Supporting information for

Phase Behavior of Soybean Phosphatidylcholine and Glycerol Dioleate in Hydrated and Dehydrated States Studied by X-Ray Scattering

Jenni Engstedt, Justas Barauskas, Vitaly Kocherbitov[a,b]

Corresponding Author:

Vitaly Kocherbitov, Biofilms – Research Center for Biointerfaces, Malmö University, SE-205 06 Malmö, Sweden, Biomedical Sciences, Faculty of Health and Society, Malmö University, SE-205 06 Malmö; Email: vitaly.kocherbitov@mau.se

Methods

SasView model equations

Core Shell Sphere

Following model provided the form factor, $P(q)$, for a spherical particle characterized with a core-shell structure. The form factor was normalized by the particle volume.

The 1D scattering intensity was calculated in the following way,

$$P(q) = \frac{scale}{V} F^2(q) + background \qquad (S1)$$

where

$$F(q) = \frac{3}{V_s}\left[V_c(\rho_c - \rho_s)\frac{\sin(qr_c) - qr_c\cos(qr_c)}{(qr_c)^3} + V_s(\rho_s - \rho_{solv})\frac{\sin(qr_s) - qr_s\cos(qr_s)}{(qr_s)^3}\right] \qquad (S2)$$

where V_s is the volume of the whole particle, V_s is the volume of the core, $r_s = radius + thickness$ is the radius of the particle, r_c is the radius of the core, ρ_c is the scattering length density of the core, ρ_s is the scattering length density of the shell, ρ_{solv}, is the scattering length density of the solvent. In eq S2, we see that both scattering from the whole particle and the core are contributing to $P(q)$. [1] For cylindrical core shell model see [2].
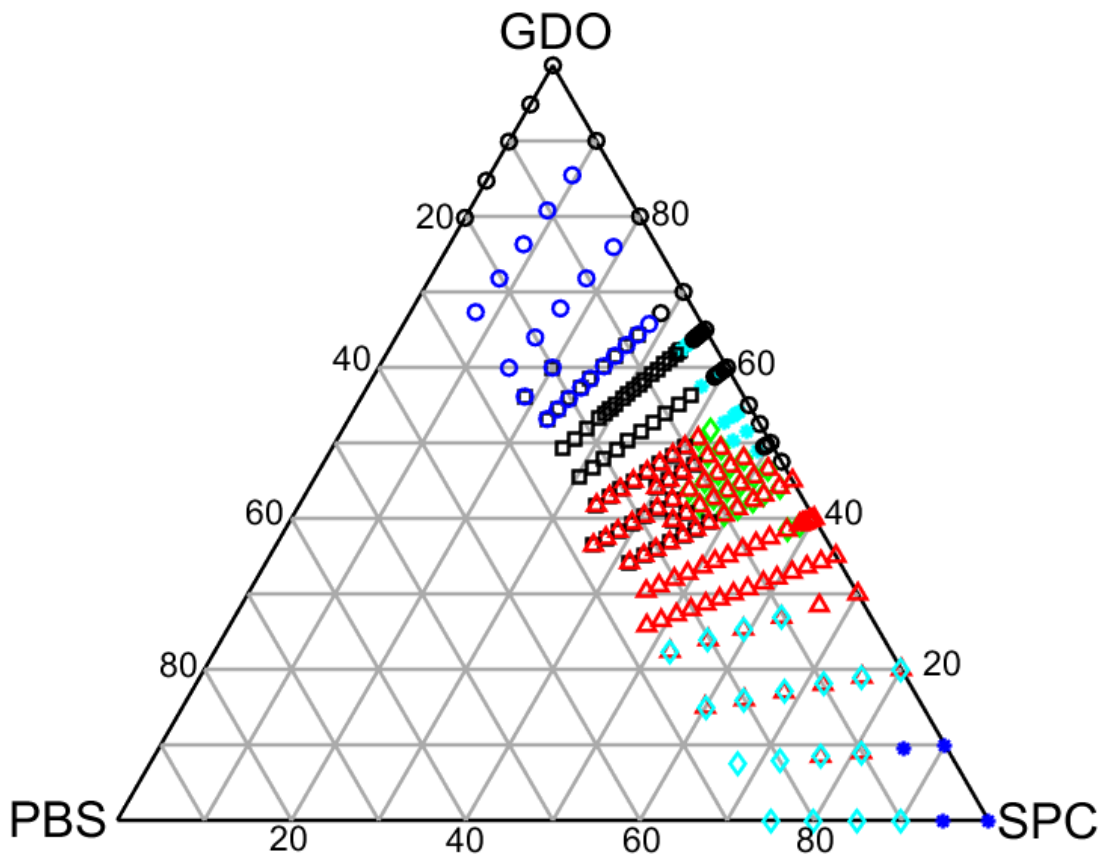
Phase diagram

Figure S1. Phase diagram aims to display all measurements done with SAXS.

Reversed micellar phase

Table S1. Parameters from modelling dry lipid mixtures with SasView using Spherical core shell model with hard sphere structure factor.

SPC/GDO ratio	Core radius (Å) [R_{core}]	Error	Shell Thickness (Å) [T_{shell}]	Error	Radius (Å) [$R_{micelle}$]	Volume fraction of spheres [φ_{sphere}]	Error	Core Volume fraction [φ_{core}]	Number of lipids in one micelle [N_{lipids}]
50/50	11.1	3.7e-3	6.9	4.0e-3	17.9	0.49	8.2e-5	0.115	41.5
45/55	11.0	7.5e-3	6.7	4.6e-3	17.7	0.49	1.4e-4	0.118	40.8
40/60	10.2	4.5e-3	6.5	5.0e-3	16.8	0.47	1.0e-4	0.107	36.5
35/65	9.8	6.6e-3	6.4	7.6e-3	16.2	0.45	1.4e-4	0.099	34.0
30/70	9.4	5.8e-3	6.4	6.8e-3	15.8	0.44	1.3e-4	0.093	32.8
25/75	8.8	9.6e-3	6.3	1.1e-2	15.2	0.42	1.7e-4	0.083	30.4
20/80	8.4	4.6e-3	6.6	5.0e-3	15.0	0.40	2.e-4	0.071	31.1
10/90	7.1	1.1e-2	6.7	1.4e-2	13.8	0.34	2.3e-4	0.047	29.1
0/100	5.1	1.2e-2	7.1	1.7e-2	12.2	0.26	5.5e-4	0.019	26.7

Limited hydration
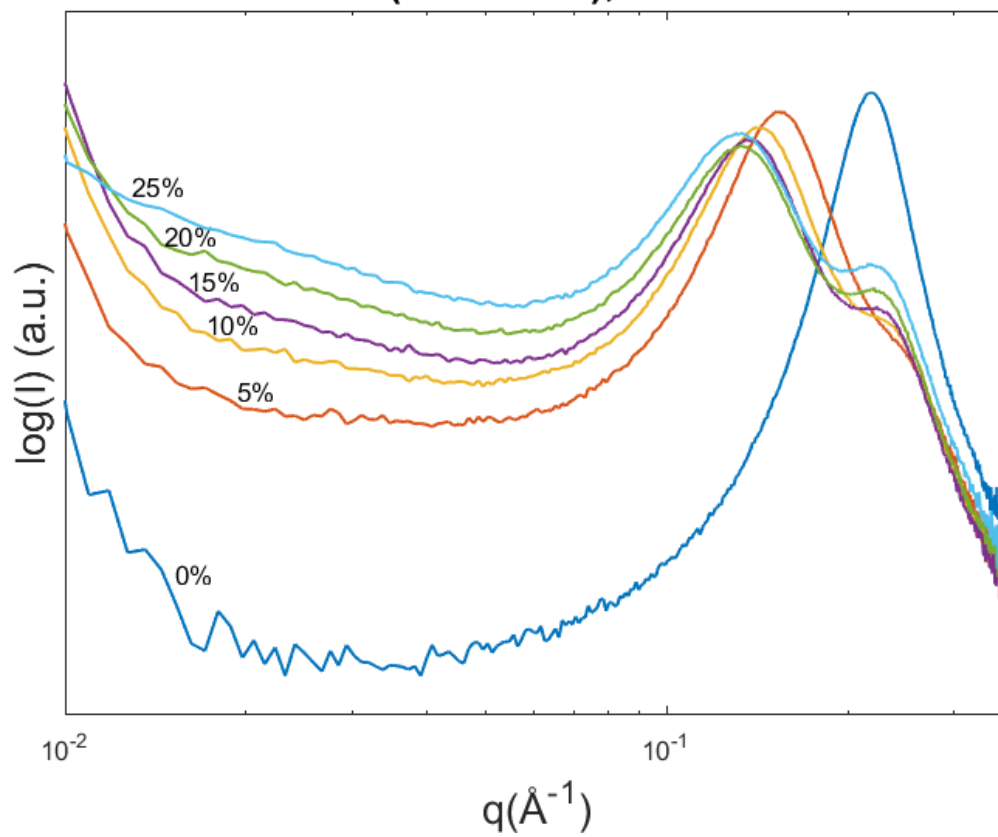**20/80 (SPC/GDO), 1% EtOH**

Figure S2. The increase in background is linear in log (I) vs log(q) coordinates.

S5

Table S2. Parameters from modelling hydrated 20/80 SPC/GDO mixture with SasView using cylindrical core shell model with sticky hard sphere structure factor.

Hydration level (*wt%*)	Core radius (Å)	Error	Shell Thickness (Å)	Error	Length of micelle (Å)	Error	Structure factor Radius (Å)	Volume fraction	Error
0	8.4	4.6e-3	6.6	5.0e-3	-	-	15.0	0.40	2.e-4
5	11.9	2.5e-1	6.8	7.3e-1	30.8	5.4e-1	24.3	0.30	1.8e-2
10	10.7	3.5e-1	9.3	1.1	30.4	0.9e-1	26.3	0.28	2.6e-2
15	9.7	2.3e-1	10.8	6.7e-1	30.6	6.4e-1	27.4	0.26	1.4e-2
20	11.5	2.1e-1	8.0	4.5e-1	43.9	2.1e-1	28.0	0.19	6.9e-3
25	11.0	2.0e-1	8.4	4.2e-1	45.3	1.6e-1	28.3	0.18	6.7e-3

Fd3m
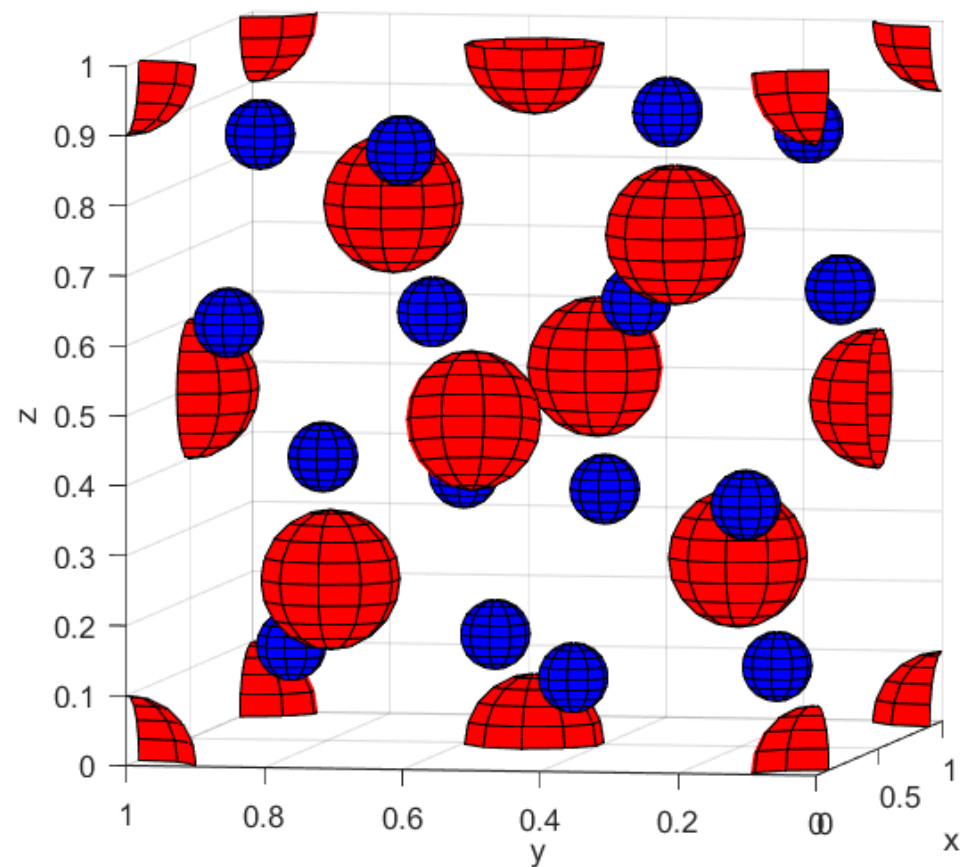
Figure S3. Fd3m structure used for calculation of scattering pattern. The coordinates of 24 micelles in the unit cell are calculated based on the structure presented by Seddon and Templer (Handbook of Biological Physics, 1995). For visualisation, sphere radii of 0.1 and 0.05 are used.
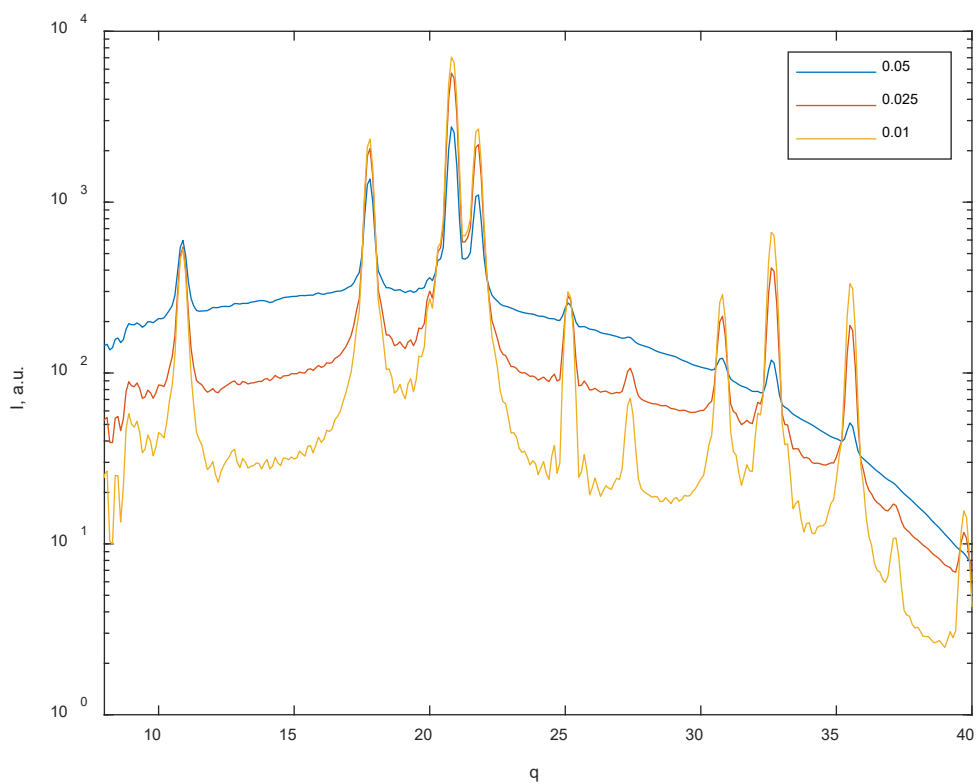
Figure S4. Scattering intensity generated by Fd3m structure consisting of $16^3 = 4096$ unit cells. All radii are taken equal, R=0.1. The legend shows standard deviations of x,y,z coordinates of spheres from their equilibrium positions.
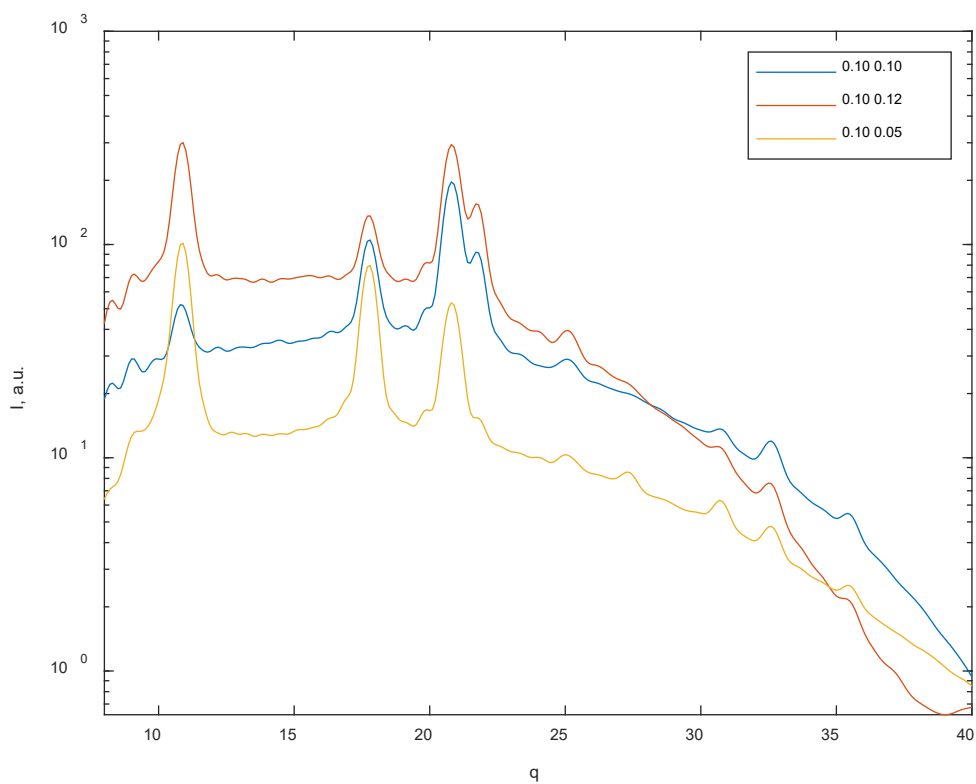
Figure S5. Scattering intensity generated by Fd3m structure consisting of $8^3 = 512$ unit cells. The standard deviations of x,y,z coordinates of spheres from their equilibrium positions are 0.05.The legend shows the values of radii. For calculation of q, the distances are normalized by the unit cell size.

Intermediate phase
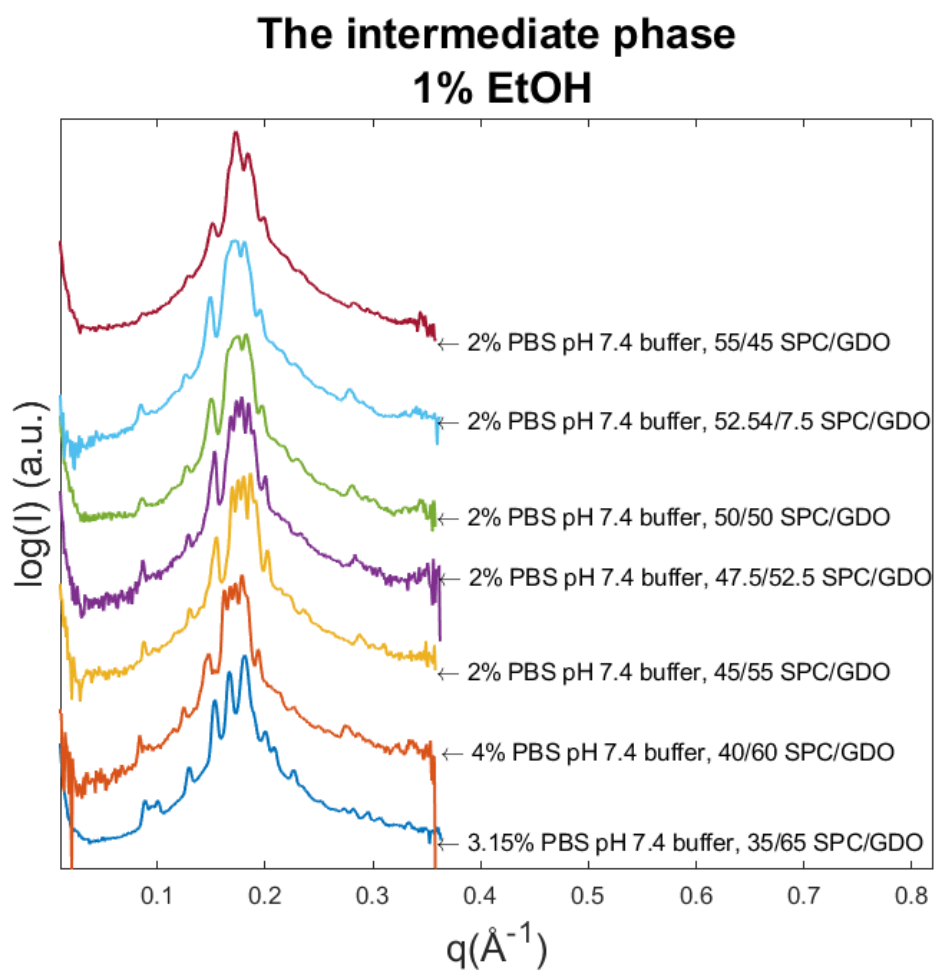
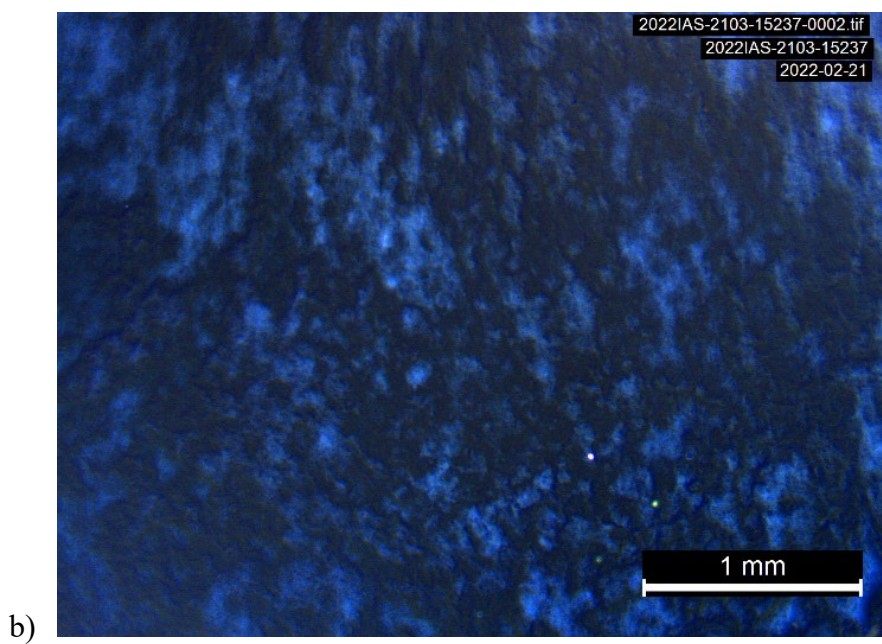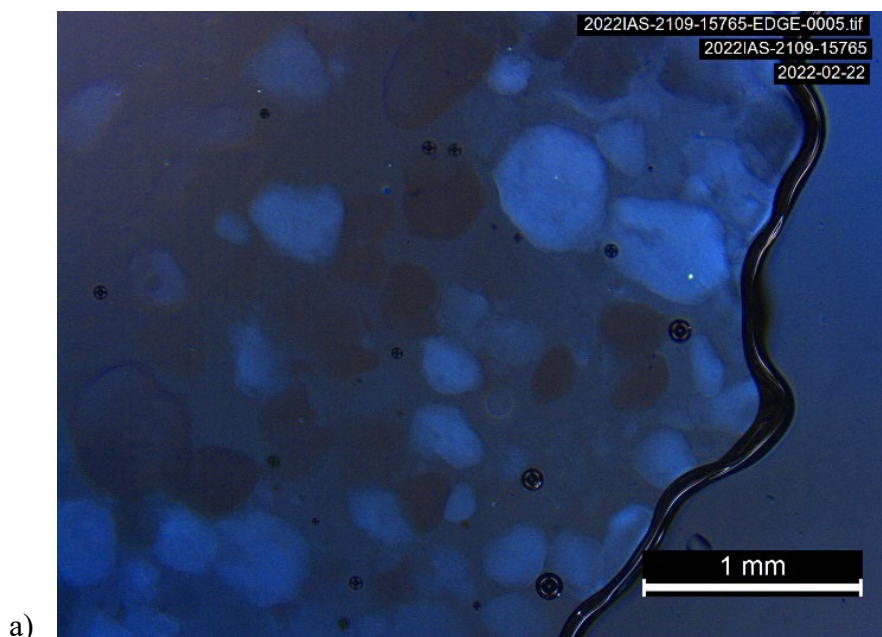**Figure S6.** Similarities between the intermediate phases.

Figure S7. Intermediate phase observed through microscope with polarized light. a) 35/65 SPC/GDO with 3.15% $H_2O^{(PBS)}$.. b) 47.5/52.5 SPC/GDO 1% $H_2O^{(PBS)}$.
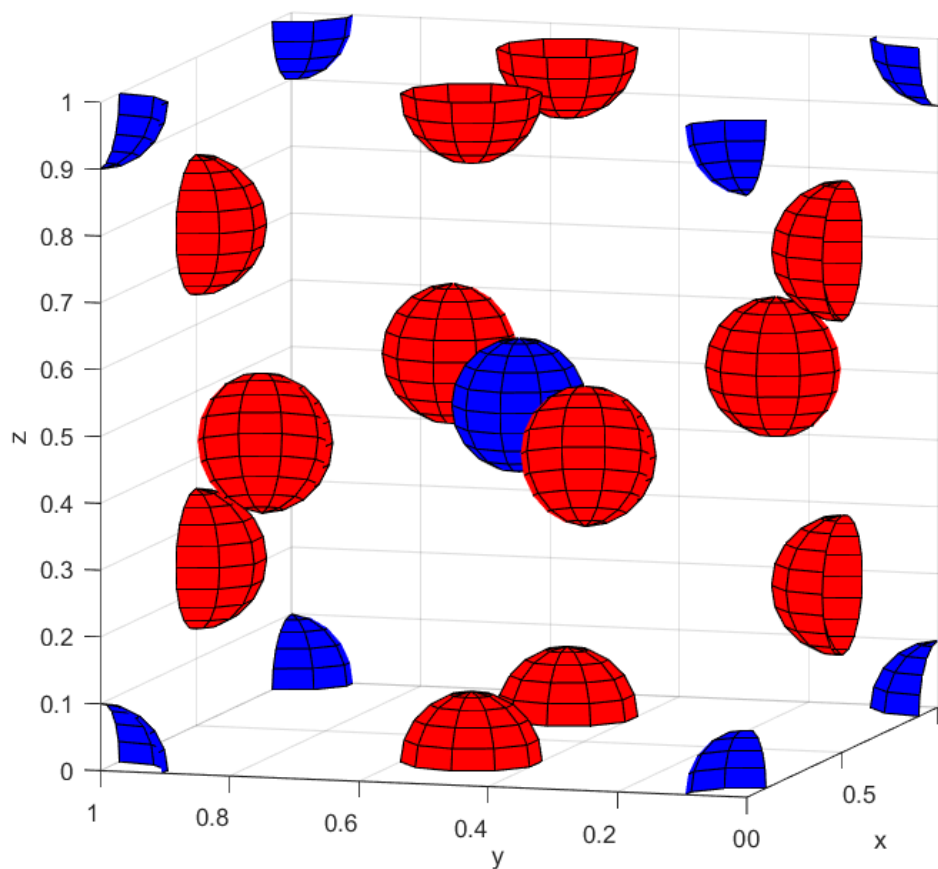
Pm3n

Figure S9. The structure of Pm3n (Q^{223}) phase. The coordinates of 8 micelles in the unit cell are calculated based on the structure presented by Vargas et al. (J. Mol. Biol., 1992, 225, 137-145)
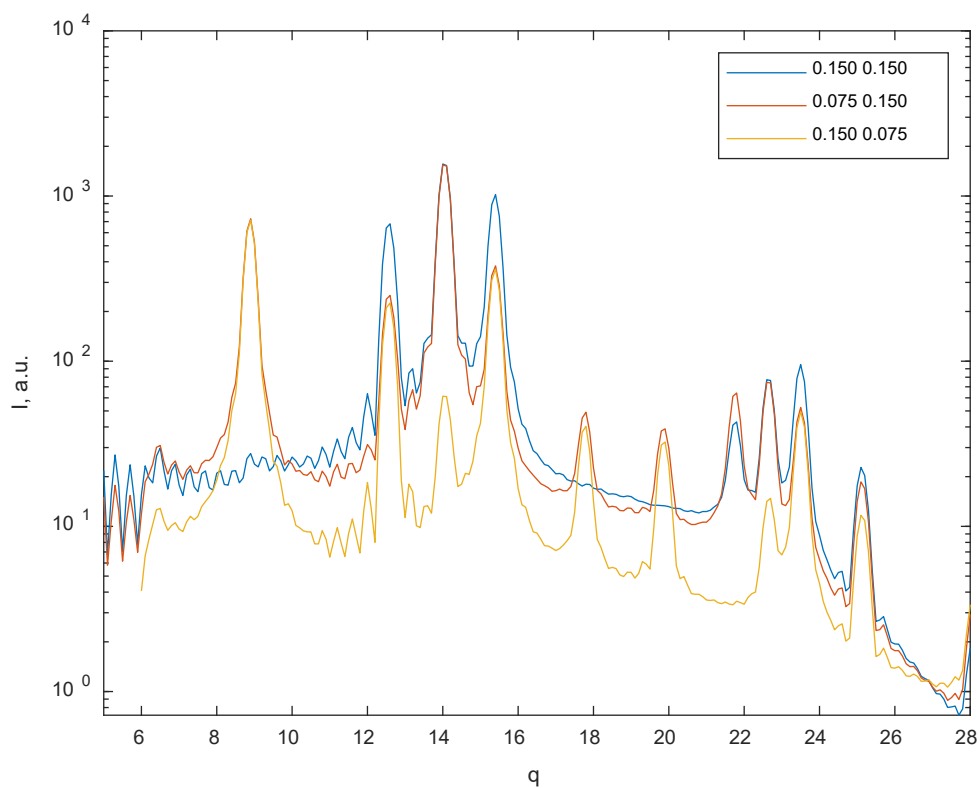
Figure S10. Scattering intensity generated by Pm3n structure consisting of $16^3 = 4096$ unit cells. Each unit cell consists of 8 spherical micelles. The legend shows the micelle radii (the radius of the micelle in 0, 0 position is shown first). The standard deviation of x,y,z coordinates of spheres is 0.025.
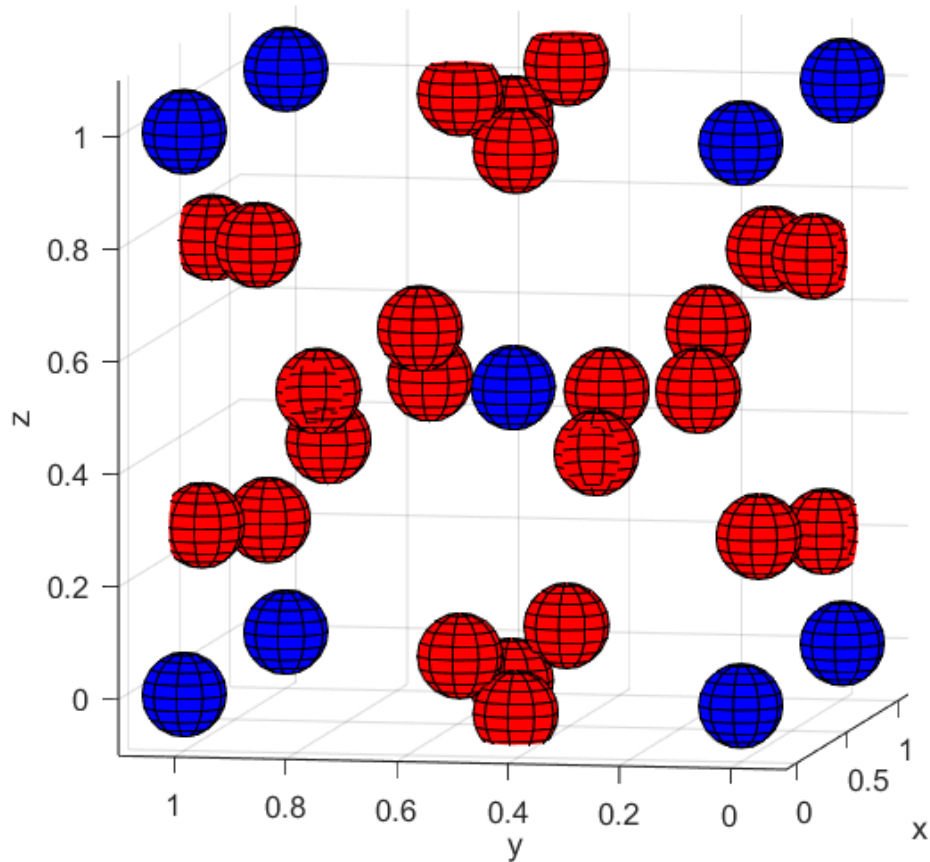
Figure S11. The structure of Pm3n (Q^{223}) phase. The coordinates of 8 micelles of the unit cell (2 spherical and 6 elongated) are calculated based on the structure presented by Fontell, 1990. The elongated micelles are modelled as two spheres placed close to each other. The total structure consists of 14 spheres per unit cell.
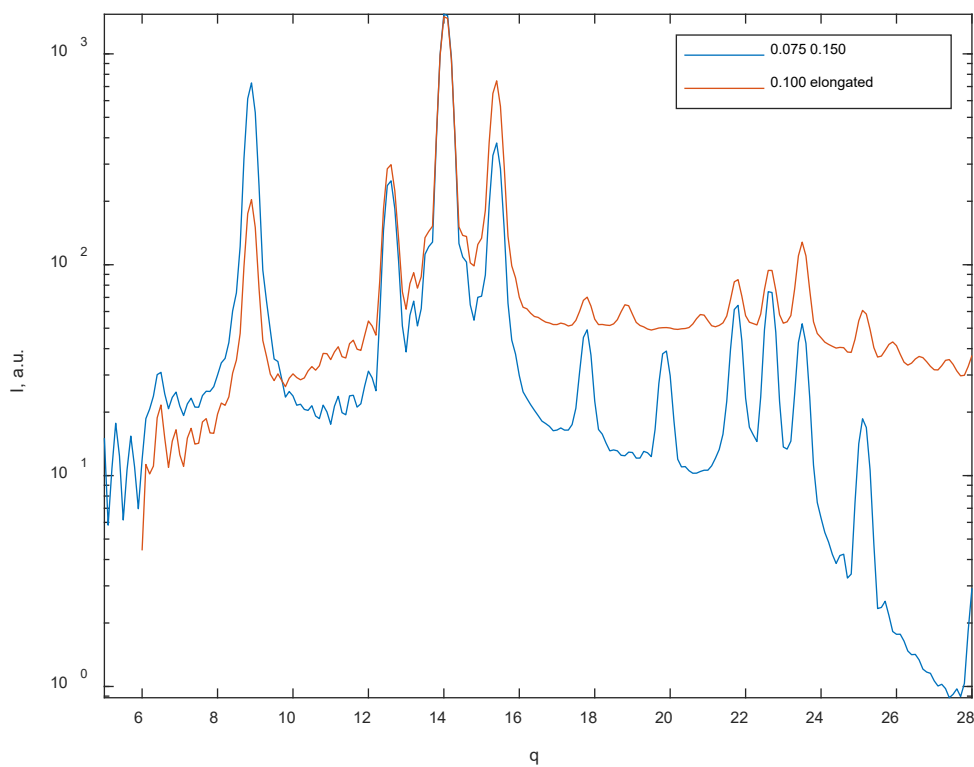
Figure S12. Scattering intensity generated by Pm3n structure consisting of $16^3 = 4096$ unit cells. Each unit cell consists of 8 spherical micelles of two different sizes (blue curve) or 2 spherical plus 6 elongated micelles (red curve). The legend shows the micelle radii used in calculations.
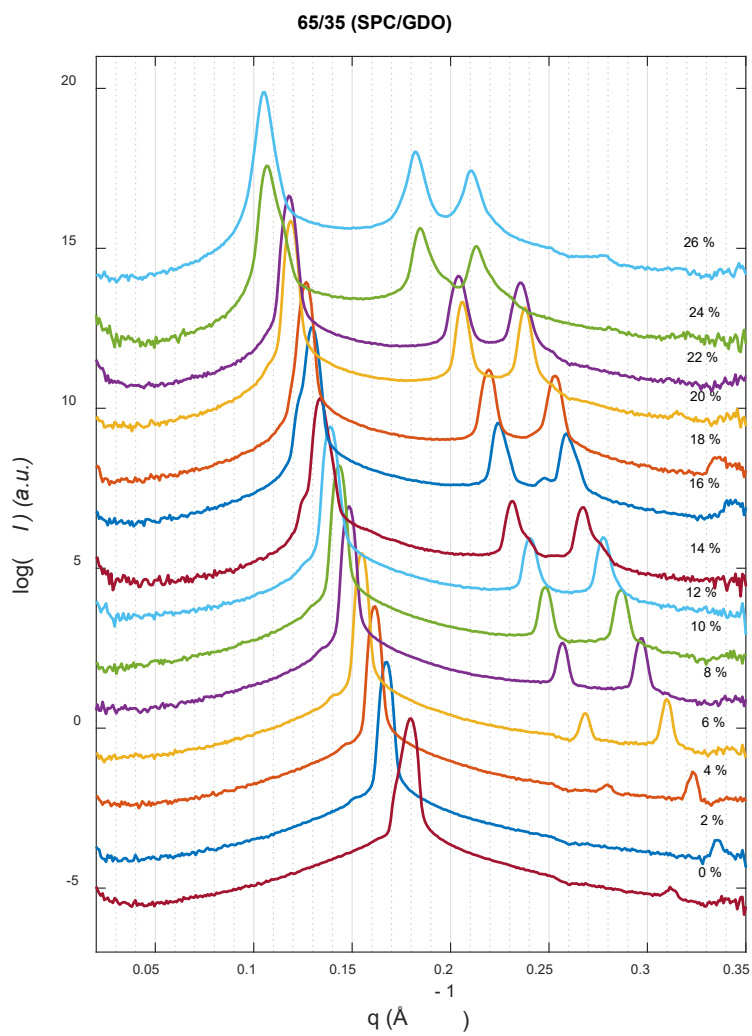
Hexagonal phase

65/35 (SPC/GDO)

Figure S13. Scattering curves generated by 2D swelling of hexagonal phase with increasing amount of $H_2O^{(PBS)}$.
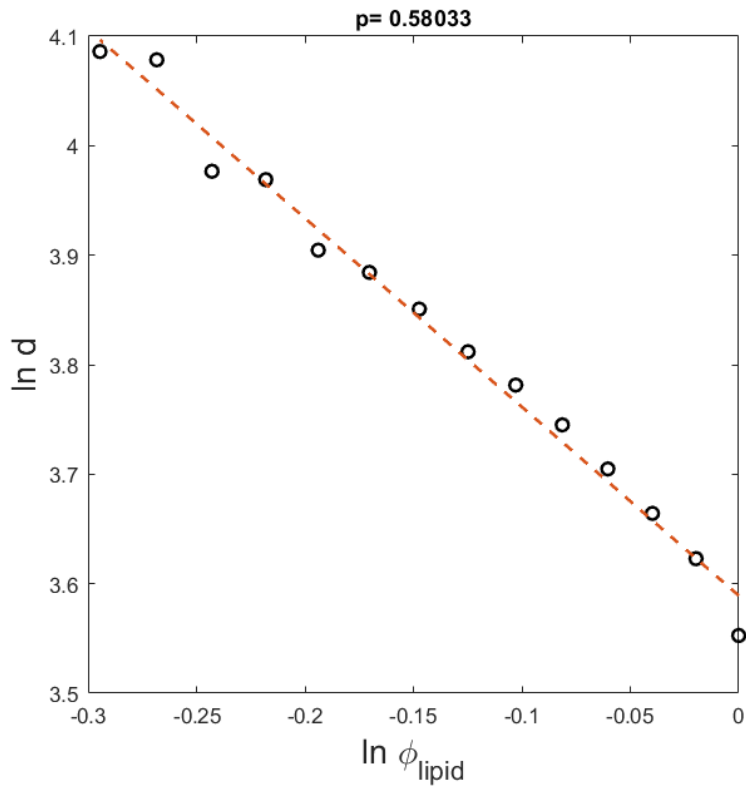
Figure S14. Swelling of hexagonal phase. In general, swelling can be described as

$\ln d = \ln d_0 - \frac{1}{p} \ln \varphi_L$. For 1D swelling the slope is expected to be 1, and 2D swelling -1/2. Here

the swelling of hexagonal phase fits to neither with a slope of -1/0.58.

Additional equations

Mole Fraction

Calculations of mole fractions for lipid 1 in the binary system:

$$x_1 = \frac{1}{1 + \frac{(\frac{1}{w_1}-1)M_1}{M_2}} \tag{S3}$$

Determination of mole fractions for lipid 1 for three component system:

$$x_1 = \frac{w_1/M_1}{w_1/M_1 + w_2/M_2 + w_3/M_3} \tag{S4}$$

References

1.   SasView. *SasView 5.0.5 documentation - Form factor for a monodisperse spherical particle with particle with a core-shell structure.* 2022; Available from: https://www.sasview.org/docs/user/models/core_shell_sphere.html.
2.   SasView. *SasView 5.0.5 documentation - Right circular cylinder with a core-shell scattering length density profile.* 2022; Available from: https://www.sasview.org/docs/user/models/core_shell_cylinder.html.