Abstracts & List of Participants

RiPPs

1st INTERNATIONAL CONFERENCE

24-26 April 2019, Granada, Spain

Invited Abstract

RiPPs in action

presenting author: Colin Hill

Colin Hill and Paul Ross (APC Microbiome Ireland, UCC)

RiPPs are fundamentally interesting molecules for many reasons, but they also have the potential to be used in a number of settings, in combating infection, as food preservatives, as shapers of complex communities, and potentially as anti-cancer drugs. In this presentation I will outline some of our work in applying RiPPs in these settings, including the treatment and prevention of bovine mastitis, engineering lantibiotics for use in food systems, the role of bacteriocins in colonisation resistance, and the potential of using molecules like nisin in treating cancer cell lines.

Invited Abstract

Exploiting the combinatorial potential of microviridin biosynthesis

presenting author: Elke Dittmann

Elke Dittmann (University of Potsdam)

Cyanobacteria feature a number of unique families of ribosomally produced and posttranslationally modified peptides (RiPPs). Among these natural products microviridins are particularly noteworthy. Microviridins are processed into a unique cage-like architecture and specifically inhibit different types of serine proteases. We have developed two technologies to produce the peptides either by heterologous expression in E.coli or via a chemoenzymatic in vitro reconstitution approach. Both technologies were used for the diversification of the peptides and optimization of their bioactivity. The work was largely inspired by the natural diversity of the peptide family and co-crystallization studies of microviridin J and trypsin and allowed to dissect protease specificity conferring moieties of microviridins. The technologies were also utilized to mine novel types of microviridins either from environmental DNA or by omitting all cultivation and DNA isolation steps using synthetic production of bioinformatically predicted microviridin types and to produce microviridins with non-canonical amino acids. I will discuss possibilities and limitations of microviridin engineering approaches. Further, I will use microviridins as a case study to highlight special features of cyanobacterial RiPP systems and provide insights into the regulation of RIPP biosynthesis in filamentous cyanobacteria. Understanding regulation and function of the peptides can provide new avenues for the genomic mining of RiPPs in the natural producers.

[1] M. Baunach & E. Dittmann, Microviridins, Comprehensive Natural Products III: Chemistry and Biology, Elsevier Science, in print.

Invited Abstract

Mechanisms of cyclization in cyanobactin biosynthesis

presenting author: Eric Schmidt

Eric W. Schmidt (University of Utah)

Cyanobactins are circular RiPPs made by cyanobacteria. Although the cyanobactin pathway has now been studied for about 15 years, there continue to be surprises in enzyme mechanisms. Here, I will focus on recent advances in biosynthesis, especially the cyclization steps.

Invited Abstract

Hypermodified peptides from strange microbes

presenting author: Joern Piel

Joern Piel (ETH Zurich, Switzerland)

The polytheonamides are among the most complex and biosynthetically distinctive natural products known to date. These potently cytotoxic peptides are derived from a ribosomal precursor processed by 49 mostly non-canonical posttranslational modifications. Since the producer 'Entotheonella factor' is a "microbial dark matter" bacterium only distantly related to cultivated organisms, and because heterologous expression hosts generated only poorly converted products, >70-step chemical syntheses have been developed to access these unique compounds. The talk presents recent studies to establish a biosynthetic platform by mining prokaryotic diversity for alternative producers. Using this system, we generated the aeronamides, new polytheonamide-type compounds with near-picomolar cytotoxicity. Fully modified aeronamides, as well as the "polygeonamides" produced from deep-rock biosphere DNA, were obtained within two days and contain the highest numbers of D-amino acids in known biomolecules. With increasing bacterial genomes being sequenced, similar host mining strategies might become feasible to access further elusive natural products from uncultivated life.

Invited Abstract

Three powerful strategies for lanthipeptide engineering

presenting author: Oscar Kuipers

Jingjing Deng, Xinghong Zhao, Auke van Heel, Manuel Montalban-Lopez, Steven Schmitt, Sven Panke, Oscar P. Kuipers (University of Groningen)

Engineering of lanthipeptides has great potential for developing new-to-nature variants with improved functional properties. Here, three totally different ways for lanthipeptide engineering will be demonstrated and their value for developing new antimicrobials as well as for understanding biosynthesis will be discussed.

1.Ring shuffling and high throughput screening by the nano-Fleming

Using 12 template lantibiotics >40 structural elements were defined and combined, using a topological layout containing 5 structural elements (e.g. rings or hinge region) acquired by combinatorial DNA synthesis. The completely new variants were expressed in *Lactococcus lactis* and screened for activity by the nano-Fleming, based on micro-alginate beads and fluorescence assisted cell sorting. Several completely novel hybrids were obtained that showed a new antimicrobial activity spectrum.

2.Directed evolution of biosynthesis enzymes to allow modification of recalcitrant residues

Using a bacterial display system and lanthipeptide variants containing a circular Strep-tag which has much higher affinity for Streptavidin than the linear Strep-tag, we attempted to improve modification of recalcitrant Ser/Thr residues by coexpressing an error-prone PCR generated mutant library of *nisB* or *nisC*. Several mutant enzymes were picked up that could modify e.g. a Ser preceded by an Asp, which wild type NisB will not do.

3.Generation of new-to-nature derivatives by incorporating Met-analogs followed by click chemistry

In this study, Four methionine analogues (Aha, Hpg, Nle and Eth) were successfully incorporated at four different positions of nisin in *L. lactis* through force feeding. LC-MS analysis showed high levels of incorporation for all these methionine analogues at defined positions. Three derivatives displayed high antimicrobial activity against *L. monocytogenes*, *Micrococcus flavus* or *L. lactis*. Overall, nisin derivatives with Aha and Hpg incorporated are being used to generate nisin conjugates by click chemistry.

Invited Abstract

Genome mining and biosynthetic study of linaridins natural products

presenting author: Qi Zhang

Qi Zhang (Fudan University)

Linaridins are a small but growing class of RiPPs. The canonic member of this family, cypemycin, possesses potent in vitro activity against mouse leukemia cells and also exhibits narrow-spectrum antibiotic activity. Using cypemycin as a model molecule, we have established a heterologous expression system and studied the structure-activity relationships involved in precursor peptide maturation. We also carried out an extensive genome-wide survey of linaridin biosynthetic genes, showing this class of natural products is widespread in nature and possesses vast structural diversity. Subsequent genome mining efforts allowed characterization of a series of novel linaridins. Some of our recent results, including those in the biosynthetic studies of the cypemycin aminovinyl-cysteine (AviCys) moiety, and some mode of action studies will also be presented.

Invited Abstract

Exploring the continuous story of lasso peptides

presenting author: Sylvie Rebuffat

Sylvie Rebuffat (Muséum national d'Histoire naturelle, MNHN - CNRS)

Lasso peptides form a continuously expanding class of ribosomally-synthesized and post-translationally modified peptides (RiPPs) produced by bacteria that are characterized by an unique entangled topology. Basically, the lasso fold consists of a macrolactam ring, which is closed via an isopeptide bond between the N-terminal amino group of the core peptide and the side-chain carboxylate of an Asp or Glu at position 7-9, and is threaded by the C-terminal tail. The structure is maintained through bulky residues acting as plugs or/and disulfide bridges. The particular post-translational modification that creates the compact [1]rotaxane lasso topology is ensured by two dedicated enzymes encoded in the peptide gene cluster. Lasso peptides bearing a multitude of further post-translational modifications, such as C-terminal methylation or (poly)phosphorylation, incorporation of D-Trp, acetylation, or citrullination have been regularly discovered in recent years, with accumulated bacterial genomes and improved genome mining tools and screening technologies. Indeed, tailoring enzymes expand the chemical diversity around the lasso scaffold and subsequently could enlarge the range of bioactivities. The first lasso peptides to be described exhibited antimicrobial activities directed against a narrow spectrum of closely related bacteria coping in their niche, as exemplified by microcin J25 from *Escherichia coli* which remained for many years the archetype of lasso peptides. Since then, and although their roles in Nature remain unknown, a range of therapeutical properties have been revealed for lasso peptides, including enzyme inhibition, receptor binding, anticancer properties, etc. An overview of the lasso peptide saga will be given, opening the scope of perspectives offered by this remarkable class of RiPPs.

Invited Abstract

Biosynthesis and Engineering of Peptide Natural Products

presenting author: Wilfred van der Donk

Wilfred van der Donk (University of Illinois)

Lanthionine-containing peptides (lanthipeptides) are RiPPs with many members displaying high antimicrobial activity against pathogenic bacteria (lantibiotics). These peptides are post-translationally modified to install multiple thioether crosslinks. During their biosynthesis, a single enzyme typically breaks 8-16 chemical bonds and forms 6-10 new bonds with high control over regio- and chemoselectivity. The defining post-translational modification of lanthipeptides is a macrocyclization step, but additional tailoring reactions also take place such as oxidation, halogenation, and reduction. This presentation will discuss investigations of the mechanisms of these remarkable catalysts, their use for the generation of non-natural cyclic peptide libraries, and the utilization of their genes for genome mining efforts.

Selected Talk 1

A new machine learning algorithm identifies previously undetected biosynthetic gene clusters for RiPPs

presenting author: Alexander Kloosterman

Alexander Kloosterman, Peter Cimermancic, Michalis Hadjithomas, Michael Fischbach, Mohamed S. Abou Donia, Gilles P. van Wezel, Marnix Medema (Leiden University)

Ribosomally synthesized and post-translationally modified peptides (RiPPs) form a diverse group of natural products. Because of the diversity of RiPPs, genome mining for novel RiPP biosynthetic gene clusters (BGCs) has proven troublesome. Most strategies target modifying enzymes common to a specific subclass of RiPPs. Although these strategies are effective at finding RiPP BGCs similar to known variants, they struggle to find novel RiPP subclasses.

We have developed a novel approach to mine genomes for RiPP BGCs, called RiPP-TIDE (RiPP Tool for Integrative Discovery and Enrichment of gene clusters). The method is based on a Support Vector Machine (SVM) model trained to predict the probability that a given gene encodes a RiPP precursor. The surrounding genes in operon with the precursor are used to form putative BGCs. We then compare BGCs with similar architecture found in multiple genomes, to determine the frequency of occurrence: the assumption is that RiPP biosynthesis will be part of “accessory” genome rather than the core genome of a genus. Using this strategy, we have identified candidate RiPP gene cluster families that are not found by other genome mining algorithms, like BAGEL and antiSMASH. One gene cluster belonging to a newly discovered gene cluster family in *Streptomyces* was studied in more detail. Overexpression of a regulator activated the gene cluster, and its product could be identified as a novel RiPP. These findings suggest that the chemical space for RiPP natural products may be significantly larger than so far anticipated, offering new opportunities for genome mining-based drug discovery.

Selected Talk 2

Uncovering the unexplored diversity and biosynthesis of thioamidated RiPPs

presenting author: Andrew Truman

Javier Santos-Aberturas, Govind Chandra, Luca Frattaruolo, Rodney Lacret, Thu H. Pham, Natalia M. Vior, Tom H. Eyles, Andrew W. Truman (John Innes Centre)

Widespread genome sequence has revealed the massive untapped potential of bacteria to make specialized metabolites, including thousands of uncharacterized RiPP biosynthetic gene clusters. However, the absence of a common biosynthetic feature across all RiPP pathways makes it difficult to identify novel RiPP pathways. To accelerate the identification of precursor peptides that lack homology to known RiPP families, we have developed RiPPER (RiPP Precursor Peptide Enhanced Recognition), a new tool for the family-independent identification of RiPP precursor peptides, and apply this methodology to search for novel thioamidated RiPPs. Thioamidation was believed to be a rare post-translational modification, which has been shown to be catalyzed by a pair of proteins (YcaO and TfuA) in Archaea. The thioviridamide-like molecules are a family of actinobacterial cytotoxic RiPPs that feature multiple thioamides, and it has been proposed that a YcaO-TfuA pair of proteins also catalyzes their formation. RiPPER was used with all actinobacterial TfuA domain proteins to identify hundreds of previously undescribed gene clusters that we hypothesize make thioamidated RiPPs, which reveals that a highly diverse landscape of precursor peptides are encoded in these gene clusters. This leads to the first rational discovery of a new family of thioamidated natural products, the thiovarsolins from *Streptomyces varsoviensis*, which were identified following transformation-associated recombination cloning of this gene cluster. I will also discuss recent findings in the biosynthesis and engineering of the thioviridamide-like molecules.

Selected Talk 3

Biosynthesis and activity of the DNA gyrase inhibitor Microcin B17: new lessons from an oldest RiPP

presenting author: Dmitry Ghilarov

Dmitry Ghilarov, Clare E.M. Stevenson, Dmitrii Y. Travin, Julia Piskunova, Marina Serebryakova, Lukasz Mazurek, Elizabeth Michalczyk, Jonathan Heddle, Anthony Maxwell, David M. Lawson, Konstantin Severinov (Malopolska Centre of Biotechnology, Jagiellonian University, Cracow, Poland)

The introduction of azole heterocycles into a peptide backbone is the principal step in the biosynthesis of numerous compounds with therapeutic potential. One of them is microcin B17 (MccB17), a bacterial topoisomerase II inhibitor whose activity depends on the conversion of selected serine and cysteine residues of the precursor peptide to oxazoles and thiazoles by the McbBCD synthetase complex. We have determined crystal structures of the McbBCD complex alone and with bound nucleotides and modified peptides, revealing distinct sites for cyclodehydratase and dehydrogenase activities. After modification, MccB17 precursor is cleaved by the *E. coli* TldD/E protease. Crystals of TldD/E reveal that the complex forms a closed spherical shell and operates like a 'molecular pencil sharpener', whereby unfolded peptides are fed through a narrow channel to access the active site of the protease, explaining why cleavage of MccB17 occurs only after the modification. The mode of action of MccB17 is similar to highly successful fluoroquinolone antibiotics and a non-ribosomal natural product albicidin. This similarity is additionally manifested in the action of specific resistance factors, pentapeptide repeat proteins, which can interact with and protect host DNA gyrase in a toxin-specific way.

A 40-years old model object, microcin B17 still presents puzzles and offers opportunities for deeper understanding and control of biosynthesis of a large group of ribosomally-synthesized natural products, as well as for development of new antibacterials.

Selected Talk 4

Big Data Genomics for RiPP Discovery and Biosynthetic Characterization

presenting author: Douglas A Mitchell

Doug Mitchell, Jonathan Tietz, Chris Schwalen, Graham Hudson, Sangeetha Ramesh, Adam DiCaprio, Arash Firouzbakht, , Brandon Burkhart (Univ. of Illinois at Urbana-Champaign)

The genomics revolution has thus far supplied us with nearly 100,000 publicly available microbial genome sequences. Mining this vast resource for biosynthetic gene clusters whose products could hold future value to humankind is trivial for non-ribosomal peptide and polyketide natural products. Nontrivial is the bioinformatic identification of ribosomally synthesized and posttranslationally modified peptides, or RiPPs. This lecture will cover our motivations, initial forays, and on-going work in how big data genomics and high-throughput mining tools can be brought to bear in identifying and cataloguing all observable members of a desired class of RiPP. Analysis of these specific datasets naturally leads to the generation of new biosynthetic hypotheses while also accelerating the discovery of new members of that RiPP class and underscoring divergent cases that are first-in-class RiPPs themselves.

Selected Talk 5

Mode of action of non-lipid II interacting lantibiotics

presenting author: Eefjan Breukink

Xiaoqi Wang, Eefjan Breukink (Membrane Biochemistry & Biophysics, Dept. of Chemistry, Science Faculty, Utrecht University)

The lantibiotic nisin has been extensively studied and it has a unique and efficient targeted pore-formation mechanism where it, together with its docking molecule Lipid II, forms pores in the bacterial membrane. The first two lantionine rings of nisin has been shown to be involved in the binding of Lipid II, and all lantibiotics that have a similar A/B ring system are likely to target Lipid II as well in the same manner. Yet, there are lantibiotics that are, as nisin, also active in the nanomolar concentration range, but lack the specific A/B ring system that would mark them as Lipid II-targeting. These lantibiotics are thus using other mechanisms, and uncovering these may unravel novel ways to tackle the antibiotic resistance problem. Two examples of such lantibiotics are under study in our lab: pep5 and epilancin 15X. Pep5 is a bacteriostatic lantibiotic that acts specifically towards staphylococci and somehow rapidly shuts down the metabolism of the target bacteria. Efforts to the elucidation of its target will be discussed/shown. Epilancin 15X dissipates the membrane potential of the target bacteria, at somewhat higher concentrations as compared to nisin, but still in the nanomolar range. Indications are there that it also uses a specific docking molecule with which it forms pores. Although we could show that it (surprisingly) interacts with Lipid II, model membrane studies showed that Lipid II itself is not likely to be the target. Our efforts to unravel this mode of action will be shown/discussed as well.

Selected Talk 6

Zn-dependent bifunctional proteases are responsible for leader peptide processing of class III lanthipeptides

presenting author: Huan Wang

Shaoming Chen, Bing Xu, Erquan Chen, Jiaqi Wang, Jingxia Lu, Stefano Donadio, Huiming Ge, and Huan Wang (Nanjing University)

Lanthipeptides are an important subfamily of ribosomally synthesized and posttranslationally modified peptides, and the removal of their N-terminal leader peptides by a designated protease(s) is a key step during maturation. Whereas proteases for class I and II lanthipeptides are well-characterized, the identity of the protease(s) responsible for class III leader processing remains unclear. Herein, we report that the class III lanthipeptide NAI-112 employs a bifunctional Zn-dependent protease, AplP, with both endo- and aminopeptidase activities to complete leader peptide removal, which is unprecedented in the biosynthesis of lanthipeptides. AplP displays a broad substrate scope in vitro by processing a number of class III leader peptides. Furthermore, our studies reveal that AplP-like proteases exist in the genomes of all class III lanthipeptide producing strains but are usually located outside the biosynthetic gene clusters. Biochemical studies show that AplP-like proteases are universally responsible for the leader removal of the corresponding lanthipeptides. In addition, AplP-like proteases are phylogenetically correlated with aminopeptidase N from Escherichia coli, and might employ a single active site to catalyze both endo- and aminopeptidyl hydrolysis. These findings solve the long-standing question as to the mechanism of leader peptide processing during class III lanthipeptide biosynthesis, and pave the way for the production and bioengineering of this class of natural products.

Selected Talk 7

New Enzyme Discovery via Lasso Peptide Genome Mining

presenting author: James Link

A. James Link (Princeton University)

Lasso peptides are a large class of RiPPs typified by their chiral rotaxane structure. The formation of lasso peptides depends on only two post translational modifications: proteolysis of the leader peptide from the core peptide and formation of an isopeptide bond between the N-terminus of the core peptide and a Glu or Asp sidechain. In this talk I will discuss our efforts in lasso peptide genome mining. In addition, I will describe how genome mining has led to the discovery of new enzymes and transporters that interact with and modify lasso peptides.

Selected Talk 8

Deciphering bottromycin biosynthesis

presenting author: Jesko Köhnke

Asfandyar Sikandar, Laura Franz, Sebastian Adam, Jesko Köhnke (Helmholtz Institute for Pharmaceutical Research Saarland)

Bottromycins were originally discovered as antibacterial peptides of unknown biosynthetic origin with promising activity against Gram-positive bacteria. The naturally occurring variant bottromycin A2 is highly effective against major Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococci (VRE). More importantly, they provide a completely new scaffold with a molecular target at the A-site of the prokaryotic 50S ribosome, not tackled by any other antibiotic used in the clinic. In 2012, the biosynthetic origin of bottromycins was finally elucidated and the responsible gene cluster published – they belong to the growing family of ribosomally synthesized and post-translationally modified peptides (RiPPs). Like all RiPPs, the biosynthesis of bottromycins starts with the expression of a precursor peptide, which contains a core peptide (the eventual natural product), and, uniquely amongst bacterial RiPPs, a follower peptide (important for enzymatic recognition of the precursor peptide). We have established the order of biosynthetic reactions leading to bottromycins through a combined approach, using in vitro biochemistry and structural biology. In the process, we have identified new YcaO enzyme chemistry (macroamidine formation), the gatekeeper of bottromycin biosynthesis and an unusual resistance protein.

Selected Talk 9

The biogenesis and evolution of branched cyclic peptides in plants

presenting author: Jing-Ke Weng

Roland Kersten, Jing-Ke Weng (Whitehead Institute/MIT)

Lyciumins are branched cyclic lyciumins initially isolated from the Chinese wolfberry Lycium barbarum. Lyciumins are protease-inhibiting peptides featuring an N-terminal pyroglutamate and a macrocyclic bond between a tryptophan-indole nitrogen and a glycine α-carbon. We report a lyciumin precursor gene from L. barbarum, which encodes a BURP domain and repetitive lyciumin precursor peptide motifs. Genome and transcriptome mining enabled by this initial finding revealed rich lyciumin genotypes and chemotypes widespread in flowering plants as well as in distantly related lycophytes. We establish a biosynthetic framework of lyciumins and demonstrate the feasibility of producing diverse natural and unnatural lyciumins in transgenic tobacco. With rapidly expanding plant genome resources, our approach will complement bioactivity-guided approaches to unlock and engineer hidden plant peptide chemistry for pharmaceutical and agrochemical applications.

Selected Talk 10

Subcellular localization of a lantibiotic biosynthesis and secretion machinery in *Lactococcus lactis*

presenting author: Jingqi Chen

Jingqi Chen, Auke J. van Heel, Oscar P. Kuipers (Groningen University)

Lantibiotics are ribosomally synthesized antimicrobial peptides secreted by mainly Gram-positive bacteria. For the lantibiotic nisin, the dehydratase NisB dehydrates specific serines and threonines in precursor nisin, and subsequently the cyclase NisC links intramolecular cysteines to the dehydrated amino acids forming thioether ring-like structures. Finally the transporter NisT exports the modified precursor nisin to the outside of the cell. The structures of nisin, NisB and NisC have been elucidated, but their subcellular localization is still unclear. Thus we characterized the subcellular localization and the assembly process of the nisin maturation and secretion machinery in *Lactococcus lactis*. We found that prenisin, NisB and NisC not only localize to cell poles, with a preference for old poles, but also co-localize at the same spots, suggesting that the nisin maturation machinery is assembled mainly at old poles. Although the polar localization of the nisin maturation complex components is independent of each other, the targeting of NisB to cell poles is earlier than that of prenisin and NisC. This indicates that NisB may play an important role in the assembly process of the nisin maturation machinery. Furthermore, we show that NisT is distributed uniformly and circumferentially, in a pattern consistent with membrane localization. It seems that the modification and subsequent secretion of nisin can occur at different places. Based on these data, we propose a model for the assembly and subcellular localization of the nisin maturation machinery and for the secretion of the modified precursor peptide in *L. lactis*.

Selected Talk 11

Mycofactocin biosynthesis: New chemistry, new molecules, but still an unknown product

presenting author: John Latham

Richard Ayikpoe, Bulat Khaliullin, John A. Latham (University of Denver)

The mycofactocin biosynthetic pathway consists of the genes *mftABCDE(F)* and also one of the most widely distributed RiPP natural product pathways. Although the structure, function, and biosynthesis of mycofactocin remains unknown, it has been predicted to be a novel peptide derived redox cofactor. Recent efforts have been made to reconstitute the in vitro activity of mft gene products and have led to the discovery of novel biochemical modifications and structures. Indeed, we established that MftC, a radical-S-adenosylmethionine protein, catalyzes the two-step modification of MftA in the presence of the RiPP recognition element, MftB. This modification yields MftA* which contains an unprecedented 3-amino-5-[(p-hydroxyphenyl)methyl]-4,4-dimethyl-2-pyrrolidione (AHDP) moiety on the C-terminus of MftA. Next, we found that the iron-dependent peptidase, MftE, liberates AHDP from MftA*. In addition, we discovered that AHDP is further modified by the FMN-dependent isomerase, MftD, to form the previously undiscovered 4-[(p-hydroxyphenyl)methyl]-5,5-dimethyl-3,4,5,6-tetrahydro-1H-pyrimidin-2-one (HDTP). Although the putative final biochemical step and the structure of the mycofactocin still remains enigmatic, the journey of discovery has led us to question the physiological role of mycofactocin.

Selected Talk 12

Investigation of the Catalysis and Substrate Binding by Class IV Lanthipeptide Synthetase Kinase Domains

presenting author: Julian Hegemann

Julian D. Hegemann, Liuqing Shi, Michael L. Gross, Wilfred A. van der Donk (University of Illinois at Urbana-Champaign)

Lanthipeptides can be subdivided into four classes, based on characteristics of their biosynthetic enzymes. While the processing enzymes of classes I and II have been thoroughly studied, less is known about lanthipeptide synthetases of classes III and IV. The latter enzymes share a three-domain arrangement, featuring an N-terminal lyase, a central kinase, and a C-terminal cyclase domain.

Recently, we reported on the interactions of the class IV lanthipeptide synthetase SgbL with its precursor peptide SgbA. It was shown that a, potentially alpha-helical, stretch of residues in the central to N-terminal segment of the SgbA leader peptide is crucial for recognition by SgbL. Furthermore, binding studies were used to demonstrate that the substrate is recognized and bound by the central kinase domain.

Based on these findings, we set out to further study how the kinase domain catalyzes the phosphorylation of the core peptide and which part of this domain is required for substrate recognition. To accomplish this, a combination of homolog identification, structure predictions, sequence alignments, mutagenesis, hydrogen-deuterium exchange (HDX) – mass spectrometry (MS), and binding assays was employed. Here, our newest findings will be presented, which allowed the identification of the leader peptide binding site and residues crucial for catalysis. Further comparisons with class III lanthipeptide synthetases were carried out to show that these features have been conserved between both classes. Concludingly, it will be discussed how these results can be used to generate class III/IV protein or precursor peptide hybrids to further study differences and similarities between both classes.

Selected Talk 13

Efficient in vivo Synthesis of Lasso Peptide Pseudomycoidin Proceeds in the Absence of Leader and Leader Peptidase

presenting author: Konstantin Severinov

Tatyana Zyubko, Marina Serebryakova, Yulia Piskunova, Leah B. Bushin, Mikhail Metelev, Mohammad R. Seyedsayamdost, Svetlana Dubiley, and Konstantin Severinov (Skolkovo Institute of Science and Technology/Rutgers University)

Threaded lasso peptides are widespread in bacteria. They are made from linear ribosomally-synthesized precursor peptides in reactions that include specific proteolytic processing at the leader-core part junction site of the precursor by a dedicated protease recognizing the leader, followed by cyclisation of the newly formed N-terminus of the core part with a side chain of internal aspartic or glutamic residue catalyzed by a lasso cyclase. The resulting branched lasso has a tail that is fixed inside the cycle formed. We characterized a new lasso peptide, pseudomycoidin, encoded by Bacillus pseudomycoides DSM 12442. The most surprising and unique feature of pseudomycoidin is that it can be produced in vivo from the core part of precursor by the lasso cyclase in the absence of leader recognizing protease. The absolute simplicity of pseudomycoidin synthesis system makes it a powerful platform to generate pseudomycoidin-based lasso-peptide libraries and to study the poorly understood process of lasso formation. Unlike other known peptides of its class, pseudomycoidin appears to be a branched unthreaded lasso. We detected two additional modifications of pseudomycoidin, phosphorylation of terminal residue that was observed on other lasso peptides, followed by glycosylation, which was not observed heretofore. We speculate that these bulky C-terminal modifications may help maintain the threaded lasso topology of the compound.

Selected Talk 14

Systematic characterization of position one variants within the lantibiotic nisin

presenting author: Lutz Schmitt

Marcel Lagedroste, Jens Reiners, Sander H.J. Smits, Lutz Schmitt (Heinrich Heine University)

Lantibiotics are a growing class of natural compounds, which possess antimicrobial activity against a broad range of Gram-positive bacteria. Their high potency against human pathogenic strains such as MRSA and VRE makes them excellent candidates as substitutes for classic antibiotics in times of increasing multidrug resistance of bacterial strains. New lantibiotics are detected in genomes and can be heterologously expressed. The functionality of these novel lantibiotics requires a systematic purification and characterization to benchmark them against for example the well-known lantibiotic nisin. Here, we used a standardized workflow to characterize lantibiotics consisting of six individual steps. The expression and secretion of the lantibiotic was performed employing the promiscuous nisin modification machinery. We mutated the first amino acid of nisin into all proteinaceous amino acids and compared their bactericidal potency against sensitive strains as well as strains expressing nisin resistance proteins. Interestingly, we can highlight four distinct groups based on the residual activity of nisin against sensitive as well as resistant *L. lactis* strains.

Selected Talk 15

Substrate promiscuity and scope of rSAM peptide epimerases

presenting author: Madlen Korneli

Madlen Korneli, Lorenz Hug, Maximilian J. Helf, David Peterhoff, Jackson Cahn, Viviane Reber, Ralf Wagner and Jörn Piel (Institute of Microbiology, Department of Biology, ETH Zürich, Switzerland)

The enzyme family of radical S-adenosyl methionine peptide epimerases (RSPEs) catalyze a regiospecific and irreversible conversion of L- to D-configured amino acid residues in ribosomally encoded peptides. This peptide substrate consists of a highly conserved N-terminal leader region important for substrate recognition and a C-terminal core region that is subject to post-translational modification. The function of RSPEs was investigated by heterologous production in E. coli utilizing an orthogonal D2O-based induction system (ODIS) combined with mass-spectrometry analysis. The collective data show that RSPEs exhibit impressive substrate promiscuity, where the epimerization pattern is mainly, but not exclusively, directed by the core peptide sequence. The combination of RSPEs' high regiospecificity, substrate promiscuity, and irreversible action make them promising tools for biotechnology applications in peptide engineering. However, the core features that are important for epimerase regiospecificity remain elusive. To date, a modest set of naturally occurring peptides substrates have been investigated, however limitations of the current ODIS method, such as sample handling, need to be overcome to achieve higher throughput. Consequently, we focused on advancing the methodical approach to enable screening of peptide libraries for gaining insight into frequency of converted peptides, as well as regional and amino acid preferences for epimerization.

Selected Talk 16

AS-48 as an example of a broad spectrum bacteriocin for clinical development

presenting author: Manuel Montalban-Lopez

Manuel Montalbán-López, Rubén Cebrián, Eva Valdivia, Manuel Martínez-Bueno, Mercedes Maqueda (Department of Microbiology, University of Granada)

The bacteriocin AS-48 is a head-to-tail cyclized antimicrobial compound produced by diverse Enterococcus species. It is an amphipathic molecule arranged in five helical segments where the positively charged residues are asymmetrically distributed. This allows the oligomeric organization in two dimer forms, namely DF-I and DF-II, where the monomers interact through hydrophobic and hydrophilic regions, respectively. Electrostatic attraction and dimer dissociation are key for membrane insertion and strongly related to the specificity of AS-48 for bacterial membranes.

Traditionally, bacteriocins are defined as narrow spectrum antimicrobials. AS-48 definitely breaks with this definition since it targets both Gram-positive and Gram-negative bacteria, although the latest require higher concentrations. The activity of AS-48 against skin and soft tissue infectious agents (Staphylococcus aureus, Cutibacterium acnes, Gardnerella vaginalis or Streptococcus agalactiae) is specially promising for topical use. Additionally, its activity against Mycobacterium tuberculosis highlights its medical potential. Interestingly, in the last years, the spectrum of AS-48 has been extended towards parasites belonging to the Trypanosomatidae, which includes relevant pathogens such as Leishmania and Trypanosoma. AS-48 can efficiently kill these protozoa. The activity is variable and depends on the life-cycle stage of the parasite and the species. Remarkably, the interaction of AS-48 with Trypanosoma brucei causes extremely rapid death at low MIC, lower than any drug in the market.

Collectively, the spectrum and potency of AS-48 together with the low immunogenicity and toxicity detected in vitro against a range of tumor and healthy cell lines, point at AS-48 as a promising candidate for clinical tests.

Selected Talk 17

Biosynthetic engineering of RiPPs

presenting author: Maria Lopatniuk

Lopatniuk Maria, Myronovskyi Maksym, Luzhetskyy Andriy (Saarland University, Saarbrücken, Germany)

The biosynthesis of RiPPs through pre-peptide provides an opportunity to alter the amino acids in the final structure generating a library of derivatives. Each single codon in a core-peptide gene corresponds to a certain amino acid in the biosynthetic product, and a site-specific codon substitution enables controlled replacement of amino acids, which in turn can alter the structure and the function of the new peptide. Therefore we decided to use two classes of RiPPs, the lanthipeptide and thiopeptide, for further derivatization experiments. The cinnamycin was used for successful incorporation of non-canonical amino acids (ncAAs) at two pre-selected positions in the cinnamycin scaffold. This is the first reported instance of the ncAAs incorporation into the natural product in streptomycetes using the orthogonal pyrrolysyl-tRNA synthetase/tRNAPyl pair from Methanosarcina barkeri. Five new cinnamycin derivatives were obtained. The combination of partial hydrolysis and MS/MS fragmentation analysis was used to verify the exact position of the incorporation events.

A rational random mutagenesis of the selected codons of the core-peptide gene was used to generate new derivatives of thioholgamide. After being modified, the new thioholgamide BGCs were expressed in S. lividans leading to the production of 24 new derivatives. The production yield of new compounds differs, depending on the incorporated amino acids. The structures of new thioholgamides were verified by MS/MS fragmentation analysis together with sequencing of the corresponding pre-peptide gene.

The structures and the biological activities of the obtained cinnamycin and thioholgamide derivatives along with the experimental details will be presented.

Selected Talk 18

Uncovering New Mechanisms in Ripp Biosynthesis

presenting author: Satish Nair

Dillon Cogan, Chayanid Ongpipattanakul, Andrea Garcia Hernandez (University of Illinois Urbana-Champaign)

Several natural products that were previously believed to have originated from non-ribosomal peptide pathways have since been demonstrated as RiPPs that are the result of very novel post-translational modifications. Here, we will describe recent results from our lab in uncovering the biosynthetic routes that elaborate these modifications. These cases provide insights into how biosynthetic pathways may be adapted to function on peptide precursors. Characterization of the biosynthetic enzymes also provide a means for identifying new RiPP natural products.

Selected Talk 19

Expansion of the natural diversity of the microviridin-like modified peptides

presenting author: Seokhee Kim

Seokhee Kim (Seoul National University)

Defining feature of the microviridin family of ribosomally synthesized and post-translationally modified peptides (RiPPs) is the distinct macrocycles that are formed by multiple -ester/amide. This type of multiple side-to-side cross-links, which is shown only in a few RiPP families such as lantipeptides and sactipeptides, has a great potential for topological diversity of macrocyclic structure, but microviridins have shown only a single cross-linking connectivity with the highly conserved core motif, TxKxPSDx(E/D)(D/E). We have demonstrated that microviridin-like RiPPs have novel sequences and cross-linking connectivity beyond the one found in microviridins. So far, we identified five new sub-groups of microviridin-like RiPPs, including plesiocins and thuringinins that have the TTxxxxEE and TxxTxxxExxD motif, respectively. They show difference in ring size, ring topology, number of -ester/amide, and number of rings, but have several common features that indicate basic mechanism of biosynthesis. We suggest that these expanded RiPP family should be designated as omega-ester containing peptides (OEPs). We also characterized the cross-reactivity of enzymes that belong to different OEP sub-groups, and found that, in contrast to some examples in lantipeptides, the cross-linking pattern was determined by enzyme reactivity as well as by core sequence. This result led us to design and synthesize multi-functional hybrid RiPPs by combinatorial application of multiple orthogonal enzyme-substrate pairs. Collectively, we demonstrated that the microviridin-like RiPPs have much higher natural diversity and present a great potential for the synthetic biology application to find novel bioactive peptides.

Selected Talk 20

Lipolanthines - Ribosomally Synthesized Lipopeptides with Anti-Staphylococcal Activity

presenting author: Vincent Wiebach

Vincent Wiebach, Roderich D. Süssmuth (Institut für Chemie, Technische Universität Berlin, Straße des 17. Juni 124, 10623 Berlin, Germany)

Due to the emergence of multiple and fully resistant pathogens, the need for novel lead structures for the development of antimicrobial drugs is of highest importance.[1] During the screening of bacterial extracts, the highly potent anti-staphylococcal lanthipeptide microvionin was isolated from a culture of Microbacterium arborescens. Microvionin is a ribosomally synthesized and post-translationally modified peptide (RiPP) which exhibits the new triamino dicarboxylic acid avionin and an unprecedented lipidation with an N-terminal fatty acid modification.[2] The microvionin (mic) gene cluster was identified, corresponding genes were assigned and suggest a combination of polyketide or fatty acid and ribosomal peptide biosynthetic pathways. The central steps of the avionin biosynthesis were reconstituted in vitro, revealing a dependence on the cooperative action of a cysteine decarboxylase (MicD) and class III lanthionine synthetase (MicKC).[3] Intensive genome mining was utilized to discover microvionin-related gene clusters in various Actinomycetes. These efforts lead to the isolation of nocavionin from Nocardia terpenica, revealing a widespread distribution of this novel lanthipeptide class, termed lipolanthines. The lipolanthines exhibit great potential as antibacterial drugs, not only due to their strong biological activity but also with regard to engineering purposes aiming for a modulation of activity and pharmacokinetic properties.

[1] M. May, Nature 2014, 509, S4–S5.

[2] V. Wiebach, A. Mainz, M.-A. J. Siegert, G. Lesquame, S. Tirat, A. Dreux-Zigha, J. Aszodi, D. Le Beller, R. D. Süssmuth, Nat. Chem. Biol. 2018, 14, 652–654.

[3] P. G. Arnison, et al., Nat. Prod. Rep. 2013, 30, 108–160.

Selected Talk 21

One-pot saturation mutagenesis of RiPP precursor peptides provides comprehensive knowledge about the determinants of substrate recognition

presenting author: Yuki Goto

Yuki Goto, Haruka Takeue, Yamato Komatsu, Alexander A. Vinogradov, Hiroaki Suga (Yuki Goto)

Mutagenesis studies on precursor peptides of RiPPs have been performed to gain insight into the substrate tolerance and specificity of RiPP modifying enzymes. Conventionally, mutated precursor peptides are often heterologously overexpressed and purified, and then subjected to in vivo or in vitro modification by RiPP enzymes to examine whether the mutations affect the enzymatic reaction. Alternatively, we have devised cell-free translation systems integrated with RiPP enzymes to achieve plasmid-free and purification-free mutagenesis of precursor peptides, which increased the throughput of the assay. Nonetheless, as mutated precursor peptides are prepared and assayed one by one in these strategies, the number of precursor variants that can be tested in a study is generally limited to several dozens. Although these strategies have allowed for an alanine-scanning or rationally designed mutagenesis of certain regions of precursors, such studies may not obtain comprehensive knowledge about the determinants of substrate recognition.

To gain a more comprehensive understanding of essential and variable residues of RiPP precursors, we here develop a new methodology that enables us to examine thousands of precursor mutants at a time. In this method, a mixed library of precursor variants is prepared and incubated with RiPP enzymes of interest, and we can evaluate how efficiently each variant in the mixture is modified by the enzymes. We have demonstrated saturation mutagenesis of an RiPP precursor peptide, that is, testing all proteinogenic amino acids at all positions in a sequence. This method has a potential to provide comprehensive knowledge of substrate tolerance and specificity of RiPP modifying enzymes, which facilitates engineering of RiPP biosynthetic pathways.

Selected Talk 22

Mechanism of the tryptophan lyase NosL

presenting author: Yvain Nicolet

Yvain Nicolet (Institut de Biologie Structurale)

The radical S-adenosyl-L-methionine tryptophan lyase uses radical-based chemistry to convert L-tryptophan into 3-methyl-2- indolic acid, a fragment in the biosynthesis of the thiopeptide antibiotic nosiheptide. This complex reaction involves several successive steps corresponding to (i) the activation by a specific hydrogen-atom abstraction, (ii) an unprecedented •CO_2^- radical migration, (iii) a cyanide fragment release, and (iv) the termination of the radical-based reaction. In vitro study of this reaction is made more difficult because the enzyme produces a significant amount of a shunt product instead of the natural product. In this presentation, we will discuss further how a combination of X-ray crystallography, electron paramagnetic resonance spectroscopy, and quantum and hybrid quantum mechanical/molecular mechanical calculations, we have made significant progresses in our understanding of the radical-based mechanism of the enzyme and how the protein matrix controls the reaction. These results pave the way to protein engineering to develop variants of the MIA fragments that could be integrated into nosiheptide in order to increase our panel of available molecules to struggle against multidrug-resistant gram positive pathogens.

Poster Number 1

Genome-led discovery and characterisation of novel RiPPs

presenting author: Alicia Russell

Alicia Russell, Rodney Lacret, Andrew Truman (John Innes Centre)

RiPPs are an important class of natural product, and bioinformatic analysis has shown that microorganisms are harbouring many more RiPP biosynthetic gene clusters (BGCs) than are currently characterised. Genome mining for novel RiPP BGCs is often hampered by poor detection of precursor peptides, which are short in sequence length and rarely annotated in genomes. Using a more targeted genome-mining approach that specifically picks out putative precursor peptides nearby target RiPP tailoring enzymes (RTEs), we have identified a novel, diverse and highly prevalent family of RiPP BGCs spanning over 200 actinobacterial genomes. All the BGCs identified contain YcaO-domain proteins, which are involved in catalysing several post-translational modifications of known RiPPs such as amidine, thioamide and oxazol(in)e formation. The presence of diverse RTEs and the sequence variation of observed precursor peptides suggest that this family of RiPP BGCs may produce several different structurally diverse molecules. We have successfully TAR cloned one such BGC from Streptomyces albus J1074 and expressed it heterologously in Streptomyces coelicolor M1146, and metabolomic analysis led to identification of the pathway product. Deletions of genes in the cluster have also confirmed the involvement of individual RTEs in biosynthesis. The compound has been purified and the structure elucidated by NMR, revealing a hexapeptide containing an oxazoline ring. The compound currently has no known bioactivity, but further targets will be explored to investigate the role of this RiPP in nature. Further work will also focus on exploring RiPP BGCs from other genera, which we predict will produce structurally distinct molecules.

Poster Number 2

Insights into Ribosomal Peptide Backbone Modification from the Structure of a YcaO-Peptide Substrate Complex

presenting author: Andi Liu

Andi Liu, Shi-Hui Dong, Nilkamal Mahanta, Doug Mitchell, Satish Nair (Department of Microbiology, University of Illinois at Urbana-Champaign)

YcaO enzymes are known to catalyze the ATP-dependent formation of azoline heterocycles, thioamides, and (macro)lactamidines on peptide substrates. These enzymes are found in multiple biosynthetic pathways, including those for several different classes of ribosomally synthesized and post-translationally modified peptides (RiPPs). However, there are major knowledge gaps in the mechanistic and structural underpinnings that govern each of the known YcaO-mediated modifications. Here, we present the first structure of any YcaO enzyme bound to its peptide substrate in the active site, specifically that from Methanocaldococcus jannaschii which is involved in the thioamidation of the alpha-subunit of methyl-coenzyme M reductase (McrA). The structural data are leveraged to identify and test the residues involved in substrate binding and catalysis by site-directed mutagenesis. We also show that thioamide-forming YcaOs can carry out the cyclodehydration of a related peptide substrate, which underscores the mechanistic conservation across the YcaO family and allows for the extrapolation of mechanistic details to azoline-forming YcaOs involved in RiPP biosynthesis. A bioinformatic survey of all YcaOs highlights the diverse sequence space in azoline-forming YcaOs and suggests their early divergence from a common ancestor. The data presented within provide a detailed molecular framework for understanding this family of enzymes, which reconcile several decades of prior data on RiPP cyclodehydratases. These studies also provide the foundational knowledge to impact our mechanistic understanding of additional RiPP biosynthetic classes.

Poster Number 3

Amidohydrolase: The gatekeeper of bottromycin biosynthesis

presenting author: Asfandyar Sikandar

Asfandyar Sikandar, Laura Franz, Okke Melse, Iris Antes,§ and Jesko Koehnke (Workgroup Structural Biology of Biosynthetic Enzymes,Helmholtz Institute for Pharmaceutical Research Saarland, Helmholtz Centre for Infection Research, Saarland University, Campus Geb. E8.1, 66123 Saarbrücken, Germany)

The ribosomally synthesized and post-translationally modified peptide (RiPP) bottromycin A2 possesses potent antimicrobial activity. Its biosynthesis involves the enzymatic formation of a macroamidine, a process previously suggested to require the concerted efforts of a YcaO enzyme (BotCD) and an amidohydrolase (BotAH) in vivo. In vitro, BotCD was shown to be sufficient for the formation of the macroamidine, but the process is reversible.

We demonstrate that BotAH is highly selective for macrocyclized precursor peptides and cleaves C-terminal of a thiazoline, thus removing the follower peptide. After follower cleavage, macroamidine formation is irreversible, indicating BotAH as the gatekeeper of bottromycin biosynthesis. This resolves the apparent disparity between the in vitro and in vivo data.The biochemical and structural characterisation BotAH along with its model with substrate will be presented.

Poster Number 4

BAGEL4: A user-friendly web server to mine RiPPs and bacteriocins

presenting author: Auke van Heel

Auke J. van Heel, A. de Jong, J. H. Viel, J. Kok and O.P. Kuipers (University of Groningen)

Interest in secondary metabolites such as RiPPs is increasing worldwide. To facilitate the research in this field we have updated our mining web server. BAGEL4 is faster than its predecessor and is now independent from ORF- calling. Gene clusters of interest are discovered using the core-peptide database and/or through HMM motifs that are present in associated context genes. The databases used for mining have been updated and extended with literature references and links to UniProt and NCBI. Additionally, we have included automated promoter and terminator prediction and the option to upload RNA expression data, which can be displayed along with the identified clusters. Further improvements include the annotation of the context genes, which is now based on a fast blast against the prokaryote part of the UniRef90 database, and the improved web-BLAST feature that dynamically loads structural data from UniProt. Overall BAGEL4 provides the user with more information through a user-friendly web- interface which simplifies data evaluation. The BAGEL4 website can be accessed at http://bagel4.molgenrug.nl/ .

Poster Number 5

Experimental evolution under cell envelope stress of nisin producing lactococci

presenting author: Beatriz Martínez

Mª Jesús López-González, Susana Escobedo, Thomas Janzen, Rute Neves, Ana Rodríguez and Beatriz Martínez (DairySafe group. IPLA-CSIC. Paseo Río Linares s/n. 33300 Villaviciosa, Asturias, Spain)

Lactococcus lactis is widely used as starter in cheese fermentations and nisin producers are often included in the formulation of protective cultures for enhancing food safety and prolonging shelf life. However, developing novel starter blends is hindered by the relatively small biodiversity within available commercial strains. In this work, we have assessed experimental evolution under cell envelope stress as means to diversify industrial *L. lactis*. Five nisin-producing *L. lactis* strains were exposed repeatedly to increasing concentrations of Lcn972, a bacteriocin that triggers the cell envelope stress response in *L. lactis*. Stable evolved mutants that retained main technological aptitudes (milk acidification, nisin production) could be selected. In general, evolved mutants were more hydrophobic, less autolytic, heat sensitive, and cross-resistance to other cell wall antimicrobials (lysozyme, bacitracin) was also observed. Loss of plasmids was detected in some cases. Genome analysis revealed distinct and shared non-synonymous mutations depending on the parental strain and were found in genes involved in stress response, detoxification modules, cell envelope biogenesis and/or nucleotide metabolism. Moreover, transcriptional studies to identify the molecular basis behind the observed phenotypes are in progress. As a whole, the results support the use of experimental evolution under cell envelope stress as a feasible strategy to diversify industrial strains.

Poster Number 6

Catalytic Leader Peptide enables the Biosynthesis of 3-thiaglutamate

presenting author: Chi Pan Ting

Chi P. Ting, Michael A. Funk, Wilfred A. van der Donk (University of Illinois at Urbana-Champaign)

Ribosomally-synthesized and post-translationally modified peptides (RiPPs) are a rapidly growing family of natural products where a genetically encoded precursor peptide is modified by tailoring enzymes. In this presentation, we report on a biosynthetic gene cluster in the plant pathogen Pseudomonas syringae (pma) that uses a ribosomal peptide as a catalytic scaffold for the biosynthesis of a small molecule natural product, 3-thiaglutamate. The pathway begins by the use of a truncated LanB enzyme that catalyzes cysteine addition to the C-terminus of the peptide, PmaA, a reaction that replaces ribosomal peptide synthesis. Next, a di-iron enzyme catalyzes the excision of the β-methylene of the added cysteine to produce an α-thioglycine residue and formate. Carboxymethylation of the α-thioglycine and proteolysis affords 3-thiaglutamate and regenerates the initial peptide (PmaA) which can undergo another round of turnover converting L-Cys to 3-thiaglutamate. This novel system expands the use of ribosomal peptides in natural product biosynthesis.

Poster Number 7

Structural studies of a LanBC complex from L. lactis in vitro

presenting author: Christina Vivien Knospe

Christina Vivien Knospe, Jens Reiners, Andre Abts, Sander H.J. Smits, Lutz Schmitt (Heinrich-Heine-University Duesseldorf)

Lantibiotics are ribosomally synthesized and post-translationally modified peptides (RiPPs) with antimicrobial activity. The best-studied lantibiotic is nisin produced and secreted by Lactococcus lactis. The prepeptide (pre-NisA) is composed of an N-terminal signal leader peptide (23 amino acids) and a C-terminal core peptide (34 amino acids), which is modified posttranslationally by a maturation complex composed of NisB (dehydratase) and NisC (cyclase) in an alternating manner. The modification includes the dehydration of Ser and Thr residues to dehydroalanine and dehydrobutyrine as well as a subsequent cyclization process of the dehydrated residues with Cys residues forming (methyl-)lanthionine rings.

Here we present the stoichiometry of the nisin maturation complex first solved by a combination of multi-angle light scattering and size exclusion chromatography (MALS-SEC) in vitro and additionally by small-angle X-ray scattering (SAXS) comprising a dimer of NisB, a monomer of NisC and a single molecule of prenisin. However, the highly conserved -FNLD- box in the leader sequence as well as the modification state of the core peptide are crucial for the recognition and formation of the maturation complex (NisB/NisC). Unmodified and dehydrated prenisin lead to complex formation, whereas fully modified prenisin forms no maturation complex indicating that all installed (methyl-)lanthionine rings function as releasing factor. The exchange of the -FNLD- box to four alanines (-AAAA-) in the leader sequence revealed no more complex formation showing its requirement for the formation of the maturation complex.

Poster Number 8

Computational analysis of the lysinoalanine cross-link formation in duramycin biosynthesis

presenting author: Claudio D. Navo

Claudio D. Navo, Linna An, Dillon P. Cogan, Satish K. Nair, Wilfred A. van der Donk, Gonzalo Jiménez-Osés (University of La Rioja)

Duramycin is a RiPP which binds phosphatidylethanolamine, a phospholipid found in mammalian and microbial cell membranes, and has been investigated as an antibiotic, a therapeutic for cystic fibrosis, or an inhibitor of viral entry. Duramycin has 19 amino acids, including five post-translational modifications: one beta-hydroxylated aspartic acid (Hya), one lanthionine, two methyllanthionines and an essential lysinoalanine (Lal) cross-link. This latter is formed by a DurN-catalyzed Michael-type addition of the side chain of a lysine residue to a dehydroalanine (Dha) and has shown to be essential for antimicrobial activity.

Very recently, our groups have reported the crystallographic structure of DurN. Surprisingly, in the structure of duramycin bound to DurN, no residues of the enzyme are near the Lal cross-link. Instead, the Hya residue of the substrate makes interactions with Lal, suggesting it acts as a base to deprotonate the reactive Lys during catalysis. Biochemical data suggest that DurN pre organizes the reactive conformation of the substrate, such that the Hya of the substrate can serve as the catalytic base for Lal formation.

A computational study combining Molecular Dynamics (MD) simulations and hybrid Quantum Mechanics/Molecular Mechanics (QM/MM) has been performed to describe the interactions between duramycin and DurN with atomic resolution. As a result of this study, a detailed mechanism and energetics for duramycin cyclization in agreement with the experimental observations, has been proposed.

Poster Number 9

Identification, cloning and heterologous expression of the gene cluster encoding RES-701-3 and RES-701-4 biosynthesis

presenting author: Daniel Oves-Costales

Daniel Oves-Costales, Marina Sánchez-Hidalgo, Jesús Martín, Olga Genilloud (Fundacion MEDINA)

RES-701-3 and RES-701-4 are two class II lasso peptides originally identified in the fermentation broth of Streptomyces sp. RE-896 which have been described as selective endothelin type B receptor antagonists. These two lasso peptides only differ in the identity of the C-terminal residue (tryptophan in RES-701-3, 7-hydroxy-tryptophan in RES-701-4), thus raising an intriguing question about the mechanism for the modification of the tryptophan residue. Here we describe the identification of their gene cluster through the genome mining of *Streptomyces caniferu*s CA-271066, its cloning and heterologous expression in *Streptomyces coelicolor* M1152, and show that the seven ORFs encoded within the gene cluster are sufficient for the biosynthesis of both lasso peptides. We propose that ResE, a protein lacking known putative conserved domains, plays a key role in the post-translational modification of the C-terminal tryptophan of RES-701-3 to afford RES-701-4. A BLASTP search with the ResE amino acid sequence shows the presence of homologues of this protein in the genomes of three other *Streptomyces* strains which also harbour the genes encoding the RES-701-3/4 precursor peptide, split-B proteins and ATP-dependent lactam synthetase required for the biosynthesis of these compounds.

Poster Number 10

Bioengineering Nisin to overcome the Nisin Resistance Protein

presenting author: Des Field

Des Field, Tony Blake, Harsh Mathur, Paula M O'Connor, Paul Cotter, Paul Ross and Colin Hill (Alimentary Pharmabiotic Centre, University College Cork)

The emergence and dissemination of antibiotic resistant bacteria is a major medical challenge. Lantibiotics are highly modified bacterially produced antimicrobial peptides that have attracted considerable interest as alternatives or adjuncts to existing antibiotics. Nisin, the most widely studied and commercially exploited lantibiotic, exhibits high efficacy against many pathogens. However, some clinically relevant bacteria express highly specific membrane-associated nisin resistance proteins. One notable example is the nisin resistance protein (NSR) that acts by cleaving the peptide bond between ring E and the adjacent serine 29, resulting in a truncated peptide with significantly less activity. We utilised a complete bank of bioengineered nisin (nisin A) producers in which the serine 29 residue has been replaced with every alternative amino acid. The nisin A S29P derivative was found to be as active as nisin A against a variety of bacterial targets but, crucially, exhibited a 20-fold increase in specific activity against a strain expressing the nisin resistance protein. Another derivative, nisin PV, exhibited similar properties but was much less prone to oxidation. This version of nisin with enhanced resistance to specific resistance mechanisms could prove a valuable tool in the fight against antibiotic resistant pathogens.

Poster Number 11

Roseocin, a Two-Component Lantibiotic from an Actinomycete

presenting author: Dipti Sareen

Dr. Dipti Sareen, Dr. Mangal Singh (Department of Biochemistry, Basic Medical Sciences Block-II, Sector-25, Panjab University, Chandigarh-160014, India)

Lantibiotics are lanthionine containing peptide natural products that belong to the class of ribosomally synthesized and post-translationally modified peptides (RiPPs). Recent expansion in the availability of microbial genome data and in silico analysis tools have accelerated the discovery of these promising alternatives to antibiotics. Following the genome-mining approach, a biosynthetic gene cluster for a putative two-component lantibiotic roseocin was identified in the genome of an Actinomycete, *Streptomyces roseosporus* NRRL 11379. Post-translationally modified lanthipeptides of this cluster were obtained by heterologous expression of the genes in *E. coli* and were in vitro reconstituted to their bioactive form for structure and bioactivity analysis.The two peptides displayed antimicrobial activity against Gram-positive bacteria in synergy only, a property reminiscent of the two-component lantibiotics. Structural characterization confirmed the installation of four lanthionine rings with a disulphide bond in the α-component and six lanthionine rings in the β-component, by the single promiscuous lanthionine synthetase. Roseocin is a two-component lantibiotic with extensive lanthionine bridging in the two constituent peptides, not observed previously.

Poster Number 12

Phasolicin – a founding member of a new group of thiazole/oxazole modified peptides inhibiting prokaryotic translation

presenting author: Dmitrii Travin

Dmitrii Travin, Mikhail Metelev, Ilya Osterman, Marina Serebryakova, Konstantin Severinov (Centre for Life Sciences, Skolkovo Institute of Science and Technology, 143026 Moscow, Russia)

Linear thiazole/oxazole-modified peptides (LAPs) comprise a subgroup of RiPPs characterized by the presence of azole cycles introduced through the cyclization of side chains of cysteine, serine and threonine residues of precursor peptide. Although the diversity of well-characterized LAPs is limited, these compounds demonstrate the antibacterial activity through several completely different mechanisms: microcin B17 is a DNA-gyrase poison, plantazolicin targets the membrane, while klebsazolicin effects prokaryotic translation. This diversity of modes of action among the peptides sharing common chemical features makes LAPs a group of special interest for the search of new antibacterials.

Here we report the characterization of a new LAP biosynthetic gene cluster present in the genome of *Rhizobium sp*. Pop5. Cultivation of this strain enabled identification of a 2364 Da peptide with three thiazole and five oxazole cycles. This compound, which we named phasolicin (PHS), demonstrated antibacterial activity against a set of strains from genera *Rhizobium, Sinorhizobium, Azorhizobium* and *Agrobacterium* including phytopathogenic *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. We established that PHS is a translation inhibitor that binds in the exit channel of the prokaryotic ribosome and obstructs the passage of the nascent peptide in a way similar to klebsazolicin. However, PHS lacks the N-terminal amidine cycle, which is strictly required for KLB action, has more azole cycles, binds further from the peptidyl transferase center of the ribosome than KLB and exhibits species-specific binding to the ribosome.

Poster Number 13

Exploration of α-Keto-β-amino Acid Chemical Applications and the Splicease Substrate Tolerance

presenting author: Edgars Lakis

Edgars Lakis, Jörn Piel (ETH Zurich)

Splicease is a radical S-adenosylmethionine (rSAM) enzyme that performs a noncanonical protein splicing and creates an α-keto-β-amino acid in ribosomally synthesized peptide. Mutagenesis studies on the precursor peptides have provided an initial insight into the Splicease substrate tolerance. The enzyme hereologous co-expression with a precursor peptide bearing non-native leader sequence does not abolish the tyramine excision in vivo. The precursor is modified even when the core sequence is fused to N-terminus of a protein, although in lesser extent. In addition, to retain the Splicease activity, only a minimal 11-residue-long core peptide is necessary. We envision, the minimal core peptide fusion could provide an orthogonal chemical handle for site-specific modification of a protein-of-interest compared to conventional strategies relying on nucleophilic amino acids.

Electrophilic β-amino-α-ketoamide moiety readily reacts with methoxamines or hydrazides to form an imine. To demonstrate a utility of the α-keto-β-amino acid, we successfully conjugated the ribosomally synthesized post-translationally modified peptide (RiPP) with biotin and fluorescein hydrazides and subsequently used in affinity purification and fluorescence tagging, respectively. However, the transient imine linkage requires acidic pH and presence of an organic solvent.

Poster Number 14

Identifying Potential RiPP Clusters Using a Neural Network Trained to Recognize Precursor Peptides

presenting author: Emzo de los Santos

Emmanuel Lorenzo de los Santos (University of Warwick)

Significant progress has been made in the past few years on the computational identification of RiPP clusters. This has been done by both identifying RiPP Tailoring Enzymes (RTEs) and RiPP Precursor Peptides. Identification of RiPP Precursor Peptides has been challenging, due to their short length, causing them to be occasionally missed by gene finding software. To address this software, such as RODEO, has been developed that uses support vector machines (SVMs) to accurately identify precursor peptide sequences from the six-frame translation of open reading frames in close proximity to RTEs. While accurate, this approach has some limitations since the SVMs are trained on features specific to a particular RiPP class; furthermore, the identification of potential RiPP clusters is limited to those of known classes. However, the success of SVMs in identifying precursor peptides presents us with a rich dataset of “high-confidence” putative precursor sequences for a variety of RiPP classes. These can be combined and used to train a neural network that can identify potential RiPP precursor peptides. I present a workflow that uses a neural network trained to identify precursor peptides as a starting point for identifying putative RiPP clusters. After putative precursor peptides are identified, the genomic environment around each precursor peptide is examined, these coding groups are clustered together based on sequence similarity. This is an alternate way to identify putative RiPP clusters starting with precursor peptides instead of RTEs, opening the possibility of discovering previously uncharacterized RiPP classes.

Poster Number 15

Synthetic biology approach to obtain novel antimicrobials with cyclic modifications

presenting author: Fangfang liu

Fangfang Liu, Auke van Heel, Oscar Kuipers (University of Groningen)

Extensive use of antibiotic substances in medicine and agriculture has resulted in an increase of antibiotic-resistant bacteria, necessitating the development of novel antimicrobial compounds from alternative sources. The ribosomally synthesized and post-translationally modified peptides (RiPPs) entails diverse natural products and their ribosomal origin enables strategies to modify the peptide sequence and to create variants with altered biological and physico-chemical properties.

To investigate circularin A, a circular bacteriocin produced by *Clostridium beijerinckii* ATCC 25752 and active against *Clostridium perfringens*, is expressed heterologously in diverse hosts. The extraordinary leader peptide (only 3 amino acids) makes this modification machinery highly attractive for biosynthetic peptide manipulations. Ultimately we want to combine different RiPP modification machineries to make new-to-nature molecules containing multiple post-translational modifications.

Poster Number 16

Making NRPS antimicrobial peptides the ribosomal way

presenting author: Fleur Ruijne

Fleur Ruijne, Oscar P. Kuipers (University of Groningen)

Natural products, like the last-resort antibiotic daptomycin, are produced by nonribosomal peptide synthetases (NRPSs) and comprise interesting structural elements for the development of novel antimicrobials with high efficacy and a relatively low probability of inducing resistance. These large multimodular enzymes produce complex cyclic lipopeptide antimicrobials in an assembly line-like fashion. Creating analogues of these complex products to improve and expand the therapeutic applications and antibacterial spectrum by altering the enzymatic modules is however challenging and laborious. Therefore, we sought to synthesize these peptides in a ribosomal way, making it possible to create analogues by simple mutagenesis of the genetically encoded peptide. In order to ribosomally synthesize these complex peptides, several post-translational modification enzymes need to be orchestrated in time and space in vivo to recognize the same precursor peptide to act upon. Recognition of the precursor peptide will be ensured by a hybrid leader peptide, which guides the enzymes to the core peptide for modification. Post-translational modifications include macrocyclization and epimerization, followed by an in vitro lipidation step. The resulting plug-and-play system of modification enzymes will allow for the facile biosynthesis of a whole variety of new-to-nature peptides, hereby aimed on the production of novel antimicrobials. Furthermore, this research will provide valuable knowledge on the mechanisms of these posttranslational modification enzymes to further aid in the development of the production of bioactive peptides. In this poster, progress and design strategies on the development of these new-to-nature NRPS antimicrobial peptide analogues will be discussed.

Poster Number 17

Discovery of MDN-0207, a novel glycosylated lanthipeptide with unusual structural features and potent antibacterial activity

presenting author: Francisco Javier Ortiz-López

Francisco Javier Ortiz-López, Daniel Carretero-Molina, Jesús Martín, Mercedes de la Cruz, Marina Sánchez, Caridad Díaz, Ignacio González, Maria-Isabel Morosini, Francisca Vicente, Fernando Reyes, Julia Deisinger, Anna Müller, Tanja, Schneider, Olga Genilloud. (Fundacion MEDINA)

Lanthipeptides are a major class of ribosomally synthesized and post-translationally modified peptides (RiPPs) characterized by the presence of amino acids lanthionine (Lan) and methyllanthionine (MeLan).

As part of our antibiotic discovery programs from microbial sources, we have isolated and structurally characterized a novel glycosylated lanthipeptide, MDN-0207, from a strain of *Streptomyces sp.* from MEDINA´s culture collection. This new lanthipeptide presents some special structural features, such as an unusually high number of D-amino acids and an unprecedented glycosylated tyrosine residue carrying a disaccharide non-previously reported in any natural product. Structural elucidation of the molecule was carried out through a combination of NMR analysis, HRMS/MS, exploratory chemistry (reduction / desulfurization) and genome mining.

MDN-0207 shows significant antibacterial activity against a panel of gram-positive human pathogens, with potent bioactivities (MIC 0.5 ug/mL) against MRSA and moderate activity against a set of clinical isolates of *Clostridium difficile* (MIC 4 ug/mL).

Gene expression bioreporters for cell wall clearly show that MDN-0207 interferes with the Lipid II biosynthesis cycle. Inhibition of enzymatic activity of Lipid II transglycosylation-involved enzymes PBPP2 and SgtB strongly support the Lipid II binding as the most plausible mode of action. The role of the disaccharide moiety in the antibacterial activity is being further investigated.

Poster Number 18

Bioinformatic mapping of the radical SAM superfamily identifies new Cα, Cβ , and Cɣ-linked thioether-containing peptides

presenting author: Graham Hudson

Graham A. Hudson, Brandon J. Burkhart, Adam J. DiCaprio, Christopher J. Schwalen, Bryce Kille, Taras V. Pogorelov, and Douglas A. Mitchell (Department of Chemistry, University of Illinois at Urbana-Champaign)

Recently developed bioinformatic tools have bolstered the discovery of ribosomally synthesized and posttranslationally modified peptides (RiPPs). Using an improved version of Rapid ORF Description & Evaluation Online (RODEO 2.0), a biosynthetic gene cluster mining algorithm, we bioinformatically mapped the sactipeptide RiPP class via the radical S-adenosylmethionine (SAM) enzymes that form the characteristic sactionine (sulfur-to-alpha carbon) crosslinks between a cysteine and an acceptor residue. Hundreds of new sactipeptide biosynthetic gene clusters were uncovered and a novel sactipeptide "huazacin" with growth-suppressive activity against Listeria monocytogenes was characterized. Bioinformatic analysis further suggested that a group of sactipeptide-like peptides heretofore referred to as SCIFFs (six cysteines in forty-five residues) might not be sactipeptides as previously thought. Indeed, the bioinformatically-identified SCIFF peptide "freyrasin" was demonstrated to contain six thioethers linked the beta carbons of six aspartate residues. Another SCIFF, thermocellin, was shown to contain a thioether crosslinked to the gamma carbon of threonine. SCIFFs feature a different paradigm of non-alpha carbon thioether linkages, as they are exclusively formed by radical SAM enzymes, as opposed to the polar chemistry employed during lanthipeptide biosynthesis. Therefore, we propose the renaming of the SCIFF family as radical non-alpha thioether peptides (ranthipeptides) to better distinguish them from the structurally-distinct sactipeptide and lanthipeptide RiPP classes. We anticipate that further exploration of both sactipeptide and ranthipeptide biosynthetic space will yield novel scaffolds as well as reveal enzymological details regarding the radical-mediated installation of peptidic thioether crosslinks including mechanism, substrate recognition, and substrate tolerance.

Poster Number 19

Substrate Recognition in type II lanthipeptide synthetases

presenting author: Imran Rahman

Imran R. Rahman, Jeella Acedo, Wilfred A. van der Donk (Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA)

Biosynthesis of class II lanthipeptides is carried out by bifunctional synthetases, known as LanMs, which perform both Ser/Thr dehydration and subsequent cyclization reactions on a linear precursor peptide to form lanthionine and methyllanthionine linkages. LanM enzymes have been found to be extraordinarily substrate tolerant, and understanding how they recognize and interact with their substrates will facilitate their application in combinatorial biosynthetic platforms. Herein, we show the results of our studies of the role of the conserved residues in class II lanthipeptide leader peptides to determine what parts of their sequences are necessary for recognition by the maturation enzymes. We conducted heterologous expression of alanine variants of selected class II lanthipeptide precursors with their respective LanMs and identified that the C-terminal portion is important for processivity. Additionally, we performed saturation transfer difference (STD) NMR and 2D NMR experiments to identify which residues are interacting strongly with the respective LanM. We further confirmed the role of each of these residues for mediating interactions by fluorescence polarization binding assays. Taken together, we will show that the C-terminal aliphatic and hydrophobic residues are critical for substrate binding by LanM enzymes.

Poster Number 20

Creation of small single ring peptides using the mersacidin modification machinery in *Lactococcus lactis*

presenting author: Jakob Hendrik Viel

Jakob Viel, Oscar Kuipers (University of Groningen)

The use of lanthipeptide modification systems to create new and better molecules is very applicative, especially in regard to its prospects in creating and improving antibiotics and other therapeutics. The freedom of design for the creation of such molecules, is limited by the properties of the enzymes used in the modification process. The modification enzyme MrsM of Class-II lanthipeptide mersacidin, a lantibiotic produced by *Bacillus amyloliquefaciens*, can install notably small and large ring structures. This property makes it a good candidate for the lanthipeptide toolbox. Here, the dehydration and cyclisation activity of MrsM was combined with the mersacidin transporter and leader-protease MrsT. MrsM was expressed with and without MrsT, in combination with a range of small precursor peptides in *L. lactis*. MrsM was found to be functional in *L. lactis*, and when co-expressed with MrsT, the modified peptides can be purified from the supernatant in their bioactive form. These results clear the way for further characterization experiments on MrsM, allowing for purposeful application of the enzyme in the future.

Poster Number 21

An Exploration of the unknown landscape of thioamidated ribosomal peptides in Actinobacteria using RiPPER

presenting author: Javier Santos Aberturas

Javier Santos-Aberturas, Govind Chandra, Luca Frattaruolo, Rodney Lacret, Thu Pham, Natalia M. Vior, Tom H. Eyles, Andrew W. Truman (Department of Molecular Microbiology, John Innes Centre, Norwich, UK)

The rational discovery of new specialized metabolites by genome mining represents a very promising strategy in the quest for new bioactive molecules with invaluable applications in medicine and agriculture. Ribosomally synthesized and post-translationally modified peptides (RiPPs) derive from genetically encoded precursor peptides and may harbour the largest amount of unexplored structural diversity among the main classes of specialized metabolites. However, RiPP gene clusters are particularly refractory to reliable bioinformatic predictions due to the absence of a common biosynthetic feature across all pathways. Here, we describe RiPPER, a new tool for the unbiased identification of RiPP precursor peptides and apply this methodology to search for novel thioamidated RiPPs in Actinobacteria. Thioamidation is an extremely rare post-translational modification that is catalyzed by a pair of proteins (YcaO and TfuA) in archaea. In Actinobacteria, the thioviridamide-like molecules are a family of cytotoxic RiPPs that feature multiple thioamides, and it has been proposed that a YcaO-TfuA pair of proteins also catalyzes their formation. Potential biosynthetic gene clusters encoding YcaO and TfuA protein pairs are common in Actinobacteria but the chemical diversity generated by these pathways is almost completely unexplored. A RiPPER analysis reveals a highly diverse landscape of precursor peptides encoded in previously undescribed gene clusters that are predicted to make thioamidated RiPPs. To illustrate this strategy, we describe the first rational discovery of a new family of thioamidated natural products, the thiovarsolins from Streptomyces varsoviensis.

Poster Number 22

CerR Promotes Cerecidin Production and Immunity in *Bacillus cereus*

presenting author: Jin Zhong

Jin Zhong, Li Zhang, Kunling Teng (Institute of Microbiology, Chinese Academy of Sciences)

Cerecidins are small lantibiotics from Bacillus cereus that were obtained using a semi-in vitro biosynthesis strategy and showed prominent antimicrobial activities against certain Gram-positive bacteria. However, the parental strain *B. cereus* As 1.1846 is incapable of producing cerecidins, most probably due to the transcriptional repression of the cerecidin gene cluster. Located in the cerecidin gene cluster, cerR encodes a putative response regulator protein that belongs to the LuxR family transcriptional regulators. CerR (84 amino acids) contains only a conserved DNA binding domain and lacks a conventional phosphorylation domain, which is rarely found in lantibiotic gene clusters. To investigate its function in cerecidin biosynthesis, cerR was constitutively expressed in B. cereus As 1.1846. Surprisingly, constitutive expression of cerR enabled the production of cerecidins and enhanced self-immunity of *B. cereus* toward cerecidins. Reverse transcription-PCR analysis and electrophoresis mobility shift assays indicated, respectively, that the cer cluster was transcribed in two transcripts (cerAM and cerRTPFE) and that CerR regulated the cerecidin gene cluster directly by binding to the two predicted promoter regions of cerA and cerR. DNase I footprinting experiments further confirmed that CerR specifically bound to the two promoter regions at a conserved inverted repeat sequence that was designated a CerR binding motif (cerR box). The present study demonstrated that CerR, as the first single-domain LuxR family transcriptional regulator, serves as a transcriptional activator in cerecidin biosynthesis and activates the cerecidin gene cluster, which was otherwise cryptic in B. cereus.

Poster Number 23

Generation of New-to-nature Nisin Derivatives with Enhanced Activity by Incorporating Non-canonical Amino Acids

presenting author: Jingjing Deng

Jingjing Deng, Jakob H. Viel, Oscar P. Kuipers (University of Groningen)

Lantibiotics are ribosomally synthesized and post-translationally modified peptide harbouring unusual posttranslationally modified amino acid residues such as lanthionines, methyllanthionines and dehydrated residues. Incorporation of non-canonical amino acids (ncAAs) into Lantibiotics have great potential to achieve broad structural diversity and biological activity. Nisin is the first discovered and the best studied lantibiotic. Besides its well-known food preservative property, nisin is effective against clinically relevant antibiotic-resistant pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant Enterococcus (VRE). In this study, Four methionine analogues (Aha, Hpg, Nle and Eth) were successfully incorporated at four different positions of nisin in *Lactococcus lactis* through force feeding. LC-MS analysis showed high levels of incorporation for all these methionine analogues at defined positions. The antimicrobial activity against MRSA, VRE, *Enterococcus faecium, Bacillus cereus, Listeria monocytogenes, L. lactis* and *Micrococcus flavus* were investigated and three derivatives displayed high antimicrobial activity against *L. monocytogenes, Micrococcus flavus or L. lactis*. Overall, Incorporation of non-canonical amino acid (ncAAs) into nisin display greatly enhanced structural diversity while retaining or even improving their antimicrobial activity against specific pathogens or Gram-positive bacteria. The genetic code of *L. lactis* are expanded by incorporating methionine analogues. Furthermore, the nisin derivatives with Aha and Hpg incorporated can be used to generate nisin conjugates by click chemistry.

Poster Number 24

Bypassing lantibiotic resistance by an effective nisin derivative

presenting author: Julia Schumacher

Zaschke-Kriesche, Lara V. Behrmann, Jens Reiners, Marcel Lagedroste and Sander H.J. Smits (Institute of Biochemistry, Heinrich-Heine-University Duesseldorf, Universitaetsstrasse 1, 40225 Düsseldorf, Germany)

The need for antibiotics is raising and antimicrobial peptides are excellent candidates to fulfill this function. The group of lantibiotics, for example, are active in the nanomolar range and target membranes of mainly Gram-positive bacteria. They bind to lipid II, inhibiting cell growth and in some cases form pores within the bacterial membrane, inducing cell death. Commercial usage of lantibiotics is, however hampered by the presence of gene clusters in human pathogenic strains which, when expressed, confer resistance. The human pathogen *Streptococcus agalactiae* COH1, expresses several lantibiotic resistance proteins resulting in resistance against for example nisin (Khosa et al. 2013).

Here we present a novel lantibiotic nisin variant lacking the last lanthionine ring named CCCCP, which is able to overcome the resistance mediated by the nisin resistance protein (NSR) from *Streptococcus agalactiae*.

We were able to express and purify CCCCP and show that the activity of CCCCP in vivo against *L. lactis* is 20 nM. The strains expressing the nisin resistance protein do not display any resistance against this nisin derivate. Furthermore, we show that CCCCP is still able to form pores in the membranes of *L. lactis*. The lack of resistance is due to the fact that NSR cannot cleave CCCCP as shown by an in vitro cleavage assay and therefore is unable to confer resistance.

S. Khosa, Z. AlKhatib and S. H. J. Smits (2013) NSR from *Streptococcus agalactiae* confers resistance against nisin and is encoded by a conserved nsr operon. Biological chemistry 394, 1543-1549

Poster Number 25

Unveiling of novel lantibiotics from genomic information of microorganisms

presenting author: Kunling Teng

Kunling Teng, Jin Zhong, Yayong Liu, Jian Wang (Institute of Microbiology, Chinese Academy of Sciences)

Lantibiotics are ribosomally synthesized and posttranslationally modified peptides with potent antimicrobial activities. Discovery of novel lantibiotics has been greatly accelerated with the soaring release of genomic information of microorganisms. Bovicin HJ50 is a unique class II lantibiotic producing by *Streptococcus bovis* HJ50 and contains one rare disulfide bridge. By using its precursor BovA as a drive sequence, three representative novel lan loci from *Clostridium perfringens* D str. JGS1721, *Bacillus cereus* As 1.348 and *B. thuringiensis* As 1.013 were identified by PCR screening. The corresponding mature lantibiotics designated perecin, cerecin, thuricin and suicin were obtained by a semi-in-vitro biosynthesis and structurally elucidated to contain a conserved disulfide bridge. Further analysis indicated that the disulfide bridge played a crucial role in maintaining the hydrophobicity of bovicin HJ50, which might facilitate it to exert antimicrobial function. Novel lantibiotic cerecidins were unveiled in Bacillus cereus strain As 1.1846 through genomic mining and PCR screening. The designated cer locus is different from that of conventional class II lantibiotics in that it included seven tandem precursor cerA genes. Two natural variants of cerecidins A1 and A7 were obtained which contained two terminal nonoverlapping thioether rings rarely found in lantibiotics. Both cerecidins A1 and A7 were active against a broad spectrum of Gram-positive bacteria. Cerecidin A7, especially its mutant Dhb13A, showed remarkable efficacy against multidrug-resistant *Staphylococcus aureus* (MDRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), and even *Streptomyces.* Recently, we also discovered new bacteriocins in the metagenome of microbes in fermented vegetables.

Poster Number 26

Antimicrobial resistance in bacterial strains isolated from mixed juice treated or not by high hydrostatic pressure.

presenting author: María José Grande Burgos

MJ. Grande, R. Lucas, J. Rodríguez López, R. P. Pulido, B. Iglesias, A. Parras y A. Galvez. (University of Jaén, Department of Health Sciences)

Introduction: From a microbiological point of view, food safety must not only take into account the presence of pathogenic, spoiling or toxin-producing microorganisms, but also the possibility that the present microbiota acts as a reservoir of genes for resistance to antimicrobial agents. In recent years, interest in ready-to-eat and minimally processed vegetable foods has increased. There are also new ways of consuming traditional products, derived from fruits, vegetables, how are mixtures of vegetable juices and fruits or milk (also known as smoothies), which in addition to improving the taste of raw materials individually allow better use of agricultural products. In the present study, a collection of 88 strains was isolated from mixed juice prepared form broccoli / Bimi broccoli, endive, natural apple juice, parsley, celery, natural lemon juice, water and stevia as a sweetener. After 16S rDNA sequencing, 27% of isolates were identified as *Pseudomonas*, 16% as *Enterobacteriae*, 18% as *Leuconostoc* and 14% as *Bacillus* in addition to other families.

This collection of identified strains were tested for resistance to different antibiotics and biocides and thus detect the presence of multiresistant strains. We were able to verify that from 88 selected strains, 46 were resistant to beta-lactam antibiotics and 40 to tetracyclines. The presence of antibiotic resistance genes and efflux pumps like *ereA*, *ereB, aad, efrB, mdeA, norA, sepA* were also tested. These results show the need for future treatments, in order to determine the resistance genes involved and determine the presence of multiresistant strains in this kind of juice.

Acknowledgements. This work was supported by grant AGL2016-77374-R (MINECO)

Poster Number 27

Identification of antimicrobial RiPPs against undesirable gut targets in Non-alcoholic fatty liver disease: A genome mining approach

presenting author: Maria Victoria Fernández Cantos

María Victoria Fernández Cantos, Diego García de la Morena, Auke J. van Heel, and Oscar P. Kuipers (Department of Molecular Genetics, University of Groningen)

The human gut has a complex microbial population of 100 trillion bacterial and archaeal cells distributed over more than 1000 species. The community is dominated by bacteria, with the predominance of Firmicutes and Bacteroides[1]. Increasing evidence has shed light on the multiple levels of associated interdependence between gut and liver. Consequently, it is not surprising that intestinal microbiome dysbiosis has been associated with different diseases, including Non-alcoholic fatty liver disease (NAFLD). This disease has become the most common liver disease in Western countries and it encompasses a spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis of the liver[2].

The Marie Sklodowska-Curie Innovative Training Network “BestTreat” is a microbiome engineering platform. It aims to investigate the complex interaction between gut microbiome and its host, giving insights about microbiome signatures for risk prediction and monitoring the disease. In parallel, new therapeutics avenues for NAFLD will be developed focusing on narrow spectrum antimicrobial compounds, such as RiPP bacteriocins, produced by GI-tract species against target bacteria. As a first step towards achieving this goal, genome mining of strains closely related to key gut species has been performed using BAGEL4 [3].

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Poster Number 28

Genome mining for ribosomally synthesized and post-translationally modified peptides (RiPPs) in MEDINA microbial collection

presenting author: Marina Sánchez-Hidalgo

Marina Sánchez-Hidalgo, Daniel Oves-Costales, Fernando Román-Hurtado, Olga Genilloud (Fundación MEDINA)

Natural products (NP) are non-essential small molecules produced by plants, microbes and invertebrates. Actinobacteria produce more than 70% of all NP scaffolds used to produce clinically-relevant anti-infective molecules. One class of NP that has gained considerable attention in recent years is the ribosomally synthesized and post-translationally modified peptides (RiPPs). RiPPs can reach a high degree of chemical diversity through extensive post-translational modifications (PTMs) of a precursor peptide that confer them a wide range of activities.

The development of next generation sequencing (NGS) technologies has reinvigorated the search for novel bioactive natural products. Genome sequencing and the continuous development of bioinformatics tools and databases for the automated scanning and annotation of secondary metabolite gene clusters has revealed that actinomycetes and many other bacteria carry the genetic potential to produce many more RiPPs and other secondary metabolites than those that are detected in laboratory conditions.

Fundación MEDINA is a non-profit research organization focused on the discovery of novel bioactive molecules from one the world's largest and most diverse microbial collections and natural products libraries, which cover the broadest chemical space. In this work, we mine the genomes of 27 talented strains from our microbial collection with antiSMASH and BAGEL and describe some of the predicted BGCs that have been associated with their produced RiPPs, as well as other cryptic RiPP BGCs.

Poster Number 29

The spliceases, creating α-keto-β-amino acid containing peptides in an unusual splicing reaction

presenting author: Marjan Verest

Marjan Verest, Jörn Piel (ETH Zürich)

Canonical ribosomally produced proteins consist of α-amino acids, up until recently the only peptides containing β-amino acids have been produced by non-ribosomal peptide synthetases. Spliceases, a novel class of radical S-adenosylmethionine (rSAM) enzymes with a SPASM domain, create α-keto-β-amino acid containing products from ribosomally produced peptides. In an unprecedented reaction, the peptide backbone is spliced at an XYG peptide motif and the equivalent of a tyramine is excised before the peptide sections are reconnected. The first splicease was discovered in the cyanobacterium Pleurocapsa sp. PCC7319, but its homologs are widespread and can be found for example in actinomycetes, archaea, and proteobacteria. The genetic environment of the spliceases is also diverse, making it hard to predict a function for the α-keto-β-amino acid moiety. Precursor peptides range in size from peptide to protein, may contain multiple XYG motifs, and gene clusters can contain multiple precursor peptides. Studying a range of spliceases will give us insights into this diverse class of peptide modifying enzymes.

Poster Number 30

Elucidation of RiPP biosynthesis in the symbiotic cyanobacterium Nostoc punctiforme

presenting author: Martin Baunach

Martin Baunach, Daniel Dehm, Julia Krumbholz, Vincent Wiebach, Roderich Süssmuth, Elke Dittmann (, Department of Microbiology, University of Potsdam, Golm, Germany)

The complex and unique life cycle of the symbiotic, filamentous cyanobacterium Nostoc punctiforme is reflected by the extraordinary high number of biosynthetic gene clusters (BGCs) encoding nonribosomal peptide synthetases, polyketide synthases, and ribosomally produced and posttranslationally modified peptides (RiPPs).[1] However, despite laborious attempts to characterize these BGCs the majority is orphan. This is especially true for the numerous predicted RiPP BGCs, for which no authentic products could be isolated so far.

To uncover the hidden natural product diversity of N. punctiforme a library of transcriptional reporter strains was constructed and screened under varying cultivation conditions.[2] Our data demonstrate that the majority of the cryptic gene clusters is not silent but expressed with regular or sporadic pattern. Cultivation of N. punctiforme using high-density fermentation overrules the spatial control and leads to a pronounced upregulation of more than 50% of the BGCs. This combination of holistic reporter-supported transcriptional analysis together with innovative cultivation techniques led to the discovery of highly unusual variants of the tricyclic ribosomal peptide microviridin that were previously elusive in N. punctiforme.[2,3]

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Poster Number 31

Functional analysis of LanI mediated lantibiotic immunity in Bacillus subtilis.

presenting author: Michael Häsler

Michael Häsler, Christoph Geiger, Sophie Marianne Korn, Peter Kötter and Karl-Dieter Entian (Michael Häsler; Goethe University of Frankfurt)

Lantibiotics subtilin and nisin are produced by Bacillus subtilis and Lactococcus lactis, respectively. To prevent toxicity of its own lantibiotic both bacteria express specific immunity proteins, including lipoproteins SpaI and NisI and ABC-transporters SpaFEG and NisFEG. In previous work peptide release assays showed that the contribution of the ABC-transporter SpaFEG to subtilin immunity is the expulsion of subtilin, most likely from the cytoplasmic membrane into the extracellular medium (Stein et al., 2005). However, little is known about the specific function of lipoproteins SpaI and NisI. Although both proteins are different in size and exhibit considerable differences in their primary sequence, our structural analysis showed that SpaI and NisI share largely the same 3D structure (Christ et al., 2012, Hacker et al., 2015). Interestingly, NisI which has twice the size of SpaI consists of two domains which both have a SpaI like 3D-structure. The 3D- structures, however, did not provide much insight into the molecular function of both lipoproteins.
Using LILBID (laser induced liquid bead ion desorption) mass spectrometry we show that subtilin interacts with SpaI monomers and dimers as well. Expression of either SpaI or NisI in a subtilin non-producing B. subtilis strain showed that both lipoproteins prevented pore-formation in a specific manner for their respective lantibiotic. Mutational analysis unraveled the structural constraints within subtilin that are needed for SpaI mediated immunity. Furthermore, the physiological function of SpaI in lantibiotic immunity could be elucidated.

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Poster Number 32

Fluorescently labeled unnatural analogs of lanthipeptide Cytolysin

presenting author: Nuria Mazo

Nuria Mazo, Imran Rahman, Wilfred A. van der Donk, Gonzalo Jiménez-Osés (University of La Rioja)

Lanthipeptides are ribosomally synthesized and post-translational modified peptides (RiPPs) which contain sulfur cross-linked lanthionine and/or methyllanthionine. These residues are formed by a Michael-type addition reaction of the sulfur atom of a cysteine to a dehydroalanine or dehydrobutyrine previously formed by enzymatic dehydration.

The number of lanthipeptides described has increased during last decades, indicating the great interest these type of antimicrobial peptides generate.

One of the most common methodologies to obtain synthetic lanthipeptides is using bacterial systems (E. Coli) to express them. However, Solid-Phase Peptide Synthesis (SPPS) is an attractive alternative for the preparation of chemically modified variants with an enormous impact over the last decades.

In this work, we have focused on cytolysin, which shows hemolytic activity against eukaryotic cells, as well as antimicrobial properties. We have developed a new SPPS-based methodology to synthesize new fluorescently labeled full-length unnatural modifications of cytolysin S (CylLS) by late-stage fast intramolecular ring-opening of cyclic sulfamidates derived from alpha- and beta-amino acids. The structural properties and antimicrobial and hemolytic activities of these derivatives in conjugation with partner lanthipeptide CylLL are been measured and compared with native CylLS.

Poster Number 33

Expression of single-ringed lanthipeptides with a Novel SyncM-modification system in Lactococcus lactis

presenting author: Patricia Arias

Patricia Arias-Orozco, Oscar Kuipers* (University of Groningen)

Lantitbiotics are ribosomally synthesized peptides that constitute a promising alternative as therapeutics against multidrug bacterial infections (Ongey and Neubauer - 2016). Within this diverse group of natural products, class II lanthipeptides are modified by bifunctional enzymes. A promiscuous class II enzyme was found in a cyanobacterium named *Prochlorococcus* MIT9313 which is able to carry out post-translational modifications on a wide range of different substrates (Y Yu – 2015). Cyanobacteria are gram-negative photosynthetic bacteria and a significant source of secondary metabolites that show many biological activities like antibacterial, anti-inflammatory etc. (Yue Mi et al. 2017). As an example of this a *Synechococcus* strain was found containing 80 different peptides and one single ProcM-like enzyme, working on different topologies of the core peptide. Taking advantage of this lanthipeptide pool and a modification gene cluster, we designed an expression system in *Lactococcus lactis* using a ProcM-like enzyme and synthesized novel engineered single ring peptides, which relatively large rings, based on the Synechococcus lanthipeptide structures. We selected a ProcM-like enzyme, called SyncM from Synechococcus (A Cubillos-Ruiz - 2017) and used wildtype precursors from the same strain to implement the expression system using the nisin promoter. Expressed peptides were analyzed by Tricine-SDS-PAGE and by Matrix assisted laser desorption time of flight MS (MALDI-TOF). Results are encouraging, as 4 different peptides were successfully produced and purified and shown to be modified by the LanM enzyme.

Poster Number 34

Uncovering the diversity of post-translationally modified peptides in cyanobacterial strains from LEGE Culture Collection

presenting author: Raquel Castelo-Branco

Raquel Castelo-Branco, Vitor Vasconcelos, David Fewer, Pedro Nuno Leão (CIIMAR - Interdisciplinary Centre of Marine and Environmental Research of the University of Porto)

Cyanobacteria are a rich source of natural products with novel structures as well as interesting biological activities. One abundant group of bioactive metabolites produced by Cyanobacteria are the Ribosomally synthesized and Post-translationally modified Peptides (RiPPs). Due to the conserved genomic arrangement of genes involved in RiPPs biosynthesis, their biosynthetic pathways can be predicted from genome sequence data making RiPPs particularly suited for genome mining strategies to discover new natural products. In this study, twenty genomes from phylogenetically diverse cyanobacterial strains belonging to Blue Biotechnology and Ecotoxicology Culture Collection (LEGEcc) were analyzed in order to evaluate their biosynthetic potential to produce RiPPs. AntiSMASH analysis identified 63 putative RiPP-encoding biosynthetic gene clusters being more abundant in strains belonging to Nostocales order. The RiPP classes identified include bacteriocins, microviridins, cyanobactins, lasso peptides and lanthipeptides, some of them showing similarity with known RiPPs whilst the majority encodes uncharacterized RiPPs. This genome mining study highlights the potential of LEGEcc as an untapped RiPPs reservoir.

Poster Number 35

Fighting Clostridium spp.: Design of new specific peptides by gene mining and synthetic biology.

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The controversial genus *Clostridium* is formed by Gram-positive, endospore forming bacteria, strictly anaerobic or aerotolerant rod-shaped cells that can be found in soil as well as in intestinal microbiota of animals and humans. Certain species as *C. tetani*, *C. botulinum*, *C. difficile* or *C. perfringens* are important human/animal pathogens which can also produce toxins that play an important role in their pathogenicity. Some of these species, such as *C. difficile*, have been reported as the most common cause of nosocomial diarrhea, resulting in significant morbidity and mortality in hospitalized patients. The resistance of the spores to antibiotics and their side effects on the gut microbiota are related with the emergence of the infection and its relapses. Lantibiotics suppose an innovative alternative for cell growth inhibition due to their dual mechanism of action and low resistance rate. The final goal of this work has been the production of new lantibiotics with high activity and specificity against *Clostridium*. With some exceptions, lantibiotics/bacteriocins are usually active against bacteria close to the producer strains. Therefore, we attempted heterologous expression of putative lantibiotic core peptides identified after genome mining of more than 500 genomes of *Clostridium*. Additionally, we have designed new chimeric lantibiotics, by a combination of some parts of the previous ones with some parts of nisin. They were cloned and expressed using the nisin modification machinery in *Lactococcus lactis*. For all they, the peptide called AMV-10 was the best, displaying a strong activity against all the *Clostridium* strains tested.

Poster Number 36

Efficient in vivo Synthesis of Lasso Peptide Pseudomycoidin Proceeds in the Absence of Leader and Leader Peptidase

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Lasso peptides are widespread in bacteria. They are made from linear ribosomally-synthesized precursor peptides in reactions that include specific proteolytic processing at the leader-core part junction site of the precursor by a dedicated protease recognizing the leader, followed by cyclisation of the newly formed N-terminus of the core part with a side chain of internal aspartic or glutamic residue catalyzed by a lasso cyclase. The resulting branched lasso has a tail that is fixed inside the cycle formed. We characterize a new lasso peptide, pseudomycoidin, encoded by *Bacillus pseudomycoides* DSM 12442. The most surprising and unique feature of pseudomycoidin is that it can be produced in vivo from the core part of precursor by the lasso cyclase in the absence of leader recognizing protease. The absolute simplicity of pseudomycoidin synthesis system shall make it a powerful model to generate pseudomycoidin-based lasso-peptide libraries and to study the poorly understood process of lasso formation. Unlike other known peptides of its class, pseudomycoidin appears to be a branched unthreaded lasso. We detected two additional modifications of pseudomycoidin, phosphorylation of terminal residue that was observed on other lasso peptides, followed by glycosylation, which was not observed heretofore. We speculate that these bulky C-terminal modifications may help maintain the threaded lasso topology of the compound.

Poster Number 37

Probing radical-mediated RiPP maturation

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Sporulation killing factor (SKF) is a ribosomally synthesized and post-translationally modified peptide (RiPP) produced by *Bacillus*. SKF contains a thioether cross-link between the α-carbon at position 40 and the thiol of Cys32, introduced by a member of the radical S-adenosyl-l-methionine (SAM) superfamily, SkfB. Radical SAM enzymes employ a 4Fe-4S cluster to bind and reductively cleave SAM to generate a 5'-deoxyadenosyl radical. SkfB utilizes this radical intermediate to abstract the α-H atom at Met40 to initiate cross-linking. In addition to the cluster that binds SAM, SkfB also has an auxiliary cluster, the function of which is not known. We demonstrate that a substrate analogue with a cyclopropylglycine (CPG) moiety replacing the wild-type Met40 side chain forgoes thioether cross-linking for an alternative radical ring opening of the CPG side chain. The ring opening reaction also takes place with a catalytically inactive SkfB variant in which the auxiliary Fe-S cluster is absent. Therefore, the CPG-containing peptide uncouples H atom abstraction from thioether bond formation, limiting the role of the auxiliary cluster to promoting thioether cross-link formation. CPG proves to be a valuable tool for uncoupling H atom abstraction from peptide modification in RiPP maturases and demonstrates potential to leverage RS enzyme reactivity to create noncanonical amino acids.

Poster Number 38

Directed evolution of lantibiotic synthetases with high throughput selection by circular Strep-tag display on the surface of *Lactococcus lactis*

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Lantibiotics are potent peptide antimicrobials that are ribosomally synthesized and post-translationally modified (RiPPs). Their ribosomal synthesis and enzymatic modifications provide excellent opportunities to design and engineer a large variety of novel antimicrobial compounds. However, one of the achilles heels in the heterologous production of novel or engineered lanthipeptides is the deficiency of dehydration and/or ring formation due to unfavorable amino acid residues in the vicinity of Ser, Thr or Cys residues. To fully exploit the potential of RiPPs as antimicrobial candidates, tailored enzymes for the dehydration of difficult residues or difficult to circularize regions in a lantibiotic are required. Here, we report a novel methodology to select potent and new NisB enzymes using direct evolution of the nisB gene. The selection of these new NisB enzymes is based on the ability to dehydrate peptides, which are difficult to dehydrate for the wild-type NisB, e.g. when unfavorable resides like Asp, Glu or Arg are present in front of the residue to be dehydrated. This was achieved by bacterial-display of linear peptide precursors with a special strep-tag fused to part of the *Lactococcus lactis* PrtP protease which has the LPXTG cell wall-anchoring motif. We introduced the error-prone PCR nisB-mutant library in the designed display system of lanthipeptides, together with a substrate with a lanthione bridged Strep-tag preceded by a 'difficult' residue like Asp in front of Thr. The selection of the improved NisB mutants was performed by a high-throughput novel streptavidin-coupled magnetic bead system. The selection of the new NisB variants is based on the ability to modify the precursor peptide and subsequently display them as mature peptides with a cyclic Strep-tag (which displays a stronger affinity for the streptavidin-coupled magnetic beads than the linear Strep-tag) on the *L. lactis* surface. Thus, we selected several improved variants of NisB that are able to dehydrate Thr residues preceded by an Asp residue.

Poster Number 39

Conotoxins and the Post-translational Modification Mechanism Research

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Conotoxins are a kind of small bioactive peptides ribosomally synthesized, which consist of 10-40 amino acid, include 2-4 disulfides. Many identified conotoxins show diverse physiological activities that target different receptors of the cell membrane, such as ion channels, primarily in neurons. Conotoxins are widely used tools in neuroscience, and have promising therapeutic applications. It is conservatively estimated that there are approximately 50,000 conotoxins could be secreted by different *Conus* species. However, only a few have been developed and utilized to date. Conotoxins are the most diversified molecules, with multiple disulfides and a high frequency of post-translational modifications (such as proteolytic processing, C-terminal amidation, disulfide bond formation, hydroxylation of proline, carboxylation of g-glutamate, cyclization of glutamine, sulfation of tyrosine, and O-glycosylation), which is closely related to the function of conotoxins. Conotoxins are a potential model system for more general understanding of the posttranslational modification of secreted polypeptides. Although, a few studies are focused on the process of conotoxins translation, folding and post-modification, the post-translational modification mechanisms of conotoxins are still not clear and there are far more questions raised than answers provided about possible mechanisms and functions of post-translational modifications in Conus. In recent years, our group has been working on the research of multiple post-translational modifications of conotoxins using the cell-free biosynthesis in vitro to clarify multiply post-translational modification mechanisms, and further broaden the development and utilization of new drugs in neuroscience research, clinical therapy.

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