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## Sub optimal $^{15}\text{N}$ 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

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Willy Vincent Bienvenut, Olivier Langella, Thierry Balliau, Filippo Rusconi, Marlène Davanture, et al.. Sub optimal  $^{15}\text{N}$  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-03815633>**

Submitted on 21 Oct 2022

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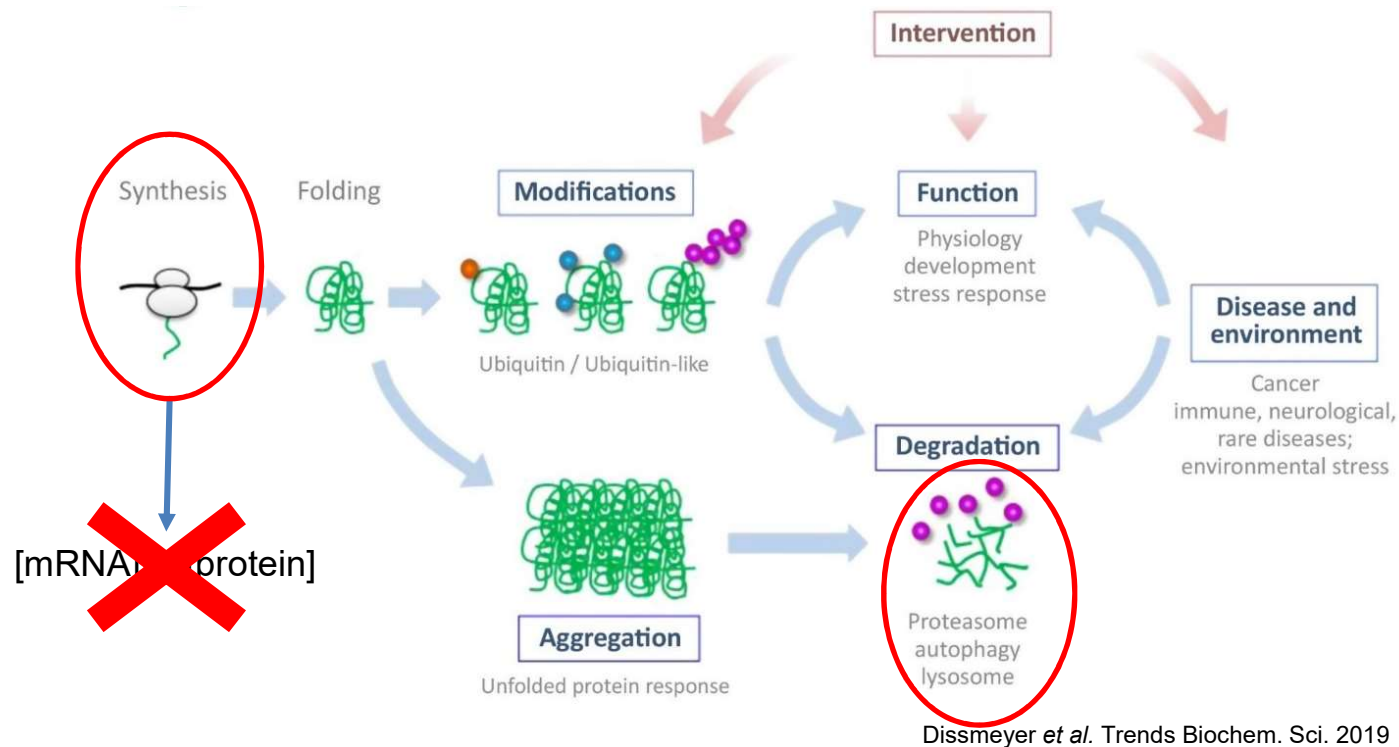
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# Sub optimal $^{15}\text{N}$ metabolic labelling in plant:

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



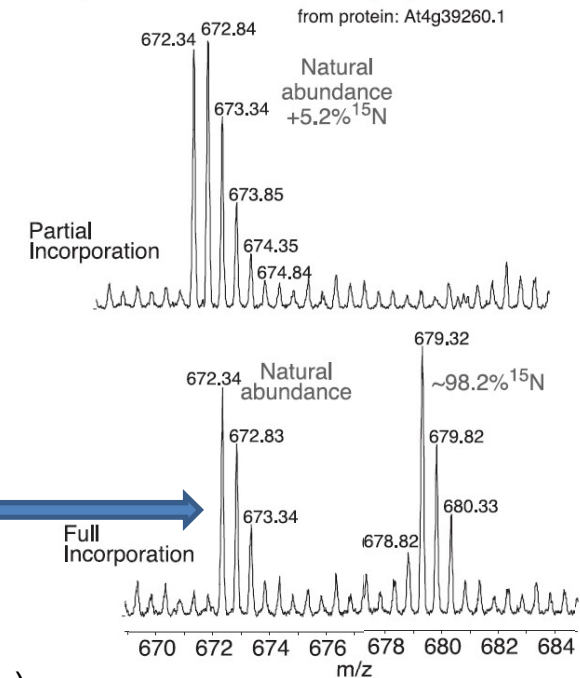
Water, CO<sub>2</sub>, fertilizers and trace elements...

C H N O S

<sup>12</sup>C → <sup>13</sup>C (CO<sub>2</sub>)

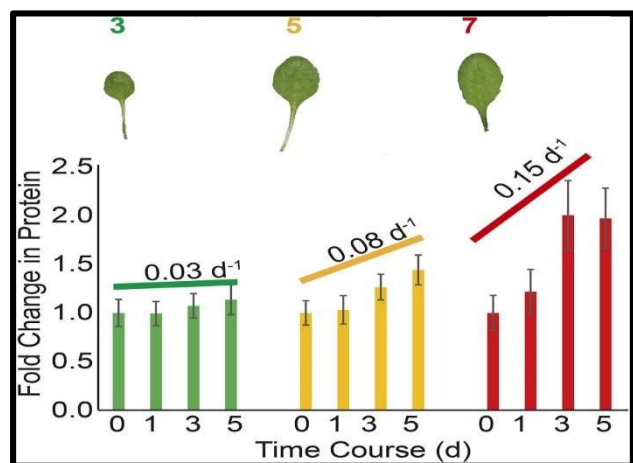
<sup>14</sup>N → <sup>15</sup>N (NH<sub>4</sub>NO<sub>3</sub>)

Peptide: TFSQFGDVIDSK (2+)



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

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

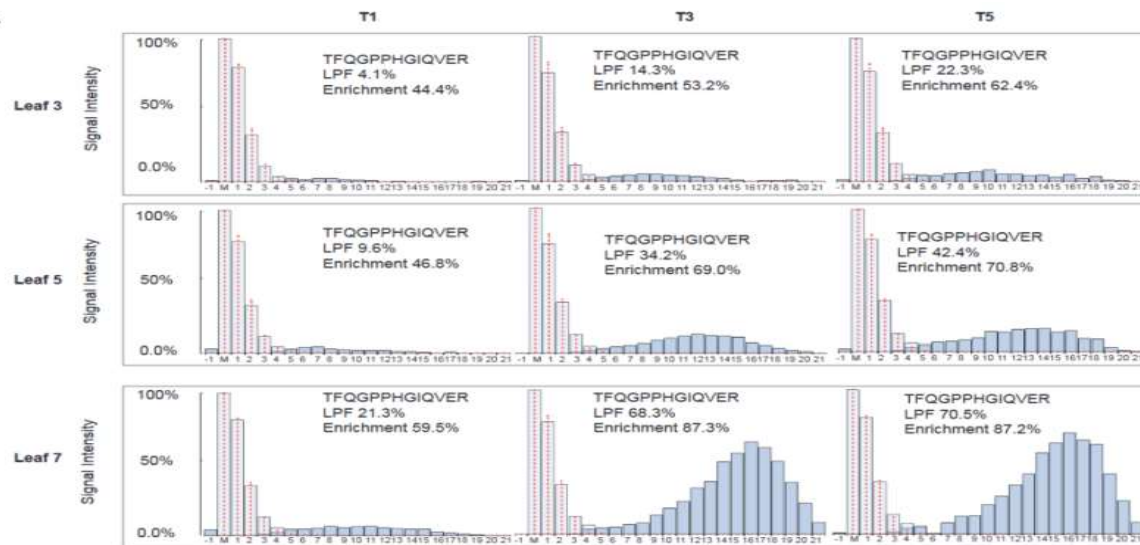


21 days old non-labelled Arabidopsis plants



Determination of the  
FCP coefficient

(Fold Change in Protein)



Determination of the LPF

(Labelled Protein Fraction)

# K<sub>D</sub> and K<sub>S</sub> 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 <sub>D</sub> = - $\frac{\ln FCP \cdot (1 - LPF)}{t}$		$-\frac{\ln 1.07 \cdot (1 - 14.3\%)}{3 \text{ d}} = 0.04 \text{ d}^{-1}$	$-\frac{\ln 1.07 \cdot (1 - 96.3\%)}{3 \text{ d}} = 1.61 \text{ d}^{-1}$
K <sub>S</sub> = $\frac{FCP - e^{-K_D t}}{1 - e^{-K_D t}} \cdot K_D$		$\frac{1.07 - e^{-0.04 \cdot 3}}{1 - e^{-0.04 \cdot 3}} \cdot 0.04 = 0.07 \text{ d}^{-1}$	$\frac{1.07 - e^{-1.61 \cdot 3}}{1 - e^{-1.61 \cdot 3}} \cdot 1.61 = 1.73 \text{ d}^{-1}$

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

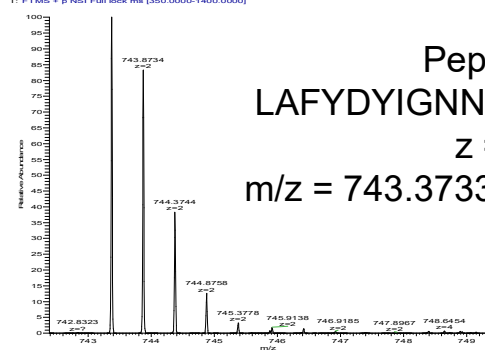
# Large scale protein turnover measurement...

- Proposed alternative
  - **Low labelling rate (5-6% vs. >70-90%  $^{15}\text{N}$  )**
    - **Advantage :**
      - **Peptide isotopic distribution is moderately modified!!!**
        - Heavy & light isotopomers overlay
        - Minor sample complexity increase
    - *Bottlenecks...*
      - **Intensive post-acquisition data processing:**
        - $^{15}\text{N}$  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...



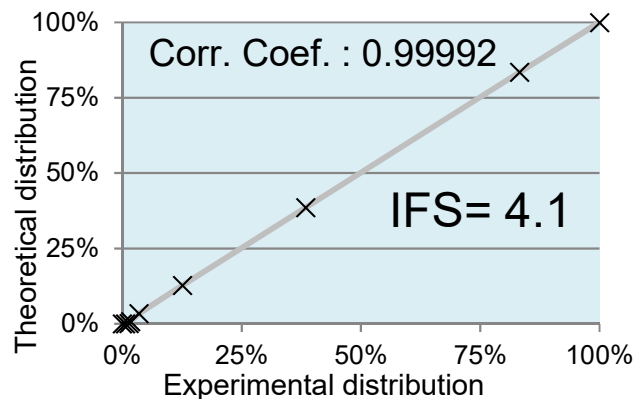
# The isotopic distribution world...

20200110\_Tcharnoz\_90\_114 #19204-19208 REF: 64.05-64.83 AV: 51 NL: 9.46E7  
T: FTMS - p MS1 Full lock ms (250.0000-1800.0000)

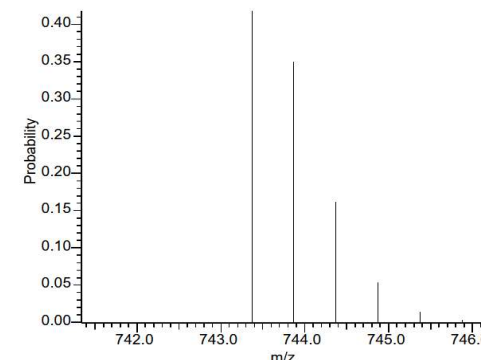


Peptide experimental isotopic distribution

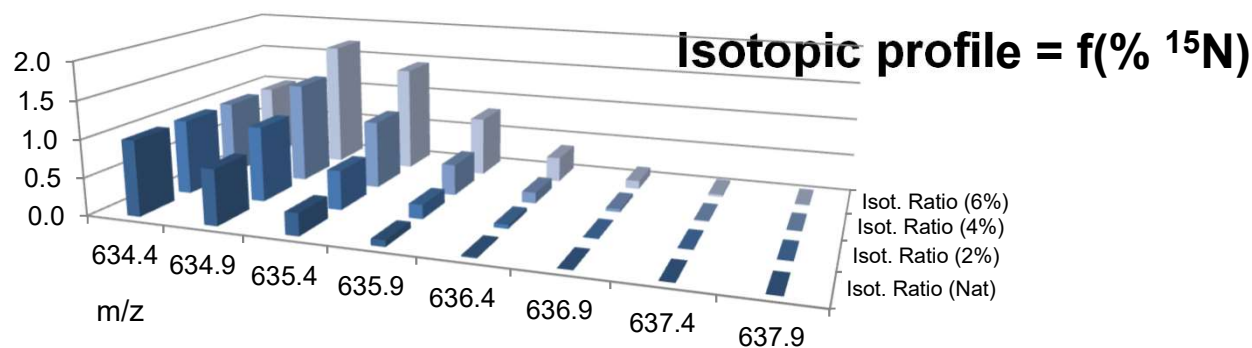
Peptide:  
LAFYDYIGNNPAK  
z = 2+  
m/z = 743.3733 Th



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



Peptide simulated isotopic distribution





# Standard samples preparation and proteins ID



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

<sup>15</sup>N labelling  
pulse

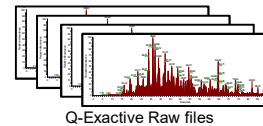
½ MS media 10%  
of <sup>15</sup>N  
(90% of <sup>14</sup>N)

Collecting  
samples at  
multiple time  
points

Standard proteomics  
sample preparation



RP-LC Q-Exactive analysis



Q-Exactive Raw files

Raw file mzML  
conversion

X!Tandem  
**X!**

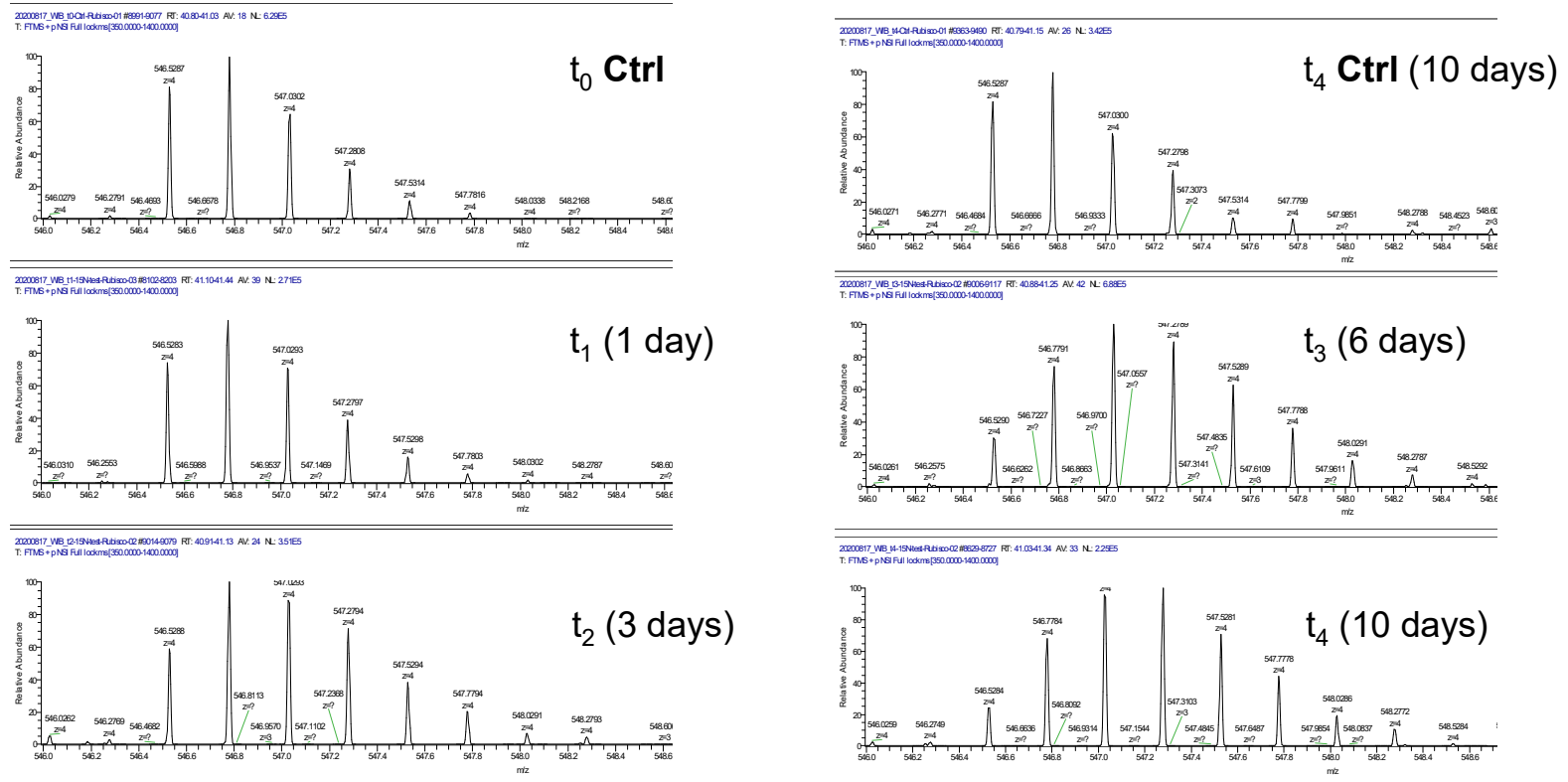
*Zm-B73-REFERENCE-  
DB.protein.fasta*



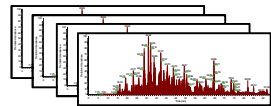
List of identified  
peptides and proteins

# Isotopic distribution metabolic labelling kinetics

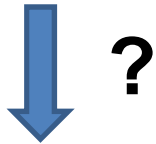
Peptide sequence: AGVKEYKLTYYTPEYETK



# Post Acquisition Data Processing

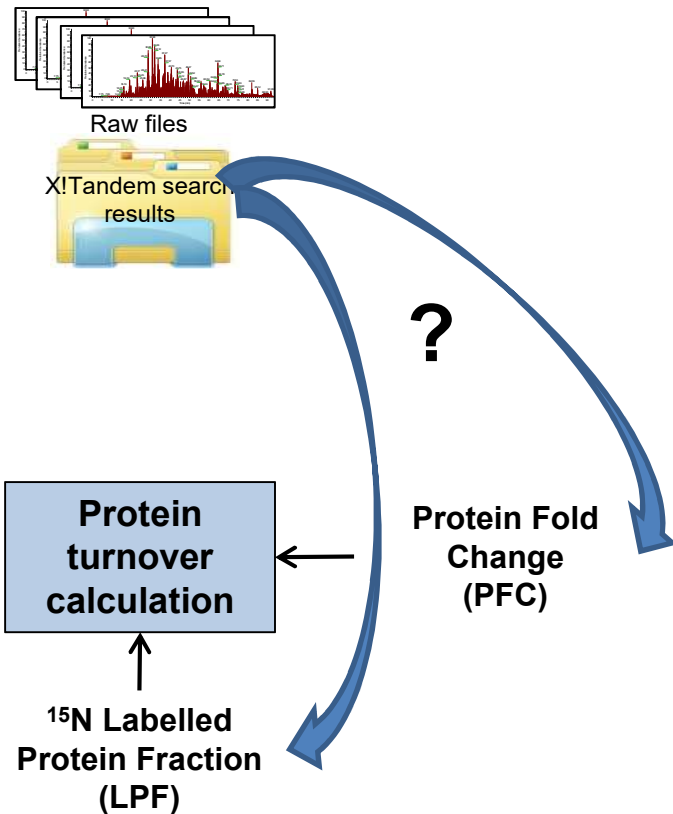


Raw files

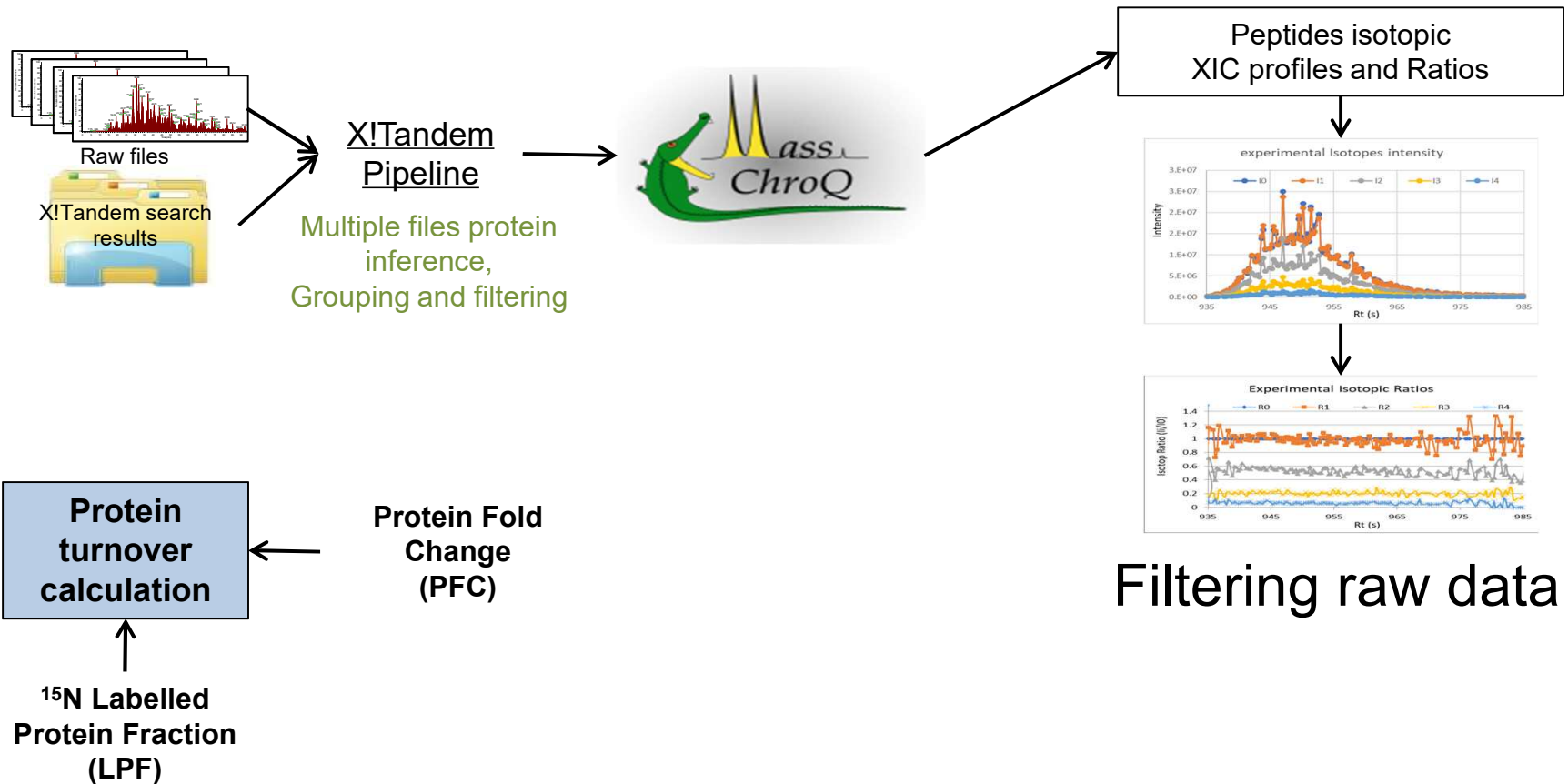


**Protein  
turnover  
calculation**

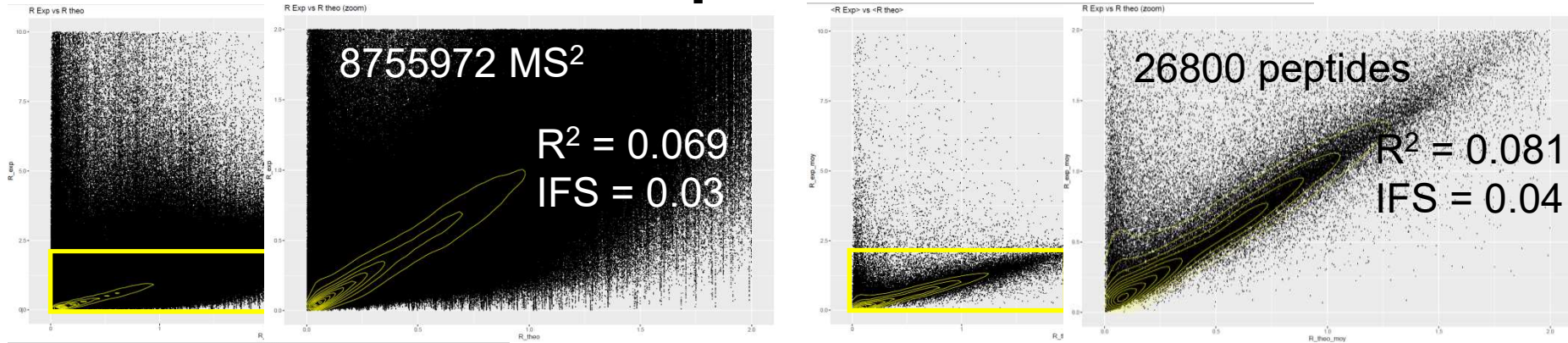
# Post Acquisition Data Processing



# Post Acquisition Data Processing

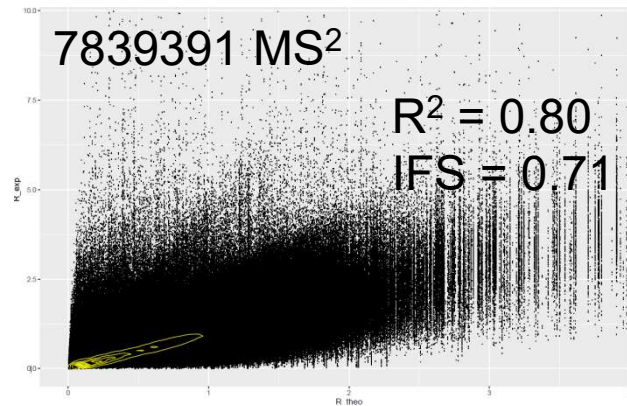


# A view Exp vs Theo Ri...



## Pre-processing

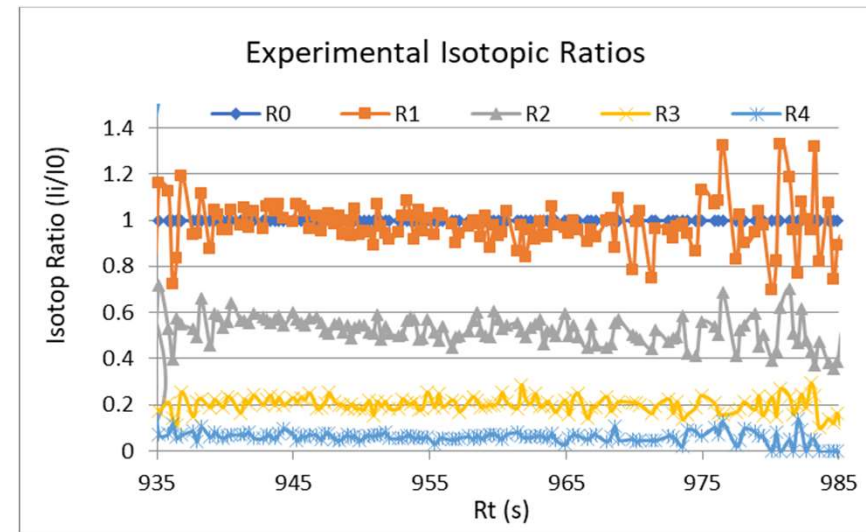
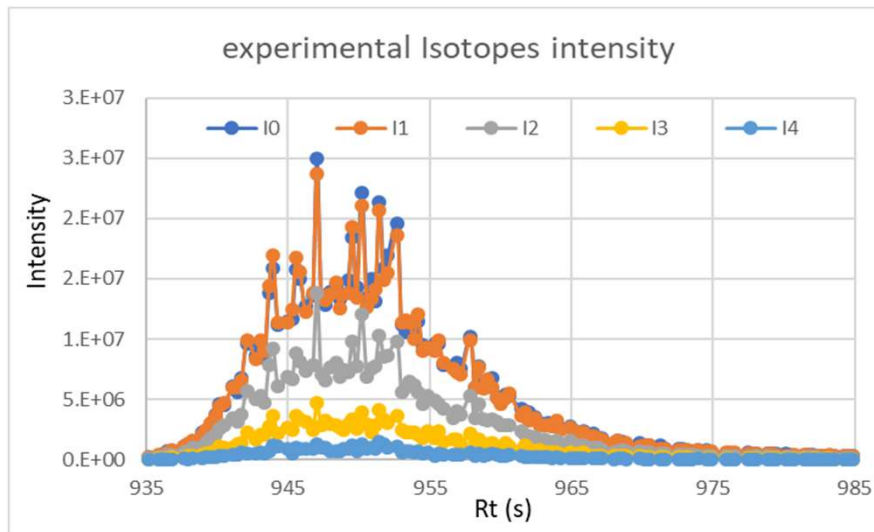
Removing outliers:  $R_{i \text{ Exp}}(\text{pepID}) < R_{i \text{ Theo}}(\text{pepID}) * 1.2$  at 6% <sup>15</sup>N





# Filtering data

## 2 levels filters



Inter-Spectrum Correlation

Peptide Ri distribution



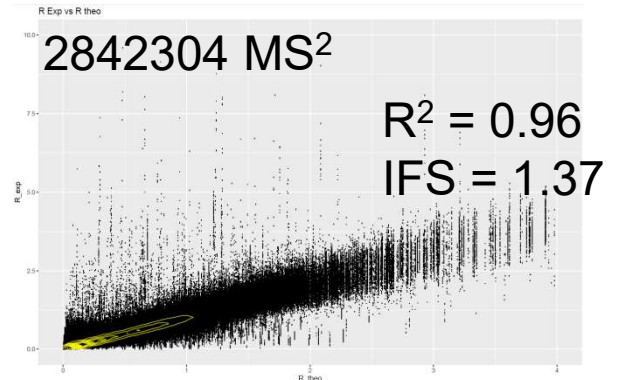
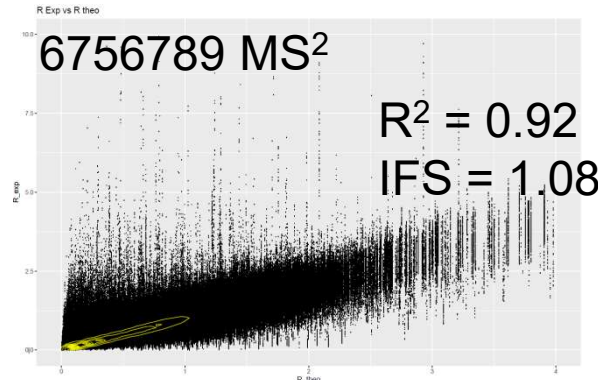
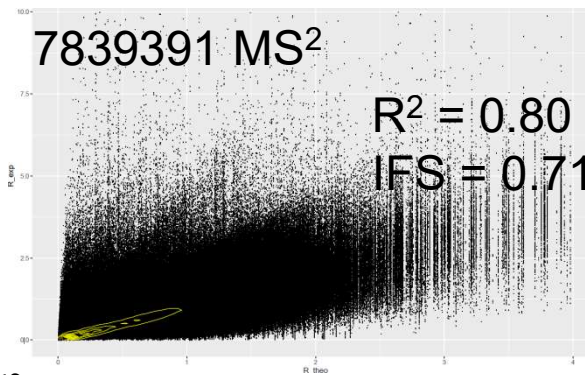
# Isotopic ratios and profile filtering

2 levels filters

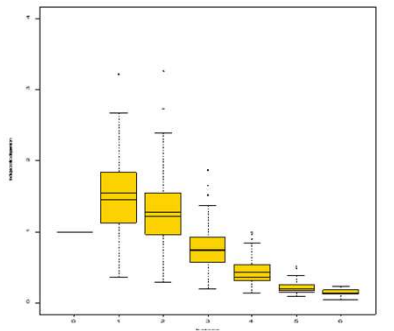
Raw ratios

Peptide( $R_i$ ) dist.

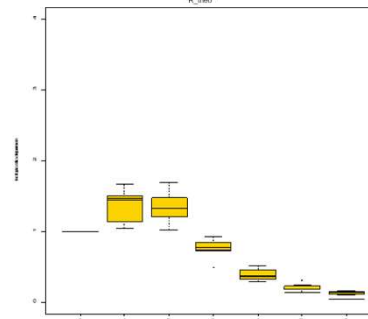
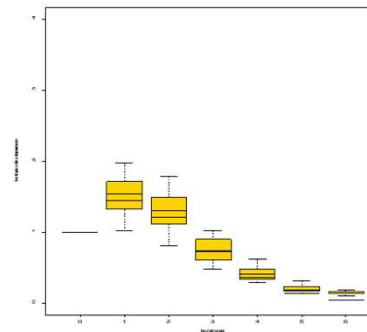
Spectrum<sup>2</sup> Corr.



Single peptide Ratios



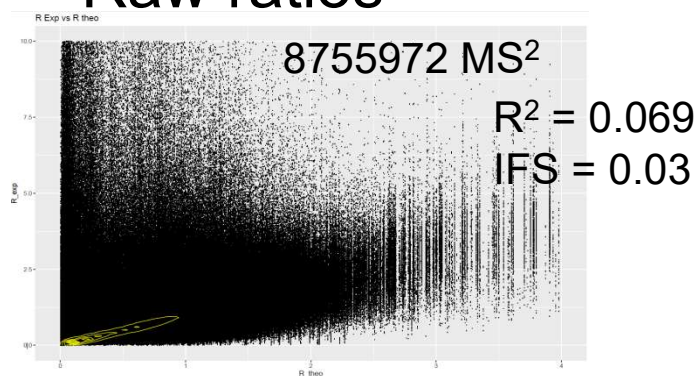
$\langle R_i \rangle - 2 SD < R_i < \langle R_i \rangle + 2 SD$



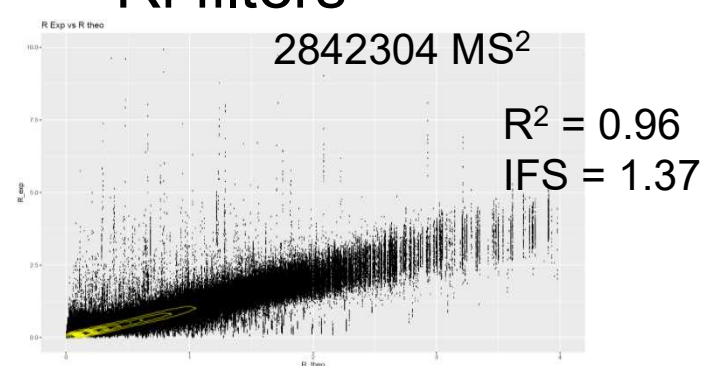
MS Corr. > 0.9

# Additional filters ...

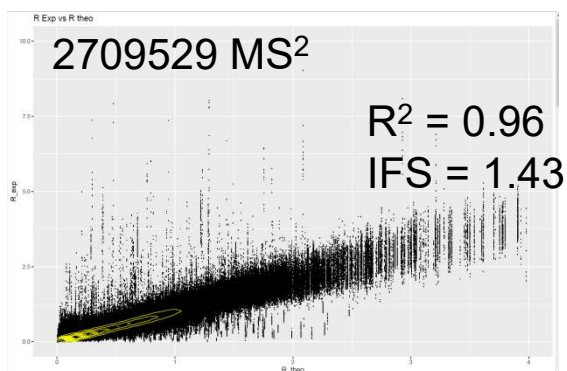
## Raw ratios



## Ri filters

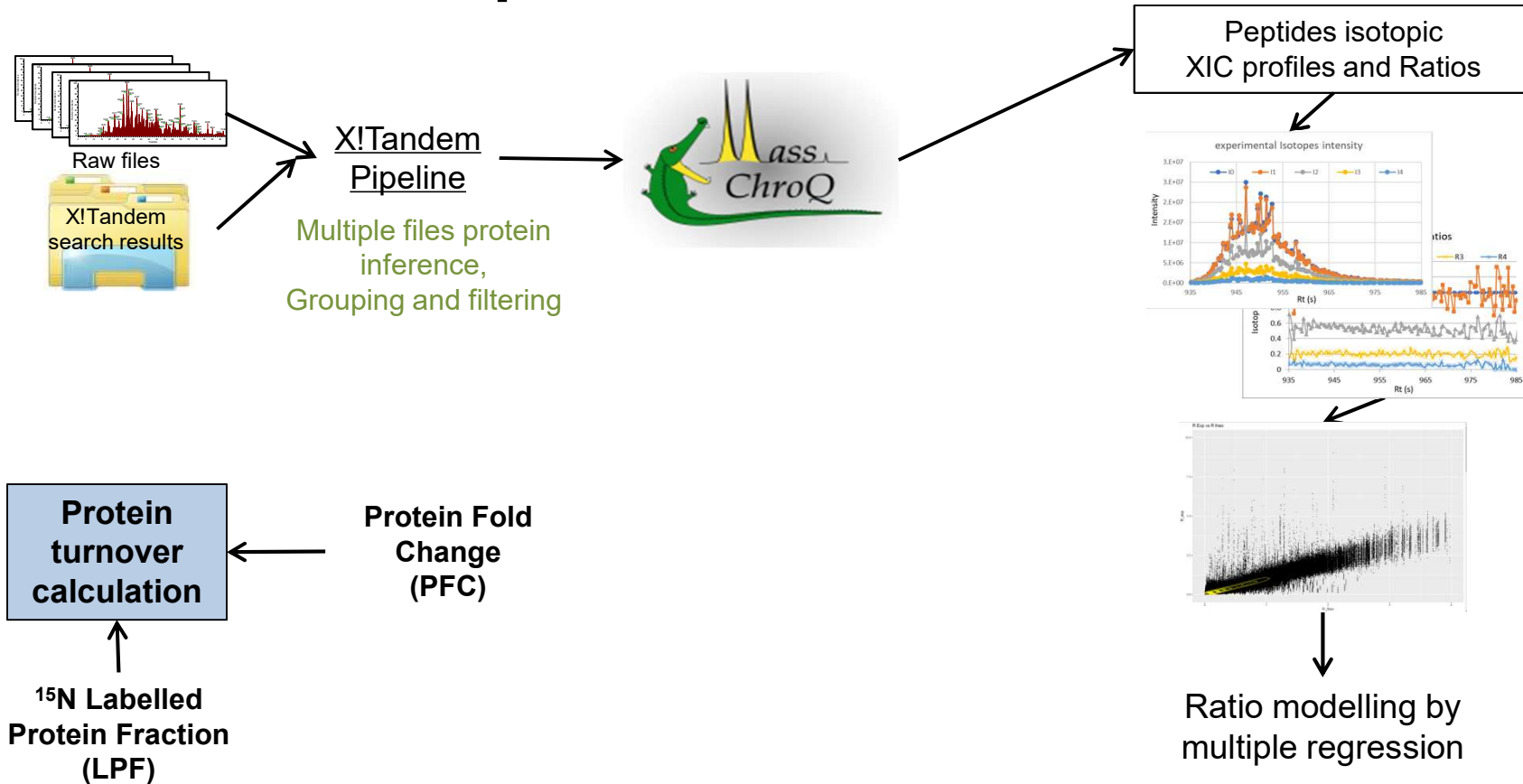


**Favouring data redundancy**



Redundant measurements  
in multiples replicates ( $\geq 2$  out of 4 MS run)  
Minimum isotopes ratios ( $\geq 4$  I)  
Minimum number of spectra ( $\geq 5$  MS<sup>2</sup>)

# Post Acquisition Data Processing



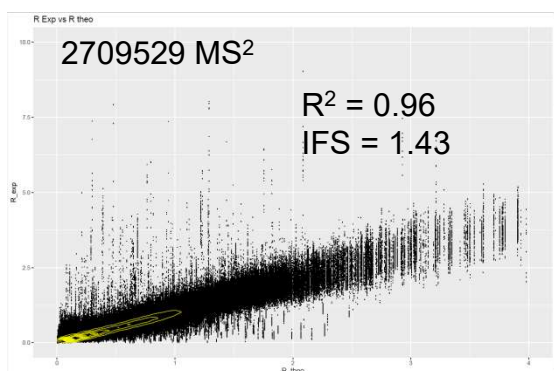
# Post Acquisition Data Processing

## Highly redundant peptide isotopic profiles

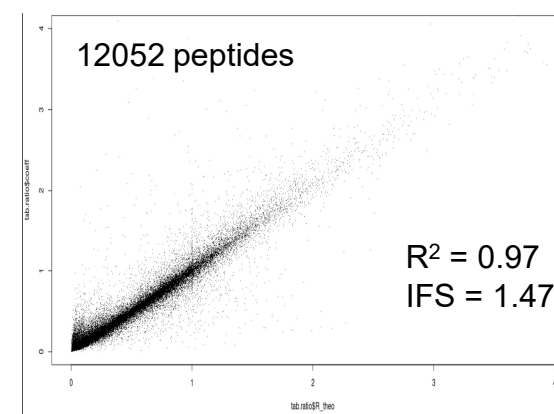
Multiple acquisitions (Triplicate at least)  
Multiple Rt (10-30 s peptide elution)  
Multiple z (2+,3+...)

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

isotope<sub>i</sub>: isotope i fixed effect  
MSrun<sub>j</sub>: MS run j fixed effect  
RT<sub>k</sub>: = retention time random effect  
ε<sub>ijk</sub>: residual

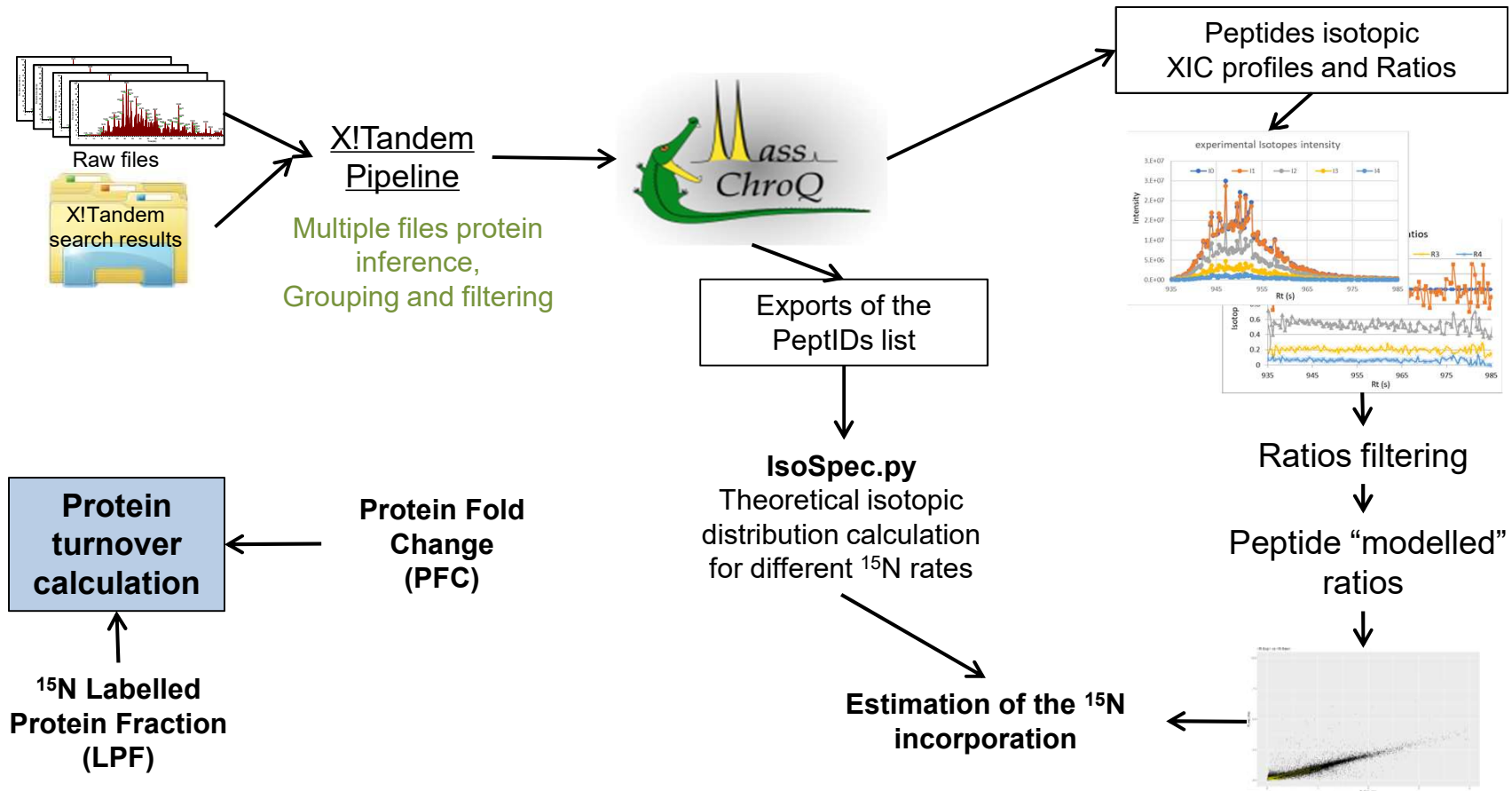


Ratio modelled by  
multiple regression



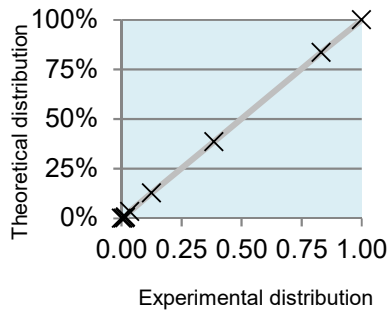
Single isotopic distribution per non-redundant peptide

# Post Acquisition Data Processing

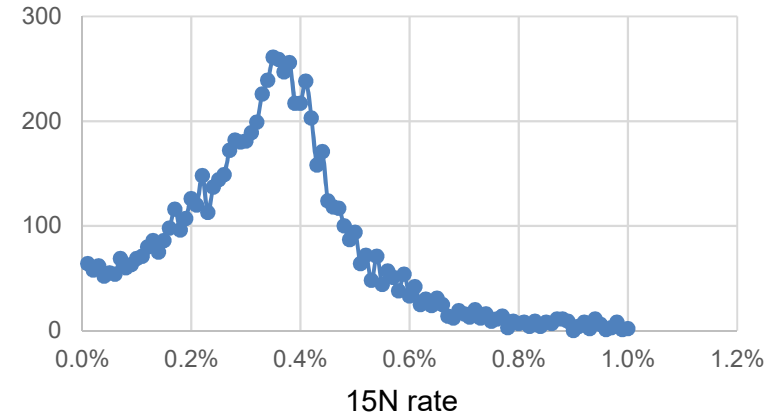
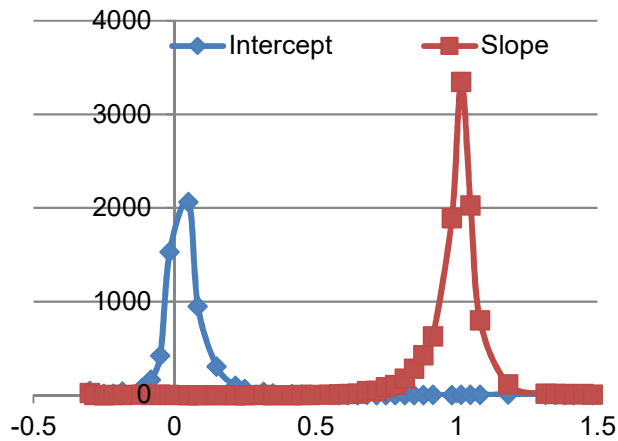
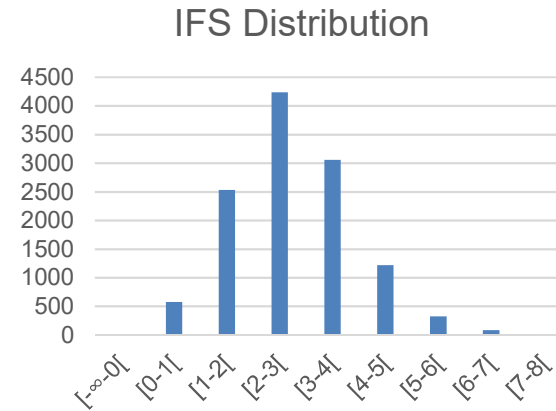


# <sup>15</sup>N rate: an iterative approach

Ex.: Ctrl sample



**IFS**  
**Slope**  
**Intercept**

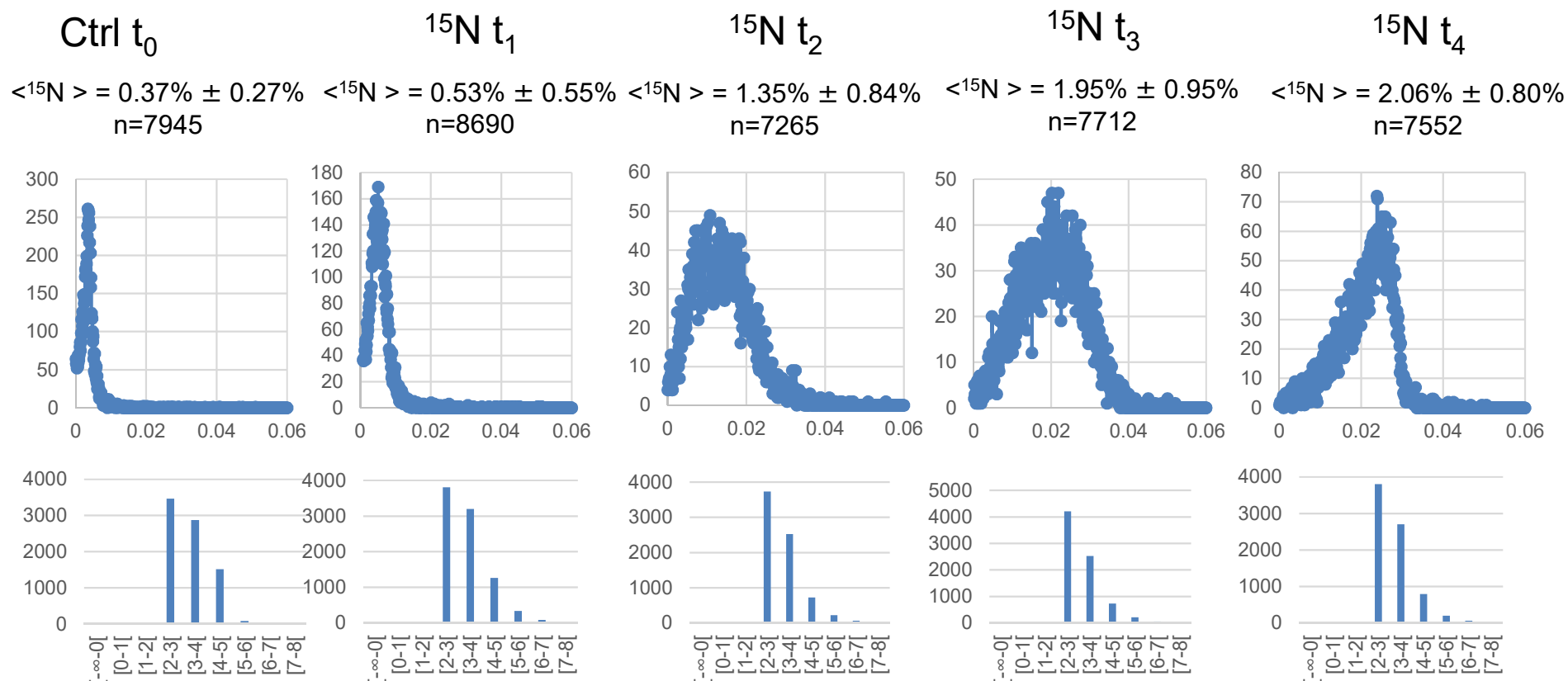


$\langle^{15}\text{N rate}\rangle = 0.37\% \pm 0.27\%$  (n=7945)



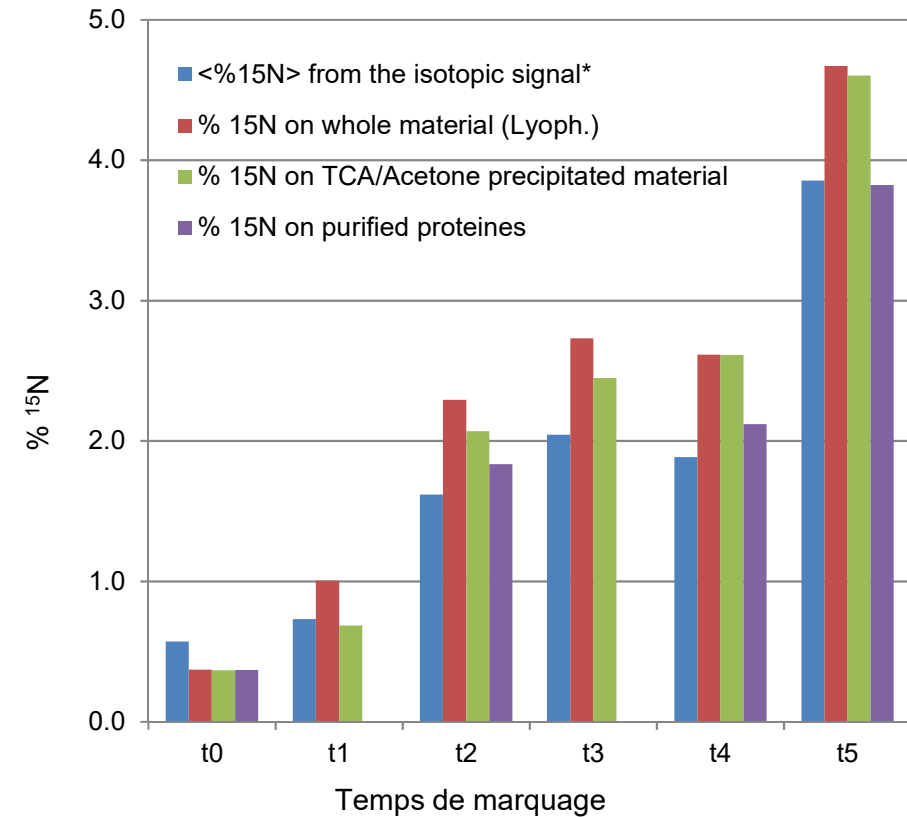
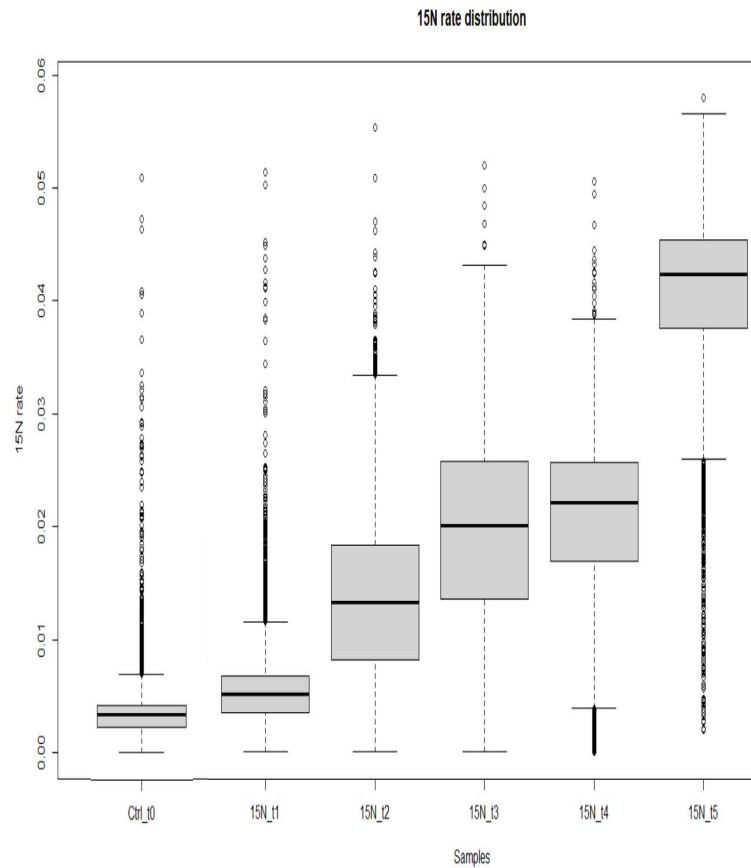
# $^{15}\text{N}$ rate evolution

## $^{15}\text{N}$ Metabolic labelling

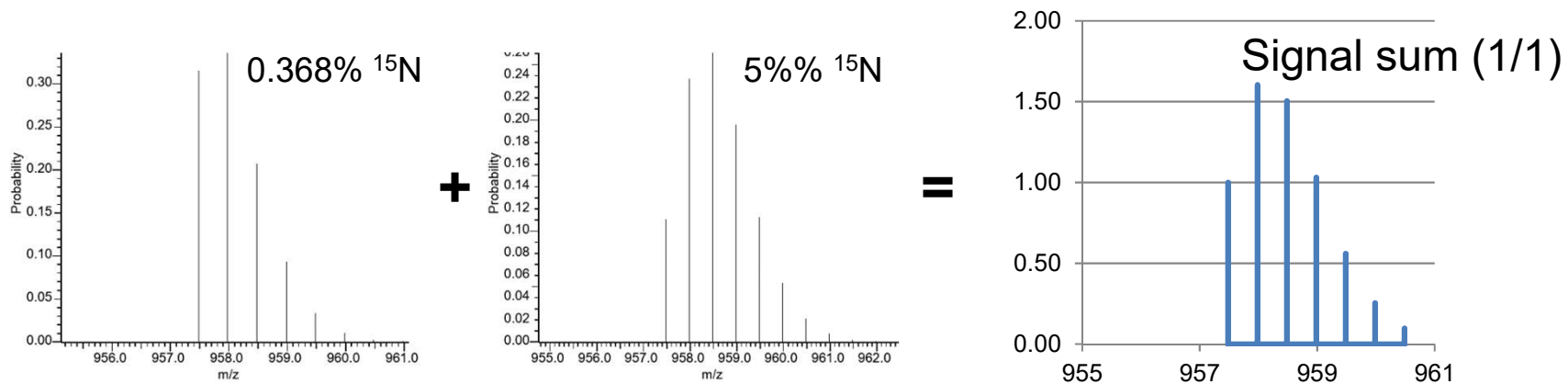




# “Gold standard” measurement



# Apparent $^{15}\text{N}$ rate to LPF



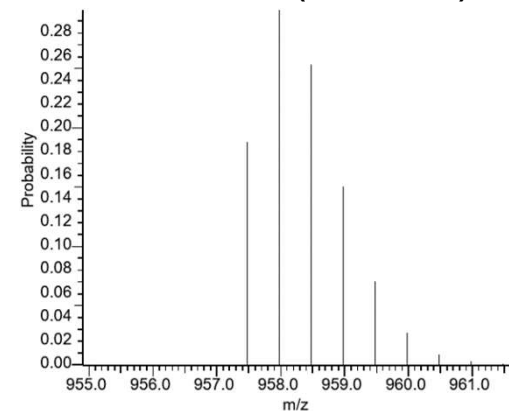
$$\text{LPF} = \frac{[\text{labelled}]}{([\text{initial}] + [\text{labelled}])}$$

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

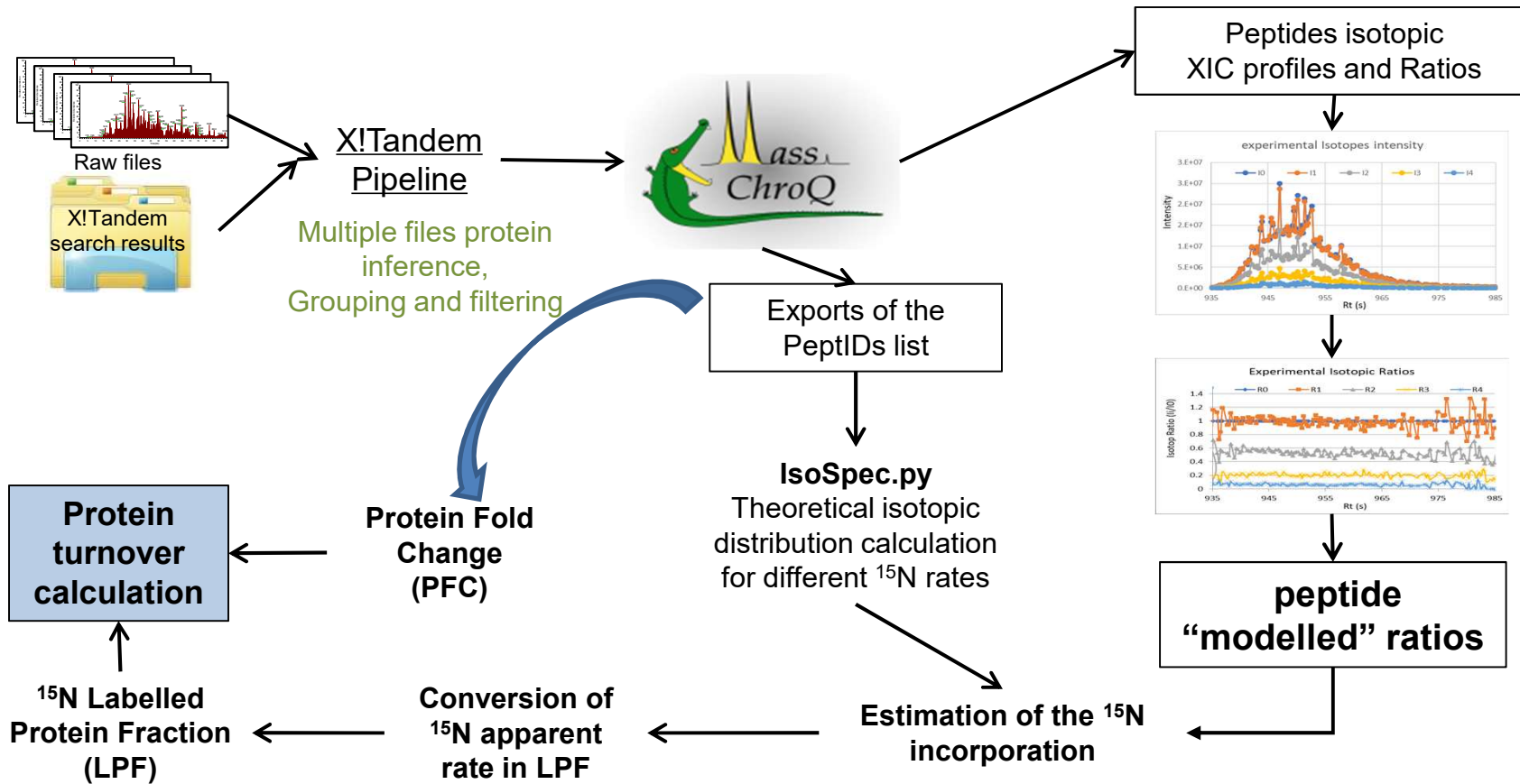
$$^{15}\text{N}_{\text{App}} = \frac{(^{15}\text{N}_{\text{Nat}} \times [\text{initial}] + ^{15}\text{N}_{\text{Max}} \times [\text{labelled}])}{([\text{initial}] + [\text{labelled}])}$$

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

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  $^{15}\text{N}$  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  $^{15}\text{N}$  labelling rates
    - Estimation of the Labelled Protein Fraction (LPF)
- In progress:
  - Improvement of the LPF accuracy
  - Protein label free quantitation even with  $^{15}\text{N}$  metabolic labeling
  - R script package under development

## Diapositive 26

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**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)

