Supplementary Information

The Pimeloyl-CoA Synthase BioW Defines a New Fold for Adenylate-Forming Enzymes

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Supplementary Results

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Supplementary Table 1. Primers used for cloning of wild type BioW and variants.

All mutants were created by primer extension with the exception of His16→Ala, for which Quick-Change mutagenesis was used. Uppercase nucleotides are the Ligation Independent Cloning (LIC) overhangs.

A. aeolicus	
BioWA-f	TACTTCCAATCCAATGCAatggatcttttcagtgtaagaatg
BioWA-r	TTATCCACTTCCAATGTTATTAttataactcaattagaactacttttt
BioW_Nde1	aagcagccgcatatggatcttttcag
BioW_Xho1	ctagctcgagttataactcaattagaa
BWAH16_F	cagaaaaacggaaaggccgtctcgggagcggaa
BWAH16_R	ctctttttttactatcctttccgctcccgagac
BWA-132A-f	gaagaggacaaggaagcgggagtaagaaccatt
BWA-132A-r	aatggttcttactcccgcttccttgtcctcttc
BWA-159A-f	gaaggctacaccctagcaactgttgacgctctg
BWA-159A-r	cagagcgtcaacagttgctagggtgtagccttc
BWA-182A-f	gcggagctttgctgggctgatgatcccgat
BWA-182A-r	atcgggatcatcagcccagcaaagctccgc
BWA-187A-f	agtgatgatcccgatgccgtcacgggatacgtt
BWA-187A-r	aacgtatcccgtgacggcatcgggatcatcact
BWA-199A-f	ggaaaggaaataggagccgtcaggataactccc
BWA-199A-r	gggagttatcctgacggctcctatttcctttcc
BWA-201A-f	gaaataggatacgtcgcgataactcccttgaag
BWA-201A-r	cttcaagggagttatcgcgacgtatcctatttc
BWA-215A-f	gatcctttgggcggagcggtttacttcgtaagc
BWA-215A-r	gcttacgaagtaaaccgctccgcccaaaggatc

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Supplementary Table 2. Data collection, phasing and refinement statistics

	Native	Pimelate (SeMet)	AMP-CPP+ Mg^{2+}+Pimelate	CoA+AMP
PDB Code	5TV5	5TV6	5TV8	5TVA
Data collection				
Space Group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell: a, b, c (Å)	56.7, 68.2, 158.4	68.5, 103.2, 113.8	68.6, 105.3, 111.9	55.7, 68.9, 156.6
Resolution (Å)[1]	50-2.5 (2.51-2.5)	50-2.45 (2.49-2.45)	48-2.55 (2.56-2.55)	50-2.25 (2.35-2.25)
Total reflections	147,152	217,857	223,869	235,772
Unique reflections	22,008	30,059	27,824	29,328
R_{sym} (%)	13.5 (56.0)	9.0 (86.0)	10.3 (93.8)	7.6 (98.8)
I/σ(I)	11.9 (3.8)	20.9 (1.8)	16.5 (2.2)	18.5 (2.6)
Completeness (%)	99.8 (63.1)	99.8 (97.5)	99.5 (85.9)	99.9 (100)
Redundancy	6.7 (6.8)	7.3 (5.6)	8.0 (6.8)	8.0 (8.3)
Refinement				
Resolution (Å)	42.0-2.5	50-2.45	25-2.55	38.1-2.25
No. reflections	21,955	27,317	26,362	29,312
R_{work} / R_{free}[2]	20.6/27.0	19.0/23.0	18.4/22.9	20.1/24.5
Number of atoms				
Protein	3,826	3,776	3,834	3,831
Ligand	-	86	84	94
Water	159	184	129	109
B-factors				
Protein	39.3	44.8	62.4	53.9
Ligand	-	44.6	70.4	55.1
Water	35.3	40.1	53.7	50.3
R.m.s deviations				
Bond lengths (Å)	0.008	0.009	0.007	0.009
Bond angles (°)	1.023	1.272	1.265	1.135

1. Highest resolution shell is shown in parenthesis.
2. 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.

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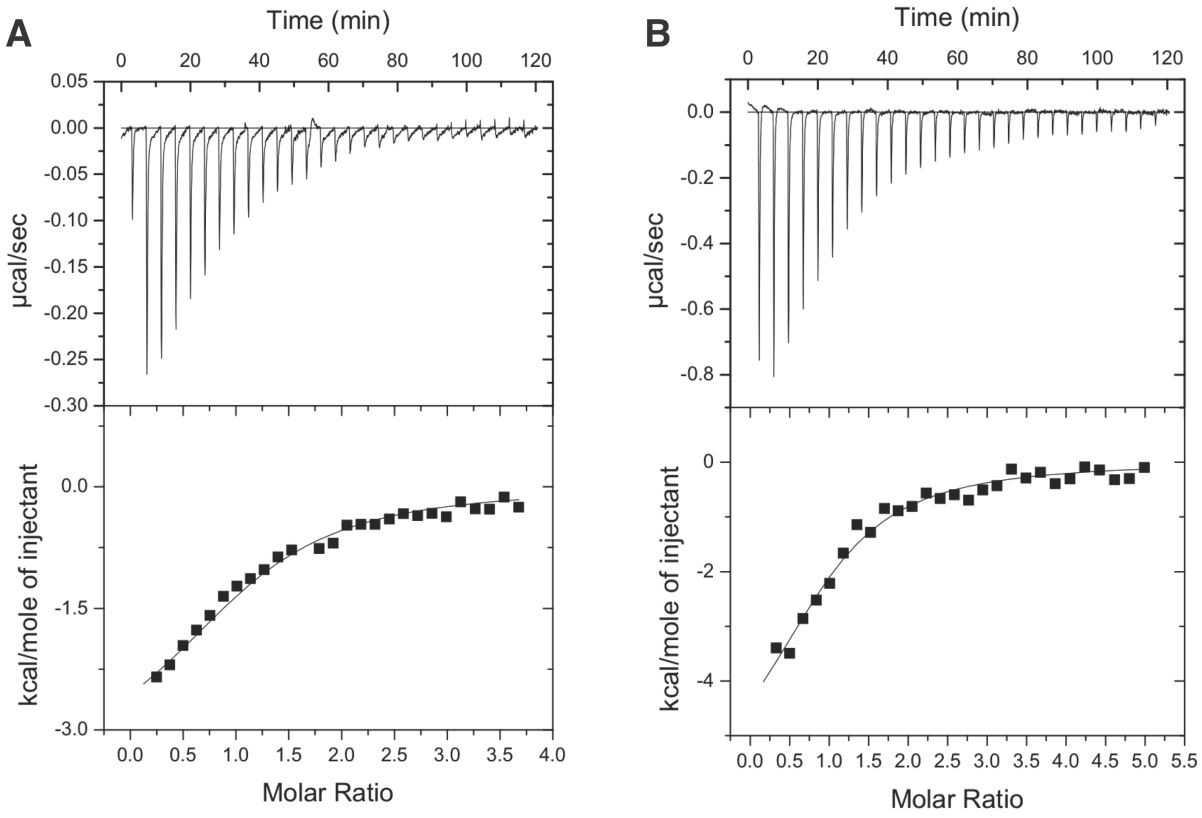

Supplementary Figure 1. Binding isotherms for BioW. Binding isotherms from ITC measurement of AaBioW binding to (A) ATP and (B) CoA.

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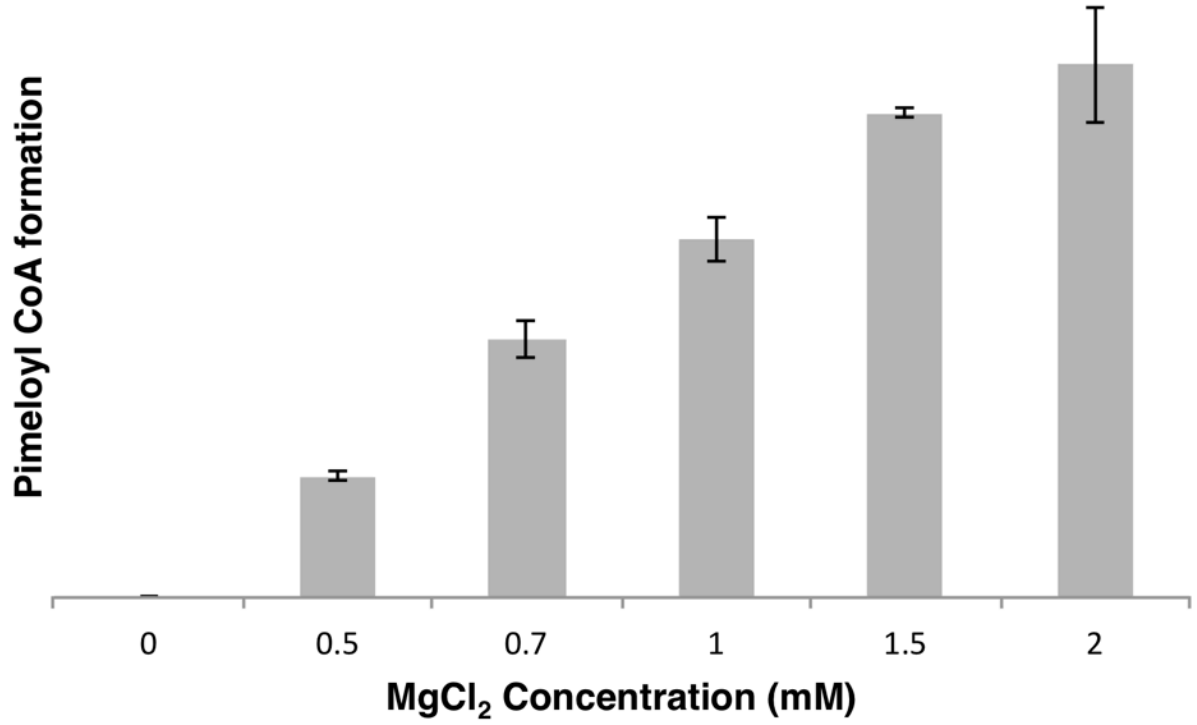

Supplementary Figure 2. Dependence of BioW activity on Mg^{2+}. Reactions to test the requirement for Mg^{2+} contained 20 mM HEPES, 0.2 mM DTT, 1 mM CoA, 1 mM ATP and 3 μM AaBioW. Reactions were done at 42°C for 6 h. A control reaction without any additional $MgCl_2$ was run with 1 mM EDTA added.

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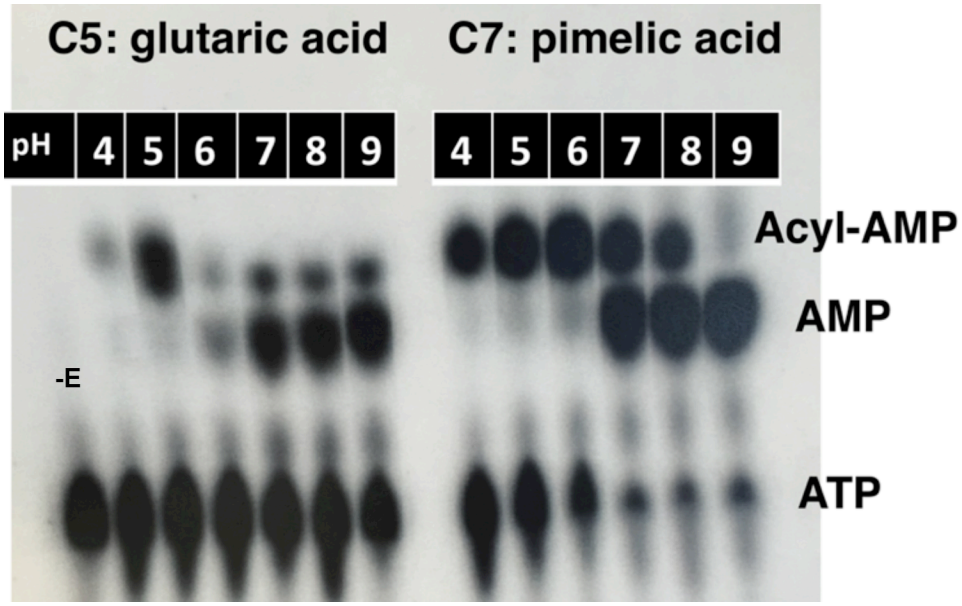

Supplementary Figure 3. Proofreading of dicarboxylate-adenylates as a function of pH. All reactions were carried out in the absence of CoA in order to determine the stability of the acyl-AMP intermediate, as determined by generation of AMP. With the cognate pimelate (C7) substrate, the acyl-AMP intermediate is hydrolyzed at pH greater than 6. For the non-cognate glutarate (C5) substrate, increased pH did not have as significant an effect on hydrolysis of the adenylate. At pH 4 the enzyme has only a low level of activity with either substrate. The left most lane lacked enzyme (-E)

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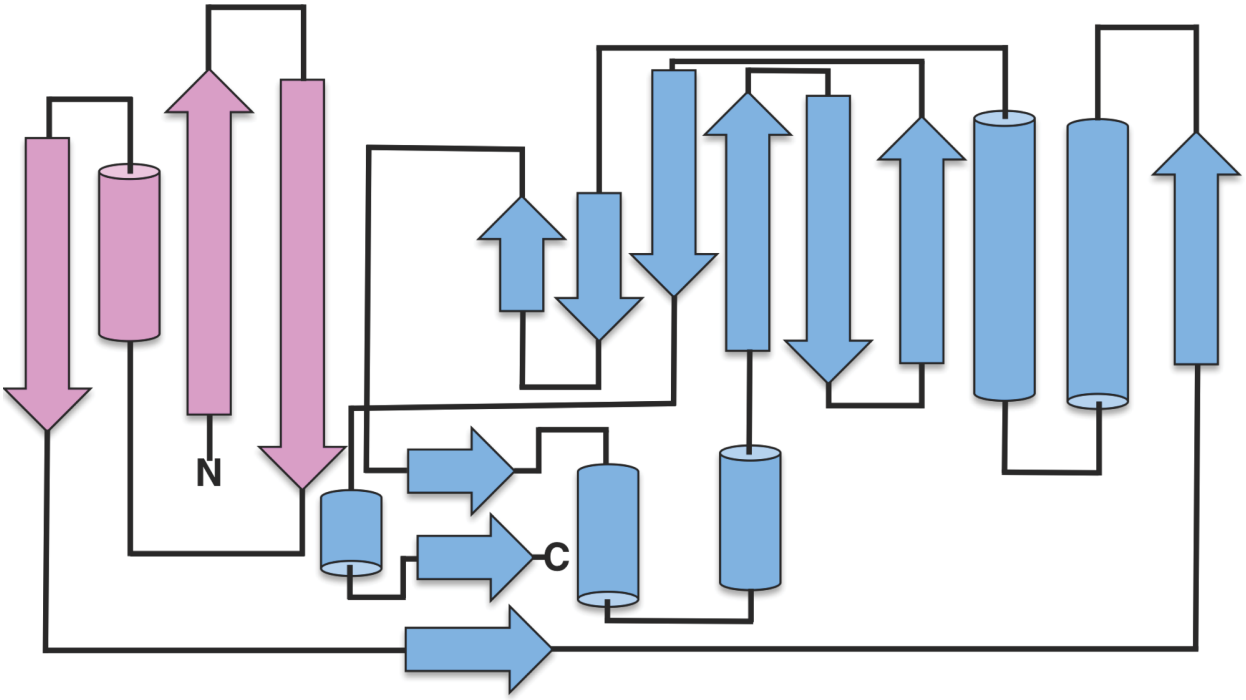

Supplementary Figure 4: **Topology of AaBioW.** Topology diagram showing the overall fold of BioW. Domains are colored using the same scheme as in Figure 3.

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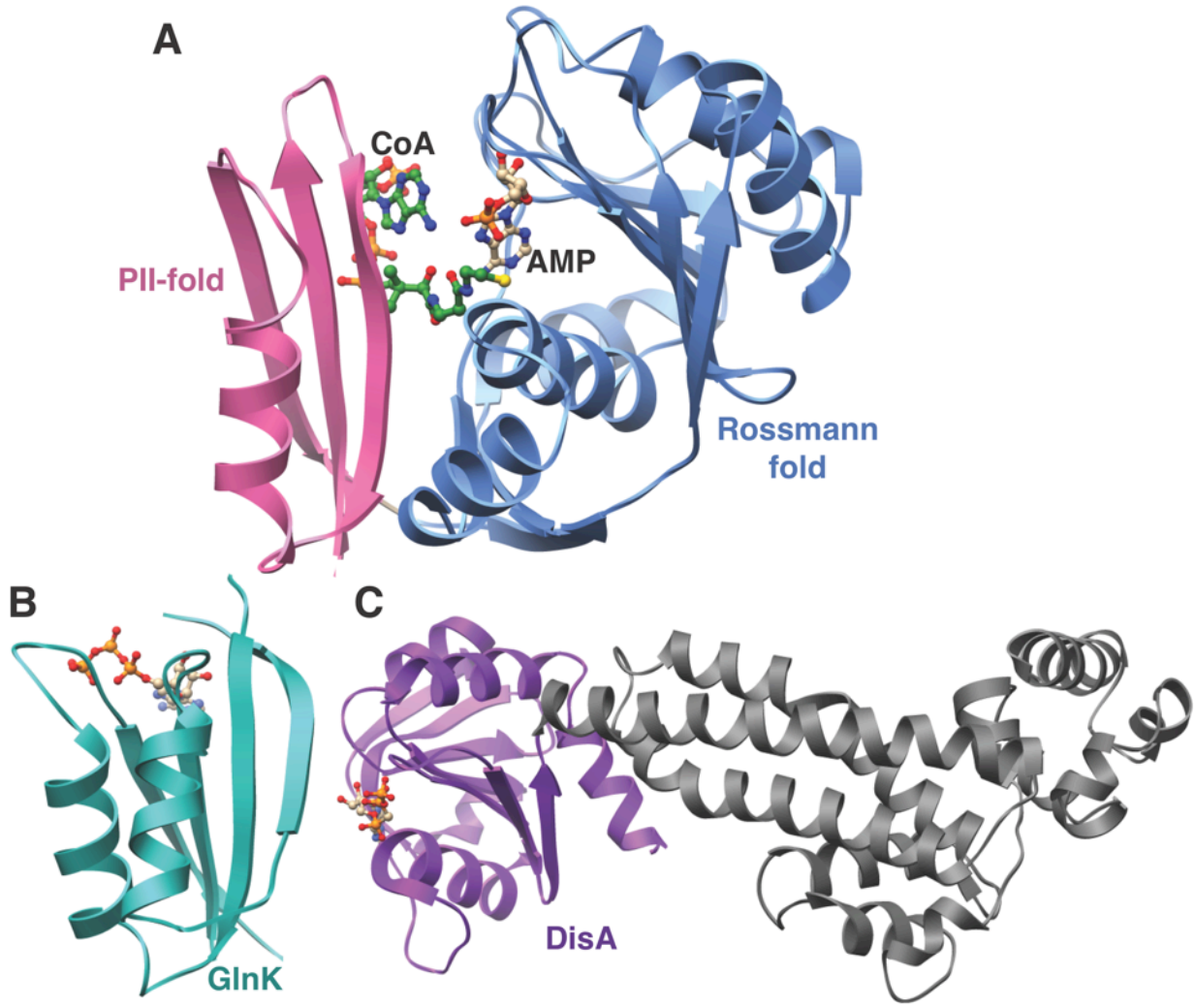

Supplementary Figure 5. Structural homologs of BioW. (A) The structure of the AaBioW complex with bound CoA and AMP. The P_{II} fold domain is colored in pink and the Rossmann fold is colored in light blue. (B) Structure of the P_{II} fold protein GlnK[1] (in cyan) in complex with ATP (PDB Code: 2GNK). (C) Structure of the diadenylate cyclase DisA[2] with bound AMP (PDB Code 3C23). The Rossmann fold domain is colored in purple.

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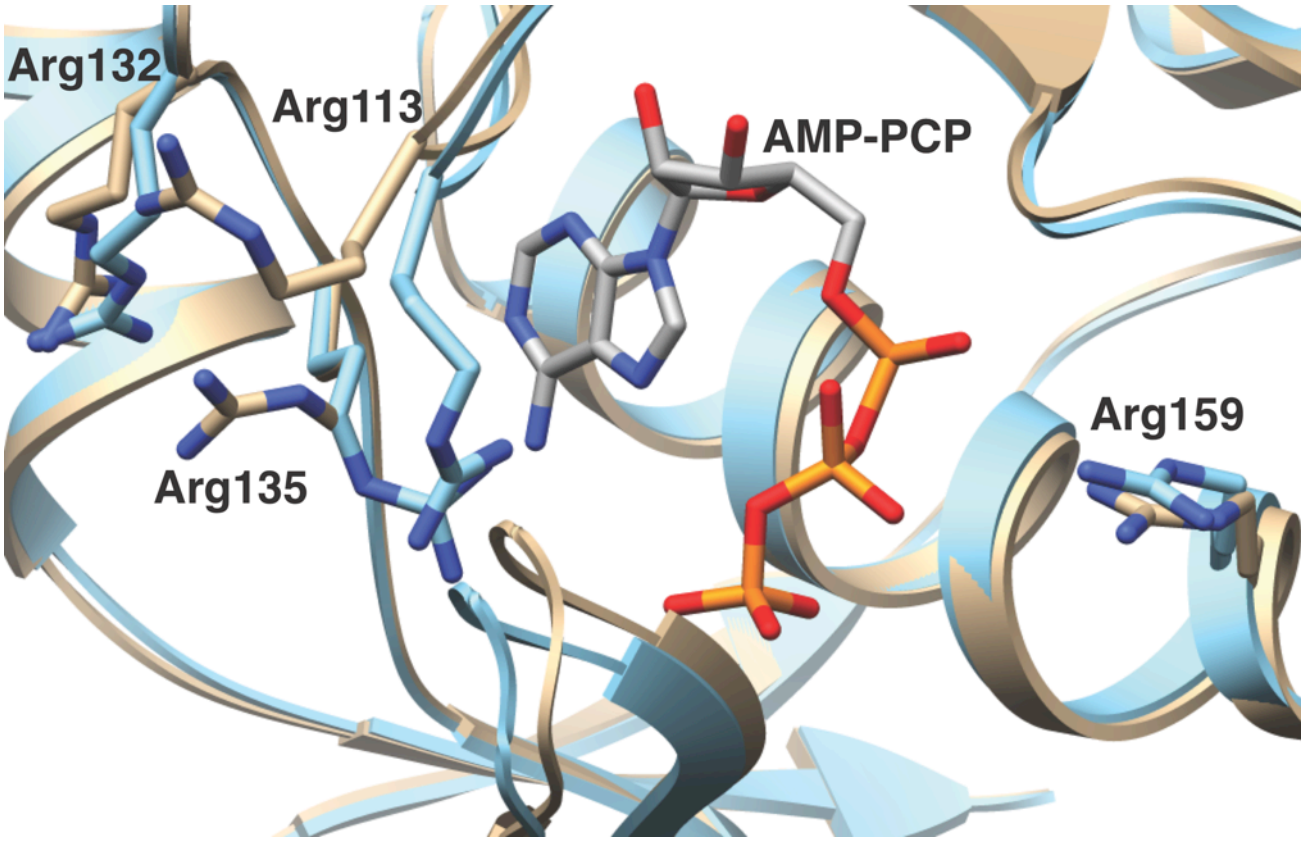

Supplementary Figure 6. Side chain movement of active site Arg side chains during catalysis. Superposition of cocrystal structures of AaBioW with bound AMP-PCP (shown in light brown) and with bound CoA (shown in cyan). Note the movement of multiple active site Arg residues between the adenylation and thioester formation complexes. These movements are necessary to accommodate binding of the different substrate ligands.

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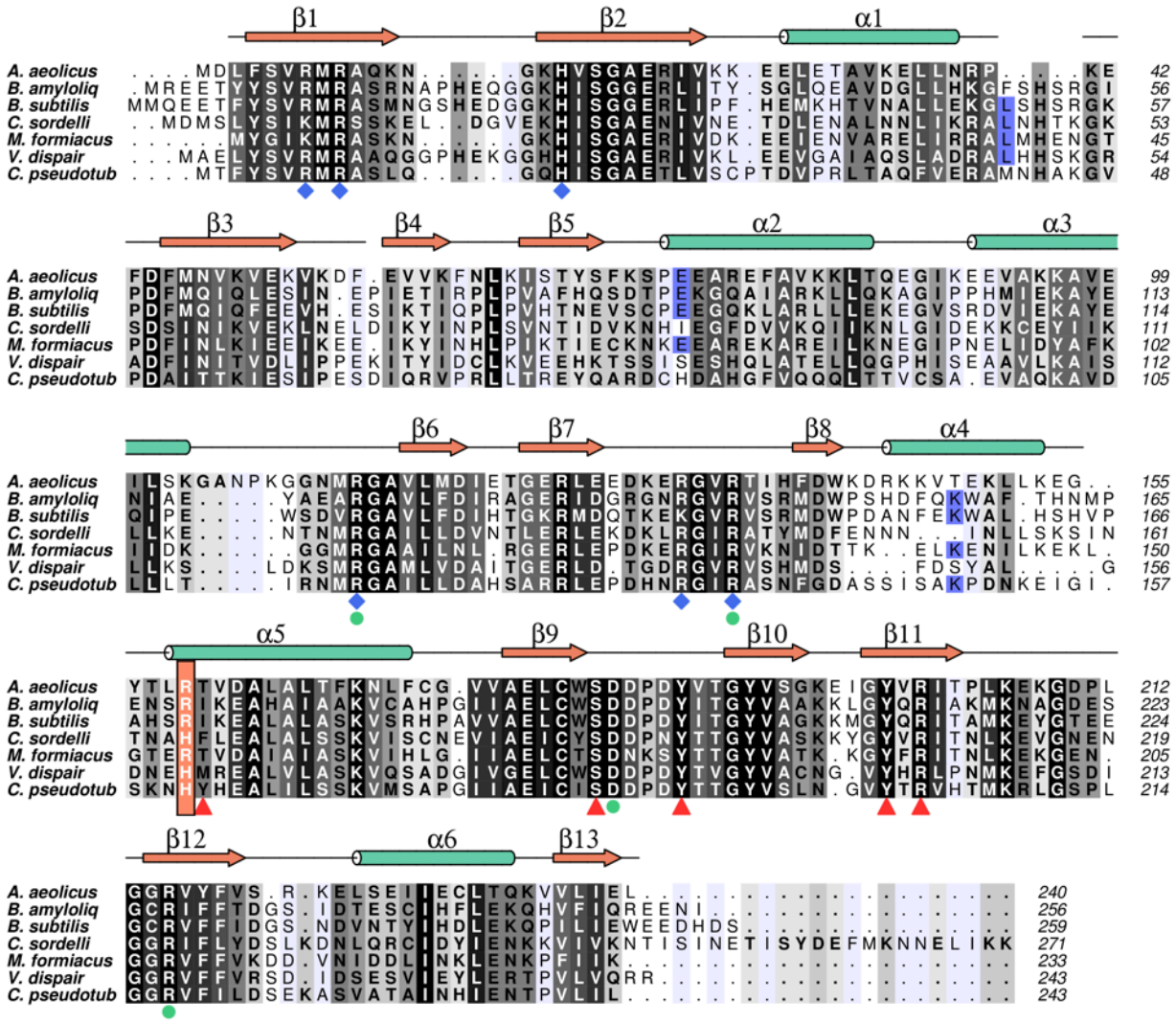

Supplementary Figure 7. Structure-based multiple sequence alignment of various BioW. Structure based alignment of the primary sequences of BioW from *Aquifex aeolicus*, *Bacillus amyloliquifaciens*, *Bacillus subtilis*, *Clostridium sordelli*, *Methanotorris formiacus*, *Veillonella dispair*, and *Corynebacterium pseudotuberculosis*. Secondary structures are derived from the crystal structure of AaBioW. The red triangles show residues that interact with pimelate, the blue diamonds show residues that engage CoA and residues demarcated with green circle interact with ATP. The gatekeeper residue Arg159 (and equivalent) is shown colored in a brown box.

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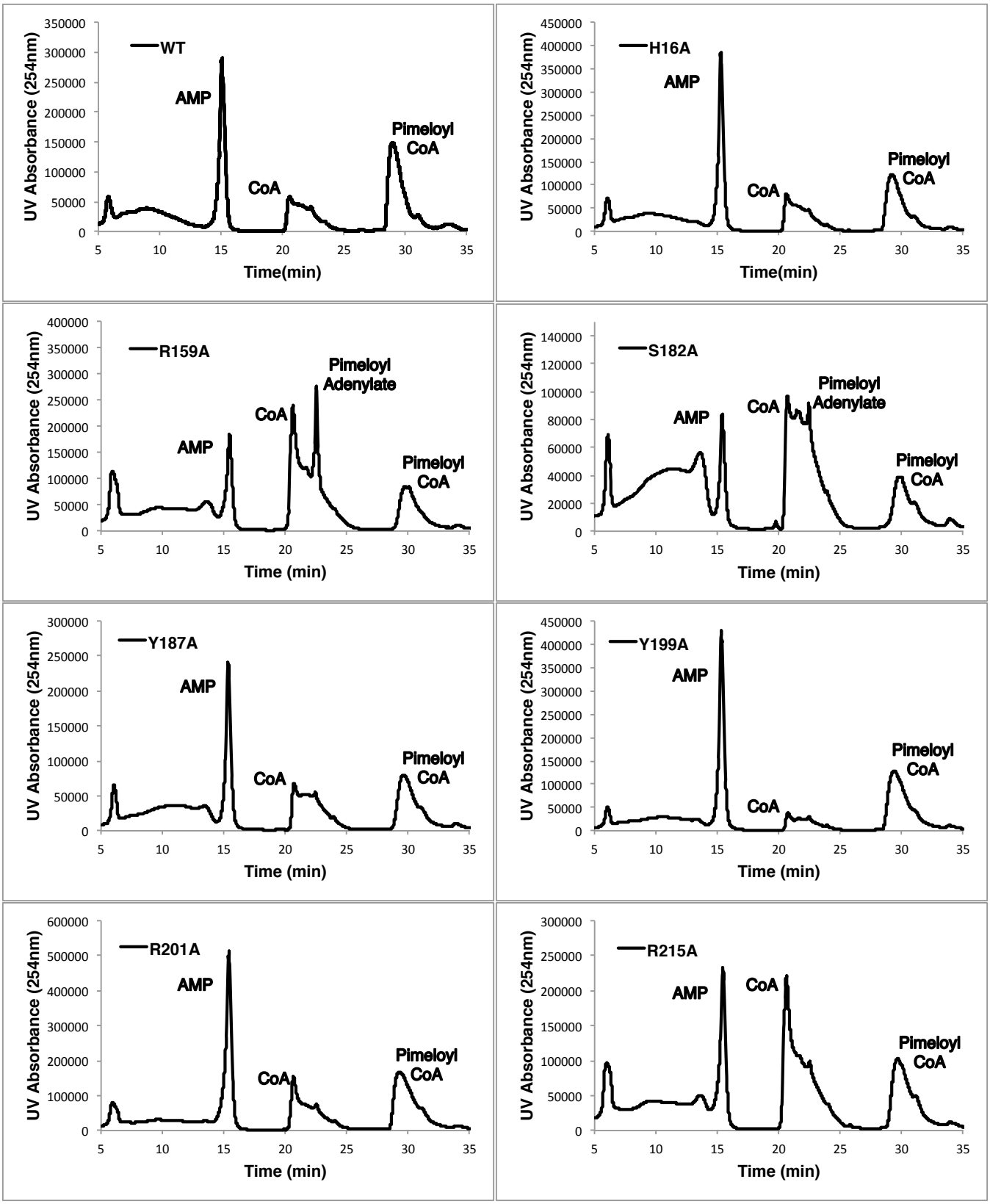

Supplementary Figure 8. Pimelic acid reactions with wild type (WT) and mutant BioW proteins. HPLC traces (absorbance at 254 nM) for reactions of wild-type and mutant AaBioW proteins with pimelic acid as the substrate. All products were confirmed by mass spectrometry.

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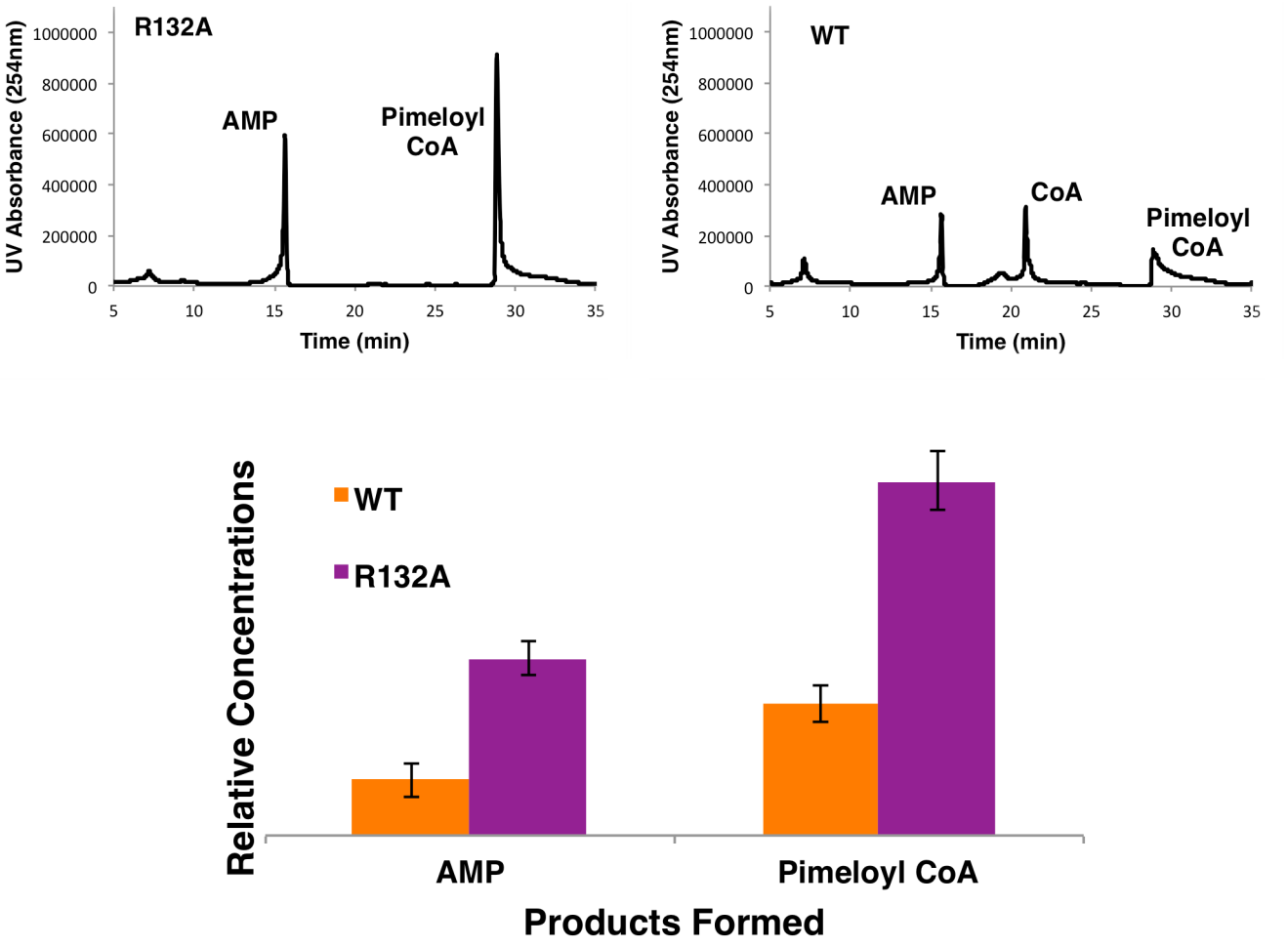

Supplementary Figure 9. Activity of the Arg132→Ala mutant with pimelate. HPLC traces (absorbance at 254 nM) for wild-type AaBioW (top left) and the Arg132→Ala (top right) mutant with pimelate. The bottom panel shows the relative amounts of both AMP and pimeloyl-CoA product formed for both enzymes. Reactions were run on buffer containing 20 mM HEPES, 100 mM NaCl, 0.2 mM DTT, 2 mM MgCl2, 1 mM ATP, 1 mM pimelate, 0.5 mM CoA and 10 μM protein for 4 hours at 50°C.

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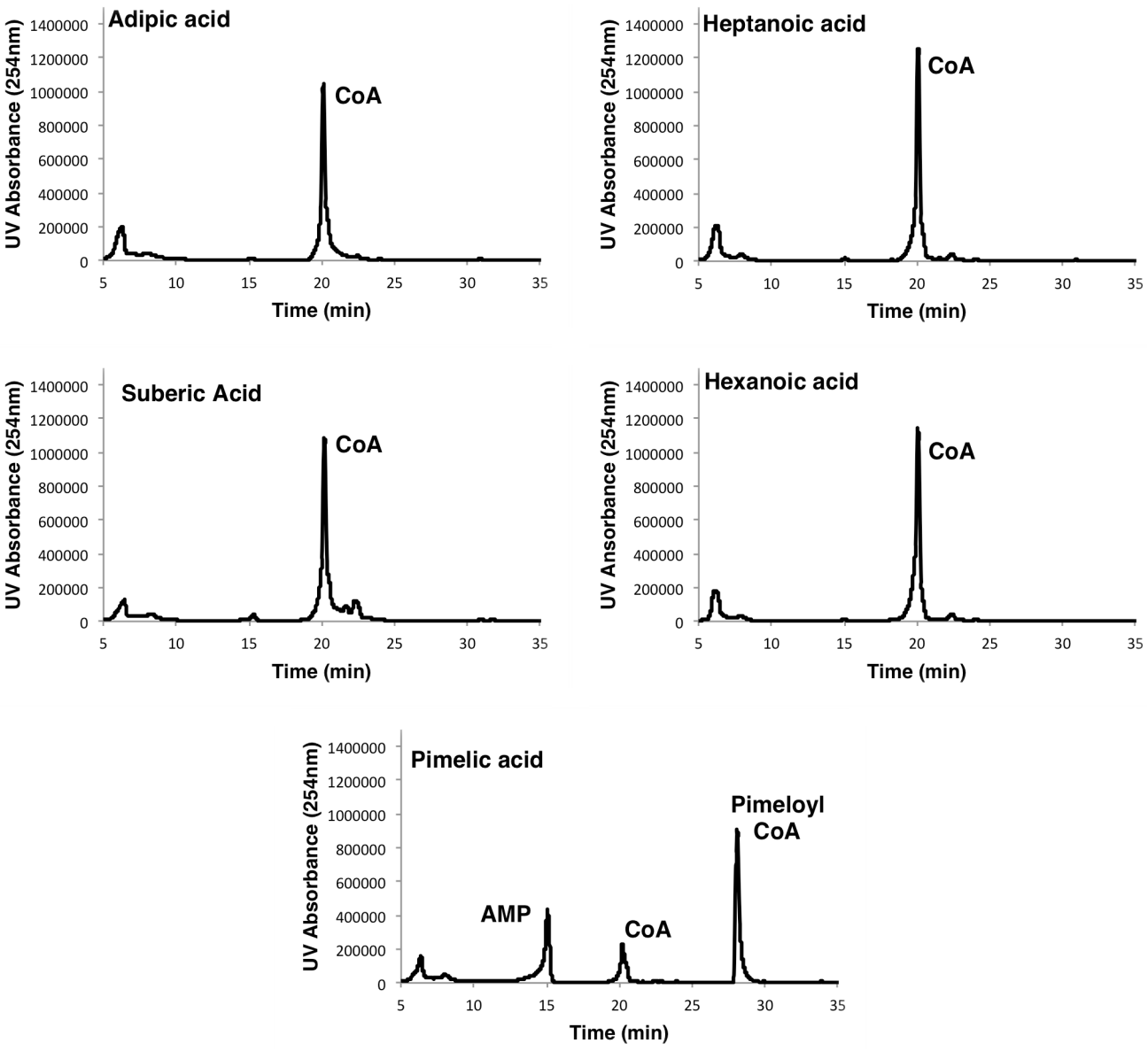

Supplementary Figure 10. Arg201→Ala mutant with substrates. HPLC traces (absorbance at 254 nM) traces for AaBioW Arg201→Ala mutant with different mono- and di-carboxylate substrates.

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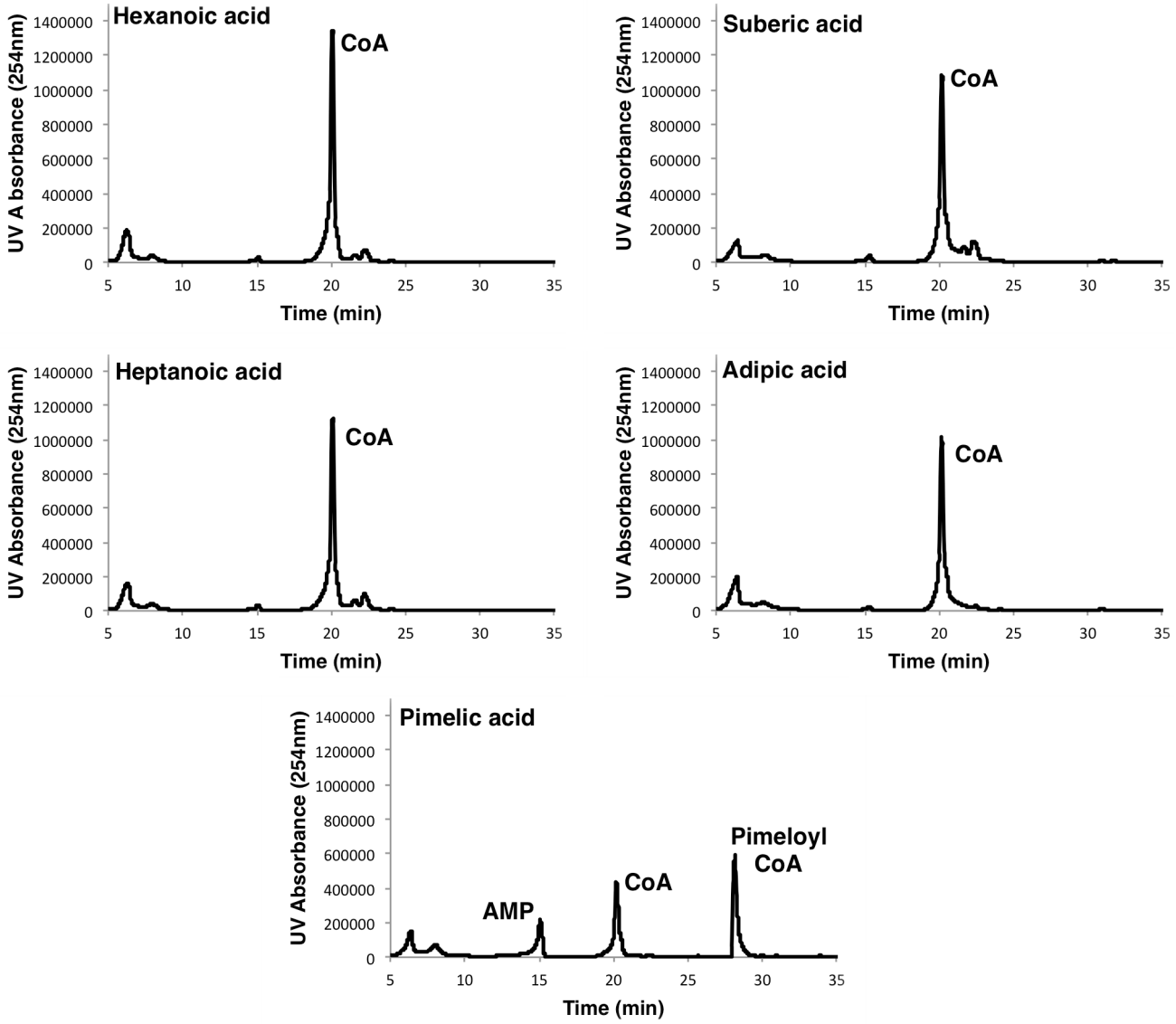

Supplementary Figure 11. Arg215→Ala mutant with substrates. HPLC traces (absorbance at 254 nM) traces for AaBioW Arg215→Ala mutant with different mono- and di-carboxylate substrates.

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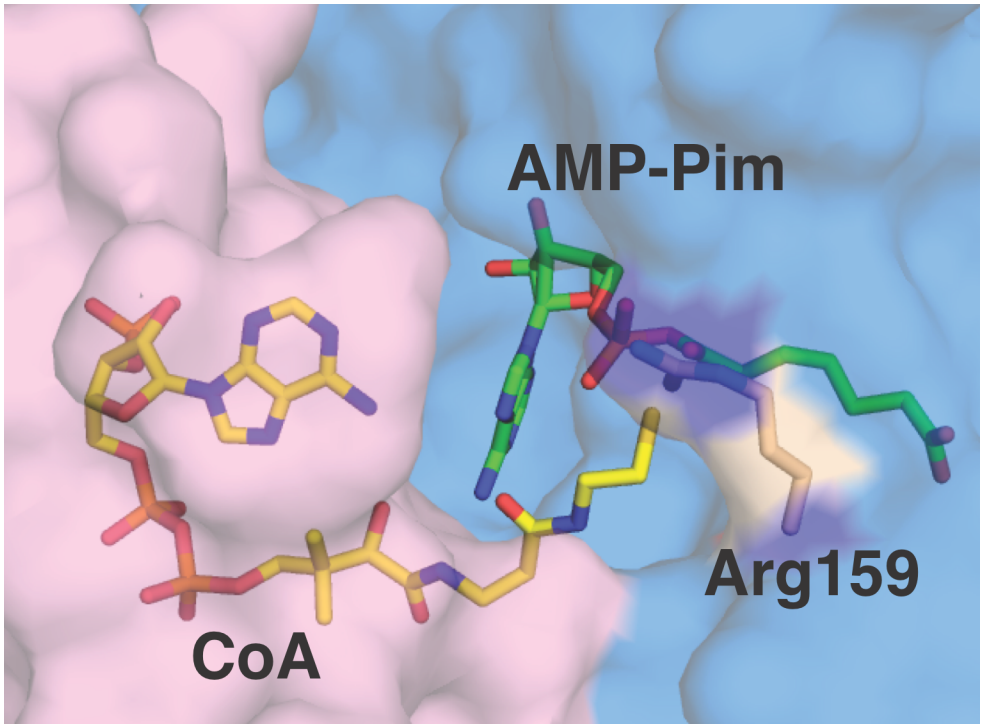

Supplementary Figure 12: **Model of thioester formation conformation**. Surface representation of the AaBioW active site based on the model from Figure5B. The P_{II} and the Rossmann fold domains are shown as pink and blue surfaces, respectively, and CoA (yellow) and pimeloyl-AMP (green) are shown as sticks.

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