Supplementary Material

A survey of substrate specificity among Auxiliary Activity Family 5 Copper Radical Oxidases

Maria E. Cleveland[1,2,3], Yann Mathieu[1,3], David Ribeaucourt[4,5,6], Mireille Haon[4], Paul Mulyk[2], Jason E. Hein[2], Michael Lafond[5], Jean-Guy Berrin[4], Harry Brumer[1,2,3,7,8]*

[1]Michael Smith Laboratories, University of British Columbia, 2185 East Mall, Vancouver, BC, V6T 1Z4, Canada;

[2]Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC, V6T 1Z1, Canada;

[3]BioProducts Institute, University of British Columbia, 2385 East Mall, Vancouver, BC, V6T 1Z4, Canada

[4]INRAE, Aix Marseille Univ, UMR 1163 Biodiversité et Biotechnologie Fongiques, 13009 Marseille, France

[5]Aix Marseille Univ, CNRS, Centrale Marseille, iSm2, Marseille, France

[6]V. Mane Fils, 620 route de Grasse, 06620 Le Bar sur Loup, France

[7]Department of Biochemistry and Molecular Biology, University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC, V6T 1Z3, Canada;

[8]Department of Botany, University of British Columbia, 3200 University Boulevard, Vancouver, BC, V6T 1Z4, Canada

Corresponding Author

*Harry Brumer: brumer@msl.ubc.ca

Supplementary Tables

Table S1. Key amino acid residues in selected AA5_2 genes compared to other characterized members.

Enzyme	Organism	GenBank/JGI mycocosm Accession	Active Site Amino Acids							Distal Amino Acids	Modularity
			Radical Stabilization	Substrate Recognition/Active Site Cavity Shape						Catalytic Efficiency	
*Fgr*GalOx	Fusarium graminearum	AAO95371	W290	F194	Q326	Y329	R330	Q406	P463	C383	CBM 32-CAT
*Cgr*AlcOx	Colletorichum graminicola	EFQ30446	F	W	G	F	M	T	L	C	CAT
*Cgr*AAO	Colletorichum graminicola	EFQ27661	Y	F	E	W	R	T	V	C	PAN 1-CAT
*Fgr*AAO	Fusarium graminearum	XP_011322138	W	F	E	Y	K	Q	P	N	CBM 32-CAT
Y1	Eurotium rubrum	XP_040635673	W	F	K	W	R	D	P	Y	CBM32-CAT
Y11	Melanomma pulvis-pyrius	KAF2798557	W	F	G	F	R	A	P	N	CBM32-CBM32-CAT
Y12	Niesslia exilis	jgi|Nieex1|772836	W	F	E	W	R	D	A	S	CBM32-CAT
Y13 *Pfe*GalOx	Penicillium fellutanum	jgi|Penfe1|382062	W	Y	Q	Y	R	Q	P	S	CBM32-CAT
Y14 *Phu*RafOx	Pseudozyma hubeiensis	XP_012186969	Y	F	A	W	R	S	G	C	UNK-CAT
Y15	Setosphaeria turcica	XP_008020456	W	F	Q	L	R	V	G	C	UNK-CAT
Y16 *Sto*AA5	Stagonospora sp.	OAK97814	W	Y	E	F	R	D	G	C	CAT
Y17	Talaromyces marneffei	XP_002148539	F	F	G	Y	R	N	V	T	UNK-CAT
Y18 *Asy*AlcOx	Aspergillus sydowii	XP_040706357	F	Y	H	D	R	V	P	C	UNK-CBM32-CAT
Y19	Torpedospora radiata	jgi|Torra1|425635	W	Y	E	F	R	Q	P	C	CBM32-CAT
Y2 *Uma*RafOx	Ustilago maydis	XP_011389156	Y	F	A	W	R	S	G	C	UNK-CAT

Y20	Metarhizium acridum	XP_007812971	W	Y	Q	Y	R	Q	P	N	CBM32-CAT
Y21	Leptosphaeria maculans	XP_003841328	W	F	S	F	H	G	G	C	CAT
Y22	Acremonium strictum	jgi|Acrst1|1377707	W	F	E	S	K	R	P	N	CBM32-CAT
Y23 *Cca*AA5	Corynespora cassiicola	PSN67470	W	A	A	N	E	H	G	C	CAT
Y24	Myrothecium inundatum	jgi|Myrin1|117638	W	F	A	Y	K	A	N	C	CBM32-CAT
Y25	Niesslia exilis	jgi|Nieex1|192818	W	F	Q	F	R	Q	P	C	CBM32-CAT
Y29 *Mre*GalOx	Mytilinidion resinicola	XP_033570565	W	F	Q	F	R	Q	P	N	CBM32-CBM32-CAT
Y3	Loramyces macrosporus	jgi|Lorma1|509879	Y	F	E	W	R	T	V	C	UNK-CAT
Y35 *Cgl*AlcOx	*Colletotrichum gloeosporioides*	jgi|Gloci1|1901294	F	W	L	F	M	T	L	C	CAT
Y36 *Pru*AA5_2 (*Pru*AlcOx)	Penicillium rubens	CAP96757	W	Y	D	Y	R	E	P	S	CBM32-CAT
Y4	Colletotrichum incanum	KZL84074	W	F	R	F	A	D	Q	N	CAT
Y40 *Fox*AlcOx	Fusarium oxysporum f. sp. conglutinans race 2 54008	FOPG_18201	W	F	D	S	K	A	Q	C	CBM32-CAT
Y41 *Fox*GalOxB	Fusarium oxysporum	FOTG_04629	W	M	Q	F	R	Q	P	C	UNK-CBM32-CAT
Y42 *Exe*GalOx	Exophiala xenobiotica	KIW55415	W	F	Q	F	R	Q	P	C	CBM32-CBM32-CAT
Y5 *Por*AlcOx	Magnaporthe oryzae	XP_003719369	F	F	G	L	Y	T	L	C	WSC-CAT
Y6	Colletotrichum zoysiae	jgi|Colzo1|587911	Y	F	G	Y	-	T	V	C	UNK-CAT
Y7	Amniculicola lignicola	KAF1999280	W	Y	D	Y	R	Q	P	N	CBM32-CBM32-CAT
Y8 *Afl*AlcOx	Aspergillus flavus	KAF7627372	W	Y	L	Y	H	E	P	S	UNK-CBM32-CAT
Y9	Didymella exigua	XP_033446128	W	F	Q	F	R	E	P	C	CBM32-CBM32-CAT
Y10	Cladonia grayi	jgi|Clagr3|10990	W	F	E	Y	R	N	V	C	CBM32-CAT

Y26	Bisporella sp.	jgi|Bissp1|110239	W	Y	Q	F	R	Q	P	N	UNK-CBM-CAT
Y27	Xanthoria parietina	jgi|Xanpa2|1578647	W	F	R	Y	R	Q	P	N	CBM32-CAT
Y28	Pseudovirgaria hyperparasitica	XP_033597585	W	F	G	V	R	E	P	N	CBM32-CBM32-CAT
Y30	Ampelomyces quisqualis	KAF1914598	Y	F	E	W	R	T	V	C	UNK-CAT
Y31	Usnea florida	jgi|Usnflo1|819633	W	F	Q	Y	R	Q	P	C	CBM32-CAT
Y32	Nectria haematococca	XP_003039318	W	F	E	Y	R	R	P	C	CBM32-CAT
Y33	Penicillium rubens	CAP97212	W	F	-	L	R	D	P	Y	CBM32-CAT
Y34	Claviceps purpurea	CCE30181	W	F	Q	Y	R	Q	P	D	CBM32-CAT
Y37	Phialophora attae	KPI34764	W	Y	K	Y	R	D	I	C	UNK-CAT

Table S2. Pairwise sequence ID/similarity matrix between characterized AA5_2 enzymes. Sequence similarity is highlighted in turquoise and sequence identity is highlighted in blue.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1. *Fox*GalOx_2 (FOTG_04629)		33.8	32.4	33.3	39.6	36.8	36.4	26.3	22.6	31.5	25.9	24.9	23.2	22.1	39.7	31.4	29.6	22.5	27.9
2. *Afl*AlcOx (KAF7627372)	46.1		48.2	50.9	43.8	38.9	49.4	36.2	32.3	45.2	35.2	36.2	33.7	31.8	51.6	42.9	28.2	31.7	35.5
3. *Pru*AA5_2A (*Pru*AlcOx) (CAP96757)	43.3	63.6		56.1	40	36.8	48.6	37.1	34.6	42.8	35.5	36.1	34.5	33.8	49	43.3	26	32.5	30.9
4. *Pfe*GalOx (jgi|Penfe1|382062)	44.8	65.9	72.1		40.6	36.6	49.6	36.4	33.7	43.1	36	36.3	35.6	32.6	47.5	45.3	26	32.1	35.2
5. *Exe*GalOx (KIW55415)	51.2	58.2	52.8	55.3		52.7	43.7	35	32.7	37.9	33.8	33.9	32.4	29.8	46.5	39.1	32	31.3	35.9
6. *Mre*GalOx (XP_033570565)	48.7	53.4	50.2	49.8	66.7		42.3	35.1	31.3	35.3	34.7	34.4	32.3	29.6	40.8	36	32.7	30.5	32.2
7. *Fox*AlcOx (FOPG_18201)	46.2	65.6	64.6	64.6	55.1	52.6		39.6	37.4	43.3	35.7	37.6	36.2	35	58.4	67.6	28.2	35.8	36
8. *Por*AlcOx (XP_003719369)	36.9	52.6	54.2	53.6	46.2	47.6	52.2		57.3	37.7	37.7	38.3	38.4	38.5	37.9	40	26	55	33.9
9. *Cgl*AlcOx (jgi|Gloci1|1901294)	31.5	46.2	49.9	49.9	42.3	41.4	50.4	68.8		31.7	36.4	36.4	46.9	44.5	33.9	36.9	23.4	79.4	29.8
10. *Asy*AlcOx (XP_040706357)	43	63.6	61.2	59.5	53.4	51	60	53.2	46.8		32.8	33.2	33.4	31.8	39.2	35.8	27.5	29.9	32.7
11. *Uma*RafOx (XP_011389156)	35.7	51.6	52.6	53.9	47.6	48.2	51.6	54.6	52.7	51.1		80.5	43.2	39.9	36.2	35.1	43.2	33.4	35.5
12. *Phu*RafOx (XP_012186969)	35.9	53.1	53.9	54.1	49.5	48.7	52.9	55.5	53	52	88.2		44.5	41	36	36.3	42.5	33.4	36.7
13. *Sto*AA5 (OAK97814)	32	46.5	50.2	48.4	42.1	43.3	49.6	53.5	65.3	46.1	57.5	58.9		62.7	34.4	36.4	27.1	41.3	36.9
14. *Cca*AA5 (PSN67470)	30.3	44.4	47.7	45.9	39.5	39.2	46.8	52.7	63.2	45.3	54.3	55.4	75.6		32.7	35.1	24.1	40.1	34
15. *Fgr*GalOx (AAO95371)	48.5	67.6	66.4	65.2	58	51.9	71.9	53.8	48.5	57.9	54.8	55.6	50.5	47.6		58	30	37.6	38.8
16. *Fgr*AAO (XP_011322138)	40.7	57.3	60.4	60.9	49.6	46.6	74.6	56.3	54.5	52.3	53.2	53.9	53.3	50.9	72.1		27.6	42.2	35.9
17. *Cgr*RafOx (EFQ36699.1)	44.3	42.1	39.8	41.1	48.6	48	40.9	39.4	34.6	41.4	52.2	52	36	34.4	40.9	37.8		25.2	32.8
18. *Cgr*AlcOx (EFQ30446)	31.6	46.2	46.9	48.1	41.5	40.3	48.5	67.5	88.4	45.3	50.2	49.8	60.8	59.1	52.1	57.2	34.7		33.4
19. *Cgr*AAO (EFQ27661.1)	40.7	54.6	51.3	54.7	51.1	49.2	52.2	50.4	44.6	50.5	53.3	54.9	48.7	47.4	54.4	51.1	47.3	47.3	

Table S3. Purification yields of expressed AA5_2 proteins from 400 mL of BMMY culture after three days of methanol feeding.

Enzyme	Purification Yield	
	mg	mg.L^{-1}
*Uma*RafOx	16	40
*Por*AlcOx	0.9	2.2
*Afl*AlcOx	17	43
*Pfe*GalOx	8.4	21
*Phu*RafOx	2.4	6.0
*Sto*AA5	2.2	5.5
*Asy*AlcOx	26	65
*Cca*AA5	3.9	9.8
*Mre*GalOx	2.0	5.0
*Cgl*AlcOx	21	53
*Pru*AlcOx	14	35
*Fox*AlcOx	2.8	7.0
*Fox*GalOxB	3.0	7.5
*Exe*GalOx	3.1	7.8

Table S4. Initial activity screens* of produced AA5_2 on galactose, raffinose and benzyl alcohol

Enzyme	Specific Activity (μmol.min^{-1}.mg^{-1})		
	Galactose (300 mM)	Raffinose (300 mM)	Benzyl Alcohol (30 mM)
*Uma*RafOx	0.19±0.01	2.78±0.27	1.11±0.07
*Por*AlcOx	0.44±0.02	0.97±0.45	4.44±0.09
*Afl*AlcOx	15.1±4.26	n.m.§	48.8±1.21
*Pfe*GalOx	41.4±4.66	27.8±0.93	11.4±2.33
*Phu*RafOx	0.04±0.02	1.89±0.45	1.00±0.09
*Asy*AlcOx	0.30±0.02	0.06±0.01	3.71±0.08
*Mre*GalOx	4.08±0.48	4.53±0.14	0.38±0.01
*Cgl*AlcOx	2.27±0.12	26.9±0.93	524 ±28.0
*Pru*AlcOx	50.1±0.63	20.2±0.89	35.5±2.72
*Fox*AlcOx	0.79±0.03	0.15±0.03	2.02±0.19
*Fox*GalOxB	68.0±0.96	50.3±4.26	6.50±0.22
*Exe*GalOx	13.8±1.04	6.52±0.15	3.45±0.27

*Measurements were performed in triplicates at 30 ˚C in 100 mM sodium-phosphate buffer at pH 8.0 using the HRP/ABTS assay. Activities were monitored using concentrations indicated within parentheses for each substrate.

§No activity detected with a specific activity limit of detection of 9 x 10^{-4} μmol.min^{-1}.mg^{-1} using 0.39 nmole of purified enzyme.

Table S5. Initial activity screens* of *Exe*GalOx and *Mre*GalOx

	Substrate	Specific Activity (μmol.min^{-1}.mg^{-1})	
		***Exe*GalOx**	***Mre*GalOx**
Carbohydrates	D-Galactose (300mM)	20.7 ±0.42	5.73 ±1.72
	D-Lactose (300mM)	6.27 ±0.45	1.93 ±0.20
	Raffinose (300mM)	9.06 ±1.45	4.28 ±1.06
	D-Mannose (300mM)	0.01 ±0.00	0.01 ±0.00
	Melibiose (300mM)	9.00 ±0.80	4.67 ±0.50
	D-Fructose (300mM)	0.08 ±0.00	n.m.§
	D-Xylose (300mM)	0.12 ±0.01	0.01 ±0.00
	D-Glucose (300mM)	0.01 ±0.00	0.01 ±0.00
	Sucrose (300mM)	n.m.§	0.02 ±0.02
	D-Ribose (300mM)	n.m.§	0.01 ±0.00
	L-Arabinose (300mM)	0.68 ±0.09	0.08 ±0.02
Polyols	Glycerol (300mM)	1.42 ±0.01	0.09 ±0.02
	Sorbitol (300mM)	0.37 ±0.00	0.01 ±0.00
	Galactitol (300mM)	0.16 ±0.00	0.01 ±0.00
	Mannitol (300mM)	NA	0.01 ±0.00
Diols	1,2-Propanediol (300mM)	0.23 ±0.03	n.m.§
	1,3-Propanediol (300mM)	0.68 ±0.03	0.01 ±0.00
	1,4-Butanediol (300mM)	0.66 ±0.01	0.01 ±0.00
	1,5-Pentanediol (300mM)	NA	n.m.§
	1,6-Hexanediol (300mM)	NA	n.m.§
Aldehyde	Methyl Glyoxal (5mM)	n.m.§	n.m.§
	Glycolaldehyde Dimer (5mM)	NA	NA
Primary alcohols	Ethanol (300mM)	0.30 ±0.01	n.m.§
	Methanol (300mM)	0.12 ±0.01	n.m.§
	1-Butanol (300mM)	0.39 ±0.02	n.m.§
	Hexanol (2.5mM)	0.38 ±0.04	0.01 ±0.00
	Octanol (2.5mM)	n.m.§	n.m.§
Aryl alcohols	Benzyl alcohol (30mM)	0.72 ±0.02	0.02 ±0.01
	Cinnamyl alcohol (2.5mM)	0.40 ±0.00	n.m.§
	4-methoxybenzyl alcohol (2.5mM)	0.40 ±0.01	0.02 ±0.02
	3-methoxybenzyl alcohol (2.5mM)	0.81 ±0.02	0.04 ±0.01
	Coniferyl alcohol (5mM)	n.m.§	n.m.§
	Veratryl alcohol (2.5mM)	1.19 ±0.02	0.05 ±0.01
	4-hydroxybenzyl alcohol (2.5mM)	0.06 ±0.01	n.m.§
	Vanillyl alcohol (2.5mM)	0.01±0.00	0.02 ±0.02
Furans	HMF (2.5mM)	0.26 ±0.05	n.m.§
	HMFCA (2.5mM)	0.31 ±0.04	n.m.§
	DFF (2.5mM)	n.m.§	n.m.§
	FFCA (2.5mM)	0.02 ±0.01	n.m.§
	Furfural (2.5mM)	NA	n.m.§

*Measurements were performed in triplicate at RT in 100 mM buffer in different buffers at different pH using the HRP/ABTS plate assay. Activities were monitored using concentrations indicated within parentheses for each substrate.

§No activity detected with a specific activity limit of detection of 9×10^{-4} μmol.min^{-1}.mg^{-1} using 0.17 nmole of purified *Exe*GalOx enzyme and of purified *Mre*GalOx enzyme.

Table S6. Initial activity screens* of *Pfe*GalOx and *Fox*GalOxB

Substrate		Specific Activity (μmol.min^{-1}.mg^{-1})	
		***Pre*GalOx**	***Fox*GalOxB**
Carbohydrates	D-Galactose (300mM)	182 ±11.6	60.7 ±5.77
	D-Lactose (300mM)	69.7 ±11.2	16.2 ±1.16
	Raffinose (300mM)	93.0 ±0.75	25.9 ±1.34
	D-Mannose (300mM)	15.4 ±1.27	0.37 ±0.09
	Melibiose (300mM)	26.7 ±11.5	59.7 ±3.46
	D-Fructose (300mM)	23.0 ±4.04	0.35 ±0.02
	D-Xylose (300mM)	114 ±22.0	0.61 ±0.06
	D-Glucose (300mM)	4.55 ±0.46	0.03 ±0.02
	Sucrose (300mM)	0.44 ±0.08	0.01 ±0.00
	D-Ribose (300mM)	45.8 ±2.40	0.85 ±0.09
	L-Arabinose (300mM)	9.02 ±0.49	2.13 ±0.07
Polyols	Glycerol (300mM)	193 ±44.9	5.07 ±0.22
	Sorbitol (300mM)	28.0 ±2.36	2.63 ±0.07
	Galactitol (300mM)	5.29 ±0.30	1.04 ±0.06
	Mannitol (300mM)	19.6 ±1.91	NA
Diols	1,2-Propanediol (300mM)	11.4 ±1.29	2.44 ±0.22
	1,3-Propanediol (300mM)	6.21 ±1.76	4.88 ±0.42
	1,4-Butanediol (300mM)	1.33 ±0.18	5.57 ±0.37
	1,5-Pentanediol (300mM)	0.66 ±0.03	4.83 ±0.15
	1,6-Hexanediol (300mM)	4.28 ±0.44	3.68 ±0.23
Aldehyde	Methyl Glyoxal (5mM)	0.43 ±0.14	0.61 ±0.02
Primary alcohols	Ethanol (300mM)	0.43 ±0.14	3.76 ±0.57
	Methanol (300mM)	1.25 ±0.02	0.89 ±0.05
	1-Butanol (300mM)	0.91 ±0.07	3.80 ±0.10
	Hexanol (2.5mM)	0.24 ±0.07	2.24 ±0.31
	Octanol (2.5mM)	0.44 ±0.17	2.00 ±0.11
Aryl alcohols	Benzyl alcohol (30mM)	20.3 ±1.59	5.84 ±0.20
	Cinnamyl alcohol (2.5mM)	6.76 ±1.02	3.60 ±0.43
	4-methoxybenzyl alcohol (2.5mM)	2.39 ±0.21	1.78 ±0.71
	3-methoxybenzyl alcohol (2.5mM)	3.76 ±0.15	6.66 ±0.30
	Coniferyl alcohol (5mM)	0.15 ±0.08	n.m.§
	Veratryl alcohol (2.5mM)	2.34 ±0.21	10.2 ±0.21
	4-hydroxybenzyl alcohol (2.5mM)	0.05 ±0.01	0.10 ±0.12
	Vanillyl alcohol (2.5mM)	0.01 ±0.00	n.m.§
Furans	HMF (2.5mM)	1.51 ±0.12	3.34 ±0.20
	HMFCA (2.5mM)	1.31 ±0.19	3.31 ±0.32
	DFF (2.5mM)	0.43 ±0.18	0.52 ±0.03
	FFCA (2.5mM)	0.30 ±0.11	0.02 ±0.00
	Furfural (2.5mM)	0.17 ±0.03	n.m.§

*Measurements were performed in triplicate at RT in 100 mM buffer in different buffers at different pH using the HRP/ABTS plate assay. Activities were monitored using concentrations indicated within parentheses for each substrate.

§No activity detected with a specific activity limit of detection of 9 x 10^{-4} μmol.min^{-1}.mg^{-1} using 0.2 nmole of purified *Pfe*GalOx enzyme and 0.13 nmole of purified *Fox*GalOxB enzyme.

Table S7. Initial activity screens* of *Uma*RafOx and *Phu*RafOx

	Substrate	Specific Activity (µmol.min^{-1}.mg^{-1})	
		***Uma*RafOx**	***Phu*RafOx**
Carbohydrates	D-Galactose (300mM)	0.17 ±0.01	0.24 ±0.02
	D-Lactose (300mM)	0.04 ±0.01	0.08 ±0.02
	Raffinose (300mM)	1.77 ±0.56	2.21 ±0.13
	D-Mannose (300mM)	0.01 ±0.00	n.m.§
	Melibiose (300mM)	0.67 ±0.06	0.41 ±0.03
	D-Fructose (300mM)	0.04 ±0.01	0.05 ±0.01
	D-Xylose (300mM)	0.01 ±0.00	n.m.§
	D-Glucose (300mM)	n.m.§	n.m.§
	Sucrose (300mM)	n.m.§	n.m.§
	D-Ribose (300mM)	0.02 ±0.00	n.m.§
	L-Arabinose (300mM)	0.08 ±0.01	0.12 ±0.02
Polyols	Glycerol (300mM)	3.22 ±0.15	5.41 ±0.58
	Sorbitol (300mM)	0.23 ±0.02	0.42 ±0.05
	Galactitol (300mM)	0.01 ±0.00	n.m.§
	Mannitol (300mM)	0.07 ±0.01	0.11 ±0.01
Diols	1,2-Propanediol (300mM)	0.28 ±0.05	0.37±0.06
	1,3-Propanediol (300mM)	0.47 ±0.17	1.23 ±0.08
	1,4-Butanediol (300mM)	0.32 ±0.11	0.23 ±0.09
	1,5-Pentanediol (300mM)	0.02 ±0.00	0.01 ±0.00
	1,6-Hexanediol (300mM)	0.02 ±0.00	0.01 ±0.00
Aldehyde	Methyl Glyoxal (5mM)	0.03 ±0.05	0.01 ±0.00
	Glycolaldehyde (5mM)	1.40 ±0.26	1.03 ±0.30
Primary alcohols	Ethanol (300mM)	0.01 ±0.00	0.02 ±0.01
	Methanol (300mM)	0.02 ±0.00	0.03 ±0.00
	1-Butanol (300mM)	0.01 ±0.00	0.01 ±0.00
	Hexanol (2.5mM)	0.01 ±0.00	n.m.§
	Octanol (2.5mM)	n.m.§	0.02 ±0.01
Aryl alcohols	Benzyl alcohol (30mM)	0.46 ±0.16	0.40 ±0.04
	Cinnamyl alcohol (2.5mM)	0.23 ±0.05	0.39 ±0.04
	4-methoxybenzyl alcohol (2.5mM)	0.04 ±0.01	0.06 ±0.04
	3-methoxybenzyl alcohol (2.5mM)	0.15 ±0.06	0.23 ±0.02
	Coniferyl alcohol (5mM)	0.01 ±0.00	0.01 ±0.01
	Veratryl alcohol (2.5mM)	0.13 ±0.00	0.14 ±0.03
	4-hydroxybenzyl alcohol (2.5mM)	0.02 ±0.01	0.02 ±0.02
	Vanillyl alcohol (2.5mM)	n.m.§	0.03 ±0.01
Furans	HMF (2.5mM)	0.12 ±0.02	0.03 ±0.01
	HMFCA (2.5mM)	0.07 ±0.01	0.01 ±0.01
	DFF (2.5mM)	0.01 ±0.01	0.04 ±0.00
	FFCA (2.5mM)	n.m.§	n.m.§
	Furfural (2.5mM)	n.m.§	0.05 ±0.03

*Measurements were performed in triplicate at RT in 100 mM buffer in different buffers at different pH using the HRP/ABTS plate assay. Activities were monitored using concentrations indicated within parentheses for each substrate.

§No activity detected with a specific activity limit of detection of 9×10^{-4} μmol.min^{-1}.mg^{-1} using 1.12 nmole of purified *Uma*RafOx enzyme and 0.23 nmole of purified *Phu*RafOx enzyme.

Table S8. Initial activity screens* of *Afl*AlcOx and *Pru*AlcOx

Substrate		Specific Activity (μmol.min^{-1}.mg^{-1})	
		***Afl*AlcOx**	***Pru*AlcOx**
Carbohydrates	D-Galactose (300mM)	65.0 ±1.41	79.9 ±29.4
	D-Lactose (300mM)	7.07 ±0.37	12.2 ±2.12
	Raffinose (300mM)	12.0 ±1.36	13.5 ±3.73
	D-Mannose (300mM)	0.14 ±0.09	2.87 ±0.76
	Melibiose (300mM)	17.4 ±1.39	8.41 ±2.32
	D-Fructose (300mM)	2.64 ±0.01	9.10 ±3.36
	D-Xylose (300mM)	3.34 ±1.63	3.70 ±1.05
	D-Glucose (300mM)	0.07 ±0.01	0.29 ±0.04
	Sucrose (300mM)	0.28 ±0.02	n.m.§
	D-Ribose (300mM)	0.45 ±0.12	4.67 ±1.78
	L-Arabinose (300mM)	1.95 ±0.55	3.20 ±0.13
Polyols	Glycerol (300mM)	35.7 ±1.50	119 ±12.7
	Sorbitol (300mM)	1.46 ±0.09	9.50 ±2.18
	Galactitol (300mM)	3.10 ±0.04	3.44 ±0.36
Diols	1,2-Propanediol (300mM)	14.5 ±0.21	38.3 ±2.38
	1,3-Propanediol (300mM)	9.51 ±0.24	34.8 ±2.71
	1,4-Butanediol (300mM)	30.9 ±6.72	18.8 ±2.43
	1,5-Pentanediol (300mM)	25.0 ±0.67	17.8 ±1.04
	1,6-Hexanediol (300mM)	25.0 ±0.25	14.0 ±3.01
Aldehyde	Methyl Glyoxal (5mM)	2.47 ±0.26	4.57 ±1.92
Primary alcohols	Ethanol (300mM)	1.51 ±0.09	3.50 ±0.21
	Methanol (300mM)	1.22 ±0.04	2.54 ±0.46
	1-Butanol (300mM)	25.0 ±2.27	12.9 ±2.08
	Hexanol (2.5mM)	0.46 ±0.11	0.95 ±0.22
	Octanol (2.5mM)	n.m.§	0.07 ±0.02
Aryl alcohols	Benzyl alcohol (30mM)	248 ±16.6	61.5 ±5.43
	Cinnamyl alcohol (2.5mM)	22.4 ±1.02	26.2 ±2.57
	4-methoxybenzyl alcohol (2.5mM)	12.1 ±0.53	8.56 ±1.30
	3-methoxybenzyl alcohol (2.5mM)	45.5 ±2.82	23.9 ±2.85
	Coniferyl alcohol (5mM)	n.m.§	4.57 ±1.92
	Veratryl alcohol (2.5mM)	9.80 ±0.32	2.61 ±0.38
	4-hydroxybenzyl alcohol (2.5mM)	2.48 ±1.16	2.51 ±3.70
	Vanillyl alcohol (2.5mM)	n.m.§	n.m.§
Furans	HMF (2.5mM)	52.8 ±3.92	28.7 ±11.2
	HMFCA (2.5mM)	8.58 ±0.89	21.7 ±2.17
	DFF (2.5mM)	0.16 ±0.01	0.46 ±0.03
	FFCA (2.5mM)	n.m.§	0.03 ±0.02
	Furfural (2.5mM)	NA	NA

*Measurements were performed in triplicate at RT in 100 mM buffer in different buffers at different pH using the HRP/ABTS plate assay. Activities were monitored using concentrations indicated within parentheses for each substrate.

§No activity detected with a specific activity limit of detection of 9 x 10^{-4} μmol.min^{-1}.mg^{-1} using 0.2 nmole of purified *Afl*AlcOx enzyme and 1.0 nmole of purified *Pru*AlcOx enzyme.

Table S9. Initial activity screens* of *Fox*AlcOx and *Asy*AlcOx

Substrate		Specific Activity (µmol.min^{-1}.mg^{-1})	
		***Fox*AlcOx**	***Asy*AlcOx**
Carbohydrates	D-Galactose (300mM)	0.83 ±0.22	0.33 ±0.11
	D-Lactose (300mM)	0.11 ±0.01	0.25 ±0.12
	Raffinose (300mM)	0.08 ±0.02	0.02 ±0.03
	D-Mannose (300mM)	0.26 ±0.03	0.01 ±0.01
	Melibiose (300mM)	0.03 ±0.01	n.m.§
	D-Fructose (300mM)	0.35 ±0.07	0.76 ±0.29
	D-Xylose (300mM)	n.m.§	n.m.§
	D-Glucose (300mM)	0.05 ±0.01	0.01 ±0.01
	Sucrose (300mM)	0.02 ±0.00	n.m.§
	D-Ribose (300mM)	n.m.§	n.m.§
	L-Arabinose (300mM)	0.01 ±0.00	n.m.§
Polyols	Glycerol (300mM)	0.15 ±0.03	5.47 ±2.24
	Sorbitol (300mM)	0.01 ±0.00	4.56 ±0.29
	Galactitol (300mM)	0.01 ±0.00	0.76 ±0.02
	Mannitol (300mM)	0.01 ±0.00	3.66 ±0.56
Diols	1,2-Propanediol (300mM)	0.05 ±0.00	1.04 ±0.16
	1,3-Propanediol (300mM)	0.06 ±0.01	1.83 ±0.42
	1,4-Butanediol (300mM)	0.16 ±0.08	1.60 ±0.44
	1,5-Pentanediol (300mM)	0.06 ±0.00	3.98 ±1.22
	1,6-Hexanediol (300mM)	0.03 ±0.00	7.41 ±1.15
Aldehyde	Methyl Glyoxal (5mM)	0.81 ±0.04	0.09 ±0.16
	Glycolaldehyde Dimer (5mM)	0.69 ±0.27	NA
Primary alcohols	Ethanol (300mM)	0.02 ±0.00	0.44 ±0.10
	Methanol (300mM)	0.01 ±0.00	0.01 ±0.01
	1-Butanol (300mM)	0.02 ±0.00	1.76 ±0.32
	Hexanol (2.5mM)	0.01 ±0.00	0.12 ±0.11
	Octanol (2.5mM)	n.m.§	n.m.§
Aryl alcohols	Benzyl alcohol (30mM)	1.85 ±0.37	11.6 ±5.83
	Cinnamyl alcohol (2.5mM)	0.10 ±0.06	3.24 ±0.73
	4-methoxybenzyl alcohol (2.5mM)	0.25 ±0.03	3.13 ±1.18
	3-methoxybenzyl alcohol (2.5mM)	0.10 ±0.02	4.20 ±0.37
	Coniferyl alcohol (5mM)	n.m.§	n.m.§
	Veratryl alcohol (2.5mM)	0.21 ±0.01	1.22 ±0.19
	4-hydroxybenzyl alcohol (2.5mM)	0.02 ±0.00	n.m.§
	Vanillyl alcohol (2.5mM)	n.m.§	n.m.§
Furans	HMF (2.5mM)	0.09 ±0.00	0.45 ±0.04
	HMFCA (2.5mM)	0.09 ±0.01	0.27 ±0.03
	DFF (2.5mM)	0.05 ±0.01	n.m.§
	FFCA (2.5mM)	0.01 ±0.00	n.m.§
	Furfural (2.5mM)	n.m.§	n.m.§

*Measurements were performed in triplicate at RT in 100 mM buffer in different buffers at different pH using the HRP/ABTS plate assay. Activities were monitored using concentrations indicated within parentheses for each substrate.

§No activity detected with a specific activity limit of detection of 9×10^{-4} μmol.min^{-1}.mg^{-1} using 0.20 nmole of purified *Fox*AlcOx enzyme and 0.20 nmole of purified *Asy*AlcOx enzyme.

Table S10. Initial activity screens* of *Por*AlcOx and *Cgl*AlcOx

Substrate		Specific Activity (μmol.min^{-1}.mg^{-1})	
		***Por*AlcOx**	***Cgl*AlcOx**
Carbohydrates	D-Galactose (300mM)	1.71 ±0.89	0.82 ±0.04
	D-Lactose (300mM)	1.16 ±0.90	0.40 ±0.02
	Raffinose (300mM)	3.00 ±0.65	61.21 ±7.27
	D-Mannose (300mM)	2.95 ±0.99	n.m.§
	Melibiose (300mM)	0.25 ±0.18	1.18 ±0.09
	D-Fructose (300mM)	3.14 ±0.90	6.15 ±1.78
	D-Xylose (300mM)	1.03 ±0.52	0.68 ±0.04
	D-Glucose (300mM)	3.04 ±0.15	0.44 ±0.03
	Sucrose (300mM)	0.29 ±0.36	0.81 ±0.04
	D-Ribose (300mM)	0.66 ±0.42	1.37 ±0.06
	L-Arabinose (300mM)	0.52 ±0.17	0.61 ±0.03
Polyols	Glycerol (300mM)	9.76 ±1.27	318 ±17.3
	Sorbitol (300mM)	10.2 ±1.38	207 ±9.06
	Galactitol (300mM)	3.62 ±0.84	80.9 ±1.86
	Mannitol (300mM)	2.69 ±0.55	NA
Diols	1,2-Propanediol (300mM)	11.2 ±1.69	393 ±11.7
	1,3-Propanediol (300mM)	11.8 ±0.74	576 ±31.0
	1,4-Butanediol (300mM)	12.0 ±0.18	698 ±42.3
	1,5-Pentanediol (300mM)	10.9 ±0.58	705 ±135
	1,6-Hexanediol (300mM)	9.94 ±0.70	657 ±71.4
Aldehyde	Methyl Glyoxal (5mM)	2.45 ±1.45	n.m.§
Primary alcohols	Ethanol (300mM)	5.87 ±0.80	496 ±32.0
	Methanol (300mM)	2.07 ±0.90	78.6 ±3.50
	1-Butanol (300mM)	6.32 ±0.82	532 ±17.6
	Hexanol (2.5mM)	4.06 ±0.87	428 ±4.66
	Octanol (2.5mM)	3.67 ±1.48	180 ±10.6
Aryl alcohols	Benzyl alcohol (30mM)	11.5 ±0.81	569 ±31.1
	Cinnamyl alcohol (2.5mM)	5.75 ±1.58	541 ±15.6
	4-methoxybenzyl alcohol (2.5mM)	6.06 ±0.80	319 ±17.9
	3-methoxybenzyl alcohol (2.5mM)	7.46 ±0.49	402 ±0.00
	Coniferyl alcohol (5mM)	n.m.§	n.m.§
	Veratryl alcohol (2.5mM)	4.49 ±0.43	160 ±11.6
	4-hydroxybenzyl alcohol (2.5mM)	1.00 ±0.15	10.2 ±1.76
	Vanillyl alcohol (2.5mM)	n.m.§	n.m.§
Furans	HMF (2.5mM)	6.81 ±1.17	690 ±18.6
	HMFCA (2.5mM)	10.2 ±0.92	272 ±29.0
	DFF (2.5mM)	1.20 ±0.12	n.m.§
	FFCA (2.5mM)	1.14 ±0.09	n.m.§
	Furfural (2.5mM)	n.m.§	NA

*Measurements were performed in triplicate at 25 ˚C in 100 mM buffer in different buffers at different pH using the HRP/ABTS assay. Activities were monitored using concentrations indicated within parentheses for each substrate.

§No activity detected with a specific activity limit of detection of 9×10^{-4} μmol.min^{-1}.mg^{-1} using 0.23 nmole of purified *Por*AlcOx enzyme and 0.03 nmole of purified *Cgl*AlcOx enzyme.

Table S11. Comparison of catalytic parameters of *Fox*AlcOx, *AflAlcOx, Pru*AAA5_2A (*Pru*AlcOx), *PfeGalOx, ExeGalOx, Mre*GalOx *and Fox*GalOxB with other enzymes acting on galactose*

Enzyme	*Galactose*			References
	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}.s^{-1}$)	
*Fox*GalOxB	24 ±1.3	81 ±1.0	**3400**	This work
*Exe*GalOx	25 ±4.0	130 ±3.7	**5200**	This work
*Mre*GalOx	64 ±4.8	32 ±0.7	**500**	This work
*Afl*AlcOx	980 ± 61	400 ±10	**410**	This work
*Pru*AlcOx	110 ±18	220 ±8.6	**2000**	This work
*Pfe*GalOx	28 ±6.7	140 ±4.5	**5000**	This work
*Fox*AlcOx	2000 ±650	8.3 ±1.7	**4.1**	This work
*Fgr*GalOx	102 ±6.4	1059 ±18.9	**10400 ±680**	[1]
R330K	895 ±85.9	208 ±10.8	**232 ±25**	[1]
M_1	43 ±2	1376 ±35	**32 000 ±1700**	[2]
M_3	54 ±7	1800 ±400	**31 ±7**	[3]
C383N	390 ±38	410 ±17	**1100 ±150**	[4]
C383S	34 ±3.6	1100 ±30	**32000 ±4500**	[4]
*Cgr*AAO	ND	ND	**13.1 ±0.8**	[5]
*Fgr*AAO	1700 ± 150	21 ± 1.0	**12**	[6]
*Fox*AAO	1600 ± 150	23 ± 1.2	**14**	[6]

* ND not determined

Table S12. Comparison of catalytic parameters of *Por*AlcOx, *Cgl*AlcOx, *Fox*AlcOx, *Afl*AlcOx, *Pru*AA5_2A (*Pru*AlcOx), *Pfe*GalOx, *Uma*RafOx, *Phu*RafOx, *Asy*AlcOx with other enzymes acting on benzyl alcohol, glycerol, butanol and 1,4-butanediol*

Enzyme	*Benzyl alcohol*			*Glycerol*			References
	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}.s^{-1}$)	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}.s^{-1}$)	
*Por*AlcOx	8.6 ±0.15	1.3 ± 0.10	**6600**	8.5 ±0.32	57 ± 8.1	**1500**	This work
*Cgl*AlcOx	350 ±20	3.6 ±0.8	**97000**	NA	NA	**NA**	This work
*Fox*AlcOx	230 ±70	24 ±4.5	**100**	NA	NA	**NA**	This work
*Afl*AlcOx	410 ±11	19 ± 1.9	**21000**	NA	NA	**NA**	This work
*Pru*AlcOx	110 ±5.0	9.2 ±1.8	**12000**	170 ±3.0	34 ±1.7	**4900**	This work
*Pfe*GalOx	NA	NA	**NA**	140 ±1.1	69 ±1.6	**2000**	This work
*Uma*RafOx	NA	NA	**NA**	2900 ±720	130 ±24	**45**	This work
*Phu*RafOx	NA	NA	**NA**	2900 ±390	56 ±3.8	**19**	This work
*Asy*AlcOx	79 ±22	17 ±1.8	**220**	700 ±80	38 ±2.0	**54**	This work
*Fgr*GalOx	ND	ND	**424 ±2**	NA	NA	**NA**	[7]
*Cgr*AlcOx	0.69 ±0.04	94 ±1	**140000**	104 ± 4	96 ± 2	**920**	[8]
*Cgr*AAO	27 ±0.9	54.5 ±0.6	**2020 ± 70**	605 ± 34	58.7 ± 0.9	**97 ± 5.6**	[5]
*Fgr*AAO	86 ± 12	37 ± 2.8	**430**	2200 ± 360	17 ± 1.2	**7.7**	[6]
*Fox*AAO	30 ± 19	31 ± 4.2	**1000**	2700 ± 403	24 ± 1.6	**8.8**	[6]
Enzyme	***Butanol***			***1,4-Butanediol***			**References**
	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}.s^{-1}$)	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}.s^{-1}$)	
*Por*AlcOx	7.9 ±0.09	4.6 ± 0.30	**1700**	9.5 ±0.51	4.3 ± 0.56	**2200**	This work
*Cgl*AlcOx	NA	NA	**NA**	330 ±13	3.9 ±0.4	**85000**	This work
*Cgr*AAO	0.68 ± 0.04	96 ± 1	**140000**	1.2 ± 0.04	97 ± 1	**83000**	[8]

* NA not assessed; ND not determined

Table S13. Comparison of catalytic parameters of *Cgl*AlcOx *and Afl*AlcOx with other enzymes acting on HMF

Enzyme	*HMF*			References
	K_M (mM)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1}.s^{-1}$)	
*Cgl*AlcOx	0.09 ±0.01	240 ±16	**2700000**	This work
*Afl*AlcOx	190 ± 20	5.0 ± 1.3	**38000**	This work
*Cgr*AAO	6.5 ±0.3	126 ± 1.5	**19400 ± 90**	[5]
*Fgr*AAO	14 ± 2.6	29 ± 1.7	**2100**	[6]
*Fox*AAO	17 ± 4.9	26 ± 2.6	**1500**	[6]
Bacterial HMFO	1.4	9.9	**7100**	[9]
*Per*AAO	1.6 ± 0.2	0.33 ± 0.01	**220 ±42**	[10]
*Mt*GLOX	20.2 ± 9.0	15.9	**982**	[11]
*Pci*GLOX1	15.66 ± 2.35	1.59 ± 0.12	**101.66 ± 0.01**	[12]
*Pci*GLOX2	5.87 ± 2.04	0.56 ± 0.09	**96.04 ±0.01**	[12]
*Pci*GLOX3	6.35 ± 1.32	0.75 ± 0.07	**118.35 ± 0.01**	[12]

Supplementary Figures

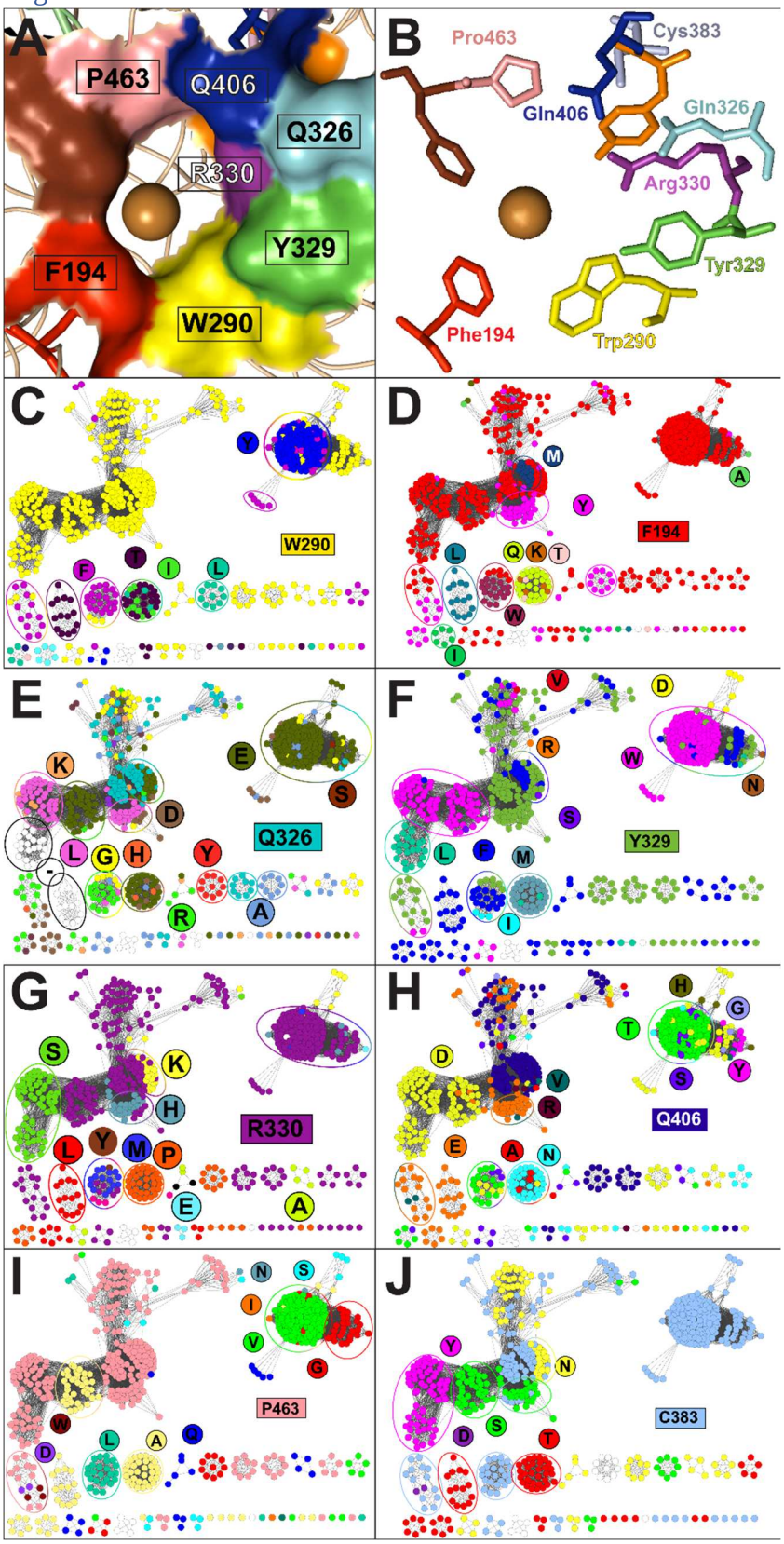


Figure S1: (A-B) Active site residues of *Fgr*GalOx involved in catalysis and/or substrate binding investigated in this study. (A) Surface representation (B) Sticks representation (C-J) Sequence similarity networks at an alignment score cut-off of 10^{-550} of 623 catalytic modules from the AA5_2 subfamily with their corresponding amino acid variability for a specific position in *Fgr*GalOx. For each panel, predicted native signal peptides and additional N-terminal modules have been removed. Each node is colored according to its variability assessed by inspecting the multiple sequence alignment used to generate the phylogenetic tree and the SSNs in Figure 1.

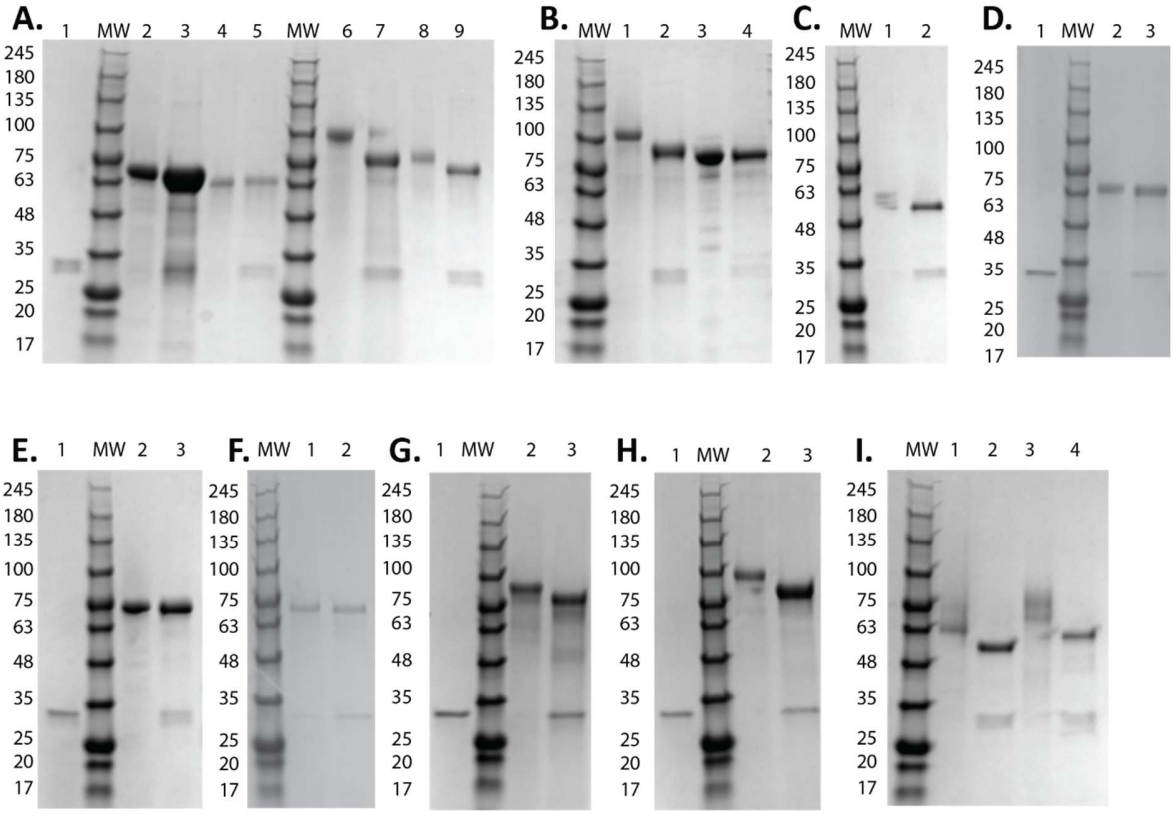


Figure S2. SDS-PAGE of recombinantly produced enzyme in *P. pastoris* and N-deglycosilation study. Aliquot of purified enzymes were N-glycosylated under denaturing conditions with pNGaseF. A. 1: pNGase, 2: native *Uma*RafOx (2.5 µg), 3: *Uma*RafOx (2.5µg) + pNGase, 4: native *Phu*RafOx, 5: *Phu*RafOx (2.5µg) + pNGase, 6: native *Alf*AlcOx (2.5 µg), 7: *Alf*AlcOx (2.5 µg) + pNGase, 8: native *Pru*AA5_2A (*Pru*AlcOx) (2.5 µg), 9: *Pru*AA5_2A (*Pru*AlcOx) (2.5 µg) + pNGase. B. 1: native *Exe*GalOx (2.5 µg), 2: *Exe*GalOx (2.5 µg) + pNGase, 3: native *Mre*GalOx (2.5 µg), 4: *Mre*GalOx (2.5 µg) + pNGase. C. 1: native *Cgl*AlcOx (2.5 µg), 2: *Cgl*AlcOx (2.5 µg) + pNGase. D. 1: pNGaseF, 2: native *Por*AlcOx (2.5 µg), 3: *Por*AlcOx (2.5 µg) + pNGase. E. 1: pNGase, 2: native *Fox*AlcOx (2.5 µg), 3: *Fox*AlcOx (2.5 µg) + pNGase. F. 1: native *Fox*GalOxB (2.5 µg), 2: *Fox*GalOxB (2.5 µg) + pNGase. G. 1: pNGase, 2: native *Pfe*GalOx (2.5 µg), 3: *Pfe*GalOx (2.5 µg) + pNGase, H. 1: pNGase, 2: native *Asy*AlcOx (2.5 µg), 3: *Asy*AlcOx (2.5 µg) + pNGase. I. 1: native *Sto*AA5 (2.5 µg), 2: *Sto*AA5 (2.5 µg) + pNGAse, 3: native *Cca*AA5 (2.5 µg), 4: *Cca*AA5 (2.5 µg) + pNGase. All gels were stained by Coomassie blue. MW = molecular weight markers, as indicated.

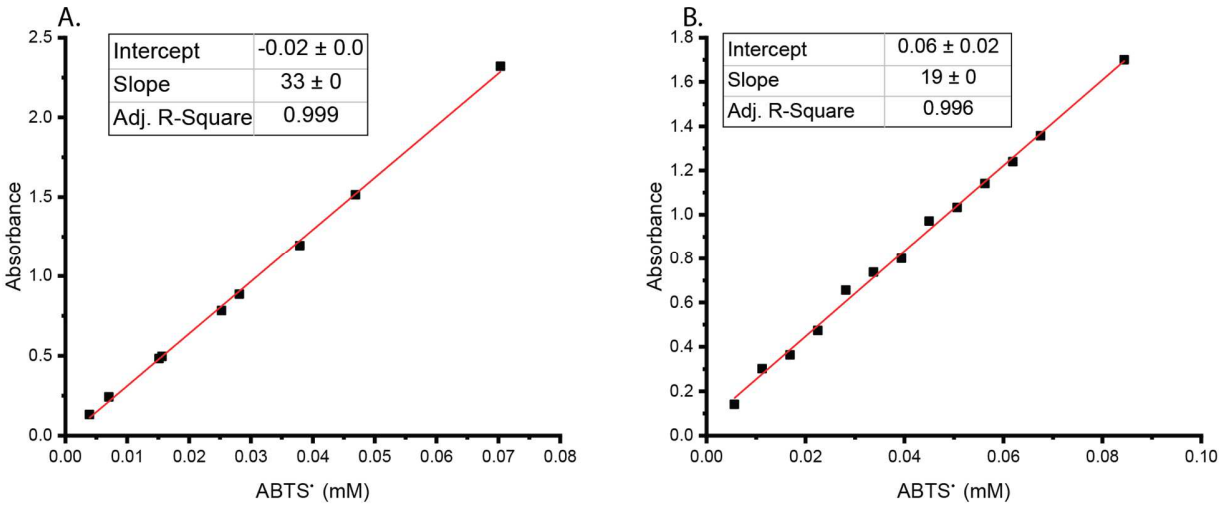


Figure S3. ABTS radical standard curve. A. Carry UV-VIS. B. BioTek Epoch microplate spectrophotometer.

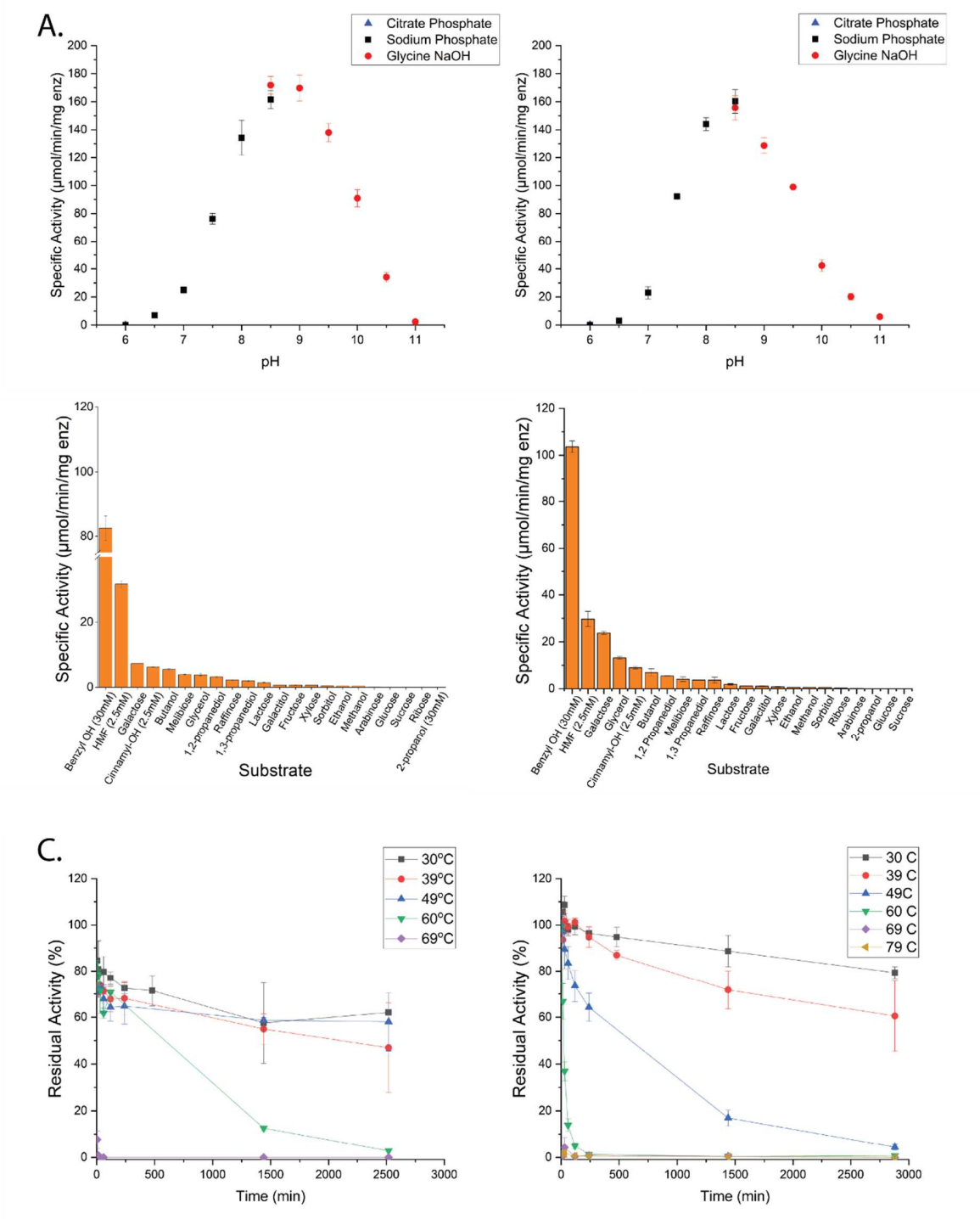
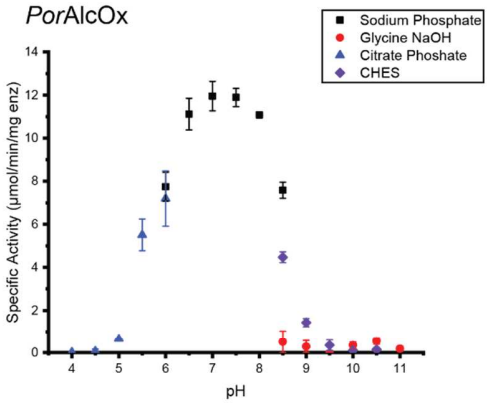
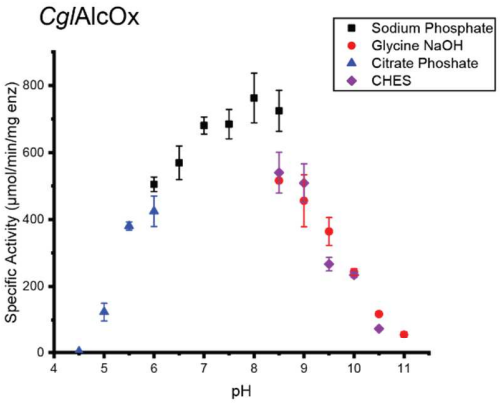
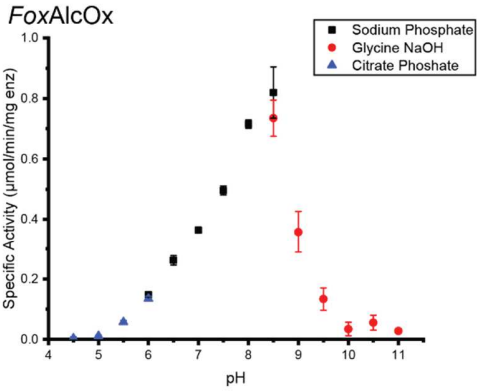
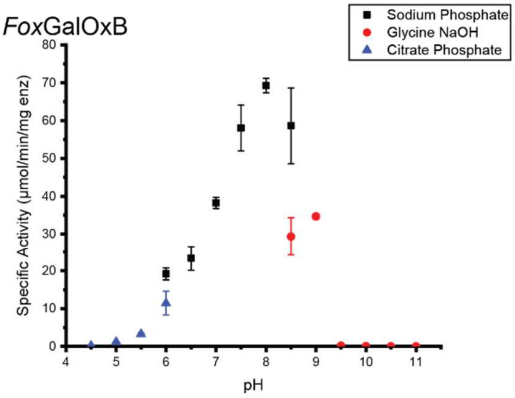


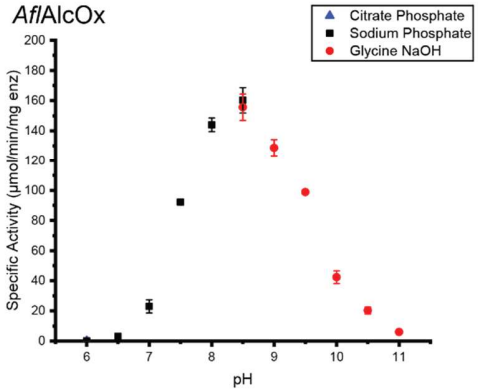
Figure S4. Proof-of-concept experiment for medium through-put HRP-ABST plate assays using *Afl*AlcOx. A. pH profiles. B. Substrate activity screens. C. Temperature stability assay. Left panels correspond to experiments performed using Cary 60 UV-VIS spectrometer while the right panel corresponds to the experiments performed using the BioTek Epoch microplate spectrophotometer.

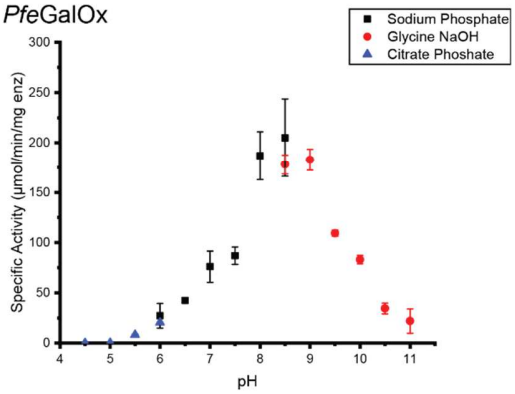

PorAlcOx
Sodium Phosphate
Glycine NaOH
Citrate Phoshate
CHES
Specific Activity (µmol/min/mg enz)
pH

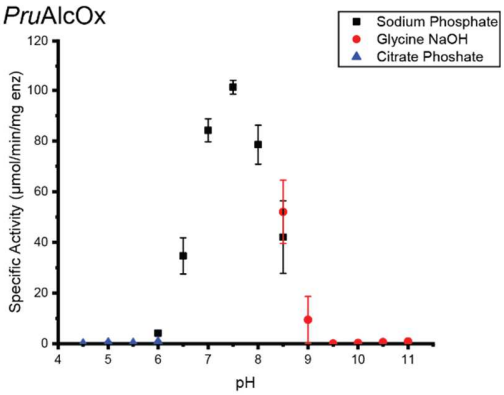

CglAlcOx
Sodium Phosphate
Glycine NaOH
Citrate Phoshate
CHES
Specific Activity (µmol/min/mg enz)
pH

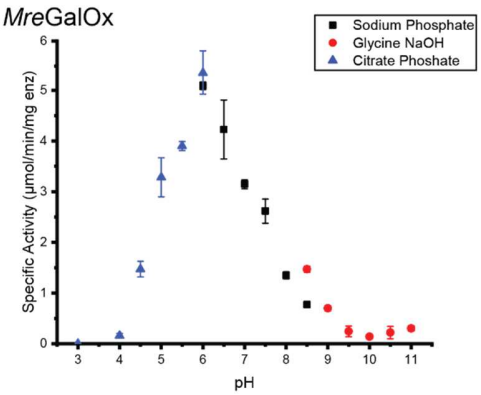

FoxAlcOx
Sodium Phosphate
Glycine NaOH
Citrate Phoshate
Specific Activity (µmol/min/mg enz)
pH


FoxGalOxB
Sodium Phosphate
Glycine NaOH
Citrate Phosphate
Specific Activity (µmol/min/mg enz)
pH


AflAlcOx
Citrate Phosphate
Sodium Phosphate
Glycine NaOH
Specific Activity (µmol/min/mg enz)
pH


PfeGalOx
Sodium Phosphate
Glycine NaOH
Citrate Phoshate
Specific Activity (µmol/min/mg enz)
pH


PruAlcOx
Sodium Phosphate
Glycine NaOH
Citrate Phoshate
Specific Activity (µmol/min/mg enz)
pH


MreGalOx
Sodium Phosphate
Glycine NaOH
Citrate Phoshate
Specific Activity (µmol/min/mg enz)
pH

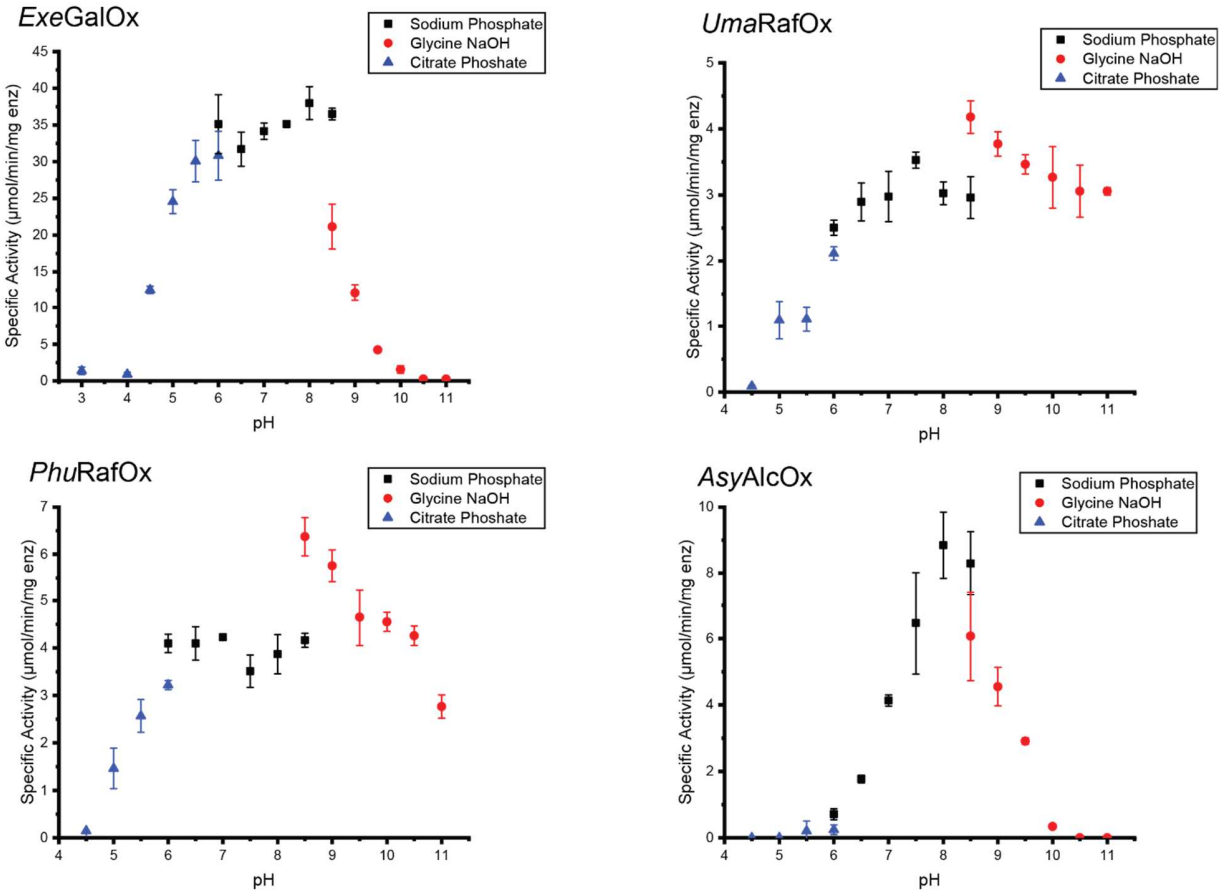
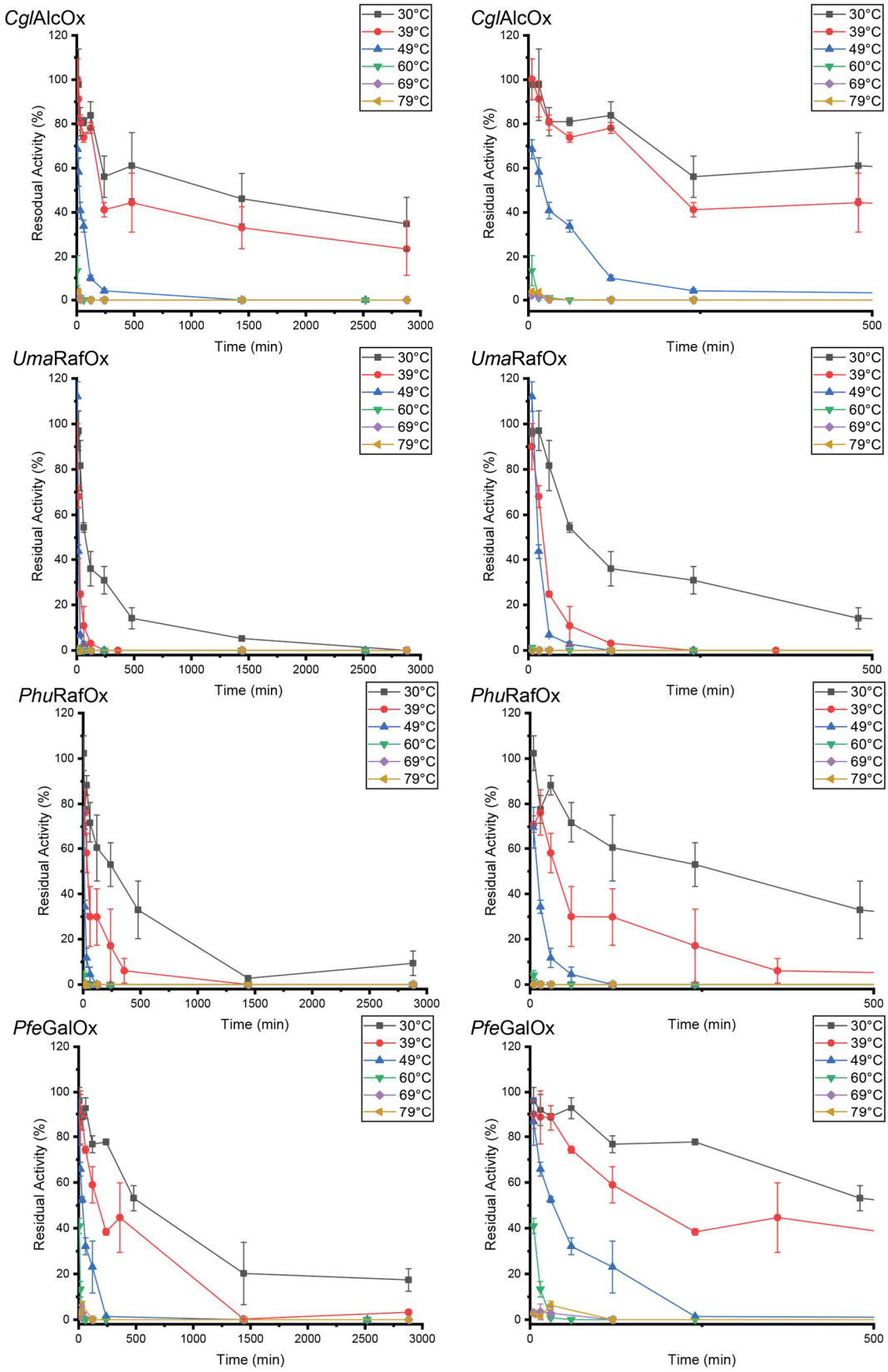
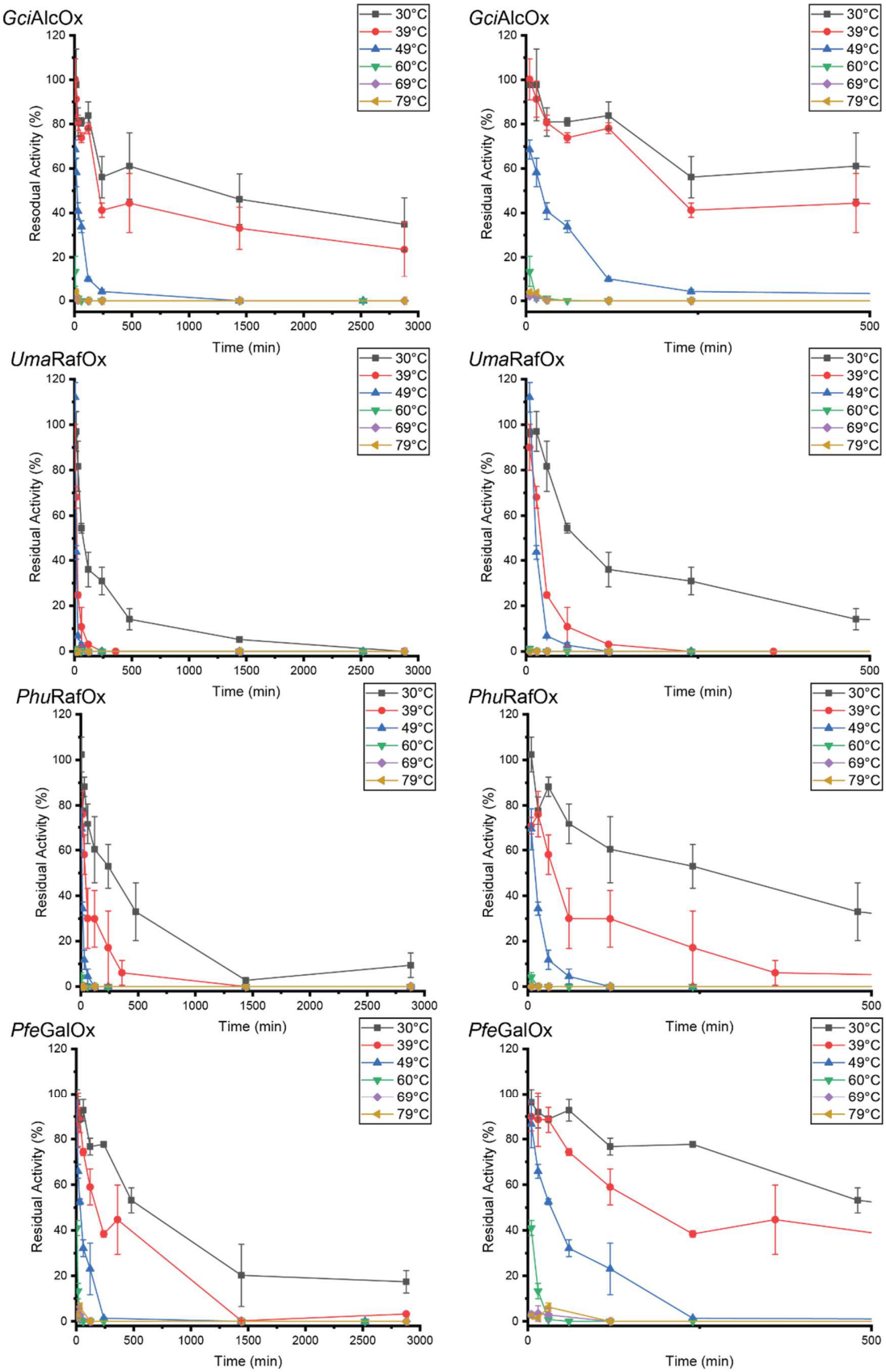


Figure S5. pH-rate profiles. pH-rate profiles were determined using the coupled HRP-ABTS assay with either 300 mM galactose, 30 mM benzyl alcohol, and 300 mM glycerol as the substrate at each pH value. Black squares for phosphate-citrate buffer, red circles for sodium phosphate buffer and blue triangles for glycine-NaOH buffer. The enzyme under study is annotated in each graph. Error bars represent standard deviations over three replicates.


CglAlcOx
Resodual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C
CglAlcOx
Residual Activity (%)
Time (min)
UmaRafOx
Residual Activity (%)
Time (min)
UmaRafOx
Residual Activity (%)
Time (min)
PhuRafOx
Residual Activity (%)
Time (min)
PhuRafOx
Residual Activity (%)
Time (min)
PfeGalOx
Residual Activity (%)
Time (min)
PfeGalOx
Residual Activity (%)
Time (min)


GciAlcOx
Residual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C
GciAlcOx
Residual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C
UmaRafOx
Residual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C
UmaRafOx
Residual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C
PhuRafOx
Residual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C
PhuRafOx
Residual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C
PfeGalOx
Residual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C
PfeGalOx
Residual Activity (%)
Time (min)
30°C
39°C
49°C
60°C
69°C
79°C

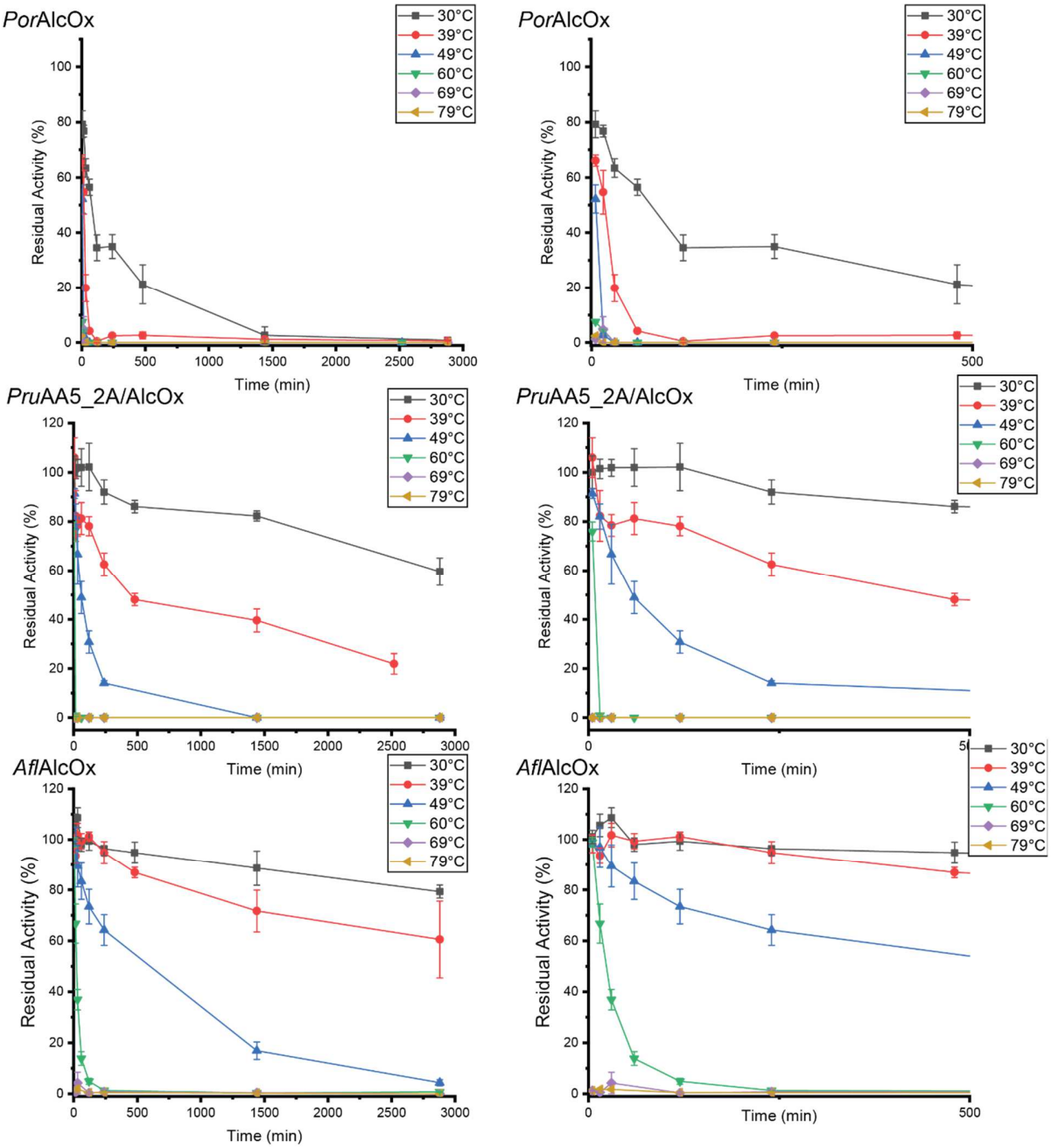


Figure S6. Temperature stability. Activity values were determined using the coupled HRP-ABTS assay with either 300 mM galactose, 30 mM benzyl alcohol and 300 mM glycerol as the substrate. The enzyme was pre-incubated at each temperature, maintained by a gradient thermocycler: 30 °C (black square), 39 °C (red circle), 49 °C (blue triangle), 60 °C (green triangle), 69 °C (purple diamond) and 79 °C (yellow side triangle). The reactions were performed at RT. The enzyme under study is annotated in each graph. Error bars represent standard deviations over duplicate measurements. Graphs in the left column contain the full data over 48 hours while on the right column is a zoomed in graph showing the first 500 min.

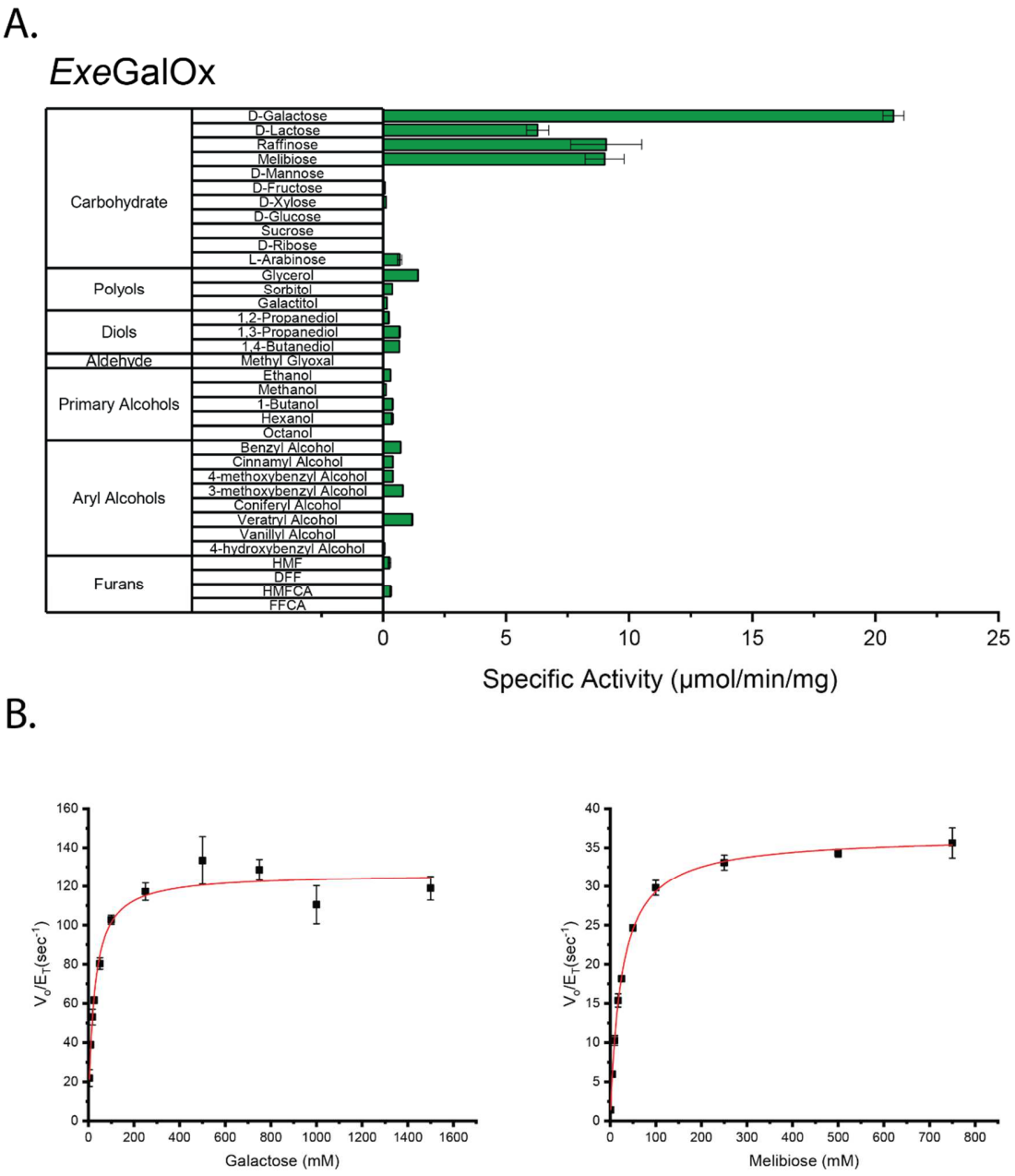
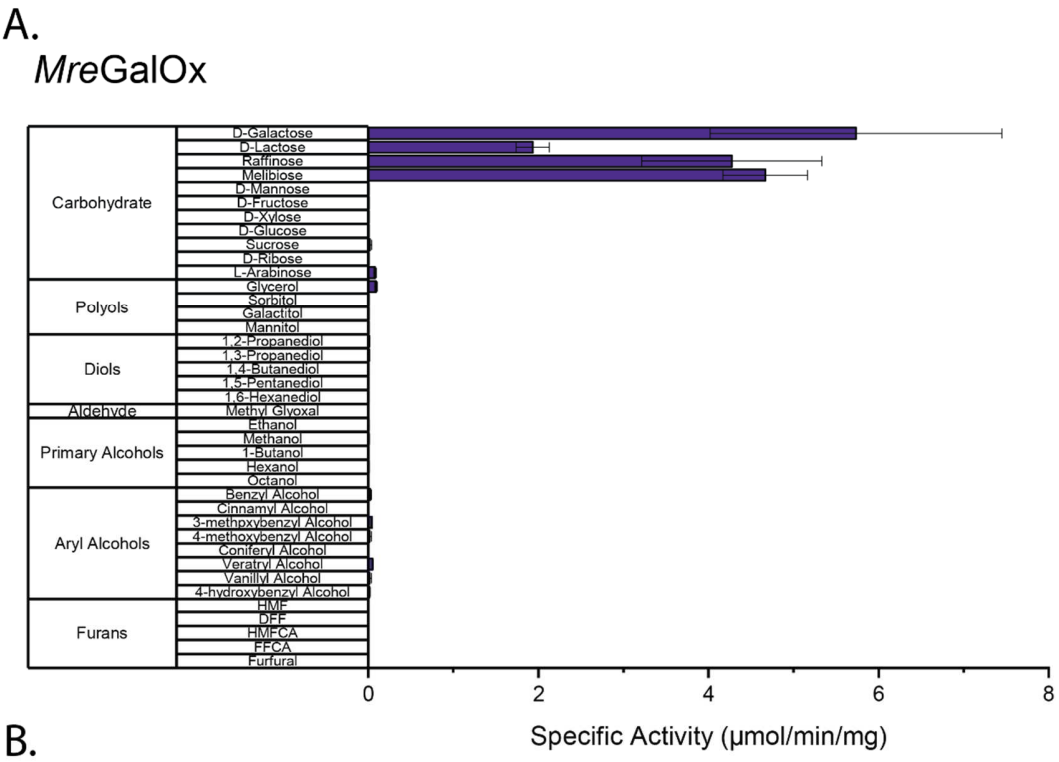


Figure S7. *Exe*GalOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.



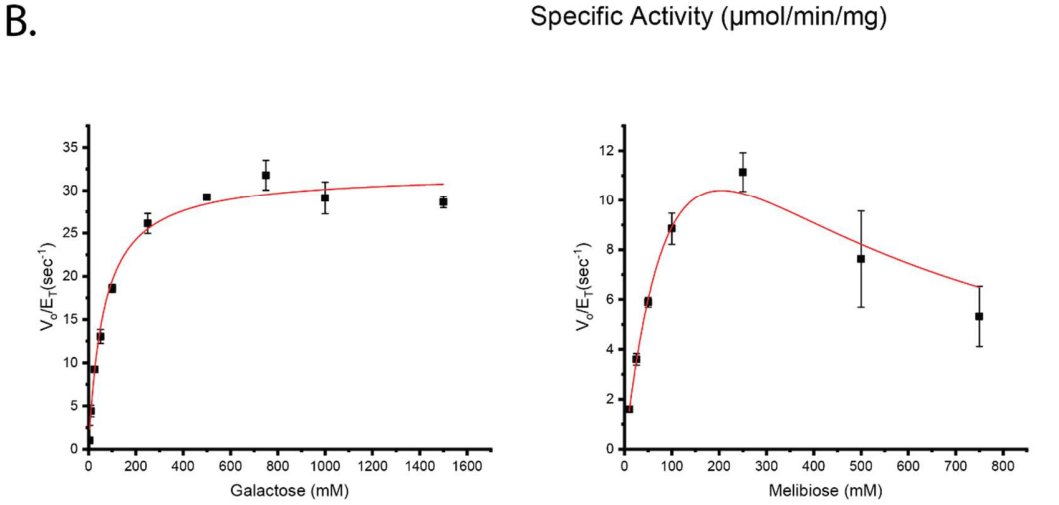


Figure S8. *Mre*GalOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Melibiose that did not display saturation kinetics due to substrate inhibition, therefore k_{cat}/K_M values were obtained from the slope of linear fittings. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

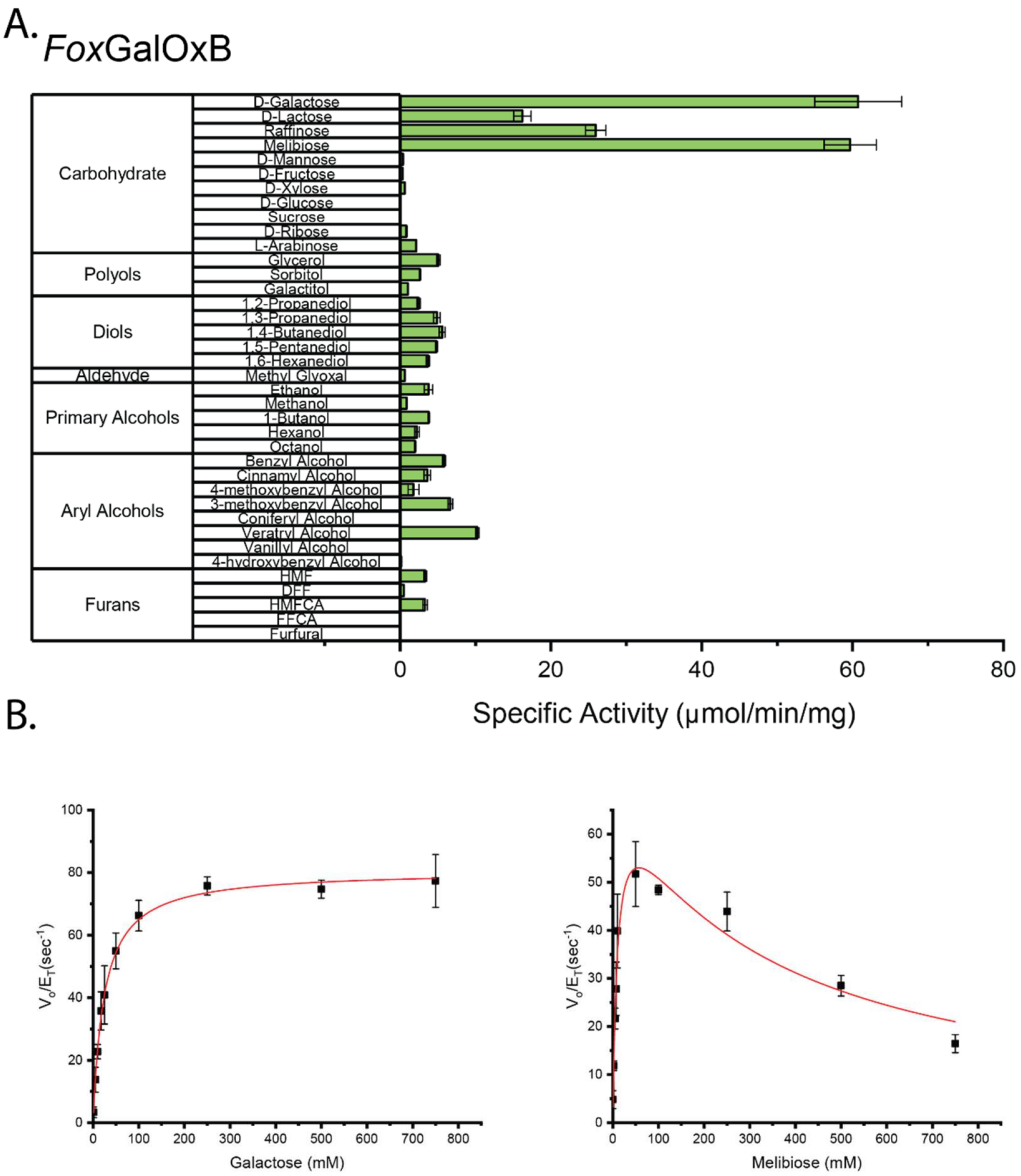


Figure S9. *Fox*GalOxB kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Melibiose that did not display saturation kinetics due to substrate inhibition, therefore k_{cat}/K_M values were obtained from the slope of linear fittings. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

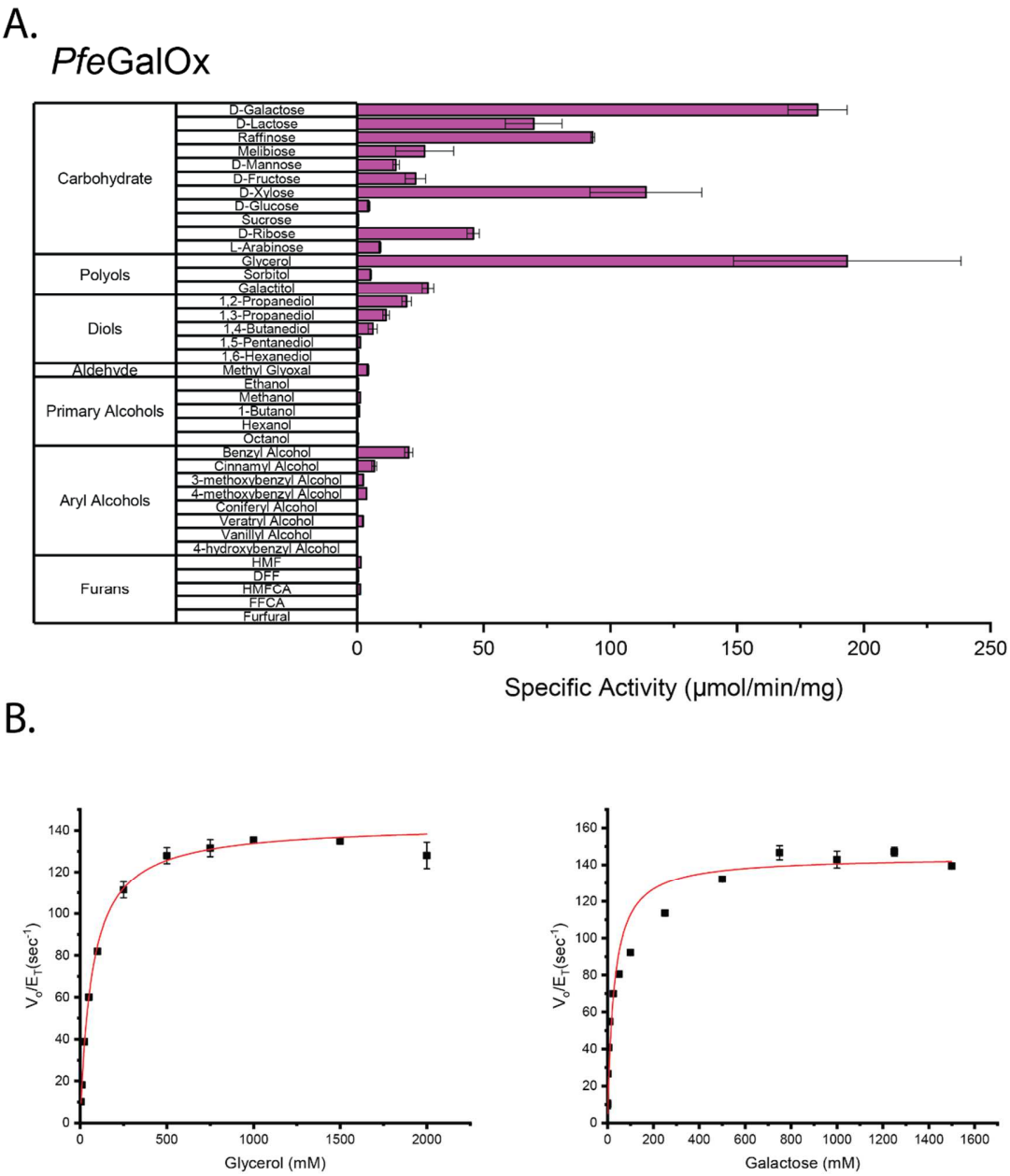
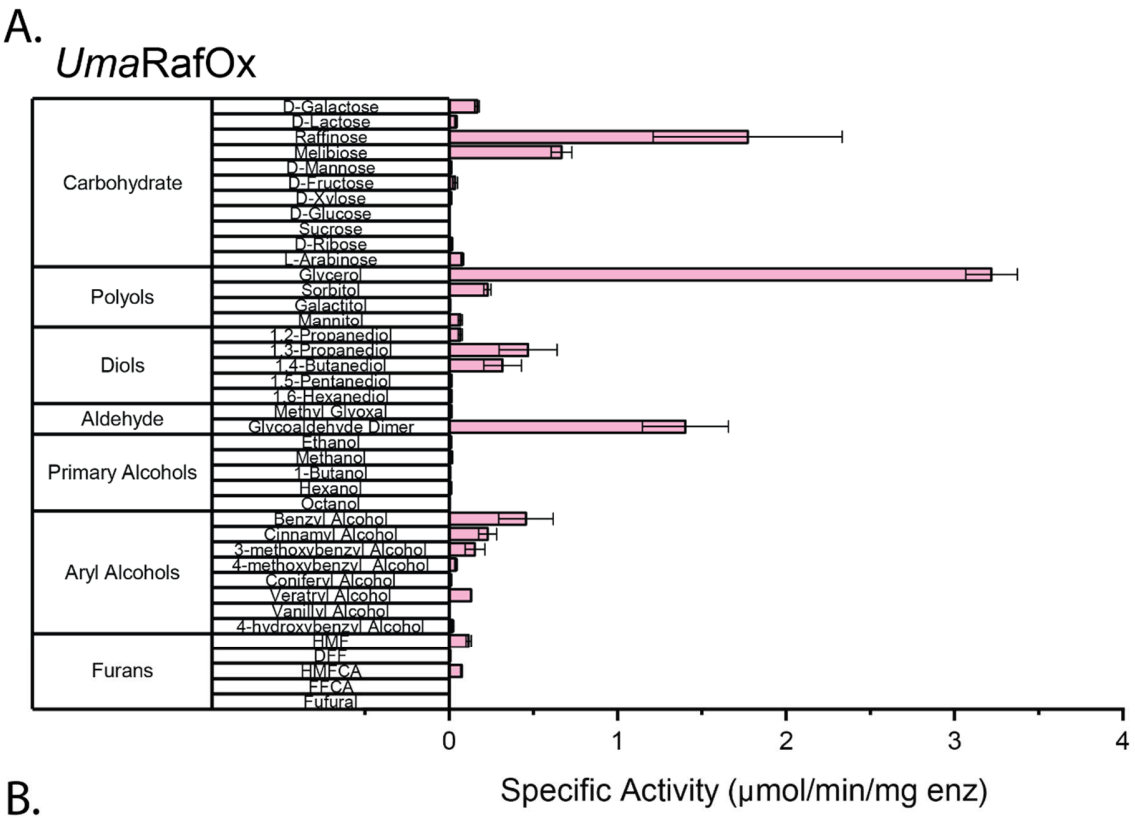


Figure S10. *Pfe*GalOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.



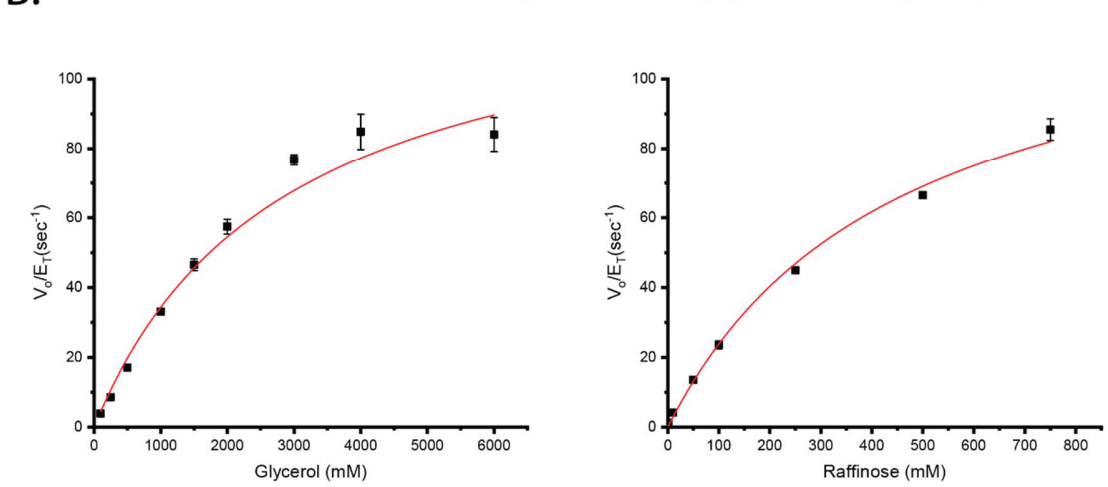


Figure S11. *Uma*RafOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

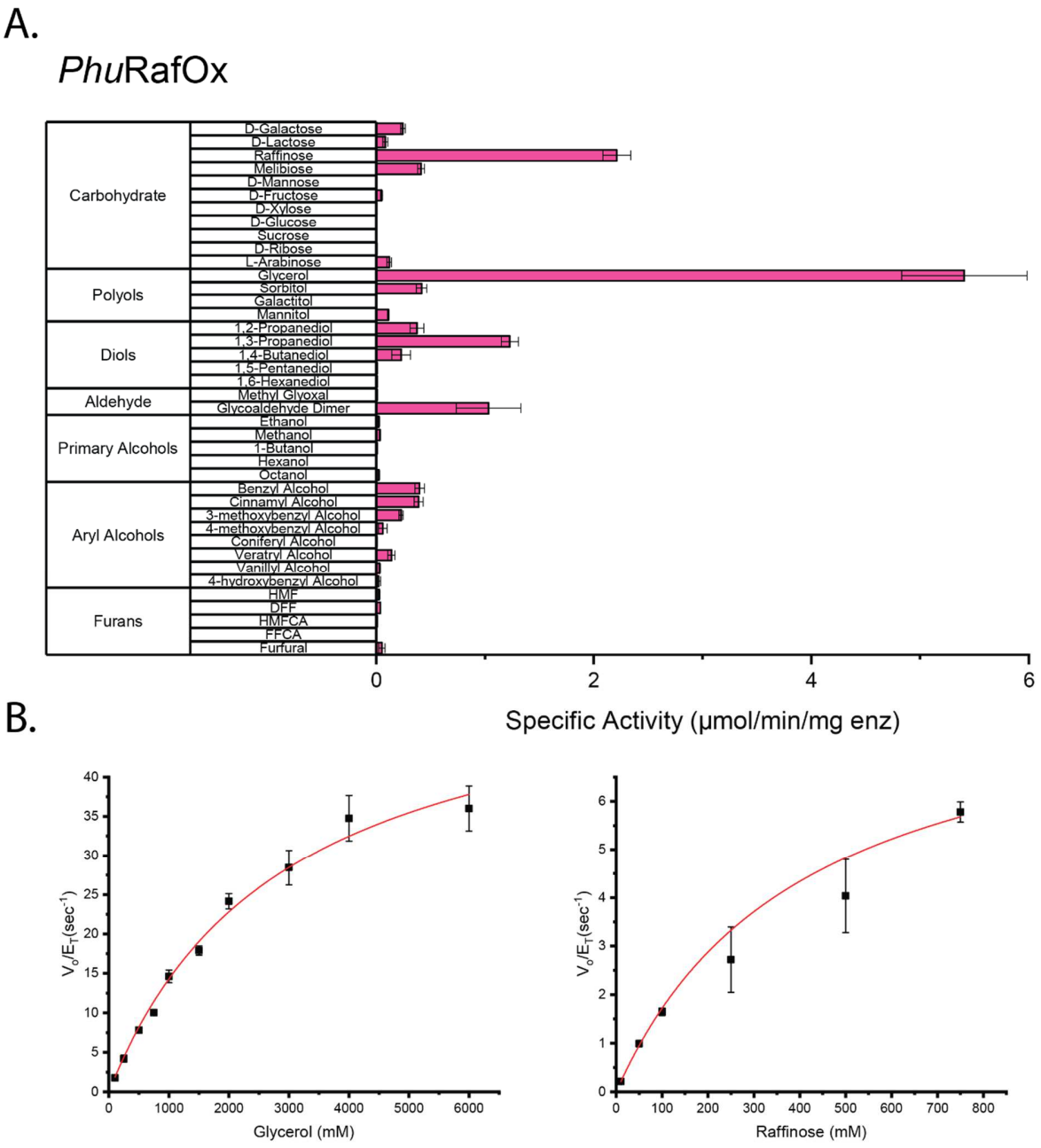
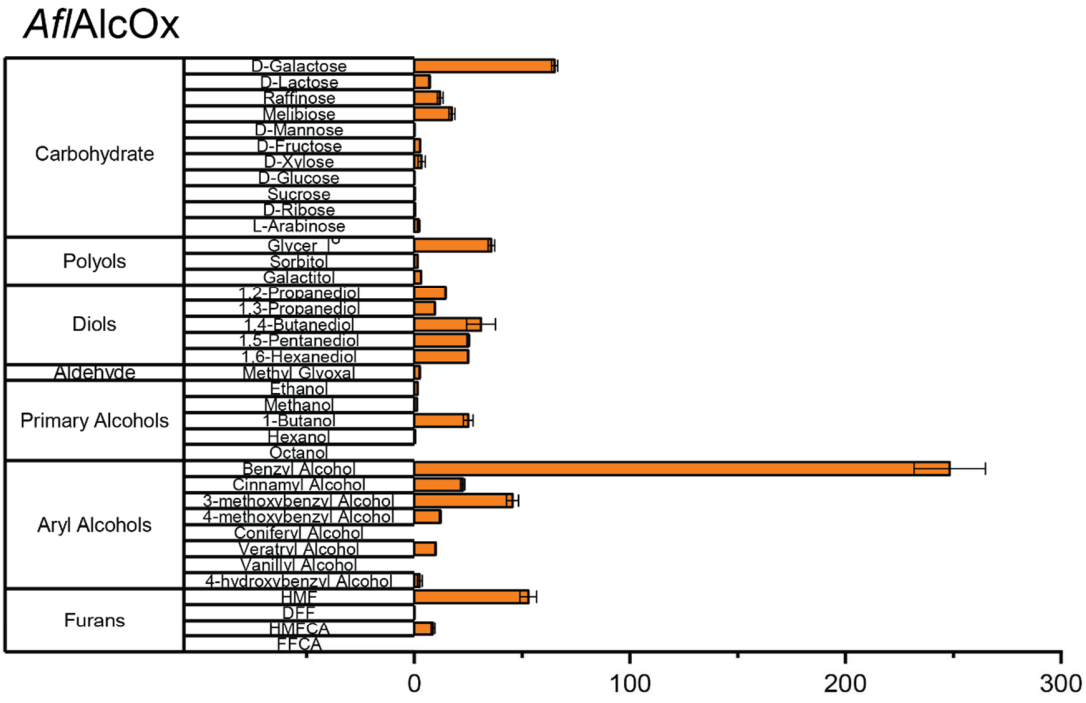


Figure S12. *Phu*RafOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

A.


AflAlcOx
Carbohydrate
D-Galactose
D-Lactose
Raffinose
Melibiose
D-Mannose
D-Fructose
D-Xylose
D-Glucose
Sucrose
D-Ribose
L-Arabinose
Polyols
Glycerol
Sorbitol
Galactitol
Diols
1,2-Propanediol
1,3-Propanediol
1,4-Butanediol
1,5-Pentanediol
1,6-Hexanediol
Aldehyde
Methyl Glyoxal
Primary Alcohols
Ethanol
Methanol
1-Butanol
Hexanol
Octanol
Aryl Alcohols
Benzyl Alcohol
Cinnamyl Alcohol
3-methoxybenzyl Alcohol
4-methoxybenzyl Alcohol
Coniferyl Alcohol
Veratryl Alcohol
Vanillyl Alcohol
4-hydroxybenzyl Alcohol
Furans
HMF
DFF
HMFCA
FFCA
0
100
200
300
Specific Activity (µmol/min/mg)

B.

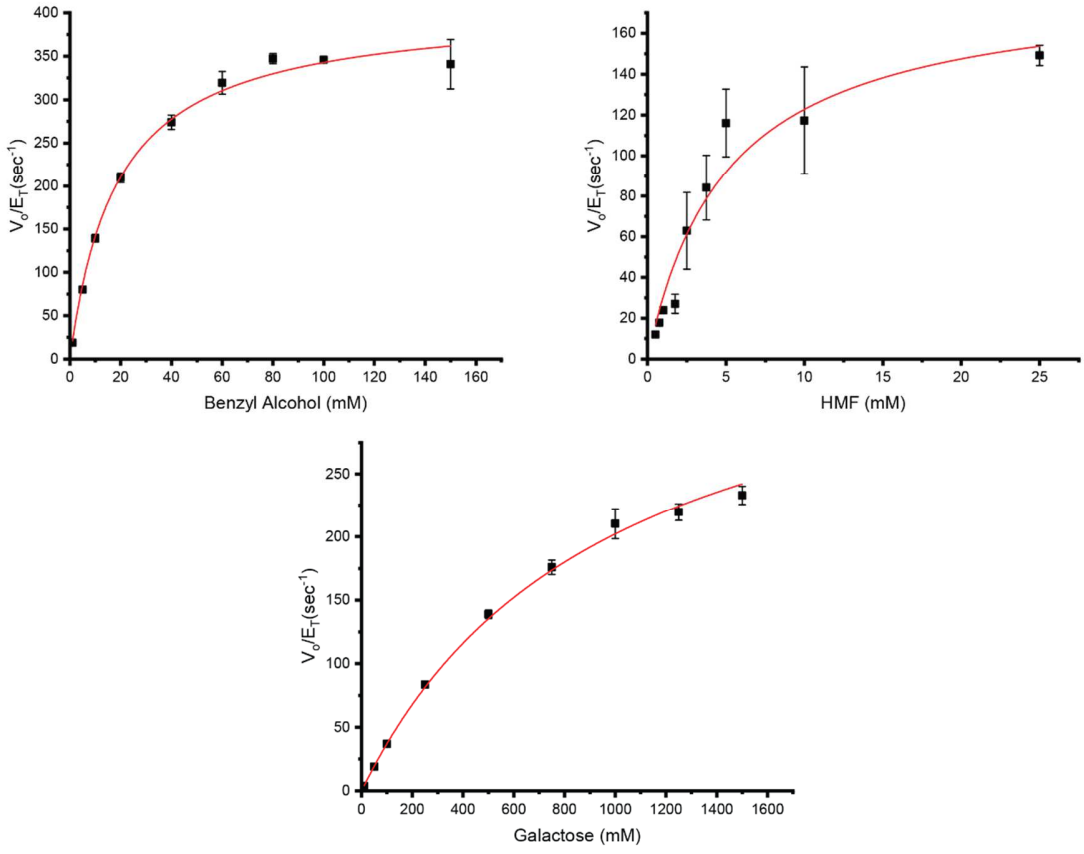
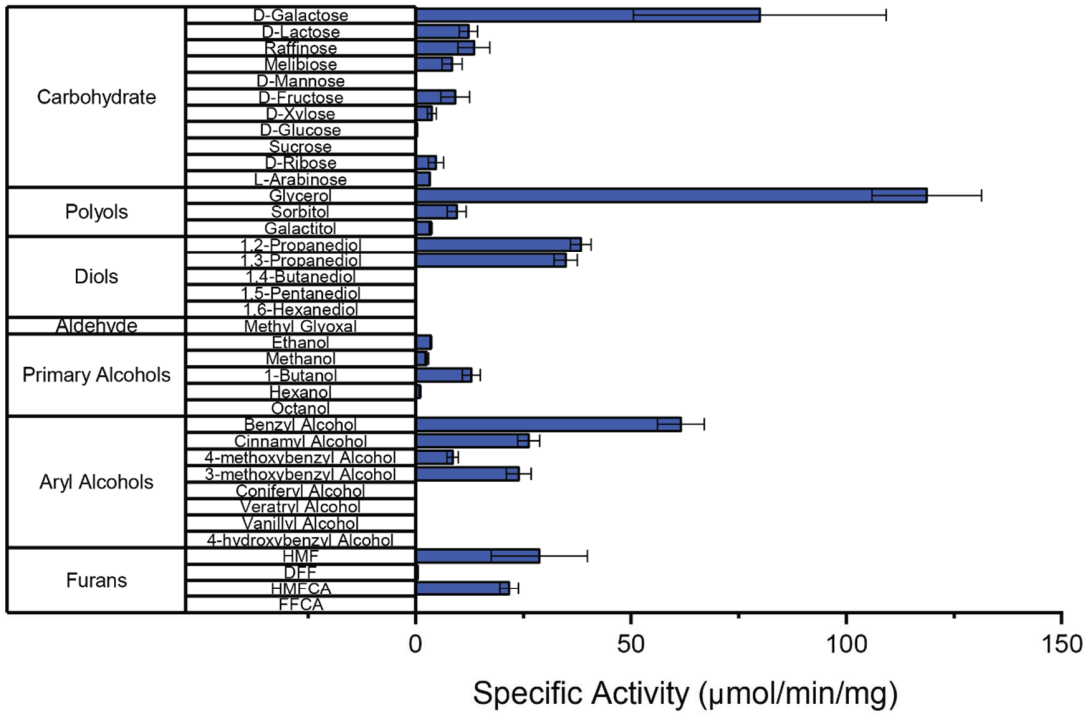

$V_o/E_T(sec^{-1})$
Benzyl Alcohol (mM)
HMF (mM)
Galactose (mM)

Figure S13. *Afl*AlcOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

A.


PruAA5_2A/AlcOx
Carbohydrate
D-Galactose
D-Lactose
Raffinose
Melibiose
D-Mannose
D-Fructose
D-Xylose
D-Glucose
Sucrose
D-Ribose
L-Arabinose
Polyols
Glycerol
Sorbitol
Galactitol
Diols
1,2-Propanediol
1,3-Propanediol
1,4-Butanediol
1,5-Pentanediol
1,6-Hexanediol
Aldehyde
Methyl Glyoxal
Primary Alcohols
Ethanol
Methanol
1-Butanol
Hexanol
Octanol
Aryl Alcohols
Benzyl Alcohol
Cinnamyl Alcohol
4-methoxybenzyl Alcohol
3-methoxybenzyl Alcohol
Coniferyl Alcohol
Veratryl Alcohol
Vanillyl Alcohol
4-hydroxybenzyl Alcohol
Furans
HMF
DFF
HMFCA
FFCA
0
50
100
150
Specific Activity (µmol/min/mg)

B.

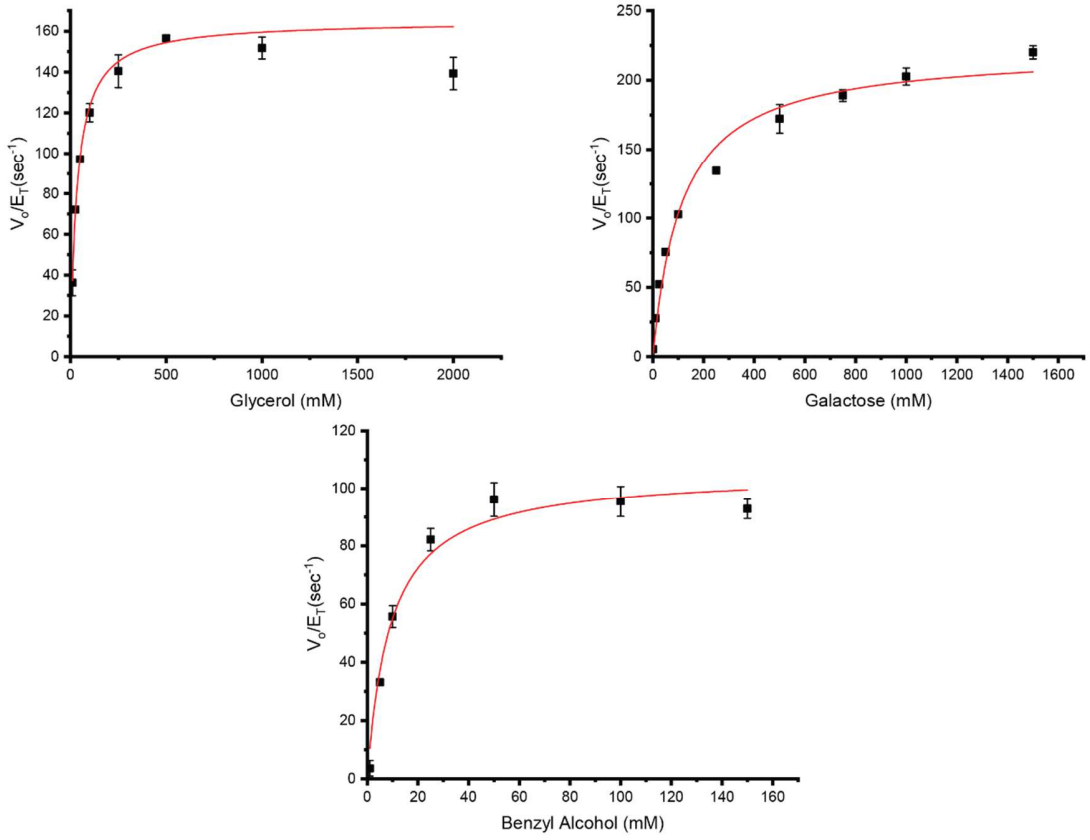

$V_o/E_T(sec^{-1})$
Glycerol (mM)
Galactose (mM)
Benzyl Alcohol (mM)

Figure S14. *Pru*AA5_2A (*Pru*AlcOx) kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

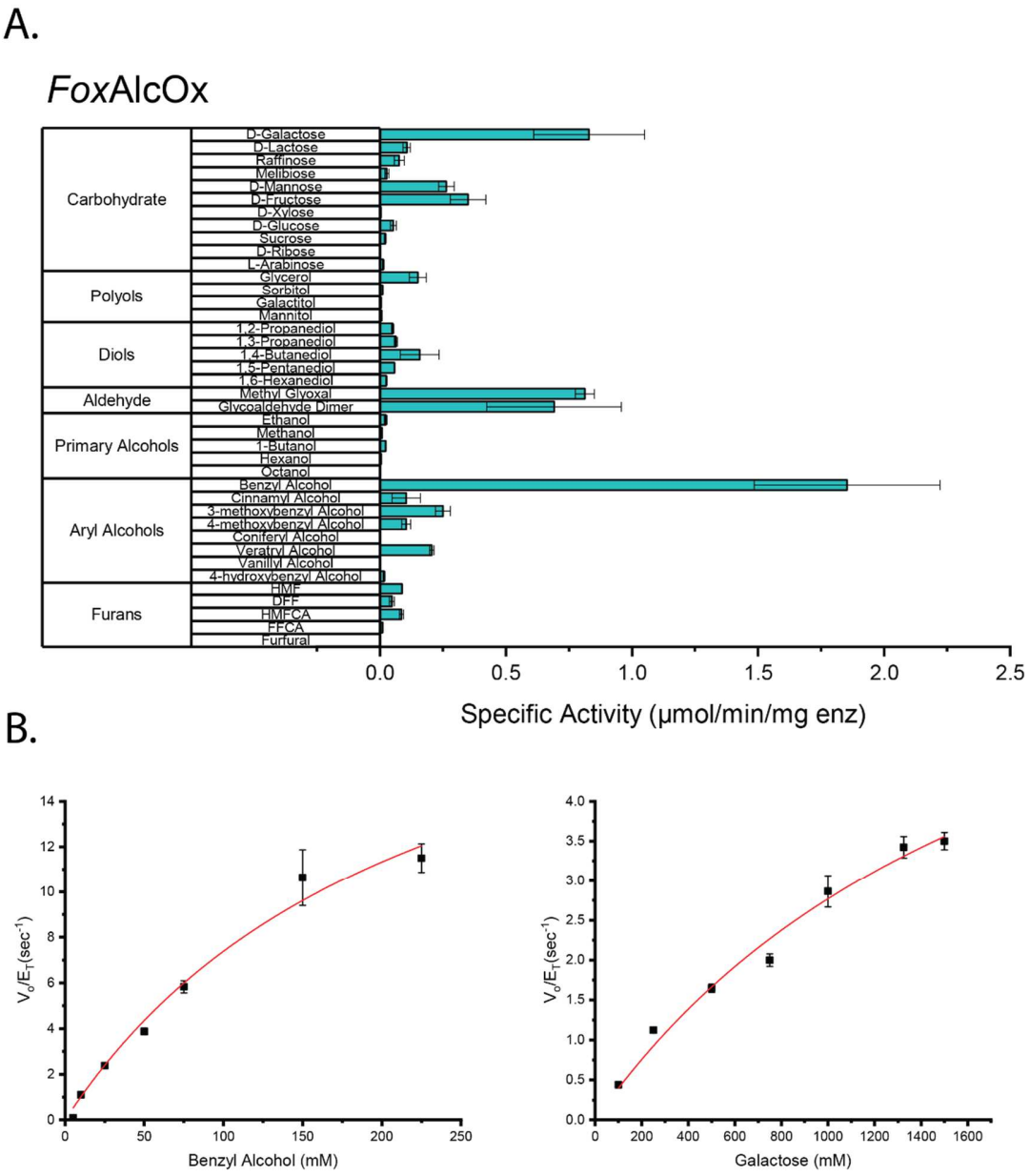
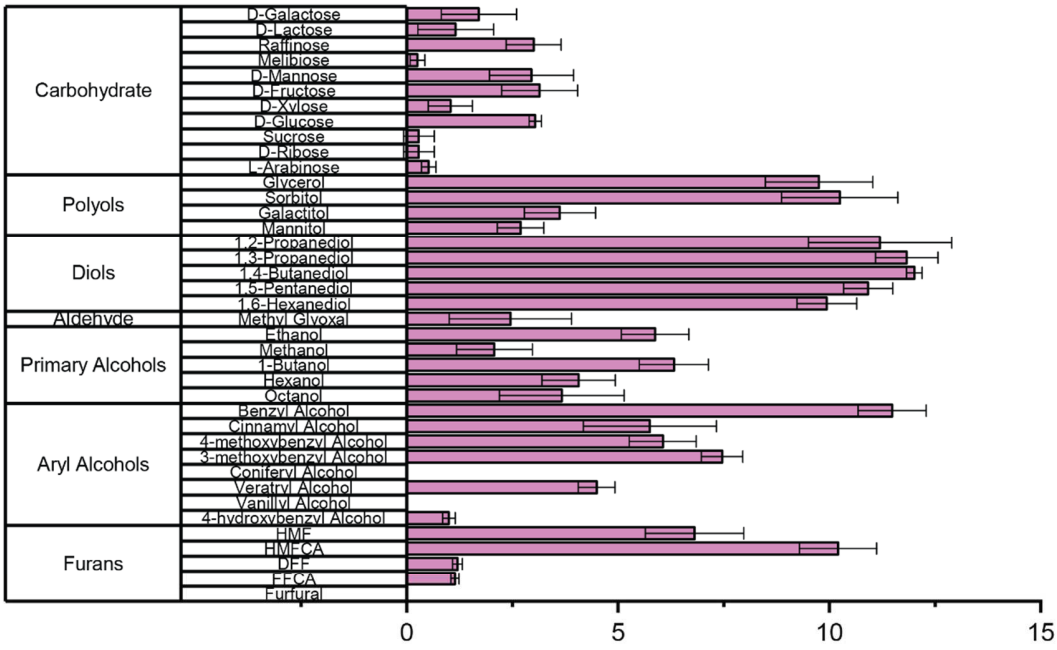


Figure S15. *Fox*AlcOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

A. *Por*AlcOx


Carbohydrate
D-Galactose
D-Lactose
Raffinose
Melibiose
D-Mannose
D-Fructose
D-Xylose
D-Glucose
Sucrose
D-Ribose
L-Arabinose
Polyols
Glycerol
Sorbitol
Galactitol
Mannitol
Diols
1,2-Propanediol
1,3-Propanediol
1,4-Butanediol
1,5-Pentanediol
1,6-Hexanediol
Aldehyde
Methyl Glyoxal
Primary Alcohols
Ethanol
Methanol
1-Butanol
Hexanol
Octanol
Aryl Alcohols
Benzyl Alcohol
Cinnamyl Alcohol
4-methoxybenzyl Alcohol
3-methoxybenzyl Alcohol
Coniferyl Alcohol
Veratryl Alcohol
Vanillyl Alcohol
4-hydroxybenzyl Alcohol
Furans
HMF
HMFCA
DFF
FFCA
Furfural
0
5
10
15
Specific Activity (µmol/min/mg enz)

B.

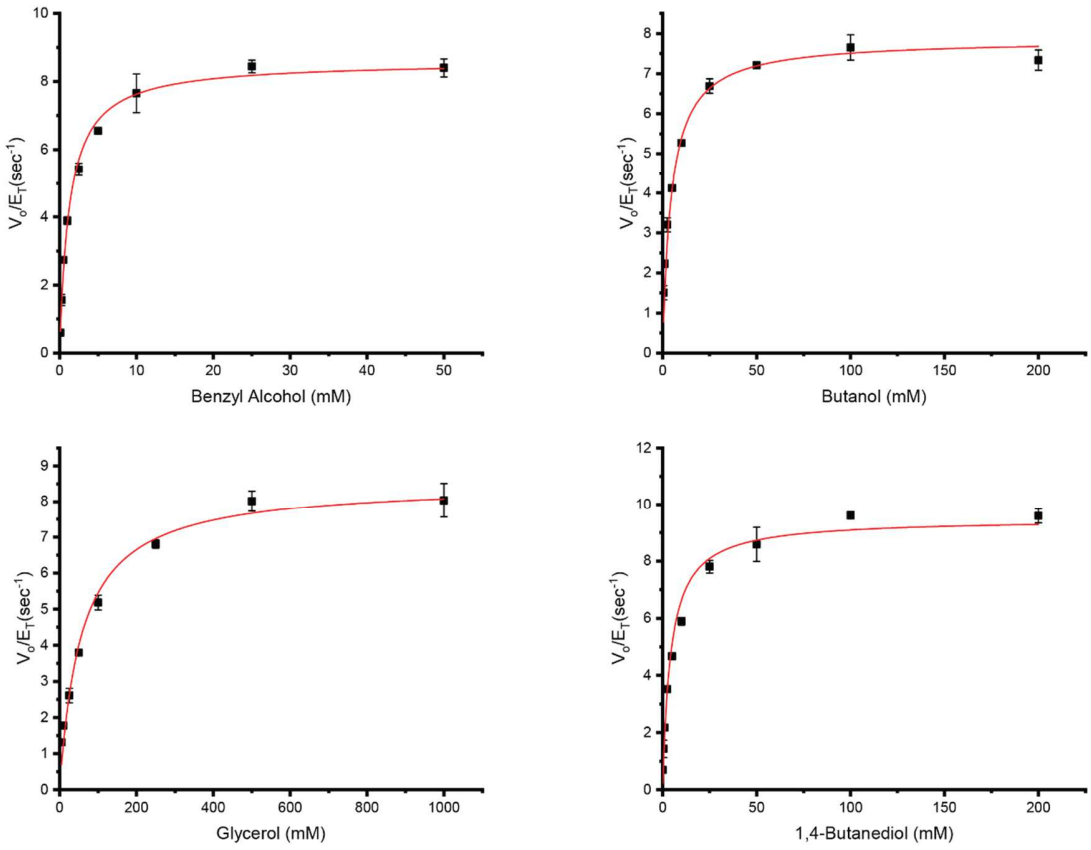
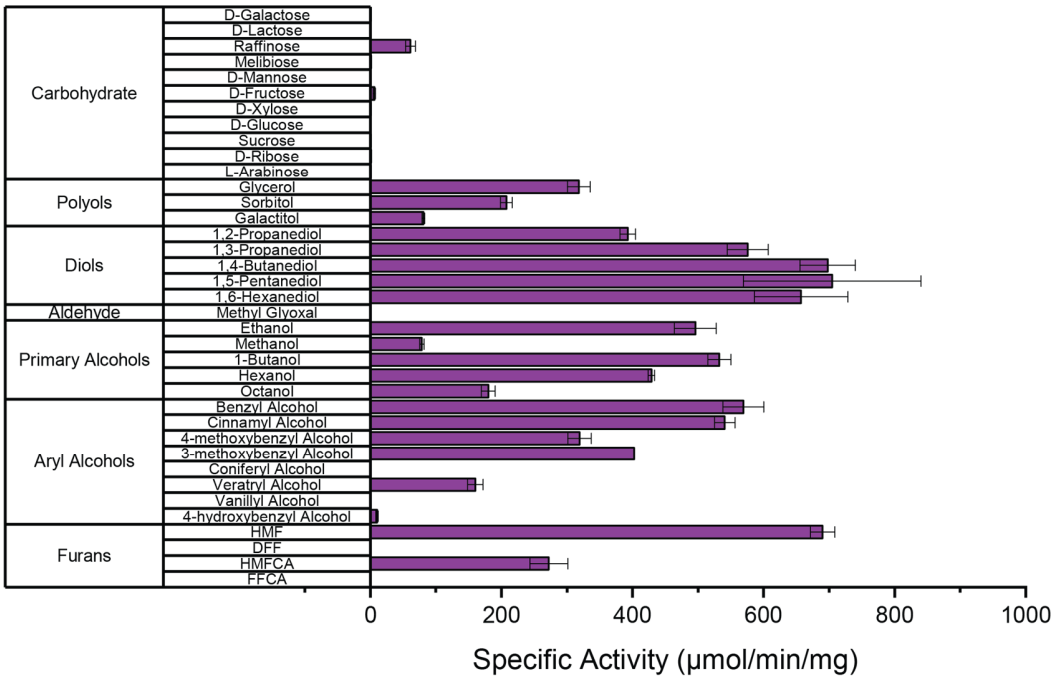

$V_o/E_T(sec^{-1})$
Benzyl Alcohol (mM)
Butanol (mM)
Glycerol (mM)
1,4-Butanediol (mM)

Figure S16. *Por*AlcOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

A.

*Cgl*AlcOx


Carbohydrate
D-Galactose
D-Lactose
Raffinose
Melibiose
D-Mannose
D-Fructose
D-Xylose
D-Glucose
Sucrose
D-Ribose
L-Arabinose
Polyols
Glycerol
Sorbitol
Galactitol
Diols
1,2-Propanediol
1,3-Propanediol
1,4-Butanediol
1,5-Pentanediol
1,6-Hexanediol
Aldehyde
Methyl Glyoxal
Primary Alcohols
Ethanol
Methanol
1-Butanol
Hexanol
Octanol
Aryl Alcohols
Benzyl Alcohol
Cinnamyl Alcohol
4-methoxybenzyl Alcohol
3-methoxybenzyl Alcohol
Coniferyl Alcohol
Veratryl Alcohol
Vanillyl Alcohol
4-hydroxybenzyl Alcohol
Furans
HMF
DFF
HMFCA
FFCA
0
200
400
600
800
1000
Specific Activity (μmol/min/mg)

B.

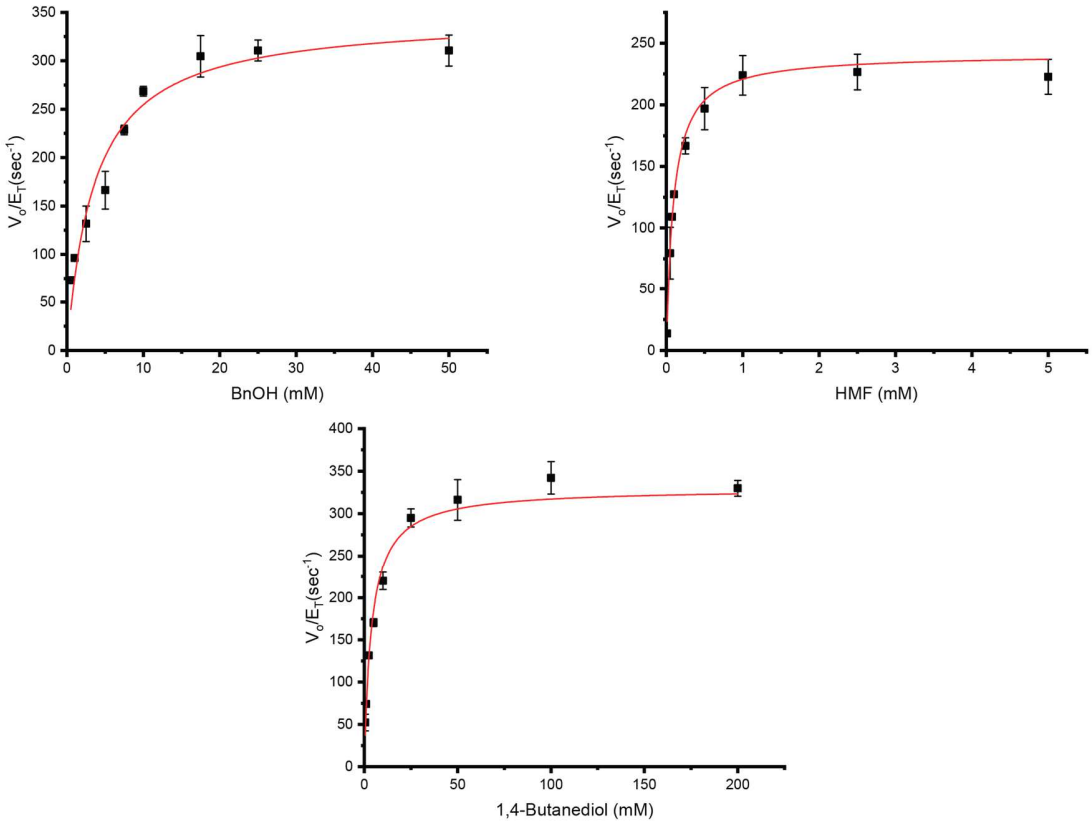

$V_o/E_T(sec^{-1})$
BnOH (mM)
HMF (mM)
1,4-Butanediol (mM)

Figure S17. *Cgl*AlcOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

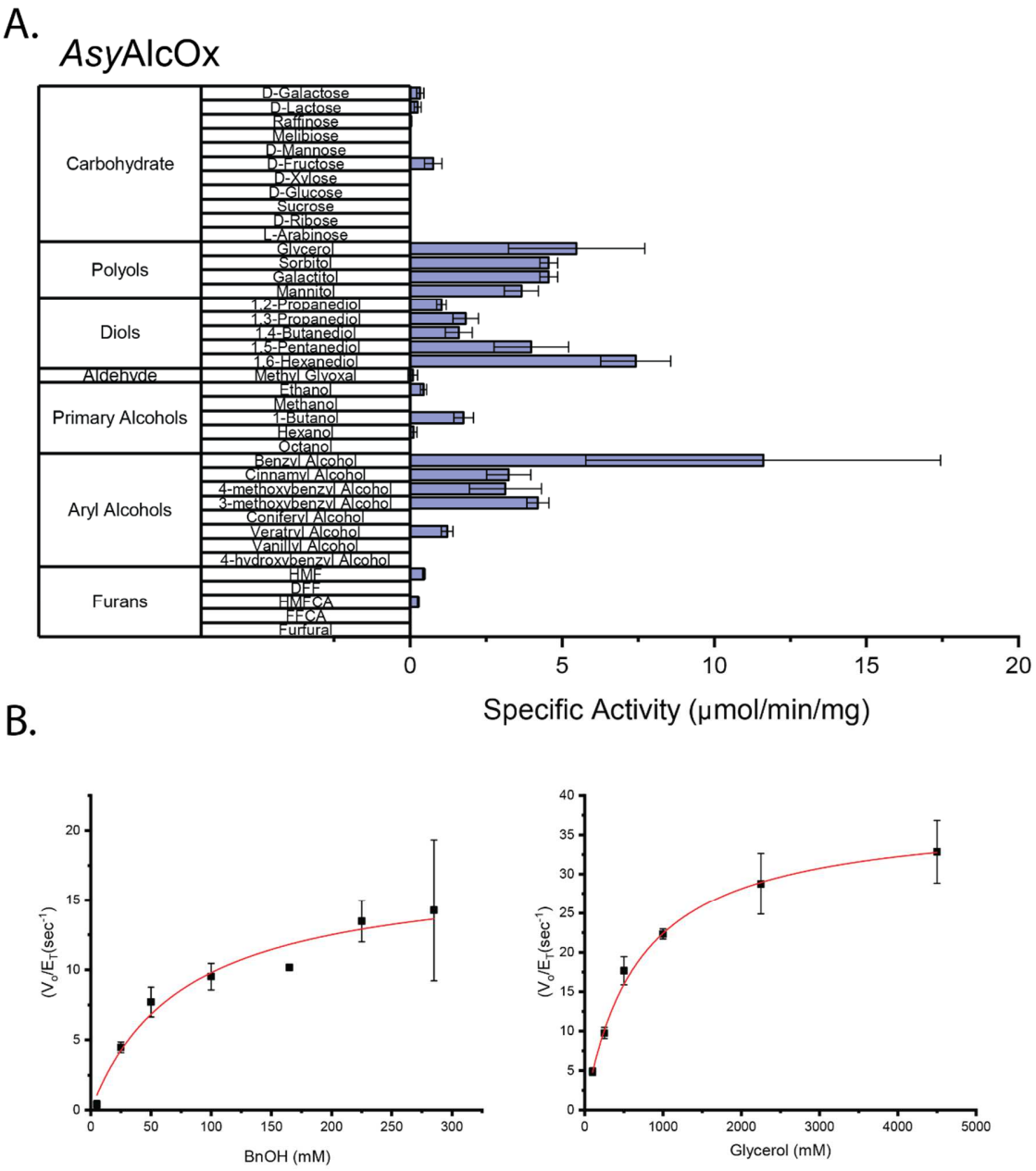


Figure S18. *Asy*AlcOx kinetics. A. Specific activities. Measurements were performed in triplicate at RT in 100 mM buffer using the HRP/ABTS assay. Activities were monitored using 300 mM for carbohydrates, polyols, diols and primary alcohols, 30 mM for benzyl alcohol and galactitol, 5 mM for methyl glyoxal and glycolaldehyde dimer, 2.5 mM for aryl alcohols and furans. B. Michaelis-Menten kinetics. Initial-rate values were measured in triplicate at each substrate concentration. Error bars represent the standard deviation from the mean values. The individual k_{cat} and K_M values were determined by performing a non-linear fitting analysis of the standard Michaelis-Menten equation to the data using OriginLab 9.55. Individual substrates are indicated in the x-axis labels and the enzyme under study is annotated in each graph.

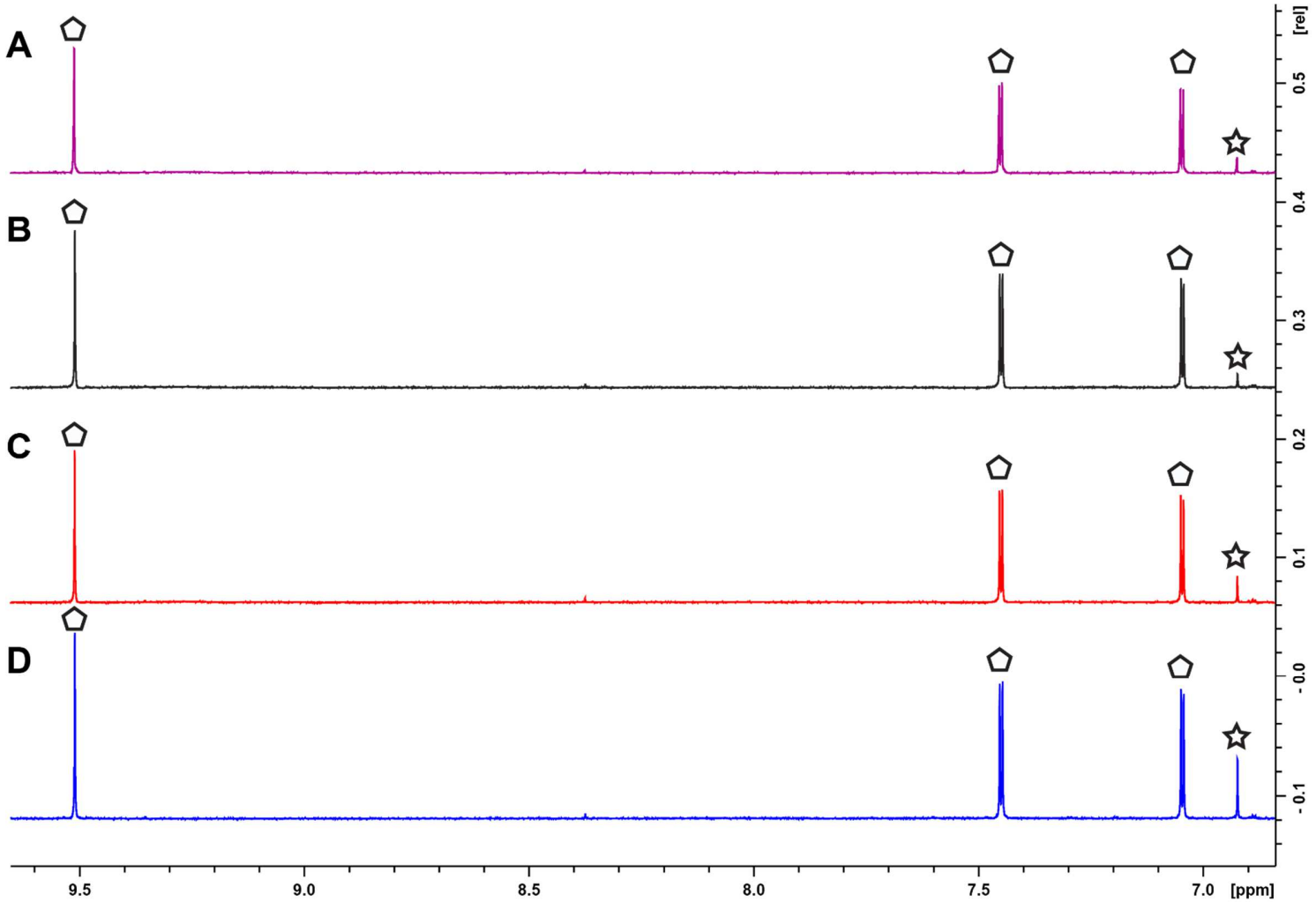


Figure S19. ^{1}H-NMR analysis of the oxidation of HMF, HMFCA, DFF and FFCA by *Cgl*AlcOx. A.HMF (magenta); B. HMFCA (black). C. DFF (red) and D. FFCA (blue). ^{1}H NMR spectra (600 MHz, 12:1 D_2O: buffer, 50 mM) of reaction product profiles after 17 h incubation with *Cgl*AlcOx in the presence of catalase and HRP with 10 mM substrate. Reactions with HMF, HMFCA and DFF showed full consumption of substrate to produce FFCA (⬠) and FDCA (☆). Reactions with FFCA (⬠) produced partial conversion to FDCA (☆).

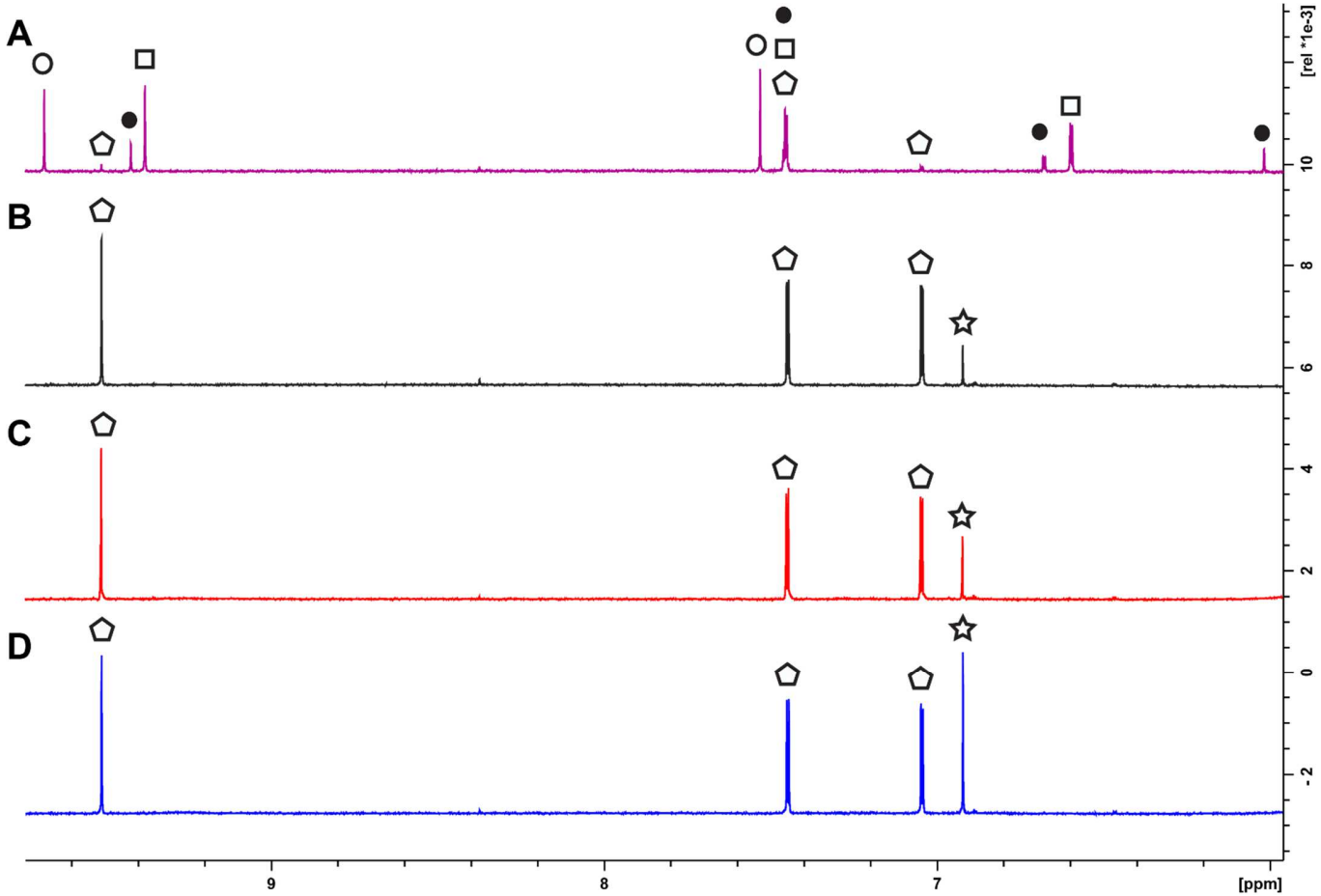


Figure S20. ^{1}H-NMR analysis of the oxidation of HMF, HMFCA, DFF and FFCA by *Por*AlcOx. A.HMF (black); B. HMFCA (blue). C. DFF (red) and D. FFCA (pink). ^{1}H NMR spectra (600 MHz, 12:1 D_2O: buffer, 50 mM) of reaction product profiles after 17 h incubation with *Por*AlcOx in the presence of catalase and HRP with 10 mM substrate. Reactions with HMF (□) showed 51 % consumption of substrate to DFF (○) and its hydrate form DFF_{hyd}(●) and FFCA (⬠). Reactions with HMFCA and DFF showed full consumption of substrate to produce FFCA (⬠) and FDCA (☆). Reactions with FFCA (⬠) produced partial conversion to FDCA (☆).

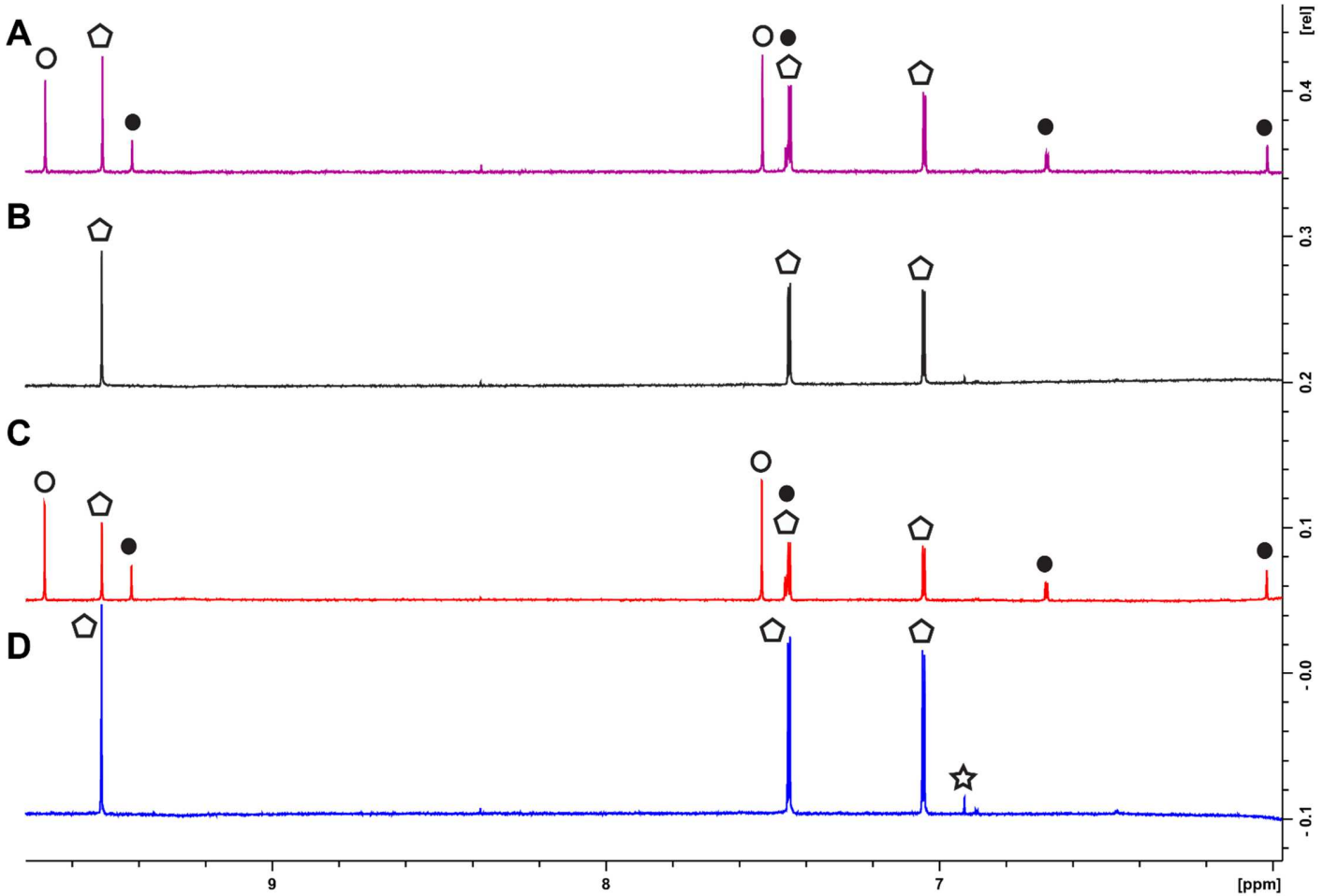


Figure S21. ^{1}H-NMR analysis of the oxidation of HMF, HMFCA, DFF and FFCA by *Pru*AA5_2A (*Pru*AlcOx). A.HMF (black); B. HMFCA (blue). C. DFF (red) and D. FFCA (pink). ^{1}H NMR spectra (600 MHz, 12:1 D_2O: buffer, 50 mM) of reaction product profiles after 17 h incubation with *Pru*AA5_2A/AlcOx in the presence of catalase and HRP with 10 mM substrate. Reactions with HMF (□) showed 100 % consumption of substrate to DFF (○) and its hydrate form DFF_{hyd}(●) and FFCA (⬠). Reactions with DFF showed partial conversion to FFCA (⬠). Reactions with HMFCA shower 100 % conversion to FFCA (⬠) while reactions with FFCA (⬠) shower low conversion to FDCA (☆).

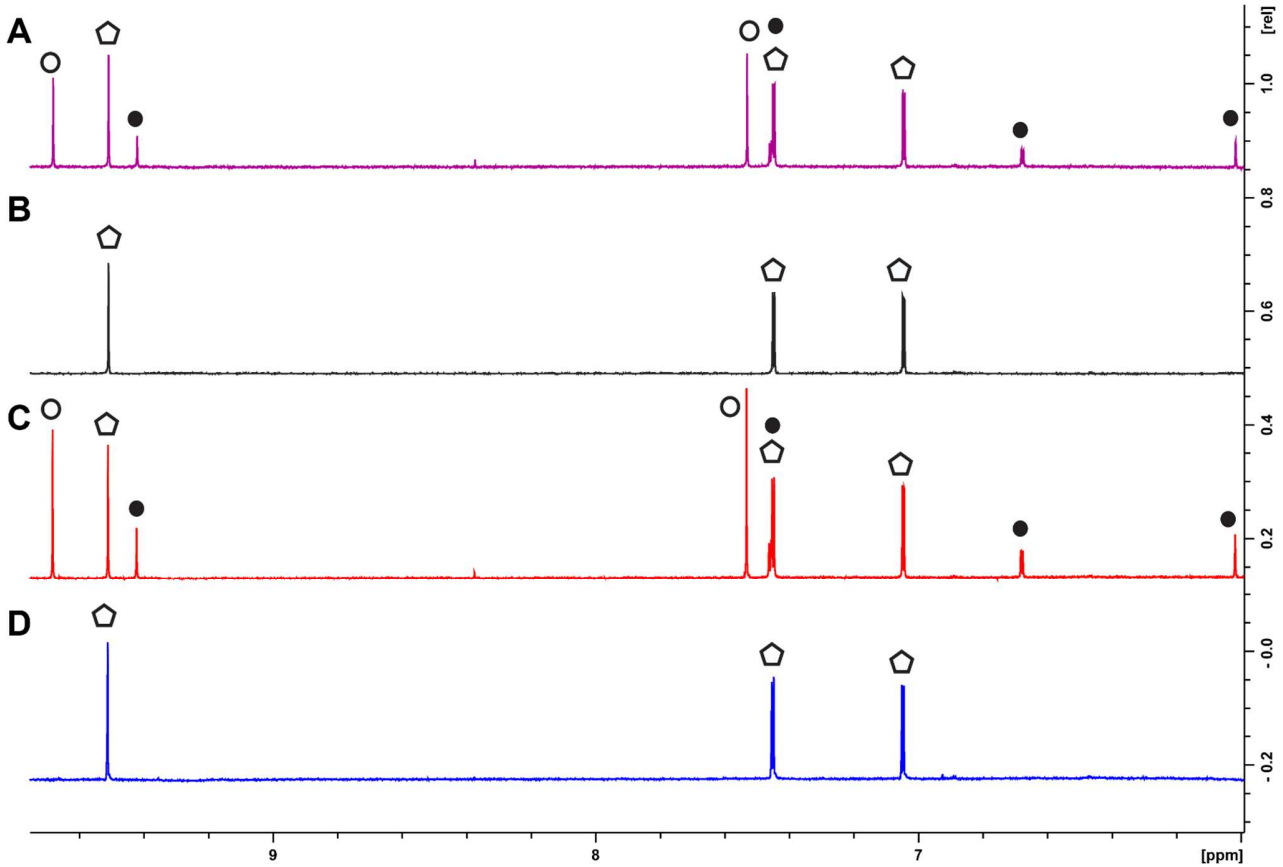


Figure S22. ^{1}H-NMR analysis of the oxidation of HMF, HMFCA, DFF and FFCA by *Afl*AlcOx. A.HMF (black); B. HMFCA (blue). C. DFF (red) and D. FFCA (pink). ^{1}H NMR spectra (600 MHz, 12:1 D_2O: buffer, 50 mM) of reaction product profiles after 17 h incubation with *Afl*AlcOx in the presence of catalase and HRP with 10 mM substrate. Reactions with HMF (□) showed 100 % consumption of substrate to DFF (○) and its hydrate form DFF_{hyd}(●) and FFCA (⬠). Reactions with DFF showed partial conversion to FFCA (⬠). Reactions with HMFCA shower 100 % conversion to FFCA (⬠) while reactions with FFCA (⬠) shower no conversion.

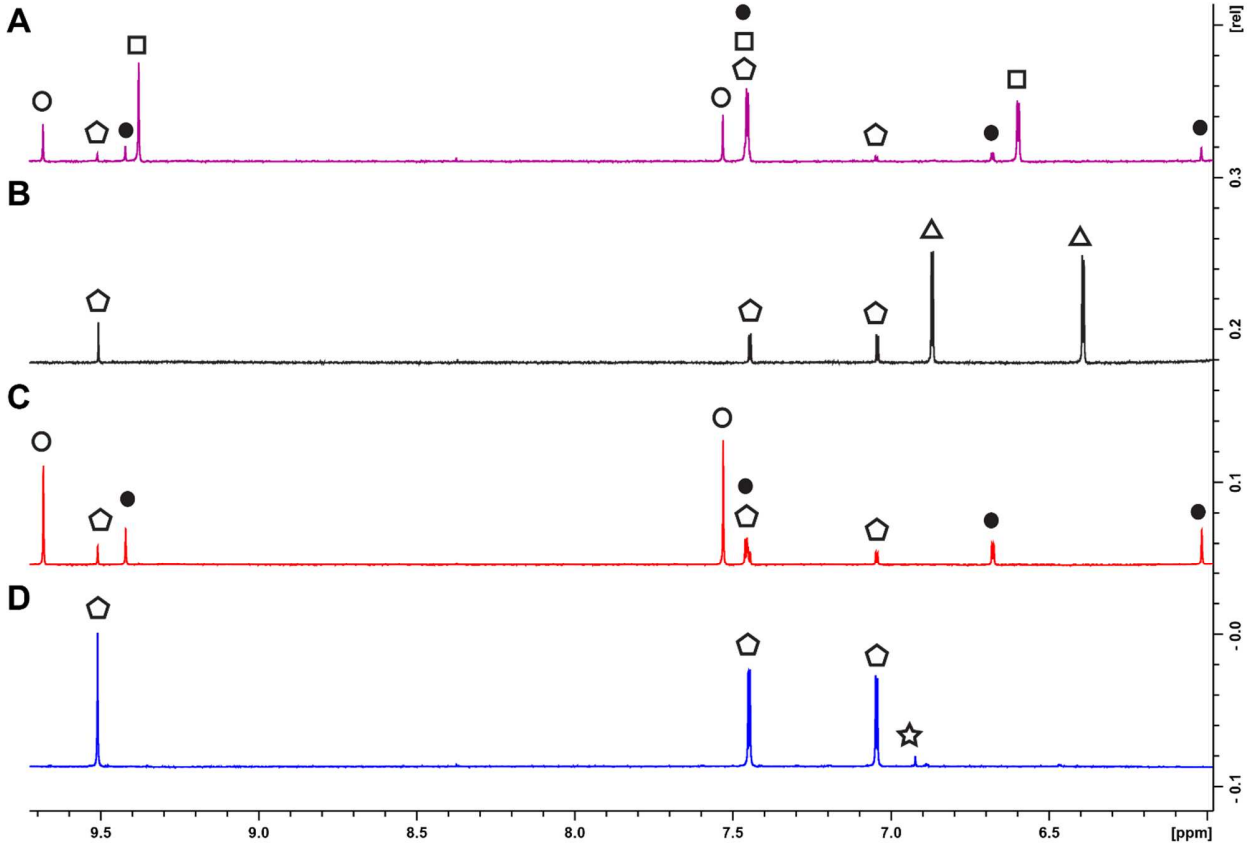
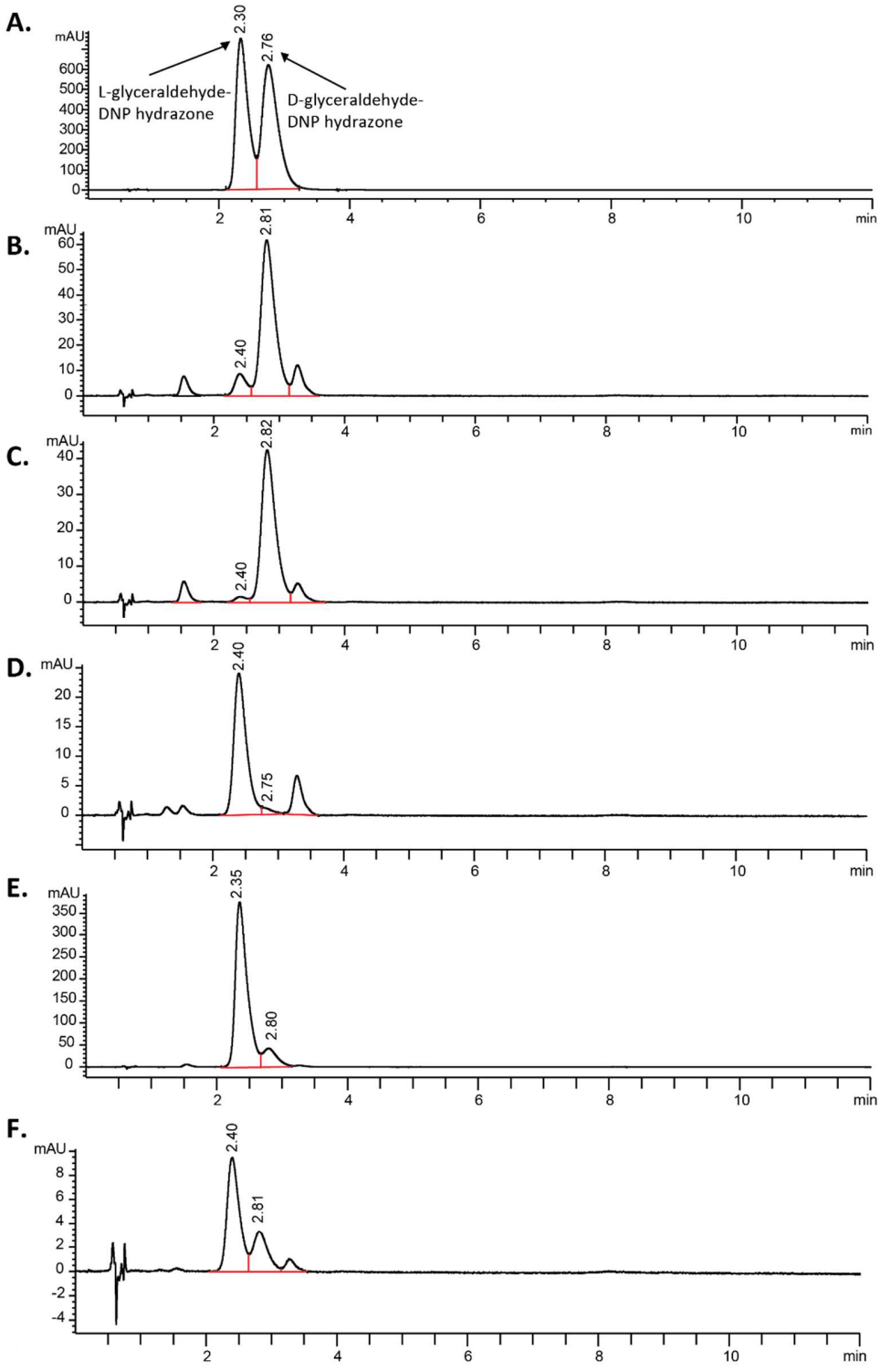


Figure S23. ^{1}H-NMR analysis of the oxidation of HMF, HMFCA, DFF and FFCA by *Fox*GalOxB. A.HMF (black); B. HMFCA (blue). C. DFF (red) and D. FFCA (pink). ^{1}H NMR spectra (600 MHz, 12:1 D_2O: buffer, 50 mM) of reaction product profiles after 17 h incubation with *Fox*GalOxB in the presence of catalase and HRP with 10 mM substrate. Reactions with HMF (□) showed 62 % consumption of substrate to DFF (○) and its hydrate form DFF_{hyd}(●) and FFCA (⬠). Reactions with HMFCA and DFF showed 17 % and 12 %, respectively, consumption of substrate to produce FFCA (⬠). Reactions with FFCA (⬠) produced partial conversion to FDCA (☆).


A.
mAU
2.30
2.76
L-glyceraldehyde-
DNP hydrazone
D-glyceraldehyde-
DNP hydrazone
min
B.
mAU
2.81
2.40
min
C.
mAU
2.82
2.40
min
D.
mAU
2.40
2.75
min
E.
mAU
2.35
2.80
min
F.
mAU
2.40
2.81
min

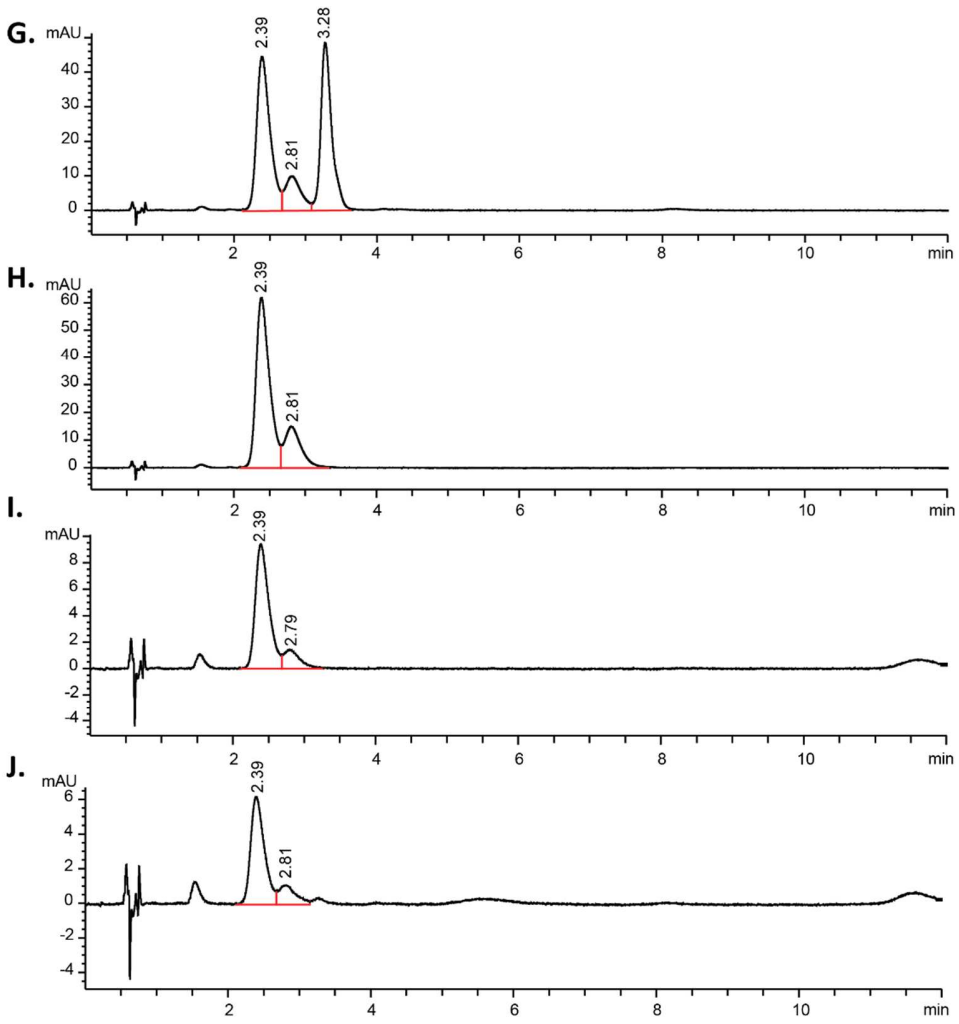


Figure S24. Stereochemistry determination of glycerol oxidation by *Por*AlcOx, *Cgl*AlcOx, *Pru*AA5_2A (*Pru*AlcOx), *Afl*AlcOx, *Pfe*GalOx, *Fox*GalOxB, *Asy*AlcOx, *Uma*RafOx and *Phu*RafOx. A. Chromatogram of *L*/*D*-glyceraldehyde-hydrazone. B. Glyceraldehyde-hydrazone composition after *Por*AlcOx oxidation of glycerol. C. Glyceraldehyde-hydrazone composition after *Cgl*AlcOx oxidation of glycerol. D. Glyceraldehyde-hydrazone composition after *Pru*AA5_2A (*Pru*AlcOx) oxidation of glycerol. E. Glyceraldehyde-hydrazone composition after *Afl*AlcOx oxidation of glycerol. F. Glyceraldehyde-hydrazone composition after *Pfe*GalOx oxidation of glycerol. G. Glyceraldehyde-hydrazone composition after *Fox*GalOxB oxidation of glycerol. H. Glyceraldehyde-hydrazone composition after *Asy*AlcOx oxidation of glycerol. I. Glyceraldehyde-hydrazone composition after *Uma*RafOx oxidation of glycerol. J. Glyceraldehyde-hydrazone composition after *Phu*RafOx oxidation of glycerol. Peaks are labelled with retention times.

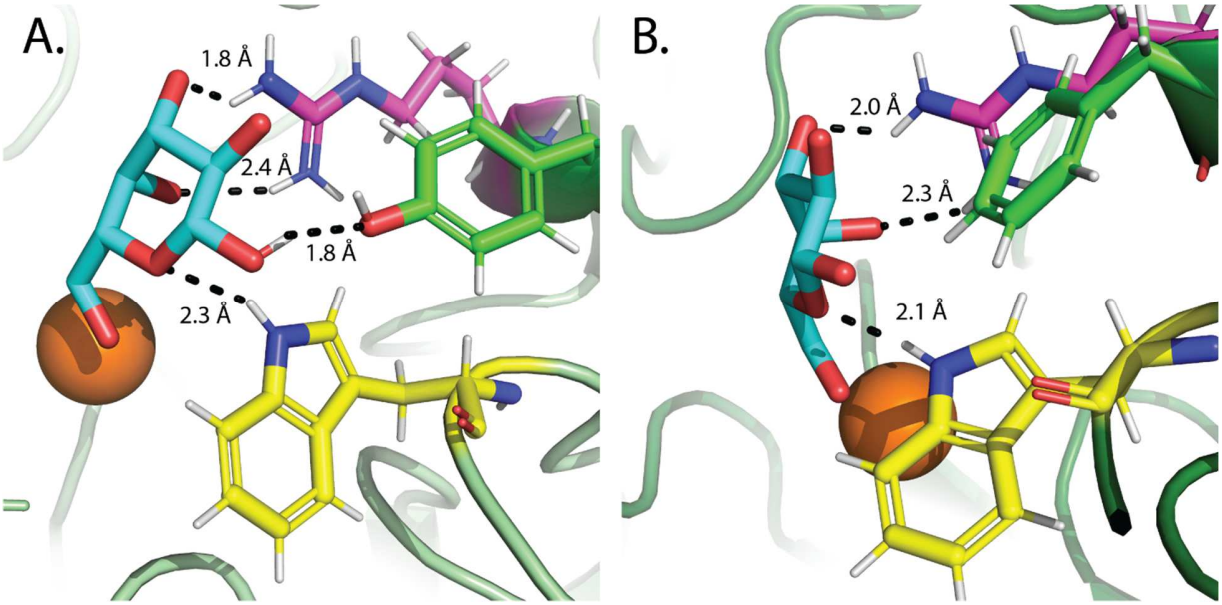


Figure S25. Molecular docking of galactose (cyan) in *Fgr*GalOx (PDB ID 1GOF)[13] (A) and Phyre2 homology models of *Exe*GalOx (B) using AutoDock Vina as implemented in Chimera. The copper atom is depicted as a dark orange sphere, while W290, Y329/F329, and R330 are depicted as yellow, green and magenta sticks respectively. Interactions with the docked substrate, are indicated in black.

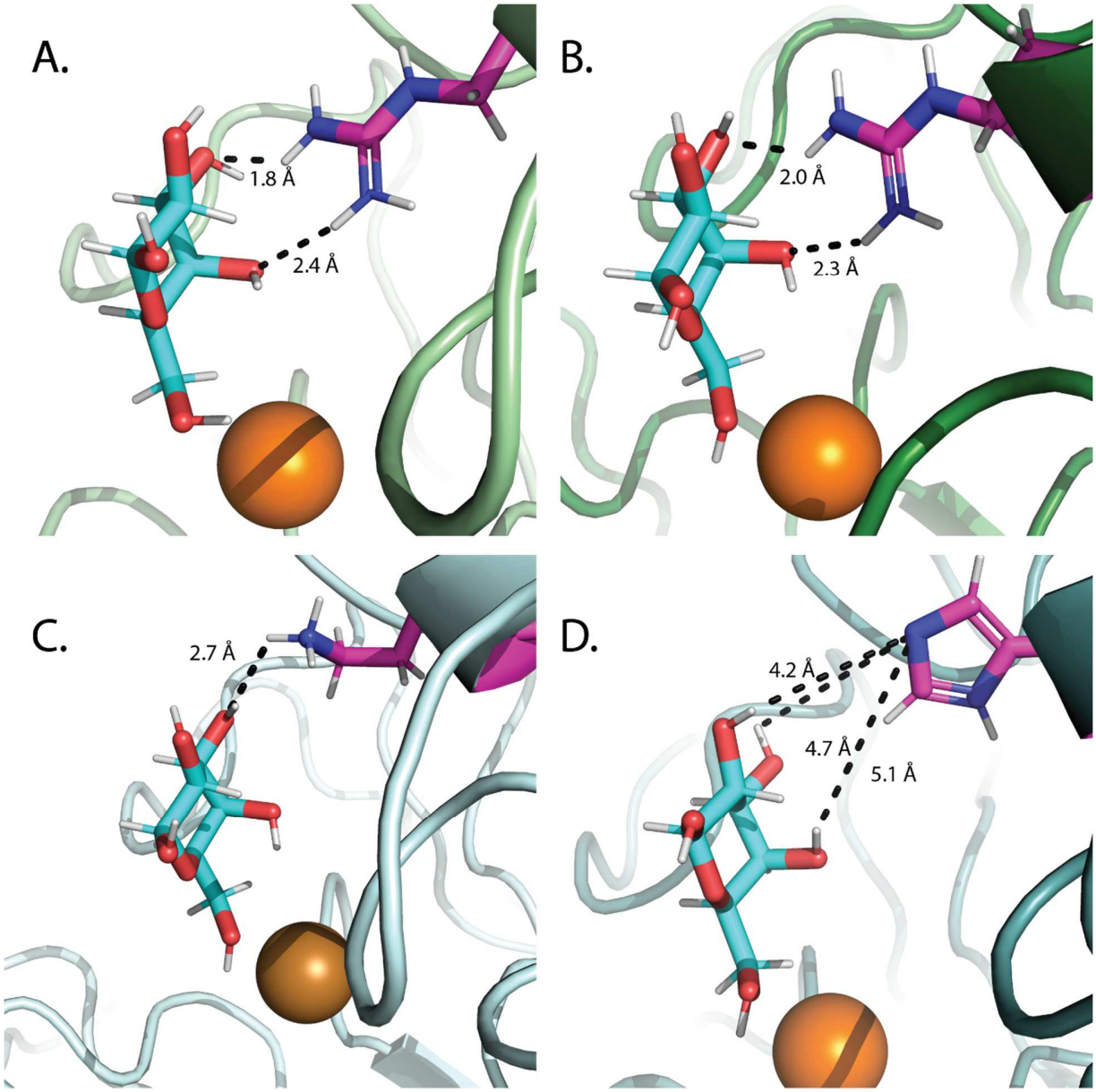


Figure S26. Molecular docking of galactose (cyan) in *Fgr*GalOx (PDB ID 1GOF)[13] (A) and Phyre2 homology models of *Exe*GalOx (B), *Fox*AlcOx (C) and *Afl*AlcOx (D) using AutoDock Vina as implemented in Chimera. The copper atom is depicted as a dark orange sphere, while R330/K330/H330 are depicted as magenta sticks. Interactions with the docked substrate, are indicated in black.

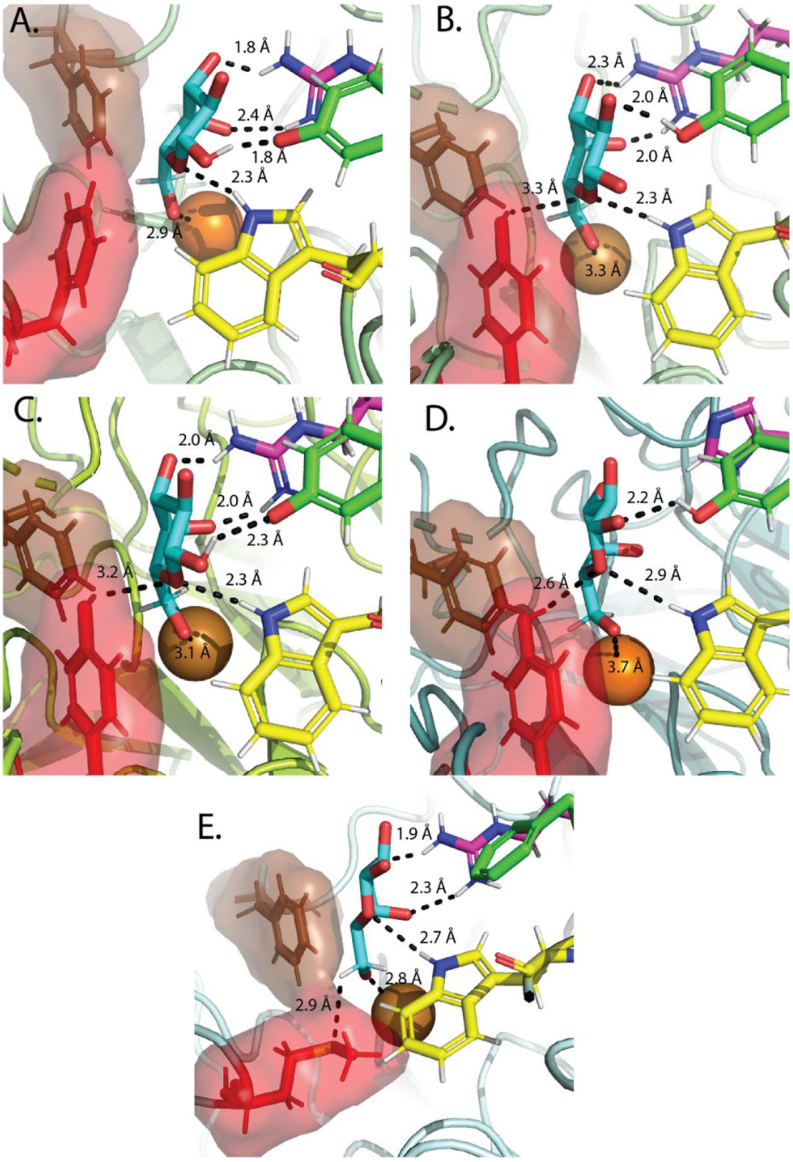


Figure S27. Molecular docking of galactose (cyan) in *Fgr*GalOx (PDB ID 1GOF)[13] (A) and Phyre2 homology models of *Pfe*GalOx (B), *Pru*AlcOx (C), *Afl*AlcOx (D) and *Fox*GalOxB (E) using AutoDock Vina as implemented in Chimera. The copper atom is depicted as a dark orange sphere, while W290, Y329, R330/H330, F494 and F194 are depicted as yellow, green, magenta, brown and red sticks respectively, including surface representation for F194 and F464. Interactions with the docked substrate, are indicated in black.

Supplemental References

1. Deacon SE, Mahmoud K, Spooner RK, Firbank SJ, Knowles PF, Phillips SEV, McPherson MJ. Enhanced Fructose Oxidase Activity in a Galactose Oxidase Variant. ChemBioChem. 2004;5(7):972-9. https://doi.org/10.1002/cbic.200300810
2. Sun L, Petrounia IP, Yagasaki M, Bandara G, Arnold FH. Expression and stabilization of galactose oxidase in Escherichia coli by directed evolution. Protein Eng, Des Sel. 2001;14(9):699-704. https://doi.org/10.1093/protein/14.9.699
3. Escalettes F, Turner NJ. Directed Evolution of Galactose Oxidase: Generation of Enantioselective Secondary Alcohol Oxidases. ChemBioChem. 2008;9(6):857-60. https://doi.org/10.1002/cbic.200700689
4. Deacon SE, McPherson MJ. Enhanced Expression and Purification of Fungal Galactose Oxidase in Escherichia coli and Use for Analysis of a Saturation Mutagenesis Library. ChemBioChem. 2011;12(4):593-601. https://doi.org/10.1002/cbic.201000634
5. Mathieu Y, Offen WA, Forget SM, Ciano L, Viborg AH, Blagova E, Henrissat B, Walton PH, Davies GJ, Brumer H. Discovery of a Fungal Copper Radical Oxidase with High Catalytic Efficiency toward 5-Hydroxymethylfurfural and Benzyl Alcohols for Bioprocessing. ACS Catal. 2020;10(5):3042-58. https://doi.org/10.1021/acscatal.9b04727
6. Cleveland M, Lafond M, Xia FR, Chung R, Mulyk P, Hein J, Brumer H. Two Fusarium Copper Radical Oxidases with High Activity on Aryl Alcohols. Biotechnol Biofuels. 2021;"in revision".
7. Whittaker MM, Whittaker JW. Catalytic Reaction Profile for Alcohol Oxidation by Galactose Oxidase. Biochemistry. 2001;40(24):7140-8. https://doi.org/10.1021/bi010303l
8. Yin D, Urresti S, Lafond M, Johnston EM, Derikvand F, Ciano L, Berrin J-G, Henrissat B, Walton PH, Davies GJ, Brumer H. Structure–function characterization reveals new catalytic diversity in the galactose oxidase and glyoxal oxidase family. Nat Commun. 2015;6(1):10197. https://doi.org/10.1038/ncomms10197
9. Dijkman WP, Fraaije MW. Discovery and Characterization of a 5-Hydroxymethylfurfural Oxidase from Methylovorus sp. Strain MP688. Appl Environ Microbiol. 2014;80(3):1082-90. https://doi.org/10.1128/AEM.03740-13
10. Carro J, Ferreira P, Rodríguez L, Prieto A, Serrano A, Balcells B, Ardá A, Jiménez-Barbero J, Gutiérrez A, Ullrich R, Hofrichter M, Martínez AT. 5-hydroxymethylfurfural conversion by fungal aryl-alcohol oxidase and unspecific peroxygenase. FEBS J. 2015;282(16):3218-29. https://doi.org/10.1111/febs.13177
11. Kadowaki MAS, Godoy MOd, Kumagai PS, Costa-Filho AJd, Mort A, Prade RA, Polikarpov I. Characterization of a New Glyoxal Oxidase from the Thermophilic Fungus *Myceliophthora thermophila* M77: Hydrogen Peroxide Production Retained in 5-Hydroxymethylfurfural Oxidation. Catalysts. 2018;8(10):476. https://doi.org/10.3390/catal8100476
12. Daou M, Yassine B, Wikee S, Record E, Duprat F, Bertrand E, Faulds CB. *Pycnoporus cinnabarinus* glyoxal oxidases display differential catalytic efficiencies on 5-hydroxymethylfurfural and its oxidized derivatives. Fungal Biol Biotechnol. 2019;6(1):4. https://doi.org/10.1186/s40694-019-0067-8
13. Ito N, Phillips SEV, Yadav KDS, Knowles PF. Crystal Structure of a Free Radical Enzyme, Galactose Oxidase. J Mol Biol. 1994;238(5):794-814. https://doi.org/10.1006/jmbi.1994.1335