New Probes to Elucidate and Capitalize on Nature's Biosynthetic Machinery

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Project description:
Natural products research plays a vital role in scientific endeavour leading to novel bioactive compounds for use in crop science and the pharmaceutical industry. An analysis of sources of new drugs from 1981-2010 indicates that 64% of new chemical entities (NCEs) were discovered from the inspiration of natural products. Furthermore, biosynthetic studies are providing fascinating insights into genetics and enzymology with the prospect of manipulating pathways to provide new bioactive natural and unnatural products combined with clean and efficient methods for their production. The focus of this project will be polyketide biosynthetic pathways which produce natural products exhibiting antimicrobial activity.

Polyketides are produced in Nature from simple building blocks which are assembled in a modular fashion by sophisticated mega-enzymes (polyketide synthases) followed by selective post assembly modifications using tailoring enzymes. These systems offer particularly valuable prospects for creating novel compounds with optimised properties cleanly and efficiently. However, the biosynthetic machinery is highly complex and to understand the exquisite selectivity is not trivial requiring a combination of state-of-the-art methodologies at the chemistry-biology interface. Often these studies are compromised by the lack of the required substrates/ probes and so non-ideal or simplified models are used. To meet these challenges recently we have combined single site 13C enrichment into key biosynthetic intermediates and 13C-observe cryoprobe technology, to enable facile monitoring of polyketide intermediate processing in an extended enzyme cascade (Angew. Chem. Int. Ed., 2019, 58, 12446). Despite the large number of carbon signal present in the system, no background 13C signal is observed from natural abundance protein signals when attached to either a single acyl carrier protein (ACP) or the dimeric 4M di-domain (86 kDa) and the 13C signal remains sharp. In a further study, combining high resolution X-ray crystallographic studies, molecular modelling and 13C-NMR analysis of whole-cell biotransformations with MupW or Mupw/MupZ over-expressing E.coli of a specifically 13C-labelled substrate, insights into the key heterocyclic ring formation of the antibiotic mupirocin have been revealed (Figure). The aim of this project is to build upon these exciting preliminary results to elucidate key features of polyketide biosynthetic pathways and enzyme mechanisms with the longer-term goal of providing new bioactive molecules (focussing on antimicrobials) and biocatalysts of widespread value. This interdisciplinary programme will include the design and synthesis of 13C-labelled probes (which in some cases will be loaded into ACPs), structural biology (X-ray crystallography and NMR), molecular modelling and biotransformations.

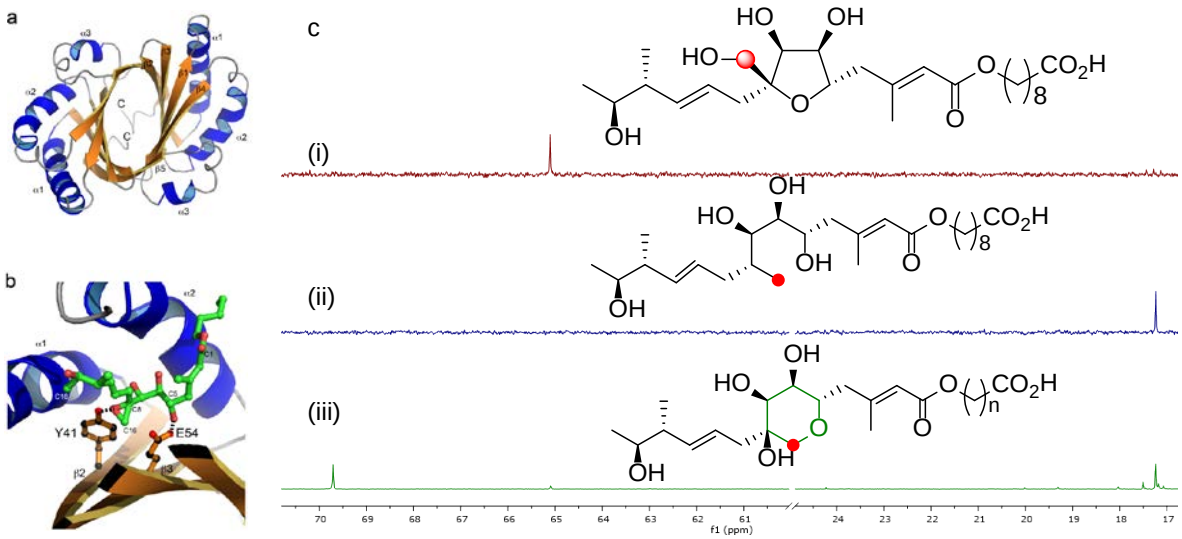

Figure: a. X-ray crystal structure of MupZ, an epoxide hydrolase in the biosynthesis of the antibiotic mupirocin; b. Molecular modelling reveals the catalytic dyad in the active site of MupZ converting the epoxide to a THP ring essential for biological activity; c. Monitoring key biotransformations in *E. coli* overexpressing (i) MupW or (iii) MupW/MupZ by ^{13}C-NMR using (ii) specifically carbon-13 labelled substrate.