Introduction

Artificial enzymes based on the LmrR scaffold offer new opportunities for catalyzing hydrazone formation and Friedel-Crafts alkylation, using non-canonical amino acids (ncAAs) like p-aminophenylalanine (pAF) engineered into the protein's large hydrophobic pocket. The Roelfes Lab has demonstrated that LmrR_pAF can efficiently catalyze these reactions.

Objectives

Experimental Validation

- To validate our predicted EVB simulations by the alanine scanning approach on the active site residues of LmrR_pAF.

Workflow

1. Mutagenesis & Cloning

- Perform site-directed mutagenesis to generate active site variants of LmrR.
- Clone the mutant LmrR genes into the pET-17b expression vector.
- Co-transform E. coli with pET-17b-LmrR and a pEVOL plasmid encoding the
- orthogonal tRNA/synthetase for ncAA incorporation.

2. Expression & Purification

- Express LmrR variants in E. coli using small-scale cultures.
- Purify proteins using Ni-NTA affinity chromatography.

3. Activity Measurement & Data Analysis

- Perform reaction assays for all LmrR variants and controls in parallel.
- Monitor product formation by UV/Vis spectroscopy or HPLC.
- Analyze assay data to determine kinetic parameters (e.g., reaction rates, k_cat,
- K_M) using standard software, enabling direct comparison of mutant activities.

Timeline

Week 1 - Mutagenesis & Cloning Generate and verify alanine mutants

Week 2 - Expression & Purification Express and purify mutants

Week 3 and 4 - Activity Assays, data analysis and run catalytic assays to finish writing the report