GlycoSpot

Enzyme screening standard

Julia Schückel
Research Scientist

The beginning

GlycoSpot
Enzyme screening standard

To harness the power and potential of **enzymes**, we need to **identify them** and **determine their activity**.

- Advances in genomics and bioinformatics enables the detection of candidate genes for relevant enzymes
- The activity of these enzymes needs to be evaluated empirically to maximize the potential of the catalysts
- The current methods are slow, expensive and require specialized equipment and personnel

Professor **William G. T. Willats**, KU
International capacity within plant cell walls, specializing in polysaccharides

Professor **Mads Clausen**, DTU
International capacity within synthetic carbohydrate chemistry

Built on years of research from two world-leading teams within plant biology and chemistry, William and Mads formed a spin-out company named GlycoSpot™ in 2014 – with the goal to solve this challenge.

The team

GlycoSpot
Enzyme screening standard

Board

Chairman, M.Sc.
Ole Kring
- CEO SMB

Members, Professor
William Willats
- Newcastle University

Professor
Mads Clausen,
- DTU

M.Sc.
Anders Jensen
- CAPNOVA

Organization

Interim CEO, MBA
Thomas Lacentra

Scientists, Ph. D
Stjepan Kracun
Julia Schückel

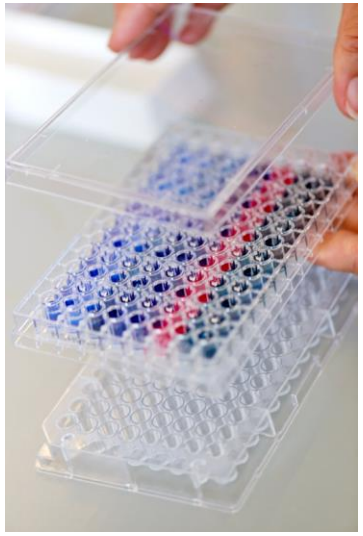
Laboratory assistants
Marta Iraburu Martinez
Rojan Demirtas
Iuliana Nita

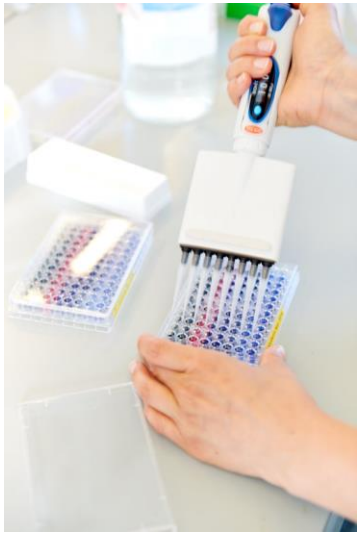
We offer

GlycoSpot

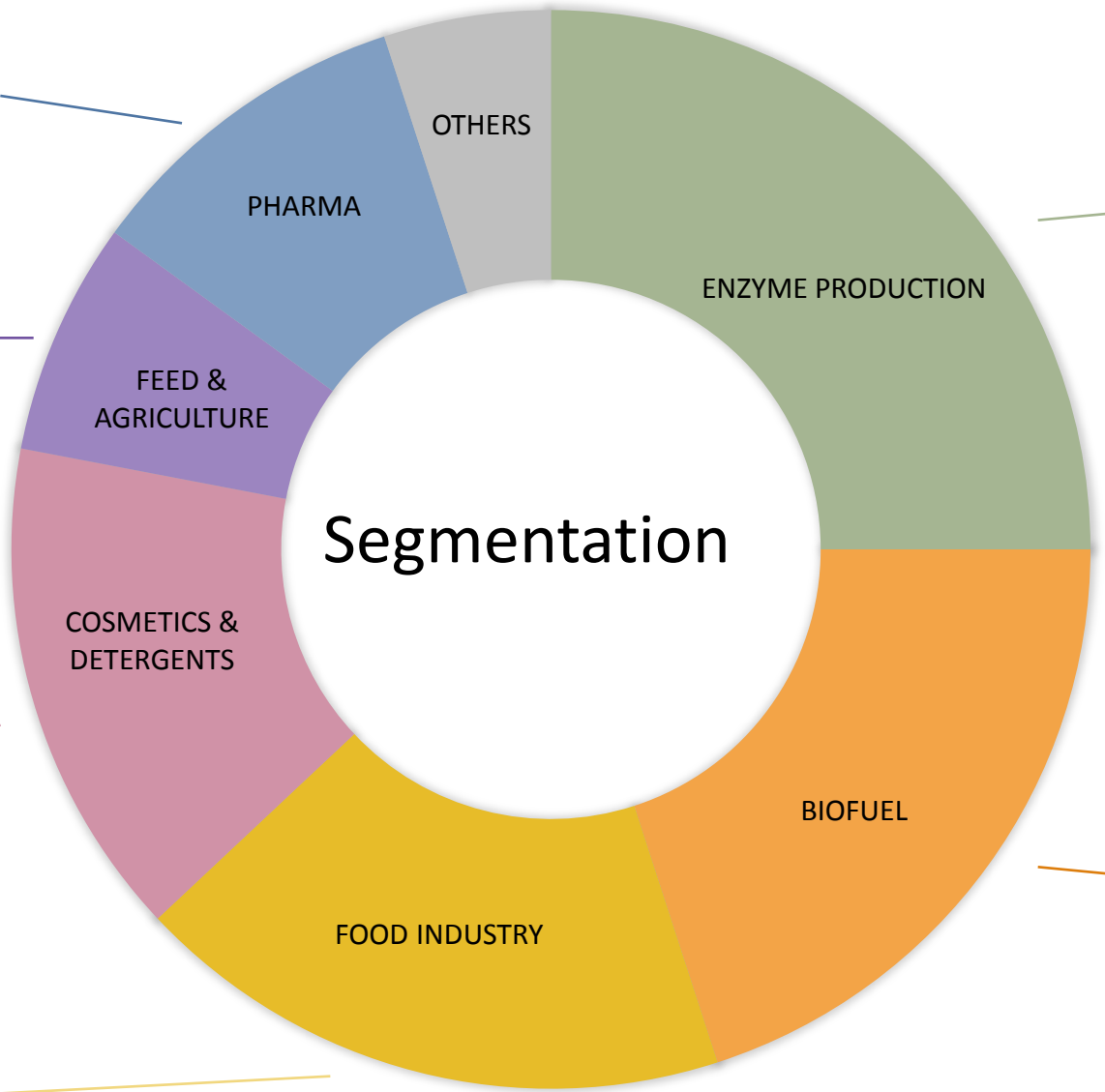
Enzyme screening standard

- Technology
- Service
- Support





GlycoSpot
Enzyme screening standard



Academic areas

Fitness supplement
Drug Development
Artificial food

Production
Discovery

Feed Industry

Personal and
Household care

Raw material
Process optimization
Advisory company

Raw material
Bread
Diary
Beverage

TECHNOLOGY

GlycoSpot 

Enzyme screening standard

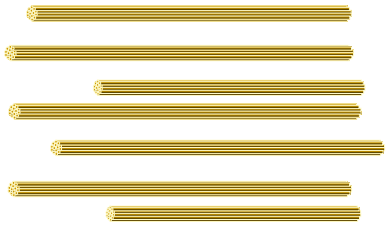
Julia Schückel
Research Scientist

Substrate source: Plant polysaccharides

GlycoSpot
Enzyme screening standard

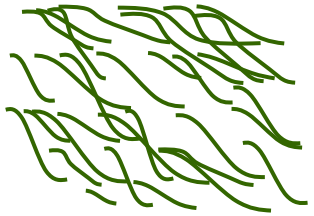
Cell wall

Cellulose



Hemicelluloses

- Mannans
- Xylans
- Xyloglucans
- Mixed linkage glucans

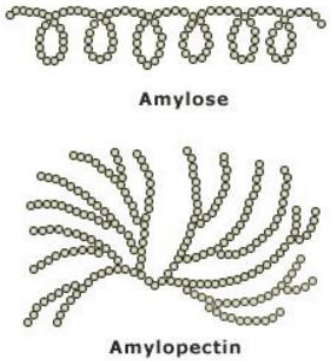


Pectins

- Homogalacturonan
- Rhamnogalacturon I
- Rhamnogalacturonan II
- Xylogalacturonan

Energy storage

Starch



New generation of chromogenic substrates

GlycoSpot
Enzyme screening standard

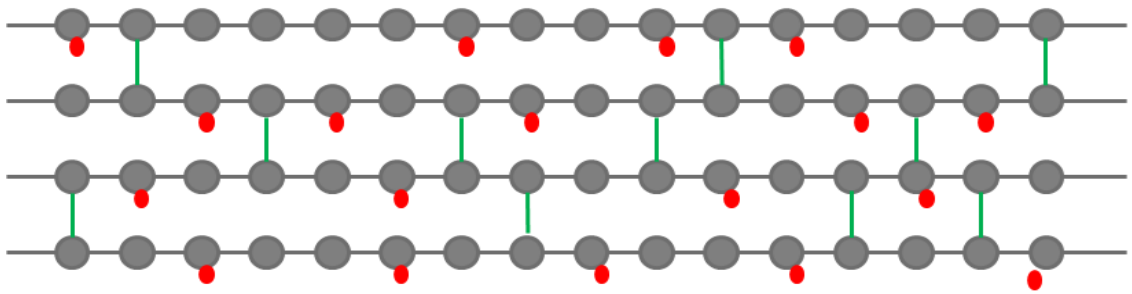
1. Insoluble **C**hromogenic **P**olymer **H**ydrogel substrate - **CPH** substrate
   - Source: pure polysaccharides
2. **I**nsoluble **C**hromogenic **B**iomass substrate - **ICB** substrate
   - Source: complex biomass material

Schückel *et al.* **2016** *J. Vis. Exp.* **115**
Kračun & Schückel *et al. Biotechnology for Biofuels* 2015, **8**:70

Patent application filed PA 2015 70311 (**2015**)
Patent application WO2015036000

Chromogenic Polymer Hydrogel (CPH) substrates

GlycoSpot
Enzyme screening standard



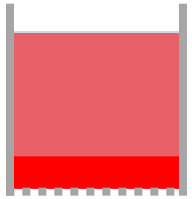
polysaccharide chain

dye

crosslinker

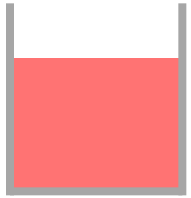
Add sample

Substrate plate
containing CPH substrate



Incubation (enzyme activity release colour)

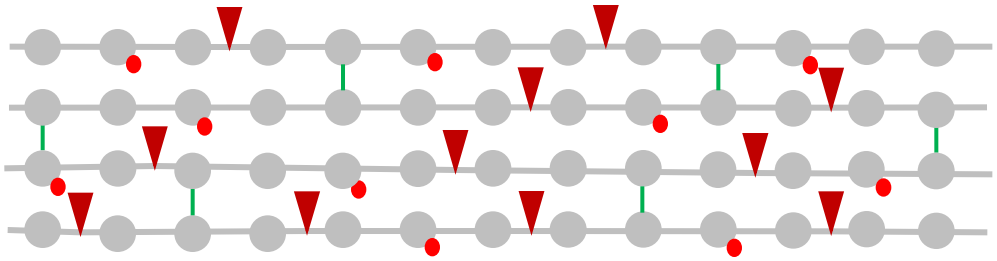
Product plate



Colour intensity proportional to enzyme activity

How does it work?

GlycoSpot
Enzyme screening standard



Active *endo*-enzyme

- Enzyme treatment releases small soluble dyed oligosaccharides, which give rise to colouration of the supernatant
- If there is colour release – the applied enzyme can degrade the polysaccharide that the substrate is made of

Chromogenic Polymer Hydrogel substrates

GlycoSpot
Enzyme screening standard

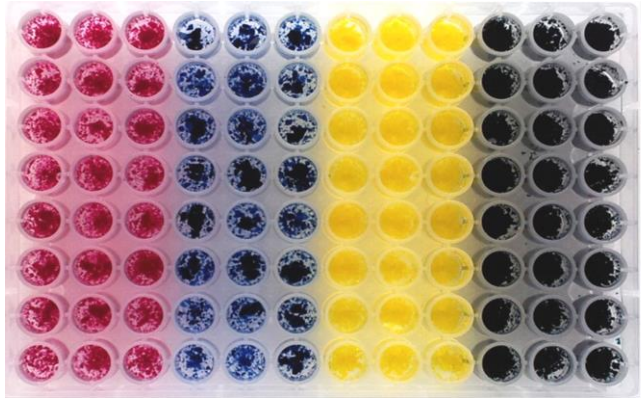


Plate with substrate CPH-galactomannan

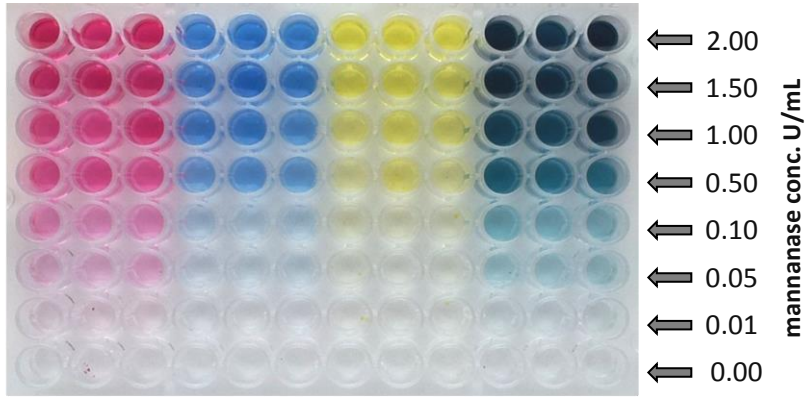
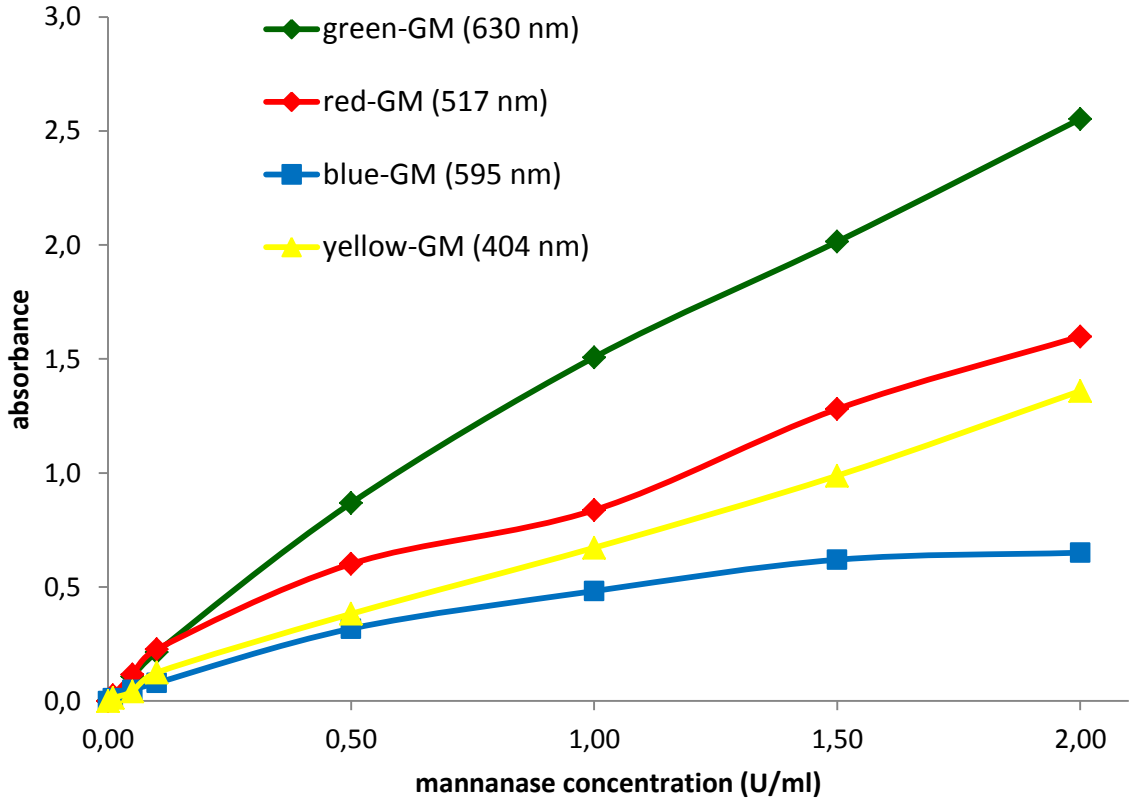


Plate with supernatant after 1h reaction

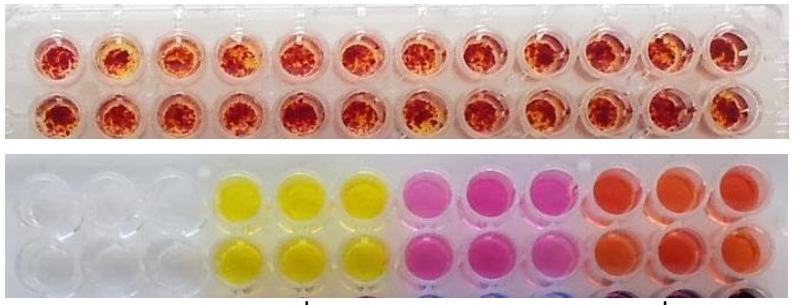


Kračun *et al. Biotechnology for Biofuels* 2015, **8**:70

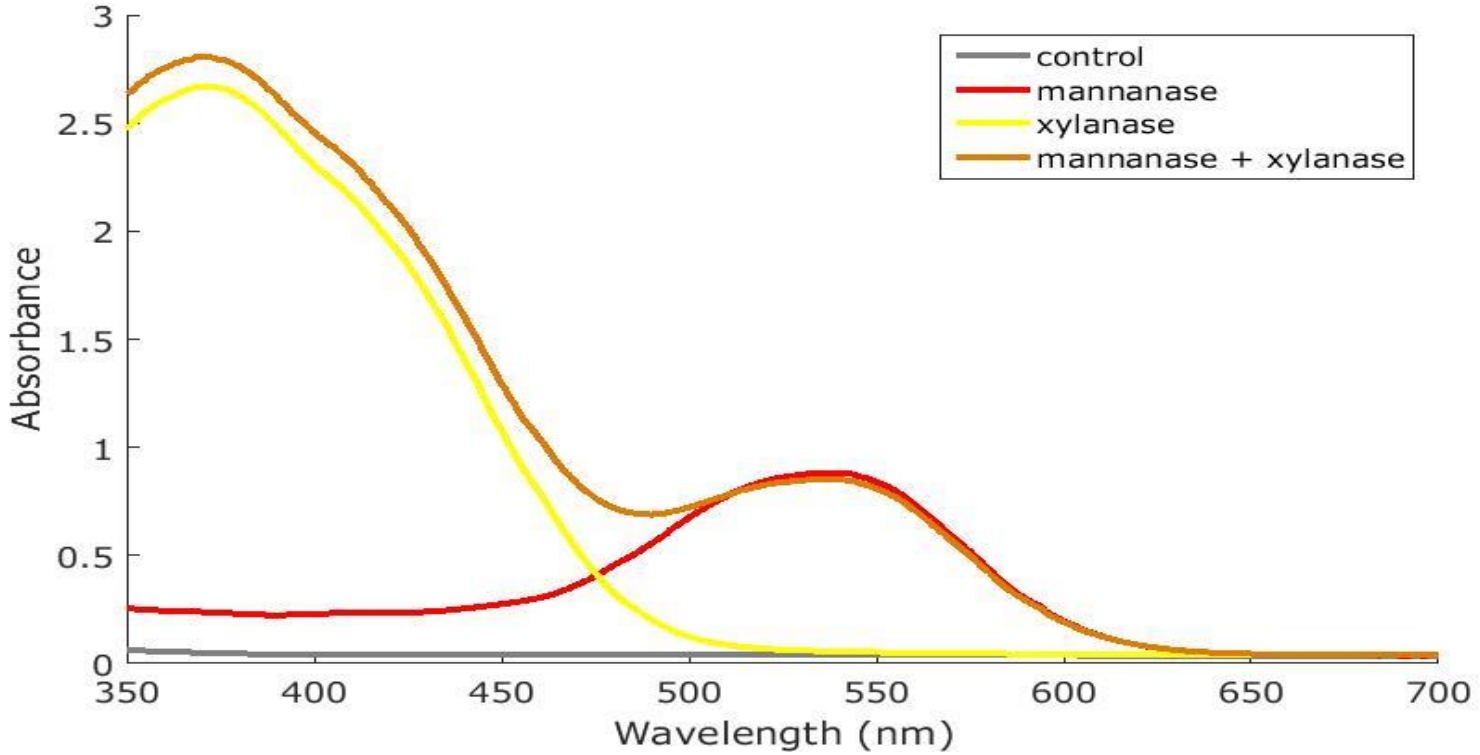
Combination of two CPH substrates

GlycoSpot
Enzyme screening standard

Xylan + GalMan



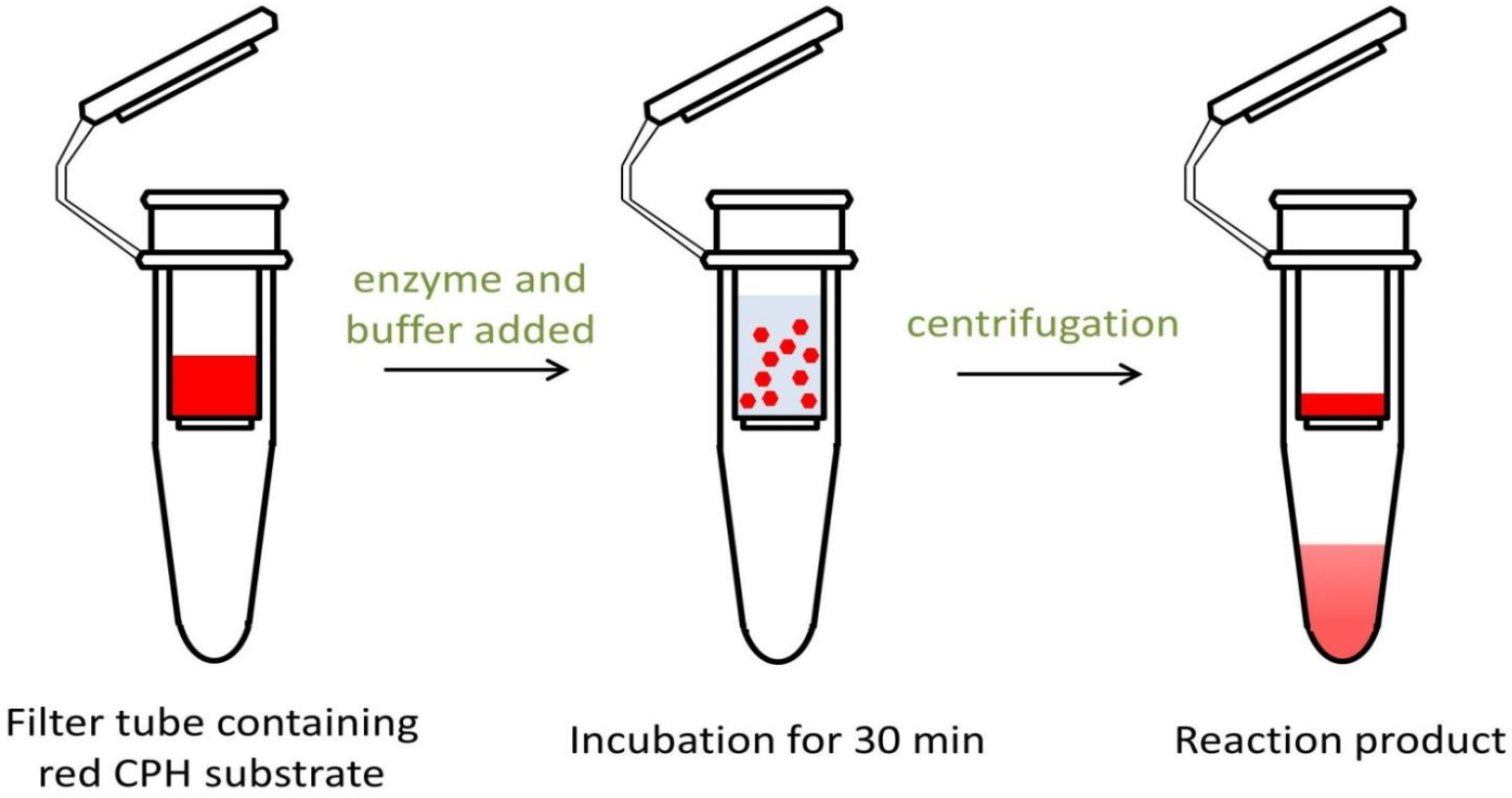
no enzyme xyl man xyl +man



Schückel *et al.* **2016** *J. Vis. Exp.* **115**

CPH substrates in tubes

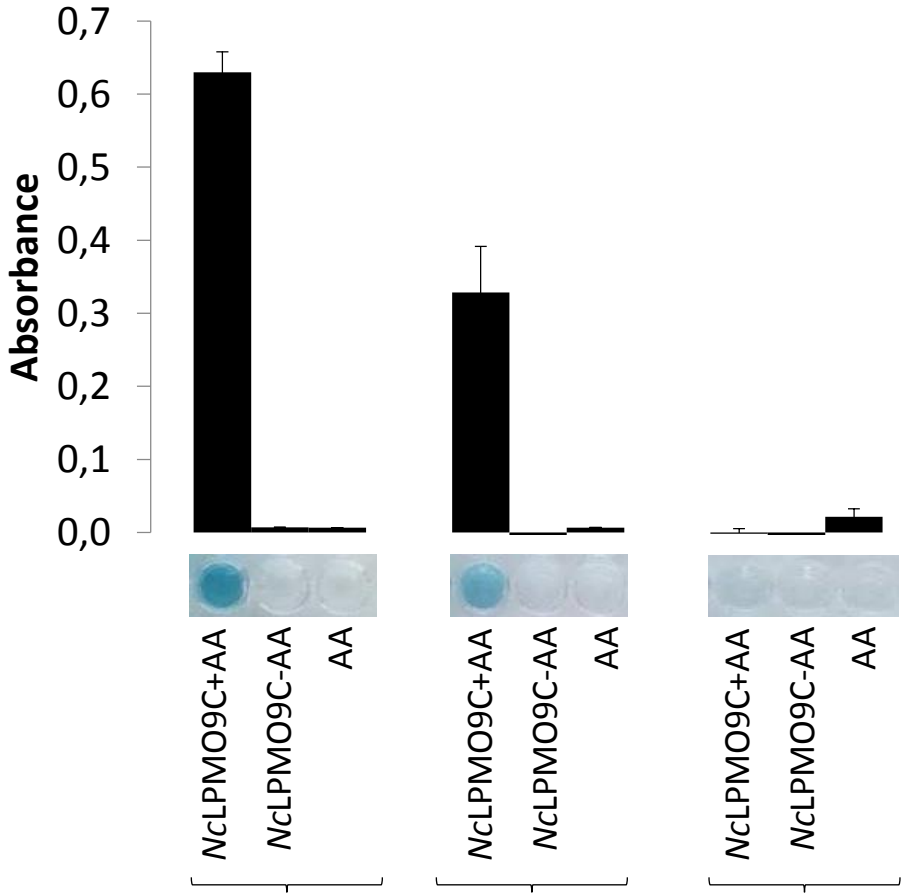
GlycoSpot
Enzyme screening standard



LPMO and protease activity

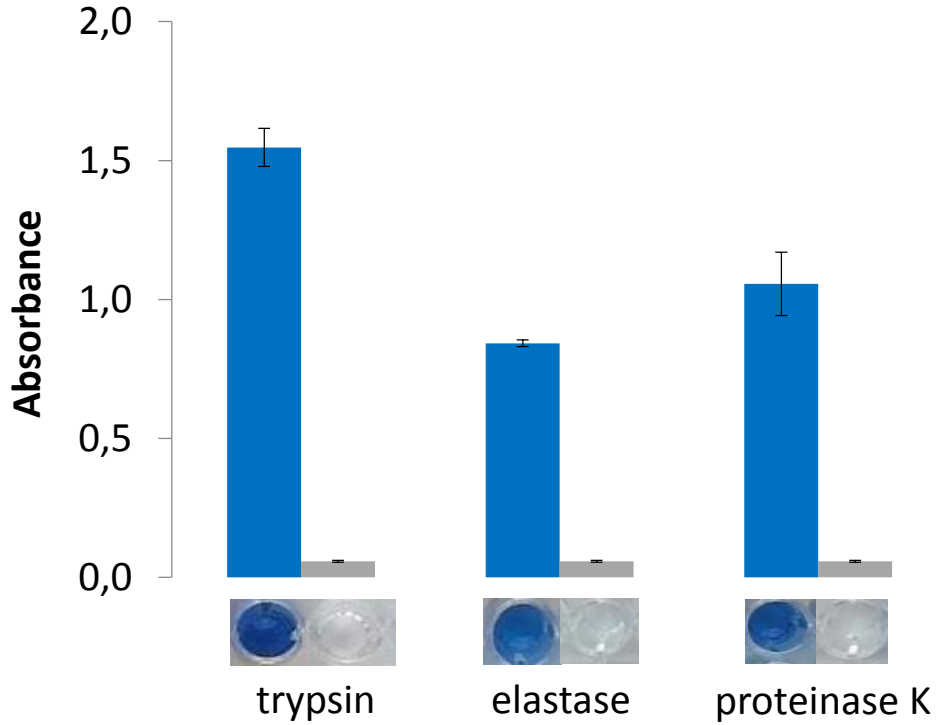
GlycoSpot
Enzyme screening standard

LPMO - lytic polysaccharide monooxygenase



Substrate: 2-HE cellulose lichenan xylan

Proteases



AA: ascorbic acid

List of CPH substrates

GlycoSpot
Enzyme screening standard

Substrate	Source			
CPH-2-hydroxyethylcellulose (CPH-2HE cellulose)	N/A	**CELLULOSE**	**PLANT**	**POLYSACCHARIDE**
CPH-amylopectin	potato	**STARCH**		
CPH-amylose	potato			
CPH-arabinoxylan	wheat	**HEMICELLULOSE**		
CPH-galactomannan	carob			
CPH-lichenan	Icelandic moss			
CPH-xylan	beechwood			
CPH-xyloglucan	*tamarind*			
CPH-β-glucan from barley	barley			
CPH-β-glucan from oat	oat			
CPH-β-glucan from yeast	yeast			
CPH-arabinan	sugar beet	**PECTIN**		
CPH-pectic galactan	potato			
CPH-rhamnogalacturonan	soy bean			
CPH-chitosan	crab shells		**OTHER**	
CPH-curdlan	*Alcaligenes faecalis*			
CPH-dextran	*Leuconostoc* spp.			
CPH-pachyman	*Poria cocos*			
CPH-pullulan	*Aureobasidium pullulans*			
CPH-casein	bovine milk			**PROTEIN**

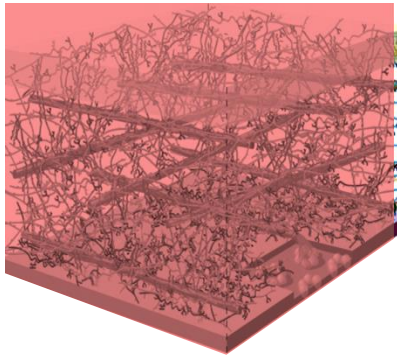
Insoluble Chromogenic Biomass (ICB) substrates

GlycoSpot
Enzyme screening standard

Homogenized plant material

synthesis

Insoluble Chromogenic Biomass

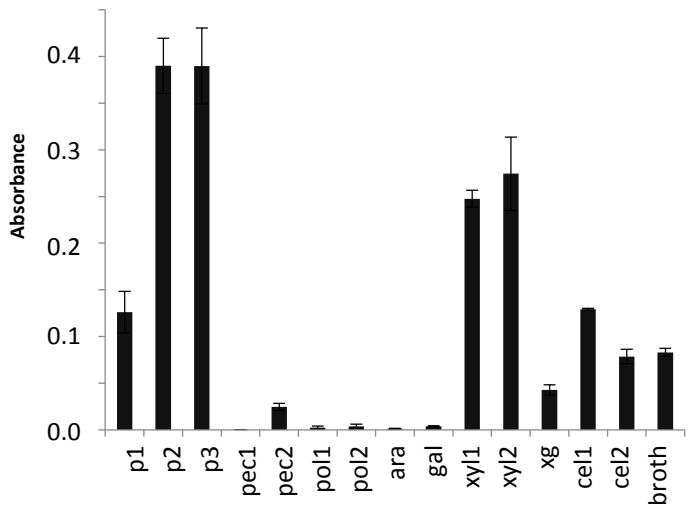


Advantage: ICB substrates are designed to address enzymatic accessibility in the context of complex biological material.

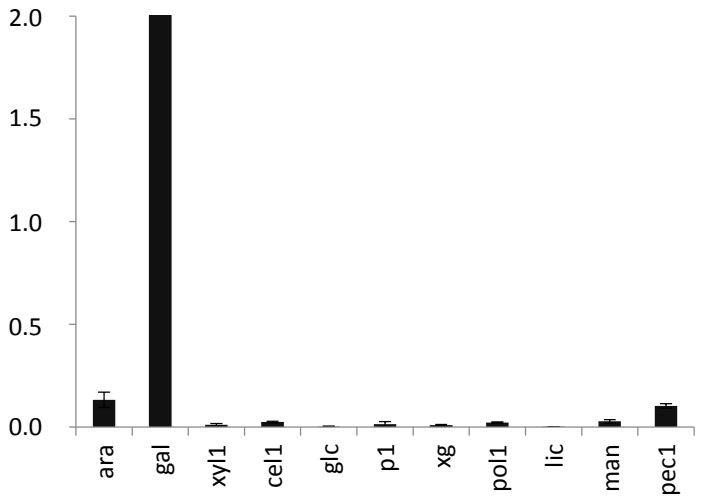
Insoluble Chromogenic Biomass (ICB) substrates

GlycoSpot
Enzyme screening standard

ICB-wheat straw



ICB-lupin seeds



p1	Pectinase (Rhizopus sp.)
p2	Pectolyase Y-23 (Aspergillus japonicus)
p3	Pectolyase (Aspergillus japonicus)
pec1	Pectate lyase (C. japonicus)
pec2	Pectate lyase (Aspergillus sp.)
pol1	endo-polygalacturonase (Aspergillus niger) M2
pol2	endo-polygalacturonase
ara	endo-arabinase
gal	endo-1,4-β-D-Galactanase
xyl1	β-xylanase,M4 (Aspergillus niger)
xyl2	endo-1,4-β-Xylanase M1 (T. viride)
xg	Xyloglucanase (GH5) (*Paenibacillus* sp.)
cel1	endo-cellulase (EGII) (*Trichoderma longibrachiatum*)
cel2	Cellulase (*Bacillus amyloliquefaciens*)
glc	endo-1,3-β-glucanase
lic	Lichenase (endo-1,3(4)-β-Glucanase) (Bacillius sp.)
man	endo-1,4 ß-Mannanase (Cellvibrio japonicus)
broth	Culture broth from *Penicillium expansum* (5d after inoculation)

Kračun *et al. Biotechnology for Biofuels* 2015, **8**:70

Industrial Applications

GlycoSpot

Enzyme screening standard

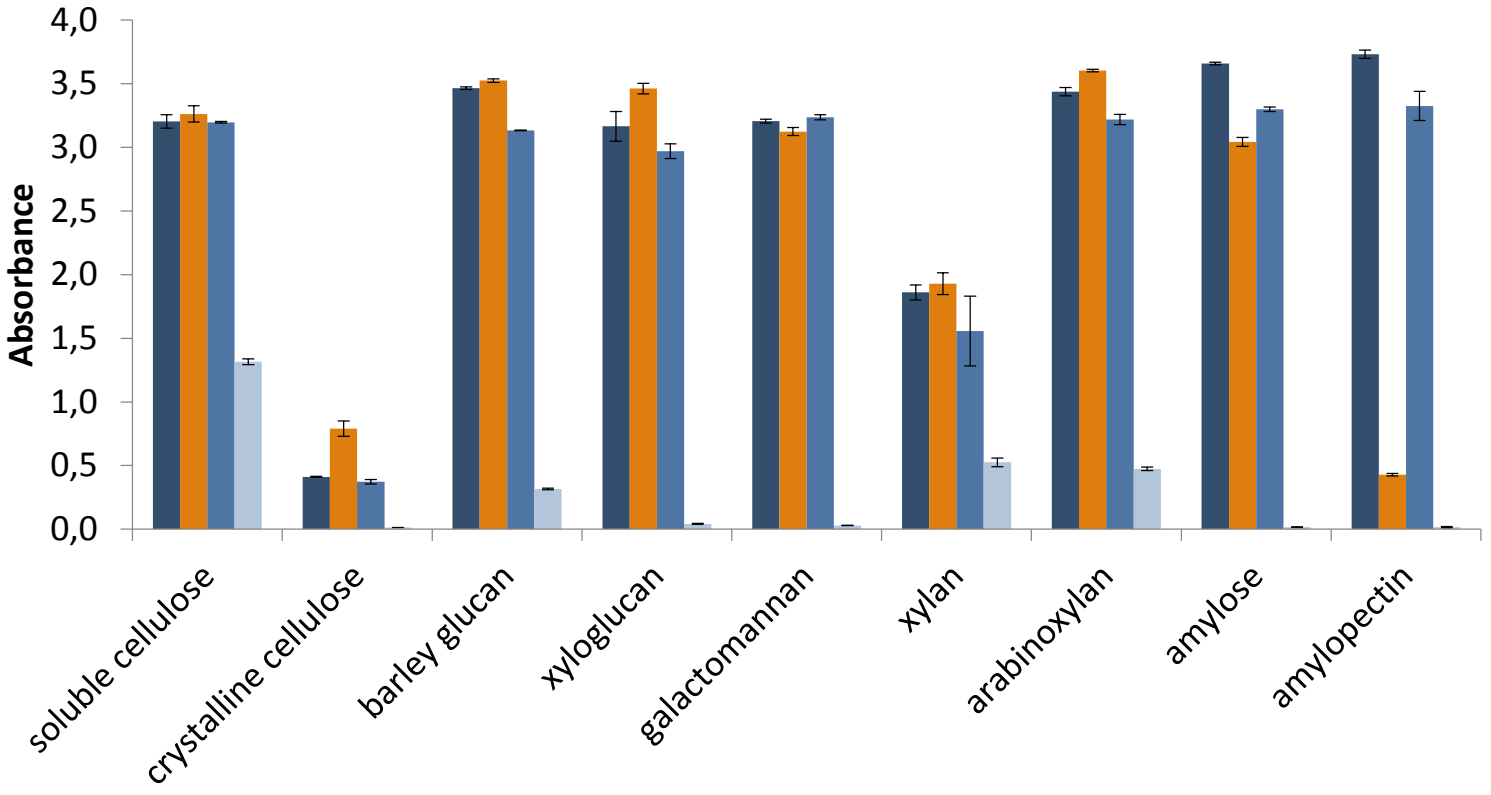
Julia Schückel
Research Scientist

Enzymes in biomass conversion

GlycoSpot
Enzyme screening standard

- Efficient enzyme cocktails can degrade biomass more effectively

- Discovery of new enzymes requires high-throughput screening of biochemical properties of new enzyme candidates



Enzyme cocktail 1

Enzyme cocktail 2

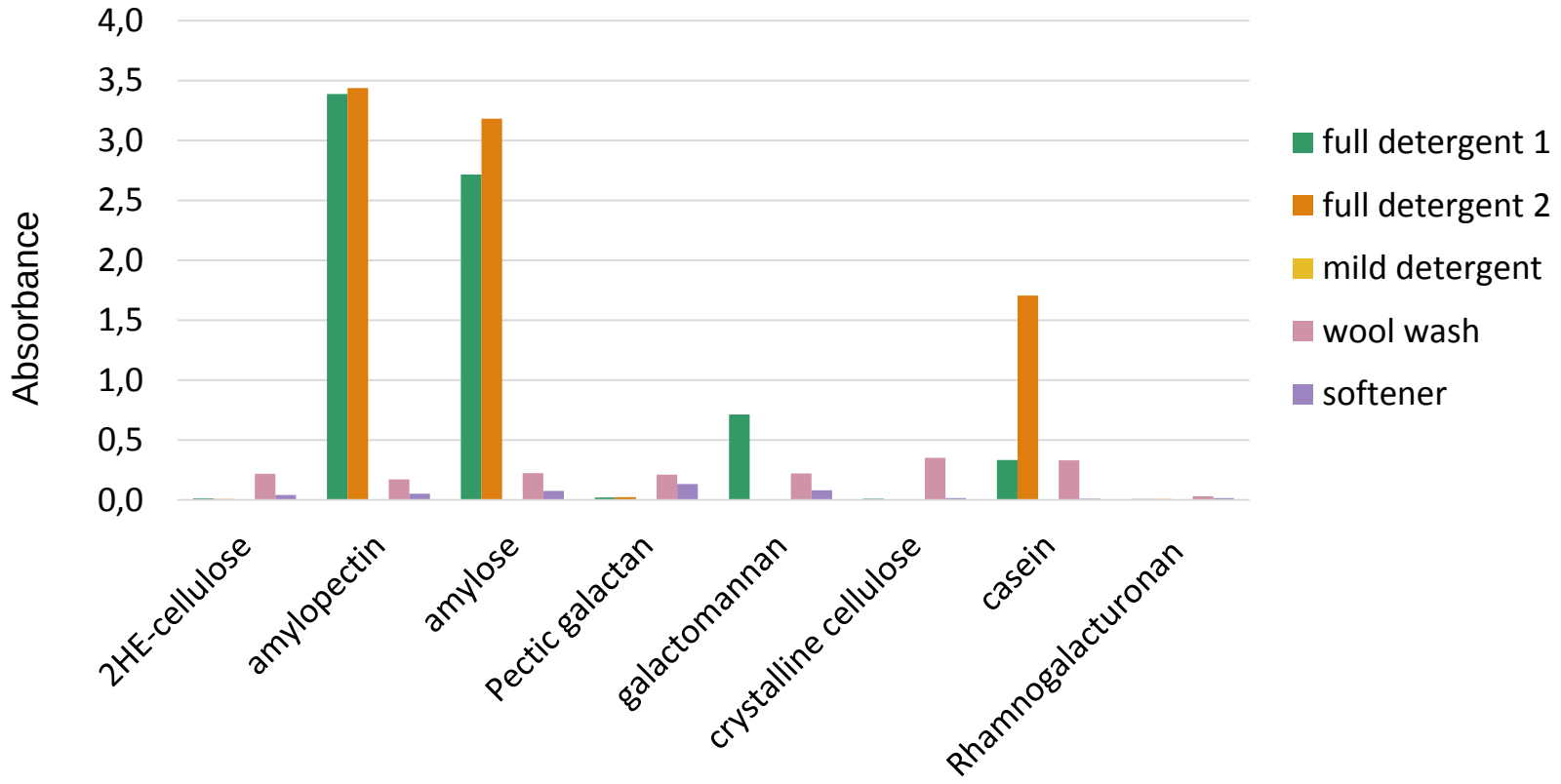
Time point 1

Time point 2

Enzymes in washing detergents

GlycoSpot
Enzyme screening standard

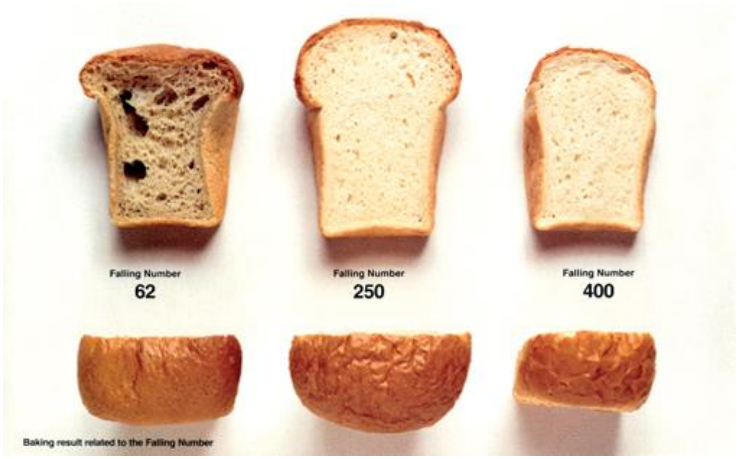
- Analysis of enzymes in various detergents
- Activity screening after storage (residual activity)



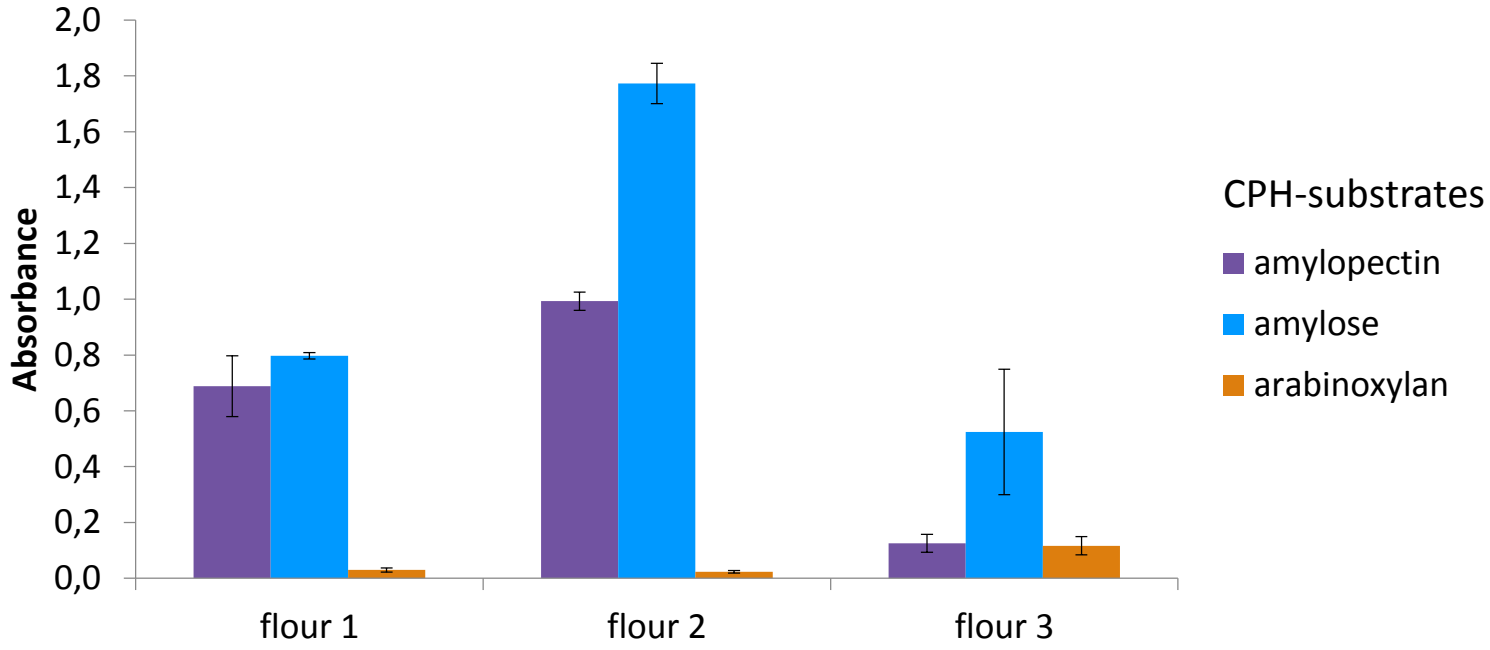
Reaction conditions: 1h at 26 ˚C

Enzymes in bread production

GlycoSpot
Enzyme screening standard



- Amylase (starch-degrading) activity in flour has direct effect on the quality of the bread
- Amylase activity now is measured through the “falling number method”



Summary

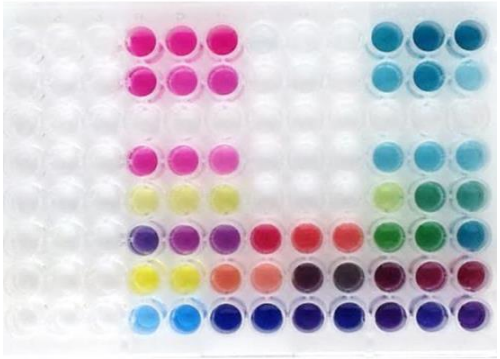
GlycoSpot
Enzyme screening standard

Two types of substrates

CPH substrate
(pure polysaccharide)

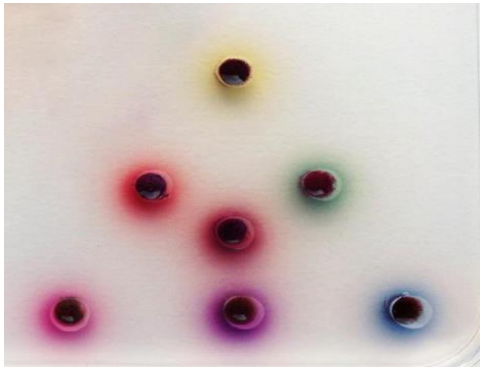
ICB substrate
(complex mixture)

Three types of assay formats



96-well plates

filter tubes

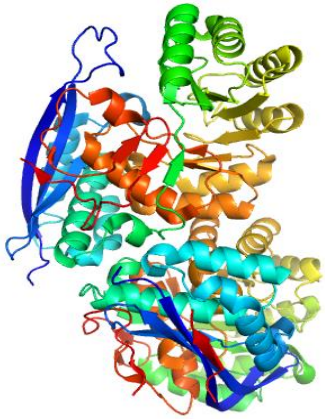


agar plates

Summary

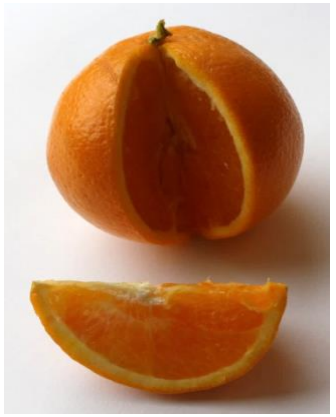
GlycoSpot
Enzyme screening standard

What have we screened?



Purified enzymes
and enzyme cocktails

Secreted enzymes from
bacteria and fungi



Endogenous plant
enzymes

Enzymes produced by termites

Saliva from various animals

Schückel *et al.* **2016** *J. Vis. Exp.* **115**

Jiménez, D.J. *et al. Appl Microbiol Biotechnol,* **2016**. doi:10.1007/s00253-016-7713-3

Kračun & Schückel *et al. Biotechnology for Biofuels* **2015**, 8:70

Mackenzie *et al. Applied and Environmental Microbiology,* **2015**, 81(1):187-95

Patent application filed PA 2015 70311 (**2015**)

Patent application WO2015036000

GlycoSpot

Enzyme screening standard