Dynamic and structural studies of lipid droplets using synchrotron light

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DYNAMIC AND STRUCTURAL STUDIES OF LIPID DROPLETS USING SYNCHROTRON LIGHT

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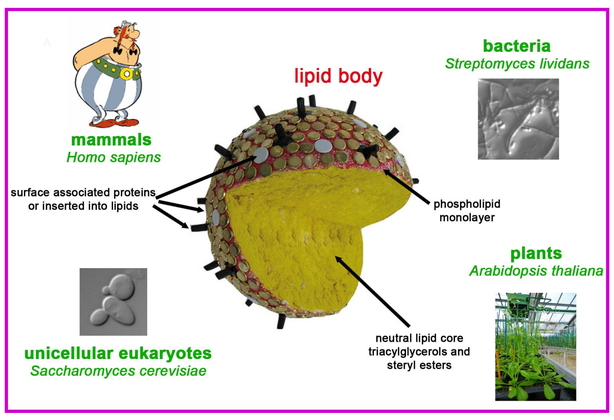
CONTEXT

Lipid droplet: a complex and dynamic organelle

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



Lipid droplets: not well known but with rising interest

From biologists

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

From medical field

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

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

From industrials

➠ **crushing** : oils for food and non food productions are extracted from seed LDs

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

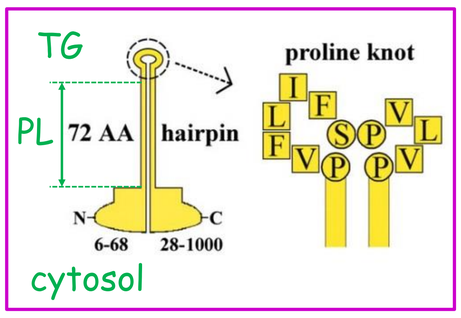
Oleosins, seed lipid body associated proteins

Oleosins, AtOle1 and AtClo1, are LD integral proteins

Predicted structure = tri-block organization [5]:

➠ 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 (PL) monolayer and/or the triacylglycerol (TG) core.



Questions and objectives

➠ Role on lipid filling

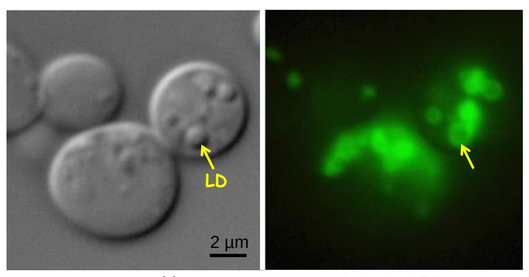
➠ Role on LD structure and stabilization

➠ Structural data on oleosins inserted into LD (natural environment)

HETEROLOGOUS EXPRESSION OF PLANT OLEOSINS IN YEAST

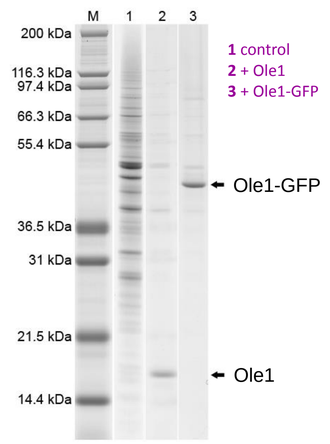
Oleosins are targeted to LDs in yeast

Photonic microscopy pictures (bright field and epifluorescence) of yeast expressing AtClo1-GFP [6].



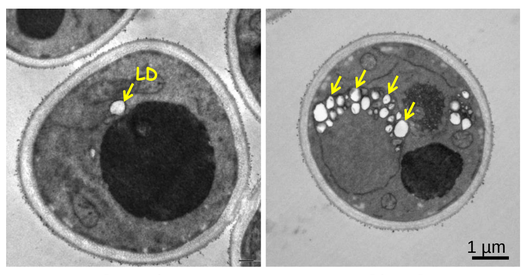
Ole1 and Clo1 become the major proteins associated with yeast LDs

SDS-PAGE protein profiles of LDs purified on sucrose gradient



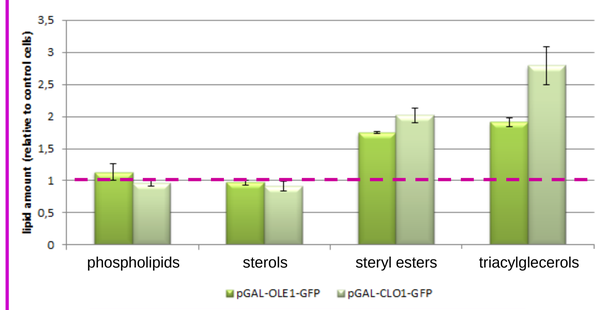
Oleosins induce LDs and lipid accumulation

Thin sections of yeasts expressing AtOle1-GFP (transmitted electron microscopy)



LDs, round and white structures, are accumulated in oleosin expressing cells

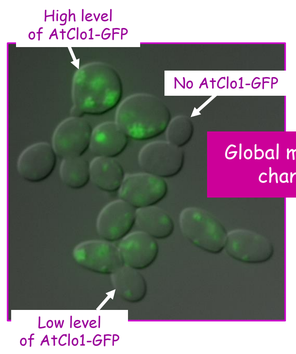
Lipid classes were analyzed using thin layer chromatography after Folch extraction



oleosins induce neutral lipid accumulation

DYNAMIC STUDY USING SYNCHROTRON FTIR

Single cell FTIR analysis on Soleil SMIS beamline revealed a link between neutral lipid and carbohydrate fluxes [7].



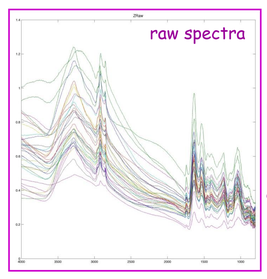
Global metabolic changes?

Brightness of Synchrotron Light + ZnSe hemisphere

SMIS

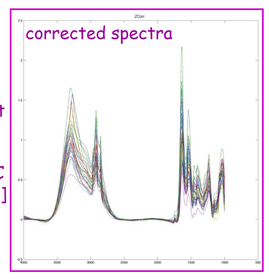
Acquisition of 50 spectra

high spectral and spatial resolution
➠ Single cell level (4 x 4µm)
➠ Heterogeneity of cell population

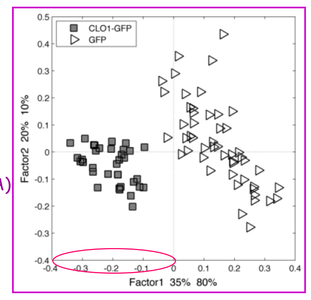


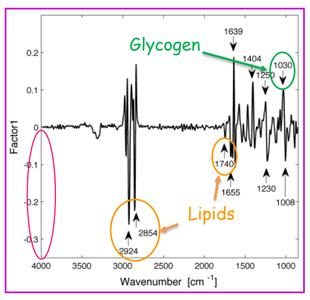
Matlab Pretreatment

RMieS-EMSC correction [8]

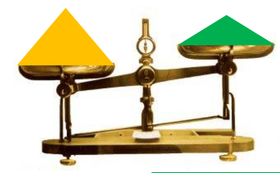


Principal Component Analysis (PCA)





Metabolic modifications were confirmed using biochemical analysis



More neutral lipids triacylglycerol and steryl esters

Less storage carbohydrates trehalose and glycogen

STRUCTURAL STUDY USING SRCD

SRCD at DISCO beamline revealed that Ole1 is mainly beta folded when inserted in LDs [9]

Summary of oleosin structural studies

Conventional approaches

➠ in **detergents**
➠ in **solvents**
➠ in **liposomes**

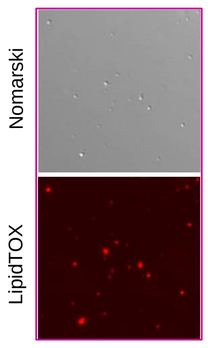
→ Results are contradictory Mainly alpha or mainly Beta

Toward natural environment

➠ in **plant LDs** but numerous proteins
➠ in **artificial LDs** but unfolded proteins

→ Not relevant

➠ in **yeast LDs with oleosin as major protein** → ?



LDs purified on sucrose gradient and observed using microscopy

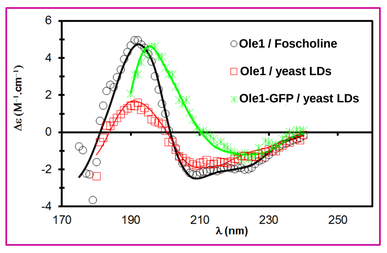
Brightness of Synchrotron light + Wide spectral range

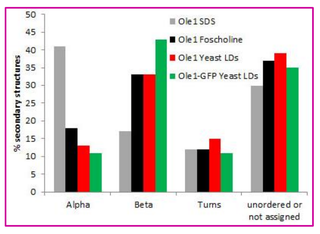
Yeast LDs with Ole1 or Ole1-GFP

DISCO

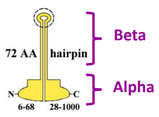
CD on turbid samples as purified LDs

First SRCD spectra on purified organelle





Hydrophobic domain of oleosins = beta sheet hairpin



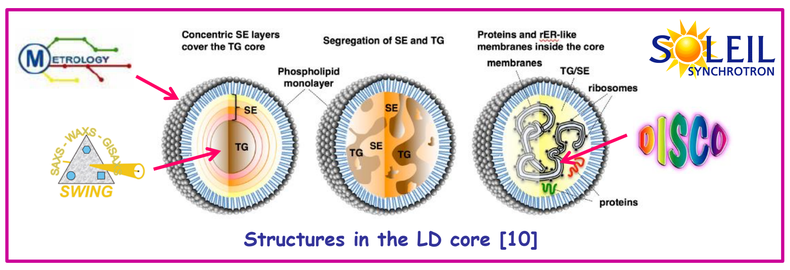
Perspectives on LD study

Lipid core organization?

➠ Small-angle X-ray scattering SAXS (SWING)
➠ Membrane fluidity using fluorescence anisotropy (DISCO)
➠ Protein mapping using Deep UV (DISCO)
➠ 3D imaging with UV (DISCO)

LD and other cell compartments?

➠ 3D imaging with UV (DISCO)



Structures in the LD core [10]

Structural studies on LD proteins

Other oleosins

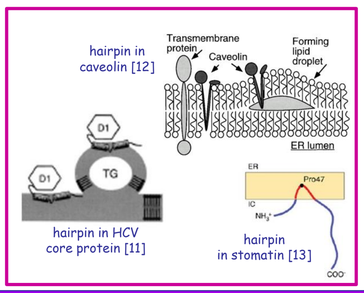
➠ SRCD on Ole3 in LDs (DISCO)
➠ Synchrotron water radiolysis footprinting of accessible amino-acids (METROLOGY)

Protein with hairpin hydrophobic core

➠ Stomatin in progress, caveolin?, GPAT4? HCV core protein?

Other LD proteins

➠ Perilipins, Apolipoproteins



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