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

Supporting Information

Protein packaging in ionic liquids mixtures: an ecofriendly approach towards improved stability of β-Lactoglobulin in cholinium-based mixed ionic liquids

Anamika Sindhu[a], Sumit Kumar[a], Dibyendu Mondal[b]*, Indra Bahadur[c], and Pannuru Venkatesu[a]*

[a.]Department of Chemistry, University of Delhi, Delhi, 110 007, India

[b.]Centre for Nano & Material Science, JAIN (deemed to be University), Jain Global Campus, Bangalore-562112, India

[c.]Department of Chemistry, School of Physical and Chemical Sciences, Material Science Innovation & Modelling (MaSIM) Focus Area, Faculty of Natural and Agricultural Sciences, North-West University (Mafikeng Campus), Private Bag X2046, Mmabatho 2735, South Africa

* Corresponding authors: m.dibyendu@jainuniversity.ac.in, dmtapu@gmail.com; venkatesup@hotmail.com; pvenkatesu@chemistry.du.ac.in; Tel: +91-11-27666646-142; Fax: +91-11-2766 6605

The determination of ΔS_m and ΔH_m values

The thermal transition curves were analysed to calculate thermodynamic parameter such as transition temperature (T_m), enthalpy change of protein unfolding (ΔH_m) and entropy change of unfolding (ΔS_m) at T_m. The value of transition temperature (T_m) at a condition where ΔG_u is zero, can be acquired by analysis of the plot of ΔG_u versus T (◦C). The slope of this plot at T_m yields the entropy change of unfolding (ΔS_m). The ΔH_m was calculated using the following equation.

$$\Delta H_m = T_m \Delta S_m$$

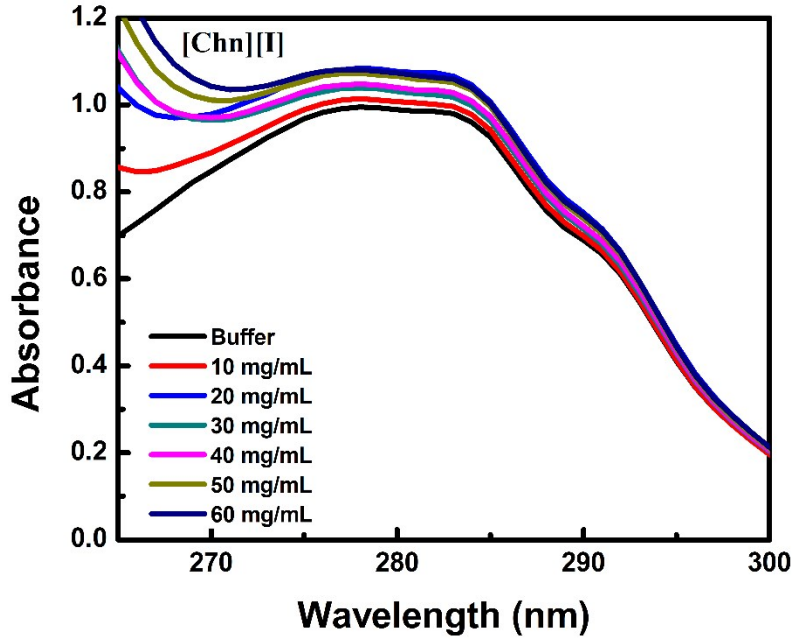

Figure. S1 UV-visible absorption spectra of β-LG in presence of varying concentrations of [Chn][I] at 25 °C.

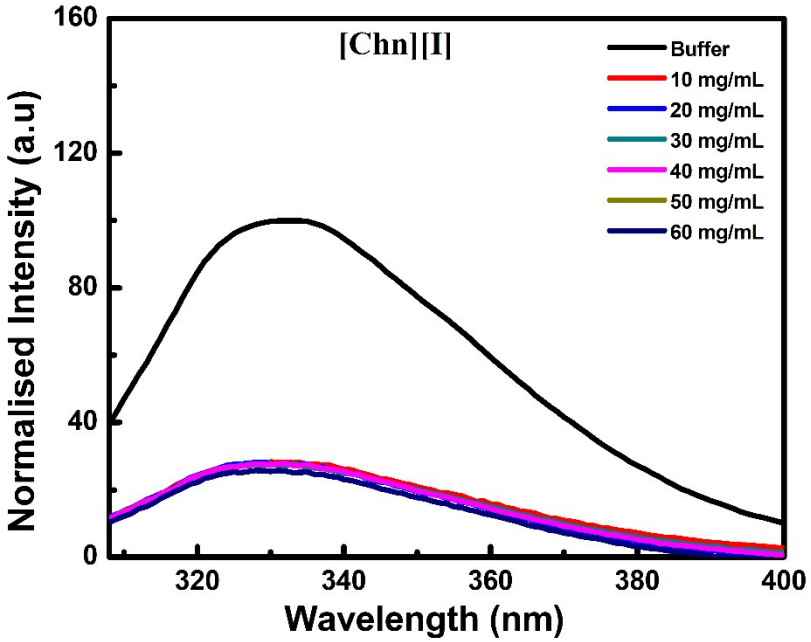

Figure. S2 Fluorescence emission spectra of β-LG in presence of varying concentrations of [Chn][I] at 25 °C.

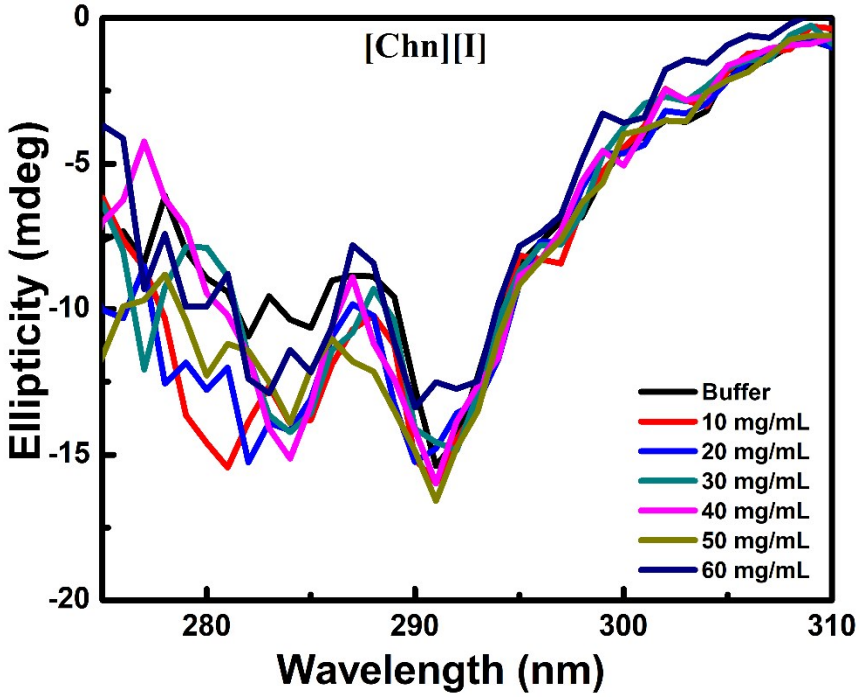

Figure S3. Near-UV CD analysis of β-LG conformation in presence of varying concentrations of [Chn][I] at 25 °C.

Table S1 Hydrodynamic Diameters (d_H) of β-LG in presence of various choline-based ILs and their mixtures

	d_H (nm)
Pure β-LG	5.6
[Chn][Ac] 10mg/mL	5.1
[Chn][Bit] 10mg/mL	6.0
[Chn][Cl] 10mg/mL	5.0
[Chn][Dhp] 10mg/mL	4.9
[Chn][I] 10mg/mL	5.5
[Chn][I] 20mg/mL	5.7
[Chn][I] 30mg/mL	6.1
[Chn][I] 40mg/mL	6.4
[Chn][I] 50mg/mL	6.5
[Chn][I] 60mg/mL	6.9
[Chn][Ac]+ [Chn][I] (1:1)	5.3
[Chn][Ac]+ [Chn][I] (1:2)	5.3
[Chn][Ac]+ [Chn][I] (1:3)	5.6
[Chn][Ac]+ [Chn][I] (1:4)	5.7
[Chn][Ac]+ [Chn][I] (1:5)	5.8

[Chn][Ac]+ [Chn][I] (1:6)	**6.0**
[Chn][Bit]+ [Chn][I] (1:1)	**6.0**
[Chn][Bit]+ [Chn][I] (1:2)	**6.1**
[Chn][Bit]+ [Chn][I] (1:3)	**6.2**
[Chn][Bit]+ [Chn][I] (1:4)	**6.0**
[Chn][Bit]+ [Chn][I] (1:5)	**6.1**
[Chn][Bit]+ [Chn][I] (1:6)	**6.2**
[Chn][Cl]+ [Chn][I] (1:1)	**5.1**
[Chn][Cl]+ [Chn][I] (1:2)	**5.1**
[Chn][Cl]+ [Chn][I] (1:3)	**5.3**
[Chn][Cl]+ [Chn][I] (1:4)	**5.8**
[Chn][Cl]+ [Chn][I] (1:5)	**5.9**
[Chn][Cl]+ [Chn][I] (1:6)	**6.0**
[Chn][Dhp]+ [Chn][I] (1:1)	**5.8**
[Chn][Dhp]+ [Chn][I] (1:2)	**5.8**
[Chn][Dhp]+ [Chn][I] (1:3)	**5.8**
[Chn][Dhp]+ [Chn][I] (1:4)	**5.9**
[Chn][Dhp]+ [Chn][I] (1:5)	**6.1**
[Chn][Dhp]+ [Chn][I] (1:6)	**6.3**

Table S2: Transition temperature (T_m), Enthalpy change of unfolding (ΔH_m) and entropy change of unfolding (ΔS_m) at T_m determined by the fluorescence analysis of thermal denaturation of β-LG in absence and presence of various choline-based ILs and their mixtures. The error in T_m does not exceed 0.1 ◦C.

	T_m (°C)	ΔS_m (kJ.mol^{-1} K^{-1})	ΔH_m (kJ.mol^{-1})
Pure β-LG	**83.86**	**1.24**	**445.90**
[Chn][Ac] 10mg/mL	**85.85**	**1.28**	**459.52**
[Chn][Bit] 10mg/mL	**88.16**	**2.72**	**982.76**
[Chn][Cl] 10mg/mL	**85.65**	**1.44**	**516.67**
[Chn][Dhp] 10mg/mL	**91.00**	**2.90**	**1056.03**
[Chn][I] 10mg/mL	**83.20**	**0.97**	**345.35**
[Chn][I] 20mg/mL	**82.35**	**1.03**	**366.16**
[Chn][I] 30mg/mL	**82.20**	**0.64**	**227.40**
[Chn][I] 40mg/mL	**80.80**	**0.55**	**194.60**
[Chn][I] 50mg/mL	**79.00**	**0.74**	**260.53**
[Chn][I] 60mg/mL	**78.10**	**0.51**	**179.13**
[Chn][Ac]+ [Chn][I] (1:1)	**84.85**	**1.18**	**422.44**
[Chn][Ac]+ [Chn][I] (1:2)	**84.65**	**1.13**	**404.31**
[Chn][Ac]+ [Chn][I] (1:3)	**84.20**	**1.10**	**393.08**
[Chn][Ac]+ [Chn][I] (1:4)	**84.00**	**0.77**	**275.00**
[Chn][Ac]+ [Chn][I] (1:5)	**82.60**	**1.08**	**384.21**
[Chn][Ac]+ [Chn][I] (1:6)	**82.50**	**0.84**	**298.74**
[Chn][Bit]+ [Chn][I] (1:1)	**87.60**	**1.50**	**540.22**
[Chn][Bit]+ [Chn][I] (1:2)	**87.00**	**0.84**	**303.03**
[Chn][Bit]+ [Chn][I] (1:3)	**87.43**	**1.10**	**396.63**
[Chn][Bit]+ [Chn][I] (1:4)	**84.65**	**0.79**	**282.66**

[Chn][Bit]+ [Chn][I] (1:5)	**80.78**	**0.77**	**272.52**
[Chn][Bit]+ [Chn][I] (1:6)	**78.63**	**0.80**	**281.42**
[Chn][Cl]+ [Chn][I] (1:1)	**85.45**	**1.19**	**426.73**
[Chn][Cl]+ [Chn][I] (1:2)	**85.20**	**1.18**	**422.85**
[Chn][Cl]+ [Chn][I] (1:3)	**85.00**	**0.98**	**350.98**
[Chn][Cl]+ [Chn][I] (1:4)	**84.85**	**0.91**	**325.78**
[Chn][Cl]+ [Chn][I] (1:5)	**84.45**	**0.70**	**250.32**
[Chn][Cl]+ [Chn][I] (1:6)	**83.27**	**0.61**	**217.41**
[Chn][Dhp]+ [Chn][I] (1:1)	**90.80**	**1.27**	**462.21**
[Chn][Dhp]+ [Chn][I] (1:2)	**88.85**	**0.94**	**340.28**
[Chn][Dhp]+ [Chn][I] (1:3)	**88.45**	**0.99**	**357.98**
[Chn][Dhp]+ [Chn][I] (1:4)	**87.65**	**0.84**	**303.07**
[Chn][Dhp]+ [Chn][I] (1:5)	**87.00**	**0.80**	**288.12**
[Chn][Dhp]+ [Chn][I] (1:6)	**86.20**	**0.71**	**255.13**