

Research Topic for the ParisTech/CSC PhD Program

**Subfield:** Structural biology

**ParisTech School:** AgroParisTech

**Title:**Engineering acyltransferases for synthetic biology of lipids

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**Short description of possible research topics for a PhD:**

50 years after the discovery of the Kennedy pathway responsible for synthesis of triacylglycerols (TAGs) in animal fats, vegetable and microbial oils, information on the structure of acyltransferases, and on the residues responsible for their specificity, is still limited. These enzymes include LPATs (lysophosphatidic acid acyltransferases), that add the middle or sn-2 fatty acid to lysophosphatidic acid, and DGATs (diacylglycerol acyltransferases) that add a fatty acid to the sn-3 position, final and committed step in TAG biosynthesis. Because of their impact on oilyield and quality, DGATs are exciting targets for food industry and green chemistry. The availability of sequenced genomes has increased the number of putative DGAT sequences, providing candidate genes for metabolic engineering. We hypothesize that the substrate specificity of most DGATs limits the incorporation of unusual fatty acids in TAGs. We will therefore search for sequences encoding acyltransferases from plants that synthesize TAGs containing exotic FAs. We will express them in various hosts, then study the activity and specificity of the encoded enzymes (wt and mutated) *in vivo* (microbial systems) and *in vitro* (enzymatic assays). We will also try to solve their 3D structure (cristallography) in order to better understand the structural basis of their specificity, allowing their rational engineering.

**Required background of the student:**

The successful candidate will have proved successful experience with protein expression and purification, kinetics measurements, and with protein structural studies. Additional knowledge on neutral lipid metabolism, although not required, will be appreciated.

**A list of 5(max.) representative publications of the group:**

- 1) Haili N, Louap J, Canonge M, Jagic F, Louis-Mondesir C, Chardot T, Briozzo P (2016) Expression of Soluble Forms of Yeast Diacylglycerol Acyltransferase 2 That Integrate a Broad Range of Saturated Fatty Acids in Triacylglycerols. PLoS One 11: e0165431
- 2) Ayme L, Jolivet P, Nicaud JM, Chardot T (2015) Molecular Characterization of the *Elaeis guineensis* Medium-Chain Fatty Acid Diacylglycerol Acyltransferase DGAT1-1 by Heterologous Expression in *Yarrowia lipolytica*. PLoS One 10: e0143113
- 3) Ayme L, Baud S, Dubreucq B, Joffre F, Chardot T (2014) Function and localization of the *Arabidopsis thaliana* diacylglycerol acyltransferase DGAT2 expressed in yeast. PLoS One 9: e92237\*
- 4) Beopoulos A, Haddouche R, Kabran P, Dulermo T, Chardot T, Nicaud JM (2012) Identification and characterization of DGA2, an acyltransferase of the DGAT1 acyl-CoA:diacylglycerol acyltransferase family in the oleaginous yeast *Yarrowia lipolytica*. New insights into the storage lipid metabolism of oleaginous yeasts. Applied Microbiology and Biotechnology 93: 1523-1537