The Laboratory of Natural Product Biosynthesis and Enzymological Medicinal Chemistry

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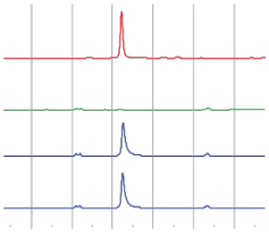
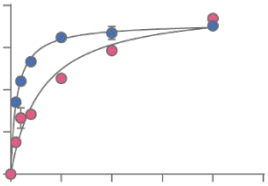
The Laboratory of Natural Product Biosynthesis and Enzymological Medicinal Chemistry Skills and Techniques

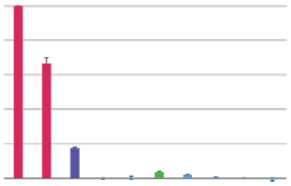
Mechanical Characterization



Function

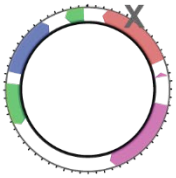
Reaction speed





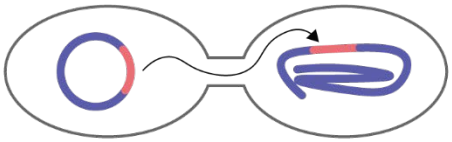
Substrates
Inhibitors

Gene/protein engineering

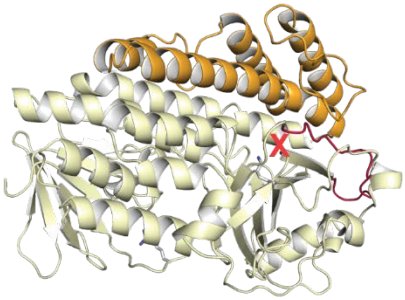


In vitro

In vivo

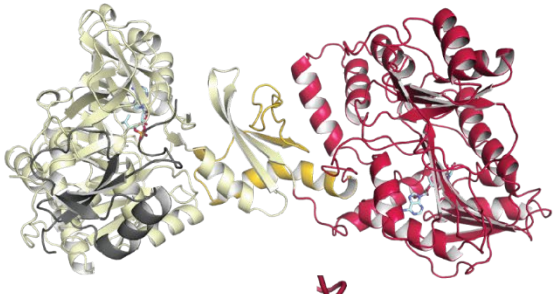


Protein

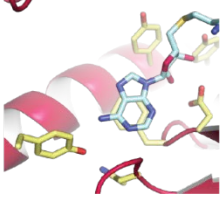


Structural Characterization

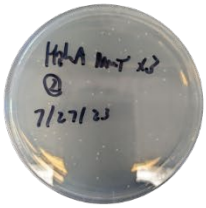
Crystallization



Structure



Antimicrobial assays



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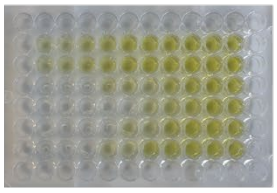
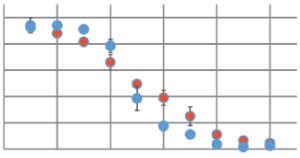
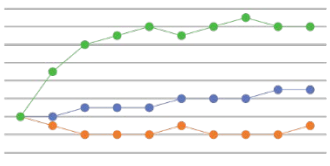




Quantitative



Broad interest: Natural product biosynthesis and the development of enzymatic tools

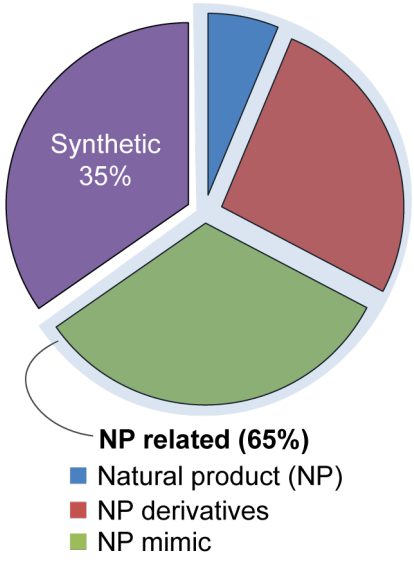


Figure 1: Source of small molecule drugs approved during 1981-2019 in USA.[1]

Natural products (NPs), secondary metabolites biosynthesized by microorganisms and plants, are a very important source of drugs and drug leads. Infectious diseases, autoimmune diseases, and other ailments have been treated by these molecules throughout human history. In ancient times, crude drugs that are dried and/or ground organism matters were used to treat these diseases. The clinical use of NPs was greatly intensified by the discovery and isolation of the first pure antibiotic penicillin in 1928.[2] NP drugs generally have high selectivity (less adverse effects) thanks to their complex chemical structures. In the past 40 years, nearly 65% of U.S. Food and Drug Administration (FDA) approved small molecule drugs have been NP-related (Figure 1).[1] However, a new threat has been appearing in recent years. According to the World Health Organization, infections by antibiotic-resistant pathogens are increasing globally and are anticipated as one of the greatest threats to human health in the future.[3] Therefore, new drug candidates for use in clinics are in high demand.

Since a small modification on the chemical structure of compounds may dramatically alter their bioactivity, bioavailability, and/or biostability, derivatization of known bioactive molecules is a promising cost- and time-effective approach to developing new drugs.[4, 5] However, it is significantly challenging to modify a specific part of the structure in a specific way by conventional organic chemistry, especially when the chemical structure of the parent compound is complex, as is the case with NPs. Therefore, enzymatic modification of bioactive compounds has gathered much attention in the drug discovery field.[6]

NIH-funded project (1R15GM151721-01; the origins of amino acid selectivity in the homologation pathway)

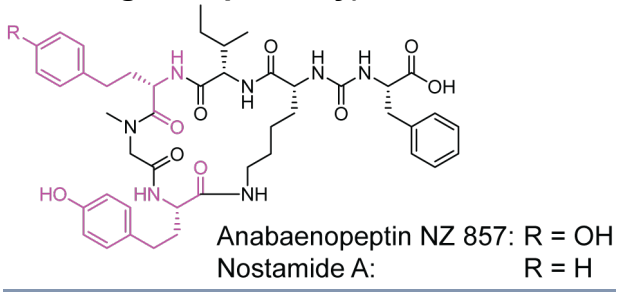


Figure 2: Structures of cyanobacterial NRPs anabaenopeptins produced by *Nostoc punctiforme* PCC 73102 (ATCC 29133). The homologated residues are shown in purple.

To use enzymes as tools for drug modification, it is essential to understand their functions. As a platform, I selected nonribosomal peptide synthetases (NRPSs) because they are one of the largest enzymatic families of NP biosynthesis; additionally, they have been shown to be flexible to engineering.[7] NRPSs are large modular proteins that contain multiple catalytic domains in each module. Each domain has a specific and distinct function, and each module is responsible for assembling one amino acid or amino acid-like building block. The orchestrated actions of modules and domains produce extremely diverse peptide NPs that are termed nonribosomal peptides (NRPs).[8] By directing the actions of these domains and modules through mutagenesis, the chemical structure of the final compound can be modified.

Pathogens develop antibiotic resistance in various ways, one of which uses enzymes to degrade or modify the target so that the molecule is no longer active. Proteases or peptidases

are often used to develop resistance in peptide NPs.[9, 10] To tackle this resistance mechanism, nature uses modified amino acids such as D-amino acid and β-amino acid as building blocks in these NPs, so that the enzymes do not recognize the NPs as their substrates to degrade. Obtaining D-amino acid is the most common strategy for microorganisms to take in NRP biosynthesis, but for that reason, many other microorganisms possess or develop resistance to D-amino acid containing NPs.[11] Therefore, it is important to develop new strategies to tackle resistance to peptide NPs. There are some found to have an uncommon modification of amino acid(s) in their structure, homologation (addition of methylene group to extend the side chain of amino acid) (Figure 2).[12] By applying the enzymatic homologation pathway to other NRPSs as a homologation tool, more biologically stable NRPs can be produced. In this project, we focus on characterizing the enzymatic homologation of aromatic amino acids, L-phenylalanine (L-Phe) and L-tyrosine (L-Tyr).

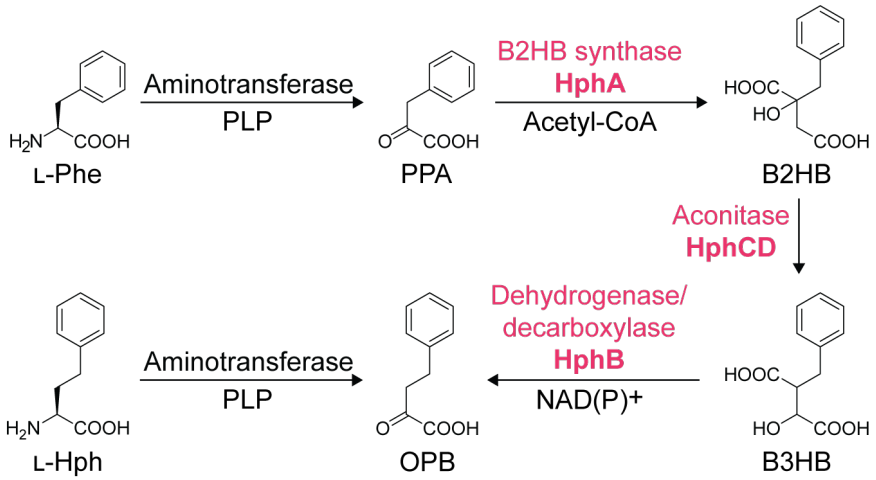


Figure 3: Proposed homologation pathway of L-Phe in the anabaenopeptin biosynthesis.[13, 14] Enzymes that carry out the transformation are indicated on top or left of the arrows. Enzymes colored red are the focus of this study. The compound abbreviations denote the following: L-Hph = homologated L-Phe, PPA = phenylpyruvic acid, B2HB = 2-benzyl-2-hydroxybutanedioic acid, B3HB = 2-benzyl-3-hydroxybutanedioic acid, and OPB = 2-oxo-4-phenylbutyric acid.

Homologation of L-Phe and L-Tyr is proposed to be catalyzed by four enzymes, which contain aminotransferase (AT), B2HB synthase, aconitase, and dehydrogenase/ decarboxylase (Figure 3).[13] Among these four enzymes, AT is very well characterized, and aromatic-amino-acid AT that is present in all microbial species catalyzes the reaction from L-Phe to phenylpyruvic acid (PPA) as well as 2-oxo-4-phenylbutyric acid (OPB) to homologated L-Phe (L-Hph).[14] However, the other three enzymes are yet to be characterized. To characterize those enzymes, my lab test the activity of the purified enzymes *in vitro* after the genes that encode these three enzymes are cloned for overexpression and purification of the enzymes from the cyanobacterium *Nostoc punctiforme* PCC73102 (ATCC 29133), which produces NRPs (anabaenopeptins) that contain homologated L-Phe and L-Tyr in the structure (Figure 2).[13] The enzymes will also be crystallized to determine the structure for structure-guided mutagenesis studies to understand the enzymes as well as expand their substrate scope.

Ultimate goals of the homologation project

The goals of this project are the development of an enzymatic homologation tool for (Figure 4):

- *Derivatization of existing NRPs*
- *Mass production of homologated amino acids*

Goal 1: as noted above, enzymes to produce NRPs are flexible to engineering, therefore the homologation pathway can be integrated into the biosynthetic pathway of an NRP with slight modifications of parent enzymes by combinatorial biosynthesis. The newly synthesized NRPs are expected to have enhanced biostability than the original molecule.

Goal 2: homologated amino acids are an important tool for designing new synthetic drugs, but the full potential has not been utilized because of the high cost of these molecules. By engineering the homologation pathway to be able to accept many other amino acids, the homologated amino acids can be mass-produced, which can be used to synthesize new drug candidates at a much lower cost.

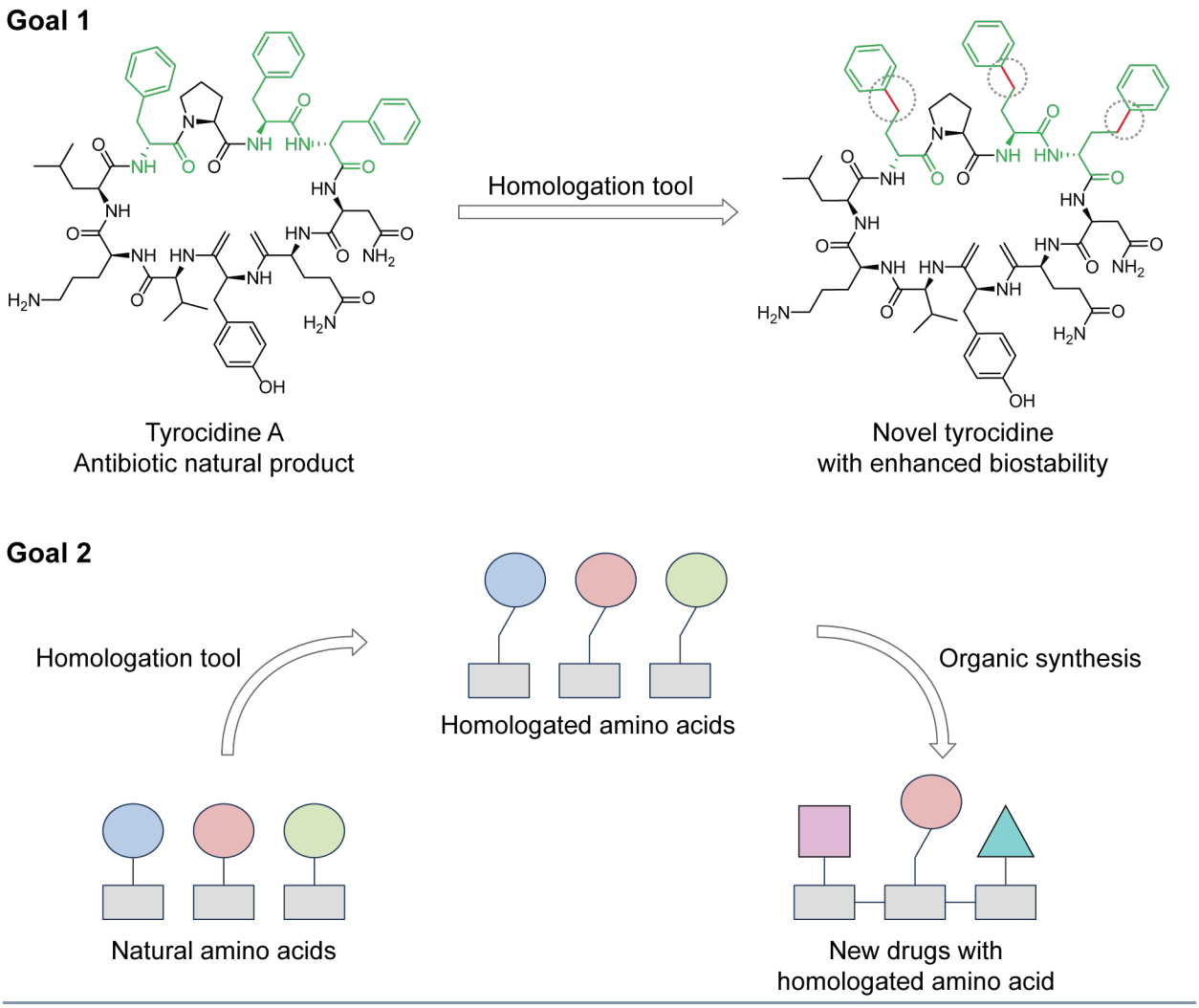


Figure 4: Goals of the homologation project.

Other projects and collaborations are going on…

Publication list of Shogo Mori, Ph.D.

Study area code: (M = mechanical characterization, S = structural characterization, E = engineering study, T = development of the enzymatic tool, D = discovery of new NPs and antimicrobial study, I = bioinformatics)

At Augusta University

1. Stewart, L. E.; Owens, S. L.; Ahmed, S. R.; Lang, R. M.; **Mori, S.** (2024) Characterization of HphA – the first enzyme in the enzymatic homologation pathway for L-phenylalanine and L-tyrosine. *ChemBioChem*. *Under revision*. (Study area: M)
2. Owens, S. L.; Ahmed, S. R.; Lang, R. M.; Stewart, L. E.; **Mori, S.** (2024) Natural Products That Contain Higher Homologated Amino Acids. *ChemBioChem*, 25(9), e202300822. (Review)

Before Augusta University

3. Lundy, T. A.†; **Mori, S.**†; Garneau-Tsodikova, S. (2020). Lessons learned in engineering interrupted adenylation domains when attempting to create trifunctional enzymes from three independent monofunctional ones. *RSC Advances., 10*, 34299-34307. (Study area: E)
4. **Mori, S.**; Garneasu-Tsodikova, S.; Tsodikov, O. V. (2020). Unimodular methylation by adenylation-thiolation domains containing an embedded methyltransferase. *J. Mol. Biol., 432(21)*, 5802-5808. (Study area: M, E)
5. Lundy, T. A.†; **Mori, S.**†; Garneau-Tsodikova, S. (2020). A thorough analysis and categorization of bacterial interrupted adenylation domains, including previously unidentified families. *RSC Chem. Biol., 1*, 233-250. **Selected as one of the 2021 Editors' Choice publications.** (Study area: I)
6. Lundy, T. A.†; **Mori, S.**†; Thamban Chandrika, N.; Garneau-Tsodikova, S. (2020). Characterization of a Unique Interrupted Adenylation Domain That Can Catalyze Three Reactions. *ACS Chem. Biol., 15(1)*, 282-289. (Study area: M)
7. **Mori, S.**†; Pang, A. H.†; Thamban Chandrika, N.; Garneau-Tsodikova, S.; Tsodikov, O. V. (2019). Unusual substrate and halide versatility of phenolic halogenase PltM. *Nat. Commun. 10*, 1255. (Study area: M, S, T)
8. Lundy, T. A.; **Mori, S.**; Garneau-Tsodikova, S. (2019). Probing the limits of interrupted adenylation domains by engineering a trifunctional enzyme capable of adenylation, *N*-, and *S*-methylation. *Org. Biomol. Chem. 17*, 1169-1175. (Study area: E)
9. **Mori, S.**; Green, K. D.; Choi, R.; Buchko, G. W.; Fried, M. G.; Garneau-Tsodikova, S. (2018). Using MbtH-like proteins to alter substrate profile of a nonribosomal peptide adenylation enzyme. *ChemBioChem*. *19(20)*, 2186-2194. (Study area: M)
10. **Mori, S.**; Garneau-Tsodikova, S. (2018). Making figures: are you taking the best approach to maximize visibility? *MedChemComm*., *9(9)*, 1399-1403. (Opinion)
11. **Mori, S.**†; Pang, H. A.†; Lundy, T. A.; Garzan, A.; Tsodikov, O. V.; Garneau-Tsodikova, S. (2018). Structural basis for backbone *N*-methylation by an interrupted adenylation domain. *Nat. Chem. Biol.*, *14(5)*, 428-430. (Study area: M, S)
12. Lundy, T. A.; **Mori, S.**; Garneau-Tsodikova, S. (2018). Engineering bifunctional enzymes capable of adenylating and selectively methylating the core or side chain of amino acids. *ACS Synth. Biol.*, *7(2)*, 399-404. (Study area: E)
13. **Mori, S.**; Garzan, A.; Tsodikov, O. V.; Garneau-Tsodikova, S. (2017) Deciphering Nature's intricate way of *N*,*S*-dimethylating L-cysteine: Sequential action of two bifunctional adenylation domains. *Biochemistry*, *56(46)*, 6087-6097. (Study area: M)
14. **Mori, S.**; Shrestha, S. K.; Fernandez, J.; Alvarez San Millan, M.; Garzan, A.; Al-Mestarihi, A. H.; Lombó, F.; Garneau-Tsodikova, S. (2017). Activation and loading of the starter unit during thiocoraline biosynthesis. *Biochemistry*, *56(34)*, 4457-4467. (Study area: M)

15. **Mori, S.**; Nepal, K.; Kelly, G. T.; Sharma, V.; Simkhada, D.; Gowda, V.; Delgado, D.; Watanabe, C. M. H. (2017). The priming of azabicycle biosynthesis in the azinomycin class of antitumor agents. *Biochemistry*, *56(6)*, 805-808. (Study area: M)
16. **Mori, S.**; Simkhada, D.; Zhang, H.; Erb, M. S.; Zhang, Y.; Williams, H.; Fedoseyenko, D.; Russell, W. K.; Kim, D.; Fleer, N.; Ealick, S.; Watanabe, C. M. H. (2016). Polyketide ring expansion mediated by a thioesterase, chain elongation and cyclization domain, in azinomycin biosynthesis: characterization of AziB and AziG. *Biochemistry*, *55(4)*, 704-714. (Study area: M)
17. **Mori, S.**; Williams, H.; Cagle, D.; Karanovich, K.; Horgen, F. D.; Smith III, R.; Watanabe, C. M. H. (2015). Macrolactone nuiapolide, isolated from a Hawaiian marine *Cyanobacterium*, exhibits anti-chemotactic activity. *Mar. Drugs*, *13(10)*, 6274-6290. (Study area: D)
18. Simkhada, D.; Zhang, H.; **Mori, S.**; Williams, H.; Watanabe, C. M. H. (2013). Activation of cryptic metabolite production through gene disruption: dimethyl furan-2,4-dicarboxylate produced by *Streptomyces sahachiroi*. *Beilstein J. Org. Chem*., *9*, 1768-1773. (Study area: E, D)
19. Foulke-Abel, J.; Agbo, H.; Zhang, H.; **Mori, S.**; Watanabe, C. M. H. (2011). Mode of action and biosynthesis of the azabicycle-containing natural products azinomycin and ficellomycin. *Nat. Prod. Rep*., *28(4)*, 693-704. (Review)

Protein Structures Deposited in PDB

1. **5WMM**: Crystal Crystal structure of an adenylation domain interrupted by a methylation domain (AMA4) from nonribosomal peptide synthetase TioS. (2018) Pang, A.H.; **Mori, S.**; Garneau-Tsodikova, S.; Tsodikov, O.V. (2.9 Å)

Patents

1. **United States Patent Application 20200299671**: Immobilized Multi-Enzymatic Halogenation System. (2020) Garneau-Tsodikova, S.; Tsodikov, O.V.; **Mori, S.**; Burkart, M.D.; La Clair, J.J.

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3. WHO *Antimicrobial resistance: global report on surveillance 2014*; World Health Organization: 2014.
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