

File name: Supplementary Information

Description: Supplementary figures and supplementary tables.

Supplementary Table 1

Structural homologs of domains AN and AC of MdcA

Structural homologs	Family	Z score	Sequence identity (%)
Domain AN			
succinyl-CoA:3-ketoacid CoA transferase (SCOT, and PDB entries 3CDK, 1O9L, 3DLX, 3RRL)	I	21	20
acetyl-CoA transferase	I	21	17
acyl-CoA transferase YdiF	I	19	20
gluconate-CoA transferase	I	18	20
succinyl-CoA:acetate CoA transferase	I	16	18
citrate lyase α subunit (citrate-ACP transferase, PDB entries 1XR4 and 2HJ0)	II	18	16
Domain AC			
4-hydroxybutyrate CoA transferase (and PDB entry 2OAS)	I	18	17
succinyl-CoA:acetate CoA transferase	I	17	13
succinyl-CoA:3-ketoacid CoA transferase	I	12	14
citrate lyase α subunit (PDB entries 2HJ0 and 1XR4)	II	17	16

Supplementary Table 2
Catalytic activities of MdcE mutants

Protein	Initial velocity¹
Wild-type	2.02×10^{-3} (1)
Q100E	2.58×10^{-4} (8)
Y102F	1.89×10^{-3} (1)
S142A	1.81×10^{-4} (11)
Q100E-Y102F	7.11×10^{-6} (280)

¹ The reaction contained 125 μ M malonyl-CoA as the substrate. The values in parentheses are the fold change compared to the activity of the wild-type enzyme.

Supplementary Table 3

Primers used for making MDC bacterial expression constructs

Gene ¹	UniProt entry ID for protein	Primers
<i>MdcA</i>	A6UY03	Forward: GCGCCATGGCGACACCGATCTCTCC Reverse: GCGGAATTCTTACCAGCTCCTGAAACG
<i>MdcC</i>	Q3K5B7	Forward: GCGCCATGGAAACCTTGTCC Reverse: GCCCTCGAGTTAGTCATGGCCGATCTCC
<i>MdcD</i>	A6UY06	Forward: GCCCATATGACTGACGTCGCCCCGCCTGC Reverse: GCCCTCGAGTTATGTTGCATCTCCTTGGCCCAGC
<i>MdcE</i>	A6UY07	Forward: GCCGAATTTCGAGCCAACCGTTCGCTTCCCGTGG Reverse: GCCAAGCTTTTACCACTGCTCGCGCAGCAG

¹MdcA, MdcD and MdcE are from *P. aeruginosa*, and MdcC from *P. fluorescens*.

Supplementary Table 4

Bacterial and fungal strains used in this study

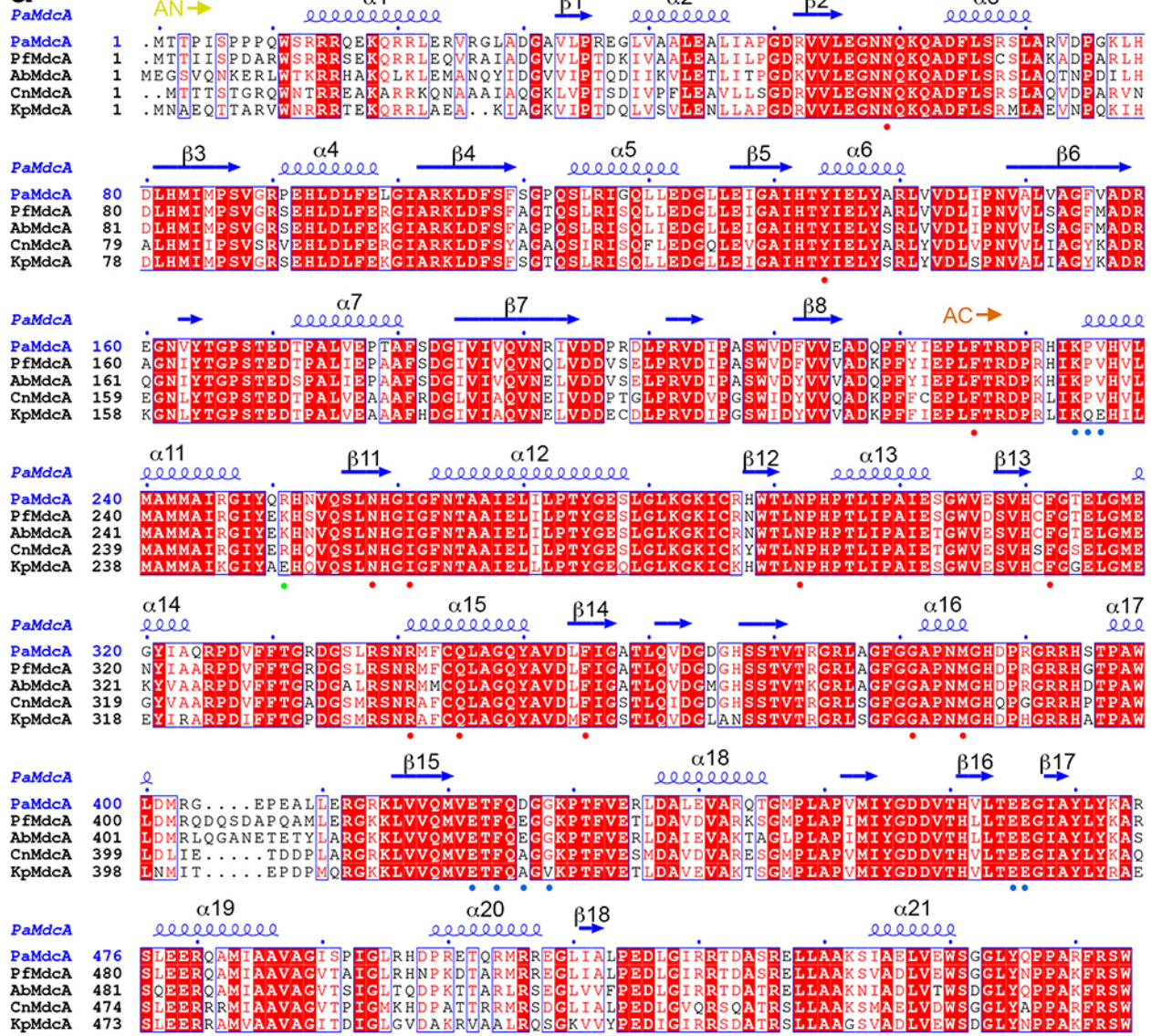
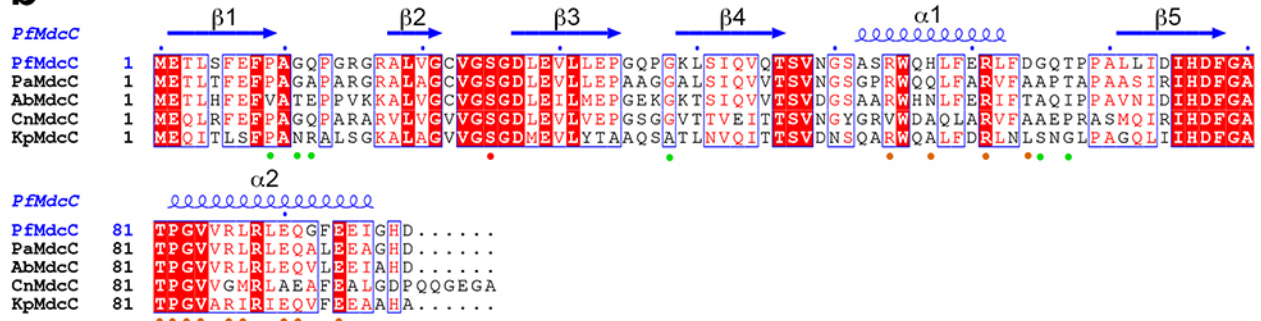
Strain	Number	Relevant characteristics	Source
<i>Pseudomonas aeruginosa</i>			
UCBPP-PA14		Clinical isolate UCBPP-PA14	Ref. 1
PA14 $\Delta mdcA$	LD2504	PA14 with deletion in PA14_02550	this study
PA14 $\Delta mdcC$	LD2538	PA14 with deletion in PA14_02570	this study
PA14 $\Delta mdcE$	LD2510	PA14 with deletion in PA14_02590	this study
PA14 $\Delta mdcC::mdcC$	LD2674	PA14 $\Delta mdcC$ with <i>mdcC</i> (PA14_02570) reinserted at site of deletion	this study
PA14 <i>mdcA</i> R341E	LD2741	PA14 containing R314E mutation in the active site of MdcA	this study
PA14 <i>mdcA</i> Δ 519-554	LD2752	PA14 containing a deletion in the coding sequence for MdcA from residues 519-554	this study
<i>Escherichia coli</i>			
UQ950		<i>E. coli</i> DH5 λ (pir) strain for cloning. F Δ (argF-lac)169 ϕ 80dlacZ58(Δ M15) <i>glnV44</i> (AS) <i>rjbD1</i> <i>gyrA96</i> (NaI ^R) <i>recA1</i> <i>endA1</i> <i>spoT1</i> <i>thi-1</i> <i>hsdR17</i> <i>deoR</i> λ pir ⁺	D. Lies, Caltech
BW29427		Donor strain for conjugation. <i>thrB1004</i> <i>pro</i> <i>thi</i> <i>rpsL</i> <i>hsdS</i> <i>lacZ</i> Δ M15 <i>RP4-1360</i> Δ (araBAD)567 Δ dapA1314:: <i>[erm pir(wt)]</i>	W. Metcalf, University of Illinois
<i>Saccharomyces cerevisiae</i>			
InvSc1		<i>MATa/MATa</i> <i>leu2/leu2</i> <i>trp1-289/trp1-289</i> <i>ura3-52/ura3-52</i> <i>his3-ΔI/his3-ΔI</i>	Invitrogen

¹ Rahme, L. G. *et al.* Common virulence factors for bacterial pathogenicity in plants and animals. *Science* **268**, 1899-1902 (1995).

Supplementary Table 5

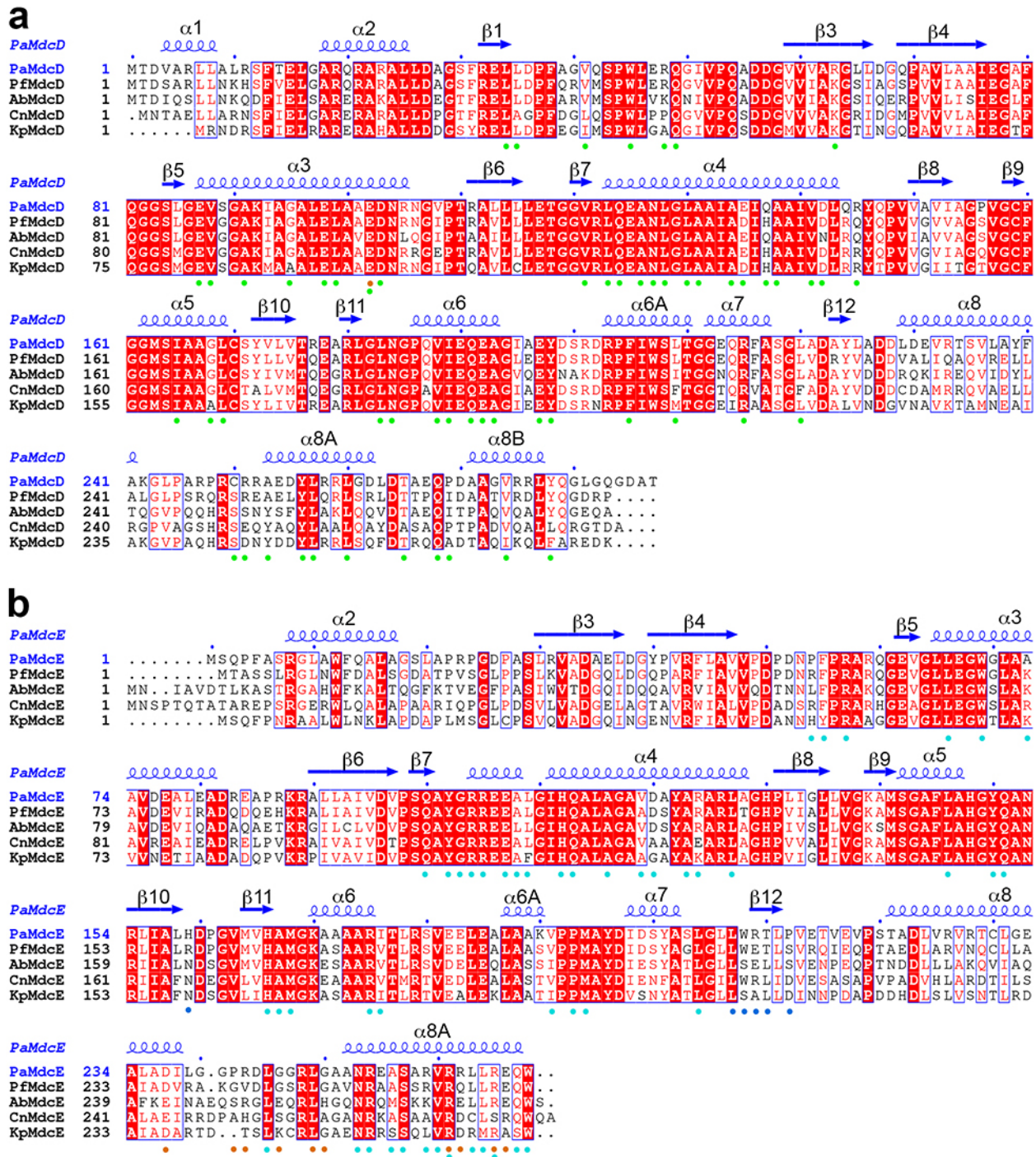
Primers used in this study

<i>ΔmdcA</i>	
ΔmdcA yeast-1	ccaggcaaattctgtttatcagaccgcttctgcgttctgaCTTGCGCTGGATCAACTCG
ΔmdcA yeast-2	agctcggcgatgctcttcgcCTTCTGGTTGTTGCCTTC
ΔmdcA yeast-3	gaaggcaacaaccagaagGCGAAGAGCATCGCCGAGCT
ΔmdcA yeast-4	ggaattgtgagcggataacaatttcacacaggaaacagctCATCTCAGAGACTCCCAGCC
<i>ΔmdcC</i>	
ΔmdcC yeast-1	ccaggcaaattctgtttatcagaccgcttctgcgttctgaGAAGGCGTGAACACCCATC
ΔmdcC yeast-2	gggagtgggcgccgaaatcGGCGGGAAATTCGAAGGTCAG
ΔmdcC yeast-3	ctgacctcgaattccccgccGATTTCGGCGCCACTCCC
ΔmdcC yeast-4	ggaattgtgagcggataacaatttcacacaggaaacagctAGTCCGAGGTTGGCTTCC
<i>ΔmdcE</i>	
ΔmdcE yeast-1	ccaggcaaattctgtttatcagaccgcttctgcgttctgatGCTGGCCATGACTGACGT
ΔmdcE yeast-2	acgcgtagctgtcgatgtcGCGCAACGAAGCAGGATC
ΔmdcE yeast-3	gatcctgcttcgttgcgcGACATCGACAGCTACGCGT
ΔmdcE yeast-4	ggaattgtgagcggataacaatttcacacaggaaacagctCGATCAGCAGGCACAAC
<i>ΔmdcC::mdcC</i>	
ΔmdcC yeast-1	ccaggcaaattctgtttatcagaccgcttctgcgttctgaGAAGGCGTGAACACCCATC
ΔmdcC yeast-4	ggaattgtgagcggataacaatttcacacaggaaacagctAGTCCGAGGTTGGCTTCC
<i>mdcA R341E</i>	
mdcA yeast-1	ccaggcaaattctgtttatcagaccgcttctgcgttctgatCCCGGCTTGATTGAACAG
mdcA yeast-2	GGCCAGTTGGCAGAACATctcATTGGAACGCAGCGAGCC
mdcA yeast-3	GGCTCGCTGCGTTCCAATgagATGTTCTGCCAACTGGCC
mdcA yeast-4	ggaattgtgagcggataacaatttcacacaggaaacagctATGGGTGTTACGCCTTC
<i>mdcA Δ519-554</i>	
mdcA del yeast-1	ccaggcaaattctgtttatcagaccgcttctgcgttctgatGTGCTGATGGCGATGATG
mdcA del yeast-2	tcgcgatggcggtcatcaGCCGAGGTCCTCGGGCAG
mdcA del yeast-3	ctgcccaggacctcggcTGATGAACGCCATCGCGA
mdcA del yeast-4	ggaattgtgagcggataacaatttcacacaggaaacagctCATCTCAGAGACTCCCAGCC

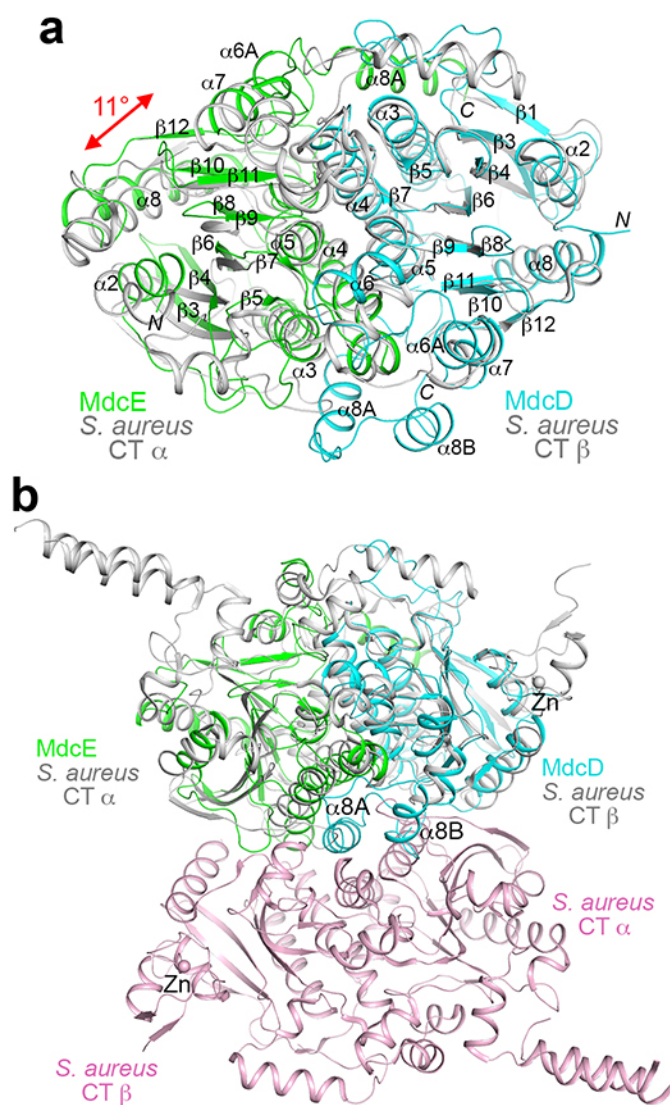
a**b**

Supplementary Fig. 1. Sequence alignment of MdcA and MdcC subunits. **(a).** Sequence alignment of MdcA. The observed secondary structure elements are shown in blue above the sequence and labeled. Residues in the active site are indicated with the red dots. MdcA residues in contact with MdcC are indicated with the blue dots, and those in contact with MdcE with the green dots. The domain boundaries are indicated with the arrowheads and labeled. Pa: *Pseudomonas aeruginosa*, Pf: *Pseudomonas fluorescens*, Ab: *Acinetobacter baumannii*, Cn: *Cupriavidus necator*, Kp: *Klebsiella pneumoniae*. **(b).** Sequence alignment of MdcC. MdcC residues in contact with MdcA are indicated with the orange dots. Modified from an output from ESPript ²

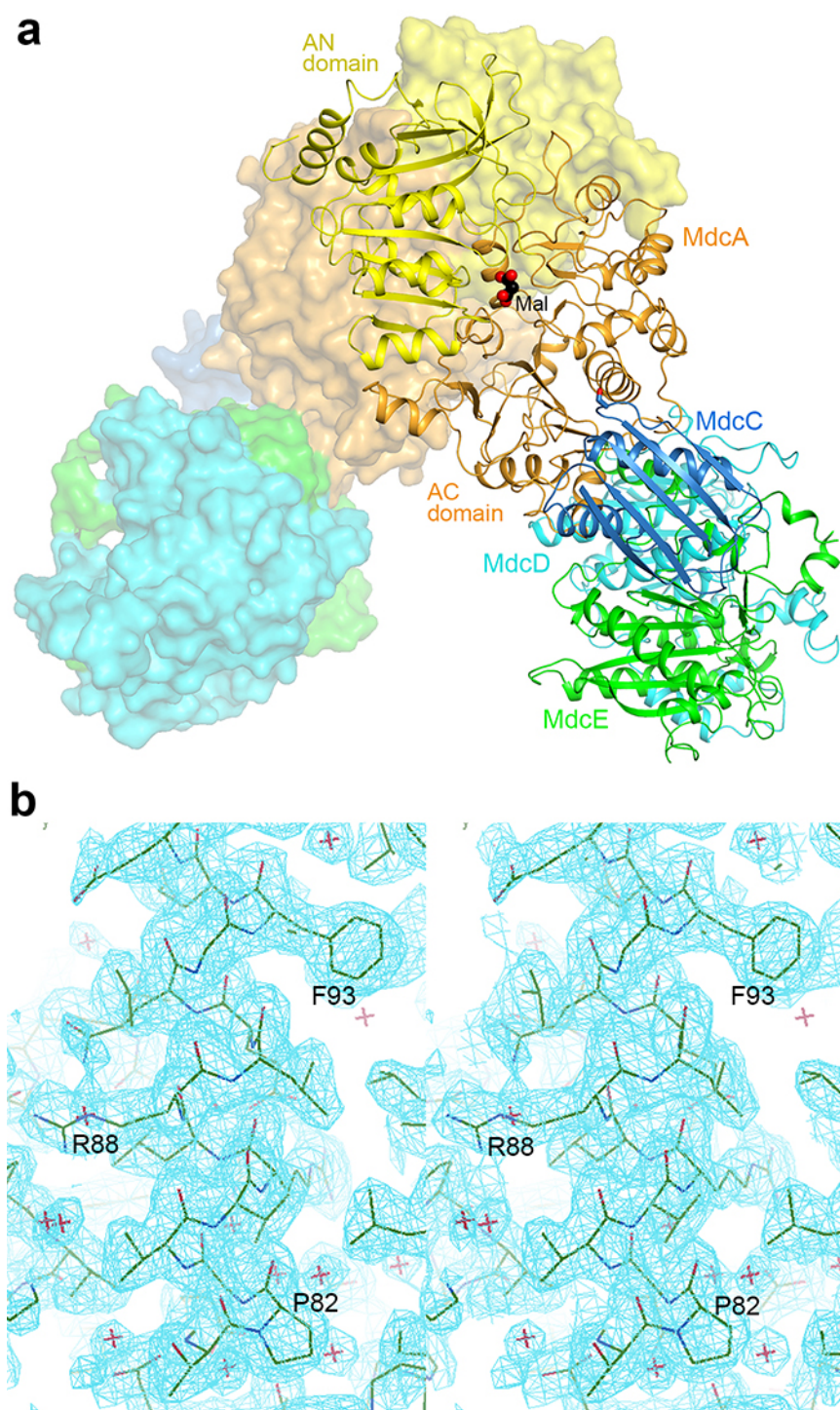
² Gouet, P., Courcelle, E., Stuart, D. I. & Metoz, F. ESPript: analysis of multiple sequence alignments in PostScript. *Bioinformatics* **15**, 305-308 (1999).



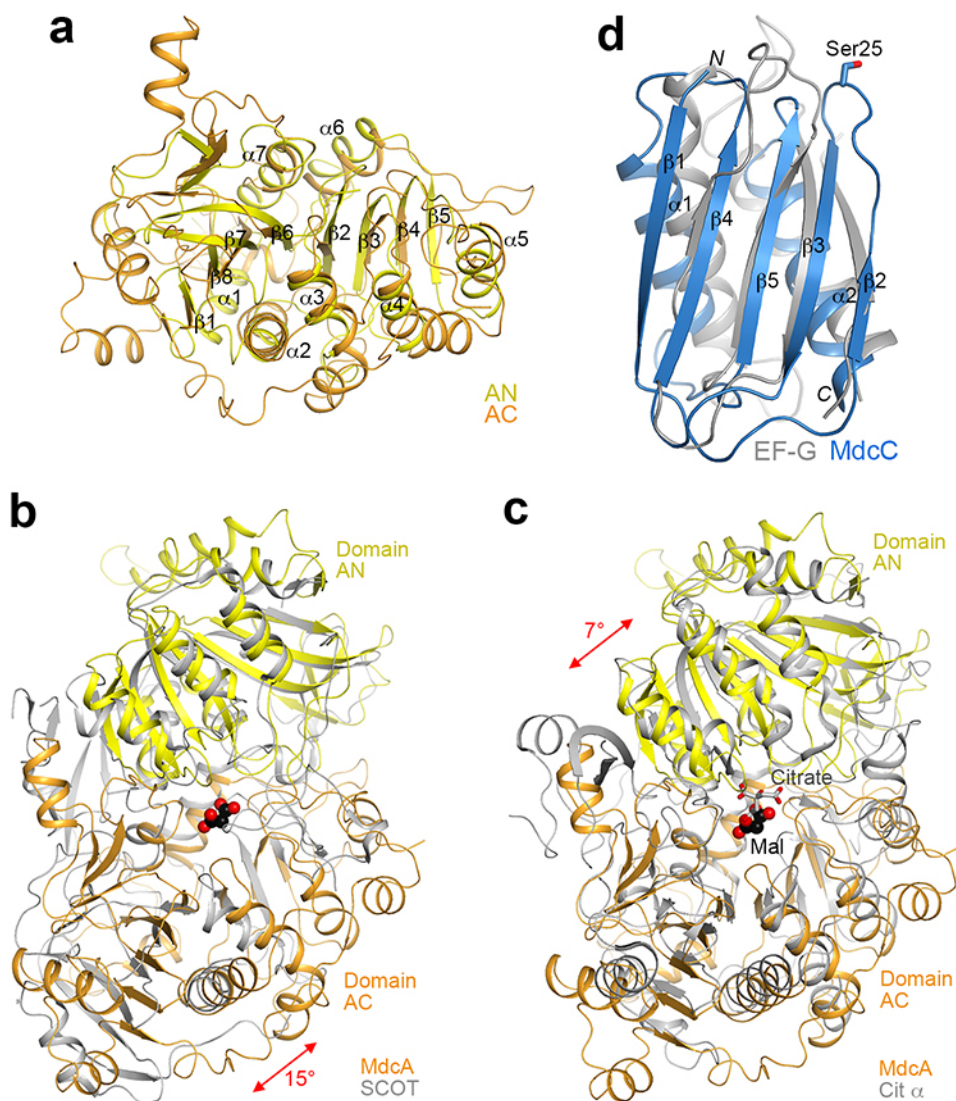
Supplementary Fig. 2. Sequence alignment of MdcD and MdcE subunits. (a). Sequence alignment of MdcD. (b). Sequence alignment of MdcE.



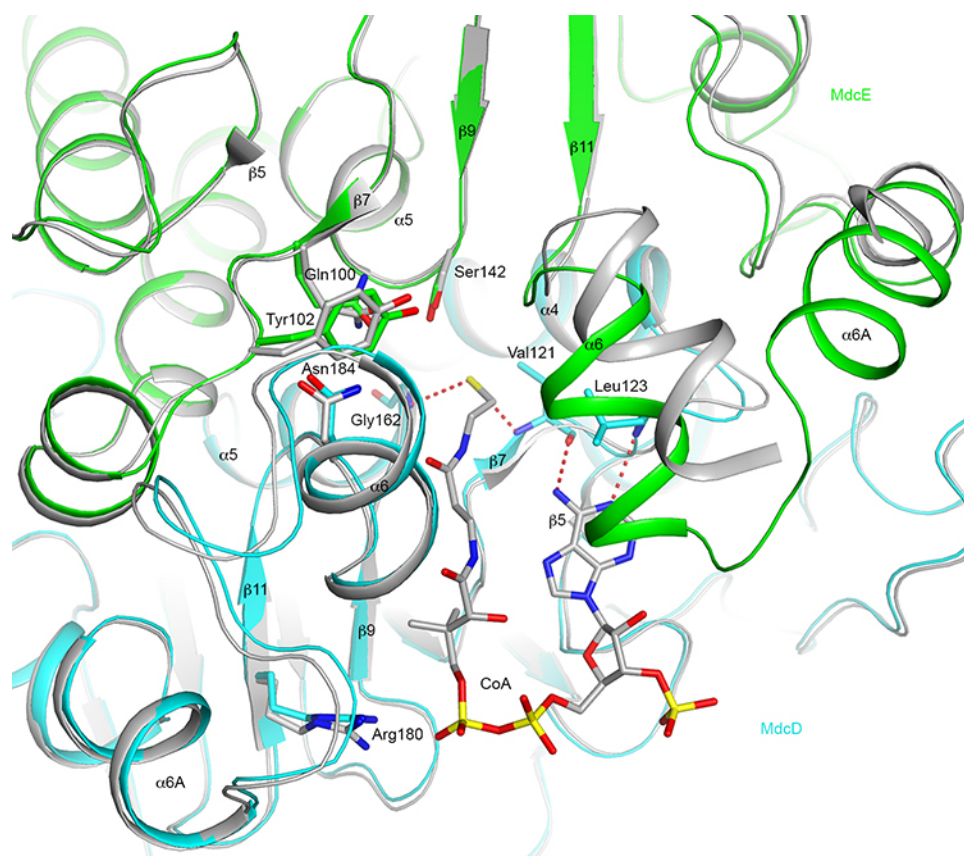
Supplementary Fig. 3. Structural comparison between MdcD-MdcE with ACC CT. **(a)** Overlay of the structure of the MdcD-MdcE complex (in color) with that of the CT $\alpha\beta$ hetero-dimer of *S. aureus* ACC (gray). The overlay is based on MdcD, and an 11° difference in the orientation of MdcE relative to the CT α subunit is observed. **(b)** Overlay of the structure of the MdcD-MdcE complex (cyan and green) with that of a CT $\alpha\beta$ hetero-dimer in the $\alpha_2\beta_2$ hetero-tetramer of *S. aureus* ACC (gray and pink). The $\alpha 8A$ and $\alpha 8B$ helices at the C-terminal end of MdcD are incompatible with the interface between the two CT $\alpha\beta$ hetero-dimers.



Supplementary Fig. 4. (a) A dimer of the MDC in the crystal. One MDC hetero-tetramer is shown as ribbons, while the other as a surface. The dimer interface is mediated solely by MdcA. MDC is a hetero-tetramer in solution. (b) Stereo view of $2F_o - F_c$ electron density at 2.2 Å resolution for a helix in MdcA, contoured at 1σ .



Supplementary Fig. 5. Structural homologs of MdcA and MdcC. **(a)** Overlay of the structures of the MdcA AN (yellow) and AC (orange) domains. **(b)** Overlay of the structure of MdcA (in color) with that of succinyl-CoA:3-ketoacid CoA transferase (SCOT, Z score 21, 20% sequence identity, gray). The overlay is based on the AN domain, and a 15° difference in the orientation of the AC domain relative to the equivalent in SCOT is observed. Malonate is shown as a ball-and-stick model (black). **(c)** Overlay of the structure of MdcA (in color) with that of citrate lyase α subunit (Cit α , Z score 18, 16% identity). The overlay is based on the AC domain, and a 7° difference in the orientation of the AN domain relative to the equivalent in citrate lyase is observed. Citrate is shown as a stick model (gray). **(d)** Overlay of the structure of MdcC (in color) with that of domain IV of elongation factor G (EF-G, gray).



Supplementary Fig. 6. Conformational changes in MdcD-MdcE during catalysis. The active site region of MdcD-MdcE in the CoA complex (in color, with the CoA in gray) is overlaid with that in the malonate complex (gray). Differences are seen for the $\alpha 6$ - $\alpha 6A$ segment of MdcE, the loop containing residues Val121 and Leu123, and the $\alpha 6$ - $\alpha 6A$ segment of MdcD.