Journées PROTÉOME VERT

A Dijon

2025

26-27 mai

UMR Agroécologie

Centre INRAE, 17 Rue Sully

Agroécologie
Dijon
Unité de Recherche

Organisées avec le soutien de :

ProFI PROTEOMICS

BAP INRAE

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SPS Saclay Plant Sciences

université PARIS-SACLAY

PROGRAMME

Lundi 26 mai

13h30-14h Accueil

14h-14h10 Présentation des journées
Par **Delphine Aimé et Karine Gallardo**, Agroécologie – Dijon

Session 1 - Animatrice : Elisabeth Jamet, LRSV-Toulouse

14h10-14h50 Bruno Guillotin, Institut des Sciences des Plantes – Paris-Saclay (IPS2)
Single cell RNAseq approaches to investigate cell identity reprogramming and mobile proteins.

14h50-15h05 Carole Pichereaux, Plateforme protéomique ProteoToul – Toulouse
La plateforme ProteoToul : Offre technologique et approches en Agrobiosciences.

15h05-15h25 Angélique Besson-Bard, Agroécologie – Dijon
Identification of S-nitroso-proteomes under physiological or stress conditions in *Arabidopsis thaliana*, *Nicotiana tabacum* and the alga *Klebsormidium nitens.*

15h25-15h45 Lorraine Pennera, LPCV – Grenoble
Identification of nickel-binding proteins in plants.

15h45-16h15 Pause

Session 2 - Animateur : Titouan Bonnot, Agroécologie-Dijon

16h15-16h55 Sébastien Aubourg, IRHS – Angers
Exploring the world of small secreted peptides in Arabidopsis.

16h55-17h15 Christian Dubos, IPSiM – Montpellier
Développement de la peptidomique au sein de la plateforme MSPP : applications à l'étude de la régulation de l'homéostasie du fer chez les végétaux.

17h15-17h35 Julie Boudet, GDEC – Clermont Ferrand
Genetic and molecular mechanisms of global warming tolerance in interaction with sulfur nutrition in bread wheat.

17h35-17h55 Willy Bienvenut, GQE – Saclay / PAPPSO
Determining Protein Turnover Rates in Tomato Fruit via ^{15}N Metabolic Labeling and Peptide Isotopic Pattern Analysis by Mass Spectrometry.

17h55-18h30 Amélie Peuteuil et Karine Gallardo, Agroécologie – Dijon
Atelier d'Intelligence Collective : Boostons notre réseau.

19h00 Buffet sur site

Mardi 27 mai

Session 3 – Animateur : Harold Duruflé, BioFora-CR ECOVID

9h00-9h40 Jean Armengaud, Plateforme ProGénoMIX, Univ. Paris-Saclay, CEA, INRAE, DMTS, SPI
Soil and sediment metaproteomics: state of the art and perspectives.

9h40-10h00 Maëla Sémery, GQE – Saclay
Integrating proteomics data into a genome-scale metabolic model to predict fluxes in maize leaf.

10h00-10h20 Thierry Chardot, IJPB – Versailles
When proteomics meets lipid droplets: approaches and innovations.

10h20-10h50 Pause

Session 4 – Animatrice : Karine Gallardo, Agroécologie-Dijon

10h50- 11h30 Claire Rosnoblet, Agroécologie – Dijon
Mobilisation de l'UPS et de la protéine CDC48 dans les interactions biotiques : approches protéomiques

11h30-11h50 Mélisande Blein-Nicolas, GQE – Saclay / PAPPSO
MCQR: A modular, flexible, and user-friendly toolbox for deep exploration, processing, and analysis of quantitative proteomics data.

11h50-12h05 Amélie Peuteuil, Agroécologie – Dijon
Restitution de l'atelier "Boostons notre réseau".

12H05-12h30 Discussion générale
Animée par **Melisande Blein-Nicolas**, GQE – Saclay / PAPPSO

12h30 Fin des journées Protéome Vert
Possibilité de manger au self du Centre INRAE de Dijon
(Paiement par chèque ou carte bancaire uniquement)

Single cell RNAseq approaches to investigate cell identity reprogramming and mobile proteins.

Bruno Guillotin [1], Ramin Rahni [2], Laura Lee[2], Kenneth Birnbaum[2]

[1] Institute of Plant Sciences of Paris-Saclay-IPS2, CNRS, FR
[2] Center for Genomics and Systems Biology, CGSB, New York University, USA.

Corresponding author: bruno.guillotin@cnrs.fr

Plants have a remarkable capacity for regeneration. In Arabidopsis, the entire root tip—housing stem cells and specialized cells like the gravity-sensing columella—can be cut off and the remnant tissue will rapidly divide and differentiate to replace these missing identities. Despite some knowledge of the molecular mechanisms driving this process, the necessity of cell division in this context is not fully understood. Here, we define a timeline of the major steps in regeneration and investigate the role of division in that time. We show that while cell cycle inhibition blocks regeneration, some partial reprogramming can still occur. Using single-cell RNA (scRNAseq) sequencing profiles and live imaging during regeneration, we found that a subset of cells near an ablation injury dramatically increases division rate by truncating G1 phase. Cells in G1 undergo a transient nuclear peak of glutathione (GSH) prior to coordinated entry into S phase, followed by rapid divisions and cellular reprogramming. We propose a model in which GSH from the outer tissues is released upon injury, licensing an exit from G1 near the wound to induce rapid cell division and reprogramming.
In the second part, I will present an ongoing project that focuses on understanding how cells communicate with each other to ensure coordinated growth and organ morphogenesis. More specifically, we identified transcription factors that can move between cells through plasmodesmata, using an approach called Block-seq. This approach relies on cell-specific, inducible blocking of plasmodesmata using a callose synthase, followed by scRNA-seq.

Keywords: Root, scRNAseq, cell identity, cell-cell communication

La plateforme ProteoToul : Offre technologique et approches en Agrobiosciences

Carole Pichereaux [1,2,3]

[1] Fédération de Recherche Agrobiosciences, Interactions et Biodiversité (FRAIB), Université de Toulouse, CNRS, Université Toulouse - Paul Sabatier (UT), Auzeville Tolosan, F-31326 France
[2] Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, Université Toulouse - Paul Sabatier (UT), Toulouse, F-31077 France
[3] Infrastructure nationale de protéomique, ProFI, FR 2048, Toulouse, France

Corresponding author: Carole.Pichereaux@ipbs.fr

ProteoToul Services (https://proteotoul.ipbs.fr/proteotoul-services), la plateforme protéomique de Toulouse est localisée à l'Institut de Pharmacologie et de Biologie Structurale (IPBS) et est dirigée par Odile Schiltz. La plateforme est ouverte à l'ensemble de la communauté scientifique du secteur public et privé. Elle appartient au réseau local des plateformes technologiques en sciences du vivant, Genotoul (https://www.genotoul.fr/), et elle fait partie de l'infrastructure nationale de protéomique française, ProFI (https://www.profiproteomics.fr/).
Carole Pichereaux, ingénieur de la Fédération de Recherche Agrobiosciences, Interactions et Biodiversité (FR AIB), spécialisée en spectrométrie de masse assure la prise en charge des projets protéomiques locaux et nationaux dans le domaine des agrobiosciences.
ProteoToul Services est équipé des dernières générations de spectromètres de masse (MS) couplés à des systèmes chromatographiques (nanoLC-MS/MS) pour l'acquisition des données et d'outils bioinformatiques dédiés pour l'analyse de ces données. Cette multiplicité d'instruments permet de proposer un large choix de stratégies à la pointe de la technologie. Ces approches innovantes seront illustrées grâce à des exemples de projets qui vont de l'identification de protéines à l'analyse de modifications post-traductionnelles, et à des analyses quantitatives différentielles pour identifier des partenaires protéiques d'une protéine cible.

Keywords: Protéomique, Spectrométrie de masse, Agrobiosciences, Analyse quantitative (BioID)

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1. Chaudron Z et al., Nitric oxide production and protein S-nitrosation in algae. Plant Science 355:112472 (2025)
2. Prévitali T *et al.,* Lotus resistance against Ralstonia is enhanced by Mesorhizobium and does not impair mutualism. New Phytol. 245(3): 1249-1262 (2025)
3. Sarrette B et al., Medicago truncatula SOBIR1 controls pathogen immunity and specificity in the Rhizobium-legume symbiosis. Plant Cell Environ. 48(1):32-50 (2025)
4. Ormancey M, *et al*., Complementary peptides represent a credible alternative to agrochemicals by activating translation of targeted proteins. Nature Communications 14(1):254 (2023)
5. Valérie Nicolas-Francès, *et al.,* S-Nitrosation of *Arabidopsis thaliana* Protein Tyrosine Phosphatase 1 Prevents Its Irreversible Oxidation by Hydrogen Peroxide. Front Plant Sci. 13 :807249 (2022)

Identification of S-nitroso-proteomes under physiological or stress conditions in Arabidopsis thaliana, Nicotiana tabacum and the alga Klebsormidium nitens

Valérie Nicolas-Francès [a], Claire Rosnoblet [a], Siham Hichami [a], Carole Pichereaux [b,c], David Wendehenne [a], and Angélique Besson-Bard [a]

[a] Université Bourgogne Europe, Institut Agro Dijon, INRAE, UMR Agroécologie, Dijon, France
[b] Fédération de Recherche (FR3450), Agrobiosciences, Interactions et Biodiversité (FRAIB), CNRS, Toulouse, France
[c] Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse UPS, CNRS, Toulouse, France

Corresponding author: angelique.besson-bard@ube.fr

Nitric oxide (NO) is an important signaling molecule in both animal and plant organisms. NO is produced under physiological conditions, but also in response to biotic and abiotic stresses, notably during the defense response of photosynthetic organisms. NO exerts its effects by regulating gene expression, as well as through 3 post-translational modifications. One of these, named S-nitrosation (or S-nitrosylation), involves the addition of a molecule of NO to a cysteine (Cys) residue to form an S-nitrosothiol bond. This modification can have an impact on the activity, localization or interaction with its partners for the targeted protein. S-nitrosation is difficult to detect because it generally affects a small proportion of the population of the targeted protein, and is reversible and labile. In recent years, a technique called « biotin-switch » has been developed in 2001 by Jaffrey and Snyder[1] to detect S-nitrosated proteins. It briefly involves replacing the NO bound to the Cys residues with a biotin tag, which is then used to identify the protein. We used this technique on different species (*Arabidopsis thaliana*, *Nicotiana tabacum* and, more recently, the green alga *Klebsormidium nitens*) under various conditions and then analyzed the samples by mass spectrometry on the Toulouse proteomics platform. This enabled us to identify hundreds of S-nitrosated proteins, and we have begun to characterize several of them[2,3,4].

Keywords: nitric oxide, S-nitrosation, *Arabidopsis thaliana*, *Nicotiana tabacum*, *Klebsormidium nitens*

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2. Astier, A. *et al.*, Nitric oxide inhibits the ATPase activity of the chaperone-like AAA+ATPase CDC48, a target for S-nitrosylation in cryptogein signaling in tobacco cells. Biochemical Journal 447, 249–260 (2012).
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Identification of nickel-binding proteins in plants

Lorraine Pennera[1], Fabienne Devime[1], Sylvie Figuet[1], Yohann Couté[2], Sylvain Merlot[3], Claude Alban[1], Stéphane Ravanel[1] and Jonathan Przybyla-Toscano[1]

[1]Univ. Grenoble Alpes, CEA, INRAE, CNRS, IRIG, LPCV, 38000 Grenoble, France
[2]Univ. Grenoble Alpes, CEA, INSERM, UA13 BGE, CNRS, CEA, Grenoble, France
[3]LRSV, UMR5546 CNRS/UPS/INPT, 31000 Toulouse, France

Corresponding author: lorraine.pennera@cea.fr

Nickel (Ni) is an essential micronutrient for plants. This element is required for the activity of urease, an enzyme involved in nitrogen metabolism (Myrach *et al*., 2017), and for some Ni-dependent glyoxalases participating in detoxification processes (Jain *et al*., 2016). Trace amounts of Ni are sufficient to support plant growth and development, whereas moderate concentrations of Ni in the soil can be toxic to most plant species. Remarkably, some plant species have evolved mechanisms to tolerate and accumulate substantial amounts of Ni in their foliar tissues when grown in Ni-rich soils (Brooks *et al.*, 1977). Among these mechanisms, we hypothesize that Ni-binding proteins (NiBP) may act as metal chelators, effectively mitigating its toxic effects. To identify novel plant NiBPs, we developed a metalloproteomic approach using the Ni-hyperaccumulator species *Noccaea caerulescens.* The leaf soluble proteome of plants challenged with a high Ni concentration was first fractionated by successive liquid chromatography steps, and the presence of Ni in protein fractions was monitored by inductively coupled plasma mass spectrometry. Then, Ni enriched protein fractions were subsequently sequenced by tandem mass spectrometry. The application of stringent selection criteria (*i.e.,* MS/MS criteria, manual reviewing, relationship with metal homeostasis, correlated with transcriptomic data) led to the identification of promising NiBP candidates. The detailed biochemical characterization of three identified proteins is currently underway to confirm their Ni-binding capacity. Further genetic analysis *in planta* will clarify the role of these newly identified NiBP in plant Ni homeostasis.

Keywords: metalloproteomics, nickel-binding proteins, hyperaccumulator species, *Noccaea caerulescens*

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Myrach, T., Zhu, A., and Witte, C.-P. (2017). The assembly of the plant urease activation complex and the essential role of the urease accessory protein G (UreG) in delivery of nickel to urease. J Biol Chem *292*, 14556–14565.

Exploring the world of small secreted peptides in Arabidopsis

Sandra Pelletier[1], Rayan Eid[1], Aurélie Dupriez[2], Thierry Balliau[3], Enora Panek[4], Sophie Aligon[1], Philippe Grappin[1], Bruno Favery[4], Harald Keller[4], Mélisande Blein-Nicolas[3], Elisabeth Jamet[2], Sébastien Aubourg[1]

[1] Université Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, 49000 Angers, France
[2] Laboratoire de Recherche en Sciences Végétales, Université de Toulouse, CNRS, Toulouse INP, 31320 Auzeville-Tolosane, France
[3] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, PAPPSO, 91190 Gif-sur-Yvette, France
[4] Université Côte d'Azur, INRAE, CNRS, UMR 1355-7254, ISA, 06903 Sophia Antipolis, France

Corresponding author: sebastien.aubourg@inrae.fr

Plant responses to biotic aggressions involve a great diversity of molecules including regulatory proteins and hormones. Among these actors, small secreted peptides (SSPs) may directly interact with pathogens or act in signalling and cell-to-cell communication through their interactions with membrane receptor complexes.
The short size of SSPs, their limited sequence conservation, their low and/or condition-specific expression and the possible post-translational modifications required for they bioactivity (including proteolytic processing of larger precursor proteins) make their identification difficult on the basis of genomic sequences. Therefore, only a small fraction of the genes liable to encode these SSPs has been characterized and their impact and diversity appears to be seriously underestimated.
Combining bioinformatics predictions, transcriptomics profiling in presence of pathogens or elicitors and peptidomics approaches, we explore the SSP gene space in *Arabidopsis thaliana* aiming to describe the fraction involved in biotic stress-response pathways. Our procedure attempts to classify Arabidopsis SSPs into families in taking into account their great variability, and to overcome the limitation of gene predictors through the iterative mining of genomic sequences with control and curation steps. The library of SSPs precursors obtained is used to identify the peptides contained in apoplastic fluids of leaves challenged with pathogens.
The functional characterization of hitherto undescribed candidates is in progress (knock-out mutants, evaluation of synthetic peptides for their elicitor or microbiocide activity) and should lead to significant advances in the discovery of new key players in danger sensing and modulation of immune responses. According to their conservation among plant species, these SSPs might be used in innovative biocontrol strategies aiming at jointly optimizing plant quality and resistance.

Keywords: phytocytokine, stress signalling, bioinformatics, peptidomics

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Développement de la peptidomique au sein de la plateforme MSPP : applications à l'étude de la régulation de l'homéostasie du fer chez les végétaux

Berger Nathalie[1], Kalra Muskan[1], Gao Fei[2], Rofidal Valérie[1], Demolombe Vincent[1], Santoni Véronique[1], Dubos Christian[1]

[1] IPSiM, CNRS, INRAE, Institut Agro, University Montpellier, 34060 Montpellier, France
[2] College of Agronomy, Hunan Agricultural University, Changsha 410128, China; Yuelushan

Corresponding author: christian.dubos@inrae.fr

Iron (Fe) is an essential micronutrient for plant growth and development whose homeostasis must be tightly regulated to avoid deficiency or excess that could be detrimental to the cells. In Arabidopsis thaliana, this mechanism is regulated by a series of transcription factors that act in an intricate regulatory network. If tremendous efforts have been deployed to decipher the molecular mechanisms that regulate iron homeostasis in plants, the nature of the long-distance signal that conveys, via the phloem sap, information on the iron status of aerial tissues to the roots in order to coordinate iron uptake with the plant needs for iron is still to be determined. With the aim to identify potential actors involved in this process, we set up peptidomic analyses of phloem sap of Arabidopsis plants grown in iron replete and iron deficiency conditions. During the presentation we will present the implementation of the method, and associated difficulties, and the results obtained.

Keywords: peptidomic, iron, Arabidopsis

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Genetic and molecular mechanisms of global warming tolerance in interaction with sulfur nutrition in bread wheat

Rosie Sangata[1], Benoit Bernay[2], Laurent Linossier[3], Emmanuelle Bancel[1], Marielle Merlino[1], Sibille Perrochon[1], David Alvarez[1], Jacques Le Gouis[1], Julie Boudet[1]

[1] Université Clermont-Auvergne, INRAE, UMR1095 GDEC, Clermont-Ferrand, France
[2] Université de Caen Normandie, Plateforme Proteogen, Caen, France
3 Limagrain Ingrédients, Riom, France

Corresponding author: julie.boudet@uca.fr

Bread wheat (*Triticum aestivum* L.) provides about 20% of the total calories and proteins in the human diet. It is mainly consumed after different types of processing which each require a specific technological quality that depends mainly on the storage protein content and composition. Climate change, including the increasing prevalence of intense climatic events, and the increasing soil sulfur deficiency due to reduced atmospheric deposition (Eriksen 2009), are expected to alter the wheat technological quality. It has been shown that sulfur nutrition helps to limit the effects of heat stress (HS) (Tao et al. 2018; Bonnot et al. 2023). The TempSo project aims to assess and understand the effects of sulfur nutrition on the adaptation of bread wheat to global warming. Its objective is to answer three questions: (i) Does sulfur nutrition mitigate the negative impact of HS on grain quality? (ii) Which molecular mechanisms are involved in the sulfur nutrition x heat stress interaction? (iii) Is there a genetic variability in responses?
Two experiments based on two wheat varieties with contrasting quality stability were carried out: one in the field under semi-controlled conditions and the other in growth chambers under controlled conditions. For this last one, six treatments were applied: two periods of HS (23/29°C night/day) application during grain filling, and corresponding controls (15/21°C), with or without sulfur application. The effects of HS and sulfur nutrition, alone or in combination, on grain quality were assessed at maturity. In order to identify the molecular actors and metabolic pathways impacted by HS and/or sulfur nutrition, -omics analyses (proteomics, RNAseq, redox status, free amino acid and hormone levels) were performed on grains harvested before and after the HS periods. These data will be integrated using tools such as the R package mixOmics.

Keywords: bread wheat, data integration, grain quality, heat stress, sulfur nutrition

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Determining Protein Turnover Rates in Tomato Fruit via ^{15}N Metabolic Labeling and Peptide Isotopic Pattern Analysis by Mass Spectrometry

Willy Bienvenut[1], Marie Helene Andrieu[2], Olivier Langella[1], Sarah Naudin[2],Thierry Bailliau[1], Sophie Colombie[2], Mélisande Blein-Nicolas[1]

[1]Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE - Le Moulon, PAPPSO,
91190, Gif-sur-Yvette, France
[2]Université de Bordeaux, INRAE, Biologie du Fruit et Pathologie, UMR 1332, Bordeaux Metabolome, MetaboHUB, PHENOME-EMPHASIS, 71 av E. Bourlaux, 33140 Villenave d'Ornon, France.

Corresponding author: willy.bienvenut@cnrs.fr

Proteostasis—the balance between protein synthesis and degradation—is essential for proper development in living organisms. Measuring protein turnover rates (PTR), defined by synthesis (Ks) and degradation (Kd) constants, provides critical insights into these regulatory mechanisms. To address this, we developed a novel proteomics approach using suboptimal ^{15}N metabolic labelling combined with mass spectrometry measurement of the peptide isotopic distribution to estimate Ks and Kd values in tomato fruit. Our strategy relies on calculating two key parameters across multiple labelling time points: the Protein Fold Change (PFC) and the Labelled Protein Fraction (LPF). These values are extracted from mass spectrometry data using an adapted version of MassChroQ, MCQR, and a custom R package. The resulting Kd constants are finally filtered to ensure reliability. We applied this pipeline to study protein turnover during tomato fruit growth, establishing a complete ^{15}N labelling protocol and validating our workflow on real datasets. This presentation will highlight the methodology, key results, and current limitations of this novel approach.

Keywords: Protein Turnover, ^{15}N metabolic labeling, bioinformatics, MassChroQ, MCQR

Acknowledgments / Funding:
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Soil and sediment metaproteomics: state of the art and perspectives

Jean Armengaud [1]

[1] Laboratory «Innovative technologies for Detection and Diagnostics», ProGénoMix IBISA platform, CEA, Bagnols-sur-Cèze, France

Corresponding author: jean.armengaud@cea.fr

Microorganisms play a fundamental role in the health of humans, animals, and plants, as well as in essential terrestrial geochemical processes. Gaining a comprehensive understanding of how microbial communities function requires detailed characterization of their taxonomic diversity and a precise description of the specific contributions of each taxon to overall molecular activity. Next-generation metaproteomics (1), enabled by the latest generation of high-resolution tandem mass spectrometers, provides deep insights into the composition and functionality of microbial communities across diverse sample types, including complex environments such as soils and sediments. This technology focused on proteins is central for functional studies of microbiota, enabling the simultaneous identification of microbial taxa, quantification of their biomass, detection of taxon-specific proteins, and analysis of their roles in ecosystem function. A critical step in any metaproteomic workflow is the selection of a reference database matching the microbial composition of the sample (2). This step is especially challenging for environmental matrices like soil and sediment, given their high diversity and heterogeneity (3). In this context, proteotyping, which uses protein sequence signatures to identify microorganisms and quantify their biomass, emerges as a powerful strategy (4). In this presentation, I will share several case studies demonstrating the potential of proteotyping, and address key challenges in the field of metaproteomics, including instrument limitations, data interpretation, and integration of results. Finally, I will outline future directions and highlight promising opportunities for advancing this transformative approach (5).

Keywords: metaproteomics, microbiome, proteins, spectrometry, soil

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Integrating proteomics data into a genome-scale metabolic model to predict fluxes in maize leaf

Maëla SÉMERY[1], Sylvain Prigent[2,3], Mélisande BLEIN-NICOLAS[1], Christine DILLMANN[1]

[1] Université Paris-Saclay, INRAE, CNRS, AgroParisTech, GQE-Le Moulon, 91190, Gif-Sur-Yvette, France[2] Université de Bordeaux, INRAE, UMR1332 BFP, 33882 Villenave d'Ornon, France
[3] Bordeaux Metabolome, MetaboHUB, PHENOME-EMPHASIS, 33140 Villenave d'Ornon, France

Corresponding author: christine.dillmann@inrae.fr

Developing drought-tolerant crop varieties is crucial for agroecological transition and requires a deeper understanding of adaptation mechanisms. Advances in high-throughput phenotyping now enable the characterization of many individuals at the transcriptome, proteome, and metabolome levels across different environments. However, linking genetic or molecular changes to phenotypic adaptations remains challenging. Data integration methods, particularly those using constraint-based metabolic models, can predict metabolic fluxes and provide new insights into the most active metabolic pathways under varying environmental conditions.
Several approaches predict fluxes across metabolic networks by applying constraints to solve stationary state equations. Some use omics data to identify fluxes that best fit experimental observations. Among these, Petrizzelli et al. proposed a method using proteomic data to constrain flux predictions, which has been tested on a simplified model of central carbon metabolism in yeast. To investigate maize adaptation to drought, we aim to predict metabolic fluxes in 254 maize hybrids grown under two irrigation conditions using available proteomic data. The first step is to adapt Petrizzelli's method to the genome-scale metabolic model of the maize leaf, the preliminary results of which will be presented here. This will provide a toolkit that helps identify the determinants of flux variation in response to drought.

Keywords: constraint-based metabolic model, quantitative proteomics, metabolic fluxes, Zea mays

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When proteomics meets lipid droplets: approaches and innovations.

Thierry Chardot[1]

[1] Université Paris-Saclay, INRAE, AgroParisTech, Institute Jean-Pierre Bourgin for Plant Sciences (IJPB), 78000, Versailles, France.

Corresponding author: thierry.chardot@inrae.fr

Lipid droplets are highly original intracellular structures found in most living organisms. In plants, they are found in seeds, leaves, and pollen. They are composed of a core of lipids, surrounded by a monolayer of phospholipids in which several proteins are found [1]. One of the major questions that has arisen is to identify the proteins associated with these organelles. In this presentation, we will focus on seed lipid droplets. The main proteins found are oleosins, caleosins, and steroleosins, although other proteins are also present. Oleosins, caleosins, and steroleosins have hydrophobic regions that enable them to anchor to the organelle. The absence of charged residues in these regions represents an obstacle to their digestion by trypsin. For this reason, we have performed several methodological developments involving the use of different proteases to obtain high coverage (particularly of the hydrophobic region) and identify proteins with certainty [2]. We were also interested in the modifications that these proteins could carry, whether on amino acids of the peptide chain [3] or the N-terminus [4]. In contrast with the abundant literature on the structure of membrane proteins, the structure and insertion of lipid droplet proteins in a phospholipid monolayer remains scarcely described, and is limited to a few old and contradictory articles. To precisely delineate cytosolic regions from those buried in the lipid matrix, we have developed structural proteomics approaches, based on the use of specific chemical reagents, and also of radicals generated by the ionization of water under the action of X-rays [5]. Developing functional and structural proteomics will be useful for identifying and tracking of lipid droplet-associated proteins, from both sequenced and non-sequenced species, and throughout their life cycle.

Keywords: Seeds, lipid droplets, oleosins, structural proteomics.

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Mobilisation de l'UPS et de la protéine CDC48 dans les interactions biotiques : approches protéomiques.

Claire Rosnoblet[1], Angélique Besson-Bard[1], Valérie Nicolas[1], Carole Pichereaux[2,3,4], Pascale Winckler[5], Hervé Bègue[1], Pauline Chatelain[1], David Wendehenne[1]

[1] Université Bourgogne Europe, Institut Agro Dijon, INRAE, UMR Agroécologie, Dijon, France
[2] Fédération de Recherche Agrobiosciences, Interactions et Biodiversité (FRAIB), Université de Toulouse, CNRS, Université Toulouse III - Paul Sabatier (UT3), Castanet Tolosan, F-31326 France
[3] Institut de Pharmacologie et de Biologie Structurale (IPBS), Université de Toulouse, CNRS, Université Toulouse III - Paul Sabatier (UT3), Toulouse, F-31077 France
[4] Infrastructure nationale de protéomique, ProFI, FR 2048, Toulouse, France
[5]Dimacell Imaging Facility, UMR PAM A02.102, L'Institut Agro Dijon, INRAe, INSERM, Université Bourgogne Europe, Université Marie & Louis Pasteur, Dijon, France.

Corresponding author: claire.rosnoblet@ube.fr

Lors d'attaques par des microorganismes pathogènes, les plantes mettent en place une cascade de signalisation finement régulée afin de lutter contre le développement de la maladie. Les acteurs moléculaires de cette réponse immunitaire gagnent à être étudiés dans l'optique d'une meilleure compréhension des réponses de défense des plantes afin à terme de limiter l'apport de produits phytosanitaires. Ainsi, lorsque les plantes répondent à une attaque pathogène, on assiste notamment à une augmentation de la synthèse protéique. Or, afin de maintenir une homéostasie protéique correcte et d'éviter un choc protéotoxique, les acteurs du système ubiquitine protéasome (UPS) sont mobilisés. Parmi ces derniers, nous avons mis en évidence que la protéine CDC48, une ATPase dont les homologues chez la levure et les mammifères ont été bien étudiés, était accumulée lors de la réponse immunitaire de cellules de tabac élicitées par la cryptogéine, un facteur de virulence synthétisé par l'oomycète *Phytophtora cryptogea*[1]. Par la suite, grâce à une approche d'immunoprécipitation de la protéine endogène suivie d'analyses par spectrométrie de masse, nous avons mis en évidence que cette protéine intervenait dans les mêmes voies de régulation intracellulaires que ses orthologues de levure et de mammifères, en assistant le protéasome dans la dégradation de protéines ubiquitinylées non directement accessibles à ce dernier. Par ailleurs, CDC48 chez le tabac interagit également avec des protéines impliquées dans la régulation Redox, hautement contrôlée dans la réponse immunitaire végétale[2]. Par la suite, à l'aide de la caractérisation d'ubiquitinomes de cellules de tabac traitées ou non par la cryptogéine et surexprimant la protéine CDC48, nous avons caractérisé la régulation de l'UPS par CDC48[3].

Keywords: CDC48, immunité des plantes, système ubiquitine protéasome

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MCQR: A modular, flexible, and user-friendly toolbox for deep exploration, processing, and analysis of quantitative proteomics data

Thierry Balliau[1], Anne Frambourg[2], Olivier Langella[1], Marie-Laure Martin[3,4,5], Michel Zivy[1], and Mélisande Blein-Nicolas[1]

[1] GQE-Le Moulon, Université Paris-Saclay, INRAE, CNRS, AgroParisTech, IDEEV — 12, route 128 — Gif-sur-Yvette — F-91272 — France
[2] Université Paris-Saclay, INRAE, ENVA, BREED, 78350, Jouy-en-Josas, France
[3] Université Paris-Saclay, CNRS, INRAE, Université Evry, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France
[4] Université de Paris Cité, Institute of Plant Sciences Paris-Saclay (IPS2), 91190, Gif sur Yvette, France
[5] Université Paris-Saclay, AgroParisTech, INRAE, UMR MIA Paris-Saclay, 91120, Palaiseau, France

Corresponding author: melisande.blein-nicolas@inrae.fr

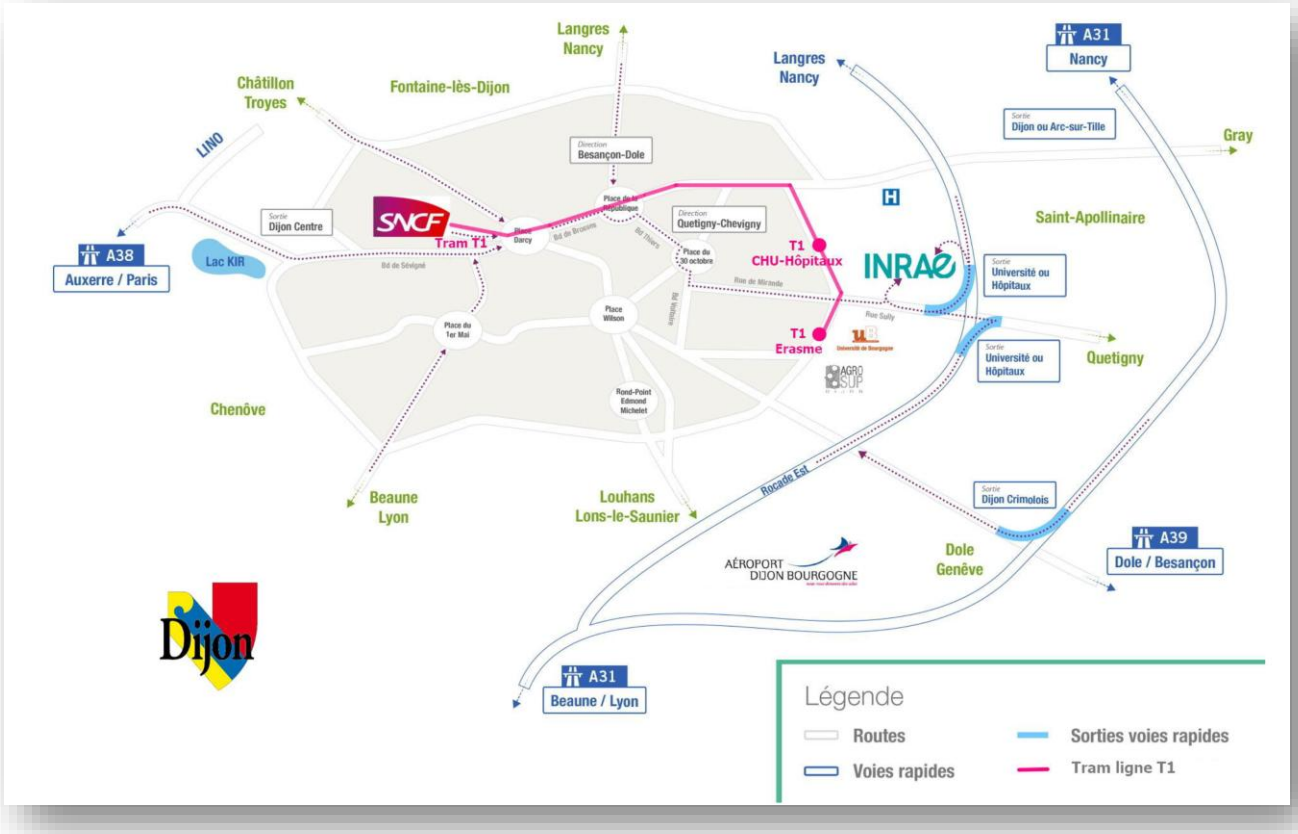
In the field of proteomics, generating biologically relevant results from mass spectrometry (MS) signals remains a challenging task. This is partly due to the fact that the computational strategies for converting MS signals into biologically interpretable data dependent heavily on the MS acquisition method. Additionally, the processing and the analysis of these data vary depending on whether the proteomic experiment was performed with or without labeling, and with or without fractionation. Several R packages have been developed for processing and analyzing MS data, but they only incorporate identification and quantification data; none of them takes into account other invaluable information collected during MS runs. To address this limitation, we introduce MCQR, an alternative R package for the in-depth exploration, processing, and analysis of quantitative proteomics data generated from either data-dependent or data-independent acquisition methods. MCQR incorporates isotope data and leverages experimental retention time measurements for quality control, data filtering, and processing. Its modular architecture offers flexibility to accommodate various types of proteomics experiments, including label-free, label-based, fractionated, or those enriched for specific post-translational modifications. Its functions, designed as simple building blocks, are user-friendly, making it easy to test parameters and methods, and to construct customized analysis scenarios. These unique features position MCQR as a comprehensive toolbox, perfectly suited to the specific needs of MS-based proteomics experiments.

Keywords: quantitative proteomics, mass spectrometry, bioinformatics, quality control, data filtering, statistical analysis

Liste et contacts des participants

Nom	Prénom	Email
Aimé	Delphine	delphine.aime@inrae.fr
Armengaud	Jean	jean.armengaud@cea.fr
Aubourg	Sébastien	sebastien.aubourg@inrae.fr
Besson-Bard	Angélique	angelique.besson-bard@inrae.fr
Bienvenut	Willy	willy.bienvenut@cnrs.fr
Blein-Nicolas	Mélisande	melisande.blein-nicolas@inrae.fr
Bonnot	Titouan	titouan.bonnot@inrae.fr
Boudet	Julie	julie.boudet@inrae.fr
Burstin	Judith	judith.burstin@inrae.fr
Chardot	Thierry	thierry.Chardot@inrae.fr
Christine	Le Signor	christine.lesignor@inrae.fr
Dubos	Christian	christian.dubos@inrae.fr
Duruflé	Harold	harold.durufle@inrae.fr
Frachon	Léa	lea.frachon@inrae.fr
Gallardo	Karine	karine.gallardo@inrae.fr
Guillotin	Bruno	bruno.guillotin@cnrs.fr
Jamet	Elisabeth	Elisabeth.jamet@univ-tlse3.fr
Jeannin	Nicolas	nicolas.jeannin@inrae.fr
Leriche	Pierre	pierre.leriche@inrae.fr
Martinet	Alix	martinet.alix@laposte.net
Maslard	Corentin	corentin.maslard@inrae.fr
Nicolas	Valérie	valerie.nicolas@ube.fr
Pennera	Lorraine	lorraine.pennera@cea.fr
Peuteuil	Amélie	amelie.peuteuil@inrae.fr
Pichereaux	Carole	carole.pichereaux@ipbs.fr
Poinssot	Benoit	benoit.poinssot@inrae.fr
Rajjou	Loïc	loic.rajjou@agroparistech.fr
Rofidal	Valerie	valerie.rofidal@inrae.fr
Rosnoblet	Claire	claire.rosnoblet@inrae.fr
Sémery	Maëla	maela.semery@inrae.fr
Tayeh	Nadim	nadim.tayeh@inrae.fr
Vernoud	Vanessa	vanessa.vernoud@inrae.fr

Plan d'accès



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