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Supporting information for article:

Crystal structure of a putative 3-hydroxypimelyl-CoA dehydrogenase, Hcd1, from *Syntrophus aciditrophicus* strain SB at 1.78 Å resolution

David M. Dinh, Leonard M. Thomas and Elizabeth A. Karr

Table S1 SDB family protein structures used for comparison in this study.

Z score, RMDS, and %AA Identity are from the DALI server and based on comparison to 7SUB, *Sa*Hcd1

PDB ID/Chain(s)	Z score	RMSD (Å)	AA % Identity
2UVD: Chains A-H	29.8-29.9	2.2-2.3	44-45
4RZH: Chains A-B	28.5-28.7	2.2	45
4JRO: Chains A-D	27.7-28.9	2.2-2.4	40-41
3SJ7: Chains A-B	28.2-28.7	2.3-2.4	39-40
1EDO: Chain A	28.2	2.2	39
2HQ1: Chain A	27.9	2.2	38
4NBT: Chains A-D	24.7-24.9	2.4-2.7	37-38

AA=amino acid

Table S2 Interfacing residues making hydrogen bonds or salt bridges at the *Sa*Hcd1 dimer interface predicted by PISA.

		Distance (Å)
Hydrogen Bonds	TYR 236 [OH] - GLU 221 [OE1]' TYR 236 [OH]' - GLU 221 [OE1]	2.94
	GLN 240 [NE2] - LYS 243 [O]' GLN 240 [NE2]' - LYS 243 [O]'	2.98
	LYS 243 [N] - GLN 240 [OE1]' LYS 243 [N]' - GLN 240 [OE1]	2.79
	ASP 245 [N] - TYR 236 [O]' ASP 245 [N]' - TYR 236 [O]	3.25
	GLY 246 [N] - TYR 236 [O]' GLY 246 [N]' - TYR 236 [O]	2.98
Salt Bridges	LYS 235' [NZ]- GLU 221 [OE2]	2.77

The interfacing atoms of each residue are indicated in the brackets and follow the atom naming convention set by the Protein Data Bank.

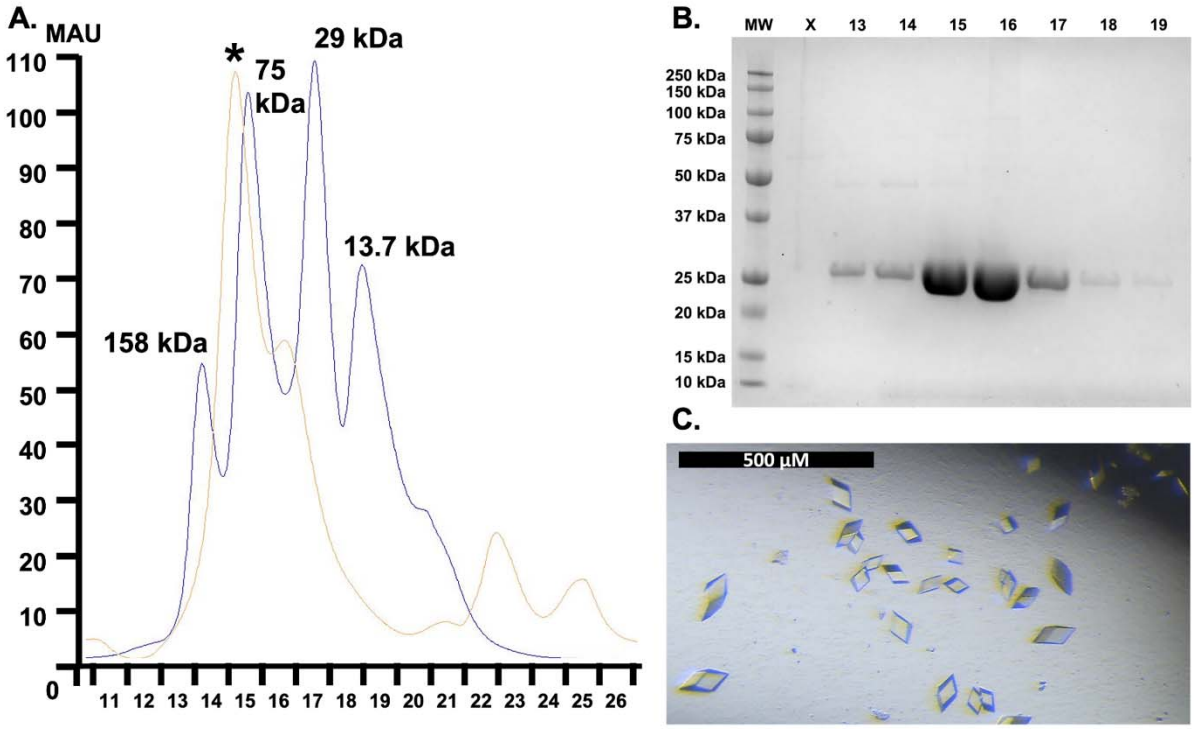

Figure S1 (A) An SEC chromatogram of *Sa*Hcd1 with overlayed standards. The orange line indicates UV absorption (mAU) over time for the *Sa*Hcd1 sample, and the blue line indicates the standards calibrated to the SEC column. The SEC standards are aldolase (158 kDa), conalbumin (75 kDa), carbonic anhydrase (29 kDa), and ribonuclease A (13.7 kDa). The asterisk indicates the elution of *Sa*Hcd1 corresponding to at least a dimer. The x-axis indicates the fraction numbers. The axes have been overlayed with new markings for ease of viewing. Additionally, the X-axis fractions 0-11 were cropped for space. (B). Representative SDS-PAGE of *Sa*Hcd1 after SEC. Molecular weight standards are marked. The predominant band corresponds to the predicted molecular weight of ~28.7 kDa. (C) Crystals of *Sa*Hcd1. Crystals were formed in 0.1 M sodium malonate (pH 4.0) and 12% (w/v) PEG 3350.

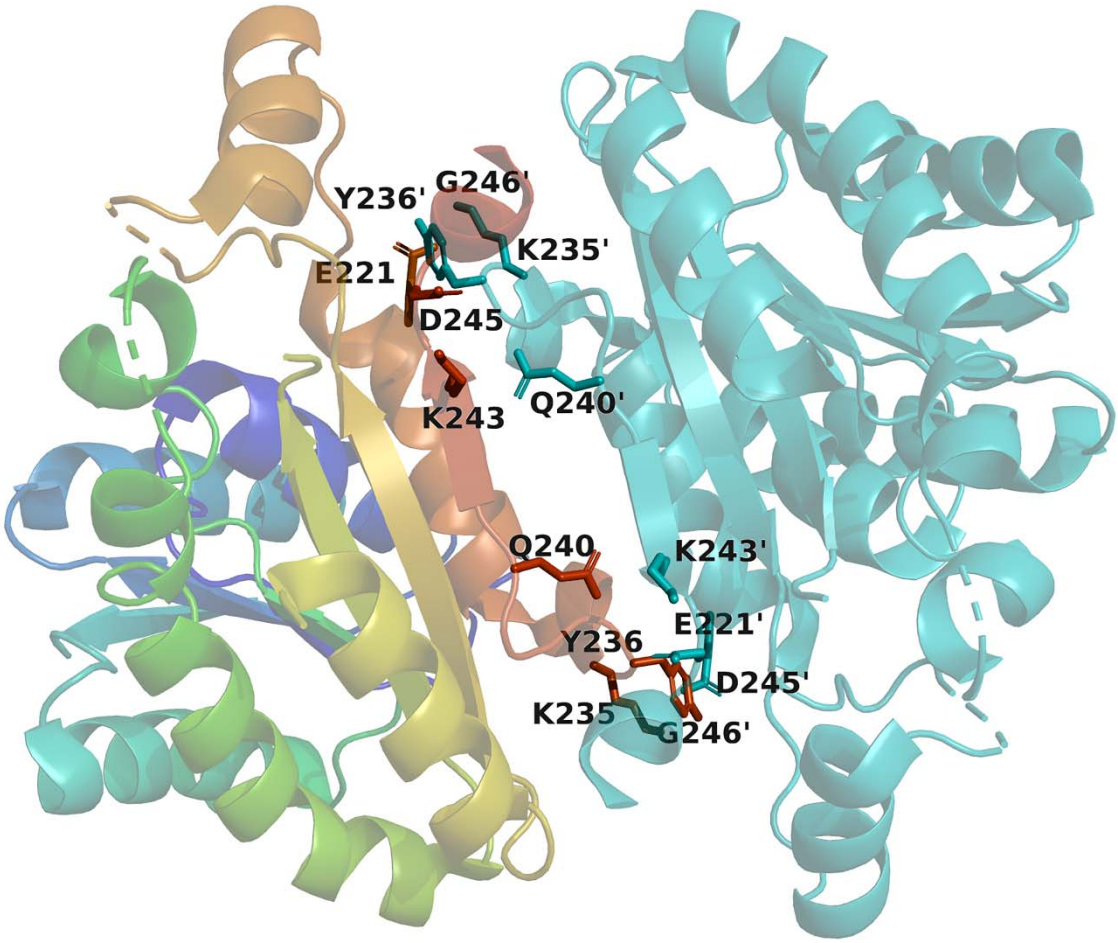

Figure S2 *Sa*Hcd1 dimer formed by symmetry expansion. The symmetry mate is shown in cyan with the original structure shown in the rainbow color scheme (N-terminus blue with red C-terminus). The side chains of residues predicted to interact at the dimer interface through hydrogen bonds or a salt bridge (K235 and E221) are labeled as sticks.

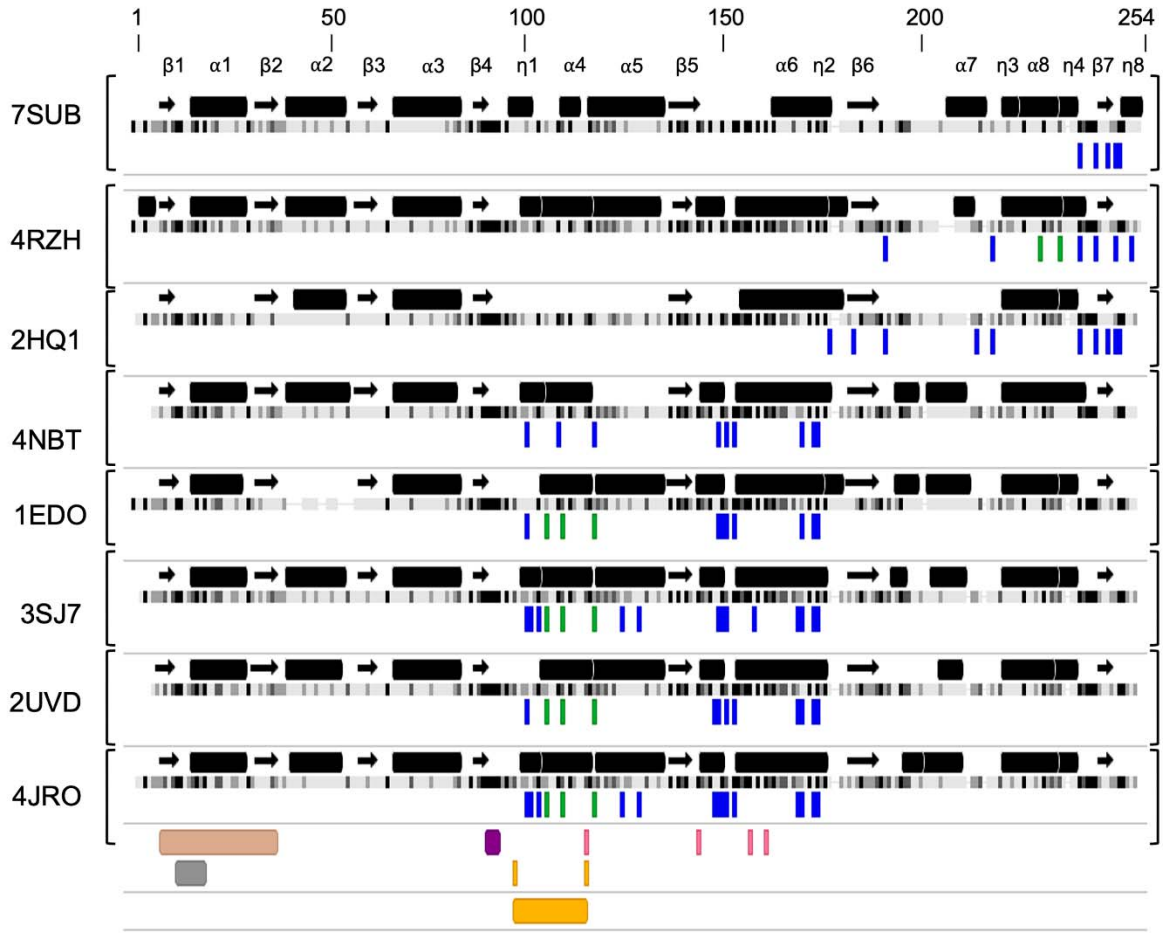

Figure S3 A pictoral representation of the full SDR-family comparison alignment. Proteins are indicated by their PDB ID: *Sa*Hcd1 (7SUB), FabG from *Synechocystis* sp. PCC 6803 (4RZH) (Liu *et al.*, 2015), ORF1438 a putative glucose/ribitol dehydrogenase from *Clostridium thermocellum* (2HQ1)(unpublished), FabG from *Acholeplasma laidlawii* (4NBT) (Javidpour *et al.*, 2014), beta-keto acyl carrier protein reductase from *Brassica napus* (with NADP+) (1EDO) (Fisher *et al.*, 2000), FabG from *Staphylococcus aureus* complex with NADPH (3SJ7) (Dutta *et al.*, 2012), OAR from *Bacillus anthracis* (BA3989) (2UVD) (Zaccai *et al.*, 2008), and FabG from *Listeria monocytogenes* (with NADP+) (4JRO) (unpublished). Secondary structures for all structures are visually depicted as arrows (β-strands) and helices (α-helices). The provided secondary structure labeling is for *Sa*Hcd1. Sequence alignment was performed using Blosum62 global alignment with free end gaps using Geneious Prime 2023.0.4 (https://www.geneious.com) (Henikoff & Henikoff, 1992). Shading is based on the Blosum62 matrix with a threshold of 1, so residues that are 100% similar are shaded black, those that are 80-100% similar are shaded charcoal, and those that are 60 to 80% similar are shaded gray. Residues involved in the dimerization interface are highlighted on each sequence within the alignment. Residues involved in hydrogen bonds at the interface are denoted by blue boxes, and those engaged in salt brides are represented by green boxes. Below the alignment, several additional features are highlighted that were also highlighted in Figure 2. The β - α - β motif is represented by a

brown rectangle. The glycine-rich motif is represented by a gray rectangle. The residues of the catalytic tetrad/triad are denoted by pink squares. Notable residues not conserved in *Sa*Hcd1 are denoted by gold squares, and a region of interest is noted by a gold rectangle. A highly conserved NNAG sequence is denoted by a purple rectangle.