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Enzyme engineering to improve lignin breakdown for the production of fine chemicals or value-added products

"This funding allowed us to create a permutation library of GcoA enzyme mutants that will help to improve the enzymes kinetics and substrate specificity and/or promiscuity," Northumbria University

PROJECT AIMS: The breakdown of lignocellulosic biomass can help the transition from fossil fuels. This project aims to improve the deconstruction of lignocellulosic biomass by improving the activity and substrate scope of two metalloenzymes, the cytochrome P450 enzyme GcoA and the multicopper oxidase CueO. This will be achieved using computational simulations to design mutants with improved activity and substrate scope that will then be tested experimentally.

OUTCOMES & NEXT STEPS:

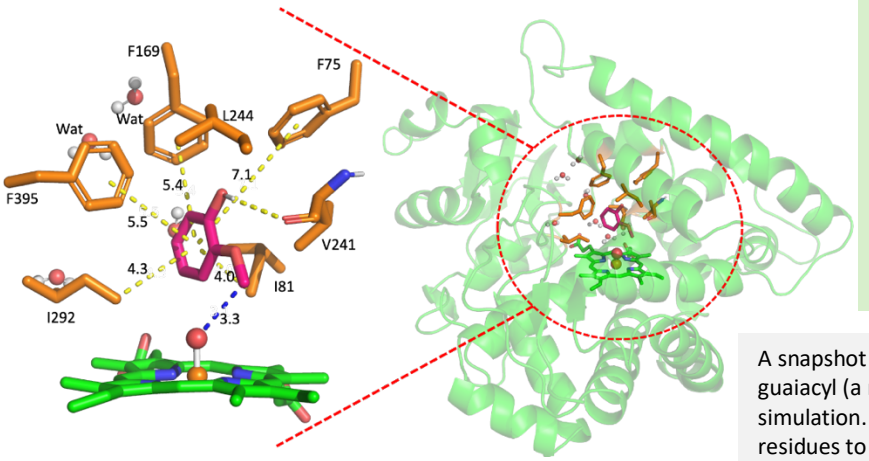
- The project is being taken forward using collaborative funds obtained from the Hub for Biotechnology in the Built Environments.
- Once GcoA mutants have been fully characterised, data will be used for a responsive mode application to investigate lignin-degrading enzymes.
- A paper explaining the significance of F169A mutation towards the valorisation of lignin monomers is under review by *Inorganic Chemistry*.
- The academic partners will present at a conference to advertise the GcoA permutation library to the research community. The library library will also form the basis of a publication.

RESULTS: The main outcome of this project was the cloning, over-expression, and purification of GcoA and CueO.

Quantum mechanics/molecular mechanics simulations were used to investigate which active-site residues of GcoA should be mutated to improve the turnover and substrate scope. Molecular dynamic simulations revealed six sites, and three mutations were postulated for each site. Given the large number of potential mutations, a statistical design of experiments approach was used. From the potential 729 residue permutations, 30 were selected using Latin hypercube sampling. Once mutagenesis had been carried out, plasmids containing the mutated sequences were expressed in *E. coli.* Twenty-seven of the mutation permutations produced soluble protein. In addition, the GcoB protein — which is required for activity of GcoA — was cloned, over-expressed, purified and assayed for activity and the reconstructed GcoAB system found to have levels of activity comparable to those reported in literature.

The expression of both CueO and GcoAB proteins was also investigated in the thermophile *Geobacillus;* this bacterium is being studied as a microbial cell factory capable of dealing with recalcitrant waste products in the built environment. *Geobacillus* was able to produce soluble and active proteins, although the protein yield was much greater in *E. coli.* Work on characterising the GcoA mutants and the effect of GcoAB and CueO protein expression on lignin breakdown is ongoing.

Change in technology readiness level: 2 to 3



A snapshot of the active site of the wild-type GcoA enzyme in complex with guaiacyl (a monometric unit of lignin) obtained from the molecular dynamic simulation. The Heme group is shown in green, the guaiacyl unit in pink and residues to be mutated are shown in orange.