Supporting Information

Laccase activation in deep eutectic solvents

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Laccase activity tests

The enzymatic reaction was carried out at 25ºC by adding 100 μL of sample in 500 μL of 0.4 mM 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 1400 μL of 50 mM citrate/100 mM phosphate buffer at pH 4.5. Since ABTS oxidation is dependent on pH and an acidic pH is necessary. One unit (U) of laccase activity is defined as the amount of enzyme able to oxidize 1 μmol of ABTS *per* minute. The oxidation of ABTS was monitored by the increase in absorbance measured in kinetic model of a UV-Vis spectrophotometer (Agilent 8453) at 420 nm (ε = 36.000 $M^{-1}cm^{-1}$). The absorbance per min was obtained from the slope of the initial linear portion of the kinetic curve. These data (absorbance versus time) were automatically measured using the kinetic mode of the spectrophotometer. Laccase activities are presented in UL^{-1}.

The laccase activity was calculated through the following equation S1:

$$\frac{U}{L} = \frac{A_{min} \times f_{dil} \times V_{rxn} \times 10^6}{\varepsilon_{420nm}} \qquad \text{(S1)}$$

Where A_{min} is the absorbance per minute (determined by linear regression); f_{dil} is the dilution factor; V_{rxn} is the volume of reaction (in mL); 10^6 is the conversion factor of M into μM; ε_{420nm} is the ABTS molar absorption coefficient.

Table S1 – Relative laccase activity in different DES at different concentrations and molar ratio. Control: laccase activity in phosphate buffer pH 7.0 (50 mM) was considered as 100 %.

DES (molar ratio)	DES aqueous solution (%)	Relative activity (%)	DES (molar ratio)	DES aqueous solution (%)	Relative activity (%)
ChCl:EtG (2:1)	10	51.4±0.4	ChDHP:EtG (2:1)	10	117±1
	25	29.7±0.2		25	82.7±0.1
	50	19.1±0.1		50	76.9±0.4
ChCl:EtG (1:1)	10	53.1±0.1	ChDHP:EtG (1:1)	10	121±1
	25	31.7±0.2		25	94.8±0.2
	50	21.2±0.1		50	77.6±0.4
ChCl:EtG (1:2)	10	63.0±0.7	ChDHP:EtG (1:2)	10	123±0.5
	25	40.2±0.5		25	93.4±0.3
	50	25.4±0.1		50	65.9±0.8
ChCl:Gly (2:1)	10	63.4±0.3	ChDHP:Gly (2:1)	10	125±1
	25	37.3±0.1		25	96.4±0.2
	50	23.5±0.4		50	85.7±2.0
ChCl:Gly (1:1)	10	74.5±0.1	ChDHP:Gly (1:1)	10	134±1
	25	39.5±0.1		25	110±6
	50	25.2±0.4		50	110±2
ChCl:Gly (1:2)	10	84.2±0.8	ChDHP:Gly (1:2)	10	124±1
	25	47.8±0.7		25	106±1
	50	31.4±0.1		50	91.4±0.2
ChCl:Ery (2:1)	10	69.1±1.2	ChDHP:Ery (2:1)	10	123±0.5
	25	38.9±1.0		25	108±1
	50	23.5±0.4		50	70.4±1
ChCl:Ery (1:1)	10	74.3±0.4	ChDHP:Ery (1:1)	10	119±2
	25	43.9±0.4		25	100±1
	50	28.4±0.1		50	66.6±0.8
ChCl:Ery (1:2)	10	87.7±1.2	ChDHP:Ery (1:2)	10	137±0.5
	25	58.0±0.2		25	133±2
	50	38.3±0.3		50	113±6
ChCl:Xyl (2:1)	10	70.0±0.7	ChDHP:Xyl (2:1)	10	146±1
	25	43.1±0.5		25	118±1
	50	25.4±0.3		50	90.3±2.8
ChCl:Xyl (1:1)	10	84.4±0.7	ChDHP:Xyl (1:1)	10	147±1
	25	50.1±1.5		25	135±1
	50	29.4±0.2		50	106±2
ChCl:Xyl (1:2)	10	97.5±1.6	ChDHP:Xyl (1:2)	10	174±1
	25	58.8±1.7		25	171±3
	50	40.5±0.4		50	147±1

ChDHC:EtG (2:1)	10	101±1	Bet:EtG (2:1)	10	110±1
	25	144±1		25	107±2
	50	153±1		50	104±2
ChDHC:EtG (1:1)	10	111±1	Bet:EtG (1:1)	10	104±1
	25	146±2		25	104±2
	50	175±1		50	105±1
ChDHC:EtG (1:2)	10	115±1	Bet:EtG (1:2)	10	106±1
	25	138±2		25	113±1
	50	145±3		50	109±1
ChDHC:Gly (2:1)	10	116±2	Bet:Gly (2:1)	10	116±1
	25	141±2		25	107±1
	50	166±1		50	105±2
ChDHC:Gly (1:1)	10	123±1	Bet:Gly (1:1)	10	105±1
	25	160±1		25	110±1
	50	183±2		50	112±1
ChDHC:Gly (1:2)	10	131±1	Bet:Gly (1:2)	10	104±2
	25	154±2		25	108±1
	50	147±2		50	112±1
ChDHC:Ery (2:1)	10	134±3	Bet:Ery (2:1)	10	111±1
	25	162±3		25	108±1
	50	196±5		50	111±1
ChDHC:Ery (1:1)	10	156±1	Bet:Ery (1:1)	10	101±1
	25	171±1		25	107±1
	50	196±4		50	112±1
ChDHC:Ery (1:2)	10	175±3	Bet:Ery (1:2)	10	112±1
	25	177±2		25	110±1
	50	126±9		50	116±1
ChDHC:Xyl (2:1)	10	156±1	Bet:Xyl (2:1)	10	118±2
	25	193±4		25	118±1
	50	201±5		50	119±1
ChDHC:Xyl (1:1)	10	112±1	Bet:Xyl (1:1)	10	113±1
	25	125±1		25	118±1
	50	137±1		50	114±1
ChDHC:Xyl (1:2)	10	125±1	Bet:Xyl (1:2)	10	114±1
	25	115±1		25	120±1
	50	139±2		50	120±1

Table S2 – Relative laccase activity in reference compound at different concentrations and molar ratio. Control: laccase activity in phosphate buffer pH 7.0 (50 mM) was considered as 100%.

Compound	Compound aqueous solution (%)	Relative activity (%)
ChCl	10	26.1±0.4
	25	15.2±0.2
	50	9.6±0.1
ChDHP	10	114±1
	25	95.4±0.8
	50	59.4±3.1
ChDHC	10	126±1
	25	149±3
	50	186±1
Bet	10	121±1
	25	113±1
	50	102±2
EtG	10	114±1
	25	114±1
	50	106±3
Ery	10	118±1
	25	118±2
	50	-
Xyl	10	117±1
	25	120±1
	50	121±1
Gly	10	119±2
	25	120±1
	50	126±1

Table S3 – Relative activity of laccase in presence of ChDHC:Xyl (2:1) at 25 wt%, ChDHP:Xyl (1:2) at 25 wt% and ChDHP:Xyl (1:2) at 10 wt% at 60ºC and -80ºC. Relative activity (%) is addressed by comparison with the free-DES phosphate buffer pH 7.0 (50 mM) (control, 100%).

Time (days)	**Relative laccase activity (%)**			
	Enzyme stored at 60ºC			
	Control	**ChDHP:Xyl (1:2) 10 wt%**	**ChDHP:Xyl (1:2) 25 wt%**	**ChDHC:Xyl (2:1) 25 wt%**
0	100	174±1	171±3	200±2
2	4.1±0.1	11.6±0.7	5.5±0.5	0
6	0	0	0	0
9	0	0	0	0
12	0	0	0	0
15	0	0	0	0
20	0	0	0	0
	Enzyme stored at -80ºC			
0	100	174±1	171±3	200±2
2	90.4±1.3	170±0.4	168±0.5	194±1.8
6	77.2±1.6	173±4.3	153±3.7	190±3.7
9	78.2±9.1	170±2.2	149±0.5	187±1.7
12	92.1±1.4	165±0.6	138±0.5	145±0.1
15	85.3±6.6	137±4.5	140±5.7	140±6.9
20	90.5±9.2	130±.7	137±6.3	135±8.1

Table S4 - Docking affinity energy and interacting amino acids residues predicted by AutoDock vinna for Laccase and the DES HBAs.

HBAs	Affinity (kcal/mol)	Type of interaction	From	To	Distance (Å)
$[Ch]^+$	-3.2	Electrostatic	$[Ch]^+$	ASP140	4.57
		Hydrogen Bond	ASP128	$[Ch]^+$	2.98
			$[Ch]^+$	ASP128	3.00
				ASP131	3.01
				ASP140	2.53
				ASP128	2.61
				TYR137	2.60
				ASP140	2.70
Betaine	-3.5	Electrostatic	Betaine	ASP444	5.18
		Hydrogen Bond	HIS402	Betaine	3.06
			ARG442		3.11
			Betaine	ARG442	2.75
					3.03
				ALA403	2.69
$[DHP]^-$	-3.8	Hydrogen Bond	HIS111	$[DHP]^-$	3.20
			SER113		3.27
			$[DHP]^-$	ALA80	2.66
				HIS111	2.68
$[DHC]^-$	-4.8	Hydrogen Bond	$[DHC]^-$	ASP206	2.16
				HIS458	2.49
					2.53
			GLY392	$[DHC]^-$	2.88
Cl^-	-1.2	-	-	-	-

Table S5 - Docking affinity energy and interacting amino acids residues predicted by AutoDock vinna for laccase and DES HBDs.

HBDs	Affinity (kcal/mol)	Type of interaction	From	To	Distance (Å)
Xylitol	-4.8	Hydrogen Bond	ALA80	Xylitol	2.96
			HIS111	Xylitol	3.22
			Xylitol	SER113	2.66
				HIS111	2.36
Glycerol	-4.2	Hydrogen Bond	ALA80	Glycerol	2.90
			HIS111		3.28
			Glycerol	SER113	2.29
				ALA80	2.71
Ethylene glycol	-3.4	Hydrogen Bond	HIS111	Ethylene glycol	3.18
			SER113		3.14
			Ethylene glycol	ALA80	2.18
				HIS111	3.03
Erythritol	-4.5	Hydrogen Bond	Erythritol	SER113	2.71
				ALA80	3.05
			HIS111	Erythritol	3.26

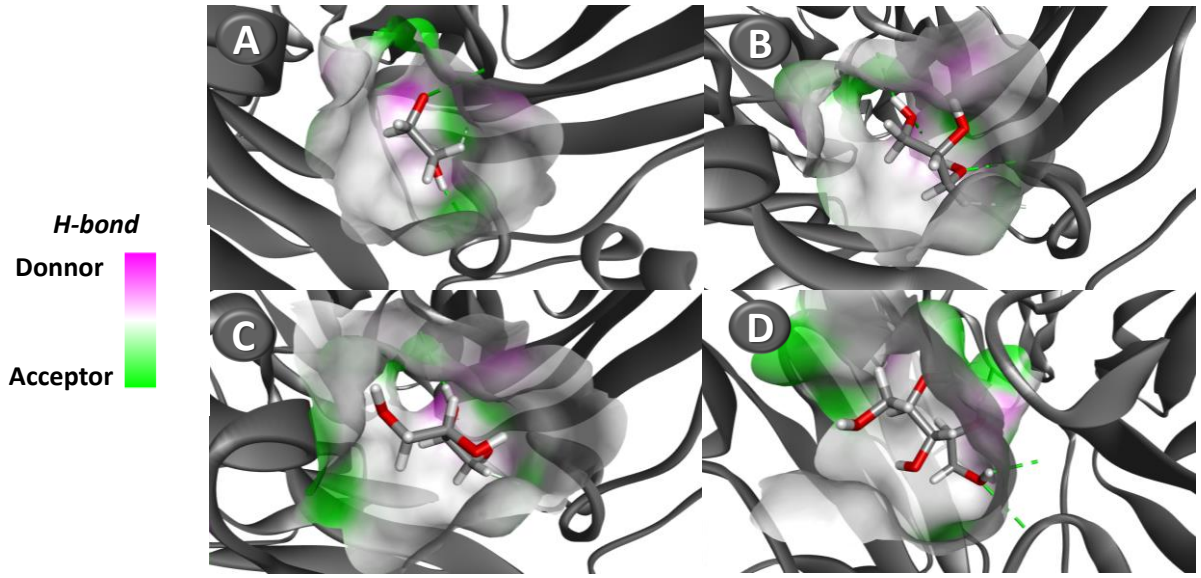

Figure S1. Docking pose with the lowest absolute value of affinity for laccase with: (A) ethylene glycol, (B) glycerol, (C) erythritol and (D) xylitol.

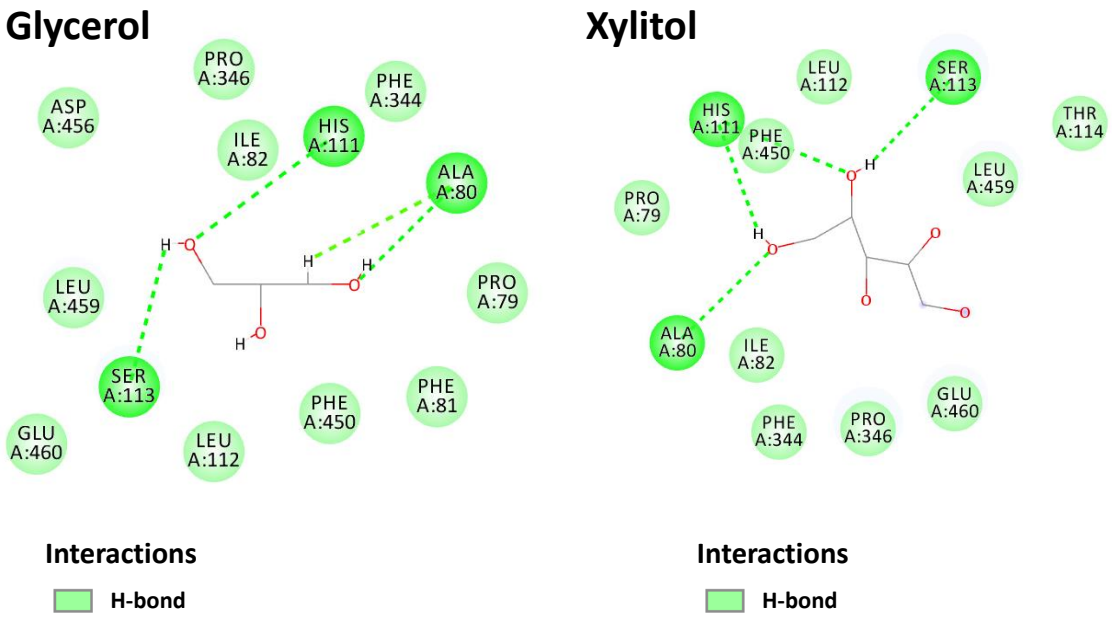

Figure S2. Molecular interaction diagrams of glycerol and xylitol and amino acids residues of laccase.

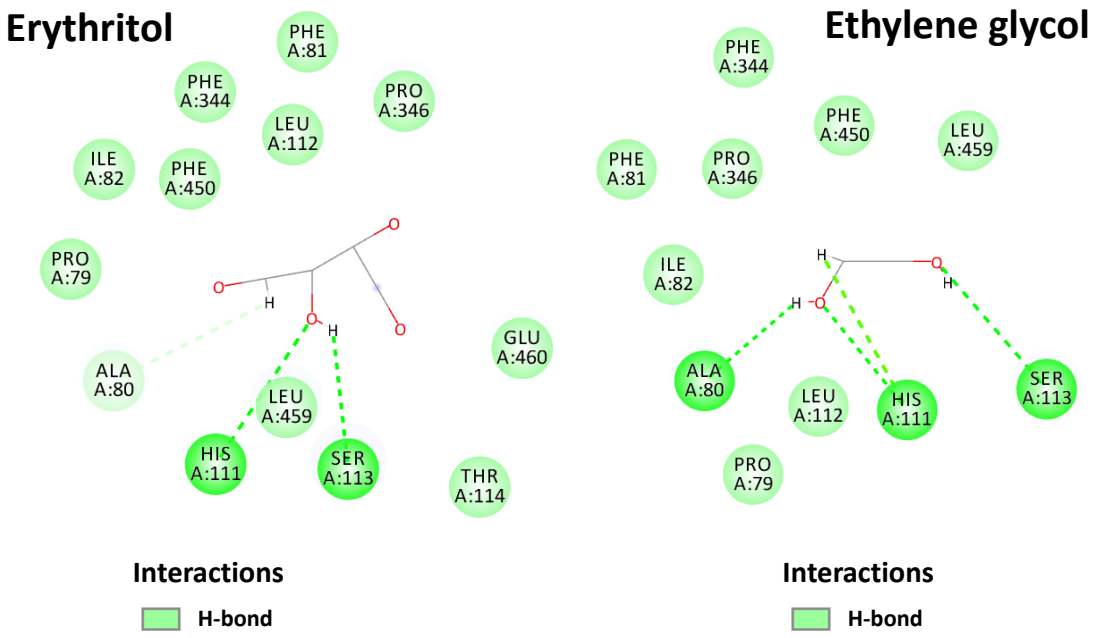

Figure S3. Molecular interaction diagrams of erythritol and ethylene glycol and amino acids residues of laccase.

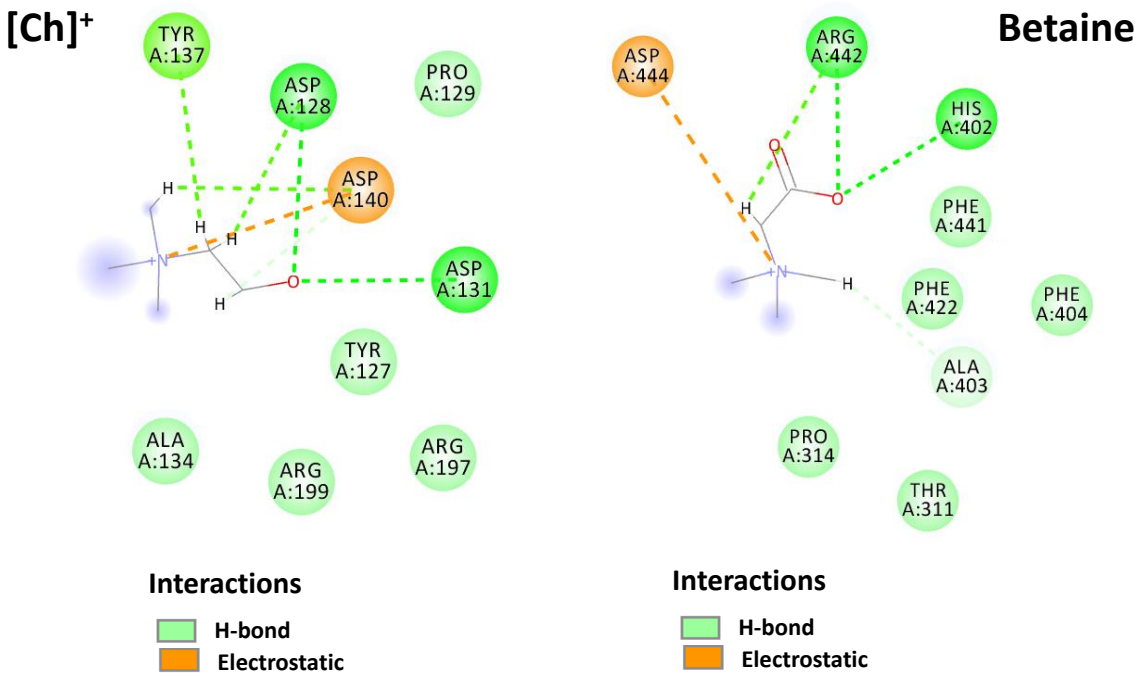

Figure S4. Molecular interaction diagrams of $[Ch]^+$ and betaine and amino acids residues of laccase.

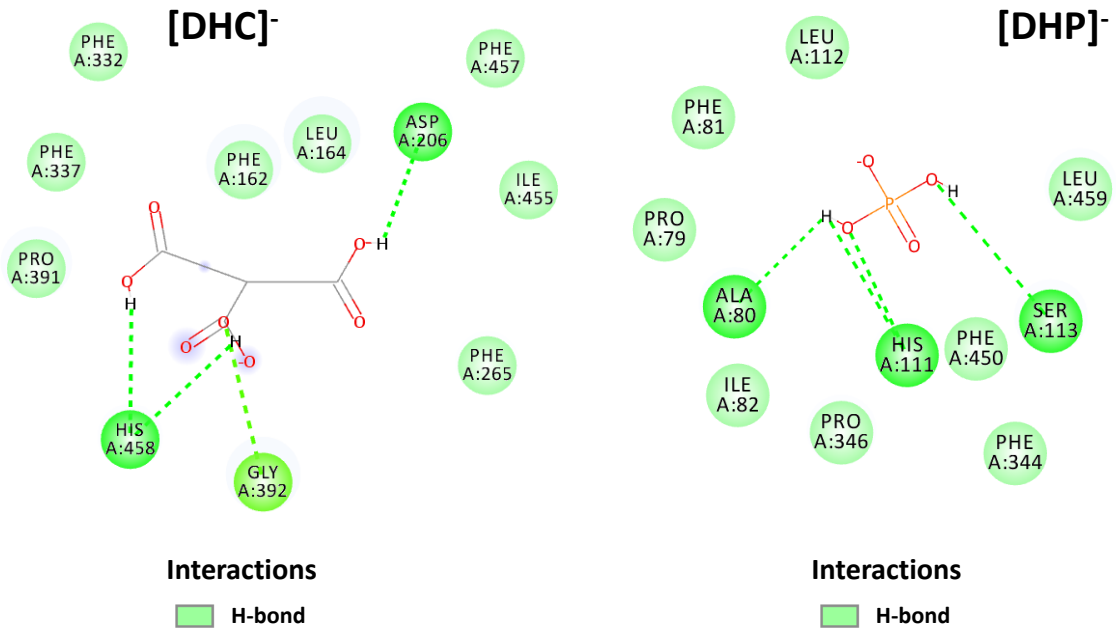

Figure S5. Molecular interaction diagrams of $[DHC]^-$ and $[DHP]^-$ and amino acids residues of laccase.