PROTEIN STRUCTURE REPORT

Crystal structure of *Saccharomyces cerevisiae* Aro8, a putative α -aminoadipate aminotransferase

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Abstract: α -Aminoadipate aminotransferase (AAA-AT) catalyzes the amination of 2-oxoadipate to α -aminoadipate in the fourth step of the α -aminoadipate pathway of lysine biosynthesis in fungi. The aromatic aminotransferase Aro8 has recently been identified as an AAA-AT in *Saccharomyces cerevisiae*. This enzyme displays broad substrate selectivity, utilizing several amino acids and 2-oxo acids as substrates. Here we report the 1.91 Å resolution crystal structure of Aro8 and compare it to AAA-AT LysN from *Thermus thermophilus* and human kynurenine aminotransferase II. Inspection of the active site of Aro8 reveals asymmetric cofactor binding with lysine-pyridoxal-5-phosphate bound within the active site of one subunit in the Aro8 homodimer and pyridoxamine phosphate and a HEPES molecule bound to the other subunit. The HEPES buffer molecule binds within the substrate-binding site of Aro8, yielding insights into the mechanism by which it recognizes multiple substrates and how this recognition differs from other AAA-AT/kynurenine aminotransferases.

Keywords: lysine biosynthesis; aminotransferase; pyridoxal 5'-phosphate; multi-substrate enzyme; aromatic aminotransferase I; X-ray crystallography

Introduction

In yeast and certain fungi, the amino acid L-lysine is synthesized via the seven-enzyme α -aminoadipate

(AAA) pathway. The fourth enzyme, α -aminoadipate aminotransferase (AAA-AT), catalyzes the pyridoxal 5'-phosphate (PLP)-dependent reversible transamination

Abbreviations: AAA, α -aminoadipate; AAA-AT, α -aminoadipate aminotransferase; GSAM, glutamate-1-semialdehyde aminotransferase; hKAT II, human KAT II; KAT, kynurenine aminotransferase; LLP, lysine-pyridoxal-5-phosphate; PPA, N-phosphopyridoxyl- α -aminoadipate; PLP, pyridoxal 5'-phosphate; PMP, pyridoxamine phosphate; SeMet, selenomethionine

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of 2-oxoadipate to generate AAA utilizing the amino donor glutamate.¹ In *Saccharomyces cerevisiae*, two proteins, termed glutamate- α -ketoadipate transaminase I and II, were found to catalyze this reaction.^{2,3} However, the identities of the genes corresponding to these enzymes remained elusive until the discovery of three *S. cerevisiae* open reading frames, YGL202W (ARO8), YER152C, and YJL060W (BNA3), which encode enzymes that harbor AAA-AT activity.⁴ Among these enzymes, Aro8 displayed the highest activity in a glutamate dehydrogenase-coupled AAA-AT assay, suggesting it may function as the primary AAA-AT in *S. cerevisiae*.

Aro8 was initially identified as aromatic aminotransferase I, an enzyme involved in the biosynthesis of phenylalanine and tyrosine.^{5,6} Aro8 belongs to a novel subgroup of class I aminotransferases comprising AAA-ATs and kynurenine aminotransferases (KATs).^{7–11} Similar to many class I aminotransferases, Aro8 exhibits broad substrate specificity. Aro8 has been reported to utilize glutamate, phenylalanine, tyrosine and tryptophan as amino donors with phenylpyruvate, hydroxyphenylpyruvate, 2-oxoglutarate and pyruvate as amino acceptors.^{5,6,12,13} In addition, it can use methionine, leucine, and AAA as donors with their corresponding 2-oxo acids as acceptors. Because Aro8 possesses activity toward AAA/2-oxoadipate and mutant *aro8* strains failed to grow in the presence of a mixture of tyrosine, tryptophan and phenylalanine, it was speculated to have an additional role as one of the glutamate/2-oxoadipate transaminases.⁵ Further evidence supporting the reclassification of Aro8 as an AAA-AT derives from a recent study in which the enzyme was shown to display greater specificity towards 2-oxoadipate compared to the aromatic amino acid substrates phenylalanine and tyrosine.¹² To gain insight into the molecular basis of its substrate selectivity, we have determined the crystal structure of Aro8 bound to its cofactor and compared it to structures of other AAA-ATs, *Thermus thermophilus* LysN, and human KAT II (hKAT II), which also recognize multiple amino acid/2-oxo-acid substrates.

Results and Discussion

Structure of Aro8

The crystal structure of full-length Aro8 was determined by selenomethionine (SeMet) single wavelength anomalous diffraction (SAD) phasing to 2.30 Å and was used as a molecular replacement model for solving the structure of the native enzyme at 1.91 Å (Table I). Aro8 crystallized with two biologically relevant homodimers composed of subunits A and B and subunits C and D, respectively, in the asymmetric unit. Each Aro8 homodimer exhibits domain-swapping with tightly associated subunits [Fig. 1(A)].

The overall structural core of Aro8 adopts a typical type-1 aminotransferase fold,^{14,15} comprising

Table I. Data Collection and Refinement Statistics

	SeMet Aro8	Native Aro8
Data collection		
Wavelength (Å)	0.97856	0.97856
Space group	<i>C2</i>	<i>C2</i>
Cell dimensions		
a, b, c, (Å)	227.5, 160.9, 70.7	227.4, 159.8, 70.6
β (°)	106.5	106.0
Resolution (Å) ^a	30.0–2.30 (2.38–2.30)	40.0–1.91 (1.98–1.91)
R_{merge} (%) ^b	9.1 (40.7)	6.5 (46.1)
$I/\sigma I$	29.9 (3.1)	36.5 (2.2)
Total reflections	751,214	782,138
Unique reflections	105,905	181,016
Redundancy	7.1 (5.3)	4.3 (2.7)
Completeness (%)	97.0 (86.9)	96.8 (78.4)
Phasing FOM	0.60	
Refinement		
Resolution range (Å)		34.25–1.91
No. of reflections		180,889
No. of atoms		16,855
Protein atoms		15,696
Cofactor/HEPES		71/75
Water atoms		1013
$R_{\text{work}}/R_{\text{free}}$ ^c		18.2/21.8
Average B-factors (Å²)		
Overall		28.6
Protein		28.8
Cofactor/HEPES		36.0/51.4
Water		24.2
RMSDs		
Bond length (Å)		0.014
Bond angles (°)		1.370
MolProbity score		1.54
Percentile ^d		94 th
Ramachandran favored (%)		96.9
Ramachandran allowed (%)		3.1
Ramachandran outliers (%)		0

^a Values in parentheses are for the highest-resolution shell.

^b $R_{\text{merge}} = \sum_{hkl} \sum_i |I_j - I| / \sum_{hkl} \sum_i I_j$ where I is the mean intensity of reflection hkl .

^c $R_{\text{work}} = \sum_{hkl} ||F_o| - |F_c|| / \sum_{hkl} |F_o|$; $R_{\text{free}} = 5.0\%$ of the total reflections.

^d For structures with a resolutions 1.91 ± 0.25 Å, $n = 11,889$.

large (residues 52–368) and small (residues 369–500) domains, both of mixed α/β topology. In addition, a helical N-terminal motif (residues 1–51) precedes the large domain of Aro8 [Fig. 1(A)]. The large domain forms a seven-stranded mixed parallel and anti-parallel β -sheet ($\beta 4$ – $\beta 5$ and $\beta 8$ – $\beta 12$) surrounded by helices ($\alpha 4$ – $\alpha 8$, $\alpha 11$, and $3_{10}6$) [Fig. 1(A,B)]. Two β -strands ($\beta 2$ – $\beta 3$) of this domain fold into an anti-parallel sheet that interacts with the corresponding β -strands of the adjacent subunit to form a four-stranded sheet at the dimer interface. This secondary structure element differs from the classical type-I aminotransferase fold that possesses an α -helix at this position, as observed in the AAA-AT LysN from *T. thermophilus*,^{11,16} [Fig. 1(C)] and has been observed in only one other

Figure 1. Crystal structure of Aro8 and comparison to other related aminotransferases. (A) Ribbon diagram of a homodimer of Aro8 with the N-terminal motif, large domain, and small domain of subunit C colored in blue, violet and cyan, and of subunit D in light orange, yellow and orange, respectively. The active site is denoted by the presence of LLP or PMP (gray sticks). (B) Illustration of subunit D with each secondary structure element labeled. PLP forms a Schiff base with the ϵ -amino group of Lys305 in this subunit (LLP, gray). (C and D) Superimposition of a subunit of Aro8 [colored as described for subunit D in (A)] with a subunit of *T. thermophilus* LysN (C) (PDB ID 3CBF, colored blue with the N-terminal region in cyan) and hKAT II (D) (PDB code 2QLR, colored violet with the N-terminal motif in magenta). An interactive view is available in the electronic version of the article. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

aminotransferase, hKAT II [Fig. 1(D)].^{7,10} Aside from this feature, the other major difference between the large domain of Aro8 and the corresponding domain in LysN and hKAT II is the presence of an extended loop containing helices α_{10} and α_{11} in Aro8 [Fig. 1(C,D) and Supporting Information Fig. S1]. The small domain follows the large domain in Aro8 and comprises a four-stranded anti-parallel β -sheet (β_{13} – β_{14} and β_{16} – β_{17}), which is, flanked on a single face by several helices (α_{13} – α_{15} and $3_{10}8$) [Fig. 1(A,B)]. The overall fold of the small domain is homologous to the structures of LysN and hKAT II, with the exception that Aro8 has additional loops, including helices $3_{10}9$, $3_{10}10$, and $3_{10}12$ [Fig. 1(C,D) and Supporting Information Fig. S1].

In contrast to the large and small domains, the N-terminal motif diverges significantly from the canonical type-1 aminotransferase fold that consists of two α -helices, with the α_2 helix occluding the entrance

to the active site within the same subunit, as exemplified in LysN [Fig. 1(C)]. Instead, the helical N-terminal region of Aro8 (α_1 – α_2 , β_1 , and $3_{10}1$ – $3_{10}2$) adopts a conformation that blocks the active site of the adjacent subunit, similar to hKAT II [Fig. 1(A,D)]. Although the domain-swapped N-terminal motif is unique to Aro8 and hKAT II, the α_2 helix in these enzymes plays the same functional role in capping the active site as the α_2 helix within an individual subunit of LysN. Closure of the α_2 helix over the active site of Aro8 is observed in subunits A and C, whereas in subunits B and D this helix is disordered (Supporting Information Fig. S2), indicating its flexibility in gating access to the active site (discussed below).

Cofactor binding

The active site of Aro8 is located in a cleft between the large and small domains of one subunit along with the N-terminal motif and large domain of the

Figure 2. The active site of Aro8. (A) Stereo diagram of the active site of subunit D [sticks, colored as described in Fig. 1(A)], in which PLP forms a Schiff base with the ϵ -amino group of Lys305 (LLP, gray). Electron density of an $F_o - F_c$ omit map contoured to 2.0σ around the LLP molecule is shown as a gray mesh. Hydrogen bonds and salt bridge interactions to LLP are depicted as orange dashes. (B) Stereo-view of the active site of subunit C [sticks, colored as described in Fig. 1(A)] bound to PMP (gray sticks) and HEPES (orange sticks). Electron density of an $F_o - F_c$ omit map around PMP and the HEPES molecule (contoured to 1.8σ) within this subunit is shown as a gray mesh. Hydrogen bonds and salt bridge interactions to PMP and HEPES are illustrated as gray and orange dashes, respectively. Certain protein residues that interact with the PMP cofactor have been omitted for clarity. Residues from the adjacent subunit are indicated by a prime ('). An interactive view is available in the electronic version of the article. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

neighboring subunit. It is distinguished by the presence of the PLP cofactor, although no PLP was included in the crystallization solution [Fig. 1(A)]. Clear electron density for the internal aldimide linkage between PLP and the Lys305 ϵ -amino group that generates lysine-pyridoxal-5-phosphate (LLP) is only observed in subunit D [Fig. 2(A)]. In subunits B and C, electron density maps suggest that pyridoxamine phosphate (PMP) and a partially ordered HEPES buffer molecule are bound within their active sites [Fig. 2(B) and Supporting Information Fig. S3(A)]. PMP, as opposed to PLP, was modeled into these subunits because the electron density observed beyond the C4' atom is out of plane with the pyridoxyl ring. This observation supports the modeling of the amine nitrogen of PMP in contrast

to the aldehyde oxygen of PLP, which should be coplanar with the pyridoxal ring due to the sp^2 hybridization of the carbonyl group. In subunit A, the electron density of the cofactor is relatively poor compared to that of the other subunits, and the aldimide linkage of LLP is not observed nor is the PLP aldehyde oxygen or the PMP amine nitrogen. It is conceivable that the Schiff base between Lys305 and PLP in subunit A has been broken, possibly due to radiation damage during data collection. Nonetheless, electron density for the pyridoxal ring system is evident in the electron density maps of the A subunit suggesting that PLP, PMP, or LLP is bound in its active site.

The binding mode of LLP in Aro8 is homologous to other fold-type 1 aminotransferases, with LLP

engaging in contacts with several highly conserved residues [Fig. 2(A)].¹⁵ The pyridine ring of LLP is stacked between Phe141 and Pro250, and the C2' atom participates in a hydrophobic interaction with Ile215. The O3 atom of LLP forms hydrogen bonds with the side chains of Tyr251 and Asn220, and the pyridine nitrogen atom in the cofactor engages in an apparent salt bridge interaction with the carboxylate group of Asp248. In addition, the phosphate moiety of LLP is stabilized by several polar contacts. These interactions include hydrogen bonds with the main chain nitrogen atoms of Asn141 and Thr142 and with the side chains of Thr142, Ser302, Ser304, and Tyr105' (the prime denotes a residue from the neighboring subunit) as well as a salt bridge interaction with the guanidinium group of Arg312. Overall, these interactions are virtually identical to those observed for LLP in the hKAT II structure, with the minor exceptions that Phe166 and Ile215 in Aro8 correspond to a tyrosine and valine, respectively, in hKAT II.^{7,10}

In subunits B and C, the cofactor PMP adopts a binding mode homologous to LLP by engaging in equivalent hydrophobic and polar contacts with Aro8 [Fig. 2(B) and Supporting Information Fig. S3(A)]. An alignment of subunits C and D of Aro8 illustrates similar conformations of the active site residues between the PMP- and LLP-bound subunits [Supporting Information Fig. S3(B)]. The phosphate moiety of each cofactor occupies the same general position and engages in an analogous network of hydrogen bonds and salt bridge interactions. However, the pyridoxal ring system rotates with the C4' atom of PMP turning $\sim 16^\circ$ away from Lys305. The amine nitrogen also faces the opposite direction, presumably positioning it to react with a 2-oxo-acid substrate [Supporting Information Fig. S3(B)]. Rotation of the pyridoxal ring system of LLP relative to that of PMP or an aldimide-linked PLP-substrate complex appears to be a common feature of AAA-ATs and KATs, as illustrated by cofactor-bound structures of LysN and hKATII that display ring relative rotations of 30° and 20° , respectively.^{7,11} In summary, LLP and PMP bind to Aro8 in a comparable fashion with little conformation change within the cofactor binding site of the enzyme.

The observation that the Aro8 homodimer comprises PMP- and LLP-bound subunits is suggestive of conformational asymmetry in the dimer. This finding is consistent with structural and biochemical studies of the *Synechococcus* glutamate-1-semialdehyde aminomutase (GSAM) whose homodimeric structure displays analogous subunit asymmetry in binding both LLP and PMP.¹⁷ Conversely, the structure of GSAM from *Bacillus subtilis* displays symmetric active sites,¹⁸ suggesting that the asymmetry in this enzyme may be species-dependent. Active site asymmetry or half-site reactivity has also been reported for other PLP-dependent aminotransferases, including alanine and

aspartate aminotransferases.^{19,20} Future studies will be needed to address the PLP/PMP content of the isolated enzyme and whether Aro8 exhibits half-site reactivity or if the asymmetry in its active sites resulted from crystal packing interactions that favored different conformations in the subunits composing the homodimer.

Substrate binding cleft

During refinement and model building, additional interpretable electronic density was observed in the active sites of subunits B and C, which could be modeled by a partially occupied HEPES molecule (Fig. 2[B]). The HEPES binds in a pocket adjacent to the PMP cofactor and interacts with residues from both subunits within the homodimer. Recognition of the sulfonate moiety of HEPES is mediated through hydrogen bonds with the side chain nitrogen of Asn220 and the backbone nitrogen of Gly43'. In addition, it forms apparent salt bridge interactions with the guanidinium group of Arg470. The piperazine ring of HEPES also forms a weak hydrophobic interaction with the side chain of Tyr105'. These residues are conserved in LysN and hKAT II and have been shown to participate in substrate binding.^{7,11,16} Superimposition of the active site of Aro8 subunit C bound to PMP and HEPES with LysN bound to *N*-phosphopyridoxyl- α -amino adipate (PPA)¹¹ and with hKAT II in a complex with PMP and kynurenine⁷ demonstrates that HEPES occupies the amino acid/2-oxo acid substrate binding cleft (Supporting Information Fig. S4). Specifically, the HEPES sulfonate moiety mimics the binding modes of the α -carboxylate groups of kynurenine and the AAA moiety of PPA by engaging in a network of hydrogen bonds and salt bridge interactions with Gly43', Asn220 and Arg470 in Aro8 that are strictly conserved in hKAT II and LysN. Based on this observation, substrates of Aro8 are predicted to bind through similar interactions with these residues.

The PPA and kynurenine substrates in LysN and hKAT II, respectively, are also recognized by a conserved arginine residue (Arg23 in LysN and Arg20 in hKAT II) (Supporting Information Figs. S1 and S4). In LysN, the δ -carboxylate group of the AAA moiety of PPA is stabilized through salt bridge interactions with the guanidinium group of Arg23 in LysN¹¹, and the corresponding residue in hKAT II, Arg20, participates in cation- π stacking with the kynurenine substrate.^{7,21} However, in Aro8, this arginine is substituted with Lys26, which resides in the flexible $\alpha 2$ helix that contacts the active site of the neighboring subunit in the homodimer (Supporting Information Fig. S1). The side chain of Lys26 in Aro8 has ambiguous electron density and could not be modeled. However, it is conceivable that this lysine residue may rearrange to play an important role in substrate recognition, analogous to

the corresponding arginine residue in LysN and hKAT II.^{7,11,16,21} A model of kynurenine bound to the active site of Aro8 displays similarities to the kynurenine-bound structure of hKAT II (Supporting Information Fig. S5) indicating that Aro8 may be able to process this substrate despite the presence of Lys26. Critical interactions including the salt-bridge and hydrogen bonds between kynurenine and Aro8 are conserved in hKATII, with the exception of the weak hydrogen bond to Tyr142 (Phe166 in Aro8). However, this model does not account for any conformational changes that might occur in the $\alpha 2$ helix upon kynurenine binding, and thus, future experiments are required to determine whether kynurenine is a bona fide substrate of Aro8.

Although the Lys26 substitution in Aro8 may not affect the binding of some substrates, it may play a large role in the binding of other substrates. For example, Aro8 is not particularly active using glutamate as a substrate, with the aromatic amino acids phenylalanine and tyrosine having higher k_{cat}/K_m values by over one and two orders of magnitude, respectively.¹² This finding is in contrast to hKAT II, which displays catalytic efficiencies within two-fold for glutamate, tyrosine, and phenylalanine.²¹ Although kinetic parameters of LysN using aromatic amino acid substrates have yet to be reported, Arg23 in LysN has an essential role in glutamate recognition, as alanine and glutamine mutations at this residue decrease the catalytic efficiency for glutamate by two to three orders of magnitude.¹⁶ The differences in amino acid preference between Aro8 and other AAA-AT/KAT enzymes may be in part due to the presence of Lys26 instead of an arginine residue in Aro8. Further structural and biochemical studies of the amino acid and 2-oxo acid specificity of Aro8 are needed to investigate the molecular basis of its substrate selectivity.

In addition to the substitution of the conserved arginine by Lys26, variations in the conformation of the N-terminal region that associates with the entrance to the active site of the adjacent subunit in the Aro8 homodimer may contribute to its substrate specificity. Previous structural studies have shown that substrate binding to LysN and hKAT II involves conformational changes within the N-terminal motif encompassing the $\alpha 2$ helix. The details of these rearrangements are described elsewhere,^{11,16,21,22} but briefly, the orientation of the $\alpha 2$ helical region changes conformation to accommodate the binding of amino acid and 2-oxo acid substrates of different sizes and polarities. Both LysN and hKAT II employ an induced-fit mechanism for substrate binding. This is in contrast to other aminotransferases including hKat I and the branched-chain amino acid aminotransferase from *Escherichia coli*, which undergo relatively few conformational changes upon substrate binding.^{11,22} Similar to LysN and hKAT II, a substrate-binding-induced closure of the $\alpha 2$ helix region is also likely to occur in

Aro8, as the N-terminal region displays several different conformations that illustrate its intrinsic flexibility (Supporting Information Fig. 2S). Remarkably, each of the four Aro8 subunits in the crystallographic asymmetric unit displays distinct conformations for the N-terminal region containing the $\alpha 2$ helix that are independent of cofactors or ligands bound within the respective active sites. For example, in the Aro8 homodimer composed of the A and B subunits, the helical region in the A subunit is disordered, leaving the B subunit active site in complex with PMP and HEPES relatively solvent-exposed. Conversely, in the homodimer comprising subunits C and D, the $\alpha 2$ helical region is ordered in subunit D and occludes the active site of the neighboring C subunit that is bound to PMP and HEPES (Supporting Information Fig. 2S). We cannot exclude the possibility that the ordering or disordering of the $\alpha 2$ helix in each subunit is due to crystal packing. Nonetheless, these observations indicate that the $\alpha 2$ helical region possesses inherent plasticity, adopting several conformations that may promote the binding of different substrates to Aro8. Co-crystal structures in complex with different amino acid and 2-oxo acid substrates will prove valuable in elucidating the role that the N-terminal $\alpha 2$ helical region plays in defining the substrate selectivity of Aro8 in comparison to related aminotransferases.

Materials and Methods

Cloning, overexpression and purification of Aro8

Full length Aro8 was amplified from yeast genomic DNA and was subcloned into a modified pET15b vector that possess a tobacco etch virus (TEV) protease cleavage site in place of the thrombin site. The protein was overexpressed in Rosetta 2 (DE3) *E. coli* cells by inducing with 0.1 mM isopropyl β -D-1-thiogalactopyranoside and growing at 25°C overnight. Aro8 was affinity purified using nickel-Sepharose (GE Healthcare) by eluting with a linear gradient of 0–500 mM imidazole in 50 mM Tris pH 8.0, 500 mM NaCl, and 5 mM β -mercaptoethanol. The N-terminal hexahistidine tag was then removed by TEV protease as previously described.²³ Aro8 protein was further purified using a Superdex 200 16/600 size exclusion column (GE Healthcare) in a buffer of 50 mM Tris, pH 8.0, 150 mM NaCl, and 1 mM Tris(2-carboxyethyl)phosphine. The concentration of purified Aro8 was determined by UV absorbance ($\epsilon_{280\text{ nm}} = 76,780$).²⁴ SeMet Aro8 was expressed in the *E. coli* strain B834 (EMD Millipore) using a modified protocol of Doublé²⁵ and was purified as described for native Aro8.

Crystallization, data collection and structure determination

Crystals of native and SeMet Aro8 were obtained by hanging drop vapor diffusion at 20°C by mixing a

1:1 ratio of 15–25 mg/mL protein with 100 mM HEPES, pH 7.0–7.4 and 1.25–1.35 M sodium citrate. Crystals were cryo-protected by freezing in their crystallization condition using 1.44 M sodium citrate. Diffraction data were collected on the Life Sciences Collaborative Access Team (LS-CAT) beamline 21-ID-G at the Advanced Photon Source Synchrotron Argonne National Labs, Argonne IL. A 2.30Å resolution SAD data set was collected using a single SeMet Aro8 crystal, and a 1.91Å data set was collected using a native Aro8 crystal. The diffraction data were processed and scaled using HKL2000.²⁶ The 22 Se atom sites were located using the program HYSS²⁷ from PHENIX.²⁸ Phasing, density modification and initial model building were completed using the AutoSol²⁹ procedure within PHENIX. The Aro8 SeMet model was further refined using Coot³⁰ and PHENIX.³¹ The high-resolution native Aro8 structure was solved using the SeMet Aro8 structure as a molecular replacement model in MOLREP,³² which was modeled and refined using Coot³⁰ and REFMAC³³ with TLS refinement.³⁴ The final model was validated in MolProbity³⁵ (Table I) and “kicked” omit maps were generated using Calculate maps in PHENIX.²⁸ Structural figures were rendered using PyMOL (Schrödinger, LLC). The Aro8 structure was deposited in the RCSB Protein Data Bank with accession code 4JE5.

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