

## **Extraction and separation of proteins by Ionic Liquid Aqueous Two-Phase System**

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### **Electronic Supplementary Information**

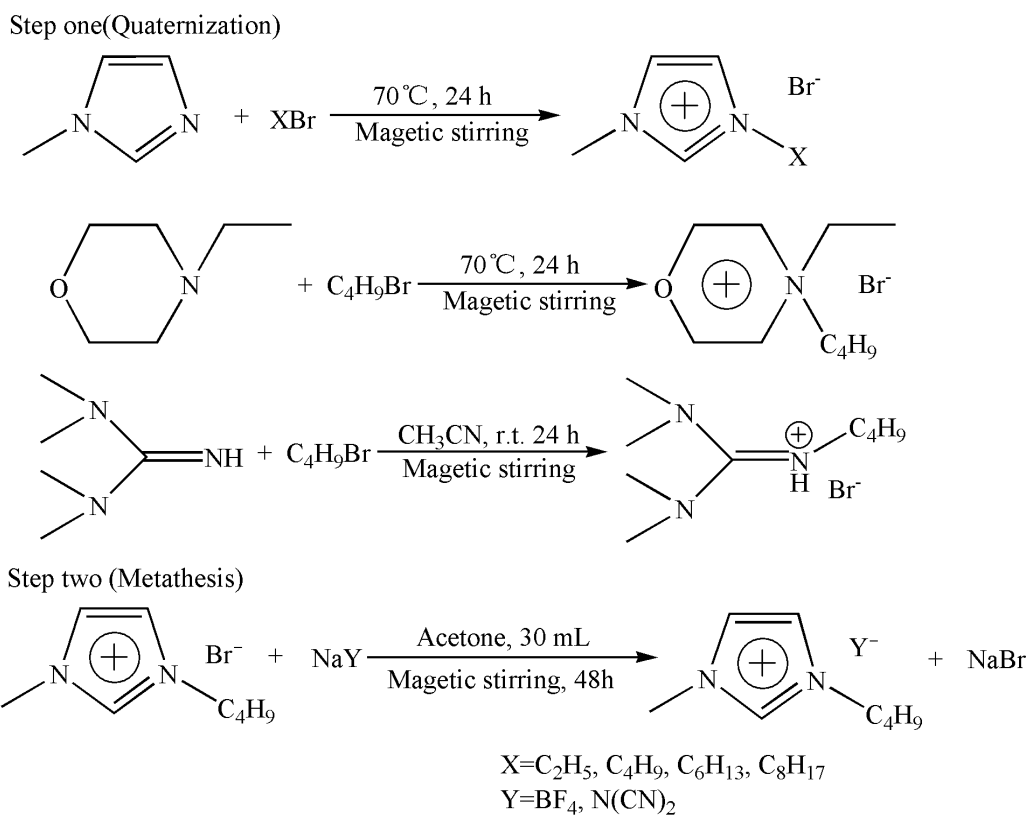


Fig. S1 Flow charts of synthesis process

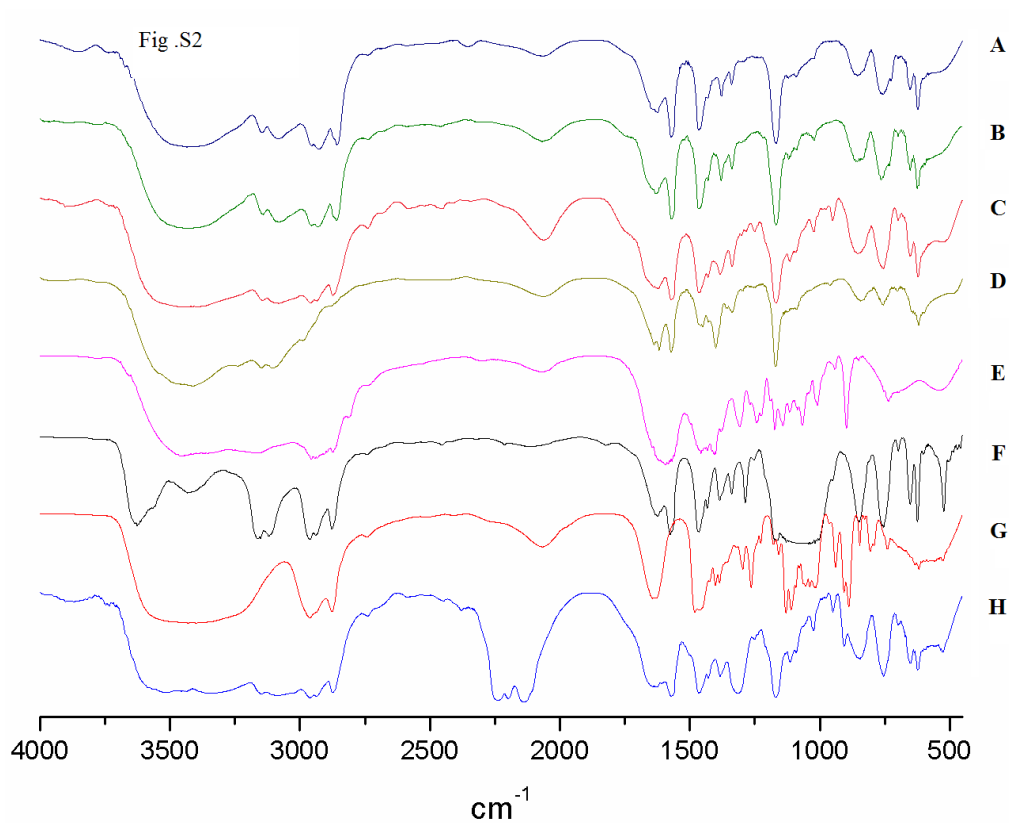


Fig. S2 Infrared spectroscopy of ILs: A. [omim]Br, B. [hmim]Br, C. [bmim]Br, D. [emim]Br, E. [btmg]Br, F. [bmim]BF<sub>4</sub>, G. [nebm]Br, H. [bmim]N(CN)<sub>2</sub>.

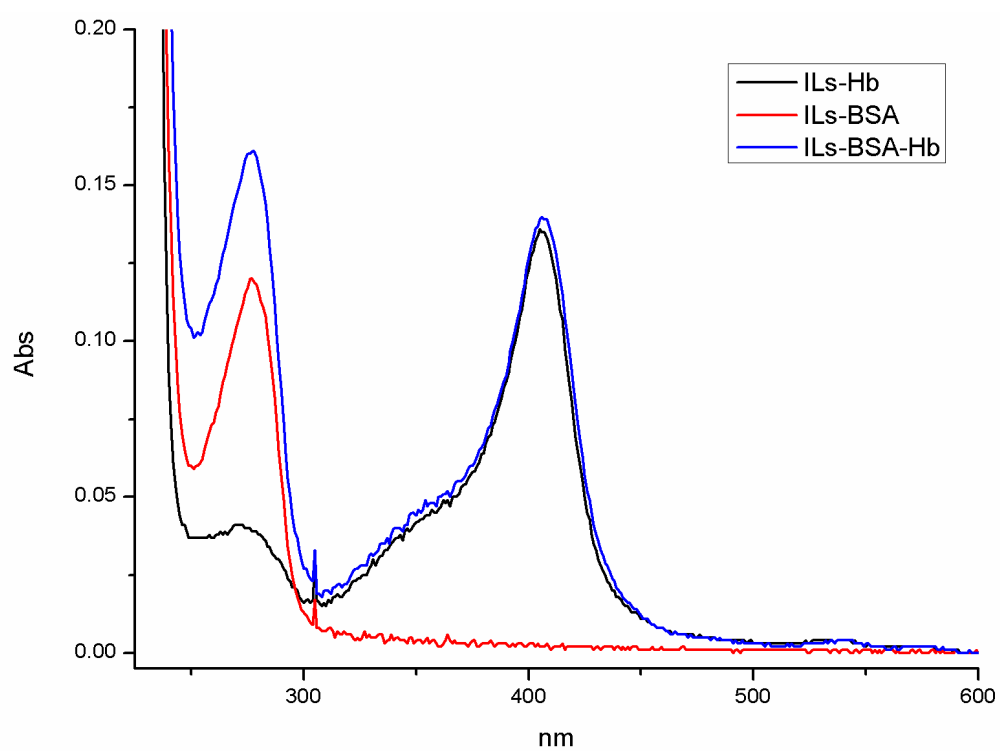


Fig. S3 UV spectrum of the mixed sample extraction

**Table S1**

**(A) <sup>1</sup>H-NMR spectra of the investigated ILs**

ILs <sup>b</sup> (D <sub>2</sub> O)	<sup>1</sup> H NMR (δ, ×10 <sup>-6</sup> ) <sup>a</sup>												
	2-H	3-H	4-H	5-H	6-H	7-H	8-H	9-H	10-H	11-H	12-H	13-H	14-H
[emim][Br]	8.72(s, 1H)	-	7.41(d, J=2.0 Hz, 1H)	7.48(s, 1H)	3.88(s, 3H)	4.23(t, J=7.2 Hz, 2H)	1.49(t, J=6.4 Hz, 3H)	-	-	-	-	-	-
[bmim]Br	8.76(s, 2H)	-	7.47(d, J=2.0 Hz, 2H)	7.52(s, 1H)	3.93(s, 4H)	4.23(t, J=7.2 Hz, 3H)	1.882(m, 3H)	1.358(q, J=7.6 Hz, 3H)	0.86(t, J=6.4 Hz, 4H)	-	-	-	-
[hmim][Br]	8.72(s, 3H)	-	7.46(d, J=2.0 Hz, 3H)	7.51(s, 1H)	3.92(s, 5H)	4.22(t, J=7.2 Hz, 4H)	1.89(m, 4H)	1.32(q, J=7.6 Hz, 4H)	1.30(d, J=2.0 Hz, 3H)	1.89(m, 4H)	0.88(t, J=6.4 Hz, 5H)	-	-
[omim][Br]	8.69(s, 4H)	-	7.45(d, J=2.0 Hz, 4H)	7.49(s, 1H)	3.90(s, 6H)	4.20(t, J=7.2 Hz, 5H)	1.87(m, 5H)	1.28(q, J=7.6 Hz, 5H)	1.25(d, J=2.0 Hz, 4H)	0.87(m, 5H)	0.85(m, 2H)	1.30(m, 2H)	0.83(t, J=6.4 Hz, 6H)
[bmim][BF <sub>4</sub> ]	8.69(s, 5H)	-	7.44(d, J=2.0 Hz, 5H)	7.49(s, 1H)	3.91(s, 7H)	4.21(t, J=7.2 Hz, 6H)	1.87(m, 6H)	1.35(q, J=7.6 Hz, 6H)	0.94(d, J=2.0 Hz, 5H)	(m, 6H)	7H)	-	-
[bmim][N(CN) <sub>2</sub> ]	8.70(s, 6H)	-	7.41(d, J=2.0 Hz, 6H)	7.46(s, 1H)	3.87(s, 8H)	4.18(t, J=7.2 Hz, 7H)	1.83(m, 7H)	1.31(q, J=7.6 Hz, 7H)	0.90(d, J=2.0 Hz, 6H)	-	-	-	-
[nebm][Br]	4.085(t, J=7.2 Hz, 3H)	3.579(t, J=7.2 Hz, 3H)	-	3.579(s, 1H)	4.085(t, J=7.2 Hz, 3H)	3.538(t, J=7.2 Hz, 3H)	1.365(t, J=6.4 Hz, 4H)	3.447(t, J=7.2 Hz, 3H)	-	1.45(q, J=7.6 Hz, 3H)	1.017(t, J=6.4 Hz, 5H)	-	-
[btmg][Br]	-	3.104(dt, J=7.7 Hz, 12H)	7.56(s, 1H)	1.83(m, 2H)	3.104(dt, J=7.7 Hz, 2H)	1.34(dt, J=14.7 Hz, 2H)	7.4 Hz, 2H)	0.93(q, J=7.3 Hz, 3H)	-	-	-	-	-

<sup>a</sup> Note: <sup>1</sup>H-NMR chemical shifts are reported downfield from trimethylsilane (TMS). Multiplicities are abbreviated as s=singlet, d=doublet, quart =quartet, t=triplet and m= multiplet.

<sup>b</sup> The eight ILs were recorded on Varian-INOVA 400 NMR spectrometry.

**(B) <sup>13</sup>C-NMR spectra of the investigated ILs**

ILs (D <sub>2</sub> O) <sup>b</sup>	<sup>13</sup> C NMR (δ, ×10 <sup>-6</sup> ) <sup>a</sup>												
	2-C	3-C	4-C	5-C	6-C	7-C	8-C	9-C	10-C	11-C	12-C	13-C	14-C
[emim][Br]	206	-	112.46	114.02	26.21	35.39	5.13	-	-	-	-	-	-
[bmim]Br	126.48	-	112.88	114.13	26.41	39.96	21.94	9.43	3.35	-	-	-	-
[hmim][Br]	128.23	-	112.81	114.07	26.33	40.19	19.79	15.63	20.97	12.42	3.89	-	-
[omim][Br]	128.94	-	115.41	116.7	28.93	42.8	22.45	18.57	21.48	21.31	24.27	15.26	6.68
[bmim][BF <sub>4</sub> ]	128.64	-	114.98	116.26	28.37	42.07	24.07	11.54	5.41	-	-	-	-
[bmim][N(CN) <sub>2</sub> ]	128.97	-	115.32	116.66	28.86	42.45	24.45	11.94	5.82	-	-	-	-
[nebm][Br]	52.997	50.327	-	50.327	52.997	47.199	5.752	50.716	15.448	11.977	-0.771	-	-
[btmg][Br]	160.536	-	39.809	31.143	29.038	19.242	13.04	-	-	-	-	-	-

<sup>a</sup> Note: <sup>13</sup>C-NMR chemical shifts are reported downfield from trimethylsilane (TMS). <sup>b</sup> The eight ILs were recorded on Varian-INOVA 400 NMR spectrometry.

**Table S2 The protein concentrations, volumes and the partition coefficients in single factor experiments**

ILs	C <sub>t</sub>	C <sub>b</sub>	D	Salt content	C <sub>t</sub>	C <sub>b</sub>	D	Sample amount	C <sub>t</sub>	C <sub>b</sub>	D	Shaking time	C <sub>t</sub>	C <sub>b</sub>	D
	(mg/ml)	(mg/ml)		(%)	(mg/ml)	(mg/ml)		(mg/ml)	(mg/ml)	(mg/ml)		(min)	(mg/ml)	(mg/ml)	
1.0mmol	3.3403	55.95014	16.7502	35%	3.2706	10.7169	3.2767	10mg/ml	0.0535	9.2729	173.3879	1 min	2.5591	6.3119	2.4664
1.5mmol	1.4801	25.3997	17.1603	40%	2.2206	13.1639	5.9280	15mg/ml	0.1539	12.5352	81.4632	2 min	1.1744	8.7591	7.4582
2.0mmol	0.5332	19.2002	36.0128	45%	2.062	13.1639	6.3840	20mg/ml	0.3571	16.2637	45.5451	4 min	1.0798	8.9222	8.2622
2.5mmol	0.4852	16.1005	33.181	50%	1.1436	15.2848	13.3658	25mg/ml	0.6327	19.6814	31.1048	6 min	0.7962	9.4117	11.8196
3.0mmol	0.306	13.9797	45.6839	55%	0.3119	16.2637	52.132	30mg/ml	0.7971	24.0312	30.1468	8 min	0.6601	10.5537	15.9872
3.5mmol	0.303	13.3271	43.9771	60%	0.7917	15.6111	19.7197	35mg/ml	1.5578	27.1382	17.4208	10 min	0.4127	10.0642	24.3828
					Lys			Hb			BSA				
Temperature	C <sub>t</sub>	C <sub>b</sub>	D	PH	Addition	C <sub>t</sub>	C <sub>b</sub>	D	C <sub>t</sub>	C <sub>b</sub>	D	C <sub>t</sub>	C <sub>b</sub>	D	
(°C)	(mg/ml)	(mg/ml)			(mg)	(mg/ml)	(mg/ml)		(mg/ml)	(mg/ml)		(mg/ml)	(mg/ml)		
20	1.7483	10.8389	4.9166	7	10	5.6759	1.1160	5.0859	3.9419	3.4505	1.1424	5.4963	1.2456	4.4124	
25	1.2795	13.1018	8.1206	8	10	5.9070	4.5667	1.2935	6.2353	1.2879	4.8412	6.4751	0.98536	6.5720	
30	0.9281	17.769	10.4916	9	10	4.8382	8.6702	0.5580	6.9409	0.9077	7.6466	7.6171	0.8619	8.8374	
35	0.718	13.1018	14.4711	10	10	4.3183	11.3657	0.3799	6.8527	0.8059	8.5028	7.1277	0.8442	8.4436	
40	0.6043	13.3075	17.4626	11	10	4.0294	12.4568	0.3235	7.2056	0.1939	37.1575	6.8014	0.6214	10.9447	
45	0.4281	14.5417	26.9343	12	10	2.6284	17.4055	0.1510	5.0004	2.2801	2.1930	5.9857	1.0167	5.8875	
				13	10	2.3539	18.1109	0.1299	4.4712	2.9824	1.4992	5.8226	1.4484	4.0199	