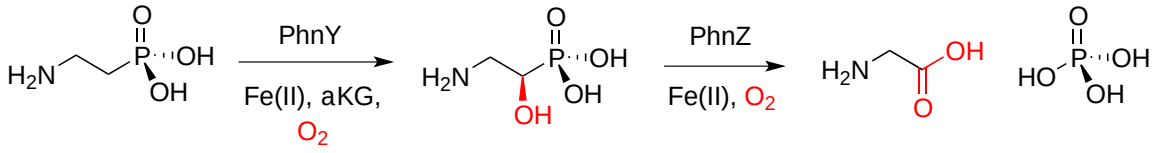
BCHM 421/422 – 2018/2019

Project #1 Outline: Inorganic phosphate (Pi) is an essential component of many biological molecules and thus is required by all life forms. However, soluble Pi is typically at low abundance in the environment. To compensate, microbes have evolved unique carbon-phosphorus bond cleaving reactions to use organophosphonates as an alternative source of Pi. The enzymes PhnY and PhnZ, found widely in marine bacteria, comprise a new pathway for oxidative CP bond cleavage. Both enzymes use iron and molecular oxygen to successively oxidize the α-carbon of 2-aminoethylphosphonic acid to produce Pi and glycine. We are interested in probing the substrate specificities and mechanisms of these enzymes and their homologs using ^{31}P-NMR spectroscopy, kinetic analysis, kinetic isotope effects, and site directed mutagenesis. A long-term goal is to engineer these enzymes to degrade phosphonates of environmental concern, such as RoundUp, the most widely used herbicide on earth.



Supervisor: David Zechel

Project Title: The mechanisms and substrate specificities of the phosphonate degrading enzymes PhnY and PhnZ

Keywords (3-5):

1. **Enzyme catalysis**
2. **phosphonate degradation**
3. **carbon-phosphorus bond cleavage**
4. **Fe(II) dependent oxygenase**

Project Goals:

- Express and purify PhnY and PhnZ homologs.
- Determine substrate specificities of PhnY and PhnZ homologs using phosphonates with different structures and ^{31}P-NMR spectroscopy.
- Identify mechanistic features through kinetic analysis and site directed mutagenesis.

Experimental Approaches:

- Expression and purification of PhnY and PhnZ in *E. coli*.
- Site directed mutagenesis.
- Assay of enzyme activity using ^{31}P-NMR spectroscopy, endpoint assays, or coupled enzyme assays.

References:

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