Supporting Information

Lysozyme conformational changes with ionic liquids: spectroscopies, small angle x-ray scattering and crystallography study

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Table S1. Weight percentage of ILs corresponding to ion concentrations of ILs in IL-water mixtures in this study.

ILs	Molecule weight	1 mol%	5 mol%	10 mol%	16.7 (17) mol%	33 mol%	100 mol%
EaN	108.1	5.7	24	40	54.6	75	99.7
BAN	136.1	7.2	28.6	45.8	60.3		
EtAN	124.1	6.6	26.6	43.4	57.9		
EaMs	141.2	7.4	29.2	46.6	61.1		
TeaMs	197.3	10	36.6	55	68.6		
ChoMs	199.3	10	36.8	55.2			
ChoAc	163.2	8.4	32.3				
ChoDHP	201.2	10.1			69.1		
TeaDHP	199.2	10			68.6		

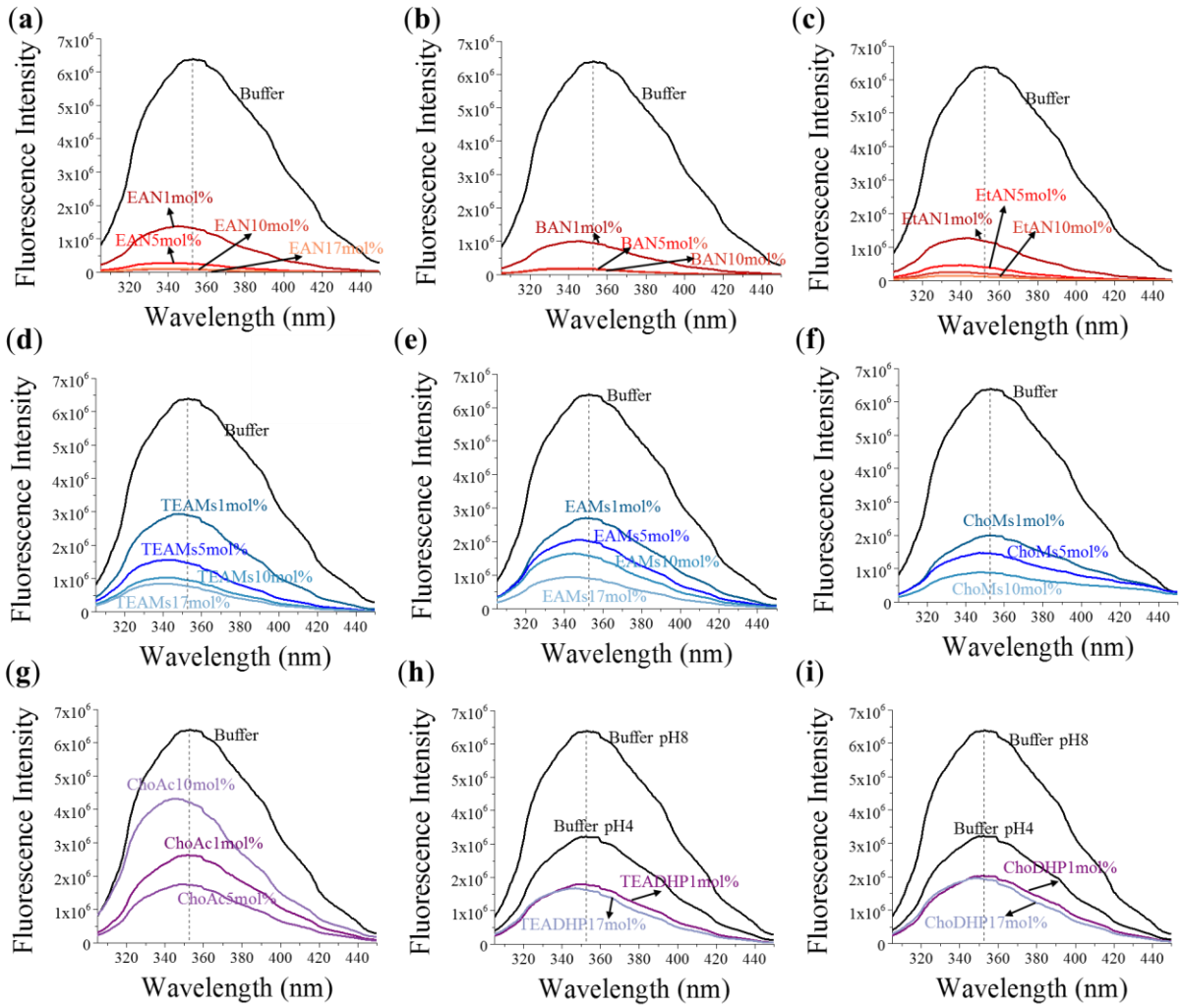

Figure S1. Fluorescence spectra of lysozymes in IL-water mixtures of a) EAN, b) BAN, c) EtAN, d) TEAMs, e) EAMs, f) ChoMs, g) ChoAc, h) TEADHP and i) ChoDHP compared to that in buffer.

Table S2. Details of SAXS parameters of data collection and analysis.

	Data collection parameters of SAXS
Instrument	Australian Synchrotron SAXS/WAXS beamline[15]
Beam Geometry	120 μm point source
Wavelength (Å)	1.033
q range (Å-1)	0.006 – 0.53
Temperature (°C)	25
Solvent	Tris buffer 100 mM and IL-water mixtures
Protein concentration (mg/mL)	5.0
Collection Mode	Well-plate coflow SAXS

Data analysis parameters

Sample name	**Guiner I(0) (cm^{-1}) [std.err]**	**Guinier R_g (Å) [std.err]**	**qR_g (max)**	**P(r) I(0) (cm^{-1})**	**P(r) R_g (Å)**	**D_{max} (Å)**
pH 4	0.013 ± 0.000065	13.53 ± 0.12	1.16	0.01	14.0	40
pH 6	0.014 ± 0.000051	15.48 ± 0.09	1.3	0.01	15.5	55
pH 7	0.011 ± 0.000044	14.50 ± 0.09	1.3	0.01	14.6	46
pH 7.5	0.014 ± 0.000049	14.64 ± 0.09	1.27	0.01	14.6	46
pH 8	0.014 ± 0.000046	14.27 ± 0.09	1.21	0.01	14.5	43
EAN1mol%	0.016 ± 0.000056	16.65 ± 0.1	1.28	0.02	17.3	75
EAN5 mol%	0.011 ± 0.000064	15.48 ± 0.14	1.30	0.01	15.6	61
EAN10 mol%	0.0092 ± 0.000051	15.32 ± 0.14	1.30	0.01	16.2	80
EAN17 mol%	0.007 ± 0.000044	14.75 ± 0.16	1.29	0.01	14.8	50
BAN1 mol%	0.016 ± 0.000053	16.3 ± 0.09	1.27	0.02	16.7	70
BAN5 mol%	0.011 ± 0.000052	15.40 ± 0.11	1.29	0.01	15.5	62
BAN10 mol%	0.011 ± 0.000052	15.09 ± 0.12	1.29	0.01	15.3	64
EtAN1 mol%	0.0014 ± 0.000051	16.19 ± 0.1	1.29	0.01	16.4	57
EtAN5 mol%	0.013 ± 0.00007	16.08 ± 0.15	1.3	0.01	16.3	59
EtAN10 mol%	0.0068 ± 0.000063	15.43 ± 0.24	1.29	0.01	15.6	56
EtAN17 mol%	0.0023 ± 0.000046	15.00 ± 0.52	1.26	0	15.4	57
EAMs1 mol%	0.014 ± 0.000055	15.83 ± 0.11	1.24	0.01	16.2	66
EAMs5 mol%	0.0079 ± 0.000044	15.49 ± 0.14	1.3	0.01	15.7	60
EAMs10 mol%	0.0049 ± 0.000038	15.07 ± 0.2	1.3	0	15.3	57
EAMs17 mol%	0.0033 ± 0.000038	14.94 ± 0.28	1.30	0	15.1	57
TEAMs1 mol%	0.0013 ± 0.000047	15.43 ± 0.09	1.28	0.01	15.5	54

Table S2. Continued.

Sample name	Guiner I(0) (cm^{-1}) [std.err]	Guinier R_g (Å) [std.err]	qR_g (max)	P(r) I(0) (cm^{-1})	P(r) R_g (Å)	D_{max} (Å)
TEAMs5 mol%	0.0098 ± 0.000043	15.39 ± 0.11	1.29	0.01	15.5	57
TEAMs10 mol%	0.0068 ± 0.000037	14.98 ± 0.13	1.29	0.01	15.1	55
TEAMs17 mol%	0.0052 ± 0.000037	14.77 ± 0.17	1.30	0.01	14.9	50
ChoMS1 mol%	0.0092 ± 0.000049	15.59 ± 0.15	1.23	0.01	15.7	56
ChoMS5 mol%	0.0081 ± 0.000048	15.89 ± 0.16	1.29	0.01	16.2	60
ChoAC1 mol%	0.01 ± 0.000044	15.37 ± 0.11	1.3	0.01	15.5	55
ChoAC5 mol%	0.01 ± 0.000042	15.49 ± 0.11	1.3	0.01	15.7	56
ChoAC10 mol%	0.0087± 0.000042	15.79± 0.13	1.3	0.01	16.1	57
TEADHP1 mol%	0.0014 ± 0.000053	16.05 ± 0.10	1.29	0.01	16.2	65
TEADHP17 mol%	0.0017 ± 0.000062	14.40 ± 0.87	1.3	0.01	16.4	63
ChoDHP1 mol%	0.0098 ± 0.000047	15.72 ± 0.13	1.29	0.01	15.7	51
ChoDHP17 mol%	0.0026 ± 0.000043	16.39 ± 0.47	1.29	0	16.3	52
EAN1 mol%-pH6	0.013 ± 0.000062	16.67 ± 0.12	1.28	0.01	17.1	64
EAN5 mol%-pH6	0.0085 ± 0.000049	15.61 ± 0.15	1.3	0.01	15.6	51
EAN10 mol%-pH6	0.006 ± 0.000043	15.17 ± 0.18	1.3	0.01	15.2	53
EAN17 mol%-pH6	0.0058 ± 0.000048	15.15 ± 0.21	1.29	0.01	15.1	50
TEAMs1 mol%-pH6	0.011 ± 0.000043	15.38 ± 0.1	1.29	0.01	15.4	53
TEAMs5 mol%-pH6	0.0086 ± 0.00004	15.14 ± 0.12	1.29	0.01	15.2	58
TEAMs10 mol%-pH6	0.0049 ± 0.000036	15.07 ± 0.18	1.3	0	15.2	53
ChoAc1 mol%-pH6	0.012 ± 0.000047	15.15 ± 0.1	1.29	0.01	15.2	54
ChoMs1 mol%-pH6	0.013 ± 0.000053	15.43 ± 0.1	1.29	0.01	15.7	61
EtAN1 mol%-pH6	0.012 ± 0.000056	16.34 ± 0.13	1.29	0.01	16.7	62

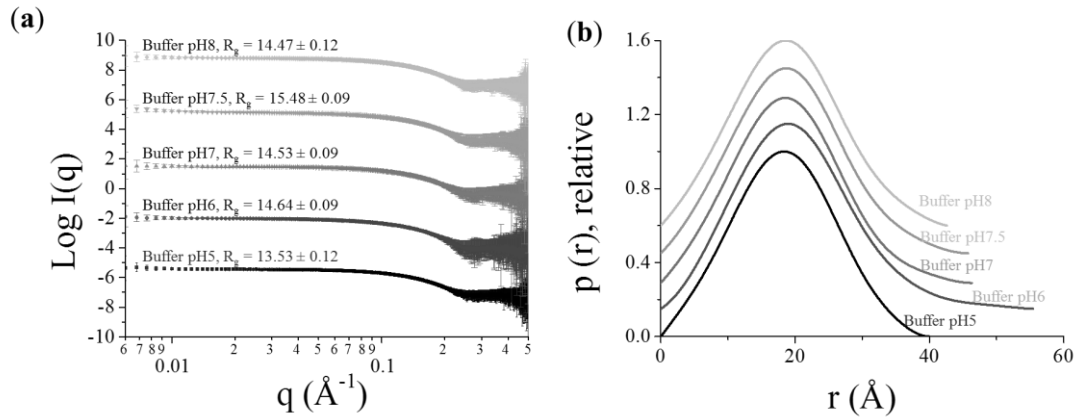

Figure S2. SAXS patterns a) and P (r) plots b) for lysozyme in Tris buffers (100 mM, from pH 8 to 7) and acetate buffers (100 mM, pH 6 and 4). The inset of a) shows the R_g values of lysozyme for each pattern. An offset was applied for easier comparison of the samples.

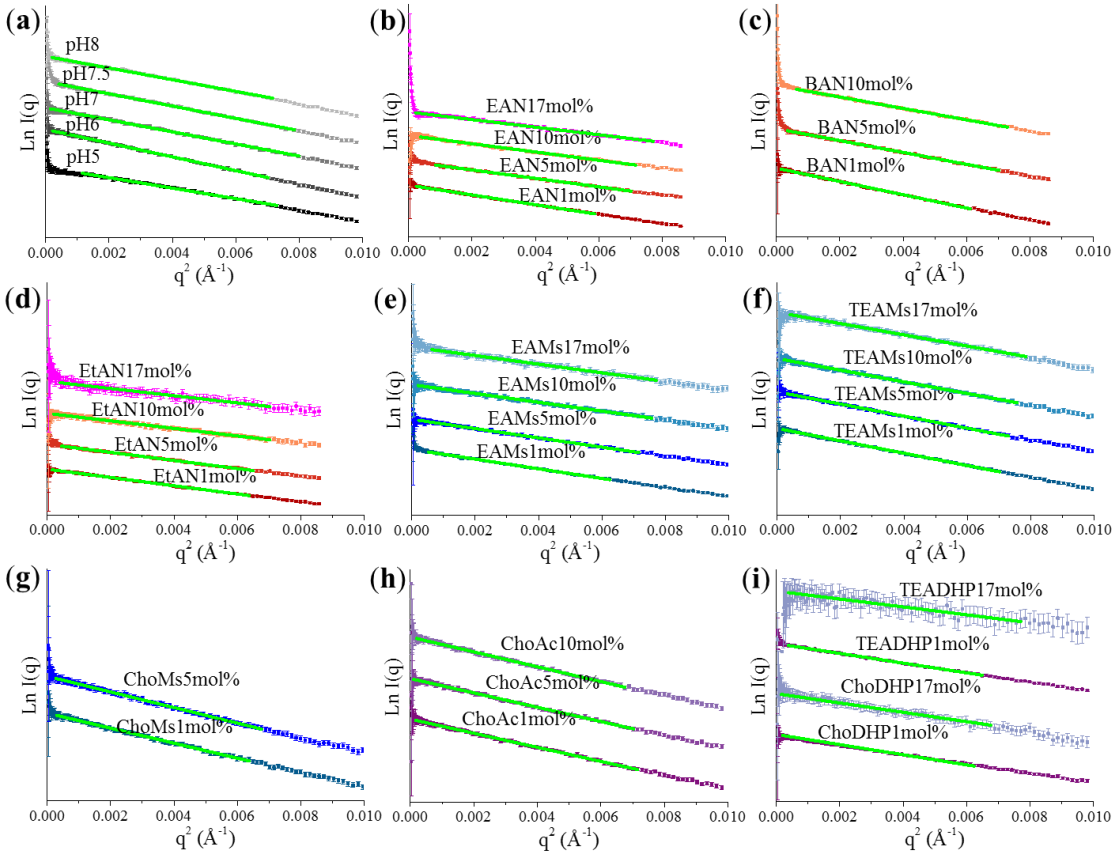

Figure S3. Guinier plots of lysozyme in buffer a) and IL-water mixtures of b) EAN, c) BAN, d) EtAN, e) TEAMs, f) EAMs, g) ChoMs, h) ChoAc and i) TEADHP and ChoDHP at different concentrations. The range for calculating R_g values was shown in green and obtained from the ATSAS 3.01 software. The detailed parameters were provided in Table S2. An offset was applied for easier comparison of the samples.

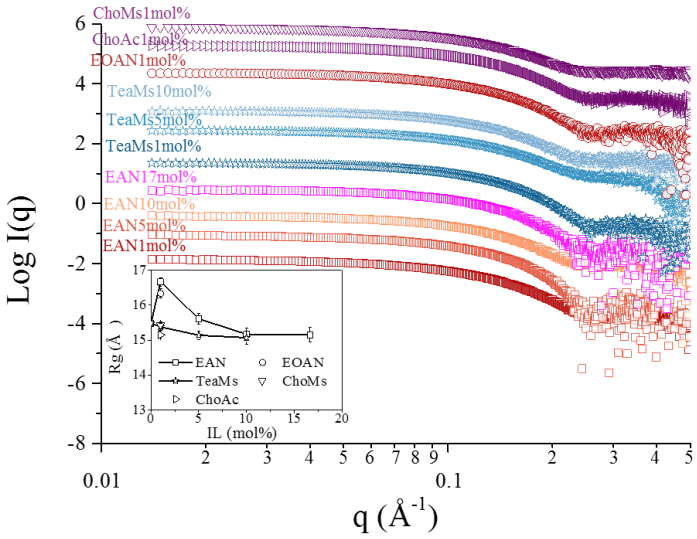

Figure S4. SAXS patterns of lysozyme in EAN, TeaM, EtAN, ChoAc, and ChoMs-water mixtures at pH 6. The inset shows the R_g values of lysozyme as a function of IL concentration. The offset was applied for comparison of each sample.

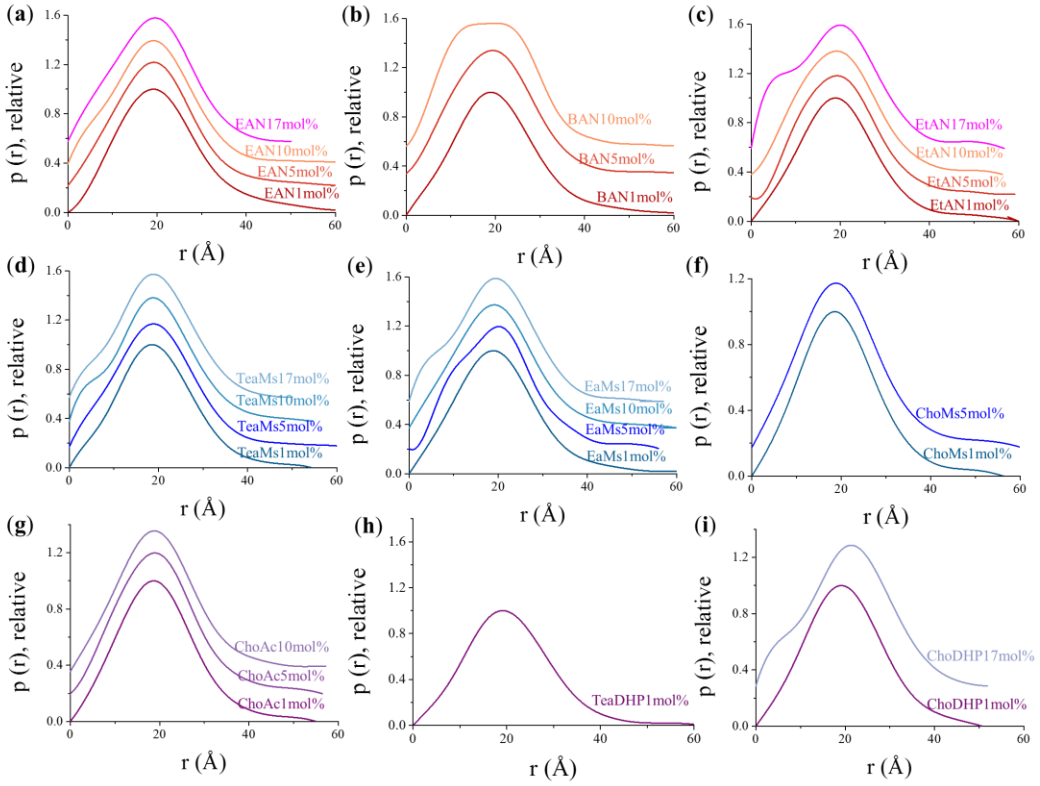

Figure S5. Distance distribution functions P(r) plots of IL-water mixtures of the plate SAXS for a) EAN, b) BAN, c) EtAN, d) TEAMs, e) EAMs, f) ChoMs, g) ChoAc, h) TEADHP and i) ChoDHP. The plots were based on Figure 4 and obtained from the ATSAS 3.01 software. An offset was applied for easier comparison of the samples.

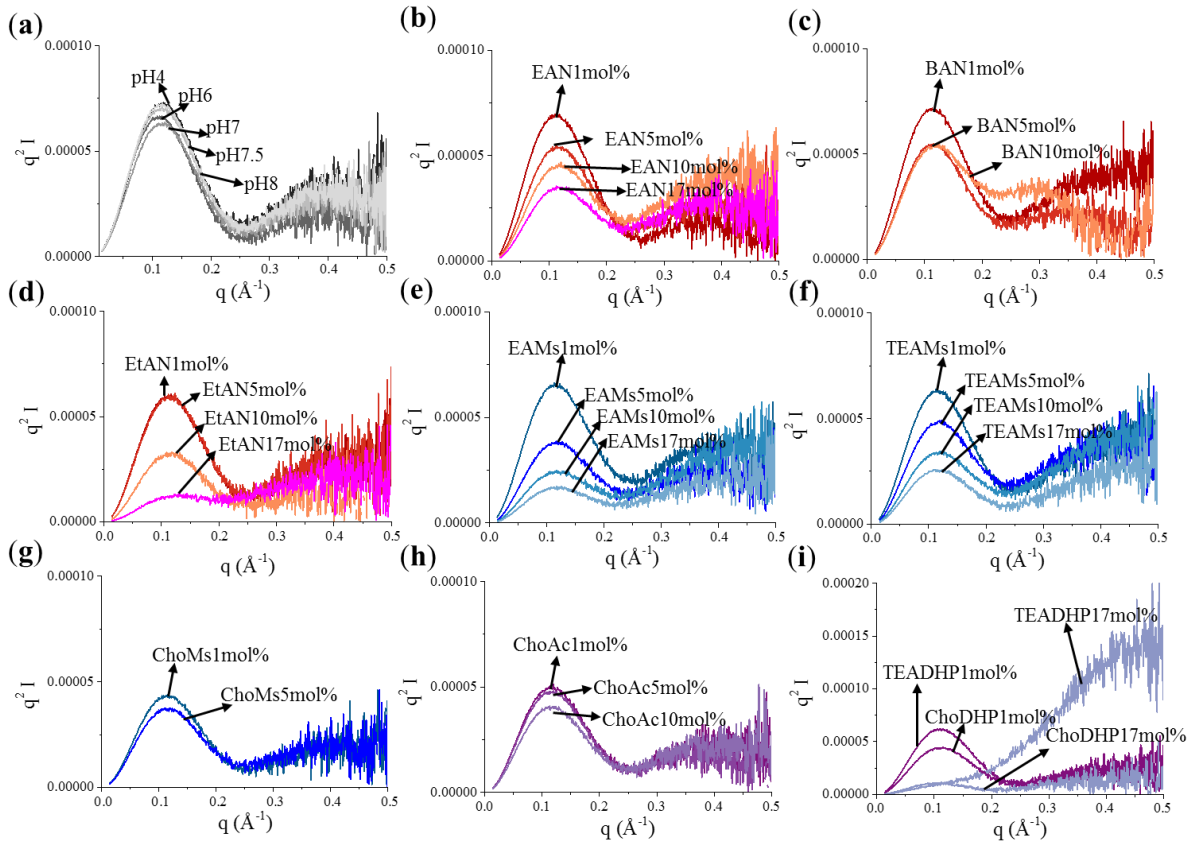

Figure S6. Kratky plots of lysozyme in buffer at different pH a) and IL-water mixtures of b) EAN, c) BAN, d) EtAN, e) TEAMs, f) EAMs, g) ChoMs, h) ChoAc and i) TEADHP and ChoDHP.

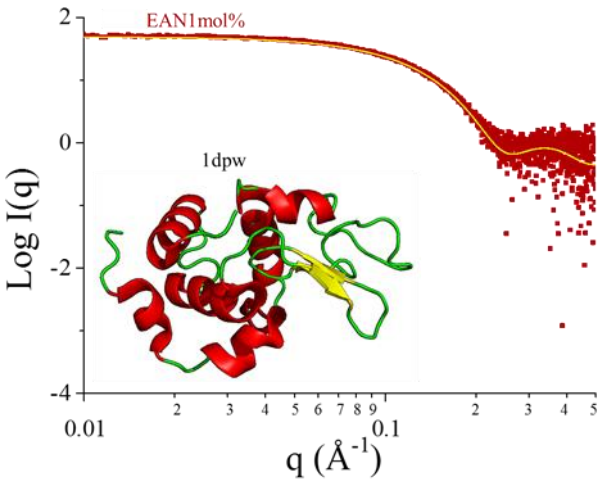

Figure S7. CRYSOL fitting of SAXS data of 1 mol% EAN with 1dpw lysozyme pdb model. The images were generated using PyMol (PyMol molecular graphics system, version 1.3; Schrödinger, LLC).

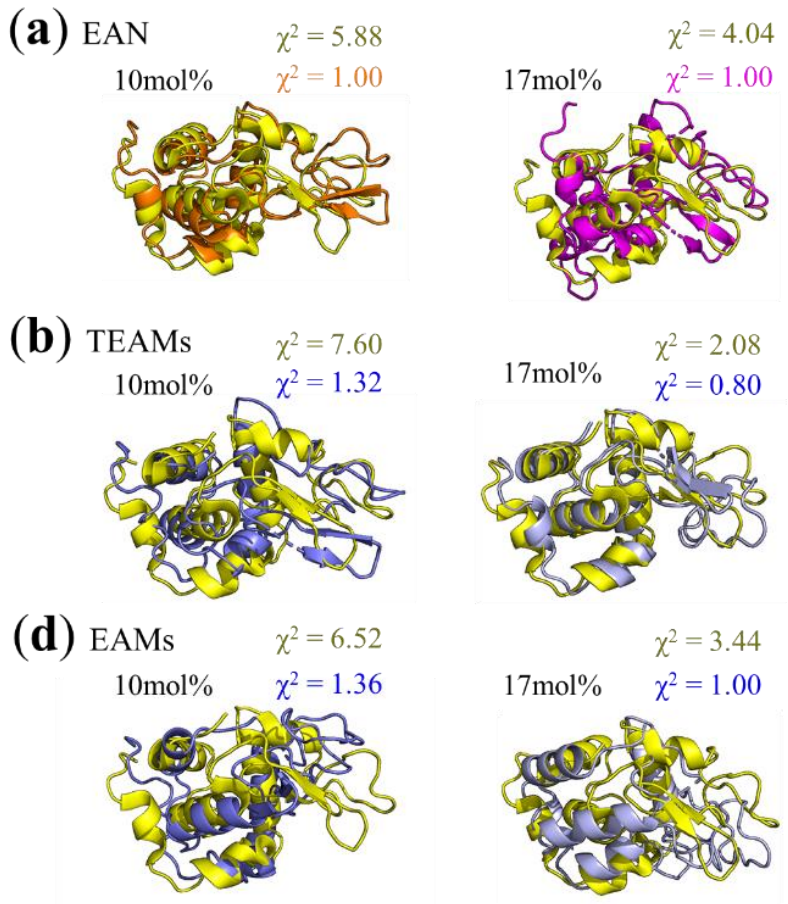

Figure S8. SREFLEX models of lysozyme in IL-water mixtures at 10 and 17 mol%, a) EAN, b) TeaMs and c) EAMs. SREFLEX models were fitted based on corresponding SAXS patterns (Figure 4) and crystal structure of lysozyme (PDB:1dpw), while the best model was selected based on χ^2 value from modellings. The initial lysozyme structure (PDB:1dpw) was aligned with the model and visualized by PyMol.

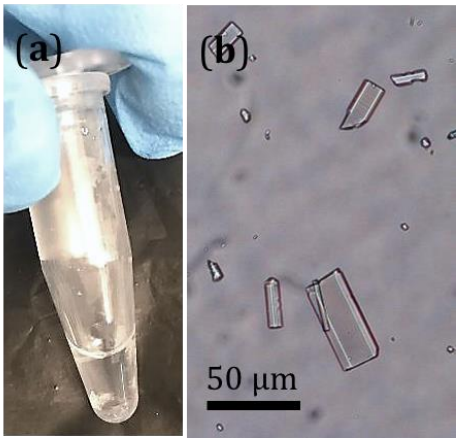

Figure S9. Lysozyme crystals a) grown using batch method in EAN (1 mol%) and b) an optical microscopy image of the crystals. Crystals formed varied in size from 50 × 10µm to a minimum of 10 × 5 µm.

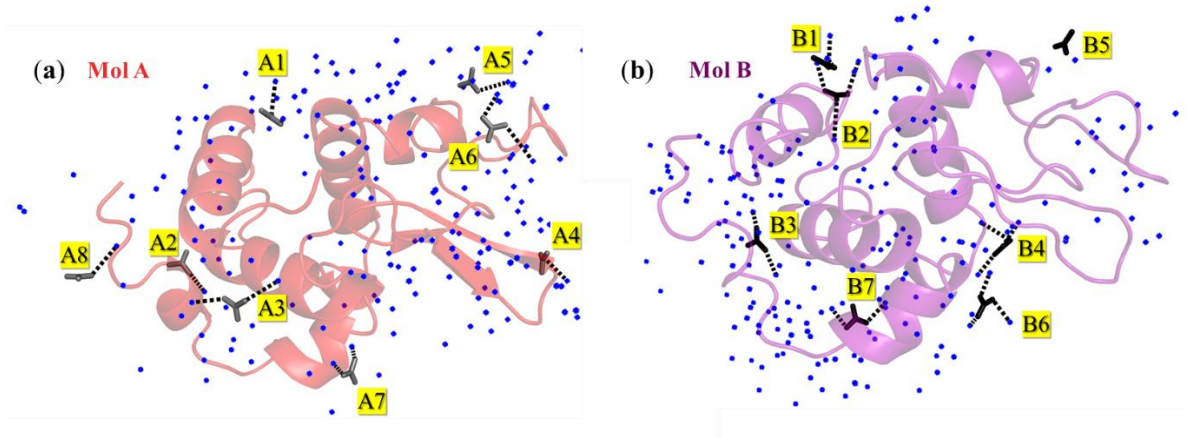

Figure S10. Interactions of nitrate ions with water molecules on the surface of lysozyme crystal in 1 mol% EAN-water mixture, the two lysozyme molecules in a unit cell including a) Mol A and b) Mol B. The IL molecules are labelled A1-A8 and B1-B7.

Table S3. Details of crystallography parameters of lysozyme crystal in EAN1mol%.

Data collection	
Crystal	Lysozyme EAN1mol%
Resolution range (Å)	42.23-1.20 (1.22-1.20)*
Space group	$P12_11$
Unit cell length (Å)	*a*=27.58, *b*=62.64, *c*=59.65
Unit cell angles (º)	*a*=90.00, *b*=90.54, *c*=90.00
Number of reflections	
Total observations	417805 (15754)
Unique reflections	60485 (2781)
Multiplicity	6.9 (6.0)
Completeness (%)	95.5 (85.2)
Mean I/σ	19.5 (3.9)
Mean CC (1/2)	0.999 (0.938)
R-pim	0.019 (0.152)
R-meas	0.050 (0.378)
R-merge	0.043 (0.313)
Refinement statistics	
Resolution range (Å)	43.23-1.20
R-work	0.145
R-free	0.179
Rotamer Outliers	0.46
Number of non-hydrogen atoms	2781
macromolecules	4060
water	254
nitrate ion	60
Protein residues	254
RMS(bonds)	0.0196
RMS(angles)	2.19
Ramachandran favored (%)	98.08
Ramachandran allowed (%)	1.57
Ramachandran outliers (%)	0.39
Rotamer outliers (%)	0.46
Clashscore	0.97
Average B-factor	15.71
macromolecules	16.03
nitrate ion	28.56
water	24.61
PDB ID	7JMU

* Statistics for highest resolution shell shown in parentheses.