Supplementary Materials

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Synthesis of ILs: Nuclear magnetic resonance (NMR) spectra

Tetrabutylphosphonium formate, [P_{4444}][HCOO]:

^{1}H NMR (D_2O, 75 MHz, δ/ppm): 0.56 (t, 12H, C**H**$_3$-), 1.16 (m, 16H, -C**H**$_2$-), 1.78 (m, 8H, -C**H**$_2$-P-), 8.08 (s, 1H, C**H** – COO).

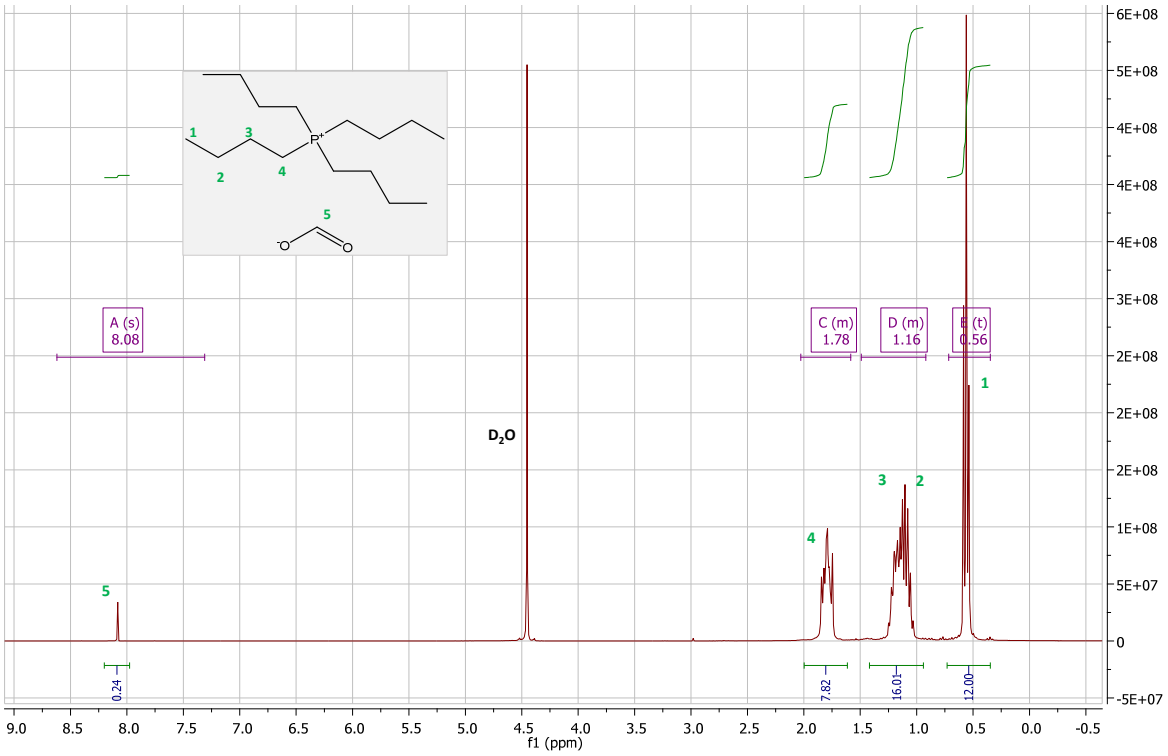

Figure S1 – ^{1}H NMR spectrum of [P_{4444}][HCOO].

Tetrabutylphosphonium acetate, [P_{4444}][CH_3COO]:

^{1}H NMR (D_2O, 75 MHz, δ/ppm): 0.55 (t, 12H, C**H**$_3$-), 1.13 (m, 16H, -C**H**$_2$-), 1.53 (s, 3**H**, -C**H**$_2$-), 1,77 (m, 8H, -C**H**$_2$-P-).

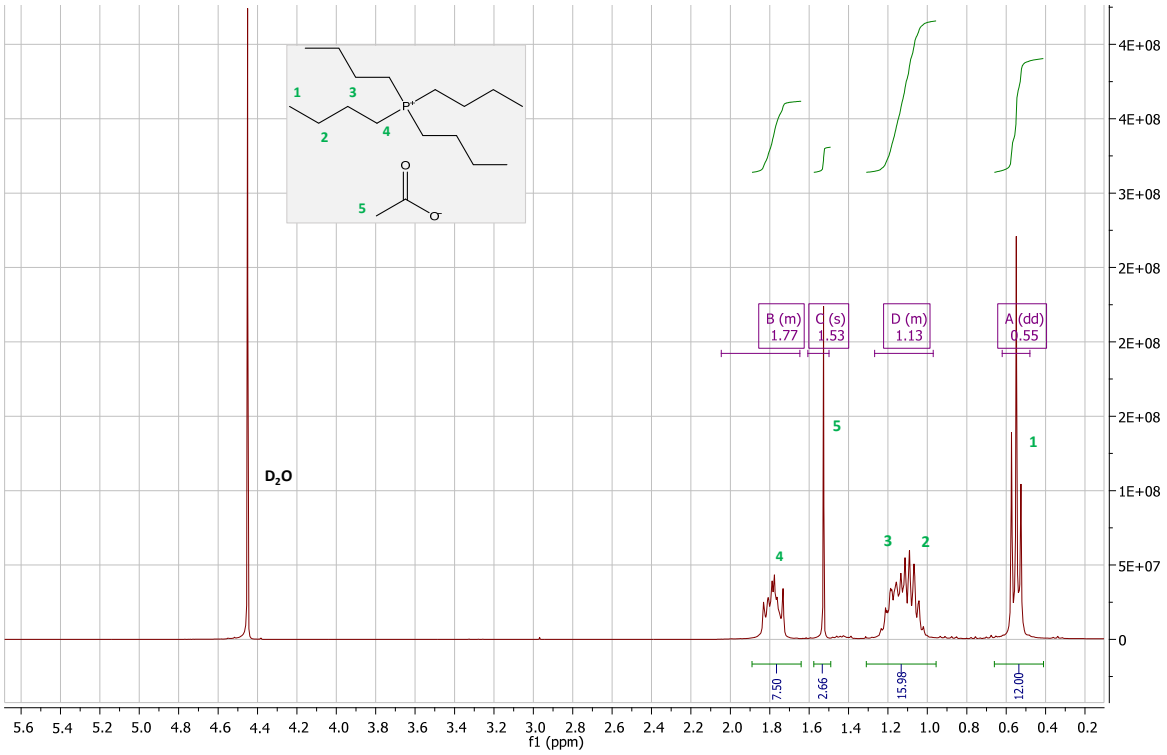

Figure S2 – ^{1}H NMR spectrum of [P_{4444}][CH_3COO].

Tetrabutylammonium formate, [N_{4444}][HCOO]:

^{1}H NMR (D_2O, 75 MHz, δ/ppm): 0.82 (t, 12H, C**H**$_3$-), 1.24 (m, 8H, -C**H**$_3$), 1.51 (m, 8H, -C**H**$_2$-), 3.07 (m, 8H, -C**H**$_2$-N-), 8.33 (s, 1H, C**H** – COO).

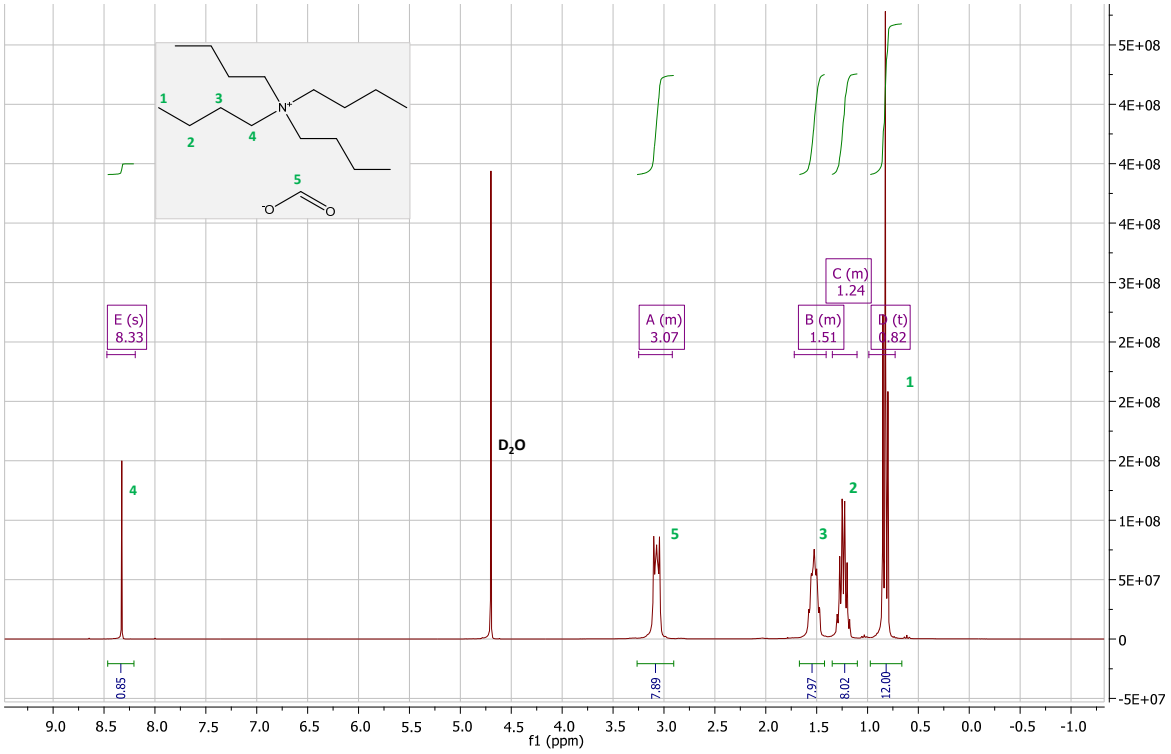

Figure S3 – ^{1}H NMR spectrum of [N_{4444}][HCOO].

Tetrabutylammonium acetate, [N_{4444}][CH_3COO]:

^{1}H NMR (D_2O, 75 MHz, δ/ppm): 0.82 (t, 12H, -C**H**$_3$), 1.23 (m, 8H, -C**H**$_2$-), 1.52 (m, 8H, -C**H**$_2$-), 1.78 (s, 3H, C**H**$_2$-CO-), 3.07 (m, 8H, -C**H**$_2$-N-).

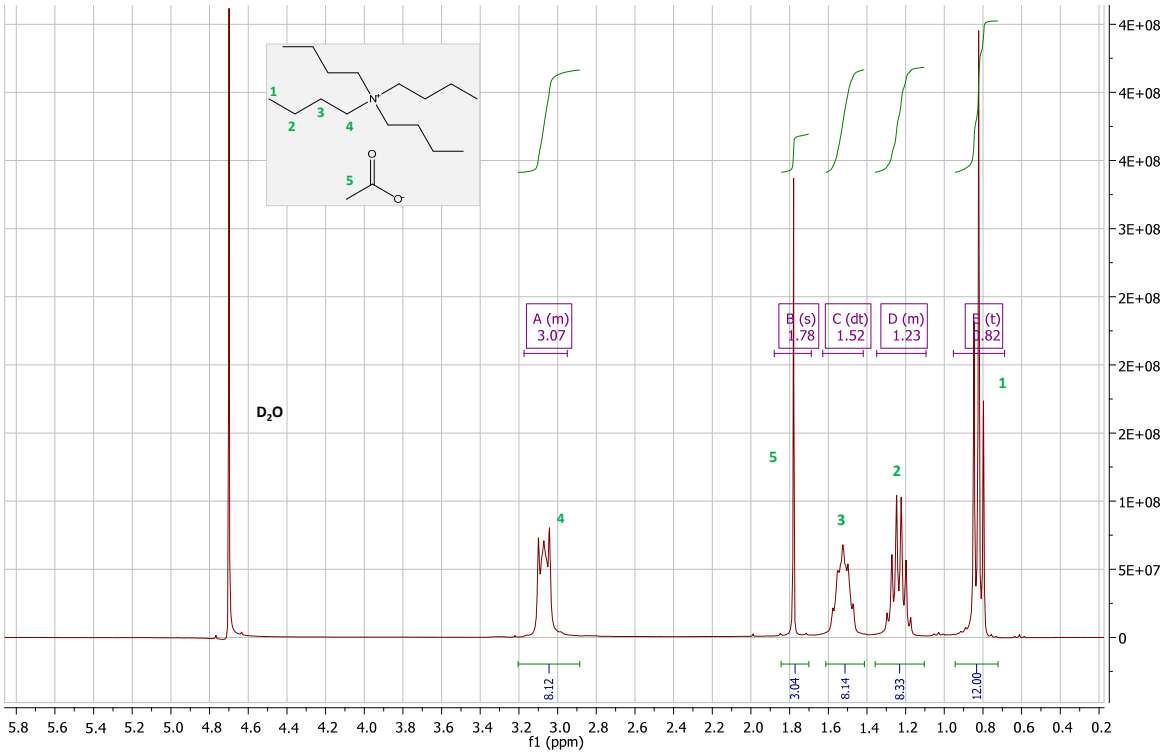

Figure S4 – ^{1}H NMR spectrum of [N_{4444}][CH_3COO].

Phase diagrams of aqueous biphasic systems

Table S1 – Experimental binodal data in weight percent (wt.%) for the ternary systems comprising IL + K_2HPO_4 + H_2O at 25 °C and atmospheric pressure.

$[P_{4444}][HCOO]$		$[P_{4444}][CH_3COO]$		$[N_{4444}][HCOO]$		$[N_{4444}][CH_3COO]$	
[IL]	[Salt]	[IL]	[Salt]	[IL]	[Salt]	[IL]	[Salt]
35.1691	5.4490	35.5932	3.7561	40.8074	2.4426	52.6928	1.3673
26.8586	6.9669	31.2548	4.4559	33.0900	4.6188	42.1895	2.8191
23.5034	8.0978	27.8273	5.6931	29.3327	5.4917	38.1318	3.8040
21.5970	8.8678	24.8217	6.8877	27.2292	6.3648	34.3294	4.9893
19.6753	9.9005	22.8133	8.3556	25.6839	7.0465	31.5019	5.5568
18.4047	10.6356	20.8526	9.5070	24.1267	7.4113	27.3984	7.0579
16.2599	11.4918	19.1209	10.8533	22.3843	8.4369	25.5928	7.5596
15.2482	11.9190	17.5101	11.3287	20.7199	9.3772	24.5210	8.1332
14.4142	12.3703	15.6797	12.1632	19.9067	9.8886	23.4534	8.6494
13.9012	12.8840	14.3802	12.8375	19.2834	10.2287	22.5100	9.1246
13.3304	13.0063	12.7553	14.0715	18.4272	10.6092	21.5213	9.8060
12.7548	13.2281	12.0606	14.4290	17.5023	11.2539	20.4229	10.6400
11.8842	13.8788	11.4743	14.8912	15.9431	12.3725	19.8638	10.8027
11.6564	14.1552	10.8315	15.2955	15.4259	12.6394	19.0971	11.2972
11.2533	14.5113	9.9008	16.2426	14.5992	13.3205	18.6589	11.4143
10.7247	14.8377	9.3743	16.5995	14.2164	13.6204	17.6985	12.2450
10.2978	14.9536	8.9120	16.7947	13.8757	13.8712	16.9196	12.8548
9.6091	15.5431	8.3248	17.4476	13.4918	13.9835	16.3937	13.1342
9.1259	15.8634	7.9796	17.5590	12.6482	14.6921	15.7895	13.6139
8.7817	15.9494	7.5069	17.9754	12.1168	15.0961	14.9020	14.5056
8.3005	16.2746	7.0445	18.3550	11.4978	15.6219	14.3997	14.9188
7.9041	16.5472	6.7863	18.4600	11.1098	15.9874	13.9135	15.3149
7.6120	16.7434	6.4822	18.7304	10.7452	16.0064	13.4830	15.6132
7.4033	16.8991	6.2180	18.9473	10.3904	16.3973	13.0648	15.9950
7.1020	17.0853	5.9080	19.1420	9.8980	16.8662	12.6597	16.2751
6.7693	17.2944	5.7320	19.1750	9.7101	17.0123	12.4115	16.4583
6.3981	17.6693	5.5820	19.2311	9.5399	17.0673	11.9820	16.8296

Table S1 (cont.) – Experimental binodal data in weight percent (wt.%) for the ternary systems comprising IL + K_2HPO_4 + H_2O at 25 °C and atmospheric pressure.

$[P_{4444}][HCOO]$		$[P_{4444}][CH_3COO]$		$[N_{4444}][HCOO]$		$[N_{4444}][CH_3COO]$	
[IL]	[Salt]	[IL]	[Salt]	[IL]	[Salt]	[IL]	[Salt]
6.0907	17.9341	5.4661	19.2685	9.2969	17.3232	11.5724	17.1908
5.8984	18.1005	5.2437	19.5589	8.9973	17.6344	11.2404	17.4821
		5.0588	19.6678	8.8686	17.7291	10.7974	17.8877
		4.7985	20.1228	8.6848	17.8849	10.5225	18.2173
		4.6455	20.2349	8.3477	18.1941	9.9202	19.1059
		4.4699	20.4634	8.1482	18.3746	7.7064	20.9842
		4.3164	20.6273	7.9399	18.5370	7.5566	21.0193
		4.1818	20.7734	7.6888	18.7887	7.2858	21.3700
		4.0323	21.0109	7.4791	19.0184	6.7998	22.1351
		3.8743	20.9984	7.1814	19.2434	6.6689	22.2512
		3.7362	21.2295	6.9828	19.4893	6.4692	22.5164
		3.6359	21.3139	6.8230	19.6143	6.2704	22.7621
				6.5616	19.9284	6.0214	23.0103
				6.3880	20.0629	5.8835	23.1041
				6.2495	20.2886	5.7844	23.1887
				6.0352	20.4913	5.6434	23.3389
				5.9144	20.6329	5.3844	23.8779
				5.7277	20.8213	5.2179	24.2330
				5.6774	20.8342	5.0717	24.1945
				5.5579	20.9484	4.9153	24.4225
				5.3441	21.2250	4.7468	24.6597
				5.2139	21.3647	4.6400	24.7377
				5.0634	21.5500		
				4.9553	21.7015		
				4.8402	21.8318		

Table S2 – Experimental binodal data in weight percent (wt.%) for the ternary systems comprising IL + $K_3C_6H_5O_7$ + H_2O at 25 °C and atmospheric pressure.

[P_{4444}][HCOO]		[P_{4444}][CH_3COO]		[N_{4444}][HCOO]		[N_{4444}][CH_3COO]	
[IL]	[Salt]	[IL]	[Salt]	[IL]	[Salt]	[IL]	[Salt]
53.7479	6.2906	61.7189	6.3598	42.7799	10.9536	43.2048	7.0781
47.1037	7.8989	54.3210	8.6140	38.2282	12.0291	42.0219	7.6076
43.3960	9.4465	33.3609	14.6900	33.5586	13.8127	40.8721	7.5239
40.0179	10.8122	29.8287	16.6166	29.9976	15.6567	35.9117	10.2004
37.2514	12.0353	26.0103	19.0718	26.6407	18.0096	33.8312	11.7778
35.0406	12.8132	22.5641	21.6804	25.0470	18.8095	30.3810	14.4390
32.6162	13.9453	19.8458	23.6747	22.8548	20.5174	27.0978	17.1965
29.7596	15.7505	17.7732	25.2821	20.9772	22.1081	23.8509	20.0031
27.2236	17.5081	15.6837	27.0571	19.1908	23.3995	22.0817	21.6358
25.3328	18.7913	14.3144	28.0871	17.9163	24.4822	20.3462	23.3295
23.5705	20.0308	13.3194	28.7676	16.7682	25.2585	18.9674	24.6848
21.7125	21.4209	12.1596	29.7981	15.5132	26.4048	17.2584	26.4113
19.6496	23.1772	11.2084	30.5835	14.1429	27.9483	15.6011	28.1010
18.3074	24.1676	10.3326	31.3395	13.2994	28.6926	13.9483	29.8276
17.4630	24.6442	9.6480	31.8361	12.5589	29.2484	12.2809	31.6501
		8.9500	32.5326	11.8453	29.9028	10.1959	34.0478
		8.4379	32.9464	11.2808	30.3531		
				10.6272	31.1642		
				10.0840	31.7182		
				9.5987	32.1993		
				8.9464	32.8357		
				8.4310	33.4680		
				7.9632	33.9199		
				7.6213	34.2267		
				7.2050	34.6713		
				6.7803	35.2808		
				6.4700	35.7045		
				6.2169	35.9971		
				6.0468	36.0959		
				5.8265	36.3372		

Table S3 – A, B and C parameters of the equation proposed by Merchuk et al.[a] and respective correlation coefficients, R^2, for the ternary systems comprising IL + salt + H_2O systems at 25 °C and atmospheric pressure.

IL	Salt	$A \pm \sigma$	$B \pm \sigma$	$10^5 (C \pm \sigma)$	R^2
[P_{4444}][HCOO]	K_2HPO_4	67.90 ± 7.09	-0.3440 ± 0.04	17.62 ± 2.39	0.999
	$C_6H_5K_3O_7$	105.56 ± 12.03	-0.2978 ± 0.04	2.25 ± 0.72	0.999
[P_{4444}][CH_3COO]	K_2HPO_4	91.44 ± 16.50	-0.4569 ± 0.07	13.53 ± 3.00	0.999
	$C_6H_5K_3O_7$	150.21 ± 23.58	-0.3727 ± 0.05	1.94 ± 0.65	0.999
[N_{4444}][HCOO]	K_2HPO_4	69.30 ± 2.54	-0.3722 ± 0.02	9.07 ± 0.72	0.999
	$C_6H_5K_3O_7$	120.02 ± 13.69	-0.3328 ± 0.03	2.01 ± 0.27	0.999
[N_{4444}][CH_3COO]	K_2HPO_4	75.74 ± 2.78	-0.3875 ± 0.02	5.53 ± 0.56	0.999
	$C_6H_5K_3O_7$	80.03 ± 5.39	-0.2454 ± 0.02	1.55 ± 0.26	0.996

[a] $[IL] = A\exp[(B \times [Salt]^{0.5}) - (C \times [Salt]^3)]$, where [IL] and [Salt] represent the IL and salt weight percent data (wt.%), respectively, and A, B, and C are the parameters obtained by regression of the experimental data. Equation reported in Merchuk, J.C.; Andrews, B.A.; Asenjo, J.A. Aqueous two-phase systems for protein separation studies on phase inversion. *J. Chromatogr. B Biomed. Appl.* **1998**, *711*, 285–293, doi:10.1016/S0378-4347(97)00594-X.

Table S4 – Experimental tie-lines (TLs) in weight percent (wt.%) and respective lengths (TLL) for ternary systems comprising IL + salt + H_2O systems at 25 °C and atmospheric pressure. Initial mixture compositions are represented as $[IL]_M$ and $[Salt]_M$, whereas IL-rich and salt-rich phases compositions are denoted by $[IL]_{IL}$, $[Salt]_{IL}$, $[IL]_{Salt}$ and $[Salt]_{Salt}$.

IL	Salt	$[IL]_{IL}$	$[Salt]_{IL}$	$[IL]_M$	$[Salt]_M$	$[IL]_{Salt}$	$[Salt]_{Salt}$	TLL
[P_{4444}][HCOO]	K_2HPO_4	36.58	3.76	24.69	28.13	0.00	79.72	84.30
	$C_6H_5K_3O_7$	38.54	10.80	24.97	27.53	0.14	57.87	60.74
[P_{4444}][CH_3COO]	K_2HPO_4	36.58	3.95	24.69	28.13	0.00	78.11	82.69
	$C_6H_5K_3O_7$	38.48	12.60	24.96	27.77	0.37	55.09	57.07
[N_{4444}][HCOO]	K_2HPO_4	36.57	2.93	24.70	28.13	0.00	80.24	85.54
	$C_6H_5K_3O_7$	42.25	9.52	24.84	27.77	0.51	53.20	60.41
[N_{4444}][CH_3COO]	K_2HPO_4	42.18	2.28	24.93	27.95	0.00	64.98	75.57
	$C_6H_5K_3O_7$	55.78	2.16	25.11	28.01	3.16	46.50	68.81

Extraction of transferrin

Table S5 – Extraction efficiencies (EE_{TF}, %) and recovery yield (Y_{TF}, %) of transferrin achieved with ternary systems comprising IL + salt + H_2O at 25 ºC with a controlled pH of ≈7.4 ± 1.

IL	Salt	$pH_{IL\text{-rich phase}}$	EE_{TF} %	Y_{TF} %
$[P_{4444}][HCOO]$	K_2HPO_4	8.19 ± 0.16	100	99.16 ± 1.56
$[P_{4444}][CH_3COO]$		7.74 ± 0.16	100	98.28 ± 0.87
$[N_{4444}][HCOO]$		7.65 ± 0.08	100	86.73 ± 1.57
$[N_{4444}][CH_3COO]$		8.16 ± 0.15	100	100.00
$[P_{4444}][HCOO]$	$C_6H_5K_3O_7$	6.73 ± 0.15	100	98.01 ± 0.98
$[P_{4444}][CH_3COO]$		6.69 ± 0.05	100	86.19 ± 0.02
$[N_{4444}][HCOO]$		6.34 ± 0.01	100	86.56 ± 0.45
$[N_{4444}][CH_3COO]$		6.98 ± 0.02	100	98.73 ± 1.59

Molecular docking

Table S6 – Docking affinity energy and interacting atoms predicted by AutoDock Vina for transferrin (pH 6.5) + ILs ions.

Compound	Affinity (kcal/mol)	Type of interaction	From	To	Distance (Å)
$[N_{4444}]^+$	-4.0	Hydrophobic	ALA658	$[N_{4444}]^+$	4.04
			$[N_{4444}]^+$	LYS657	3.71
$[P_{4444}]^+$	-3.5	Electrostatic	$[P_{4444}]^+$	ASP477	4.93
		Hydrophobic		ARG475	4.51
$[CH_3COO]^-$	-3.4	Electrostatic	ARG644	$[CH_3COO]^-$	4.71
		Hydrogen Bond	ASN411		2.20
			GLU423		2.04
			ASN584		2.50
			ARG644		2.16
$[HCOO]^-$	-3.0	Hydrogen Bond	ARG456	$[HCOO]^-$	1.92
					2.22
			GLY459		2.05
			TYR517		2.31

Table S7 – Docking affinity energy and interacting atoms predicted by AutoDock Vina for transferrin (pH 8) + ILs ions.

Compound	Affinity (kcal/mol)	Type of interaction	From	To	Distance (Å)
$[N_{4444}]^+$	-3.8	Hydrophobic	$[N_{4444}]^+$	PRO508	4.70
			PHE395	$[N_{4444}]^+$	4.50
		Electrostatic	$[N_{4444}]^+$	GLU375	4.39
		Hydrogen Bond			3.41
$[P_{4444}]^+$	-4.0	Hydrophobic	PHE186	$[P_{4444}]^+$	5.28
$[CH_3COO]^-$	-3.3	Electrostatic	ARG644	$[CH_3COO]^-$	4.67
		Hydrogen Bond	ASN411		2.07
			GLU423		1.99
			ASN584		2.64
			ARG644		2.39
					2.07
$[HCOO]^-$	-3.1	Hydrogen Bond	ALA458	$[HCOO]^-$	2.16
			GLY459		2.02
			TYR517		2.30
			$[HCOO]^-$	THR452	2.96