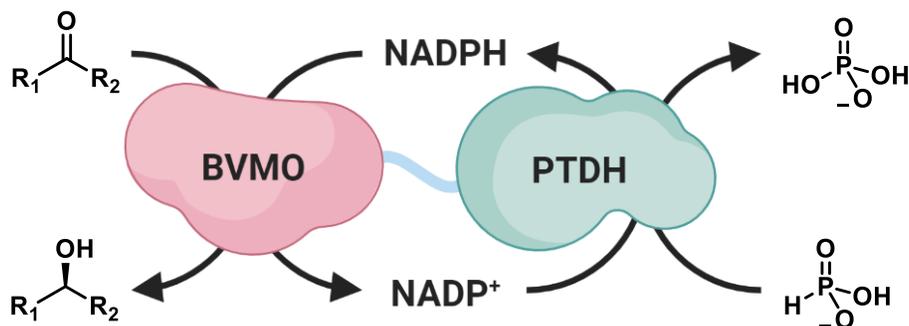


BCHM 421/422 – 2020/2021

Project Title: Engineering a 'self-sufficient' Baeyer-Villiger monooxygenase

Supervisor: Dr. Graeme Howe

Project Outline: Enzymes can accelerate chemical reactions by as much as 10^{23} times over the rate of the corresponding spontaneous processes. As these proteinaceous species are environmentally benign, the ability to exploit enzymes as catalysts for useful chemical reactions would revolutionize the \$52-billion-dollar chemical industry. This project aims to engineer a Baeyer-Villiger monooxygenase (BVMO) for use as a biocatalyst in the production of valuable chiral alcohols. BVMOs have already found some traction in industrial settings, but these enzymes are often plagued by poor stability, modest catalytic activities, and the need for extremely expensive FAD and NADPH cofactors. We will use bioinformatics to find novel BVMOs from thermophilic organisms. Identified thermostable BVMOs will be used as starting points for subsequent engineering efforts. We will circumvent the issues of instability and modest catalytic activity by using directed evolution to engineer a robust, more efficient BVMO variant. To avoid the need to stoichiometric amounts of the expensive cofactors, we will engineer fusion proteins that link our BVMO variant to a cofactor regeneration enzyme like phosphite dehydrogenase (PTDH). The resulting fusion protein will be a 'self-sufficient' enzyme with two domains: one that stereospecifically reduces ketones to alcohols and another that regenerates the necessary cofactors (see below). This BVMO-PTDH fusion will be sufficiently stable and catalytically active to allow our new biocatalyst to be tested for use in industrially relevant conditions for the production of valuable chiral alcohols from cheap, achiral ketone starting materials.



Project Goals:

- Identify BVMO variants from thermostable organisms
- Confirm function of putative BVMOs through *in vitro* assays
- Engineer a BVMO with optimal thermostability and catalytic efficiency
- Generate a bifunctional fusion protein that effectively functions as a 'self-sufficient' BVMO
- Fine-tune resulting self-sufficient BVMO to optimally produce valuable chiral alcohols from achiral building blocks

Experimental Approaches:

- Bioinformatics (BLAST, EFI-EST, InterPro, Clustal Omega, Phyre2.0, etc.)
- Molecular biology (cloning, PCR, gel electrophoresis, protein expression/purification, etc.)
- Enzymology (*in vitro* kinetic assays, thermal shift assays, site-directed mutagenesis, directed evolution)
- Organic/green chemistry (flash chromatography, distillation, switchable solvents, etc.)

References:

1. Leisch, H.; Morley, K.; Lau, P. C. K. *Chem. Rev.* **2011**, *111*, 4165.