Supporting Information

Integrated extraction-preservation strategies for RNA using biobased ionic liquids

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EXPERIMENTAL SECTION

Ionic liquids synthesis. An aqueous solution of each amino acid was added drop-wise to [Ch][OH] methanol solution with a slight equimolar excess. The reaction mixture was kept at room temperature (25 °C) under inert atmosphere and continuous stirring for at least 24 h. The synthesized ILs were washed with acetonitrile/methanol (7:3, v/v) to remove unreacted amino acids. Excess solvent and water were then removed under reduced pressure using a rotary evaporator (60 °C, 2 h) and high vacuum (60 °C, 72 h). The dried ILs were finally collected and stored under dark and refrigerated conditions.

AA-IL-based ABS phases identification. The IL and PPG-rich phase were identified by conductivity (50 µL of each ABS phase were diluted in 5 mL of distilled water) using an electrode from Mettler Toledo (Lab 731-ISM). The phase with a higher conductivity corresponds to the IL-rich phase.

Bacterial lysate preparation from *Escherichia coli* DH5α cultivations. The bacterial lysate sample was obtained from a cell culture of *Escherichia coli* DH5α, and as previously described.[1] Cell growth was carried out in shake flasks at 37 °C and 250 rpm containing 250 mL of Terrific Broth medium (12 g/L tryptone, 24 g/L yeast extract, 4 mL/L glycerol, 0.017 M KH_2PO_4, 0.072 M K_2HPO_4). The bacterial growth was suspended at the beginning of the logarithmic decline phase, approximately after 8 h (OD_{600} ± 5.4). Cells were recovered by centrifugation (4,000 × g, 10 min, 4 °C), and the bacterial pellets were stored at -20 °C. Bacterial pellets were lysed based on the protocol described by Chomczynski and co-workers[1]. Briefly, 50 mL of bacterial pellets were resuspended in 5 mL of denaturing cell lysis solution (4 M guanidine thiocyanate; 25 mM sodium citrate, pH 7.0; 0.5 % (m/v) N-laurosylsarcosine and 0.1 M β-mercaptoethanol) to perform lysis and then incubated on ice for 10 min. After incubation, the suspension was centrifuged at 19,000 × g and 4 °C, for 30 min at 4 °C

and the soluble nucleic acids present in the supernatant was concentrated by the addition of 5 mL of ice-cold isopropanol. The precipitate was recovered by centrifugation at 16,000 × g for 20 min at 4 °C. The supernatant was discarded, and the resulting pellet was washed with 2.5 mL of 75 % ethanol and incubated at room temperature for 10 min.[2, 3] After centrifugation at 16,000 × g for 5 min at 4 °C, the air-dried pellet was dissolved in 2 mL of 0.05% DEPC-treated water and incubated for 10 min at 60 °C to ensure complete solubilization.

Figures/Tables

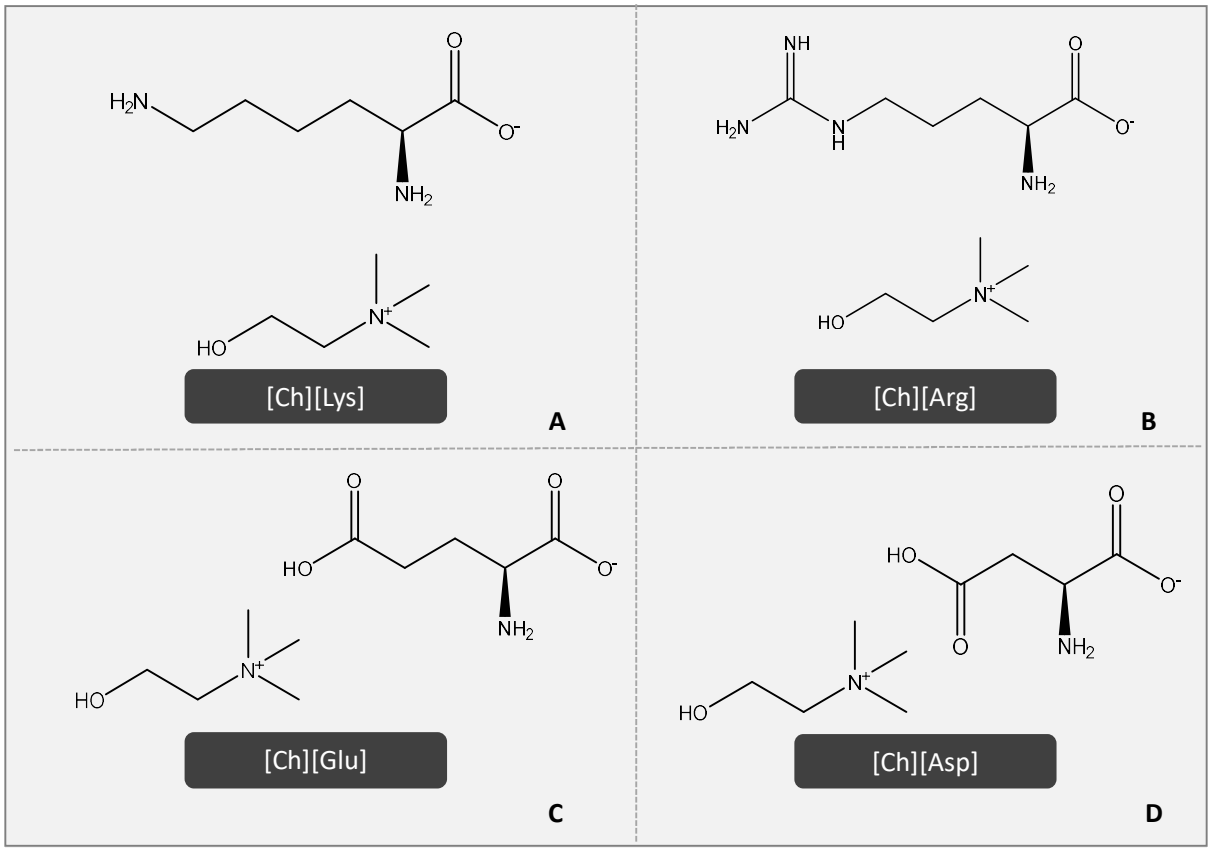

Figure S1 – Chemical structures of the studied AA-ILs.

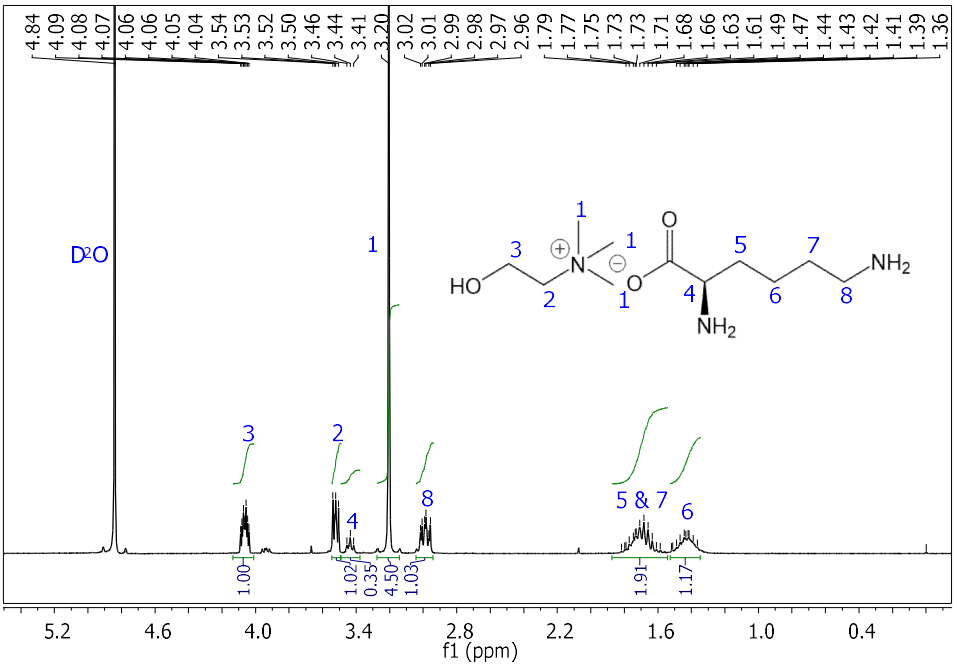

Figure S2 – **[Ch][Lys]** - ^{1}H NMR (D_2O, 75 MHz, δ/ppm): 1.36 (m, 2H, -C**H**$_2$-), 1.61-1.79 (m, 4H, -C**H**$_2$-), 2.96 (m, 2H, -C**H**$_2$-N-), 3.20 (s, 9H, -N-C**H**$_3$), 3.41 (t, 1H, -CO-C**H**-N-), 3.50 (m, 2H, -C**H**$_2$-N-), 4.04 (m, 2H, -C**H**$_2$-OH). Elemental analysis: Calculated C 52.99, H 10.91, N 16.85; Found C 50.17, H 10.33, N 13.86.

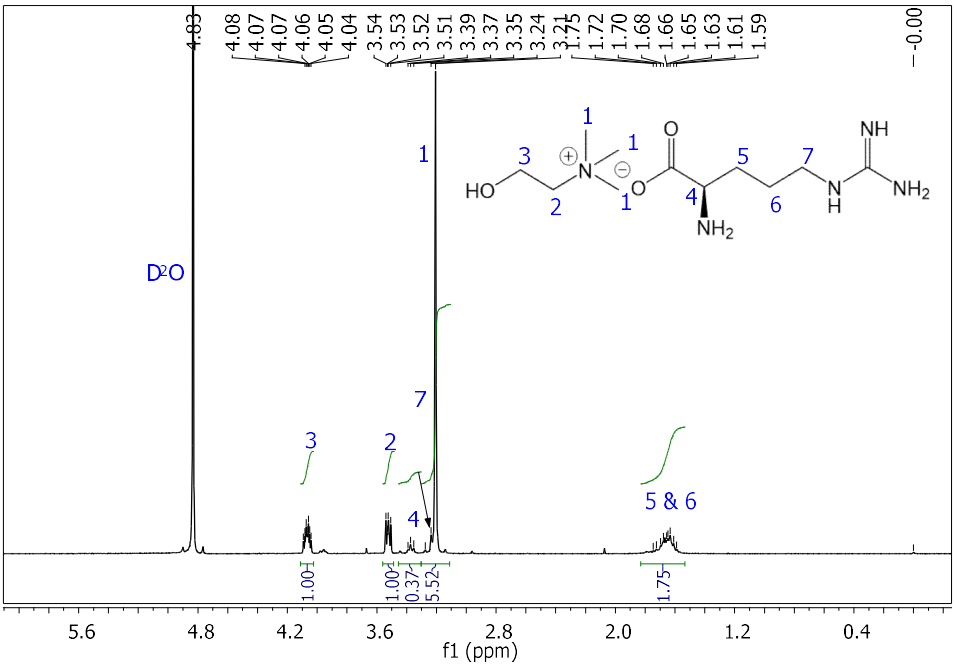

Figure S3 – **[Ch][Arg]** - ^{1}H NMR (D_2O, 75 MHz, δ/ppm): 1.59-1.75 (m, 4H, -CH_2-), 3.21 (s, 9H, -CH_3), 3.24 (s, 2H, -CH_2-N-), 3.35 (t, 1H, -CO-CH-N-), 3.51 (q, 2H, -CH_2-N-), 4.04 (m, 2H, -CH_2-OH). Elemental analysis: Calculated C 47.63, H 9.81, N 25.25; Found C 47.73, H 7.94, N 19.91.

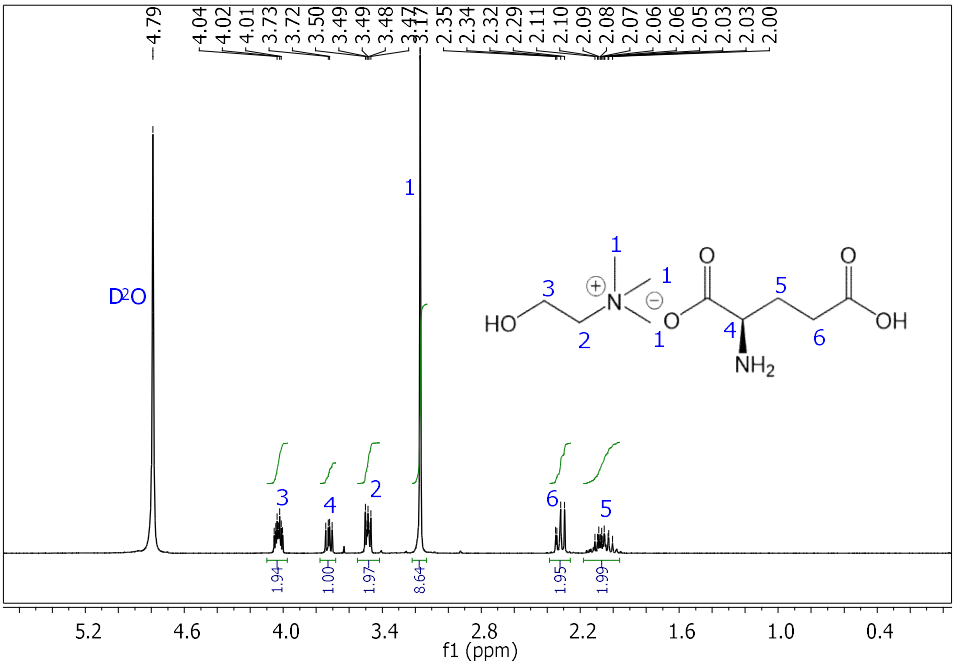

Figure S4 – **[Ch][Glu]** - ^{1}H NMR (D_2O, 75 MHz, δ/ppm): 2.00 (m, 2H, -C**H**$_2$-), 2.29 (dd, 2H, -C**H**$_2$-), 3.17 (s, 9H, -C**H**$_3$), 3.47 (m, 2H, -C**H**$_2$-N-), 3.72 (q, 1H, -C**H**-NH_2), 4.01 (m, 2H, -C**H**$_2$-OH). Elemental analysis: Calculated C 47.99, H 8.86, N 11.19; Found C 47.71, H 8.80, N 10.22.

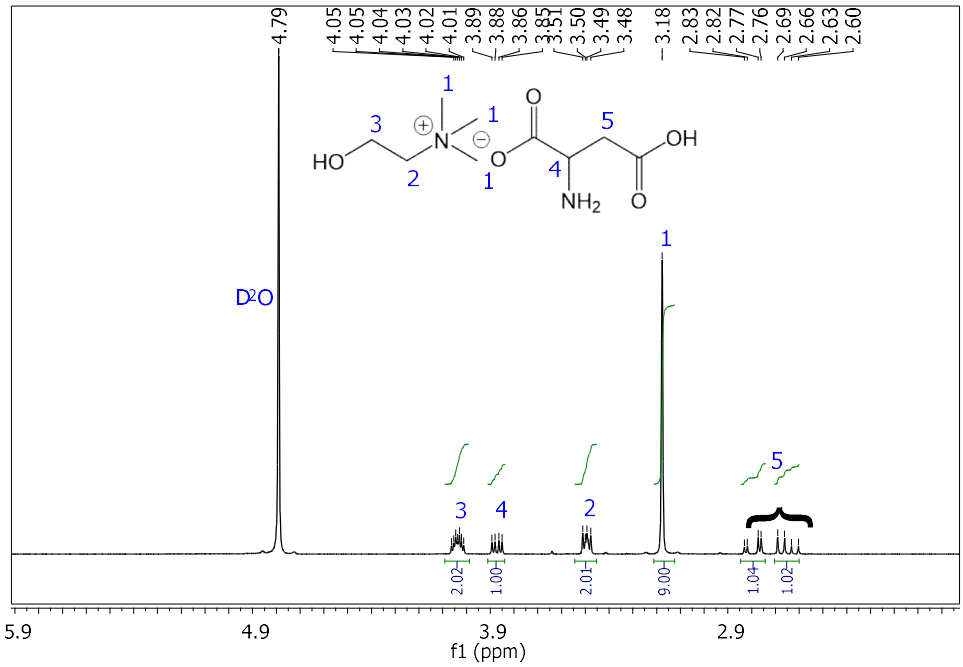

Figure S5 – **[Ch][Asp]** - ^{1}H NMR (D_2O, 75 MHz, δ/ppm): 2.60 (dd, 1H, -C**H**$_2$-), 2.76 (dd, 1H, -C**H**$_2$-), 3.18 (s, 9H, -N-C**H**$_3$), 3.48 (m, 2H, -C**H**$_2$-N-), 3.85 (q, 1H, -CO-C**H**-NH$_2$), 4.01 (m, 2H, -C**H**$_2$-OH). Elemental analysis: Calculated C 45.75, H 8.53, N 11.86; Found C 45.43, H 8.86, N 11.75.

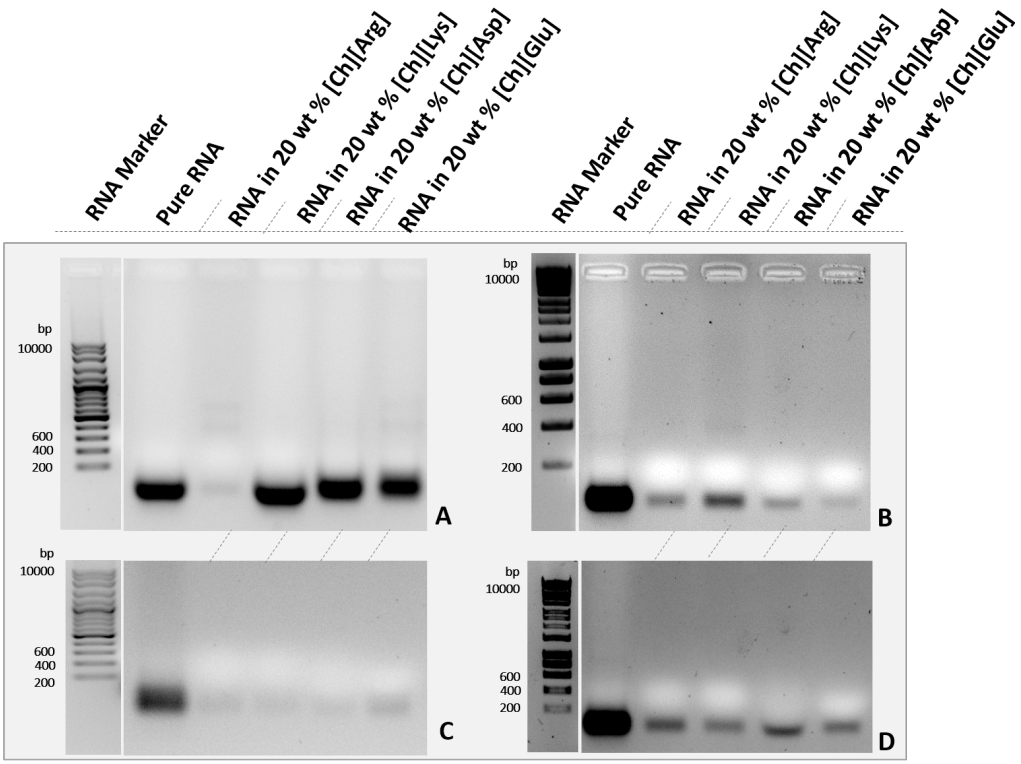

Figure S6 – Agarose gel electrophoresis corresponding to RNA recovery tests from the IL-rich phase using a protocol based on alcohol addition; **A** – precipitation with ethanol; **B** – precipitation with propan-2-ol; **C** – precipitation with propan-1-ol; and **D** – precipitation with butanol.

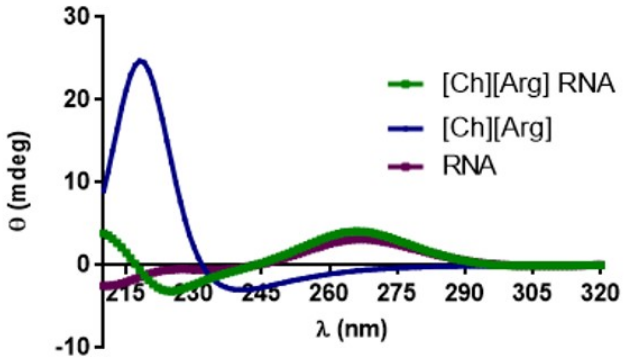

Figure S7 – Representative CD spectra of [Ch][Arg] (blue), RNA dissolved in 20 wt % [Ch][Arg] (green) and RNA in 0.05 % DEPC-treated water (purple).

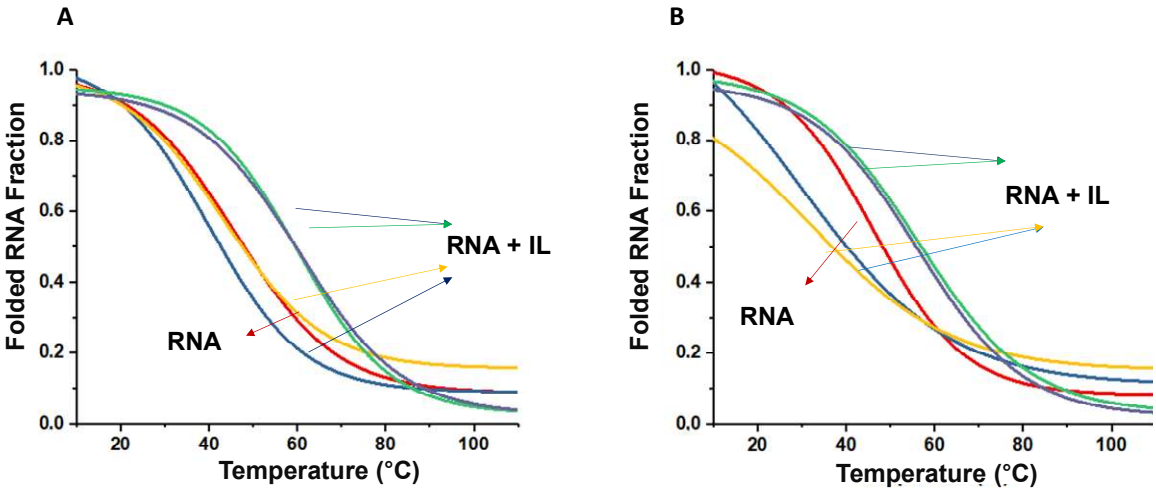

Figure S8 – CD melting curves (265 nm) of RNA in 0.05 % DEPC-treated water, after incubation with ILs for 1 hour (A) and after 15 days (B). RNA in 0.05 % DEPC-treated water: – Red. RNA in the presence of 20 wt % ILs: [Ch][Lys] – yellow; [Ch][Arg] – blue; [Ch][Glu] – green; and [Ch][Asp] –purple.

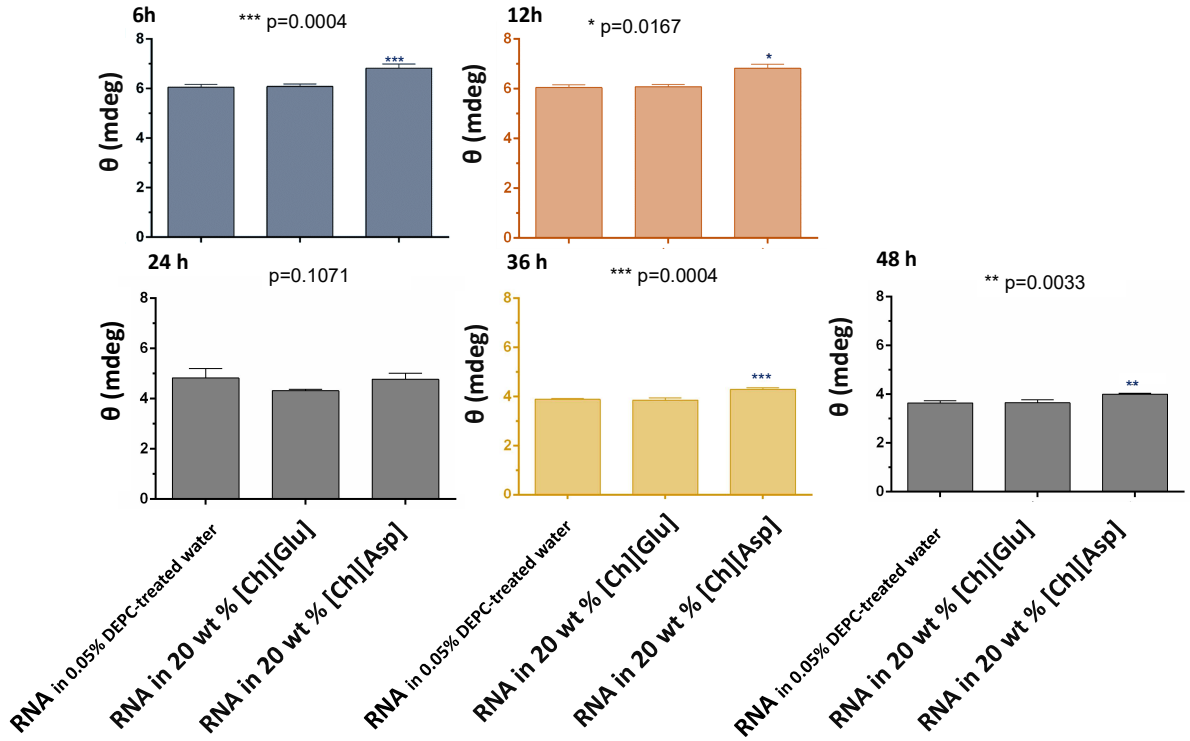

Figure S9 – RNA average ellipticity at 265 nm dissolved in 0.05 % DEPC-treated water and in aqueous solutions of 20 wt % of [Ch][Glu] or [Ch][Asp], in presence of 1 % (v/v) FBS. Distinct incubation periods were studied: 6, 12, 24, 36 and 48 h.

Table S1 – Weight fraction data of the binodal curves of ABS formed by PPG400 (w_1) + AA-IL (w_2) + H_2O at 25 °C and atmospheric pressure.

[Ch][Lys]		[Ch][Arg]		[Ch][Glu]		[Ch][Asp]	
w_1	w_2	w_1	w_2	w_1	w_2	w_1	w_2
49.84	3.32	73.74	1.76	57.06	21.39	53.12	6.02
43.77	4.11	62.99	3.24	54.36	22.61	50.02	7.45
35.44	5.52	58.85	4.04	42.95	26.65	44.63	7.89
29.60	7.10	44.27	7.26	42.62	27.16	42.45	8.69
24.58	8.67	34.25	9.57	42.62	27.17	39.30	8.89
20.23	10.69	29.40	10.74	36.45	30.92	37.45	9.76
17.48	11.67	26.25	12.34	33.30	34.49	34.95	10.07
14.61	13.36	22.42	14.92	28.92	39.26	32.66	10.74
14.20	13.48	18.90	17.73	25.19	40.48	31.06	11.01
12.88	14.56	16.80	19.12	24.85	40.71	29.13	11.73
11.84	14.93	12.37	22.59			20.07	14.84
10.04	17.36					18.13	16.03
						16.11	17.62
						14.33	19.48
						13.03	20.29
						11.89	21.09

Table S2 – *A*, *B* and *C* parameters of the equation proposed by Merchuk *et al.*[4] and respective correlation coefficients, R^2, for the ABS formed by PPG 400, AA-IL, and water at 25 °C.

[Ch][AA]	$A \pm \sigma$	$B \pm \sigma$	10^5 ($C \pm \sigma$)	R^2
[Ch][Lys]	145.9 ± 20.6	-0.63 ± 0.06	2.14 ± 1.27	0.998
[Ch][Arg]	122.6 ± 14.7	-0.40 ± 0.05	4.57 ± 2.32	0.999
[Ch][Glu]	238.7 ± 24.8	-0.70 ± 0.06	0.09 ± 1.46	0.993
[Ch][Asp]	230.5 ± 71.1	-0.60 ± 0.12	3.78 ± 3.01	0.962

Table S3 – Weight fraction compositions for the PPG 400 + AA-IL + H_2O systems at 25 °C, and respective values of tie-line length (TLL). Initial mixture compositions are represented as $[PPG]_M$ and $[IL]_M$, whereas $[PPG]_{PGG}$ and $[IL]_{PGG}$ are the composition of PPG400 and IL at the PPG-rich phase, respectively, and vice-versa. Conductivity values of the top ($Cond_{TOP}$) and bottom phase ($Cond_{BOT}$) are also presented.

[Ch][AA]	Weight fraction composition (wt %)								
	PPG_{PPG}	IL_{PPG}	$Cond_{Top}$ (μ/cm)	PPG_M	IL_M	PPG_{IL}	IL_{IL}	$Cond_{BOT}$ (μ/cm)	TLL
[Ch][Lys]	93.31	0.47	18.72	19.88	20.2	4.83	24.19	550.19	92.57
[Ch][Arg]	96.4	0.35	119.51	20.01	19.96	11	22.27	1146.80	88.17
[Ch][Glu]	90.96	1.94	14.10	19.89	20.14	8.46	23.06	576.07	85.15
[Ch][Asp]	90.23	2.42	140.04	19.99	20.00	8.16	22.92	576.07	84.60

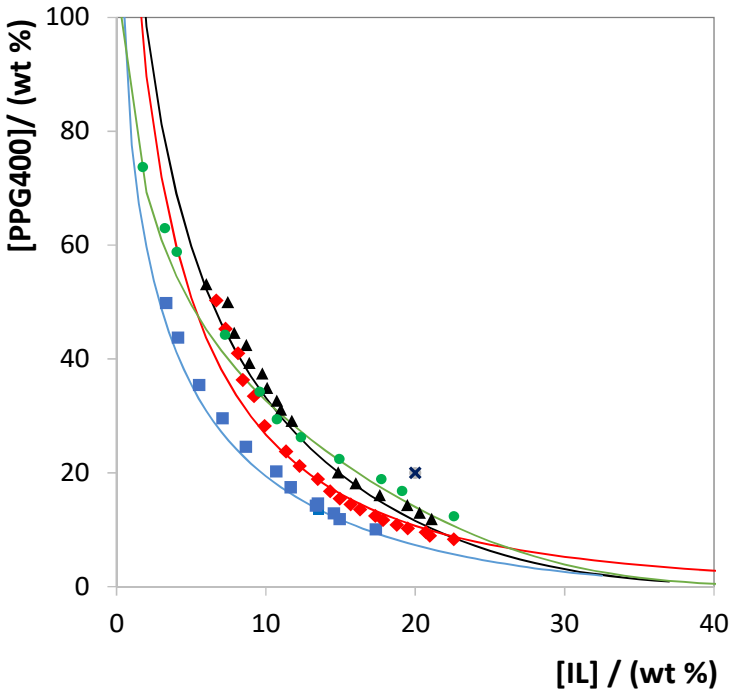

Figure S10 – Phase diagrams of ABS composed of PPG 400 + AA-ILs + H_2O and respective fitting (-)[4] at 25 °C: [Ch][Lys] (■); [Ch][Arg] (●); [Ch][Glu] (◆); [Ch][Asp] (▲). Mixture point selected for the RNA extraction assays (×).

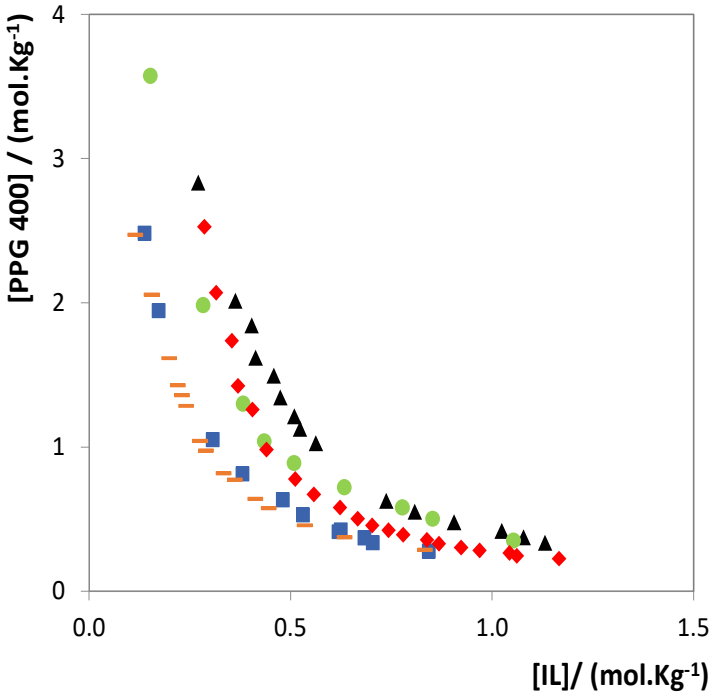

Figure S11 – Phase diagrams in molality units of ABS composed of PPG 400 + AA-IL + H_2O at 25 °C: [Ch][Lys] (■); [Ch][Arg] (●); [Ch][Glu] (◆); [Ch][Asp] (▲). Binodal curve for the ABS formed by PPG 400 + [Ch][Lys] + H_2O from the literature (—)[5].

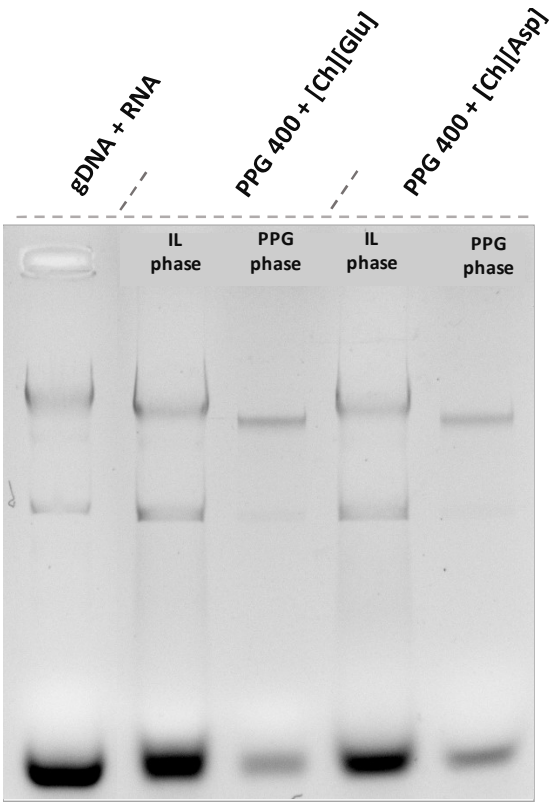

Figure S12 – Agarose gel electrophoresis of the ABS coexisting phases with 20 wt % PPG 400 + 20 wt % AA-IL + 60 wt % bacterial lysate (gDNA +RNA). The initial lysate sample mainly containing RNA and genomic DNA was also analyzed.

References

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