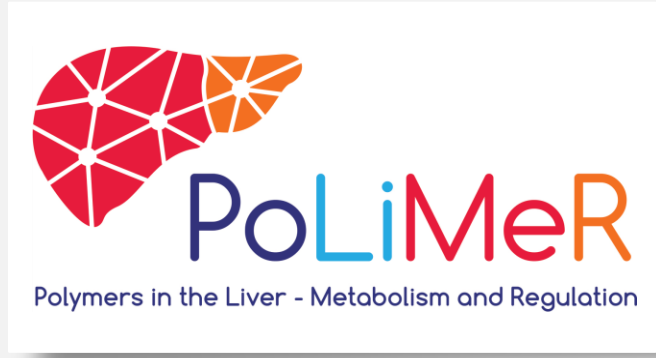
PoLiMeR ASC1: Analytical Techniques for the Carbohydrates



Filothei Tzounidou: PhD Student

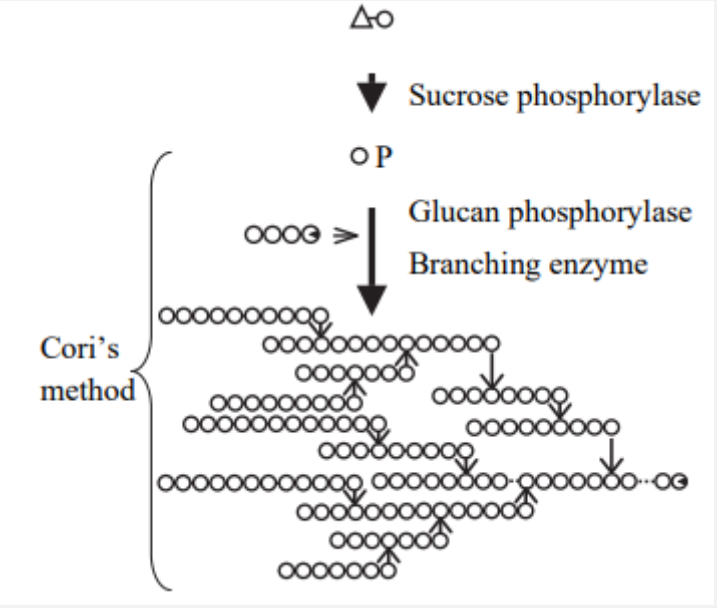
Professor Rob Field: Primary Supervisor

Chromatography & Mass Spec

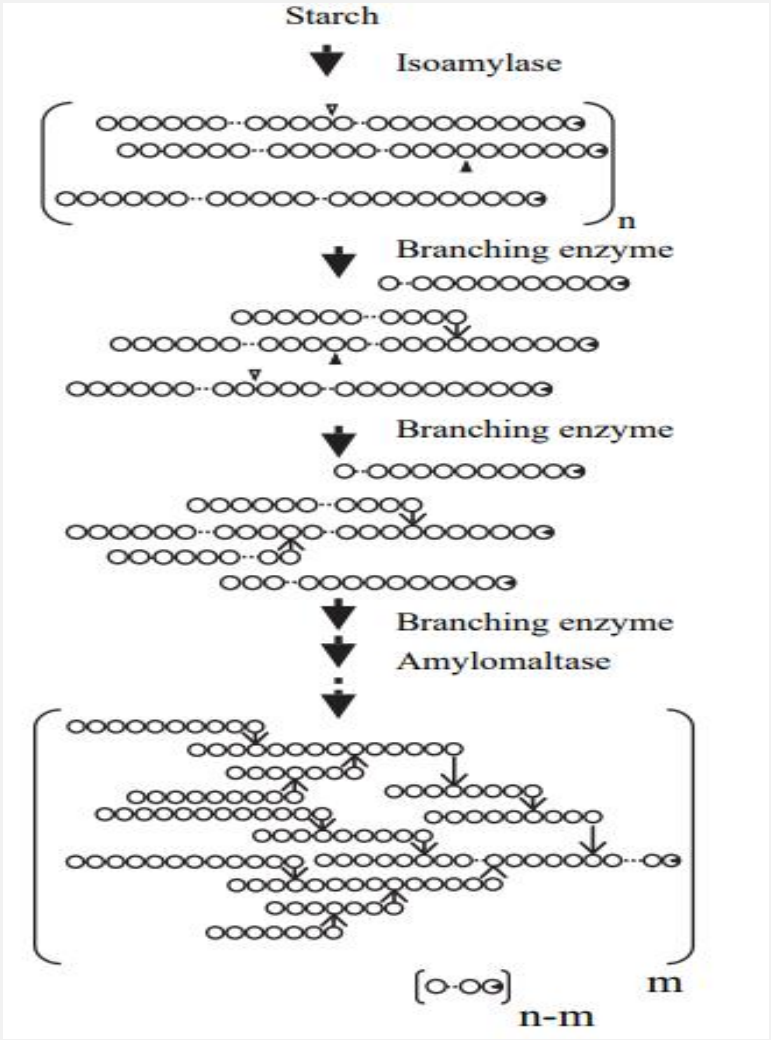
- High-performance Anion Exchange Chromatography coupled with Pulsed Amperometric Detection (HPAE-PAD)
- Ion mobility spectrometry (IMS/MS)

In vitro synthesis

I. De novo synthesis



II. Degradative synthesis from starch



- Mass: **MALDI- ToF**
- Chain-length distribution: **HPAEC-PAD**
- Ratio of branch points: **Reducing ends assay**
- Approach the determination of branching position by: **IMS/MS**

HPAE-PAD

Carbohydrates

- Weakly acidic nature
- pKa in the range of 12–13
- pH > pKa of the carbohydrate -> ionize

Column

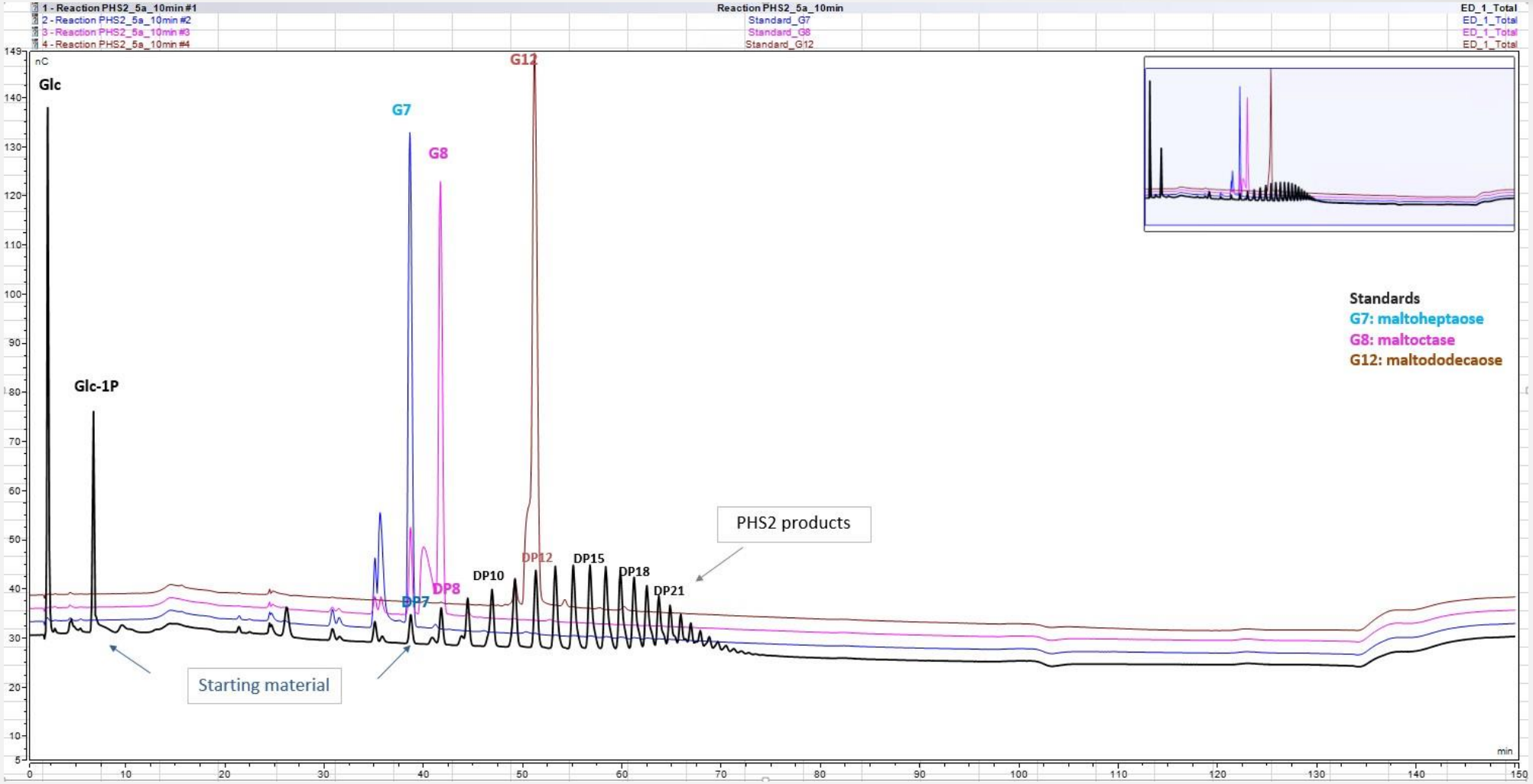
Using strong anion exchange stationary phases to bind the ionized carbohydrates

Eluent

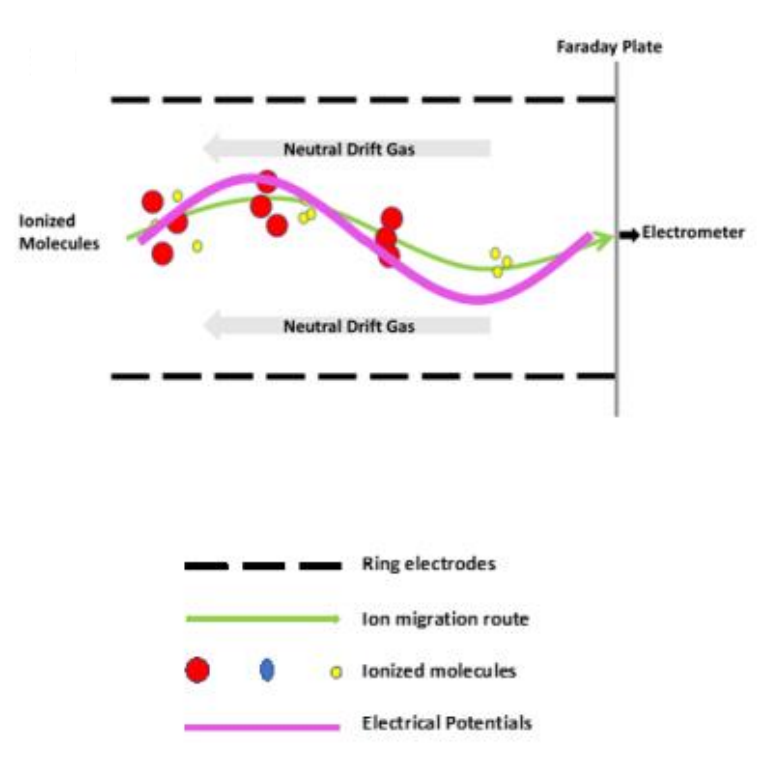
Separations require hydroxide-based eluents (NaOH, KOH)
Complex carbohydrates, improved by using stronger eluent (NaOH/ NaOAc)

Detection

Potentials are applied on gold working electrodes (WE) surface that carbohydrates pass and thus they oxidized and generate a current that can be measured by the reference electrodes(RE) (dc amperometry)


1 - Reaction PHS2_5a_10min #1
2 - Reaction PHS2_5a_10min #2
3 - Reaction PHS2_5a_10min #3
4 - Reaction PHS2_5a_10min #4
Reaction PHS2_5a_10min
Standard_G7
Standard_G8
Standard_G12
ED_1_Total
nC
Glc
G7
G8
G12
Glc-1P
DP7
DP8
DP10
DP12
DP15
DP18
DP21
PHS2 products
Starting material
Standards
G7: maltoheptaose
G8: maltoctase
G12: maltododecaose
min

Ion mobility spectrometry (IMS/MS)



Travelling wave ion mobility spectrometry (TWIMS)

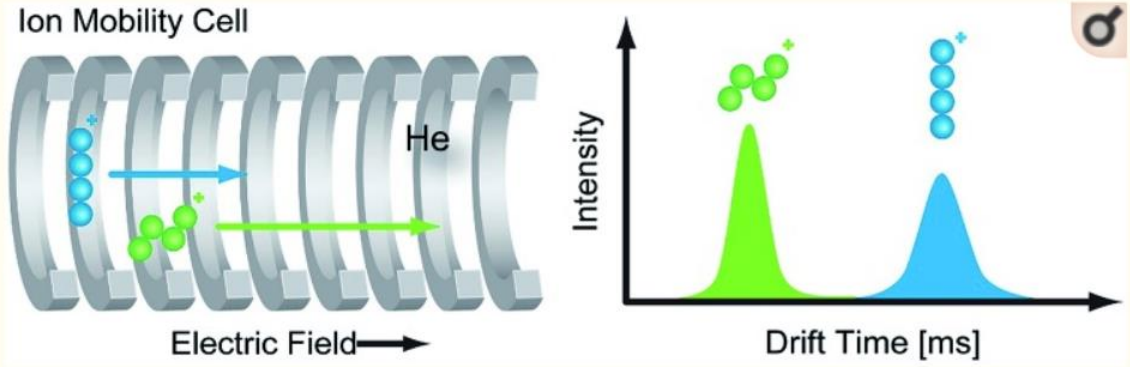


Fig. 2

Principle of ion mobility spectrometry (IMS). In the ion mobility cell ions are separated according to their charge, size, and shape. The IM cell is filled with a neutral buffer gas such as helium or nitrogen. Gas-phase ions travel through the IM cell, guided by a weak electric field, in which larger ions (blue) undergo more collisions with the buffer gas and thus have longer drift times compared to more compact (green) ions.

Thank you!