SUPPLEMENTARY INFORMATION

Measuring the effect of ionic liquids on laccase activity using a simple, parallel method

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The supplementary information describes the synthesis of the ionic liquids and spectra for the reaction mixtures.

Materials

All thiocyanate ionic liquids, $[C_4mim][N(CN)_2]$, $[C_2mim][C_1(OC_2)_3OSO_3]$,$[C_4mim][C_1(OC_2)_3OSO_3]$, $[C_4eim][TFA]$ and $[C_4eim][TFA]$ were gifts from Merck at the curtsey of Dr. Will Pitner. The phosphonium ILs were a gift from CYTEC. All trialkylamines, dimethyl and diethyl sulphates and alkyl halides were purchased from Aldrich. All $[BF_4]$[1] ILs and $[C_4mim][C_1CO_2]$[2] were synthesised following stadard methdods.

Methods

Preparation of Ionic Liquids

The following salts were known and they were synthesised using known procedures:[3-15] $[C_2mim][C_8OSO_3]$, $[C_4mim][C_1OSO_3]$, $[C_4mim][lactate]$, $[C_4mim]I$, $[C_4mim][C_1COO]$, $[C_4mim]Cl$, $[C_8mim]Cl$, $[C_4mim][C_2OSO_3]$, $[C_4mim][C_3OSO_3]$, $[C_4mim][C_2OC_2OSO_3]$, $[C_4mim][C_1OC_2OSO_3]$, $[C_6mim]Br$, $[C_4eim]$ Br and$[C_6mim]I$.

General preparation for 1-alkyl-3-methylimidazolium halides

All 1-alkyl-3-methylimidazolium halides were synthesised by treating 1-methylimidazole with the appropriate haloalkane using known methodology.[4, 6]

Saccharin-based salts

Saccharin based ionic liquids were synthesised according to known procedures.[10, 16]

Analytical data:

$[C4m_{\beta}py][sacch]$: ^{1}**H** **δ**($CDCl_3$) 8.26-7.57(8H,m,ArH),4.84(2H,t,Py^+CH_2), 2.59(3H,s,CH_3), 1.97(2H,m,CH_2), 1.35(2H,m,CH_2), 0.89(3H,m,CH_3)

ESIMS: M^+(cation; calc 150.1283, obs. 150.1293); M^+(anion; calc 181.9912, obs. 181.9889)

General preparation of alkylsulfate salts

Dialkylsulfate (0.2 mol) was added dropwise to the appropriate trialkylamine (0.2 mol) and stirred at room temperature overnight. No solvent was used in this reaction. The resulting viscous ionic liquid was shown to have undergone a complete and clean reaction by ^{1}H NMR spectroscopy.

Alkyl substitution at the methyl or ethyl sulfate anion was carried out by a *trans*-esterification procedure, with the appropriate alcohol, developed by Wasserscheid.[7, 17, 18]

Analytical data for alkylsulfate ionic liquids are given below:

$[N_{1\,1\,2\,4}][C_2OSO_3]$: **δ** ($D_2O$) 4.07(2H,q,$CH_2$-OS), 2.32(2H,q,$CH_2N^+$), 2.22(2H,m,$CH_2N^+$), 3.00(6H,s,$N^+Me_2$), 1.70(2H,m,$CH_2$), 1.28(3H,s,$OCH_2\mathbf{CH_3}$), 1.23(2H,m, CH_2), 0.92(3H,t,CH_3)

ESIMS: M^+(cation; mass 130.1568, calc. 130.1596); M^+(anion; calc 124.9909, obs. 124.9896)

$[N_{2\,(2O2O1)3}][C_2OSO_3]$: δ ($CDCl_3$) 4.07(2H,q,$CH_2$-OS),3.92(6H,brm,6x$OCH_2$), 3.77(6H,brm,6x$OCH_2$), 3.5(8H,m,6x$OCH_2$ & CH_2N^+), 3.34(9H,s,3xOCH_3), 1.83(3H,t,CH_3), 1.27(3H,t,CH_3)

ESIMS: M^+(cation; mass 352.2699, calc. Mass 352.2691); M^+(anion, obs.125.1101, calc. 124.9909)

$[N_{1\,(2OH)3}]$ $[C_1OSO_3]$: δ D_2O **δ** ^{1}H NMR (D_2O, 300 MHz) δ: 4.05 (6H, m,O-CH_2), 3.73 (3H, s, OCH_3), 3.66 (6H, t, N-CH_2), 3.24 (3H, s, N-CH_3),

ESMS: M^+(cation; obs 164.1239, calc. 164.1287); M^+(anion, obs 111.0111 , calc. 110.9752)

General preparation for dicyanamide salts

$[C_{10}mim][N(CN)_2]$[16], $[C_4eim][N(CN)_2]$[16], $[C_6py][N(CN)_2]$[19] and $[P_{6,6,6,14}]$ $[N(CN)_2]$[20] were prepared according to standard methods.

$[C_4mim][N(CN)_2]$ and $[C_4m_{\beta}py]$ $[N(CN)_2]$ were known and they were prepared by a method developed by MacFarlane *et al.*[21, 22] Both $[N_{2\ 4\ (2OH)2}][N(CN)_2]$ and $[N_{1\,1\,4\,2OH}][N(CN)_2]$ were also prepared utilising the same method which involves mixing equimolar aqueous solutions of the desired [cation]Br and silver(I) dicyanamide. The resulting slurry was stirred in the dark for 18 h, and then silver(I) bromide was collected by filtration to give a clear aqueous filtrate. Water was removed under reduced pressure and the resulting ionic liquids were dried at 60-70 ^{o}C under vacuum (~0.1-0.5 bar) . The halide content in the dicyanamide ionic liquids was determined to be in the range 1.1-1.46 wt. %.

$[N_{2\,4\,(2OH)2}][N(CN)_2]$: ^{1}H δ D_2O **(**4.07(4H,t,CH_2O), 3.59(6H,m,3xCH_2N^+), 3.44(2H,m,CH_2), 1.77(2H,m,CH_2), 1.45(2H,m,CH_2), 1.38(2H,m,CH_2), 1.02(3H,s,CH_3)

ESIMS: M^+(cation; obs. 190.1827, calc. 190.1807) ;M^+(anion, obs. 66.0088 , calc. 66.0092)

$[N_{1\,1\,4\,2OH}][N(CN)_2]$: ^{1}H δ D_2O **(**4.01(2H,m,CH_2O), 3.46(2H,m,CH_2N^+), 3.37-3.31(2H,m, CH_2N^+), 3.1(6H,s,NMe_2), 1.72(2H,m,CH_2), 1.35(2H,m,CH_2), 0.92(3H,t,CH_3)

ESIMS: M^+(cation; obs. 146.1524, calc. 146.1545) ;M^+(anion, obs. 66.0089, calc. 66.0092)

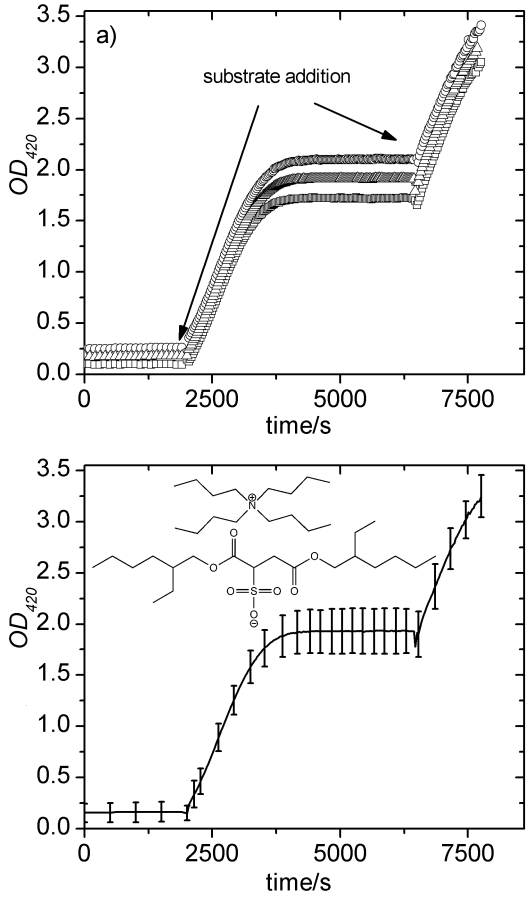

Fig. S1 Time course of laccase catalyzed ABTS oxidation in the presence of $[N_{4\,4\,4\,4}][AOT]$. The upper plot shows the raw data for each replicate and the lower plot averages and error bars.

General procedure for synthesis of bistriflamides

$[C_4eim][NTf_2]$, $[C_{10}mim][NTf_2]$, $[C_6py]$ $[NTf_2]$ and $[P_{6\,6\,6\,14}]$ $[NTf_2]$ are all known.[16, 23, 24] The synthesis of $[N_{1\,2\,2\,2O2O\text{-quinoline}}]$ $[NTf_2]$ will be published elsewhere.

$[N_{1\,1\,4\,10}]$ $[NTf_2]$: This IL was synthesised by treating N,N-dimethylbutyl amine with 1-bromooctane and 1-bromodecane to obtain the corresponding bromide salts. These salts were dissolved in dichloromethne and shaken with 1.1 eq. of Li bistriflamide in water. The organic layers were washed four times with water, filtered and solvent removed under high vacuum (~0.1-0.5 bar) to give final products.

$[N_{1\,1\,4\,10}][NTf_2]$: ^{1}**H δ** ($CDCl_3$) 3.58-3.51(4H,m,2xCH_2N^+), 3.42(6H,s,Me_2N^+), 1.71(4H,brm,2xCH_2), 1.45(2H,m,CH_2), 1.36-1.25(14H,brm,7xCH_2), 1.01(3H,t,CH_3), 0.88(3H,t,CH_3)

ESIMS: M^+(cation; calc. 242.2848, obs. 242.2797); M^+(anion; calc. 279.9173, obs. 279.9091)

General procedure for synthesis of docusate ionic liquids

$[C_4mim][AOT]$, $[C_4eim][AOT]$ and $[N_{4\,4\,4\,4}]$ $[AOT]$ are known.[25, 26] The rest of the docusate (AOT) ionic liquids were synthesised according to a procedure developed by Davis *et al* [27]. An equimolar mixture of Na[AOT] and 1-alkyl-3-methylpyridinium halides or phosphonium halides were dissolved in dichloromethane/diethyl ether and stirred for 24 h at room temperature. A high speed (8000 rpm) centrifuge was employed to facilitate the removal of precipitated sodium halides form dichloromethane/ diethyl ether solutions of ionic liquids before filtering them through a short silica plug. Solvents were removed on a Rotovaporator to produce docusate ionic liquids. The solvent residues from all docusate ionic liquids were removed under vacuum (~0.1-0.5 bar) at 70-80 °C.

$[C_4m_\beta py][AOT]$

^{1}**H δ** ($CDCl_3$) 9.14 (1H, s,ArH), 9.07 (1H, d, ArH), 8.20 (1H, d, ArH), 8.00(1H, t, ArH), 4.80 (2H, t, N-CH_2), 4.17(1H, dd, SCH), 4.1-3.9(4H,m, OCH), 3.2-3.1(2H, m, CH_2), 2.62(3H, s, Ar-CH_3), 1.98(2H, pt, CH_2), 1.62-1.2(18H, m, 9CH_2), 1.1(3H,t, CH_3), 0.87(12H, t, 4xCH_3)

LSIMS: (C_4-3-mpy + H; Calc. 150.1283, obs. 150.1270), (AOT anion; Calc. 421.2260, obs. 421.2275)

$[C_6m_\beta py][AOT]$

^{1}**H δ** ($CDCl_3$) 9.10 (1H, s,ArH), 9.01 (1H, d, ArH), 8.21 (1H, d, ArH), 8.00(1H, t, ArH), 4.76 (2H, t, N-CH_2), 4.13(1H, dd, SCH), 4.14-3.94(4H, m, OCH), 3.22-3.11(2H, m, CH_2), 2.60(3H, s, Ar-CH_3), 1.98(2H, pt, CH_2), 1.61-1.26(22H, m, 11xCH_2), 0.85(15H, m, 5xCH_3)

ESIMS: 178.1542 (C_6-3-mpy + H; Calc. 178.1576), 421.2271 (AOT anion; Calc. 421.2260)

$[C_8m_\beta py][AOT]$:

^{1}**H δ** ($CDCl_3$) 9.12 (1H, s, ArH), 9.04 (1H, d, ArH), 8.21 (1H, d, ArH), 8.00(1H, t, ArH), 4.77 (2H, t, N-CH_2), 4.17(1H, dd, SCH), 4.15-4.06(4H,m, OCH), 3.22-3.11(2H, m, CH_2), 2.62(3H, s, Ar-CH_3), 1.98(2H, pt, CH_2), 1.61-1.26(26H, m, 13xCH_2), 0.86(15H, m, 5xCH_3)

ESIMS: (C_8-3-mpy + H; Calc. 207.1909; obs.207.1918), (AOT anion; Calc. 421.2260, obs. 421.2280)

$[C_{10}mim][AOT]$:

^{1}**H δ** ($CDCl_3$) 9.50 (1H, s, ArH), 7.61 (1H, d, ArH), 7.44 (1H, d, ArH), 4.24(2H, m, CH_2Im), 4.13(1H, dd, SCH), 4.13-3.90(7H,m, OCH & CH_3N+), 3.22-3.09(2H, m, CH_2), 1.87(2H, pt, CH_2), 1.63-1.26(30H, m, 13xCH_2), 0.86(15H, m, 5xCH_3)

ESIMS: (C_{10}mim; Calc. 223.2174; obs. 223.2162), (AOT anion; Calc. 421.2260, obs. 421.2274)

$[P_{6\,6\,6\,14}][AOT]$: ^{1}**H δ** ($CDCl_3$) 4.13-3.89(5H,m,2xOCH_2 & CH), 3.32(1H,dd,CH), 3.13(1H,dd,CH), 2.27(8H,brm,4xCH_2-P^+), 1.50(14H,brm,7xCH_2), 1.31-1.26(46H,brm,23xCH_2), 0.91-0.88(24H,brm,8xCH_3)

ESIMS: M^+(cation; obs. 483.4982, calc. 483.5059) ;M^+(anion, obs. 421.2182, calc. 421.2260)

$[P_{8\,8\,8\,14}][AOT]$: ^{1}**H δ** ($CDCl_3$) 4.08-3.87(5H,m,2xOCH_2 & CH), 3.23(1H,dd,CH), 3.10(1H,dd,CH), 2.32(8H,brm,4xCH_2-P^+), 1.50(20H,brm,10xCH_2), 1.24(28H,brm,14xCH_2), 0.96(12H,t,4xCH3), 0.86(12H,t,4xCH3)

ESIMS: M^+(cation; 567.5967, calc. 567.5998) ; M^+(anion, obs. 421.2161, calc. 421.2260)

General procedure for synthesis of diisooctylphosphinate ionic liquids

Diisooctylphosphinate ionic liquids were synthesised by

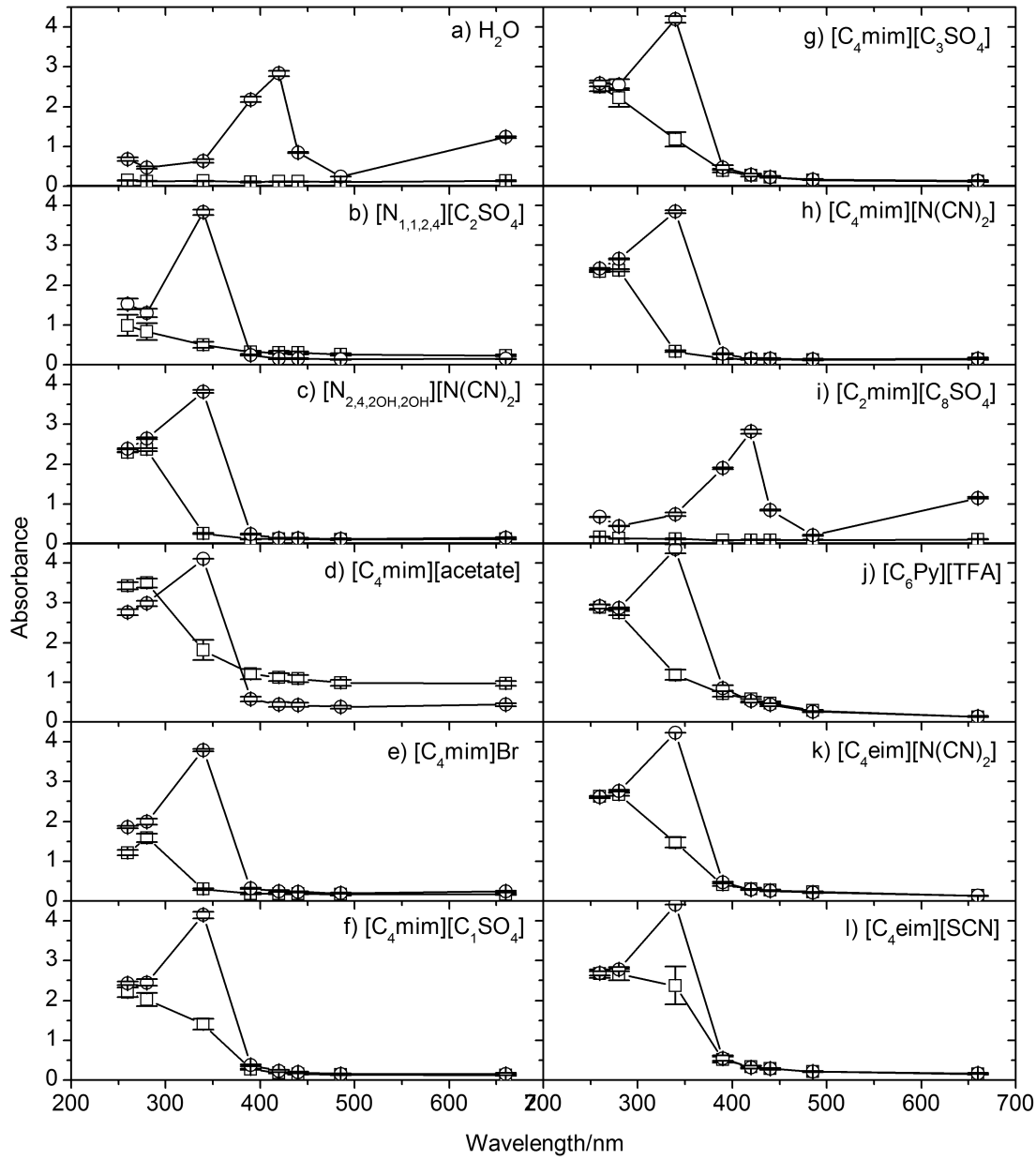

Fig. S2 Discontinuous spectra of reaction mixtures were measured before addition of ABTS (squares) and after completion of the reaction (circles) using the filters available for the plate reader (260, 280, 340, 390, 420, 440, 485, 660 nm). The samples contained citric acid buffer (pH 4.5), LTV laccase and 20 % of: a) H_2O, b) $[N_{1\ 1\ 2\ 4}][C_2OSO_3]$, c) $[N_{2,4,2OH,2OH}][N(CN)_2]$, d) $[C_4mim][acetate]$, e) $[C_4mim]Br$, f) $[C_4mim][C_1OSO_3]$, g) $[C_4mim][C_3OSO_3]$, h) $[C_4mim][N(CN)_2]$, i) $[C_2mim][C_8OSO_3]$, j) $[C_6Py][TFA]$, k) $[C_4eim][N(CN)_2]$, l) $[C_4eim][SCN]$. A peak at 340 nm indicates ABTS and a peak at 420 nm indicates oxidized ABTS. Each value represents the average of triplicate experiments; the error bars show the 95% confidence interval of the average.

treating equimolar quantities of sodium diisooctylphosphinate (50 mM) with the corresponding [cation]Cl (50 mM) in dry propanone (400 cm^3) and then heating under reflux overnight. The cooled reaction mixture was filtered to remove NaCl, and propanone from the resulting filtrate was removed on a rotary evaporator and final traces of the solvent were removed under vacuum (~0.1-0.5 bar) at 60 ^oC.

$[N_{1\ 8\ 8\ 8}][(^iC_8O)_2PO_2]$: ^1H δ ($CDCl_3$) 3.43-3.40(6H,m,3xCH_2N^+), 3.33(3H,s, CH_3N^+), 1.98(2H, brH, 2xPCH), 1.66(4H,m,2xCH2), 1.50(2H, brm, 2xPCH), 1.41 (2H, brq,**CH**-CH_3), 1.37-1.23(16H, brm, 8xCH_2), 1.12(8H,overlapping m,2xCH_3 & CH_2) , 0.91(18H,overlapping s,6xCH_3), 0.87 (9H,overlapping m, 3xCH_3)

ESIMS: M^+(cation; obs. 368.4238, calc. 368.4256); M^+(anion, obs. 289.2286, calc. 289.2296)

Quality control experiments

Assay verification for biphasic systems

The proposed photometric screening assay utilizes a vertical light path. In biphasic systems the light will therefore pass through both phases and might be affected by the interface or be scattered due to emulsion formation. Fig. S1 shows the enzyme activity in a biphasic system in individual replicate experiments (a), as well as the averages including error bars (b), as typically used to present the data throughout the paper. It can be seen in the stagnant fraction of the graph that the relative noise between consecutive measurements in each individual replicate experiment is small. The slopes of the individual replicates are also identical, but are offset between the replicates due to differences in the initial and final readings of the replicates. This might be due to imprecision in measuring the amount of ionic liquid due to the high viscosity and is fully reflected in the error bars. Generally the figure shows that the proposed assay is suitable to characterize the rate of reaction in biphasic systems; the only imprecision is in the extent of the reaction.

Spectroscopic verification of experimental procedure

ABTS was used as a photometrically traceable substrate for laccase. ABTS has an absorbance maximum at 340 nm and the oxidized form has its maximum at 420 nm, allowing the rate of oxidation to be measured. The spectra of selected ionic liquids were measured in the presence of ABTS to check for possible interference with the absorbance measurements and hence the rate determinations. Fig. S2 shows discontinuous UV-Vis spectra recorded with the FLUOstar Optima Microplate Reader (BMG Labtech Ltd., UK) that was used for the kinetic assay. The optical system of the plate reader is filter based, hence the limited wavelengths available. The spectra shown in Fig. S2 are of ionic liquids that inhibited the laccase completely except for $[C_2mim][C_8OSO_3]$ (i) and an ionic liquid-free system (a) as positive controls. A peak of oxidized ABTS can be seen for the positive controls at 420 nm (a), while the unoxidized substrate, ABTS, could not be detected in the control at 340 nm. In all other cases only unoxidized ABTS can be observed at 340 nm. These finding show that no unnoticed oxidation of ABTS occurred in the cases shown in Fig. S2. There was no absorbance at 340 nm in (a) but all except (i) had strong absorbance at 340 nm, indicating that the ABTS had not been oxidized. Some ILs also absorbed at 340 nm (*e.g.* (l)), but the peak of unoxidized ABTS was still clearly visible at the end of the reaction time, and no new peak at 420 nm appeared. Therefore it can be concluded that the tested ILs do not interfere with the optical measurements.

Notes and references

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