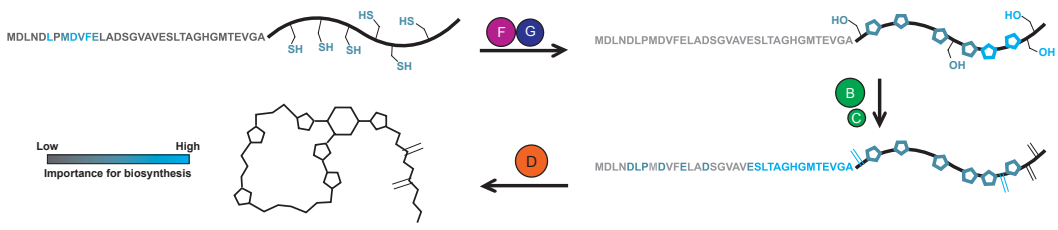
Expanding Thiopeptide Chemical Space Through in vitro Enzymatic Synthesis

Graham Hudson and Douglas A. Mitchell

Thiopeptides are a class of macrocyclic, post-translationally modified peptide natural products that includes potent antibiotics such as thiostrepton, GE2270A, and thiomuracin. Thiopeptides routinely display nanomolar activity against a variety of clinically-relevant pathogens including methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococci. Despite their impressive in vitro profile, thiopeptides remain underutilized in clinical settings due to poor physicochemical properties and challenging chemical syntheses. We have reconstituted the core biosynthetic machinery from thiomuracin biosynthesis and have optimized this system to produce multi-milligram quantities of bioactive macrocycle. Reconstitution has enabled in-depth studies of each enzymatic transformation on this pathway to reveal reaction order, timing, and scope of substrate specificity. Armed with this information, we are now poised to harness this system to generate analogs with enhanced properties/potency as well as functionalizable moieties for chemo- and site-selective derivatization.



Towards the Development of Analyte Specific Photoacoustic Probes for *in vivo* Nitric Oxide Imaging

Christopher Reinhardt and Jefferson Chan

Nitric oxide (NO) is a metastable free radical with several important biological functions. Most notably NO plays a key role in the regulation of vasodilation and in macrophage mediated immune response. Moreover, the dysregulation of NO has been attributed to inflammation, neurodegenerative diseases, and cancer. For all of these reasons, it is crucial to develop technologies for the quantification of NO in vivo. Only with a fundamental understanding of NO biology can novel therapeutics and diagnostics be developed. To date, NO has been successfully imaged using fluorescence, chemiluminescence, EPR spectroscopy, and amperometry, however these methods suffer from poor resolution, complex instrumentation and/or insufficient imaging depths, thereby hindering their clinical applications.

Photoacoustic imaging, an emerging non-invasive imaging modality, relies on the ability of light excited small molecules to release heat through non-radiative relaxation. The resulting rapid expansion can be measured as sound and is correlated to the photoacoustic probe's concentration. Since sound scatters three orders of magnitude less than light within tissue, photoacoustic imaging is capable of generating high-resolution images at centimeter depths. We report the synthesis and characterization of the first analyte specific photoacoustic probe for NO, APNO-1. APNO-1 allows for the molecular information accessible by fluorescent imaging at clinically relevant depths.