BINDING PROTEINS FOR GLYCOPROTEIN ANALYSIS

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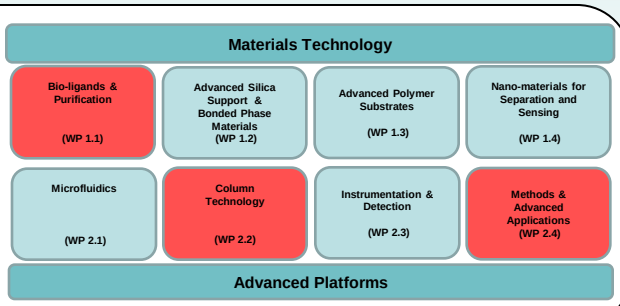
Aims:

- The cloning, expression, purification and characterisation of recombinant prokaryotic glycolytic enzymes
- The mutagenesis of prokaryotic glycolytic enzymes to generate novel recombinant carbohydrate binding proteins
- The characterisation of the binding profile of the novel recombinant carbohydrate binding proteins



1. Intro:

The overall objective of this project is to generate novel carbohydrate binding proteins for use in glycoprotein analysis which are amenable to large scale production. The approach used here is the modification of prokaryotic glycolytic enzymes. The enzymes being studied are from *Photorhabdus luminescens* and are chitobiases (chb) which are a subset of glycolytic enzymes. These enzymes recognize and cleave N-Acetylglucosamine (GlcNAc) dimers to GlcNAc monomers.

We will eliminate their enzymatic activity while hoping they still retain their binding capabilities. The resulting novel carbohydrate binding proteins will have huge commercial potential in the field of glycoanalysis.

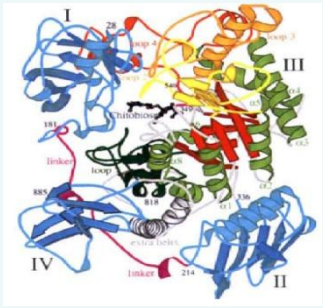


Figure 1: *A chitobiase from Serratia marcescens. A chb from Serratia marcescens has already been cloned and purified. Its catalytic activity has been removed and it has been shown to bind glycans in solution [Tews et al 1996, Prag et al 2000]. Our specific objective is to look at a homologue of this chb from Photorhabdus luminescens and use the work on Serratia marcescens as a guide.*

2. Cloning, Expression and Purification of a chb from P. *luminescens*

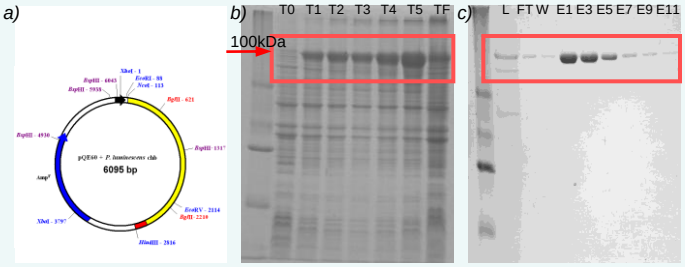


Figure 2: *a) Construct map. Chb PCR product (yellow) was inserted into a pQE60 vector as an NcoI-BamHI fragment. This vector adds a C terminal His tag (red) to its product. The vector also contains ampicillin resistance (blue) for selection. This product was subsequently transformed into Escherichia coli XL10Gold for expression. b) Samples from E. coli cultures containing the expression vectors were taken every hour for 5 hours and lysed by sonication. SDS-PAGE was used to examine expression of chb c) IMAC purification of chb*

3. Enzymatic activity of the chb chitobiase compared with commercial counterpart

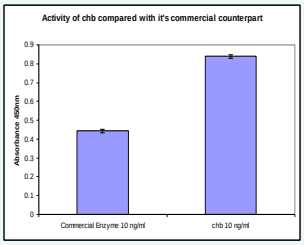


Figure 3: *Colorimetric Assay to test activity of chb enzyme. The substrate 4-Nitrophenol N-acetyl β D glucosaminide is cleaved by the enzyme to release a coloured compound which can be detected.*

4. Generation of a novel non-hydrolytic chb carbohydrate-binding protein.

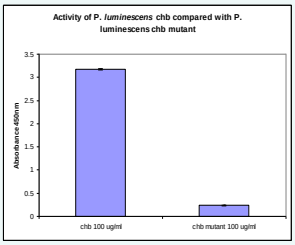


Figure 4: *Mutagenesis of recombinant chb results in abolition of catalytic activity.*

5. ELLA analysis detects binding of chb mutant to GlcNAc residues

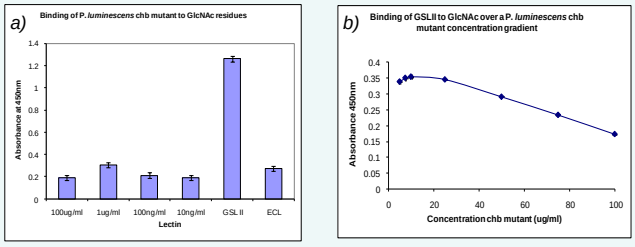


Figure 5: *a) ELLA used to detect binding to GLcNAc residues using GSLII as a positive control and ECL as a negative control. No binding by chb mutant to GlcNAc residues was detected however this could be due to an inability to detect a signal rather than no binding occurring. b) Competitive assay using a constant concentration of GSLII with an increasing concentration of chb mutant. Indirectly shows that chb mutant is binding to GlcNAc residues.*

6. Future Work

- Enable detection of chb mutant in ELLAs – use of linker, longer His tag or biotinylation
- Further characterization of binding once detection is enabled
- Random mutagenesis to alter binding specificities/affinities

7. Project outputs

Oral Presentations

- **School of Biotechnology Seminar Series,** DCU, April 2011

Poster presentations

- **School of Biotechnology Research Day,** DCU, January 2011
- **ISSC Review Meeting,** DCU, June 2010
- **3rd Annual Meeting of Glycoscience Ireland**, UCD, August 2010.

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