GENETICALLY ENHANCED RECOMBINANT LECTINS FOR GLYCO-SELECTIVE ANALYSIS AND PURIFICATION

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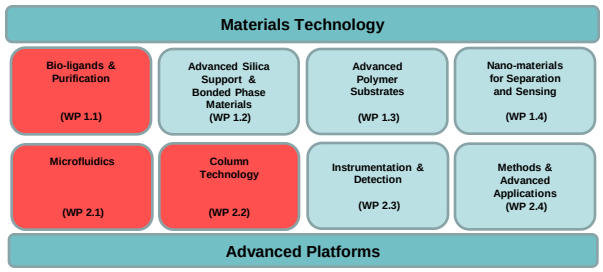
Aims:

- Generation of a library of recombinant prokaryotic lectins (RPL's) through random mutagenesis of the carbohydrate binding sites of bacterial lectins.
- Characterisation of mutant lectins with respect to structure and specificity
- Provision of mutant RPL's with enhanced affinity and/or altered specificity, alongside wild-type RPL's, for glycoprotein analysis and purification in WP 2.1 and WP 2.2.



1. Random Mutagenesis of Prokaryotic Lectins

RPL-Gal1 is derived from a bacterial lectin with a specificity for terminal α-linked galactose. It was cloned into an *E. coli* expression vector with purification tags at either the N- or C-terminus, then expressed and purified.

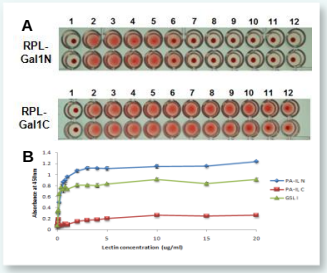


Figure 1: (A) *Haemagglutination assay comparing RPL-Gal1 tagged at the N- (RPL-Gal1N) or C-terminus (RPL-Gal1C). RPL-Gal1C is the most active of the two lectins. However, only RPL-Gal1N is detectable in an Enzyme Linked Lectin Assay (ELLA)* **(B)**.

Since a high throughput assay is required for screening RPL-Gal1 mutants, further studies were performed using RPL-Gal1N.

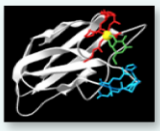


Figure 2: *RPL-Gal1 monomer with a single bound galactose molecule (green). Galactose binds directly to a calcium ion in the binding site (yellow). Calcium binding amino acids are coloured red. Mutagenesis experiments were designed to alter the residues coloured blue.*

2. High-Throughput RPL Mutant Expression & Screening

Random mutagenesis within the nucleotide sequence encoding the RPL-Gal1 carbohydrate binding site was performed, resulting in approximately 1000 *Escherichia coli* KRX transformants. High-throughput protein expression of selected RPL-Gal1 mutants was performed through a 96-deepwell format array with an *E. coli* strain harbouring mutant clones. *In-situ* chemical lysis facilitated screening of the entire array through ELLA.

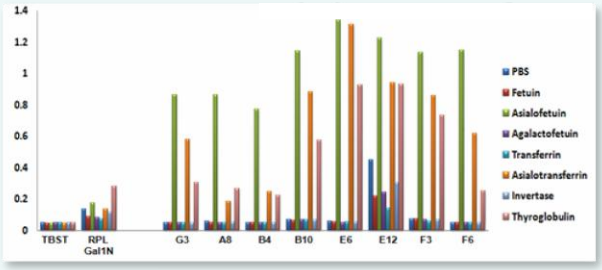


Figure 2: *ELLA analysis of selected mutants from clone library demonstrating altered carbohydrate specificity. The RPL mutants were screened against a panel of glycoproteins displaying a range of glycan structures.*

3. Functional Evaluation of RPL Mutants

Neoglycoproteins, BSA conjugated to Gal-α-1,3-Gal or Gal-β-1,4-GlcNAc, were used to determine the specificity and affinity of the most active mutants. Affinity analysis was performed through lectin dilution ELLA and through competitive carbohydrate inhibition ELLA.

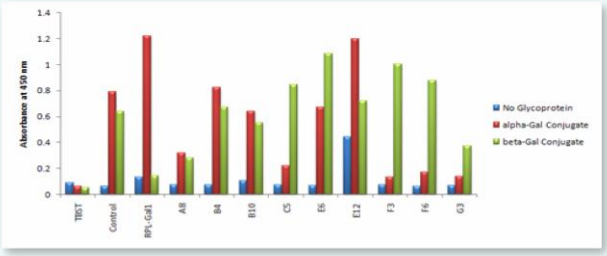


Figure 4: *Investigation of RPL-Gal mutants by ELLA using neoglycoproteins. The parent lectin, RPL-Gal1, is specific for terminal α-linked galactose, but the mutants show a capacity to interact with α- or β-linked galactose (RPL-Gal1 MB10, RPL-Gal1 MB4) or display a switch in specificity for β-linked galactose (. RPL-Gal1 MC5, RPL-Gal1 MF6).*

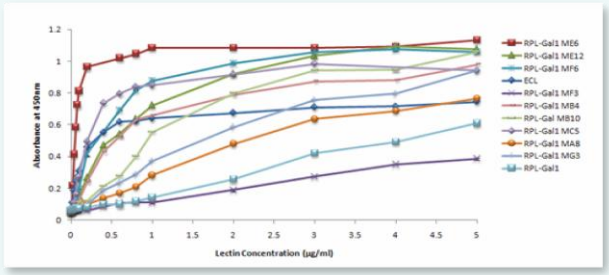


Figure 5: *Association constants of the lectins on the Lactosamine-BSA neoglycoconjugate were determined by ELLA. Binding affinities on this substrate range from 55 ng/mL (RPL-Gal1 ME6) to 2.8 mg/mL (RPL-Gal1).*

4. Project outputs

- 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"

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