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

Supporting Information

Fortification of thermal and structural stability of hemoglobin by choline chloride-based deep eutectic solvents.

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This supporting information contains 10 Figures and 2 Tables on 8 pages

Two state unfolding mechanism:

The two-state unfolding mechanism (shown in equation 1) is used for understanding the thermal unfolding of the protein. The native and denatured state of the protein is referred to as folded and unfolded states respectively. Equation 2 is used to find the fraction of unfolded protein. Y, is referred to as the intensity of protein, which is used to find the fraction of folded (f_f) and unfolded *(*f_u) protein at any temperature 'T'. The Experimentally observed intensities of native (Y_f) and denaturant (Y_u) states are calculated by the extrapolations of the pre- and post-transition base-lines of the thermal denaturation curve using the linear fitting method. Gibbs free energy ($\Delta_{fu}G$) is obtained using equation 5, where R is the universal gas constant and T is the absolute temperature. At equilibrium, the $\Delta_{fu}G$ value is always zero. Thus, the temperature at which this turns out to be zero is said to be the T_m (transition temperature) for protein. The equilibrium constant (K) for the transition is calculated using equation 4.

$$N \leftrightharpoons D \quad (1)$$

$$f_u = (Y_f - Y) / (Y_f - Y_u) \quad (2)$$

$$f_u + f_f = 1 \quad (3)$$

$$K = f_u / f_f = (Y_f - Y) / (Y - Y_u) \quad (4)$$

$$\Delta_{fu}G = -RTlnK \quad (5)$$

$$\Delta_{fu}H=T_m\Delta_{fu}S \quad (6)$$

The other thermodynamic parameters at T_m can be obtained by analysis of the plot of $\Delta_{fu}G$ versus T. The slope of this plot at T_m gives the entropy change of unfolding ($\Delta_{fu}S$). The $\Delta_{fu}H$ value was calculated using equation 6. ΔC_p value was calculated using the Gibbs-Helmholtz equation (equation 7).

$$\Delta_{fu}G(T)=\Delta_{fu}H(T_m)\,[1-(T/T_m)]+\Delta C_p[(T-T_m)-T\ln(T/T_m)] \quad (7)$$

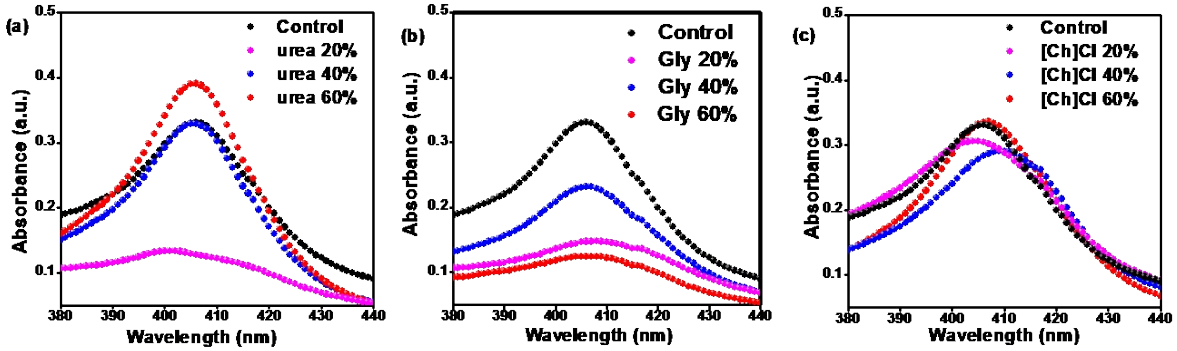

Figure 1S. Absorption spectra of Hb in the presence of varying concentrations of (a) urea (b) Gly (c) [Ch]Cl in 10 mM Tris-HCl buffer of pH 7 (Control) at 25 °C.

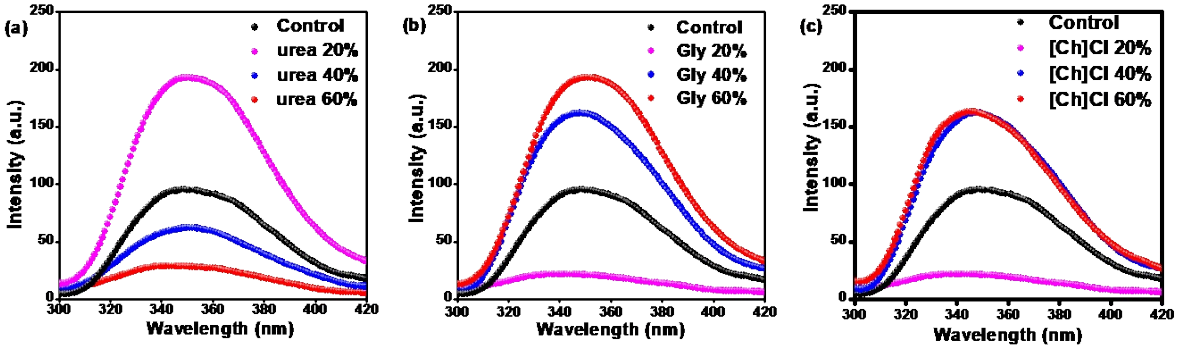

Figure 2S. Steady-state fluorescence spectra of Hb in the presence of varying concentrations of (a) urea (b) Gly (c) [Ch]Cl in 10 mM Tris-HCl buffer of pH 7 (Control) at 25 °C.

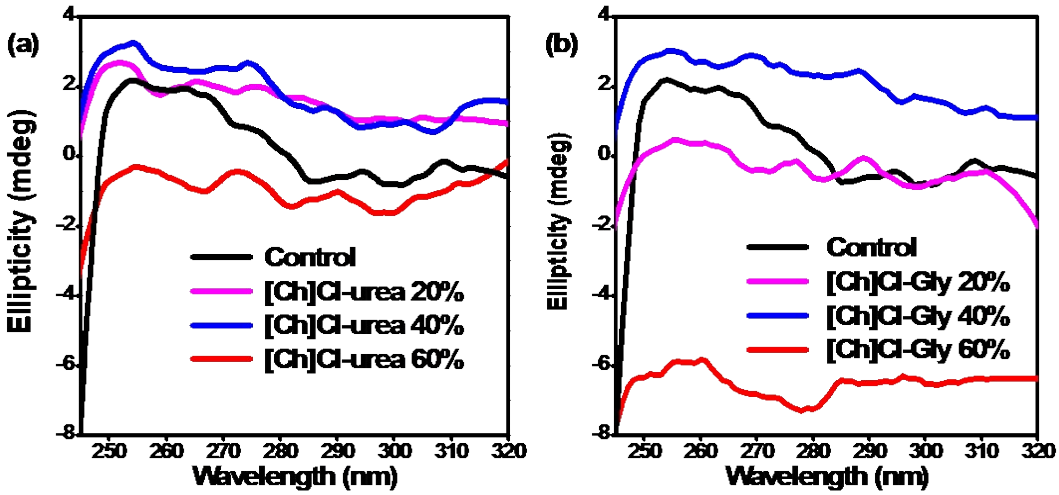

Figure 3S. Near-UV CD spectra of Hb in the presence of varying concentrations of DESs (a) DES 1 and (b) DES 2 in 10 mM Tris-HCl buffer of pH 7 (Control) at 25 °C.

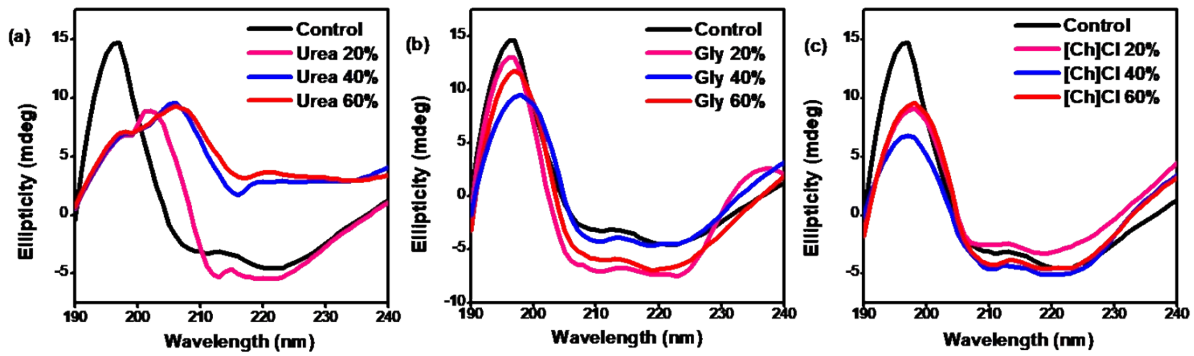

Figure 4S. Far-UV CD spectra of Hb in the presence of varying concentrations of (a) urea (b) Gly (c) [Ch]Cl in 10 mM Tris-HCl buffer of pH 7 (Control) at 25 °C.

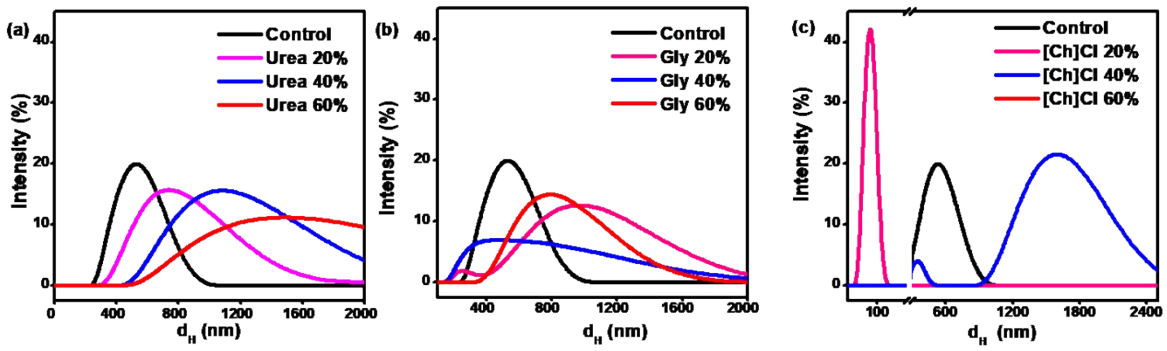

Figure 5S. The size (d_H) distribution of the light scattered intensity by Hb in the presence of a varying concentration of (a) urea (b) Gly (c) [Ch]Cl in 10 mM Tris-HCl buffer of pH 7 (Control) at 25 °C.

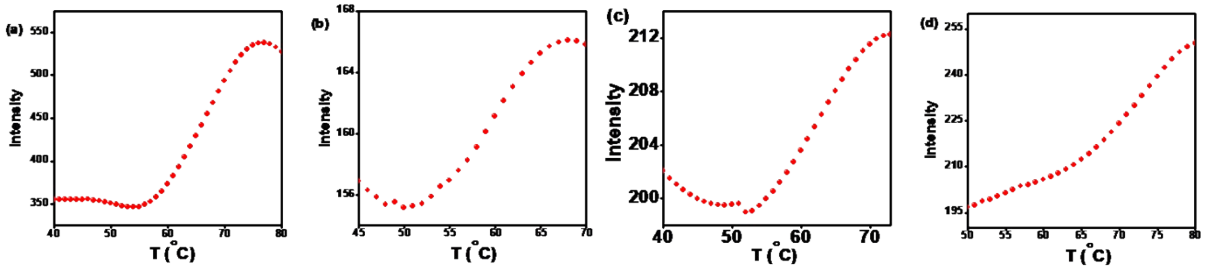

Figure 6S. Plot of Intensity versus Temperature (T (°C)) for Hb in presence of DES 1 at (a) 0 (b) 20 % (c) 40 % and (d) 60 % concentration in the presence of Tris-HCl buffer pH=7.0.

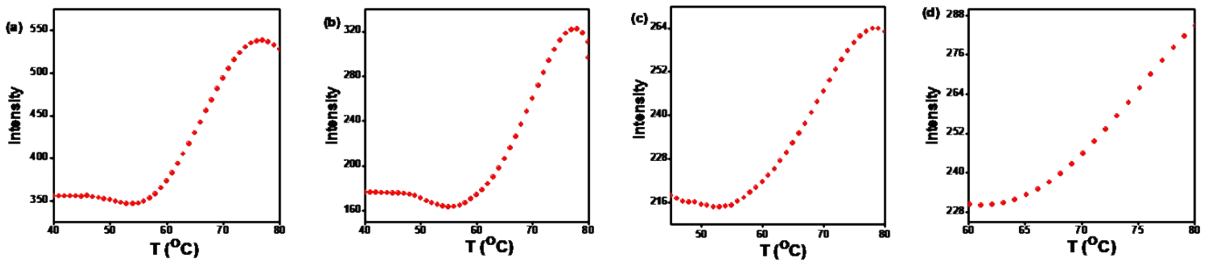

Figure 7S. Plot of Intensity versus Temperature (T (°C)) for Hb in presence of DES 2 at (a) 0 (b) 20 % (c) 40 % and (d) 60 % concentration in the presence of Tris-HCl buffer pH=7.0.

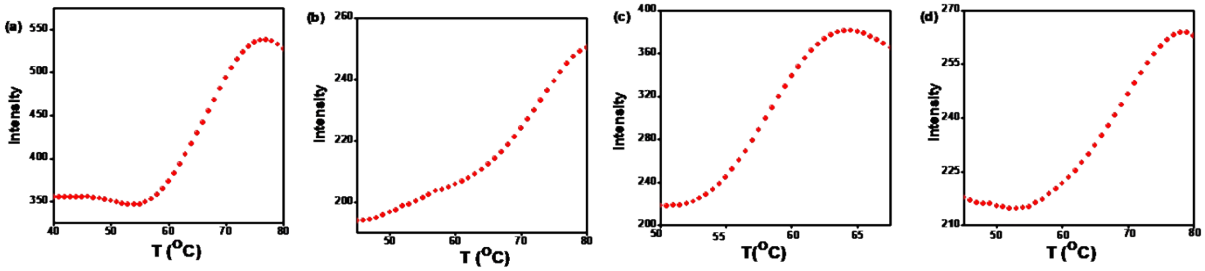

Figure 8S. Plot of Intensity versus Temperature (T (°C)) for Hb in presence of [Ch]Cl at (a) 0 (b) 20 % (c) 40 % and (d) 60 % concentration in the presence of Tris-HCl buffer pH=7.0.

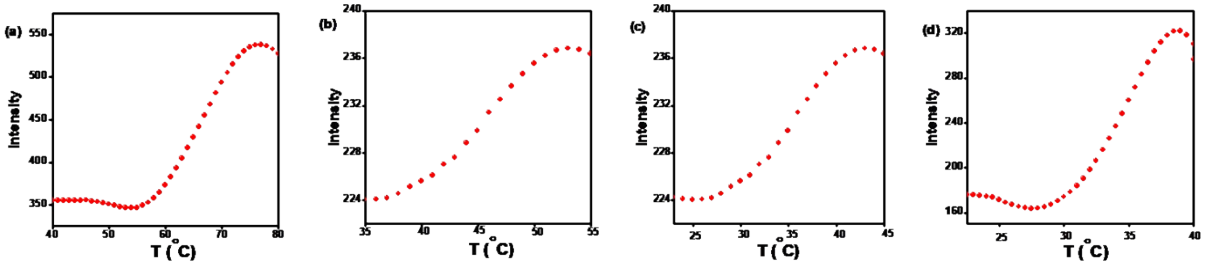

Figure 9S. Plot of Intensity versus Temperature (T (°C)) for Hb in presence of urea at (a) 0 (b) 20 % (c) 40 % and (d) 60 % concentration in the presence of Tris-HCl buffer pH=7.0.

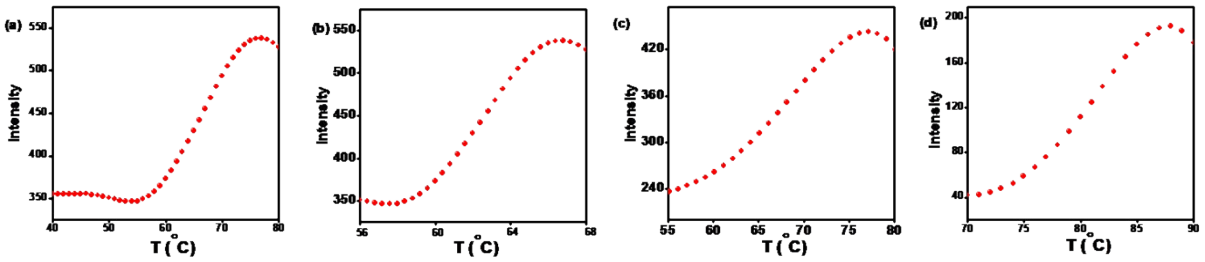

Figure 10S. Plot of Intensity versus Temperature (T (°C)) for Hb in presence of Gly at (a) 0 (b) 20 % (c) 40 % and (d) 60 % concentration in the presence of Tris-HCl buffer pH=7.0.

Table S1. Fraction secondary structure of Hb in presence of DESs and their components.

Solvent-System	α-Helix	β-pleated	Random coil	Unordered
Control	0.6	0.12	0.12	0.16
DES 1	0.62	0.08	0.2	0.1
DES 2	0.68	0.14	0.1	0.08
[Ch]Cl	0.62	0.22	0.14	0.1
Urea	0.52	0.16	0.16	0.11
Glycerol	0.64	0.11	0.11	0.09

Table S2. Thermodynamic parameters T_m, $\Delta_{fu}G$, $\Delta_{fu}S$, $\Delta_{fu}H$, ΔC_p of DES 1, DES 2, Urea, Gly, and [Ch]Cl.

Concentration of DES 1 (% by mass)	**T_m (°C)**	**$\Delta_{fu}G$ (Kcal/mol)**	**$\Delta_{fu}S$ (Kcal/mol K)**	**$\Delta_{fu}H$ (Kcal/mol)**	**ΔC_p (Kcal/mol K)**
0	67.2±0.2	1.06024	0.26	88.4	3.64089
20%	64.1±0.7	1.03516	0.16	53.92	2.23841
40%	67.3±0.7	1.06024	0.51	142.8	5.29776
60%	72.3±0.4	1.18365	0.42	176.07	5.41702

Concentration of DES 2 (% by mass)	**T_m (°C)**	**$\Delta_{fu}G$ (Kcal/mol)**	**$\Delta_{fu}S$ (Kcal/mol K)**	**$\Delta_{fu}H$ (Kcal/mol)**	**ΔC_p (Kcal/mol K)**
0	67.2±0.2	1.06024	0.26	88.4	3.64089
20%	69.1±0.4	0.98166	0.45	153	6.5969
40%	67.3±0.5	1.19184	0.479	163.8	6.76149
60%	77.3±0.8	1.47177	0.558	195.3	6.83879
Concentration of Urea (% by mass)	**T_m (°C)**	**$\Delta_{fu}G$ (Kcal/mol)**	**$\Delta_{fu}S$ (Kcal/mol K)**	**$\Delta_{fu}H$ (Kcal/mol)**	**ΔC_p (Kcal/mol K)**
0	67.2±0.2	1.06024	0.26	88.4	3.64089
20%	46.5±0.8	0.93198	0.08	6.38	1.5527
40%	38.4±0.4	0.20206	0.02	12.44	1.05815
60%	36.3±0.5	0.13232	0.04	24.7	0.21025
Concentration of Glycerol (% by mass)	**T_m (°C)**	**$\Delta_{fu}G$ (Kcal/mol)**	**$\Delta_{fu}S$ (Kcal/mol)**	**$\Delta_{fu}H$ (Kcal/mol)**	**ΔC_p (Kcal/mol K)**
0	67.2±0.2	1.06024	0.26	88.4	3.64089
20%	62.5±0.6	0.91263	0.128	42.88	1.19138
40%	70.4±0.3	1.08842	0.106	36.358	1.80096
60%	80.5±0.4	1.32034	0.46	162.38	7.4696
Concentration of [Ch]Cl (% by mass)	**T_m (°C)**	**$\Delta_{fu}G$ (Kcal/mol)**	**$\Delta_{fu}S$ (Kcal/mol K)**	**$\Delta_{fu}H$ (Kcal/mol)**	**ΔC_p (Kcal/mol K)**
0	67.2±0.2	1.06024	0.26	88.4	3.64089
20%	67.4±0.4	1.06024	0.198	67.32	2.67932
40%	60.4±0.6	0.19466	0.05	16.65	0.81543
60%	64.5±0.8	0.19912	0.06	20.22	0.91134