The Nature of modularity in polyketide synthases: a computational design approach to understand and engineer biological assembly lines

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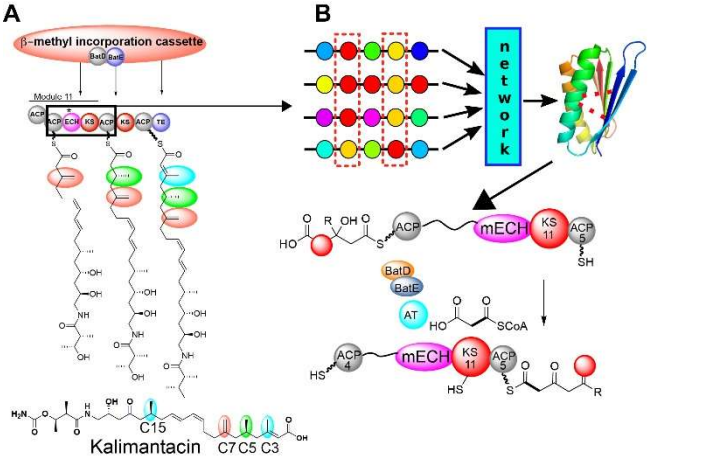
Project description:

Polyketide synthases are large protein complexes, with single chains in the range of thousands of amino acids, responsible for the synthesis of a broad range of secondary metabolism compounds and in particular antibiotics.

Kalimantacin is a polyketide antibiotic isolated from Alcaligenes sp. YL-02632S and Pseudomonas fluorescens that shows high selectivity for Staphylococcal species, which represent a large fraction of hospital infections linked to rise of multiple antibiotic resistance. Until now, no resistant strains have been reported, indicating that kalimantacin and its derivatives could be new potentially specific antibiotics. The synthesis of kalamantacin and polyketides in general is a complex process that requires the coordination of multiple enzymes. In these systems, the functional protein domains are arranged as a modular assembly line where the precursor polyketide is progressively extended and modified. The modular structure shows a way to engineer polyketides by swapping modules and therefore alter the structure of the final product in a predictable way. This approach has been attempted in the before with limited success due to the lack of structural information. However, the recent development of structure prediction and the contribution of machine learning and evolutionary information has made tackling this problem possible.

In this project we will combine computational methods for structure prediction and design to understand how a kalimantacin module is organized and engineer the interface between specific enzymes to allow a plug and play design approach, where modules can be added and swapped to control the final product structure. The designed modules will be experimentally assessed for their ability to synthesize the desired compounds using NMR spectroscopy and this information will inform further optimization and design of new compounds. This project will combine experimental skills in molecular biology, protein biochemistry, enzymology, NMR spectroscopy, with computational skills in programming, data analysis, molecular modelling and protein design.



A. Schematic for part of the pathway producing the compound kalimantacin. In red, green and blue, a group of the β-branches that are incorporated.
B. Design based on interaction networks from multiple sequence alignment, coevolution information and structural constraints. Linker and protein interface design will be assayed using our ^{13}C NMR based probe that can follow polyketide assembly (red dot) and NMR based structural assays.

The goal is to develop universal interfaces between modules and a general method for rapid design of compatible interfaces. These results will allow design of new kalimantacin-derived antibiotics and extend this approach to other polyketide synthases, finally tapping into the potential of modular biosynthesis.

References: Walker et al. (2019) Control of beta-branching in kalimantacin biosynthesis: Application of 13C NMR to polyketide programming. Angew. Chemie. Int. Ed. 58, 12446-12450. Fage et al. (2020) The kalimantacin polyketide antibiotics inhibit fatty acid biosynthesis in Staphylococcus aureus by targeting the enoyl-acyl carrier protein binding site of FabI. Angew. Chemie. Int. Ed. 59, 10549-10556.