Probing enzyme reaction cascades in antibiotic biosynthesis at atomic resolution by NMR

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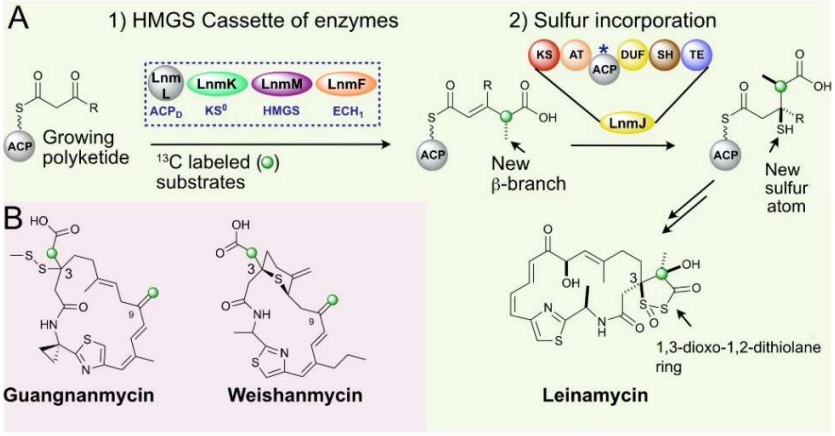
Project description:



Polyketide biosynthetic pathways generate vast numbers of diverse compounds that represent one of the largest collections of chemical structures with biological activities and high commercial value (eg cholesterol-lowering, statins with a global market value of $25 billion). How are they formed? Well, Henry Ford may be credited with inventing the car assembly line in the early 1900s, but he was beaten to it by microbial biosynthetic pathways. Polyketides are generated by polyketide synthases, many of which are giant assemblies of multi-modular polypeptides harbouring multiple sequential catalytic domains. The evolution of the natural product via simple carbon building blocks can progress in a systematic, in-cis, pathway dictated by the linear arrangement of enzymes in the covalently linked modules but critically can recruit free-standing in-trans partners at critical junctures to perform additional chemistry. Many questions remain about how these synthases control the in-cis and in-trans balance and and is a cornerstone of future synthetic biology and bio-engineering efforts of these pathways towards high-value 'non-natural' products.

This project's main aim is to advance our understanding of these enzyme cascades as applied to leinamycin, a sulfur containing anti-tumour polyketide antibiotic (Figure panel A). The focus of the project will be to explore how a group of enzymes in this pathway work together to exquisitely control incorporation of a sulfur containing ring that is critical for biological potency. Synthetically this would be challenging but Nature achieves this cleanly and efficiently. This clever manipulation is also observed in the related biologically active weishamycins and guangnanmycins (Figure panel B).

To unpick the complex function of this mutli-enzyme cascade, we have designed a new tool that combines NMR and chemical synthesis. This allows us to essentially introduce a micro-antenna (a carbon-13 label) within a molecule and NMR lets us look at or "tune into" this signal. Hence the fate of a particular molecule can be followed in reconstructed enzymes assemblies in close to real time.

The aim of the PhD is to investigate the structure and function of several of the components from these sulfur incorporating pathways and characterize new molecular transformations, including an intriguing 'domain of unknown function' that is predicted to be involved in forming a critical C-S bond.

Crucially, with this synthetic biology project you will learn techniques encompassing structural biology, NMR, X-ray crystallography, microbiology and molecular modelling/design as well as work with synthetic chemists, offering a wide array of avenues to explore.