

Development of Manufacturing Capability for Rare Sugar Nucleotides

Primary contact for the team

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Team

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Summary

Plant cell walls are an intricate assembly of polysaccharides and phenolic compounds. There is a significant interest in the use of plant cell walls as a source of energy and to extract compounds which may have industrial application. One of the biggest hurdles in the development of cell wall derived products is our poor understanding of cell wall biosynthesis. Synthesis of polysaccharides occurs mainly through the activity of Glycosyltransferase (GT) enzymes that transfer an activated nucleotide-sugar onto a specific growing polysaccharide acceptor. The ability to manufacture sugar nucleotides is therefore of profound importance to the development of novel technologies for study and engineering of polysaccharide biosynthesis. While many sugar nucleotides are commercially available, some of the key sugar nucleotides employed in the synthesis of pectin are not available. Better understanding of cell wall biosynthesis, enabled through experiments using manufactured sugar nucleotides, may enable us to engineer the polysaccharides to have desired properties. This project will focus on pectin, which is a heterogenous polysaccharide found in plant primary cell walls. Pectin is abundant in food waste and as a highly negatively charged polymer it might find industrial application. We will aim to develop a synthetic biology toolbox to synthesise nucleotide sugars, which is required for in vitro analysis of pectin biosynthesis enzymes and is currently not available commercially.

Proposal

This project will aim to develop both synthetic biology and hardware tools to better understand and utilise the pectic polysaccharides.

We propose to develop the technology to manufacture a range of novel sugar nucleotides that are presently not available commercially. This will include sugar nucleotides that are required to study pectin biosynthesis enzymes. Sugar nucleotide biosynthesis occurs in the Golgi apparatus and is performed by a range of different enzymes. As a part of our project we propose to synthesise GoldenGate compatible modules encoding sugar nucleotide biosynthetic enzymes and express those in *E. coli*. Expressed enzymes will be used to convert commercially available sugar nucleotides into sugar nucleotides that are not available commercially, or that are prohibitively expensive. Sugar nucleotides will then be purified through a combination of enzyme treatments and chromatographic separations.

By doing so we will enable further study into the structure and function of glycosyl transferases and the manufacture of novel pectic saccharides.

The successful completion of this project will be guaranteed by a wide skillset available in the team:

Dr Tom Simmons – founder and CEO of Cambridge Glycoscience and an industrial partner in the project. Following seven years as an academic carbohydrate biochemist, Tom has extensive expertise in carbohydrate analysis and purification. Cambridge Glycoscience will be responsible for the conversion of purification and characterisation of sugar nucleotides.

Jan Lyczakowski – a 3rd year PhD student with Prof Paul Dupree at the Department of Biochemistry, University of Cambridge. Jan, has already successfully completed one OpenPlant Fund project and presented developed hardware at the OpenPlant stall during the Cambridge University Science Festival. He has expertise in GoldenGate cloning, enzyme expression and entrapment. Will be responsible for cloning of biosynthesis enzymes.

Dr Henry Temple – an OpenPlant funded post-doctoral researcher working with Prof. Paul Dupree at the Department of Biochemistry, University of Cambridge. Henry has extensive expertise in biosynthesis of nucleotide-sugars, GoldenGate cloning and bacterial expression systems. He has successfully expressed some nucleotide sugar biosynthesis genes as a part of his PhD. Henry will be primarily responsible for cloning of sugar nucleotides biosynthesis enzymes and their expression.

Benefits and outcomes

Thanks to the expertise and enthusiasm of the team this project is highly feasible and likely to be completed within 6 months. This project falls within the remit of OpenPlant deliverables, especially the carbohydrate engineering part of the research centre. As a part of our work we will seek to engage glycobiochemists working at the John Innes Centre. We believe that developed tools and products will be of high interest for multiple groups working in the cell wall biology field. Moreover, we think that this project will enable the OpenPlant community to engage with an industrial partner, Cambridge Glycoscience. This collaboration may initiate new industrial project from both Cambridge and Norwich partners.

The specific outcomes of this project will provide following benefits:

- Better techniques to probe the substrate specificities of glycosyl transferases
- Ability to perform *in vitro* synthesis of pectic polysaccharides

Sponsor for the research and cost centre

Prof Paul Dupree (pd101@cam.ac.uk) – Department of Biochemistry, University of Cambridge

Cost code PHZJ/297

I confirm that I have the full support of the sponsor listed above and that they can be added to the OpenPlant Fund mailing list to receive project updates (to which they can unsubscribe at any time).

Budget

DNA synthesis - £1500

Gene cloning and expression - £500

Purchase of sugar nucleotides - £500

Testing of synthesised sugar nucleotides - £500

Purification and characterisation of sugar nucleotides - £1000