Supplementary Information

Chorismatase mechanisms reveal fundamentally different types of reaction in a single conserved protein fold

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Material and Methods

Production and purification of chorismate and isochorismate. Production and purification of chorismate from *E. coli* fermentation supernatants, as well as the *in vitro* production and purification of isochorismate using a two-enzyme cascade, were carried out as described previously.¹

Site-directed mutagenesis. FkbO variants were constructed using the QuikChange method. 50 ng of pET28a-FkbO as a template was amplified using 500 nM of site-directed mutagenesis primers, 3% DMSO and Phusion Flash PCR Master Mix (NEB) in a volume of 50 µL. QuikChange primers: *FkbO_A331C-fwd* 5'-TTG CAC ACC GAC ATA TGC CGC GAG GAT CTG CTC-3', *FkbO_A331C-rev* 5'-GAG CAG ATC CTC GCG GCA TAT GTC GGT GTG CAA-3'. All other FkbO variants were constructed using synthetic gene fragments (see below).

Hyg5 variants were constructed using the overlap extension method. In the first step, two overlapping fragments were amplified using 50 ng of pET28a-Hyg5 as a template with 500 nM of the corresponding primers, 3% DMSO and Phusion Flash PCR Master Mix (NEB) in a volume of 50 µL. In step two, 50 ng of each linear fragment was incubated using 3% DMSO and Phusion Flash PCR Master Mix. After 10 cycles, 500 nM of primers *Hyg5-fwd* and *Hyg5-rev* were added to the reaction mixture to start the amplification. For the generation of the double mutant pET28a-Hyg5_G240A/C327A, pET28a-Hyg5_G240A was used as a template. The resulting linear fragments harboring the desired mutation were digested using NdeI and XhoI and ligated into pET28a. Overlap extension primers: *Hyg5-fwd* 5'-TATATATA CAT ATG AAC CGT CAT CG-3', *Hyg5-rev* 5'-TATATA CTC GAG CAT GAC CAC GCC-3', *Hyg5_G240A-fwd* 5'-GTC TTC GTG TCC GCG ACC GCC AGC GTG-3', *Hyg5_G240A-rev* 5'-CAC GCT GGC GGT CGC GGA CAC GAA GAC-3', *Hyg5_C327A-fwd* 5'-ACG GTG GAC GTC GCC CGC TCC GAT CTG-3', *Hyg5_C327A-rev* 5'-CAG ATC GGA GCG GGC GAC GTC CAC CGT-3', *Hyg5_C327S-fwd* 5'-ACG GTG GAC GTC AGC CGC TCC GAT CTG - 3', *Hyg5_C327S-rev* 5'-CAG ATC GGA GCG GCT GAC GTC CAC CGT-3', *Hyg5_E334Q-fwd* 5'-GAT CTG GTG CAG ATC GAG GGC GTG-3', *Hyg5_E334Q-rev* 5'-CAC GCC CTC GAT CTG CAC CAG ATC-3'. Hyg5_G240A was generated by partial gene synthesis, as described below.

Generation of chorismatase variants using partial gene synthesis. Gene fragments of FkbO_A244G and FkbO_A244G/A331C harboring the desired mutation were synthesized (GeneArt, life technologies; Frankfurt, Germany) with restriction sites for AgeI (5'-) and EcoRI (-3'). Gene fragments

and pET28a-FkbO were digested using the corresponding restriction endonucleases and ligated, yielding pET28a-FkbO_A244G and pET28a-FkbO_A244G/C327A.

A gene fragment of Hyg5_G240A harboring the desired mutation was synthesized (GeneArt, life technologies; Frankfurt, Germany) with restriction sites for EagI (5'-) and XhoI (-3') and ligated into pET28a, yielding pET28a-Hyg5_G240A.

All variants were confirmed by sequencing (GATC Biotech; Konstanz, Germany).

Expression and purification. The heterologous expression and purification of chorismatases and variants was performed as described previously: Hyg5 variants were expressed and purified analogous to the protocol described for FkbO.^{2,3} Expression and purification of the isochorismate synthase EntC was performed as described previously.¹

Chorismatase assay. The assay employed for all variants and the wild-type chorismatases was carried out as described previously for FkbO and Hyg5.¹ Due to the low activity of some of the variants, the reaction time was increased up to 20 h. The reaction was stopped by removing the enzymes (spin filtration, 10 kDa cutoff) and the supernatant was analyzed by LC-MS or HPLC. An assay run under the same conditions without the addition of chorismatase served as a negative control.

Chorismatase assay in ¹⁸O-labeled water. 1 mM chorismate was dissolved in Tris buffer (100 mM, pH 7.0) containing ¹⁸O-labeled water (97%) and incubated with FkbO or Hyg5 (100 µg/mL) at RT for 2 h. To avoid ¹⁸O-label exchange from the product pyruvate *via* acetalization,⁴ an LDH-coupled assay was used, where the pyruvate formed was immediately converted with NADH-dependent LDH into lactate. The reaction was stopped by removing the enzymes (spin filtration, 10 kDa cutoff) and the supernatant was analyzed by LC-MS. An assay run under the same conditions without the addition of chorismatase served as a negative control; in addition, reactions with and without chorismatase were carried out in nonlabeled water.

Chorismatase assay with chorismate selectively ¹⁸O-labeled at the C4-OH position. The assay was performed under the same conditions (+ 10 mM MgCl₂), with the same control reactions, as described above for the chorismatase assay using ¹⁸O-labeled water. 1 mM isochorismate was used as a substrate, and was partially converted into selectively ¹⁸O-labeled chorismate using the isochorismate synthase EntC (100 µg/mL). Due to the fact that chorismatases are not able to convert isochorismate, all isochorismate was converted into selectively labeled chorismate and subsequently to the

chorismatase products when incubated with FkbO or Hyg5.¹ The reaction was stopped by removing the enzymes (spin filtration, 10 kDa cutoff) and the supernatant was analyzed by LC-MS.

Assay of Hyg5 with potential intermediates. The assay was performed as described previously for Hyg5 using either 3,4-*trans*-CHD or enoyl benzoate as a substrate instead of chorismate.¹ Due to possible lower activity with these compounds, the reaction time was increased to 20 h. The reaction was stopped by removing the enzymes (spin filtration, 10 kDa cutoff) and analyzed by HPLC. Assays run under the same conditions without addition of Hyg5 served as a negative control, and the reaction of chorismate with Hyg5 as a positive control. Enoyl benzoate was donated by Dr Barrie Wilkinson (John Innes Center, Norwich, UK), for which we are grateful.

Analytical methods. For enzyme activity assays, HPLC(-MS) analysis was carried out on an Agilent 1100 HPLC system equipped with an RP18 column (Eclipse XDB, 5 μ m, 12.5 cm, 4.6 mm, Agilent) using water and acetonitrile (each containing 0.1% TFA for HPLC or 0.1% formic acid for LC-MS) as mobile phase, as described previously for HPLC analysis.¹ For MS analysis, the LC system was coupled with an ion-source mass spectrometer (Api2000, Applied Biosystems) monitoring masses between 50 and 400 amu in the negative mode.

For assays using labeled solvents, LC-MS analysis was carried out on the same system equipped with an Hilic column (X-Bridge BEH Amide, 2.5 μ m, 10 cm, 2.1 mm, Waters) using 10 mM ammonium acetate buffer (pH 6.8) and acetonitrile as mobile phase. Separation was achieved using a stepwise gradient [starting conditions: 95% acetonitrile, 0.3 mL/min, 3 min; linear gradient I: up to 40% acetonitrile, 0.3 mL/min, 13 min; isocratic gradient: 40% acetonitrile, 0.3 mL/min, 18.5 min; linear gradient II: up to 95% acetonitrile, 0.3 mL/min, 20 min; final conditions: 95% acetonitrile, 0.3 mL/min, 35 min; retention times: 3-HBA – 2.6–3.9 min, pyruvate – 4.4–5.6 min (not monitored in DAD), lactate – 9.8–10.8 min (not monitored in DAD), 3,4-*trans*-CHD – 10.5 min, chorismate – 11.9 min, isochorismate – 12.4 min, NADH – 12.7 min, NAD⁺ – 13.2 min]. For MS analysis, the LC system was coupled with an ion-source mass spectrometer (Api2000, Applied Biosystems) monitoring masses from 50 to 112 and 113.4 and 400 amu in the negative mode [m/z (nonlabeled/labeled): 3-HBA – 137/138/139 amu, pyruvate – 87/88 amu, lactate – 89/91 amu, 3,4-*trans*-CHD – 155/157 amu, chorismate – 225/227 amu, isochorismate – 225/227 amu].⁵

Circular Dichroism (CD) spectroscopy of chorismatase and variants. To confirm the correct folding of the chorismatase variants, CD spectra of the enzymes (10 μ M) were recorded in Tris buffer (100 mM, pH 7.0) between 215 and 300 nm on a spectropolarimeter (J-810, Jasco). Spectra of the wild type and variants were compared *via* superposition of the spectra using the SpectraMax software (Jasco).

Crystallization of Hyg5 and the Hyg5 variant C327S^{Hyg5}. After purification with Ni-NTA affinity chromatography, Hyg5 and C327S^{Hyg5} were further purified using size exclusion chromatography (Superdex 75) in 20 mM Tris, 100 mM NaCl and 1 mM TCEP at pH 7.5. Prior to crystallization, Hyg5 was concentrated to 2.5–3 mg/mL and afterwards supplemented with 15 mM 3-(2-carboxyethyl) benzoate.^{2,3} Hyg5 crystals were obtained *via* hanging-drop vapor diffusion at 18 °C by equilibration against a reservoir solution of 0.1 M MES pH 6.5, 0.2 M ammonium sulfate and 20–25% PEG 5000 MME (1:1 ratio of protein to reservoir solution). Well-diffracting protein crystals were obtained after a period of 1 week. C327S^{Hyg5} was concentrated to 2 mg/mL and afterwards supplemented with 15 mM 3-HBA. C327S^{Hyg5} was crystallized using the hanging-drop diffusion method in 0.1 M MES pH 6.5–7, 0.2 M ammonium sulfate and 20% PEG 5000 MME.

Data collection and structure determination. Diffraction data for Hyg5 and C327S^{Hyg5} were collected at the beamline PXI (XO6SA) with a wavelength of 1.001 Å and at a temperature of 100 K [Swiss Light Source, Paul Scherrer Institute (PSI), Villigen, Switzerland]. Data reduction was carried out with the X-ray Detector Software (XDS Program Package).^{6,7} The structure for Hyg5 was solved with FkbO (*PDB: ID 4bps*)³ as a molecular replacement model using PHASER. The first model for Hyg5 was built using AUTOBUILD and, later, manually using COOT.⁸ The preliminary model was iteratively completed within several rounds of rebuilding and runs of phenix.refine.⁹ Coordinates and restraints of the 3-(2-carboxyethyl) benzoate (ID: 3EB) were the same as in *PDB ID 4bps*. The structure was refined at 1.9 Å resolution and the model yielded an R-factor and R-free of 0.1319 and 0.1646, respectively. The diffraction data were processed in the space group *P*32. The crystal was twinned with 50% twinning obeying twin law $-h,-k,l$. The overall quaternary structure was similar to the chorismatase FkbO: 3-(2-carboxyethyl) benzoate is found in the active site of two chains, whereas the active site of one chain is empty. 93% of the residues were in the favored region of the Ramachandran plot, while 6.09% were in the allowed region and 0.91% were Ramachandran outliers. For C327S^{Hyg5}, the Hyg5 structure was used as a molecular replacement model. C327S^{Hyg5} was co-crystallized with 3-HBA in its active site. For C327S^{Hyg5}, first a rigid body refinement was carried out with successive refinement cycles in

phenix.refine. Geometry restraints for 3-HBA were obtained with phenix.eLBOW.¹⁰ The C327S^{Hyg5} crystal diffracted to 2.75 Å; the diffraction data were processed in space group *P1*-21-1. Three chains in the asymmetric unit were obtained and the model was refined to an R-factor and R-free of 0.2530 and 0.3081, respectively. 96% of the C327S^{Hyg5} residues were favored in the Ramachandran plot and 2.5% were in the allowed region, while 1.5% were Ramachandran outliers. The structures were evaluated using the MolProbity server (<http://molprobity.biochem.duke.edu>). Data collection and refinement statistics are detailed in **Table S1**. Figures were prepared using UCSF Chimera.¹¹

Phylogenetic studies. A pblast search using the amino acid sequences of FkbO from *Streptomyces hygroscopicus* subsp. *ascomyceticus*, Hyg5 from *Streptomyces hygroscopicus* and XanB2 from *Xanthomonas campestris* pv. *campestris* were used as templates.¹² In total 134 hits with an E-value < e⁻⁹⁰ were used for an amino acid based multiple sequence alignment; based on this alignment a phylogenetic tree (Jukes-Cantor model, Neighbor-Joining method, no outgroups) was created using Geneious Tree Builder of Geneious version 8 (<http://www.geneious.com>).¹³

Supplementary Fig. 1: Multiple Alignment of chorismatases Conserved amino acids in all three chorismatases are highlighted in grey. Amino acids that are part of the active site are labeled with an asterisk (*). The two amino acids that are suggested to be responsible for the product selectivity in CH-FkbO and CH-Hyg5 are highlighted in cyan. The amino acid that is suggested to be responsible for the unselectivity of the CH-XanB2 reaction is highlighted in green.

Fkb0:	MTDAGRQGRVEALSISVTAPYCRFEKTGSPDLE-GDETVLGLIEHGTGHTDVSIVDGA	PR	: 59			
RapK:	-----MRQLTPPVTA	YCRFEKLGASDLD-GDETL	LG	VIEHRTGHTGVS	LAEGCPR	: 50
Hyg5:	-----MNPSSLVLNGLTSYFENGRARVVPVGRN	IL	GVVNYASVCEYPTLDHGYPE	: 51		
Cuv10:	-----MNPSSLVLNGLTSYFENGRAGVPPAGRN	IL	GVVNYASVCEYPTLDHGYPE	: 51		
XanB2_camp:	-----MTAPTLQPNQTVRHPRLQVDYVAQTD	PSTLLQDAQMLAVFGFGDAAPRLDD--PR	: 53			
XanB2_oryz:	-----MTASQAQTTHAVRHPRLQVDYVDQTD	PAVLLADPQVLAVFGFGDAAPRLHD--PR	: 53			
Fkb0:	TAVHTTTTRDDEAFTEVWHAQRPVESGMDNGIAWARTDAYLFGVVRTGES-GRYAD	ATAAL	: 118			
RapK:	TAVHTTTTREDESFAEAWHAEGPKESSRHDGVAVARTPDYLF	GVARVPEG-GRYAAGTAAV	: 109			
Hyg5:	LEINMVAPTAEPFAEVWVTDAEAEHGERDGITYAHDGEYFFCAGRPPT-GRYTEATRAA	: 110				
Cuv10:	LAINMVAPTAEPFAEVWVTDAEAEHGERDGITYAHDGEYFFCAGRPPT-GRYTEATRAA	: 110				
XanB2_camp:	YLRVPLQPHYAADVLEVWRTDAPVRSGRDGNIAWSSDGR	LQFGVIEIDEQEVEIEEAAAAKA	: 113			
XanB2_oryz:	YLRVPLQPHYQASVLEVWRTAASVRSGREGNIAWSSDGH	LHFGVIEIDEQDLIDIEEAAAAKA	: 113			
Fkb0:	YTNV	FQLTRSLGYPL	LARTWNYVSGINTTNADGLE	VYRDF	FCVGRAQALDEGGIDPATMPA	: 178
RapK:	YTGI	FDLIGTLGYPS	LARTWNYVSGINTPNADGLE	VYRDF	FCVGRAEALDARGIDPATMPA	: 169
Hyg5:	YVTMFEL	LEEF	GYSSVFRMWNFIGDINRDNAEGMEVYRDF	FCRGRAEAFEQCRLEFDQFPA	: 170	
Cuv10:	YVTMFEL	LEEF	GYSSVFRMWNFIGDINRDNAEGMEVYRDF	FCRGRAEAFEQCRLEFDQFPA	: 170	
XanB2_camp:	YAEITAFVSGSQTPRLLRIWNYLDAITL	SGSD-RERYRQFCVGRARGLG--AFDVAQLPA	: 170			
XanB2_oryz:	YAEITAFVSGSQTPRLLRIWNYLDAITL	SGSD-RERYRQFCVGRARGLG--AFDTAQLPA	: 170			
Fkb0:	ATGIG-AHGGG	ITCVFLAARGGVRINIENPAVLTAHHYPTTYGPRPPVFARATWLG	PPPE-	: 236		
RapK:	ATGIG-AHGARI	TCYFIAARAGDRVNMENPAVLTAHRYPQRYGPRPPVFS	GH	LALAAG--	: 226	
Hyg5:	ATGIG-SRGGG	IAFYLLACRSGGHVHIENPRQVPAYHYPKRYGPRAPRFARATY	LPSRAA	: 229		
Cuv10:	ATGIG-SRGGG	IAFYLLACRSGGHVHIENPRQVPAYHYPKRYGPRAPRFARATY	LPSRAA	: 229		
XanB2_camp:	ATAVGR	CDARI	IQIYWLAAADAGTPLENPRQVSAYNYPRQYGPQPPSFAR	MLPPAGG-	: 229	
XanB2_oryz:	ATAVGR	CDDERI	IQIYWLAAANAGMPLENPRQVSAYNYPRQYGPQPPSFAR	MLPPAGS-	: 229	
Fkb0:	---GGRLF	ISATAGILGHRTVHHG	DTV	GQCEVALDNMARVIGAENLRRHGVQR	GHVLADV	: 293
RapK:	---GGRLF	VSATAGIVGQETVHHG	DVA	AQCEVSL	ENIARVIGAENLGRHGLRRGYALADV	: 283
Hyg5:	DGVGGQ	VFVSGTASVLGHETAHEGDLVKQ	CLALENIELVISGGNLA	AHGISAGHGLTAL	: 289	
Cuv10:	DGVGGQ	VFVSGTASVLGHETAHEGDLVKQ	CLALENIELVISGGNLA	AHGISAGHGLTAL	: 289	
XanB2_camp:	---DMPL	LLSGTAAVVG	HASMHTGQLLAQLEETFANFDALLGAARQHAPGLPAQ	FGAG--	: 284	
XanB2_oryz:	---DMPL	LLSGTAAVVG	HASMHTGQLLAQLEETFANFDALLGAARLHASALPAQ	FGAG--	: 284	
Fkb0:	DHLKVYVR	RREDL	DTVRRVCAARLSSTA	AVALLHTDIA	REDLLVEIEGMVA	: 344
RapK:	DHLKVYVR	HREDI	STVRRICAERLSREATVAVLHTDIA	RTDLLVEIEGVVA	: 334	
Hyg5:	RNIKVYVR	RESDVPAVREICREAFSPDADIVYLTVDV	CRSDLLVEIEGVVM	: 340		
Cuv10:	RNIKVYVR	RAEDVPAVREICREAFSPDADIVYLTVDV	CRSDLLVEIEGVVM	: 340		
XanB2_camp:	TRLKVYVR	ERDDL	PKVAQALDARFGDAVPRLLH	AVICRDELA	VEIDGVHG	: 335
XanB2_oryz:	TRLKVYVR	ERDDL	PKVAQALDARFGDAVPRLLH	AVICRDELA	VEIDGVHG	: 335

Accession code: FkbO from *Streptomyces hygroscopicus* subsp. *ascomyceticus*: AAF86394.1
 RapK from *Streptomyces rapamycinicus* NRRL 5491: AGP59510.1
 Hyg5 from *Streptomyces hygroscopicus*: AAC38060.1
 Cuv10 from *Streptomyces* sp. LZ35: AGO98693.1
 XanB2_camp from *Xanthomonas campestris* pv. *campestris*: AAY51142.1
 XanB2_oryz from *Xanthomonas oryzae*: WP_024744263.1

Supplementary Fig. 2: Putative intermediates of the CH-Hyg5 subfamily reaction and HPLC analysis of chorismatase assays with enoyl benzoate and 3,4-*trans*-CHD. A) Potential intermediates. B) Enoyl benzoate as a reference (0 h). Enoyl benzoate after 20 h incubation with Hyg5, and double amount of enoyl benzoate after 20 h incubation with Hyg5. C) 3,4-*trans*-CHD as a reference (0 h). 3,4-*trans*-CHD after 20 h incubation without Hyg5, and 3,4-*trans*-CHD after 20 h incubation with Hyg5. The retention time of 3-HBA would be 16 min.

Supplementary Fig. 3: HPLC analysis of chorismatase assays and CD spectra (variants and wild-type enzymes of FkbO and Hyg5). A) Chorismate as a reference and products of the wild-type enzyme and variants of FkbO. B) Chorismate as a reference and products of the wild-type enzyme and variants of Hyg5. C) CD spectra of the wild-type enzyme and variants of FkbO. D) CD spectra of the wild-type enzyme and variants of Hyg5.

C **D**

Supplementary Fig. 4: LC-MS analysis of chorismatase assays

A) in H_2^{18}O (FkbO and Hyg5). i) Total ion count (TIC) of important mass to charge ratios (m/z ; FkbO assay). ii) m/z in detail for the relevant peaks of a: $m/z = 89$ – nonlabeled lactate; $m/z = 91$ – labeled lactate; $m/z = 155$ – nonlabeled 3,4-*trans*-CHD; $m/z = 157$ – labeled 3,4-*trans*-CHD. iii) Total ion count (TIC) of important mass to charge ratios (m/z ; Hyg5 assay). iv) m/z in detail for the relevant peaks of c: $m/z = 89$ – nonlabeled lactate; $m/z = 91$ – labeled lactate; $m/z = 137$ – nonlabeled 3-HBA; $m/z = 139$ – labeled 3-HBA.

B) in H₂¹⁸O with 4-¹⁸O-labeled chorismate (FkbO and Hyg5). i) Total ion count (TIC) of important mass to charge ratios (m/z ; FkbO assay). ii) m/z in detail for the relevant peaks of a: $m/z = 89$ – nonlabeled lactate; $m/z = 91$ – labeled lactate; $m/z = 155$ – nonlabeled 3,4-*trans*-CHD; $m/z = 157$ – labeled 3,4-*trans*-CHD. iii) Total ion count (TIC) of important mass to charge ratios (m/z ; Hyg5 assay). iv) m/z in detail for the relevant peaks of c: $m/z = 89$ – nonlabeled lactate; $m/z = 91$ – labeled lactate; $m/z = 137$ – nonlabeled 3-HBA; $m/z = 139$ – labeled 3-HBA.

C) in H₂O (FkbO and Hyg5). i) Total ion count (TIC) of important mass to charge ratios (m/z ; FkbO assay). ii) m/z in detail for the relevant peaks of a: $m/z = 89$ – nonlabeled lactate; $m/z = 155$ – nonlabeled 3,4-*trans*-CHD. iii) Total ion count (TIC) of important mass to charge ratios (m/z ; Hyg5 assay). iv) m/z in detail for the relevant peaks of c: $m/z = 89$ – nonlabeled lactate; $m/z = 137$ – nonlabeled 3-HBA.

Supplementary Fig. 5: Structure and active site of the chorismatase Hyg5 and Hyg5 variant C327S. A) Stereo view of the three-domain organization of Hyg5: N-terminal domain (yellow), central domain (green) and C-terminal domain (cyan). Loops 1–3 of the C-terminal domain (blue) form the binding cavity for the competitive inhibitor 3-(2-carboxyethyl)benzoate (Hyg5) or 3-HBA (variant C327S) B) Stereo view of the amino acids that form the active site of Hyg5 and their interactions with ligand 3-(2-carboxyethyl)benzoate (Hyg5) or 3-HBA (variant C327S) C) Stereo view of Fo-Fc simulated annealing omit maps for 3-(2-carboxyethyl)benzoate (Hyg5) contoured at a sigma level of 1.5 and 3-HBA (variant C327S) contoured at a sigma level of 1.0. D) Chorismate and wild-type enzyme Hyg5 as references for product formation of C327S^{Hyg5}.

A

B

C

Supplementary Table 1: Data collection and refinement statistics (molecular replacement) of Hyg5 and variant C327S^{Hyg5}

	Hyg5	C327S ^{Hyg5}
	PDB-ID: 5AG3	PDB-ID: 5A3K
Data collection		
Space group	<i>P</i> 32	<i>P</i> 1-21-1
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	116.18, 116.18, 70.43	63.77, 127.21, 81.89
α , β , γ (°)	90.00, 90.00, 120.00	90.00, 104.85, 90.00
Resolution (Å)	44.81-1.898(1.966-1.898) *	44.27-2.753(2.851-2.753)
<i>R</i> _{sym} or <i>R</i> _{merge}	0.08411(1.171)	0.2841(2.219)
<i>I</i> / σ <i>I</i>	12.50(0.88)	5.15(0.64)
Completeness (%)	99.80(98.11)	94.46(98.80)
Redundancy	5.0(3.5)	3.2(3.2)
Refinement		
Resolution (Å)	1.898	2.753
No. reflections	420788(29193)	99130(10430)
<i>R</i> _{work} / <i>R</i> _{free}	0.1319 / 0.1646	0.2530(0.3507) / 0.3081(0.3947)
No. atoms	9138	7982
Protein	7760	7763
Ligand/ion	67	45
Water	1311	174
<i>B</i> -factors		
Protein	40.90	66.20
Ligand/ion	40.40	67.40
Water	42.70	43.70
R.m.s. deviations		
Bond lengths (Å)	0.014	0.003
Bond angles (°)	1.47	0.82

*Values in parentheses are for the highest-resolution shell.

Supplementary Fig. 6: Phylogenetic tree of the chorismatase subfamilies. The amino acids postulated to be responsible for the distinction between the subfamilies are found in all sequences; only the putative XanB2-subfamily sequences from *Stenotrophomonas* species (*) feature an cysteine instead of an alanine in the XanB2-defining position. Details regarding the construction of the tree are given in the experimental section.

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