

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

Willy Vincent Bienvenut, Liam Le Goffic, Olivier Langella, Thierry Balliau,

Melisande Blein-Nicolas

▶ To cite this version:

Willy Vincent Bienvenut, Liam Le Goffic, Olivier Langella, Thierry Balliau, Melisande Blein-Nicolas. Sub optimal 15N metabolic labelling in plant: A new way to perform large scale protein turnovers determination using the peptides isotopic distribution. Analytics 2022, Sep 2022, Nantes, France. hal-03773594

HAL Id: hal-03773594 https://hal.science/hal-03773594

Submitted on 9 Sep 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.



Distributed under a Creative Commons Attribution 4.0 International License

P163

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

BIENVENUT W. V., LE GOFFIC L., LANGELLA O., BALLIAU T., BLEIN-NICOLAS M. GQE, IDEEV, CNRS, Gif sur Yvette, France

Abstract

Proteostasis is defined as the processes required maintaining the equilibrium between protein synthesis and degradation. Tight regulations from gene transcription to post-translational proteins modifications of these processes are required in cell cycle and survival to balance cellular development and environmental stresses. It has been shown repeatedly that the abundance of proteins only moderately correlates with that of transcripts. While the determination of protein turnover using pulsed SILAC metabolic labelling is popular for large scale proteomics investigations. Although used with small mammals (e.g. mice), such approach remains difficult with whole organisms and almost impossible with autotroph species like plants. Alternatively, it has been proposed to use inorganic sources of 15N isotopes that could be metabolically incorporated to de novo synthetized amino acids but full 15N protein labelling is almost impossible due to the high amino acid recycling rate in plant.

Instead of reaching complete 14N/15N exchange as previously done by Millar et al.^{1,2}, we decide to perform a 15N metabolic pulse at very low incorporation yield (around 5 % 15N enrichment). This limited incorporation rate offers some advantages of which a limited increase of mass spectrometry signal complexity. Indeed, the 15N-labelled products moderately modify the peptide natural isotopic distribution and both labelled and unlabelled product appears as an overlapping isotopic distribution. Then, the recorded signal is extensively processed to filter out the outliers. Finally, the filtered signal is finally used to first perform label free protein quantitation which provide the "Protein Fold Change" (PFC) evolution over time. Second, the experimental peptide isotopic distribution is used to determine the apparent 15N labelling rate and the evolution of the 15N "Labelled Protein Fraction" (LPF) over the labelling time.

3 days labelling Initial **Ctrl** 1 day labelling Ctrl after 10 days **Processing for LPF determination**

Data processing

Thanks to our local bioinformatics developments including i2MassChroQ, MassChroQ and the MCQR package, we propose an approach of MS signal processing that make it possible to determine the fold change in protein (FCP) and the labelled protein fraction (LPF). Both values (FCP & LPF) are key elements and are required to estimate the turnover rate individually for each protein³ at the proteomic scale. Furthermore, this presentation highlights that accurate isotopic distribution measurement is possible and could be used from ordinary largescale proteomics LC-MS/MS instruments to provide an interesting alternative to determine protein turnover determination at the proteome scale

Sample production, preparation and analysis





Endocarp Seeds and gel Placenta Carpel septum

Detailed protocol is available in reference 4: - Photoperiod: 16h/8h (Day/Night) using natural light or additional LED light - Hygrometry : 60-80% - Temperature: - Day: 23-28°C / Night: 16-19°C - Feeding solution depending the developmental stage (Nutritive solution table)



Processing for PFC determination

zone

Nutritive solutions:

Feeding solution	Description	KNO₃ (mM)	K ₂ SO ₄ (mM)	KH ₂ PO ₄ (mM)	Ca(NO ₃) ₂ (mM)	Ca(¹⁵ NO ₃) ₂ (mM)	MgSO₄ (mM)	Oligo Elements
Initial	Up to the 1st flower	3.5	1	2	6	-	2	х
End growth	Fruit development	4	1.5	1.5	4	-	1.5	Х
Labelling	¹⁵ N labelling	4	1.5	1.5	3.4	0.6	1.5	Х

Sample production

- Fixe developmental stage: 12 days after anthesis
- Labelling for different times: 1-12 days - 3-6 fruits for each labelling time point from different plants - Samples collected from the equatorial zone of
- the fruit and reduced to small pieces of 2x10 mm - Liquid nitrogen frozen and stored at -80°C - Stainless steel ball (10 mm) grinding for 1mn at 25 Hz

Protein digestion:

Sampling

Protein extraction: Proteins were extracted by phenol extraction using a protocol described in reference (6) using 100mg of the tomato frozen powder previous prepared.

Proteins pellet was subject to reduction and alkylation with iodoacetamide before tryptic digestion in 50 mM ammonium bicarbonate. The resulting peptides were desalted by solid phase extraction using polymeric C_{18} columns.

Sample analysis and data processing for peptide/protein identification



HPLC separation nanoLC ultra 2D (Eksigent) Biosphere C_{18} pre-column (5um, 12 nm 360/100 um, 20 mm) and column (3um, 12 nm, 360/75 um, 300 mm) Linear gradient from 5% to 70% buffer B in 75 min, 300nl/min buffer A: 0.1% FA in water; buffer B: 0.1% FA in acetonitrile



Acquisition with Xcalibur[™] v4.0 with (1) MS scan 350-1400 m/z range at 75000 resolution (2) MS/MS scan at 17500 resolution. Step 2 repeated for the eight most intense ions detected in step 1 with precursor charge step of 2⁺, 3⁺ and 4⁺, dynamic exclusion of 50 s

Protein degradation and

synthesis constants calculation



Evolution of the peptide ¹⁵N rate (in %) during



% 15N per individual peptide Evolution of the unlabelled protein fraction



0.4 0.6 0.8 (1- LPF) or unlabelled fraction (averaged per non redundant protein) Kd are calculated using the equation proposed by li et al. (3) for the proteins with a FCP range in between]-0.449, 0.449[across the labelling process. For the calculation, Kd is defined as 1 since no significant differences could be associated to this range.

The Kd could also be plotted as a function of the labelling time (graph on the right). Since FCP = 1, the slope is equal to the Kd. A validation of the Kd is based to a comparison of the linear regression slope (Kd) to the linear model passing through 0 (best correlation hypothesis). As an example, protein Solyc01g005240 with a Kd = 0.0473 day⁻¹

whereas protein Solyc01g049650 with a Kd = 0.0344 day⁻¹ is not retained due to the strong divergence between the 2 models.

 $K_{\rm D}$ distribution (1455 proteins)



Kd (day-1



the different labelling times (from t0 to t11 days)

Protein degradation rate constant (3)

 $K_{D} = -\frac{\ln FCP \cdot (1 - LPF)}{1}$

FCP: Fold Change in Protein \rightarrow variation of the protein abundance during the experiment \rightarrow Processing based on the MCQR package

Protein synthesis rate constant (3)

K_S FCP - e^{-K_D.t}

LPF: Labelled Protein Fraction \rightarrow Fraction of the ¹⁵N labelled peptide/protein out of all material \rightarrow Processing based on the MassChroq and dedicated R scripts

Since all fruits are collected at 12 days (at the same developmental stage), we do not expect any difference in protein amount. In such condition, which is equivalent to a « stationary phase » the PFC must be identical whatever the labelling time and then, Kd = Ks.

Acknowledgments

Many thanks to Sarah Naudin, Marie-Helène Andrieu, Sophie Colombié from the META team at the BFP for the production of the biological samples, Anne Marmagne, Cléline Masclaux-Daubresse from the SATURN team at the IJPB for the ICMS ¹⁵N measurement and their contribution to the development of this project.

The authors and the META team thank the BAP department of the INRAE for the PROOFER grant related to this project.



- \rightarrow Low ¹⁵N labelling (<6%) is compatible with standard LC-MS acquisition with low loss of efficiency for peptides/proteins characterisation.
- → Variations of the isotopic pattern provide relevant information for differential proteins quantitation
- → Kinetic constants for proteins synthesis and degradation could be determine with such approach
- → Collected data could be used directly for both LPF and FCP determination

1) Salih, K. J. et al. Biochem J 2020, 477, 3019-3032.

2) Nelson, C. J. et al. Plant Physiol 2014, 166, 91-108.

3) Li, L. et al. Plant Cell 2017, 29, 207-228

 \rightarrow Some improvement are required especially for data acquisition to improve:

0.12

- Signal stability and reproducibility
- Peptide separation (HPLC and/or ionic mobility)
- \rightarrow To develop some scoring scheme able to:
 - Strengthen the robustness of the LPF
 - Validate/invalidate the final Kd/Ks calculation



4) Andrieu M.H. et al. NOV'AEE Numéro Régulier #05.juin 2022 5) Sénécaut N. Ph D. thesis manuscritpt; Université Paris Cité: 22 juin 2022 6) Belouah, I. et al. *Data in brief* **2020**, *28*, 105015











