Supplementary Material

CRYSTAL STRUCTURES OF *STAPHYLOCOCCUS EPIDERMIDIS* MEVALONATE DIPHOSPHATE DECARBOXYLASE BOUND TO INHIBITORY ANALOGS REVEAL NEW INSIGHT INTO SUBSTRATE BINDING AND CATALYSIS*

Michael L. Barta[‡,1], D. Andrew Skaff[§,1], William J. McWhorter[‡], Timothy J. Herdendorf[§], Henry M. Miziorko[§,2], and Brian V. Geisbrecht[‡,2]

From Division of Cell Biology and Biophysics[‡], Molecular Biology and Biochemistry[§], School of Biological Sciences, University of Missouri-Kansas City; Kansas City, Missouri 64110

Running Head: Inhibitor–mevalonate diphosphate decarboxylase structures

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. **Complete structure-based multiple sequence alignment of MDD proteins.** Alignment was generated using ClustalW. Numbers above the sequences correspond to *S. epidermidis* MDD. Red stars below the sequences correspond to invariant amino acid side chains involved in DPGP and FMVAPP interaction, while the green star represents the single variable active site residue.

Fig. S2. **Analytical size-exclusion chromatography of recombinant *S. epidermidis* MDD.** *A*, Purified MDD was injected onto a Tricorn 10/300 Superdex 200 (GE Bioscience) analytical size-exclusion chromatography column and its retention time was compared to a series of globular protein standards (BioRad). *B*, Size-exclusion calibration curve for four globular protein standards. The apparent molecular weight (*M.W.*) for MDD was estimated from observed elution volume (*E.V.*) using the equation: $M.W. = 29{,}649e^{-0.431 \times E.V.}$. For an elution volume of 14.05 ml, this yields an apparent molecular weight of 64 kDa.

SUPPLEMENTARY TABLES

Tables S1 and S2. **Intermolecular contacts between *S. epidermidis* MDD and the inhibitors DPGP and FMVAPP.** Distances for polar contacts between selected atoms of the MDD protein and inhibitors DPGP (Table S1) and FMVAPP (Table S2). In Table S2, distances are shown for both the wild-type and $Ser^{192} \rightarrow Ala$ forms of MDD bound to FMVAPP.

FIG. S1

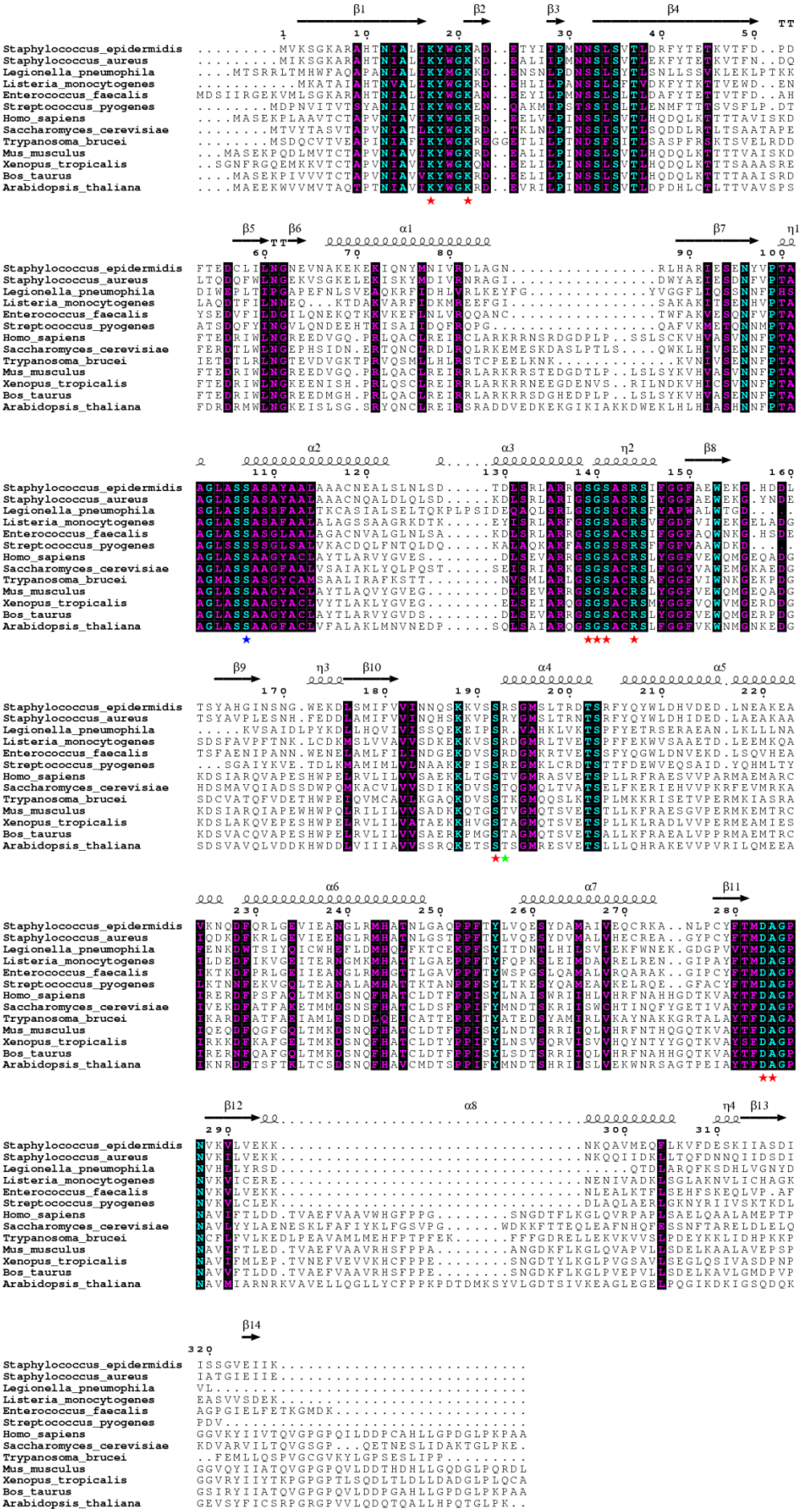
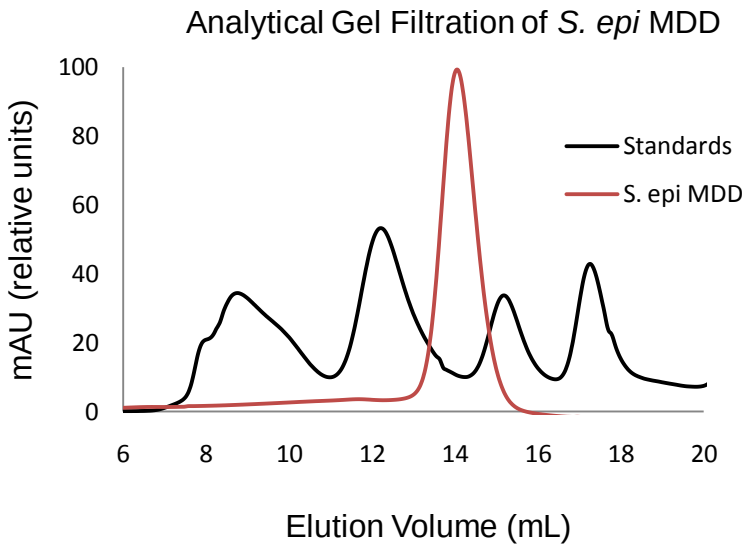
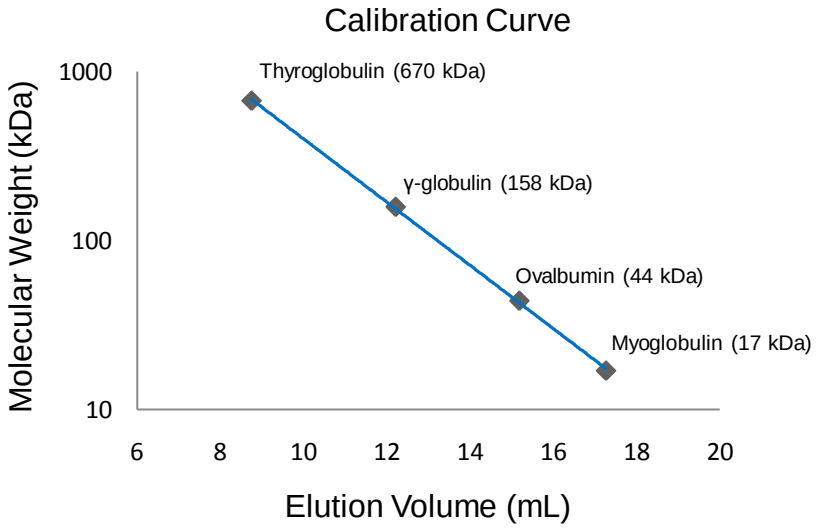

Staphylococcus_epidermidis
Staphylococcus_aureus
Legionella_pneumophila
Listeria_monocytogenes
Enterococcus_faecalis
Streptococcus_pyogenes
Homo_sapiens
Saccharomyces_cerevisiae
Trypanosoma_brucei
Mus_musculus
Xenopus_tropicalis
Bos_taurus
Arabidopsis_thaliana

FIG. S2

A


Analytical Gel Filtration of S. epi MDD
mAU (relative units)
100
80
60
40
20
0
6
8
10
12
14
16
18
20
Elution Volume (mL)
Standards
S. epi MDD

B


Calibration Curve
Molecular Weight (kDa)
1000
100
10
Thyroglobulin (670 kDa)
γ-globulin (158 kDa)
Ovalbumin (44 kDa)
Myoglobulin (17 kDa)
6
8
10
12
14
16
18
20
Elution Volume (mL)

Supplemental Tables 1 & 2

Table S1. MDD + DPGP Contacts

Enzyme		Distance (Å)	Inhibitor	
Amino Acid	Atom	DPGP	Atom	Group
Tyr18	OH	2.7	OAF	β-phosphoryl
Lys21	NZ	2.6	OAC	β-phosphoryl
Ser107	OG	2.9	OAD	α-phosphoryl
Ser139	OG	3.1	OAH	α-phosphoryl
Ser139	OG	3.1	OAG	β-phosphoryl
Gly140	N	2.9	OAF	β-phosphoryl
Ser141	OG	2.6	OAH	α-phosphoryl
Ser141	N	3.1	OAD	α-phosphoryl
Arg144	NH2	2.8	O	Carboxylate
Arg144	NH1	3.1	O	Carboxylate
Ser192	OG	3.0	OAM	α-phosphoryl
Ser192	OG	3.2	OAB	C2-hydroxyl
Arg193	NH2	2.9	OAG	β-phosphoryl
Arg193	NE	2.8	OAC	β-phosphoryl

Table S2. MDD + FMVAPP Contacts

Enzyme		Distance (Å)		Inhibitor	
Amino Acid	Atom	FMVAPP	$Ser^{192}\rightarrow Ala$	Atom	Group
Tyr18	OH	2.8	2.7	OAF	β-phosphoryl
Tyr18	N	2.9	2.8	OAD	Carboxylate
Lys21	NZ	2.8	2.7	OAB	β-phosphoryl
Ser139	OG	3.2	3.0	OAC	α-phosphoryl
Ser139	OG	2.6	2.7	OAG	β-phosphoryl
Gly140	N	2.8	2.8	OAF	β-phosphoryl
Ser141	OG	2.8	2.7	OAC	α-phosphoryl
Ser141	N	3.0	3.0	OAC	α-phosphoryl
Arg144	NH2	2.9	3.0	OAA	Carboxylate
Arg144	NH1	3.1	3.1	OAD	Carboxylate
Ser192	OG	2.8	N/A	OAH	α-phosphoryl
Arg193	NH2	3.1	3.0	OAG	β-phosphoryl
Arg193	NE	2.7	2.7	OAB	β-phosphoryl
Asp283	OD	3.4	3.6	OAE	C3-hydroxyl
Ala284	N	3.1	3.1	FAI	Fluoromethyl