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Selective interaction of the major milk proteins, casein micelles, onto polar lipid bilayers: role of phase and charge investigated using AFM

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Background

The study of interactions between biological membrane lipids and proteins has gained growing interest since they are crucial in different biological functions (eg. as cell signaling, trafficking...) or in the control of membranes' physical stability or functional properties. Among biological membranes, the one enveloping the milk fat globules (MFGM) is of special interest as the interface with the surrounding bulk of milk proteins or with

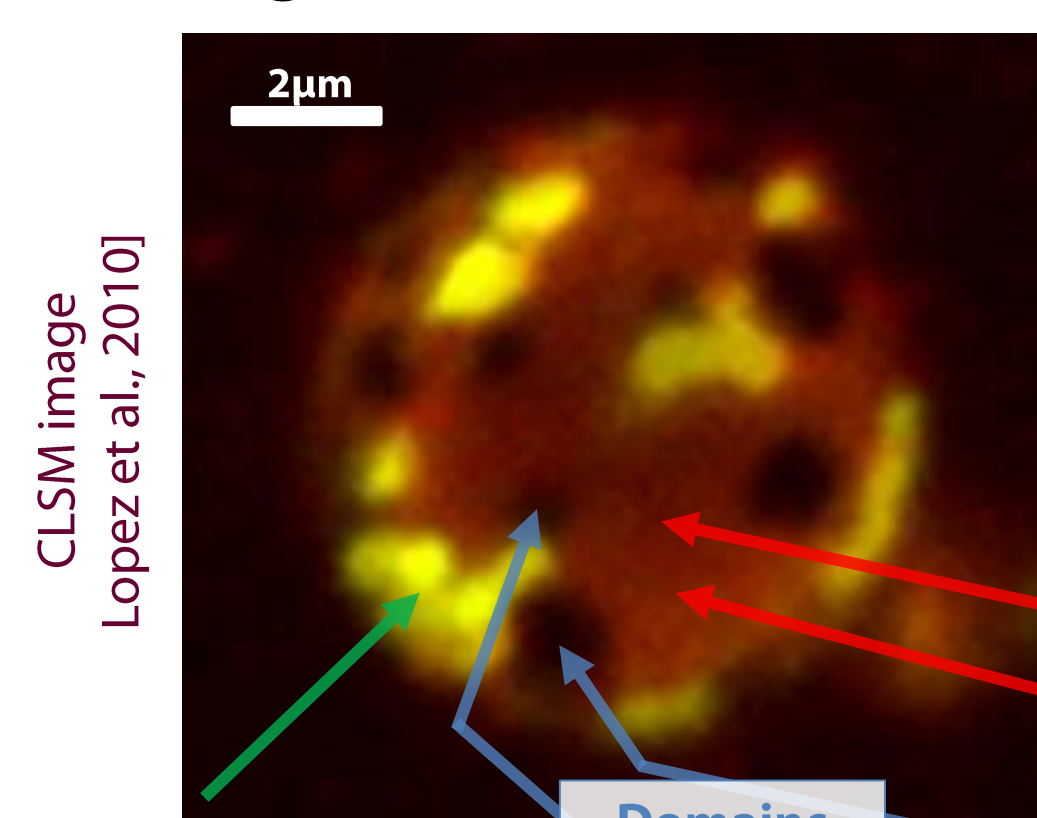
lipolytic enzymes and tissues of the gastro-intestinal tract during digestion. The MFGM exhibits lateral heterogeneities resulting from the different phase states and/or head-group charges of the polar lipids.

In the present study, our goal was to determine how the phase and charge states of MFGM polar lipids drive their interaction with casein micelles, as the major milk protein assembly.

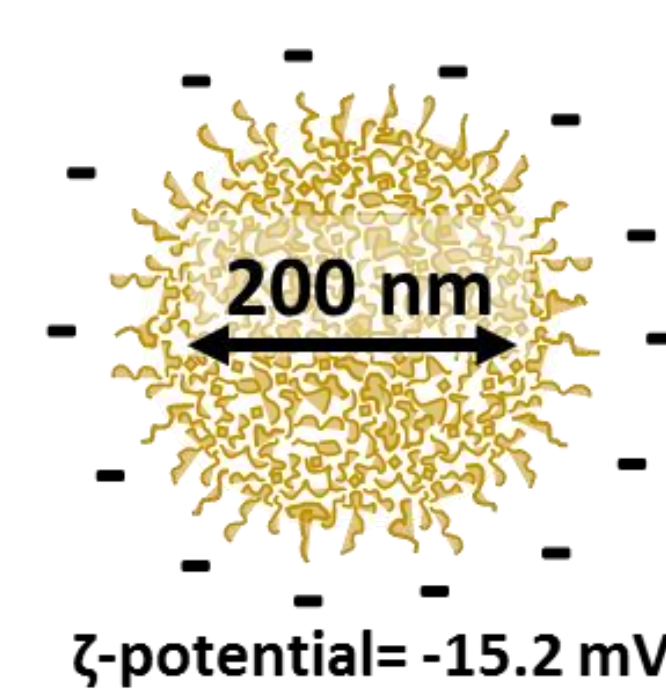
Strategy

MFGM polar lipids are characterized by the structure of their acyl chains and the charge of their head-groups: saturated lipids, such as milk SM (MSM), show high transition temperature ($T_m \sim 35^\circ\text{C}$) and are present in gel phase domains at $T < T_m$, or in liquid-ordered (l_o) phase domains in the presence of cholesterol (at $T >$ or $< T_m$); while unsaturated lipids such as DOPC, DOPS, PI have low T_m ($< 0^\circ\text{C}$) and are present in fluid phase. MSM and DOPC show zwitterionic head-groups while PS and PI are anionic.

Milk fat globule enveloped by a biological membrane (MFGM)



Casein micelles

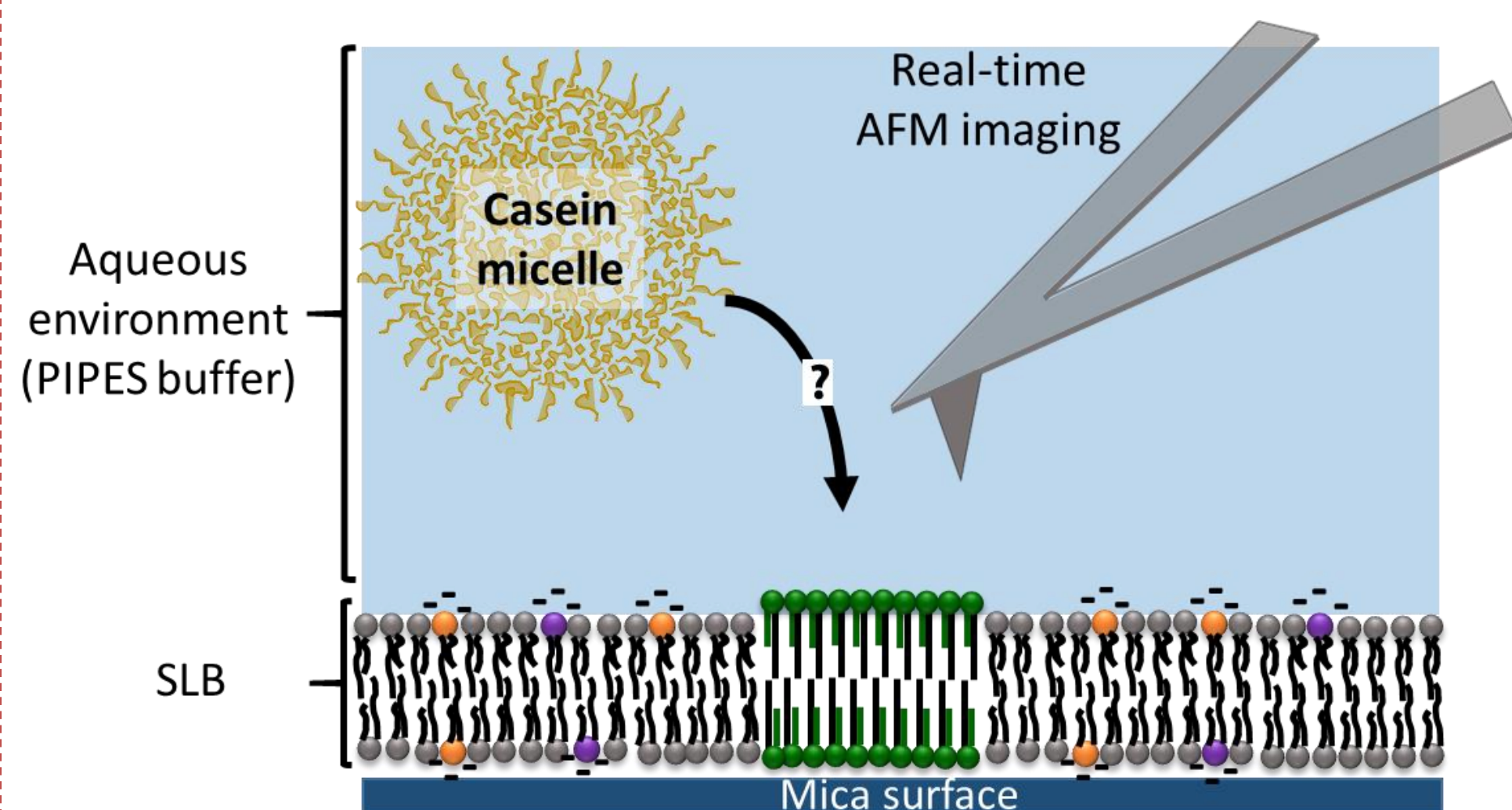


	MSM/Chol (l_o)	MSM (gel)	DOPC (Fluid)	DOPC/DOPS/PI (Fluid)
Molecular area ($\text{\AA}^2/\text{molecule}$)	40.0	52.1	69.1	67.9
ζ -potential (mV)	+0.4	+4.2	+1.1	-9.6

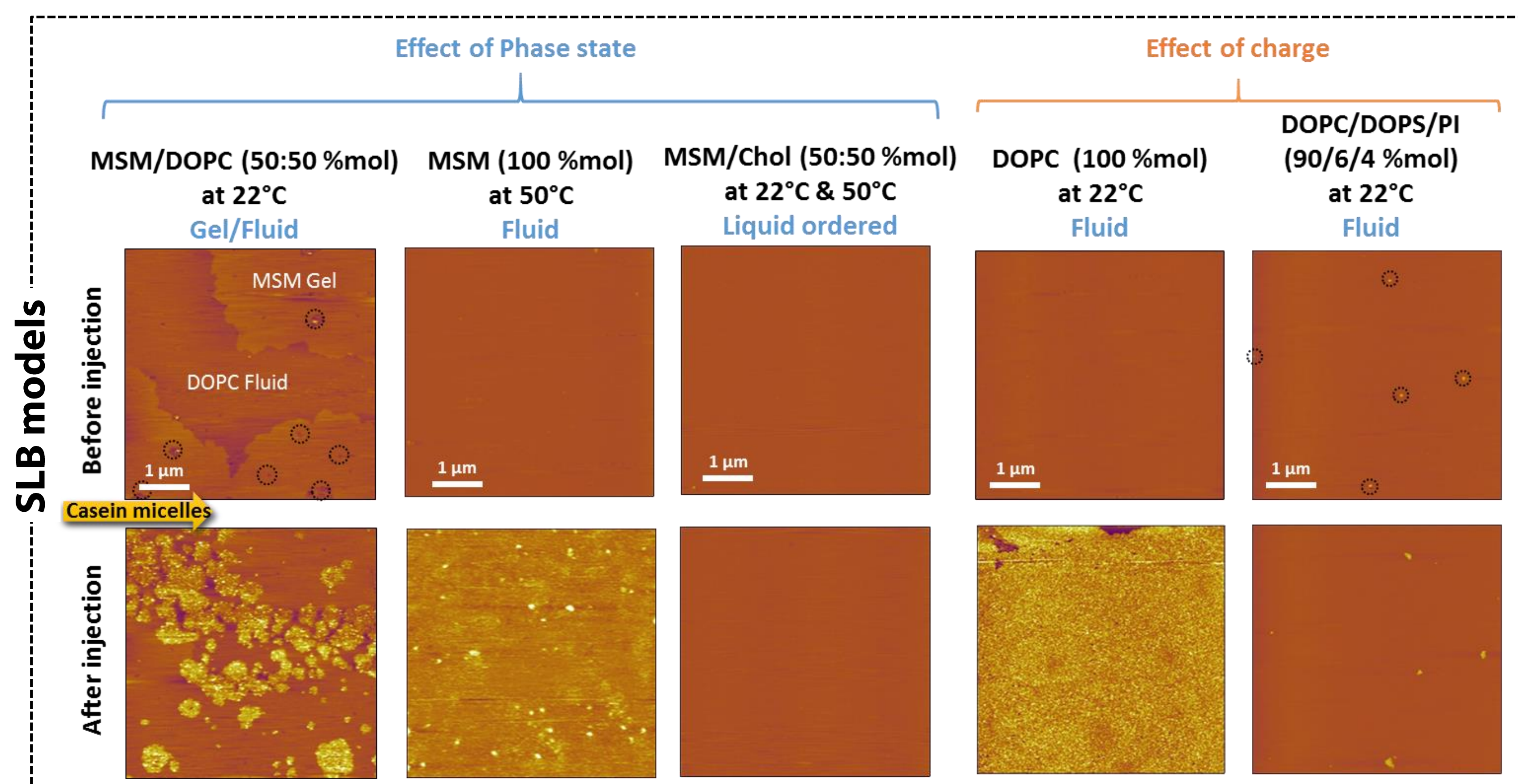
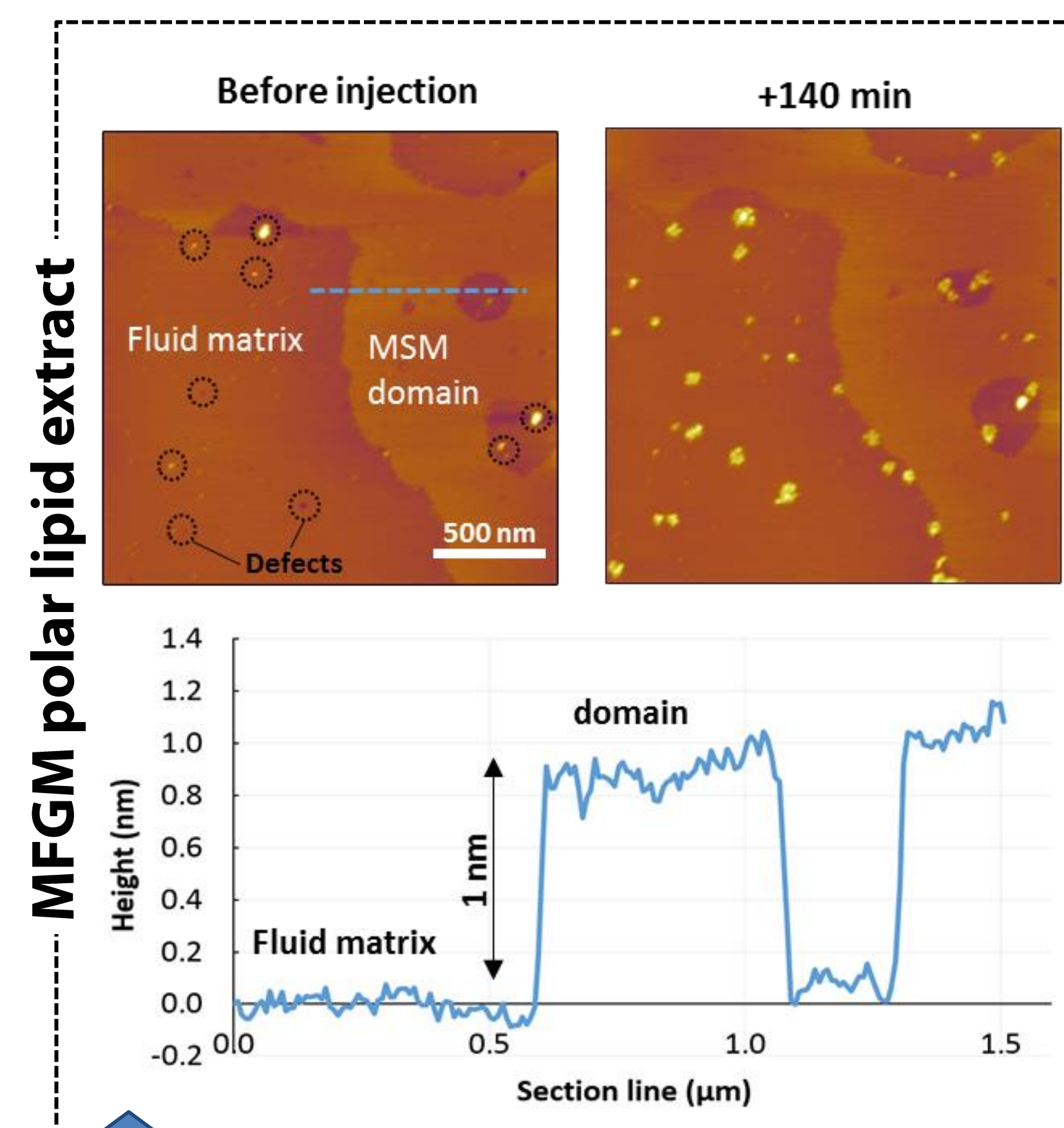
Physical characterization of lipids: Molecular area of the different lipids was measured at a surface pressure of 25 mN/m, and their zeta-potential in PIPES buffer (2mM Ca, pH 6.7).

AFM imaging

Real-time atomic force microscopy (AFM) imaging was used for monitoring the interaction of the negatively charged casein micelles with hydrated supported lipid bilayers (SLB) composed of MFGM lipid extract and of simplified SLB models.



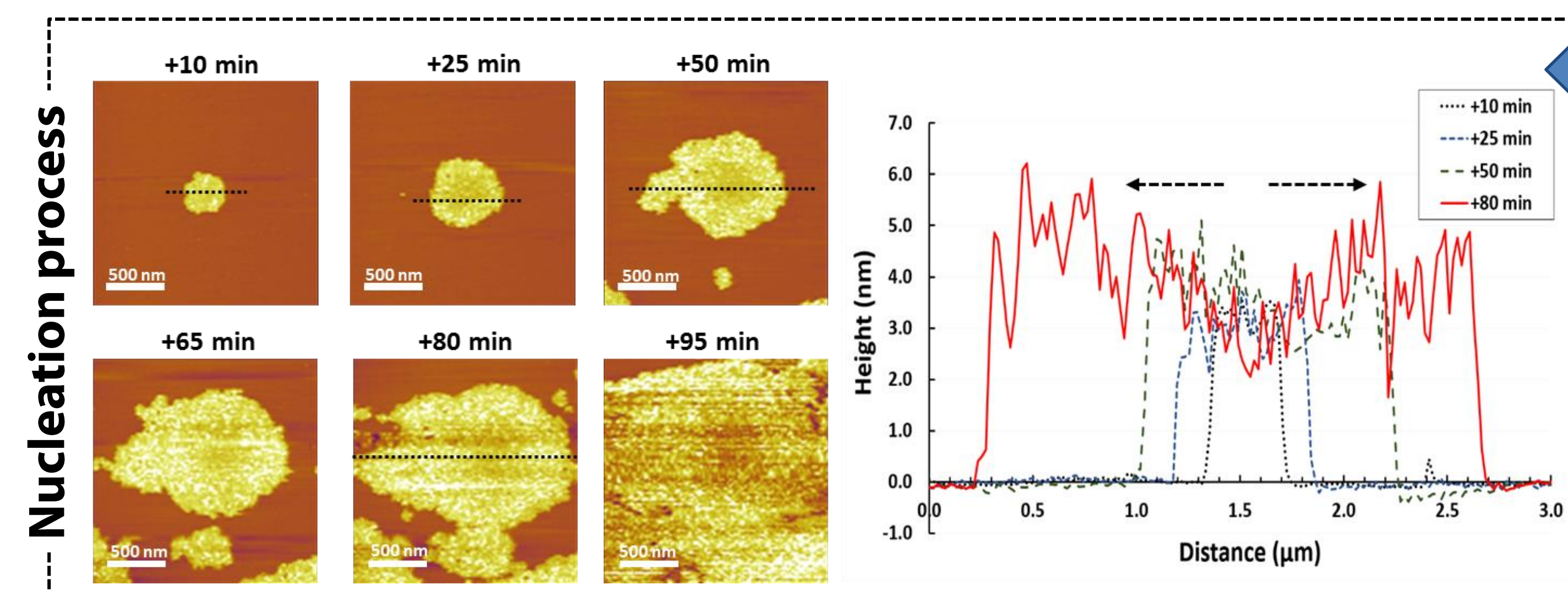
Results



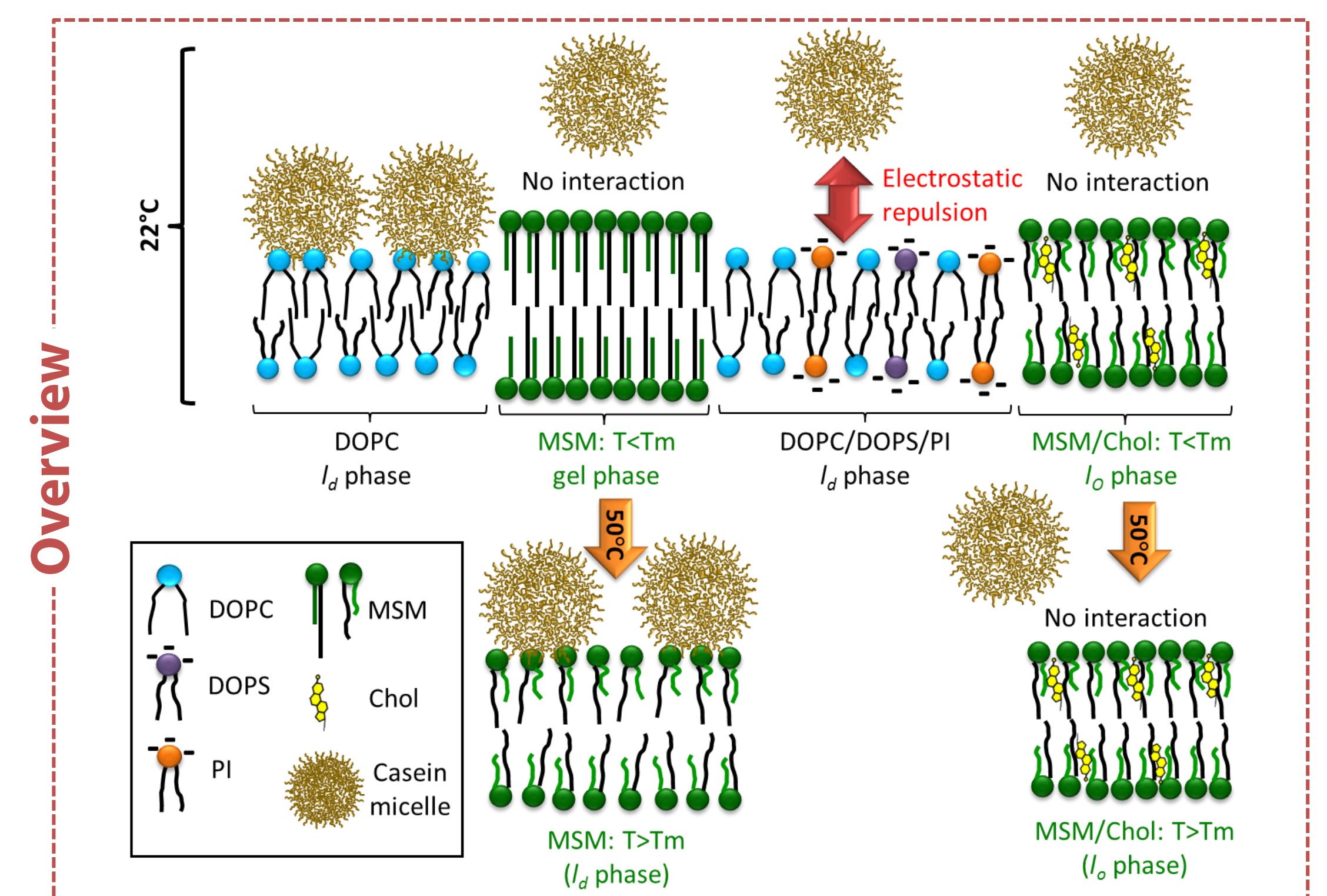
Casein micelles:

- Do not interact with lipid mixtures showing low molecular area (ie. MSM at 22 °C & MSM/Chol at 22 and 50 °C) neither with negatively charged mixtures (ie. DOPC/DOPS/PI).
- Do interact with zwitterionic lipids showing high molecular area (ie. DOPC at 22°C and MSM at 50°C).

Casein micelles interact only with defects, but not with the gel phase domains neither with the fluid matrix of MFGM at 22°C ($T < T_m$ of MSM).



The interaction of casein micelles with SLB of DOPC occurred preferentially through nucleation growth process.



Highlights

- The interaction of casein micelles with MFGM is hindered by the high lateral packing density of MSM-rich domains (gel and l_o phases) and the presence of anionic lipids (PS and PI) in the fluid matrix.
- These results open new perspectives for the design of polar lipid coated emulsion droplets and liposomes with variable capacity for protein adsorption.

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