Supporting Information for

Direct Insight into the Nonclassical Hydrophobic Effect in Bile Salt:β-Cyclodextrin Interaction: Role of Hydrophobicity in Governing the Prototropism of a Biological Photosensitizer

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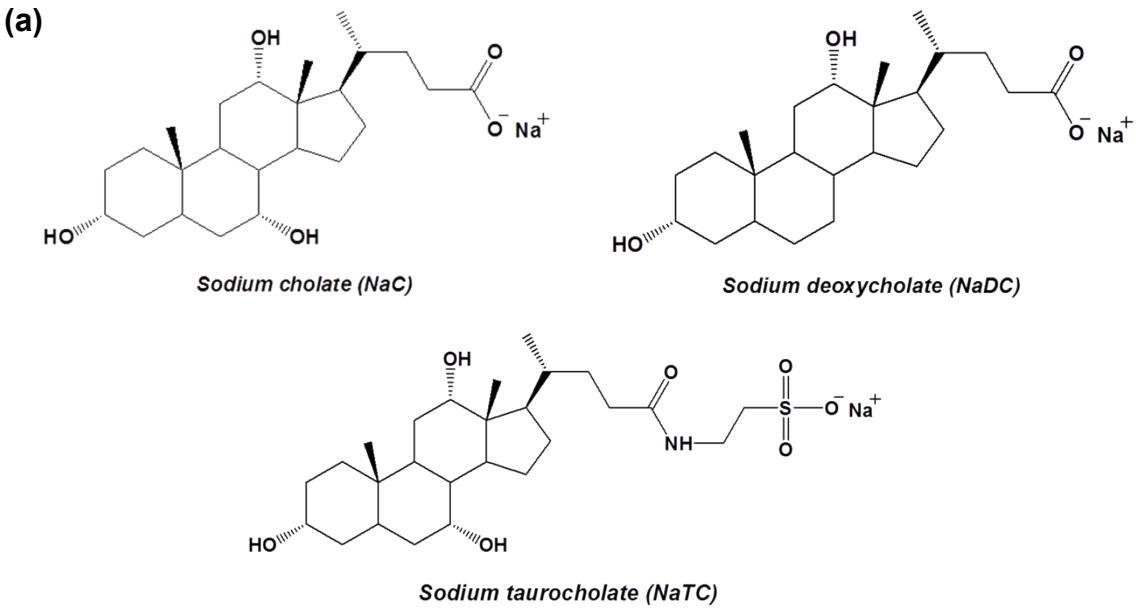
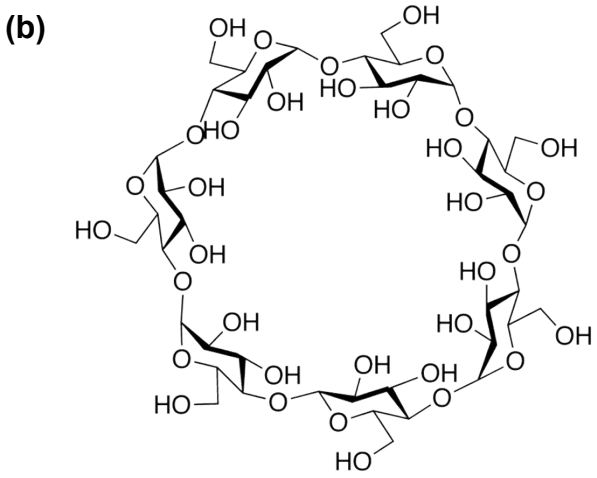
Scheme S1: Schematic Structures of (a) Bile Salts under Investigation and (b) β-Cyclodextrin





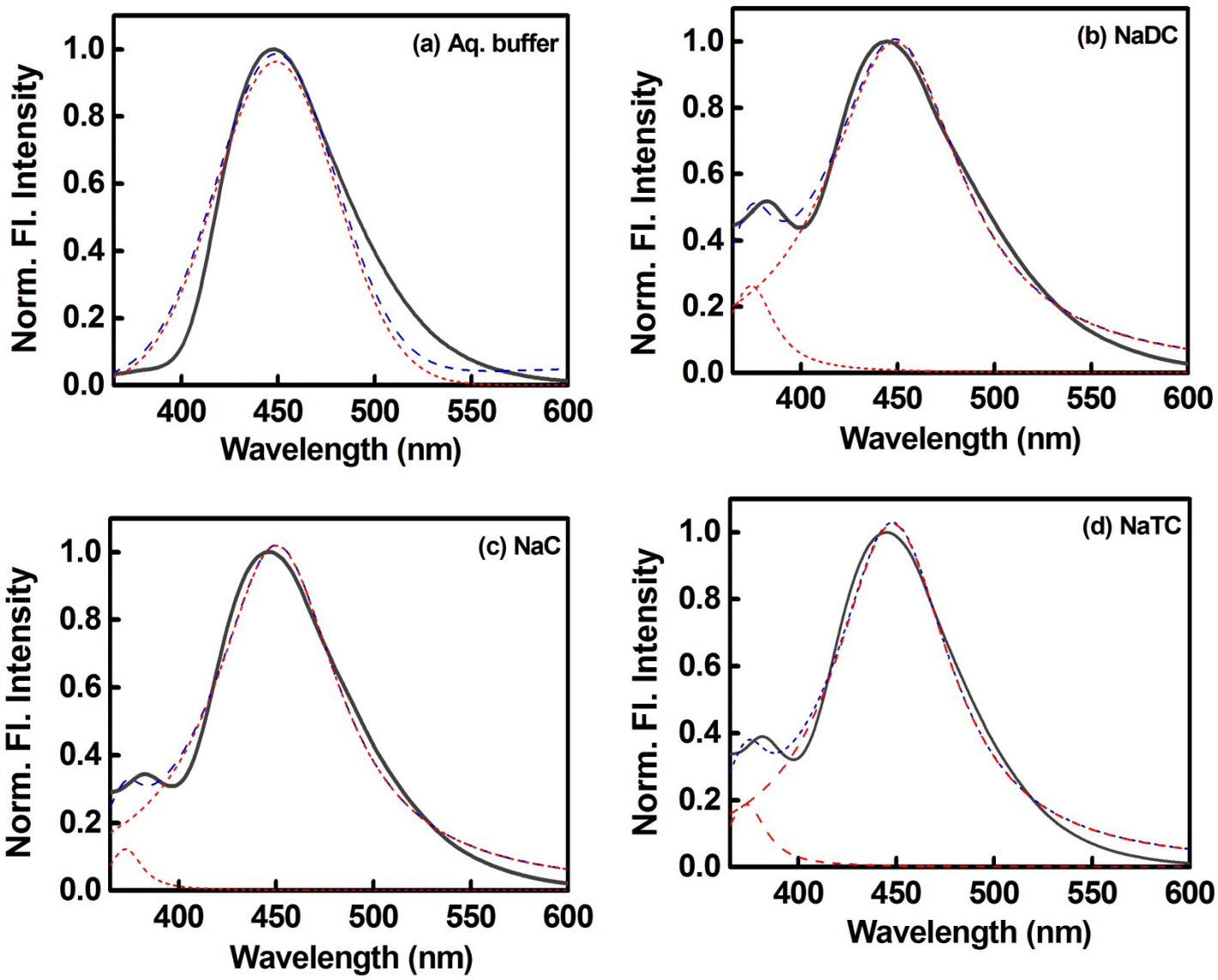


Figure S1: Deconvoluted emission spectral profiles of NHM in various environments as specified in the figure legends (namely, (a) in aqueous buffer, (b) in NaDC, (c) in NaC, and (d) NaTC). The bold gray lines designate the experimental profiles; the red dotted lines designate the resolved bands into individual Gaussian components and the blue dashed lines are the simulated spectra based on the resolved Gaussian bands.

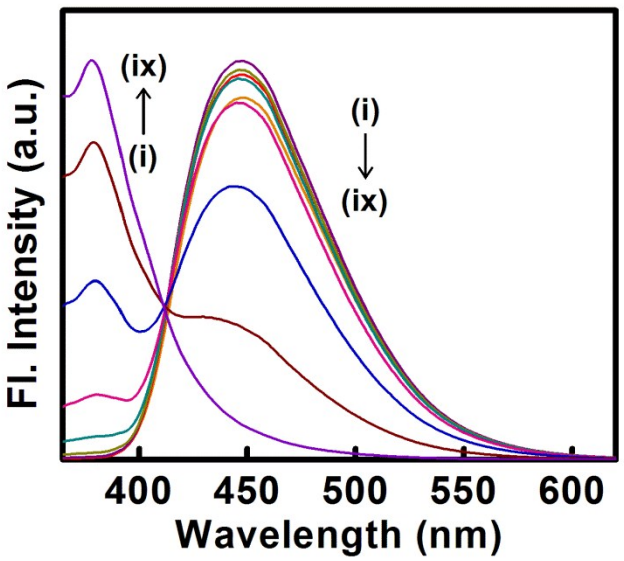


Figure S2: Modulation in emission spectral profiles of NHM (*ca.* 2.0 μM, λ_{ex} = 340 nm) as a function of varying polarity of the medium. Curves (i) → (ix) correspond to vol. % water/1,4-dioxane = 0/100, 10/90, 20/80, 30/70, 40/60, 50/50, 60/40, 70/30, 90/10. λ_{ex} = 340 nm.

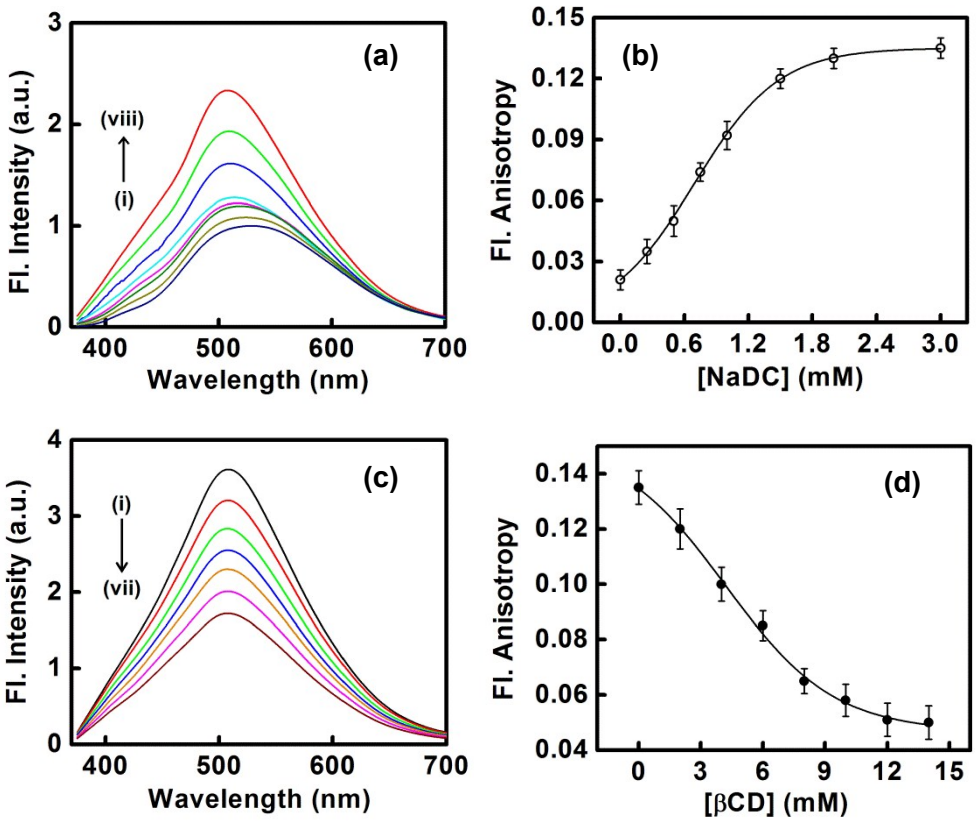


Figure S3: (a) Examples of fluorescence spectra of tetracycline (TC) with increasing concentration of NaDC (i → viii: 0.0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0 mM NaDC). Enhancement of fluorescence intensity indicates the binding interaction of the drug (TC) with the bile salt aggregate. (b) Variation of fluorescence anisotropy of TC with added NaDC. (c) Examples of fluorescence spectra of NaDC-bound TC upon treatment of βCD ((i → vii: 0.0, 2.0, 4.0, 6.0, 10.0, 12.0, 14.0 mM βCD). The release of the drug from the bile salt encapsulated state is indicated by the reduction of fluorescence intensity in the presence of βCD. (d) Variation of fluorescence anisotropy of NaDC-bound TC with added βCD.

The interaction of tetracycline (TC) with NaDC results in discernible fluorescence intensity enhancement of the drug coupled with a significant blue-shift (~ 22 nm) with increasing concentration of the bile salt (Figure S3a). This can be adequately rationalized on the basis of entrapment of the drug molecules within the bile salt aggregates. The blue-shift of the emission wavelength signifies a decrease of hydrophilicity of the microenvironment in the immediate vicinity of the drug molecules within the NaDC aggregates. The entrapment of the drug within the bile salt aggregate is further indicated by the increase in fluorescence anisotropy with added NaDC (Figure S3b).

Incremental addition of βCD to NaDC-bound drug is found to result in decrease of fluorescence intensity of TC (Figure S3c) as can be rationalized on the basis of release of the NaDC-bound drug following bile salt:βCD inclusion complex formation. The decrease in fluorescence anisotropy of NaDC-bound drug with added βCD (Figure S3d) further corroborates this.

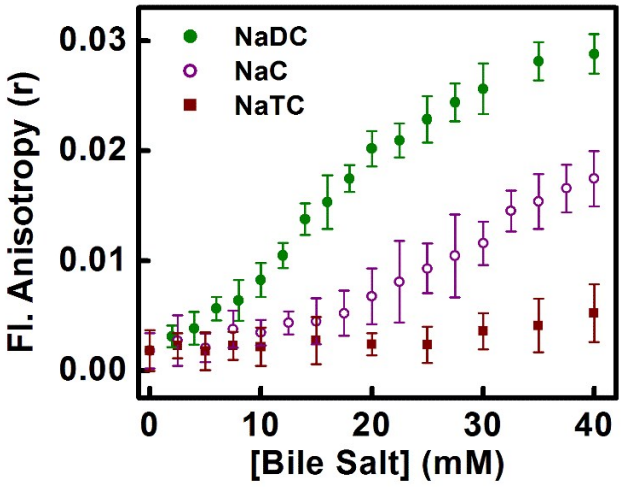


Figure S4: Variation of steady-state fluorescence anisotropy of NHM with increasing concentrations of NaDC (–●–), NaC (–○–), and NaTC (–■–). Each data point is an average of 15 individual measurements. The error bars are within the marker symbols if not apparent.

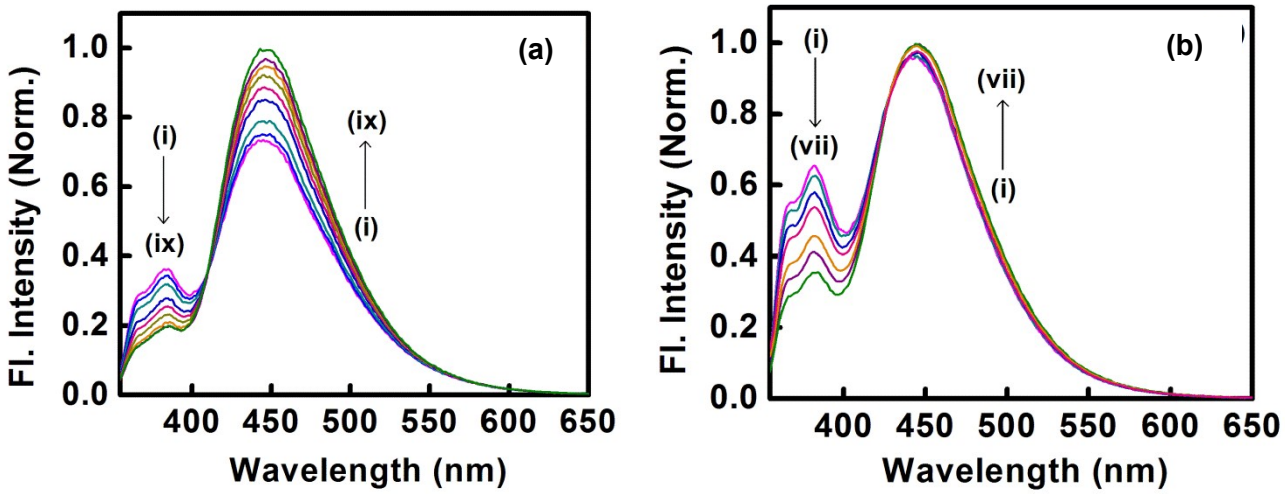


Figure S5: Emission spectra of (a) NaC-bound NHM and (b) NaTC-bound NHM (*ca.* 2.0 μM, λ_{ex} = 340 nm) in the presence of varying concentrations of βCD. Curves (i) → (ix) correspond to [βCD] = 0, 1, 3, 5, 6.5, 8, 10, 12, 17 mM in (a), Curves (i) → (vii) correspond to [βCD] = 0, 3, 5, 10, 14, 17, 20 mM in (b).

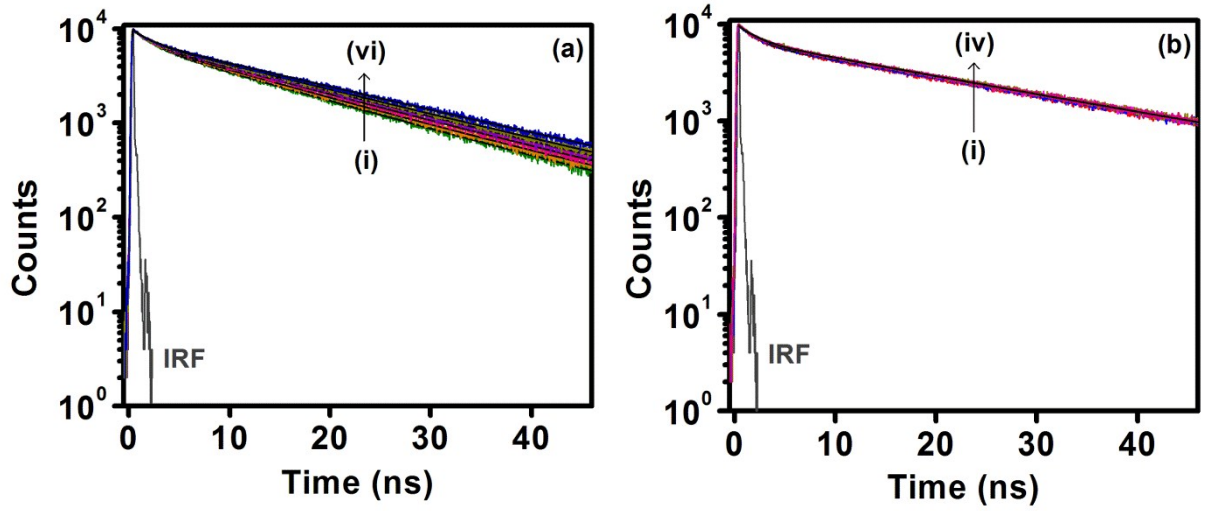


Figure S6: Time-resolved fluorescence decay transients of (a) NaC-bound NHM and (b) NaTC-bound NHM in the presence of varying concentrations of βCD. Curves (i) → (vi) correspond to [βCD] = 0, 4, 5, 8, 10, 17 mM in (a), Curves (i) → (vii) correspond to [βCD] = 0, 5, 8, 16 mM in (b).

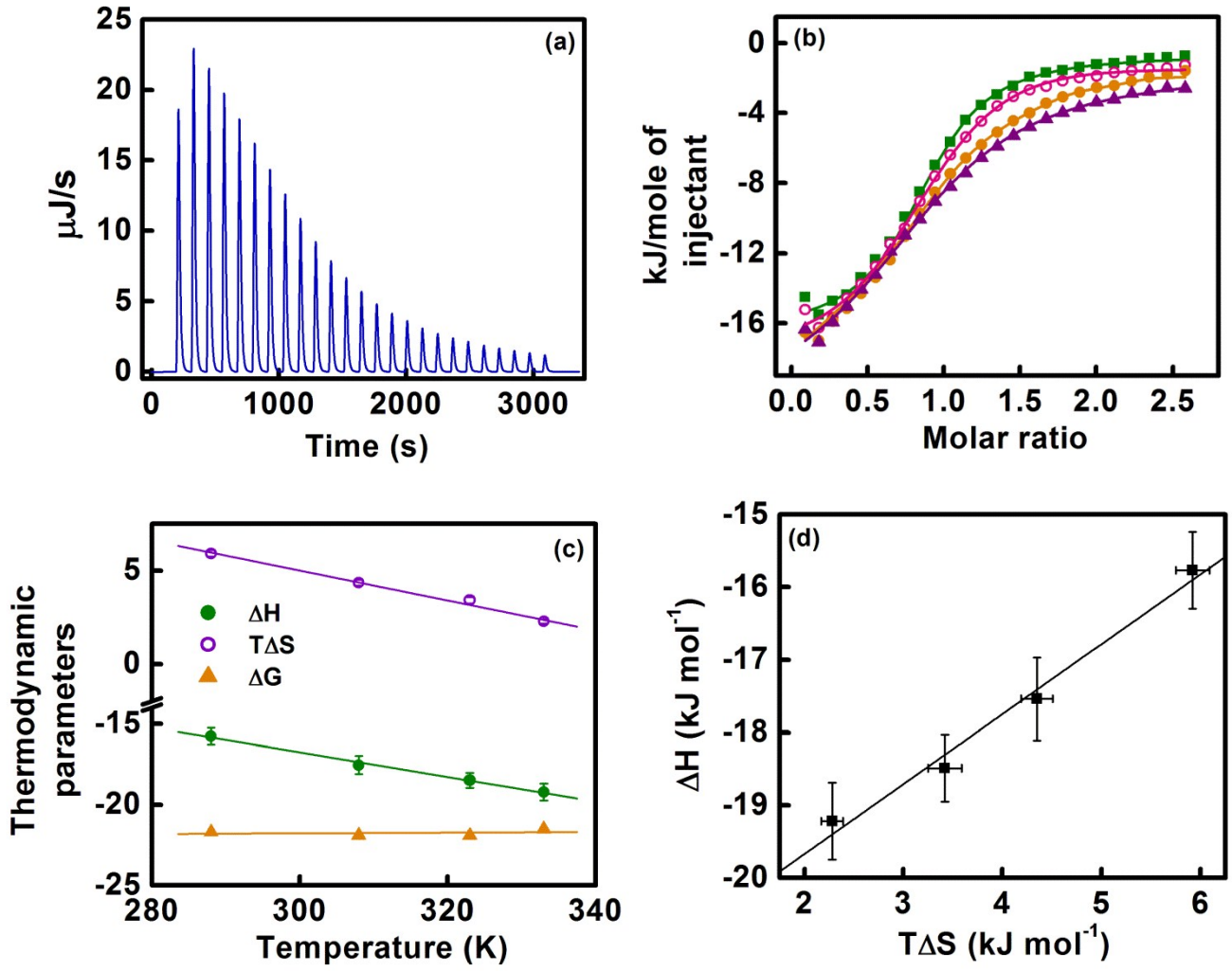


Figure S7: Representative ITC profile for the titration of NaC with βCD. (a) Integrated heat burst curve after correction of heat of dilution at 288 K. (b) The ITC enthalpograms obtained at various temperatures (288 K: –■–, 308 K: –○–, 323 K: –●–, 333 K: –▲–). (c) Plot of variation of relevant thermodynamic parameters (ΔH in kJ mol^{-1}: –●–, TΔS in kJ mol^{-1}: –○–, ΔG in kJ mol^{-1}: –▲–) for NaC:βCD interaction. (d) Variation of ΔH with TΔS for NaC:βCD interaction (the goodness of fit parameter for the linear regression to the experimental data points = 0.99). The error bars are within the symbol if not apparently visible.

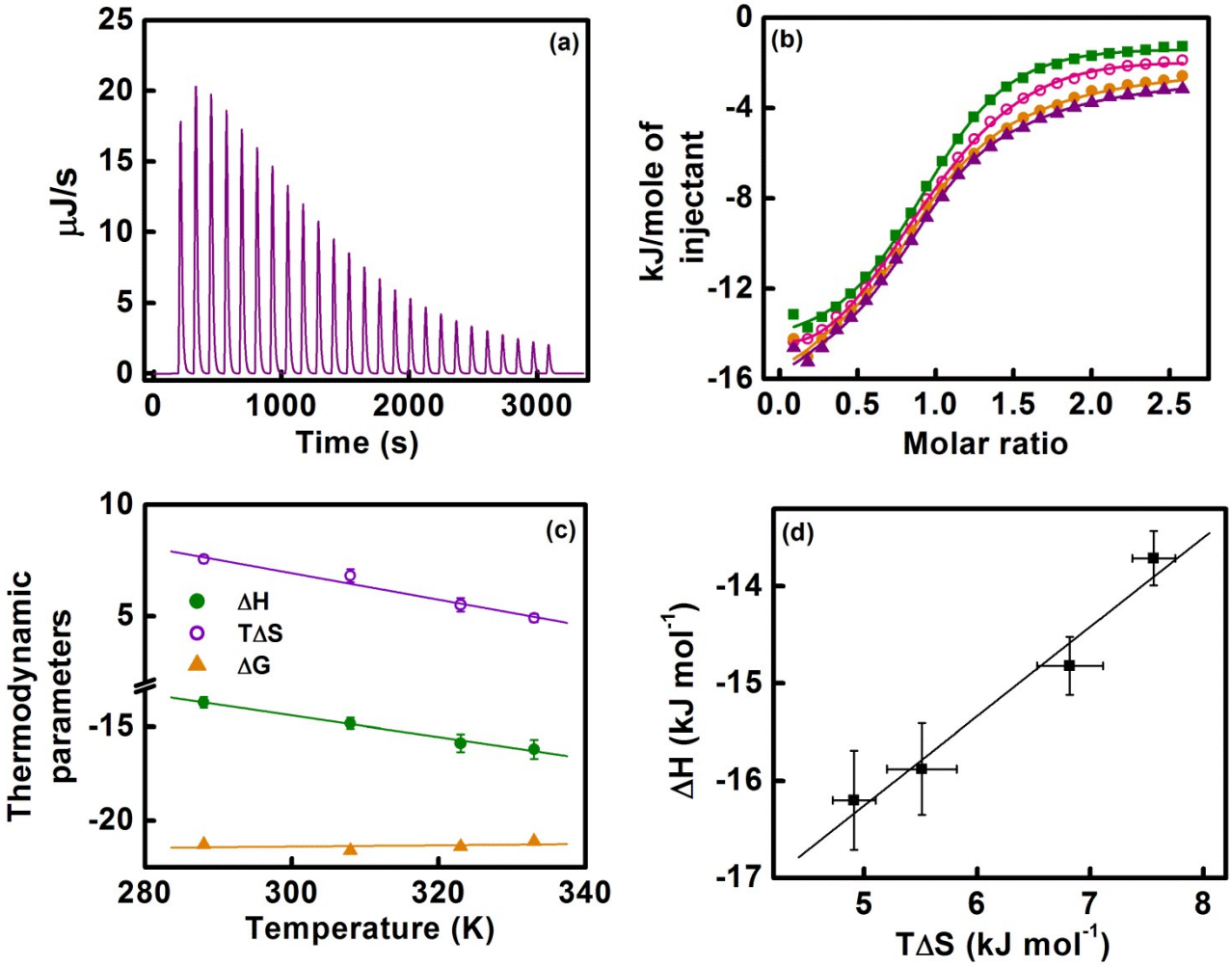


Figure S8: Representative ITC profile for the titration of NaTC with βCD. (a) Integrated heat burst curve after correction of heat of dilution at 288 K. (b) The ITC enthalpograms obtained at various temperatures (288 K: –■–, 308 K: –○–, 323 K: –●–, 333 K: –▲–). (c) Plot of variation of relevant thermodynamic parameters (ΔH in kJ mol^{-1}: –●–, TΔS in kJ mol^{-1}: –○–, ΔG in kJ mol^{-1}: –▲–) for NaTC:βCD interaction. (d) Variation of ΔH with TΔS for NaTC:βCD interaction (the goodness of fit parameter for the linear regression to the experimental data points = 0.98). The error bars are within the symbol if not apparently visible.

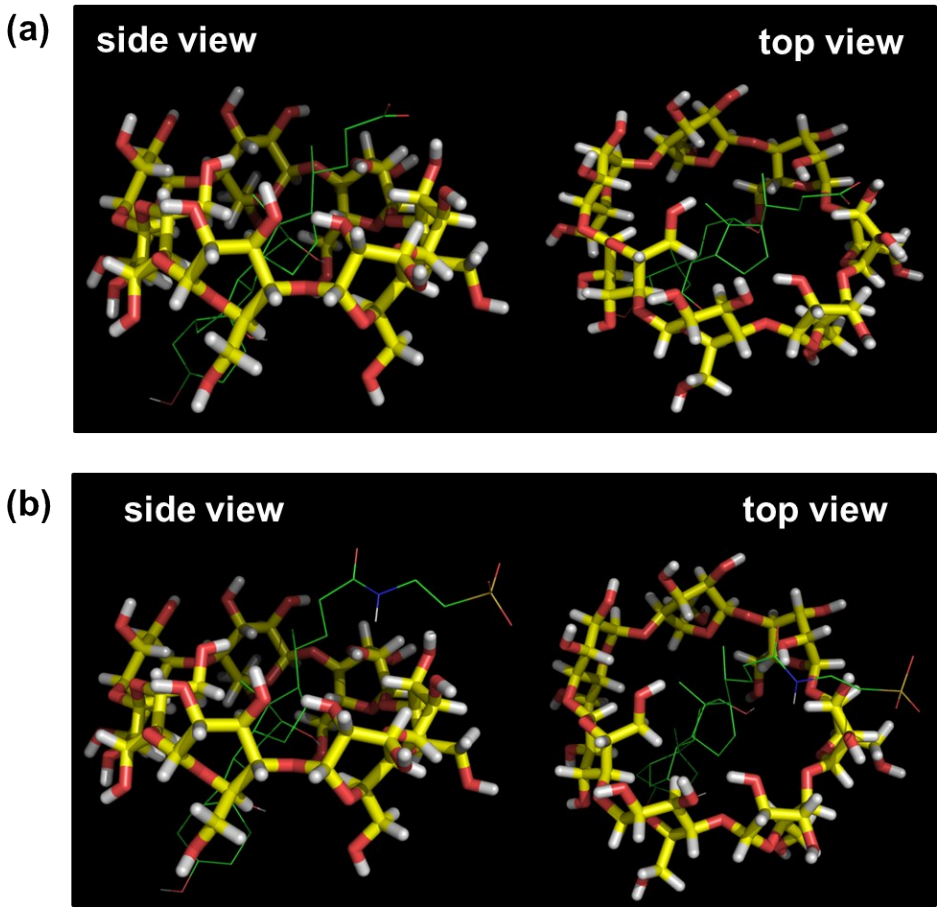


Figure S9: Stereo view of minimum energy docked conformation of (a) NaC and (b) NaTC with βCD.

Table S1: Time-Resolved Fluorescence Decay Parameters of NHM with Added Bile Salts

Environment	τ_1[a] (ns)	τ_2[a] (ns)	α_1	α_2	χ^2
Aq. buffer	21.59	-	1.00	-	1.01
[NaDC] (mM)					
0.25	20.85	2.14	0.98	0.02	1.01
5	20.21	2.12	0.91	0.09	1.05
7.5	18.76	2.06	0.85	0.15	1.05
10	18.12	2.06	0.72	0.28	1.08
12.5	14.43	2.03	0.62	0.38	1.05
15	13.9	2.02	0.56	0.44	1.07
17.5	12.83	1.98	0.54	0.46	1.06
20	11.42	1.98	0.49	0.51	1.07
[NaC] (mM)					
5	19.78	2.17	0.93	0.07	0.99
10	18.28	2.02	0.87	0.13	1.07
15	16.98	1.96	0.81	0.19	1.06
20	15.67	2.07	0.75	0.25	0.99
25	14.20	1.98	0.72	0.28	0.99
[NaTC] (mM)					
5	21.29	2.32	0.95	0.05	0.99
10	21.18	2.35	0.90	0.10	1.07
15	21.14	2.27	0.88	0.12	1.06
20	21.02	2.18	0.87	0.13	1.06
25	20.51	2.22	0.84	0.16	1.01
30	20.43	2.25	0.80	0.20	1.02
40	20.12	1.90	0.79	0.21	0.99

[a]±4%.

Table S2: Time-Resolved Fluorescence Decay Parameters of Bile Salt-Bound NHM with Added βCD

Environment	τ_1[a] (ns)	τ_2[a] (ns)	α_1	α_2	χ^2
[βCD] (mM)					
20 mM NaDC	11.42	1.98	0.49	0.51	1.07
+ 0.5 mM βCD	12.60	2.24	0.56	0.44	1.01
+ 3 mM βCD	13.06	2.12	0.57	0.43	1.05
+ 5 mM βCD	13.79	1.92	0.60	0.40	1.04
+ 8 mM βCD	14.87	1.84	0.64	0.36	1.05
+ 10 mM βCD	16.03	1.67	0.66	0.34	1.08
+ 12 mM βCD	16.90	1.36	0.70	0.30	1.07
+ 14 mM βCD	17.80	1.27	0.75	0.25	1.03
+ 20 mM βCD	18.61	1.08	0.80	0.20	1.02
25 mM NaC	14.20	1.98	0.72	0.28	0.99
+ 4 mM βCD	14.63	1.92	0.75	0.25	1.04
+ 5 mM βCD	15.24	1.75	0.79	0.21	1.05
+ 8 mM βCD	15.82	1.79	0.81	0.19	1.03
+ 10 mM βCD	16.40	1.72	0.82	0.18	1.07
+ 14 mM βCD	16.98	1.65	0.84	0.16	1.02
+ 17 mM βCD	17.25	1.67	0.85	0.15	1.09
40 mM NaTC	20.12	1.9	0.79	0.21	0.99
+ 3 mM βCD	20.07	1.81	0.80	0.20	1.05
+ 5 mM βCD	20.01	1.85	0.81	0.19	1.08
+ 8 mM βCD	19.92	1.85	0.83	0.17	1.01
+ 16 mM βCD	19.82	1.90	0.84	0.16	1.04
+ 20 mM βCD	19.77	1.80	0.85	0.15	1.02

[a]±4%.

Experimental

Materials

The drug NHM, the bile salts, namely, NaDC, NaC, and NaTC, β-cyclodextrin (βCD) (Scheme S1) and potassium iodide (KI) were used as procured from Sigma-Aldrich Chemical Co., USA. Phosphate buffer was obtained from Sigma-Aldrich Chemical Co., USA and 10 mM phosphate buffer of pH 7.40 was prepared from it in deionized triply distilled Milli pore water.

Instrumentation and Methods

Steady-State Spectral Measurements. The absorption and fluorescence spectra were acquired on a Cary 100 UV-vis spectrophotometer, and Fluorolog 3-111 fluorometer, respectively. All spectroscopic measurements are appropriately background corrected and carried out with freshly prepared solutions maintaining a low concentration (~ 2.0 μM) of the drug (NHM) in order to minimize the possibilities of reabsorption and/or inner filter effects.

Steady-State Fluorescence Anisotropy Measurement. Steady-state fluorescence anisotropy (*r*) measurements were carried out on the same Fluorolog 3-111 fluorometer. The fluorescence anisotropy (*r*) is defined as:[S1]

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad \text{(S1)}$$

$$G = \frac{I_{HV}}{I_{HH}} \quad \text{(S2)}$$

Here, $I_{VV(H)}$ is the emission intensity collected with vertically (horizontally) oriented emission polarizer following photoexcitation by vertically polarized light. *G* is the correction factor for the detector sensitivity of the instrument.[S1]

Time-Resolved Fluorescence Decay. Fluorescence lifetimes were obtained by the method of Time Correlated Single-Photon Counting (TCSPC). The samples were excited at λ_{ex} =

375 nm using picosecond laser diode (IBH-NanoLED-375L) as the light source and the signals were collected using a Hamamatsu MCP Photomultiplier (Model R-3809U-50) at magic angle polarization of 54.7^{o} to minimize the contribution from fluorescence depolarization.[S1] The decays were analyzed to extract the fluorescence lifetime parameters on DAS-6 decay analysis software. The goodness of the fits was evaluated by the χ^2 criteria (negating fitting analysis returning χ^2 beyond the range $1.10 \leq \chi^2 \leq 0.99$) and distribution of the residuals of the fitted functions to the raw data. The multiexponential fluorescence decay (I(t)) is described as:[S1]

$$I(t) = \sum_i \alpha_i exp\left(-t/\tau_i\right) \quad \text{(S3)}$$

in which α_i represents the pre-exponential factor (amplitude) corresponding to the i^{th} decay time constant, τ_i.[S1]

Time-Resolved Fluorescence Anisotropy Decay. For time-resolved fluorescence anisotropy decay measurements, the polarized fluorescence decays collected at parallel [$^{I_{\parallel}(t)}$] and perpendicular [$^{I_{\perp}(t)}$] emission polarizations following photoexcitation by vertically polarized light were first collected at the emission maxima of the samples under investigation. The anisotropy decay function, $r(t)$ was then constructed according to the following expression:[S1]

$$r(t) = \frac{I_{\parallel}(t) - GI_{\perp}(t)}{I_{\parallel}(t) + 2GI_{\perp}(t)} \quad \text{(S4)}$$

Here the grating factor (G) was determined from long time tail matching technique.[S1]

Isothermal Titration Calorimetry (ITC). The thermodynamics of the interaction of the bile salts with βCD was investigated by isothermal titration calorimetry (ITC) carried out on a Nano ITC, TA Instrument. A total of 25 aliquots of βCD solution was injected (in 2 μL interval) from a rotating syringe (300 rpm) into the sample chamber containing the bile salt solution at an interval of 120 s between each injection. Both the βCD and bile salt solutions were properly

degassed prior to measurements in order to eliminate the possibility of bubble formation during titration. In a control experiment within identical experimental window the heat of dilution was evaluated for titration of βCD into aqueous phosphate buffer. The raw heat change data characterizing the given interaction were determined from appropriate correction of heat of dilution and then analyzed on NanoAnalyze software (version 2.4.1) provided with the instrument according to a model for one set of binding sites.[S2,S3]

Herein, it could be pertinent to state that according to the convention of our instrument (that is, Nano ITC, TA Instrument) an exothermic or endothermic process is reflected through upward or downward heat burst curve, respectively.[S3] It is to emphasize that the upward/downward trend of the raw ITC data is not universal, whereas the sign of ΔH as obtained from fitting of the experimental data must be regarded as the genuine thermodynamic signature and we have interpreted our data accordingly.

Molecular Docking Simulation. The three-dimensional structure of βCD was obtained from the Protein Data Bank having PDB code: 2Y4S.[S4] The docking simulation was carried out with the AutoDock 4.2[S5] suite of programs. The three-dimensional structure of the bile salts (NaC, NaDC and NaTC) was generated from their optimized geometries (DFT/B3LYP/6-31G(d,p)) obtained from calculation on Gaussian 03W[S6] software package. The docking parameters used were as follows: grid size along *X*-, *Y*-, and *Z*-axis: 80, 80 and 80 Å, respectively; grid-spacing: 0.375 Å; GA population size = 150; maximum number of energy evaluations = 250000; GA crossover mode = two points. The minimum binding energy conformer was searched out of 10 different conformations for each docking simulation. The visualization of the docked conformations was performed on PyMOL software package.[S7]

Deconvolution of Spectra. The deconvolution of the fluorescence spectral profile(s) into individual Gaussian components was performed using the Marquardt-Levenberg algorithm as implemented in MS Origin 7 following the method described elsewhere.[S8]

References:

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