

Supplementary Material

Sustainable depletion of high-abundance serum proteins using aqueous biphasic systems based on glycine-betaine analogue ionic liquids for the detection of masked biomarkers

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Methods

Synthesis of AGB-ILs

To synthesize AGB-ILs, the general synthetic route schematized in Figure S1 was followed. Specific experimental procedures for each of the AGB-ILs investigated are explained below.

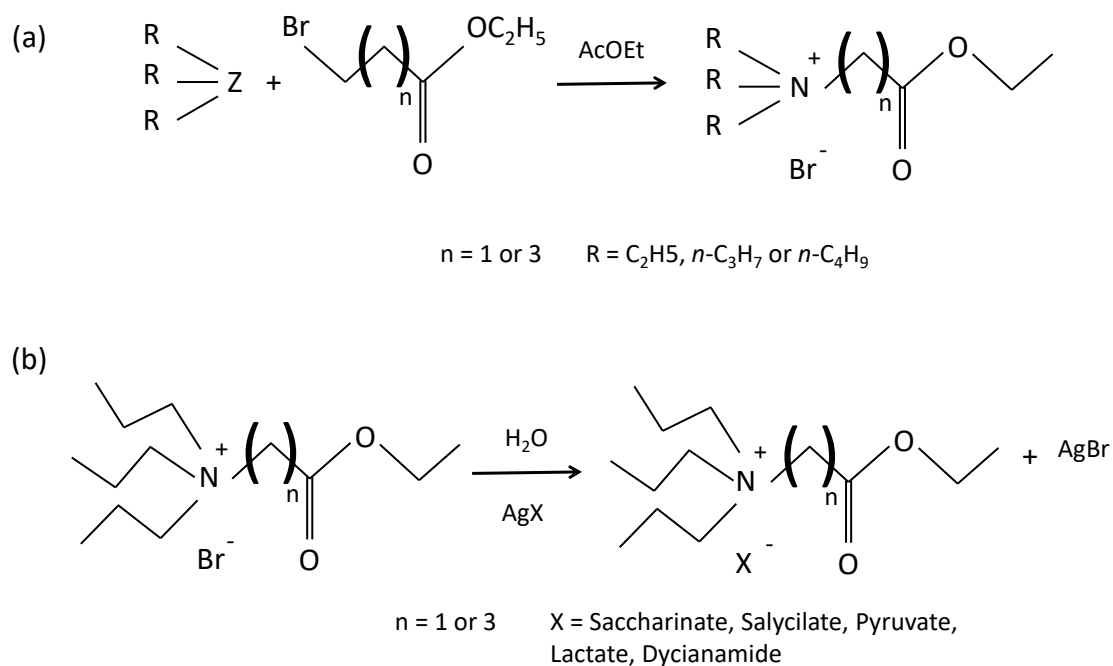


Figure S1. Synthesis pathway for AGB-ILs. (a) Preparation of the bromide AGB-IL precursors. (b) Anion metathesis to obtain the final AGB-ILs.

***N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium saccharinate ([Pr₃NC₄][Sac])**

N,N,N-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium bromide ([Pr₃NC₄]Br) was synthesized adopting well-established protocols reported by us [1]. [Pr₃NC₄][Sac] was synthesized by the following reaction: [Pr₃NC₄]Br (16 g, 0.05 mol) in water (150 mL) was added silver saccharinate (17.4 g, 0.06 mol) while stirring. The mixture was refluxed for 12 h and then stirred at room temperature for 2 h. AgBr precipitate was filtered and washed with ethyl acetate and ethyl ether, then discarded. The resulting supernatant was subsequently dried under vacuum to remove the organic solvents and water.

***N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium lactate ([Pr₃NC₄][Lac])**

[Pr₃NC₄][Sac] was synthesized by the following reaction: [Pr₃NC₄]Br (16 g, 0.05 mol) in water (150 mL) was added silver lactate (11.8 g, 0.06 mol) while stirring. The mixture was refluxed for 12 h and then stirred at room temperature for 2 h. AgBr precipitate was filtered and washed with ethyl acetate and ethyl ether, then discarded. The resulting supernatant was subsequently dried under vacuum to remove the organic solvents and water.

***N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium pyruvate ([Pr₃NC₄][Pyr])**

[Pr₃NC₄][Pyr] was synthesized by the following reaction: [Pr₃NC₄]Br (16 g, 0.05 mol) in water (150 mL) was added silver pyruvate (11.6 g, 0.06 mol) while stirring. The mixture was refluxed for 12 h and then stirred at room temperature for 2 h. AgBr precipitate was filtered and washed with ethyl acetate and ethyl ether, then discarded. The resulting supernatant was subsequently dried under vacuum to remove the organic solvents and water.

***N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium dicyanamide ([Pr₃NC₄][Dca])**

[Pr₃NC₄][Dca] was synthesized by the following reaction: [Pr₃NC₄]Br (16 g, 0.05 mol) in water (150 mL) was added silver dicyanamide (10.4 g, 0.06 mol) while stirring. The mixture was refluxed for 12 h and then stirred at room temperature for 2 h. AgBr precipitate was filtered and washed with ethyl acetate and ethyl ether, then discarded. The resulting supernatant was subsequently dried under vacuum to remove the organic solvents and water.

***N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium salicylate ([Pr₃NC₄][Sal])**

[Pr₃NC₄][Sal] was synthesized by the following reaction: [Pr₃NC₄]Br (16 g, 0.05 mol) in water (150 mL) was added silver salicylate (14.7 g, 0.06 mol) while stirring. The mixture was refluxed for 12 h and then stirred at room temperature for 2 h. AgBr precipitate was filtered and washed with ethyl acetate and ethyl ether, then discarded. The resulting supernatant was subsequently dried under vacuum to remove the organic solvents and water.

***N,N,N*-tri(*n*-propyl)(2-ethoxy-2-oxoethyl)-1-ammonium salicylate ([Pr₃NC₂][Sal])**

N,N,N-tri(*n*-propyl)(2-ethoxy-2-oxoethyl)-1-ammonium bromide ([Pr₃NC₂]Br) was synthesized according to previously reported protocols [2]. [Pr₃NC₂][Sal] was synthesized by the following reaction: [Pr₃NC₂]Br (14.1 g, 0.05 mol) in water (150 mL) was added silver salicylate (14.7 g, 0.06 mol) while stirring. The mixture was refluxed for 12 h and then stirred at room temperature for 2 h. AgBr precipitate was filtered and washed with ethyl acetate and ethyl ether, then discarded. The resulting supernatant was subsequently dried under vacuum to remove the organic solvents and water.

Protein quantification by SE-HPLC-UV

Table S1. Analytical performance parameters obtained from external calibration curves by SE-HPLC-UV.

Protein	IgG	HSA	Transferrin
Calibration range (g·L⁻¹)	0.1 – 1	0.5 – 5	0.5 – 10
Correlation coefficient (R²)	0.9991	0.9972	0.9925
Limit of detection, LOD (mg·L⁻¹)^a	37	432	1208
Limit of quantification, LOQ (mg·L⁻¹)^b	112	1310	3659

^{a, b} LOD and LOQ were calculated as $3.3 \times (\sigma/S)$ and $10 \times (\sigma/S)$, respectively, where σ is the residual standard deviation and S is the slope of the calibration curve.

Results

AGB-ILs characterization

Table S2. Characterization of *N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium saccharinate ([Pr₃NC₄][Sac]).

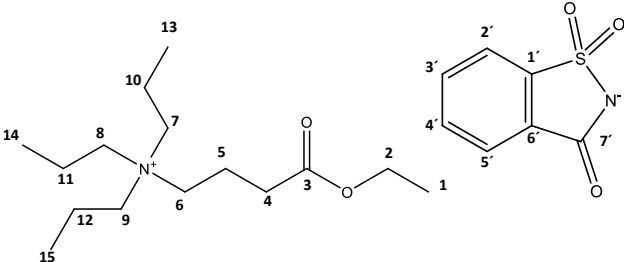
Chemical structure	
¹H NMR (300 MHz, DMSO)	δ 0.89 (9 H, t, <i>J</i> =7.2 Hz, H13, H14, H15), 1.20 (3 H, t, <i>J</i> =7.1 Hz, H1), 1.56-1.69 (6 H, m, H10, H11, H12), 1.79-1.89 (2 H, m, H5), 2.42 (2 H, t, <i>J</i> =6.8 Hz, H4), 3.11-3.20 (8 H, m, H6, H7, H8, H9) 4.08 (2 H, q, <i>J</i> =7.1 Hz, H2), 7.61 (2H, m, H2', H5'), 7.68 (2H, m, H3', H4').
¹³C NMR (75,47 MHz, DMSO)	δ 10.9 (C13, C14, C15), 14.6 (C1), 15.3 (C10, C11, C12), 17.2 (C5), 30.2 (C4), 59.8 (C6, C7, C8, C9), 60.6 (C2), 119.6 (C2'), 123.0 (C5'), 131.6 (C6'), 132.3 (C4'), 135.0 (C3'), 145.6 (C1'), 168.0 (C7'), 172.4 (C3).
Elemental Analysis	C ₂₂ H ₃₆ N ₂ O ₅ S Calculated: C 59.9%, H 8.2%, N 6.3%, O 18.1%, S 7.2%. Experimental: C 56.1, H 8.2%, %, N 6.2%, O 7.0%.
Molecular Weight	440.60 g/mol
Melting Point	98.51 °C
Temperature of Decomposition	178.81 °C

Table S3. Characterization of *N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium lactate ([Pr₃NC₄][Lac]).

Chemical structure	
¹H NMR (300 MHz, DMSO)	δ 0.90 (9 H, t, $J=7.2$ Hz, H13, H14, H15), 1.04 (3 H, d, $J=7.0$ Hz, H1'), 1.20 (3H, q, $J=7.1$ Hz, H1), 1.22 (1 H, q, $J=7.1$ Hz, H2'), 1.57-1.69 (6 H, m, H10, H11, H12), 1.79-1.81 (2 H, m, H5), 2.42 (2 H, t, $J=6.8$ Hz, H4), 3.12-3.20 (8 H, m, H6, H7, H8, H9) 4.08 (2 H, q, $J=7.1$ Hz, H2).
¹³C NMR (75.47 MHz, DMSO)	δ 11.1 (C13, C14, C15), 14.4 (C1), 15.2 (C10, C11, C12), 22.4 (C1'), 30.4 (C5), 31.2 (C4), 59.6 (C7, C8, C9), 61.1 (C6), 67.6 (C2), 154.1 (C2'), 172.7 (C3, C3').
Elemental Analysis	C ₁₈ H ₃₇ NO ₅ Calculated: C 62.2%, H 10.7%, N 4.0%, O 23.0%. Experimental: C 62.8, H 10.0%, %, N 3.7%.
Molecular Weight	347.50 g/mol
Melting Point	33.25 °C
Temperature of Decomposition	189.64 °C

Table S4. Characterization of *N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium pyruvate ([Pr₃NC₄][Pyr]).

Chemical structure	
¹H NMR (300 MHz, DMSO)	δ 0.90 (9 H, t, <i>J</i> =7.2 Hz, H13, H14, H15), 1.20 (3 H, t, <i>J</i> =7.1 Hz, H1), 1.57-1.70 (6 H, m, H10, H11, H12), 1.79-1.81 (2 H, m, H5), 2.00 (3H, s, H1'), 2.42 (2 H, t, <i>J</i> =6.8 Hz, H4), 3.12-3.20 (8 H, m, H6, H7, H8, H9) 4.08 (2 H, q, <i>J</i> =7.1 Hz, H2).
¹³C NMR (75.47 MHz, DMSO)	δ 11.1 (C13, C14, C15), 14.4 (C1), 15.2 (C10, C11, C12), 17.2 (C5), 28.9 (C1'), 30.2 (C4), 59.7 (C6, C7, C8, C9), 60.7 (C2), 172.4 (C3, C3'), 207.6 (C2').
Elemental Analysis	C ₁₈ H ₃₅ NO ₅ Calculated: C 62.5%, H 10.2%, N 4.0%, O 23.1%. Experimental: C 49.5, H 8.9%, %, N 4.7%.
Molecular Weight	345.48 g/mol
Melting Point	30.58 °C
Temperature of Decomposition	172.33 °C

Table S5. Characterization of *N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium dicyanamide ([Pr₃NC₄][Dca]).

Chemical structure	
¹H NMR (300 MHz, DMSO)	δ 0.90 (9 H, t, <i>J</i> =7.2 Hz, H13, H14, H15), 1.20 (3 H, t, <i>J</i> =7.1 Hz, H1), 1.57-1.70 (6 H, m, H10, H11, H12), 1.79-1.81 (2 H, m, H5), 2.43 (2 H, t, <i>J</i> =7.2 Hz, H4), 3.12-3.20 (8 H, m, H6, H7, H8, H9) 4.08 (2 H, q, <i>J</i> =7.2 Hz, H2).
¹³C NMR (75.47 MHz, DMSO)	δ 11.1 (C13, C14, C15), 14.4 (C1), 15.2 (C10, C11, C12), 17.2 (C5), 30.2 (C4), 59.7 (C6, C7, C8, C9), 60.7 (C2), 120.0 (C1'), 172.4 (C3).
Elemental Analysis	C ₁₇ H ₃₂ N ₄ O ₂ Calculated: C 62.9%, H 9.9%, N 17.2%, O 9.8%. Experimental: C 60.1, H 9.8%, N 16.5%.
Molecular Weight	324.47 g/mol
Melting Point	35.99 °C
Temperature of Decomposition	156.59 °C

Table S6. Characterization of *N,N,N*-tri(*n*-propyl)(4-ethoxy-4-oxobutyl)-1-ammonium salicylate ([Pr₃NC₄][Sal]).

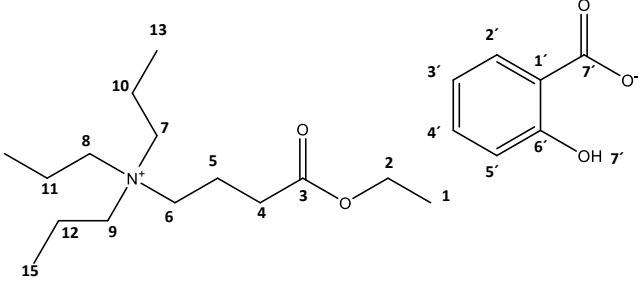
<p>Chemical structure</p>	
<p>¹H NMR (300 MHz, DMSO)</p>	<p>δ 0.89 (9 H, t, <i>J</i>=7.2 Hz, H13, H14, H15), 1.20 (3H, t, <i>J</i>=7.1 Hz, H1), 1.56-1.68 (6 H, m, H10, H11, H12), 1.79-1.89 (2 H, m, H5), 2.40 (2 H, t, <i>J</i>=7.1 Hz, H4), 3.11-3.20 (8 H, m, H6, H7, H8, H9) 4.08 (2 H, q, <i>J</i>=7.1 Hz, H2), 6.54 (1H, dd, <i>J</i>=7.4, 1.2 Hz, H3'), 6.59 (1H, dd, <i>J</i>=8.1, 1.0 Hz, H5'), 7.10 (1H, ddd, <i>J</i>= 8.1, 7.1, 1.9, H4'), 7.63 (1H, dd, <i>J</i>=7.6, 1.9 Hz, H2').</p>
<p>¹³C NMR (75.47 MHz, DMSO)</p>	<p>δ 11.1 (C13, C14, C15), 14.4 (C1), 15.1 (C10, C11, C12), 17.2 (C5), 30.2 (C4), 59.7 (C1'), 60.8 (C5), 116.0 (C5'), 116.2 (C3'), 121.5 (C1'), 130.2 (C4'), 131.8 (C2'), 163.6 (C6'), 171.6 (C3), 172.7 (C1'').</p>
<p>Elemental Analysis</p>	<p>C₂₂H₃₇NO₅ Calculated: C 66.8%, H 9.4%, N 3.5%, O 20.2%. Experimental: C 61.4, H 9.6%, %, N 3.2%.</p>
<p>Molecular Weight</p>	<p>395.54 g/mol</p>
<p>Melting Point</p>	<p>60.78 °C</p>
<p>Temperature of Decomposition</p>	<p>151.23 °C</p>

Table S7. Characterization of *N,N,N*-tri(*n*-propyl)(2-ethoxy-2-oxoethyl)-1-ammonium salicylate ([Pr₃NC₂][Sal]).

Chemical structure	
¹H NMR (300 MHz, DMSO)	δ 0.88 (9 H, t, $J=7.2$ Hz, H11, H12, H13), 1.24 (3H, t, $J=7.1$ Hz, H1), 1.59-1.72 (6 H, m, H8, H9, H10), 3.35-3.40 (6 H, m, H2) 4.23 (2 H, q, $J=7.1$ Hz, H2), 4.36 (2H, s, H4), 6.56 (1H, dd, $J=7.4, 1.1$ Hz, H3'), 6.60 (1H, dd, $J=8.1, 0.8$ Hz, H5'), 7.12 (1H, ddd, $J= 8.1, 7.1, 1.9$, H4'), 7.64 (1H, dd, $J=7.6, 1.7$ Hz, H2').
¹³C NMR (75.47 MHz, DMSO)	δ 10.9 (C11, C12, C13), 14.2 (C1), 15.5 (C8, C9, C10), 61.5 (C4), 62.5 (C2,C5, C6, C7), 116.2 (C5'), 116.3 (C3'), 120.8 (C1'), 130.3 (C2'), 131.8 (C4'), 163.6 (C6'), 165.2 (C3) 171.6 (C1'').
Elemental Analysis	(C ₂₀ H ₃₃ NO ₅) Calculated: C 65.3%, H 9.0%, N 3.8%, O 21.7%. Experimental: C 62.8, H 8.9%, %, N 3.7%.
Molecular Weight	367.49 g/mol
Melting Point	50.76 °C
Temperature of Decomposition	176.97 °C

Table S8. EC₅₀ values (mg·L⁻¹) with the respective 95% confidence limits (lower limit; upper limit) of the studied AGB-ILs after 5, 15 and 30 minutes of exposure of the marine bacterium *Vibrio fischeri*.

AGB-IL	EC₅₀ (mg·L⁻¹) at 5 min	(lower limit; upper limit)
[Pr ₃ NC ₄][Pyr]	957.8	775.4; 1140.3
[Pr ₃ NC ₄][Lac]	1778.5	1392.4; 2164.6
[Pr ₃ NC ₂][Sal]	333.0	282.8; 383.2
[Pr ₃ NC ₄][Sal]	163.6	113.6; 214.0
[Pr ₃ NC ₄][Sac]	833.7	282.3; 1385.1
[Pr ₃ NC ₄][Dca]	1835.1	1560.7; 2109.5
AGB-IL	EC₅₀ (mg·L⁻¹) at 15 min	(lower limit; upper limit)
[Pr ₃ NC ₄][Pyr]	722.5	582.6; 862.3
[Pr ₃ NC ₄][Lac]	1669.0	1365.6; 1972.4
[Pr ₃ NC ₂][Sal]	318.3	248.7; 387.8
[Pr ₃ NC ₄][Sal]	133.2	99.9; 166.4
[Pr ₃ NC ₄][Sac]	113.8	2.7; 224.9
[Pr ₃ NC ₄][Dca]	1016.8	974.3; 1059.4
AGB-IL	EC₅₀ (mg·L⁻¹) at 30 min	(lower limit; upper limit)
[Pr ₃ NC ₄][Pyr]	646.5	478.2; 814.8
[Pr ₃ NC ₄][Lac]	1713.5	1412.5; 2014.5
[Pr ₃ NC ₂][Sal]	304.7	239.4; 370.0
[Pr ₃ NC ₄][Sal]	137.5	92.1; 182.9
[Pr ₃ NC ₄][Sac]	38.6	10.9; 66.3
[Pr ₃ NC ₄][Dca]	672.3	302.5; 1042.1

AGB-IL-based ABS ternary phase diagrams

Table S9. Experimental weight percent data for ternary systems composed of AGB-IL (1) sharing the same anion and cationic core but differing in the alkyl chain length of the cation + $K_3C_6H_5O_7/C_6H_8O_7$ (2) + H_2O at 25 °C.

[Pr₃NC₄][Sal]		[Pr₃NC₂][Sal]	
100w₁	100w₂	100w₁	100w₂
68.963	0.961	63.426	3.602
62.076	1.847	54.584	4.453
59.345	2.595	47.568	5.162
55.627	3.192	43.422	5.999
51.132	3.680	39.607	6.649
47.180	4.160	37.519	7.355
44.902	4.566	34.857	7.772
42.803	5.019	32.916	8.301
40.931	5.341	31.254	8.788
39.043	5.699	29.760	9.316
37.478	6.057	28.023	9.427
36.139	6.321	26.660	9.955
34.579	6.599	25.525	10.166
33.766	6.970	24.644	10.531
32.589	7.126	23.453	10.853
31.310	7.316	22.506	11.121
30.666	7.604	21.627	11.394
29.628	7.688	20.828	11.582
28.635	7.860	20.059	11.783
28.139	8.054	19.361	11.998
27.219	8.176	18.727	12.197
26.415	8.312	18.119	12.343
25.959	8.534	17.556	12.479
25.169	8.725	17.120	12.674
24.574	8.884	16.535	12.939
23.937	9.035	15.788	13.153
23.392	9.177	15.549	13.349
22.804	9.256	15.234	13.588
22.287	9.377	14.827	13.637
21.779	9.436	14.495	13.785
21.737	9.738	14.101	13.822
21.013	9.986	13.723	13.920
20.435	10.087	13.495	14.044

20.168	10.165	13.113	14.270
19.863	10.232	12.776	14.346
19.600	10.283	12.484	14.422
19.310	10.408	12.242	14.443
19.036	10.530	11.948	14.490
18.771	10.617	11.635	14.795
18.513	10.663	11.423	14.832
18.245	10.743	11.258	14.844
17.809	10.772	11.055	14.881
17.587	10.878	10.842	14.935
17.364	10.994	10.717	15.074
17.146	11.094	10.542	15.145
16.935	11.215	10.342	15.180
16.725	11.311	10.224	15.277
16.379	11.269	10.075	15.374
16.218	11.344	9.873	15.496
16.052	11.397	9.660	15.504
15.889	11.458	9.583	15.531
15.745	11.516	9.499	15.657
15.464	11.488	9.377	15.698
15.292	11.563	9.262	15.732
15.144	11.602	9.063	15.959
14.954	11.766	8.922	15.958
14.779	11.868	8.734	16.111
14.629	11.970	8.574	16.185
14.490	12.031	8.341	16.290
14.334	12.097	8.140	16.432
14.117	12.107	7.964	16.575
13.976	12.178	7.857	16.613
13.869	12.279	7.694	16.676
13.672	12.327	7.604	16.711
13.546	12.413	7.490	16.730
13.371	12.437	7.418	16.823
13.182	12.611	7.290	16.920
13.015	12.606	7.188	17.012
12.868	12.609	7.079	17.119
12.757	12.641	6.920	17.349
12.676	12.693	6.816	17.307
12.565	12.762	6.745	17.305
12.476	12.842	6.710	17.328
12.327	12.868	6.654	17.415

12.214	12.913	6.576	17.557
12.115	12.954	6.517	17.518
12.001	13.008	6.483	17.527
11.896	13.055	6.441	17.580
11.805	13.088	6.397	17.641
11.701	13.145	6.349	17.695
11.629	13.204	6.283	17.713
11.475	13.186	6.243	17.757
11.408	13.237	6.203	17.809
11.239	13.339	6.148	17.881
11.084	13.436	6.106	17.941
10.899	13.507	6.046	17.978
10.702	13.691	5.993	18.012
10.462	13.811	5.884	18.343
10.262	13.908		
10.096	13.962		
9.923	14.087		

Table S10. Experimental weight percent data for the ternary systems composed of AGB-IL (1) with a common cation and different anions + $K_3C_6H_5O_7/C_6H_8O_7$ (2) + H_2O at 25 °C and under atmospheric pressure.

[Pr ₃ NC ₄][Lac]		[Pr ₃ NC ₄][Dca]		[Pr ₃ NC ₄][Pyr]		[Pr ₃ NC ₄][Sac]	
100w ₁	100w ₂	100w ₁	100w ₂	100w ₁	100w ₂	100w ₁	100w ₂
54.633	10.399	70.322	0.506	34.046	26.369	58.403	0.516
50.605	12.069	56.293	1.081	32.658	27.454	51.549	1.328
47.311	12.874	44.247	1.472	30.827	28.060	45.777	1.808
44.876	13.864	39.884	1.480	29.131	28.354	40.895	2.288
42.044	15.416	36.306	1.840	27.785	28.452	37.451	3.156
38.018	17.146	33.305	2.016	26.921	29.193	34.358	4.869
35.643	18.465	31.123	2.151	25.800	29.709	27.706	6.334
33.885	19.373	70.322	0.506	24.628	29.932	25.881	8.066
31.650	20.522	56.293	1.081	23.850	30.601	23.979	10.197
28.991	22.111	44.247	1.472	23.330	30.861	22.724	11.416
26.350	23.847	39.884	1.480	22.868	30.958	21.403	12.846
24.700	24.817	36.306	1.840	22.301	31.389	20.179	14.017
22.809	25.548	33.305	2.016	21.759	31.987	19.012	15.148
21.505	26.388	31.123	2.151	21.266	32.188	17.884	16.364
20.042	27.355	28.264	2.963	20.678	32.659	16.867	17.551
18.680	28.287	25.178	3.511	20.086	33.456	16.103	18.282
17.687	28.977	22.629	3.736	19.504	33.956	15.228	19.188
16.682	29.690	20.001	4.450	18.955	34.312	14.508	20.063
15.758	30.305	18.081	4.790	18.456	34.781	13.887	20.782

14.991	30.874	28.264	2.963	17.780	35.101	13.172	21.600
14.322	31.366	25.178	3.511	17.380	35.561	12.623	22.187
13.038	32.332	8.152	9.026	17.022	36.058	12.065	22.785
12.398	32.853	7.806	9.355	16.420	36.343	11.533	23.406
11.891	33.245	7.718	9.434	16.176	36.400	11.043	23.993
11.487	33.537	7.929	9.446	15.958	36.530	10.583	24.544
10.904	34.037	7.840	9.782	15.659	37.055	10.163	25.095
10.478	34.391	7.834	9.952	15.344	37.502	9.724	25.738
9.656	35.147	7.643	10.551	15.095	37.486	9.399	26.131
8.835	35.916	7.484	10.800	14.801	37.842	9.076	26.522
		7.376	11.110	14.663	37.932	8.798	26.946
		7.389	11.297	14.395	38.273	8.163	27.746
		7.254	12.790	14.221	38.338	7.856	28.190
		7.110	11.833	13.995	38.692	7.566	28.630
		6.932	13.348	13.656	38.625	7.324	28.962
		6.863	13.917	13.479	38.976	7.090	29.282
		6.869	14.556	13.106	39.668	6.840	29.694
		6.474	15.091	12.908	39.969		
		6.228	15.583	12.758	39.920		
		5.514	17.046	12.472	40.406		
		5.634	17.902	12.294	40.632		
		4.995	18.918	12.119	40.948		
				11.684	41.189		
				11.502	41.554		
				11.379	41.508		
				11.236	41.830		
				11.065	42.018		
				10.923	42.209		
				10.775	42.322		

Table S11. Correlation parameters of Merchuk Equation used to describe the experimental binodal data at 25 °C and pH 7.

AGB-IL	Salt	$A \pm \sigma$	$B \pm \sigma$	$10^5 (C \pm \sigma)$	R^2
[Pr ₃ NC ₄][Pyr]	K ₃ C ₆ H ₅ O ₇ /C ₆ H ₈ O ₇	91.64 ± 1.465	-0.290 ± 0.007	2.78 ± 0.17	0.9904
[Pr ₃ NC ₄][Lac]		128.16 ± 9.66	-0.268 ± 0.021	2.31 ± 0.01	0.9995
[Pr ₃ NC ₂][Sal]		138.70 ± 8.83	-0.472 ± 0.027	0.21 ± 1.39	0.9985
[Pr ₃ NC ₄][Sal]		108.30 ± 1.87	-0.403 ± 0.009	0.35 ± 1.08	0.9951
[Pr ₃ NC ₄][Sac]		93.89 ± 2.70	-0.212 ± 0.020	3.11 ± 0.36	0.9939
[Pr ₃ NC ₄][Dca]		126.75 ± 5.91	-0.875 ± 0.036	0.19 ± 5.50	0.9926

Table S12. Experimental TLs and TLLs of the ABS composed of IL + K₃C₆H₅O₇/C₆H₈O₇ + H₂O at 25 °C and pH 7. The mixture composition common to all ILs (30/30 wt% IL/salt) is highlighted in gray to enable direct comparison of TLLs and their relation to ABS formation efficiency.

Weight fraction composition / (wt%)							
IL	[IL] _{IL}	[salt] _{IL}	[IL] _M	[salt] _M	[IL] _{salt}	[salt] _{salt}	TLL
[MepyrNC ₄]Br ^a	57.83	4.15	30.25	24.63	4.05	44.07	66.97
	59.63	3.66	30.38	29.17	0.79	54.98	78.07
[Et ₃ NC ₄]Br ^a	58.23	2.38	34.65	20.05	2.85	43.86	69.20
	76.07	0.09	30.02	29.57	1.51	47.83	88.54
[Pr ₃ NC ₄]Br ^a	66.80	0.56	39.73	20.18	0.30	48.75	82.13
	72.38	0.20	30.22	29.57	0.19	50.51	87.96
[Bu ₃ NC ₄]Br ^a	60.31	2.09	25.33	19.69	1.39	31.73	65.95
	62.76	1.80	29.64	29.53	9.88E ⁻⁰⁵	54.35	81.85
[Pr ₃ NC ₄][Pyr]	53.85	3.35	30.02	19.98	2.93	38.89	62.10
	68.09	1.05	30.26	30.31	0.15	53.60	85.89
[Pr ₃ NC ₄][Lac]	57.88	8.49	32.93	22.38	9.07	35.67	55.87
	67.62	5.62	30.00	29.31	1.84	47.04	77.73
[Pr ₃ NC ₂][Sal]	67.09	2.35	29.56	29.61	2.90E ⁻¹²	51.08	82.92
	61.34	2.95	29.01	16.16	0.11	27.97	66.14
[Pr ₃ NC ₄][Sal]	71.14	1.08	19.81	20.59	4.60E ⁻⁰³	28.12	76.10
	79.33	0.60	29.52	29.75	5.12E ⁻¹⁶	47.03	91.92
[Pr ₃ NC ₄][Sac]	55.17	6.12	30.16	20.06	13.79	29.18	47.37
	80.30	0.54	29.62	29.99	0.94	46.65	91.79
[Pr ₃ NC ₄][Dca]	64.59	0.59	20.11	15.63	2.15	21.70	65.91
	80.33	0.27	30.54	29.17	0.32	46.71	92.51

^a Data retrieved from Pereira et al [1].

Depletion efficiencies

Table S13. Depletion efficiencies of HSA ($DE_{\text{HSA}}\%$) and IgG ($DE_{\text{IgG}}\%$) obtained using AGB-IL-based ABS composed of 30 wt% of AGB-IL + 60 wt% of citrate buffer solution ($\text{K}_3\text{C}_6\text{H}_5\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at 50 wt%, pH 7) + 10 wt% of human serum at 25 °C. Experiments were performed at least in duplicate ($n \geq 2$); no standard deviations (σ) are reported due to quantitative depletion in all replicates.

AGB-IL	$DE_{\text{HSA}}\% \pm \sigma$	$DE_{\text{IgG}}\% \pm \sigma$
[MepyrNC ₄]Br	100	100
[Et ₃ NC ₄]Br	100	100
[Pr ₃ NC ₄]Br	100	100
[Bu ₃ NC ₄]Br	100	100
[Pr ₃ NC ₄][Pyr]	100	100
[Pr ₃ NC ₄][Lac]	100	100
[Pr ₃ NC ₂][Sal]	100	100
[Pr ₃ NC ₄][Sal]	100	100
[Pr ₃ NC ₄][Sac]	100	100
[Pr ₃ NC ₄][Dca]	100	100

Transferrin extraction and detection

Table S14. Recovery yield ($RY_{\text{TF}}\%$) and concentration ($[\text{TF}]_{\text{depleted serum}}$, $\text{g}\cdot\text{L}^{-1}$) of transferrin in serum determined by SE-HPLC after high-abundance proteins depletion using the system composed of 30 wt% of AGB-IL + 60 wt% of $\text{K}_3\text{C}_6\text{H}_5\text{O}_7/\text{C}_6\text{H}_8\text{O}_7$ at 50 wt%, pH 7 + 10 wt% of human serum. The concentration of transferrin in the original serum sample is $1.8 \pm 0.1 \text{ g}\cdot\text{L}^{-1}$ as determined by ELISA and was used to determine the relative error of the analysis.

$RY_{\text{TF}}\% \pm \sigma$	$[\text{TF}]_{\text{depleted serum}} \pm \sigma (\text{g}\cdot\text{L}^{-1})$	Relative error (%)
78 ± 3	1.42 ± 0.06	22

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