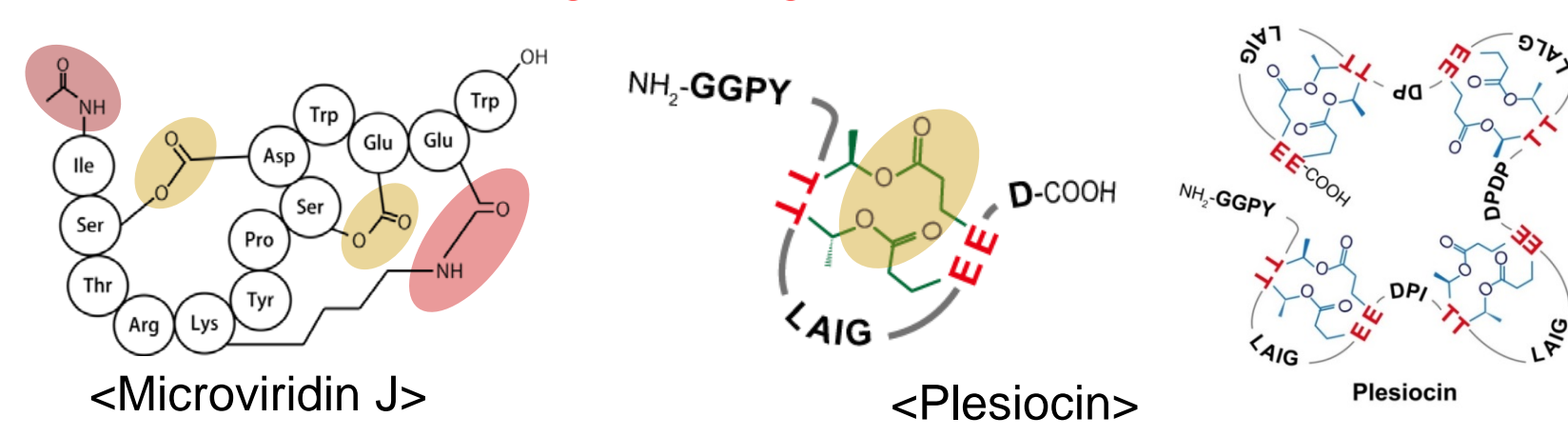
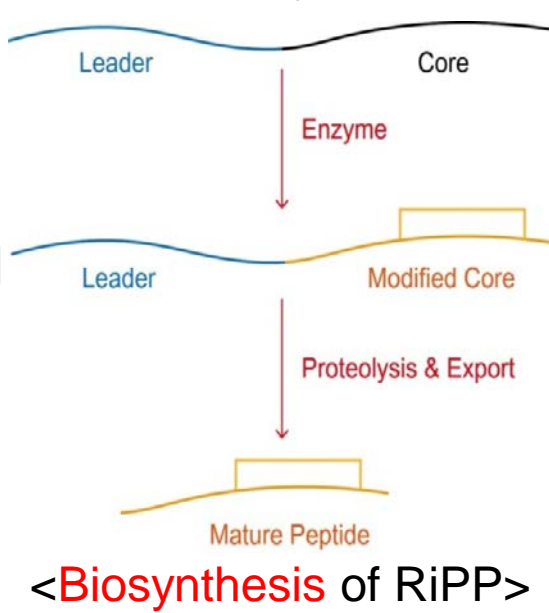


Introduction

What is RiPP?

Biosynthetic logic of RiPP is relatively **simple** and **amenable to bioengineering**.

Ribosomally synthesized
Post-translationally modified
Peptides



Microviridin, a subfamily in RiPP, can be a good model system to study due to its **simple PTM**

Phylogenetic analysis

Groups in Mv-like RiPPs

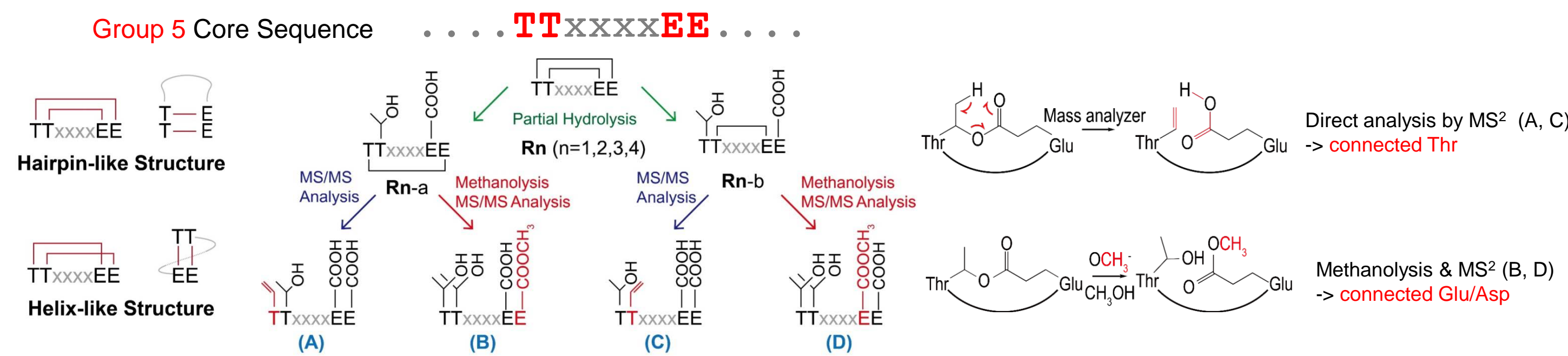
Various sequences, but **distinct** consensus are found on Microviridin-like RiPPs.



Connectivity analysis

Recent Studies

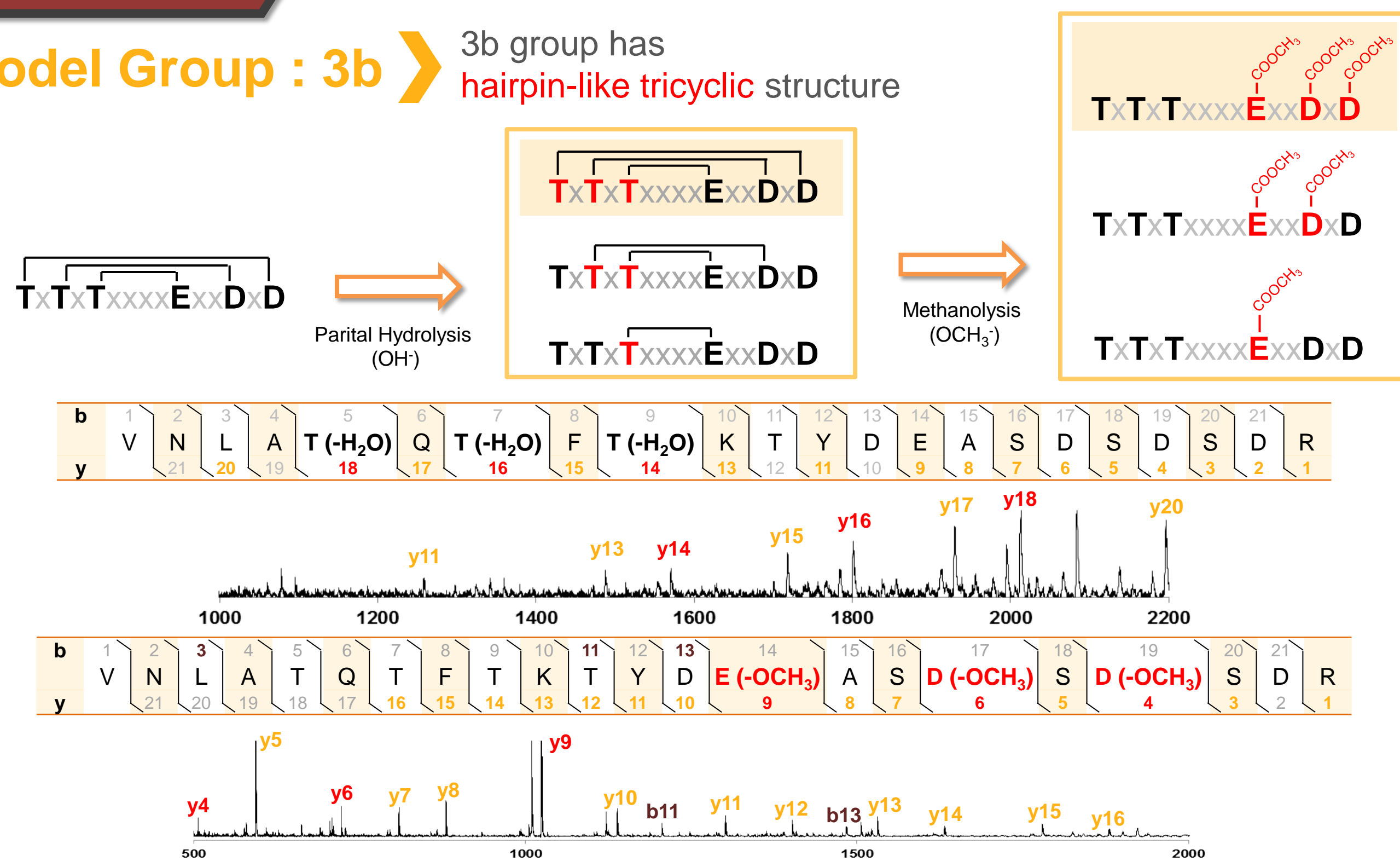
Connectivity of Mv-like RiPPs can be determined by combining **MS²** and **hydrolysis / methanolysis**.



Connectivity of microviridin-like RiPPs can be determined by **MS²** and **hydrolysis / methanolysis**

Model Group : 3b

3b group has **hairpin-like tricyclic** structure

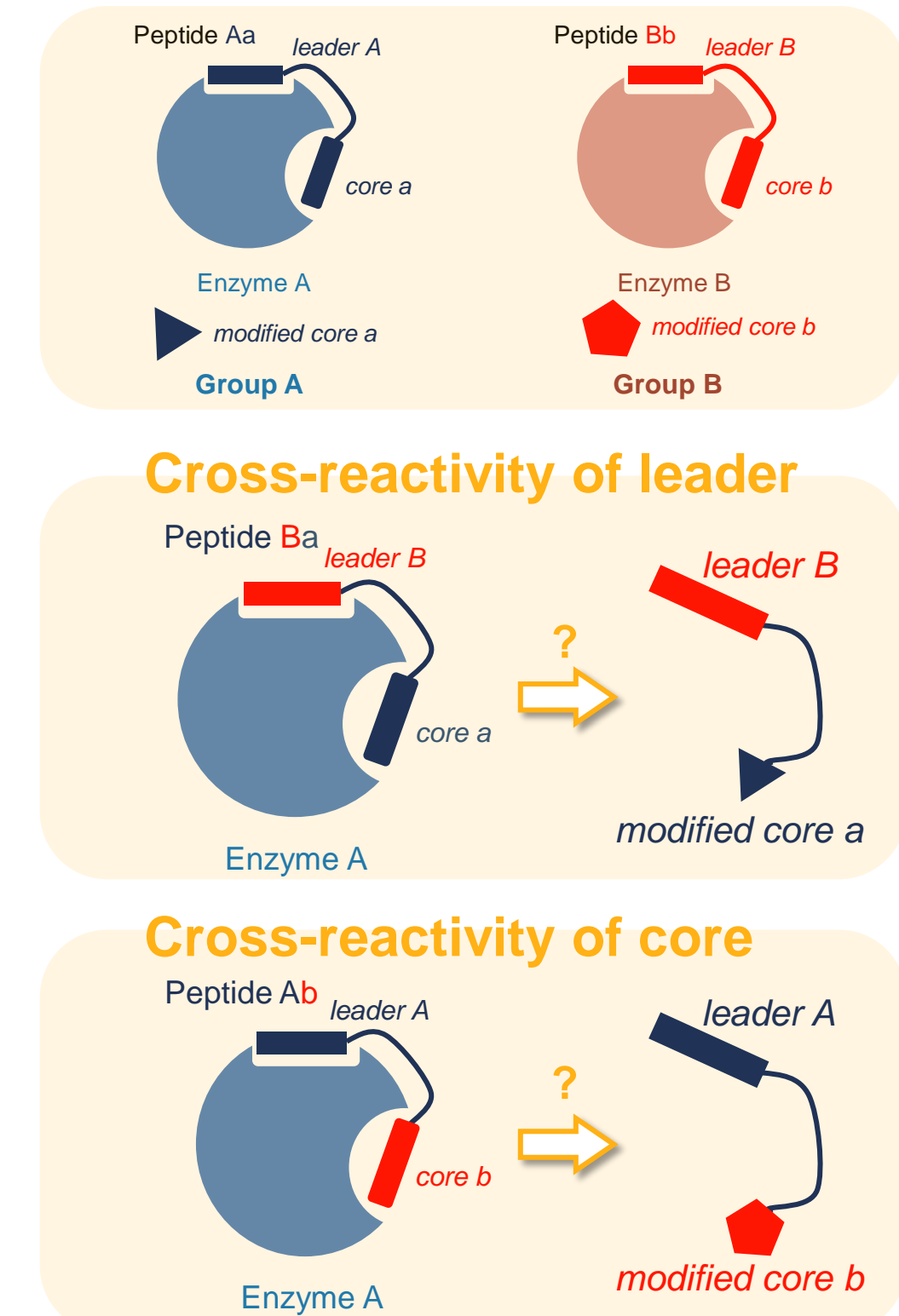


Cross-reactivity

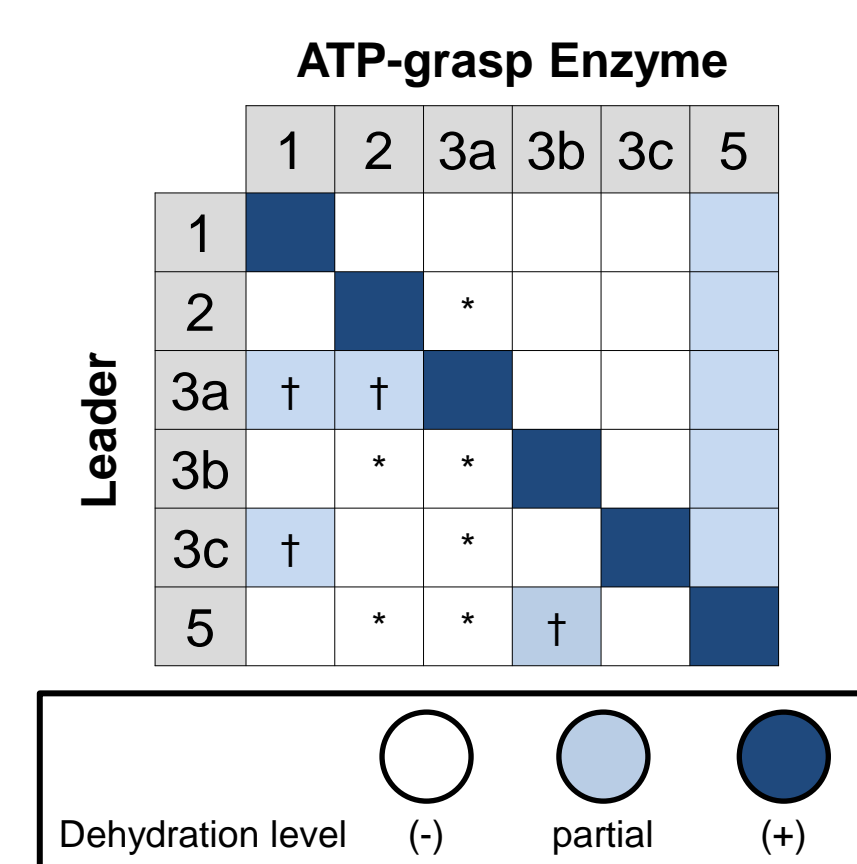
Cross-reactivity?

Result Summary

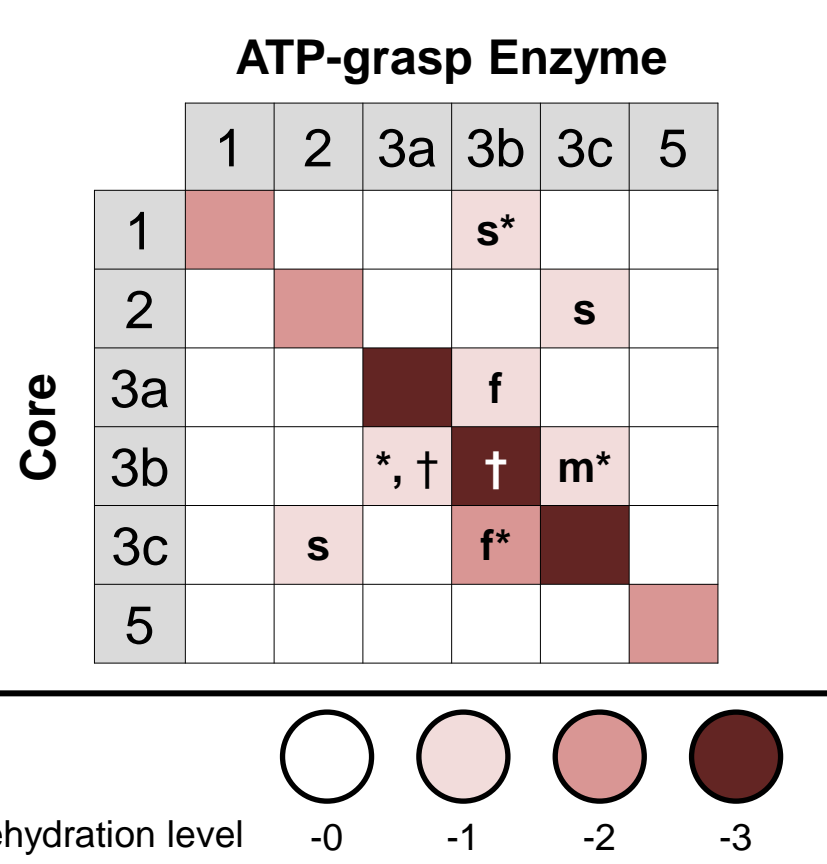
There are **6 orthogonal biosynthetic enzymes** in Mv-like RiPPs



Cross-reactivity of leader



Cross-reactivity of core



* : ATP-grasp enzyme was not even co-expressed
† : final product was not observed

f : faster than native
m : comparable to native
s : slower than native

* : Product has non-native connectivity
† : Product with 1 more crosslinking was slightly observed

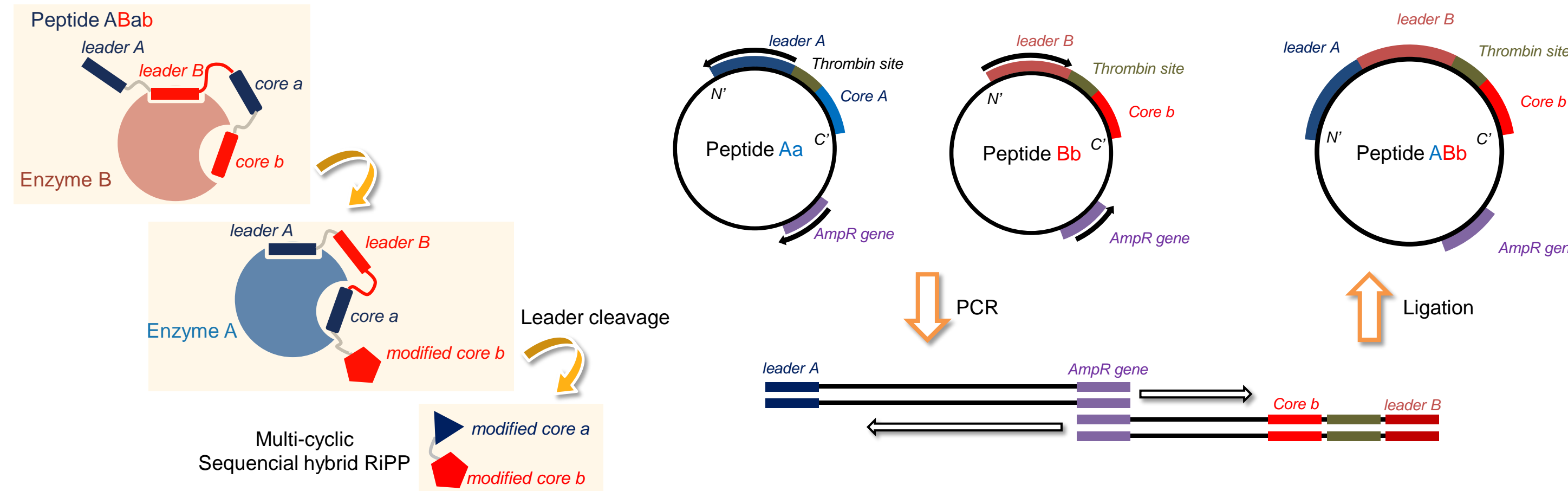
This is the first case that **macrocycles can be cross-unreactive** in same class of RiPP (e.g., lanthipeptide – highly cross-reactive)

Hybrid Mv-like RiPP

Hybrid RiPP?

Cloning Strategy

Overlap extension PCR



Preliminary Result

Sequential hybridization of Mv-like RiPPs is not always successful

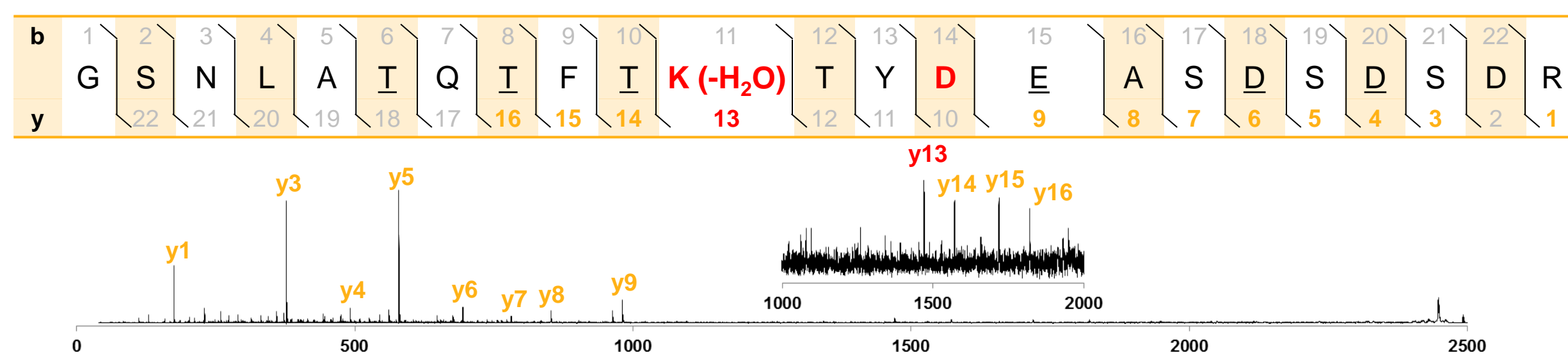
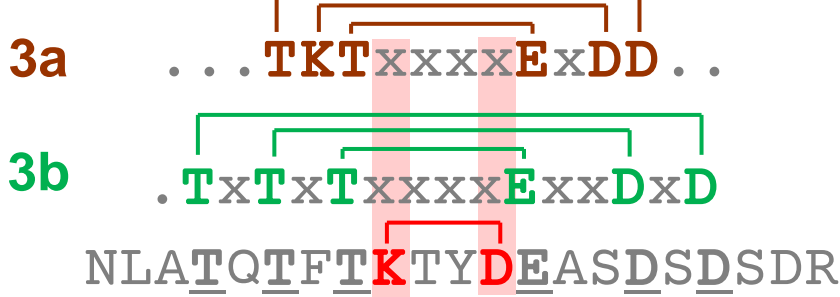
| Construct | 1 | 5 | Combined |
|-----------|--------|----|----------|
| 1_5 | 0 | -2 | -2 |
| 5_1 | -2 | -2 | -4 |
| 5_1x | 0 ~ -2 | -2 | -2 ~ -4 |

Linker between leaders = GS
Linker between leader and core = LVPR GS (Thrombin site)
Linker between cores = DPGS (DP for HCOOH digest)
A_B = (N') A leader – B leader – A core – B core
x = VTGGKG LVPRGS as linker between leader and core

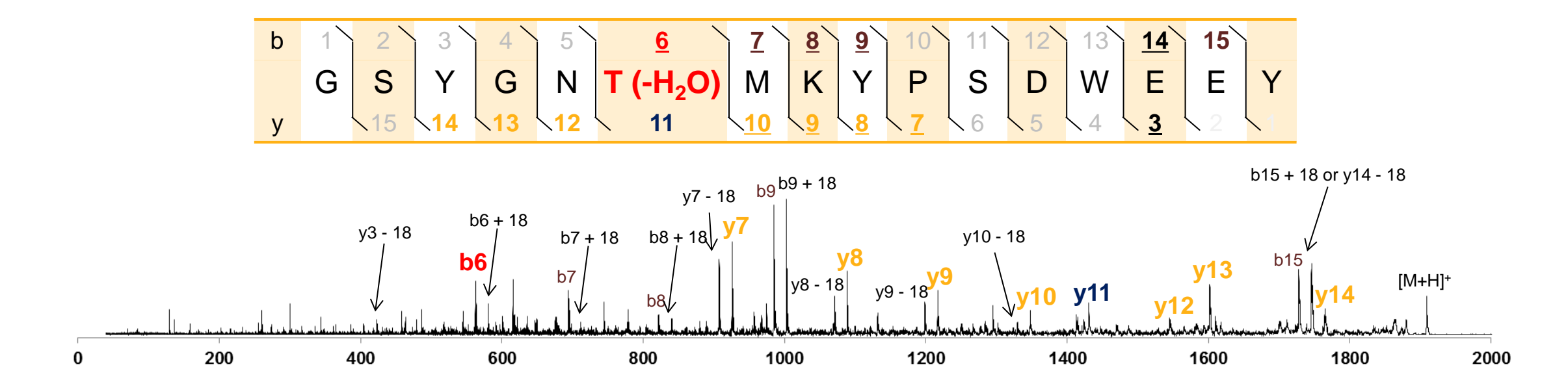
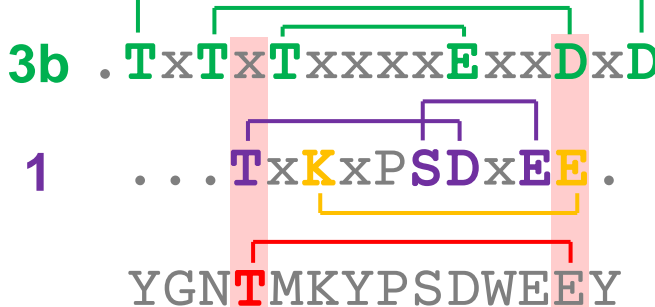
- Crosslinking reactions by each enzyme is **completely orthogonal**
- Linker between leader and core of native precursor is **important for efficient crosslinking reaction** by enzyme
- Sequential hybridization of multiple macrocycles from various RiPPs also might be difficult due to low efficiency

Connectivities of core peptides crosslinked by noncognate enzyme

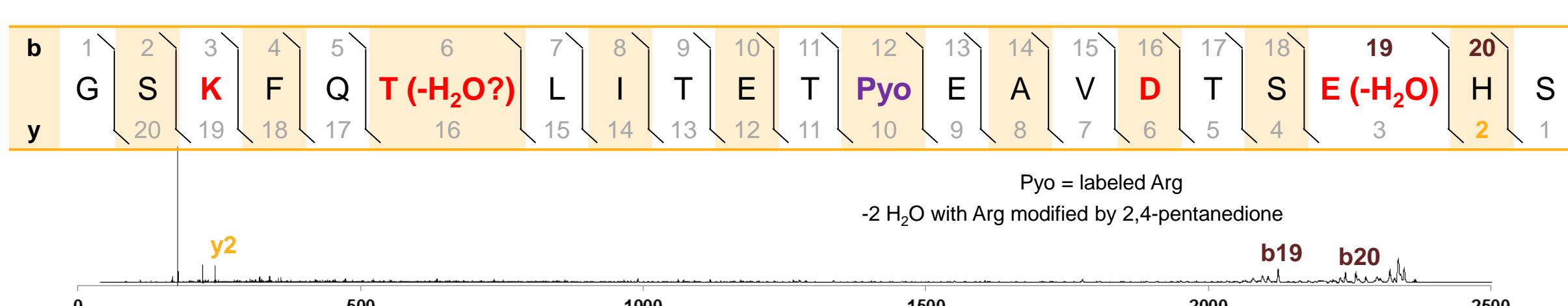
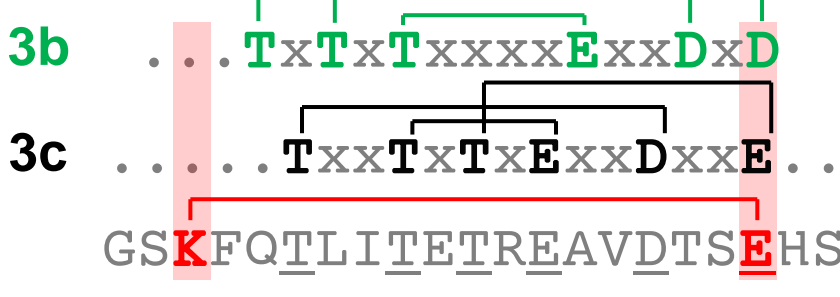
(1) core = 3b / enzyme = 3a



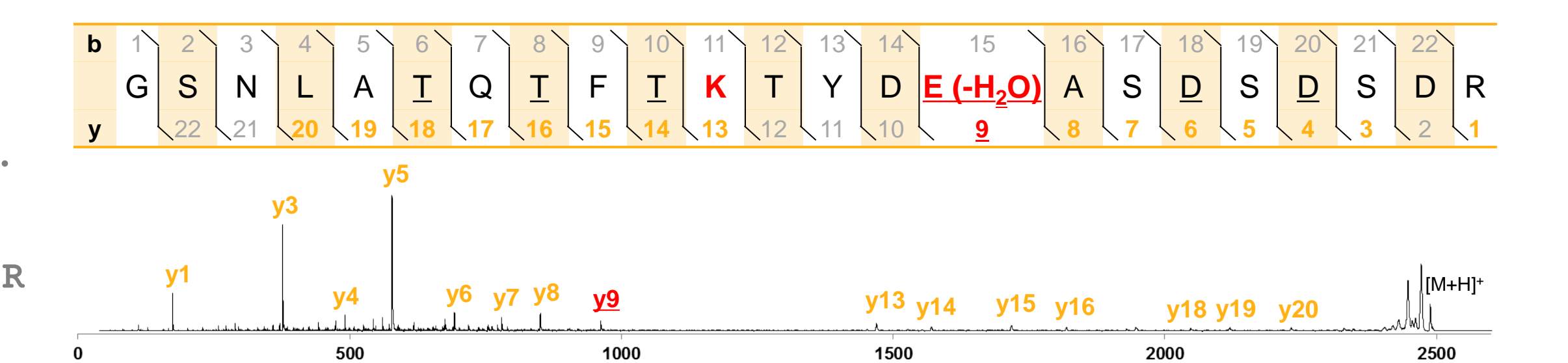
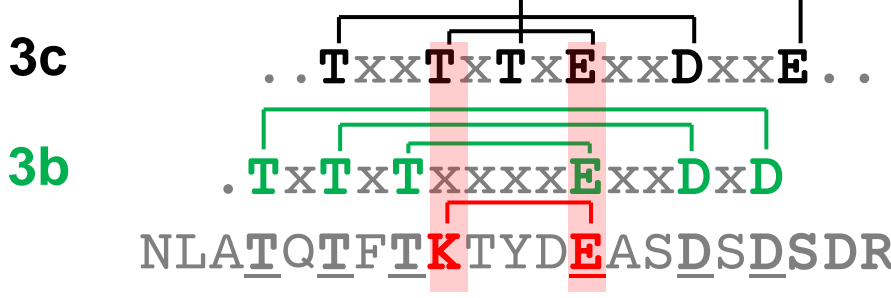
(2) core = 1 / enzyme = 3b



(3) core = 3c / enzyme = 3b

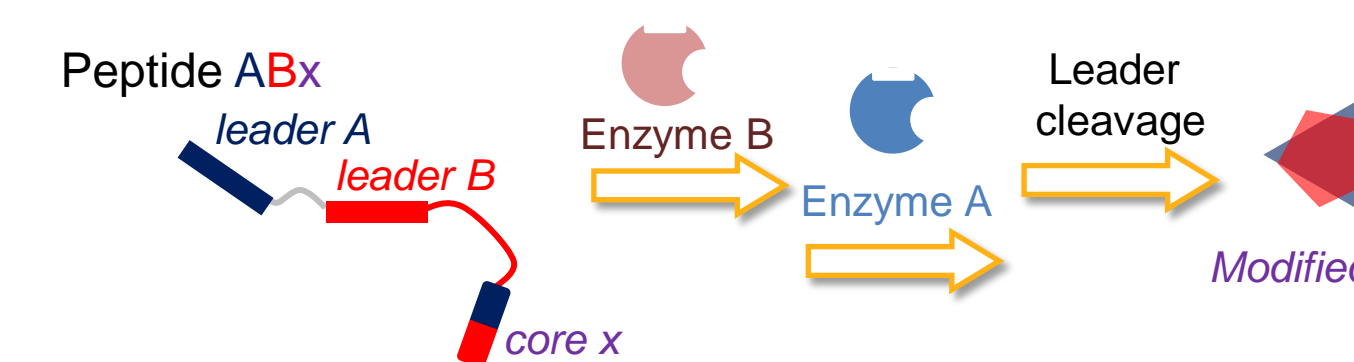


(4) core = 3b / enzyme = 3c

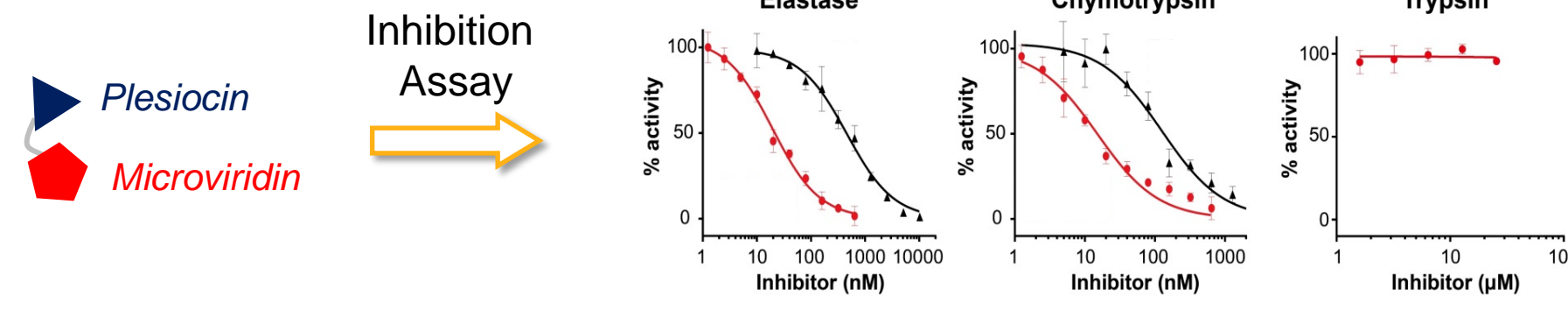


Future work

Overlap hybrid RiPP



Multiple Protease-targeting inhibitor development



Can sequential hybridized Mv-like RiPPs be used as **universal** serine-protease inhibitor?

Reference

- Angew. Chem. Int. Ed., **2008**, 47, 7756-7759
Nat. Chem. Biol., **2016**, 12, 973-979
Cell Chem. Biol., **2011**, 18, 1413-1421
Biochemistry, **2017**, 56, 4927-4930
Nat. Prod. Rep., **2013**, 30, 108-160
Proc. Natl. Acad. Sci., **2012**, 109, 18361-18366