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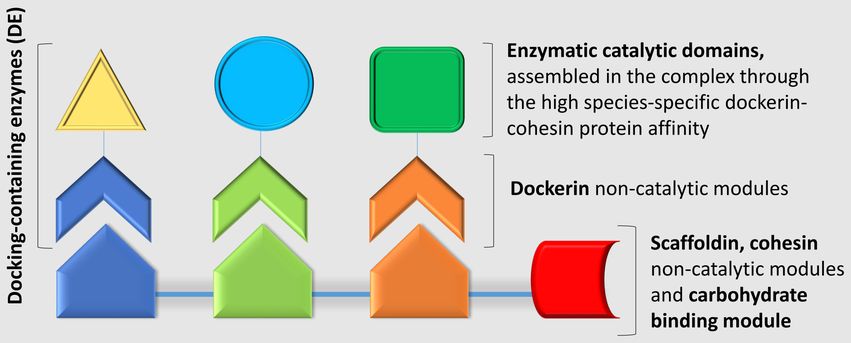
ON THE WAY TO CREATE DESIGNER XYLANOSOMES

1. Introduction

DESIGNER XYLANOSOMES

- **Inspired by natural cellulosomes**, multicatalytic enzyme complexes found in anaerobic cellulolytic organisms.
- Cellulosomes contain **different enzyme specificities** able to hydrolyse the complex hemicellulosic and cellulosic fractions of biomass.
- The engineered **Designer Xylanosomes** of this study aim to either degrade **arabinoxylan** to **completion** or produce **arabinoxylan-oligosaccharides** with specific structures and interesting properties for **fine-chemicals** as prebiotics applications, for example.

Schematic view of a modular Designer Xylanosome:

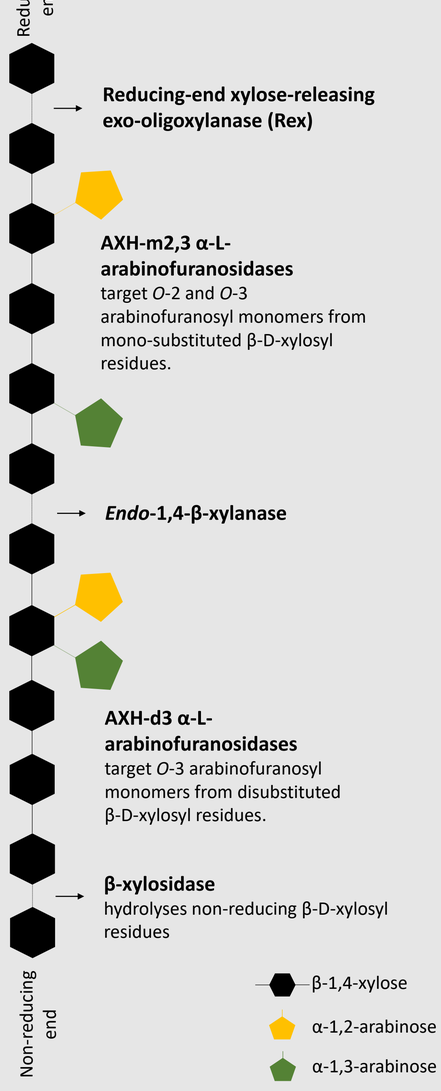


2. Inspiration

Processing of residual biomass as raw materials provides **economic and ecological benefits** due to its **biorenewability**.

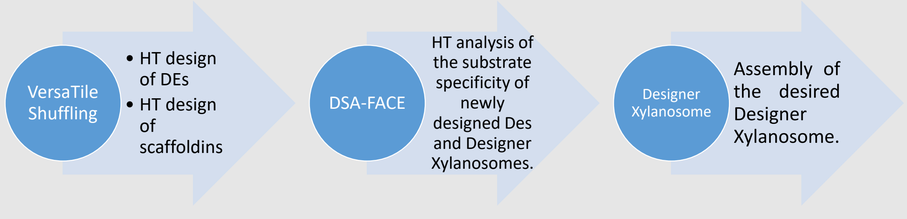
Arabinoxylan is largely found in cereal grains and grass cell walls.

Arabinoxylans are **complex substrates** and its hydrolysis requires the combination of **diverse enzymatic activities**.



3. Research Goal

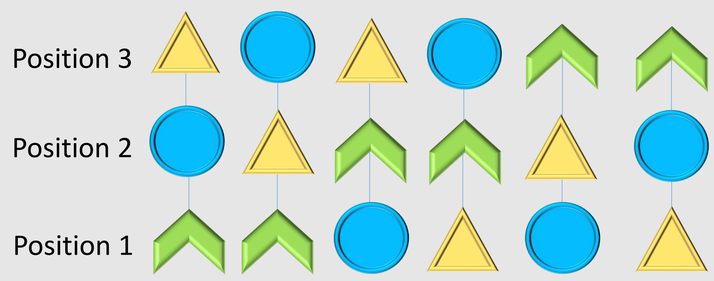
Platform for the high-throughput (HT) design and analysis of new multi-catalytic enzyme complexes that can selectively speed up the hydrolysis of arabinoxylan for different applications.



4. Experimental set-up and Results

A. DESIGN OF 96 CUSTOMIZED MULTICATALYTIC DEs BY VERSATILE SHUFFLING

- **VersaTile Shuffling**, shuffling technology which allows to assemble non-homologous catalytic and/or non-catalytic modules in HT.
- **Multicatalytic DE,** docking enzyme consisting of 1 dockerin and 2 enzymatic catalytic modules. For this case there are 6 possible final DEs constructs:



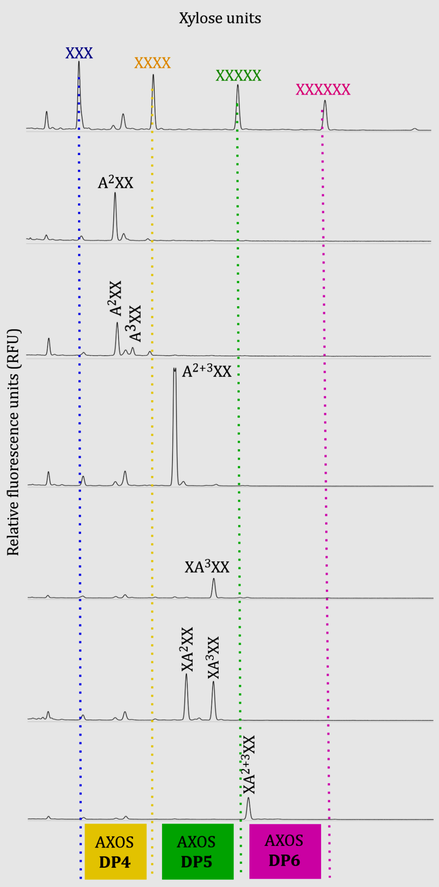
- **96 Multicatalytic DEs** were constructed in **one day**:

Modules used for construction of 96 multicatalytic DEs		
Catalytic modules		**Dockerin non-catalytic modules**
Endo-xylanases from *Thermobifida fusca*	GH10 Xyl10A	*Clostridium thermocellum* Cel48S dockerin (Type I)
	GH10 Xyl10B	*Clostridium cellulolyticum* 5A dockerin (Type I)
	GH11 Xyl11A	*Clostridium thermocellum* xDocA dockerin (Type II)
β-xylosidases from *Bifidobacterium adolescentis*	GH120 XylB	*Ruminococcus Flavefaciens* 44A dockerin (Type III)
	GH43 XylC	
	GH8 RexA	
α-L-arabinofuranosidases from *Bifidobacterium adolescentis*	GH43 AbfA AXH-m2,3	
	GH43 AbfA AxhD3	

B. ANALYSIS OF THE SUBSTRATE SPECIFICITY OF THE DEs CREATED BY DNA SEQUENCER-AIDED FLUOROPHORE-ASSISTED CARBOHYDRATE ELECTROPHORESIS (DSA-FACE)

- **DSA-FACE,** highly sensitive technique (pM range) for the analysis of the substrate specificity of arabinoxylan-active enzymes in HT.

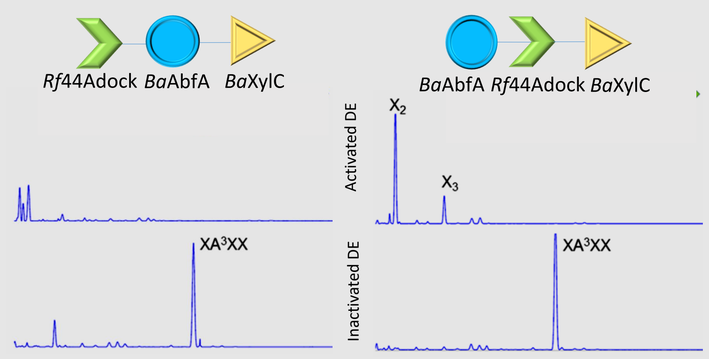
a. DSA-FACE is able to **resolve isomeric AXOS:**



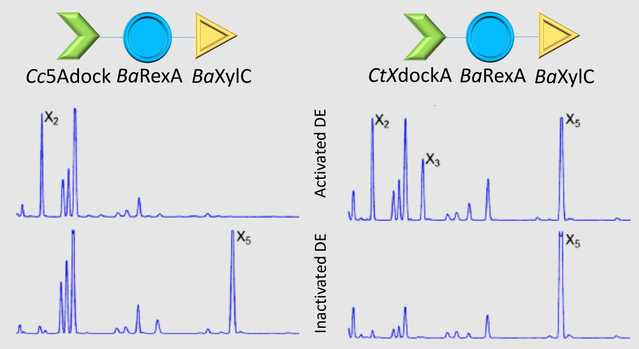
b. Enzymatic reactions with DEs and AXOS

Enzymatic reaction between DE and 10 µM XA^3XX/X_5. pH 6, 50 °C, 16 h
2 nM sugar were analysed by DSA-FACE.

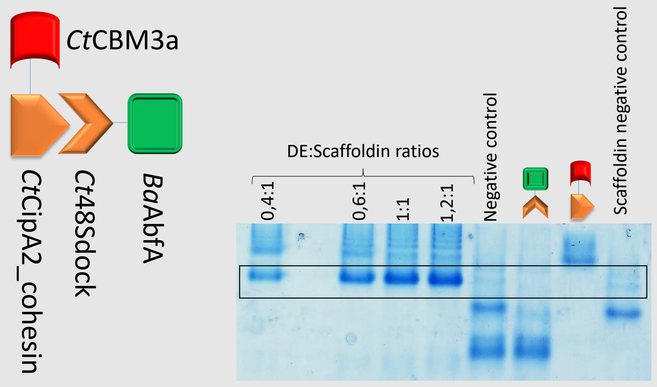
Order of modules in DE **matters:**



Type of dockerin in DE **matters:**



C. ASSEMBLY OF A DESIGNER XYLANOSOME
- assessed by Native-Page



5. Conclusions

- 96 modular DEs can be constructed, expressed and their substrate specificity analysed in 7 working days.
- Different modules position/composition in the final DEs may have interesting properties for degradation of arabinoxylan.
- A faster/more efficient technique for the analysis of the successful assembly a high number of putative Designer Xylanosomes needs to be investigated.

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