Tailoring the partitioning of proteins using ionic liquids as adjuvants in polymer-polymer aqueous biphasic systems

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Supporting Information

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Phase diagrams

Table S1 Experimental data of the binodal curves for the ABS composed of PEG 6000 + Dextran 450-550 +H_2O at 298 K.

Without IL	
[Dextran] / (wt%)	**[PEG] / (wt%)**
20.54	1.10
18.99	1.32
17.40	1.78
15.19	2.06
13.78	2.09
12.86	2.41
12.07	2.66
11.06	2.94
10.25	3.09
9.80	3.14
9.53	3.21
9.36	3.30
8.93	3.44
8.56	3.58
7.75	3.76
7.23	4.00
6.66	4.11
6.02	4.33
5.59	5.06
5.05	5.25

Table S2 Experimental data of the binodal curves for the ABS composed of PEG 6000 + Dextran 450-550 + 5 wt% IL + H_2O at 298 K.

$[C_4mim]Cl$		**$[C_4mpyr]Cl$**		**$[C_4mpip]Cl$**		**$[N_{4444}]Cl$**		**$[P_{4444}]Cl$**	
[Dextran] / (wt%)	[PEG] / (wt%)	[Dextran] / (wt%)	[PEG] / (wt%)	[Dextran] / (wt%)	[PEG] / (wt%)	[Dextran] / (wt%)	[PEG] / (wt%)	[Dextran] / (wt%)	[PEG] / (wt%)
19.94	1.07	12.87	2.55	10.58	2.86	14.16	2.11	10.26	3.06
17.51	1.45	9.47	3.22	7.87	3.92	12.57	2.72	7.89	3.24
15.62	2.26	8.11	3.98	6.47	4.01	9.62	3.26	6.42	3.64
13.21	2.76	6.71	4.06	5.23	4.19	6.90	4.17	5.76	3.94
11.44	2.74	5.43	4.49	4.71	4.66	4.87	5.03	4.92	4.65
10.89	2.95	4.55	4.95	4.24	4.60	3.86	4.80	4.18	4.81
10.27	3.07	3.95	4.98	3.94	4.76	2.67	5.60	3.50	4.74
9.84	3.23	3.49	5.05	3.57	4.85	2.13	5.69	3.01	5.12
9.27	3.56	3.21	5.08	3.25	5.00	1.76	5.56	2.59	5.26
8.44	3.86	3.03	5.24	2.97	5.09	1.52	5.86	2.10	5.49
7.72	4.03	2.83	5.26	2.71	5.17	1.30	5.89	1.83	5.51
7.15	4.27	2.53	5.30	2.41	5.35	1.14	5.878	1.40	5.70
6.47	4.46	2.24	5.57	2.10	5.61	0.92	5.79	1.16	6.51
5.87	4.63	1.92	5.67	1.80	5.73				
5.31	4.85	1.67	5.85	1.58	5.89				
4.92	4.80	1.45	5.90	1.36	6.12				
4.53	5.05	1.28	5.92	1.15	6.30				
4.15	5.31	1.09	6.20	0.93	6.36				
3.78	5.19	0.92	6.20	0.74	6.85				
3.49	5.28								
3.05	5.61								
2.71	5.76								
2.36	5.69								
2.09	5.94								

Table S3 Experimental data of the binodal curves for the ABS composed of PEG 6000 + Dextran 450-550 + 10 wt% IL + H_2O at 298 K.

$[C_4mim]Cl$		**$[C_4mpyr]Cl$**		**$[C_4mpip]Cl$**		**$[N_{4444}]Cl$**		**$[P_{4444}]Cl$**	
[Dextran] / (wt%)	[PEG] / (wt%)	[Dextran] / (wt%)	[PEG] / (wt%)	[Dextran] / (wt%)	[PEG] / (wt%)	[Dextran] / (wt%)	[PEG] / (wt%)	[Dextran] / (wt%)	[PEG] / (wt%)
15.71	2.21	11.17	2.16	16.53	2.21	17.47	1.81	6.25	2.97
10.89	3.35	9.84	2.53	10.58	3.55	14.16	2.31	5.19	3.37
9.14	3.51	9.19	3.09	5.48	4.22	11.88	2.63	4.46	3.80
8.42	3.66	8.58	3.31	4.71	4.84	9.71	3.31	3.70	4.07
8.05	3.68	7.63	3.31	3.92	4.81	8.36	3.47	3.20	4.48
7.70	3.91	6.81	3.68	3.67	4.83	7.06	3.83	2.79	4.48
7.13	4.04	5.92	3.92	3.46	4.84	5.44	4.33	2.52	4.50
6.76	4.14	4.82	4.31	3.23	5.13	4.95	4.56	2.27	4.60
6.28	4.27	3.86	4.57	2.99	5.04	4.28	4.93	1.99	4.69
5.78	4.49	3.04	4.72	2.77	5.12	3.51	4.69	1.76	4.87
5.25	4.69	2.58	5.14	2.53	5.23	3.09	5.67	1.51	5.01
4.96	4.76	2.07	5.07	2.31	5.44	2.29	5.57	1.27	5.26
4.56	5.06	1.89	5.22	2.12	5.56	1.71	6.03	1.00	5.55
4.18	5.19	1.67	5.46	1.92	5.46	1.29	6.02	0.79	5.70
3.75	5.36	1.45	5.74	1.77	5.62	1.01	6.05	0.61	5.80
3.37	5.34	1.22	5.87	1.62	5.72	0.74	6.44		
3.05	5.42	1.01	6.07	1.42	6.04				
2.81	5.42	0.78	6.81	1.17	6.17				
2.58	5.57			0.92	6.50				
2.33	5.72			0.73	6.83				
2.03	5.94								
1.72	6.03								
1.46	6.07								
1.26	6.21								

Table S4 Adjusted parameters obtained by the regression of the experimental data by Eq (1) for the ABS composed of PEG 6000 + Dextran 450-550 + H_2O at 298 K.

IL	Parameters		
Without IL	r^2	0.99	**StdErr**
	A	16.30	1.24
	B	-5.14E-01	2.90E-02
	C	3.26E-05	1.02E-05

Table S5 Adjusted parameters obtained by the regression of the experimental data by Eq (1) for ABS composed of PEG 6000 +DEX 450-550 + 5 or 10 wt% IL + H_2O at 298 K.

5 wt% IL				10 wt% IL			
IL	Parameters		StdErr	IL	Parameters		StdErr
[C_4mim]Cl	r^2	0.99		[C_4mim]Cl	r^2	0.98	
	A	8.87	0.356		A	8.87	0.18
	B	-0.27	0.02		B	-2.88E-01	1.07E-02
	C	0.00	0.00		C	2.13E-05	6.59E-06
[C_4mpyr]Cl	r^2	0.98		[C_4mpyr]Cl	r^2	0.98	
	A	8.25	0.21		A	7.76	0.31
	B	-2.70E-01	1.53E-02		B	-2.22E-01	2.65E-02
	C	1.97E-05	7.36E-06		C	3.00E-04	5.78E-06
[C_4mpip]Cl	r^2	0.99		[C_4mpip]Cl	r^2	0.99	
	A	8.76	0.15		A	8.23	0.20
	B	-3.11E-01	1.07E-02		B	-2.70E-01	1.53E-02
	C	1.30E-05	5.03E-06		C	4.89E-05	1.08E-06
[P_{4444}]Cl	r^2	0.98		[P_{4444}]Cl	r^2	0.99	
	A	8.97	0.40		A	7.38	0.18
	B	-3.36E-01	2.49E-02		B	-3.01E-01	1.93E-02
	C	2.66E-05	8.27E-06		C	5.00E-04	1.00E-05
[N_{4444}]Cl	r^2	0.99		[N_{4444}]Cl	r^2	0.98	
	A	7.98	0.32		A	8.44	0.31
	B	-2.59E-01	3.03E-02		B	-2.85E-01	2.11E-02
	C	4.16E-05	5.15E-05		C	5.45E-05	1.76E-06

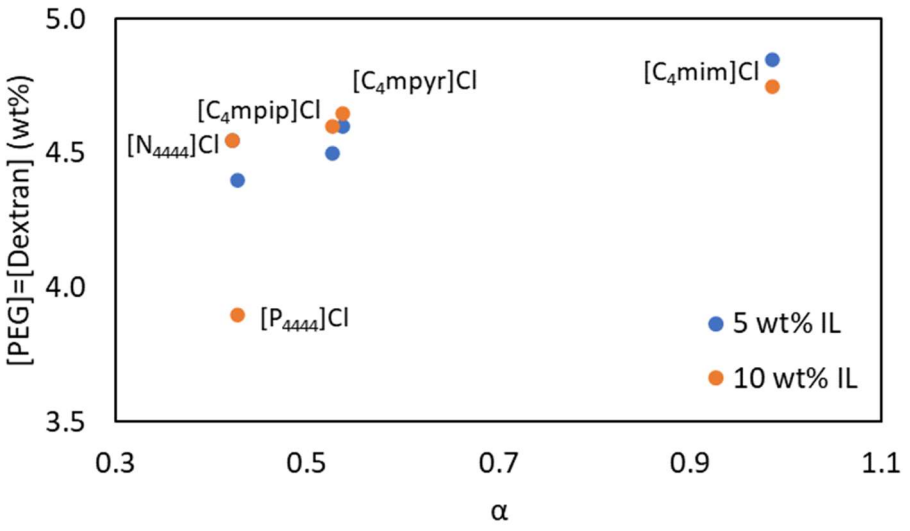

Figure S1 Values at each binodal curve in which [PEG]=[Dextran] (wt%) *versus* the predicted hydrogen-bond acidity of ionic liquids (α) by COSMO-RS.

Partition of ILs

Table S6 Partition coefficients of IL (K_{IL}) in ABS composed of 5 wt% PEG + 10 wt% Dextran + 5 wt% IL + 80 wt% water and 5 wt% PEG +10 wt% Dextran + 10 wt% IL + 75 wt% water

	IL	K_{IL}	Standard Deviation
5 wt% IL	**[C_4mim]Cl**	1.140	0.009
	[C_4mpyr]Cl	1.152	0.015
	[C_4mpip]Cl	1.152	0.020
	[N_{4444}]Cl	1.359	0.041
	[P_{4444}]Cl	1.243	0.002
10 wt% IL	**[C_4mim]Cl**	1.142	0.017
	[C_4mpyr]Cl	1.082	0.030
	[C_4mpip]Cl	1.120	0.019
	[N_{4444}]Cl	1.476	0.190
	[P_{4444}]Cl	1.312	0.045

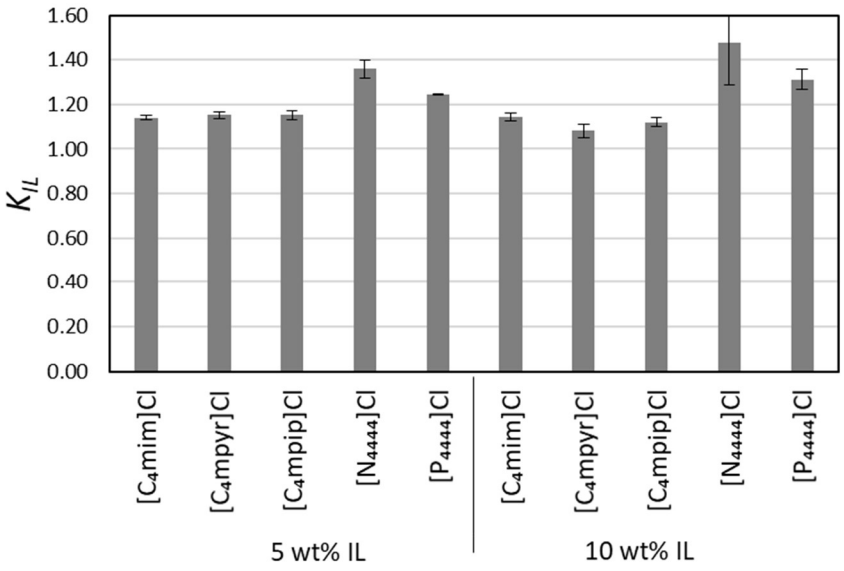

Figure S2 Partition coefficients of IL (K_{IL}) in ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran + 5 wt% IL + 80 wt% H_2O and 5 wt% PEG 6000 +10 wt% Dextran + 10 wt% IL + 75 wt% H_2O.

Proteins

Table S7 Partition coefficients of IgG, BSA and Cyt C in ABS composed of 5 wt% PEG + 10 wt% Dextran + 5 wt% IL + 80 wt% protein aqueous solution and 5 wt% PEG +10 wt% Dextran + 10 wt% IL + 75 wt% protein aqueous solution.

IL		BSA		IgG		Cyt C	
		K_{Prot}	Standard Deviation	K_{Prot}	Standard Deviation	K_{Prot}	Standard Deviation
Without IL		0.130	0.014	0.175	0.023	1.065	0.024
5 wt% IL	**[C_4mim]Cl**	0.182	0.025	0.163	0.025	0.032	0.032
	[C_4mpyr]Cl	0.155	0.036	0.163	0.004	0.039	0.089
	[C_4mpip]Cl	0.170	0.027	0.281	0.002	0.009	0.009
	[N_{4444}]Cl	0.474	0.019	0.227	0.040	0.008	0.079
	[P_{4444}]Cl	0.264	0.052	0.053	0.014	0.028	0.028
10 wt% IL	**[C_4mim]Cl**	0.150	0.000	0.197	0.035	0.009	0.089
	[C_4mpyr]Cl	0.151	0.006	0.174	0.012	0.040	0.040
	[C_4mpip]Cl	0.186	0.013	0.244	0.036	0.048	0.048
	[N_{4444}]Cl	0.359	0.019	0.172	0.044	0.000	0.000
	[P_{4444}]Cl					0.041	0.071

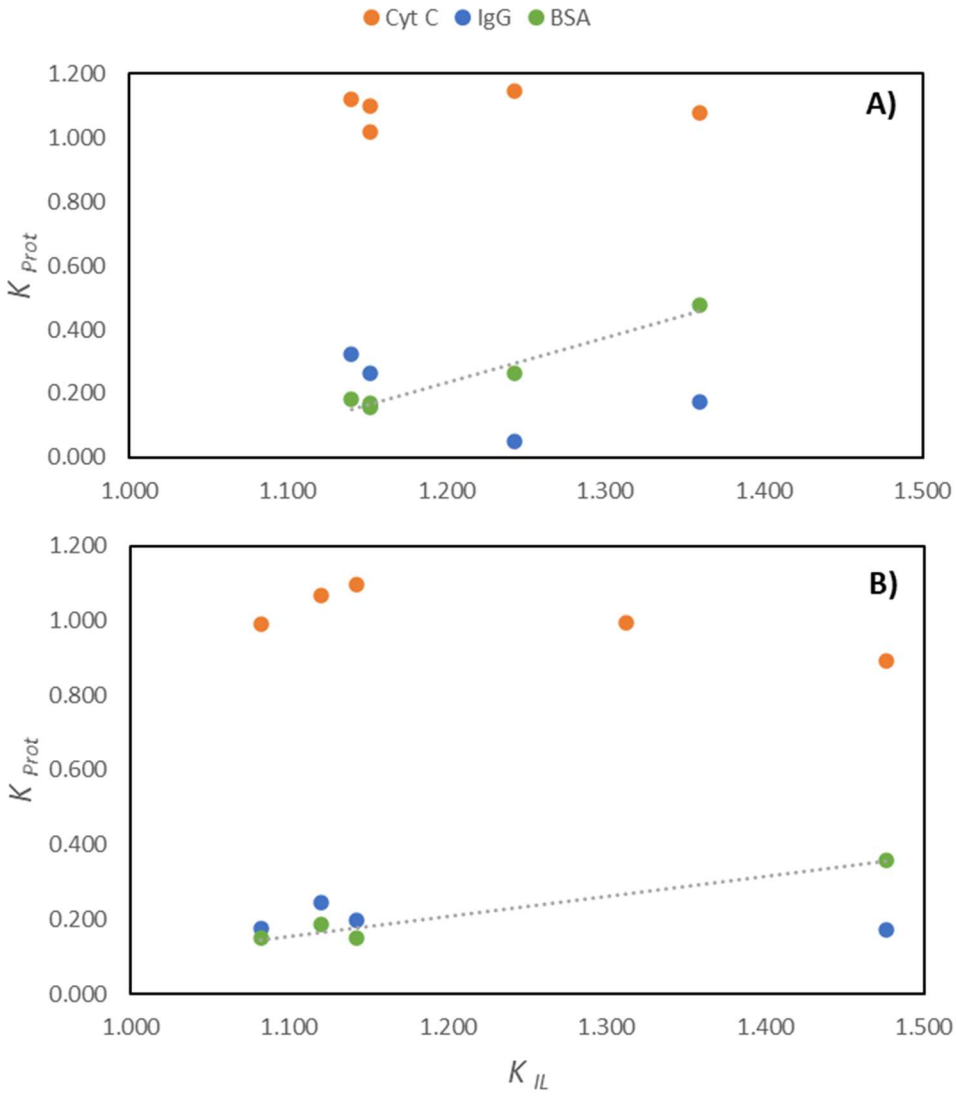

Figure S3 K_{Prot} *versus* K_{IL}. A) ABS composed of 5 wt% if IL; B) ABS composed of 10 wt% of IL.

Table S8 Partition coefficients of IgG, BSA and Cyt C in ABS composed of 22 wt% PEG 400 + 18 wt% $(NH_4)_2SO_4$ + 5 wt% IL + 55wt% protein aqueous solution.

IL		BSA		IgG		Cyt C	
		K_{Prot}	Standard Deviation	K_{Prot}	Standard Deviation	K_{Prot}	Standard Deviation
Without IL		∞	-	∞	-	0.181	0.122
5 wt% IL	[C_4mim]Cl	∞	-	∞	-	1.195	0.006
	[N_{4444}]Cl	∞	-	∞	-	0.951	0.018

Molecular Docking

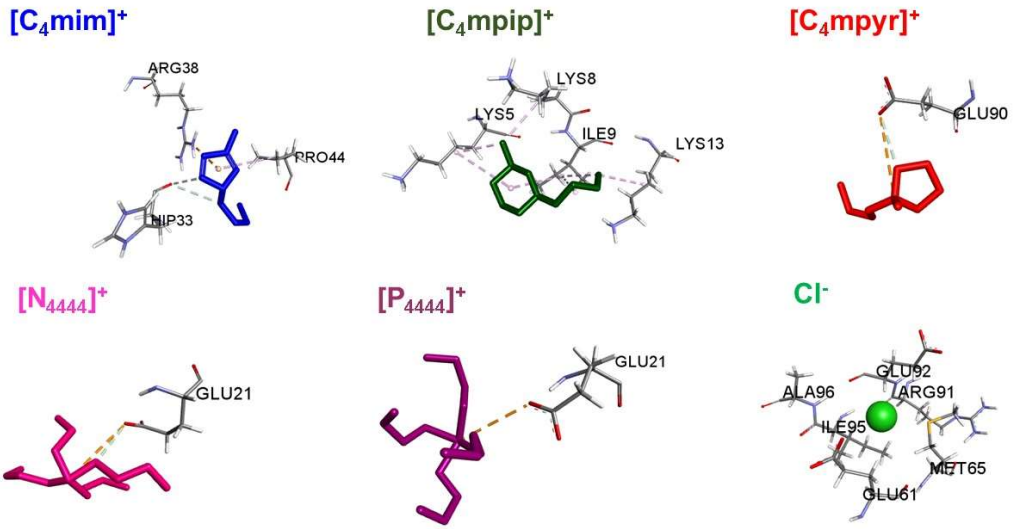

Figure S4 3D molecular interaction diagrams of Cyt C with IL cations $[C_4mim]^+$, $[C_4mpyr]^+$, $[C_4mpip]^+$, $[N_{4444}]^+$ and $[P_{4444}]^+$ and IL anion Cl^-.

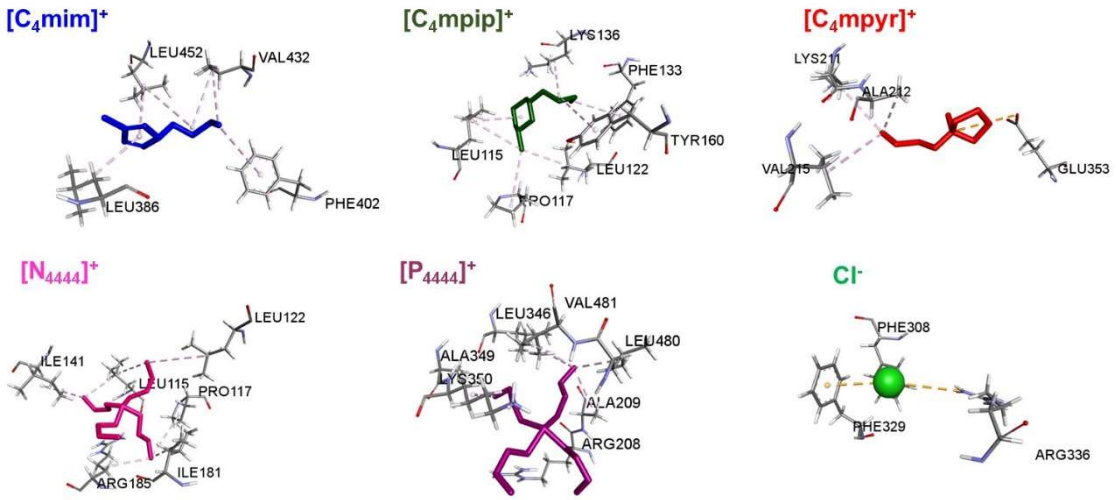

Figure S5 3D molecular interaction diagrams of BSA with IL cations $[C_4mim]^+$, $[C_4mpyr]^+$, $[C_4mpip]^+$, $[N_{4444}]^+$ and $[P_{4444}]^+$ and IL anion Cl^-.

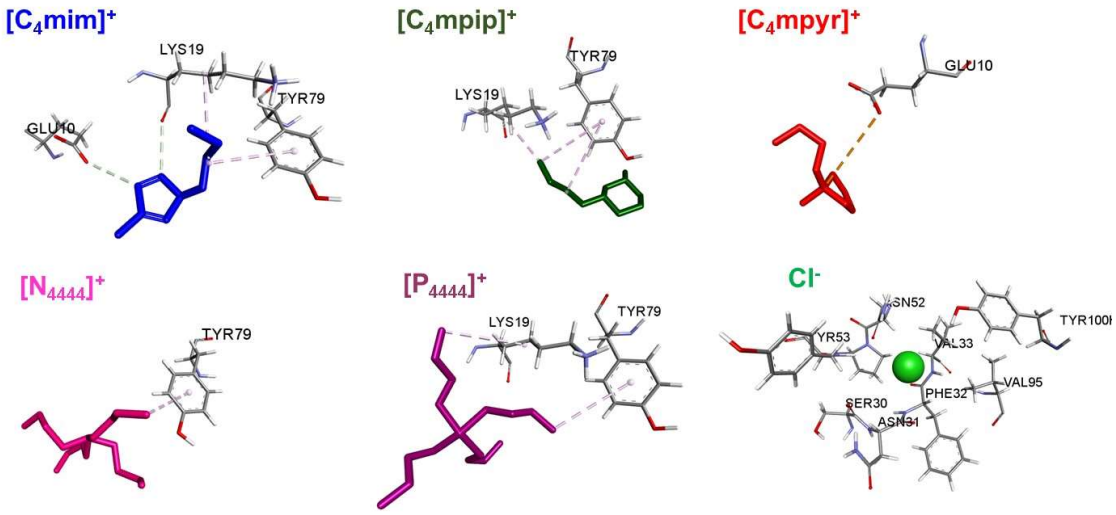

Figure S6 3D molecular interaction diagrams of IgG with IL cations $[C_4mim]^+$, $[C_4mpyr]^+$, $[C_4mpip]^+$, $[N_{4444}]^+$ and $[P_{4444}]^+$ and IL anion Cl^-.

Table S9 Docking affinity energy and interacting atoms predicted by AutoDock Vina for Cyt C and IL ions.

Compound	Affinity (kcal/mol)	Type of interaction	From	To	Distance (Å)
$[C_4mim]^+$	-3.0	Hydrogen Bond	$[C_4mim]^+$	Histidine 33	3.76
					3.50
		Electrostatic	Arginine 38	$[C_4mim]^+$	4.05
		Hydrophobic	$[C_4mim]^+$	Proline 44	5.31
$[C_4mpyr]^+$	-3.3	Electrostatic	$[C_4mpyr]^+$	Glutamic Acid 90	5.16
		Hydrogen Bond			3.77
$[C_4mpip]^+$	-3.9	Hydrophobic	Lysine 5	$[C_4mpip]^+$	4.90
			Isoleucine 9		4.83
			$[C_4mpip]^+$	Lysine 5	3.97
				Lysine 8	3.87
				Isoleucine 9	5.05
					4.05
				Lysine 13	4.34
$[N_{4444}]^+$	-2.6	Electrostatic	$[N_{4444}]^+$	Glutamic Acid 21	4.93
		Hydrogen Bond			3.70
$[P_{4444}]^+$	-3.1	Electrostatic	$[P_{4444}]^+$		4.12
Cl^-	-0.9	-	-	-	-

Table S10 Docking affinity energy and interacting atoms predicted by AutoDock Vina for BSA and IL ions.

Compound	Affinity (kcal/mol)	Type of interaction	From	To	Distance (Å)
$[C_4mim]^+$	-4.8	Hydrophobic	Valine 432	$[C_4mim]^+$	4.55
			$[C_4mim]^+$	Valine 432	4.97
				Leucine 452	5.09
			Leucine 386	$[C_4mim]^+$	4.72
			Phenylalanine 402		5.14
			Leucine 452		4.81
$[C_4mpyr]^+$	-4.7	Electrostatic	$[C_4mpyr]^+$	Glutamic Acid 353	5.22
		Hydrophobic	Alanine 212	$[C_4mpyr]^+$	4.06
			$[C_4mpyr]^+$	Lysine 211	5.07
				Valine 215	4.56
$[C_4mpip]^+$	-5.0		Leucine 115	$[C_4mpip]^+$	4.10
			Lysine 136		4.27
			$[C_4mpip]^+$	Leucine 115	4.50
				Proline 117	4.42
				Leucine 122	4.38
			Phenylalanine 133	$[C_4mpip]^+$	5.16
			Tyrosine 160		5.26
$[N_{4444}]^+$	-5.8	Hydrophobic	$[N_{4444}]^+$	Leucine 115	4.20
				Leucine 122	4.95
				Proline 117	4.77
				Isoleucine 181	4.46
				Arginine 185	4.30
				Leucine 115	4.32
				Isoleucine 141	4.85
$[P_{4444}]^+$	-4.1		Alanine 209	$[P_{4444}]^+$	4.11
			Alanine 349		3.60
			$[P_{4444}]^+$	Leucine 346	4.43
				Leucine 480	4.19
				Valine 481	5.22
				Lysine 350	5.10
Cl^-	-1.5	Electrostatic	Arginine 336	Cl^-	4.86
			Cl^-	Phenylalanine 308	4.04
				Phenylalanine 329	3.83

Table S11 Docking affinity energy and interacting atoms predicted by AutoDock Vina for IgG and IL ions.

Compound	Affinity (kcal/mol)	Type of interaction	From	To	Distance (Å)
$[C_4mim]^+$	-2.5	Hydrogen Bond	$[C_4mim]^+$	Lysine 19	3.32
				Glutamic Acid 10	3.75
		Hydrophobic	Lysine 19	$[C_4mim]^+$	4.73
			Tyrosine 79		4.91
$[C_4mpyr]^+$	-2.6	Electrostatic	$[C_4mpyr]^+$	Glutamic Acid 10	5.34
$[C_4mpip]^+$	-3.0	Hydrophobic	$[C_4mpip]^+$	Lysine 19	4.09
			Tyrosine 79	$[C_4mpip]^+$	5.11
					4.85
$[N_{4444}]^+$	-2.6				5.02
$[P_{4444}]^+$	-2.0		$[P_{4444}]^+$	Lysine 19	4.49
			Tyrosine 79	$[P_{4444}]^+$	5.05
Cl^-	-0.7	-	-	-	-

Selectivity

Table S13 Selectivity between proteins in polymer-polymer ABS with ILs at 5 and 10 wt%. ABS for which the selectivity is higher when employing ILs, when directly comparing with the respective system without IL, are provided in bold.

IL	Proteins	Selectivity	
Without IL	Cyt C/IgG	6.08	
	Cyt C/BSA	8.18	
	IgG/BSA	1.34	
		5 wt% IL	**10 wt% IL**
$[C_4mim]Cl$	Cyt C/IgG	3.44	5.59
	Cyt C/BSA	6.14	7.31
	IgG/BSA	**1.78**	1.31
$[C_4mpyr]Cl$	Cyt C/IgG	**6.75**	5.70
	Cyt C/BSA	7.08	6.59
	IgG/BSA	1.05	1.16
$[C_4mpip]Cl$	Cyt C/IgG	3.83	4.37
	Cyt C/BSA	5.97	5.74
	IgG/BSA	**1.56**	1.31
$[N_{4444}]Cl$	Cyt C/IgG	**6.20**	5.20
	Cyt C/BSA	2.74	2.50
	IgG/BSA	0.37	0.48
$[P_{4444}]Cl$	Cyt C/IgG	**21.7**	
	Cyt C/BSA	4.33	
	IgG/BSA	0.20	

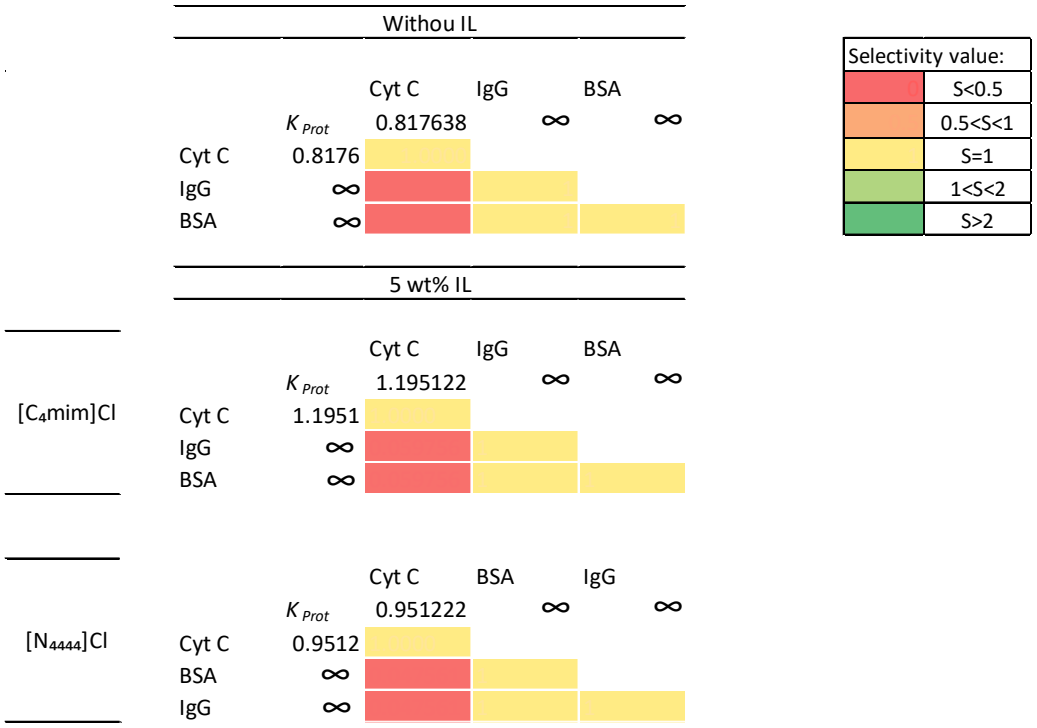

Figure S7 Selectivity of proteins in ABS composed of composed of 22 wt% PEG 400 + 18 wt% $(NH_4)_2SO_4$ + 5 wt% IL + 55wt% protein aqueous solution. ∞ means total protein partition to the top phase.

Table S14 Selectivity between proteins in polymer-salt ABS with ILs at 5 wt%.

IL	Proteins	Selectivity
Without IL	Cyt C/IgG	<0.5
	Cyt C/BSA	<0.5
	IgG/BSA	1
[C4mim]Cl	Cyt C/IgG	<0.5
	Cyt C/BSA	<0.5
	IgG/BSA	1
[N4444]Cl	Cyt C/IgG	<0.5
	Cyt C/BSA	<0.5
	IgG/BSA	1

Proteins Mixture

Table S15 Partition Coefficient (K_{Prot}) of IgG, BSA and Cyt C in ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran+ 5 wt% IL + 80 wt% proteins mixture aqueous solution.

IL		BSA		IgG		Cyt C	
		K_{Prot}	Standard Deviation	K_{Prot}	Standard Deviation	K_{Prot}	Standard Deviation
Without IL		0.063	0.013	0.089	0.026	1.193	0.028
5 wt% IL	**[C_4mim]Cl**	0.288	0.048	0.387	0.030	1.046	0.035
	[N_{4444}]Cl	0.269	0.044	0.262	0.070	1.118	0.006

Extraction efficiency

The extraction efficiency (EE_{Prot}) of each protein (i) to the bottom phase was determined according to Eq. S1:

$$EE_{Prot}\,\% = \frac{[Prot]_i\,(bottom) \times W(Bottom-phase)}{[Prot]_i\,(bottom) \times W(Bottom-phase) + [Prot]_i\,(top)\,W(Top-phase)} * 100 \qquad \text{(S1)}$$

Table S16 Extraction efficiency (EE_{prot}) of IgG, BSA and Cyt C in ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran + 5 wt% IL + 80 wt% proteins mixture aqueous solution.

IL		BSA		IgG		Cyt C	
		EE_{Prot} (%)	Standard Deviation	EE_{Prot} (%)	Standard Deviation	EE_{Prot} (%)	Standard Deviation
Without IL		95.17	1.20	95.28	1.42	60.20	1.14
5 wt% IL	**[C_4mim]Cl**	81.78	1.82	82.16	1.44	63.00	1.26
	[N_{4444}]Cl	79.73	3.35	88.27	2.59	63.52	0.64

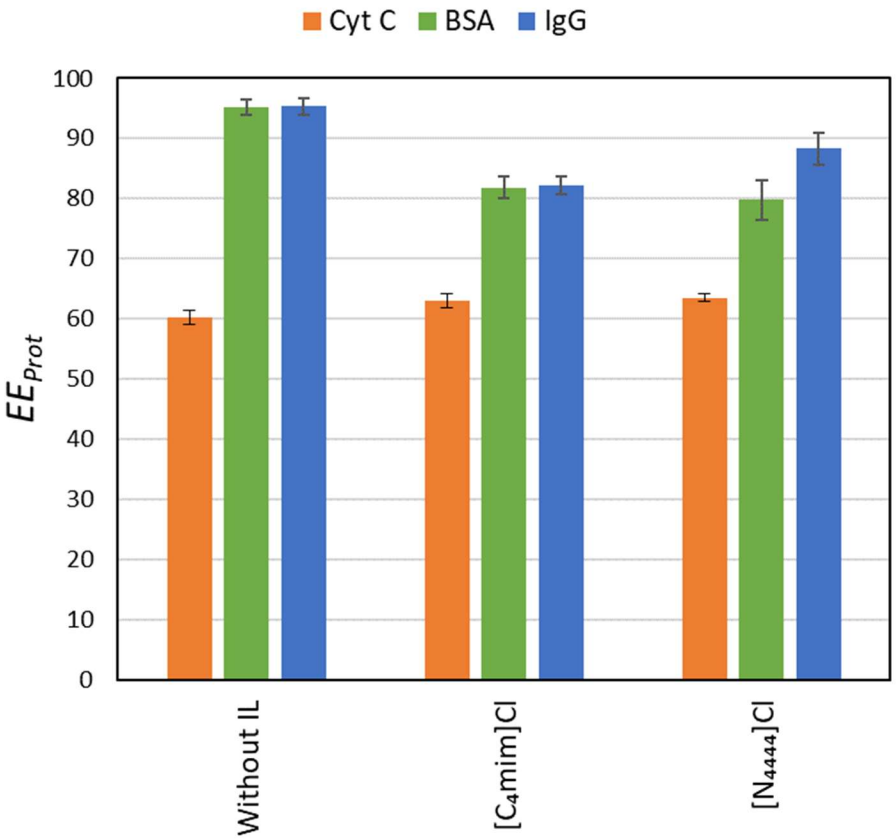

Figure S8 Extraction efficiency of each protein (EE_{Prot}) to the bottom-phase (Dextran-rich phase) in ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran + 5 wt% IL + 80 wt% protein aqueous solution (individual protein and mixture of the three proteins): Cyt C ( ); BSA ( ); IgG ( ).

SE-HPLC chromatograms

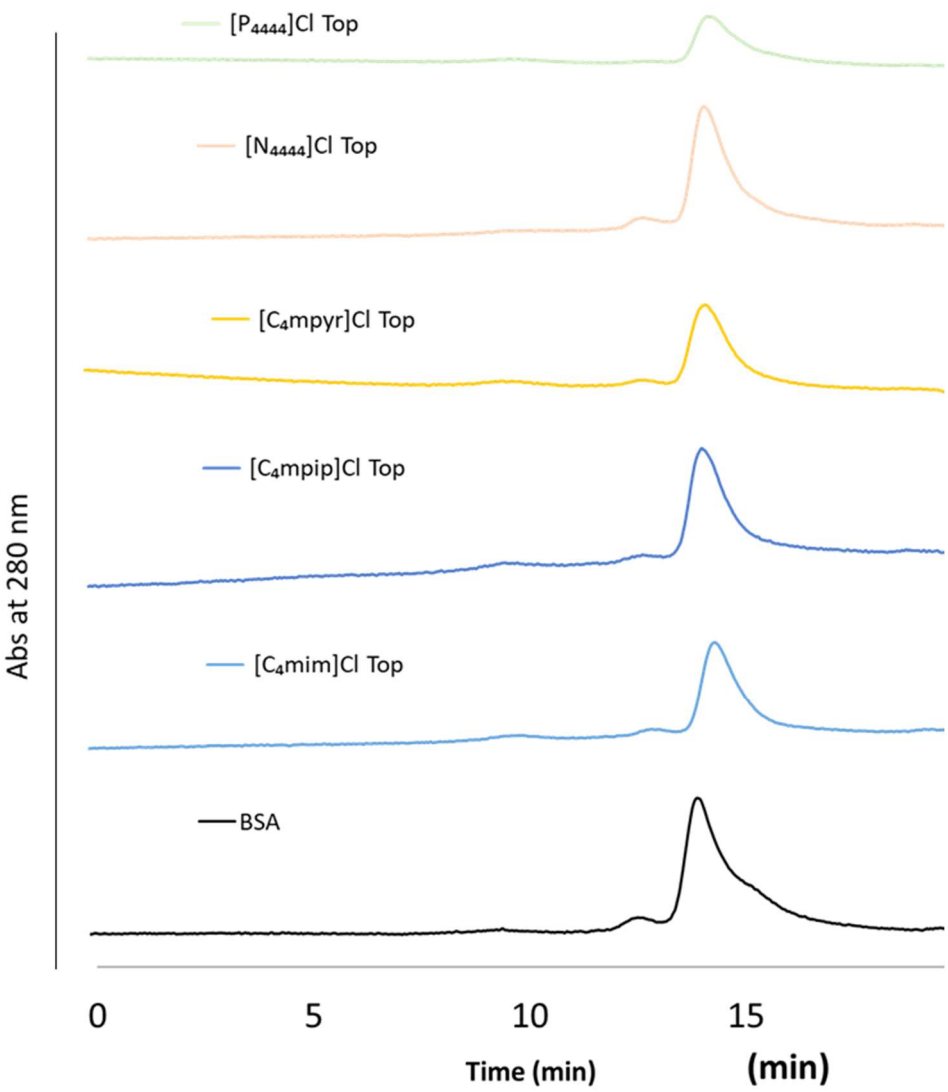

Figure S9 Size-exclusion chromatograms of BSA and of the phases after BSA partition in the ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran + 5 wt% IL + 80 wt% protein aqueous solution. The BSA peak is characterized by a retention time around 13.8 min.

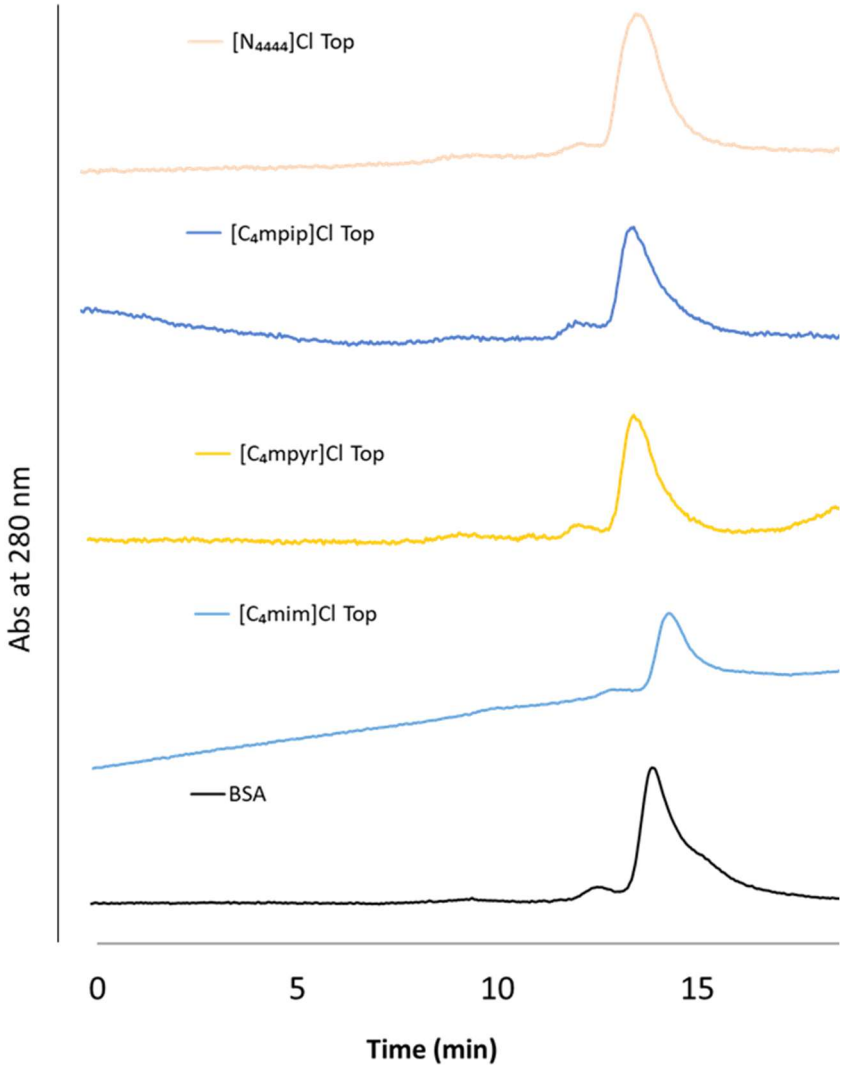

Figure S10 Size-exclusion chromatograms of BSA and of the phases after BSA partition in the ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran + 10 wt% IL + 75 wt% protein aqueous solution. The BSA peak is characterized by a retention time around 13.8 min.

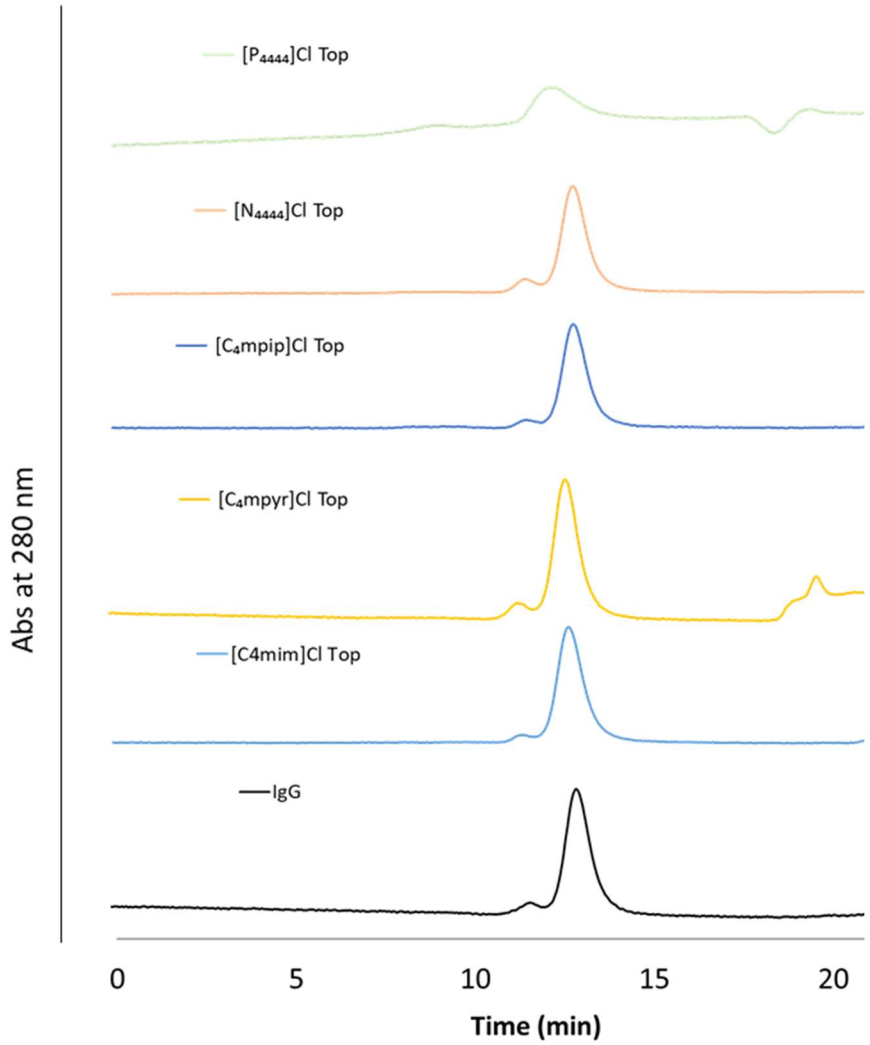

Figure S11 Size-exclusion chromatograms of IgG and of the phases after IgG partition in ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran + 5 wt% IL + 80 wt% protein aqueous solution. The IgG peak is characterized by a retention time around 12.7 min.

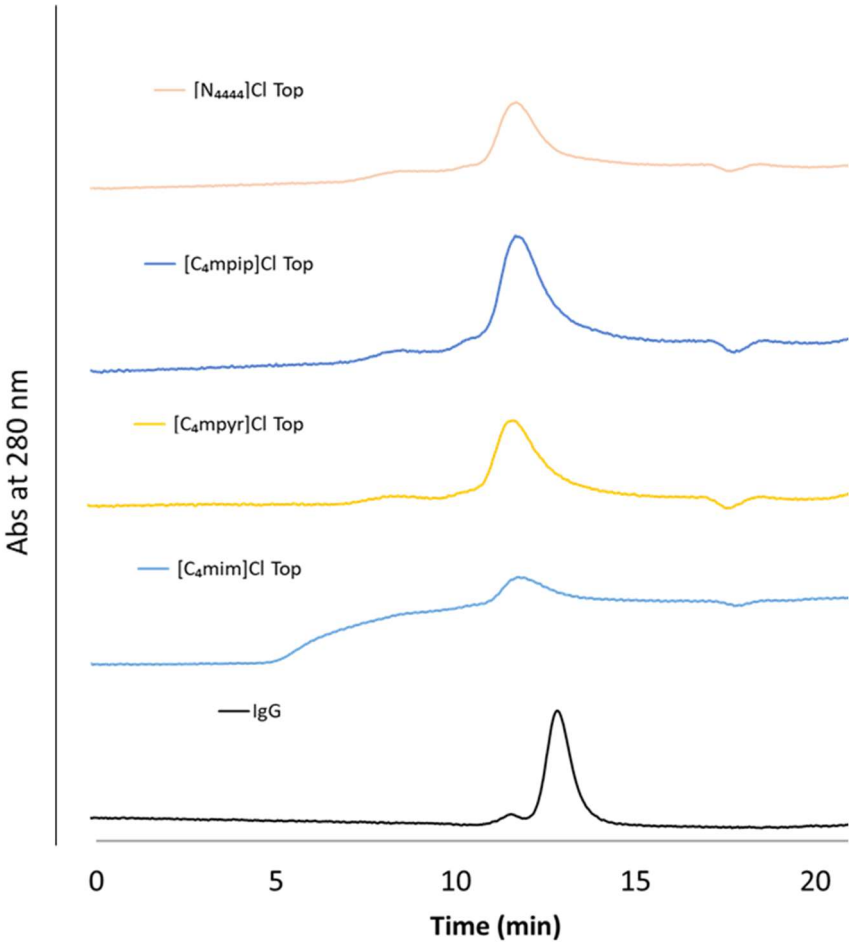

Figure S12 Size-exclusion chromatograms of IgG and of the phases after IgG partition in ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran + 10 wt% IL + 75 wt% protein aqueous solution. The IgG peak is characterized by a retention time around 12.7 min.

Cyt C Enzymatic Activity

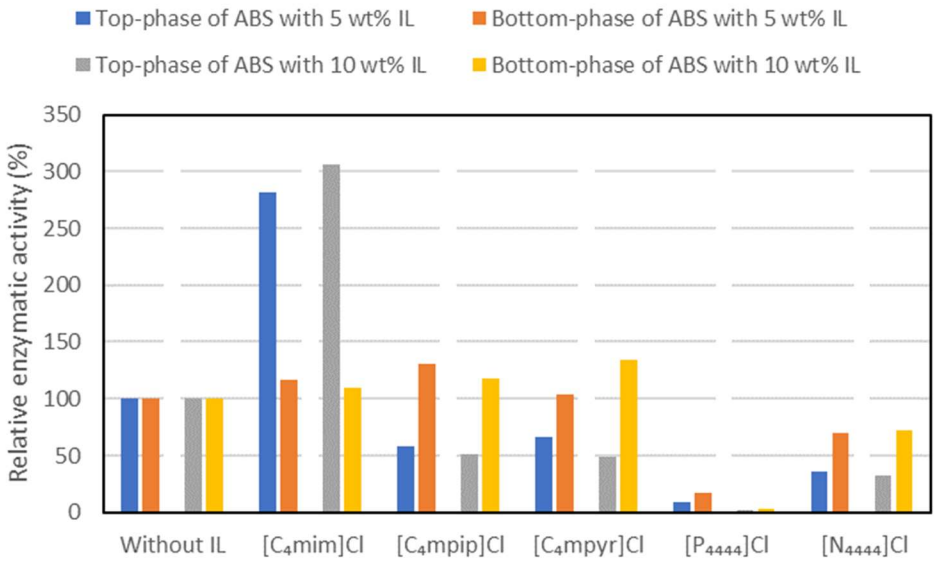

Figure S13 Relative enzymatic activity of Cyt C in ABS composed of 5 wt% PEG 6000 + 10 wt% Dextran + 5 or 10 wt% IL + 80 or 75 wt% protein aqueous solution. The relative enzyme activity was calculated considering the phases of ABS without IL as 100 % of enzymatic activity.