Supporting Information Available

Chemoenzymatic Approaches for Streamlined Detection of Active Site Modifications on Thiotemplate Assembly Lines Using Mass Spectrometry

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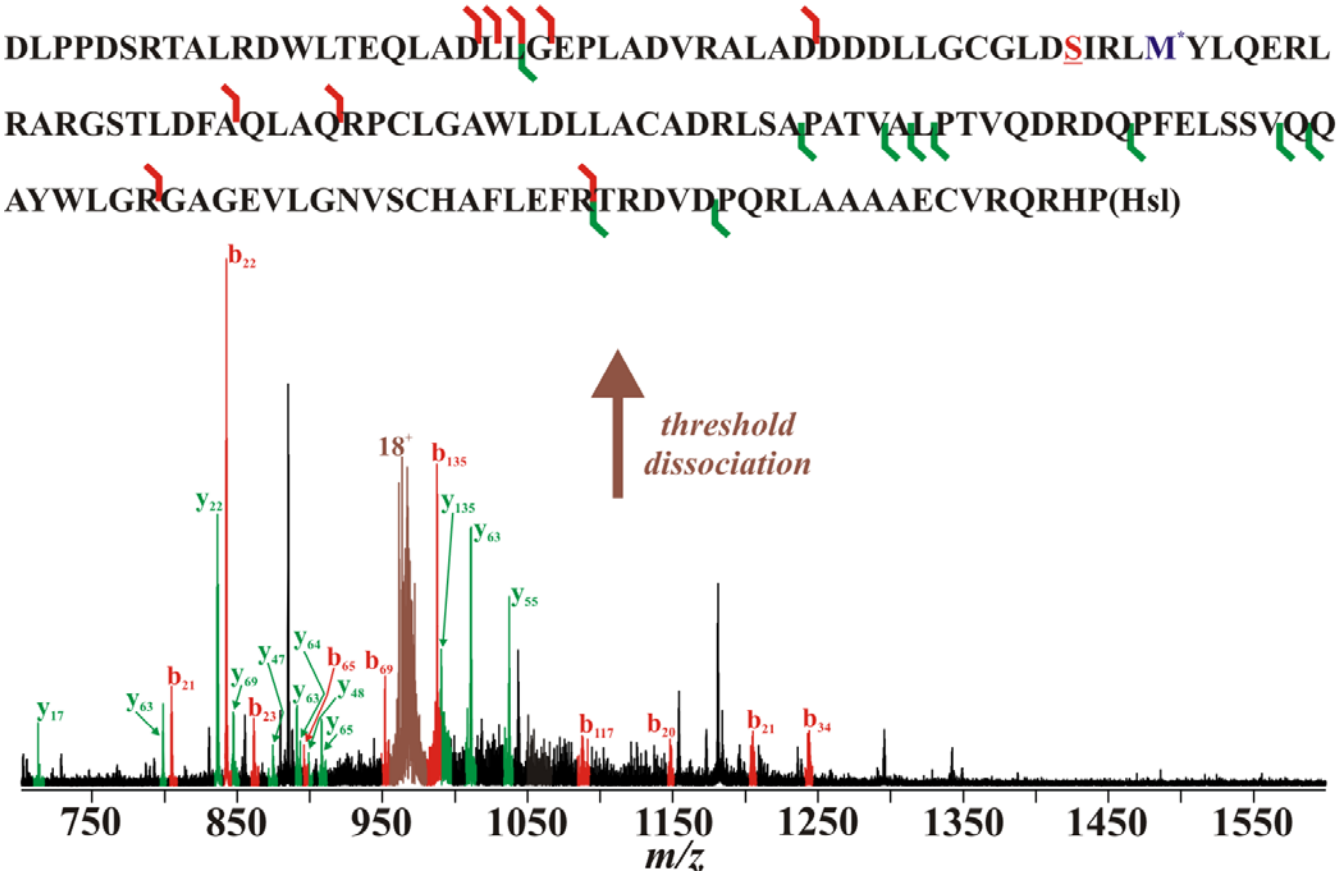
Synthesis and Characterization of Coenzyme A Analogues

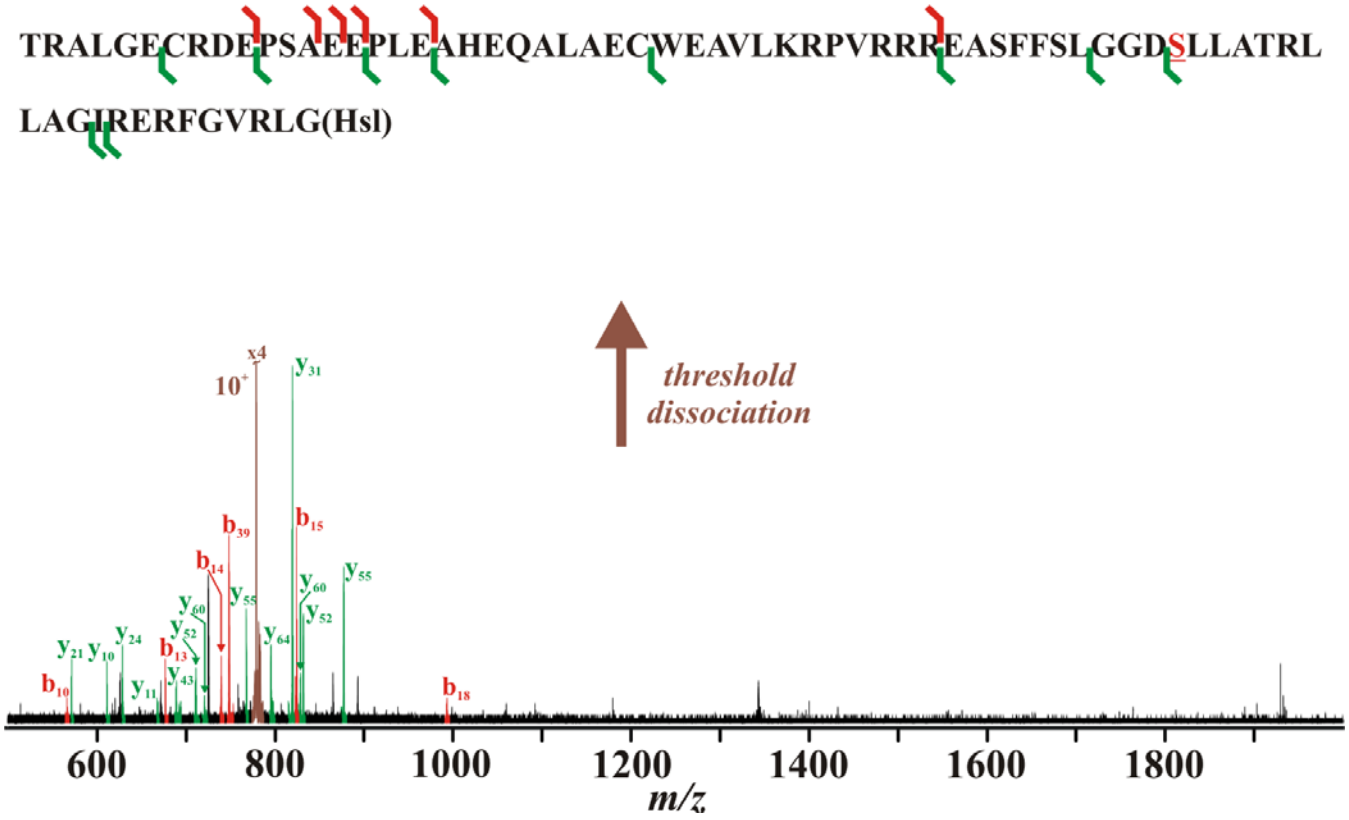
Synthesis of biotin-maleimidyl-*S*-CoA was accomplished according to the protocols described in references 22 and 25. To a solution of biotin maleimide (Pierce) (10 mg, 0.019 mmol) in 300 µL DMSO, coenzyme A (Sigma) (18.2 mg, 0.023 mmol) in 2 mL 50 mM MES acetate pH 6.0 was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then purified by preparative HPLC on a reversed-phase C18 column with a gradient of 0-60% acetonitrile in 0.1% TFA/water over 35 min. The yield was near 100% as judged by HPLC. The purified compound was lyophilized and the identity was confirmed by LC-MS operating in the positive ion mode: calc'd for $C_{44}H_{71}N_{12}O_{23}P_3S_2$: 1293.2 observed: 1292.8

Synthesis of pyrene-maleimidyl-*S*-CoA was accomplished by combining coenzyme A (Sigma) and pyrene-maleimide (Molecular Probes) in a 1:1 molar ratio in 2 mL of 100 mM MES pH 6. The reaction proceeded in the dark at room temperature for 30 min. Unreacted dye was removed by extracting 3x into EtOAc. The remaining material was divided into aliquots and stored at -80 ^{o}C. The yield was near 100% as judged by HPLC (see biotin-maleimidyl-*S*-CoA purification protocol above). The purified compound was characterized using MALDI-TOF operating in the negative ion mode and observed with lithium adducts, likely from the coenzyme A stock: calc'd for $C_{41}H_{43}LiN_8O_{18}P_3S$: 1067.8 observed: 1067.7

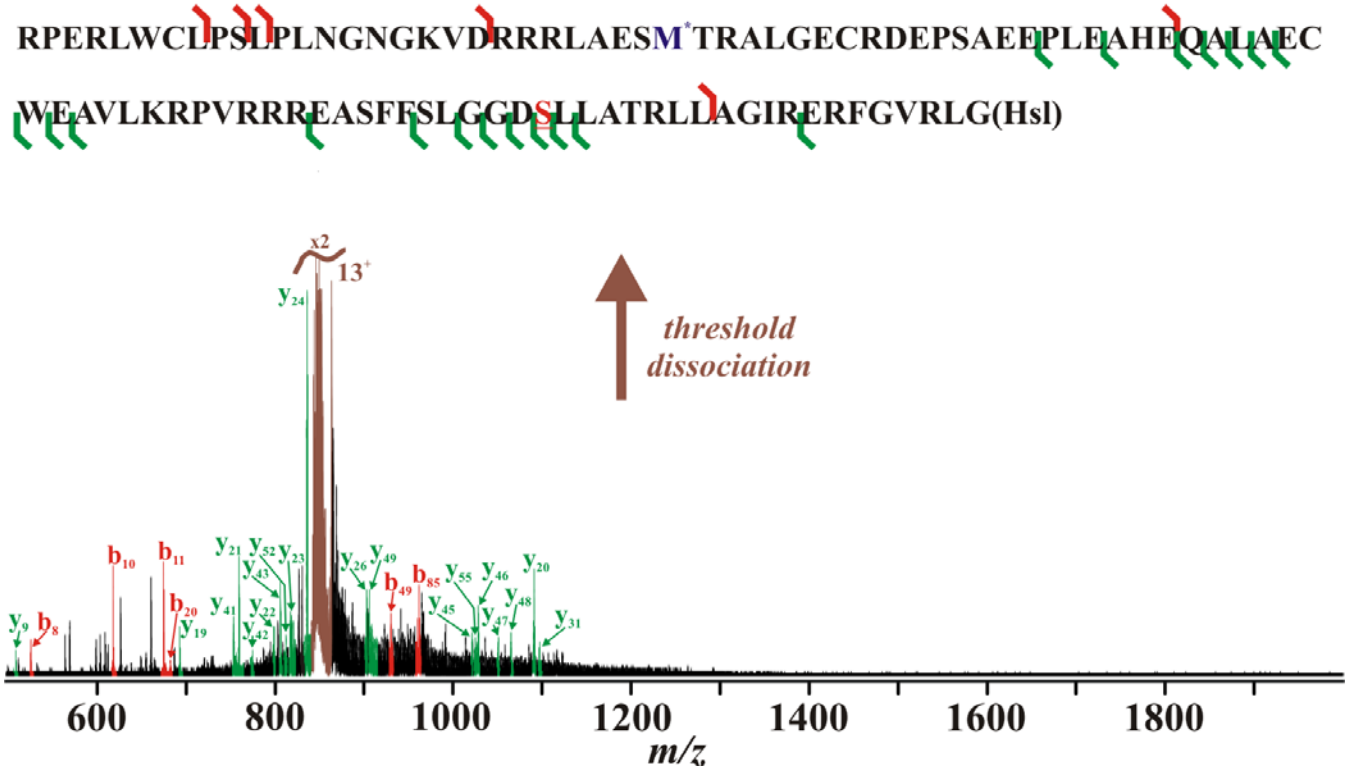
Synthesis of BODIPY-FL-N-(2-aminoethyl)-maleimidyl-*S*-coenzyme A was accomplished as denoted in reference 22. Coenzyme A (2.5 mM) and BODIPY-FL-N-(2-aminoethyl)-maleimide (2.5 mM) were added to 100 mM MES pH 6 / 100 mM MgOAc pH 6 / 15% DMSO to a final volume of 2 mL. The mixture was vortexed, and then placed at 4 ^{o}C in the dark for 30 min. After an additional 10 min incubation at room temperature, the reaction was assessed by silica gel TLC (using 5:2:4 *n*-butanol:$C_2H_3O_2$:H_2O) and the yield was determined to be near 100%. The small amount of unreacted dye was removed by extracting 3x into EtOAc. The remaining material was divided into 20 µL aliquots and frozen at -80 ^{o}C. The compound was characterized using fast atom bombardment (FAB) mass spectrometry in the negative ion mode: calc'd for $C_{41}H_{52}BN_{11}O_{19}F_2P_3S$: 1176.7 observed: 1176.5



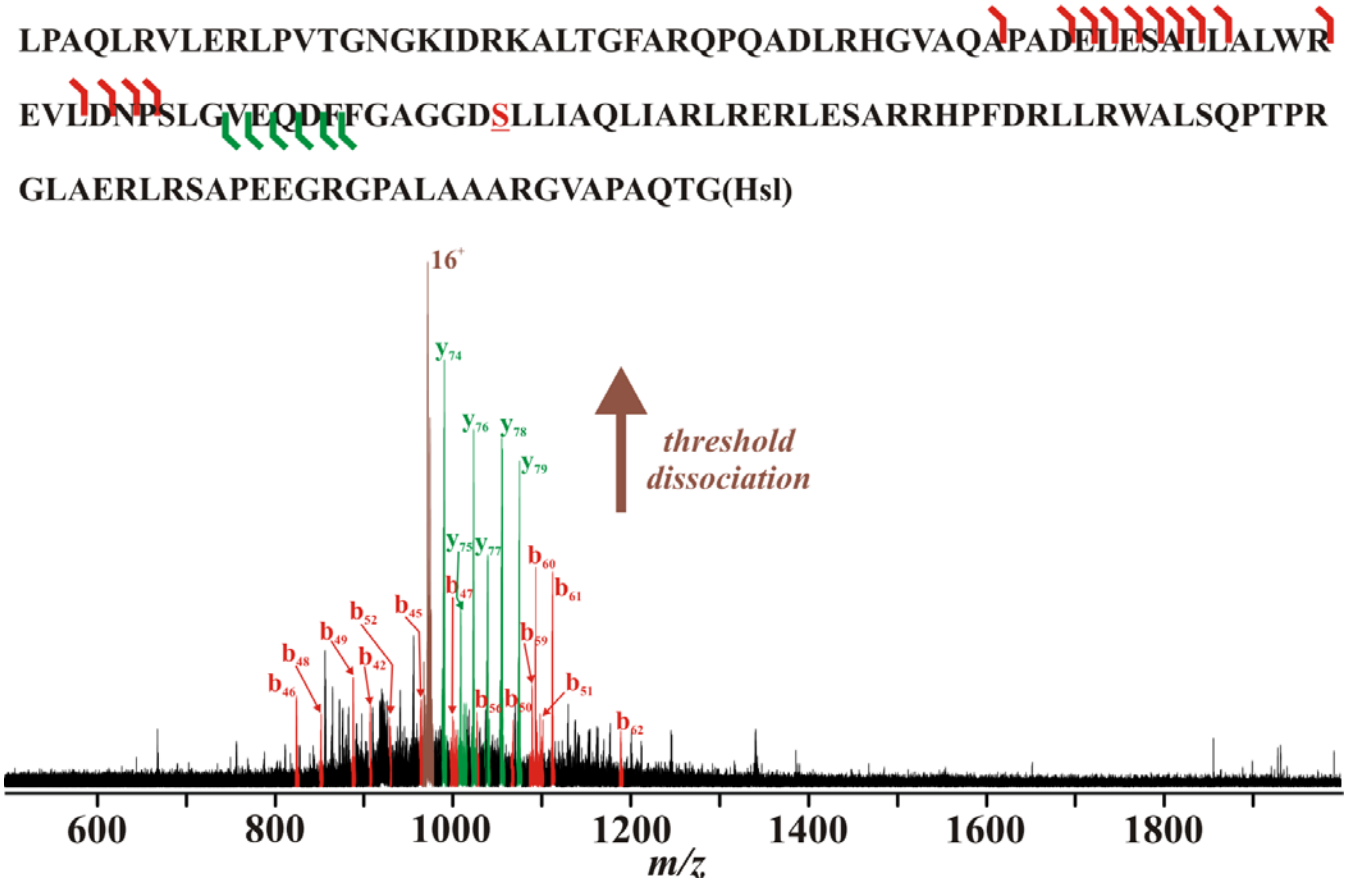
Supporting Figure 1: Infrared photodissociation of the unexhaustive CNBr peptide harboring the active site serine of the PchE ArCP thiolation domain. The active site serine is denoted in underlined red bold text in the sequence above. The (*) on the methionine indicates a site of oxidation. The notation (Hsl) denotes a homoserine lactone. Tandem mass spectrometry allowed for the observation of 9 b-ions and 10 y-ions, with 1 four-amino acid sequence tag (b_{20} - b_{23}).



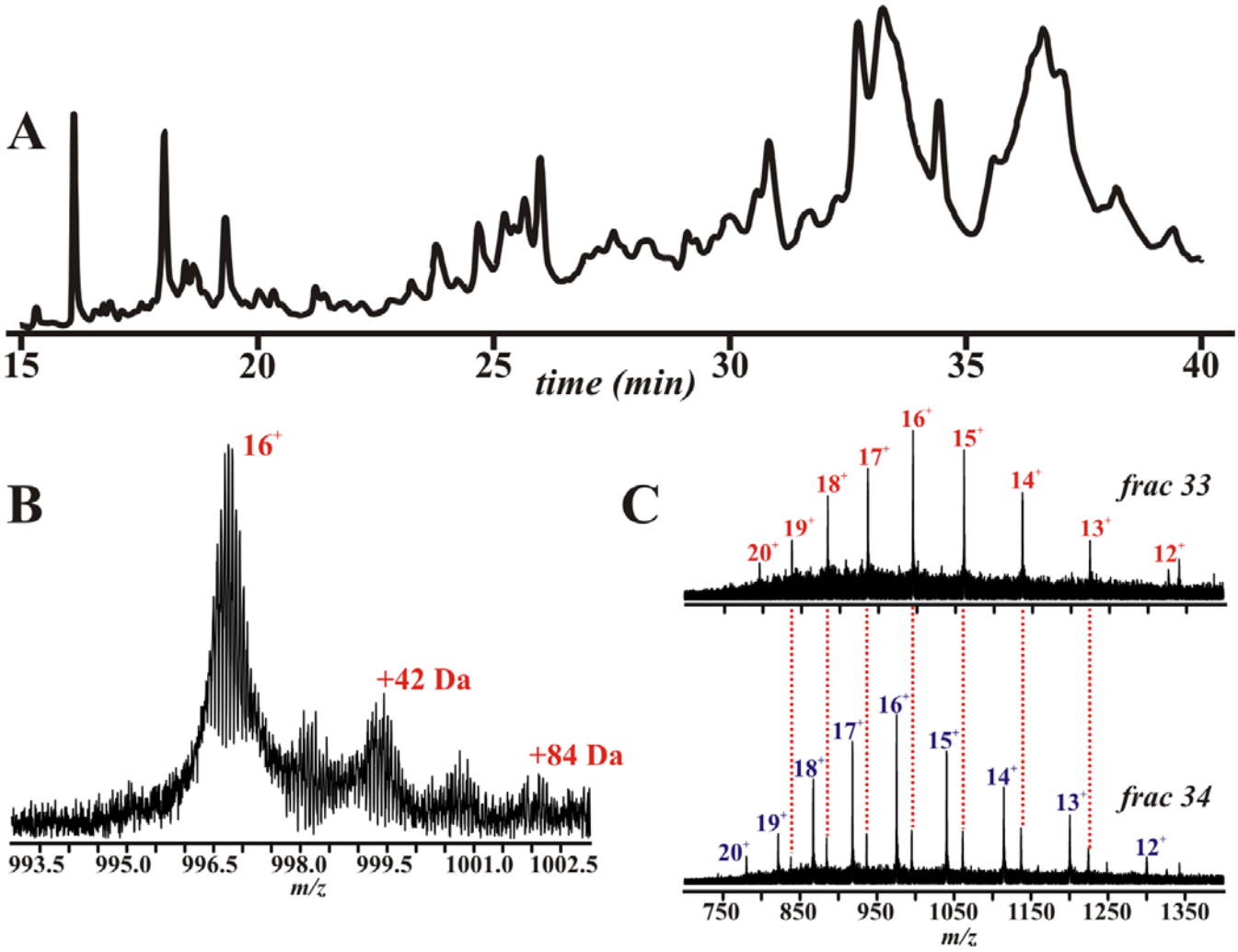
Supporting Figure 2: Infrared photodissociation of the exhaustive CNBr peptide harboring the active site serine of the PchE PCP1 thiolation domain. The active site serine is denoted in underlined red bold text in the sequence above. The (Hsl) denotes a homoserine lactone. Tandem mass spectrometry allowed for the observation of 6 b-ions and 10 y-ions, with 1 three-amino acid sequence tag (b_{13} - b_{15}).



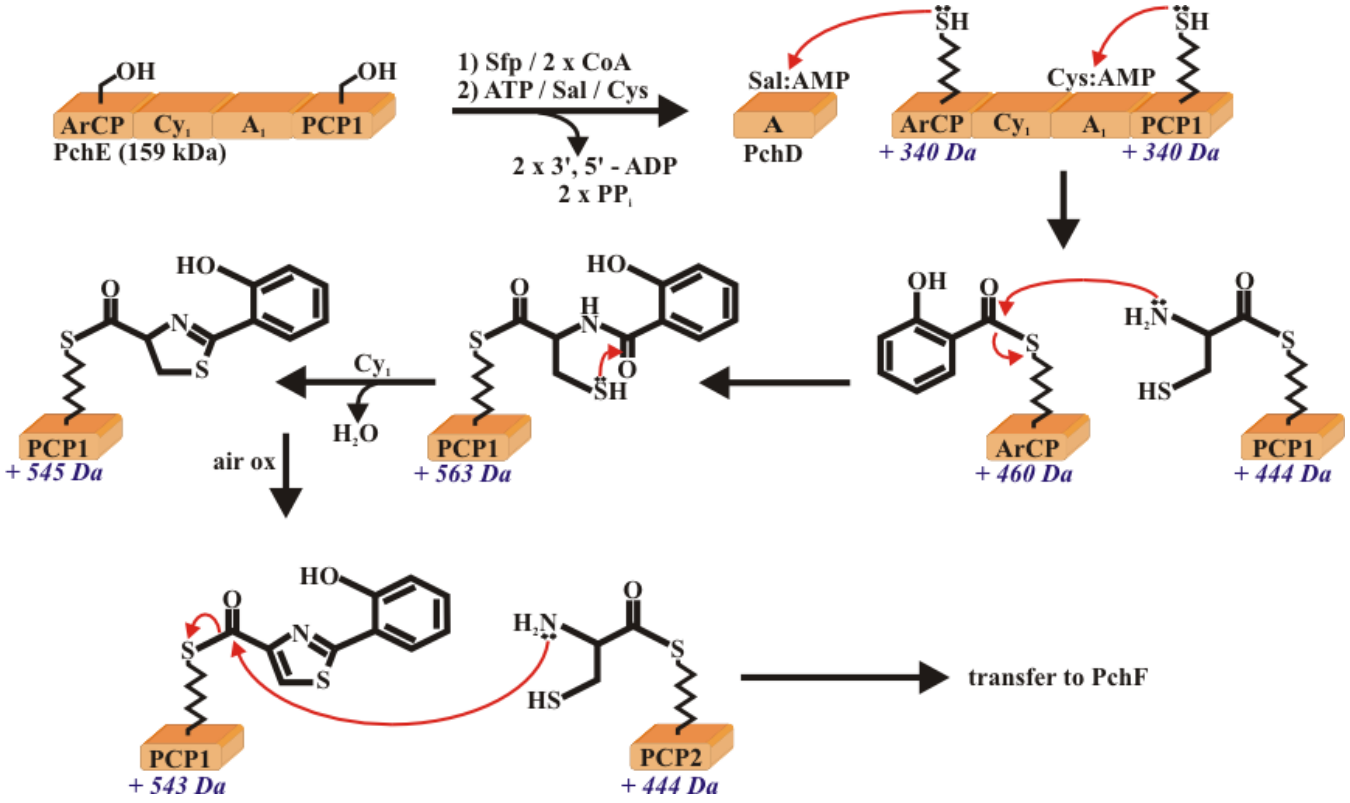
Supporting Figure 3: Infrared photodissociation of the unexhaustive CNBr peptide harboring the active site serine of the PchE PCP1 thiolation domain. The active site serine is denoted in underlined red bold text in the sequence above. The (Hsl) denotes a homoserine lactone. The (*) indicates an oxidized methionine. Tandem mass spectrometry allowed for the observation of 6 b-ions and 19 y-ions, with 1 six-amino acid sequence tag (y_{19} - y_{24}).



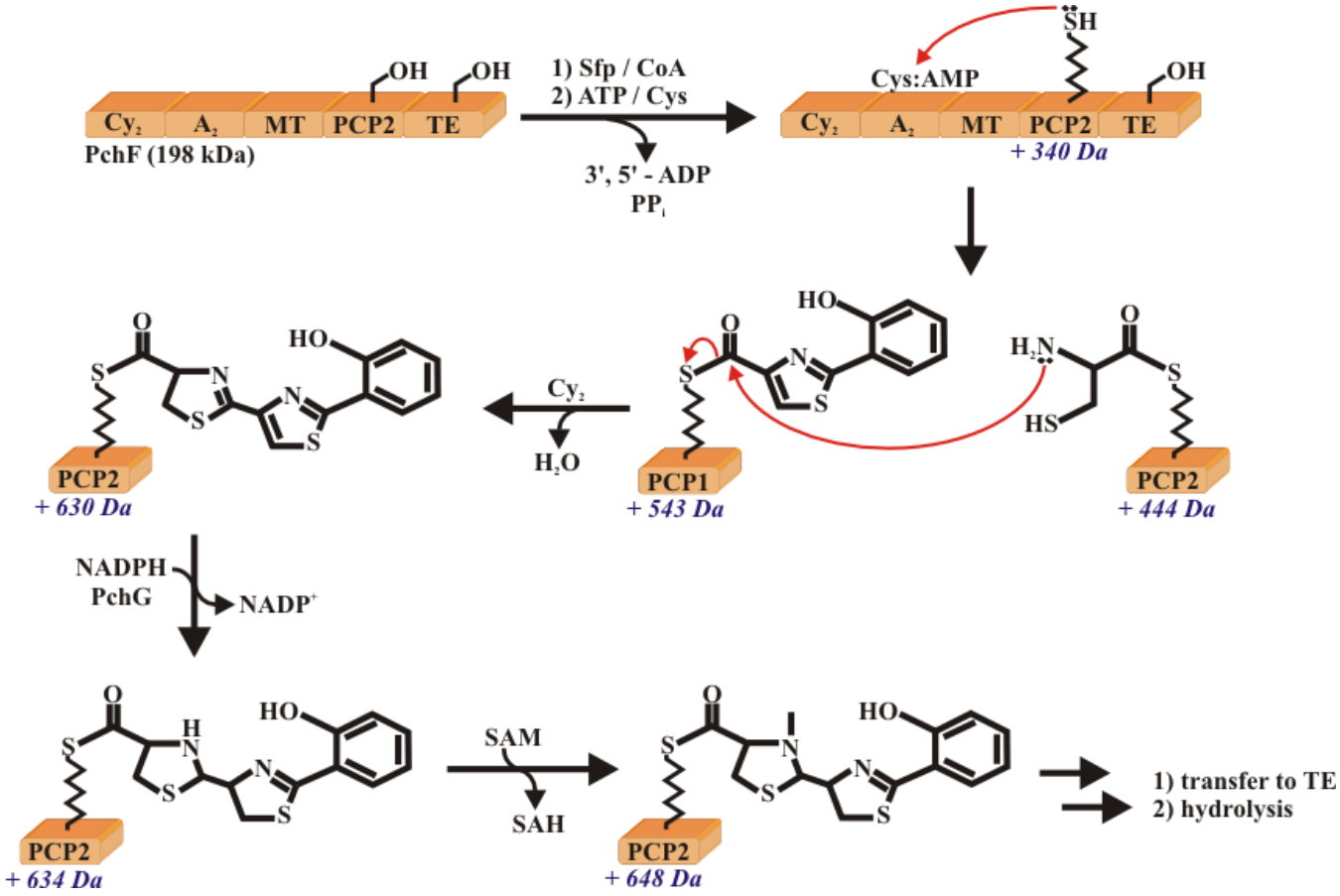
Supporting Figure 4: Infrared photodissociation of the exhaustive CNBr peptide harboring the active site serine of the PchF PCP2 thiolation domain. The active site serine is denoted in underlined red bold text in the sequence above. The (Hsl) denotes a homoserine lactone. Tandem mass spectrometry allowed for the observation of 14 b-ions and 6 y-ions, with 1 eight-amino acid sequence tag (b_{45} - b_{52}).



Supporting Figure 5: Loading PchF with CoA, acetyl-*S*-CoA and acetoacetyl-*S*-CoA in equimolar concentrations. (A) The UV trace at 220 nm of the C4 reversed phase separation. (B) The mass shift profile for the exhaustive PCP2 active site peptide. Only a +42 Da mass shift was observed at low abundance relative to the holo form. (C) Digestion of the apo-form PchF. The top panel represents chromatographic fraction #33, and consists solely of holo-PCP2. The bottom panel represents chromatographic fraction #34, and consists of both apo and holo forms. These data demonstrate that PchF undergoes *in vivo* phosphopantetheinylation during expression, which accounts for the low incorporation of the short acyl-chain CoA's.



Supporting Figure 6: Mechanism of intermediate formation on PchE. After priming the enzyme with phosphopantetheinyl cofactors, PchE and PchD activate and acylate salicylic acid onto the ArCP domain and L-cysteine onto the PCP1 domain. The Cy_1 domain subsequently catalyzes cyclodehydration to the HPT-*S*-PCP1 complex intermediate prior to transfer to PchF. The mass shifts induced at each step are indicated.



Supporting Figure 7: Mechanism of pyochelin formation on PchF. After successful phosphopantetheinylation and acylation with L-cysteine, the Cy_2 domain catalyzes condensation and cyclodehydration of the HPT-*S*-PCP1 moiety onto the L-cysteinyl-*S*-PCP2, creating the HPTT-*S*-PCP2 intermediate. PchG subsequently reduces the proximal thiazoline ring to its thiazolidine oxidation state, which can prevent formation of the oxazole on the distal thiazoline ring. The thiazolidine ring is subsequently N-methylated by the MT domain and SAM prior to transfer to the TE domain and hydrolysis. The mass shifts for each step are denoted.