Supporting Information

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SI Materials and Methods

Media. The defined medium was M9 supplemented at final concentrations with 0.8% (vol/vol) glycerol, 0.1% (wt/vol), and vitamin-assay casamino acids (Difco) (1). Minimal medium contained 0.4% (wt/vol) glucose in place of the glycerol and amino acids. Medium 2XYT contained 16 g of tryptone, 10 g of yeast extract, and 5 g of NaCl per liter; agar was added to 1.5% (wt/vol). Antibiotics were used at the following concentrations: kanamycin sulfate, 50 $\mu g \cdot mL^{-1}$; chloramphenicol, 30 $\mu g \cdot mL^{-1}$; spectinomycin sulfate, 100 $\mu g \cdot mL^{-1}$.

Cloning, Protein Expression, and Purification. Preparation of plasmids encoding C-terminally hexahistidine-tagged derivatives of wild-type and the S82A BioH proteins encoded in vector pET28b+ (Novagen) have been described previously (1). Single, double, triple, and quadruple BioH mutants designed to disrupt acyl carrier protein (ACP) binding were generated from the C-terminally hexahistidine-tagged wild-type protein by standard site-directed mutagenesis protocols (1). The primers used were BioH R138A (5′-AGT GAT GAT TTT CAG GCT ACA GTG GAG CGG TTC-3′ and 5′-CTG AAA ATC ATC ACT GAG TTG CTG CTG AAA TCC-3′); BioH R142A (5′-CAG CGT ACA GTG GAG GCG TTC CTG GCG TTA CAA-3′ and 5′-CTC CAC TGT ACG CTG AAA ATC ATC ACT GAG TTG-3′); BioH R155A (5′-GGG ACT GAA ACG GCG GCC CAG GAT GCG CGG GCG-3′ and 5′-CGC CGT TTC AGT CCC CAT GGT TTG TAA CGC CAG-3′); BioH R159A (5′-GCG CGC CAG GAT GCG GCG GCG TTG AAG AAA ACC-3′ and 5′-CGC ATC CTG GCG CGC CGT TTC AGT CCC CAT GGT-3′); and BioH R159A/R155A (5′-GGG ACT GAA ACG GCG GCC CAG GAT GCG GCG GCG-3′ and 5′-AGT GAT GAT TTT CAG GCT ACA GTG GAG GCG TTC-3′). All constructs were verified by DNA sequencing by ATGC Inc.

Wild-type and mutant BioH enzymes used in this study for crystallization and biochemical assays all carried a C-terminal fusion of hexahistidine tag (unless stated otherwise) and were purified identically. Clones were transformed into *E. coli* BL21(DE3) and cultures were grown in LB growth medium supplemented with 50 μg/mL kanamycin sulfate. Two liters of culture was grown at 37 °C until an OD at 600 nm of 0.6, and protein production was induced by addition of 0.3 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). The culture was then shifted to 18 °C and further grown for 16 h. The cells were collected by centrifugation and resuspended in 20 mM Tris·HCl (pH 8.0), 500 mM NaCl, and 10% (wt/vol) glycerol buffer, and lysed by multiple passages through a C5 Avestin cell homogenizer. The lysate was centrifuged and the supernatant was applied to a 5-mL HisTrap (GE Biosciences) column equilibrated in the cell harvest buffer. The column was extensively washed with 20 mM Tris·HCl (pH 8.0), 1 M NaCl, and 30 mM imidazole buffer, and eluted by a linear gradient to 250 mM imidazole. Protein purity was determined by SDS/PAGE, and greater than 95% pure fractions were applied to a Superdex-75 size-exclusion chromatography column equilibrated with 20 mM Hepes (pH 7.5) and 100 mM KCl buffer. The peak fractions corresponding to the BioH monomer were pooled and concentrated. This procedure routinely yielded greater than 30 mg of highly pure protein per liter of culture. The protein concentrations were determined by Bradford assay (Bio-Rad).

Crystallization. The complex of pimeloyl-ACP methyl ester (Me-pimeloyl-ACP) with BioH S82A was crystallized by sparse matrix screening, followed by optimization of screening hits. For crystallization, the BioH S82A mutant enzyme was concentrated to 0.6 mM and mixed in a 1:1.2 molar ratio with the Me-pimeloyl-ACP substrate. The complex was incubated at room temperature for 2 h. Crystallization screening was performed with various commercial crystallization screens by mixing 1 μL of protein complex with 1 μL of mother liquor in 48-well hanging-drop trays. Trays were incubated at 9 °C and periodically monitored for crystal growth. Initial crystallization conditions were identified and refined to yield reproducible crystallization conditions: 28–32% (wt/vol) PEG1500, 5–10% (wt/vol) glycerol at 20 °C. The crystals obtained were briefly soaked in mother liquor supplemented with 20% (wt/vol) glycerol and vitrified in liquid nitrogen for X-ray diffraction data collection at Life Sciences Collaborative Access Team, Sector 21, Argonne National Laboratory.

Phasing and Structure Determination. A fourfold redundant data set was collected from a single crystal of BioH S82A mutant enzyme in complex with Me-pimeloyl-ACP to a limiting resolution of 2.05 Å using a Mar 300 CCD detector at Life Sciences Collaborative Access Team 21ID-D, Argonne National Laboratory. Unit cell parameters were determined using iMOSLFM (2) and used as a reference for indexing using XDS (3). The structure of BioH S82A in complex with Me-pimeloyl-ACP substrate was solved by molecular replacement using an ensemble search model consisting of wild-type *E. coli* BioH crystal structure (PDB ID code 1M33) (4) and *E. coli* ACP derived from PDB ID code 3EJB (5). For each component of the ensemble search model, all water molecules and ligand atoms were removed. The solution obtained by the program Phaser (6) was imported into ARP/wARP (7), which allowed for a majority of residues to be automatically built. Cross-validation used 5% of the data in the calculation of the free R factor (8). The remaining model, including the phosphopantetheine arm and pimeloyl-methyl ester, was built in manually when the free R factor dropped below 30% using Coot (9). Solvent molecules were built by ARP/wARP solvent procedure and manually verified. The restraints definition for the phosphopantetheinylated pimeloyl-methyl ester was generated by Web-based PRODRG2 server (10). Structure refinement was carried out by the Phenix refinement procedure (11). The stereochemistry of the model was monitored throughout the course of refinement using MolProbity (12). Relevant data collection and refinement statistics are provided in Table S1.

In Vivo Complementation Assays. Strain STL243 (MG1655 Δ*bioH*::FRT Δ*pcnB*::cat) was constructed by phage P1 transduction of strain SLL24 (1) with a lysate grown on MG1655 Δ*pcnB*::*cat* (a gift of Alexander Smith of the J.E.C. laboratory). Strain STL243 was transformed with plasmid pET28b carrying either the wild-type *bioH* gene or a mutant gene. The resulting strains were maintained in spectinomycin and kanamycin throughout the experiment. The strains were inoculated at 30 °C overnight in 5 mL of M9 medium containing 0.8% (wt/vol) glycerol (M9 glycerol) and 1 nM biotin. A total of 1 mL of the cultures were collected, washed three times with M9 glycerol to remove biotin, and resuspended in 1 mL of M9 glycerol. Cell density was normalized to OD at 600 nm of 1. A total of 10 μL of the cells were then used to inoculate 1.5 mL of M9 glycerol. The growth analysis was performed on Bioscreen C (Growth Curves USA) using a proprietary 100-well microplate. Each well contained 250 μL of the cell suspension. Growth was monitored by OD at 600 nm at 37 °C for 24 h with high agitation rate. The growth curve of each strain was plotted based on the mean OD from five replicates using OriginPro 8.6. SEs were all less than 2% of mean ODs.

Growth phenotypes were also tested by colony formation on solid medium (Fig. S6).

Construction of Plasmids Encoding Biotinylation Peptide-Tagged BioF and BioH, Protein Purification, and the Order-of-Addition Assay. In prior work, a plasmid (pSTL6) encoding a version of BioH with a C-terminal hexahistidine tag in pET28b was constructed (1). An analogous pET28b plasmid (pSTL3) encoding a C-terminal hexahistidine-tagged BioF was made in the same manner as the BioH plasmid using the following oligonucleotide primers: BioF forward (5′-GGC AGC ATC ATG AGC TGG CAG GAG AAA ATC-3′) and BioF reverse (5′-CAA TGG CTT GTC TCG AGA CCG TTG CCA TGC-3′). Both genes contained an XhoI site at the junction of the coding and tag sequences, which was used together to replace the C-terminal hexahistidine tags with a sequence encoding a peptide sequence (Pep-85) that becomes biotinylated by the *E. coli* BirA biotin protein ligase. Two oligonucleotides PEP-85 top (5′-TCG AGG GAG GAC TGA ACG ACA TCT TTG AAG CGC AAA AAA TCG AAT GGC ATT AAG CTT GC-3′) and PEP85 btm (5′-TCA GCA AGC TTA ATG CCA TTC GAT TTT TTG CGC TTC AAA GAT GTC GTT CAG TCC TCC C-3′) were annealed to give a double-stranded cassette having overhanging XhoI and BlpI ends. The BioH-encoding plasmid was digested with BlpI (located 50 bp downstream of the termination codon) and XhoI and ligated to the cassette to give plasmid pCY890, which encoded the BioH–Pep-85 fusion. DNA sequencing showed the construct had the expected sequence. Similar manipulations could not be done with the BioF plasmid because the *bioF* gene contains a BlpI site. Hence the BioH plasmid was digested with XhoI and ClaI (which cuts in the vector kanamycin resistance gene), and the smaller of the two resulting fragments containing the Pep-85 tag was ligated to the larger fragment of pSTL3 cut with the same enzymes to give the BioF–Pep-85 fusion protein expression plasmid pCY889. Plasmids pCY889 and pCY890 encoded active proteins, as shown by their abilities to allow growth of Δ*bioF* and Δ*bioH* strains, respectively, in the absence of biotin.

Plasmids pCY889 and pCY890 were used to transform strain Tuner (DE3) carrying additional copies of *birA* (the *E. coli* gene encoding biotin protein ligase) encoded on a second plasmid, pCY216 (13). The resulting strains were maintained in chloramphenicol and kanamycin throughout the experiment. For expression of BioF and BioH, the strains were inoculated in 5 mL of LB at 30 °C overnight, and subcultured at 1:100 ratio in 250 mL of 2XYT medium. The cells were grown at 37 °C until OD at 600 nm reached 0.6. Arabinose was added to 0.02% (wt/vol) to induce expression of BirA for 30 min, and then IPTG was added to 0.1 mM to induce expression of BioF or BioH for 3 h. The cells were collected by centrifugation, resuspended in lysis buffer [100 mM sodium phosphate (pH 6.5), 150 mM NaCl, 10% (wt/vol) glycerol, and 1 mM Tris(2-carboxyethyl)phosphine (TCEP)], and lysed by French press treatment at 17,500 psi. Ammonium sulfate was added slowly to the soluble cell extract to 55% (wt/vol) saturation under constant stirring on ice. After 30 min of stirring, protein pellet was collected by centrifugation at 20,000 × *g*. The pellet was washed three times, each with 20 mL of lysis buffer containing 55% (wt/vol) ammonium sulfate. The pellet was then dissolved in lysis buffer and biotin-tagged BioF or BioH was purified by monomeric avidin agarose (Pierce). The agarose was washed with 30 column volumes of lysis buffer, and the biotin-tagged proteins were eluted with lysis buffer containing 4 mM biotin. The proteins were dialyzed extensively in lysis buffer to remove biotin and concentrated using Amicon Ultra centrifugal filters (Millipore). Protein concentrations were determined by Bradford assay (Bio-Rad) using bovine serum globulin (Pierce) as standard.

The BioH-BioF order-of-addition assay allows in vitro conversion of Me-pimeloyl-ACP to dethiobiotin (DTB) using pure BioH, BioF, BioA, and BioD enzymes. The assay was divided into two reactions. In the first reaction, Me-pimeloyl-ACP was incubated with BioH, BioF, or both plus the required substrates to give pimeloyl-ACP, 7-keto-8-aminopelargonic acid (KAPA) methyl ester, or KAPA as potential products. In the second reaction, these products were converted to DTB by adding the other three enzymes. The first reaction (50 μL) contained assay premix [50 mM 3-(*N*-morpholino)propanesulfonic acid (pH 7.5), 100 mM NaCl, 10 mM $MgCl_2$, 10% (wt/vol) glycerol, 2.5 mM DTT, 10 mM L-Ala, and 100 μM pyridoxal-5′-phosphate], 10 μM Me-pimeloyl-ACP, and 1 nM of either biotin-tagged BioH or biotin-tagged BioF. The reaction was incubated at 37 °C for 1 h. The biotin-tagged proteins were removed by incubation with 25 μL of streptavidin agarose (Novagen; binding capacity of 2 nmol of biotin per 25 μL) on ice for 30 min. The agarose bead was separated from the supernatant using a spin filter. The second reaction (100 μL) contained 50 μL of the supernatant, assay premix, 2 mM potassium bicarbonate, 2.5 mM ATP, 2.5 mM SAM, 10 nM BioA, 10 nM BioD, and 1 nM of either BioH or BioF. The reaction was incubated at 37 °C for 1 h and quenched by boiling for 10 min. A total of 20 μL of each reaction was spotted on DTB bioassay plates containing strain ER90 as described previously (1).

Synthesis of Me-Pimeloyl-ACP. Enzymatic preparation of Me-pimeloyl-ACP was adapted from the methods of Lin et al. (1). Briefly, the acylation reaction contained 50 mM Tris·HCl (pH 8.5), 10 mM $MgCl_2$, 0.5 mM TCEP, 5 mM ATP, 5 mM Me pimelate, 1 mM ACP, and 50 μM AasS. The reactions were incubated at 37 °C for 1.5 h. Me-pimeloyl-ACP was purified by ion-exchange chromatography using Vivapure D spin columns (GE Healthcare Life Sciences). The reaction mixture was diluted five-fold in binding buffer [25 mM 4-morpholineethanesulfonic acid (pH 6), 10% (wt/vol) (vol/vol) glycerol, and 1 mM TCEP] containing 100 mM LiCl, and loaded into the column. The column was washed with binding buffer containing 250 mM LiCl, and the ACP was eluted in binding buffer containing 500 mM LiCl. Me-pimeloyl-ACP was desalted by dialysis in binding buffer, and concentrated using Amicon Ultra centrifugal filter (Millipore). The ACP product was analyzed in a conformationally sensitive electrophoretic mobility shift assay in 20% polyacrylamide gels containing 2 M urea at 130 V for 3.5 h. ACP was stained with Coomassie Blue R250. Other acyl-ACPs were prepared by the same method.

Me-Pimeloyl-ACP Hydrolysis Assay. The reactions contained 100 mM sodium phosphate (pH 7), 200 μM Me-pimeloyl-ACP, and 5 nM BioH. The hydrolysis reaction was monitored at seven time-points over a course of 20 min using the following protocol. A premix of buffer and ACP substrate, without BioH, was incubated at 37 °C for 1 min. The hydrolysis reaction was initiated by adding BioH. At each time point, the reaction was sampled, immediately quenched by addition of an equal volume of 10 M urea, and stored on dry ice until analysis. The reaction samples were mixed with loading dye [100 mM 2-(*N*-morpholino)ethanesulfonic acid (pH 6) and 70% (wt/vol) glycerol] at 2:1 ratio before loading into the gel (1).

4-Nitrophenyl Acetate Hydrolysis Assay. The hydrolysis of 4-nitrophenyl acetate by BioH produced 4-nitrophenol, which was monitored by OD at 405 nm. The 500-μL reaction contained 100 mM Mes (pH 6.5), 10 mM 4-nitrophenyl acetate, 10% (vol/vol) dimethyl sulfoxide, and 10 nM BioH. Kinetic analysis was performed at room temperature in a Beckman Coulter DU 800 spectrophotometer. The reaction was equilibrated without BioH for 1 min and 20 s, initiated by adding BioH, and monitored continuously for a total of 7 min. Mean reaction rates and SEs were determined from three replicate experiments.

Electrophoretic Gel-Shift Assay of BioH–ACP Complex Formation. BioH S82A was incubated with various molar ratios of Me-pimeloyl-ACP to form a complex, which was detected by EMSA;

briefly, a 10-μL reaction consisting of 10 μM BioH and 10 μM, 20 μM, 50 μM, or 100 μM of a methyl dicarboxylate-ACP in 50 mM Hepes (pH 7.5) containing 150 mM KCl. The reaction was incubated at 37 °C for 10 min, mixed with 5 μL of loading dye (1.5× Tris-borate-EDTA buffer in 60% (wt/vol) glycerol), and loaded into a 15-well 6% DNA Retardation Gel (Invitrogen). BioH monomer and the complex were separated by electrophoresis in Tris-glycine buffer (10 mM Tris, 100 mM glycine) at 70 V for 100 min at room temperature. The proteins were visualized by staining with Coomassie Blue R250. Me-glutaryl-ACP and Me-azelayl-ACP were assayed in parallel to determine the effect of acyl chain length on BioH binding. The S82A versions of the BioH Arg mutants were assayed by the same method.

Bacterial Strains. *Escherichia coli* strains ER90 (14, 15), YFJ239 (16), DK574/pJT93 (17), and STL24 (1) were described previously. BL21(DE3) was from Invitrogen and Tuner (DE3) was from Novagen.

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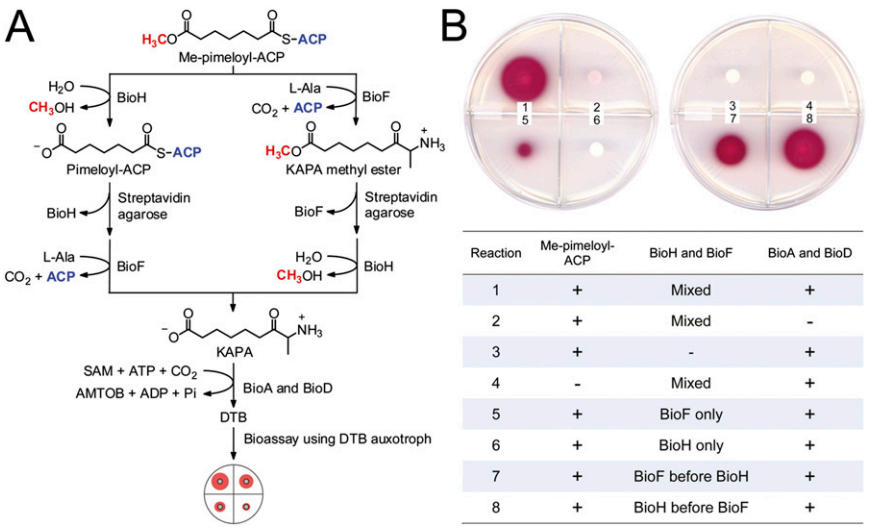

Reaction	Me-pimeloyl-ACP	BioH and BioF	BioA and BioD
1	+	Mixed	+
2	+	Mixed	-
3	+	-	+
4	-	Mixed	+
5	+	BioF only	+
6	+	BioH only	+
7	+	BioF before BioH	+
8	+	BioH before BioF	+

Fig. S1. In vitro order-of-addition assay. (*A*) Me-pimeloyl-ACP was the substrate for in vitro synthesis of KAPA by two putative routes, producing either pimeloyl-ACP or KAPA methyl ester as intermediates. KAPA would eventually be converted by the BioA and BioD reactions to DTB for bioassay. BioH and BioF were biotin-tagged to allow their quantitative removal by binding to streptavidin agarose before addition of the second enzyme. (*B*) The production of DTB, via the synthesis of KAPA, was visualized on the bioassay plate as described in *Materials and Methods*. AMTOB, *S*-adenosyl-2-oxo-4-methylthiobutyric acid.

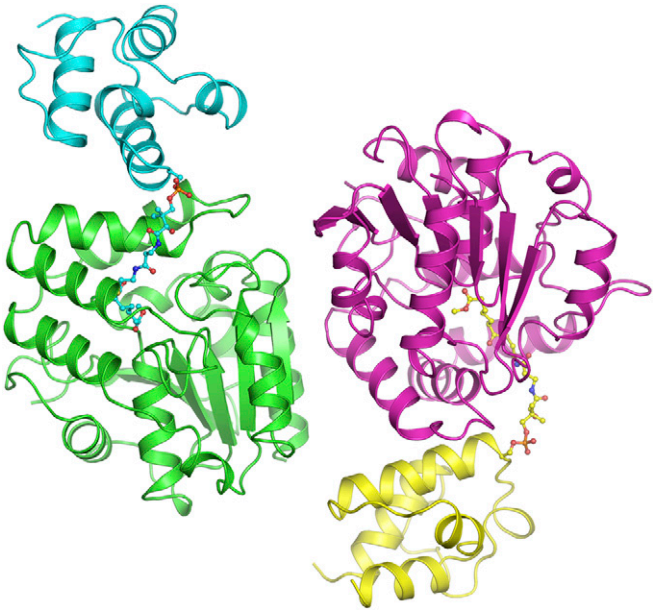

Fig. S2. Asymmetric unit organization for the BioH S82A cocrystal structure in complex with Me-pimeloyl-ACP. The BioH–ACP complexes are shown in cartoon representation. The phosphopantetheinylated pimeloyl methyl ester is shown in stick-ball representation with the carbon atoms colored as the corresponding ACP cartoon.

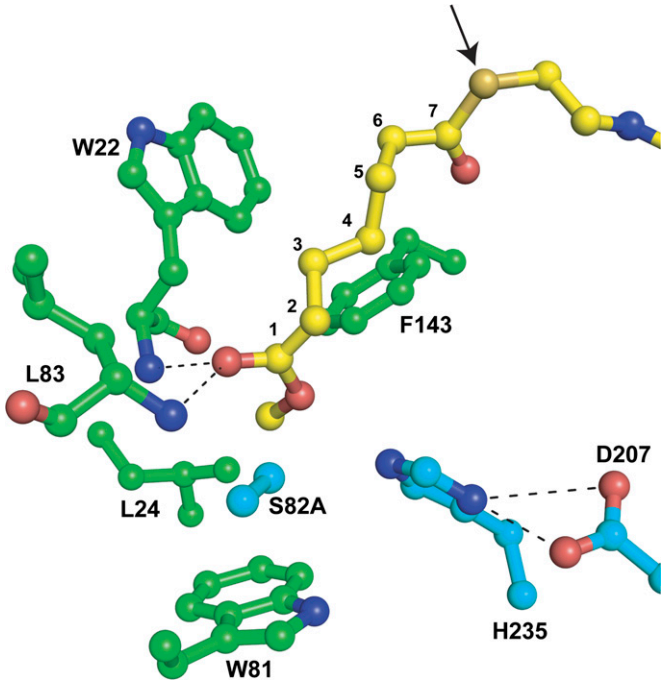

Fig. S3. BioH catalytic triad and interactions stabilizing the oxyanion hole. The catalytic triad residues are shown with their carbon atoms colored cyan. The phosphopantetheinylated pimeloyl methyl ester is shown with carbon atoms colored yellow. Additional residues making contacts with the ester moiety are shown with carbon atoms in green. The thioester sulfur atom is marked by an arrow, and the carbon atoms of the seven-carbon pimeloyl chain are numbered. Hydrogen bonds are shown as black dashed lines.

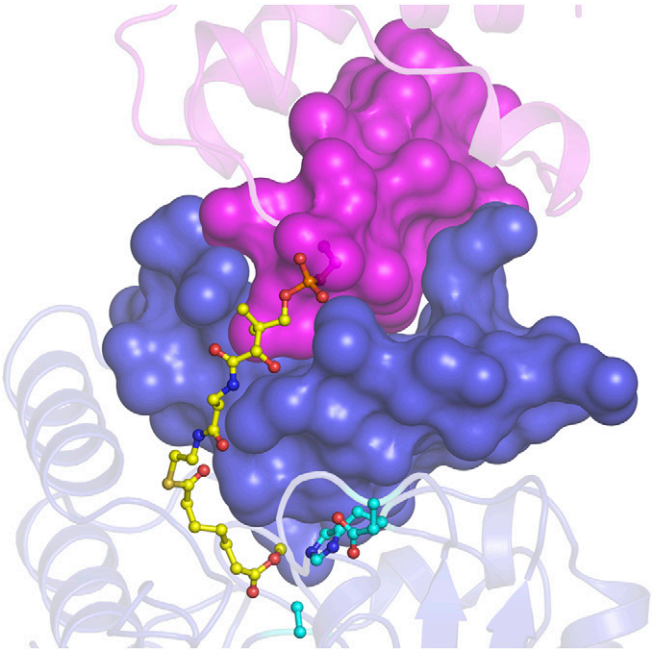

Fig. S4. Interactions between ACP-α2 and the BioH capping helices. The ACP-α2 helix and the BioH capping helices are shown in surface representation and colored pink and blue, respectively. The phosphopantetheinylated pimeloyl methyl ester and the side chains of BioH catalytic triad residues are shown in stick-ball representation with the respective carbon atoms colored yellow and cyan, respectively.

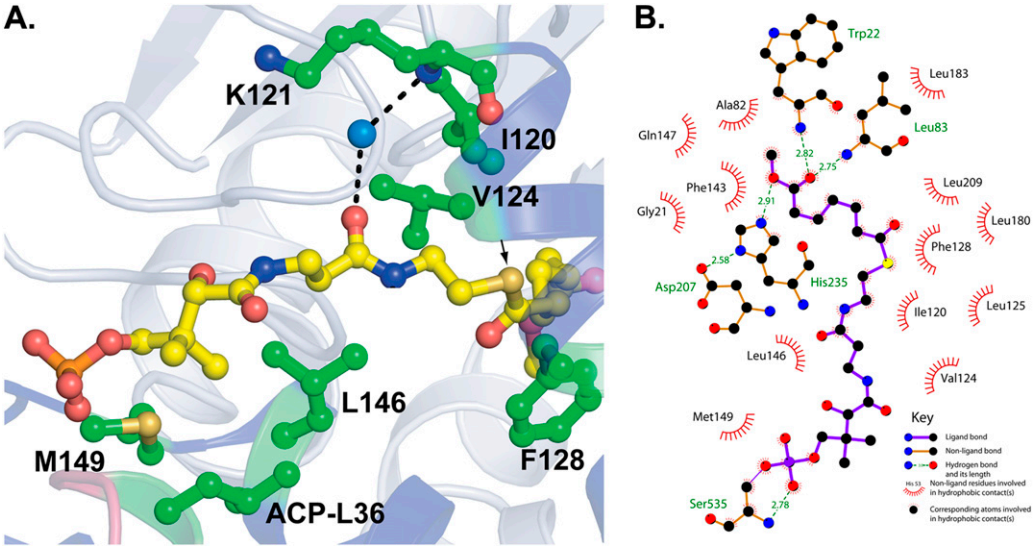

Fig. S5. Interactions of the phosphopantetheine arm of the substrate with BioH. (*A*) BioH and ACP are shown in cartoon representation and colored blue and pink respectively. The BioH capping helices are colored darker than the core domain. The phosphopantetheine-linked pimeloyl methyl ester is shown in ball-stick representation with carbon atoms in yellow. Sulfur atoms are colored golden. The arrow indicates the thioester sulfur atom. Enzyme residues are shown in ball-stick representation and with carbon atoms colored green. A water molecule is shown as a blue sphere. Hydrogen bond interactions are shown as black dashes. Note that the water molecule is present in only one of the two complexes within the crystallographic asymmetric unit. (*B*) 2D representation of binding interactions of the phosphopantetheine-pimeloyl-methyl ester with BioH S82A. This figure was generated by the program LigPlot (18).

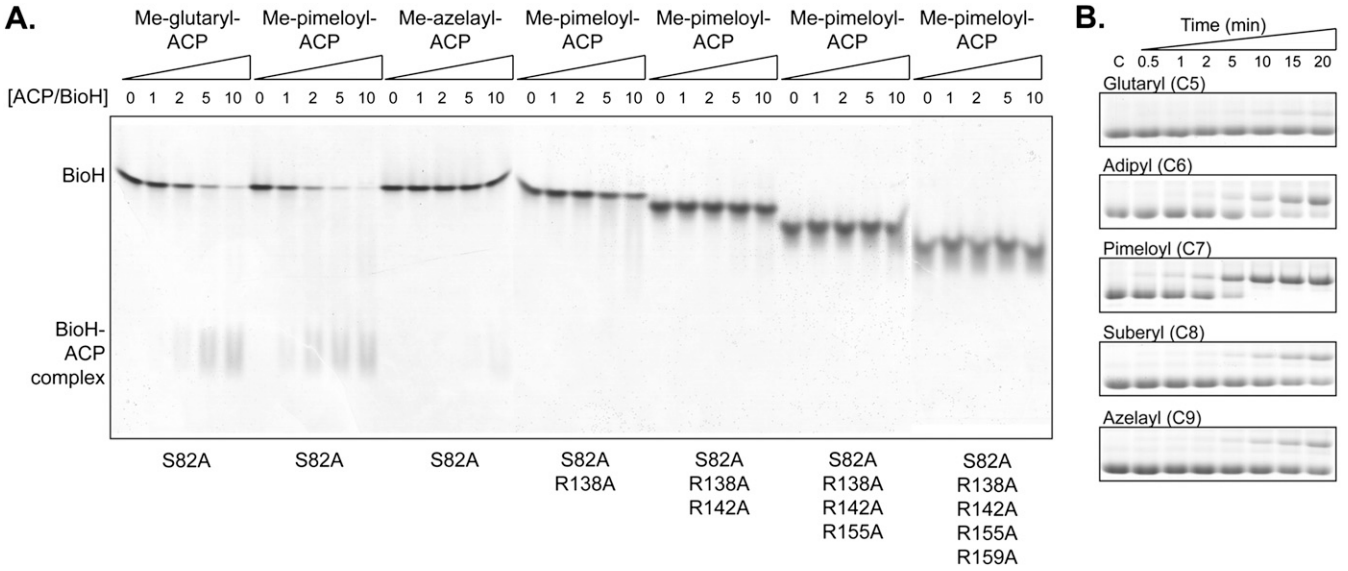

Fig. S6. Formation of BioH S82A–Me-pimeloyl-ACP complexes and specificity of BioH-catalyzed hydrolysis. (*A*) Binding of Me-pimeloyl-ACP to the various BioH S82A proteins was assayed by EMSA as described in *SI Materials and Methods*. The binding of Me-pimeloyl-ACP resulted in the formation of the faster-migrating BioH–ACP complex and the disappearance of BioH S82A monomer, in a [ACP/BioH] molar ratio-dependent manner. Quantitation of the BioH bands using ImageJ gave K_d values of 3.1 and 19.1 μM for BioH S82A binding to Me-pimeloyl-ACP and Me-glutaryl-ACP, respectively. No quantifiable binding was observed with Me-azelayl-ACP. Substitution of alanine for the BioH arginine residues at positions 138, 142, 155, and 156 significantly reduced binding to Me-pimeloyl-ACP, but increased the rate of migration of BioH due to loss of positive charge. Because of the small size and low pI of ACP, the acyl-ACPs migrated through these high-porosity gels and were not observed. Note also that the highly acidic ACP stains much more poorly with Coomassie Blue than BioH, and hence the staining of the shifted species seems almost entirely to reflect BioH staining. (*B*) Wild-type BioH-catalyzed hydrolysis of ACP thioesters of the methyl esters of glutarate, adipate, pimelate, suberate, and azelate. The product is the slower-migrating ω-carboxyl-ACP, which can be resolved from the substrates in a destabilizing urea-PAGE system as described in *SI Materials and Methods*.

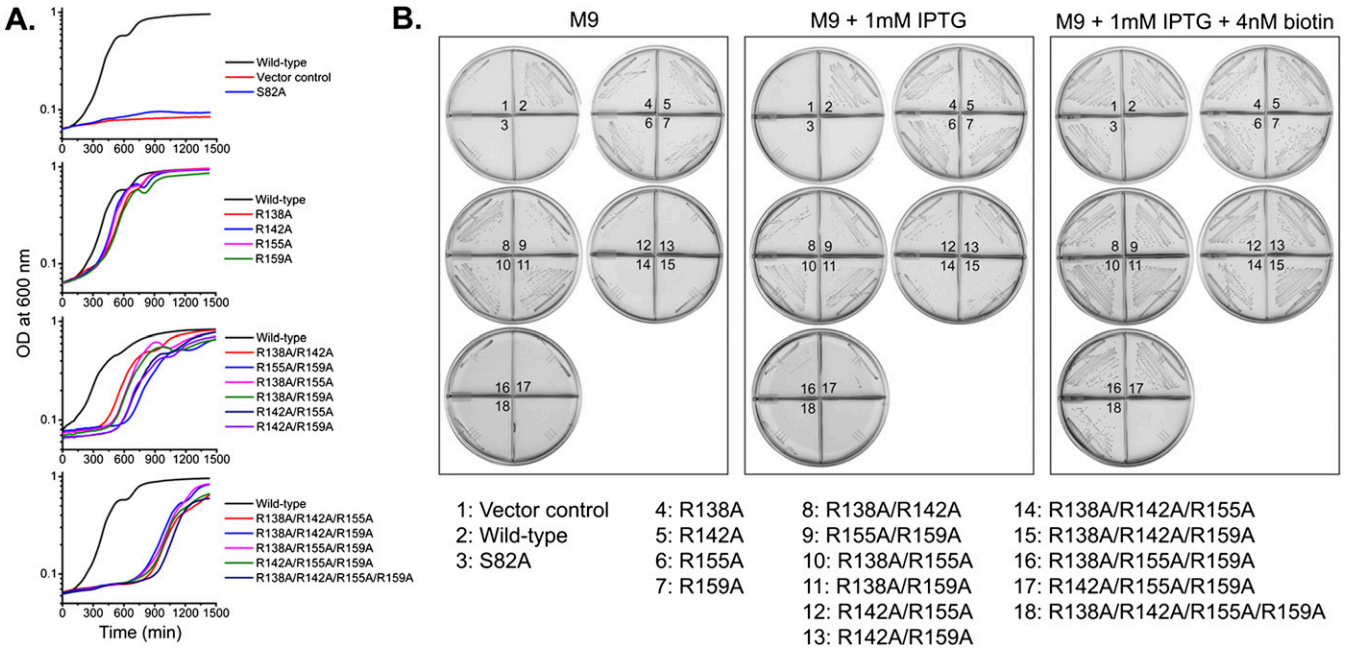

Fig. S7. Alanine substitutions for residues R138, R142, R155, and R159 reduced BioH activity in vivo as assayed by growth curves (*A*) and colony formation (*B*). The effect of Ala substitutions on BioH activity was investigated by assaying complementation of a Δ*bioH* strain. The chromosomal *bioH* gene of the host strain was deleted, and a plasmid encoding BioH was required to complete biotin biosynthesis and sustain cell growth. (*A*) The strain carrying the empty vector or that expressing the S82A mutant protein failed to grow. Exponential growth of cultures expressing the single Ala substitution mutants was slightly delayed. These cultures required more time to accumulate sufficient biotin to support fatty acid synthesis. The lag became more apparent in the double mutants and increased to nearly 10 h in the triple and quadruple mutants. (*B*) Complementation by expression of BioH mutant proteins assayed by colony formation on M9 minimal agar plates.

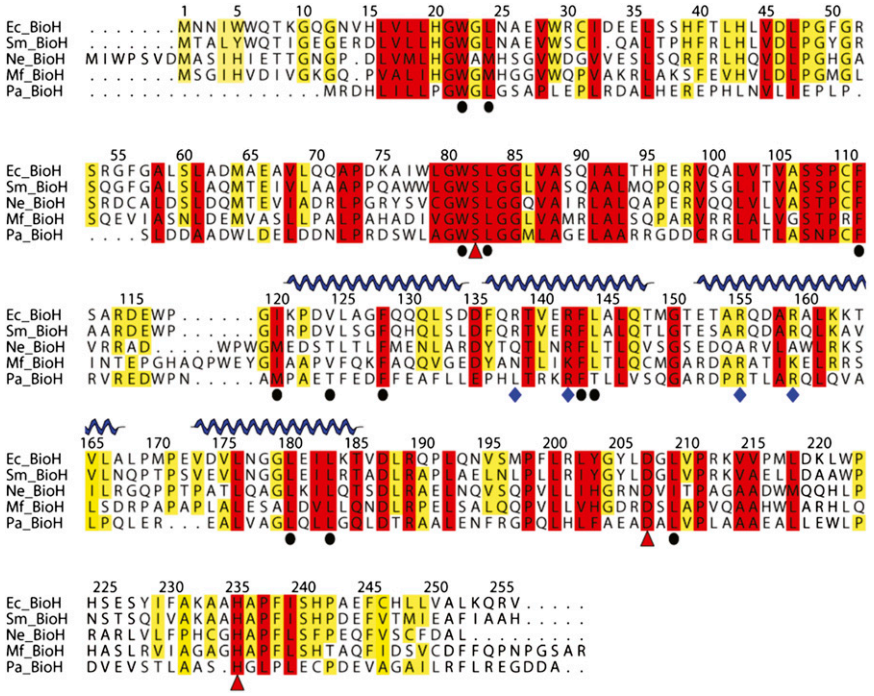

Fig. S8. Structure based primary sequence alignment of *E. coli* BioH with homologs demonstrate conservation of catalytically relevant residues. Residues comprising of the esterase catalytic triad are marked by a triangle (▲) under the residues; residues implicated for ACP binding are marked by a diamond (♦) under the residues, and residues which form the hydrophobic channel for directing the pimeloyl methyl ester toward the catalytic site, and for coordinating the methyl ester are marked by a circle (●) under the residues. The four helices of the BioH capping domain are shown by a cartoon above the residues. Residue numbering is that of *E. coli* BioH. Primary sequence alignment was generated by ClustalW (19) and imported into ALINE (20) for figure generation. Ec_BioH, BioH sequence from *E. coli*; Mf_BioH, BioH sequence from *Methylobacillus flagellates* KT; Ne_BioH, BioH sequence from *Nitrosomonas europaea*; Pa_BioH, BioH sequence from *Pseudomonas aeruginosa*; Sm_BioH, BioH sequence from *Serratia marcescens*. Note that Ec_BioH and Sm_BioH lie outside their respective *bio* operons, whereas Ne_BioH, Mf_BioH, and Pa_BioH lie within their respective *bio* operons.

Table S1. Data collection, phasing, and refinement statistics

	BioH S82A–Me-Pim-ACP
Data collection	
Cell dimensions	
Space group	P1
a, b, c, Å	57.08, 57.21, 60.95
α, β, γ, °	101.99, 90.10, 112.74
Resolution (Å)*	40.00–2.05 (2.10–2.05)
Total reflections	232,039
Unique reflections	84,546
R_{sym} (%)	9.4 (64.1)
I/σ(I)	9.43 (2.84)
Completeness (%)	97.2 (96.7)
Redundancy	3.7 (3.6)
Refinement	
Resolution, Å	35.13–2.05 (2.10–2.05)
No. reflections	42,533
R_{work}/R_{free} †	0.192/0.236
No. of atoms	
Protein	5,142
Ligand	66
Water	224
B-factors‡	
BioH/ACP/ligand (chain A + chain B)	40.80/66.45/54.3
BioH/ACP/ligand (chain C + chain D)	42.81/67.58/56.4
Water	46.01
Ramachandran analysis	
Favored	97.2%
Allowed	2.5%
Outliers	0.3%
Rmsd	
Bond lengths, Å	0.010
Bond angles, °	1.195

*Highest-resolution shell is shown in parenthesis.

†R-factor = $\Sigma(|F_{obs}| - k|F_{calc}|)/\Sigma|F_{obs}|$ and R-free is the R value for a test set of reflections consisting of a random 5% of the diffraction data not used in refinement.

‡B-factors for each of the two BioH-S82A Me-ACP-pimeloyl complexes are listed. B-factors are provided as BioH-S82A/ACP/phosphopantetheine-pimeloyl-methyl ester.