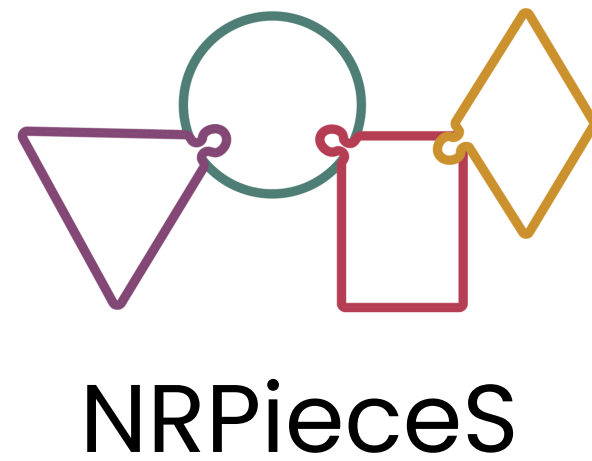
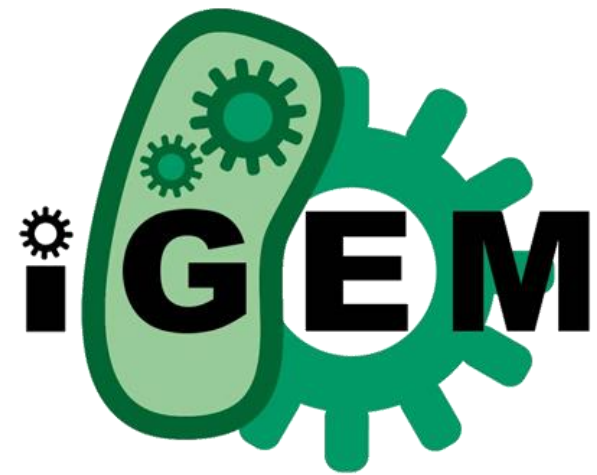


Fighting Antimicrobial Resistance with Non-Ribosomal Peptide Synthetases

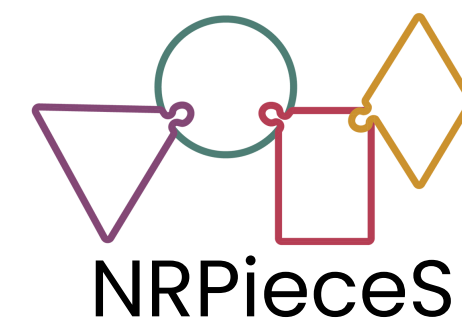


iGEM Team Marburg 2025

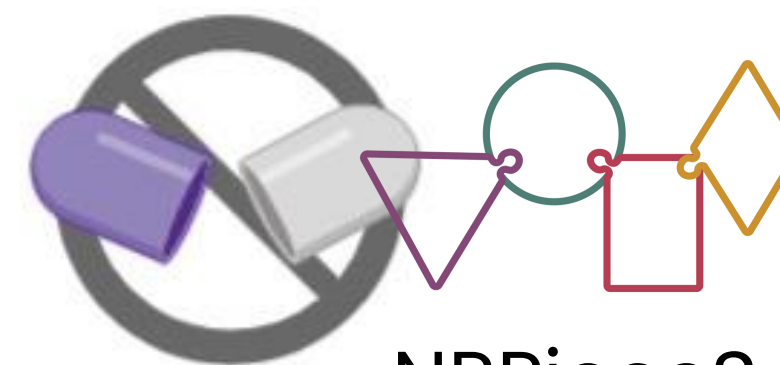
Hesen Trade & Invest



Just a Cut?



Discovery of Penicillin



Discovery void

NRPieceS

1910

1928

1945

1975

1987

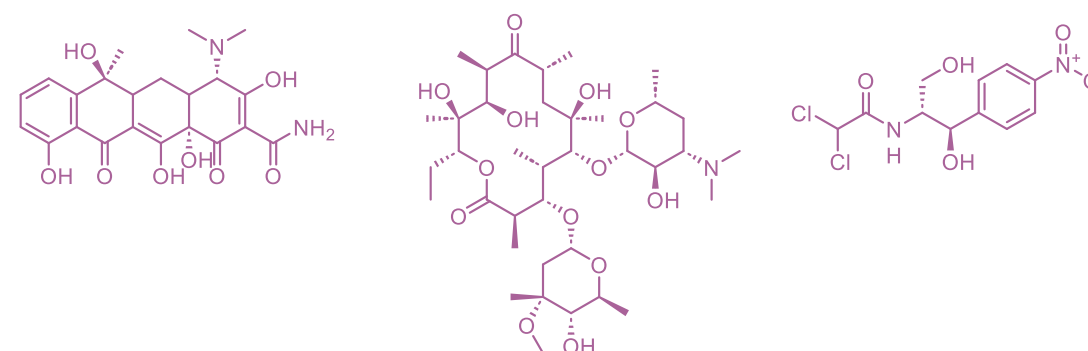
2025

2050

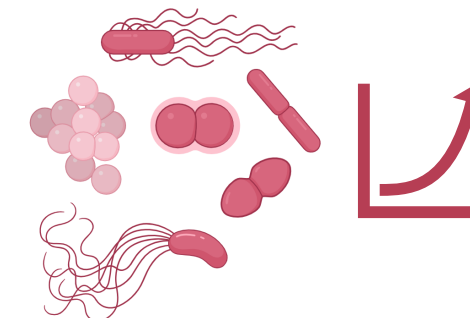
Infections are No. 1
cause of death



Golden age of antibiotics



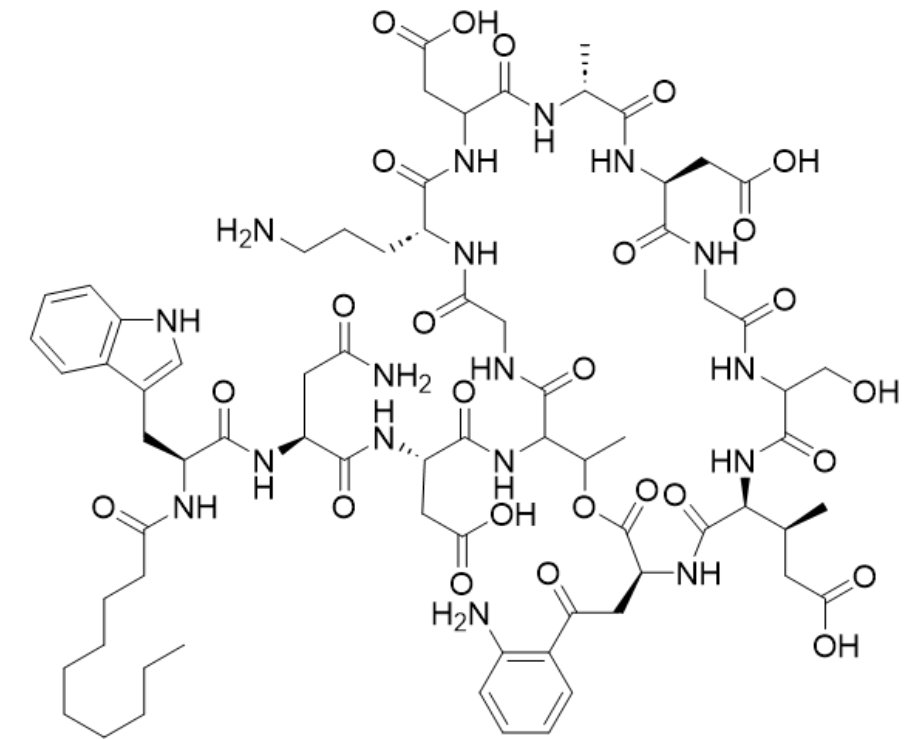
Increase of
resistant bacteria



10 million deaths
per year



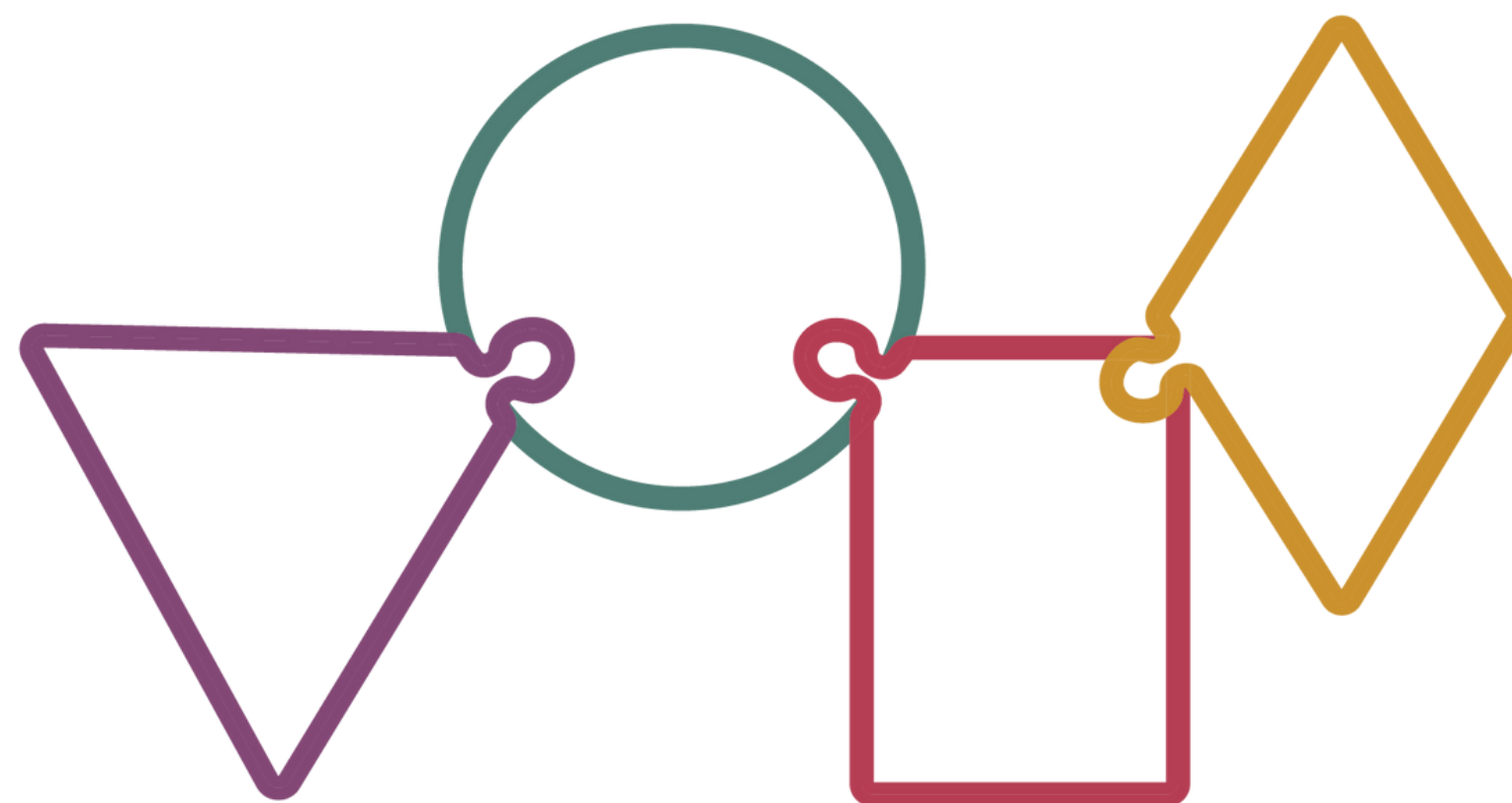
NRPieces



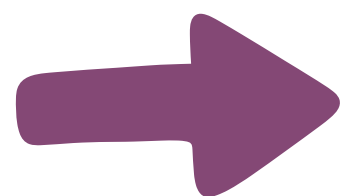
Daptomycin

Produced by Non-Ribosomal Peptide Synthetases (NRPS)

We need new ways to discover antibiotics!

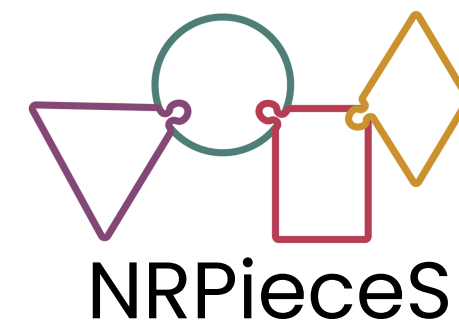


NRPPieces



Sustainable, high-throughput antibiotic
discovery via **engineered NRPS**

What are Non-Ribosomal Peptide Synthetases?

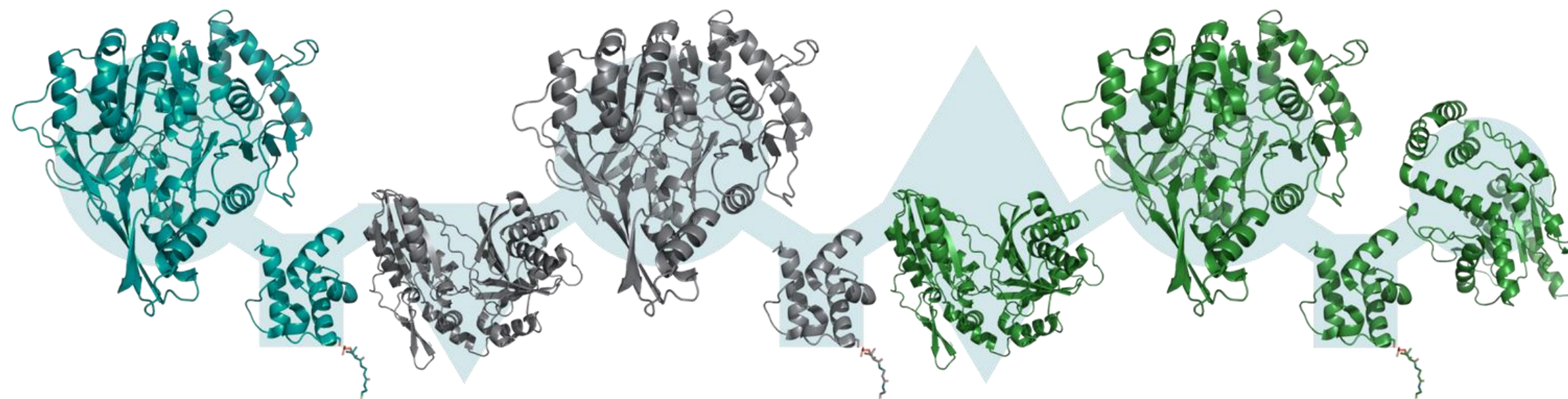


Module 1

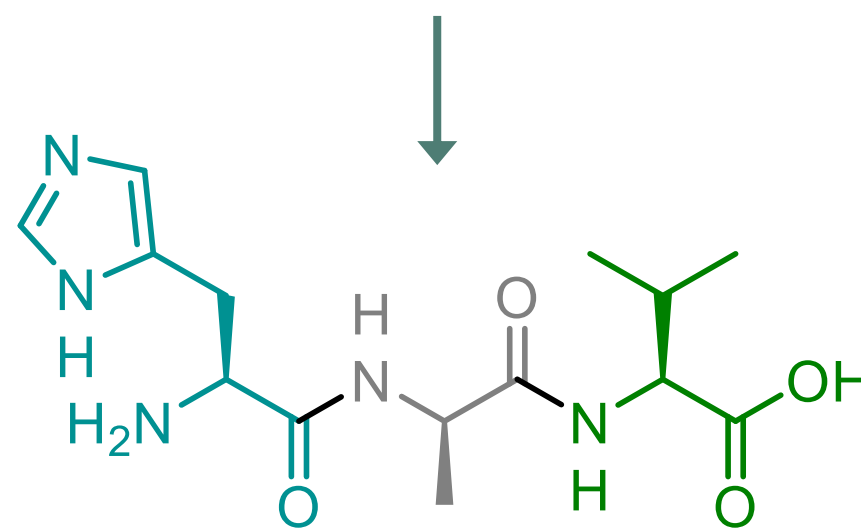
Module 2

Module 3

NRPS

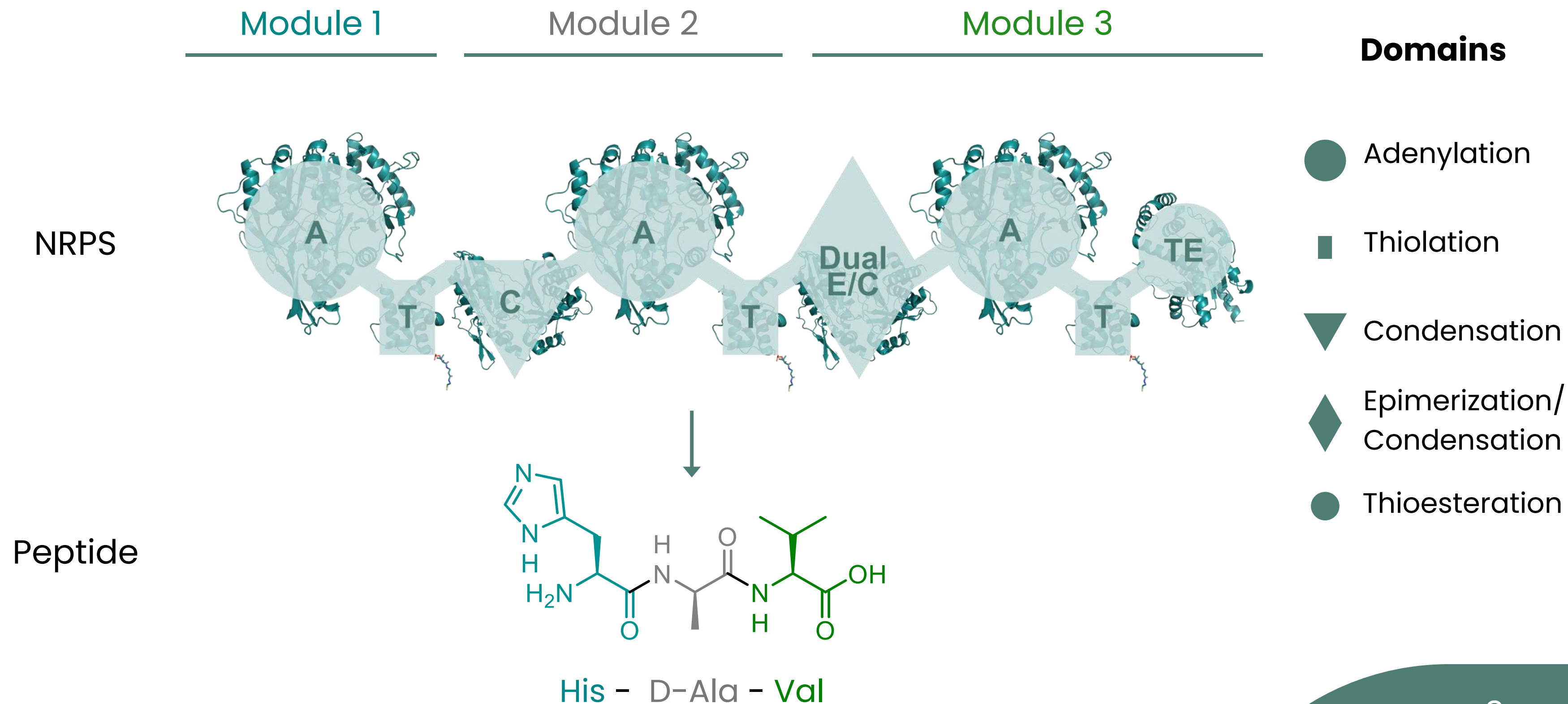
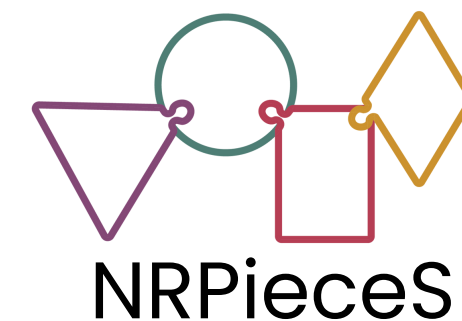


Peptide

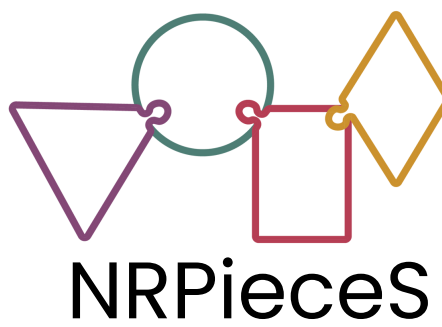


His - D-Ala - Val

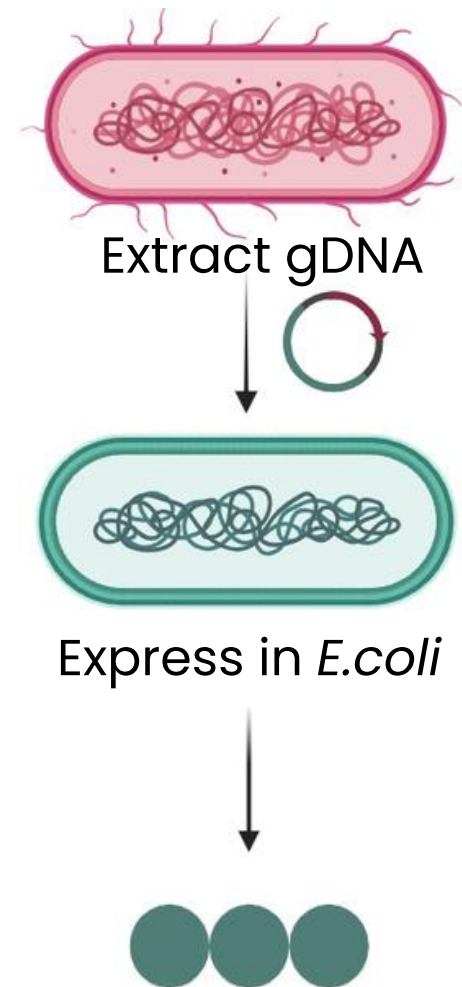
What are Non-Ribosomal Peptide Synthetases?



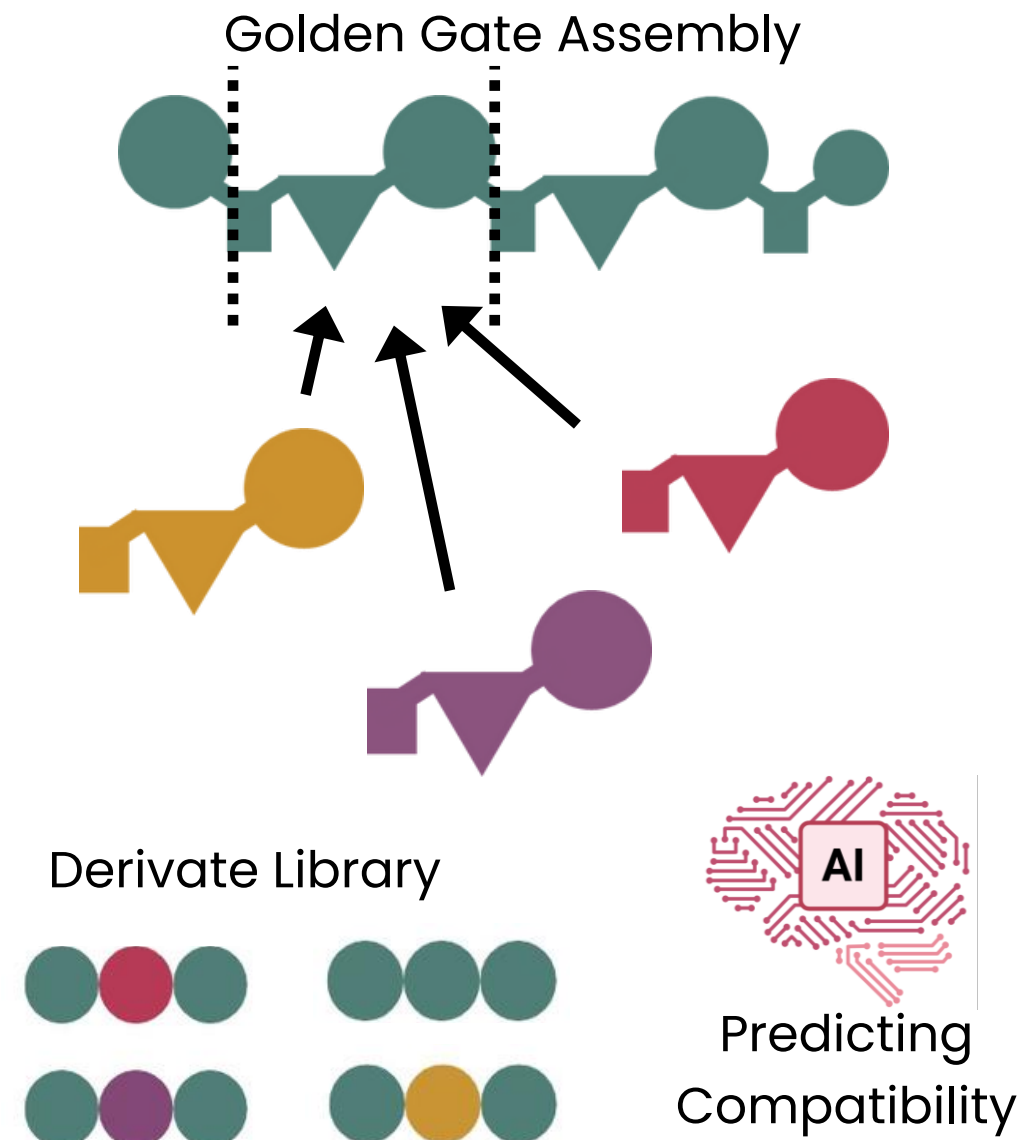
Reinventing Antibiotic Discovery with NRPS



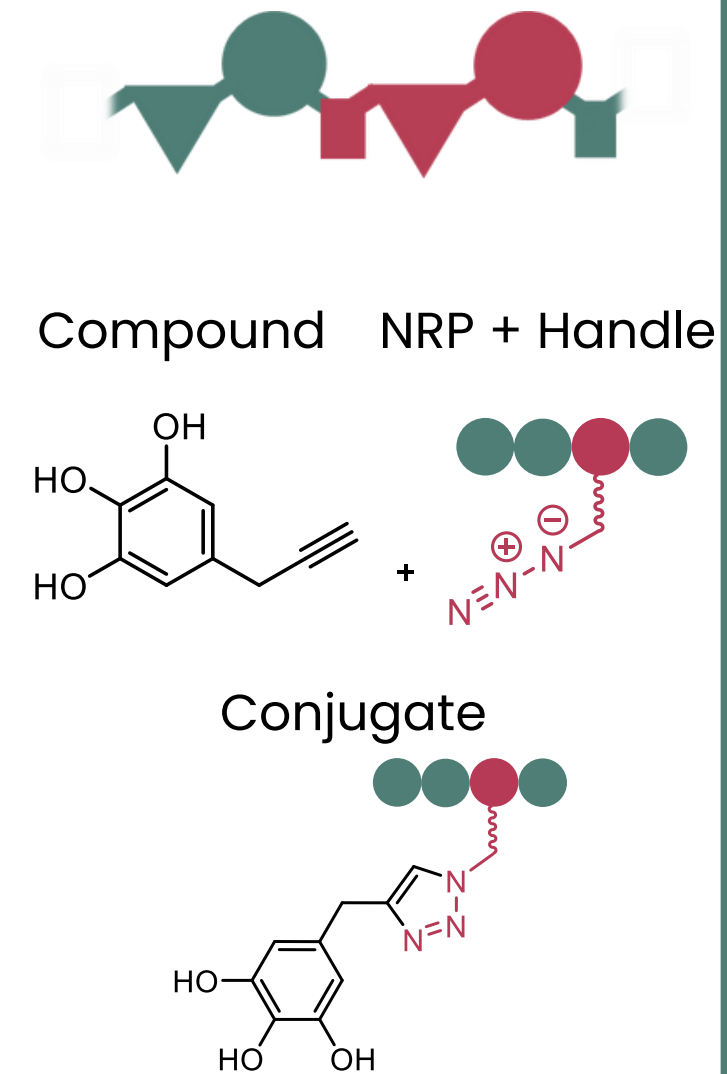
Heterologous Expression



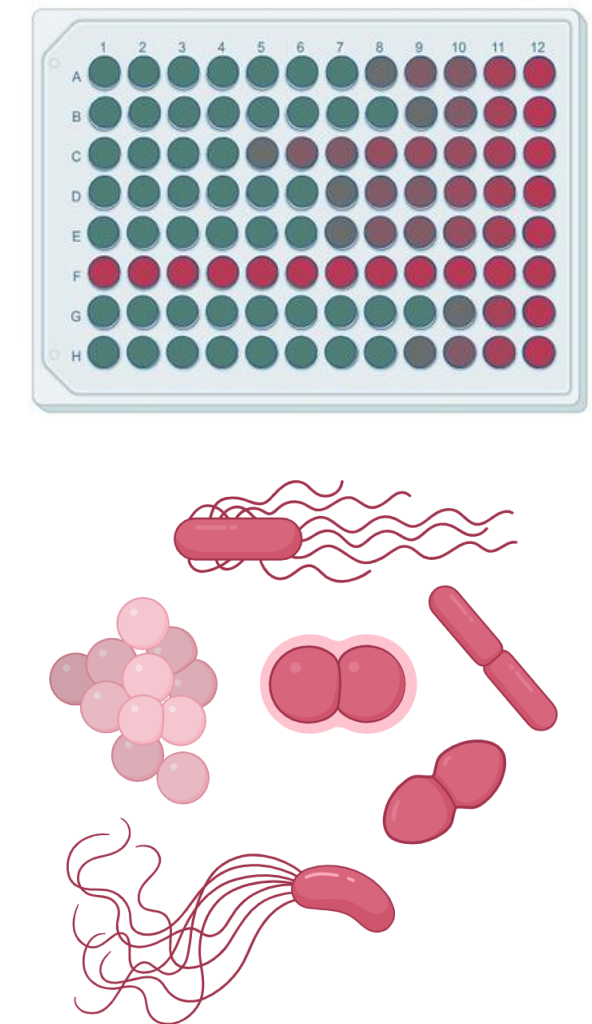
Derivatization



Drug Delivery



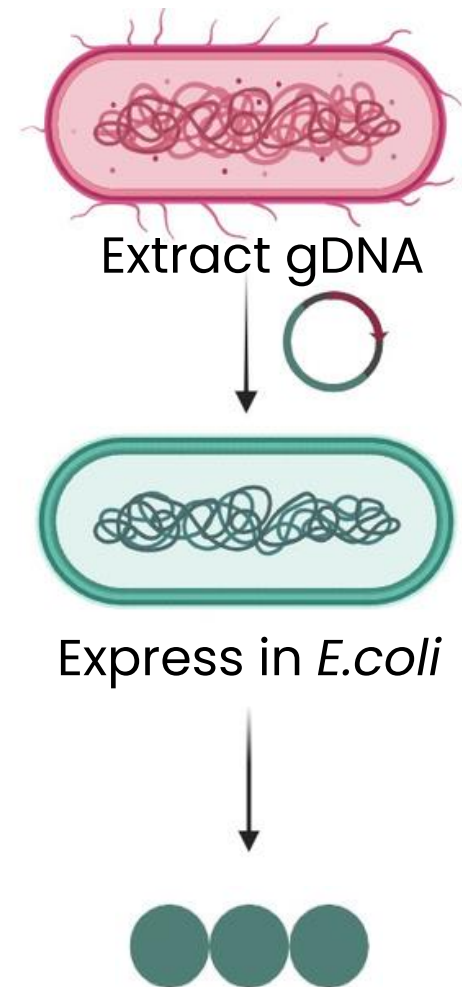
Activity Testing



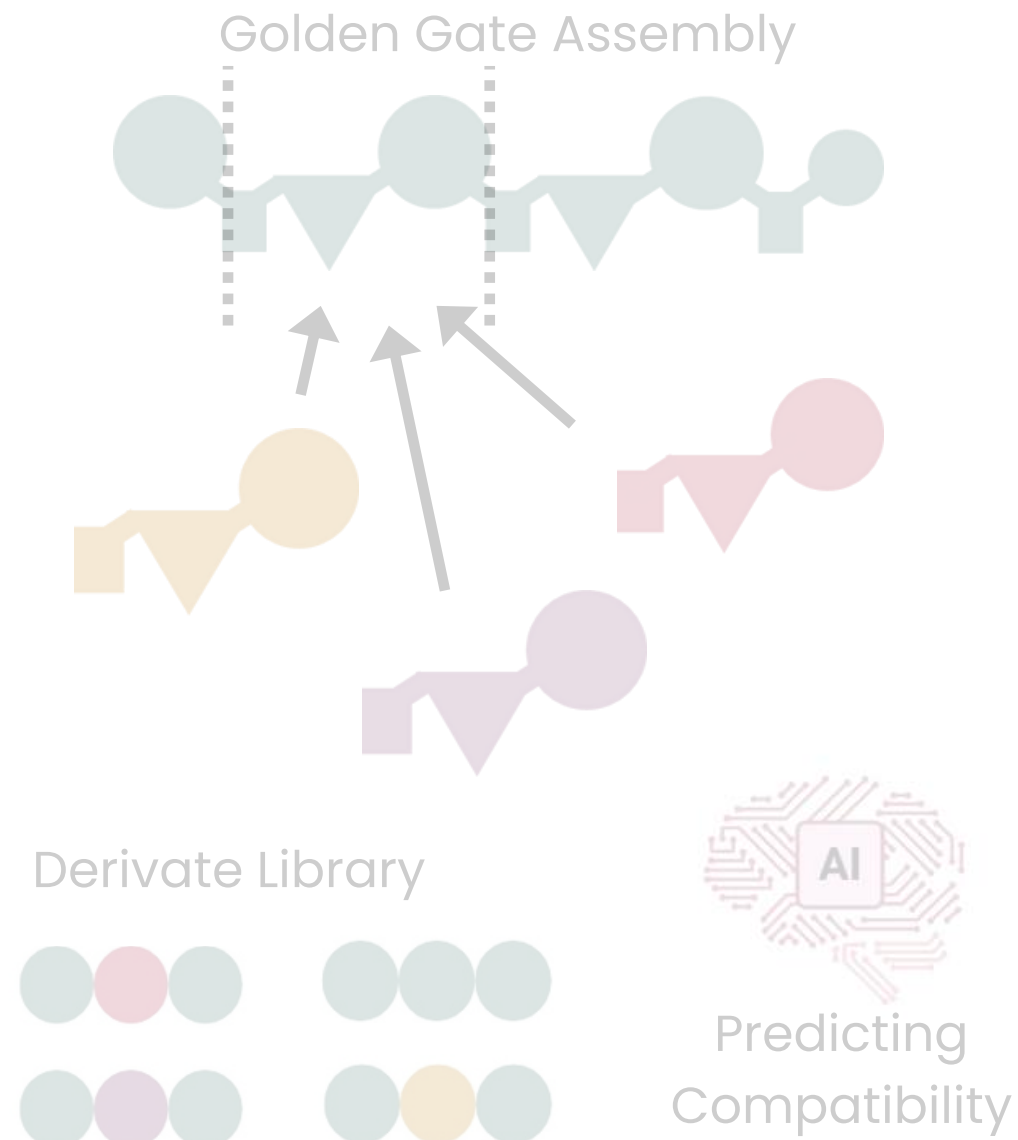
Reinventing Antibiotic Discovery with NRPS



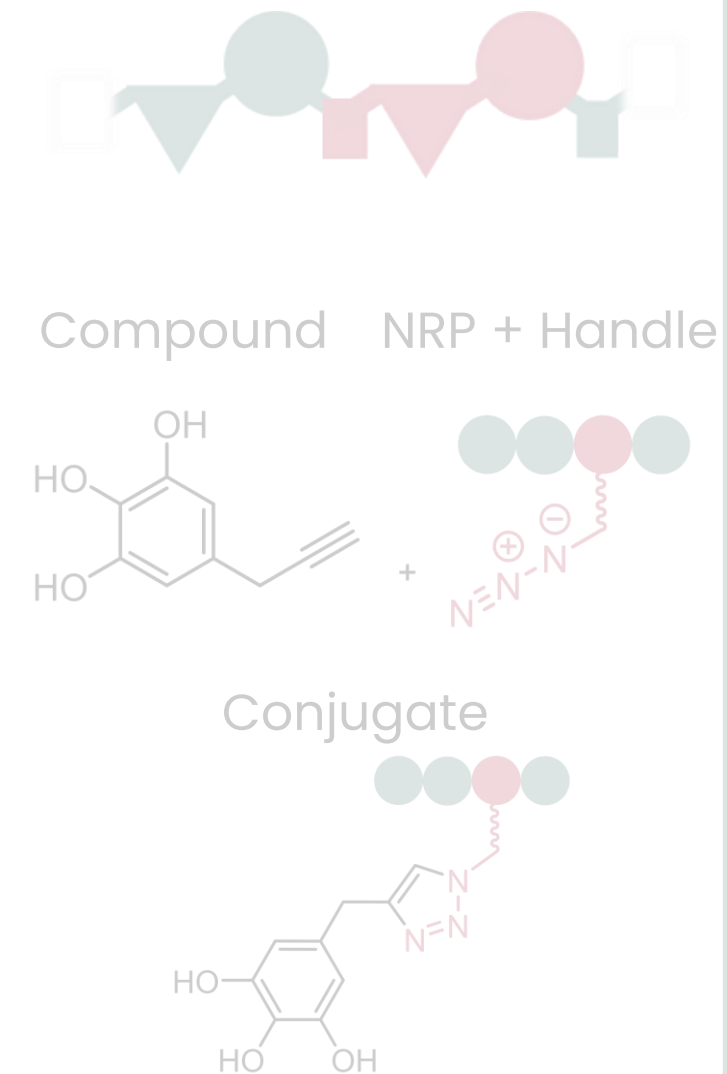
Heterologous Expression



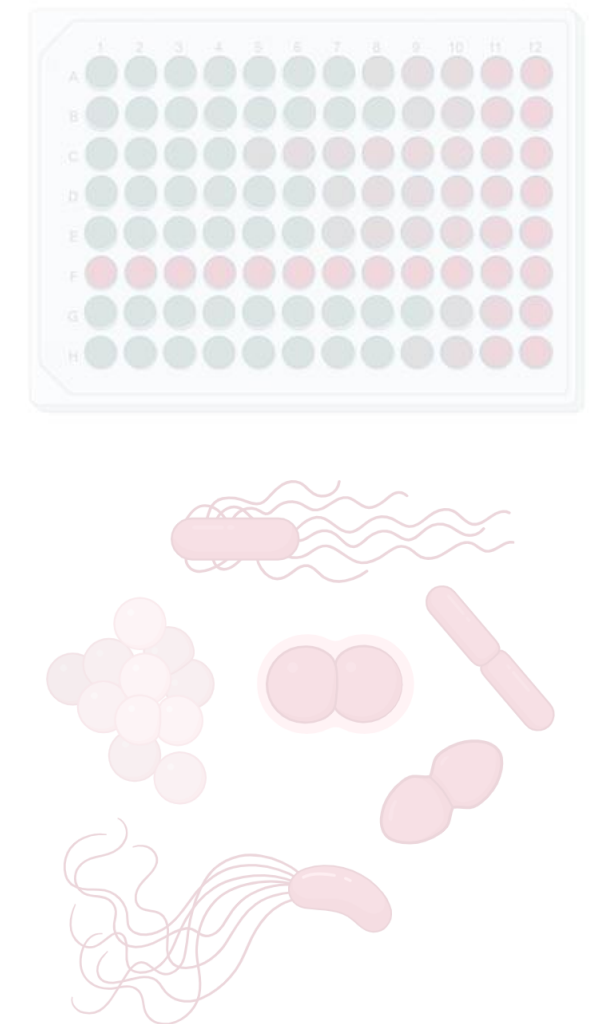
Derivatization



Drug Delivery



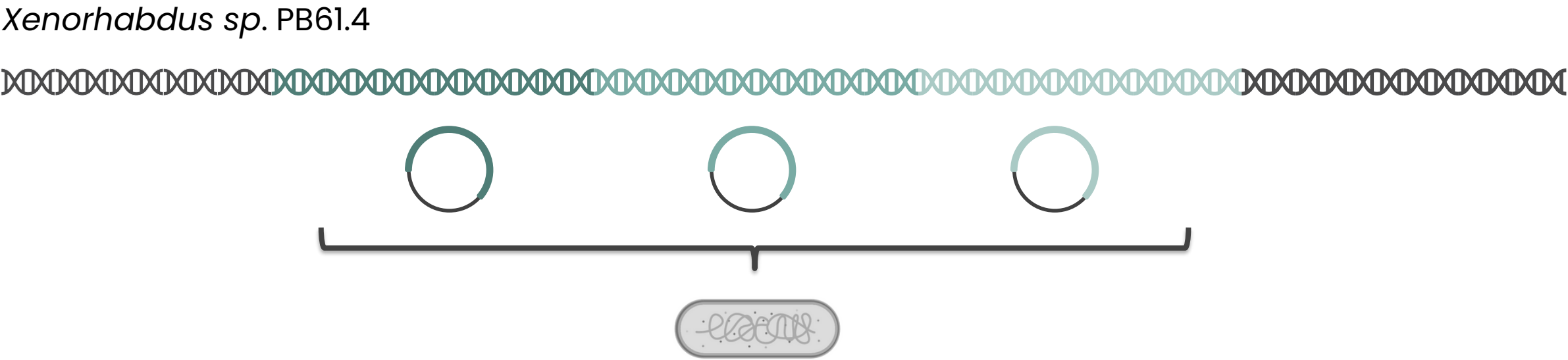
Activity Testing



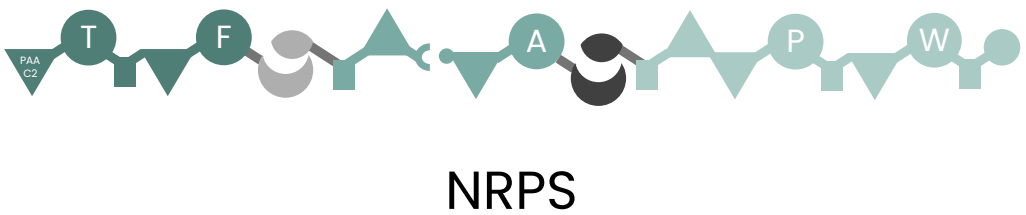
Heterologous Expression



Split cluster into plasmids
for expression



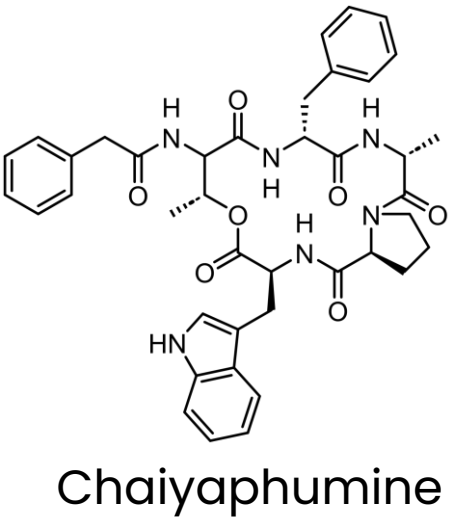
NRPS expression
in *E. coli*



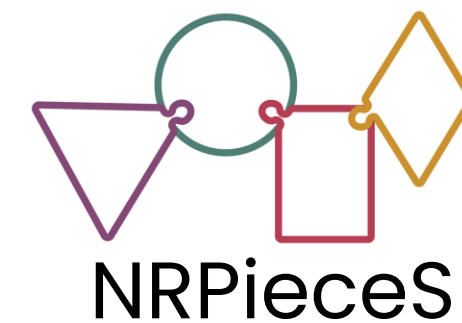
NRPS is assembled by **inteins**.



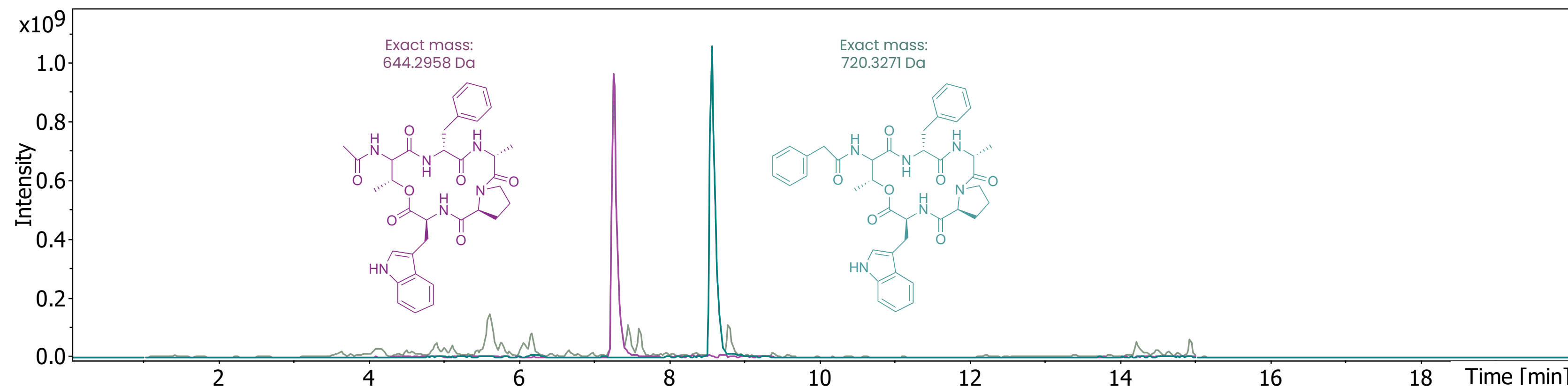
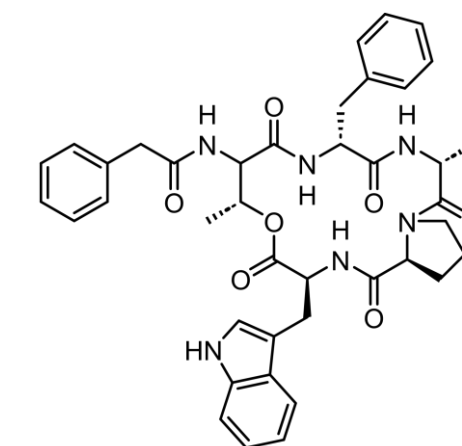
Peptide
production



Chaiyaphumine is producible in *E. coli*



Chaiyaphumine



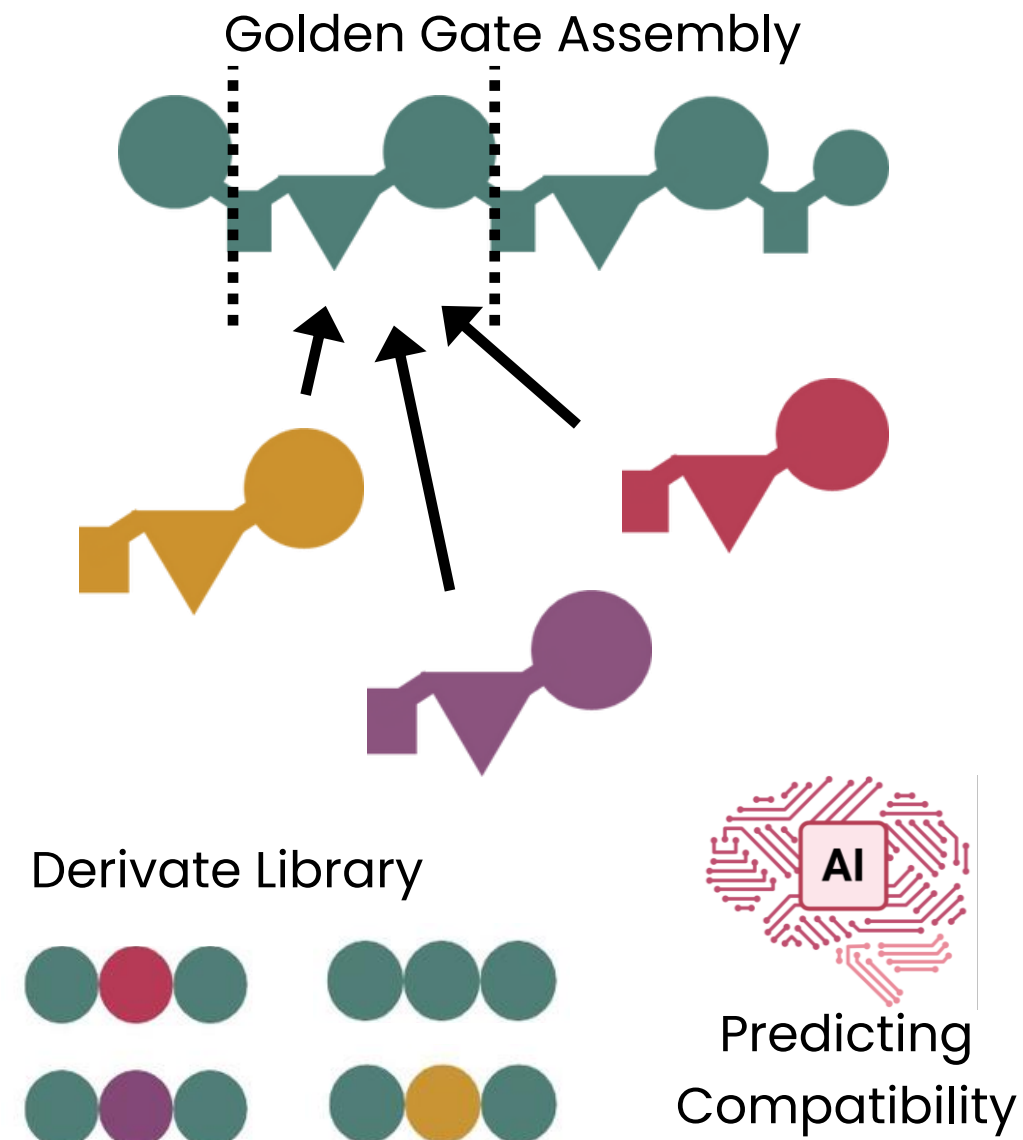
Peptide Production is analyzed by LC-MS.

Reinventing Antibiotic Discovery with NRPS

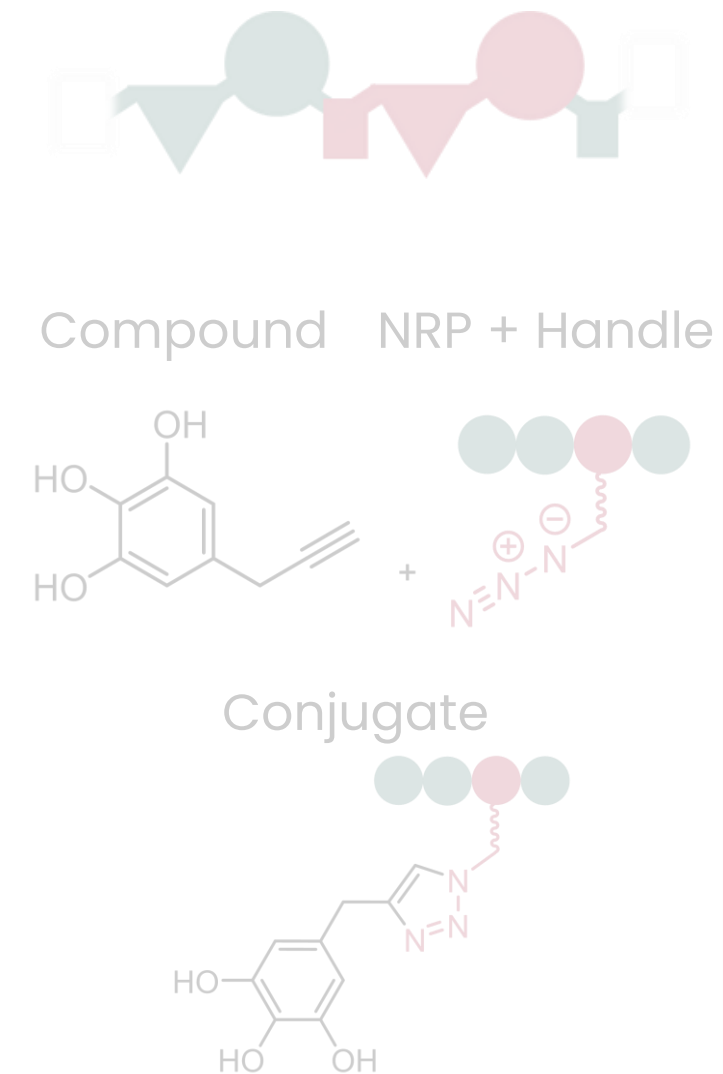
Heterologous Expression



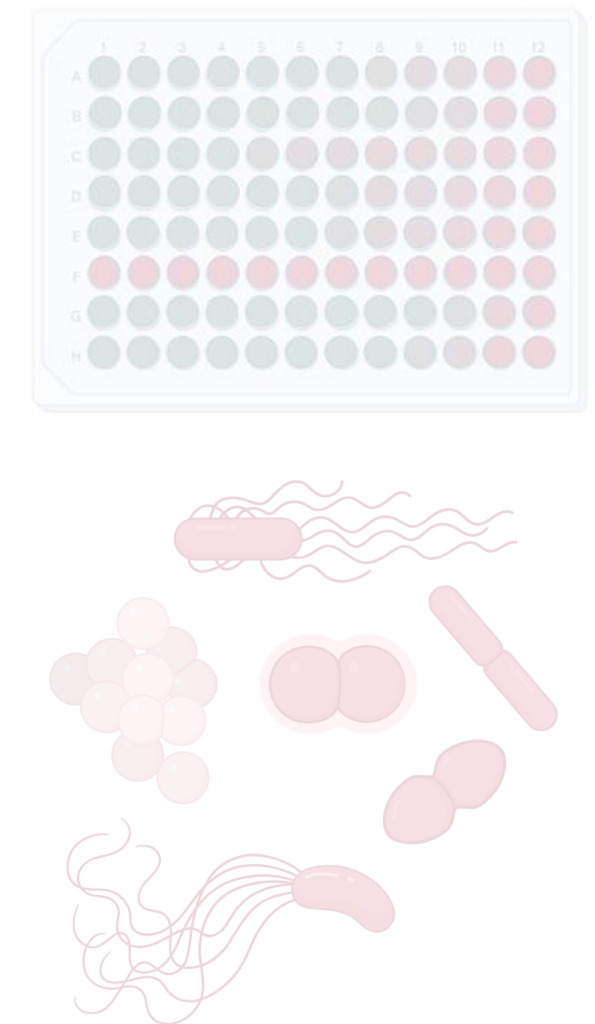
Derivatization



Drug Delivery



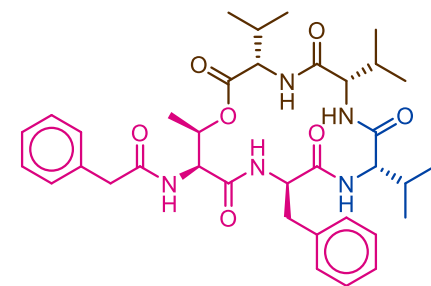
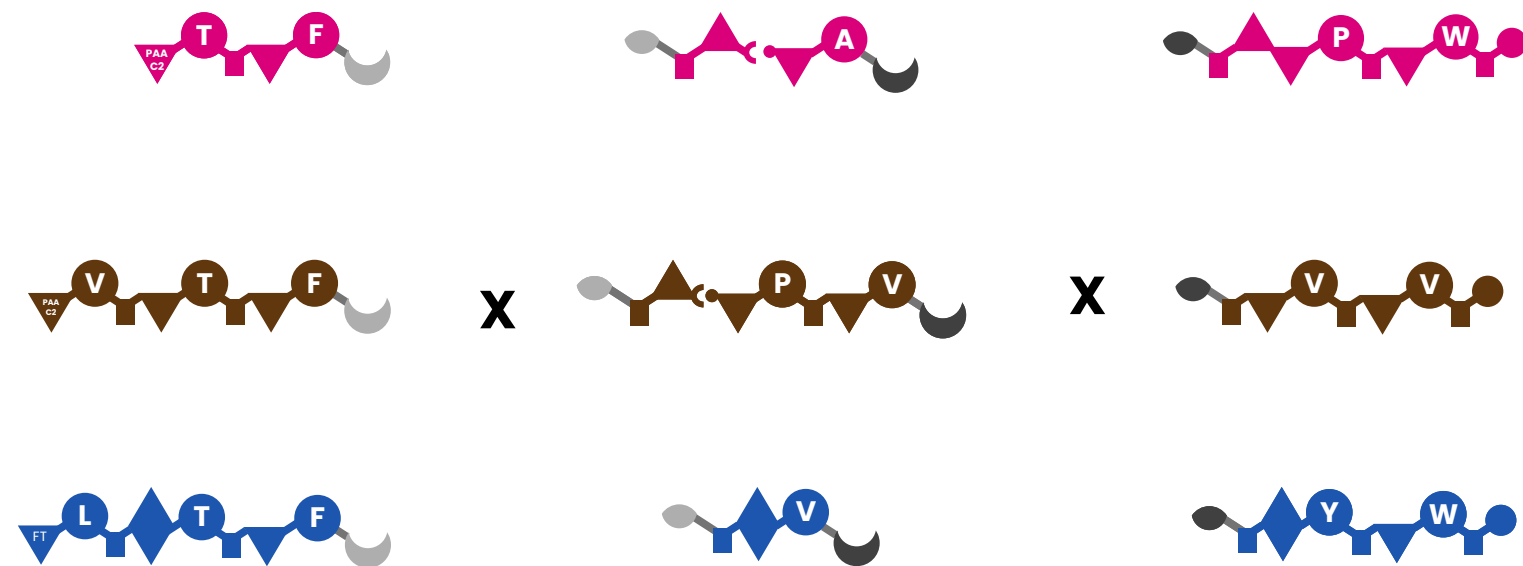
Activity Testing



NRP Derivatization – 2 strategies

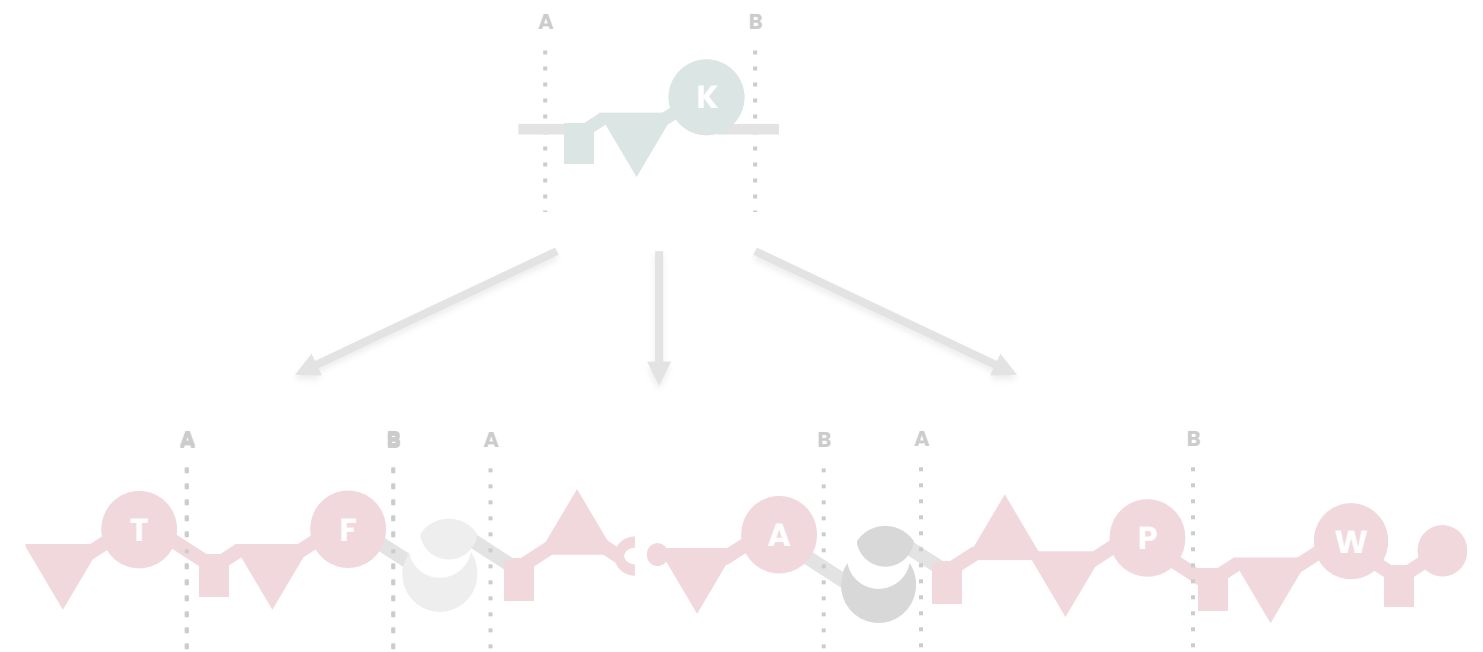


Intein Shuffling



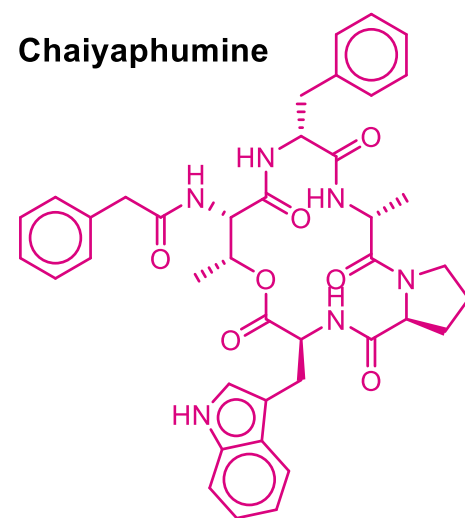
Novel NRPS – novel peptide!

Module Exchange

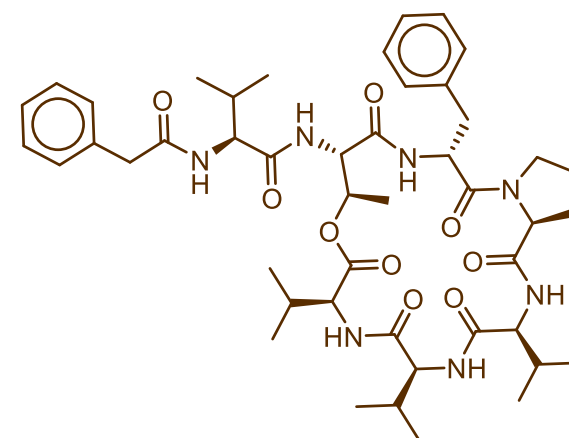


NRP Derivatization by Intein Shuffling

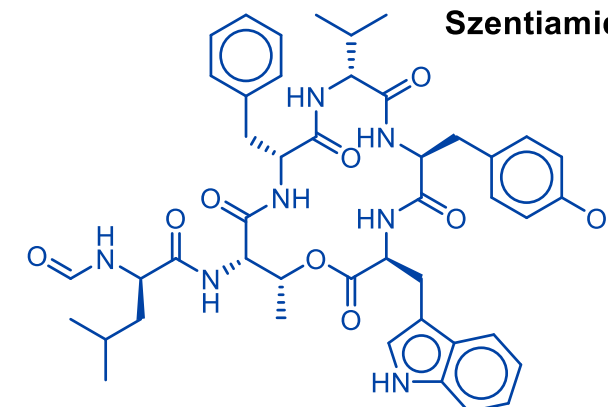
Chaiyaphumine



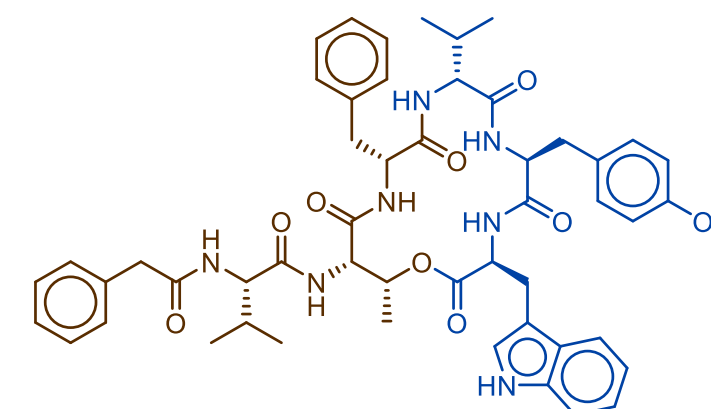
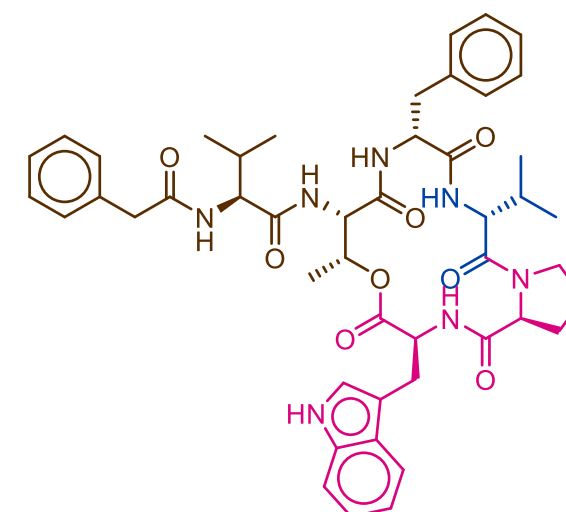
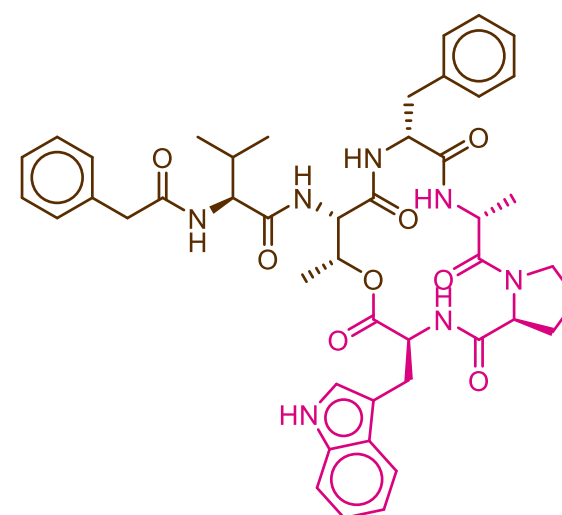
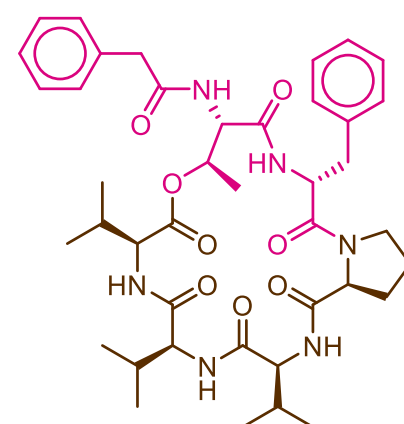
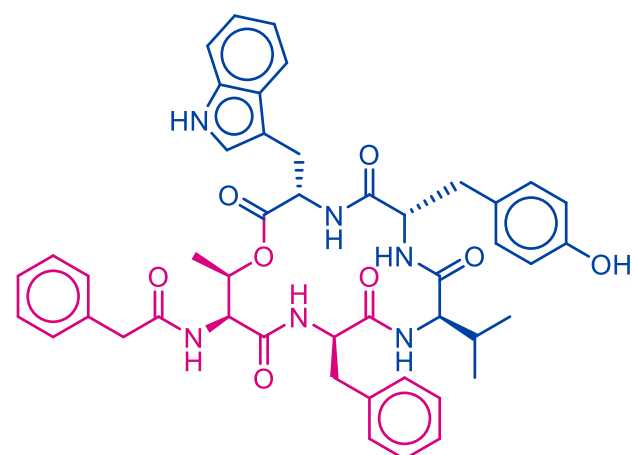
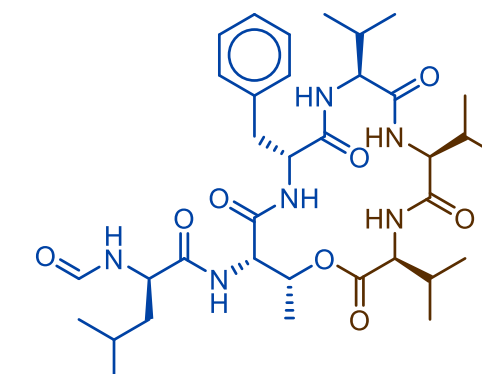
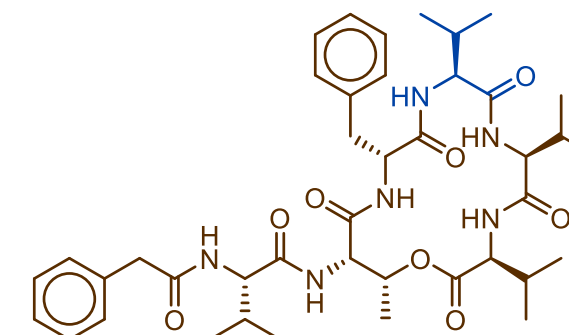
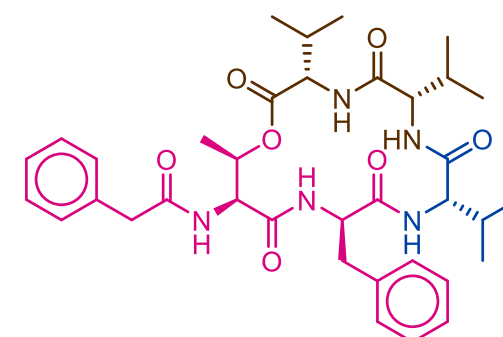
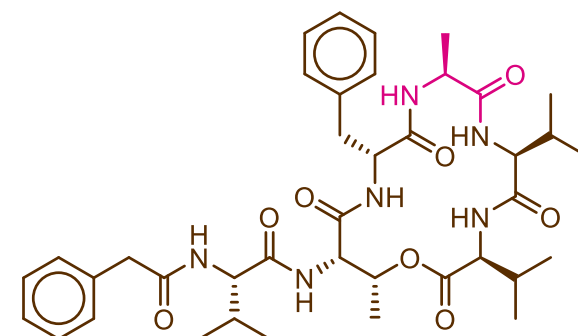
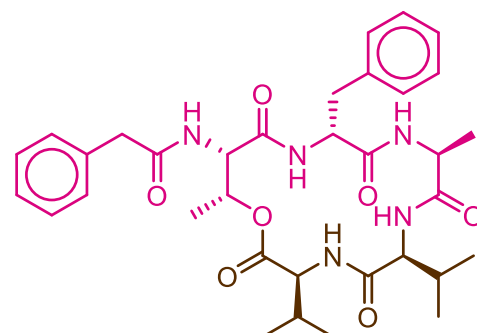
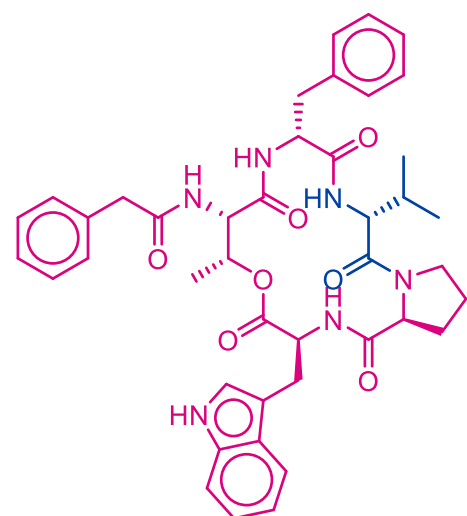
Xentrivalpeptide



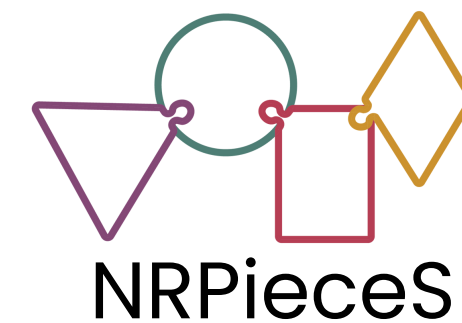
Szentiamid



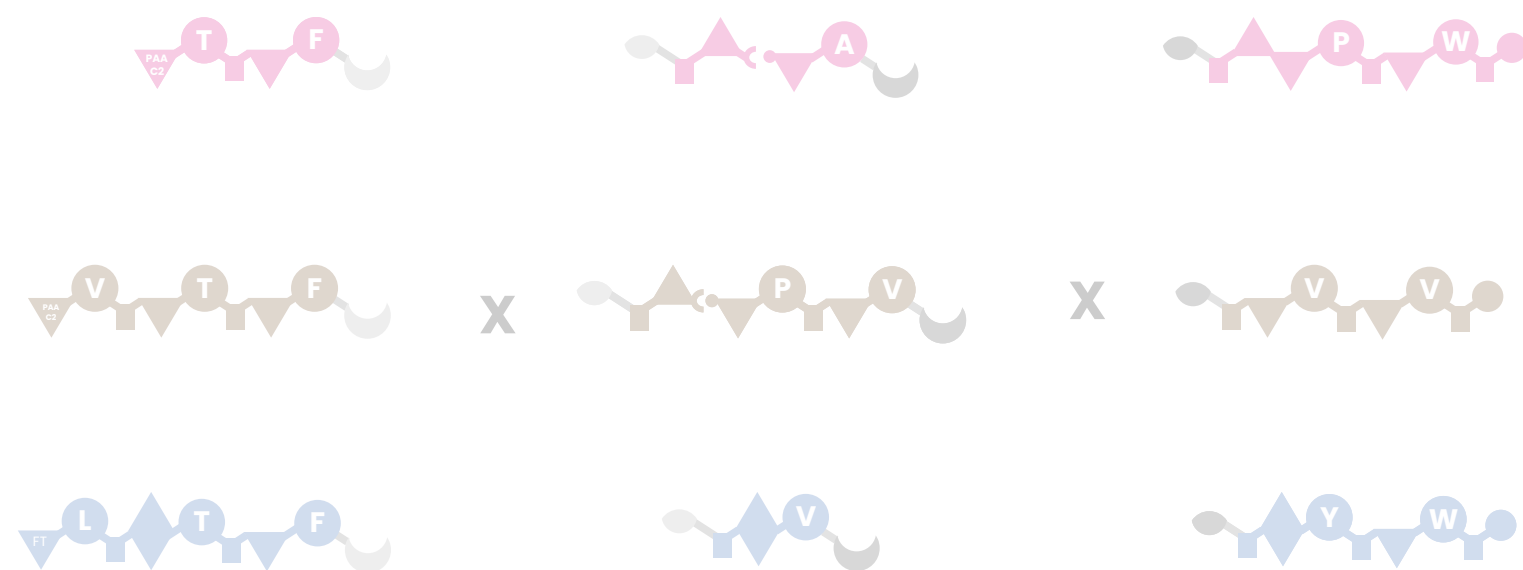
11 new peptides



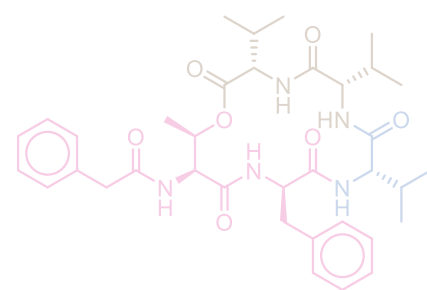
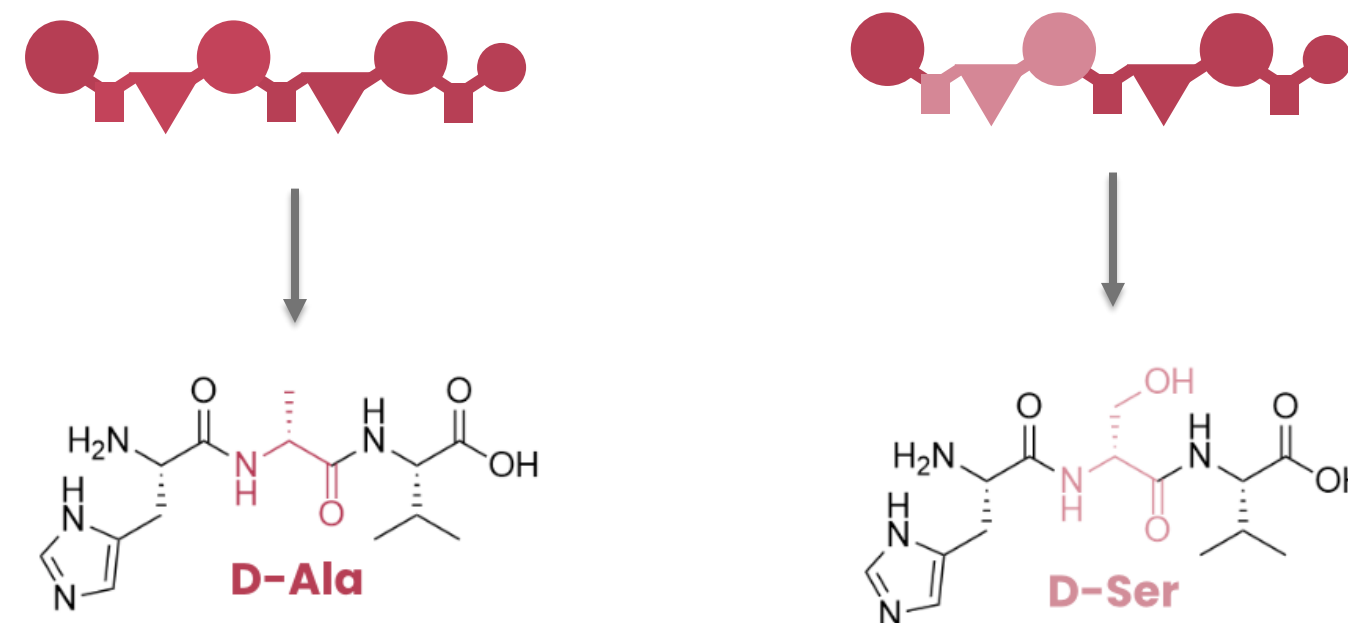
NRP Derivatization – 2 strategies



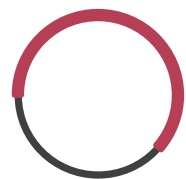
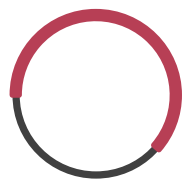
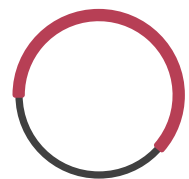
Intein Shuffling



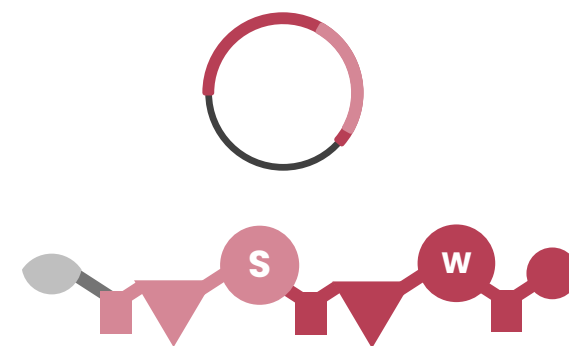
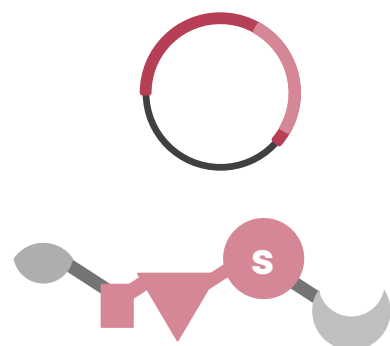
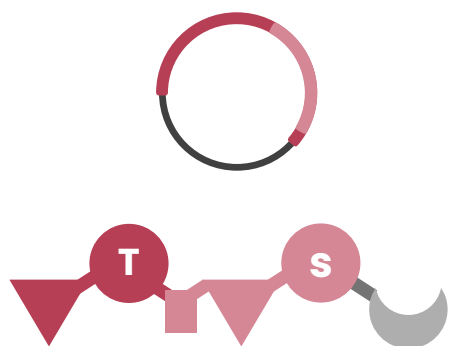
Module Exchange



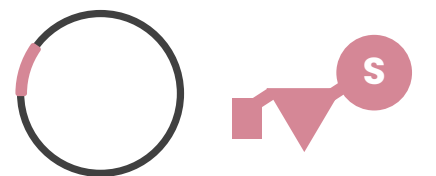
NRP Derivatization by Module Exchange



Golden Gate Cloning

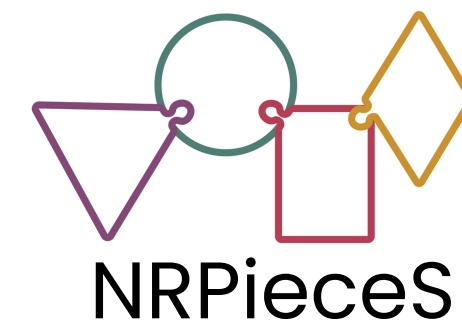


NRPieces
Exchange modules

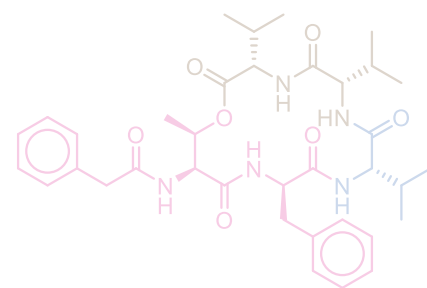
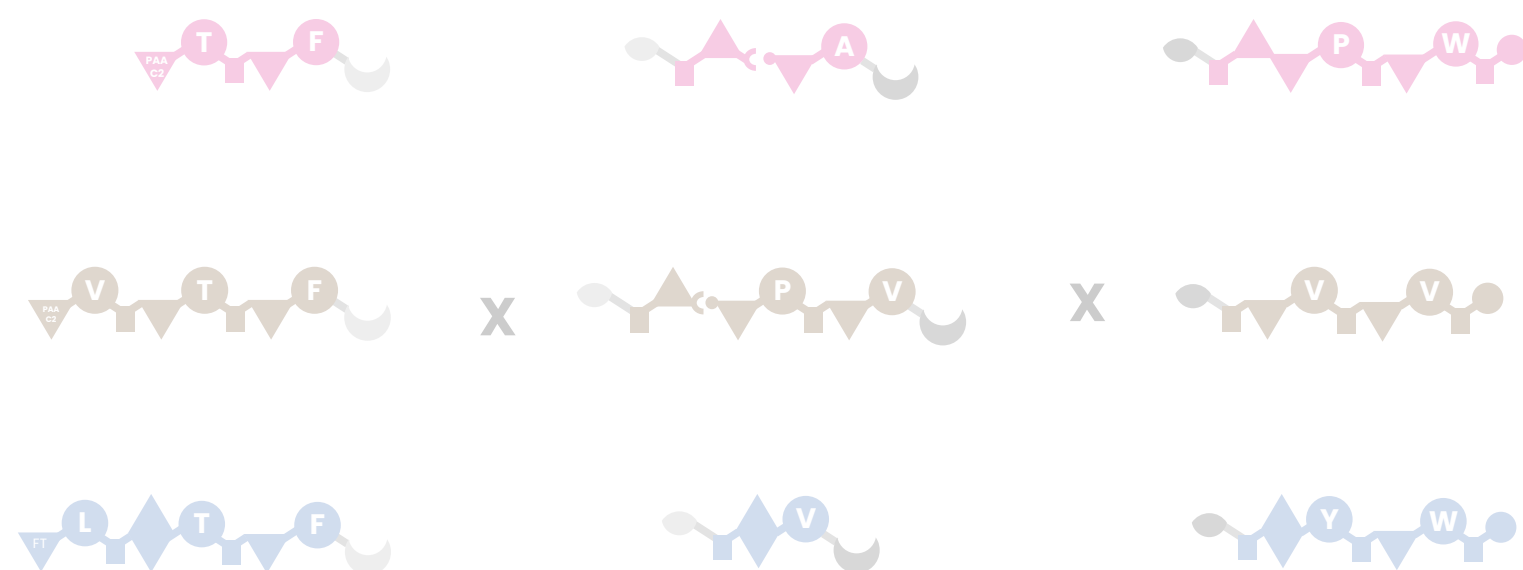


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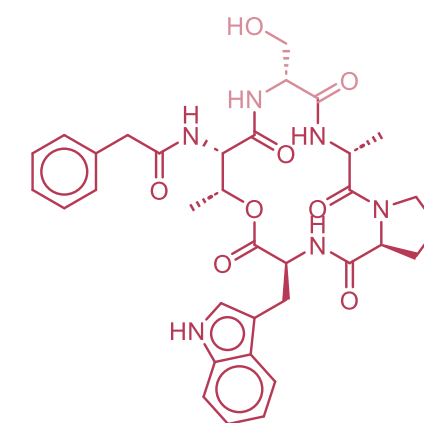
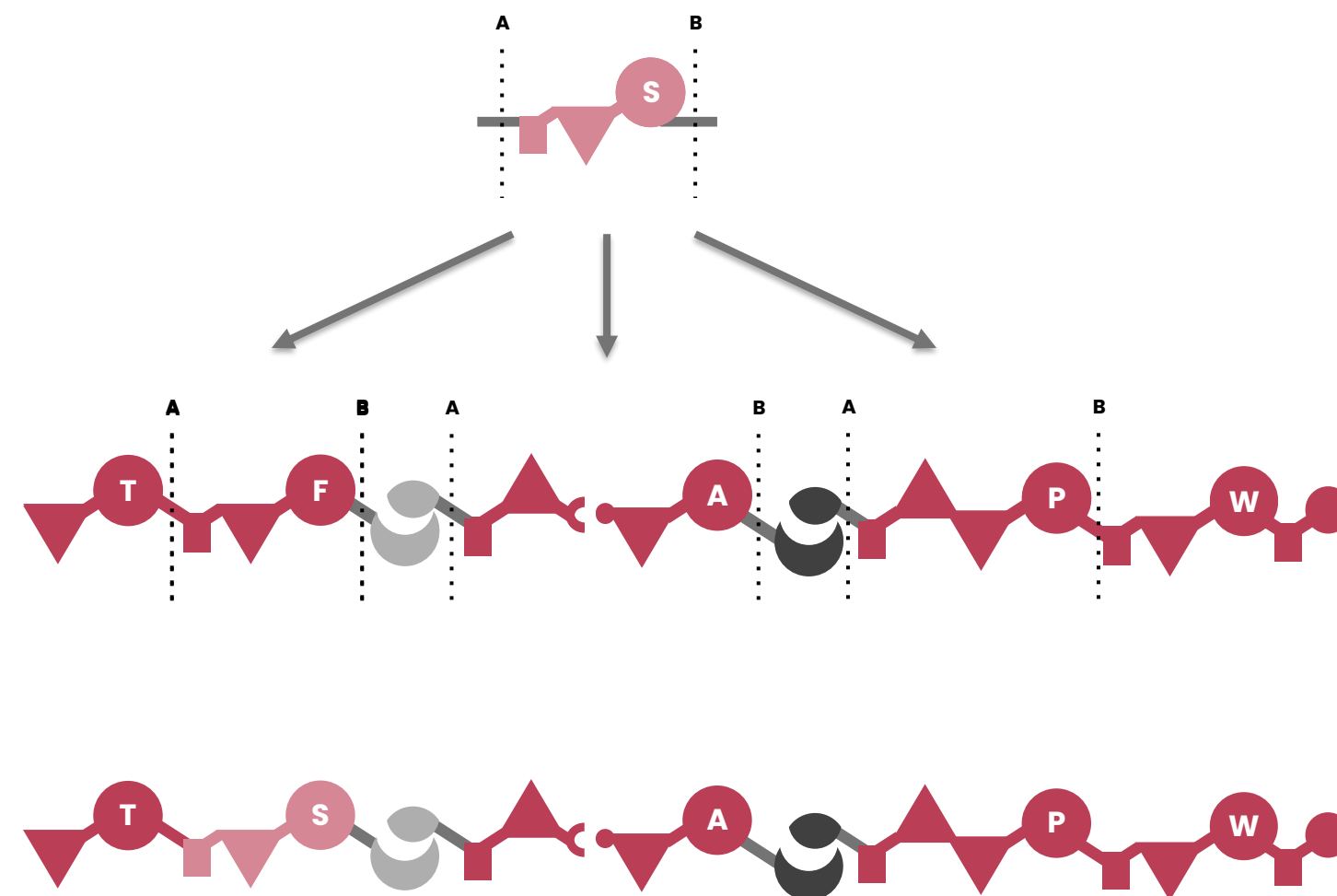
NRP Derivatization – 2 strategies



Intein Shuffling



Module Exchange

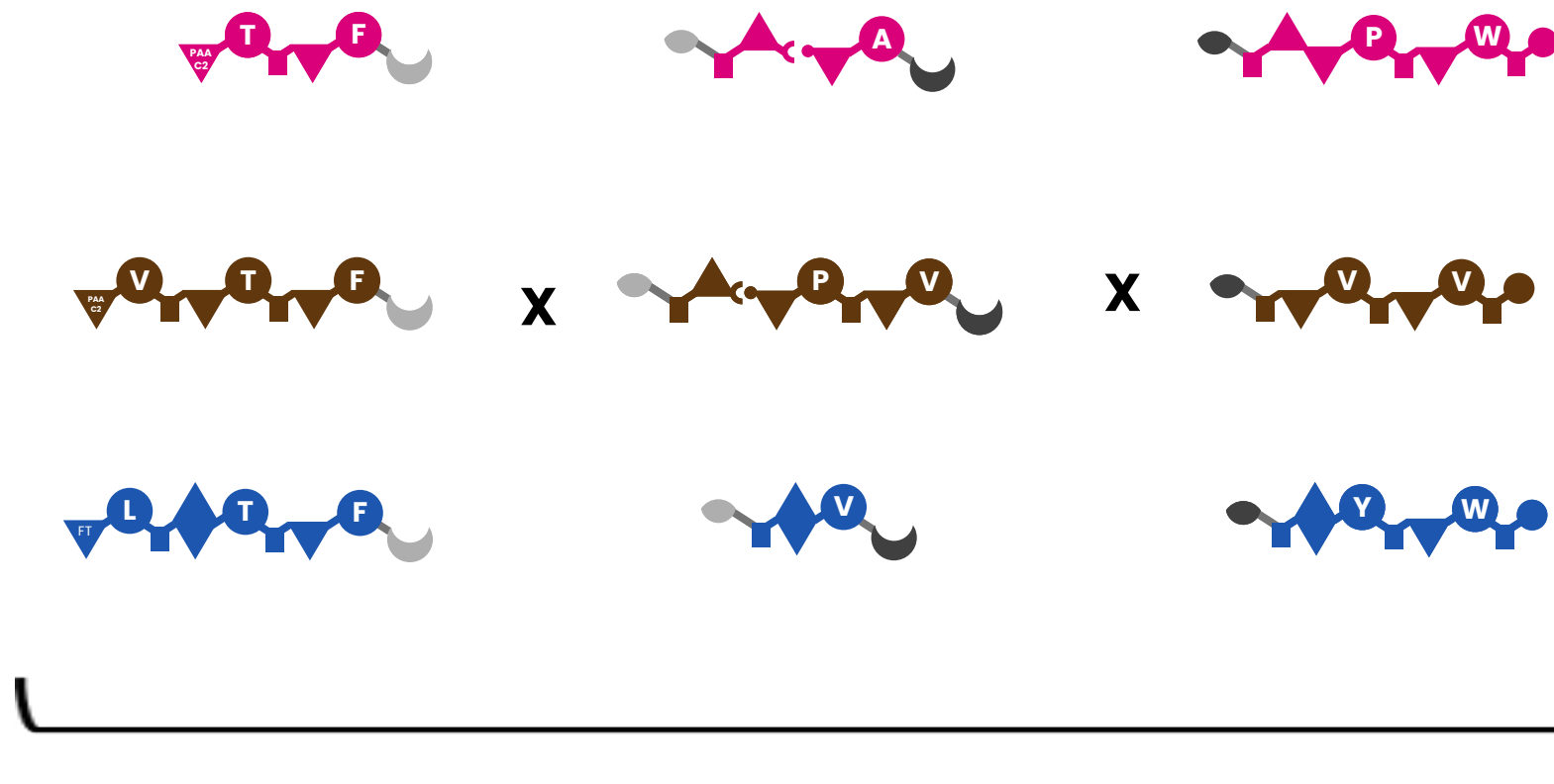


Novel NRPS – novel peptide!

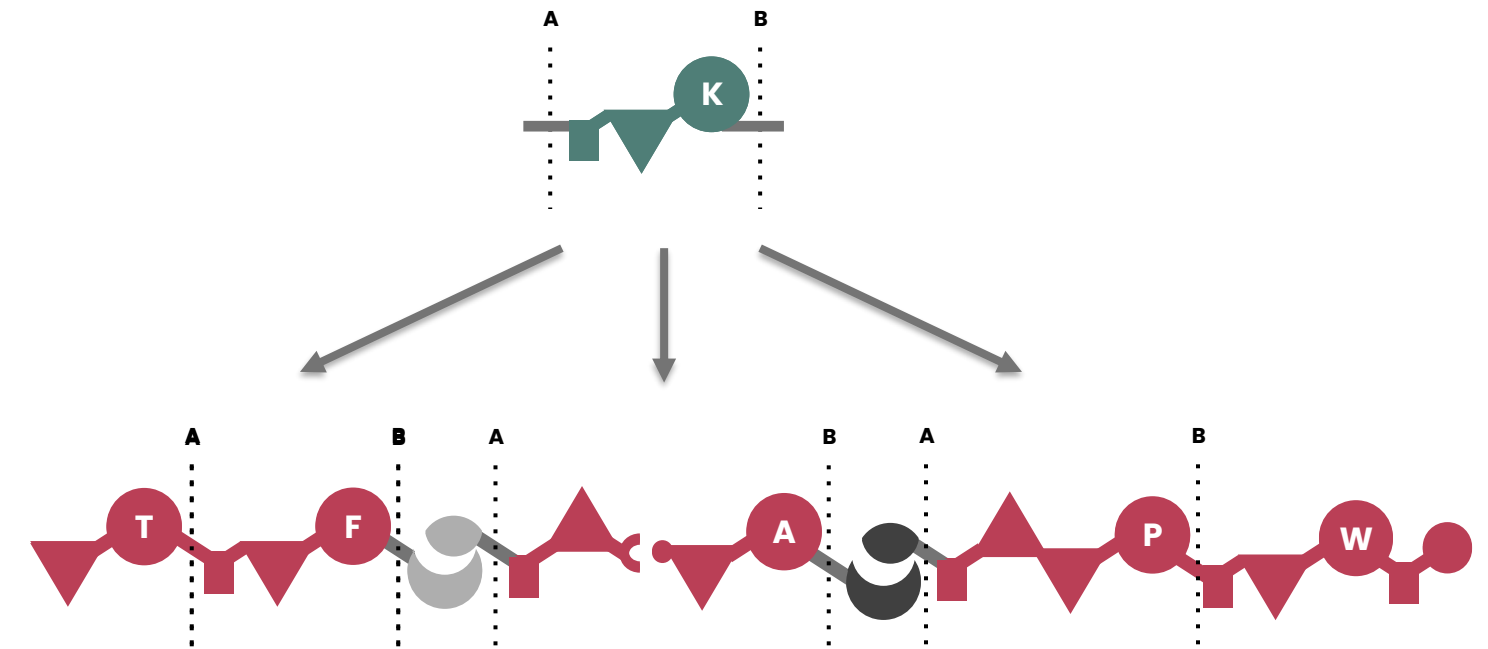
NRP Derivatization – 2 strategies



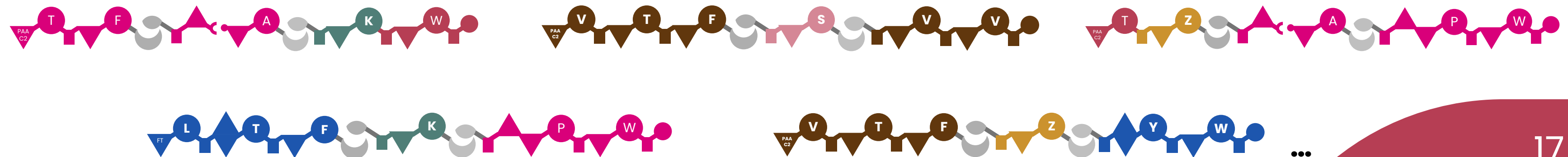
Intein Shuffling



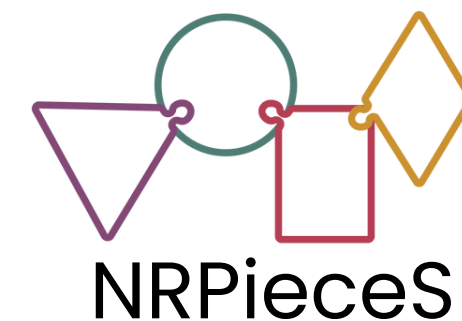
Module Exchange



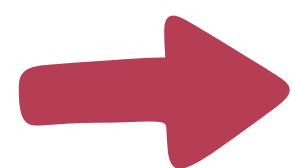
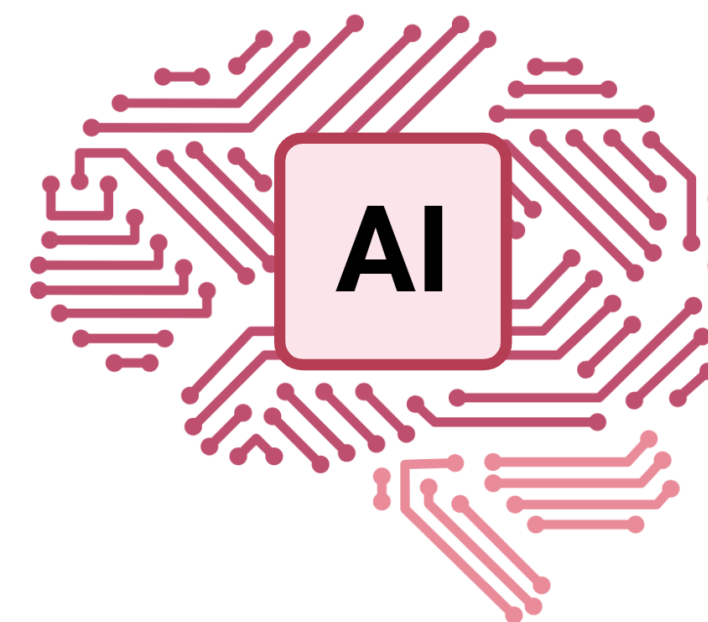
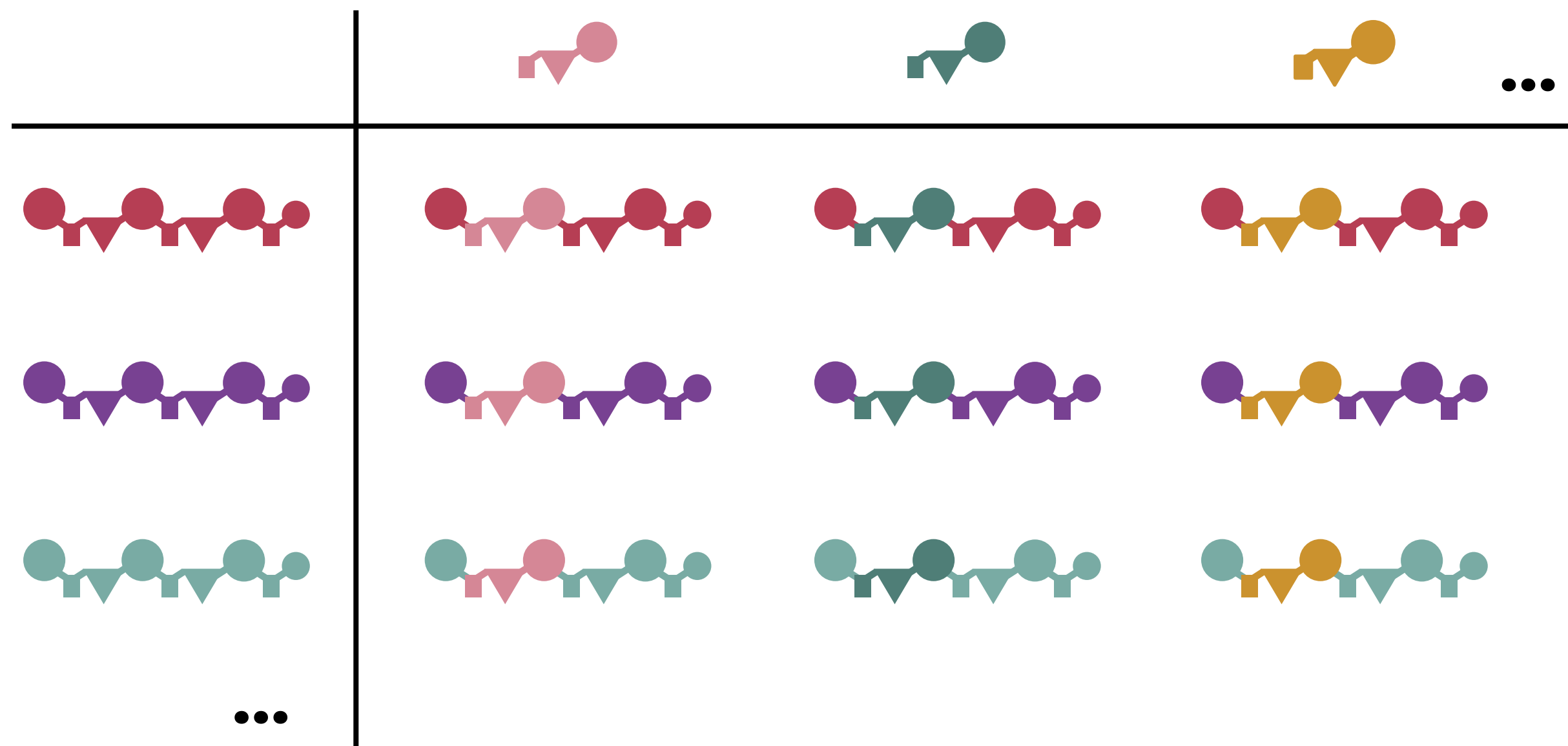
Numerous new peptides!



NRP Derivatization

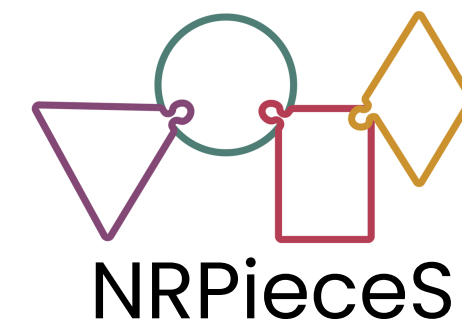


Not all combinations work together & produce a peptide.



Too many combinations to test them all...

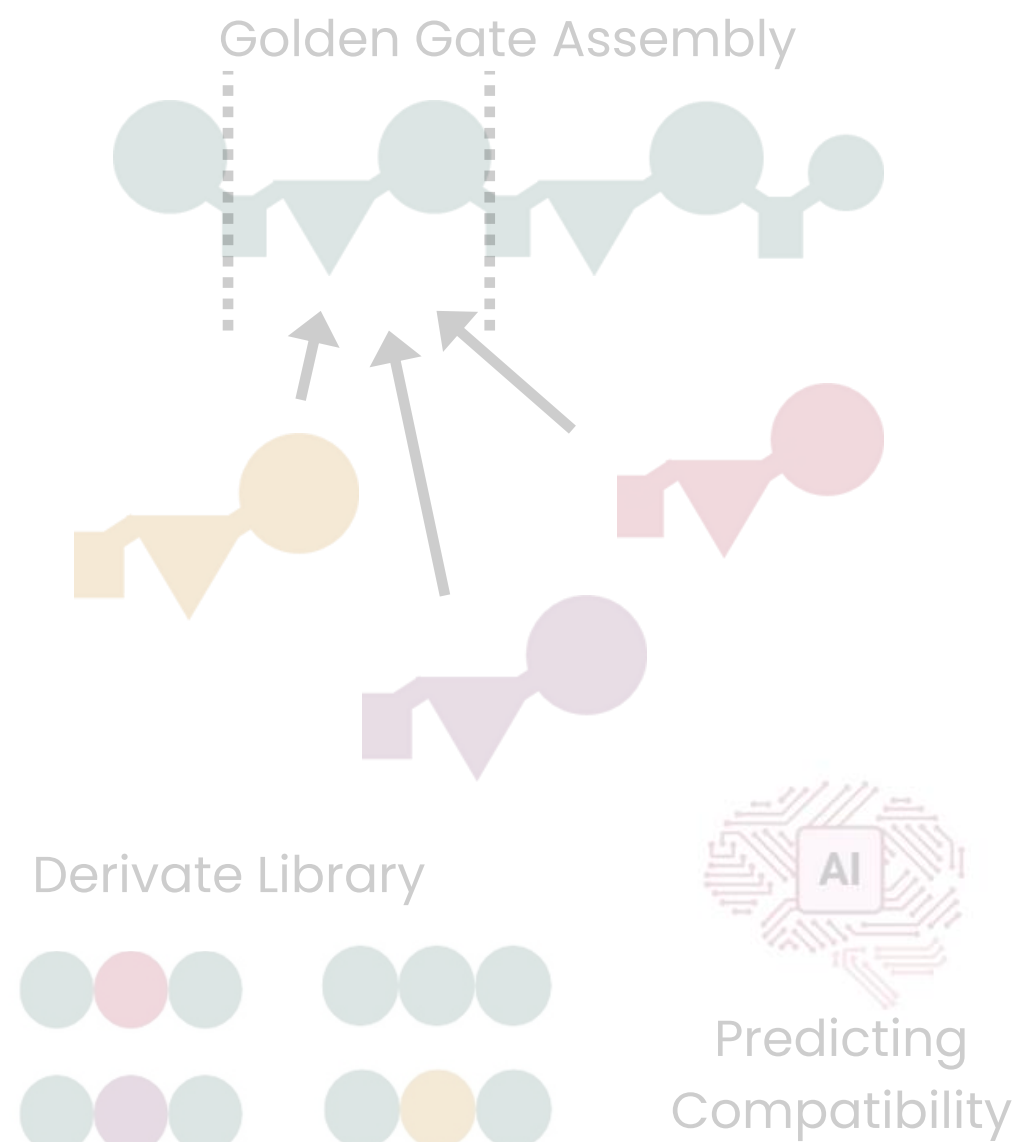
Reinventing Antibiotic Discovery with NRPS



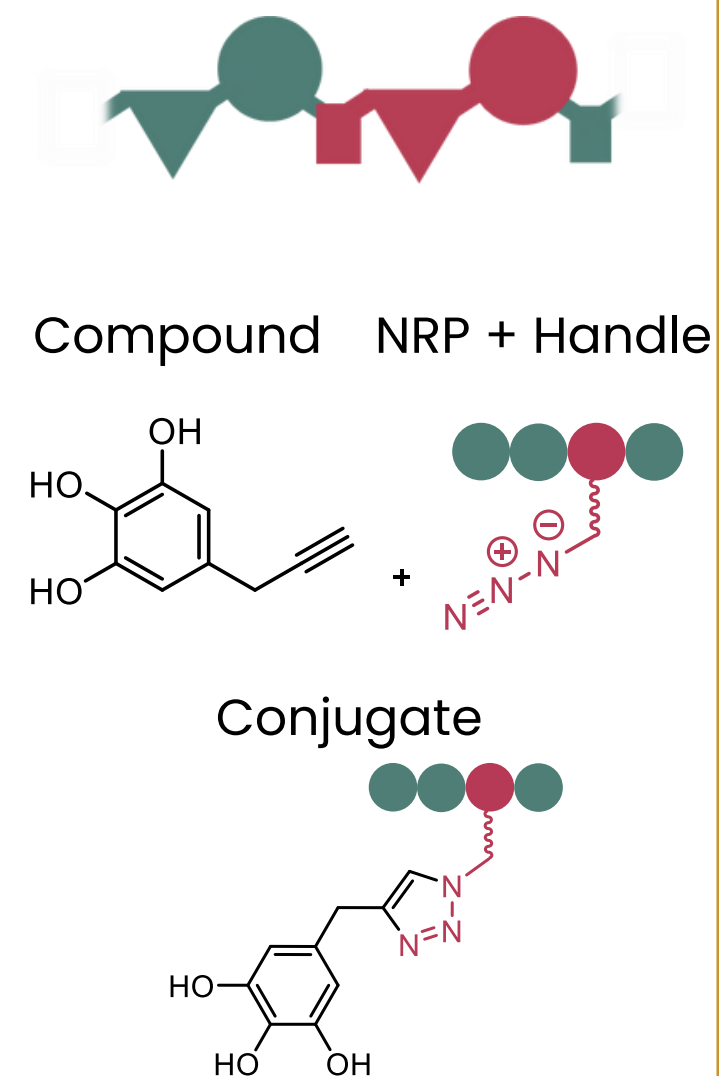
Heterologous Expression



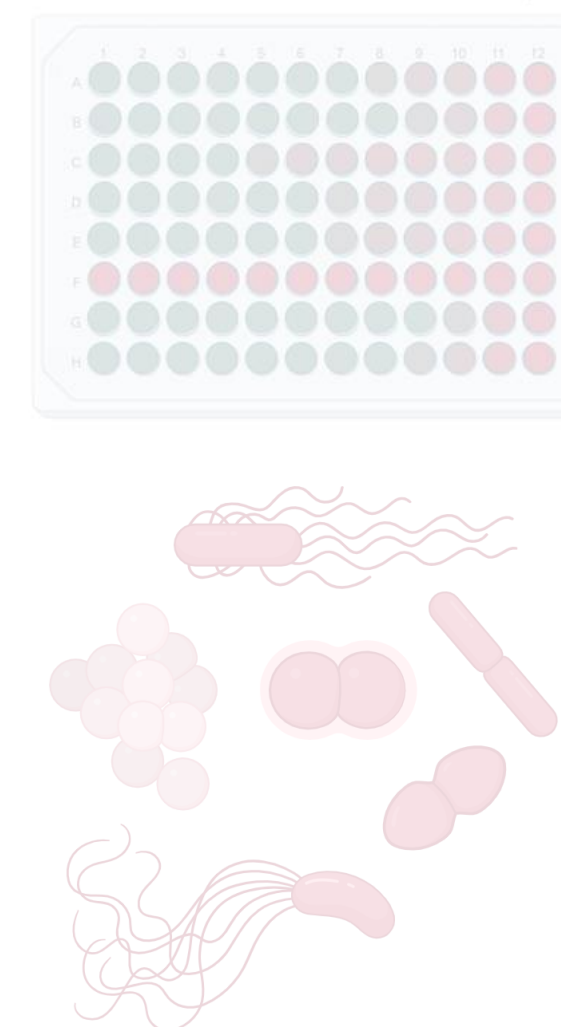
Derivatization



Drug Delivery



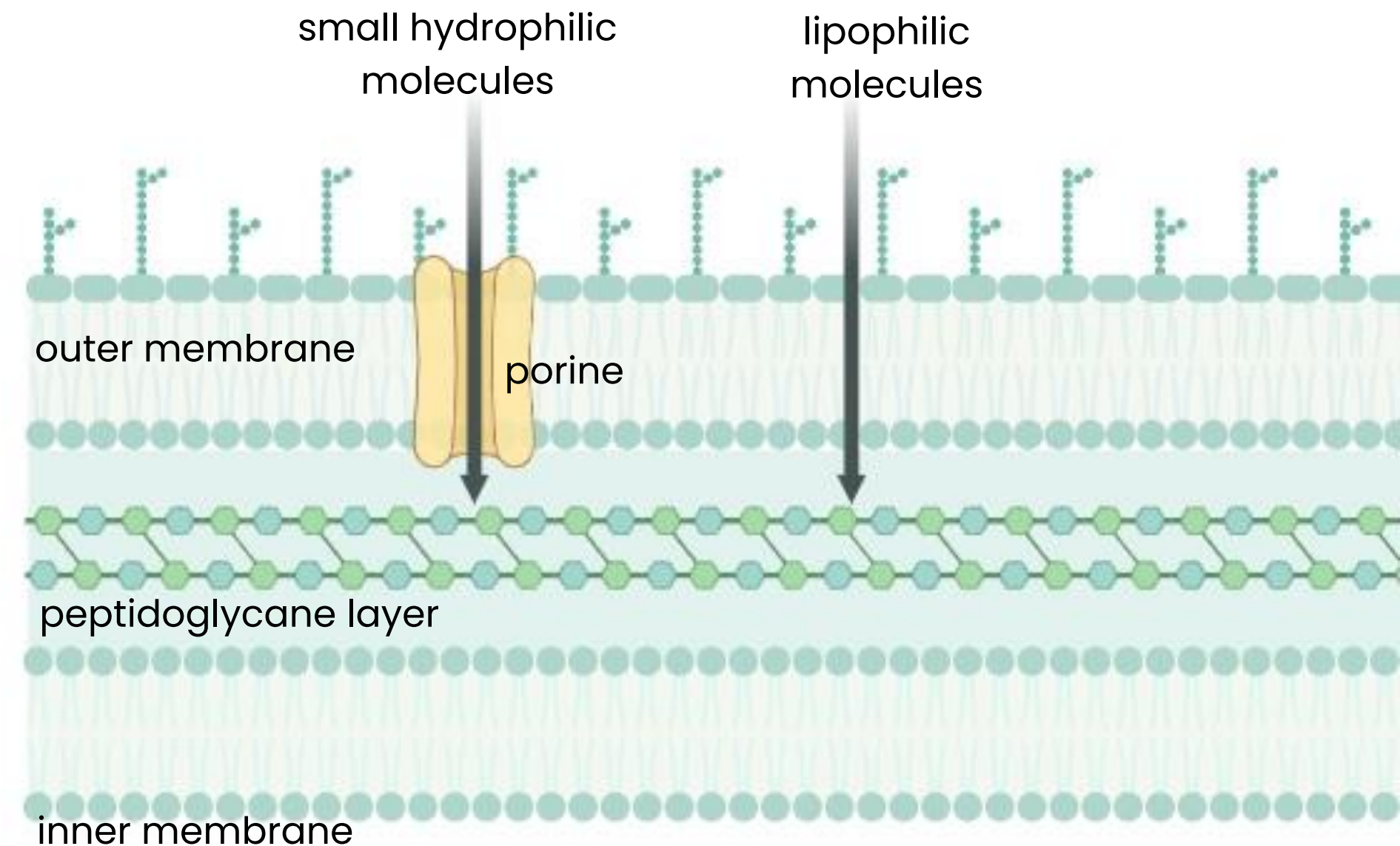
Activity Testing



The Outer Membrane (OM) - A Robust Barrier

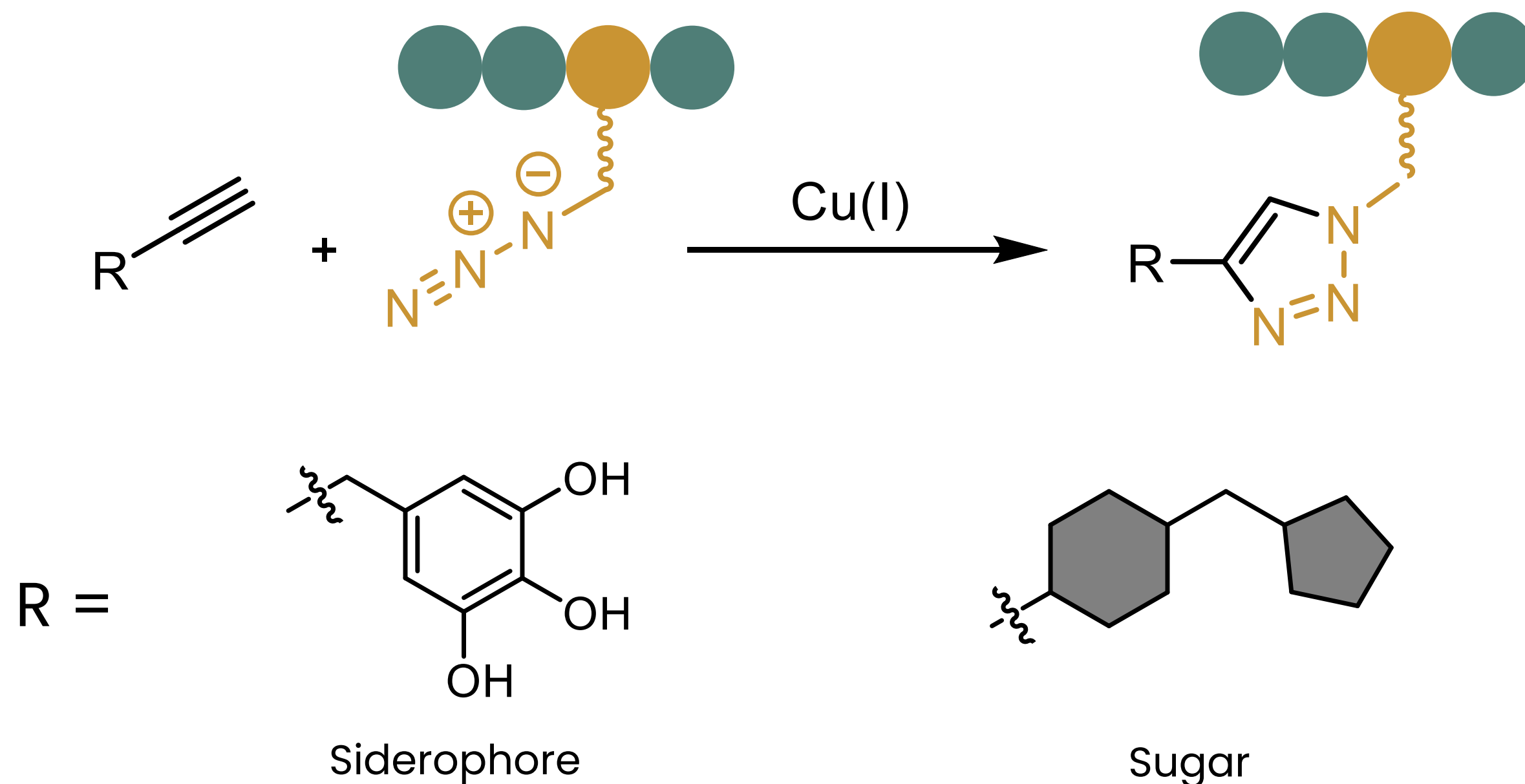


- Bottleneck of most NRP Antibiotics: Target **only Gram-(+)** bacteria
- OM of Gram-(-) bacteria = robust permeability barrier



Reshaping Antibiotic Delivery

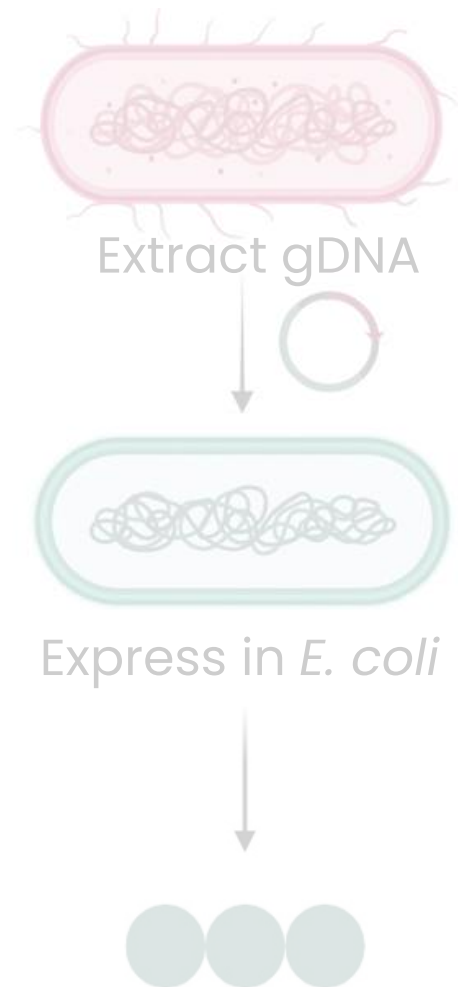
Amino acid with a "handle"



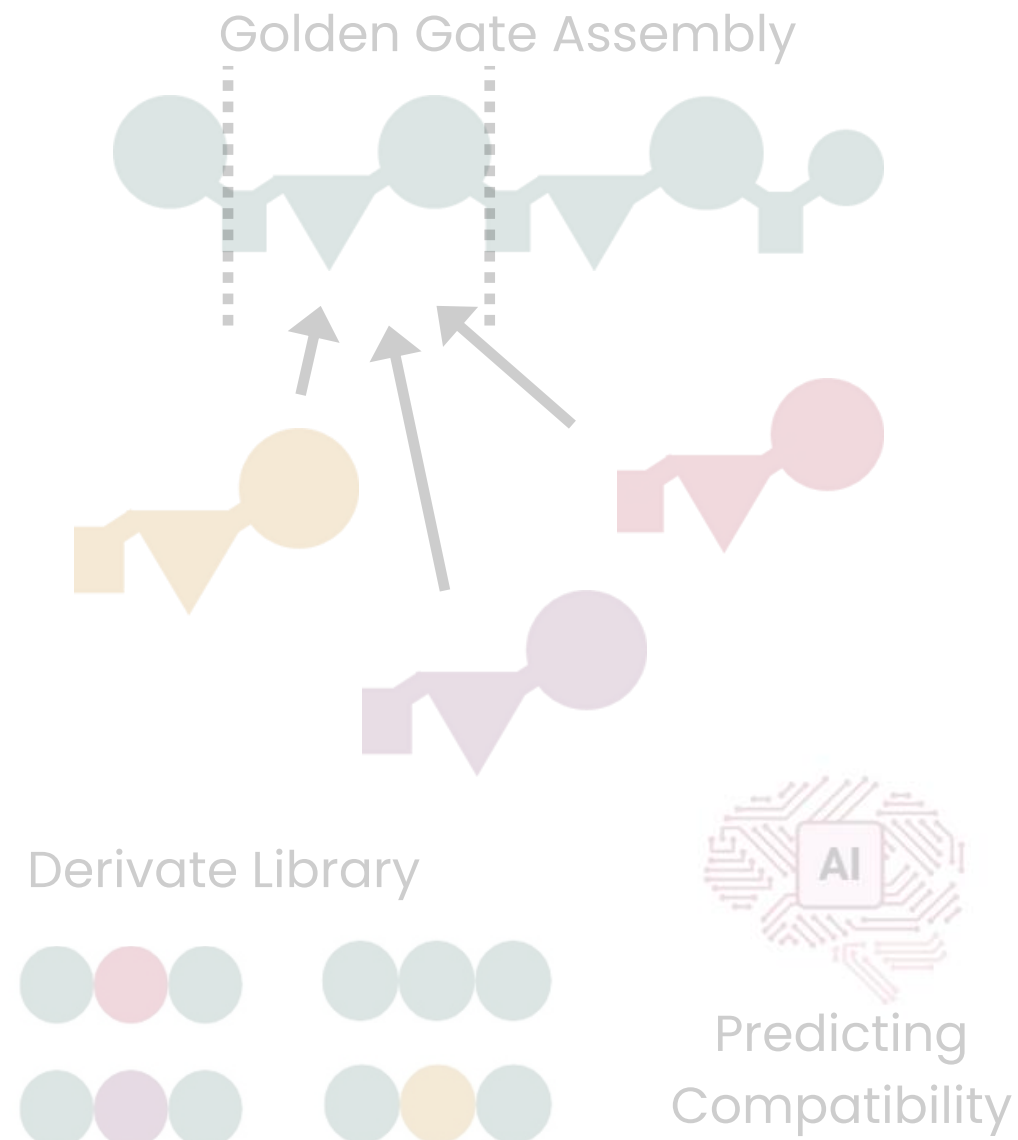
Conjugation to siderophores or nutrients for enhanced uptake

Reinventing Antibiotic Discovery with NRPS

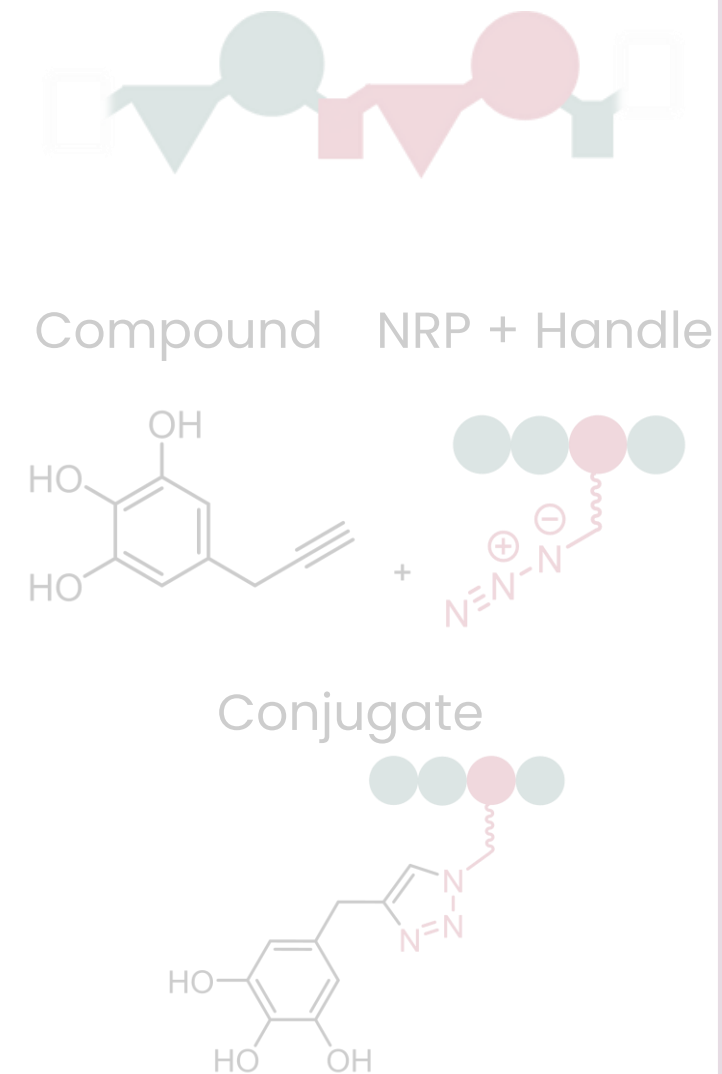
Heterologous Expression



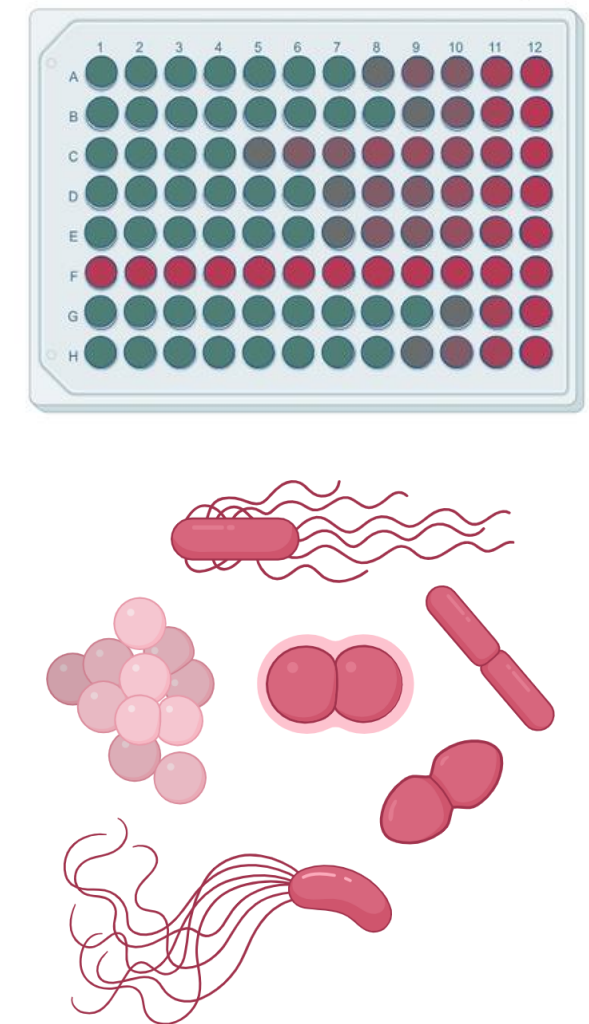
Derivatization



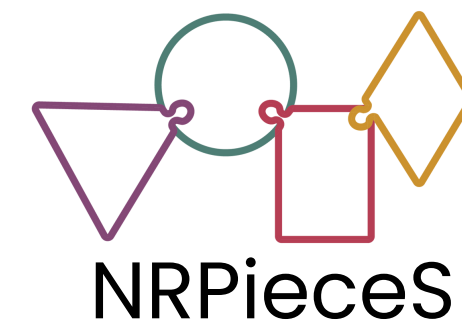
Drug Delivery



Activity Testing



Summary



Let's discover a peptide antibiotic!

1. NRPS Design



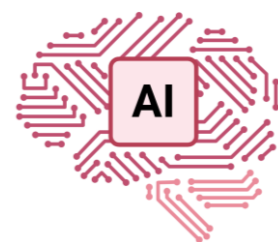
Native NRPS



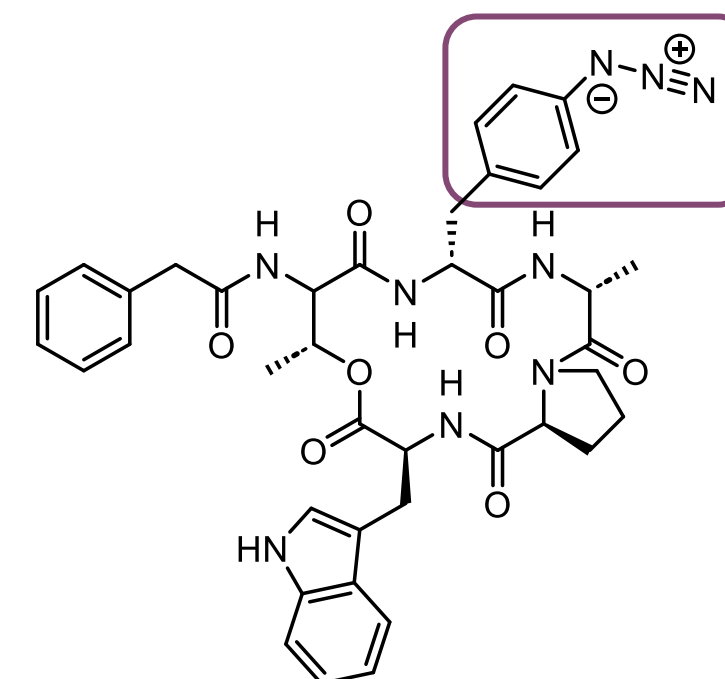
NRPiece

No

Redesign

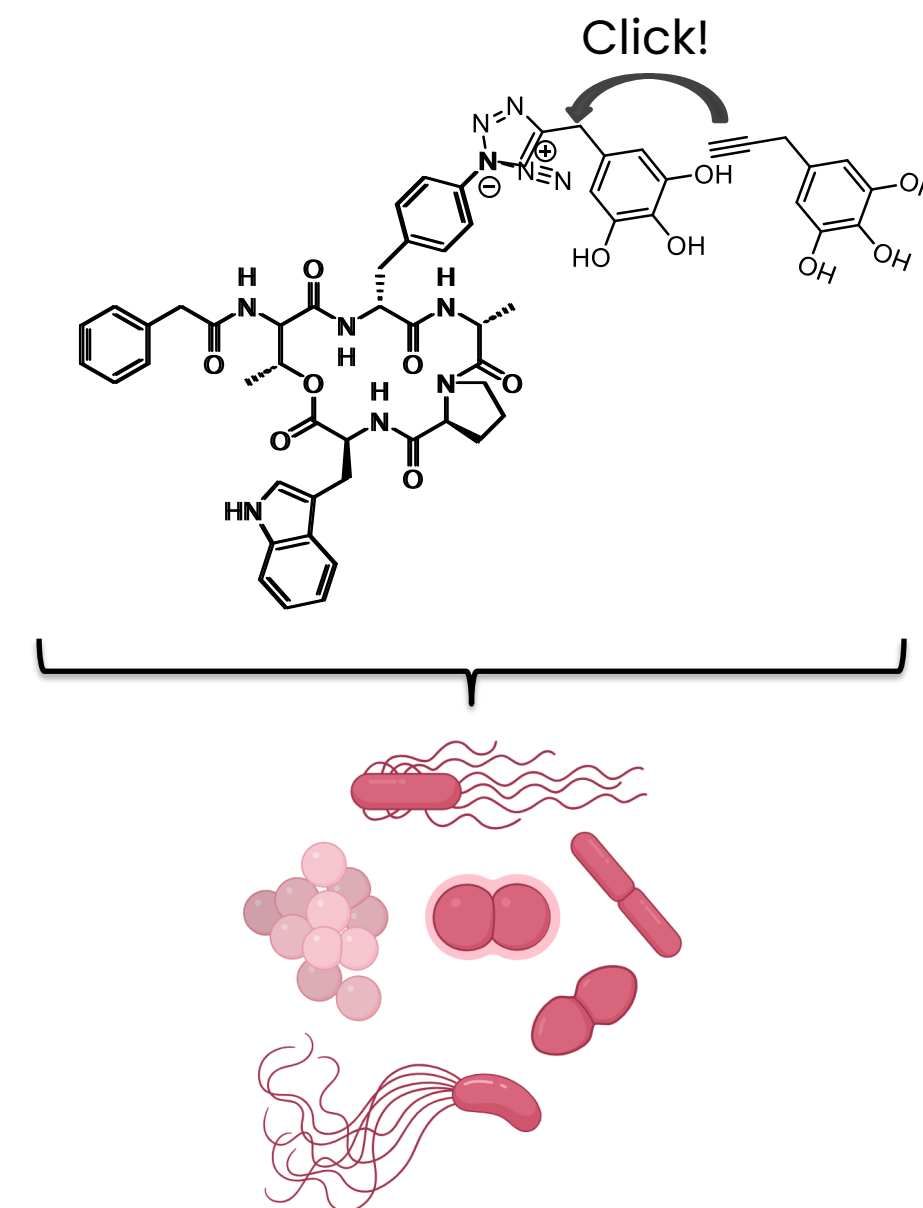


Yes

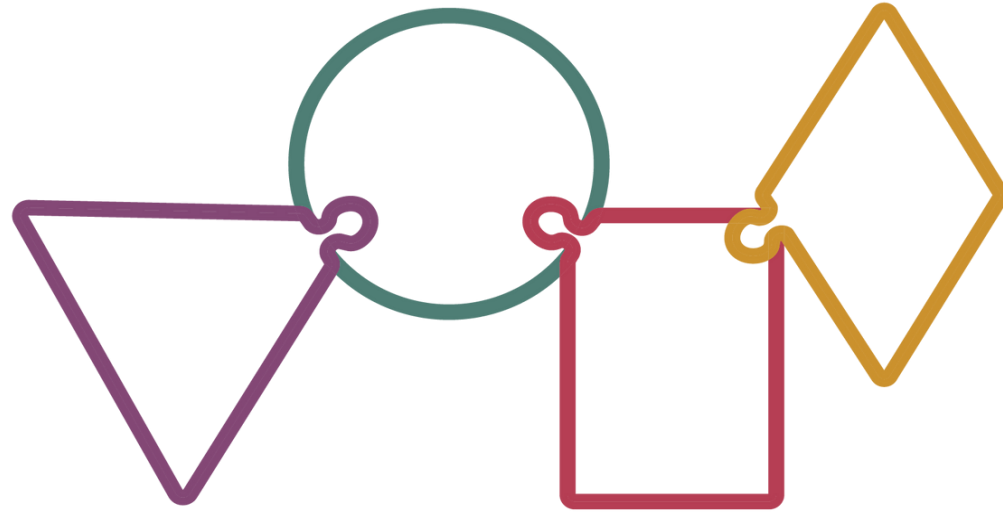


3. Synthesis

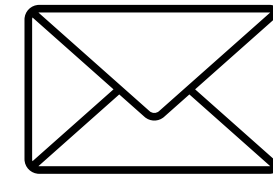
4. Drug Delivery



Thank you for your attention!



NRPieces



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This project is only possible thanks to our sponsors:



Ulrich Neumann-Stiftung



AG Voll



AG Bode



AG Thanbichler



Department of Chemistry



Department of Pharmacy



AG Sogaard



AG Höfer



AG Erb



AG Randau



Questions?



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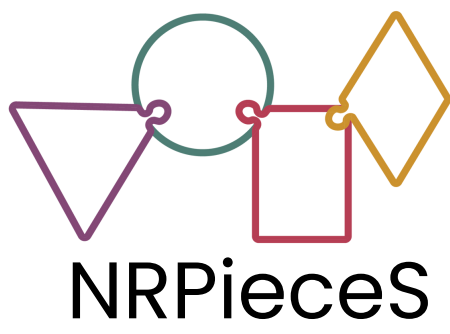
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iGEM Team 2025 Marburg



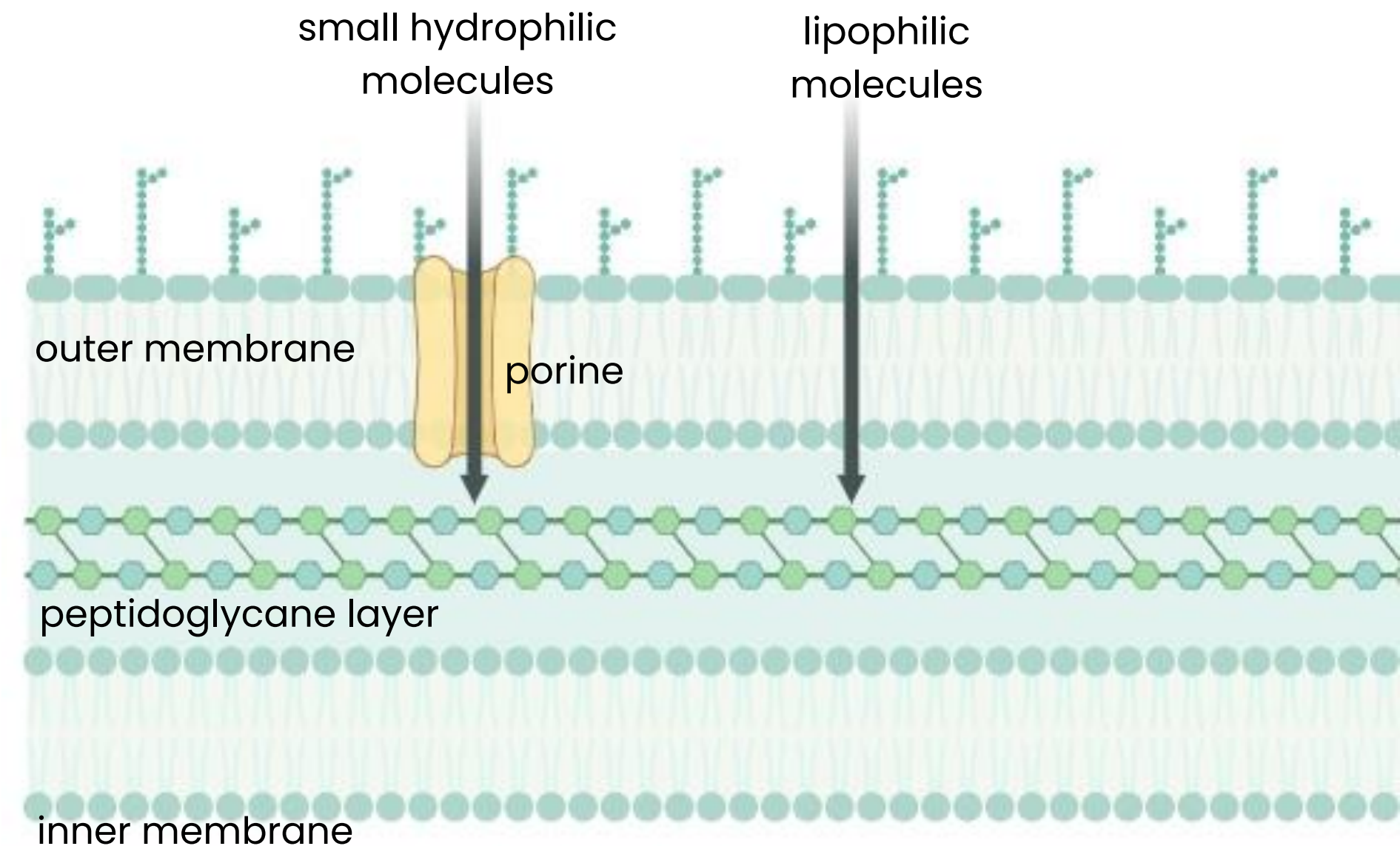
Supplemental slides



The Outer Membrane (OM) - A Robust Barrier



- Bottleneck of most NRP Antibiotics: Target **only Gram-(+)** bacteria
- OM of Gram-(-) bacteria = robust permeability barrier

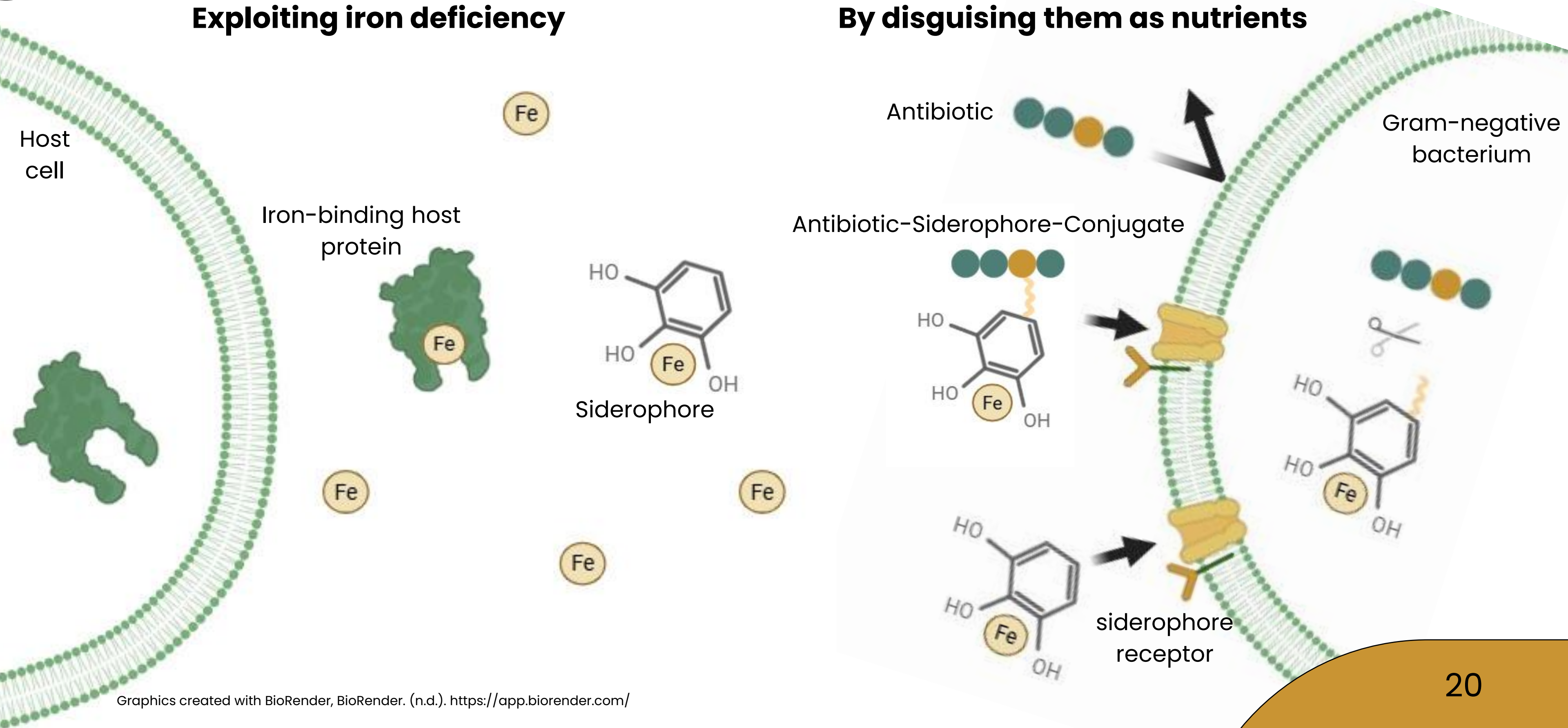


Facilitated Diffusion: Trojan Horse Strategy

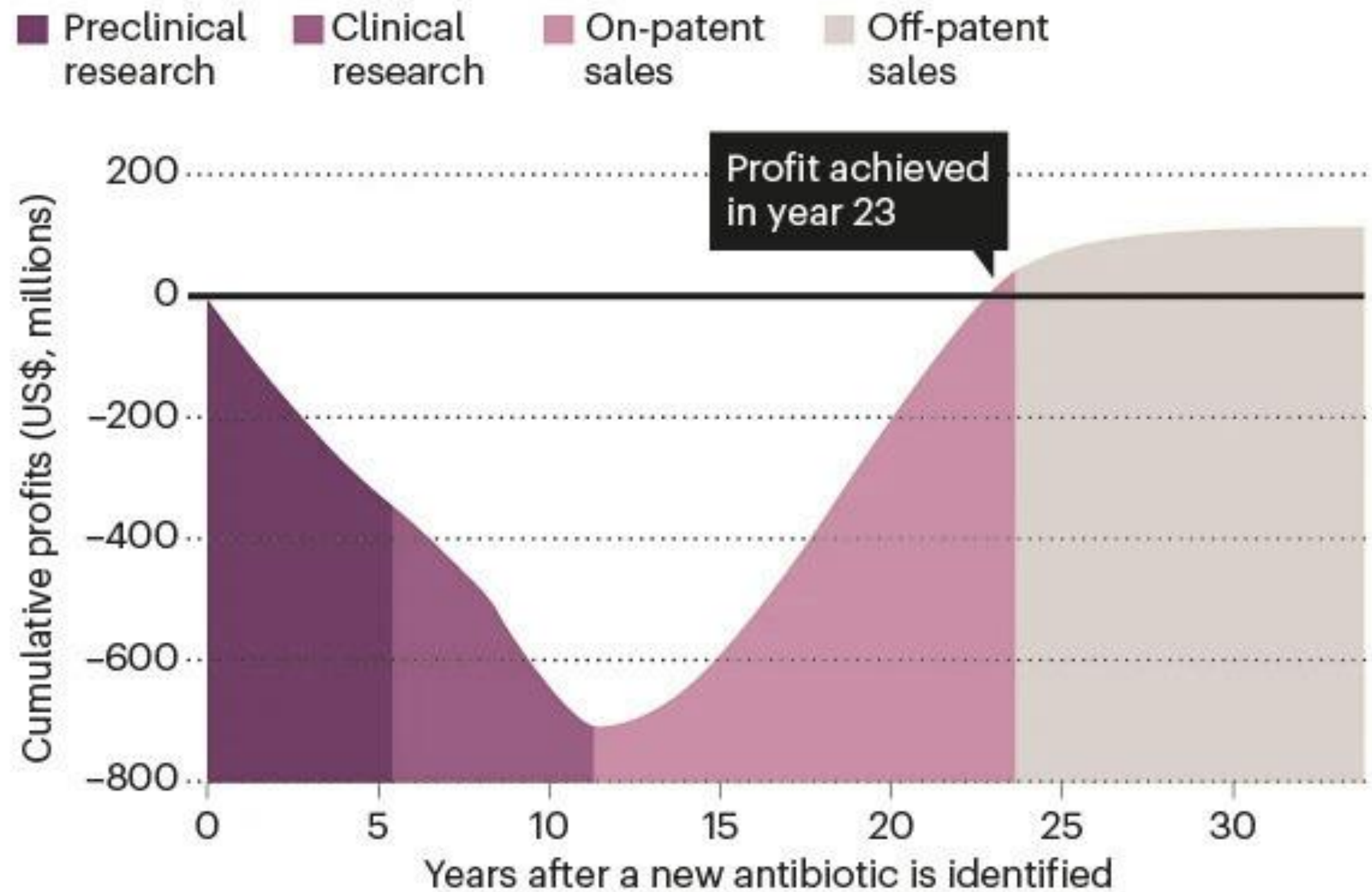


Exploiting iron deficiency

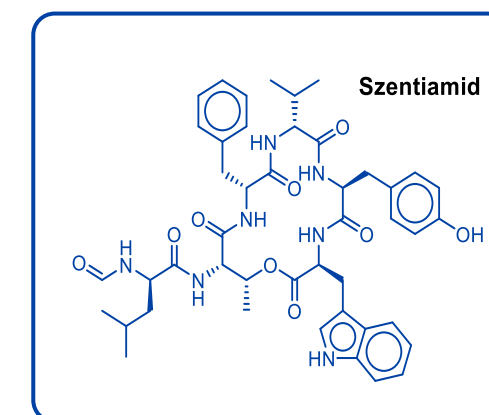
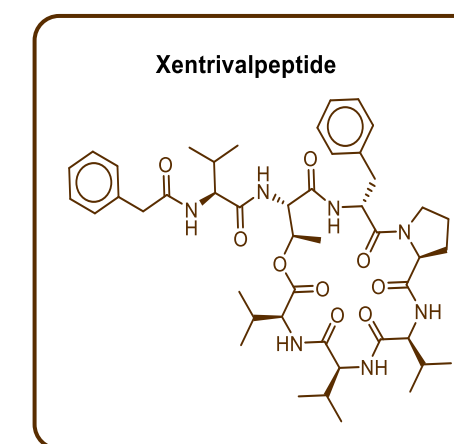
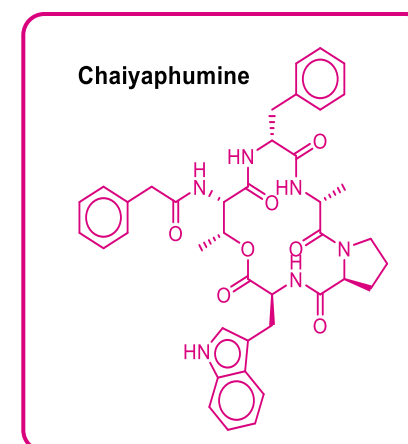
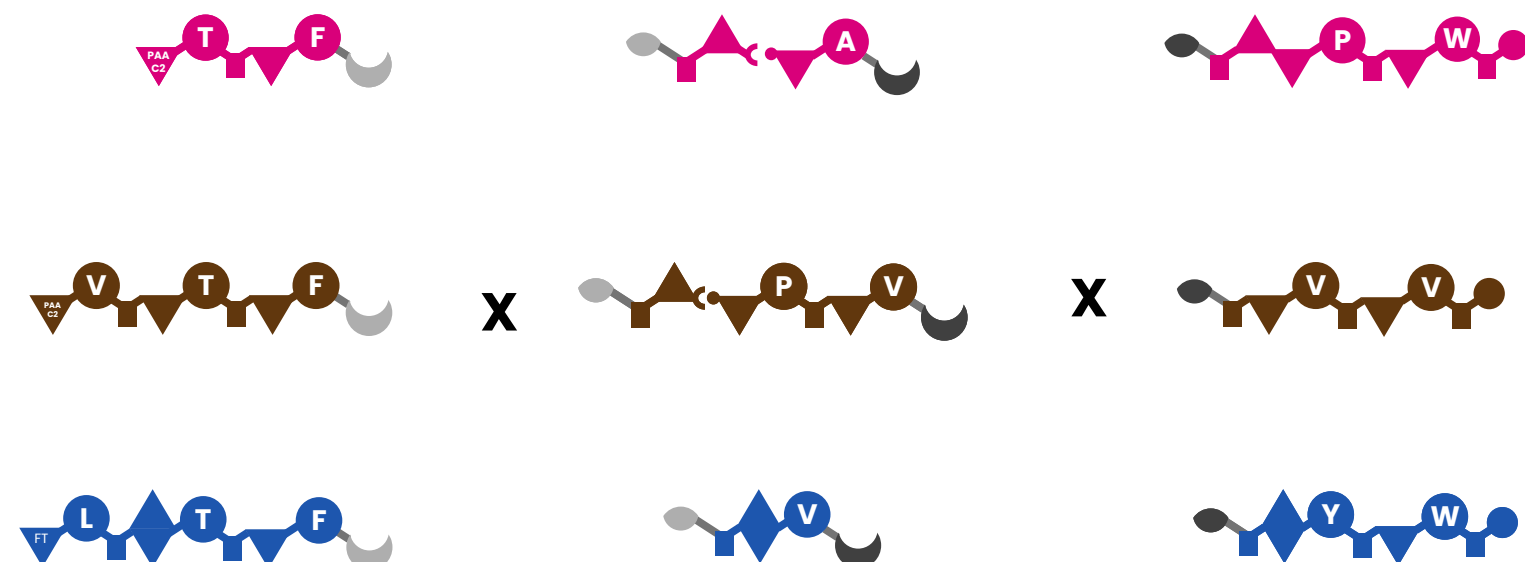
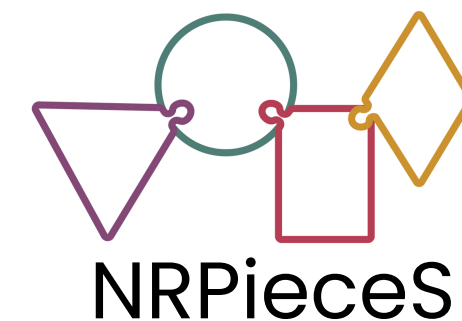
By disguising them as nutrients



What slows down development? - Low Profits

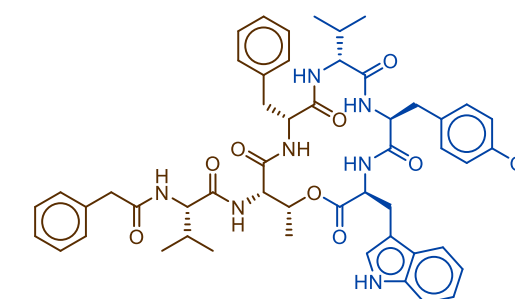
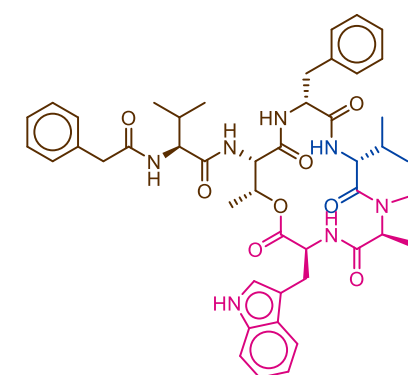
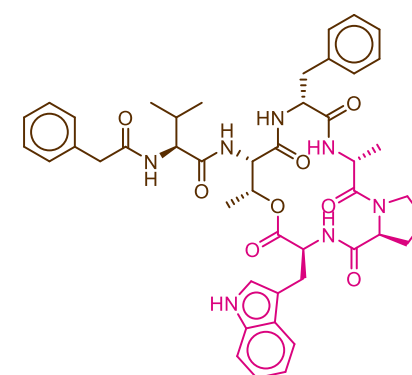
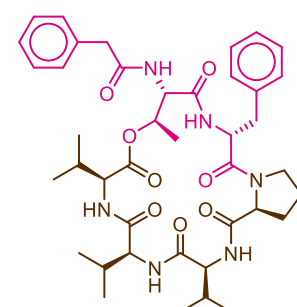
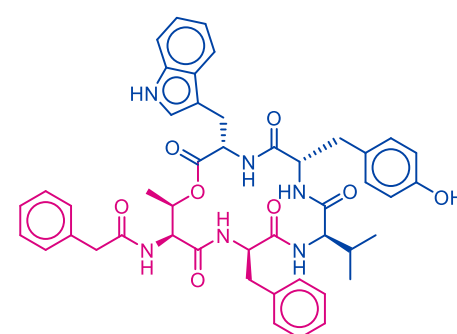
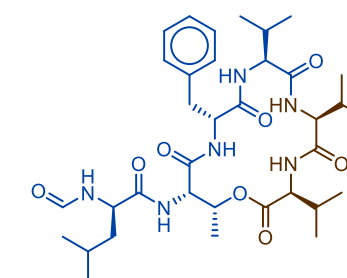
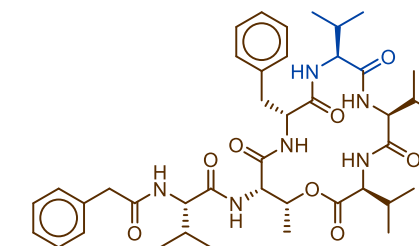
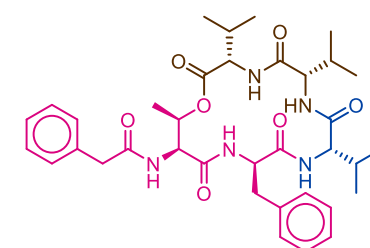
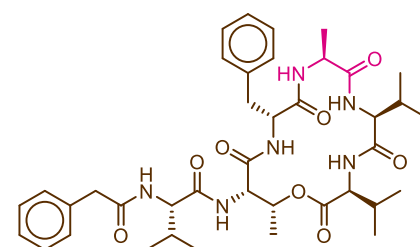
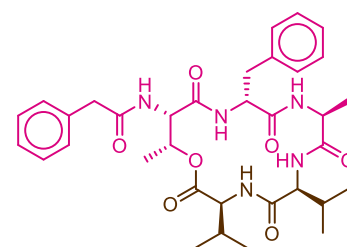
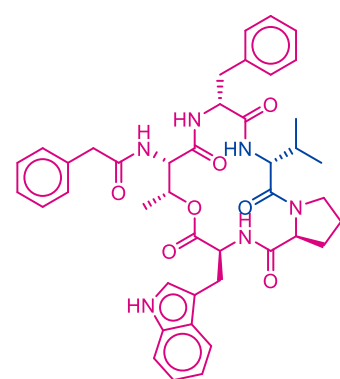


NRP Derivatization by Intein Shuffling

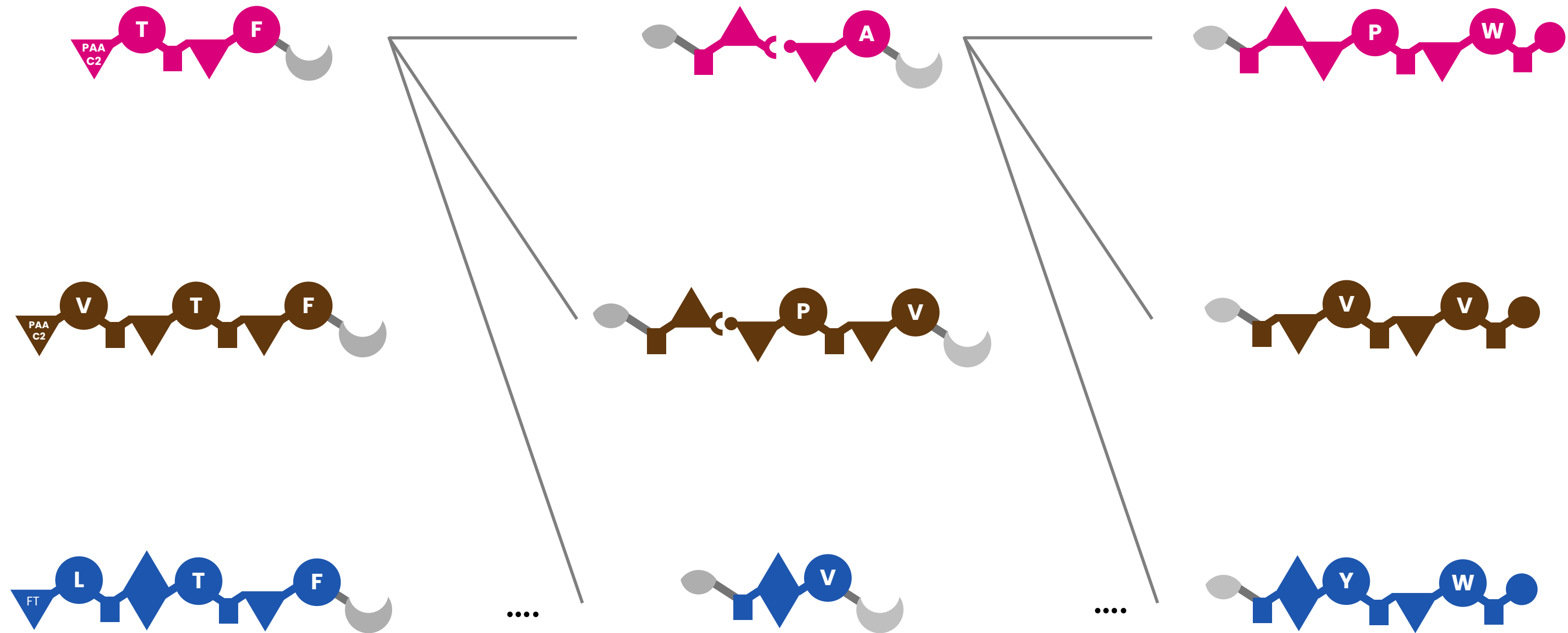


Combinations of 3 native NRPS

11 new peptides



NRP Derivatization by Intein Shuffling

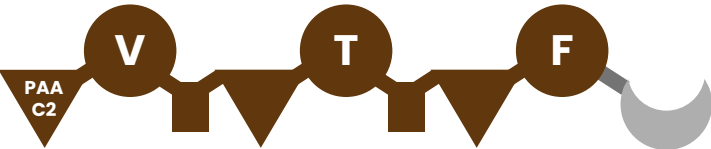
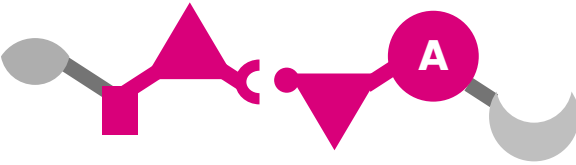


Chaiyaphumine Xentrivalpeptide Szentiamid A

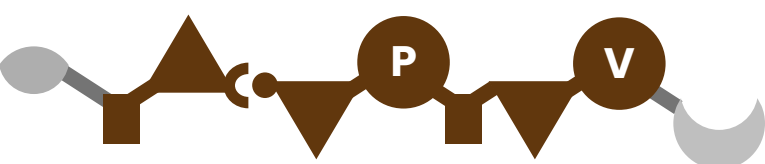
NRP Derivatization by Intein Shuffling



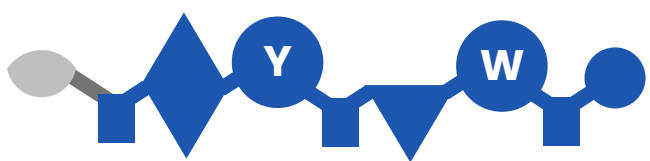
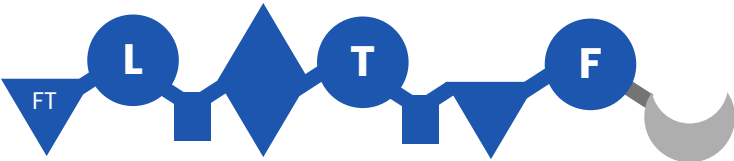
27 new NRPS



X



X



Chaiyaphumine



Xentrivalpeptide



Szentiamid A