

## **Supporting Information**

### **Structural and Thermodynamic Studies on the Interaction of Iminium and Alkanolamine Forms of Sanguinarine with Hemoglobin**

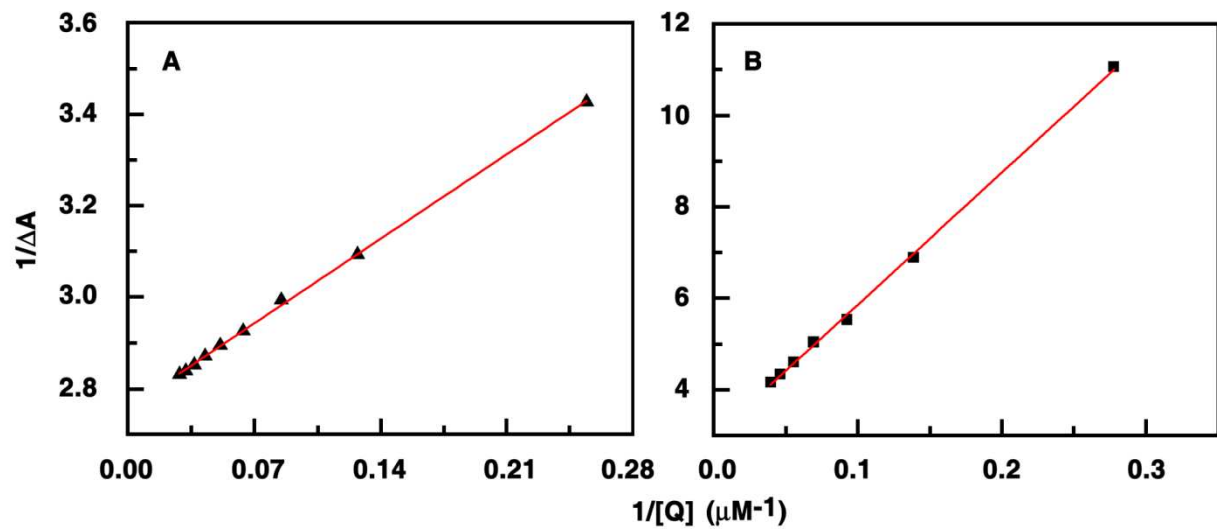
Soumitra Hazra and Gopinatha Suresh Kumar\*  
Biophysical Chemistry Laboratory, Chemistry Division  
CSIR-Indian Institute of Chemical Biology  
Kolkata 700 032, India

\*To whom correspondence should be addressed.

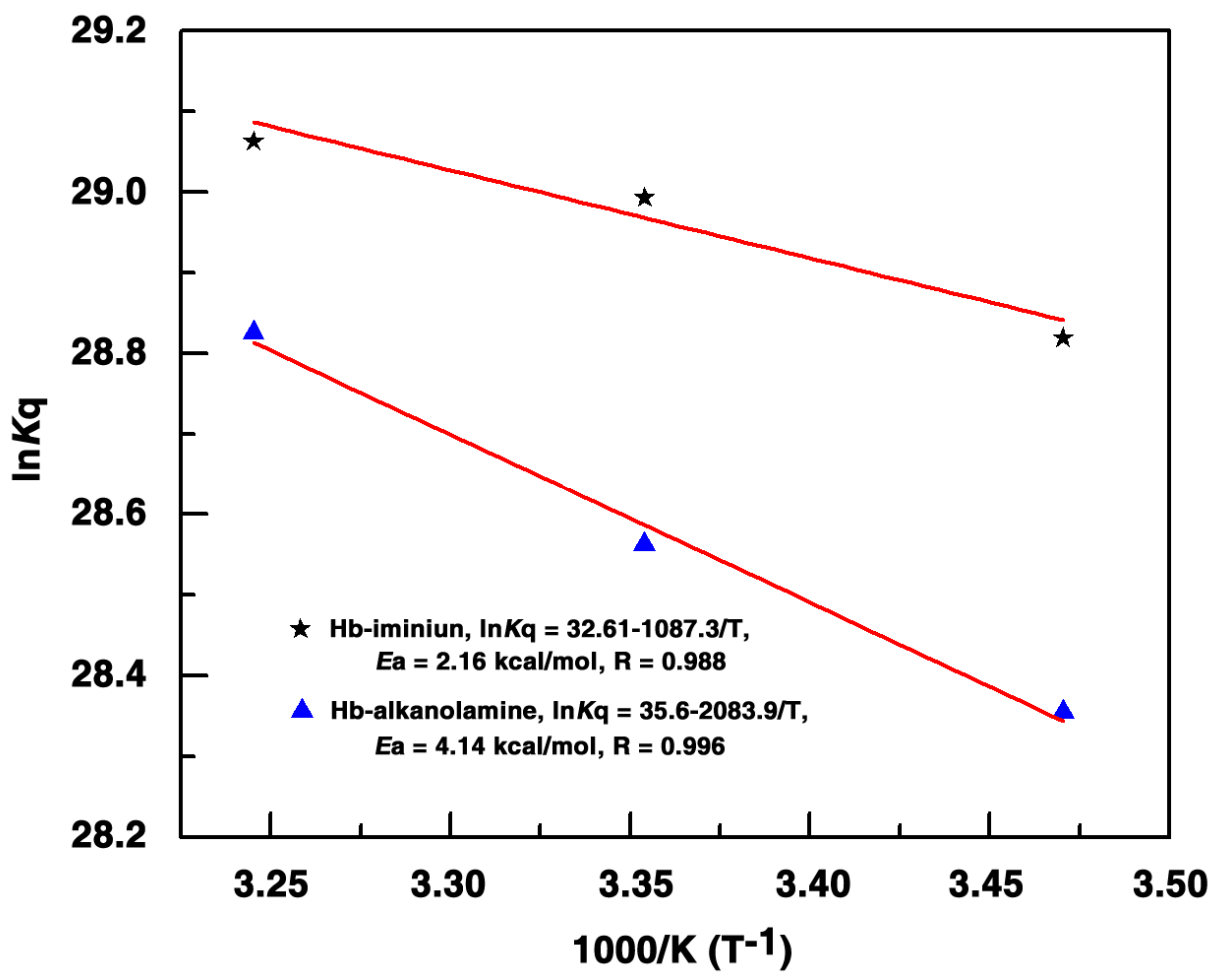
Phone: +91 33 2472 4049 / 2499 5723

Fax: +91 33 2473 0284 / 5197

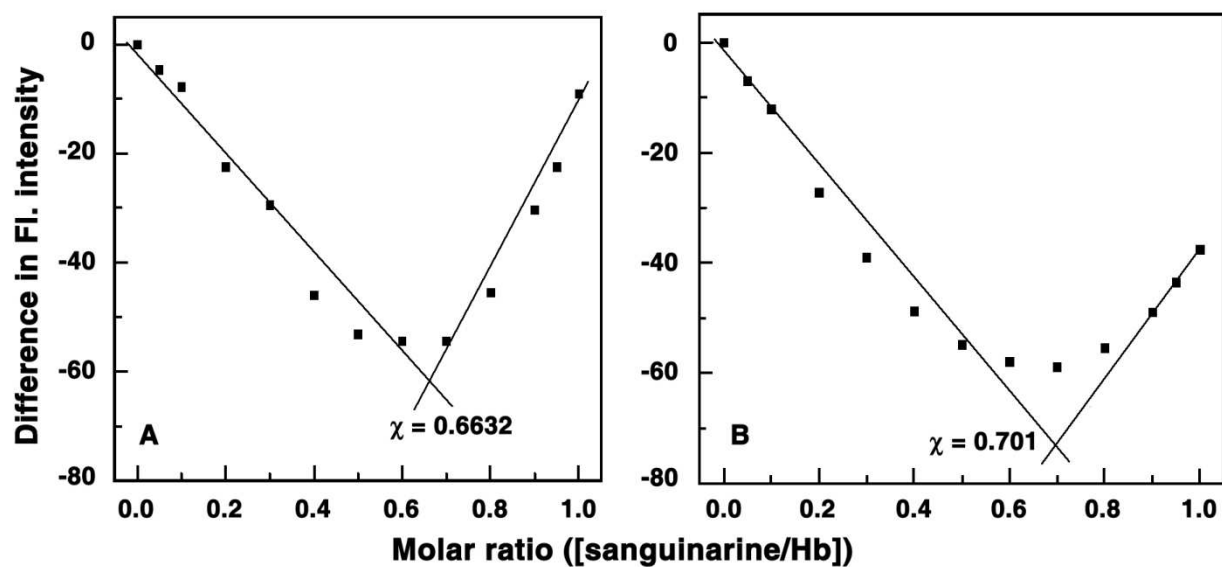
e-mail: [gskumar@iicb.res.in](mailto:gskumar@iicb.res.in) / [gskumar@csiriicb.in](mailto:gskumar@csiriicb.in)



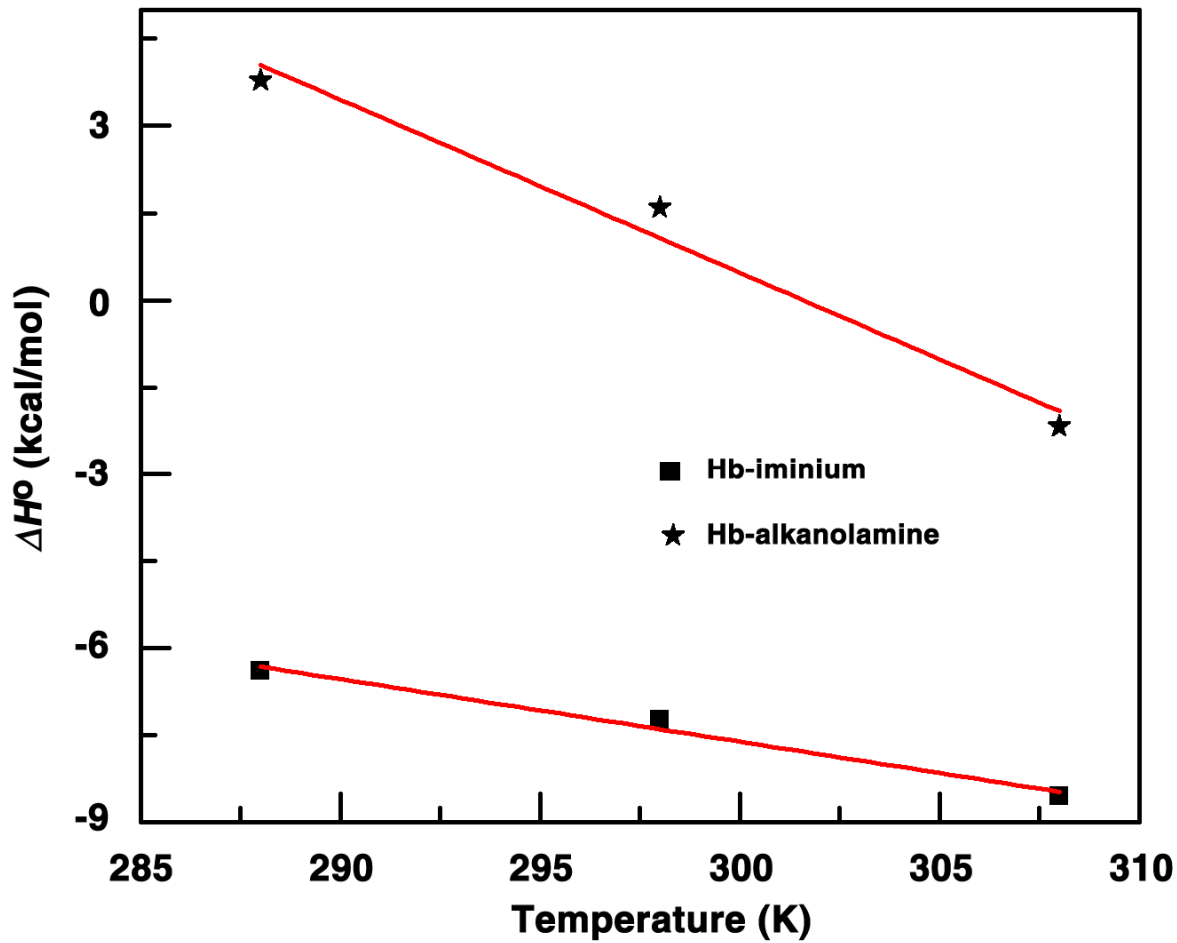
**Figure S1.** Benesi-Hildebrand plots for the binding of Hb with (A) iminium and (B) alkanolamine.



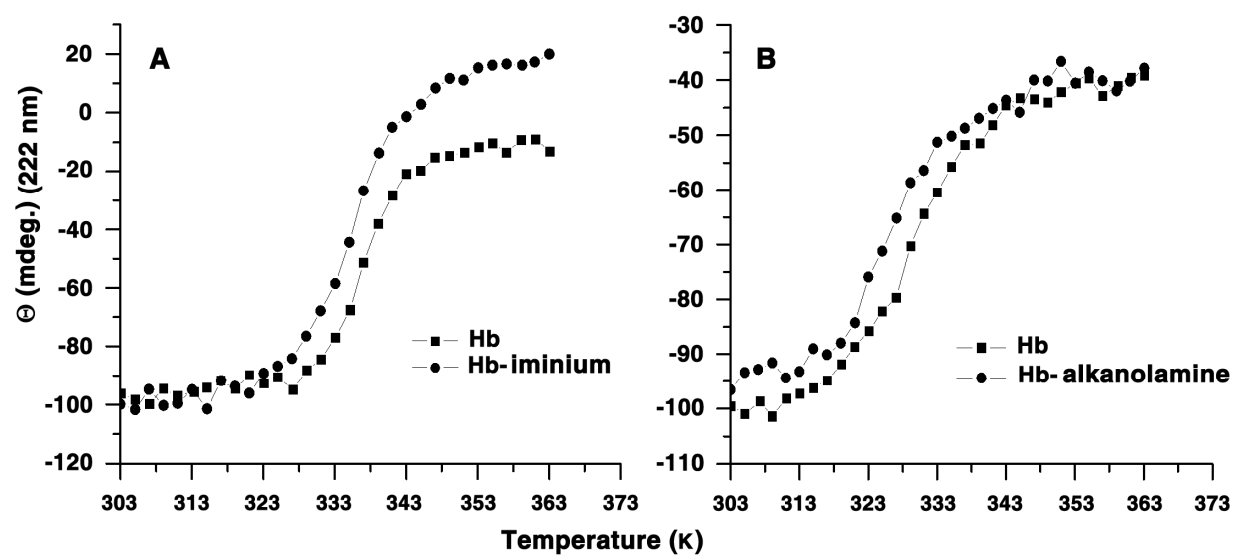
**Figure S2.** Arrhenius plots of (A) Hb-iminium and (B) Hb-alkanolamine system.  $E_a$  is the activation energy of the quenching process.  $R$  is the correlation coefficient for Arrhenius plots.



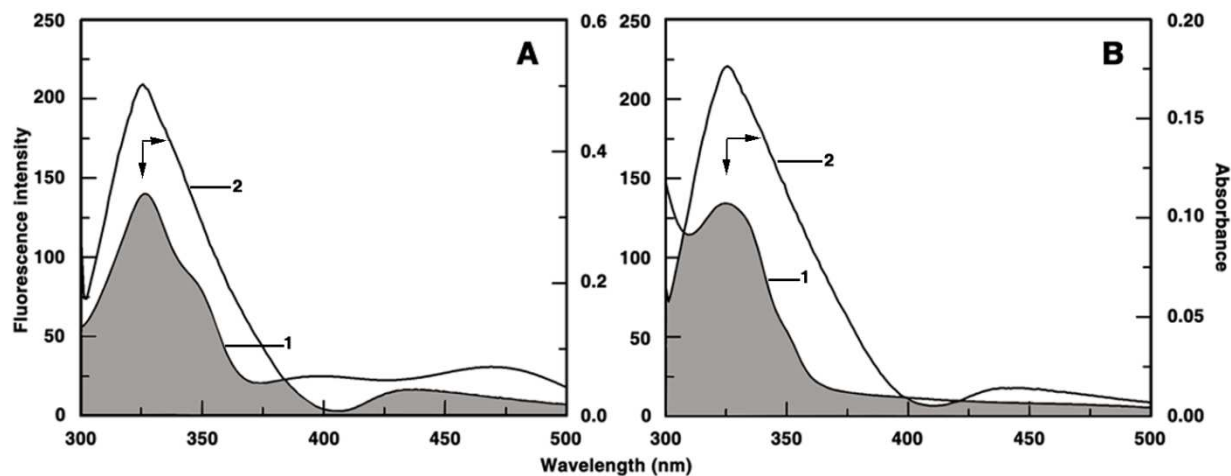
**Figure S3.** Job plot for the binding of sanguinarine (A) iminium and (B) alkanolamine forms to the hemoglobin. Fluorescence quenching of hemoglobin was monitored at 329 nm while excitation wavelength was 295 nm.



**Figure S4.** Plot of variations of enthalpy *versus* temperature for Hb-iminium (square) and Hb-alkanolamine (star) complexation.



**Figure S5.** Changes in ellipticity at 222nm of (A) Hb (2  $\mu$ M), Hb-iminium (1:5) (pH =6.2) and (B) Hb (2  $\mu$ M), Hb-alkanolamine (1:5) complex (pH =9.2) as a function of temperature.



**Figure S6.** Overlap (shaded portion) of the Hb fluorescence spectra (donor) and absorption spectra (acceptor) of sanguinarine (A) iminium and (B) alkanolamine. In panel (A) and (B) curve 1 represent absorption spectra of (A) iminium and (B) alkanolamine and curve 2 represent the fluorescence spectrum of Hb at pH 6.2 and 9.2, respectively. The excitation of Hb was done at 295 nm. The ratio of the concentration of [Hb]:[sanguinarine] = 1:1.

**Table S1:** Thermodynamic parameters derived from ITC experiments for the sanguinarine–ANS systems at 25°C.

pH	System	Binding constant ( $K_b$ )	( $\Delta H^0$ ) cal/mol	( $T\Delta S^0$ ) cal/mol	( $\Delta G^0$ ) cal/mol
6.2	ANS-Sanguinarine iminium	$(8.29 \pm 0.12) \times 10^4$	$-2970 \pm 91.22$	3725.0	$-6695.0 \pm 91.22$
9.2	ANS-Sanguinarine alkanolamine	$(1.99 \pm 0.16) \times 10^4$	$-664.1 \pm 78.28$	5185.2	$-5849.3 \pm 78.28$