



HAL
open science

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

Willy Vincent Bienvenut, Olivier Langella, Thierry Balliau, Filippo Rusconi, Marlène Davanture, Melisande Blein-Nicolas

► **To cite this version:**

Willy Vincent Bienvenut, Olivier Langella, Thierry Balliau, Filippo Rusconi, Marlène Davanture, et al.. Sub optimal ^{15}N metabolic labelling in plant: A new way to perform large scale protein turnovers determination using the peptides isotopic distribution.. ProteoVilamoura, Spanish, French and Portuguese Proteomics Societies, May 2022, VilaMoura, Portugal. hal-03815633

HAL Id: hal-03815633

<https://hal.science/hal-03815633v1>

Submitted on 21 Oct 2022

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.

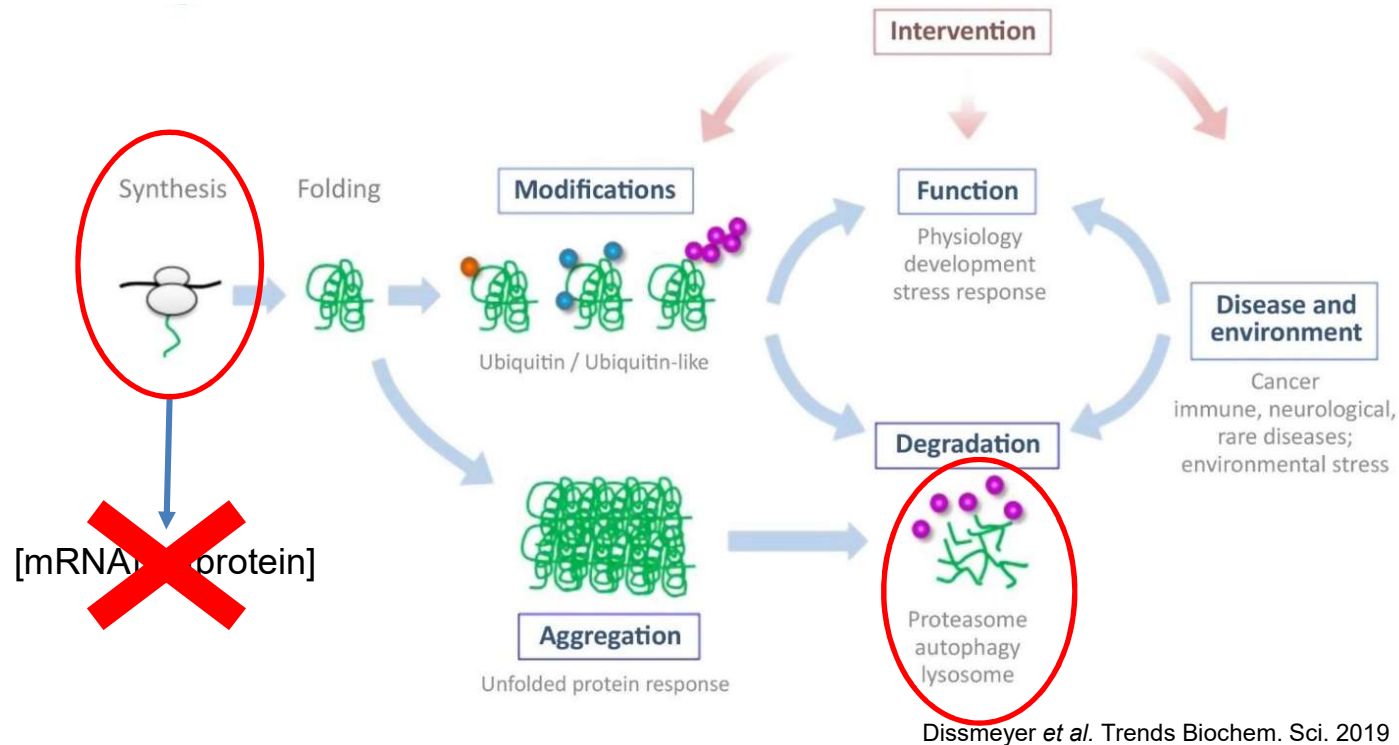
Public Domain

Sub optimal ^{15}N metabolic labelling in plant:

A new way to perform large scale protein turnovers determination using the peptides isotopic distribution

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

Proteostasis and large scale protein turnover rate determination



of protein Half-life and Turnover rate
Influence directly the Protein abundance

What about phototrophic organisms

Not possible to use
heavy-labelled amino acid



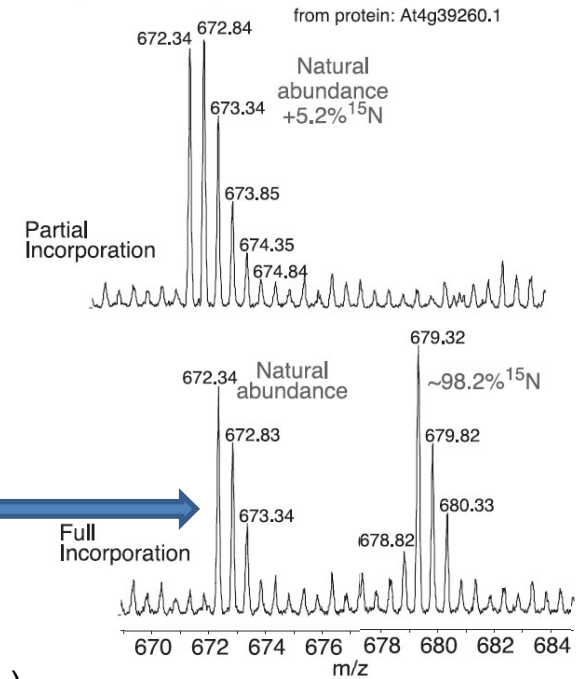
Water, CO₂, fertilizers and trace elements...

C H N O S

¹²C → ¹³C (CO₂)

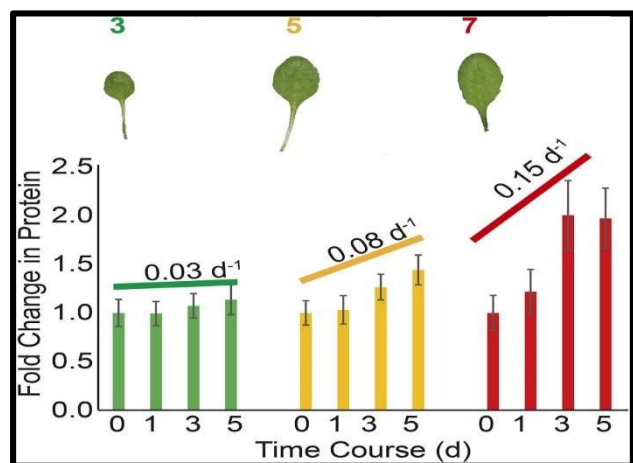
¹⁴N → ¹⁵N (NH₄NO₃)

Peptide: TFSQFGDVIDSK (2+)



Huttlin et al. *Mol. Cell. Proteomics* 2017

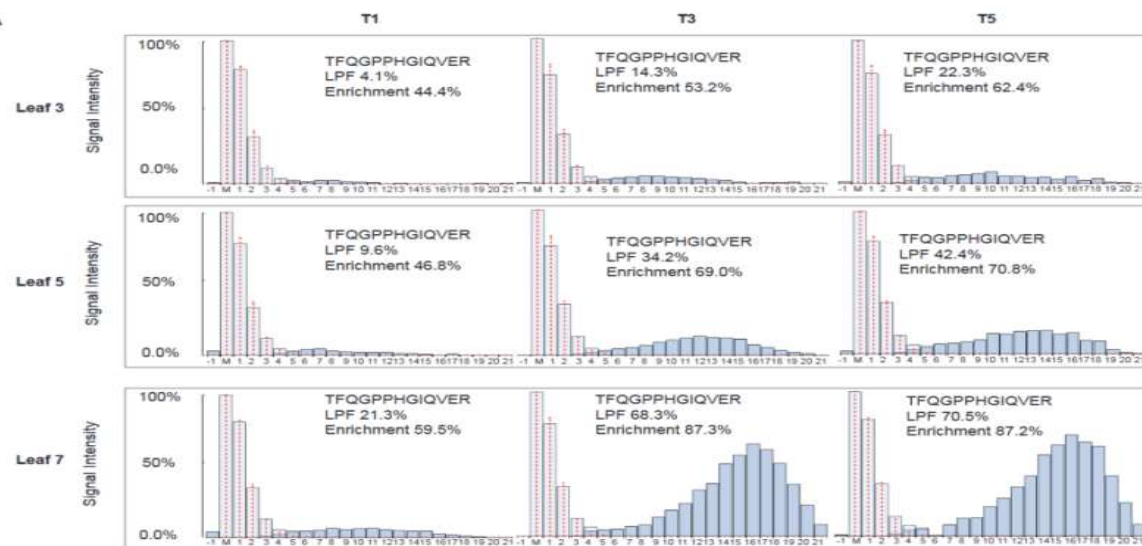
Li *et al.* approach, *Plant Cell* 2017; 29:207-228



21 days old non-labelled Arabidopsis plants

Determination of the
FCP coefficient

(Fold Change in Protein)



Determination of the LPF

(Labelled Protein Fraction)

K_D and K_S calculation

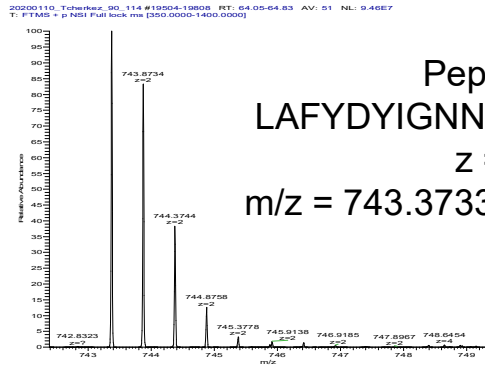
		RBCL (TFQGPPHGIQVER)	THI1 (MGPTFAMMISGQK)
Fold Change in Protein Abundance (FCP)	T0	1	1
	T3	1.07	1.07
Labelled Protein Abundance (LPF)	T0	0	0
	T3	14.3%	96.3%
$K_D = - \frac{\ln FCP \cdot (1 - LPF)}{t}$		$- \frac{\ln 1.07 \cdot (1 - 14.3\%)}{3 \text{ d}} = 0.04 \text{ d}^{-1}$	$- \frac{\ln 1.07 \cdot (1 - 96.3\%)}{3 \text{ d}} = 1.61 \text{ d}^{-1}$
$\frac{K_S}{A} = \frac{FCP - e^{-K_D \cdot t}}{1 - e^{-K_D \cdot t}} \cdot K_D$		$\frac{1.07 - e^{-0.04 \cdot 3}}{1 - e^{-0.04 \cdot 3}} \cdot 0.04 = 0.07 \text{ d}^{-1}$	$\frac{1.07 - e^{-1.61 \cdot 3}}{1 - e^{-1.61 \cdot 3}} \cdot 1.61 = 1.73 \text{ d}^{-1}$

- Millar et al. method
 - High data-processing
 - Scripts not available
 - ...
- Alternative approach... ?
 - Adapted to our local tools
 - Adaptable to field investigations
 - Low cost
 - ...

Large scale protein turnover measurement...

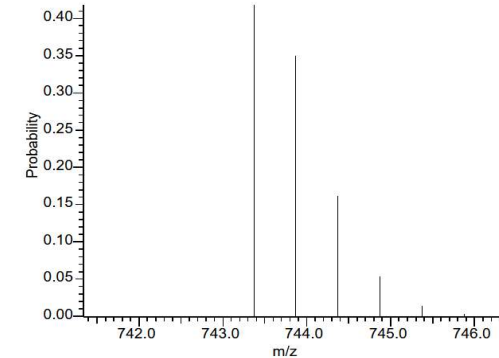
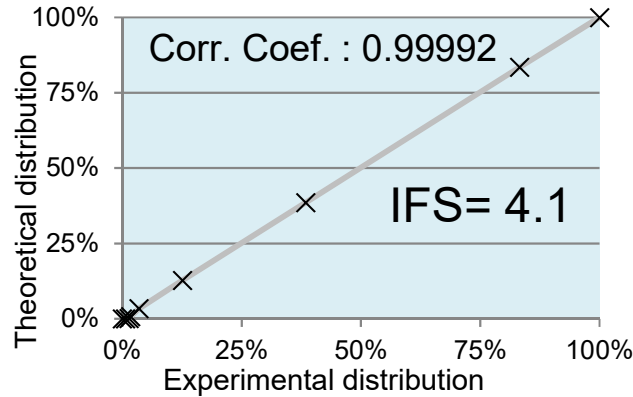
- Proposed alternative
 - **Low labelling rate (5-6% vs. >70-90% ^{15}N)**
 - **Advantage :**
 - **Peptide isotopic distribution is moderately modified!!!**
 - Heavy & light isotopomers overlay
 - Minor sample complexity increase
 - *Bottlenecks...*
 - **Intensive post-acquisition data processing:**
 - ^{15}N labelling yield # peptide isotopic distribution
 - **Protein amount variation** (label free quantitation)
- Processing based on our **open sources softwares**
 - **X!Tandem Pipeline, MassChroQ, MCQR (R package),...**
 - Additional R and Python processing scripts under development...

The isotopic distribution world...



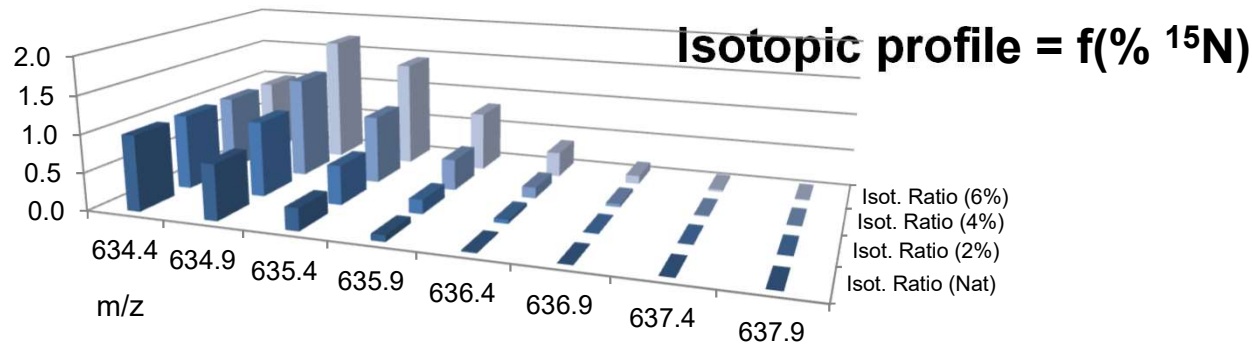
Peptide experimental isotopic distribution

Peptide:
LAFYDYIGNNPAK
z = 2+
m/z = 743.3733 Th



Peptide simulated isotopic distribution

Isotope Fitting Score (IFS) = $-\log(1-\text{Corr.})$



Standard samples preparation and proteins ID



Z. maize B73 in potting soil;
Normal growth until leaf 6

¹⁵N labelling
pulse

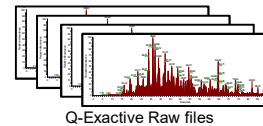
½ MS media 10%
of ¹⁵N
(90% of ¹⁴N)

Collecting
samples at
multiple time
points

Standard proteomics
sample preparation



RP-LC Q-Exactive analysis



Q-Exactive Raw files

Raw file mzML
conversion



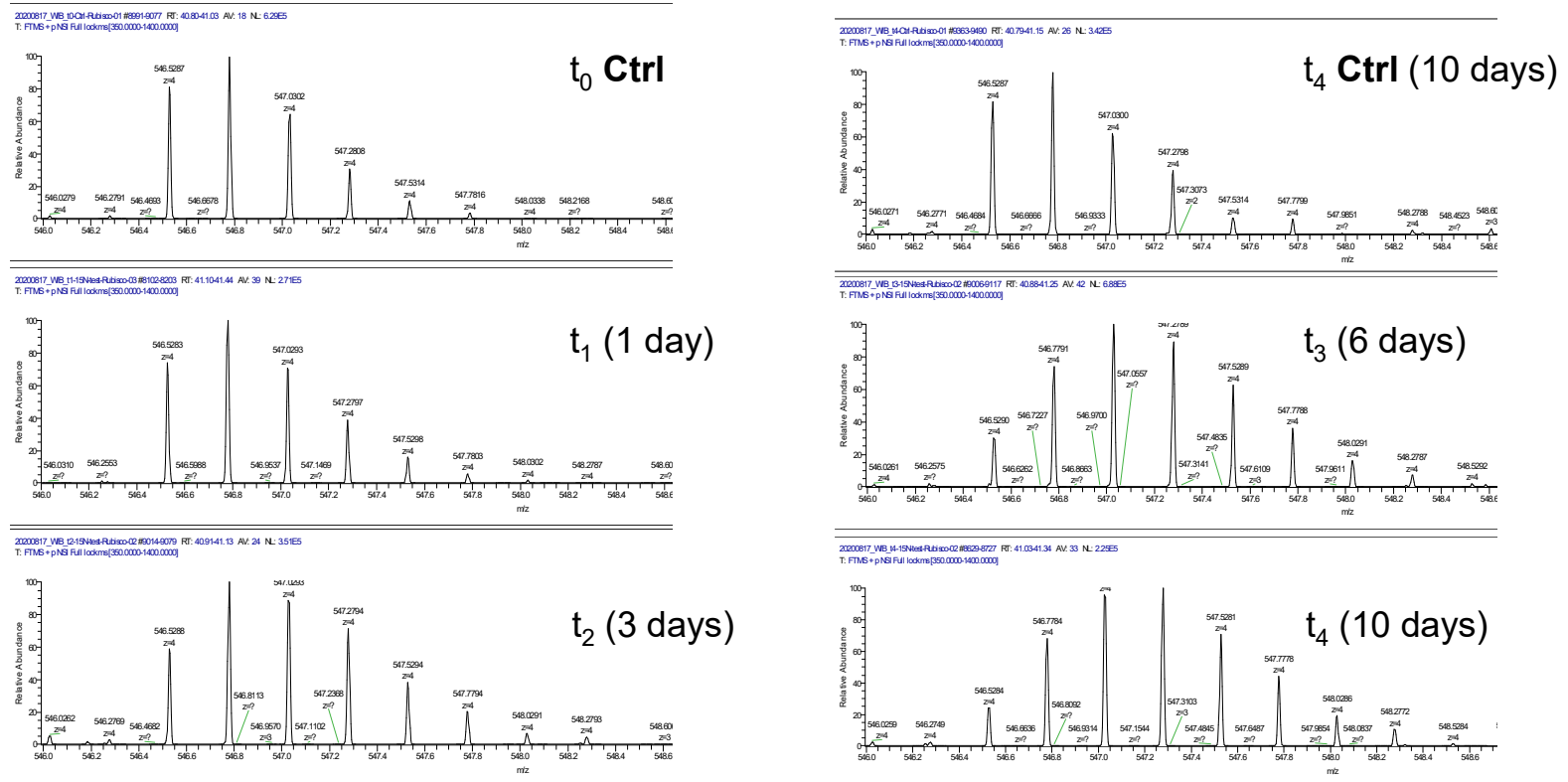
*Zm-B73-REFERENCE-
DB.protein.fasta*



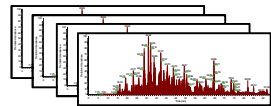
List of identified
peptides and proteins

Isotopic distribution metabolic labelling kinetics

Peptide sequence: AGVKEYKLTYYTPEYETK



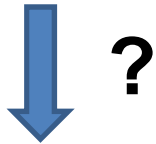
Post Acquisition Data Processing



Raw files

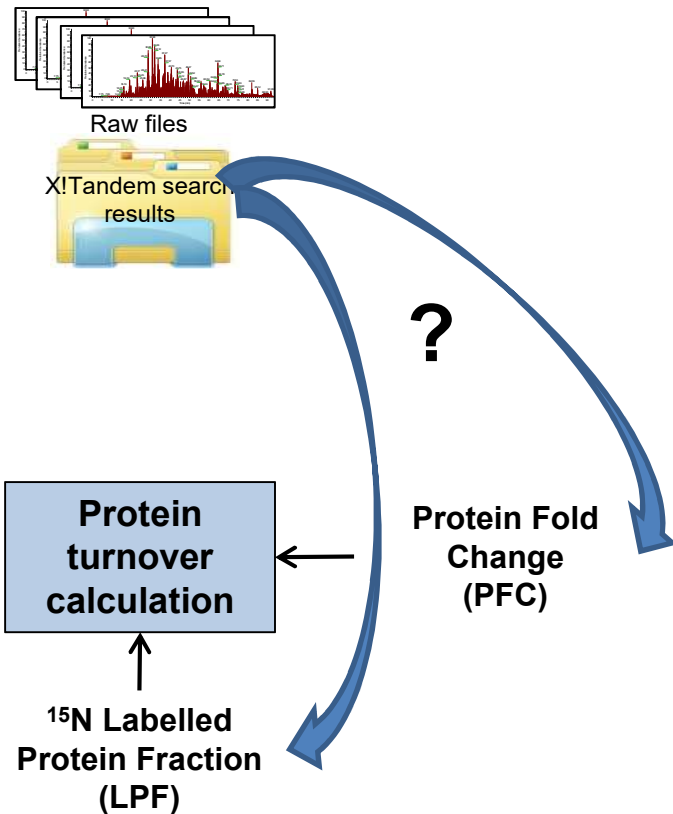


X!Tandem search
results

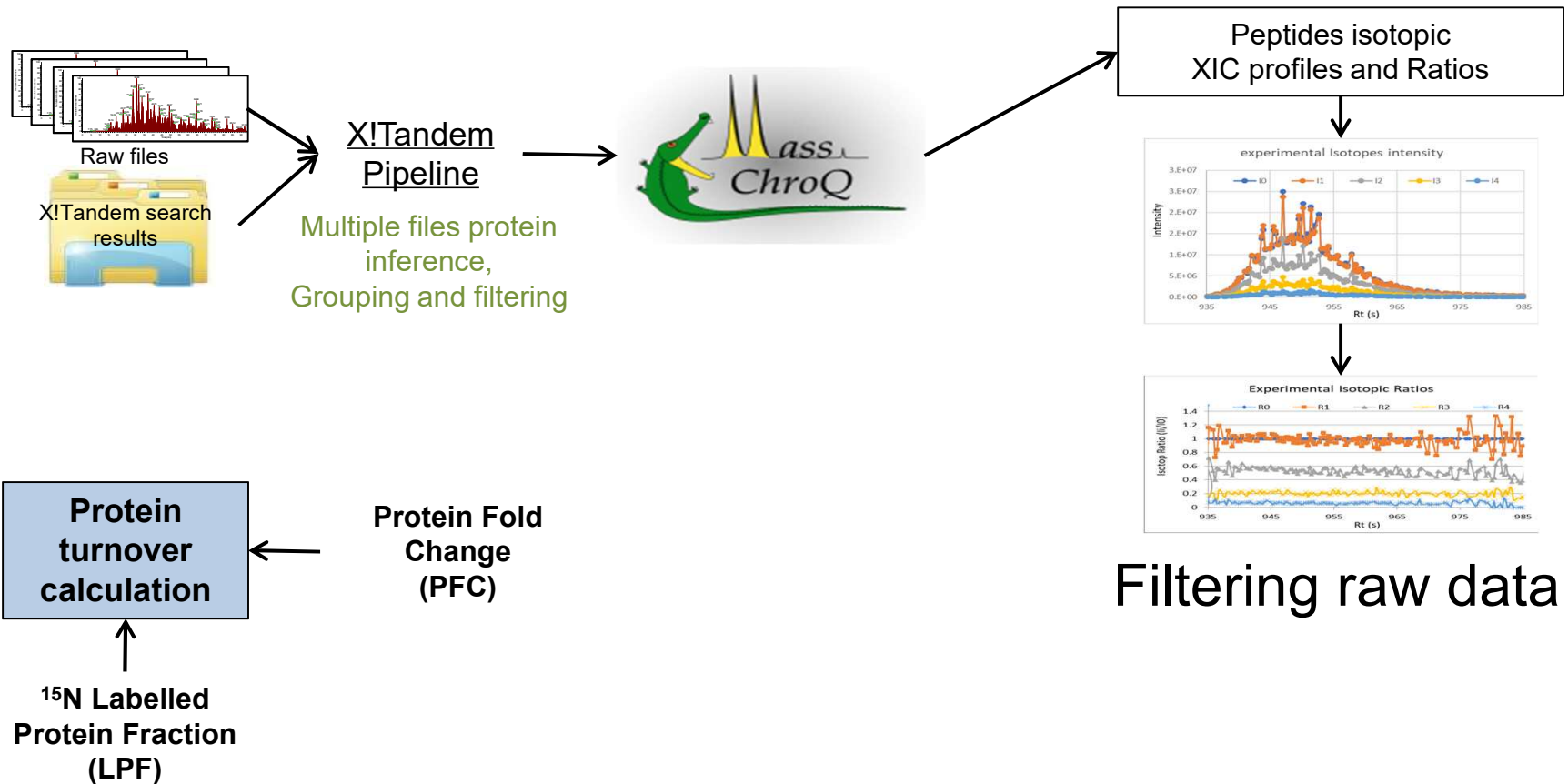


**Protein
turnover
calculation**

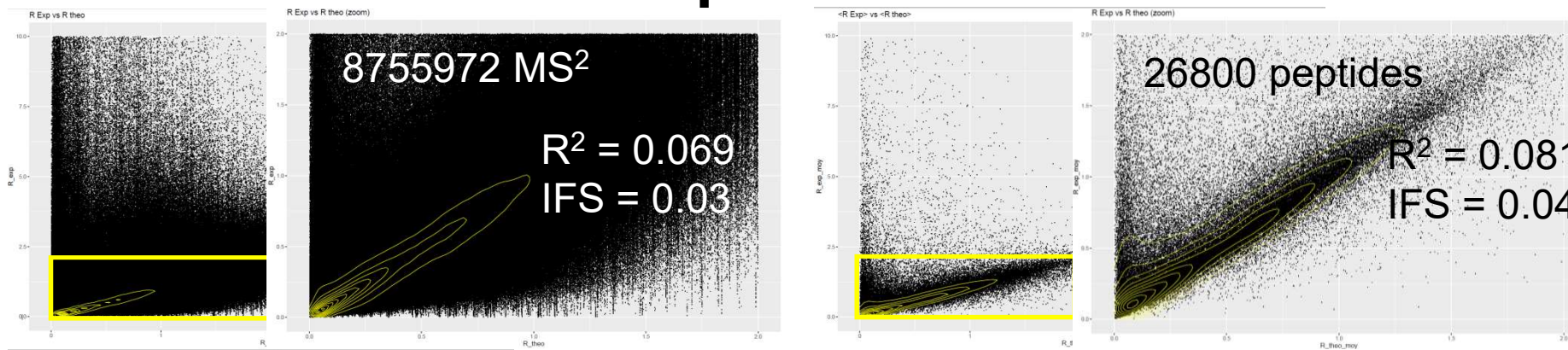
Post Acquisition Data Processing



Post Acquisition Data Processing

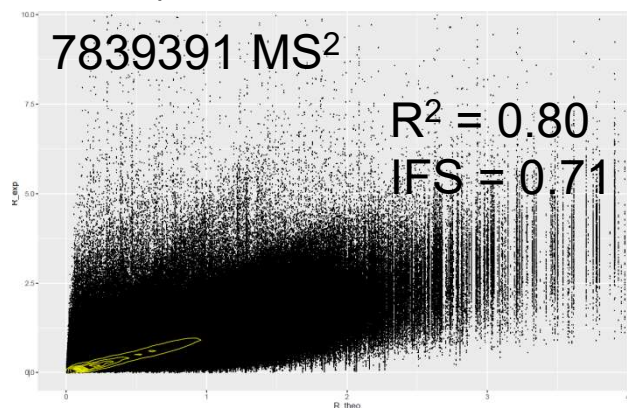


A view Exp vs Theo Ri...



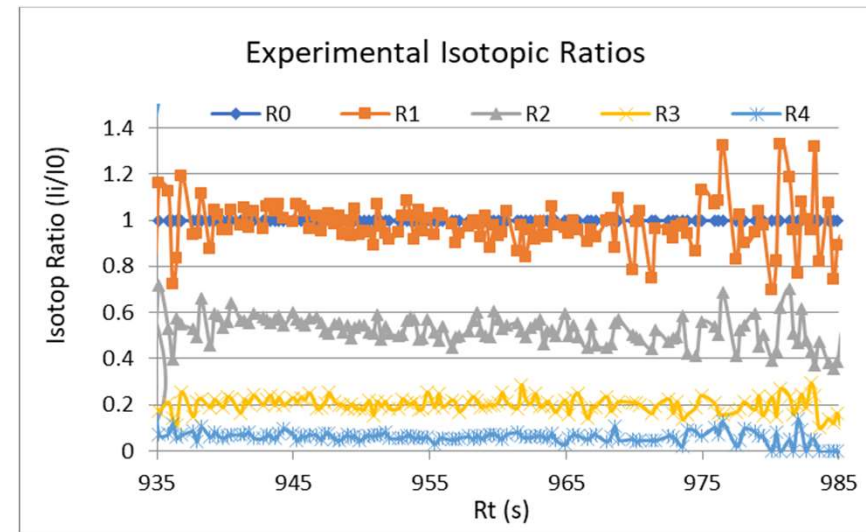
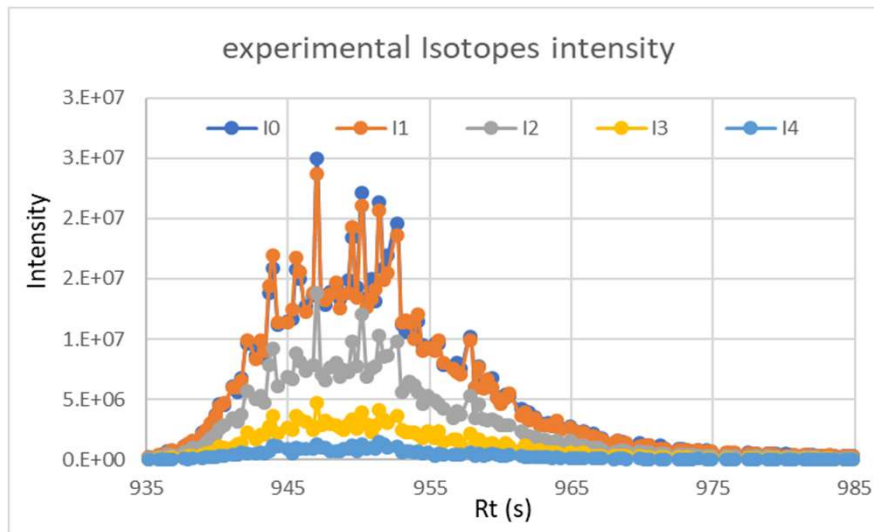
Pre-processing

Removing outliers: $R_{i \text{ Exp}}(\text{pepID}) < R_{i \text{ Theo}}(\text{pepID}) * 1.2$ at 6% ¹⁵N



Filtering data

2 levels filters



Inter-Spectrum Correlation

Peptide Ri distribution

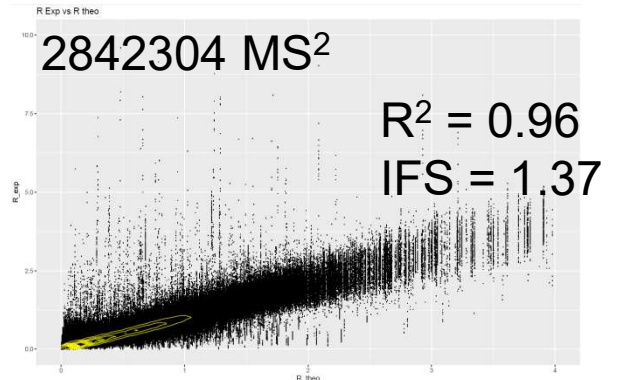
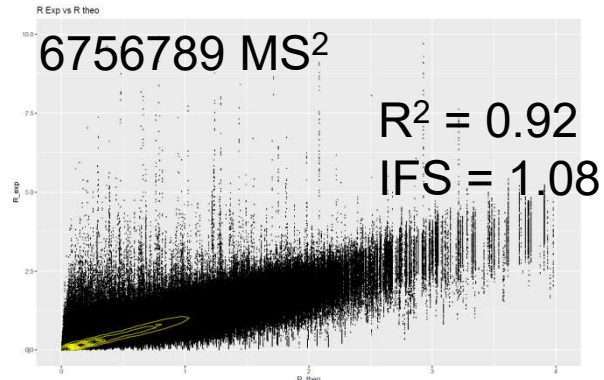
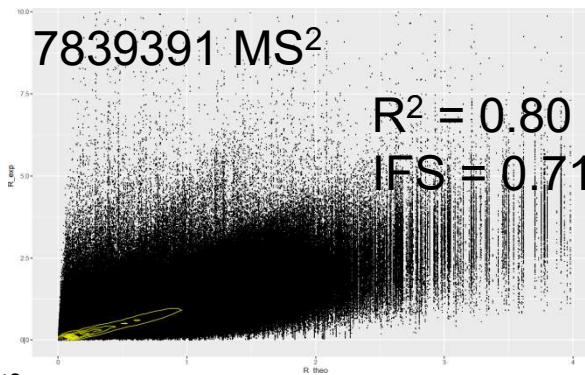
Isotopic ratios and profile filtering

2 levels filters

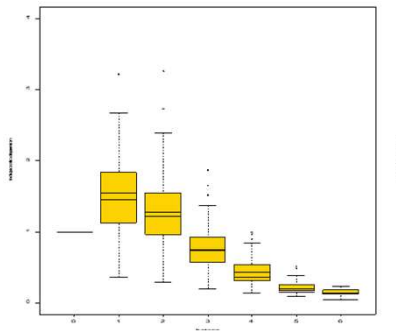
Raw ratios

Peptide(R_i) dist.

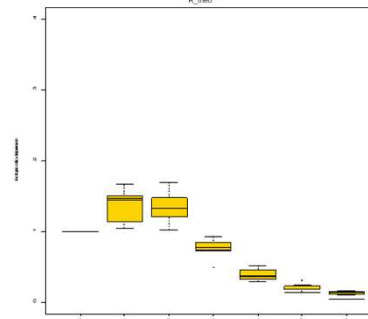
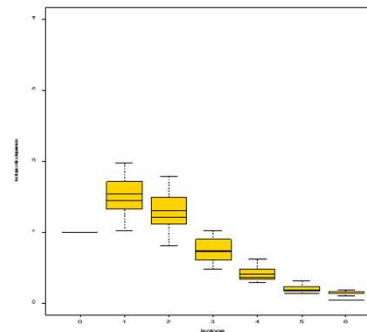
Spectrum² Corr.



Single peptide Ratios



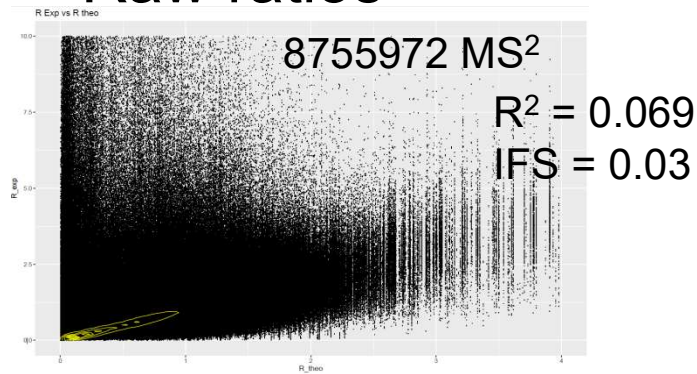
$\langle R_i \rangle - 2 SD < R_i < \langle R_i \rangle + 2 SD$



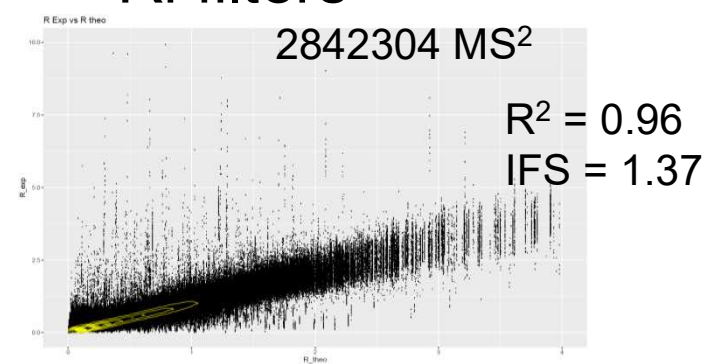
MS Corr. > 0.9

Additional filters ...

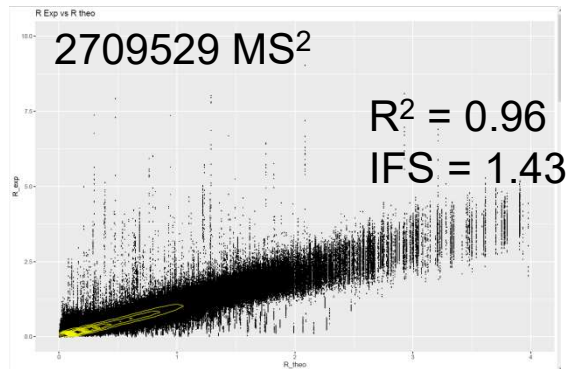
Raw ratios



Ri filters

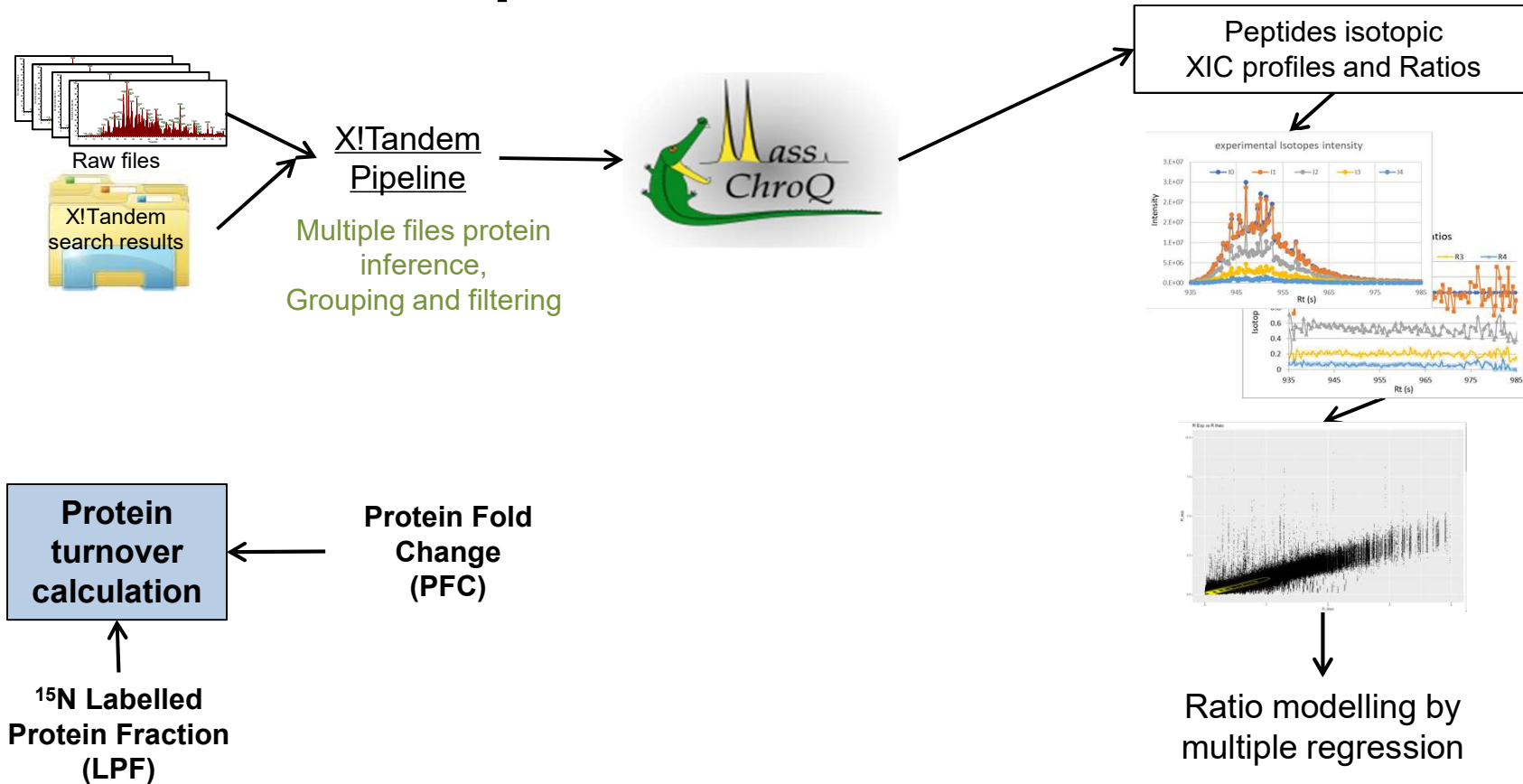


Favouring data redundancy



Redundant measurements
in multiples replicates (≥ 2 out of 4 MS run)
Minimum isotopes ratios (≥ 4 I)
Minimum number of spectra (≥ 5 MS²)

Post Acquisition Data Processing



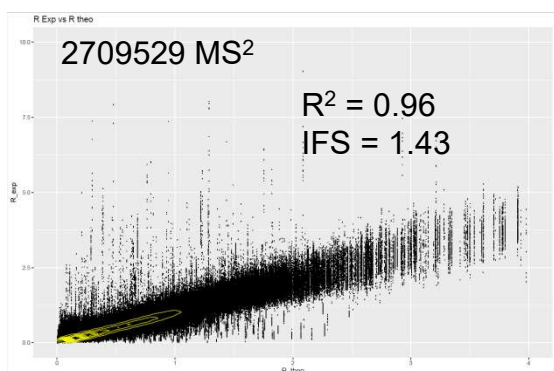
Post Acquisition Data Processing

Highly redundant peptide isotopic profiles

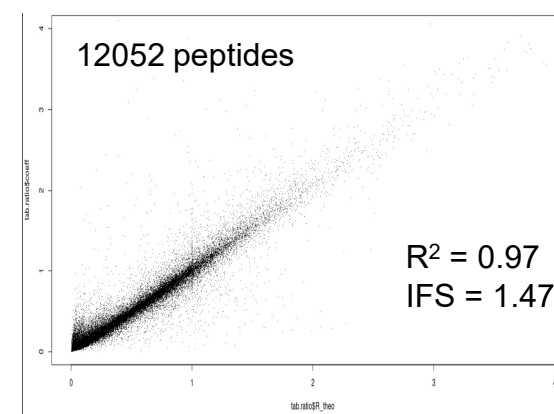
Multiple acquisitions (Triplicate at least)
Multiple Rt (10-30 s peptide elution)
Multiple z (2+,3+...)

$$\log(R_{ijk}) = \text{isotope}_i + \text{MSrun}_j + \text{RT}_k + \varepsilon_{ijk}$$

isotope_i: isotope i fixed effect
MSrun_j: MS run j fixed effect
RT_k: = retention time random effect
ε_{ijk}: residual

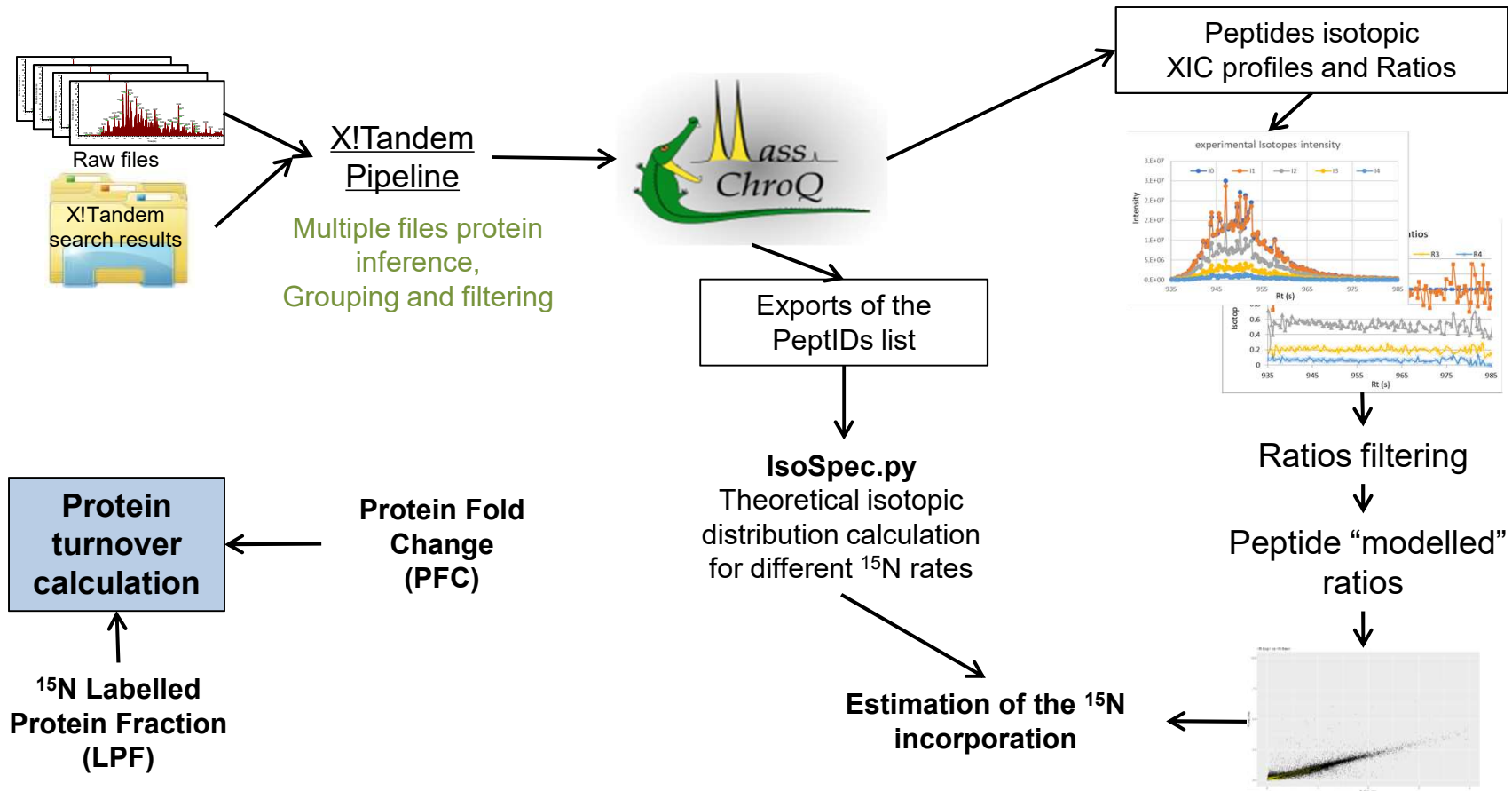


Ratio modelled by
multiple regression



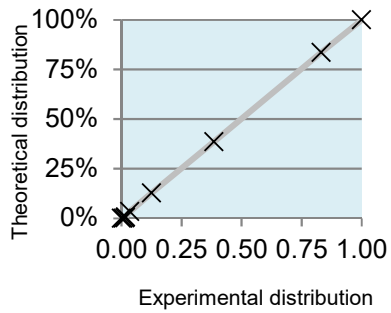
Single isotopic distribution per non-redundant peptide

Post Acquisition Data Processing

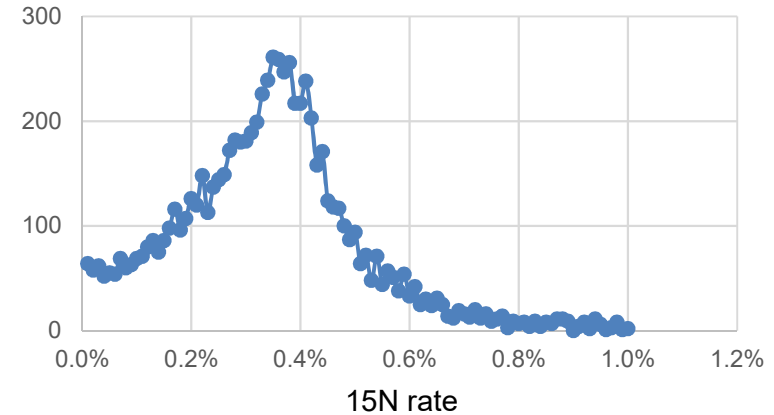
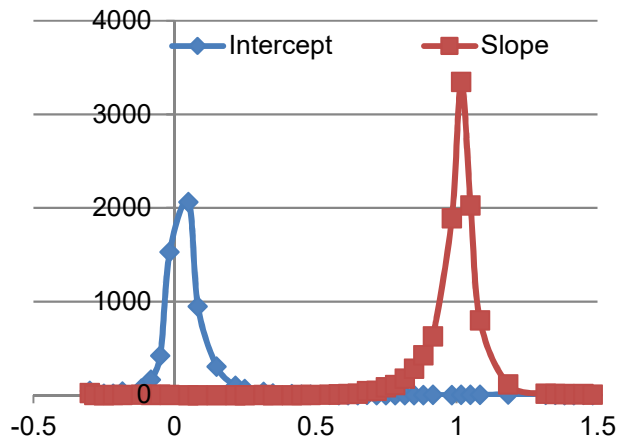
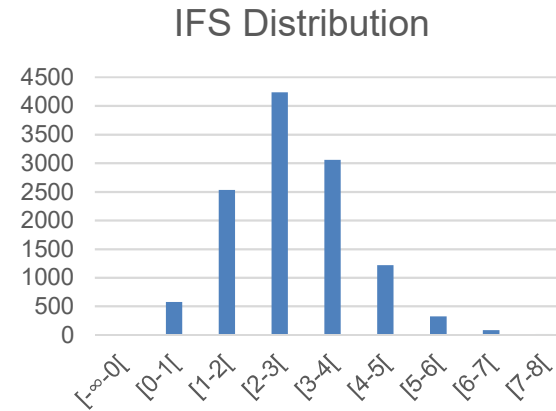


¹⁵N rate: an iterative approach

Ex.: Ctrl sample



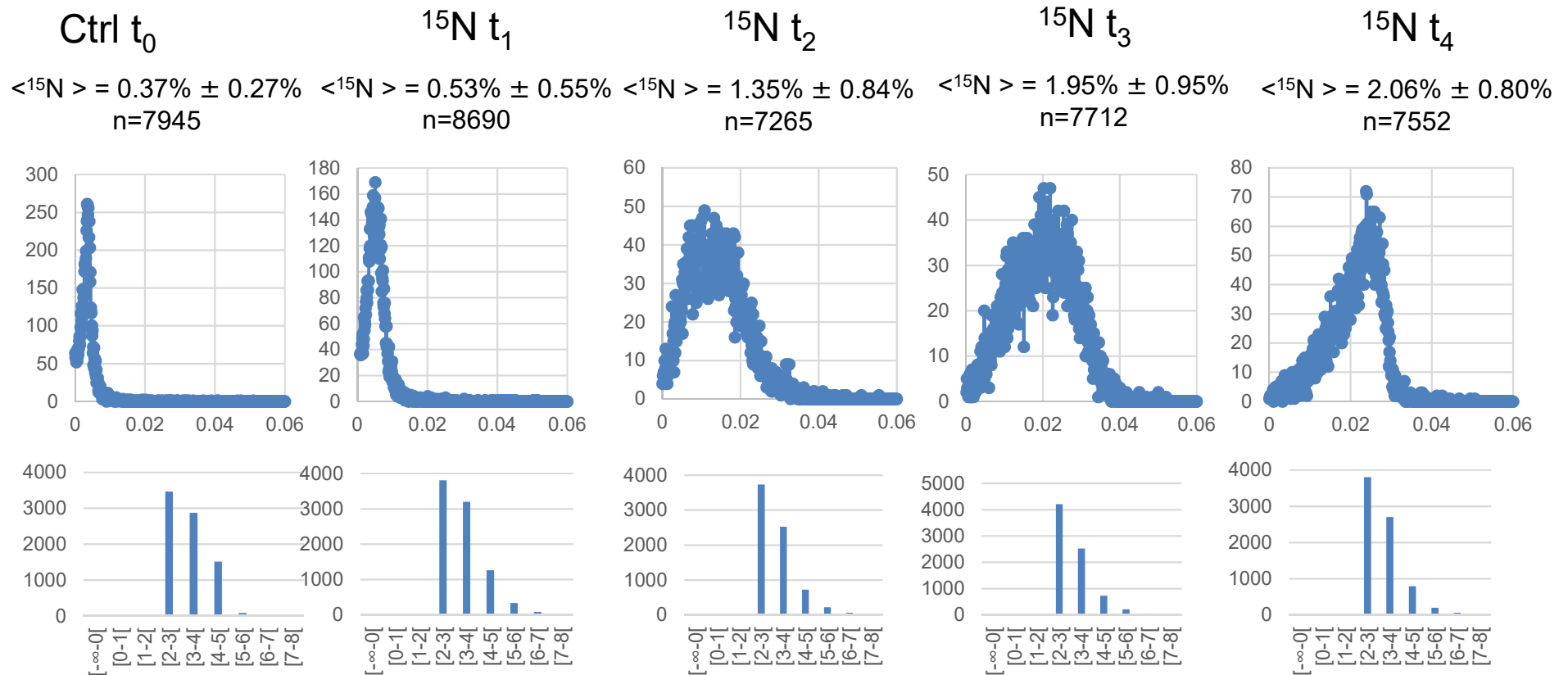
IFS
Slope
Intercept



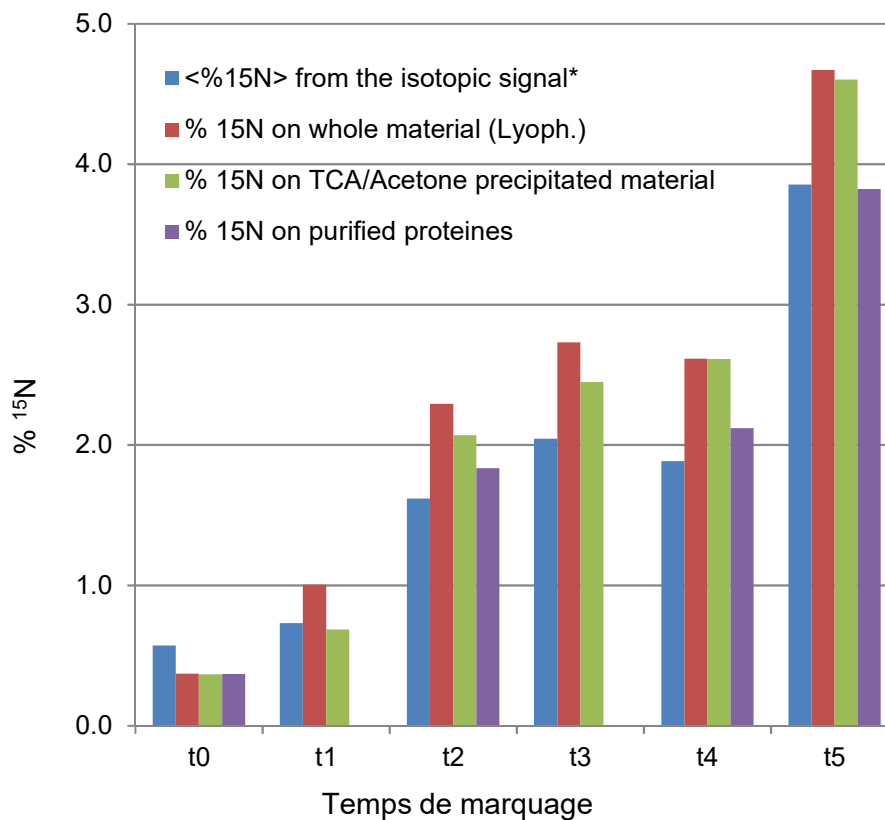
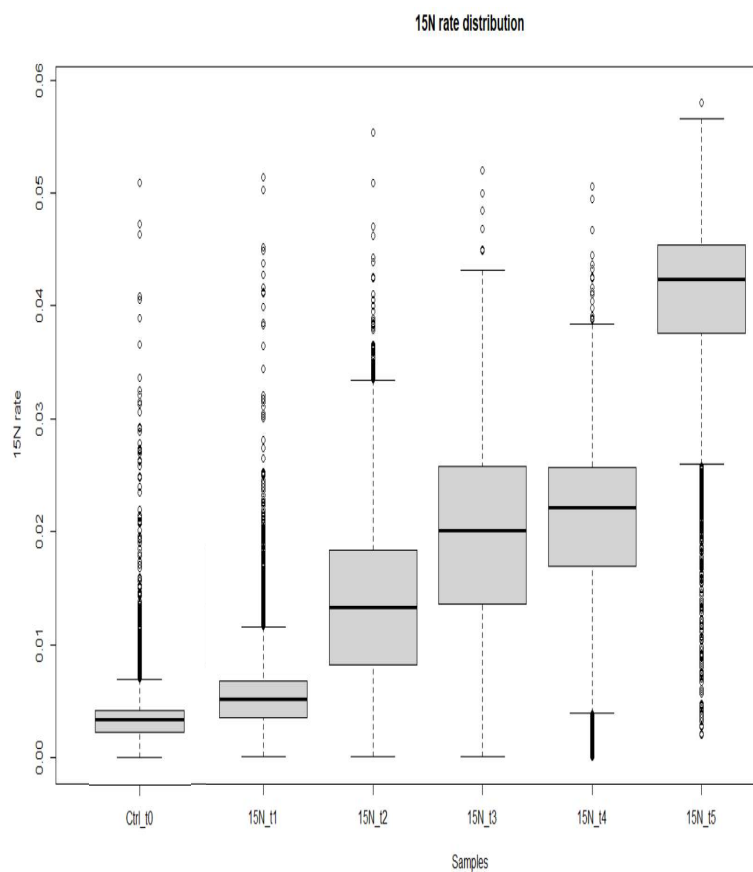
$\langle^{15}\text{N rate}\rangle = 0.37\% \pm 0.27\%$ (n=7945)

^{15}N rate evolution

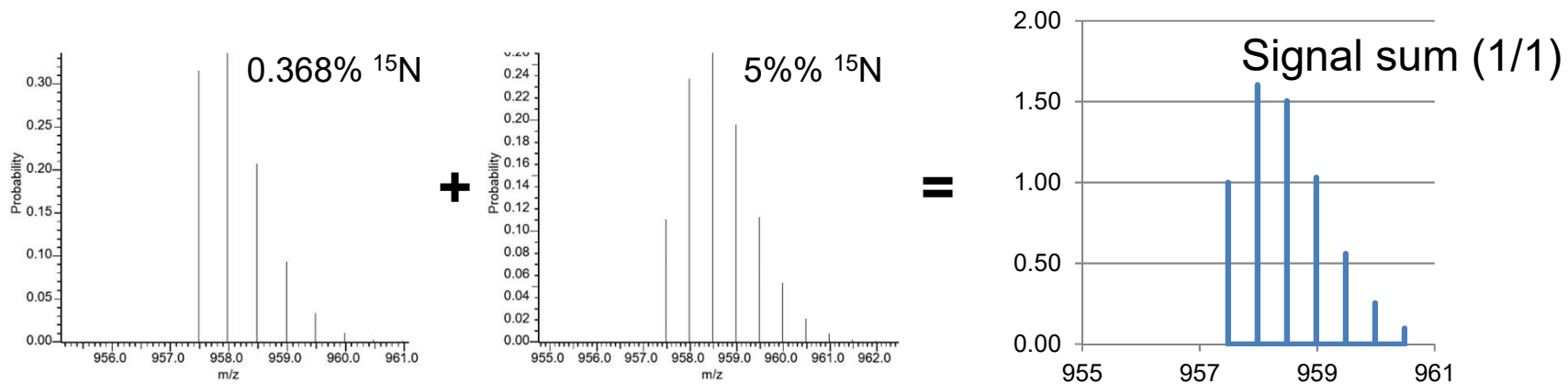
^{15}N Metabolic labelling



“Gold standard” measurement



Apparent ^{15}N rate to LPF



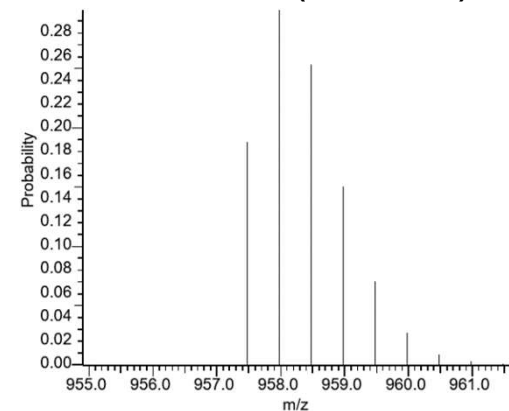
$$\text{LPF} = \frac{[\text{labelled}]}{([\text{initial}] + [\text{labelled}])}$$

$$([\text{initial}] + [\text{labelled}]) = 1$$

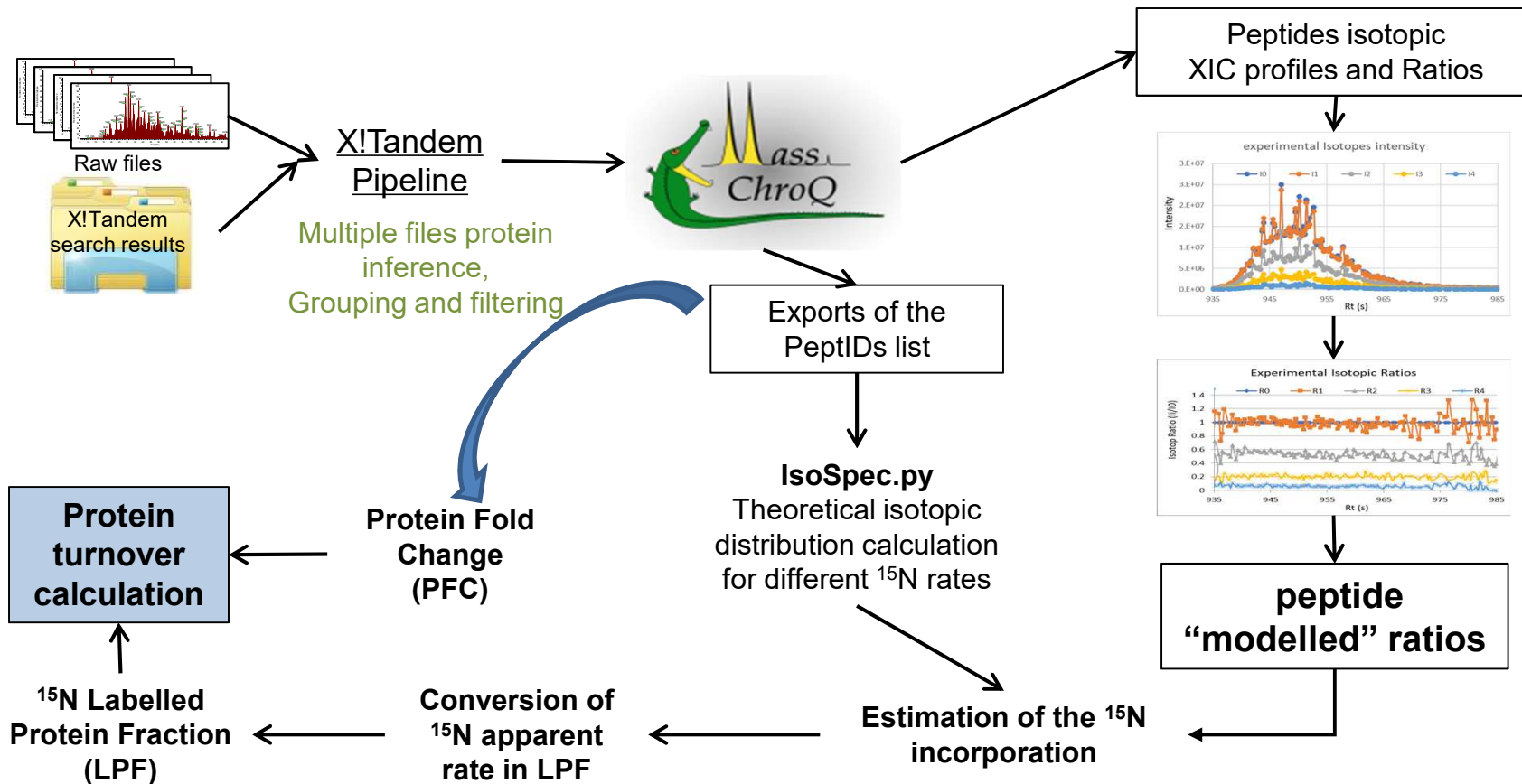
$$^{15}\text{N}_{\text{App}} = \frac{(^{15}\text{N}_{\text{Nat}} \times [\text{initial}] + ^{15}\text{N}_{\text{Max}} \times [\text{labelled}])}{([\text{initial}] + [\text{labelled}])}$$

$$\text{LPF} = \frac{(^{15}\text{N}_{\text{App}} - ^{15}\text{N}_{\text{Nat}})}{(^{15}\text{N}_{\text{Max}} - ^{15}\text{N}_{\text{Nat}})}$$

Theoretical (2.68 %)



Post Acquisition Data Processing



MCQR

- A set of objects and functions for R to handle MassChroQ results:
 - Label free quantitation
 - Developed for natural isotopic abundance!
- Requires some adaptations
 - Isotope selection
 - Fine tuning...

To conclude...

- Low ^{15}N metabolic labelling is compatible with:
 - Peptide characterisation and protein identification
 - Determination of the Protein Fold Change (MCQR)
- Data post acquisition processing provides:
 - Reliable experimental isotopic distributions
 - Evaluate the ^{15}N labelling rates
 - Estimation of the Labelled Protein Fraction (LPF)
- In progress:
 - Improvement of the LPF accuracy
 - Protein label free quantitation even with ^{15}N metabolic labeling
 - R script package under development

Diapositive 26

W6

Willy, 04/05/2022

Acknowledgement's

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

Collaboration with

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

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

