

Supporting Information

Robust ω -Transaminases by Computational Stabilization of the Subunit Interface

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Table S1. Data collection and refinement values.

	<i>PjTA-R4</i>	<i>PjTA-R6</i>
Data collection		
Beamline	In-house	ESRF/ID30A-3
Wavelength (Å)	1.54179	0.96770
Space group	P4 ₃	P4 ₃
Unit cell dimensions a,b,c, (Å)	98.1, 98.1, 119.3	98.9, 98.9, 120.3
Resolution (Å) ^A	49.03 – 1.95 (1.99-1.95)	45.74 – 1.85 (1.88-1.85)
Total observations	607461 (31957)	584793 (29557)
Unique reflections	82063 (4522)	98464 (4860)
$\langle I/\sigma \rangle^A$	16.7 (2.5)	13.0 (1.5)
CC _(1/2)	0.999 (0.743)	0.999 (0.569)
Completeness (%) ^A	100.0 (100.0)	100.0 (100.0)
R _{merge} (%) ^{A, B}	9.0 (70.1)	6.9 (120.8)
Refinement		
R _{work} /R _{free} (%) ^B	14.0 / 16.9	15.6 / 18.6
Number of non-H atoms		
Protein	6991 (2 chains)	6984 (2 chains)
Solvent	638	534
Cofactor	62 (2 PLP)	64 (2 PLP)
Average B (Å ²)	18.2	32.0
RMSD		
Bond lengths (Å)	0.009	0.006
Bond angles (°)	1.5	1.4
Ramachandran plot % favoured, outliers	93.06, 1.25	93.7, 1.3
PDB entry	6TB0	6TB1

^A Values in parentheses correspond to highest resolution shell.

^B R_{free} is calculated as R_{work} using 5% of all reflections randomly chosen, which were excluded from structure refinement.

Table S2. Potentially stabilizing mutations predicted by Rosetta and FoldX energy calculations.

Mutation	$\Delta\Delta G^{\text{Predicted}}$ (kJ/mol/subunit)			ΔT_m^{app} (°C) ^A	Location	B-factor rank	Stricter energy criteria passed
	FoldX	Rosetta row 3	Rosetta row 16				
S6K	0.4	-2.7	-4.6	0.5	Surface	1	-
P9A	9.0	-4.3	3.9	2.0	Surface	54	-
P9K	3.4	-7.4	5.4	1.5	Surface	54	-
P9R	3.1	-5.0	12.1	0.5	Surface	54	-
I13K	-3.4	4.8	13.4	0.0	Surface	82	-
N22D	-9.3	8.5	3.4	0.0	Surface	88	-
N22E	-3.8	12.9	5.4	-0.5	Surface	88	-
A23L	-9.2	0.9	-16.8	0.0	Surface	107	+
H26F	-3.3	5.1	9.2	0.0	Surface	84	-
H26M	-2.6	11.6	16.1	0.0	Surface	84	-
E28K	-2.2	-8.2	-8.0	0.5	Surface	4	-
E35L	-4.3	-13.4	-12.0	-0.5	Surface	39	+
E38K	1.2	-9.9	-7.5	1.5	Surface	25	-
E38Q	0.3	-5.5	-6.4	2.0	Surface	25	-
E38N	1.3	-3.0	-0.4	-0.5	Surface	25	-
E38A	3.0	-9.1	-2.8	0.5	Surface	25	-
E38H	1.6	-6.6	0.2	0.0	Surface	25	-
E38S	-0.9	-10.5	-3.2	-0.5	Surface	25	-
D45F	-6.4	-8.8	-11.1	-1.0	Surface	44	+
D45I	-6.1	-5.7	-9.0	0.5	Surface	44	+
D45K	-4.4	-1.1	6.1	-1.5	Surface	44	-
D45V	-3.2	-2.5	-2.6	0.0	Surface	44	-
D45W	-4.5	-15.9	-12.8	-0.5	Surface	44	+
D45Y	-7.1	-11.5	-12.0	-1.5	Surface	44	+
Q46K	-0.2	-3.0	5.0	0.5	Surface	20	-
Q46N	0.1	-4.3	4.4	-0.5	Surface	20	-
G49A	-0.5	-4.0	-5.7	-2.5	Surface	233	-
G49P	-6.7	6.4	-11.0	-0.5	Surface	233	+
N66D	-9.5	14.6	17.2	No Signal	Surface	243	-
E74I	-7.8	-11.2	-15.3	5.5	Surf & Interf	83	+
N78K	-0.1	-4.1	3.8	1.0	Surf & Interf	164	-
F86W	2.9	-12.9	-13.3	4.0	Interface	267	-
S87D	-3.0	1.1	-3.1	7.0	Interface	138	-
S87H	-2.0	-15.1	-15.7	1.0	Interface	138	-
S87N	-4.1	-9.9	-6.8	1.5	Interface	138	-
S87Q	-3.8	-4.8	2.8	1.0	Interface	138	-
K89Q	3.4	-3.7	-13.3	0.5	Interface	184	-
K89A	1.2	-4.4	-7.6	2.0	Interface	184	-
K89F	-6.2	-19.0	-23.5	6.0	Interface	184	+
K89L	-3.7	1.1	-11.1	4.5	Interface	184	-
K89M	-6.0	-5.9	-13.0	3.5	Interface	184	+
K89W	8.2	-15.9	-29.4	3.5	Interface	184	-
K89Y	-0.8	-16.6	-19.6	4.0	Interface	184	-
H91F	4.8	-4.1	-8.6	-1.0	Surface	259	-
S94A	-2.0	-9.4	-12.9	8.0	Interface	276	-
E99K	-2.1	-7.6	10.5	-2.0	Surface	118	-
E103A	3.1	-4.1	2.8	-0.5	Surface	36	-
E103K	0.1	-6.5	5.6	0.0	Surface	36	-

Mutation	$\Delta\Delta G^{\text{Predicted}}$ (kJ/mol/subunit)			ΔT_m^{app} (°C) ^A	Location	B-factor rank	Stricter energy criteria passed
	FoldX	Rosetta row 3	Rosetta row 16				
E103S	3.6	-3.5	6.1	No Signal	Surface	36	-
M109F	-0.1	-9.6	-19.5	-1.5	Surface	200	-
M109Y	7.9	-6.8	-0.5	-1.0	Surface	200	-
S110Q	-3.8	-4.7	2.3	1.0	Surface	211	-
N116L	-8.8	-2.3	-8.9	-1.0	Interface	422	+
G118A	-4.2	-6.6	-5.7	-1.5	Interface	454	-
G118E	-8.6	31.1	43.2	-0.5	Interface	454	-
G118L	-9.0	23.7	23.4	-0.5	Interface	454	-
G118M	-10.3	7.0	11.5	-1.5	Interface	454	-
G118P	-14.2	-1.4	-12.1	-1.5	Interface	454	+
G118Y	-9.5	24.6	24.6	-4.5	Interface	454	-
M128F	-5.0	-15.5	-14.8	4.5	Interface	269	+
M128L	2.4	-7.8	-3.6	-0.5	Interface	269	-
M128W	9.2	-10.6	5.6	0.5	Interface	269	-
M128Y	-1.5	-10.8	-8.8	0.0	Interface	269	-
Y131F	-3.1	12.2	8.0	-1.5	Surface	176	-
N133A	-2.6	0.1	1.4	No Signal	Surface	145	-
P139K	5.6	-3.2	4.5	1.0	Surface	94	-
A140K	-4.1	-2.2	-0.8	0.0	Surface	131	-
A140Q	-3.1	-1.1	6.3	No Signal	Surface	131	-
N149G	-3.0	1.8	2.7	2.5	Surface	294	-
N149K	-2.8	4.6	5.7	No Signal	Surface	294	-
N149R	-5.3	4.1	16.5	0.5	Surface	294	-
G150A	-5.1	-10.5	-3.0	0.0	Buried	437	-
G150S	-1.8	-9.4	-2.3	No Signal	Buried	437	-
H152F	-6.8	4.2	-1.6	-1.0	Buried	428	-
H152Y	-2.7	4.5	0.9	-2.0	Buried	428	-
I154D	12.0	-3.2	-0.8	4.0	Interface	322	-
I154N	13.3	-7.0	-5.7	3.5	Interface	322	-
I154V	3.1	-10.3	-5.2	9.0	Interface	322	-
G166S	-0.8	-6.6	1.1	-0.5	Surface	142	-
Q168K	-8.0	9.2	14.4	0.0	Surface	197	-
G182P	-5.9	5.7	-10.3	-1.5	Surface	280	+
G182S	-3.6	-3.4	-0.3	-3.0	Surface	280	-
C183P	-5.6	3.1	-0.3	-7.5	Surface	250	-
Y187F	-3.7	1.4	-4.9	-1.3	Surface	225	-
Y187M	-3.6	15.2	14.1	-1.5	Surface	225	-
F189Y	1.8	-2.8	3.9	0.5	Surface	112	-
L191A	0.8	1.0	-2.9	-0.5	Surface	8	-
A192P	-3.7	-5.6	-7.0	0.5	Surface	7	-
E196P	-3.6	7.1	-0.4	-0.5	Surface	41	-
H198A	1.6	-4.6	-4.4	0.5	Surface	11	-
H198Q	-0.9	-5.0	-4.1	0.5	Surface	11	-
H198R	-0.9	-3.8	3.8	0.0	Surface	11	-
D201N	0.8	-4.8	0.9	-0.5	Surface	69	-
V205R	-2.9	0.9	-0.5	-2.5	Surface	80	-
E206Y	-5.7	-5.6	-10.7	0.5	Surface	98	+
K210L	-5.0	-5.0	-5.1	-0.5	Surface	34	-
T218K	1.3	-2.7	1.8	0.0	Surface	122	-
M228L	-1.1	-8.7	2.1	No Signal	Buried	238	-

Mutation	$\Delta\Delta G^{\text{Predicted}}$ (kJ/mol/subunit)			ΔT_m^{app} (°C) ^A	Location	B-factor rank	Stricter energy criteria passed
	FoldX	Rosetta row 3	Rosetta row 16				
V235R	-3.4	10.1	13.8	-0.5	Surface	282	-
R238H	-3.1	7.4	7.7	0.5	Surface	30	-
E242A	0.1	-3.8	-1.4	0.5	Surface	32	-
E242K	-0.6	-4.6	-4.0	-0.8	Surface	32	-
E242P	-4.1	7.2	2.4	-0.5	Surface	32	-
C248L	-8.8	-10.4	-19.6	-1.0	Surface	158	+
C248M	-2.8	0.7	9.7	0.5	Surface	158	-
D252K	-0.9	-3.6	-4.7	0.0	Surface	46	-
D252N	-0.6	-7.7	-4.3	No signal	Surface	46	-
V255M	-4.7	9.9	-5.4	-2.0	Surface	220	-
V255L	-5.8	5.3	-3.2	-2.0	Surface	220	-
D258N	5.1	-8.2	-11.1	0.0	Buried	444	-
V260I	-2.1	-4.1	-8.8	-0.5	Buried	448	-
V260L	-1.3	-2.7	-2.5	-1.0	Buried	448	-
C262L	-6.3	6.3	11.3	0.0	Buried	373	-
C262M	-5.0	-0.6	6.3	0.0	Buried	373	-
C262P	-5.9	1.3	-1.5	0.0	Buried	373	-
G263A	-0.8	-6.5	-8.6	0.5	Buried	404	-
Q269H	0.3	-5.6	-2.0	0.5	Surface	109	-
Q269N	0.3	-4.2	0.8	0.5	Surface	109	-
Q269S	2.2	-4.2	1.3	No signal	Surface	109	-
S273A	-0.9	-7.0	-0.9	0.0	Surface	265	-
Q279K	-1.4	-3.8	-0.4	-4.0	Surface	74	-
Q279N	0.5	-3.6	-1.2	-0.5	Surface	74	-
Q279H	-0.5	-5.9	5.3	No Signal	Surface	74	-
M283I	1.0	-3.8	4.1	-3.0	Buried	335	-
Q288H	19.4	-6.2	-10.2	No signal	Buried	389	-
N302D	-5.6	10.4	12.0	0.5	Surface	219	-
A303P	-4.3	4.1	-2.9	0.5	Surface	133	-
A303Q	-2.5	1.9	1.3	No signal	Surface	133	-
P304A	7.5	-4.8	-4.4	-1.0	Surface	106	-
V305F	-3.0	13.1	1.6	-0.5	Surface	189	-
V305M	-4.8	12.9	7.0	0.0	Surface	189	-
E307H	2.9	-3.6	-10.5	0.0	Surface	75	-
E307Q	0.5	-5.3	-3.7	0.0	Surface	75	-
D311N	-1.3	-4.4	-2.1	-0.5	Surface	18	-
D311Q	0.6	-3.9	4.0	No signal	Surface	18	-
Q312K	-3.3	2.5	8.3	0.5	Surface	22	-
Q314K	-1.5	-3.9	0.6	0.0	Surface	15	-
L319F	-6.9	-4.9	-12.9	4.0	Interface	117	+
L319Y	-2.9	-5.9	-13.0	-0.5	Interface	117	-
T324L	-2.8	9.5	6.8	-1.5	Interface	256	-
G325W	9.6	-2.8	-4.3	-3.5	Interface	320	-
G325Y	7.8	-5.1	-15.6	-1.0	Interface	320	-
A333Q	-2.3	-2.8	-3.9	-0.5	Surface	330	-
A333H	-3.0	2.9	-1.4	-0.5	Surface	330	-
E345A	0.3	-4.8	-3.5	-2.5	Surface	66	-
E345K	-4.5	-9.6	-6.4	-3.0	Surface	66	-
E345M	-5.8	-7.2	-8.8	-2.0	Surface	66	+
E345N	-1.6	-5.5	-7.1	-3.0	Surface	66	-

Mutation	$\Delta\Delta G^{\text{Predicted}}$ (kJ/mol/subunit)			ΔT_m^{app} (°C) ^A	Location	B-factor rank	Stricter energy criteria passed
	FoldX	Rosetta row 3	Rosetta row 16				
S346G	-3.8	0.2	-1.6	No signal	Surface	121	-
S346K	-7.3	-8.1	-7.6	0.0	Surface	121	-
S346R	-3.3	-3.1	1.8	-0.5	Surface	121	-
V348I	-3.3	8.7	7.0	-1.0	Surface	203	-
H350W	-5.2	-10.7	-10.1	0.5	Surface	190	+
Q353K	-0.1	-3.6	1.4	0.8	Surface	119	-
R359I	-5.8	-4.8	-13.0	-0.3	Surface	47	+
R359L	-7.9	-10.8	-14.4	0.3	Surface	47	+
S360A	-1.1	-4.7	-9.8	-0.8	Surface	104	-
S360K	-2.6	-6.0	-3.7	-0.8	Surface	104	-
Q363K	-3.3	-4.0	-2.4	-0.5	Surface	93	-
D367K	-0.5	-4.7	-0.5	0.5	Surface	155	-
D367N	0.6	-5.6	-11.3	0.5	Surface	155	-
D367T	1.9	-4.7	-6.5	No signal	Surface	155	-
D388N	5.2	-6.2	-2.5	0.0	Surface	115	-
V390Q	-3.0	5.7	12.3	0.0	Surface	99	-
V390P	-3.4	30.5	2.9	No signal	Surface	99	-
S391K	1.8	-3.5	-5.4	-0.5	Surface	151	-
K392G	-2.9	-2.3	-0.3	0.0	Surface	185	-
A393R	-3.9	4.6	7.1	1.5	Surface	201	-
Q396K	-4.6	-13.1	-2.9	-0.5	Surface	174	-
Q396E	-0.4	-3.1	2.6	-0.5	Surface	174	-
Q396G	-0.7	-16.8	-6.6	-0.5	Surface	174	-
Q396R	-4.9	-12.8	3.9	No signal	Surface	174	-
Q396S	-1.3	-4.6	0.7	-0.5	Surface	174	-
A397R	-2.7	4.0	8.3	0.0	Surface	182	-
L398P	-3.5	-3.8	-5.5	-0.5	Surface	135	-
L398Y	-3.2	1.9	-3.2	-1.5	Surface	135	-
T400F	-2.8	15.5	2.6	No signal	Surface	321	-
R403A	-0.1	-2.8	-8.5	0.5	Surface	140	-
M405F	6.3	-13.1	-10.0	-0.5	Buried	287	-
M405H	11.3	-6.8	-9.6	0.0	Buried	287	-
M405L	1.9	-4.0	-4.5	-0.5	Buried	287	-
M405W	23.8	-11.9	-0.8	No signal	Buried	287	-
G407A	-3.9	-9.4	-6.1	0.0	Surface	383	-
G407F	-4.5	-6.8	-9.0	0.0	Surface	383	+
G407I	-5.0	-12.4	-9.7	0.0	Surface	383	+
G407N	-3.6	-7.6	-4.0	-0.3	Surface	383	-
G407S	-3.6	-4.2	3.6	No signal	Surface	383	-
G407Y	-4.5	-8.3	-13.2	0.0	Surface	383	+
A409M	-4.1	0.6	3.4	-1.5	Surface	408	-
Q410H	-2.6	0.6	0.5	0.5	Surface	240	-
E411K	-0.6	-2.7	2.5	0.0	Surface	86	-
E411Q	-1.1	-4.9	2.4	0.0	Surface	86	-
A418P	-4.5	7.2	-2.5	0.5	Surface	299	-
M419F	14.3	-3.1	-2.7	0.5	Surface	206	-
M419I	3.5	-6.2	-1.7	1.0	Surface	206	-
M419L	2.3	-4.2	5.3	1.5	Surface	206	-
V423I	-3.0	3.4	2.7	No signal	Buried	365	-
V423L	-3.3	8.9	10.7	-0.5	Buried	365	-

Mutation	$\Delta\Delta G^{\text{Predicted}}$ (kJ/mol/subunit)			ΔT_m^{app} (°C) ^A	Location	B-factor rank	Stricter energy criteria passed
	FoldX	Rosetta row 3	Rosetta row 16				
N432D	-7.3	10.7	12.9	0.0	Surface	251	-
E433A	5.0	-9.4	-1.9	0.0	Surface	70	-
E433K	2.5	-8.7	-6.5	0.5	Surface	70	-
E433P	-1.5	-4.5	-5.7	0.5	Surface	70	-
E433Q	2.2	-3.2	-1.9	0.0	Surface	70	-
G437A	-3.6	-8.4	-1.1	0.0	Surface	306	-
G437K	-5.1	-12.2	-5.3	0.0	Surface	306	-
G437N	-0.8	-5.9	-3.2	-1.0	Surface	306	-
G437Y	-9.4	-11.1	-16.1	0.5	Surface	306	+
R442K	-5.9	0.2	1.2	0.0	Surface	307	-
D448W	-6.5	-16.5	-12.6	-2.0	Surface	148	+
D448R	-5.0	-10.0	-5.6	-0.5	Surface	148	-
D448A	-2.5	-9.5	-10.5	-1.0	Surface	148	-
D448H	-2.5	-6.2	-6.2	-1.5	Surface	148	-
D448K	-4.6	-11.4	-5.7	-1.0	Surface	148	-
D448N	-2.0	-6.3	-5.9	-1.0	Surface	148	-
D448Q	-3.2	-8.1	-8.8	-1.0	Surface	148	-
D448S	-0.2	-3.9	1.0	-1.0	Surface	148	-
T450L	-11.3	-4.7	-14.6	-1.5	Surface	325	+
T450V	-6.3	-5.0	-6.6	0.0	Surface	325	-
T451F	-3.2	-3.9	-5.5	-0.5	Surface	217	-
T451K	-4.7	-3.6	-5.2	0.0	Surface	217	-
T451Q	-2.7	-1.7	-4.9	0.0	Surface	217	-
T451R	-3.9	-3.2	-1.6	No signal	Surface	217	-
T451W	-4.9	-9.5	-12.8	0.0	Surface	217	+
Q452K	-0.7	-4.0	1.7	0.0	Surface	43	-
Q452A	1.2	-2.8	1.8	0.0	Surface	43	-
V454L	-2.8	20.3	18.5	-0.5	Surface	116	-

^A T_m^{app} of wild-type *PjTA* (WT) is 62 °C.

Table S3. Success rates amongst point mutations in FRESCO libraries.

Enzyme	Number of mutations ^A		Fraction	Reference
	Tested	Stabilizing	successful (%)	
Haloalkane dehalogenase LinB	109	11	10	Floor et al. ¹
Halohydrin dehalogenase HheC	218	29	13	Arabnejad et al. ²
HMF oxidase	140	17	12	Martin et al. ³
Limonene epoxide hydrolase	64	11	17	Wijma et al. ⁴
Peptide amidase	120 ^B	12 ^B	10	Wu et al. ⁽⁵⁾
Xylanase	105	10	10	Bu et al. ⁽⁶⁾
Cyclohexanone monooxygenase	128	16	13	Fürst et al. ⁽⁷⁾
Transaminase (global)	236 ^B	32 ^B	13	This study
Transaminase (subunit interface)	34	19	56	This study

^A Number of mutations examined in wild-type template.

^B Includes mutations suggested by consensus analysis.

Table S4. Effect of protein structure on the predictions by Rosetta and FoldX energy calculations.

X-ray structure	$\Delta\Delta G^{\text{Predicted}}$ (kJ/mol/subunit) by FoldX			$\Delta\Delta G^{\text{Predicted}}$ (kJ/mol/subunit) by Rosetta Row3		
	6G4B	6G4C	6G4D	6G4B	6G4C	6G4D
P9A	8.9	9.2	8.8	-5.6	-1.8 ^A	-5.6
P9K	2.7	3.0	4.5	-8.8	-5.1	-8.3
E38K	0.7	1.7	1.3	-10.3	-10.7	-8.8
E38Q	-0.2	0.5	0.5	-5.9	-5.7	-5.0
A60V	10.3	8.8	7.4	7.8	6.3	5.9
E74I	-6.8	-8.8	-7.7	-11.4	-11.3	-11.0
N78K	0.2	-0.7	0.3	-4.2	-3.7	-4.4
F86W	2.7	2.9	3.2	-12.7	-12.9	-13.1
S87D	-3.0	-2.7	-3.2	1.1	1.4	0.8
S87H	-2.0	-2.1	-2.0	-14.8	-14.9	-15.5
S87N	-3.9	-3.9	-4.4	-10.0	-9.4	-10.1
S87Q	-3.6	-4.0	-3.9	-4.9	-5.6	-3.8
K89A	0.7	1.7	1.1	-4.5	-4.4	-4.2
K89F	-6.9	-5.3	-6.3	-18.9	-18.3	-19.7
K89L	-3.7	-3.7	-3.7	1.6	2.5	-0.7
K89M	-6.5	-5.9	-5.5	-5.9	-5.9	-5.8
K89W	6.7	9.1	8.7	-16.3	-15.0	-16.5
K89Y	0.2	1.0	-3.5 ^B	-16.3	-15.6	-17.8
S94A	-2.2	-1.7	-2.0	-9.5	-9.3	-9.3
S110Q	-3.1	-4.6	-3.6	-4.1	-5.3	-4.8
M128F	-4.9	-5.3	-4.7	-15.7	-15.0	-15.9
P139K	4.2	6.0	6.5	-2.9	-2.4 ^A	-4.3
N149G	-1.7	-2.7	-4.7	1.7	1.0	2.6
I154D	11.3	13.8	10.8	-1.9 ^A	-3.0	-4.7
I154N	12.1	14.6	13.2	-6.1	-7.0	-8.0
I154V	3.2	4.1	1.9	-8.7	-10.3	-12.0
L227V	12.6	14.2	14.6	10.0	11.1	10.1
L319F	-6.8	-6.8	-7.0	-4.8	-5.6	-4.3
A393R	-3.7	-4.3	-3.8	4.6	4.9	4.4
M419I	3.4	4.3	2.9	-4.7	-5.7	-8.1
M419L	2.5	1.1	3.3	-3.0	-4.2	-5.3

^A With this value for $\Delta\Delta G^{\text{Predicted}}$, the mutation would have fallen outside the -2.5 kJ/mol/subunit criterion and would not have been selected for experimental characterization.

^B With this value for $\Delta\Delta G^{\text{Predicted}}$ the mutation would have fallen inside the criterion for FoldX, unlike with the averaging over three structures. However, this mutation was selected by Rosetta with all three X-ray structures. Thus, this would not have influenced the outcome of the selection procedure.

Table S5. Potentially stabilizing mutations selected by phylogenetic analysis and subjected to experimental verification.

Mutation	Location	ΔT_m^{app} ($^{\circ}\text{C}$) ^A
A60V	Interface	4
F77M	Interface	-0.5
I154V	Interface	9
L227V	Surface	1
C248L	Surface	0.5
I256V	Buried	0.5
S290T	Interface	-1
A297S	Buried	-6
A303D	Surface	No signal
N432T	Surface	0

^A T_m^{app} of WT is 62 $^{\circ}\text{C}$.

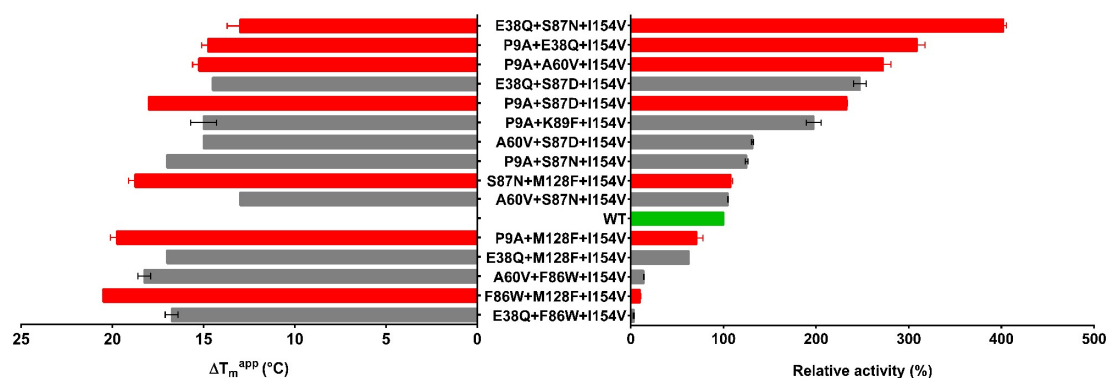


Figure S1. Activities and ΔT_m^{app} of *Pj*TA triple mutants. Activities were determined by measuring acetophenone formation from (*S*)-1-PEA as described in Materials and Methods (WT: 13 U/mg, 1 U equals 1 μmol product former per min). WT is marked with a green bar. Triple mutants shown with red bars were selected as templates for construction of four-fold mutants.

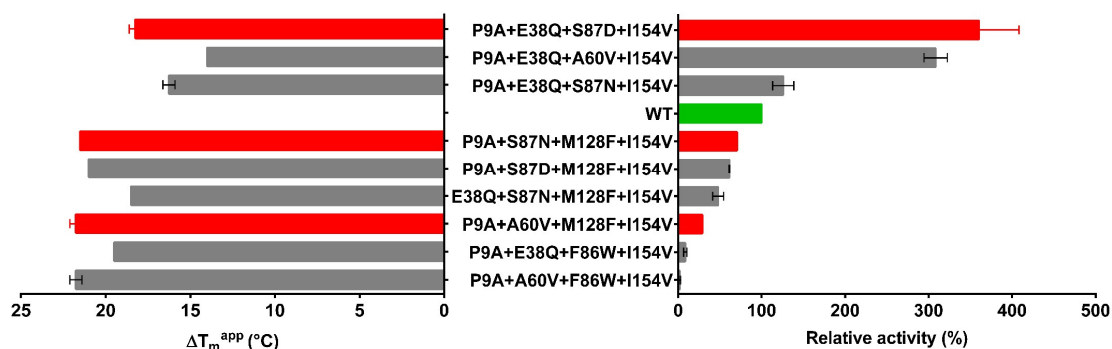


Figure S2. Activities and ΔT_m^{app} of *PjTA* four-fold mutants. Activities were determined by measuring acetophenone formation from (*S*)-1-PEA as described under Materials and Methods. Wild-type *PjTA* (WT) is shown as a green bar. Four-fold mutants shown with red bars were selected as templates to construct five-fold mutants.

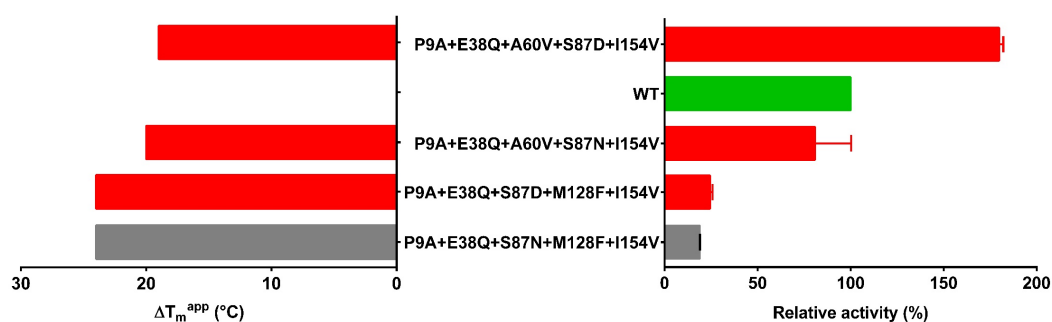


Figure S3. Activities and ΔT_m^{app} of *PjTA* five-fold mutants. Activities were determined by measuring acetophenone formation from (*S*)-1-PEA as described under Materials and Methods. WT is marked as green bar. Five-fold mutants with red color were selected as templates for six-fold mutants.

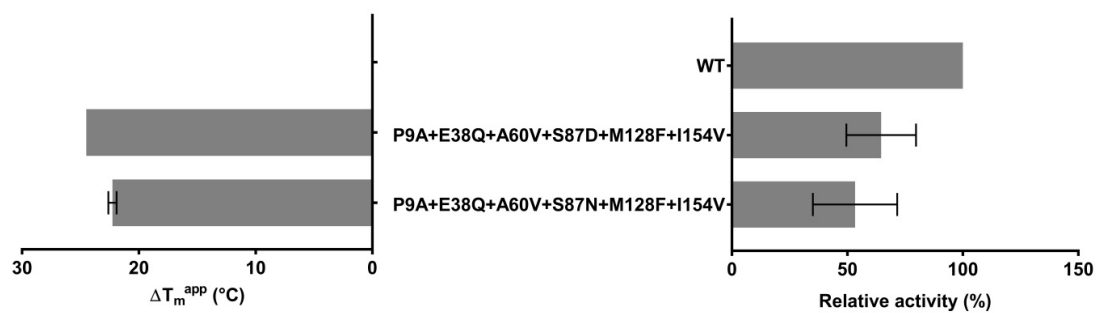


Figure S4. Activities and ΔT_m^{app} of *PjTA* six-fold mutants. Activities were determined by measuring acetophenone formation from (*S*)-1-PEA as described under Materials and Methods.

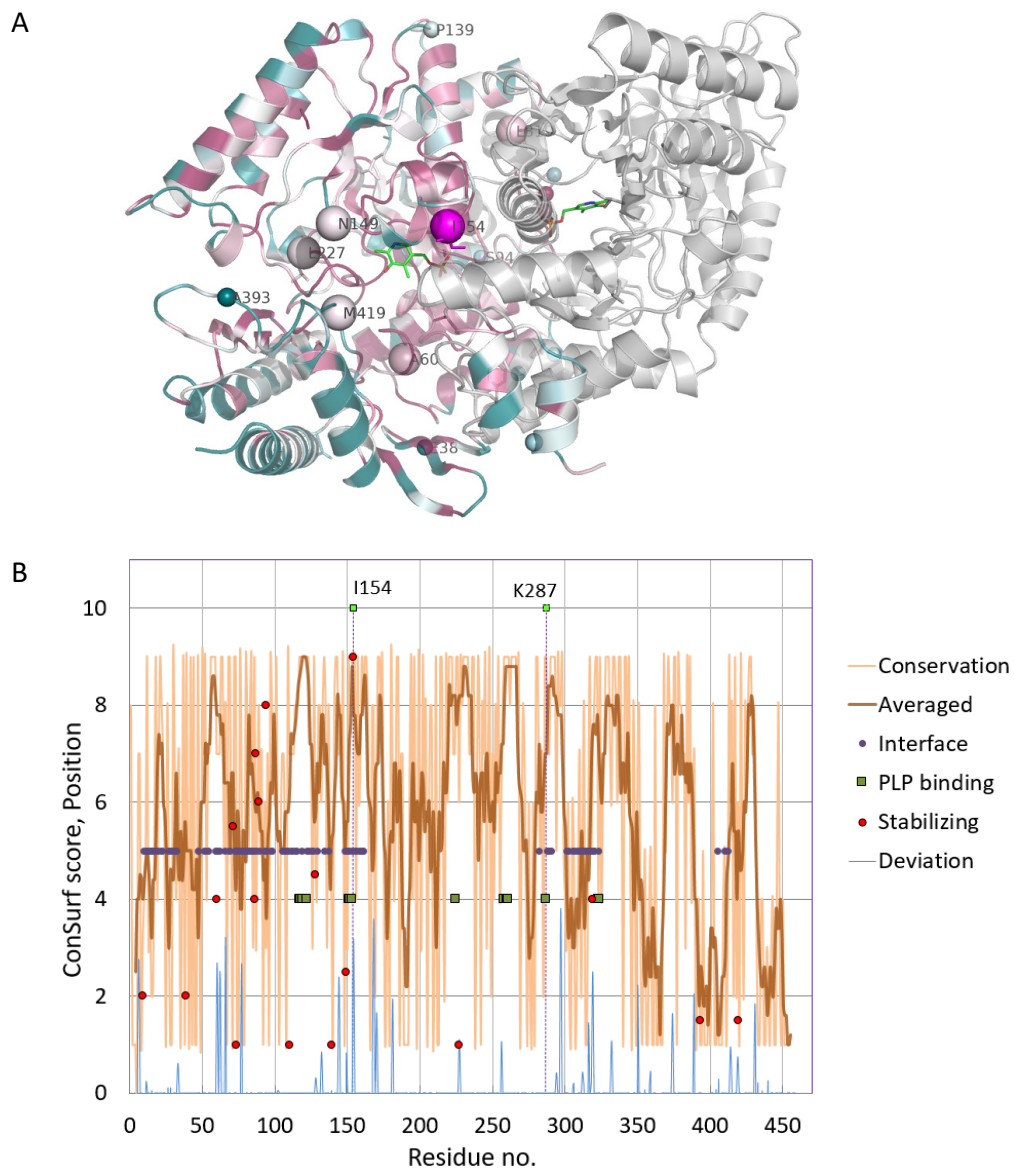


Figure S5. Mapping sequence conservation and stabilizing mutations. A set of 1000 fold-type I PLP sequences homologous to *PjTA* (NCBI non-redundant protein sequence database) was aligned (Clustal omega), duplicates were removed by JalView8 (90% identity cutoff),⁽⁸⁾ and the remaining sequences were analyzed by ConSurf.⁽⁹⁾ **A**) *PjTA* structure (pdb 6G4B) with the A chain colored turquoise to maroon by ConSurf score. PLP groups are shown as green sticks, Ile154 is colored magenta. $\text{C}\alpha$ atoms of stabilizing mutated residues are marked with spheres ($\Delta T_m^{\text{app}} = +1^\circ\text{C}$ cutoff), and larger spheres in case of positions in *PjTA* that show above average deviation from consensus (positive ConSurf score). **B**) distribution of highly conserved positions and regions along the sequence. Colors: light brown, ConSurf score of all positions; brown, ConSurf score averaged over a 5 amino acid sliding window; purple, interface residues; green marks, residues contacting the PLP cofactor; red dots, stabilizing mutations (ΔT_m^{app} plotted, $^\circ\text{C}$); blue lines, deviation from consensus (arbitrary units proportional to ConSurf score). The positions of Ile154 and the internal aldimine Lys287 are also marked.

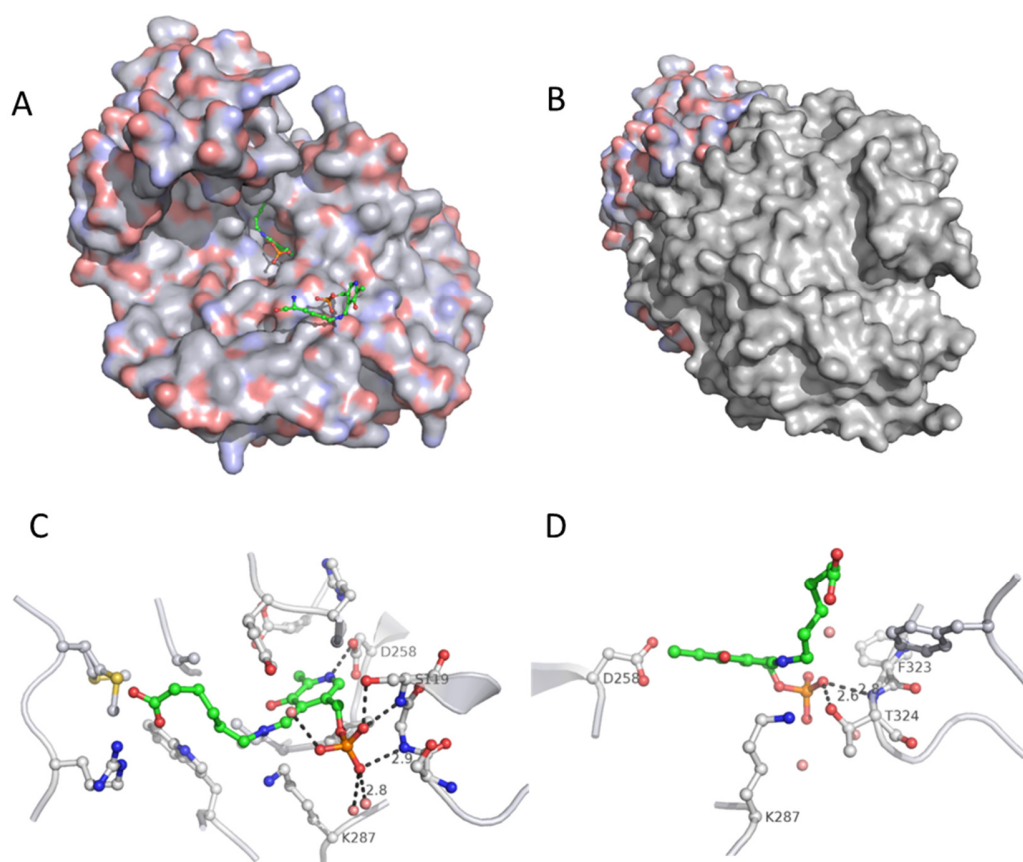


Figure S6. Intersubunit binding of PLP cofactors in *PjTA*. **A)** position of the PLP cofactors (external aldimine with 6-aminohexanoic acid, green) at the protein surface of the *PjTA* A monomer. **B)** The PLP binding sites covered by the B monomer (grey). **C)** Hydrogen bonding interactions between the PLP phosphate group and residues of the A subunit; **D)** Interactions between the PLP phosphate group and residues of the B subunit. Distances in Å.

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