From antibiotic discovery to cell morphogenesis
Or why work on *Streptomyces* now

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Secondary metabolites derived from filamentous bacteria of the genus Actinomycetes have transformed our current health care. They produce a multitude of medically relevant molecules, from anticancer compounds to immunomodulators to antibiotics [1]. The application of modern genomic techniques has accelerated the discovery of specialised secondary metabolites. I will discuss an example of how a bioinformatic informed analysis resulted in the isolation of three chemically diverse specialised secondary metabolites from the extremophile *Amycolatopsis sp.* DEM30355: tatiomicin, vancoresmycin and kanglemycin A [2,3].

Although Actinomycetes are used in a diverse range of industrial applications, we lack a detailed understanding of their cell morphogenesis. The best studied organisms and valuable antibiotic producers are from the genus *Streptomyces. Streptomyces* grow by the tip extension and from complicated hyphae structures. The essential tropomyosin-like protein DivIVA promotes tip extension and branch formation[4], though the exact molecular mechanism of its activity and its binding partners are unknown.

We are setting out to understand on molecular level how tip growth and branching occurs in *Streptomyces* through a combination of genetic, biochemical and microscopy techniques. We aim to identify genes and proteins involved in this process by initially utilising two complementary techniques. The first technique involves a genetic lethal overexpression screen using the CRISPR/Cas9 technology while the second is a novel proximity labelling technique called Turbo-ID. In this assay a promiscuous version of the biotin ligase BirA is genetically fused to the protein of interest. Proteins in close proximity are biotinylated and can be subsequently identified by mass spectrometry. We will further dissect the binding sites on DivIVA conducting a full alanine screen of a DivIVA copy in trans. The native copy of DivIVA will be targeted by an inducible protein degradation system. This will allow us to conduct modifications on DivIVA, which would normally not be viable.

I hope to demonstrate that *Streptomyces* are not only talented producers of secondary metabolites but also fascinating microorganism on which to study cell morphogenesis.

1 Berdy, J. (2005). Bioactive microbial metabolites. *J Antibiot (Tokyo), 58*(1), 1-26.

2 Kepplinger, B., Morton-Laing, S., Seistrup, K. H., Marrs, E. C. L., Hopkins, A. P., Perry, J. D., Allenby, N. E. E. (2018). Mode of Action and Heterologous Expression of the Natural Product Antibiotic Vancoresmycin. *ACS Chem Biol, 13*(1), 207-214

3 Mosaei, H.*, Molodtsov, V.*, Kepplinger, B.*, Harbottle, J., Moon, C. W., Jeeves, R. E., Zenkin, N. (2018). Mode of Action of Kanglemycin A, an Ansamycin Natural Product that Is Active against Rifampicin-Resistant Mycobacterium tuberculosis. *Mol Cell, 72*(2), 263-274 e265.
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4 Flardh, K. (2003). Essential role of DivIVA in polar growth and morphogenesis in Streptomyces coelicolor A3(2). *Mol Microbiol, 49*(6), 1523-1536.