

SUPPLEMENTAL INFORMATION

Crystal structure of an (*R*)-selective ω-transaminase from *Aspergillus terreus*

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Table S1. Sequences of primers used for cloning and mutagenesis in this study. Sequences of restriction sites are underlined, start and stop codon are indicated with boxes and mutated bases are highlighted in bold letters.

Primer name (mutation)	Sequence
AT-ωTA_for(<i>Nco</i> I)	AATCACC <u>ATG</u> GCGTCTATGGACAAAGTATTCGC
AT-ωTA_rev(<i>Xho</i> I-Stop)	AATCACTCGAGATTACGTTTCGTTGTAGTCGATTTCAAGG
AT-ωTA_for(<i>Nde</i> I)	AATCACAT <u>ATG</u> GCGTCTATGGACAAAGTATTCGC
AT-ωTA_rev(<i>Hind</i> III)	AATCAAAGCTTA <u>TTA</u> ATTACGTTTCGTTGTAGTCGATTTTC
AT-ωTA-Y60A_for	GCACTCTGACCTGACTGCAGACGTTCCGTCTGTATG
AT-ωTA-Y60A_rev	CATACAGACGGAACGTCT GC AGTCAGGTCAGAGTGC
AT-ωTA-Y60F_for	CTCTGACCTGACTT TC GACGTTCCGTCTG
AT-ωTA-Y60F_rev	CAGACGGAACGT CG AAAGTCAGGTCAGAG
AT-ωTA-R128A_for	GTCTGAAAGGCGTT GCT GGTACTCGTCCG
AT-ωTA-R128A_rev	CGGACGAGTACCAGCAACGCCTTTTCAGAC
AT-ωTA-W184A_for	GTAAAAACCTGCAG GCG GGCGACCTGGTTC
AT-ωTA-W184A_rev	GAACCAGGTCG CCCG CCTGCAGGTTTTTAAC
AT-ωTA-W184F_for	GTAAAAACCTGCAGT TCG GCGACCTGGTTCGTG
AT-ωTA-W184F_rev	CACGAACCAGGTCG CCG AACTGCAGGTTTTTAAC

Table S2. (Conserved) amino acids within a 12 Å range from the cofactor PLP in AT- ω TA.

distance [Å]	chain	residue	conserved
4	A	THR-59	*
4	A	TYR-60	*
4	A	ARG-79	*
4	A	SER-83	*
4	A	THR-178	
4	A	VAL-179	
4	A	LYS-180	
4	A	ASN-181	
4	A	TRP-184	
4	A	GLU-213	*
4	A	GLY-214	*
4	A	GLY-216	*
4	A	PHE-217	*
4	A	ASN-218	*
4	A	LEU-235	
4	A	GLY-237	
4	A	VAL-238	*
4	A	THR-239	*
4	A	THR-274	*
4	A	THR-275	*
6	A	LEU-58	*
6	A	ASP-61	*
6	A	VAL-62	*
6	A	HIS-76	*
6	A	LEU-87	*
6	A	ARG-169	
6	A	ASP-176	
6	A	PRO-177	
6	A	LEU-182	
6	A	LEU-187	
6	A	SER-215	*
6	A	GLN-236	
6	A	ARG-240	*
6	A	CYS-273	*
6	A	ALA-276	*
8	A	PRO-63	*
8	A	SER-64	*
8	A	LEU-80	*
8	A	ALA-82	*
8	A	CYS-84	*
8	A	LYS-86	*
8	A	PHE-115	*

8	A	GLU-117	*
8	A	LEU-118	*
8	A	VAL-120	*
8	A	VAL-149	
8	A	ILE-175	
8	A	GLN-183	
8	A	ASP-186	
8	A	THR-212	*
8	A	ILE-219	*
8	A	GLY-233	
8	A	VAL-234	
8	A	LYS-241	*
8	A	SER-242	*
8	A	GLY-277	
8	A	GLY-278	*
8	A	MET-280	*
8	A	TYR-306	
8	B	HIS-55	
12	A	GLY-52	
12	A	HIS-55	
12	A	SER-56	*
12	A	ASP-57	
12	A	VAL-65	*
12	A	PHE-71	*
12	A	ARG-72	*
12	A	LEU-73	*
12	A	ASP-74	*
12	A	ASP-75	*
12	A	ILE-77	*
12	A	THR-78	*
12	A	GLU-81	*
12	A	THR-85	*
12	A	ARG-88	*
12	A	LEU-89	
12	A	ALA-114	*
12	A	VAL-116	*
12	A	ILE-119	*
12	A	THR-121	*
12	A	ARG-122	*
12	A	ASN-138	*
12	A	TYR-140	*
12	A	PHE-142	*
12	A	GLN-144	*
12	A	TYR-146	

12	A	VAL-147	
12	A	TRP-148	
12	A	MET-150	
12	A	ARG-168	
12	A	VAL-170	
12	A	PRO-171	
12	A	PRO-172	
12	A	ALA-174	
12	A	GLY-185	
12	A	VAL-188	
12	A	ARG-189	
12	A	GLY-190	
12	A	MET-191	
12	A	TYR-201	*
12	A	PRO-202	
12	A	PHE-203	*
12	A	LEU-204	
12	A	THR-205	*
12	A	LEU-211	*
12	A	VAL-220	*
12	A	THR-229	*
12	A	PRO-230	*
12	A	ASP-231	*
12	A	ARG-232	
12	A	VAL-243	*
12	A	ILE-244	*
12	A	ASN-245	*
12	A	PHE-271	*
12	A	MET-272	*
12	A	ILE-279	*
12	A	ILE-302	
12	A	TRP-307	
12	A	MET-309	
12	A	HIS-310	
12	B	GLN-51	
12	B	PHE-53	
12	B	MET-54	
12	B	SER-56	*
12	B	ARG-128	
12	B	GLN-183	
12	B	GLY-185	
12	B	ARG-189	
>12	A	-	ASN-218



Figure S1. Output of the Swiss-Model Template Identification module

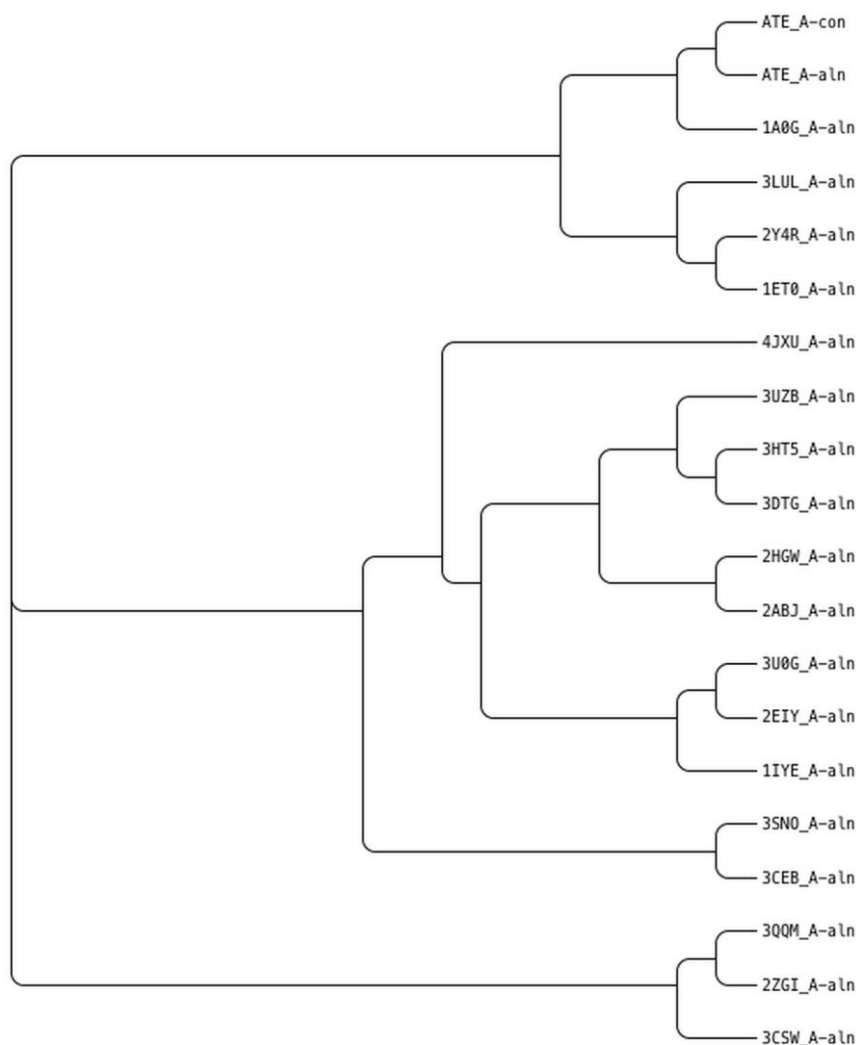


Figure S2. Cladogram of selected fold IV aminotransferases: AT- ω TA, D-ATA: 1A0G from *Bacillus* sp YM-1, BCATs: 1IYE from *E. coli*, 2EIY from *Thermus thermophilus*, 3HT5 from *Mycobacterium tuberculosis*, 3DTG from *Mycobacterium smegmatis*, 3UZB from *Deionococcus radiodurans*, 3U0G from *Burkholderia pseudomallei*, 2ABJ and 2HGW from human, ADCLs: 1ETO from *E. coli*, 2Y4R from *Pseudomonas aeruginosa*, and a few putative aminotransferases: 2ZGI from *Thermus thermophilus* HB8, 3CEB from *Haemophilus somnus*, 3CSW from *Thermotoga maritima*, 3LUL from *Legionella pneumophila*, 3QQM from *Mesorhizobium loti*, 3SNO from *Corynebacterium glutamicum*, 4JXU from *Sinorhizobium meliloti*.

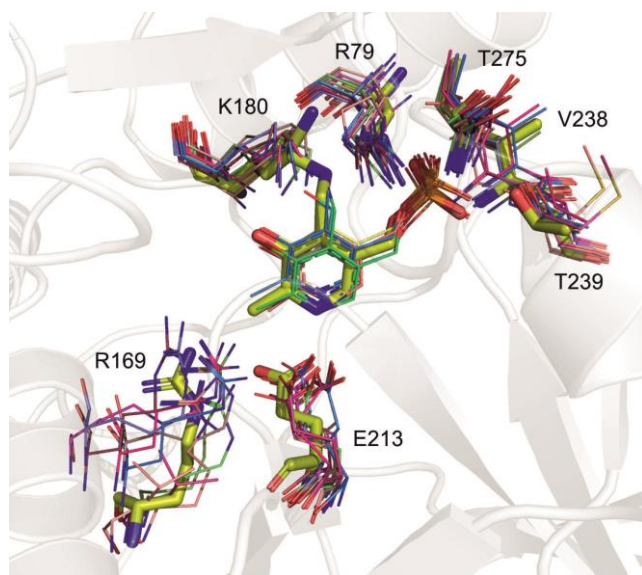


Figure S5. Alignment of PLP and PLP-coordinating amino acids of AT- ω TA (light green sticks) with other members of fold IV family (lines of various colours). The figure was prepared using the program PyMOL.

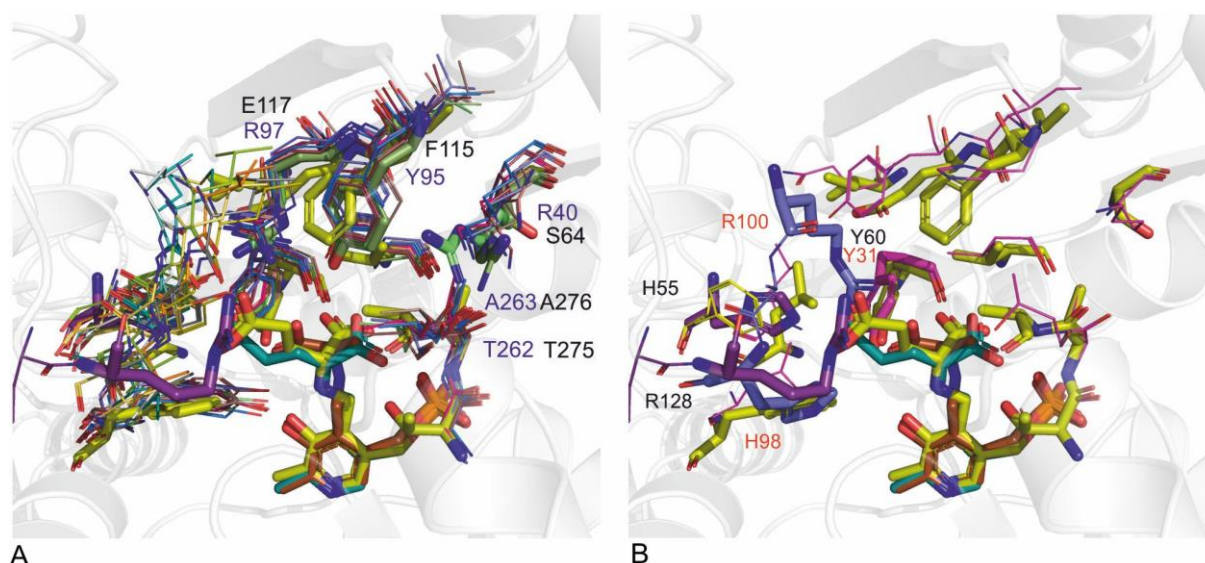


Figure S6. Alignment of active site amino acids of AT- ω TA with other members of the fold IV family (A: BCATs, B: D-ATA). Light green sticks: amino acids and L-Glu-aldimine of AT- ω TA, turquoise: L-Glu-aldimine bound in BCAT, brown: D-Ala-aldimine bound in D-ATA. Other amino acids depicted as sticks: conserved amino acids of either BCAT or D-ATA involved in α -carboxylate binding. Labelling: black: AT- ω TA, blue: BCAT, red: D-ATA. The figures were prepared using the program PyMOL.

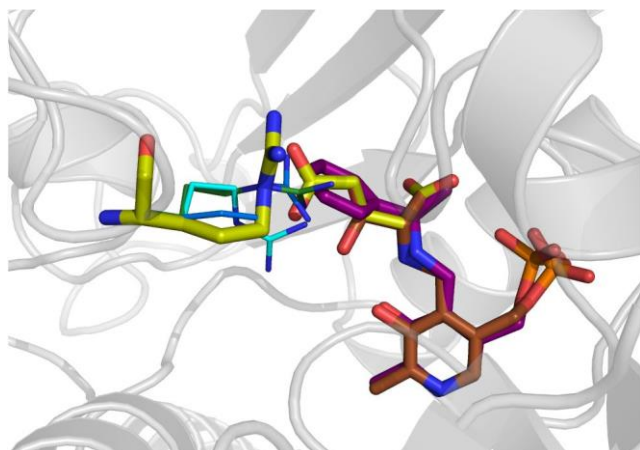


Figure S7. Different rotamers of arginine 128 in AT- ω TA. Grey: Cartoon depiction of the AT- ω TA backbone, light green stick depiction: original conformation of R128 in AT- ω TA; blue, turquoise and green lines: alternative rotamers that move R128 closer to the α -carbonyl of a docked D-Ala-aldimine (brown) and docked acetophenone-aldimine (purple); L-glutamate-aldimine also depicted in light green. The figure was prepared using the program PyMOL.