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## Sub optimal $^{15}\text{N}$ metabolic labelling in plant to determine protein turnovers: A new look at the isotopic distribution

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## Introduction

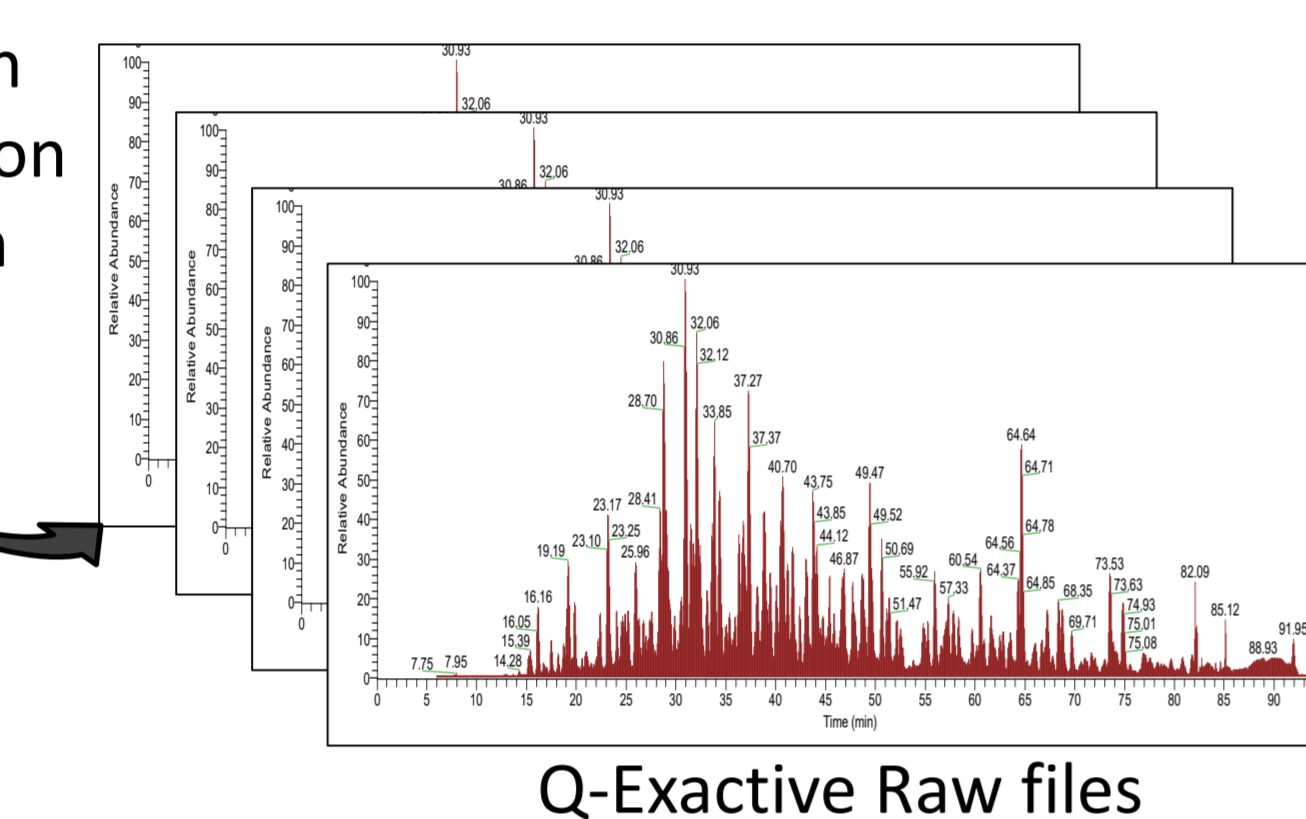
Protein turnover is a crucial information required to better understand proteostasis and the processes required maintaining the equilibrium between protein synthesis and degradation to balance cellular development and/or environmental stresses. The determination of protein turnover using pulsed SILAC metabolic labelling is popular but, such approach remains difficult with whole organisms and impossible with autotroph species like plants. Alternatively, inorganic sources of <sup>15</sup>N could be metabolically incorporated to *de novo* synthesized amino acids although <sup>15</sup>N incorporation rates remains sub-optimal. Instead of reaching high <sup>15</sup>N labelling yield (close to 100%), we decide to remain at very low incorporation rates (maximum 5-6%). At such level, the neo-peptides isotopic distribution is only moderately modified compared to the peptides natural isotopic distribution. Both labelled and unlabelled products appear as an overlapping isotopic cluster which increases very moderately the peptide mixture complexity during LC-MS/MS analyses. Then, the accurate measurement of the experimental isotopic distribution is mandatory to determine the <sup>15</sup>N yield observed for each characterised peptide over time. Post-acquisition data processing is performed using our free and open sources software including X!TandemPipeline<sup>1</sup>, MassChroQ<sup>2</sup> and "R" specific script<sup>3</sup> already available and/or under development to determine the fold change in protein (FCP) and the labelled protein fraction (LPF) which are required to estimate large scale protein turnover rate.

## Samples preparation and proteins identification

Species: *Zea maize* B73  
Growth in potting soil  
Normal growth until leaf 6  
<sup>15</sup>N labelling pulse using 200 ml of ½ MS media containing 10% of <sup>15</sup>N (NH<sub>4</sub>NO<sub>3</sub>)  
Collecting 5-6 cm of the F6 leaves at 1, 3, 6, 10 and 15 days



Protein extraction  
Reduction/Alkylation  
Trypsin Digestion



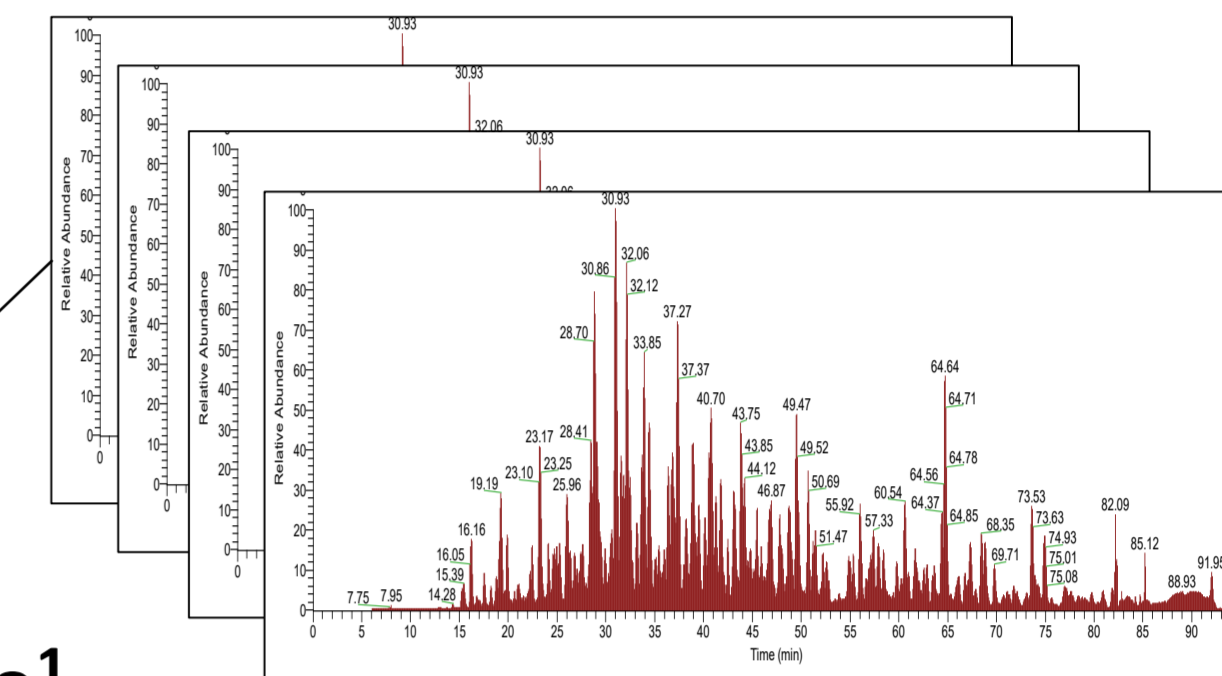
List of X!Tandem characterised peptides and proteins for each acquired raw file



Zm-B73-REFERENCE-DB.protein

Mconvert  
mzML files  
conversion

## Post Acquisition data processing



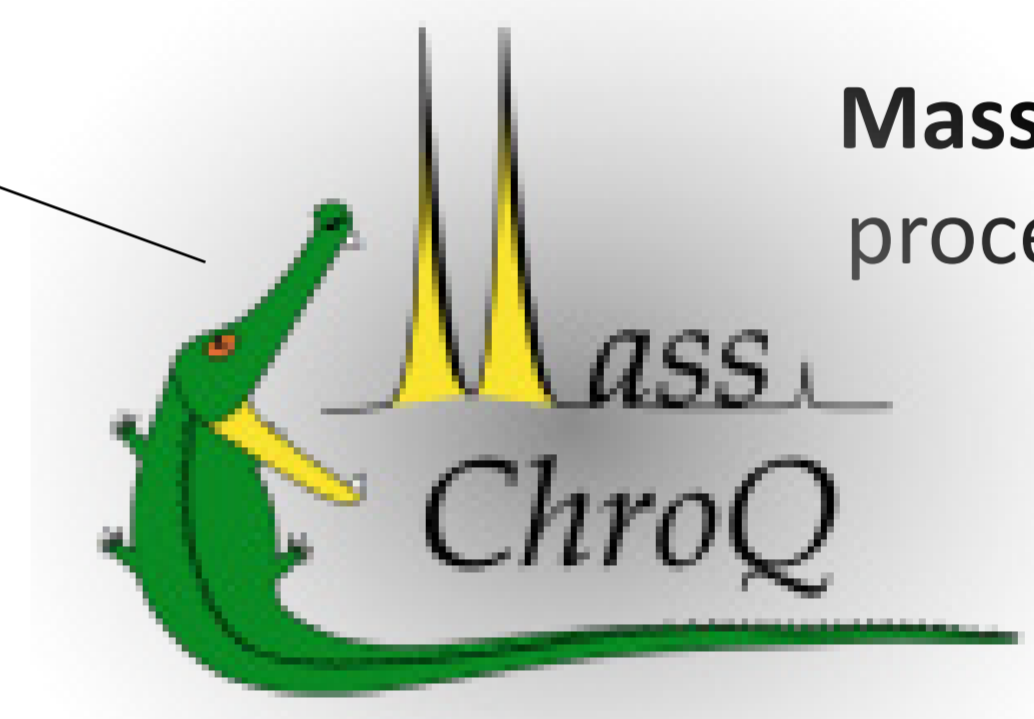
Raw files

X!Tandem Pipeline<sup>1</sup>

Multiple files protein inference, grouping and filtering

MCQR (R package<sup>3</sup>)

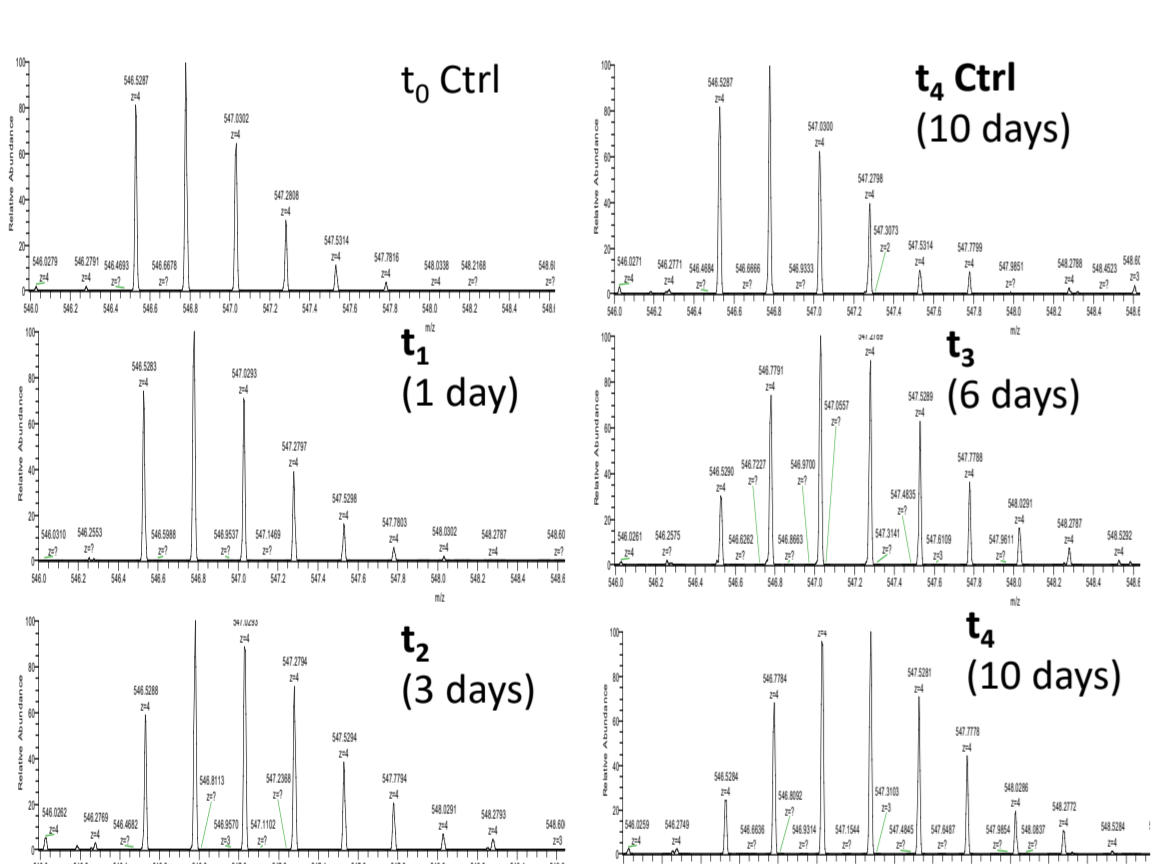
Differential protein quantification:



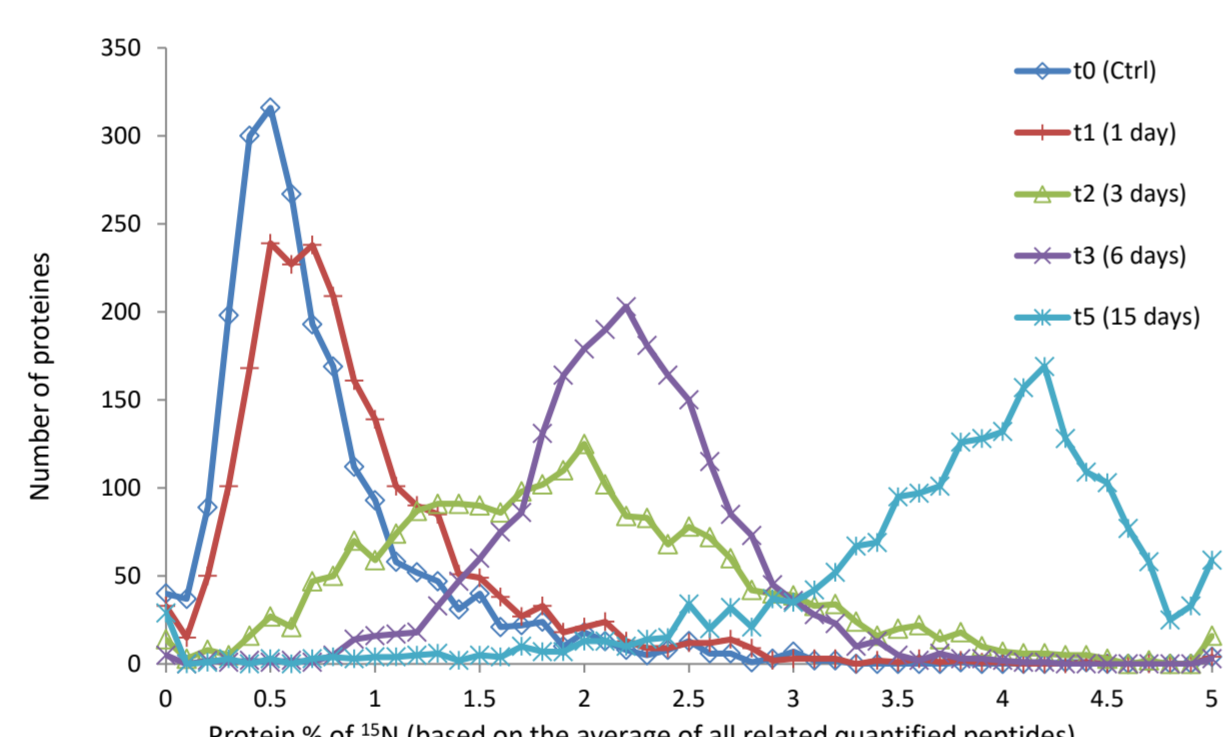
MassChroQ processing<sup>2</sup>

Determination of the Protein Fold Change (PFC)

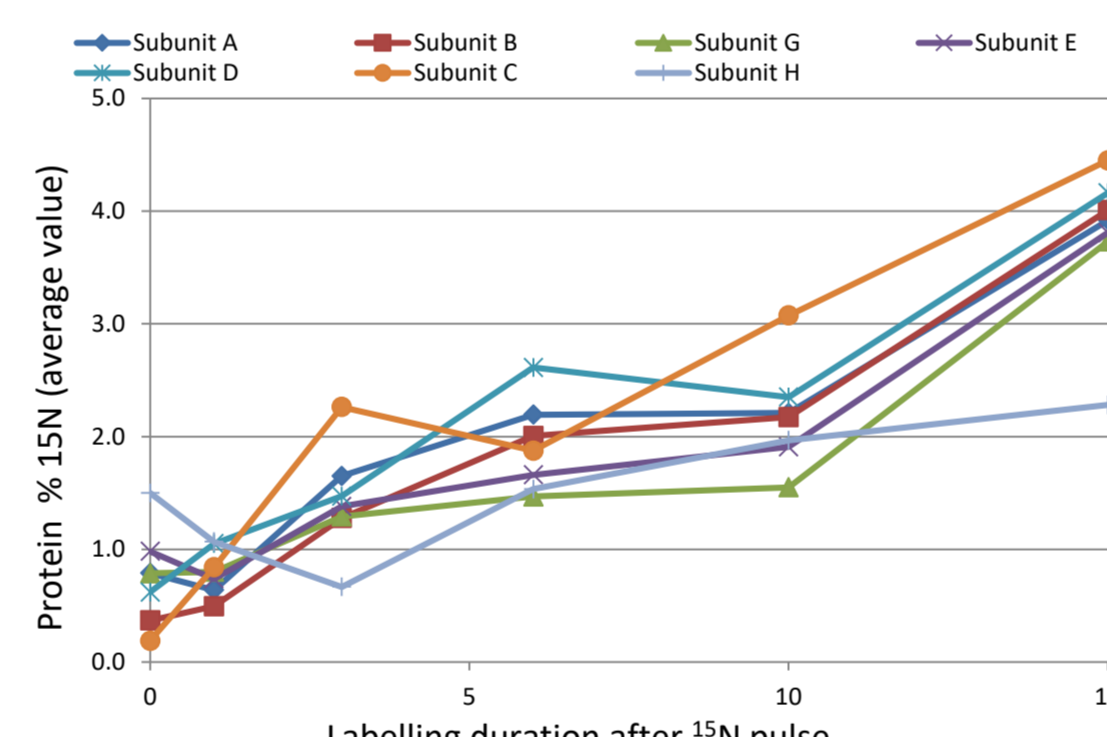
## Work in progress and result



Peptide isotopic distribution time lapse at different labelling times



Experimental <sup>15</sup>N ratio profiles for the characterised proteins at different labelling times



<sup>15</sup>N metabolic labelling yield observed for V-type ATPases complex protein subunits at different labelling times

Large scale proteins synthesis/degradation rates and ½ life determination

Protein turnover calculation<sup>5-6</sup> (based on PFC and LPF)

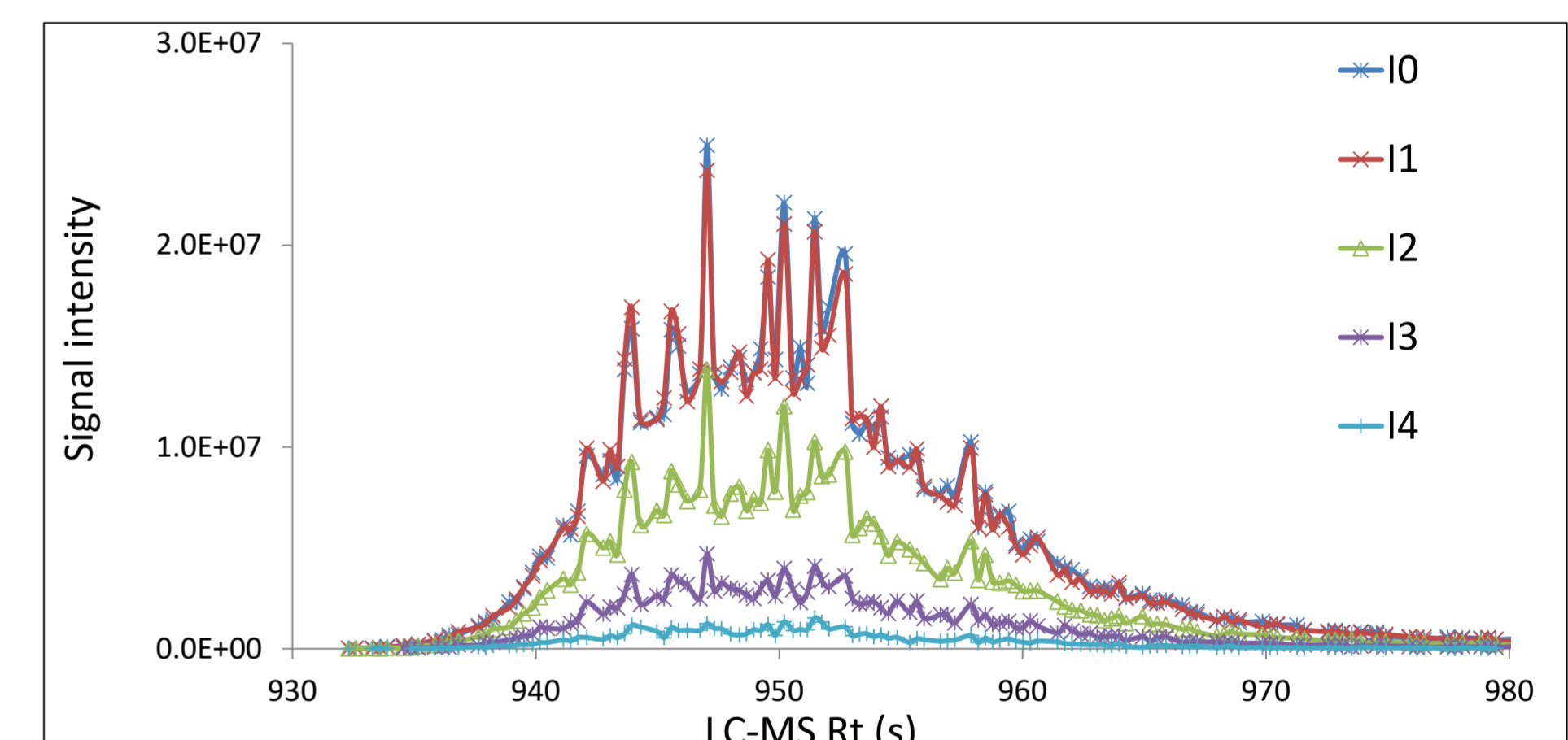
<sup>15</sup>N Labelled Protein Fraction (LPF) determination

Exports of the PeptIDs list

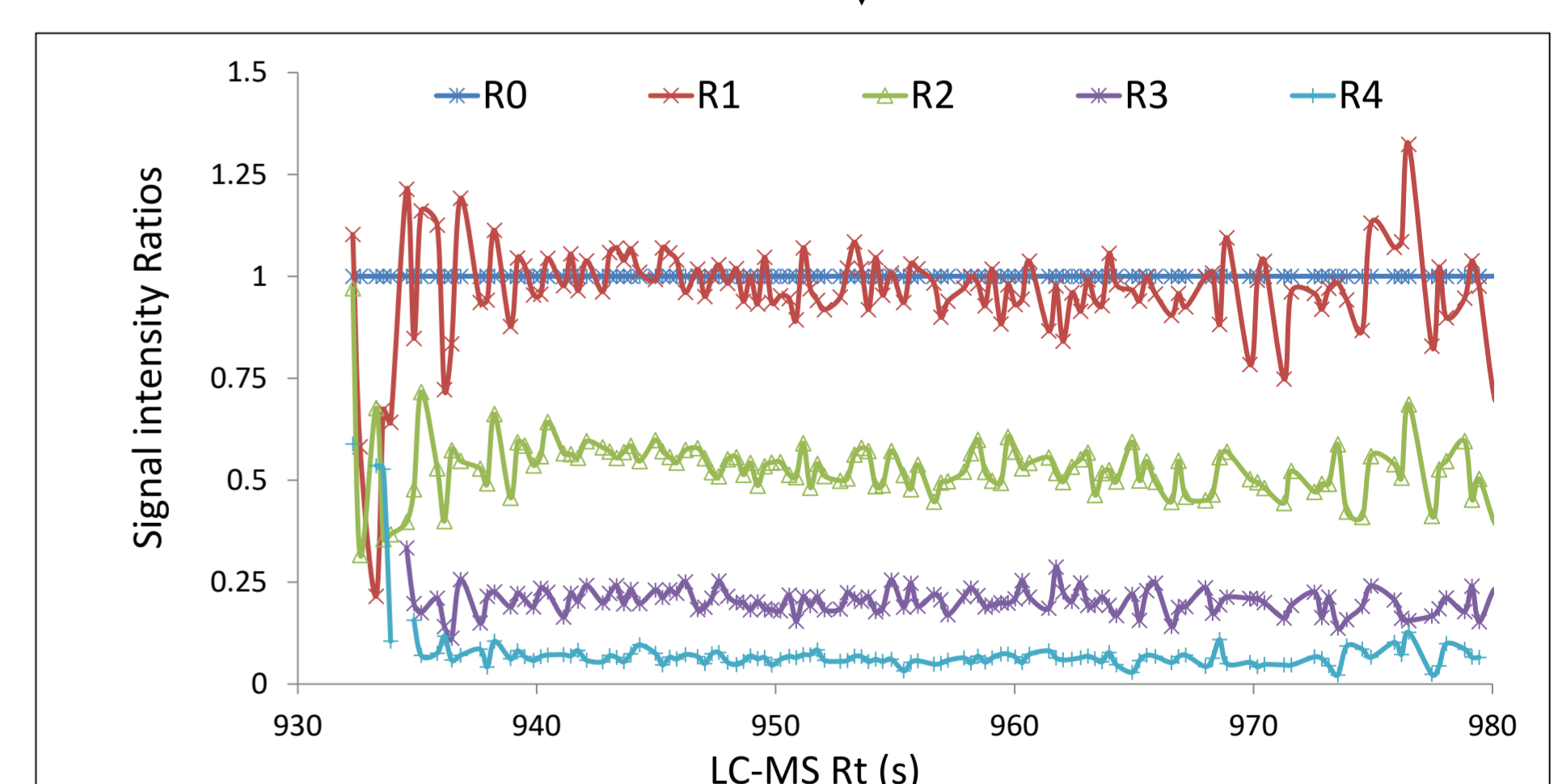
IsoSpec.py<sup>4</sup>

Theoretical isotopic distribution calculation (multiple <sup>15</sup>N rate)

Peptides isotopic XIC profiles extraction

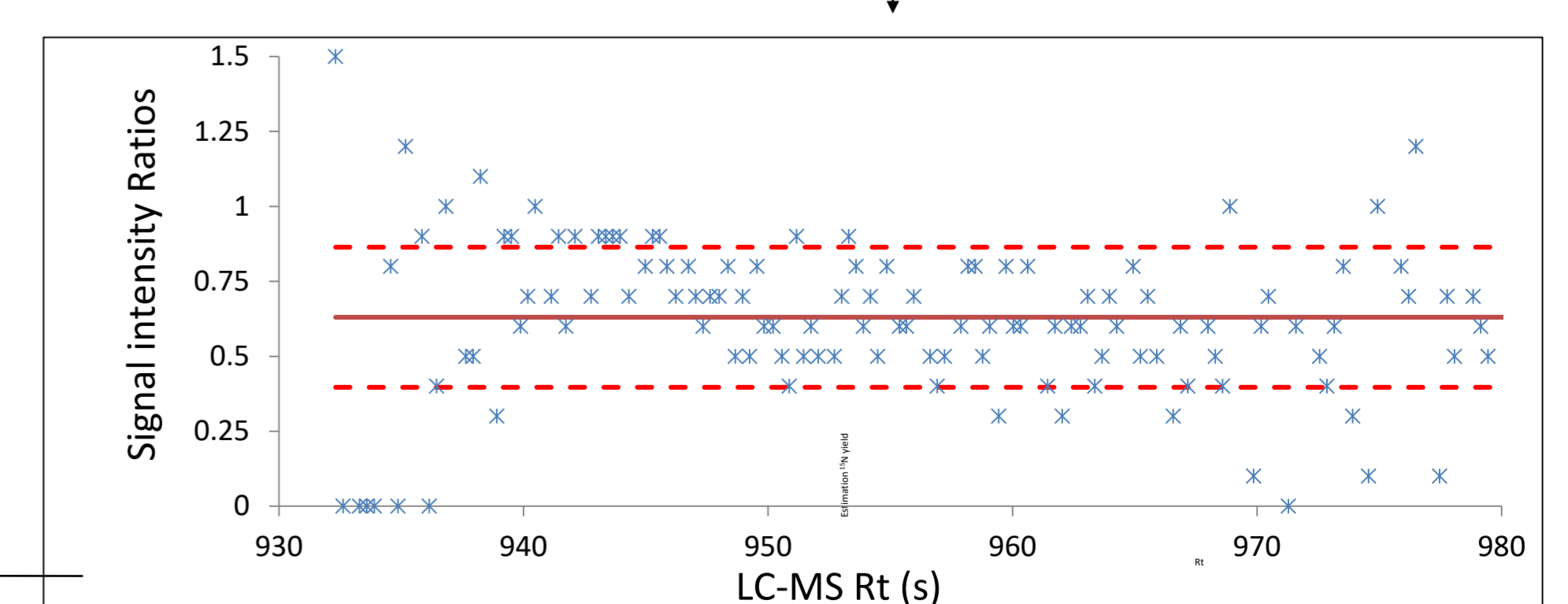


Calculation of the Experimental isotopic ratios



Estimation of the <sup>15</sup>N incorporation

And outliers suppression



Estimation of the <sup>15</sup>N labelling rate by comparison of the experimental vs. theoretical isotopic ratios using the multiple occurrences collected during the RP-LC elution; Average value obtained after outliers suppression:  $\langle \%^{15}\text{N} \rangle = 0.63 \pm 0.23$  for the pepa1a4 from sample collected at t1

## Conclusions

Partial and low <sup>15</sup>N metabolic labelling is compatible with :

- Plant growth even in soil or open fields
- Peptide/protein characterisation
- Determination of the Protein Fold Change (MCQR)

Data post acquisition processing provides:

- Reliable experimental isotopic distributions including
- <sup>15</sup>N labelling rates evaluation
- Estimation of the Labelled Protein Fraction (LPF)

In progress and under development:

- Improvement of the LPF accuracy using data redundancy
- Publicly available data processing R script

## References

- 1) Langella O. *et al. J Proteome Res.* 2017; 16(2):494-503
- 2) Valot B. *et al. Proteomics* 2011; 17:3572-7
- 3) MCQR (R package): <https://forgemia.inra.fr/pappso/mcqr>
- 4) Łącki M.K. *et al. Anal Chem.* 2020; 92(14): 9472-5
- 5) Li L. *et al. Plant Cell.* 2017; 29(2): 207-28
- 6) Sadygov R.G. *et al. Anal Chem.* 2020; 92): 14747-53