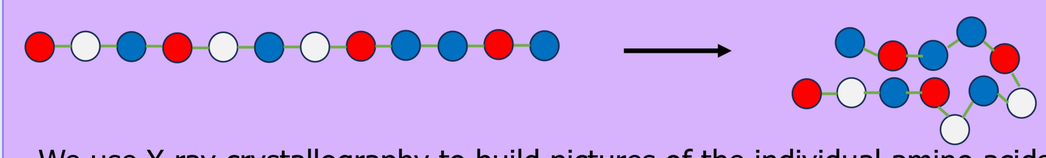
Testing 'Prometheus' - An analytical computer program to predict how proteins fold and form connections.

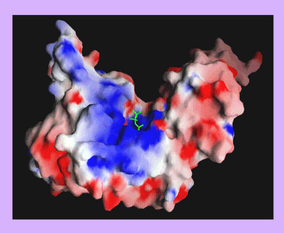
Introduction

Proteins are fundamental biological molecules responsible for running the processes in our body that keep us alive. Proteins are made up of a linear chain of amino acids that fold up to give each protein its unique structure.

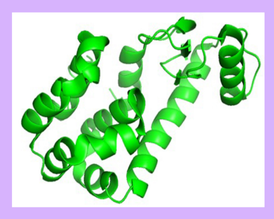


We use X-ray crystallography to build pictures of the individual amino acids that combine to give each protein its unique structure. Despite having these incredibly precise images of the components of each protein we do not yet fully understand how proteins are held together, or why they want to interact with other proteins.

Model of a protein made using X-ray crystallography.

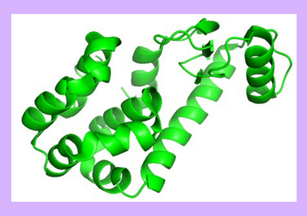


Model of theT4 lysozyme protein from X-ray crystallography with only the backbone of the protein shown.



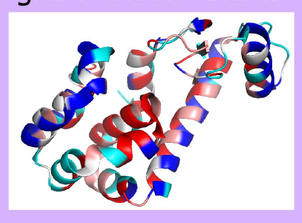
We want to understand how individual amino acids of a protein help to make the overall shape that the protein adopts.

Lawrence Williams (Rutgers) believes rules govern why individual amino acids in a linear protein sequence interact causing the protein to fold up into a three-dimensional shape. His program – Prometheus – explains which amino acids of a protein want to bind to each other. The more they want to bind, the more red the program colours them.



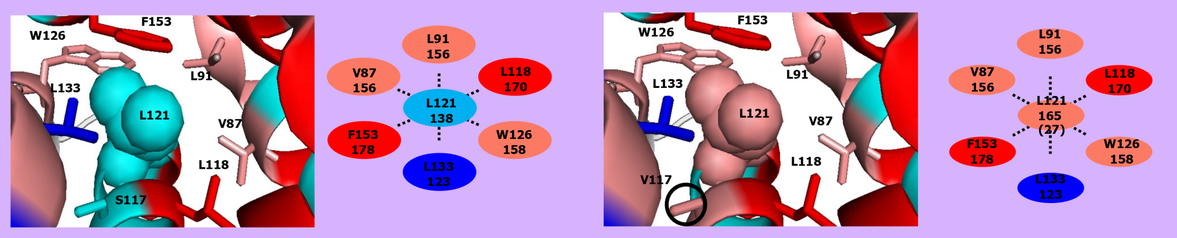
Professor Lawrence Williams

Prometheus



Brian Matthews identified a single amino acid change in the protein T4 lysozyme that switched the 117th amino acid from a serine to a valine. This increased the stability of the protein. The protein five degrees more stable when heated. The reason was not fully understood!

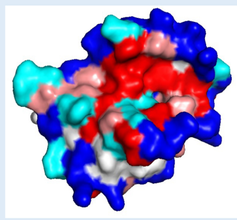
Prometheus predicts that Serine 117 actually affects amino acid 121, leucine, increasing its interaction with neighbouring amino acids increasing protein stability.



A T4 lysozyme model, showing mutation site S117V and its surrounding amino acids in stick and ball graphics. If S117 is changed to V117, L121 changes colour in Prometheus and becomes more reactive.

Aims: We want to understand if Prometheus can predict the desire of individual amino acids in a protein to interact with molecules. If this is the case, could it predict the amino acids in disease targets that will bind to drugs.

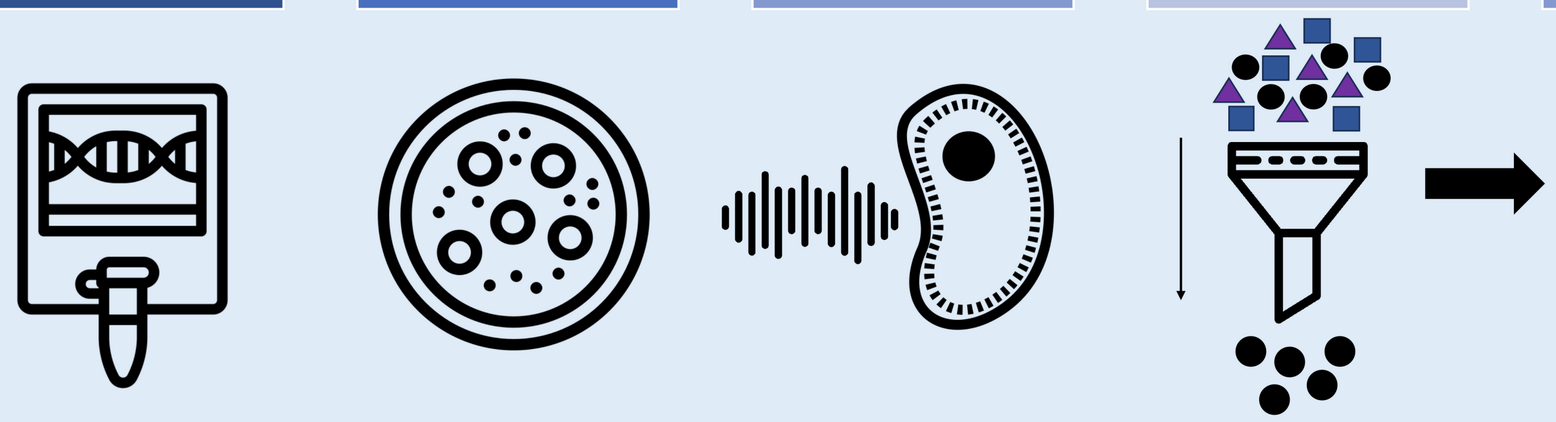
We are introducing single amino acid changes into the protein T4 lysozyme that have predicted effects by Prometheus. We are purifying the mutated proteins and then measuring the temperature that this new protein unfolds at to test the predictions.

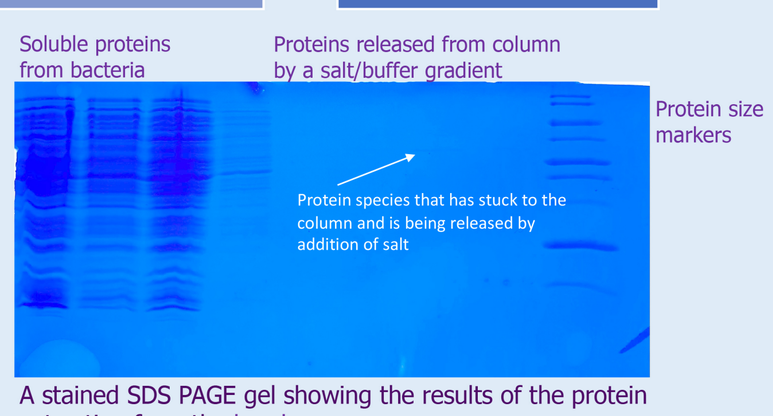


Structure of candidate drug target protein with predicted pocket for binding, coloured using Prometheus. Red amino acids indicate possible sites for drugs to bind.

Methodology

Introduce mutations into wild-type (WT) DNA using PCR to make mutated DNA → Insert WT DNA into competent cells to express protein, grow, then add a chemical inducer to force cells to make our desired protein → Break cells open using sonication (high frequency sound waves), centrifuge to remove cell debris and DNA → Extract WT protein from the cells using a specialised bead that attracts the T4 lysozyme, wash then extract the lysozyme with a salt/buffer gradient → Examine extraction fractions with a polyacrylamide gel → Perform thermal denaturation trials to asses protein stability (see Figure 6), compare to mutated protein



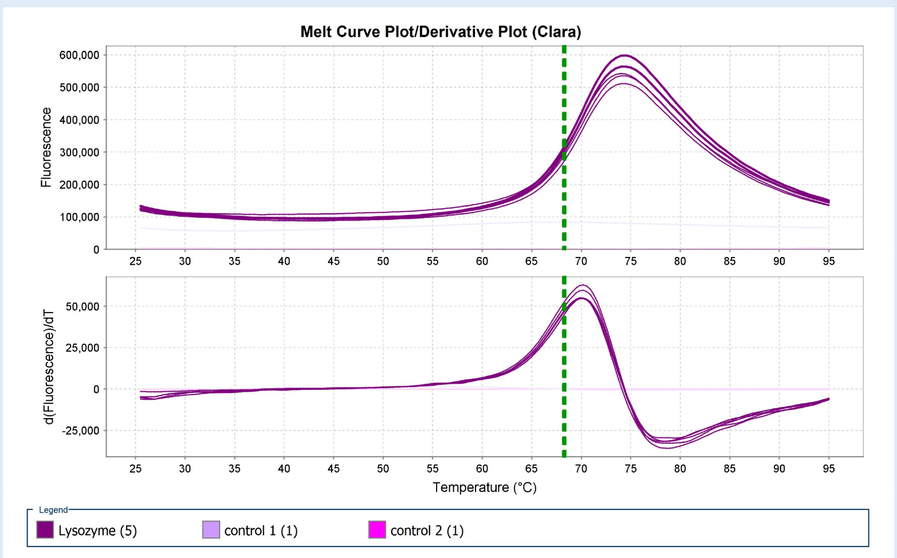


A stained SDS PAGE gel showing the results of the protein extraction from the beads.

Optimisation and Results

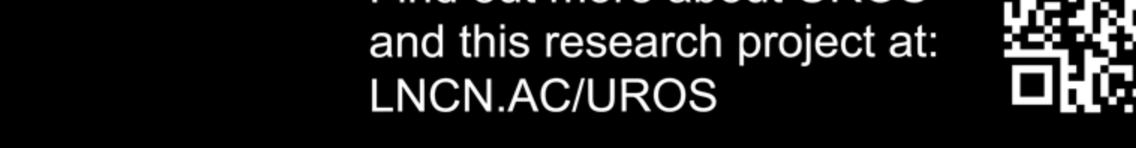
We were able to successfully our mutation fragments individually into the template DNA, particularly the changes of arginines at positions 95 and 96 of T4 lysozyme into histidines. Williams predicts these changes will enhance the protein's stability. We had to adapt and optimise our process multiple times during the duration of the project, for example adjusting the denaturation temperature of the PCR or experimenting with different bacterial cells to see which expressed the protein best.

We measured protein stability by heating wild type lysozyme protein in the presence of a fluorescent dye, SPYRO Orange.



A thermal denaturation curves depicting the fluorescence of a wild type lysozyme protein (upper graph) and the first derivative of that data (lower graph). The temperature of thermal denaturation over 5 repeats was 68.33 +/- 0.04 °C.

In a thermal denaturation assay, protein denaturation is measured via an increase in flurescence over a slow increase of temperature. As the protein is heated up, it unfolds and exposes hydrophobic residues to which the dye, SPYRO Orange, can then attach. As it attaches, it becomes fluorescent, which can then be measured in a qPCR machine. Below is a visual representation of the protein (black circles) unfolding and the dye (green squares) attaching and subsequently becoming fluorescent.



Discussion

The melting temperature (Tm) indicates that the stability is very high, as expected. Assays are now ongoing with T4 lysozyme and the mutants we have introduced, to allow their comparison. Prometheus predicts differential affects for the various amino acids that we are introducing into the T4 lysozyme. Our full data set will help validate the ability of Prometheus to understand the structure of proteins and there desire/ability to interact.

Baase, W. A., Liu, L., Tronrud, D. E., & Matthews, B. W. (2010) Lessons from the lysozyme of phage T4. *Protein Science*, 19(4).
Ferraboschi, P., Ciceri, S., & Grisenti, P. (2021) Applications of lysozyme, an innate immune defense factor, as an alternative antibiotic. *Antibiotics,* 10(12).
Matthews, B. W., & Remington, S. J. (1974) The three dimensional structure of the lysozyme from bacteriophage T4. *Proceedings of the National Academy of Sciences of the United States of America*, *71*(10).
Williams, L. J., Schendt, B. J., Fritz, Z. R., Attali, Y., Lavroff, R. H., & Yarmush, M. L. (2019) A protein interaction free energy model based on amino acid residue contributions: Assessment of point mutation stability of T4 lysozyme. *TECHNOLOGY*, *07*(01/02).

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