

Linked studies of secondary metabolism using small and large molecule mass spectrometry

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Many natural products with antibacterial, antibiotic and anticancer properties are synthesized by large, modular enzymes known as non-ribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs). These enzymes utilize covalent catalysis (a thio-template mechanism) to synthesize natural products composed of (amino) acyl units in an assembly line fashion. A common feature of these biosynthetic pathways is the tethering of the growing natural product to carrier proteins (thiolation (T) domains) distributed throughout the biosynthetic machinery through a phosphopantetheinyl (PPant) arm post-translationally donated from coenzyme A. The large size of these enzymes (often >500 kDa) makes recombinant over expression and *in vitro* analysis often intractable; the need to synthesize complex substrates or radio-labeled compounds needed for standard biochemical assays can also prove difficult. Fourier transform mass spectrometry (FTMS) has already proven an invaluable tool for analysis of NRPS and PKS biosynthesis, as monomer unit loading, condensation and tailoring events can be measured as mass shifts to the T domain. Applications of a new MS assay, the PPant ejection assay, will be highlighted that makes use of a common fragment ion generated from the loss of the PPant arm from a T domain peptide during tandem MS. This assay shows promise for reducing the effort involved for *in vitro* analysis of NRPS and PKS biosynthetic enzymes through far more efficient detection of PPant ejection products - even in complex peptide mixtures. Examples from the gramicidin S system *in vitro* and in the native producer (*Bacillus brevis*), will be described along with other systems under study in our laboratory.