USING *E. COLI* NFSA AS A MODEL TO IMPROVE OUR UNDERSTANDING OF ENZYME ENGINEERING

Kelsi Hall, School of Biological Sciences, Victoria University of Wellington, New Zealand
kelsi.hall@vuw.ac.nz
Katherine Robins, School of Biological Sciences, Victoria University of Wellington, New Zealand
Abby Sharrock, School of Biological Sciences, Victoria University of Wellington, New Zealand
Wayne Patrick, School of Biological Sciences, Victoria University of Wellington, New Zealand
Jeff Mumm, Wilmer Eye Institute, John Hopkins University, Baltimore, Maryland, USA.
David Ackerley, School of Biological Sciences, Victoria University of Wellington, New Zealand

Key Words: nitroreductase, directed evolution, epistasis, cell-ablation

There is a substantial gap between the levels of enzyme activity that nature can achieve and those that scientists can evolve in the lab. This suggests that conventional directed evolution techniques involving incremental improvements in enzyme activity may frequently fail to ascend even local fitness maxima. This is most likely due to the difficulty for step-wise evolutionary approaches in effectively retaining mutations that are beneficial in combination with one another, but on an individual basis are neutral or deleterious (i.e., exhibit positive epistasis). We sought to determine whether a superior enzyme identified using a simultaneous mass site directed mutagenesis approach could have been identified using a step-wise approach. We conducted simultaneous mass randomisation of eight key active site residues in *Escherichia coli* NfsA, a nitroreductase enzyme that has diverse applications in biotechnology. Using degenerate codons, we generated a diverse library containing 394 million unique variants. We then applied a powerful positive selection using chloramphenicol which is toxic to *E. coli* but can be detoxified via nitro-reduction. This has enabled us to recover a diverse range of highly active nitroreductase variants. For two of the most active variants, we have created all possible combinations of single mutations. This allowed us to examine whether a step-wise mutagenesis pathway could have also yielded these enzymes. As anticipated, we identified complex epistatic interactions between residues in these enzyme variants. We have also investigated the “black-box” effect of enzyme engineering, examining the consequences that evolving NfsA towards one specialist activity had on the other promiscuous activities of NfsA. Variants generated in this study have also had practical applications, in particular for targeted cell ablation in zebrafish. We have identified NfsA variants that are highly active with nil-bystander prodrugs that can selectively ablate nitroreductase expressing cells without harm to adjacent cells. In ongoing work, our lead variants are being evaluated for their utility in transgenic zebrafish models of degenerative disease.