Supporting Information

Supramolecular Assembly of Hoechst-33258 with Cucurbit[7]uril Macrocycle

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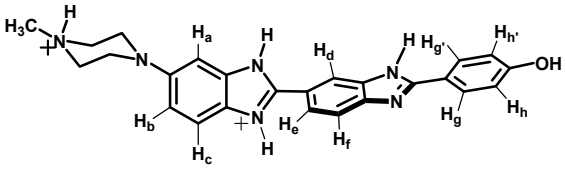
Details of NMR Characterization



Structure S1: Chemical structure of H33258 at pH 4.5

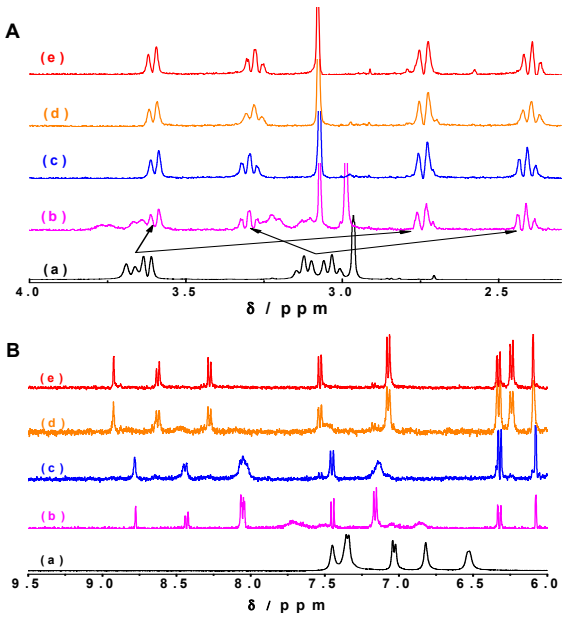


Figure 7 (as provided in the main text): ^{1}H NMR titration spectra of H33258 dye at pH 4.5 with the addition of molar equivalents of CB7: 0 (**a**); 0.5 (**b**); 1.0 (**c**); 1.6 (**d**); 2.2 (**e**) shown in the aliphatic region (**A**) and aromatic region (**B**).

Efforts were made to identify the marked protons (Structure S1) to understand the change in their chemical environment due to complexation. At pH 4.5, as provided in Fig.7, the aliphatic region identifies the NCH_3 and piperazinyl protons, however, the concomitant changes in the aromatic region of the spectra are not straightforward. At this moment it is quite difficult to assign these signals, based on the integrations and the splitting patterns of the well resolved peaks we attribute the singlet at 6.08 ppm to protons H_a and the doublets at 6.32 and 7.45ppm to the protons H_b/H_c (Structure S1). These protons will be greatly affected by the interaction of Hoechst-33258 with CB7 through the N-methyl piperazinyl benimidazole part. We anticipate that the interaction of Hoechst-33258 with CB7 causes the deaggregation which causes the clear separation and downfields shift of the aromatic protons of the dye. Inclusion inside the hydrophobic cavity of CB7 in principle, render the upfield shift of the proton resonance. A close look at the geometry optimized structures (Fig.8) reveal that the benzimidazole part of the N-methyl piperazinyl benimidazole unit is partly embedded inside the CB7 cavity. Based on this logic we attribute the singlet at 6.08 ppm to protons H_a and the doublets at 6.32 and 7.45ppm to the protons H_b and H_c respectively. Finally addition of 2.2 equivalent CB7 rather simplified the ^{1}H-NMR spectra which clearly show all the individual aromatic proton resonances.

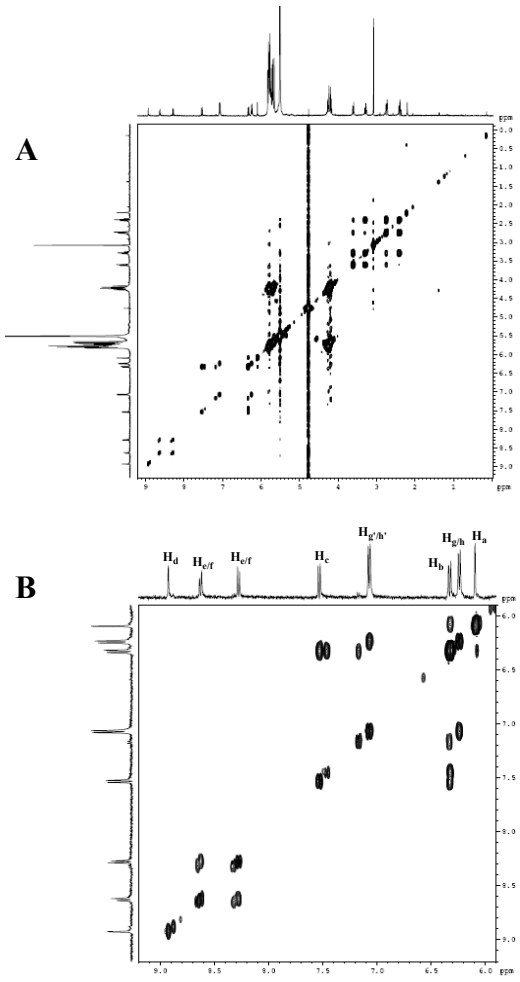


Figure S1: The full ^{1}H-^{1}HCOSY for Hoechst-33258-CB7 complex at pH 4.5 (**A**) and the aromatic region enlarged (**B**).:

The 2D ^{1}H-^{1}H COSY in presence of 2.2 equivalent of CB7 at pH 4.5 shows correlation between the pairs of aromatic protons appearing at 6.24 and 7.07ppm, 6.32 and 7.53ppm, 8.28 and 8.63ppm. Based on the splitting pattern and integration we attribute the doublets at 6.24 and 7.07ppm to phenolic protons, H_g/H_h, the doublets at 8.28 and 8.63ppm to the H_e/H_f and the singlet at 8.92ppm to H_d. H_e/H_f, H_d and H_c protons show a slightly upfield shift with respect to their individual positions in presence of one equivalent of CB7 along with the downfield shift of the phenolic protons H_g/H_h. These observations clearly indicate that at high concentration of CB7 (~2.2eq.) the host CB7 form ternary inclusion complex with inclusion of the phenol benzimidazole unit of Hoechst-33258 by the second CB7 molecule. Based on the ^{1}H-NMR spectroscopic measurements we anticipate that the phenolic unit is embedded in the hydrophobic cavity of CB7 leading a downfield shift and the phenol-benzimidazole part resides in the desheilding region of the carbonyl portal of CB7 rendering the upfield shift of the respective proton resonances.

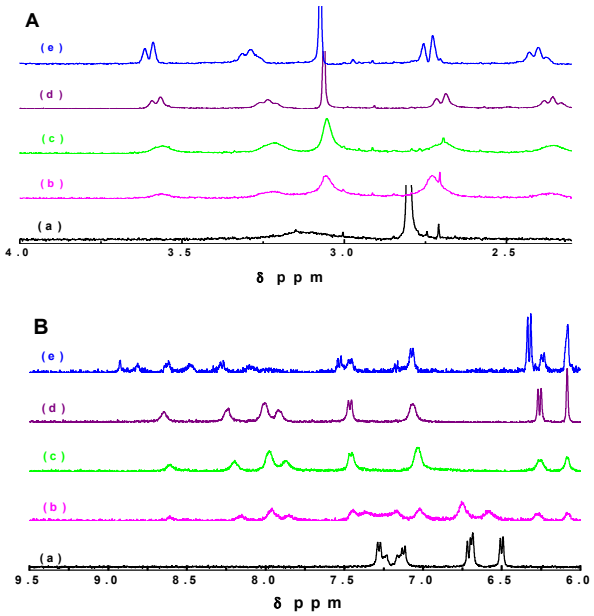


Figure S2: ^{1}H NMR titration spectra of H33258 dye at pH 7 with the addition of molar equivalents of CB7: 0 (**a**); 0.5 (**b**); 1.0 (**c**); 1.6 (**d**); 2.2 (**e**) shown in the aliphatic region (**A**) and aromatic region (**B**).

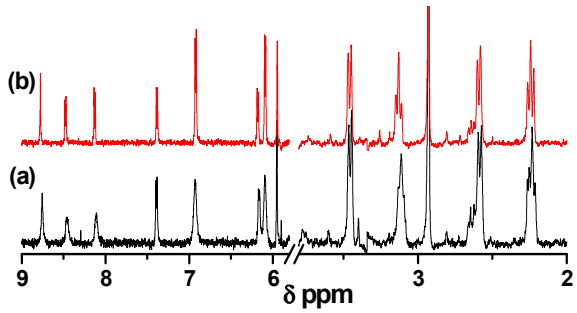


Figure S3: ^{1}H NMR titration spectra of H33258 dye at pH 7 (**a**) and 4.5 (**b**) in the presence of about 20 molar equivalents of CB7, indicating similar structural orientation for both the complexes.

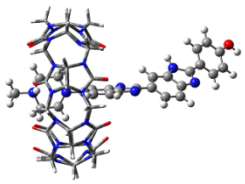
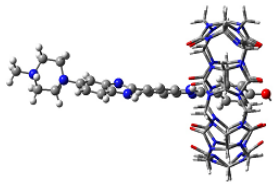
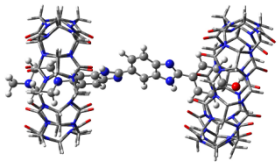
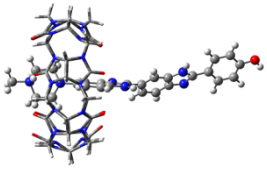
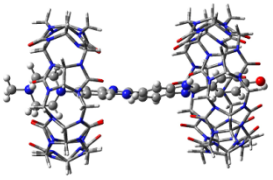
CB7 + H33258 (+1) **(pH 7)** **(CB7 at piperazine side)** **ΔH_f = - 46.53 kcal/mol**	
CB7 + H33258 (+1) **(pH 7)** **(CB7 at phenol side)** **ΔH_f = - 9 kcal/mol**	
2CB7 + H33258 (+1) **(pH 7)** **ΔH_f = - 47.96 kcal/mol**	
CB7 + H33258 (+2) **(pH 4.5)** **ΔH_f = -88 kcal/mol**	
2CB7 + H33258 (+2) **(pH 4.5)** **ΔH_f = -105.58 kcal/mol**	

Figure.S4: Geometry optimized structures of CB7-H33258 complex in different protonated forms.

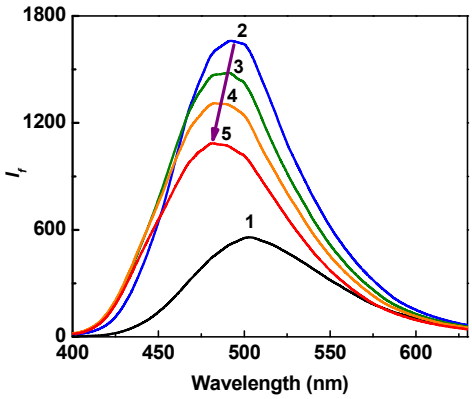


Figure.S5: Fluorescence intensity measured at pH 4.5 for H33258 (1), CB7-H33258 complex (2) and solution of (2) in presence of [$CaCl_2$]/μM: 50 (3); 100 (4); 1000 (5); indicating the dissociation of the 2:1 complex to 1:1 stoichiometry.

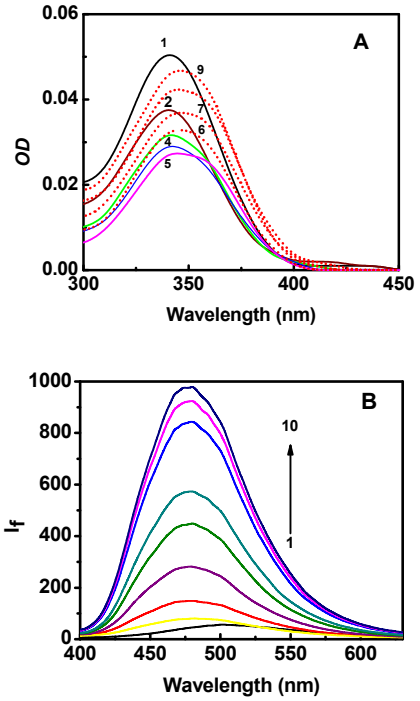


Figure S6: Absorption (**A**) and Emission (**B**) spectra of H33258 (~1μM) recorded in solution at pH 9 with increasing concentration of CB7. (**A**) H33258 with [CB]/μM; 0(1); 1 (2); 1.5 (3); 2 (4); 4.5 (5); 6.5 (6); 12.5 (7); 18.5 (8); 23 (9). (**B**) H33258 with [CB]/μM; 0 (1); 1 (2); 1.5 (3); 2 (4); 4.5 (5); 6.5 (6); 12.5 (7); 18.5 (8); 23 (9).

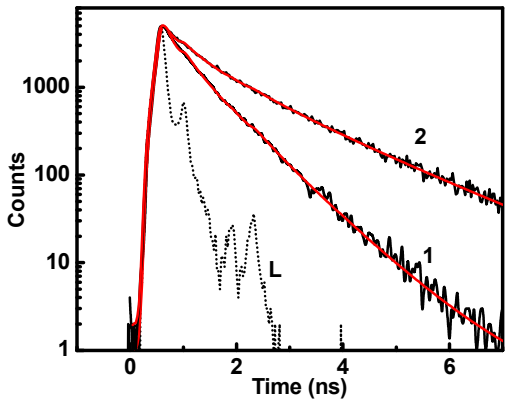


Figure.S7:Decay traces of H33258 at 500 nm (~1μM) in solutions at pH 9 (1) and with the addition of ~200μM of CB7 (2). L represents lamp profile.