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SUPPORTING INFORMATION

Stereoelectronic effects in the reaction of aromatic substrates catalysed by *Halomonas elongata* transaminase and its mutants

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***Table S.1**: retention times of ketones, aldehydes, and amines in isocratic run with 25% acetonitrile/75% water with TFA 0.1% v/v at 25 °C with a flow rate of 1 mL/min (Supelcosil LC-18-T column (250 mm x 4.6 mm, 5 µm particle size; Supelco, Sigma-Aldrich, Germany)*

Ketones/Aldehydes	Retention time	Amines	Retention time
Acetophenone	12.2 min	(*S*)-Phenylethylamine	4.3 min
p-Nitroacetophenone	16.2 min	(*S*)-p-Nitrophenylethanamine	4.8 min
m-Nitroacetophenone	15.1 min	(*S*)-m-Nitrophenylethanamine	4.7 min
o-Nitroacetophenone	12.9 min	(S)-o-Nitrophenylethanamine	4.4 min
p-Fluoroacetophenone	14.3 min	(*S*)-p-Fluorophenylethanamine	4.6 min
m-Fluoroacetophenone	14.5 min	(*S*)-m-Fluorophenylethanamine	4.6 min
o-Fluoroacetophenone	14.7 min	(*S*)-o-Fluorophenylethanamine	4.2 min
p-Trifluoroacetophenone	39.4 min	n.a.	-
m-Trifluoroacetophenone	46.6 min	n.a.	-
o-Trifluoroacetophenone	35.9 min	n.a.	-
p-Methoxyacetophenone	14.8 min	(*S*)-p-Methoxyphenylethanamine	4.6 min
m-Methoxyacetophenone	14.4 min	(*S*)-m-Methoxyphenylethanamine	4.7 min
o-Methoxyacetophenone	15.9 min	(*S*)-o-Methoxyphenylethanamine	5.1 min
p-Methylacetophenone	22.2 min	(*S*)-p-Methylphenylethanamine	5.9 min
m-Methylacetophenone	22.1 min	n.a.	-
o-Methylacetophenone	22.7 min	n.a.	-
Benzaldehyde	10.6 min	phenylmethanamine	3.7 min
p-Nitrobenzaldehyde	12.2 min	*p*-Nitrophenylmethanamine	4.2 min
m-Nitrobenzaldehyde	12.0 min	*m*-Nitrophenylmethanamine	4.1 min
o-Nitrobenzaldehyde	12.3 min	*o*-Nitrophenylmethanamine	3.7 min
p-Fluorobenzaldehyde	12.3 min	*p*-Fluorophenylmethanamine	4.1 min
m-Fluorobenzaldehyde	13.5 min	*m*-Fluorophenylmethanamine	4.0 min
o-Fluorobenzaldehyde	12.2 min	*o*-Fluorophenylmethanamine	3.7 min
p-Trifluorobenzaldehyde	41.2 min	*p*-Trifluorophenylmethanamine	7.2 min
m-Trifluorobenzaldehyde	38.8 min	*m*-Trifluorophenylmethanamine	7.1 min
o-Trifluorobenzaldehyde	39.8 min	*o*-Trifluorophenylmethanamine	5.4 min
p-Methoxybenzaldehyde	16.2 min	*p*-Methoxyphenylmethanamine	4.3 min
m-Methoxybenzaldehyde	16.8 min	*m*-Methoxyphenylmethanamine	4.2 min
o-Methoxybenzaldehyde	15.6 min	*o*-Methoxyphenylmethanamine	4.2 min
p-Methylbenzaldehyde	18.7 min	*p*-Methylphenylmethanamine	4.8 min
m-Methylbenzaldehyde	20.4 min	*m*-Methylphenylmethanamine	4.7 min
o-Methylbenzaldehyde	19.4 min	*o*-Methylphenylmethanamine	4.4 min

2 – Transaminases' stability

Stability of transaminases in the reaction conditions tested was determined by spectrophotometric enzyme assay performed with phosphate buffer 50 mM pH 8 in the presence of pyridoxal 5'-phosphate (PLP) 0.1 mM, (*S*)-PEA 25 mM and Pyruvate 25 mM. The analysis of the residual activity was carried out at 25 °C and 100% corresponds to the activity before incubation (3.99 ± 0.02 U/mg for wild type enzyme, 4.43 ± 0.02 U/mg for W56G, 0.50 ± 0.01 for Y149F, 0.06 ± 0.01 U/mg for F84A and 0.43 ± 0.02 for I258A). Each reaction was performed in triplicates and the results reported as the average of the data obtained.

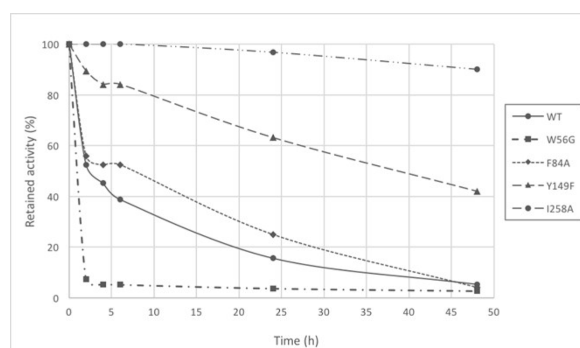

Figure S.1: *temperature stability profile of HEWT WT and mutants*

3 – In silico mutations evaluation

Table S.2 shows the docking energies (expressed in Kcal/mol) for the WT enzyme and different mutants at each position calculated against four different molecules. The natural substrate (*S*)-PEA was used as reference compound to tune the methodology; subsequently the *ortho*-, *meta*-, and *para*-nitro substituted acetophenones were tested to identify the more plausible mutations able enhance binding of such substrates.

Table S.2: *Substrates' docking energies (Kcal/mol) determined by the simulation software Autodock Vina.*

Position	**Mutation**	**(*S*)-PEA**	***o-NO$_2$***	***m-NO$_2$***	***p-NO$_2$***
W56	-	-7.5	-4.2	-5.4	-3.1
	G	**-7.6**	**-8.3**	**-7.3**	**-7.6**
	A	-7.5	-6.7	-6.4	-6.7
	L	-7.2	-6.3	-6.3	-6.2
	F	-7.6	-5-5	-5.7	-5.2
F84	-	-7.5	-4.2	-5.4	-3.1
	G	-	-7-6	-6.9	-4.1
	A	**-3.0**	**-8.0**	**-7.5**	**-4.3**
	L	-6.7	-6.1	-6.4	-4.0
	W	-6.7	-5-0	-6.0	-2.5
Y149	**-**	-7.5	-4.2	*-5.4*	*-3.1*
	G	-7.4	-5.7	*-6.3*	*-4.4*
	A	-7.1	-5.8	*-6.2*	*-4.1*
	F	**-7.2**	**-5.1**	**-5.6**	**-3.4**
I258	**-**	-7.5	-4.2	-5.4	-3.1
	G	-6.2	-6.5	-7.0	-7.3
	A	**-6.8**	**-6.6**	**-7.3**	**-7.4**
	V	-7.2	-6.2	-5.1	-4.2