Supplementary Materials

Biocatalyzed Reactions towards Functional Food Components 4-Alkylcatechols and Their Analogues

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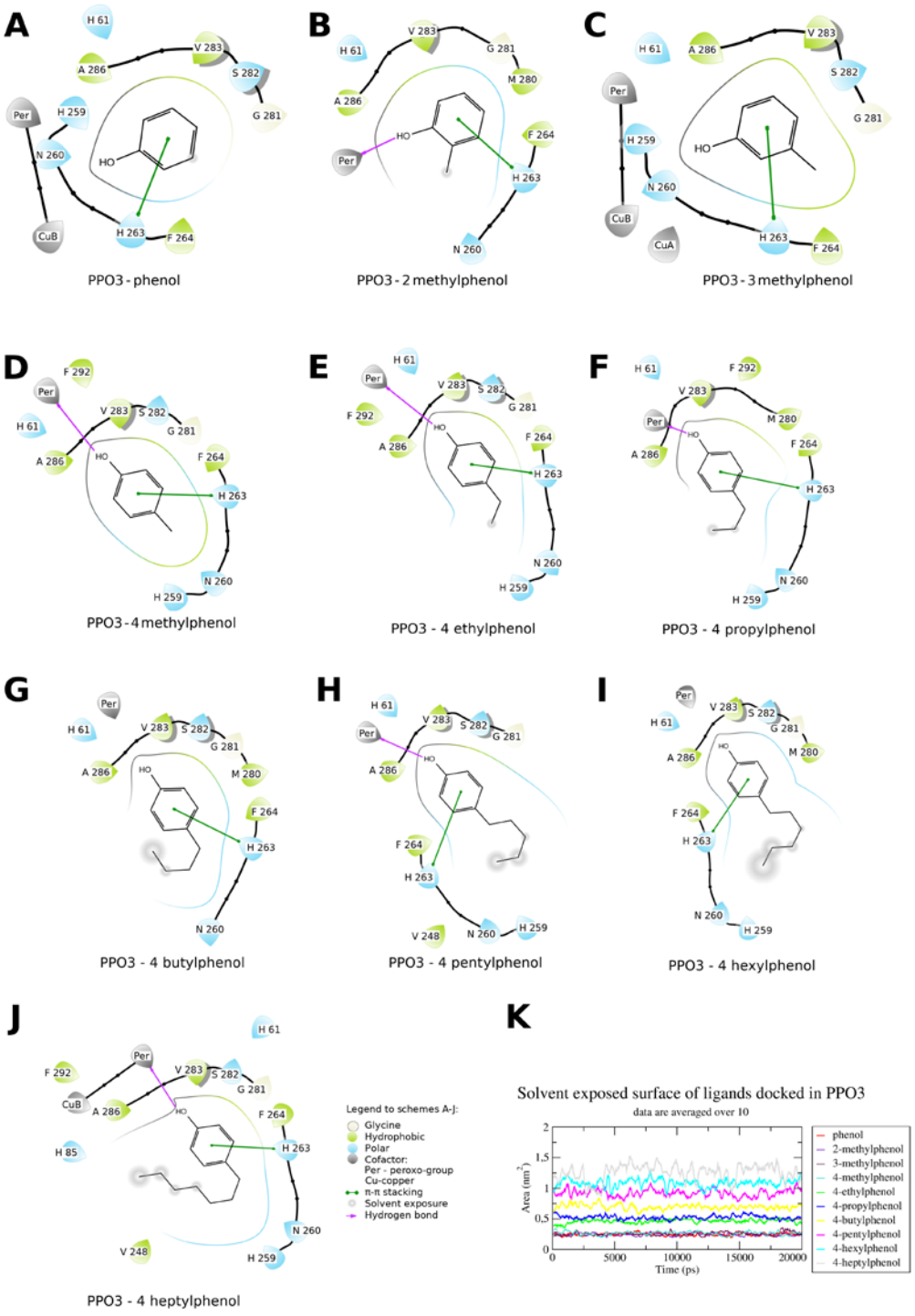


Figure S1. (A-J) Schemes of ligands in the active site of PPO3 after 20 ns of molecular dynamics (MD) simulations. Amino acid residues and cofactor atoms within 0.3 nm distance from the ligands are shown by drops and labeled. Schemes are done in Schrödinger software. Legend is shown in the last figure (J). (K) Change in solvent-exposed surface of ligands during 20 ns of MD simulations in the active site of PPO3. Graphics is made with Xmgrace.

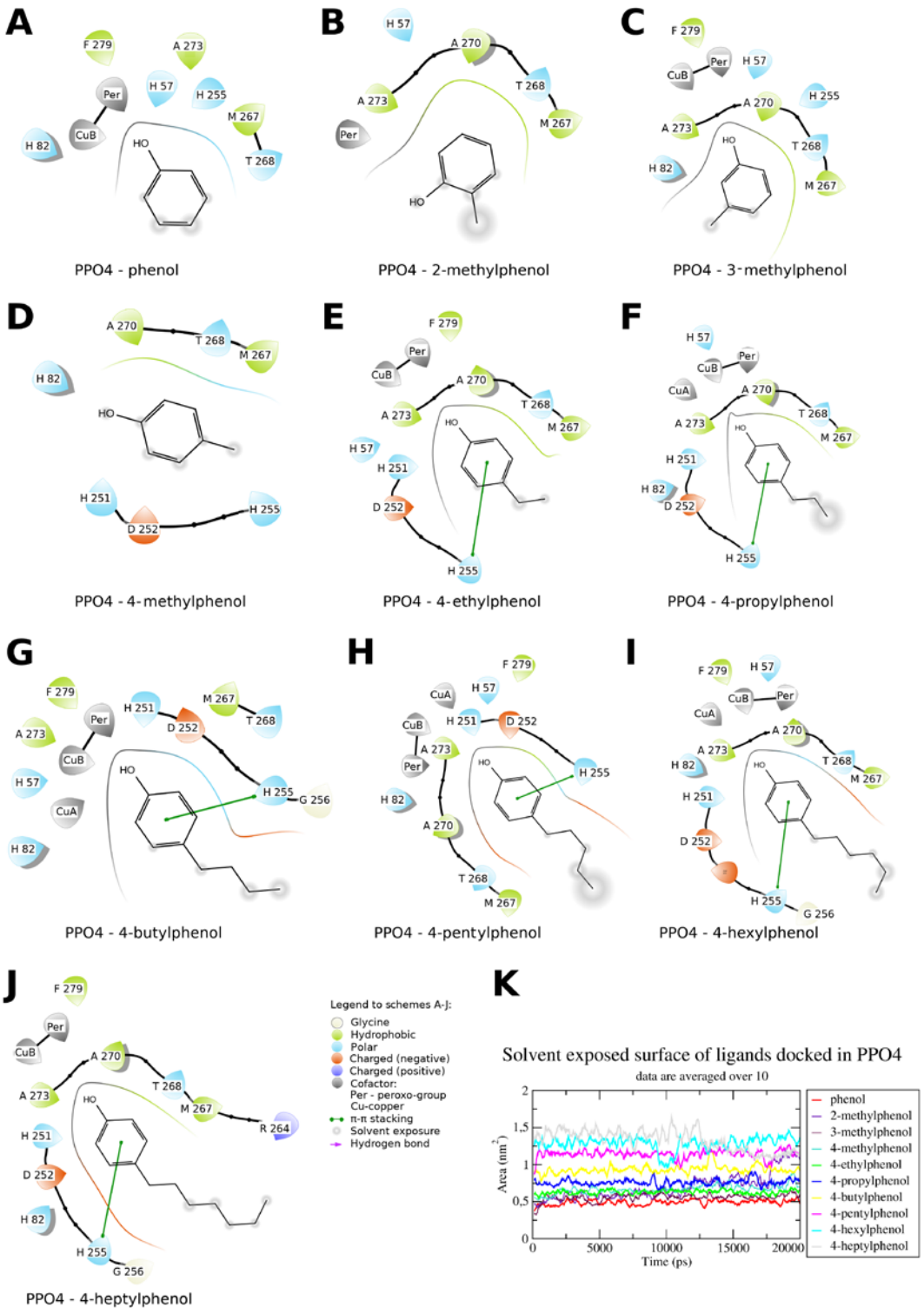


Figure S2. (A-J) Schemes of ligands in the active site of PPO4 after 20 ns of MD simulations. The orientation of 2-methylphenol (B) is only shown for 10 ns snapshot, as the ligand left the active site of PPO4 later. Amino acid residues and cofactor atoms within 0.3 nm distance from ligands are shown by drops and labeled. Schemes are done in Schrödinger software. Legend is shown in the last figure (J). (K) Change in solvent-exposed surface of ligands during 20 ns of MD simulations in the active site of PPO4. The graphics is made with Xmgrace.

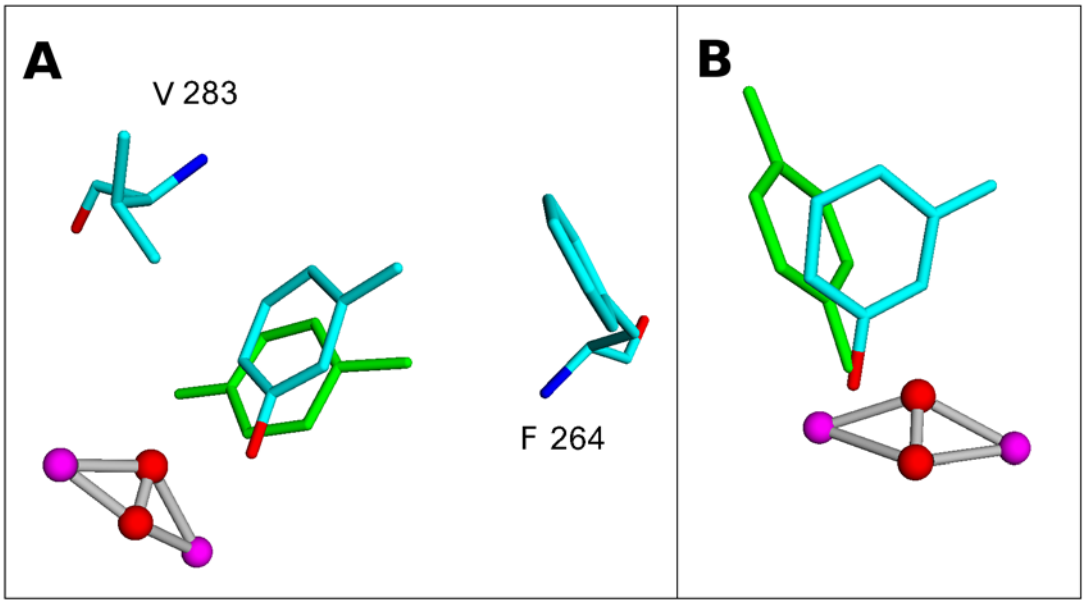


Figure S3. (A) Alignment of corresponding positions of 4-methylphenol (green) and 3-methylphenol (element color) in the active site of PPO3 after 20 ns of MD simulations. F264 and V283 participating in hydrophobic interactions with the substrate are shown. (B) Alignment of the corresponding positions of 4-methylphenol (green) and 3-methylphenol (element) in the active site of PPO4 after 20 ns of MD simulations.

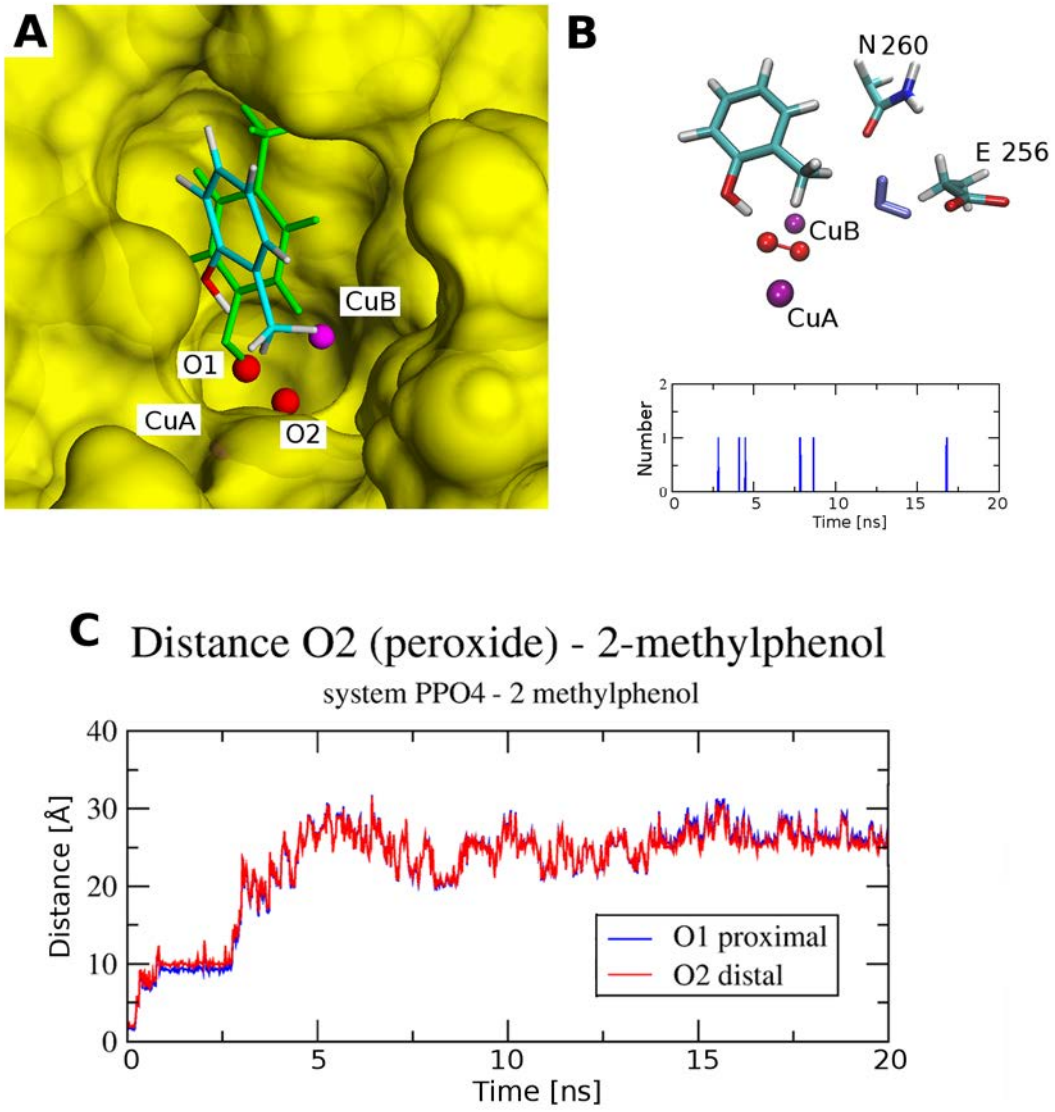


Figure S4. (A) Equilibrated orientation of 2-methylphenol in the active site of PPO3 (element color) overlayed with orientation of 4-methylphenol (green). PPO3 amino acid residues are shown by molecular surface (yellow). (B) Water penetration close to cofactor in the complex of PPO3 with 2-methylphenol. Residues N260 and E256 which could participate in activating a water molecule to deprotonate the substrate [27] are shown after 20 ns of MD. Cofactor is shown by ball representation. Water molecule found during MD simulation within 5.2 Å from cofactor Cu and 4 Å from Asn260 are shown. Number of these water molecules during MD is calculated in the graphics. (C) Increase in distance to peroxide oxygens in PPO4-phenol and PPO4-2-methylphenol complexes as a result of ligand rotation (within 20 ns). The ligand left the active site after 2.5 ns.

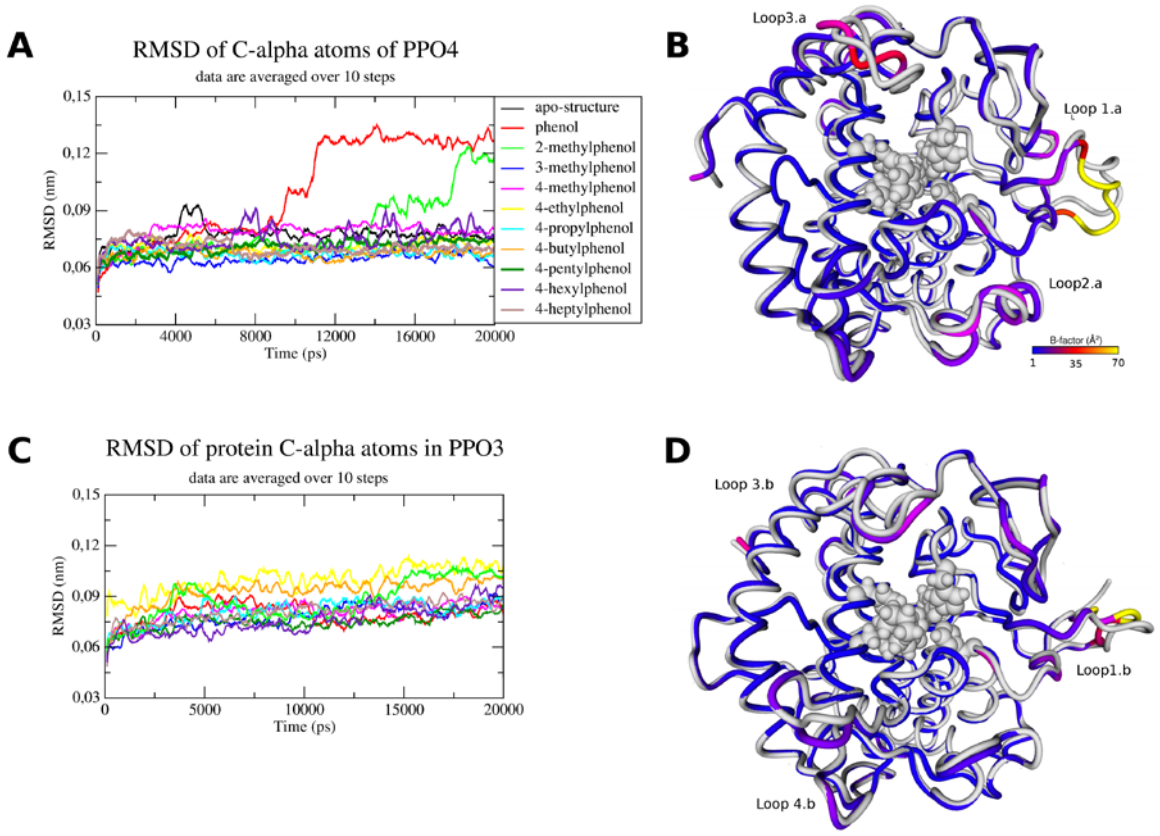


Figure S5. (A,C) Root-mean-square displacement (RMSD) of protein C-α atoms calculated during 20 ns of MD simulations of (A) PPO4 and (C) PPO3. Data are averaged over 10 simulation snapshots. The legend in (A) also holds for (C). (B,D) Overlay of average structure from MD simulations of (B) PPO4 – phenol system and (D) PPO3 – 4-butylphenol system. Systems showing higher RMSD in (A, C) were selected for comparison. Structures of proteins after 20 ns of MD are colored according to B-factor (blue with value of 0 is rigid, yellow (75) is more flexible). B-factor (temperature factor) was calculated as root means square fluctuation of each residue during MD from the position found in the crystal structure. Ligands, cofactors and active site His residues from initial structure are shown in ball representation. Most flexible regions include loop 1.a (residues 65-75), 2.a (residues 178-185) and 3.a (residues 235-244) in PPO4 (B) and loops 1.b (residues 69-78) and 3.b (residues 243-255) in PPO3 (D). Loop 4.b (residues 269-276) is significantly shorter in PPO4 than in PPO3.

Table S1. Retention times (RTs) and local absorption maxima of phenol and alkyl phenols **1a**–**7a** and the corresponding catechols **1b**–**7b** in analytical HPLC [1].

Compound [2]	Mobile phase (MeCN/water) [3]	Retention time (min)	Local spectral maximum (nm)
Phenol	20/80	1.38	277
Catechol	20/80	0.725	281
1a	20/80	1.96	277
1b	20/80	1.04	281
2a	30/70	1.90	277
2b	30/70	1.01	281
3a	30/70	3.56	277
3b	30/70	2.00	281
4a	40/60	2.62	277
4b	40/60	1.35	281
5a	40/60	4.60	277
5b	40/60	2.11	281
6a	50/50	2.96	277
6b	50/50	1.55	281
7a	50/50	4.76	277
7b	50/50	2.32	281

MeCN = acetonitrile. [1] The HPLC system was Shimadzu Prominence (see main text for details) equipped with a Chromolith SpeedRod RP-18 column (50 x 4.6 mm; Merck). The analysis was performed at a flow rate of 2.0 mL min^{-1} and 34 °C. [2] See Scheme 2 (main text) for structures. [3] The mobile phase contained 0.1% H_3PO_4.

Table S2. MS data of compounds **1b**–**7b** [1].

Compound [2]	MS-ESI *m*/*z*
1b	$[M-H]^-$ calcd for $C_7H_7O_2$ 123.1, found 123
2b	$[M-H]^-$ calcd for $C_8H_9O_2$ 138.1, found 138 $[M+HCOO]^-$ calcd for $C_9H_{11}O_4$ 183.1, found 183
3b	$[M-H]^-$ calcd for $C_9H_{11}O_2$ 151.1, found 151 $[M+HCOO]^-$ calcd for $C_{10}H_{13}O_4$ 197.1, found 197 $[M+Cl]^-$ calcd for $C_9H_{11}O_2Cl$ 187.1, found 187 $[M-H+H_2O]^-$ calcd for $C_9H_{13}O_3$ 169.1, found 169
4b	$[M-H]^-$ calcd for $C_{10}H_{13}O_2$ 165.2, found 165 $[M+HCOO]^-$ calcd for $C_{11}H_{15}O_4$ 211.2, found 211 $[M+Cl]^-$ calcd for $C_{10}H_{13}O_2Cl$ 201.2, found 201 $[M-H+H_2O]^-$ calcd for $C_{10}H_{15}O_3$ 184.2, found 184
5b	$[M-H]^-$ calcd for $C_{11}H_{15}O_2$ 179.1, found 179 $[M+Cl]^-$ calcd for $C_{11}H_{15}O_2Cl$ 215.1, found 215 $[M+HCOO]^-$ calcd for $C_{12}H_{17}O_4$ 225.1, found 225
6b	$[M+Cl]^-$ calcd for $C_{12}H_{18}O_2Cl$ 229.09, found 229
7b	[M+Cl]- calcd for $C_{13}H_{20}O_2Cl$ 243.14, found 243

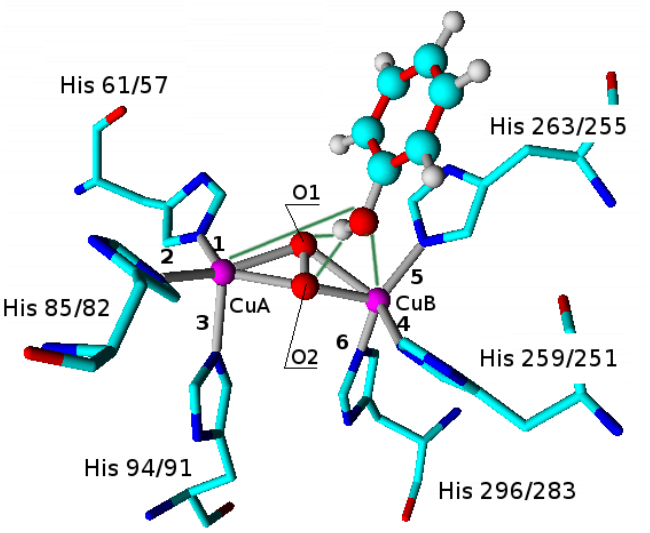
[1] See main text for details of the LC-MS system. [2] See Scheme 2 (main text) for structures.

Table S3. Average distances of the hydroxyl hydrogens in alkylphenols (ligands) from the oxygen atoms of the $[Cu_2O_2]^{2+}$ cofactor in tyrosinases PPO3 and PPO4.

Ligand	**Average distance (Å)**			
	PPO3		**PPO4**	
	proximal O (O1 in pdb)	**distal O (O2 in pdb)**	**proximal O**	**distal O**
phenol	1.70	1.80	1.83	1.97
2-methylphenol	1.86	2.80	-	-
3-methylphenol	1.76	2.10	1.78	2.12
4-methylphenol (**1a**)	1.60	2.60	1.96	1.95
4-ethylphenol (**2a**)	1.60	2.70	1.9	1.71
4-*n*-propylphenol (**3a**)	1.60	2.70	1.86	1.71
4-*n*-butylphenol (**4a**)	1.56	2.68	1.95	1.71
4-*n*-pentylphenol (**5a**)	1.62	2.60	1.83	1.7
4-*n*-hexylphenol (**6a**)	1.64	2.25	1.81	1.72
4-*n*-heptylphenol (**7a**)	1.59	2.67	1.81	1.69

Table S4. Average distances between CuA or CuB and the coordinating His residues in PPO3 and PPO4 isoenzymes.

Analyzed distance	Average value (Å)	Schematic representation of the analyzed distances
PPO3		
Cu – His 61	2.1 ± 0.05	
CuA – His 85	2.08 ± 0.03	
CuA – His 94	2.2 ± 0.1	
CuB – His 259	2.065 ± 0.08	
CuB – His 263	2.08 ± 0.05	
CuB – His 296	2.06-±-0.01	
PPO4		
CuA – His 57	2.101 ± 0.01	
CuA – His 82	2.107 ± 0.005	
CuA – His 91	2.09 ± 0.012	
CuB – His 251	1.962 ± 0.01	
CuB – His 255	2.029 ± 0.07	
CuB – His 283	2.000 ± 0.017	



Coordinating bonds formed by enzyme and cofactor are shown as grey sticks, calculated distances between cofactor – ligand are shown in dark green (exemplified for PPO3 and phenol as ligand).