Supplementary information

Switching the substrate preference of fungal aryl-alcohol oxidase: Towards stereoselective oxidation of secondary benzyl alcohols

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This Supplementary information includes **Tables S1-S3**, **Figs. S1-S4**, and supplementary **references**

Table S1. Spectroscopic properties of AAO and the nine variants analyzed, in 50 mM phosphate, pH 6.0.

	$\lambda^{band\ I}$ (nm)	$\lambda^{band\ II}$ (nm)	$\varepsilon^{band\ I}$ ($M^{-1}cm^{-1}$) [a]
AAO	386	463	11050[1]
Y92F	386	463	10044[2]
I500A	386	457	9925
I500M	384	458	9609
I500W	388	461	9668
F501A	387	462	10389[3]
F501W	387	462	9944[3]
L315A/I500A	385	458	9904
I391A/I500A	386	458	10089
I500M/F501W	386	460	9290

[a]Taken from literature[1-3] or estimated here.

Table S2. Chromatographic conditions for secondary alcohol resolution by chiral HPLC.

	n-Hexane/ isopropanol	*R* (min)	*S* (min)	Standard (RT, min)
1-(*p*-Methoxyphenyl)-ethanol	98:2	18.8	20.0	2-phenyl-2-propanol (9.1)
1-Phenyl-1-propanol	98:2	10.1	11.6	2-phenyl-2-propanol (9.1)
1-Phenyl-2-methypropanol	99:1	12.0	12.5	4-methoxythioanisole (8.8)

Table S3. Oxidation rate (k_{obs}), conversion yield, and (*R*)-1-(*p*-methoxyphenyl)-ethanol *ee* in 24-h reactions of (±)1-(*p*-methoxyphenyl)-ethanol (5 mM) with AAO and nine variants (5 µM) in 50 mM phosphate, pH 6.0, at 25ºC.

	k_{obs} (min^{-1})	Conversion (%)[a]	*ee* (%)
AAO	3.8	34	51
Y92F	1.8	20	25
I500A	57.8	50	100
I500M	-	50	100
I500W	0	0	0
F501A	7.5	46	87
F501W	1.9	7	15
L315A/I500A	4.1	37	59
I391A/I500A	0.6	8	9
I500M/F501W	-	50	100

[a]Referred to the racemic mixture

Supplementary references

1. F. J. Ruiz-Dueñas, P. Ferreira, M. J. Martínez and A. T. Martínez, *Protein Express. Purif.*, 2006, **45,** 191-199.
2. P. Ferreira, A. Hernández-Ortega, K. Borrelli, F. Lucas, B. Herguedas, V. Guallar, A. T. Martínez and M. Medina, *FEBS J.*, 2015, **282,** 3091-3106.
3. A. Hernández-Ortega, F. Lucas, P. Ferreira, M. Medina, V. Guallar and A. T. Martínez, *J. Biol. Chem.*, 2011, **286,** 41105-41114.

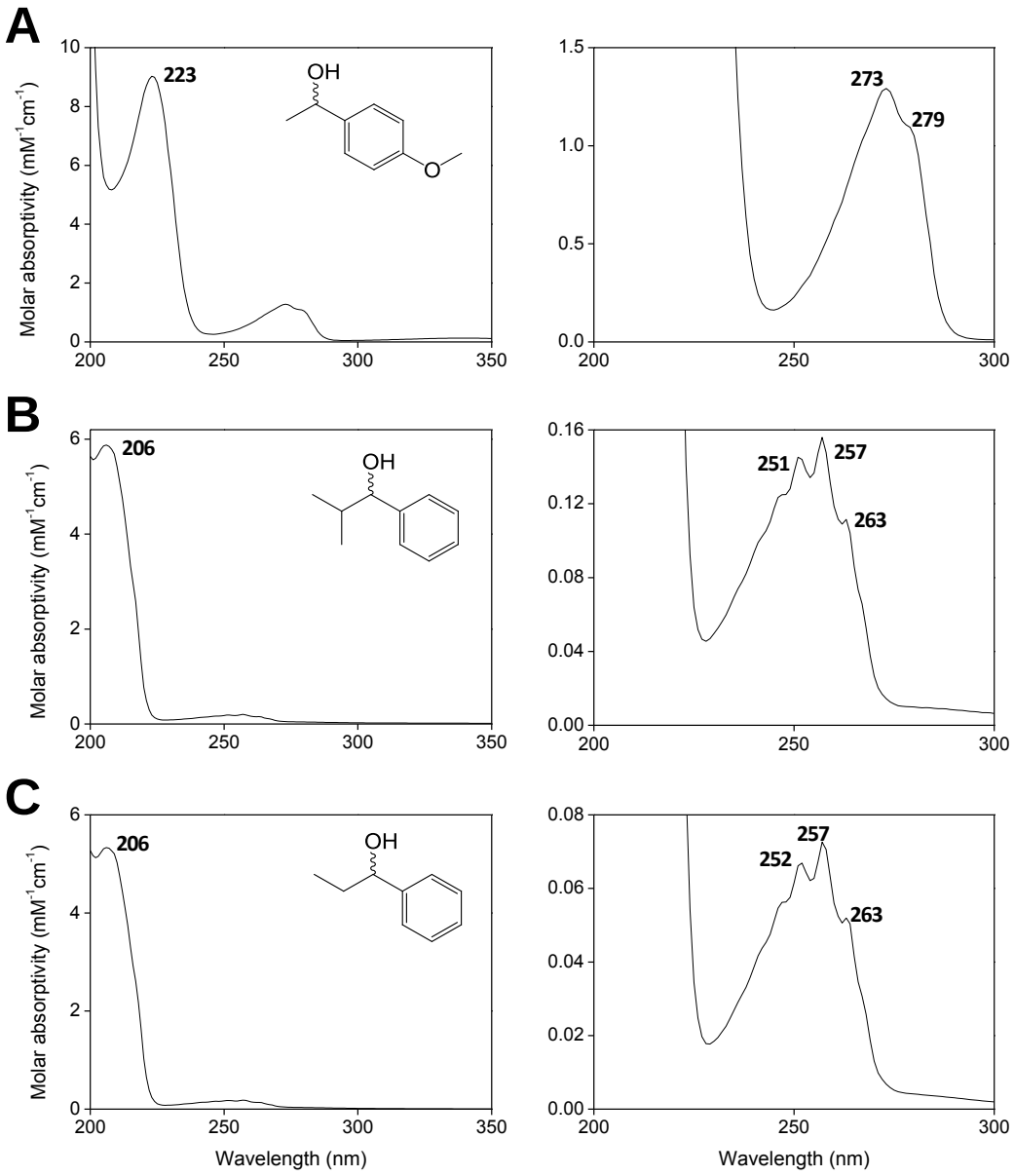

Fig. S1. Molar absorptivity spectra of (±)1-(*p*-methoxyphenyl)-ethanol (**A**), (±)1-phenyl-1-propanol (**B**) and (±)1-phenyl-2-methylpropanol (**C**) in the ultraviolet region (*left*) and enlarged 250-280 nm peak (*right*).

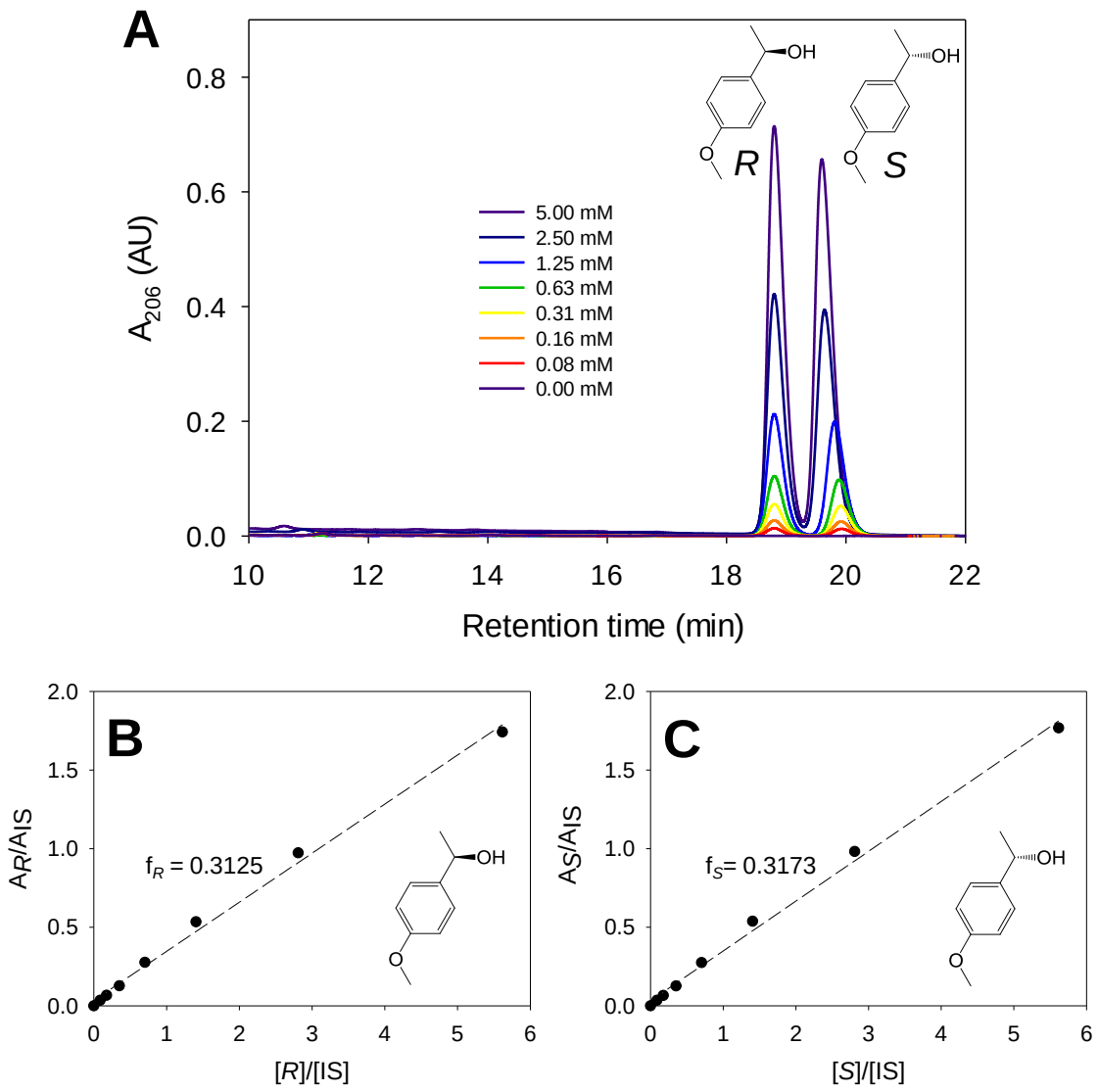

Fig. S2. Separation of different concentrations of (±)1-(*p*-methoxyphenyl)-ethanol by chiral HPLC (**A**) and calibration curves of *R* (**B**) and *S* (**C**) enantiomers yielding response factors (f_R and f_S) using an internal standard (IS), 2-phenyl-2-propanol (not shown).

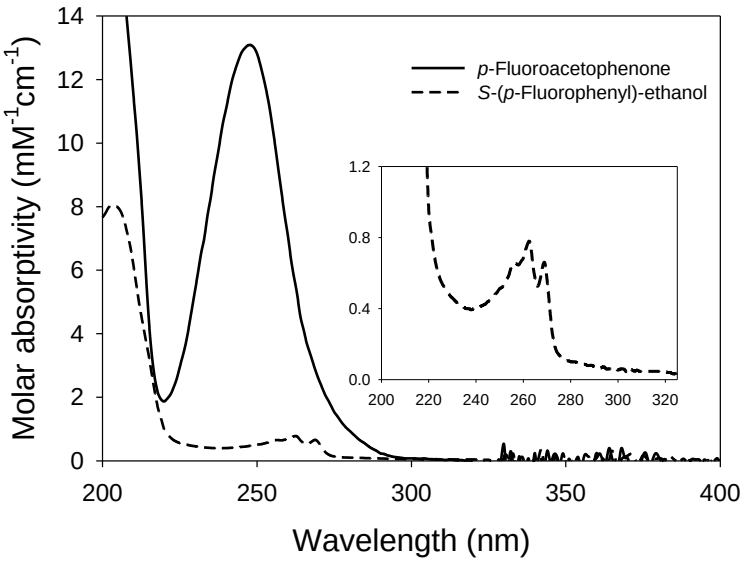

Fig. S3. Molar absorptivity spectra of (*S*)-1-(*p*-fluorophenyl)-ethanol (*dashed line*) and *p*-fluoroacetophenone (*solid line*). The inset shows an enlargement of the alcohol spectrum.

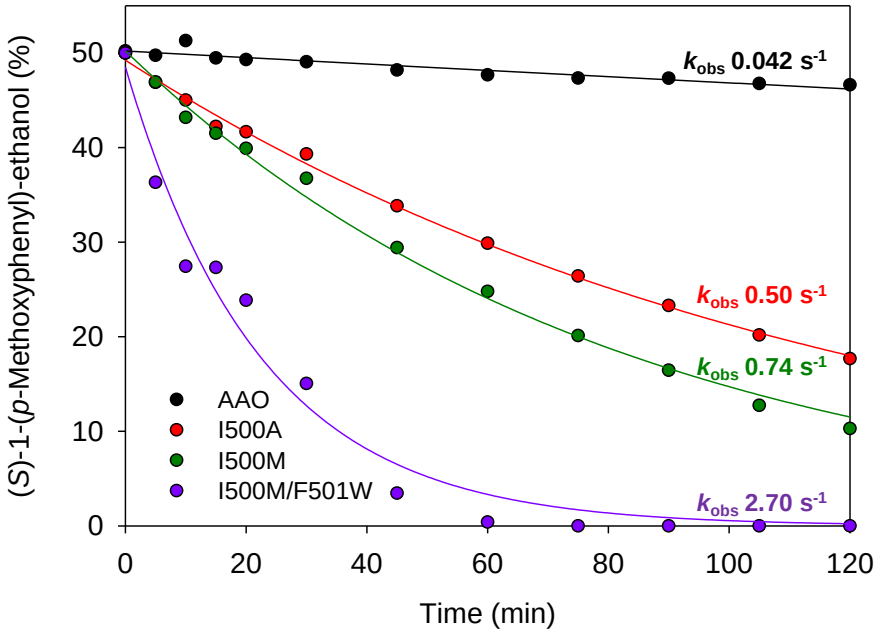

Fig. S4. Oxidation rates of (*S*)-1-(*p*-methoxyphenyl)-ethanol by AAO and three selected variants during 2-h incubation. Reactions between alcohol (2.5 mM, racemic mixture) and enzyme (2.5 µM) were performed in 50 mM phosphate, pH 6.0, at 25ºC.