Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information for

Contrasting Effects of pH on the Modulation of Structural Integrity of Hemoglobin Induced by Sodium Deoxycholate

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Table S1: Variation of Secondary Structure of Hb at Different pH with Increasing Concentrations of the NaDC

NaDC (mM)	α-Helix[#]	β-Sheet[#]	Random coil[#]
pH 9.0			
0	83.2	1.3	15.5
2.5	80.3	1.6	18.1
3.8	77.1	2.1	20.8
5.0	73.7	2.7	23.6
8.5	67.8	3.1	29.1
14	66.2	3.5	30.3
pH 7.4			
0	71.3	2.5	26.2
1.65	67.5	2.9	29.6
3.3	64.7	3.5	31.8
4.9	62.9	3.9	33.2
9.6	61.7	4.2	34.1
pH 3.2			
0	10.4	31.0	58.6
0.066	15.3	29.1	55.6
0.20	30.0	26.2	43.8
0.32	39.4	22.5	38.1
0.57	41.7	20.6	37.7
0.65	53.4	18.5	28.1

[#] ± 2%

Table S2: The Binding Regions Obtained from Interactions of Hb-NaDC at Different pH

Method	pH 9.0		pH 7.4	
	C_1 (mM)	C_2 (mM)	C_1 (mM)	C_2 (mM)
Absorption of the Soret band	2.88±0.07	7.73±0.25	2.02±0.10	4.88±0.17
Emission maxima of Trp	3.17±0.11	8.49±0.31	2.63±0.12	6.80±0.21
Average lifetime of Trp	2.28±0.08	6.87±0.27	2.24±0.09	6.02±0.24

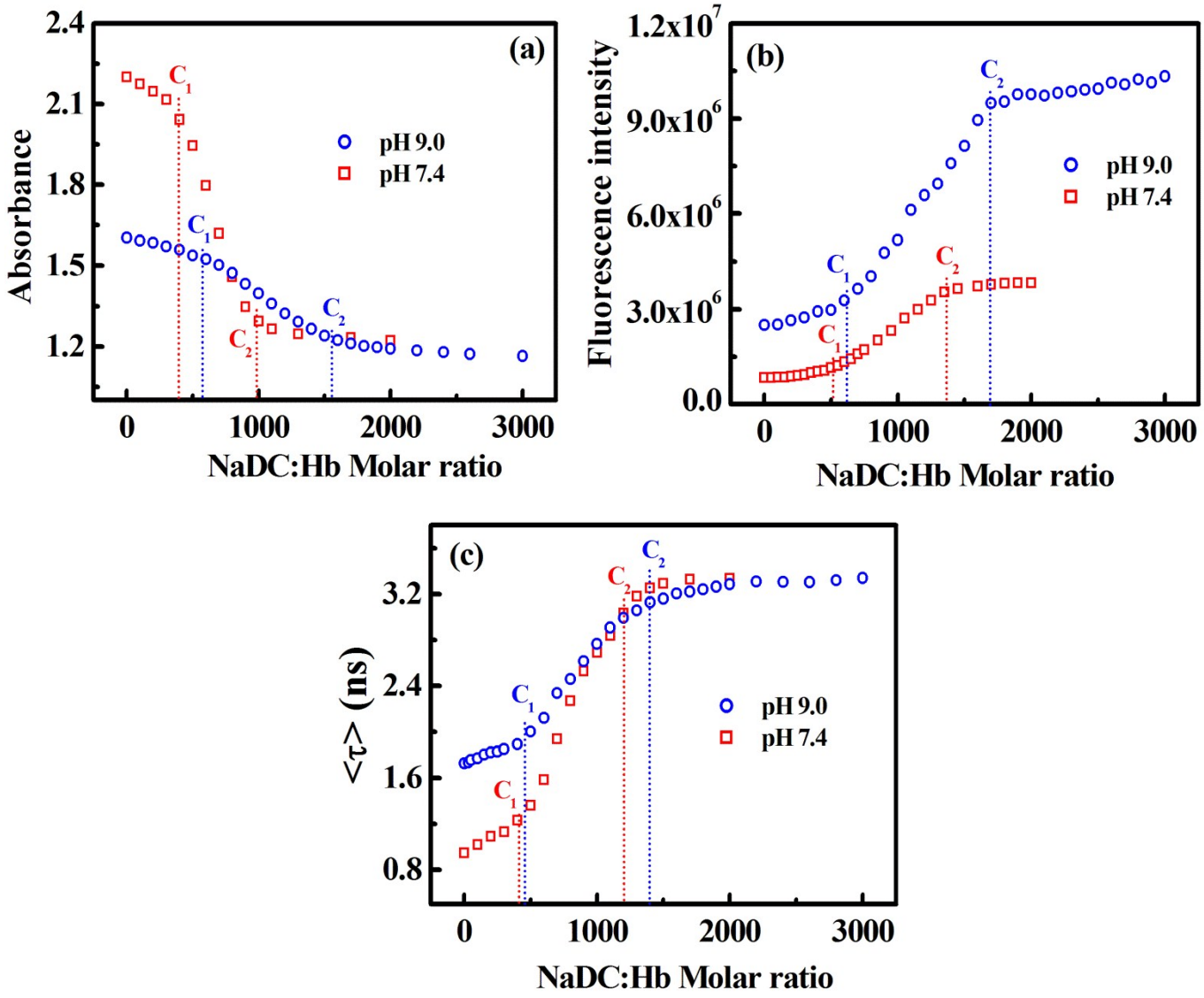

Figure S1. (a) Variation of absorbance of Soret band with varying NaDC:Hb molar ratios, (b) variation of the fluorescence intensity of Hb with varying NaDC:Hb molar ratios and (c) variation of the average lifetimes of Hb with varying NaDC:Hb molar ratios at pH 9.0, blue circle and pH 7.4, red square.

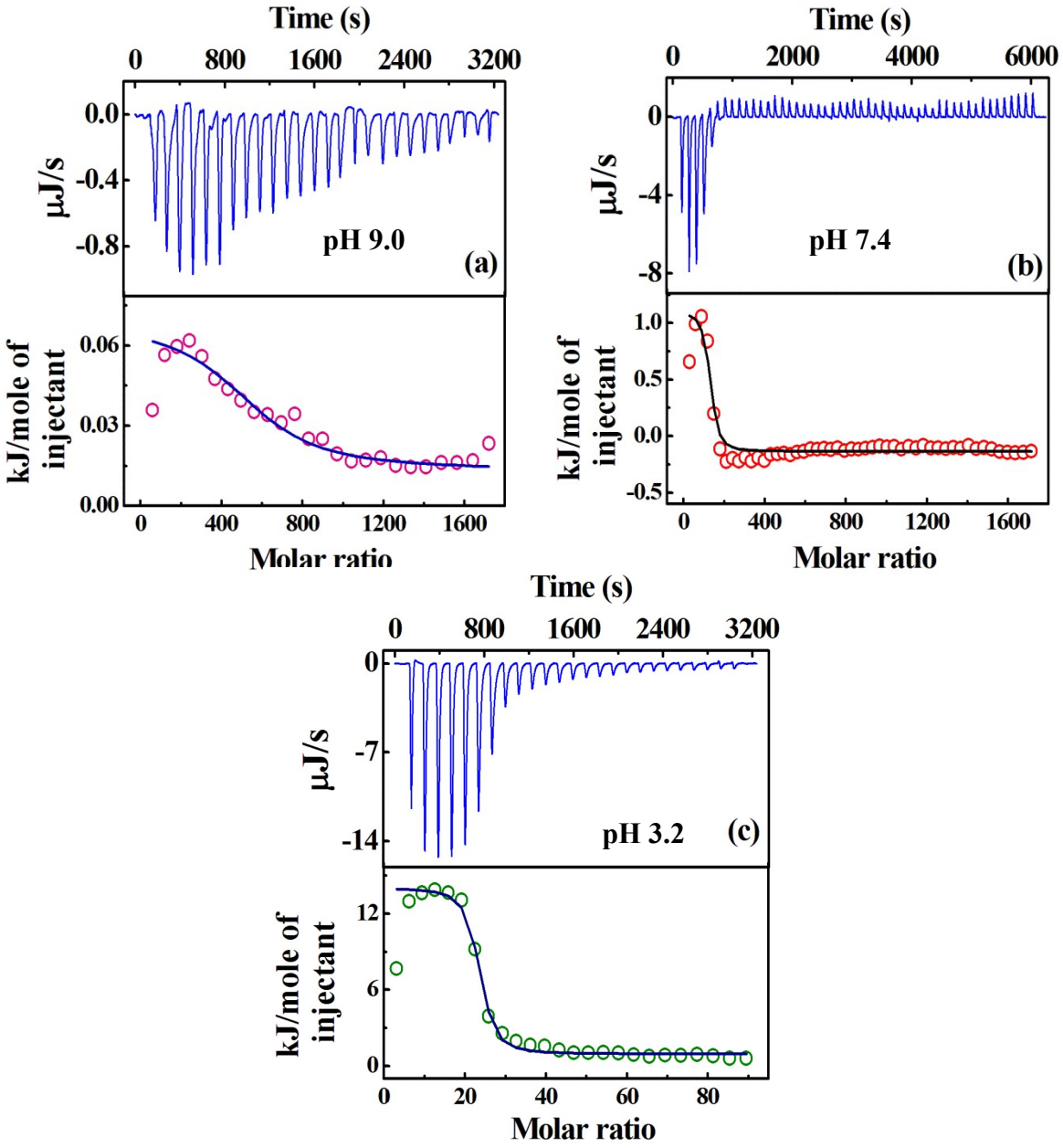

Figure S2. ITC profiles for the titration of Hb with NaDC (a) at pH 9.0, (b) at pH 7.4 and (c) at pH 3.2 at 25°C. (Top) A representative ITC profile showing the raw data for the integrated heat change (after appropriate correction for heat of dilution). (Bottom) ITC enthalpograms obtained at 25°C, along with the best fit lines (solid lines) according to a single set of sites binding model. According to the power convention of the instrument the exothermic (endothermic) process is shown by upward (downward) heat burst curve. See the Instrumentation and Methods section for details of the power sign convention.

Experimental Section

Materials

Hemoglobin (Hb), sodium deoxycholate (NaDC), Tris buffer, sodium citrate and citric acid were used as procured from Sigma-Aldrich Chemical Co., USA. All solutions were prepared in triply distilled deionized Milli-Q water. The concentration of Hb was 5 μM for CD spectroscopy, steady-state absorption/fluorescence and time-resolved experiments, whereas it was 50 μM for ITC experiments.

Instrumentation and Methods

Circular Dichroism (CD) Measurements. The CD spectra were recorded on a JASCO J-815 spectropolarimeter using a cylindrical cuvette of 0.1 cm path length at 25 °C. Each CD spectrum is an average of four successive scans collected at a 50 nm/min scan rate with an appropriately corrected baseline.

Steady-State Spectral Measurements. The absorption and fluorescence spectra were recorded on a Cary 100 UV-vis spectrophotometer, and Fluorolog 3-111 fluorometer, respectively. All spectroscopic measurements are appropriately background corrected.

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} = 295 nm using picosecond laser diode (IBH-NanoLED-295L) 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.[1] The decays were analyzed to extract the fluorescence lifetime parameters on DAS-6 decay analysis software. The multiexponential fluorescence decay (I (t)) is described as:[1]

$$I(t) = \sum_i \alpha_i exp\left(-t/\tau_i\right) \quad (1)$$

in which α_i represents the pre-exponential factor (amplitude) corresponding to the i^{th} decay time constant, τ_i.[1]

Isothermal Titration Calorimetry (ITC). The calorimetric titration of NaDC with hemoglobin (at different pH) was carried out on a Nano ITC, TA Instrument. For pH 9.0 and 3.2 a total of 25 aliquots of NaDC solution were injected at 2 μL interval from a rotating syringe (300 rpm) into the sample chamber containing 50 μM hemoglobin solution, whereas in case of pH 7.4, NaDC solution was added in aliquots of 50 in 1 μL interval from a rotating syringe. The concentrations of NaDC used in ITC studied are 250 mM at pH 7.4 and 9.0 and 14 mM at pH 3.2. The interval between each injection is 120s. The raw heat change data associated with the interactions 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.[2-4]

Herein, it could be pertinent to state that according to the convention of Nano ITC, TA Instrument an exothermic or endothermic process is revealed through upward or downward heat burst curve, respectively.[2,5] It is important to note 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 is considered as the genuine thermodynamic signature and we have interpreted our data accordingly.

References:

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