A novel starch binding laccase from the wheat pathogen *Zymoseptoria tritici* highlights the functional diversity of ascomycete laccases

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Table S1 List of *Zymoseptoria tritici* (*Ztr*Lac1A) and 6 other homologues with bimodular organizations

	Locus number	Length	Identity (Catalytic domain)	CBM	Linker length
Zymoseptoria tritici, *Ztr*Lac1A	XP003852363	723	100%	110	12
Zymoseptoria brevis, *Zbr*Lac1A	KJX94131	723	99%	110	12
Sphaerulina musiva, *Smu*Lac1A	XP_016758816	742	69%	108	32
Pseudocercospora musae, *Pmu*Lac1A	KXT09849	746	68%	107	32
Mycosphaerella eumusae, *Meu*Lac1A	KXS98121	719	69%	107	17
Pseudocercospora fijiensis, *Pfi*Lac1A	XP_007930185	587	68%	--	--
Dothistroma septosporum, *Dse*Lac1A	EME38505	729	68%	107	12
Daldinia eschscholtzii, *Des*Lac1A	AEX97872	418	60%	--	--
Baudoinia panamericana, *Bpa*Lac1A	XP_007679364	703	62%	108	17

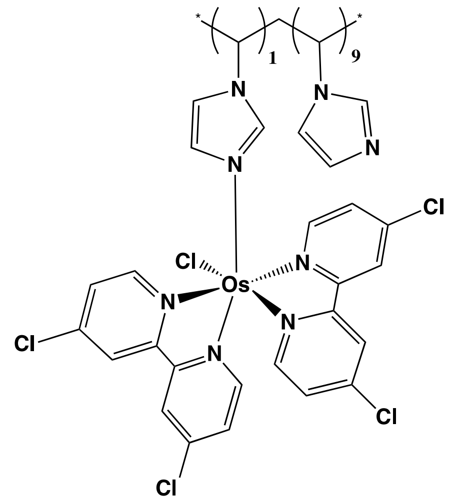


Fig. S1 Chemical structure of the osmium polymer [Os(4,4'-dichloro-2,2'-bipyridine)2(poly vinylimidazole)10Cl]·Cl with formal potentials $E°'$= 0.549 V *vs.* SHE

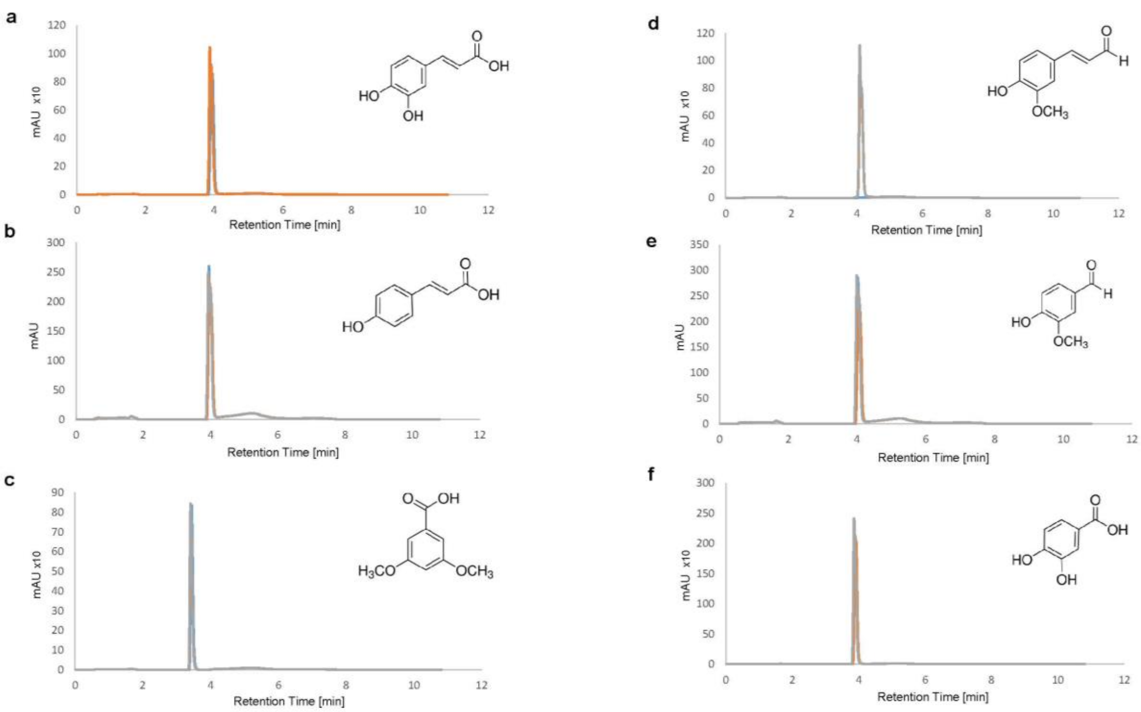
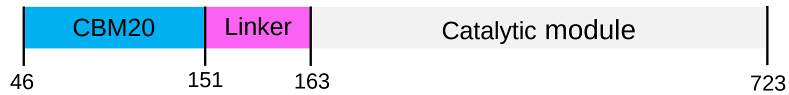


Fig. S2 HPLC ultimate profiles of different aromatic substrates assayed for *Ztr*Lac1A activity screening. The substrates are: (**a**) caffeic acid, (**b**) *p*-coumaric acid, (**c**) 3,5 dimethoxybenzoic acid, (**d**) coniferyl aldehyde, (**e**) vanillin and (**f**) 3,4 dihydroxybenzoic acid. All reactions were conducted in 0.5 ml for 45 and 90 min depicted in orange and blue respectively and controls are shown in grey. The reaction mixtures were analyzed by high performance liquid chromatography (HPLC3000), using reversed phase column C-18 (Kinetex LC column 100 mm x 4.6 mm, paricle size 2.6 µm). The elution was performed using a gradient of solvent A: water/TFA (100:0.01; v/v), solvent B: acetonitrile/TFA (100:0.01; v/v) and detection was conducted at 285 and 322 nm. The steps of gradient were linear from 0-4% of B for 5 min, 4-30% of B for 2 min and 30-100% of B for 2 min eluted at flowrate of 1.0 ml/min at room temperature

a



M R L H S I F N F L A L A A P I F A T V L P S Y D Y S Q R S E E V S A Q H L E S R Q S N A G
S I P V T F N L L A Q T G W G E S I Q V A G S T D E L G N W A A A N A V A L T A A Q Y T A G
N P L W S I T V D L P V G Q S F S Y K F I R I S V D G T V I W E D D P D R F Y T V P G N G
A T A A T I S N S W Q A R P **T T S** A **T** R **T** A **T S T** A P I A S S T C T N G P T S R G C W T S P
F S I N T D F D Q N W P I T G R T V S Y N F D I T **N** I T L A P D G F A R P V F A V N G Q Y P
G P T I F A N W G D T I Q V T V Q N S L Q H **N** G T S I H W H G M R M Y H A N G Q D G V P
G V T E C P I A P G R S K T Y T L L A T Q Y G T S W Y H S H F S S Q Y G D G I L G P I V I N
G P A S A N Y D I D L G A L P M T D W Y Y P T V M T T A A R A M H A N A L A P T A D N G L I
N G T M V S T S G N R G A Y A K T T L T A G K K H R L R F I N T S V D N H F M V G L D G
H I M T V I S A D F V P V E P Y **N** A S W I F I G I G Q R Y D V V I S A D Q T P G R Y W F R A
E V Q D S A G C G A N F Q N G N I R S I F S Y S S L P S N T P T L P T S S P T P Y T Q R C
S D E T S L T P V W N S Y V P R G R I P D A S S F S E L S I A I **N** Q S T A A D G K L T L Y W
Q V **N** G T P L R A D W E K P S V M T V R D G K T F P K A S N M I T F D D D D E D E W T Y
I L I T T G Q G S P F N V N I P H P I H L H G H D F Y V L G T G T S A W R D A D R Q G L N
Y E D P V R R D V A M L P S E G W L A I A W R R D N P G V W L V H C H I A W H A D E G F
A V Q F L E S P D K M L D V A P L P G D F D D Q C A A W S D F Y Q D G L Y K Q S D S G V

b

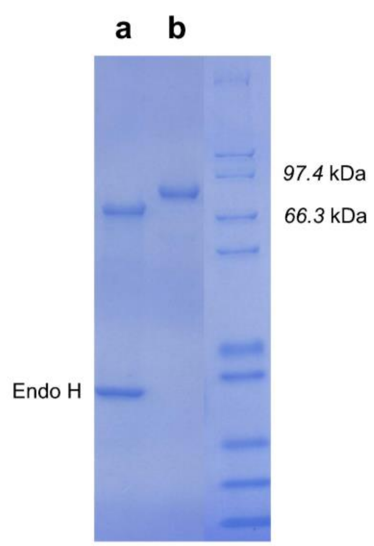


Fig. S3 (**a**) Laccase catalytic module, carbohydrate binding module and linker are colored in grey, blue and pink, respectively. Asparagine residues predicted to be *N*-glycosylated are colored in blue and those with more than 0.6 potential indicated with underline. (**b**) Purified *Ztr*Lac1A analyzed using SDS-PAGE after EndoH treatment A and before EndoH treatment B. The Mark 12 protein standard (Invitrogen) is in right lane

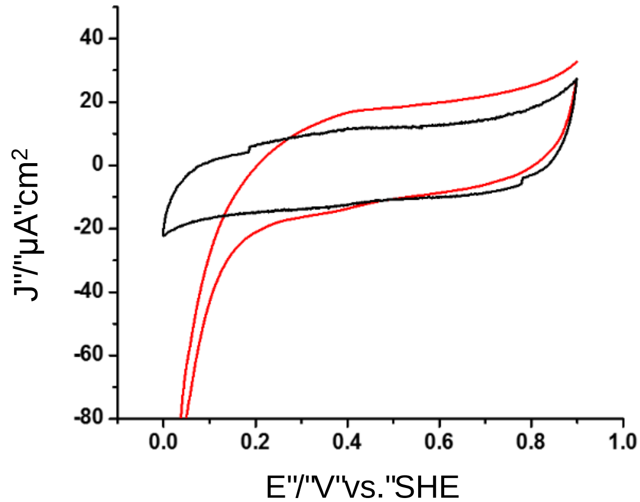
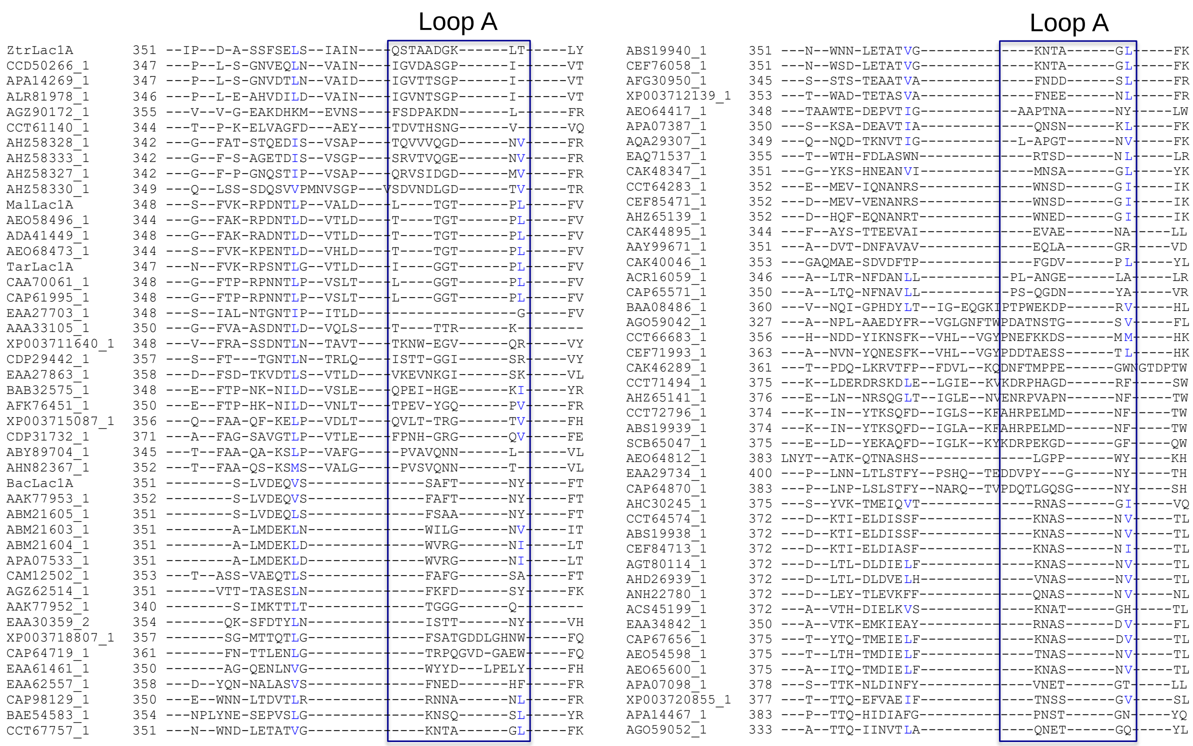


Fig. S4 Voltammograms recorded with naked graphite low-density electrode in N_2 (black line) and in the presence of O_2 (red line); Conditions: 0.1 M acetate buffer pH 4 in 0.1 M $NaClO_4$; scan rate 10 mVs^{-1}

a



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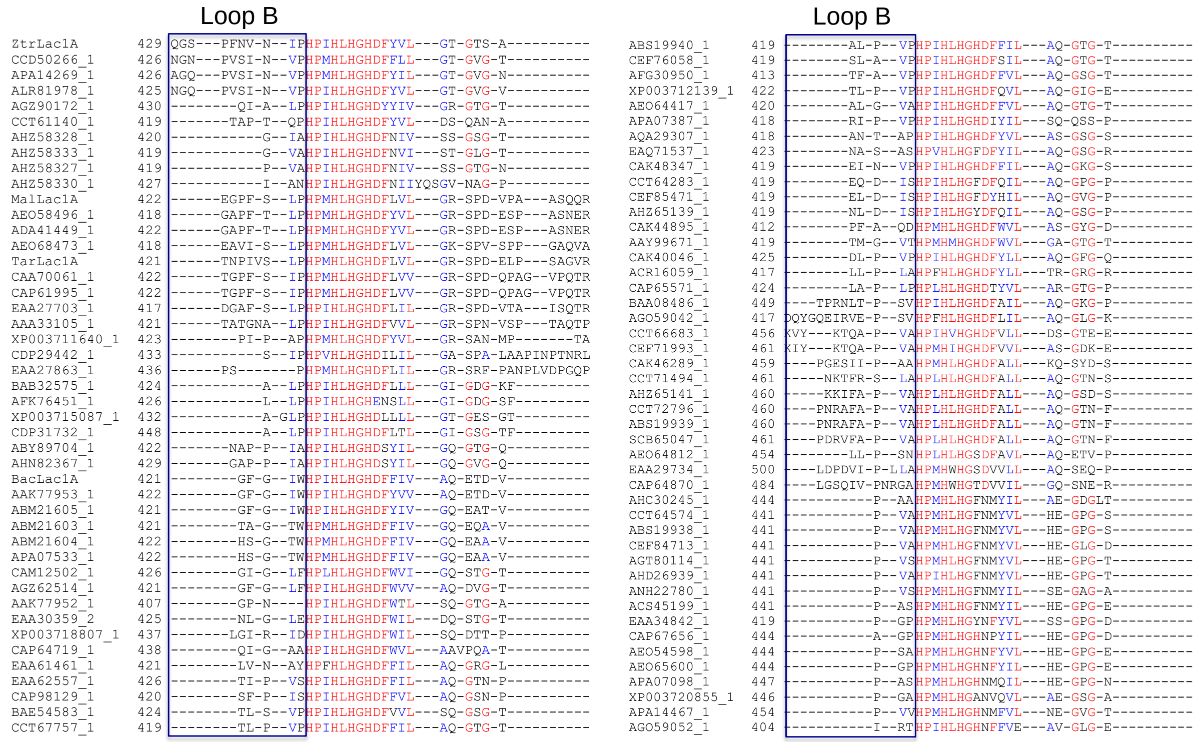

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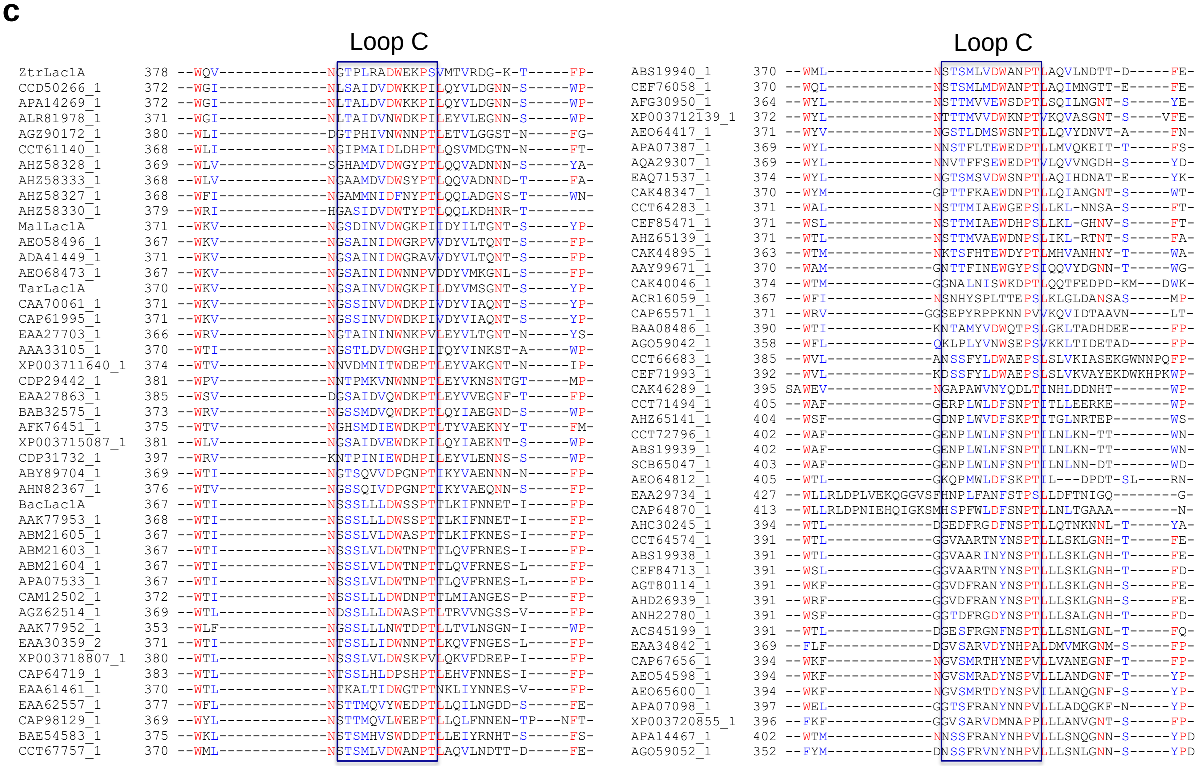
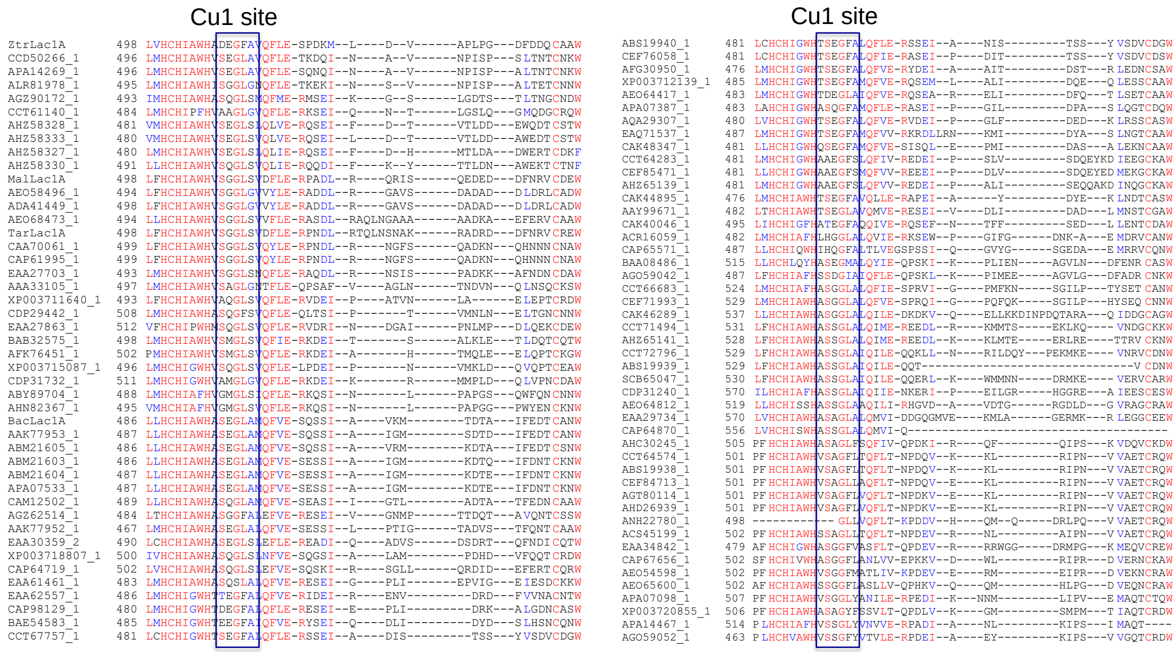


d



e

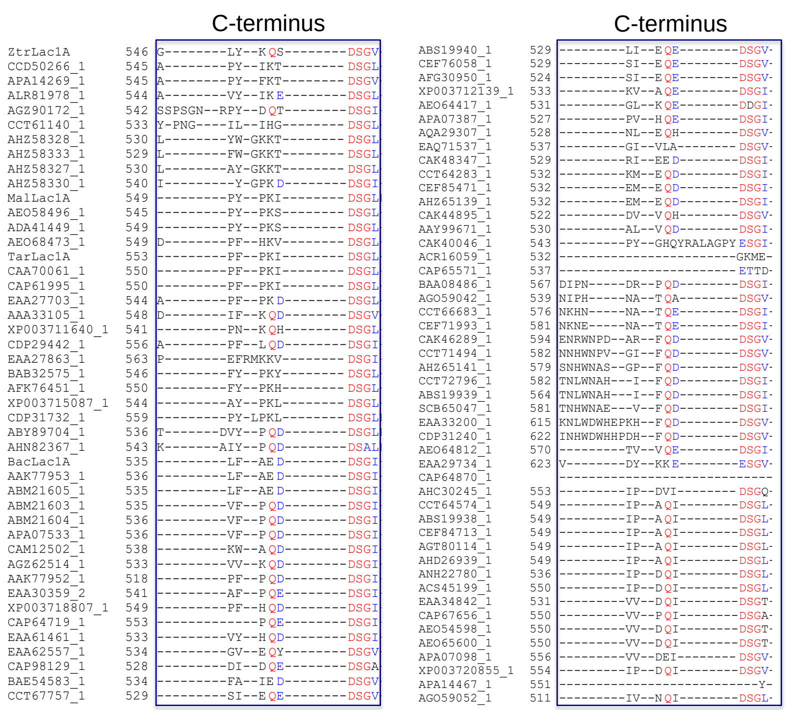


Fig. S5 Sequence alignment of *Ztr*Lac1A (catalytic module) along representative members of AA1 subfamily 3 deposited in CAZy database. All three loops A, B, C, Cu1 site ligands as well as the C-terminus regions are depicted in boxes. Fully conserved residues are in red letters, semi-conserved in blue and non-conserved in black. Accession numbers are shown except *Ztr*Lac1A, *Mal*Lac1A, *Tar*Lac1A and *Bac*Lac1A

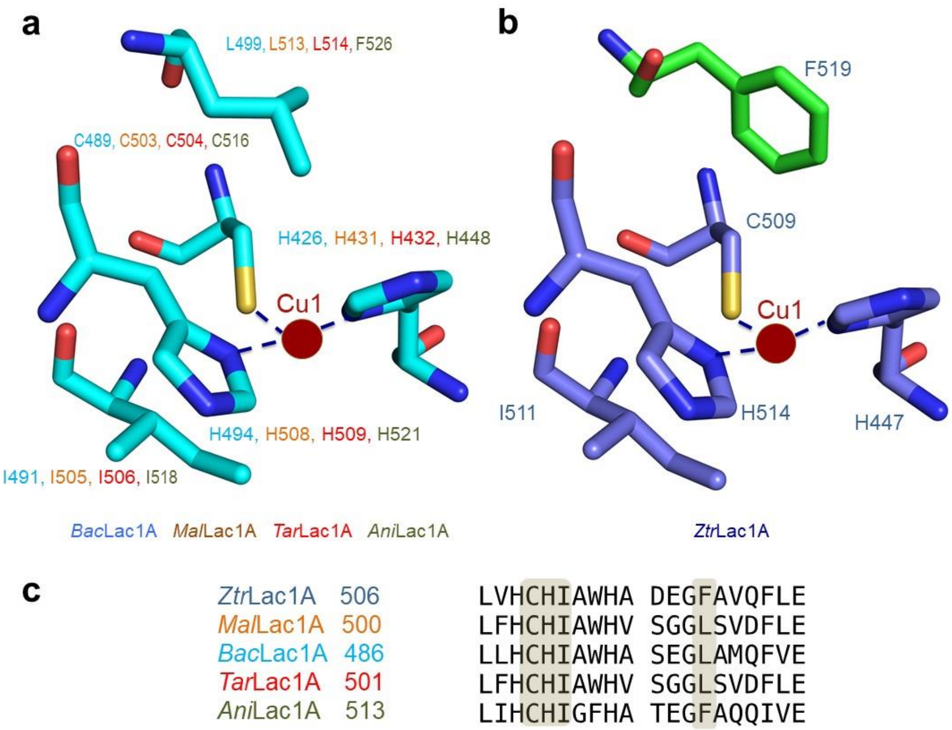


Fig. S6 Architecture of Cu1 copper site in *Bac*Lac1A, *Mal*Lac1A, *Tar*Lac1A and *Ani*Lac1A (**a**) and *Ztr*Lac1A (**b**). The Cu1 copper ions are depicted as brown spheres and their coordination bonds are shown in black dashed lines. The structure of *Bac*Lac1A PDB entry 3SQR was used [41]. (**c**) Structural sequence alignment of *Ztr*Lac1A with *Mal*Lac1A, *Tar*Lac1A, *Bac*Lac1A and *Ani*Lac1A

References

41. Osipov E, Polyakov K, Kittl R, Shleev S, Dorovatovsky P, Tikhonova T, Hann S, Ludwig R, Popov V. Effect of the L499M mutation of the ascomycetous *Botrytis aclada* laccase on redox potential and catalytic properties. Acta Crystallogr D Biol Crystallogr. 2014;70:2913−2923.