

Mechanistic Dissection and Engineering of Modular Polyketide Synthases

Project ID: 281

Supervisory team

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Project description: Polyketide biosynthetic pathways produce a remarkably diverse set of bioactive molecules with substantial benefit to society, from widely used cholesterol-lowering agents to new antimicrobial leads. Many are assembled by modular polyketide synthases (PKSs): large enzymes in which multiple catalytic domains act in sequence. Products can be built within one polypeptide (in cis) and can also recruit free partner enzymes (in trans) to add further chemistry. Understanding how PKSs coordinate the in-cis and in-trans steps is fundamental to the synthetic biology and bioengineering aimed at delivering high-value natural and designed products. Our approach treats these systems as coordinated, tuneable assemblies whose behaviour can be measured, modelled, and ultimately reused to make molecules that meet health and sustainability needs. This project focuses on the leinamycin biosynthetic pathway. Leinamycin displays antibiotic and anti-proliferative activity, and a sulfur-containing ring within the molecule is essential for activity. The sequence of the catalytic steps that generate this unusual and fascinating bacterial natural product remains elusive, however, and we will draw comparisons to related systems such as the weishamycins and guangnanmycins to help understand how this molecule is produced. We will express and purify both discrete enzyme domains and multi-modular synthase proteins, together with key enzymes from representative systems (for example, kalimantacin and ambruticin), to enable in-vitro reconstitution, mutational analysis, and biophysical characterisation. To investigate these multi-enzyme assemblies, we will use a combination of NMR and mass spectrometry (MS)-based analysis alongside targeted synthesis. By synthetically introducing a micro-antenna (a carbon-13 label) within a molecule, NMR lets us “tune into” this signal. Using this technique, the fate of a particular molecule can be followed in reconstructed enzyme assemblies in close to real time. High-resolution MS allows us to detect and quantify intermediates and products to confirm sulfur-incorporating transformations and to map flux through competing routes. The aim of the PhD is to define structure and function relationships across sulfur-installing enzymes and to characterise new transformations, including the role of a domain of unknown function predicted to form a key carbon-sulfur bond.

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.