Re-engineering the building blocks of bacterial natural product assembly lines

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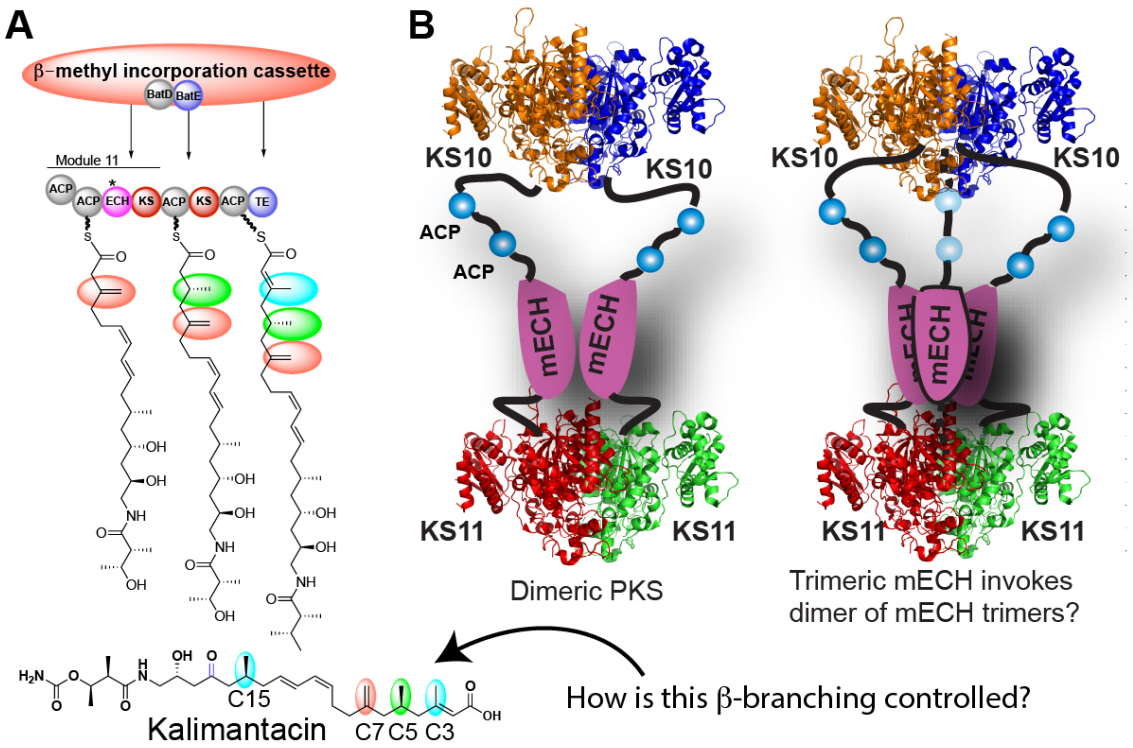
Project description:
Polyketide biosynthetic pathways generate vast numbers of diverse compounds that represent one of the largest collections of chemical structures with biological activities and high commercial value, many of which are already exploited across a spectrum of applications. How are they 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 (and by many millions of years!). Polyketides are generated by type I modular polyketide synthases, sophisticated biosynthetic mega-enzymes, like the assembly of enzymes shown opposite, which may be rationally manipulated to deliver functionally optimised products. At their extreme, these pathways involve 50-100 proteins with tight control over the order in which they act, their interactions and their interdependencies. How all of this is controlled, however, remains elusive and our laboratory strives to understand these principles.

This project aims to advance our understanding of these assemblies as applied to kalimantacin, an anti-MRSA antibiotic (see Figure Panel A). The focus of the project will be to manipulate a very specific part of this pathway which exquisitely controls how kalimantacin is modified by branching of the carbon skeleton. These (beta)-branches are formed from methyl or exo-methylene groups that are appended in several different ways along kalimantacin (shown in red, green and blue) by a single dedicated cassette of enzymes. Synthetically this would be challenging but this clever molecular trick is extremely important as the branches are critical for the anti-bacterial potency of kalimantacin. We now have preliminary data on how the branching might be controlled (Walker et al. Angew. Chemie Int. Ed. 2019, 58, 12446-12450) or how the system is programmed to make each branch. In this PhD project key components of the systems (ECH or Enoyl CoA hydratase domains) will be engineered to explore if we can reverse the order of beta-branch incorporation and hence apply production line engineering approaches to delivering new chemical entities. In addition the trimeric quaternary structure of ECH domains is incompatible with current models for polyketide synthases so a second part of the project will be to study how these modules assemble and you will learn to apply state of the art Cryo-EM techniques to this challenge.

Crucially, this synthetic biology project will draw on techniques encompassing structural biology, NMR, Cryo-EM, collaboration with synthetic chemists, microbiology and molecular modelling/design offering a wide array of avenues for the student to explore.



A. Schematic for part of the pathway producing the compound kalimantacin, highlighting the synthetic complexity achieved by the multi-modular assembly line and in red, green and blue, a group of the β-branches that are incorporated. **B. Left.** Canonical dimeric arrangement of the β-branching junction arranged around a dimeric mECH. **Right.** Adding a trimeric mECH would require at least a second trimer to maintain symmetry.