Electronic supplementary information (ESI)

NMR investigations on binding and dynamics of imidazolium-based ionic liquids with HEWL

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Figure captions

Fig. S1 CD spectra of HEWL in the absence and presence of ILs. The concentration of each IL and HEWL used were 2 mM and 10 μM, respectively.

Fig. S2 Stock plot of ^{1}H NMR spectra of aqueous HEWL (50 μM) in the absence and presence of ILs: control (Blue), IL1 (Red), IL2 (Green) and IL3 (Magenta). The concentration of ILs used were 5 mM in each case.

Fig. S3 Variable-temperature selective and non-selective spin-lattice relaxation rates (R_{1SEL} and R_{1NS}) for aqueous IL2 (5 mM) in the presence of HEWL (50 μM). Proton resonance of α-CH_2 (H1′) of IL2 was chosen.

Fig. S4 ^{35}Cl NMR spectra of (A) IL3 and (B) NaCl recorded with (i) 0 μM, (ii) 5 μM, (iii) 25 μM and (iv) 50 μM concentrations of HEWL at 25°C.

Fig. S5 (A) Residues showing significant (deep blue spheres) and moderate (light blue spheres) chemical shift perturbations (CSPs) observed in IL1 titration with HEWL labelled on crystal structure (PDB ID: 2vb1). (B) Specific residue stretch (G104-V109) showing interaction with IL2 is highlighted in blue colour on crystal structure of HEWL. Secondary structural elements: helix, sheet and loops are coloured in red, yellow and green, respectively.

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Table S2. Selective and nonselective spin relaxation rates (R_{1NS} and R_{1SEL}) of IL (5 mM)/HEWL/D_2O mixtures against [HEWL] at 25°C. Proton resonances of $–OCH_3$ (H1′) of IL1, α-CH_2 (H1′) of IL2 and α-CH_2 (H7) of IL3 were chosen.

Table S3. ^1H Self-diffusion coefficient of water (at 25°C) in aqueous HEWL-IL mixtures. Here the [HEWL] was fixed at 5 mM, while [IL] was varied.

Table S4. Spin-lattice relaxation rates of IL (5 mM)/HEWL (50 μM)/D_2O mixtures measured with (R_1irr) and without (R_1non-irr) irradiation using DIRECTION NMR (at 25°C).

Table S5. Spin relaxation rates of IL3 (^{35}Cl) and NaCl (both ^{23}Na and ^{35}Cl) as a function of [HEWL] at 25°C.

Circular Dichroism (CD)

CD measurements were carried out by using JASCO J-815 (B047461168) spectropolarimeter at 25°C. CD spectra were recorded for 500 μL HEWL-IL mixtures with concentrations of 10 μM HEWL and 2 mM ligand in water using a quartz cell with path length of 10 mm. Here the concentration of ILs were limited due to high HT voltage saturation at concentrations higher than 2 mM. CD data were collected from 190 to 260 nm (far UV-region) with an average of three scans. The band width and response time were chosen as 1 nm and 1 s, respectively.

Impact of ILs on secondary structure of HEWL

Before attempting ligand based NMR studies, CD measurements were carried out on HEWL as well as in the presence of ILs to monitor the influence of chosen ILs on the protein stability in terms of secondary structural changes.[1-4] The related CD data and observations are provided in Fig. S1. CD spectra of HEWL show a characteristic helical structure, negative double minima at 208 and 220 nm (in the far UV region) in the absence and presence of ILs. For IL3, the far-UV CD spectrum of HEWL is very similar to that of native HEWL. A feeble change in the CD pattern is noticed for IL1, where molar ellipticity is shifted to positive side indicating a slight change in secondary structure. However, more positive shift tendency of IL2 indicates a moderate change in the secondary structure of HEWL relative to IL1, which could be attributed to the presence of longer alkyl (octyl chain) in IL2. It may be concluded that the secondary structural conformation of HEWL is not significantly influenced by the concentration of ILs used, albeit the moderate decrease of molar ellipticity observed for IL2. CD results further verified with the analysis of ^{1}H NMR spectra (vide infra).

^{1}H NMR spectra of HEWL (recorded in 90% H_2O and 10% D_2O) in the presence of individual IL1, IL2 and IL3 have been recorded with good signal to noise ratio for protein

(~20000 scans), with a view to probe the changes in HEWL secondary structure induced by ILs (Fig. S2). It is clear that the amide resonances of HEWL in the region from 6 – 11 ppm do not reflect any loss of secondary structures in protein at the give concentrations of ILs used in this study (Fig. S2).

For protein-based NMR experiments, ^{1}H-^{15}N HSQC has been utilized to gauge the protein stability by monitoring amide region (~ 6 to 11 ppm in ^{1}H dimension). Even at the highest concentration of ILs under study, we did not notice any notable collapse in –NH region which indicates that the secondary structure of HEWL is still retained. Based on the above, we would like to mention that the protein stability issue is clearly verified and we are confident that the protein is stable during our NMR measurements. Such ^{1}H-^{15}N HSQC NMR reports to verify the protein stability in terms of secondary structure of four bundle helix protein *Im*7 in the presence of imidazolium ILs were also discussed in earlier publication by Figueiredo et. al.[5]

The CD results taken together with those of ^{1}H NMR clearly supports the native structure of HEWL is not significantly perturbed by studied ILs. However, a residual perturbation from native structure is noticed for IL2 by CD, while such a remark is not evident from NMR. All the above observations render the protein stability, which is an essential pre-requisite for the success of protein-ligand study.

References

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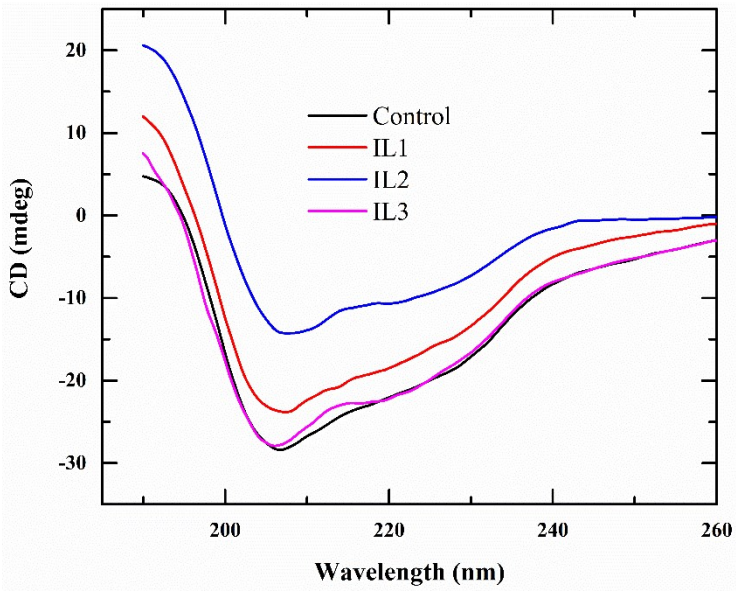

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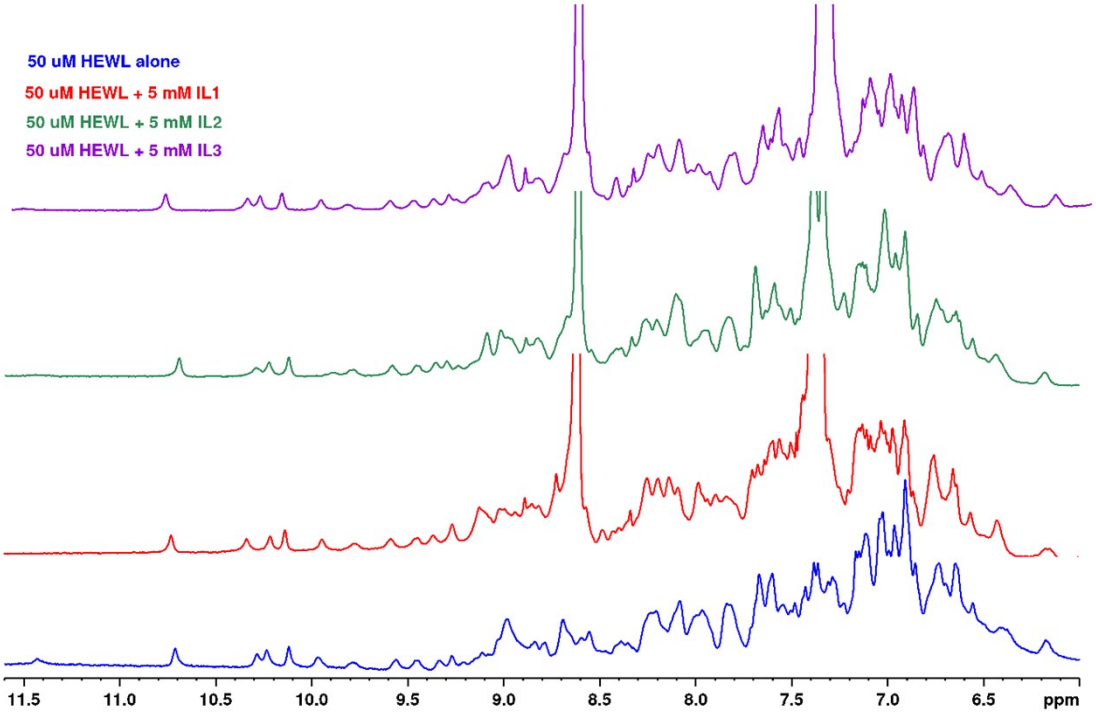

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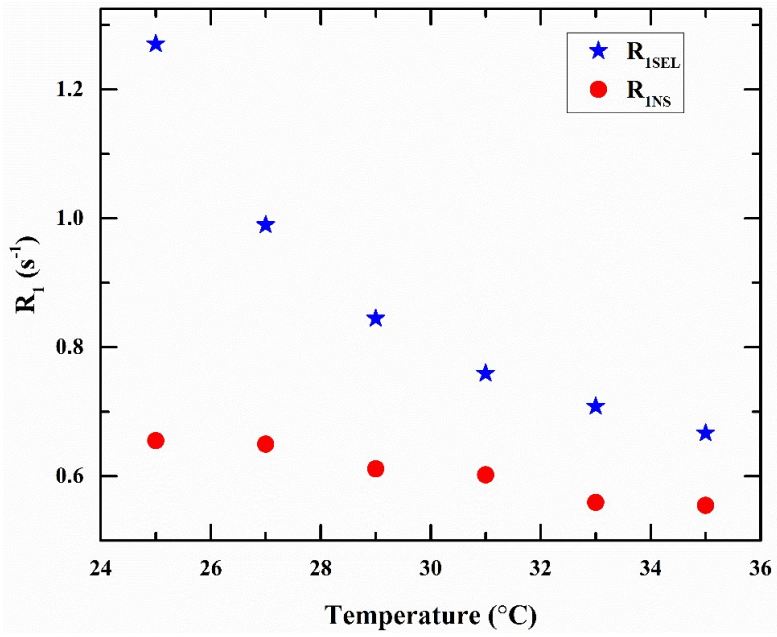

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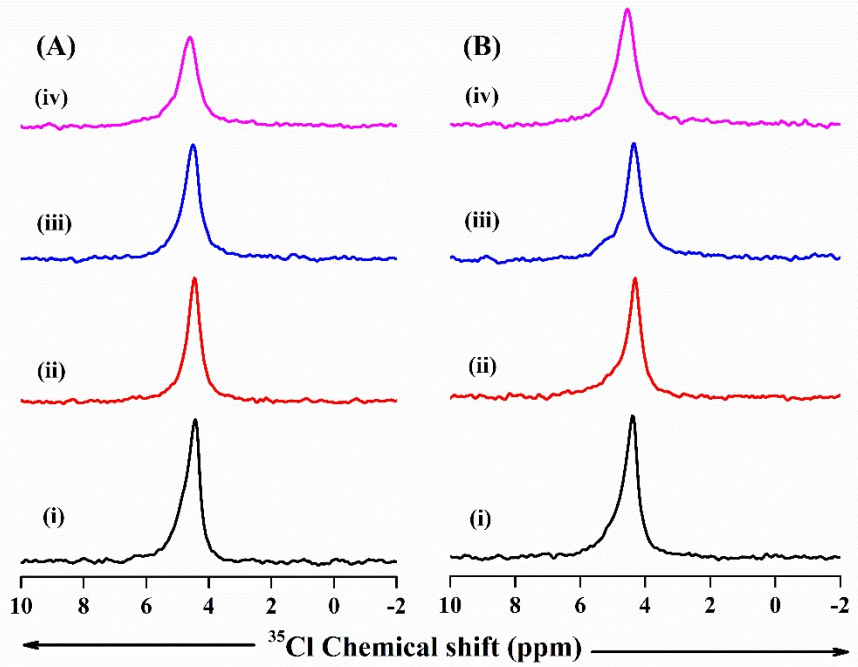

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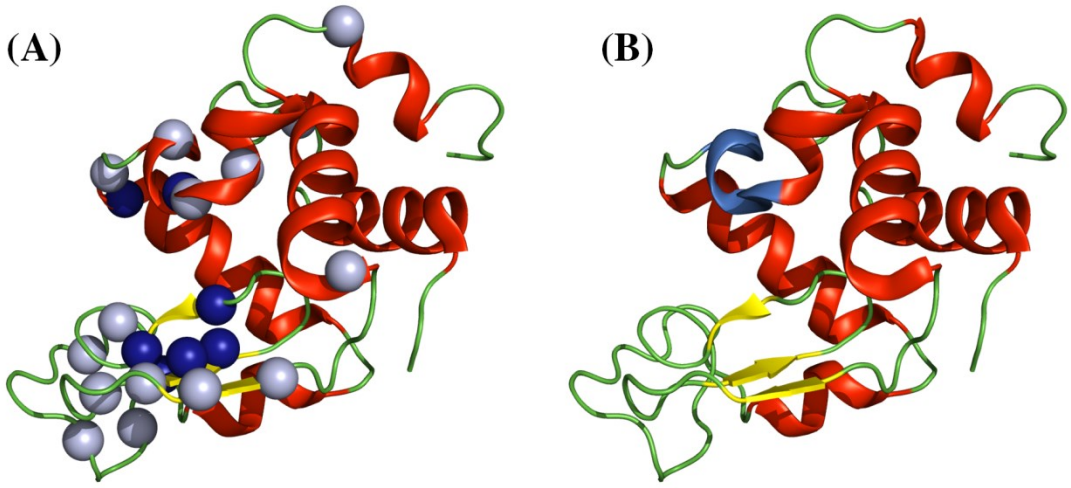

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Table S1. pH data measured on various HEWL/IL/water mixtures.

S. No.	HEWL	IL	Solvent	Measured pH
1	-	-	90% H_2O/10% D_2O^*	7.2
2	5 mM	-	90% H_2O/10% D_2O	3.95
3	-	IL1 (50 mM)	90% H_2O/10% D_2O	3.52
4	-	IL2 (10 mM)	90% H_2O/10% D_2O	4.14
5	-	IL3 (20 mM)	90% H_2O/10% D_2O	5.49
6	5 mM	IL1 (50 mM)	90% H_2O/10% D_2O	3.96
7	5 mM	IL2 (10 mM)	90% H_2O/10% D_2O	4.0
8	5 mM	IL3 (20 mM)	90% H_2O/10% D_2O	4.01

*Uncorrected for deuterium isotope effect

Table S2. Selective and nonselective spin relaxation rates (R_{1NS} and R_{1SEL}) of IL (5 mM)/HEWL/D_2O mixtures against [HEWL] at 25˚C. Proton resonances of –OCH_3 (H1′) of IL1, α-CH_2 (H1′) of IL2 and α-CH_2 (H7) of IL3 were chosen.

HEWL	IL1		HEWL	IL2		HEWL	IL3	
[μM]	R_{1NS}	R_{1SEL}	μM	R_{1NS}	R_{1SEL}	μM	R_{1NS}	R_{1SEL}
0	0.1184	0.118	0	0.55	0.488	0	0.39	0.353
10	0.120	0.117	1	0.532	0.512	10	0.389	0.340
30	0.124	0.118	2.5	0.567	0.513	30	0.38	0.358
50	0.122	0.115	10	0.565	0.522	50	0.39	0.357
70	0.125	0.118	15	0.56	0.52	70	0.395	0.36
90	0.124	0.120	30	0.60	0.55	90	0.40	0.37
110	0.127	0.120	50	0.62	0.57	110	0.40	0.37
140	0.123	0.125	70	0.560	0.57	140	0.407	0.37
170	0.133	0.126	90	0.565	0.60	170	0.425	0.383
200	0.137	0.134	110	0.597	0.62	200	0.42	0.387
230	0.140	0.134	140	0.632	0.66	230	0.42	0.380
260	0.142	0.134	170	0.657	0.71	260	0.42	0.395
300	0.140	0.133	200	0.65	0.832	-	-	-
-	-	-	230	0.666	0.870	-	-	-
-	-	-	260	0.656	0.98	-	-	-
-	-	-	300	0.701	1.332	-	-	-

Table S3. ^{1}H Self-diffusion coefficient of water (at 25°C) in aqueous HEWL-IL mixtures. Here the [HEWL] was fixed at 5 mM, while [IL] was varied.

S. No.	Mixture	$D/10^{-9}$ (m^2s^{-1})
1	HEWL/D_2O	1.744
2	HEWL/IL1 (0.5 mM)/D_2O	1.776
3	HEWL/IL1 (5 mM)/D_2O	1.762
4	HEWL/IL1 (10 mM)/D_2O	1.728
5	HEWL/IL2 (0.5 mM)/D_2O	1.751
6	HEWL/IL2 (5 mM)/D_2O	1.786
7	HEWL/IL2 (10 mM)/D_2O	1.764
8	HEWL/IL3 (0.5 mM)/D_2O	1.793
9	HEWL/IL3 (5 mM)/D_2O	1.773
10	HEWL/IL3 (10 mM)/D_2O	1.818

Table S4. Spin-lattice relaxation rates of IL (5 mM)/HEWL (50 μM)/D_2O mixtures measured with (R_1irr) and without (R_1non-irr) irradiation using DIRECTION NMR (at 25˚C).

IL1			IL2			IL3		
Proton	Off R_1 (s^{-1})	On R_1 (s^{-1})	Proton	Off R_1 (s^{-1})	On R_1 (s^{-1})	Proton	Off R_1 (s^{-1})	On R_1 (s^{-1})
				Cation				
H4	0.131	0.135	H4	0.129	0.129	H4	0.129	0.132
H5	0.113	0.118	H5	0.111	0.111	H5	0.113	0.115
H6	0.255	0.258	H6	0.257	0.263	H6	0.251	0.262
H7	0.38	0.384	H7	0.393	0.414	H7	0.367	0.414
H8	0.373	0.375	H8	0.381	0.383	H8	0.368	0.375
H9	0.338	0.343	H9	--	--	H9	0.335	0.340
H10	0.310	0.321	H10	0.310	0.315	H10	0.308	0.312
				Anion				
H1'	0.117	0.121	H8′	0.376	0.413	-	-	-
-	-	-	$(CH_2)_n$	0.480	0.492	-	-	-
-	-	-	H2′	0.688	0.706	-	-	-
-	-	-	H1′	0.527	0.597	-	-	-

Table S5. Spin relaxation rates of IL3 (^{35}Cl) and NaCl (both ^{23}Na and ^{35}Cl) as a function of [HEWL] at 25˚C.

HEWL [μM]	R_1 (s^{-1})		
	$^{35}Cl^a$	$^{23}Na^b$	$^{35}Cl^c$
0	28.00	16.37	24.89
	(0.4)	(0.10)	(0.7)
5	34.10	16.35	25.75
	(1.15)	(0.08)	(0.89)
25	41.68	16.38	41.80
	(1.25)	(0.08)	(1.67)
50	52.80	16.12	49.18
	(1.84)	(0.11)	(1.13)

[a]1-butyl 3-methylimidazolium chloride (IL3)
[b, c] Sodium Chloride (NaCl)