Supporting Information for

Effect of Biological Confinement on the Photophysics and Dynamics of a Proton-Transfer Phototautomer: An Exploration of Excitation and Emission Wavelength-Dependent Photophysics of the Protein-Bound Drug

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Section S1: Absorption and Emission Spectra of 5ClSA in Various Environments.

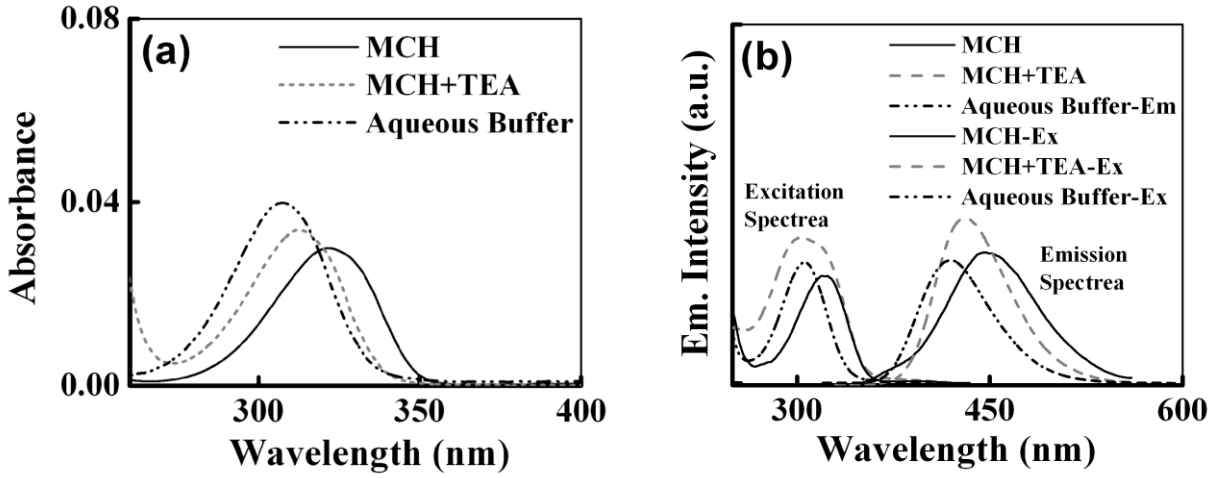
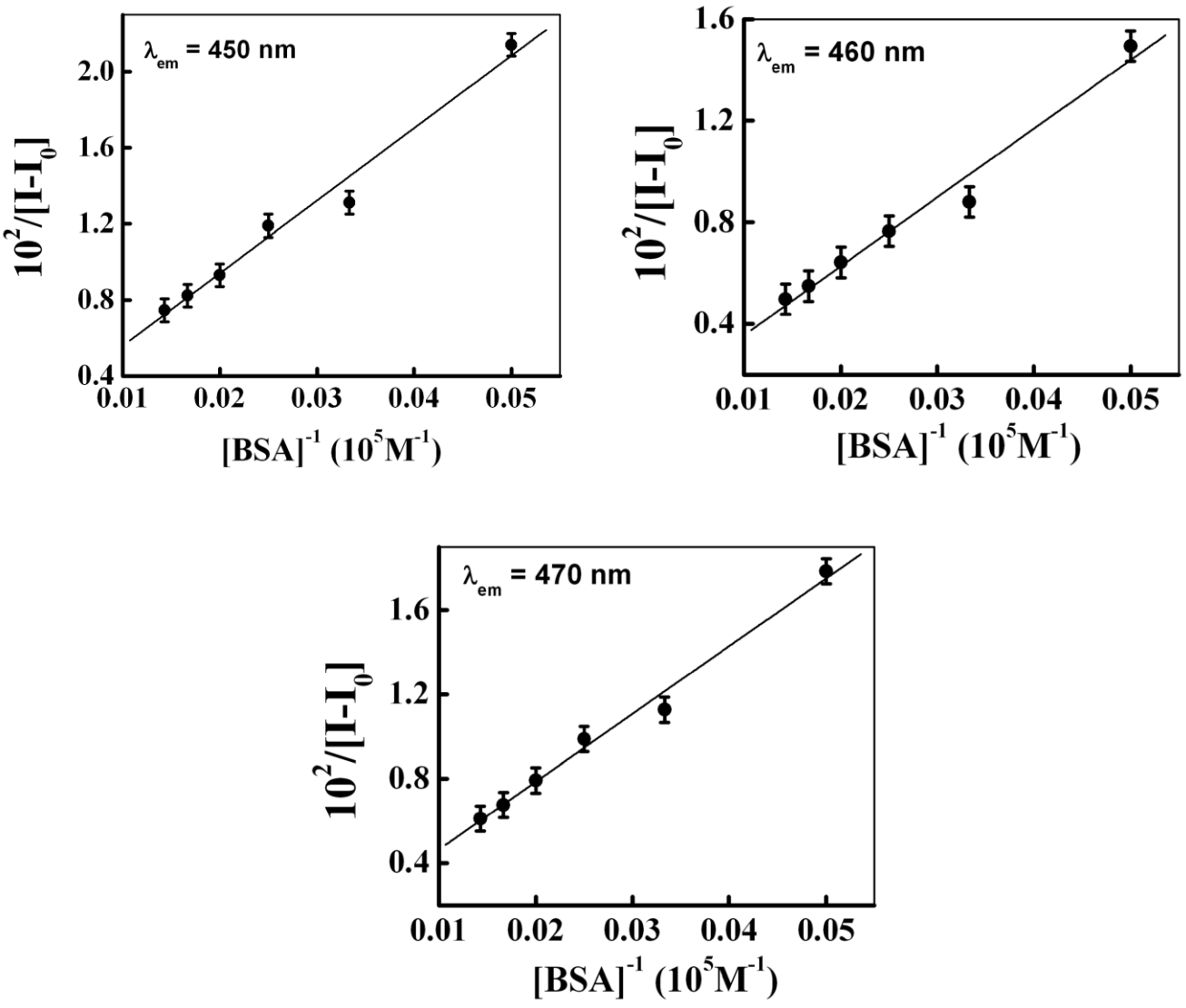


Figure S1: (a) Representative absorption spectral profile of 5ClSA in MCH solvent (solid, black), in MCH solvent in the presence of base TEA (dot, grey) and in aqueous buffer medium (dashed dot dot, black). (b) Representative emission and excitation spectral profile of 5ClSA in MCH solvent (solid, black), in MCH solvent in the presence of base TEA (dash, grey) and in aqueous buffer medium (dash dot dot, black).



Section S2: Benesi-Hildebrand plots for the drug–protein binding interaction from emission intensity data taken at various wavelengths as specified in the figure legends yield binding constant, *K* (± 10%) = 4.71 × 10^2 M^{-1} for λ_{em} = 450 nm, 3.16× 10^2 M^{-1} for λ_{em} = 460 nm and 4.46 × 10^2 M^{-1} for λ_{em} = 470 nm.

Section S3. Excitation-Emission Matrix Spectroscopy.

Excitation-Emission Matrix Spectroscopy (EEMS), or 3D fluorescence spectroscopy, is recently finding its application in a variety of fields.[1-4] The technique can be employed as a simple device to yield information regarding the conformational change of protein in the course of the probe-protein binding interaction through a comprehensive display of the fluorescence characteristics of the sample under study both as a function of excitation and emission wavelengths simultaneously. Figure S1 displays the 3D fluorescence spectra of (a) BSA alone, and (b) BSA:5ClSA composite system with [5ClSA] = 12.0 μM. The modulation in the 3D fluorescence spectra with increasing 5ClSA concentration probably indicates the presence of binding interaction between the protein and the probe. The 3D fluorescence spectra of BSA (Figure S1a) exhibits two detectable peaks denoted as peak 1 and peak 2 in the figure (Peak A is due to Rayleigh scattering and peak B is due to the second harmonic scattering). Peak 1 (λ_{ex} ~ 280 nm, λ_{em} ~ 350 nm) is attributed to both the Trp and Tyr residues of BSA since at λ_{ex} ~ 280 nm the fluorescence spectra of protein is known to be characterized by intrinsic fluorescence of both Trp and Tyr, the fluorescence of phenylalanine (Phe) residue being negligible.[1-4] Peak 2 (λ_{ex} ~ 240 nm, λ_{em} ~ 340 nm) is essentially characterized by the fluorescence properties of the polypeptide backbone structures of BSA.[1-4] A direct comparison of Figures S1a and b distinctly reveals the modulation in 3D fluorescence characteristics of BSA as a result of interaction with the probe in terms of emission intensity reduction of peak 1 and peak 2, however, to differential extents. The intensity ratio of peak 1 of BSA alone and BSA:5ClSA composite system changes as 1:0.62 in Figure S1b ([5ClSA] = 12.0 μM), while the same for peak 2 changes as 1:0.6 in Figure S1b ([5ClSA] = 12.0

μM). Since the two peaks in BSA (Figure S1a) originate from different types of residues, the observed difference in the extent of their perturbation upon interaction with a given concentration of 5ClSA (all other experimental conditions and instrumental settings conserved) suggests for specific sort of interaction between BSA and 5ClSA resulting in some micro-environmental and conformational changes in BSA. Thus the present set of observations corroborate to previous findings.(The red-shifted band at λ_{em} ~ 440 nm in Figure S1b emanates from direct excitation of the drug molecule as is also illustrated in the inset figure which shows the development of the red-shifted drug fluorescence following excitation at 295 nm).

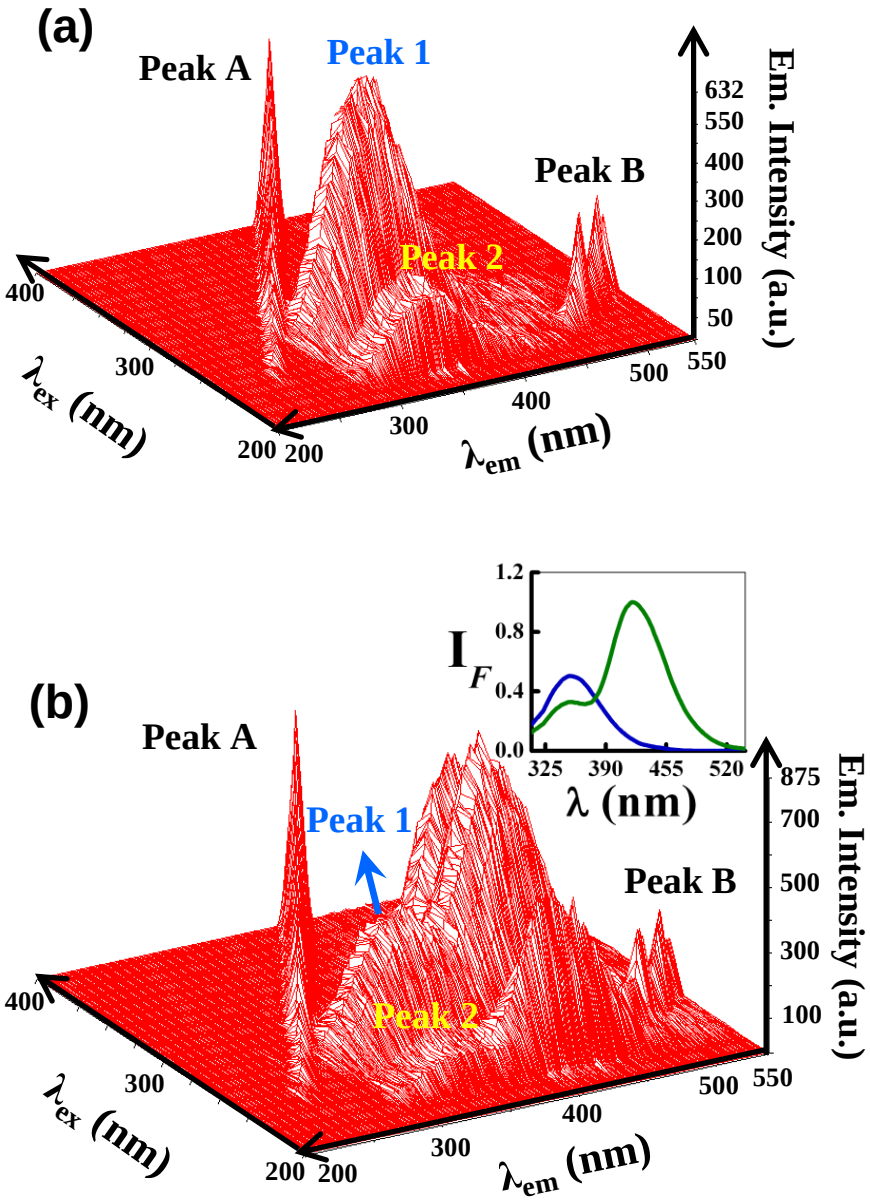


Figure S2: Excitation Emission Matrix Spectra (EEMS) of (a) BSA alone and (b) 5ClSA:BSA composite system ([5ClSA] = 12.0 μM). The inset in panel (b) shows the normal emission spectra of BSA alone (blue) and in the presence of 12.0 μM 5ClSA (green) (λ_{ex} = 295 nm).

Section S4: Constant Wavelength Synchronous Fluorescence Spectroscopy (CWSFS).

The results of synchronous fluorescence spectra for BSA in the presence of varying drug concentrations are shown in Figure S3. Synchronous fluorescence spectroscopy (SFS) is a multidimensional fluorescence technique, which has become an increasingly active subject of research ever since its introduction by Lloyd in 1971.[8] The constant wavelength SFS technique involves simultaneous scanning of the excitation and the emission monochromators while maintaining a constant wavelength interval between them. According to the theory conjured up by Miller,[9] when the wavelength interval ($\Delta\lambda$) is fixed at $\Delta\lambda$ = 60 nm and $\Delta\lambda$ = 15 nm, the SFS yields characteristic information of the Trp and Tyr residue of the protein, respectively.[1,8-13] Figure S3 shows representative synchronous fluorescence spectral profiles of the protein with increasing drug concentration for $\Delta\lambda$ = 15 nm (Figure S3a) and $\Delta\lambda$ = 60 nm (Figure S3b).

It is pertinent in this context to explore the relative variation (I/I_0) of synchronous fluorescence intensity of BSA with varying drug concentration for both $\Delta\lambda$ = 60 nm and $\Delta\lambda$ = 15 nm (Figure S3c). It is seen in the figure that for both the cases, CWSFS ($\Delta\lambda$ = 60 nm and $\Delta\lambda$ = 15 nm) exhibits notable reduction of synchronous fluorescence intensity of BSA with increasing drug concentration, however, the slope being higher for $\Delta\lambda$ = 60 nm compared to that of $\Delta\lambda$ = 15 nm. This implies that the drug is probably located closer to Trp residue compared to Tyr residue.[8-13] This inference is well supported from blind docking simulation result which shows a Trp residue (Trp-212 in hydrophobic subdomain IIA) in near vicinity of the drug within the binding site in the protein.

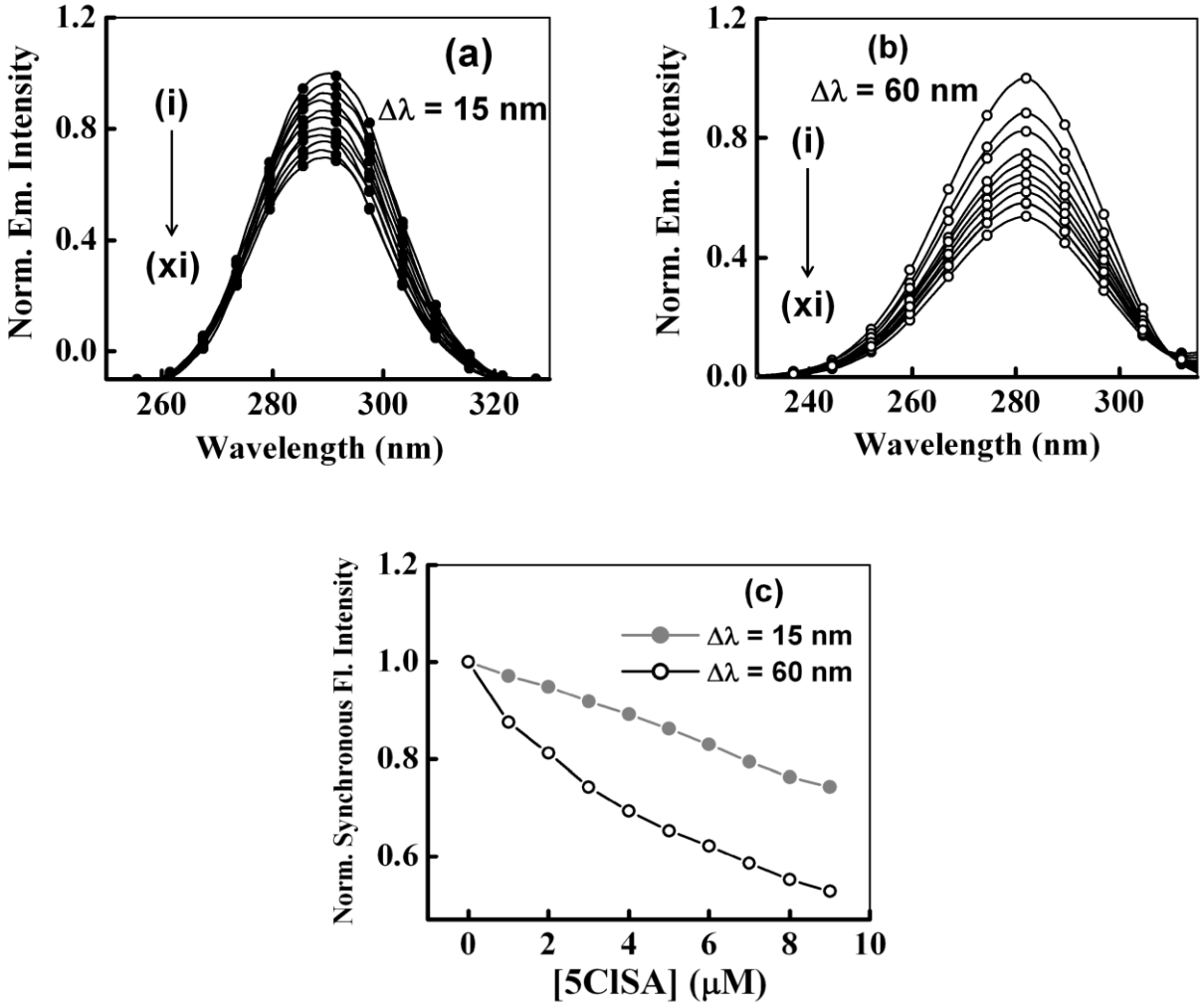


Figure S3: Representative synchronous fluorescence profile of the protein (BSA) in the presence of increasing drug concentration for (a) $\Delta\lambda$ = 15 nm and (b) $\Delta\lambda$ = 60 nm. Variation of normalized (I_0/I) synchronous fluorescence intensity of BSA as a function of increasing drug concentration for $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm as indicated in the figure legend. Here I_0 denotes the fluorescence intensity of the protein in the absence of the drug and I terms denote the same with subsequently increasing drug concentration. [BSA] = 4.0 μM, T = 300 K and pH = 7.40.

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