E3B The Elements of Bioremediation, Biomanufacturing & Bioenergy Metals in Biology

Durham University · University of Kent · Manchester · Biotechnology and Biological Sciences Research Council

PROJECT PARTNERS: Warsipreet Singh and Paul James, Northumbria University; James Finnegan, Prozomix

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Northumbria University NEWCASTLE

PROZOMIX Recombinant Enzymes and Proteins

Improving ethylene production using non-heme iron-containing ethylene-forming enzymes

"This funding helped the computational study of the mechanism of ethylene-forming enzymes as well as enabling the cloning, over-expression, and assay of the enzyme at Northumbria University"

PROJECT AIMS: Ethylene is used as feedstock for making a wide range of essential materials including plastics, textiles, solvents, fibres, detergents and foams. Ethylene is mainly obtained by the cracking of fossil fuels. Microorganisms that express ethylene-forming enzymes represent a sustainable pathway to ethylene production from lingocellulose biomass. The overall aim of the project is to improve the ethylene production of the ethylene-forming enzymes from P. syringae pv. phaseolicola PK2 using computational and experimental approaches.

OUTCOMES & NEXT STEPS:

- The project is being taken forward using funds from the Hub for Biotechnology in the Built Environments.
- The enzyme will be biochemically characterised to study properties such as thermal stability, pH stability and substrate specificities.
- Data from this study will be combined with data from BIVE3B006 and used for a future funding application

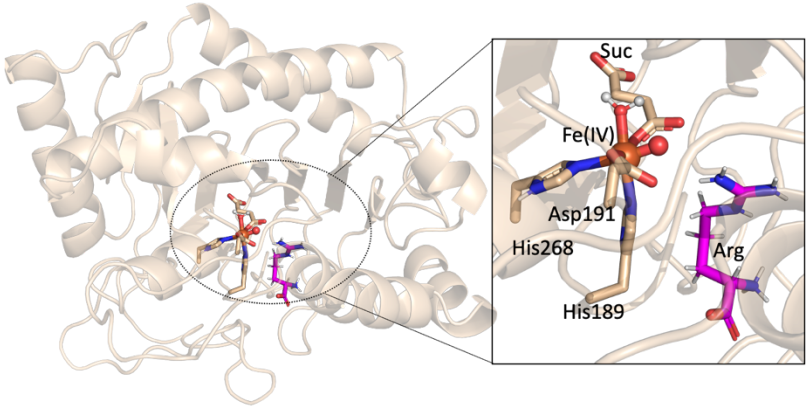

Figure 1. QM/MM optimised structure of the ethylene forming enzyme in Fe(IV)=O intermediate state. The succinate and co-substrate arginine are shown in wheat and magenta colours.

RESULTS: Since the active site of the ethylene-forming enzyme (EFE) contains iron, the researchers performed quantum mechanical calculations to obtain the active site parameters to run classical molecular dynamics simulations. The molecular dynamic simulations and quantum mechanics and molecular mechanics (QMMM) calculations of the wild-type EFE in complex with succinate and co-substrate L-arginine were performed in the Fe(IV)=O intermediate state. (Figure 1). The data obtained from simulations were used to predict mutants such as which can improve the improve ethylene production.

The main outcomes of this project was the development of parameters for the non-heme active site, understanding of how active-site residues stablise oxoglutarate and L-arginine in EFE. The wild-type EFE was cloned, overexpressed and purified. A high expression level was achieved using an auto-induction media. The enzyme was further purified after affinity chromatography using size-exclusion chromatography, revealing the enzyme to be a monomer in solution.

Moving forward, the plan is to test enzymes carrying mutations suggested by computational modelling to improve ethylene production in this newly developed assay system. The mutation which will be tested experimentally are Asp33Val, Phe283Leu and Lys100Ala.

Change in technology readiness level: 2 to 3