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Development of novel substrate preferences and improved performance of carbohydrate oxidases for functionalization of polysaccharides

I graduated with the degree M.Sc.Eng. (Biotechnology) from Aarhus University, Denmark, in early 2012, after finalizing my thesis work at the Laboratory of Bioprocess Engineering at Aalto University. Preceding the master thesis, I spent a semester as an exchange student at Lund University in Sweden, where I found appetite to continue my studies abroad. Coming from a background with Engineering, Molecular Biology and Biochemistry (Biotechnology altogether), I was introduced to the concept of Biorefineries and carbohydrate active enzymes (cazy.org) through Aalto University professors and researchers. From the Biotechnology Programme at Aarhus University my interest is enzymes, and now particular enzymes that act by oxidizing polysaccharides:

It is anticipated that the future chemical and polymer production will be based on functionalization and derivatization of non-consumable biomass, including lignocellulose residues from agricultural and forest industries. The complex structure of lignocellulose comprises cellulose, hemicellulose and lignin, which makes up the fibrous fraction of agricultural plants and wood. While potential applications of biomass derived polymers and fibres are plentiful, it will be important to develop sophisticated techniques for tailoring biopolymer chemistry to meet the requirements of new application concepts.

Oxidation of carbohydrates is performed by a class of enzymes called carbohydrate oxidoreductases, (oxidases and reductases). They catalyze the oxidation of hydroxyl groups to form lactone, which spontaneously hydrolyses to carboxylic acid or a keto or aldehyde group by using different types of electron accepters. Most known carbohydrate oxidases act on C-1 hydroxyls of mono- and disaccharides, and have received much attention because they can be used in analytical biochemistry and in the production of food and pharmaceuticals. However, the only oxidase reported so far to act on primary hydroxyls is galactose oxidase (GaOx, EC 1.1.3.9), which is a 68 kDa radical copper oxidase secreted by the fungus *Fusarium spp.* The catalyzed reaction of GaOx is the oxidation of D-galactose to galacto-hexodialose, which is the oxidation of the C-6 hydroxyl group to the corresponding aldehyde. A great advantage and application potential of GaOx is its ability to act on oligo- and polysaccharides, where it oxidizes terminal galactose residues. In fact, the most important factors for novelty of oxidases for the biomass material application are their regioselectivity and ability to act on polymeric substrates, and because GaOx naturally possess these properties make it a promising basis for further enhancements.

In addition, several C-1 acting oligosaccharide oxidases (OOX) have been reported in the literature in recent years. Glucooligosaccharide oxidase (GOOX) from *Acremonium strictum* oxidizes glucose, maltose, lactose, cellobiose and cello- and malto-oligosaccharides. Interestingly structural analysis of GOOX shows an active site that conform an open carbohydrate binding groove which allows accommodation of longer-chain oligosaccharides. From an application point of view, GaOx and GOOX will form an excellent catalyst pair for polysaccharide derivatization.

To fully exploit the potential of Galactose oxidase this project aims to develop a novel galactose oxidase to selectively create reactive carbonyl and carboxyl functionalities within oligo- and polysaccharides. Accordingly the specific research objectives are to:

1. Improve the performance of carbohydrate oxidases on polysaccharides through construction of chimeric proteins that contain selected carbohydrate-binding modules.
2. Gain oxidase activity on arabinose through site-specific mutagenesis of galactose oxidase.
3. Characterize putative galactose oxidases and oligosaccharide oxidases obtained from bioinformatics screens