



Success and bottlenecks of large scale plant protein turnover measurement

Willy Vincent Bienvenut

► To cite this version:

Willy Vincent Bienvenut. Success and bottlenecks of large scale plant protein turnover measurement: A tomato fruit maturation case study. 5th InPPO conference, May 2023, Thessalonique, Greece. hal-04358470

HAL Id: hal-04358470

<https://hal.science/hal-04358470>

Submitted on 21 Dec 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License



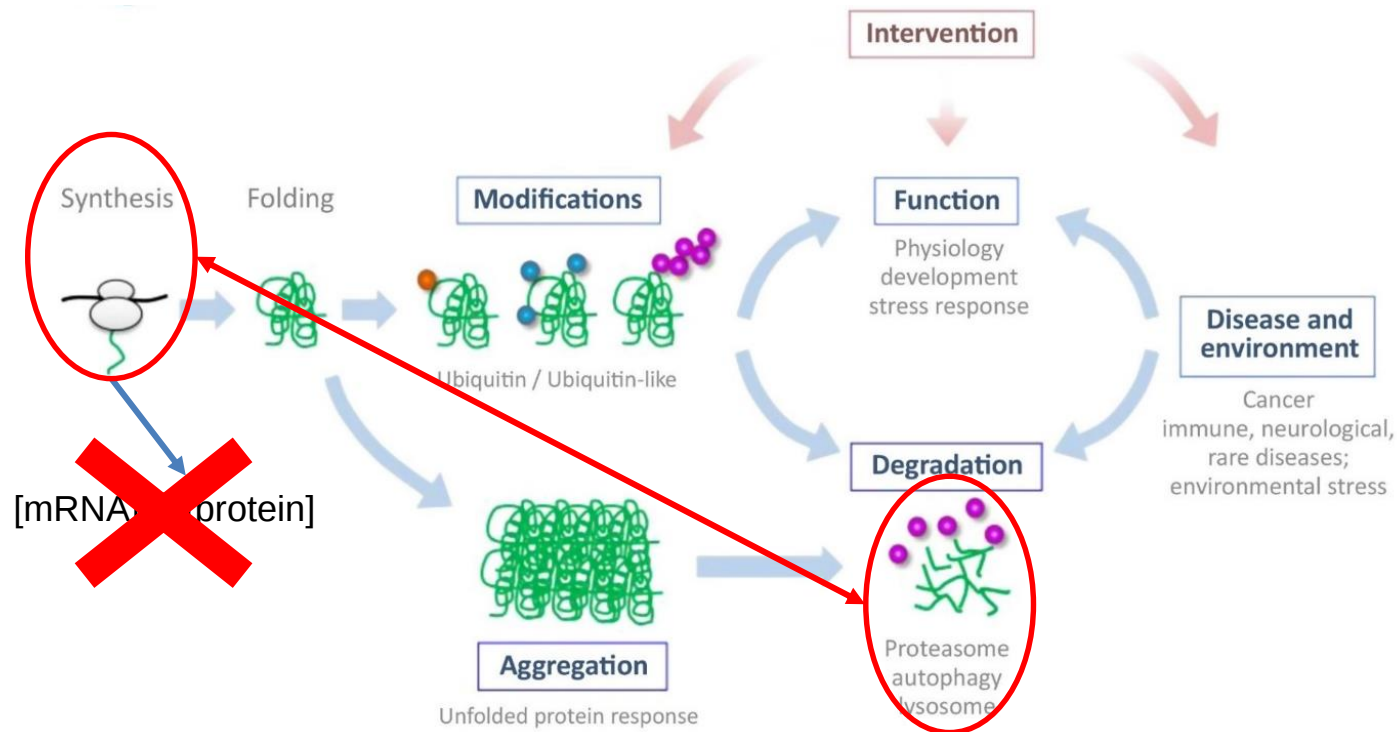
Génétique
Quantitative
et Évolution
Le Moulon



Success and bottlenecks of large scale plant protein turnover measurement: a tomato fruit maturation case study

Willy Bienvenut, IDEEV, GQE, Gif/Yvette, France

Proteostasis and large scale protein turnover rate determination



Dissmeyer et al. Trends Biochem. Sci. 2019

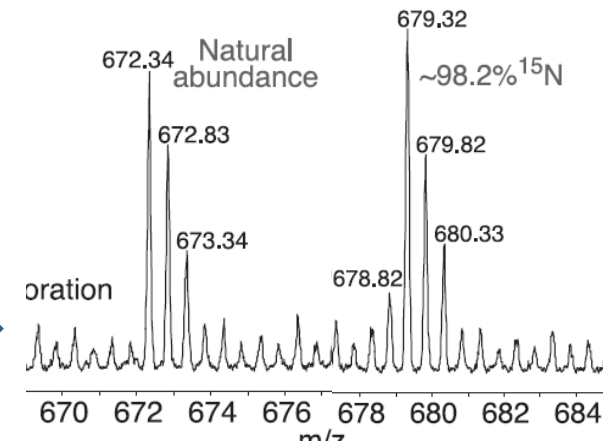
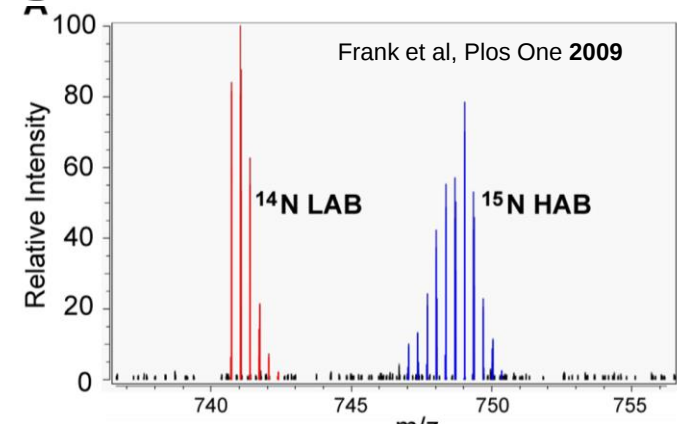
Influence of **protein Half-life and Turnover rate**
in **protein abundance variability**

Protein turnover in phototrophic organisms...



S. Lycopersicum
Var. Moneymaker
hydroponic culture
In mineral substrate

Water, CO₂,
fertilizers,
trace
elements...



¹²C ¹³C (CO₂)

¹⁴N ¹⁵N (NH₄ / NO₃)

Usual K_d/K_s equations

Very few technical approaches proposed for plants...

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

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

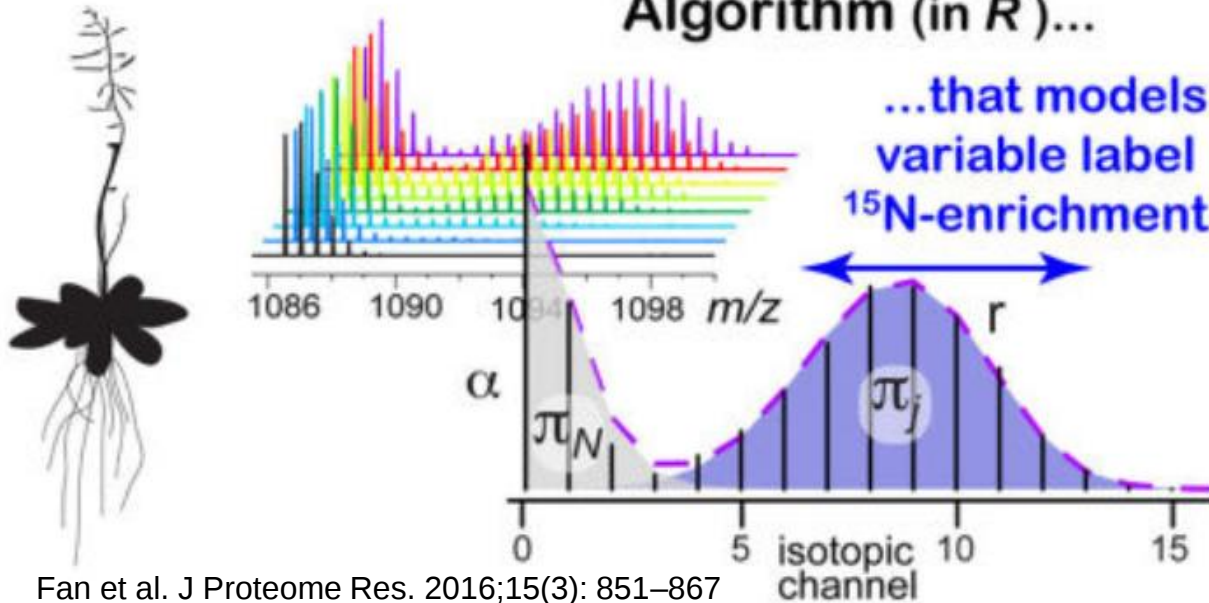
Determination of the:

FCP: Fold Change in Protein

LPF: Labelled Protein Fraction

Is that simple...?

A Proteome-Scale Protein Turnover Algorithm (in R)...



- High data processing
- Some scripts not available
- N labelled peptides are not compatible with MS characterisation:
 - Most of the acquired data are useless

An alternative approach... ?

- Adapted to the processing tools available locally
 - i2MassChroQ (X!Tandem identification tool and inference filtering interface)
 - MassChroq (peptide quantitation)
 - MCQR (Protein quantitation & statistics R package)
- Providing a dedicated pipeline to perform large scale protein turnover measurement
- Reducing data complexity (MS) and cost (^{15}N) ...

Determination of the FCP coefficient
(Fold Change in Protein)



Label-free quantitation
MCQR tool

Adapted version compatible with ^{15}N labelling

Determination of the LPF
(Labelled Protein Fraction)



Based on the isotopic distribution
**MassChroQ &
dedicated R scripts**

Isotopic distribution: a new way to explore...

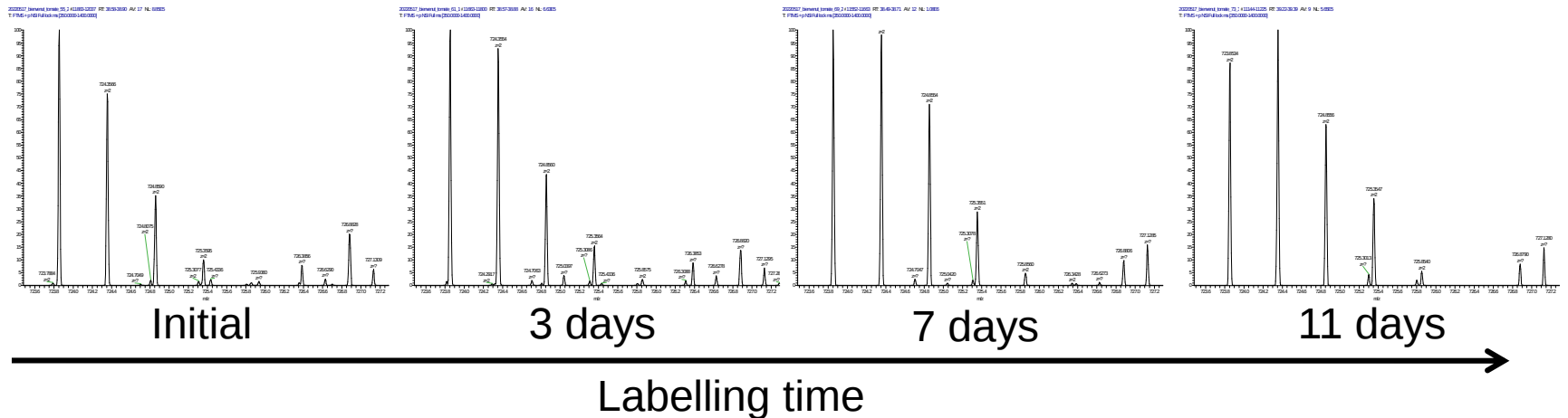
S. Lycopersicum
Var. money Maker
hydroponic culture
In coconuts fiber



5-10% ^{15}N
enriched media
→

Metabolic
labelling pulse

Standard proteomics
sample processing



How to estimate the LPF ?

$$LPF = 1 - \frac{R_1^{Exp} - R_1^{15N}}{R_1^{Nat} - R_1^{15N}}$$

$$R_1 = \frac{I_1}{I_0}$$

Exp: experimental measurement

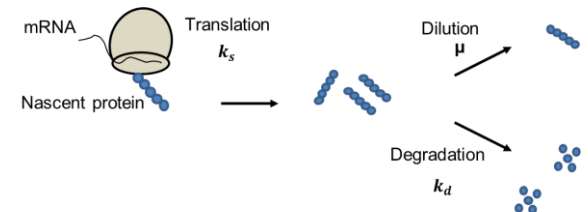
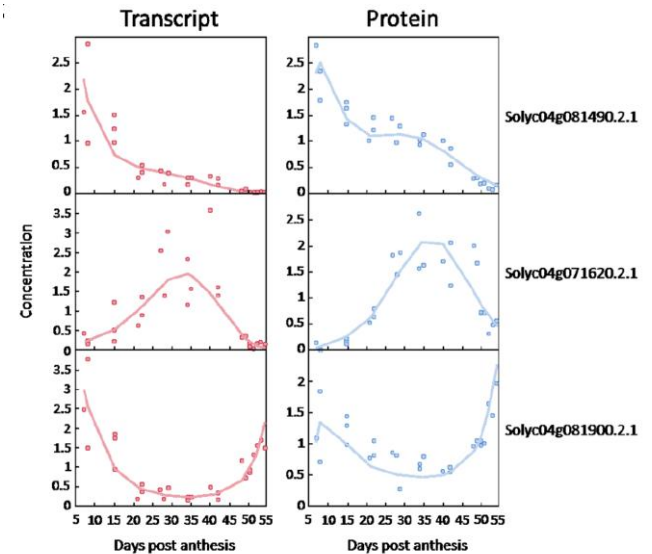
¹⁵N: Max ¹⁵N incorporation at t[∞] (Theo/Calc)

Nat: Natural isotopic distribution (Theo/Calc)

The PROOFER project:

exPeRimental validatiOn OF protEin tuRnover

- Protein turnover model developed on tomato fruit
 - Belouah *et al.* 2019, J. Proteomics
- Protein turnover model using:
 - Transcriptomics data:
 - Absolute quantitation of 22877 transcripts with spikes in the experimental RNAseq
 - Proteomics data:
 - XIC absolute quantification of 2490 proteins using peptide intensity modelling



$$\frac{dp(t)}{dt} = k_s r(t) - (k_d + \mu(t))p(t)$$

Samples preparation and proteins ID



- * All samples collected at 12 days post anthesis
- * Labelling time was variable (0- 12 days)

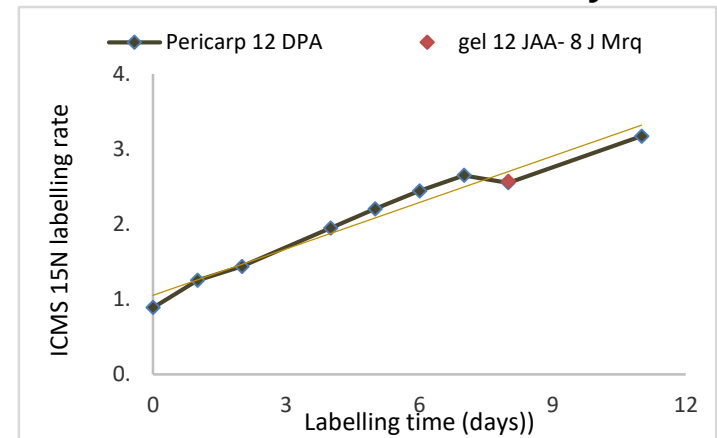
Tomato developmental stages



Samples collected at multiple ^{15}N labelling time points

Standard proteomics sample preparation

Evolution of the ^{15}N rate by ICMS



i2MassChroQ

X!Tandem

X!

REFERENCE-DB.protein.fasta

List of identified

peptides and proteins

MassChroQ
Label free quantitation
& Isotopic distribution exports

Isotopic

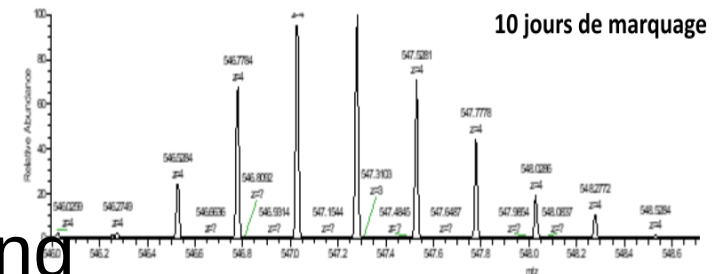
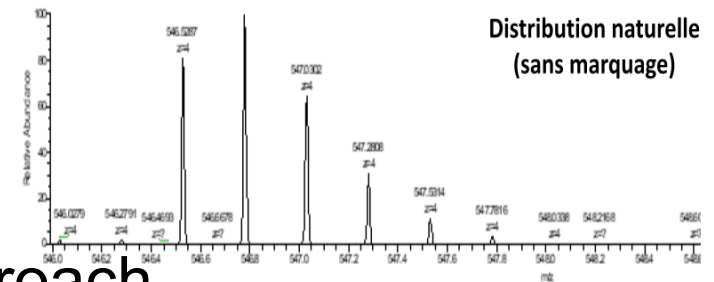
profiles

MCQR: R package for quantitation

How to determine the Fold Change in Protein (FCP)

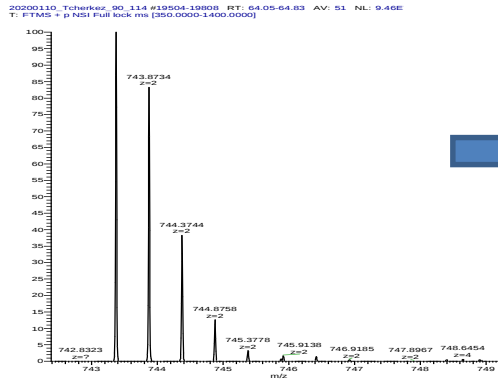
- Drawbacks:
 - Based on 1st or most intense isotope
 - ¹⁵N metabolic labelling
modify the isotopic distribution
- Modified processes:
 - Intensity normalisation
 - Alternative “missing value” approach
 -
- New **IsoProt_MCQR** script
 - Compatible with ¹⁵N labelling

séquence du peptide : AGVKEYKLTYYTPEYETK



Display of the isotopic ratios

Peptide experimental isotopic distribution



$$\rightarrow R_i = \frac{I_i}{I_0}$$

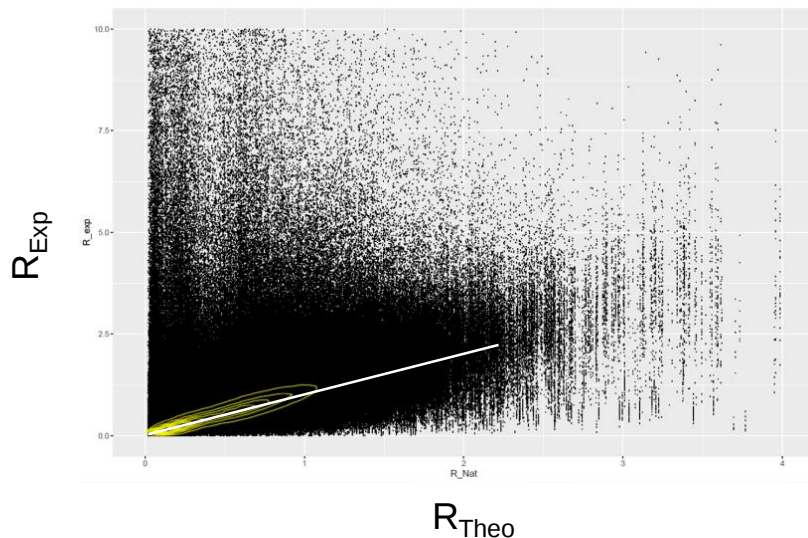
Experimental Intensity

Extracted by MassChroQ
Converted in R_i by R script

Theoretical R_i calculation

IsoSpec python library by M. Lacki;
adapted by P. Rusconi

Example for the Ctrl sample at t=0



5.190.167 R_i
106.219 peptides profiles
29,840 nr pepIDs

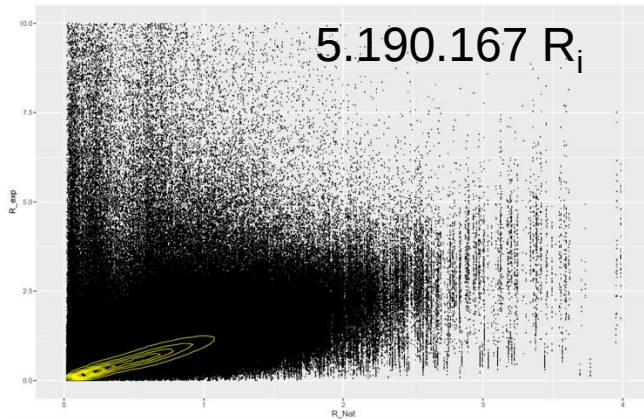
$$R_{\text{exp}} = 0.76 \cdot R_{\text{theo}} + 0.39, R^2 = 0.02, \text{IFS} = 0.01$$

Filtering the raw data

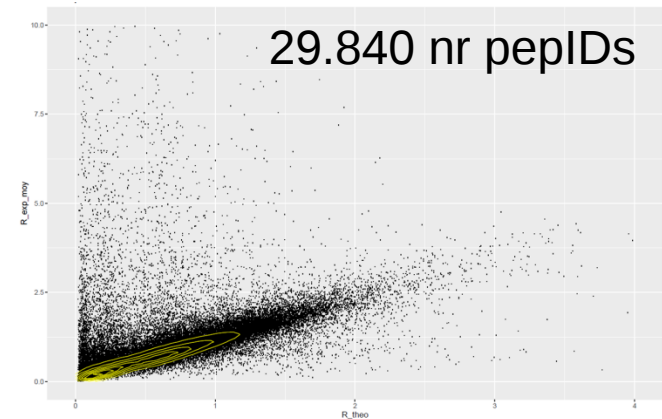
- Minimum number of isotopes:
 - Usually 4 isotops min (I_0 - I_3)
- Maximum ^{15}N incorporation rate:
 - based on theo. Max. ^{15}N rate isotopic distribution
- R_i dispersion:
 - $\bar{A} \pm 1$ SD range retained (Theo. 70% retained)
- **Minimum data redundancy:**
 - Min 2/3 Anal. Rep.,
 - >4 isotopic profiles / Rep,
 - >3 Isotopes / isotopic profil
 - ...

Filtering the raw data (initial vs. final)

$$R_{\text{exp}} = 0.76 \cdot R_{\text{theo}} + 0.39, R^2 = 0.02, \text{IFS} = 0.01$$

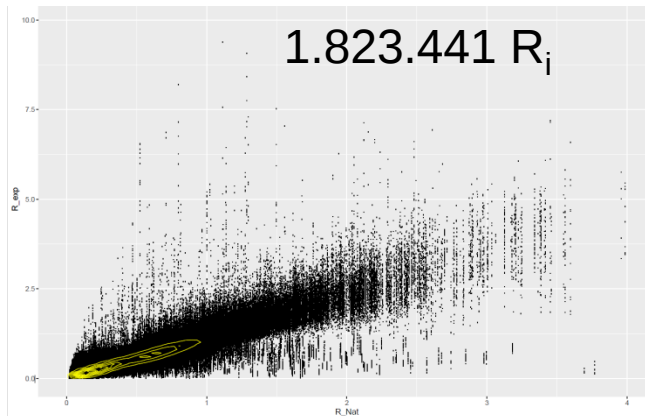


$$R_{\text{exp}} = 0.75 \cdot R_{\text{theo}} + 0.43, R^2 = 0.07, \text{IFS} = 0.03$$

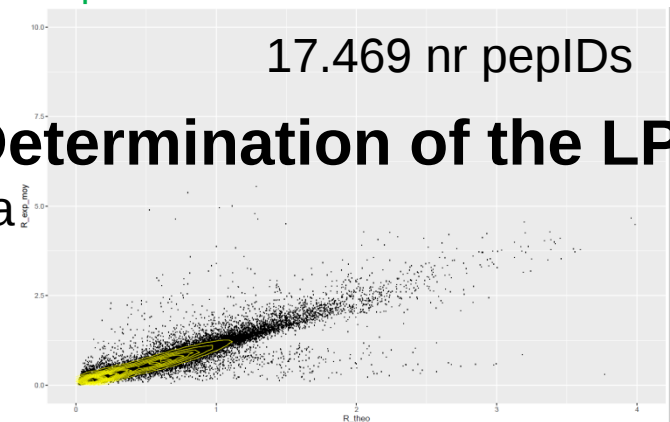


Initial data

$$R_{\text{exp}} = 1.01 \cdot R_{\text{theo}} + 0.05, R^2 = 0.95, \text{IFS} = 1.27$$

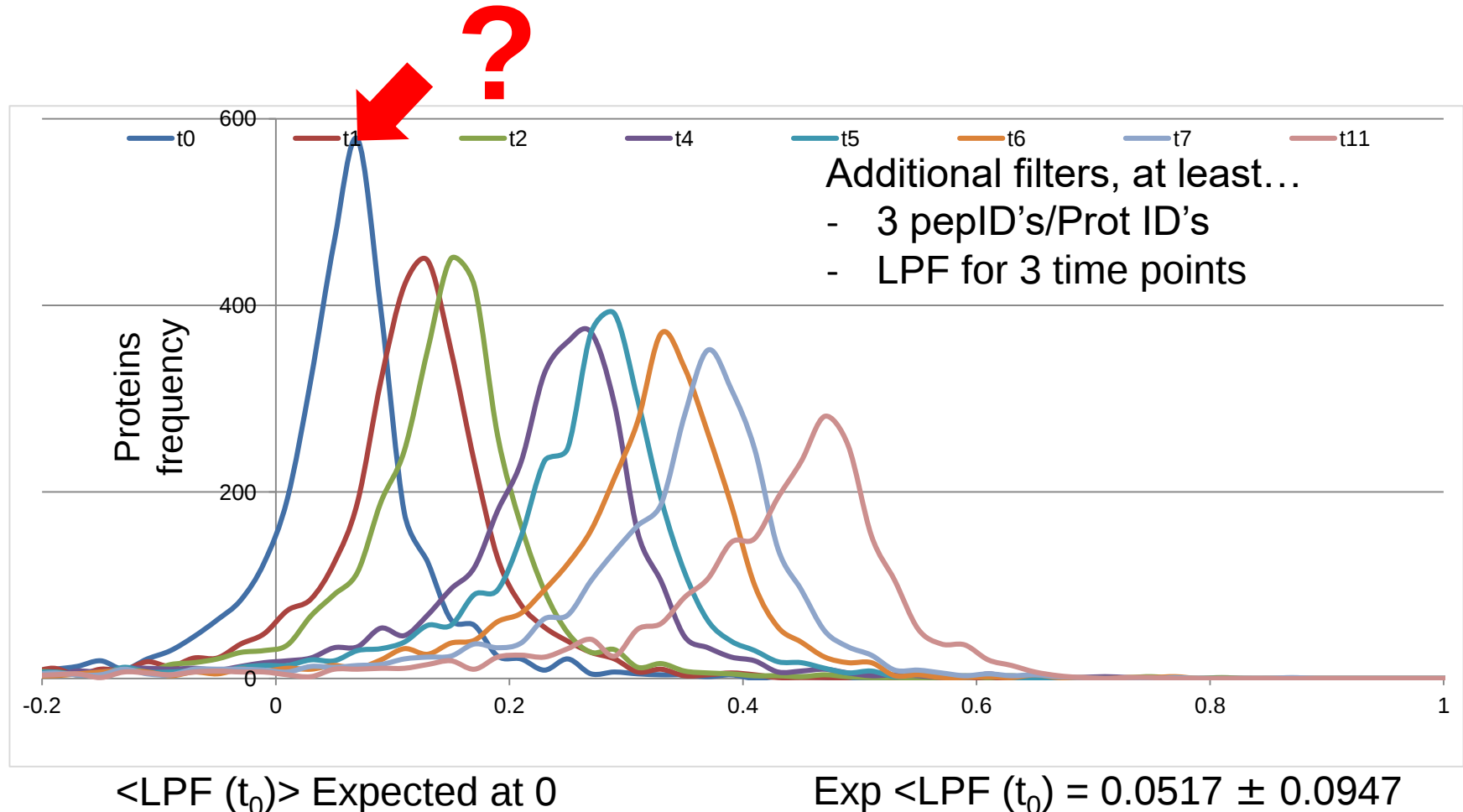


$$R_{\text{exp}} = 1.00 \cdot R_{\text{theo}} + 0.06, R^2 = 0.94, \text{IFS} = 1.21$$



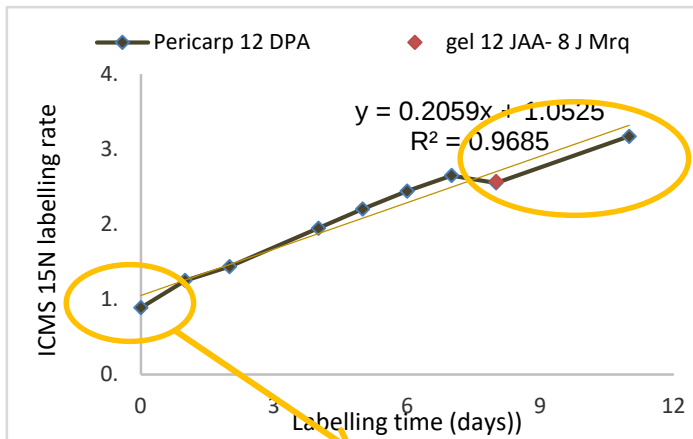
Determination of the LPF
Filtered data

Protein LPFs distribution



Experimental drawbacks...

Evolution of the ^{15}N rate by ICMS



Natural ^{15}N rate = 0.364%

Technical problem related to automatic
plant watering system during the WE
Labelling time + 1 days

Tomato developmental stages

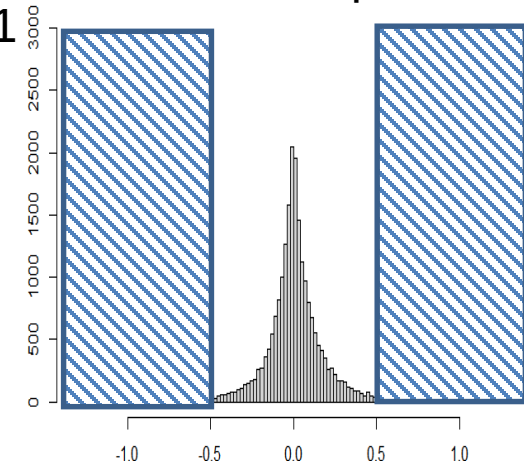


All samples were collected
at 12 days post anthesis

Labelling time was variable (0- 12 days)

By definition: 2511/2973 proteins
FCP = 1

Selection of
proteins with
no significant
variation in
quantity



Determination of the K_d/K_s

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

Since $FCP = 1$

$$K_d(t) = \ln(1-LPF)/t$$

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



$$K_s = K_d$$

Av. individual values

$$\langle K_d \rangle = 0.0528 \pm 0.0077 \text{ d}^{-1}$$

Classic linear reg.

$$K_d = 0.0536 \text{ d}^{-1}, R^2 = 0.987$$

Reg. line through the origin

$$K_d = 0.0538 \text{ d}^{-1}$$

Av. individual values

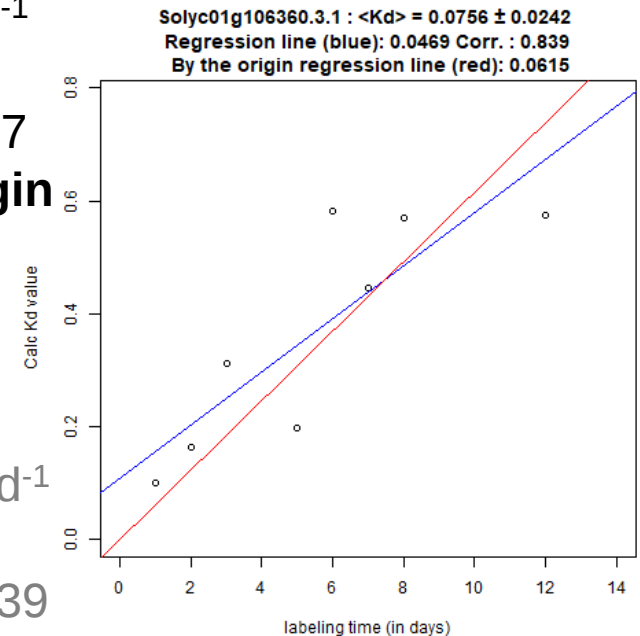
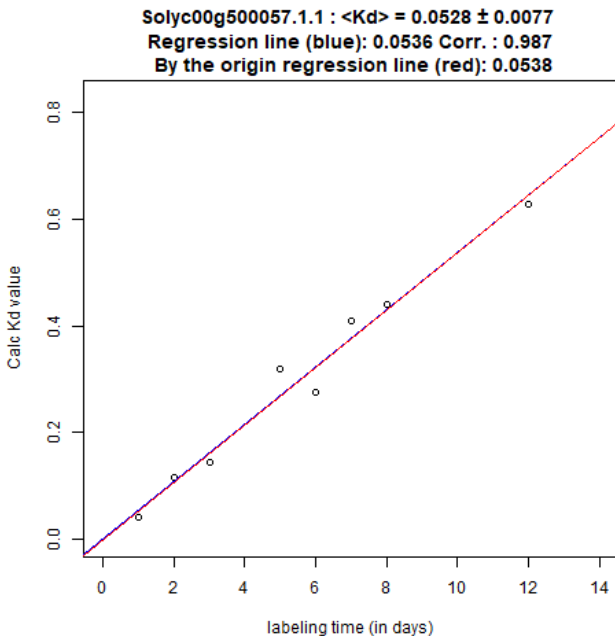
$$\langle K_d \rangle = 0.0756 \pm 0.0242 \text{ d}^{-1}$$

Classic linear reg.

$$K_d = 0.0469 \text{ d}^{-1}, R^2 = 0.839$$

Reg. line through the origin

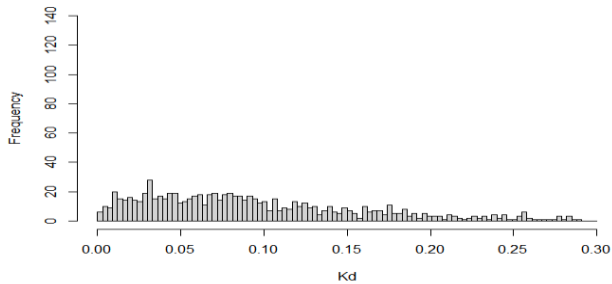
$$K_d = 0.0615 \text{ d}^{-1}$$



Results:

Predicted K_d (Belouah *et al.* model) vs. Exp K_d

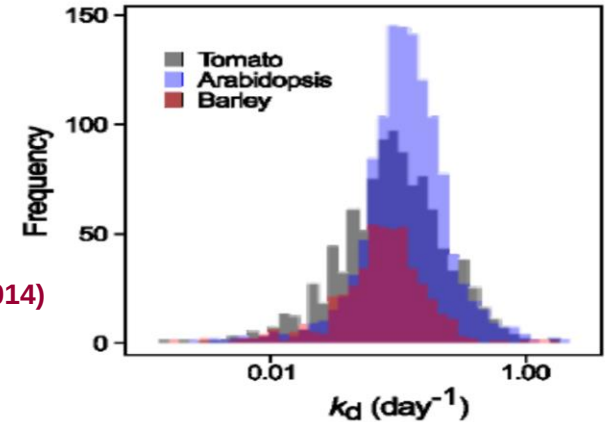
distribution des K_d (modèle)



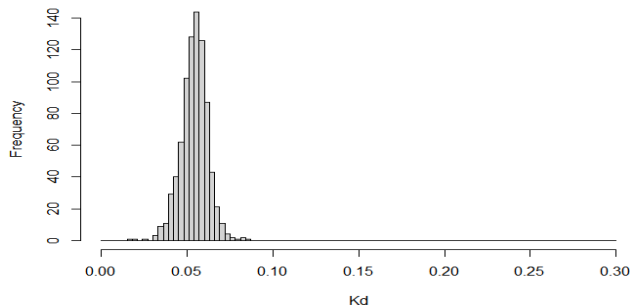
Distribution of 1103 predicted k_d of the tomato fruit : 0,113 day

Arabidopsis leaf median 0.113 day⁻¹,
~ 9 days (1228 k_d from Li *et al.*, 2017)

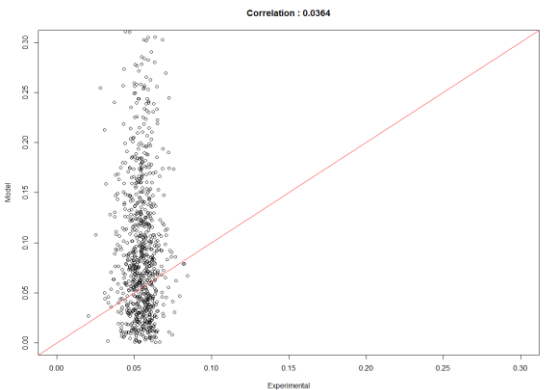
Barley leaf median 0.076 day⁻¹,
~13 days (505 k_d from Nelson *et al.*, 2014)



distribution des K_d (expérimentaux)



Averaged individual values
 $\langle K_d \rangle = 0.0601 \pm 0.0121 \text{ d}^{-1}$



Similar $\langle K_d \rangle$ value but with a much more restricted range
Strong discrepancies between the model and Exp. values

Conclusions

- Partial ^{15}N metabolic labelling:
 - compliant with LPF estimation
 - compatible with ^{15}N labelling
- Calculation of $K_d(K_s)$ is possible
- Restricted K_d range may be related to:
 - The sampling plan (too short, 1-10 days)
 - Probable influence of the metabolic lag time (28h)

Questioning...

- Some additional investigations are required to understand the results discrepancies...
- What about a model using mRNA & Protein quantitative values to determine protein turnover...
- Some major bias in Millar's processing pipelines (related to the FCP)...
- Too few reliable studies in plant for an accurate comparison of the results

Acknowledgement's

Liam Le Goffic
Thomas Welfelé
Olivier Langella
Thierry Balliau
Filippo Rusconi,
Marlène Davanture,
Mélisande Blein-Nicolas

Collaboration with

C. Masclau-Daubresse & A. Marmagne
(IJPB, Versailles)

S. Colombié & Y. Gibon
(BFP, Bordeaux)



PROOFER grant (BAP department INRAE)