Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information

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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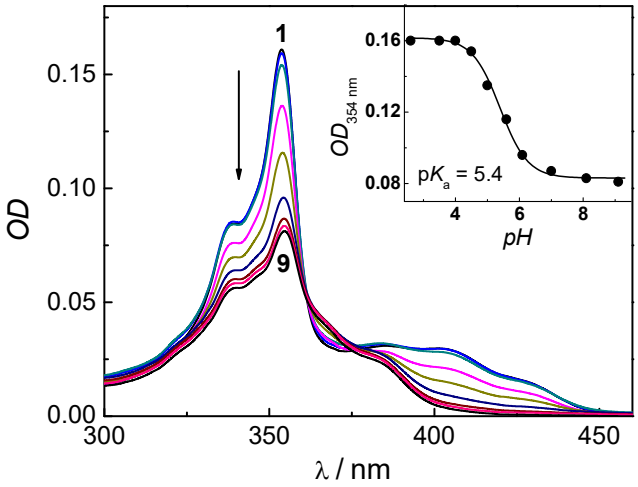

Figure S1. Absorption spectra of acridine (2.45×10^{-6} M) in water at different *pH*s. (1) 2.6, (2) 4.0, (3) 4.5, (4) 5.0, (5) 5.6, (6) 6.1, (7) 7.0, (8) 8.1 and (9) 9.1. Inset: variation in absorbance with pH at 354 nm.

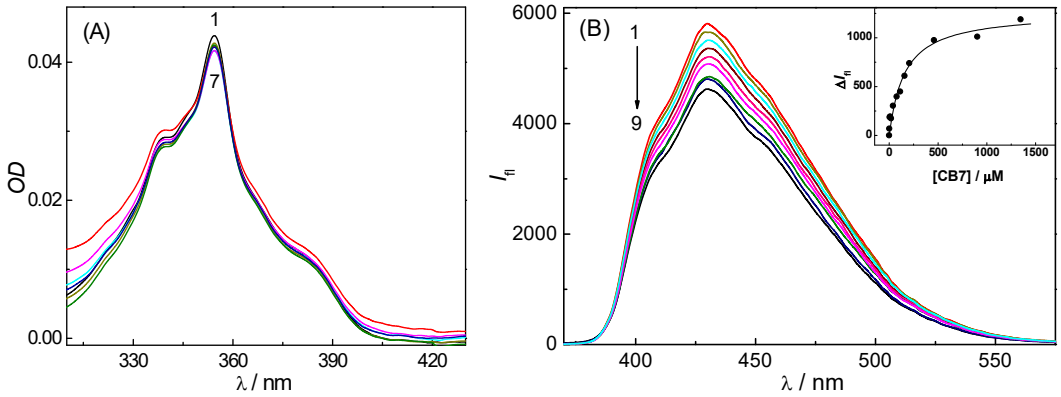

Figure S2. (A) Absorption spectra of Ac (4.5×10^{-6} M) in aqueous solution at different CB7 concentrations at pH 11. [CB7]/μM: (1) 0, (2) 5, (3) 20, (4) 77, (5) 113, (6) 157 and (7) 206. (B) Fluorescence spectra of Ac (4.5×10^{-6} M) in aqueous solution at different CB7 concentrations at pH 11. [CB7]/mM: (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.

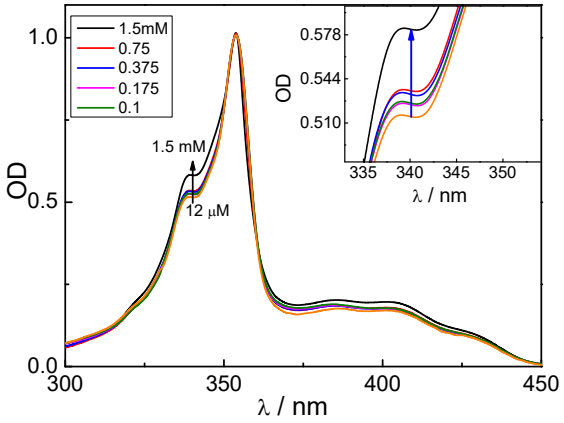

Figure S3. CB7 Concentration dependent normalized absorption spectra of acridine dye at pH ~3.5.

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]

$$I_f = I^0_{Dye}\frac{[Dye]_{eq}}{[Dye]_0} + I^{\infty}_{CB7:Dye}\frac{[CB7:Dye]_{eq}}{[Dye]_0} \qquad \text{(S1)}$$

Where, I^0_{Dye} and $I^{\infty}_{CB7:Dye}$ are the extrapolated fluorescence intensities of the uncomplexed and complexed form of the dye, respectively, $[\mathrm{Dye}]_0$ and $[\mathrm{CB7}]_0$ 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.[2,3] For fitting, the change in the fluorescence intensity (ΔI_f^{λ}) was plotted against the total host concentration and the obtained titration curve was fitted according to the rearranged eq. S2:[1, 3]

$$\Delta I_f^{\lambda} = \left(1 - \frac{[Dye]_{eq}}{[Dye]_0}\right)\left(I_{CB7:Dye}^{\infty} - I_{Dye}^{0}\right) \tag{S2}$$

where, $[Dye]_{eq}$ is expressed as

$$[Dye]_{eq} = \{K_{eq}[Dye]_0 - K_{eq}[SCX6]_0 - 1 + \sqrt{(K_{eq}[Dye]_0 + K_{eq}[CB7]_0 + 1)^2 - 4K_{eq}^2[Dye]_0[CB7]_0}\} / 2K_{eq} \tag{S3}$$

<u>Method M2</u>: 1:2 host: guest binding model[4]

$$\text{CB + Dye} \xrightleftharpoons{K_1} \text{CB : Dye} \tag{S4a}$$

$$\text{CB : Dye + Dye} \xrightleftharpoons{K_2} \text{CB : (Dye)}_2 \tag{S4b}$$

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

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} and I_{ggh}, respectively):

$$I = x_g\, I_g + x_{gh}\, I_{gh} + 2\, x_{ggh}\, I_{ggh} \tag{S5}$$

x_g, x_{gh} 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:

$$x_g = \frac{[G]}{[G]_0} \tag{S6}$$

$$x_{gh} = \frac{[HG]}{[G]_0} \qquad \Rightarrow x_{gh} = \frac{K_1 [H]_0}{1 + K_1 [G] + K_1 K_2 [G]^2} \cdot \frac{[G]}{[G]_0} \tag{S7}$$

$$x_{gh} = \frac{[HG_2]}{[G]_0} \qquad \Rightarrow x_{ggh} = \frac{K_1 K_2 [H]_0}{1 + K_1 [G] + K_1 K_2 [G]^2} \cdot \frac{[G]^2}{[G]_0} \tag{S8}$$

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

$$I = \frac{[G]}{[G]_0} \cdot \frac{\left(1 + K_1 [G] + K_1 K_2 [G]^2\right) I_g + K_1 [H]_0 I_{gh} + 2 K_1 K_2 [H]_0 [G] I_{ggh}}{1 + K_1 [G] + K_1 K_2 [G]^2} \tag{S9}$$

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

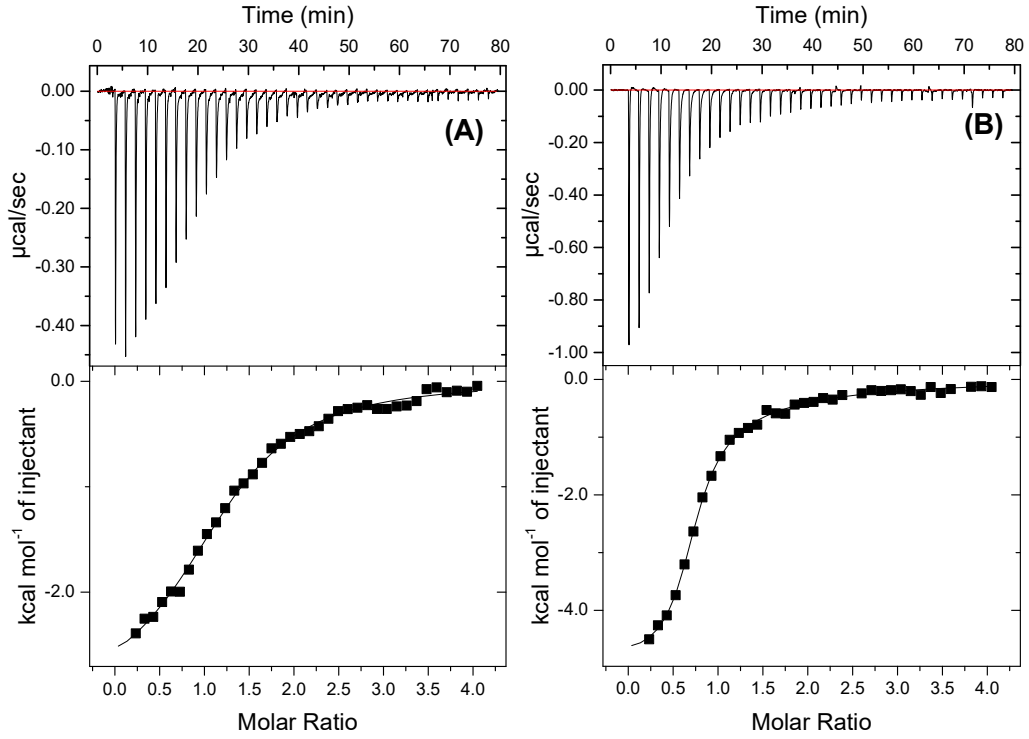

Figure S4. (A) ITC isotherm for titration of AcH^+ with CB7 in water at 25 °C. (B) ITC isotherm for titration of CB8 with AcH^+ in water at 25 °C.

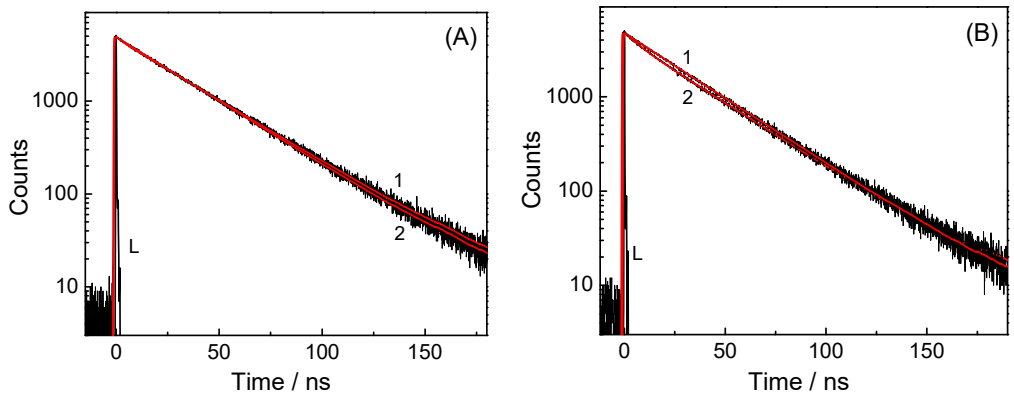

Figure S5. Decay traces of AcH^+ in solutions at pH ~3.5 in the absence (1) and presence (2) of 250 μM CB7 (A) and 70 μM CB8 (B). 'L' represents excitation lamp profile. λ_{ex} = 374 nm.

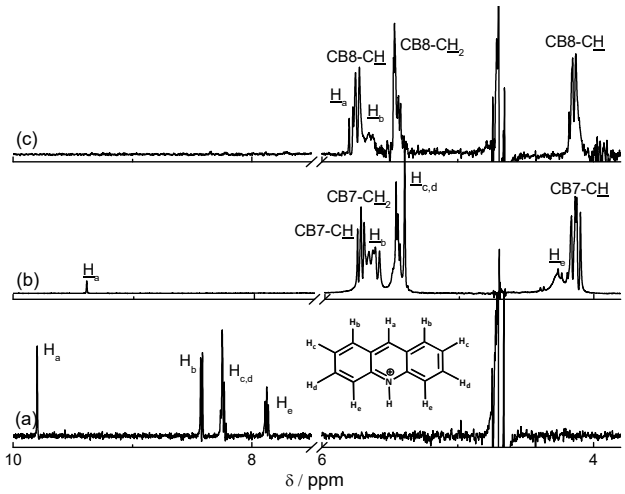

Figure S6. ^{1}H NMR spectra (500 MHz) of ~100 μM acridine dye in the absence (a) and in the presence (b) of 1mM CB7 and (c) 80 μM CB8 in D_2O at pD 4.5. Inset: Pictorial representation of ACH^+.

Method M3:

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

$$A_{obs} = \frac{A^{\infty}_{AcH^+}}{\{1+10^{pH-pK_a}\}} + \frac{A^{\infty}_{Ac}}{\{1+10^{pK_a-pH}\}} \qquad \text{(S10)}$$

where A_{obs} is the observed absorbance at any pH, and $A^{\infty}_{AcH^+}$ and A^{∞}_{Ac} are the extrapolated absorbances of the AcH^+ and Ac forms, respectively. From this analysis, the pK_a value of the dye in its ground state was found to be 5.4 ± 0.1, which matches well with the reported value.[3, 5]

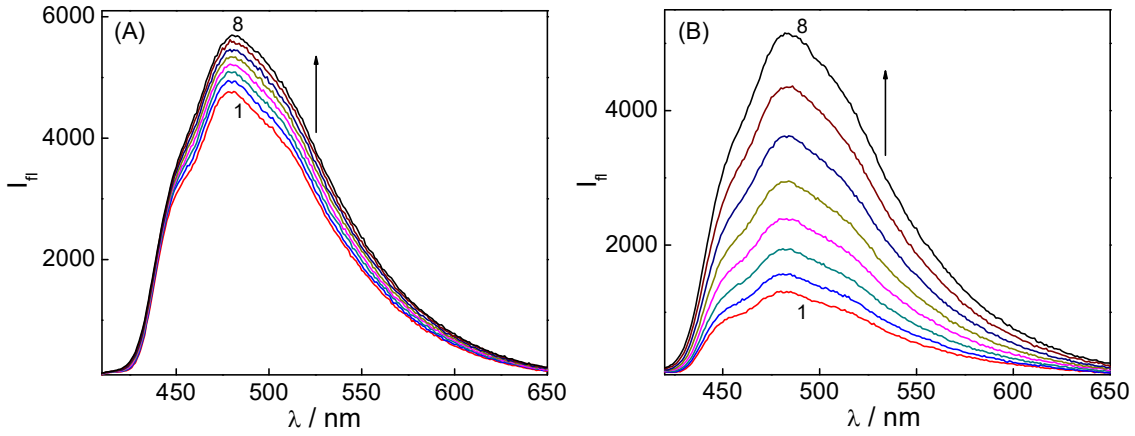

Figure S7. Fluorescence spectra of CB7:AcH^+ complex **(A)** and CB8:AcH^+ complex **(B)** with increasing temperature. T/ °C: (1) 20, (2) 30, (3) 40, (4) 50, (5) 60, (6) 70 and (8) 80.

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

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