

SUPPLEMENTARY FIGURES

Figure S1. The milk fat globule membrane structure and lipid composition of milk polar lipid extracts determined by HPLC (classes of polar lipids) and GC (acyl chain lengths).

Figure S2. Procedure of AFM image analysis using FIJI [35].

Figure S3. Evolution of Surface pressure (circles; π , mN.m^{-1}) and Ellipsometric angle (triangles; Δ , $^\circ$) as a function of the sub-phase molar concentration of rDGL (panel A). Graph presented in panel B shows the evolution of Ellipsometric angle (Δ , $^\circ$) versus Surface pressure (π , mN.m^{-1}). Values determined at least in duplicate following rDGL adsorption at the air/water interface.

Figure S4. Langmuir-Schaefer transfer of rDGL/lipid monolayer onto hydrophobic mica support and liquid cell AFM imaging.

To prepare the hydrophobic mica, a DPPC monolayer was transferred by Langmuir-Blodgett method onto freshly cleaved mica disc at high surface pressure (35 mN.m^{-1}). After two days of dehydration, the coated mica could be used. The sealed (bottom side) AFM Teflon chamber is placed at the bottom of the trough. At the end of the lipase absorption kinetic (2 hours), the interfacial film was collected by lowering horizontally the hydrophobic mica disc at very low speed (0.5 mm/min) using the dipper of Nima technology (England). After contact with the film, the sample was maintained blocked on the sealed chamber. Then all system was reverted and the chamber was placed on the AFM (Molecular Imaging, Pico+, Plus, Scientec, France). The sample was maintained hydrated all the time. The liquid level of the chamber could be readjusted with 10 mM sodium acetate buffer ($\text{pH } 5$) containing 100 mM NaCl and 20 mM CaCl_2 . Imaging was carried out in contact mode at room temperature as described in section 2.5.

Figure S5. 3D surface representation models of rDGL in its open conformation. Panels A and B show top views of rDGL with the enzyme oriented parallel to the lipid interface (page plane) as previously deduced from structural analogies with pancreatic lipase [4]. In panels A, hydrophobic and basic

residues are shown in white and blue color, respectively, while other residues are shown in dark grey. Electrostatic surface potential of open rDGL 3D structure (panels B) was calculated at pH 5. The electrostatic surface potentials were displayed color-coded onto a van der Waals surface using the PyMOL Molecular Graphics System (version 1.3, Schrödinger, LLC). Red and blue colors represent net negative and positive charges, while white color represents overall neutral positions, respectively.

Figure S1.

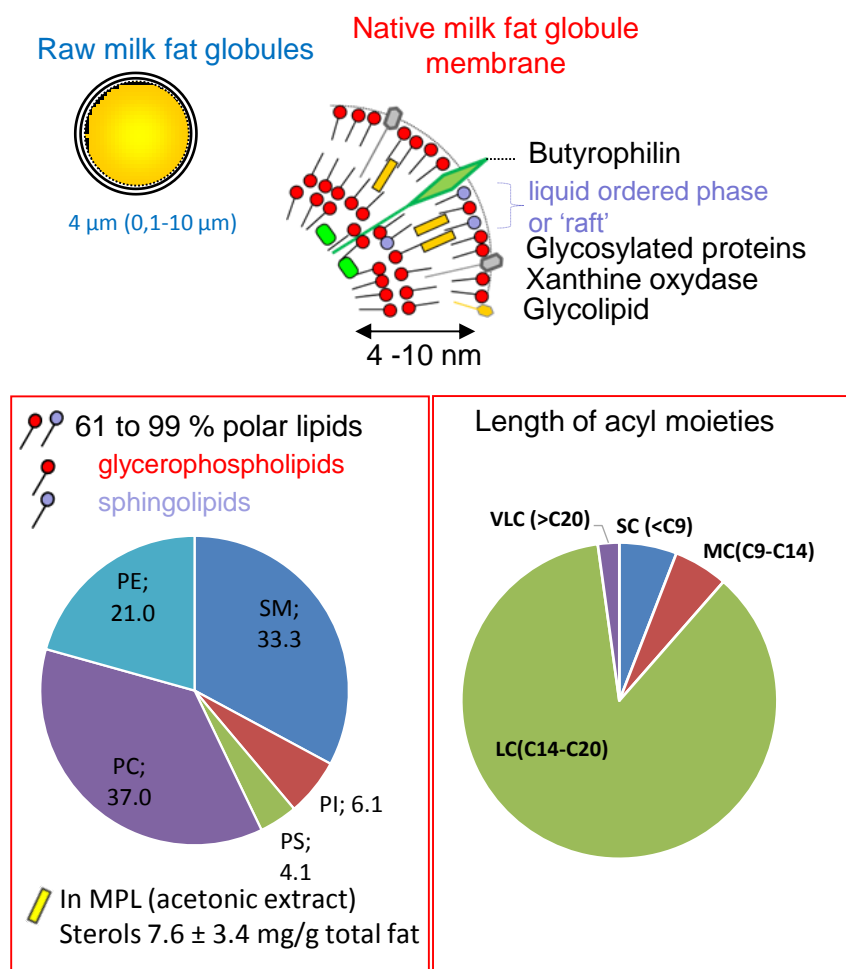
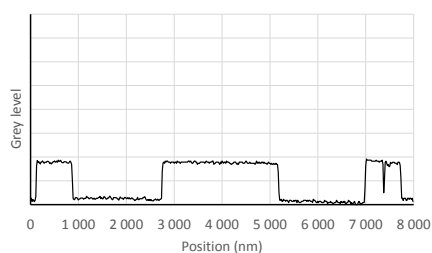
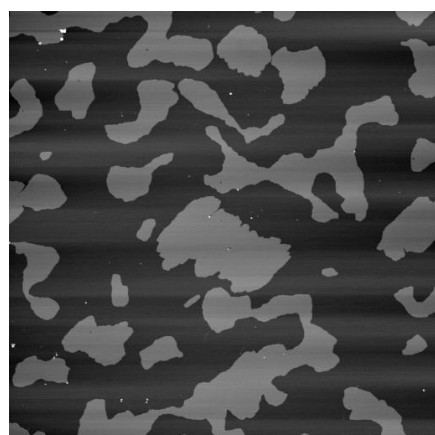
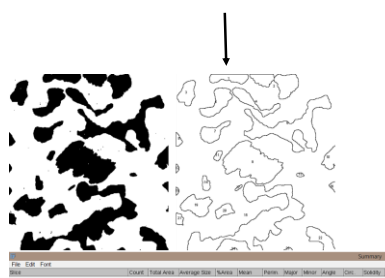


Figure S2.

In the absence of lipase

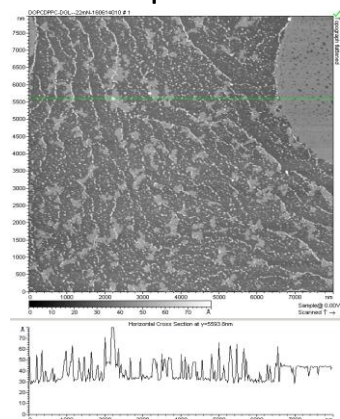


analysis / plot profile / gray limits of LC domains



crop / threshold / analysis of particles

In the presence of lipase



3 main ranges of heights distinguished on profile:

- h3 ≤ LC domains
- h2 ~ 3-4 nm, gray levels 115-130
- h1 > 5 nm, gray levels 130-255

Crop to exclude very large LC domains
Threshold at the given gray level
Limit on particle size range to exclude for
instance fragments of LC domain in h2
(particles $< 0.05 \mu\text{m}^2$)
Analysis of particles

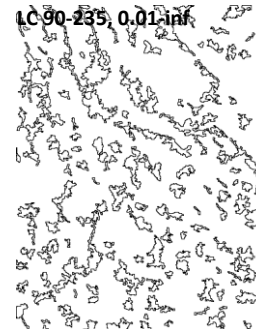
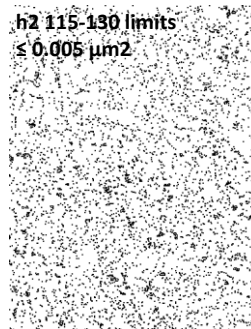
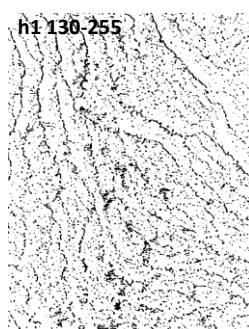


Figure S3.

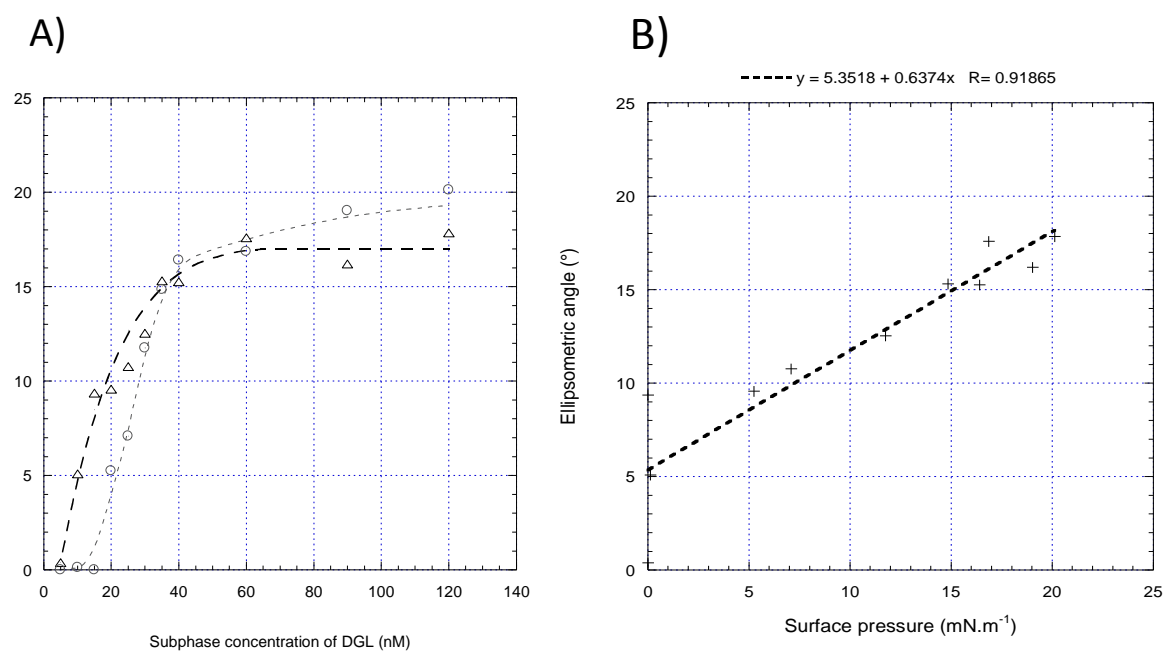
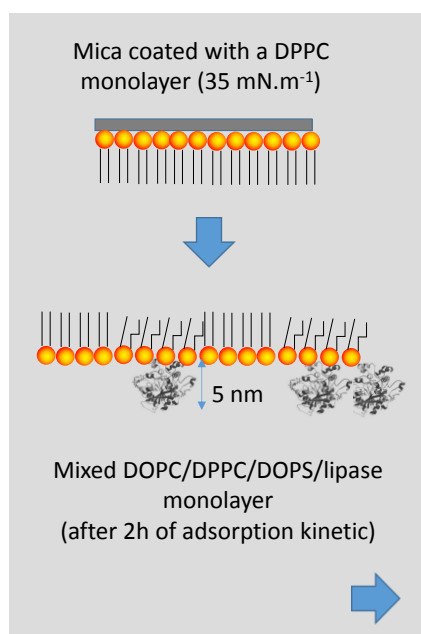
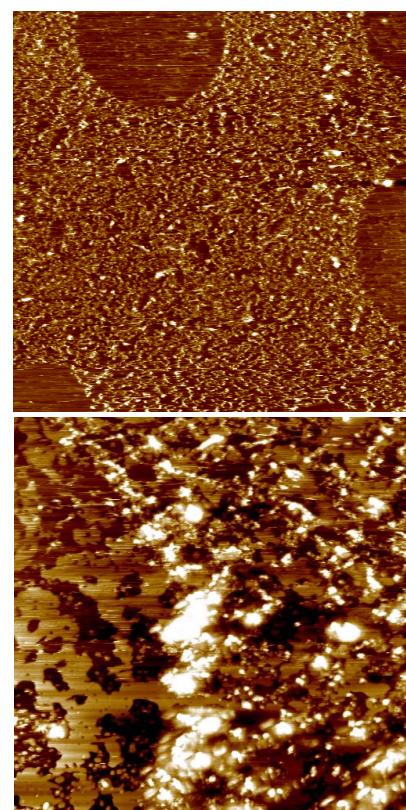
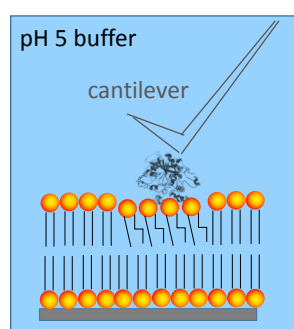


Figure S4.

Langmuir-Schaefer transfer



AFM observation in liquid cell



$8 \times 8 \mu\text{m}^2$ (above) and $2.5 \times 2.5 \mu\text{m}^2$ (below), $\Delta z = 10 \text{ nm}$ corresponding AFM image

Figure S5.

