The Laboratory of Natural Product Biosynthesis and Enzymological Medicinal Chemistry

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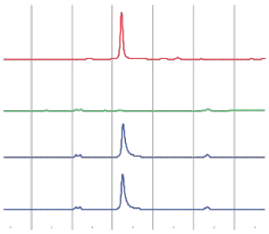
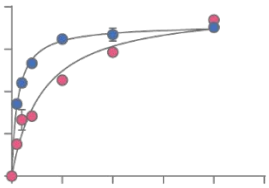
The Laboratory of Natural Product Biosynthesis and Enzymological Medicinal Chemistry Skills and Techniques

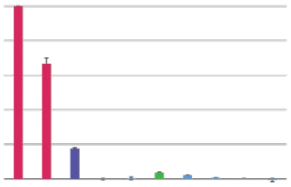
Functional Characterization



Function

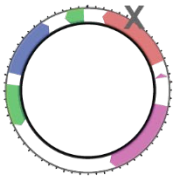
Reaction speed





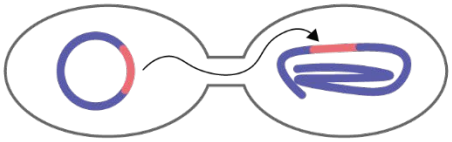
Substrates
Inhibitors

Gene/protein engineering

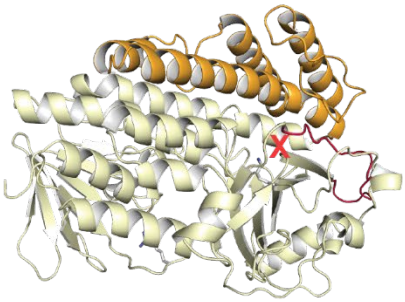


In vitro

In vivo

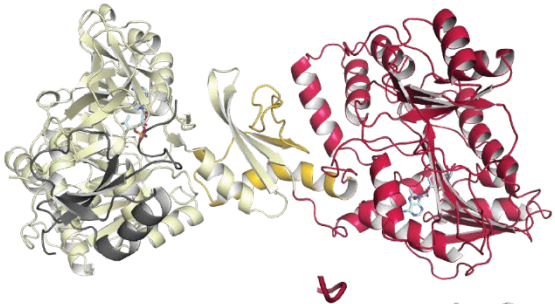


Protein

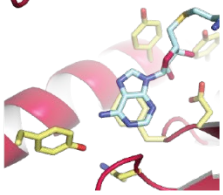


Structural Characterization

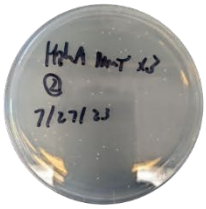
Crystallization



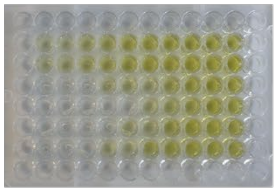
Structure

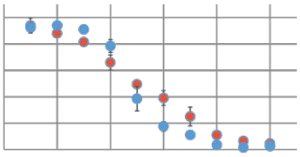


Antimicrobial assays

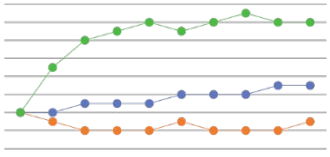


Qualitative





Quantitative



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

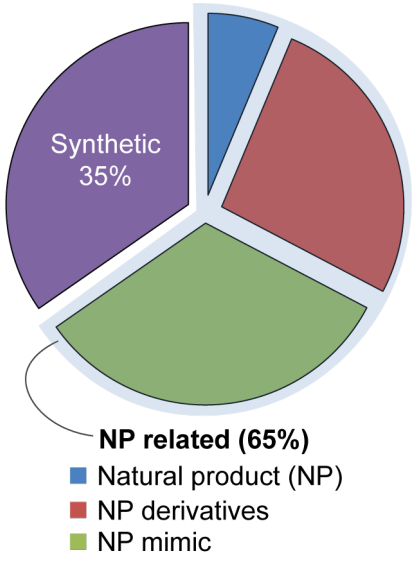


Figure 1: Source of small molecule drugs approved during 1981-2019 in USA.[1]

Natural products (NPs), which are secondary metabolites produced 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 materials were used for treatment. The clinical application of NPs was greatly intensified by the discovery and isolation of the first pure antibiotic penicillin in 1928.[2] NP-based drugs generally exhibit high selectivity (less adverse effects) due to their complex chemical structures. Over the past 40 years, nearly 65% of U.S. Food and Drug Administration (FDA) approved small molecule drugs have been related to NPs (Figure 1).[1] However, a new threat has emerged in recent years. According to the World Health Organization, infections by antibiotic-resistant pathogens are rising globally and are anticipated to become one of the greatest threats to human health in the future.[3] Therefore, there is a growing demand for new drug candidates for clinical use..

Since even small modifications on the chemical structure of compounds can dramatically affect 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 particular 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 gained considerable attention in the drug discovery field.[6]

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

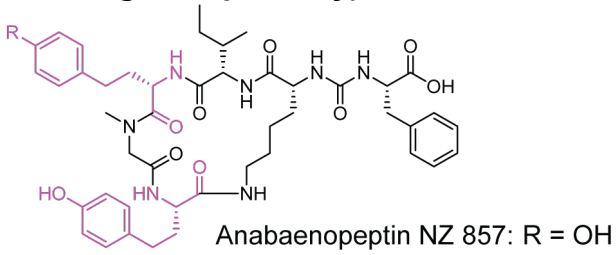


Figure 2: Structures of cyanobacterial NRPs anabaenopeptins produced by *Nostoc punctiforme* PCC 73102 (ATCC 29133). The homologated residues are shown in purple.

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

Pathogens develop antibiotic resistance through various mechanisms, one of which involves the use of enzymes to degrade or modify the target molecule, rendering it inactive. Proteases or peptidases are often utilized by pathogens to develop resistance against peptide NPs.[9, 10] To tackle this resistance mechanism, nature employs modified amino acids, such as D-amino acid and β-amino acid, as building blocks in these NPs. This modification helps ensure that the enzymes do not recognize the NPs as 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 have developed or acquired resistance to NPs that contain D-amino acids.[11] Therefore, it is important to develop new strategies to combat resistance to peptide NPs. Some NPs exhibit uncommon modifications to their amino acid structures, such as homologation: the addition of a methylene group to extend the side chain of an amino acid (Figure 2).[12] By applying the enzymatic homologation pathway to other NRPSs as a homologation tool, it is possible to produce more biologically stable NRP variants. In this project, we focus on characterizing the enzymatic homologation of the aromatic amino acids, L-phenylalanine (L-Phe) and L-tyrosine (L-Tyr).

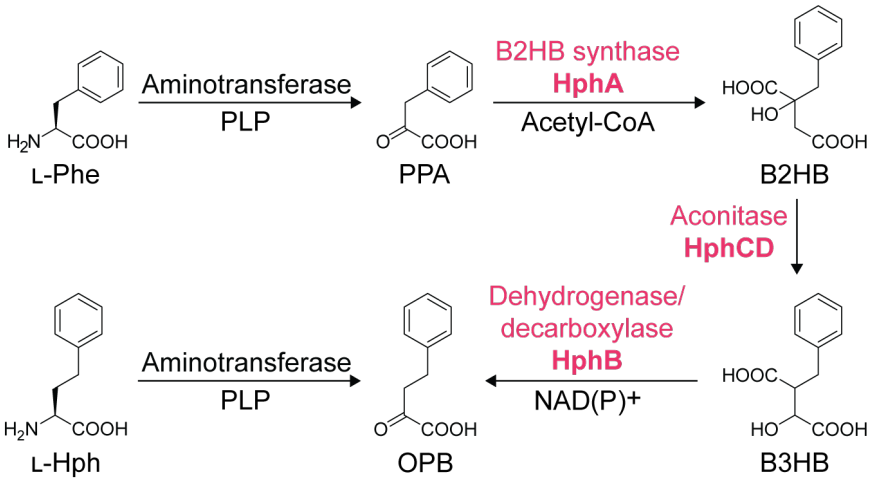


Figure 3: Proposed homologation pathway of L-Phe in the anabaenopeptin biosynthesis.[13, 14] Enzymes that carry out the transformation are indicated on top or left of the arrows. Enzymes colored red are the focus of this study. The compound abbreviations denote the following: L-Hph = homologated L-Phe,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

The homologation of L-Phe and L-Tyr has been proposed to be catalyzed by four enzymes, which contain aminotransferase (AT), B2HB synthase, aconitase, and dehydrogenase/decarboxylase (Figure 3).[13] Among these enzymes, AT is well characterized, and aromatic-amino-acid AT that is present in all microbial species catalyzes the conversion of L-Phe to phenylpyruvic acid (PPA) and the transformation of 2-oxo-4-phenylbutyric acid (OPB) to homologated L-Phe (L-Hph).[14] However, the other three enzymes are yet to be characterized. To study these enzymes, my lab tests the activity of the purified enzymes *in vitro* after cloning the genes that encode them for overexpression and purification of the enzymes from the cyanobacterium *Nostoc punctiforme* PCC73102 (ATCC 29133). This cyanobacterium produces NRPs (anabaenopeptins) that incorporate homologated L-Phe and L-Tyr in their structures (Figure 2).[13] The enzymes will also be crystallized to determine the structure, enabling structure-guided mutagenesis studies to understand the enzymes and 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 discussed above, enzymes involved in the production of NRPs are adaptable for engineering. This flexibility allows for the integration of the homologation pathway into the biosynthetic pathway of an NRP, with only minor modifications to the parent enzymes through combinatorial biosynthesis. The newly synthesized NRPs are expected to exhibit enhanced biostability compared to the original molecule.

Goal 2: Homologated amino acids serve as a valuable tool for designing new synthetic drugs, but their full potential has not yet been utilized due to the high costs associated with these molecules. By engineering the homologation pathway to accommodate a wider variety of amino acids, it is possible to mass-produce homologated amino acids. This development would facilitate the synthesis of new drug candidates at significantly lower costs.

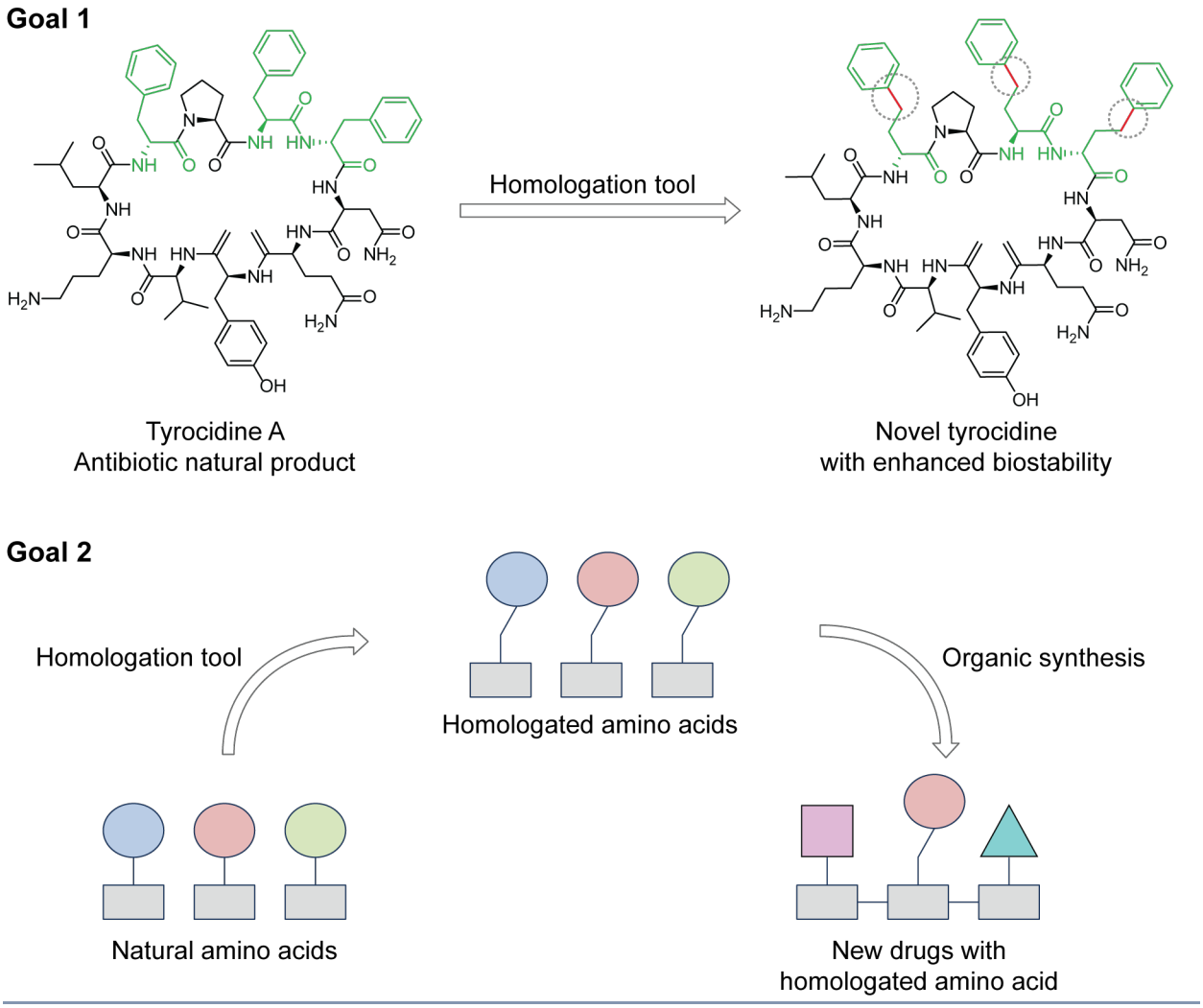


Figure 4: Goals of the homologation project.

Other projects and collaborations are going on…

Publication list of Shogo Mori, Ph.D.

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

At Augusta University

1. Nkosi, A. K.; Girgis, A. S.; Samir, A.; Morsy M. A.; Shaban, A. M.; Fayad, W.; Soliman, A. A. F.; Williams, C.; **Mori, S.**; Khanna L.; Verbeck G.; Panda, S. S. (2026) Multifunctional Curcumin Inspired 3,5 Diarylidene 4 Piperidones: Design, Synthesis, Biological Evaluation, and Computational Mechanistic Studies. *Pharmaceuticals*, *submitted*. (Study area: O)
2. Lang Harman, R. M.; Blackstone, H. G.; Aruna, F. O.; Patel, S. R.; Shin, M.; NeSmith, R. K.; Dickson, D. B.; Spencer, A. C.; **Mori, S.** (2026). Amenability of the Gatekeeper Enzyme HphA to Engineering in the Homologation Pathway of L-Phenylalanine and L-Tyrosine through Homology-Based Site-Directed Mutagenesis. *ACS Omega*, 11(8), 13789-13798. (Study area: E)
    a. *Preprint*: Lang Harman, R. M.; Blackstone, G.; Aruna, F. O.; Patel, S. R.; Shin, M.; NeSmith, R. K.; Dickson, D. B.; Spencer, A. C.; **Mori, S.** (2025). Amenability to Engineering of the Homologation Enzyme, HphA, through Homologous-Based Site-Directed Mutagenesis. *bioRxiv*, doi: 10.64898/2025.12.01.691582. (Study area: E)
3. Buchanan, D.; **Mori, S.**; Chadli, A.; Panda, S. S. (2025) Natural Cyclic Peptides: Synthetic Strategies and Biomedical Applications. *Biomedicines*, *12(1)*, 240. (Review)
4. 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*, 25(16), e202400369. (Study area: F)
5. 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

6. 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)
7. **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: F, E)
8. 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)
9. 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: F)
10. **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: F, S, T)
11. 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)

12. **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: F)
13. **Mori, S.**; Garneau-Tsodikova, S. (2018). Making figures: are you taking the best approach to maximize visibility? *MedChemComm*., *9(9)*, 1399-1403. (Opinion)
14. **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: F, S)
15. 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)
16. **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: F)
17. **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: F)
18. **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: F)
19. **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: F)
20. **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)
21. 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)
22. 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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14. Cho, B. K.; Seo, J. H.; Kang, T. W.; Kim, B. G., Asymmetric synthesis of L-homophenylalanine by equilibrium-shift using recombinant aromatic L-amino acid transaminase. *Biotechnol Bioeng* **2003,** *83* (2), 226-34.