

Synchrotron Multimodal Methods for Lipid Droplets *in vitro* and *in cellulo* Studies

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ABSTRACT

In all organisms, storage lipids are maintained in the cytoplasm in specialized organelles called lipid droplets (LDs). These nanoparticles consist mainly of a core of neutral lipids (triacylglycerols and/or steryl esters) enclosed in a monolayer of phospholipids, and contain a number of proteins which vary considerably with the species. More than inert fat balls, LDs are complex organelles and their abnormal dynamics is associated with several diseases (virus-induced steatosis, diabetes, atherosclerosis and myopathies). In the context of green chemistry, LDs are promising sources of lipid-derived molecules for chemistry, food, medicine and cosmetics. For these reasons, understanding LD structure and way of life is of major importance.

Combination of synchrotron radiation techniques enable us to go deeper into the comprehension of LD biology. We investigated the LD structure, from surface proteins to internal lipid core using two models, plant and yeast [1, 2]. First, we obtained structural data on oleosin, a seed LD surface protein, using SRCD [3] and proteomics after X-ray footprinting [4, 5]. The results obtained revealed a beta hairpin folding of the protein when inserted in lipids, its native environment. Second, LD core structure was studied after LD purification using small angle X-Ray scattering upon temperature variations, or *in cellulo* using a large set of label free synchrotron techniques. We performed transmission X-ray microscopy on vitrified yeast and deep ultra violet on living yeast. We confirmed the ultrastructural lipid heterogeneity of LD and its concentric layer organization.

Our objectives are to go further in LD study in a preserved environment. 1) Towards more resolution in intact seeds, from the organ scale to the cellular and intracellular scales, using innovative micro- and nano-computed tomography. 2) Towards more dynamics using DUV imaging of living cells in microfluidic devices.

REFERENCES

1. Froissard, M., *et al.*, Heterologous expression of AtC1o1, a plant oil body protein, induces lipid accumulation in yeast. *FEMS Yeast Res.*, 2009. 9(3): p. 428-38.
2. Jamme, F., *et al.*, Single Cell Synchrotron FT-IR Microspectroscopy Reveals a Link between Neutral Lipid and Storage Carbohydrate Fluxes in *S. cerevisiae*. *PLoS One*, 2013. 8(9): p. e74421.
3. Vindigni, J.D., *et al.*, Fold of an oleosin targeted to cellular oil bodies. *Biochim Biophys Acta*, 2013. 1828(8): p. 1881-88.
4. Baud, A., *et al.*, SOLEIL shining on the solution-state structure of biomacromolecules by synchrotron X-ray footprinting at the Metrology beamline ». *J. Synchr. Rad.* 2017. 24 (3): p. 576-85.
5. Jolivet, P., *et al.*, Structural Proteomics: Topology and Relative Accessibility of Plant Lipid Droplet Associated Proteins ». *J. of Proteomics*, 2017. 169: p. 87-98.