

Multimode binding and stimuli responsive displacement of acridine orange dye complexed with *p*-sulfonatocalix[4/6]arene macrocycles

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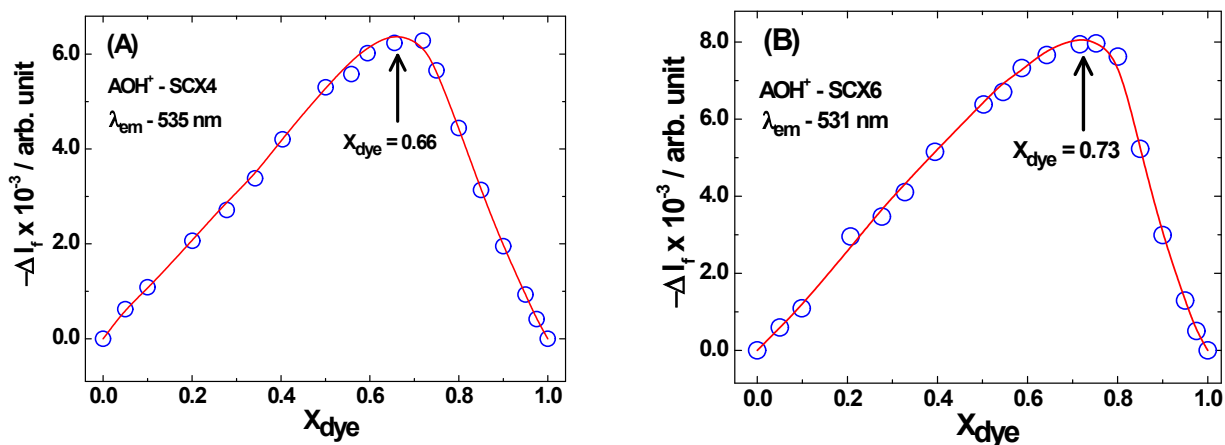
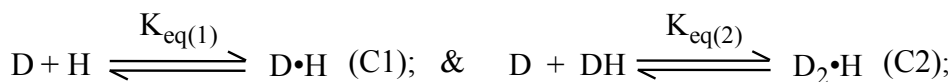


Figure S1. Job's plots for **(A)** AOH⁺-SCX4 system and **(B)** AOH⁺-SCX6 system, obtained from the fluorescence changes ($\Delta I_f = I_{\text{AOH}^+/\text{SCX}_n} - I_{\text{AOH}^+}$) monitored as a function of the mole fraction of the dye (X_{dye}). Sum of the concentrations of the dye and the host was kept as 10 μM .

Note S1: Binding equation for sequential formation 1:1 and 2:1 dye to host complexes

Complexation equilibria for the sequential formation 1:1 and 2:1 dye to host complexes in a system would be represented as,



From mass balance, we have,

$$[D]_0 = [D] + [C1] + 2[C2] \tag{1}$$

and $[H]_0 = [H] + [C1] + [C2]$ (2)

Considering the two complexation equilibria, we have,

$$K_{\text{eq}(1)} = \frac{[C1]}{[D][H]} \quad \text{and} \quad K_{\text{eq}(2)} = \frac{[C2]}{[D][C1]} = \frac{[C2]}{K_{\text{eq}(1)}[D]^2[H]} \tag{3}$$

Therefore, using mass balance for host (eq. 2) we can express concentration of C1 as,

$$\begin{aligned} [C1] &= K_{\text{eq}(1)}[D][H] = K_{\text{eq}(1)}[D]\{[H]_0 - [C1] - [C2]\} \\ &= K_{\text{eq}(1)}[D][H]_0 - K_{\text{eq}(1)}[D][C1] - K_{\text{eq}(1)}[D][C2] \\ &= K_{\text{eq}(1)}[D][H]_0 - K_{\text{eq}(1)}[D][C1] - K_{\text{eq}(1)}K_{\text{eq}(2)}[D]^2[C1] \end{aligned}$$

Or, $[C1] + K_{\text{eq}(1)}[D][C1] + K_{\text{eq}(1)}K_{\text{eq}(2)}[D]^2[C1] = K_{\text{eq}(1)}[D][H]_0$

Or, $[C1] = \frac{K_{\text{eq}(1)}[D][H]_0}{\{+ K_{\text{eq}(1)}[D] + K_{\text{eq}(1)}K_{\text{eq}(2)}[D]^2\}}$ (4)

Following similar procedure, we can have.

$$\begin{aligned}
[C2] &= K_{eq(2)}[D][C1] = K_{eq(2)}[D] \left\{ \frac{K_{eq(1)}[D][H]_0}{+ K_{eq(1)}[D] + K_{eq(1)}K_{eq(2)}[D]^2} \right\} \\
&= \frac{K_{eq(1)}K_{eq(2)}[D]^2[H]_0}{\left\{ + K_{eq(1)}[D] + K_{eq(1)}K_{eq(2)}[D]^2 \right\}}
\end{aligned} \tag{5}$$

Now, using mass balance for the dye (eq. 1), we can write,

$$[D]_0 = [D] + [C1] + 2[C2]$$

$$\text{Or, } [D]_0 = [D] + \frac{K_{eq(1)}[D][H]_0}{\left\{ + K_{eq(1)}[D] + K_{eq(1)}K_{eq(2)}[D]^2 \right\}} + 2 \frac{K_{eq(1)}K_{eq(2)}[D]^2[H]_0}{\left\{ + K_{eq(1)}[D] + K_{eq(1)}K_{eq(2)}[D]^2 \right\}} \tag{6}$$

$$\text{Or, } [D] = [D]_0 - \frac{K_{eq(1)}[D][H]_0}{\left\{ + K_{eq(1)}[D] + K_{eq(1)}K_{eq(2)}[D]^2 \right\}} - 2 \frac{K_{eq(1)}K_{eq(2)}[D]^2[H]_0}{\left\{ + K_{eq(1)}[D] + K_{eq(1)}K_{eq(2)}[D]^2 \right\}}$$

$$\text{Or, } [D] \left\{ + K_{eq(1)}[D] + K_{eq(1)}K_{eq(2)}[D]^2 \right\} = [D]_0 \left\{ + K_{eq(1)}[D] + K_{eq(1)}K_{eq(2)}[D]^2 \right\} - K_{eq(1)}[D][H]_0 - 2K_{eq(1)}K_{eq(2)}[D]^2[H]_0$$

$$\text{Or, } [D] + K_{eq(1)}[D]^2 + K_{eq(1)}K_{eq(2)}[D]^3 = [D]_0 + K_{eq(1)}[D]_0[D] + K_{eq(1)}K_{eq(2)}[D]_0[D]^2 - K_{eq(1)}[D][H]_0 - 2K_{eq(1)}K_{eq(2)}[D]^2[H]_0$$

$$\text{Or, } K_{eq(1)}K_{eq(2)}[D]^3 + K_{eq(1)}[D]^2 - K_{eq(1)}K_{eq(2)}[D]_0[D]^2 + 2K_{eq(1)}K_{eq(2)}[D]^2[H]_0 + K_{eq(1)}[D][H]_0 - K_{eq(1)}[D]_0[D] + [D] = [D]_0$$

$$\text{Or, } K_{eq(1)}K_{eq(2)}[D]^3 + K_{eq(1)} \left\{ 2K_{eq(2)}[H]_0 - K_{eq(2)}[D]_0 + 1 \right\} [D]^2 + \left\{ K_{eq(1)}[H]_0 - K_{eq(1)}[D]_0 + 1 \right\} [D] - [D]_0 = 0 \tag{7}$$

Eq. 7 can be written in the simplified form as,

$$a[D]^3 + b[D]^2 + c[D] + d = 0 \tag{8}$$

where,

$$a = K_{eq(1)}K_{eq(2)} \tag{8a}$$

$$b = K_{eq(1)} \left\{ 2K_{eq(2)}[H]_0 - K_{eq(2)}[D]_0 + 1 \right\} \tag{8b}$$

$$c = \left\{ K_{eq(1)}[H]_0 - K_{eq(1)}[D]_0 + 1 \right\} \tag{8c}$$

$$\text{and } d = -[D]_0 \tag{8d}$$

Since eq. 7 (or 8) is a 3rd order equation, solving this equation to obtain the exact solution for [D] at any stage of the titration is a very difficult task. Accordingly, explicit analysis of the observed fluorescence titration data was not possible in this study. Therefore, to get an idea about the relative binding strengths in the studied AOH⁺-SCXn systems, observed titration data for both the dye-host systems were analyzed only approximately, following a simplified mathematical equation, as discussed below in Note S2.

Note S2: Simplified binding equation for sequential formation of 1:1 and 2:1 dye to host complexes

For the sequential formation 1:1 and 2:1 dye to host complexes, we can simply write,

$$K_{eq(1)} = \frac{[C1]}{[D][H]} \Rightarrow [C1] = K_{eq(1)}[D][H] \quad (9)$$

$$K_{eq(2)} = \frac{[C2]}{[D][C1]} = \frac{[C2]}{K_{eq(1)}[D]^2[H]} \Rightarrow [C2] = K_{eq(1)}K_{eq(2)}[D]^2[H] \quad (10)$$

From the mass balance for the dye in the solution, we have,

$$[D]_0 = [D] + [C1] + 2[C2] = [D] + K_{eq(1)}[D][H] + 2K_{eq(1)}K_{eq(2)}[D]^2[H] \quad (11)$$

Now applying a simplified condition that the equilibrium host concentration [H] is very similar to the total host concentration $[H]_0$ used in the solution, we can write eq. 11 in the simplified form as,

$$2K_{eq(1)}K_{eq(2)}[H]_0[D]^2 + (1 + K_{eq(1)}[H]_0)[D] - [D]_0 = 0 \quad (12)$$

And thereby, the free dye concentration [D] in the solution would be given as,

$$[D] = \frac{-\left(1 + K_{eq(1)}[H]_0\right) + \left(1 + 2K_{eq(1)}[H]_0 + K_{eq(1)}^2[H]_0^2 + 8K_{eq(1)}K_{eq(2)}[H]_0[D]_0\right)^{1/2}}{4K_{eq(1)}K_{eq(2)}[H]_0} \quad (13)$$

In the titration experiment, the observed fluorescence intensity at any stage would be the sum of contribution from all the emissive species. Therefore we can write,

$$I_{obs} = I_D + I_{C1} + I_{C2} = k_D[D] + k_{C1}[C1] + k_{C2}[C2] \quad (14)$$

Here I_D , I_{C1} and I_{C2} are the intensity contributions from D, C1 and C2 species, respectively. It can be assumed further that the intensity contributions of the respective emissive species would be proportional to their concentrations present in the experimental solution. Therefore, we can write,

$$I_0 = k_D[D]_0, \quad I_{C1}^\infty = k_{C1}[C1]_\infty = k_{C1}[D]_0, \quad \text{and} \quad I_{C2}^\infty = k_{C2}[C2]_\infty = k_{C2}[D]_0 / 2$$

$$\text{Or,} \quad k_D = \frac{I_0}{[D]_0}, \quad k_{C1} = \frac{I_{C1}^\infty}{[D]_0} \quad \text{and} \quad k_{C2} = \frac{2I_{C2}^\infty}{[D]_0} \quad (15)$$

Therefore eq. 14 can be rewritten as,

$$\begin{aligned} I &= I_0 \frac{[D]}{[D]_0} + I_{C1}^\infty \frac{[C1]}{[D]_0} + 2I_{C2}^\infty \frac{[C2]}{[D]_0} \\ &= I_0 \frac{([D]_0 - [C1] - 2[C2])}{[D]_0} + I_{DH}^\infty \frac{[C1]}{[D]_0} + 2I_{D_2H}^\infty \frac{[C2]}{[D]_0} \\ &= I_0 + \left(I_{DH}^\infty - I_0\right) \frac{[C1]}{[D]_0} + 2\left(I_{D_2H}^\infty - I_0\right) \frac{[C2]}{[D]_0} \end{aligned}$$

$$\text{Or, } (I - I_0) = (I_{DH}^\infty - I_0) \frac{[C1]}{[D]_0} + 2(I_{D_2H}^\infty - I_0) \frac{[C2]}{[D]_0}$$

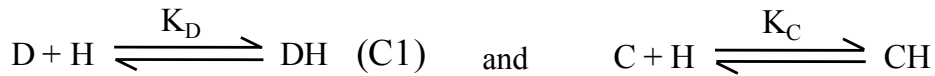
$$\text{Or, } \Delta I = \Delta I_{C1}^\infty \frac{K_{eq(1)}[D][H]_0}{[D]_0} + 2\Delta I_{C2}^\infty \frac{K_{eq(1)}K_{eq(2)}[D]^2[H]_0}{[D]_0} \quad (16)$$

Here, [D] is to be considered as given by eq. 13. This is the simplified mathematical relation used to fit the fluorescence titration data for the studied dye-host systems just to get an approximate idea about the relative binding strengths between AOH⁺-SCX4 and AOH⁺-SCX6 systems, though the applied approximation in deriving the above relation is not truly valid for the titration data especially at the lower host concentration region.

Note S3: Analysis of dye displacement results following fluorescence titration

Note S3.1: Dye displacement studies and relevant mathematical formulation

In the cases of dye displacement experiments, we need to deal with the independent complex formations of both the dye (D) and the competitive binder (C) with the host (H). In these cases, considering only the very simplest complex formations of D and C with H, we can represent these systems as,



Under this simplified considerations, at any stage of the dye displacement study, the mass balance for the dye (D), competitive binder (C) and host (H) would be given as,

$$[C1]_0 = [D]_\infty = [C1] + [D] \quad (1)$$

$$[C]_0 = [C] + [CH] \quad (2)$$

$$[H]_0 = [H] + [C1] + [CH] \quad (3)$$

where [C1]₀, [D]_∞ and [H]₀ represent the initial C1 concentration, total dye concentration displaced finally and total host concentration used in the solution.

For the formation of the C1 complex, we can write,

$$K_D = \frac{[C1]}{[D][H]} = \frac{[C1]}{\{[C1]_0 - [C1]\}[H]}$$

$$\text{Or, } [C1] = K_D \{ [C1]_0 - [C1] \} [H] = K_D [C1]_0 [H] - K_D [C1] [H]$$

$$\text{Or, } [C1] = \frac{K_D [C1]_0 [H]}{1 + K_D [H]} \quad (4)$$

Similarly, For the formation of the CH complex, we can write,

$$K_C = \frac{[CH]}{[C][H]} = \frac{[CH]}{\{[C]_0 - [CH]\}[H]}$$

$$\text{Or, } [CH] = K_C \{ [C]_0 - [CH] \} [H] = K_C [C]_0 [H] - K_C [CH] [H]$$

$$\text{Or, } [\text{CH}] = \frac{K_C[\text{C}]_0[\text{H}]}{1 + K_C[\text{H}]} \quad (5)$$

Therefore, substituting eq. 4 and 5 into eq. 3 we can have,

$$[\text{H}]_0 = [\text{H}] + \frac{K_D[\text{C1}]_0[\text{H}]}{1 + K_D[\text{H}]} + \frac{K_C[\text{C}]_0[\text{H}]}{1 + K_C[\text{H}]}$$

$$\text{Or, } [\text{H}]_0 = \frac{[\text{H}](1 + K_D[\text{H}])(1 + K_C[\text{H}]) + K_D[\text{C1}]_0[\text{H}](1 + K_C[\text{H}]) + K_C[\text{C}]_0[\text{H}](1 + K_D[\text{H}])}{(1 + K_D[\text{H}])(1 + K_C[\text{H}])}$$

$$\text{Or, } [\text{H}]_0 (1 + K_D[\text{H}] + K_C[\text{H}] + K_D K_C [\text{H}]^2) = [\text{H}] (1 + K_D[\text{H}] + K_C[\text{H}] + K_D K_C [\text{H}]^2) + K_D[\text{C1}]_0[\text{H}] + K_D K_C [\text{C1}]_0[\text{H}]^2 + K_C[\text{C}]_0[\text{H}] + K_D K_C [\text{C}]_0[\text{H}]^2$$

$$\text{Or, } [\text{H}]_0 + K_D K_C [\text{H}]_0 [\text{H}]^2 + \{K_D[\text{H}]_0 + K_C[\text{H}]_0\}[\text{H}] = [\text{H}] + K_D K_C [\text{H}]^3 + \{K_D + K_C\}[\text{H}]^2 + \{K_D K_C [\text{C1}]_0 + K_D K_C [\text{C}]_0\}[\text{H}]^2 + \{K_D[\text{C1}]_0 + K_C[\text{C}]_0\}[\text{H}]$$

$$\text{Or, } K_D K_C [\text{H}]^3 + \{K_D + K_C + K_D K_C ([\text{C1}]_0 + [\text{C}]_0 - [\text{H}]_0)\}[\text{H}]^2 + \{1 + K_D[\text{C1}]_0 + K_C[\text{C}]_0 - K_D[\text{H}]_0 - K_C[\text{H}]_0\}[\text{H}] - [\text{H}]_0 = 0$$

$$\text{Or, } K_D K_C [\text{H}]^3 + \{K_D + K_C + K_D K_C ([\text{C1}]_0 + [\text{C}]_0 - [\text{H}]_0)\}[\text{H}]^2 + \{1 + K_D([\text{C1}]_0 - [\text{H}]_0) + K_C([\text{C}]_0 - [\text{H}]_0)\}[\text{H}] - [\text{H}]_0 = 0 \quad (6)$$

This is a cubic equation, which can be written in its simple form as,

$$a[\text{H}]^3 + b[\text{H}]^2 + c[\text{H}] + d = 0 \quad (6a)$$

where, $a = K_D K_C$

$$b = \{K_D + K_C + K_D K_C ([\text{C1}]_0 + [\text{C}]_0 - [\text{H}]_0)\}$$

$$c = \{1 + K_D([\text{C1}]_0 - [\text{H}]_0) + K_C([\text{C}]_0 - [\text{H}]_0)\}$$

$$d = -[\text{H}]_0$$

The discussion above shows that the consideration of only the very simplest complex formations of D and C with H also leads to a cubic equation, which is beyond our scope to solve for an exact solution of [H]. In reality, for the AOH⁺-SCX_n systems, not only the DH complexes but also the D₂H complexes are formed in the solution. Therefore, the true representation of the AOH⁺-SCX_n systems will effectively lead to a 4th order equation and thus will make the situation much more difficult to be treated for an exact solution of [H]. Thus, for the analysis of the fluorescence titration data from the dye displacement measurements in the AOH⁺-SCX_n systems, we were compelled to apply an extreme approximation to obtain only a reasonable fit to the observed titration data and thus to estimate the binding strengths of the dye and the competitive binder with the SCX4 and SCX6 hosts only roughly for a relative comparison, as are discussed in the next two subsections.

Note S3.2: Simplified relation for the correlation of fluorescence titration data from dye displacement studies

In the dye displacement studies involving AOH⁺-SCX_n systems, applying a drastic approximation that the dye binds to the hosts only with a single mode of complexation, i.e. as the C1 complex

only, as considered in section Note S3.1, and considering that on addition of the reasonably high concentrations of the competitive binder $[C]_0$ in the solution, the equilibrium concentration of C1 complex will become much less than that of CH complex (i.e. $[C1] \ll [CH]$), the mass balance for the host concentration in the solution can be expressed as,

$$[H]_0 = [H] + [CH] + [C1] \approx [H] + [CH] \quad (7)$$

Further, at any stage of the dye displacement studies, the equilibrium concentration of the competitive binder $[C]$ can be considered to be very similar to the total concentration $[C]_0$ of the competitive binder used in the solution. Following the above considerations and expressing the concentration $[CH]$ as given by eq. 5, we can rewrite eq. 7 as,

$$[H]_0 = [H] + \frac{K_c[C]_0[H]}{1 + K_c[H]}$$

$$\text{Or, } [H]_0 + K_c[H]_0[H] = [H] + K_c[H]^2 + K_c[C]_0[H]$$

$$\text{Or } K_c[H]^2 + \{1 + K_c[C]_0 - K_c[H]_0\}H - [H]_0 = 0 \quad (8)$$

This is a quadratic equation and thus can be solved easily. The solution of eq. 8 that provides us a physically meaningful result for equilibrium $[H]$ concentration at any given concentration of the competitive binder $[C]_0$ is expressed as,

$$[H] = \frac{-\{1 + K_c[C]_0 - K_c[H]_0\} + \sqrt{\{1 + K_c[C]_0 - K_c[H]_0\}^2 + 4K_c[H]_0}}{2K_c} \quad (9)$$

Now considering the actual dye displacement experiments where it is assumed that only C1 type of complexes are formed, there will be only two emissive species in the solution, i.e. the free dye (D) and the C1 complex. Therefore, the observed fluorescence intensity (I_{obs}) at any given state of the measurements in the dye displacement studies can be expressed as,

$$I_{\text{obs}} = I_{[C1]_0} \frac{[C1]}{[C1]_0} + I_{[D]_\infty} \frac{[D]}{[D]_\infty} = I_{[C1]_0} \frac{[C1]}{[C1]_0} + I_{[D]_\infty} \frac{[D]}{[C1]_0} \quad (10)$$

where $I_{[C1]_0}$ is the initial intensity when all the dyes were bound to the host and $I_{[D]_\infty}$ is the final intensity when all the dye would be displaced from C1 as the free dye through the competitive binding of C with the host. Expressing the concentration $[CH]$ as given by eq. 5, we can write,

$$\frac{[C1]}{[C1]_0} = \frac{K_D[H]}{1 + K_D[H]}$$

$$\text{and } \frac{[D]}{[D]_\infty} = \frac{[D]}{[C1]_0} = 1 - \frac{[C1]}{[C1]_0} = 1 - \frac{K_D[H]}{1 + K_D[H]} = \frac{1}{1 + K_D[H]}$$

Therefore, eq. 10 can be rewritten as,

$$\text{Or, } I_{\text{obs}} = I_{[C1]_0} \frac{K_D[H]}{1 + K_D[H]} + I_{[D]_\infty} \frac{1}{1 + K_D[H]}$$

$$\text{Or, } I_{\text{obs}} = \frac{I_{[C1]_0} K_D[H] + I_{[D]_\infty}}{1 + K_D[H]}$$

$$\text{Or, } (I_{\text{obs}} - I_{[C1]_0}) = \frac{I_{[C1]_0} K_D [H] + I_{[D]_{\infty}}}{1 + K_D [H]} - I_{[C1]_0} = \frac{I_{[C1]_0} K_D [H] + I_{[D]_{\infty}} - I_{[C1]_0} - I_{[C1]_0} K_D [H]}{1 + K_D [H]}$$

$$\text{Or, } (I_{\text{obs}} - I_{[C1]_0}) = (I_{[D]_{\infty}} - I_{[C1]_0}) \left(\frac{1}{1 + K_D [H]} \right)$$

$$\text{Or, } \Delta I_f = \Delta I_{\infty} \left(\frac{1}{1 + K_D [H]} \right) \quad (11)$$

where, $\Delta I_f = (I_{\text{obs}} - I_{[C1]_0})$ and $\Delta I_{\infty} = (I_{[D]_{\infty}} - I_{[C1]_0})$. As the equilibrium host concentration $[H]$ can be approximately estimated by using eq. 9, we can evidently fit the fluorescence titration data in the dye displacement studies reasonably at least by using eq. 11 to estimate the approximate binding strengths of the dye and the competitive binder with the SCX4 and SCX6 hosts, for their relative comparisons. In the present analysis we used the K_D values similar to the $K_{\text{eq}(2)}$ values obtained before for the respective AOH^+ -SCXn systems, as the initial reduction in the fluorescence intensity for the starting solution in the dye displacement studies were effectively due to the host assisted dimerization of the dye.

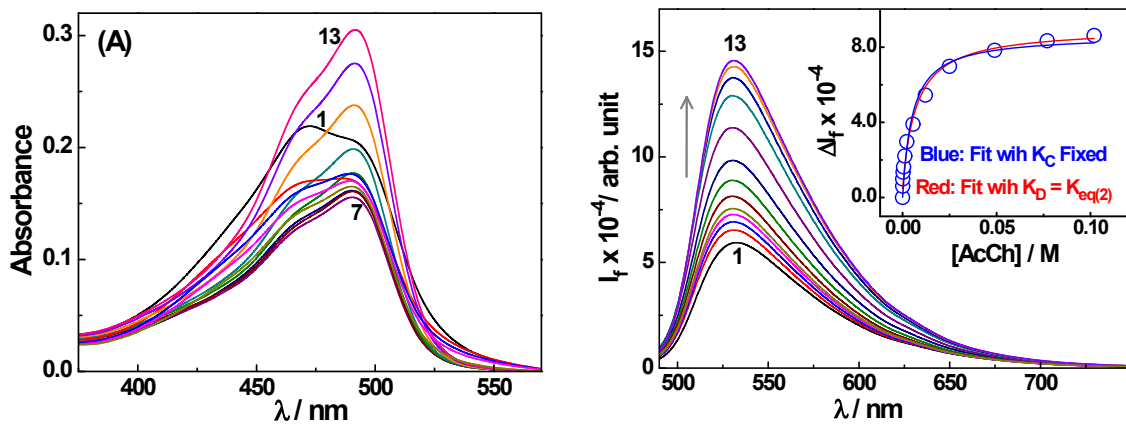


Figure S2. (A) Changes in the absorption spectra for the AOH^+ -SCX4 system (7 μM dye and 10 μM host) with increasing AcCh concentration. $[\text{AcCh}]/\text{mM}$ for spectra 1-13 are: 0, 0.036, 0.108, 0.215, 0.551, 1.22, 2.23, 5.55, 12.1, 25.0, 49.0, 77.0 and 102, respectively. (B) Changes in the fluorescence spectra of the same AOH^+ -SCX4 system with similarly changing AcCh concentration as in the case of absorption studies. **Inset:** Intensity changes at 531 nm with the increasing concentration of AcCh. Circles are the experimental data points and continuous curves are the fits to the data following eq. 6 and 7; Red: Considering K_D similar to the respective $K_{\text{eq}(2)}$ value and Blue: Considering K_C fixed as the reported value in the literature.^{6,13,56,72}

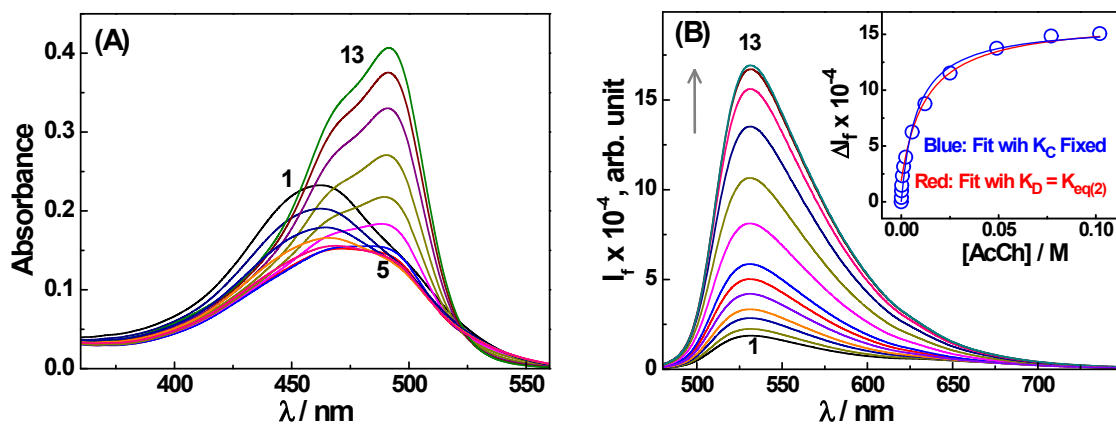


Figure S3. (A) Changes in the absorption spectra for the AOH⁺-SCX6 system (6.9 μM dye and 3.6 μM host) with increasing AcCh concentration. [AcCh]/mM for spectra 1-13 are: 0, 0.036, 0.108, 0.215, 0.551, 1.22, 2.23, 5.55, 12.1, 25.0, 49.0, 77.0 and 102, respectively. (B) Changes in the fluorescence spectra of the same AOH⁺-SCX4 system with similarly changing AcCh concentration as in the case of absorption studies. **Inset:** Intensity changes at 531 nm with the increasing concentration of AcCh. Circles are the experimental data points and continuous curves are the fits to the data following eq. 6 and 7; Red: Considering K_D similar to the respective $K_{eq(2)}$ value and Blue: Considering K_C fixed as the reported value in the literature.^{6,13,56,72}

Table S1. Comparison of the K_C values from the present study with those reported earlier in the literature for the interaction of the competitive binders AD and AcCh with SCX_n hosts.

Chemical Systems	K_C / M^{-1} (From the literature; ref. no. as in main Paper)	K_C / M^{-1} (With assumption of K_D similar to $K_{eq(2)}$)	K_C / M^{-1} (with K_D as obtained by fixing K_C for AcCh- SCX _n cases)
AcCh-SCX4	1.2×10 ⁵ (ref. 6) 1.0×10 ⁵ (ref. 13 & 56) 5.0×10 ⁴ (ref. 72)	2.7×10 ³ (Highly under-estimated K_C value)	1.2×10 ⁵ (fixed as in ref. 6); (Estimated K_D in this case is 6.1×10 ⁷ M ⁻¹)
AcCh-SCX6	7.9×10 ⁴ (ref. 72)	6.8×10 ² (Highly under-estimated K_C value)	7.9×10 ⁴ (fixed as in ref. 72) (Estimated K_D in this case is 1.7×10 ⁸ M ⁻¹)
AD-SCX4	Not available in the literature	5.5×10 ²	2.0×10 ⁴ (Obtained on using K_D fixed to 6.1×10 ⁷ M ⁻¹ , as obtained from the fit of the AcCh-AOH ⁺ -SCX4 data listed above)
AD-SCX6	Not available in the literature	2.4×10 ²	1.5×10 ⁴ (Obtained on using K_D fixed to 1.7×10 ⁸ M ⁻¹ , as obtained from the fit of the AcCh-AOH ⁺ -SCX6 data listed above)