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2D DPPC Based Emulsion-like Structures Stabilized by Silica Nanoparticles

Eduardo Guzmán,[†] Davide Orsi,[‡] Luigi Cristofolini,^{‡†} Libero Liggieri,[†] Francesca Ravera^{†,}*

[†] Consiglio Nazionale delle Ricerche-Istituto per l'Energetica e le Interfasi, U.O.S. Genova (CNR-
IENI). Via De Marini 6, 16149-Genova (Italy)

[‡] Dipartimento di Fisica e Scienze della Terra, Università degli Studi di Parma.

Viale Usberti 7/A, 43100-Parma (Italy)

ABSTRACT

We studied the mechanical and structural properties of mixed surface layers composed by 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and silica nanoparticles (NPs). These layers are obtained by spreading a DPPC Langmuir monolayer on a colloidal silica dispersions. The transfer/incorporation of NPs into the DPPC monolayer, driven by electrostatic interactions, alters the molecular orientation, the mechanisms of domain formation and consequently the phase behavior of the surface layer during compression. The investigation of these systems by means of complementary techniques (Langmuir trough, Fluorescence microscopy, Ellipsometry and SEM) shows that the incorporated NPs preferentially distribute along the liquid expanded phase of DPPC. The layer assumes the stable and homogeneous bi-dimensional structure of a 2D analogous of a Pickering emulsion. In fact, the presence of particles provides a circular shape to the DPPC domains and stabilise them against growth and coalescence during the monolayer compression.

1. INTRODUCTION

The interaction between nanoparticles (NPs) and adsorbed or spread surfactant layers at liquid interfaces is relevant in several scientific and technological fields such as material science, biomaterials, emulsion-foam technology, pharmaceuticals and nano-medicine.¹⁻⁴

The potential effects of NP on the mechanical and physicochemical properties of surfactant adsorbed layers are well known from previous studies based on surface tension and rheological measurements.⁵ On the other side insoluble surfactant layers have been widely investigated in presence of NP as Langmuir monolayers spread on nanoparticle dispersions. This experimental approach is especially effective to assess the properties of biologically relevant systems such as mixtures of lipids.

In this context, NPs of different chemical composition, size and hydrophobicity/philicity, have been investigated as interacting with lipid monolayers, containing 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) as main component.⁶⁻¹¹ These studies have evidenced that NPs may significantly alter the phase behavior of the surface layer and its structure,¹²⁻¹⁵ because of their interaction with the lipid molecules which induces changes in the surface molecular packing. Such interactions may have hydrophobic or electrostatic origin depending on the chemical nature of the particles.¹⁰

The same type of interactions is also responsible of the ability of DPPC to bind to several biological relevant molecules if they are present in the water phase, e.g. interaction with interface-related proteins such as myelin basic protein (MBP, a protein deemed to be involved in degenerative neuronal diseases) was demonstrated for DPPC as well as for other phospholipid monolayers.¹⁶ At the same time, incorporation of DNA in DPPC monolayers was indeed

demonstrated by different techniques,¹⁷ including x-ray reflectivity¹⁷ and could be exploited for therapeutic as well as for biosensing purposes.¹⁸

The work presented here focuses on the properties of a mixed DPPC – silica nanoparticles layer obtained by spreading a DPPC monolayer at the surface of dispersions of colloidal silica. Under these conditions, in fact, the Silica NPs tend to be incorporated into the DPPC monolayers driven by electrostatic interactions.^{19,20}

This system has been investigated in refs. 12, 13 by Langmuir trough measurements and Brewster Angle Microscopy (BAM), in the framework of studies on the nanoparticle impact on the pulmonary surfactant functionality. In those studies, as well as in many other toxicological investigations, the choice of DPPC is justified by the fact that it is the main component of bio-systems such as lung surfactant or cell membranes.²¹ Nevertheless, the motivation of the present work is related to the development of innovative soft materials being the DPPC and silica representative of organic-inorganic mixed systems undergoing, under certain conditions, interesting processes of surface re-organization.

The analysis reported in the following is based on diverse surface diagnostics implemented in the Langmuir trough technique, such as Fluorescence microscopy and Ellipsometry, combined with Scanning Electron Microscopy (SEM) of Langmuir-Schaefer (LS) films obtained from the mixed monolayers. Those techniques proved successful in identifying the morphology, and therefore also in the interpretation of the mechanical properties, of similar interfacial systems, realized by a completely different route, i.e. 2D interfacial gels formed by Langmuir monolayers of gold nanoparticles.²²⁻²⁴ In that case, the steps leading to the formation of the gel could be identified by epi-microscopy²² and those could be used to provide an explanation of the observed dynamical properties^{23,24}

The results obtained here by this deeper characterization, discussed in view of the previous thermodynamic and rheological findings, provide new insights into the stable and homogeneous bi-dimensional structures obtained as well as on their rheological properties.

2. MATERIALS

1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC) was purchased from Sigma (Germany) at 99 % purity and used without further purification. DPPC spreading solutions (1 mg/mL) were prepared using HPLC grade chloroform (Sigma, Germany).

Colloidal silica dispersions were obtained by diluting commercial samples (Levasil-Stark, Germany). Three different samples were used, Levasil 300/30, 200/30 and 50/50, presenting three different BET values, 300, 200, 50 cm²/g respectively, corresponding to different average diameters. As declared by the producer and confirmed in previous studies on the same systems,^{5,25} these commercial dispersions are characterized by a very high stability, without stabilizing additive, due to an important negative surface charge obtained during the specific production process. The surface tension measurements of these silica dispersions provided in fact the same value of the pure water, i. e. 72.5 mN/m, constant over several hours.

The bare silica dispersions have been also characterized by dynamic light scattering (DLS) and ζ -potential measurements which confirmed their good stability and provided the particle size distribution and the particle surface charge. It was found $d=9.0, 15.0, 60.0 \pm 0.2$ nm, as average particle diameters, in agreement with the nominal values by the BET data i.e. 300, 200 and 50 cm²/g, respectively, and a ζ -potential of -42 ± 4 mV in agreement with what found in ref. 25.

Water for all the experiments was purified by a multi-cartridge Elix plus Milli-Q (Millipore) system, providing a resistivity greater than 18 M Ω ·cm.

3. METHODS

A conventional Langmuir trough of a total area $A_t = 243 \text{ cm}^2$ (KSV Minitrough, Finland) was used, equipped with two Delrin[®] barriers allowing for symmetric compression of the surface layer and with a Whilhelmy plate tensiometer for the surface pressure measurement.

The mixed layers were obtained by spreading at the surface of the aqueous silica dispersions a volume of 30 μL of DPPC solution in chloroform at a concentration of 1.36 mM.

In this way the spread DPPC monolayer, after evaporation of the solvent, presented a density $\Gamma^0 = 10 \text{ }\mu\text{mol}/\text{m}^2$. All the colloidal silica dispersions used as sub-phase were at a concentration of 1 wt% and their volume was approximately 200 cm^3 .

As discussed in ref. 12, after a time of about 1 hour, appropriate for the complete evaporation of the solvent and the transfer of silica NP from the sub-phase to the surface, the layer can be assumed at equilibrium. In all the experiments presented here the compression of the surface layer was performed at a rate of 2 cm^2/min , corresponding to 0.25 $\text{nm}^2\text{molecule}^{-1} \text{ min}^{-1}$ which is sufficiently low to ensure the equilibrium conditions during the layer compression.

The 2D morphology of the monolayers was investigated by Fluorescence microscopy using a Nikon Eclipse-Ti optical microscope (Nikon Corp., Japan) with a 20x objective coupled to a fluorescence module. A small quantity (1 mol %) of fluorescence marked lipid NBD-PC (1-palmitoyl-2-{12-[(7-nitro-2-1,3-benzoxadizole-4-yl)amino]dodecanoyl}phosphatidylcholine), purchased by Avanti Polar Lipids, USA, was added to the DPPC solution used for the spreading. The images analysis was done using our own software developed in the Matlab computing environment²² as well as the freeware software Image.²⁶

For Ellipsometry measurements a multipurpose apparatus (Multiskop Optrel, Germany) ²⁷ was used with a single wavelength of 632.8 nm. This technique was employed to evaluate the thickness of the mixed layer at water/air interface under various compression, always in the Drude approximation. The optical model adopted was that described in details in ref. 17. This specific apparatus provides an average thickness value calculated on a spot of area of few square millimeters.

Langmuir-Schaeffer films of the monolayers were deposited onto silicon wafers and have been analysed by Scanning Electron Microscopy (SEM-FEG Zeiss SUPRA™ 40), a field-emission SEM with improved spatial resolution (5-10 nm) and minimized sample charging/damage.

All the measurements presented in this study were performed at a controlled temperature of 22 °C.

4. RESULTS AND DISCUSSION

DPPC is a zwitterionic natural surfactant that can be spread on an aqueous sub-phase as a Langmuir monolayer with the trimethyl ammonium headgroup protruding into the water. Thus the attachment of silica NPs to the DPPC monolayer is driven by electrostatic interactions. Silica particles which are well dispersible in water due to their negative surface charge, may be captured and incorporated in the layer affecting its phase behavior and forming mixed layers with particular thermodynamic and structural properties.

The compression isotherm and the quasi equilibrium dilational elasticity of the mixed DPPC - Silica NP with intermediate average diameter (Levasil 200/30) were previously obtained in Ref. 12. In Figure 1 the analogue compression isotherms obtained for Silica dispersions at different NP sizes are reported, compared with that of pure DPPC.

As already discussed in ref. 12, the presence of NPs affects the principal characteristics of the well known Surface Pressure-Area (Π -A) isotherm of the pure DPPC^{28,29} which is the typical plateau in the surface pressure, corresponding to the coexistence of Liquid Expanded (LE) and Liquid Condensed (LC) phases, and reduces the collapse surface pressure. These effects, observed also here independently of the size of the Silica particles, can be explained by the NP incorporation in the monolayer. Such incorporation also explains the surface pressure increase at smaller compression degrees, or higher surface areas, as due to the increased effective DPPC surface concentration or excluded area effect.³⁰

For pure DPPC, the coexistence region is characterized by the nucleation and growth of bean-shaped domains that coalesce during compression to form a total condensed phase. As pointed out in ref. 12 by BAM, in presence of particles these domains assume a different, less elongated, shape and their area is almost one order of magnitude smaller compared to those found for DPPC spread at the pure water surface.

In this work the fluorescence microscopy technique is used to obtain a more accurate characterisation of this mixed biphasic layers. As shown below, using this techniques provides new insights on how the 2D structure of the layer evolves during the compression. The fluorescence probe added in small quantity (around 1% of the total molar content of the lipid solution) to the spreading solution of DPPC, preferentially distributes inside the liquid expanded phase.³¹ This provides a highly improved visualisation of the domains due to the increased contrast with the condensed region.

Figure 2 shows some examples of surface textures of DPPC monolayers in presence of silica nanoparticles as obtained by fluorescence microscopy for Silica NP with intermediate average diameter (Levasil 200/30), at different compression degrees corresponding to different values of surface pressures.

For all the silica particle sizes the presence of well defined circular domains is observed for surface pressure higher than 7 mN/m, corresponding to the onset of the LE-LC coexistence phase of the pure DPPC.

The rounder shape of the LC domains and their reduced size in presence of NPs, has also been observed for other negatively charged nanoparticles.⁹ To understand this effect it is important to consider that the main factor driving the transfer into the DPPC monolayer of NPs is their electrostatic interaction with the cationic lipid group. The presence of NPs in the sub-layer tends to reduce the dipolar repulsion in the condensed state which is responsible of the elongated shape of DPPC domains.^{32,33} As a consequence the role of the line tension becomes more important in the minimization of the free energy at the border of the domains, making more circular their shape. On the other side the penetration of NPs in the monolayer induces a hydrophobic mismatch between the lipid molecules which reduces the DPPC molecular packing and, as a matter of fact, hinders the nucleation and the growth of LC domains. The combination of these two effects favours the persistence of a big number of circular shaped domains during the compression.

The average radius and area of the domains, R_D and A_D , as obtained by the image analysis are reported in Figure 3. It is evident that the size of the domains increases for low surface pressure till reaching a plateau value for $\Pi > 25$ mN/m. The comparison of these A_D data with those of pure DPPC obtained by Nag and Keough³¹ and Arriaga et al.,³⁴ reported in Figure 3b, clearly points out the reduction of the average domain size induced by the NPs. These results confirm that the presence of NPs avoids the growth of the domains above a threshold value which corresponds to the size of the initial nucleus of condensed phase observed for pure DPPC. In addition, the transition to a total condensed phase, occurring in pure DPPC monolayers around $\Pi \sim 25$ mN/m, is not observed here.

The dependence on the surface pressure of the fraction of area occupied by the dispersed condensed phase, Φ , reported in Figure 4 shows the achievement of a maximum coverage of about 50 -55 % of the total area of the monolayer for surface pressures larger than 35-40 mN/m. Similar trends, with the achievement of a limit fraction of the dispersed condensed phase, have been reported also for emulsions³⁵ and two dimensional foams.³⁶

The slight increasing trend of the number of domains per unit area, n_D , during the compression, reported in Figure 4b, is also significant because proves that the domains coalescence is avoided by the presence of particles. This means also that for surface pressure lower than 25 mN/m, the area of the expanded phase is reduced during compression, while the average domain area slightly increases. The further increase of Φ before the achievement of the plateau is only due to the reduction of the expanded phase. The rather steep rise of the surface pressure at $A/A_0 < 0,5$ is then due to the resistance of the expanded phase rich of particles and the hindering of the domain growth for effect of the NP presence. This also explains the negligible variation of Φ for surface pressure larger than 40 mN/m being very low, $\Delta A/A_0 \sim 0.05$, the variation of the total surface area corresponding to a rise of surface tension from 40 to 60 mN/m.

Further information on the structural characteristics of these mixed surface layers can be obtained by the analysis of the domain size distributions. In Figures 5 and 6, some examples of such distributions are reported obtained for different values of the surface pressure and sizes of the Silica particles. As a general finding this analysis evidences the mono-modal character of the size distribution in the whole compression range. It is worth noticing that for pure DPPC monolayers, the size distributions obtained by Arriaga et al.³⁴ present, on the contrary, a bimodal character where the smaller size detected is close to those of the domains here observed.

The size distribution of this kind of surface layers, at the equilibrium, is expected to be determined by the balance between the line tension, λ , and the effect of the dipole density difference, Δm , between the two phases.³² According to the theoretical approach proposed by Lee et al.,³⁷ assuming isolated circular domains of radius R_D , a simplified model can be obtained which provides the following expression for their size distribution,

$$N(R_D) = \left\{ N_0 \exp \left[-\beta \left(\frac{R_0}{R_D} - 1 \right)^2 \right] \right\}^{R_D^2 / R_0^2} \quad (1)$$

where

$$\beta = \frac{(\Delta m)^2 R_0}{4\epsilon k_B T} \quad (2)$$

N_0 is the number of domains of radius R_0 , with the lowest energy, β is related to the width of the distribution and ϵ is the dielectric permittivity of water. The values of N_0 , β and R_0 obtained by fitting Eq. (1) to the experimental data provide an estimation of λ and Δm from Eq. (2) and assuming $\lambda = (K_B T \beta / \pi R_0) \ln[4R_0 / e^3 \delta]$, where δ is the molecular cut-off distance with a typical value of 0.5 nm.³⁸

The values so obtained of λ and Δm are reported in Figure 7. They are rather constant versus the surface pressure and in the range of those typically reported for lipid monolayers and bilayers.^{38,39}

The values of λ found with other techniques,^{40,41} or with molecular dynamics simulation,⁴² for pure DPPC domains and mixtures of lipids, are about one order of magnitude higher than those found here in presence of silica particles. This suggests us to consider the DPPC decorated silica NP as linactant species,⁴³ and advance the hypothesis that such particles, mainly distributed in the expanded phase, tend to cover the border of the condensed domains preventing their coalescence.

The preferred distribution of NP in the expanded external phase is confirmed by the analysis of the SEM images of Langmuir-Schaeffer (LS) films transferred at controlled surface pressure. SEM analysis, beside providing images with higher magnification, allows a good visualisation of the phase rich in silica NP, due to the electronic contrast. As shown in Figure 8, apart some inclusions in the interior of the condensed domains, most nanoparticles are rather homogeneously distributed in the surrounding expanded phase. That allows one to conclude that the dispersed condensed phase is constituted by the LC domains and the matrix is the LE phase rich in DPPC decorated particles.

The results obtained by Ellipsometry for these mixed layers at different degree of layer compression are significant to further corroborate this scenario. In Figure 9 the thickness of the mixed layers obtained with the different Silica dispersions is reported as a function of the surface pressure compared with that of the pure DPPC monolayer. Also in this case the size of the silica particles does not appreciably influence the results, apart that for the smallest particles (Levasil 300/30) the thickness maintains slightly lower. We remember here that the thickness, h , obtained by ellipsometry is the result averaged on a few square millimeters portion of the mixed two phase layer. In principle, height inhomogeneities on the nanometric scale should be dealt with within an Effective Medium Approximation, such as the Lorentz-Lorenz one.⁴⁴ However, given the reduced value of the film thickness, which never exceeds the Drude approximation, we can model the rough layer with an “average” homogeneous layer whose thickness h is expressed as a combination of the matrix and domain thicknesses, h_E and h_D , respectively, that is

$$h = \Phi h_D + (1 - \Phi) h_E .$$

The thickness of the domains, h_D , can be assumed to equal that of the condensed phase of pure DPPC, that is the value at high surface pressure reported in Figure 9a. Thus the thickness of the matrix phase, or expanded phase, h_E , can be calculated according to

$$h_E = \frac{h - h_D \Phi}{1 - \Phi} \quad (3)$$

In Figure 9b h_E versus the surface pressure is reported as calculated by Eq. (3) with Φ obtained by the fluorescence images (Figure 6). It is interesting to notice that h_E , obtained for Levasil 300/30 at high layer compression, assumes the value of the particle diameter (9 nm), which is in agreement with the hypothesis of a matrix composed by a compact single particle layer.

For both the other two dispersions the maximum h_E is slightly smaller than the particle size but appreciably larger than the condensed domains.

In general these results allow considering the mixed layer as a network composed by DPPC condensed domains dispersed in a matrix mainly composed by particles that above a certain compression ($\Pi > 30$ mN/m) becomes a compact mono distributed particle layer.

Assuming this scenario, at low surface pressure the LC phase is expected to grow, due to the transfer of free lipid molecules from the expanded phase, or by coalescing with the neighbour LC domains, until they are totally surrounded by DPPC-decorated particles which prevent their further growth. That means that the layer obtained by compression may be considered as the 2D analogous of a Pickering emulsion, where the DPPC-decorated particles in the expanded phase stabilise the condensed DPPC domains against growth and coalescence during the monolayer compression.

According to this interpretation and using the line tension and the other characteristics of the layer here found out, a deeper interpretation of the quasi-equilibrium dilational elasticities of the mixed layers previously obtained,¹² can be advanced.

Figure 10 shows the quasi-equilibrium dilational elasticity of the monolayer, as calculated from the $\Pi - A$ isotherm, found in ref. 13 for Levasil 200/30 silica dispersions. Coherently with the disappearance of the plateau corresponding to the LE-LC phase transition, in presence of NPs the

dilational modulus presents a minimum much less pronounced compared with the typical minimum of pure DPPC, followed by a continuous increase with the compression advancement. In fact, the exchange of material between the two coexisting phases, responsible of the quasi zero elasticities, found in this zone for pure DPPC, is strongly reduced by the presence of particles. The other effect induced by the NP penetration is the strong reduction, of about four times, of the elasticity of the highly compressed phase, indicative of a less rigid layer.

An expression for the dilational elasticity, E , of a biphasic surface, like that here investigated, can be written in terms of the elasticities, E_D and E_E , of the dispersed condensed domains and the expanded phase (matrix), respectively, ⁴⁵

$$\frac{1}{E} = \frac{\Phi}{E_D - \lambda/2R_D} + \frac{1 - \Phi}{E_E} \quad (4)$$

Eq. (4) is obtained by assuming domains of constant radius R_D and writing the variation of the total area A as $\delta A = \delta A_E + 2\pi N_D R_D \delta R_D$. with A_E the area of the expanded phase and N_D the number of domains. The surface tensions σ_D and σ_E of the condensed and expanded phases, respectively, are assumed to be related to the line tension through $\sigma_D = \sigma_E - \lambda/R_D$.

From Eq. (4) an expression of the elasticity of the expanded mixed phase ε_E can be derived,

$$E_E = \frac{E(1 - \Phi)}{1 - E \Phi / \left(E_D - \frac{\lambda}{2R_D} \right)} \quad (5)$$

E_E versus the surface pressure, can be evaluated by Eq. (5), using for E_D the value of the elasticity of the condensed pure DPPC phase (the maximum in Figure 10), for E the values of the mixed system obtained for $\Pi > 7$ mN/m, corresponding to the merging of the condensed domains, and for Φ and R_D those obtained by fluorescence images. The values of E_E reported in Figure 10 are coincident with the measured E for $\Pi < 10$ mN/m that means that the matrix phase behaves like

the mixed DPPC-NP expanded phase (first smaller maximum in the diagram). After that, increasing the surface pressure, when the formation of domains becomes important, E_E increases more slowly with the surface pressure and maintains lower than E for all the compression,

5. CONCLUSIONS

The study reported here shows that the penetration of Silica NPs to lipid monolayers, mediated by electrostatic interactions, may lead to the formation of rather ordered networks at the water/air surfaces. The employment of complementary diagnostic techniques for the analysis of spread monolayers and the comparison with the previously obtained rheological results on similar mixed layers, allowed us to advance in the characterisation of these complex mixed layers.

For the here investigated DPPC-Silica NPs monolayers it has been evidenced during the layer compression the transition from a homogeneous expanded phase to a 2D emulsion-like structure.

In fact, these results show that colloidal silica particles, with particle sizes ranging from 9 to 60 nm, incorporated in the spread DPPC monolayer, prevent the reaching of a totally condensed phase. From the clear visualisation provided by the Fluorescence analysis, it is evident the network-like texture of the monolayer with the presence of small and circular LC domains surrounded by a LE also at high compression degree. The expanded phase, as it was possible to establish by other diagnostics analysis, mainly SEM and Ellipsometry, is rich in Silica nanoparticles which have an important role in stabilizing the network, avoiding domain growth and coalescence.

The results presented here, even if obtained for specific model materials (silica NP and DPPC) provide new insights into the supra-molecular organisation of mixed nanoparticle-lipid layers and suggest the extension of this study towards other different nanomaterials

This kind of 2D ordered structures whose the formation is simply driven by the interactions between lipids and inorganic solid particles, could be potentially employed in various fields of applications such as pharmaceuticals and biomedical utilisation. The observed structures may be, for example, utilised as templates or precursors for surface nano-patterning or for the production of 2D membranes with controlled geometrical characteristics. Other examples can be found in the field of drug delivery, in fact, hybrid silica-lipids nanomaterials have been proposed to be used as carriers for the controlled release of drugs.^{46,47} The study reported here may be relevant to understand and tune the structure of similar nanocapsules.

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Figure Captions:

Figure 1: $\Pi - A$ isotherm of mixed DPPC - Silica nanoparticle monolayers, obtained by spreading DPPC on dispersions of Silica particles at different sizes d , and Π -A isotherm of pure DPPC as found in ref. 12.

Figure 2. Fluorescence microscopy images ($278 \times 208 \mu\text{m}^2$) of mixed silica nanoparticle DPPC monolayers at different compression degrees or surface pressure Π .

Figure 3: Geometrical characteristics against the surface pressure of the biphasic layer obtained by the analysis of the fluorescence images, in presence of Silica NPs, at different particle sizes d : (a) average radius of the condensed domains; (b) average area of the condensed domains (\bullet) compared to those of DPPC spread on pure water, reported by Arriaga et al. (\circ)³⁴ and Nag et al. (Δ).³¹ Results obtained from the analysis at least of five independent images. The lines are guides for the eye.

Figure 4: Area fraction occupied by the condensed domains in the mixed Silica NP – DPPC layers (a), and number of condensed domains per unit area (b), obtained by the analysis of the fluorescence images, as a function of the surface pressure and for different particle sizes d . Results obtained from the analysis at least of five independent images. The lines are guides for the eye.

Figure 5: Domain size distribution obtained by the analysis of the fluorescence images of mixed layers containing silica particle with $d=15 \text{ nm}$, at different compression degree or surface pressure Π . The best fit distributions (solid lines) are obtained by using Eq. (1).

Figure 6: Domain size distributions (above) and corresponding fluorescence images ($278 \times 208 \mu\text{m}^2$) of mixed layers (below), with silica particles of different sizes d , and at the same compression degree corresponding to $\Pi=15 \text{ mN/m}$. The best fit distributions (solid lines) are obtained by using Eq. (1).

Figure 7: Line tension (a) and dipole density difference (b) against the surface pressure obtained from the best fit parameters from the analysis of the domain size distribution (Eqs. 1 and 2) for different particle sizes d .

Figure 8: SEM image ($180 \times 150 \mu\text{m}^2$) of a Langmuir-Schaeffer film obtained by transferring a mixed DPPC – Silica nanoparticle layer with $d=15 \text{ nm}$, at controlled $\Pi = 20 \text{ mN/m}$.

Figure 9: Thickness of the spread layers obtained by Ellipsometric measurements for mixed DPPC Silica particles layers, at different particle sizes d , (a), and the corresponding thickness of the matrix layers surrounding the condensed domains, calculated by Eq. 3 (b). Lines are guides for the eye.

Figure 10: Quasi equilibrium dilational elasticity of pure DPPC and mixed DPPC-Silica NP layers ($d=15 \text{ nm}$) as found in ref. 13 compared with the elasticity of the matrix layer surrounding the condensed domains, calculated by Eq. 5. Lines are guides for the eye.

