Modulation in the Acidity Constant of Acridine Dye with Cucurbiturils: Stimuli-Responsive $pK_a$ Tuning and Dye Relocation into Live Cells

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Figure S1. Absorption spectra of acridine ($2.45 \times 10^{-6}$ M) in water at different pHs. (1) 2.6, (2) 4.0, (3) 4.5, (4) 5.0, (5) 5.6, (6) 6.1, (7) 7.0, (8) 8.1 and (9) 9.1. Inset: variation in absorbance with pH at 354 nm.
Figure S2. (A) Absorption spectra of Ac (4.5\times10^{-6} \text{ M}) in aqueous solution at different CB7 concentrations at pH 11. [CB7]/\mu\text{M}: (1) 0, (2) 5, (3) 20, (4) 77, (5) 113, (6) 157 and (7) 206. (B) Fluorescence spectra of Ac (4.5\times10^{-6} \text{ M}) in aqueous solution at different CB7 concentrations at pH 11. [CB7]/\mu\text{M}: (1) 0.0, (2) 0.02, (3) 0.04, (4) 0.11, (5) 0.16, (6) 0.21, (7) 0.46, (8) 0.90 and (9) 1.35.

Figure S3. CB7 Concentration dependent normalized absorption spectra of acridine dye at pH ~3.5.

Method M1: 1:1 host: guest binding model

In the present systems, the binding constants ($K$) for the different forms of the dye with the CB7 host were estimated at suitable pH conditions by the fluorescence titration method assuming 1:1 complexation stoichiometry according to eq. S1, which afforded satisfactory fitting results.$^{1-3}$

$$I_f = I_{Dye}^0 \frac{[Dye]_{eq}}{[Dye]_0} + I_{CB7:Dye}^\infty \frac{[CB7:Dye]_{eq}}{[Dye]_0}$$

(S1)

Where, $I_{Dye}^0$ and $I_{CB7:Dye}^\infty$ are the extrapolated fluorescence intensities of the uncomplexed and complexed form of the dye, respectively, $[Dye]_0$ and $[CB7]_0$ are the respective total concentrations of dye (AcH\(^+\), Ac forms, as applicable) and CB7...
host, and \([Dye]_{eq}\) is the concentration of uncomplexed dye in the solution. Exchange of the dye during its excited-state lifetime (<31 ns), i.e., the conversion of the uncomplexed dye to the complexed one or vice versa, can be excluded since the corresponding rate constants are very small for macrocyclic host molecules. For fitting, the change in the fluorescence intensity \((\Delta I_f^2)\) was plotted against the total host concentration and the obtained titration curve was fitted according to the rearranged eq. S2:

\[
\Delta I_f^2 = \left(1 - \frac{[Dye]_{eq}}{[Dye]_0}\right) \left(I_{CB: \text{Dye}}^0 - I_{Dye}^0\right)
\]

(S2)

where, \([Dye]_{eq}\) is expressed as

\[
[Dye]_{eq} = \left(K_{eq}[Dye]_0 - K_{eq}[SCX6]_0 - 1 + \sqrt{(K_{eq}[Dye]_0 + K_{eq}[CB7]_0 + 1)^2 - 4K_{eq}^2[Dye]_0 [CB7]_0}\right) / 2K_{eq}
\]

(S3)

Method M2: 1:2 host: guest binding model

\[
\begin{align*}
\text{CB} + \text{Dye} & \quad \xleftrightarrow{K_1} \quad \text{CB} : \text{Dye} \\
\text{CB} : \text{Dye} + \text{Dye} & \quad \xleftrightarrow{K_2} \quad \text{CB} : (\text{Dye})_2
\end{align*}
\]

(S4a)

\[
K_{(\text{ternary complex})} = K_1 \times K_2
\]

(S4b)

The fluorescence intensity of the system, \(I\), is a function of the intensities of the free guest \((I_g)\) and the 1:1 and 1:2 host-guest complexes \((I_{gh} \text{ and } I_{ggh})\), respectively:

\[
I = x_g I_g + x_{gh} I_{gh} + 2 x_{ggh} I_{ggh}
\]

(S5)

\(x_g, x_{gh} \text{ and } x_{ggh}\) are the mole fractions of the free guest and the 1:1 and 1:2 host-guest complexes.

The mole fractions are further defined as follows:

\[
x_g = \frac{[G]}{[G]_0}
\]

(S6)

\[
x_{gh} = \frac{[HG]}{[G]_0} \quad \Rightarrow x_{gh} = \frac{K_1 [H]_0}{1 + K_1 [G] + K_1 K_2 [G]^2} \cdot \frac{[G]}{[G]_0}
\]

(S7)
Thus, the fluorescence intensity of the system is obtained through substitution of eq. (S6-S8) into eq. (S5).

$$I = \frac{[G]}{[G]_0} \left( \frac{K_1 K_2 [H]_0}{1 + K_1 [G] + K_1 K_2 [G]^2} \right) I_{gh} + \frac{K_1 K_2 [H]_0 [G]}{1 + K_1 [G] + K_1 K_2 [G]^2} I_{ggh}$$

(S9)

The $K$ value of CB8:(AcH$^+$)$_2$ has been estimated from the binding isotherm (inset of Fig. 3B) by using eq. S9.

Figure S4. (A) ITC isotherm for titration of AcH$^+$ with CB7 in water at 25 °C. (B) ITC isotherm for titration of CB8 with AcH$^+$ in water at 25 °C.
Figure S5. Decay traces of AcH⁺ in solutions at pH ~3.5 in the absence (1) and presence (2) of 250 μM CB7 (A) and 70 μM CB8 (B). ‘L’ represents excitation lamp profile. λ_{ex} = 374 nm.

Figure S6. ¹H NMR spectra (500 MHz) of ~100 μM acridine dye in the absence (a) and in the presence (b) of 1mM CB7 and (c) 80 μM CB8 in D₂O at pD 4.5. Inset: Pictorial representation of ACH⁺.

Method M3:
The pH-dependent absorbance changes at 355 nm (inset of Fig. S1) were fitted according to the following relation (eq. S10),³

\[
A_{obs} = \frac{A_{AcH}^{\infty}}{1+10^{pK_a-pH}} + \frac{A_{Ac}^{\infty}}{1+10^{pK_a-pH}} \tag{S10}
\]

where \(A_{obs}\) is the observed absorbance at any pH, and \(A_{AcH}^{\infty}\) and \(A_{Ac}^{\infty}\) are the extrapolated absorbances of the AcH⁺ and Ac forms, respectively. From this analysis, the p\(K_a\) value of the dye in its ground state was found to be 5.4 ± 0.1, which matches well with the reported value.³⁵
Figure S7. Fluorescence spectra of CB7:AcH⁺ complex (A) and CB8:AcH⁺ complex (B) with increasing temperature. T(°C): (1) 20, (2) 30, (3) 40, (4) 50, (5) 60, (6) 70 and (8) 80.

Figure S8. Fluorescence microscopic images recorded from CHO cell lines at pH 7.4 after treating them with uncomplexed acridine dye (10 μM) (a); acridine dye (10μM)-CB7 (1mM) (b); and acridine dye-CB7 (1mM)-AD (100μM) (c).

References