Electronic Supplementary Information

Sustainable strategies based on glycine-betaine analogues ionic liquids for the recovery of monoclonal antibodies from cell culture supernatants

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Production of anti-human interleukin-8 (anti-IL-8) monoclonal antibodies:

Anti-human interleukin-8 (anti-IL-8) monoclonal antibodies were produced in-house by a CHO DP-12 clone#1934 (ATCC CRL-12445) using DHFR minus/methotrexate selection system, obtained from the American Type Culture Collection (LGC Standards, Middlesex, UK). CHO DP-12 cells were grown in a mixture of 75 % (v/v) of serum-free media formulated with 0.1 % Pluronic® F-68 and without L-glutamine, phenol red, hypoxanthine, or thymidine (ProCHO™5, Lonza Group Ltd, Belgium), and 25 % (v/v) of Dulbecco's modified Eagle's medium (DMEM), supplemented with 10 % (v/v) of ultra-low IgG fetal bovine serum (FBS). ProCHO™5 formulation contains 4 $mmol \cdot L^{-1}$ L-glutamine (Gibco®, Carlsbad, CA), 2.1 $g \cdot L^{-1}$ $NaHCO_3$ (Sigma–Aldrich), 10 $mg \cdot L^{-1}$ recombinant human insulin (Lonza), 0.07 % (v/v) lipids (Lonza), 1 % (v/v) antibiotics (100 $U \cdot mL^{-1}$ penicillin and 100 $\mu g \cdot mL^{-1}$ streptomycin) (Gibco®) and 200 nM methotrexate (Sigma). DMEM was formulated to contain 4 $mmol \cdot L^{-1}$ of L-glutamine, 4.5 $g \cdot L^{-1}$ of D-glucose, 1 $mmol \cdot L^{-1}$ of sodium pyruvate, 1.5 $g \cdot L^{-1}$ of $NaHCO_3$, 2 $mg \cdot L^{-1}$ of recombinant human insulin, 35 $mg \cdot L^{-1}$ of L-proline (all acquired at Sigma), 0.1 % (v/v) of a trace element A, 0.1 % (v/v) of a trace element B (both from Cellgro®, Manassas, VA, USA), and 1 % (v/v) of antibiotics (100 $U \cdot mL^{-1}$ of penicillin and 100 $\mu g \cdot mL^{-1}$ of streptomycin from Gibco®). The composition of trace element A includes 1.60 $mg \cdot L^{-1}$ of $CuSO_4 \cdot 5H_2O$, 863.00 $mg \cdot L^{-1}$ of $ZnSO_4 \cdot 7H_2O$, 17.30 $mg \cdot L^{-1}$ of selenite·2Na, and 1155.10 $mg \cdot L^{-1}$ of ferric citrate, while the trace element B is composed of 0.17 $mg \cdot L^{-1}$ of $MnSO_4 \cdot H_2O$, 140.00 $mg \cdot L^{-1}$ of $Na_2SiO_3 \cdot 9H_2O$, 1.24 $mg \cdot L^{-1}$ of molybdic acid, ammonium salt, 0.65 $mg \cdot L^{-1}$ of NH_4VO_3, 0.13 $mg \cdot L^{-1}$ of $NiSO_4 \cdot 6H_2O$, and 0.12 $mg \cdot L^{-1}$ of $SnCl_2$.

Cultures were carried out in T-75 flasks (BD Falcon, Franklin Lakes, NJ) at 37 (±1) ºC and 5 % CO_2 with an initial cell density of 2.1×10^6 $cells \cdot mL^{-1}$. Cell passages were performed every 4 days in a laminar flow chamber. Cell supernatants were centrifuged in BD Falcon™ tubes at 175 × g for 7 min, collected and storage at -20 ºC. This culture was maintained for several months, with the mAbs concentration varying between 40.5 and 99.4 $mg \cdot L^{-1}$. The produced anti-IL-8 mAb has an isoelectric point (pI) of 9.3 [1].

Phase diagrams, tie-lines and tie-line lengths determination:

The determination of the binodal curves was performed using the cloud point titration method at 25 (± 1) °C and atmospheric pressure, where the mixture compositions were gravimetrically determined. IL aqueous solutions with concentrations ranging between 60 wt% and 80 wt% were prepared. To these solutions, a 40 wt% K_2HPO_4/KH_2PO_4 aqueous solution at pH 7.0 was added, allowing the identification of a cloud point corresponding to the biphasic system, followed by the addition of water up to the identification of clear solutions, which correspond to the monophasic region. The experimental binodal curves were adjusted by the equation proposed by Merchuk et al. [2]:

$$[\mathrm{IL}] = A.\mathrm{e}^{B.[\mathrm{salt}]^{0.5} - C.[\mathrm{salt}]^3} \quad \text{(S1)}$$

where [IL] and [salt] correspond the IL and phosphate salt weight fraction percentages, respectively, and the coefficients A, B and C are fitting parameters determined using the SigmaPlot 11.0 software.

The TLs were determined by the resolution of the following equations (S2 – S5), allowing to obtain the concentrations of IL and salt in both top and bottom phases:

$$[IL]_{TOP} = A \times exp[B[salt]_{TOP}^{0.5} - C[salt]_{TOP}^{3}] \quad \text{(S2)}$$

$$[IL]_{BOT} = A \times exp[B[salt]_{BOT}^{0.5} - C[salt]_{BOT}^{3}] \quad \text{(S3)}$$

$$[\mathrm{IL}]_{TOP} = \frac{[IL]_M}{VR} - \left(\frac{1-VR}{VR}\right)[IL]_{BOT} \quad \text{(S4)}$$

$$[\mathrm{salt}]_{TOP} = \frac{[salt]_M}{VR} - \left(\frac{1-VR}{VR}\right)[salt]_{BOT} \quad \text{(S5)}$$

where the terms TOP, BOT, and M represents the top phase, bottom phase and the mixture point, respectively. The VR parameter represents the ratio between the top phase weight and the total system weight.

Tie-line lengths (TLLs), which give indication on the coexisting phases compositions difference, were calculated according to Equation S6:

$$\mathrm{TLL} = \sqrt{([IL]_{TOP} - [IL]_{BOT})^2 + ([salt]_{TOP} - [salt]_{BOT})^2} \quad \text{(S6)}$$

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) assays:

To address the proteins profile and the stability/integrity of mAbs, a volume of 25 μL of each prepared sample was applied in a 12 % acrylamide gel, prepared from a 40 % acrylamide/bis stock solution (29:1) from Bio-Rad, and ran at 90 mV using a running buffer containing 192 mM glycine, 25 mM Tris, and 0.1 % (w/v) SDS at pH 8.3. The molecular weight standard used was Precision Plus Protein™ Dual Color Standards from BioRad. Gels were stained with 0.1 % (w/v) Coomassie Brilliant Blue R-250 from Pharmacia AB Laboratory Separations® (Uppsala, Sweden), 30 % (v/v) ethanol, 10 % (v/v) acetic acid and water, in an orbital shaker at 40 °C and moderate velocity during 1 hour. Gels were then distained using a solution containing 30 % (v/v) ethanol and 10 % (v/v) acetic acid, in an orbital shaker at 25 °C and moderate velocity, until background colour disappeared. Finally, gels were stored in milli-Q water at room temperature, until digital images of the gels were acquired using a calibrated densitometer GS-800 from Bio-Rad and analyzed with the informatics tool Quantity One 4.6 also from Bio-Rad.

Competitive enzyme linked immunosorbent assays (ELISA):

In order to address the anti-IL-8 specific activity, a 96-well ELISA plate from a Quantikine® Human IL-8/CXCL8 kit from R&D systems (Minnneapolis, MN, USA). The plate was coated with 100 μL assay diluent followed by the addition of 50 μL of 1 $mg \cdot L^{-1}$ of human IL-8 standard from Sigma-Aldrich. The plate was left for incubating for two hours at room temperature. After this period, each well was aspirated and washed for four consecutive times with wash buffer. Then, 100 μL of each sample containing anti-IL-8 mAbs was added to the wells, and were left to incubate for one hour at room temperature, being further washed as previously described. 100 μL of anti-IL-8 conjugate was added to all wells and incubated for one hour at room temperature, and after washed again as previously described. 200 μL of substrate solution was then added to the wells and incubated for 30 minutes at room temperature (protected from the light with a plate sealer). Finally, 50 μL of stop solution was added to end the reaction, and the absorbance was measured at 450 nm and 540 nm in a SYNERGY|HT microplate reader, BioTek. Final results were calculated based on the subtraction of the absorbance at 450 nm for the obtained at 570 nm, to correct optical deviations from the plate.

Table S1. Experimental binodal weight fraction data for the system composed of $[Et_3NC_4]Br$ + K_2HPO_4/KH_2PO_4 (pH = 7) + H_2O at 25°C and atmospheric pressure.

w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}	w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}
51.7158	1.3968	46.8874	21.4961	13.3615	65.1424
49.1841	2.0115	48.8044	20.4072	14.1098	65.4829
45.3876	2.4604	52.1519	19.3642	14.9166	65.7192
43.6260	2.8236	53.5503	18.5393	15.4864	65.9743
41.8363	3.2445	54.9192	17.6821	16.0854	66.2325
39.2609	4.4043	56.3348	17.2613	16.4102	66.3285
37.3440	5.1186	57.5374	16.7545	16.7801	66.4654
35.5340	5.7994	58.6666	16.1590	17.2296	66.6114
33.5615	6.7174	59.7211	15.6074	17.6410	66.7516
32.1482	7.2128	60.6389	15.0983	18.0283	66.8734
30.5871	7.9867	61.4262	14.6943	18.3353	66.9703
29.4386	8.5074	62.0540	14.2288	18.6864	67.0849
28.1514	9.1755	62.6732	13.7727	19.0652	67.1622
26.6557	10.0327	63.3115	13.4202	19.3552	67.2246
25.2733	10.8654	63.8613	13.0383	19.6609	67.3007
23.9932	11.6712	64.3356	12.6732	19.9368	67.3900
22.7115	12.5375	64.7510	12.3236	20.2188	67.4576

Table S2. Experimental binodal weight fraction data for the system composed of $[Pr_3NC_4]Br$ + K_2HPO_4/KH_2PO_4 (pH = 7) + H_2O at 25°C and atmospheric pressure.

w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}	w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}
46.7054	2.1985	51.0961	17.0550	13.4711	69.4740
42.1058	2.7180	55.1762	16.6708	13.6951	69.6341
39.7763	3.0260	57.1977	16.2161	14.0025	69.7814
38.3309	3.5038	58.1653	15.7029	14.4182	69.8789
36.3250	3.8135	59.8615	15.2808	14.6902	70.0290
35.0851	4.3104	60.6045	14.8764	14.9377	70.1859
34.0362	4.6568	61.3069	14.5101	15.1736	70.3164
32.8430	5.1607	61.9963	14.0598	15.5842	70.3559
31.7858	5.4230	62.7912	13.7562	15.7541	70.4897
30.7993	5.8231	63.3776	13.4437	15.9700	70.5863
29.7088	6.2842	64.0070	13.1126	16.2745	70.6129
28.4249	7.1453	64.4298	12.8116	16.4715	70.7169
26.8219	7.7220	65.4561	12.5339	16.6910	70.7750
26.1978	7.8928	65.9095	12.3199	16.9234	70.7567
25.1770	8.4974	66.3256	12.0934	17.1225	70.7841
24.4584	8.8353	66.7063	11.9741	17.1840	70.8419
23.6273	9.4027	66.9701	11.7659	17.3459	70.8882
23.0131	9.6684	67.3185	11.6193	17.4225	70.9582
22.2044	10.1700	67.6256	11.3859	17.6797	70.9344
21.4931	10.6041	67.9028	11.2426	17.7580	70.9994
20.8855	10.9855	68.1289	11.0875	17.8563	71.0562
20.2877	11.3269	68.3854	10.8142	18.1385	71.0472
19.6855	11.6886	68.6259	10.5802	18.3009	71.1189
19.1518	12.0150	68.8332	10.3550	18.4575	71.1875
18.5498	12.4290	69.0212	10.0686	18.7651	71.1663
17.9196	12.9447	69.1357	9.8677	18.9253	71.2070
17.5541	13.0904	69.3555	9.6748	19.0543	71.2710

Table S3. Experimental binodal weight fraction data for the system composed of $[Bu_3NC_4]Br$ + K_2HPO_4/KH_2PO_4 (pH = 7) + H_2O at 25°C and atmospheric pressure.

w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}	w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}
51.9191	1.5923	46.4886	19.9229	10.6083	69.4688
46.9127	2.2425	50.8448	19.6559	10.7165	69.6276
43.5194	2.8259	53.6547	19.3711	10.8592	69.7697
40.6580	3.3347	56.0073	19.1145	10.9999	69.8857
38.7496	3.7627	57.4877	18.8703	11.1478	69.9819
36.9253	4.1905	58.8841	18.6285	11.2366	70.1349
35.3648	4.5575	60.0777	18.3906	11.3605	70.2489
34.3087	4.9972	60.6941	18.0247	11.6521	70.3232
33.0009	5.3309	61.6682	17.7833	11.7297	70.4870
31.9095	5.6870	62.4036	17.5645	11.8503	70.5852
30.8285	5.9424	63.2291	17.4554	11.7549	70.7897
29.9970	6.2751	63.7279	17.1115	11.8054	71.0831
29.3602	6.6332	64.0066	16.8963	11.9177	71.1860
28.5857	6.8980	64.5163	16.7101	12.0118	71.2781
27.8617	7.1895	64.9489	16.4733	12.1863	71.3404
26.7656	7.5591	65.6753	16.2437	12.3013	71.4550
26.2042	7.7500	66.0458	16.0542	12.3871	71.5587
25.6010	7.9565	66.4425	15.7384	12.6363	71.6253
24.9938	8.2821	66.7241	15.5206	12.7421	71.7373
24.5268	8.5354	66.9378	15.3861	12.7600	71.8539
24.0878	8.7532	67.1590	15.2391	12.8375	71.9233
23.5363	8.8867	67.5770	15.0648	12.9345	72.0007
23.1539	9.0881	67.7581	14.8445	13.1257	72.0298
22.7397	9.3094	67.9509	14.6358	13.1686	72.1956
21.7278	9.8415	68.4306	14.4703	13.2813	72.2484
21.3484	9.8776	68.7740	14.2562	13.4088	72.3350
21.0914	10.0179	68.8906	14.0149	13.6488	72.3362
20.8433	10.1843	68.9724	13.8367	13.6957	72.4676
20.5244	10.3452	69.1304	13.5768	13.8901	72.5331
20.2125	10.4667	69.3208	13.4128	14.0051	72.5821

w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}	w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}
13.2620	14.1119	72.6261	10.3766	16.0386	73.5849
13.1412	14.1659	72.6929	10.1812	16.2248	73.5940
12.9857	14.2291	72.7852	10.0949	16.2673	73.6377
12.8099	14.3801	72.8099	10.0124	16.3119	73.6757
12.6944	14.4451	72.8605	9.8769	16.4291	73.6940
12.5847	14.4919	72.9235	9.7809	16.5342	73.6849
12.4572	14.6170	72.9258	9.6936	16.5702	73.7362
12.3302	14.6830	72.9868	9.5839	16.6483	73.7678
12.1484	14.8117	73.0398	9.4728	16.7449	73.7824
12.0215	14.9158	73.0627	9.3885	16.8200	73.7915
11.9090	14.9821	73.1089	9.3028	16.8918	73.8054
11.7858	15.0514	73.1628	9.1908	16.9694	73.8399
11.6448	15.0975	73.2577	9.0704	17.0956	73.8340
11.5268	15.1950	73.2783	8.9516	17.2253	73.8231
11.4269	15.2454	73.3276	8.8276	17.2695	73.9029
11.3341	15.3180	73.3479	8.7369	17.3473	73.9158
11.1809	15.4807	73.3384	8.6171	17.4598	73.9230
10.9538	15.6209	73.4253	8.5204	17.5681	73.9114
10.8184	15.7586	73.4230	8.3911	17.7123	73.8965
10.7250	15.8024	73.4726	8.2205	17.8465	73.9330
10.6031	15.9322	73.4647	8.1151	17.9201	73.9647
10.4472	16.0085	73.5443			

Table S4. Experimental binodal weight fraction data for the system composed of [MepyrNC$_4$]Br + K_2HPO_4/KH_2PO_4 (pH = 7) + H_2O at 25°C and atmospheric pressure.

w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}
58.1075	1.6606	40.2319
51.1023	2.2806	46.6171
47.4332	2.9757	49.5912
45.6564	3.5523	50.7913
43.1322	4.0567	52.8111
41.4770	4.6154	53.9075
40.1201	5.0927	54.7873
38.8002	5.4909	55.7089
37.0268	6.3923	56.5809
35.7334	6.7845	57.4821
34.0957	7.6148	58.2894
32.9499	8.0007	59.0495
31.4460	8.9373	59.6166
29.9522	9.6941	60.3537
28.2156	10.7641	61.0203
26.8084	11.5994	61.5922
25.5219	12.3816	62.0965
24.0855	13.3985	62.5160
22.9392	14.1729	62.8879
21.7332	15.0486	63.2182
20.9443	15.5108	63.5448
19.6848	16.5412	63.7739
18.7191	17.1825	64.0984
17.7646	17.9354	64.3000
16.9757	18.5246	64.4997
16.2394	19.0688	64.6917
15.5946	19.6120	64.7933
14.8761	20.1964	64.9275
14.2163	20.7115	65.0722

Table S5. Experimental binodal weight fraction data for the system composed of $[C_4mim]Br$ + K_2HPO_4/KH_2PO_4 (pH = 7) + H_2O at 25°C and atmospheric pressure.

w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}
47.8896	2.8782	49.2322
44.6709	3.3364	51.9927
42.7620	3.9148	53.3232
40.6202	4.8294	54.5504
38.5897	5.7409	55.6694
36.6716	6.5095	56.8190
34.9949	7.1748	57.8303
33.4899	7.6854	58.8248
31.7325	8.6182	59.6493
30.2865	9.5007	60.2128
28.8790	10.2398	60.8811
27.6987	10.8714	61.4299
26.0237	12.0395	61.9367
24.7303	12.8324	62.4373
23.2340	13.8948	62.8711
21.9085	14.8360	63.2554
20.6594	15.7425	63.5981
19.4208	16.7565	63.8227
18.6038	17.3371	64.0591
17.6196	18.1176	64.2628
16.7150	18.8506	64.4344
16.0224	19.3893	64.5883
15.1673	20.1299	64.7028

Table S6. Experimental binodal weight fraction data for the system composed of $[N_{4444}]Br$ + K_2HPO_4/KH_2PO_4 (pH = 7) + H_2O at 25°C and atmospheric pressure.

w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}	w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}
49.9689	2.4034	47.6277	18.0533	11.5277	70.4189
44.1506	2.6017	53.2478	17.7389	11.6696	70.5915
41.5556	3.4015	55.0429	17.4655	11.7658	70.7687
37.8742	3.9722	58.1536	16.9962	12.1610	70.8428
35.7664	4.2466	59.9870	16.4493	12.4598	71.0909
34.0903	4.6862	61.2235	16.0400	12.6959	71.2640
32.7900	5.1248	62.0852	15.6227	12.9768	71.4005
31.2127	5.5757	63.2117	15.3555	13.0590	71.5854
29.8797	5.9235	64.1968	15.1463	13.1795	71.6742
29.1839	6.1756	64.6405	14.9276	13.3432	71.7292
28.5238	6.5591	64.9171	14.7185	13.5051	71.7764
27.2931	6.9072	65.7997	14.2949	13.7538	71.9514
26.6248	7.1952	66.1800	14.0990	13.9120	71.9891
25.9214	7.5842	66.4944	13.7383	14.0869	72.1748
25.3178	7.8177	66.8645	13.5464	14.2548	72.1988
24.6583	8.0711	67.2706	13.2829	14.4597	72.2574
24.0394	8.3259	67.6347	13.0889	14.5421	72.3690
23.5126	8.5586	67.9288	12.9008	14.6227	72.4765
22.9492	8.8461	68.2046	12.6184	14.8947	72.4869
22.4423	9.0995	68.4582	12.4582	14.9839	72.5579
22.0426	9.2601	68.6973	12.1939	15.2358	72.5703
21.3796	9.7366	68.8838	11.9768	15.4133	72.6099
20.8133	9.9356	69.2511	11.8414	15.4239	72.7347
20.3810	10.2482	69.3708	11.6420	15.6514	72.7066
20.0379	10.4119	69.5502	11.4691	15.6472	72.8837
19.6974	10.5982	69.7044	11.3481	15.7390	72.9130
19.3656	10.7446	69.8898	11.2308	15.8197	72.9495
18.9979	10.9113	70.0907	11.0741	15.9457	72.9802
18.6544	11.1490	70.1967	10.9616	16.0328	73.0056
18.3464	11.3659	70.2877	10.8320	16.1466	73.0214

w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}	w_{IL}	$w_{K_2HPO_4/KH_2PO_4}$	w_{H_2O}
10.6485	16.3437	73.0078	9.5551	17.1341	73.3108
10.5295	16.4137	73.0568	9.3807	17.3017	73.3176
10.3581	16.5640	73.0779	9.2694	17.3650	73.3656
10.2351	16.5659	73.1990	9.1144	17.5130	73.3726
10.0696	16.7696	73.1608	8.9721	17.6519	73.3760
9.9792	16.7621	73.2587	8.8628	17.6816	73.4556
9.8453	16.8889	73.2658	8.7199	17.8158	73.4643
9.7277	16.9738	73.2985			

Table S7. Correlation parameters used to describe the experimental binodal curve of the ternary system composed of IL + K_2HPO_4/KH_2PO_4 pH 7 + H_2O through Equation S1.

IL	A ± σ	B ± σ	C ± σ (× 10^{-4})	R^2
[Et_3NC_4]Br	82.968 ± 2.194	-0.353 ± 0.014	0.338 ± 0.078	0.9892
[Pr_3NC_4]Br	85.806 ± 1.860	-0.415 ± 0.010	0.475 ± 0.057	0.9944
[Bu_3NC_4]Br	87.536 ± 0.418	-0.415 ± 0.002	1.141 ± 0.001	0.9996
[MepyrNC$_4$]Br	85.693 ± 1.246	-0.331 ± 0.007	0.297 ± 0.033	0.9979
[C_4mim]Br	84.974 ± 2.374	-0.330 ± 0.013	0.281 ± 0.060	0.9945
[N_{4444}]Br	101.442 ± 1.689	-0.486 ± 0.007	0.649 ± 0.039	0.9975

Table S8. Experimental data of TLs and TLLs for the ternary systems composed of IL + K_2HPO_4/KH_2PO_4 pH 7 + H_2O. [IL] and [salt] correspond to the compositions of IL and salt, respectively, while content of water corresponds to the amount required to reach 100 wt%. The subscripts TOP, M and BOT refers to the top (IL-rich) phase, mixture point and bottom (salt-rich) phase, respectively. The ratio (VR) between the top and bottom phases weight is also denoted.

IL	Mass fraction composition (wt%)						TLL	VR
	$[IL]_{TOP}$	$[salt]_{TOP}$	$[IL]_{M}$	$[salt]_{M}$	$[IL]_{BOT}$	$[salt]_{BOT}$		
$[Et_3NC_4]Br$	37.427	5.023	24.989	15.103	3.434	32.572	44.851	0.634
	38.959	4.540	27.350	15.141	1.231	28.992	51.091	0.692
	43.016	3.442	30.196	14.897	0.816	41.147	56.590	0.696
$[Pr_3NC_4]Br$	34.655	4.723	19.840	15.172	4.494	25.996	36.908	0.509
	39.272	3.530	22.023	15.402	3.186	28.367	43.808	0.522
	40.731	3.212	24.978	14.992	1.584	32.487	48.883	0.598
	44.985	2.417	29.910	17.730	0.025	48.086	64.086	0.665
	55.047	1.144	40.190	17.480	4.828×10^{-5}	61.669	81.814	0.730
$[Bu_3NC_4]Br$	34.048	5.022	14.974	14.941	5.778	19.723	31.864	0.325
	43.734	2.778	20.034	14.923	2.387	23.9666	46.460	0.427
	49.522	1.880	25.312	14.941	0.878	27.679	55.062	0.491
	53.548	1.401	29.790	17.750	0.014	37.811	64.742	0.556
	65.722	0.477	39.900	17.560	3.460×10^{-4}	43.956	78.802	0.607
$[MepyrNC_4]Br$	36.792	6.398	24.961	14.978	8.764	26.711	34.599	0.578
	37.478	6.132	27.014	15.346	2.597	36.845	46.475	0.700
	42.197	4.539	29.944	14.898	2.010	38.513	52.623	0.695
$[C_4mim]Br$	32.578	8.190	25.046	14.998	4.198	33.842	38.255	0.735
	35.532	6.853	27.357	15.010	1.540	40.769	48.018	0.760
	39.931	5.195	29.673	15.407	0.853	44.098	55.140	0.738
	54.397	1.830	38.850	17.410	0.015	60.073	79.685	0.732
$[N_{4444}]Br$	25.441	7.752	14.775	15.032	5.963	21.046	23.582	0.452
	32.537	5.380	19.950	15.022	1.466	29.182	39.140	0.595
	44.451	2.873	25.058	15.180	1.134	30.363	51.303	0.552

Table S9. Performance of ABS composed of IL + K_2HPO_4/KH_2PO_4 pH 7 + H_2O for the purification of anti-IL-8 mAbs directly from CHO cell culture supernatant given by the recovery yield ($\%Yield_{IgG}$) and purification factor ($\%Purity_{IgG}$).

25 wt% IL + 15 wt% K_2HPO_4/KH_2PO_4 pH 7 + 22.5 wt% H_2O + 37.5 wt% CHO cell culture supernatant		
IL	$\%Yield_{IgG}$ ± σ	PF ± σ
[Et_3NC_4]Br	92.3 ± 0.6	1.0 ± 0.0
[Pr_3NC_4]Br	97.3 ± 1.9	1.3 ± 0.1
[Bu_3NC_4]Br	100.0 ± 8.0	1.5 ± 0.1
[MepyrNC$_4$]Br	97.0 ± 6.5	1.1 ± 0.1
[C_4mim]Br	73.1 ± 3.4	1.2 ± 0.1
[N_{4444}]Br	73.1 ± 4.4	1.0 ± 0.2
30 wt% IL + 15 wt% K_2HPO_4/KH_2PO_4 pH 7 + 17.5 wt% H_2O + 37.5 wt% CHO cell culture supernatant		
IL	$\%Yield_{IgG}$ ± σ	PF ± σ
[Pr_3NC_4]Br	19.8 ± 1.9	1.0 ± 0.2
[Bu_3NC_4]Br	100.0 ± 3.4	1.6 ± 0.3
[MepyrNC$_4$]Br	61.3 ± 6.1	0.8 ± 0.1
[C_4mim]Br	100.0 ± 6.8	1.3 ± 0.1
40 wt% IL + 15 wt% K_2HPO_4/KH_2PO_4 pH 7 + 7.5 wt% H_2O + 37.5 wt% CHO cell culture supernatant		
IL	$\%Yield_{IgG}$ ± σ	PF ± σ
[Pr_3NC_4]Br	49.8 ± 1.1	0.8 ± 0.0
[Bu_3NC_4]Br	60.4 ± 5.2	0.8 ± 0.1
[C_4mim]Br	100.0 ± 8.1	1.3 ± 0.2

Table S10. Water content (in weigth fraction percentade; determined by the TL data) in the top (IL-rich) phase of the systems evaluated for the extraction and purification of anti-IL-8 mAbs directly from CHO cell culture supernatant using ABS composed of 25, 30 and 40 wt% IL + 15 wt% K_2HPO_4/KH_2PO_4 pH 7 + H_2O.

IL	25 wt% IL	30 wt% IL	40 wt% IL
$[Et_3NC_4]Br$	57.550	-	-
$[Pr_3NC_4]Br$	56.057	52.598	43.810
$[Bu_3NC_4]Br$	48.598	45.050	33.801
$[MepyrNC_4]Br$	56.810	53.260	-
$[C_4mim]Br$	59.232	54.870	43.773
$[N_{4444}]Br$	52.676	-	-

Table S11. Performance parameters of the mAbs purification two-step process based on: (a) an ABS step using a system composed of 40 wt% $[Bu_3NC_4]Br$ + 15 wt% K_2HPO_4/KH_2PO_4 pH 7 + 7.5 H_2O + 37.5 wt% CHO cell culture supernatant and (b) an ultrafiltration (UF) step. Parameters evaluated: mAbs concentration ([IgG]), recovery yield ($\%Yield_{IgG}$), purity level ($\%Purity_{IgG}$), purification factor (PF), and activity of mAbs ($\%Activity_{anti\text{-}IL\text{-}8}$) (whenever applicable). Overall processes refers to the final product obtained by the two-step platforms based on the following unit operations: (i) TPP (TOP phase) + UF (retentate); (ii) TPP (TOP phase) + UF (filtrate); (iii) TPP (Precipitate) + UF (retentate); (iv) TPP (Precipitate) + UF (filtrate).

		[IgG] ± σ ($mg \cdot L^{-1}$)	$\%Yield_{IgG}$ ± σ	$\%Purity_{IgG}$ ± σ	PF ± σ	$\%Activity_{anti\text{-}IL\text{-}8}$
Supernatant		99.4	-	22.7	-	90.6 ± 1.3
ABS	**TOP phase**	42.4 ± 5.0	60.4 ± 5.2	17.4 ± 2.0	0.8 ± 0.1	90.5 ± 2.0
UF	**Retentate**	19.7 ± 3.4	47.3 ± 13.5	32.8 ± 1.5	1.4 ± 0.1	66.4 ± 2.4
	Filtrate	23.4 ± 5.7	45.1 ± 16.1	29.8 ± 5.7	1.3 ± 0.1	
ABS	**Precipitate**	46.4 ± 3.3	41.0 ± 2.6	60.9 ± 2.0	2.7 ± 0.1	74.7 ± 3.2
UF	**Retentate**	24.5 ± 2.7	52.7 ± 2.2	63.5 ± 1.8	2.8 ± 0.1	52.7 ± 3.7
	Filtrate	8.3 ± 1.3	18.1 ± 4.0	67.2 ± 1.8	3.0 ± 0.1	
Overall process (i)		19.7 ± 3.4	28.6 ± 18.7	32.8 ± 1.5	1.4 ± 0.1	-
Overall process (ii)		23.4 ± 5.7	27.2 ± 21.3	29.8 ± 5.7	1.3 ± 0.1	-
Overall process (iii)		24.5 ± 2.7	21.6 ± 4.8	63.5 ± 1.8	2.8 ± 0.1	-
Overall process (iv)		8.3 ± 1.3	7.4 ± 6.6	67.2 ± 1.8	3.0 ± 0.1	-

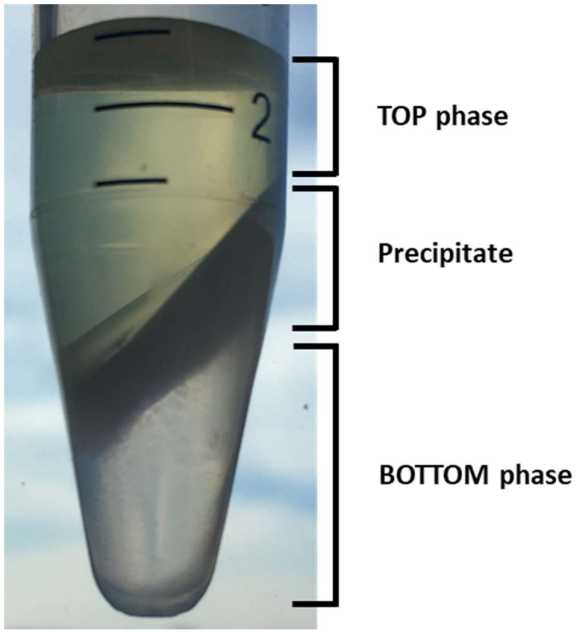

Figure S1. Macroscopic aspect of the ABS composed of 40 wt% $[Bu_3NC_4]Br$ + 15 wt% KH_2PO_4/K_2HPO_4 pH 7 + 22.5 wt% H_2O + 37.5 wt% CHO cell culture supernatant, where the top phase corresponds to the IL-rich phase and the bottom phase to the salt-rich phase. The precipitate rich in proteins is at the interface.

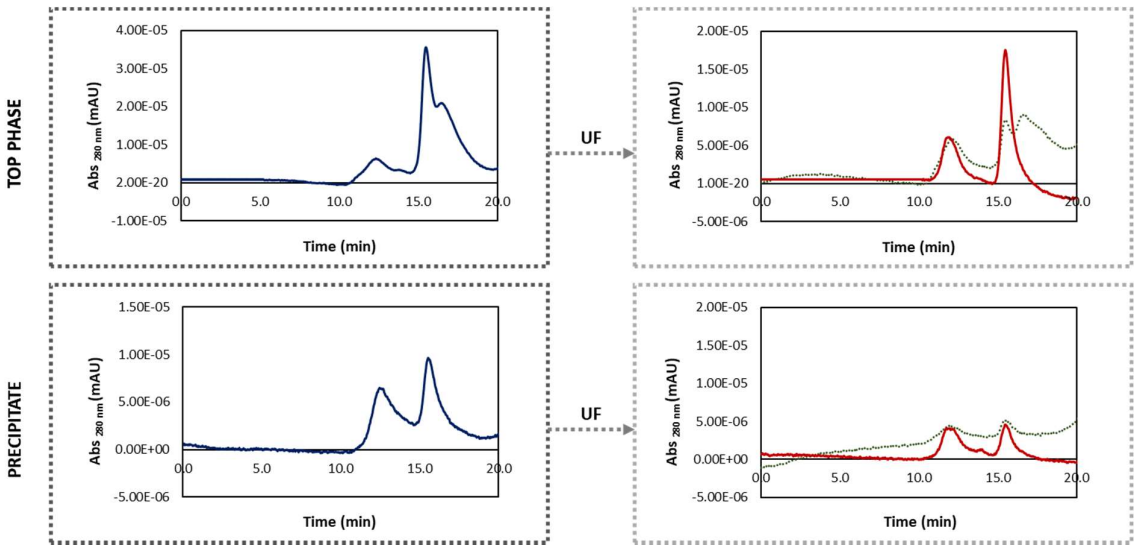

Figure S2. SE-HPLC chromatographic profiles of mAbs purification in the top phase/precipitate of ABS composed of 40 wt% $[Bu_3NC_4]Br$ + 15 wt% KH_2PO_4/K_2HPO_4 pH 7 + 7.5 wt% H_2O + 37.5 wt% CHO cell culture supernatant (–), and after an ultrafiltration step of both samples: retentate (–) and filtrate (··).

Table S12. Performance parameters of the mAbs purification two-step process after the IL recycling. Recovery yields ($\%Yield_{IgG}$) and purity levels ($\%Purity_{IgG}$) determined in the three cycles, for the top phase and precipitate in the TPP and UF steps.

			$\%Yield_{IgG} \pm \sigma$	$\%Purity_{IgG} \pm \sigma$
1st cycle	**TOP phase**	**TPP**	60.4 ± 5.2	17.4 ± 2.0
		UF	47.3 ± 13.5	32.8 ± 1.5
	Precipitate	**TPP**	41.0 ± 2.6	60.9 ± 2.0
		UF	52.7 ± 2.2	63.5 ± 1.8
2nd cycle	**TOP phase**	**TPP**	73.4 ± 15.8	17.0 ± 0.4
		UF	47.1 ± 4.6	34.2 ± 5.7
	Precipitate	**TPP**	41.1 ± 18.8	64.9 ± 8.6
		UF	62.4 ± 2.5	59.8 ± 6.7
3rd cycle	**TOP phase**	**TPP**	58.3 ± 5.3	17.9 ± 3.2
		UF	46.4 ± 5.3	34.7 ± 4.2
	Precipitate	**TPP**	40.7 ± 7.6	60.2 ± 7.8
		UF	55.9 ± 2.7	64.6 ± 4.3

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