Electronic Supplementary Material (ESI) for CrystEngComm

Electronic Supplementary Information

Hofmeister effects of ionic liquids in protein crystallization: direct and water-mediated interactions

Magdalena Kowacz*[a], Abhik Mukhopadhyay[b], Ana Luísa Carvalho[b], José M. S. S. Esperança[a], Maria J. Romão[b], Luís Paulo N. Rebelo*[a]

[a]Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

[b]REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

*magda@itqb.un.pt; luis.rebelo@itqb.unl.pt

Table S1. Details of data collection statistics for lysozyme

Dataset	Lysozyme – [Ch][Ac]
X-ray source	Soleil (Proxima I)
Crystal data	
Unit cell parameters (Å)	*a*=78.35 *b*=78.35 *c*=36.90
Space group	$P4_32_12$
Molecules per ASU	1
Mosaicity	0.28
Matthews coefficient ($Å^3$ Da^{-1})	1.98
Solvent content (%)	38.01

Max. resolution (Å)	1.50
Data collection and processing	
Resolution limits (Å)	39.17-1.50 (1.51 – 1.50)
Wavelength (Å)	0.8856
No. of observed reflections	132855 (19918)
No. of unique reflections	20915 (3118)
R_{sym}	5.8 (60.0)
R_{meas}	6.2 (62.0)
R_{mrgd-F}	11.6 (50.9)
Completeness (%)	99.6 (100)
$<I/\sigma I>$	19.33 (3.19)
Refinement statistics	
Resolution (Å)	30.17-1.50
Reflections used	17978
R_{work} (%)	16.75
R_{free}[a] (%)	21.09
Number of water molecules	172
Temperature factors (Å^2)	
Average for protein atoms	18.04
Average for choline	33.27
Average for acetate	33.53
Average for solvent	33.15

Ramachandran plot: Residues other than Gly and Pro in :	
Most favored regions (number)	125
Additional allowed regions (number))	4
Disallowed regions (number)	0
PDB code	4AGA

Table S2. Data collection and refinement statistics for Ribonuclease A

Dataset	Ribonuclease
Xray source	Soleil (Proxima I)
Crystal data	
Unit cell parameters (Å) (°)	*A*=64.00 *b*=64.00 *c*=63.61
Molecules per ASU	1
Mosaicity	0.28
Matthews coefficient ($Å^3$ Da^{-1})	2.70
Solvent content (%)	54.41
Max. resolution (Å)	1.58
Data collection and processing	
Space group	$P3_221$

Resolution limits (Å)	31.81-1.58 (1.67-1.58)
Wavelength (Å)	0.8856
No. of observed reflections	70218 (10743)
No. of unique reflections	19704 (2972)
R_{pim}	0.019(0.025)
R_{mrgd}	0.036(0.047)
Completeness (%)	93.7(98.2)
$<I/\sigma I>$	26.7(19.2)
Refinement statistics	
Resolution (Å)	55.43-1.58
Reflections used	18627
R_{work} (%)	16.24
R_{free}[a] (%)	19.56
Number of water molecules	172
Ramachandran plot[b]: Residues other than Gly and Pro in :	
Most favored regions (number)	117
Additional allowed regions (number))	4
Disallowed regions (number)	0
PDB code	4AO1

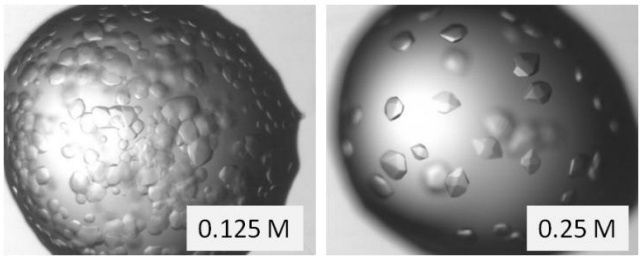

Fig. S1 Effect of increasing concentration of [Ch][C_1SO_3] on RNase crystallization at a given concentration of protein and inorganic precipitants (20 mg/ml and 1/4 reservoir solution, respectively).

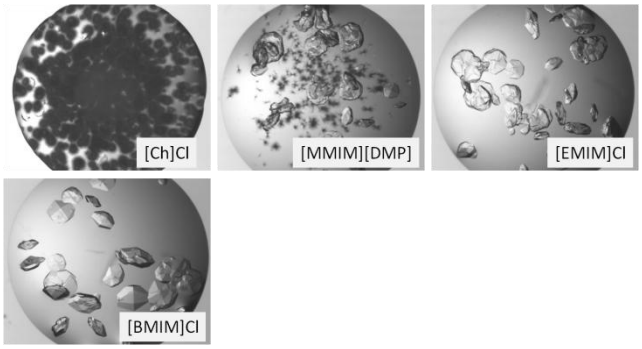

Fig. S2 Effect of ILs on the crystallization of lysozyme at 0.06 M concentration of the IL. Note that the effect of [MMIM][DMP] is not directly comparable within this series due to change of anion. Nevertheless, the nucleation density of the lysozyme crystals in the presence of [MMIM][DMP] is always lower than in the presence of any [Ch]-based ILs and higher than in the presence of any [EMIM]- or [BMIM]-based IL (irrespectively of the anion).

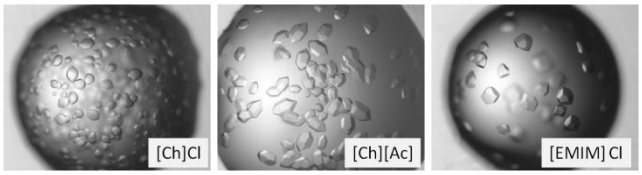

Fig. S3 Effect at a given concentration (0.25 M) of particular ionic liquids on RNase crystallization (at fixed concentration of the protein and inorganic precipitants, here 20 mg/ml and 1/4 of the reservoir solution).

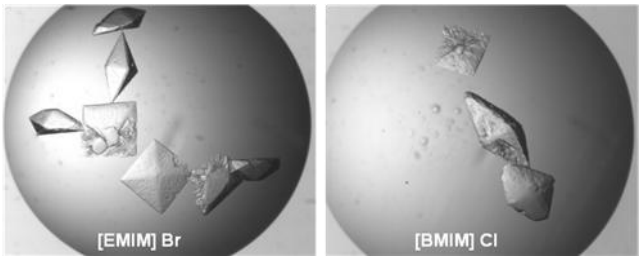

Fig. S4 Dissolution of the originally regular and smooth lysozyme crystals grown in the presence of 0.25 M of initial concentration of [EMIM]Br and [BMIM]Cl.

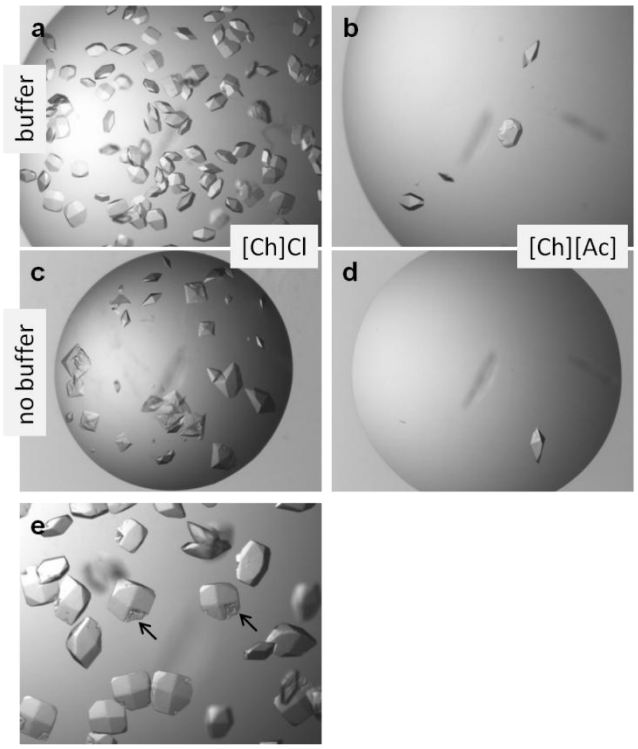

Fig. S5 Lysozyme crystals grown at 4°C in 1 M solution of a) [Ch]Cl and b) [Ch][Ac] in the presence of 25 mM sodium acetate buffer (pH = 4.57) and in 1 M solution of: c) [Ch]Cl and d) [Ch][Ac] without buffer (in either reservoir or protein solution). Image e) presents the partial dissolution of the crystals grown in a buffered solution of 1 M [Ch]Cl after placing the crystals at 20°C.