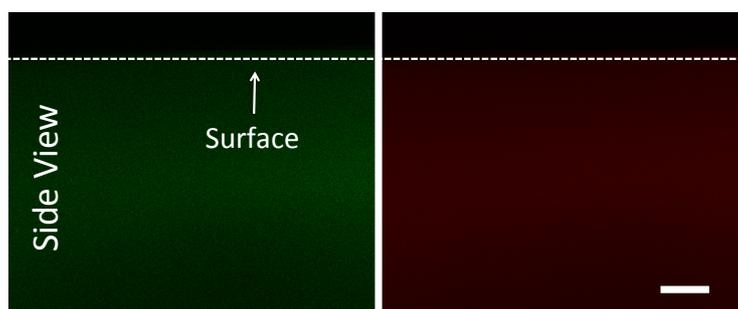


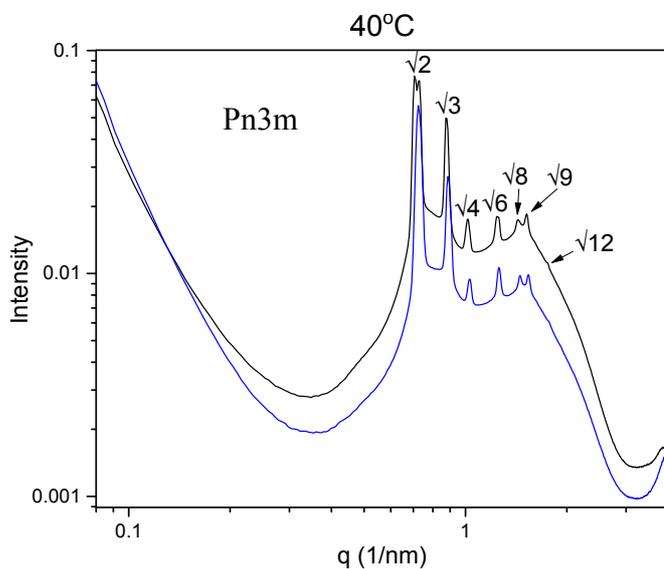
Electronic Supplementary Information (ESI) for

**Temperature responsive lipid liquid crystal layers with embedded nanogels**

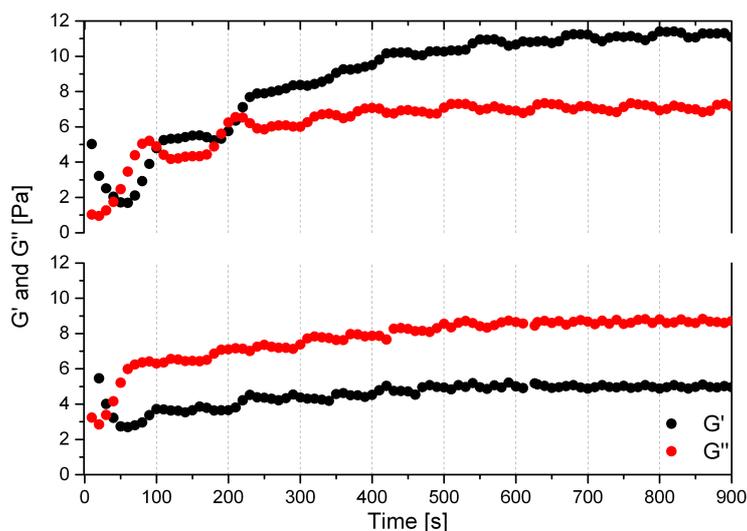
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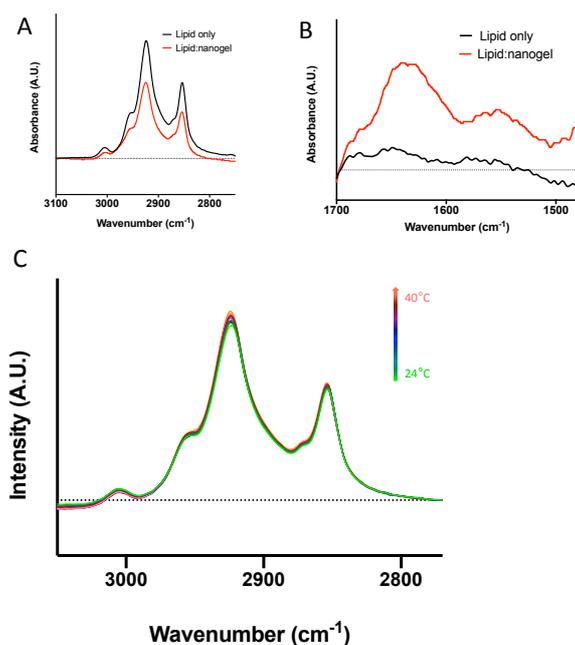
**Figure S1.** Confocal fluorescence xz images of a lipid only (GMO-50:DGMO, 40:60 wt ratio) layer at 25 °C showing the side view of the layer with the red (lipid probe) and green (aqueous probe) channels overlaid. Scale bar 20  $\mu\text{m}$ .



**Figure S2.** Comparison of SAXS profiles of a pure lipid phase (black line) and a nanogel:lipid phase (blue line) in excess water at 40 °C. The arrows show the indexing of the corresponding peaks.



**Figure S3.** Time experiments performed at 0.05 rad/s and strain 1% for the lipid system with (bottom) and without (top) particles at 25 °C. The experiments were performed in excess water, please refer to the text for experimental details.  $G'$  and  $G''$  absolute values should not be taken in account.



**Figure S4.** Comparison of ATR-FTIR spectra of hydrated surface layers of pure lipid (black lines, GMO-50:DGMO, 40:60 wt ratio) and with nanogels (red lines, 10wt% nanogel in GMO-50:DGMO, 40:60 wt ratio). The C–H stretching (A) and C=O and stretching (B) regions are shown. (C) ATR-FTIR spectra showing the C–H stretching region of the spectra arising from a pure lipid film (GMO-50:DGMO, 40:60 wt ratio) in excess water measured over a temperature range of 24 °C to 40 °C. No change is observed in the asymmetric  $\text{CH}_2$  stretch peak with increasing temperature for the lipid-only sample.

## **Experimental Section**

*Materials.* Diglycerol monooleate (DGMO) was obtained from Danisco A/S (Brabrand, Denmark). It is composed by 88 % of diglycerol monoester and 4.9% free glycerol and polyglycerols, in which the main fatty acid component is oleic acid (90.7%), followed by linoleic (4.2 %), saturated (2.9 %), eicosenoic (1.2 %) and linolenic (0.8 %) acids. Capmul GMO-50 (GMO-50, Lot No. 140721-6) was purchased from Abitec (Janesville, WI). GMO-50 is a blend of 64.52% monoacylglycerol, 31.70% diacylglycerol and 3.78% triacylglycerol. The fatty acid constituents are 86.1 % oleic, 5.7 % linoleic, 4.0 % palmitic and 3.2 % stearic acid. Ethanol (99.7%) was purchased from Solveco AB (Rosersberg, Sweden). Water was purified to a resistivity of 18.2 M $\Omega$ -cm at 25 °C using the MilliQ system (Millipore).

*Preparation of polymer nanogels.* Poly(N-isopropylacrylamide), pNIPAM, nanogels were synthesized via precipitation polymerization in the presence of a surfactant. 0.193 g sodium dodecyl sulfate was dissolved in 96 g of water in a three-necked round-bottomed flask. 1.48 g re-crystalized N-isopropylacrylamide was added to the reaction mixture together with 0.065 g of the cross-linker N,N'-methylenebis(acrylamide). Thereafter the reaction mixture was bubbled with argon for 30 min before the temperature was increased to 70 °C. 0.055 g of potassium persulfate dissolved in 2 g of water was added to the reaction mixture to initiate the polymerization. The reaction was left to proceed for 6 h under a constant argon atmosphere and at a constant stirring rate of 250 rpm. The solution was left to cool down over night under constant stirring. The next day the solution was filtered and put in a dialysis bag. The dialysis water was replaced once a day for two weeks until the conductivity was found to be below 1  $\mu$ S/cm.

*Preparation of lipid formulations.* A stock solution of 85 wt % lipids (composed by GMO-50/DGMO at 40/60 wt ratio) and 15 wt % ethanol was prepared, sealed and left mixing for 24h in a roller mixer. Afterwards, pNIPAM nanogel particles were added to the stock solution at a concentration of 10 wt%. Finally, samples were mixed for 24h in a roller mixer, followed by centrifugation up-and-down (when needed) until they looked homogeneous by visual inspection and under polarized light. These formulations were further mixed with excess water for the SAXS measurements. Hence, small amounts of the lipid-nanogel mixtures were added to milliQ water (<95 wt%) followed by roller mixing for 7 days at room temperature prior to measurements.

*Deposition of lipid layers.* Lipid and lipid-nanogel formulations were diluted with ethanol to a lipid concentration of 20wt% prior to deposition. The dilute formulations were dropped onto the silica surfaces (40-50  $\mu$ L for  $\sim 1$  cm<sup>2</sup> cover glass samples) to cover almost the whole surface. As the solubility and temperature transition of pNIPAM polymers is known to be strongly affected by even small amounts of ethanol,<sup>19-21</sup> care was taken to allow the ethanol to fully evaporate in a dust-free chamber. The drop-casting procedure resulted in a visually glossy and smooth lipid layer on the surface, which was immersed in excess water and sealed into the sample holder, unless otherwise specified.

*Small Angle X-ray Scattering (SAXS).* Bulk lipid mixtures with and without PNIPAM nanogel were measured at the I911-4 SAXS beamline at the MAX IV Laboratory (Lund University, Sweden). Measurements were performed at seven different temperatures (from 25 °C to 40 °C, with intervals of 2.5 °C) with exposure times of 60 s. Samples were confined between kapton windows in a steel sample holder. The

experiments were performed at a wavelength of 0.91 Å and the scattered intensity was recorded using a Pilatus 1M 2D detector (DECTRIS Ltd., Baden-Daettwil, Switzerland). The sample-to-detector distance (1919 mm) and detector positions were calibrated with Silver behenate. Fit2D software was employed to analyze the two dimensional SAXS profiles.

*Confocal Fluorescence Microscopy and Fluorescence Correlation Spectroscopy (FCS).* Imaging and FCS measurements of the lipid-based surface structures were performed using a Leica TCS SP2 confocal microscope, operated in the inverted mode, using a 63x1.2 numerical aperture water immersion objective. The samples were contained in a single well sample holder with a round cover glass (diameter of 15 mm) and hydrated with 2 mL of water. Images were taken with a resolution of 512 x 512 pixels using a 400 Hz bidirectional scan with each scanning line averaged 4 times. Lipid formulations were labeled with 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) for the lipid phase (0.01 mol%) and Rhodamine 110 for the water phase (100 nM). A 488 nm Ar ion (at 50% tube power ) and a 561 nm DPSS laser were used to excite the green fluorescence of Rhodamine 110 and the red fluorescence of Rhodamine B-labelled lipid, respectively. The fluorescence emission was acquired with two PMT in the 498 nm - 530 nm and in the 571 nm - 630 nm ranges for Rhodamine B and Rhodamine 110, respectively. For FCS the fluorescence emission was acquired with a ISS module (ISS, Inc. 1602 Newton Drive Champaign, IL, USA) equipped with two APD with 500– 530 nm (where the fluorescence emission of Rhodamine 110 was acquired) and 607–683 nm (where the fluorescence emission of Rhodamine B-labelled lipid was acquired) band pass, respectively.

*Rheology.* The rheological measurements were carried out on an Anton Paar Physica MCR 301 instrument equipped with a cone–plate geometry (50 mm, 1°). The temperature was controlled by a Peltier system. The layer formation with and without particles was estimated by using a rheometer. The experiment has been performed in a non-conventional way and absolute  $G'$  and  $G''$  values cannot be taken in consideration, however the  $G'$  and  $G''$  behaviours and the respective reciprocal values of each other allows several considerations. The sample was loaded on the bottom plate of the rheometer and after 15 minutes an excess of water was added allowing the formation of the gel; at the same time the cone plate was moved into the measure position and the time experiment was started. The value of frequency and strain in order to perform an experiment in the linear viscoelastic regime were previously estimated after the film formation. Here the time formation of the film was observed with and without particles. First, in layers containing nanogels the viscous modulus dominates the elastic ones leading to the conclusion that it cannot be addressed as a film but most likely as an heterogeneous viscous sample, while without particles after 300 s from the starting time of the experiment  $G'$  dominates indicating the film formation. The initial oscillation in  $G'$  and  $G''$  for the system without particles could be due to a spatial anisotropic formation of the gel along the gap.

*Attenuated Total Reflectance FTIR (ATR-FTIR).* ATR-FTIR spectra were recorded using a ThermoNicolet Nexus instrument (Madison, WI, USA) that was fitted with a mercury cadmium telluride detector and an air dryer to purge the instrument of water vapour and carbon dioxide. The layers were prepared on Piranha-cleaned silicon crystals and fitted into an ATR flow cell accessory (Specac, Kent, UK) with the temperature controlled via top and bottom plate with a (Julabo CF40). The FTIR

spectra of the lipid layers over a range of temperatures were recorded at a resolution of  $4\text{ cm}^{-1}$  where a total of 128 individual scans were recorded and added into a single spectrum using OMNIC software (Thermo Nicolet Nexus, Madison, WI, USA). The spectra were not smoothed. Bands arising from the background were eliminated from the sample spectra by subtracting a spectrum of the sample cell, including the silicon crystal, in  $\text{D}_2\text{O}$  prior to lipid layer formation.

**Abbreviations.** LC, liquid crystalline; GMO, Glycerol Monooleate; DGMO, diglycerol monooleate; Pnm3, cubic phase crystal space group; DLS, dynamic light scattering; ATR-FTIR, attenuated total reflectance Fourier transform infrared spectroscopy; SAXS, small angle X-ray scattering.

### **Authors' contributions**

APD coordinated the study, participated in the design of the study, carried out parts of the lab work, participated in data analysis, and drafted the manuscript; CH and LC carried the ATR-FTIR measurements and data analysis; MV and MW carried out parts of the lab work including the sample preparation and the SAXS measurements; CM carried out the microscopy and FCS measurements and data analysis; LG carried out the rheology measurements and data analysis; SL performed the SAXS measurements; SN synthesized and characterized the nanogel particles; DB, GP and JB participated in the design of the study and helped draft the manuscript; TN conceived of the study and helped draft the manuscript. All authors gave final approval for publication.

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