SUPPLEMENTARY INFORMATION

Structure and Function of a Complex Between Chorismate Mutase and DAHP Synthase: Efficiency Boost for the Junior Partner

Severin Sasso[1,3], Mats Ökvist[2,3], Kathrin Roderer[1], Marianne Gamper[1], Giosiana Codoni[1], Ute Krengel[2,*] and Peter Kast[1,*]

[1]Laboratory of Organic Chemistry, ETH Zurich, CH-8093 Zurich, Switzerland and
[2]Department of Chemistry, University of Oslo, N-0315 Oslo, Norway.
[3]These authors contributed equally to this work.

*Corresponding authors:

U Krengel, Department of Chemistry, University of Oslo, N-0315 Oslo, Norway. Tel.: +47 22 85 5461; Fax: +47 22 85 5441; E-mail: ute.krengel@kjemi.uio.no

and

P Kast, Laboratory of Organic Chemistry, ETH Zurich, HCI F333, Wolfgang-Pauli-Strasse 10, CH-8093 Zurich, Switzerland; Tel.: +41 44 632 2908; Fax: +41 44 633 1326; E-mail: kast@org.chem.ethz.ch

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Supplementary Table SI Genetic complementation[a]

Plasmid	Encoded CM[b]	Growth on M9c +F	Growth on M9c +FY
pKTCMM-H	MtCM	+	+
pKTCM-H	MtCM+15	+	+
pMG246	MtCM-4	+	+
pMG212H-W (positive control)[c]	BsCM	+	+
pMG212H-0 (negative control)[c]	none	0	+

[a]Chorismate mutase (CM)-deficient *E. coli* KA12/pKIMP-UAUC cells (Kast *et al*, 1996) transformed with the indicated plasmid were streaked out onto M9c agar plates (Kast *et al*, 2000), supplemented either with 20 μg/ml phenylalanine (M9c +F) or with both 20 μg/ml phenylalanine and 20 μg/ml tyrosine (M9c +FY). 133 μM IPTG (isopropyl *β*-D-thiogalacto-pyranoside) were added to activate the auxiliary functions on pKIMP-UAUC. Addition of salicylate, the inducer of the *sal* promoter present on the plasmids, was not necessary, indicating that basal gene expression suffices for complementation. On the other hand, the observation that salicylate impairs growth of the pKTCMM-H, pKTCM-H, and pMG246 transformants (data not shown) can presumably be explained by toxicity at very high expression levels. The plates were incubated at 30 °C for 5 days; +, good growth; 0, no growth.

[b]MtCM, MtCM+15, and MtCM-4 refer to MtCM versions starting at different positions in the open reading frame, resulting in a 90, 105, and 86 residue protein, respectively. BsCM, monofunctional *Bacillus subtilis* CM of the AroH class encoded by *aroH* (Gamper *et al*, 2000).

[c]Control plasmids were described previously (Gamper *et al*, 2000).

Supplementary Table SII Protein-ligand interactions: hydrogen bonds and van der Waals contacts to transition state analog **3** in the ternary complex (PDB ID 2W1A)[a]

Inhibitor **3** atom	Protein/Water atom	Distance [Å]
O1	Arg35 Nη1	3.3
O1	Ser39 Cβ	3.5
O1	Ser39 Oγ	2.6
O1	Leu81 Cδ2	3.6
O2	Ile21 Cδ1	3.9
O2	Arg35 Nη2	3.0
O2	Val62 Cγ2	3.9
O2	Leu81 Cδ2	3.6
O2	Wat1 O	2.8
O3	Arg18' Cγ	3.6
O3	Arg18' Nε	3.0
O3	Ile42 Cγ2	3.9
O3	Arg58 Cγ	3.6
O3	Arg58 Nε	3.8
O3	Wat1 O	3.0
O4	Arg 18' Cζ	3.7
O4	Arg18' Nη2	3.0
O4	Ile42 Cγ2	3.9
O4	Arg46 Cζ	3.5
O4	Arg46 Nη1	3.1
O4	Arg46 Nη2	3.2
O5	Leu54 Cα	3.4
O5	Val55 Cα	4.0
O5	Val55 N	3.0
O5	Val55 O	3.8
O5	Glu59 Cγ	3.2
O5	Glu59 Oε2	2.7
O7	Arg46 Nη2	3.1
O7	Wat2 O	2.7
C1	Ser39 Oγ	3.6
C1	Wat1 O	3.8
C2	Wat1 O	3.6
C2	Arg58 Cγ	3.8
C2	Leu81 Cδ2	3.9
C3	Arg58 Cγ	3.6
C3	Glu59 Cγ	3.5
C4	Arg46 Nη2	3.9
C4	Val55 N	3.7
C4	Glu59 Oε2	3.8
C5	Arg46 Nη2	3.8
C5	Wat2 O	3.2
C6	Ser39 Oγ	3.5
C6	Arg85 Cγ	3.6
C6	Wat2 O	3.5
C8	Ile42 Cγ2	3.6
C8	Arg46 Nη2	3.9
C8	Wat2 O	3.6
C9	Wat1 O	3.4
C9	Ser39 Oγ	3.4
C10	Arg35 Nη1	3.8
C10	Ser39 Oγ	3.3
C10	Leu81 Cδ2	3.4
C10	Wat1 O	3.6
C11	Arg18' Nε	3.6
C11	Ile42 Cγ2	3.6
C11	Arg46 Nη1	3.9

[a]All closest contacts with distances ≤ 4.0 Å are listed, based on an average of the two copies of the active site in the asymmetric unit. An apostrophe (') indicates that the residue is from a symmetry-related molecule in the crystal structure.

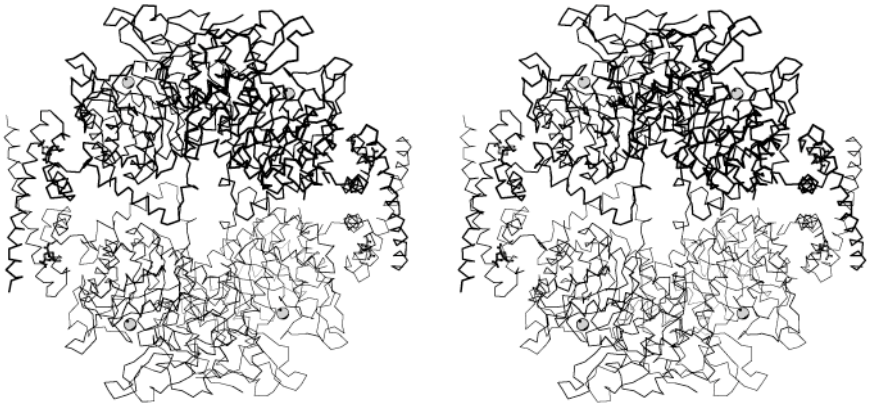
Supplementary Table SIII Statistical details of the data presented in Table III

	P value[a]	
Amino acid added	100 nM MtCM	30 nM MtCM + 300 nM MtDS
200 μM Phe	0.64	0.0002
200 μM Tyr	0.27	0.0028
100 μM Tyr + 100 μM Phe	0.80	0.0031[b]
200 μM Trp	0.25	0.69

[a]The mean values and standard deviations reported in Table III of the main manuscript were compared to the values of the corresponding control measurement without addition of amino acids. It was assumed that the underlying populations were normally distributed. *P* values were calculated with the conventional unpaired two-tailed t-test using the program GraphPad (http://www.graphpad.com/quickcalcs/ttest1.cfm).

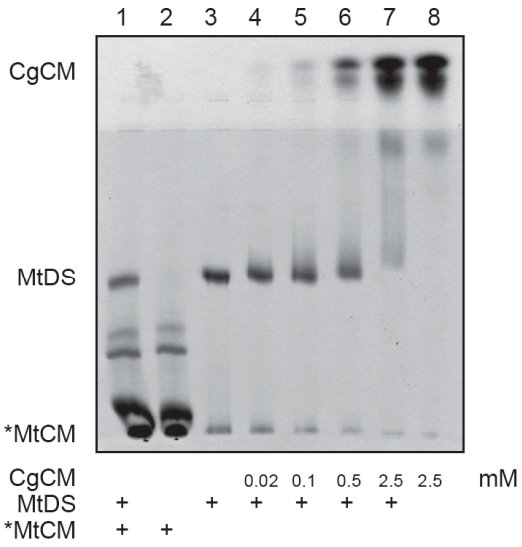
[b]As the variance differed significantly from the variance of the corresponding control measurement according to Snedecor's F-test, Welch's t-test was used in this case.

Supplementary Figure S1



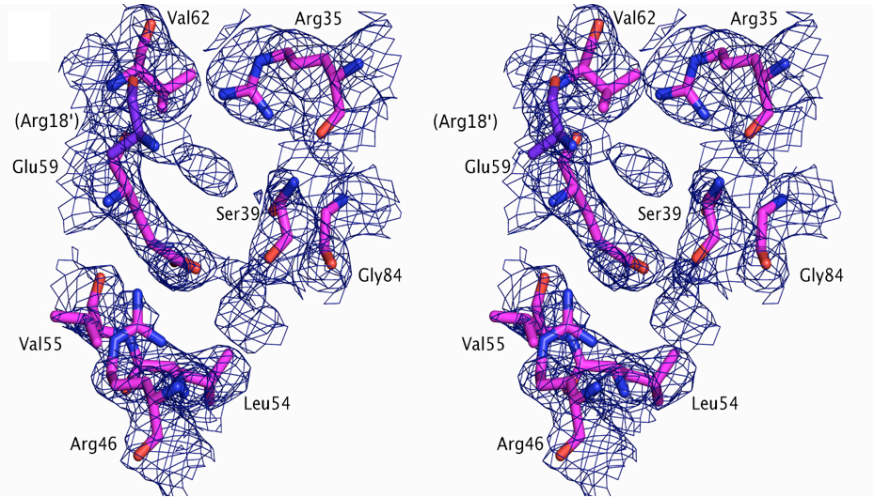
Supplementary Figure S1 Stereo picture of the backbone trace of the ternary MtCM-MtDS complex with bound transition state analog **3** (PDB ID 2W1A). MtDS, which exhibits a TIM barrel fold, forms a homotetramer (center), which is adorned on opposite sides by MtCM homodimers completing the heterooctameric complex.

Supplementary Figure S2



Supplementary Figure S2 Interaction of MtDS with the heterologous CgCM probed by band shift experiments using native PAGE. MtDS was present at 0.02 mM concentration where indicated (+). Increasing concentrations (0.02 to 2.5 mM) of CgCM lead to a progressive MtDS bandshift (lanes 4 to 7) compared to MtDS alone (lane 3), compatible with formation of a heterologous CgCM-MtDS complex. That the MtDS bandshift is not just the result of the high protein concentration employed was shown by the lack of an effect of 2.5 mM *MtCM [exported $AroQ_{\gamma}$ subclass CM from *M. tuberculosis* (Sasso *et al*, 2005); lanes 1 and 2] on the migration behavior of MtDS (compare lanes 1 and 3).

Supplementary Figure S3



Supplementary Figure S3 Stereo figure showing MtCM active site details of the MtCM-MtDS complex (PDB ID 2W19, magenta/purple carbons) without **3** (2Fo-Fc simulated annealed composite OMIT map contoured at 1.5 σ). Arg18' in parentheses indicates that this residue (purple) has been modeled as an alanine due to lack of electron density.

SUPPLEMENTARY METHODS

Construction of plasmids

Restriction endonucleases and DNA-modifying enzymes were, in general, purchased from New England Biolabs (Ipswich, MA, USA), Stratagene (La Jolla, CA, USA), or Sigma-Aldrich (Buchs, Switzerland) and used as suggested by the manufacturer. All DNA fragments generated by the polymerase chain reaction (PCR) were always verified after cloning by DNA sequencing over their entire length.

Expression plasmid pKTCM-H, containing the *aroQ*$_\delta$ gene (Rv0948c) starting with the first possible start codon, encoding the protein variant MtCM+15 without His tag, and controlled by a dual *sal*/T7 promoter, was constructed by PCR amplifying *aroQ*$_\delta$. The PCR involved *Mycobacterium tuberculosis* chromosomal DNA as the template and primers 206-MTCM2S (ACCGATGTCATATGCGTCCAGAACCCCCACATCA; restriction site used for cloning underlined) and 203-MTCM2CN (CGATACACTAGTTATTAGTGACCGAGGCGGCCACGGCCCAAT) (40 cycles of 95 °C for 1 min, 47 °C for 2 min, 72 °C for 5 min; followed by 72 °C for 7 min). The 323 bp *Nde*I/*Spe*I PCR fragment was ligated to the 4529 bp *Nde*I/*Spe*I fragment of pMG211 (Sasso *et al*, 2005), yielding pKTCM-H (4852 bp).

Expression plasmid pKTCMM-H, containing *aroQ*$_\delta$ starting with the second possible start codon, encoding the MtCM protein without His tag, was constructed by amplifying *aroQ*$_\delta$ with pKTCM-H as the template and primers 296-MID-S (CAAACATATGAACCTGGAAATGCT) and 203-MTCM2CN (95 °C for 2 min; 30 cycles of 95 °C for 30 s, 51 °C for 30 s, 72 °C for 1 min; followed by 72 °C for 10 min). The 278 bp *Nde*I/*Spe*I PCR fragment was ligated to the 4529 bp *Nde*I/*Spe*I fragment of pMG211, yielding pKTCMM-H (4807 bp).

Expression plasmid pKTCM-HC, containing the wild-type *aroQ*$_\delta$ gene starting from the first possible start codon and encoding MtCM+15 with a C-terminal His tag, was constructed by PCR-amplifying *aroQ*$_\delta$ from *M. tuberculosis* chromosomal DNA with primers 206-MTCM2S and 204-MTCM2N (CGATAACTCGAGGTGACCGAGGCGGCCACGGCCCAAT) (40 cycles of 95 °C for 1 min, 47 °C for 2 min, 72 °C for 5 min; followed by 72 °C for 7 min). The PCR product was digested either with *Nde*I/*Bgl*I to yield a 182 bp fragment, or

with *Bgl*I/*Xho*I to yield a 136 bp fragment. Both fragments were used in a three-fragment ligation with the 4561 bp *Nde*I/*Xho*I fragment of pMG211 to give pKTCM-HC (4879 bp).

Plasmid pMG246 is identical to pKTCMM-H, except that the $aroQ_{\delta}$ gene starts with the third possible start codon. For the construction of pMG246 (4795 bp) the wild-type gene on pKTCM-HC was amplified with primers 202-MTCM2S (ACCGATGTCATATGCTCGAG-TCCCAACCT) and 203-MTCM2CN (95 °C for 1 min; 25 cycles of 95 °C for 1 min, 43 °C for 1 min, 72 °C for 1 min; followed by 72 °C for 7 min). After *Nde*I and *Spe*I digestion, the 266 bp fragment was ligated to the 4529 bp *Nde*I/*Spe*I fragment of pMG211.

Expression plasmid pKTDS-H containing the *aroG* gene (Rv2178c) encoding MtDS without His tag was constructed by amplifying *aroG* with *M. tuberculosis* chromosomal DNA as the template and primers 260-DSMT1S (CGATGACATATGAACTGGACCGTCG-ACAT) and 262-DSMT3N (ACACTAGTTATTAGTCCCGCAGCATCTCC) (95 °C for 2 min; 30 cycles of 95 °C for 30 s, 51 °C for 30 s, 72 °C for 2 min; followed by 72 °C for 10 min). The 1394 bp *Nde*I/*Spe*I PCR fragment was ligated to the 4529 bp *Nde*I/*Spe*I fragment of pMG211, yielding pKTDS-H (5923 bp).

Expression plasmid pKTDS-HN containing *aroG* encoding MtDS with N-terminal His tag (sequence Met-His_6-Ser-Ser-Gly fused to the initial Met) was constructed by amplifying the *aroG* gene with pKTDS-H as the template and primers 269-DSMT4S (CAGTGTCAT-ATGCACCATCATCATCATCATTCTTCTGGTATGAACTGGACCGTCGACATA) and 262-DSMT3N (95 °C for 2 min; 35 cycles of 95 °C for 30 s, 40 °C for 30 s, 72 °C for 2 min; followed by 72 °C for 10 min). The 1424 bp *Nde*I/*Spe*I PCR fragment was ligated to the 4529 bp *Nde*I/*Spe*I fragment of pMG211, yielding pKTDS-HN (5953 bp).

Plasmid pKIMP-UM containing *pheA* (Rv3838c) encoding the prephenate dehydratase from *M. tuberculosis* (MtPDT) was constructed by amplifying the *pheA* gene with *M. tuber-culosis* chromosomal DNA as the template and primers 183-MPHEA-5' (GCTGCTCCA-TGGTGCGTATCGCTTACCT) and 184-MPHEA-3' (CATCATGGGCCCGCGGCCATTA-GCTTATGCTTGCGCCCCCT) (95 °C for 1 min; 40 cycles of 95 °C for 1 min, 51 °C for 2 min, 72 °C for 3 min; followed by 72 °C for 7 min). The 980 bp *Nco*I/*Bsp*120I PCR frag-ment was ligated to the 2521 bp *Bsp*HI/*Bsp*120I fragment of pKIMP-UAUC (Kast *et al*, 1996), yielding pKIMP-UM (3501 bp).

The high-copy number plasmid pMG213H-W is, like pMG211, a derivative of pKSS (Kast, 1994). Both pMG213H-W and pMG211 feature the dual-promoter system described previously (Gamper *et al*, 2000) with a *sal* and T7 promoter in tandem. The T7 promoter is controlled by the *lac* repressor (*lacI* is carried on pMG213H-W and pMG211) and thus IPTG-inducible. The *sal* promoter is inducible by salicylate due to the presence on the plasmids of the *nahR* gene (encoding the activator protein). Both pMG213H-W and pMG211 also carry an ampicillin resistance gene and a T7 terminator. For the construction of pMG213H-W (4943 bp), a 414 bp *Nde*I/*Spe*I-digested PCR fragment carrying *aroH* (identical to the fragment described previously for the ultimate step of the construction of the moderate-copy version pMG212H-W) (Gamper *et al*, 2000) was ligated to a 4529 bp *Nde*I/*Spe*I fragment identical to the one of pMG211. Thereby, the C-terminal His-tag sequence (present in pMG211) was deleted and replaced by an *aroH* gene version (Gamper *et al*, 2000) encoding wild-type BsCM with an N-terminal His tag fused as Met-His_6-Ser-Ser-Gly to the second Met of BsCM (yielding protein H-AroH).

Expression plasmid pMG213H-Mtu containing *pheA* encoding MtPDT with N-terminal His tag was constructed by amplifying the *pheA* gene with pKIMP-UM as the template and primers 194-MTPDT-5' (ACCGATGTCATATGCACCATCATCATCATCATTCTTCTGG-TATGGTGCGTATCGCTTACCT) and 195-MTPDT-3' (TTCCACTAGTCTTATGCTTGC-GCCCCCT) (95 °C for 1 min; 25 cycles of 95 °C for 1 min, 51 °C for 2 min, 72 °C for 3 min; followed by 72 °C for 7 min). The 1000 bp *Nde*I/*Spe*I PCR fragment was ligated to the 4529 bp *Nde*I/*Spe*I fragment of pMG213H-W, yielding pMG213H-Mtu (5529 bp).

The CgCM production plasmid pKCGCM-HN (4837 bp) encodes a 100 amino acid version of CgCM, which contains an N-terminal Met-His_6-Ser-Ser-Gly tag fused to 90 genuine CgCM residues including its native C-terminus (Cgl0853; http://beta.uniprot.org/uniprot/Q8NS29). It was constructed by ligating the 799 bp *Sac*I/*Sac*II fragment of pKCGCM-N5a with the 4038 bp *Sac*I/*Sac*II fragment of pKCGCM-H. pKCGCM-N5a (4837 bp) has the same structure as pKCGCM-HN, except for an A to G point mutation at position 230 of the assigned open reading frame Cgl0853 stemming from a PCR artefact, which was subsequently corrected for pKCGCM-HN. pKCGCM-N5a was constructed by ligation of the 4529 bp pMG211 *Nde*I/*Spe*I fragment with the 308 bp fragment generated by *Nde*I/*Spe*I digestion of the 320 bp fragment obtained by PCR with primers 338-CCM1HS (ATTCATATGCACCATCATCATCATCATTCTTCTGGTATGCCTTCTGGCA-CGGATGACCCATT) and 339-CCM1N (AGGACTAGTTATTATCCGAGTTTTCCGCGT-

CCCAT) on template pKCGCM-HC (10 min 94 °C; 25 cycles of 30 s 94 °C, 30 s 57 °C, 1 min 30 s 72 °C; followed by 10 min at 72 °C). pKCGCM-H (4807 bp) carries the gene encoding the untagged 90 C-terminal residues of CgCM (Cgl0853). It was assembled from the 4529 bp pMG211 *Nde*I/*Spe*I fragment and the 278 bp *Nde*I/*Spe*I fragment of the PCR product (290 bp) obtained with primers 339-CCM1N and 340-CCM2S (ATTCATATGCCT-TCTGGCACGGATGACCCATT) on template pKCGCM-HC using the same conditions as for pKCGCM-N5a. pKCGCM-HC (4873 bp), which carries a gene variant encoding the 90 genuine CgCM residues as in pKCGCM-HN plus an additional 13 amino acids at its N-terminus and a Leu-Glu-His_6-tag at its C-terminus, was assembled by ligating the 4561 bp *Nde*I/*Xho*I fragment of pMG211 with a 312 bp *Nde*I/*Xho*I-digested PCR fragment. The PCR was carried out with primers 313-CGCM1S (CCAGATCATATGTGCATGACTAATGCAG-GTGA) and 314-CGCM2N (GGGATCTCGAGTCCGAGTTTTCCGCGTCCCA) on *Corynebacterium glutamicum* ATCC 13032 chromosomal DNA (90 °C for 2 min; 40 cycles of 95 °C for 30 s, 49 °C for 30 s, 72 °C for 2 min; followed by 72 °C for 7 min).

Plasmids pKTCMM-HN-G86A (4837 bp), pKTCMM-HN-R87A (4837 bp), pKTCMM-HN-L88A (4837 bp), and pKTCMM-HN-L88* (4828 bp) were used for the production of N-terminally His-tagged MtCM versions (the tag is fused as Met-His_6-Ser-Ser-Gly to the second possible start methionine) carrying mutations in the C-terminal region. pKTCMM-HN (4837 bp) encodes the corresponding 100-residue wild-type protein. The constructs were obtained by PCR amplification (94 °C for 1 min; 30 cycles of 94 °C for 1 min, 55 °C (50 °C for pKTCMM-HN) for 1 min, 72 °C for 1 min; followed by 72 °C for 7 min) of the $aroQ_{\delta}$ gene on pKTCMM-H, using as forward primer 354-MTCMM-HNfw (ACGGATGACATATGCACCATCATCATCACCACTCTTCGGGTATGAACCTGGAAA-TGCTCGAG). The corresponding reverse primers were 357-G86Arv (AACAACTAGTTAT-TAGTGACCGAGGCGAGCACGGCCCAA), 358-R87Arv (AACAACTAGTTATTAGTG-ACCGAGAGCGCCACGGCCCAA), 355-L88Arv (AACAACTAGTTATTAGTGACCAG-CGCGGCCACGGCCCAA), 356-L88*rv (ACAACTAGTTATTAGCGGCCACGGCCCAA-TCGCAAA), and 131-TERM (CCCTCAAGACCCGTTTAGA) for plasmids pKTCMM-HN-G86A, pKTCMM-HN-R87A, pKTCMM-HN-L88A, pKTCMM-HN-L88*, and pKTCMM-HN (wild type), respectively. Every PCR product was digested with *Nde*I/*Spe*I, and the 308 bp (299 bp for plasmid pKTCMM-HN-L88*) fragments were ligated to the calf intestine phosphatase (New England Biolabs)-dephosphorylated 4529 bp *Nde*I/*Spe*I fragment of pMG242-0. pMG242-0 (5860 bp) is a derivative of pMG211 that contains a 1331 bp

stuffer fragment between *Nde*I and *Spe*I (unpublished data, 2005). The 4529 bp *Nde*I/*Spe*I vector fragment used for the cloning is identical to the one of pMG211.

Plasmid pKTCMM-HN-R46K (4837 bp) for production of the N-terminally His-tagged R46K MtCM mutant was assembled from the same 4529 bp *Nde*I/*Spe*I fragment and a 308 bp *Nde*I/*Spe*I-digested PCR product. The latter was obtained by overlap extension of two PCR fragments, since the mutation needed to be introduced into the middle of the gene. Applying the PCR temperature regime described above for the C-terminal mutants, and pKTCMM-H as template, the 5' gene fragment was produced with primer pair 354-MTCMM-HNfw and 361-R46Krv (GCCATTTTGGCCTTGCCGATGGCCTT), and the 3' fragment with primers 359-R46Kfw (GGCAAGGCCAAAATGGCGTCCGGTGGC) and 131-TERM. Overlap extension was accomplished by subjecting the purified, annealing 5' and 3' fragments to a primer extension reaction in the absence of external primers (95°C for 1 min; 3 cycles of 95 °C for 30 s, 48 °C for 1 min, 72 °C for 10 min; 72 °C for 7 min), followed by addition of the appropriate amplification primers 131-TERM and 352-CTLIB-S (CCTTGTTCATATGCAC-CATCATCATCACCACTCTT) and PCR amplification (95 °C for 1 min, 30 cycles of 95 °C for 30 s, 50 °C for 1 min, 72 °C for 30 s; followed by 72 °C for 7 min).

Overproduction and purification of **M. tuberculosis** *chorismate mutase (MtCM) and DAHP synthase (MtDS)*

Details to the preparation of wild-type MtCM (without a tag) and His-tagged MtDS using transformants KA13/pKTCMM-H and KA13/pKTDS-HN, respectively, will be published elsewhere (M Ökvist, S Sasso, K Roderer, P Kast and U Krengel, in preparation).

N-terminally His-tagged MtCM mutants were produced in strain KA13 (MacBeath and Kast, 1998; MacBeath *et al*, 1998), transformed with one of the plasmids pKTCMM-HN-R46K, pKTCMM-HN-G86A, pKTCMM-HN-R87A, pKTCMM-HN-L88A, or pKTCMM-HN-L88*. The corresponding wild-type MtCM control encoded on pKTCMM-HN was prepared in parallel. All MtCM variants were overproduced by growing the corresponding transformants in LB +Amp$^{150\ \mu g/ml}$ medium in shake flasks at 30 °C (230 rpm) to exponential phase ($0.3 \leq OD_{600} \leq 0.6$), induction with 0.5 mM IPTG, and further incubation overnight. A cell lysate was prepared and the proteins were purified by Ni-NTA agarose chromatography

(QIAGEN AG, Hombrechtikon, Switzerland) as published previously for His-tagged *MtCM (Sasso *et al*, 2005). Yields ranged between 8 and 33 mg/L culture volume.

Overproduction and purification of **M. tuberculosis** *prephenate dehydratase (MtPDT)*

The MtPDT with an N-terminal His tag was overproduced by growing KA13/pMG213H-Mtu cells as described above for the His-tagged MtCM mutants, but with 2% (w/v) glucose present. Protein purification followed a protocol described earlier for His-tagged *MtCM (Sasso *et al*, 2005), except that 1 mM β-mercaptoethanol was present in all solutions to keep the protein reduced. Yields were typically 4 mg/L culture volume.

Overproduction and purification of **C. glutamicum** *chorismate mutase (CgCM)*

The $AroQ_{\delta}$ subclass CM from *C. glutamicum* carrying an N-terminal His tag was overproduced by growing KA13/pKCGCM-HN transformants as described above for KA13/pMG213H-Mtu. Purification followed the protocol described earlier for His-tagged *MtCM (Sasso *et al*, 2005), except that instead of potassium phosphate buffer, 20 mM BTP (1,3-bis[tris(hydroxymethyl)methylamino]propane) buffer, pH 7.0, was used. Yields were between 20 and 70 mg/L culture volume.

Mass spectrometry of purified proteins

Solutions of purified untagged MtCM and His-tagged CgCM or MtDS were desalted for mass spectrometry by several cycles of diluting with 0.1% acetic acid and concentrating with Microcon YM-3 ultrafiltration devices (Millipore, Billerica, MA, USA). His-tagged MtCM variants (10 μM) were desalted through a NAP-5 column (GE Healthcare, Otelfingen, Switzerland) that was eluted with 0.1% acetic acid. MALDI MS of MtCM was performed on an Ultraflex II TOF spectrometer (Bruker, Bremen, Germany), and ESI MS of MtDS was performed on a Micromass Q-TOF Ultima spectrometer (Waters, Manchester, UK). For the calculation of M_r from the primary sequence, the Wisconsin program package from Accelrys (San Diego, CA, USA) was used.

Determination of protein concentration

Protein concentrations were determined by the Bradford assay (Bradford, 1976) with bovine serum albumin as the standard. For MtCM and MtDS, the Bradford assay was calibrated with the results from quantitative amino acid analysis, which was carried out at the Functional Genomics Center Zurich, Switzerland. For this purpose, ca. 10 μg of purified protein in 20 mM BTP, pH 7.0, (MtCM) or in BTP++ (MtDS) at a concentration of at least 0.7 μg/μl was lyophilized, and gas-phase hydrolysis was performed for 22 h at 110 °C. BTP++ is 20 mM BTP, pH 7.5, 150 mM NaCl, 0.5 mM TCEP [tris(2-carboxyethyl)phosphine hydrochloride], 0.2 mM phosphoenolpyruvate, 0.1 mM $MnCl_2$ (Webby *et al*, 2005). The hydrolyzed sample was dissolved in 60 to 300 μl of 50 mM HCl containing the internal standards norvaline and sarcosine at a concentration of 50 μM, and 1 μl was analyzed on an Amino Quant amino acid analyzer (Agilent, Santa Clara, CA, USA) using *o*-phthalaldehyde/Fmoc chemistry with fluorescence detection. The system was calibrated by a three-point calibration using amino acid standard mixtures of 9, 22.5, and 90 μM.

CM activity assays and determination of the apparent complex dissociation constant

In the presence of MtPDT, CM activity was detected using a discontinuous CM assay (Cotton and Gibson, 1965). The reactions were started by adding 0.3 mM chorismate to the enzymes to be tested in 50 mM cold potassium phosphate, pH 7.5. Samples were incubated at 37 °C for 0, 5, and 10 min. The reaction was quenched with 1 volume of 1 M HCl and incubation for 20 min at 37 °C. Four volumes of 1 M NaOH were added, and the absorption was measured at 320 nm ($\varepsilon_{320\,(phenylpyruvate)}$ = 17500 M^{-1} cm^{-1}) after removal of air bubbles by centrifugation.

In all other cases (i.e., no MtPDT present), CM activity was assessed by a continuous assay by following chorismate consumption at 274 nm (ε_{274} = 2630 M^{-1} cm^{-1}) or at 310 nm (ε_{310} = 370 M^{-1} cm^{-1}). Continuous CM assays were performed at 30 °C and pH 7.5 either in 50 mM BTP, in BTP+ (50 mM BTP, additionally containing 0.5 mM TCEP, 0.2 mM phosphoenolpyruvate, and 0.1 mM $MnCl_2$), or in 50 mM potassium phosphate. The catalytic rate constant k_{cat} was calculated per CM active site, assuming that the number of active sites corresponds to the number of polypeptides. Initial velocities were fitted to the Michaelis-Menten equation (Segel, 1976).

The apparent MtCM-MtDS dissociation constant $K_{d,app}$ for a 1:1 stoichiometry (equation 1), was derived from equation 2, which is an adaptation from previous work (Taira and Benkovic, 1988):

$$K_{d,app} = \frac{[CM][DS]}{[CM \cdot DS]} \quad (1)$$

$$v = v_{CM} + \frac{[CM \cdot DS](v_{CM \cdot DS} - v_{CM})}{2[CM]_0} \quad (2)$$

where [CM] and [DS] are the concentrations of free CM and free DAHP synthase, respectively, [CM·DS] is the concentration of the complex, v_{CM}, v_{DS} and $v_{CM \cdot DS}$ are the corresponding velocities, and $[CM]_0$ is the total CM concentration. Equation 2 can be transformed into:

$$v = v_{CM} + \left([CM]_0 + [DS]_0 + K_{d,app} - \sqrt{\left([CM]_0 + [DS]_0 + K_{d,app} \right)^2 - 4[CM]_0[DS]_0} \right) \cdot \frac{v_{CM \cdot DS} - v_{CM}}{2[CM]_0} \quad (3)$$

where $[DS]_0$ is the total DAHP synthase concentration. Equation 3 was used to fit the titration data (fitting parameters: $v_{CM \cdot DS}$ and $K_{d,app}$), substituting the velocities by the kinetically derived catalytic efficiencies k_{cat}/K_m. Iterative curve fitting was performed with the program KaleidaGraph (Synergy Software, Reading, PA, USA).

Native PAGE

For native polyacrylamide gelelectrophoresis, the PhastSystem and consumables from GE Healthcare were used. The 12.5% homogeneous gel (stacking zone: 6% polyacrylamide, 3% crosslinking; separation zone: 12.5% polyacrylamide, 2% crosslinking) contained 0.112 M acetate and 0.112 M Tris, pH 6.5. Buffer strips contained 0.25 M Tris and 0.88 M L-alanine in 3% agarose, resulting in a pH of 8.8 in the gel during separation. Proteins were separated according to the method described by the manufacturer in Separation Technique File No. 120 and stopped after 140 to 160 accumulated volt hours.

Crystallography

In addition to the crystals described in the main text, crystals of MtCM without malate have also been obtained under conditions described recently (PDB ID: 2QBV) (Kim *et al*, 2008). However, the resulting structure was not fully refined as its quality was inferior to the un-

liganded structure deposited in the PDB. Attempts to either soak MtCM crystals or co-crystallize MtCM with the transition state analog inhibitor **3** (Figure 1A of main text) have so far been unsuccessful.

For malate-bound MtCM, a data set was collected to a resolution of 1.65 Å, covering 90° of reciprocal space in 0.5° steps on a Rigaku 007HF X-ray generator with an R-axis IV image plate detector using one crystal with the approximate dimensions of 0.05 × 0.05 × 0.05 mm^3. For the MtCM-MtDS complex, data sets to 2.15 Å and 2.35 Å resolution were collected for the binary and ternary complex (with transition state analog **3**), respectively. The data were collected in two passes at beamline ID14-3 at the ESRF, from rod-shaped crystals with a cross section of 0.05 × 0.05 mm^2.

The structures were determined by Molecular Replacement with the program Phaser (McCoy *et al*, 2005) implemented in the CCP4 suite of programs (Bailey, 1994), using a protomer from the previously solved MtDS structure (PDB ID: 2B7O) (Webby *et al*, 2005) as a search model. Initial maps showed extra density into which the helices of MtCM could be built. We later solved the MtCM-malate structure by molecular replacement using the MtCM structure from the ternary complex as a model. Initial refinement was conducted with the refine component of the *PHENIX* program package (Adams *et al*, 2002) and included simulated annealing to reduce model bias. Later rounds of refinement and rebuilding were carried out alternatingly with Refmac5 (Murshudov *et al*, 1999) and Coot (Emsley and Cowtan, 2004).

The structure of the unliganded MtCM-MtDS complex showed that the MtDS manganese center had been damaged, either before cryo-protection and flash cooling of the crystals or by exposure to the high-energy X-ray beam during the diffraction experiment. Addition of 2-4 mM $MnCl_2$ to the crystallization conditions and 5 mM of the reducing agent tris(2-carboxyethyl)phosphine hydrochloride (TCEP) to the protein sample and more moderate X-ray exposure was tested to overcome this problem. However, the occupancy of MtCM was found to be reduced (to roughly 50-75%) in the resulting structures and also in an alternative attempt, where excess MtCM (2:1 final ratio) was used. It is unclear if this was due to the changed crystallization conditions or due to sample decomposition.

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