Improving on Nature: protein engineering and design

Sasha Derrington, Claire Windle, Laura Cross, Christopher Rowley, Anna Polyakova,
Chi Trinh, Adam Nelson, Arwen Pearson and Alan Berry

Introduction

We are using protein engineering and directed evolution in a wide range of projects to seek to create new enzymes with altered catalysis, to engineer new molecular machines, to create complex macromolecules, to interfere and engineer new orthogonal organelles and to screen vast libraries of directed evolution variants for non-natural catalytic function.

Modifying the activity of *N*-acetylneuraminic acid lyase using non- natural amino acids

N-Acetylneuraminic acid lyase (NAL) catalyses the reversible aldol condensation of *N*-acetyl-D-mannosamine and pyruvate to form *N*-acetylneuraminic acid, through a mechanism that involves the formation of a Schiff base with a lysine residue at position 165. This enzyme has previously been the focus of many traditional directed evolution experiments. Now by using a chemical mutagenesis strategy it has been possible to incorporate non-natural amino acids into the active site of NAL.

An in depth study of the enzyme K165 γ-thialysine has been carried out. The non-natural amino acid γ-thialysine was incorporated into the enzyme by inserting a cysteine residue at position 165 by site directed mutagenesis then subsequent reaction of the cysteine with 2,5-dibromohexan-1,6-diamide (diBr) to form dehydroalanine (dha). The dehydroalanine containing protein can then undergo a conjugate addition of a thiol, in this case aminoethanethiol, to create the non-natural amino acid (figure 1). This method of conversion has also been shown to work at many other positions within the protein and with a wide variety of thiols.

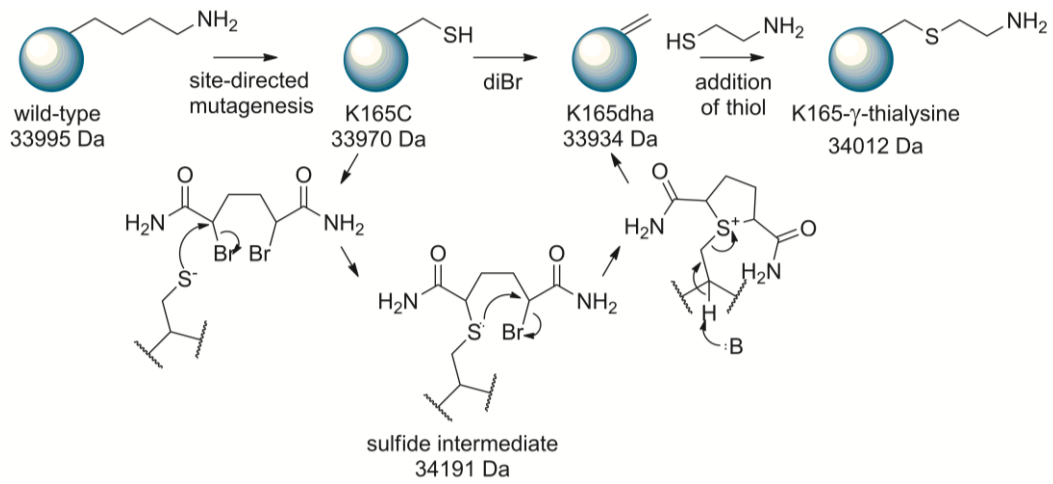

Figure 1: conversion of wild-type NAL into K165 γ-thialysine by chemical mutagenesis.

K165 γ-thialysine has been produced at a large enough scale for crystallographic and in-depth kinetic studies. The X-ray structure showed the K165 γ-thialysine active site to be practically identical to that of the wild-type, while kinetic analyses showed the activity of the enzyme to be much lower. Determination of the kinetic parameters for the reaction at a range of pH values showed the pH optima of K165 γ-thialysine NAL had been shifted from that of the wild-type. The inclusion of the sulphur in the γ-thialysine side chain at position 165 therefore unbalances the pKas in the active site of the enzyme, resulting in lower catalytic rates.

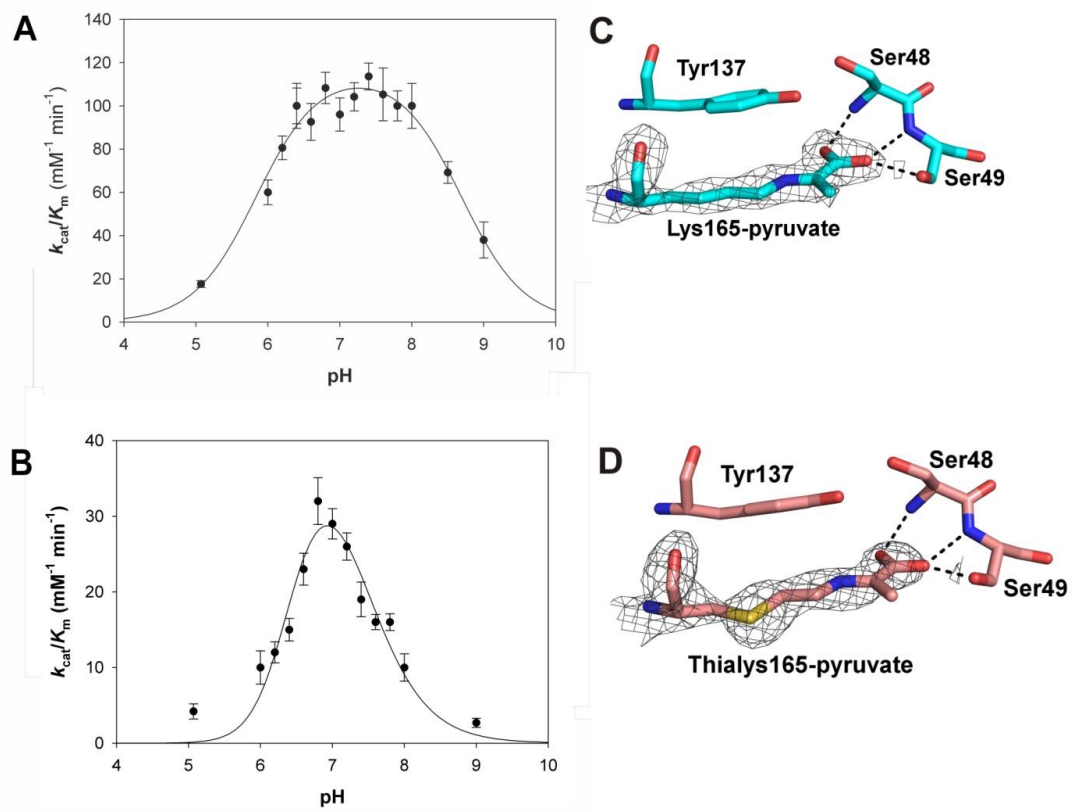

Figure 2: pH curves for **(a)** wild-type and **(b)** K165 γ-thialysine NAL. **(c)** and **(d)** the active site structures of the Schiff base complexes of wild-type and K165 γ-thialysine NAL.

Polyketide synthases and non-ribosomal peptide synthases.

Polyketide synthases (PKS) and non-ribosomal peptide synthases (NRPS) are large multi-modular proteins responsible for the formation of polyketides (PK) and non-ribosomal peptides (NRP), respectively. The compounds produced as secondary metabolites often have additional properties useful to us in everyday life. They are produced in an assembly-like fashion, adding small units together to form a larger compound. Our interests lie in the structure-function relationships of these enzymes that allow the selection of building blocks to make vastly different compounds. In the future we will edit this selection in a predictable manner.

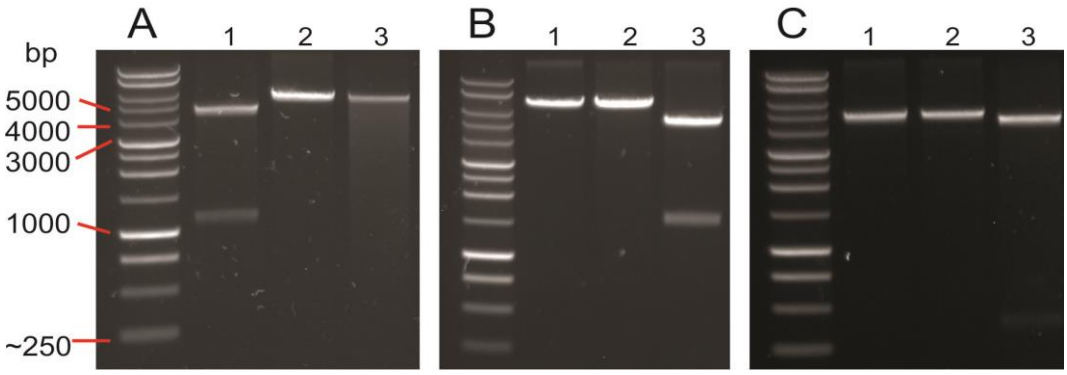

Figure 3: agarose gel showing successful cloning of *idm* genes which encode proteins responsible for starter unit biosynthesis. **(a)** *idmI* and pKK223-3 (~1.2kb) double (1) and single (2 & 3) restriction digests. **(b)** *idmJ* and pETDUET (~1.5kb) single (1 & 2) and double (3) restriction digests. **(c)** *idmK* and pKK223 3 (~300bp) single (1 & 2) and double (3) restriction digests.

Initial work has focussed on cloning and expression of proteins responsible for the formation of a starting unit of a PK/NRP. We intend to structurally characterise the proteins and any interactions formed, in preparation for attempting to engineer the natural substrate specificity.

Creating novel binding motifs for orthogonal receptors

This project looks at the interaction between the peroxisomal receptor PEX5 and the signal sequence PTS1 which allows targeting of proteins to the peroxisome. Mutated PTS1 sequences with be synthesised and screened against a library of PEX5 mutants with the aim to develop an orthogonal PEX5-PTS1 binding interaction.

mRNA display

A new project is using mRNA display methods to screen vast libraries of enzyme variants for novel activities.

Funding

Our work is funded by BBSRC, the Wellcome Trust and the Leverhulme Trust.

Collaborators

Leeds: P. Stockley, A. Baker, A. Cuming and S. Warriner.