Electronic Supporting Information

Cucurbit[7]uril Inclusion Complexation as a Supramolecular Strategy for Color Stabilization of Anthocyanin Model Compounds

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Synthesis.

MMF⁺ was synthesized as previously described.[1] B-TMF was synthesized based on previous literature[2, 3] by the condensation reaction between equimolar quantities (0.65 mmol) of 2-hydroxy-4-methoxy-benzaldehyde and 3,4-dimethoxy-acetophenone dissolved in glacial acetic acid (10 mL) in a round bottom flask. All chemicals used were reagent grade and the reaction was carried out under constant stirring at room temperature. Dry gaseous HCl (generated from sulfuric acid and NaCl) was bubbled into the reaction flask throughout the reaction and the progress of the reaction was followed by color changes from yellow to intense red until no further color change was observed. Diethyl ether (15 mL) was added to the reaction mixture to precipitate the product, which was filtered and washed with diethyl ether (2 x 5 mL). Yield: 87 mg (35%), ^{1}H NMR (500 MHz, CD$_3$OD) δ/ppm: 4.05 (s, 3H), 4.06 (s, 3H), 4.18 (s, 3H), 7.34 (d, J = 8.7 Hz, 1H), 7.55 (dd, J = 9.0, 2.4 Hz, 1H), 7.92-7.93 (m, 1H), 8.02 (d, J = 2.3 Hz, 1H), 8.20 (d, J = 9.0 Hz, 1H), 8.29 (dd, J = 8.7, 2.3 Hz, 1H), 8.52 (d, J = 8.8 Hz, 1H), 9.16 (d, J = 8.8 Hz, 1H). ^{13}C NMR (125 MHz, CH$_3$OD) δ/ppm: 57.0, 57.3, 58.1, 101.7, 112.3, 113.7, 115.0, 122.8, 127.7, 133.1, 154.8; some non-hydrogenated carbons were not detected. HRMS (ESI in methanol, positive mode) m/z: calcd for C$_{18}$H$_{17}$O$_4$ 297.112135 found: 297.1136. El. Anal. C$_{18}$H$_{17}$O$_4$Cl•3 H$_2$O: calculated/%: C 55.89, H 5.99; found/%: C 56.07, H 5.92.
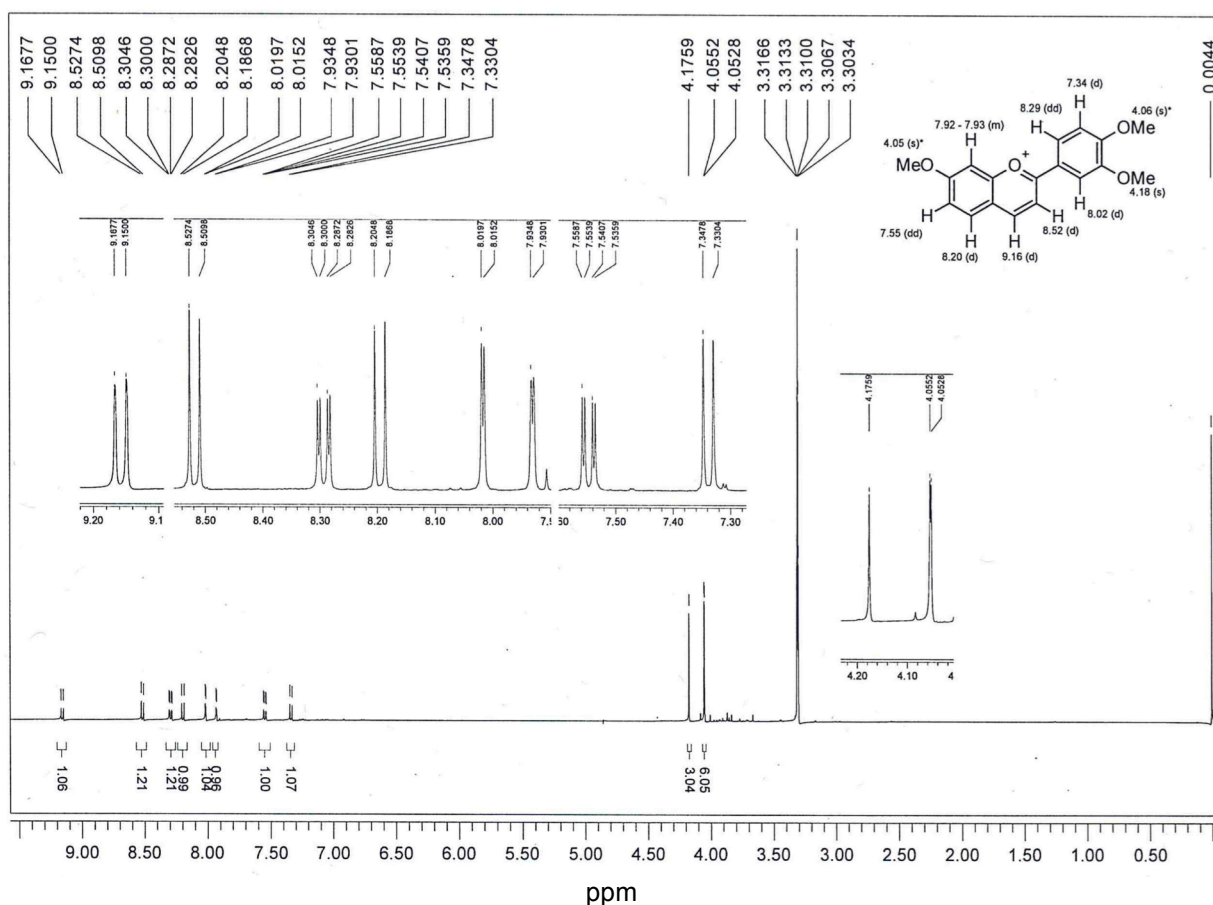

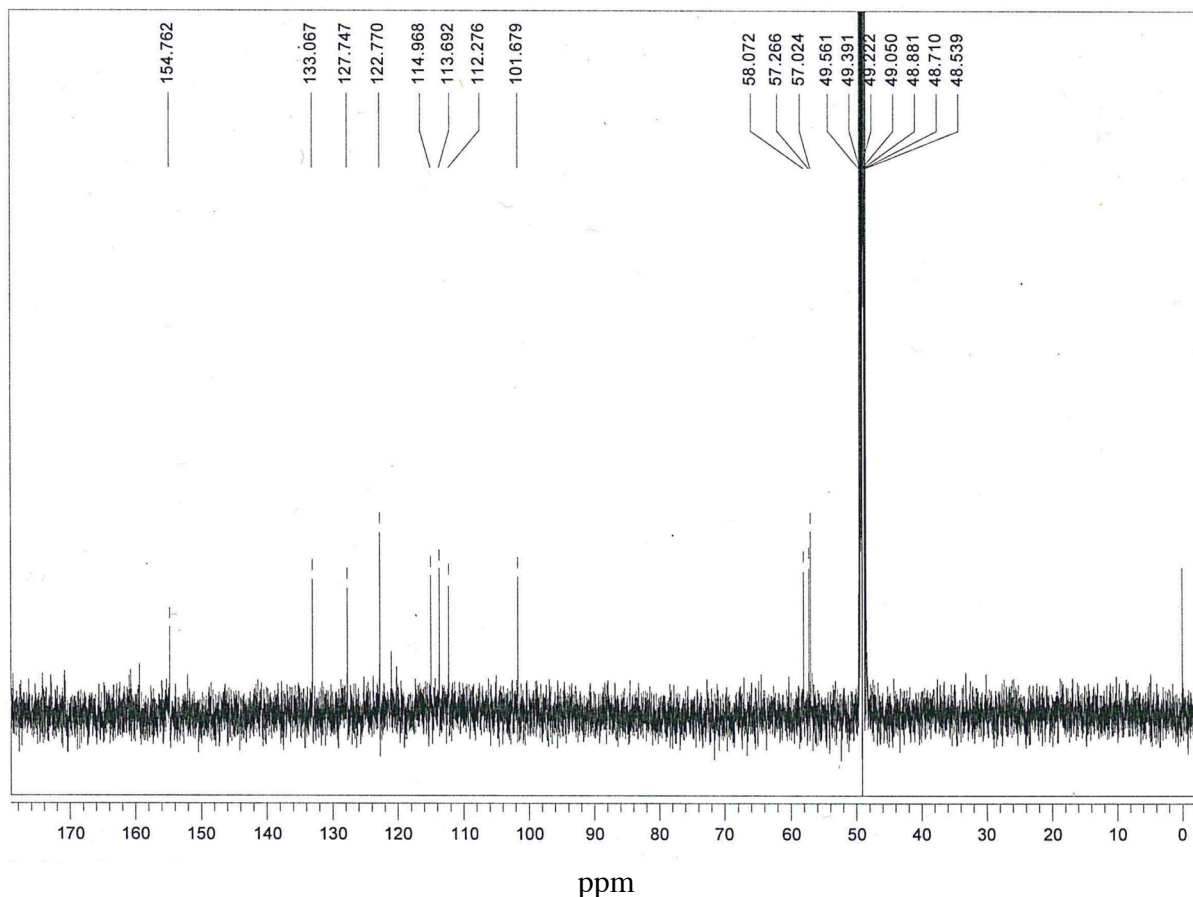**Figure S1.** ^{1}H NMR of B-TMF in CH$_3$OD (500 MHz). Assignment of the two peaks denoted as * is ambiguous.

Figure S2. ^{13}C NMR spectra of B-TMF in CH$_3$OD (125 MHz).

CB[7] was synthesized using a modified procedure based on previous reports.[4-6] All chemicals used in the synthesis were reagent grade. The modified procedure is the following: Glycoluril (10.0 g, 70.4 mmol, 1.0 eq.) and powdered paraformaldehyde (4.0 g, 133.3 mmol, 1.9 eq.) were added to a 100 mL two-necked round bottom flask. A volume of 20 mL of 20% (v/v) H$_2$SO$_4$ (95-95%) in HCl (36-38%) was added to the flask and the solution was stirred vigorously until a gel formed. The reaction was heated at 80 °C for 3 h followed by 5 h heating at 95-105 °C. The homogenous solution was cooled to RT and was then poured into 150 mL of methanol. The resulting suspension was stirred further followed by the gradual addition of water (100-120 mL). After water addition the suspension was stirred overnight and then filtered. The filtrate was precipitated with methanol (150 mL), the suspension was filtered and the solid was washed with methanol. This solid was dissolved in water and re-precipitated with methanol to remove mineral acids. The solid obtained from the latter step was dissolved in 20% aqueous glycerol (1.0 g solid: 19 mL water:glycerol) and was heated under stirring for 5 h at 70-80 °C. Insoluble material was removed by filtration. Methanol was added to the filtrate to precipitate CB[5] and CB[7]. Separation of CB[5] from CB[7] was achieved by repeated re-precipitation from water/methanol (1g solid:16-18 mL water:2-3 mL methanol). If any CB[6] was shown to be present (by ESI-MS) then 1.6 g of solid was dissolved in 30 mL water through which a 1:1 methanol:acetone mixture was diffused for 3-4 h. The turbid solution was filtered. Methanol was added to the filtrate leading to the precipitation of CB[7]. Final removal of small amounts of CB[5] was achieved by refluxing 1.1 g of solid dissolved in a water methanol mixture (1:1, 75 mL) for 10 h. The

resultant suspension was cooled to RT, filtered and washed with methanol. The solid was dried under vaccum for 12 h at 80 °C. CB[7] was characterized by ^{13}C NMR and ESI-MS based on literature data.[4] An aqueous sample of CB[7] was titrated by measuring the changes in the absorbance of the cobaltocenium cation at 261 nm (ε_{261} = 34,200 M^{-1} cm^{-1} for Cob$^+$),[7] and a purity of 95% for CB[7] was established right after drying the sample under vacuum. Solid CB[7] absorbs water over time and CB[7] stock solutions were regularly titrated with the cobaltocenium ion to establish the actual CB[7] concentration prior to performing quantitative experiments.

Fitting of binding isotherms.

The fluorescence intensity of the guest Fl$^+$ at various CB[7] concentrations was determined by integration of the corrected emission spectra between 500 and 700 nm, while for MMF$^+$ the spectra were integrated between 430 and 650 nm. The guest and CB[7] concentrations were corrected for dilution before the data were fit using the Scientist 3 program. Numerical fits were employed for 1:1 or 1:2 (guest:CB[7]) binding models, where the definitions for the total concentration of B-TMF and MMF$^+$ are different because of the hydration of Fl$^+$ in the case of B-TMF and no such reaction in the case of MMF$^+$.

1:1 binding model: The definitions of terms are:

K_{ap} = equilibrium constant between Fl$^+$ and the sum of B, C_Z and C_E. This parameter is not used in the case of MMF$^+$.

K_{11} = 1:1 equilibrium constant

[G] = free Fl$^+$ or MMF$^+$ concentration

[G]$_T$ = total concentration of B-TMF or MMF$^+$

[CB] = free CB[7] concentration

[CB]$_T$ = total concentration of CB[7]

[G@CB] = concentration of 1:1 complex

Φ_f = emission quantum yield for G

Φ_1 = emission quantum yield for G@CB

I = fluorescence intensity (experimental observable)

The equations used for the fit are:

$I = \Phi_f \, [\text{G}] + \Phi_1 \, [\text{G@CB}]$ (S1)

$K_{11} \, [\text{G}] \, [\text{CB}] = [\text{G@CB}]$ (S2)

$[\text{G}]_T = [\text{G}] + [\text{G@CB}] + [\text{G}]/K_{ap}$ (S3)

In the case of MMF$^+$: $[\text{G}]_T = [\text{G}] + [\text{G@CB}]$ (S3a)

$[\text{CB}]_T = [\text{CB}] + [\text{G@CB}]$ (S4)

1:2 binding model: Additional definitions of terms are:

K_{12} = 1:2 equilibrium constant

[G@CB$_2$] = concentration of 1:2 complex

Φ_2 = emission quantum yield for G@CB$_2$

The equations used for the fit are:

$I = \Phi_f \, [\text{G}] + \Phi_1 \, [\text{G@CB}] + \Phi_2 \, [\text{G@CB}_2]$ (S5)

$K_{11} \, [\text{G}] \, [\text{CB}] = [\text{G@CB}]$ (S2)

$$K_{12} \text{ [G@CB] [CB]} = \text{[G@CB}_2\text{]} \tag{S6}$$
$$\text{[G]}_T = \text{[G]} + \text{[G@CB]} + \text{[G@CB}_2\text{]} + \text{[G]}/K_{ap} \tag{S7}$$
In the case of MMF$^+$: $\text{[G]}_T = \text{[G]} + \text{[G@CB]} + \text{[G@CB}_2\text{]}$ \hfill (S7a)
$$\text{[CB]}_T = \text{[CB]} + \text{[G@CB]} + 2\text{[G@CB}_2\text{]} \tag{S8}$$

Determination of the apparent hydration constant for Fl$^+$

Fl$^+$ is colored and has an absorption maximum at 470 nm, while the other species (B, C_z, C_E) are colorless and have absorption maxima around 380 nm. The equilibrium between Fl$^+$ and the sum of the hydrated from B and the chalcones (C_z and C_E) (eq. S9 where Z = B + C_z + C_E) corresponds to an apparent pK value (pK_{ap}) defined by K_{ap} (eq. S10).

$$Fl^+ + H_2O \rightleftarrows Z + H^+ \tag{S9}$$

$$K_{ap} = \frac{\text{[Z] [H}^+\text{]}}{\text{[Fl}^+\text{]}} \tag{S10}$$

Absorption spectra for B-TMF were determined at different pH values. The absorbance at low pH values corresponds to the absorbance of Fl$^+$ only, while at pH 6.04 only the species in Z were present. The relative mole fraction of Fl$^+$ at different pH values was calculated from the absorbance at 470 nm at each pH (A) and the absorbance at the lowest pH (A_{low}) and the highest pH (A_{high}) employed (eq. S11).

$$x_{Fl} = \frac{A - A_{high}}{A_{low} - A_{high}} \tag{S11}$$

The relationship between the molar fraction of Fl$^+$ and pH is given by:

$$\log\left(\frac{x_{Fl}}{1 - x_{Fl}}\right) = pK_{ap} - pH \tag{S12}$$

The dependence of log ($x_{Fl}/(1 - x_{Fl})$) with pH was fit to a linear function (eq. S12). For the data collected after 24 h, the slope was -1.02 ± 0.07 and the intercept was 3.1 ± 0.2 (Fig. 1 in the paper). The value of pK_{ap} calculated from the slope and intercept is 3.0 ± 0.3 (at the pK_a, the value of log ($x_{Fl}/(1 - x_{Fl})$) is zero). The data collected after 2 h led to a slope and intercept of -0.8 ± 0.1 and 2.5 ± 0.3, leading to a pK_{ap} value of 3.1 ± 0.5. The pK_{ap} value recovered from the spectra collected after 2 h is within the experimental error of the value collected after 24 h. This result indicates that the contribution of C_E to the total equilibrium was small. A pK_h from 2.5 to 3.3 was used for the fits of the binding isotherms to estimate the value for K_{21} (see below).

Stabilization of Fl⁺ in the presence of CB[7].

The stabilization of Fl^+ in the presence of CB[7] was studied by inducing a pH jump from pH 3 to pH 5.5. The same pattern of stabilization occurred as was observed at pH 4.3 (Fig. 2 in the paper), with a partial decrease of the absorbance of Fl^+ in the presence of 12 μM CB[7], and complete inhibition of the decomposition of Fl^+ in the presence of 120 μM CB[7] (Fig. S3).
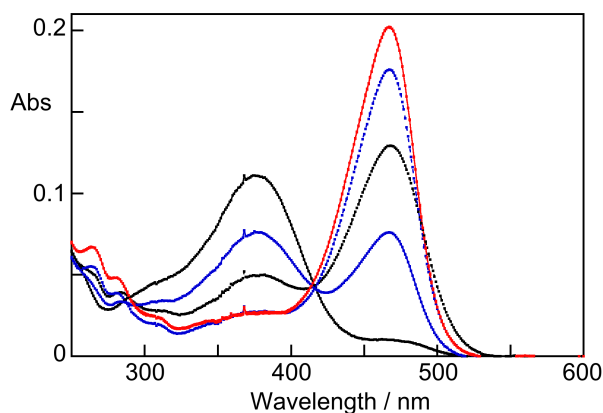

Figure S3. Absorption spectra of B-TMF (5 μM) at pH 5.5 immediately after addition of B-TMF (dashed lines) and after 7 h (solid lines) in the absence of CB[7] (black) and presence of 12 μM (blue) and 120 μM CB[7] (red, the dashed and solid lines are overlaid).

The stabilization of Fl^+ by CB[7] was also investigated by fluorescence. The emission of Fl^+ in water is weak and forms B, C_z and C_E of B-TMF do not fluoresce. Therefore, fluorescence is diagnostic for the presence of CB[7] complexes containing Fl^+. B-TMF was injected into aqueous solutions at pH 4.3 in the absence of CB[7] and in the presence of 12 μM and 120 μM CB[7] (Fig. S4). Immediately after the addition of B-TMF the weak emission of Fl^+ in water was observed and the emission of Fl^+ in the presence of both CB[7] concentrations was strong. After 30 min the emission for Fl^+ in water and in the presence of 12 μM CB[7] decreased compared to the emission in the presence of 120 μM CB[7]. This trend continued for the measurements after 60 min of B-TMF addition. These results show that, at the highest CB[7] concentration, Fl^+ is stabilized against hydration to form B.

A second experiment was performed in which B-TMF was added to an aqueous solution at pH 4.3 and was incubated for 3 h, after which 12 μM or 120 μM CB[7] were added to the solution. During the incubation period Fl^+ was hydrated to form B and C_Z, and some C_E. Fluorescence spectra right after the addition of CB[7] showed an increase in the emission intensity in the presence of 120 μM of the host while only a modest increase was observed in the presence of 12 μM of CB[7] (Fig. S5). The intensity in the presence of 120 μM CB[7] increased further when the spectra were recorded after 80 min. This result shows that addition of CB[7] shifts the equilibrium from B to Fl^+ and this shift is more prominent at the higher CB[7] concentration.
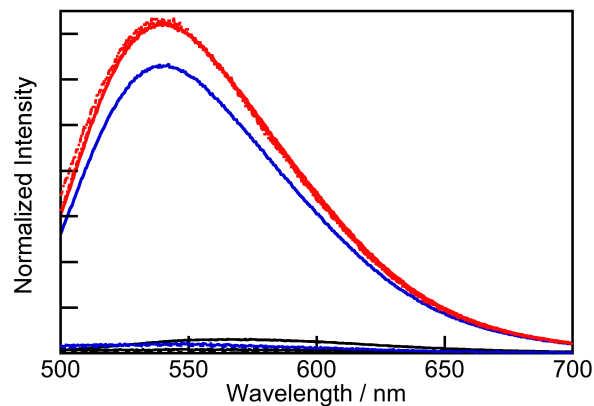
Figure S4. Fluorescence spectra for B-TMF (5 μM) at pH 4.3 immediately after addition of B-TMF (solid lines) and after 30 or 60 min (dashed lines). All spectra in water and in the presence of 12 μM CB[7] at 30 and 60 min have low intensities. The spectra in the presence of 120 μM CB[7] were normalized at 541 nm (red). The intensities for B-TMF in water (black) and in the presence of 12 μM CB[7] (blue) are relative to the normalized intensity in the presence of 120 μM CB[7].
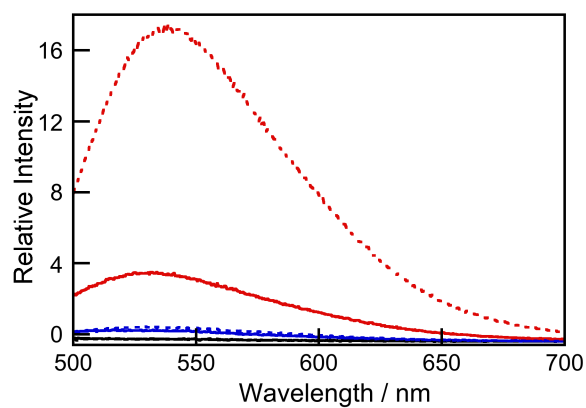

Figure S5. Fluorescence spectra for a solution of B-TMF (5 μM) initially incubated for 3 h in water at pH 4.3 (black) followed by the addition of 12 μM (blue) or 120 μM of CB[7] (red) recorded right after the addition of CB[7] (solid lines) and 80 min after the addition of CB[7] (dashed lines).

Binding isotherms of B-TMF and MMF⁺ with CB[7]

The fluorescence intensities of B-TMF and MMF$^+$ increased with increasing concentrations of CB[7] (Fig. S6).
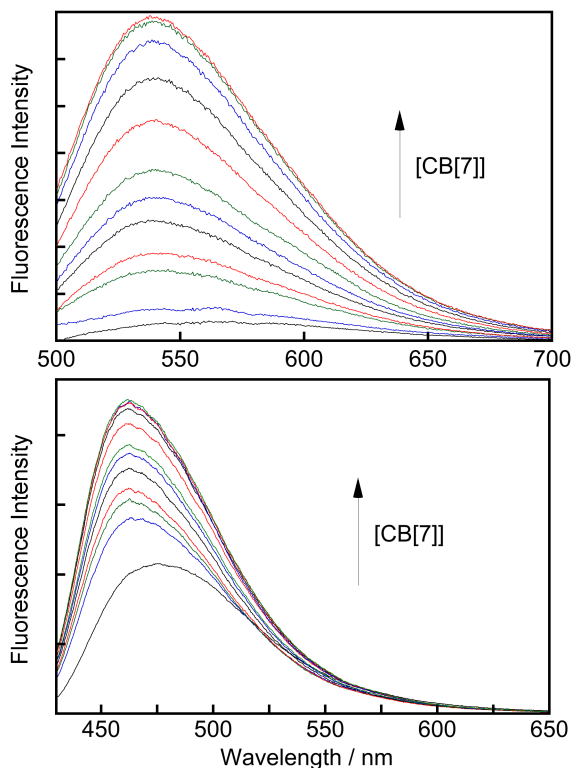

Figure S6. Top: Fluorescence spectra of B-TMF (0.2 μM) in water ([HCl] = 1 mM) in the presence of different concentrations of CB[7] (from the bottom to the top: 0, 0.18, 0.54, 0.73, 1.1, 1.5, 2.0, 3.6, 7.2, 18, 34, 78 μM). Bottom: Fluorescence spectra of MMF$^+$ (0.2 μM) in water ([HCl] = 1 mM) in the presence of different concentrations of CB[7] (from the bottom to the top: 0, 0.23, 0.46, 0.68, 0.91, 1.1, 1.4, 2.1, 3.6, 6.8, 13 and 22 μM.

The model used for the analysis for the binding of B-TMF with CB[7] includes the pK_{ap} value (see above), because in the presence of 1.0 mM HCl, B-TMF is present as a 1:1 mixture between Fl$^+$ and the sum of B, C_E and C_Z. The solution was at equilibrium between Fl$^+$, and B, C_E and C_Z at the start of the binding isotherm studies. The fluorescence intensity was measured after sequential injections of the CB[7] stock solution into the cell containing B-TMF. The sample was kept in the sample holder of the fluorimeter to achieve the required temperature after each injection and, during this time, the equilibrium between Fl$^+$ and B was established; the contribution due to the changes in concentrations of C_E and C_Z was considered to be minor. Therefore, the model employed assumed a constant Fl$^+$/(B + C_E + C_Z) ratio in water.

The binding isotherm for B-TMF with CB[7] did not fit to a model assuming a 1:1 binding stoichiometry (Fig. 5 in the paper), but an adequate fit was obtained when assuming sequential 1:1 and 1:2 B-TMF:CB[7] stoichiometries. Nonetheless, the errors in the best-fit values of K_{11} and K_{12} were large because the difference in the emission quantum yields of B-TMF in the 1:1 and 1:2 complexes was small. These quantum yields are unknown and therefore could not be fixed in the numerical fit. The value for K_{11} recovered from the kinetic studies (1.5×10^6 M^{-1}) was fixed and a fit with good residuals was obtained (Fig. 5 in the paper). The results were qualitatively the same when pK_{ap} values of 2.5, 3.0 or 3.3 were employed in the sense of obtaining poor fits for a 1:1 stoichiometry and good fits when 1:1 and 1:2 complexes were assumed to be present. The value for K_{12} was estimated to be lower than 3×10^4 M^{-1}.

Numerical fits of the isotherm for the binding of MMF$^+$ with CB[7] showed systematic deviations when a 1:1 complexation stoichiometry was assumed (Fig. S7), while the fit for the sequential formation of 1:1 and 1:2 MMF$^+$:CB[7] complexes was adequate. The recovered values for the sequential mechanism were $(9 \pm 2) \times 10^5$ M^{-1} for K_{11} and $(8 \pm 5) \times 10^5$ M^{-1} for K_{12}.
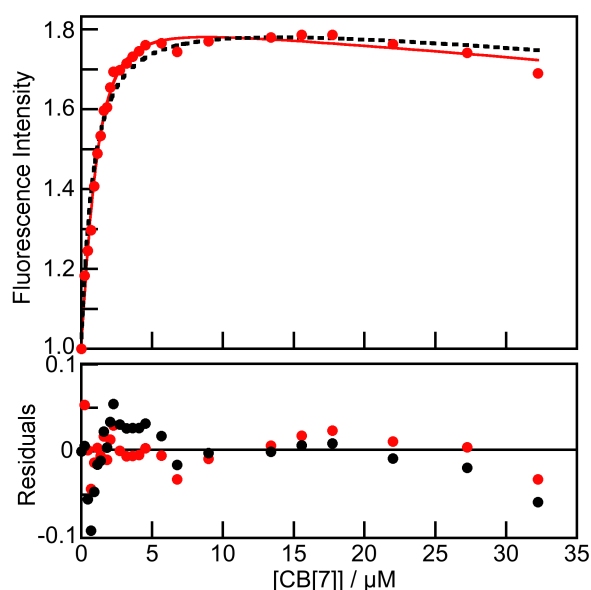

Figure S7. Top: Fit of the binding isotherm for MMF$^+$ (0.2 μM) with CB[7] to a 1:1 stoichiometry (black, dashed line) and to sequential 1:1 and 1:2 MMF$^+$:CB[7] stoichiometries (red, solid line). Bottom: residuals between the data and calculated values for the fit assuming a 1:1 stoichiometry (black) and sequential 1:1 and 1:2 stoichiometries (red).

References.

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