Structural study of unconventional proteins, the membrane Hairpin Proteins, using DISCO light.

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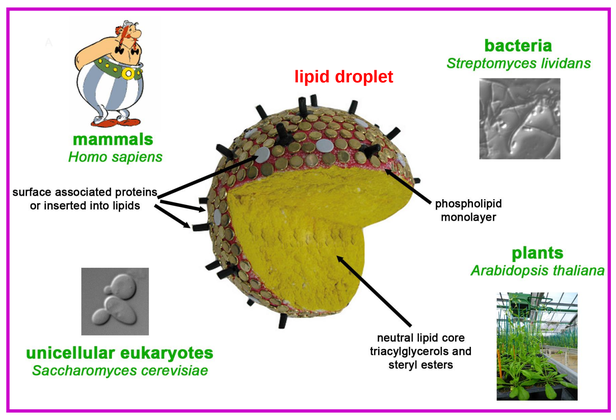
CONTEXT

Lipid droplet: a complex and dynamic organelle

LD is not an inert fat depot but a dynamic organelle which regulates cell metabolism and signaling [1]



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].

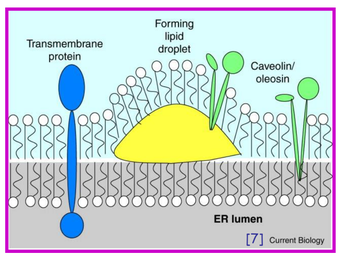
Industrials

- **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]

Hairpin proteins associated with lipid droplets

Many hairpin proteins are associated with LD.

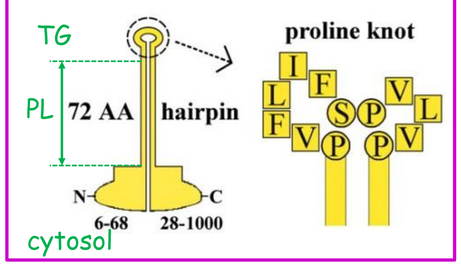
- Hepatitis C virus Core Protein: Organization of viral particle in mammalian host cell [5]?
- Stomatin: Link between milk LD size and stomatin content in goat [6].
- Caveolin: Regulation of caveolin trafficking in mammalian cells [7]?
- Oleosins and Caleosin: Role in LD biogenesis and stabilization in plants [8]



Predicted structure = tri-block organization :

- variable N-terminal and C-terminal part, exposed at the surface and in contact with the cytosol
- central part inserted into the phospholipid (PL) monolayer and/or the triacylglycerol (TG) core.

Oleosin[9]



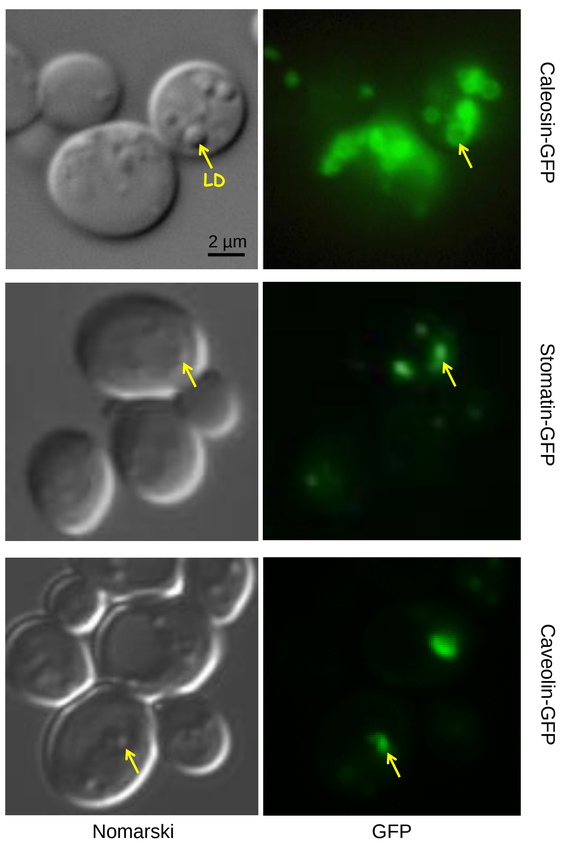
Questions and objectives

- Role on lipid filling
- Role on LD structure and stabilization
- Structural data on hairpin proteins inserted into LD (natural environment)

HETEROLOGOUS EXPRESSION OF HAIRPIN PROTEINS IN YEAST

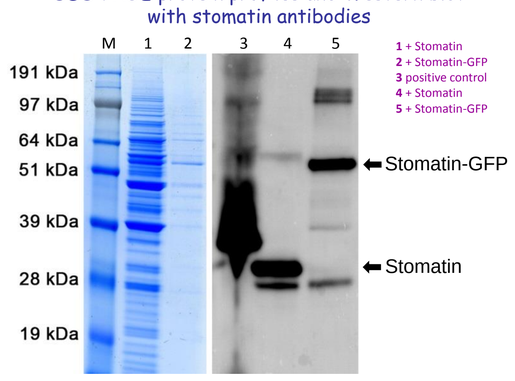
Hairpin proteins are targeted to LDs in yeast

Photonic microscopy (bright field and epifluorescence) of yeast expressing Caleosin-GFP [10], Stomatin-GFP or Caveolin-GFP.



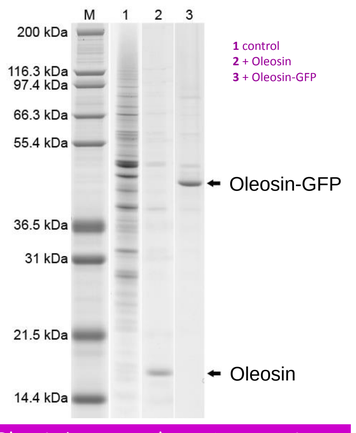
Hairpin proteins are associated with LDs in cells

SDS-PAGE protein profiles and Western blot with stomatin antibodies



Hairpin proteins are associated with purified LDs

SDS-PAGE protein profiles



Oleosin becomes the major protein associated with yeast LDs

STRUCTURAL STUDY OF OLEOSIN USING SRCD AT DISCO

Summary of oleosin structural studies

Conventional approaches

- in **detergents**
- in **solvents**
- in **liposomes**

→ Results are contradictory Mainly alpha or mainly beta [11, 12]

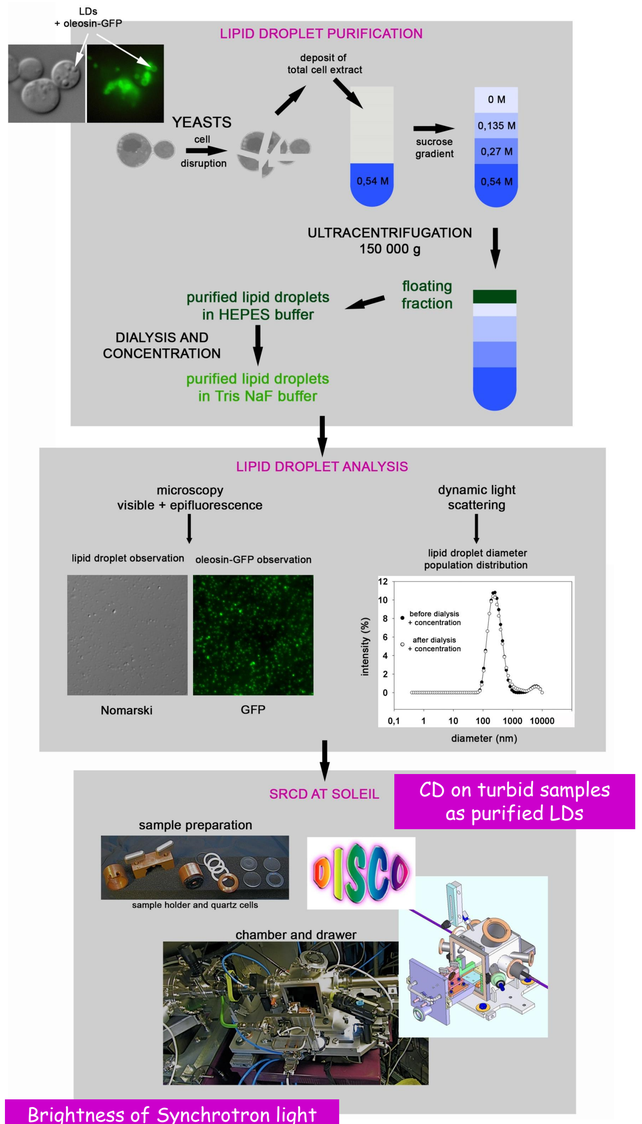
Toward natural environment

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

→ Not relevant

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

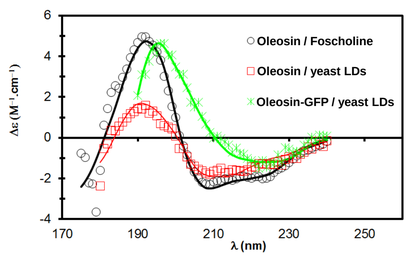
LD purification protocol for SRCD



Brightness of Synchrotron light + Wide spectral range

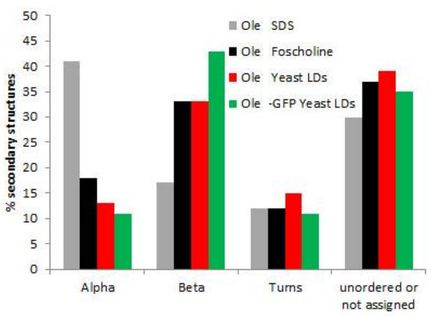
Oleosins are mainly beta folded when inserted in LDs [13]

Spectra were obtained using Suprasil quartz cells of 100 200 and 500 µm path length. Protein concentrations were in the 0.1 to 0.5 g.L^{-1} range.

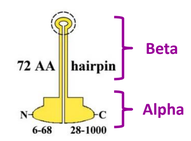


First SRCD spectra on purified organelle

Data processing and secondary structure determination were performed using the CDtool software and the ContinLL method on DICHROWEB

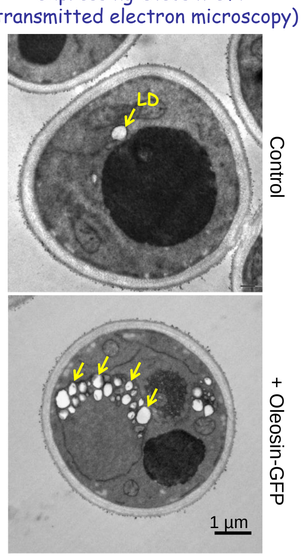


Hydrophobic domain of oleosins = beta sheet hairpin



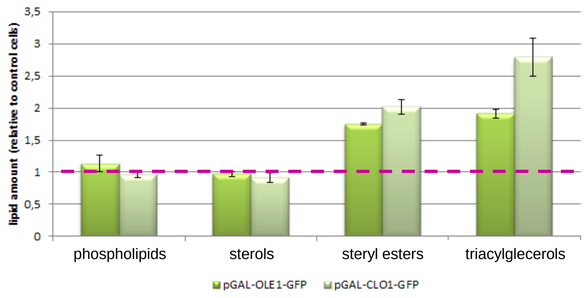
Oleosins induce LD and lipid accumulation

Thin sections of yeasts expressing Oleosin-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

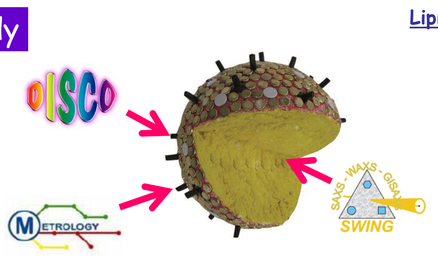


oleosins induce neutral lipid accumulation

Perspectives on LD study

Hairpin protein?

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



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)

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