



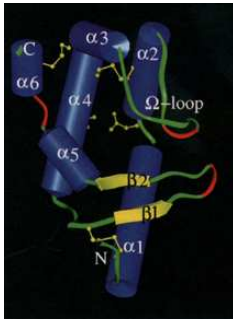
QUANTITATIVE PROTEOMICS REVEALS A DYNAMIC ASSOCIATION OF PROTEINS TO DETERGENT RESISTANT MEMBRANES UPON ELICITOR SIGNALING IN TOBACCO

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Tobacco - Cryptogein model



Cryptogein = small protein of 10 kDa synthesized by the oomycete *P. cryptogea* non host for tobacco.

Inoculation with *Phytophthora parasitica*

disease



Pre-treatment with cryptogein

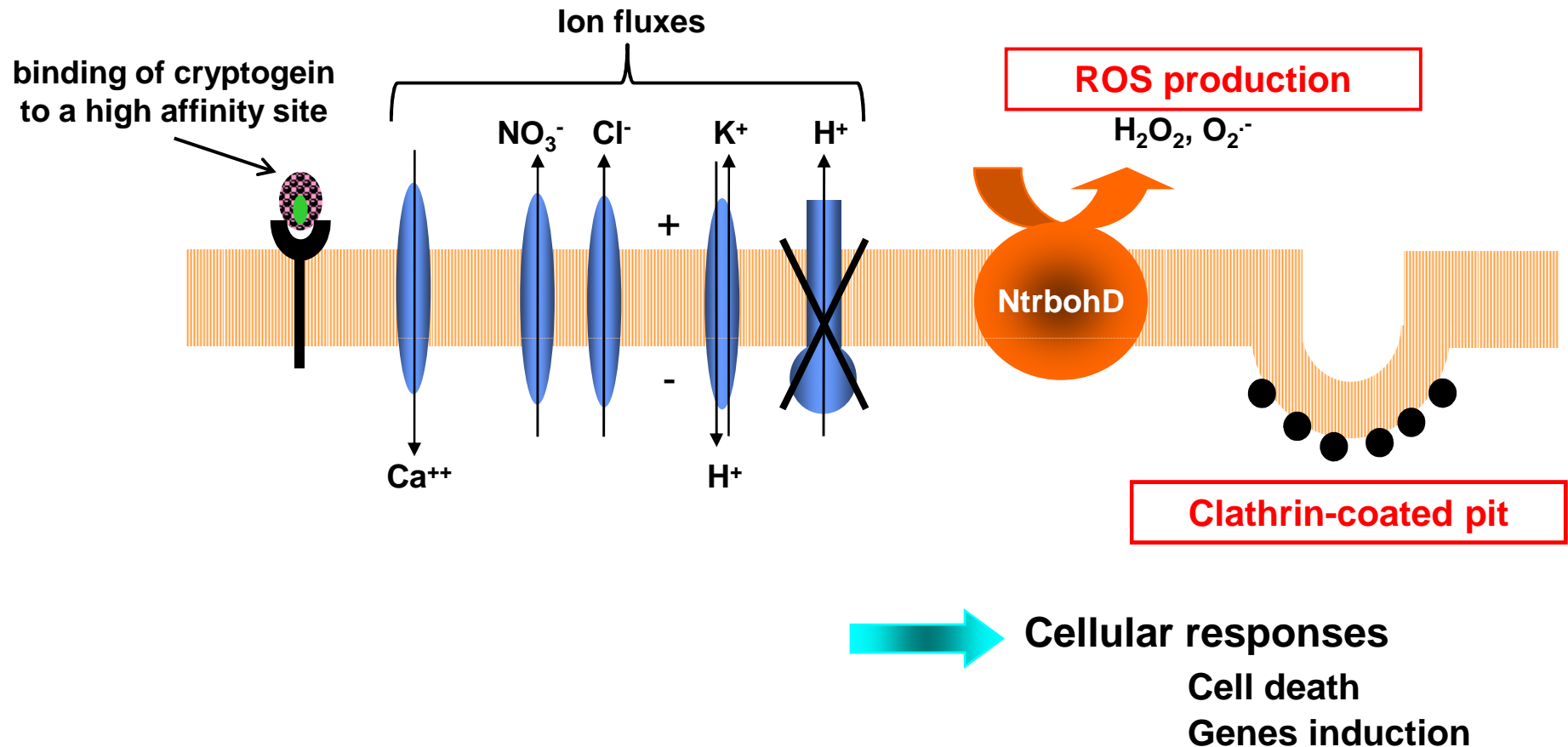


Necrosis
Hypersensitive
Response

Systemic
Acquired Resistance

This model is favourable to understand mechanisms of defence signalling

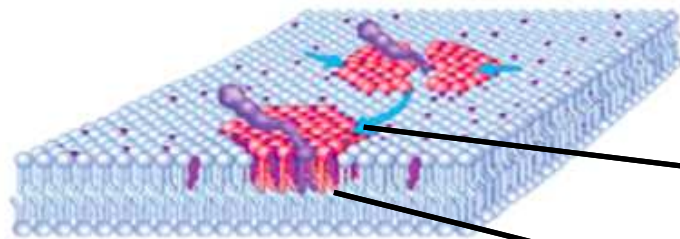
Early signalling triggered by cryptogein on plasma membrane



These very early events involve proteins located on the plasma membrane

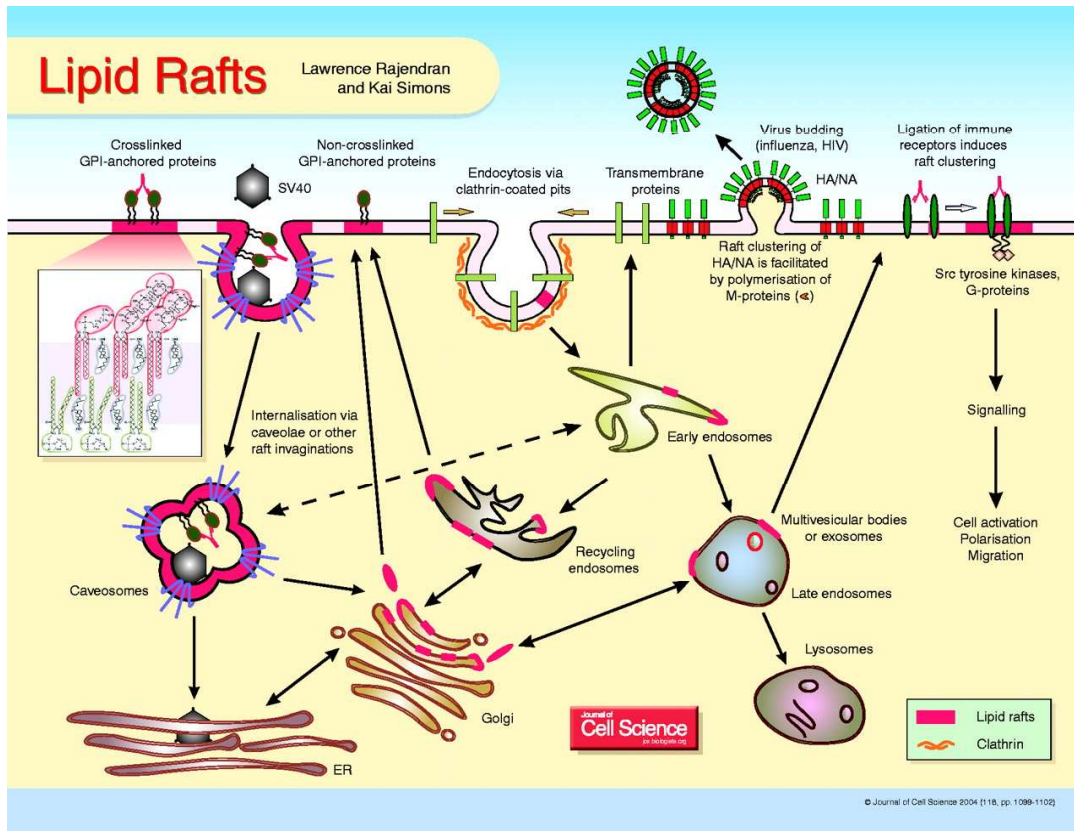
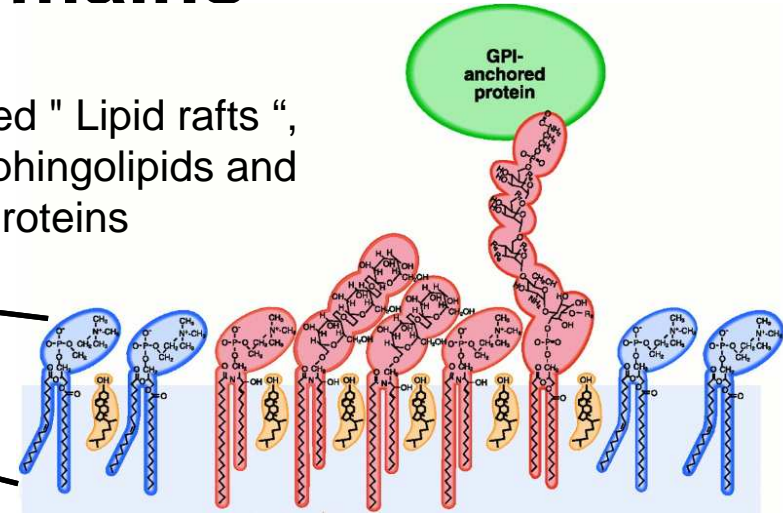
→ particular organization ?

Animal microdomains



lipids forming the PM are spatially organized

Some domains, called " Lipid rafts ", are rich in sterols, sphingolipids and signaling proteins



Compact structure



DRM
=

Detergent Resistant Membranes

Lipid Raft involved in many cellular process such as :

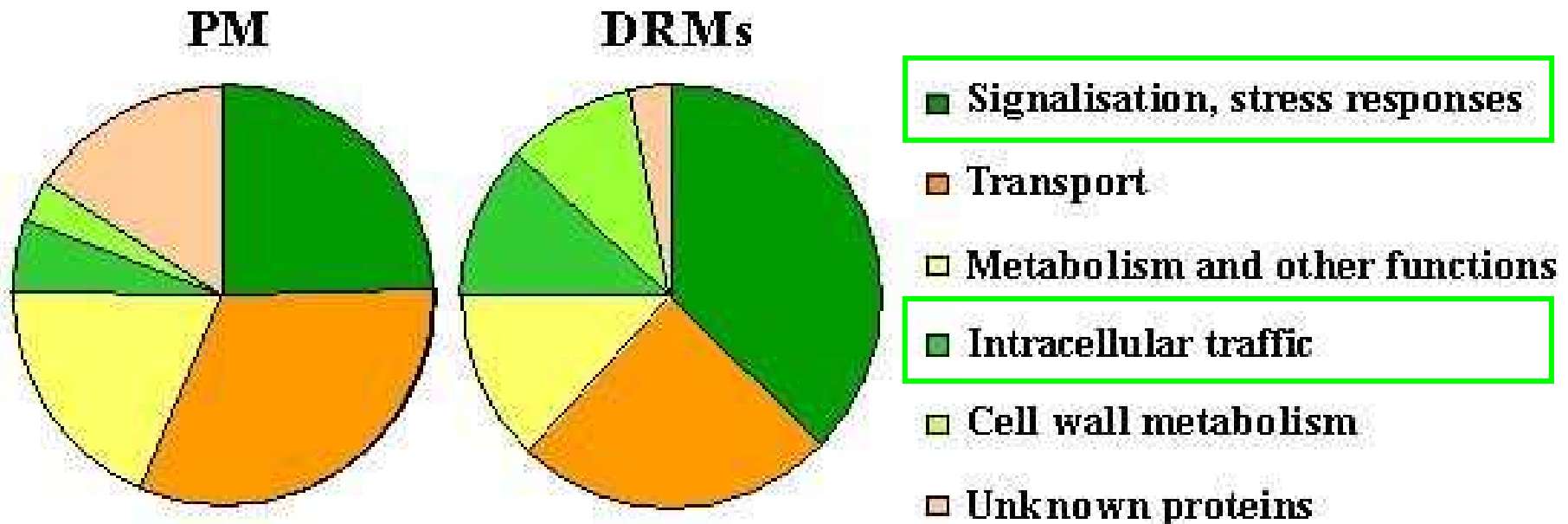
- Endocytosis
- Virus budding
- immune receptor signalling

Microdomains in tobacco?

- Detergent resistant membranes were purified from PM of tobacco leaves and cells. This fraction is enriched in sterols and sphingolipids (Mongrand *et al*, 2004)

As in animals, plant microdomains have a particular lipid composition

- Proteomic study  **145 DRM-associated proteins**



(Morel *et al.*, Mol Cell Proteomics 2006)

As in animals, plant microdomains are enriched in signaling proteins

Microdomains : **dynamic** protein **reservoirs** involved in the implementation of defense mechanisms?

Comparative analysis of DRM proteome from cells treated or not by cryptogein



Identification of potential candidates whose association to DRMs
change in response to cryptogein

Identify new potential actors of early cryptogein signaling

**Test the hypothesis that association/ dissociation of proteins to
microdomains is a signaling element activated by cryptogein**

For this, we need to have a quantitative proteomic approach

Workflow on Tobacco cells

N14
labelled



N15
labelled

+ Cry
(T)

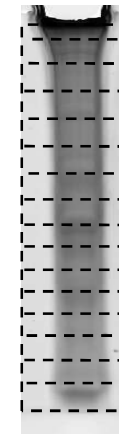
- Cry
(C)

Combining

DRM Purification

Separation of proteins
by 1D electrophoresis

N14T/N15C



Band 1

Band 20

Incubation 5 min with cryptogein

N15
labelled



N14
labelled

+ Cry
(T)

- Cry
(C)

Reverse experiment

Combining

DRM Purification

Separation of proteins
by 1D electrophoresis

N14C/N15T



Band 21

Band 40

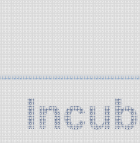
Samples undergo a trypsin digestion and
an LC-MS / MS analysis on OrbiTrap

Workflow on Tobacco cells

N14
labelled

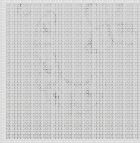


N15
labelled



Incubation

N15
labelled



N14
labelled



Very high amount of generated data



Need to have an automated methodology to
analyze these experiments



Validation of the software developed by the
proteomic platform of Toulouse
P2-087

Samples undergo a trypsin digestion and
an LC-MS / MS analysis on Orbitrap

Separation of proteins
by 1D electrophoresis

Band 1

Band 20

Separation of proteins
by 1D electrophoresis

Band 21

Band 40

MFPaQ : Mascot File Parsing and Quantification



Mascot File Parsing and Quantification - 4.0.0 *beta 1*

Home

Configuration

Experiment manager

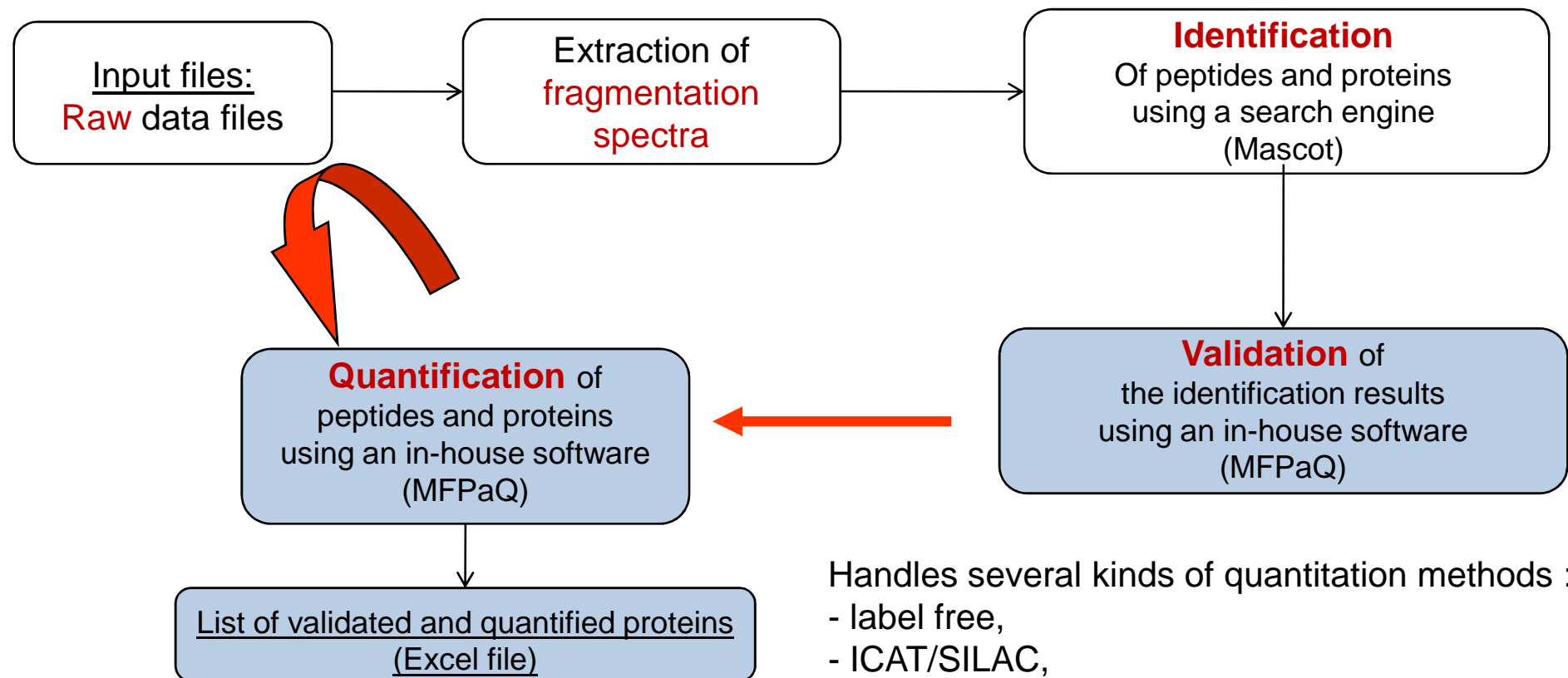
Quantification

Login : emma

Password :

Connected !

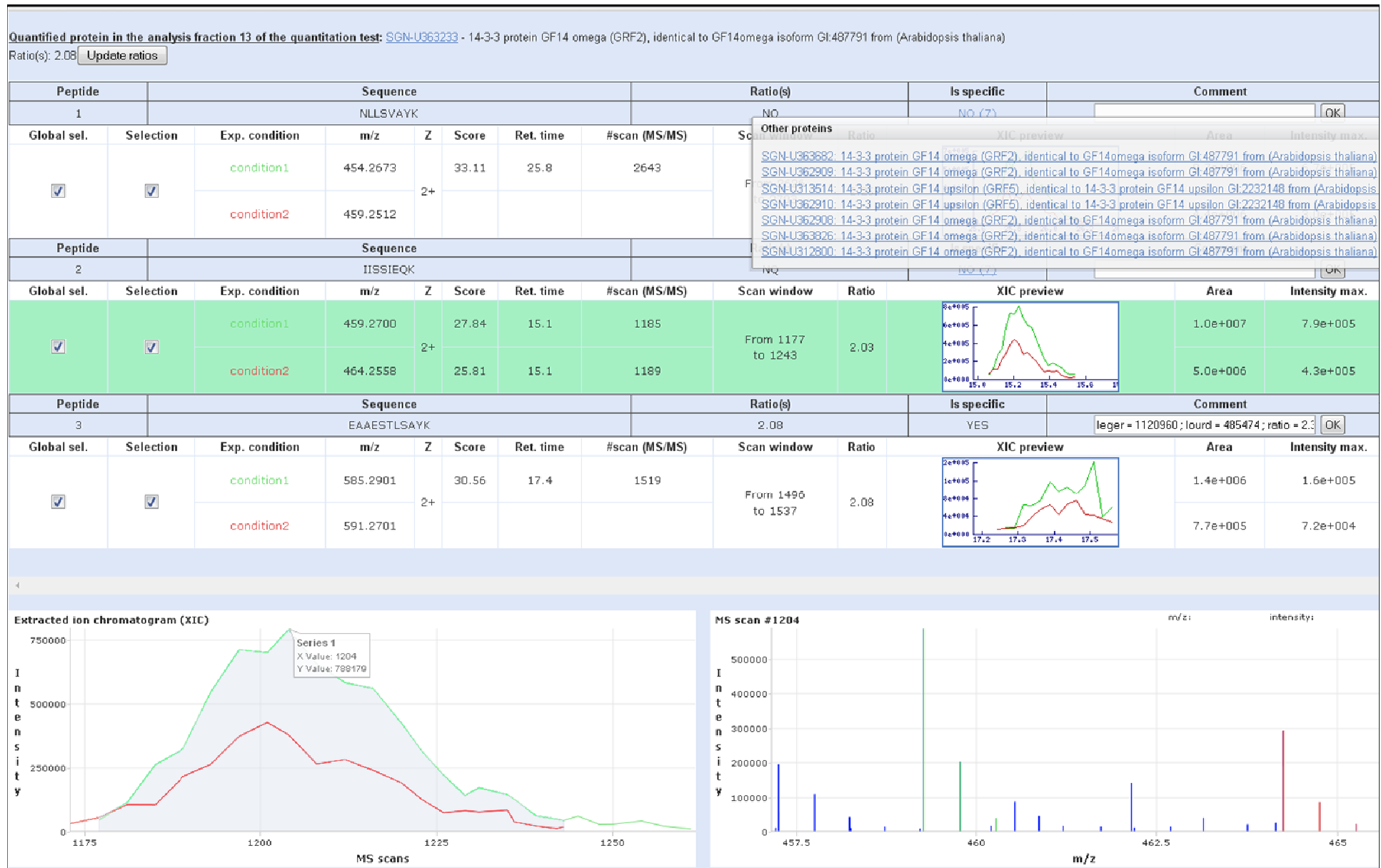
Current user : emma



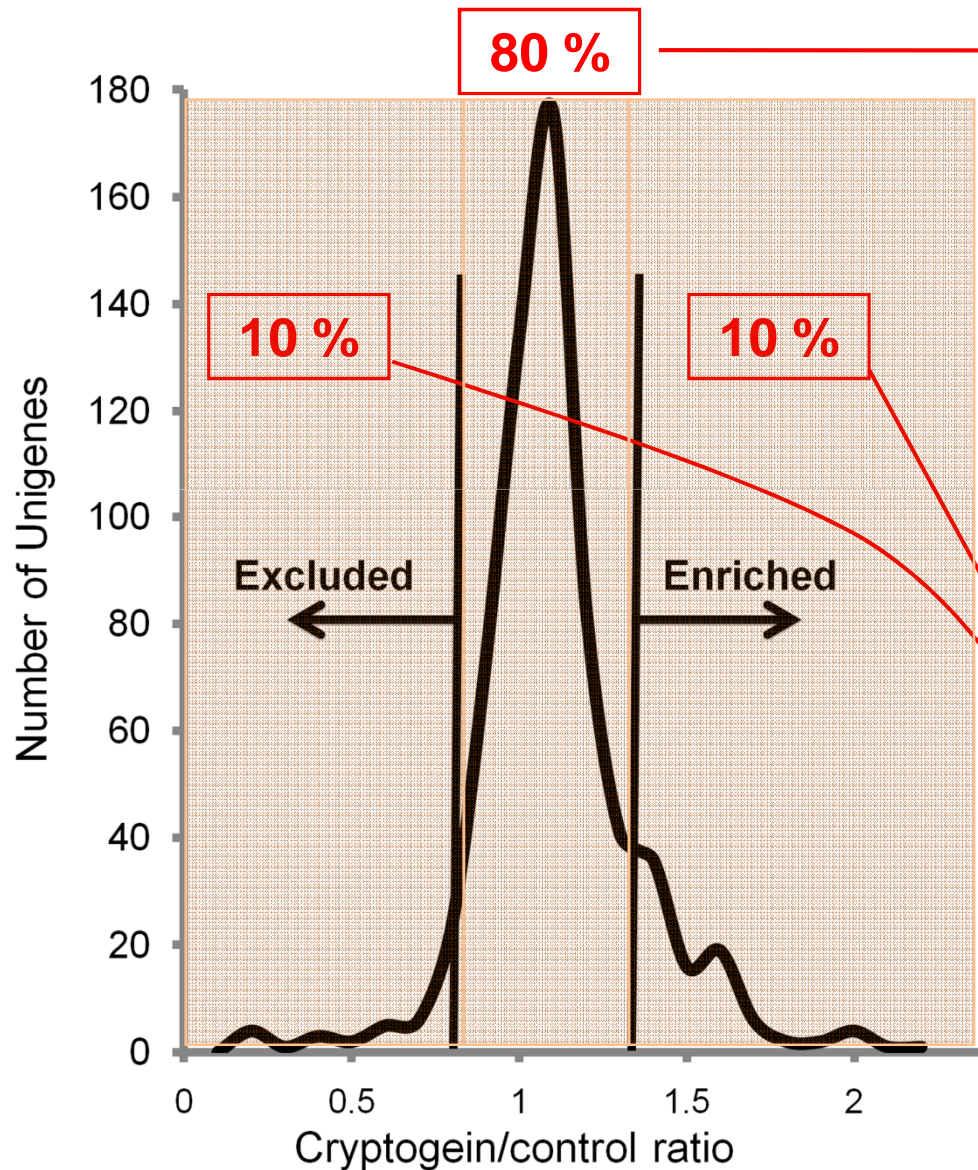
Handles several kinds of quantitation methods :

- label free,
- ICAT/SILAC,
- **14N/15N labelling.**

MFPaQ quantification of the 14-3-3 protein



How did we select enriched or excluded proteins ?



80 % of proteins exhibited a ratio close to the median values



Most of proteins are not enriched or excluded in DRM in response to cryptogeen

Analysis of proteins exhibiting Cry / control ratio in the highest 10 % or lowest 10 % in both experiments

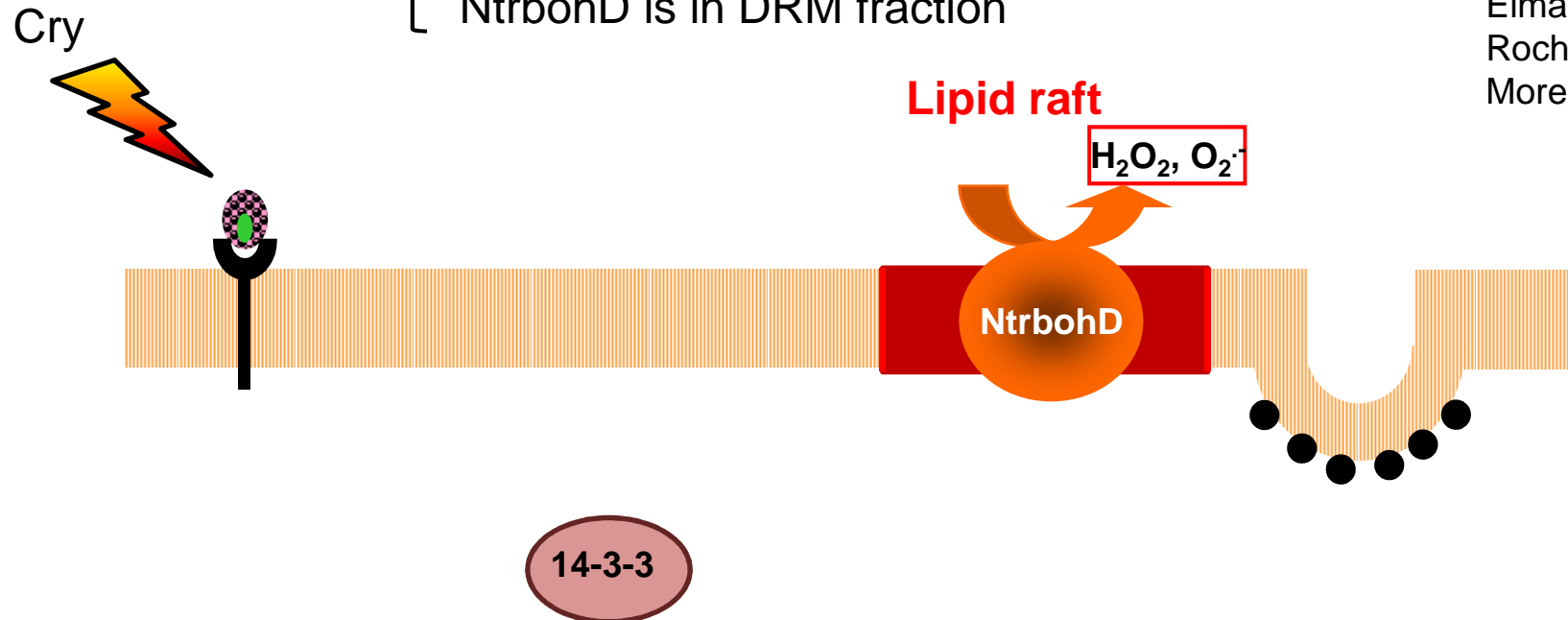
Proteins enriched in microdomains in response to cryptogeiin

Acc . N.	Sp.	Protein name	Experiment		Reverse experiment	
			Global ratio	Number of peptides	Global ratio	Number of peptides
signalization						
Gi:3023189	N.t.	14-3-3 C	1.43	5	2.15	4

previous studies

- 14-3-3 interacts with NtrbohD
- 14-3-3 is a positive regulator of ROS production
- NtrbohD is in DRM fraction

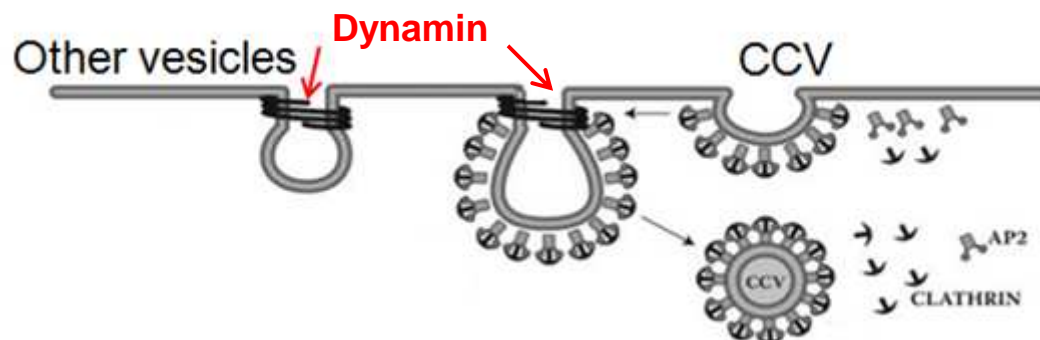
Elmayan *et al*, 2007
 Roche *et al*, 200
 Morel *et al*, 2006



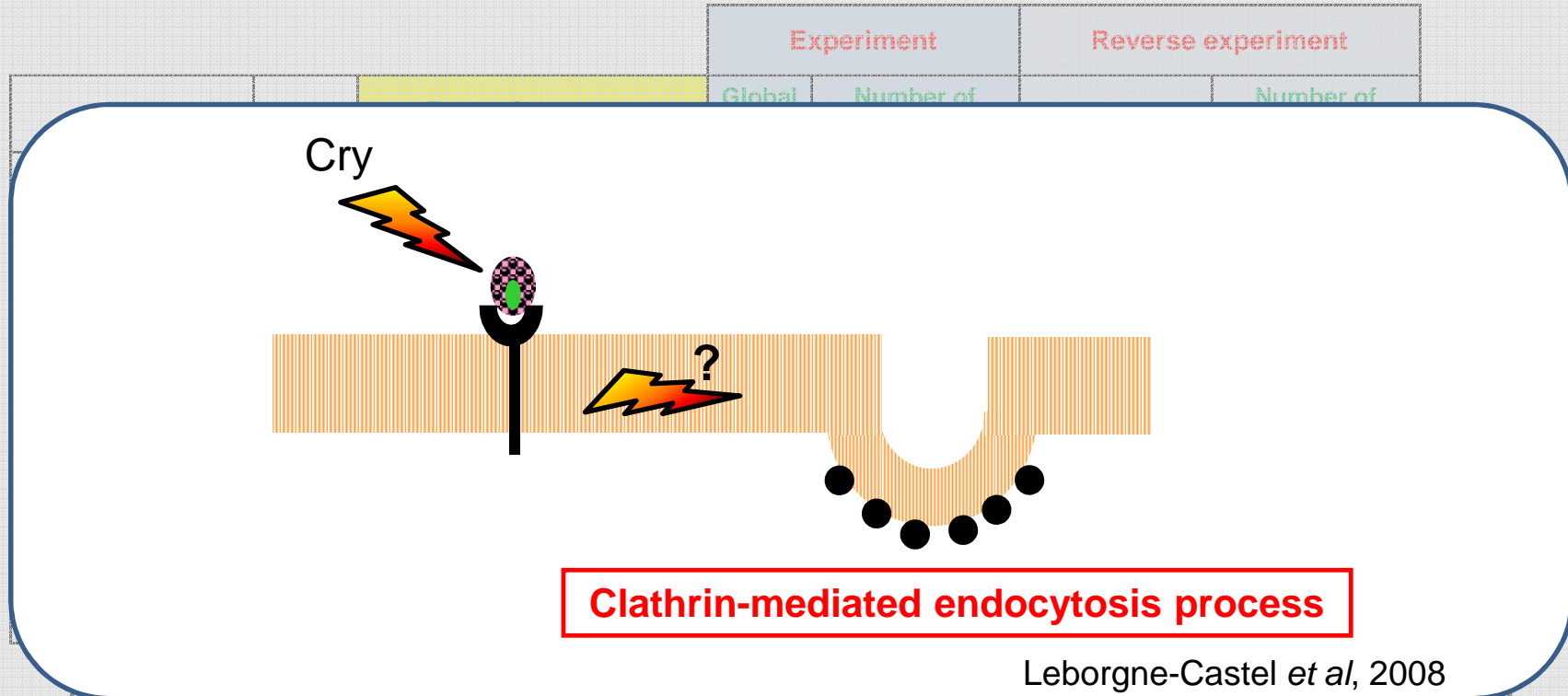
Proteins excluded from microdomains in response to cryptogein

Acc . N.	Sp.	Protein name	Experiment		Reverse experiment	
			Global ratio	Number of peptides	Global ratio	Number of peptides
Vesicular trafficking						
Gi:5931765	N.t.	Dynamin-1A	0.41	13	0.68	20
Gi:18411520	A.t.	Dynamin-1E	0.45	15	0.71	12
Gi:15218486	A.t.	Dynamin-2A	0.394	5	0.79	4
Gi:15218837	A.t.	Dynamin-2B	0.524	4	0.73	2

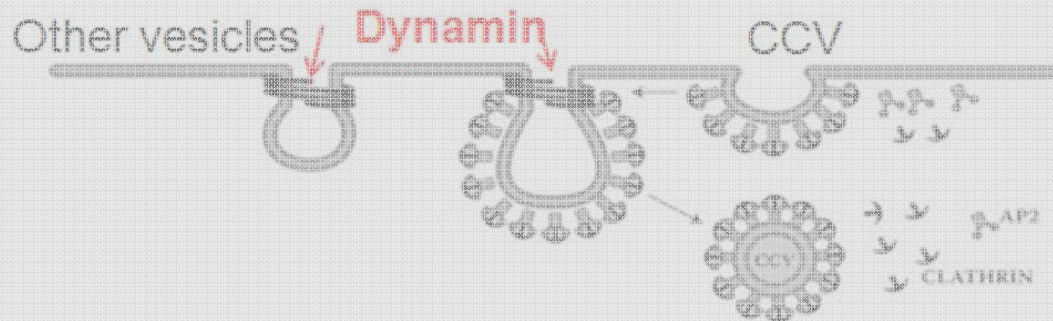
Dynamins = Large GTPase involved in the scission of vesicles from membranes.



Proteins excluded in microdomains in response to cryptogen ?

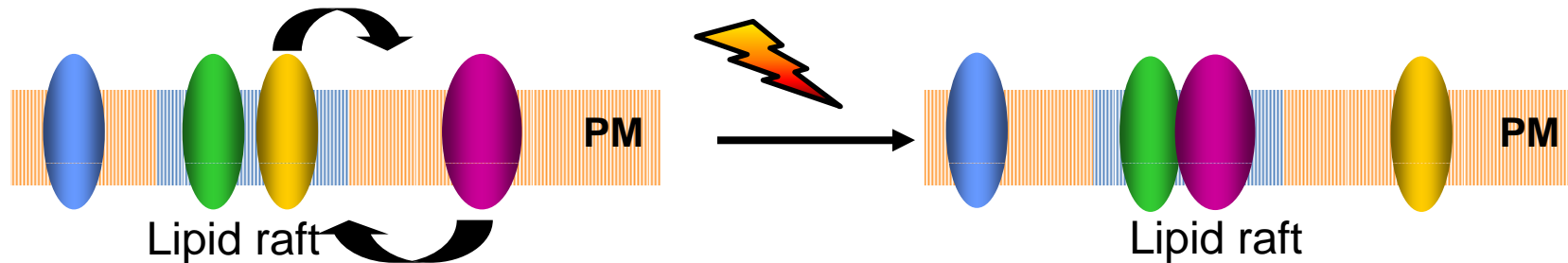


Dynamins = Large GTPase involved in the scission of nascent vesicles from parent membranes.



Conclusion

- Validation of the N14/N15 quantitation module of the MFPaQ software
- First evidence of a dynamic association of proteins to microdomains in plants upon a biological stimulus



- Function of such domains and of their dynamics during signal transduction processes occurring in plant defenses.

(Stanislas *et al*, MCP, 2009)

Prospects

- **Check involvement of these proteins in the signaling pathway**
 - By functional genomic approach
 - **antisense construct** and effects on the different steps of the signal transduction
- **Visualize the hypothesis that association/dissociation of proteins to microdomains is a signaling event triggered by cryptogein**
 - By cell biology
 - **Native promoter - cDNA - XFP construct** in order to follow by confocal and electronic microscopy the dynamic of these proteins localization in response to cryptogein



These constructs are ongoing



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- Proteomic platform of Toulouse



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In particular

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Johanne Morel
Françoise Simon-Plas



**THANKS
FOR YOUR ATTENTION**