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Lasso Peptides: An Intriguing Class of Bacterial Natural Products

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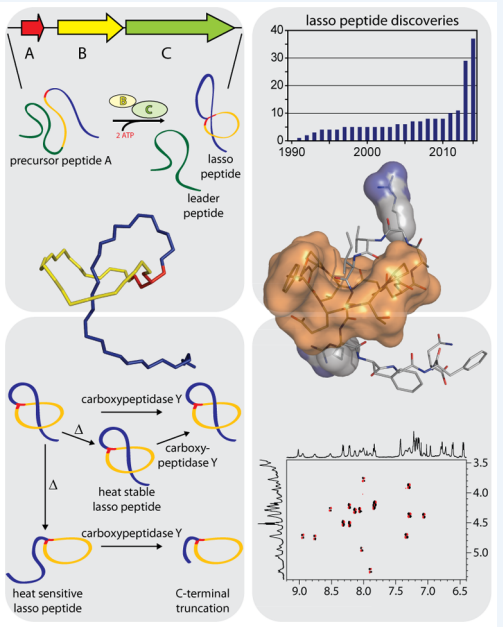
Supporting Information

CONSPECTUS: Natural products of peptidic origin often represent a rich source of medically relevant compounds. The synthesis of such polypeptides in nature is either initiated by deciphering the genetic code on the ribosome during the translation process or driven by ribosome-independent processes. In the latter case, highly modified bioactive peptides are assembled by multimodular enzymes designated as nonribosomal peptide synthetases (NRPS) that act as a protein-template to generate chemically diverse peptides. On the other hand, the ribosome-dependent strategy, although relying strictly on the 20−22 proteinogenic amino acids, generates structural diversity by extensive post-translational-modification. This strategy seems to be highly distributed in all kingdoms of life. One example for this is the lasso peptides, which are an emerging class of ribosomally assembled and post-translationally modified peptides (RiPPs) from bacteria that were first described in 1991.



A wide range of interesting biological activities are known for these compounds, including antimicrobial, enzyme inhibitory, and receptor antagonistic activities. Since 2008, genome mining approaches allowed the targeted isolation and characterization of such molecules and helped to better understand this compound class and their biosynthesis. Their defining structural feature is a macrolactam ring that is threaded by the C-terminal tail and held in position by sterically demanding residues above and below the ring, resulting in a unique topology that is reminiscent of a lariat knot. The ring closure is achieved by an isopeptide bond formed between the N-terminal α-amino group of a glycine, alanine, serine, or cysteine and the carboxylic acid side chain of an aspartate or glutamate, which can be located at positions 7, 8, or 9 of the amino acid sequence.

In this Account, we discuss the newest findings about these compounds, their biosynthesis, and their physicochemical properties. This includes the suggested mechanism through which the precursor peptide is enzymatically processed into a mature lasso peptide and crucial residues for enzymatic recognition. Furthermore, we highlight new insights considering the protease and thermal stability of lasso peptides and discuss why seven amino acid residue rings are likely to be the lower limit feasible for this compound class. To elucidate their fascinating three-dimensional structures, NMR spectroscopy is commonly employed. Therefore, the general methodology to elucidate these structures by NMR will be discussed and pitfalls for these approaches are highlighted. In addition, new tools provided by recent investigations to assess and prove the lasso topology without a complete structure elucidation will be summarized. These include techniques like ion mobility−mass spectrometry and a combined approach of thermal and carboxypeptidase treatment with subsequent LC-MS analysis. Nevertheless, even though much was learned about these compounds in recent years, their true native function and the exact enzymatic mechanism of their maturation remain elusive.

1. INTRODUCTION

Lasso peptides are natural products that are found throughout the bacterial domain and exhibit a versatile array of biological functions.[1−21] They belong to the class of ribosomally assembled and post-translationally modified peptides (RiPPs), meaning that their precursor peptides are gene-encoded and matured by a set of processing enzymes. What distinguishes lasso peptides from other RiPPs is their unique topology. On the most basic structural level, they consist of a macrolactam ring comprised of seven to nine residues and a linear C-terminal peptide tail. The ring is formed between the N-terminal α-amino group and an aspartate or glutamate side chain. Complexity is gained by threading of the C-terminal tail through the ring, yielding a structure reminiscent of a lariat knot and explaining their name. The lasso topology is predominantly stabilized by steric interactions, which are sometimes, but not necessarily, assisted by the presence of

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disulfide bridges. 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. The lasso peptide family is further subdivided into three classes, depending on the number of disulfide bonds present (Figure 1). In this Account, we give a concise overview of lasso peptides starting from their initial discovery up to the current state.

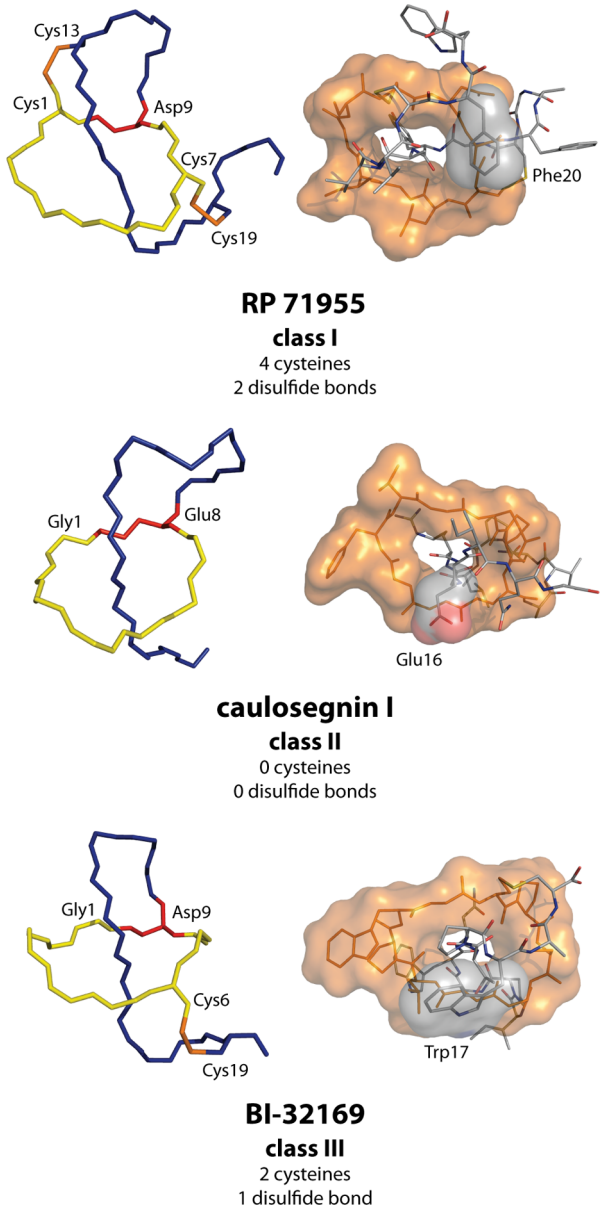
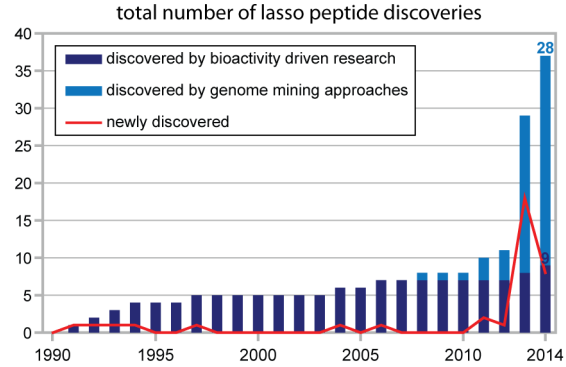
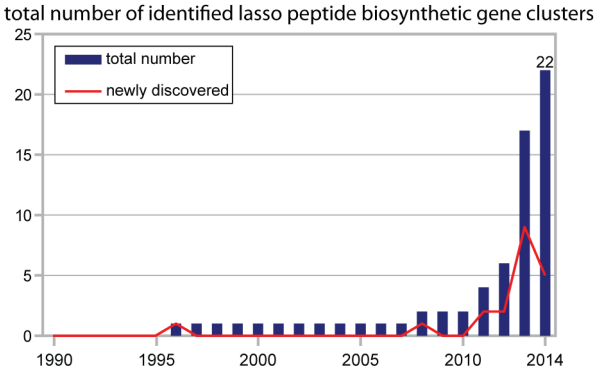


Figure 1. Different lasso peptide classes. Exemplary structures of RP 71955,[5] caulosegnin I,[22] and BI-32169[23] are shown. Left side, lasso peptide backbones. Right side, surface representations of ring and plug residues.

2. DISCOVERY, BIOLOGICAL ACTIVITIES, AND BIOSYNTHESIS

The first lasso peptide was discovered in 1991.[1] Since then, a total of 38 lasso peptides (not counting rediscoveries) have been described.[1−4,6−10,12−14,16,17,19,22,24−31] From these, 35 belong to class II, two to class I, and one to class III. In the history of lasso peptide discoveries, a line can be drawn at 2008. Before this particular year, all lasso peptides were reported in the course of activity-driven compound isolations and, as such, discoveries of new lasso peptides happened largely by chance.[1−4,6−10,12,13,24] This changed in 2008 with the report of capistruin, the first lasso peptide to be isolated by a genome mining approach.[14] Since then, the number of lasso peptides discovered by such approaches has steadily increased (Figure 2).[21,22,25−28,30,31]





known biological activities

lasso peptide	inhibitor/antagonist of	antimicrobial
anantin	atrial natriuretic factor	-
BI-32169	glucagon receptor	-
capistruin	Gram-negative RNA polymerase	yes
lariatin	-	yes
lassomycin	ClpC1	yes
microcin J25	Gram-negative RNA polymerase	yes
propeptin	prolyl endopeptidase	yes
RES-701 type	endothelin type-B receptor	-
siamycin type	HIV fusion and replication, myosin light chain kinase	yes
streptomonomicin	-	yes
sungsanpin	A549 lung cancer cell line	-

Figure 2. Overview of lasso peptide discoveries from 1991 to 2014[1−4,6−10,12−14,16,17,19,22,24−31] and identified biological activities (see also Supplementary Table S1).[1−14,16,17,19,20,32,33]

Historically, microcin J25 (MccJ25) is considered to be a kind of archetype, since it was not only one of the earliest discovered lasso peptides but also the first whose biosynthetic gene cluster was identified (Figure 3).[34] This cluster (*mcjABCD*) was found on a plasmid and proven to be sufficient for MccJ25 production by heterologous expression.[34] In general, all so far known lasso peptides originated from either proteobacterial or actinobacterial sources (Figure 4),

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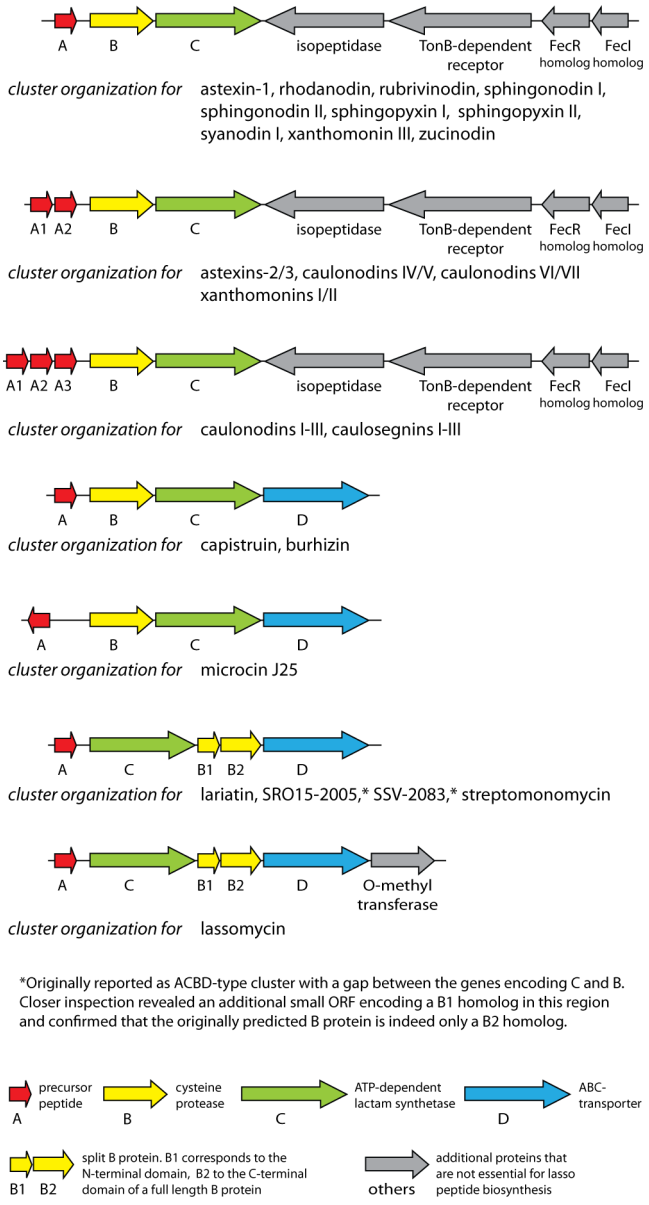

Figure 3. Organizations of known functional lasso peptide biosynthetic gene clusters.[14,17,19,22,25−31,34,35]

even though genome mining approaches have revealed putative biosynthetic gene clusters in other phyla as well.[21,26−28]

The biological activities hitherto identified for lasso peptides encompass a wide variety of functions (Figure 2). Antimicrobial activity appears to be the most common and in some cases the molecular targets have been identified.[2,6,8−11,13,14,17,19] These are the Gram-negative RNA polymerase (RNAP) for MccJ25 and capistruin,[11,43] as well as the hsp100 family chaperone ClpC1 from *Mycobacterium tuberculosis* for lassomycin.[17] Considering that MccJ25 is only produced in the stationary growth phase,[44] it was suggested that lasso peptides could generally function as antimicrobial agents that are produced when nutrient scarcity sets in, granting their hosts an advantage against competitors. However, such a function may only be true for a few lasso peptides, because the majority of recently isolated lasso peptides do not exhibit any observable inhibitory capacities.[22,26,27,30]

Interestingly, most of the recently discovered lasso peptides are produced by clusters that are lacking a homologue of the immunity-conferring ABC-transporter McjD and instead feature other highly conserved adjacent genes (Figure 3).[22,26−31,45] These encode, for example, putative peptidases, which in the case of the astexin systems were shown to act as lasso peptide specific isopeptidases. The astexin isopeptidases were also proven to be highly selective, that is, they were only acting on the lasso peptides produced by the adjacent clusters, while being completely inert toward others.[28]

Therefore, it might be possible that the actual roles of some lasso peptides are linked to their genetic surroundings. Clusters containing ABC-transporters might produce antimicrobials; clusters adjacent to isopeptidase genes might have another function. Currently, it is unclear what this function might be, but in most cases the isopeptidase genes are part of a cluster that contains genes coding for a putative TonB-dependent receptor and a σ-/anti-σ-factor pair. Based on this similarity to elements needed for siderophore uptake and transcription regulation of the corresponding genes, a similar scavenger function was proposed for lasso peptides connected to isopeptidases.[28]

However, there is so far no experimental proof of a lasso peptide that selectively binds another small molecule. Alternatively, the combination of a dedicated uptake and degradation machinery could hint toward a signaling system, where the lasso peptide is first recognized and imported by the TonB-dependent receptor and subsequently linearized by the isopeptidase. In this scenario, either the lasso or the resulting linear peptide would act as signaling molecule. Of course, these suggestions are merely based on observed homologies, but future research might soon reveal more information about these systems.

From a functional perspective, it is worth taking a closer look at the molecular targets of MccJ25 and lassomycin. In both cases, bacteria that spontaneously developed resistances were analyzed. This data led to the identification of ClpC1 as the target for lassomycin. ClpC1 is an ATPase that interacts with the ClpP1P2 proteolytic complex to mediate targeted protein breakdown. It was shown that binding of lassomycin both stimulates ATPase activity and at the same time uncouples ATP hydrolysis from the ClpP1P2 mediated proteolysis.[17] In case of MccJ25, the first results of the target identification were not as clear. Initially, all identified MccJ25-resistant clones carried mutations in genes encoding the TonB-dependent receptor FhuA, which recognizes and imports the iron-loaded siderophore ferrichrome, or other proteins involved in the TonB-uptake machinery, which provides the energy for dedicated import processes by transduction of proton motive force of the cytoplasmic membrane. Only later, mutants were found that showed alterations in the gene encoding the RNAP.[11] This makes sense since a mutation in the RNAP can easily be lethal, while a mutation in the ferrichrome import protein complex is much more easily tolerated and can deter MccJ25 to hijack this siderophore uptake machinery.

Subsequent *in vitro* experiments confirmed that MccJ25 was able to inhibit Gram-negative RNAPs, most likely by plugging the NTP-uptake channel, while this was not observed for Gram-positive RNAPs.[11,46,47] Additional experiments with capistruin yielded similar results.[43] Interestingly, a second mode of action is reported for MccJ25, and this plays an important role in its activity against highly sensitive *Salmonella* species and *Escherichia coli* species overexpressing FhuA. This

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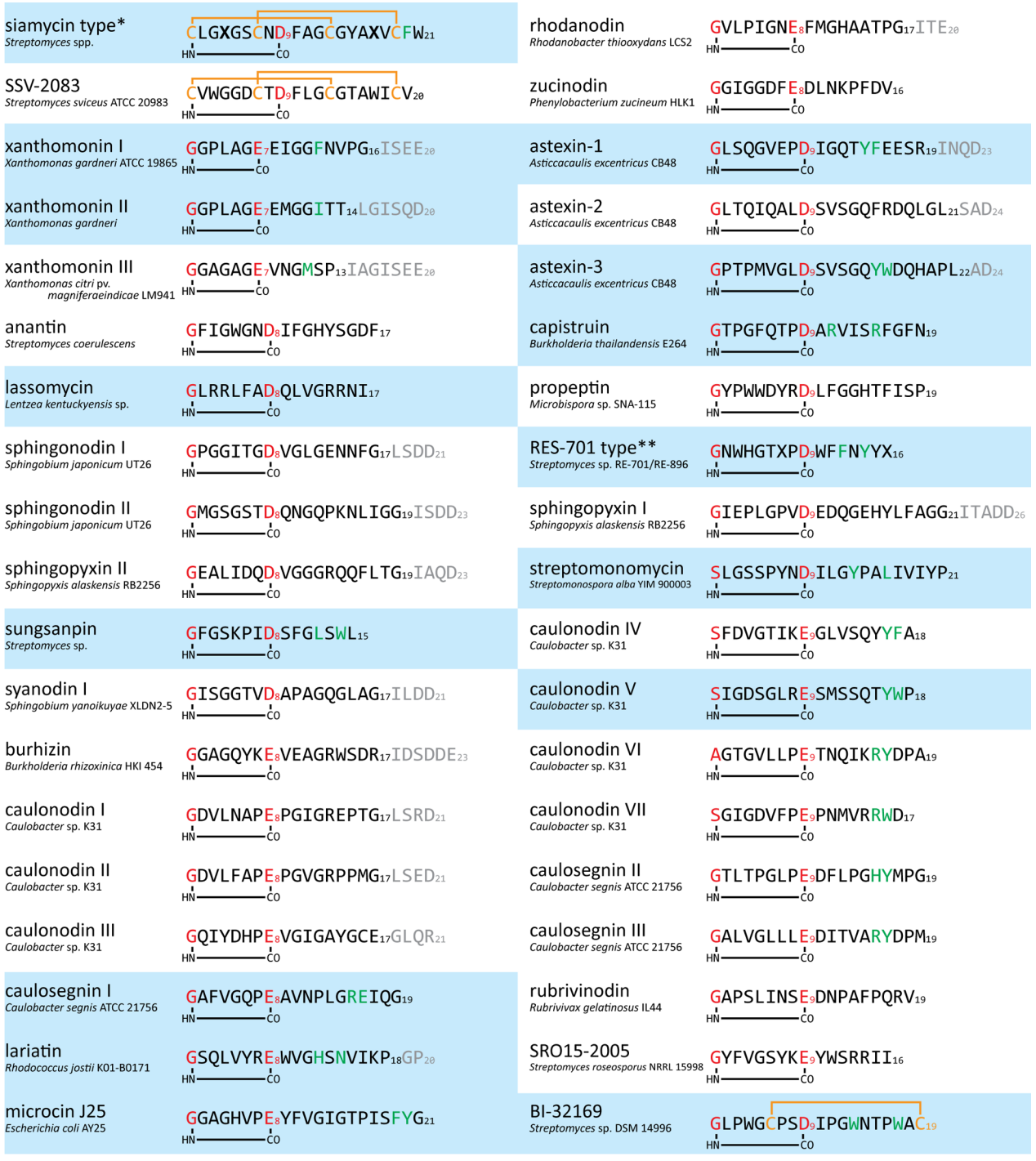

Figure 4. Overview over all known lasso peptides sorted by class and ring size.[1−10,12−14,16,17,19,22−31,36−42]

activity was proven to be completely independent of RNAP inhibition, because it is also observed in strains with resistant RNAP variants as long as intracellular concentrations become high enough. In these cases, MccJ25 appears to interact with the membrane and thereby depolarizes it and decreases oxygen consumption. Apparently, this is caused by MccJ25-triggered

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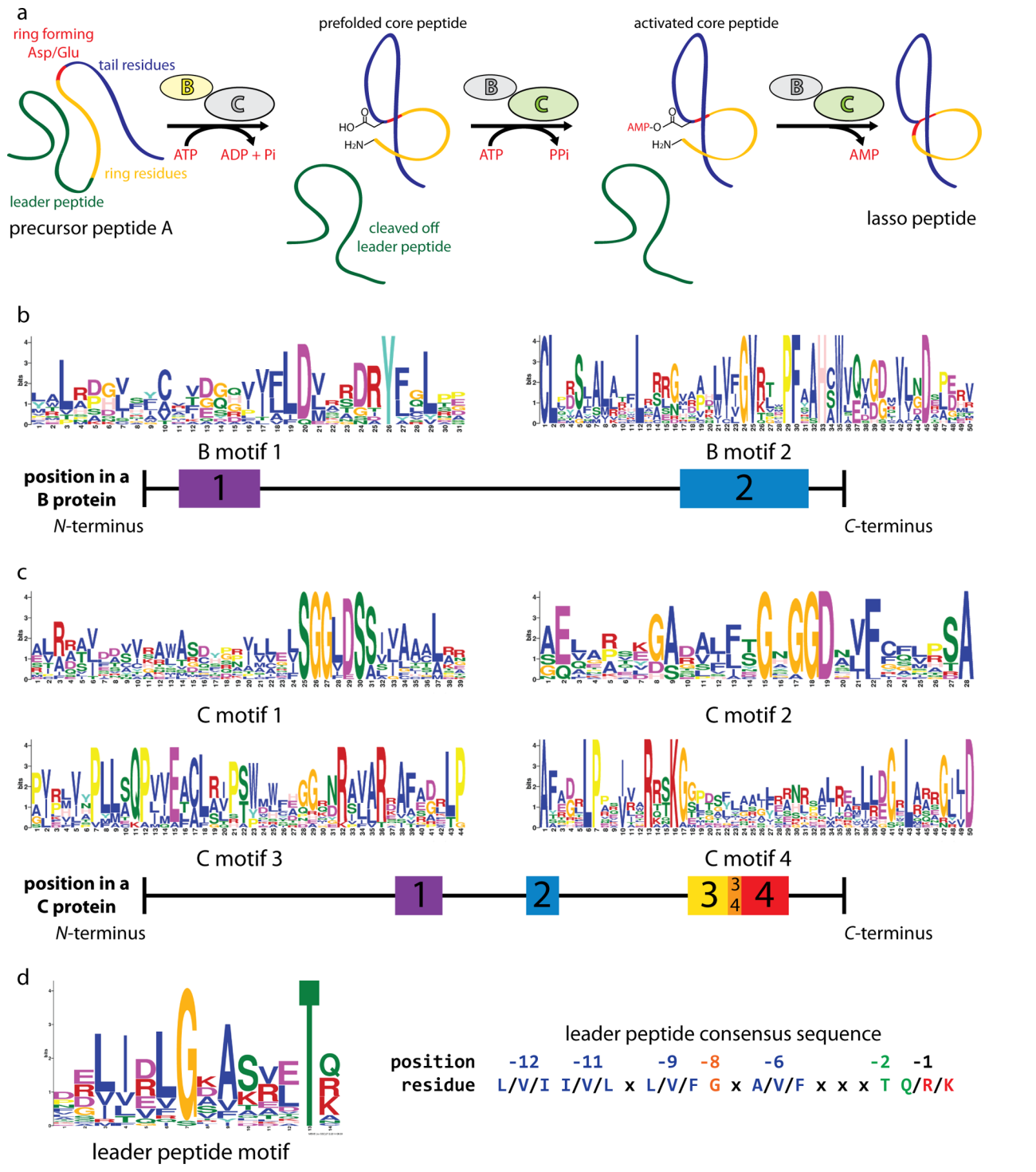

Figure 5. (a) Proposed mechanism of lasso peptide biosynthesis[27,49] and conserved motifs in (b) B proteins,[27] (c) C proteins,[27] (d) and proteobacterial leader peptides.[31]

superoxide production, though the underlying mechanism is not yet understood.[32,33]

The recently elucidated crystal structure of MccJ25 bound to FhuA shows how MccJ25 mimics the binding of ferrichrome and thereby is able to enter the cells.[48] Compared with MccJ25, capistruin does not fit within the FhuA binding pocket and cannot exhibit the observed interactions crucial for selective binding. Hence, it is most likely unable to enter the cells in this way and might instead show a preference for another receptor. This could also explain why capistruin is able to inhibit the *E. coli* RNAP *in vitro* with a potency comparable to MccJ25 but shows a much weaker antimicrobial activity against MccJ25-sensitive *E. coli* strains.[43,48]

In general, lasso peptide production requires at least three genes encoding a precursor peptide A, a cysteine protease B, and an ATP-dependent lactam synthetase C. Gene clusters might contain additional genes, but so far no system was proven to be in need of an additional enzyme to produce mature lasso peptides.[26−28,49] The only exceptions to some extent are the clusters that produce lariatin, lassomycin,

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streptomonomicin, and a series of putative ones identified by genome mining studies, which contain an additional small open reading frame (ORF) shown to be essential for lariatin biosynthesis.[17,19,26−28,35] Still, bioinformatic analysis of the corresponding small proteins showed that apparently in these systems an evolutionary event led to a split of the B protein into two separate ORFs, encoding the N-terminal and C-terminal domains of full length B proteins.[27,28] To highlight the genetic origin of split B proteins, we suggest a common nomenclature, in which the small proteins originating from the N-terminal domain are called B1 and those from the C-terminal domain are named B2 (Figure 3).

In vitro studies were performed with the MccJ25 biosynthesis system. In this system, McjA is first processed by the ATP-dependent cysteine protease McjB, which cleaves off the leader peptide. McjC was shown to be an adenylate-forming enzyme that catalyzes the subsequent isopeptide bond formation between the N-terminal glycine of the core peptide and the Glu8 side chain at the cost of one additional ATP. These studies have further shown that McjB and McjC are interdependent and thereby act as an ATP-dependent synthethase that processes McjA into mature MccJ25, which is then exported by the ABC-transporter McjD.[45,49] The observed ATP-dependency for McjB was quite unexpected, since it should not be needed for its protease activity. Because B proteins carry the catalytic triad typical of cysteine proteases in their C-terminal domain, it is proposed that the N-terminal domain is most likely the site of ATP binding. While not proven yet, the N-terminal domain is suggested to act in a chaperone-like manner to mediate the prefolding during maturation, because the threading cannot occur after the ring formation.[27,49] A proposed mechanism for lasso peptide biosynthesis alongside a representation of conserved motifs of the processing enzymes is shown in Figure 5a−c.

Because only little information from *in vitro* experiments is available, the way the enzymes interact with precursor peptides is only poorly understood. Nevertheless, *in vivo* studies utilizing mutagenesis have helped to understand sequence-specific factors that are important for enzymatic processing.[22,29−31,50−53] The most crucial residues in the core peptide seem to be those located at position 1 and the ring forming aspartate/glutamate. The position 1 residue is typically a glycine (class II/III) or cysteine (class I) and exchange leads to drastic decrease, if not complete abolishment of lasso peptide production.[22,29−31,50,53]

The previous paradigm that all class II lasso peptides require a glycine at this position was recently proven wrong because caulonodins IV−VII feature Ala1 or Ser1 and only poorly tolerate a Gly1 substitution.[31] Therefore, it seems that there are indeed biosynthetic machineries that developed a different preference for the position 1 residue, even though genome mining studies suggest that there is a definite bias to Gly1 in the plethora of putative biosynthetic gene clusters identified.[26−28,31]

The threonine at the penultimate position of the leader region was hitherto found in every precursor peptide and in all tested instances was only poorly amenable to exchanges, with structurally similar residues being tolerated best.[22,29−31,50,52] A recent study furthermore identified a conserved region in the leader sequences of proteobacterial precursor peptides that is also essential for effective processing (Figure 5d).[31]

An aspect that has so far been rather neglected is the postsynthetic modification of lasso peptides. This is probably because most characterized clusters only contain genes encoding precursor peptides and processing enzymes, while lacking ORFs that could encode putative tailoring enzymes. Still, genome mining studies uncovered a series of gene clusters containing ORFs that were not seen before in the context of lasso peptides.[27,28] Hence, further investigation might reveal whether these are indeed enzymes capable of lasso peptide tailoring.

Interestingly, there are most likely two instances reported where a tailored lasso peptide was isolated. The first instance is lassomycin, which features a methylated C-terminus and whose gene cluster contains a putative O-methyltransferase that is likely responsible for introducing this modification, although experimental proof is still missing.[17] The other instance could have been observed in context of the RES-701 family, where the only difference between RES-701-1 and -2 or RES-701-3 and -4 is the identity of the C-terminal residue, which is either tryptophan (RES-701-1/3) or 7-hydroxy-tryptophan (RES-701-2/4).[4] Because such a modification is unlikely to occur chemically during extraction/purification, it is reasonable to assume that it was introduced enzymatically before isolation. Because the biosynthetic gene clusters of these lasso peptides are still unknown, it is for future research to decide whether this hypothesis holds true.

3. PHYSICOCHEMICAL PROPERTIES

Until recently, the physicochemical properties of lasso peptides were not widely investigated. This gave rise to hypotheses about lasso peptide structure and stability that were proven wrong and revised accordingly in the last couple of years. These include that all lasso peptides were assumed to have an extraordinary thermal stability, the presumed possible ring sizes, and, as discussed above, which position 1 residues are tolerated during biosynthesis.[22,27,30,31]

The hypothesis regarding the thermal stability of lasso peptides originated from studies on MccJ25, where it was shown to exhibit its antimicrobial activity even after it was autoclaved.[2] Therefore, it was expected that all lasso peptides would behave this way due to their shared topology. However, during the characterization of caulosegnins I−III, it was observed in LC-MS analyses that after thermal treatment of caulosegnins I and III a new peak appeared at a different retention time but featuring the same mass.[22] Since the mass was unaltered, a nonchemical conversion had to have occurred, which for a lasso peptide is the unthreading from the lasso to the branched-cyclic topology. After this observation, subsequent studies showed thermal sensitivity for several new lasso peptides, highlighting that even though some are resistant against denaturing by high temperatures, this is not necessarily true for every lasso peptide.[22,27−29,31] To prove that thermal treatment converts a lasso to a branched-cyclic peptide, the compound can be treated with carboxypeptidases before and after incubation at elevated temperatures. Normally, the untreated lasso peptide shows no or much less truncation than the branched-cyclic analog that arises from heat-associated unthreading (Figure 6).[22,27,29,31]

This behavior is useful for characterization of newly isolated lasso peptides, because it can be employed to provide evidence for a lasso topology without having to perform a complete structure elucidation. Nonetheless, the criteria for thermal stability or sensitivity are still poorly understood. This is especially highlighted by comparison of caulosegnins II and III. While the former is very stable even at 95 °C, the latter readily unthreads at elevated temperatures. This is even more

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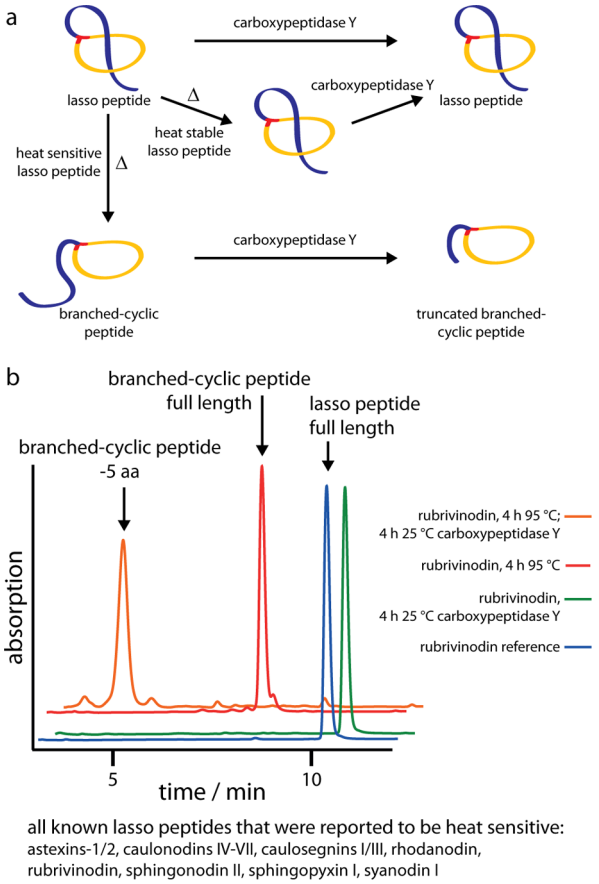

Figure 6. Thermal stability and carboxypeptidase Y assays shown (a) schematically and (b) employing rubrivinodin.[22,27−29,31]

intriguing considering that these compounds are produced by the same biosynthetic machinery, have identical ring sizes, have similar primary structures, and were both shown to employ tyrosine as lower plug (Figure 4).[22] Therefore, these compounds might be used in future studies to shed some light on which factors influence a lasso peptide's resistance to high temperatures.

Regarding their ring sizes, nearly all discovered lasso peptides contain either eight or nine amino acid residue rings.[1−4,6−10,12−14,16,17,19,22,24−29,31] Because these ring sizes were the only ones observed until recently, it was suggested that no others were feasible for threaded macrolactam rings: ten-residue rings were deemed to be too big to allow steric maintenance, while seven-residue rings were assumed to be too small to allow threading. The latter assumption was refuted with the report of xanthomonins I−III, which feature rings formed between Gly1 and Glu7.[30] These seven amino acid rings not only confer high thermal stability but also approach the lower size limit of threaded macrocycles found in the structurally similar (albeit unlike lasso peptides chemically accessible) group of [2]rotaxanes. Since in these cases the macrocycles comprise 20 atoms,[54,55] it seems unlikely that a lasso peptide could feature a threaded macrolactam ring consisting of merely six amino acids (19−20 atoms).

The fundamental reasoning behind this is that the aforementioned [2]rotaxanes possess strictly linear macrocycles and threading moieties, while lasso peptides have to rely on peptide bonds, which have branching in the form of carboxyl groups and side chains.[30,54,55]

In general, the lasso scaffold also confers high stability against proteases.[22,27,29−31,50] MccJ25 is an especially intriguing example in this regard. While the large loop region of MccJ25 seems to be generally accessible to proteases, for example, thermolysin, the ring and tail are so tightly associated that they remain interlocked even when they are no longer covalently connected, which can be observed in both solution and gas phase.[56] Because of this tremendous protease and thermal stability, recent studies investigated the potential of MccJ25 in epitope grafting efforts, resulting in the promising proof-of-principle creation of integrin antagonists through mutation of MccJ25 (Figure 7).[15,18]

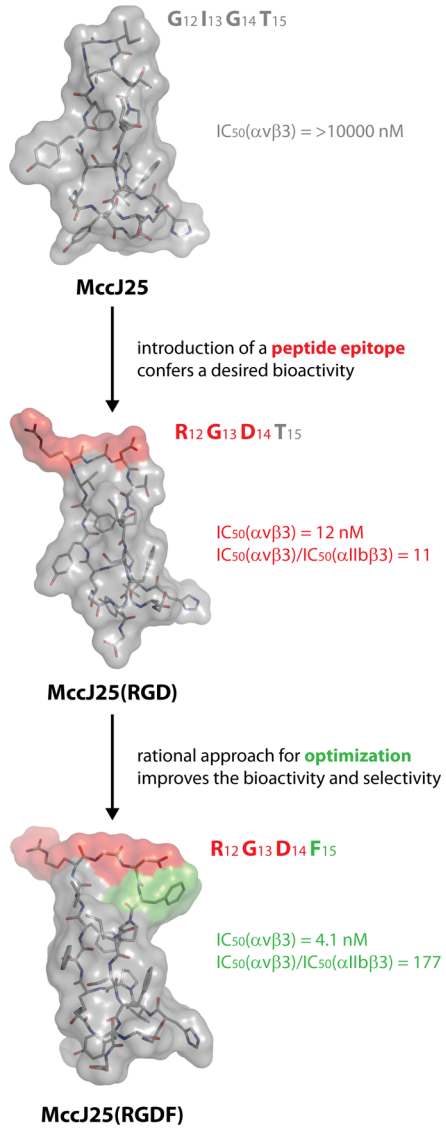

Figure 7. Schematic depiction of an epitope grafting approach employing MccJ25 with the creation of an $\alpha v\beta 3$ integrin antagonist shown as example.[15,18]

4. STRUCTURE ELUCIDATION

Structure elucidation of lasso peptides is traditionally accomplished by NMR spectroscopy, but there are also two crystal structures known. Intriguingly, the conditions under which these two compounds, BI-32169 and xanthomonin I, were crystallized are quite different.[30,42] For xanthomonin I, a

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saturated solution in water was applied to standard crystallization screens, whereas the quite hydrophobic BI-32169 had to be dissolved in pure DMSO. Therefore, xanthomonin I was crystallized like a protein, while BI-32169 was crystallized in a way more typical of small organic molecules. In the latter case, the reduction of solubility caused by mixing a DMSO stock solution with an aqueous crystallization condition probably already facilitated the crystal formation.

For NMR spectroscopy, D_2O/H_2O (1:9), methanol-d_3, and DMSO-d_6 are most commonly used as solvents.[5,6,13−19,22,23,26,28−31,36−41,57] Generally, it is of great importance for successful and reliable structure elucidation that the backbone NH-signals show a broad chemical shift dispersion instead of being clustered in a narrow region. This not only significantly facilitates the interpretation of the spectrum but also is a sign for rigid and defined structures, which is typical for the lasso fold, while clustering often corresponds to random coiled structures, which would rather suggest the presence of a branched-cyclic topology (Figure 8).[29,57] Moreover, thorough assignment of the NOE contacts is

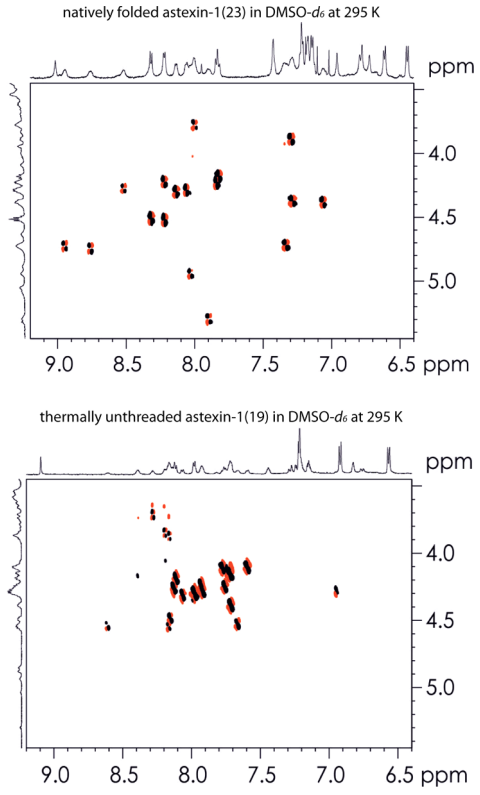

Figure 8. DQF-COSY spectra of astexin-1(**23**) in lasso topology and thermally unthreaded astexin-1(**19**).[29]

mandatory, because negligence in this concern not only is overall detrimental but could wrongfully yield a branched-cyclic instead of a lasso structure (or vice versa) in the final structure calculations. Furthermore, characteristic nonstandard long-range NOEs of the types d_{NN}, $d_{\alpha N}$, $d_{\alpha\alpha}$, $d_{\beta N}$, and $d_{\gamma N}$ are typically found between the isopeptide bond forming residues, between the threading moieties and the ring, and, if present, for short β-sheets. For correct signal assignment, investigation of the temperature dependency of single signals has proven to be quite helpful. Signals that show little or no dependency often correspond to groups that are shielded from the surrounding solvent, which therefore commonly also exhibit a low H/D-exchange rate.[57] For lasso peptides, these signals belong to residues that are buried within the ring or are involved in ring formation. On the basis of 2D-NMR spectra, a family of 15−20 low energy structures is usually calculated to represent the structure in solution. In summary, the correct, careful, and thorough signal assignment and interpretation might be very time-consuming but nevertheless is absolutely essential for obtaining an accurate and reliable three-dimensional structure and therefore should be considered accordingly when investigating these interesting natural products.[57]

Finally, a very recent study presented an elegant way to differentiate between lasso and branched-cyclic peptides via ion mobility−mass spectrometry (IM-MS). At high charge states, the observed collision cross sections for lasso peptides were significantly smaller than for their branched-cyclic analogs and thereby allowed the authors to distinguish between the two topologies.[58] Based on these results, IM-MS could be employed for a faster initial assessment of the present topology in future studies of novel putative lasso peptides.

5. CONCLUSION

In recent years, research on lasso peptides has made significant progress. Particularly, after the first reported discovery through directed genome mining in 2008,[14] these approaches have much developed and improved at least for clusters of proteobacterial origin and thereby greatly increased the number of known lasso peptides.[22,26−28,30,31] As a side effect, much was discovered about their structural features and stability properties, and previously assumed limits of this compound class, for example, the feasible ring sizes or the possible position 1 residues, were overhauled and had to be revised.[22,30,31] In light of all of this new information, it becomes obvious that the so far best characterized representative of this RiPP family, MccJ25, is quite extraordinary indeed. It has a unique cluster arrangement and an exceptionally large loop and is until today the only known lasso peptide whose ring and tail region were shown to remain associated even after their covalent connection is broken.[56] Nevertheless, this also means that many of the assumptions and basic principles derived from corresponding studies have to be reevaluated and reassessed in regard to being generally true for other lasso peptides as well.

The next goals in this field of research become apparent. First, it becomes very clear that the established protocols for heterologous production in *E. coli* seem to be confined to clusters from proteobacteria and are less viable for clusters from other phyla, despite the large variety of putative biosynthetic gene clusters they harbor, as determined by genome mining.[21,26−28] Therefore, a new methodology has to be devised and established to tap into this rich source of hitherto uncharacterized lasso peptides and all the new discoveries that may come alongside. Second, an appreciably sized library of diverse lasso peptides is now available from heterologous production, although their actual function remains elusive.[14,22,26−31,52] Hence, future research should try to elucidate their role in nature, that is, when and why they are produced and how they fulfill their biological functions on a molecular level.

Furthermore, investigation of the potential of lasso peptide scaffolds in medicinal applications has just begun and seems very promising so far.[15,18] These efforts should be continued to

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assess whether lasso peptide based therapeutics are indeed feasible as a concept.

In summary, lasso peptides comprise a fascinating fold within the RiPP superfamily, about which very little is still known despite the broad distribution of biosynthetic gene clusters throughout the bacterial domain, as apparent through genome mining. The evolutionary dispersion and conservation of these compounds in bacteria highlights that they may indeed fulfill important functions, thereby emphasizing the need to further study these natural products and shed more light on their biosynthesis and roles in nature.

■ ASSOCIATED CONTENT

Supporting Information

More detailed overview of lasso peptide bioactivities. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.accounts.5b00156.

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