Xylanase assisted Mass Spectrometry Fingerprinting of Acetylated Glucuronoxylans (GX) – *the potential of AP-Maldi-ITMS*

Department of Food and Environmental Sciences

Sun-Li Chong

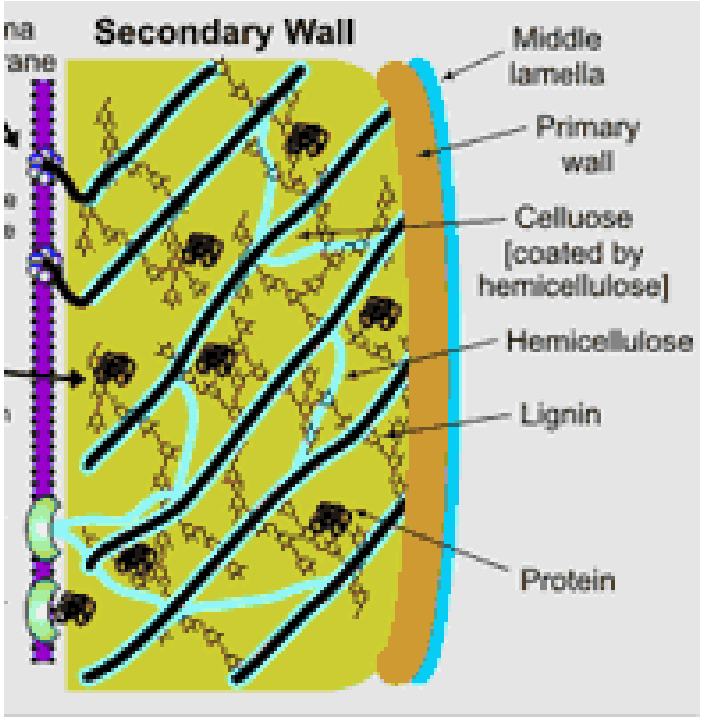
COST FP0901 meeting

Characterisation of raw biomass and processed materials for biorefinery, bioenergy and biofuels production

Paris, January 25th-26th 2011

Secondary cell wall

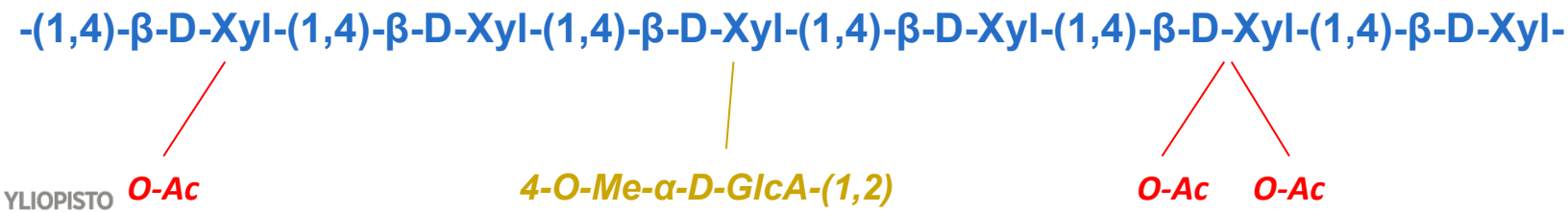
- The thickest layer in cell wall biomass
- Secondary cell wall in wood tissue was predominantly comprised of
  - Cellulose
  - Lignin
  - Hemicellulose
    - Glucuronoxylan
    - Arabinoglucuronoxylan
    - (galacto)glucomannan



Secondary cell wall model
Cellulose bundles, orientated at same angle, were embeded in the network of hemicellulose and lignin.
http://www.ccrc.uga.edu/~mao/intro/ouline.htm

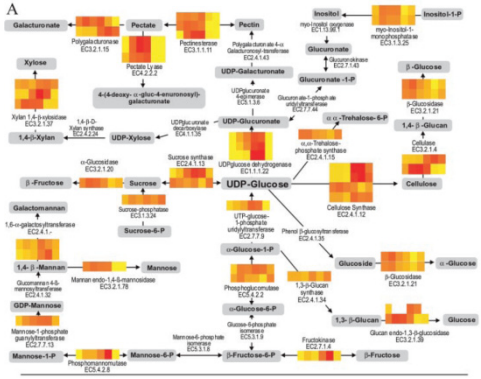
Glucuronoxylan (GX) in hardwood

- Most abundant hemicellulose in hardwood (30%)
- Consisting linear (1→4) linked β-D-xylopyranosyl residues and randomly substituted by 4-*O*-methylglucuronic acid (meGlcA) /glucuronic acid (GlcA) and acetyl groups (Ac).
- *in vivo* GX modification:
  - Affects fiber quality
    - Chemical bleaching to remove GX – economic and environmental cost
  - Challenge for bioenergy production – better saccharification
    - complete hydrolysis of GX requires various hydrolytic enzymes



GX Bioynthesis and Isolation for structural analysis

- Good understanding of GX biosynthesis in secondary wall is required for *in vivo* fibre engineering
  - Alteration of GX via genetic approach to understand roles and networking of key enzymes in the GX biosynthesis.



Hertzberg 2001

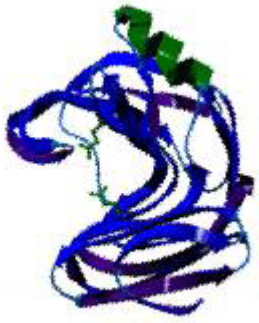
- More refined method to isolate GX from plant biomass for structural analysis is required.
  - Conventional alkaline isolation method causing deacetylation (less informative)

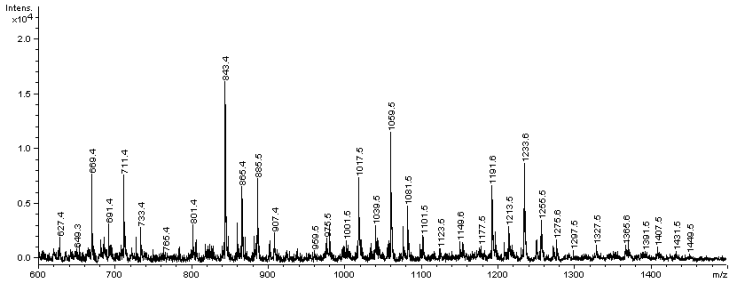
-(1,4)-β-D-Xyl-(1,4)-β-D-Xyl-(1,4)-β-D-Xyl-(1,4)-β-D-Xyl-(1,4)-β-D-Xyl-(1,4)-β-D-Xyl-

O-Ac *4-O-Me-α-D-GlcA-(1,2)* *O-Ac* *O-Ac*

Aim

- To obtain fingerprinting spectra of oligosaccharides liberated directly from wood (acetylated glucuronoxylan) by combining xylanase hydrolysis and AP-MALDI mass spectrometry detection.

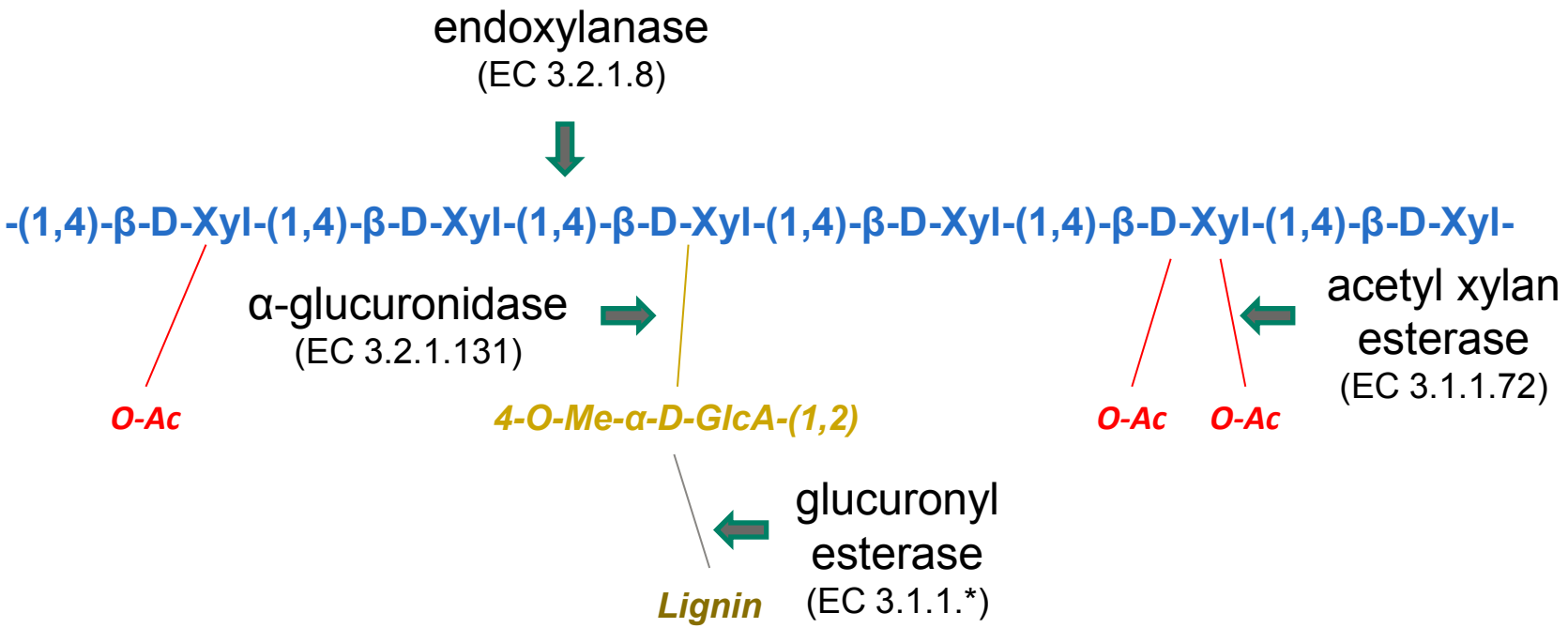




Background

- Enzymes are specific in their hydrolytic action
- Endoxylanases hydrolyze randomly xylans and their action is hindered by side groups such as meGlcA and Ac => structural fragments
- Mass spectrometry is a sensitive method which requires small amount of sample

Enzymatic hydrolysis of GX



Sample treatment

Young woody poplar stems were ground and washed in hot ethanol

↓

30mg alcohol insoluble residues (AIR) incubated at pH 5 with the *GH10 endo-1,4-β-D-xylanase of *Aspergillus aculeatus* (10,000 nkat/g AIR) for 24h

↓

The liberated XOS were purified using graphitized carbon columns

↓

The neutral XOS were eluted in 50% (v/v) acetonitrile

The acidic XOS were eluted in 50% (v/v) acetonitrile in 0.1% (v/v) TFA

↓

AP-MALDI-ITMS detection
Matrix: DHB (10mg/ml)

* Gift from Novozymes

AP-Maldi-ITMS

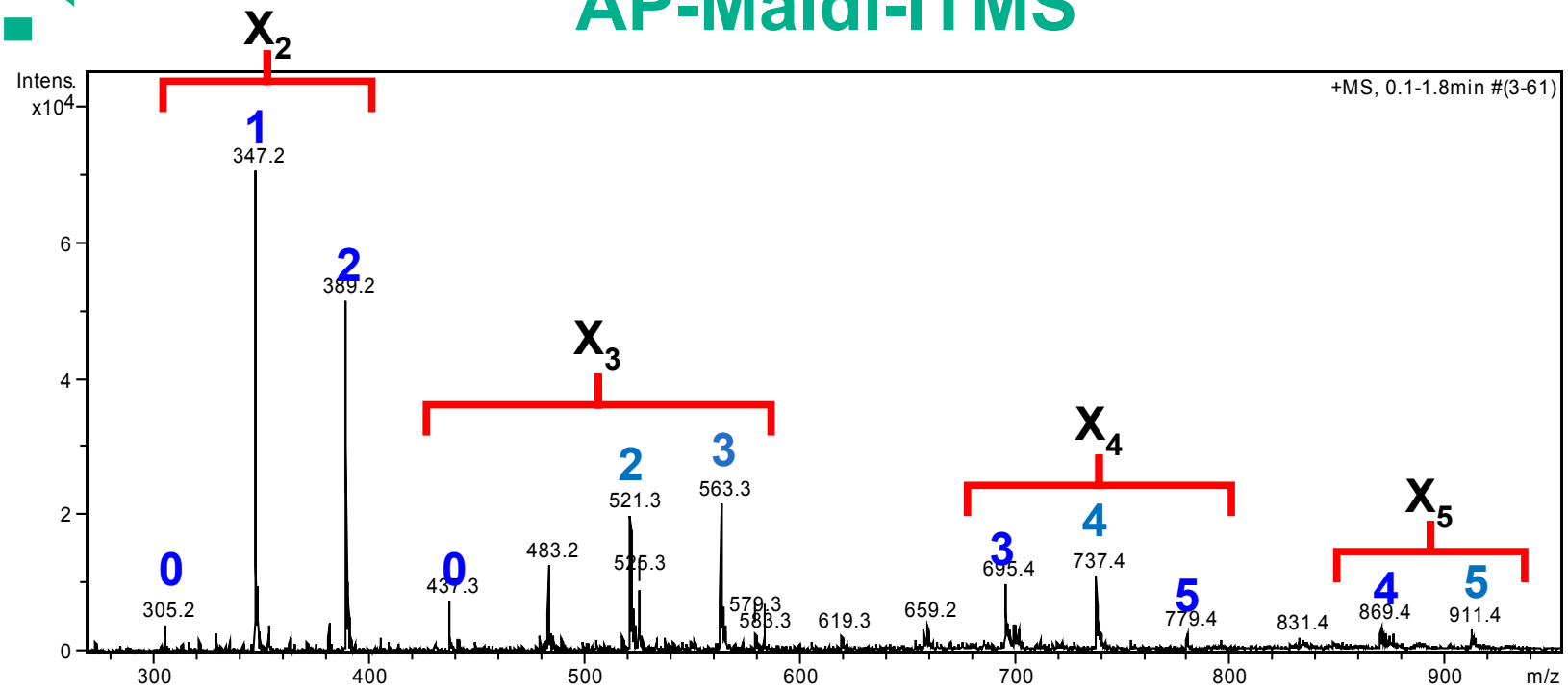
- Atmospheric Pressured-Matrix Assisted Laser Desorption Ionization-Ion Trap Mass Spectrometer
  - Ionisation of molecule analytes at ambient pressure (Maldi-TOF => vacuum)
  - Less metastable fragmentation (analyte ions were cooled down due to collision interactions with surrounding gas) (Moyer 2003)
    - Sialylated carbohydrates were not required to be derivatised (Zaia 2004)
- Developed in about 10 years ago
  - Almost no report on the analysis of plant derived oligosaccharides were found.
  - Vacuum Maldi-TOF was generally used.
- Advantages:
  - Able to determine mass and structure of biomolecules in one system.
  - Interchangeable with ESI/ APPI/ APCI

AP-Maldi-ITMS Setting

- Nitrogen Laser source: 337nm
- Laser Pulse Energy: 264µJ
- Mass analyser: ion trap
  - Standard mass range: *m/z* 50-2000
  - Extended mass range: up to *m/z* 4000
- Calibration
  - Manufacturer supplied ESI tuning mix (m/z: 118.2 – 2121.7)
- Performance test
  - Custom made acidic XOS (UXX), m/z = 627.17

Neutral XOS (Ac substituted)

AP-Maldi-ITMS

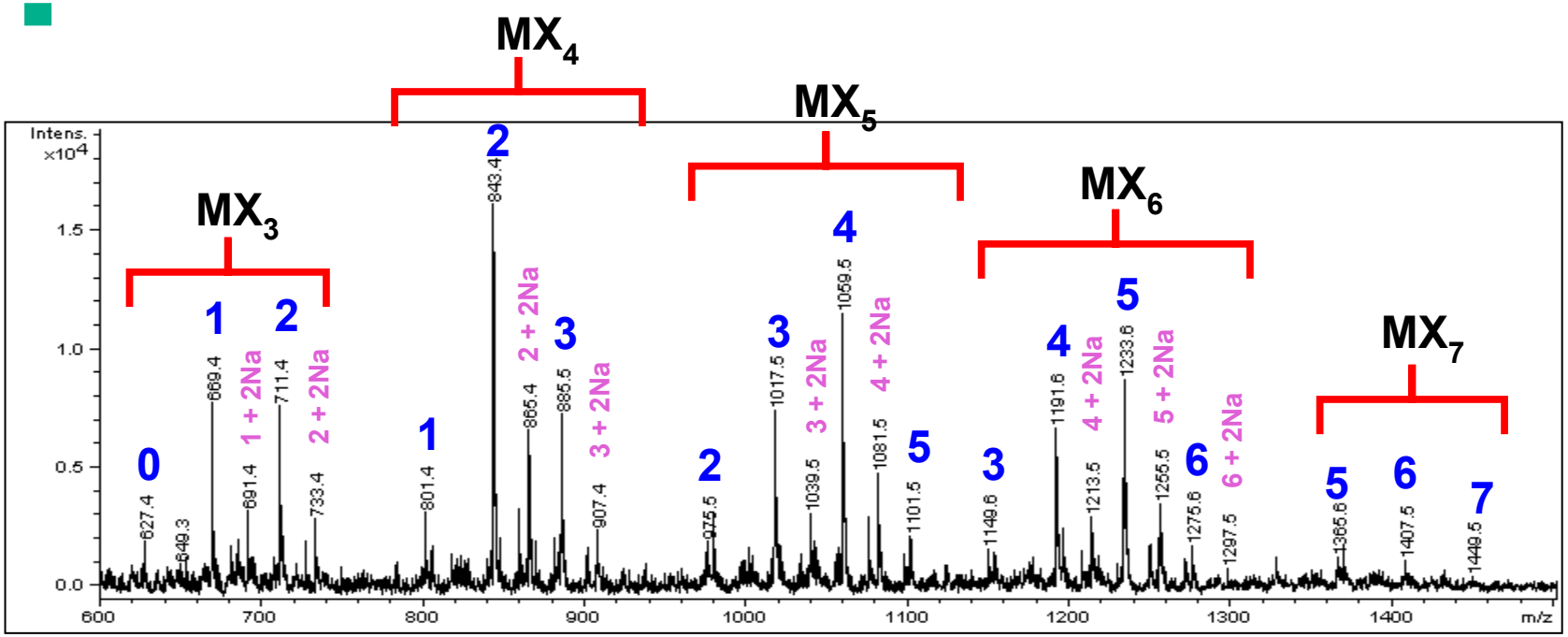


Numeric symbol = number of acetyl groups

- Detection: positive mode; Na+ adduct
- XOS (X_2-X_5) were detected and they were mostly acetylated.
- Main peaks: X_2 carried 1 and 2 acetyl groups.
- Non acetylated X_2 and X_3 were observed.

Acidic XOS (Ac and meGlcA substituted)

AP-Maldi-ITMS

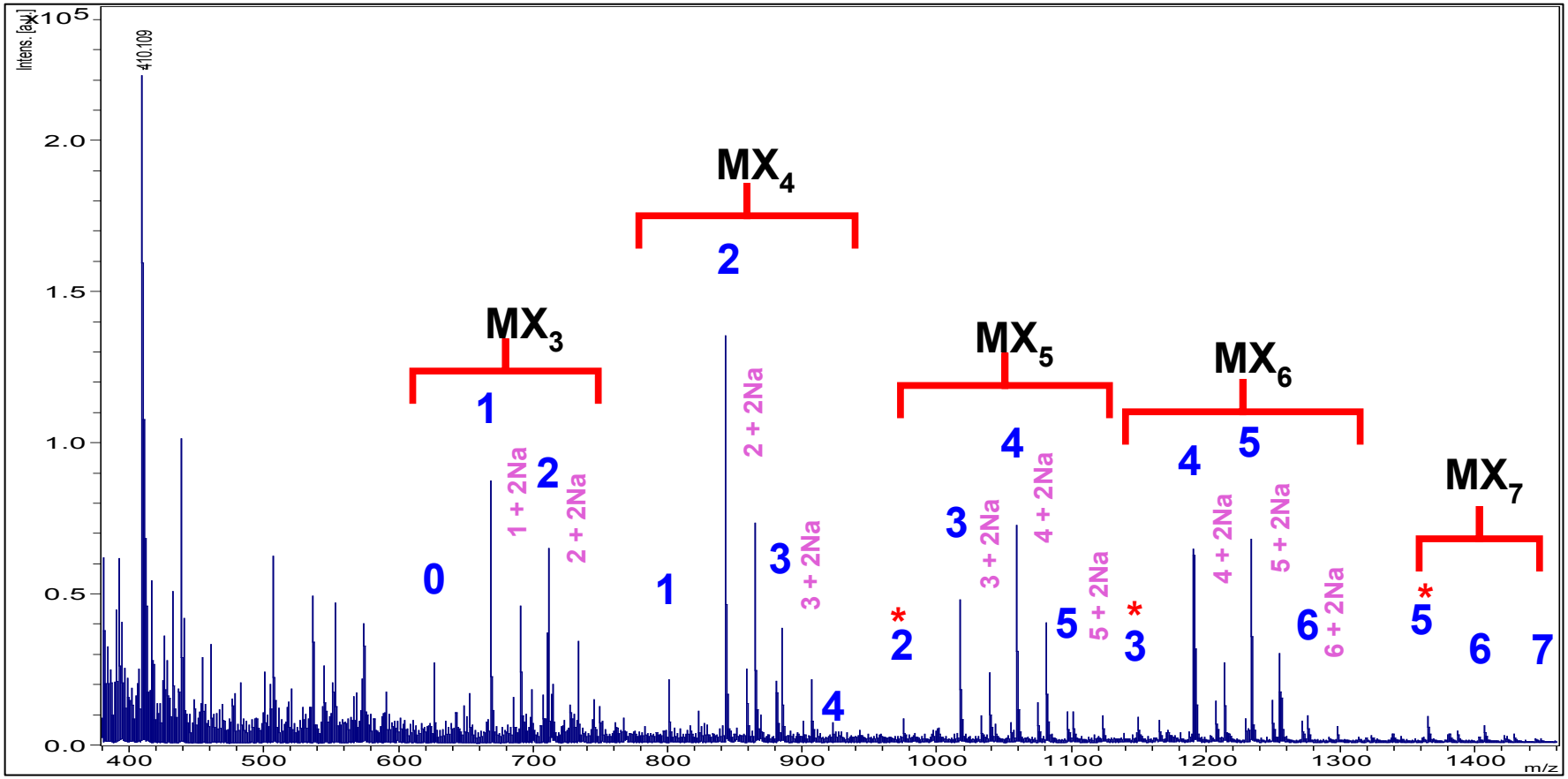


MX: MeGlcA α(1→2) linked XOS
Numeric symbol = number of acetyl groups; Na = Sodium

- Detection: positive mode; Na+ & [2Na-H]+ adducts
- MeGlcA-XOS (ranges from 3-7 Xyls) were substituted with single MeGlcA and acetylated at different level.
- Non acetylated MeGlcA-X_3 was observed.

Acidic XOS (Ac and meGlcA substituted)

Vacuum Maldi-TOF



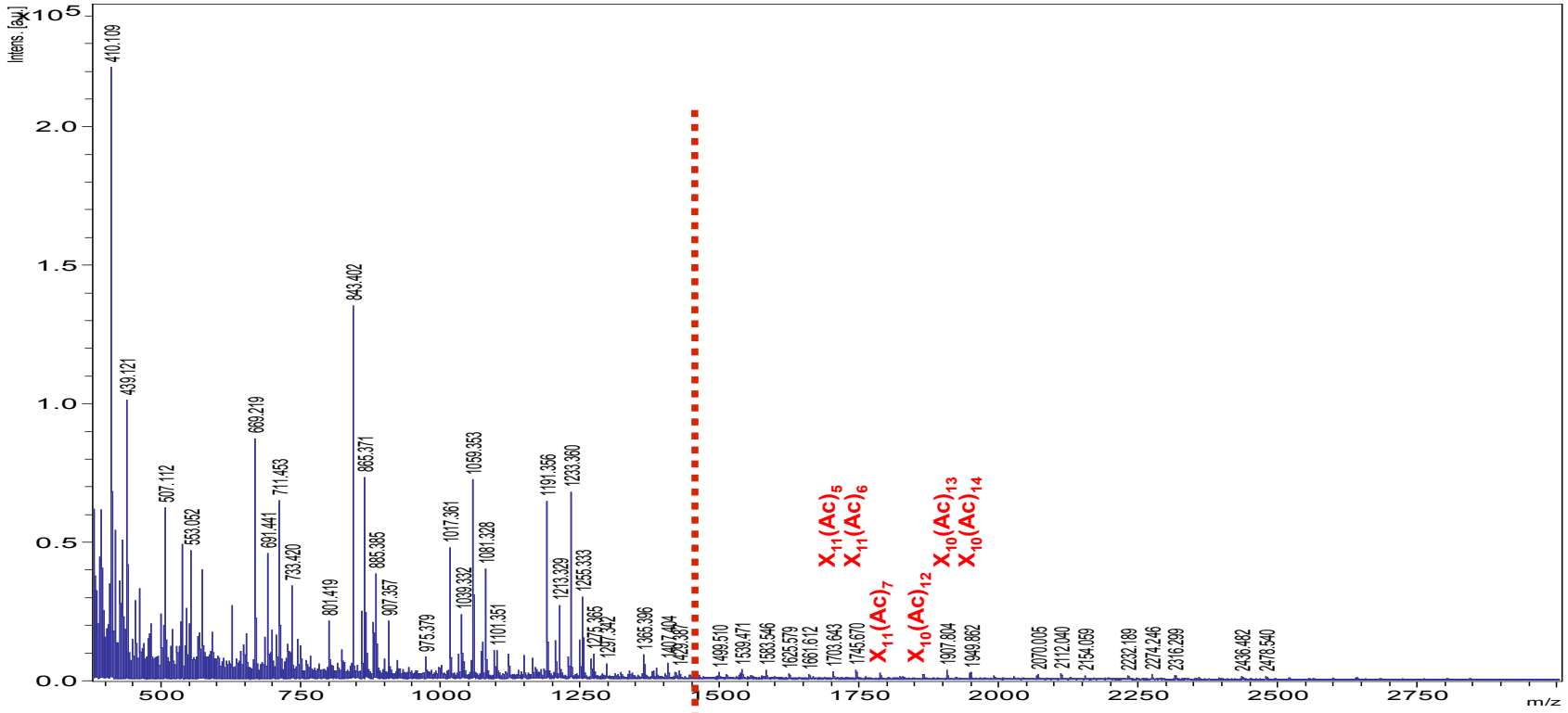
- Same ion profiles was obtained in comparison to AP-Maldi-ITMS.

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

- Laser source: 337 nm, 50 Hz; Accereration voltage: 25kV; 1000-2000 shoots were averaged
- Calibration: Manufacturer supplied protein standard (*m/z*:1000-3000)

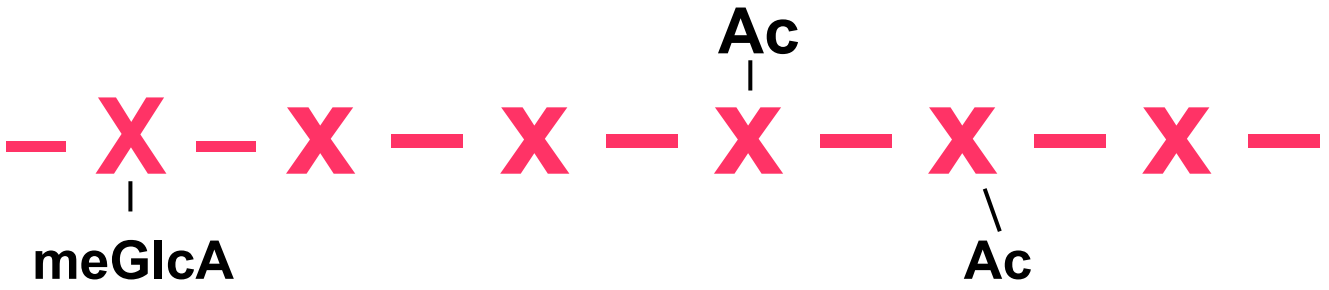
Acidic XOS (Ac and meGlcA substituted)

Vacuum Maldi-TOF



- Better sensitivity observed in vacuum Maldi-TOF
- Larger neutral XOS are detected in the acidic fraction
- No significant acidic XOS undetected by AP-Maldi-MS

To prove the method



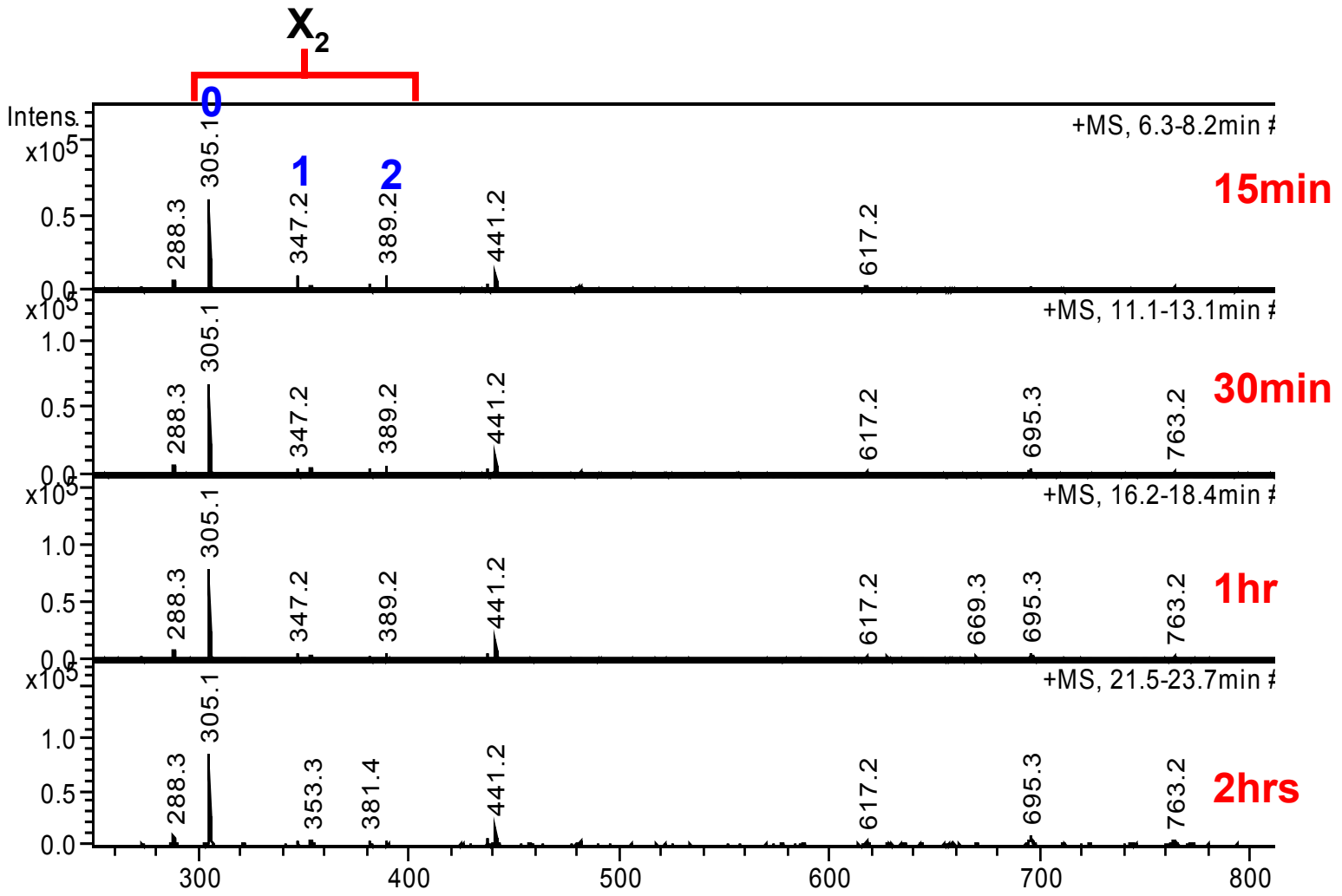
meGlcA : 4-O-methyl-glucuronic acid
Ac: acetyl group

Chemical modification – partial deacetylation

Chemical structural changes (50mM NaOH, pH 12.7)
-15min, 30min, 1hr and 2hrs

Poplar Wild Type

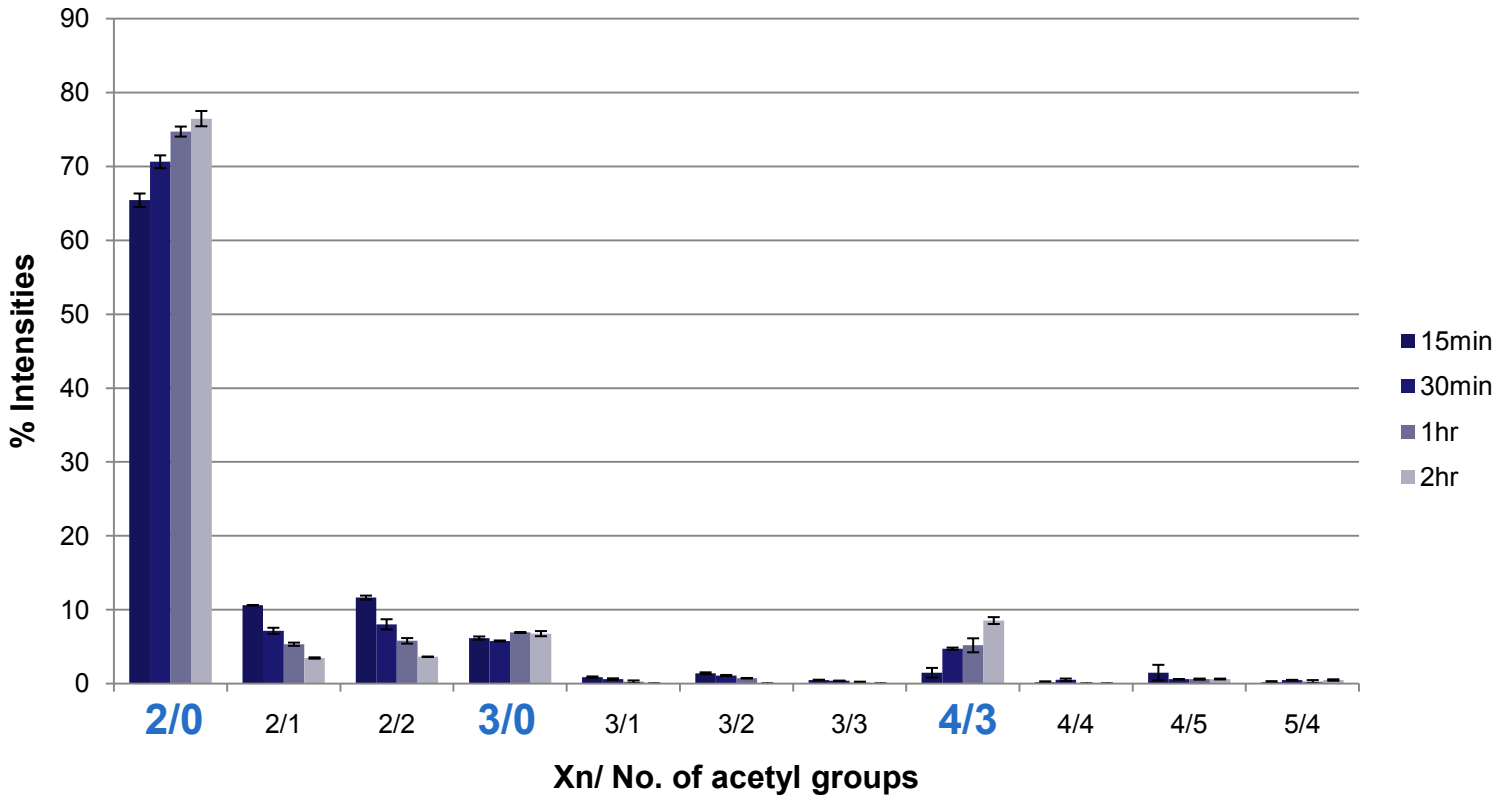
Neutral XOS (After deacetylation)



Poplar Wild Type

Neutral XOS (After deacetylation)

Comparison of % intensities in time series

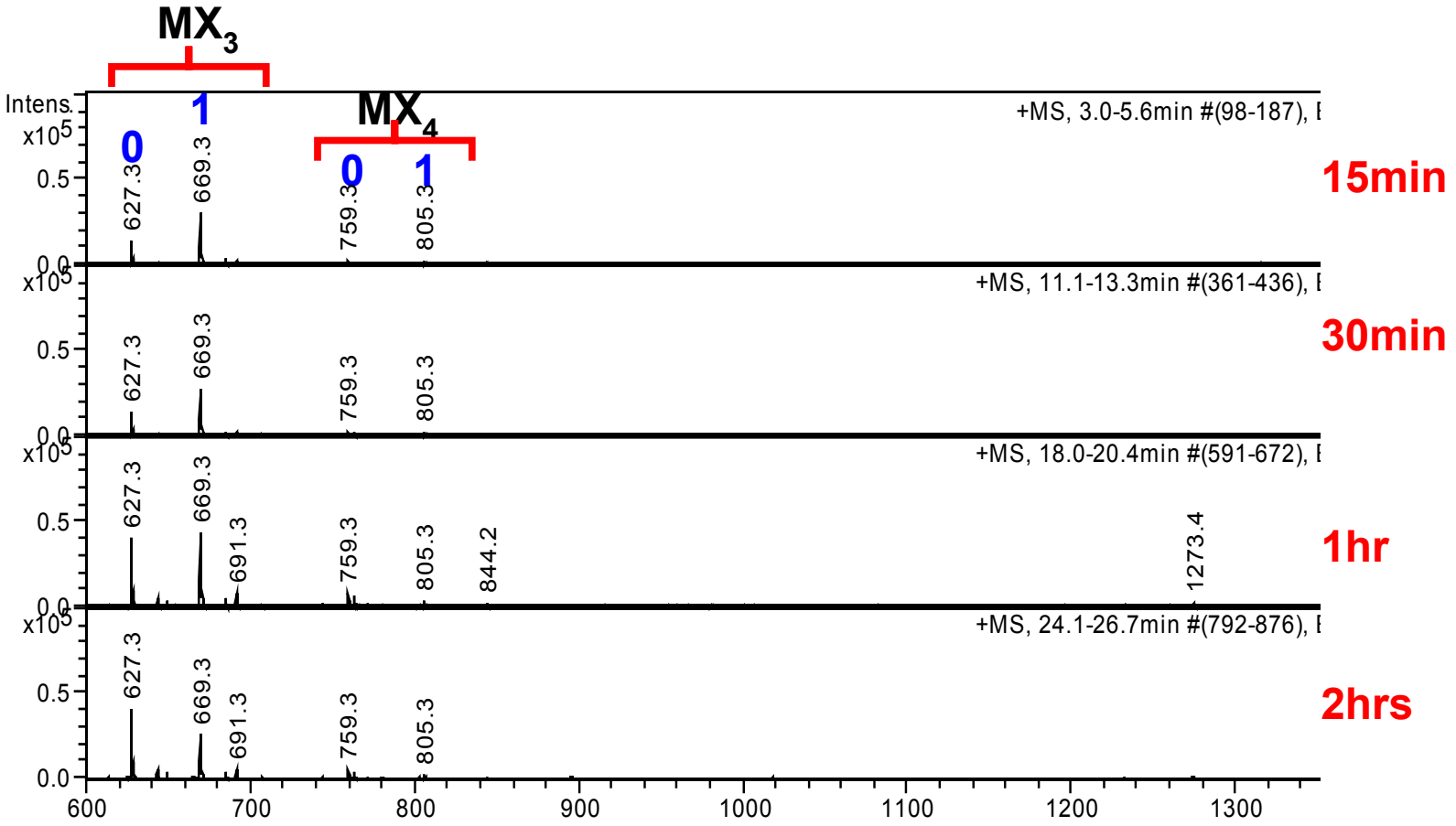


The non acetylated X_2 & X_3 were increased

The X_2 and X_3 carried 1-2 acetyl groups were decreased

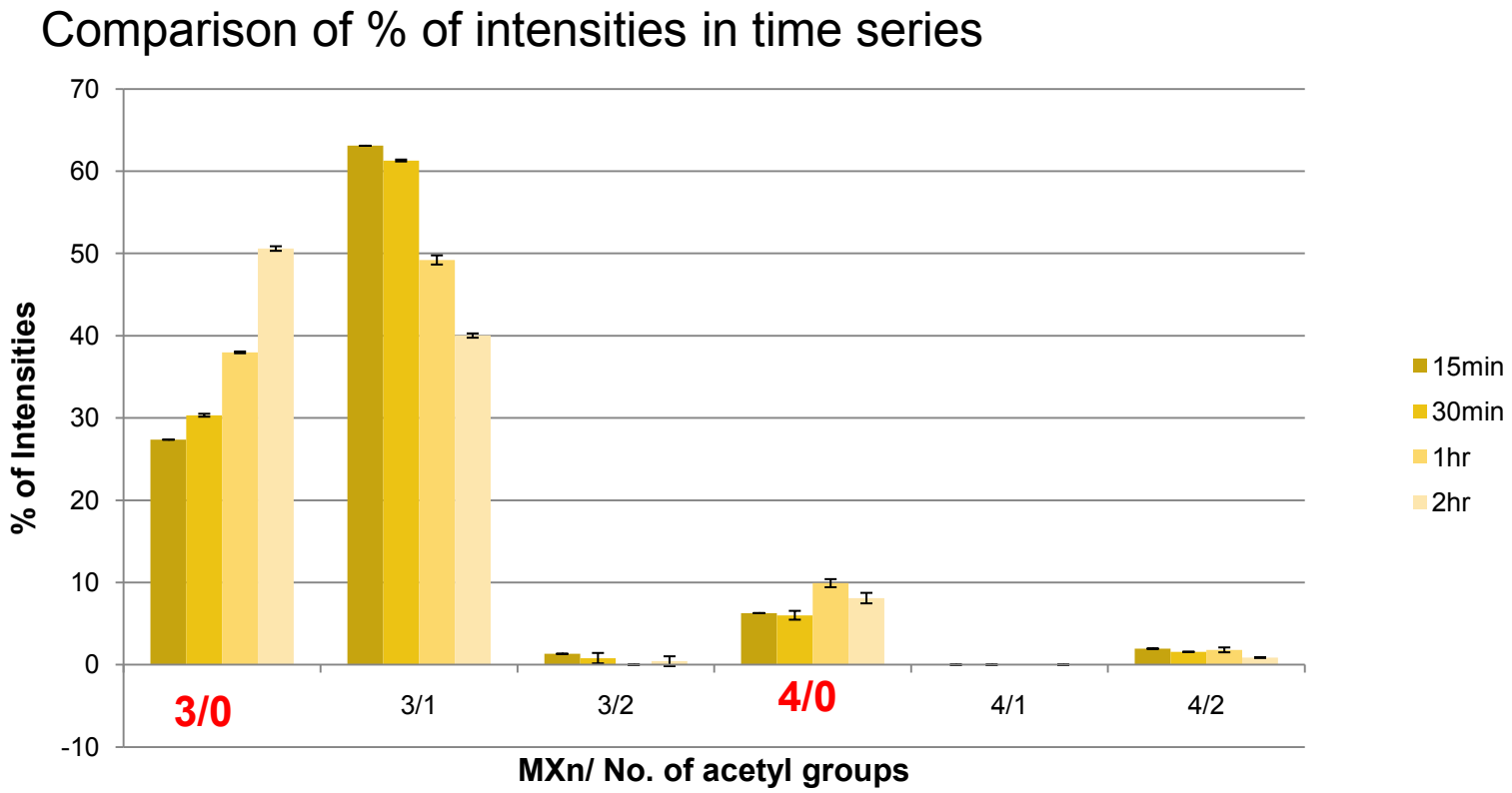
Poplar Wild Type

Acidic XOS (After deacetylation)



Poplar Wild Type

Acidic XOS (After deacetylation)

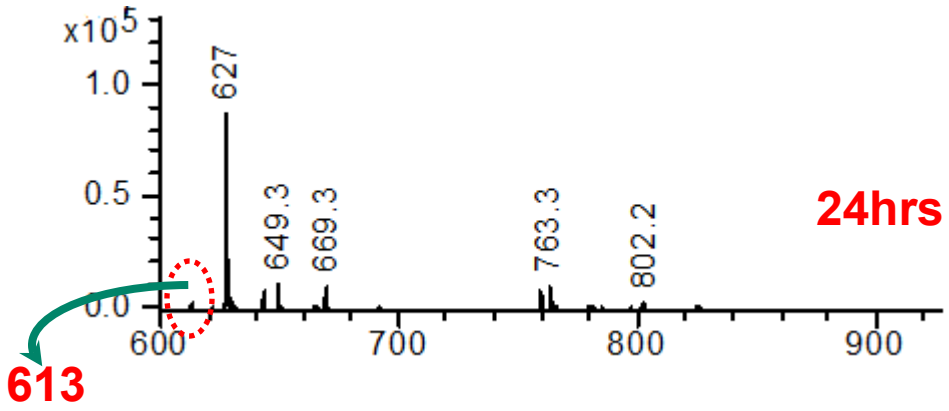


The non acetylated MX_3 & MX_4 were increased

The MX_3 carried one acetyl groups was decreased

Structural Elucidation of m/z 627 and 613

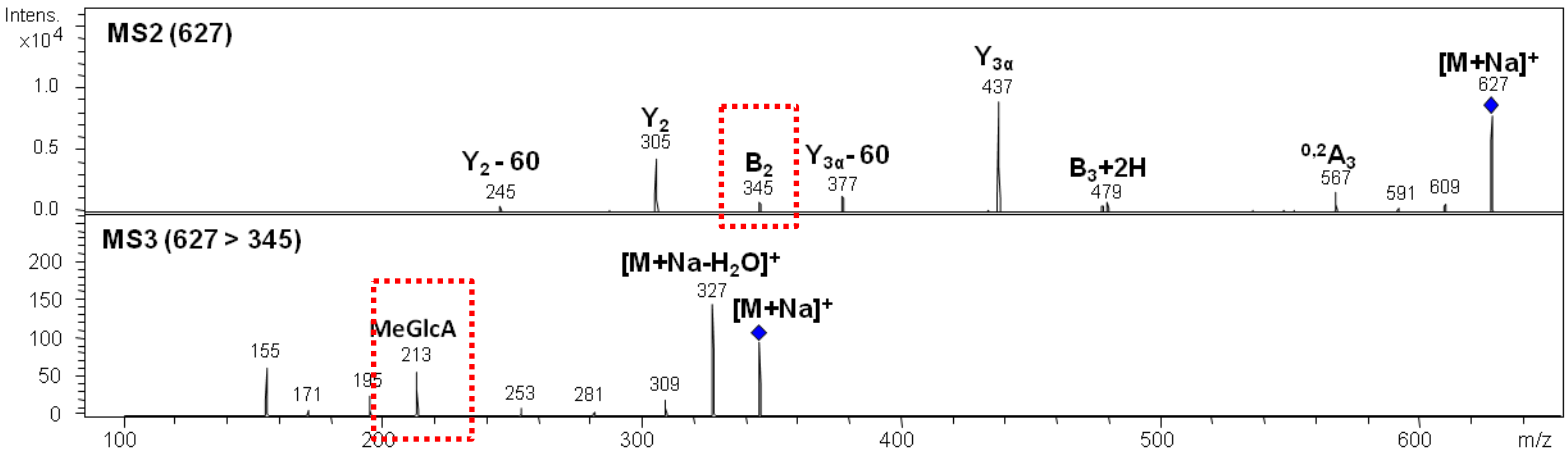
Non-methylated glucuronic acids present in young poplar wood stem?

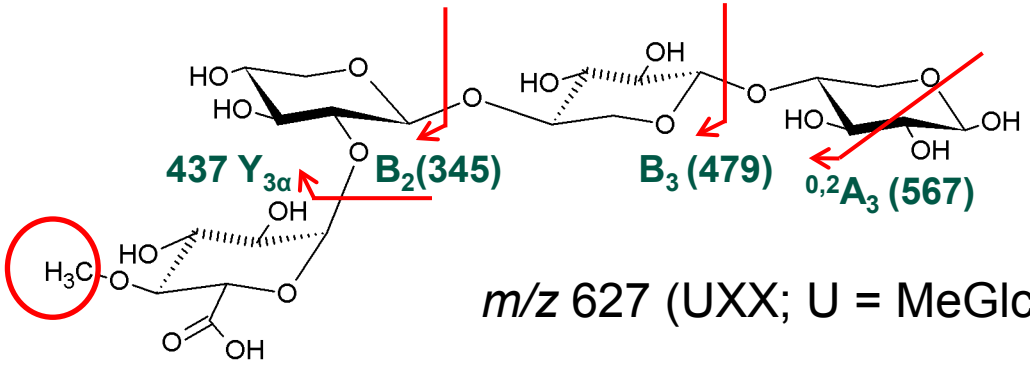


24hrs of deacetylation

Structural Elucidation of m/z 627

AP-Maldi-ITMS Tandem MS

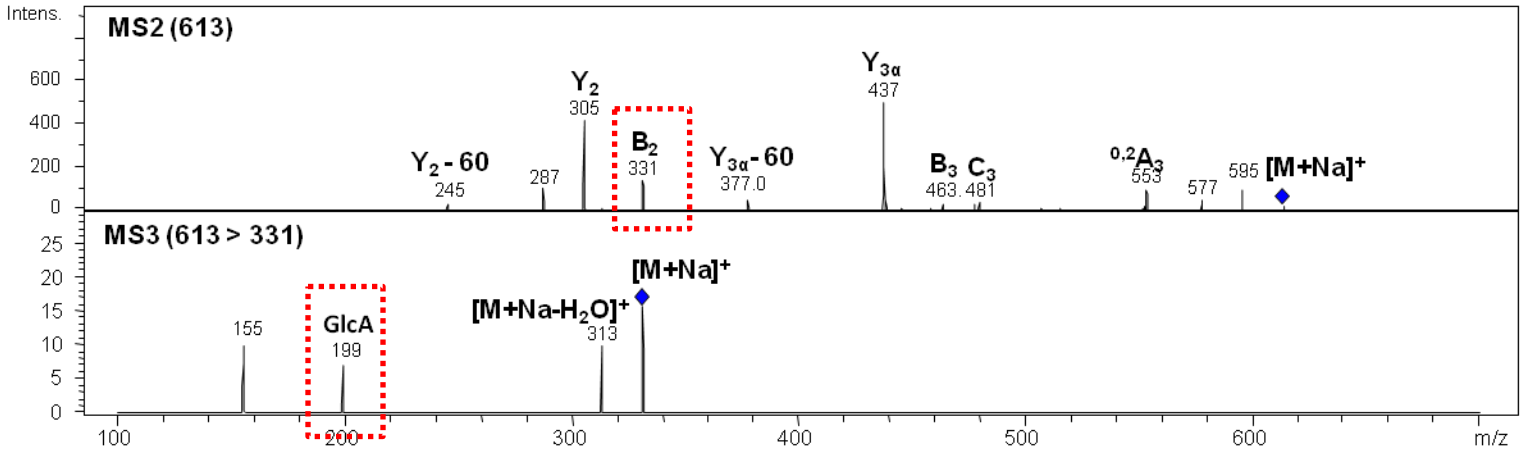


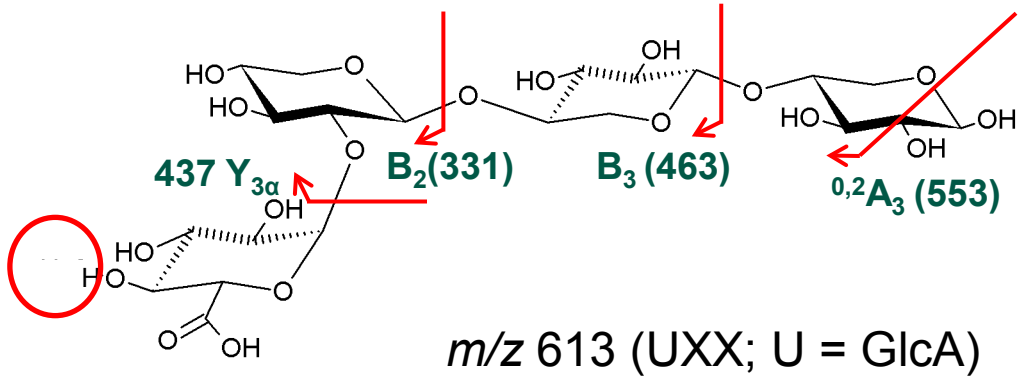


m/z 627 (UXX; U = MeGlcA)

Structural Elucidation of m/z 613

AP-Maldi-ITMS Tandem MS





m/z 613 (UXX; U = GlcA)

Conclusions

- The AP-Maldi-ITMS has shown to be the potential tool to determine mass and structure of plant derived oligosaccharides.
- The fingerprinting mass spectra of acetylated neutral and acidic XOS were obtained from wood stems.
- The relative abundances of XOS can be compared between wood species, transgenic plants etc. in order to obtain information on the structure (substitution) of xylans.

Acknowledgement

University of Helsinki

Dept. of Food & Environmental Sciences

Dr. Sanna Koutaniemi

Dr. Päivi Tuomainen (Docent)

Prof. Maija Tenkanen

Faculty of Pharmacy

Teemu Nissilä

Dr. Raimo Ketola (Docent)

Institute of Biotechnology (Protein Chemistry)

Gunilla Rönnholm

Dr. Nisse Kalkkinen

Umeå Plant Science Centre

Dr. Marta Derba-Maceluch

Prof. Ewa Mellerowicz

Funding €€

Postgraduate studies

- Academy of Finland
- Glycoscience graduate School