

## ENZYMATIC APPROACHES IN THE PREPARATION OF OLIGOSACCHARIDIC PROBES

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In the course of the preparation of new oligosaccharidic probes for studies on protein-sugar interactions, several methodologies have been developed to overcome the challenging chemical synthesis of these molecules.

The yields of enzymatic syntheses of oligosaccharides by retaining glycoside hydrolases rarely exceed 50% because of hydrolysis of the newly formed glycosidic linkage. Protein engineering of retaining  $\beta$ -D-glycoside hydrolases and the 'glycosynthase' concept originally developed by S. Withers on  $\beta$ -D-glucosidase [Mackenzie, 1998] have enabled us to prepare, in almost quantitative yields, oligosaccharides of the  $\beta$ -(1 $\rightarrow$ 4),  $\beta$ -(1 $\rightarrow$ 3) and ( $\beta$ -1 $\rightarrow$ 3:1 $\rightarrow$ 4) series by site-directed mutagenesis of genes encoding the corresponding *endo*- $\beta$ -D-glucanases. [Fort, 2000, Fairweather, 2003, Faijes, 2001]. The efficiency of this approach will be demonstrated by the preparation of a library of xylogluco-oligosaccharides as probes for xyloglucan active enzymes.

As an alternative strategy, *in vivo* synthesis of oligosaccharides in recombinant *E. coli* has also been developed. When *E. coli* cells: *i*) co-express several heterologous glycosyltransferases, *ii*) possess the machinery required for sugar nucleotide synthesis and *iii*) acceptor molecules are present, complex oligosaccharides were produced intracellularly on a gram scale. [Dumon, 2004, Antoine, 2005] Examples of the synthesis of fucosyl- and sialyl-oligosaccharides in different series will be given.

### References

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