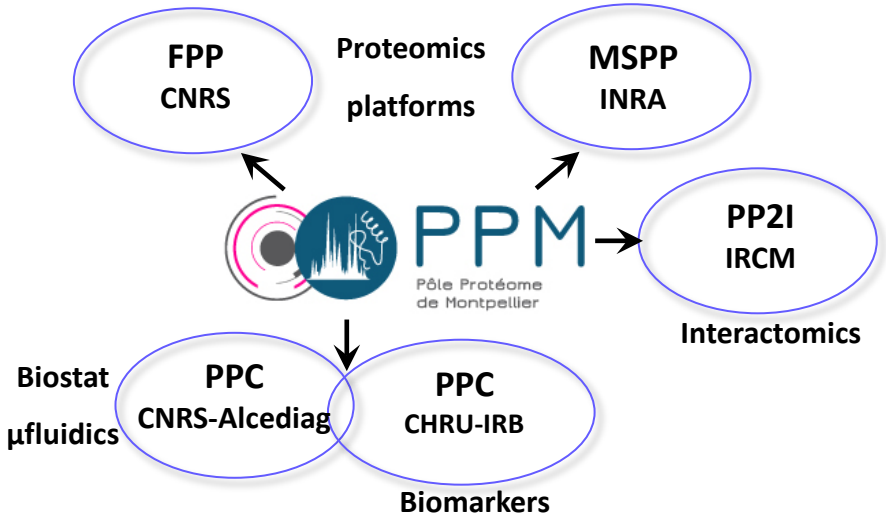
Plate-forme de spectrométrie de masse protéomique

MSPP
Mass Spectrometry Proteomics Platform

Contact: umr-bpmp-mspp@supagro.inra.fr

Local



Network

Mass Prot' INRA

protéome vert
green proteome

sfeap
société française d'électrophorèse & d'analyse protéomique

Label

→ IBiSA (Infrastructures en Biologie Santé et Agronomie)

→ ISO9001:2015

MSPP – skills

Pipeline HPLC-spectro

nanoLC-Q-TOF
Maxis impact HD
(Bruker)

nanoLC-Q-Orbitrap
Q-Exactive+
(Thermo)

MS analyses

1	2	3	4
Identification	**Large-scale and label-free quantification**	**Targeted approach (Parallel Reaction Monitoring)**	**Post-translational modification**
→ proteome → interactome	→ quantitative analysis of proteomes	→ to identify minor proteins → semi absolute quantification	→ phosphorylation → ubiquitylation

Biochemistry

Enrichment
- phospho-pept
- Ubiquitylated-pept

Bioinformatical analysis of data

MATRIX SCIENCE
Mascot

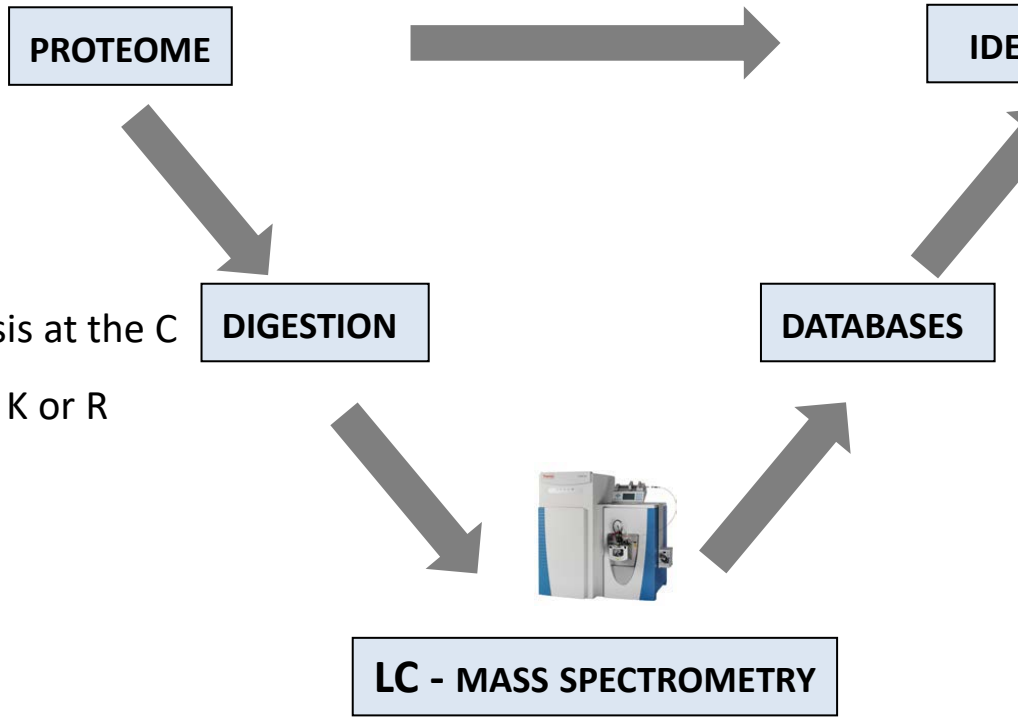
MaxQuant

Skyline

X!Tandem

MassChroq

Principle of proteomic strategy - bottom up



Trypsin: hydrolysis at the C terminus of K or R

By comparing the experimental data to existing data banks, masses identify the peptides in the sample

Accurate measurement of

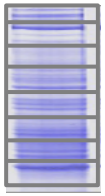
- **mass of peptides (MS) and**
- **mass of peptide fragments (MS/MS)**

Root membrane proteome

Microsome Purification

Microsome stripping

Protein fractionation



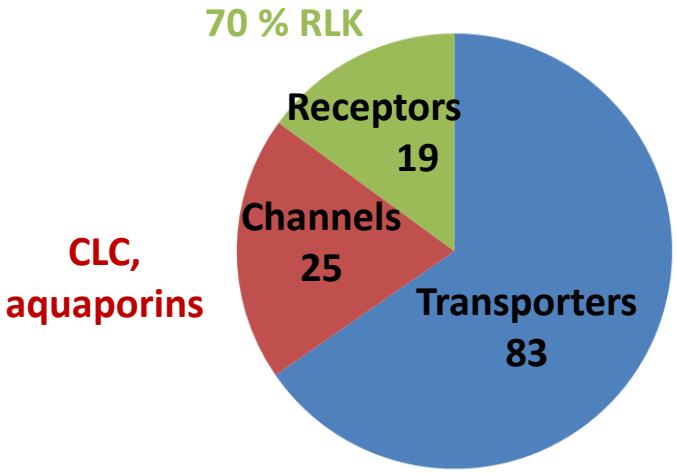
Digestion

Identification LC-MS/MS

2000 Proteins

800 Transmembrane proteins

130 Transporters/receptors/channels



Shazad et al. unpublished

Interactome - case of aquaporin

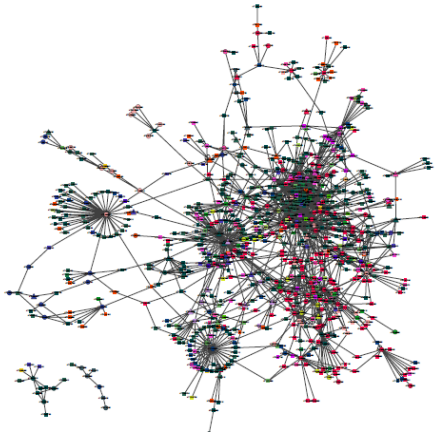
Plants expressing GFP-aquaporin

→ Immuno-purification with anti-GFP

↓ MS identification of interactants

↓ 400 proteins

Cytoscape analysis →

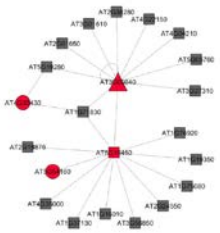


Bellati et al. MCP 2016
Champeyroux et al. submitted

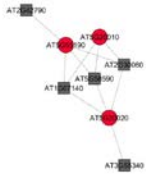
Aquaporin network (883 protéines / 1620 liaisons)

↓

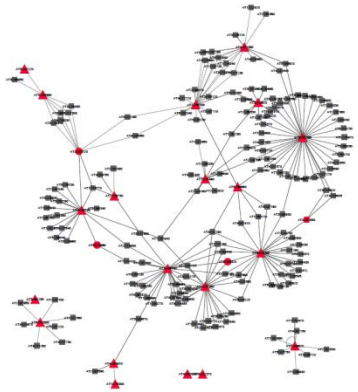
New regulatory mechanisms identified by clusters



Brassinosteroid



GTPase

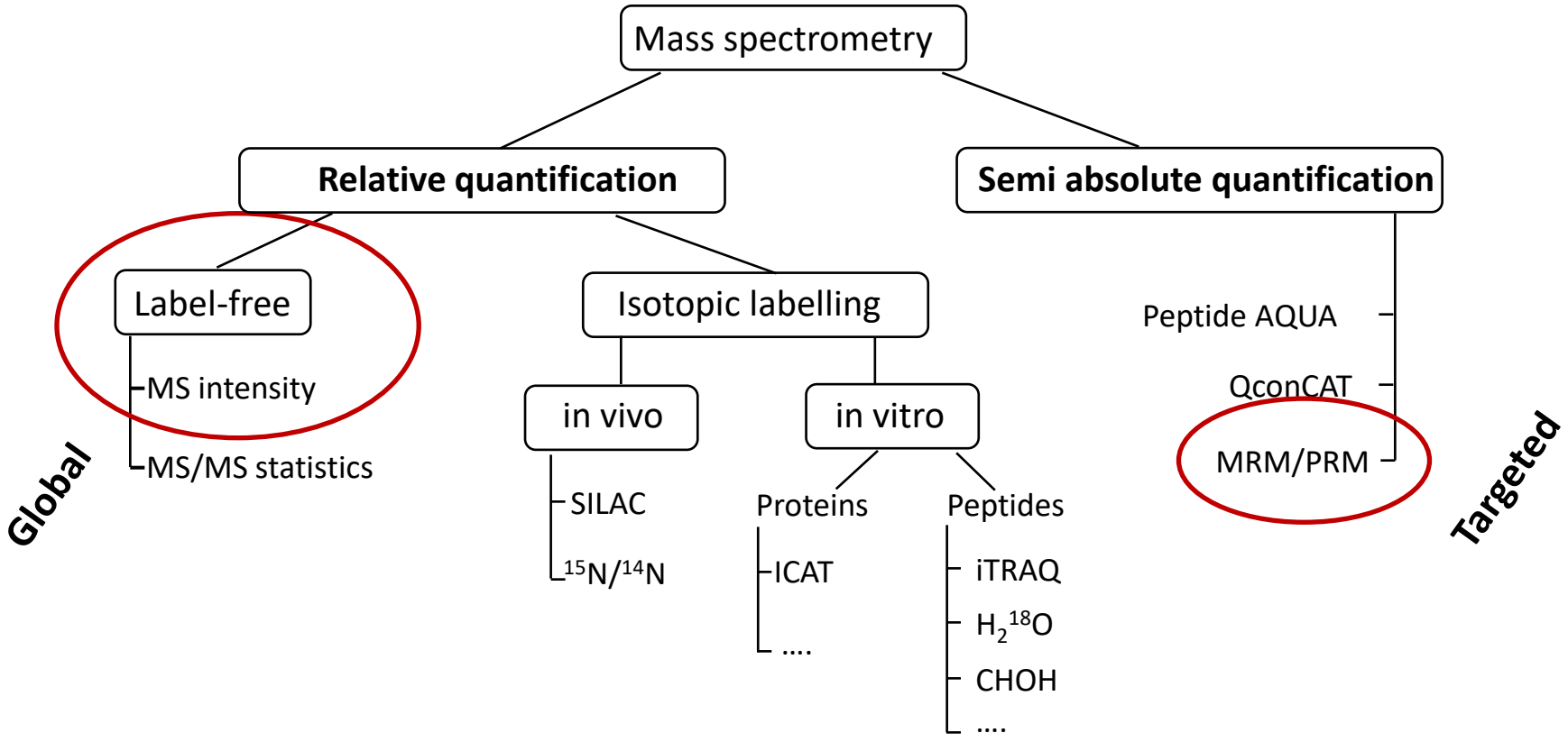


NaCl network

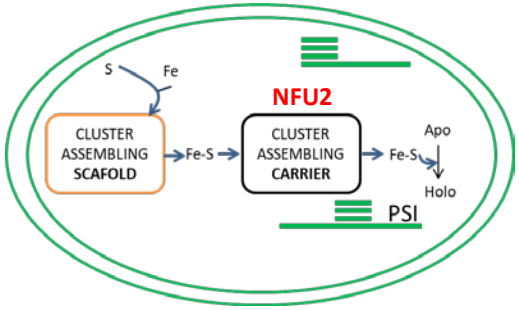
A role in the regulation of PIP function ?

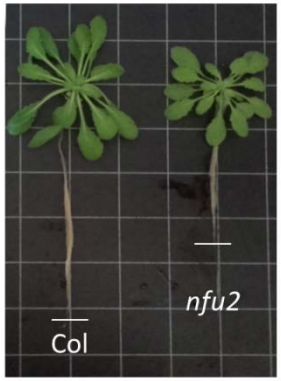
NaCl interferes with proteins involved in endomembrane trafficking

MS-based-quantitative methods

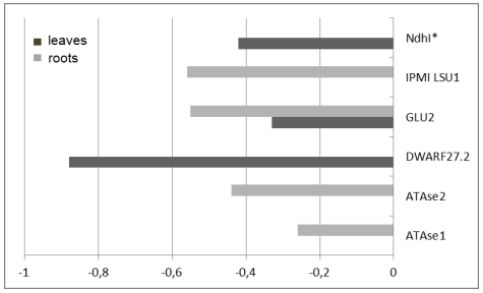


Fe-S cluster transfer in chloroplast





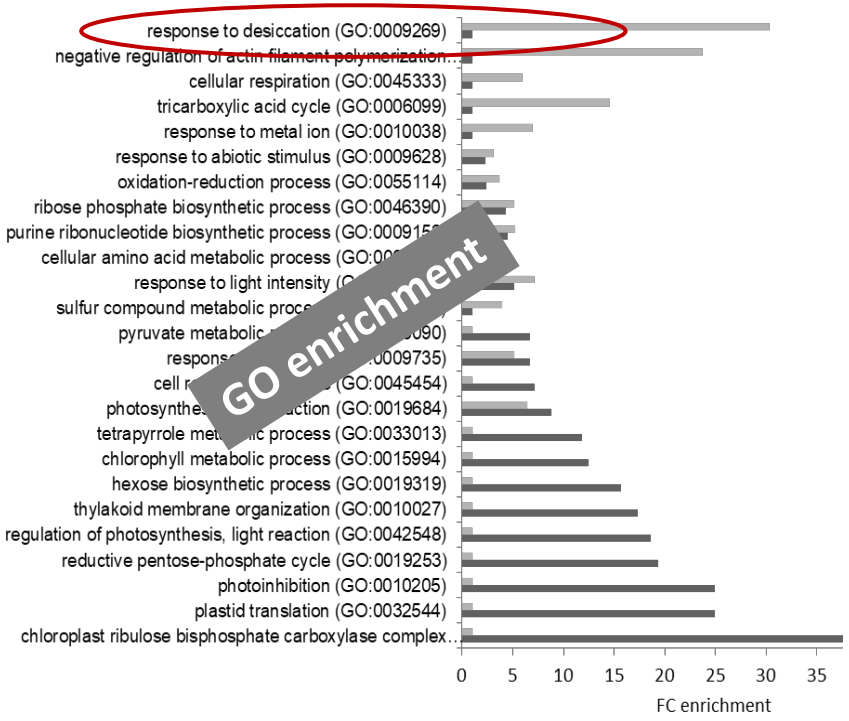
Objective 1: to identify specific targets of NFU2 by label-free quantitative analysis of WT and nfu2 proteomes



33 among 50 targets were quantifiable

→ the abondance of 7 targets decreased in *nfu2*

Objective 2: to identify novel functions affected by NFU2



→ Sensitivity of *nfu2* to dessication

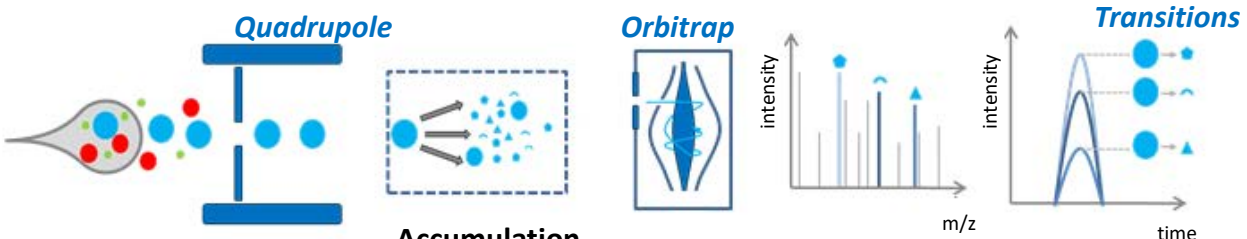
→ Decreased ABA content in *nfu2*

Berger et al., in preparation

Parallel Reaction Monitoring to identify low-abundant proteins

➡ Orbitrap technology

Q-Orbitrap Exactive+

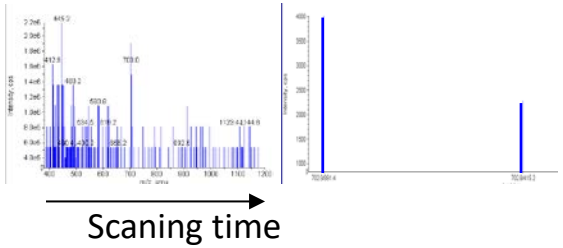


ESI | Mass filter | Accumulation in C-Trap HCD fragmentation | Analyzer | MS/MS spectra | PRM trace

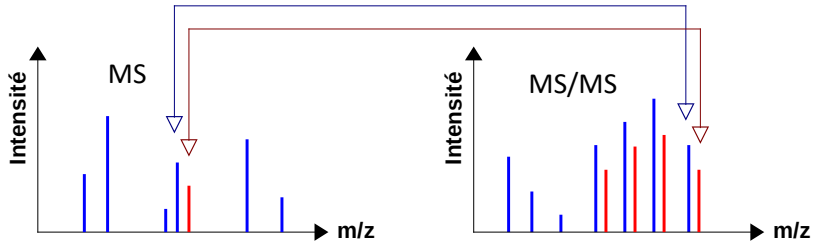
➡ Known protein sequence

Labeled peptides | same sequence as the peptide of interest
with a mass increment due to isotopic labeling
strictly similar MS behavior

→ High specificity and sensitivity



→ Precise relative quantification



$$\text{ratio} = \frac{\text{L (endogenous)}}{\text{H (labeled)}}$$

Ethylene receptors in WT and Never Ripe mutant tomato fruit

(coll. C Chervin, Toulouse)

Strategy

IMG MG Br Br8

↓

Membrane protein extraction

↓

Protein fractionation

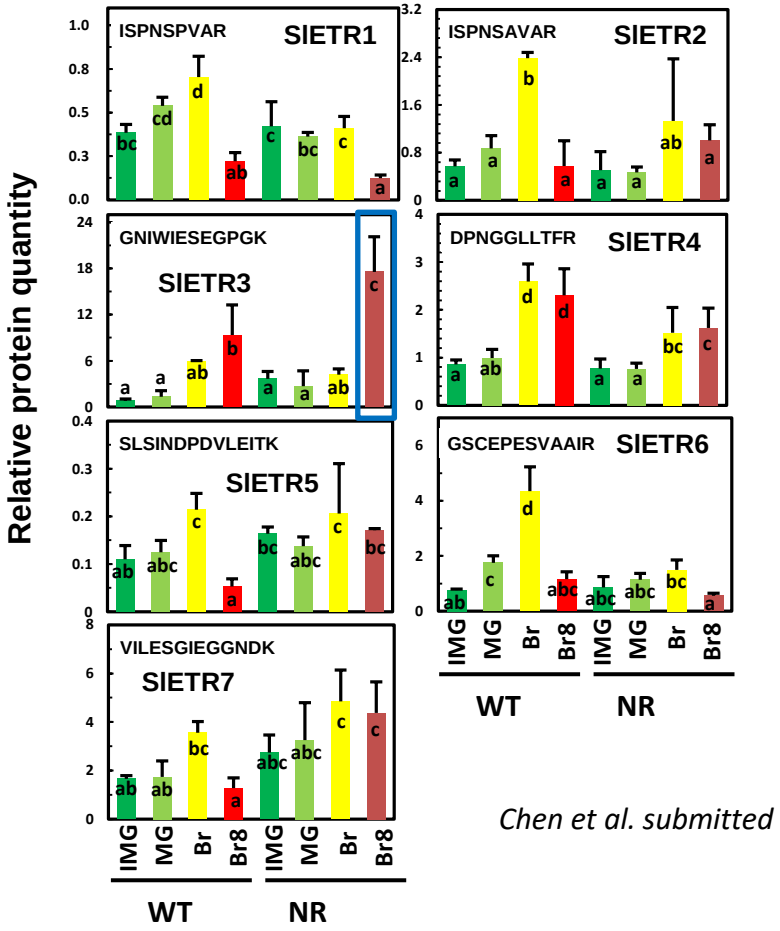
↓ Trypsin digestion

PRM analysis with spiked labelled ETR peptides

Q-EXACTIVE +

- Design of ETR peptides
- PRM parameters optimisation
- Calibration curve
- Relative quantification curve

Identification and quantification all ETRs



Chen et al. submitted

Never Ripe
- → **accumulation of mutated and inactive ETR3**
- → **blocking of the ethylene signaling pathway**
- → **orange tomato**

> than 200 PTM classes

Table 1. Some common and important post-translational modifications

PTM type	ΔMass[a] (Da)	Stability[b]
Phosphorylation		
pTyr	+80	+++
pSer, pThr	+80	+/++
Acetylation	+42	+++
Methylation	+14	+++
Acylation, fatty acid modification		
Farnesyl	+204	+++
Myristoyl	+210	+++
Palmitoyl	+238	+/++
etc.		
Glycosylation		
N-linked	>800	+/++
O-linked	203, >800	+/++
GPI anchor	>1,000	++
Hydroxyproline	+16	+++
Sulfation (sTyr)	+80	+
Disulfide bond formation	–2	++
Deamidation	+1	+++
Pyroglutamic acid	–17	+++
Ubiquitination	>1,000	+/++
Nitration of tyrosine	+45	+/++

[a]A more comprehensive list of PTM Δmass values can be found at: http://www.abrf.org
[b]Stability: + labile in tandem mass spectrometry, ++ moderately stable; +++ stable.

From Mann and Jensen (2003) Nature Biotech.

etc ...

Membrane phospho-proteome

Root membrane fraction

↓

Protein digestion

↓

Phospho-peptide enrichment (titane column)

↓

Maxis ← **MS analysis** → **Q-Exactive+**

Vialaret et al. Proteomics 2014

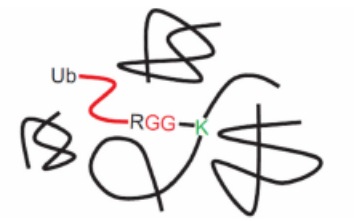
500	**Phospho-peptides**	**950**
350	**Phospho-proteins**	**575**

Shazad et al. unpublished

↑ spectrometers performances ⇨ ↑ phospho-sites identification

Ubiquitination

Strategy

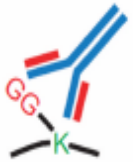


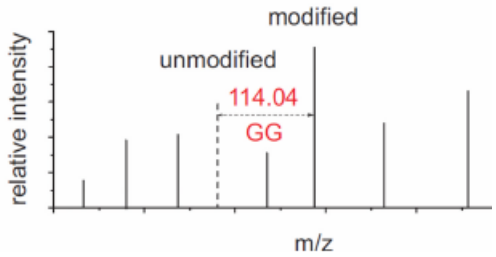
Protein lysate

Trypsin digestion

Ubiquitin remnant-containing peptide

Immuno - precipitation



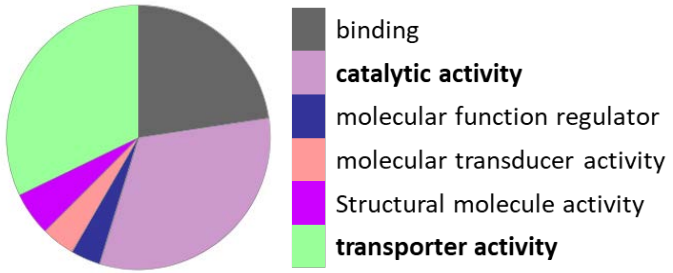


Preliminary results

Root microsomes → 650 Ubi-peptides

↕

300 Ubi-proteins (50 % with TMD) ↔



Perspectives

Label-free quantification → comparisons of softwares

PRM → precise quantification

Post-translational modifications

→ quantification of ubiquitination

→ other PTM ?

Other development ?

Communication - formation

Formation Inter-régionale CNRS-Inserm-INRA

- ✓ Protéomique et étude des modifications post-traductionnelles

14-17 juin 2016 , Le Lazaret, Sète

- ✓ Analyses protéomiques quantitatives: choix de méthodes et analyses de données

28/05-01/06 2018 , Le Lazaret, Sète

INRA- MassProt network

- ✓ De la préparation des échantillons à l'interprétation, validation et intégration des résultats; stratégies et aspects pratiques . 13-16 juin 2017 Cannes Mandelieu

Biocampus

BPMP

- ✓ Thematic school MISTRAL
- ✓ Internal (soon: initiation to proteomics)
- ✓ Students (M2, L3)

MSPP – operating mode

STEPS

- 1st meeting: technical feasibility of the project /risks/cost → « CR mise en place projet »
- Regular follow-up of the project → « CR d'étapes »
- Valorization

COST

Different prices according to

- **delivery *vs* collaboration**
- **academic lab. *vs* BPMP**
- **private company**

Cost/run

	Identif	Quanti	Phos	Q+Phos
Delivery	150 €			
Coll. academic lab.	85 €	105 €	125€	145€
Coll. BPMP	**50€**	**65 €**	**75€**	**85€**
Private company	480 €			

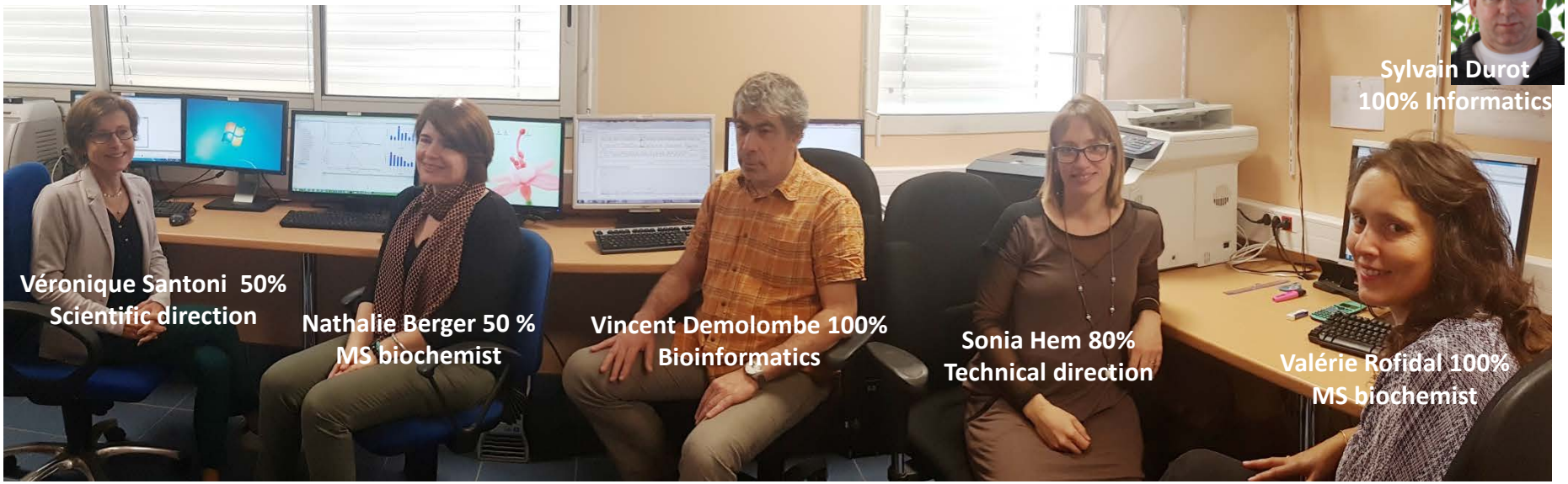
40 % discount

TIMING

up to 20 samples : **4-6 weeks for identification**

6-8 weeks for label-free quantification

MSPP team



Sylvain Durot
100% Informatics