GENETICALLY ENHANCED RECOMBINANT LECTINS FOR GLYCO-SELECTIVE ANALYSIS AND PURIFICATION

Damien Keogh1, Ruth Larragy1,2, Roisin Thompson3, Michael O’Connell4, Brendan O’Connor1,2, Paul Clarke2
1. School of Biotechnology, Dublin City University, Dublin, Ireland
2. Irish Separation Science Cluster, Dublin City University, Dublin, Ireland

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-Ga1 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-Ga1 tagged at the N- (RPL-Ga1N) or C-terminus (RPL-Ga1C). RPL-Ga1C is the most active of the two lectins. However, only RPL-Ga1N is detectable in an Enzyme Linked Lectin Assay (ELLA) (B).

Since a high throughput assay is required for screening RPL-Ga1 mutants, further studies were performed using RPL-Ga1N.

Figure 2: RPL-Ga1 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-Ga1 carbohydrate binding site was performed, resulting in approximately 1000 Escherichia coli KRX transformants. High-throughput protein expression of selected RPL-Ga1 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 Ga1-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-Ga1 mutants by ELLA using neoglycoproteins. The parent lectin, RPL-Ga1, is specific for terminal α-linked galactose, but the mutants show a capacity to interact with α- or β-linked galactose (RPL-Ga1 MB10, RPL-Ga1 MB4) or display a switch in specificity for β-linked galactose (RPL-Ga1 MC5, RPL-Ga1 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-Ga1 ME6) to 2.8 mg/mL (RPL-Ga1).

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”

This research has been funded by Science Foundation Ireland under grant number 08/SRC/1412 and by Dublin City University Office of the Vice-President for Research.