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Sub optimal ¹⁵N metabolic labelling in plant: A new way to perform large scale protein turnovers determination using the peptides isotopic distribution

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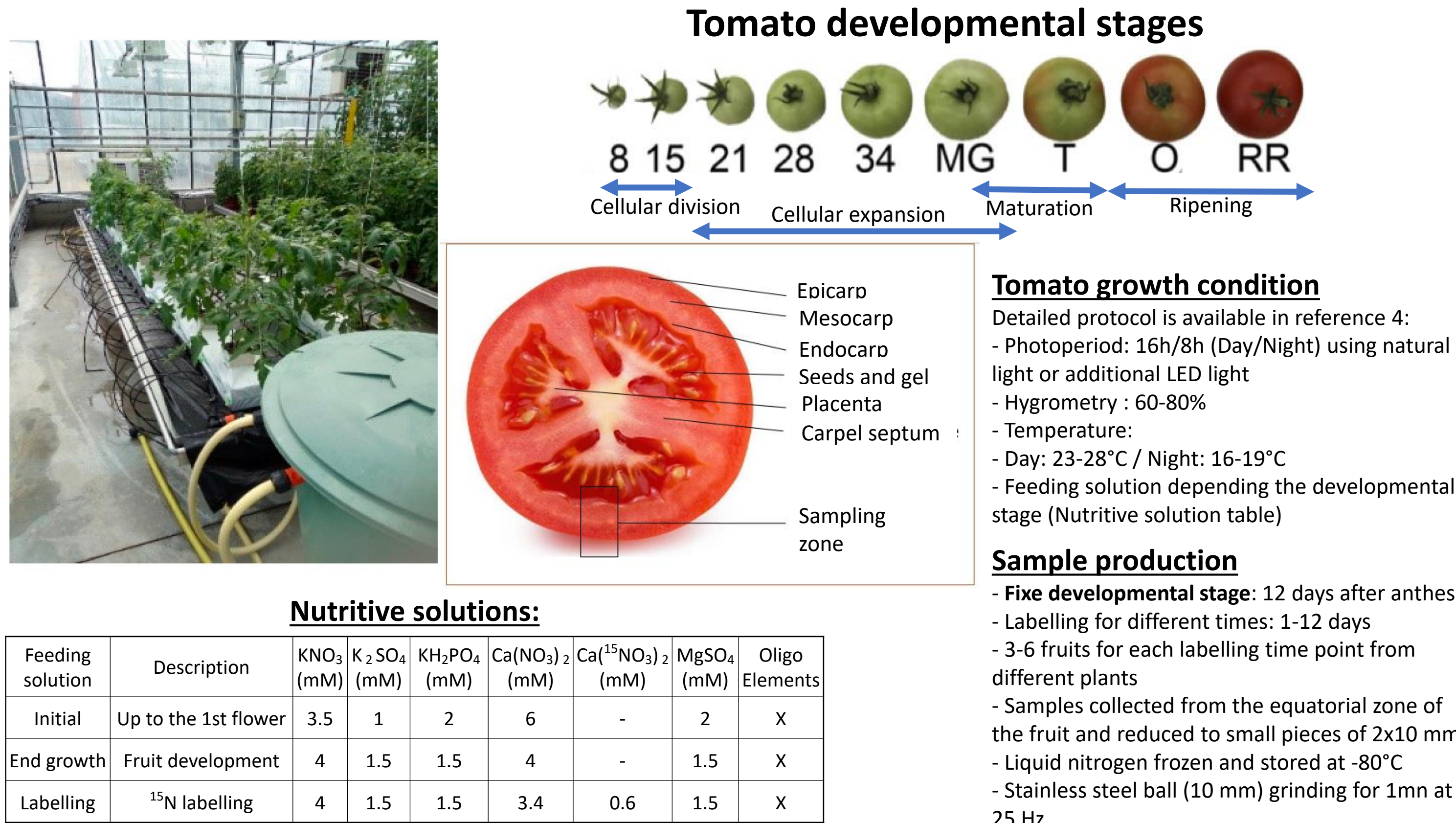
Abstract

Proteostasis is defined as the processes required maintaining the equilibrium between protein synthesis and degradation. Tight regulations from gene transcription to post-translational proteins modifications of these processes are required in cell cycle and survival to balance cellular development and environmental stresses. It has been shown repeatedly that the abundance of proteins only moderately correlates with that of transcripts. While the determination of protein turnover using pulsed SILAC metabolic labelling is popular for large scale proteomics investigations. Although used with small mammals (e.g. mice), such approach remains difficult with whole organisms and almost impossible with autotroph species like plants. Alternatively, it has been proposed to use inorganic sources of ¹⁵N isotopes that could be metabolically incorporated to de novo synthesized amino acids but full ¹⁵N protein labelling is almost impossible due to the high amino acid recycling rate in plant.

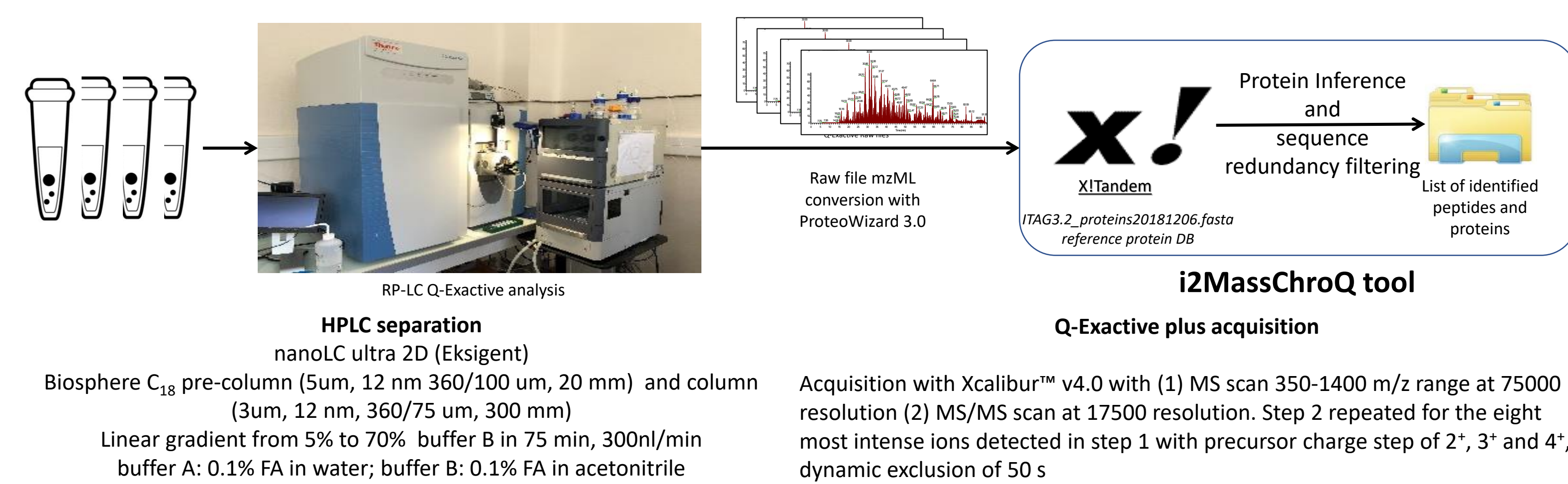
Instead of reaching complete ¹⁴N/¹⁵N exchange as previously done by Millar et al.^{1,2}, we decide to perform a ¹⁵N metabolic pulse at very low incorporation yield (around 5% ¹⁵N enrichment). This limited incorporation rate offers some advantages of which a limited increase of mass spectrometry signal complexity. Indeed, the ¹⁵N-labelled products moderately modify the peptide natural isotopic distribution and both labelled and unlabelled product appears as an overlapping isotopic distribution. Then, the recorded signal is extensively processed to filter out the outliers. Finally, the filtered signal is finally used to first perform label free protein quantitation which provide the "Protein Fold Change" (PFC) evolution over time. Second, the experimental peptide isotopic distribution is used to determine the apparent ¹⁵N labelling rate and the evolution of the ¹⁵N "Labelled Protein Fraction" (LPF) over the labelling time.

Thanks to our local bioinformatics developments including i2MassChroQ, MassChroQ and the MCQR package, we propose an approach of MS signal processing that make it possible to determine the fold change in protein (FCP) and the labelled protein fraction (LPF). Both values (FCP & LPF) are key elements and are required to estimate the turnover rate individually for each protein³ at the proteomic scale. Furthermore, this presentation highlights that accurate isotopic distribution measurement is possible and could be used from ordinary large-scale proteomics LC-MS/MS instruments to provide an interesting alternative to determine protein turnover determination at the proteome scale

Sample production, preparation and analysis



Sample analysis and data processing for peptide/protein identification



Protein degradation and synthesis constants calculation

$$K_D = -\frac{\ln FCP \cdot (1 - LPF)}{t}$$

FCP: Fold Change in Protein
→ variation of the protein abundance during the experiment
→ Processing based on the MCQR package

$$K_S = \frac{FCP - e^{-K_D \cdot t}}{1 - e^{-K_D \cdot t}} \cdot K_D$$

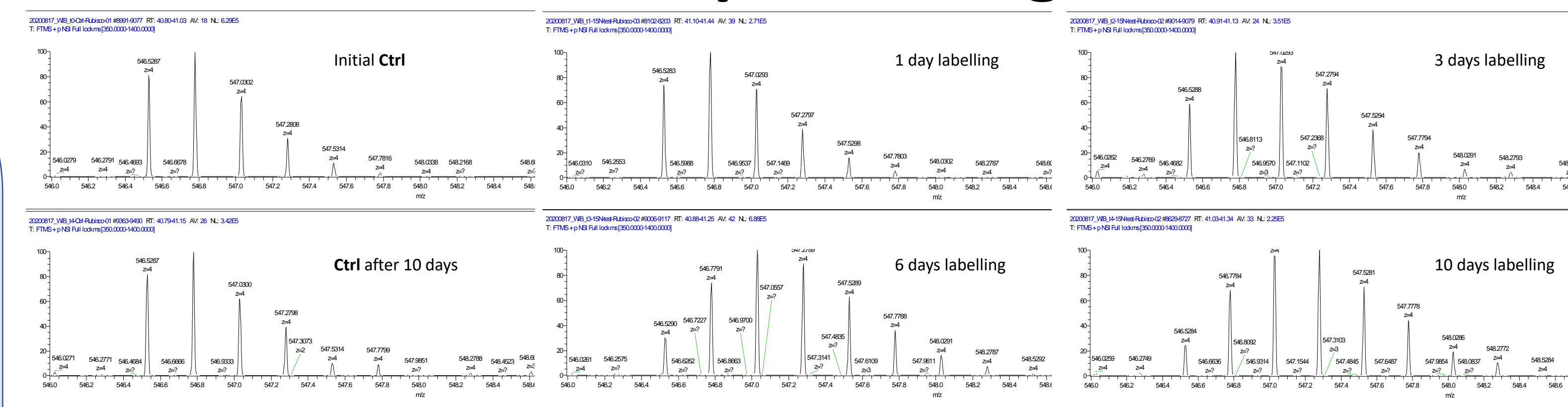
LPF: Labelled Protein Fraction
→ Fraction of the ¹⁵N labelled peptide/protein out of all material
→ Processing based on the MassChroQ and dedicated R scripts

Since all fruits are collected at 12 days (at the same developmental stage), we do not expect any difference in protein amount. In such condition, which is equivalent to a « stationary phase » the PFC must be identical whatever the labelling time and then, K_d = K_s.

Acknowledgments

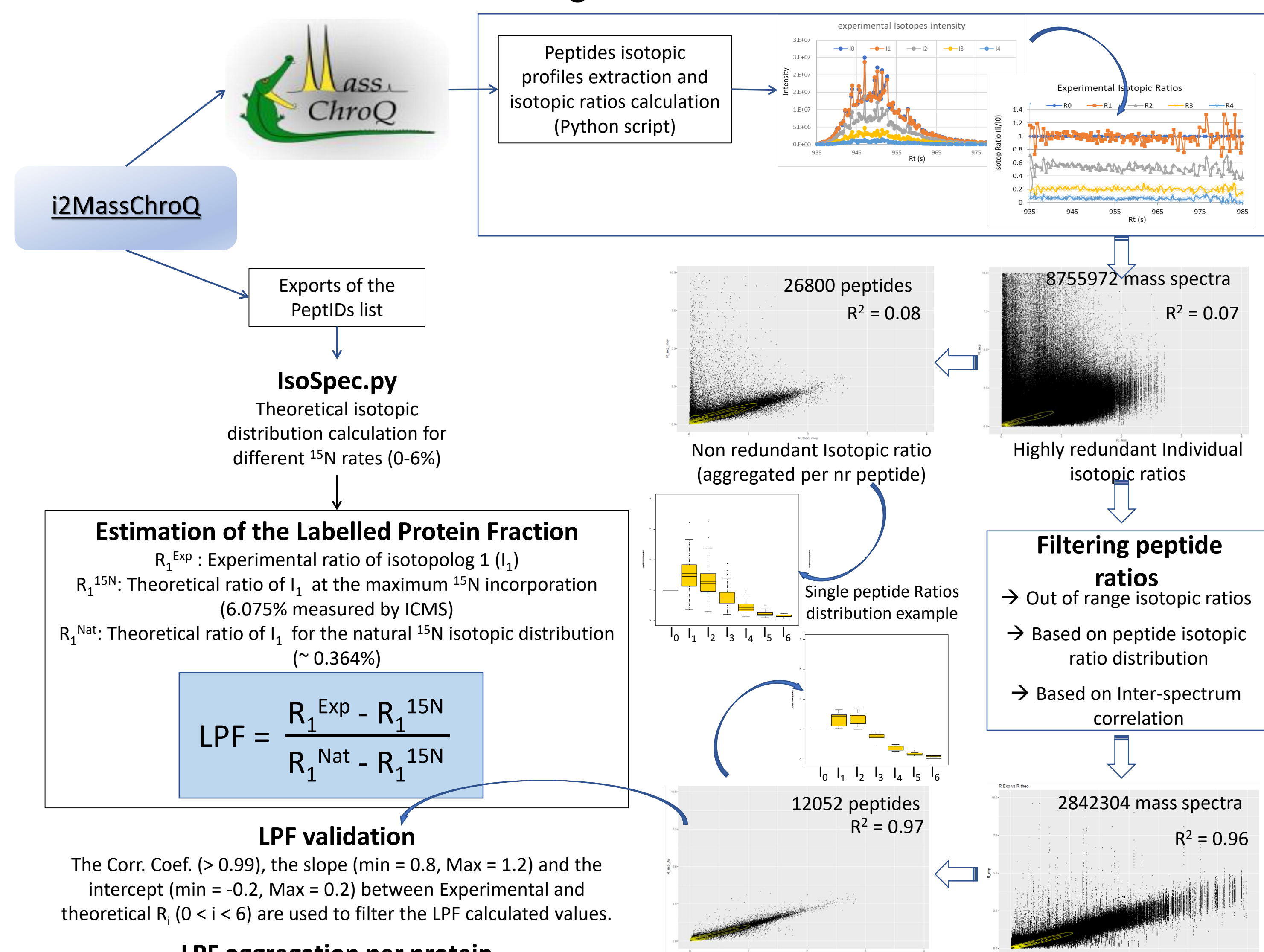
Many thanks to Sarah Naudin, Marie-Hélène Andrieu, Sophie Colombié from the META team at the BFP for the production of the biological samples, Anne Marmagne, Cléline Masclaux-Daubresse from the SATURN team at the IJBP for the ICMS ¹⁵N measurement and their contribution to the development of this project. The authors and the META team thank the BAP department of the INRAE for the PROOFER grant related to this project.

Data processing

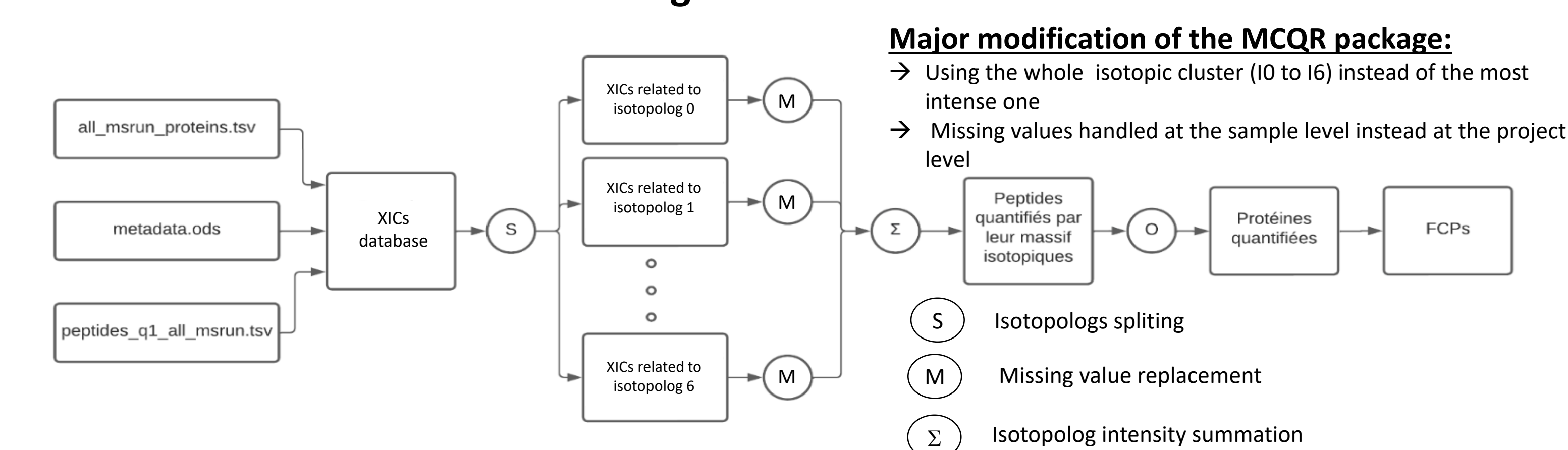


Example of the evolution of the isotopic pattern during the ¹⁵N incorporation (low incorporation rate with 4.5% ¹⁵N enrichment) for the peptide AGVKEYKLYTPEYETK

Processing for LPF determination

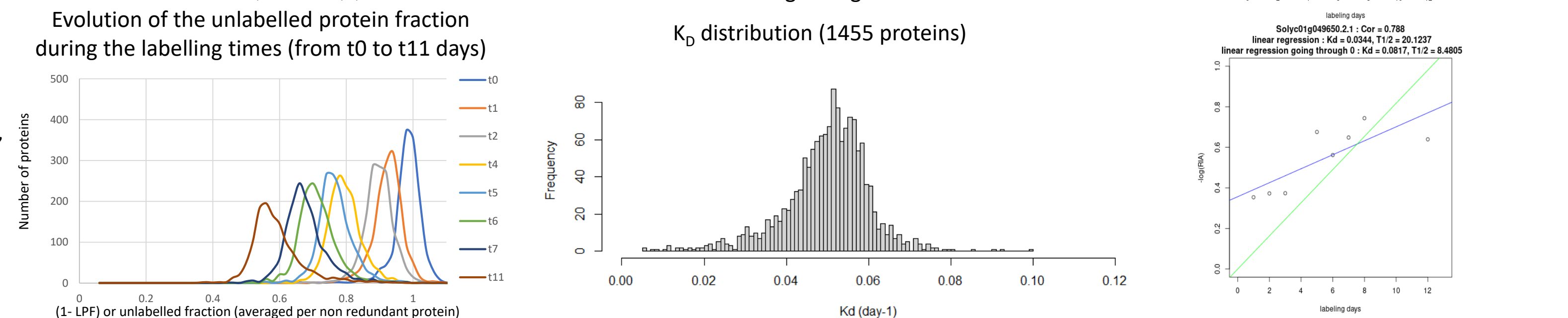
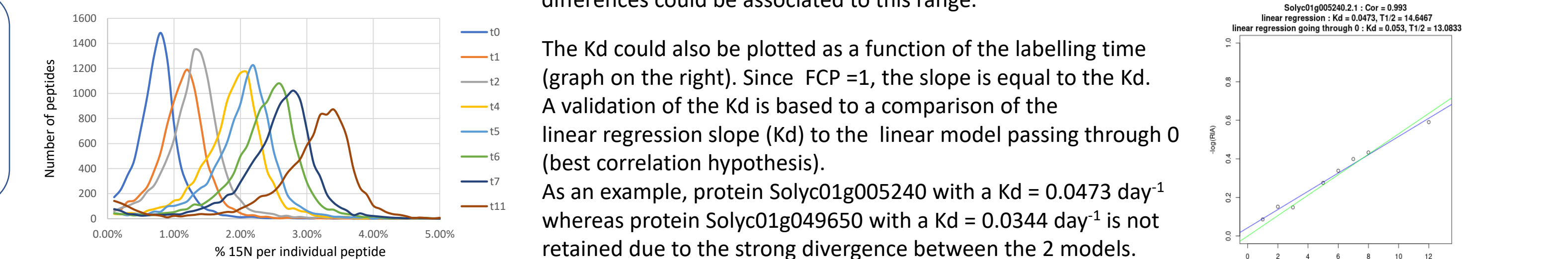


Processing for PFC determination



Results

K_d are calculated using the equation proposed by Li et al. (3) for the proteins with a FCP range in between]0.449, 0.449] across the labelling process. For the calculation, K_d is defined as 1 since no significant differences could be associated to this range.



Conclusions

- Low ¹⁵N labelling (<6%) is compatible with standard LC-MS acquisition with low loss of efficiency for peptides/proteins characterisation.
- Variations of the isotopic pattern provide relevant information for differential proteins quantitation
- Kinetic constants for proteins synthesis and degradation could be determined with such approach
- Collected data could be used directly for both LPF and FCP determination
- Some improvement are required especially for data acquisition to improve:
 - Signal stability and reproducibility
 - Peptide separation (HPLC and/or ionic mobility)
- To develop some scoring scheme able to:
 - Strengthen the robustness of the LPF
 - Validate/invalidate the final K_d/K_s calculation

References

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