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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

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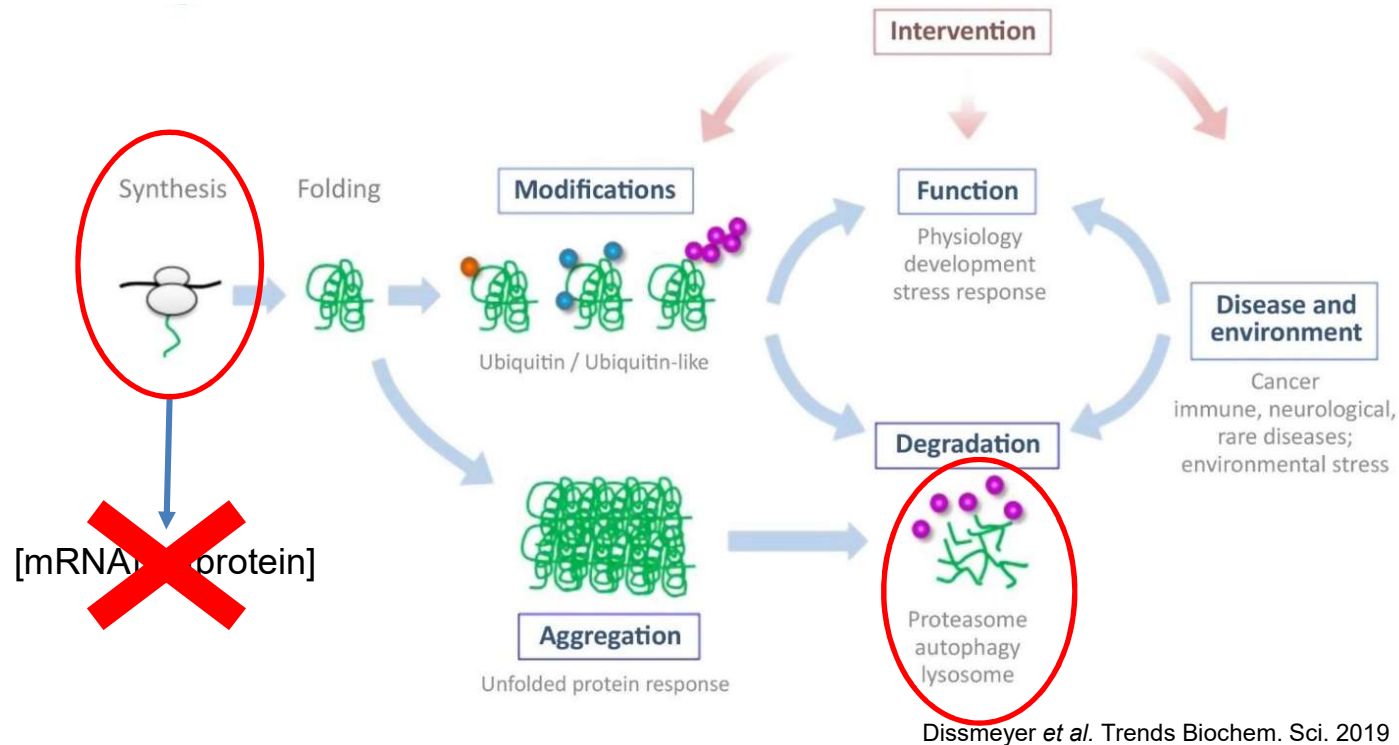
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Sub optimal ^{15}N metabolic labelling in plant:

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



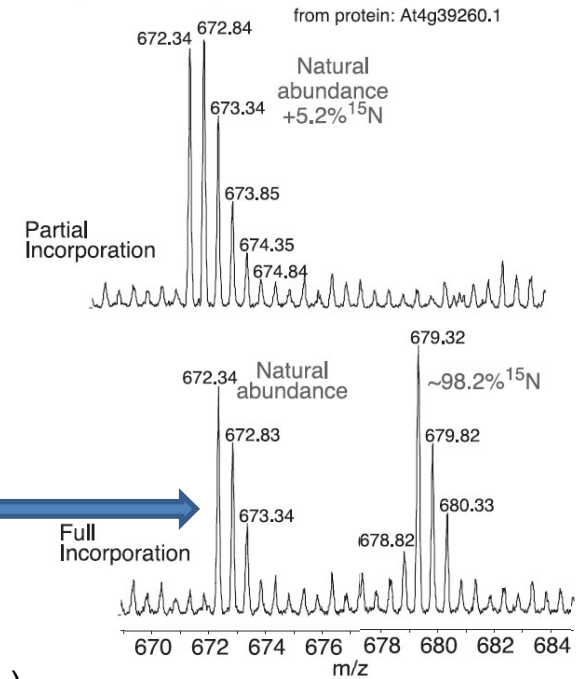
Water, CO₂, fertilizers and trace elements...

C H N O S

¹²C → ¹³C (CO₂)

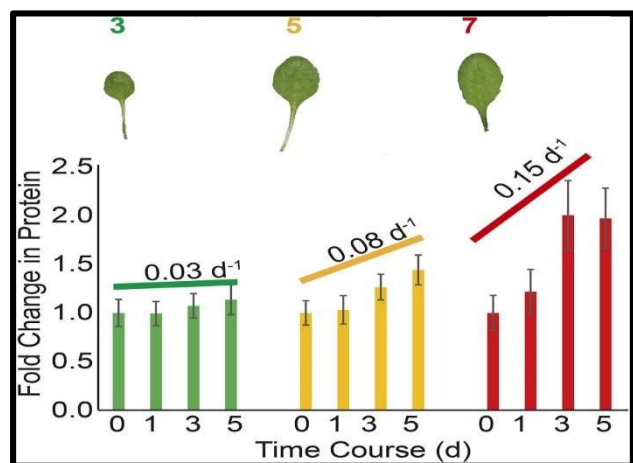
¹⁴N → ¹⁵N (NH₄NO₃)

Peptide: TFSQFGDVIDSK (2+)



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

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

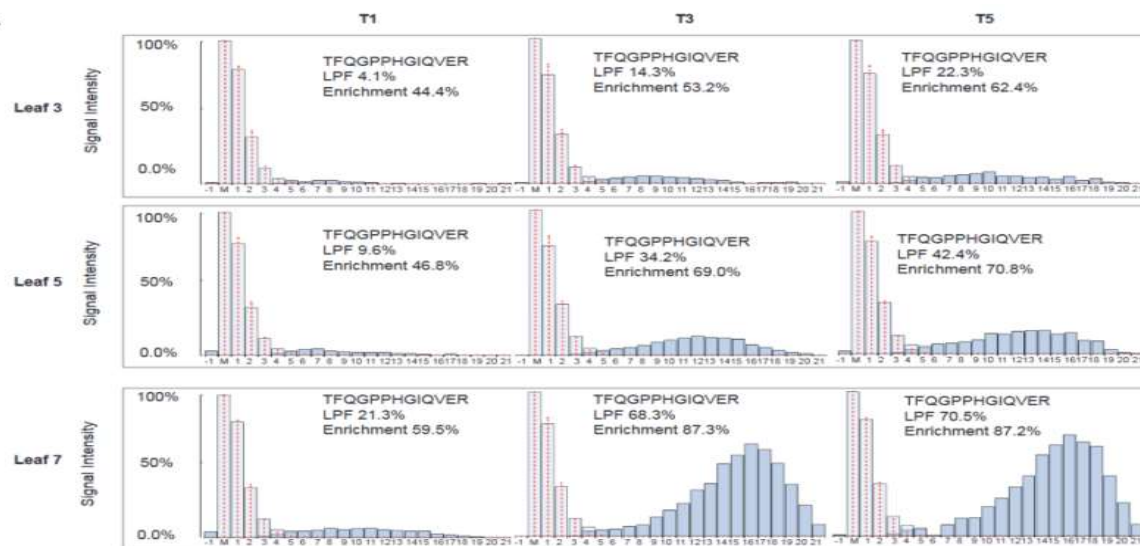


21 days old non-labelled Arabidopsis plants



Determination of the
FCP coefficient

(Fold Change in Protein)



Determination of the LPF

(Labelled Protein Fraction)

K_D and K_S calculation

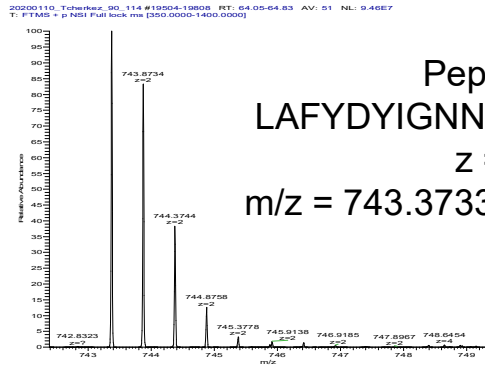
		RBCL (TFQGPPHGIQVER)	THI1 (MGPTFAMMISGQK)
Fold Change in Protein Abundance (FCP)	T0	1	1
	T3	1.07	1.07
Labelled Protein Abundance (LPF)	T0	0	0
	T3	14.3%	96.3%
K _D = - $\frac{\ln FCP \cdot (1 - LPF)}{t}$		$-\frac{\ln 1.07 \cdot (1 - 14.3\%)}{3 \text{ d}} = 0.04 \text{ d}^{-1}$	$-\frac{\ln 1.07 \cdot (1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

- 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...

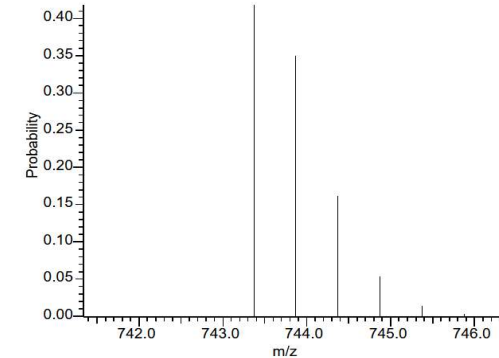
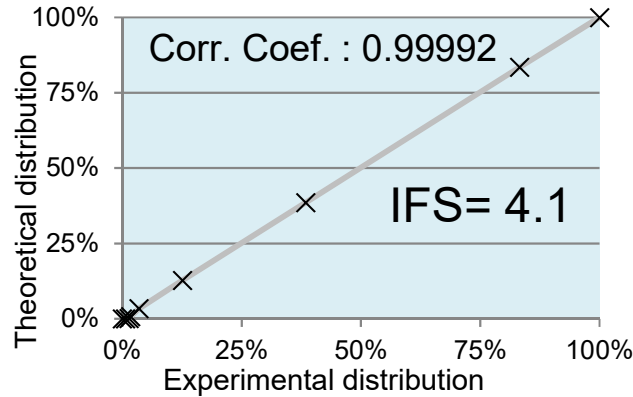
- Proposed alternative
 - **Low labelling rate (5-6% vs. >70-90% ^{15}N)**
 - **Advantage :**
 - **Peptide isotopic distribution is moderately modified!!!**
 - Heavy & light isotopomers overlay
 - Minor sample complexity increase
 - *Bottlenecks...*
 - **Intensive post-acquisition data processing:**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



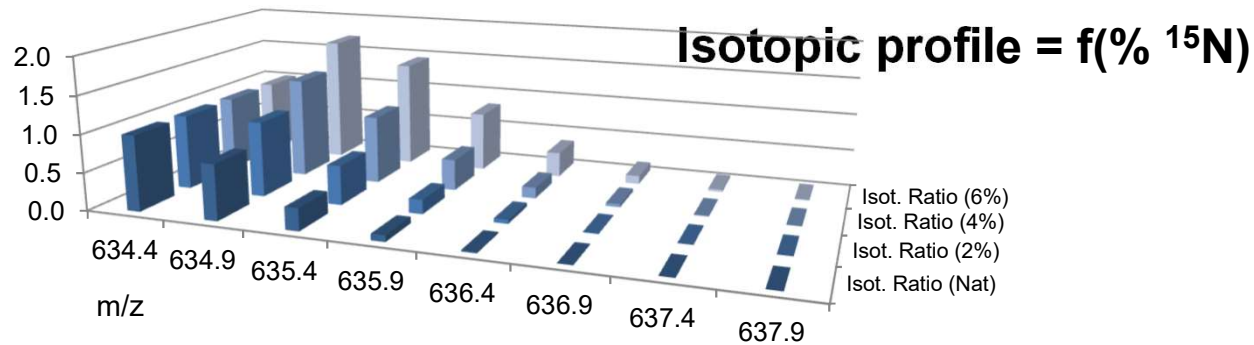
Peptide experimental isotopic distribution

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

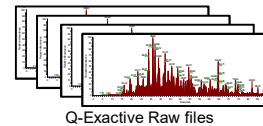
½ MS media 10%
of ¹⁵N
(90% of ¹⁴N)

Collecting
samples at
multiple time
points

Standard proteomics
sample preparation



RP-LC Q-Exactive analysis



Q-Exactive Raw files

Raw file mzML
conversion

X!Tandem
X!

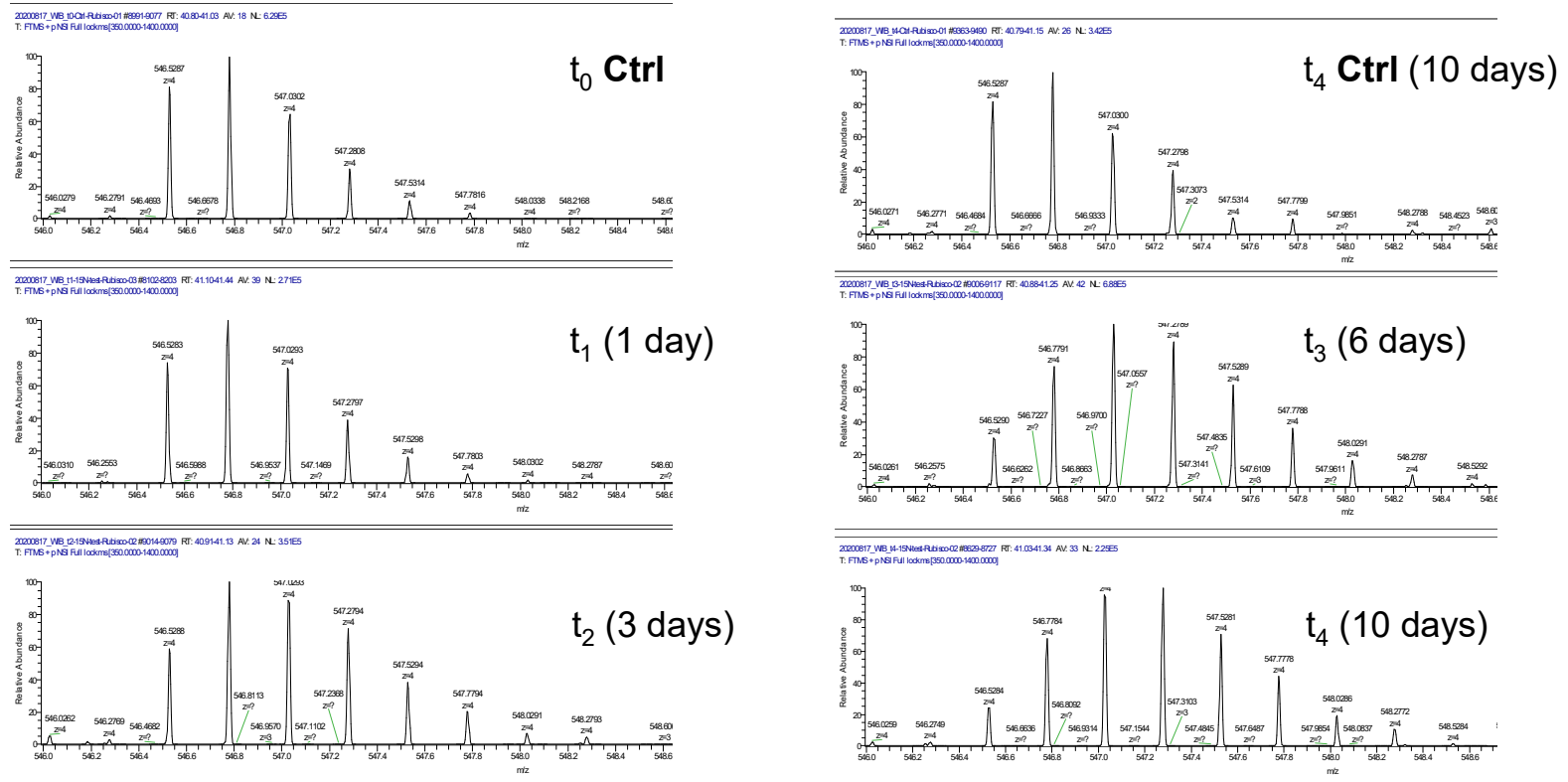
*Zm-B73-REFERENCE-
DB.protein.fasta*



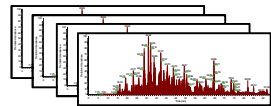
List of identified
peptides and proteins

Isotopic distribution metabolic labelling kinetics

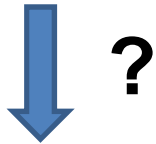
Peptide sequence: AGVKEYKLTYYTPEYETK



Post Acquisition Data Processing

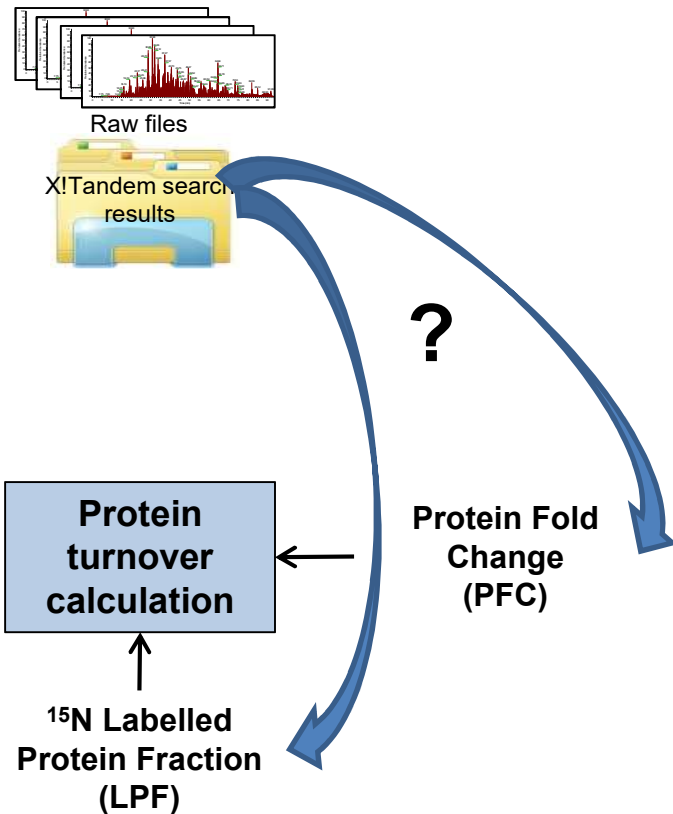


Raw files

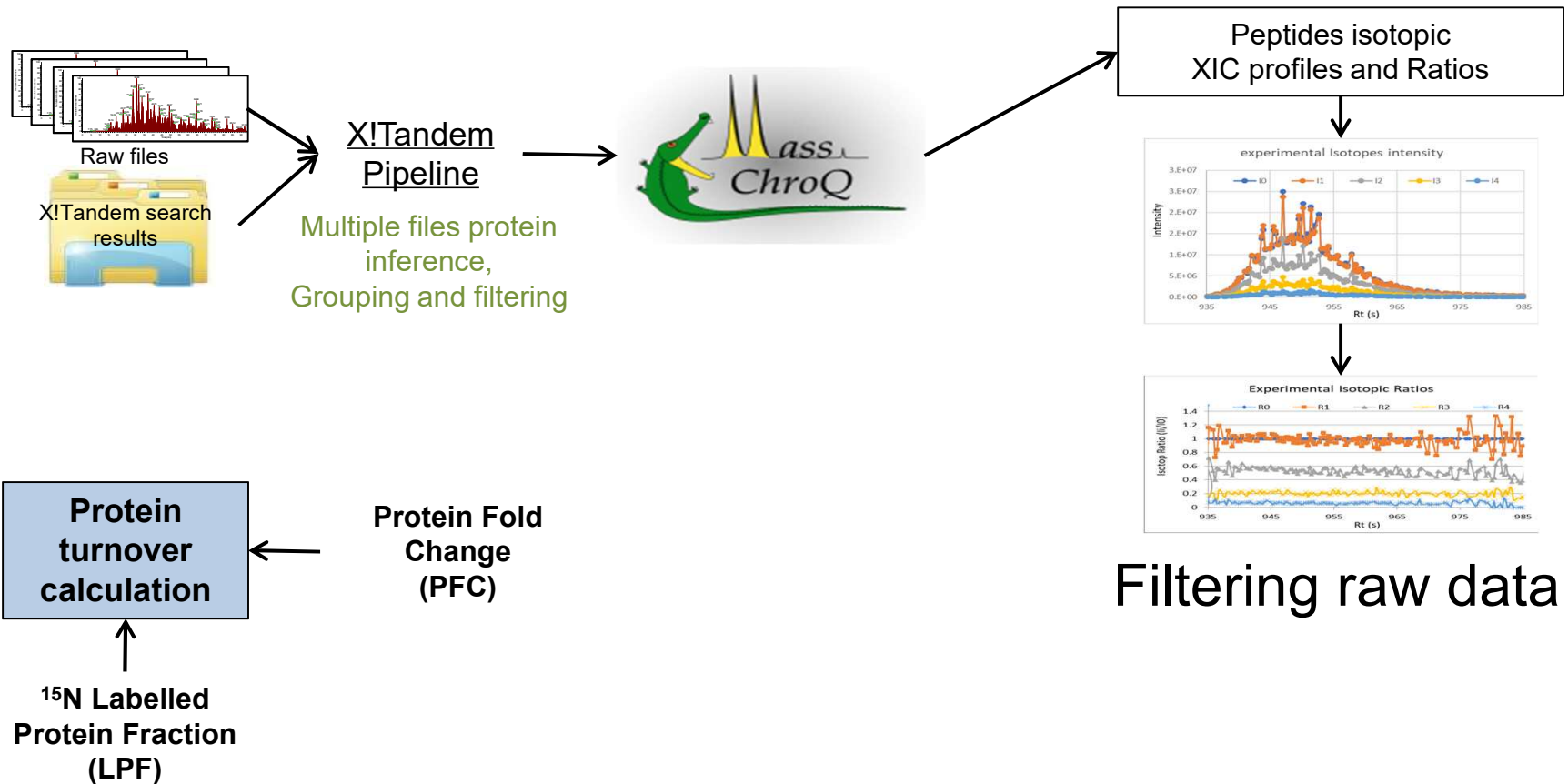


Protein
turnover
calculation

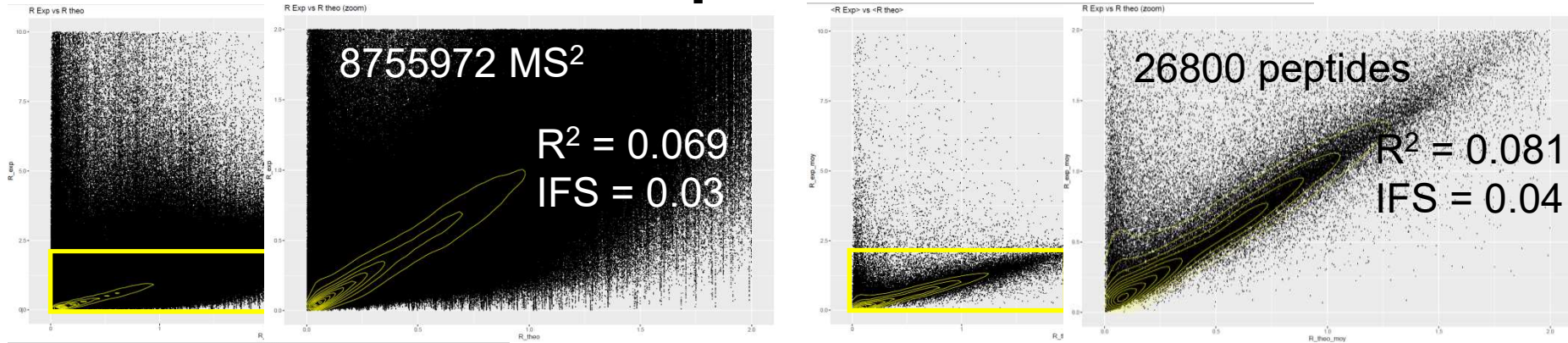
Post Acquisition Data Processing



Post Acquisition Data Processing

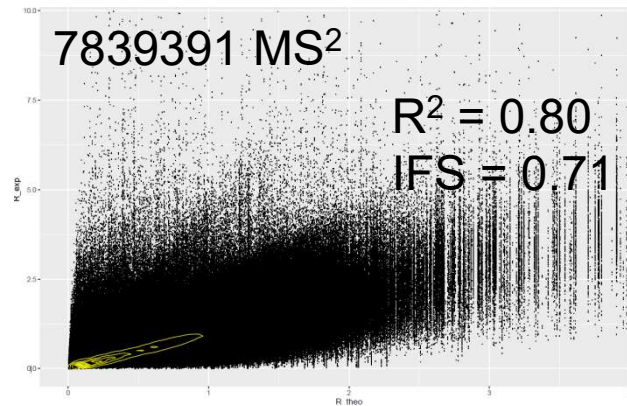


A view Exp vs Theo Ri...



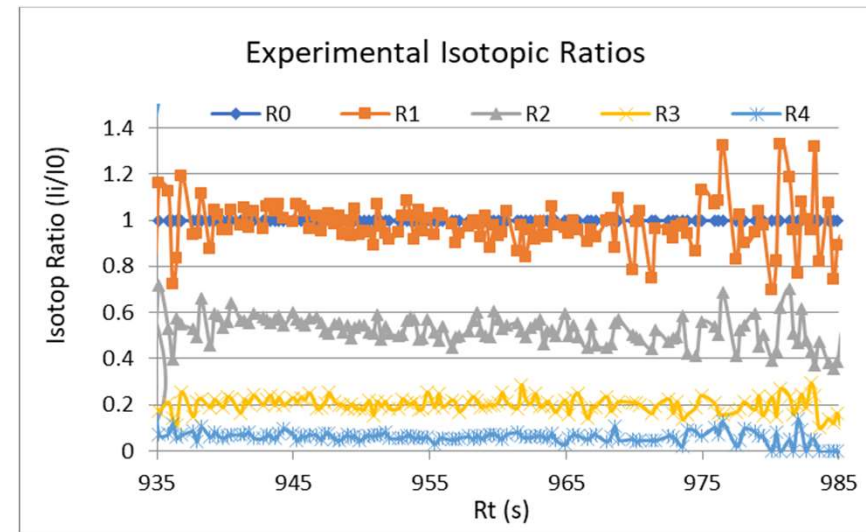
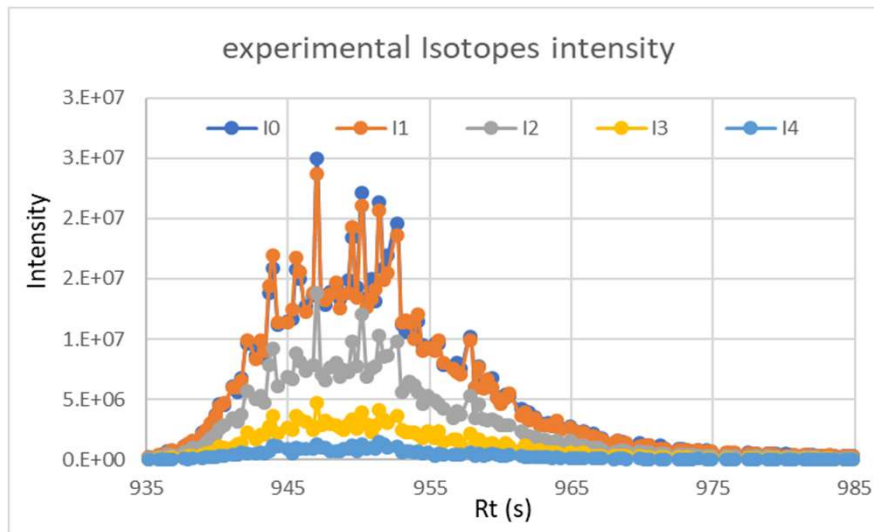
Pre-processing

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



Filtering data

2 levels filters



Inter-Spectrum Correlation

Peptide Ri distribution

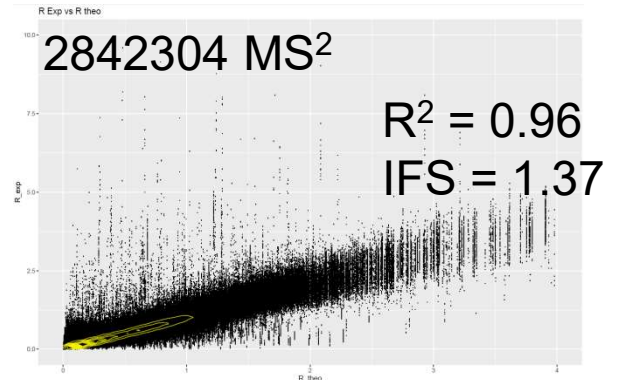
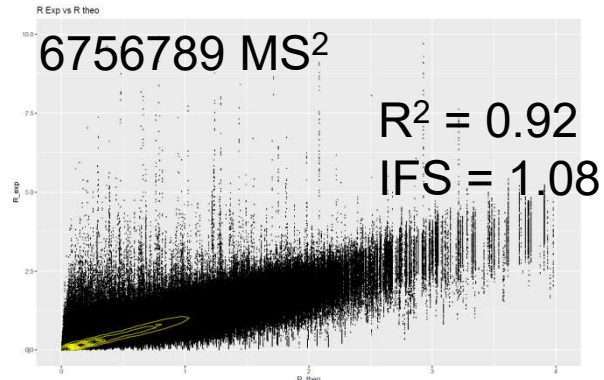
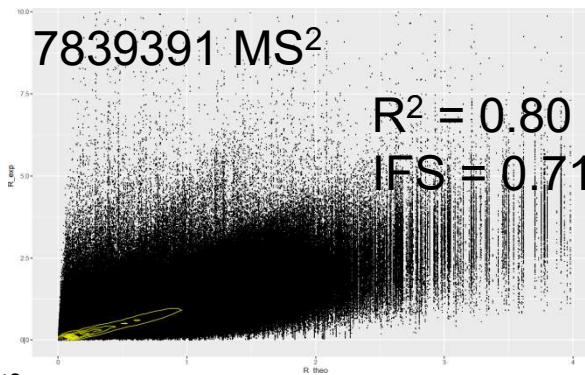
Isotopic ratios and profile filtering

2 levels filters

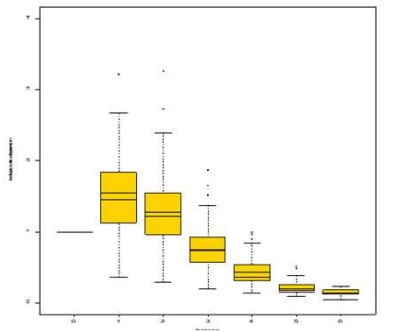
Raw ratios

Peptide(R_i) dist.

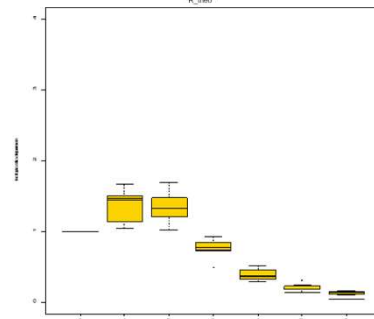
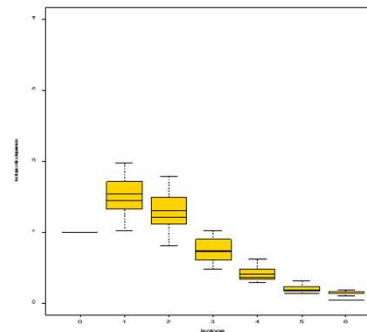
Spectrum² Corr.



Single peptide Ratios



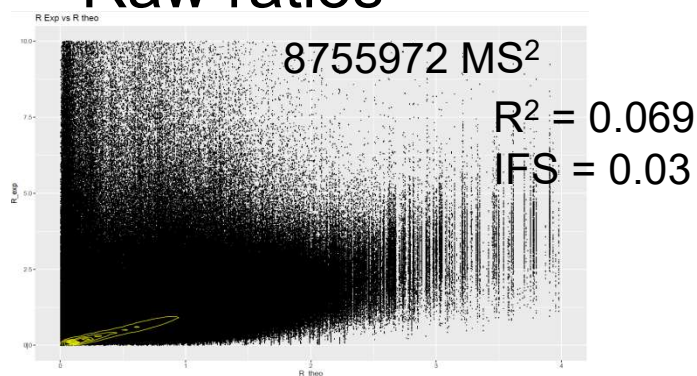
$\langle R_i \rangle - 2 SD < R_i < \langle R_i \rangle + 2 SD$



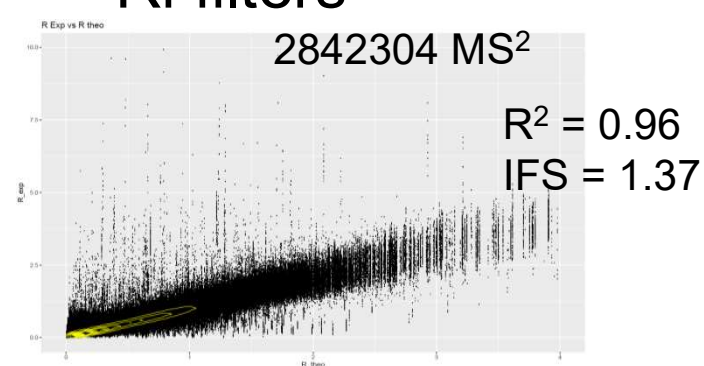
MS Corr. > 0.9

Additional filters ...

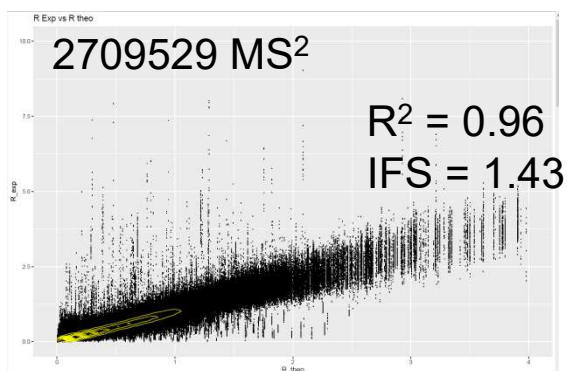
Raw ratios



Ri filters

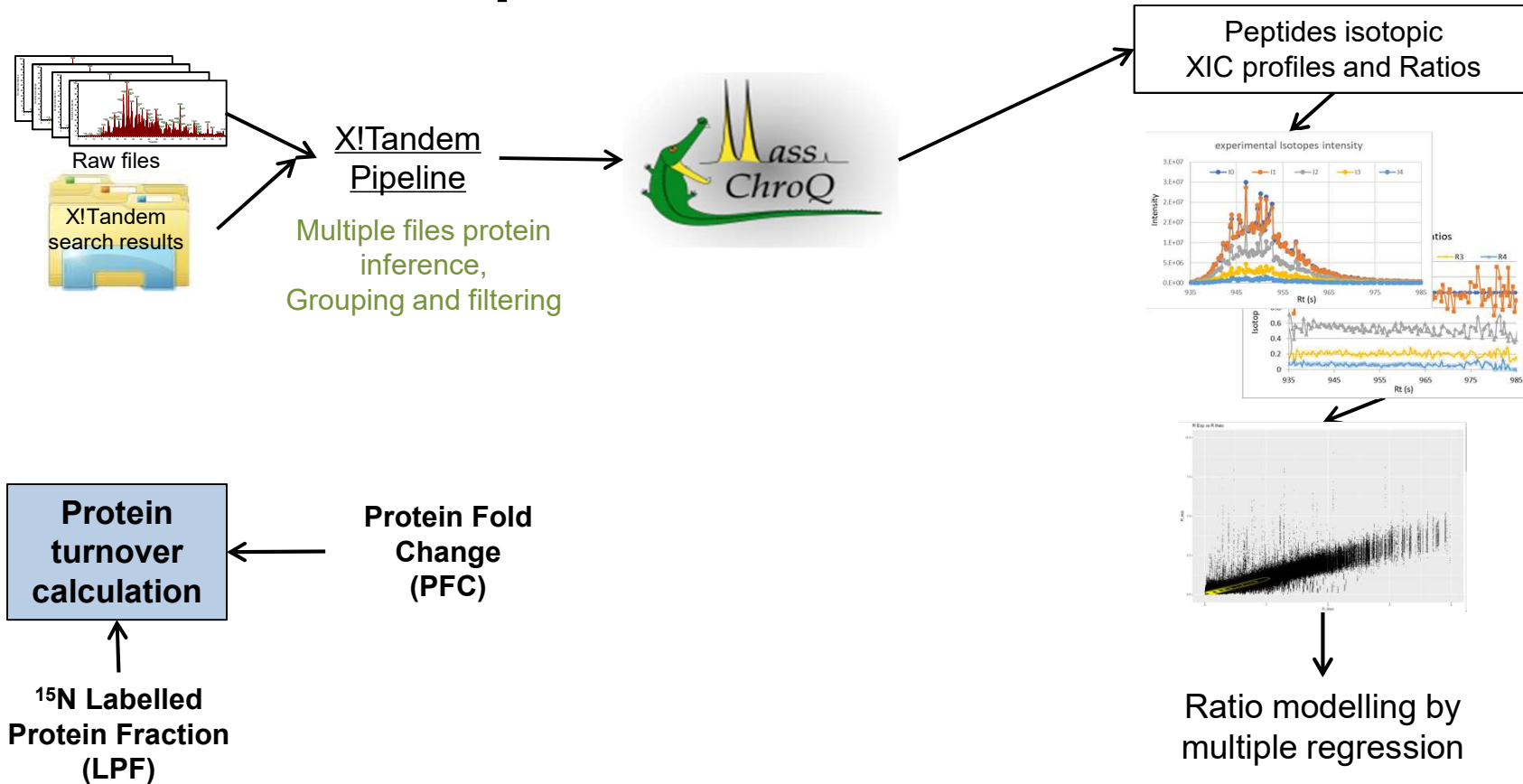


Favouring data redundancy



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



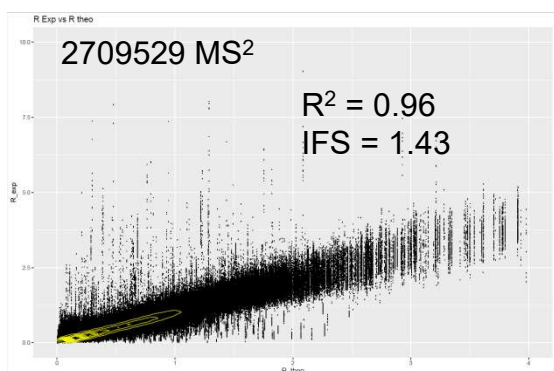
Post Acquisition Data Processing

Highly redundant peptide isotopic profiles

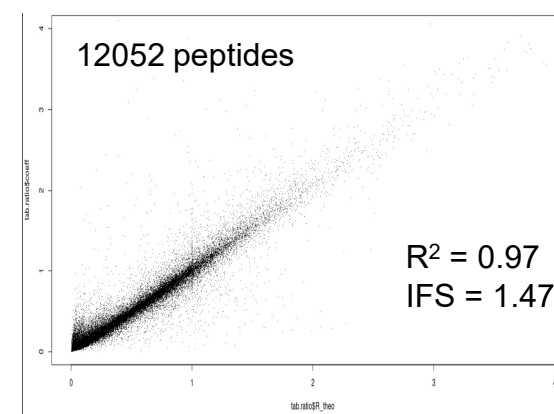
Multiple acquisitions (Triplicate at least)
Multiple Rt (10-30 s peptide elution)
Multiple z (2+,3+...)

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

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

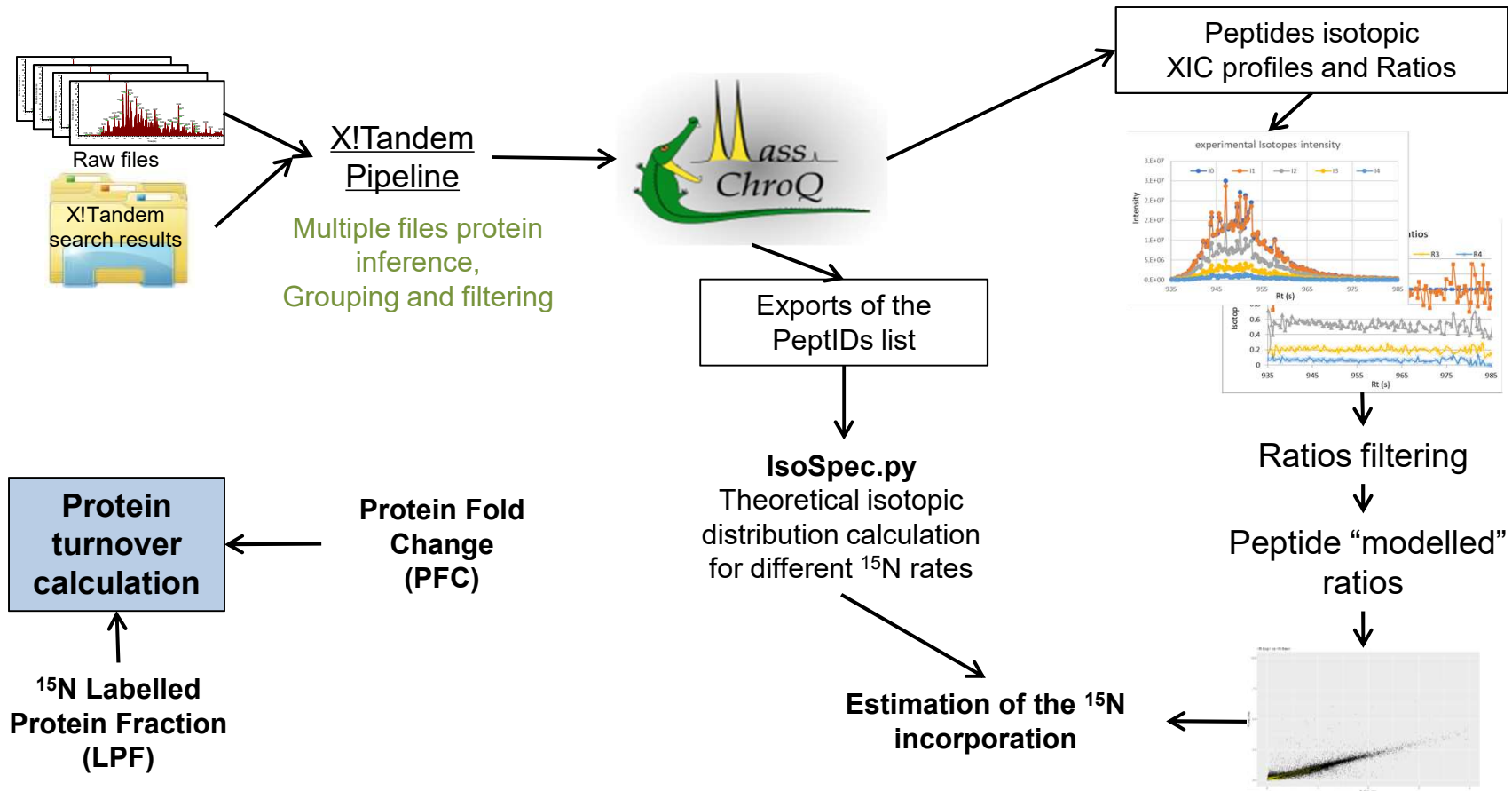


Ratio modelled by
multiple regression



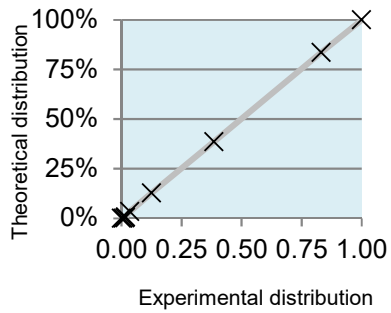
Single isotopic distribution per non-redundant peptide

Post Acquisition Data Processing

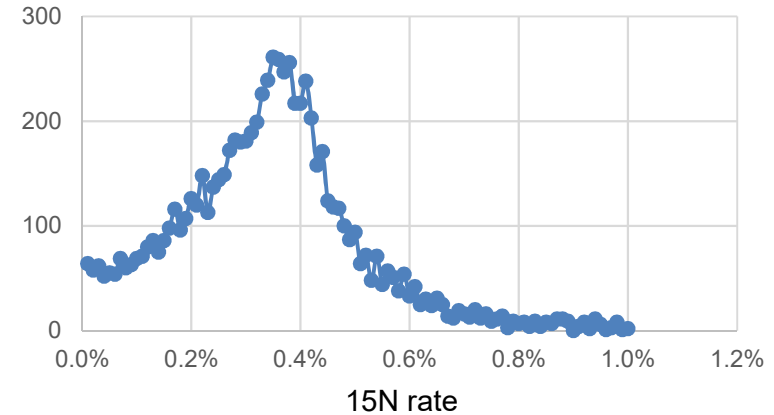
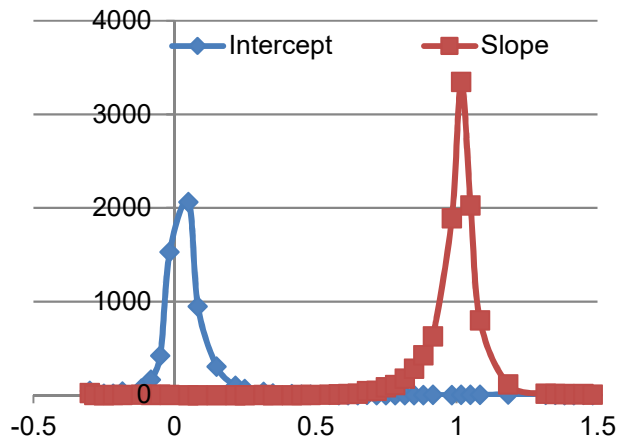
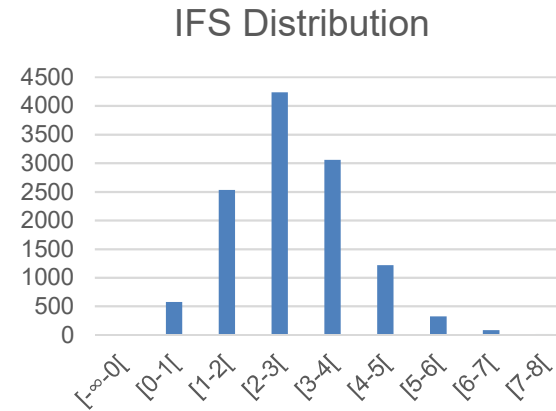


¹⁵N rate: an iterative approach

Ex.: Ctrl sample



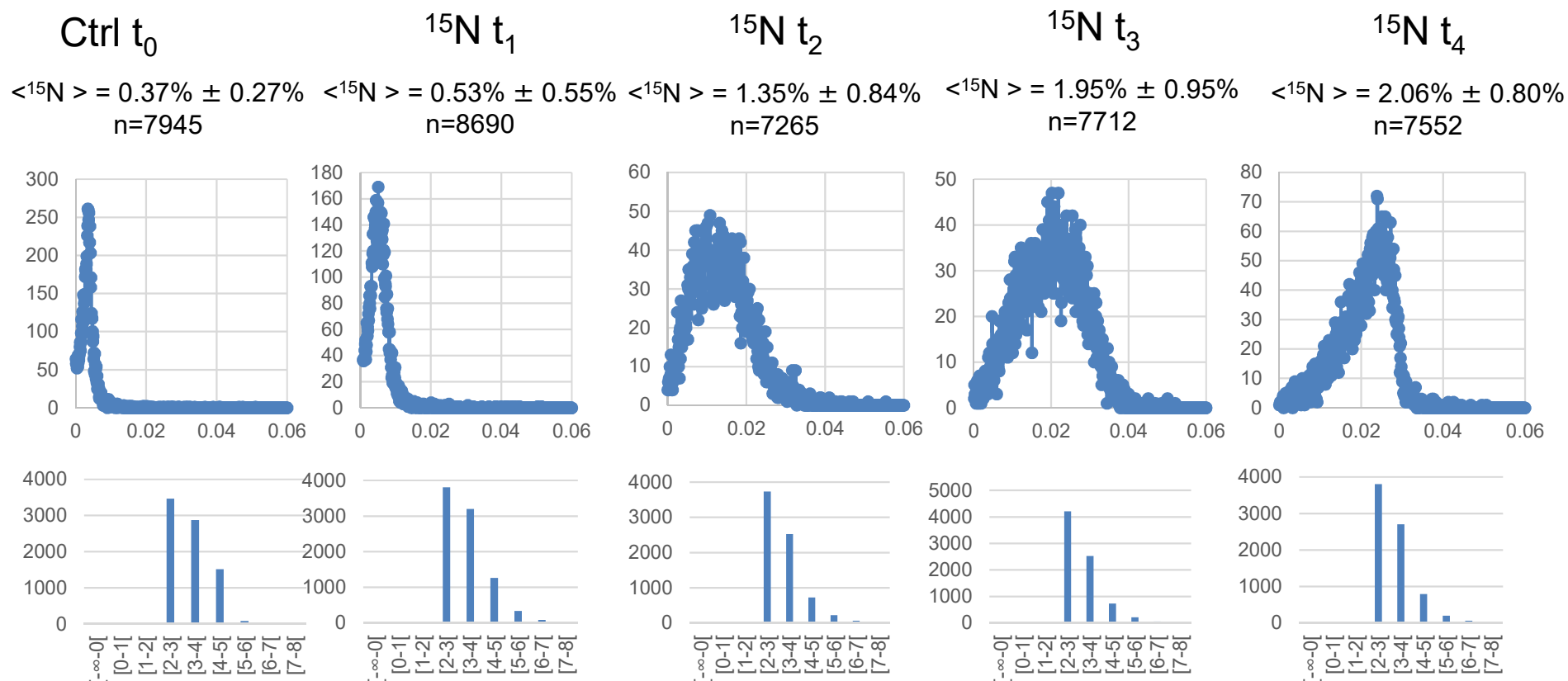
IFS
Slope
Intercept



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

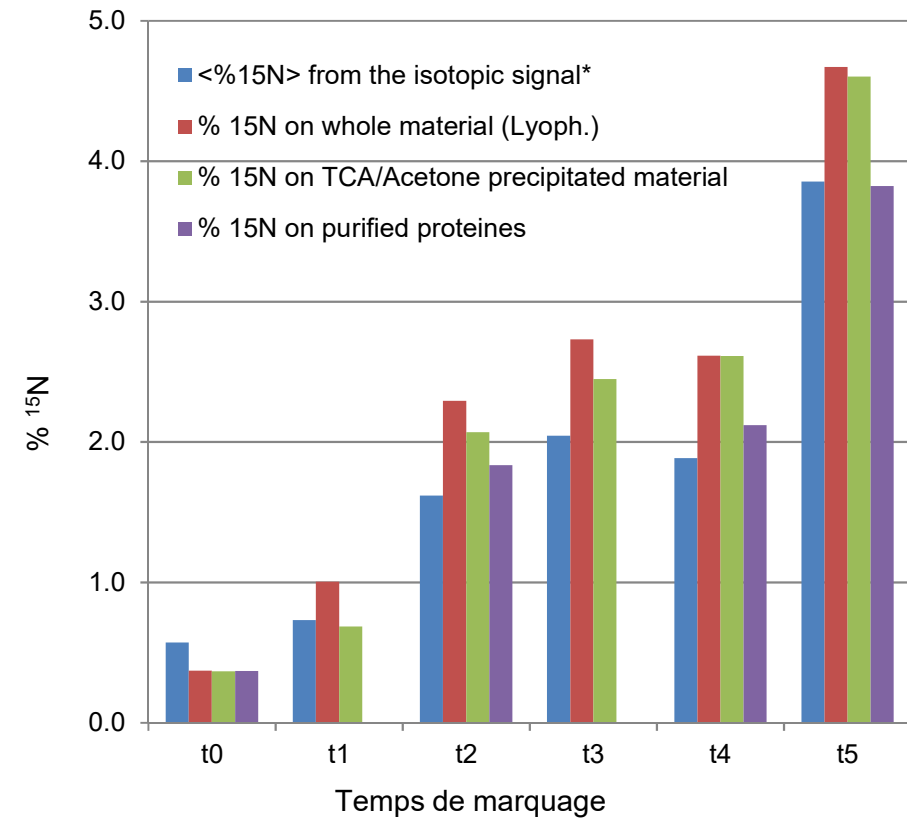
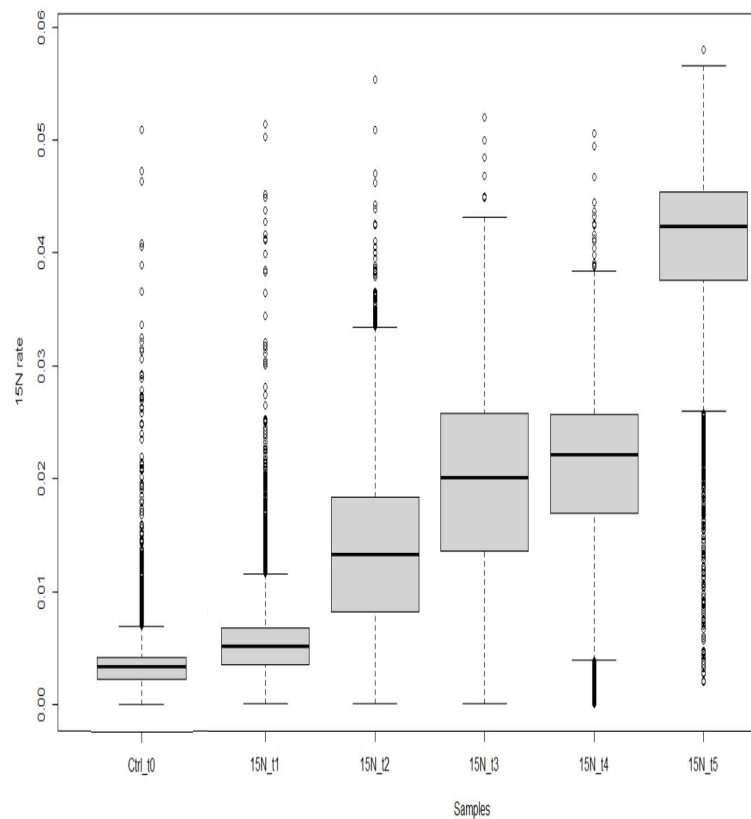
^{15}N rate evolution

^{15}N Metabolic labelling

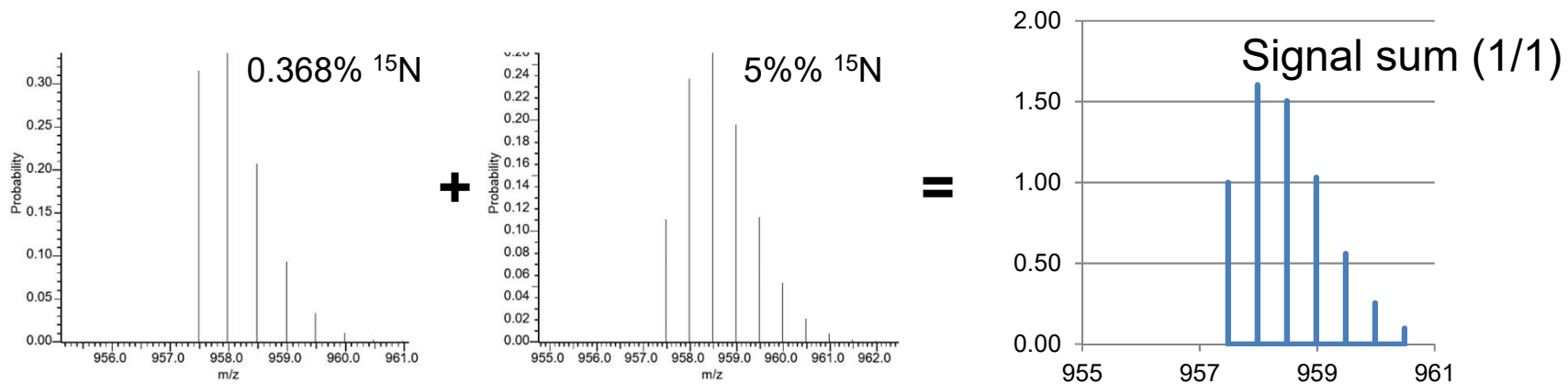


“Gold standard” measurement

15N rate distribution



Apparent ^{15}N rate to LPF



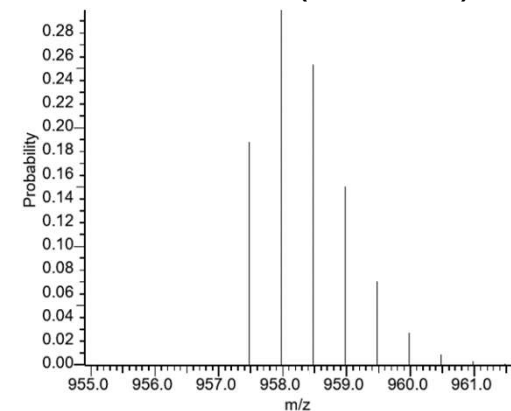
$$\text{LPF} = \frac{[\text{labelled}]}{([\text{initial}] + [\text{labelled}])}$$

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

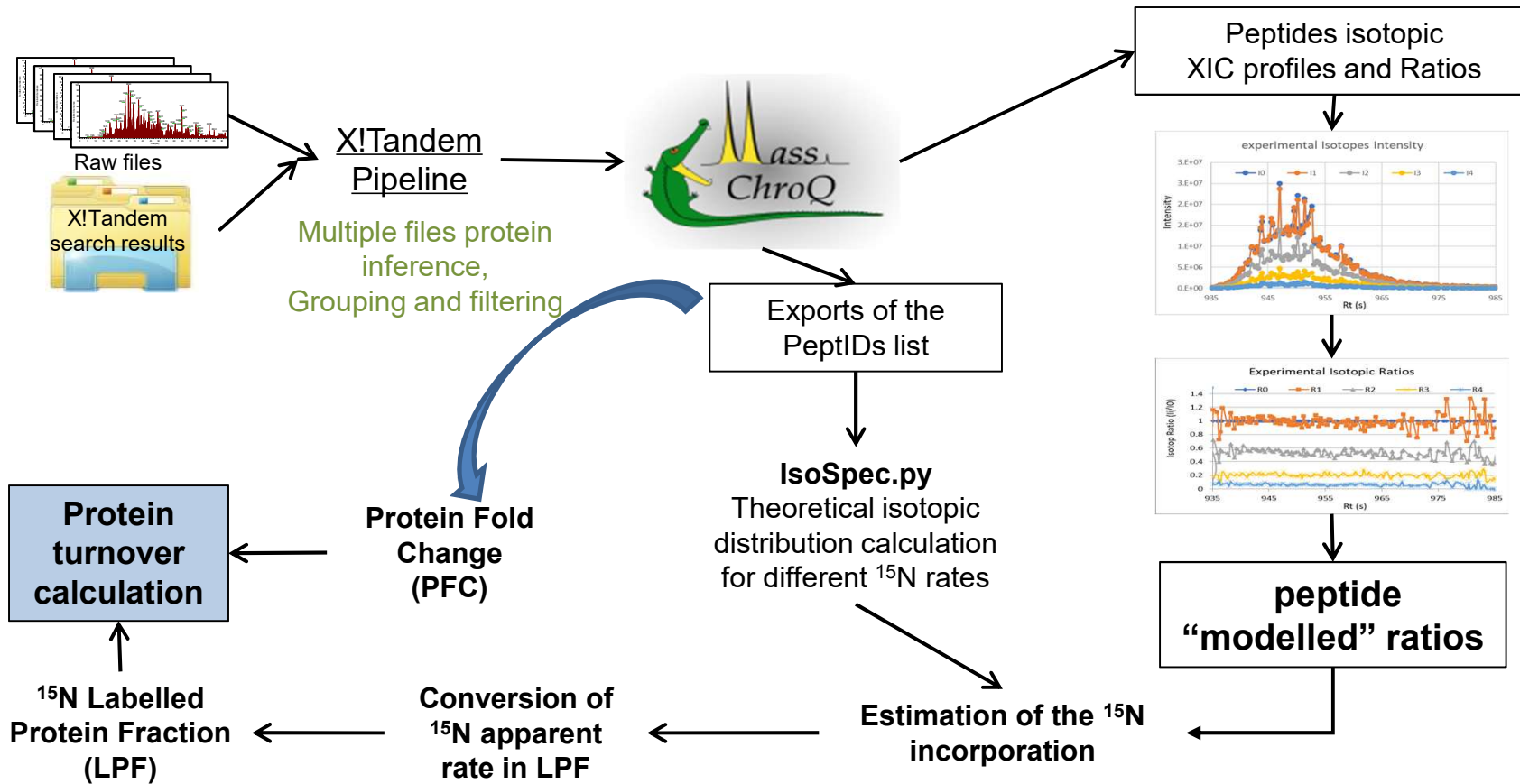
$$^{15}\text{N}_{\text{App}} = \frac{(^{15}\text{N}_{\text{Nat}} \times [\text{initial}] + ^{15}\text{N}_{\text{Max}} \times [\text{labelled}])}{([\text{initial}] + [\text{labelled}])}$$

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

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

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(BFP, Bordeaux)

