# Computation-guided engineering of distal mutations in an artificial enzyme

This project aligns closely with my current research on glutathione peroxidase (GPX), where I have observed that distal mutations significantly influence catalytic activity despite being located far from the active site. The opportunity to work on a well-established artificial enzyme system like LmrR, which has been extensively characterized both computationally and experimentally, will provide me with invaluable insights and practical skills directly relevant to my thesis. Moreover, this project embodies a cutting-edge approach that integrates computational predictions, molecular dynamics, directed evolution, and detailed kinetic and stability assays-a multidisciplinary methodology that I am eager to master. Learning these techniques in a leading lab will greatly enhance my ability to innovate in enzyme engineering and deepen my understanding of allosteric regulation and protein dynamics.

**Learning**

Pathway Mapping Techniques: I will gain hands-on experience with computational and experimental methods for identifying and validating allosteric communication pathways that are directly transferable to my own research on evolutionary trajectories in GPX and other enzymes.
Computational Tools for Mutation Design: I will learn to use state-of-the-art computational platforms Zymspot, to predict and rationalize the effects of distal mutations.
Experimental Characterization: I will acquire expertise in enzyme kinetics and thermostability assays.

**Contribution**

I will leverage my research experience to compare how distal single and combination of mutations and the incorporation of selenocysteine (Sec) as a non-natural amino acid affect enzyme catalysis in the artificial enzyme developed in the lab. By introducing Sec at specific positions, I aim to explore how this unique amino acid-known for broadening substrate specificity and altering catalytic properties-can reveal new aspects of allosteric communication and activity enhancement in different protein scaffolds. This approach will help determine whether the benefits of Sec observed in natural selenoproteins can be translated to artificial enzymes.
Using algorithms like greedy search, I will explore the “fitness landscape” of the artificial enzyme that is, a map of how different mutations and their combinations affect activity. The goal is to find the shortest or most effective sequence of mutations that leads from the starting enzyme to a highly active and stable variant, while avoiding mutations that might destabilize the protein or reduce its function
I will be actively involved in analysing experimental and computational results, and in preparing manuscripts or presentations, thereby contributing to the lab’s scientific output

**What I Will Achieve**

Experimental validation of novel distal mutations that enhance enzyme activity and stability.
Novel insights into how distal mutations can be harnessed to optimize enzyme function, informed by both directed evolution and evolutionary pathway analysis.
A comparative, cross-disciplinary framework for studying allosteric networks, strengthening both my thesis and the lab’s research program, which might also lead to joint publications