Yeast lipid bodies under sunlights, dynamic and structural studies using SMIS and DISCO beamlines

Jean-David J.-D. Vindigni, Yann Gohon, Roselyne Tâche, Frederic Jamme, Alexandre A. Giuliani, Franck Wien, Thierry Chardot, Pierre Briozzo, Marine Froissard

YEAST LIPID BODIES UNDER SUNLIGHTS
DYNAMIC AND STRUCTURAL STUDIES USING SMIS AND DISCO BEAMLINES

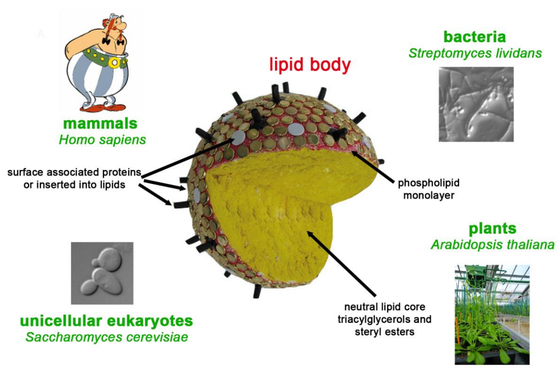
Jean-David Vindigni[1], Yann Gohon[1], Roselyne Tâche[1], Alexandre Giuliani[2,3], Frédéric Jamme[2,3], Franck Wien[2], Thierry Chardot[1], Pierre Briozzo[1], Marine Froissard[1]

[1] Dynamique et Structure des Corps Lipidiques, UMR 1318 IJPB, INRA AgroParisTech, 78 026 Versailles;
[2] Synchrotron SOLEIL, 91 192 Gif-sur-Yvette; [3] CEPIA, U1008, INRA, 44 316 Nantes

CONTEXT

Lipid body: a complex and dynamic organelle

In cells, neutral lipids (triglycerides and steryl esters) are stored in organelles called **lipid bodies (LB)** [1]. They are present in all organisms, from bacteria to plants and animals.



Lipid bodies: not well known but with rising interest

From biologists

➟ LB is not an inert fat depot but a **dynamic organelle** which regulates cell metabolism and signaling

From medical field

➟ LBs have a crucial role in **diseases with increasing prevalence** (obesity, diabetes) [2]

➟ **Oleosins** (from peanut and hazelnut), seed LB associated proteins are **allergens** [3].

From industrials

➟ **crushing** : oils for food and non food (biofuel and green chemistry) productions are extratcted from seed LBs

➟ **food processing industry, cosmetic and health** : oleosins harbor interfacial properties and could be use as **emulsifying agents or in drug delivery systems** [4]

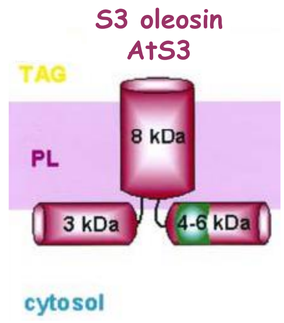
Oleosins, seed lipid body associated proteins

Oleosins are LB integral proteins

Predicted structure = tri-block organization :

➟ variable N-terminal and C-terminal part, exposed at the surface and in contact with the cytosol

➟ highly hydrophobic central part inserted into the phospholipid monolayer and/or the TAG core.



Questions and objectives

➟ Role on lipid filling

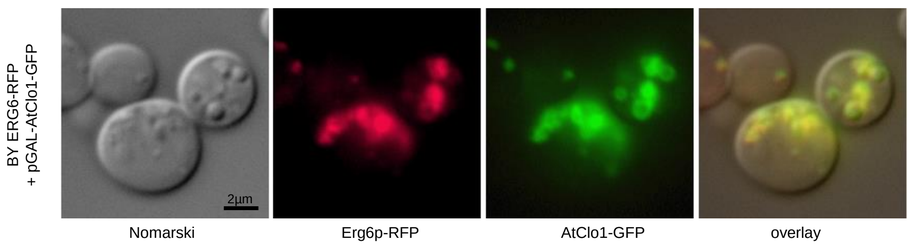
➟ Role on LB structure and stabilization

➟ Structural data on oleosins inserted into LB (natural environment)

RESULTS

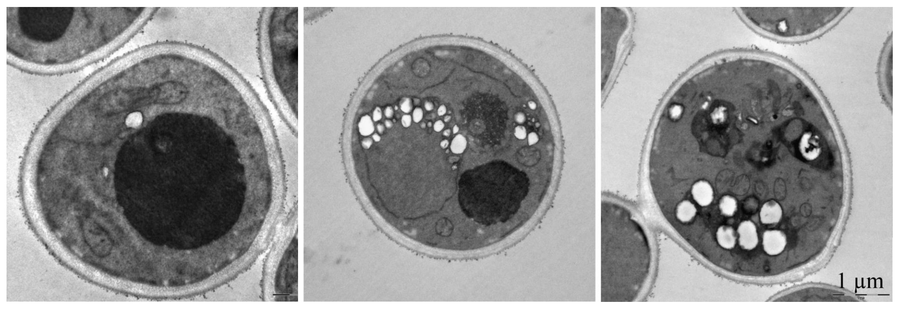
Oleosins are targeted to lipid bodies in *S. cerevisiae*

Photonic microscopy pictures (bright field and epifluorescence) of yeast expressing Erg6p-RFP (lipid body Delta(24)-sterol C-methyltransferase) and AtClo1-GFP [5].



Oleosins induce neutral lipid accumulation in yeast

Thin sections of yeasts expressing AtS3-GFP or AtClo1-GFP (transmitted electron microscopy)

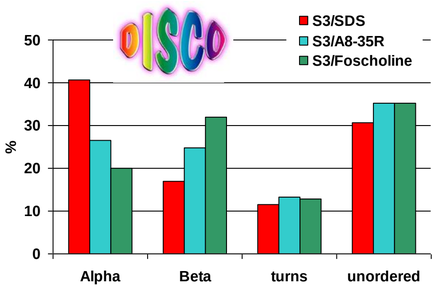


Lipid bodies, round and white structures, are more abundant in cells expressing oleosins when compared to the control cells.

STRUCTURAL STUDIES

AtS3 oleosin structures in surfactants are contradictory

Using SRCD on DISCO beamline, we obtained the structure of AtS3 oleosin solubilized in various surfactants, 2% SDS, Amphipol 0,5% A8-35R and 10 mM Foscholine [6]



What is the fold of AtS3 in a natural environment?

Artificial LB with AtS3 → No SRCD spectra recordable

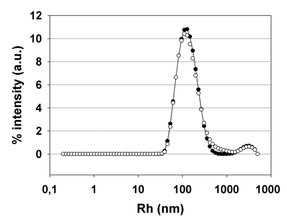
LB purified from *A. thaliana* seed → No SRCD spectra recordable

AtS3 oleosin is massively associated with lipid bodies in yeast

Purified LBs are analyzed:

➟ using microscopy

➟ using dynamic light scattering



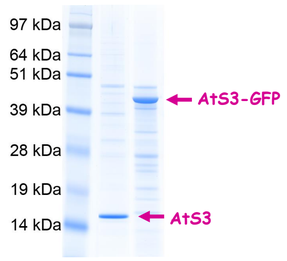
Yeast LB are suitable for SRCD spectra acquisition

➟ with oleosin as major protein

➟ 220 nm average diameter

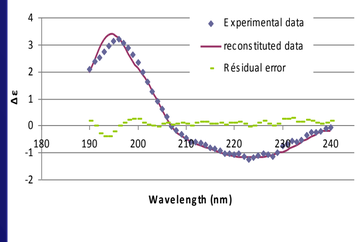
➟ in 10mM Tris 70 mM NaF buffer

The associated proteins are analyzed by SDS PAGE

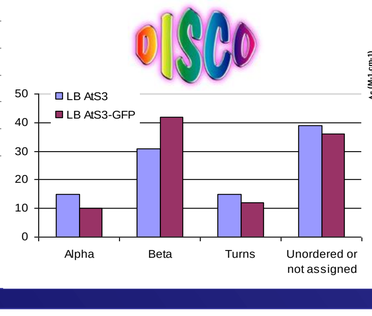


AtS3 oleosin structure in natural environment is mainly beta

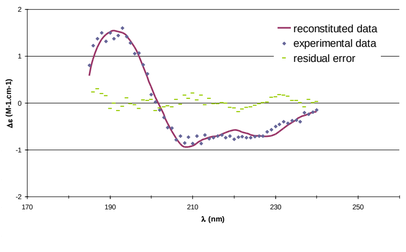
AtS3-GFP in LB



we obtained SRCD spectra on native LB in solution



AtS3 in LB



Conclusion and perspectives of structural studies

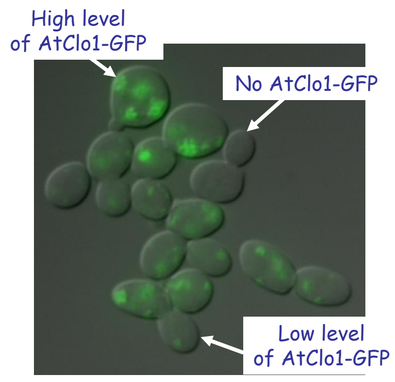
➟ We obtained the **first SRCD data on whole organelle** and **information on the fold of AtS3 oleosin in a natural environment**, lipid bodies. We observed that the fold in LB was the same as the fold of AtS3 solubilized in Foscholine

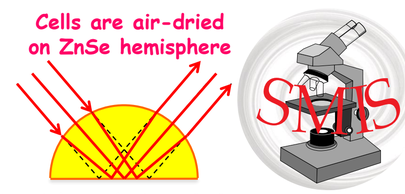
➟ We would validate our results using **SRCD-FTIR coupled analysis** on dry films. We obtained the first spectra on DISCO and SMIS in December 2011.

BIBLIOGRAPHIE [1] Murphy (2011). Protoplasma. [2] Bostrom et al. (2007). Nat. Cell Biol. 9, 1286. [3] Pons et al. (2002). Allergy. 57, 88. [4] Capuano et al. (2007). Biotechnol. Adv. 25, 203. [5] Froissard et al. (2009). FEMS Yeast Res. 9, 428. [6] Gohon et al. (2011). Biochim. Biophys. Acta. 1808, 706. [7] Bassan et al. (2010). The Analyst. 135, 268.

DYNAMIC STUDIES

Neutral lipid content heterogeneity revealed by single cell FTIR analysis

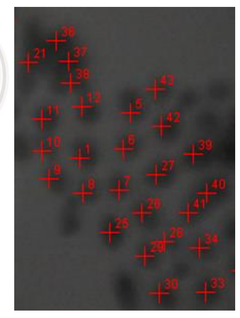


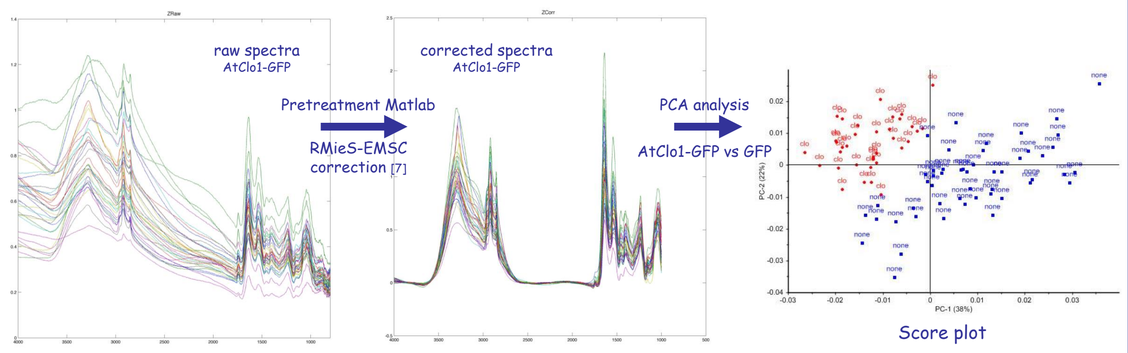


Spatial resolution: 4x4 µm

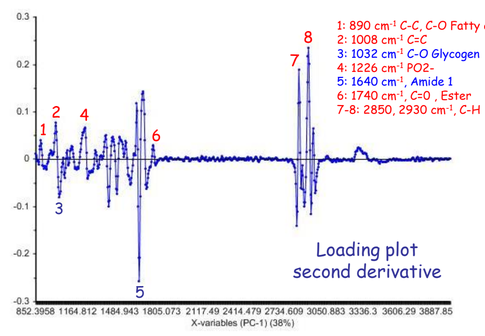
➟ **Single cell spectra acquisition**

➟ **Fluorescence acquisition**

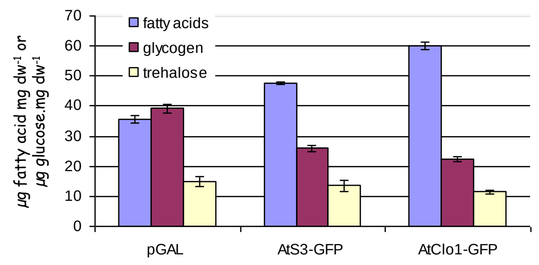




Neutral lipid increase is correlated with global metabolic modifications



Metabolic modifications were detected using FTIR and confirmed by biochemical analysis



Conclusion and perspectives of dynamic studies

➟ Using ZnSe hemisphere and synchrotron radiation, we obtained **yeast single cell FTIR spectra**. We confirmed the **cell heterogeneity** observed with fluorescence microscopy.

➟ We observed that lipid accumulation induces global metabolic modifications. These results were confirmed by biochemical analysis and revealed a **link between storage lipid and storage carbohydrate fluxes**

➟ Now, we will determine if these metabolic modifications are the consequence of **pathway regulations at the transcription level** by conducting a transcriptomic analysis of yeast cells