

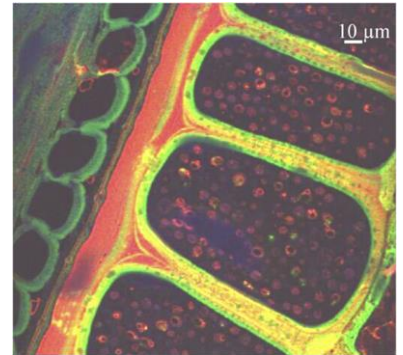


Immunomodulatory Activities of Wheat Arabinoxylan Hydrolyzates

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Kathy Christianson

Time Slips and Visiting Scientists

Yasuyuki Nishitsuji
Angela Ostenson
Tuba Turkmen
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Kaitlyn Peterson



Wheat Quality Laboratory



NDSU Wheat
Quality Lab

Project Leader
Dr. Senay Simsek

Survey: Regional
Quality Report

Export Cargo
Survey

Nursery Samples:
Breeding Programs

Research Projects



Including Field
Plots

Established 1907

Wheat Quality Laboratory



1. Kernel Quality



2. Milling Quality



3. Flour Quality



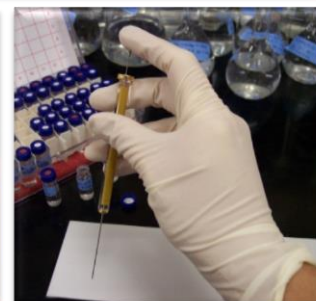
4. Dough Quality



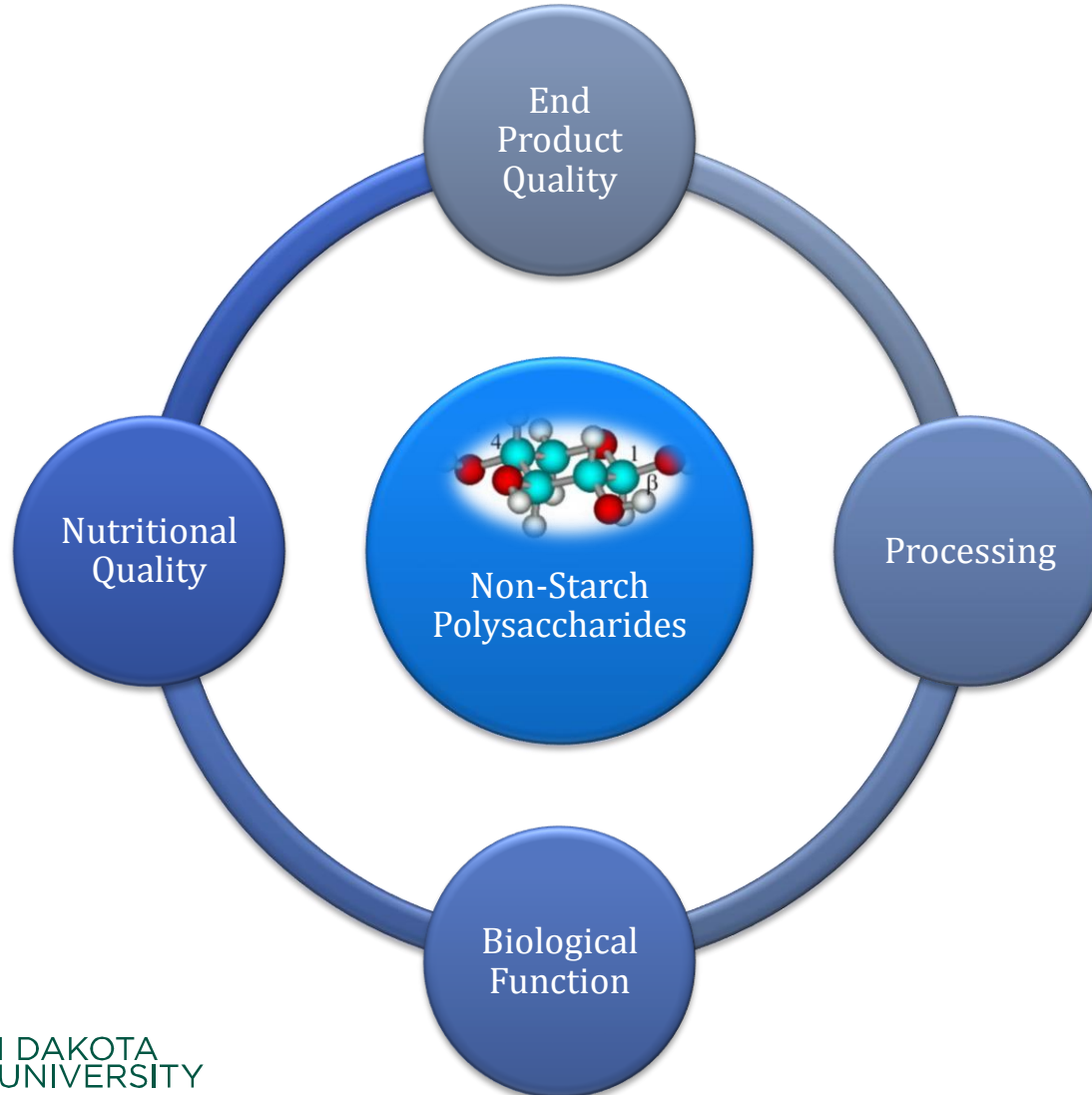
5. Baking Quality

Analytical Chemistry Laboratory

Analysis of
Sugars
Polysaccharides
Starch
Wheat Proteins
Mycotoxins

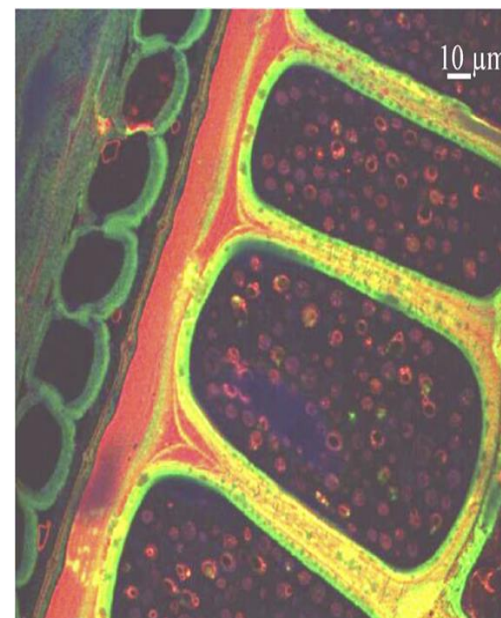


Non-Starch Polysaccharides

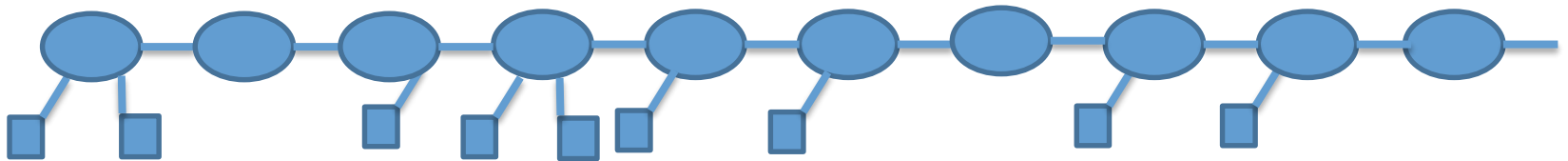
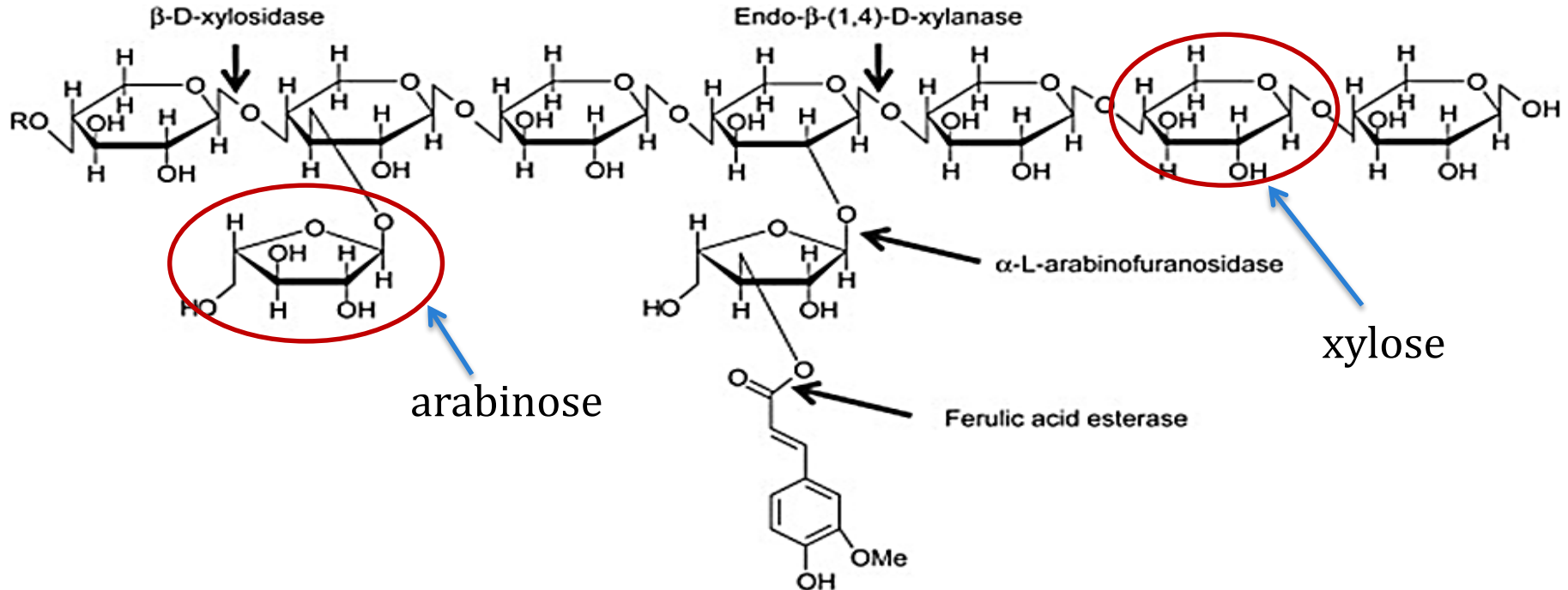


Arabinoxylans (AX)

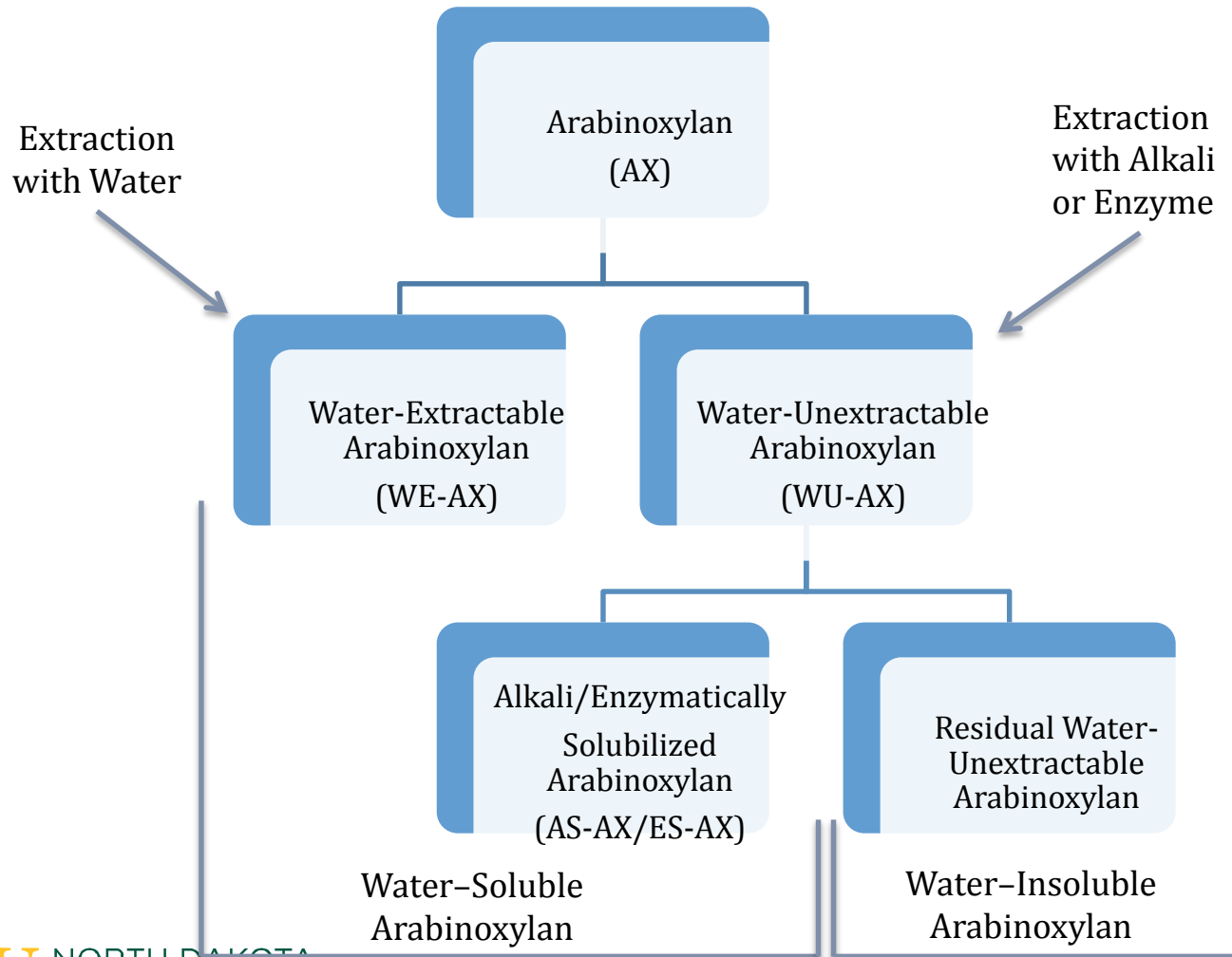
- AX are the main non-starch polysaccharide in cereals and grasses
- It is the predominant polysaccharide in the cell wall of wheat grain
 - 5.8-6.4% of grain



Structure of Arabinoxylans



Classification of AX



Immunity

- Immunity
- Immune system
- Inflammation



<http://effectivehealth.ca/staying-healthy-cold-flu-season/>

Immunity

- Immunity → resistance to disease
- Immune system → collection of cells, tissues and molecules that mediate immunity
- Inflammation → a response of the innate immune system that serves as a protective mechanism against infection and tissue damage



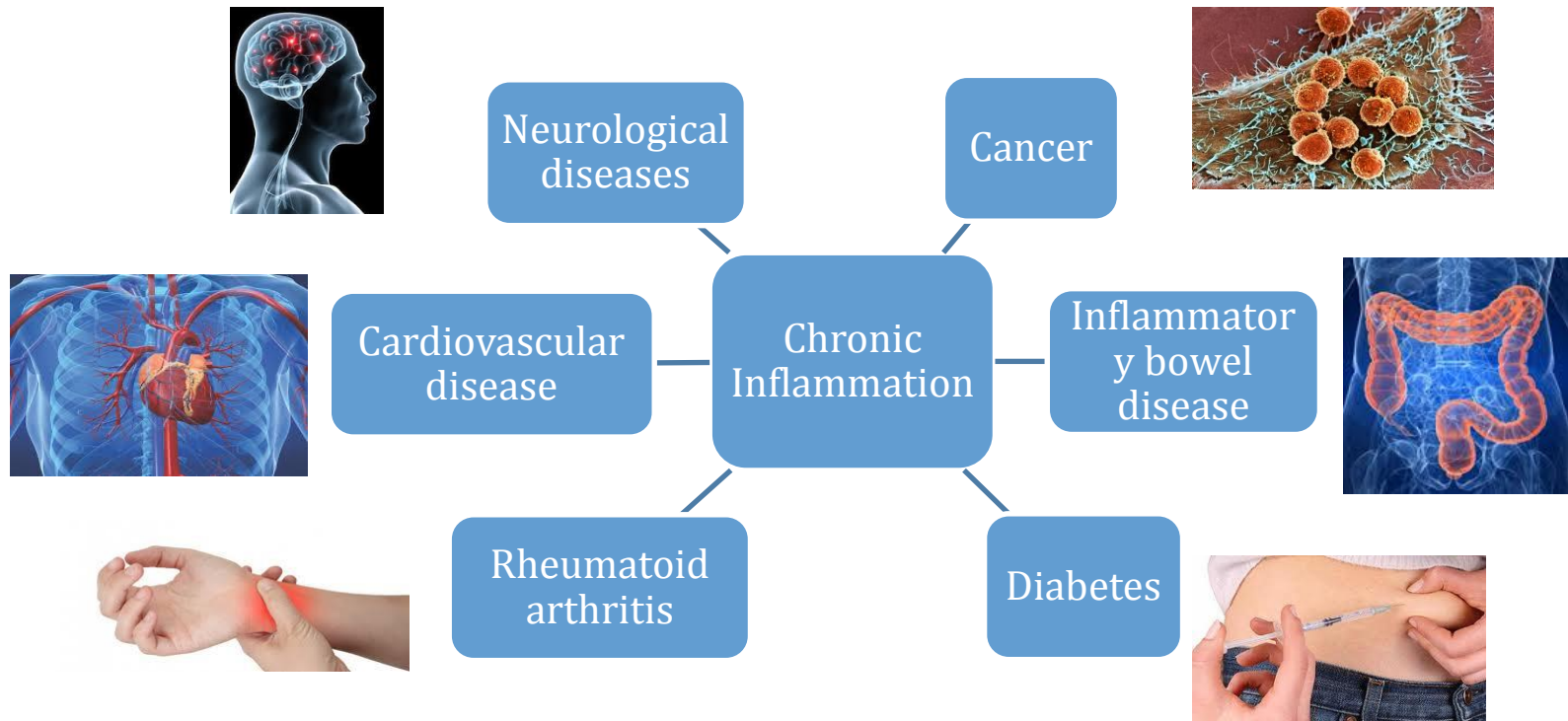
<http://effectivehealth.ca/staying-healthy-cold-flu-season/>

- Healthy immune system
- Inflammation

} Healthy individual



<http://everydayhealthforlife.com/immune-system-boosters/>



<http://www.grandislandphysicaltherapy.com/>

<http://www1.imperial.ac.uk/nhli/cardio/>

<http://www.healthline.com/health-slideshow/arthritis-wrist>

<http://www.mskcc.org/blog/>

<http://www.millersurgery.co.uk/procedures/>

<http://mdweightworx.com/mdweightworx/type-2-diabetes/>

Immunomodulators

- Immunomodulators → compounds that alter the inflammatory response
- Plant polysaccharides?



<http://www.sweetadditions.net/food-drinks/>

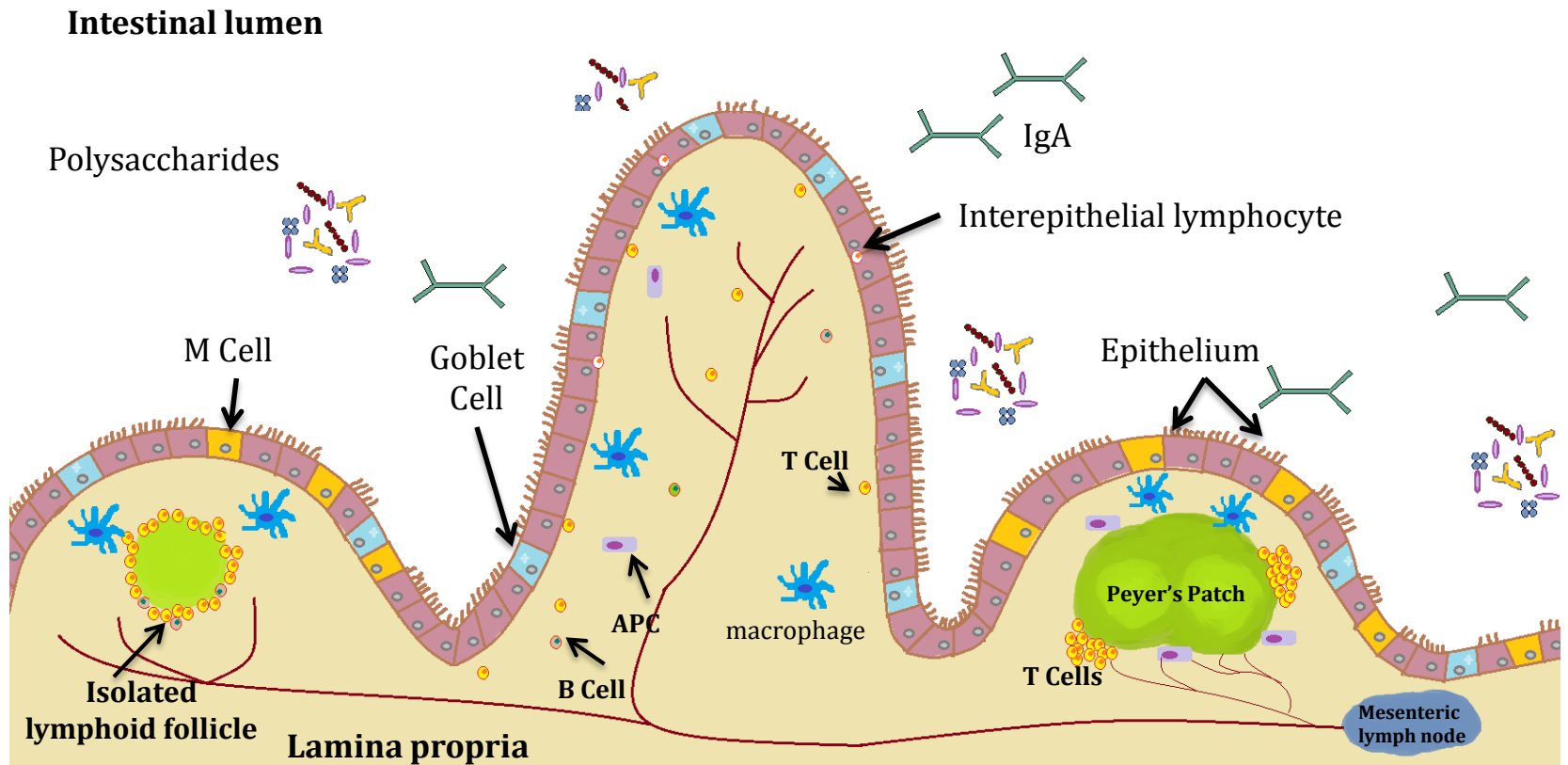
Immunomodulators

- Immunomodulators → compounds that alter the inflammatory response
- Polysaccharides → possible immunomodulators
- Polysaccharides are capable of directly stimulating the intestinal epithelial monocytes
 - Leads to immunological outcomes



<http://www.sweetadditions.net/food-drinks/>

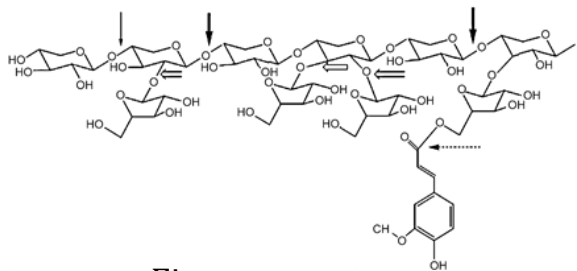
The gut-associated lymphoid tissues (GALT)



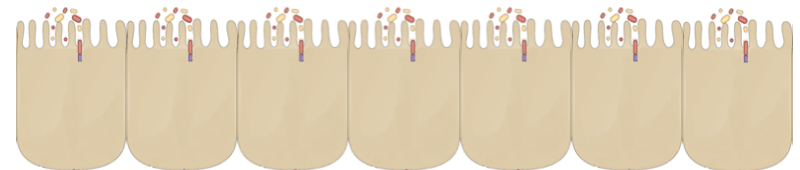
Adapted from Paeschke and Aimutis (2010)

Need Statement

- Immune response in mice and chicken
- Lack of understanding on.....



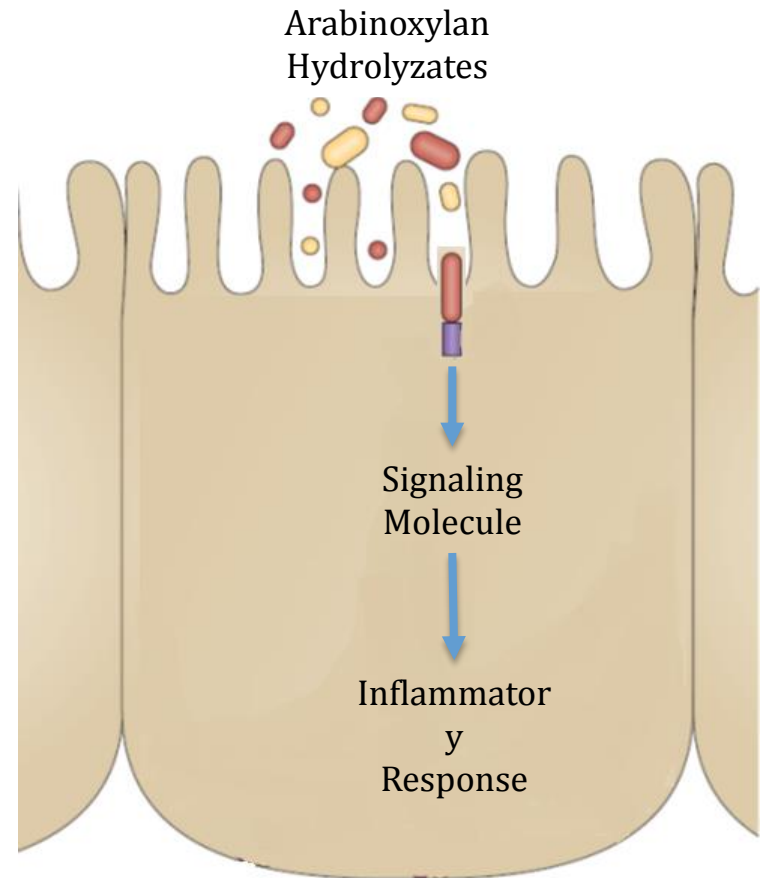
Fine structure



Cellular level

Hypothesis

AX hydrolyzates have
structurally driven
immunomodulatory
properties

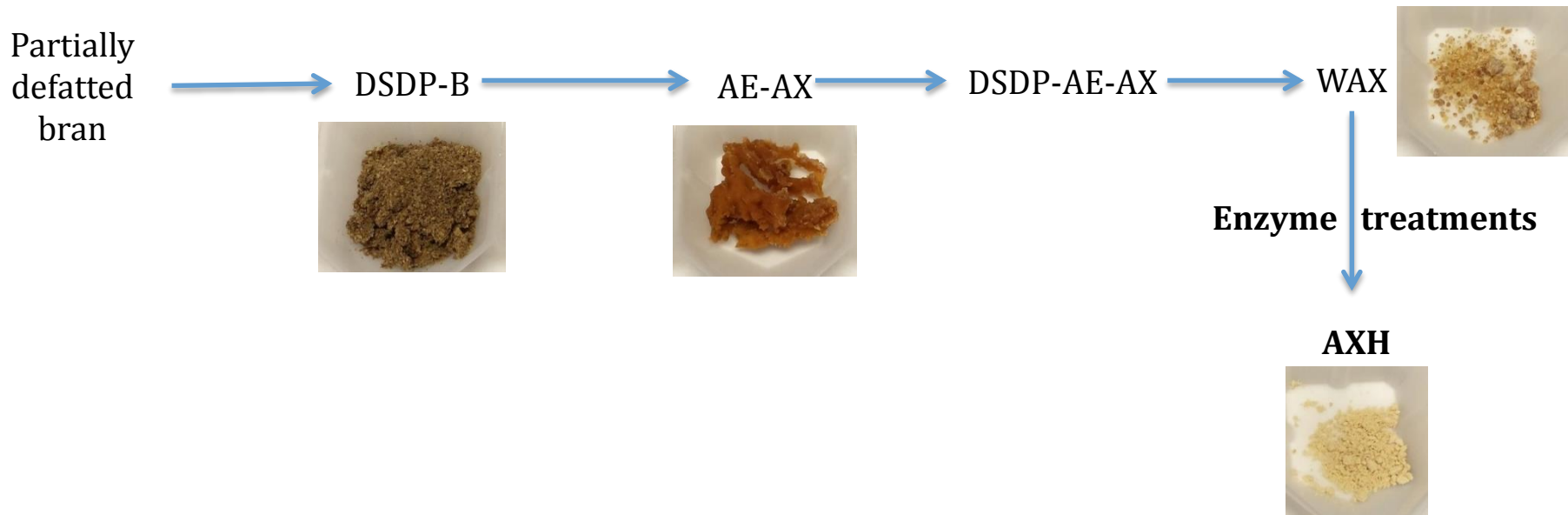


Adapted from Artis (2008)

Objectives

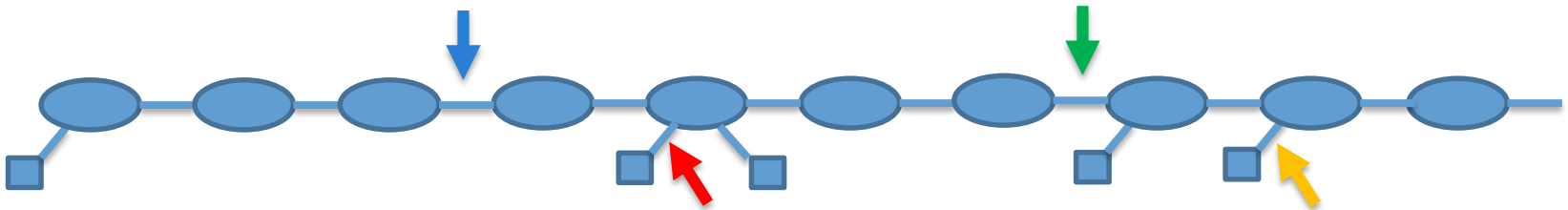
- Production and characterization of enzymatically tailored arabinoxylan hydrolyzates
- Evaluation of immunomodulatory activity with respect to structure

Production of Arabinoxylan Hydrolyzates from Wheat Bran

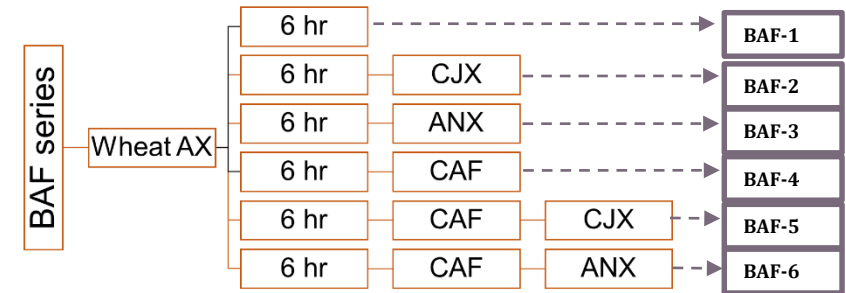
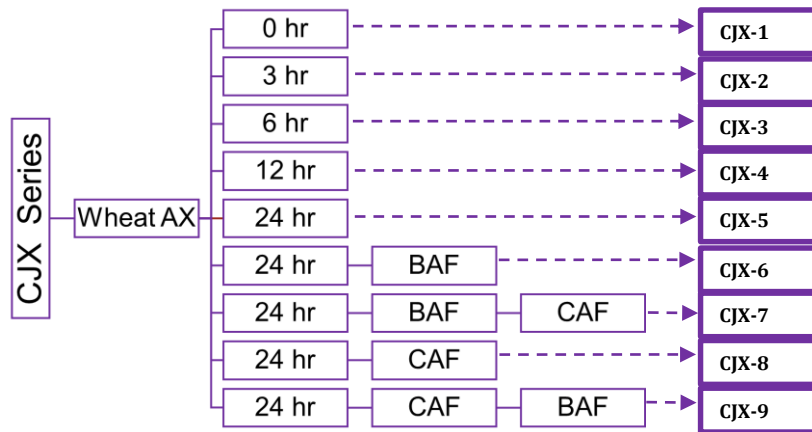
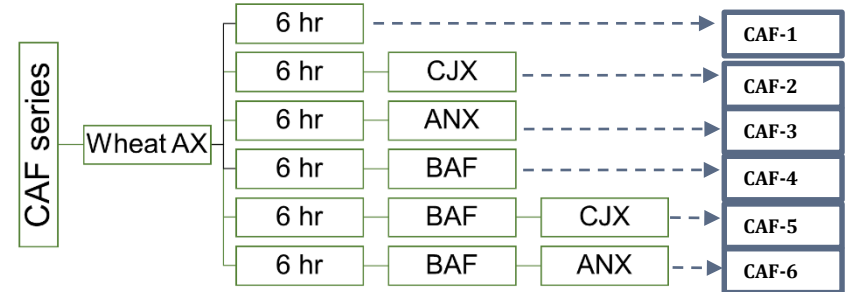
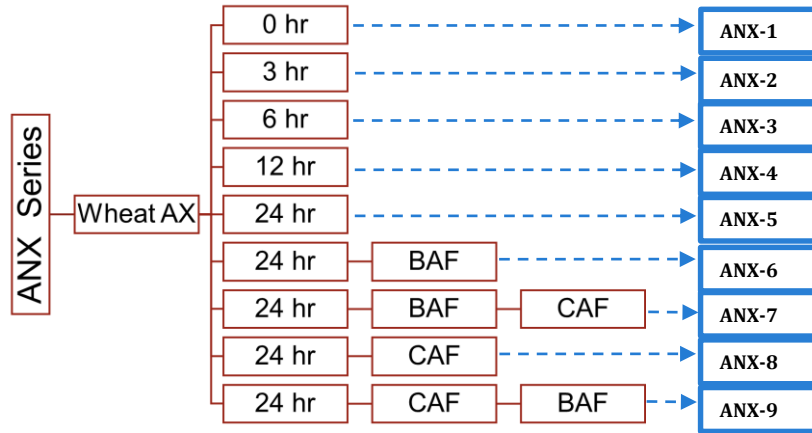


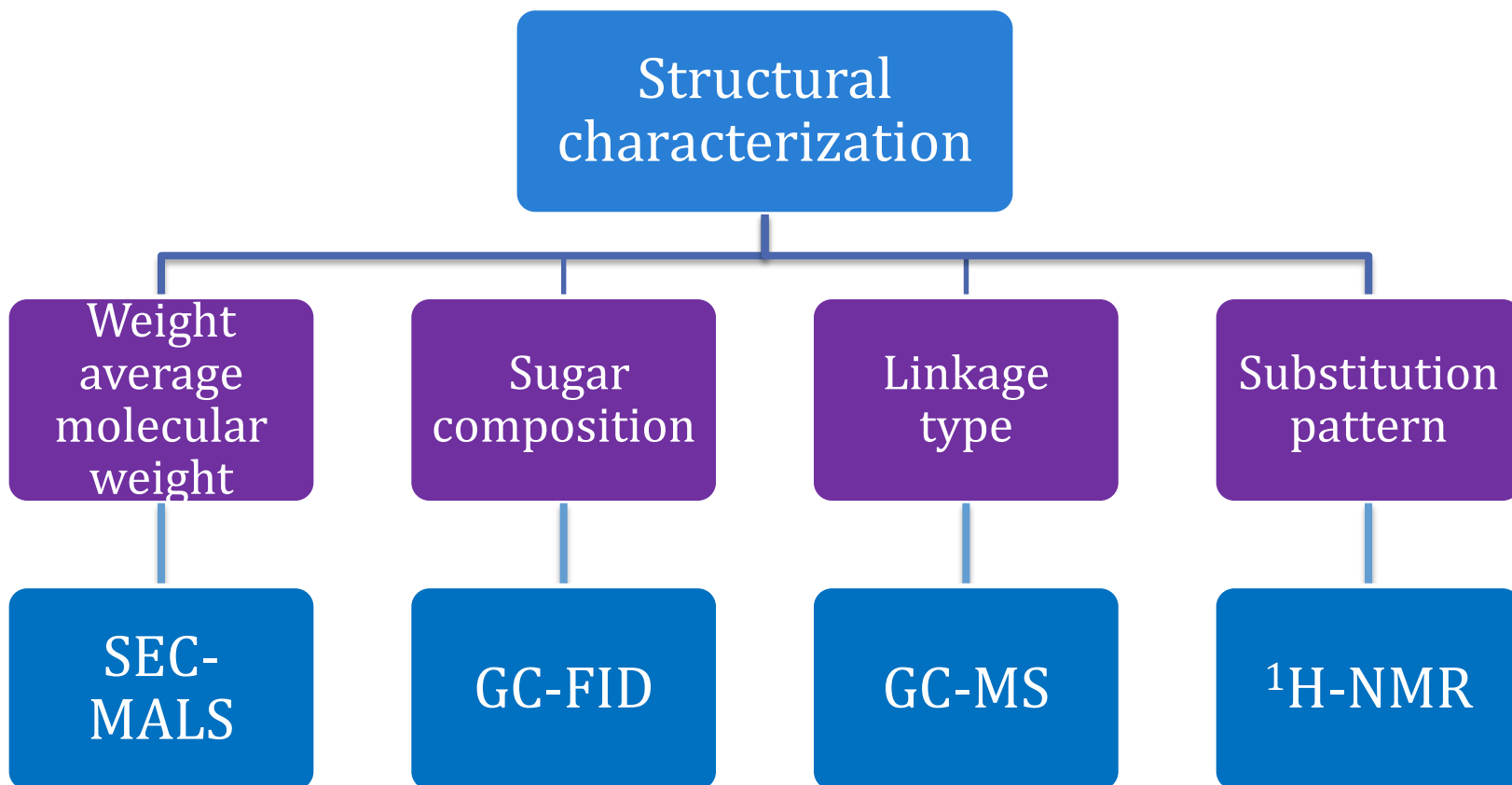
The Enzymes Used

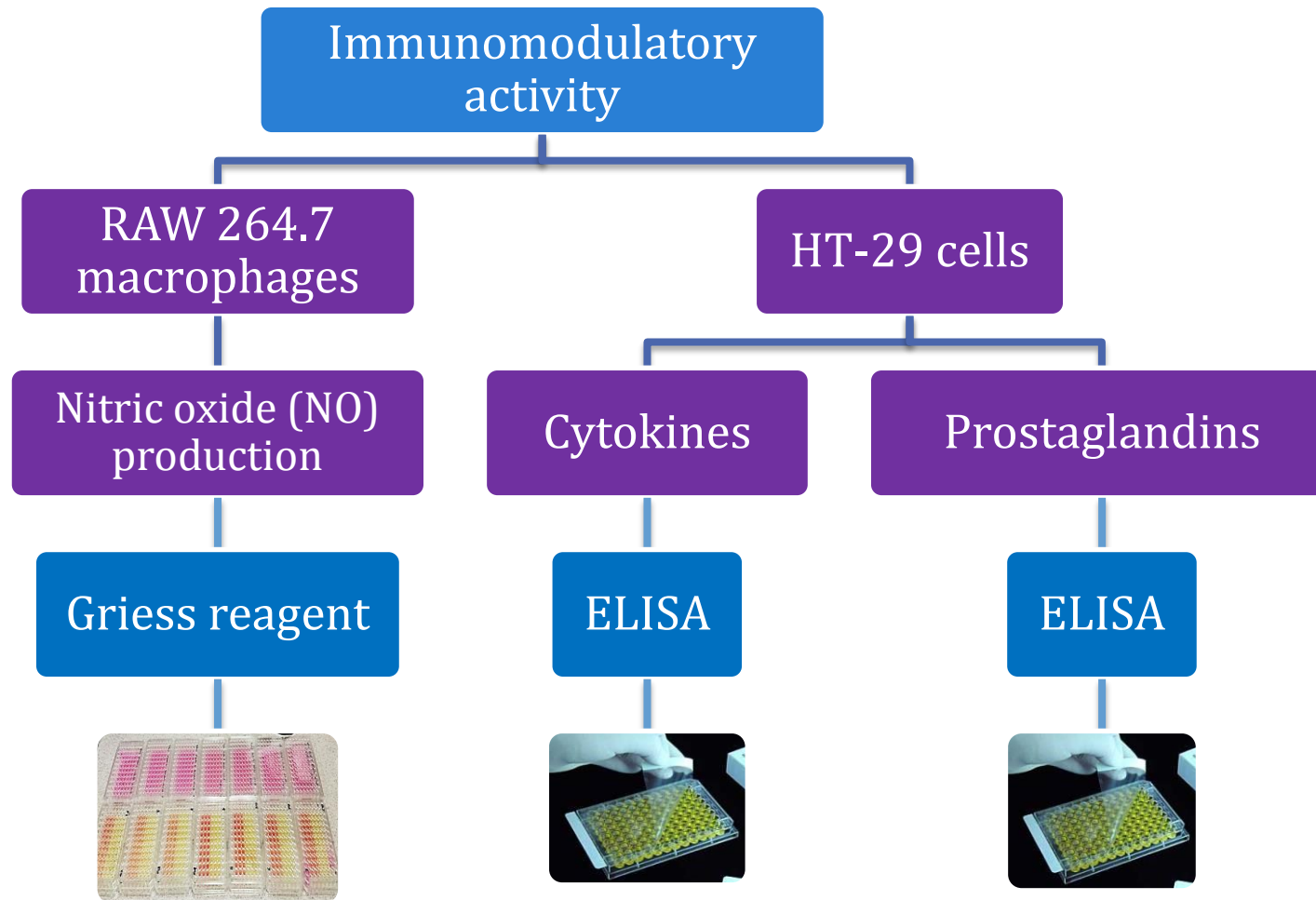
- Endo-1,4- β -Xylanase M4 (*A. niger*)(GH11)-**ANX**
- Endo-1,4- β -Xylanase (*Cellvibrio japonicus*)(GH10)-**CJX**
- α -L-Arabinofuranosidase (*Clostridium thermocellum*)(GH51)-**CAF**
- α -L-Arabinofuranosidase (*Bifidobacterium adolescentis*)(GH43)-**BAF**



Production of Enzymatically Tailored AX Hydrolyzates

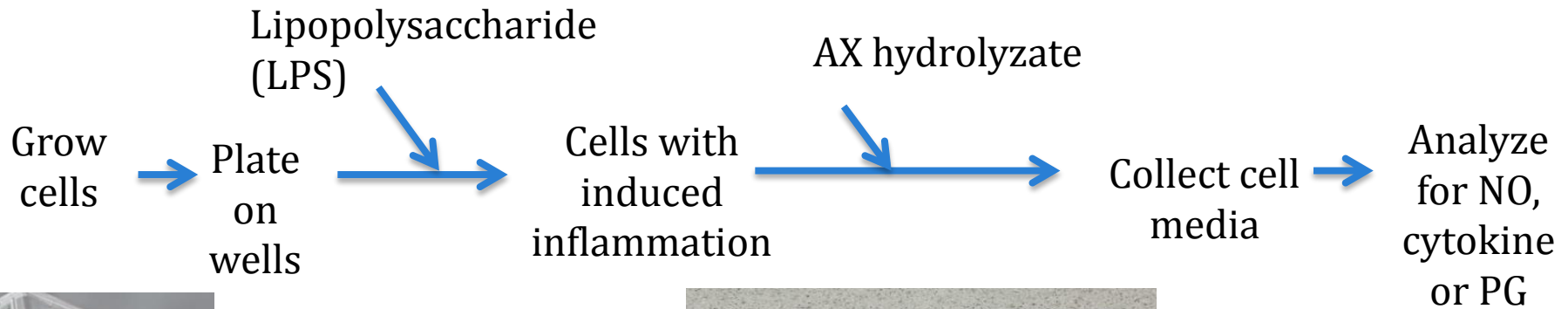






http://shop.midsci.com/productdetail/M50/100-SEAL-PLT/SealPlate_Sealing_Film,_ELISA,_Non-Sterile,_100/pk/

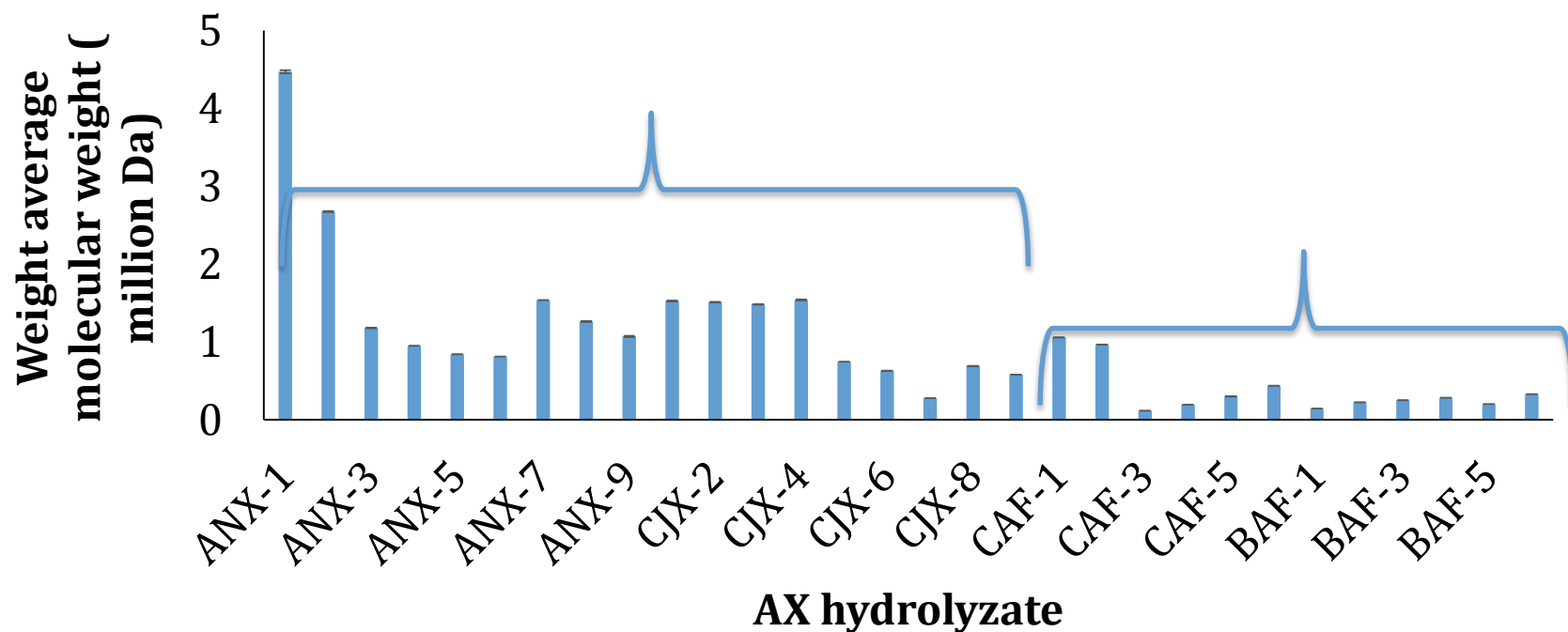
General Procedure



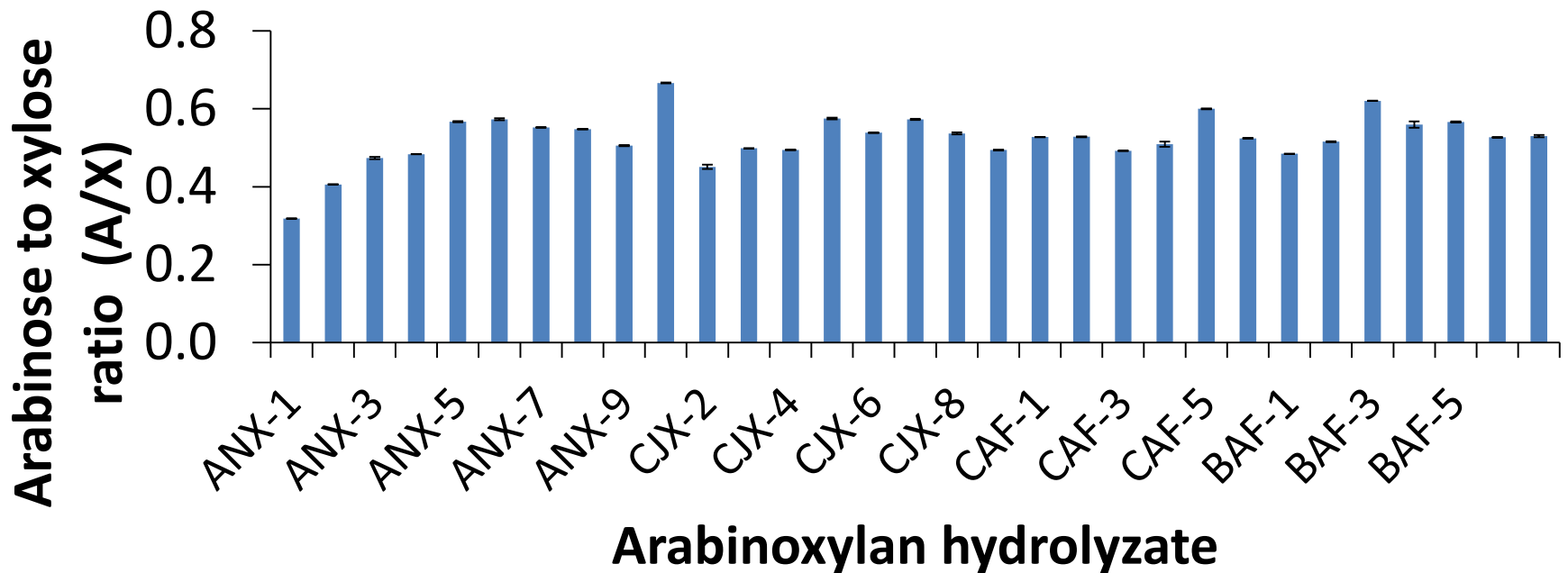
http://technologyinscience.blogspot.com/2008_07_01_archive.html#.VDDTthbbGSo

Structural Characterization

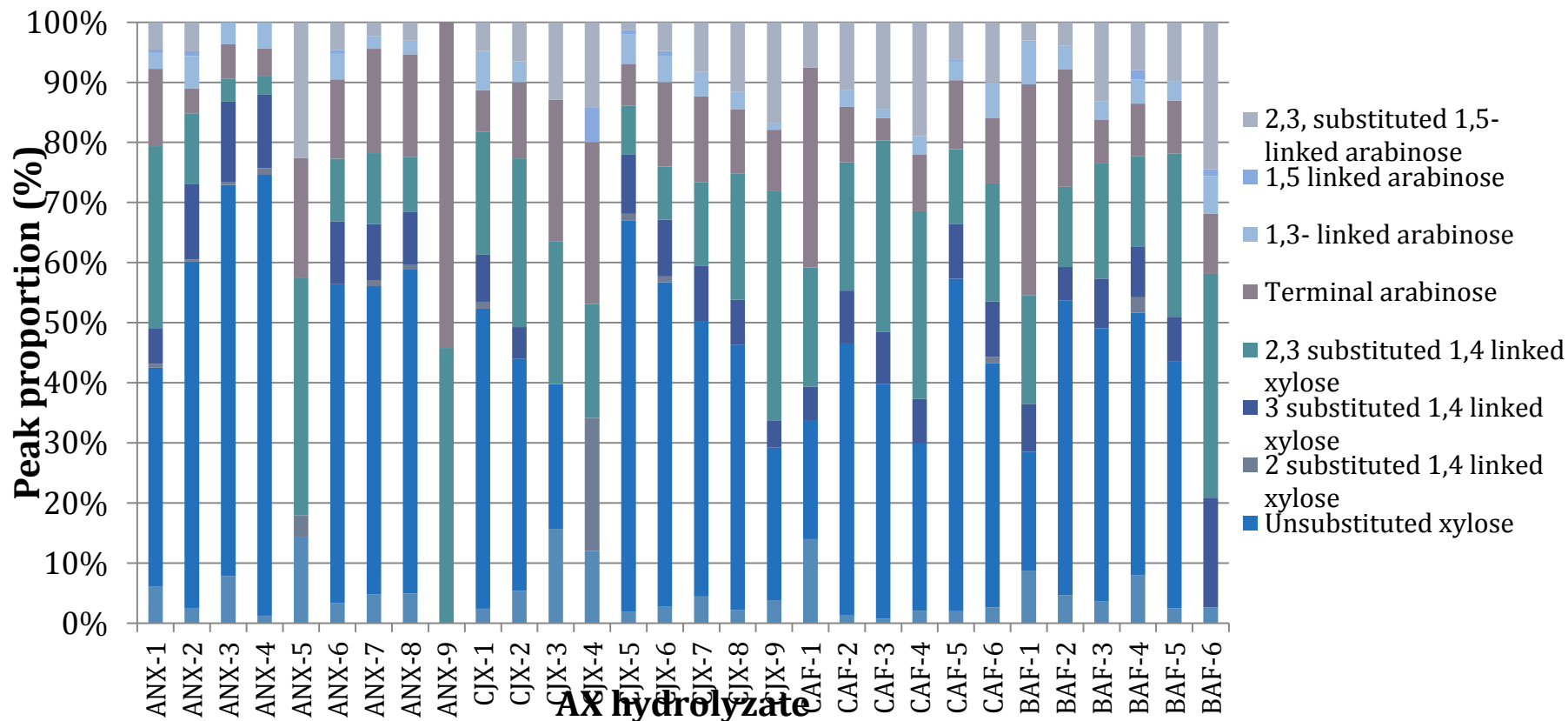
Weight Average Mw of AX Hydrolyzates Determined by SEC-MALS



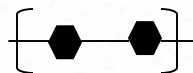
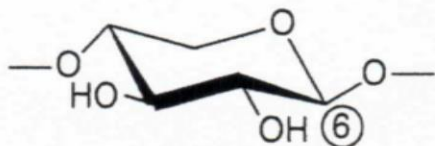
Arabinose to Xylose Ratio



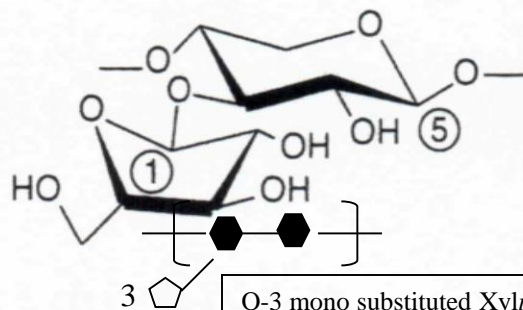
Linkage Analysis Determined by GC-MS



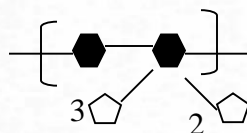
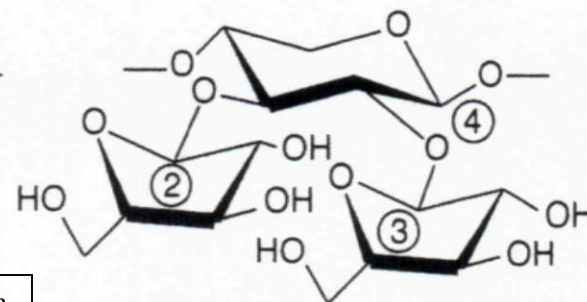
^1H NMR



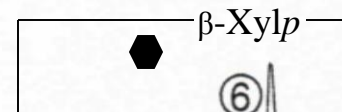
Unsubstituted Xylp



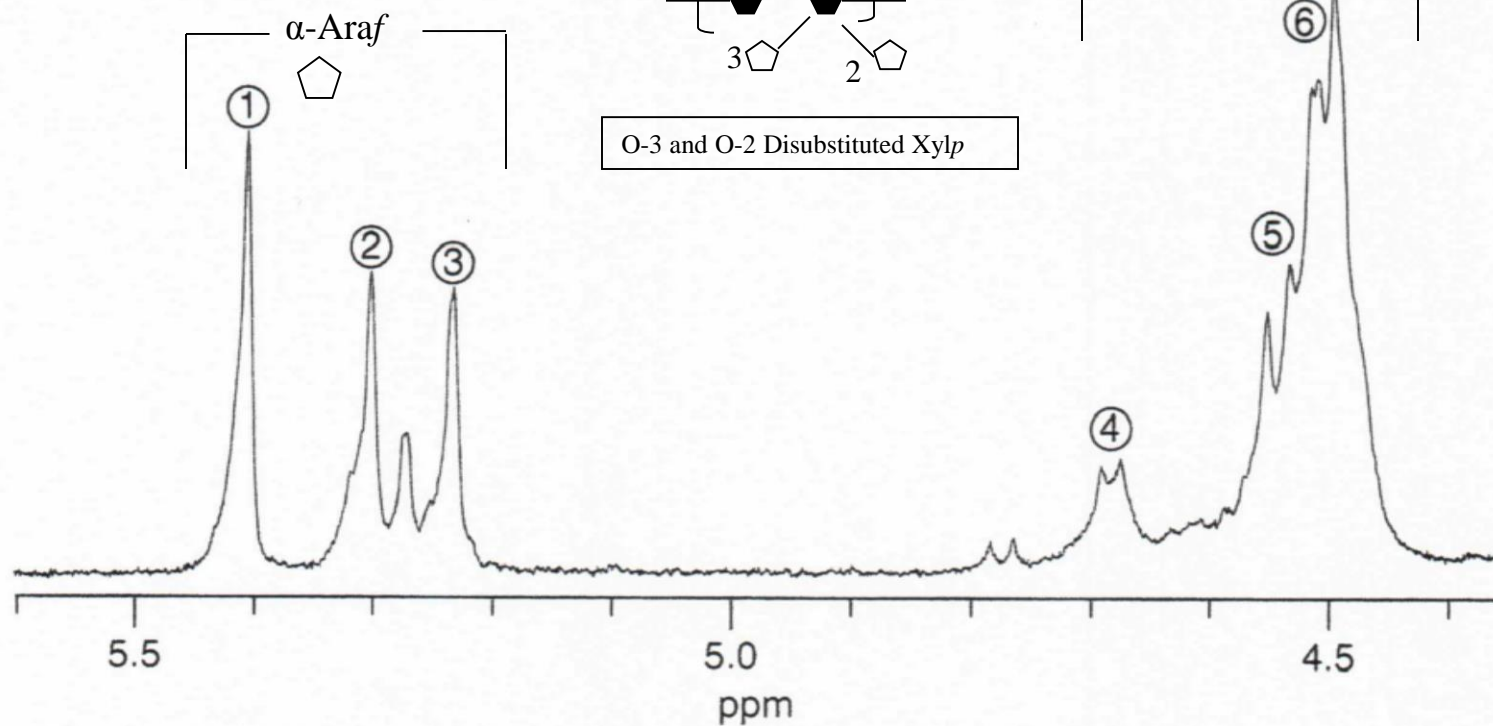
O-3 mono substituted Xylp



O-3 and O-2 Disubstituted Xylp



β -Xylp



^1H NMR Resonance Area

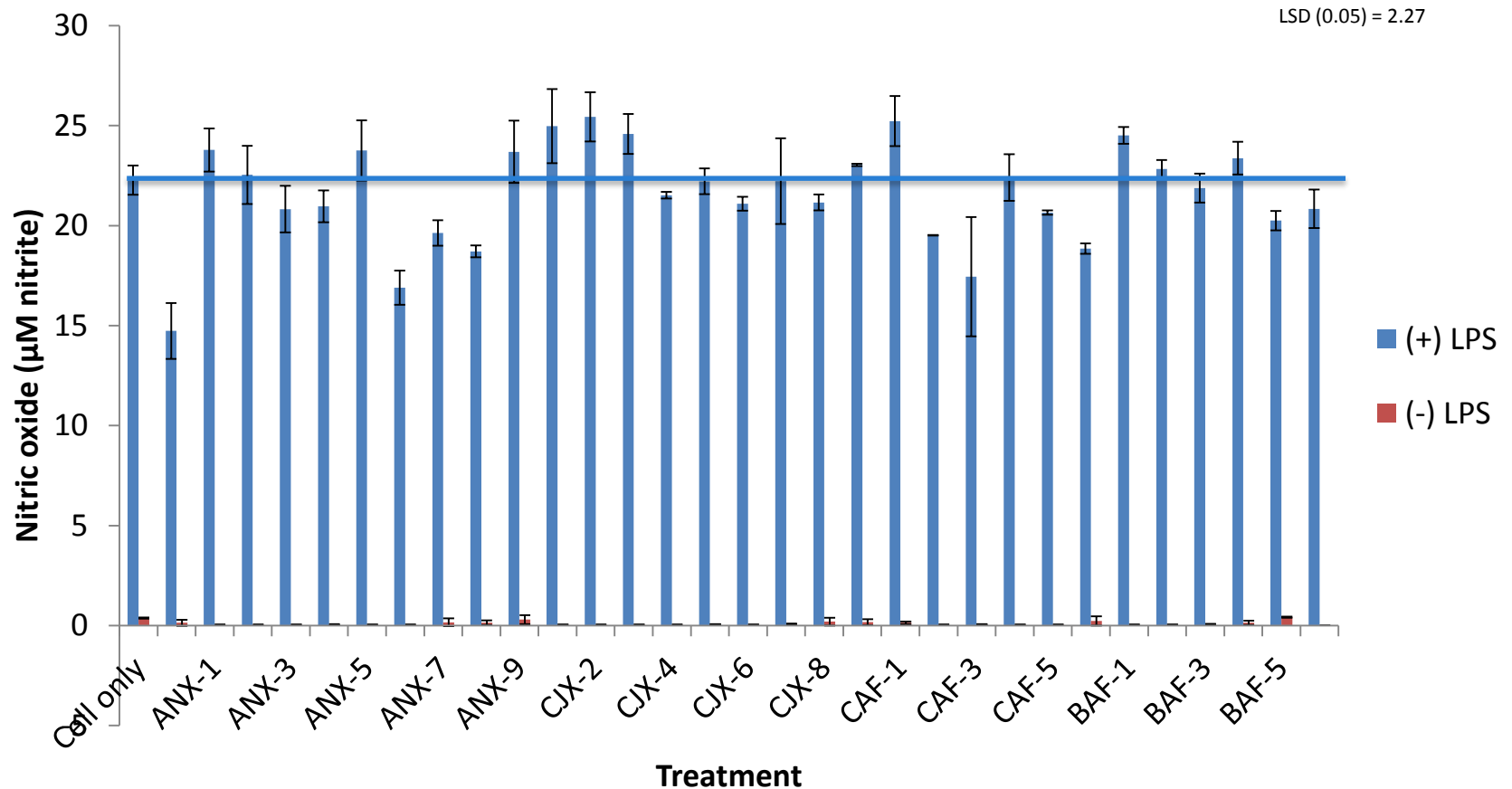
	% Total Resonance Area					
AX hydrolyzate	Resonance 1	Resonance 2	Resonance 3	Resonance 4	Resonance 5	Resonance 6
ANX-6	14.3	13.3	10.2	11.2	13.5	37.6
ANX-7	15.2	11.5	9.9	9.2	14.1	40.2
CAF-6	13.1	9.3	8.9	11.5	13.2	44.0
BAF-5	12.6	10.6	9.2	7.5	13.5	46.6

Overall Results with Structural Analysis

- Ten-fold reduction in the molecular weight in the AX molecule compared to its starting substrate
- The CJX series resulted in lower molecular weights compared to ANX series
- CAF series yielded larger polysaccharides compared to the BAF series
- Information derived about each enzymes could benefit decision making regarding the application of these enzymes

Immunomodulatory Properties

LPS induced inflammation with respect to NO



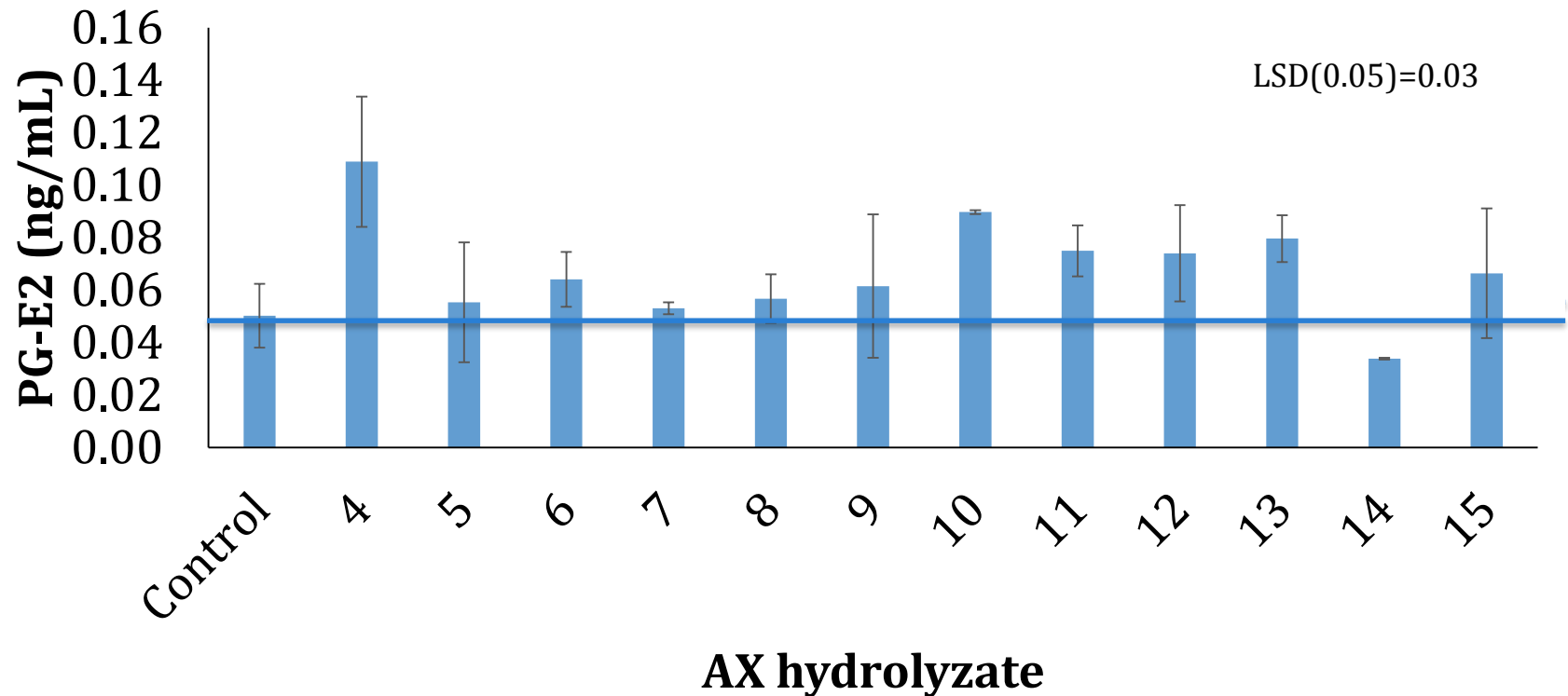
AXH with anti-inflammatory and pro-inflammatory properties*

Anti-inflammatory properties	Percent decrease in NO production compared to control (%)	Pro-inflammatory properties	Percent increase in NO production compared to control (%)
ANX-6	24.1	CJX-2	14.2
CAF-3	21.7	CAF-1	13.3
ANX-8	16.0	CJX-1	12.2
CAF-6	15.4	CJX-3	10.4
CAF-2	12.4		
ANX-7	11.9		

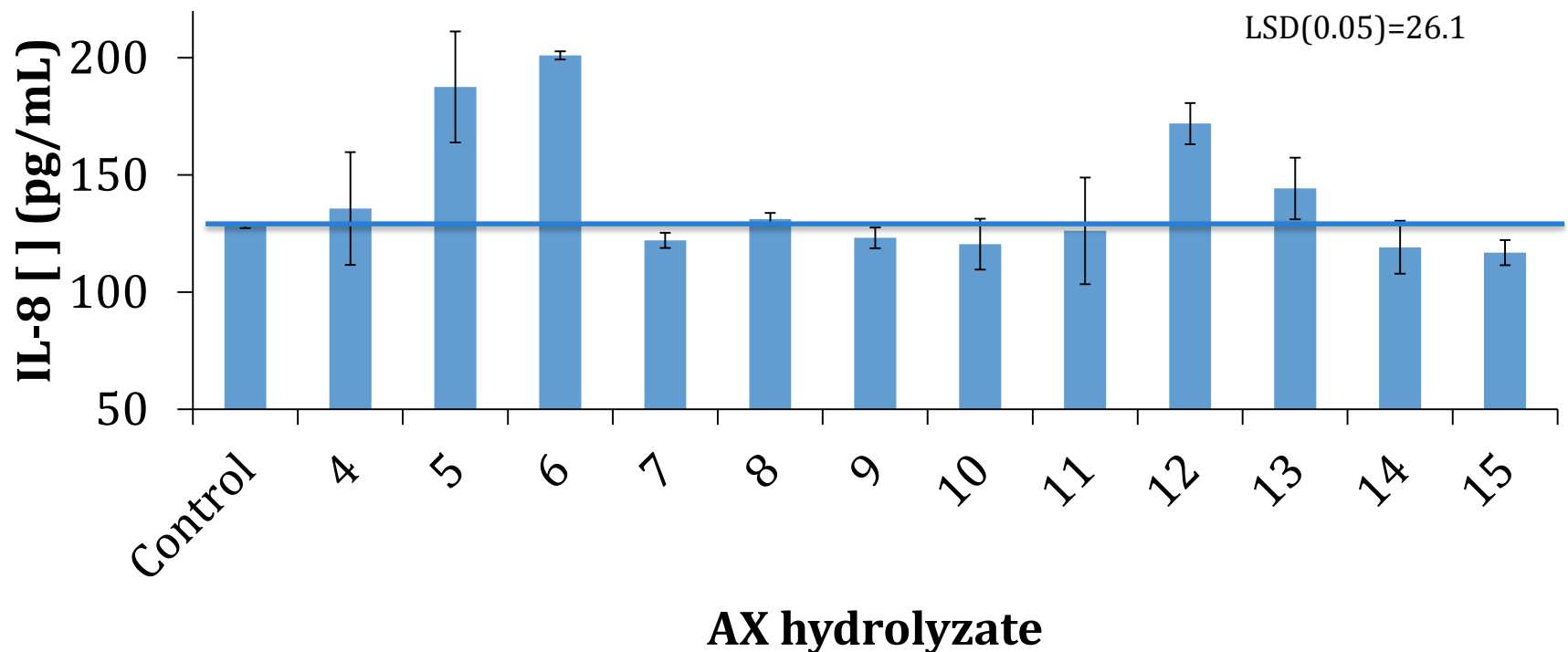
*The AXH are presented in their descending order of potency

- Twelve AX hydrolyzates were selected for further evaluation based on NO assay
 - Tested on intestinal epithelial cell line HT-29 stimulated with LPS

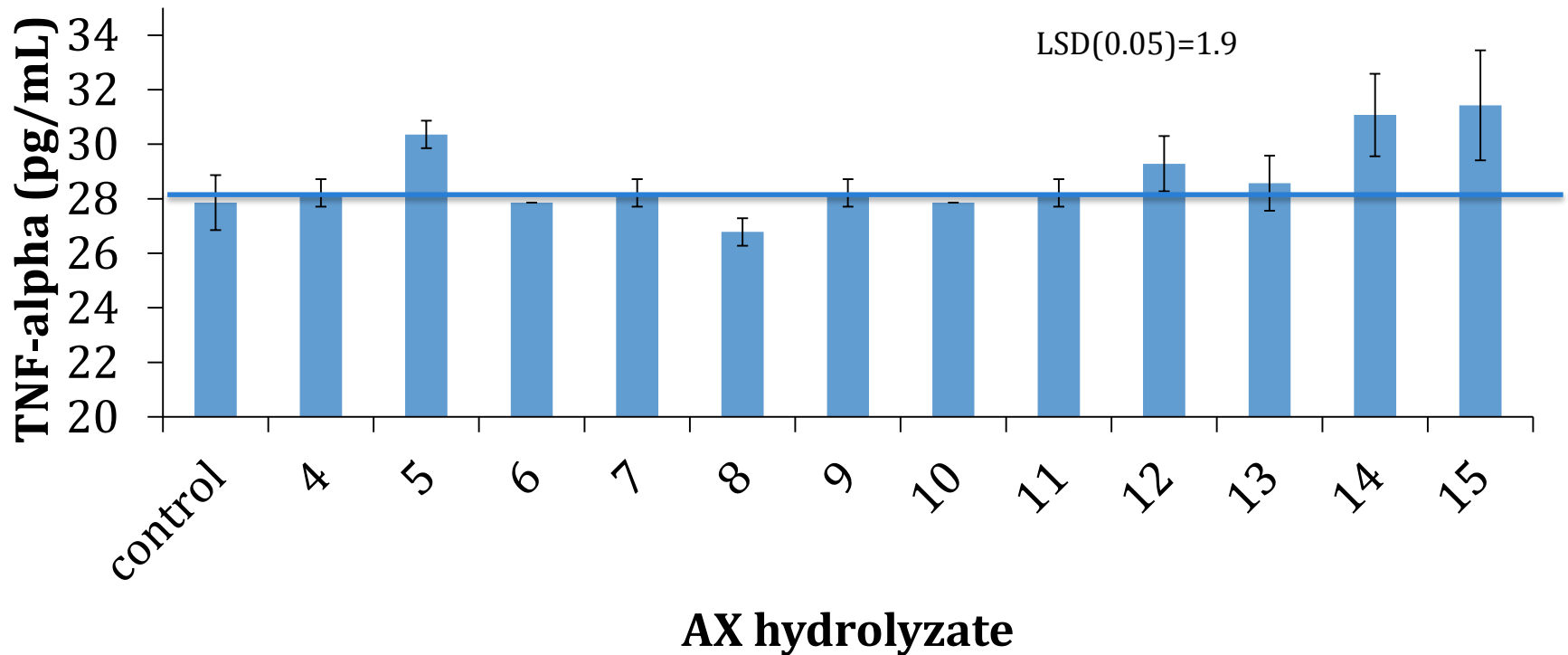
Effect of selected AX hydrolyzates on **prostaglandin** production in LPS induced HT-29 cells



Effect of selected AX hydrolyzates on **IL-8** production in LPS induced HT-29 cells



Effect of selected AX hydrolyzates on TNF-alpha production in LPS induced HT-29 cells



Immunomodulatory properties of AX hydrolyzates

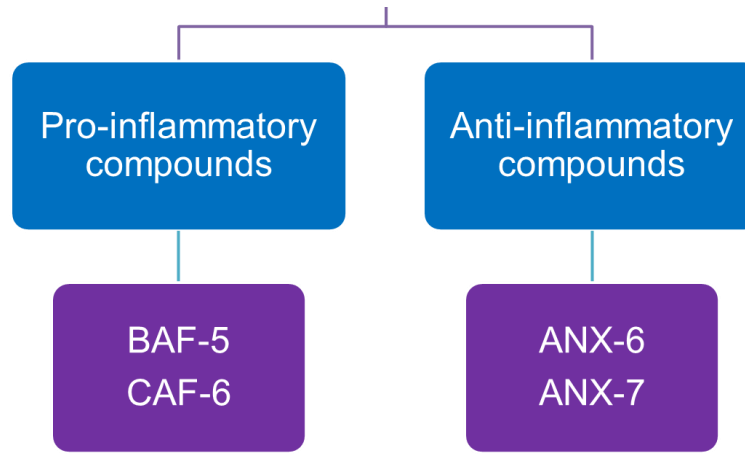
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graph TD; A[Immunomodulatory properties of AX hydrolyzates] --> B[Pro-inflammatory compounds]; A --> C[Anti-inflammatory compounds]; B --> D[BAF-5]; B --> E[CAF-6]; C --> F[ANX-6]; C --> G[ANX-7];
```

Pro-
inflammatory
compounds

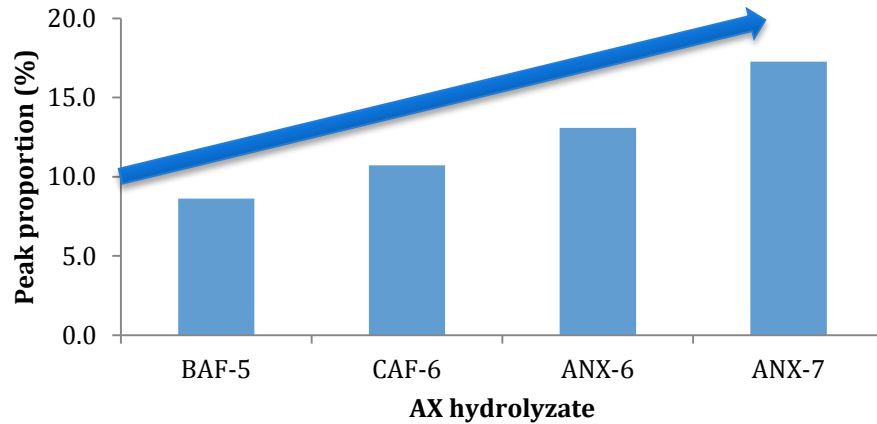
BAF-5
CAF-6

Anti-
inflammatory
compounds

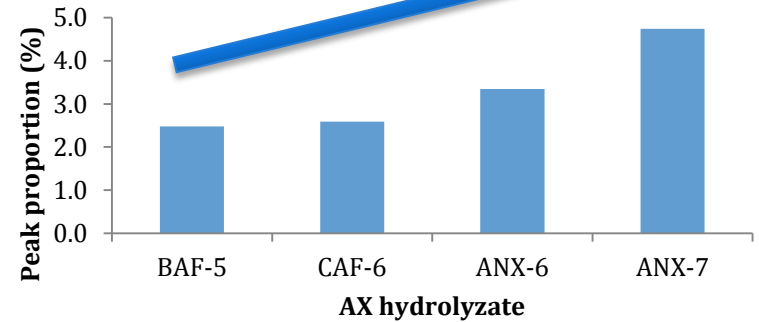
ANX-6
ANX-7

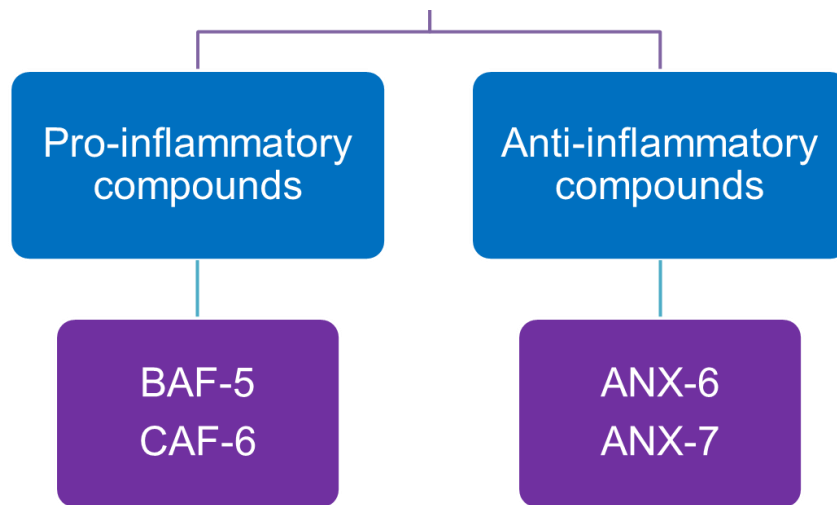


Terminal Arabinose

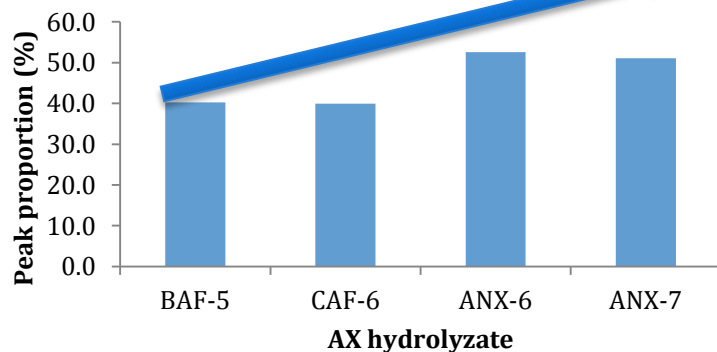


Terminal Xylose

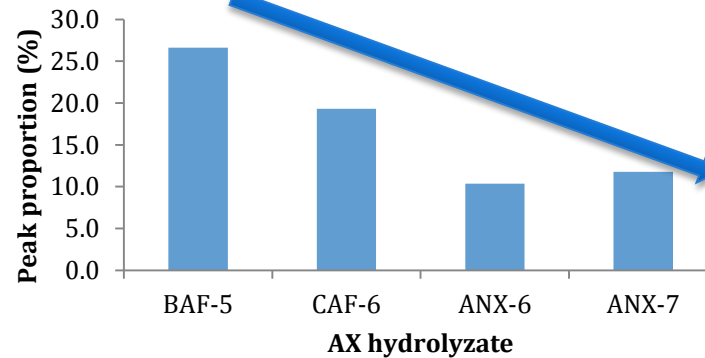


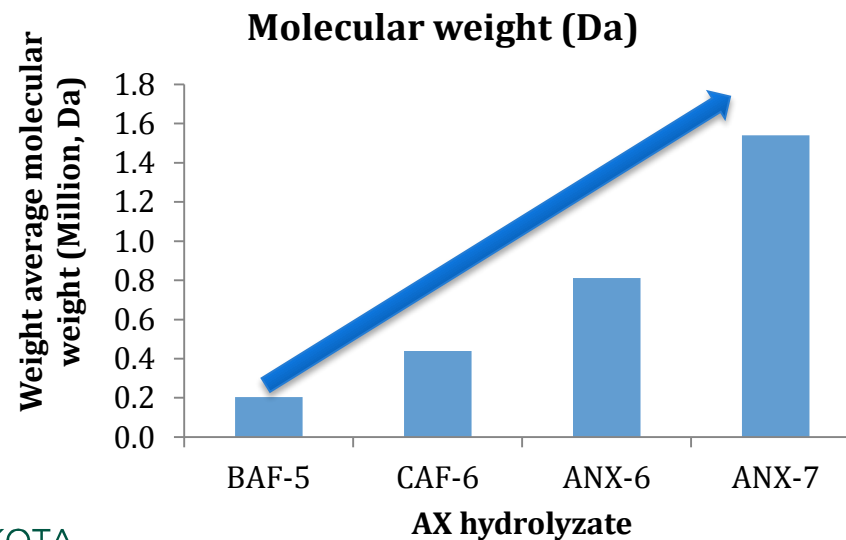
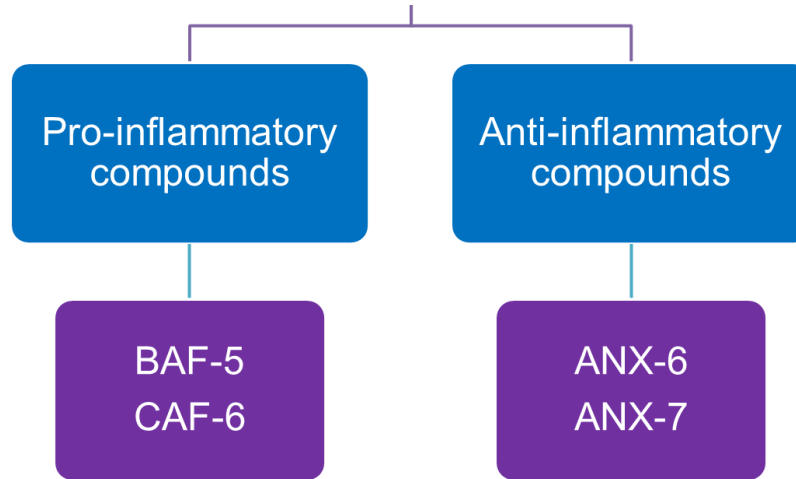


Unsubstituted xylose



Disubstituted xylose





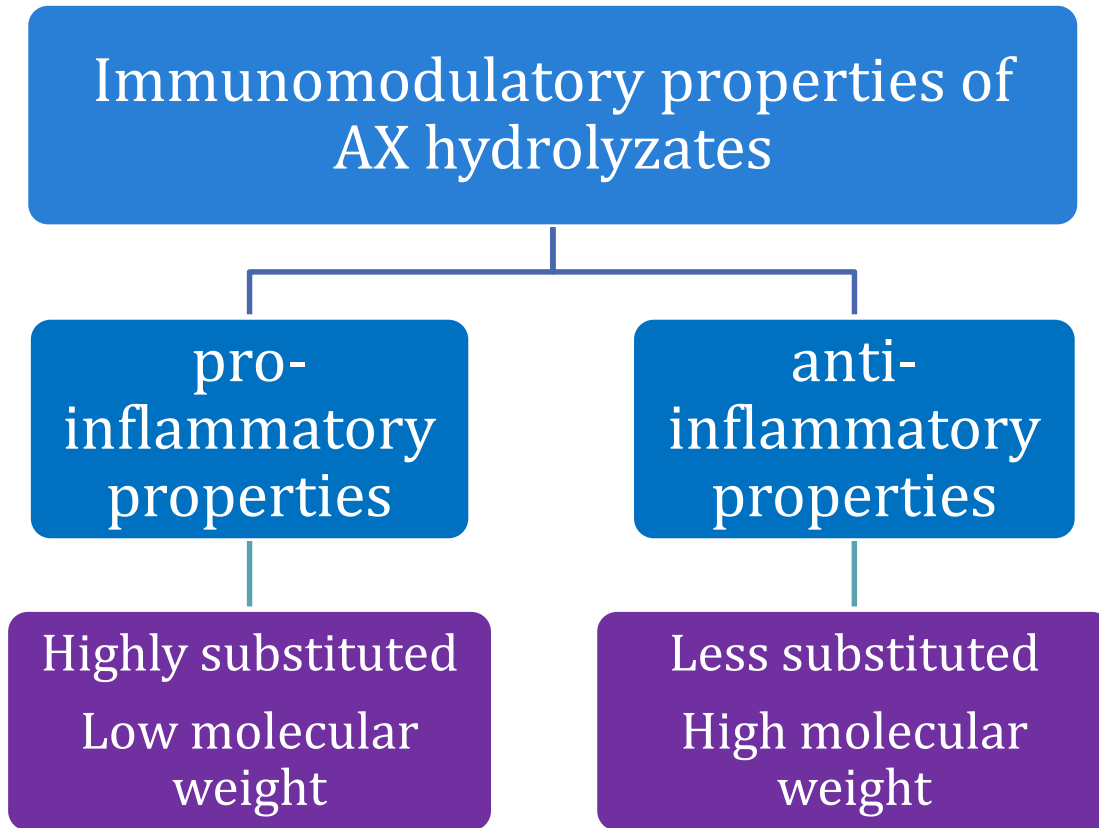
Conclusions

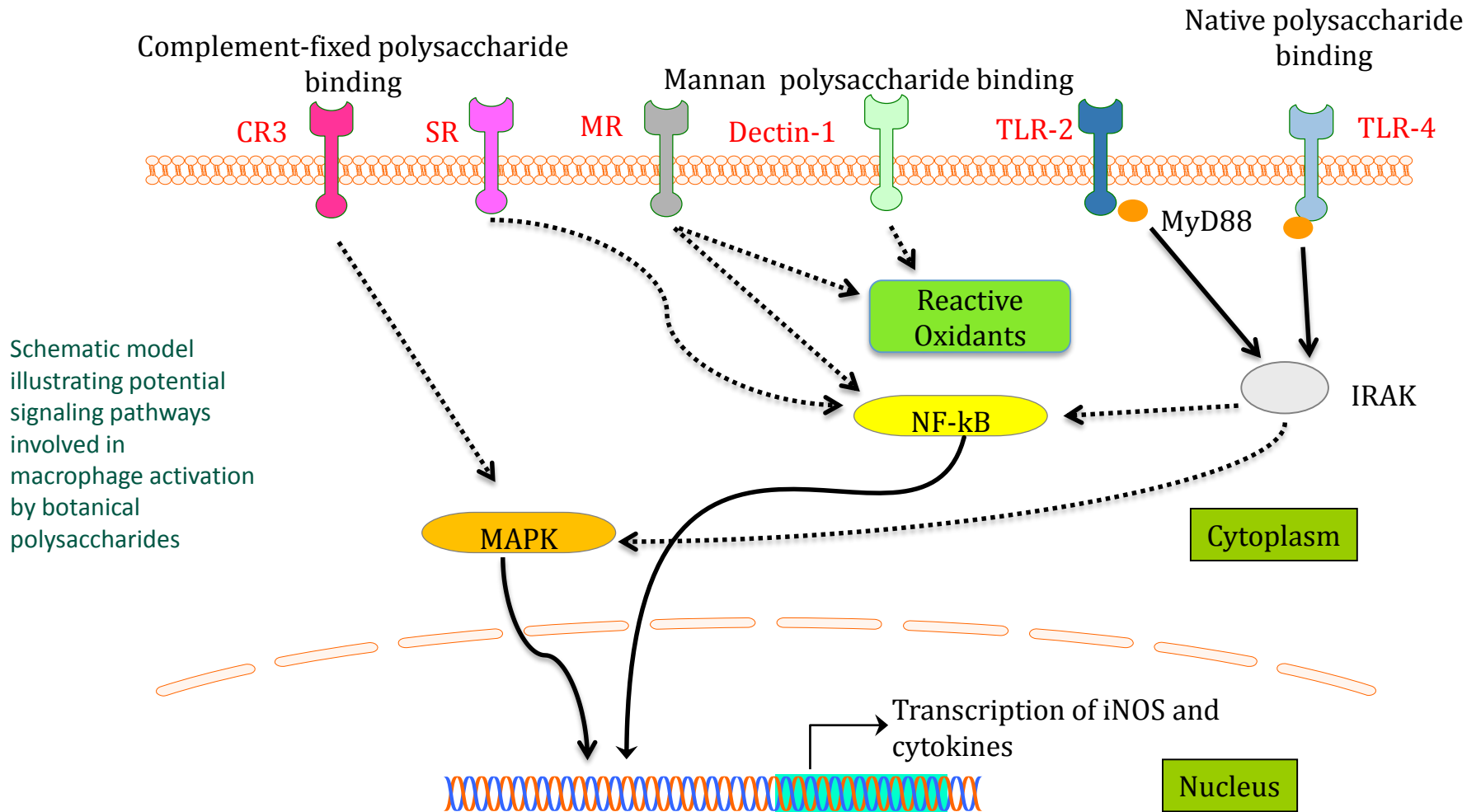
The enzymes being used can be used to produce structurally different arabinoxylan hydrolyzates

Wheat bran derived AX hydrolyzates possess diverse immunomodulatory properties

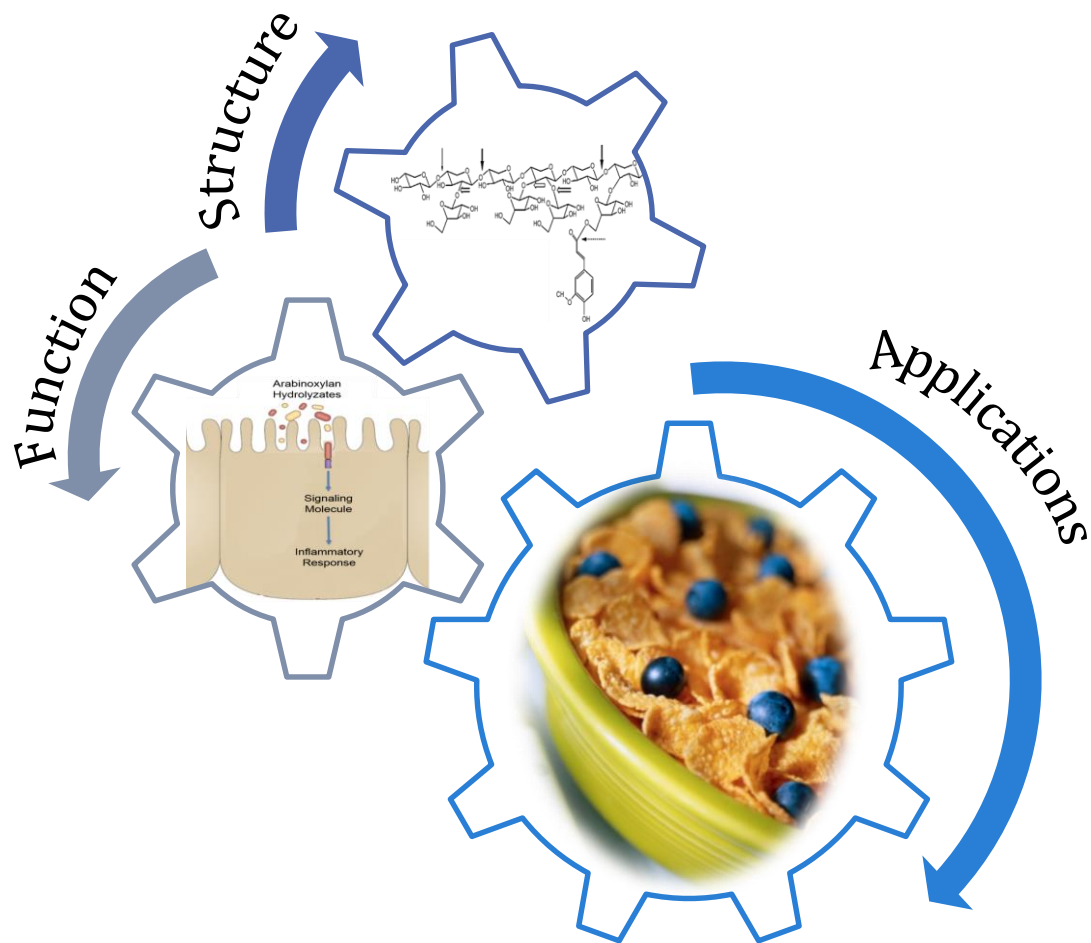
Fine structural details seemed to be related to the immunomodulatory properties

Conclusions





The Big Picture



Future research for immunomodulatory studies

- Use AXH with more specific structural details: Bacteroidetes growth/ immunomodulatory properties
- AX oligosaccharides: Bacterial growth/ immunomodulation
- Dose dependence of AXH as immunomodulators
- Cell culture models with cytokine induced inflammation
- Evaluate the fermentation products of AXH
- AXH on common probiotics: *Bifidobacteria* and *Lactobacillus*
- In vivo experiments to understand structure function relationship of AXH



Questions

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