

Lasso Peptides: Structure, Function, Biosynthesis, and Engineering



Lasso peptides as an emerging class of therapeutic peptides

Structural elucidation of lasso peptides

Chemical synthesis approaches

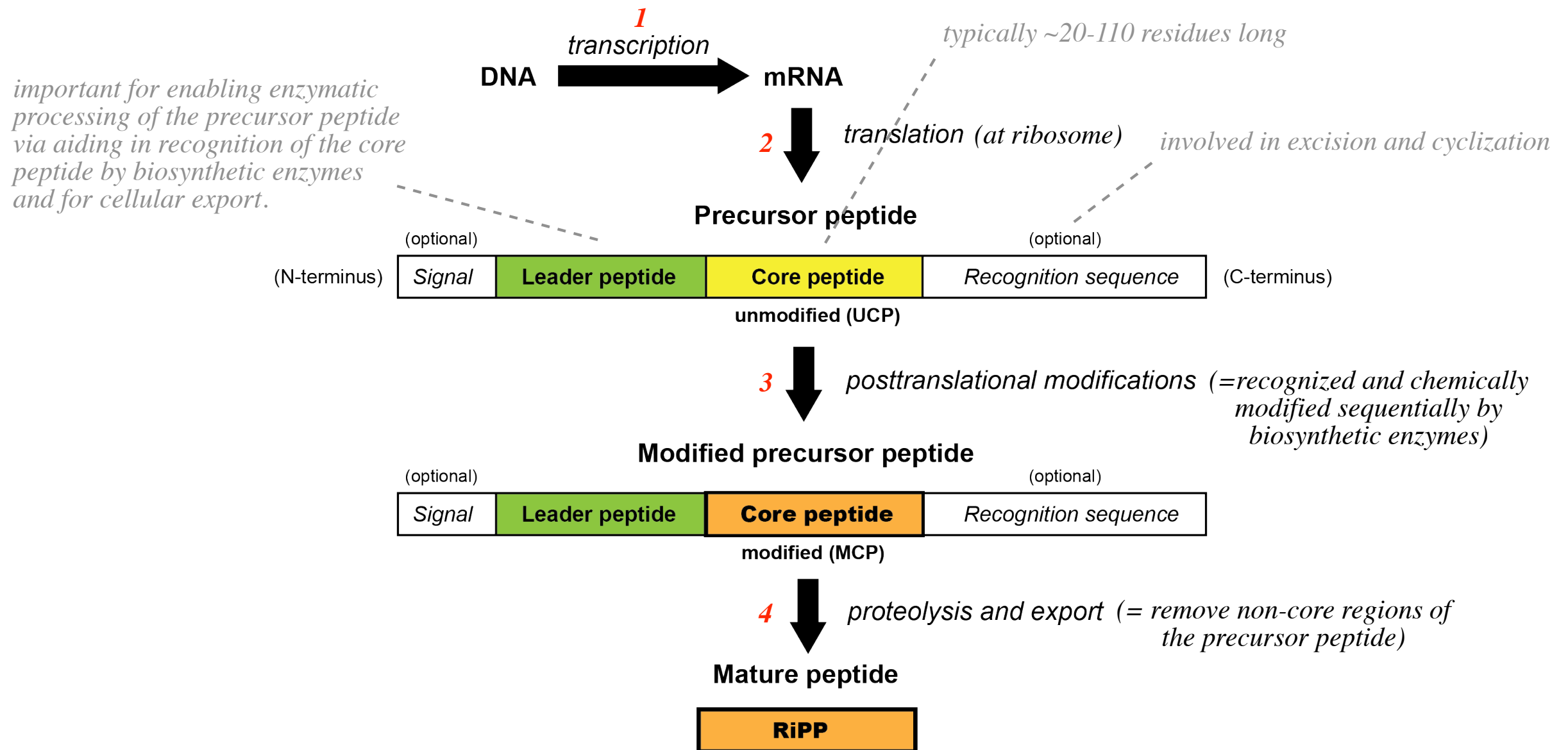
MccJ25 - a model for studies

Ximing Li

Acc. Chem. Res. **2015**, 48, 1909–1919; *Org. Biomol. Chem.* **2018**, 16, 5065–5080;
Nat. Prod. Rep. **2012**, 29, 996–1006; *Front. Bioeng. Biotechnol.* **2020**, 8, 571165.

Ribosomally synthesized and post-translationally modified peptides (RiPPs), or ribosomal natural products:

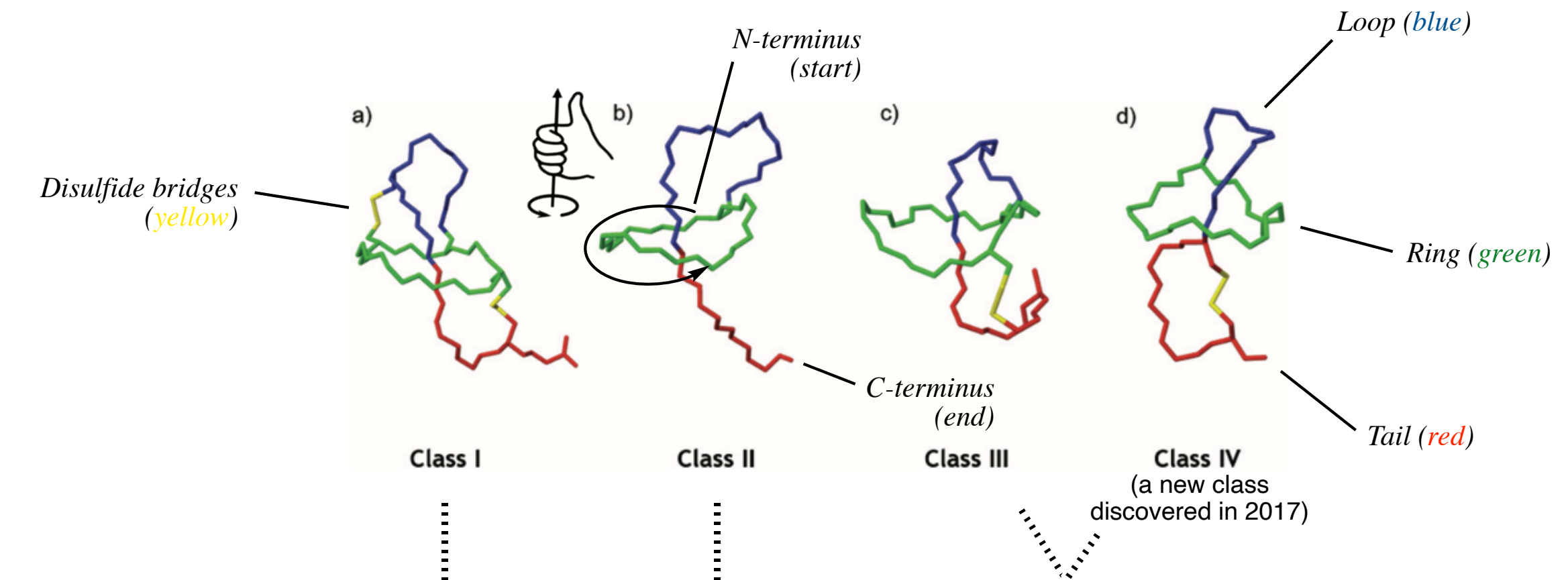
- are ribosomally-produced;
- undergo subsequent enzymatic post-translational modification;
- share the general scheme of:



- consisting of 20+ sub-classes: Autoinducing peptides; Bacterial Head-to-Tail Cyclized Peptides; Glycocins; **Lasso peptides**...

Characteristics of lasso peptides:

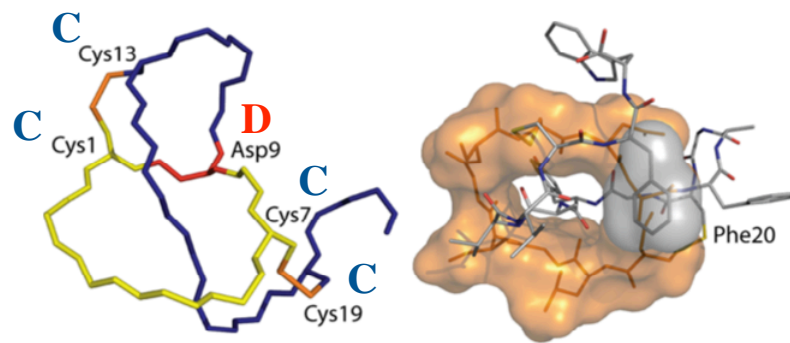
- C-terminus thread through an N-terminal macrolactam ring in a right-handed conformation;
- consisting of 15-26 proteinogenic amino acids and sharing an N-terminal 7- to 9-residue macrolactam ring;
- mostly carrying Gly, Cys, Ser or Ala at the N-terminus;
- ring is formed between the N-terminal alpha-amino group and the side chain of an Asp or Glu.



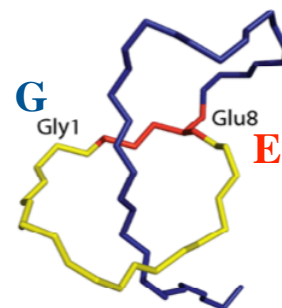
<u>Presence and number of disulfide bonds:</u>	2 disulfide bonds: 1 involves N-terminal Cys 1 connects ring to the tail	0 disulfide bond; lasso topology is stabilized by steric interactions	1 disulfide bond, either: connects the ring to the tail (Class III); or presents in the tail (Class IV)
<u>Extracted from</u>	actinobacteria(G+) only	mostly proteobacteria(G-)	actinobacteria(G+) only
<u># Discovered</u>	2	35	1 each
<u>Chirality</u>	all known lasso peptides for which structures have been solved exhibit the right-hand conformation		

Characteristics of lasso peptides:

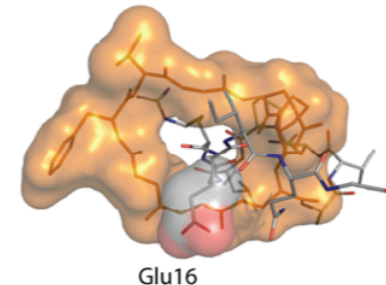
- C-terminus thread through an N-terminal macrolactam ring in a right-handed conformation;
- consisting of 15-26 proteinogenic amino acids and sharing an N-terminal 7- to 9-residue macrolactam ring;
- mostly carrying Gly/G, Cys/C, Ser/S or Ala/A at the N-terminus;
- ring is formed between the N-terminal alpha-amino group and the side chain of an Asp/D or Glu/E.



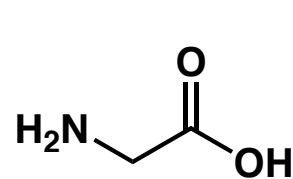
RP 71955
class I
4 cysteines
2 disulfide bonds



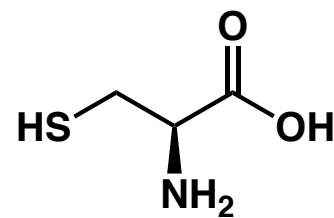
caulosegnin I
class II
0 cysteines
0 disulfide bonds



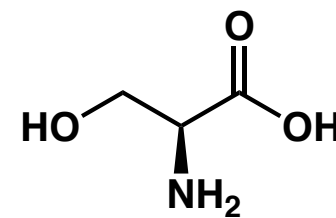
BI-32169
class III
2 cysteines
1 disulfide bond



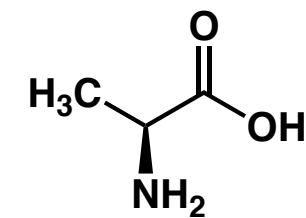
Glycine (Gly, **G**)



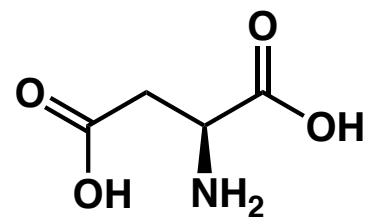
L-Cysteine (Cys, **C**)



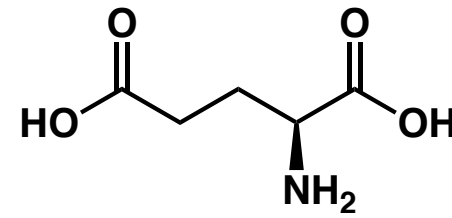
L-Serine (Ser, **S**)



L-Alanine (Ala, **A**)



L-Aspartic acid (Asp, **D**)



L-Glutamic acid (Glu, **E**)

Part of known lasso peptides:

“The steric stabilization occurs between the ring and the side chains of specific residues located in the C-terminal region, the so-called **plugs**. Positioned above and below the ring, the plugs maintain the knotted topology.”

siamycin type*

Streptomyces spp.



SSV-2083

Streptomyces svaceus ATCC 20983



xanthomonin I

Xanthomonas gardneri ATCC 19865



xanthomonin II

Xanthomonas gardneri



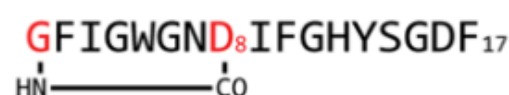
xanthomonin III

Xanthomonas citri pv.
magniferaeindicae LM941



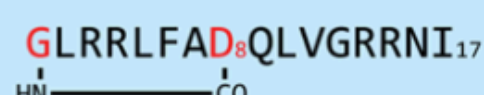
anantin

Streptomyces coeruleus



lassomycin

Lentzea kentuckyensis sp.



sphingonodin I

Sphingobium japonicum UT26



sphingonodin II

Sphingobium japonicum UT26



sphingopyxin II

Sphingopyxis alaskensis RB2256



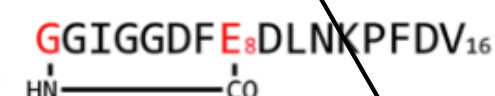
rhodanodin

Rhodanobacter thiooxydans LCS2



zucinodin

Phenylobacterium zucineum HLK1



astexin-1

Asticcacaulis excentricus CB48



astexin-2

Asticcacaulis excentricus CB48



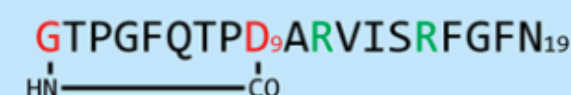
astexin-3

Asticcacaulis excentricus CB48



capistruin

Burkholderia thailandensis E264



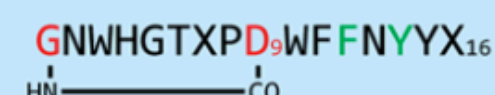
propeptin

Microbispora sp. SNA-115



RES-701 type**

Streptomyces sp. RE-701/RE-896



sphingopyxin I

Sphingopyxis alaskensis RB2256



streptomomycin

Streptomonospora alba YIM 90003



= 3D structure determined by NMR or X-ray



= 3D structure not known

G = residues involved in macrolactam ring formation

C = residues involved in disulfide bridge formation

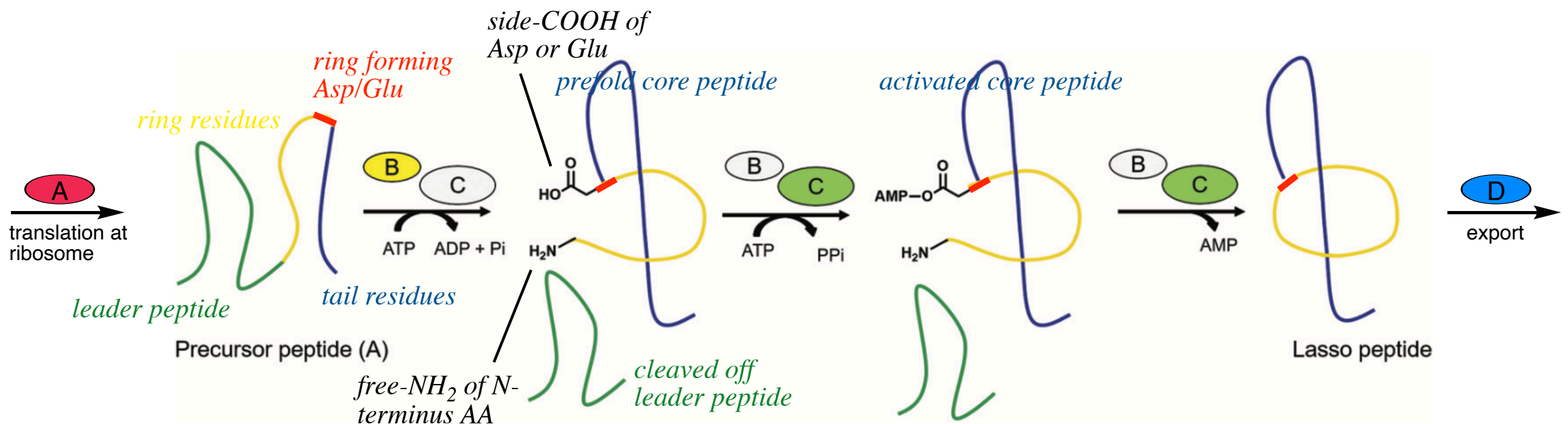
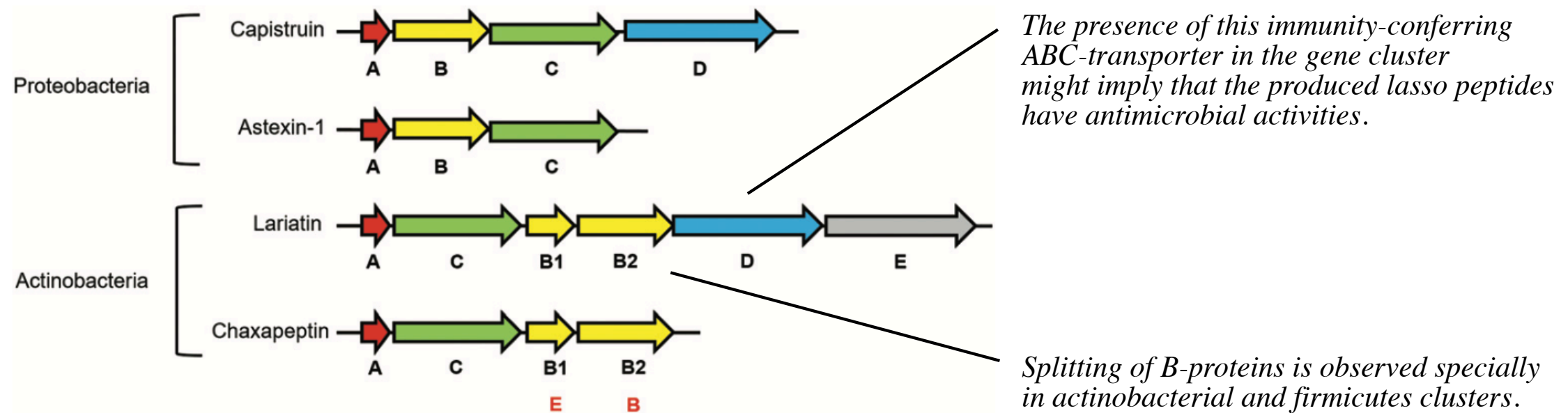
W = residues acting as plugs

D = residues typically cleaved off in main product before isolation

Biosynthesis of lasso peptides:

The biosynthetic gene clusters of lasso peptides consist of at least three genes, which encode

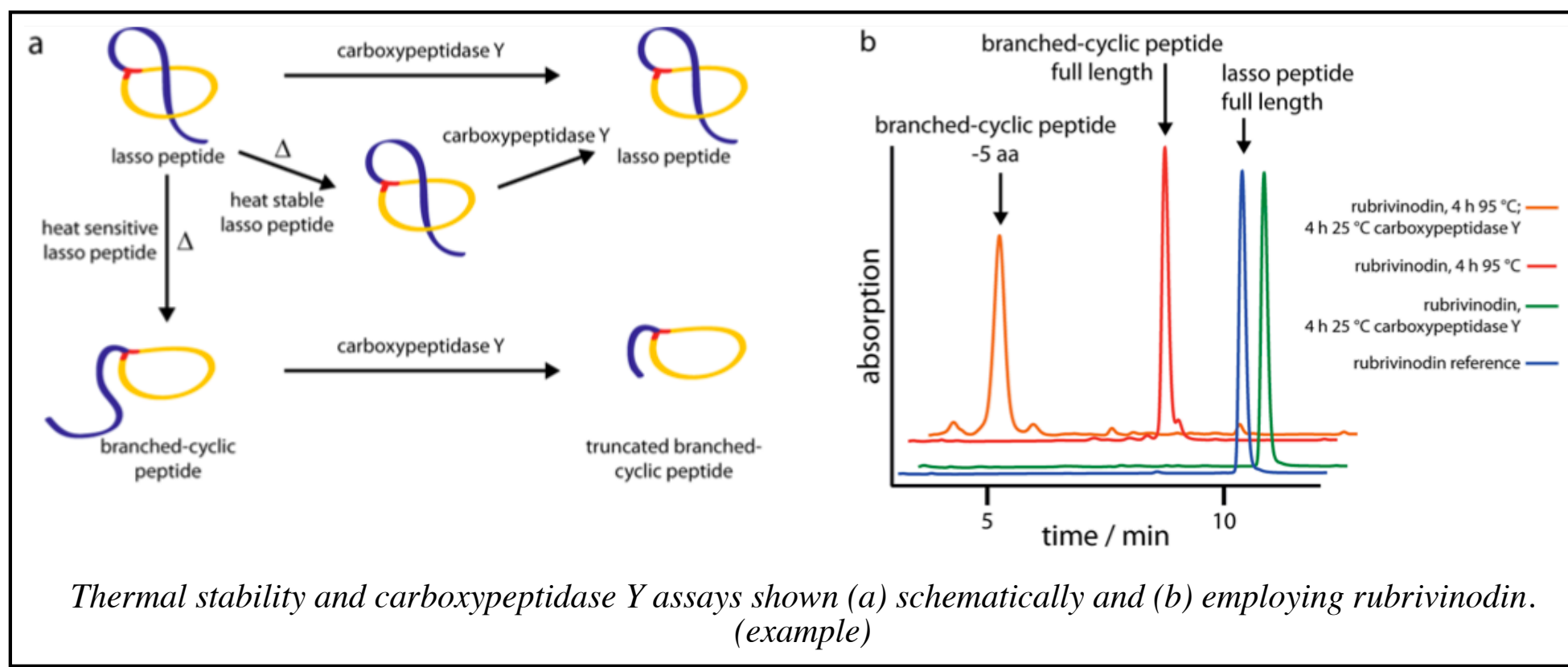
- the precursor peptide (A, recall RiPPs);
- an ATP-dependent cysteine protease (B) with homology to transglutaminase;
- an ATP-dependent macrolactam synthetase (C) with homology to asparagine synthetase;
- sometimes, a fourth gene encoding an ABC-transporter (D).



Physicochemical properties and structural elucidation:

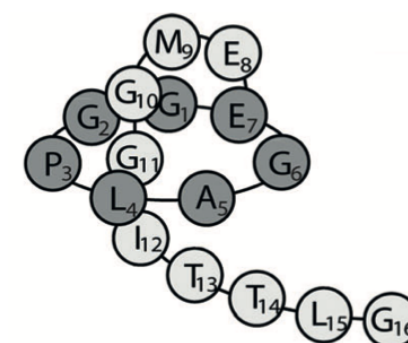
Thermal stability and protease stability...

- Hypothesis: extraordinary thermal stability, also high stability against proteases.
- Reasoning: rigid and folded structure.
- Fact: thermal stability (not always). protease stability (yes).



Ring size...

- Hypothesis: all lasso peptides contain either 8 or 9 amino acid residue rings.
- Reasoning: 10-residue seems to be too big to allow steric maintenance, while 7-residue rings seem too small to allow threading.
- Fact: xanthomonins I-III, 7 amino acid rings (also with high thermal stability)



Xanthomonin II
 ACIE. 2014, 53, 2230–2234

The criteria for these physicochemical properties are still poorly understood.

Physicochemical properties and structural elucidation:

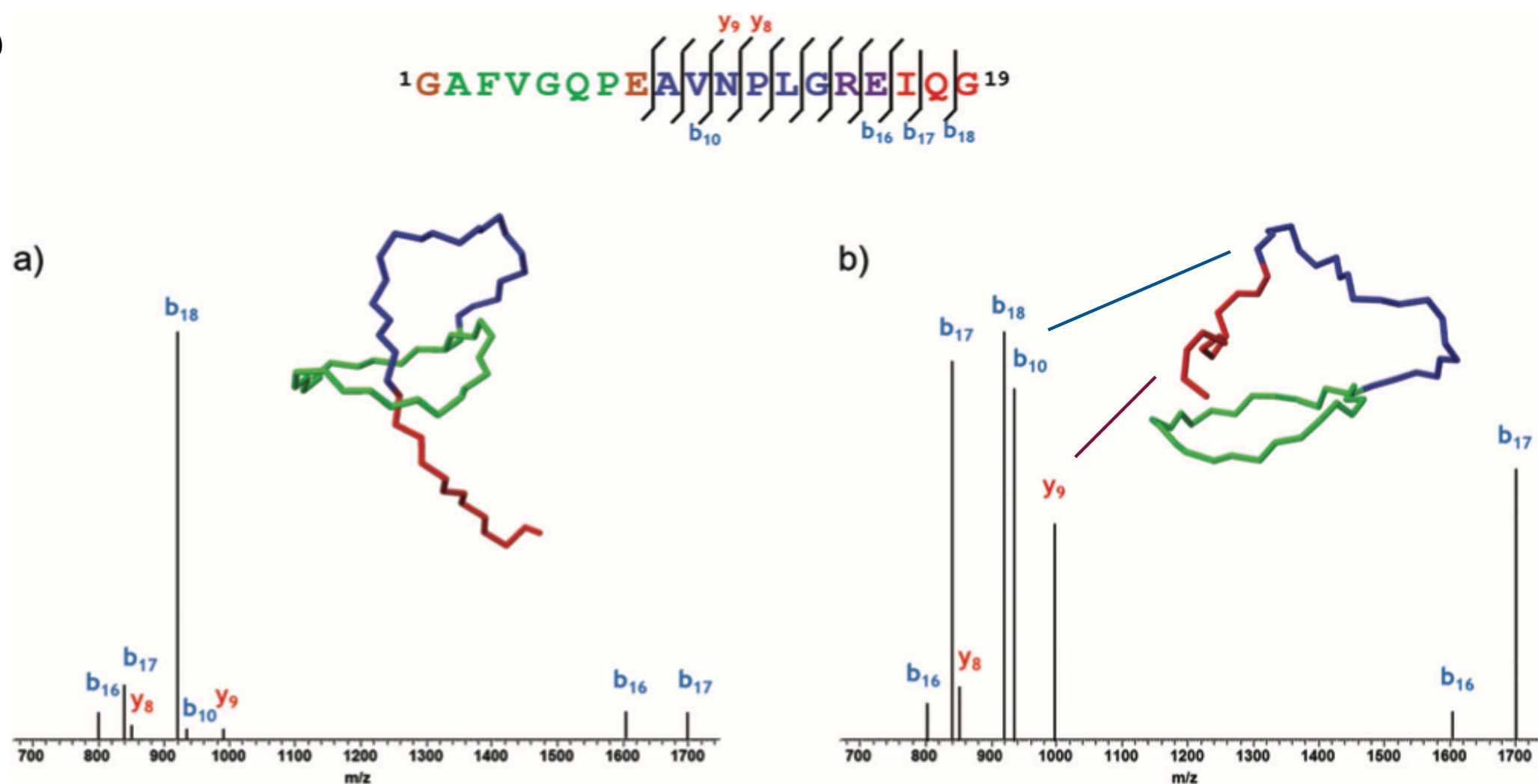
Most common technique used to characterize lasso peptides:

NMR - powerful but complicated, sometimes result's vague.

X-ray - no general condition developed for lasso peptide crystallization.

MS² - useful when combined with thermal and enzymatic treatments, providing preliminary information about the location of the ring based on the fact that canonical fragmentation in lasso peptides will occur only in the loop and tail regions of the peptide.

(example)



MS² spectra of the main fragmentations of (a) caulosegnin I (PDB code 2XL6) and (b) its branched-cyclic topoisomer. The fragmentations in black correspond to the doubly charged ions. The amino acid plugs are shown in purple and the amino acids involved in the macrolactam ring formation is brown. The ring residues are shown in green, amino acid belonging to the loop in blue, and the amino acids in the tail in red.

Chemical synthesis of lasso peptides:

Attractive candidates for drug development:

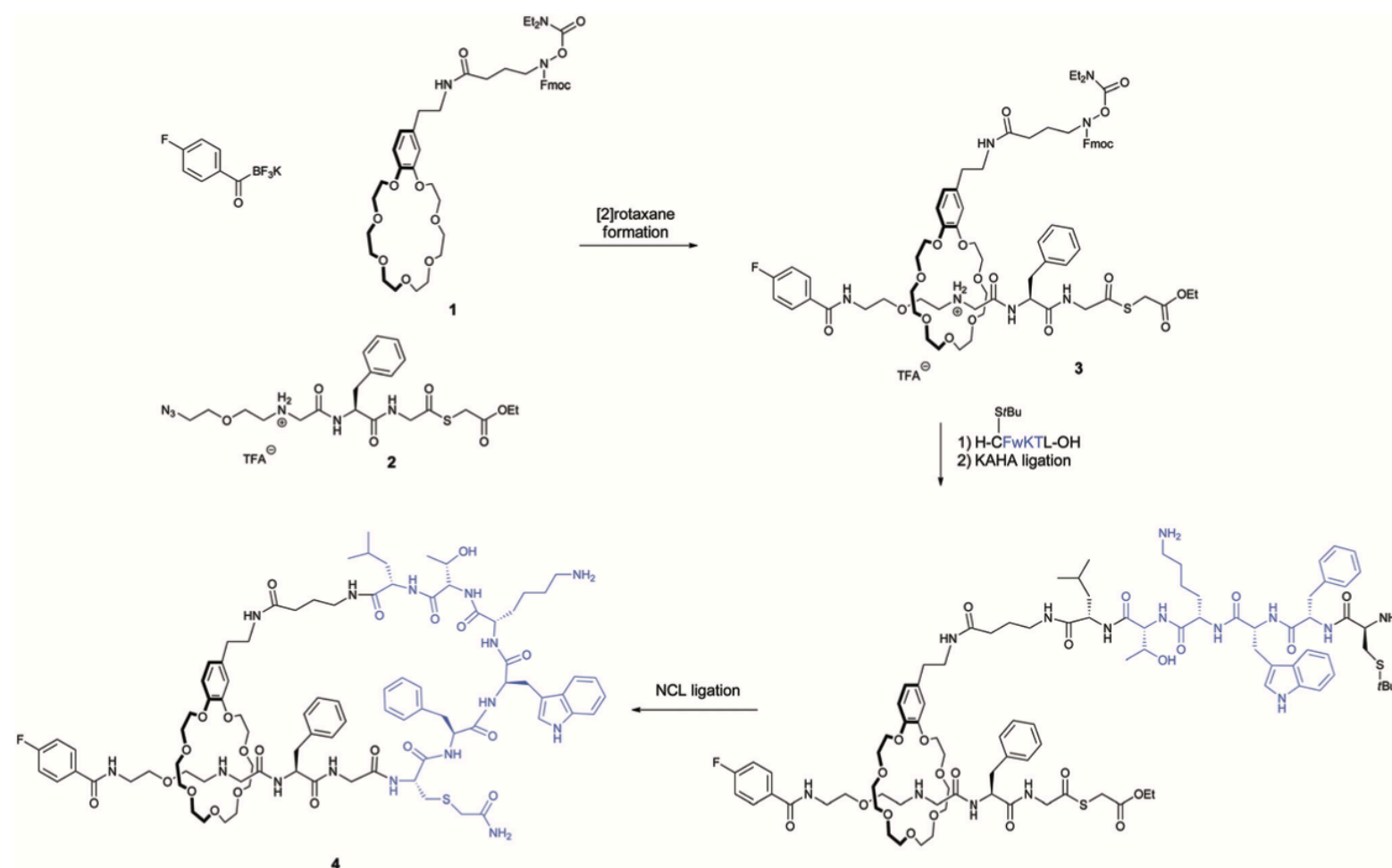
- diverse functionality
- high stability -> ideal scaffolds for epitope grafting

Most efforts on synthetic strategies for lasso peptides are based on either:

- imitation of rotaxane and catanane self-entangled structure



“Hey, would you mind taking a quick sculpture of me and my family?”



Lasso peptide-based rotaxane, prepared from a 21-crown-7 ether and secondary dialkylammonium.

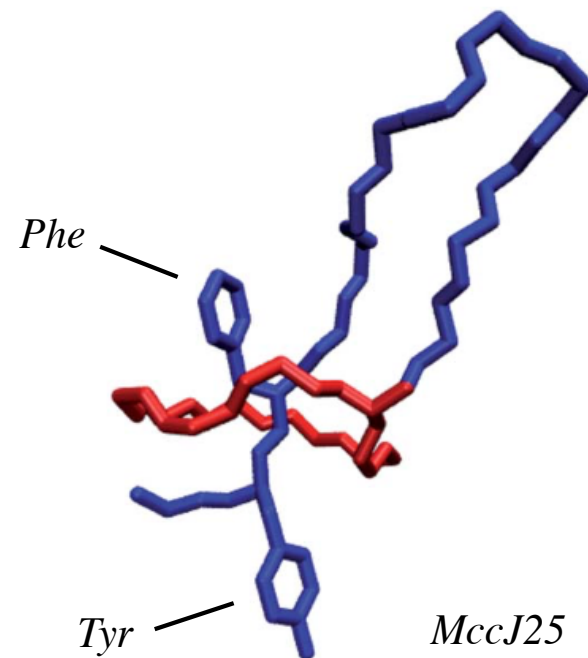
Chem. Sci. **2017**, 8, 2878–2884

- chemoenzymatic approaches (mostly difficult due to large entropic barriers or regio-/stereo-selectivity issues)

No lasso peptide has been synthesized to date using chemical protocols

- highly challenging synthetic routes
- folding of peptides disturbs the necessary *preorganization* between the components to be assembled into an interlocked lasso structure

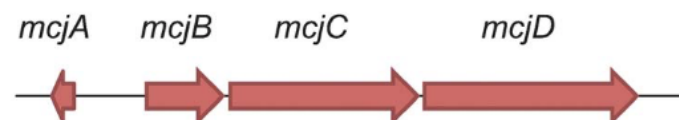
A model for studies - Microcin J25 (MccJ25):



MccJ25

*Isolated from feces of newborn infant,
Instituto de Química Biológica, Uruguay, 1992*

- Structure: extreme example of “steric lock” - perfect stability
- Function: active against several Gram(-) bacterial species including *E. coli*, *Salmonella*, and *Shigella*, with its MoA studied extensively



*Schematics of the gene clusters
for MccJ25*

- McjA: 58 amino acids, precursor protein
- McjB: 208 amino acids, processing enzyme (protease)
- McjC: 513 amino acids, processing enzyme (cyclization)
- McjD: 580 amino acids, export protein (ABC transporter)

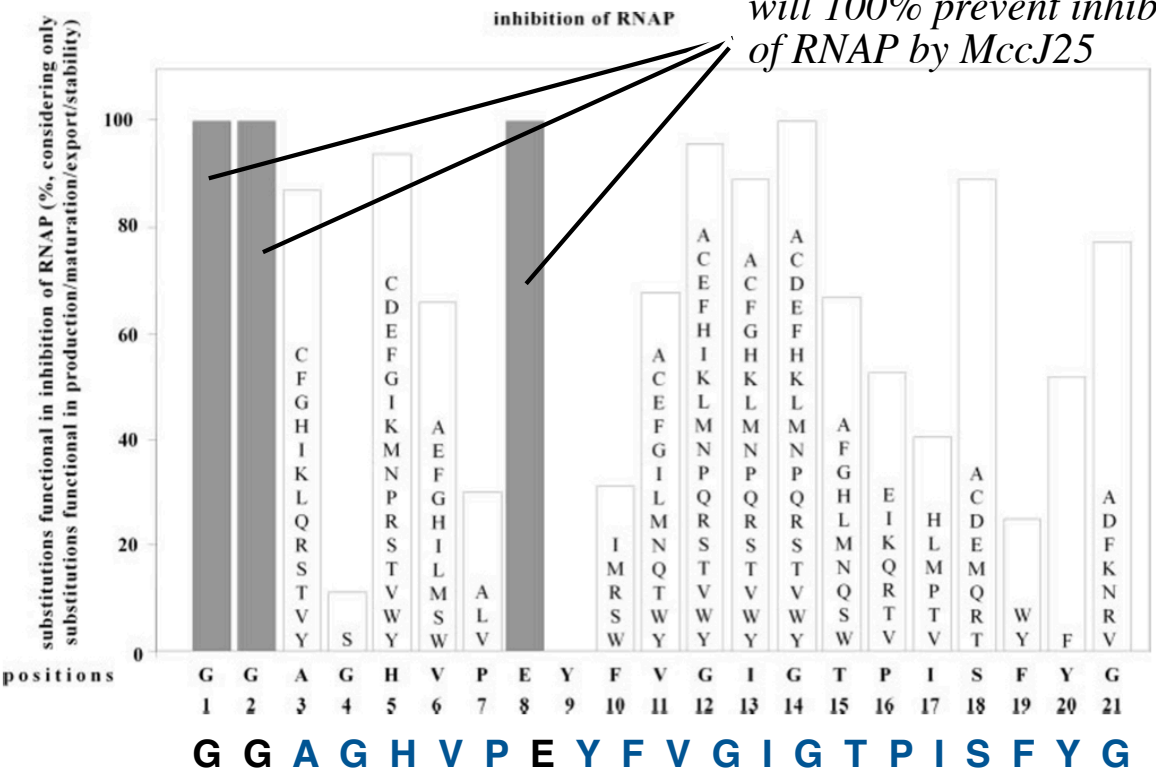
McjA **MIKHFHFNKLSSGKKNNVPS****PAKGV****IQIKKSASQLTKGGAGHVPEYFVGIGTPISFYG**
Minimal McjA **MKSASQLTKGGAGHVPEYFVGIGTPISFYG**

- Only 8 residues in the leader sequence are strictly required for MccJ25 maturation as detectable by an antibacterial assay.

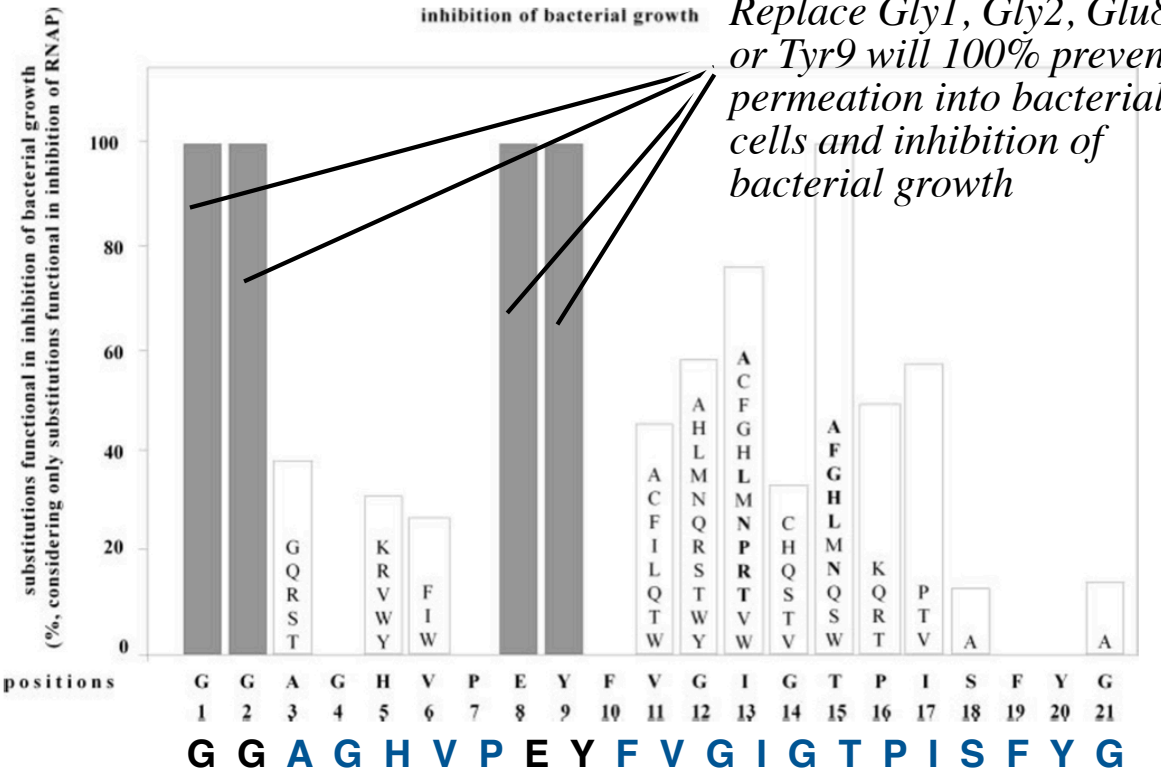
*The **N-terminal portions** of the MccJ25 leader peptides are dispensable in vivo.
(The full length McjA is compared to the minimal sequences needed for correct processing of the precursors in vivo.)*

A model for studies - Microcin J25 (MccJ25):

Effects of single-amino acid substitutions in MccJ25 on inhibition of RNAP.
Data are presented for 242 single-amino acid substitution derivatives of MccJ25, comprising all substitutions shown to be competent for production/ maturation/ export/ stability. The sequence of MccJ25 is shown at the bottom. The heights of bars indicate the percentage of tested amino acid substitutions that **do not prevent inhibition of RNAP by MccJ25**. Letters within bars list amino acid substitutions that do not prevent inhibition of RNAP by MccJ25.



Effects of single-amino acid substitutions in MccJ25 mutants on permeation into bacterial cells and inhibition of bacterial growth. Data are presented for 155 single-amino acid substitution derivatives of MccJ25, comprising all substitutions shown to be competent for production/ maturation/ export/ stability and competent for inhibition of RNAP. The sequence of MccJ25 is shown at the bottom. The heights of bars indicate the percentage of tested amino acid substitutions that **do not prevent permeation into bacterial cells and inhibition of bacterial growth**. Letters within bars list amino acid substitutions that do not prevent permeation into bacterial cells and inhibition of bacterial growth.



McjA MIKHFHFNKLSSGKKNNVPSPAKGV IQIKKSASQLTKGGAGHVPEYFVGIGTPI SFYG
Minimal McjA MKSASQLTKGGAGHVPEYFVGIGTPI SFYG

The C-terminal portions of the McjA precursor protein

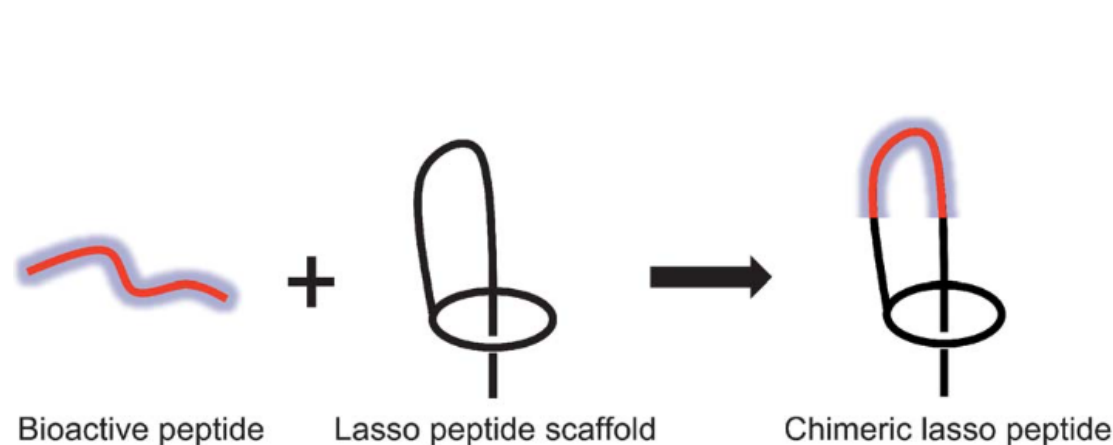
- Only 3 residues of McjA are strictly essential for bio-synthesis, maturation, and export: Gly1 and Glu8 that form the amide bond defining the ring, and 1 adjacent residue Gly2.

A model for studies - Microcin J25 (MccJ25):

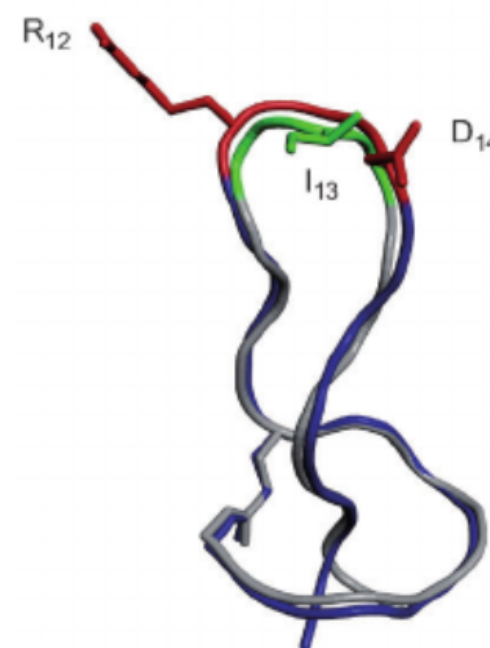
Given the large diversity of lasso variants of MccJ25, they appear to be promising molecular scaffolds.

Example of use of the lasso peptide structure for presenting a bioactive epitope:

- Grafting an integrin-binding motif RGD onto MccJ25 by substituting a tripeptide sequence in the tail-loop region with Arg-Gly-Asp.



Since the loop region of MccJ25 has been demonstrated to be amenable to redesign, the loop (or portions thereof) can be replaced with peptide sequences to generate chimeric structures.



Structural alignment of MccJ25 (gray) and MccJ25 RGD (blue). The grafted RGD sequence is highlighted as red sticks.