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## Large scale plant protein turnover measurement

Willy Vincent Bienvenut, Marie-Hélène Andrieu, Olivier Langella, Thierry Balliau, Sophie Colombie, Melisande Blein-Nicolas

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Willy Bienvenu<sup>1</sup>, Marie Helene Andrieu<sup>2</sup>, Olivier Langella<sup>1</sup>, Thierry Bailliau<sup>1</sup>, Sophie Colombie<sup>2</sup>, Mélisande Blein-Nicolas<sup>1</sup>

1) Université Paris-Saclay, CNRS, INRAE, AgroParisTech, GQE - Le Moulon, PAPPSO, 91190 Gif-sur-Yvette, France; 2) Université de Bordeaux, INRAE, Biologie du Fruit et Pathologie, Bordeaux Metabolome, MetaboHUB, PHENOME-EMPHASIS, 71 av. E. Bourlaux, 33140 Villenave d'Ornon, France.

Email corresponding author: willy.bienvenu@universite-paris-saclay.fr

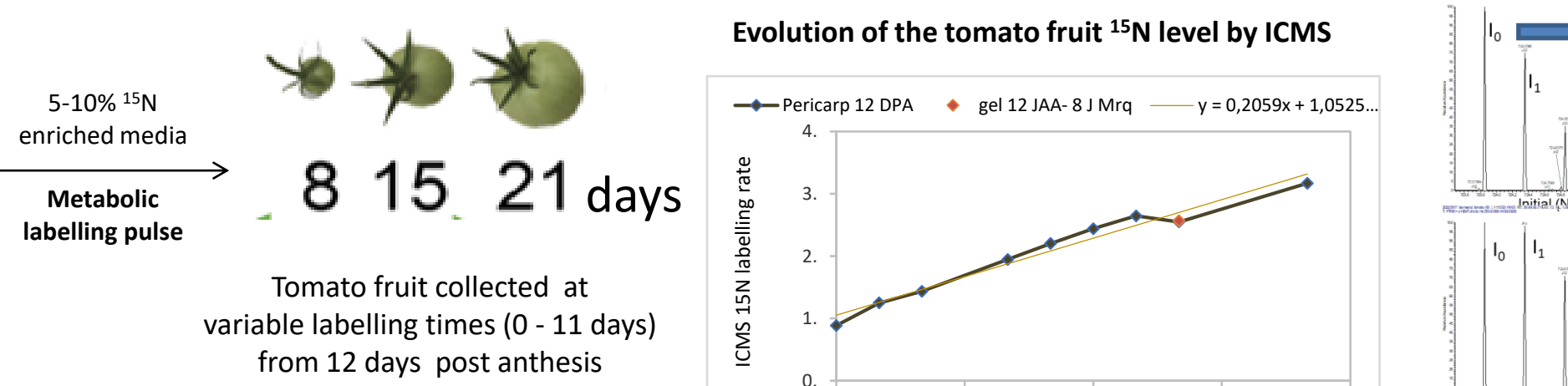
## ABSTRACT

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 <sup>15</sup>N 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 <sup>15</sup>N-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 <sup>15</sup>N 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 <sup>15</sup>N labelling. Then we prepared and analyzed the collected samples at different time points after the <sup>15</sup>N 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.



*S. Lycopersicon*  
 Var. money Maker  
 hydroponic culture in coconuts fiber

## Plant Growth & <sup>15</sup>N metabolic labelling evolution Ref 1



## Sample preparation Ref 2, LC-MS/MS and analysis



## $K_D/K_S$ calculation & Equations Ref 3

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

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

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

## Fold Change in Protein

FCP determined with the 15N-MCQ R package  
 (Adapted version of the MCQ R package Ref 5 using multiple isotopes)

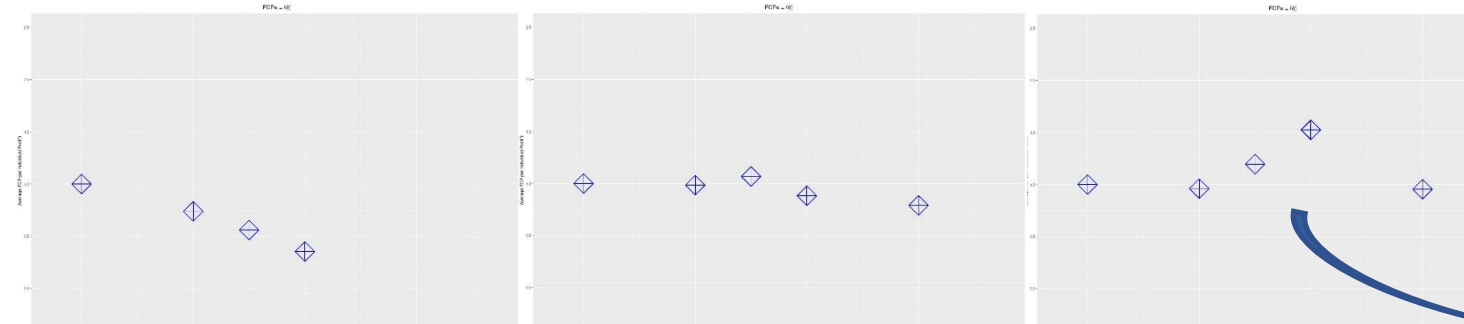
- Quantitation done using all isotopes (up to 6, not less than 4)
- Normalized (median) on the sum of the isotopic intensities
- Selected peptides must be identified in all samples
- Min. 2 peptides per protein (shared peptides are filtered out)
- XIC cross correlation > 0.5

## LPF calculation from peptide isotopic distribution Ref 4

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

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

Examples of single protein FCP evolution determined by 15N-MCQ R package



## 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

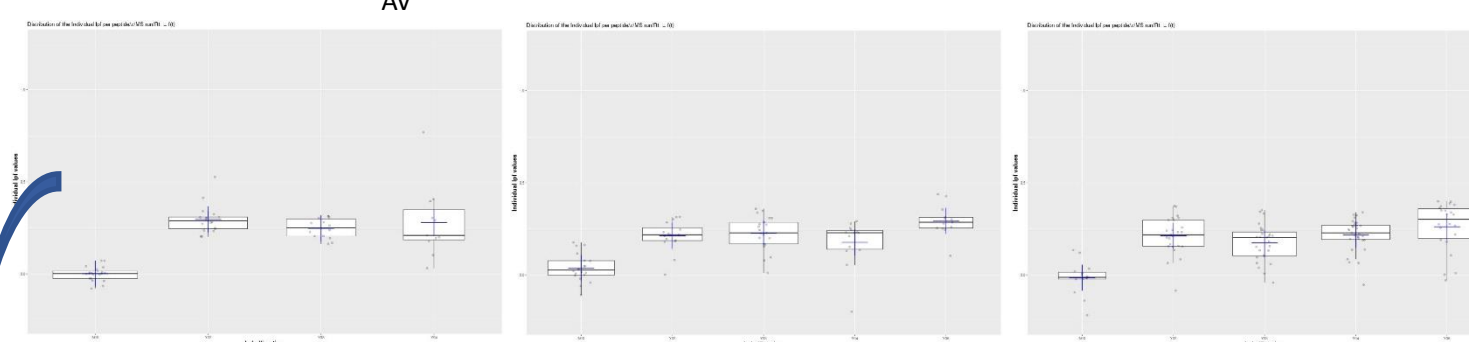
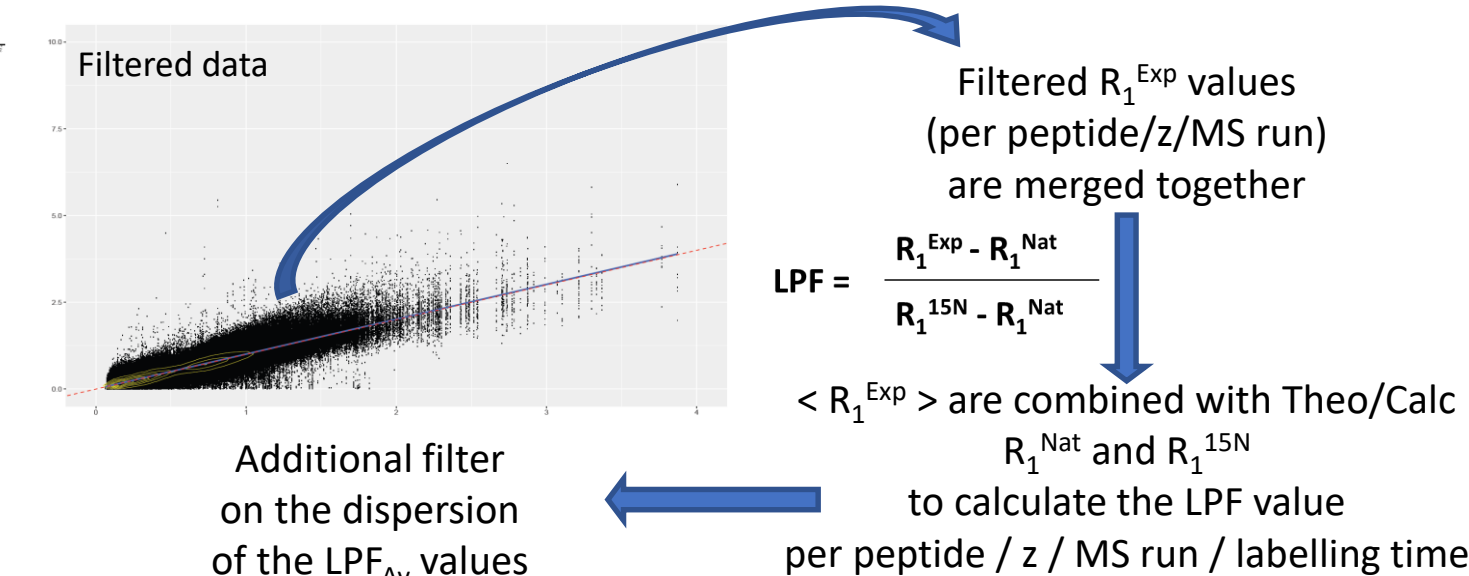
## Labelled Protein Fraction

### Isotopic profile extraction based on MassChroQ Ref 6 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



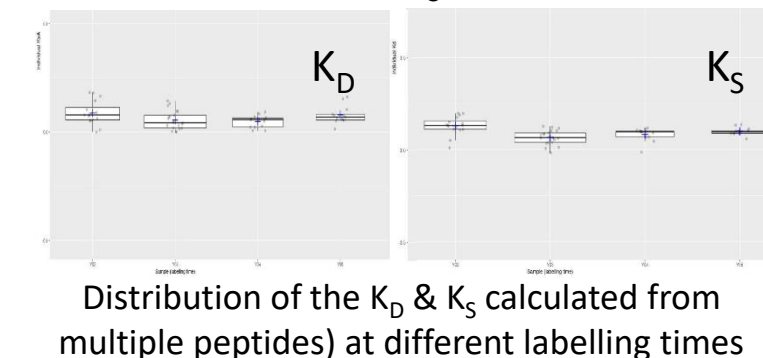
Examples of protein LPF's distributions associated to three MCQR quantified proteins at the different labelling times

### Protein $K_D$ Calculation LPF / PFC = f(t)

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

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

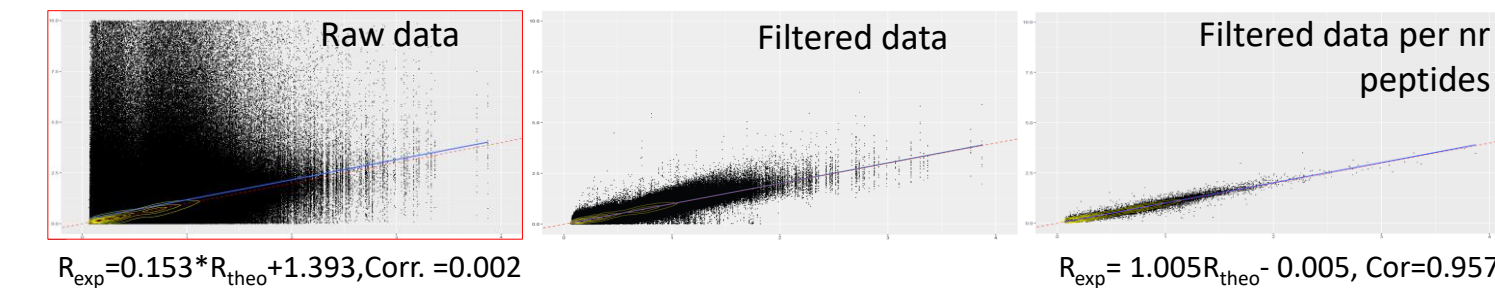
### Protein $K_d$ & $K_s$ distributions



Direct access to the poster

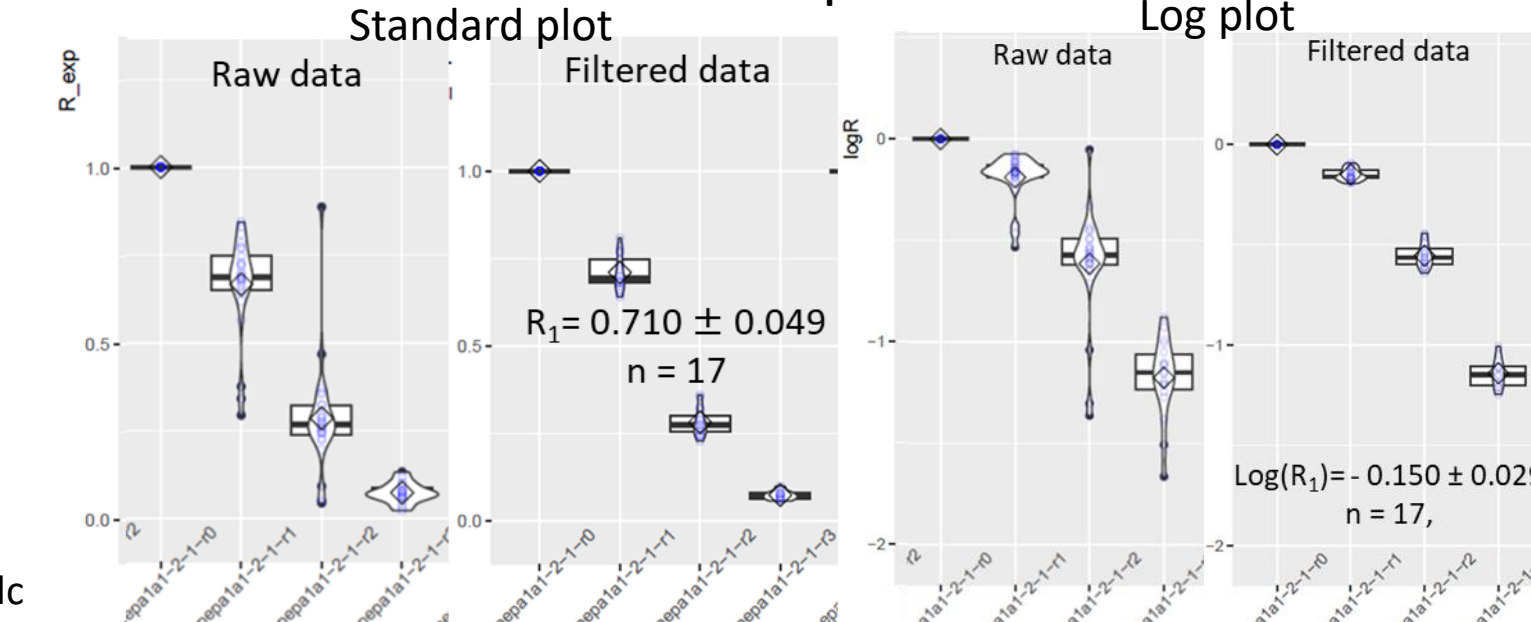
## Theoretical Isotopic distribution calculation Ref 7

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

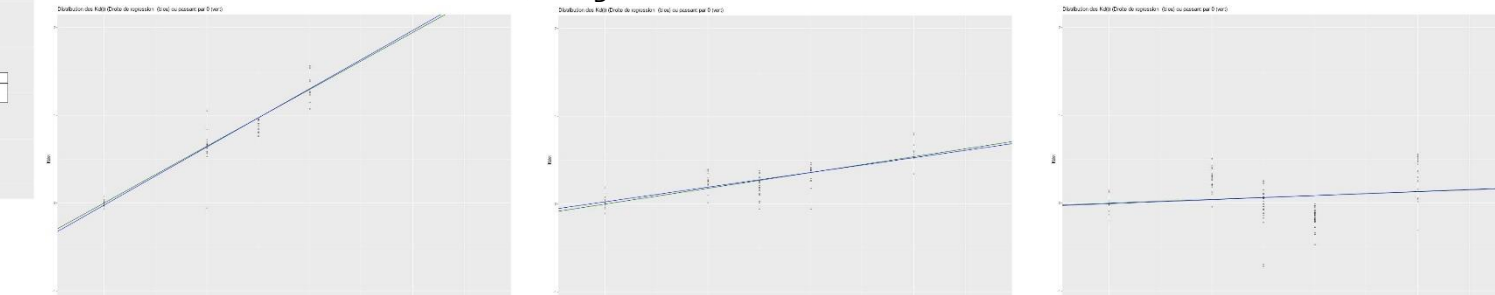
## Examples of the filters effects on redundant $R_1^{\text{Exp}}$ distribution



Applied filters are efficient in outliers suppression and  $R_1^{\text{Exp}}$  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, bleu line is the conventional linear regression (with intercept)

## Conclusions

### Proof of concept:

Determination of the  $K_D$  &  $K_S$  using plant metabolic <sup>15</sup>N labelling & measurement of peptide isotopic distribution

### Drawbacks:

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