PROCESS-SCALE GLYCOPROTEIN PURIFICATION

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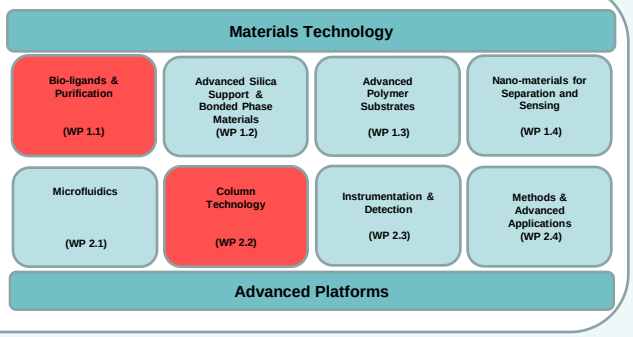
AIMS:

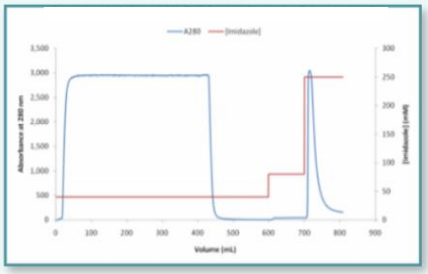
A selection of prokaryotic lectins with a variety of glycan specificities and affinities have been identified, cloned, expressed in *Escherichia coli* and characterised. The aims of this project are to:

- express the lectins at 1L scale to produce sufficient quantities for immobilisation studies (~100 mg)
- immobilise the lectins on Sepharose
- evaluate lectin performance on column by monitoring their ability to reproducibly capture and elute glycoprotein glycoforms.



1. Scaled Production of RPLs

Recombinant bacterial lectins (RPL's) with binding specificities for galactose (RPL-Gal) and mannose (RPL-Man) were chosen for immobilisation experiments. Genetically incorporated affinity tags facilitate rapid downstream purification and provide products of consistent quality.



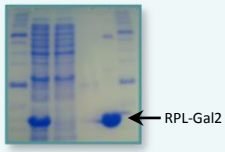


Figure 1: *Single step purification of RPL-Gal2 by IMAC. Approximately 150 mg RPL-Gal2 can be recovered from 1 L culture.*

2. Preparation of Lectin-Affinity Resins

Purified RPLs were immobilised on cyanogen bromide- and epoxy-activated Sepharose beads via intrinsic lysine residues. Commercially available lectins were also immobilised on sepharose beads to produce columns for comparison.

Lectin	Resin Volume (mL)	Total Protein Added (mg)	Total Protein Bound (mg)	Immobilisation Density (mg.mL^{-1})	Immobilisation Density (μmol.mL^{-1})
ECL	2	32.4	19.8	9.9	0.38
RPL-Gal2	1.5	40	39.5	26.3	1.75
RPL-Gal1ME6	2	117.0	39.6	19.8	1.42
RPL-Gal1 MF3	1.7	40	39.2	23.0	1.64
RPL-Man1	2	30	30	15.0	1.1 5

3. Evaluation of Lectin-Affinity Resins: determination of load capacity, reproducibility and optimal elution conditions

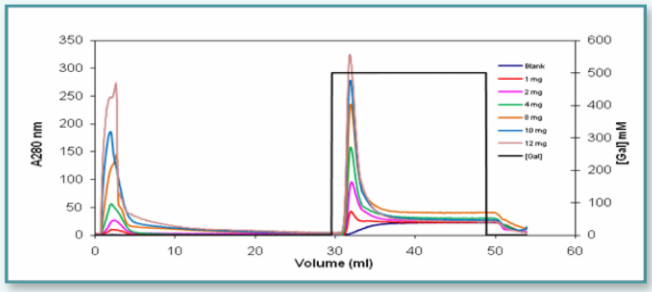


Figure 2: *Load capacity experiments established that 6 mg of asialofetuin can be injected onto an RPL-Gal2 column without overloading the column.*

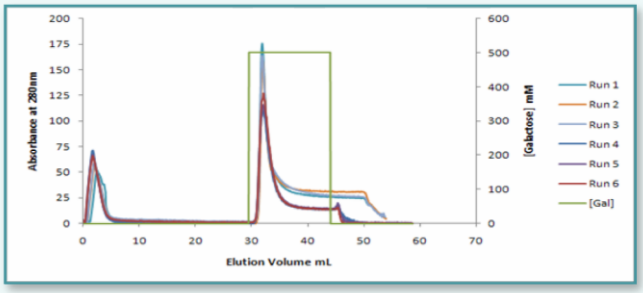


Figure 3: *Repeat injections of 4 mg asialofetuin were performed on two 1 mL RPL-Gal2 prepared from different batches of lectin. Overlay plots show good reproducibility between columns and excellent reproducibility on each column (Runs1-3 & Runs4-6, respectively).*

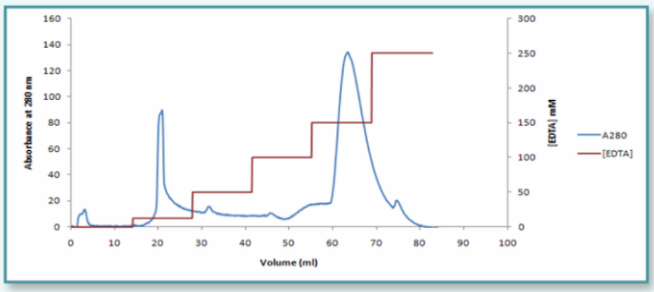


Figure 4: *Separation of glucose oxidase glycoforms by an RPL-Man1 using an EDTA step gradient. The RPL's are Ca^{2+} dependent allowing bound species to be eluted using either EDTA or a specific sugar.*

4. Project outputs

Commercialisation Activity

- This work is encompassed in a patent application which is in preparation.
- Publication will be withheld pending patent submission.
- An Enterprise Ireland Commercialisation grant has been awarded to Dr Paul Clarke to commercialise this research: CF 2011 1052 "ProLegere – Glycoseparation Solutions for the Life Science Industries"

Journal Articles

Roisin Thompson, Aileen Creavin, Michael O'Connell, Brendan O'Connor, Paul Clarke. **Analytical Biochemistry**; 413 (**2011**), 114-122.

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