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

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Introduction

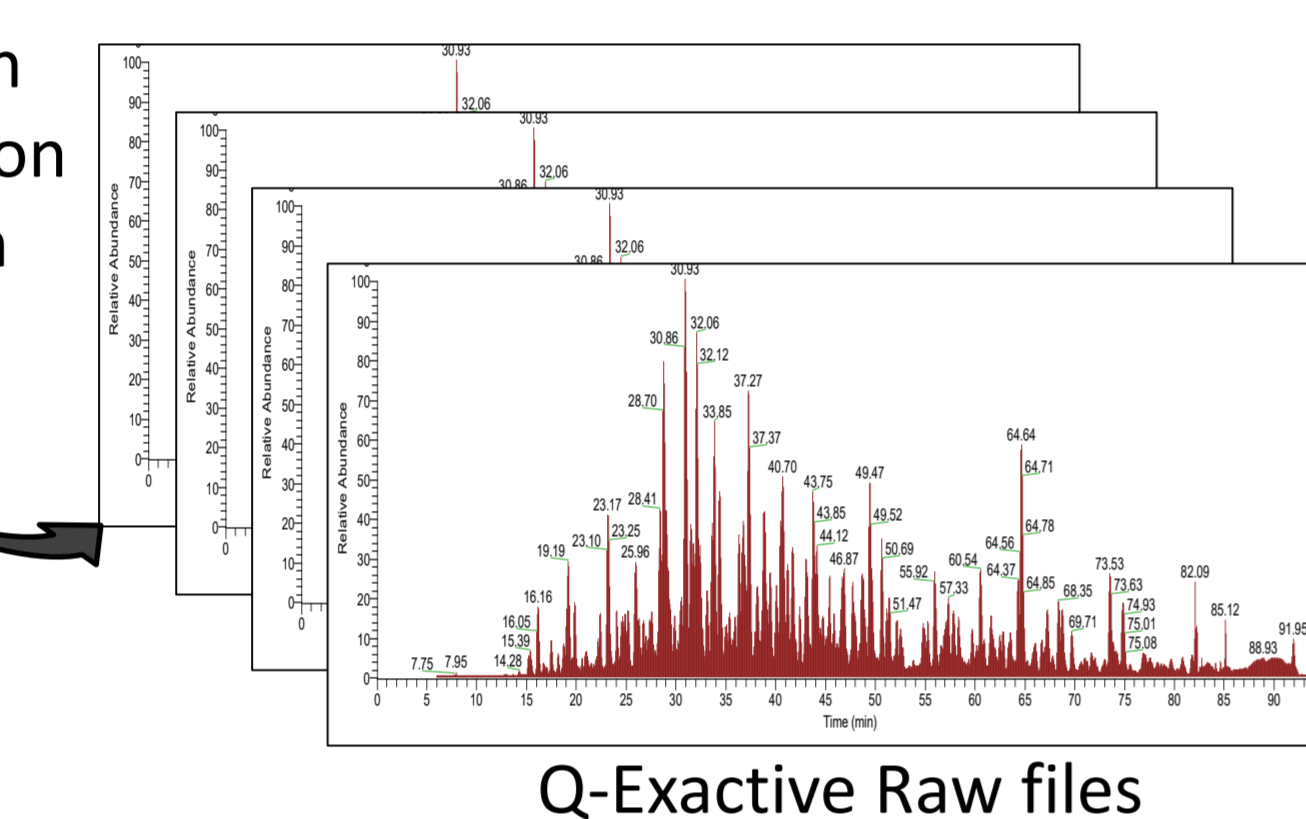
Protein turnover is a crucial information required to better understand proteostasis and the processes required maintaining the equilibrium between protein synthesis and degradation to balance cellular development and/or environmental stresses. The determination of protein turnover using pulsed SILAC metabolic labelling is popular but, such approach remains difficult with whole organisms and impossible with autotroph species like plants. Alternatively, inorganic sources of ¹⁵N could be metabolically incorporated to *de novo* synthesized amino acids although ¹⁵N incorporation rates remains sub-optimal. Instead of reaching high ¹⁵N 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 ¹⁵N yield observed for each characterised peptide over time. Post-acquisition data processing is performed using our free and open sources software including X!TandemPipeline¹, MassChroQ² and "R" specific script³ 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
¹⁵N labelling pulse using 200 ml of ½ MS media containing 10% of ¹⁵N (NH₄NO₃)
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

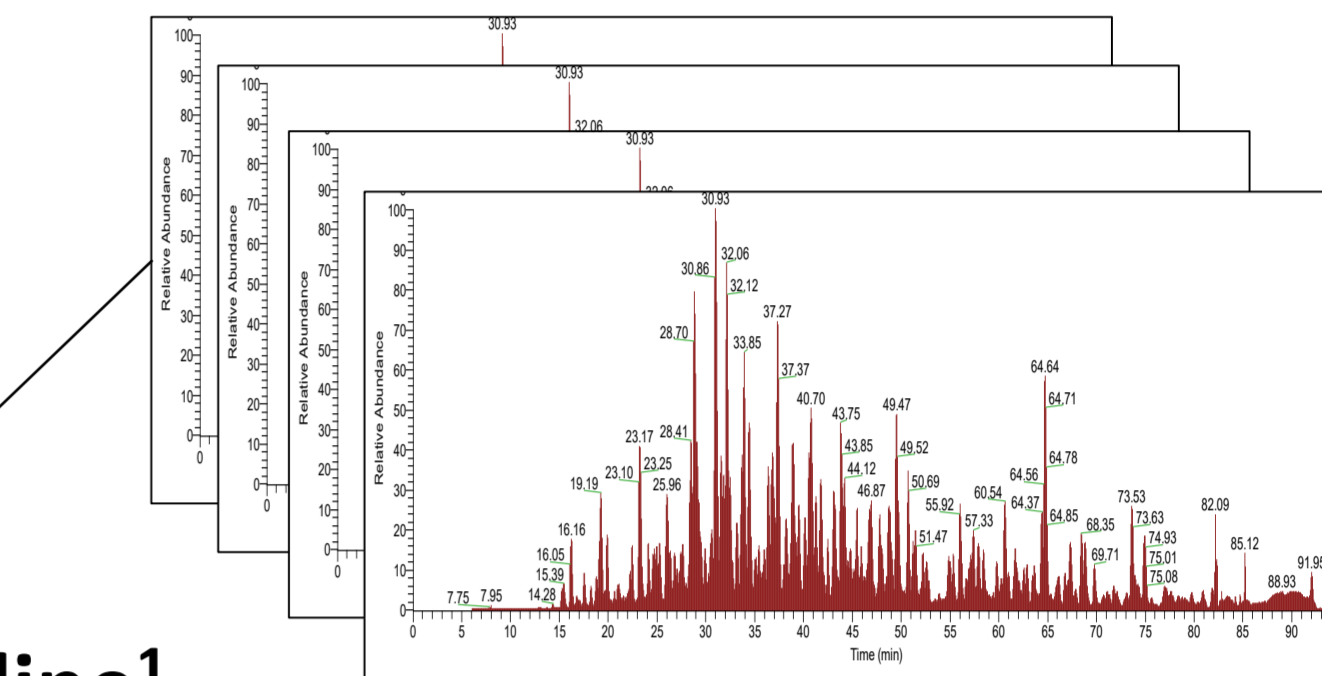
Mconvert
mzML files
conversion

Post Acquisition data processing



X!Tandem Pipeline¹

Multiple files | protein inference, grouping and filtering

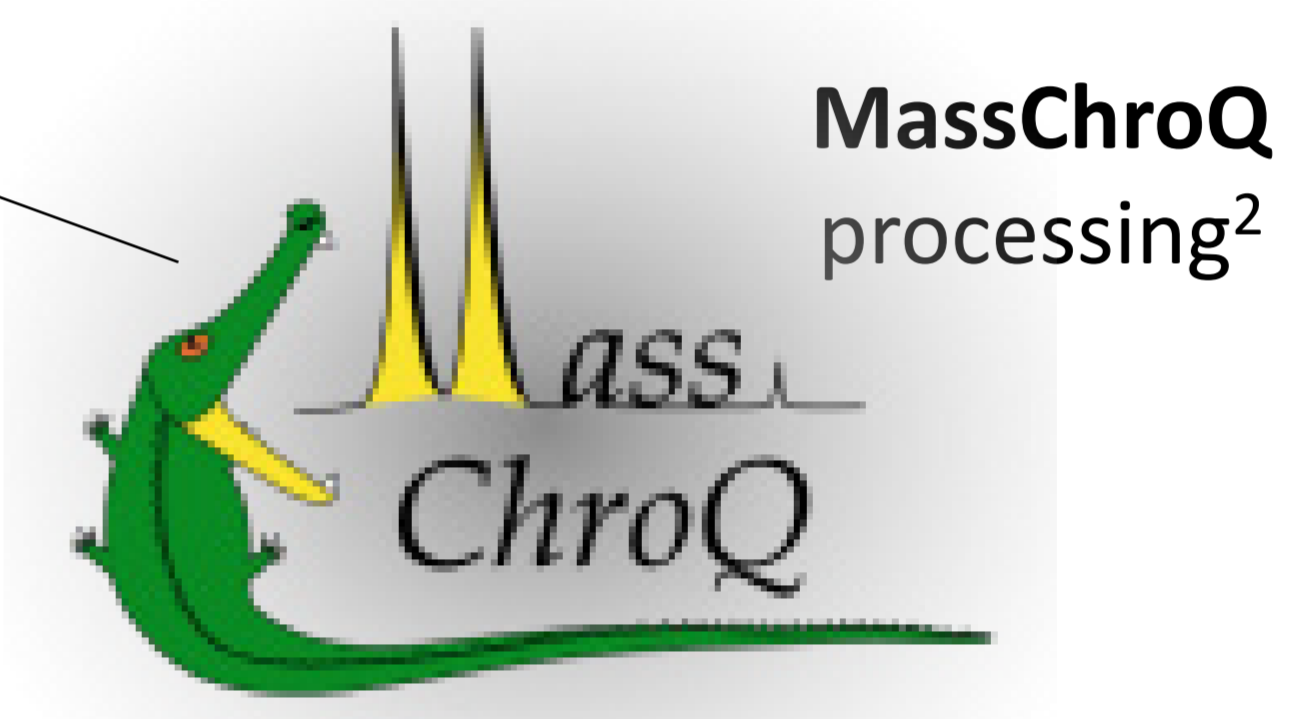


Raw files

MCQR
(R package³)

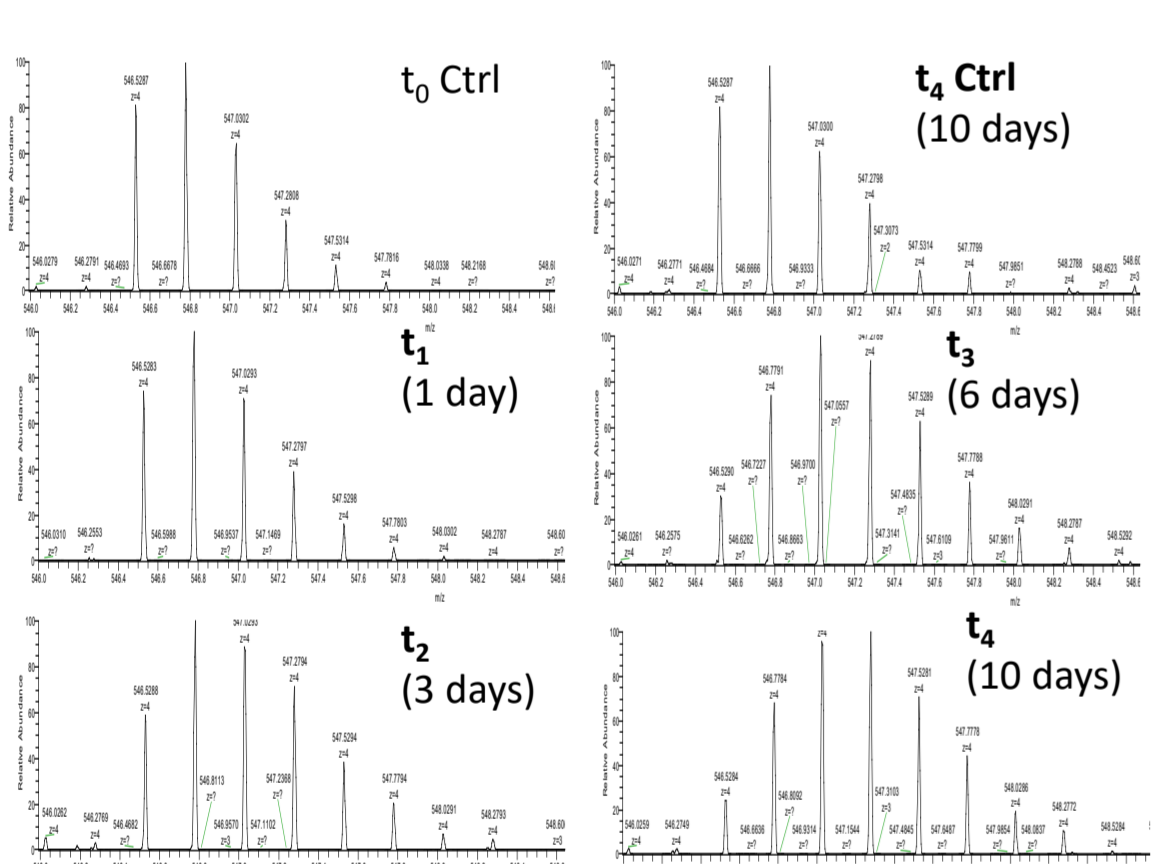
Differential protein quantification:

Determination of the Protein Fold Change (PFC)

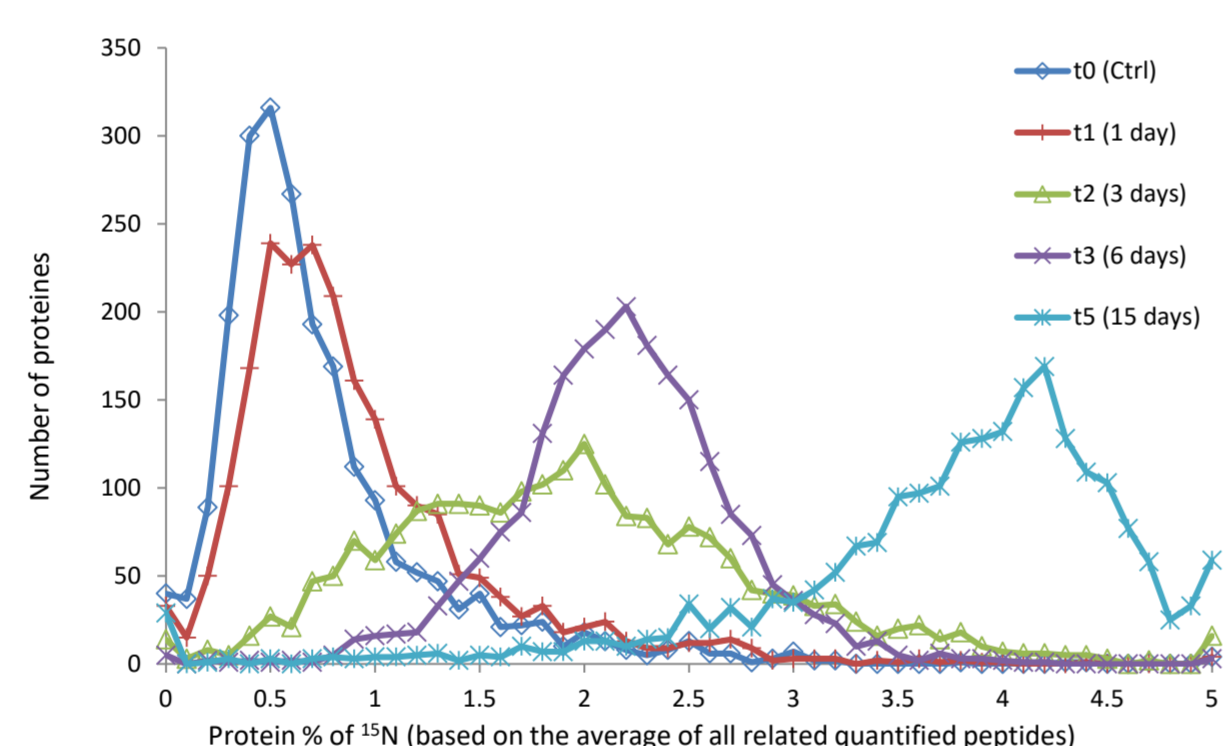


MassChroQ processing²

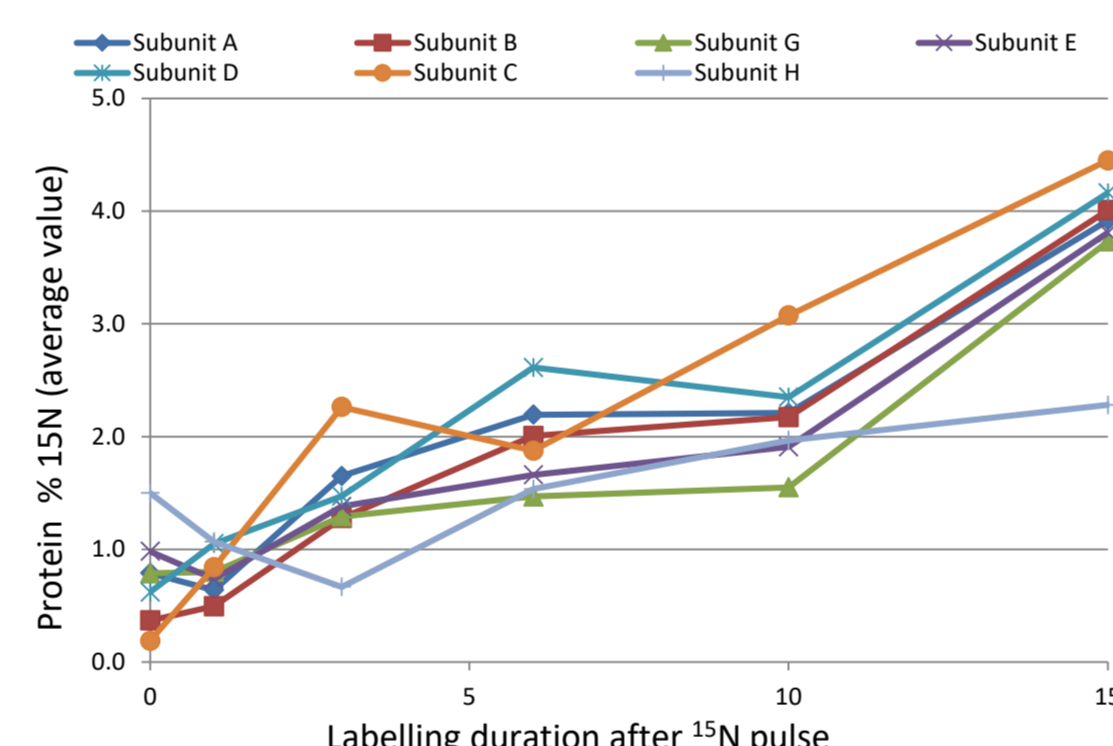
Work in progress and result



Peptide isotopic distribution time lapse at different labelling times



Experimental ¹⁵N ratio profiles for the characterised proteins at different labelling times



¹⁵N 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 calculation⁵⁻⁶
(based on PFC and LPF)

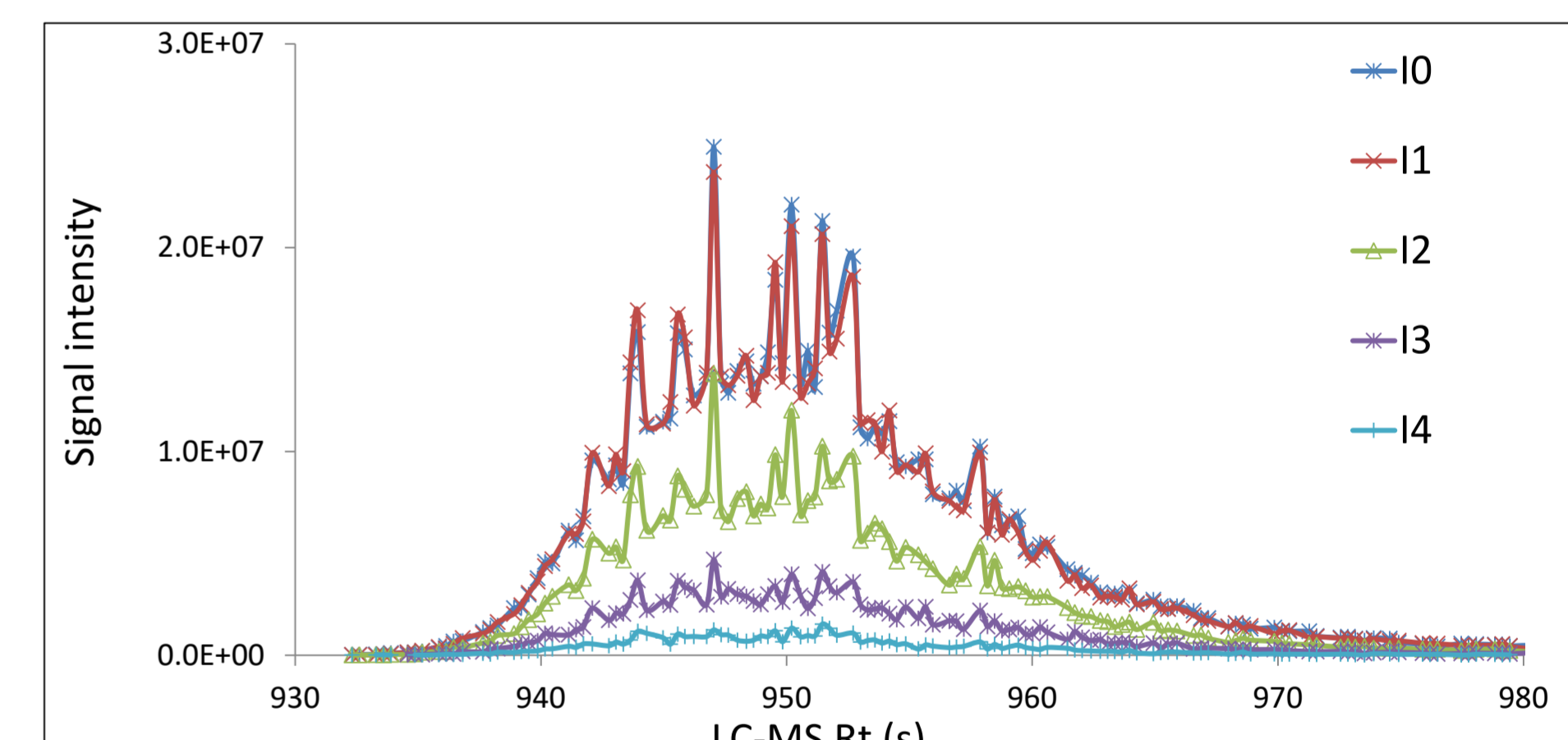
¹⁵N Labelled Protein Fraction (LPF) determination

Exports of the PeptIDs list

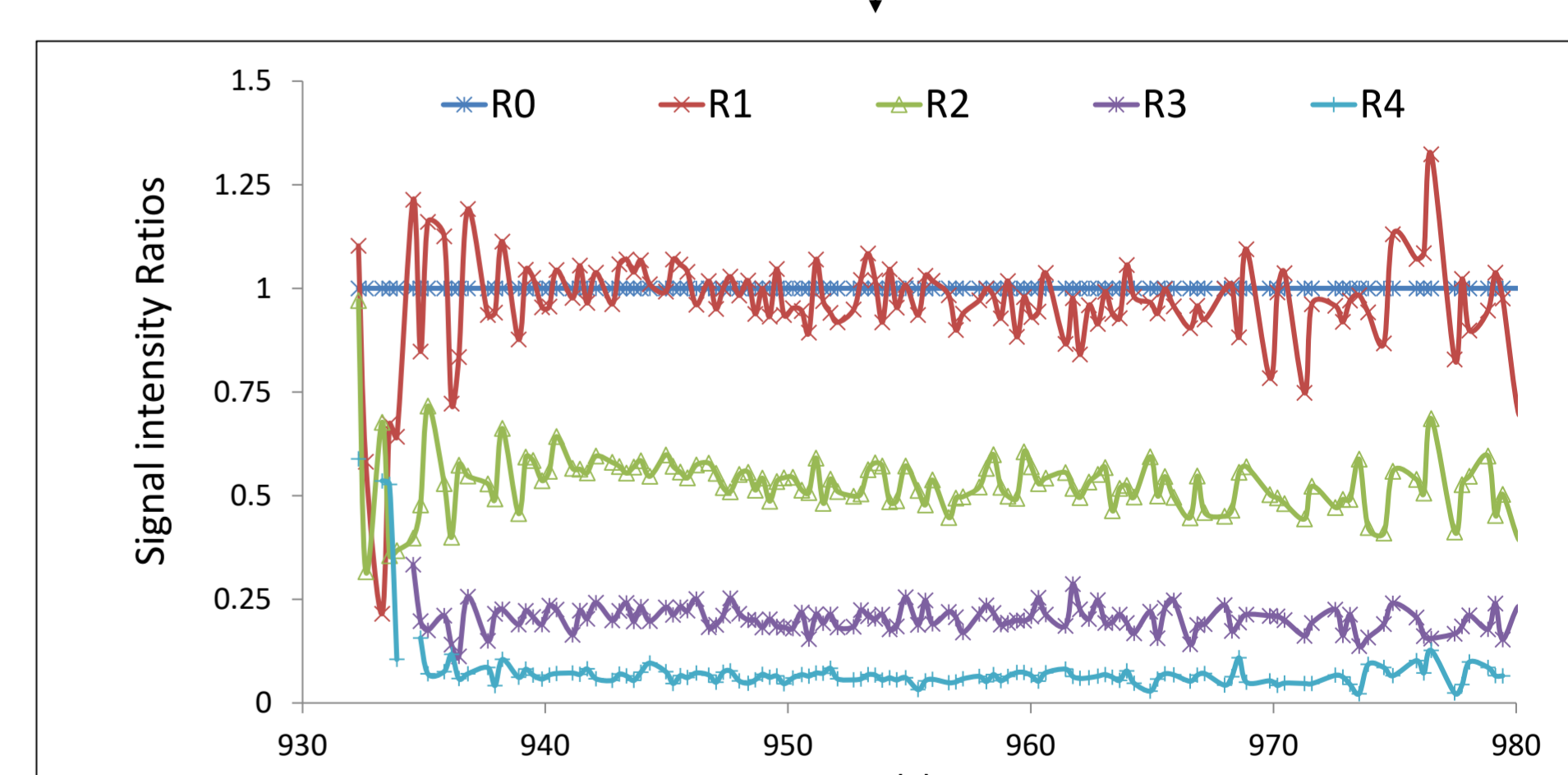
IsoSpec.py⁴

Theoretical isotopic distribution calculation (multiple ¹⁵N rate)

Peptides isotopic XIC profiles extraction

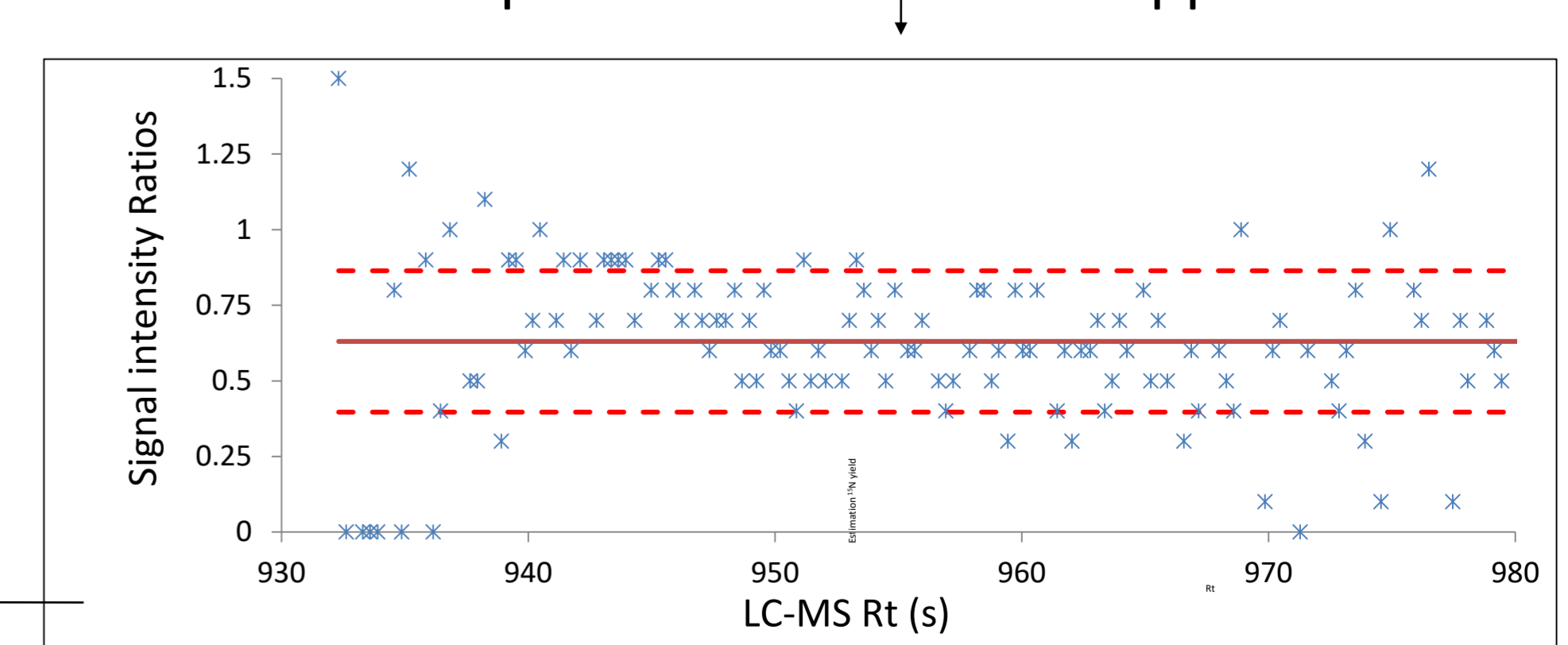


Calculation of the Experimental isotopic ratios



Estimation of the ¹⁵N incorporation

And outliers suppression



Estimation of the ¹⁵N 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 ¹⁵N 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
- ¹⁵N 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