Pressure-induced interdigitation in bicellar mixtures containing anionic lipid

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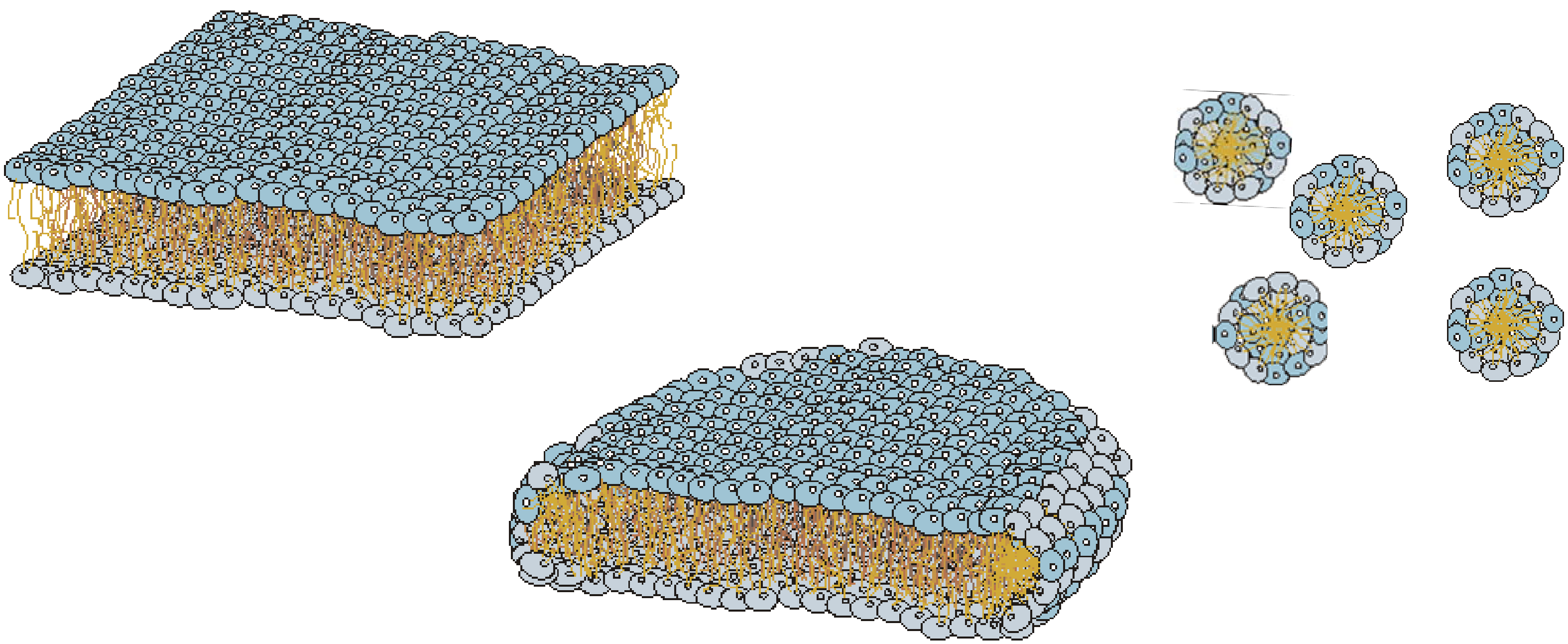
Lipid assemblies reflect balance between interactions:

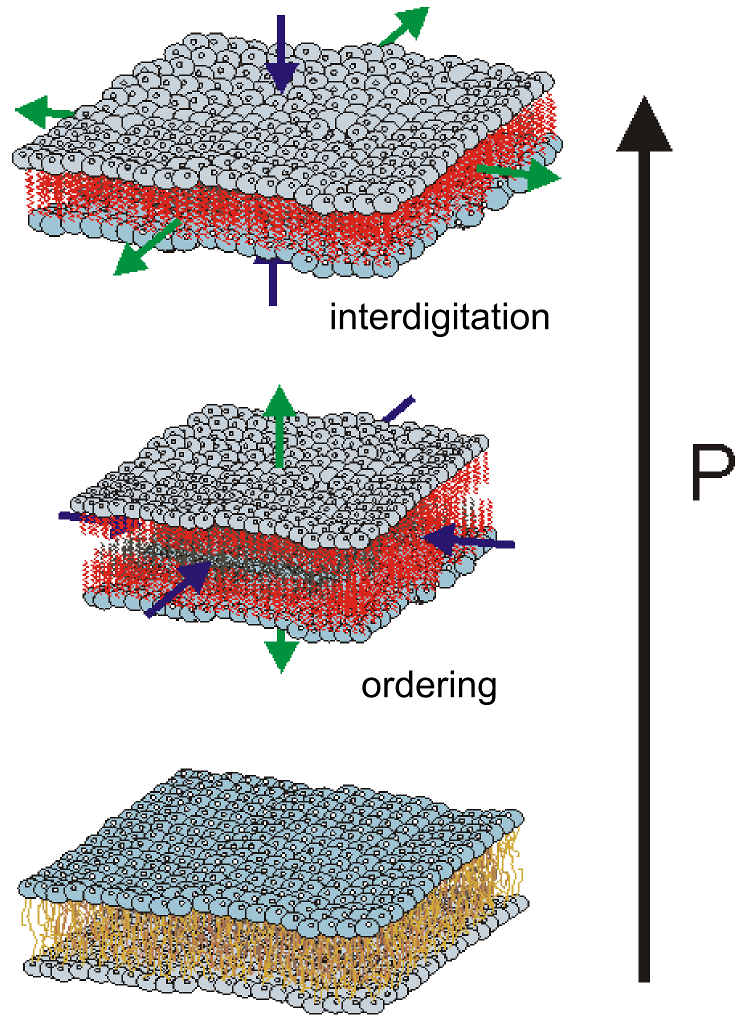
- at the headgroup-water interface
- in the hydrophobic interior



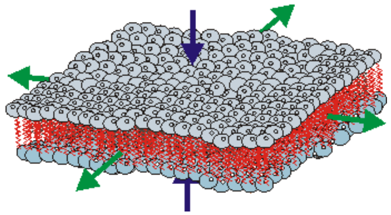
Bilayers are anisotropic

Response to hydrostatic pressure reflects competition between:

- lateral compression
  - chain ordering
- perpendicular compression
  - bilayer thinning

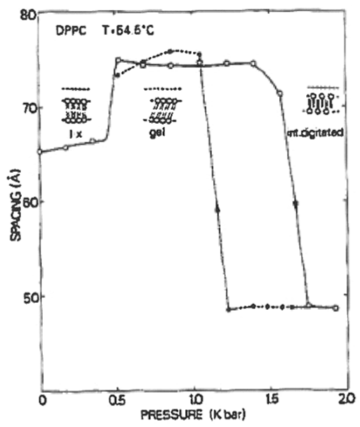


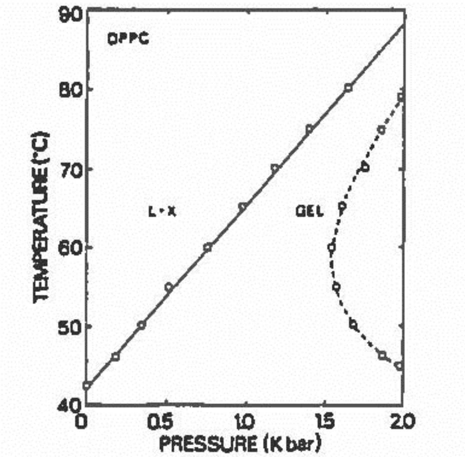
Pressure-induced interdigitation



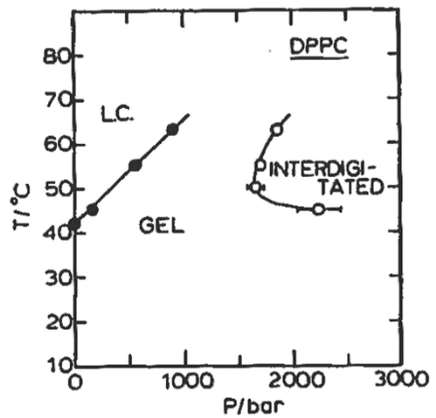
Observed with neutrons

- Braganza and Worcester (1986) *Biochemistry* **25**, 2591

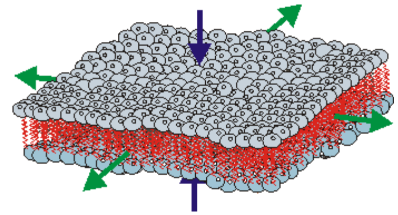




- Winter and Pilgrim (1989) *Ber. Bunsenges. Phys. Chem.* **93**, 708



Pressure-induced interdigitation

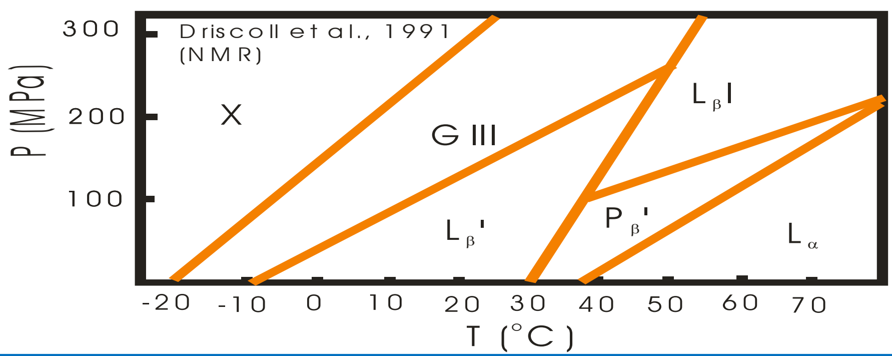


Observed by vibrational spectroscopy

- Wong, Siminovitch, and Mantsch (1988) *Biochim. Biophys. Acta* 947, 139

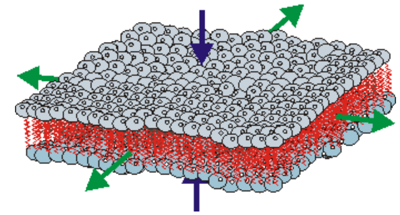
Observed by ^{2}H NMR

- Driscoll, Jonas, and Jonas (1991) *Chem. Phys. Lipids* **58**, 97

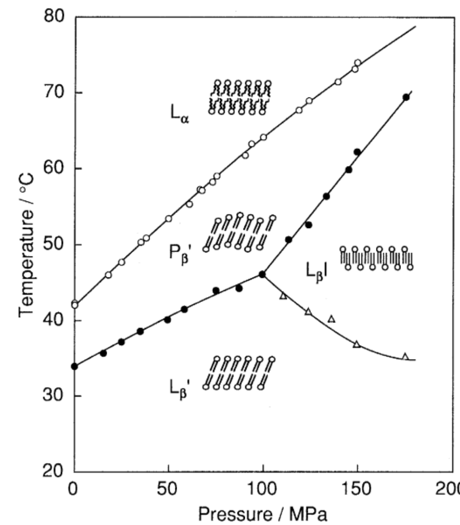


(adapted)

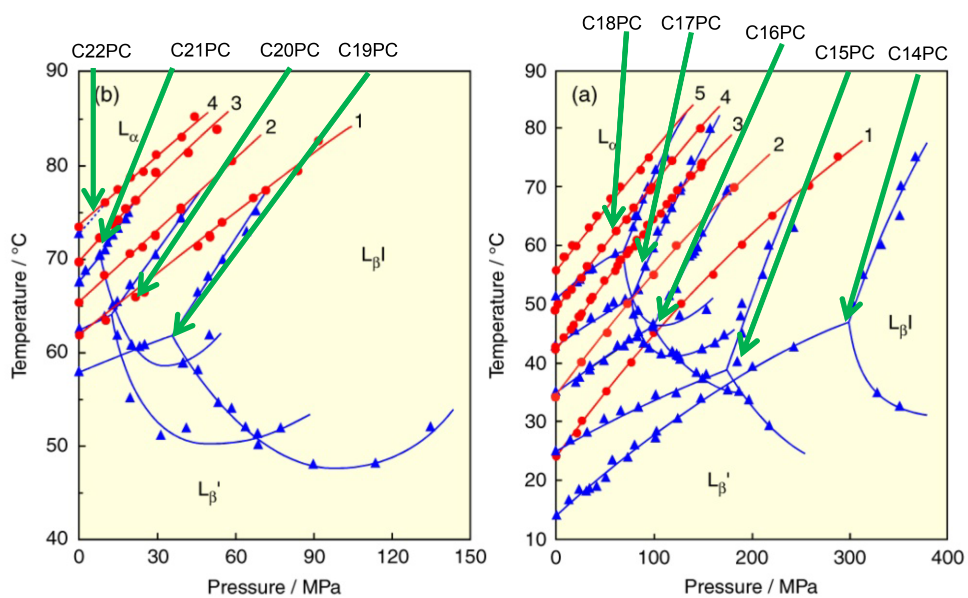
Pressure-induced interdigitation



Observed by light transmission

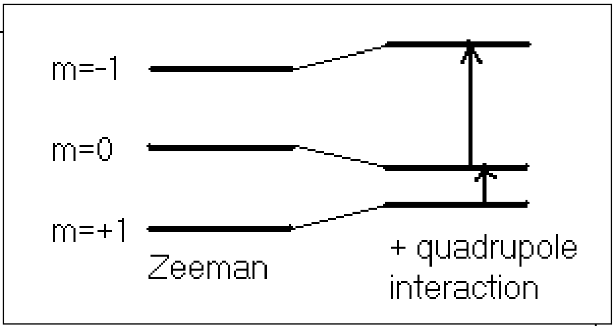


Maruyama, Hata, Matsuki, and Kaneshina (1997) *Biochim. Biophys. Acta* **1325**, 272



Matsuki, Goto, Tada, and Tamai (2013) *Int. J. Mol. Sci.* **14**, 2282.

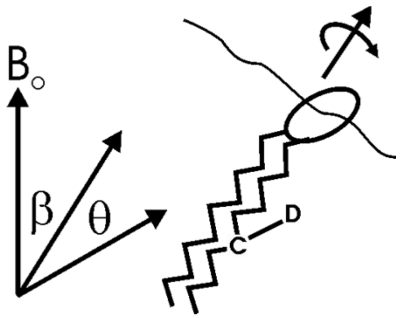
^{2}H-NMR on chain-deuterated lipid

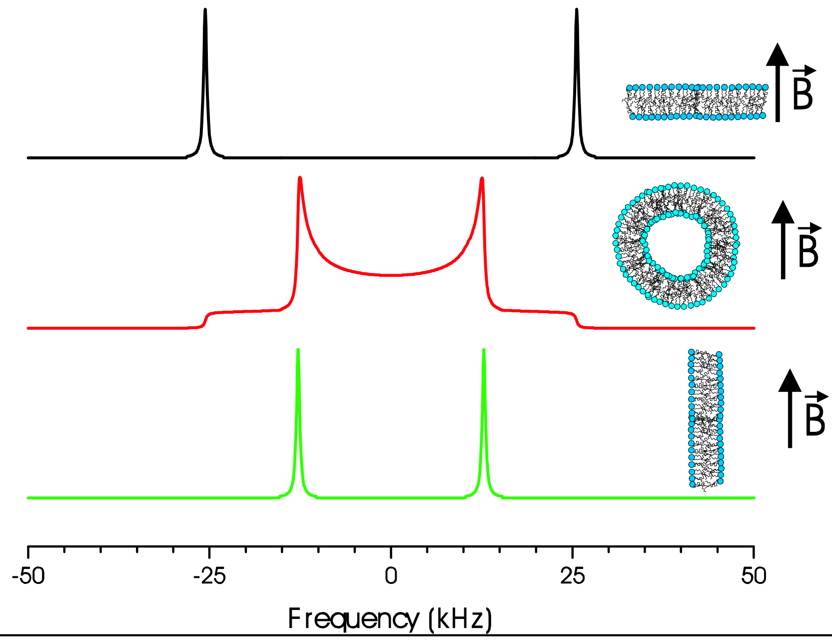


Orientation-dependent quadrupole interaction

Orientational order parameter : $S_{CD} = \langle 3\cos^2\theta - 1 \rangle / 2$

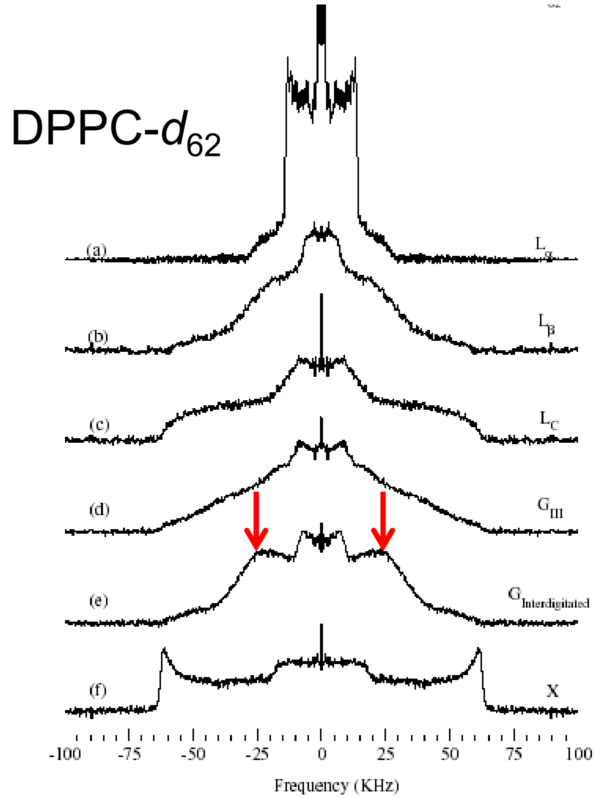
Doublet splitting: $\Delta\nu \approx 125\,\mathrm{kHz} \times (3\cos^2\beta - 1) \times S_{CD}$

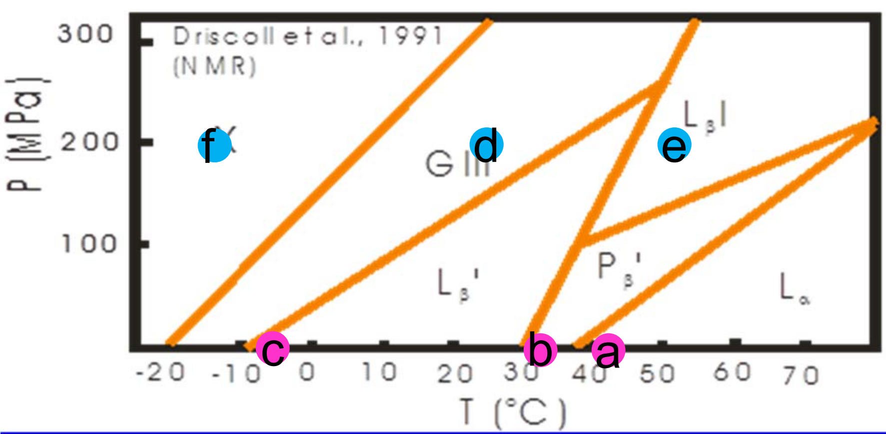




Effect of chain motion:

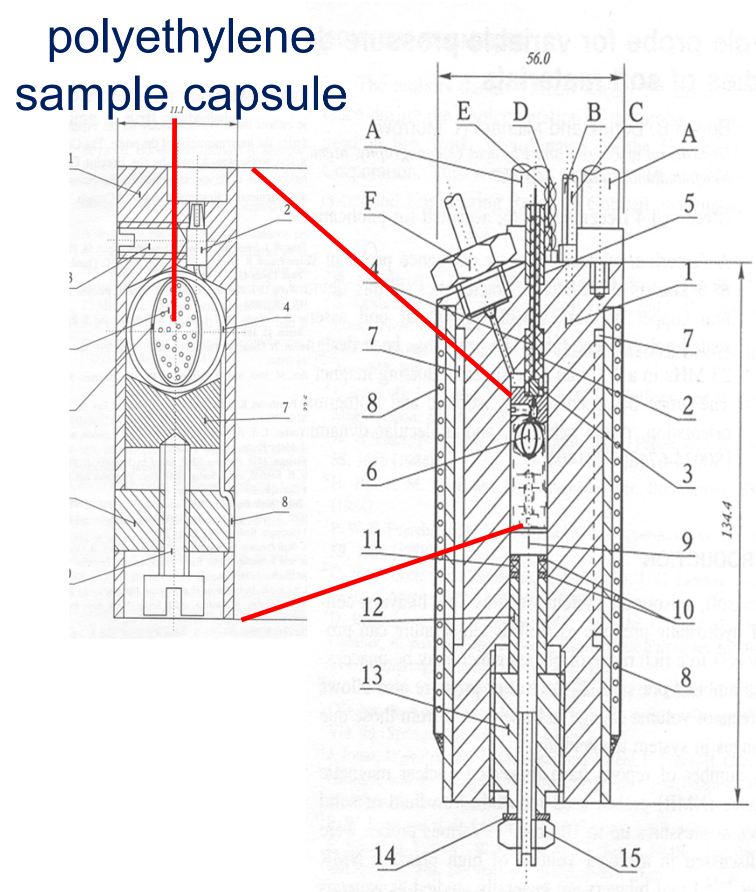
- larger amplitude → smaller splitting
- more constrained → larger splitting



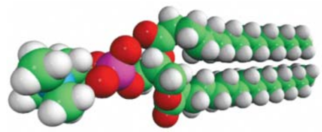


(a) L_α; 41°C, ambient pressure
(b) P_β' (gel); 31°C, ambient pressure
(c) L_C (subgel); -6°C, ambient pressure
(d) GIII; 24°C, 196 MPa
(e) L_βI (interdigitated gel); 51°C, 196 MPa
(f) L_C phase; -15°C, 196 MPa

Variable-pressure Probe (Bonev)

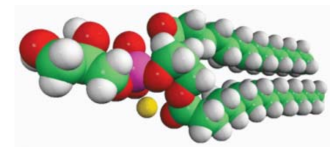


DPPC-d_{62} and DPPG-d_{62} spectra at 85 MPa

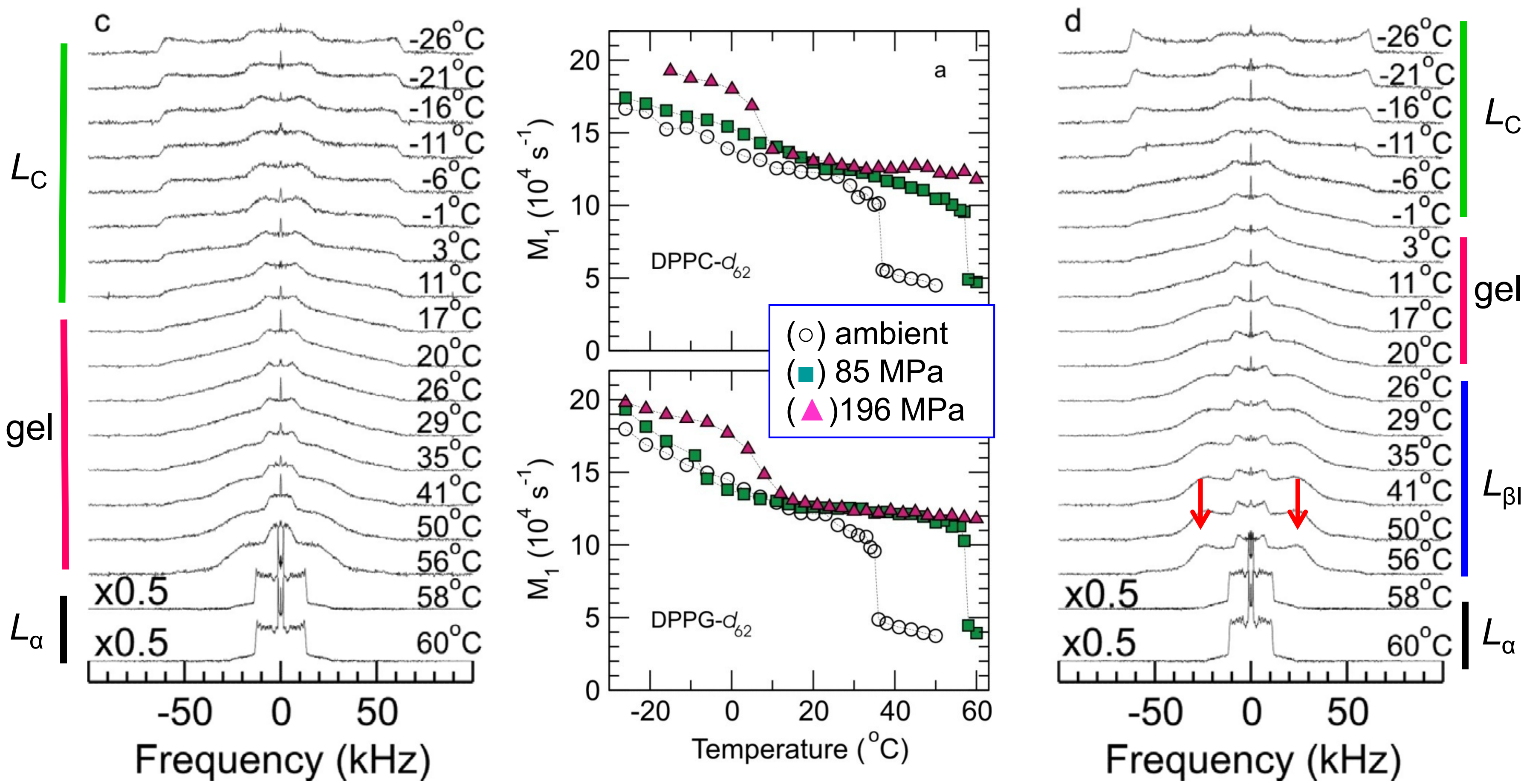


DPPC-d_{62}

Singh et al. (2008) *Eur. Biophys. J.* **37**, 783

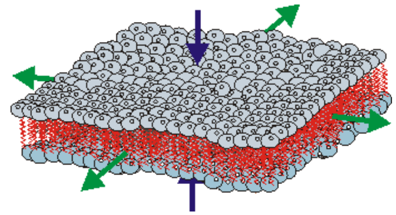


DPPG-d_{62}



Ordered phases just below “main” transition are different

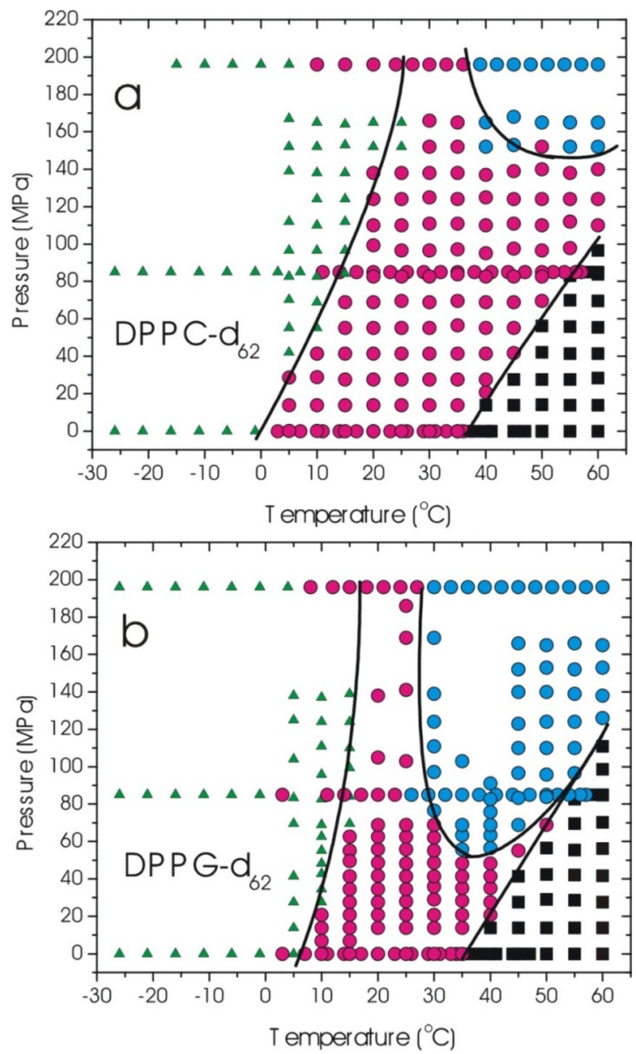
Pressure-induced interdigitation



Singh et al. (2008) *Eur. Biophys. J.* **37**, 783

Phase diagrams

- Anionic headgroup lowers minimum interdigitation pressure



(■) L_α
(●) gel
(●) $L_{\beta I}$
(▲) L_C

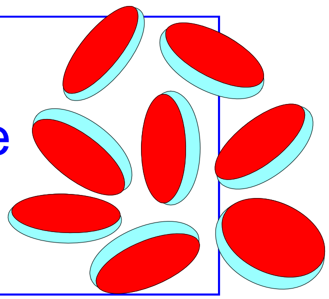
Mixtures of short-chain & long chain lipids (Bicellar mixtures)

Example: DMPC/DHPC (di-14:0-PC/di-6:0-PC)

Does high pressure behaviour reflect "edges" and chain-length mismatch?

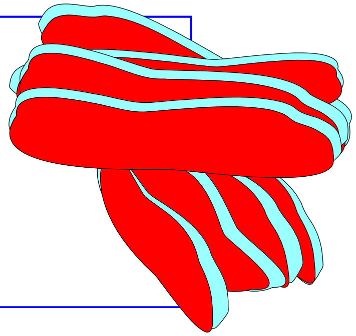
Low T (<~23°C):

- Bicelles: short chain lipids on edge
- Rapid isotropic reorientation



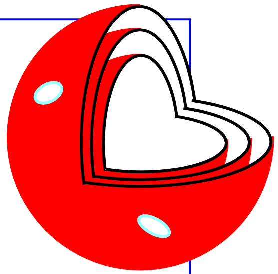
Intermediate T (~24-35°C):

- Nematic wormlike micelle phase
- Short chain lipids on edges
- Magnetically orientable, viscous

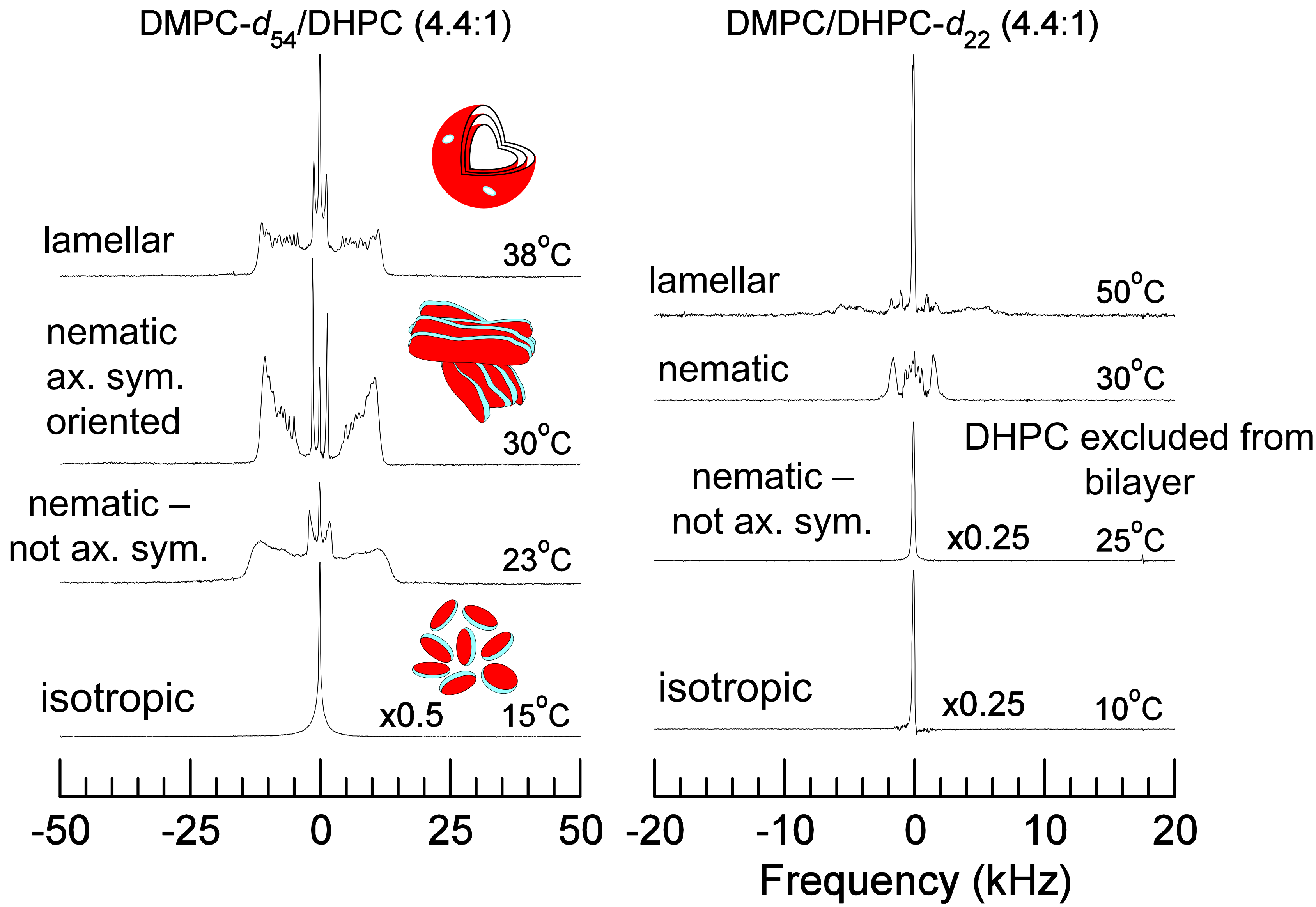


High T (>~36°):

- Multilamellar bilayer vesicle phase
- Short chain lipids on pore rims?

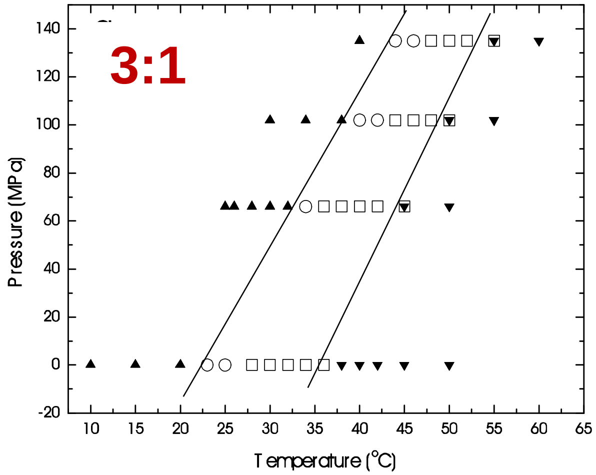


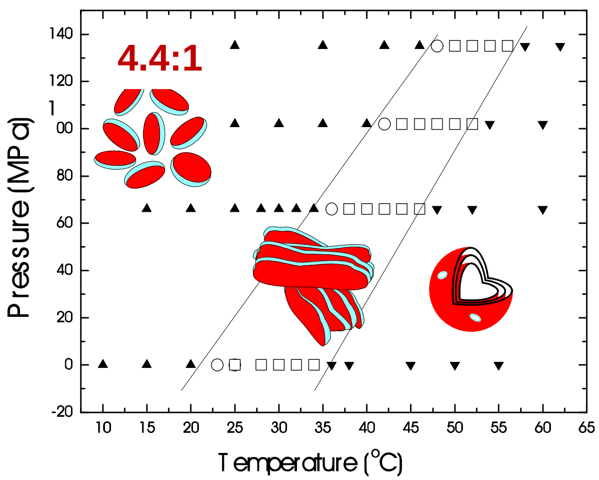
Characteristic Spectra



Uddin & Morrow (2010) *Langmuir* **26**,12104

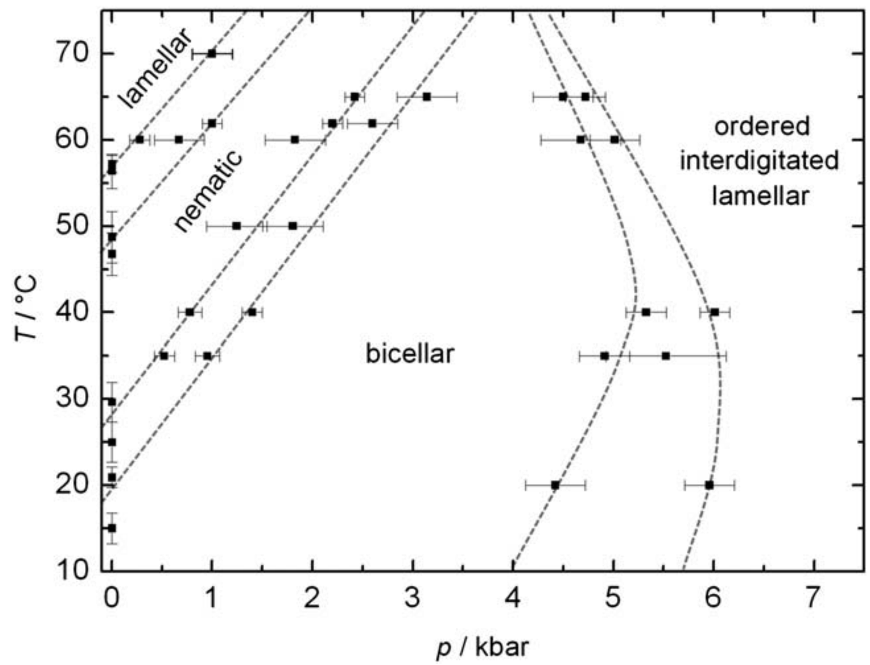
DMPC/DHPC P-T phase diagrams





Using ^{2}H NMR

Uddin & Morrow (2010) *Langmuir* **26**,12104



Using SAXS and FTIR

Jeworrek, Uelner, and Winter (2011) *Soft Matter* **7**, 2709

- Note persistence of nematic to high pressure

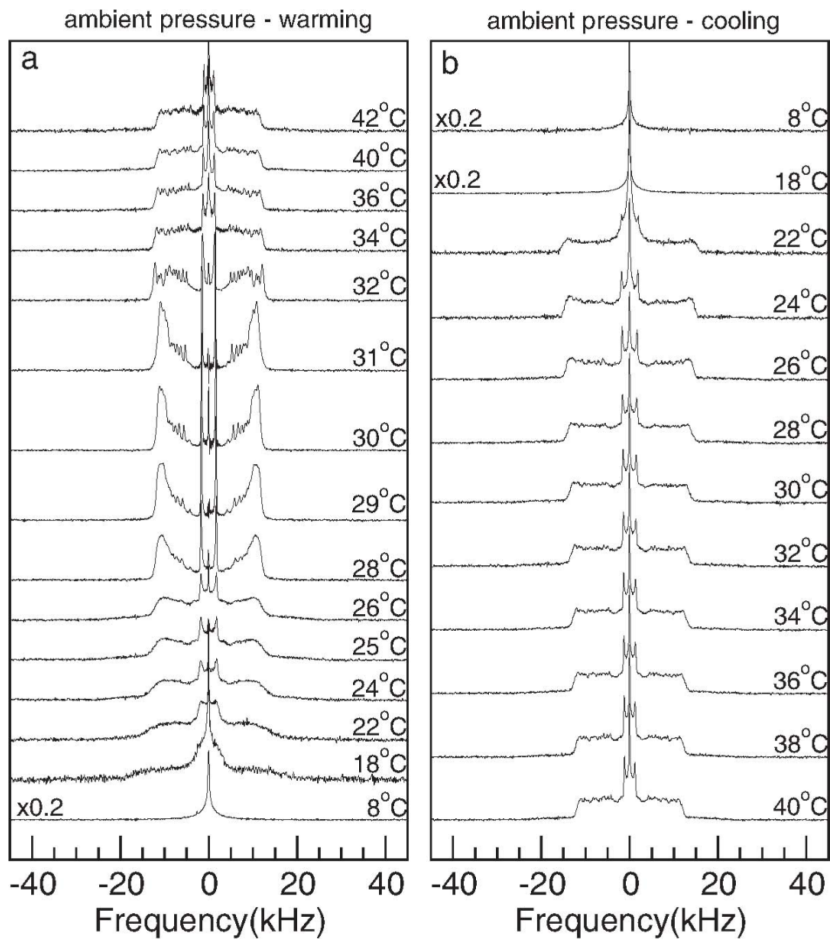
How does anionic lipid change mixture behaviour?

DMPC-d_{54}/DMPG/DHPC (3:1:1)

At ambient pressure:

- DMPG modifies "orientable phase" temperature range
- Oriented-to-lamellar transition more distinct
  - Lamellar more MLV-like
- Orientable phase suppressed on cooling

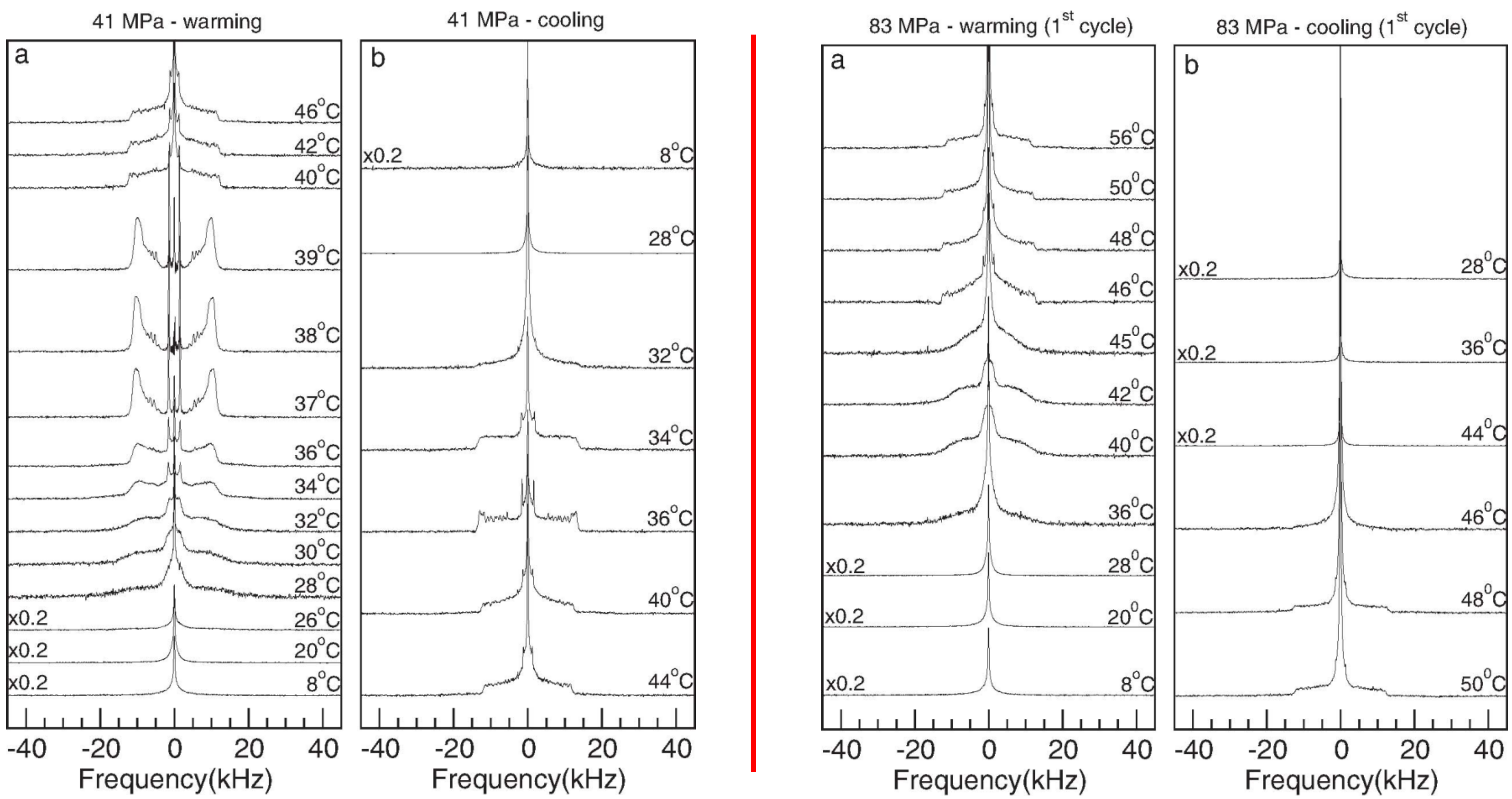
Rahmani et al. (2013) *Langmuir* **29**, 13481



How does anionic lipid change mixture behaviour?

DMPC-d_{54}/DMPG/DHPC (3:1:1)

Rahmani et al. (2013) *Langmuir* **29**, 13481

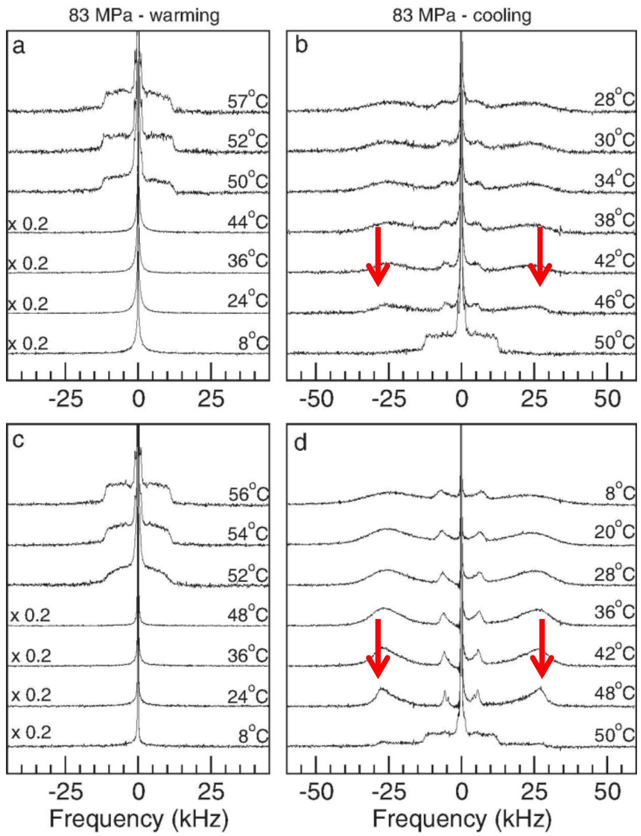


- Aside from shift, behaviour at 41 MPa similar to ambient.
- No orientation seen on heating at 83 MPa.

How does anionic lipid change mixture behaviour?

Rahmani et al. (2013) *Langmuir* **29**, 13481

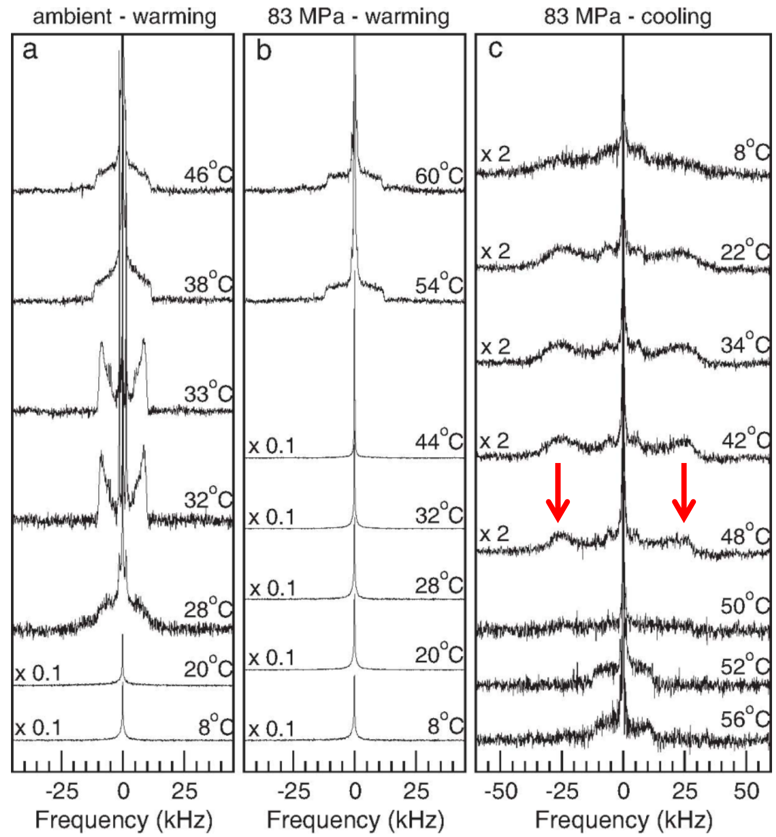
DMPC-d_{54}/DMPG/DHPC (3:1:1)



For 2nd cycle at 83 MPa, see intensity at ~±28 kHz.

- Interdigitation.

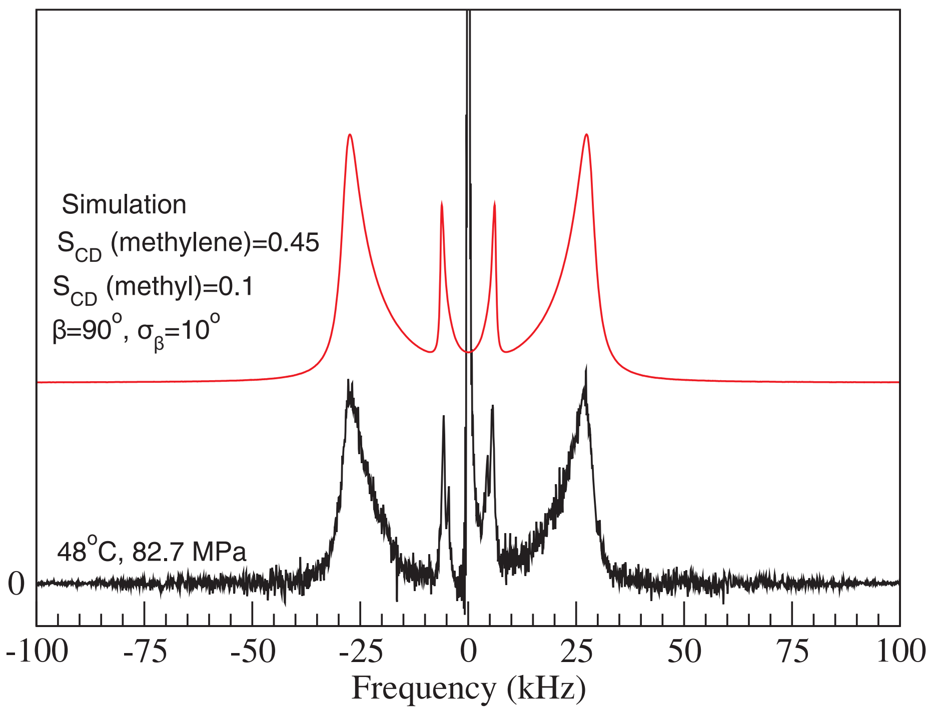
DMPC/DMPG-d_{54}/DHPC (3:1:1)

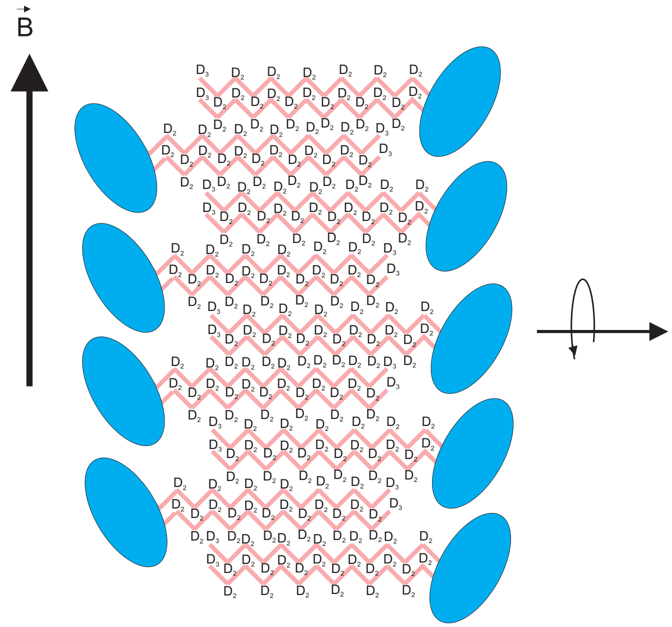


Same behaviour when DMPG deuterated.

- Not a demixing effect

^2H NMR Spectrum - Interdigitated gel





S_{CD}=0.5 corresponds to CD bonds perpendicular to rigid chain axis

Can simulate assuming:

- high, uniform chain order
- axially symmetric reorientation about oriented bilayer normal

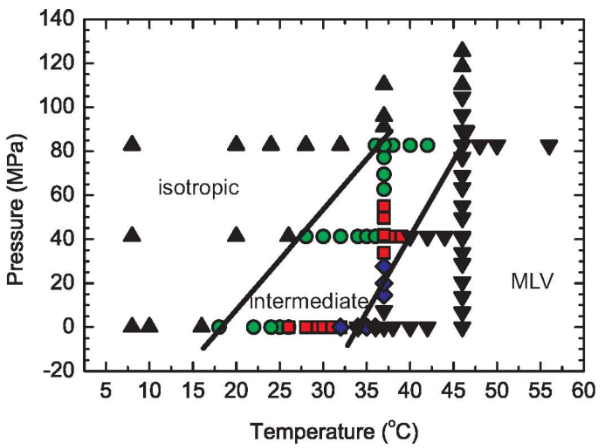
Isothermal Pressurization and Depressurzation

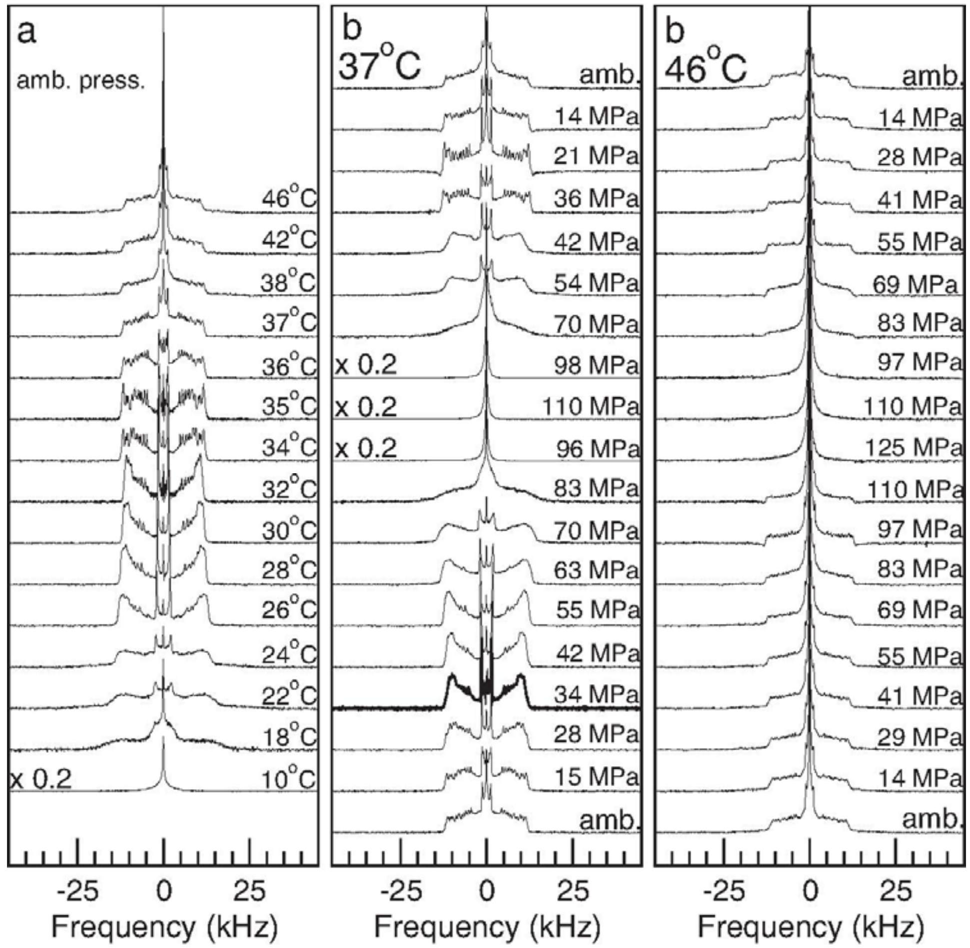
DMPC-d_{54}/DMPG/DHPC (3:1:1)

Isothermal pressurization and depressurization at 37°C (to 110 MPa) and 46°C (to 125 MPa)

- No interdigitation

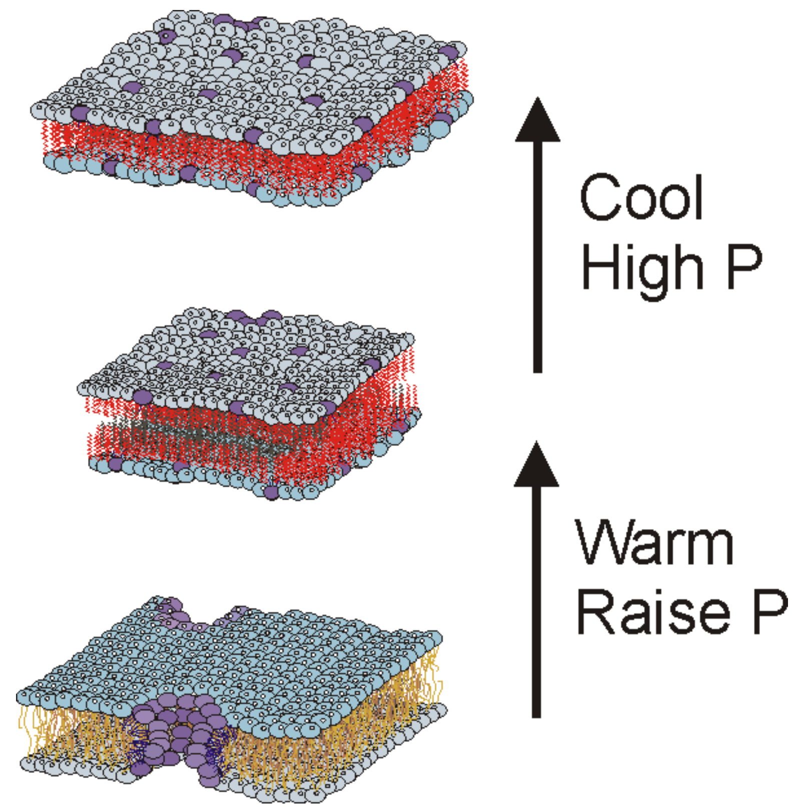
Interdigitation is not an equilibrium transition under these conditions!





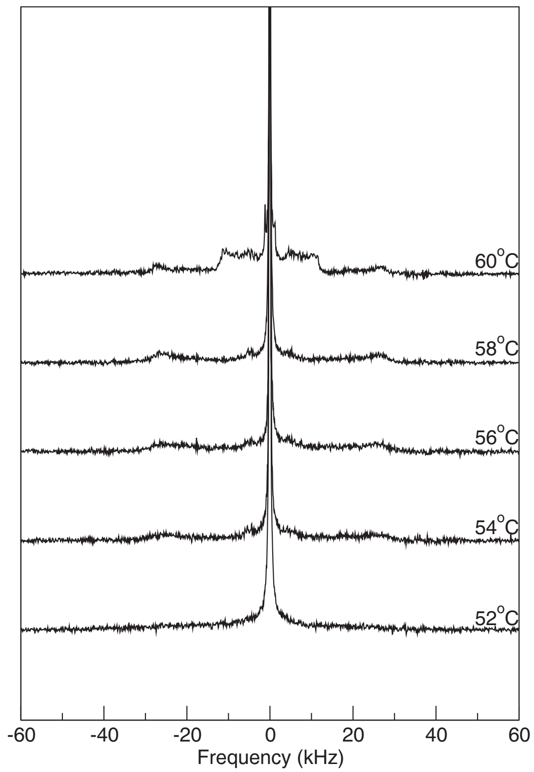
Kinetic trapping?

- DHPC lines pores in lamellar phase
- Raising T increases DHPC mixing with longer chain lipids
- On cooling at high P, longer chains order.
  - likely inhibits DHPC lateral diffusion and reaggregation
  - non-equilibrium distribution of the short chain lipid
- Trapped DHPC perturbs long chain packing
  - promotes interdigitation
- Interdigitation likely precludes DHPC reaggregation



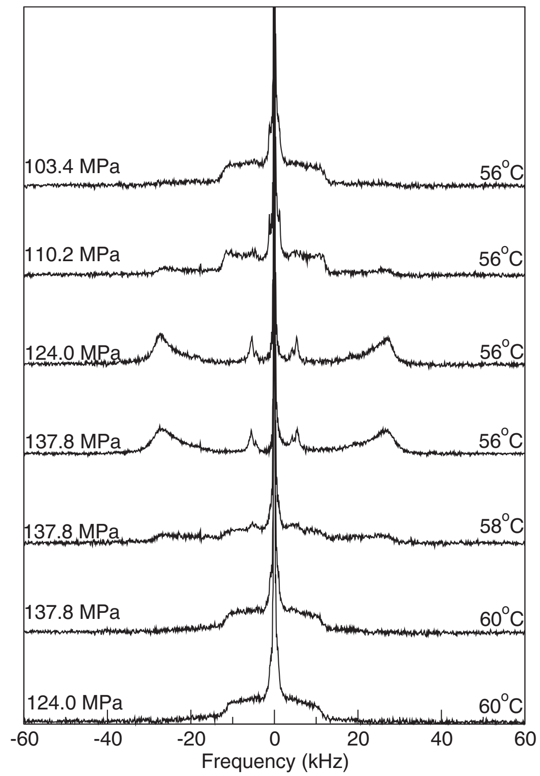
Equilibrium interdigitation in DMPC-d_{54}/DMPG/DHPC (3:1:1)?

Isobaric warming



Isotropic to interdigitated to lamellar on warming at 138 MPa

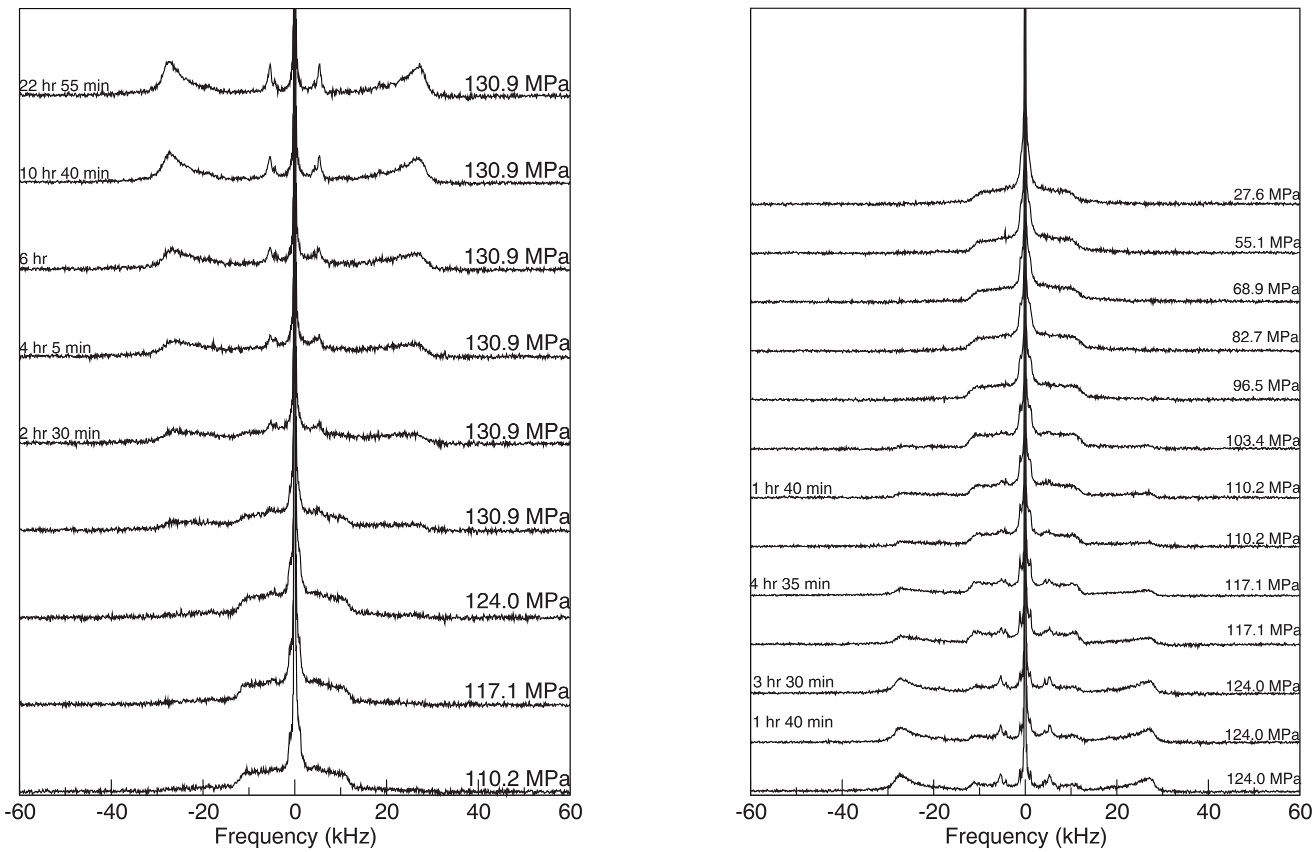
Return to lamellar on cooling



Lamellar to interdigitated to lamellar on cooling and varying pressure

Equilibrium interdigitation in DMPC-d_{54}/DMPG/DHPC (3:1:1)?

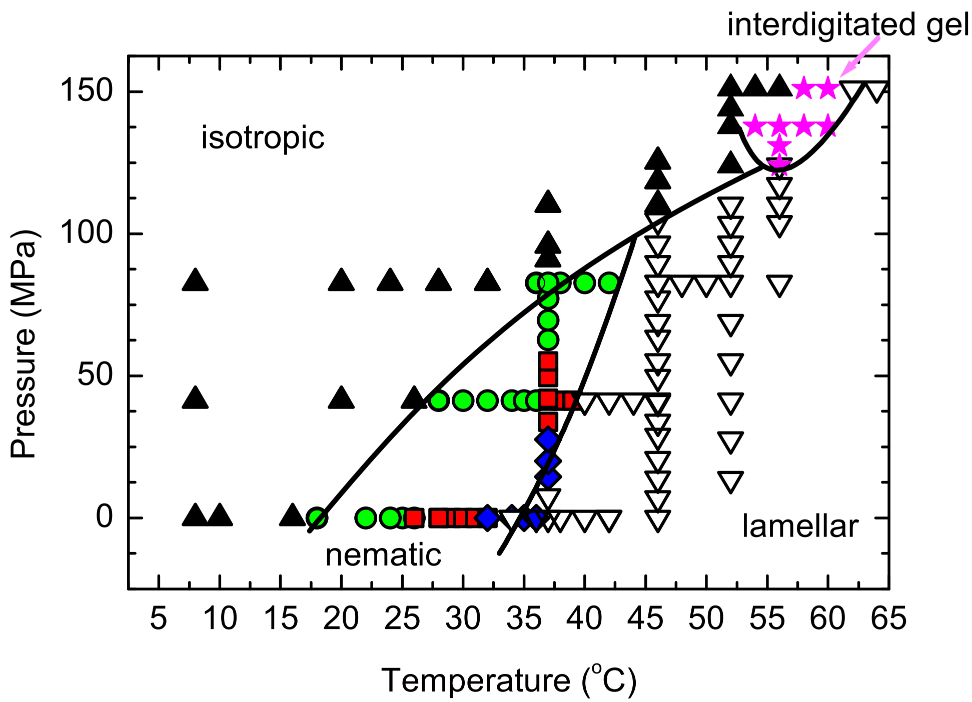
Pressurize and Depressurize at 56°C



Reversible interdigitation at 56°and 125-130 MPa

Phase diagram

(Based on morphologies seen while warming or raising pressure)



- No magnetic orientation seen above ~100 MPa
- Persistent interdigitation still seen when cooling from "equilibrium" interdigitation region (pink stars) of phase diagram

Summary

- Anionic lipid lowers minimum interdigitation pressure
  - cf. 400 MPa for DMPC/DHPC by Winter group
- Magnetic orientation not seen above 100 Mpa
  - How does anionic lipid affect dP/dT for orientable-to-extended lamellar transition?
- Cooling at high pressure promotes “non-equilibrium” interdigitation
  - can persist to low pressure and low temperature
  - Possibly “kinetic trapping” of a non-equilibrium lateral distribution of short chain lipid

Collaborators:	Valerie Booth (Biochemistry) – lung surfactant, AMPs Anand Yethiraj (Physics) – bicellar material, microgel
Current Undergrad:	Collin Knight – bicellar material
Current Grad:	Ashkan Rahmani – bicelles at high pressure Gagandeep Kaur Sandhu (Morrow,Booth) - AMPs Suhad Sbeih (Morrow, Yethiraj) - mirogels
Recent Undergrad:	Lauren MacEachern – bicellar material Alex Sylvester (Morrow,Booth) – peptides in bicelles Stephen Ratighan – DLS on vesicles Pamela Rennie (WISE student) William Whelan (Morrow,Booth) – DSC on bacteria Alanna Flynn (Morrow) - bicellar material
Recent Grad:	Dharamaraju Palleboina (Morrow, Booth) - lung surfactant James Pius (Booth, Morrow) - antimicrobial peptides

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