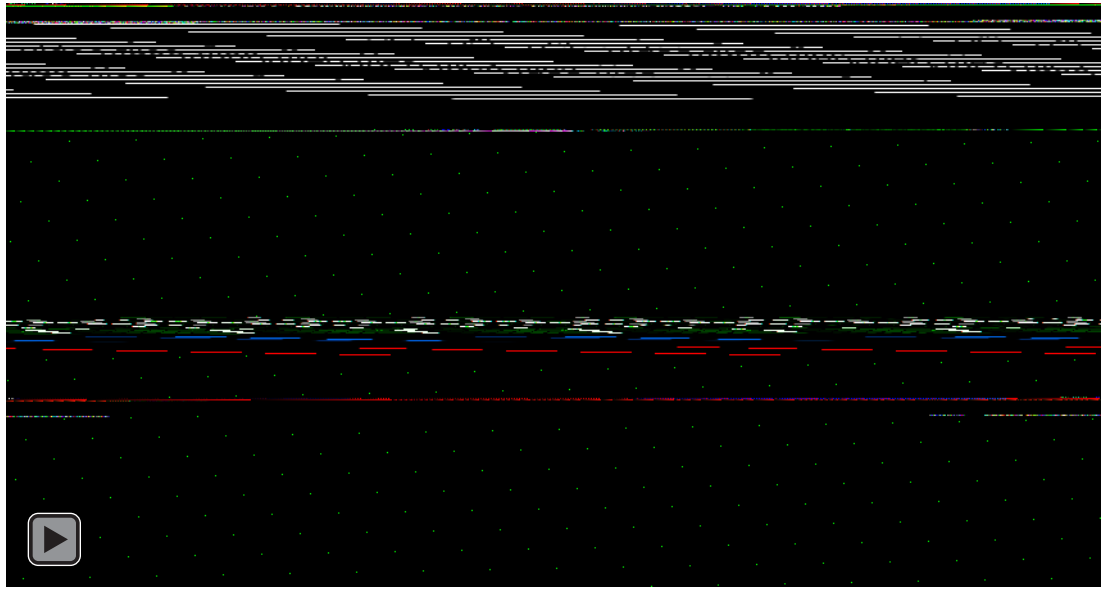
Introduction

Characterisation of the samples by mass: **M**atrix **A**ssisted **L**aser **D**esorption **I**onisation – **T**ime **o**f **F**light (MALDI-TOF)

How does it work?

- ✓ Samples are transformed into ions using a laser energy absorbing matrix
- ✓ Ionised samples are separated by their mass inside a flight tube before they are detected by a detector
- ✓ It can be used for proteomics, metabolomics, glycobiology, microbiology, etc.



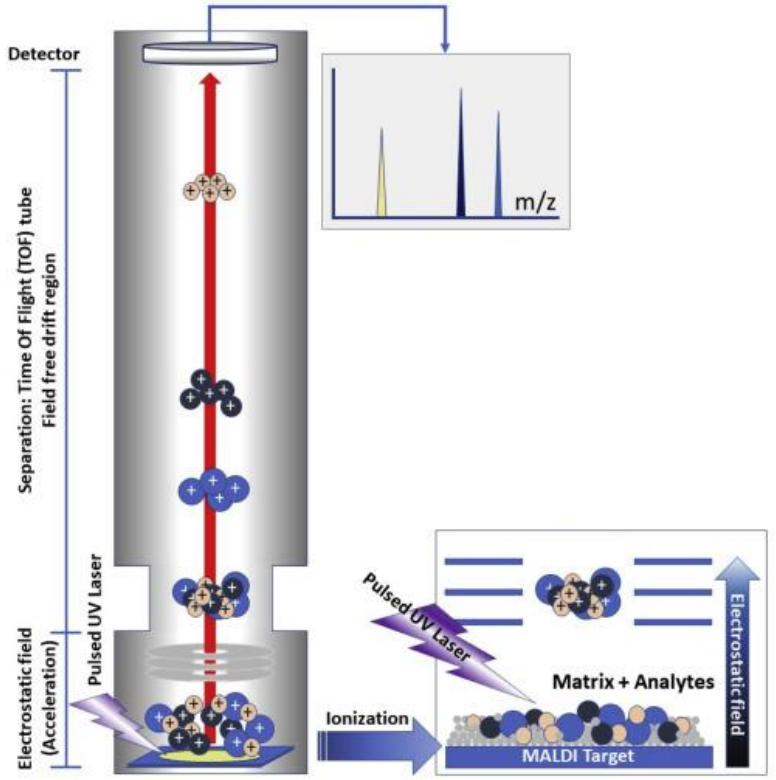
Characterisation of the samples by mass: **M**atrix **A**ssisted **L**aser **D**esorption **I**onisation – **T**ime **o**f **F**light (MALDI-TOF)

What is the matrix?

- A matrix is an organic compound that enhances/facilitates the ionization of the sample

What type of matrix can be used?

- ✓ Be capable of co-crystallization with the analyte
- ✓ High absorptivity coefficient at the UV wavelength of 337.7 nm emitted by the MALDI laser
- ✓ Be chemically non-reactive with the analyte
- ✓ Must not sublimate at a pressure of 70 mTorr - the pressure maintained within the sample chamber of the MALDI source



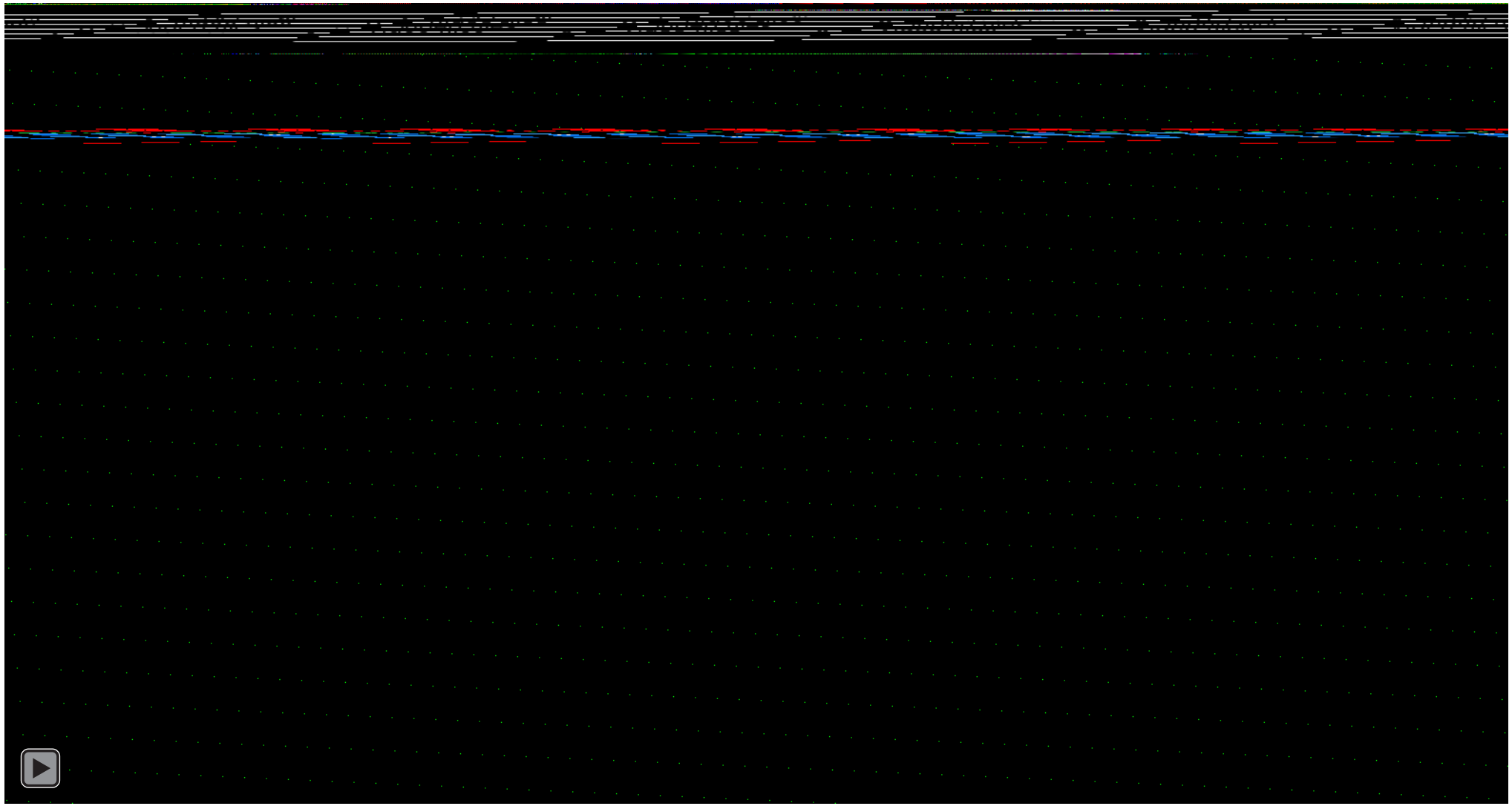
Introduction

Characterisation of the samples by mass: **M**atrix **A**ssisted **L**aser **D**esorption **I**onisation – **T**ime **o**f **F**light (MALDI-TOF)

…How is the sample prepared?

…How is a MALDI spectra recorded?

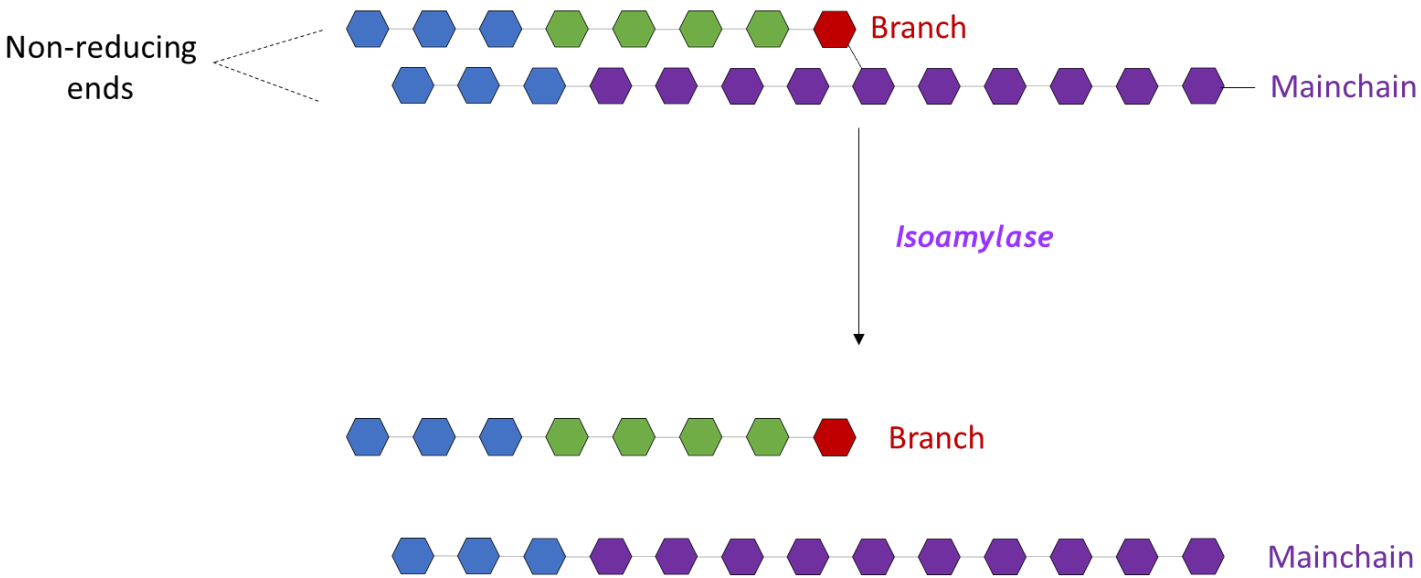
How to perform MALDI-TOF



Application to glycogen

Characterisation of the samples by mass: **M**atrix **A**ssisted **L**aser **D**esorption **I**onisation – **T**ime **o**f **F**light (MALDI-TOF)

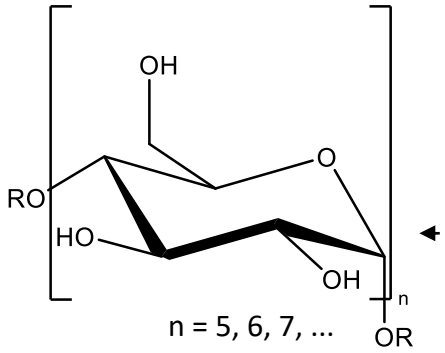
How can we use MALDI with glycogen?

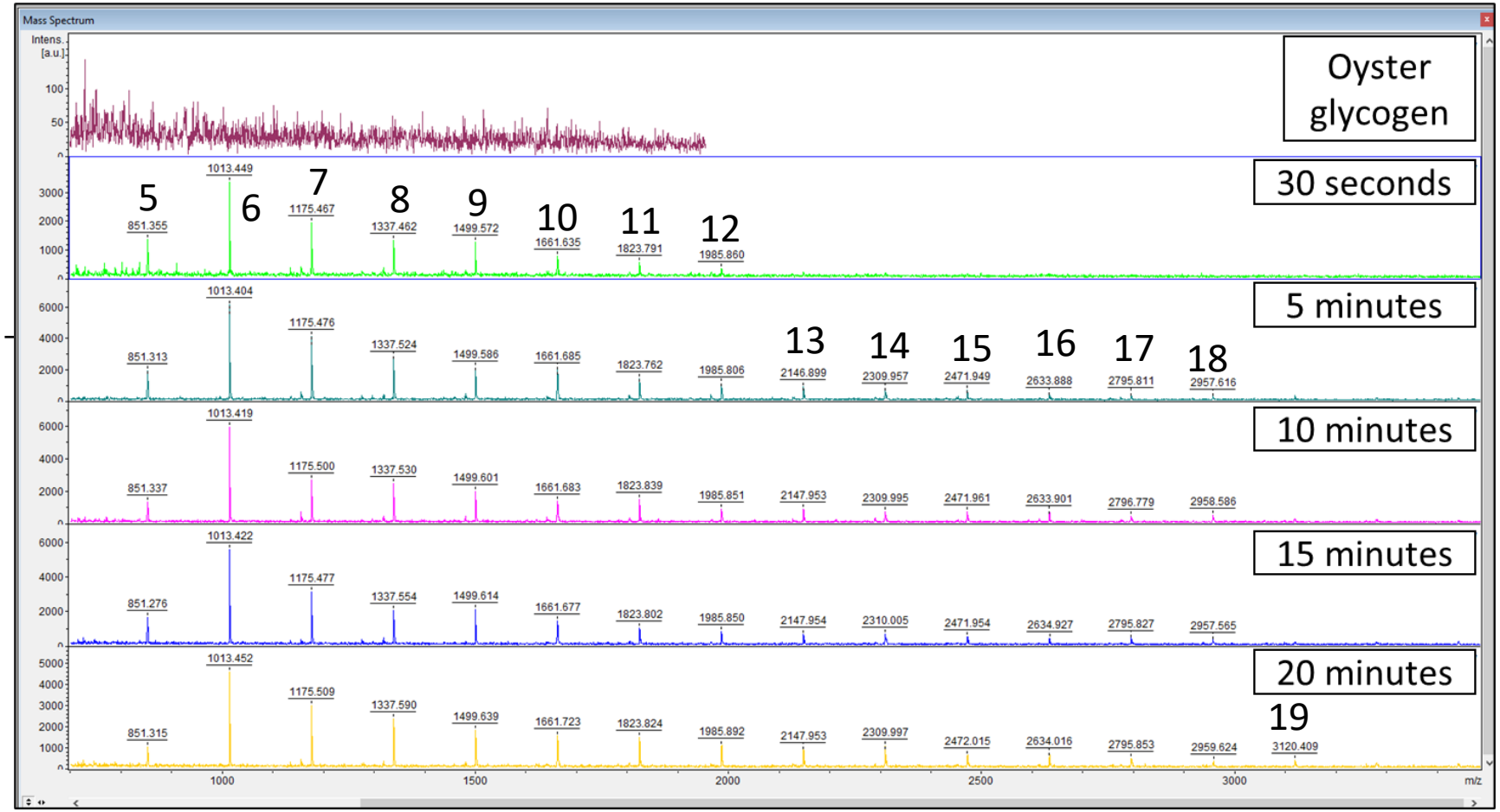


- ✓ Analyse the **mass of the products** released by debranching activity
- ✓ Identify the **length of branches** that characterise the surface of glycogen molecules
- ✓ Map a **putative structure of glycogen** in combination with other analytical techniques
- ✓ **Impact of GSDs** on glycogen structure

Application to glycogen

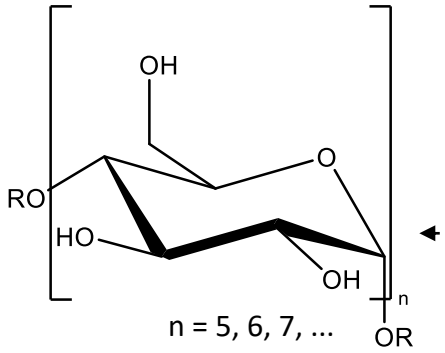
*Oligosaccharides released by debranching of **oyster glycogen** with isoamylase in 20 minutes*

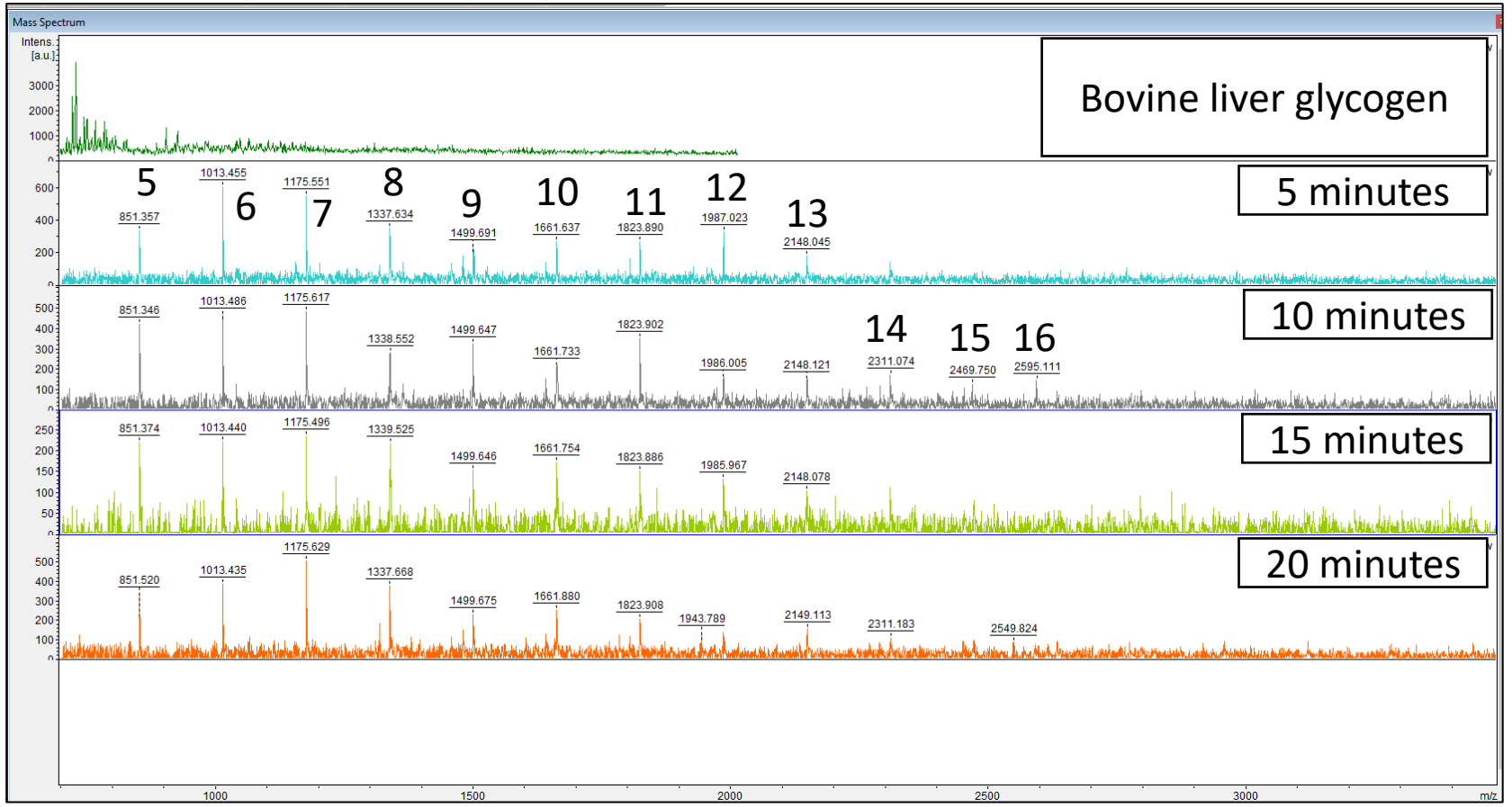




Application to glycogen

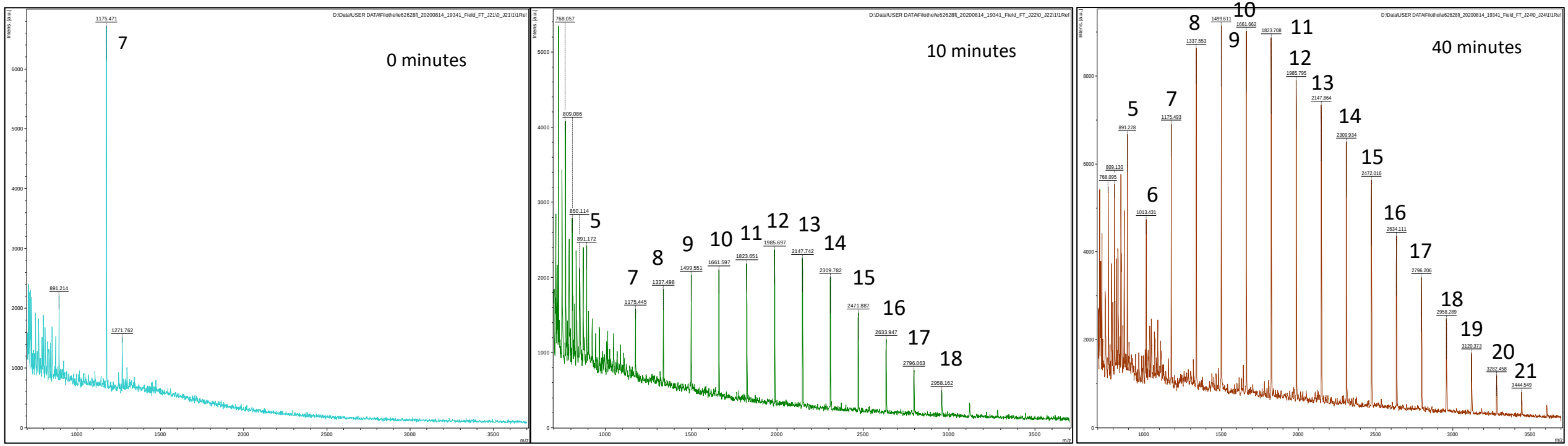
*Oligosaccharides released by debranching of **bovine liver glycogen** with isoamylase in 20 minutes*





Application to glycogen

*Oligosaccharides synthesised by **de novo synthesis** of oligosaccharides from maltoheptaose*



Comparison with debranched products to understand the structure of native glycogen

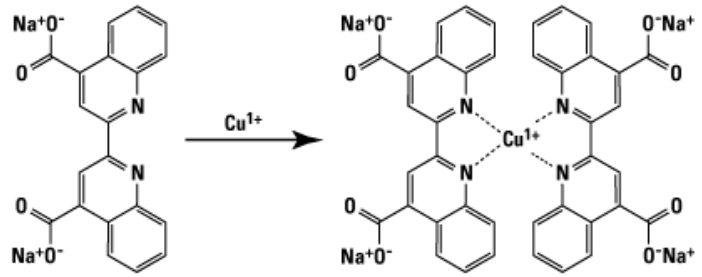
Characterisation of the samples by quantification of reducing end sugars: Bicinchoninic acid assay **(BCA)**

How does it work?

- ✓ Samples with reducing residues (e.g. amino acid or glucose polymers) takes part in a redox reaction:

  Reducing residues + Cu^{2+} → Oxidised residues + Cu^{+1}

  Cu^{+1} + 2BCA → Complex BCA-Cu^{+1} (purple, Abs_{560})



Bicinchoninic Acid (BCA) sodium salt

BCA-Copper Reaction

- ✓ The absorbance is proportional to the concentration of reducing residues converted into oxidised residues

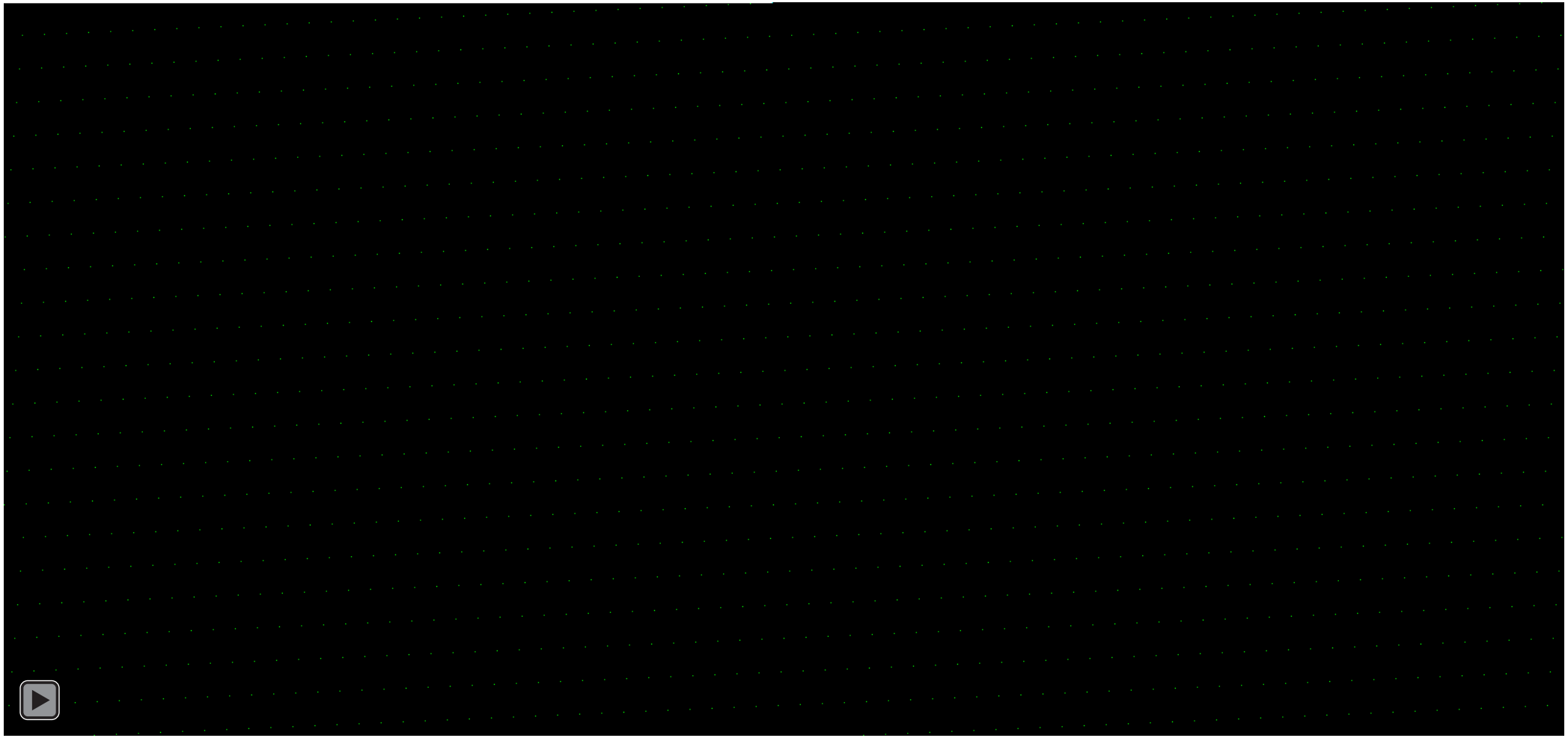
- ✓ High sensitivity towards glucose polymers and proteins
- ✓ Easy to perform

Characterisation of the samples by quantification of reducing end sugars: Bicinchoninic acid assay **(BCA)**

…How is it performed?

How to perform BCA assay

Characterisation of the samples by quantification of reducing end sugars: Bicinchoninic acid assay **(BCA)**



Application to glycogen

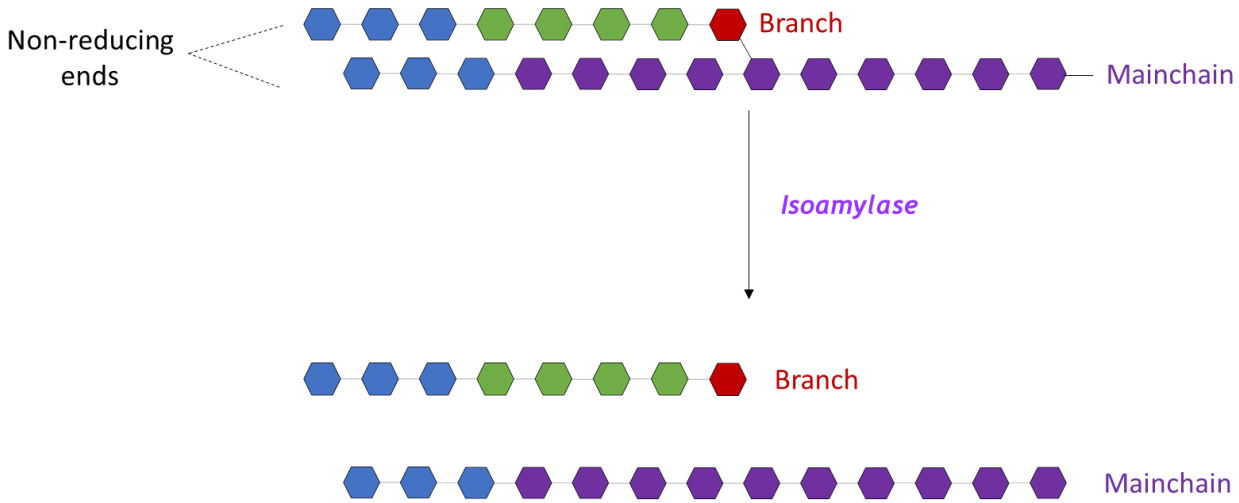
Characterisation of the samples by quantification of reducing end sugars: Bicinchoninic acid assay **(BCA)**

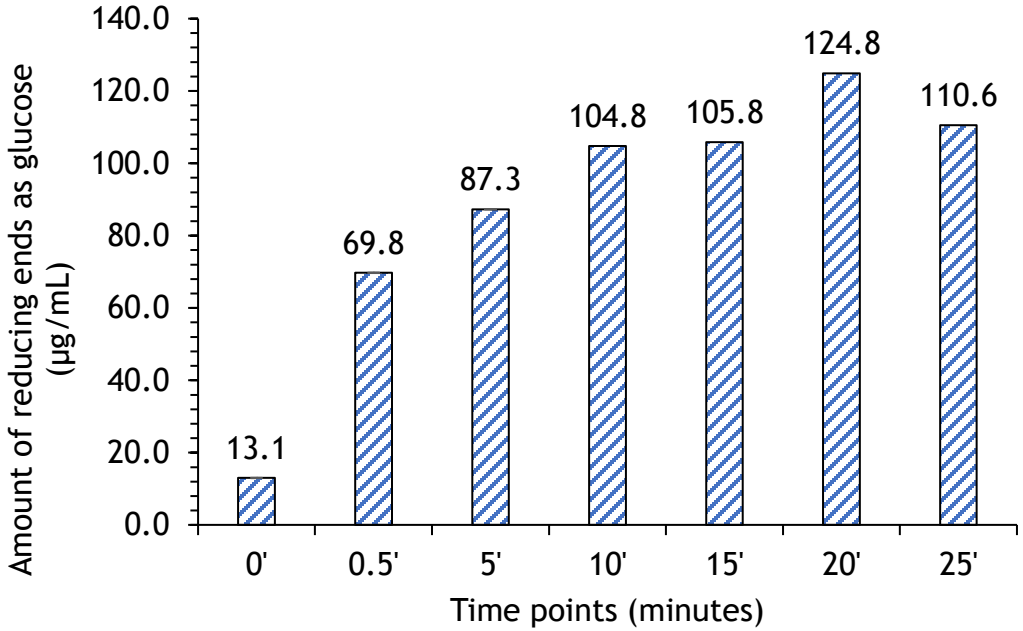
How can we use BCA with glycogen?

- ✓ **Quantify the products** released during the debranching of glycogen
- ✓ Identify when the enzymatic cleavage is over
- ✓ Determine the **degree of branching** of glycogen
- ✓ **Impact of GSDs** on glucose availability

Characterisation of the samples by quantification of reducing end sugars: Bicinchoninic acid assay **(BCA)**

Quantification of the branches *cleaved during debranching*





Sum up

Length of the branches from the surface to the core

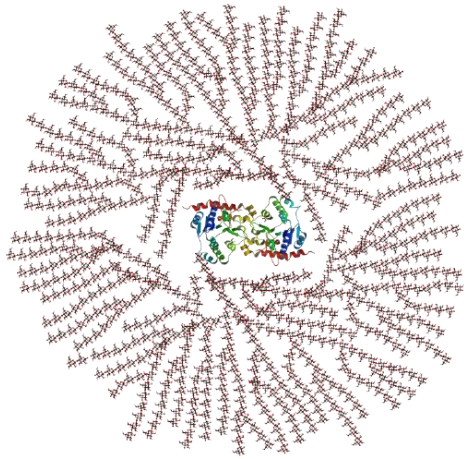
MALDI-TOF TLC

Quantification of the products released

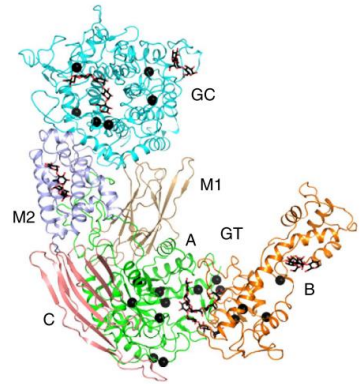
BCA assay

Quantity of glucose produced by debranching glycogen

Combination of other techniques (i.e. phenol-sulfuric acid assay)



Map the structure of glycogen



Identify the effects of GSDs mutation on glycogen and glycogen-active enzymes