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Large scale plant protein turnover measurement

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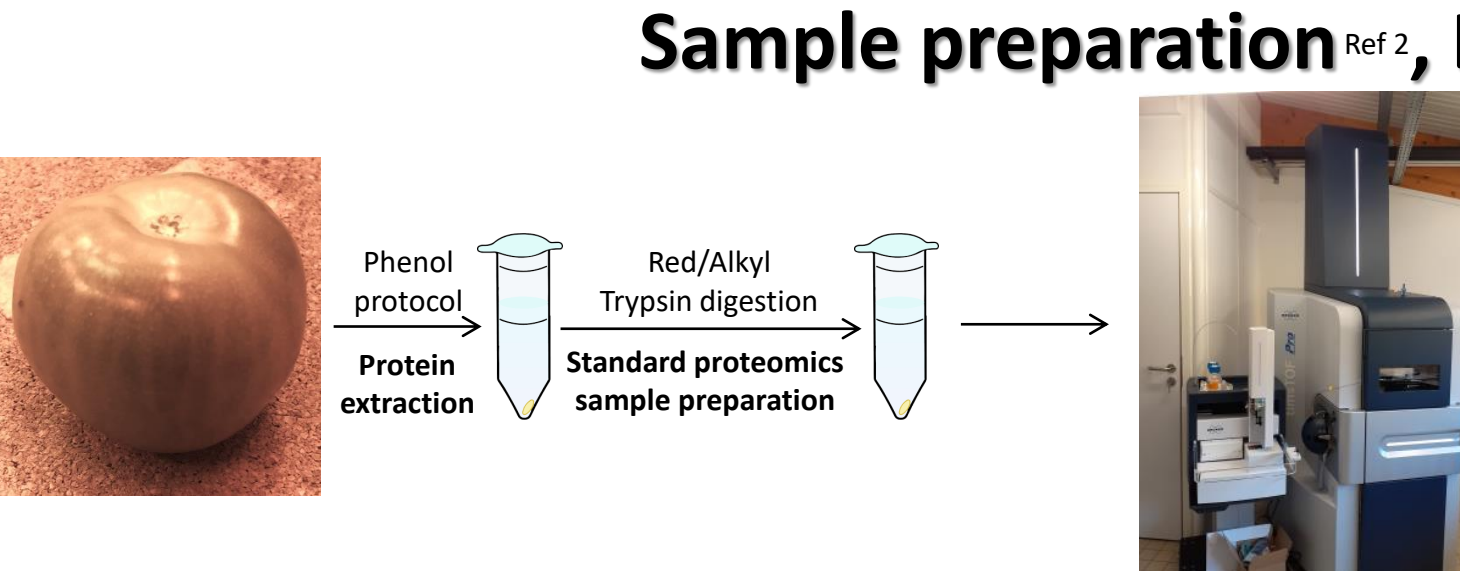
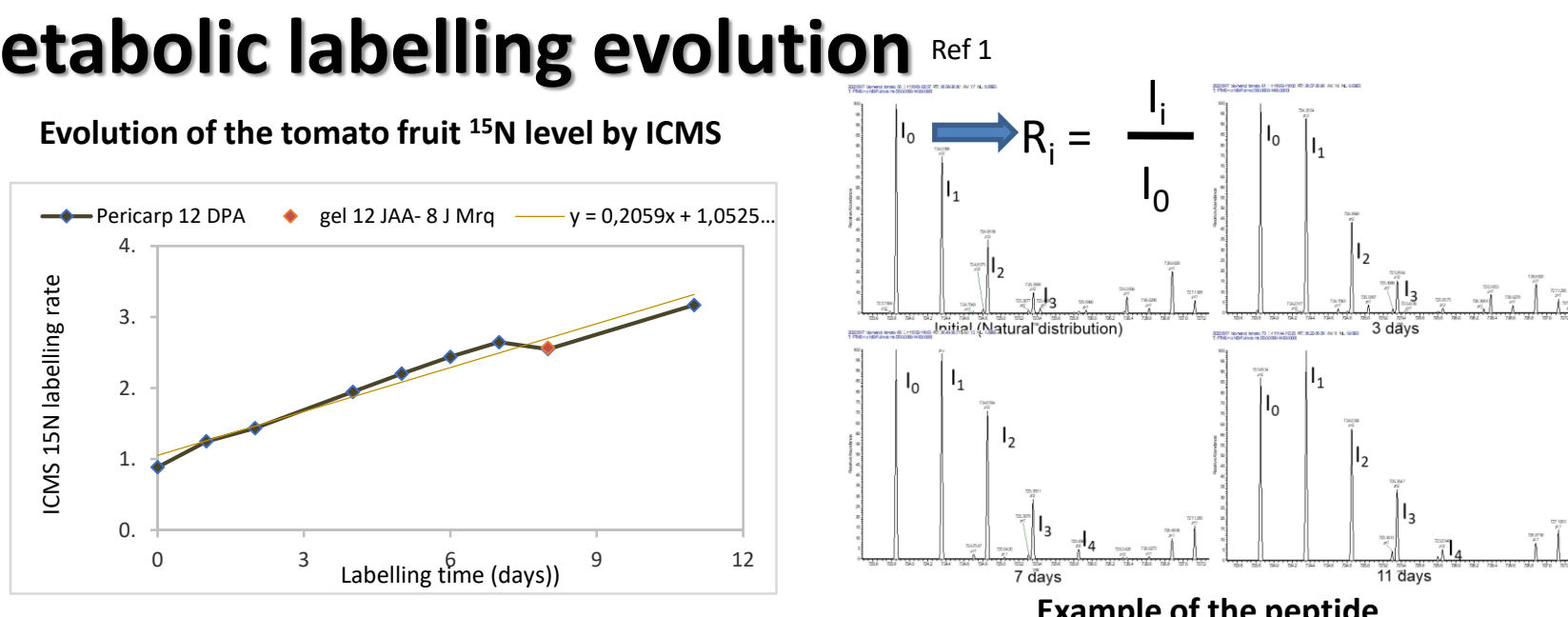
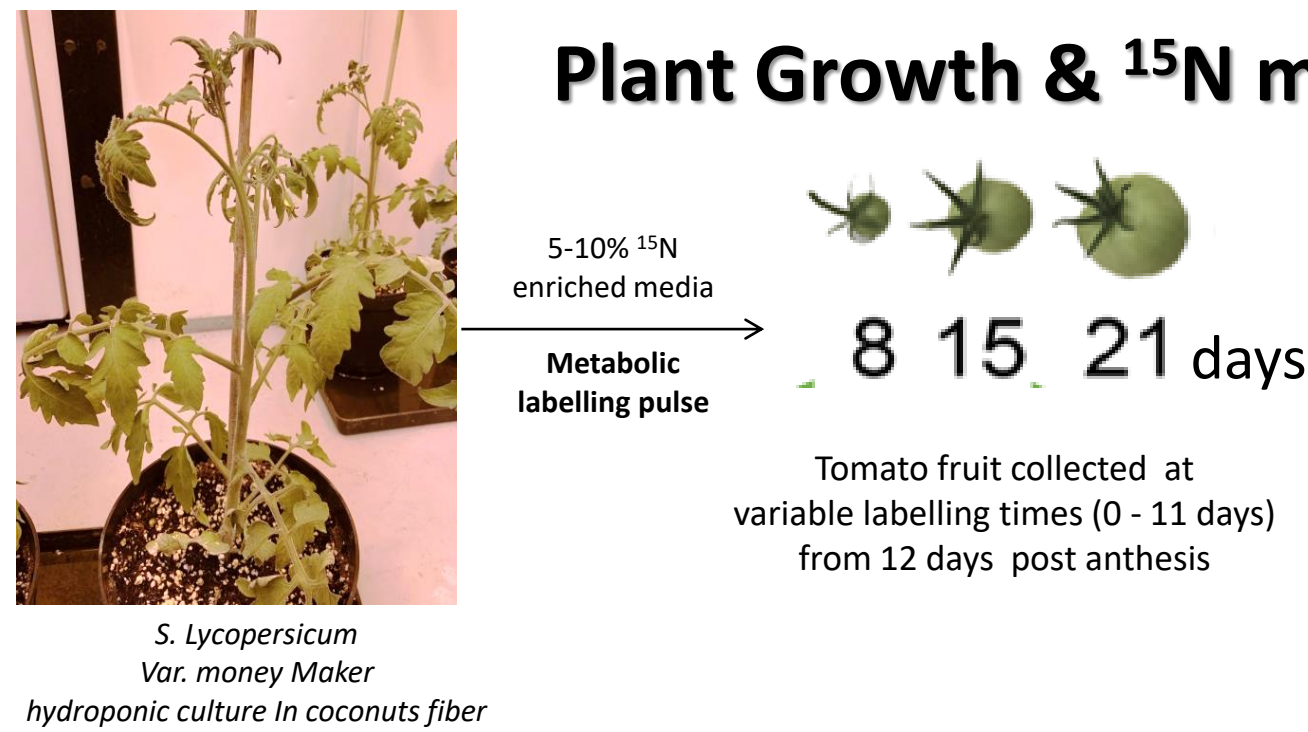
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ABSTRACT

Proteostasis is a key element among the molecular mechanisms required to maintain the equilibrium between protein synthesis and degradation during organism's development. The protein turnover rate (PTR) is the parameter to survey to better understand proteostasis mechanisms which are associated to protein synthesis (K_s) and degradation (K_d) constants. Presently, PTR measurement based on pulse SILAC labelling are not compatible with autotroph species. Then, we developed a novel large scale proteomics strategy based on suboptimal ¹⁵N metabolic labelling to calculate K_s and K_d constants in plants. Our approach essentially requires to determine the "protein fold change" (FCP) and the "protein labelled fraction" (LPF) at several labelling kinetics time points. These two values are calculated from mass spectrometry data after adapting MassChroQ, a peptide quantification software and MCQR, an R package dedicated to the statistical analysis of proteomics quantification. First, our developments take into account the peptide ¹⁵N-modified isotopic distribution to perform robust label-free quantitation to determine the FCP for each characterized peptide/protein. Second, we used the variation of the peptide isotopic distribution to estimate the LPF and its evolution during the ¹⁵N kinetic assay. Finally, we filtered the calculated K_d constants using statistical approaches. We tested this processing pipeline in a project dedicated to study tomato fruit growth and maturation. To this end, we first setup a methodology for tomato fruit ¹⁵N labelling. Then we prepared and analyzed the collected samples at different time points after the ¹⁵N starting pulse. Finally, we used the raw mass spectrometry data to test our processing pipeline. This initial application provides an excellent opportunity to improve our processing pipeline and the results, successes and bottlenecks will be detailed more extensively during this presentation.



Sample preparation, LC-MS/MS and analysis

K_D/K_S calculation & Equations

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

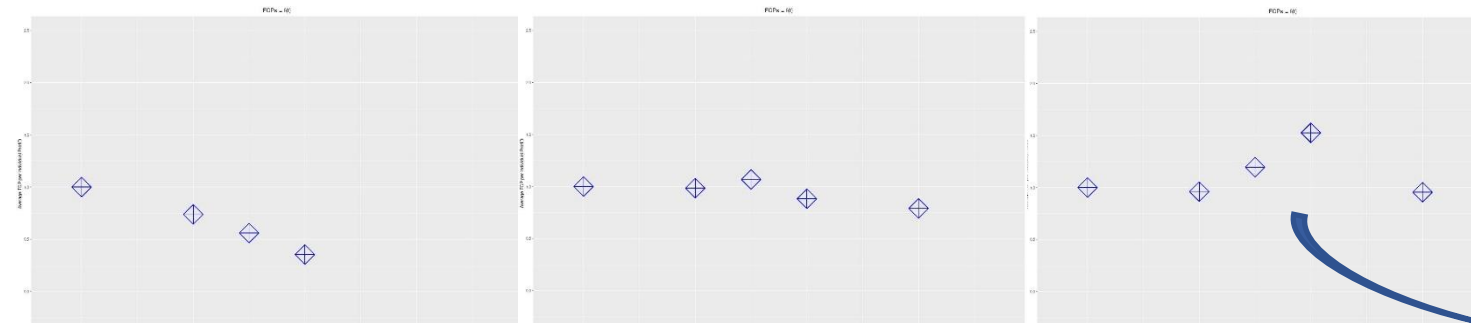
$$K_S / A = \frac{\text{FCP} - e^{-K_D * t}}{1 - e^{-K_D * t}} * K_D$$

K_S / A : Relative Synthesis constant
 K_D : Degradation constant
 LPF: Labelled Protein Fraction
 FCP: Fold Change in Protein)
 t: duration in days

LPF calculation from peptide isotopic distribution

$$\text{LPF} = \frac{R_1^{\text{Exp}} - R_1^{\text{Nat}}}{R_1^{15\text{N}} - R_1^{\text{Nat}}}$$

R_1^{Exp} : Experimental ratio R_1/R_0
 R_1^{Nat} : Theoretical R_1 for natural isotopic abundance
 $R_1^{15\text{N}}$: Theoretical R_1 for ¹⁵N max incorporation (medium ¹⁵N yield)



Fold Change in Protein

FCP determined with the 15N-MCQR package (Adapted version of the MCQR package using multiple isotopes)

- Quantitation done using all isotopes (up to 6, not less than 4)
- Normalized (median) on the sum of the isotopic intensities
- Selected peptides must be identified in all samples
- Min. 2 peptides per protein (shared peptides are filtered out)
- XIC cross correlation > 0.5

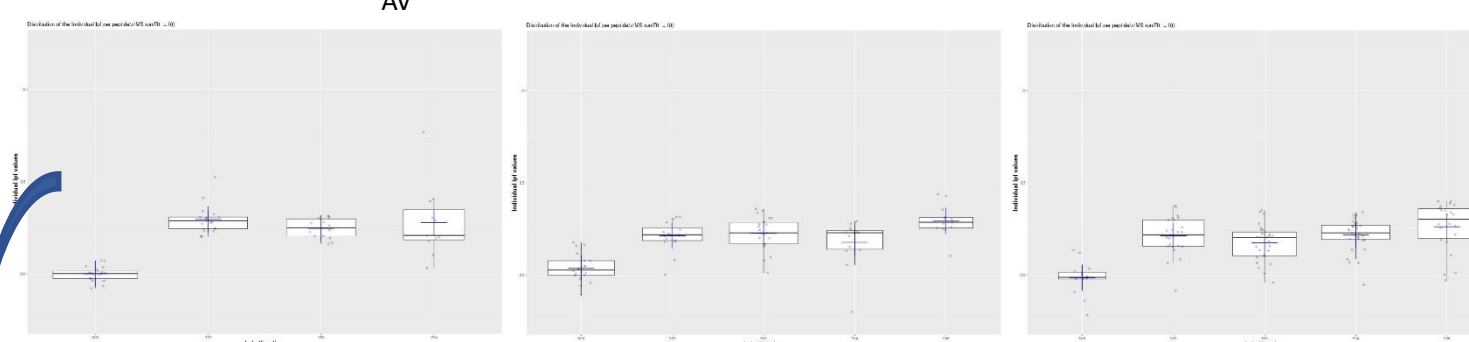
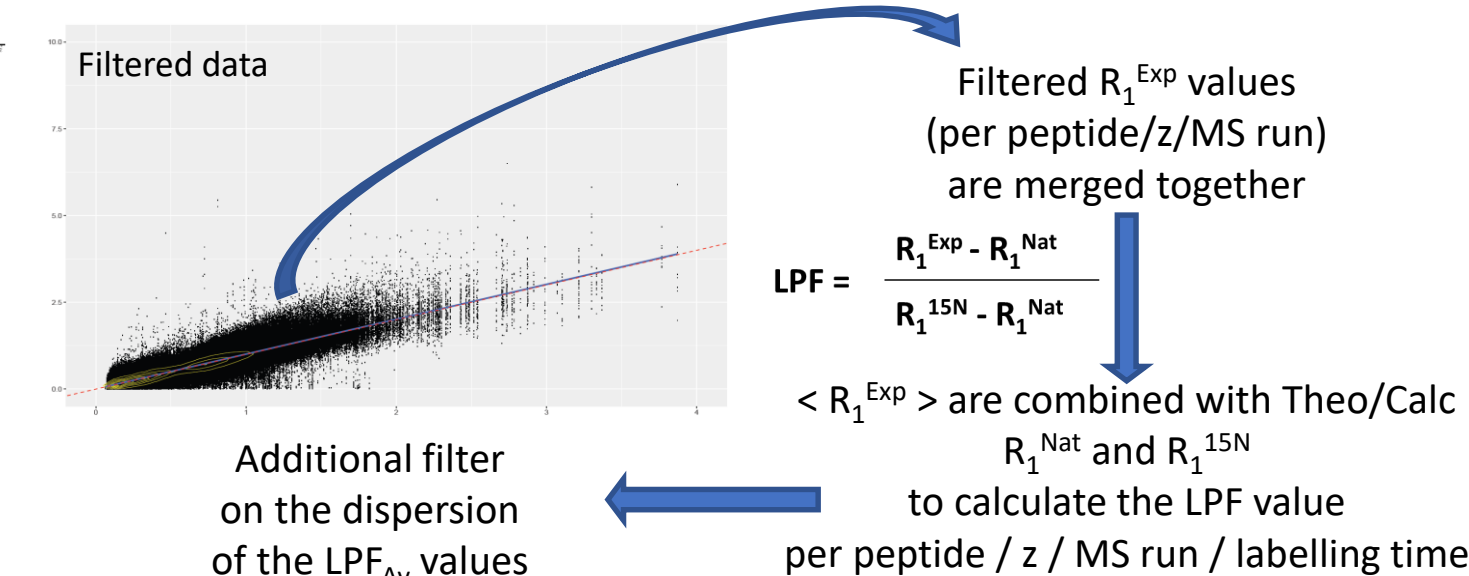
References
 Ref 1: Andrieux *et al.* NOV'AE, 2022; 5: 1-11
 Ref 2: Faurobert *et al.* Meth. Mol. Biol. 2007; 355: 9-14
 Ref 3: Nelson *et al.* J. Proteome Res, 2013; 12-7: 3449-59
 Ref 4: Sénécaut N. *et al.* J Proteome Res. 2021; 20(3): 1476-1487
 Ref 5: <https://forgemia.inra.fr/pappso/mcqr>
 Ref 6: Valot *et al.* Proteomics, 2011; 11(17): 3572-7
 Ref 7: Lacki *et al.* Anal. Chem. 2020; 92(14): 9472-5

Labelled Protein Fraction

Isotopic profile extraction based on MassChroQ export files

- All characterized "peptide / z / isotope / MS run" XICs are exported using MassChroQ tool
- Collected data are filtered using an R script taking care of:
- Number of isotopes: usually $I_0 - I_3$ (I_0 & I_1 required for LPF calc.)
 - "Peptide / z / MS run" LC-Rt window
 - Minimum intensity threshold
 - Isotopic distribution cross-correlation along the peptide Rt window
 - Data redundancy:
 - * Technical replicates
 - * Number of isotopic profiles / replicate
 - * Number of isotopes / isotopic profile
 - * Number of peptides / protein

Data processing to determine LPF per individual protein



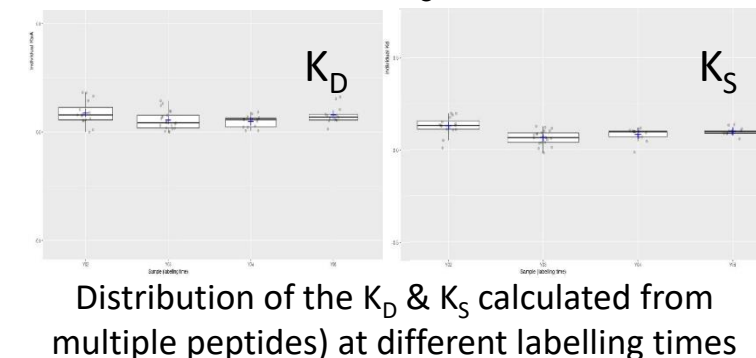
Examples of protein LPF's distributions associated to three MCQR quantified proteins at the different labelling times

Protein K_D Calculation LPF / PFC = f(t)

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

$$K_S / A = \frac{\text{FCP} - e^{-K_D * t}}{1 - e^{-K_D * t}} * K_D$$

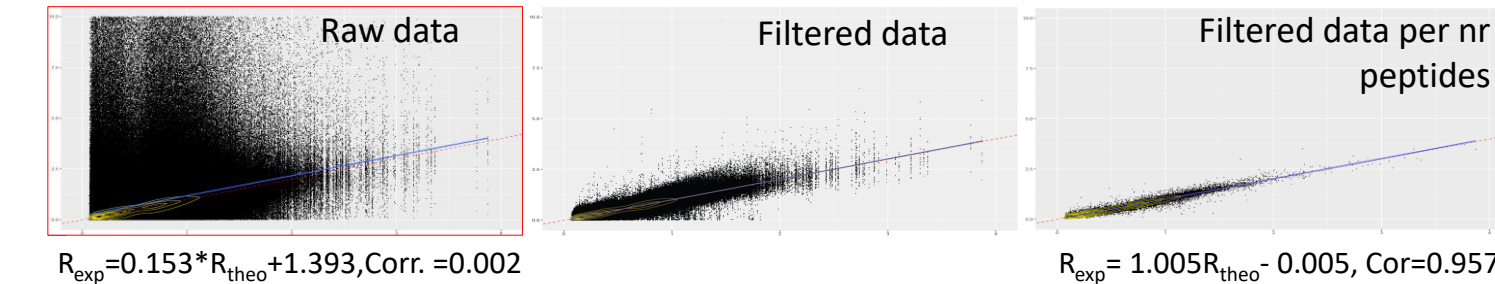
Protein K_d & K_s distributions



Direct access to the poster

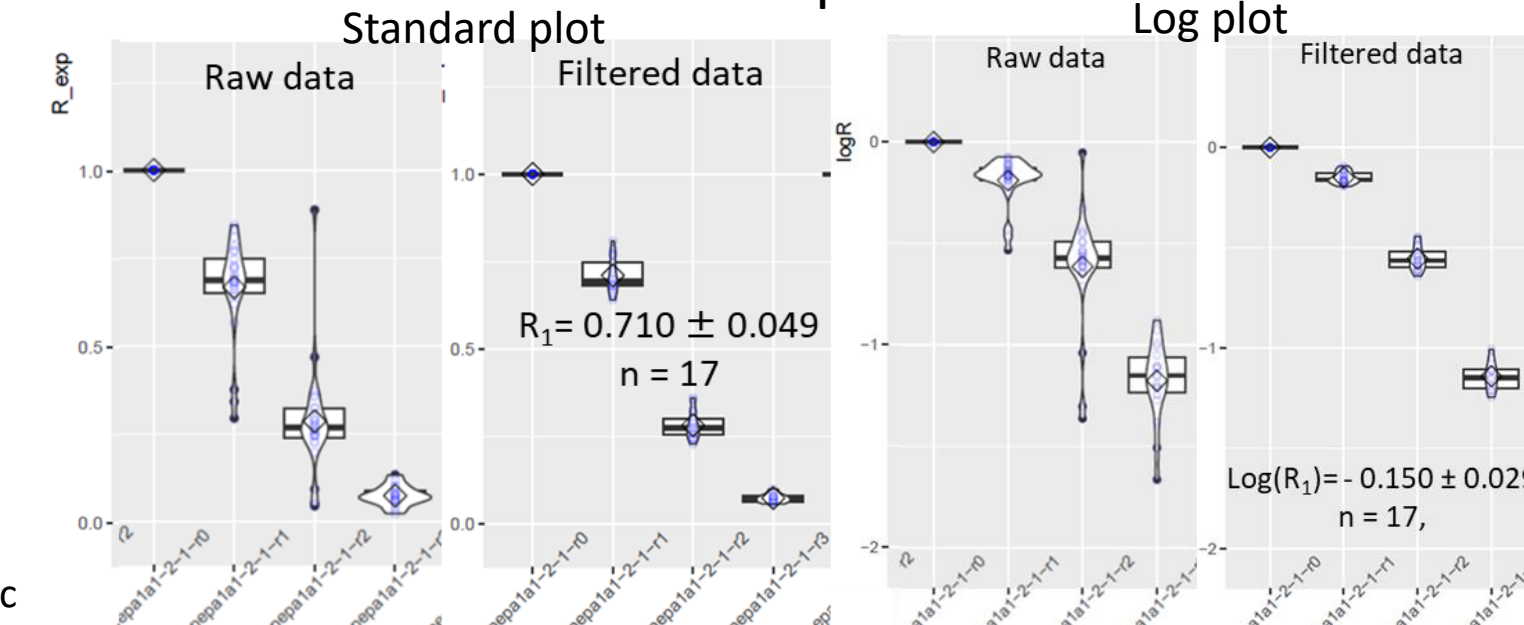
Theoretical Isotopic distribution calculation

Calculation of the peptide isotopic distribution are performed using the isospec library and used for data visualization (R_1^{Nat}) and $R_1^{15\text{N}}$



Plot of the Exp. R_1 vs Theo. R_1 (Natural isotopic distribution): A) raw data (8,872,811 R_1 associated to 163,037 peptides profiles, 60,593 nr pepIDs); B) Filtered data; C) Unique nr. peptides (filtered data)

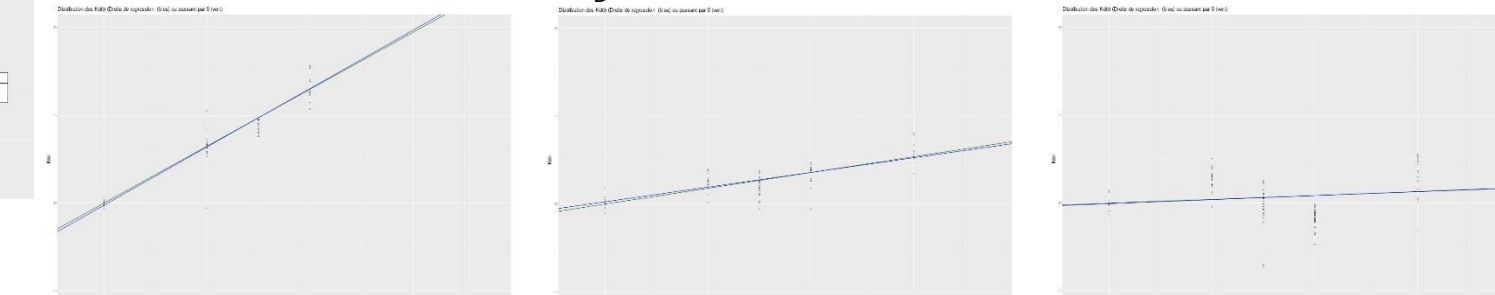
Examples of the filters effects on redundant R_1^{Exp} distribution



Applied filters are efficient in outliers suppression and R_1^{Exp} centroiding of the collected experimental values

K_D validation tests

K_D could be estimated from the labelling kinetics measurement as $y = K_D(t)$ with no (or minor) intercept



Protein K_D 's as a function of the labelling times; Red line is the linear regression with no intercept, blue line is the conventional linear regression (with intercept)

Conclusions

Proof of concept:

Determination of the K_D & K_S using plant metabolic ¹⁵N labelling & measurement of peptide isotopic distribution

Drawbacks:

High data processing, filtering and validation
 Some issues with isotopic measurement reproducibility with usual proteomics dedicated instruments