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

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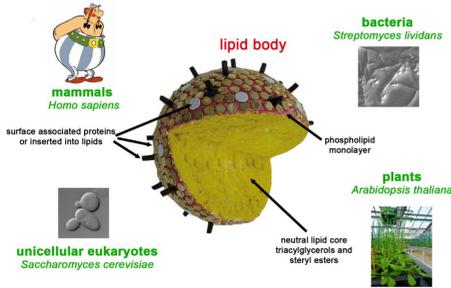
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## CONTEXT

### 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

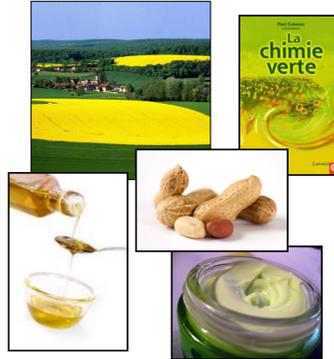
→ LB is not an inert fat depot but a **dynamic organelle** which regulates cell metabolism and signaling

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 extracted from seed LBs  
 → **food processing industry, cosmetic and health**: oleosins harbor interfacial properties and could be used 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.



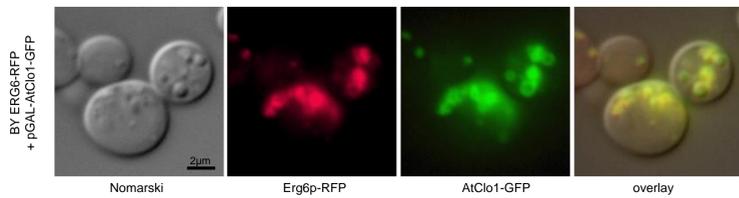
Questions and objectives

→ Role on lipid filling  
 → Role on LB structure and stabilization  
 → Structural data on oleosins inserted into LB (natural environment)

## RESULTS

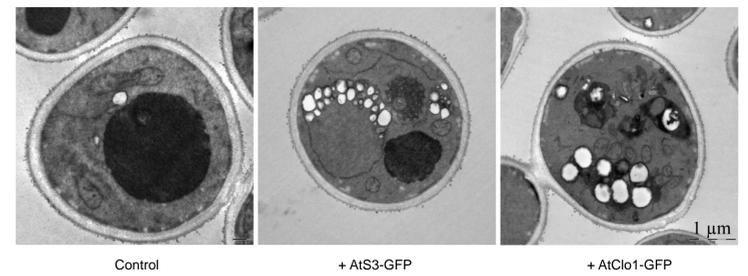
### Oleosins are targeted to lipid bodies in *S. cerevisiae*

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)

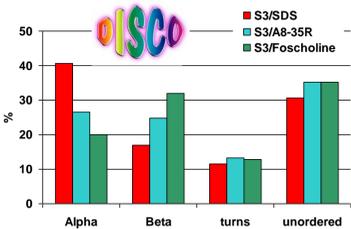


Lipid bodies, round and white structures, are more abundant in cells expressing oleosins when compared to the control cells.

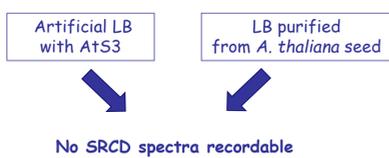
## STRUCTURAL STUDIES

### 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]

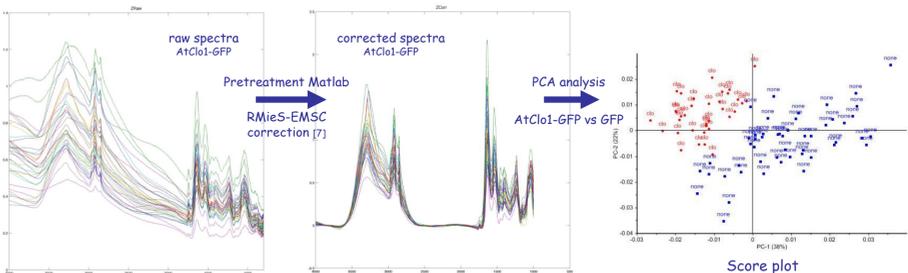
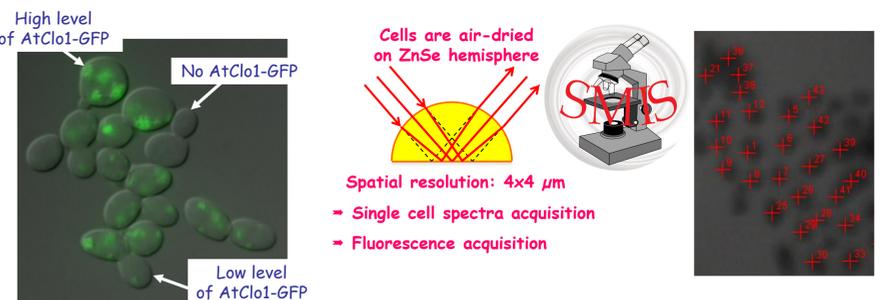


What is the fold of AtS3 in a natural environment?



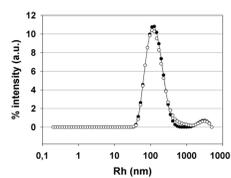
## DYNAMIC STUDIES

### Neutral lipid content heterogeneity revealed by single cell FTIR analysis



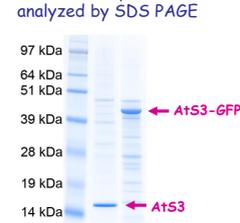
### AtS3 oleosin is massively associated with lipid bodies in yeast

Purified LBs are analyzed:  
 → using microscopy  
 → using dynamic light scattering

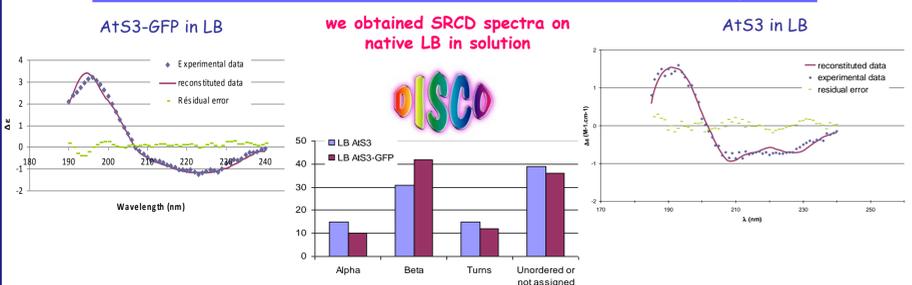


Yeast LB are suitable for SRCD spectra acquisition  
 → with oleosin as major protein  
 → 220 nm average diameter  
 → in 10mM Tris 70 mM NaF buffer

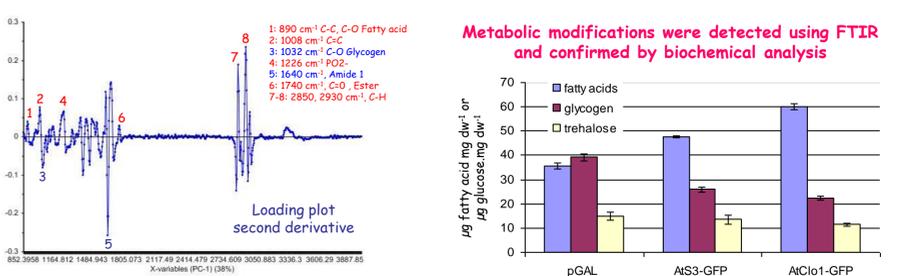
The associated proteins are analyzed by SDS PAGE



### AtS3 oleosin structure in natural environment is mainly beta



### Neutral lipid increase is correlated with global metabolic modifications



### 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.

### 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