} \) recovered from the binding study when the same \( K_E \) value was employed in both studies (Table 3.6). This observation indicates that the results from the kinetic study and binding study are self-consistent.
3.5 Discussion

3.5.1 Chrial recognition observed by the binding study

The steady-state spectra for NpOH free in water, the steady-state spectra for NpOH bound as 1:1 complex, the singlet-excited state lifetimes for NpOH free in water, the singlet-excited lifetimes for NpOH bound as 1:1 complex and the quenching rate constants for the triplet-excited states of NpOH free in water are the same for both NpOH enantiomers. These observations indicated that the photophysical properties of NpOH free in water or NpOH bound as 1:1 complex are the same for both NpOH enantiomers. The quenching rate constants for the triplet-excited states of NpOH bound as 1:1 complexes are the same for both NpOH enantiomers, indicating that β-CD provides the same protection to both NpOH enantiomers in 1:1 complexes. Moreover, the values of $K_{11}$ are the same for both NpOH enantiomers. These observations indicate that the photophysics study cannot differentiate the complexation process for the formation of NpOH-β-CD 1:1 complexes for both NpOH enantiomers.

The steady-state fluorescence spectra for the NpOH/β-CD system at high concentration of NpOH are different for both NpOH enantiomers. More excimer emission was observed for (R)-NpOH than for (S)-NpOH indicating that the chiral recognition can be observed for the formation of NpOH-β-CD 2:2 complexes. Moreover, the steady-state spectra of the $EE$ complex are different from the spectra of other NpOH species ($Np$, $E$, $N$, $EN$ and $NN$). Therefore, the photophysics study was focused on the process of the formation of 2:2 complexes, especially $EE$ complexes, to study the chiral recognition in the NpOH-β-CD system.
The approach to determine the relative concentrations of 2:2 complexes was conducted since the dependence of the relative concentrations of 2:2 complexes with the concentrations of $\beta$-CD can be employed in the binding study. The relative concentrations of $EN$ and $NN$ complexes were determined by the LFP experiments. The results indicated that the concentration of $EN$ and $NN$ complexes for (R)-NpOH, within the experimental errors, is the same as that for (S)-NpOH. In other words, the chiral recognition for the formation of 2:2 complexes cannot be observed by the LFP experiments. The relative concentrations of $EE$ complexes were determined by the steady-state experiments and employed in the binding study. The results show that the range of $K_{EE}$ values for (R)-NpOH is always higher than that for (S)-NpOH, although the value of $K_{EE}$ cannot be determined. The fact that more $EE$ complexes were formed for (R)-NpOH is also confirmed by the results from the SPC experiments. The value of $A_3$, which is related to the fraction of NpOH bound as 2:2 complexes (see Section a.4), is higher for (R)-NpOH than for (S)-NpOH.

The binding study on the formation of the $EE$ complexes discovered the chiral recognition not only for the formation of $EE$ complexes, but also for the formation of 1:1 complexes. The values of $C_{EE}$ should be similar for both NpOH enantiomers since the lifetimes of the singlet-excited state NpOH in the 2:2 complex were very similar for both NpOH enantiomers. For the same value of $C_{EE}$ recovered, the value of the corresponding $K_e$ for (R)-NpOH is always larger than that for (S)-NpOH (shown in Figure 3.13). Moreover, the value of $K_N$ for (R)-NpOH should be smaller than that for (S)-NpOH since the values of $K_{11}$ are almost the same for both NpOH enantiomers. It is worth noting that
the value of $K_E$ for (S)-NpOH should be lower than 650 M$^{-1}$ since the lowest value of $C_{EE}$ for (S)-NpOH was $1.43 \times 10^4$ according to the lowest value of $C_{EE}$ for (R)-NpOH.

### 3.5.2 Chiral recognition observed by the dynamic study

The entry and exit rate constants for NpOHs with β-CD as 1:1 complexes were previously determined to be $2.9 \times 10^8$ M$^{-1}$s$^{-1}$ and $1.8 \times 10^5$ s$^{-1}$, respectively, i.e. the dynamics for the formation of 1:1 complex occur within 6 µs no matter how low the concentration of reagents are. Moreover, the dynamics for the formation of 2:2 complexes occur on millisecond time scales. As a result, the dynamics observed in the stopped flow experiments relates to the formation of 2:2 complexes.

The equilibria for the formation of 1:1 and 2:2 complexes are coupled. As a result, the equilibrium constants for the formation of 1:1 complexes ($K_{11}$) are required to determine the rate constants for the formation of 2:2 complexes from the stopped flow experiments.

The fact that only one relaxation process was observed suggests that the dynamics for the formation of $EE$, $EN$ and $NN$ 2:2 complexes occur on similar time scales. In other words, the rate constants for the formation of different 2:2 complexes are similar; otherwise there should be more than one differentiable kinetic process for the formation of different 2:2 complexes. The simulations were conducted to confirm this argument.

According to the simulation studied under three conditions as aforementioned in Section 3.2.11, when the difference between $k_{EE}^+$ and the association rate constants for other 2:2 complexes ($\Delta k_{22}^+$) corresponds to 25% of $k_{EE}^+$, the differentiation of two kinetic relaxation processes is observable for the simulated traces with the same signal-to-noise ratios as observed for the kinetic measurements (see Figure 3.14). Moreover, when the difference between $k_{EE}^-$ and the dissociation rate constants for other 2:2 complexes ($\Delta k_{22}^-$)
corresponds to 20% of $k_{\text{EE}}^{+}$, the differentiation of two kinetic relaxation processes is observable. It is worth noticing that the limiting values of $\Delta k_{-}^{22}$ or $\Delta k_{-}^{-22}$ determined are different when the simulations were conducted under three different conditions. For example, the limiting value of $\Delta k_{+}^{22}$ corresponds to 5% of $k_{+}^{\text{EE}}$ when the simulations were conducted under the condition that $K_{E}$ is equal to $K_{N}$ (Figure 3.5). The values reported above (i.e. 25% of $k_{+}^{\text{EE}}$ and 20% of $k_{-}^{\text{EE}}$ for $\Delta k_{+}^{22}$ and $\Delta k_{-}^{-22}$, respectively) are the highest values determined.

The chiral recognition for the formation of 2:2 complexes was observed in stopped flow experiments. The dissociation rate constants ($k_{-}^{\text{EE}}$) for the 2:2 complexes at 10 °C were determined as 111 s$^{-1}$ and 163 s$^{-1}$ for (R)- and (S)-NpOH, respectively. The values were consistent with the range (20 – 2500 s$^{-1}$) estimated in previous work$^{203}$. The value of $k_{-}^{\text{EE}}$ for (R)-NpOH is 46.8% lower than that for (S)-NpOH. The lower $k_{-}^{\text{EE}}$ for (R)-NpOH is consistent with the fact that more EE complexes were formed for (R)-NpOH.

The value of $k_{+}^{\text{EE}}$ was related to the ratio of $K_{N}$ to $K_{E}$ (c in equation 3.50) and $k_{+}^{22}$. Since the value of c was not the same for both NpOH enantiomers, the comparison of $k_{+}^{\text{EE}}$ for both NpOH enantiomers was not practical. However, the range of association rate constants ($k_{+}^{\text{EE}}$) for the EE complex at 10 °C were close for both NpOH enantiomers.

$\beta$-CD has been employed as a chiral selector since 1983.$^{264,265}$ Moreover, the main mechanisms for the chiral recognition by $\beta$-CD were studied quite well as a lock-and-key mechanism.$^{122}$ However, no study has reported the chiral recognition of $\beta$-CD from a kinetic point of view. In this study, chiral recognition was observed for the first time for the complexation dynamics of a CD host-guest system. The results which can only be
reached by the kinetic study support the mechanism for the chiral recognition observed for the formation of 2:2 complexes in binding study, i.e. more $EE$ complex formed in (R)-NpOH / β-CD system is due to the lower value of dissociation rate constants for $EE$ complex in (R)-NpOH / β-CD system. Furthermore, the results discovered by the kinetic study indicate that the equilibrium binding constants are similar for the formation of all three 2:2 complexes. This information was a crucial prerequisite for the binding study performed on the formation of 2:2 complex and led to the conclusion that in NpOH/β-CD system, more $E$ complexes and less $N$ complexes are formed for (R)-NpOH than for (S)-NpOH. This example shows that kinetic study can provide unique information to discover the chiral recognition for the formation of 1:1 complexes, which cannot even be observed by the equilibrium binding study.
4 Dynamics of Pyrene Incorporation into Octa-Acid Nanocapsules

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4.1 Introduction

4.1.1 The binding of pyrene to octa acid

The complexation of Octa-Acid (OA, Scheme 1.5) with pyrene (Py, Scheme 4.1) was studied by Gibb's and Ramamurthy's groups. The formation of an OA-Py 2:1 complex was confirmed by NMR and steady-state fluorescence experiments. Moreover, the dynamics for the formation of OA-Py 2:1 complex were slow on the NMR time scale. The polarity of the cavity of OA capsules is similar to benzene, since the shape of the fluorescence emission spectra of Py, which is sensitive to the environment of Py, are the same for Py bound to OA and for Py in benzene. The opening-closing process of OA capsules was studied by quenching of the excited state of Py with $O_2$. The oxygen-quenching rate constant for the excited state of Py located inside the OA capsules ($5 \times 10^5$ M$^{-1}$s$^{-1}$) is $3 \times 10^3$ times lower than that for the excited state of Py free in buffer. This observation, as discussed by the authors, is due to the opening-closing process of OA capsules, which is the rate limiting step for the oxygen-quenching process.
Some preliminary studies were conducted by Carla Santos de Oliveira in our research group. The rate constants for the singlet-excited state of Py quenched by I⁻ were determined in the absence and presence of OA. The value of the iodide-quenching rate constant for the excited state of Py bound to OA ($5.6 \times 10^5$ M$^{-1}$s$^{-1}$) is much lower than that for the excited state of Py free in buffer ($9.7 \times 10^8$ M$^{-1}$s$^{-1}$). The lifetimes of the singlet-excited state of Py were determined under different experimental conditions. The lifetime of the singlet-excited state of Py bound to OA in the air-saturated solution (354 ns) is close to that in the nitrogen-purged solution (364 ns) and that in the oxygen-purged solution (343 ns). Comparatively, the lifetime of the singlet-excited state of Py free in buffer in the air-saturated solution (141 ns) is much shorter than that in the nitrogen-purged solution (205 ns) and is much longer than that in the oxygen-purged solution (61 ns). These observations clearly show that the accessibility of quencher (I⁻ or O$_2$) to the excited state of Py bound to OA is largely limited by the OA host molecules, indicating that Py is located inside of the cavity of the OA capsule when complexed.

4.1.2 Objectives

The first objective of this work was to determine the binding isotherm for the OA-Py complexes, which was employed to determine the host-guest stoichiometries and the corresponding equilibrium constants for the OA-Py complexes.
The second objective of this work was to study the binding dynamics of Py with OA to provide mechanistic insights into the binding affinities of Py to OA. The kinetic scheme with all relevant reactions for the Py/OA system is shown in Scheme 4.2.

\[ \text{OA} + \text{Py} \rightleftharpoons \text{Py} \cdot \text{OA} \]  
\[ \text{OA} + \text{Py} \cdot \text{OA} \rightleftharpoons \text{OA} \cdot \text{Py} \cdot \text{OA} \]  

Scheme 4.2. Reactions for the binding of Py with OA in the presence of buffer.

### 4.2 Experimental Section

#### 4.2.1 Instrumentation

UV-Vis absorption spectra were recorded by using a Varian Cary 100 spectrophotometer at room temperature. A PTI QM-2 fluorometer was employed to measure steady-state fluorescence emission spectra and steady-state fluorescence excitation spectra. As the emission spectra were collected, the excitation wavelength was set to 334 nm or 340 nm and the emission light was recorded in the wavelength range of 350 to 500 nm. As the excitation spectra were collected, the emission wavelength was set to 390 nm and the excitation light was scanned in the wavelength range of 280 nm to 360 nm. The excitation and emission slit bandwidths on the monochromators were set to 2
The values of the excitation wavelength for the fluorometer mentioned in this chapter were corrected by comparing the difference of peak positions between the fluorescence excitation spectra determined for a Py sample by the fluorometer and the absorption spectra determined for the same Py sample by the absorption spectrophotometer. Control experiments were performed by collecting the corresponding spectra of the baseline solutions. The baseline solutions were the buffer solutions for the qualitative study of the photophysical properties of Py in the absence and presence of OA. In the case of the determination of the binding isotherm of the OA/Py complexes, the baseline solutions were the solutions containing all chemicals with the exception of Py. All fluorescence spectra were corrected by subtracting the baseline spectrum.

Time-resolved fluorescence decays were measured with an Edinburgh OB 920 single photon counting (SPC) system. The excitation source was a 335 nm pulsed light emitting diode (EPLED330 – Edinburgh instruments Ltd.). The bandpass for the emission monochromator was ca. 16 nm (2 mm slits). The excitation wavelength was set to 391 nm. The stop counts were set to 2,000, and the accumulation of data stopped when the highest counts at a channel reached 2,000. The instrument response function (IRF) was collected with a Ludox (Aldrich) solution by scattering light at 335 nm. The IRF was used to deconvolute the lamp profile from the fluorescence decays.

Quartz cells with dimensions of 10 mm × 10 mm were employed for steady-state and time-resolved fluorescence experiments. The samples for both experiments were thermostated before data collection at 20.0 ± 0.1 °C for at least 15 min using a circulating water bath.
A SX20 stopped flow system (Applied Photophysics) was employed to measure the kinetics for the formation of the OA/Py complex using the fluorescence detection mode. The excitation source was a mercury-xenon lamp (L2382 – Hamamatsu Photonics). The excitation wavelength was 334 nm or 340 nm with a monochromator slit bandwidth of 0.19 nm (0.04 mm slits). The values of the excitation wavelength for the stopped flow system mentioned in this chapter were corrected by comparing the position of the principle emission lines of the mercury-xenon lamp with the peak positions of the fluorescence excitation spectra determined for the deionized water by the stopped flow system. The emission was collected at 90 degrees with respect to the excitation beam by selecting the emission wavelengths by means of an interference filter with the center wavelength of 405 nm (FWHM of 38 nm, Melles Griot, lot 13637 (ANDV7248)). The samples contained in the two injection syringes were thermostated for at least 15 min at 20 °C. A mixing ratio of 1:1 for the OA and Py solutions was employed. Therefore, the final concentrations of OA and Py were half of the concentrations prepared for the solutions contained in the syringes. The concentrations of chemicals mentioned below are the final concentrations of chemicals unless otherwise stated. The detection photomultiplier (PMT) voltage was adjusted so that the highest expected emission intensity was at 6 V or lower. The same photomultiplier voltage was used throughout one experiment to ensure that intensities at different concentrations of OA or Py can be compared. At least 20 kinetic traces were averaged to increase the signal-to-noise ratio. The following control experiments were performed: (i) Injection of buffer against buffer to determine the zero intensity value for the system to be subtracted from intensity values for solutions containing Py but not OA. (ii) Injection of OA against buffer to determine
the baseline value to be subtracted from all intensities measured for solutions containing OA. The values for control experiments (i) and (ii) are very similar. (iii) Injection of Py against buffer to determine the emission intensity of Py in the absence of OA. All other experimental conditions, such as the concentrations of buffer and the PMT voltage, were kept the same for both control experiments and experiments where OA solutions were mixed with Py solutions.

4.2.2 Materials

OA samples (C₉₆O₃₂H₆₄·4H₂O) were synthesized by Gibb’s group as previously described.¹⁶¹ OA samples, sodium tetraborate (Na₂B₄O₇·10H₂O, BDH, ≥ 98%), and methanol (Caledon Laboratories, spectra grade, > 99.8%) were used without further purification. Pyrene (Py) was purchased from Aldrich (99%) and recrystallized at least twice from ethanol:water 90%:10% mixture. Py was checked for the presence of fluorescent impurities by measuring the fluorescence decay of a 0.5 µM aqueous solution of Py in water. The decay was mono-exponential indicating that no other emissive species were present in solution. Deionized water (Barnstead NANOpure deionizing systems ≥ 17.8 MΩ cm⁻¹) was employed for all samples.

4.2.3 Solution preparation

The 10 mM borate buffer solutions (mentioned hereafter as "buffer" in this chapter) were prepared by dissolving the appropriate amount of solid sodium tetraborate in water. The OA stock solutions (555 µM) were prepared by dissolving the appropriate amount of solid OA in buffer. The Py stock solution (1 mM) was prepared by dissolving the appropriate amount of solid Py into methanol. The solutions with lower concentrations of species (e.g. Py and OA) were prepared by injecting the corresponding stock solutions
into buffer. Aerated samples were used for all experiments because the consistent deaeration for the stopped flow experiment is difficult to achieve leading to a larger uncertainty in the fluorophore’s emission intensity.

4.2.4 Time-resolved fluorescence

The fluorescence decays were fitted to a sum of exponentials (Equation 4.3) using the Edinburgh software (version 3, Edinburgh Instruments Ltd.) to determine the lifetimes ($\tau_i$) and the pre-exponential factors ($A_i$). The quality of each fit was judged by the residuals plot and the value of the reduced chi-squared parameter ($\chi^2$). The number of exponentials for each fit was increased until the residuals were random and the $\chi^2$ values were between 0.9 and 1.2.\textsuperscript{241} The relative contribution of the $i$th component to the total fluorescence intensity was defined by Equation 4.4.

\[
I_t = I_0 \times \sum_{i=1}^{n} \left[ A_i \times e^{-\frac{t}{\tau_i}} \right] \quad (4.3)
\]

\[
F_i = \frac{\tau_i \times A_i}{\sum_{i=1}^{n} (\tau_i \times A_i)} \quad (4.4)
\]

4.2.5 Data treatment for the binding isotherm of the OA/Py complex

Steady-state fluorescence emission spectra were determined for solutions of Py in the absence and presence of OA. Spectra were corrected by subtracting a baseline which corresponds to a spectrum for a solution containing all chemicals with the exception of the fluorophore (Py). The correction was to separate the fluorescence emission of Py from Raman emission and the fluorescence emission of impurities presented in OA. The corrected spectra were integrated between 350 nm and 500 nm and the integrated
intensity for each spectrum ($I_{flu}^*$) was related to the instrumental settings, such as the excitation light intensity and the bandwidth. Therefore, the values of $I_{flu}^*$ varied between experiments performed on different days. The values of $I_{flu}^*$ were divided by the integrated intensity for Py in the absence of OA ($I_{flu,0}^*$) to determine the normalized intensity for each spectrum ($I_{flu}$). The values of $I_{flu}$, i.e. the normalized $I_{flu}^*$, are independent of the instrumental settings mentioned above. The dependence of $I_{flu}$ on the concentration of OA was employed to study the binding isotherm of the 2:1 OA-Py complex (see Section 4.2.6).

### 4.2.6 Binding isotherms for 2:1 OA-Py complexes

The dependence of $I_{flu}$ on the OA concentration was fitted numerically using Scientist 3 from Micromath. Briefly, an equation is defined for the measured variable, i.e. the fluorescence emission intensity for each concentration of OA and Py ($I_{flu}$ stated in Section 4.2.5), as a function of emission efficiencies ($C_{Py}$ and $C_{2:1}$) and concentration of Py in the aqueous phase ([G]) or bound to OA as OA-Py 2:1 complex ([H$_2$G]). The relationship between the concentrations of various Py and OA species is defined by the overall equilibrium binding constant for the OA-Py 2:1 complex ($\beta_{2:1}$ in Reaction 4.5) and the mass balance equations.

\[
\text{Py} + 2\text{OA} \xrightleftharpoons{\beta_{2:1}} \text{OA} \cdot \text{Py} \cdot \text{OA}
\]  

(4.5)

The model employed in Scientist 3 (model 1) is listed as follows:

The total concentration of OA ([H$_T$]) is defined as the independent variable. The equilibrium concentration of free host ([H]), free guest ([G]), 2:1 complex ([H$_2$G]) and
the fluorescence emission intensity \( I_{\text{Flu}} \) are defined as the dependent variables. The emission efficiency for the 2:1 complex \( (C_{21}) \) and the overall equilibrium binding constant for the 2:1 complex \( (\beta_{21}) \) are defined as parameters in the model. \( R \), the ratio of the fluorescence emission intensity of Py in the absence of OA \( (I_{\text{flu},0}) \) to the total concentration of Py \( ([G]_T) \), is a constant for each independent experiment.

Equations for model 1 are listed as follows:

\[
\begin{align*}
[H_2G] &= \beta_{21} \times [G] \times [H]^2 \quad (4.6) \\
[H] &= [H]_T - 2 \times [H_2G] \quad (4.7) \\
[G] &= [G]_T - [H_2G] \quad (4.8) \\
I_{\text{flu}} &= R \times ([G] + C_{21} \times [H_2G]) \quad (4.9)
\end{align*}
\]

where \( R = \frac{I_{\text{flu},0}}{[G]_T} \quad (4.10) \)

Ranges for the dependent variables are listed as follows:

\[
\begin{align*}
0 < [H] < [H]_T \quad (4.11) \\
0 < [G] < [G]_T \quad (4.12) \\
0 < [H_2G] < [G]_T \quad (4.13)
\end{align*}
\]

4.2.7 Data treatment for stopped flow experiments

The stopped-flow traces were determined as the intensity change of the fluorescence emission over time. The stopped-flow traces provided the kinetic information of the complexation between Py and OA as well as the information to determine the equilibrium constant for the formation of the OA-Py 1:1 complex.

The stopped-flow traces were treated by two methods to obtain the kinetic information: (i) the exponential curve fitting method\(^\text{198}\) and (ii) the second-order global analysis
method. When the stopped-flow traces were studied by the exponential curve fitting method, the traces obtained for different concentrations of OA were fitted with a mono-exponential function (n = 1) or with a function equal to a sum of exponentials (Equation 4.14) if necessary. The quality of each fit was judged by the residuals plot. The number of exponentials for each fit was increased until the residuals were random.

\[ I_t = I_{SF}^* + \sum_{i=1}^{n} [I_{x,i}(1 - e^{-k_{obs,i}t})] \]  

where \( I_{SF}^* \) is the fluorescence intensity at time 0, \( I_{x,i} \) and \( k_{obs,i} \) are respectively the pre-exponential factor and the relaxation rate constant (i.e. inverse of the relaxation time of the system) for each kinetic process \( i \).

The values of \( I_{SF}^* \) for each trace were corrected by subtracting the fluorescence intensity for a mixture containing all chemicals with the exception of the fluorophore (Py). The corrected fluorescence intensities were then divided by the corrected fluorescence intensity for Py in the absence of OA to determine the normalized fluorescence intensity at time 0 for each trace \( (I_{SF}) \). The dependence of \( I_{SF} \) on the concentration of OA was employed to study the binding isotherm of the OA-Py 1:1 complex (see Section 4.2.8).

When the stopped-flow traces were studied by the second-order global analysis method, the traces determined for different concentrations of OA were simultaneously fitted with the non-linear least-squares regression method using Pro-Kineticist II (Applied Photophysics Ltd). The fitting models were derived according to the reaction scheme proposed (Scheme 4.2). The equilibrium constant for the formation of 1:1 OA-Py complex \( (K_{11}) \), the association \( (k_{a}^{22}) \) and dissociation rate constants \( (k_{d}^{22}) \) for the
formation of the 2:1 OA-Py complex were the global parameters in the common fitting models. These global parameters were shared by all traces during the fitting and were varied to minimize the sum of the chi-squared values for all traces.

The stopped-flow traces were treated by the following method to ensure that the traces determined on different days can be compared. The intensity of the fluorescence emission determined in the stopped flow experiments corresponds to the output voltage of PMT, which is related to the instrumental settings, such as the excitation light intensity, the voltage applied to PMT and the bandwidth of the monochromators. Therefore, the kinetic traces determined on different days or determined by using different excitation wavelengths cannot be compared directly. The kinetic traces were corrected by subtracting a baseline which corresponds to the fluorescence intensity for a mixture containing all chemicals with the exception of the fluorophore (Py). The corrected kinetic traces were then divided by the corrected fluorescence intensity for Py in the absence of OA, i.e. the corrected kinetic traces were normalized. The fluorescence intensities for each time point \( t \) in the normalized traces \( I_{\text{SF}}^t \) are independent of the instrumental settings and only depend on the emission efficiencies \( C_i \) of each species \( i \) and the concentration of the corresponding species \( [i] \).

### 4.2.8 Binding isotherms for 1:1 complexes

The dependence of the fluorescence intensities \( I_{\text{SF}} \) stated in Section 4.2.7 or \( I_{\text{flu}} \) stated in Section 4.2.5) on the CB[7] concentration was fitted numerically using Scientist 3 from Micromath. Briefly, an equation is defined for the measured variable, i.e. the fluorescence intensity for each concentration of OA and Py \( I_{11} \), representing \( I_{\text{SF}} \) or \( I_{\text{flu}} \), as a function of emission efficiencies \( C_{\text{py}} \) and \( C_{11} \) and concentration of Py in the aqueous
phase ([G]) or bound to OA as 1:1 complex ([HG]). The emission efficiencies ($C_i^0$) are related to the fluorescence quantum yields for each species and instrumental settings, such as the excitation light intensity and bandwidth of the monochromators. The magnitude for the $C_i^0$ values varied between experiments performed on different days. However, relative $C_i$ values, the ratios of $C_i^0$ to $C_{np}^0$, are constant for each Py species. The emission efficiencies discussed below are the relative emission efficiencies unless otherwise stated. The relationship between the concentrations of various Py and OA species is defined by the equilibrium binding constant for the 1:1 complex ($K_{11}$) and the mass balance equations.

The model employed in Scientist 3 (model 2) is listed as follows:

The total concentration of OA ([H]$_T$) is defined as the independent variable. The equilibrium concentration of free host ([H]), free guest ([G]), 1:1 complex ([HG]) and $I_{11}$ are defined as the dependent variables. The emission efficiency for the 1:1 complex ($C_{11}$) and the equilibrium binding constant for the 1:1 complex ($K_{11}$) are defined as the parameters in the model. $R$, the ratio of the fluorescence intensity of Py in the absence of OA ($I_{11,0}$) to the total concentration of Py ([G]$_T$), is a constant for each independent experiment.

Equations for model 2 are listed as follows:

$$[\text{HG}] = K_{11} \times [\text{G}] \times [\text{H}]$$
(4.15)

$$[\text{H}] = [\text{H}]_T - [\text{HG}]$$
(4.16)

$$[\text{G}] = [\text{G}]_T - [\text{HG}]$$
(4.17)

$$I_{11} = R \times ([\text{G}] + C_{11} \times [\text{HG}])$$
(4.18)
where \( R = \frac{I_{11p}}{[G]_T} \)  

(4.19)

Ranges for the dependent variables are listed as follows:

\[
0 < [H] < [H]_T \quad (4.20) \\
0 < [G] < [G]_T \quad (4.21) \\
0 < [HG] < [G]_T \quad (4.22)
\]

4.3 Results

4.3.1 UV-Vis spectra of pyrene in the absence and presence of octa acid

Bathochromic shifts were observed in the absorption spectra of Py with the addition of OA into the solution. This observation supports the formation of a complex between OA and Py and indicates that the cavity of OA provides a less polar environment for Py than the aqueous phase does. A maximum shift of 5 nm was observed for the bathochromic shift when all free Py was bound to OA, e.g. the absorption peak shift from 335 nm to 340 nm.

![Absorption spectra for the buffer solutions of 0.2 \( \mu \text{M} \) Py in the absence (red) and presence of 4 \( \mu \text{M} \) OA (blue). The spectra were corrected by subtracting a baseline which corresponds to the absorbance intensity for a mixture containing all chemicals with the exception of the fluorophore (Py).](image-url)
4.3.2 Steady-state and time-resolved fluorescence

The fluorescence decay for 0.2 µM Py in the absence of OA follows a mono-exponential decays with a lifetime of 130.9 ± 0.5 ns. The fluorescence decay for 0.2 µM Py in the presence of 4 µM OA could not be adequately fitted to a mono-exponential function, but fitted well to the sum of two exponentials, where one lifetime was very short (1 ± 0.2 ns) and the other lifetime was 361 ± 0.1 ns according to two independent experiments. The contribution to the total fluorescence intensity of the component with the lifetime of 361 ns is very high (99.6% as determined by Equation 4.4). As a result, the lifetime of 361 ns is assigned to the OA/Py complex, while the lifetime of 1 ns may be assigned to the OA sample. The fluorescence excitation spectra of Py in the absence and presence of OA were determined (Figure 4.2). The bathochromic shift of ca. 6 nm, e.g. 334.5 nm – 340 nm, was observed for Py in the presence of OA. This observation supports the formation of OA/Py complex. The peaks around 334.5 nm for Py free in buffer and around 340 nm for Py bound to OA are narrow (FWHM of ca. 6 nm). The excitation efficiency at 334.5 nm for Py free in buffer is higher than that for Py bound to OA while the excitation efficiency at 340 nm for Py free in buffer is much lower than that for Py bound to OA. Consequently, the fluorescence emission intensity for Py free in buffer is higher than that of Py bound to OA when the excitation wavelength was set to 335 nm (Figure 4.3); the fluorescence emission intensity of Py free in buffer is much lower than that of Py bound to OA when the excitation wavelength was set to 340 nm (Figure 4.4).
Figure 4.2. Fluorescence excitation spectra for the buffer solutions of 0.2 µM Py in the absence (red) and presence of 4 µM OA (blue). The emission wavelength was set at 390 nm.

Figure 4.3. Fluorescence emission spectra for the buffer solutions of 0.2 µM Py in the absence (red) and presence of 4 µM OA (blue). The excitation wavelength was set at 335 nm.
Figure 4.4. Fluorescence emission spectra for the buffer solutions of 0.2 µM Py in the absence (red) and presence of 4 µM OA (blue). The excitation wavelength was set at 340 nm.

Each fluorescence emission spectrum in Figure 4.3 and Figure 4.4 was normalized to an intensity of 1 at a wavelength of maximum fluorescence (Figure 4.5). The bathochromic shifts of ca. 3 nm, e.g. 369 nm – 371.75 nm and 380.5 nm – 383 nm, were observed when Py were bound to OA. The shape of the fluorescence emission spectra for Py free in buffer is different from that for Py bound to OA. It is well known that the intensity of the 0-0 band (i.e. peak I at 369 nm for Py free in buffer and at 372 nm for Py bound to OA) is sensitive to the polarity of the environment of Py while the intensity of peak III is not affected by the polarity of the environment of Py. As a result, the ratio of intensities for peak I and peak III ($I_1/I_3$) can be employed to study the polarity of the environment of Py. The value of $I_1/I_3$ was determined as $1.74 \pm 0.03$ for Py free in buffer according to two independent experiments, which is closed to the values previously reported, e.g. 1.70 reported by Kaanumalle et al, 1.59 reported by Kalyanasundaram et al and 1.87 reported by Dong et al. The value of $I_1/I_3$ for Py bound to OA ($0.91 \pm 0.01$), which was determined according to two independent experiments, is similar as the
value for Py in n-octanoic acid (0.91) or for Py in n-octanol (0.92).\textsuperscript{269,270} Moreover, this value of $I_1/I_3$ for Py bound to OA is much lower than that for Py in water. These observations indicate that the polarity of the cavity of OA is much lower than that of water. It is worth noticing that the value of $I_1/I_3$ for Py bound to OA reported in this work is lower than the value of 1.01 reported by Kaanumalle et al.\textsuperscript{168} This difference may be due to different experimental conditions chosen by these two research groups. The concentration ratios of OA to Py was 20:1 in this work. Under this experimental condition, all Py was bound to OA. However, the concentration ratios of OA to Py was 2:1 in the work of Kaanumalle et al, which leads to the partial binding of Py to OA.

![Figure 4.5](image)

**Figure 4.5.** Normalized fluorescence emission spectra for the buffer solutions of 0.2 µM Py in the absence (red and green) and presence of 4 µM OA (blue and black). The spectra were normalized to an intensity of 1 at a wavelength of maximum fluorescence. The excitation wavelengths were set at 335 nm (green and black) or at 340 nm (red and blue).

### 4.3.3 Determination of the overall equilibrium constants for the 2:1 complexes

The fluorescence intensities of Py were enhanced in the presence of OA when the excitation wavelength was set to 340 nm (Figure 4.6).
Figure 4.6. The dependence of the fluorescence emission spectra of Py with the concentration of OA. [Py] = 0.2 µM; [OA] from bottom to top are: 0 µM, 0.09 µM, 0.28 µM, 0.46 µM, 0.65 µM, 0.83 µM, 1.02 µM, 1.48 µM and 5.13 µM. Excitation wavelength was set at 340 nm.

The dependence of the fluorescence intensities ($I_{flu}$) with the concentration of OA was determined and was fitted with Model 1 for the formation of OA-Py 2:1 complex (see Section 4.2.6). The model 1 fits the data very well (Figure 4.7), indicating that the model of the formation of OA-Py 2:1 complex is suitable to describe the complexation of OA and Py at equilibrium. The overall equilibrium binding constant for the formation of OA•Py•OA complex was determined as $(3.19 \pm 0.06) \times 10^{12}$ M$^{-2}$ according to two independent experiments. Furthermore, the dependence $I_{flu}$ with the concentration of OA was fitted with Model 2 for the formation of OA-Py 1:1 complex (see Section 4.2.8). The fitting curve (the blue curve in Figure 4.7) deviates from the data points, indicating that the model of the formation of OA•Py is not the right model to describe the complexation of OA and Py at equilibrium.
4.3.4 Stopped flow experiments

The emission change over time was measured in stopped flow experiments and the collected spectra region, 292.8 nm – 367.2 nm, relates to the emission from Py free in buffer and bound to OA. The kinetic traces in Figure 4.8 were determined for 0.2 µM Py mixing with 3 µM OA using different excitation wavelengths. The timescale for the stopped flow experiments was set to 2 s. The fluorescence intensity (blue curve in Figure 4.8) increased with time when the excitation wavelength was set to 334 nm. Comparatively, the fluorescence intensity (red curve in Figure 4.8) decreased with time when the excitation wavelength was set to 340 nm. This observation is consistent with the observation in the steady-state experiments that the excitation efficiency at 334 nm for Py is higher than that for Py bound to OA while the excitation efficiency at 340 nm for Py is lower than that for Py bound to OA (See Section 4.3.2).
Figure 4.8. Stopped-flow traces for Py mixing with 3 µM OA (red and blue) and for Py mixing with buffer (green and black). [Py] = 0.2 µM. The excitation wavelengths were set to 334 nm (red and black) or 340 nm (blue and green). The traces determined at the same excitation wavelength were normalized by dividing them by the fluorescence emission intensity of Py in the absence of OA at the same excitation wavelength.

Both traces (red and blue curves in Figure 4.8) reached a limiting value within 1 s and were fitted well with a mono-exponential function (Equation 4.23, which was derived from Equation 4.14). The residuals between the fitting curves and the experiment data are randomly scattered around 0 (Figure 4.9), indicating that only one kinetic relaxation process is observed for each trace. The relaxation rate constant (\(k_{\text{obs}}\) in Equation 4.23) were determined for each trace. The value of \(k_{\text{obs}}\) recovered for the trace determined with the excitation wavelength at 340 nm (8.4 ± 0.5 s\(^{-1}\)) is closed to the one recovered for the trace determined with the excitation wavelength at 334 nm (8.9 ± 0.1 s\(^{-1}\)). This observation indicates that the kinetic relaxation processes observed in both traces are the same. Moreover, the kinetic traces for Py mixing with OA did not start at the intercept of the kinetic trace for Py mixing with buffer. This observation indicates that some of the dynamics for the formation of the OA/Py complexes occurred within the dead time of the
stopped flow equipment (1 ms). The signal-to-noise ratio for the trace determined with
the excitation wavelength at 334 nm is better than that for the trace determined with the
excitation wavelength at 340 nm. This observation is due to the high excitation light
intensity at 334 nm, which is one of the principle emission lines of the mercury-xenon
lamp. Therefore, the following experiments were conducted with the excitation
wavelength at 334 nm to achieve good signal-to-noise ratio for the kinetic traces.

\[ I_i = I_{SR}^* + I_a \left( 1 - e^{-kt}\right) \]  

(4.23)

Figure 4.9. Residuals between the fitting curve and the experimental data for 0.2 \( \mu \text{M} \) Py
mixing with 3 \( \mu \text{M} \) OA. The experimental data were fitted with a mono-exponential
function. Blue: Residuals for the experimental data determined at 340 nm. Red: Residuals
for the experimental data determined at 334 nm.

The stopped flow experiments were conducted by mixing 0.2 \( \mu \text{M} \) Py with different
concentrations of OA (Figure 4.10). The kinetic trace \( a \) and \( g \), which were respectively
determined for Py mixing with buffer and for buffer mixing with buffer, are horizontal
lines. The intercept of trace \( a \) is related to the sum of the fluorescence intensity for Py in
the absence of OA and the baseline intensity for buffer while that of trace \( g \) is related to
the baseline intensity for buffer. Each kinetic trace \( i \) (\( i = b \) to \( f \)) was studied by the
exponential curve fitting method (see section 4.2.7) to check how many kinetic relaxation processes occurred on the stopped flow time scale. Each kinetic trace \(i\) (\(i = b\) to \(f\)) is fitted well with a mono-exponential function (Equation 4.23) and the residuals between the experimental data and the fitting curve are randomly scattered around 0 (e.g. Figure 4.11 and Figure 4.12). This observation indicates that there is only one kinetic relaxation process occurring on the 10 s time scale for each trace, i.e. only one reaction is slow on the stopped flow time scale. The fluorescence intensity at equilibrium for each trace decreases with the increase of the concentration of OA. This observation is consistent with the results observed in the steady-state fluorescence experiments (See section 4.3.2).

![Figure 4.10](image_url)

Figure 4.10. Stopped-flow traces for Py mixing with buffer (a), for Py mixing with OA (b – f) and for buffer mixing with buffer (g). The excitation wavelengths were set to 340 nm. [Py] = 0.2 µM. [OA] = 0.5 µM (b, red), 1 µM (c, blue), 2 µM (d, green), 3 µM (e, red) and 4 µM (f, blue).
Figure 4.11. Stopped-flow trace \( f \) fitted with a mono-exponential function. Top panel: Experimental trace for 0.2 \( \mu \)M Py mixing with 4 \( \mu \)M OA (red), the fitting curve (black). Bottom panel: Residuals between the fitting curve and the experimental data (blue). Only the first 1 s of the trace \( f \) is shown in the figure for better visibility.

Figure 4.12. Stopped-flow trace \( b \) fitted with a mono-exponential function. Top panel: Experimental trace for 0.2 \( \mu \)M Py mixing with 0.5 \( \mu \)M OA (red), the fitting curve (black). Bottom panel: Residuals between the fitting curve and the experimental data (blue).

The kinetic traces \( i \) (\( i = b \) to \( f \)) start at intensities different from the intercept of the kinetic trace \( a \), indicating that a pre-equilibrium kinetic process occurred within 1 ms for each kinetic trace (see Figure 4.13, which shows the kinetic traces in the first 0.6 s after the mixing of Py and OA). The fluorescence intensity at time 0 for each trace decreases with the increase of the concentration of OA, indicating the formation of a Py species.
within the dead time of the stopped flow equipments. The fluorescence intensity at time 0 ($I_{SF}^*$) and the relaxation rate constant ($k_{obs}$) were determined for each kinetic trace. Furthermore, the values of $I_{SF}^*$ were employed to determine the values of $I_{SF}$ (see section 2.2.7) and the dependence of $I_{SF}$ with the concentration of OA were employed to determine the equilibrium constant for the OA-Py 1:1 complex.

![Figure 4.13. Stopped-flow traces for Py mixing with buffer (a) and for Py mixing with OA (b – f). The excitation wavelengths were set to 340 nm. [Py] = 0.2 µM. [OA] = 0.5 µM (b, red), 1 µM (c, blue), 2 µM (d, green), 3 µM (e, red) and 4 µM (f, blue). This figure is the expanded view of the first 0.6 s of the traces shown in Figure 4.10](image)

4.3.5 Determination of the equilibrium constants for the OA-Py 1:1 complexes

Two-step kinetics were observed for the complexation of OA and Py in the stopped flow experiments. The first step was sufficiently fast to be viewed as a pre-equilibrium process while the second step was slow on the stopped flow time scale. The first step led to the formation of a Py species, which reacted further to generate the OA-Py 2:1 complex at the equilibrium. Therefore, the Py species generated in the first step was assigned to the OA-Py 1:1 complex.
The dependence of $I_{sf}$ with the concentration of OA was determined and was fitted with Model 2 to determine the equilibrium constant for the formation of OA-Py 1:1 complex (see Section 4.2.8). The model 2 fits the data very well (Figure 4.14), indicating that the model of the formation of the OA•Py complex is suitable to describe the complexation of OA and Py within the dead time of the stopped flow equipment. The equilibrium constant for the formation of the OA•Py complex was determined as $(4.5 \pm 0.6) \times 10^5 \text{ M}^{-1}$ from two independent experiments.

Figure 4.14. Binding isotherm for the complexation of Py (0.2 µM) with OA in buffer. The fit of the 1:1 binding isotherm was obtained using Scientist and the residuals between the fit (black curve) and the experimental data (black dots in the top panel) are shown in the bottom panel. The recovered $K_{11}$ value is $(4.5 \pm 0.6) \times 10^5 \text{ M}^{-1}$ from two independent experiments.

### 4.3.6 The second-order global analysis

A two-step reaction scheme (Scheme 4.2) was employed to build up a global analysis model (Model 3) in Pro-Kineticist II to fit the kinetic traces. The reaction for the formation of the OA•Py complex (Reaction 4.1) is sufficiently fast to be viewed as a pre-equilibrium process. Therefore, the equilibrium binding constant ($K_{11}$) can be recovered for Reaction 4.1 while the rate constants for this pre-equilibrium processes cannot. The
reaction for the formation of the OA•Py•OA complex (Reaction 4.2) is slow on the stopped flow time scale. Consequently, the association (\(k_{21}^+\)) and the dissociation (\(k_{21}^-\)) rate constants for Reaction 4.2 were recovered. The kinetic traces for 0.2 \(\mu\)M Py mixing with different concentration of OA (Figure 4.10) were fitted in the second-order global analysis. The value of \(K_{11}\) was fixed to \(4.5 \times 10^5\) M\(^{-1}\) (determined as aforementioned in Section 4.3.5) and other two parameters (i.e. \(k_{21}^+\) and \(k_{21}^-\)) were treated as free parameters. The residuals between the fitting curve and the experimental data are randomly scattered around 0, indicating that Model 3 is suitable to fit the data determined for 0.2 \(\mu\)M Py mixing with OA. The values of \(k_{21}^+\) recovered from two independent experiments, i.e. \((2.59 \pm 0.09) \times 10^6\) M\(^{-1}\)s\(^{-1}\) and \((2.39 \pm 0.09) \times 10^6\) M\(^{-1}\)s\(^{-1}\), are close to each other. However, the values of \(k_{21}^-\) recovered from two independent experiments, \((5 \times 10^5 \pm 0.03)\) s\(^{-1}\) and \((0.111 \pm 0.007)\) s\(^{-1}\), are quite different. The values of \(k_{21}^-\) recovered from two independent experiments are both small with large errors, indicating that the dissociation process of OA•Py•OA complex contributes little to the kinetic relaxation process observed in Figure 4.10. Therefore, the simplified model (Model 4, i.e. Scheme 4.3) was employed to fit the kinetic traces. The value of \(K_{11}\) was fixed to \(4.5 \times 10^5\) M\(^{-1}\) and \(k_{21}^+\) was treated as a free parameter. The residuals between the fitting curve and the experimental data are randomly scattered around 0 (as shown in Figure 4.15), indicating that Model 4 is suitable to fit the data determined for 0.2 \(\mu\)M Py mixing with OA. The values of \(k_{21}^+\) recovered from two independent experiments, i.e. \((2.58 \pm 0.07) \times 10^6\) M\(^{-1}\)s\(^{-1}\) and \((2.63 \pm 0.02) \times 10^6\) M\(^{-1}\)s\(^{-1}\), are similar as the values determined by using Model 3. This observation supports the argument that the contribution of the dissociation process
of OA•Py•OA complex is very small to the kinetic relaxation process observed in Figure 4.10.

\[
\begin{align*}
\text{OA} & \quad + \quad \text{Py} \quad \overset{K_{11}}{\rightleftharpoons} \quad \text{Py} \cdot \text{OA} \\
\text{OA} & \quad + \quad \text{Py} \cdot \text{OA} \quad \overset{k_{21}^+}{\rightarrow} \quad \text{OA} \cdot \text{Py} \cdot \text{OA}
\end{align*}
\]  \tag{4.24}

\[
\begin{align*}
\text{OA} & \quad + \quad \text{Py} \quad \overset{k_{11}^-}{\rightarrow} \quad \text{Py} \cdot \text{OA} \\
\text{OA} & \quad + \quad \text{Py} \cdot \text{OA} \quad \overset{k_{21}^+}{\rightarrow} \quad \text{OA} \cdot \text{Py} \cdot \text{OA}
\end{align*}
\]  \tag{4.25}

Scheme 4.3. Reactions for the binding of Py with OA in the presence of buffer.

Figure 4.15. Residuals between the experimental data and the fit to the second-order global fitting model (Model 4, i.e. Scheme 4.3). The stopped-flow traces for OA mixing with Py were shown in Figure 4.10. [Py] = 0.2 µM; [OA] from top to bottom are: 0.5 µM, 1 µM, 2 µM, 3 µM and 4 µM.

The value of \( k_{21}^+ \), i.e. \((2.61 \pm 0.04) \times 10^6 \text{ M}^{-1} \text{s}^{-1} \) according to two independent experiments, is determined by fixing the value of \( K_{11} \) as \( 4.5 \times 10^5 \text{ M}^{-1} \). The error of \( k_{21}^+ \) is quite small. However, the error of \( k_{21}^+ \) may be underestimated since the uncertainty of the
value of $K_{11}$ was not taken in account during the global fitting process. Therefore, the highest and the lowest limits for $K_{11}$ were individually fixed in the global fitting process and the corresponding values of $k_{21}^+$ were determined (Table 4.1). The residuals between the experimental data and calculated data were randomly around 0 for all the global fittings. The values of $k_{21}^+$ were determined as $(2.6 \pm 0.2) \times 10^6$ M$^{-1}$s$^{-1}$ and $(2.7 \pm 0.2) \times 10^6$ M$^{-1}$s$^{-1}$ from two independent experiments, leading to an average of $k_{21}^+$ as $(2.6 \pm 0.2) \times 10^6$ M$^{-1}$s$^{-1}$.

Table 4.1. Values of the association rate constants for the formation of OA•Py•OA complex ($k_{21}^+$) recovered from the global fitting and the corresponding values of the equilibrium constants for the formation of OA•Py complex ($K_{11}$) fixed in the global fitted process.

<table>
<thead>
<tr>
<th>$K_{11}$ fixed / 10$^5$ M$^{-1}$</th>
<th>$k_{21}^+$ recovered / 10$^6$ M$^{-1}$ s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>trial I</td>
</tr>
<tr>
<td>3.9</td>
<td>2.79 ± 0.07</td>
</tr>
<tr>
<td>4.5</td>
<td>2.58 ± 0.07</td>
</tr>
<tr>
<td>5.1</td>
<td>2.44 ± 0.06</td>
</tr>
</tbody>
</table>

*The errors were recovered by globally fitting the kinetic traces (shown in Figure 4.10) with Model 4.

The value of $K_{21}$, i.e. the equilibrium constant for the formation of OA•Py•OA (Reaction 4.2), was recovered as $(7 \pm 1) \times 10^6$ M$^{-1}$ using Equation 4.26. The value of $k_{21}^-$ was recovered as $2.7 \pm 0.4$ s$^{-1}$ using Equation 4.27. The value of $\beta_{21}, K_{11}, K_{21}, k_{21}^+$ and $k_{21}^-$ were listed in Table 4.2.
\[ K_{21} = \frac{\beta_{21}}{K_{11}} \]  
\[ k_{21}^- = \frac{K_{11} \times k_{21}^+}{\beta_{21}} \]

Table 4.2. Values of \( \beta_{21}, K_{11}, K_{21}, k_{21}^+ \) and \( k_{21}^- \) recovered in this work

<table>
<thead>
<tr>
<th>Parameters</th>
<th>( \beta_{21} / 10^{-12} \text{ M}^2 )</th>
<th>( K_{11} / 10^5 \text{ M}^{-1} )</th>
<th>( K_{21} / 10^6 \text{ M}^{-1} )</th>
<th>( k_{21}^+ / 10^6 \text{ M}^{-1} \text{s}^{-1} )</th>
<th>( k_{21}^- / \text{s}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td>3.19 ± 0.06</td>
<td>4.5 ± 0.6</td>
<td>7 ± 1</td>
<td>2.6 ± 0.2</td>
<td>0.37 ± 0.06</td>
</tr>
</tbody>
</table>

\(^a\)The values and errors of \( \beta_{21}, K_{11} \) and \( k_{21}^+ \) were all recovered from two independent experiments. The values and errors of \( K_{21} \) were recovered using Equation 4.26. The values and errors of \( k_{21}^- \) were recovered using Equation 4.27.

### 4.4 Discussion

#### 4.4.1 The effect of OA binding on the photophysical properties of Py

The bathochromic shifts were observed in the absorption, the fluorescence excitation and the fluorescence emission spectra of Py when Py was bound to OA. Moreover, the bathochromic shifts were ca. 5 nm in the absorption and the fluorescence excitation spectra. The bathochromic shift in the absorption spectra for Py in the OA host system is large compared with that for Py in other host systems, e.g. Py in the sodium dodecylsulphate micelle system (ca. 1 nm),\(^{267}\) Py in the sodium cholate host system (ca. 2.5 nm),\(^{271}\) and Py in the bovine serum albumin host system (ca. 4 nm).\(^{272,273}\)

The bathochromic shift of 5 nm in the fluorescence excitation spectra is large. Moreover, the excitation peaks around 334.5 nm for Py free in buffer and around 340 nm for Py bound to OA are narrow with FWHM of ca. 6 nm. As a result, the change of the fluorescence emission intensities upon the binding of Py to OA is strongly dependent on the excitation wavelength.
The shape of fluorescence emission spectra for Py bound to OA is very different from that for Py free in buffer. The value of $I_1/I_3$ of Py, which is very sensitive to the polarity of the environment of Py, was determined as 0.91 and 1.74 for Py bound to OA and for Py free in buffer, respectively. These observations indicates that the cavity of OA provided Py a non-polar environment which is very different from the aqueous phase.

4.4.2 The binding affinity of Py to OA

The dependence of the fluorescence intensities of Py with the concentration of OA can be fitted well with the model of the formation of OA-Py 2:1 complex instead of the model of the formation of OA-Py 1:1 complex. This observation supports the mechanism of the formation of OA-Py 2:1 complex at equilibrium.

Two-step kinetics were observed for the complexation of OA and Py in the stopped flow experiments. The first step led to the offset of the fluorescence intensity at time 0 for each kinetic trace away from the fluorescence intensity for the mixing of Py with buffer. The equilibrium constants for the formation of OA-Py 1:1 complex ($K_{11}$) were determined by studying the dependence of the offset of the fluorescence intensity at time 0 with the concentration of OA. The equilibrium constant for the formation of OA-Py 2:1 complex ($K_{21}$) can be determined by dividing the overall equilibrium constant for the complexation of OA and Py ($\beta_{21}$) by $K_{11}$. The equilibrium constants for the formation of OA-Py 1:1 complex and for the formation of 2:1 OA-Py complex ($K_{21}$) are $(4.5 \pm 0.6) \times 10^5$ M$^{-1}$ and $(7 \pm 1) \times 10^6$ M$^{-1}$, respectively. The value of $K_{11}$ is close to the value recovered for the formation of OA-acyclic carboxylic acids 1:1 complex, e.g. $1.09 \times 10^5$ M$^{-1}$ for OA•decanoate, $2.80 \times 10^5$ M$^{-1}$ for OA•3-noradamantane carboxylate. The
values of $K_{11}$ and $K_{21}$ are respectively much higher than the corresponding values (ca. 280 M$^{-1}$ for $K_{11}$ and ca. 100 M$^{-1}$ – 170 M$^{-1}$ for $K_{21}$) in the $\gamma$-CD-Py host-guest system.$^{274,275}$

4.4.3 The binding dynamics of Py with OA

The first step observed for the complexation of OA and Py in the stopped flow experiments was sufficiently fast to be viewed as a pre-equilibrium process while the second step was slow on the stopped flow time scale. In other words, the dynamics for the formation of high-order OA-Pyrene complex was much slower than that for the formation of 1:1 OA-Pyrene complex. The same trend was also observed in $\gamma$-CD-Pyrene system.$^{274}$ The association rate constant for the formation of OA-Py 2:1 complex ($k_{21}^+$, $(2.6 \pm 0.2) \times 10^6$ M$^{-1}$s$^{-1}$) was determined by globally studying the kinetic relaxation processes of all traces with the two-step OA-Py complexation model. The dissociation rate constant for the formation of OA-Py 2:1 complex ($k_{21}^-$, $2.7 \pm 0.4$ s$^{-1}$) was determined by dividing $K_{21}$ by $k_{21}^+$. The value of $k_{21}^+$ for the OA-Py 2:1 complex is much higher than that for the $\gamma$-CD-Py 2:1 complex ($< 200$ M$^{-1}$s$^{-1}$) while the value of $k_{21}^-$ for the OA-Py 2:1 complex is closed to that for the $\gamma$-CD-Py 2:1 complex ($< 2$ s$^{-1}$).$^{274}$ As a result, the equilibrium constant for the formation of OA-Py 2:1 complex is much higher than that for the formation of $\gamma$-CD-Py 2:1 complex. In other words, the binding kinetic study, from the dynamic point of view, provided further mechanistic information to explain the high binding affinity of OA•Py to OA.

It is worth noticing that OA can form aggregates in the presence of the inorganic salts, e.g. 50 mM sodium tetraborate leads to the aggregation of OA at the concentration of 1 mM.$^{164}$ In this study, the concentrations of OA ($< 6 \mu$M) and sodium tetraborate (10 mM)
were low to avoid the aggregation of OA. Moreover, the aggregation of OA should lead to the observation of two kinetic relaxation process for each kinetic trace, since the aggregation process for OA and the complexation process of OA with Py are coupled. Only one kinetic relaxation process was observed for each kinetic trace in the stopped flow experiment, indicating that the aggregation of OA did not occur in the OA-Py system under the experimental conditions employed in this study.

Ramamurthy's group determined the quenching rate constants of the excited state of Py by oxygen.\textsuperscript{218} The value of oxygen-quenching rate constant for the excited state of OA•Py•OA ($5 \times 10^5$ M$^{-1}$s$^{-1}$) is much lower than that for the excited state of Py free in buffer ($1.6 \times 10^9$ M$^{-1}$s$^{-1}$). They suggested that this observation was due to the opening-closing process of the OA capsule, which was the rate limiting step in the oxygen-quenching process for the excited state of Py inside the OA capsule. However, the opening-closing process of the OA capsule they studied does not represent the dissociation process of the OA capsule, since the OA capsule can open partially to let $O_2$ move into the cavity of OA rather than completely dissociate.
Summary

The kinetic studies were conducted in three host-guest systems, i.e. the CB[7]-NpAmH⁺ system, the β-CD-NpOH system and the OA-Py system. The results showed that the kinetic studies provided crucial information to build up the mechanism for the complexation of host and guest molecules in each system.

The kinetic studies on the CB[7]-NpAmH⁺ system were introduced in Chapter 2. The addition of co-cations in the CB[7]-NpAmH⁺ system led to the formation of CB[7]•M⁺ and M⁺•CB[7]•M⁺, which decreased the concentration of CB[7] species available for binding with NpAmH⁺. Consequently, the kinetic process for the binding of the CB[7] species to NpAmH⁺ was retarded to the extent that the kinetics was suitable to be studied in the stopped flow experiments and the overall equilibrium constants for the complexation between CB[7] species and NpAmH⁺ was decreased with the addition of co-cations. Only CB[7] free in water can bind to NpAmH⁺ while CB[7]•M⁺ or M⁺•CB[7]•M⁺ cannot. This mechanism for the complexation of the CB[7] species and NpAmH⁺ can be only supported by the kinetic studies. The association rate constant for CB[7] binding to NpAmH⁺ is ca. $6.3 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ while the dissociation rate constant is ca. $55 \text{ s}^{-1}$, leading to a very high equilibrium binding constant of CB[7] to NpAmH⁺.

The kinetic studies on the CB[7]-NpAmH⁺ system can be extended to other CB[n]-guest systems. The guest molecules with different size, shape and charge distribution may bind to different CB[n] species (e.g. CB[n] free in water or CB[n]•M⁺) through different mechanism (e.g. the flip-flop mechanism or the mechanism that the guest moves into the CB[n] cavity directly). The kinetic studies for the complexation between different guests
and CB[n] will lead to the understanding how the molecular recognition mechanism and the binding dynamics of CB[n] with guests are affected by the molecular structures of the host and the guest molecules.

The methodology was developed to determine the equilibrium and rate constants for the complexation between CB[n] and non-fluorescent guests (e.g. Na⁺ and H₃O⁺ in this work). The kinetic process for the binding of the CB[7] species to Na⁺ or H₃O⁺ was sufficiently fast to be viewed as a pre-equilibrium process and the equilibrium constants for the complexation between CB[7] and M⁺ were determined. This methodology can be developed to study the complexation between CB[n] and non-fluorescent guests with much slower rate constants. Consequently, not only the equilibrium constants but also the rate constants for the complexation between CB[n] and non-fluorescent guests can be determined.

The kinetic studies on the β-CD-NpOH system were introduced in Chapter 3. The reactions between the enantiomers of NpOH and β-CD lead to the formation of two kinds of 1:1 complexes (E and N complex) and three kinds of 2:2 complexes (EE, EN and NN complex), where the difference between E and N is the alignment of NpOH inside the β-CD cavity. The formation of the 2:2 complex, which occurred on the millisecond time scale at 10 °C, was studied by stopped flow. The association rate constant for the formation of the 2:2 complex are in the same range for both NpOH enantiomers while the dissociation rate constant for the formation of the 2:2 complex are quite different. The value of this dissociation rate constant for (R)-NpOH is 46.8% lower than that for (S)-NpOH. As a result, more 2:2 complexes were formed for (R)-NpOH than for (S)-NpOH. Furthermore, the kinetic study indicated that the equilibrium binding constants are similar
for the formation of all three 2:2 complexes. This observation was a crucial prerequisite for the conclusion that more $E$ complexes and less $N$ complexes were formed for (R)-NpOH than for (S)-NpOH. In other words, the kinetic study provides unique information to discover the chiral recognition for the formation of 1:1 complexes, which cannot be observed in the equilibrium binding study.

This example shows that chiral recognition due to subtle structural changes can lead to large differences being observed for the host-guest dynamics. This finding is important for the design of supramolecular structures where the function is dynamic in nature. For example, in catalysis chiral recognition may be achieved even if the thermodynamics would not show significant chiral discrimination. The kinetic studies can be extended to the chiral recognition of guest with the modified cyclodextrins with much larger chiral recognition effect, which could provide useful information to help understand the mechanism of the chiral recognition process and to help design the functionalized CD.

The kinetic studies on the OA-Py system were introduced in Chapter 4. The kinetic studies supported the two-step successive complexation reaction model. The kinetic process for the formation of OA-Py 1:1 complex was sufficiently fast to be viewed as a pre-equilibrium process while the kinetic process for the formation of OA-Py 2:1 complex was slow on the stopped flow time scale. The equilibrium constant for the formation of OA-Py 1:1 complex and the rate constants for the formation of OA-Py 2:1 complex were determined. It is worth noticing that the equilibrium constant for the formation of OA-Py 1:1 complex can only be determined by the kinetic studies since the equilibrium binding study was not suitable to determine the equilibrium constant for the formation of intermediate (i.e. OA-Py 1:1 complex).
The kinetic studies can be conducted on the complexation between OA and different guest molecules. OA capsule can bind to a wide range of guest molecules to form OA-guest 1:1, 2:1 or 2:2 complexes and the type of complexes is mainly dependent on the sizes, the hydrophobicities or the polarities of the guests. The kinetic studies could help to understand the formation of the OA-guest complexes with different stoichiometries from the dynamic point of view.
Reference


Appendix

a.1. The relationship between the overall equilibrium binding constant \( (\beta_{11}) \) and the equilibrium binding constants for CB[7] binding to NpAmH\(^+\) or co-cations

The derivation was conducted by using the NpAmH\(^+\)/CB[7]/Na\(^+\) system as an example.

The overall equilibrium constant \( (\beta_{11}) \) for the binding of NpAmH\(^+\) to CB[7] can be defined by Equation A.1, which was derived from Equation 2.8.

\[
\beta_{11} = \frac{[HG]}{[H][G]} \tag{A.1}
\]

where \([HG]\), \([H]\) and \([G]\) were the concentration of the NpAmH\(^+\)/CB[7] complex, the concentration of CB[7] species which do not bind with NpAmH\(^+\) and the concentration of NpAmH\(^+\) species which do not bind with CB[7], respectively. The value of \([HG]\), \([H]\) and \([G]\) can be defined by Equations A.2, A.3 and A.4 according to Scheme 2.3.

\[
[HG] = [\text{NpAmH}^+ \@ \text{CB[7]}]_{\text{eq}} \tag{A.2}
\]

\[
[H] = [\text{CB[7]}]_{\text{eq}} + [\text{CB[7]} \cdot \text{Na}^+]_{\text{eq}} + [\text{Na}^+ \cdot \text{CB[7]} \cdot \text{Na}^+]_{\text{eq}} \tag{A.3}
\]

\[
[G] = [\text{NpAmH}^+]_{\text{eq}} \tag{A.4}
\]

\[
K_{01} = \frac{[\text{CB[7]} \cdot \text{Na}^+]_{\text{eq}}}{[\text{CB[7]}]_{\text{eq}}[\text{Na}^+]_{\text{eq}}} \tag{A.5}
\]

\[
K_{02} = \frac{[\text{Na}^+ \cdot \text{CB[7]} \cdot \text{Na}^+]_{\text{eq}}}{[\text{CB[7]} \cdot \text{Na}^+]_{\text{eq}}[\text{Na}^+]_{\text{eq}}} \tag{A.6}
\]

Equation A.7 can be derived from Equations A.3, A.5 and A.6.

\[
[H] = [\text{CB[7]}]_{\text{eq}} \left(1 + K_{01}[\text{Na}^+] + K_{01}K_{02}[\text{Na}^+]^2\right) \tag{A.7}
\]

Equation A.8 can be derived from Equations A.1, A.2, A.4 and A.7.
\[
\beta_{11} = \frac{[\text{NpAmH}^+@\text{CB}[7]]_{\text{eq}}}{[\text{NpAmH}^+]_{\text{eq}} [\text{CB}[7]]_{\text{eq}} \left( 1 + K_{01}[\text{Na}^+] + K_{01}K_{02}[\text{Na}^+]^2 \right)} 
\]  
(A.8)

Equation A.9 can be derived from Equation 2.18.

\[
K_{001} = \frac{[\text{NpAmH}^+@\text{CB}[7]]_{\text{eq}}}{[\text{NpAmH}^+]_{\text{eq}} [\text{CB}[7]]_{\text{eq}}} 
\]  
(A.9)

As a result, Equation A.10 can be derived from Equations A.8 and A.9.

\[
\beta_{11} = \frac{K_{001}}{1 + K_{01}[\text{Na}^+] + K_{01}K_{02}[\text{Na}^+]^2} 
\]  
(A.10)

Equation A.10 is derived for NpAmH+/CB[7]/Na\(^+\) system. Moreover, Equation A.11 can be derived by the same method for NpAmH+/CB[7]/H\(_3\)O\(^+\) system.

\[
\beta_{11} = \frac{K_{001}}{1 + K_1[H_3O^+] + K_1K_2[H_3O^+]^2} 
\]  
(A.11)

a.2. The derivation for the relationship between the observed rate constants \((k_{\text{obs}})\) and the concentration of CB[7] according to the mechanism proposed in Scheme 2.3

Equation A.12 can be derived for the reaction 2.18.

\[
\frac{d[\text{NpAmH}^+@\text{CB}[7]]}{dt} = k_{001}([\text{CB}[7]][\text{NpAmH}^+]) - k_{001}^{-1}[\text{NpAmH}^+@\text{CB}[7]] 
\]  
(A.12)

Equation A.13 can be derived from Equation A.12 where the symbol \([i]\) with a subscript “eq” corresponds to the concentration of species \(i\) in equilibrium and the symbol \(\Delta[i]\) corresponds to the difference between the concentration of species \(i\) at time \(t\) and the concentration of species \(i\) in equilibrium.
\[
\frac{d\left([\text{NpAmH}^\star@\text{CB}[7]]_{\text{eq}} + \Delta\left([\text{NpAmH}^\star@\text{CB}[7]\right]\right)}{dt} = \\
+k_{001}^+\left([\text{CB}[7]]_{\text{eq}} + \Delta[\text{CB}[7]]\right)\left([\text{NpAmH}^+]_{\text{eq}} + \Delta[\text{NpAmH}^+]\right) \\
-k_{001}^-\left([\text{NpAmH}^\star@\text{CB}[7]_{\text{eq}} + \Delta[\text{NpAmH}^\star@\text{CB}[7]]\right) \\
\]

The term \( \frac{d\left([\text{NpAmH}^\star@\text{CB}[7]]\right)}{dt} \) in Equation A.12 is equal to 0 at the equilibrium state and Equation A.14 is derived.

\[
k_{001}^+\left([\text{CB}[7]]_{\text{eq}}\right)\left([\text{NpAmH}^+]_{\text{eq}}\right) = k_{001}^-\left([\text{NpAmH}^\star@\text{CB}[7]]_{\text{eq}}\right) \quad (A.14)
\]

Equation A.15 is derived from Equations A.13 and A.14.

\[
\frac{d\left(\Delta[\text{NpAmH}^\star@\text{CB}[7]]\right)}{dt} = k_{001}^+\Delta[\text{CB}[7]]\left[\text{NpAmH}^+\right]_{\text{eq}} \\
+k_{001}^\star[\text{CB}[7]]_{\text{eq}}\Delta[\text{NpAmH}^+] - k_{001}^-\Delta[\text{NpAmH}^\star@\text{CB}[7]] \\
\]

The following derivation is conducted to study the relationship between three variables \( \Delta[\text{NpAmH}^+] \), \( \Delta[\text{CB}[7]] \) and \( \Delta[\text{NpAmH}^\star@\text{CB}[7]] \) so that the first two variables can be substituted by the last variable in Equation A.15.

The reaction 2.16 was a pre-equilibrium process on the stopped flow time scale. As a result, Equations A.16 – A.18 can be derived.

\[
[\text{CB}[7] \cdot \text{Na}^+] = K_{0i}[\text{CB}[7]][\text{Na}^+] \quad (A.16)
\]

\[
[\text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} + \Delta[\text{CB}[7] \cdot \text{Na}^+] = K_{0i}\left([\text{Na}^+]_{\text{eq}} + \Delta[\text{Na}^+]\right) \\
\times\left([\text{CB}[7]]_{\text{eq}} + \Delta[\text{CB}[7]]\right) \\
[\text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} = K_{0i}[\text{CB}[7]]_{\text{eq}}[\text{Na}^+]_{\text{eq}} \quad (A.17)
\]

Equation A.19 can be derived from Equations A.17 and A.18.

\[
\Delta[\text{CB}[7] \cdot \text{Na}^+] = K_{0i}[\text{CB}[7]]_{\text{eq}}\Delta[\text{Na}^+] + K_{0i}[\text{Na}^+]_{\text{eq}}\Delta[\text{CB}[7]] \quad (A.19)
\]
The reaction 2.17 was a pre-equilibrium process on the stopped flow time scale. As a result, Equations A.20 – A.22 can be derived.

\[
[\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+] = K_{o1} [\text{CB}[7] \cdot \text{Na}^+] [\text{Na}^+] \\
[\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+ \text{eq}] + \Delta [\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+] = K_{o2} \left([\text{Na}^+]_{\text{eq}} + \Delta [\text{Na}^+]\right) \times \left([\text{CB}[7] \cdot \text{Na}^+ \text{eq}] + \Delta [\text{CB}[7] \cdot \text{Na}^+]\right) \\
[\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+ \text{eq}] = K_{o2} [\text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} [\text{Na}^+]_{\text{eq}}
\]

Equation A.23 can be derived from Equations A.21 and A.22.

\[
\Delta [\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+] = K_{o2} [\text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} \Delta [\text{Na}^+] + K_{o2} [\text{Na}^+]_{\text{eq}} \Delta [\text{CB}[7] \cdot \text{Na}^+]
\]

The mass balance equation A.24 for the sodium species can be derived from the reactions 2.16 and 2.17.

\[
\Delta [\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+] + \Delta [\text{CB}[7] \cdot \text{Na}^+] + \Delta [\text{Na}^+] = 0
\]

The mass balance equation A.25 for the CB[7] species can be derived from the reactions 2.16, 2.17 and 2.18.

\[
\Delta [\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+] + \Delta [\text{CB}[7] \cdot \text{Na}^+] + \Delta [\text{CB}[7]] + \Delta [\text{NpAmH}^+ @ \text{CB}[7]] = 0
\]

There are four equations (A.19, A.23, A.24 and A.25) with five \( \Delta [i] \) (i.e. \( \Delta [\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+] \), \( \Delta [\text{CB}[7] \cdot \text{Na}^+] \), \( \Delta [\text{Na}^+] \), \( \Delta [\text{CB}[7]] \) and \( \Delta [\text{NpAmH}^+ @ \text{CB}[7]] \)). Therefore, each \( \Delta [i] \) can be expressed in terms of one of the other four \( \Delta [i] \), e.g. \( \Delta [\text{CB}[7]] \) can be expressed in terms of \( \Delta [\text{NpAmH}^+ @ \text{CB}[7]] \) by Equation A.26.

\[
\Delta [\text{CB}[7]] = -R \times \Delta [\text{NpAmH}^+ @ \text{CB}[7]]
\]

where

\[
R = \frac{1 + K_{o1}[\text{CB}[7]]_{\text{eq}} + K_{o2}[\text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} + K_{o1}K_{o2}[\text{CB}[7]]_{\text{eq}} [\text{Na}^+]_{\text{eq}}}{1 + K_{o2}[\text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} + \left(K_{o1} + K_{o1}K_{o2}[\text{Na}^+]_{\text{eq}}\right) [\text{CB}[7]]_{\text{eq}} + [\text{Na}^+]_{\text{eq}}}
\]

The mass balance equation A.28 for the NpAmH\(^+\) species can be derived from the reactions 2.18.
\[
\Delta [\text{NpAmH}^+] = -\Delta [\text{NpAmH}^+ @ \text{CB}[7]] 
\] (A.28)

Equation A.29 can be derived from Equations A.15, A.26 and A.28.

\[
\frac{d\left(\Delta [\text{NpAmH}^+ @ \text{CB}[7]]\right)}{dt} = -k_{001}^* \times R \times [\text{NpAmH}^+]_{\text{eq}} \Delta [\text{NpAmH}^+ @ \text{CB}[7]] 
\] (A.29)

\[-k_{001}^* [\text{CB}[7]]_{\text{eq}} \Delta [\text{NpAmH}^+ @ \text{CB}[7]] - k_{001}^- \Delta [\text{NpAmH}^+ @ \text{CB}[7]]\]

The observed rate constant \(k_{\text{obs}}\) can then be defined (Equation A.30).

\[
k_{\text{obs}} = k_{001}^* \left([\text{CB}[7]]_{\text{eq}} + R \times [\text{NpAmH}^+]_{\text{eq}}\right) + k_{001}^- 
\] (A.30)

Equation A.27 for \(R\) can be simplified as Equation A.31 according to Equations A.32, A.33, A.34 and A.35.

\[
R = \frac{1}{1 + K_{01}[\text{Na}^+]_{\text{eq}} + K_{02} \times [\text{Na}^+]_{\text{eq}}^2} 
\] (A.31)

\[K_{01}[\text{CB}[7]]_{\text{eq}} \ll 1 \] (A.32)

\[K_{02}[\text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} \ll 1 \] (A.33)

\[[\text{Na}^+]_{\text{eq}} \gg [\text{CB}[7]]_{\text{eq}}\] (A.34)

\[K_{01} K_{02}[\text{CB}[7]]_{\text{eq}} [\text{Na}^+]_{\text{eq}} \ll 1 \] (A.35)

The mass balance for \text{CB}[7] species was defined by Equation A.36.

\[
[\text{CB}[7]]_T = [\text{CB}[7]]_{\text{eq}} + [\text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} + [\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+]_{\text{eq}} 
\] (A.36)

Equation A.37 can be derived from Equations A.18, A.36 and A.38.

\[
[\text{CB}[7]]_{\text{eq}} = \frac{1}{1 + K_{01} \times [\text{Na}^+]_{\text{eq}} + K_{01} K_{02} \times [\text{Na}^+]_{\text{eq}}^2 \times [\text{CB}[7]]_T} 
\] (A.37)

\[K_{02} = \frac{[\text{Na}^+ \cdot \text{CB}[7] \cdot \text{Na}^+]}{[\text{CB}[7] \cdot \text{Na}^+] [\text{Na}^+]}\] (A.38)

Equation A.39 can be derived from Equations A.30, A.31, A.37 and A.38.

\[
k_{\text{obs}} = k_{001}^* \frac{1}{1 + K_{01} \times [\text{Na}^+]_{\text{eq}} + K_{01} K_{02} \times [\text{Na}^+]_{\text{eq}}^2 \times \left([\text{NpAmH}^+]_{\text{eq}} + [\text{CB}[7]]_T\right)} + k_{001}^- 
\] (A.39)
Equation 2.31 can be derived from Equation A.39 under the experimental condition where Equation A.40 is valid.

\[ [\text{CB[7]}]_t \gg [\text{NpAmH}^+]_{\text{eq}} \] (A.40)

### a.3. The relationship between emission efficiencies and the lifetime of the singlet-excited state for the NpOH species

The statement that the ratio of \( C_i \) can be determined as the ratio of \( \tau_i \) for the \( E \) and \( N \) complex was crucial for the derivation of equation 3.37 from equation 3.36 as aforementioned. The following derivation shows how this statement was achieved.

The fluorescence emission from each species \( i \) in the steady-state fluorescence experiments can be studied on the basis of equation A.41 where \( I_{F,i} \), \( I_{abs,i} \) and \( \Phi_{F,i} \) are the rate of emission of photons, the rate of absorption of photons and the fluorescence quantum yield for each species \( i \).

\[ I_{F,i} = I_{abs,i} \Phi_{F,i} \] (A.41)

\( I_{abs,i} \) can be related to the the rate of incidence of photons \( (I_0) \) and the absorbance of species \( i \) \( (Abs_i) \) by equation A.42. The exponential term in equation A.42 can be linearized when the value of \( Abs_i \) is lower than 0.15 (equation A.43).

\[ I_{abs,i} = I_0 \left(1 - 10^{-Abs_i} \right) \] (A.42)
\[ 1 - 10^{-Abs_i} = 2.205 \times Abs_i \] (A.43)

Since the absorbance of 5 µM NpOH was less than 0.05, equation A.44 can be derived from equation A.41. Equation A.47 was derived from equation A.44 by substituting \( Abs_i \) and \( \Phi_{F,j} \) on the basis of the definitions in equations A.45 and A.46. The molar extinction coefficient \( (\epsilon_i) \), the pathlength \( (l) \), the rate constant for the fluorescence from the singlet-
excited species $i$ ($k_{F,i}$) and the lifetime for the singlet-excited species $i$ ($\tau_i$) were constant for each species $i$, while the parameter $I_0$ was related to instrumental settings, such as the excitation light intensity and bandwidth.

$$I_{F,i} = I_0 \times 2.205 \times Abs_i \times \Phi_{F,i}$$  \hspace{1cm} (A.44)

$$Abs_i = \varepsilon_i \times l \times [i]$$  \hspace{1cm} (A.45)

$$\Phi_{F,i} = k_{F,i} \times \tau_i$$  \hspace{1cm} (A.46)

$$I_{F,i} = I_0 \times 2.205 \times \varepsilon_i \times l \times [i] \times k_{F,i} \times \tau_i$$  \hspace{1cm} (A.47)

The values of $I_{F,j}$ were related to instrumental settings, such as the excitation light intensity and bandwidth, however, the ratio of $I_F$ was independent of instrumental settings for the species $i$ and $j$ present in the same solution (Equation A.48).

$$\frac{I_{F,i}}{I_{F,j}} = \frac{\varepsilon_i \times k_{F,i} \times \tau_i \times [i]}{\varepsilon_j \times k_{F,j} \times \tau_j \times [j]}$$  \hspace{1cm} (A.48)

The fluorescence intensity for each species ($I_{Mon,i}$) determined from steady-state fluorescence experiments is related to the emission efficiency ($C_i$) and the concentration of each species ($[i]$) (Equation A.49). The ratio of $I_{Mon}$ values, which is independent of instrumental settings, is equal to the ratio of $I_F$ values for species $i$ and $j$ present in the same solution (Equation A.50). Equation A.51 can be derived from equation A.49 and A.50.

$$I_{Mon,i} = C_i \times [i]$$  \hspace{1cm} (A.49)

$$\frac{I_{Mon,i}}{I_{Mon,j}} = \frac{\varepsilon_i \times k_{F,i} \times \tau_i \times [i]}{\varepsilon_j \times k_{F,j} \times \tau_j \times [j]}$$  \hspace{1cm} (A.50)

$$\frac{C_i}{C_j} = \frac{\varepsilon_i \times k_{F,i} \times \tau_i}{\varepsilon_j \times k_{F,j} \times \tau_j}$$  \hspace{1cm} (A.51)

The rate constant $k_F$ is related to the refractive index of the medium, the oscillator strength and the wavenumber corresponding to the maximum wavelength of absorption
In general, the oscillator strength of fluorescence dyes are insensitive to the environment where the dyes are located. The absorption spectra for both NpOH enantiomers in the presence of β-CD had the same shape indicating that the values of $\varepsilon_i$ were the same for the both NpOH enantiomers. The fluorescence spectra for $Np$, $E$, $N$, $EN$ and $NN$ for both NpOH enantiomers had the same shape indicating that the value of $\nu_0$ for each species was the same. As a result, the values of $k_{F,i}$ were the same for both NpOH enantiomers free in water ($Np$), or in the complex with β-CD ($E$, $N$, $EN$ and $NN$). Therefore the conclusion was reached that the ratio of $C_i$ was only dependent on the ratio of $\tau_i$ for $Np$, $E$, $N$, $EN$ and $NN$ (Equation A.52).

$$\frac{C_i}{C_j} = \frac{\tau_i}{\tau_j}$$ (A.52)

It is worth noting that the emission spectra of the $EE$ complex had a different shape from the spectra of the other NpOH species ($Np$, $E$, $N$, $EN$ and $NN$). This observation suggests that $\nu_0$, $\varepsilon_i$ and $k_{F,i}$ for the $EE$ complex were different from those for the other NpOH species. As a result, the ratio of $C_i$ cannot be related to the ratio of $\tau_i$ values when the comparison is conducted between the EE complex and the other NpOH species. However, the emission spectra of the $EE$ complex determined in the TRES experiments (see Section 3.4.3) had the same shape for both NpOH enantiomers. This observation indicated that $\nu_0$, $\varepsilon_i$ and $k_{F,i}$ for the $EE$ complex were the same for both NpOH enantiomers. So the ratio of $C_i$ was only dependent on the ratio of $\tau_i$ when the comparison was conducted between both NpOH enantiomers in the $EE$ complex. This conclusion was employed to analyze the ratio of $C_i$ for both NpOH enantiomers in the $EE$ complex in the discussion section.
**a.4. Analysis of the pre-exponential factors in the time-resolved fluorescence experiments**

The fluorescence decays determined in SPC experiments can be fitted to a sum of exponentials (Equation 3.7) when there are several species $i$ with different singlet-excited states lifetimes. The fluorescence intensities for each species $i$ can be related to the pre-exponential factor $A_i$ and singlet-excited state lifetime $\tau_i$ by equation A.53. Equation A.54 is derived from equation A.53.

\[
\frac{I_{F,i}}{I_{F,j}} = \frac{\int_0^\infty A_i \times e^{-\frac{t}{\tau_i}}}{\int_0^\infty A_j \times e^{-\frac{t}{\tau_j}}}
\]

(A.53)

\[
\frac{I_{F,i}}{I_{F,j}} = \frac{A_i \tau_i}{A_j \tau_j}
\]

(A.54)

Equation A.55 can be derived by associating equations A.48 and A.54.

\[
\frac{A_i}{A_j} = g_{i,j} \times \left[ \begin{array}{c} i \\ j \end{array} \right]
\]

(A.55)

where

\[
g_{i,j} = \frac{\epsilon_i \times k_{\text{F,i}}}{\epsilon_j \times k_{\text{F,j}}}
\]

(A.56)

**a.5. Analysis of the pre-exponential factors in the LFP experiments**

The decays of the triplet-excited states of species determined in LFP experiments were fitted to a sum of exponentials (Equation A.57) when there were several species $i$ with different triplet-excited states lifetimes.

\[
\Delta A = a_0 + \sum_{i=1}^{n} [a_i \times e^{-k_{\text{obs,i}}^{\text{lp}}}] 
\]

(A.57)

where $a_i$ and $k_{\text{obs,i}}^{\text{lp}}$ are the pre-exponential factor and rate constant for the decay of the triplet-excited states of the species $i$, respectively.
The values of \( a_i \) are related to the concentration of the species \( i \) ([\( i \)]). The following derivation shows how this statement was achieved.

The values of \( a_i \) can be related to initial concentrations of the triplet-excited states of the species \( i \) \([i^3]_0\) by equation A.58.

\[
a_i = [i^3]_0 \times \Delta \varepsilon_{420,i}
\] (A.58)

where \( \Delta \varepsilon_{\text{exc}} \) is the difference in the extinction coefficients of the triplet-excited states and the ground states of the species \( i \) at 420 nm, the wavelength where the decay traces were recorded.

\([i^3]_0\) can be related to the amount of absorbed photons ((\( h\nu_{\text{abs},i} \)) and the quantum yield of the intersystem crossing from \( S_1 \) to \( T_1 \) (\( \Phi_{\text{ST},i} \)) for species \( i \) as shown in equation A.59.

\[
[i^3] = [h\nu_{\text{abs},i}] \times \Phi_{\text{ST},i}
\] (A.59)

The amount of absorbed photons can be related to the amount of incident photons for each laser pulse ([\( h\nu \)]_0) and the absorbance of species \( i \) (\( \text{Abs}_i \)) by equation A.60.

\[
[h\nu_{\text{abs},i}] = [h\nu]_0 \times (1 - 10^{-\text{Abs}_i})
\] (A.60)

Since \([i^3]_0\) is much lower than the concentration of the ground state of the species \( i \), the change of \( \text{Abs}_i \) due to the formation of the triplet-excited states of the species \( i \) is low. Therefore, the exponential term in Equation A.60 can be linearized according to Equation A.43. Equation A.61 can be derived by associating equations A.43, A.45 and A.60.

\[
[h\nu_{\text{abs},i}] = [h\nu]_0 \times 2.205 \times \varepsilon_{266,i} \times l \times [i]
\] (A.61)

where \( \varepsilon_{266,i} \), \([i]\) are the extinction coefficient at 266 nm and the concentration of species \( i \), respectively.
The values of \( a_i \) can be related to the concentration of species \( i \) by equation A.62 which was derived by associating equations A.58, A.59 and A.61.

\[
a_i = [h\nu]_0 \times 2.205 \times l \times \phi_{ST,i} \times \varepsilon_{266,i} \times \Delta \varepsilon_{420,i} \times [i] \quad \text{(A.62)}
\]

The amount of incident photons for each laser pulse ([\( h\nu \]_0)) and the light pathlength (\( l \)) are related to the instrumental settings. The triplet quantum yield (\( \phi_{ST,i} \)) for the NpOH species \( i \) may not be markedly dependent on the environment where the NpOH species \( i \) is located, as \( \phi_{ST,i} \) for naphthalene species is independent of the environment where the naphthalene molecules are located. The absorption spectra for both NpOH enantiomers in the presence of \( \beta \)-CD had the same shape, which indicated that the values of \( \varepsilon_{266,i} \) were the same for the both NpOH enantiomers. Therefore, the values of the parameter \( R_{22,i} \) in equation A.63 could be assumed to be the same for both NpOH enantiomers in the \( NN \) and \( EN \) complexes when determined under the same experimental settings, i.e. the same power of the excited laser pulses and the same power of the monitoring beam.

\[
R_{22,i} = [h\nu]_0 \times 2.205 \times l \times \phi_{ST,i} \times \varepsilon_{266,i} \quad \text{(A.63)}
\]

The ratio of \( a_i \) for (R)-NpOH in the \( NN \) and \( EN \) complexes (\( a_{2R} \)) to \( a_i \) for (S)-NpOH in the \( NN \) and \( EN \) complexes (\( a_{2S} \)) can be related to the ratio of the concentration of (R)-NpOH in the \( NN \) and \( EN \) complexes (\( [\text{NN}]_R + [\text{EN}]_R \)) to the concentration of (S)-NpOH in the \( NN \) and \( EN \) complexes (\( [\text{NN}]_S + [\text{EN}]_S \)) as shown in equation A.64.

\[
\frac{a_{22,R}}{a_{22,S}} = \frac{\Delta \varepsilon_{420,R}}{\Delta \varepsilon_{420,S}} \times \frac{[\text{NN}]_R + [\text{EN}]_R}{[\text{NN}]_S + [\text{EN}]_S} \quad \text{(A.64)}
\]