

## Electronic Supporting Information

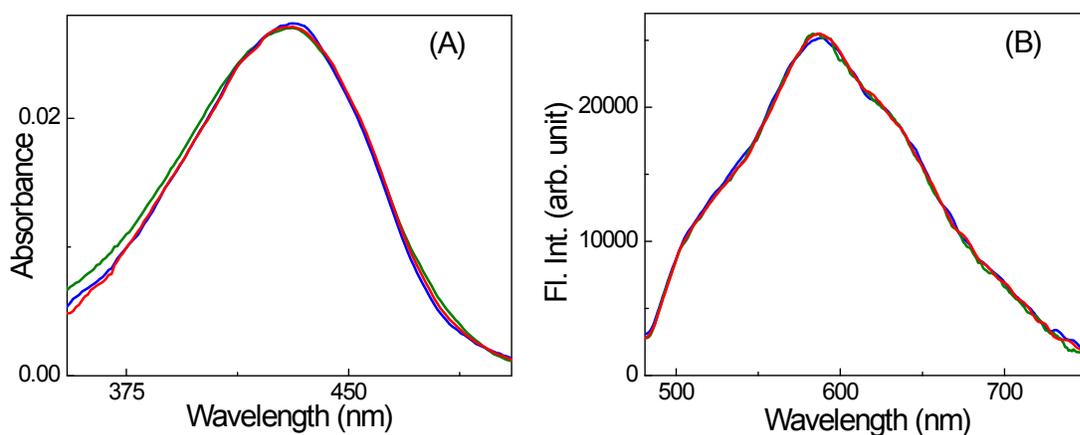
### **Inhibition of the Prototropic Tautomerism in Chrysazine by *p*-Sulfonatocalixarene Hosts**

Poojan Milan Gharat,<sup>a</sup> Dilip Kumar Maity,<sup>b</sup> Haridas Pal,<sup>a,b,\*</sup> and Sharmistha Dutta Choudhury<sup>a,b,\*</sup>

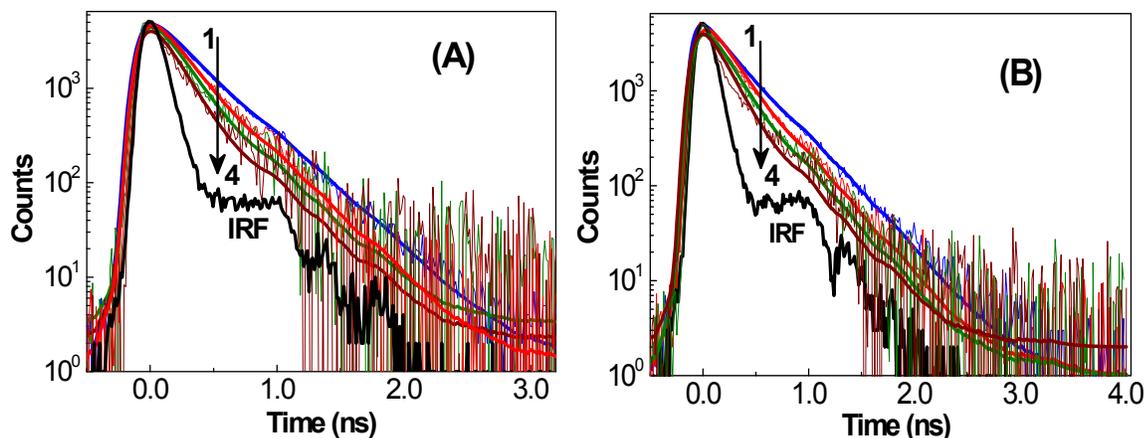
<sup>a</sup>Radiation & Photochemistry Division, Bhabha Atomic Research Centre, Mumbai 400085, India.

<sup>b</sup>Homi Bhabha National Institute, Training School Complex, Anushaktinagar, Mumbai 400094, India.

E-mail: hpal@barc.gov.in, sharmidc@barc.gov.in



**Figure S1.** (A) Absorption and (B) emission spectra of CZ in aqueous solutions at pH 3 (blue), 5 (green) and 8 (red).



**Figure S2.** Fluorescence decay traces of CZ in the presence of 0, 10, 17 and 30 mM SCX4 (1-4) measured at (A) 515 nm (B) 585 nm. Excitation wavelength is 406 nm. IRF is the instrument response function.

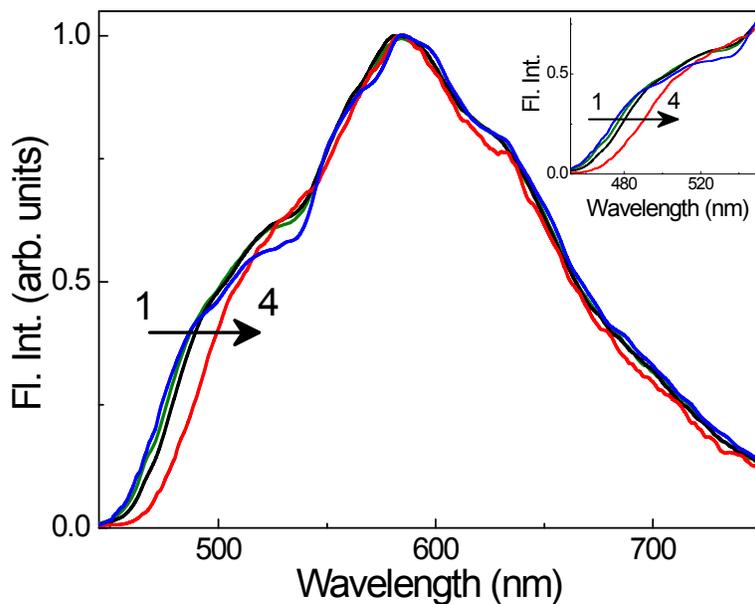
### **Note S1**

In aqueous solution, the excited state populations of  $N^*$  and  $T^*$  forms of CZ show reasonably fast single exponential decay kinetics with  $\tau_f$  value of 0.29 ns. The exactly matching  $\tau_f$  values of the  $N^*$  and  $T^*$  forms of the dye suggests the existence of a kinetic equilibrium between the two tautomeric forms in their excited state within the time scale probed by the present TCSPC setup. In the presence of SCXn, the fluorescence decay traces are seen to have a major contribution from a substantially fast decay component along with a small contribution of a longer decay tail (not shown here). We observed that this long decay tail arises from the hosts SCXn themselves. This intrinsic emission of the hosts, being very weak, does not cause any significant changes in the steady-state fluorescence spectra of CZ. However, the weak emission from the two hosts can show their presence in the observed decay traces measured for both  $N^*$  and  $T^*$  forms of the dye. Therefore, the measured decay of the dye in the presence of SCXn is simply due to the sum of the individual contributions of the dye emission and the SCXn intrinsic emission. Since, the longer decay tails observed in the decay traces of the dye in presence of SCXn are very similar to the decay tails observed for the intrinsic emission of the SCXn, we could extract the corrected decays of the dye by tail matching the experimentally observed decays of the SCXn-CZ systems with the decays of the SCXn intrinsic emission and subsequent subtraction of the latter from the former decays. The corrected decay traces thus obtained for the

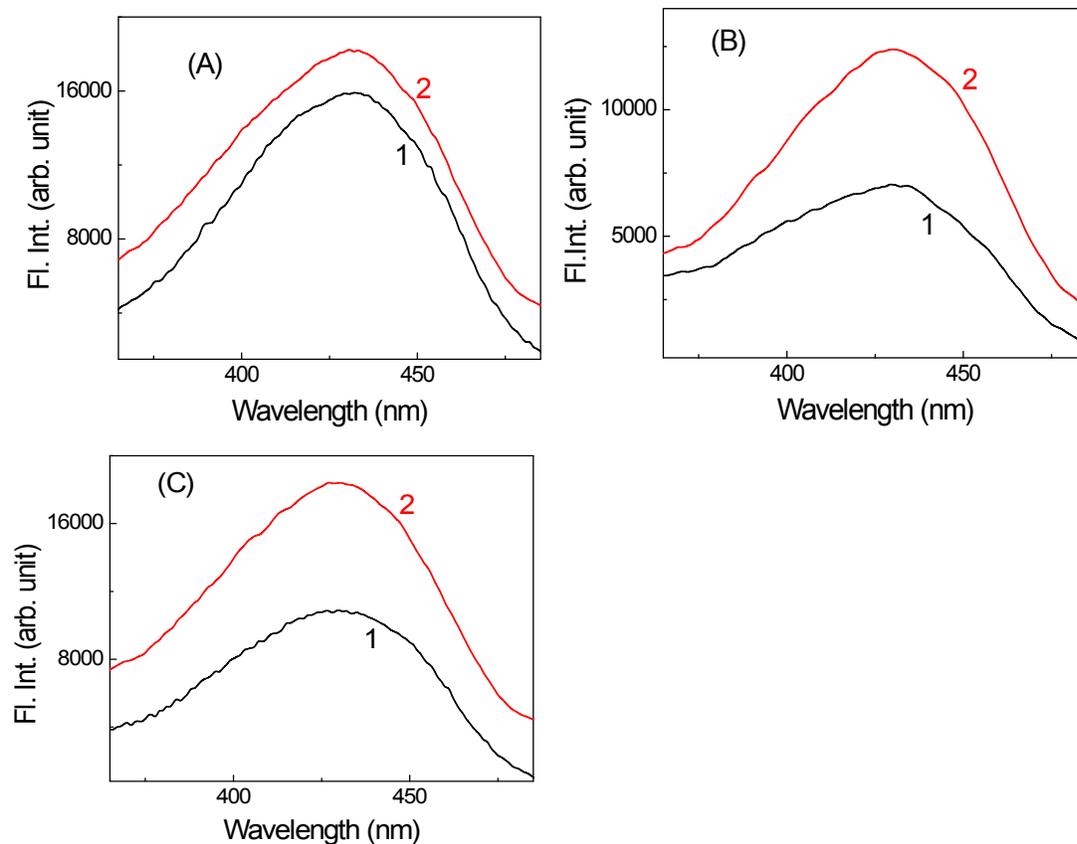
N\* and T\* forms of the dye in the presence of SCX4 are presented in Figures S2A and B, respectively, and the fitted lifetimes are given in Table S1.

**Table S1** Excited state lifetimes of CZ at various concentrations of SCX4.

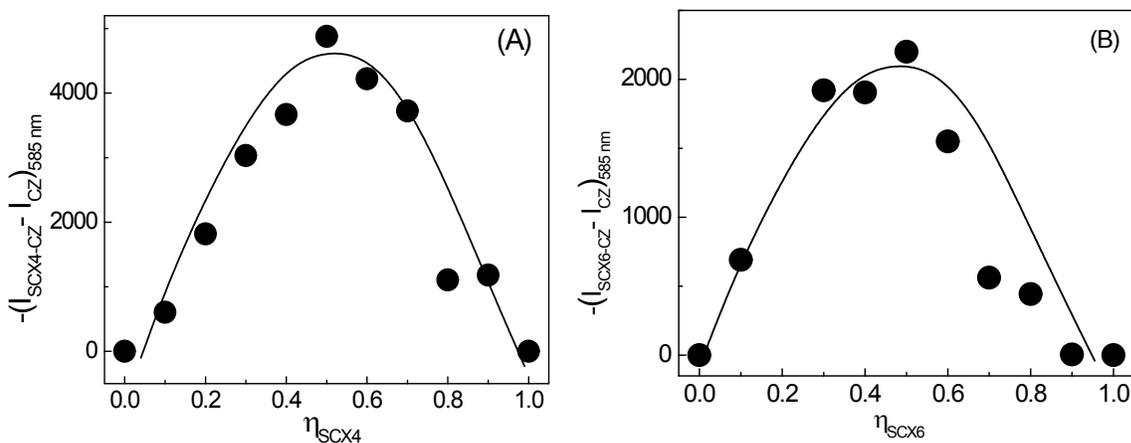
[SCX4] (mM)	$\tau_{f, 515 \text{ nm}}$ (ns)	$\tau_{f, 585 \text{ nm}}$ (ns)
0	0.29	0.29
10	0.23	0.24
17	0.21	0.22
30	0.18	0.18



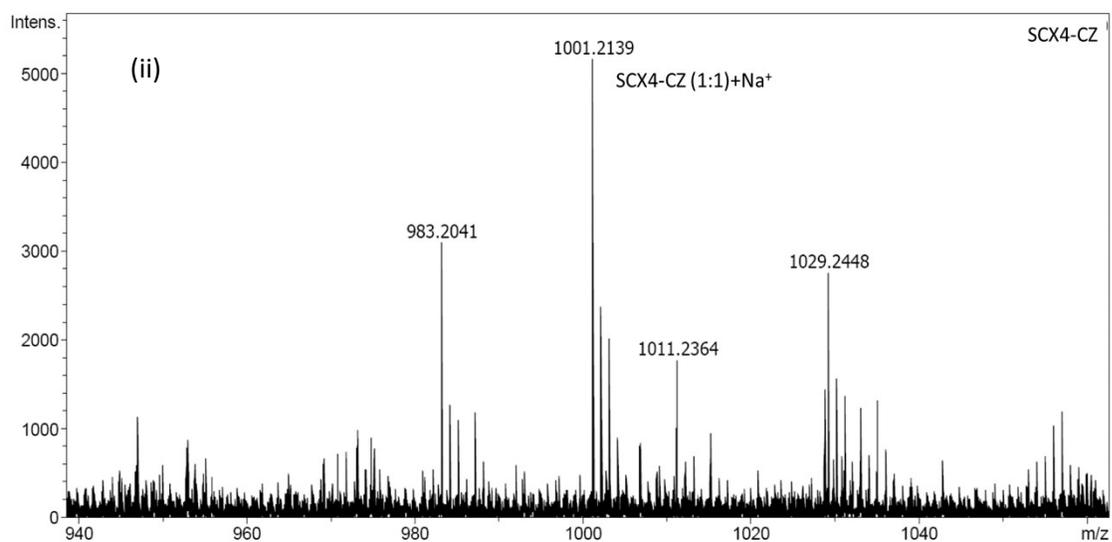
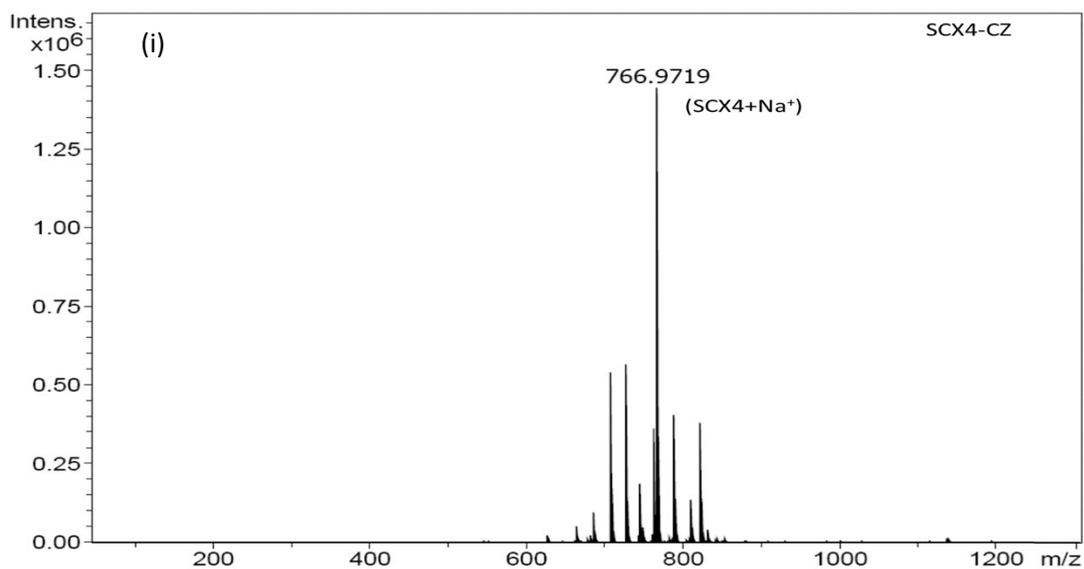
**Figure S3.** Normalized emission spectra of CZ in cyclohexane (1, blue), ethylacetate (2, green), acetonitrile (3, black), and water (red). Inset shows an enlarged view of the same to depict the shift in the LWEB (N\* emission) with increasing polarity of the solvent medium. Excitation wavelength is 415 nm.



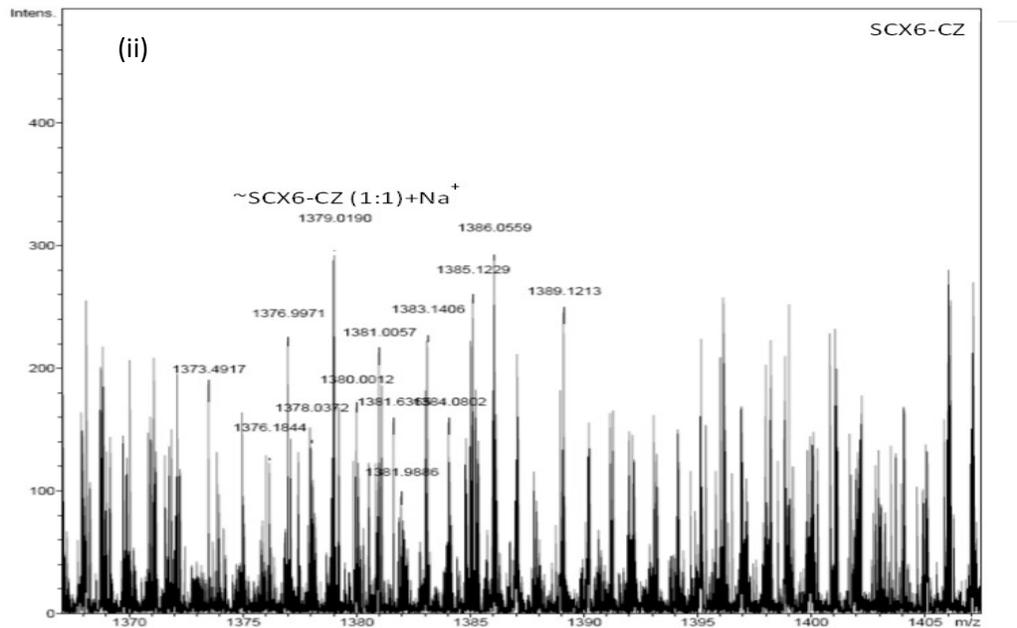
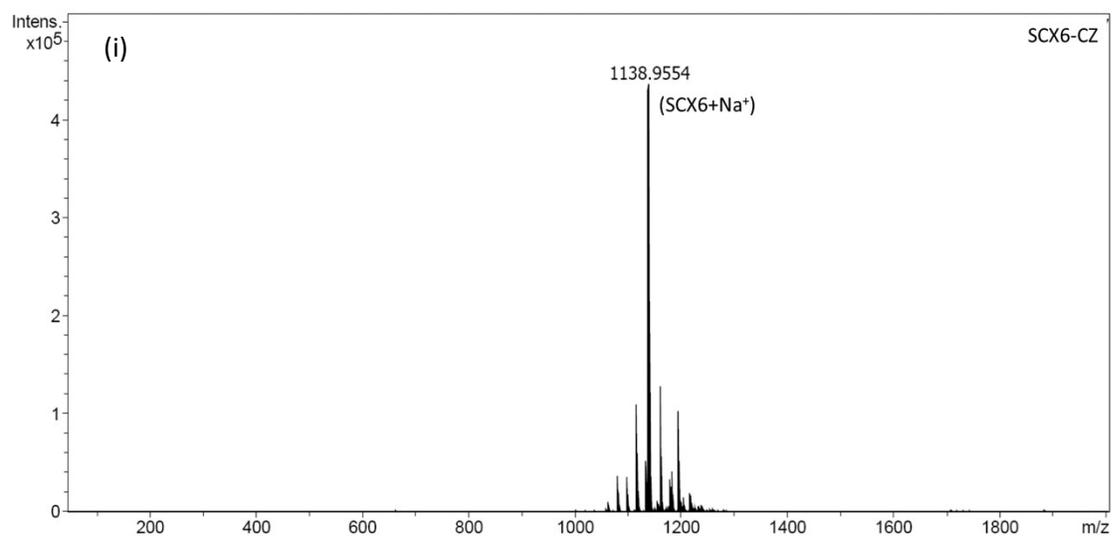
**Figure S4.** Excitation spectra of CZ in aqueous solutions monitored at 515 nm (1, black) and 585 nm (2, red) in the absence of host (A) in the presence of 31 mM SCX4 (B) and in the presence of 20 mM SCX6 (C).



**Figure S5.** Job plots for (A) SCX4-CZ and (B) SCX6-CZ system obtained from the changes in the fluorescence intensities on complex formation. While the measurement for the SCX4-CZ system was carried out at room temperature (25 °C), the measurement for the SCX6-CZ system was carried out at a lower temperature (20 °C) to obtain reliable result.



**Figure S6.** (A) ESI-MS for SCX4-CZ system. The major peak corresponds to the free host SCX4 (i). The presence of very weak host-guest complexes with 1:1 stoichiometry can be approximately identified in the enlarged view shown in the bottom panel (ii).



**Figure S6.** (A) ESI-MS for SCX6-CZ system. The major peak corresponds to the free host SCX6 (i). The presence of very weak host-guest complexes with 1:1 stoichiometry can be approximately identified in the enlarged view shown in the bottom panel (ii).

## Note S2.

### Calculation of binding constants for SCX<sub>n</sub>-CZ systems

By considering 1:1 host-guest complex formation, the binding equilibrium for the normal form of the dye can be presented as



where, H= Host, N = Normal form of the free dye and C<sub>N</sub> = Normal form of the complexed dye

$$\therefore K_{eq} = \frac{[C_N]}{[N][H]} \quad (S2)$$

If K<sub>eq</sub> is not large and the host concentration [H]<sub>0</sub> is much higher than the guest concentration [N]<sub>0</sub>, then one can assume that the equilibrium host concentration in the solution is effectively the same as the actually added host concentration, i.e., [H]≈[H]<sub>0</sub>.

$$\therefore K_{eq} = \frac{[C_N]}{[N][H]_0} = \frac{[C_N]}{([N]_0 - [C_N])[H]_0} \quad (S3)$$

$$\therefore \frac{[C_N]}{[N]_0} = \frac{K_{eq}[H]_0}{1 + K_{eq}[H]_0} \quad (S4)$$

The fluorescence intensity observed at 515 nm can be considered as

$$I_{N(\text{obs})} = I_{N(\text{free})} + I_{C_N} \quad (S5)$$

where, I<sub>N(free)</sub> and I<sub>C<sub>N</sub></sub> are the fluorescence intensities due to the normal form of the free and bound dye, respectively.

Since I<sub>N(free)</sub> and I<sub>C<sub>N</sub></sub> will be proportional to the concentration of N and C<sub>N</sub> respectively, in the solution, one can write within approximation that,

$$I_{N(\text{free})} = K_1[N] \text{ and } I_{C_N} = K_2 [C_N]$$

where K<sub>1</sub> and K<sub>2</sub> are the proportionality constants that would be the functions of fluorescence yields as well as the absorption coefficients at the excitation wavelength of the normal form of the free and bound dye, respectively.

Considering, I<sub>N</sub><sup>0</sup> and I<sub>N</sub><sup>∞</sup> to be the fluorescence intensity in the absence of any H and in the situation when all the N has been converted to C<sub>N</sub>, one can write,

$$I_N^0 = K_1[N]_0 \text{ or } K_1 = \frac{I_N^0}{[N]_0} \text{ and } I_N^\infty = K_2[C_N] = K_2[N]_0 \text{ or } K_2 = \frac{I_N^\infty}{[N]_0}$$

Thus, eq. S5 can be rewritten as,

$$I_{N(\text{obs})} = I_N^0 \frac{[N]}{[N_0]} + I_N^\infty \frac{[C_N]}{[N_0]} \quad (\text{S6})$$

$$\text{or, } I_{N(\text{obs})} = I_N^0 \frac{[N]_0 - [C_N]}{[N_0]} + I_N^\infty \frac{[C_N]}{[N_0]} \quad (\text{S7})$$

$$\text{or, } (I_{N(\text{obs})} - I_N^0) = \frac{[C_N]}{[N_0]} (I_N^\infty - I_N^0) \quad (\text{S8})$$

Using eq.S4, eq. S8 can be rewritten as,

$$(I_{N(\text{obs})} - I_N^0) = (I_N^\infty - I_N^0) \frac{K_{\text{eq}}[H]_0}{1 + K_{\text{eq}}[H]_0} \quad (\text{S9})$$

$$\text{or, } \Delta I_N = \Delta I_N^\infty \frac{K_{\text{eq}}[H]_0}{1 + K_{\text{eq}}[H]_0} \quad (\text{S10})$$

Similarly, the fluorescence intensity observed at 585 nm can be given as,

$$I_{T(\text{obs})} = I_{T(\text{free})} + I_{C_T} \quad (\text{S11})$$

where,  $I_{T(\text{free})}$  and  $I_{C_T}$  are the fluorescence intensities at 585 nm due to free and complexed dye respectively.

$$\text{Also, } I_{T(\text{free})} = K_1'[T] \text{ and } I_{C_T} = K_2'[C_T]$$

where  $K_1'$  and  $K_2'$  are the proportionality constants that would be the functions of the fluorescence yield of the T form of the free and bound dye respectively.

In addition to the host-guest binding equilibrium, the two other equilibria that exist in the present systems, due to the prototropic tautomerism of the free and bound forms of excited CZ are expressed as follows:



where,  $K_T$  and  $K_T'$  are the equilibrium constants for the tautomerization reaction between  $N^*$  and  $T^*$  forms of free and SCXn bound CZ at zero and infinite host concentrations, respectively.

Thus,

$$I_{T(\text{obs})} = K_1'[T] + K_2'[C_T] \text{ or } I_{T(\text{obs})} = K_1'K_T[N] + K_2'K_T'[C_N] \quad (\text{S14})$$

If  $I_T^0$  &  $I_T^\infty$  be the fluorescence intensity at 585 nm in absence of any host and at the situations when all N is complexed by the hosts, respectively, then,

$$I_T^0 = K'_1 K_T [N]_0 \text{ or } K'_1 K_T = \frac{I_T^0}{[N]_0} \quad (\text{S15})$$

$$\text{and } I_{C_T}^\infty = K'_2 K'_T [C_N^\infty] = K'_2 K'_T [N]_0 \text{ or } K'_2 K'_T = \frac{I_T^\infty}{[N]_0} \quad (\text{S16})$$

Thus, eq. S14 can be rewritten as,

$$I_{T(\text{obs})} = I_T^0 \frac{[N]}{[N_0]} + I_N^\infty \frac{[C_N]}{[N_0]} \text{ or } I_{T(\text{obs})} = I_T^0 \frac{[N]_0 - [C_N]}{[N_0]} + I_N^\infty \frac{[C_N]}{[N_0]} \quad (\text{S17})$$

Thus, as before,

$$(I_{T(\text{obs})} - I_T^0) = (I_T^\infty - I_T^0) \frac{K_{\text{eq}}[H]_0}{1 + K_{\text{eq}}[H]_0} \quad (\text{S18})$$

$$\text{or } \Delta I_T = \Delta I_T^\infty \frac{K_{\text{eq}}[H]_0}{1 + K_{\text{eq}}[H]_0} \quad (\text{S19})$$

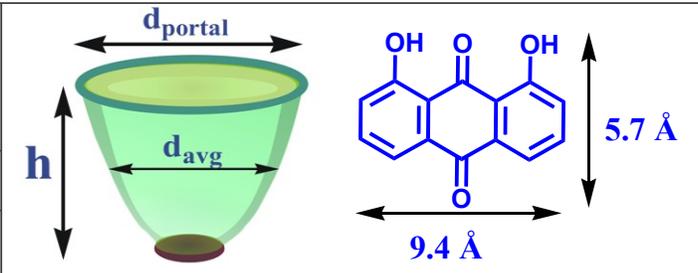
Considering that the absorption spectrum of CZ does not change to a very large extent in the presence of the hosts and assuming that both  $N^*$  and  $T^*$  forms of CZ are equally quenched by

SCXn, one can simply have,  $\frac{K'_2}{K_2} = \frac{K'_1}{K_1}$  or,  $\frac{K'_2}{K'_1} = \frac{K_2}{K_1}$ .

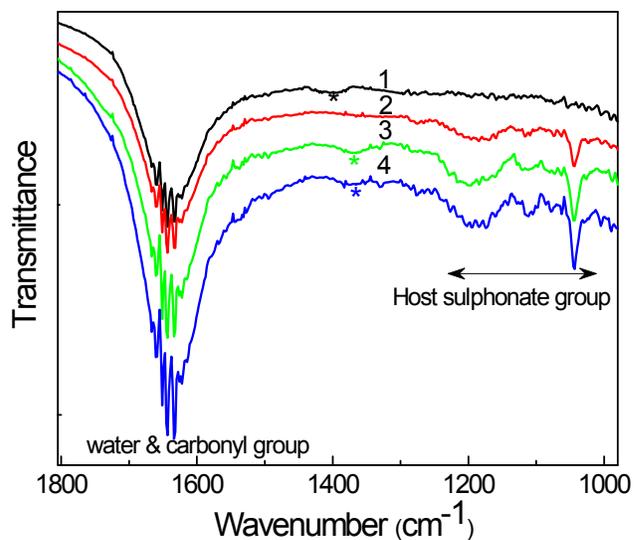
$$\therefore K_T = \frac{[T]}{[N]} = \frac{K_2 I_T^0}{K_1 I_N^0} \text{ or, } K_T \propto \frac{I_T^0}{I_N^0} \quad (\text{S20})$$

$$\text{and } K'_T = \frac{[C_T]}{[C_N]} = \frac{K'_2 I_T^\infty}{K'_1 I_N^\infty} \text{ or, } K'_T \propto \frac{I_T^\infty}{I_N^\infty} \quad (\text{S21})$$

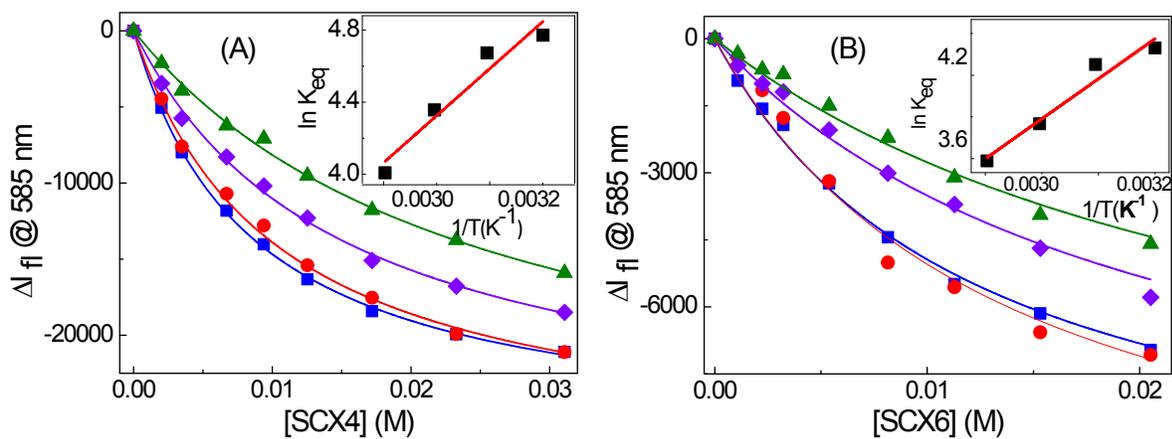
**Table S2** Approximate dimensions of the SCXn hosts and CZ.

Host	Average Cavity Diameter $d_{\text{avg}}$ (Å) <sup>a</sup>	Upper Rim Diameter $d_{\text{portal}}$ (Å) <sup>a</sup>	Height $h$ (Å) <sup>a</sup>	
SCX4	~3.0	~11.0	~7.0	
SCX6	~7.6	~16.0	~7.0	

<sup>a</sup> Refs. 1-3 and from present geometry optimization studies



**Figure S7.** FTIR spectra of (1) CZ, (2) SCXn hosts (SCX4 and SCX6 have similar features), (3) SCX4-CZ and (4) SCX6-CZ, in aqueous medium. Peaks indicated by (\*) correspond to the OH bending mode of CZ. The assignments of the IR peaks are according to Refs. 4-8.



**Figure S8.** Binding isotherms for (A) SCX4-CZ and (B) SCX6-CZ monitored at 585 nm at 30°C (■), 40°C (●), 50°C (◆) and 60°C (▲). Insets show the corresponding van't Hoff plots.

**Table S3** Binding constants for the SCXn-CZ systems at various temperatures obtained by following the changes in the fluorescence intensity at 515 and 585 nm.

System	SCX4-CZ		SCX6-CZ	
Temp (°C)	$K_{eq} (M^{-1})$		$K_{eq} (M^{-1})$	
	Obtained by fitting to eq. S10 (eq. 4 in the main text)	Obtained by fitting to eq S19 (eq. 5 in the main text)	Obtained by fitting to eq. S10 (eq. 4 in the main text)	Obtained by fitting to eq. S19 (eq. 5 in the main text)
30	135	118	79	81
40	118	107	68	72
50	99	78	48	47
60	69	56	34	36

## References

1. W. Yang and M. M. de Villiers, *Eur. J. Pharm. Biopharm.*, 2004, **58**, 629.
2. P. M. Gharat, S. Joseph, M. Sundararajan, S. Dutta Choudhury and H. Pal, *Org. Biomol. Chem.*, 2016, **14**, 11480.
3. V. Ramamurthy and S. Gupta, *Chem. Soc. Rev.*, 2015, **44**, 119.
4. N. N. Valand, M. B. Patel and S. K. Menon, *RSC Adv.*, 2015, **5**, 8739.
5. J. M. Davey, C. O. Too, S. F. Ralph, L. A. P. Kane-Maguire and G. G. Wallace, *Macromolecules*, 2000, **33**, 7044.
6. S. F. Chin, M. Makha, C. L. Raston and M. Saunders, *Chem. Commun.*, 2007, 1948.
7. J. Bandara, J.A. Mielczarski, J. Kiwi, *Applied Catalysis B: Environmental*, 2001, **34**, 307.
8. D. Shchukin, S. Poznyak, A. Kulak, P. Pichat, *J. Photochem. Photobiol. A*, 2004, **162**, 423.