Insight into secondary structure of Arabidopsis thaliana seed oleosins

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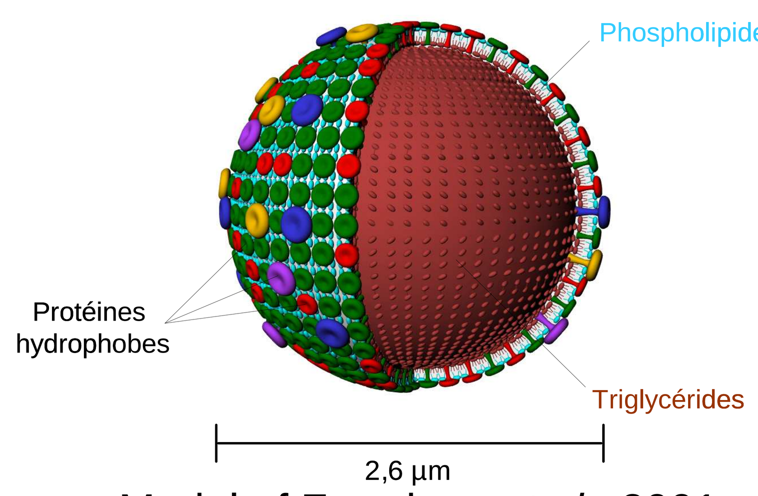
Introduction

Oléosomes store energy



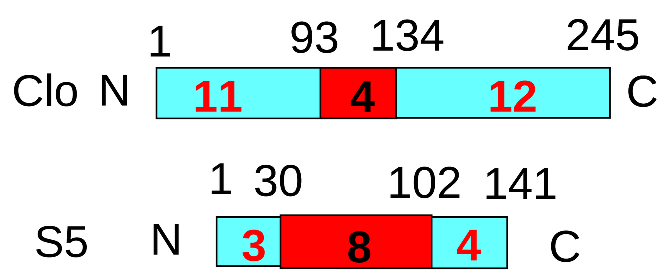
Model of Frandsen *et al*., 2001.

Oleaginous seeds store energy in oil bodies called oleosomes.

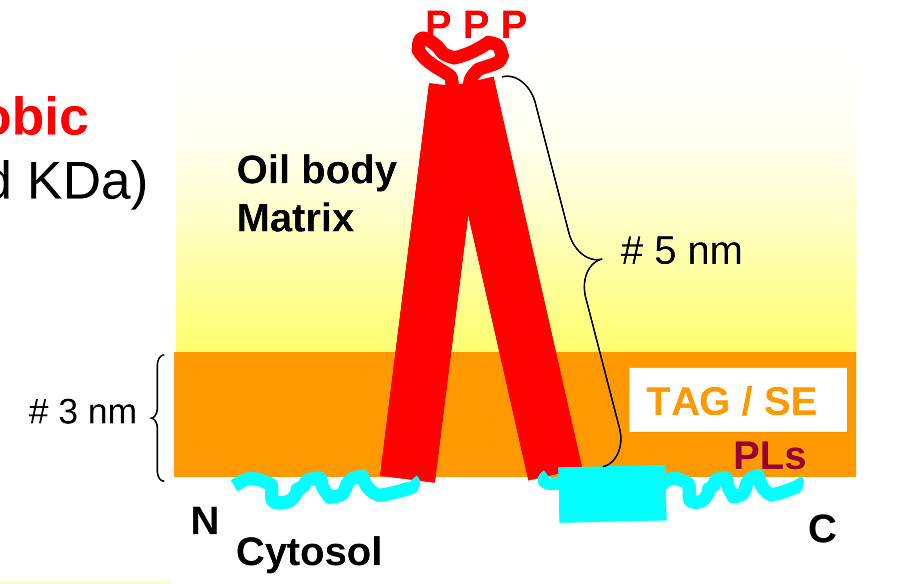
Their triacylglycerol (TAG) core is wrapped in a **phospholipid** (PL) **monolayer** containing several **integral proteins** such as **caleosin (Clo)** and **S5 oleosin**.

Oleosomes Integral proteins

Triblock repartition of amino acids.
The size of **hydrophilic** and **hydrophobic** domains is shown (number of residus and KDa)

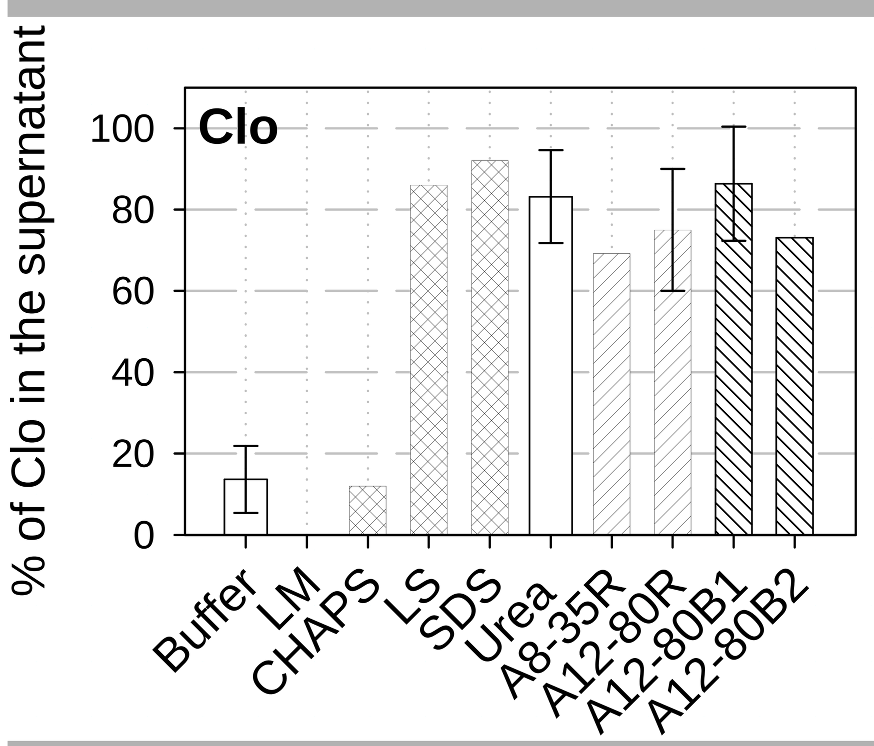


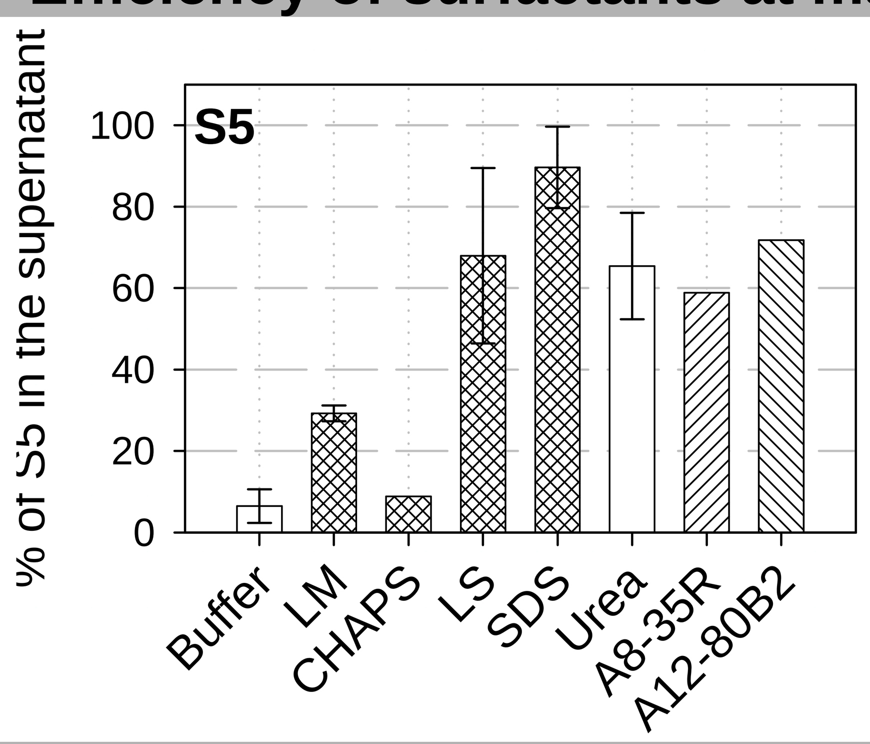
Model of Huang, 1996.



As Oleosins are highly hydrophobic proteins, little is known about their structure

Efficiency of surfactants at maintaining Clo and S5 in solution

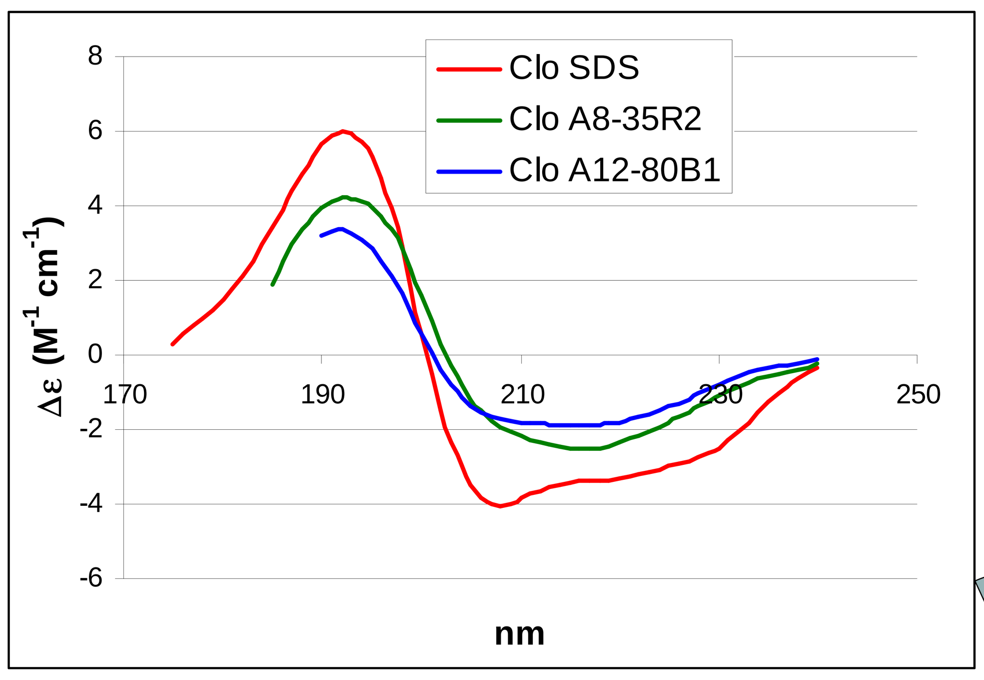




Controls
Detergents
Amphiphatic polymers: **amphipols**

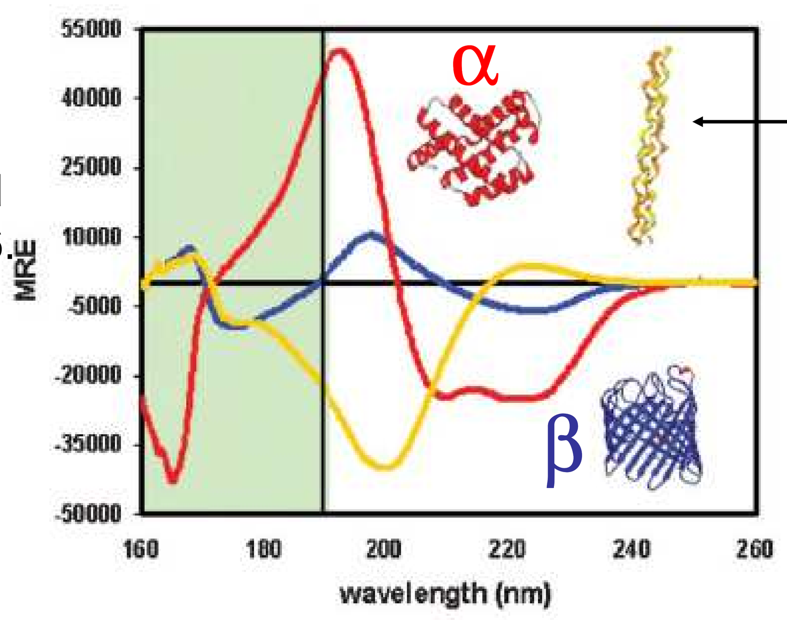
Only charged detergents (*i.e.* SDS and LS) and all amphipols were efficient at maintaining S5 and Clo in solution

Synchrotron Radiation Circular Dichroism (SRCD) on solubilized Clo and S5



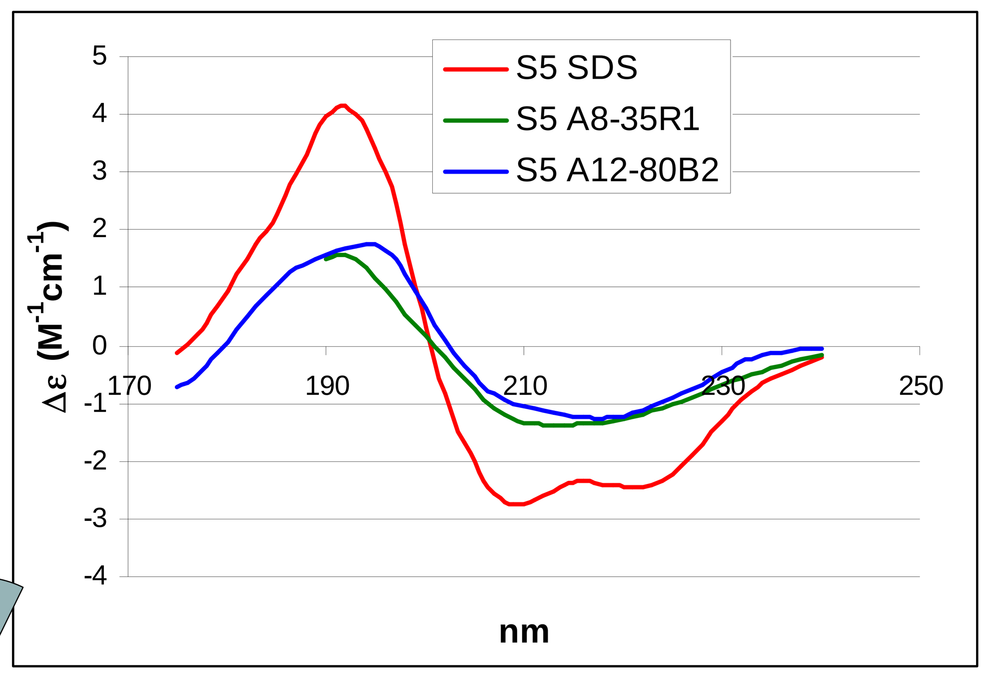
SRCD spectra acquisition

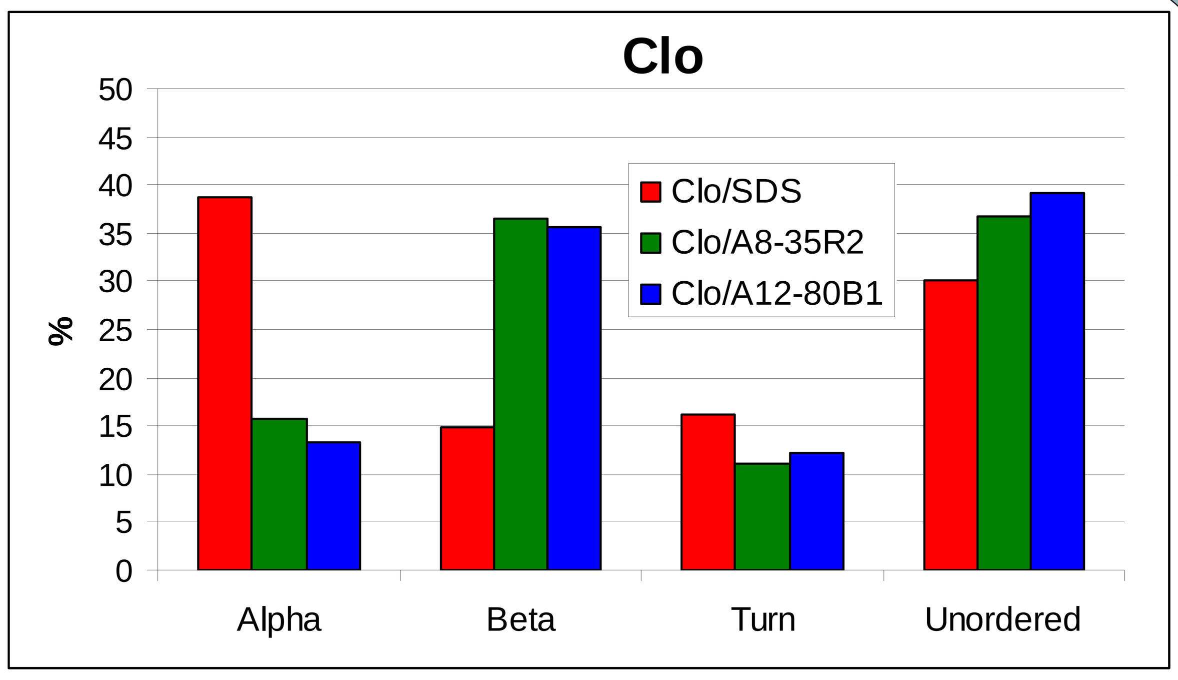
Miles and Wallace., 2006.



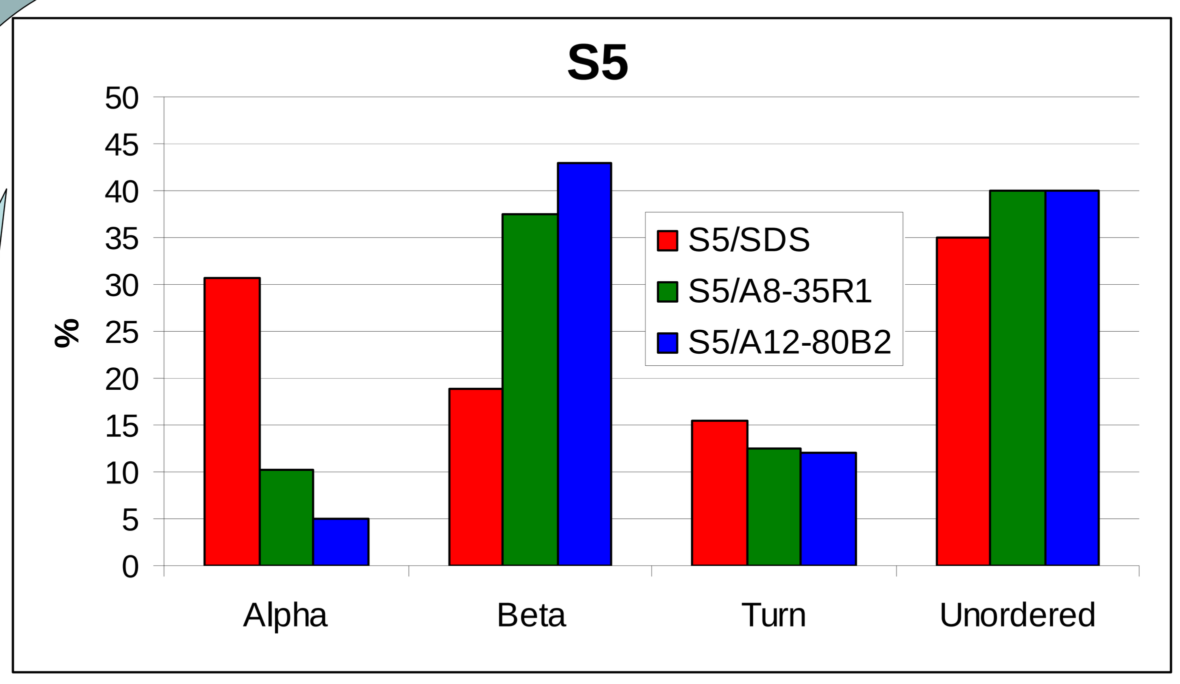
Neither α nor β

deconvolution





In complex with an amphipol, Clo and S5 contain more β and less α secondary structures features than with SDS.



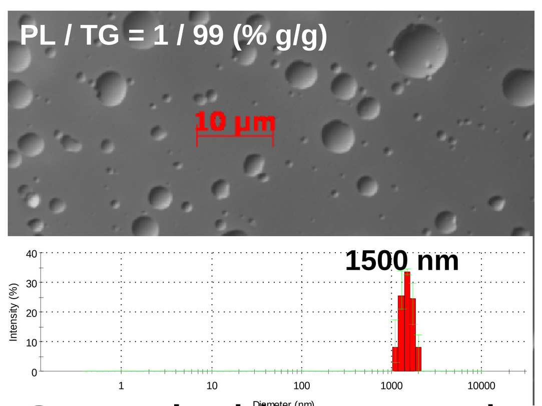
What is the secondary structure of oleosins in a natural environment?

Studies of oleosins inserted into whole oleosomes

Artificial oleosomes

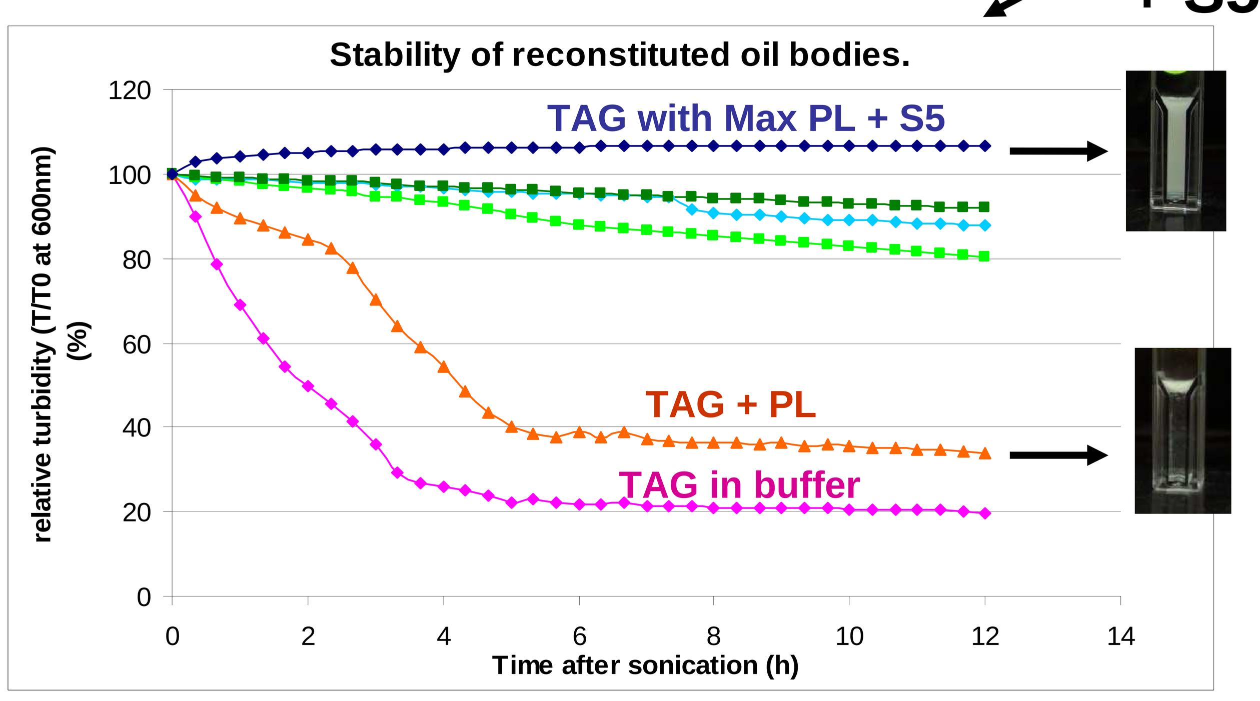
Reconstituted with 3 components:
- Oleosine / PL / TAG.

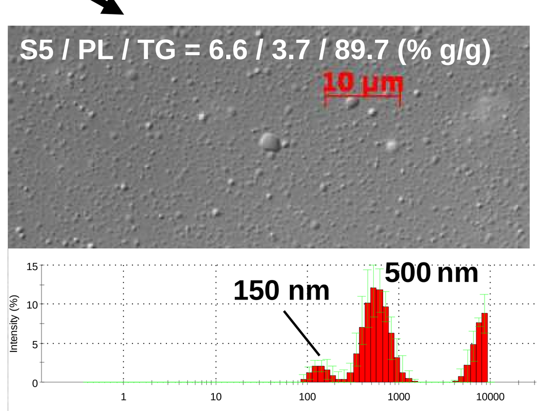
Optimized amount of S5 and PL **stabilized** the emulsion and **reduced** oleosome size



Control without protein

+ S5

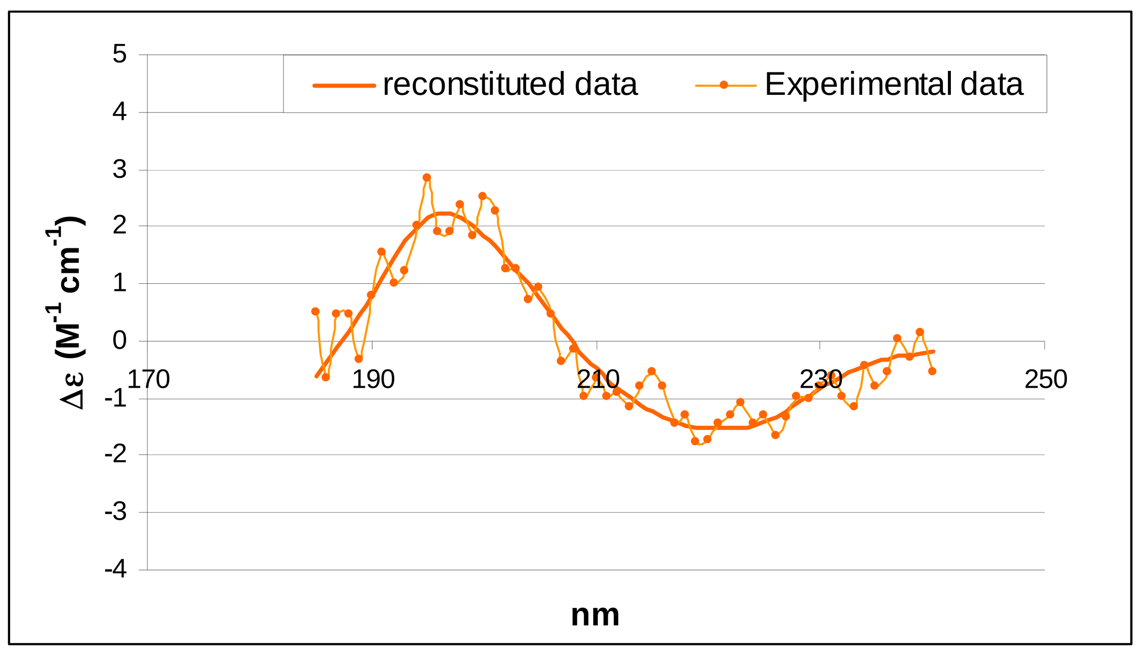




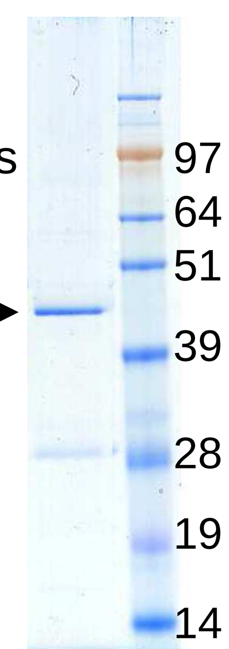
Protein CD signal in artificial oleosomes is blurred by light scattering.

Vegetalized yeast oleosomes

Some of our first attemps of SRCD on yeast oleosomes containing oleosin **GFPtag–S3** protein provided a **weak protein–like signal.**



SDS-PAGE of purified yeast oleosomes



Previous CD attempts on oleosomes in the litterature were unsuccessfull (Li *et al.,* 2002).

Next approach will consist in improving such a signal and obtaining one with oleosine S3 inserted alone into yeast oleosomes.

Frandsen GI *et al., Physiol. Plant.* (2001) 112: 301-307 ; **Huang HAC,** *Plant. Physiol.* (1996) 110: 1055-61 ; **Li M.** *et al., J. Biol. Chem.* (2002) 277: 37888-95 ; **Miles AJ, Wallace BA,** *Chem. Soc. Rev*. (2006) 35: 39-51 ; **Withmore L, Wallace BA,** *Biopolymers* (2008) 89: 392-400.
SRCD data were processed using CD tools program: **Lees JG** *et al., Anal. Biochem.* (2004) 332: 285-289.
Secondary structure prediction was achieved using Contin method with SP175 reference sets. **Lee JG** *et al*., bioinformatics (2006) 22: 1955-62.