Supporting Information

Evaluating self-buffering ionic liquids for biotechnological applications

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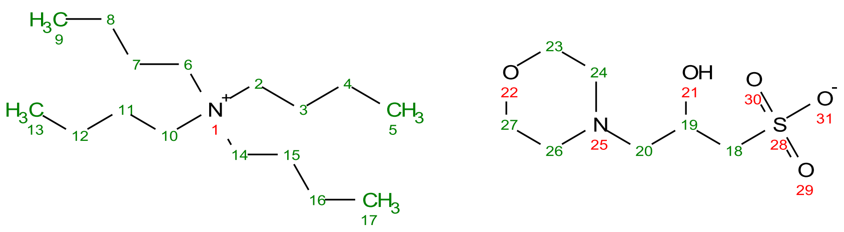
Section S1. Experimental methods

Synthesis of GB-ILs

Hydroxide solution of tetrabutylammonium, tetrabutylphosphonium and cholinium, respectively, were added slowly to an aqueous solution slightly in excess of GBs equimolar. After gentle stirring for 12 hours at room temperature, the water content in the reaction mixture was evaporated under sufficient pressure reduction and heat from evaporator bath at 60 ºC using a rotary evaporator (Heidolph, model Laborota 4003 c/w rotavac vario vacuum pump), until a highly viscous liquid was obtained. The crude product was mixed with equal volume of solvent: a mixture of acetonitrile and methanol in 1:1 volume ratio was used for tetrabutyl-ammonium and -phosphonium-based GB-ILs, whereas pure methanol was used for cholinium-based GB-ILs. Then it was stirred vigorously for 2 hours at room temperature. The mixture was filtered at least twice to remove any free acid content. Afterwards, the filtrate was evaporated for the solvent removal and processed under high vacuum with constant stirring for a minimum period of 48 hours at 60 ºC or at molten state if the product was obtained as a solid salt at room temperature. The water mass fraction content in the synthesized GB-ILs was determined by coulometric Karl Fischer titration (Metrohm, model 831) and was less than 0.1 % by mass in all samples. The chemical structure of GB-ILs was confirmed by ^{1}H and ^{13}C NMR spectroscopy (Bruker, model AMX 300) operating at 300 and 75 MHz, respectively.

Table S1. ^{1}H and ^{13}C NMR analysis of GB-ILs.

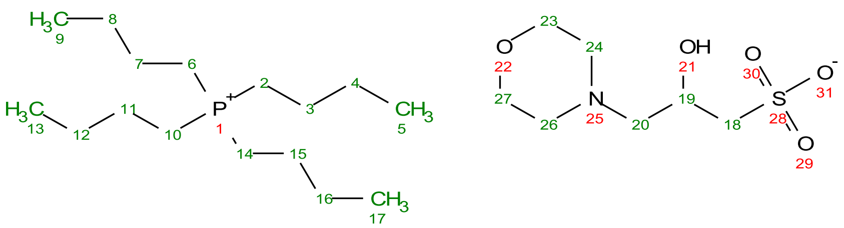
[$N_{4,4,4,4}$][MOPSO]



^{1}H NMR (300 MHz, D_2O) δ 0.93 (t, J = 7.3 Hz, 12H, 5, 9, 13, 17), 1.34 (h, J = 7.3 Hz, 8H, 4, 8, 12, 16), 1.63 (p, J = 7.1 Hz, 8H, 3, 7, 11, 15), 2.51 – 2.70 (m, 6H, 20, 24, 26), 2.93 – 3.08 (m, 2H, 18), 3.18 (t, J = 7.1 Hz, 8H, 2, 6, 10, 14), 3.74 (t, J = 4.8 Hz, 4H, 23, 27), 4.22 – 4.35 (m, 1H, 19).

^{13}C NMR (75 MHz, D_2O) δ 12.89 (5, 9, 13, 17), 19.12 (4, 8, 12, 16), 23.10 (3, 7, 11, 15), 53.00 (24, 26), 55.85 (18), 58.01 (2, 6, 10, 14), 63.14 (20), 64.27 (19), 66.07 (23, 27).

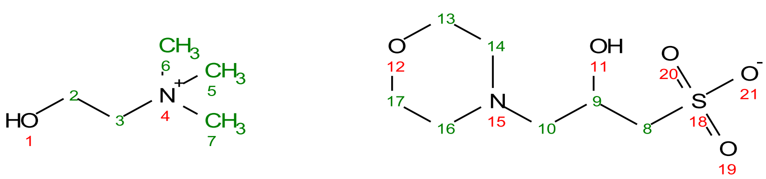
[$P_{4,4,4,4}$][MOPSO]



^{1}H NMR (300 MHz, D_2O) δ 0.92 (t, J = 7.1 Hz, 12H, 5, 9, 13, 17), 1.36 – 1.66 (m, 16H, 3, 4, 7, 8, 11, 12, 15, 16), 1.99 – 2.31 (m, 8H, 2, 6, 10, 14), 2.50 – 2.71 (m, 6H, 20, 24, 26), 2.93 – 3.12 (m, 2H, 18), 3.75 (t, J = 4.8 Hz, 4H, 23, 27), 4.19 – 4.44 (m, 1H, 19).

^{13}C NMR (75 MHz, D_2O) δ 12.65 (5, 9, 13, 17), 17.92 (2, 6, 10, 14), 23.20 (3, 4, 7, 8, 11, 12, 15, 16), 53.03 (24, 26), 55.87 (18), 63.14 (20), 64.30 (19), 66.08 (23, 27).

[Ch][MOPSO]

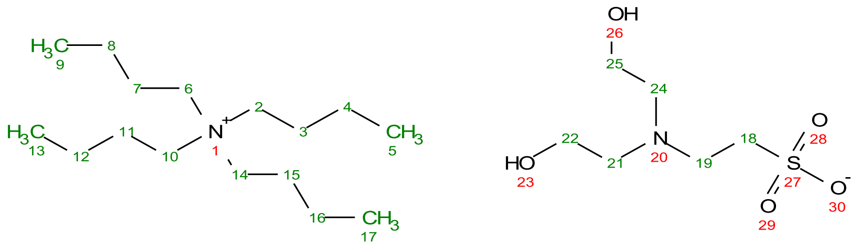


^{1}H NMR (300 MHz, D_2O) δ 2.51 – 2.66 (m, 6H, 10, 14, 16), 2.95 – 3.11 (m, 2H, 8), 3.18 (s, 9H, 5, 6, 7), 3.45 – 3.53 (m, 2H, 3), 3.75 (t, J = 4.8 Hz, 4H, 13, 17), 3.95 – 4.10 (m, 2H, 2), 4.23 – 4.38 (m, 1H, 9).

^{13}C NMR (75 MHz, D_2O) δ 52.98 (5, 6, 7), 53.84 (14, 16), 55.58 (8), 55.82 (2), 63.13 (10), 64.16 (9), 65.99 (13, 17), 67.36 (3).

Table S1. Continued.

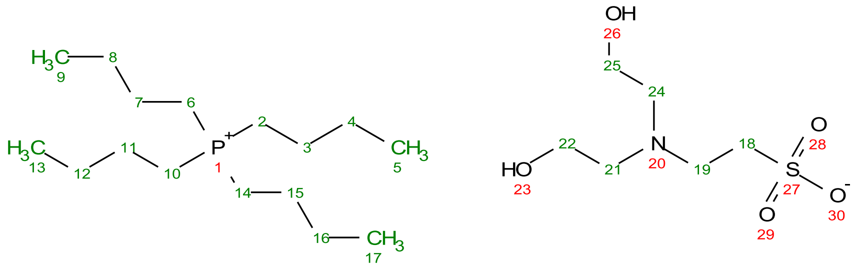
$[N_{4,4,4,4}]$[BES]



^{1}H NMR (300 MHz, D_2O) δ 0.93 (t, *J* = 7.4 Hz, 12H, 5, 9, 13, 17), 1.35 (h, *J* = 7.3 Hz, 8H, 4, 8, 12, 16), 1.61 (p, *J* = 7.2 Hz, 8H, 3, 7, 11, 15), 2.70 (t, *J* = 6.1 Hz, 4H, 21, 24), 2.95 – 3.10 (m, 4H, 18, 19), 3.17 (t, *J* = 7.2 Hz, 8H, 2, 6, 10, 14), 3.66 (t, *J* = 6.1 Hz, 4H, 22, 25).

^{13}C NMR (75 MHz, D_2O) δ 12.95 (5, 9, 13, 17), 19.16 (4, 8, 12, 16), 23.10 (3, 7, 11, 15), 47.01 (18), 48.79 (19), 54.91(21, 24), 58.06 (2, 6, 10, 14), 58.63 (22, 25).

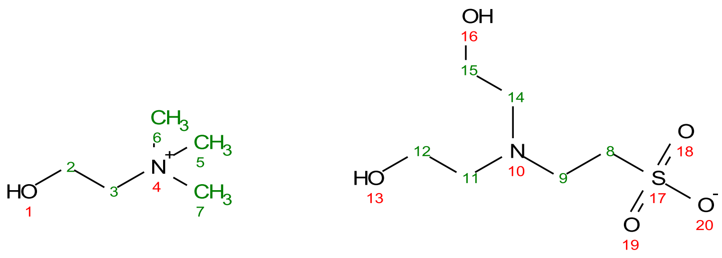
$[P_{4,4,4,4}]$[BES]



^{1}H NMR (300 MHz, D_2O) δ 0.90 (t, *J* = 7.0 Hz, 12H, 5, 9, 13, 17), 1.33 – 1.62 (m, 16H, 3, 4, 7, 8, 11, 12, 15, 16), 2.05 – 2.23 (m, 8H, 2, 6, 10, 14), 2.70 (t, *J* = 6.1 Hz, 4H, 21, 24), 2.93 – 3.13 (m, 4H, 18, 19), 3.66 (t, *J* = 6.1 Hz, 4H, 22, 25).

^{13}C NMR (75 MHz, D_2O) δ 12.56 (5, 9, 13, 17), 17.88 (2, 6, 10, 14), 23.37 (3, 4, 7, 8, 11, 12, 15, 16), 46.88 (18), 48.67 (19), 54.82 (21, 24), 58.57 (22, 25).

[Ch][BES]

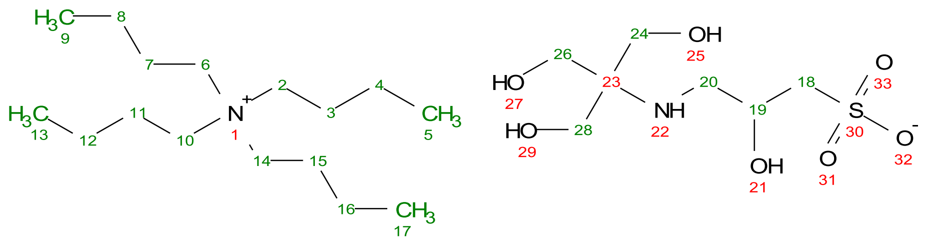


^{1}H NMR (300 MHz, D_2O) δ 2.74 (t, *J* = 6.0 Hz, 4H, 11, 14), 2.97 – 3.12 (m, 4H, 8, 9), 3.17 (s, 9H, 5, 6, 7), 3.39 – 3.56 (m, 2H, 3), 3.68 (t, *J* = 6.0 Hz, 4H, 12, 15), 3.97 – 4.10 (m, 2H, 2).

^{13}C NMR (75 MHz, D_2O) δ 46.83 (8), 48.74 (9), 53.87 (5, 6, 7), 54.85(11, 14), 55.57 (2), 58.44 (12, 15), 67.39 (3).

Table S1. Continued.

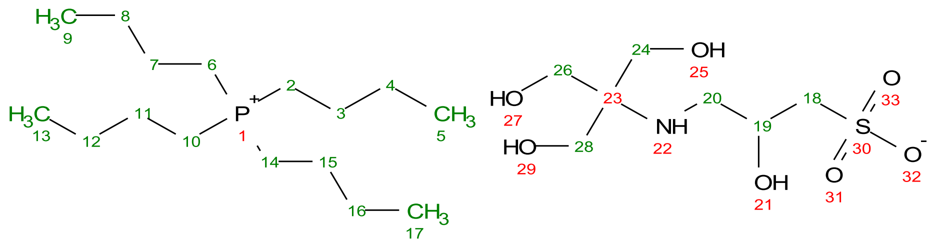
[$N_{4,4,4,4}$][TAPSO]



^{1}H NMR (300 MHz, D_2O) δ 0.92 (t, *J* = 7.3 Hz, 12H, 5, 9, 13, 17), 1.33 (h, *J* = 7.4 Hz, 8H, 4, 8, 12, 16), 1.62 (p, *J* = 7.5 Hz, 8H, 3, 7, 11, 15), 2.68 (dd, *J* = 12.1, 8.7 Hz, 1H, 20'), 2.83 (dd, *J* = 12.1, 3.5 Hz, 1H, 20''), 3.05 (dd, *J* = 5.9, 2.7 Hz, 2H, 18), 3.17 (t, *J* = 7.5 Hz, 8H, 2, 6, 10, 14), 3.54 (d, *J* = 1.8 Hz, 6H, 24, 26, 28), 4.11 (dddd, *J* = 8.7, 6.6, 5.3, 3.5 Hz, 1H, 19).

^{13}C NMR (75 MHz, D_2O) δ 12.79 (5, 9, 13, 17), 19.09 (4, 8, 12, 16), 23.07 (3, 7, 11, 15), 45.81 (20), 55.44 (18), 58.03 (2, 6, 10, 14), 59.71 (24, 26, 28), 60.44 (23), 67.18 (19).

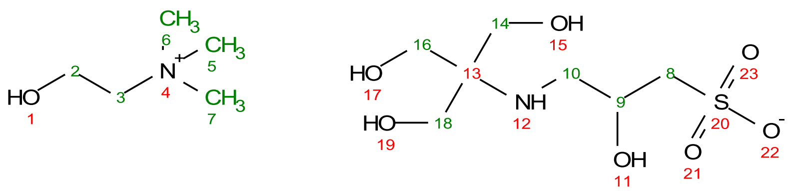
[$P_{4,4,4,4}$][TAPSO]



^{1}H NMR (300 MHz, D_2O) δ 0.89 (t, *J* = 7.1 Hz, 12H, 5, 9, 13, 17), 1.34 – 1.61 (m, 16H, 3, 4, 7, 8, 11, 12, 15, 16), 2.05 – 2.23 (m, 8H, 2, 6, 10, 14), 2.67 (dd, *J* = 12.1, 8.7 Hz, 1H, 20'), 2.83 (dd, *J* = 12.0, 3.5 Hz, 1H, 20''), 3.05 (dd, *J* = 5.8, 2.7 Hz, 2H, 18), 3.54 (d, *J* = 1.8 Hz, 6H, 24, 26, 28), 4.11 (dddd, *J* = 8.6, 6.5, 5.2, 3.5 Hz, 1H, 19).

^{13}C NMR (75 MHz, D_2O) δ 12.50 (5, 9, 13, 17), 17.89 (2, 6, 10, 14), 23.35 (3, 4, 7, 8, 11, 12, 15, 16), 45.82 (20), 55.44 (18), 59.68 (24, 26, 28), 60.47 (23), 67.20 (19).

[Ch][TAPSO]

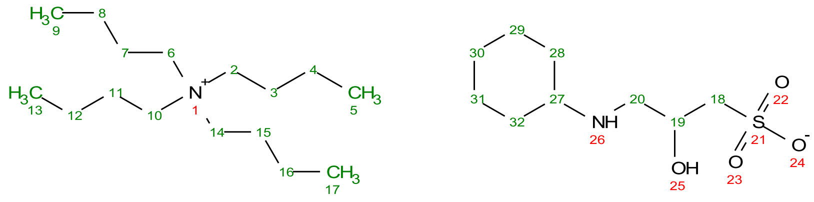


^{1}H NMR (300 MHz, D_2O) δ 2.74 (dd, *J* = 12.1, 8.8 Hz, 1H, 10'), 2.90 (dd, *J* = 12.2, 3.5 Hz, 1H, 10''), 3.08 (dd, *J* = 5.9, 2.6 Hz, 2H, 8), 3.18 (s, 9H, 5, 6, 7), 3.49 (m, 2H, 3), 3.57 (d, *J* = 1.7 Hz, 6H, 14, 16, 18), 3.95 – 4.09 (m, 2H, 2), 4.14 (dddd, *J* = 8.7, 6.6, 5.2, 3.4 Hz, 1H, 9).

^{13}C NMR (75 MHz, D_2O) δ 45.89 (10), 53.83 (5, 6, 7), 55.43 (8), 55.58 (2), 60.17 (14, 16, 18), 60.42 (13), 67.31 (9), 67.39 (3).

Table S1. Continued.

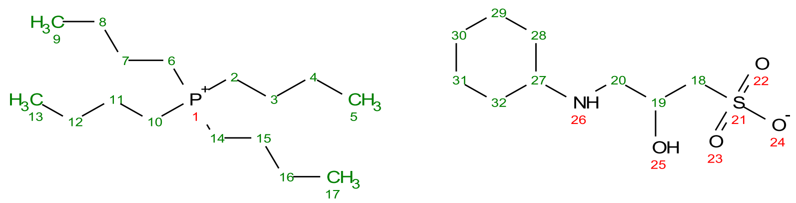
[$N_{4,4,4,4}$][CAPSO]



^{1}H NMR (300 MHz, D_2O) δ 0.93 (t, *J* = 7.3 Hz, 12H, 5, 9, 13, 17), 0.97 – 1.28 (m, 6H, 29, 30, 31), 1.35 (h, *J* = 7.3 Hz, 8H, 4, 8, 12, 16), 1.61 (p, *J* = 7.9 Hz, 8H, 3, 7, 11, 15), 1.69 – 1.92 (m, 4H, 28, 32), 2.39 – 2.52 (m, 1H, 27), 2.61 (dd, *J* = 12.6, 8.7 Hz, 1H, 20), 2.84 (dd, *J* = 12.6, 3.6 Hz, 1H, 20), 2.96 – 3.05 (m, 2H, 18), 3.17 (t, *J* = 7.9 Hz, 8H, 2, 6, 10, 14), 4.08 – 4.22 (m, 1H, 19).

^{13}C NMR (75 MHz, D_2O) δ 12.91 (5, 9, 13, 17), 19.16 (4, 8, 12, 16), 23.11(3, 7, 11, 15), 24.63 (29, 31), 25.65 (30), 32.14 (28, 32), 50.49 (20), 55.70 (18), 55.81 (27), 58.09 (2, 6, 10, 14), 66.34 (19).

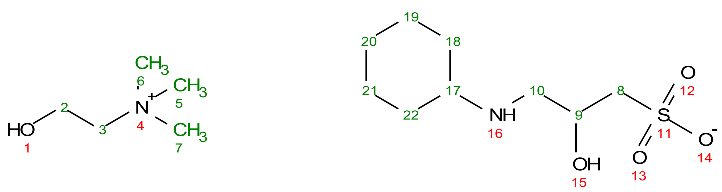
[$P_{4,4,4,4}$][CAPSO]



^{1}H NMR (300 MHz, D_2O) δ 0.90 (t, *J* = 7.1 Hz, 12H, 5, 9, 13, 17), 0.96 – 1.34 (m, 6H, 29, 30, 31), 1.36 – 1.60 (m, 16H, 3, 4, 7, 8, 11, 12, 15, 16), 1.65 – 1.90 (m, 4H, 28, 32), 2.03 – 2.23 (m, 8H, 2, 6, 10, 14), 2.39 – 2.55 (m, 1H, 27), 2.62 (dd, *J* = 12.7, 8.8 Hz, 1H, 20), 2.84 (dd, *J* = 12.7, 3.6 Hz, 1H, 20), 2.97 – 3.08 (m, 2H, 18), 4.08 – 4.23 (m, 1H, 19).

^{13}C NMR (75 MHz, D_2O) δ 12.57 (5, 9, 13, 17), 17.59 (2, 6, 10, 14), 23.90 (3, 4, 7, 8, 11, 12, 15, 16), 26.09 (29, 30, 31), 31.93 (28, 32), 50.38 (20), 55.63 (18), 55.79 (27), 66.25 (19).

[Ch][CAPSO]



^{1}H NMR (300 MHz, D_2O) δ 0.93 – 1.31 (m, 5H, 19, 20, 21'), 1.54 – 1.64 (m, 1H, 21''), 1.66 – 1.93 (m, 4H, 18, 22), 2.42 – 2.54 (m, 1H, 17), 2.64 (dd, *J* = 12.7, 8.8 Hz, 1H, 10), 2.85 (dd, *J* = 12.7, 3.6 Hz, 1H, 10), 2.98 – 3.06 (m, 2H, 8), 3.17 (s, 9H, 5, 6, 7), 3.44 – 3.54 (m, 2H, 3), 3.97 – 4.09 (m, 2H, 2), 4.12 – 4.22 (m, 1H, 9).

^{13}C NMR (75 MHz, D_2O) δ 24.51 (19, 21), 25.52 (20), 31.71 (18, 22), 50.32 (10), 53.85 (5, 6, 7), 55.55 (8), 55.58 (17), 55.80 (2), 66.19 (9), 67.39 (3).

Table S2. Buffering properties of GBs and GB-ILs (0.05 M) at (25 and 37) ºC.

GBs/ GB-ILs	25 ºC			37 ºC		
	mid-point pH	buffer region	Buffer capacity, β*	mid-point pH	buffer region	Buffer capacity, β*
MOPSO	6.9	5.9-7.9	0.0243	6.7	5.7-7.6	0.0242
[$N_{4,4,4,4}$][MOPSO]	6.9	5.9-7.9	0.0242	6.7	5.7-7.6	0.0235
[$P_{4,4,4,4}$][MOPSO]	6.9	5.9-7.9	0.0239	6.7	5.7-7.6	0.0235
[Ch][MOPSO]	6.9	5.9-7.8	0.0243	6.7	5.7-7.6	0.0243
BES	7.1	6.1-8.2	0.0238	7.0	5.8-7.8	0.0238
[$N_{4,4,4,4}$][BES]	7.1	6.2-8.2	0.0234	6.9	5.8-7.8	0.0241
[$P_{4,4,4,4}$][BES]	7.1	6.2-8.2	0.0244	6.9	5.8-7.8	0.0242
[Ch][BES]	7.1	6.1-8.2	0.0244	6.9	5.8-7.8	0.0251
TAPSO	7.7	6.6-8.7	0.0235	7.4	6.3-8.4	0.0236
[$N_{4,4,4,4}$][TAPSO]	7.6	6.5-8.7	0.0234	7.3	6.2-8.3	0.0238
[$P_{4,4,4,4}$][TAPSO]	7.6	6.5-8.7	0.0227	7.3	6.2-8.3	0.0239
[Ch][TAPSO]	7.7	6.7-8.7	0.0238	7.3	6.3-8.3	0.0234
CAPSO	9.8	8.8-11.4	0.0243	9.5	8.7-10.3	0.0251
[$N_{4,4,4,4}$][CAPSO]	9.8	8.9-11.3	0.0234	9.4	8.5-10.2	0.0248
[$P_{4,4,4,4}$][CAPSO]	9.8	8.7-11.5	0.0241	9.4	8.5-10.2	0.0241
[Ch][CAPSO]	9.9	8.8-11.4	0.0234	9.4	8.5-10.2	0.0236

*Buffer capacity, β, is expressed as the relationship between the increment of strong base or acid added and the final pH increment, as described in the below eq.:

$$\beta = \frac{\mathrm{d}C_\mathrm{b}}{\mathrm{d}\,(pH)} = -\frac{\mathrm{d}C_\mathrm{a}}{\mathrm{d}\,(pH)}$$

where C_b and C_a, are the number of moles *per* liter of monoprotic strong base or acid added.

Table S3. EC_{50} values ($mg \cdot L^{-1}$) estimated following 5, 15, 30 min of exposure of the luminescent marine bacteria *Vibrio fischeri* to GB-ILs, with the respective 95% confidence lower and upper limits.

GBs/GB-ILs	EC_{50} ($mg \cdot L^{-1}$) 5 min (lower limit; upper limit)	EC_{50} ($mg \cdot L^{-1}$) 15 min (lower limit; upper limit)	EC_{50} ($mg \cdot L^{-1}$) 30 min (lower limit; upper limit)
MOPSO	1096.73 (497.99; 1695.46)	1135.61 (431.30; 1789.91)	1245.47 (597.79; 1893.32)
[$N_{4,4,4,4}$][MOPSO]	723.97 (574.39; 873.56)	362.84 (280.11; 445.58)	244.80 (198.99; 290.62)
[$P_{4,4,4,4}$][MOPSO]	275.08 (232.58; 317.59)	198.46 (185.93; 210.99)	160.90 (152.98; 168.82)
[Ch][MOPSO]	6365.31 (5658.05; 7072.56)	5649.66 (5205.97; 6093.35)	6132.40 (5155.83; 7108.98)
BES	1621.23 (1035.34; 2207.13)	1721.78 (1062.27; 2381.29)	2225.00 (1640.73; 2809.27)
[$N_{4,4,4,4}$][BES]	631.12 (489.84; 772.39)	344.06 (316.28; 371.84)	244.42 (241.68; 247.17)
[$P_{4,4,4,4}$][BES]	289.97 (265.84; 314.09)	166.63 (144.62; 188.63)	126.96 (117.47; 136.45)
[Ch][BES]	9666.90 (8926.68; 10407.13)	7267.36 (5290.71; 9244.00)	7327.89 (4941.16; 9714.62)
TAPSO	836.92 (670.89; 1002.94)	717.91 (534.50; 901.31)	965.42 (675.02; 1255.83)
[$N_{4,4,4,4}$][TAPSO]	492.89 (391.95; 593.83)	249.94 (213.28; 286.60)	175.26 (165.27; 185.26)
[$P_{4,4,4,4}$][TAPSO]	256.24 (232.69; 279.79)	157.77 (145.86; 170.06)	115.58 (101.41; 129.74)
[Ch][TAPSO]	4344.42 (2691.21; 5997.63)	2594.12 (1746.96; 3441.28)	3439.25 (2385.24; 4493.26)
CAPSO	1522.27 (1002.01; 2042.52)	1792.08 (1128.08; 2456.09)	3068.88 (494.40; 4643.66)
[$N_{4,4,4,4}$][CAPSO]	133.22 (125.06; 141.37)	112.89 (98.18; 127.60)	104.66 (102.16; 107.16)
[$P_{4,4,4,4}$][CAPSO]	176.46 (161.56; 191.35)	140.08 (122.68; 157.49)	113.08 (103.02; 123.14)
[Ch][CAPSO]	20426.35 (19799.70; 21053.00)	18980.45 (17899.99; 20060.91)	19504.46 (17453.97; 21554.94)

Table S4. EC_{50} values (mmol·L^{-1}) estimated following 5, 15, 30 min of exposure of the luminescent marine bacteria *Vibrio fischeri* to GB-ILs, with the respective 95% confidence lower and upper limits.

GBs/GB-ILs	EC_{50} (mmol·L^{-1}) 5 min (lower limit; upper limit)	EC_{50} (mmol·L^{-1}) 15 min (lower limit; upper limit)	EC_{50} (mmol·L^{-1}) 30 min (lower limit; upper limit)
MOPSO	4.87 (2.21; 7.53)	5.04 (1.91; 7.95)	5.53 (2.65; 8.41)
[$N_{4,4,4,4}$][MOPSO]	1.55 (1.23; 1.87)	0.78 (0.60; 0.95)	0.52 (0.43; 0.62)
[$P_{4,4,4,4}$][MOPSO]	0.57 (0.48; 0.66)	0.41 (0.38; 0.44)	0.33 (0.32; 0.35)
[Ch][MOPSO]	19.38 (17.23; 21.53)	17.20 (15.85; 18.55)	18.67 (15.70; 21.65)
BES	7.60 (4.86; 10.35)	8.07 (4.98; 11.17)	10.43 (7.69; 13.17)
[$N_{4,4,4,4}$][BES]	1.39 (1.08; 1.70)	0.76 (0.70; 0.82)	0.54 (0.53; 0.54)
[$P_{4,4,4,4}$][BES]	0.61 (0.56; 0.67)	0.35 (0.31; 0.40)	0.27 (0.25; 0.29)
[Ch][BES]	30.55 (28.21; 32.89)	22.97 (16.72; 29.21)	23.16 (15.62; 30.70)
TAPSO	3.23 (2.59; 3.87)	2.77 (2.06; 3.48)	3.72 (2.60; 4.84)
[$N_{4,4,4,4}$][TAPSO]	0.98 (0.78; 1.19)	0.50 (0.43; 0.57)	0.35 (0.33; 0.37)
[$P_{4,4,4,4}$][TAPSO]	0.49 (0.45; 0.54)	0.30 (0.28; 0.33)	0.22 (0.20; 0.25)
[Ch][TAPSO]	11.99 (7.43; 16.55)	7.16 (4.82; 9.49)	9.49 (6.58; 12.40)
CAPSO	6.41 (4.22; 8.61)	7.55 (4.75; 10.35)	12.93 (2.08; 19.57)
[$N_{4,4,4,4}$][CAPSO]	0.28 (0.26; 0.30)	0.24 (0.21; 0.27)	0.22 (0.21; 0.22)
[$P_{4,4,4,4}$][CAPSO]	0.36 (0.33; 0.39)	0.28 (0.25; 0.32)	0.23 (0.21; 0.25)
[Ch][CAPSO]	59.99 (58.15; 61.83)	55.75 (52.57; 58.92)	57.28 (51.26; 63.31)

Table S5. Experimental weight fraction data for the system composed of GB-ILs (1) + $C_6H_5K_3O_7$ (2) +H_2O at (25 ± 1) °C.

$[N_{4,4,4,4}]$[MOPSO]				$[P_{4,4,4,4}]$[MOPSO]				$[N_{4,4,4,4}]$[BES]	
100 w1	100 w2	100 w1	100 w2	100 w1	100 w2	100 w1	100 w2	100 w1	100 w2
67.49	1.47	8.93	29.81	69.15	0.93	14.88	22.17	83.92	0.53
63.15	2.38	7.77	31.00	67.30	1.32	14.61	22.31	77.95	1.13
59.67	2.87	6.18	32.82	65.01	1.78	14.22	22.58	66.55	1.74
56.75	3.32	4.86	34.56	61.08	2.34	13.94	22.78	63.72	2.19
55.28	3.67	3.58	36.61	58.78	2.62	13.63	23.00	61.40	2.73
53.61	4.19	2.37	39.10	56.83	3.14	13.39	23.14	59.72	3.14
52.16	4.59			55.05	3.55	12.87	23.51	58.26	3.59
50.91	4.96			53.68	3.99	12.67	23.63	56.18	3.98
49.45	5.49			51.95	4.38	12.53	23.73	55.07	4.39
47.86	6.09			50.45	4.74	12.31	23.86	53.06	5.12
46.16	6.71			49.17	5.18	12.00	24.11	51.53	5.49
45.00	7.22			48.04	5.68	11.78	24.26	50.41	5.80
44.14	7.53			46.03	6.35	11.60	24.37	49.50	6.13
41.97	8.47			44.38	7.04	11.39	24.53	48.02	6.78
40.48	9.15			42.94	7.62	11.14	24.75	47.07	7.18
38.58	10.07			42.02	7.90	10.85	25.01	45.61	7.84
36.60	11.17			40.74	8.42	10.61	25.21	44.78	8.14
34.41	12.37			39.73	8.89	10.45	25.33	43.37	8.74
32.46	13.44			38.54	9.46	10.28	25.41	42.25	9.22
30.39	14.66			37.50	9.93	10.05	25.61	41.01	9.82
29.64	15.09			36.62	10.31	9.83	25.81	39.96	10.33
28.67	15.66			35.40	10.86	9.50	26.14	38.61	11.05
27.99	16.04			34.43	11.27	9.17	26.29	37.49	11.64
26.64	16.89			33.49	11.69	9.00	26.43	36.33	12.24
25.97	17.33			32.28	12.33	8.84	26.57	34.62	13.25
25.29	17.72			31.10	12.95	8.60	26.81	33.18	14.06
24.98	17.90			30.02	13.52	8.43	26.86	31.82	14.86
24.61	18.12			29.16	13.96	8.01	27.26	30.16	15.88
23.94	18.58			27.96	14.61	7.79	27.48	28.87	16.67
23.61	18.74			27.10	15.05	7.59	27.70	26.94	17.93
22.56	19.43			26.35	15.47	7.32	28.01	25.44	18.90
21.76	19.95			25.31	16.03	6.95	28.39	23.82	20.00
21.46	20.15			24.57	16.45	6.67	28.65	22.40	20.95
20.30	20.75			24.07	16.70	6.49	28.85	21.23	21.73
19.66	21.22			23.87	16.76	6.26	29.10	19.83	22.71
19.17	21.58			23.50	16.96	5.92	29.51	18.42	23.71
18.22	22.24			22.86	17.35	4.92	30.60	17.03	24.73
17.70	22.62			22.32	17.63	3.55	32.32	15.31	26.03
17.05	23.10			21.61	18.07	2.54	34.01	13.52	28.10
16.77	23.31			20.98	18.38	1.96	35.31	10.51	31.01
16.50	23.53			20.35	18.75	1.09	37.90	8.34	33.41
15.63	24.16			19.88	19.00			6.38	35.91
15.13	24.53			19.35	19.34				
14.80	24.79			18.96	19.54				
14.34	25.12			18.51	19.79				
14.02	25.36			17.90	20.18				
13.66	25.67			17.67	20.24				
13.20	26.01			17.07	20.61				
12.47	26.61			16.60	20.94				
11.92	27.07			15.88	21.56				
10.91	27.97			15.54	21.77				
10.04	28.74			15.42	21.80				

Table S5. Continued.

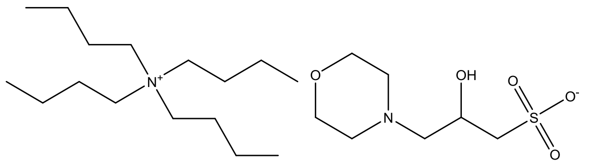
$[P_{4,4,4,4}][BES]$		$[N_{4,4,4,4}][TAPSO]$		$[P_{4,4,4,4}][TAPSO]$		$[N_{4,4,4,4}][CAPSO]$		$[P_{4,4,4,4}][CAPSO]$	
100 w1	100 w2	100 w1	100 w2	100 w1	100 w2	100 w1	100 w2	100 w1	100 w2
69.92	0.58	60.22	5.13	61.39	4.26	66.93	0.94	68.29	0.95
67.38	1.22	57.72	5.94	59.58	4.78	60.86	1.88	59.48	2.45
65.19	1.77	55.18	6.85	57.39	5.36	58.83	2.05	54.90	3.12
63.48	2.30	52.42	7.90	54.52	6.45	55.24	2.71	49.49	4.00
58.68	3.20	49.66	9.00	52.11	7.30	52.53	3.60	46.33	4.31
56.15	3.57	47.11	10.11	50.00	8.07	48.75	4.36	43.64	5.31
54.68	3.99	43.58	11.81	47.55	9.08	46.63	4.70	40.92	5.78
52.96	4.40	40.35	13.49	45.06	10.14	44.83	5.39	38.17	6.14
50.65	5.31	35.98	15.91	42.87	11.11	42.17	5.84	36.61	6.60
49.14	5.68	32.44	18.02	39.91	12.59	40.51	6.28	34.90	7.07
47.12	6.45	29.21	20.43	37.29	13.91	39.03	6.90	32.94	7.36
45.81	6.75	26.00	22.05	35.00	15.14	37.57	7.35	31.68	7.81
43.93	7.53	24.83	22.95	31.57	17.10	35.87	8.11	30.68	8.16
42.11	8.28	23.26	24.00	26.88	19.91	34.56	8.52	29.33	8.70
39.95	9.35	19.98	26.31	19.64	24.58	33.34	8.93	28.38	9.08
38.27	10.10	18.28	27.51	9.90	32.03	32.26	9.27	27.16	9.43
36.53	10.95	15.59	29.59	3.94	39.25	31.71	9.36	25.84	9.65
34.50	12.03	14.52	30.46			30.57	9.62	23.23	10.52
32.74	12.99	11.17	33.77			29.26	10.28	20.48	11.81
31.23	13.80	7.01	38.01			28.36	10.55	17.18	13.02
29.69	14.68					27.18	11.13	14.05	14.11
28.12	15.60					26.41	11.36	9.93	16.12
26.49	16.59					25.39	11.75	7.84	17.23
24.84	17.61					24.72	11.90	4.86	20.77
23.58	18.39					23.81	12.40		
22.27	19.22					23.21	12.54		
21.00	20.04					22.43	13.00		
19.85	20.78					21.99	13.08		
18.76	21.50					21.54	13.19		
17.82	22.10					21.03	13.51		
17.02	22.59					19.80	13.96		
16.03	23.27					18.62	14.40		
15.09	24.12					18.25	14.54		
14.39	24.61					16.95	15.05		
13.56	25.23					14.37	16.12		
12.71	25.88					12.73	16.87		
12.19	26.28					11.27	17.59		
11.31	26.99					9.34	18.66		
10.80	27.31					6.81	19.89		
9.96	28.03								
8.87	29.01								
7.62	30.10								
5.48	32.33								
3.58	35.01								
2.91	36.18								
1.68	39.01								

Table S6. Correlation constants obtained by the regression of the experimental binodal data through the application of eq. 2 and respective standard deviation, σ, and correlation, R^2, for the studied systems.

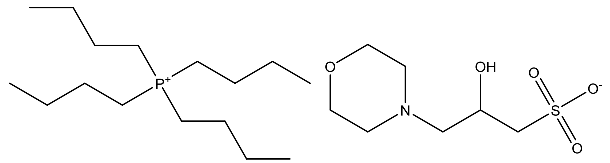
GB-ILs	A ± σ	B ± σ	10^5 (C ± σ)	R^2
[$N_{4,4,4,4}$][MOPSO]	93.3295 ± 0.3509	-0.2671 ± 0.0016	3.3529 ± 0.0393	0.9998
[$P_{4,4,4,4}$][MOPSO]	91.3766 ± 0.2435	-0.2678 ± 0.0012	5.1057 ± 0.0343	0.9998
[$N_{4,4,4,4}$][BES]	100.3352 ± 1.0301	-0.2814 ± 0.0047	2.3098 ± 0.1526	0.9973
[$P_{4,4,4,4}$][BES]	88.9790 ± 0.8679	-0.2484 ± 0.0045	4.0644 ± 0.1393	0.9983
[$N_{4,4,4,4}$][TAPSO]	106.5887 ± 0.6798	-0.2497 ± 0.0022	2.1524 ± 0.0317	0.9999
[$P_{4,4,4,4}$][TAPSO]	104.4748 ± 0.5119	-0.2552 ± 0.0018	2.7769 ± 0.0411	0.9999
[$N_{4,4,4,4}$][CAPSO]	90.6941 ± 0.7220	-0.2989 ± 0.0040	15.3076 ± 0.3686	0.9991
[$P_{4,4,4,4}$][CAPSO]	102.1415 ± 2.8588	-0.3709 ± 0.0144	21.0389 ± 1.9571	0.9942

Table S7. H-bond formation tendency of various anions of GB-ILs.

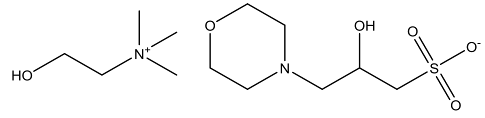
Anions	No. of H-bond acceptor	No. of H-bond donor
$[MOPSO]^-$	6	1
$[BES]^-$	6	2
$[TAPSO]^-$	8	5
$[CAPSO]^-$	5	2



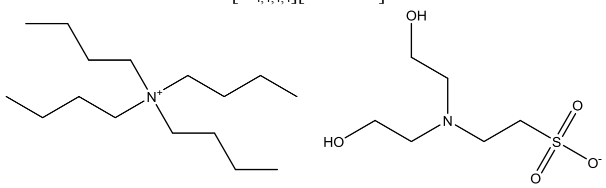
Tetrabutylammonium
2-hydroxy-3-morpholinopropanesulfonate
[$N_{4,4,4,4}$][MOPSO]



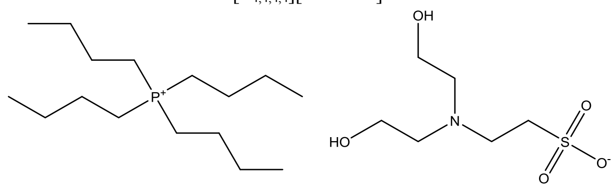
Tetrabutylphosphonium
2-hydroxy-3-morpholinopropanesulfonate
[$P_{4,4,4,4}$][MOPSO]



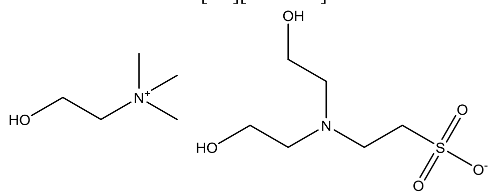
Cholinium
2-hydroxy-3-morpholinopropanesulfonate
[Ch][MOPSO]



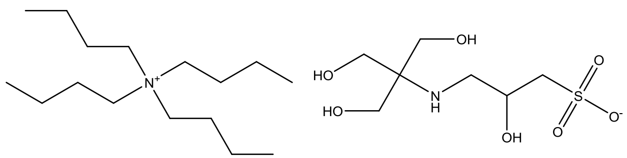
Tetrabutylammonium 2-(bis(2-hydroxyethyl)amino)ethanesulfonate
[$N_{4,4,4,4}$][BES]



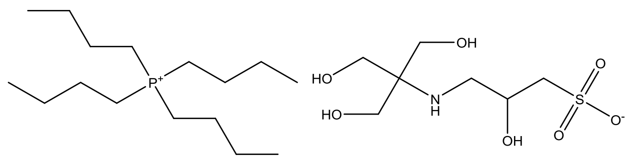
Tetrabutylphosphonium 2-(bis(2-hydroxyethyl)amino)ethanesulfonate
[$P_{4,4,4,4}$][BES]



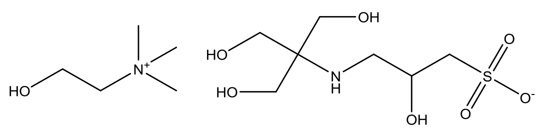
Cholinium 2-(bis(2-hydroxyethyl)amino)ethanesulfonate
[Ch][BES]



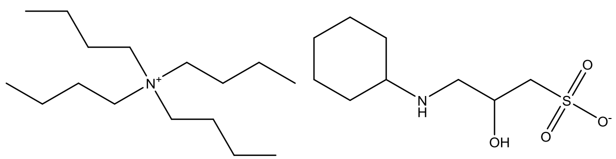
Tetrabutylammonium
N-[Tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonate
[$N_{4,4,4,4}$][TAPSO]



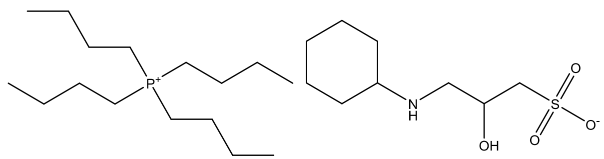
Tetrabutylphosphonium
N-[Tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonate
[$P_{4,4,4,4}$][TAPSO]



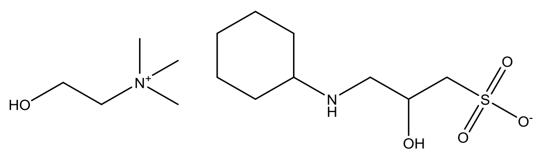
Cholinium N-[Tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonate
[Ch][TAPSO]



Tetrabutylammonium
3-(cyclohexylamino)-2-hydroxypropanesulfonate
[$N_{4,4,4,4}$][CAPSO]

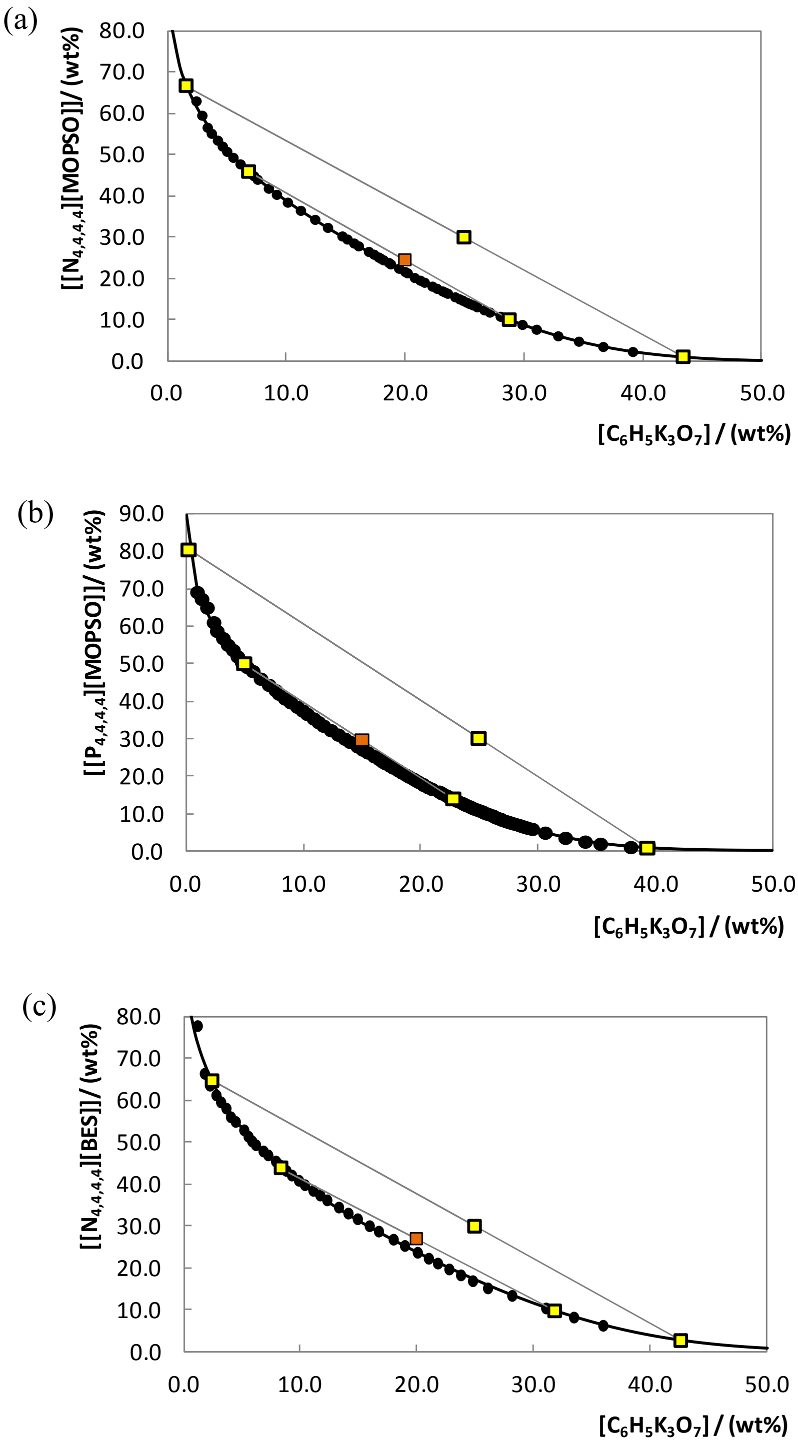


Tetrabutylphosphonium
3-(cyclohexylamino)-2-hydroxypropanesulfonate
[$P_{4,4,4,4}$][CAPSO]



Cholinium
3-(cyclohexylamino)-2-hydroxypropanesulfonate
[Ch][CAPSO]

Figure S1. Chemical structures, full names and acronyms of the synthesized GB-ILs.


(a)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
[[N4,4,4,4][MOPSO]]/ (wt%)
0.0
10.0
20.0
30.0
40.0
50.0
[C6H5K3O7] / (wt%)
(b)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
[[P4,4,4,4][MOPSO]]/ (wt%)
0.0
10.0
20.0
30.0
40.0
50.0
[C6H5K3O7] / (wt%)
(c)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
[[N4,4,4,4][BES]]/ (wt%)
0.0
10.0
20.0
30.0
40.0
50.0
[C6H5K3O7] / (wt%)

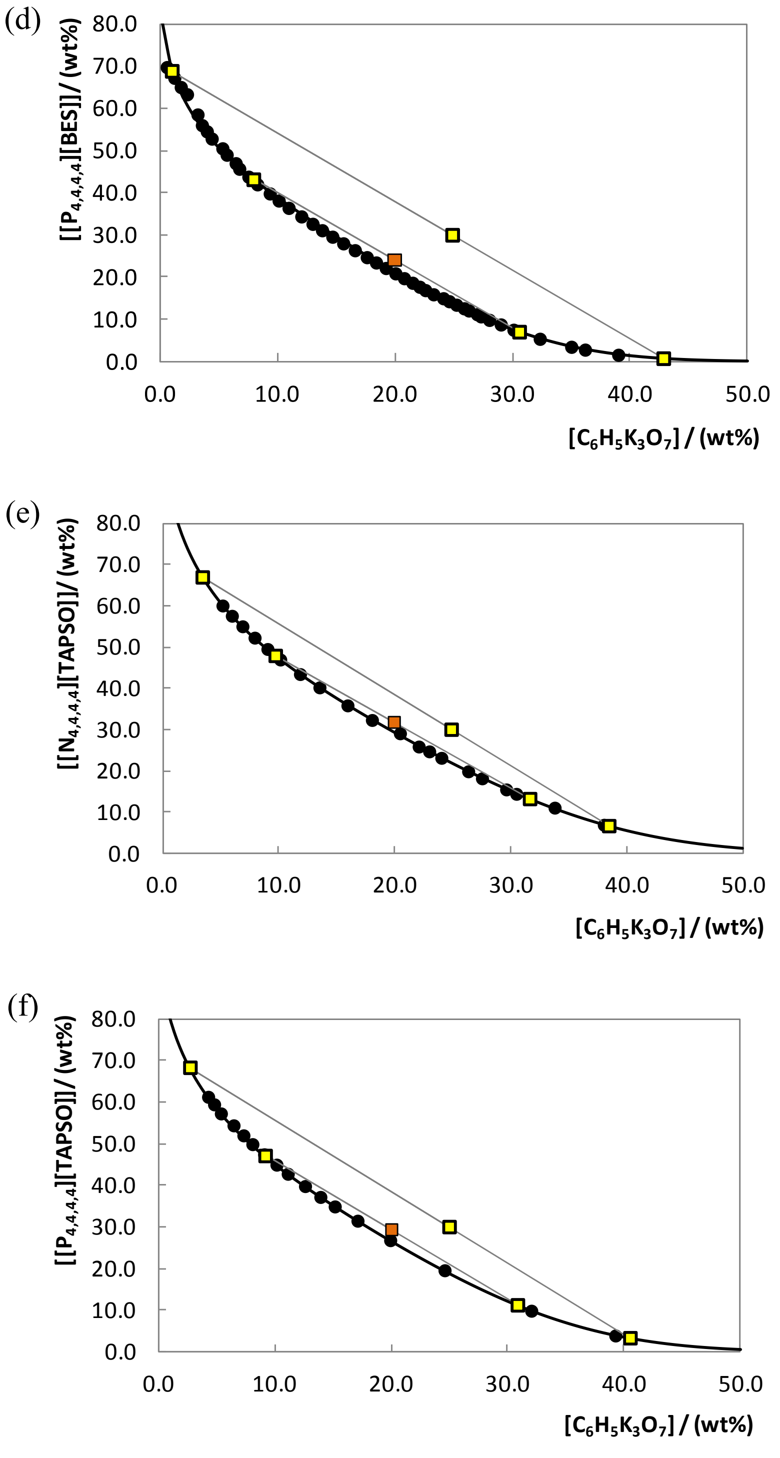


Figure S2. Ternary phase diagrams of systems consisting of (a) $[N_{4,4,4,4}]$[MOPSO], (b) $[P_{4,4,4,4}]$[MOPSO], (c) $[N_{4,4,4,4}]$[BES], (d) $[P_{4,4,4,4}]$[BES], (e) $[N_{4,4,4,4}]$[TAPSO], (f) $[P_{4,4,4,4}]$[TAPSO], + $K_3C_6H_5O_7$ + water at 25 ± 1 (ºC): (●) binodal curve data, (□) TL data, (−) adjusted binodal data through Eq.(2), (■) mixture compositions for lipase partition.

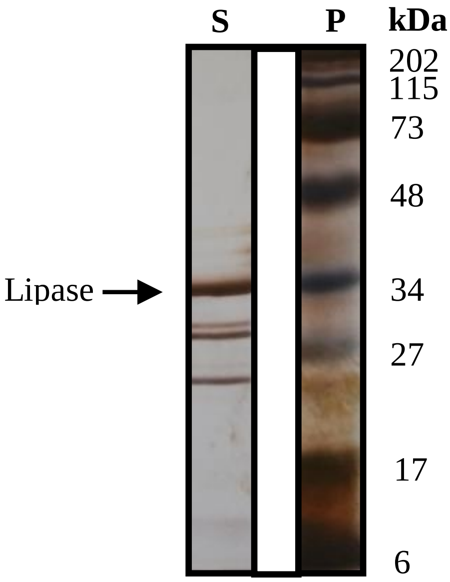


Figure S3. Sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) patterns of commercial lipase from *P. cepacia*: Lane P: molecular mass standard (6-202 kDa), Lane S: commercial lipase from *P. cepacia*; 12% acrylamide gel with silver staining.