# Natural Product Discovery and Development in the Genomic Era

# Sunday, January 21

## 12:00 PM - 6:00 PM Registration

Grand Ballroom Foyer, lobby level

## 5:00 PM - 6:00 PM Keynote Speech: Nigel Mouncey, Director Joint Genome Institute "New Voyages to Explore the Natural Product Galaxy"

Salons F-G, lobby level

## 6:00 PM - 7:00 PM Welcome Reception

Salons A-E, lobby level

## 6:00 PM - 8:00 PM Session: PS1: Poster Session 1

Salons A-E, lobby level

**P1** Sonogashira diversification of unprotected halotryptophans, halotryptohan containing tripeptides; and generation of a new to nature bromo-natural product and its diversification in water

*C. Cartmell*[*], *University of St. Andrews, Strathkinness, United Kingdom*

The blending together of synthetic chemistry and natural product biosynthesis provides a potentially powerful route to new natural product analogues[2]. Cystargamide is a structurally interesting lipo-depsi peptide containing a 5-hydroxy tryptophan as well as a 2, 3-epoxydecanoyl fatty acid chain[3]. We envisaged that by installing a sufficiently reactive handle (e.g. a C-Br bond) and developing compatible mild aqueous chemistries, biosynthesis of the halo-cystargamides and its subsequent chemical modification can be achieved. Precursor directed biosynthesis (PDB) provides a great potential for the production of new natural product analogues by exploiting the natural promiscuity of the enzymes involved in the biosynthesis of natural product. Using PDB method, we achieved the incorporation of various chloro/bromo-tryptophans and generated a series of halogenated analogues of cystargamide. 6-Br-cystargamide was subsequently diversified using aqueous cross coupling chemistries such as the Sonogashira reaction to obtain new derivatives of cystargamide. The installation of bromo/chloro handle also provided an excellent analytical handle for investigation of all metabolites by LC-MS/MS. With knowledge gained from the fragmentation of the natural cystargamide, we analysed the $MS^n$ data to identify and characterise the new natural products analogues produced via the Sonogashira reaction[1].

References

1. J. Corr, S. V. Sharma, C. Pubill-Ulldemolins, *et al. Chem Sci.,* **8**, 2039-2046, (2017)

## **P3** Identification and functional analysis of the aspergillic acid gene cluster in *Aspergillus flavus*

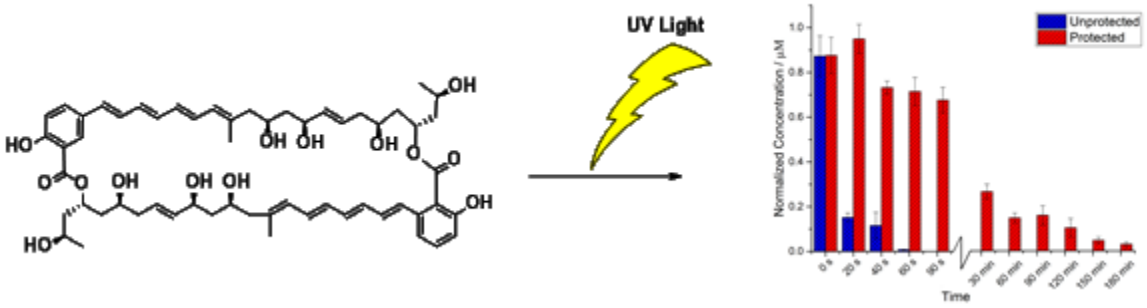
*M. Lebar*, J. Cary, R. Majumdar, C. Carter-Wientjes, B. Mack and Q. Wei, USDA, New Orleans, LA, USA; V. Uka, University of Ghent, Ghent, Belgium; S. DeSaeger, University of Ghent, Gent, Belgium; J. Di Mavungu, University of Gent, Gent, Belgium*

*Aspergillus flavus* can colonize important food staples and produce aflatoxins, a group of toxic and carcinogenic secondary metabolites. *In silico* analysis of the *A. flavus* genome has revealed 56 gene clusters predicted to be involved in the biosynthesis of secondary metabolites. *A. flavus* secondary metabolites produced during infection of maize seed are of particular interest, especially with respect to their roles in the biology of the fungus. A predicted nonribosomal peptide synthetase-like (NRPS-like) gene, designated *asaC* (AFLA_023020), present in the uncharacterized *A. flavus* secondary metabolite gene cluster 11 was shown to be expressed during the earliest stages of maize kernel infection. Cluster 11 was shown to be composed of six additional genes encoding a number of putative decorating enzymes as well as a transporter and transcription factor. LC-MS analysis of extracts from knockout mutants of these genes showed that they were responsible for the synthesis of the previously characterized antimicrobial mycotoxin aspergillic acid. Extracts of the *asaC* mutant showed no production of aspergillic acid or its precursors. Knockout of the cluster P450 oxidoreductase afforded a pyrazinone metabolite, the aspergillic acid precursor deoxyaspergillic acid. The hydroxamic acid functional group in aspergillic acid allows the molecule to bind to iron resulting in the production of a red pigment in *A. flavus* previously identified as ferriaspergillin. We observed significantly less fungal growth on maize kernels infected with *A. flavus* Δ*asaC* compared to *A. flavus* control and are currently investigating the role aspergillic acid plays in maize kernel infection, possibly through iron sequestration.

## **P5** Photoprotection of the potent polyene antibiotic Marinomycin

*C. Bailey*, J. Zarins-Tutt, M. Agbo, M. Gan, E. Abraham, R. Hamed and R. Goss, University of St Andrews, St Andrews, United Kingdom; A.D. Taboada and G. Mackenzie, Sporomex Ltd, Bridlington, United Kingdom; A. Evans, Queen's University, Kingston, ON, Canada*

Bioactive polyenes are abundant in nature showing a broad range of chemical diversity and biological activities. Despite the promising bioactivity of many isolated polyenes, the inherent instability of polyene systems towards photoisomerization continues to deter their development towards potential lead drug candidates. Marinomycin is a particularly noteworthy, but photolabile, polyene antibiotic with excellent activity against MRSA and VRSA.[1] We envisaged that microencapsulating polyenes using a chemically inert, biocompatible, non-toxic natural polymers could be utilised to improve photostability and as a drug carrier. We have demonstrated a striking level of photoprotection (t1/2 extended from 8 seconds – 34 minutes, when exposed directly to 312 nm UV radiation at 48 W) using a natural biopolymer. Our results show promise for the clinical development and administration of polyenes, such as marinomycin, that would otherwise be too unstable to consider. Additionally a serendipitous discovery revealed that our biopolymer could be used to selectively extract culture broths containing marinomycin providing higher recovery than extraction with conventional XAD resins.




**References**

H. C. Kwon, C. A. Kauffman, P. R. Jensen and W. Fenical, *J. Am. Chem. Soc.*, 2006, **128**, 1622–1632.

## **P7** Biochemical role of multicore precursor peptides in a RiPP pathway

*W. Gu*, D. Sardar and E. Schmidt, University of Utah, Salt Lake City, UT, USA*

Cyanobactin pathways are diversity-generating biosynthetic pathways that can process hypervariable substrates. Each cyanobactin precursor peptide contains multiple core sequences encoding different natural products, which is not common in RiPPs. The role of these multicore precursor peptides remains unclear. Preliminary *in vivo* data suggests that the cassette position influences the production yield. Here, we perform biochemical experiments that define the role of multiple cassettes in cyanobactin RiPP biosynthesis. Investigation of the mechanisms behind this recognition will help us better control cyanobactin production for potential therapeutic applications.

## **P9** Biosynthetic and genome mining approaches to identify triazine containing natural products

*K. Almabruk and B. Philmus, Oregon State University, Corvallis, OR, USA; M. Fenwick and S. Ealick, Cornell University, Ithaca, NY, USA*

Triazine containing natural products are of interest because of their attractive biological activities and the unique chemistry involved in their biosynthesis. One of the most studied examples is toxoflavin, which has been isolated from plant pathogenic strains of *Burkholderia*, and plays a key role in virulence. Genome mining has revealed that triazine natural product gene clusters are found in pseudomonads and actinobacteria suggesting that triazine-containing natural products are more prevalent than previously realized. In this study, we present our results from precursor labeling studies in an effort to shed light on the complex biosynthetic steps involved in forming the N-N-bond of the triazine moiety during toxoflavin biosynthesis. We also discuss the crystallographic and the in vitro characterization of two N-methyltransferases that use 1,6-didesmethyltoxoflavin (1,6-DDMT) as a substrate.

## **P11** Metagenomics-guided natural product discovery and engineering in bacteria

*T. Shock, X. Yang*, D. Chandran, J. Shock, O. Liu and J. Kim, Radiant Genomics, Emeryville, CA, USA*

Metagenomics continues to play an increasingly important role in the discovery of natural products. With the combination of next-generation sequencing, bioinformatics, genetic engineering and natural product chemistry, previously-inaccessible chemical diversity in our environment are being gradually uncovered. These technologies also circumvent the constraints of traditional culture-based natural product discovery, allowing for targeted approaches in heterologous hosts. This work focuses on recent progress in pipeline development and process engineering in a startup-scale industrial setting.

## **P13** Autometa: Automated extraction of microbial genomes from shotgun metagenomes

*I. Miller*, E. Rees, J. Ross, I. Miller, J. Baxa, J. Lopera, R. Kerby, F. Rey and J. Kwan, University of Wisconsin - Madison, Madison, WI, USA*

Culture-independent sequencing (metagenomics) is a powerful, high resolution technique enabling the study of microbial communities *in situ*. With modern sequencing technology and bioinformatics, individual genomes can be assembled and extracted directly from environmental samples containing complex microbial communities by a process known as metagenomic "binning." However, available binning programs suffer from methodological and practical shortcomings, such as the requirement of human pattern recognition, which is inherently unscalable, low-throughput, and poorly reproducible. Some methods also require the assembly of pooled samples, which can lead to poor assemblies in the case of inter-sample strain variability. We therefore devised a fully-automated pipeline, termed "Autometa," which incorporates machine learning principles to separate pure microbial genomes from single shotgun metagenomes. Autometa uses Barnes-Hut Stochastic Neighbor Embedding to analyze 5-mer frequency in the contiguous sequences (i.e., "contigs") produced by *de novo* metagenomic assembly. The DBSCAN algorithm is then used to identify groups of contigs (i.e., genome "bins") with congruent 5-mer frequency patterns by optimizing cluster boundaries based on purity of genome bins, measured by the presence of gene markers known to occur as single copies in isolated strains. Clusters are then further expanded using a decision tree classifier. In preliminary tests, Autometa recovered more pure and complete genomes from simulated, synthetic, and environmental metagenomic samples as compared to available programs such as MaxBin, MetaBAT, and MyCC. We will present examples of applying Autometa to study the characteristics of natural product producers in complex microbiomes.

## **P15** Engineering a polyketide synthase for *in vivo* production of adipic acid and beta-ketoadipic acid

*B. Pang*, J. Barajas and J.D. Keasling, Joint BioEnergy Institute, Emeryville, CA, USA*

Polyketides have remarkable structural diversity that mostly arises from combinatorial utilization and template-directed elongation of limited building blocks by type I modular polyketide synthase (PKS). Engineering of these multi-domain enzymes has the potential to produce an enormous variety of novel, rationally designed compounds, including many important commodity chemicals. Here, we engineered a module from borrelidin PKS to synthesize two important dicarboxylic acids, adipic acid and beta-ketoadipic acid (the precursor of levulinic acid), in *E. coli*. To accomplish this, we engineered the reductive loop of first extension module of borrelidin PKS, toward two different functions: by inactivating the native KR domain to afford the beta-keto product, or by swapping the KR domain to a full reductive loop for the saturated product (reported result of our group). For producing the free acids, a thioesterase (TE) was added respectively. Using these two engineered PKSs with succinyl-SNAc as starter unit for *in vitro* reactions, anticipated product was detected correspondingly. Furthermore, in order to produce these products *in vivo* without feeding SNAc, different substrates were tested for the *in vitro* assays and showed succinyl-CoA, an important intermediate in the citric cycle, could substitute succinyl-SNAc as starter unit. Finally, *E. coli* harboring the engineered PKSs produced adipic acid and beta-ketoadipic acid respectively. The engineered PKSs using succinyl-CoA as starter unit have great potentialities of producing more kinds of important dicarboxylic acids through PKS domain swapping and assembly line extension, blazing a new production path for these important commodity chemicals, which mainly originate from petroleum feedstocks currently.

## **P17** Peptide geranylation by an ABBA prenyltransferase

*M. Morita*, D. Sardar, Z. Lin and E. Schmidt, University of Utah, Salt Lake City, UT, USA; Y. Hao and S. Nair, University of Illinois at Urbana-Champaign, Urbana, IL, USA; J. Jokela and K. Sivonen, University of Helsinki, Helsinki, Finland*

Preneylation represents a key matuation step in biosynthesis of cyanobactins, which are a family of ribosomally synthesized and posttranslationally modified peptides (RiPPs). Among cyanobactins, amino acids including Ser/Thr/Trp are often prenylated by DMAPP via forward or reverse prenylation. Although most of them have $C_5$ prenyl groups, piricyclamides from a fresh-water cyanobacterium were proposed to harbor a $C_{10}$ geranyl group. In the biosynthetic pathway of piricyclamides, a gene *pirF* was proposed to encode a putative geranyltransferase; however, the enzymatic function and the compound identity were unknown. Here we characterized PirF as a Tyr *O*-geranyltransferase, synthesized piricyclamide enzymatically, and determined its structure by NMR analyses. Although the synthesized piricyclamide was a mixture of two regioisomers, one of them was identical to what was produced by the cyanobacterium. Beyond Tyr, PirF accepted several phenolic compounds as substrates, suggesting a potential of this enzyme to provide chemical diversity and drug-like property for broad small molecules. In contrast to the broad substrate tolerance, PirF selectively accepted GPP ($C_{10}$) among $C_5$-$C_{20}$ isoprenoid donors, while a homologous enzyme, PagF from another RiPP pathway, accepted DMAPP ($C_5$). To understand a rationale for the donor specificities, we determined the crystal structure of PirF in complex with an analog of GPP. The structural difference between PirF and PagF led us to identify a key residue to select isoprenoid donors. Mutation and kinetic analyses demonstrated that engineered PagF catalyzed geranylation rather than prenylation. This presentation will focus on structure elucidation of piricyclamide, biochemical basis of PirF, and engineering of RiPP prenyltransferases.

## **P19** Gel free targeted cloning of large biosynthetic gene clusters

*D. Mead*, D. Johnson, J. MacDonald, P. Brumm and R. Stankey, Varigen Biosciences, Madison, WI, USA*

The genome sequencing revolution and corresponding development of biosynthetic gene cluster (BGC) prediction and analysis tools (*e.g.*, antiSMASH) have resulted in a wealth of new biosynthetic potential for further examination. Once a BGC of interest is identified, isolating a physical DNA clone for expression, refactoring, and other analyses can be a slow, expensive process. Classical methods of cloning can take months to complete, and gene synthesis is expensive and can be stymied by GC-rich and/or repetitive sequence. Here we describe a rapid technique to directly clone large BGCs from genomic DNA without using gels or agarose plugs. Using CRISPR-Cas9 on intact genomic DNA, we targeted cuts to regions flanking BGCs of interest. Linearized *Streptomyces* BAC expression vectors with overlaps matching the BGC cut sites were prepared using PCR, and the vector and restricted DNA were assembled and transformed. We tested three BGCs from *Streptomyces coelicolor* of sizes of 21, 34, and 59 kb, and achieved successful cloning rates of 83% (5/6), 100% (14/14), and 20% (4/20) respectively. Starting with genomic isolation from a cell pellet, this technique takes ~5 days to generate a verified BGC shuttle vector, which is directly ready for heterologous expression studies. These results indicate that any sequenced biosynthetic gene cluster can be cloned intact from complex genomes and heterologously expressed to produce secondary metabolites, thereby expanding our available resources for natural product discovery.

## **P21** Mining and expressing biosynthetic gene clusters from soil metagenomes

*D. Mead*, Varigen Biosciences, Madison, WI, USA; J. Zhou, A. Pereira and M.R. Liles, Auburn University, Auburn, AL, USA; S. Monsma, Lucigen Corporation, Middleton, WI, USA; K. Degnes and A. Wentzel, SINTEF Materials and Chemistry, Trondheim, Norway*

Soil microorganisms contain vast reservoirs of bioactive natural products; however, the majority of them are recalcitrant to cultivation in the lab. In this study a large-insert soil metagenomic clone library (~110 kb and 19,200 clones) was constructed from an agricultural soil using a broad host range shuttle BAC vector. Pathway containing clones were identified using a 3D NGS pooling method in which plates, rows and columns were separately pooled and sequenced. Contigs were assembled from each pool and bioinformatically screened for secondary metabolite gene clusters using antiSMASH4.0. 593 clones containing a PKS and/or non-ribosomal peptide synthetase pathway among 1,516 total biosynthetic pathways were identified. These pathways are very divergent from known clusters, with the %G+C content varying from 34 to 79% and the nearest BLAST hit of keto-synthase domains ranging from 19 to

95% amino acid identity. New clades of keto-synthase domains were also found. 320 pathway-containing BAC clones were conjugally transferred into *Streptomyces coelicolor* M1154 and screened for the synthesis of antibacterial compounds against methicillin-resistant *Staphylococcus aureus* or the fungal pathogen *Cryptococcus neoformans* or human cancer cell lines, followed by LC/MS characterization of active clones to identify metabolites with bioactivity. The antibiosis hit rate was >10% for the bacterial and fungal screens. These results indicate that highly novel biosynthetic clusters can be cloned intact from complex metagenomes and heterologously expressed to produce secondary metabolites, thereby expanding our available resources or natural product discovery.

## **P23** Combined metabolomic and genomic approaches to antibiotic discovery

*E. Abraham*, Y. Wang and R. Goss, University of St. Andrews, Fife, United Kingdom*

Natural products provide an unparalleled starting point for drug discovery. The majority of natural product compounds in clinical use have been derived from microbial sources. However, as the same highly potent compound can be produced by many different microbes, there is always a risk of rediscovering the same antibiotic using traditional bacterial screening approaches.

However, genome sequencing data have revealed that only a small proportion of microbial biosynthetic capability has been tapped and excitingly there are many more natural products waiting to be discovered.[1,2,3]

We are employing a state-of-the-art approach for antibiotic discovery where we are combining genomics and metabolomics to identify novel antibiotics. We are reading the genomes of actinomycete bacteria to identify signature genes for natural products such as hybrid non-ribosomal peptide synthetases, polyketide synthases and lantibiotics. We then target and clone biosynthetic gene clusters that are very different from known gene clusters, and therefore likely to produce novel antibiotics, for heterologous expression. By identifying and discounting known compounds from further analysis, we focus efforts on pursuing the compounds that are likely to show the highest novelty. Cloning biosynthetic gene clusters can be very challenging and we are therefore also exploring different cloning methods including using cosmid libraries, BAC libraries and Gibson Assembly.

1. R. *Goss et al., Nat. Prod. Rep*., 2017
2. J.S. Zarins-Tutt, E. Abraham *et al*., *Prog. Mol. Subcell. Biol*., 2017
3. C. Bailey, E. Abraham *et al*., in *Chemical Biology of Natural Products*, ed. D. J Newman, G. M. Cragg and P. Grothaus, CRC Press, 2017, chapter 4

## **P25** Secondary metabolite genome mining of the rare actinomycete *Streptoalloteichus sp.* NAI 85712

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*of Science and Technology (KAIST), Daejeon, Korea, Republic of (South); T. Weber, The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby, Denmark*

Bacteria and in particular actinomycetes produce a notable array of bioactive molecules widely used as antibiotics. Due to the decrease of discovery of novel molecules from *Streptomyce*s, rare actinomycetes and rational metabolic engineering approaches represent a source of novel bioactive products with a broad range pharmacological activities. In our study the biosynthetic potential of *Streptoalloteichus sp.* NAI 85712 a rare actinomycetes was investigated. *Streptoalloteichus sp.* NAI 85712 was isolated in screening against Acinetobacter. Ribosomal 16S gene sequencing suggests a close similarity between *Streptoalloteichus sp.* NAI 85712 and *Streptoalloteichus tenebrarius* (99%) the producer strain of several important antibiotic compounds, including apramycin carbamoyl tobramycin and kanamycin B (W. Hong and S. Yan 2012). Whole-genome sequencing of *Streptoalloteichus sp.* NAI 85712 was performed by using PacBio and Illumina sequencing. The draft genome was annotated by using the automatic annotation pipeline Prokka. Additional functional annotation was performed using the RASTtk server. A total of 6656 coding sequences (CDSs), with 6,569 predicted genes, 71 tRNA genes, 15 rRNA operons, 1 tmRNA and a G+C content of 72.4%. A genome mining analysis showed that *Streptoalloteichus sp.* genome contains 29 biosynthetic gene clusters (BGCs), including two non-ribosomal peptide synthetase (NRPS) BGC, one type I polyketide synthase (PKS) BGC, two type II PKS BGCs, one type III PKS, two hybrid PKS-NRPS and three Aminoglycoside BGCs. A hybrid NRPS-PKS showed 72% similarity to gene cluster for caerulomycin biosynthesis in *Actinoalloteichus cyanogriseus* WH1-2216-6. The production of aminoglycosides and caerulomycin was detected by HPLC and by growth inhibition assay.

## **P27** Expansion of chemical space in saccharothiorides: Precursor-directed *in situ* synthesis (PDSS) in a rare actinomycete *Saccharothrix* sp.

*S. Lu, S. Nishimura and H. Kakeya*[*]*, Kyoto University, Kyoto, Japan; M. Ito and T. Kato, MicroBioPharm Japan Co., Ltd., Shizuoka, Japan*

Rare actinomycets are expected to be a rich source of natural products with novel chemical structures and biological activities. In our screening program for microbial metabolites, we discovered novel 10-membered macrolides from a rare actinomycete *Saccharothrix* sp. A1506, designated as saccharothriolides A-F.[1,2] A structure-activity relationship (SAR) study revealed that the substituent at C-7 in saccharothriolides affects cytotoxicity towards human fibrosarcoma HT1080 cells.

To obtain further insights into the SAR on saccharothriolides, we developed a precursor-directed *in situ* synthesis (PDSS) method for generating analogs. PDSS method combines biosynthesis, which routinely generates complex precursors, and *in situ* synthesis by Michael reaction, which allows an unlimited range of simple nucleophilic reagents. This simplicity of PDSS is attractive to generate novel “unnatural products”, as it does not necessarily require identification of the biosynthetic gene cluster and purification of precursors. Four methoxyaniline-substituted analogs and aminophenol-substituted analog at C-7 (saccharothriolides G-K) were synthesized by using the PDSS method.[3,4] All chemical structures were assigned by detailed spectroscopic analysis, and the absolute configurations were established on the basis of CD spectra analysis or optical rotation. The SAR study using saccharothriolides G-K confirmed the importance of the OH substituent at C-2” for cytotoxicity in HT1080 cells.

We will also present successful isolation of a predicted common biosynthetic precursor pre-saccharothriolide X for saccharothriolides A-C.

Lu, S. *et al*. *Chem. Commun*. 51, 8074 (2015); 2) Lu, S. *et al. J. Nat. Prod.* 79, 1891 (2016); 3) Lu, S. *et al*. *J. Antibiot*. 70, 718 (2017); 4) Lu, S. *et al*. submitted.

## **P29** Cell-free extracts of *Serratia marcescens* as seed treatments for control of the oomycete plant pathogen *Pythium ultimum*

*D. Roberts*[*]*, L. McKenna, J. Buyer, K. Selmer and J. Maul, USDA-ARS, Sustainable Agricultural Systems Laboratory, Beltsville, MD, USA*

We are developing natural products for control of soil-borne plant pathogens as environmentally benign disease control tactics are needed for sustainable agriculture. Prior work demonstrated that seed

treatment with cell-free ethanol extracts of *Serratia marcescens* isolate N4-5 were as effective as the commercial pesticide Thiram for control of damping-off of cucumber caused by the oomycete pathogen *Pythium ultimum* in potting mix and several natural soils. A combined genetic and biochemical approach is being used to identify the compounds in these ethanol extracts responsible for disease control. Ethanol extracts of eight mini-Tn5 Km mutants of isolate N4-5 were determined to be reduced, or deficient, in control of damping-off of cucumber caused by this pathogen in growth chamber assays. These mutants have been sequenced and this sequence is being used to identify genes involved in biosynthesis of compounds involved in disease control. Additionally, TLC-MS and LC-MS is being used to characterize the metabolite profiles of ethanol extracts from isolate N4-5 and the mutants. Ethanol extracts from isolate N4-5 contain a large amount of prodigiosin and several other compounds. Purified prodigiosin when applied as a seed treatment does not control this disease.

## **P31** Investigating the regiochemistry of an unusual bacterial Acyl-CoA dehydrogenase

*J. Blake-Hedges*, J. Chen and R. Krishna, University of California, Berkeley, Berkeley, CA, USA; J.H. Pereira, L. Chan, D.C.J. Petzold, D.L. Katz, D.P.D. Adams and J.D. Keasling, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Acyl-CoA Dehydrogenases (ACADs) are a large family of flavoenzymes that catalyze the oxidation of fully saturated acyl-CoA thioesters to their α,β-unsaturated counterparts. Although most notably involved in fatty acid β-oxidation processes, ACADs are also implicated in the biosynthesis of certain bacterial secondary metabolites. The work presented here details the characterization of a bacterial ACAD, TcsD, involved in the biosynthesis of the clinically-relevant *Streptomyces* polyketide metabolite FK506 (tacrolimus). Specifically, TcsD catalyzes the formation of the γ,δ-olefin of the extender unit allylmalonyl-CoA which is incorporated into the FK506 polyketide scaffold at the C21 position. Herein, we interrogate the unusual regiochemistry of this transformation. We present biochemical data from experiments using natural substrates and substrate analogs to probe the mechanism of TcsD. In addition, we report high resolution (1.5 Angstroms) structural data from the first solved crystal structure of this enzyme. We use this combination of biochemical and structural data to propose a logical mechanism by which TcsD controls this novel ACAD activity.

## **P33** Towards the discovery and characterization of novel stress-sensitizing natural product anthelmintics.

*E.Y. Song*, J. Knox, Y. Jiang, J. Wang and P.J. Roy, University of Toronto, Department of Molecular Genetics, Toronto, ON, Canada; J. Nodwell, University of Toronto, Department of Biochemistry, Toronto, ON, Canada*

The viability of an individual is contingent on its ability to overcome stress. Parasitic worms, which infect over 25% of all humans and common livestock species, are no different. During infection of vertebrate hosts, parasites must successfully circumvent challenges such as the release of reactive oxygen species by the host's immune system and increased temperatures in the host's body. With the rise of helminths resistant to currently used anthelmintics, new drugs are needed to lessen the burden on the global economy and public health. We have produced and screened a custom bacterial extract library containing uncharacterized natural products to identify novel molecules that suppress parasites' ability to thrive in stressful environments. Streptomycetes are known to produce many potent antibiotics, antifungals, and anthelmintics; however, the cryptic secondary metabolome of streptomycetes remains relatively uncharacterized. Here, we aim to exploit this rich source of bioactive molecules by chemical induction of cryptic secondary metabolism. We have screened these induced extracts for suppression of GFP reporters in the free-living nematode *Caenorhabditis elegans* that are expressed in response to various stressors. Our validated hits are likely to be cryptic secondary metabolites with bioactivity in the worm in

the context of a specific stress response mechanism. Not only will these hits be powerful small molecule tools, but will also be lead molecules for anthelmintics that decrease parasites' ability to respond to stress. We will purify and characterize the natural product(s) responsible for this effect, and elucidate their mechanism of action.

## **P35** Genetic optimization of multi-gene natural product biosynthesis

*S.Y. Hsu*, University of Minnesota-Twin Cities, Minneapolis, MN, USA*

Natural product as the resource for drug discovery was thought to be depleted until the bacterial genome sequencing data tells us otherwise. It has been shown that bacterial genomes harbor unprecedented amount of biosynthetic gene clusters (BGCs) potentially encoding novel compounds. However, tuning expression of these large BGCs in a heterologous host require tedious optimization on a case-by-case basis. I will present a high-throughput DNA assembly pipeline for high GC organisms aiming to rapidly reconstitute BGCs by refactoring and assemble DNA fragments. As a proof of concept, a small library of synthetic gene clusters was constructed to encode *ent*-atisanoic acid (eAA), a late-stage intermediate of a neuroprotectant. We successfully controlled the relative expression level of individual genes, identified the tailoring enzymes required for the oxidation as well as demonstrated the utility of this DNA assembly pipeline. Next, we rationally optimized isoprenoid biosynthesis by perturbing relative expression of eight enzymes in the methylerythritol phosphate (MEP) pathway. One of the mathematical optimization principles called Plackett-Burman design was used to guide the optimization effort for this eight-gene system. Total of 125 synthetic gene clusters encoding MEP pathway is required to fully screen the effects of expression of each gene on the output measured by isoprenoid titer. Preliminary analysis showed that the eAA production titer was improved from 50 mg/L to ~500 mg/L from the library constructed with DoE principle. In sum, the DNA assembly pipeline will become a powerful tool to fuel future rational optimization efforts of multi-gene systems, including large BGCs.

## **P37** Colorants production by filamentous fungi: a statistical study

*B.R. Zaccarim, F. de Oliveira and V.D.C. Santos-Ebinuma*, Univ. Estadual Paulista-UNESP, Araraquara, Brazil; M.F.S. Teixeira, Universidade Federal do Amazonas, Manaus, Brazil*

*Talaromyces amestolkiae* produces berkelic acid, mitorubrin, mitorubrinic acid, mitorubrinol, *Monascus* red azaphilone pigments, pestalacin A, a purpactin and vermicellin. These red colorants are of interest for the industry as they are stable and non-toxic and can be used as food colorants. So, considering the industrial importance of natural colorants, this work study the variation of nutrients from culture media and pH through three factorial designs aiming to increase the production of natural colorants by *T. amestolkiae* in orbital shaker. The influence of the independent variables, glucose, meat peptone, meat extract and pH, on the response red colorants production was investigated. Firstly, experiments were carried out employing a $2^4$ full factorial design. From this factorial design, pH and meat peptone were the most significant effects on colorants production. Secondly, two $2^2$ central composite designs were performed to evaluate the influence of pH and meat peptone in the production of red colorants while glucose and meat extract were kept constant at 30 g/L and 1 g/L, respectively. Medium improvement by factorial designs reached a red colorants production of $4.61UA_{490nm}$ which represents about 3-fold increase in red colorants absorbance compared to initial conditions. In conclusion, the results have shown that *T. amestolkiae* can be an effective producer of natural colorants and further experiments in bioreactor will be performed to establish it at industrial scale.

## **P39** Multi-OMICS analysis for the discovery of novel secondary metabolites

*H. Otani* and S. Deutsch, DOE Joint Genome Institute, Walnut Creek, CA, USA; K. Louie, B. Bowen and T. Northen, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

Actinobacteria are rich sources of bioactive compounds such as antibiotics. Recent advances in genomic sequencing and bioinformatics have revealed that this group of bacteria have the potential to produce a much higher number of chemical compounds than those already discovered, indicating that the majority

of biosynthetic pathways are silent under standard laboratory growth conditions. It has been hypothesized that biosynthetic gene cluster activation requires environmental cues such as stress or presence of other organisms.
Here, we used co-cultivation approach to activate such silent biosynthetic pathways. Of >100 Actinobacterial strains that we have recently sequenced, we selected 5 species based on their phylogenetic distribution and the number of predicted biosynthetic gene clusters that they harbor. Comparative exometabolome analysis using LC-MS revealed overproduction of 34 metabolites specifically under the co-culture. Molecular network analysis based on MS/MS fragmentation patterns, classified these metabolites into 15 groups. Subsequent database search revealed that the majority of the metabolites are likely to correspond to yet unidentified compounds. Overlaying transcriptome analysis on the same co-cultures using RNA-sequencing has the potential of revealing biosynthetic gene clusters associated with the novel compounds. We have already observed the up-regulation of an NRPS biosynthetic gene cluster specifically under the co-culture conditions at which one of the compounds was produced. Additional RNAseq experiments are being performed to increase the number of cluster-molecule associations.

## **P41** Evaluating engineering bottlenecks in polyketide synthase reductive loop swaps to generate biofuels and bioproducts

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The biosynthetic logic of polyketide synthases (PKSs) has traditionally been harnessed to generate a diverse range of bioactive therapeutic natural products. However, due to the ability to precisely tailor molecular structure through this biosynthetic logic, they have great potential for synthetic biology applications in other areas, which have been largely underexplored. One of these applications is to generate biofuels and bioproducts, however many biofuels and bioproducts contain saturated carbon backbones, whereas many tractable PKSs do not harbor a full casette of reductive domains. Thus, to generate these valuable molecules, the ability to reliably toggle between organizations of reductive domains is of high utility. To evaluate which features are important for reductive loop exchanges, we systematically performed reductive loop swaps on a model PKS, the first module of the lipomycin PKS fused to the erythromycin TE (LipPKS). To tease apart which features are important for successful reductive loop swaps, we evaluated the substrate structure and its impact on incomplete reductive “stalling,” as well as the stereochemical distribution of stalled products. This led us to gain a better understanding how to successfully engineer expanded reductive domains in a PKS architecture which natively only harbors a ketoreductase domain.

## **P43** Inducible actinomycin heterocycle causes changes in activity and shown in multiple actinomycin families

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The actinomycin scaffold has been well studied for its ability to bind DNA and interfere with mRNA production. Since the discovery of the parent compound, Actinomycin D, in 1964, several other families of actinomycins have been discovered. Some of these contain β-ring heterocycles and other rearrangements to the β-ring that drastically change the biological activity of these compounds. However, it remains unknown how such rearrangements occur *in vivo*. By investigating two similar families of actinomycins—Z-type and Y-type—we have shown progress towards inducing these activity-changing rearrangements *in vitro* as well offer a hypothesis for how such a rearrangement would affect the activity of these compounds.

## **P45** Natural product biosynthesis in lichen fungi part 1: Annotation of hidden biosynthetic gene clusters

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Lichen are a symbiotic association between a fungal partner (mycobiont) and an algal partner (photobiont). Lichen have evolved the ability to produce a wide array of biologically active natural products and are an underdeveloped source of new bioactive secondary metabolites. For example, usnic acid is a well-known natural polyketide produced by numerous strains of lichen and has been shown to have a wide array of biological activity with applications ranging from potential new therapeutics to materials science. We have recently sequenced the genome of the fungal partner of the lichen *Cladonia uncialis* and have identified the gene cluster responsible for usnic acid biosynthesis. In addition, we have identified a gene cluster responsible for the biosynthesis of a halogenated isocoumarin polyketide. We have also annotated an additional 28 gene clusters that appear to code for the production of polyketide natural products. We will describe the assignment, by homology to known genes from non-lichen fungi, of function to several of these gene clusters. However, we will also describe the large number of biosynthetic gene clusters that appear to have no known homology to previously reported genes. This suggests that these gene clusters may code for the biosynthesis of novel natural products. These results have led us to conclude that lichen contain the potential for a diverse number of new chemical structures which may provide lead compounds for development into new therapeutics and other applications.

## **P47** Biochemical characterization of oxime formation during phosphonocystoximic acid biosynthesis

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Phosphonocystoximate (PnCys) is a natural product containing an unusual oxime moiety produced by *Streptomyces* sp. S-481. The hydroxylated congener, hydroxyphosphonocystoximate (hPnCys), is produced by *Streptomyces regensis* NRRL WC-3744. We proposed that a Fe(II)- and 2-oxoglutarate-dependent dioxygenase (HpxV) within the hPnCys gene cluster, but absent in the PnCys gene cluster, forms the hydroxylated product. Characterization of HpxV *in vitro* revealed that the likely substrate for this enzyme is 2-aminoethylphosphonate (2AEPn), an early intermediate in the biosynthesis of hPnCys. HpxV hydroxylates 2AEPn, forming (*S*)-1-hydroxy-2AEPn (1H2AEPn). Bioinformatics analyses of the PnCys and hPnCys gene clusters identified PcxL (S-481) and HpxL (WC-3744) as putative FAD-NAD(P)H-dependent oxygenases that share little homology to known oxime-forming enzymes. Because S-481 lacks an HpxV homolog, we hypothesized that 2AEPn would be the substrate for PcxL, while (*S*)-1H2AEPn would be the substrate for HpxL. Surprisingly, *in vitro* both HpxL and PcxL preferentially catalyzed the *N*-oxygenation of 1H2AEPn over 2AEPn, as evident by the $K_M$ of the enzyme being ten-fold lower for 1H2AEPn. The enzymes also showed different substrate preferences when using enantiomerically pure (*S*)- or (*R*)-1H2AEPn as substrate. HpxL, as expected, preferentially oxidized (*S*)-1H2AEPn, the product of HpxV; however, PcxL preferentially oxidized (*R*)-1H2AEPn. Both *N*-oxidases, whether using 2AEPn or 1H2AEPn as substrate, generate three products in vitro: the (*E*)- and (*Z*)-aldoxime as well as a product containing a nitro functional group. The *N*-oxidases also have broad substrate tolerance, as they can oxidize the amine of eight other phosphonic acids containing a primary amine, as well as phosphoethanolamine and aspartic acid.

## **P49** Application of antifungal polyene post-PKS biosynthesis in rare actinomycetes *Pseudonocardia autotrophica*

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Polyene macrolides including nystatin A1 and amphotericin B have been one of the major antifungal antibiotics for several decades. However, its therapeutic application has been restricted due to severe side effects including nephrotoxicity. To overcome these limitations, various chemical and biological studies to modify the polyene structure have been conducted to develop less-toxic polyene antifungals. In our previous works, NPP (Nystatin-like *Pseudonocardia* Polyene) A1, harboring a unique di-sugar moiety named mycosaminyl-*N*-acetyl-glucosamine was identified to exhibit higher solubility and reduced hemolytic toxicity compared to nystatin A1. Through engineering of post-PKS modification steps, we developed two novel NPP A1 analogues, NPP A2 by *nppL* inactivation and NPP A3 by *nppY* replacement to *nypY*. To verify therapeutic indexes of these analogues, novel crystallization recovery methods were developed. NPP A2 exhibited 2 times higher antifungal activity and 1.8 times higher hemolytic toxicity than those of NPP A1, highlighting the importance of C10 hydroxylation toward the pharmacological activities of disaccharide-containing polyenes. Meanwhile, NPP A3 showed reduced *in-vitro* antifungal activity than nystatin A1 and exhibited higher nephrotoxical activities against human hepatocytes, suggesting that not only the number of sugar residues but also the kind of extended second sugar moiety could affect biological activities of polyene macrolides.

## **P51** Biosynthesis and sustainable supplies of the potent anti-proliferative agent Lasonolide A

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Lasonolide A (LSA), isolated from the Caribbean marine sponge Forcepia, potently inhibits the cell growth at nanomolar to picomolar concentrations of breast, leukemia, lung, melanoma and prostate cancers with a unique mode of action that is distinctly different from current therapeutics. LSA contains an interesting chemistry including a macrolide ring, highly substituted dual tetrahydropyran rings, diene, stereochemical centers and an exomethylene containing side chain. LSA presents low levels in the sponge (~5 mg per kilo gram of fresh sponge) and sponge itself is rare and known to occur in two places. The chemical synthesis of LSA is either tedious, long or complicated with low overall yields and is yet economically feasible for sustainable supply of LSA. The structure of LSA strongly suggests its origin from microorganisms associated with the sponge. The potency and unprecedent mode of action suggest LSA as a promising new anticancer drug candidate. NCI has expressed strong interest in conducting preclinical evaluation of LSA. However, the limited supply due to natural scarcity and the lack of an economically feasible synthesis has prevented it from such evaluation studies. Here, we report our attempt to address the supply of LSA via identifying and expressing the biosynthetic pathway of LSA. A metagenomic library from the sponge Forcepia has been constructed and screened extensively. We have explored the LSA biosynthetic routes based on sequence data obtained from cloned fosmids. In addition to metagenomic approach, screening of microorganisms derived from the Forcepia sponge indicates a probable production of LSA by cultivable microbes.

## **P53** Human-associated streptomycetes – novel biotope, novel perspectives for bioactive compounds isolation

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Recently, numerous studies of human microbiome suggest that streptomycetes are common colonizers of human bodies - the respiratory tract, skin, uterus. Their function is unclear, however, some works show correlation of their incidence with chronic inflammatory diseases - psoriasis and chronic obstacle pulmonary disease. Generally, they are viewed as colonizers, perhaps able to control the microbiome

composition due to their secondary metabolites. Only two species cause actinomycetoma (*S. somaliensis, S. sudanensis*).
We suppose that colonization of human tissues requires specific metabolic adaptations. In collaboration with clinical laboratories we have collected several tens of human-associated streptomycetes. Most of them come from the lower respiratory tract, mostly from people with chronic respiratory diseases, and were isolated as accompanying microbes during the selective cultivation for mycobacteria. Taxonomically they fall into various species.

Basic characterization of the strains aimed in detection of specific factors or metabolites produced by the strains that can either modulate behavior of human cells (e.g. suppress the immune response) or affect other respiratory flora or pathogens. Our data from coculture experiments with human immune cells and respiratory pathogens suggest their anti-inflammatory effects, production of potent antibiotics, sometimes with highly species-specific activities, and also of factors affecting behavior of pathogens. These were best documented in interactions with *Pseudomonas aeruginosa* (PSAE), where some increase its hemolytic activities, motility and antibiotic production of PSAE. Most of the human-associated streptomycetes are strongly β-hemolytic, with remarkably higher incidence of β-hemolytic features than in soil-originated strains.

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## **P55** Novel biosynthetic genes from the streptomycete secondary metabolism as genetic tags in screening for new producers

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Searching for new antibiotics to combat multi-resistant human pathogens is of a high priority. Microbial natural products are an immense source for novel therapeutics - antiinfectives, anti-inflammatory, and immunosuppresive drugs. Analyses of actinomycete genomes revealed an amazing number of gene clusters potentially governing biosynthesis of secondary metabolites not discovered by conventional bioactivity screening.
We have built up an actinomycete strain collection from various environments (nearly 2000 strains), in which we sought for the presence of new biosynthetic clusters. Here we present a genome-guided natural products discovery method, combining PCR screening and genome mining. It is based on the diversity and phylogeny of genes encoding cyclizing 5-aminolevulinic acid synthases (cALASs, *hem*A gene products) and genes for a subgroup of polyketide synthases producing short carbon-chains in streptomycetes-related strains. The first gene is directly connected with the production of secondary metabolites carrying the C5N unit, 2-amino-3-hydroxycyclopent-2-enone, with biological activities attractive for future use in medicine and agriculture. PCR screening revealed 226 *hem*A gene-carrying strains from 1,500 tested, with 87% putatively encoding cALAS. Phyllogenetic analysis of *hem*A homologs revealed a strain clustering according to putative types of metabolic product, which could be used to select producers of specific C5N compound classes. Supporting information was acquired through analysis of actinomycete genomic sequence data available in GenBank and further genetic or metabolic characterization of selected strains.

Our results underline the importance of environmental and evolutionary data in the design of efficient techniques for identification of novel producers.

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## **P57** Genome mining of the *Streptomyces* sp. and prospecting for natural products related to antimicrobial activity

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Actinobacteria are known as important producers of natural products with bioactive properties, such as antimicrobials, antitumorals, antivirals, among others. Bacteria of the genus *Streptomyces* are responsible for the production of several classes of antimicrobials. The aims of this study were to use the genome mining strategy to prospect the production of natural products associated with the antimicrobial activity of the *Streptomyces* sp. isolated from Antarctica. The bacterium was isolated from *Salp* sp., collected in King George Island, Antarctica. The phylogenetic analysis of the 16S rRNA gene related the bacterium to the genus *Streptomyces*. Crude extracts were obtained after 30 days of cultivation, using ethyl acetate solvent, and tested for antimicrobial activity and inhibited Gram-positive, Gram-negative bacteria and *Candida albicans*, with MIC hanging from 0.25 to 2.0 $mg.mL^{-1}$. The genome sequencing was performed using HiSeq2500 system and assembled resulting in sequences with a total length of 6,968,465 bp in 49 contigs. The analysis of secondary metabolites by antiSMASH indicated 28 clusters, 3 related to antifungal and antibacterial. The NaPDoS tool indicated 33 clusters associated to the KS domain and 51 associated to the C domain, among them, 5 antibacterial and 2 antifungals. The BAGEL tool allowed to find biosynthetic pathways related to bacteriocins, lathinpeptide and thiopeptide. The potential compounds indicated in the genomic analysis, such as Tylosin and Candicidin, effective against Gram-positive bacteria and *Candida albicans*, respectively, corroborate with the activity found in the antimicrobial assays. This study shows the potential of Antactica *Streptomyces* sp. to produce natural products.

## **P59** Enzyme oxidation of different proanthocyanidin-derived phenolic flavan-3-ol-adducts can inhibit biofilm-forming properties of pathogen and non-pathogens bacteria

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Biofilm-producing bacteria are associated with infectious pathologies that cause great health concern. Many of these bacteria increase their virulence and survival thanks to the formation of this mechanical barrier. However, it should be noted that certain beneficial bacteria may also generate biofilms of high stability. An example of such property are certain *Lactobacilli* strains, commonly used in the food industry as probiotics. Therefore, a major challenge is to look for agents (or combinations thereof) that selectively regulate the phenotype in pathogens and non-pathogens. In this work we prepared phenolic flavan-3-ol-adducts from natural proanthocyanidins as building blocks for the synthesis of new theaflavin-like and thearubigin-like oxidation products that can modulate biofilm formation by *Listeria monocytogenes, Pseudomonas aeruginosa, Helicobacter pylori* and *Staphyloccocus aureus* or to promote biofilm formation in non pathogen bacteria such as *Lactobacillus sp*. Semi-synthetic phenolic flavan-3-ol adducts were oxidized through enzymatic and non-enzymatic strategies. Further, bio-active oxidized products were purified by chromatographic methods (CPC, HPLC). Oxidation process was investigated by EPR and HPLC-ESI-MS/MS methods. Biofilm inhibitory properties were tested using pathogen and non pathogen bacteria with crystal violet and resazurin assays. Pyrogallol and resorcinol-derived adducts inhibit biofilm formation in a concentration-dependent manner, particularly in pathogenic bacteria. Interestingly, *E. coli K-12* (non-pathogen) seem to be unaffected by these compounds. Moreover, biofilm-formation in *Lactobacillus fermentum* was promoted. Therefore, these oxidation moieties are a Hit and could be the starting point to isolate new drug candidate and leads molecules with adequate drug-likeness to be used as adjuvant for modulate biofilm production.

## **P61** Genetic toolbox for reliable gene expression and efficient recombineering in *Pseudomonas putida* KT2440

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*Pseudomonas putida* is a promising bacterial host for producing natural products, such as polyketides and nonribosomal peptides. In order to efficiently engineer *P. putida*, researchers need a genetic toolbox consisting of plasmids and/or chromosomal integration systems, characterized promoters, and a technique for editing the genome. Past reports have described several constitutive promoter libraries, a

suite of broad host range plasmids that replicate in *P. putida*, and several genome editing methods. We have characterized a set of inducible promoters and discovered that IPTG-inducible promoter systems have poor dynamic range due to overexpression of the LacI repressor. By replacing the promoter driving lacI expression with weaker promoters, we increased the dynamic range of IPTG-inducible promoters in *P. putida* KT2440. Upon discovering that gene expression from a plasmid is unpredictable when using a high copy mutant of the BBR1 origin, we determined the copy numbers of several broad host range origins and found that plasmid copy numbers are significantly higher in *P. putida* KT2440 than in *E. coli*. Lastly, we have developed a λRed/Cas9 recombineering method in *P. putida* KT2440 using the genetic tools that we characterized. We generated four scarless deletions, two of which we were unable to create previously using other genome editing techniques.

## **P63** Bacterium-triggered remodeling of fungal chromatin identifies BasR, a novel key regulator of fungal natural product biosynthesis

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The genera *Streptomyces* and *Aspergillus* are known for their variety of secondary metabolites (SMs), which possess a multitude of functions. Among the bacteria of particular interest are *Streptomyces rapamycinicus* (NRRL 5491) and *Streptomyces iranensis* HM35 (DSM 41954). We could demonstrate that these microorganisms specifically induce SM formation in filamentous fungi, *e.g.* the orsellinic acid (*ors*) gene cluster in the model fungus *Aspergillus nidulans* [1,2] and the fumicycline gene cluster in the human pathogenic fungus *Aspergillus fumigatus* [1,3]. Previously, we discovered that this induction was dependent on the GcnE histone acetyltransferase catalyzing histone H3 acetylation [4]. To study regulatory mechanisms underlying this interspecies crosstalk we carried out a genome-wide analysis of chromatin acetylation changes during this microbial interaction and related these to changes in the fungal transcriptome. Our results reveal that only a functional interaction with *S. rapamycinicus* changes the chromatin landscape and activates amino acid cross-pathway control in the fungus [5]. We identified the Myb-type like transcription factor BasR as a novel regulatory node required for bacteria-triggered SM production and show that its function is conserved in other *Aspergillus* species [5].
Also, by generation of a Tn5-based transposon mutant library of *S. iranensis*, we have obtained first hints on possible bacterial genetic elements involved in triggering the silent gene clusters in *A. nidulans*.

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## **P65** Mining *Streptacidiphilus* species as a potential source of novel bioactive natural products

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Actinomycetes produce some two thirds of all known antibiotics, and of those the majority is produced by members of the genus *Streptomyces*. While Streptomyces is a very large polyphyletic genus, the other members of the *Streptomycetaceae*, *Kitasatospora* and *Streptacidiphilus*, are far less well characterised.

In this work we explore *Streptacidiphilus*, which was taxonomically characterised in 2003, as a potential source of new antimicrobials. Research on acidophilic actinomycetes, including *Streptacidiphilus*, has shown that they are great potential sources of novel secondary metabolites, including antifungal agents. We obtained over 40 *Streptacidiphilus* species from wood decay samples, three of which have been sequenced. In addition to these three species, five other strains were selected based on their antimicrobial activity. An antifungal assay based on a luciferase reporter was developed to screen for molecules that target the fungal cell wall, using *Aspergillus niger* as the target strain. Initial screening of the antifungal activity of the extracts of the selected *Streptacidiphilus* revealed various activities, which were dereplicated using LC-UV-MS/MS. Based on LC-MS and NMR data, *Streptacidiphilus* strain P02-A3a produced a family of compounds with strong antifungal activity that had not been described previously. The strain was then grown under different culturing conditions to optimise the yield of its natural products, and the optimised cultivation condition was used for scaling up and further purification of the molecules. Our data on the phylogeny and metabolic biodiversity of the new *Streptacidiphilus* strains will be presented, including our most recent data on a novel family of antifungals.

## **P67** A new machine learning algorithm identifies previously undetected biosynthetic gene clusters for RiPPs

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Ribosomally synthesized and post-translationally modified peptides (RiPPs) form a diverse group of natural products, which occur in all branches of life. They can be divided in various sub-classes, such as lantibiotics and lasso peptides, which display unique modifications in their final structure. Biosynthesis of different RiPPs follows similar principles. First, a small precursor gene is ribosomally translated to a precursor peptide. This peptide is extensively modified, and then cleaved to form the final product. The necessary enzymes are typically encoded by genes grouped together in a biosynthetic gene cluster (BGC). Genome mining strategies of these gene clusters target the modifying enzymes, as the RiPP precursors themselves show high diversity in sequence. Although this strategy is effective at finding RiPP BGCs similar to known variants, novel RiPP variants are not found.
We have developed a novel approach to mine genomes for RiPP BGCs. 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 of the precursor are added to form putative BGCs. We also determine the frequency of occurrence of these genes: the assumption is that a BGC will not be part of the core genome of a genus. Using this strategy, we have identified many uncharacterized BGCs that are not found by other algorithms like BAGEL and antiSMASH. These findings suggest that the chemical space for RiPPs may be larger than so far anticipated, offering new opportunities for genome mining-based drug discovery.

## **P69** Biosynthesis of thiocarboxylic acid-containing natural products in bacteria

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Thiocarboxylate acid-containing natural products are rare and their biosynthesis and biological significance remain unknown. Thiocarboxylate analogues of platensimycin (PTM) and platencin (PTN), antibacterial natural products, were recently discovered. Here, we report the identification of a “thioacid cassette” encoding two proteins, PtmA3 and PtmU4, responsible for carboxylate activation by coenzyme A (CoA) and sulfur transfer, respectively. Gene inactivation of *ptmA3*, *ptmU4*, and genes encoding the

sulfur-carrier protein system in the PTM–PTN overproducing strain SB12029 revealed their roles in thioPTM and thioPTN production, supporting thioPTM and thioPTN as the bona fide products of the *ptm* and *ptn* biosynthetic gene clusters. Both thioPTM and thioPTN retained strong antibacterial activities and bind tightly to FabF and FabH, the fatty acid biosynthesis targets of PTM and PTN. The thioacid cassette is prevalent in the genomes of bacteria, implicating that thiocarboxylate acid-containing natural products are an underappreciated class of natural products. The broad substrate promiscuity of the thioacid cassette provides opportunities to generate libraries of thioacids for future drug discovery efforts.

## **P71** Biosynthesis of *cis,cis*-muconic acid from a pathway-engineered *Corynebacterium glutamicum*

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Petroleum-based chemical industry faces drawback of environmental problems such as resource depletion, energy crisis, and global warming. Alternatively, environment-friendly and cost-effective biotechnological processes using engineered microorganisms are gaining attention. *Cis,cis*-muconic acid (CCM) is a promising platform chemical for a wide range of industrial applications; production of adipic acid and other biodegradable intermediates for nylon and plastic industry. Here we describe biosynthesis of CCM using a pathway-engineered *Corynebacterium glutamicum*, which is widely used for industrial production of amino acids. Based on analysis of shikimate pathway, *C. glutamicum* has been suggested to be able to synthesize CCM from glucose in the presence of a foreign protocatechuate (PCA) decarboxylase gene introduced. The codon-optimized foreign PCA gene was introduced under the strong promoter in *C. glutamicum*, followed by deletion of several bypass genes to maximize CCM accumulation. The pathway-engineered *C. glutamicum* successfully produced significant amount of CCM and proved to be a valuable cell factory for CCM biosynthesis.

**Key words:** *Corynebacterium glutamicum*, Muconic acid, Pathway engineering

**References:**

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## **P73** *Streptomyces a*rtificial chromosome system for heterologous expression of large-sized natural product biosynthetic gene cluster

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Recently, heterologous expression of biosynthetic gene cluster has become an essential strategy for titer improvement and pathway engineering of various potentially-valuable natural products. However, precise cloning and efficient overexpression of an entire biosynthetic gene cluster remains challenging due to ineffectiveness of current genetic systems in manipulating large-sized gene clusters for heterologous as well as homologous expression. Here, a versatile *Escherichia coli-Streptomyces* shuttle bacterial artificial chromosomal (BAC) conjugation vector, pSBAC, was used along with a cluster tandem integration approach to carry out homologous and heterologous overexpression of large 60-kb pikromycin (PIK) biosynthetic gene cluster and 80-kb tautomycetin (TMC) biosynthetic gene cluster. pSBAC-driven tandem integration of PIK biosynthetic gene cluster in heterologous host resulted in enhanced PIK production. Meanwhile, introduction of TMC biosynthetic gene cluster in TMC non-producing strains has resulted in similar amount of TMC production yield. Moreover, over-expression of TMC biosynthetic gene cluster in an original producing strain and recombinant *S. coelicolor* dramatically increased TMC production. In this study, the utility of the pSBAC system as a precise cloning tool for large-sized biosynthetic gene clusters has been established through heterologous expression of large-sized biosynthetic gene cluster. Moreover, this pSBAC-driven heterologous expression strategy was confirmed to be an ideal approach

for production of low and inconsistent natural products, implying that this strategy could be employed for development of a custom-designed overexpression scheme of natural product biosynthetic gene clusters in actinomycetes.

## **P75** Structural features and domain movements controlling substrate binding and cofactor specificity in class II HMG-CoA reductase

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The key mevalonate pathway enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (HMGR) uses the cofactor NAD(P)H to reduce HMG-CoA to mevalonate in the production of countless metabolites and natural products. Although HMGR inhibition by statin drugs is well understood, several mechanistic details of HMGR catalysis remain unresolved, and the structural basis for the wide range in cofactor specificity for either NADH or NADPH among HMGRs from different organisms is also unknown. Here, we present crystal structures of HMGR from *Streptococcus pneumoniae* (SpHMGR) alongside kinetic data on the enzyme's cofactor preferences. Our structure of SpHMGR bound with its kinetically preferred NADPH cofactor suggests how NADPH-specific binding and recognition are achieved. In addition, our structure of HMG-CoA-bound SpHMGR reveals large, previously unknown conformational domain movements that control HMGR substrate binding and enable cofactor exchange without intermediate release during the catalytic cycle. Taken together, this work provides critical new insights into both the HMGR reaction mechanism and the structural basis of cofactor specificity.

## **P77** Optimization of the green fluorescent protein (GFP) production using *Escherichia coli* BL21 as producer strain

*V.D.C. Santos-Ebinuma*, C. Lopes, S.R. Valentini and J.F. Brandão Pereira, Univ. Estadual Paulista-UNESP, Araraquara, Brazil*

The Green fluorescent protein (GFP) is a globular protein naturally produced by *Aequorea jellyfish* that has excellent properties to act as biosensor and biomarker. Recently, due to biotechnological advances, the responsible gene of GFP expression has been cloned and expressed into a wide range of heterologous system, in particular, *Escherichia coli* that is one of the most used. This protein can be used as biomarker in molecular biology and cell development studies, or as a biosensor in health, food and environmental control fields. Thus, considering the biotechnological importance of GFP as biomarker, in this work it was evaluated and optimized the GFP production in recombinant *E. coli* BL21 (DE3) [pLysS, pET28(a)]. The influence of agitation rate, induction time, and concentration of the inductor [isopropyl-β-1-D-thiogalactopyranoside (IPTG)] were studied. It was demonstrated that only agitation rate and induction time were significant, while, the IPTG concentration had negligible effect on the GFP production. High levels of GFP production (319.5 mg/L) were attained after the optimization, and further studies regarding the production in bioreactor will be carried out. In conclusion, the results have shown that *E. coli* BL21 can be an effective heterologous system for the GFP production, being a very promising system for the production at industrial scale.

**Acknowledgements:** This work was co-funded by FAPESP and FCT through the project 2014/19793-3.

## **P79** A functionally critical structural motif in the N-terminus of a modular polyketide synthase

*S. Yuzawa*, C. Bailey and J. Barajas, Lawrence Berkeley National Laboratory, Berkeley, CA, USA; L. Katz and J. Keasling, University of California, Berkeley, Berkeley, CA, USA*

Streptomyces genomes have a high G + C content and typically use an ATG or GTG codon to initiate protein synthesis. Although gene-identification tools perform well in low GC genomes, it is known that the accuracy in predicting a translational start site (TSS) is much lower for high GC genomes. Modular polyketide synthases (PKSs) are polymerases that utilize acyl-CoAs as substrates, which are responsible for the biosynthesis of a large number of clinically important natural products, and many of them originate

from Streptomyces. Using a Streptomyces-derived modular PKS as a model, we experimentally investigated the effects of employing alternative TSSs using a heterologous host. We inspected the N-terminal region of a native gene and found several potential start codons that may be used as TSSs. One of the TSSs employed boosted the protein level by >10-fold and the product yield by >10-fold compared to the originally annotated start codon in GenBank. Interestingly, a structural model of the PKS indicated the presence of a structural motif in the N-terminus, which may explain the observed different protein levels together with the amino acid sequence. This structure was also found in most modular PKSs that utilize non-carboxylated starter acyl-CoA substrates. This finding could be used in conjunction with start-codon prediction software to increase the accuracy of finding native start sites in modular PKS genes.

## **P81** A distributive peptide cyclase processes multiple core peptides within a single precursor peptide

*Y. Ding*, Y. Zhang and S. Bruner, University of Florida, Gainesville, FL, USA*

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are a rapidly growing family of natural products. Their biosynthesis follows a common scheme where the leader peptide of a precursor peptide guides biosynthetic enzymes to process the single core peptide. Here we describe a unique biosynthetic example as the processing of multiple core peptides within a single polypeptide precursor. In a cyanobacterial microviridin pathway, an ATP grasp ligase (named as AMdnC) installs up to two macrolactones on each of the three core peptides within the precursor peptide AMdnA. The enzyme catalysis occurs in a distributive fashion and follows an unstrict *N*-to-*C* overall directionality, unprecedented among RiPPs processing enzymes. Furthermore, the processing of AMdnA variants carrying one to four core peptides demonstrates the evolution of microviridn biosynthesis. Collectively, our results reveal a distinct biosynthetic logic of RiPPs, opening up the possibility of modular production via synthetic biology approaches.

## **P83** Salinipeptins: Integrated genomic and chemical approaches reveal D-amino acid-containing ribosomally synthesized and post-translationally modified peptides (RiPPs) from a Great Salt Lake *Streptomyces* sp.

*Z. Shang*, I. Yang and W. Fenical, Scripps Institution of Oceanography, University of California San Diego, San Diego, CA, USA; J. Winter and C. Kauffman, Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT, USA*

Analysis of the full genome of a unique, highly saline *Streptomyces* sp. strain GSL6C, isolated from The Great Salt Lake, revealed a gene cluster encoding the biosynthesis of the salinipeptins, novel members of the rare linarindin family of ribosomally synthesized and post-translationally modified peptides (RiPPs). The amino acid residues in salinipeptins A-D were suggested by genome annotation, which were confidently defined using a range of spectroscopic techniques (1D and 2D high-resolution nuclear magnetic resonance spectroscopy and tandem mass spectrometry) and chemical derivatization approaches (Marfey's and GITC analyses). The three-dimensional structure of salinipeptin A was established for the first time based on observed nuclear overhauser effect (NOE) correlations. The salinipeptins are unprecedented linaridins bearing nine D amino acids, which are rarely observed in RiPP natural products. Whole genome scanning of GSL6C did not return any homologs of the reported genes (e.g. *PoyD*, *CypI*) responsible for amino acid epimerization in RiPPs, inferring new epimerases may be involve in the conversion of L- to D-amino acids. In addition, the *N*-oxide and dimethylimidazolidin-4-one moieties in salinipeptins B and C, which are modified from *N*,*N*-dimethylalanine are uncommon in natural products. An investigation into the genes responsible for these posttranslational modifications is ongoing. Our study demonstrates that integration of genomic information early in chemical analysis significantly facilitates the discovery and structure characterization of novel microbial secondary metabolites.

# Monday, January 22

# 7:00 AM - 8:00 AM Breakfast

Flamingo/Sandpiper Deck

# 7:00 AM - 4:00 PM Registration

Grand Ballroom Foyer, lobby level

# 8:00 AM - 11:30 AM Session: 1: Natural products in microbiomes/environment

**Conveners:** **Helge Bode**, Goethe University, Frankfurt, Germany and **Yeo Joon Yoon**, Ewah Woman's University, Seoul, Korea, Republic of (South)

Salons F-G, lobby level

## 8:00 AM S1: Mining the dark: Metabolic novelty from elusive sources

*J. Piel*, ETH Zurich, Zurich, Switzerland*

Most areas of the bacterial tree of life are functionally uncharacterized. These regions include numerous deep-branching taxa that lack cultivated representatives and live in diverse habitats. Our lab uses metagenomic and single-cell-based mining strategies to investigate whether this remarkable taxonomic and ecological diversity is a resource for metabolic novelty. We have previously reported uncultured 'Entotheonella' symbionts of the sponge *Theonella swinhoei* as rich source of bioactive and biosynthetically unusual compounds. These bacteria were assigned to a newly proposed candidate phylum named 'Tectomicrobia' and contain large genomes with dozens of biosynthetic gene clusters. This talk presents new data from broader studies on the metabolism of sponge-associated bacteria. They confirmed 'Entotheonella' as talented producer taxon with a rich and diverse chemistry comparable to that of streptomycetes, but they also revealed the existence of further, unrelated producers. These data suggest fundamental contributions of sponge microbiomes to the natural product repertoire of their hosts and validate microbial dark matter as a resource of distinct chemistry. While most of the gene clusters had no counterparts in known cultured bacteria, we also identified unprecedented, mechanistically surprising enzymes that are widespread in more conventional prokaryotes, indicating the existence of large natural product families with as-yet unknown ecological and pharmacological functions.

## 8:30 AM S2: Lifestyles of the small and obscure

*E. Schmidt*, University of Utah, Salt Lake City, UT, USA*

The defensive chemistry of marine animals is rich and varied. Much of the remaining chemical and biological diversity is found in smaller animals, which have traditionally posed a technical challenge. Small animals are therefore untapped reservoirs of drug-like compounds and biosynthetic pathways. Many of the compounds and pathways (but not all) originate in symbiotic bacteria, which also vary greatly between organisms. This makes it possible to use a biosynthetic and metagenomic approach to access new compounds. Examples from tunicates and mollusks will be described.

## 9:00 AM S3: Host-associated microbes as a source of new antimicrobials

*M. Chevrette*, Dept of Genetics, University of Wisconsin-Madison, Madison, WI, USA*

The diversity of life is shaped by interactions between species and at the microbial scale, these interactions are mediated by chemistry. While mining the secondary metabolism of Actinobacteria for antagonistic interactions has proven a fruitful source of antimicrobial compounds, comparative studies have revealed the majority of biosynthetic potential to be specific to a strain or species suggesting the importance of ecological pressures across different evolutionary scales on secondary metabolic content and chemistry. Well-documented symbioses between Actinobacteria and eukaryotic organisms include fungus-growing ants, beetles, beewolf wasps, plants, and sponges, among others. These systems offer an informative ecological and evolutionary framework in which to study molecular interactions, particularly how secondary metabolites operate in defense and communication, as well as the opportunity for novel antimicrobial discovery. Here, we present two examples of antimicrobial discovery from Actinobacteria associated with eukaryotic hosts: i) the discovery of keyicin, an antibiotic active against multidrug resistant Gram-positive bacteria from a sea squirt microbiome, and ii) a systematic assessment of the antimicrobial potential of insect-associated *Streptomyces*. Co-culture and comparative metabolomics of sympatric bacteria from a sea squirt microbial community led to the production and subsequent genomic and structural characterization of the antibiotic keyicin. To more broadly assess host-associated Actinobacteria as a source of antimicrobials, we extensively sampled *Streptomyces* associated with diverse insect hosts. Through inhibition assays, genomics, and metabolomics we identified insect-associated *Streptomyces* as a prolific source of compounds active against multidrug resistant human pathogens.

## **9:30 AM** Break

## **10:00 AM** S4: Discovery of novel bioactive polyketides/non-ribosomal peptides from microbes

*W. Zhang*[*]*, UC Berkeley, Berkeley, CA, USA*

In addition to the role of therapeutic agents, many natural products are used by their producing organisms to access information about both the intracellular physiological status and extracellular environment, and control complex cellular processes such as virulence, morphological differentiation, stress response, and metal acquisition. Identification of these bioactive natural products through targeted genome mining could reveal compounds with new molecular scaffolds and functionalities that were missed during traditional cytotoxicity screening and provide new insights on the role of encoding biosynthetic enzymes and the biological function of natural products in their native environment. For example, using both *in vivo* heterologous reconstitution and *in vitro* biochemical analyses, we have revealed that a putative lipopeptide biosynthetic gene cluster conserved in pathogenic mycobacteria is responsible for synthesizing a new family of isonitrile lipopeptides through an unprecedented mechanism for isonitrile biosynthesis. Our results have further suggested that this biosynthetic gene cluster plays a role in metal transport, and thus have shed light on a new metal transport system that is crucial for virulence of pathogenic mycobacteria. We have also uncovered a novel family of polyketides native to the anaerobic bacterium *Clostridium acetobutylicum*, an organism well-known for its historical use in ABE fermentation. Through mutational analysis and chemical complementation assays, we have demonstrated that these polyketides act as chemical triggers of sporulation and granulose accumulation in this strain. This study showcases a novel strategy of manipulating the secondary metabolism of an organism to improve traits significant for industrial applications.

## **10:30 AM** S5: Peptides and natural products made by microbes and men

*H. Bode*[*]*, Goethe University Frankfurt, Frankfurt am Main, Germany*

The model bacteria *Xenorhabdus* and *Photorhabdus* live in symbiosis with nematodes and together they are able to infect and kill insects and produce a huge variety of natural products with different biological activities including antibiotics and cytotoxic compounds.
We have recently identified a global regulator that allows us to shut-down all natural product biosynthesis pathways that can be combined with promoter exchange approaches of selected biosynthesis gene

clusters to specifically produce only the desired natural product class and therefore allows the determination of its bioactivity directly from the crude extract without isolation of the compounds.

Since it would be also desirable to increase the chemical diversity of natural products beyond the ones found in nature, we have additionally identified rules for the efficient modification of natural non-ribosomal peptide synthetases (NRPS) yielding non-natural derivatives of the original peptides. These rules can also be applied for the design and assembly of completely non-natural NRPS systems that result in the production of novel peptides (e.g. linear, cyclic, containing acyl moieties and/or L- or D-amino acids) in very good yields of up to 100 mg/L. In contrast to previous approaches of NRPS engineering, we hardly observe a drop in the production titer. We are currently expanding our technology also to NRPS-PKS hybrids and are confident that the whole process can be automated to produce libraries of non-natural natural products. First small libraries have already been created in *E. coli*.

### **11:00 AM** S6: Isolation, characterization, and redesign of disaccharide-containing antifungal polyene NPP in *Pseudonocardia autotrophica*

*E.S. Kim*, Inha University, Incheon, Korea, Republic of (South)*

Invasive fungal infections are becoming a serious problem in human health, especially for the immunocompromised patients as a result of HIV infection, anticancer therapy, or organ transplantation. Although polyenes including nystatin and amphotericin showed broad-spectrum fungicidal activity and low incidence of fungal resistance, high toxicity and low water solubility limit their broad clinical application. In recent years, genome mining has become a promising strategy for design of new analogues of polyene with improved pharmacological properties.

Previously, a rare actinomycetes called *Pseudonocardia autotrophica* was determined to contain a cryptic polyene biosynthetic gene cluster through a polyene CYP-specific genome screening strategy, and then a novel nystatin-like polyene (NPP) A1 containing a disaccharide, mycosamine (a1-4)-N-acetyl-2-aminoglucose was identified. NPP A1 showed significantly higher solubility and lower hemolytic activity than nystatin A1 which contains a single sugar mycosamine.

Here, we report the complete NPP A1 biosynthetic gene cluster and pathway including unique post-PKS modification steps in *P. autotrophica*. Various engineered NPP analogues were generated through biosynthetic gene cluster manipulation, followed by evaluation of their efficacies, pharmacokinetics, and toxicities. These results suggest that NPP derivatives could be promising candidates for further development into a pharmacologically-improved and less-toxic polyene antifungal antibiotic.

Lee et al. Structural analysis and biosynthetic engineering of a solubility-improved and less-hemolytic nystatin-like polyene in *Pseudonocardia autotrophica.* Appl Microbiol Biotechnol. 2012.

Kim et al. Post-PKS tailoring steps of a disaccharide-containing polyene NPP in *Pseudonocardia autotrophica.* PLoS One. 2015.

Kim et al. Redesign of antifungal polyene glycosylation: engineered biosynthesis of disaccharide-modified NPP. Appl Microbiol Biotechnol. 2017.

# 11:30 AM - 1:00 PM Lunch

Flamingo/Sandpiper Deck

# 1:00 PM - 4:30 PM Session: 2: Chemical biology of natural products

**Conveners:** **Jae Kyung Sohng**, Sun Moon University, Asan-si, Tangjeong-myeon, Korea, Republic of (South) and **Rebecca Butcher**, University of Florida, Gainesville, FL, USA

Salons F-G, lobby level

## 1:00 PM S7: Discovery and biosynthesis of hybrid polyketide-nonribosomal peptides in nematodes

*R. Butcher*, University of Florida, Gainesville, FL, USA*

Polyketides and nonribosomal peptides are well-known for their antibiotic, antifungal, immunosuppressant, anti-parasitic, and anti-cancer activities. These structurally complex natural products are biosynthesized by polyketide synthases (PKSs) and nonribosomal synthetases (NRPSs), which function in either an assembly-line or iterative manner. Although polyketides and nonribosomal peptides are produced by many species of bacteria and fungi, they are extremely rare in metazoans. The genome of the nematode *Caenorhabditis elegans* encodes a huge, multi-module, hybrid PKS/NRPS (PKS-1) and a multi-module NRPS (NRPS-1). Using comparative metabolomics and NMR spectroscopy, we have identified and elucidated the chemical structure of nemamide, a hybrid polyketide-nonribosomal peptide that is biosynthesized by PKS-1/NRPS-1 using both iterative and assembly-line mechanisms. We have used CRISPR-Cas9 to make specific mutations in *pks-1* and *nrps-1* to investigate the roles of specific enzymatic domains in nemamide biosynthesis. We have also used comparative metabolomics to identify intermediates in the biosynthetic pathway. Furthermore, we have identified many additional enzymes encoded across the *C. elegans* genome that work in trans in nemamide biosynthesis. Our results elucidate the mechanism of polyketide-nonribosomal peptide biosynthesis in the context of a complex animal system. We have also shown that nemamide is produced specifically in the canal-associated neurons under starvation conditions, downregulates insulin signaling, and promotes survival during starvation. Our results uncover a novel mechanism by which animals respond to nutrient fluctuations to extend survival. Homologs of PKS-1 and NRPS-1 are present in most nematode species, including parasitic ones, and thus, nemamide likely plays an evolutionarily conserved role across nematode species.

## 1:30 PM S8: Natural products as an inspiration for novel chemistry and biology

*B. Shen*, Department of Chemistry, Department of Molecular Medicine, Natural Products Library Initiative at The Scripps Research Institute, The Scripps Research Institute, Jupiter, FL, USA*

Natural products are among the best sources of drugs and drug leads, and natural product biosynthesis continues to inspire new chemistry and biology. Advance made in the last two decades in connecting natural products to the genes that encode their biosynthesis has fundamentally changed the landscape of natural products research. Platensiymcin and platencin, two natural products that are potent and selective inhibitors of bacterial and mammalian fatty acid synthases, will be discussed to argue that thiocarboxylic acid as an alternative pharmacophore and thiocarboxylic acid-containing natural products as an underappreciated family should be considered in future drug discovery effort. Dynemicin, uncialamycin, tiancimycin, and yangpumicin, a growing family of anthraquinone-fused enediyne natural products, will be discussed to showcase Natue’s ingenuity in evoluing mechanisms to protect itself from some of the most cytotoxic natural products known to date.

## 2:00 PM S9: Exploring new protein targets of natural products with label-free based target identification and validation

*H.J. Kwon*, Department of Biotechnology/Yonsei University, Seoul, Korea, Republic of (South)*

Identifying protein targets of natural products is a crucial step in the development of natural products to treat human diseases. Target identification methods usually consist of probes linked to natural products (tagged natural products) to effectively pull-down target proteins based on high affinity. However, the drawback to this conventional method is that probe synthesis step is required, and the tagged natural product may lose or change its functionality. Here, we propose DARTS (drug affinity response target stability) combined with LC/MS/MS method (DARTS-MS) to resolve these drawbacks. DARTS is a target identification method of label-free natural product that depends on the target protein stability when bound

to a natural product. Here, when a natural product interacts with a protein target, the latter is more stable than the unbound protein and consequently less susceptible to proteolytic effects. The conformational changes that are induced upon interacting with the natural product thermodynamically stabilize the protein structure. This interaction information of natural product and target protein facilitates structure based better drug development and functional annotation of target protein as well. Furthermore, integration of Matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) technology will enable to validate the interaction of label-free natural product with target protein in tissue that harnesses the preclinical studies of natural products in respect with their efficacy, toxicity, and pharmacokinetics. In this presentation, recent advances for target identification of natural products towards functional and translational applications will be presented by introducing our case studies of FK506 and other natural products.

## **2:30 PM** Break

## **3:00 PM** S10: Genome-wide target identification of natural products

*J. Wong*[*]*, Novartis Institutes for BioMedical Research, Basel, Switzerland*

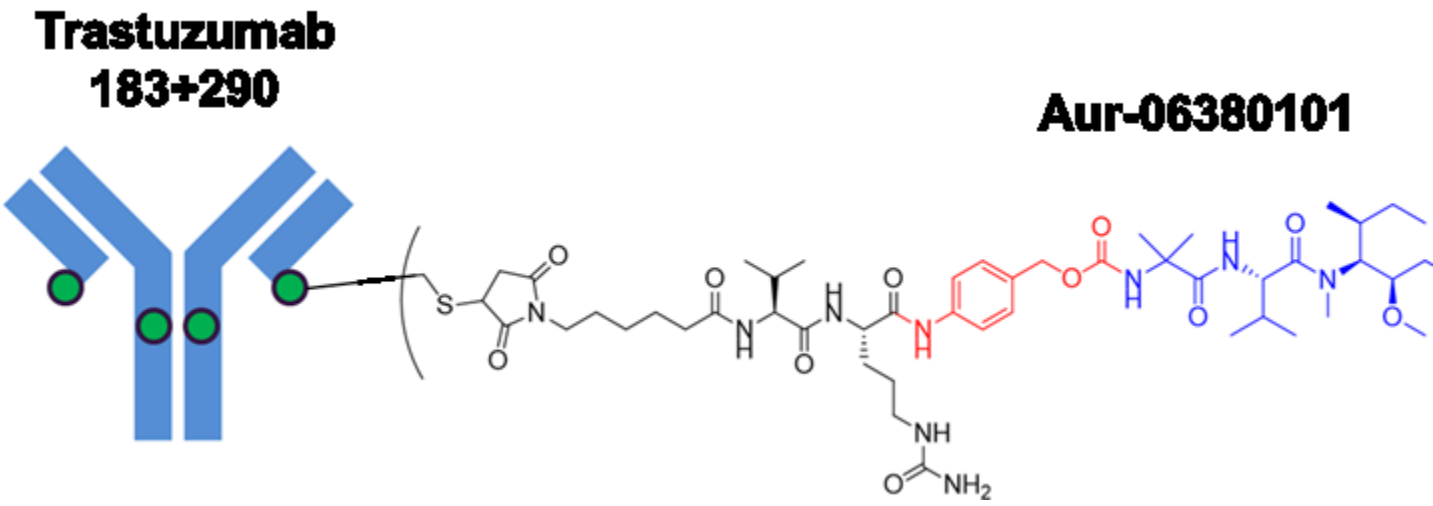
With the recent shift in the pharmaceutical industry towards phenotypic assays, the need for target identification activities is increasing. Indeed, a major challenge related to compounds identified in such complex assays remains the identification of their binding target(s) and subsequently, elucidation of their mechanism of action. A powerful and unbiased approach to get insights into the biology modulated by bioactive probes is chemogenomic profiling. Until recently, genome-wide, high resolution experiments of this nature have been restricted to fungal systems due to the lack of mammalian genome-wide deletion collections. We will show that a CRISPR/Cas9 system enables the generation of transient homo- and heterozygous deletion libraries and that it allows for the identification of efficacy targets and pathways mediating hypersensitivity and resistance relevant to the compound mechanism of action. How the studies on target and pathway identification of new natural products rationalize their valuable role in modern drug discovery will be illustrated through a few examples.

## **3:30 PM** S11: The discovery of PF-06804103, a next-generation anti-Her2 antibody drug conjugate (ADC)

*E. Graziani*[*]*, Pfizer Inc., Groton, CT, USA*

Her2-targeted antibody therapy is a clinically proven approach to treating breast cancer that can be made more efficacious by the attachment of a cytotoxic payload, resulting in an antibody-drug conjugate (ADC). We set out to design and test a Her2- targeting ADC that would show improved efficacy against tumors with low to moderate Her2 expression, improve safety by reducing off-target toxicity, and demonstrate efficacy in tumors that have become resistant to first generation anti-Her2 mAbs and ADCs such as T-DM1 (trastuzumab conjugated to the maytansinoid emtansine via a thioether linkage).

We employed a cleavable linker to ensure endosomal escape in order to avoid the degradative environment of the lysosome. Further, we optimized an auristatin-based payload for improved potency via a rational, structure-based drug design approach. Third, we optimized the sites of conjugation to maximize linker-payload stability and ADC exposure, via rational design and an empirical approach using chromatographic behavior as a surrogate for ADC lipophilicity. Surprisingly, this hypothesis did not reveal the ADC with the most improved exposure; it was only by finding the combination of improved antibody exposure and linker-payload stability that gave rise to the lowest free payload concentrations in circulation (payload Cmax) that a correlation with improved safety was revealed. The ADC resulting from these design criteria, PF-06804103, has demonstrated efficacy in multiple pre-clinical models including T-DM1 resistant models, optimized pharmacokinetic properties, and improved safety relative to earlier generation of Her2- targeted ADCs.




**4:00 PM** S12: Alternative biosynthetic pathways for primary metabolites in microorganisms are targets for specific antibiotics?

*T. Dairi*, Graduate School of Engineering, Hokkaido University, Sapporo, Japan*

The biosynthetic pathways of primary metabolites have been established with model microorganisms such as Escherichia coli and Saccharomyces cerevisiae. For a long time, the biosynthetic routes established were believed to be common among all microorganisms. However, we now realize that some microorganisms possess alternative biosynthetic pathways since genome data base has enabled us to examine the presence or absence of orthologs of the genes responsible for known biosynthetic pathways. We are interested in an alternative biosynthetic pathway for primary metabolites in microorganisms and previously reported that some microorganisms including *Helicobacter pylori*, which causes gastric carcinoma, utilized the futalosine pathway for menaquinone biosynthesis (***Science***, **321**, 1670-1673, 2008). Moreover, we recently reported that some microorganisms including *Xanthomonas oryzae,* which causes bacterial blight of rice, biosynthesizes the peptidoglycan by the unique two enzymes, XOO_1319 and XOO_1320, which catalyze ligation of L-Glu to UDP-MurNAc-L-Ala and epimerization of the terminal L-Glu of the product, respectively (***J. Am. Chem. Soc***. **139**, 4243-4245, 2017).

We have been screening compounds that inhibit these new pathway (enzymes) for development of specific antibiotics and pesticides.

# 5:00 PM - 7:00 PM Session: PS2: Poster Session 2

Salons A-E, lobby level

**P2** A rare ether-catalyzing cytochrome P450 in the biosynthesis of platensimycin

*J. Rudolf*, L. Dong and B. Shen, The Scripps Research Institute, Jupiter, FL, USA*

Platensimycin (PTM) and platencin (PTN) are highly functionalized bacterial diterpenoid natural products that target bacterial and mammalian fatty acid synthases. The biosynthetic pathways of PTM and PTN diverge at the cyclization of *ent*-copalyl diphosphate. Two distinct type I diterpene synthases form the skeletons of PTM and PTN, (16*R*)-*ent*-kauran-16-ol and *ent*-atiserene, respectively. Before *ent*-kauranol resumes its parallel biosynthesis with *ent*-atiserene, it is processed by a cytochrome P450 to form the

characteristic 11*S*,16*S*-ether linkage of PTM. The isolation of 16*R*-hydroxyl intermediates and congeners from the PTM producing strain suggests that ether formation goes through an 11*S*,16*R*-diol intermediate, due to the necessary inversion of stereochemistry at C-16. First, one $^{18}O$ atom of $^{18}O_2$ was found to be incorporated in an in vitro labeling experiment supporting that the ether oxygen in PTM originates from molecular oxygen. Next, we tested a series of alternate diterpenoid substrates with PtmO5 in vitro and found PtmO5 is a promiscuous P450. Isolation and structural characterization of the enzymatic products revealed insights into the mechanism of P450 ether formation including the regio- and stereoselectivity of PtmO5.

## **P4** Genome mining of biosynthetic gene clusters in fungal hyperparasites

*G. Harm*, J. Kalaitzis, A. Papanicolaou and M. Moffitt, Western Sydney University, Campbelltown, Australia; W. Cuddy, University of Sydney, Sydney, Australia*

## **P6** Discovery of the tyrobetaine natural products and their biosynthetic gene cluster via metabologenomics

*E. Parkinson*, J. Kemball, S. Zhukovsky and W. Metcalf, University of Illinois at Urbana-Champaign, Urbana, IL, USA; J. Hudson, A.W. Goering, R. McClure, R.J. Thomson and N.L. Kelleher, Northwestern University, Evanston, IL, USA; K.S. Ju, The Ohio State University, Columbus, OH, USA*

Natural products have long served as rich sources of antibiotics, but traditional discovery methods of screening crude extracts for novel natural products are now often unsuccessful due to high rates of rediscovery. More recently, genetic approaches for natural product discovery have shown some promise, but progress has been slow due to the difficulty of identifying unique clusters and the issue of cryptic gene clusters. To address the challenge of natural product discovery, the Metcalf laboratory (University of Illinois at Urbana-Champaign) in collaboration with the Kelleher laboratory (Northwestern University) have developed a method called *metabologenomics* that combines genomic and mass spectrometric data to allow untargeted discovery of new natural products. This method allowed for identification, isolation, and structural elucidation of two novel nonribosomal peptide natural products. Both of these compounds contain a highly unusual trimethylated N-terminus along with the unnatural amino acid 3-hydroxyleucine. These new natural products differ in that one is halogenated (hereafter referred to as chlorotyrobetaine) and the other (tyrobetaine) is not. Utilizing the metabologenomics method along with heterologous expression, the biosynthetic gene cluster responsible for production of tyrobetaine was identified. Interestingly, the gene responsible for the halogenation of chlorotyrobetaine appears to be present on a different cluster, suggesting that either the halogenase or the initial adenylation domain of the nonribosomal peptide synthetase is promiscuous. The discovery of tyrobetaine, chlorotyrobetaine, and their associated biosynthetic gene clusters demonstrates the great power of the metabologenomics method for the discovery of new natural products that could help to refill the antibiotic pipeline.

## **P8** Biosynthetic study on rare sulfonamide natural products SB-203207 and SB-203208

*Z. Hu*, Lab of Natural Products Chemistry, Graduate School of Pharmaceutical Science, The University of Tokyo, Tokyo, Japan, T. Awakawa, Lab of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan and I. Abe, Graduate School of Pharmaceutical Science, The University of Tokyo, Tokyo, Japan*

SB-203207 and SB-203208 are two isoleucyl tRNA synthetase inhibitors isolated from *Streptomyces* sp. NCIMB40513. These compounds are structurally related to altemicidin, an acaricidal and antitumor compound, and possess unusual 6-azaindene monoterpene ring and sulfonamide group. Although total synthesis of SB-203207 was achieved, its biosynthetic pathway remains unknown. To elucidate the biosynthetic mechanism of these compounds, two biosynthetic gene clusters in the producer strain were heterologously expressed in *Streptomyces lividans* step by step. The results revealed that the first cluster

is responsible for the production of altemicidin while the second cluster is responsible for the transfer of isoleucine onto the sulfonamide group and methylphenyalanine onto the hydroxyl group of altemicidin to afford SB-203208. Furthermore, *in vitro* assay of Orf-1 (tRNA synthetase like protein) with isoleucyl tRNA and altemicidin revealed that Orf-1 cannot accept ATP and isoleucine to give isoleucyl-AMP but acts as an amide synthase in this transfer reaction, indicating that Orf-1 was evolved to be a biosynthetic enzyme from house keeping proteins. We also characterized the transfer of methylphenylalanine by Orf-2 (AMP-ligase), Orf-3 (Acyltransferase) and Orf-11 (Carrier protein), that Orf-3 requires carrier protein-tethered methylphenylalanine as the acyl donor and altemicidin as the acceptor for the ester bond formation. Currently, we are investigating the biosynthetic pathway of sulfonamide through *in vitro* assay and gene deletion study.

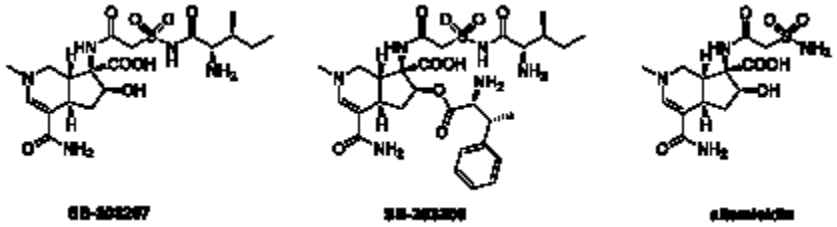


Figure **1** Structures of SB-203207, SB-203208, altemicidin

## **P10** Genetic and *in vivo* functional analysis of Ble (Orf12) from the clavulanic acid biosynthetic pathway of *Streptomyces clavuligerus*

*S. Srivastava, K. King, N. AbuSara, C. Malayny, J. Wilson and K. Tahlan*, Memorial University of Newfoundland, St. John's, NF, Canada*

Clavulanic acid is produced by *Streptomyces clavuligerus* and is a potent inhibitor of β-lactamases, enzymes that hydrolyze and inactivate conventional β-lactam antibiotics such as penicillins and cephalosporins. It differs from all other known clavams due to its unique 5*R* stereochemistry, which is also responsible for its bioactivity. Clavulanic acid and the 5*S* clavams share a common biosynthetic pathway in *S. clavuligerus* and precursors leading up to the penultimate step during clavulanic acid biosynthesis also have 5*S* stereochemistry. As to how and when the stereochemical change from 5*S* to 5*R* takes place during production has been a long standing question, with many different hypotheses being proposed. One such hypothesis involves the product of *orf12* (or Ble) from the clavulanic acid gene cluster, which resembles class A β-lactamases and has an additional N-terminal domain resembling steroid isomerases/cyclases. Previous reports from other groups have shown that Ble exhibits some *in vitro* β-lactam esterase activity (*Acta Crystallogr D Biol Crystallogr. 2013.69:1567-79*), the implications of which on clavulanic acid biosynthesis is not clear. We conducted extensive *in vivo* mutagenesis, expression and complementation studies to show that Ble is most likely a bonafide enzyme from the clavulanic acid biosynthetic pathway of *S. clavuligerus*. It is intriguing that a protein related to β-lactamases would be involved in the biosynthesis of a β-lactamase inhibitor, the implications of which will also be discussed.

## **P12** Genomics driven discovery of natural products from rare actinomycetes *Nocardia* spp. CS682

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*Nocardia* sp. CS682, was isolated from Korea and characterized as prominent producer of nargenicin A1. Nargenicin A1 exhibits significant antimicrobial potential against various Gram positive bacteria including methicillin resistant *Staphylococcus aureus* (MRSA). By whole genome sequencing and annotation, different gene clusters corresponding to various secondary metabolites were identified from Nocardia sp. CS682. The comprehensive analysis of metabolites led to elucidation of structure of such secondary metabolites. In the meantime the functional characterization of genes in biosynthetic gene cluster and characterization of metabolite profile provided insight on mechanism of biogenesis of nargenicin A1 in

*Nocardia* sp. CS682. Concurrently on the basis of information of biosynthetic mechanism, the gene mutation and gene combination strategies were employed for generating different novel derivatives of nargenicin A1. All the derivatives were structurally elucidated by mass spectrometric and NMR spectroscopic analysis as par need. The biological properties of such novel derivatives were accessed by evaluation of antimicrobial and anticancer activities.

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## **P14** Biosynthesis of natural and non-natural glycosides of genistein.

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## **P16** Production of coronafacoyl phytotoxins involves a novel biosynthetic pathway in the plant pathogen *Streptomyces scabiei*

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Phytotoxic natural products have been shown to play an important role in the ability of phytopathogenic microorganisms to cause disease. They can allow the producing organism to evade pant defense responses during host colonization and infection. The coronafacoyl phytotoxins are a family of natural products that are biosynthesised by phylogenetically distinct plant pathogenic bacteria and which function as virulence factors for these organisms. One of the most studied coronafacoyl phytotoxins is coronatine (COR), which is produced by the phytopathogenic bacterium *Pseudomonas syringae*. COR functions as a phytohormone mimic and allows manipulation of host defence signalling pathways during infection. COR and other coronafacoyl phytotoxins consist of the polyketide compound coronafacic acid (CFA) linked via an amide bond to different amino acid or amino acid analogs. Recent studies from our lab have demonstrated that the potato common scab pathogen *Streptomyces scabiei* produces the coronafacoyl phytotoxin *N*-coronafacoyl-L-isoleucine, and gene deletion analyses have shown that production of the CFA moiety in this organism involves a novel biosynthetic pathway as compared to P. syringae. In addition, we provide evidence that this novel pathway is conserved in other Actinobacteria that are predicted to produce coronafacoyl phytotoxins but not in other phytotoxin – producing organisms. We additionally reveal that production of coronafacoyl phytotoxins may occur in both plant pathogenetic and non-pathogenetic bacteria based on genomic analyses. This suggests that production of these natural products may be more prevalent than previously realized and that their role for the producing organism may not be limited to host-pathogen interactions.

## **P18** Discovery of novel antibiotics from rare-actinomycetes using combined-culture strategy

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Actinomycetes have a large potential to produce antibiotics, and much of efforts for natural product discovery were focused on *Streptomyces* species. On the other hand, rare-actinomycetes (= non-*Streptomyces*) are less-exploited resource for natural product discovery. However, recent bacterial genome sequencing projects revealed that some rare actinomycetes possess comparable levels of natural product biosynthetic gene clusters (NPGCs) in their genomes, and the most parts of their biosynthetic genes were not expressed under the standard culture conditions. We previously showed that mycolic acid-containing bacterium (MACB) efficiently induces secondary metabolite production in some *Streptomyces* strains by co-culture, and we described this method as “combined-culture”. However, the application of “combined-culture” method to rare actinomycetes was quite limited. Therefore, we applied combined-culture method to our collection of rare actinomycetes to obtain novel antibiotics from them. As the strains for combined-culture screening, we chose three orders of rare-actinomycetes (*Pseudonocardiales, Streptosporangiales*, and *Micromonosporales*), which were reported to possess large amount of NPGCs. As a result, we found that MACB (*Tsukamurella pulmonis* TP-B0596) activated the production of secondary metabolites in approximately 30% of tested strains by co-culture. Furthermore, we could identify five novel compounds from combined-culture of three rare-actinomycete strains. In conclusion, the “combined-culture” method is effective tool for activating silent biosynthetic genes present in rare-actinomycetes, and we could access novel bioactive compounds from them.

## **P20** Expanding chemical diversity in the Sirenas Biochemometrics Platform

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An increasing challenge in the field of natural products research is the discovery of novel chemistry possessing biologically relevant activity. One solution is to diversify the chemical source by collecting taxonomically distinct organisms or isolating and culturing new bacterial strains. However, these options tend to be difficult, costly, and often result in redundancies. An alternative strategy is to expand or maximize the chemical extraction process and increase chemical diversity by optimizing workflows and the discovery process itself. For example, polar organic molecules, broadly defined as those that are not retained under standard reverse-phase HPLC conditions, are frequently overlooked in most natural product drug discovery workflows due to the difficulty in their isolation, as well as the consistent lack of activity in their associated fractions during bioassay guided pipelines and cellular-based assays. Therefore, the therapeutic potential of this area of chemical space is relatively unexplored. Sirenas recently implemented discovery methods to process and fractionate polar organic fractions originating from the extracted aqueous layer. These methods, after rigorous development, include SPE using a hydrophilic/lipophilic balanced sorbent for sample preparation and subsequent HPLC under conditions suitable for the separation of these types of molecules. Complimented with proprietary software and informatics that integrate chemical and biological data for each molecule, these methods have shown promise in the identification of new chemistry with activity against *Mycobacterium tuberculosis*.

## **P22** Nature as the ultimate combinatorial biosynthetic chemist: Discovery of the Leinamycin family of natural products

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Nature’s ability to generate diverse natural products from simple building blocks has inspired combinatorial biosynthesis. Knowledge-based approach to combinatorial biosynthesis has allowed the production of designer analogues by rational metabolic pathway engineering. While successful, structural

alterations are limited, with the designer analogues often produced in significantly compromised titers. Discovery-based approach to combinatorial biosynthesis complements the knowledge-based approach by exploring the vast combinatorial biosynthesis repertoire found in Nature. Here we showcase the discovery-based approach to combinatorial biosynthesis by targeting the DUF-SH didomain, specific for sulfur incorporation from the leinamycin (LNM) biosynthetic machinery, to discover the LNM family of natural products. By mining bacterial genomes from public databases and the actinomycetes strain collection at The Scripps Research Institute, we discovered 49 potential producers that could be grouped into 18 distinct clades based on phylogenetic analysis of the DUF-SH didomains. Further analysis of the representative genomes from each of the clades identified 28 distinct *lnm*-type gene clusters. Structural diversities encoded by the LNM-type biosynthetic machineries were predicted based on bioinformatics and confirmed by in vitro characterization of selected adenylation proteins and isolation and structural elucidation of the guangnanmycins and weishanmycins. These findings demonstrate the power of the discovery-based approach to combinatorial biosynthesis for natural product discovery and structural diversity and highlight Nature as the ultimate combinatorial biosynthetic chemist. Comparative analysis of the newly discovered LNM-type biosynthetic machineries provides outstanding opportunities to dissect Nature's biosynthetic strategies and apply these findings to the practices of combinatorial biosynthesis for natural product structural diversity.

## **P24** Deciphering the role of genes involved in ethylmalonyl-CoA supply and tailoring reactions in Kirromycin biosynthesis in *Streptomyces collinus* Tü 365

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Kirromycin is a potent inhibitor of protein biosynthesis in prokaryotes as it binds to the elongation factor Tu, leading to conformational changes and ultimately stalling of the bacterial ribosome. The linear molecule is synthesized by a hybrid PKS-I/NRPS and the biosynthetic gene cluster comprises 26 genes spanning an 82 kb DNA region. A combination of *in silico* bioinformatic predictions and *in vitro* mutational studies have revealed the role of core genes involved in biosynthesis of kirromycin, however, genes involved in precursor supply and tailoring reactions remained to be fully characterized in order to fill the gaps in the pathway[1,2].

In this study, the functions of the putative crotonyl-CoA reductase/carboxylase (CCR) KirN and the tailoring enzymes KirM, KirHVI, KirOI and KirOII were investigated by genetic manipulation. Derivatives were detected in each mutant involved in tailoring reactions and in the case of the KirN mutant a lowered kirromycin production was observed.

Our genetic studies have allowed us to clarify the putative roles of all enzymes studied here, ultimately allowing us to fill many of the missing gaps in the biosynthetic pathway of kirromycin. Furthermore, this collection of mutants serves as a toolbox for production of new derivatives of the original molecule kirromycin and with this a better understanding of the potential of complex polyketides in production of antibiotics with new mode of actions.

## **P26** Arsinothricin, a novel organoarsenical antibiotic and its resistance mechanism

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Due to the dramatic increase in bacterial antimicrobial resistance (AMR), discovery and development of new antibiotics is an urgent need. Organoarsenicals have been used as antimicrobials more than a century since Paul Ehrlich's magic bullet Salvarsan. Recently a rice rhizosphere bacterium was shown to produce a novel natural product containing arsenic, arsinothricin (AST), a mimetic of the herbicidal antibiotic phosphinothricin (PT). Here we show that AST is a broad-spectrum antibiotic effective against both Gram-negative and Gram-positive bacteria and its antimicrobial activity is 30-time more effective than PT and retained even in the presence of glutamate that reverses PT. These results suggest that AST is a considerably more potent antibiotic than PT, thus we propose that this novel As-containing antibiotic has the potential to provide a countermeasure to combat AMR. The occurrence of antibiotic resistance is inevitable. Here we demonstrate that, ArsN, *N*-acetyltransferase encoded by *arsN* gene widely distributed in bacterial *ars* (arsenic resistance) operons, confers high-level resistance to AST and low resistance to PT, indicating that ArsN is an AST-selective *N*-acetyltransferase. We crystallized ArsN and solved the structures for both the apo and AST-bound forms, which provided insights into the substrate selectivity of the enzyme. These results will lead to schemes to reverse bacterial resistance to AST, making it a more effective antibiotic against the growing health treat of AMR.

## **P28** sequestration as a new mechanism for self-resistance to enediyne antitumor antibiotics

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The enediyne antibiotics show great structural variety and astonishing bioactivity. The 9- and 10-membered enediyne-producing strains, respectively, employ the apoproteins and the self-sacrifice proteins against the enediyne toxins. However, the mechanism for resistance to the anthraquinone-fused enediynes remained unclear. In this study, the self-resistance mechanism utilized in this class of microbes is revealed. Bioinformatics analysis shows that the genes encoding TnmS1/TnmS2/TnmS3, UcmS1/UcmS2/UcmS3, and DynE14/DynE15, respectively, in the biosynthetic gene clusters of anthraquinone-fused enediynes, tiancimycin (TNM), uncialamycin (UCM), and dynemicin (DYN), belong to the glyoxalase/bleomycin resistance protein/dioxygenase superfamily that may be involved in antibiotic resistance. *In vivo* resistance assays, together with *in vitro* protein-ligand binding assays, provide evidence of antibiotic sequestration as a major mechanism for self-resistance to anthraquinone-fused enediynes. Finally, the binding site of TNM was mapped by the crystal structures of TnmS1/TnmS2/TnmS3. Taken together, these finding provide a new mechanism and a missing piece for self-resistance to a set of enediyne antitumor antibiotics.

## **P30** Phenazine antibiotics via divergent biosynthesis pathways

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Entomopathogenic bacteria of the genus *Xenorhabdus* living in symbiosis with nematodes of the genus *Steinernema* are able to produce a huge diversity of toxic proteins as well as natural products as signaling molecules and virulence factors to maintain nematode development and protect the insect cadaver from food competitors. Two phenazine biosynthetic gene clusters (BGCs) including a silent one have been identified in *Xenorhabdus szentirmaii*, and are subdivided into four modules based on their involvement in four different classes of phenazine derivatives. The biosynthesis of simple hydroxyl- and carboxyl-substituted phenazines (occurring mainly in *Pseudomonas*) were generated by pairing with two monooxygenases, which were further characterized by *in vitro* assay, resulting in the production of the cytotoxic pigment iodinin. More complex phenazines like griseoluteic acid (mostly distributed in *Streptomyces*) were produced by enzymes encoded in the core set of genes of the second BGC activated via promoter exchange to reveal their function. Serendipitously, *in vivo* characterization of the free-

standing NRPS enzymes catalyzing ester-bond formation revealed the production of phenazine-amino acid derivatives active against *E. coli*. Preliminary structure-activity relationship study indicated that the activity was significantly promoted by the 9-methoxyl group. The unusual modular character of these BGCs might be a good example of a stepwise chemical diversification as a response to diverse environments with different other (micro)organisms.

## **P32** Scope and utility of trans-acyltransferases for natural product diversification

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Polyketides are a diverse class of pharmaceutically relevant natural products, whose biological activities include antibiotic, antitumor, and immunosuppressant properties, among others. These compounds are structurally complex, yet they are biosynthesized from a modest pool of small, simple building blocks. Large enzyme complexes known as polyketide synthases (PKSs) are responsible for intricately stitching together these building blocks in a predictable fashion to make the biologically active natural product. PKSs are organized into modules, where each module incorporates a single building block into the final product. This modular templated biosynthesis, enables engineering to program these pathways to produce molecules with improved or modified biological activity. Each module contains an acyltransferase (AT) domain, which is responsible for selecting the appropriate building block and is therefore often the target of engineering through either mutagenesis or entire domain replacement. An alternative approach is to abolish the activity of the natural in-line AT and utilize a discrete AT, known as a *trans*-AT, to introduce a different building block at a programmed position within the pathway. This approach has had limited success but has been hindered by faulty protein-protein interactions and the narrow substrate scope of known *trans*-ATs. Herein, we describe the previously unknown substrate promiscuity of a *trans*-AT from a PKS/NRPS hybrid pathway and probe the molecular basis of its broad specificity. In addition, we describe efforts towards engineering a highly specific *trans*-AT to broaden its substrate scope. Cumulatively, our results can be leveraged for the production of novel polyketide analogues.

## **P34** Chemical perturbation of fruit fly behaviour and development by *Streptomyces.*

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The genus *Streptomyces* is a valuable source of antibiotics and selective modulators of pathway targets. Environmental isolates of 56 strains were cultured and screened using novel assays against multicellular model eukaryotic organisms. 12% of extracts had a wide range of bioactivities that perturbed the behaviour and development of *D. melanogaster*. WAC-288, a strain of interest produced a molecule with starvation-induced larvicidal activity. In addition, the biosynthetic cluster of a volatile organic compound 2-methylisoborneol (2-MIB) was identified which induced a concentration dependent attraction-aversion response. These results describe an antagonistic relationship between *Streptomyces* and insects. Furthermore, this study expands the conventional target diversity of natural products produced by *Streptomyces* and provides novel tools to identify unique bioactive compounds against a wide range of multicellular eukaryotic organisms.

## **P36** Application of bioelectrochemical system for enhancement of 3-hydroxypropionic acid production by regulation of intracellular redox balance

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In spite of the recent advances of genetic and metabolic engineering, the regulation of intracellular redox state for moderating the thermodynamic limitation remains a challenge to develop a bioprocess for platform chemical productions. The aeration and addition of the oxidant/reductant are common strategies

for controlling the cellular redox state ($NAD^+$/NADH). Various chemical oxidants and reductants have been applied in anaerobic condition; however increase of operational cost and inhibition by toxic byproducts makes application less feasible. Bioelectrochemical system (BES) is a novel technology for the regulation of intracellular redox balance via electrochemical reactions between bacteria and electrode. 3-hydroxypropionic acid (3-HP) is commercially and industrially valuable platform chemicals which can be produced by glycerol fermentation; however the conversion of 3-HP is required for $NAD^+$ regeneration from NADH anaerobically. In this report, we applied BES for enhancement of 3-HP production through anaerobic $NAD^+$ regeneration. The recombinant *Klebsiella pneumoniae* L17 overexpressing aldehyde dehydrogenase (AldH) presented 1.8-fold increased 3-HP production yield in BES compared to that of non-BES, also central metabolic shift was found by activation of anaerobic respiration. This is the first report of 3-HP productivity enhancement by the application of BES. The results can provide a strategy of overcoming thermodynamic barrier of fermentation and various biorefinery process using BES by the regulation of bacterial redox balance.

## **P38** Acetate production from carbon monoxide by syntrophic interaction between *Citrobacter amalonaticus* Y19 and *Sporomusa ovata*

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Biological volatile acids production from inorganic carbon such as $CO_2$ and CO through chemosynthesis has been of great interest. However, biological CO conversion is difficult because of its high toxicity and low reducing equivalent. According to previous studies, mixed bacterial community consisted of homoacetogen ($CO_2 + H_2 \rightarrow$ acetate) and water-gas shift reaction catalyzing bacteria ($CO \rightarrow CO_2 + H_2$) presented higher conversion efficiency compared to single strains which have activity of both reactions. We hypothesized that syntrophic interaction (or 'division of labor') between different species is more important in biological CO conversion to produce volatile fatty acids, rather than single bacteria. To demonstrate the hypothesis, defined co-culture fermentation of *Citrobacter amalonaticus* Y19 (water-gas shift reaction catalyzing bacteria) and *Sporomusa ovata* (homoacetogen) was examined for CO conversion. Our results showed that significantly higher CO conversion and acetate production were identified in co-culture fermentation with *C. amalonaticus* Y19 and *S. ovata* compared to that of mono-culture fermentation. CO consumption and acetate production rate increased with electron shuttle, and redox equivalent transfer between two species was identified. The results suggested the model of putative carbon monoxide conversion of mixed culture of homoacetogen and water-gas shift reaction catalyzing bacteria.

## **P40** Examination of clavulanic acid biosynthesis using a comparative genomics approach

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Clavulanic acid is a clinically used inhibitor of certain β-lactamases, enzymes which are responsible for causing resistance to the penicillin and cephalosporin class of antibiotics. Clavulanic acid is industrially produced by fermenting *Streptomyces clavuligerus* and the biosynthetic pathway leading to its production has been partially elucidated, but details regarding the exact boundaries of the gene cluster(s) involved is still lacking. In addition to *S. clavuligerus*, a few other *Streptomyces* species also have the capacity to produce clavulanic acid and the genome sequences of certain microorganisms have been found to contain homologues of the clavulanic acid gene cluster; however the latter are unable to produce the metabolite. Therefore, we sequenced the genomes of isolates capable of producing clavulanic acid and using a comparative genomics/bioinformatics approach we explored the possibility of identifying the core set of genes that are involved in the production of this important secondary metabolite. Our results provide insights into possible reasons as to why some clavulanic acid like gene clusters are silent in

certain *Streptomyces* species. In addition, we also examined the detailed composition and organization of the respective gene clusters, which can provide avenues to identify metabolites related to clavulanic acid.

## **P42** Unlocking Hidden Reservoirs of Microbial Natural Products

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Microbial natural products have been the source of the majority of modern antibiotic and chemotherapeutic drugs and remain an important reservoir of therapeutic molecules. It is estimated that we have accessed less than 1 % of these natural resources largely from either our inability to detect and identify these within complex biological extracts or from the producing strain's lack of production when grown under laboratory conditions. We hypothesize that producing organisms use their natural products to respond to environmental cues from competing organisms, to nutrients and other physical factors and that natural products induced from the application of these cues can be prioritized through comparative metabolomic methods. Here we quantify the global metabolomic and secondary metabolite response to stimuli from 21 diverse strains of actinomycetes exposed to six stimuli conditions. This work identified a number of known natural products that responded strongly to stimuli as well as a novel aminopolyol induced through mixed culture in a rare streptosporangeum strain. The comparative metabolomics methods used in this study are well suited to prioritize secondary metabolites for isolation in a discovery pipeline and may additionally enable the connection between activation stimuli and genetic regulatory elements, resulting in more targeted methods of natural product activation.

## **P44** Engineered production of novel bleomycin analogues by combinatorial biosynthesis

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The bleomycins (BLMs), a family of glycopeptide antibiotics produced by several *Streptomyces* species, are currently used clinically in combination with a number of other agents for the treatment of several types of tumors. Other members of the BLM family include tallysomycins (TLMs), phleomycins and zorbamycin (ZBM). We have previously cloned and characterized the biosynthetic gene clusters for BLMs from *Streptomyces verticillus* ATCC15003, TLMs from *Streptoalloteichus hindustanus* E465-94 ATCC31158, and ZBM from *Streptomyces flavoviridis* SB9001. Applications of combinatorial biosynthesis strategies to the three biosynthetic machineries enabled the engineered production of several BLM analogues with unique structural characteristics and varying DNA cleavage activities, thereby providing an outstanding opportunity to study the structure–activity relationship (SAR) for the BLM family of anticancer drugs. We now report the engineered production of a new BLM–TLM–ZBM hybrid metabolite, named 6′-deoxy-TLM H-1, which consists of the 22-desmethyl-BLM aglycone, the TLM A C-terminal amine and the ZBM disaccharide, by heterologous expression of the *zbmGL* genes from the ZBM biosynthetic gene cluster in the *Streptoalloteichus hindustanus* Δ*tlmH* mutant strain SB8005. Evaluation of the DNA cleavage activities of 6′-deoxy-TLM H-1 as a measurement for its potential anticancer activity, in comparison with TLM H-1 and BLM A2, reveals new insight into the SAR of BLM family of anticancer drugs.

## **P46** Comparative studies of the biosynthetic pathways for anthraquinone-fused enediynes

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Using the genome mining approach, we recently discovered two novel 10-membered enediyne natural products, tiancimycin (TNM) and yangpumicin (YPM). Like dynemicin (DYN) and uncialamycin (UCM), TNM and YPM also feature an enediyne core fused to an anthraquinone moiety. These enediynes exhibit extraordinary cytotoxicity against a broad panel of cancer cell lines and UCM is currently undergoing preclinical investigation. However, little is known about their biosynthetic pathways. Comparative studies of these gene clusters enabled us to prioritize genes for genetic manipulation. Two tailoring genes, *tnmH* (encoding a SAM-dependent methyltransferase) and *tnmL* (encoding a cytochrome P450 monooxygenase), which are present in the *tnm* gene cluster but absent in the *ucm* gene cluster, were selected for gene inactivation. Five new enediyne intermediates (TNM B, TNM C, TNM C2, TNM D, and TNM E), together with many cycloaromatized congeners, were isolated and characterized from the fermentation broth of *Streptomyces* sp. CB03234 wild-type strain and its Δ*tnmH* and Δ*tnmL* mutants. Interestingly, all the congeners from the Δ*tnmH* and Δ*tnmL* mutants possess an unexpected side chain attached to the enediyne core, indicating that the biosynthesis of anthraquinone-fused enediynes is more complicated than we have anticipated. Based on the structures of the isolated congeners, we can propose a unified biosynthetic pathway for the anthraquinone-fused enediynes.

## **P48** Development of a general heterologous expression host for cyanobacterial natural product production.

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Cyanobacteria, particularly marine strains, are prolific producers of bioactive metabolites that utilize interesting biosynthetic logic. Despite the number of potent biologically active compounds, to date only one cyanobacterial compound (dolastatin 10) has been successfully developed as a drug lead (monomethyl auristatin E). Despite isolating significant quantities of bioactive compounds for structural determination and initial biological screening from cyanobacteria, obtaining the larger quantities necessary for *in vitro* mechanism of action studies, and biological studies (animal and human) is hampered by the slow growth rate, inability to genetically manipulate, difficulty in large scale cultivation, and low compound yields of many cyanobacterial strains. These problems could be overcome with the development of a general heterologous expression host for cyanobacterial natural products. Here we describe our progress toward developing the model cyanobacterium *Anabaena* sp. PCC 7120 toward this goal. We have used *Anabaena* 7120 to produce lyngbyatoxin A as a proof of concept molecule showing that *Anabaena* 7120 can recognize promoters from marine cyanobacteria, translate and properly post-translationally modify non-ribosomal peptide synthetases, and produce the desired natural product. We find that simple genetic manipulations and growth condition modification can greatly increase the yield of lyngbyatoxin A in this heterologous host. We also show that *Anabaena* 7120 can recognize promoters from a variety of cyanobacterial natural product gene clusters. We will also present our progress toward genome mining and identification of the cyanobacterial natural products coibamide A and fischerellin A.

## **P50** Natural products discovery from un- and under-explored Actinomycetes strain collection at The Scripps Research Institution (TSRI)

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Natural products possess enormous structural and chemical diversity that is unsurpassed by any synthetic libraries, remain the best sources of drugs and drug leads, but are significantly underrepresented in current small molecule libraries. The Natural Products Library Initiative (NPLI) at the Scripps Research Institute (TRSI) aims at constructing a natural products library with unique chemical and structural diversity that complements the small-molecule collection at TSRI. The current library at TSRI consists of Actinomycetales that are isolated from unexplored or underexplored ecological niches and unavailable in public strain collections (strain collection), their corresponding genomic DNA (gDNA

collection), crude microbial extracts, medium-pressure liquid chromatography fractions, and purified natural products with fully assigned structures. Traditional methods, including bioactivity-guided and chemical profiling-guided approaches, as well as genome mining methods, are used for discovery of novel natural products.

## **P52** Searching for a natural product inhibitor of the tetracycline efflux pump TetA

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Over the past decade antibiotic resistance has been increasing at an alarming rate. The continuous use of antibiotics has resulted in the emergence of resistant bacteria, thereby rendering many of the available treatments for infectious diseases ineffective. However, while there is an acute need for the discovery of new antimicrobial compounds, there is a major lack of new antibiotics coming to market. A solution to this problem is the development of compounds capable of inhibiting the resistance mechanisms of bacteria. When used in conjunction with an antibiotic, the inhibitor blocks the resistance element to allow for the drug to target the bacteria. This approach provides opportunity for the rescue of antibiotics that are no longer used in clinical settings, including tetracyclines. Tetracyclines once exhibited activity against a wide range of bacteria, and had been extensively used in the past to treat a variety of infections. Today, tetracyclines have limited use due to the development of resistance mechanisms, including bacterial efflux pumps capable of exporting drug from the cell. The identification of an efflux pump inhibitor would aid in restoring the efficacy of this “broad-spectrum” antibiotic class. Using high throughput screening, a novel assay has been devised to screen the Wright Lab’s in-house collection of over 10,000 natural product extracts derived from environmental organisms. With this platform we ultimately hope to find a natural molecule capable of targeting the TetA efflux pump in order to rescue the legacy of tetracycline antibiotics.

## **P54** An unknown regulatory system controls intrinsic rifamycin resistance in Actinobacteria

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Rifamycins are ansamycin natural products synthesized by a variety of soil actinobacteria which possess potent antibacterial activity. Rifampicin and rifabutin (semi-synthetic rifamycins) are both World Health Organization (WHO) essential medicines which are crucial to the successful treatment of *Mycobacterium tuberculosis*. Rifamycin antibiotics bind the β-subunit (RpoB) of prokaryotic RNA polymerase and inhibit transcription. Despite the ubiquity of their target among bacteria, rapid development of resistance has prevented widespread clinical use of these compounds. High level resistance to these drugs can be acquired by a single amino acid substitution in RpoB. Despite the availability of this quick and easy route to resistance, a wealth of rifamycin inactivating enzymes are present in environmental Actinobacteria. Rifamycin phosphotransferases, ADP-ribosyltransferases, glycosyltransferases, and monooxygenases have all been characterized. Intriguingly, the genes encoding these inactivating enzymes seem to be induced specifically by rifamycins. This process is known to require a *cis*-regulatory DNA motif termed the rifamycin associated element (RAE), which is present in the promoter region of rifamycin inactivating enzymes across the Actinobacteria. We posit that the RAE is a binding site for a regulatory protein. Preliminary data suggests this system functions via a de-repressible mechanism. We aim to exploit this fact by designing of a two-step genetic selection and screen based on de-repressible phenotypes to isolate mutants in this regulatory pathway. This strategy will allow for the identification of regulatory protein(s) associated with this pathway. Elucidating the mechanism by which environmental Actinobacteria sense and degrade rifamycin antibiotics.

## **P56** Milbemycins production from *Streptomyces avermitilis* through engineering of avermectin biosynthetic pathway

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Milbemycins are 16-membered macrolides, produced from *Streptomyces hygroscopicus* subsp. *aureoaureolacrim* and *Streptomyces bingchenggensis*. They are structurally related with avermectin produced from *Streptomyces avermitilis*. Milbemycins have been known to be very potent acaricidal, insecticidal, and anthelmintic agent with low toxicity. Since milbemycins are commercially important and resistance to the avermectins and their derivatives is increasing, it is crucial to develop an efficient combinatorial biosynthesis system to produce the milbemycins and novel analogs in large quantities. In an attempt to construct a *S. avermiltilis* strain that produces milbemycins, AveA1 and module 7 in AveA3 of avermectin polyketide synthase (PKS) from *S. avermitilis* SA-01 were replaced with MilA1 and module 7 in MilA3 from milbemycin PKS, respectively. The titers of total milbemycins produced by engineered *S. avermitilis* was 291.5mg/l and the major products were C5-*O*-methylated milbemycins B2, B3, and G with milbemycin A3, A4 and D in small amounts. Subsequent inactivation of the C5-*O*-methyltransferase AveD resulted in the production of milbemycin A3/A4 approximately 225mg/l in the flask and 377mg/l in the 5l fermenter culture with trace amounts of milbemycin D. In this study, it was demonstrated that the production of milbemycin was only slightly decreased by engineering avermectin biosynthetic pathway. Application of the same strategy in the higher producing industrial strain will further increase the desired product titer and also allow generation of novel milbemycin analogs with improved properties in sufficient amounts for further development.

## **P58** Regulatory and mutational analysis of the biosynthetic gene cluster for lugdunomycin, a novel antibiotic with unprecedented chemical architecture

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The introduction of major new classes of natural product-based antibacterial agents has come to a near standstill while antimicrobial resistance is rising sharply. The approval of daptomycin in 2003 marked the launch of the first new natural product-based antibiotic in decades. We have recently discovered a novel compound, called lugdunomycin, produced by *Streptomyces sp*. QL37. The compound is derived from the well-known angucyclines. The structure of lugdunomycin has been confirmed by NMR, high-resolution mass spectrometry and X-ray crystallography. Our work is focused on understanding the complex biosynthetic pathway for lugdunomycin biosynthesis, and to unravel the transcriptional control of the biosynthetic gene cluster. Lugdunomycin is derived from the angucycline backbone by complex enzymatic reactions. A Baeyer-Villiger oxidative cleavage in the quinone ring of the angucycline backbone is the likely first step in the rearrangement of angucyclines to generate the lugdunomycins. Besides lugdunomycin, 27 other rearranged and/or unrearranged angucyclines were identified in *Streptomyces sp*. QL37. This study starts from the perspective of the type II polyketide biosynthetic gene cluster that is responsible for angucycline and lugdunomycin biosynthesis. Gene disruption strategies using homologous recombination have been employed to elucidate the function of the biosynthetic and regulatory genes. *Streptomyces sp.* QL37 produces very low amounts of lugdunomycin and the compound is not produced in submerged cultures, despite testing a wide range of different culturing conditions. Strategies to improve the production of the compound by engineering of the regulatory network will also be discussed.

## **P60** High-yield production of herbicidal thaxtomins and analogs in a nonpathogenic *Streptomyces* host

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Thaxtomins are virulent factors of several plant pathogenic *Streptomyces* strains. Because of their potent herbicidal activity, environmental compatibility and biodegradability, thaxtomins are EPA-approved green herbicides. However, the low yield of thaxtomins in native *Streptomyces* producers limits their wide applications in agriculture. Synthetic biology is an emerging principle and has demonstrated its successes in producing a wide variety of natural and unnatural chemicals. Here, we describe the high-yield production of thaxtomins and the synthesis of one unnatural analog through mutasynthesis in a heterologous host *S. albus* J1074. The thaxtomin gene cluster from *S. scabiei* 87.22 was cloned and expressed in *S. albus* J1074 on a self-replicative plasmid and on its chromosome. The production of thaxtomins and nitro-tryptophan analogs was detected. When cultured in the minimal medium TMDc, the yield of thaxtomin A from *S. albus* J1074 was 10 times higher than *S. scabiei* 87.22, and optimization of the medium resulted in the highest yield of over 150 mg/L. Engineering the thaxtomin cluster led to the production of multiple biosynthetic intermediates important to the chemical synthesis of new analogs. Further, the versatility of the biosynthetic system was revealed in the mutasynthesis as the production of 5-F-thaxtomin A whose structure was determined by a combination of MS and NMR analysis. Both natural and unnatural thaxtomins demonstrated weak anticancer activity toward Jurkat and PC-3 cancer cell lines and potent herbicidal activity in radish seedling assays. These results indicated that *S. albus* J1074 has the potential to overproduce thaxtomins and thereof, fostering their agricultural applications.

## **P62** Uncovering the biosynthetic origins of cyclic phenolic natural products in pelagophyte algae.

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The chrysophaentins are cyclic phenolic natural products isolated from the colonial marine pelagophyte alga *Chrysophaeum taylori*, collected in the US Virgin Islands. These compounds inhibit the essential bacterial cell division protein FtsZ, a promising but unexploited target for antibiotic drug development, and show excellent therapeutic potential. However, chemical synthesis has so far been unable to provide a route for production of the drugs. We aim to use a synthetic biology approach to the preparation of the chrysophaentins, or advanced intermediates, by identifying the genes responsible for their production in a laboratory-cultivated strain of *C. taylori*, which we have shown to produce novel chrysophaentin analogues. In our biosynthetic hypothesis, these molecules derive from the action of a type III polyketide synthase on a phenylalanine derivative, followed by phenol coupling catalyzed by a cytochrome P450. We will sequence the genome of *C. taylori*, and characterize the complement of these gene families identified within.

## **P64** Enediyne diversification by a cofactor-promiscuous methyltransferase

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SAM-dependent methyltransferases are the most prevalent catalysts in biology to perform chemo- and regio-selective alkylations, which play diverse roles ranging from modulating gene expression to tailoring secondary metabolite structures. Previous reports have demonstrated that certain methyltransferases have cofactor promiscuity and can accept SAM analogs. In this study, we present a structural and functional characterization of TnmH, a phenolic O-methyltransferase involved in tailoring the anthraquinone ring of the tiancimycins. We demonstrate its broad substrate scope and elucidate the timing of methylation in the biosynthesis of the tiancimycins. TnmH demonstrates cofactor promiscuity towards SAM analogs, as well as the ability to completely turnover non-native substrates and cofactors in preparative scale enzymatic reactions. We facilitate turnover by including S-adenosylhomocysteine

nucleosidase from E. coli to alleviate co-product inhibition. This study sets the stage to use TnmH to generate tiancimycin analogs and bioconjugates.

## **P66** Identification and heterologous expression of the whole valinomycin gene cluster from the endophytic strain *Streptomyces* sp. CBMAI 2042

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Actinobacteria are a phylum of gram-positive terrestrial or aquatic bacteria of significant importance for pharmaceutical industry and academic research due to their great potential to biosynthesize different types of natural products, including antibiotics and anticancer agents. *Streptomyces* is the largest bacterial genera from this phylum and several sequenced genomes have revealed an abundance of gene clusters codifying enzymes from secondary metabolism demonstrating an underestimated potential to produce important bioactive metabolites. Among the most useful natural products, those biosynthesized by nonribosomal peptides synthetases (NRPS) and polyketide synthases (PKS) share both high molecular complexity and therapeutic activity.
The NRPS gene cluster encoding the biosynthesis of a cyclic dodecadepsipeptide ionophore, valinomycin, was annotated from the whole genome sequencing of the endophytic strain *Streptomyces* sp. CBMAI 2042 isolated from *Citrus* ssp. The association of fermentation process, molecular networking and mass spectrometry imaging revealed the production of the ionophore by the endophytic strain. Additionally, the heterologous expression of the entire valinomycin gene cluster in *S. coelicolor* host was successfully promoted. Although the valinomycin titer resulting from these experiments (2 mg $L^{-1}$) is still modest compared to the one achieved after optimization of enzyme-based high cell fed-batch cultivation in heterologous *E. coli* (10 mg $L^{-1}$), this represents the first example of whole VLM gene cluster heterologous expression in *Streptomyces*. The heterologous host *Streptomyces* carrying the whole enzymatic arsenal required to promote the biosynthesis of VLM provides a versatile and unique platform for production of new valinomycin derivatives in future studies.

## **P68** Enzymatic substitution of amides with thioamides on peptidic substrates

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Ribosomally synthesized post-translationally modified peptides (RiPPs) are a class of natural products that feature various degrees of enzymatic tailoring to a precursor peptide. The resulting chemical structures impart a diversity of bioactivities within this natural product class. Sulfur substitution of peptide backbone amide oxygens is a form of modification present in several RiPPs and methyl-coenzyme M reductase (MCR), the hallmark enzyme for anaerobic methanogenesis. Despite the significant impact thioamides are expected to exert on the conformational dynamics of a peptide or protein, their biosynthetic installation remains enigmatic. A recent study has shown that *ycaO* and *tfuA* genes are responsible for this modification on MCR. Analogous to azoline formation catalyzed by some YcaO homologs, we hypothesized that the YcaO protein installs the thioamide onto its peptidic substrate by the ATP-dependent activation of the amide backbone. *In vitro* reconstitution studies have revealed that YcaO proteins from methanogens indeed catalyze thioamide substitution on MCR-derived peptides in the presence of ATP and inorganic sulfide and that the TfuA partner protein appears to facilitate the reaction. The YcaO from *Methanopyrus kandleri* was shown to bind ATP in a manner characteristic to the YcaO family by crystallography study, which supported the ATP-dependent activation mechanism. Substrate tolerance and engagement of the YcaO was investigated by mass spectrometry-based enzymatic assays and fluorescent polarization-based binding studies. With the biochemical capability established, we subsequently surveyed the available bacterial and archaeal genomes for TfuA-associated YcaO-encoding biosynthetic gene clusters, which has revealed thioamidated peptides are likely severely underrepresented as RiPP class.

## **P70** Analysis of dichloroisoeverninic acid biosynthesis in pursuit of novel everninomicin analogs

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The everninomicins are complex oligosaccharides with broad antimicrobial activity produced by *Micromonospora carbonacea*. However, toxicity issues and an inability to easily access analogs stifled their previous development. Recent crystal and cryo-EM structures of everninomicin A bound to the bacterial ribosome provide a detailed map of the vital interactions responsible for the everninomicins' activity. The structures reveal crucial interactions between the 50S subunit and the aromatic dichloroisoeverninic acid (DCE) moiety of everninomicin. All natural everninomicins contain at least one iterative type I polyketide synthase (iPKS)-derived DCE moiety. We propose to access everninomicins with derivatized aromatic moieties via the biosynthetic machinery. In order to gain a better understanding of DCE biosynthesis, we deleted the four putatively associated genes: an iPKS, an O-methyltransferase (O-MT), a flavin-dependent halogenase (FDH), and a *trans* acyltransferase (AT). Functional analysis of these genes confirmed their assignment and provided seven novel everninomicin metabolites. These results demonstrate that the iPKS EvdD3 is responsible for the biosynthesis of the DCE core scaffold, orsellinic acid. Orsellinic acid is transferred to the terminal D-olivose sugar residue by AT EvdD1 to be subsequently tailored by O-MT EvdM5 and FDH EvdD2 to yield DCE. This work also revealed that transfer of the evernitrose sugar to D-olivose requires the presence of the nearly complete DCE ring. Current work is focused on *in vitro* analysis of the DCE-associated enzymes to evaluate incorporation of non-natural substrates. This work will further elucidate the biosynthesis of the everninomicins and provide novel analogs to revitalize this potent class of antibiotics.

## **P72** Investigations of the biosynthesis and signaling function of salinipostin, an unusual phosphotriester gamma-butyrolactone compound from *Salinispora*

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Salinipostins are anti-malarial compounds from the marine actinomycete, *Salinispora pacifica*. These molecules possess a unique structure consisting of a rare phosphotriester and a gamma-butyrolactone ring that resembles signaling molecules such as A-factor from *Streptomyces griseus.* In *S. griseus*, A-factor regulates morphological development and secondary metabolism via the A-factor signaling cascade. The structural similarities between salinipostin and A-factor suggested that salinipostins play a similar regulatory role in the genus *Salinispora*, evoking the hypothesis that the salinipostin is the first signaling molecule identified in *Salinispora*. We identified a key A-factor-like biosynthesis gene (A-factor synthase, *spt9*) within biosynthetic operon that we suspect is the biosynthetic gene cluster of salinipostin. To confirm the assignment of the salinipostin BGC and investigate its function, we deleted *spt9* in three species of *Salinispora*, which abolished salinipostin production. LC-MS chemical profile analyses using wild-type and salinipostin-deficient mutants indicated the function of the salinispostin as a regulator of the secondary metabolism. Our investigations of salinipostin biosynthesis have validated the BGC and lead to a proposed biosynthetic pathway. In further investigations, we expressed soluble enzymes in heterologous hosts and chemically synthesized substrates. With these resources, the early stage of biosynthesis constructing the gamma-butyrolactone structure was recapitulated *in vitro* starting from Spt9 reaction with SNAC mimic of beta-ketoacyl-ACP and dihydroxyacetone phosphate. Interrogation of salinipostins may represent a key step in unlocking the biosynthetic potential in *Salinispora*, and also study of biosynthetic pathway will enhance our basic understanding of the biochemistry responsible for the construction of a rare phosphotriester motif.

## **P74** Production of novel glycosylated macrolactam analogues of the macrolide antibiotic YC-17 by chemoenzymatic synthesis

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YC-17, a 12-membered ring macrolide antibiotic produced from *Streptomyces venezuelae* ATCC 15439, consists of polyketide macrolactone 10-deoxymethynolide attached with D-desosamine. A combined approach including chemical synthesis and engineered cell-based generation of unnatural deoxyhexose sugar and aglycone biotransformation was used to generate a range of biologically active macrolactam analogues of YC-17. First, a macrolactam analogue of the YC-17 aglycone 10-dml, aza-(10-dml) (AZDM), was chemically synthesized and an engineered strain of *S. venezuelae* with its substrate-flexible glycosyltransferase was used to assemble the new glycosylated compounds. The *S. venezuelae* YJ028 mutant strain in which the entire biosynthetic gene cluster encoding the pikromycin PKS and desosamine biosynthetic enzymes were deleted was chosen as the biotransformation host, and different deoxy sugar biosynthetic gene cassettes and the genes encoding DesVII/DesVIII were expressed. Due to inherent flexibility, DesVII/DesVIII can transfer a range of structurally diverse TDP-sugars, demonstrating their potential for the structural diversification of macrolide antibiotics both *in vitro* and *in vivo*. As a result, engineered strains of *S. venezue*lae capable of generating desosamine and varied alternative sugar moieties produced eight novel macrolactam analogues of YC-17. Some of the novel AZDM glycosides present improved *in vitro* antibacterial activities against both erythromycin-susceptible and erythromycin-resistant pathogens and metabolic stabilities compared to erythromycin. These results demonstrate the successful application combining chemical synthesis with flexible glycosylation enzymes to generate a variety of novel glycosylated macrolactam analogues of YC-17 with potent antibiotic activity for drug discovery and development.

## **P76** Antifungal activity of FK506 derivatives produced by engineering post-PKS modification steps

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FK506 is a 23-membered macrolide polyketide and used as a immunosuppressant drug for preventing the rejection of organ transplants. Furthermore, FK506 possesses various pharmaceutical potentials, including antifungal, neuroprotective, and neuroregenerative activities. A hybrid polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) system is responsible for the biosynthesis of the macrolide ring. This ring is further modified via remarkable two parallel pathways of post-PKS modification steps including C-9 oxidation by FkbD and 31-*O*-methylation by FkbM. The novel FK506 analogues, 31-*O*-demethyl-FK506, 9-deoxo-FK506, 9-deoxo-31-*O*-demethyl-FK506 and 9-deoxo-prolyl-FK506, were produced in *fkbD* or *fkbM* deletion mutant strain of *Streptomyces sp.* KCTC11604BP. FK506 derivatives were tested for fungal infections caused by *Cryptococcus neoformans*. Antifungal activities of all derivatives were maintained for *C. neoformans*. Especially, 31-*O*-demethyl-FK506 displayed significantly increased activity compared to FK506. Furthermore, these analogues did not have any neurotoxicity *in vitro*, but have remarkably lower immunosuppressive activity than FK506. These results would contribute to the development of new antifungal agents from FK506 with reduced immunosuppressive activity and toxicity. Overall, this study provides the potential of biosynthetic engineering approaches to generate improved bioactive molecules.

## **P78** GC-MS metabolite profiling of siderophore-producing *Bacillus subtilis* rhizobacteria isolated from maize (*Zea mays* L.) rhizosphere

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Plant growth promoting rhizobacteria (PGPR) strain A1 identified as *Bacillus subtilis* through 16S rDNA gene sequencing was assayed for its metabolite production using GC-MS technique. This siderophore producing bacteria was tested against fungal pathogen *Fusarium graminearum* and bacterial pathogens *Enterococcus faecalis* and *Bacillus cereus*. It showed considerable resistance to these pathogens. The strain has also been previously tested for its plant growth promoting ability on maize plants. The metabolites in the form of volatile organic compounds (VOCs) produced were isolated and analyzed with Gas chromatography-mass spectrometry (GC-MS) using eight solvents for extraction. The solvents used are chloroform, ethyl acetate, diethyl ether, n-hexane, methanol, butanol, petroleum ether, and benzene. Two metabolites were identified from butanol extract while benzene, ethyl acetate and hexane all show one notable bioactive metabolite. Petroleum ether and methanol have the largest return of metabolites with five and fifteen respectively. Chloroform and diethyl ether did not detect any hit in their GC-MS chromatograms. The detected volatile compounds could be chemically grouped into ketones, alcohols, aldehydes, pyrazines, acids, esters, pyridines and benzene compounds. This result identifies the metabolite produced by *Bacillus subtilis* after successful trial in the field.

## **P80** Improvement of crude extract yield of *Streptomyces* sp 796.1 with different carbon sources

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Production of secondary metabolites used in drugs is a characteristic of the genus *Streptomyces.* From a starfish an isolate identified as *Streptomyces* sp 796.1 with antiproliferative activity against nine tumor cell lines. However, the yield of the crude extract is 100 mg/L being about 50% for the bioactive fraction. The objective of this study was to evaluate the effect of the carbon source on the extract yield. A triplicate fermentation test was performed using R2A supplemented with artificial sea water as the base broth, the carbon source (dextrose 0.05%) being modified by L-arabinose 0.05% w/v, glycerol 1% v/v and starch 1% w/v. The chemical profiles obtained by mass spectrometry (UHPLC-ESI-MS) of the crude extracts with modified carbon source, the control and the bioactive fraction were compared. The masses of the control crude extract were 100% detected in the starch broth, 93% in L-arabinose and 78% in glycerol, whereas 91% of the masses of the bioactive fraction were detected in all extracts. However, the yield of the Starch broth was 33 mg/L, but the broth with glycerol increased the yield respect to the control (430 mg/L aprox). This indicates that the synthesis of the secondary metabolites with antiproliferative activity in this microorganism is not affected by the carbon source, with glycerol being the best alternative carbon source to improve yield.

## **P82** Effect of temperature and pH on the metabolism of *Escherichia coli* for bioactive molecules.

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Biological systems are known to be robust and adaptable to the culture environment and such robustness is inherent in the biochemical and genetic networks. The overall regulation mechanism was clarified under both pH down-shift and temperature up-shift based on fermentation characteristics and gene transcript levels. Upon temperature up-shift down regulation of glucose uptake rate corresponds to the down regulation of *ptsG* caused by the up-regulation of *mlc* gene expression. More acetate was formed with lower biomass yield and less glucose consumption rate at pH 5.5 as compared to the case at pH 7.0 under both aerobic and anaerobic conditions. The gene expressions indicate that the down- regulation of the glucose uptake rate corresponds to the down-regulation of *ptsG* gene expression caused by the up-regulation of *mlc* gene which is under positive control of Crp. In accordance with up-regulation of *arcA* gene expression at acidic condition, the expressions of the TCA cycle-related genes such as *icdA* and *gltA*, and the respiratory chain gene *cyoA* were down-regulated, whereas *cydB* gene expression was up-regulated. The decreased activity of TCA cycle caused more acetate formation at lower pH. The research

on stress response of a microorganism contributes to the variety of practical applications such as temperature-induced heterologous protein production simultaneous saccharification and fermentation (SSF) etc

## **P84** Cell-free synthetic biology for natural product bio-discovery

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Cell-free synthetic biology is an emerging field that utilizes clarified lysates, or cell-free systems, for conducting expression from DNA to protein. These systems can be thought of as open factories that conserve the cell's ability to conduct complex biochemistry but allow for addition and subtraction of necessary cofactors to drive reaction completion. We present Synvitrobio's cell-free synthetic biology platform for natural product bio-discovery. In particular, we discuss Synvitrobio's efforts to express functional ribosomal synthesized and post-translationally modified peptides (RiPPs) in its cell-free systems and corresponding antibiotic activity, and work characterizing the technical feasibility of expression of different natural product clusters in its cell-free platform.

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## **P86** Toward an enzyme-coupled, bioorthogonal platform for methyltransferases

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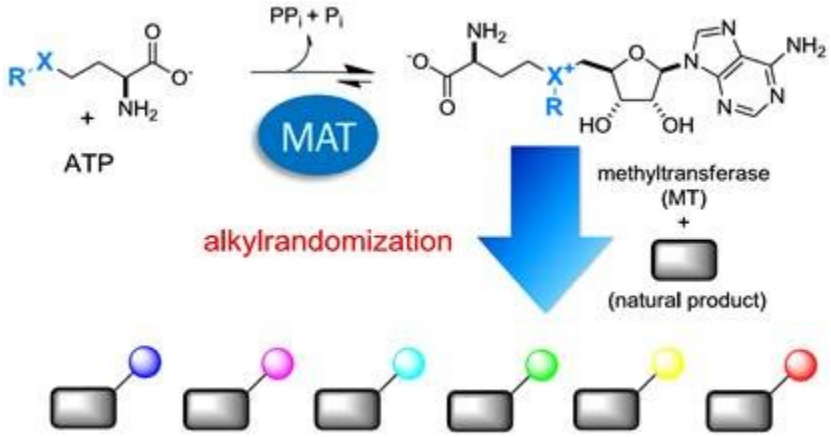
Methyl group transfer from *S*-adenosyl-l-methionine (AdoMet) to various substrates including DNA, proteins, and natural products (NPs), is accomplished by methyltransferases (MTs). Analogs of AdoMet, bearing an alternative *S*-alkyl group can be exploited, in the context of an array of wild-type MT-catalyzed reactions, to differentially alkylate DNA, proteins, and NPs. This technology provides a means to elucidate MT targets by the MT-mediated installation of chemoselective handles from AdoMet analogs to biologically relevant molecules and affords researchers a fresh route to diversify NP scaffolds by permitting the differential alkylation of chemical sites vulnerable to NP MTs that are unreactive to traditional, synthetic organic chemistry alkylation protocols. The full potential of this technology is stifled by several impediments largely deriving from the AdoMet-based reagents, including the instability, membrane impermeability, poor synthetic yield and resulting diastereomeric mixtures. To circumvent these main liabilities, we present novel chemoenzymatic strategies that employ methionine adenosyltransferases (MATs) and methionine (Met) analogs to synthesize AdoMet analogs *in vitro*. Unstable AdoMet analogs are simultaneously utilized in a one-pot reaction by MTs for the alkylrandomization of NP scaffolds. As cell membranes are permeable to Met analogs, this also sets the stage for cell-based and potentially even *in vivo* applications. We will also present the use of Met and ATP isosteres in the context of MAT-catalyzed reactions toward the generation of highly stable AdoMet analogs and their downstream utilization by MTs. Finally, we will present the development, use, and results of a high-throughput screen to identify mutant-MAT/Met-analog pairs suitable for postliminary bioorthogonal applications.




## **P88** Discovery, Pathway Engineering and Characterization of a Novel Rapamycin/FK506 Family Member by an Integrated Genome Mining Platform

*E. Pan*, Warp Drive Bio. Inc., Cambridge, MA, USA*

Natural products derived from polyketide synthases (PKS) represent an important class of commercially and medicinally relevant natural products, e.g., the antibiotic erythromycin, the immunosuppressants rapamycin and FK506, the anthelminthic agent avermectin, and the insecticide spinosad. To discover new natural products including PKS-derived compounds, Warp Drive Bio has assembled an enormous proprietary and searchable database of microbial genomes, many of which contain both known and novel biosynthetic gene clusters. To date, our database is comprised of sequence of ~135,000 genomes, estimated to encode the biosynthesis of ~3.5 million compounds. In our genome mining campaign, we identified WDB-002, a cyclic polyketide/NRPS natural product, by searching for biosynthetic clusters related to FK506 and Rapamycin. We describe methods to engineer production of WDB-002 in native and heterologous host systems by the overexpression of a non-cognate positive regulator. The structure of WDB-002 was determined by 1D- and 2D-NMR combined with mass spectrometry, and FKBP12-binding was confirmed by multiple assays. We describe the unexpected biosynthetic features of WDB-002 as compared to Rapamycin and FK506

## **P90** Elucidating the biosynthesis of novel nucleoside antibiotic structural features

*J. Overbay* and S.G. Van Lanen, University of Kentucky, Lexington, KY, USA*

Drug-resistant bacterial pathogens are rapidly becoming a widespread problem in the United States and across the globe. Meanwhile, new antibiotics entering the clinic are alarmingly scarce. Highly-modified nucleoside antibiotics, a class of natural products, target MraY bacterial translocase I. MraY is a clinically unexploited enzyme target, essential in peptidoglycan cell wall biosynthesis. This class of secondary metabolites have strong antibacterial activity against Gram-positive pathogens, yet are greatly underexplored. A subset of nucleoside antibiotics contains a characteristic 5'-C-glycyluridine (GlyU) core. The newly discovered nucleoside antibiotic, sphaerimicin, has a novel scaffold that makes it distinct from the GlyU-containing nucleosides known to date. In sphaerimicin, the GlyU core is ultimately appended to a dihydroxylated piperidine that is bridged to the 5'' amine of a 5-amino-5-deoxyribosyl moiety. Additionally, sphaerimicin features a unique 3'-sulfate. Our study will investigate these modifications, which we propose are partially achieved via incorporation of the 3'-sulfate using a novel aryl sulfatase, as well as significant scaffold extension with an aminotransferase and transketolase. Importantly, elucidating how this scaffold is biosynthesized may lead to discovery of new enzymatic chemistries that will power innovative chemoenzymatic synthesis and genome mining to uncover new natural products.

### **P92** The biosynthetic mechanism of the antibiotic Capuramycin

*E. Yan*, Pharmaceutical sciences, LEXINGTON, KY, USA and S.G. Van Lanen, University of Kentucky, Lexington, KY, USA*

Natural products have played an important role in the discovery of antibacterial agents since the introduction of penicillin in the 1940s. Until 2012, natural products or derivatives of natural products contributed about 75% of the total FDA-approved antibacterial agents. However, the discovery of novel antibiotics has dramatically decreased over the last few decades while infectious disease, and notably tuberculosis (TB), remains a major threat to global health. Thus, the discovery and development of new antibiotics are urgently needed. Capuramycin, was a kind of nucleoside antibiotics discovered in screening programs for new antibiotics in 1980s. Capuramycin-type antibiotics include A-500359s, A-503083s, and A-102395. The biosynthetic gene cluster and pathway for A-500359 and A-503083 have been identified and characterized. But the biosynthesis mechanism for A-102395 has not been fully resolved. The function of several of the gene products is difficult to predict based solely on sequence analysis, which is perhaps not unexpected since the structure of the capuramycin family of antibiotics consists of several novel chemical features. We hope to characterize the function of gene cluster of A-102395 and provide new insight into the resistance and biosynthetic strategies for the capuramycin-type antibiotics.

# Tuesday, January 23

## 7:00 AM - 8:00 AM Breakfast

Flamingo/Sandpiper Deck

## 7:00 AM - 4:00 PM Registration

Grand Ballroom Foyer, lobby level

## 8:00 AM - 11:45 AM Session: 3: New tools driving discovery of bioactive natural products

**Conveners:** **Rolf Mueller**, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbrucken, Germany and **Tom Ramseier**, Dow AgroSciences LLC, Indianapolis, IN, USA

Salons F-G, lobby level

### **8:00 AM** S13: Lessons learned from mining 10,000 actinobacterial genomes for phosphonic acid natural products

*W. Metcalf*, University of Illinois, Urbana, IL, USA*

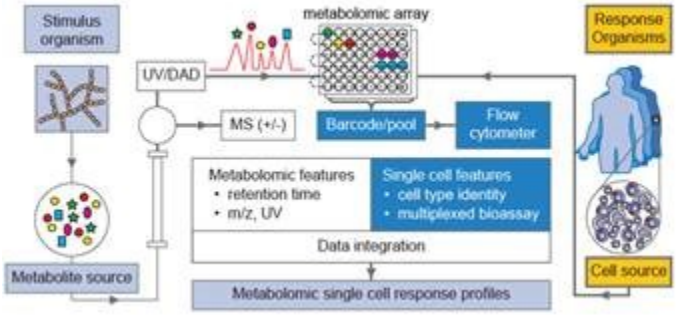
Microbially produced natural products encompass an astounding array of small molecules with useful, and often medically relevant, properties. Accordingly, approximately 75% of the currently used antibiotics are derived from these microbial metabolites, while nearly 60% of anti-cancer drugs have a similar origin. Despite these historical successes, traditional screening programs have been severely curtailed due to dramatically declining numbers of promising new candidates. Nevertheless, analysis of microbial genomes suggests that hundreds of thousands of high-value natural products have yet to be identified.

These seemingly contradictory facts led many to adopt “genome-mining” as an alternative natural product discovery strategy. While it is clear that this approach can be fruitful, the underlying assumptions that would justify widespread adoption of this technology have never been rigorously established. Over the past decade we have systematically addressed these issues through a genome-mining campaign to discover phosphonic acid natural products in over 10,000 diverse actinobacteria. The lessons learned from this project strongly validate the genome-mining approach, while at the same time highlighting key obstacles that must be overcome before genome-mining can become as the discovery method of choice.

## **8:30 AM** S14: Prioritizing the ‘embarrassment of riches’ in genome mining campaigns via Multiplexed Activity Metabolomics

*B.O. Bachmann*, Vanderbilt University, Nashville, TN, USA*

Microbial genomic studies have revealed a vast untapped potential in natural product producing bacteria and fungi. This reality has stimulated development of a wide variety of strategies to unlock this potential, including biosynthetic pathway refactoring, heterologous expression, and chemical or biological stimulation of producing organisms. While prioritization of a product of a biosynthetic gene cluster via predictive structural analysis is a powerful approach to new compound discovery, the ultimate bioactivity of such compounds remains more speculative, rendering the isolation and identification of such compounds a shoot-first-ask-questions-later proposition. This is due to the fact that the labor of isolating and structurally elucidating lead compounds continues to represent the major bottleneck in secondary metabolite discovery. Herein we describe a method to assess the activity of metabolites produced from genome mining campaigns from within the metabolomes of the producing strains, prior to their isolation. We describe an ultra-high throughput method to assess the activity of metabolites within a comprehensive metabolome in which we analyze the effects of metabolites against a heterogeneous biopsied mixture of diseased and healthy human cells (primary cells) derived from cancer patients at single cell resolution, and across a broad panel of cell status markers. This method, which we call Multiplexed Activity Metabolomics, assays a given metabolomic inventory against a cell source, assaying against multiple targets in multiple cell types, to identify bioeffectors within a given metabolome. This provides a means to prioritize upregulated metabolites from within genome mining strains by determining their potential as clinically interesting bioeffectors.




## **9:00 AM** S15: Overcoming strain engineering challenges to increase production of UK-2A, the precursor of the Fungicide Inatreq™ Active

*T. Ramseier*, E. Miller, K. Hill, J. Hu and B. Raman, Dow AgroSciences LLC, Indianapolis, IN, USA*

Actinobacteria produce secondary metabolites that serve a variety of functions and a subset of these molecules have agricultural applications. *Streptomyces sp. 517-02* for instance naturally generates a fungicidally active compound called UK-2A, which can be converted into Inatreq™ active through chemical modification to enhance its activity against the plant pathogen *Zymoseptoria tritici*. To achieve commercially relevant fermentation productivities for UK-2A, several avenues were pursued including mutagenesis and high-throughput (HTP) screening, fermentation process optimization, and targeted genetic engineering of *Streptomyces sp. 517-02*. For the latter, biosynthesis bottlenecks were identified using various approaches including UK-2A biosynthetic gene overexpression and precursor feeding experiments. Native promoter identification using omics approaches allowed temporal gene expression suitable for enhanced precursor production and hence UK-2A production. Specific challenges associated with achieving these goals along with the means by which they were overcome to increase UK-2A production will be discussed.

## 9:30 AM Break

## 9:45 AM S16: Harnessing genomic information to engineer the biosynthesis of novel natural products

*D. Gray*, Warp Drive Bio, Cambridge, MA, USA*

Natural products derived from polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS) represent an important class of commercially and medicinally relevant natural products. To discover new natural products including PKS- and NRPS-derived compounds, Warp Drive Bio has assembled an enormous proprietary and searchable database of microbial genomes, many of which contain both known and novel biosynthetic gene clusters. To date, our database is comprised of sequence of ~135,000 genomes, encoding the biosynthesis of ~3.5 million compounds. Many of the biosynthetic gene clusters contained in this database have yet to be reported in the literature, and thus Warp Drive's genomic database offers an innovative, unprecedented opportunity to discover entirely new scaffolds for drug development.

The presentation will focus on recent progress toward engineering natural product clusters as part of Warp Drive's "genomes to molecule" platform. To address the challenge of activating clusters that are 'OFF' or lowly expressed, we have developed several strategies to turn clusters 'ON' to boost compound production. We will also report progress on engineering new analogs of polyketide natural products by novel engineering strategies at the domain and module level. To illustrate the broad capacity of the "genomes to molecule" platform to discovery novel chemistry with high therapeutic potential, the discovery of novel Rapamycin/FK506-family members and novel antibiotic biosynthetic clusters will be discussed.

## 10:15 AM S17: Employing stable isotope labeling to connect gene clusters and their cognate products; A systems approach

*R. Linington*, Department of Chemistry, Simon Fraser University, Burnaby, BC, Canada*

Despite continued advances in our understanding of the structure and function of microbial biosynthetic gene clusters, relating novel clusters to their cognate products remains a challenging and time-consuming process. Improvements in heterologous expression systems and recent developments in controlling regulatory domains (e.g. CRISPR-Cas9) are providing the field with new tools in this area. While powerful, these methods are typically best suited to the investigation of individual clusters, and require separate biochemical manipulations for each cluster to be studied.

Stable isotope labeling has long been employed as a tool for characterizing processes in microbial biosynthesis. Traditionally this approach has been used either to determine the biosynthetic origin of a specific compound, or to investigate the mechanistic processes by which a particular functional group is produced in nature. By combining recent advances in untargeted mass spectrometry-based metabolomics and parallel stable isotope labeling methods, our laboratory is developing new tools to relate all expressed gene clusters to their associated products in a scalable, high-throughput manner. Preliminary results from this new platform will be presented.

## 10:45 AM S18: Discovery and development tools for (myxo)bacterial anti-infectives

*R. Mueller*, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Saarbrucken, Germany*

Microbial natural products continue to play a major role in drug discovery efforts. Genome analyses and genome mining efforts as well as biodiversity analyses indicate that large parts of the microbial secondary metabolome are currently untapped. However, the number of novel and exciting seondary metabolites reported in the literature is rather disappointing and rediscovery rates of known compounds has become a serious obstacle in the field. I will describe our efforts to a) isolate and analyse previously uncultured

myxobacteria via secondary metabolomics (1), b) employ innovative production setups to trigger production of novel metabolites (2), c) en route make best use of genomic information including genome mining efforts (3,4) and mode of action analyses (5) and d) develop and employ synthetic biotechnlogy tools to optimise compounds and productivity (6).


1. Hoffmann, T. et al (2017) Bacterial versus chemical diversity: The phylogeny paradigm in microbial natural product discovery, Nat. Commun., accepted.
2. Wagner, S. et al (2017) Covalent lectin inhibition an application in bacterial biofilm imaging, Angew. Chem. Int. Ed., accepted.
3. Viehrig, K. et al (2017) Structure and biosynthesis of crocagins: Polycyclic postranslationally modified ribosomal peptides from *Chondromyces crocatus,* Angew.Chem.Int.Ed., 56(26),7407-10.
4. Hüttel, S. et al (2017) Discovery and total synthesis of natural cystobactamid derivatives with superior activity against Gram-negative pathogens, Angew.Chem.Int.Ed., 56(41):12760-64.
5. Fu, C. et al (2017) Carolacton is a potent myxobacterial inhibitor of folate biosynthesis via specific interaction with FolD/MTHFD, Nat.Commun., 8(1):1529.
6. Sucipto, H. et al (2017) Heterologous production of myxobacterial α-pyrone antibiotics in *Myxococcus xanthus,* Metab.Eng.*,* 44:160-170.


### **11:15 AM** S19: The human microbiota as source of the cyclic thiazolidine peptide antibiotic lugdunin


*B. Krismer*[*]*, Dept. of Microbiology, University of Tubingen, Tubingen, Germany*


Within the last decades, numerous new sources have been exploited in the search for new compounds and producers. Interestingly, the human microbiota was completely neglected, although these bacteria get in closest contact with human pathogens. Recent studies indicated that the human microbiota contains a wealth of biosynthetic gene clusters for secondary metabolites with unknown potential. We investigated the human nose, which is the primary habitat of the human pathogen *Staphylococcus aureus*, for the presence of other Staphylococci with inhibitory potential. The screening indicated that more than 80 % of all staphylococcal isolates produced growth-inhibiting metabolites, which were active against non-*Staphylococcus* species, whereas only two were active against *S. aureus*. Further investigation of one of these producer strains, *Staphylococcus lugdunensis*, revealed the essentiality of a by then unknown NRPS operon for the production of the anti-*S. aureus* activity. Regulated overexpression, purification and structure elucidation of the active molecule disclosed an unknown cyclic thiazolidine-containing peptide antibiotic. This peptide, termed lugdunin, is bactericidal against major pathogens, effective in animal models, and not prone to causing development of resistance in S. aureus. Analysis of numerous *S. lugdunensis* isolates showed that lugdunin production is a species trait and two independent human studies confirmed that human nasal colonization by *S. lugdunensis* is associated with a significantly reduced *S. aureus* carriage rate. Overall, the identification of *S. lugdunensis* and further nasal species or strains with antibacterial properties against *S. aureus* highlight the potential of the human microbiota as a source for new compounds.

# 11:30 AM - 1:00 PM Lunch

Flamingo/Sandpiper Deck

# 1:00 PM - 4:45 PM Session: 4: Enzymatic reaction mechanisms in natural product biosynthesis

**Conveners:** **Ikuro Abe**, Graduate School of Pharmaceutical Science, The University of Tokyo, Tokyo, Japan and **Rebecca Goss**, University of St. Andrews, Fife, United Kingdom

Salons F-G, lobby level

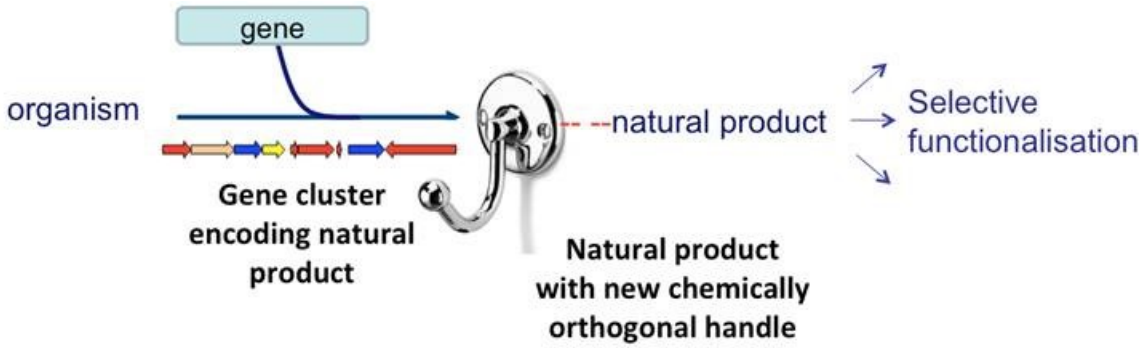
## 1:00 PM S20: Co-dependent enzyme complexes of oxidatively rearranged polyketide natural products

*J. Rohr*, University of Kentucky, Lexington, KY, USA*
Co
Jurgen Rohr

Gilvocarcin V and Mithramycin are natural product anti-cancer drugs produced by various streptomycetes. They are polyketide drugs, decorated with deoxysugars, and the assembly of their polyketide cores is catalyzed by a type-2 polyketide synthase (PKS) complex. In-depth studies of their biosyntheses focused on post-PKS tailoring steps, and revealed several unique enzymes, with interesting structures, multiple functions and intriguing co-dependencies. This led to a new hypothesis about post-PKS assembly requiring post-PKS enzyme complexes. The results of the biosynthetic investigations also outline the way for the development of new drug analogues, both through manipulation of the biosynthesis and through chemo-enzymatic methods.

## 1:30 PM S21: Living GenoChemetics: Blending synthetic chemistry and synthetic biology in vivo to enable access to new to nature natural products

*R. Goss*, University of St Andrews, St Andrews, United Kingdom*




***GenoChemetics :*** *gene insertion enables the installation of a reactive and chemically orthogonal handle into a natural product, permitting its selective functionalization and diversification*

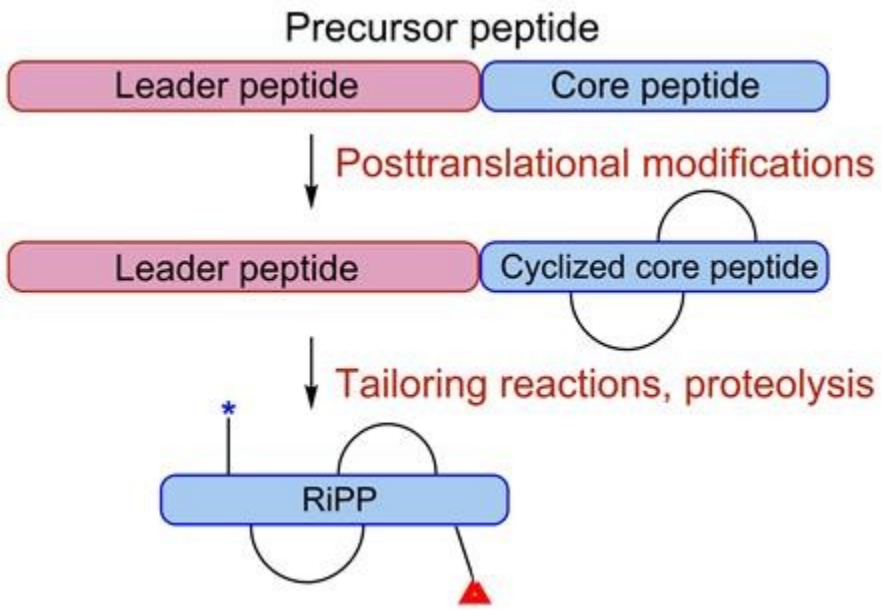
Natural products represent a treasure trove of medicinally relevant compounds: over the past 3 decades over 70% of antimicrobials and over 60% of antitumor agents entering clinical trials have been based on natural products. Generation of natural product analogues is an important area Traditional approaches of analogue generation such as total synthesis and semi-synthesis have limitations. [1, 2]

We have pioneered a new concept in which a gene is introduced to an organism and coerced to work in concert with an existing biosynthetic pathway. This installs a chemical handle that enables selective derivatisation of the natural product.[3] The selective modification of the new to nature natural product in the presence of the living cells that produce it will also be discussed.

Additionally the talk will reflect upon the structure and function of an enzyme that mediates the formation of an unusual 3'dehydroxy nucleoside.[6]

## 2:00 PM S22: Biosynthesis and engineering of cyclic peptide antibiotics

*W. van der Donk*, Howard Hughes Medical Institute and the University of Illinois at Urbana-Champaign, Urbana, IL, USA*




The genome sequencing efforts of the first decade of the 21st century have revealed that ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a very large class of cyclic peptide natural products. These molecules are produced in all three domains of life, their biosynthetic genes are ubiquitous in the currently sequenced genomes, and their structural diversity is vast. Lanthionine-containing peptides (lanthipeptides) are examples of this growing class and many members are highly effective peptide-derived antimicrobial agents that display nanomolar minimal inhibitory concentrations (MICs) 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. After installation of the crosslinks, tailoring reactions often decorate the final product. This presentation will discuss investigations of the mechanisms of these remarkable catalysts.

## 2:30 PM Break

## 2:45 PM S23: Biosynthesis of β-amino acid containing macrolactam antibiotics

*F. Kudo*, Tokyo Institute of Technology, Tokyo, Japan*

Maclolactam antibiotics with a unique β-amino acid at the starter moiety of polyketide show various biological activities such as antitumor (vicenistain, hitachimycin), antifungal (fluvirucin), antivirus (fluvirucin), antimicrobial (cremimycin), and modulator of anti-apoptotic oncoprotein Bcl-xL (incednine). Exchanging the β-amino acid moiety by engineering the biosynthetic machinery would be an attractive way to expand the structural diversity of this class of antibiotics, which may lead to novel drug candidates. So far, we have characterized a common β-amino acid incorporation mechanism, in which β-amino acid is selectively activated and transferred to a carrier protein (CP) to give β-aminoacyl-CP by an adenylation enzyme. The formed β-aminoacyl-CP is further aminoacylated to prevent undesired lactam formation during polyketide elongation process. The dipeptide moiety of the formed dipeptidyl-CP is then selectively transferred to the loading module of polyketide synthase (PKS). After the polyketide chain elongation, the terminal aminoacyl moiety is removed before the macrolactam formation. Based on the biosynthetic knowledge, mutational biosynthesis can be conducted to produce new macrolactams. However, the strict substrate specificities of the enzymes are usually problematic toward the efficient production. To overcome the situation, the substrate recognition mechanism should be understood to manipulate the

biosynthetic enzymes and machineries. In this presentation, the substrate recognition mechanism with enzyme structural analysis will be discussed.

## **3:15 PM** S24: tbd

*B. Wilkinson*, John Innes Centre, Norwich, United Kingdom*

tba

## **3:45 PM** S25: N-terminal nucleophile-hydrolase activity of gamma-glutamyltranspeptidase homologs in Actinobacteria for carbon-carbon bond cleavage

*G. Zhong and W. Liu*, Shanghai Institute of Organic Chemistry, Shanghai, China*

Gamma-glutamyltranspeptidases (gamma-GTs), which catalyze the transfer/hydrolysis of gamma-glutamyl from the thiol glutathione (GSH), GSH S-conjugates or glutamine, are ubiquitous enzymes in living organisms and play critical roles in antioxidant defense, detoxification and inflammatory processes. These enzymes belong to the superfamily of N-terminal nucleophile (Ntn)-hydrolase fold proteins, and exhibit the activity for post-translational autocatalytic cleavage of a peptide precursor to form a functional heterodimer in which a newly released N-terminal residue acts as a nucleophile and mediates amide bond hydrolysis through the formation of an acyl-nucleophile enzyme intermediate. Intriguingly, gamma-GT homologs have been found to be widely present in the organisms that do not involve GSH metabolism, such as Gram-positive actinobacteria. Herein, we demonstrate that the formation of 4-Alkyl-L-(dehydro)proline residues, the non-proteinogenic alpha-amino acids that serve as vital components of many bioactive metabolites found in actinobacteria, involves unprecedented Ntn-hydrolase activity of gamma-GT homolog for C-C bond cleavage. The related enzymes share a key Thr residue, which acts as an internal nucleophile for protein hydrolysis and then as a newly released N-terminal nucleophile for carboxylate side-chain processing through the generation of an oxalyl-Thr enzyme intermediate.

## **4:15 PM** S26: Unusual enzyme reactions in fungal meroterpenoid biosynthesis

*I. Abe*, Graduate School of Pharmaceutical Science, The University of Tokyo, Tokyo, Japan*

Meroterpenoids are hybrid natural products that are partially derived from terpenoids, and those from fungi exhibit an extremely wide range of structural diversity and biological activities. Recent advances in genome sequencing technologies and development of tools for biosynthetic studies have allowed the discovery of many biosynthetic gene clusters for fungal meroterpenoids and intensive researches at genetic and enzymatic level. We have been working on the meroterpenoids derived from a simple aromatic precursor, 3,5-dimethylorsellinic acid (DMOA), and discovered several fascinating enzymes that catalyze drastic structural rearrangement which dramatically increase structural complexity of the molecules. For example, multifunctional, non-heme iron dependent oxygenases are the key components in the austinol and the paraherquonin pathway, in which the enzymes are responsible for the construction of the spiro-lactone and cycloheptadiene moiety, respectively. On the other hand, the terretonin biosynthesis involves a cytochrome P450 and an isomerase, which work collaboratively to perform the unprecedented ring expansion reaction to afford the terretonin scaffold. This presentation will focus on molecular basis for the unusual ring reconstruction reactions in fungal meroterpenoid biogenesis.

# 6:00 PM - 7:00 PM Banquet Reception

Flamingo/Sandpiper Deck

## 7:00 PM - 9:00 PM Banquet: Banquet Speaker Arnold Demain, Drew University

Salons F-G, lobby level

# Wednesday, January 24

## 7:00 AM - 8:00 AM Breakfast

Grand Ballroom Foyer, lobby level

## 7:00 AM - 3:00 PM Registration

Grand Ballroom Foyer, lobby level

## 8:00 AM - 11:45 AM Session: 5: Big data in natural product discovery

**Conveners:** **Neil L. Kelleher**, Northwestern University, Evanston, IL, USA

Salons F-G, lobby level

### 8:00 AM S27: The antiSMASH platform: A comprehensive framework for secondary metabolite genome mining and analysis

*T. Weber*, The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby, Denmark*

With the availability of cheap and easy-to-obtain microbial whole genome sequences, *in silico* genome mining has become an indispensable tool to complement the classical chemistry-centred approach to identify and characterize novel secondary / specialized metabolites. Since the initial release in 2011, the open source genome mining pipeline *antiSMASH*[1] (https://antismash.secondarymetabolites.org) has become one of the most widely used tool to provide such analyses to the scientific public. *antiSMASH* is developed in a large international collaboration coordinated by Wageningen University (M. Medema) and DTU (T. Weber / K.Blin) by contributors from all over the world.

Here, I will present the principles behind *antiSMASH* and give a short preview on the upcoming version 5.

Recently, *antiSMASH* has been extended by several closely linked tools and databases, such as the *antismash database*[2] (https://antismash-db.secondarymetabolites.org) which provides easy access to precomputed antiSMASH annotation for thousands of bacterial genomes, or CRISPy-web[3] (https://crispy.secondarymetabolites.org), which allows interactive design of single-guide RNAs (sgRNAs) for CRISPR/Cas9 applications.

In addition to being an indispensable resource for mining for new compounds, the availability of huge numbers of bacterial genomes and knowledge about the biosynthetic gene clusters provides data for further studies, such as the origin and dissemination of antimicrobial resistance genes, which are often part of the BGCs[4].

References

[1] Blin, K., et al., 2017, Nucleic Acids Res. 45:W36-W41

[2] Blin, K., et al., 2017, Nucleic Acids Res. 45:D555-D559
[3] Blin, K., et al., 2016, Synth. Syst. Biotechnol. 1:118-121
[4] Jiang, X., et al., 2017, Nat. Commun. 8:15784

## 8:30 AM S28: Machine driven discovery of antibiotics

*N.A. Magarvey*, McMaster University, Hamilton, ON, Canada*

Antibiotics discovered from an era defined through bioactivity guided fractionation are increasingly becoming subject to clinical resistance. Revealing novel antibiotic scaffolds and realization of agents with differentiated mechanisms lacking cross-resistance is now the main challenge to ward off drug-resistant superbugs. The increase in, and capacities to, sequence microbes and microbiomes provides information that may reveal unknown chemical dark matter. Increasing details of natural product biosynthetic logic and availability of microbial genomic sequence data are supplying the information backdrop to fuel a transition in how natural antibiotic discovery is done. In this talk, a focus will be placed on the enabling tools and technologies to survey antibiotic production in this genomic data and define antibiotics with new modes of action. Much of these technologies represent a convergence of big data frameworks, artificial intelligence with the microbial genomics, metabolomics and small molecule structure elucidation.

## 9:00 AM S29: No pain, no gain: Eliciting microbial cryptic metabolites using cytotoxic small molecules

*M.R. Seyedsayamdost*, Princeton University, Princeton, NJ, USA*

Microbial secondary metabolites serve as a dominant source of pharmaceutical compounds and comprise some of our most celebrated cures. Recent studies, however, have been plagued by the frequent rediscovery of old molecules. The underlying reason is that the vast majority of secondary metabolite biosynthetic gene clusters in a given bacterium are not significantly expressed, when cultured under standard laboratory conditions. These so-called ‘silent’ or ‘cryptic’ gene clusters represent a large reservoir of bioactive molecules and methods that access them would have a profound impact on natural products research and thereby on drug discovery. In this talk, I will present new strategies that my group has developed for activating silent biosynthetic gene clusters. Application of these approaches to a diverse array of bacteria has unveiled not only the products of silent gene clusters, but also small molecule elicitors, which in most cases are growth-inhibitory or antibiotic in nature. These insights have led to the idea that old antibiotics may be used to find new, cryptic ones. In combination with emerging profiling methodologies, our efforts are beginning to provide a detailed picture of the cryptic secondary metabolomes of bacteria and their chemogenetic modulation by cytotoxic small molecules.

## 9:30 AM Break

## 9:45 AM S30: A new tool for mining ribosomal natural products

*D. Mitchell*, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Ribosomally synthesized and post-translationally modified peptide (RiPP) natural products are attractive for genome-driven discovery and re-engineering, but limitations in bioinformatic methods and exponentially increasing genomic data make large-scale mining difficult. This talk will focus on RODEO (Rapid ORF Description and Evaluation Online**), which leverages machine learning to identify natural product biosynthetic gene clusters and predict RiPP precursor peptides. We initially focused on lasso peptides, which display intriguing physiochemical properties and bioactivities, but their hypervariability rendered them challenging prospects for automated mining. Our approach yielded the most comprehensive mapping of the lasso peptide space and increased the size of the family by more than an order of magnitude. We characterized the structures and bioactivities of six new lasso peptides based on predicted structural novelty. These combined insights significantly expanded the knowledge of lasso peptides, and more broadly, provided a framework for future genome-mining efforts. RODEO2.0, which

has just been released, has the additional capability of scoring thiopeptides, sactipeptides, and certain classes of lanthipeptides. Newly emerging molecules and insight from these efforts will be briefly discussed at the end of the lecture.
**Check out our tutorials, source code, documentation, and web tool for RODEO2.0 at:
**http://www.ripprodeo.org**

## **10:15 AM** S31: Surveying thousands of Actinomycete genomes for novel biosynthetic clusters

*K. Robison*, Warp Drive Bio, Cambridge, MA, USA*

Natural products and their derivatives account for approximately 50% of all small molecule pharmaceuticals. Warp Drive Bio is engaged in the discovery of novel natural products to address critical unmet medical needs, applying a strategy of sequencing and bioinformatically-mining *Actinomycete* genomes on an unprecedented scale. We have surveyed 135,000 genomes from *Streptomyces* and rare *Actinomycetes*. Over the course of this effort, we have characterized in detail a select set of 148 genomes, many sequenced to a single contig. Technical aspects of short-read vs. long contiguity sequencing in GC-rich genomes, with implications for distinct search strategies for novel biosynthetic chemistry, will be discussed. An overview of the cluster content of these 148 genomes will be presented, as well as preliminary analyses of the richness of the entire 135,000 genome set, with a focus on the discovery of novel Rapamycin/FK506-family biosynthetic clusters.

## **10:45 AM** S32: Extracting more from images: Targeting diseases & characterizing compounds via morphological profiling

*A. Carpenter*, The Broad Institute, Cambridge, MA, USA*

Images contain rich information about the state of cells, tissues, and organisms. We work with biomedical researchers around the world to extract quantitative information from images, particularly in high-content screening experiments involving physiologically relevant model systems. As the biological systems and phenotypes of interest become more complex, so are the computational approaches needed to properly extract the information of interest; we continue to bridge the gap between biologists' needs and the latest in computational science, such as deep learning.
Beyond measuring features that biologists specify, we extract more from images through *profiling* experiments using the Cell Painting assay, where thousands of morphological features are measured from each cell's image. We are working to harvest similarities in these "profiles" for identifying compound mechanism of action, grouping similar genes, identifying the functional impact of cancer-associated alleles, discovering disease-associated phenotypes, and identifying novel therapeutics. Ultimately, we aim to make perturbations in cell morphology as computable as genomics data.

All novel algorithms and approaches from our laboratory are released as open-source software, including CellProfiler, CellProfiler Analyst, and cytominer.

## **11:15 AM** S33: Scalable platforms for large scale discovery of microbial natural products

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Bacteria and fungi offer many new compounds yet to be discovered and exploited as leads for drug discovery campaigns. The genomics era marshaled in a wealth of information about the natural product biosynthetic arsenals of both, promising vast new collections of fine chemicals. However, tools to convert

that genomic knowledge into the promised wealth of new molecules have only recently begun to produce the required “big data.” Leveraging genome sequencing and modern mass spectrometry with accurate mass, two new and scalable approaches to identification of natural products and their biosynthetic gene clusters have been developed. For actinobacteria, the technique of “metabologenomics” has been reported and led to discovery of compounds like tambromycin, harboring a new amino acid and antiproliferative activity (*ACS Cent Sci.* 2016, 2(2):99-108.). As the co-developer of this technology will be presenting this area, **this talk will focus on our solution for fungi, a platform called “FAC-MS”.** Using FAC-MS we uncovered 15 new systems (majority were cryptic) across three fungal species, including valactamide - made by a new PKS/NRPS biosynthetic gene cluster (*Clevenger et al.,* cover article in August, 2017 issue of *Nat. Chem. Biol.*: 13 (8), 895-901). We seek to leverage these interpreted genomes, new tools and their data clouds to deliver renewable, reliable sources for the discovery of new natural products. Our hope is that both academic labs and the pharmaceutical industry will benefit from these approaches, with the latter able to re-engage and resource a new era in natural products over the coming decades.

# 11:30 AM - 1:00 PM Lunch

Flamingo/Sandpiper Deck

# 1:00 PM - 4:45 PM Session: 6: Natural product discovery and regulation

**Conveners:** **Jennifer Leeds**, Novartis Institutes for BioMedical Research, Emeryville, CA, USA and **Gerard Wright**, McMaster University, Hamilton, ON, Canada

Salons F-G, lobby level

## 1:00 PM S34: Phylogeny guided natural product discovery

*G. Wright*, McMaster University, Hamilton, ON, Canada*

Natural product discovery has traditionally been performed either through a 'function first' activity guided purification approach or by a 'molecule first' strategy that seeks to describe the compound inventory of a biological sample in a fashion that is agnostic to function. These strategies have done much to inform our understanding of natural product structure and function but are generally divorced from their genomic context of the associated biosynthetic programs. An alternative method to explore natural product diversity is through a 'genes first' approach. In this case natural products are identified from their biosynthetic clusters located on the chromosome. Compound purification and characterization then follows using a number of strategies such as cluster capture methods. A refinement of this approach uses biosynthetic gene phylogeny to identify and explore clades of natural products. Our work using phylogeny guided natural product identification will be presented with a focus on compounds related to glycopeptide antibiotics. Using this approach we have identified new chemical scaffolds and variants with novel biochemical functions.

## 1:30 PM S35: Streptomyces antibiotics: from biological insights to new drugs

*C. Wu, H.U. van der Heul and G.P. van Wezel*, Molecular Biotechnology, Institute of Biology, Leiden University, Leiden, Netherlands; Y.H. Choi, Natural Product Laboratory, Institute of Biology, Leiden, Netherlands*

The increase of antimicrobial resistance (AMR) means that bacterial infections again form a major threat to human health, and new approaches for drug discovery are needed to replenish the antibiotic pipelines.

Sequencing the genomes of actinomycetes revealed that their producing capacity has been grossly underestimated, with many gene clusters that are silent or poorly expressed under routine laboratory conditions. Novel insights into the complex regulatory pathways that control growth and antibiotic production of actinomycetes is therefore required, and in particular to uncover the triggers and cues that elicit antibiotic production in the soil as well as in the laboratory. Such approaches should be based on among others ecological insights, systems and synthetic biology and genomic mining approaches. This can then be applied for the discovery of novel antimicrobials. For one, where BigPharma has routinely screened bacteria in isolation, in nature bacteria live in complex communities with other microbes, protozoa and plants, and these often competitive interactions may well elicit specific responses involving the production of natural products. My lab has set up a novel drug-discovery pipeline, whereby microbial biodiversity is combined with approaches to activate sleeping antibiotics and NMR-based metabolomics technology that allows identifying novel antimicrobial compounds that are produced in complex mixtures. This allows rapid dereplication and initial structure elucidation of the bioactivity of interest. Application of this approach identified many novel molecules, including some with novel chemical scaffolds. The challenges and possible solutions to find new antimicrobial drugs will be discussed.

## **2:00 PM** S36: Translational regulation in *Streptomyces tsukubaensis*

*B.K. Cho*, Korea Advanced Institute of Science and Technology, Daejeon, Korea, Republic of (South)*

*Streptomyces tsukubaensis* NRRL 18488 is the preferred strain for the production of the immunosuppressant agent tacrolimus (FK506). To fully exploit its genetic potential, the systematic understanding of its secondary metabolism and related regulatory mechanisms is required. Toward this end, we completed the *S. tsukubaensis* 7.9 Mb linear genome sequence including integration with multi-omics measurements. Along with accurate re-annotation of the FK506 gene cluster, a total of 2,389 transcription start sites were determined via primary transcriptome analysis. Integrated analysis of transcriptome and translatome data revealed that secondary metabolite gene clusters, especially the FK506 cluster, undergo translational control with decreased translational efficiency according to growth. This comprehensive genome-scale analysis provides insight into secondary metabolism translational regulation in *S. tsukubaensis*.

## **2:30 PM** Break

## **2:45 PM** S37: LYS228, A novel monobactam to treat infections caused by multi-drug resistant Enterobacteriaceae

*J. Leeds*, Novartis Institutes for BioMedical Research, Emeryville, CA, USA*

β-lactams were among the first natural product antibiotics to be used clinically. Many semi-synthetic and fully synthetic derivatives and analogs of natural β-lactams have been used as human therapeutics for decades. However, all clinically used β-lactams are susceptible to clinically-relevant β-lactamases. Here we describe the optimization of novel monobactams leading to LYS228, which is currently in early clinical development. LYS228 is the first single agent β-lactam that retains potency in the presence of all known classes of β-lactamases, with potent activity against carbapenem-resistant enterobacteriacease, including serine-carbapenemase and metallo β-lactamase producing strains.

## **3:15 PM** S38: Breaking the silence: New strategies for discovering novel natural products

*H. Zhao*, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Microorganisms are a major source of new therapeutic agents. My group has been developing new genomics-driven, synthetic biology-enabled strategies to discover and produce novel natural products from sequenced genomes and metagenomes. One strategy is to refactor target cryptic gene clusters in heterologous hosts. As proof of concept, we used this strategy to awaken the silent polyketide

spectinabilin pathway from *Streptomyces spectabilis* in *Streptomyces lividans* and activate a cryptic pathway containing a polyketide synthase-non-ribosomal peptide synthetases from *Streptomyces grieseus* in *Streptomyces lividans*, which led to the discovery of two novel tetramic acid natural products that have never been reported in literature. To increase the throughput, we are establishing a fully integrated robotic system to automate all the steps in gene cluster refactoring and product detection. A second strategy is to activate the target cryptic gene clusters in their native hosts by knocking-in strong promoters upstream of the target cryptic gene clusters using a CRISPR/Cas9 system. We successfully activated more than 10 cryptic gene clusters from five different Streptomyces and uncovered a number of novel natural products. A third distinct yet complementary strategy is to express the uncharacterized biosynthetic gene clusters in heterologous hosts using a direct cloning method based on artificial restriction enzymes.

## **3:45 PM** S39: Exploring new approaches to stimulating specialized metabolism

*M. Elliot*[*]*, McMaster University, Hamilton, ON, Canada*

*Streptomyces* bacteria are prolific producers of specialized metabolites, including the majority of naturally produced antibiotics. Intriguingly, these bacteria have the genetic capacity to make far greater numbers of specialized metabolites than have ever been detected. The barrier to accessing these metabolites appears to lie in their expression: many of these metabolic genes are simply not transcribed under laboratory conditions. There is tremendous interest in developing strategies to stimulate the production of these ‘cryptic’ compounds, as they represent a reservoir of potentially novel, biologically active molecules. We are taking a multi-faceted approach to activating cryptic metabolite production, including manipulating *Streptomyces* growth (and metabolism), and developing broadly applicable genetic tools. We recently discovered a new mode of *Streptomyces* growth known as 'exploration'. We have since discovered that exploring cultures deviate not only from their traditional developmental cycle, but also have an altered metabolic profile and a unique ability to modulate the growth and behavior of other microbes. In parallel, we have identified a novel metabolic regulator that appears to function as a metabolic ‘gatekeeper’ in the streptomycetes. This regulator silences the expression of multiple metabolic clusters, and its deletion leads to a profound upregulation of cryptic metabolic gene expression and dramatically increased specialized metabolic output. We have leveraged this understanding to develop genetic constructs that knock-down the activity of this regulator, and can be used to stimulate new antibiotic production in any streptomycete of interest.

## **4:15 PM** S40: Discovery, properties and biosynthesis of pseudouridimycin

*S. Donadio*[*]*, NAICONS Srl, Milano, Italy*

Pseudouridimycin (PUM) is a novel antibiotic that inhibits bacterial RNAP through a different binding site and different mechanism than rifamycins and lipiarmycins, has no cross-resistance with rifamycins and lipiarmycins, and has a spontaneous resistance rate less than one-tenth that of rifamycins and lipiarmycins. PUM exhibits antibacterial activity against drug-sensitive, drug-resistant, and multi-drug-resistant bacterial pathogens and can clear streptococcal infections in mice. PUM is a nucleoside-analog inhibitor that binds to the RNAP active-center NTP addition site competing with UTP for occupancy, making Watson-Crick H-bonded contacts with an adenine base in DNA template and H-bonded interactions with the nascent RNA product and RNAP. Because most RNAP residues that interact with PUM are essential residues that cannot be substituted without loss of RNAP activity, the PUM resistance spectrum is small and the PUM resistance rate is low. PUM is produced by *Streptomyces* sp. and consists of a formamidinylated, *N*-hydroxylated Gly-Gln dipeptide conjugated to 5'-amino-pseudouridine. PUM is part of the broader group of peptidyl nucleoside antibiotics, which interfere with different biological processes, including bacterial peptidoglycan biosynthesis, bacterial teichoic-acid biosynthesis, bacterial and fungal protein synthesis, and fungal chitin synthesis. Unlike most nucleoside antibiotics which are *N*-nucleosides, PUM is a *C*-nucleoside and the first compound to target RNA polymerase. The *pum* gene cluster contains fifteen genes, and PUM analysis of PUM biosynthesis reveals three unexpected features:

(1) production of free pseudouridine by a dedicated pseudouridine synthase; (2) nucleoside activation by specialized oxidoreductases and aminotransferases; and (3) peptide-bond formation by amide ligases.