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On the thermotropic and magnetotropic phase behavior of lipid liquid crystals containing magnetic nanoparticles

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Materials and Methods

S.1- Materials

Fe(III)acetylacetonate, 1,2-hexadecanediol, oleylamine, oleic acid, diphenylether, ethanol and mixture of hexane employed for the synthesis of SPIONs, were purchased from Sigma Aldrich (St. Louis MO), the same for Glyceryl Monooleate (GMO) and Pluronic F-127.

S.2- Synthesis of magnetic nanoparticles

Iron oxide nanoparticles were synthesized according to the protocol reported by Wang et al.[1]. Briefly, 0.71 g $Fe(acac)_3$ (2 mmol) were dissolved in 20 mL of phenyl ether with 2 mL of oleic acid (6 mmol) and 2 mL of oleylamine (4 mmol) under nitrogen atmosphere and vigorous stirring. 1,2-hexadecanediol (2.58g, 10 mmol) was then added. The solution was heated at 210 °C, refluxed for 2 h and then cooled down to RT. Ethanol was added to the dispersion and the precipitate collected, washed with ethanol to remove residual precursors and side products from the synthesis and redispersed in 20 mL hexane in the presence of 75 mM of both oleic acid and oleylamine. A stable dispersion of the SPIONs with a hydrophobic coating of oleic acid and oleylamine in hexane was obtained.

S.3- Preparation of bulk and disperse cubic phase

The preparation of bulk cubic phase with or without SPIONs was carried out according to the following procedure. First, 30 mg of GMO were weighted in a 2mL glass vial. For GMO/SPIONs systems, the appropriate volume of SPIONs dispersion was then added. About 1 mL hexane was used to solubilize the mixture and then the solvent was removed with a gentle

nitrogen flux. GMO or GMO/SPIONs systems were left under vacuum overnight sheltered from light. The dry film was then hydrated with 30 μL Milli-Q water and the sample was then centrifuged at least 5 times altering a cycle with cap facing upward with another with cap facing downward.

For the preparation of cubosomes, first GMO or GMO-SPIONs film was obtained, as previously described. 8 mg of Pluronic F-127 were then added to the dry film and the mixture was heated in a water bath at 70 °C for 5' to melt the Pluronic F-127 and then vortexed for 5'. Five cycles of heating-vortexing were carried out and then 500 μL of preheated H_2O at 70 °C were added. The dispersion was then sonicated in a bath-sonicator at 59 kHz and 100% power for 6 h, to homogenize the system.

S.4- Small-Angle X-ray Scattering (SAXS) Hecus

SAXS measurements were carried out on a S3-MICRO SAXS/WAXS instrument (HECUS GmbH, Graz, Austria) which consists of a GeniX microfocus X-ray Sealed Cu Ka source (Xenocs, Grenoble, France) power 50 W which provides a detector focused X-ray beam with k = 0.1542 nm Cu Ka line. The instrument is equipped with two one-dimensional (1D) position sensitive detectors, (HECUS 1D-PSD-50 M system) each detector is 50 mm long (spatial resolution 54 lm/channel, 1024 channels) and cover the SAXS q-range ($0.003 < q < 0.6$ Å^{-1}) and the WAXS q-range ($1.2 < q < 1.9$ Å^{-1}). The temperature was controlled by means of a Peltier TCCS-3 Hecus. SAXS curves of bulk cubic phase were recorded at 25-30-35-40-45-50 °C in a solid sample-holder. Dispersion of SPIONs were recorded in a glass capillary.

S.5- Small-Angle X-ray Scattering (SAXS) Elettra Synchrotron

Analysis of cubosomes and magnetocubosomes were carried out at SAXS beamline of synchrotron radiation Elettra, Trieste, Italy operated at 2 GeV and 300 mA ring current. The experiments were carried with $\lambda = 1.5a$ Å and SAXS signal was detected with Pilatus 3 1M detector in q-range from 0.008 $Å^{-1}$ to 0.45 $Å^{-1}$. Thermic behavior of colloidal dispersion of cubosomes and magnetocubosomes were carried out through thermostat from 25 °C to 49 °C increasing the temperature of 2 °C each step. Equilibration time at each temperature was 5 minutes. SAXS curves were recorded in a glass capillary for cubosomes and magnetocubosomes dispersions and in a solid sample-holder for the cubic phases.

S.6- SAXS analysis

Equation (1) was used to calculate lattice parameter of cubic and hexagonal phase:

$$q = \left(\frac{2\pi}{d}\right)\sqrt{h^2 + k^2 + l^2} \qquad (S1)$$

Eq. (2) was used to calculate water channel radii r_w in cubic phase while eq (3) was used to calculate volume water fraction:

$$r_w = \sqrt{(-\sigma/2\pi\chi)}d - l_c \qquad (S2)$$

$$\varphi_w = 1 - 2A_0\left(\frac{l_c}{d}\right) - \frac{4}{3}\pi\chi\left(\frac{l_c}{d}\right)^3 \qquad (S3)$$

Equation (4)[2] and (5)[3] describe water channel radii of hexagonal phase and water volume fraction respectively:

$$r_w = \frac{0.5256d - l_c}{0.994} \qquad (S4)$$

$$\varphi_w = \frac{2\pi r_w^2}{\sqrt{3}d^2} \qquad (S5)$$

We assumed that l_c into the range of 25-50 °C, is constant and assume value of about 17 Å. Moreover, to evaluate water fraction both for cubic and hexagonal phase we assumed that %w/w of Fe_3O_4 negligible.

S.7- LF-AMF specifications

A sinusoidal magnetic field was generated in the gap of a broken toroidal magnet carrying a solenoid through which an alternating electric current (AC) from a tone generator was led as described elsewhere[4]. The samples to be treated with LF-AMF were placed in the middle of the gap. Due to the design of the experimental apparatus, the magnetic field inside the cell is not isotropic. During the experiments, the field frequency was set at 6.22 kHz. Magnetic field values of magnet range from 100-330 mT with 10 V and 8 A.

Supplementary Figures

S.8- SAXS characterization of magnetic nanoparticles

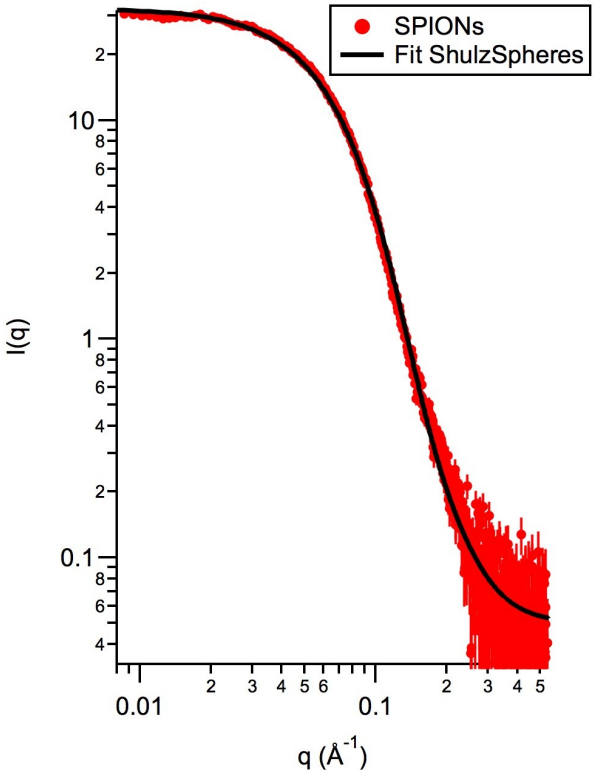

Figure S1: SAXS curve of magnetic nanoparticles disperse in hexane diluted 1:3 (red) fitted by polidisperse Shultz Sphere model (black line).

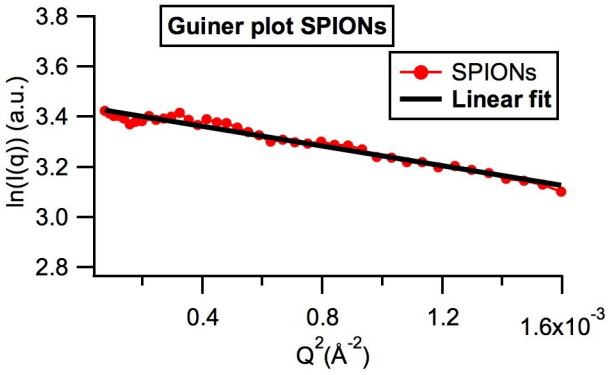

Figure S2: SAXS curves of magnetic nanoparticles with linear fit in Guiner region.

To investigate the dimension of magnetic nanoparticles synthesized as reported in S.2 section, we diluted 1:3 in hexane magnetic nanoparticles. We employed "SphereSchultz Model" by NIST to estimate the radius and polidispersity of SPIONs, which were found equal to 20±3 Å and 0.35 respectively. Moreover, the SAXS curve was analyzed with the Guiner limit law finding a gyration radius of 24 Å. In the hypothesis of spherical and monodisperse nanoparticles, the average radius of the SPIONs derived from Guinier plot is R= 31 Å. This result, corrected for the polidispersity of the colloidal dispersion[5], is about 18 Å, and this is fully consistent with the radius obtained from the "SphereSchultz Model" analysis.

S.9- Lattice parameters of GMO/H_2O systems and SAXS curves of GMO/H_2O/oleic acid-oleylamine

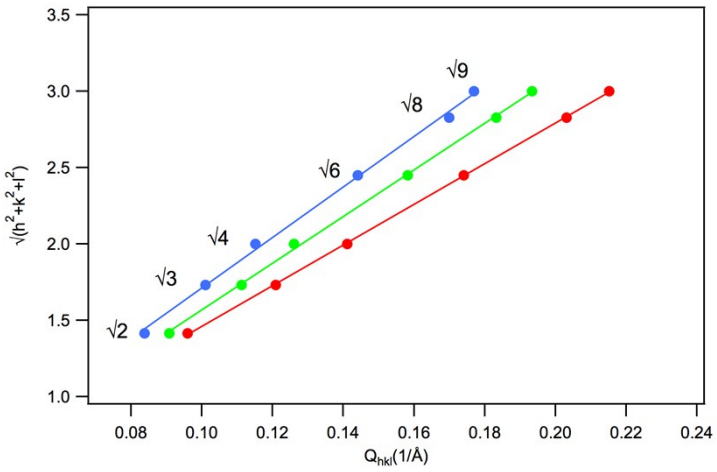

Figure S3: SAXS analysis of GMO/H_2O mesophases at 25 °C (blue line and markers), 35 °C (green line and markers) and 50 °C (red line and markers): square root of the sum of the h,k,l Miller indexes of each peak vs q-peak position (blue, green, red circles); linear fit of the experimental data to calculate lattice parameters the different mesophases (blue, green, red line).

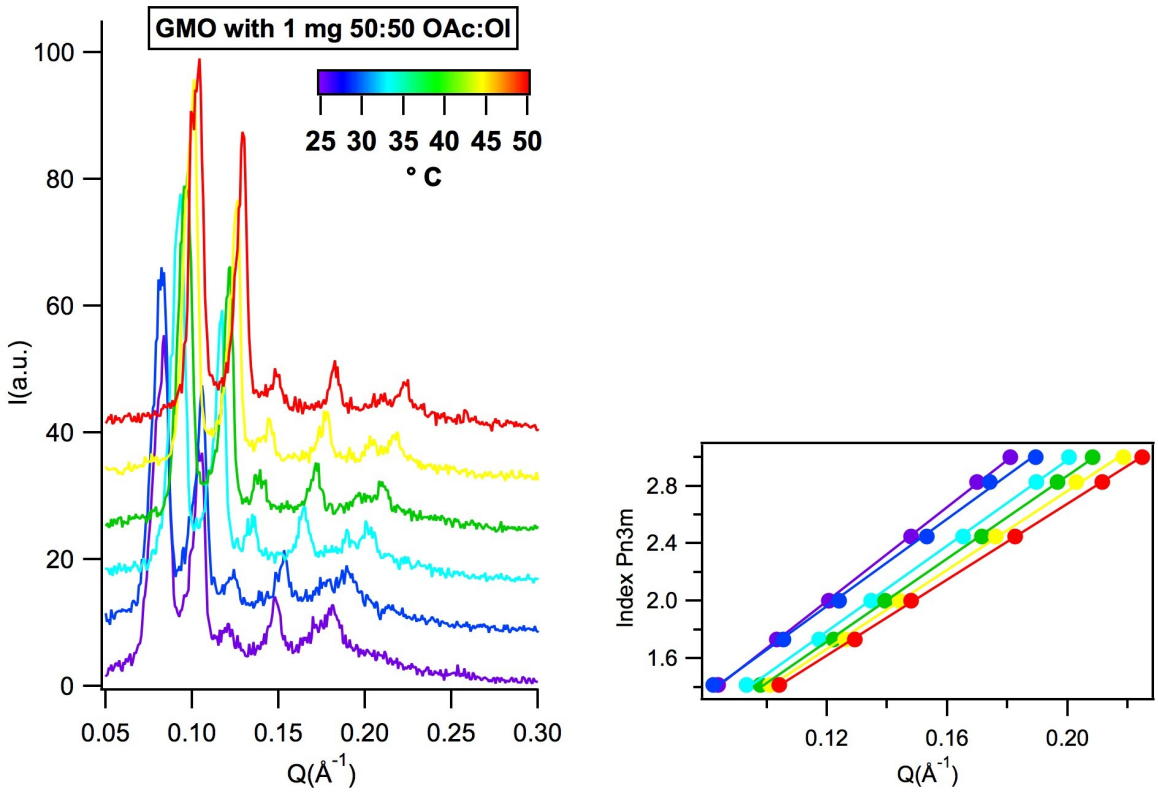

Figure S4: SAXS curves of GMO assembled with 1 mg of a mixture Oleic Acid/Oleylamine at 25-30-35-40-45-50 °C (respectively violet, blue, cyan, green, yellow and red) and Miller index on Q_{max} to determine the trend of Pn3m lattice parameter with temperature. No phase transition was observed in this system.

Table S1. Lattice parameters, water channel radii and water volume fraction of GMO assembled with 1 mg of a mixture 50:50 of Oleic acid and oleylamine

T (°C)	Lattice parameter (Å)	Water channel radii (Å)	Water volume fraction
25	103	23	0.40
30	95	20	0.36
35	94	20	0.36
40	91	19	0.34
45	86	17	0.31
50	83	15	0.29

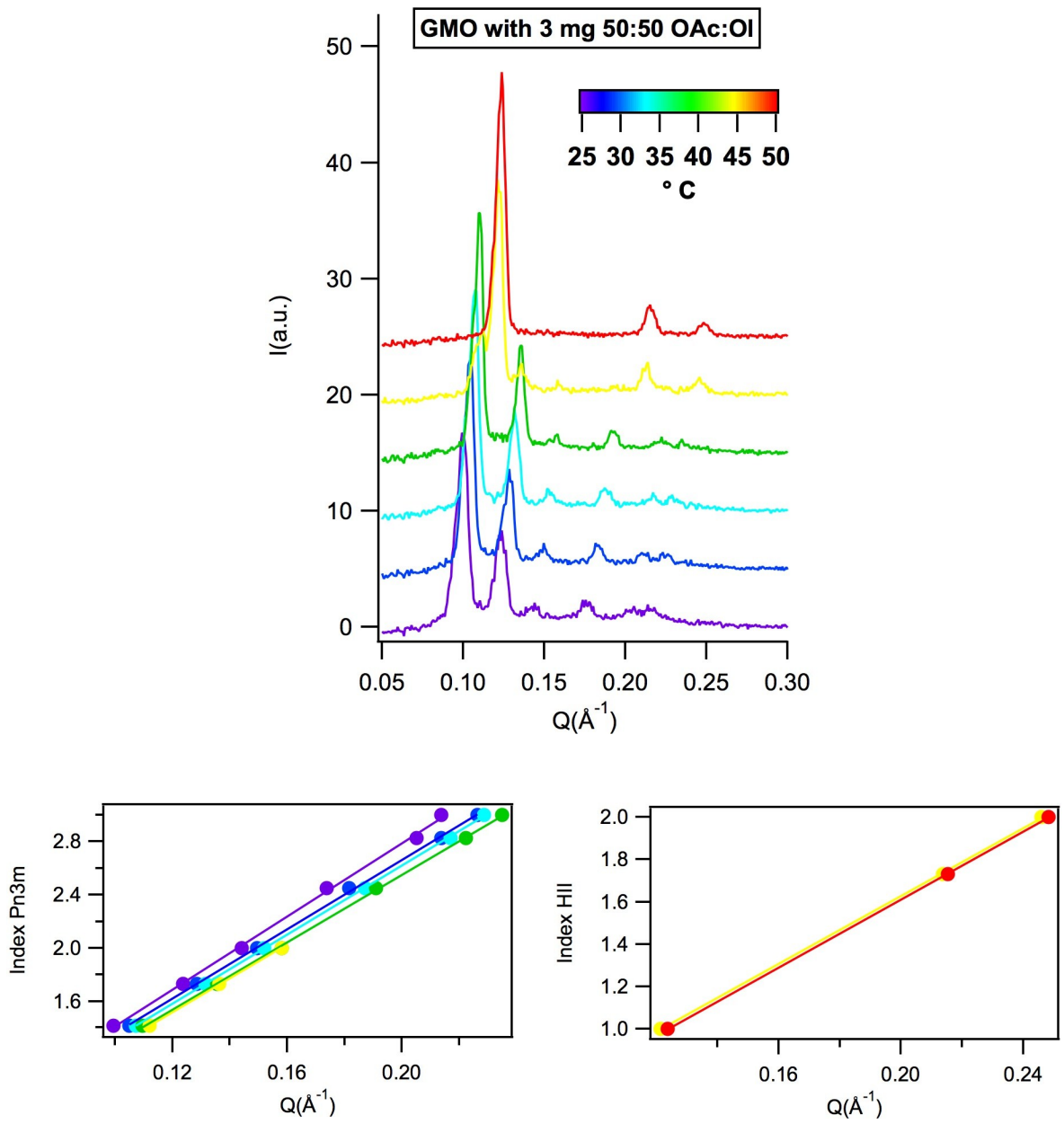

Figure S5: SAXS curves of GMO assembled with 3 mg of a mixture Oleic Acid/Oleylamine and Miller index on Q_{max} to determine the trend of the lattice parameter both for cubic (violet, blue, cyan, green and yellow) and hexagonal phase (yellow and red line) with temperature in the 25-50 °C temperature range. At 45°C the coexistence of cubic and hexagonal phases was detected. Only at 50 °C a pure inverse hexagonal phase was detected.

Table S2. Lattice parameters, water channel radii and waer volume fraction of GMO assembled with 3 mg of a mixture 50:50 of Oleic acid and oleylamine.

T (°C)	Lattice parameter (Å)	Water channel radii (Å)	Water volume fraction
25	86	17	0.31
30	82	15	0.28
35	82	15	0.28
40	80	14	0.26
45	80 (Pn3m)	14	0.26
	50 (H_{II})	9	0.13
50	50	9	0.13

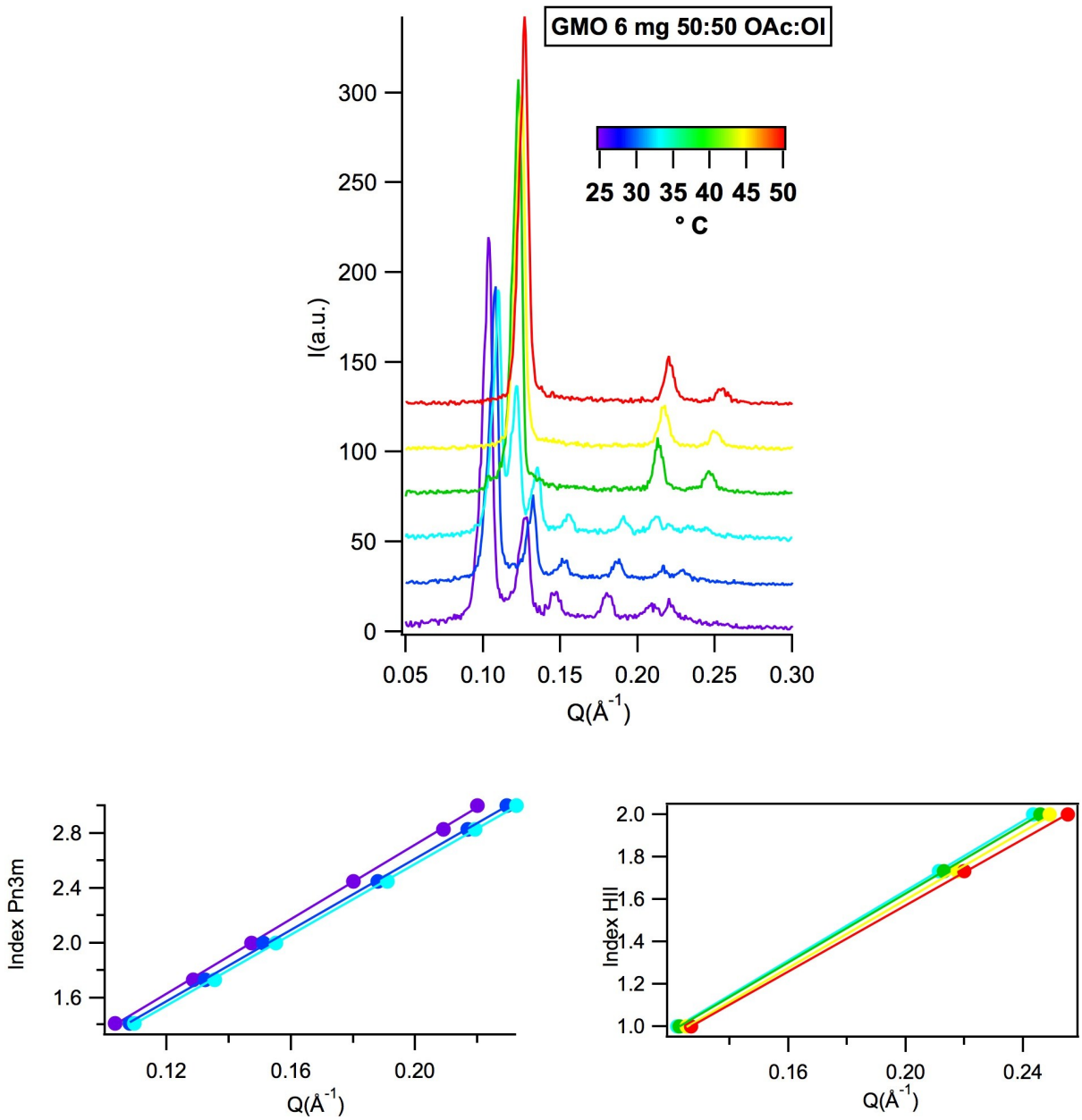

Figure S6: SAXS curves of GMO assembled with 6 mg of a mixture Oleic Acid/Oleylamine and Miller index on Q_{max} to determine the trend of the lattice parameter both for cubic (violet, blue and cyan) and hexagonal phase (cyan, green and yellow and red line) with temperature in the 25-50 °C temperature range. At 35°C the coexistence of cubic and hexagonal phases was detected. From 40 °C a pure inverse hexagonal phase was detected.

Table S3. Lattice parameters, water channel radii and waer volume fraction of GMO assembled with 6 mg of a mixture 50:50 of oleic acid and oleylamine.

T (°C)	Lattice parameter (Å)	Water channel radii (Å)	Water volume fraction
25	85	16	0.3
30	82	15	0.28
35	81 (Pn3m)	15	0.27
	52(H_{II})	10.4	0.14
40	51	9.8	0.14
45	51	9.8	0.14
50	49	8.8	0.12

S.10- SAXS curves of GMO/H_2O/SPIONs

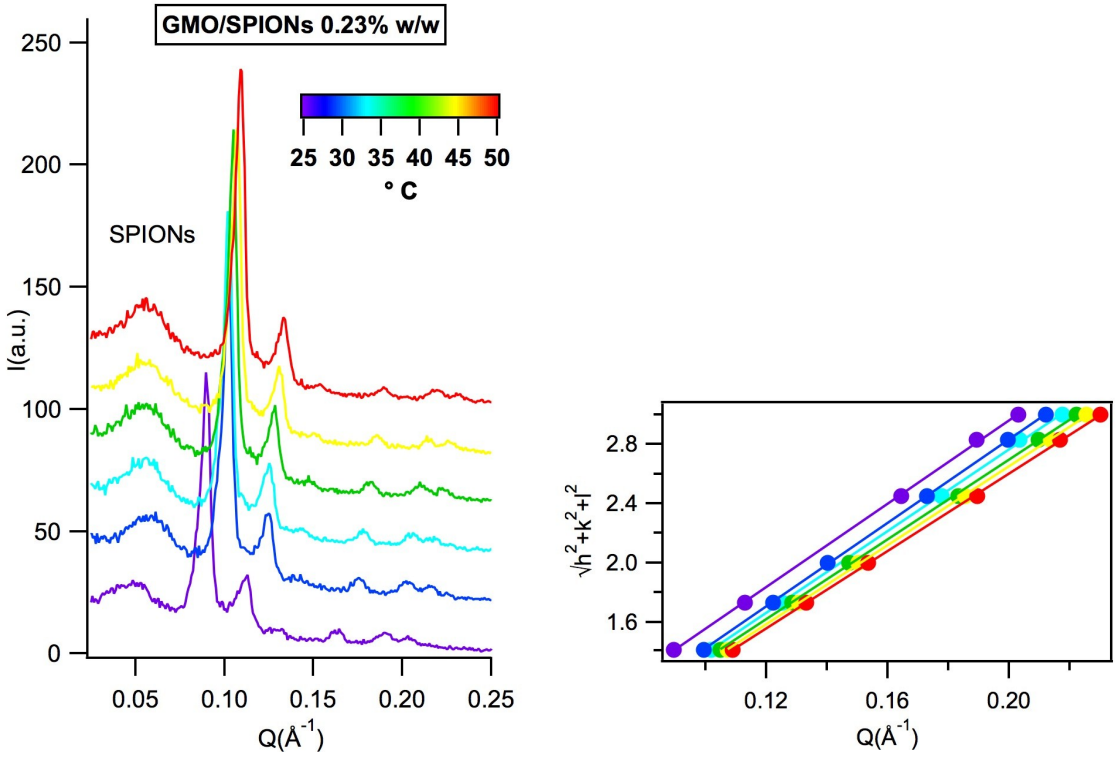

Figure S7: SAXS curves of GMO assembled with 0.23% w/w Fe_3O_4 (mixture of OAc/Ol is 1.33 mg) and Miller index on Q_{max} to determine the variation of the lattice parameter for cubic phase with temperature in the 25-50 °C temperature range. No phase transition with this amount of SPIONs was detected.

Table S4. Lattice parameters, water channel radii and waer volume fraction of GMO assembled with 0.23% SPIONs at increasing temperatures.

T (°C)	Lattice parameter (Å)	Water channel radii (Å)	Water volume fraction
25	88	17	0.32
30	88	17	0.32
35	86	16.6	0.31
40	84	15.8	0.29
45	84	15.8	0.29
50	82	15	0.28

Table S5. Lattice parameters, water channel radii and waer volume fraction of GMO assembled with 0.47% SPIONs at increasing temperatures.

T (°C)	Lattice parameter (Å)	Water channel radii (Å)	Water volume fraction
25	87 (Pn3m)	17	0.31
30	86 (Pn3m)	16.6	0.31
	55 (H_{II})	12	0.17
35	85 (Pn3m)	16	0.3
	54 (H_{II})	11	0.16
40	53 (H_{II})	11	0.15
45	52 (H_{II})	10	0.14
50	51 (H_{II})	9.8	0.14

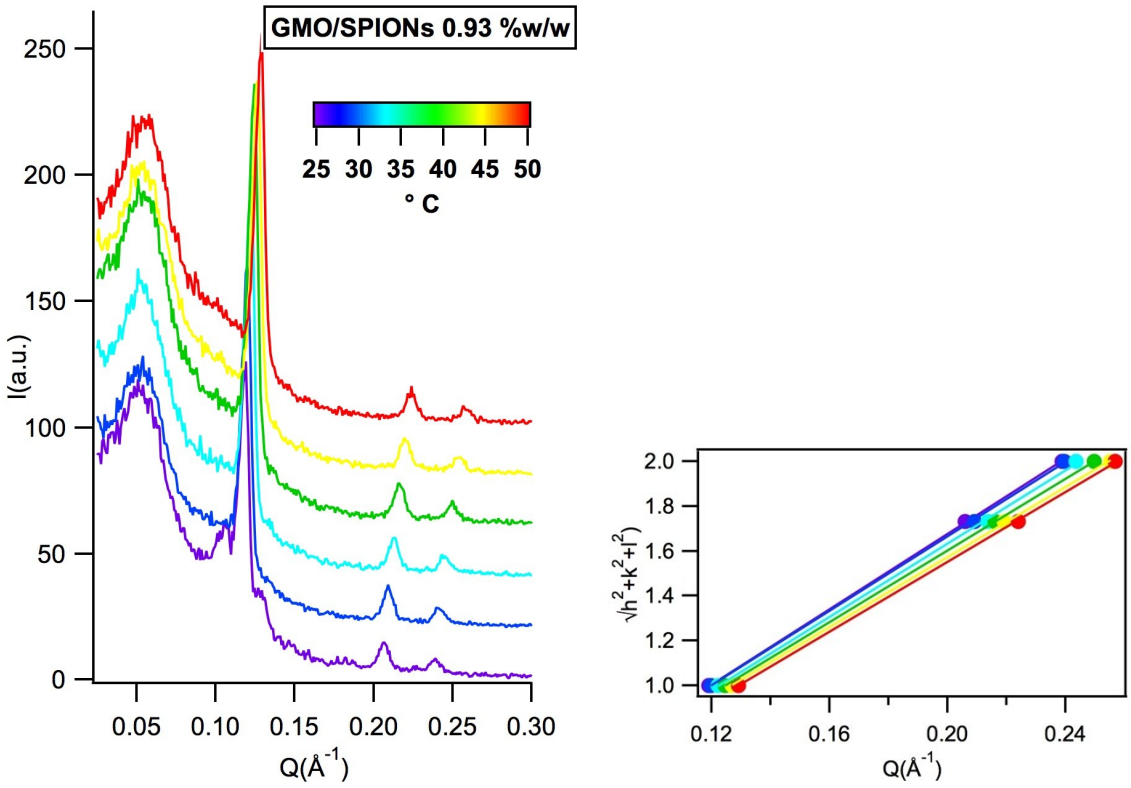

Figure S8: SAXS curves of GMO assembled with 0.93% w/w Fe_3O_4 (5.33 mg of a mixture OAc/Ol) and Miller index on Q_{max} to determine the trend of the lattice parameter for the inverse hexagonal phase with temperature in the 25-50 °C temperature range.

Table S6. Lattice parameters, water channel radii and water volume fraction of GMO assembled with 0.93% SPIONs at increasing temperatures.

T (°C)	Lattice parameter (Å)	Water channel radii (Å)	Water volume fraction
25	53	11	0.15
30	52	10	0.14
35	51	9.8	0.13
40	50	9.3	0.13
45	49	8.8	0.12
50	49	8.8	0.12

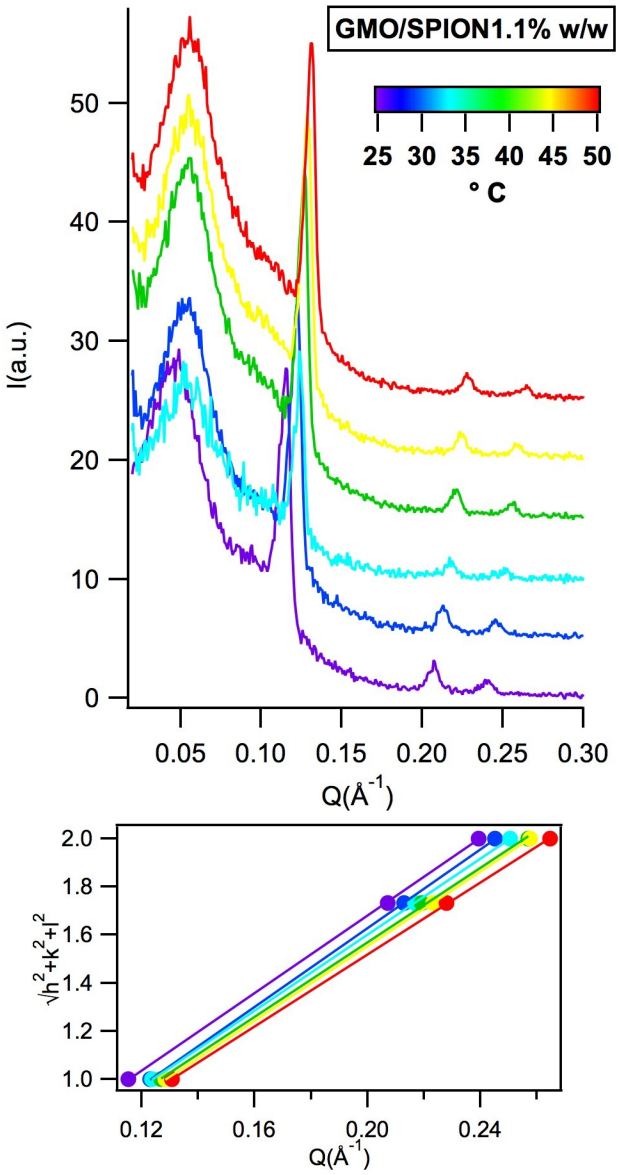

Figure S9: SAXS curves of GMO assembled with 1.1% w/w Fe_3O_4 (6.13 mg of a mixture OAc/Ol) and Miller index on Q_{max} to determine the trend of the lattice parameter for the inverse hexagonal phase with temperature in the 25-50 °C temperature rang.

Table S7. Lattice parameters, water channel radii and water volume fraction of GMO assembled with 1.1% SPIONs at increasing temperatures.

T (°C)	Lattice parameter (Å)	Water channel radii (Å)	Water volume fraction
25	50	9.3	0.13

30	51	9.8	0.14
35	49	8.8	0.12
40	49	8.8	0.12
45	49	8.8	0.12
50	47	7.7	0.1

S.11- Temperature dependence of GMO/H_2O/SPIONs lattice parameters

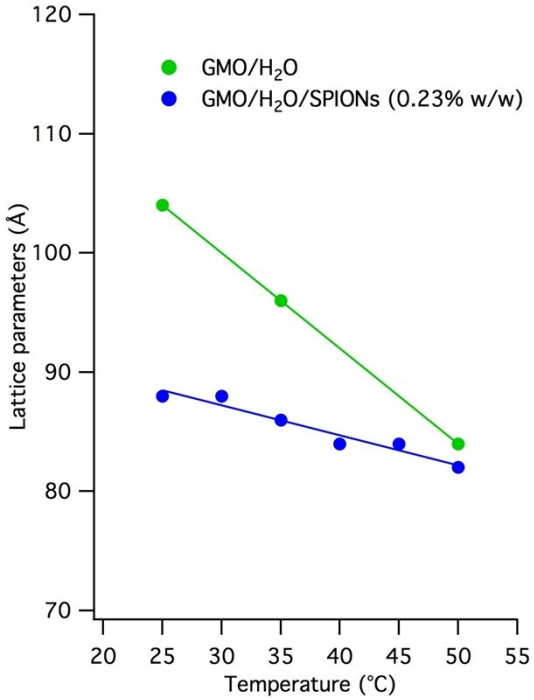

Figure S10: Linear fit of lattice parameters in GMO/H_2O system (green line) and GMO/H_2O/SPIONs (0.23% w/w) (blue line) on temperature. Both the systems have the same cubic structure (Pn3m).

S.12- DLS analysis of cubosomes and magnetocubosomes

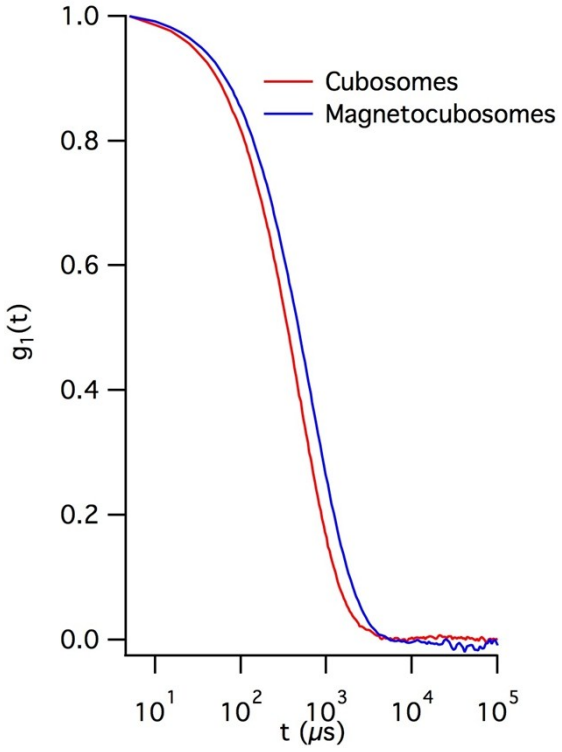

Figure S11: Dynamic Light Scattering (DLS) curves of cubosomes and magnetocubosomes water dispersion diluted 1:500 before the measurement.

Table S8. Hydrodynamic diameter and polydispersity of cubosomes and magnetocubosomes. DLS curves analyzed through a cumulant analysis stopped to the second order.

T (°C)	Hydrodynamic diameter (nm)	Polydispersity
Cubosomes	240	0.19
Magnetocubosomes	260	0.12

S.13- Temperature dependence of cubosomes and magnetocubosomes lattice parameters

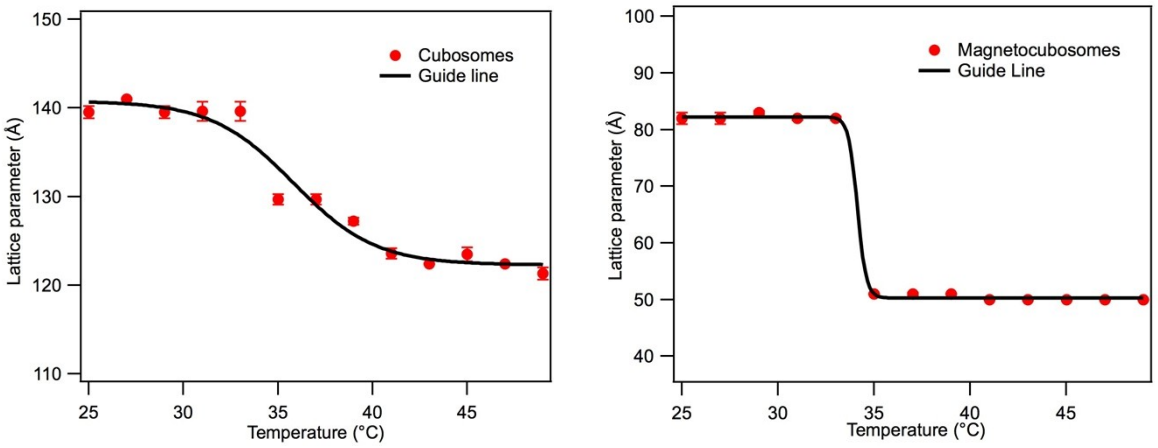

Figure S12: Trend of lattice parameters of cubosomes and magnetocubosomes with temperature in the 25-50°C temperature range, estimated from SAXS data. The lattice parameter *d* decreases following a sigmoidal-like trend. The overall lattice parameter decrease is of about 2 nm for cubosomes and 3 nm for magnetocubosomes in the 25°C-50°C temperature range. Magnetocubosomes present a phase transition Pn3m-H_{II} very close to the physiological temperature.

S.14- Derivation of Equation 4 of the main text

In the framework of Helfrich theory, the free energy of elastic curvature of a lipid bilayer g_C can be expressed as:

$$g_C = 2\kappa^B(H - H_0^B)^2 + \kappa_G^B K \quad (S6)$$

where κ^B and κ_G^B are the bending and Gaussian elastic moduli respectively, H and K are the mean and Gaussian curvatures and H_0^B is the spontaneous curvature of the bilayer. Considering that for a symmetric lipid bilayer $H_0^B = 0$, the H_{II} phase is characterized by a Gaussian curvature K=0 and that the mean curvature for a Pn3m at the mid-plane H=0, we can write equation (6) for the hexagonal and cubic phases as follows:

$$g_c(H_{II}) = 2\kappa_B H^2 \quad (S7)$$

$$g_C(Pn3m) = \kappa_G^B K \quad (S8)$$

If we express the bending and Gaussian elastic moduli for a bilayer in terms of the corresponding terms for a monolayer, we obtain:

$$\kappa_B = 2\kappa \quad (S9)$$

$$\kappa_G^B = 2(\kappa_G - 2H_0\kappa l_c) \quad (S10)$$

and can rewrite (7) and (8) as follows:

$$g_c(H_{II}) = 4\kappa H^2 \quad (S11)$$

$$g_C(Pn3m) = 2(\kappa_G - 2H_0\kappa l_c)K \quad (S12)$$

Considering a cubic-to-hexagonal phase transition, we can write the variation of elastic curvature as:

$$\Delta g_C = 4\kappa H_{H_{II}}^2 - 2K_{Pn3m}(\kappa_G - 2H_0\kappa l_c) \quad (S13)$$

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(1) Wang, L.; Luo, J.; Fan, Q.; Suzuki, M.; Suzuki, I. S.; Engelhard, M. H.; Lin, Y.; Kim, N.; Wang, J. Q.; Zhong, C. J. *J. Phys. Chem. B* **2005**, *109* (46), 21593.

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