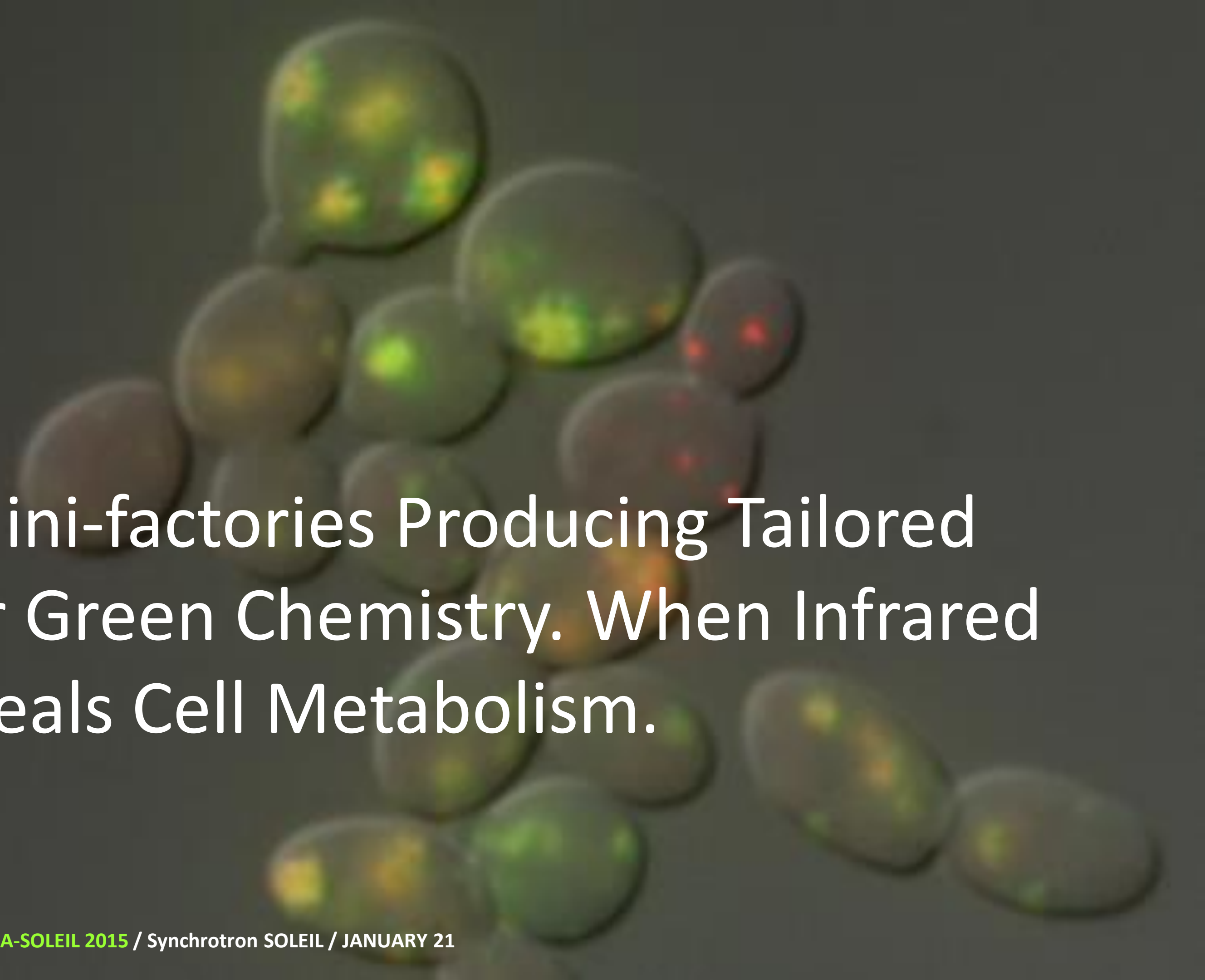
Yeasts: Mini-factories Producing Tailored Lipids For Green Chemistry. When Infrared Light Reveals Cell Metabolism

Marine Froissard



Yeasts: Mini-factories Producing Tailored Lipids For Green Chemistry. When Infrared Light Reveals Cell Metabolism.

Marine FROISSARD / INRA-SOLEIL 2015 / Synchrotron SOLEIL / JANUARY 21

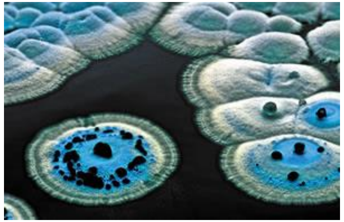
LIPIDS ARE STORED IN LIPID DROPLETS (LDs)

Mammals
Homo sapiens

From Asterix official website

Christelle CEBO
INRA GABI

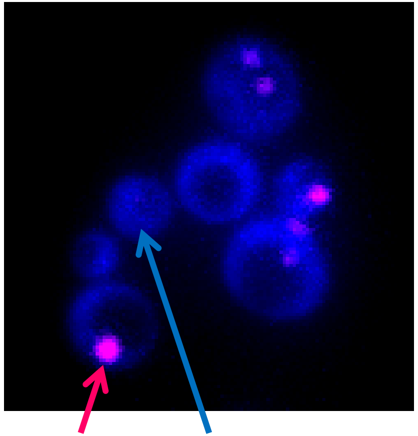
Bacteria
***Streptomyces* sp.**



Fron Thompson et al. Genome Biology 2002

Marie-Joëlle VIROLLE
CNRS IGM

Unicellular eukaryotes
Saccharomyces cerevisiae



Frédéric JAMME
Matthieu REFREGIERS

Plants
Brassica napus

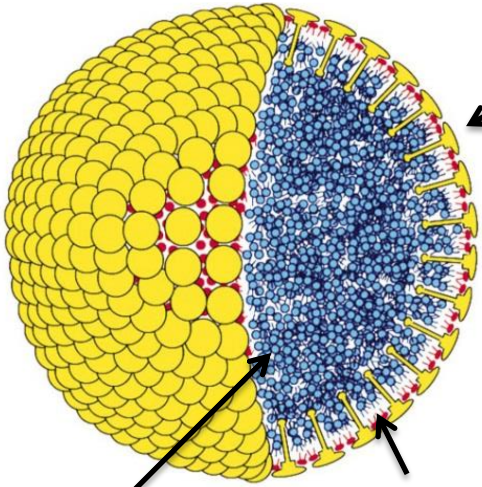
Jean WEBER / © INRA

LIPIDS ARE STORED IN LIPID DROPLETS (LD)

Plants

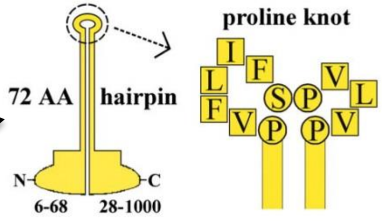
Arabidopsis thaliana / Brassica napus

seed lipid droplet = oleosome



oleosin

caleosin



From Hsieh et al. (2004) Plant Physiol

Lipid core = triacylglycerols and sterol esters

phospholipid monolayer

Predicted structure = tri-block organization

- variable N- and C- termini
- hydrophobic central domain

WHY STUDYING LDs?

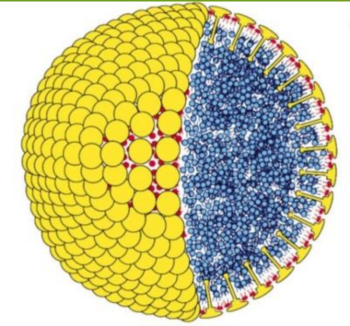
Understanding the dynamics

➡ **Oils** for food, biofuel and green chemistry are extracted from LDs

BUT

➡LDs have a crucial role in **diseases with increasing prevalence** (obesity, diabetes).

filling



mobilization

stabilization

WHY STUDYING LDs AND ASSOCIATED PROTEINS?

Valorization of LDs and associated proteins

➡ **food processing industry, cosmetic and health** : LDs are natural emulsions and **nanovectors**, and oleosins can form **suprastructures**

BUT

➡**Oleosins** (from peanut and hazelnut) are **allergens**

Biotechnol. Prog. 2005, 21, 1297–1301 1297

Elevating Bioavailability of Cyclosporine A via Encapsulation in Artificial Oil Bodies Stabilized by Caleosin

Miles C. M. Chen,† Jui-Ling Wang,‡ and Jason T. C. Tzen*,†

Graduate institute of Biotechnology, National Chung-Hsing University, Taichung, Taiwan, and Department of Physiology, National Yang-Ming University, Medical College, Taipei, Taiwan

PNAS

Self-assembly of tunable protein suprastructures from recombinant oleosin

Kevin B. Vargo[a], Ranganath Parthasarathy[a], and Daniel A. Hammer[a,b,1]

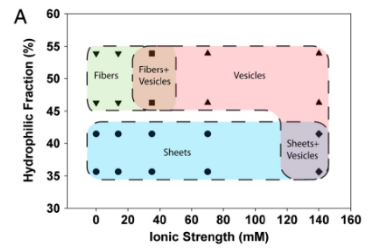
[a]Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA 19104; and [b]Bioengineering, University of Pennsylvania, Philadelphia, PA 19104

Edited by* David A. Tirrell, California Institute of Technology, Pasadena, CA, and approved June 5, 2012 (received for review April 3, 2012)

Using recombinant amphiphilic proteins to self-assemble suprastructures would allow precise control over surfactant chemistry and the facile incorporation of biological functionality. We used cryo-TEM to confirm self-assembled structures from recombinantly produced mutants of the naturally occurring sunflower protein, oleosin. We studied the phase behavior of protein self-assembly

although the direct visualization of a bilayer membrane or vesicular encapsulation has not been explicitly shown (12).

While a number of naturally occurring proteins, such as hydrophobins (13), oleosins (14), latherin (15), and ranaspumin (16), are known to stabilize interfaces, only oleosins are structurally reminiscent of a chain surfactant. Oleosins are a family of plant



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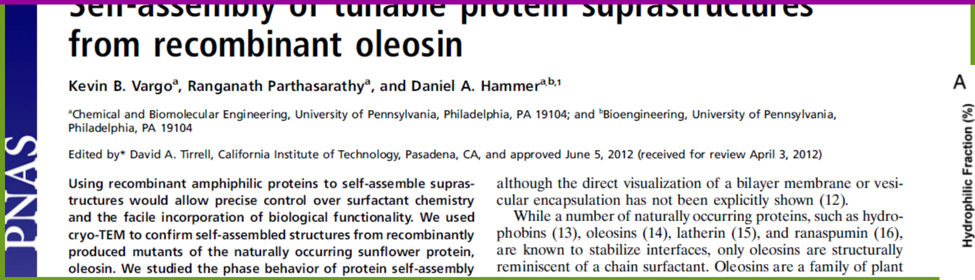
➡ **food processing industry, cosmetic and health** : LDs are natural emulsions and **nanovectors**, and oleosins can form **suprastructures**

BUT

➡

T. Chardot talk
Structural studies of proteins inserted in a half membrane using SOLEIL synchrotron Light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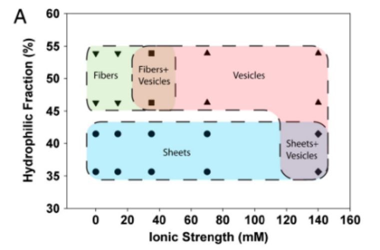

WHY STUDYING LDs AND ASSOCIATED PROTEINS?

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➡Ol

US 2012/0301932 A1

DU PONT DE NEMOURS AND CO

Nov. 29, 2012

EXPRESSION OF CALEOSIN IN RECOMBINANT OLEAGINOUS MICROORGANISMS TO INCREASE OIL CONTENT THEREIN

[0001] This application claims the benefit of U.S. Provisional Application No. 61/490,337, filed May 26, 2011, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention is in the field of biotechnology. More specifically, this invention pertains to recombinant oleaginous microorganisms that are capable of producing more oil due to the expression of a caleosin polypeptide.

BACKGROUND OF THE INVENTION

[0003] Microorganisms such as filamentous fungi, yeast and algae produce a variety of lipids, including fatty acyls,

However, there is still a need for recombinant oleaginous microorganisms having increased oil content relative to the oil of currently known strains.

[0006] U.S. Pat. No. 7,256,014 discloses that the expression of at least one plant oleosin gene in a microbial cell engineered to produce a hydrophobic/lipophilic compound, such as a carotenoid, significantly increases the overall titer of the compound.

[0007] Froissard et al. (*FEMS Yeast Res*. 9:428-438, 2009) disclose that the non-oleaginous yeast, *Saccharomyces cerevisiae*, transformed with a heterologous gene encoding a caleosin polypeptide (*Arabidopsis thaliana* caleosin 1, AtClo1), exhibited an increase in the number and size of lipid bodies and accumulated more fatty acids than the parent strain.

[0008] However, there are no reports of recombinant oleaginous microorganisms transformed with a gene encoding a caleosin polypeptide to increase the oil content of such recombinant microbial cells.

1297

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Department of

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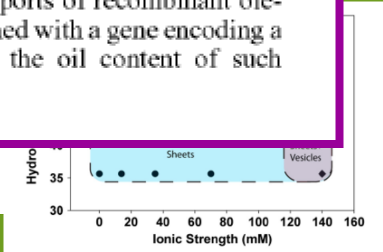
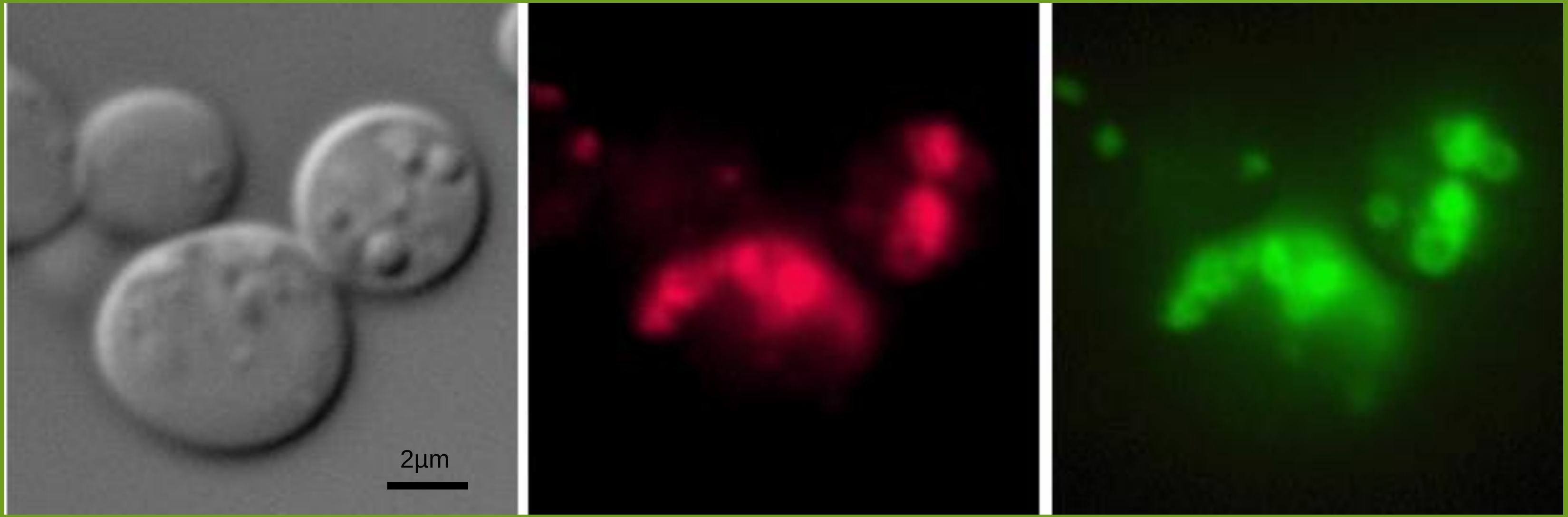


HETEROLOGOUS EXPRESSION OF OLEOSINS IN YEAST

Ole1 and Clo1 oleosins are targeted to LDs in yeast

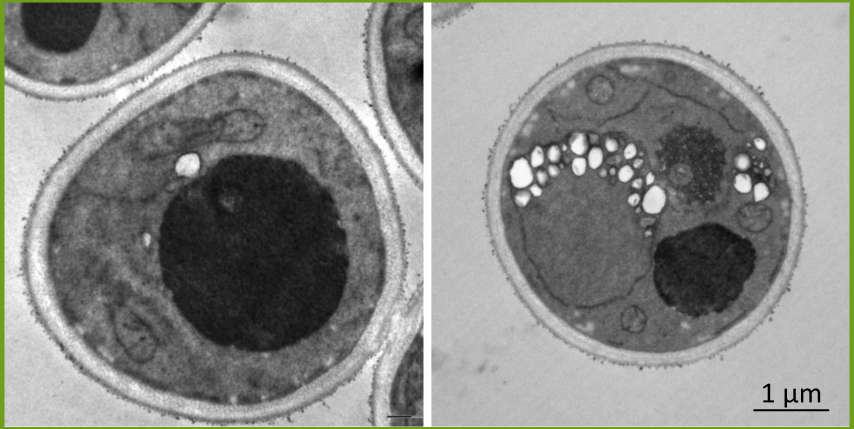


Nomarski | Erg6p-RFP | AtClo1-GFP

Froissard *et al*. 2009. FEMS Yeast Research
Boulard and Froissard. 2012. Techniques de l'Ingénieur. re209

HETEROLOGOUS EXPRESSION OF OLEOSINS IN YEAST

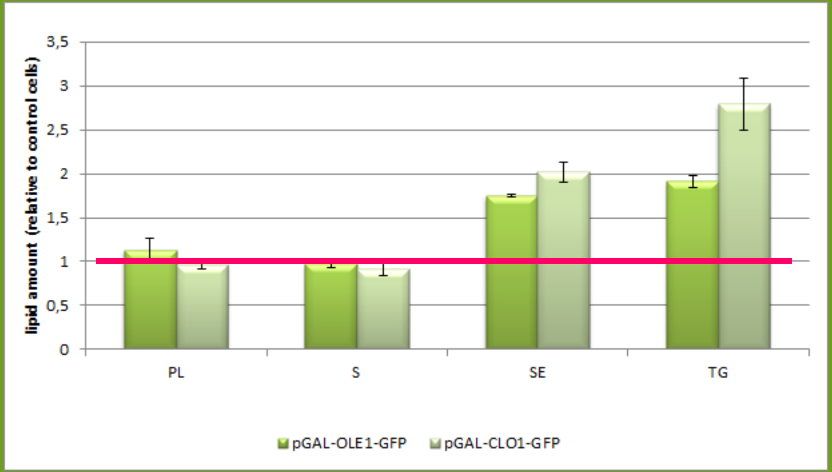
Oleosins induce LD proliferation



Control + AtOle1-GFP

Christine LONGIN
Sophie CHAT

oleosins induce triacylglycerols (TG) and steryl esters (SE) accumulation



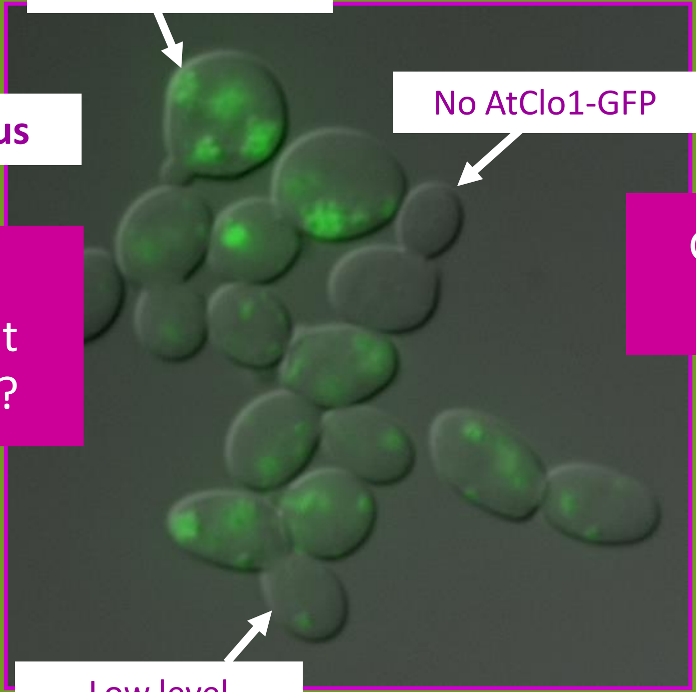
Froissard *et al*. 2009. FEMS Yeast Research
Boulard and Froissard. 2012. Techniques de l'Ingénieur. re209

METABOLISM EXPLORATION USING SYNCHROTRON FTIR

But population is heterogeneous

Metabolism heterogeneity at single cell level ?

Lipid accumulation Min and Max ?



Global metabolic changes?

SCIENCE & IMPACT

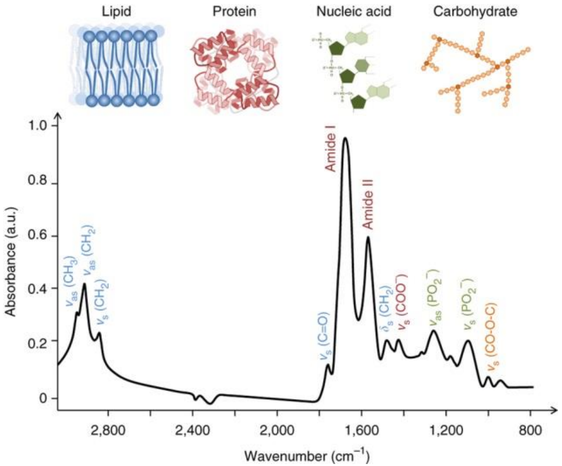
METABOLISM EXPLORATION USING SYNCHROTRON FTIR

= **S**pectroscopie et **M**icroscopie **I**nfrarouge avec **S**ynchrotron

FTIR = Vibrational spectroscopy

Spectral fingerprints of biological macromolecules

➡ **Cell metabolism overview**



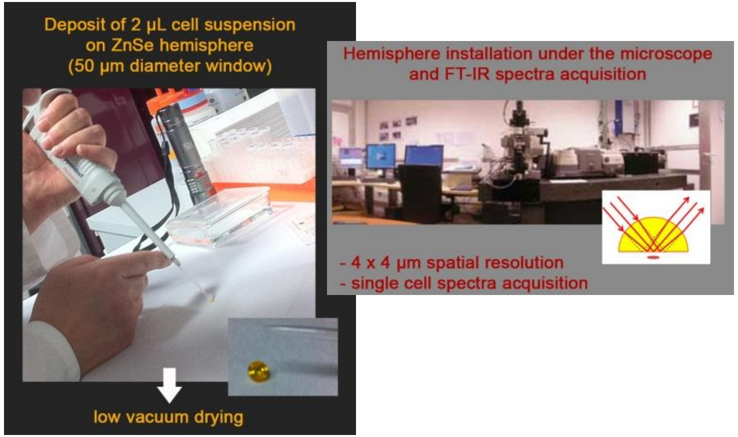
From Baker et al. Nature Protocols. 2014

+

Brightness of Synchrotron Light

+ ZnSe hemisphere

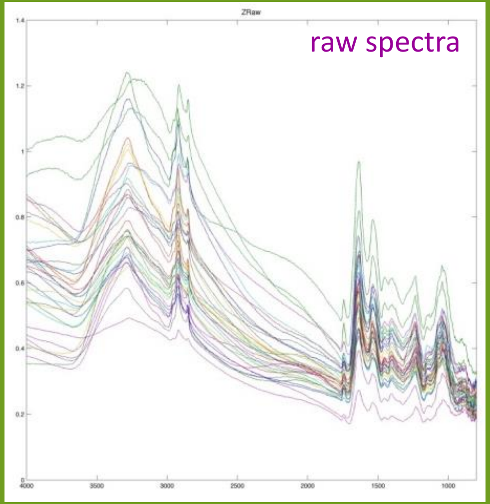
high spectral and spatial resolution



METABOLISM EXPLORATION USING SYNCHROTRON FTIR

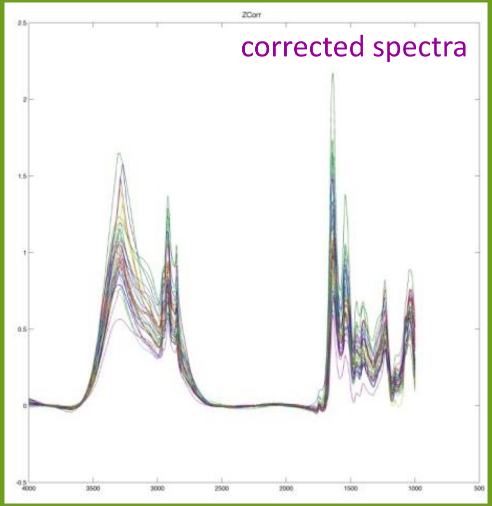
SMIS = **S**pectroscopie et **M**icroscopie **I**nfrarouge avec **S**ynchrotron

Acquisition of 50 spectra



Matlab Pretreatment

RMieS-EMSC correction



Spectra baseline deformation due to light scattering

- Round shape of cells
- Small size of cells
- Wavelenght range of mid-infrared

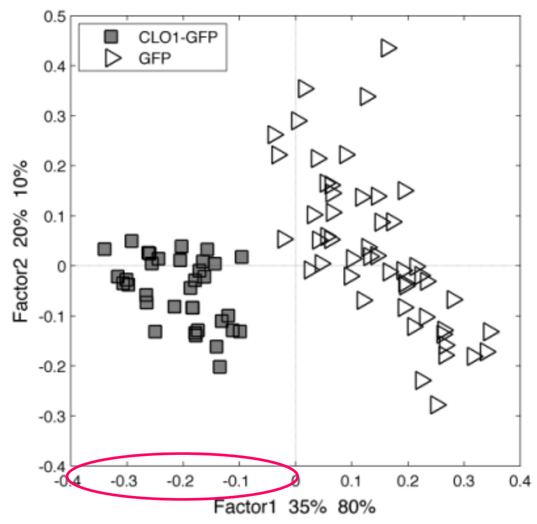
Frédéric JAMME

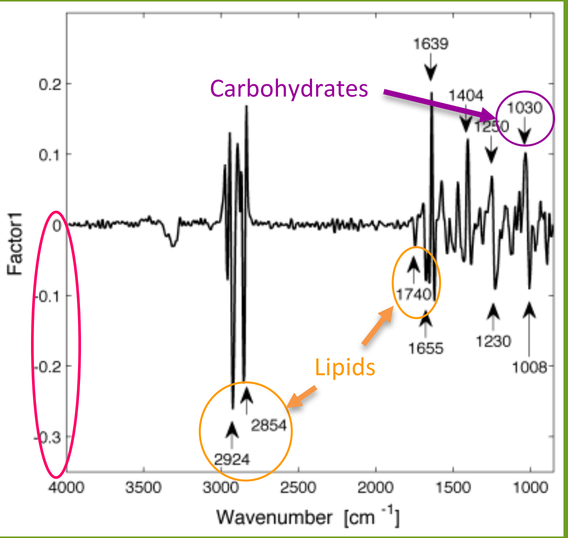
METABOLISM EXPLORATION USING SYNCHROTRON FTIR

= **S**pectroscopie et **M**icroscopie **I**nfrarouge avec **S**ynchrotron

Frédéric JAMME

Principal Component Analysis (PCA)





Population separation among Factor 1

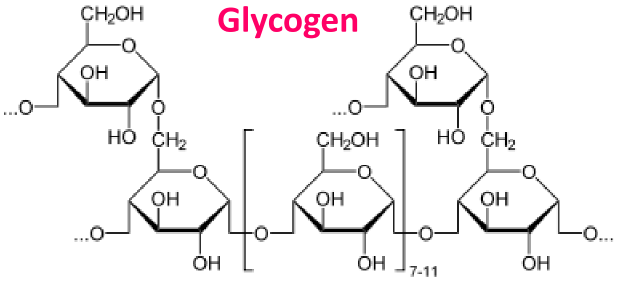
More lipids and less carbohydrates in AtCLO1-GFP expressing cells

Jamme *et al*. 2013. PLOS ONE.

METABOLISM EXPLORATION USING SYNCHROTRON FTIR

Metabolic modifications were confirmed using biochemical analysis

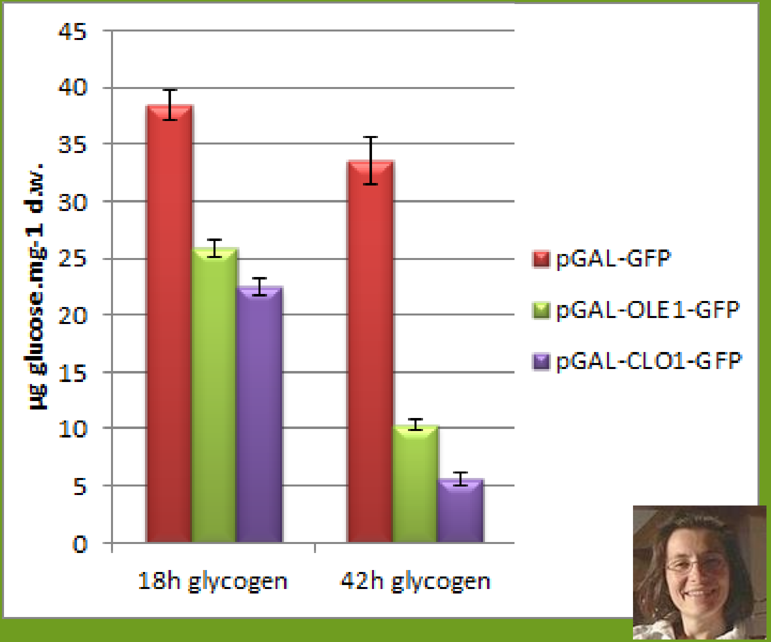
Glycogen



α-amyloglucosidase at 57° C

Free glucose

Quantification by high-performance anion-exchange chromatography with pulse amperometric detection (HPAEC-PAD)



Valérie MECHIN
INRA IJPB

Jamme *et al*. 2013. PLOS ONE.

ADDITIONAL INFORMATION

SOLEIL communication

INRA communication

INRA en lumière, 5 ans de partenariat avec SOLEIL

THANKS TO

Institut Jean-Pierre Bourgin

Dynamique et Structure des Corps Lipidiques (Actual)
Thierry CHARDOT, INRA (Head)
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Yann GOHON, AgroParisTech
Isabelle BOUCHEZ, INRA
Carine DERUYFFELAERE, INRA
Franjo JAGIC, INRA
Michel CANONGE, AgroParisTech
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Bernard CINTRAT, AgroParisTech
Zita PURKRTOVA CDD CAER DGA
Jean-David VINDIGNI (PhD, past)

Différentiation et Polarité Cellulaire (Future)
Jean-Denis FAURE, AgroParisTech (Head)

SOLEIL

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Frank WIEN
Valérie ROUAM
Bertrand CINQUIN

SMIS
Paul DUMAS (Beamline leader)
Frédéric JAMME

and SOLEIL staff

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