Journées du PROTEOME VERT

10 et 11 juin 2024

Institut Agro Montpellier

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BAP

Programme

Lundi 10 juin

13h30-14h00 Accueil

14h00-14h40 **Thomas Schiex** (MIAT, Toulouse)
Designing new proteins with artificial intelligence

14h40-15h00 **Mélisande Blein-Nicolas** (GQE, Saclay)
Integration of phenomic, proteomic, and genomic data into a multi-scale network unravels missing heritability for maize response to water deficit

15h00-15h20 **Thomas Berthelier** (LRSV, Toulouse)
Back to water: the case of *Potamogeton nodosus*

15h20-15h30 Flash Talk - **Harold Duruflé** (BioFora, Orléans)
Présentation du consortium TIMe-ID : Travail Interdisciplinaire sur les Méthodologies d'Intégration de Données temporelles en biologie.

15h30-15h40 Flash Talk - **Willy Bienvenut** (GQE, Saclay)
La metaP au service de la rhizosphère : Envisageable ?

15h40-15h50 Flash Talk - **Laurencia Fera** (GQE, Saclay)
Optimizing the Sample Preparation by Easy Extraction and Digestion (SPEED) protocol: responding oxidation challenges in plant proteomics »

15h50-16h20 Pause

16h20-17h00 **Karine Gallardo** (Agroécologie, Dijon)
Les protéines des graines de légumineuses : état des connaissances et recherches en cours dans LETSPROSEED

17h00-17h20 **Thierry Chardot** (IJPB, Versailles)
On the fate of lipid and protein reserves during Chia (*Saliva hispanica* L.) seeds germination and seedling growth

17h20-17h40 **Olivier Langella** (GQE, Saclay)
Full DDA quantitative proteomics with the i2MassChroQ software project

17h40-18h00 **Madeleine Sugano** (Eco&Sols, Montpellier)
Modifications du protéome d'un champignon ectomycorhizien par la présence de sa plante-hôte : importance pour les échanges nutritionnels champignon-plante

19h Buffet

Mardi 11 juin

9h00-9h40 **Géraldine Jean** (LS2N, Nantes)

Les apports de l'algorithmique dans l'identification des peptides et l'inférence des protéines

9h40-9h50 Flash Talk - **David Landry** (LIPME, Toulouse)

Proteomics approaches to decipher the Nod-independent signaling

9h50-10h00 Flash Talk - **Mathilde Decourcelle** (BCM, PPM, Montpellier)

Phosphoprotéomique à partir de matériel biologique critique : vers un protocole global et optimisé

10h00-10h30 Pause

10h30-11h10 **Bertrand Fabre** (LRSV, Toulouse)

Microprotein: ghosts within the proteome

11h10-11h30 **Aurélie Dupriez** (LRSV, Toulouse)

Plant signaling peptides: Theme and Variations

11h30-11h50 **Véronique Santoni** (IPSiM, Montpellier)

Roles of the protein kinase *At*SnRK2.4 in response to abscisic acid

11h50-12h10 **Laurence Lejay** (IPSiM, Montpellier)

Post-translational regulation of the root nitrate transporter NRT2.1 in Arabidopsis thaliana

12h10-12h30 Discussion générale

Designing new proteins with artificial intelligence

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Computational Protein Design aims at designing (or redesigning) proteins to grant existing proteins or new proteins targeted functions/properties. This talk will show how, in the last few years, the traditional Computational Protein Design toolbox has been largely reinvented by original neural architectures or through the direct adaptation of Generative AI architectures targeted at text (such as ChatGPT) or image (such as Dall-E) generation.
While these architectures seem to be able to design new stable scaffolds far more reliably than the previous generation of computational protein design tools, designing a protein sequence/structure for a targeted function often requires to impose specific constraints/checks on the generated sequence/structure, which is often non trivial using Deep Learning alone. This can be easier using hybrid AI design technology, mixing Deep Learning, Automated Reasoning and Molecular Modeling.
This will be illustrated on various recent protein design examples, from binders to enzymes, including some of our own designs, with associated experimental characterization.

Key words: protein engineering, computational protein design, generative AI, deep learning, automated reasoning, molecular modeling

Integration of phenomic, proteomic, and genomic data into a multi-scale network unravels missing heritability for maize response to water deficit

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The evolution of maize yields under water deficit conditions is of particular concern in the context of climate change and human population growth. However, the multiplicity and versatility of drought-response mechanisms make the design of new drought-tolerant varieties a complex task that would greatly benefit from a better understanding of the genotype-phenotype relationship. To provide novel insight into this relationship, we aimed to build a multiscale network that best explained the genetic variance of the genotype x water deficit interaction of six drought-responsive ecophysiological traits (biomass, leaf area, water use, water use efficiency, transpiration rate, and stomatal conductance).

To address this issue, we implemented an original systems biology method combining genome-wide association study, network inference, and statistical modeling on a multi-omic dataset, including phenotypic, proteomic, and genomic data acquired from 254 maize hybrids grown under well-watered (WW) and water deficit (WD) conditions. This dataset was supplemented with plasticity indices calculated as the WD/WW log ratios for protein abundances and WD/WW ratios for phenotypic traits.

Using our method, we were able to build a multiscale network that increased the explained portion of the genotype x water availability interaction variance of ecophysiological traits by 20 points as compared to a classical GWAS (84% vs. 65%). This network included 63 proteins linked to six ecophysiological traits, and 531 unique loci associated, whether directly or indirectly, with ecophysiological traits. The proteins in the network were involved in signaling, protein folding, and oxidation-reduction processes. Of the loci involved in the network, three were located in genomic regions detected only by the protein analysis and contributed to more than 7 points of the total part of the GxW variance captured. These genomic regions provide a list of candidate genes, several of which can physically interact with the proteins involved in the network.

Overall, our results show that multi-omics data integration can be an efficient way to capture missing heritability for complex phenotypic traits and identify new candidate genes related to drought response.

Back to water: the case of *Potamogeton nodosus*

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450 MY ago, terrestrial plants emerged from aquatic environments[1,2]. This transition was made possible by evolutionary innovations that led to the development of specific traits such as cuticle, stomata, tracheids, and seeds, enabling them to adapt to life on land. More recently, some of these angiosperms have returned to an aquatic life by implementing new strategies[3]. This return to aquatic life was made possible, among other adaptations, by a remodeling of the extracellular matrix including the cell wall and the cuticle. The primary plant cell wall is composed of a polysaccharide portion (cellulose, hemicelluloses, and pectins) and a protein portion that gives it its dynamics.
To investigate the mechanisms allowing this transition back to aquatic life, we have selected *Potamogeton nodosus* as one of our model species. This native plant of French River and collected in the Garonne River, exhibits heterophylly, *i.e.* a morphological distinction between its floating and submerged leaves. After sequencing and assembling the genome of this plant, we conducted a transcriptomic and a cell wall proteomic study. Microscopy was also employed to facilitate the characterization of the differences observed between the two types of leaves. These different analyses allowed to select the CW-related proteins to highlight the adaptive differences existing between submerged and floating leaves. Some examples will be discussed.

References:
1 Nishiyama, T. et al. 2018 Cell 174, 448-464.e24.
2 Delaux, P.M. et al. 2012 Perspectives in Plant Ecology, Evolution and Systematics 14, 49-59.
3 Rascio, N. 2002 Critical Reviews in Plant Sciences 21, 401-427.

Présentation du consortium TIMe-ID : Travail Interdisciplinaire sur les Méthodologies d'Intégration de Données temporelles en biologie

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La question d'intégration de données hétérogènes est aujourd'hui abordée par de multiples communautés provenant de différentes disciplines scientifiques. Cependant, l'intégration de données dites temporelles ou longitudinales en biologie reste un front de science et nécessite le développement de méthodes opérationnelles. Partager nos besoins respectifs de développement de méthodes et de production de données en faisant le point sur ce qui existe semble aujourd'hui nécessaire.
Ce consortium vise à réunir diverses communautés disciplinaires dans le but d'identifier les manques et lacunes méthodologiques et scientifiques dans ce domaine. L'objectif est de faire émerger un projet scientifique, s'appuyant sur la mise au point d'un dispositif expérimental, répondant autant à un objectif de développement et de test de méthodes que de production de connaissances biologiques, sur un système vivant dynamique.

La metaP au service de la rhizosphère: Envisageable ?

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La métaprotéomique (MetaP) est un outil de choix dans la compréhension du microbiote humain et de son interaction avec son hôte. Les progrès instrumentaux et de traitement des données permettent maintenant de mieux mettre en évidence des liens entre certaines maladies tel que la maladie de Crohn et la composition du microbiote. Considérant que les plantes ont une influence sur la sélection du microbiote de la rhizosphère, notamment pour leur développement et/ou leur adaptation aux stress biotiques et abiotiques, il apparaît que ce type d'étude pourrait être transposé aux plantes pour mieux comprendre l'interdépendance ente la plante et le microbiote de la rhizosphère. Il est donc nécessaire de pouvoir extraire les protéines contenues dans le sol et/ou à l'interface avec les racines ce qui représente un challenge compte tenue de la complexité du milieu et des contaminants contenus (matière organique en décomposition, insectes, invertébrés...). Cette présentation à pour objectif de présenter nos premiers tests d'extraction et d'analyse par spectrométrie de masse d'échantillons de terres et des résultats tel que les espèces du microbiote de la rhizosphère que nous avons pu identifier.

Optimizing the Sample Preparation by Easy Extraction and Digestion (SPEED) protocol: Responding oxidation challenges in plant proteomics

Laurencia Fera[1], Shabnam Huseynova[1], Marlène Davanture[1], Thierry Balliau[1], Mélisande BleinNicolas[1]

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A new universal method for the rapid extraction and digestion of proteins, named "Sample Preparation by Easy Extraction and Digestion" (SPEED), has recently emerged [1]. This method consists of three steps: acidification (adding trifluoroacetic acid (TFA)), neutralization (adding tris(hydroxymethyl)aminomethane (TRIS)), and digestion. This protocol addresses several challenges in proteomics analysis by mass spectrometry, such as being a detergent-free method and allowing a very rapid sample processing. Its efficacy has been proven in animal and bacterial cells. However, this protocol has not yet been tested on plant cells. The PAPPSO team, has begun testing this protocol on *Arabidopsis thaliana* but has encountered some challenges because the oxidation levels in the leaves were much higher than in conventional extraction methods (approximately 80% vs less than 20%, respectively). The main aim of my internship is to optimize the SPEED protocol to reduce this oxidation level. To resolve this issue, I have employed two different strategies: first, I added dithiothreitol (DTT) to 2M TRIS, and then I tested various scavengers known for their antioxidant properties. Regarding the first option, it did not decrease the oxidation level, as for the scavengers, we used three different types: L-methionine, glutathione, and L-ascorbic acid at 200 mM, applied at two different times during the protocol. This experiment is currently underway. In conclusion, because of its simplicity and speed of execution, SPEED holds the potential to significantly improve the daily work of plant scientists in proteomics. The next step to further enhance this protocol in plants is to test it on a larger number of samples to assess its suitability for high-throughput proteomics analysis.

[1] Doellinger et al. (2020) Sample Preparation by Easy Extraction and Digestion (SPEED) - A Universal, Rapid, and Detergent-free Protocol for Proteomics Based on Acid Extraction. Mol Cell Proteom.(1):209-222. doi: 10.1074/mcp.TIR119.001616.

Les protéines des graines de légumineuses : état des connaissances et recherches en cours dans LETSPROSEED

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Le développement des cultures de légumineuses à graines représente un levier majeur pour accélérer la transition agroécologique et nutritionnelle. Grâce à leur capacité à accumuler des protéines dans leurs graines sans dépendre de l'utilisation d'engrais azotés, les légumineuses contribuent à réduire l'empreinte environnementale. Cependant, la qualité des protéines est influencée par divers facteurs intrinsèques et extrinsèques à la graine, tels que les conditions environnementales, la disponibilité en nutriments dans le sol, et les molécules non protéiques présentes dans les graines, l'ensemble affectant leur fonctionnalité, digestibilité et caractéristiques sensorielles. Dans ce contexte, le projet LETSPROSEED* a pour objectif de produire de nouvelles connaissances afin d'optimiser la nutrition des légumineuses à graines (féverole, pois, soja), en vue de stabiliser voire améliorer la quantité et qualité des protéines. Il vise également à identifier les molécules de la graine impactant la qualité des protéines, à mettre en œuvre des stratégies pour réduire leur présence et à étudier leur interaction avec les stress biotiques et abiotiques. Ainsi, ce projet transdisciplinaire a pour ambition de développer des leviers agronomiques, génétiques et technologiques visant à améliorer l'utilisation des protéines de légumineuses pour l'alimentation humaine, tout en préservant la performance des variétés au champ. Les recherches transversales se concentrent principalement sur la féverole pour aller jusqu'au développement de prototypes d'analogues de produits laitiers. Les travaux sont répartis en différents volets : l'amélioration du rendement et de la qualité au champ (WP1), l'amélioration génétique de la qualité nutritionnelle et organoleptique des graines (WP2), et l'optimisation de la transformation des graines en ingrédients, avec des études sur la fonctionnalité des protéines (WP3), les interactions entre protéines végétales, polyphénols et protéines salivaires, qui peuvent impacter la perception sensorielle et la digestibilité des ingrédients et des produits formulés (WP4), l'allergénicité des protéines, la nutrition et les effets santé des protéines de féveroles fermentées en terme de métabolisme, de microbiote et de prévention des allergies alimentaires (WP5). Les métabolites spécialisés occupent une place centrale dans le projet car ils peuvent avoir un impact négatif sur la fonctionnalité des protéines, leur digestibilité, ainsi que sur les propriétés organoleptiques. Cependant, certains métabolites spécialisés sont également reconnus pour jouer un rôle crucial dans la résistance au stress. Ces recherches devraient approfondir notre compréhension du compromis entre l'optimisation de la qualité des protéines et la capacité des plantes et des graines à résister aux stress biotiques et abiotiques, donc à maintenir une production suffisante. Ces connaissances permettront de préciser les caractères et les gènes à cibler dans le cadre d'une sélection variétale axée sur l'amélioration des protéines de légumineuses pour l'alimentation humaine.

*LETSPROSEED (2023-2028, https://letsproseed.hub.inrae.fr/), projet financé par l'ANR au titre de France 2030 (n°ANR-22-PLEG-0002), intitulé « Accroitre la consommation des protéines de légumineuses en améliorant leur qualité et leur transformation sans compromettre la résistance aux stress ». Les partenaires : INRAE (UMR Agroécologie, CSGA, IJPB, IPS2, UMR PhAN, UMR SPO/PROBE-PFP, UMR STLO, UR BIA), Université de Caen Normandie (UMR EVA), Institut Agro Dijon (UMR PAM), Agri Obtentions, Bel, Danone, Soredab/Savencia, Terres Inovia, Via Végétale/Teraxion. Projet labellisé Vitagora et coordonné par UMR Agroécologie

On the fate of lipid and protein reserves during Chia *(Saliva hispanica* L.*)* seeds germination and seedling growth.

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Chia (*Salvia hispanica* L.) is an annual herb of the Labiatae family native to Central America. The French chia industry is organized around the Oruro variety, grown in the country's southwest. Chia seed is interesting for human food and nutrition purposes first due to its richness in oil (>30%) and protein (19%). It is rich in both ω3 and ω6 fatty acids with a high ω3/ ω6 ratio making it a valuable source of nutrients. Seeds store their reserves in different specialized organelles, namely lipid droplets (LDs), and protein bodies. Yet, the mobilization and availability of these nutrients for the organism remain challenging. Therefore, we aimed to investigate the impact of time course germination as a natural process to achieve this goal. During germination, dry seeds move from a quiescent forward to an active metabolic state that allows the development of seedlings. Thus, we examined the biochemical and metabolic changes during germination leading to seed reserve breakdown and mobilization. Firstly, the different forms of storage proteins accumulated in mature seeds were identified and monitored for evolution during germination using proteomic approaches. Secondly, we studied the fate of reserve lipids in chia seeds using lipidomic approaches. The seed lipid classes separation and identification by respectively planar chromatography and LC-MS. revealed their remodeling during the germination process. Finally, LDs were purified from mature and germinating chia seeds, and their lipid and protein composition was determined. This contribution highlighted the potential of the germination process and opened the way for chia seed reserve mobilization and their better valorization in human food and nutrition.

Acknowledgments
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Full DDA quantitative proteomics with the i2MassChroQ software project

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Mass spectrometers in the timsTOF line of instruments from the Bruker vendor have become a popular choice in the field of proteomics as of late. The amount of data generated by these instruments is very large, in part due to the number of ion mobility slots (up to 1000) that collectively make a single full scan mass spectrum. Although Bruker provides a free data-handling dynamic link library (dll, only for MS Windows), they also have provided the bio-informatics community with the technical specification of their file format.

We set out to implement a full-featured timsTOF data reader for use with the DDA proteomics i2MassChroQ software that we develop at our PAPPSO facility. In this presentation, we describe the latest features developed in i2MassChroQ with a particular emphasis on the specific optimizations brought to it in the context of quantitative proteomics with timsTOF native data.

Our work brings measurable improvements to such quantitative proteomics analyses as compared to MSFragger (FragPipe), the most performant software currently available for free. We benchmarked i2MassChroQ against MSFragger by comparing the full proteomics workflow with two publicly available data sets. We show that i2MassChroQ has a better capability at XIC extraction as measured by smaller numbers of missing data with respect to the competing software. From a pure quantitative proteomics stand point, i2MassChroQ shows a larger dynamic range of protein quantitation results along with a greater number of quantified proteins in all the samples of each data set.

Modifications du protéome d'un champignon ectomycorhizien par la présence de sa plante-hôte : importance pour les échanges nutritionnels champignon-plante.

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Les associations mycorhiziennes entre les racines des plantes et les champignons du sol sont caractérisées par des échanges de nutriments entre les deux partenaires. Ces échanges s'effectuent au niveau de l'interface cellules fongiques/cellules racinaires où il n'y a pas de communication directe entre les deux types de cellules. Dans les ectomycorhizes, cette interface d'échange est localisée dans le cortex racinaire et est appelée « réseau de Hartig ». Nous avons émis l'hypothèse que dans le réseau de Hartig, certaines protéines fongiques, en particulier les transporteurs d'éléments minéraux comme le potassium (K), le phosphate (Pi) ou l'azote (N), pourraient être modifiées quantitativement ou qualitativement via des modifications post-traductionnelles. Pour aborder cette question nous avons utilisé l'association entre le pin maritime (*Pinus pinaster*) et le basidiomycète ectomycorhizien *Hebeloma cylindrosporum.* Nous avons étudié le protéome du champignon, dont le génome est séquencé, en fonction de la présence/absence de la plante-hôte et du statut P du champignon (plus/moins P). Pour cela, les thalles ont d'abord été cultivés en conditions plus ou moins P puis ont été incubés dans un milieu très simplifié, en présence ou en absence du pin maritime. Ce système d'incubation *in vitro* permet de mimer l'espace apoplasmique du réseau de Hartig des ectomycorhizes, zone très difficile à étudier autrement. Après 48 h d'incubation, les thalles ont été collectés et les protéines totales extraites. Le protéome a été analysé pour les différentes conditions, ainsi que son degré de phosphorylation. Les résultats obtenus montrent que la présence du pin maritime modifie la quantité de protéines et/ou leur degré de phosphorylation. L'ampleur des modifications dépend du statut P du champignon, avec beaucoup plus de modifications lorsqu'il est préalablement carencé en Pi. Par exemple, l'abondance de 52 accessions est modifiée en -P contre 18 en +P. De même, la présence des racines de pin pendant ces 48 h induit la phosphorylation de nombreux transporteurs, dont deux transporteurs de Pi et de peptides, suggérant que la plante-hôte pourrait effectivement modifier les propriétés des transporteurs fongiques pour moduler les flux de nutriments dans le réseau de Hartig.

Keywords : Relation plante-champignon, *Hebeloma cylindrosporum*, *Pinus pinaster*, protéome fongique, phosphorylation, transporteurs fongiques.

Les apports de l'algorithmique dans l'identification des peptides et l'inférence des protéines

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Proteomics approaches to decipher the Nod-independent signaling

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Legumes develop a mutualistic interaction with nitrogen-fixing rhizobia known as root-nodule (RN) symbiosis. Establishment of the RN symbiosis is well-described and in most cases relies on mutual recognition through a specific molecular dialogue[1]. Plant plasma membrane receptors named LysM-RLKs are essential to perceive Nod factors (NF) as bacterial signals[2]. This leads to the formation of a new specialized organ on the root, which is called nodule[1]. Recently, Aesc*hynomene* evenia, which is nodulated by *Bradyrhizobium* strains lacking the *nod* genes, has emerged as a legume model for NF-independent RN symbiosis[2,3]. Nodules appear along the roots as well as along the stem[2]. Recently, a forward genetic screen following random mutagenesis has allowed the identification of new plant actors required for the early steps of this atypical RN symbiosis. Two proteins, a receptor-like cytoplasmic kinase (AeRLCK2) and a cysteine-rich receptor kinase (AeCRK) are essential for the establishment of this symbiosis[5,6]. Such proteins are key players in plant signaling pathways and often involved in receptor complexes. For AeRLCK2 and AeCRK, we first showed that they physically interact and have a intracellular kinase domains. Next, we used a proximity-labelling approach (TurboID-based experiments) to decipher their proximal proteomes in the presence/absence of *Bradyrhizobium*. We successfully identified potential protein partners and are currently investigating their symbiotic involvement through functional studies. Furthermore, we demonstrated that the strong kinase activity of AeCRK trans-phosphorylates AeRLCK2. To reveal other phosphorylation targets of AeCRK, we are setting up phosphoproteomic experiments in the presence/absence of *Bradyrhizobium*. This project will help us to decipher the signaling pathway required to mediate the Nod-independent symbiosis.

Keywords: Symbiosis, Nod-independent, Proximity-labelling, Phosphoproteomic

1. Roy, S. et al. Celebrating 20 Years of Genetic Discoveries in Legume Nodulation and Symbiotic Nitrogen Fixation. Plant Cell 32, 15–41 (2020).
2. Giraud, E. et al. Legumes symbioses: absence of Nod genes in photosynthetic bradyrhizobia. Science 316, 1307–1312 (2007).
3. Arrighi, J. F. et al. Aeschynomene evenia, a model plant for studying the molecular genetics of the nod-independent rhizobium-legume symbiosis. Mol Plant Microbe Interact 25, 851–861 (2012).
4. Horta Araújo, N., Nouwen, N. & Arrighi, J.-F. Nodulating another way: what can we learn from lateral root base (LRB) nodulation in legumes? J Exp Bot (2024).
5. Quilbé, J. et al. A mutant-based analysis of the establishment of Nod-independent symbiosis in the legume Aeschynomene evenia. Plant Physiol 190, 1400–1417 (2022).
6. Quilbé, J. et al. Genetics of nodulation in Aeschynomene evenia uncovers mechanisms of the rhizobium–legume symbiosis. Nat Commun 12, (2021).

Phosphoprotéomique à partir de matériel biologique critique : vers un protocole global et optimisé

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Pour les approches de phosphoprotéomique, en particulier pour des recherches de modifications sur Tyrosine, il est nécessaire de partir d'une quantité importante de matériel de départ. Ces résidus jouent un rôle majeur dans les voies de signalisation mais leur très faible abondance rend leur analyse par spectrométrie de masse difficile. En partant de la quantité la plus limitée possible de matériel biologique, le défi consiste à combiner et optimiser différentes approches de préparations pré-analytiques d'un même échantillon afin d'obtenir un workflow pour une analyse simultanée et complète des protéomes et phosphoprotéomes, incluant spécifiquement les modifications post-traductionnelles de type phosphotyrosine.

Microproteins: ghosts within the proteome

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Recent studies have shown that hundreds of small proteins were occulted when protein coding genes were annotated. These proteins, called microproteins, short open reading frame encoded peptides or alternative proteins, have failed to be annotated notably due to the short length of their open reading frame (less than 100 codons) or the enforced rule establishing that messenger RNAs (mRNAs) are monocystronic in eukaryotes. Several microproteins were shown to be biologically active molecules and seem to be involved in a wide range of biological functions. However, genome wide exploration of the microproteome is still limited to a few species. This is mainly due to their absence in canonical protein databases (*e.g.,* UniProtKB or TAIR) as well as their low expression level. In this presentation, I will discuss state-of-the-art mass spectrometry-based approaches for the identification of microproteins, as well as their advantages and challenges. I will notably describe a few deep mass spectrometry-based workflows which enabled the identification of hundreds of microproteins in several species.

Keywords: Microproteins; Short open reading frame-encoded peptides; Alternative proteins; Mass spectrometry

Plant signaling peptides: Theme and Variations

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Plant cell walls are extracellular matrices involved in cell-to-cell adhesion, plant support and signaling. They are constituted of complex networks of polysaccharides and structural proteins that can be rearranged during development and in response to environmental cues thanks to the activity of cell wall proteins (CWPs). These CWPs belong to diverse families among which proteases able to release signaling peptides from small pro-proteins. This study aims at identifying such peptides in their native form. *Arabidopsis thaliana* was used as a model plant and extracts from healthy plants and of plants treated with a bacterial elicitor (fgl22) were analyzed. Different strategies have been used to isolate fractions enriched in small proteins and peptides: (i) either extracellular fluids were extracted by low-speed centrifugation of leaves vacuum-infiltrated with a low molarity buffer; or (ii) total peptides were extracted with trichloroacetic acid (TCA) from ground frozen leaves. In the former case, small proteins were selected by centrifugation through a cut-off column with a size-exclusion of 10 kDa and there was no tryptic digestion. In the latter case, a partial tryptic digestion was applied. The proteins were identified using both the Araport11 database and a home-made database collecting known small proteins predicted to be secreted as well as newly-annotated ones based on prediction of short open reading frames and/or sequence homology. Among the identified proteins, we found Cys-rich proteins, SCOOP, RALF and CLE precursors. The length of the peptides was highly variable. In addition, we could localize the positions of hydroxyproline (Hyp) residues on some peptides and there were also found to be variable. These results will be discussed considering (i) proteolytic events occurring *in planta* or during the extraction procedure or (ii) regulatory mechanisms. Moreover, the identified peptides will be compared to those who have been shown to be active when synthesized and applied on plant organs.
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Keywords: cell wall, native peptide, peptidomics, proline hydroxylation

Roles for a protein kinase *At*SnRK2.4 in response to abscisic acid

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Abscisic acid (ABA) regulates various aspects of plant physiology, including adaptive responses to abiotic and biotic stresses. Growth regulation by ABA is both promotive and inhibitive, depending on the context, such as concentrations, tissues, and environmental conditions. ABA is perceived by receptors acting in concert with protein phosphatases 2C (PP2Cs). Such complexes release SnRK2 protein kinases from the latent inhibition by PP2C allowing the SnRK2-mediated phosphorylation and regulation of downstream targets. In Arabidopsis, protein kinases from SnRK2 class III and I are described to be mainly ABA- and osmotic responsive, respectively. However, we recently showed that SnRK2.4, a member of class I, is involved in the response of root hydraulic conductivity in resting conditions and under low ABA treament (Shahzad et al. Plant J. 2024, 117:264). In a root phenotyping experiment we also recently showed that the decreased lateral root density under low ABA exposure depended on SnRK2.4. To get insights into the molecular mechanisms regulated by SnRK2.4, we compared the proteome and phosphoproteomes of wild-type plants and of *snrk2.4* mutant plants in resting conditions and upon a 1 µM ABA treatment. Partially overlapping substrates were retrieved in both conditions suggesting that SnRK2.4 is already active in resting condition, putatively due to low level of endogenous ABA. This proteomic study revealed that SnRK2;4 could i) interfere with protein translation, ii) regulate transporters involved in the plant hydromineral nutrition iii) induce a cascade of protein kinases including AGC kinases, MAPKs, CDKs and LRKs and iv) alter the phosphorylation of plasmodesmata proteins, thus, putatively modulating cell-to-cell communication. Hypotheses are discussed whereby, under low ABA cellular content, SnRK2.4 would interfere with auxin circulation across plasmodesmata by favoring callose deposition, contributing *in fine* to regulate the lateral root density.

Post-translational regulation of the root nitrate transporter NRT2.1 in *Arabidopsis thaliana*

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Nitrate is an essential macronutrient for plant growth. In addition, it has a signaling function triggering specific adaptations in metabolism and root development. In *A. thaliana*, the transporter NRT2.1 is known to have a central role for root nitrate uptake and it could also play a role in signaling for root development in response to nitrate. Over the past few years, we combined different approach to study the regulation of NRT2.1 at the protein level. It started to draw a complex picture for NRT2.1 post-translational regulations involving phosphorylation, interaction with protein partners and cleavage of the C-terminal part.
To further investigate the role of NRT2.1 C-terminus processing, we produced transgenic plants with truncated forms of NRT2.1. This revealed an essential sequence for NRT2.1 activity located between the residues 494 and 513. Using a phospho-proteomic approach, we found that this sequence contains one phosphorylation site, at serine 501, which can inactivate NRT2.1 function, when mimicking the constitutive phosphorylation of this residue in transgenic plants. We are now exploring the role of this phosphorylation site in both the regulation of root nitrate uptake and in nitrate signaling.

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