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Lipase activity on milk fat globules in a mini-digester to simulate neonatal gastro-intestinal digestion using DISCO beamline

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In their native state, milk lipids are present in the form of dispersed droplets called Milk Fat Globules (MFG, diameter 0.1-20 μm , average = 4 μm in bovine milk) [1,2]. The native MFG is constituted by a triglyceride core covered with an external trilayered membrane inherited from its secretory past. This membrane is mainly based on polar lipids (glycerophospholipids, sphingolipids and glycosphingolipids), proteins (25 % of total membrane with high proportion of glycoproteins and enzymes), neutral lipids and minor components. The major biological function of the MFG is to deliver energy to the mammal newborn, for that, the MFG has to be hydrolyzed in the gastro-intestinal tract by lipases, in successive steps. The lipases should interact with the MFG membrane and diffuse in the supramolecular object. The aim of this work is to determine the mechanism involved in the enzymatic hydrolysis along the digestive process.

Indeed, we used millifluidic cells designed to bloc MFG and injected successively each lipase involved in the digestion process. Thank to the UV-microscope developed on the DISCO beamline, the autofluorescence of tryptophan (and tyrosine) present in the lipase amino-acid sequences had allowed the protein observation without external labeling.

The gastric and intestinal neonatal disintegration of milk fat globules has been approached through transmission images recording and analysis of the number of globules and their state of aggregation/coalescence during digestion (approached via diameter, size, mode and specific surface evolution against time).

In this presentation, the first results of these experiments will be showed. Mainly, in the stomach, the gastric lipases bind the MFG surface. Then, the MFG decreased in size in the intestinal phase releasing the intact lipid membrane suggesting that the core is hydrolyzed through the external membrane.

[1] C. Bourlieu, S. Bouhallab, and C. Lopez. Biocatalyzed modifications of milk lipids: applications and potentialities, *Trends in Food Science & Technology*, 20 (2009) 458-469.

[2] C. Bourlieu, and M-C. Michalski. Structure–function relationship of the milk fat globule. *Current Opinion in Clinical Nutrition and Metabolic Care* 18[2], 118-127. 2015.