New insights into the chemistry and biochemistry of vanadium-dependent enzymes

Nature has developed efficient enzymatic strategies to introduce halogen atoms into chemical scaffolds to modulate their physical properties and biological activities. Site-specific vanadium-dependent haloperoxidases (VHPOs) are a unique enzyme family that employ a histidine-coordinated vanadate ion and co-substrate hydrogen peroxide to oxidize aqueous halide ions and install them in a regio- and stereospecific manner on organic substrates. Although hundreds of novel VHPO homologs have been identified across diverse bacteria, only a small number have been extensively characterized. However, the majority have been involved in the construction of meroterpenoid antibiotics effective against Gram-positive bacterial pathogens. We are extensively investigating the role of VHPOs in natural product biosynthesis to discover novel and bioactive chemical scaffolds and enable downstream biocatalytic applications.

This seminar will highlight our recent efforts characterizing one unique subfamily of VHPOs that modulate the bioactivity of the alkyl quinolone (AQ) quorum sensing molecules. AQs play significant roles in human pathogen *Pseudomonas aeruginosa* virulence, but also help to shape microbial communities through their use as signaling molecules and bactericides. Through an interdisciplinary approach involving protein biochemistry, synthetic organic chemistry, analytical chemistry, microbiology, and bioinformatics, we have identified tens of novel VHPO homologs from diverse Gram-negative and Gram-positive bacteria that regioselectively brominate AQ substrates. This halogenation decreases the antimicrobial effectiveness of these modified AQs against the host VHPO producer and permits us to ask ecological questions about the significance of these enzymes in phylogenetically diverse bacteria. Moreover, AQ-halogenating VHPOs display many advantageous biochemical and biophysical properties over their actinobacterial homologs. Preliminary site-directed mutagenesis, activity assays, and single particle cryo-EM analyses have provided valuable insight into the structural and functional dynamics at play during catalysis. Overall, we aim to improve the understanding of this unique family of halogenases within both natural product biosynthesis and microbial chemical ecology.