Project by Frank Schulz

Proofreading functions in the biosynthesis of reduced polyketides

Polyketide natural products are widely used as drugs in medicinal applications and as research tools to address biological and medicinal questions in basic research. Many polyketides are structurally highly complex and pose challenges for academic research. The fact that more than half of all clinically used antiinfectives and antitumor drugs are based upon or inspired by natural products, many of which are polyketides, highlights the importance of new research projects in this area, in particular with a view on spreading antibiotic resistances and increasing cancer incidence rates in the population.

A major goal in the work on natural products is their derivatization to render them applicable for medicinal testing. Their structural complexity causes this to be a highly demanding objective, which is targeted in our group using a blend of biological and chemical strategies. An integral part of our work is the mechanistic investigation of the enzymatic machinery in type I polyketide synthases (PKS), the pivotal enzymes in the biosynthesis of reduced polyketides in bacteria. The objective is to accurately predict mutations in PKS that induce a change in the catalytic behavior and thus an equally predictable structural change in the final natural product. By means of point mutations in all reductive domain types (ketoreductases, dehydratases, and enoylreductases) as well as acyltransferase domains, we biosynthesized libraries of different polyketides through engineered fermentation in different Actinomycetes [1-4].

The most important limitation in our and related work by other groups is the observed drop in fermentation yield typically associated with manipulations of the PKS. By using point-mutations instead of domain or module swappings, this drop was less drastic and the predictability of the experiments was increased in comparison to previous experiments. However, a number of polyketide derivatives were produced in insufficient quantities for biological testing. As the point mutations did not show a negative structural effect on the PKS, we assumed this to be the result of an intrinsic substrate specificity of the involved enzymes [5]. To interrogate the mechanism of substrate specificity in PKS is challenging, as the gene clusters are exceptionally large and the involved enzymes are multifunctional. Experiments from other groups and us indicate complex substrate recognition mechanisms in the megadalton-sized PKS.

In the framework of the Research Training Group “MiCon”, these substrate recognition mechanisms are to be investigated *in vitro* and *in vivo*. Bioinformatics-guided state-of-the-art genome editing techniques will be combined with targeted metabolomics by LC-HRMS and natural product isolation.

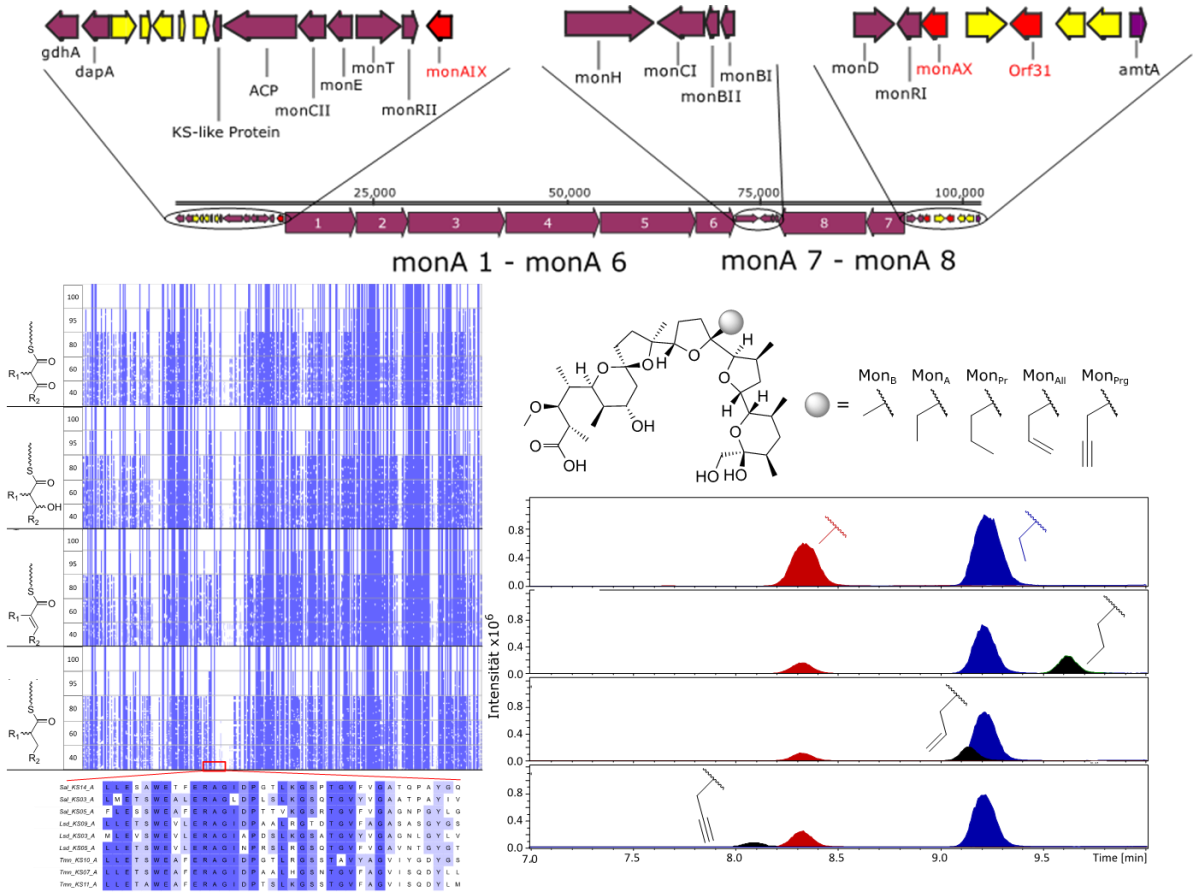

Figure: Organisation of a biosynthetic gene cluster (top). Multiple sequence alignments guide mutagenesis experiments (lower left) and LCMS-analytics aids the identification of natural product derivatives.

The conclusive goal is to advance the combinatorial biosynthesis of bioactive polyketide libraries as a new toolbox in medicinal chemistry - a research goal with great potential for the future of drug discovery that has not been achieved to date. It will be shown whether or not the bottleneck in this endeavour, substrate specificity in PKS, can be relaxed by point mutagenesis of dedicated enzymatic domains.

Relevant own publications

1. S. Kushnir, U. Sundermann, S. Yahiaoui, A. Brockmeyer, P. Janning, F. 2012 "Minimally invasive mutagenesis gives rise to a biosynthetic polyketide library" *Angew. Chem. Int. Ed.* 51:10664-10669.
2. U. Sundermann, K. Bravo-Rodriguez, S. Klopries, S. Kushnir, H. Gomez, E. Sanchez-Garcia, F. Schulz. 2013 "Enzyme-directed mutasynthesis: a combined experimental and theoretical approach to substrate recognition of a polyketide synthase." *ACS Chem. Biol.*, 8: 443–450
3. K. Bravo-Rodriguez, S. Klopries, K. R. M. Koopmans, U. Sundermann, S. Yahiaoui, J. Arens, S. Kushnir, F. Schulz, E. Sanchez-Garcia, 2015 "Substrate flexibility of a mutated acyltransferase domain and implications for polyketide biosynthesis" *Cell Chem. Biol.* 22:1425-1430.
4. M. Grote, F. Schulz, 2019 "Exploring the Promiscuous Enzymatic Activation of Non-natural Polyketide Extender Units In Vitro and In Vivo for Monensin Biosynthesis" *ChemBioChem*, 20, 1183.
5. M. Grote, S. Kushnir, N. Pryk, D. Möller, J. Erver, A. Ismail-Ali, F. Schulz, 2019 "Identification of crucial bottlenecks in engineered polyketide biosynthesis" *Org. Biomol. Chem*., 17, 26 (cover page), 6374-6385