Electronic Supplementary Information

Pressure – temperature phase behaviour of natural sphingomyelin extracts

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Additional small- and wide- angle X-ray diffraction results

Egg yolk sphingomyelin (EYSM) SAXS patterns

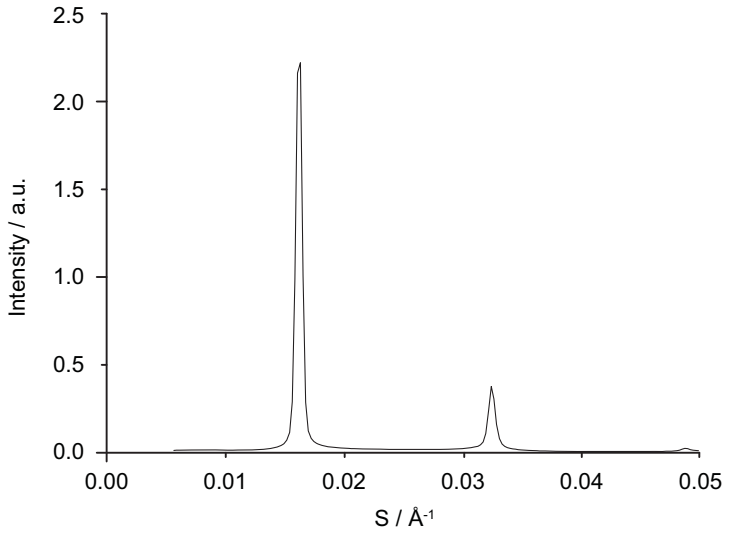

Fig. S1 Small-angle diffraction pattern from the fluid lamellar phase of EYSM at 53 °C and atmospheric pressure

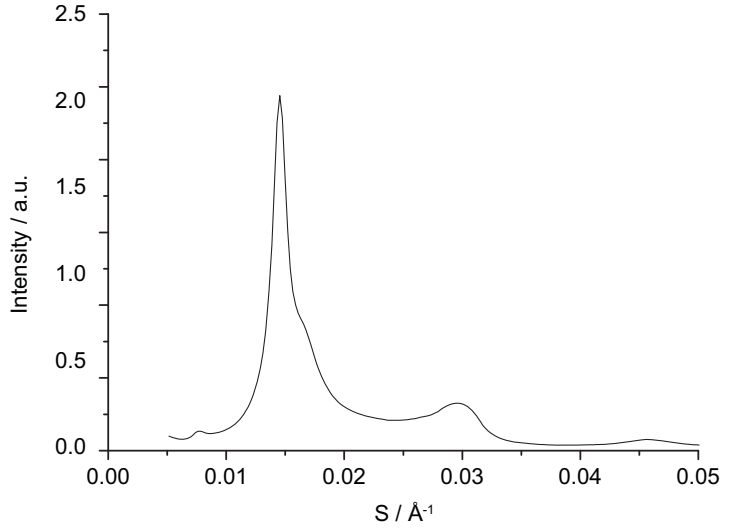

Fig. S2 Small-angle diffraction pattern from the ripple gel phase of EYSM at 24.5 °C and atmospheric pressure

Milk sphingomyelin (MSM) SAXS patterns

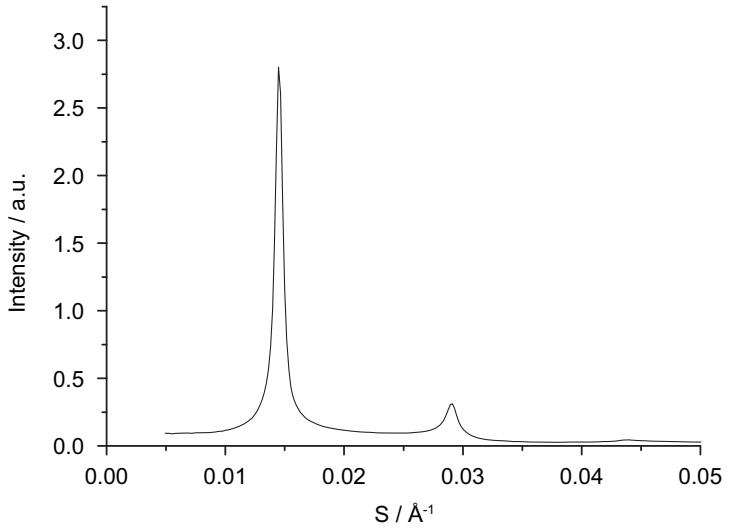

Fig. S3 Small-angle diffraction pattern from the fluid lamellar phase of MSM at 42.5 °C and atmospheric pressure

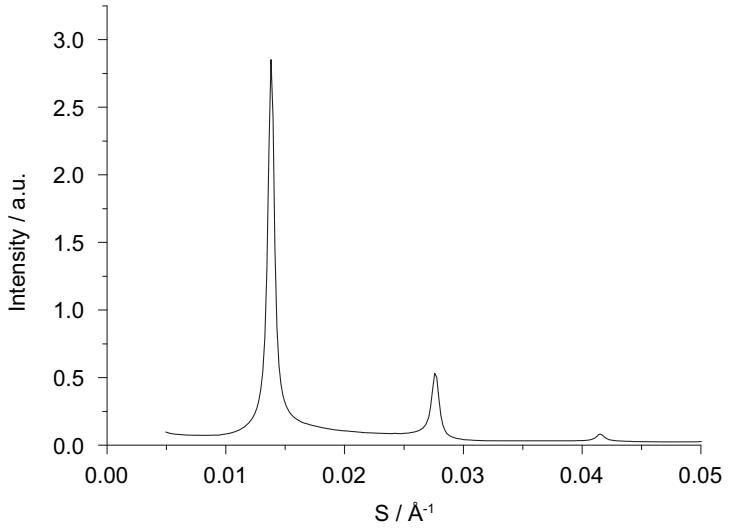

Fig. S4 Small-angle diffraction pattern from the lamellar gel phase of MSM at 25.1 °C and atmospheric pressure

WAXS patterns from the BBSM, EYSM and MSM gel phases

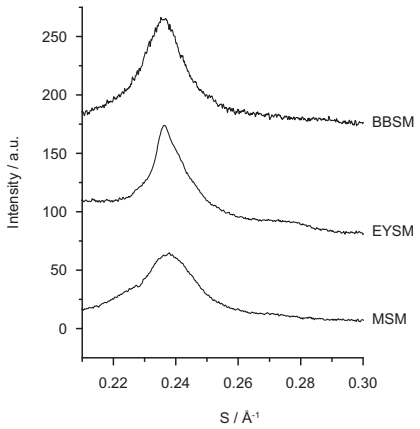

Fig. S5 Wide-angle diffraction patterns from the gel phases of BBSM, EYSM and MSM at 5 °C and atmospheric pressure (acquired with in-house Guinier camera)

Variation of the BBSM tilted ripple gel lattice parameters with temperature and pressure

(Note: Lattice parameters could only be measured at temperatures above the chain melting transition temperature, when the sample undergoes a pressure induced phase transformation to the gel structure)

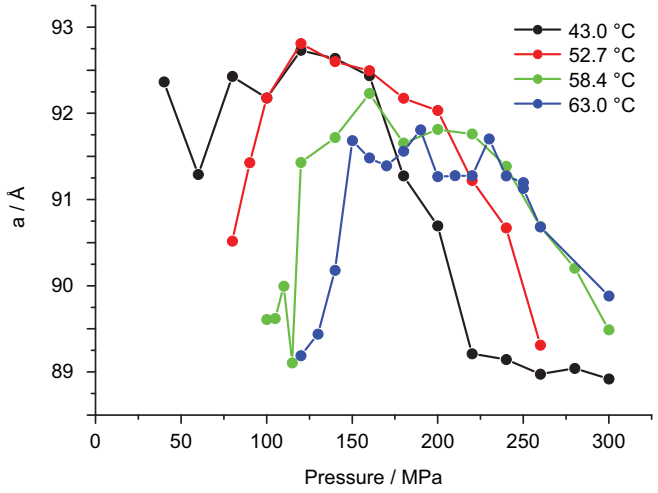

Fig. S6 Pressure dependence of the BBSM ripple gel phase *a* lattice parameter at a range of temperatures

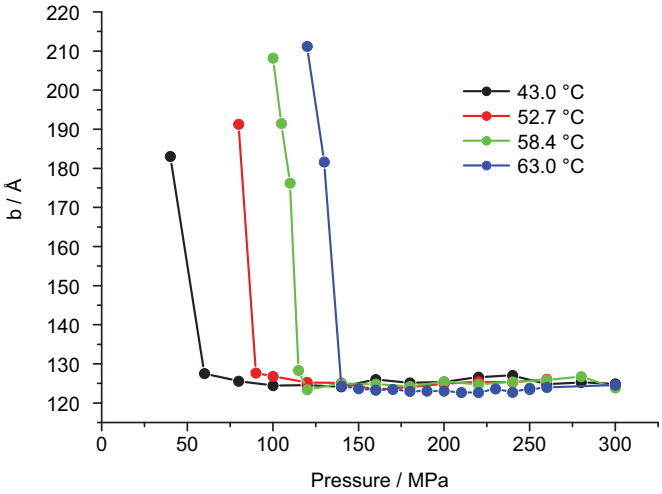

Fig. S7 Pressure dependence of the BBSM ripple gel phase *b* lattice parameter at a range of temperatures

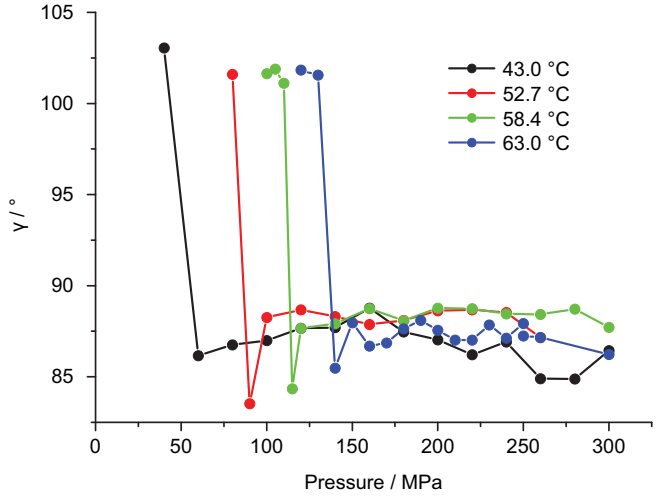

Fig. S8 Pressure dependence of the BBSM ripple gel phase *γ* lattice parameter at a range of temperatures

Variation of BBSM, EYSM and MSM layer spacing with temperature and pressure

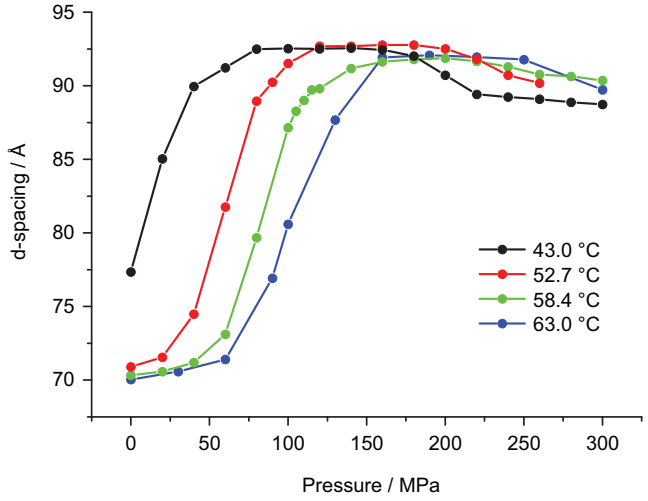

Fig. S9 Pressure dependence of the BBSM layer spacing at a range of temperatures

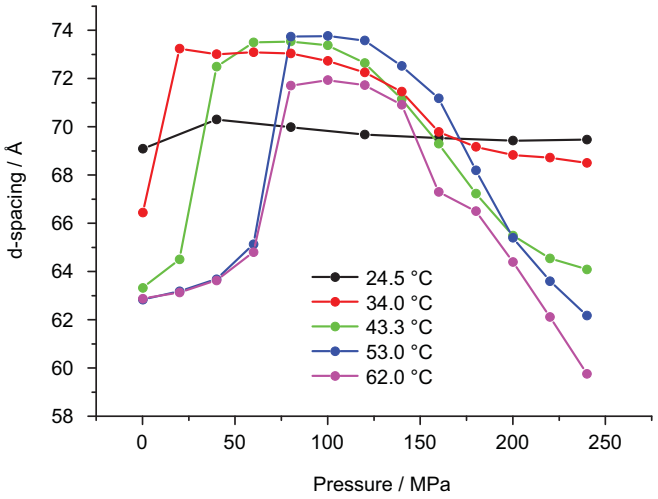

Fig. S10 Pressure dependence of the EYSM layer spacing at a range of temperatures

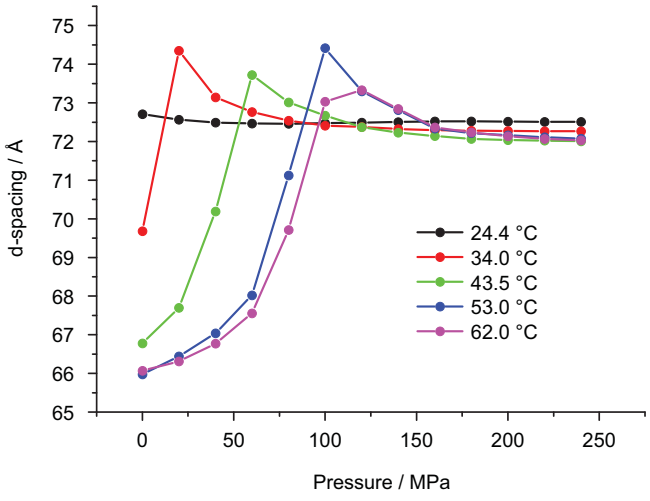

Fig. S11 Pressure dependence of the MSM layer spacing at a range of temperatures

Pressure – temperature points measured

The high pressure synchrotron SAXS experiments which contributed to the pressure – temperature phase diagrams were carried out under the following conditions:

Extract	Temperature / °C	Pressure range / MPa	Pressure intervals / MPa
BBSM	10.5	1 - 3000	100
	15.0	1 - 3000	100
	24.0	1 - 3000	200
	33.5	1 - 3000	200
	43.0	1 - 3000	200
	52.7	1- 2600	200
	58.4	1 - 3000	200
	63.0	1 - 3000	100
EYSM	24.5	1 - 2400	200
	34.0	1 - 2400	200
	43.3	1 - 2400	200
	53.0	1 - 2400	200
	62.0	1 - 2400	200
MSM	24.4	1 - 2400	200
	34.0	1 - 2400	200
	43.5	1 - 2400	200
	53.0	1 - 2400	200
	62.0	1 - 2400	200

Table S1 Pressures and temperature used during high pressure SAXS measurements which contributed to the pressure – temperature phase diagrams.

Nuclear Magnetic Resonance (NMR) acquisition parameters

All NMR data were acquired on a Bruker DRX 600 MHz spectrometer operating at 14.09 T with a ^{1}H resonance of 600.1 MHz and ^{31}P resonance of 242.9 MHz. Standard Bruker pulse programs were used with the parameters summarised below in Table S1.

	^{31}P static	**^{31}P MAS**
Pulse programme	Single pulse	Single pulse
No scans	2048	128
TD	2048	5430
Sweep width	48 kHz	48 kHz
Acquisition time	21ms	56ms
Relaxation delay	3 s	3 s
Decoupling	^{1}H	^{1}H
X-pulse length	2 µs	2 µs
X-power level	0 dB	0 dB
^{1}H-pulse length	3.6 µs	3.6 µs
^{1}H-power level	12 dB	12 dB
Lorentzian broadening	100 Hz	10 Hz
MAS spin rate	-	3-5 kHz

Table S2 Standard parameters used in NMR experiments.

All NMR data were acquired using 4mm rotors except the ^{31}P data for milk sphingomyelin (MSM) which used a 2.5 mm rotor. A much larger number of scans were required for these experiments due to the smaller sample size used. The static ^{31}P MSM experiments used 4096 scans while the ^{31}P MAS experiments used 1024 scans.

Variable temperature (VT) experiments were performed by passing the bearing gas through a chiller unit and then a heating probe before reaching the sample. All samples were heat cycled across the T_m (by heating to at least 55 °C and allowing to cool to room temperature) before beginning the experiment and allowed to equilibrate for 5 minutes at each new temperature prior to data acquisition.

Ripple phase parameter fitting

The ripple phase has two repeat distances, the interlamellar distance (*a*) and the ripple period/frequency (*b*) (fig. 3.2). The angle between these (*γ*) can also vary, giving an oblique lattice. The absolute values of these parameters varies but *b* is usually greater than *a*, for DPPC the following values have been reported *a* = 71 Å, *b* = 136 Å, *γ* = 95°.[SR1] The combination of these three variables leads to the large number of diffraction peaks seen in the small angle x-ray diffraction pattern.

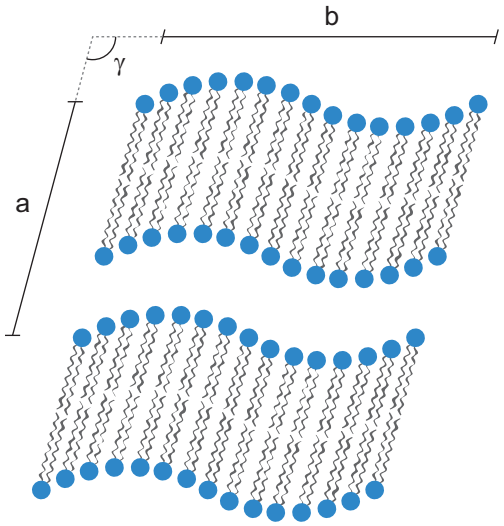

Fig. S12 Cartoon illustrating the lattice parameters of the ripple phase.

The ripple phase is usually tilted with respect to the bilayer normal. This means that unlike the flat lamellar phases, the reciprocal spacing measured from the diffraction pattern cannot be directly converted to the real spacing. Instead the three lattice parameters *a*, *b*, and γ must be calculated by the method described below.

For an oblique lattice the reciprocal lattice is given by the following [SR2] :

$$S^2 = h^2 a^{*2} + k^2 b^{*2} + 2hka^* b^* \cos\gamma^* \qquad \text{Equation S1}$$

Where:

$$a^* = \frac{1}{a \sin\gamma} \qquad \text{Equation S2}$$

$$b^* = \frac{1}{b \sin\gamma} \qquad \text{Equation S3}$$

$$\gamma^* = 180° - \gamma \qquad \text{Equation S4}$$

Once the diffraction peaks have been assigned the equations above can be solved to find a, b and γ. For the (0,1) peak, equation S1 becomes:

$$S_{(0,1)}^2 = 0^2 a^{*2} + 1^2 b^{*2} + 2 \times 0 \times 1 \times a^* b^* \cos\gamma^*$$

$$S_{(0,1)}^2 = b^{*2}$$

For the (1,0) peak :

$$S_{(1,0)}^2 = 1^2 a^{*2} + 0^2 b^{*2} + 2 \times 1 \times 0 \times a^* b^* \cos\gamma^*$$

$$S_{(1,0)}^2 = a^{*2}$$

These values can then be substituted into equation S1 for the (1,1) peak and solved to find *γ*.

$$S_{(1,1)}^2 = 1^2 a^{*2} + 1^2 b^{*2} + 2 \times 1 \times 1 \times a^* b^* \cos\gamma^*$$

$$\gamma^* = \cos^{-1}\left(\frac{S^2 - a^{*2} - b^{*2}}{2 \times a^* b^*}\right)$$

$$\gamma = 180° - \gamma^*$$

This value of *γ* can then be substituted into equations S2 and S3 to find *a* and *b*.

Supplementary references

SR1. H. Yao, S. Matuoka, B. Tenchov and I. Hatta, *Biophys. J.*, 1991, **59**, 252-255.

SR2. A. Tardieu, V. Luzzati and F. C. Reman, *Journal of Molecular Biology*, 1972, **75**, 711-718.