Supporting Information

Investigating the Stuffed Methyltransferase Domain of PchF in Pyochelin Biosynthesis

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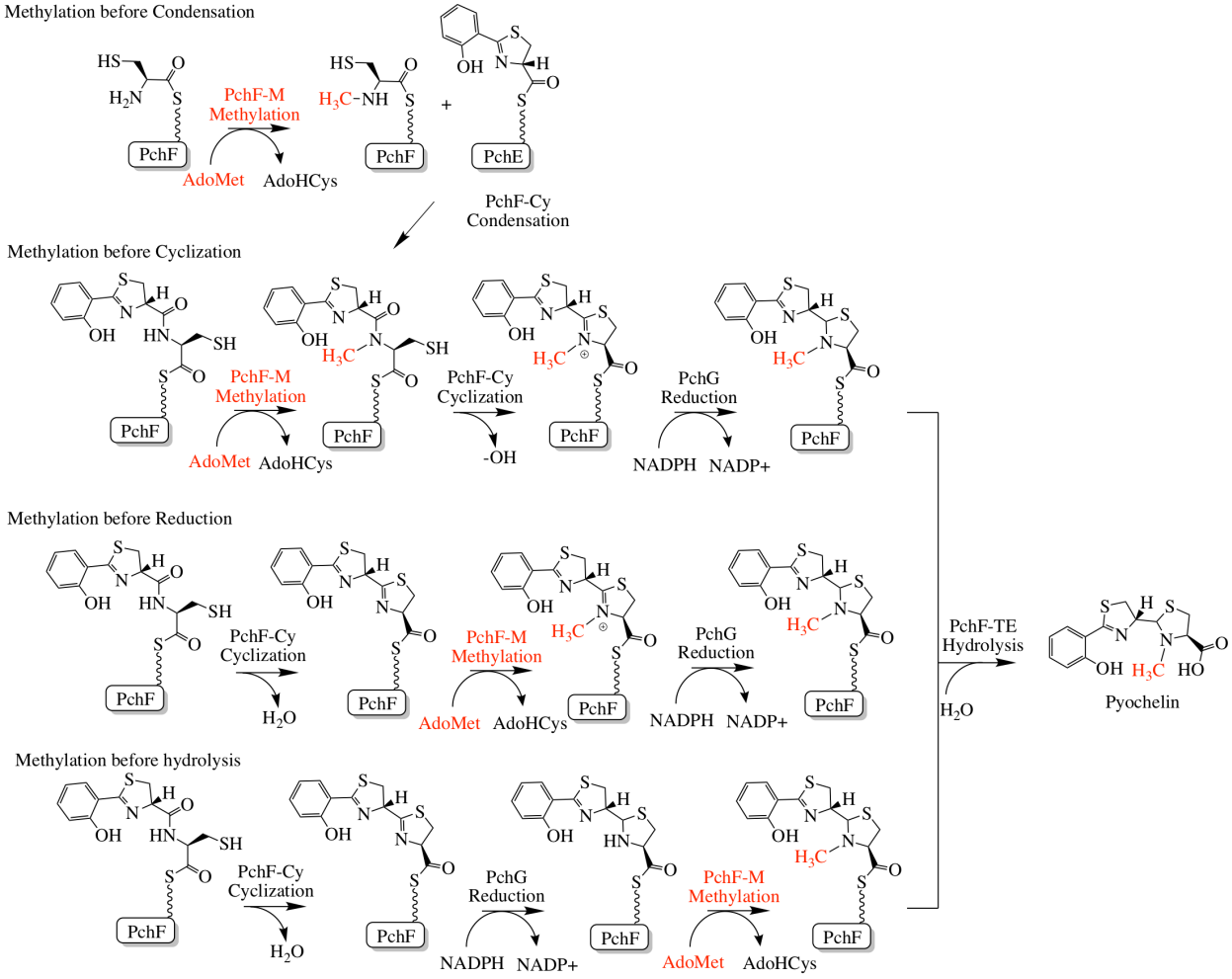

Figure S1: Possible biosynthetic strategies for PchF. AdoMet dependent methylation is proposed to take place as the last tailoring step before hydrolysis and the release of the mature peptide; however previous evidence is contradictory. Four potential biosynthetic pathways are shown for methyl transfer: before condensation, before cyclization, before reduction, or directly before hydrolysis. The methyltransferase chemistry is highlighted in red.

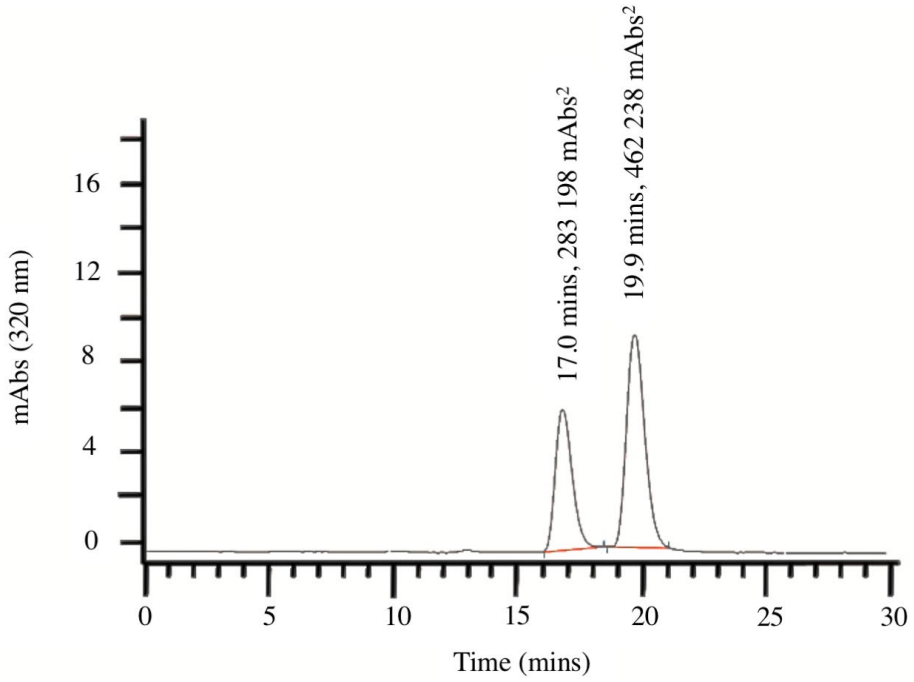

Figure S2: Separation of $HPT_{ox}T_{red}$-CO_2Et diastereomers on CHIRALCEL OD-H column. Separation conditions: 1 mL/min, Hexane:Isopropanol (90:10), absorbance monitored at 320 nm. $HPT_{ox}T_{red}$-CO_2Et exists as a diastereomer (38:62) eluting at 17.0 mins and 19.9 mins.

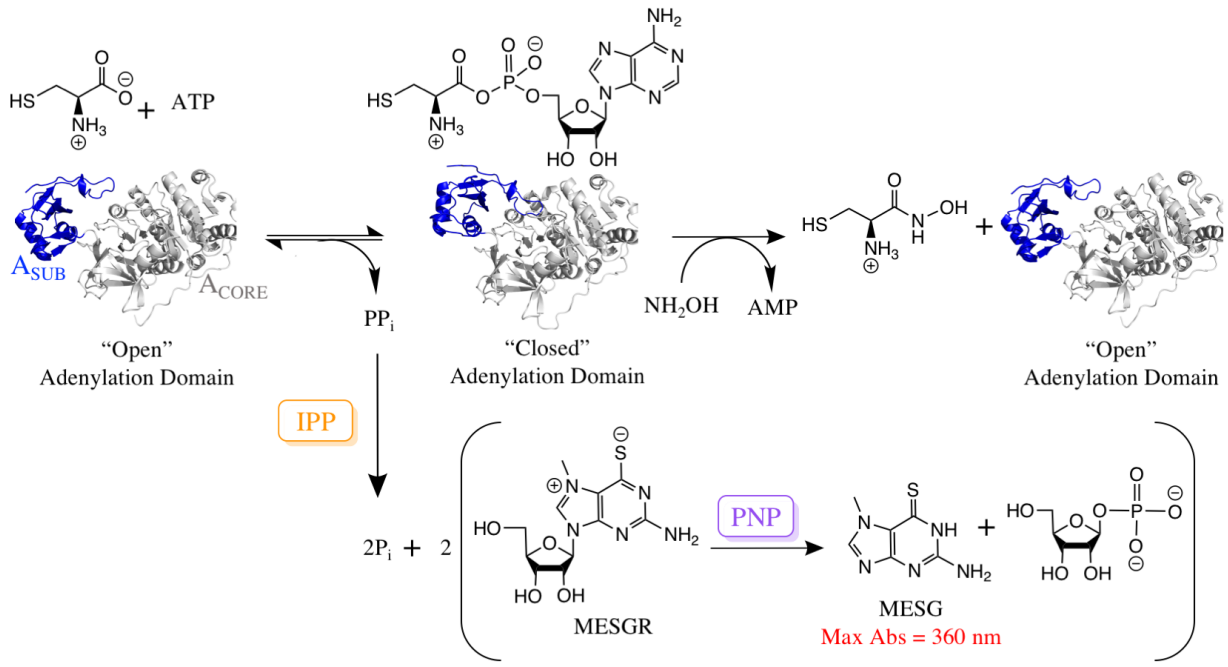

Figure S3: Adenylation assay (based on the assay designed by the Aldrich laboratory[1, 2]). The adenylation domain of PchF is specific for L-cysteine, forming an aminoacyl-AMP bond and releasing pyrophosphate (PP_i). The native protein is post-translationally modified with coenzyme A, generating a 4'phosphopantetheinyl (Ppant) on a conserved serine in the thiolation (T) domain. The high energy aminoacyl-adenylate bond is used to link the cysteine substrate to the Ppant arm of the T-domain. In this assay, the recombinant PchF variants are not post-translationally modified with the Ppant stalling the enzyme after the formation of the aminoacyl-AMP intermediate, with the adenylation domain trapped in the "closed" conformation. Opening of the active site and release of pyrophosphate (PP_i) can be promoted by addition of the nucleophile surrogate hydroxylamine, allowing the adenylation activity to enter the steady state. Inorganic pyrophosphatase (IPP) converts one pyrophosphate (PP_i) molecule to two inorganic phosphate (P_i) molecules. Purine nucleoside phosphorylase (PNP) catalyzes the phosphorylation of 7-methylthioguanosine (MesGR), generating ribose 1-phosphate and the chromogenic 7-methylthioguanine (MesG), which is monitored at 360 nm (bottom). Two molecules of MESG are generated for every one aminoacyl-AMP formation. Structures of LgrA[3] from gramicidin biosynthesis are used to illustrate the open (PDB: 5ES5) and closed (PDB: 5ES5) A-domains. The A_{core} subdomain is gray and the smaller C-terminal A_{sub} is blue.

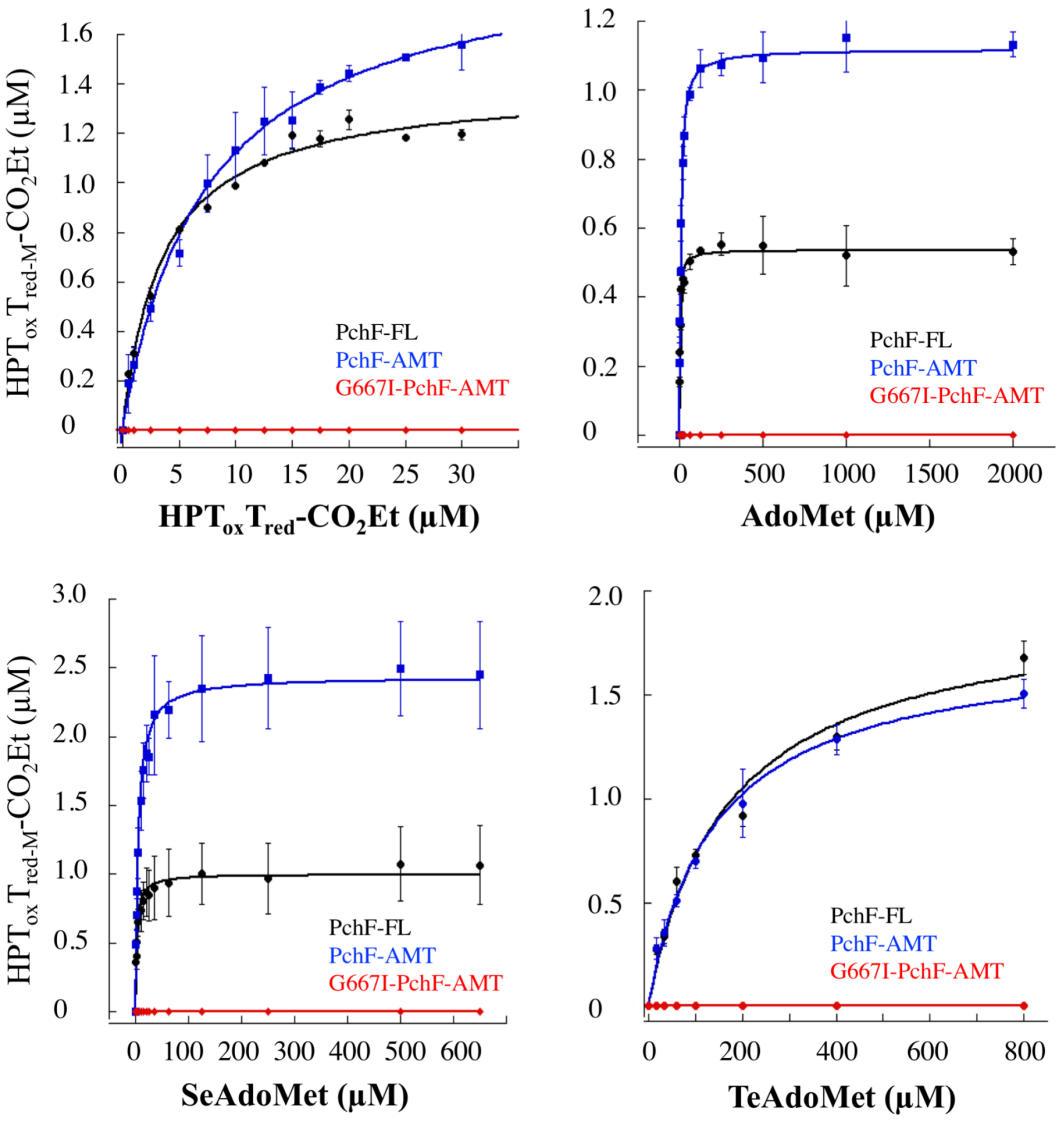

Figure S4: Methylated Product Formation Assays. Methyltransferase assay of PchF-FL, PchF-AMT, and G667I-PchF-AMT. Michaelis-Menten steady-state kinetic plots of (A) $HPT_{ox}T_{red}$-CO_2Et, (B) AdoMet, (C) SeAdoMet, (D) TeAdoMet.

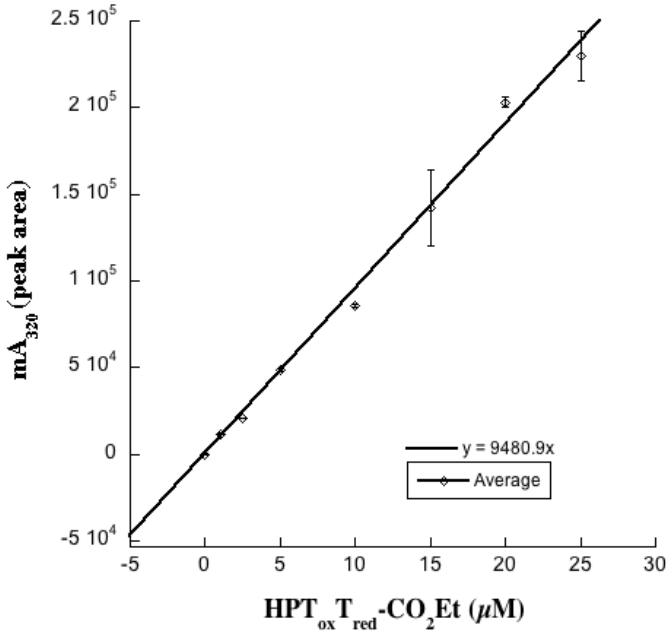

Figure S5: $HPT_{ox}T_{red\text{-}M}$-CO_2Et standard curve. $HPT_{ox}T_{red\text{-}M}$-CO_2Et standard curve was produced by running methyltransferase assays to completion at concentrations varying from 0-25 μM $HPT_{ox}T_{red}$-CO_2Et. Reactions were performed in triplicate. The slope, 9480 mAU/μM, was used to convert mAU to concentration of $HPT_{ox}T_{red\text{-}M}$-CO_2Et.

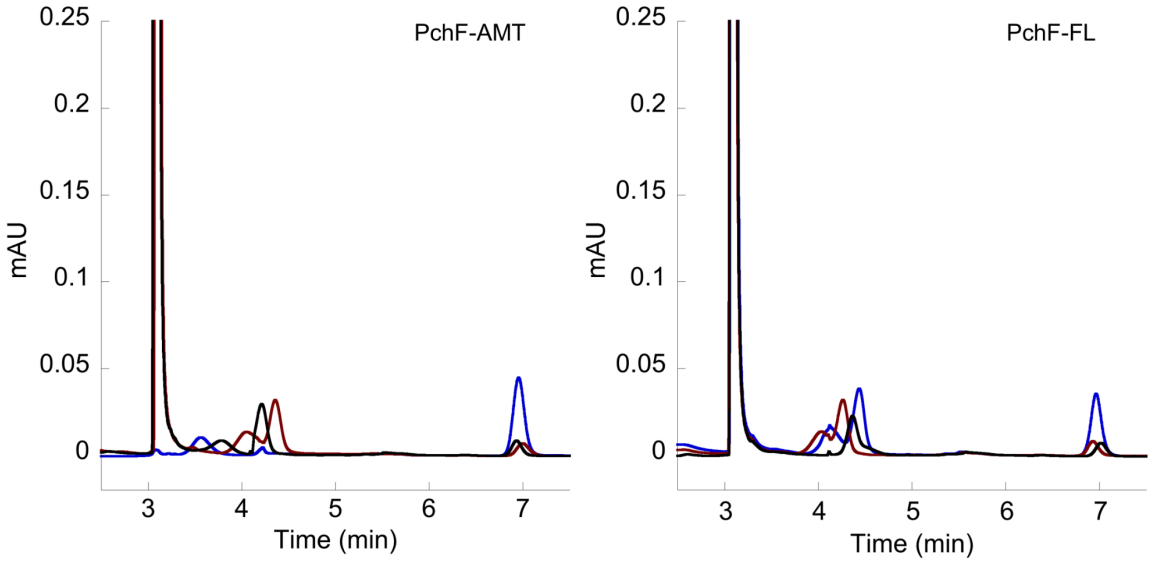

Figure S6: S-adenosylmethionine Formation Assay UPLC traces. UPLC traces of AdoHCys formation (7.0 mins) for PchF-AMT and PchF-FL reactions with no substrate (black), 3 mM L-Cys-OEt (red), and 30 μM $HPT_{ox}T_{red}$-CO_2Et (blue).

[1] Duckworth, B. P., Wilson, D. J., and Aldrich, C. C. (2016) Measurement of Nonribosomal Peptide Synthetase Adenylation Domain Activity Using a Continuous Hydroxylamine Release Assay, *Methods Mol Biol 1401*, 53-61.
[2] Wilson, D. J., and Aldrich, C. C. (2010) A continuous kinetic assay for adenylation enzyme activity and inhibition, *Anal Biochem 404*, 56-63.
[3] Reimer, J. M., Aloise, M. N., Harrison, P. M., and Schmeing, T. M. (2016) Synthetic cycle of the initiation module of a formylating nonribosomal peptide synthetase, *Nature 529*, 239-242.