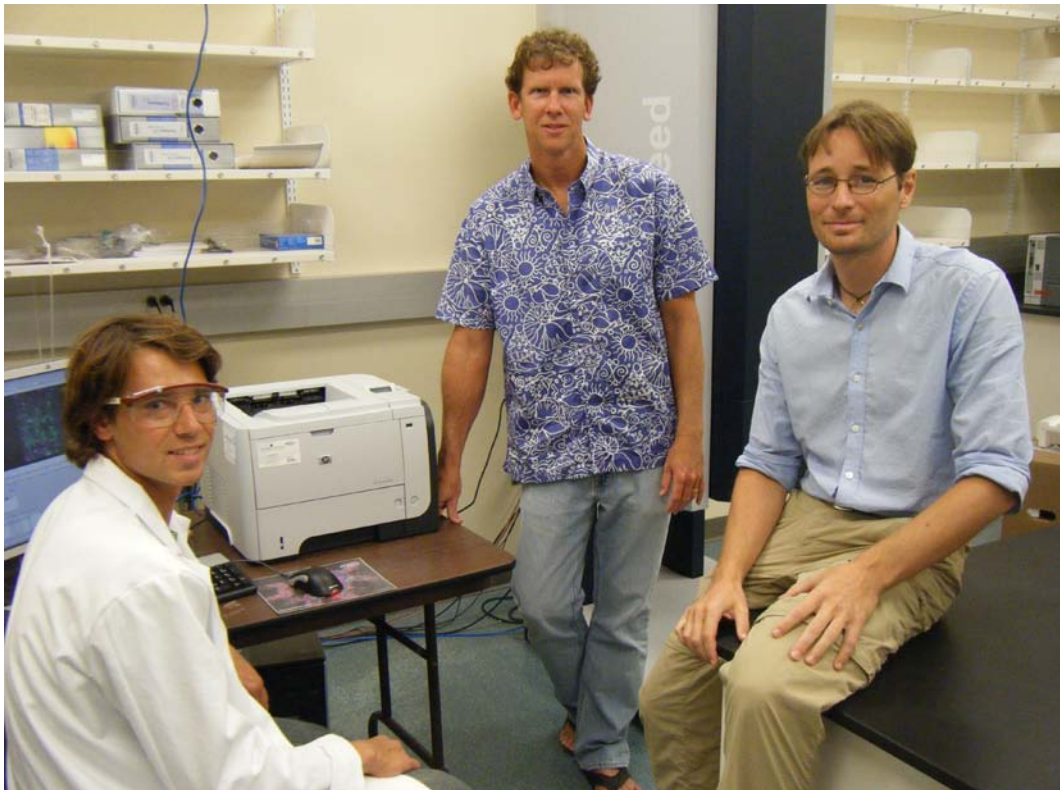
Behind the Scenes in Pharmacognosy: Interdisciplinary Insight

Dr. Amy Keller

This November, ASP member Dr. Pieter Dorrestein and ASP President Brad Moore published results of their laboratory's interdisciplinary work on the use of mass spectrometry and genomic techniques in the Proceedings of the National Academy of Sciences. As Dr. Dorrestein tells the Newsletter *below, this work featured a student now pursuing a Ph.D. in the labs of both Drs. Dorrestein and Moore. In addition to describing a new approach to natural product discovery and characterization, Dr. Dorrestein highlights the potential applications of glycogenomics. Please read the original article, "Glycogenomics as a mass spectrometry-guided genome-mining method for microbial glycosylated molecules,"* Proc Natl Acad Sci. *November 19, 2013;19;110(47):E4407-1.*

How did you become interested in linking genomic analysis to bioactive compounds, and how does this methodology work?

This idea was developed in my lab with Mr. Roland Kersten, a Diploma Arbeiten student from Germany, before he became a joint Ph.D. student with me and Dr. Moore. Our labs together were the ideal interdisciplinary environment where Mr. Kersten could accomplish the work, due to my lab's strength in natural product mass spectrometry (MS) and the Moore lab's leadership in natural product genome mining. Mr. Kersten started to use MS/MS patterns to connect the signatures to the gene clusters, and started with peptidic natural products; he continued to work on this during his PhD. Mr. Kersten showed that we could connect MS/MS fragmentation patterns to the genes that biosynthesize natural product peptides, both ribosomal encoded and by non-ribosomal peptide syntheses.



Mr. Roland Kersten, Dr. Brad Moore, and Dr. Pieter Dorrestein.

We published this work on peptidogenomics in 2011 in *Nature Chemical Biology*. It turned out that matching MS/MS signatures to non-ribosomal peptides required less information than the ribosomal encoded counterparts, as there are few gene clusters that would match this class of biosynthesis. With this realization that minimal MS/MS information is needed to match gene clusters found on a bacterial genome, we set out to look if this held true for other classes of natural products. Although we expected this to work with polyketides, isoprenoids, lipids, and others, the current paper approaches glycosylated natural products since glycosylations are often key to the biological activity of natural products and may be the largest class of natural products for which the fewest members have been characterized.

It turns out that a typical genome only has a few specific sugar biosynthetic enzymes and we used this limited search space to our advantage to create a glycogenetic code, a table of expected MS/MS mass shifts that correlate with specific sugar biosynthetic enzymes. We could then use the glycogenic code to predict the candidate sugars from the genome and the way they fragment in MS/MS

continued on page 13

continued from page 12

when attached to natural products. If these signatures are found on the MS/MS pattern we have a putative match between a molecule and the genes responsible for making them. Once a putative match is made, an iterative analysis between the biosynthetic enzymes and the MS/MS data will further solidify the match. Finally if a good match is obtained, the genes can be knocked out of organisms that are genetically tractable and/or isolation of the molecules is possible for nuclear magnetic resonance (NMR) analysis.

Who in your laboratory carried out the research?
Although Mr. Kersten was the driver of this project, others were involved. Dr. Nadine Ziemert, Dr. David Gonzalez, Dr. Victor Nizet, and Dr. Brendan Duggan also contributed to NMR analysis, genomic analysis, creation of knock-out models, and antimicrobial activity analysis.

Could you provide a brief explanation of the work and results in your own words?
The key contribution in this paper is the creation of a glycogenic code that enables one to efficiently connect MS/MS signatures to glycosylated natural products and find the genes that are responsible for their biosynthesis.

In addition to characterizing microbial glycosylated natural products, what other applications might this approach have in natural product science and health research in general?
In general, MS/MS signatures can be used to organize and classify molecules that can be detected by mass spectrometry irrespective if they are specialized metabolites, my preferred term for natural products due to their unique function or primary metabolites. Here we show that this can be done with glycosylated natural products, but can be readily extended to natural products that are only sugars, such as amino glycosides. I hope this paper will engage other investigators in different biological areas (e.g. plant, humans, animal, coral) to explore their glycogenic potential. Perhaps it will also engage informaticians and computer scientists to create an automated workflow where one can upload the genome with the LC-MS/MS data to obtain the best correlations of MS/MS data to the genes responsible for the biosynthesis. This would be truly exceptional.

What is a favorite nonscientific activity of your lab?
We have our annual swim and BBQ around the Scripps Pier Day.

What is your lab's motto or slogan?
If you are afraid of breaking the instruments, you will not get data (but work out a way to repair the instrument if it breaks on you while working on it).

What is your greatest extravagance in the lab?
We thrive on the creativity from our lab members. Be daring and not afraid to fail. Only ideas that are truly novel should be considered, especially if the outcome cannot be readily predicted and/or if they are MS based tools that are generally applicable to our understanding of the functional roles of specialized metabolites. If the idea is good, we will find a way to do the experiments. ■

We thrive on the creativity from our lab members. Be daring and not afraid to fail. Only ideas that are truly novel should be considered, especially if the outcome cannot be readily predicted and/or if they are MS based tools that are generally applicable to our understanding of the functional roles of specialized metabolites. If the idea is good, we will find a way to do the experiments.