Supplementary Material

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

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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 Ser¹⁹² \rightarrow Ala forms of MDD bound to FMVAPP.

FIG. S1

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

Staphylococcus_epidermidis Staphylococcus_aureus Legionella_pneumophila Listeria_monocytogenes Enterococcus_faecalis Streptococcus_pyogenes sapiens Homo Saccharomyces_cerevisiae Trypanosoma brucei Mus musculus Mus_musculus Xenopus_tropicalis Bos_taurus Arabidopsis_thaliana

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

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DAG

DAG

RFNAHHGDTK TINQFYGETI AYNAKKGRTA

320 ISSGVEIIK. IATGIEIIE VL. EASVVSDEK. AGPGIELFETKGMDK. AGPGIELFETKGMDK. GGVKYIIVTQVGPGPQILDDPCAHLLGPDGLPKPAA KDVARVILTQVGSGP..QETNESLIDAKTGLPKE. .FEMLLQSPVGCGVKYLGPSESLIPP. GGVQYIIATQVGPGPQLDDTHDHLGQDGLPQRDL GGVRYIIVTKPGPGFTLSQDLTLDLDADGLPLQCA GSIRYIINTQVGPGPQLDDPGAHLLGPDGLPKPAA GEVSYFICSRPGRGPVVLQDQTQALLHPQTGLPK.

β14

FIG. S2



Supplemental Tables 1 & 2

Table S1. MDD + DPGP Contacts

Table S2. MDD + FMVAPP Contacts

Enzyme)	Distance (Å)		Inhibitor
Amino Acid	Atom	DPGP	Atom	Group
Tyr18	ОН	2.7	OAF	β-phosphoryl
Lys21	NZ	2.6	OAC	β-phosphoryl
Ser107	OG	2.9	OAD	a-phosphoryl
Ser139	OG	3.1	ОАН	a-phosphoryl
Ser139	OG	3.1	OAG	β-phosphoryl
Gly140	N	2.9	OAF	β-phosphoryl
Ser141	OG	2.6	OAH	a-phosphoryl
Ser141	N	3.1	OAD	a-phosphoryl
Arg144	NH2	2.8	0	Carboxylate
Arg144	NH1	3.1	0	Carboxylate
Ser192	OG	3.0	OAM	a-phosphoryl
Ser192	OG	3.2	OAB	C2-hydroxyl
Arg193	NH2	2.9	OAG	β-phosphoryl
Arg193	NE	2.8	OAC	β-phosphoryl

Enzyme	9	Dista	nce (Å)	Inhibitor						
Amino Acid	Atom	FMVAPP	Ser ¹⁹² →Ala	Atom	Group					
Tyr18	ОН	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	a-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	a-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					