# New insight into biophysics of lipid membranes with high resolution IXS

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# Fast and slow dynamics in a cell membrane

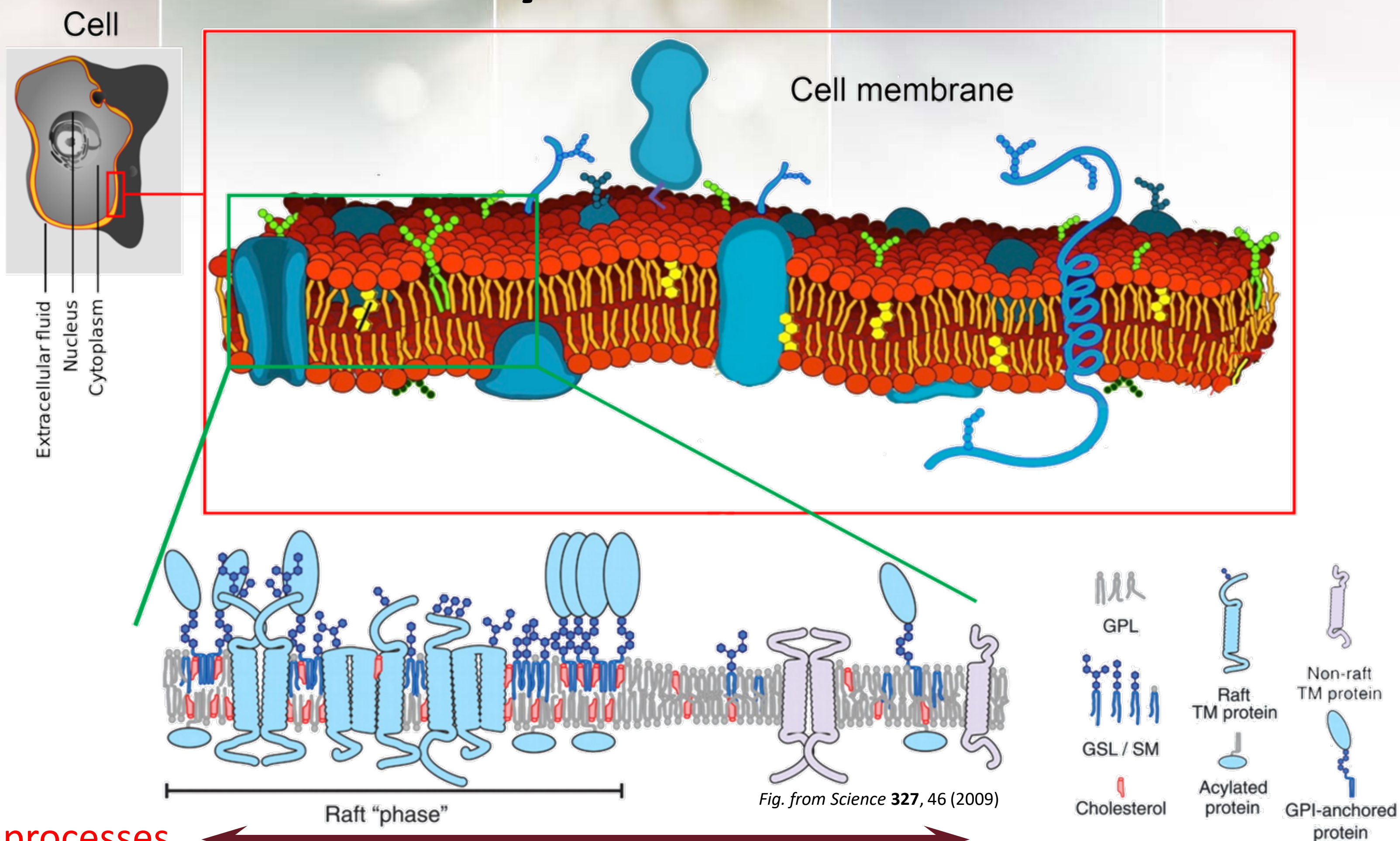



*Fig. from Science* **327**, 46 (2009)

Dynamic processes in a cell membrane is virtually impossible to study due to its complexity

**XPCS**
Slow dynamics on mesoscale:
**seconds to μs**

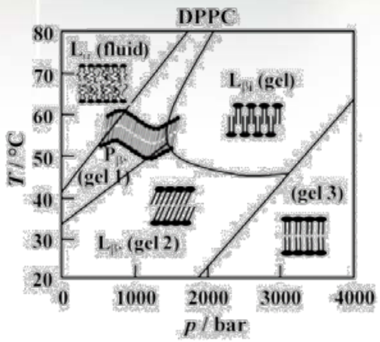
**Slow dynamics:**
Lipid domain (rafts) formation and diffusion, anomalous subdiffusion

**Ultrafast dynamics:**
Protein and lipid diffusion, lateral and transmembrane transport

**IXS**
Fast dynamics on molecular scale:
**picoseconds**

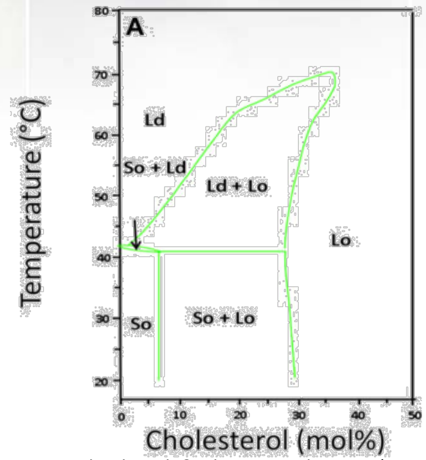
# Model membranes as a test bed

## Single lipid



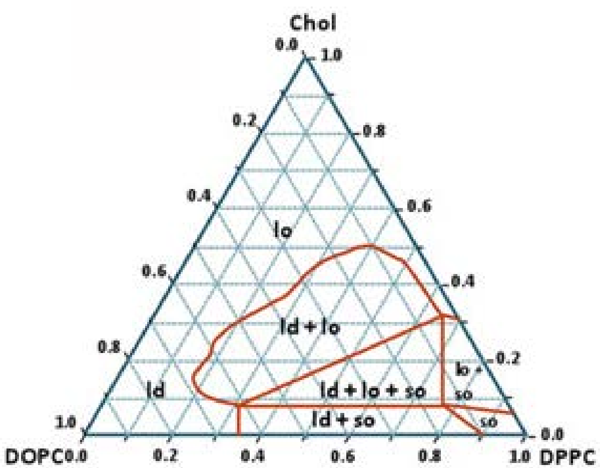

R. Winter, C. Jeworrek, Soft Matter, 2009, 5, 3157

## Binary systems (DPPC-Chol)



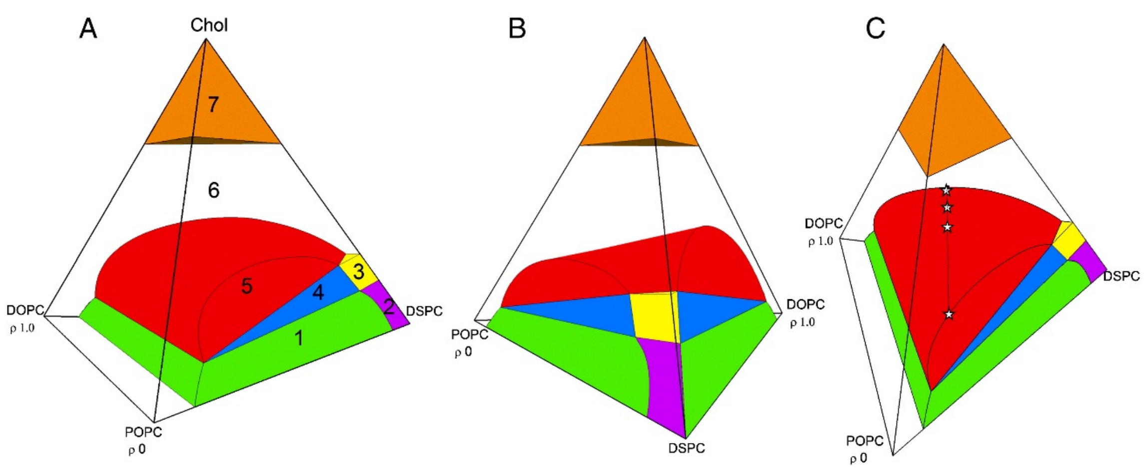

de Almeida&Joly, Front. Plant. Sci (2014)

## Ternary systems



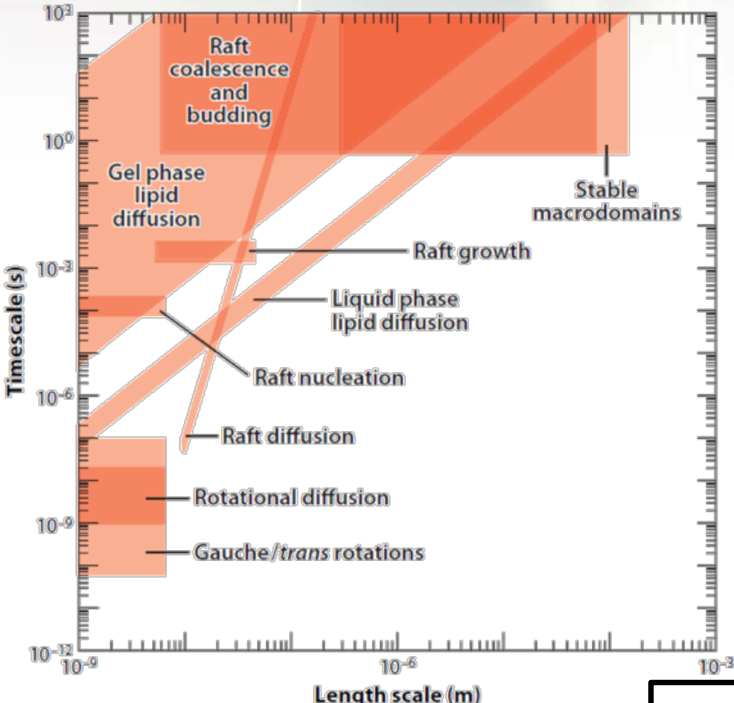

de Almeida&Joly, Front. Plant. Sci (2014)

## Quaternary phase diagram

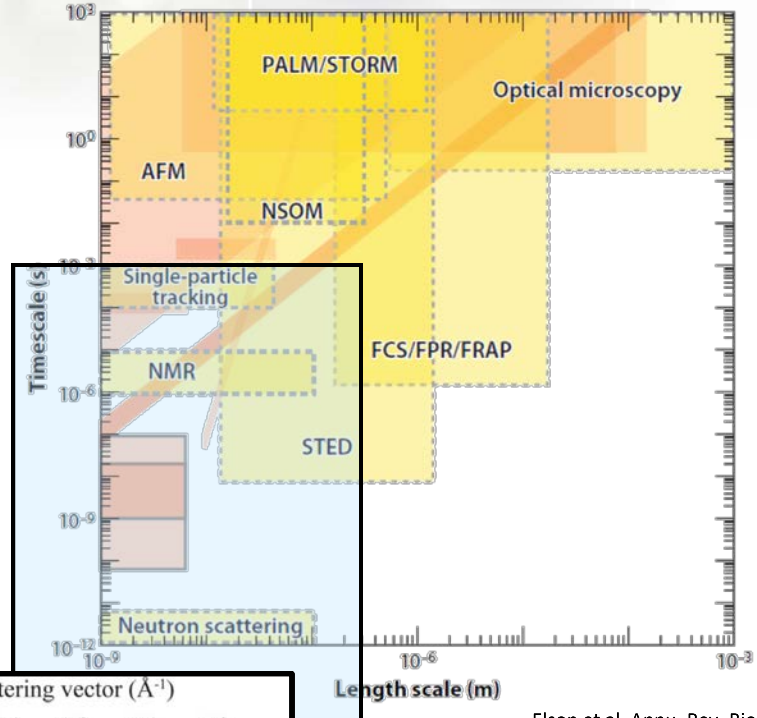


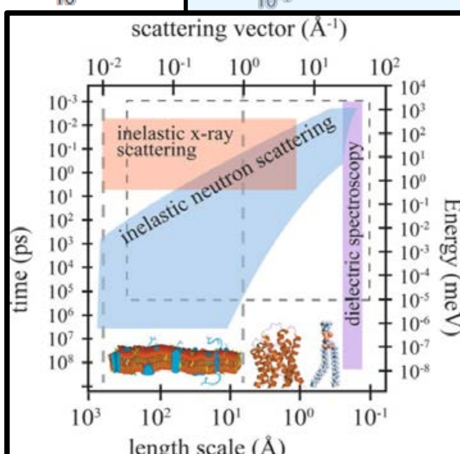

G. Feigenson, Cornell U

# Model membranes as a test bed




Predicted and observed phenomena in lipid layers span broad length and timescales



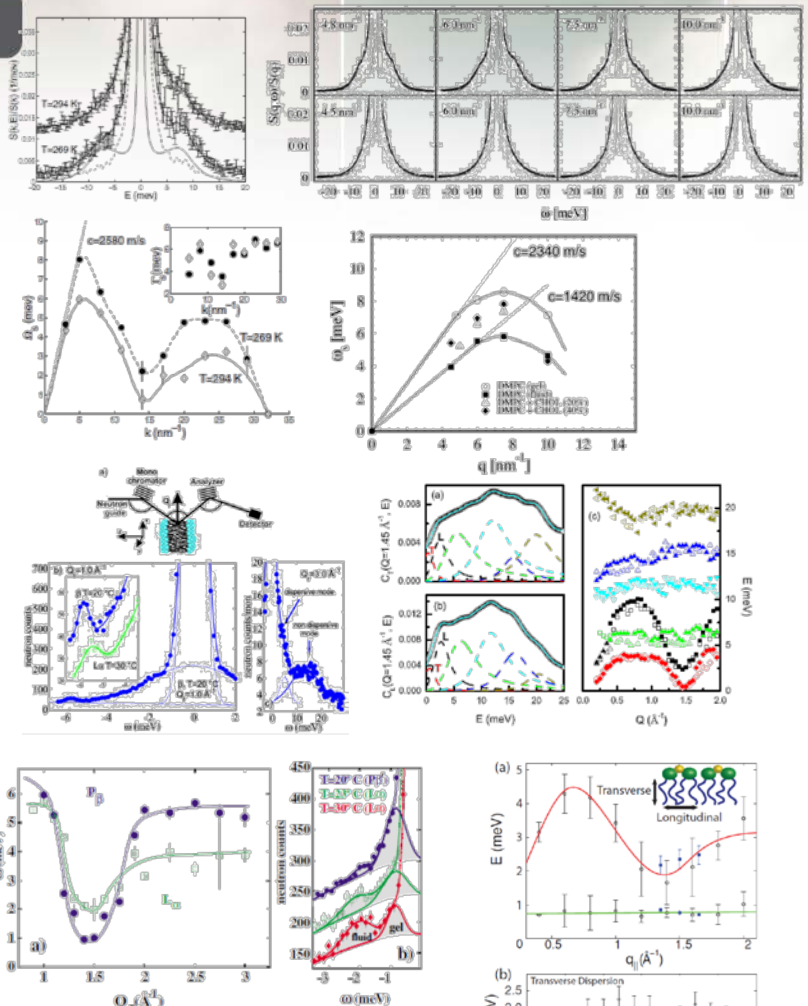



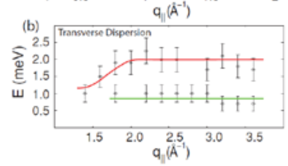

Elson et al. Annu. Rev. Biophys. 2010. 39:207–26

Experimental techniques cover most of “large” and relatively “slow” lipid behavior.

Still do not adequately determine “small” and “fast”

# Previous efforts to study fast dynamics





S.H. Chen et al. Phys. Rev. Lett. **86** (2001) 740
M. Tarek et al. Phys. Rev. Lett. **87** (2001) 238101
T. Weiss et al. Biophys. J. **84** (2003) 3767
M. C. Rheinstadter et al. Phys. Rev. Lett. **93** (2004) 108107
V. Conti Nibali et al. Phys.Rev. E **89** 050301(R) (2014)
M.Kaye, et al., Phys.Rev.E **83** 050907(R) (2011)

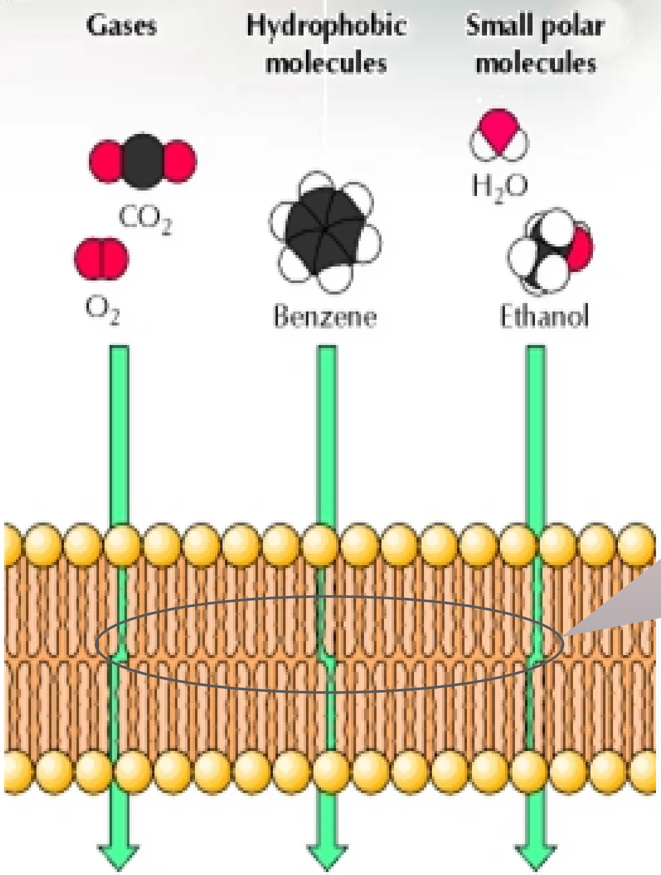

- Different parts of lipid molecules contribute to different excitations, which all can, in principle, be probed by inelastic scattering
- Generalized three effective eigenmode (GTEE) theory to fit the IXS/INS data:

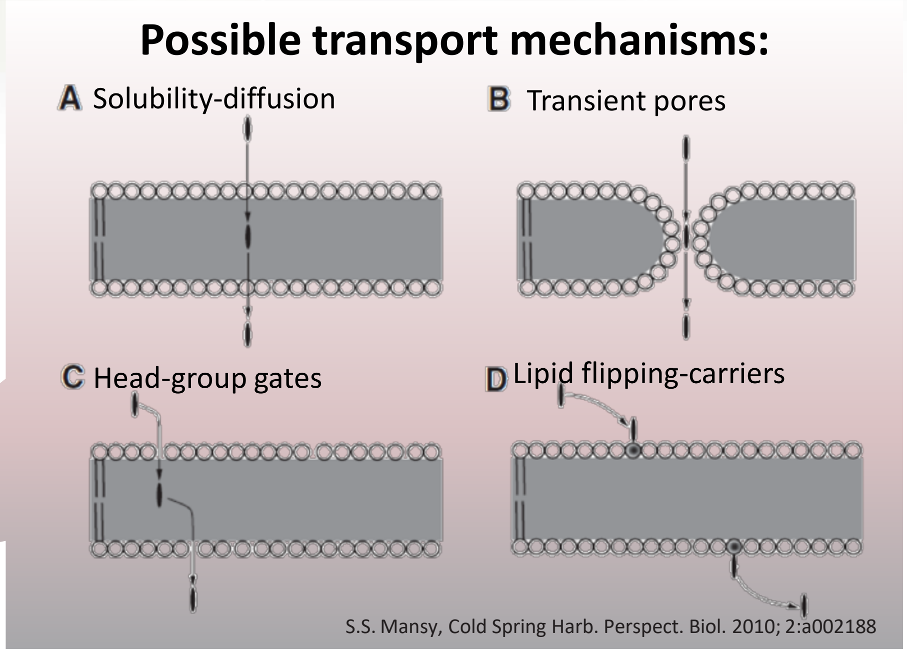
$$\frac{S(Q,\omega)}{S(Q)} = \frac{1}{\pi}\left[A_0\frac{\Gamma_h}{\omega^2+\Gamma_h^2} + A_s\left(\frac{\gamma_s + b(\omega+\omega_s)}{(\omega+\omega_s)^2+\gamma_s^2} + \frac{\gamma_s - b(\omega-\omega_s)}{(\omega-\omega_s)^2+\gamma_s^2}\right)\right].$$

- GTEE → treats the data in terms of simple liquids which is an oversimplification

Very few processes take place on ps time scale: trans-gauche isomerization, density fluctuations, ultra-fast diffusion

# Passive transport mechanisms



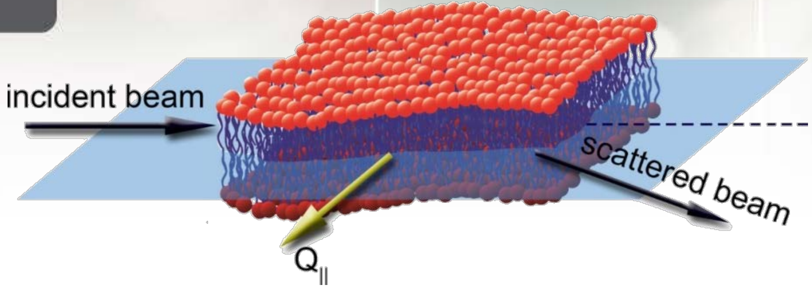

IXS is perfect to study ultrafast **thermally-triggered** phonon-mediated transport mechanisms

DPPC lipid → main constituent of pulmonary surfactant which determines $O_2$ transfer rate in lungs

Transient Pore Model
***for unsaturated lipids***

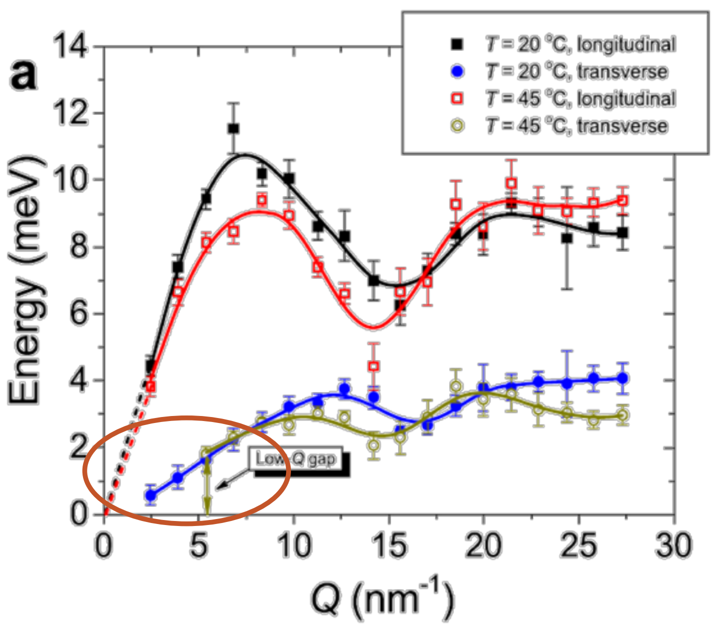
Packing Defect Model
***for saturated lipids***

# IXS measurements: DPPC lipid

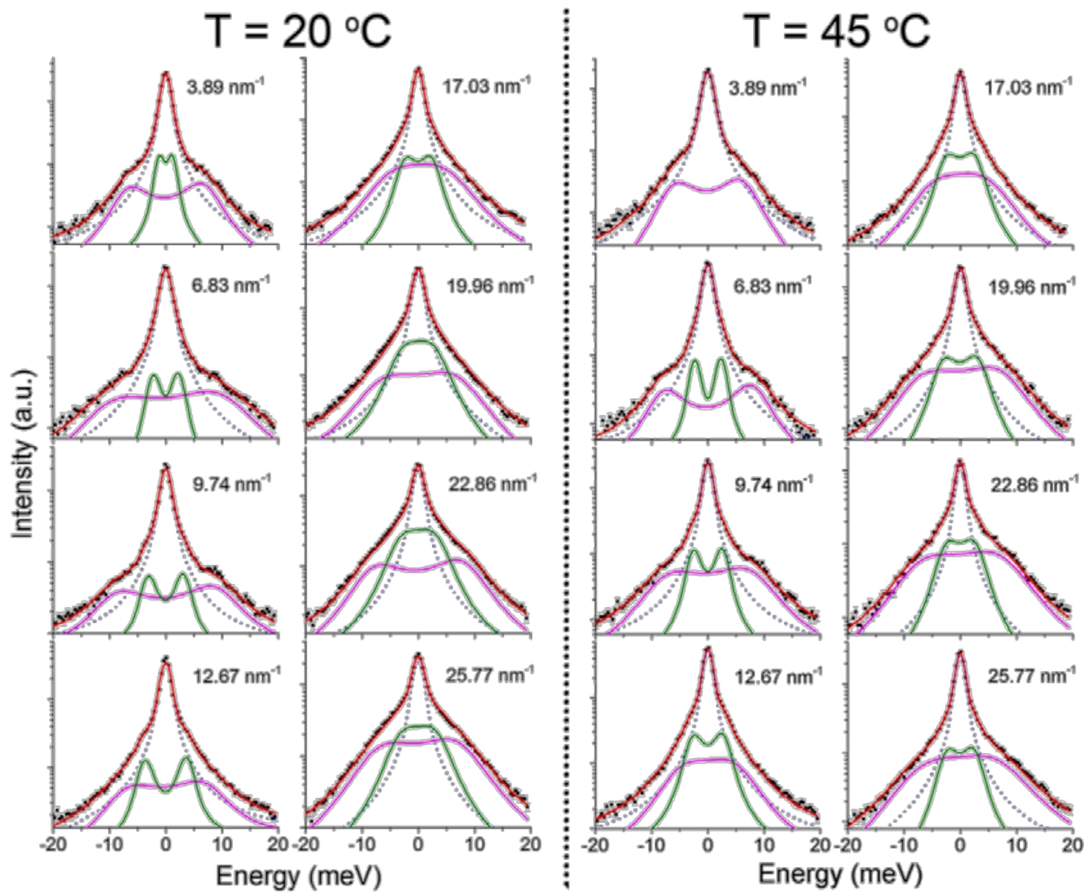



- DPPC main transition temperature: 41 °C
- Measured at 20 °C and 45 °C; E = 21.78 KeV
- Saturated water vapor

DPPC spin-coated on diamond substrate → *stack of thousands of lipid bilayers*




M. Zhernenkov et al. *Nat. Commun.* **7**, 11575 (2016)




**The discovery of the low-Q phononic gap in TA mode!**

# Low-Q phononic gap in TA mode

Low-Q phononic gap

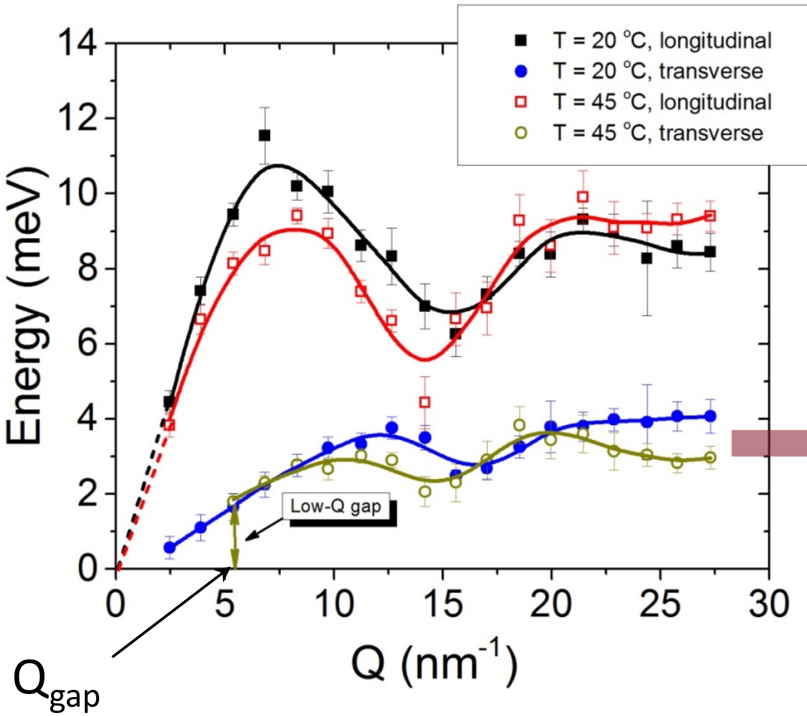
The cut-off value $Q_{gap}$
*(at which gap occurs)*

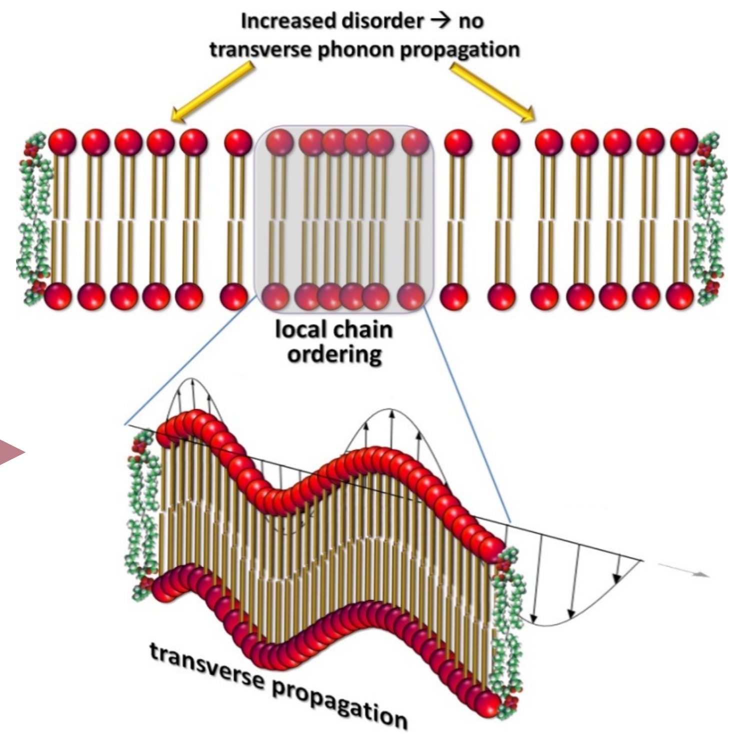
No phonon propagation at $Q < Q_{gap}$

Characteristic length-scale $d_{gap} = 2\pi/Q_{gap}$ of a local "order"
*(no phonons at smaller Q → more disorder on long scale! )*

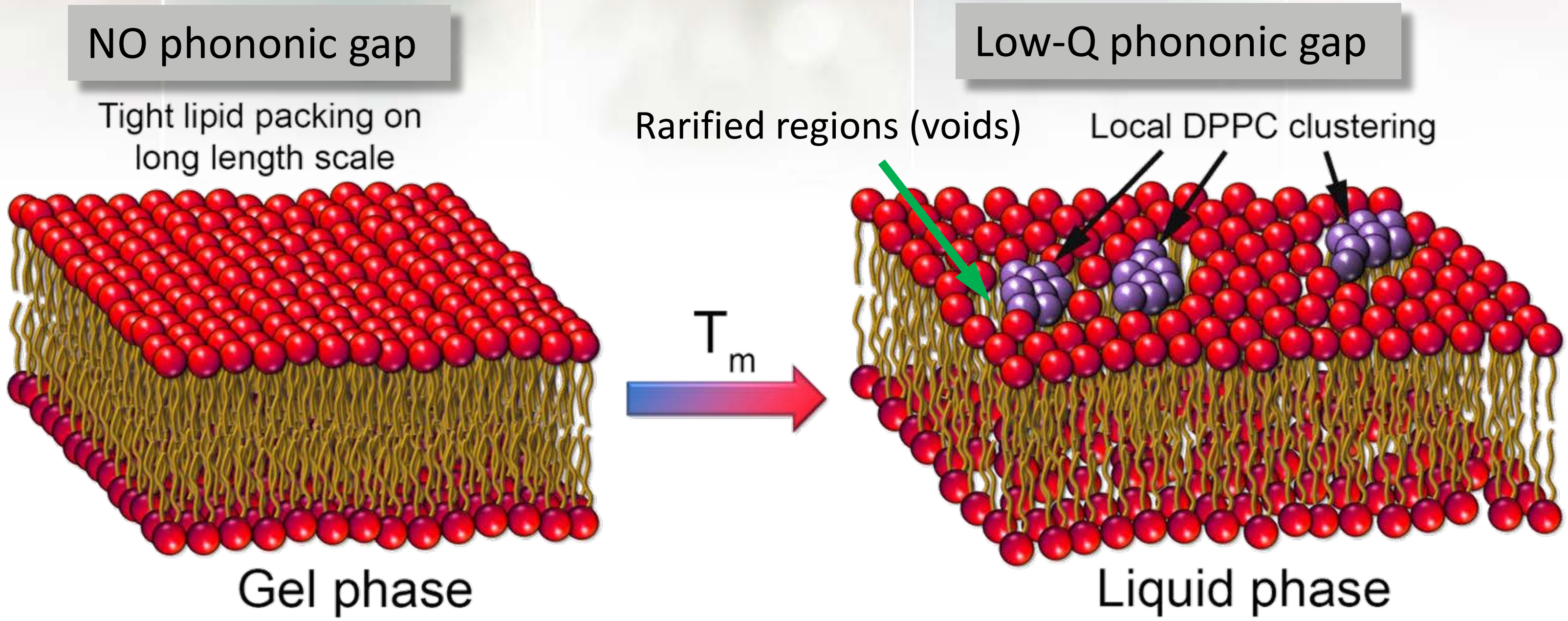
D. Bolmatov, *et al. Ann. Phys.* ***363****, 221-242 (2015)*

D. Bolmatov, *et al. J. Phys. Chem. Lett.* ***6****, 3048-3053 (2015)*

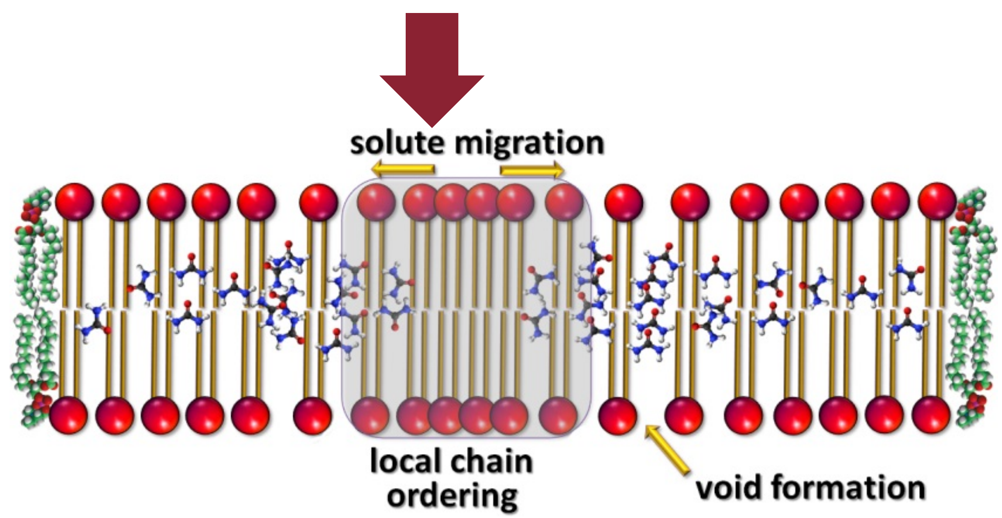


# Phonon-mediated nm-scale clustering




- the cluster size $d_{gap} = 2\pi/Q_{gap}$ is ~ 1.1-1.6 nm
- Lifetime: ~1 ps (agrees well with common assumptions – ***a few ps***)




M. Zhernenkov et al. *Nat. Commun.* **7**, 11575 (2016)

# Phonon-mediated nm-scale clustering

**Theory of solute diffusion through a membrane:**

- ultra-fast "hopping", or "rattling" between thermally-triggered voids
- partition coefficient strongly depends on the local chain ordering → solute exclusion within the region
- Potential formation of water fingers inside voids → proton translocation through membrane

Adv. Drug Deliv. Rev. **58**, 1357–1378 (2006)
J. Am. Chem. Soc. **117**, 4118–4129 (1995)
Cold Spring Harb. Perspect. Biol. (2010), 2, a002188

We observe:

- ✓ nm-scaled short-lived molecular clusters → local chain ordering, or density fluctuations
- ✓ Increased disorder beyond the cluster size → indication of the transient voids formation
- ✓ Size and the life time of the clusters agrees well with the theory prediction

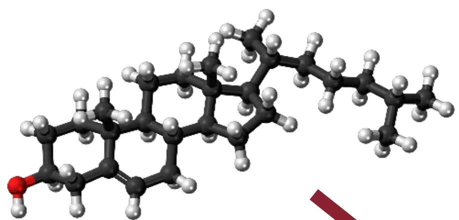
# DPPC-Cholesterol binary mixtures

Found in every cell of human body

Can make up to 50% of a cell membrane

Required for phase separation and formation of rafts (dynamic “glue”)

Cholesterol in a cell



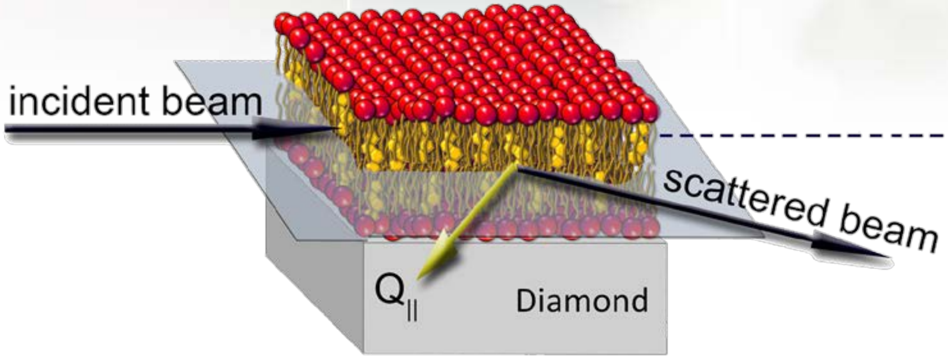
helps maintain the integrity of the membranes

facilitates cell signaling

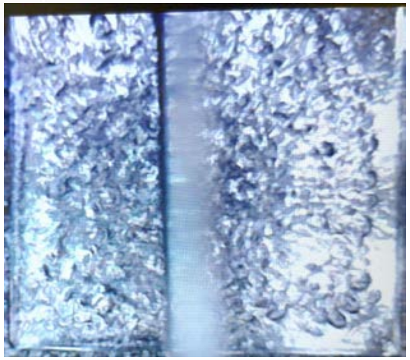
secures proteins in the membrane

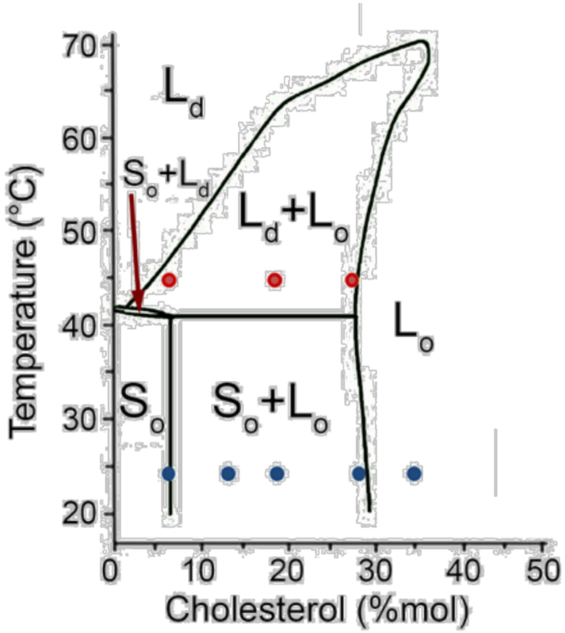
maintains the fluidity of cell membranes

# IXS measurements: DPPC-Chol

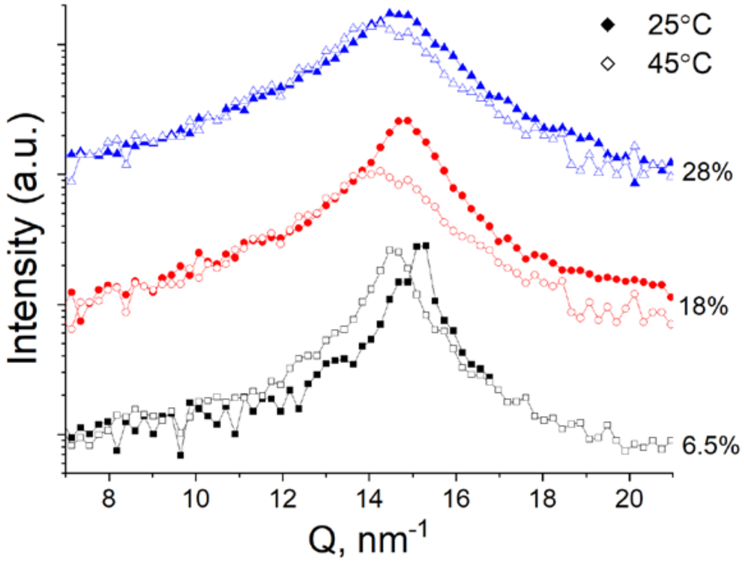



Top view of a lipid multilayer on diamond



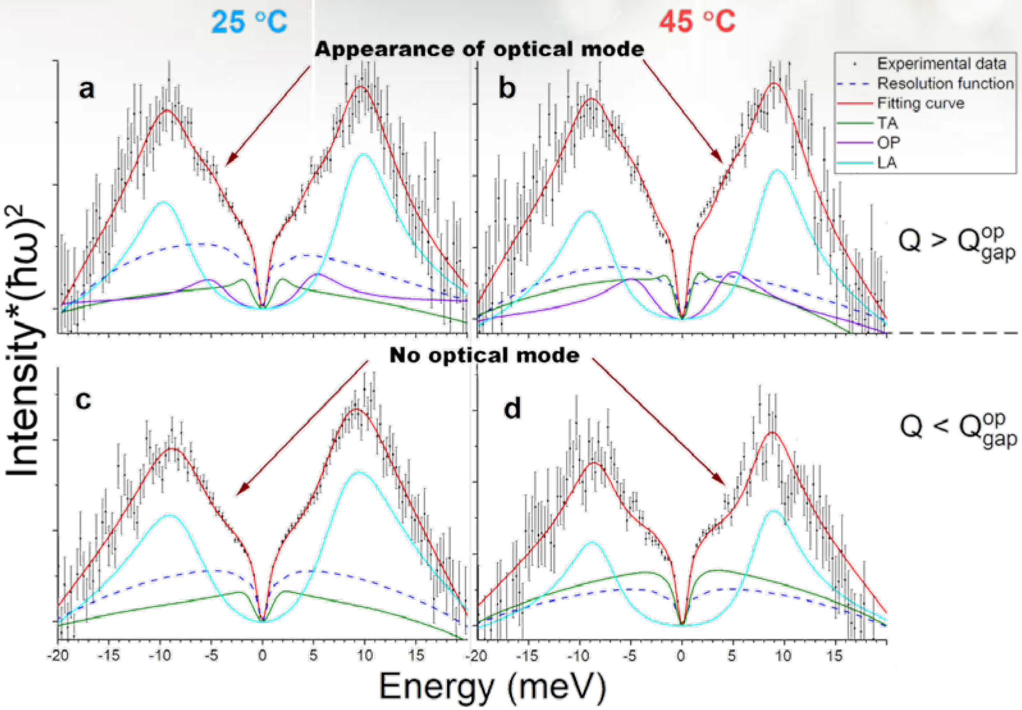



De Almeida & Joly, Front. Plant Sci. (2014)

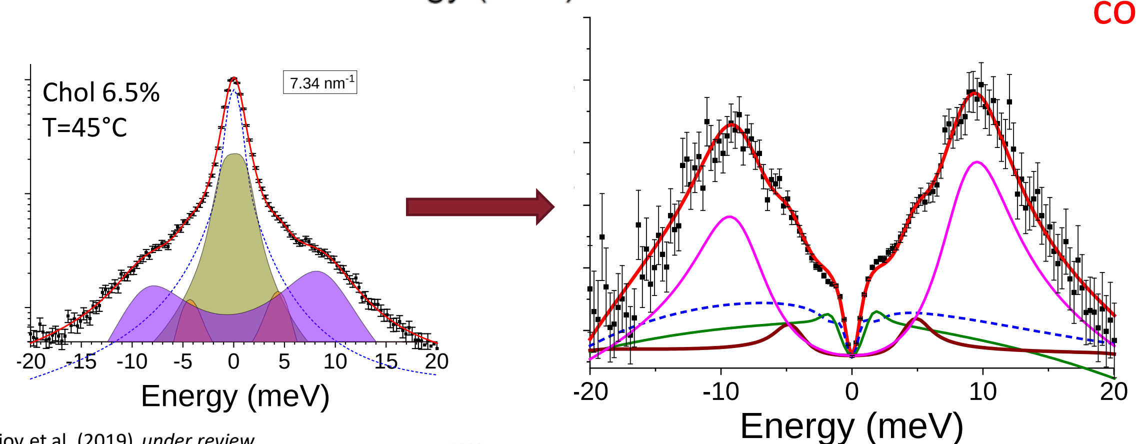


# IXS phonon current spectra

IXS currents for Chol(28%)

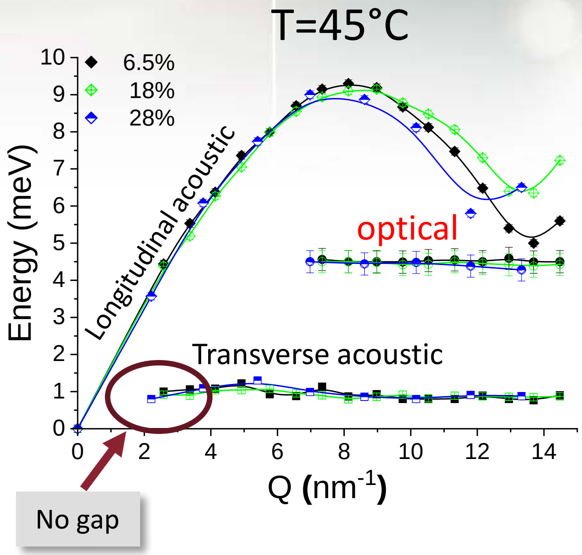


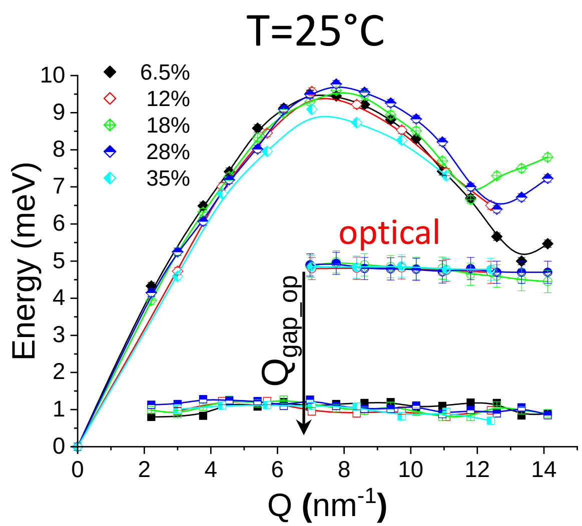

- Two or more “particles” per “unit cell” should generate the optical mode(s) due to out-of-phase movements
- Optical mode emerges at $Q_{gap\_op} \geq 7$ nm-[1] for all temperatures
- Transverse mode shows no $Q_{gap\_ta}$ within measured Q-range (in contrast to pure DPPC!)




D. Soloviov et al. (2019) *under review.*

# DPPC-Cholesterol binary mixtures



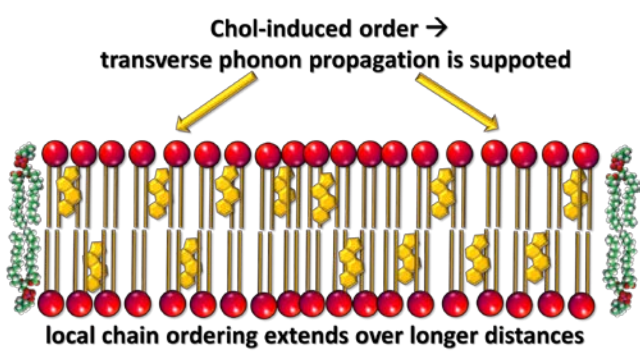

**Low-Q phononic gap in OP mode!**

- Longitudinal acoustic (LA) → compression waves (sound) propagating in lipids, no $Q_{gap}$ allowed
- Transverse acoustic (TA) → oscillatory movements of lipids due to shear restoring forces (short range)

No measurable $Q_{gap}$ in TA mode

Cholesterol adds rigidity + local ordering

Reduced diffusion rates




D. Soloviov et al. (2019) *under review.*

# Origin of phononic gap in optical mode

Optical and acoustic vibrations in linear diatomic chain

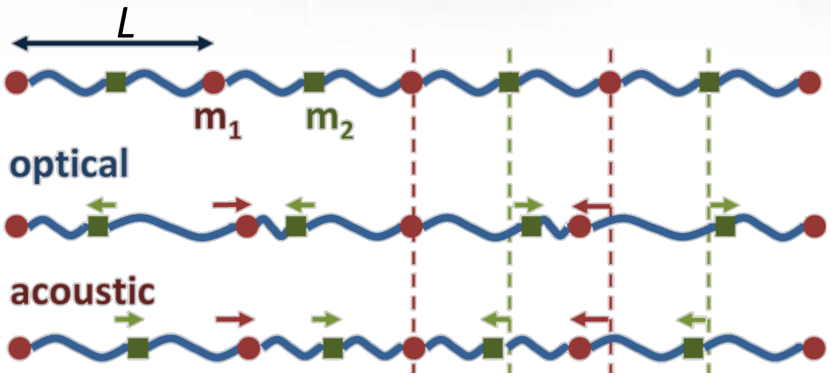
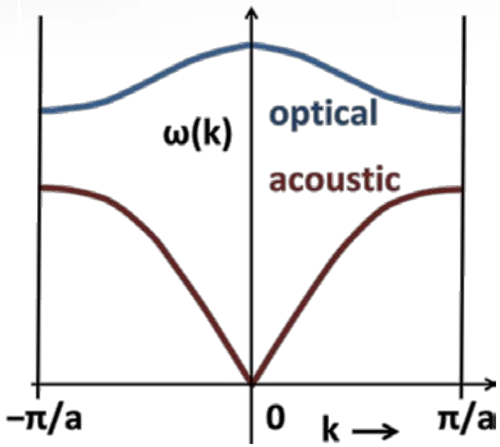



Image credit: Wikipedia




Optical mode frequency:

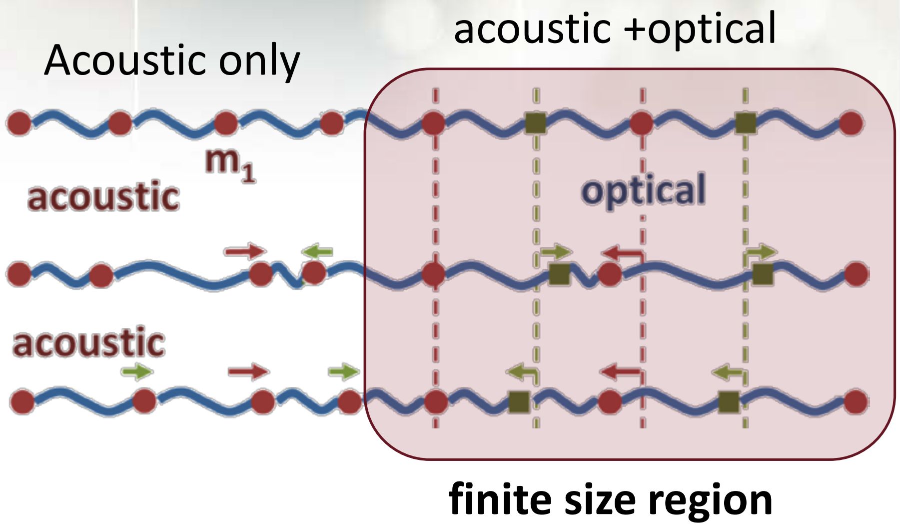
$$\nu^2 = \alpha\left(\frac{1}{M_1}+\frac{1}{M_2}\right) + \alpha\sqrt{\left(\frac{1}{M_1}+\frac{1}{M_2}\right)^2 - \frac{4\sin^2(QL)}{M_1 M_2}}$$

at Q→0 $\quad \nu^2 \sim 2\alpha\left(\frac{1}{M_1}+\frac{1}{M_2}\right)$

$\alpha$ - interparticle force constant

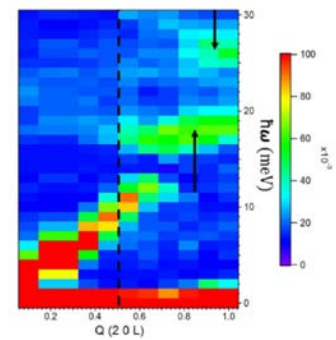
Under a continuous medium approximation (Q→0)
the optical mode cannot exhibit the phononic gap $Q_{gap}$!

# Phononic gap in optical mode: finite size effect



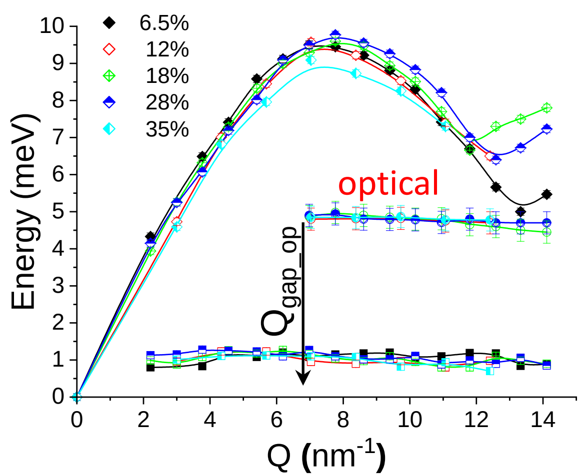

## Recent example

Finite size effect on phonons of the quasi-one-dimensional system $SrCuO_2$: doping breaks symmetry and suppresses optical mode at low Q




D. Bounoua et al. Physica B 536 (2018) 323–326

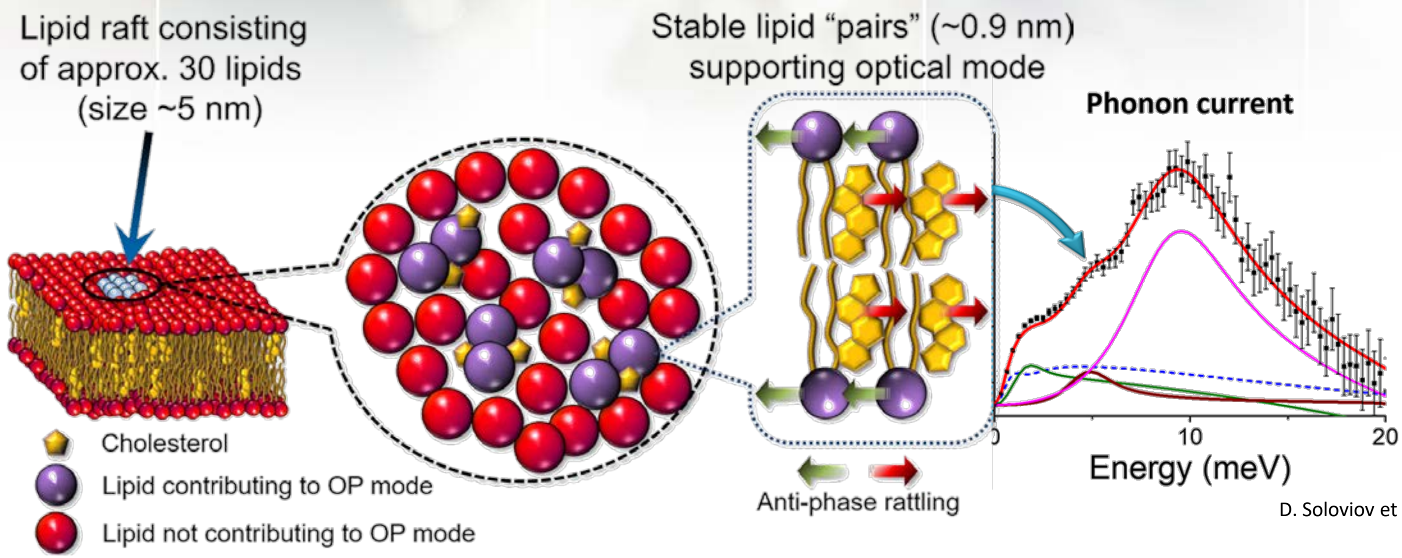
## DPPC-Cholesterol case




D. Soloviov et al. (2019) *under review.*

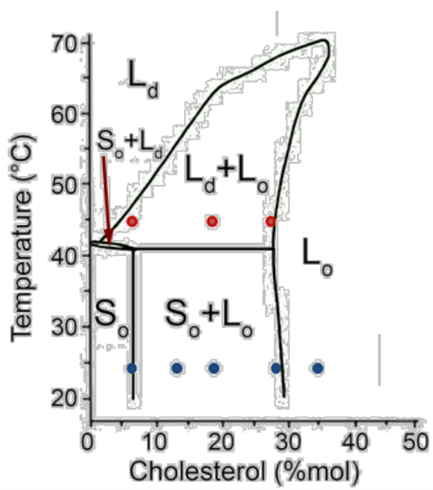
- Two lipid “particles” per “unit cell”
- $Q_{gap\_op}$ in lipids → finite size regions throughout the sample supporting optical mode
- Size of the region: $2\pi/Q_{gap\_op}$ ~ 9 Å or several lipids in diameter!

# Phononic gap in optical mode: DPPC-Chol “pairs”




D. Soloviov et al. (2019) *under review.*

**Lo phase → set of discrete patches with 2-3 coupled lipid pairs**




De Almeida & Joly, Front. Plant Sci. (2014)

- OP mode (~5meV) observed when $L_o$ phase is present
- Optical phonon energy estimation for DPPC-Chol pair: 4-7.5 meV
  - DPPC-Chol interaction energy (1-3.6 kT)*
  - Simple Hooke’s law
  - $\nu^2 \sim 2\alpha(\frac{1}{M_1} + \frac{1}{M_2})$

*Heberle, F.; Feigenson, G. Cold Spring Harb. Perspect. Biol. (2011)
Zeno, W. et al. Langmuir (2016)

# Ternary systems: DOPC/POPC-based

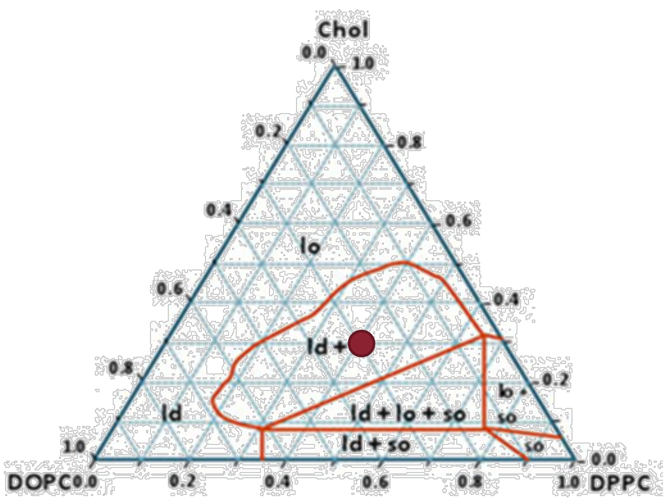
Ternary systems

3 lipid "particles"

3 combinations of "pairs"
*(but not all "pairs" are equally favored!)*

More optical modes allowed

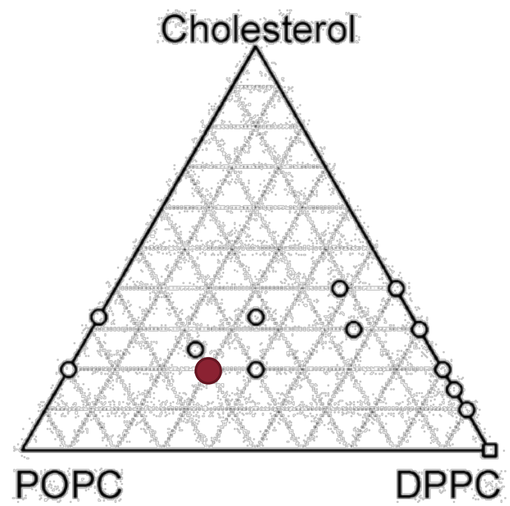
*Expectation: in reality, optical modes should be mixed in soft matter*




de Almeida&Joly, Front. Plant. Sci (2014)

DOPC(3):DPPC(4):Chol(3)

Lo+Ld

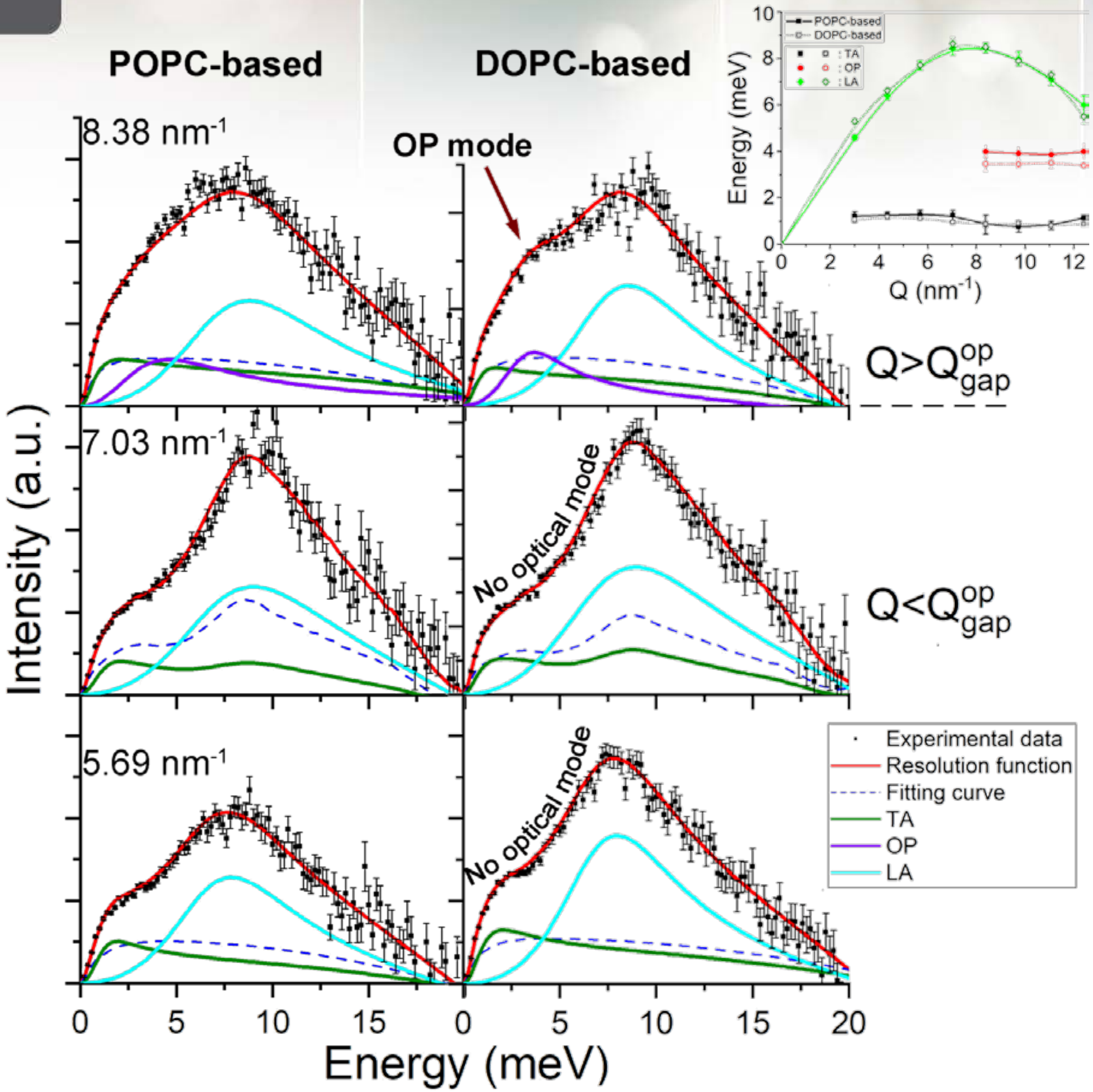



Veatch&Keller, Phys.Rev.Lett. (2005)

POPC(5):DPPC(3):Chol(2)

Coexisting liquid domains are not previously observed

# IXS: ternary systems at 37°C

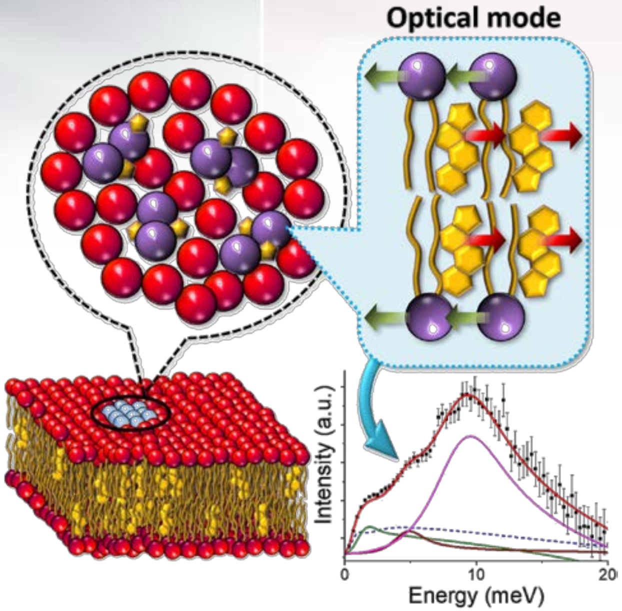



- Optical mode emerges at $Q_{gap\_op} \geq 8.3$ nm-[1]
- OP energy: 3.5-3.9meV
- Size of the region: $2\pi/Q_{gap\_op}$ ~ 7.5 Å or several lipids in diameter
- OP energy estimation for DOPC/POPC-DPPC pairs: 1.6 meV (unfavorable pairs)
- Unsaturated lipids reduce the size of discrete patches!
- No patches in Ld phase with excess of unsaturated lipids

D. Soloviov et al. (2019) *under review.*

# Summary

**Observations:**




- Multicomponent phase separated membranes exhibit optical modes
- Optical modes are supported on a short length scale → indication of discrete nano-patches (*finite size effect*), or lipid "pairs"

  (*only groups of two+ lipids can support optical mode!*)

- Optical mode only observed in phase coexistence, or when $L_o$ phase is present

**Hypothesis:** in phase separated domains, a single domain with typical sizes of 10-200 nm on nanoscale consists of a set of mobile coupled discrete areas <1 nm in diameter. The large domains form when "pairs" transiently coalesce into larger entities.

- In multicomponent mixtures: different combination of "pairs" can couple due to the selective affinity between different lipid species

# Acknowledgements

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THANK YOU!