Exploring Pactamycin Biosynthesis

by
Jessica Roland

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in BioResource Research
(Honors Scholar)

Presented March 9, 2018
Commencement June 2018

AN ABSTRACT OF THE THESIS OF

Jessica Roland for the degree of Honors Baccalaureate of Science in BioResource Research presented on March 9, 2018. Title: Exploring Pactamycin Biosynthesis.

Abstract approved:_____

Taifo Mahmud

Pactamycin, produced by the soil bacterium *Streptomyces pactum*, is a cytotoxic antibiotic that belongs to the aminocyclitol family of natural products. This antibiotic has shown various forms of biological activity, including antibacterial, antifungal, antimalarial, and antitumor activities. Despite its outstanding biological activity, the mode of formation of its pharmacophore (the aminocyclitol unit) remains elusive. The PtmN (an oxidoreductase) and PtmA (an aminotransferase) proteins are hypothesized to be involved in the initial steps in the biosynthesis of the aminocyclitol unit. The *ptmA* and *ptmN* genes were cloned into the pRSET B expression vector and transferred into *Escherichia coli* BL21 pLysS. SDS-PAGE gel electrophoresis showed that the transformed *E. coli* strains were able to produce soluble PtmA or PtmN. Crude extracts of the bacterial cells containing soluble PtmA or PtmN proteins were tested for their enzymatic activity. HPLC was used to analyze the products. The results showed that a new compound was produced in the reaction containing PtmA and PtmN. However, the identity of the product has not been established. Further experimentation is needed to confirm the activity of the enzyme.

Key Words: Pactamycin, *Streptomyces pactum,* Biosynthesis

Corresponding e-mail address: rolandje@oregonstate.edu

Exploring Pactamycin Biosynthesis

by
Jessica Roland

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in BioResouce Research
(Honors Scholar)

Presented March 9, 2018
Commencement June 2018

Honors Baccalaureate of Science in BioResource Research project of Jessica Roland presented on March 9, 2018.

APPROVED:

_____

Taifo Mahmud, representing Department of Pharmaceutical Sciences

_____

Dr. Indira Rajagopal, representing Department of Biochemistry & Biophysics

_____

Dr. Kevin Gable, representing Department of Chemistry

_____

Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

_____

Jessica Roland, Author

Acknowledgement

I would like to thank Dr. Taifo Mahmud for agreeing to let me work in his lab. Despite my minimal scientific knowledge when I first entered his lab, he has practice patience and persistence while helping me to grow into a better scientist. Dr. Mahmud is passionate about teaching his students and has taught me the incredible importance of truly understanding a concept and the integrity of scientific research. I am also thankful for post doctorate student Dr. Mostafa Abugrain for consistently answering my questions about research and for teaching me the crucial wet lab techniques for this project. Dr. Mostafa Abugrain gave me the foundation I needed in order to take part in the exploration of pactamycin biosynthesis. Additionally, I am grateful for the assistance provided by the other members of the lab, including Abdullah Alanzi, Auday Eida, Andrew Osborn, Corey Brumsted, Cem Celik, Hattan Alharbi, Ananiya Demessie, and Poojitha Jujjuri. Furthermore, I largely appreciate Dr. Kate Field and secondary mentors Dr. Kevin Gable and Dr. Indira Rajagopal for serving on the committee and supporting my endeavors regarding this project. I would also like to thank my advisor Wanda Crannell for her support and academic advice that has helped me to succeed scholastically. Lastly, I would like to thank my friends and family for their love and encouragement.

Abstract

Pactamycin, produced by the soil bacterium *Streptomyces pactum*, is a cytotoxic antibiotic that belongs to the aminocyclitol family of natural products. This antibiotic has shown various forms of biological activity, including antibacterial, antifungal, antimalarial, and antitumor activities. Despite its outstanding biological activity, the mode of formation of its pharmacophore (the aminocyclitol unit) remains elusive. The PtmN (an oxidoreductase) and PtmA (an aminotransferase) proteins are hypothesized to be involved in the initial steps in the biosynthesis of the aminocyclitol unit. The *ptmA* and *ptmN* genes were cloned into the pRSET B expression vector and transferred into *Escherichia coli* BL21 pLysS. SDS-PAGE gel electrophoresis showed that the transformed *E. coli* strains were able to produce soluble PtmA or PtmN. Crude extracts of the bacterial cells containing soluble PtmA or PtmN proteins were tested for their enzymatic activity. HPLC was used to analyze the products. The results showed that a new compound was produced in the reaction containing PtmA and PtmN. However, the identity of the product has not been established. Further experimentation is needed to confirm the activity of the enzyme.

Introduction

For decades, pactamycin has intrigued scientists with its complex structure and potent cytotoxicity. Pactamycin consists of a center aminocyclopentitol ring, 3-aminoacetophenone, 6-methylsalicylate (6-MSA), and N,N-dimethylurea moieties.[1] This natural product belongs to the aminocyclitol family of natural products and originates from the soil bacterium *Streptomyces pactum (*Figure 1).[2] Pactamycin has demonstrated various forms of biological activity, including antibacterial, antifungal, antimalarial, and antitumor activities.[3] Recent studies have concluded that pactamycin terminates cell growth by inhibiting tRNA translocation and disrupting protein synthesis by selectively interacting with the E site of the 30S ribosomal subunit.[4] This cytotoxic antibiotic has been sought for use as a clinical drug, but has never been used because of its potent and broad spectrum toxicity. With the intention of reducing and channeling the cytotoxicity of pactamycin, the main research objective was to first understand the biosynthetic pathway to pactamycin, and then biosynthetically produce pactamycin analogues that could potentially be used for clinical treatment.

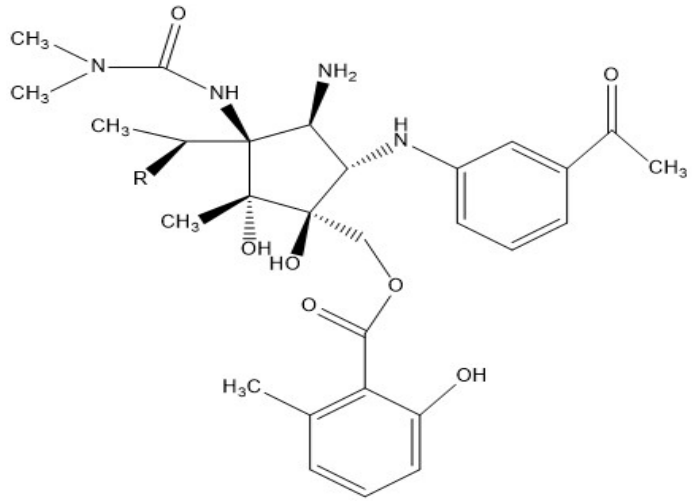


Figure 1: Chemical structure of pactamycin. It consists of a center aminocyclopentitol ring, 3-aminoacetophenone, 6-methylsalicylate (6-MSA), and N,N-dimethylurea moieties.

Synthetic organic chemistry, genetic engineering, and biosynthetic approaches have all been used in studying pactamycin. This has included the use of *S. pactum* mutants to produce a series of novel pactamycin analogues, e.g., TM-025 and TM-026.[5] Some of these analogues showed reduced cytotoxicity, less antibacterial activity, and increased selectivity against *Plasmodium falciparum*, the causative agent of Malaria.[5] Other pactamycin analogues have shown antitumor activity by inhibiting cell proliferation through inducing p53, a tumor suppressor.[6]

The pactamycin (*pct*) biosynthetic gene cluster was identified by sequencing an 86 kb continuous chromosomal DNA region of *S. pactum*.[7] Three metabolic pathways—the shikimate pathway, the amino sugar metabolic pathway, and the acetate pathway—are responsible for the formation of the 3-aminoacetophenone moiety, the aminocyclopentitol moiety, and the 6-methylsalicylic acid (6MSA), respectively, which make up the molecular structure of pactamycin.[8] While the precursor and the mode of formation of the 3-aminoacetophenone and the 6-methylsalicylic acid moieties are known, the mode of formation of the aminocyclopentitol moiety remains elusive.

A modified sugar precursor is hypothesized to play a role in the formation of the aminocyclopentitol moiety (Figure 2).[9] In this pathway, enzymes including PtmA (an aminotransferase), PtmB (a carbamoyltransferase), PtmN (an oxidoreductase), and PtmG (a deacetylase) are predicted to facilitate the modification of UDP-*N*-acetyl-glucosamine to a putative precursor of the aminocyclopentitol unit. Although the putative functions of PtmA, PtmB, PtmN, and PtmG can be predicted from their amino acid sequences, the order in which

the reactions are catalyzed remains unclear. It is postulated that the precursor *N*-acetylglucosamine is either in its nucleotidylated form (e.g., UDP-GlcNac, Figure 2) or is tethered to an acyl carrier protein (ACP) via a phosphorpantetheinyl moiety.[10]

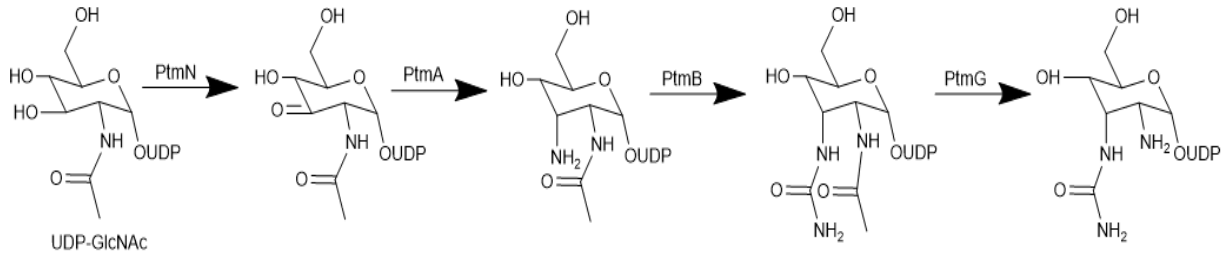


Figure 2. Proposed pathway to a modified sugar precursor used for the formation of the aminocyclopentitol moiety.

To test these possibilities, both UDP-GlcNAc and *N*-acetylglucosaminyl- 3-[3-aminophenyl]3-oxopropionyl *N*-acetylcysteamine (glycosylated β-Keto-SNAC, Figure 3), which mimics the ACP-bound substrate, were tested. It is hypothesized that the first step of the pathway involves oxidation of the sugar 3-OH, catalyzed by the PtmN enzyme. The oxidized product undergoes an aminotransferase reaction, catalyzed by the PtmA enzyme. The transaminated product undergoes a carbamoyl transferase reaction, catalyzed by the PtmB enzyme, where the amino group from the previous step connects to the incoming carbamoyl group. PtmG then hydrolyzes the acetyl group to give a free C-2 amine.

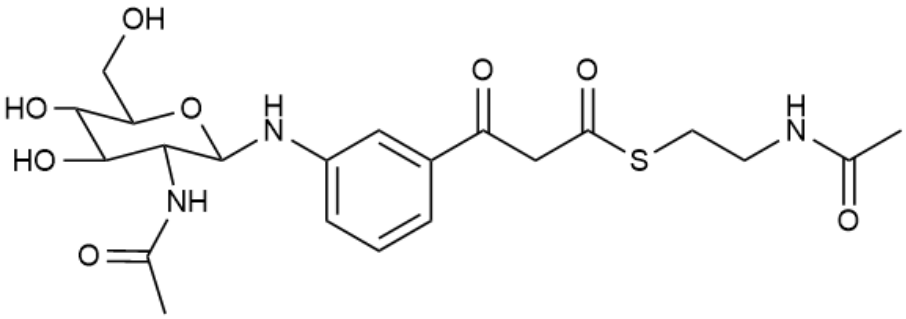


Figure 3. Chemical structure of *N*-acetylglucosaminly-3-[3-aminophenyl]3-oxopropionyl *N*-acetylcysteamine (glycosylated β-Keto-SNAC) used in the experiments.

The main objectives of this project were to produce soluble proteins PtmA and PtmN—the enzymes that are proposed to be the first two catalytic steps in the hypothesized pathway—by cloning the genes, overexpressing the genes in *E. coli*, and purifying the encoded proteins. As a preliminary study, the crude extracts of *E. coli* containing soluble PtmA or PtmN were tested for their enzymatic activity, and the products were analyzed by using HPLC.

Results and Discussion

To characterize the function of PtmA and PtmN, the *ptmA* and *ptmN* genes were cloned into the pRSET B expression vector, and the genes were overexpressed in *E. coli* BL21 pLysS. Crude extracts of the bacterial cells containing soluble PtmA or PtmN proteins were tested for enzymatic activity in eight varying experiments. HPLC was used to assay for the products in each experiment.

Sodium dodecyl sulfate polyacrylamide (SDS-PAGE) gel electrophoresis was used to determine the molecular weight of the soluble PtmA and PtmN proteins before testing for enzymatic activity (Figure 4). For both PtmA and PtmN proteins, the induced cells were lysed and centrifuged. The supernatants containing soluble PtmA or PtmN proteins were collected and tested for enzymatic activity.

The SDS-PAGE gel was ripped on the lower left corner; however, soluble PtmA and PtmN proteins were present in lanes three and five, which contained the supernatant of PtmA and PtmN.

The actual weights of PtmA and PtmN are 42.5 kDa and 36.8 kDa, respectively, and the measured weights were 43.12 kDa and 38.72 kDa.

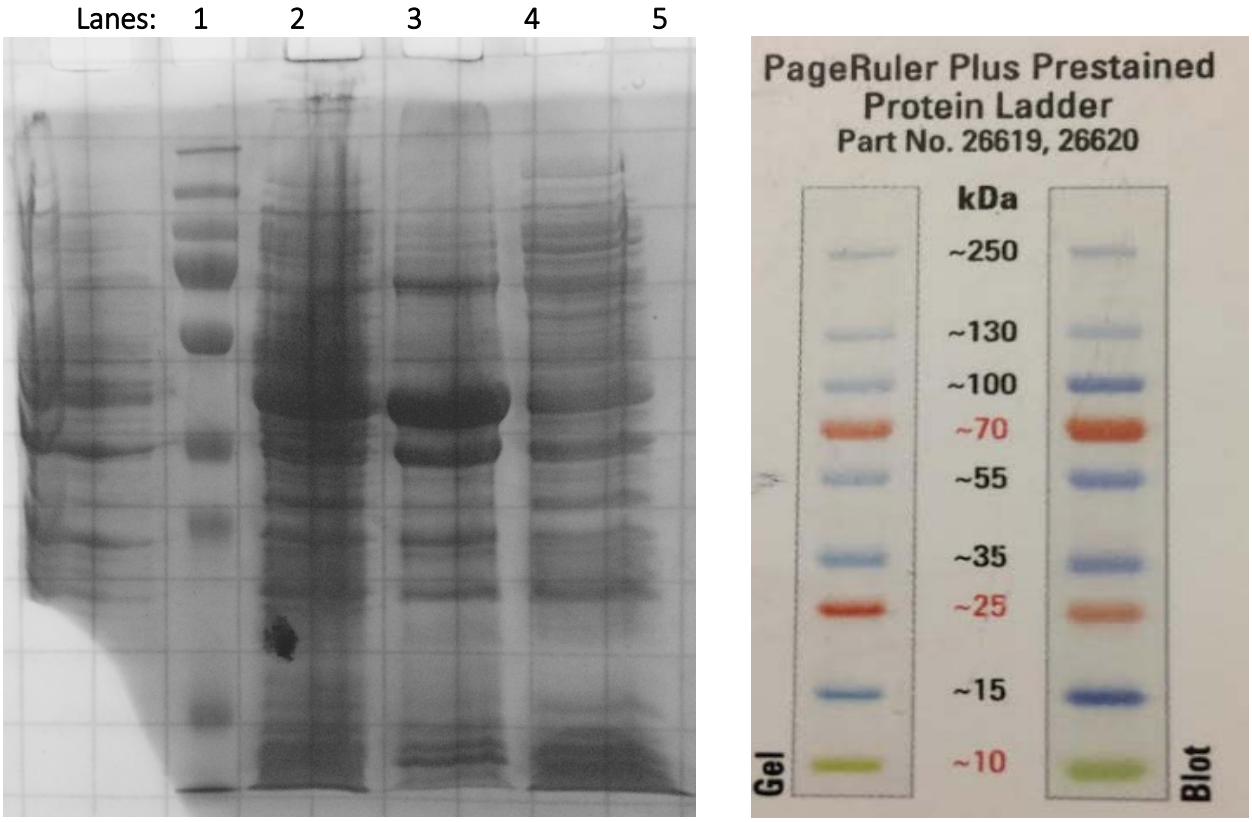


Figure 4. Left: SDS-PAGE gel of PtmA and PtmN soluble and insoluble proteins. Right: Page Ruler™ Plus Pre-stained Protein molecular weight standard. Lane 1: insoluble proteins from *ptmA* over-expressed *E. coli* . Lane 2: Page Ruler™ Plus Pre-stained Protein molecular weight standard. Lane 3: soluble proteins from *ptmA* over-expressed *E. coli*. Lane 4: insoluble proteins from *ptmN* over-expressed *E. coli*. Lane 5: soluble proteins from *ptmN* over-expressed *E. coli.*

The soluble PtmA and PtmN proteins, located in the supernatant, were used to complete a series of enzymatic reactions (**Table 1**). The substrate UDP-GlcNAc was first tested in the eight enzymatic reactions. It was predicted that PtmN requires a cofactor, NAD^+ or $NADP^+$, for the oxidoreductase reaction, and PtmA requires cofactors Pyridoxal Phosphate (PLP) and L-glutamate for the aminotransferase reaction.[11] An empty pRSET B vector was used as a negative control, and a Tris HCl buffer with pH 7.5 was used as a buffer solution.

Experiment I of the enzymatic reaction included all ingredients, except for the empty vector serving as a negative control; a positive result of both oxidoreductase and aminotransferase was predicted, since all co-factors for reactions were present. No enzymatic activity in experiment II of the enzymatic reaction was expected, since neither protein was added; the empty vector was used as a negative control. In experiment III, all cofactors required for oxidoreductase were added, and the empty vector was added instead of the PtmN enzyme to serve as a negative control for the positive oxidoreductase reaction expected as the result for experiment IV. The PtmN protein and all cofactors required for oxidoreductase were used in experiment IV. Experiment V and VI used only one oxidoreductase cofactor, $NADP^+$ and NAD^+ respectively, as a test to see if both co-factors were required for the oxidoreductase reaction to occur. Experiment VII and VIII included the PLP and L-glutamate cofactors required for aminotransferase, along with one oxidoreductase co-factor each, $NADP^+$ and NAD^+ respectively.

However, HPLC chromatograms did not show any new compounds produced in the reaction containing PtmA, PtmN, or both PtmA and PtmN. The results suggested that UDP-GlcNAc may not be the right substrate.

Ingredients:	Stock Solution Concentration:	I	II	III	IV	V	VI	VII	VIII
SNAC	100mM	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)
PtmN	---	10µL	X	X	20µL	20µL	20µL	10µL	10µL
PtmA	---	10µL	X	X	X	X	X	10µL	10µL
Empty Vector	---	X	20µL	20µL	X	X	X	X	X
NAD^+	100mM	1µL (2mM)	1µL (2mM)	1µL (2mM)	1µL (2mM)	X	1µL (2mM)	X	1µL (2mM)
$NADP^+$	100mM	1µL (2mM)	1µL (2mM)	1µL (2mM)	1µL (2mM)	1µL (2mM)	X	1µL (2mM)	X
PLP	100mM	1µL (2mM)	1µL (2mM)	X	X	X	X	1µL (2mM)	1µL (2mM)
L-Glutamate	100mM	5µL (10mM)	5µL (10mM)	X	X	X	X	5µL (10mM)	5µL (10mM)
Tris buffer (pH 7.5)	50mM	20µL	20µL	26µL	26µL	27µL	27µL	21µL	21µL
	TOTAL VOLUME:	50µL	50µL	50µL	50µL	50µL	50µL	50µL	50µL

Table 1. Experiments I through VIII, including all ingredients used in each enzymatic reaction.

Another possible substrate, *N*-acetylglucosaminyl- 3-[3-aminophenyl]3-oxopropionyl *N*-acetylcysteamine (glycosylated β-Keto-SNAC), was then used in each of the eight reactions as a substrate for the enzymatic activity.

HPLC was used to analyze the products of the enzymatic reactions at various wavelengths: 216 nm, 230 nm, 254 nm, 330 nm. The HPLC chromatograms for experiments one through eight were analyzed for enzymatic activity at wavelength 216 nm, Figures 5-12. The HPLC chromatograms for experiments one through eight at wavelengths 230 nm, 254 nm, and 330 nm, are located in the supplemental information section.

A positive result in experiment I of both oxidoreductase and aminotransferase was predicted, since both proteins and all co-factors for reactions were present. A peak at 10.5 minutes in experiment one was present, potentially representing oxidoreductase and aminotransferase

activity; however, it was similar to a peak present in the negative control in experiment II at nine minutes. A peak at 16 minutes in experiments III, IV, V, and VI, was also observed, potentially representing background activities as a result of not using purified proteins.

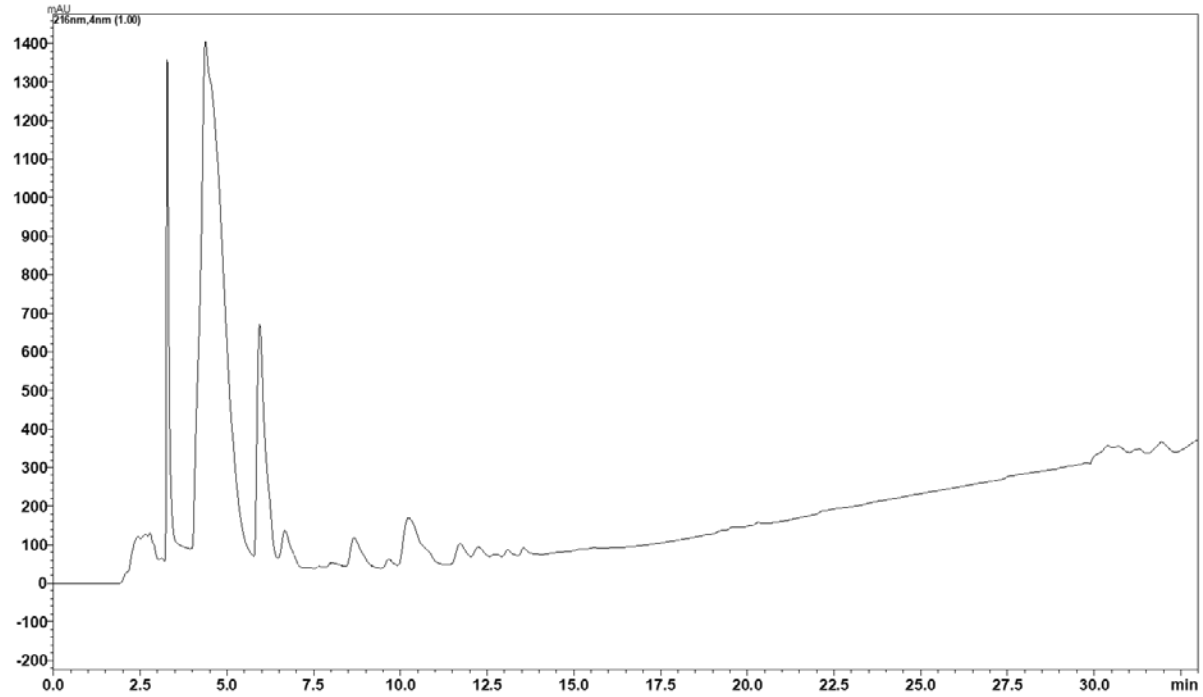


Figure 5. Experiment I: included all ingredients, except for empty vector. We expected to see products of oxidoreductase and aminotransferase reactions.

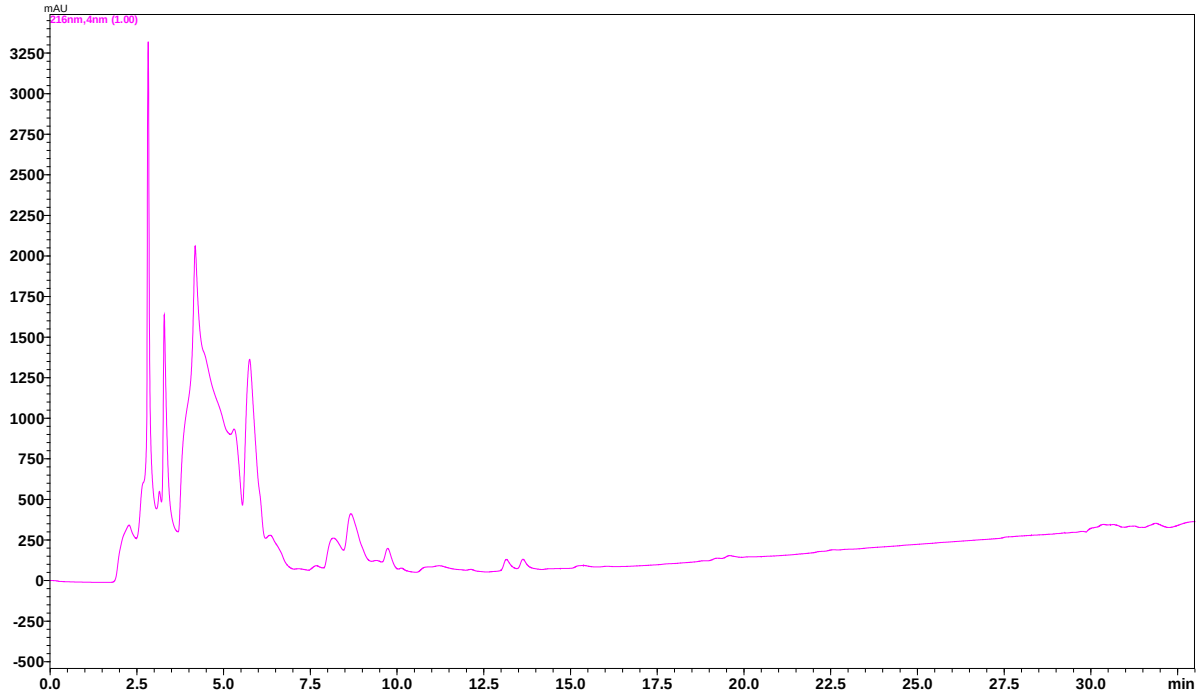


Figure 6. Experiment II: included all ingredients, except for proteins. We expected to see no enzymatic activity, as this was our negative control.

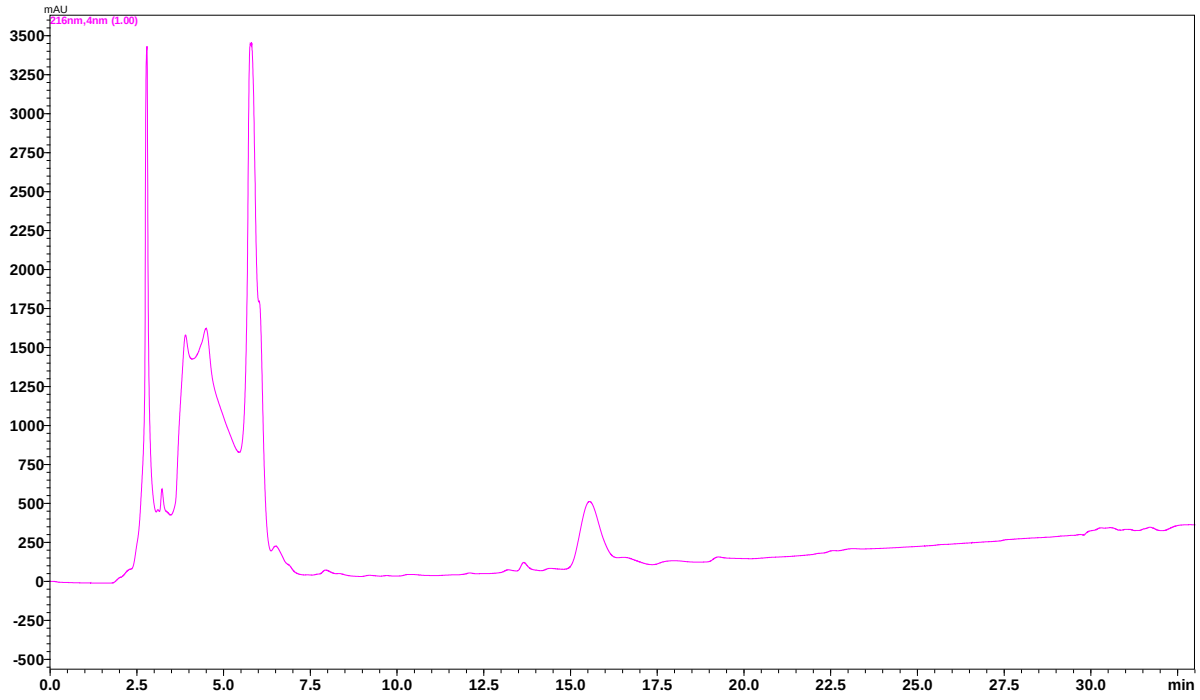


Figure 7. Experiment III: included all cofactors required for oxidoreductase, but included the empty vector instead of the PtmN enzyme, to serve as a negative control for a positive oxidoreductase reaction. We expected to see no enzymatic activity.

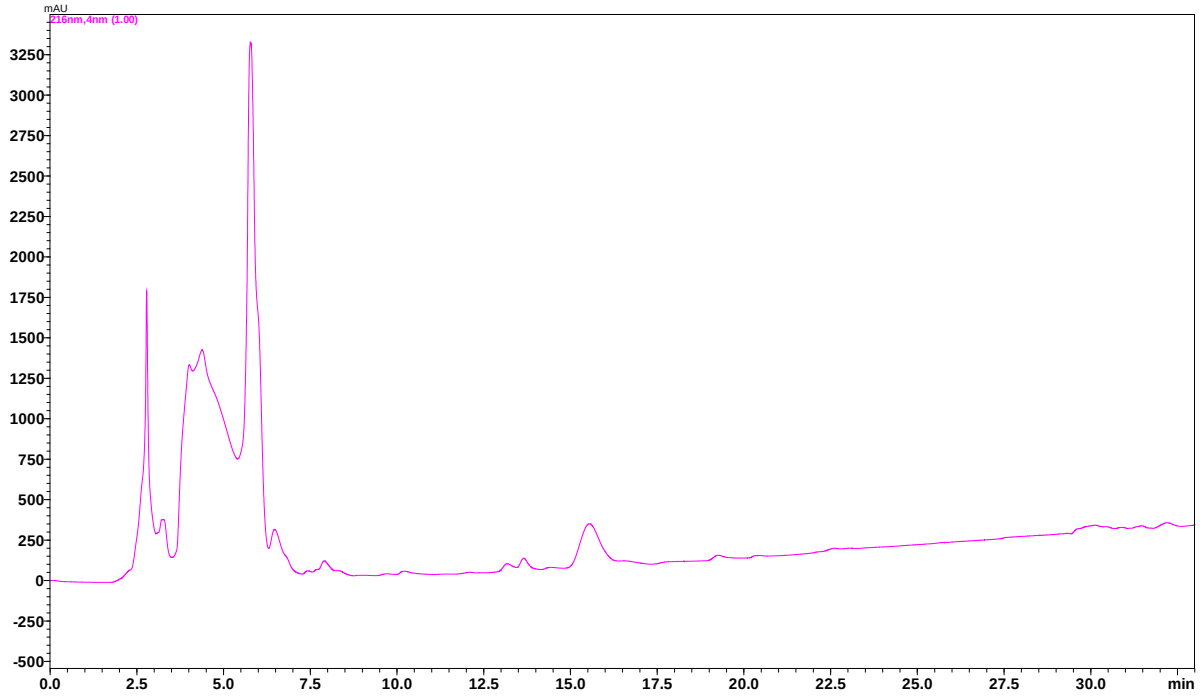


Figure 8. Experiment IV: included the PtmN protein and all cofactors required for oxidoreductase in experiment four. We expected to see products of the oxidoreductase reaction.

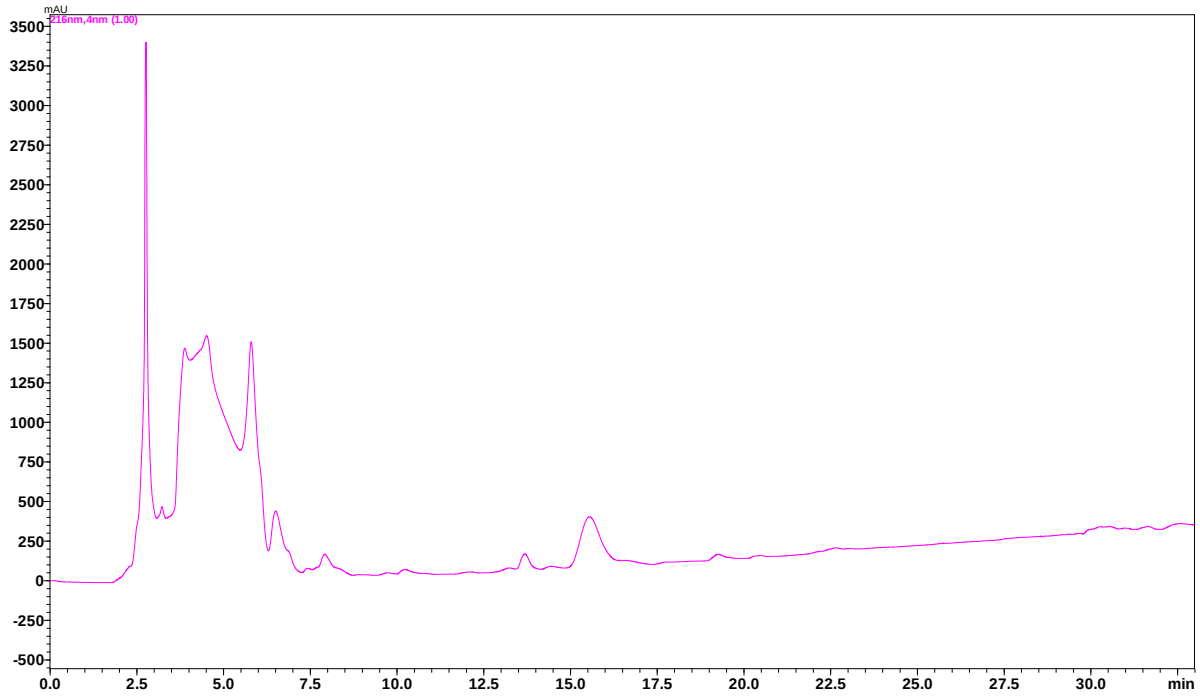


Figure 9. Experiment V: Included only one oxidoreductase cofactor, $NADP^+$, and the corresponding protein, PtmN, as a test to see if both co-factors are required for the oxidoreductase reaction to occur.

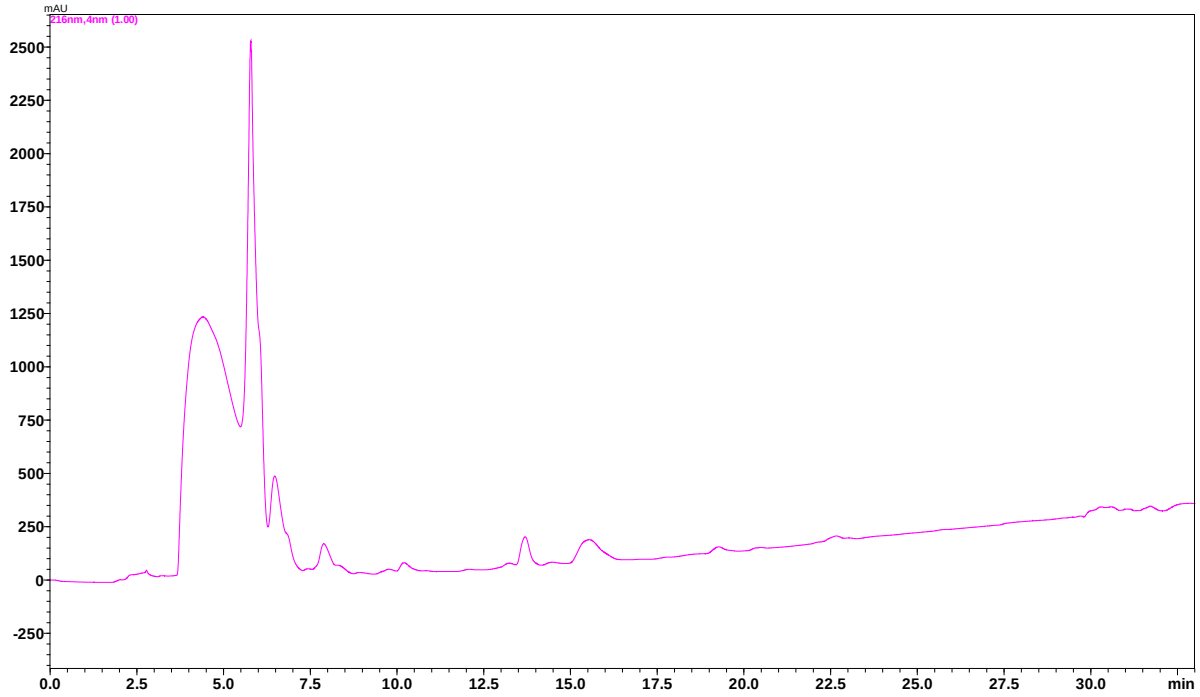


Figure 10. Experiment VI: Included only one oxidoreductase cofactor, NAD^+, and the corresponding protein, PtmN, as a test to see if both co-factors are required for the oxidoreductase reaction to occur.

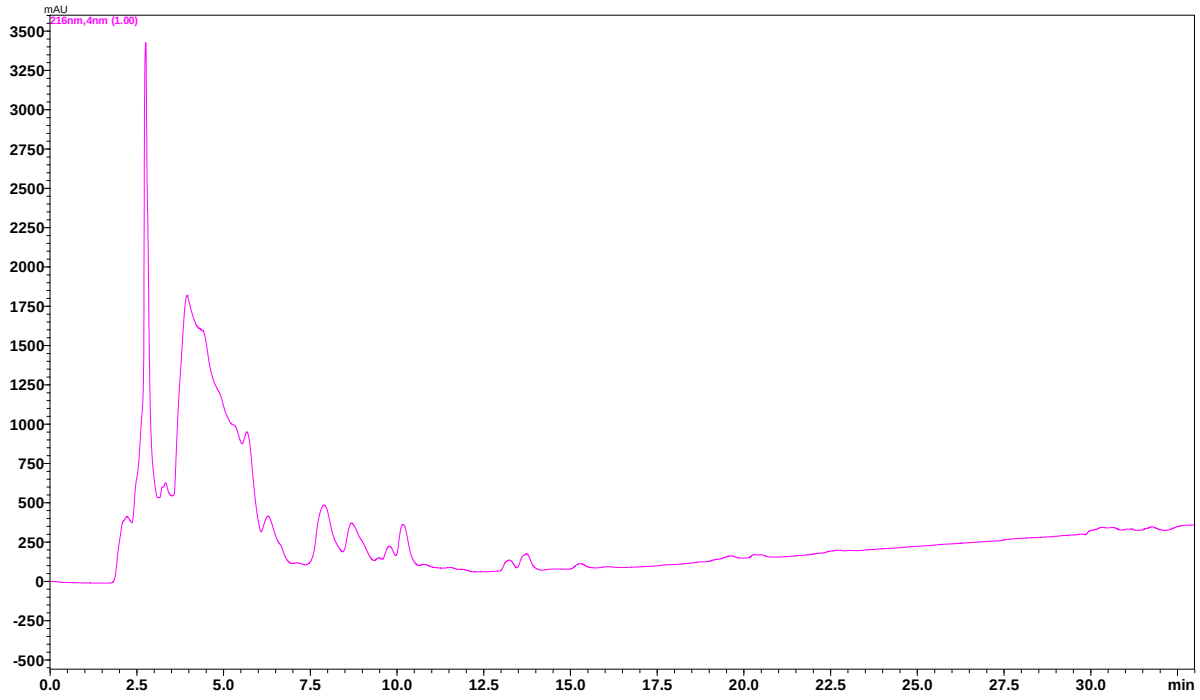


Figure 11. Experiment VII: included the Pyridoxal Phosphate (PLP) and L-Glutamate cofactors required for aminotransferase, both proteins, and one oxidoreductase co-factor: $NADP^+$. We expected to see products of the aminotransferase reaction.

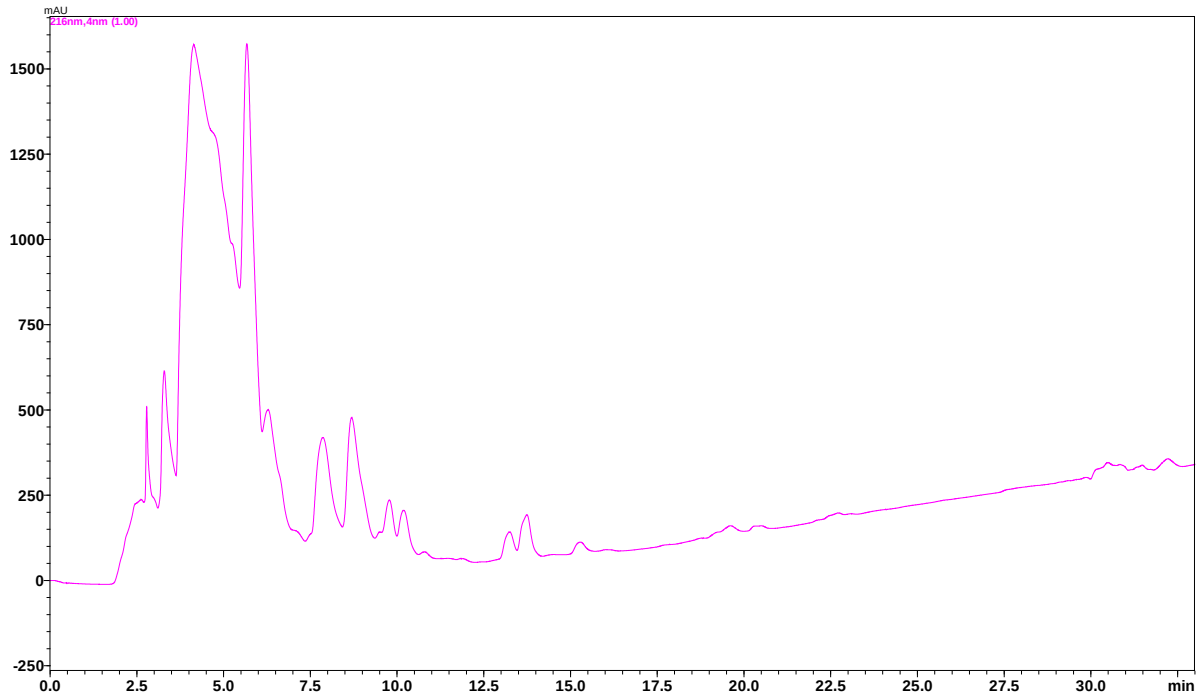


Figure 12. Experiment VIII: included the Pyridoxal Phosphate (PLP) and L-Glutamate cofactors required for aminotransferase, both proteins, and one oxidoreductase co-factor: NAD^+. We expected to see products of the aminotransferase reaction.

Conclusion

The *ptmA* and *ptmN* genes were cloned into the pRSET B expression vector, and the genes were overexpressed in *E. coli* BL21 pLysS. Crude extracts of the bacterial cells containing soluble PtmA or PtmN proteins were tested for enzymatic activity, using HPLC to assay for the products. A peak at 10.5 minutes, potentially representing oxidoreductase and aminotransferase activity, was present in experiment one, which included all ingredients except for the empty vector. The peak in experiment one was similar to a peak shown in the negative control in experiment two at nine minutes. A peak was present at 16 minutes in experiments three, four, five, and six, potentially representing background activities as a result of not using purified proteins.

Next steps of this project include purifying the PtmA and PtmN proteins by using a nickel NTA column, dialysis bag to remove imidazole, and an SDS-PAGE protein gel to confirm protein identification by molecular weight. The proteins would be boiled and would serve as a negative control in enzymatic reactions and HPLC assay. Purified proteins would eliminate background enzymatic activity caused by other proteins found in the *E. coli* competent cell.

If oxidoreductase and aminotransferase products are detected through HPLC assay, the products may be further characterized. Additional enzymes in the proposed pathway, such as PtmB and PtmG, would be cloned into expression vectors, overexpressed in *E. coli*, and assayed by HPLC.

Experimental Section

Gene Cloning

Genomic DNA of *S. pactum* ATCC 27456 was previously prepared by using the DNeasy Tissue Kit. DNA fragments were recovered from an agarose gel by using the QIAquick Gel Extraction Kit (QIAGEN). The Center for Genome Research and Biocomputing (CGRB) at Oregon State University determined the nucleotide sequences of the *ptmA* and *ptmN* gene fragments. Polymerase Chain Reaction was used to amplify the two genes of interest, *ptmA* and *ptmN*, by performing 35 cycles using a Mastercycler gradient thermocycler (manufactured by Eppendorf), High fidelity *Taq* DNA polymerase (manufactured by Invitrogen), oligodeoxyribonucleotides (manufactured by Sigma Aldrich Genosys) and primer pairs (manufactured by Invitrogen), see Table 2. All following DNA manipulations were performed according to standard protocols (Kieser et al., 2000; Sambrook and Russell, 2001), including restriction digestion by enzymes EcoR1 and BamH1 (manufactured by Promega) and ligation of the gene fragments into the pRSET B expression vector (manufactured by Novagen), see Figure 8.

	Forward Primer Sequences	Reverse Primer Sequence
PtmA Primers	5'- GGAAGATCTACATATGC GGTACGAGCCCTG	5'-CCGCAATTCTCAGCC GGCGGCGTCCGCCCAG
PtmN Primers	5'-GGAAGATCTACATATGACC CGCGAGAAGCCGAT	5'-CCGCAATTCTCAGGTCACC GCCGGGGCCGC

Table 2. PtmA and PtmN forward and reverse primers for the pRSET B vector. Restriction sites are underlined.

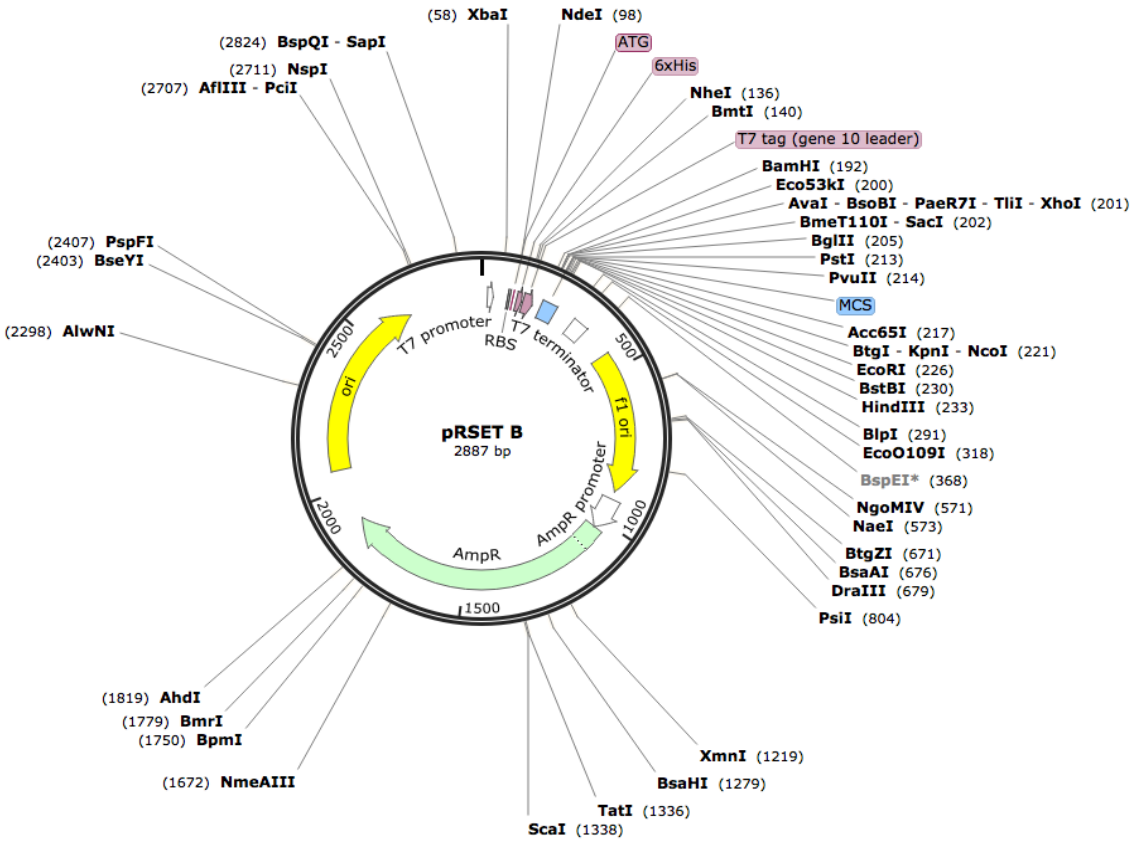


Figure 8. pRSET B Expression Vector used for digestion and ligation of the *ptmA* and *ptmN* genes.

The ligation products were transferred into *E. coli* BL21 pLysS competent cells (manufactured by Invitrogen) via heat shock. LB media, containing 100 µg/mL chloramphenicol and 100 µg/mL ampicillin, was separately inoculated with the *ptmA* and *ptmN* transferred E. coli cells. Bacteria were grown at 30℃ with shaking at 250 rpm until an OD_{600} of 0.6 was reached. The *ptmA* and *ptmN* transferred E. coli cell cultures were induced with 100 µL of 0.1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) and overexpressed with shaking at 250 rpm for 15 hours at 16℃ .

Cell Lysis

The overexpressed cells were centrifuged, isolated from supernatant, suspended with 40mM Potassium Phosphate buffer, and sonicated by Microson™ ultrasonic cell disruptor for five sets

of 30 seconds sonication and 30 second rests. The proteins were separated using a 0.5mm, 7 percent acrylamide SDS-PAGE gel that was ran in a Running Buffer (containing water, Tris base, glycerin, and sodium dodecyl sulfate) at 15 volts for 10 minutes, followed by 30 volts for 30 minutes. The gel was stained to confirm the approximate weights of soluble proteins PtmA and PtmN. The Page Ruler™ Plus Pre-stained Protein molecular weight standard was used as a comparison to measure the weights of the proteins in the SDS-PAGE gel.

Enzymatic Reaction

To detect products of the oxidoreductase and aminotransferase reactions, the crude PtmN and PtmA proteins were reacted with the substrate UDP-GlcNAc (manufactured by Sigma Aldrich Genosys) or β-Keto-SNAc (synthesized in the lab of Dr. Taifo Mahmud by graduate student Corey Brumsted), and coenzymes listed in **Table 1**, in a buffer solution of 50 mM Tris HCL buffer, pH 7.5. These reactions took place at 30℃ for three hours. The reactions were kept on ice for ten minutes, quenched by methanol, and kept at 4℃ for 20 minutes before assay by HPLC.

Ingredients:	Stock Solution Concentration:	I	II	III	IV	V	VI	VII	VIII
SNAC	100mM	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)	2µL (4mM)
PtmN	---	10µL	X	X	20µL	20µL	20µL	10µL	10µL
PtmA	---	10µL	X	X	X	X	X	10µL	10µL
Empty Vector	---	X	20µL	20µL	X	X	X	X	X
NAD^+	100mM	1µL (2mM)	1µL (2mM)	1µL (2mM)	1µL (2mM)	X	1µL (2mM)	X	1µL (2mM)
$NADP^+$	100mM	1µL (2mM)	1µL (2mM)	1µL (2mM)	1µL (2mM)	1µL (2mM)	X	1µL (2mM)	X
PLP	100mM	1µL (2mM)	1µL (2mM)	X	X	X	X	1µL (2mM)	1µL (2mM)
L-Glutamate	100mM	5µL (10mM)	5µL (10mM)	X	X	X	X	5µL (10mM)	5µL (10mM)
Tris buffer (pH 7.5)	50mM	20µL	20µL	26µL	26µL	27µL	27µL	21µL	21µL
	TOTAL VOLUME:	50µL	50µL	50µL	50µL	50µL	50µL	50µL	50µL

Table 1. Experiments I through VIII, including all ingredients used in each enzymatic reaction.

Enzymatic Reaction Analysis

HPLC was run for the SNAC substrate using a 0.5-100 gradient of methanol and a 10 mM ammonium formate (NH_4HCO_2) buffer solution for a duration of 33 minutes with an isocratic flow rate of 0.700 mL/min. The empty pRSET B vector served as the negative control, and the enzymes PtmN and PtmA were not purified. The HPLC chromatograms were analyzed at wavelengths 216 nm, 230 nm, 254 nm, 330 nm, due to the various UV absorptions of the substrate and cofactors, as seen in Table 3. The aminated product was expected to have a greater retention time than its substrate, since the aminated product would be less polar. The retention time of the substrate and cofactors is unknown, since they were not tested with this buffer solution.

Compound	UV Absorption Peak
SNAC Substrate	Unknown
GlcNAc Substrate	210nm
NAD^+	340nm
$NADP^+$	340nm
PLP	320nm
L-Glutamate	200nm

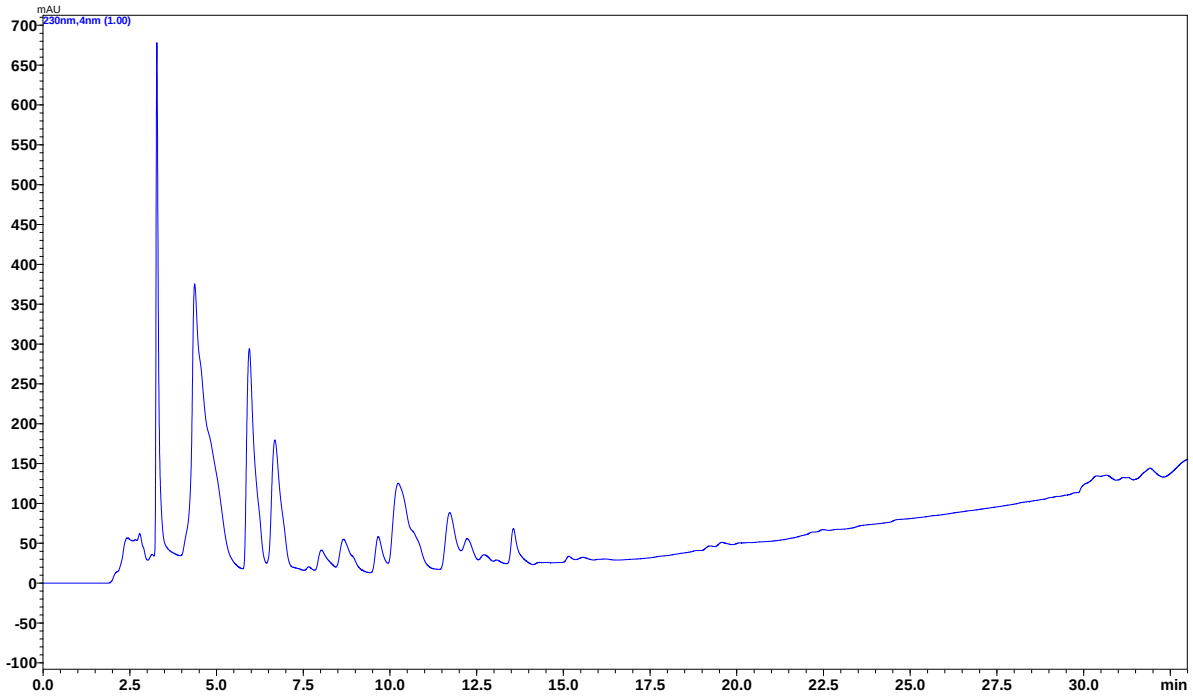
Table 3: Various UV absorptions of the substrate and cofactors, based on previous literature.

References

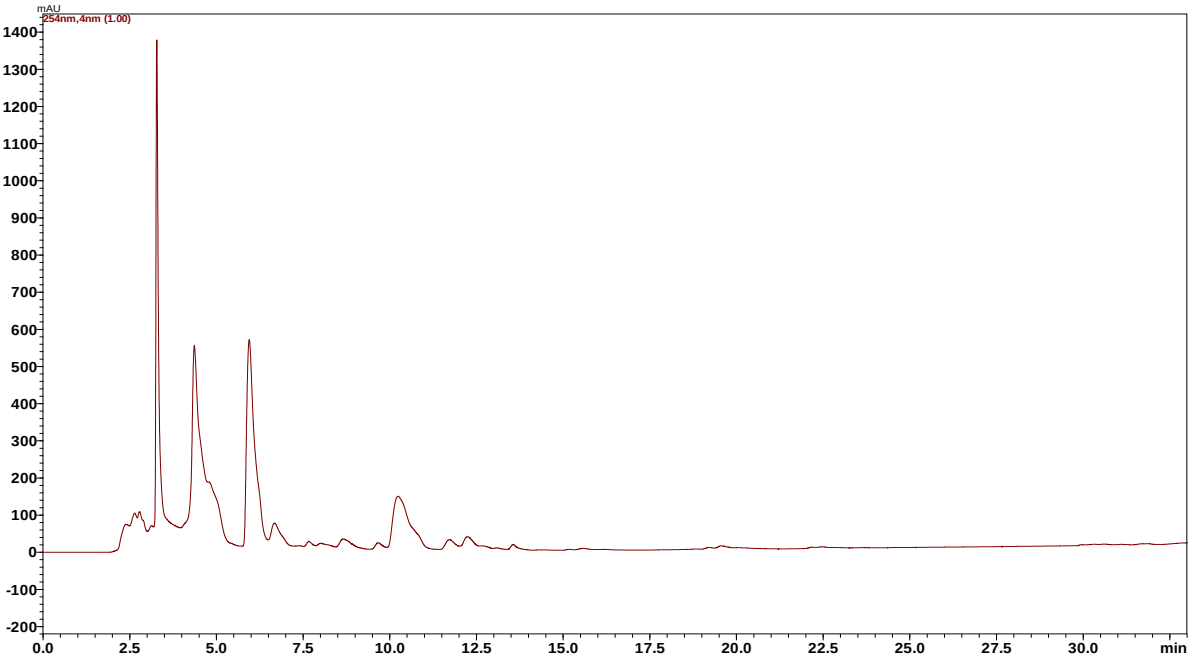
1. Kudo, F.; Kasama, Y.; Hirayama, T.; Eguchi, T. *The Journal of Antibiotics* 2007, *60*(8), 492.
2. Bhuyan, B. *Journal of Applied Microbiology* 1962, 10(4), 302 – 304.
3. Mahmud, T. 3. Pactamycin analogs and methods of use*. Patent WO2012018854A8* 2002.
4. Brodersen, D. E.; Clemons, W. M.; Carter, A. P.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Cell* 2000, *103*(7), 1143.
5. Lu, W.; Roongsawang, N.; Mahmud, T. *Chemistry & Biology* 2011, *18*(4), 425.
6. Guha, G.; Lu, W.; Li, S.; Liang, X.; Kulesz-Martin, M. F.; Mahmud, T.; Indra, A. K.; Ganguli-Indra, G. *Plos One* 2015, *10*(5).
7. Ito, T.; Roongsawang, N.; Shirasaka, N.; Lu, W.; Flatt, P. M.; Kasanah, N.; Miranda, C.; Mahmud, T. *ChemBioChem* 2009, *10*(13), 2253.
8. Abugrain, M. E.; Lu, W.; Li, Y.; Serrill, J. D.; Brumsted, C. J.; Osborn, A. R.; Alani, A.; Ishmael, J. E.; Kelly, J. X.; Mahmud, T. *ChemBioChem* 2016, *17*(17), 1579.
9. Rinehart, K. L.; Potgieter, M.; Delaware, D. L.; Seto, H. *Journal of the American Chemical Society* 1981, *103*(8), 2099.
10. Abugrain, M. E.; Brumsted, C. J.; Osborn, A. R.; Philmus, B.; Mahmud, T. *ACS Chemical Biology* 2017, *12*(2), 362.
11. Hirayama, A.; Eguchi, T.; Kudo, F. *ChemBioChem* 2013, *14*(10), 1198.

Supplemental Information

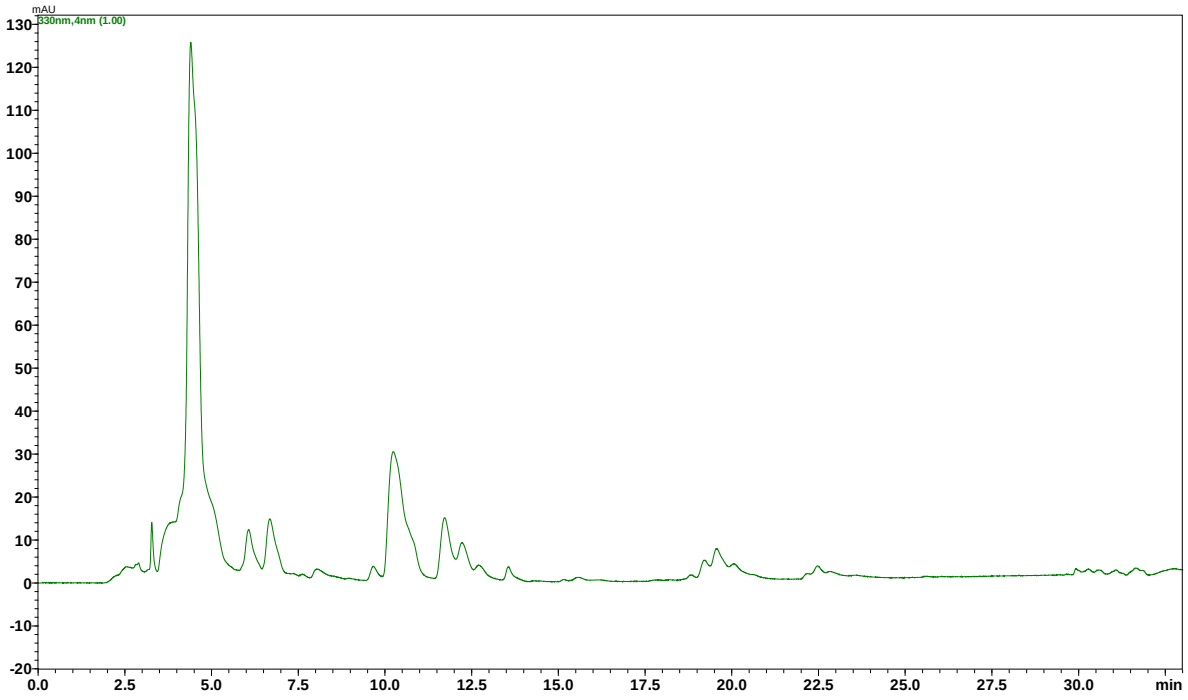
Experiment One: **230nm**; included all ingredients, except for empty vector. We expected to see products of oxidoreductase and aminotransferase reactions.



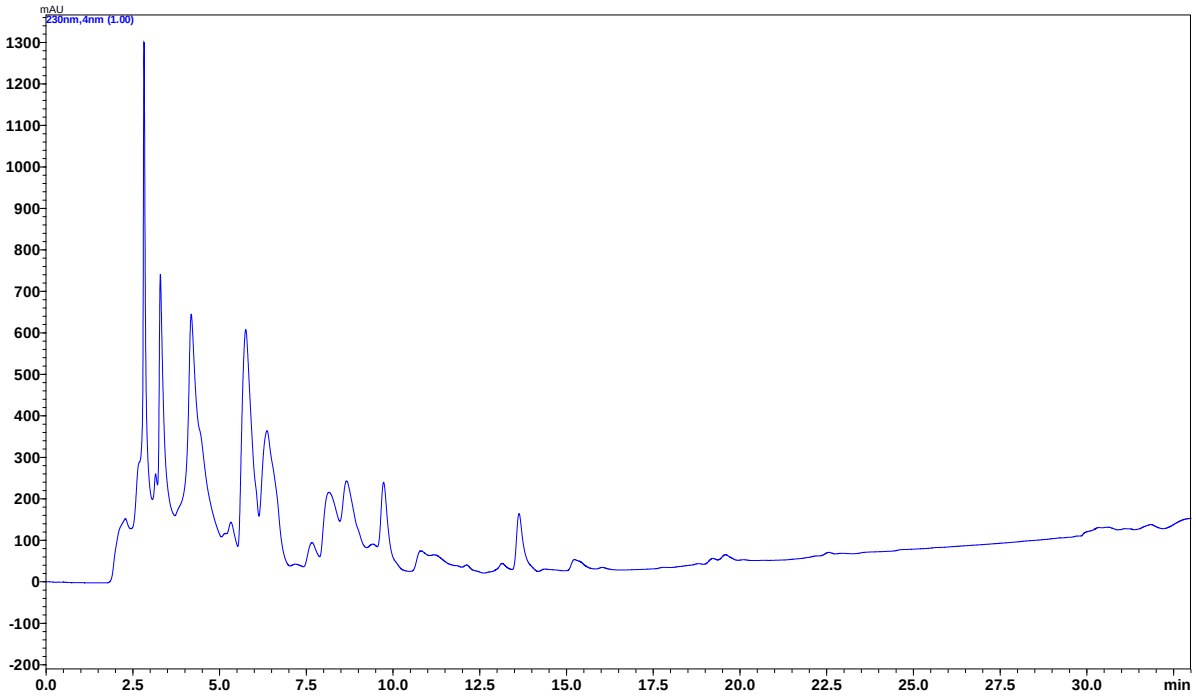
Experiment One: **254nm**; included all ingredients, except for empty vector. We expected to see products of oxidoreductase and aminotransferase reactions.



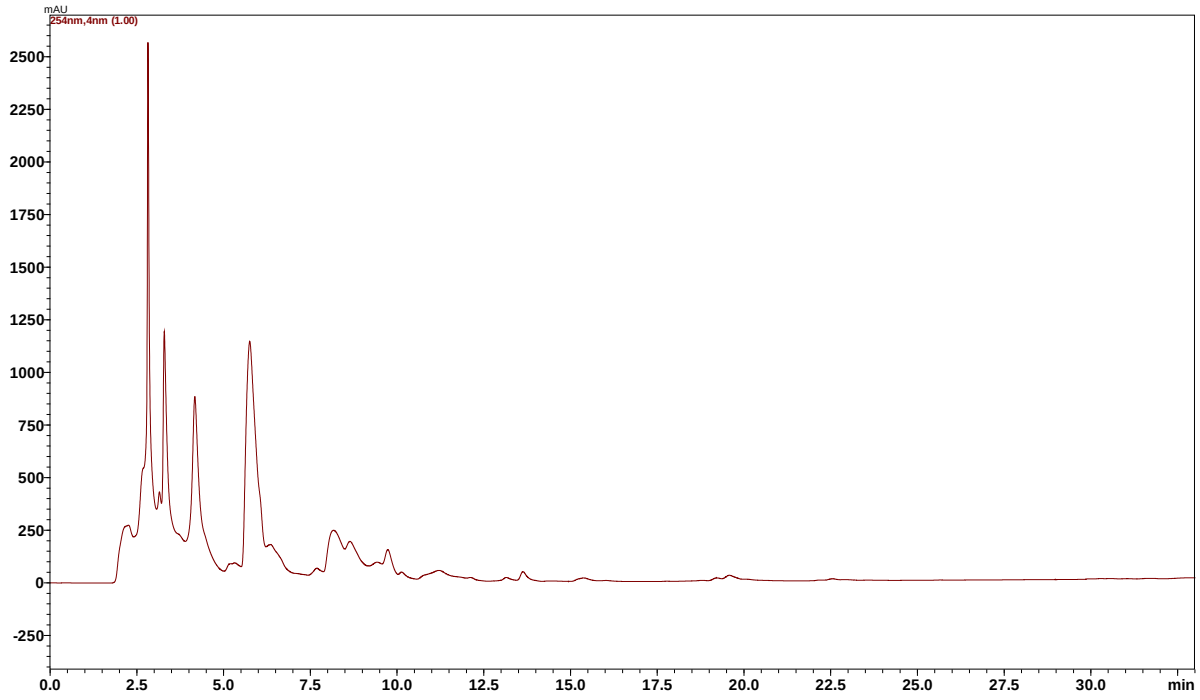
Experiment One: **330nm**; included all ingredients, except for empty vector. We expected to see products of oxidoreductase and aminotransferase reactions.



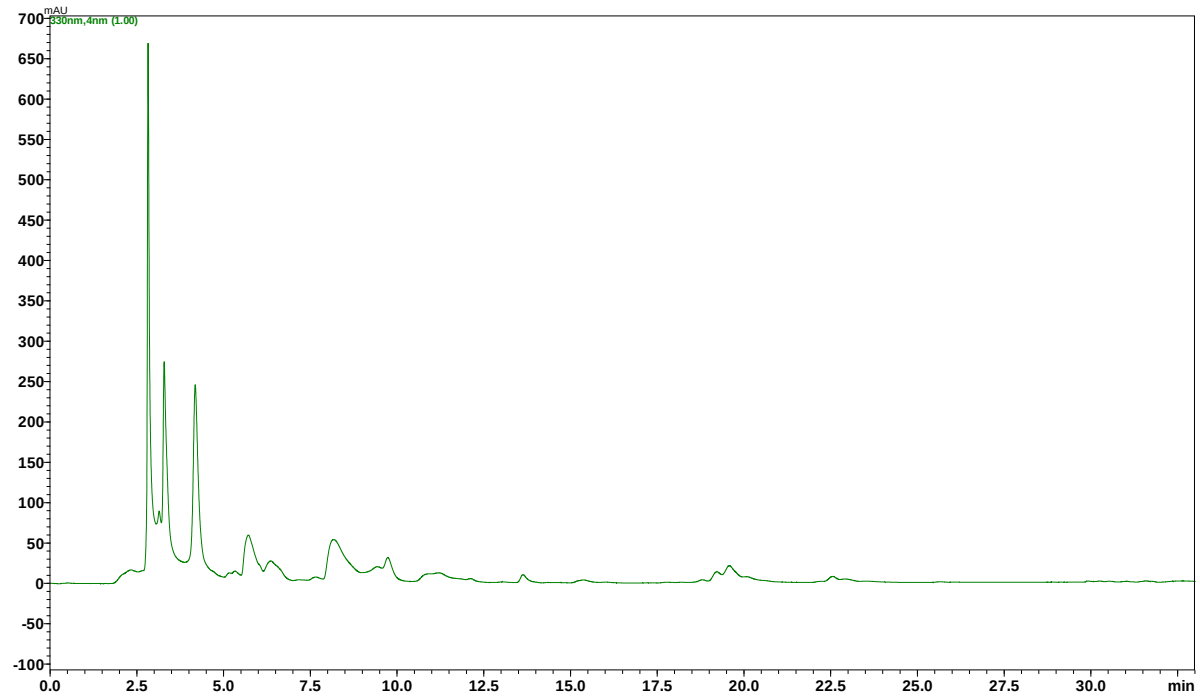
Experiment Two: **230nm;** included all ingredients, except for proteins. We expected to see no enzymatic activity, as this was our negative control.



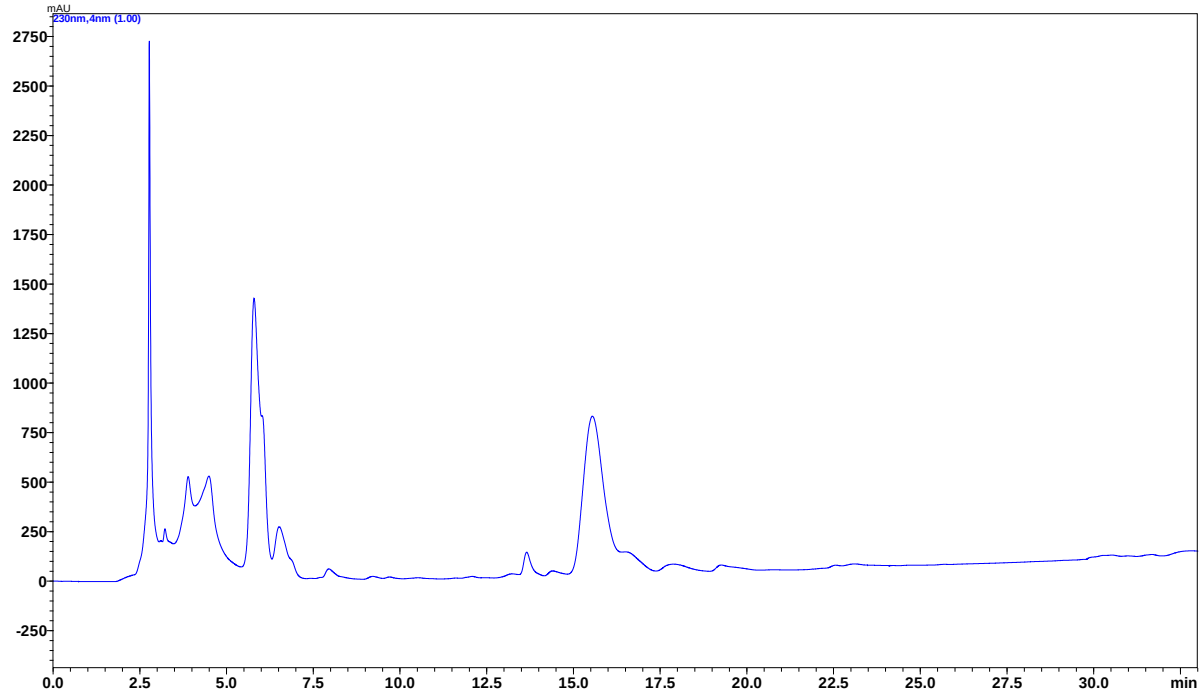
Experiment Two: **254nm;** included all ingredients, except for proteins. We expected to see no enzymatic activity, as this was our negative control.



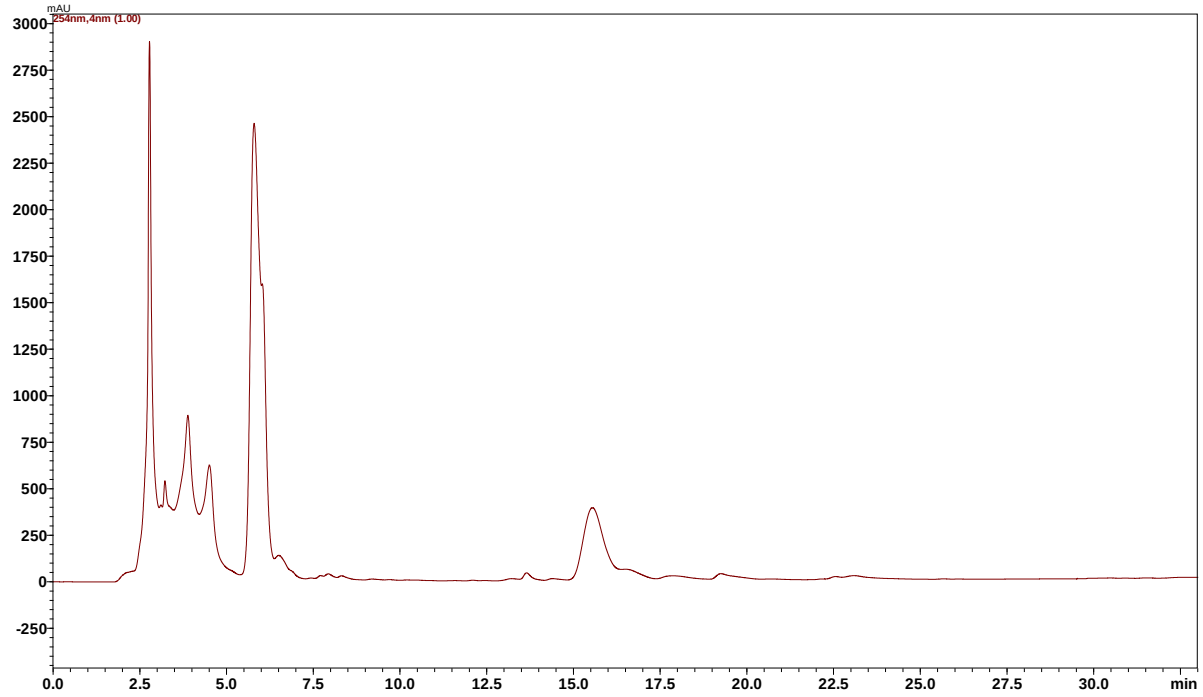
Experiment Two: **330nm;** included all ingredients, except for proteins. We expected to see no enzymatic activity, as this was our negative control.



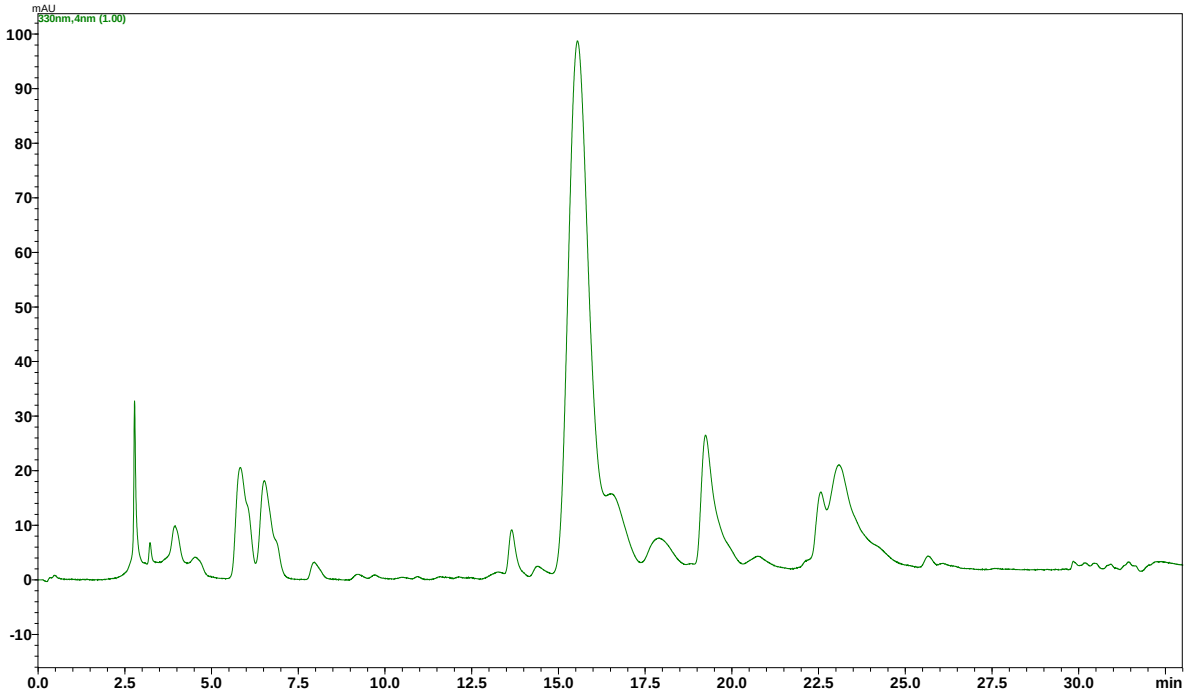
Experiment Three: 230nm; included all cofactors required for oxidoreductase, but included the empty vector instead of the PtmN enzyme, to serve as a negative control for a positive oxidoreductase reaction. We expected to see no enzymatic activity.



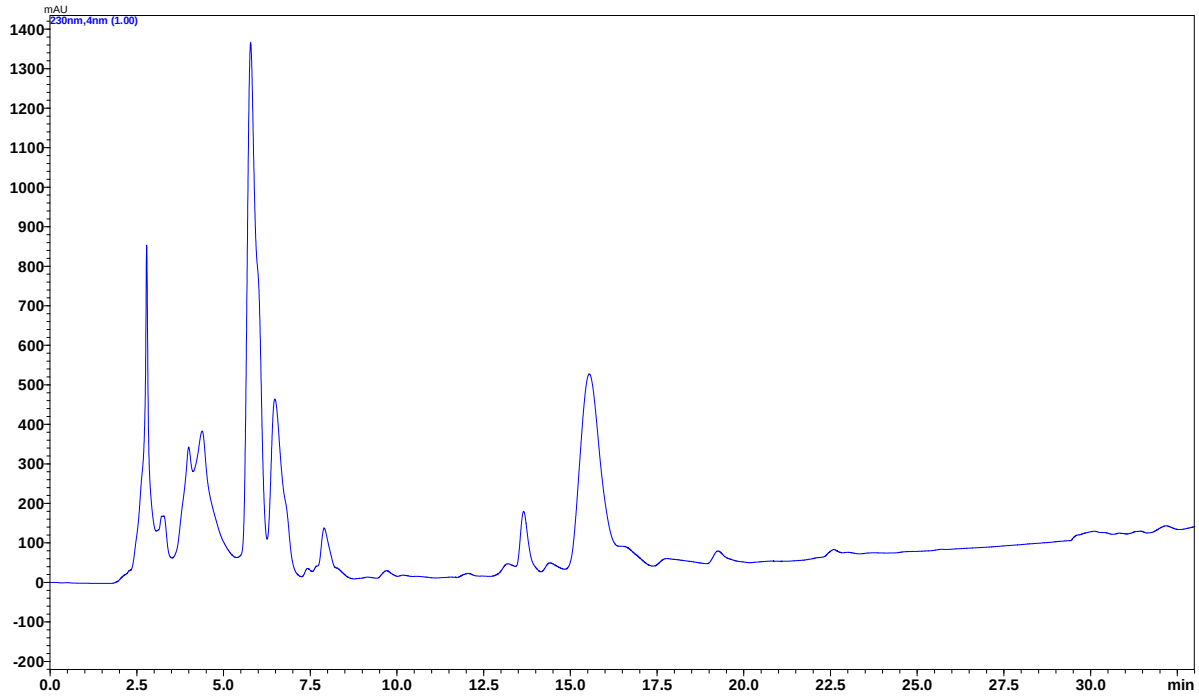
Experiment Three: 254nm; included all cofactors required for oxidoreductase, but included the empty vector instead of the PtmN enzyme, to serve as a negative control for a positive oxidoreductase reaction. We expected to see no enzymatic activity.



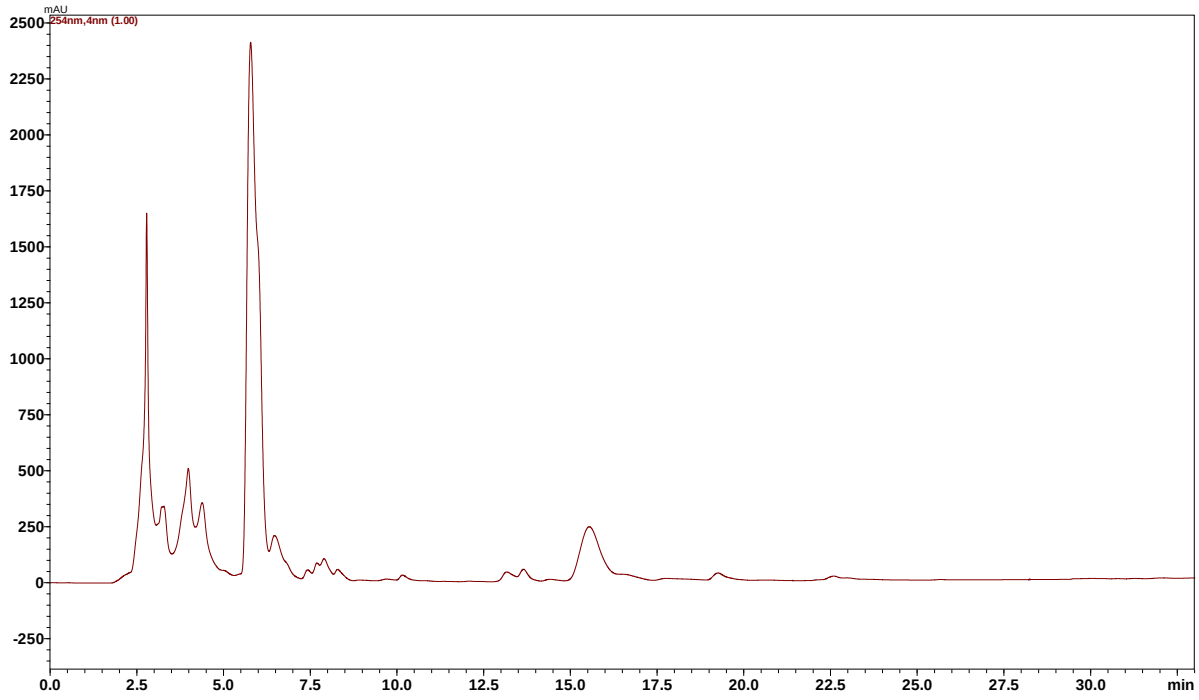
Experiment Three: **330nm**; included all cofactors required for oxidoreductase, but included the empty vector instead of the PtmN enzyme, to serve as a negative control for a positive oxidoreductase reaction. We expected to see no enzymatic activity.



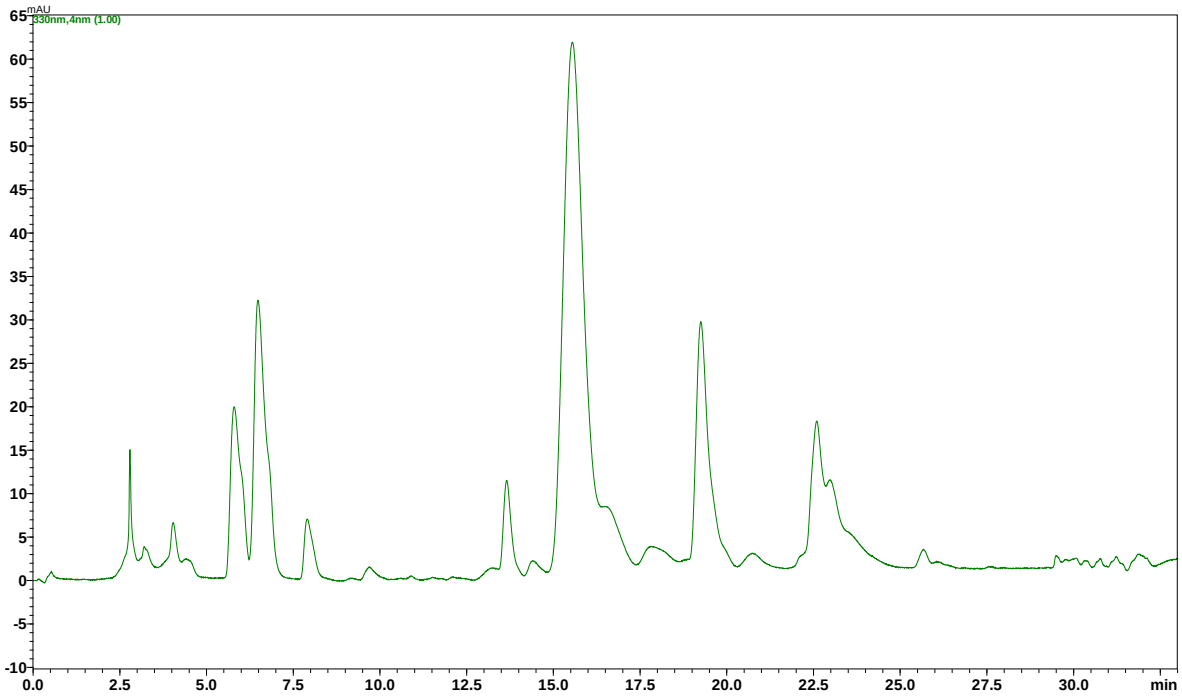
Experiment Four: **230nm**; included the PtmN protein and all cofactors required for oxidoreductase in experiment four. We expected to see products of the oxidoreductase reaction.



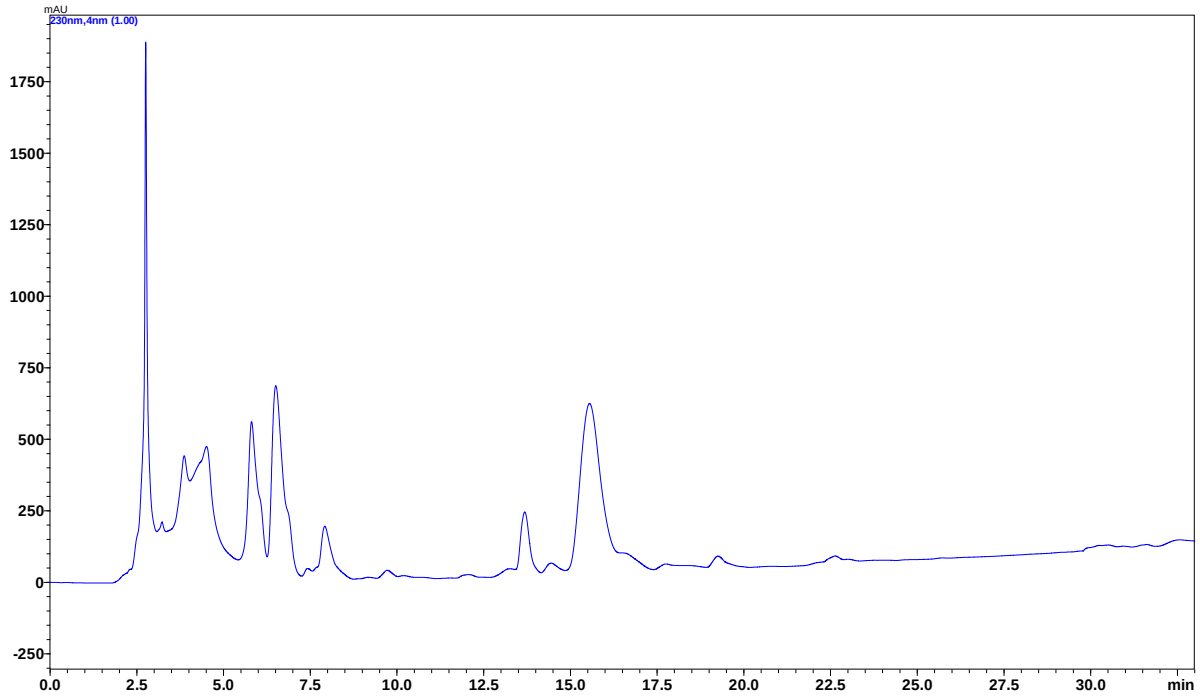
Experiment Four: 254nm; included the PtmN protein and all cofactors required for oxidoreductase in experiment four. We expected to see products of the oxidoreductase reaction.



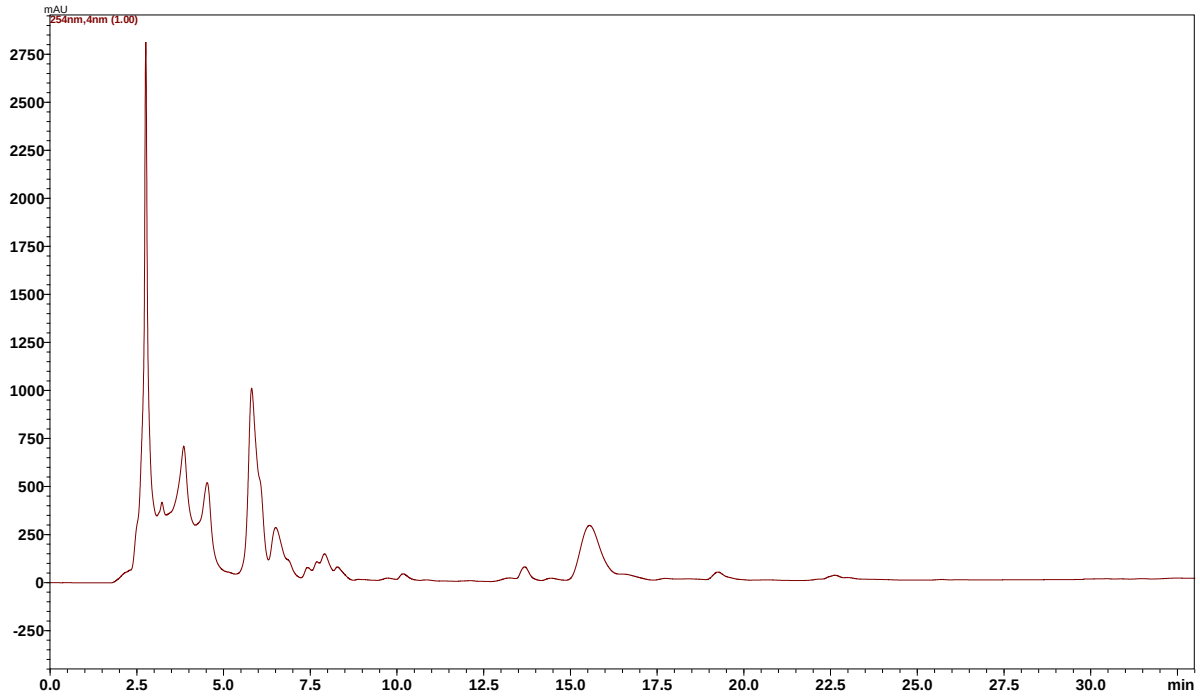
Experiment Four: 330nm; included the PtmN protein and all cofactors required for oxidoreductase in experiment four. We expected to see products of the oxidoreductase reaction.



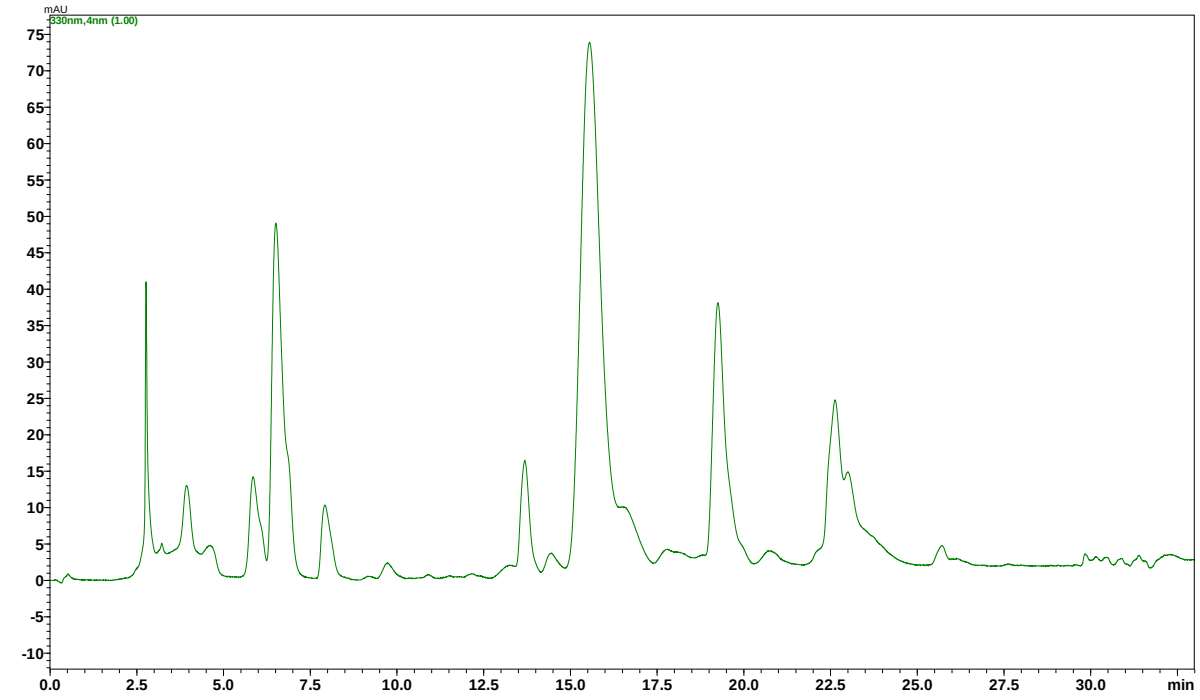
Experiment Five: 230nm; Included only one oxidoreductase cofactor, $NADP^+$, and the corresponding protein, PtmN, as a test to see if both co-factors are required for the oxidoreductase reaction to occur.



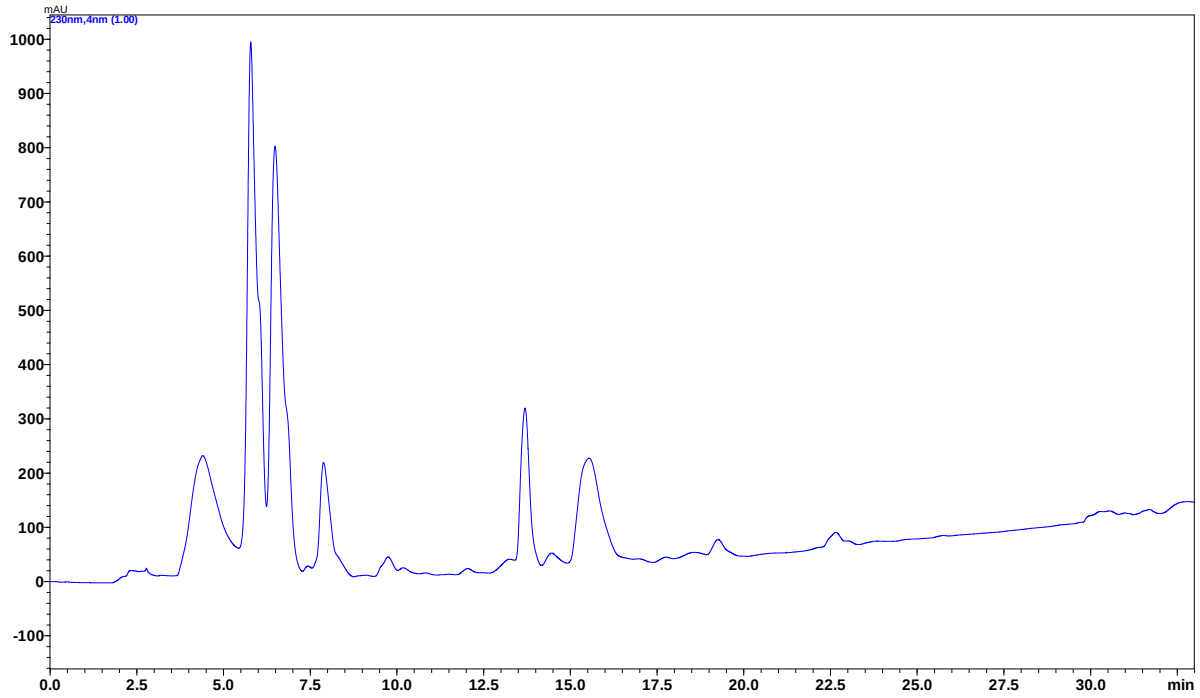
Experiment Five: 254nm; Included only one oxidoreductase cofactor, $NADP^+$, and the corresponding protein, PtmN, as a test to see if both co-factors are required for the oxidoreductase reaction to occur.



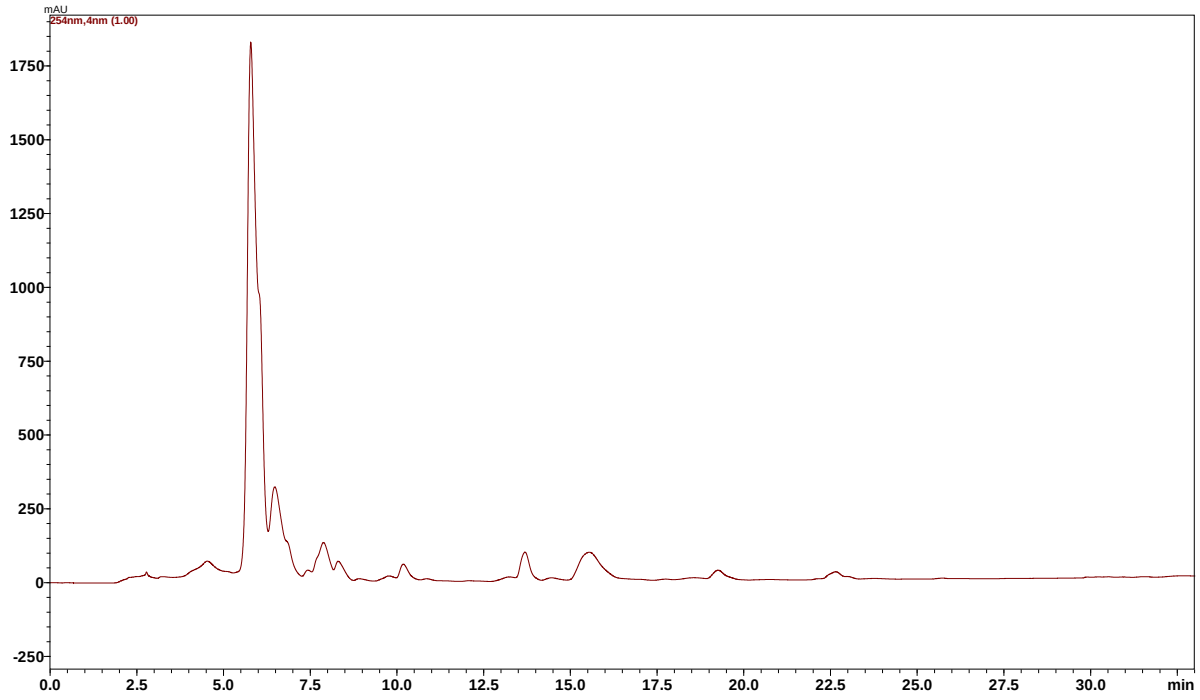
Experiment Five: 330nm; Included only one oxidoreductase cofactor, $NADP^+$, and the corresponding protein, PtmN, as a test to see if both co-factors are required for the oxidoreductase reaction to occur.



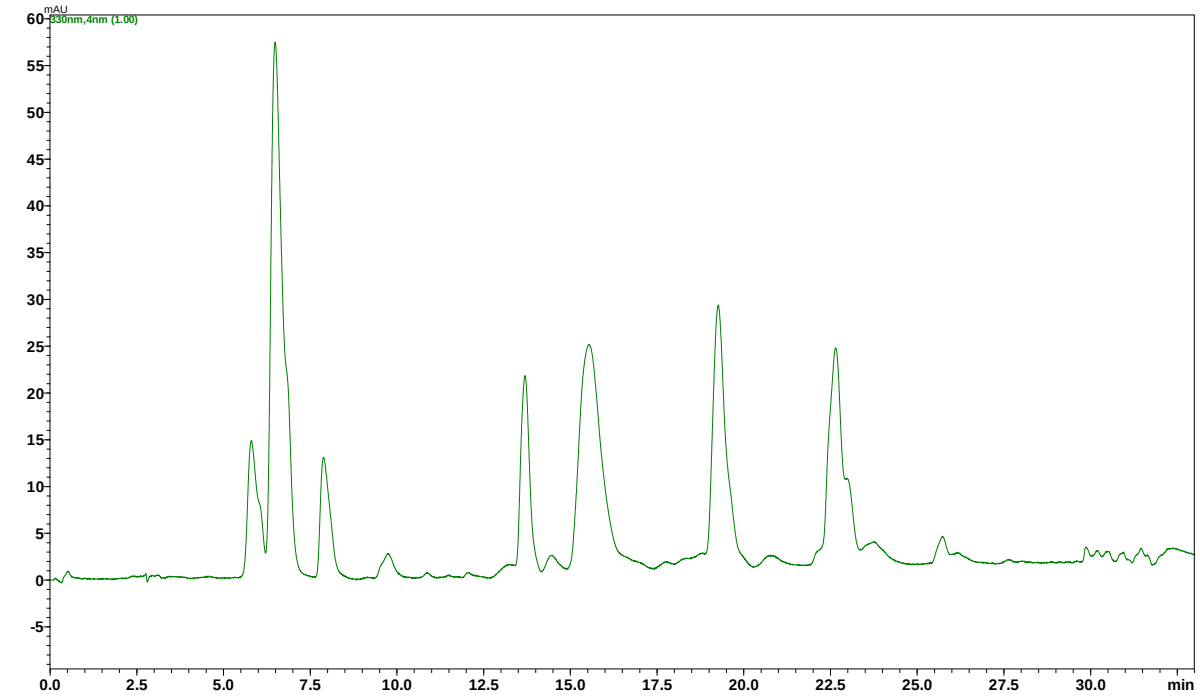
Experiment Six: 230nm; included only one oxidoreductase cofactor, NAD^+, and the corresponding protein, PtmN, as a test to see if both co-factors are required for the oxidoreductase reaction to occur.



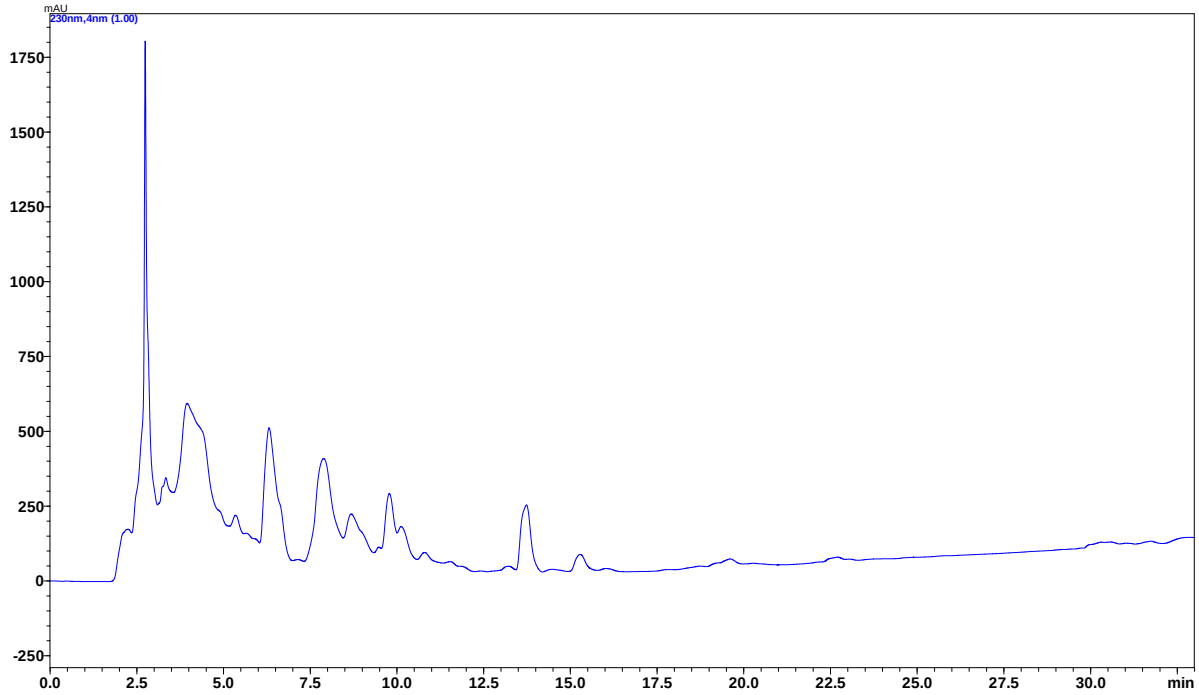
Experiment Six: 254nm; included only one oxidoreductase cofactor, NAD^+, and the corresponding protein, PtmN, as a test to see if both co-factors are required for the oxidoreductase reaction to occur.



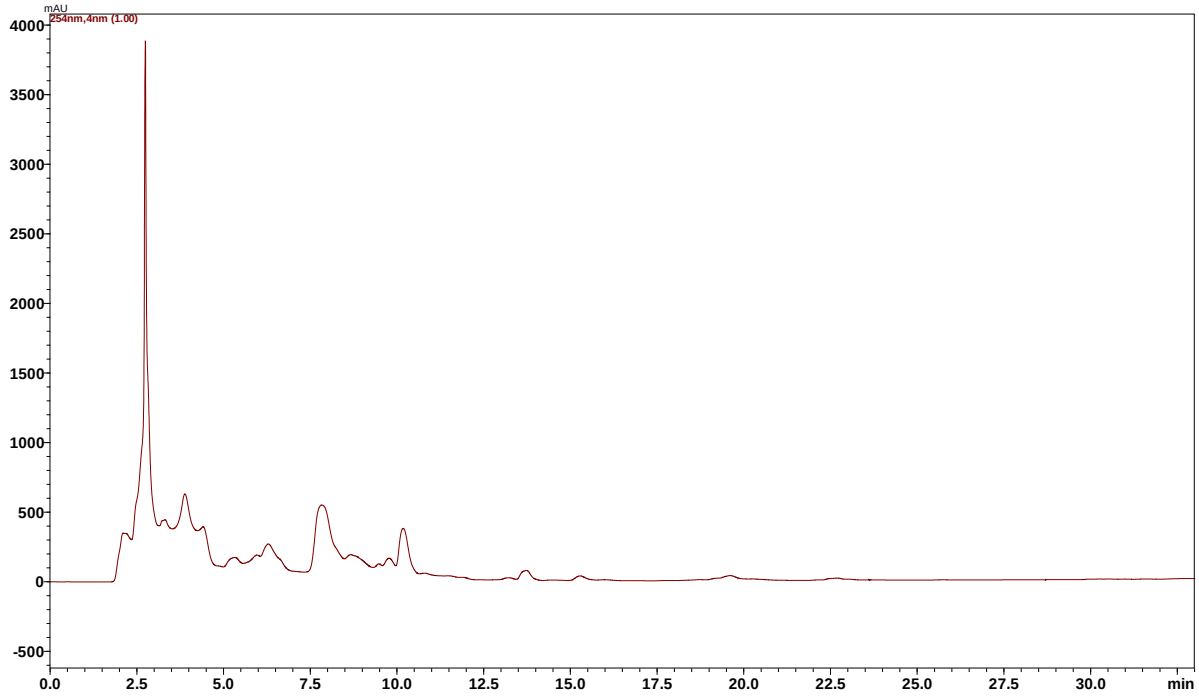
Experiment Six: 330nm; included only one oxidoreductase cofactor, NAD^+, and the corresponding protein, PtmN, as a test to see if both co-factors are required for the oxidoreductase reaction to occur.



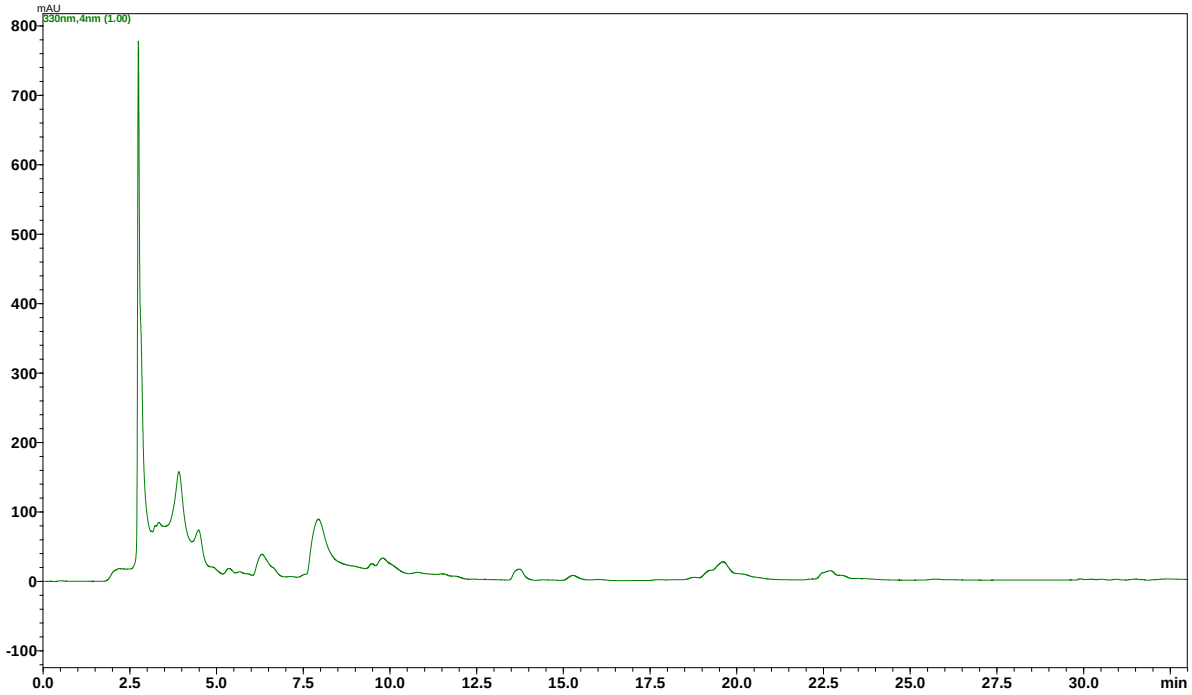
Experiment Seven: 230nm; included the Pyridoxal Phosphate (PLP) and L-Glutamate cofactors required for aminotransferase, both proteins, and one oxidoreductase co-factor: $NADP^+$. We expected to see products of the aminotransferase reaction.



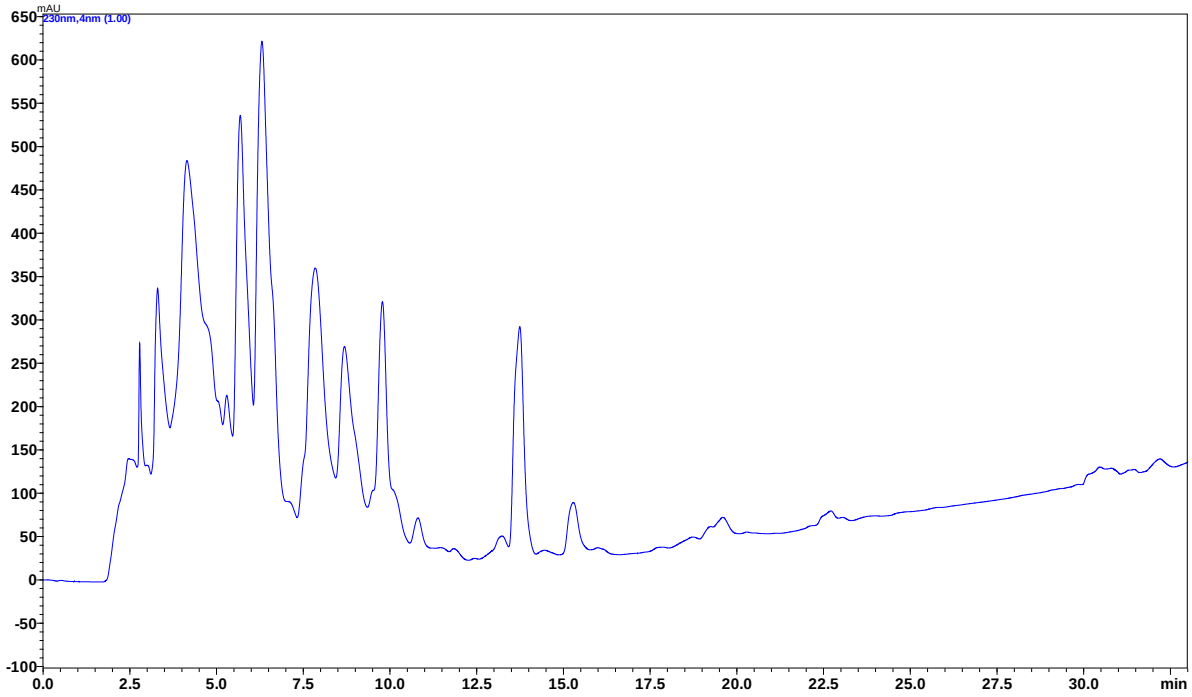
Experiment Seven: 254nm; included the Pyridoxal Phosphate (PLP) and L-Glutamate cofactors required for aminotransferase, both proteins, and one oxidoreductase co-factor: $NADP^+$. We expected to see products of the aminotransferase reaction.



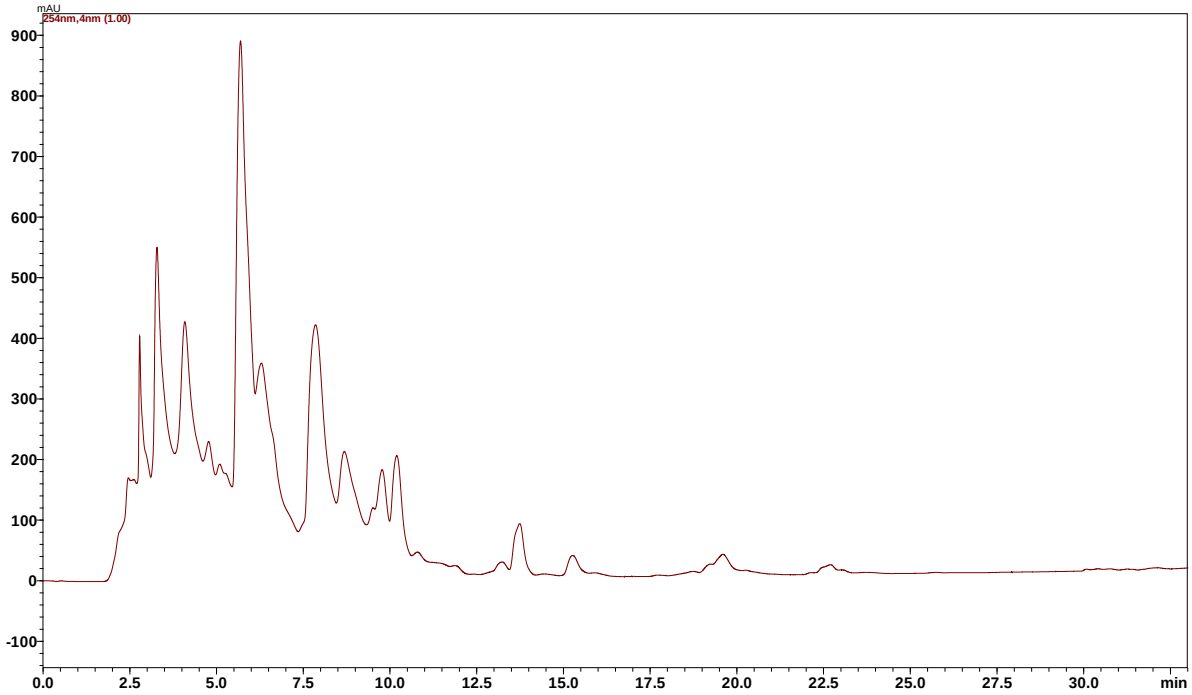
Experiment Seven: 330nm; included the Pyridoxal Phosphate (PLP) and L-Glutamate cofactors required for aminotransferase, both proteins, and one oxidoreductase co-factor: $NADP^+$. We expected to see products of the aminotransferase reaction.



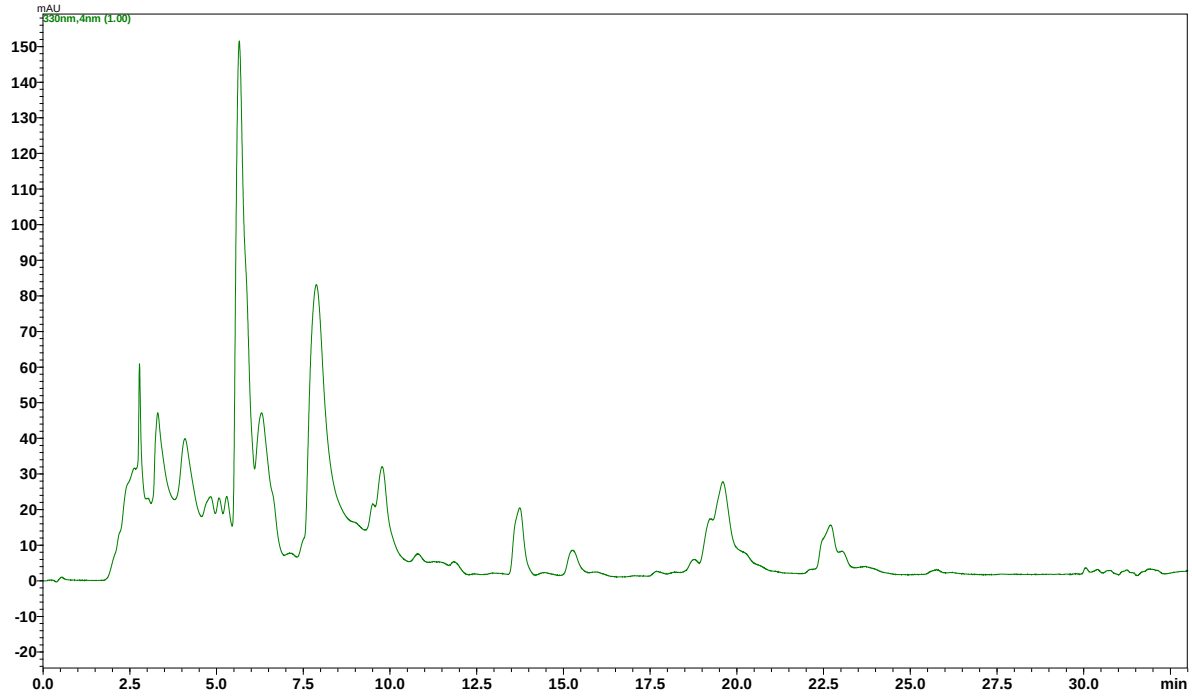
Experiment Eight: 230nm; included the Pyridoxal Phosphate (PLP) and L-Glutamate cofactors required for aminotransferase, both proteins, and one oxidoreductase co-factor: NAD^+. We expected to see products of the aminotransferase reaction.



Experiment Eight: 254nm; included the Pyridoxal Phosphate (PLP) and L-Glutamate cofactors required for aminotransferase, both proteins, and one oxidoreductase co-factor: NAD^+. We expected to see products of the aminotransferase reaction.



Experiment Eight: 330nm; included the Pyridoxal Phosphate (PLP) and L-Glutamate cofactors required for aminotransferase, both proteins, and one oxidoreductase co-factor: NAD^+. We expected to see products of the aminotransferase reaction.



PtmA Amino Acid Sequence

MRYEPWRALALWGEEEAAAALEVVRSRSLFRYYGPDLGHRTDAFERAFAELAGVPHTVAVSSGTAALTAAMV
GLGIPEGAEVIVPAVTFVASVGAVVAARGVPVFAEVDDTLTLDPAKLEELVTERTWGVMPVHLANVAADMDP
ILEVARRHGLRVIEDAAQAAGVSYRGRPVGGIGDAGAFSFQLDKNITAGEGGAVTVTDADVYDRVARYQDQG
GQFTTSKGATRGTADHPPFIGANLRMTELTAAILSVQLPRLVPLCKRLRDVARQVRAETAGLPLQWRRLPDEE
GSGGDLTFFTESRLEARRVVGALTAAGIPAHTMYQGQTVTSNRAVREGRTPWGVAWERPPRFRASEGYLGR
SVTVGLGAAMTDEDVDTIVATLRSAWADAAG

PtmA Base Pair Sequence (1176 bp)

ATGCGGTACGAGCCCTGGCGGGCCCTGGCACTGTGGGGGGAGGAGGAGGCCGCCGCGGCGCTGGAGG
TCGTCAGGTCCCGCTCGCTGTTCCGGTACTACGGGCCGGACCTGGGCCACCGCACCGACGCCTTCGAGC
GCGCCTTCGCCGAGCTGGCCGGGGTCCCGCACACCGTCGCGGTCTCCTCCGGGACGGCCGCGCTCACCG
CCGCCATGGTGGGCCTGGGCATCCCCGAGGGGGCCGAGGTCATCGTGCCCGCGGTGACCTTCGTGGCC
AGCGTCGGCGCGGTCGTCGCCGCCCGCGGCGTGCCGGTCTTCGCCGAGGTGGACGACACCCTCACCCTG
GACCCGGCGAAGCTGGAGGAGCTGGTCACCGAGCGCACCTGGGGCGTGATGCCGGTCCACCTGGCCAA
CGTGGCCGCCGACATGGACCCGATCCTGGAGGTGGCGCGCCGGCACGGCCTGCGGGTGATCGAGGACG
CCGCCCAGGCCGCCGGGGTGTCCTACCGCGGCCGGCCGGTGGGCGGCATCGGCGACGCCGGGGCGTTC
AGCTTCCAGCTCGACAAGAACATCACCGCCGGCGAGGGCGGCGCGGTCACCGTCACCGACGCCGACGTC
TACGACCGGGTGGCCCGCTACCAGGACCAGGGCGGCCAGTTCACCACCTCCAAGGGCGCCACCCGGGG
CACCGCCGACCACCCGCCGTTCATCGGCGCCAACCTGCGGATGACCGAACTGACCGCGGCCATCCTCTCG
GTGCAGCTGCCCCGGCTGGTGCCGCTGTGCAAGCGGCTGCGCGACGTGGCCCGCCAGGTGCGCGCCGA
GACCGCCGGGCTGCCCCTCCAGTGGCGGCGGCTGCCCGACGAGGAGGGCTCCGGCGGCGACCTCACCT
TCTTCACCGAGTCCCGGCTGGAGGCCCGCCGCGTGGTCGGCGCCCTCACCGCCGCCGGCATCCCGGCGC
ACACCATGTACCAGGGGCAGACCGTCACCTCCAACCGCGCGGTGCGGGAGGGACGCACCCCCTGGGGC
GTGGCCTGGGAGCGCCCGCCGCGGTTCCGGGCCAGCGAGGGCTACCTGGGCCGCTCGGTCACCGTCGG
GCTGGGCGCCGCGATGACCGACGAGGACGTGGACACCATCGTCGCCACCCTCCGCTCGGCCTGGGCGG
ACGCCGCCGGCTGA

PtmN Amino Acid Sequence

MTREKPIRFAAVGAGRVFQRYHLPCVDARDDVELVGLVDADADRAASVAAGRPGVWTGTDVARLIREARPD
ALSVCTPNDAHAAPVLAALDAGIPVLCEKPLAATVDEARRMAEHPAAAELLAVNMPFRCHSLTAPFAEAAGK
GAQRVEVSFVTPGNRVWRACTPWYGDARRAGGGALLDLGPHAIDLLMTVFGHPDVEACTVNAEGVEEQAE
LQLSFQGLPATIRIDRAARRMETAVTVTTADGAHVLDLRRNELRLADGTVRQGADRPELAAISAFFDAVTGAA
TGAAGAAGDGPAAGGAAGTSGADAAGAGATGVTGAGAVGAREALAVQLVVDEAYRRARGAAPAVT

PtmN Base Pair Sequence (1056 bp)

ATGACCCGCGAGAAGCCGATCCGGTTCGCCGCGGTGGGGGCCGGGCGGGTGTTCCAGCGCTACCACCT
GCCCTGCGTCGACGCCCGGGACGACGTGGAACTGGTGGGGCTGGTGGACGCCGACGCGGACCGGGCG
GCGTCCGTCGCGGCCGGCCGGCCGGGGGTGTGGACCGGCACCGACGTCGCGCGGCTGATCCGCGAGG
CCCGGCCGGACGCGCTCAGCGTCTGCACCCCCAACGACGCCCACGCCGCGCCGGTGCTGGCCGCGCTGG
ACGCCGGTATCCCGGTGCTCTGCGAGAAACCCCTGGCCGCCACGGTGGACGAGGCGCGGCGGATGGCC
GAGCACCCGGCCGCGGCGGAGCTGCTGGCGGTGAACATGCCGTTCCGCTGCCACTCGCTGACCGCGCCG
TTCGCCGAGGCGGCCGGCAAGGGCGCGCAGCGGGTGGAGGTCTCCTTCGTCACCCCCGGCAACCGGGT
GTGGCGGGCCTGCACCCCCTGGTACGGCGACGCCCGGCGGGCCGGCGGCGGCGCCCTGCTGGACCTCG
GTCCGCACGCCATCGACCTGCTGATGACCGTCTTCGGCCATCCGGACGTCGAGGCGTGCACGGTGAACG
CCGAGGGGGTGGAGGAACAGGCCGAGCTCCAACTGTCCTTCCAGGGCCTGCCGGCCACGATCCGGATC
GACCGGGCCGCCCGCCGGATGGAGACCGCGGTGACCGTCACCACGGCCGACGGCGCGCACGTGCTGGA
CCTGCGGCGCAACGAGCTGCGGCTCGCCGACGGCACCGTCCGGCAGGGCGCCGACCGCCCGGAACTCG
CCGCGATCTCCGCGTTCTTCGACGCGGTGACCGGCGCGGCGACCGGCGCGGCGGGGGCCGCTGGGGAC
GGCCCGGCGGCTGGCGGTGCGGCCGGCACGTCCGGAGCGGATGCGGCCGGTGCGGGCGCGACCGGTG
TGACCGGGGCGGGGGCGGTGGGCGCCCGGGAGGCGCTCGCGGTCCAGCTCGTCGTGGACGAGGCCTA
CCGCCGCGCCCGGGGCGCGGCCCCGGCGGTGACCTGA