Electronic Supporting Information

Determination of the kinetics underlying the pKₐ shift for 2-aminoanthracenium cation binding with cucurbit[7]uril

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The synthesis of CB[7] was adapted from previous procedures. Reagent grade chemicals were used in all steps of the synthesis and purification, except for the spectral grade methanol that was used in the final purification steps. Glycoluril (10.0 g, 1 equiv) and powdered formaldehyde (4.0 g, 1.9 equiv) were magnetically stirred to ensure homogeneous mixing in a two-necked 100 mL round bottom flask attached to a condenser and a CaCl₂ guard tube. After heating the solid at 50 °C for 5–10 min, 20 mL of an acid mixture of 20% H₂SO₄ in HCl was added dropwise to the solid while stirring. The reaction mixture formed a hard solid upon vigorous stirring. After addition of acid, the temperature was raised to 80 °C, and the hard solid dissolved slowly to give a clear solution. After heating the reaction mixture at 80 °C for 3 h, the temperature was raised again to 95–105 °C, and the reaction mixture was heated for another 5 h. After cooling to room temperature, the reaction mixture was poured slowly into 150 mL of cold methanol (in an ice bath) with vigorous stirring and was left standing for 30 min to completely precipitate the CB[n]s. This heterogeneous suspension was then filtered, and the solid from the filtration was mixed with 120 mL of water. This water suspension was stirred vigorously at 40 °C for 4 h and then filtered while hot. Caution was taken not to exceed a temperature of 60 °C while heating the solution, as this resulted in a waxy hard solid after filtration. The solid from the filtration was stirred with water twice more at 40 °C for 4 h to extract more CB[n]s. The filtrate contained the desired CB[7]. Of the three filtrate fractions, the second and third had a higher percentage of CB[7], determined through titration. The combined second and third filtrate fractions were cooled to room temperature, added to 150 mL of methanol, and refrigerated overnight. The methanol–water suspension was filtered and washed well with plenty of methanol. The air-dried solid obtained from the second and third fractions of the filtrate (2.0 g) from this filtration was characterized by ESI-MS. The solid was titrated and contained 60–75% CB[7]. The solid obtained from the first filtrate fraction had only 20–30% CB[7] and was discarded. The solid containing 60–75% CB[7] obtained as described above was further purified as follows: The solid (2.0 g) was stirred with 50 mL of water for 1 h. This water suspension was filtered, and the residue was stirred with another 30 mL of water for 1 h and filtered again. The filtrate fractions were combined, added to 120 mL of methanol, and refrigerated overnight for complete precipitation of CB[n]s. This water–methanol suspension was filtered, and the residue was washed with plenty of methanol to remove mineral acids. The reprecipitation of CB[7] from water using methanol was repeated to remove CB[5] from CB[7]. The solid from the filtration (1.0 g) was dissolved in the minimum required amount of water with heating (45–50 °C). Methanol was added dropwise to this hot solution until the solution turned slightly turbid. The turbid solution was filtered while hot, and the insoluble solid (mainly CB[6]) was discarded. The filtrate was cooled to room temperature and methanol (1:1.5 water–methanol volume ratio) was added to reprecipitate CB[7]. This suspension was filtered after overnight refrigeration. The solid from the filtration (700 mg) was refluxed in 50 mL of a methanol–water mixture (1.3:1 by volume) for 10 h. The resultant suspension was cooled to room temperature, filtered, and washed with methanol. The solid obtained was vacuum dried at 80 °C for 12 h. The resultant solid (500 mg) was characterized by ESI-MS, C NMR, and H NMR. The mass spectrometry data did not show the presence of any CB[6], but did show traces of CB[5]. The C NMR results showed the presence of less than 3% CB[5]. Immediately after vacuum drying, a titration with cobaltaccenium cation showed that the sample contained 90% CB[7].
2. Model for the fitting of binding isotherms and binding isotherms at different pH values

The data were fit using the Scientist 3 software. The model defined to determine the overall equilibrium binding constants for AH⁺/A with CB[7] at different pH values is as follows: [CB[7]]ₜ denotes the total concentration of CB[7] and [G]ₜ denotes the total concentration of guest, which is the sum of protonated (AH⁺) and deprotonated forms (A) of the guest. Both [CB[7]]ₜ and [G]ₜ are defined as independent variables. The equilibrium concentrations of free CB[7] ([CB[7]]ₑq), free guest ([G]ₑq), the 1:1 complex ([G@CB[7]]ₑq), and the fluorescence intensity (I) are defined as dependent variables. R corresponds to the ratio of the fluorescence intensity of the guest in the absence of CB[7] (I₀) to the total guest concentration, while C₁₁ is the ratio of the emission efficiencies of the guest in the complex and in water, and β₁₁ is the overall equilibrium binding constant for the 1:1 complex. R, C₁₁, and β₁₁ were set as parameters. Note that an overall binding constant was determined since the equilibria between Na⁺ cations and CB[7] are not formally accounted for (see manuscript for description).

The following equations are used in the model:

\[
[G@CB[7]]ₑq = β₁₁ × [G]ₑq × [CB[7]]ₑq \quad \text{(eq. S1)}
\]

\[
[CB[7]]ₑq = [CB[7]]ₜ - [G@CB[7]]ₑq \quad \text{(eq. S2)}
\]

\[
[G]ₑq = [G]ₜ - [G@CB[7]]ₑq \quad \text{(eq. S3)}
\]

\[
I = R × ( [G]ₑq + C₁₁ × [G@CB[7]]ₑq ) \quad \text{(eq. S4)}
\]

where

\[
R = \frac{I₀}{[G]ₜ} \quad \text{(eq. S5)}
\]

The concentration constraints for the dependent variables are as follows:

\[
0 < [CB[7]]ₑq < [CB[7]]ₜ \quad \text{(eq. S6)}
\]

\[
0 < [G]ₑq < [G]ₜ \quad \text{(eq. S7)}
\]

\[
0 < [G@CB[7]]ₑq < [G]ₜ \quad \text{(eq. S8)}
\]

The binding isotherms determined at pH values of 2.0, 3.8, 5.0, and 5.5 were fit to the numerical model described above. The residuals for the fit of the “green” intensity changes with CB[7] concentration were always smaller than the corresponding residuals for the “blue” emission. However, in all cases the relative residuals were less than 2% of the maximum intensity values measured. The individual values for the overall binding constants (β₁₁) determined from the
changes in the “blue” and “green” emission at the different pH values studied are shown in Table S1.

Figure S1. Binding isotherms and residuals at pH 3.8 (top), 5.0 (middle), and 5.5 (bottom) for “blue” (left) and “green” (right) emission determined for the binding of AH’/A (1 µM) with CB[7].
Table S1. Overall equilibrium constants for the binding of A/AH\(^+\) with CB[7] determined from the changes in the “blue” and “green” emission intensities at different pH values.\(^a\)

<table>
<thead>
<tr>
<th>pH</th>
<th>(\beta_{11} / 10^5) M(^{-1}) (“green”)</th>
<th>(\beta_{11} / 10^5) M(^{-1}) (“blue”)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0</td>
<td>4.92 ± 0.09</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>3.8</td>
<td>2.86 ± 0.03</td>
<td>2.80 ± 0.06</td>
</tr>
<tr>
<td>5.0</td>
<td>0.36 ± 0.01</td>
<td>0.340 ± 0.004</td>
</tr>
<tr>
<td>5.5</td>
<td>0.155 ± 0.006</td>
<td>0.148 ± 0.006</td>
</tr>
</tbody>
</table>

\(^a\), errors correspond to those recovered from the numerical fit of the data.

Derivation of the relationship between the equilibrium constants in Scheme 1 in the paper.

\[
K_a = \frac{[A][H^+]}{[AH^+]} 
\]  
(eq. S9)

\[
K_{a}^{CB} = \frac{[A@CB[7]][H^+]}{[AH^+@CB[7]]} 
\]  
(eq. S10)

\[
\beta_{11}^{AH} = \frac{[AH^+@CB[7]]}{[AH^+][CB[7]]} 
\]  
(eq. S11)

\[
\beta_{11}^{A} = \frac{[A@CB[7]]}{[A][CB[7]]} 
\]  
(eq. S12)

Combining equations S9, S10, S11, and S12 and expressing the acid dissociation constants as p\(K_a\) values leads to the following equation:

\[
\frac{10^{-pK_a^{CB}}}{10^{-pK_a}} = \frac{\beta_{11}^A}{\beta_{11}^{AH}} 
\]  
(eq. S13)
The binding of A with CB[7] was studied at pH 12. An increase in the emission intensity was observed when CB[7] was added (Fig. S2). However, the binding is weak, and the binding isotherm only shows minor curvature and no saturation of the emission intensity, which would indicate that all guest A was bound to CB[7]. The saturation value also provides a measure of the increase in the emission quantum yield for A@CB[7] when compared with free A. The lack of saturation leaves this value undetermined. For this reason, the values of $\beta_{11}^A$ and the quantum yield for A in the complex are correlated, and the fit of the binding isotherm does not lead to unique values for $\beta_{11}^A$. The binding isotherm was fit by fixing $\beta_{11}^A$ to progressively higher values (Fig. S3). The fits are adequate for $\beta_{11}^A$ values from 100 to 700 M$^{-1}$, whereas for higher overall binding constants, systematic deviations from the experimental data were observed. Therefore, the value of $\beta_{11}^A$ is smaller than 700 M$^{-1}$.

Figure S2. Left: Emission spectra for A at pH 12 in the presence of increasing concentrations of CB[7] from 0 to 145 µM. Right: Dependence of the emission intensity of A with the CB[7] concentration.

Figure S3. Fitting of the binding isotherm for A with CB[7] determined at pH 12 by fixing the $\beta_{11}^A$ value to 100 (a), 400 (b), 700 (c), 900 (d), and 1500 M$^{-1}$ (e).
3. Singlet excited state lifetimes for AH+/A in water and in the presence of CB[7]

The emission kinetics for AH+ (1 µM) in water was measured at 510 nm because AH+ adiabatically deprotonates to form excited A, which emits at this wavelength (Fig. S4). The kinetics shows a growth, which is related to the formation of excited A from excited AH+, followed by the decay of A (Table S2). In the presence of 2.6 µM CB[7], where 50% AH+ is bound to CB[7], the emission at 510 nm showed the same time profile as in the absence of CB[7]. This result indicates that the emission in the presence of CB[7] corresponds to the excitation of AH+ in water, which then forms excited A.

The emission of AH+ at 410 nm was measured in the presence of 16 µM CB[7], where all AH+ is bound, and the emission decay was mono-exponential (Fig. S4 and Table S2). At 2.6 µM CB[7], the decay showed two lifetimes; the short lifetime is related to the deprotonation of AH+ in water and the second lifetime is related to the decay of excited AH+ in the AH+@CB[7] complex.

![Figure S4.](image)

**Figure S4.** Left: Kinetics for the emission of AH+ at 410 nm at pH 2.0 in the presence of 2.6 µM (black) and 16 µM (red) CB[7]. Right: Kinetics for the emission of A at 510 nm at pH 2.0 in the absence (black) and presence of 2.6 µM (red) CB[7]. The instrument response function is shown in blue.

**Table S2.** The fluorescence lifetimes (τ) and pre-exponential factors (A) for AH+ at pH 2.0 in the absence and presence of different concentrations of CB[7].

<table>
<thead>
<tr>
<th>[CB[7]] / µM</th>
<th>Emission at 510 nm (“green”)</th>
<th>Emission at 410 nm (“blue”)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>τ₁ / ns (A₁)</td>
<td>τ₂ / ns (A₂)</td>
</tr>
<tr>
<td>0</td>
<td>1.1 ± 0.1</td>
<td>24.6 ± 0.1</td>
</tr>
<tr>
<td>2.6</td>
<td>1.1 ± 0.1</td>
<td>24.5 ± 0.1</td>
</tr>
<tr>
<td>16</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a, the errors for the pre-exponential factors are ± 0.02.

4. Kinetics for AH+/A binding to CB[7] and models used for fitting

The kinetics was measured by stopped-flow at different pH values for the “green” (Fig. S5) and “blue” emission (Fig. S6). At all pH values, a decrease was observed for the “green” emission, while a corresponding increase was observed for the “blue” emission. The amplitude of the kinetics decreased as the pH was raised, consistent with the smaller amount of AH+/A that is bound to CB[7] at the higher pH values.
A small offset was on occasion observed between the control experiment in the absence of CB[7] and all samples containing CB[7] at pH 2.0 and 3.8. The magnitude of the offset was not consistent between experiments and did not increase when the CB[7] concentration was raised. In addition, the amplitude recovered from the fits is close to the experimental amplitude, suggesting that the small offset is due to the presence of the fast component at the low pH values and not a different process that occurs within the mixing time of the stopped-flow experiment. In the latter case, the offset should have increased when the CB[7] concentration was raised because any such process would depend on the host concentration.

**Figure S5.** Stopped-flow traces measured for the “green” intensity change when mixing AH⁺/A (1 µM) with different CB[7] concentrations: Top left and right: a. 0, b. 3, c. 5, d. 7, e. 9, f. 11, and g. 13 µM. Bottom left and right: a. 0, b. 5, c. 8, d. 11, e. 14, f. 17, and g. 20 µM. The pH values are: Top left: 2.0, top right: 3.8, bottom left: 5.0, and bottom right: 5.5.
Figure S6. Stopped-flow traces measured for the “blue” intensity change when mixing AH⁺/A (1 μM) with different CB[7] concentrations: Top left and right: a. 0, b. 3, c. 5, d. 7, e. 9, f. 11, and g. 13 μM. Bottom left and right: a. 0, b. 5, c. 8, d. 11, e. 14, f. 17, and g. 20 μM. The pH values are: Top left: 2.0, top right: 3.8, bottom left: 5.0, and bottom right: 5.5.
The amplitude of the emission intensity change in the stopped-flow experiment at 0.2 s for the highest CB[7] concentration used in the kinetic experiments was normalized to the amplitude at the same CB[7] concentration in the fluorescence steady-state experiments. The normalized intensities derived from the stopped-flow experiments are the same as the intensities from the binding isotherm experiments (Fig. S7 and S8), indicating that equilibration was achieved within the 0.2 s time scale of the kinetic experiments. The correspondence between the intensities is worse at the higher pH values because the signal-to-noise ratio is lower for the kinetic experiments.

Figure S7. Comparison of the changes in the equilibrium “green” emission intensity for 1 µM A/AH⁺ with CB[7] concentration obtained from the steady-state measurements (black) and stopped-flow kinetic traces (red) at pH values of 2.0 (top left), 3.8 (top right), 5.0 (bottom left), and 5.5 (bottom right).
Figure S8. Comparison of the changes in the equilibrium “blue” emission intensity for 1 µM A/AH+ with CB[7] concentration obtained from the steady-state measurements (black) and stopped-flow kinetic traces (red) at pH values of 2.0 (top left), 3.8 (top right), 5.0 (bottom left), and 5.5 (bottom right).

The dependence of the observed rate constant for the mixing of AH+/A at pH 4.3 with CB[7] was measured in solutions where the pH was adjusted by the addition of HCl or in another solution buffered with sodium acetate. The pH was chosen such that the buffering capacity was adequate at a Na+ cation concentration of 20 mM. The kinetics was measured for the “green” emission and the data were fit to a mono-exponential function.

Figure S9. The dependence of the observed rate constant on CB[7] concentration for the complexation of AH+/A (1 µM) for experiments performed in the presence of NaCl/HCl (black) or sodium acetate buffer (red) at pH 4.3.
The kinetics at pH 2.0 were fit to a mono-exponential fit with incrementally longer start times until random residuals between the experimental data and the fit were obtained and the value for $k_{\text{obs2}}$ did not change (Fig. S10). The values for $k_{\text{obs2}}$ when determined from the systematic fit or when left as a free parameter were the same.

![Figure S10](image)

**Figure S10.** Kinetic trace (a) for the mixing of 1 µM AH$^+$ with 9 µM CB[7] at pH 2.0, and the residuals between the trace and the calculated fit of the trace to a mono-exponential function when the fit was started incrementally at 2 (b, $k_{\text{obs}} = 51$ s$^{-1}$), 10 (c, $k_{\text{obs}} = 49$ s$^{-1}$), 20 (d, $k_{\text{obs}} = 47$ s$^{-1}$), and 25 ms (e, $k_{\text{obs}} = 47$ s$^{-1}$).

**Table S3.** Observed rate constants for one set of experiments performed at pH 2.0 in the “green” region recovered from the fits of the kinetic traces to a sum of two exponentials when $k_{\text{obs2}}$ was fixed to the value obtained from the systematic fits and when left as a free parameter.

<table>
<thead>
<tr>
<th>[CB[7]] / µM</th>
<th>Observed rate constants when $k_{\text{obs2}}$ was fixed from systematic fits</th>
<th>Observed rate constants when $k_{\text{obs1}}$ and $k_{\text{obs2}}$ are free parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{\text{obs1}}$ / s$^{-1}$</td>
<td>$k_{\text{obs2}}$ (fixed) / s$^{-1}$</td>
</tr>
<tr>
<td>3</td>
<td>108 ± 4</td>
<td>24.1 ± 0.1</td>
</tr>
<tr>
<td>5</td>
<td>138 ± 3</td>
<td>32.1 ± 0.1</td>
</tr>
<tr>
<td>7</td>
<td>150 ± 2</td>
<td>40.4 ± 0.1</td>
</tr>
<tr>
<td>9</td>
<td>167 ± 2</td>
<td>47.1 ± 0.1</td>
</tr>
<tr>
<td>11</td>
<td>170 ± 2</td>
<td>53.2 ± 0.1</td>
</tr>
<tr>
<td>13</td>
<td>178 ± 1</td>
<td>58.9 ± 0.1</td>
</tr>
</tbody>
</table>
The kinetics at pH 2.0 was analyzed using the mechanism shown in Figure 3c in the paper. The fit of individual experiments to Equation 5 in the paper for the $k_{obs2}$ dependence with the CB[7] concentration is shown in Figure S11 and the values for the recovered parameters are shown in Table S4.

**Figure S11.** Fits to Equation 5 in the paper for the dependence of $k_{obs2}$ with CB[7] concentration obtained from two sets of experiments performed in the “green” region (black and blue) and one set of experiment performed in the “blue” region (red) at pH 2.0.

**Table S4.** Results from the fits shown in Figure S11 for the $k_{obs2}$ dependence with the CB[7] concentration at pH 2.0.

<table>
<thead>
<tr>
<th>Emission</th>
<th>$k_+^{AH}$ / 10$^6$ M$^{-1}$ s$^{-1}$</th>
<th>$k_-^{AH}$ / s$^{-1}$</th>
<th>$\beta_y$ / 10$^4$ M$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green (expt 1)</td>
<td>5.4 ± 0.3</td>
<td>9.2 ± 0.9</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>Green (expt 2)</td>
<td>4.7 ± 0.6</td>
<td>11 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Blue</td>
<td>4.7 ± 0.5</td>
<td>11 ± 1</td>
<td>2.7 ± 0.8</td>
</tr>
</tbody>
</table>

**Figure S12.** Dependence of $k_{obs1}$ with CB[7] concentration at pH 2.0 recovered from the fits of the stopped-flow traces to the sum of two exponentials by fixing $k_{obs2}$ to the value obtained from the systematic fits. The data points correspond to the experiments performed in the “green” (black, blue) and “blue” region (red).
The model for the global analysis fit of the kinetic data at pH 5.5 is shown in Scheme S1. The value for $\beta_{11}^A$ was set to 390 M$^{-1}$, while the value for $k_-(\text{AH})$ was set to 10 s$^{-1}$. The data from two independent experiments, each with kinetics measured for six CB[7] concentrations, were simultaneously fit to the model. The residuals at all CB[7] concentrations were random (Fig. S13).

**Scheme S1.** Model used for the fit of the kinetic data measured at pH 5.5 for the mixing of $\text{A/AH}^+$ with CB[7].

**Figure S13.** Residuals for the fits of the stopped-flow traces for the mixing of $\text{A/AH}^+$ with CB[7] at pH 5.5 using the global analysis model described in Scheme S1. The concentrations of CB[7] from the top to the bottom are: 20, 17, 14, 11, 8, and 5 µM.
The contribution of the amplitude of the pre-exponential factor for the fast component of the kinetics of AH$^+$ mixing with CB[7] is smaller at pH 3.8 than at pH 2.0 (Table S5).

**Table S5.** Pre-exponential factors for $k_{obs1}$ at pH 2.0 and 3.8 from the fits of the kinetic traces to the sum of two exponentials by fixing $k_{obs2}$, and the difference between the two values.

<table>
<thead>
<tr>
<th>[CB[7]] / µM</th>
<th>Pre-exponential factors</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 2.0</td>
<td>pH 3.8</td>
</tr>
<tr>
<td>3</td>
<td>0.057</td>
<td>0.059</td>
</tr>
<tr>
<td>5</td>
<td>0.093</td>
<td>0.083</td>
</tr>
<tr>
<td>7</td>
<td>0.118</td>
<td>0.095</td>
</tr>
<tr>
<td>9</td>
<td>0.154</td>
<td>0.117</td>
</tr>
<tr>
<td>11</td>
<td>0.185</td>
<td>0.139</td>
</tr>
<tr>
<td>13</td>
<td>0.240</td>
<td>0.163</td>
</tr>
</tbody>
</table>

5. **References**