Supporting Information

Cucurbit[7]uril assisted ultraviolet to visible fluorescence switch of a heart medicine

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Experimental section:

Milrinone (MIR), cucurbit[7]uril (CB7) were purchased from Sigma Aldrich, and used without further purification. Millipore water was used for sample preparation. CB7 was gradually added to the solution containing MIR, and the solution was gently shaken after each addition of CB7 until complete solubilization took place. Moreover, we have given 20 minutes equilibration time for each addition of CB7. Fine adjustment of pH was done by drop wise addition of diluted hydrochloric acid (Merck, India, 90% pure) for lower pH range and 0.1 M sodium hydroxide (SRL, India, 98% pure) solution for the higher pH range. pH of the solution was measured by using pH-1500 (Eutech Instruments) and it was further cross checked by silicon micro sensor pocket sized pH meter (ISFETCOM Co. Ltd., Japan).

Absorbance measurements were performed on Perkin-Elmer UV-visible spectrophotometer (Lambda-45), and steady-state fluorescence spectra were recorded in FluoroMax-4 spectrofluorimeter (Horiba Scientific, USA). All time-resolved fluorescence measurements (both life-time as well as anisotropy) were measured on a time correlated single photon counting (TCSPC) spectrometer (Horiba Jobin Yvon IBH, U.K.). The detail description of the instrument is described elsewhere.[1] Briefly, in the present work a 375 nm diode laser (1 MHz repetition rate) was used as the excitation source and MCP-PMT detector was used for collecting fluorescence signal. The instrument response function for this experimental set-up is ~90 ps. The analysis of lifetime was done by IBH DAS6 analysis software, in which a reconvolution procedure was used to analyze the observed decays using a proper instrument response function obtained by replacing the sample cell with light scattering solution (suspension of Ludox colloidal solution). The fluorescence decays were analyzed as a sum of exponentials as

$$I(t) = \sum_i a_i \exp(-t/\tau_i) \qquad (1)$$

where, $I(t)$ is the time dependent fluorescence intensity, and a_i and τ_i are the pre-exponential factor and the fluorescence lifetime for the i^{th} component of the fluorescence decay, respectively. The quality of the fits and the multi-exponential nature of the decays were evaluated by the reduced chi-square (χ^2) values and the distribution of the weighted residuals among the data channels. For a good fit, the χ^2 value was close to unity, and the weighted residuals were distributed randomly among the data channels.

For time-resolved fluorescence anisotropy decay measurements, the polarized fluorescence decays for the parallel [$I_{||}$ (t)] and perpendicular [$I_{\perp}$(t)] emission polarizations with respect to the vertical excitation polarization were first collected at the emission maxima of the probe (either peak at 390 nm or peak at 500 nm). Then, the anisotropy decay function $r(t)$ was constructed from these $I_{||}$ (t) and $I_{\perp}$(t) decays using the following equation,

$$r(t) = \frac{I_{//}(t) - GI_{\perp}(t)}{I_{//}(t) + 2GI_{\perp}(t)} \qquad (2)$$

where G is the correction factor for the detector sensitivity to the polarization detection of the emission. The anisotropy decay fitting was done using the following functional form,

$$r(t) = r_0[a_{1r}\exp(-t/\tau_{1r}) + a_{2r}\exp(-t/\tau_{2r})] \qquad (3)$$

in which r_0 is the limiting anisotropy that describes the inherent depolarization of the fluorophore and a_{ir} is the pre-exponential term that renders the fraction of the i^{th} rotational relaxation time, i.e., τ_{ir}. The quality of each fitting was judged by χ^2 values and the visual inspection of the residuals.

The computational study comprised of docking followed by semi-empirical quantum chemical (PM3MM) calculations. The crystal structure of CB7 was obtained from the Cambridge Structural Database (CSD).[2] Initially, all the chemical structures (host and guest) were geometry optimized using density functional theory (DFT) using B3LYP functional with 3-21G basis set on gaussian09 software.[3] The molecular docking study was performed by means of the AutoDock 4.2 software package using Auto Dock Tools 1.5.4 (ADT).[4] Polar hydrogen atoms and Kollman charges were added to the input files. The Lamarckian Genetic Algorithm (LGA) with a local search was used as a search method.[5] Docking analysis was performed by using the cluster of a root-mean-square deviation (rmsd) of 2.0 Å. During docking, the host (CB7) was kept rigid and the guest (milrinone) was flexible. Here it is pertinent to mention that we have used cationic form of the drug during docking, as inside the nano-cavity of CB7 the cationic form generates. The docking results were analyzed and visualized by using ADT. After 100 runs, the best conformer was ranked according to the docking energy and geometry matching. The final docked structure was fully optimized on the gaussian09 software package using PM3MM method.

Table S1a. Fluorescence decay transients of milrinone in presence of CB7 (0 to 500 μM) collected at 390 nm (λ_{ex} = 375 nm).

Name	τ_1	τ_2	τ_3	a_1	a_2	a_3	$\chi 2$	τ_{avg} [#]
MIR in water	0.097	2.61	-	0.99	0.01	-	0.99	0.131
16 μM of CB7	0.07	3.14	0.455	0.95	0.01	0.04	1.06	0.119
50 μM of CB7	0.052	3.43	0.373	0.87	0.02	0.11	1.18	0.158
100 μM of CB7	0.035	4.34	0.684	0.92	0.02	0.06	1.13	0.152
200 μM of CB7	0.027	5.11	1.139	0.94	0.02	0.04	1.16	0.156
300 μM of CB7	0.032	5.37	1.255	0.92	0.02	0.06	1.13	0.233
500 μM of CB7	0.031	5.43	1.333	0.9	0.03	0.07	1.17	0.289

[#] $\tau_{avg} = a_1\tau_1 + a_2\tau_2$

Table S1b. Fluorescence decay transients of milrinone in presence of CB7 (50 to 500 μM) collected at 500 nm (λ_{ex} = 375 nm).

Name	τ_1	τ_2	τ_3	a_1	a_2	a_3	$\chi 2$	τ_{avg} [#]
50 μM of CB7	0.061	6.997	1.685	0.5	0.05	0.45	1.04	1.104
100 μM of CB7	0.099	7.129	1.691	0.36	0.05	0.59	1.1	1.428
500 μM of CB7	0.108	7.634	1.8	0.4	0.09	0.51	1.1	1.675

[#] $\tau_{avg} = a_1\tau_1 + a_2\tau_2$

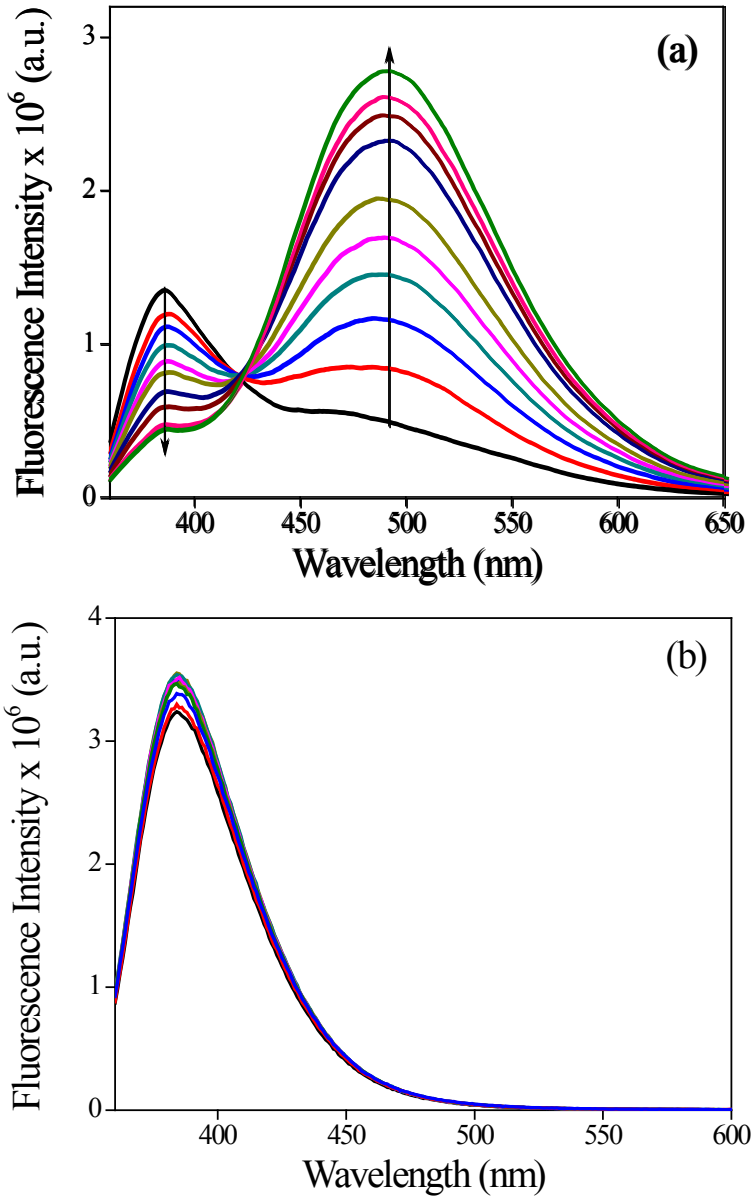

Figure S1. Emission spectra of milrinone at pH 4 (a) and pH 10 (b) in presence of CB7 (0 to 500 uM), λ_{ex} = 340 nm.

Note S1. The binding constant is calculated by using linear Benesi-Hildebrand (BH) equation,[6]

$$\frac{1}{(I-I_0)} = \frac{1}{(I_1-I_0)} + \frac{1}{(I_1-I_0)K[CB7]}$$

where I_0, I and I_1 denote the fluorescence intensity of milrinone in pure water, in presence of CB7 and in the complex, respectively, and K is the binding constant for complexation.

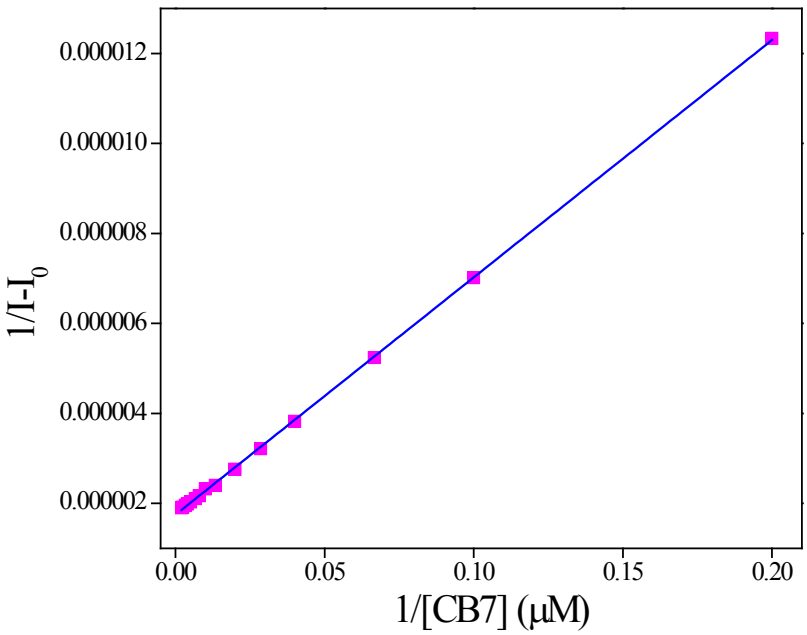

Figure S2. BH plot analysis for the complexation of milrinone with CB7 in water.

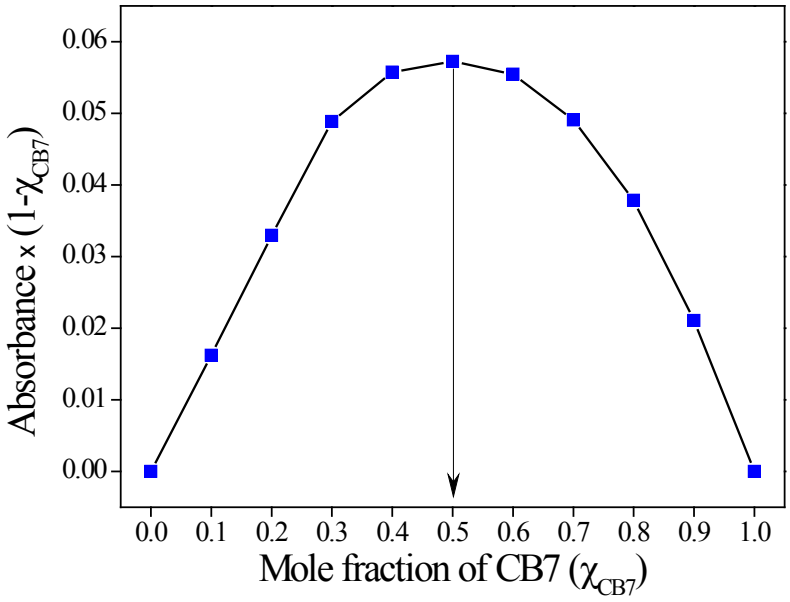

Figure S3. Job's method of continuous variation.

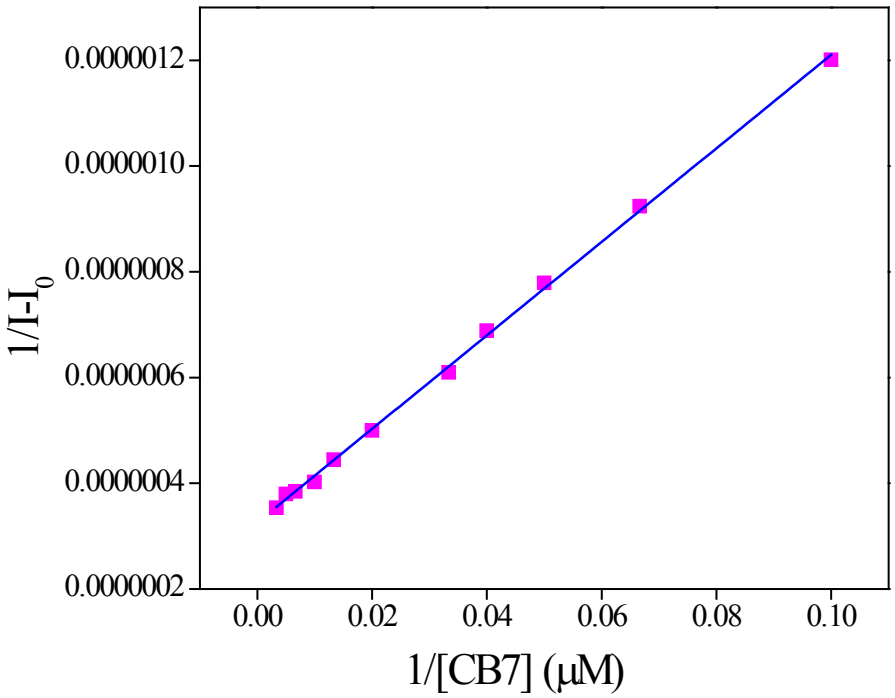

Figure S4. BH plot analysis for the complexation of milrinone with CB7 at pH 4.

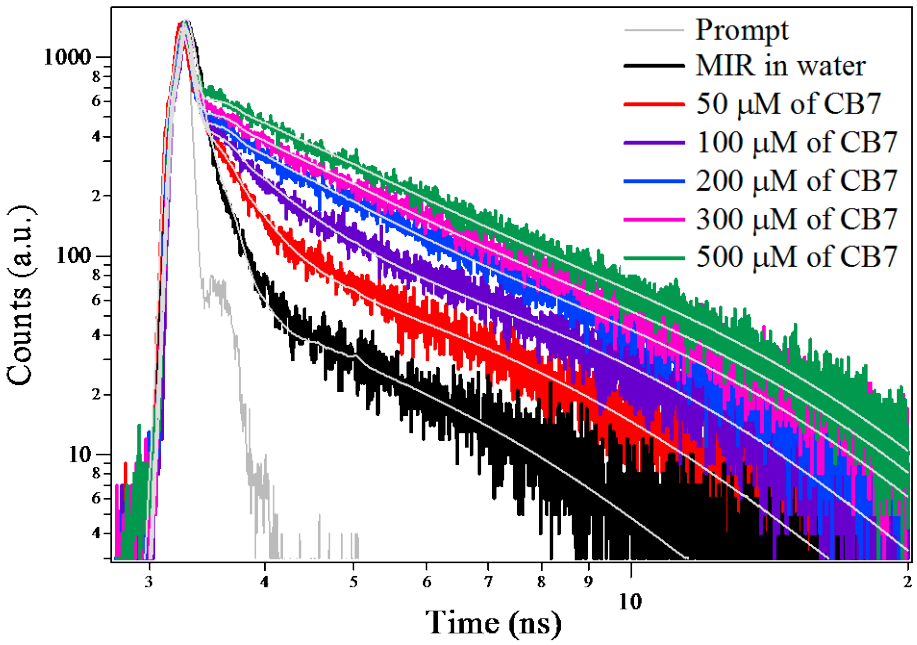

Figure S5. Fluorescence decay transients of milrinone in water and in presence of CB7 (collected at 390 nm and excited at 375 nm).

Note S2. According to the Stokes-Einstein relationship[7] hydrodynamic radius of the inclusion complex can be calculated from rotational relaxation time (τ_r) using the equation given below.

$\tau_r = 1/(6D_r)$ where $D_r = RT/6V\eta$

Here, D_r rotational diffusion coefficient, V is the hydrodynamic molecular volume of the complex, η is the viscosity of the medium (water), and T is the absolute temperature (298 K). For the 1:1 inclusion complex between MIR and CB7, a rough estimation of the hydrodynamic radius can be obtained by considering the complex as an effective sphere.

References

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