Application of inelastic scattering to study biomembranes: latest results and challenges

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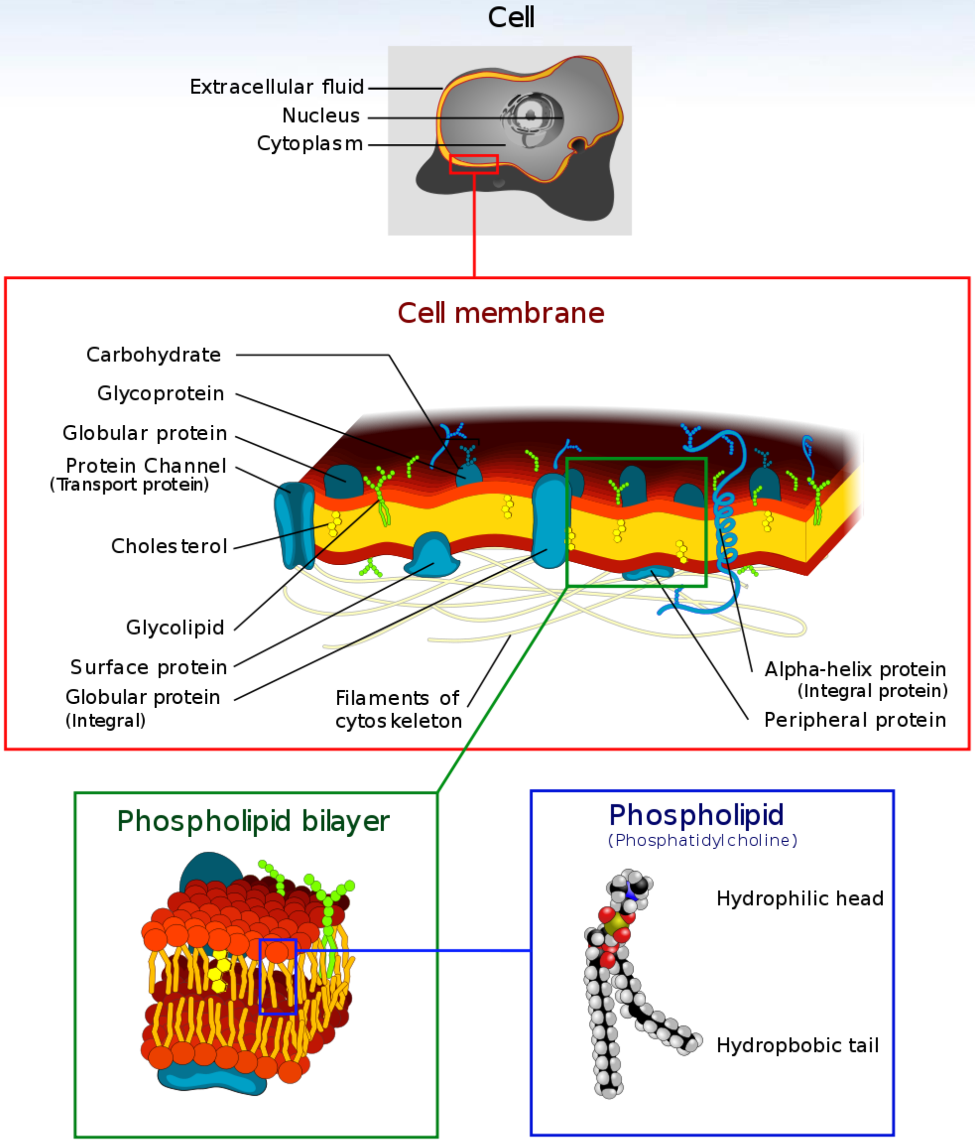
Cell membrane structure



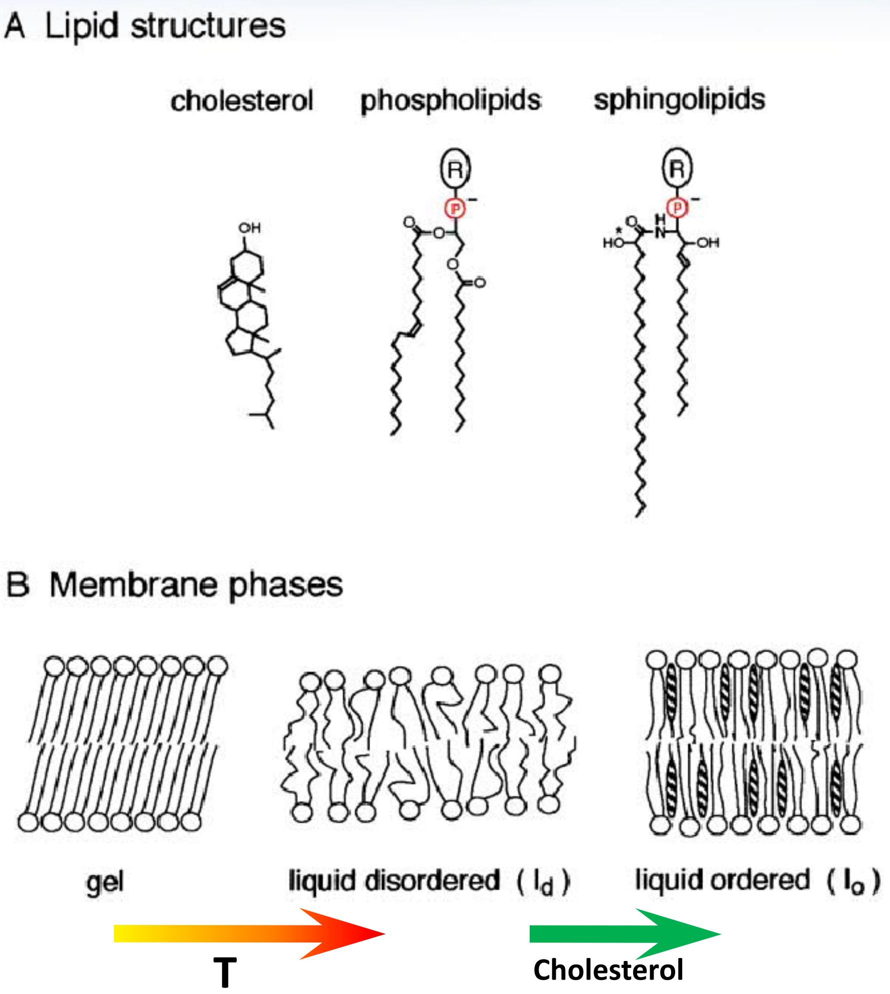
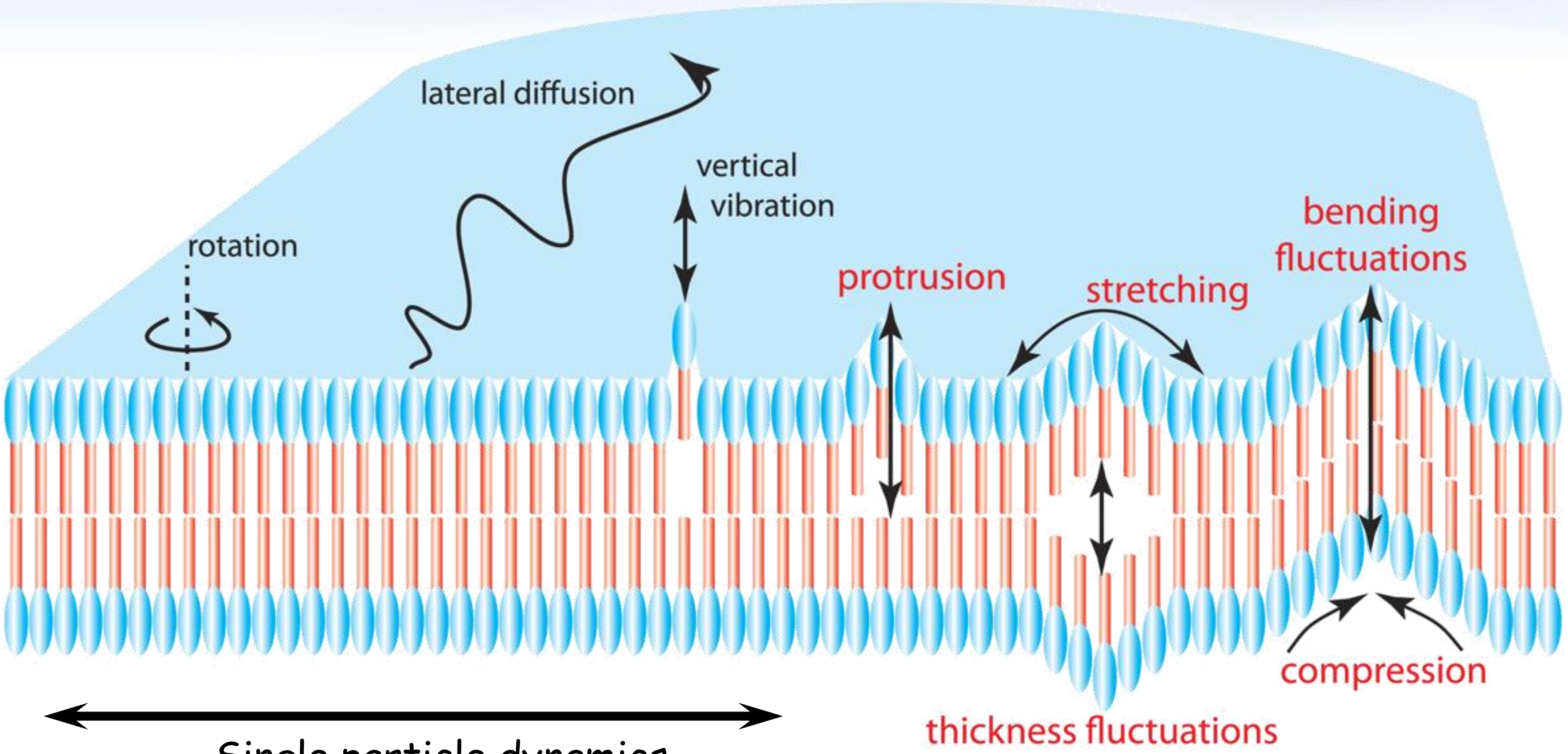


Fig. from S. Murno, Cell **115** (2003) 377

Membrane dynamics

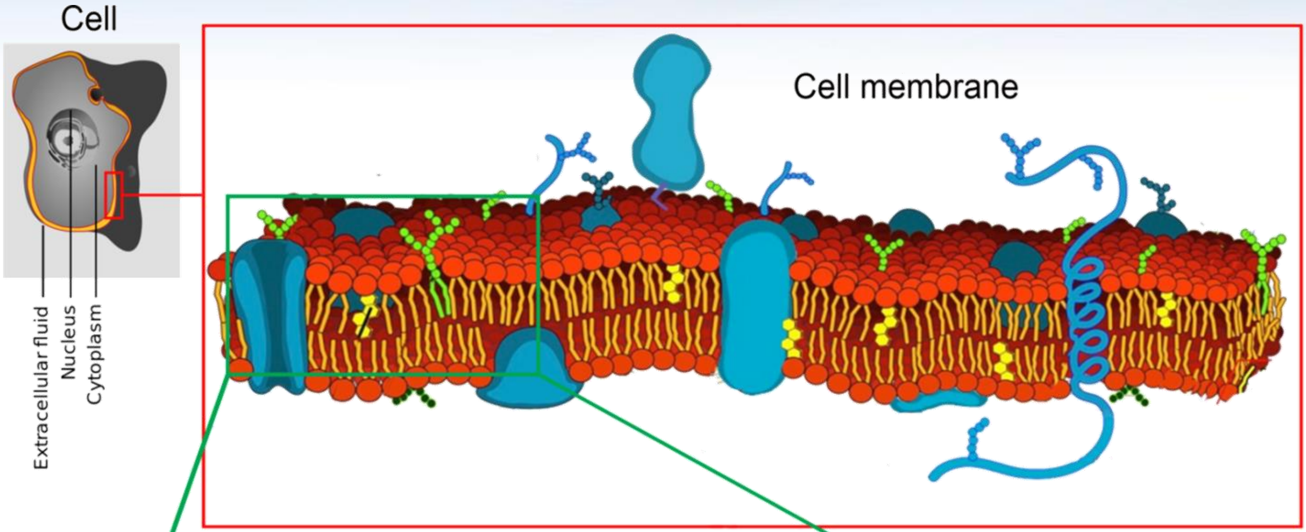


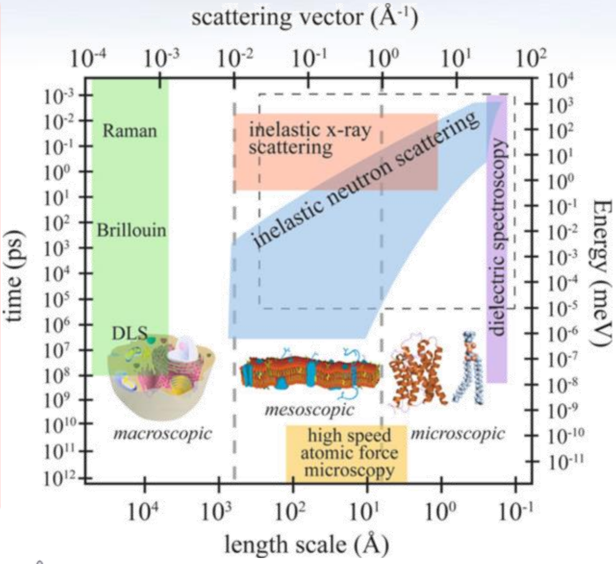
Single particle dynamics

Collective dynamics

Credit: Nagao/NIST, 12NCNR007

Slow and fast dynamics in cell membranes





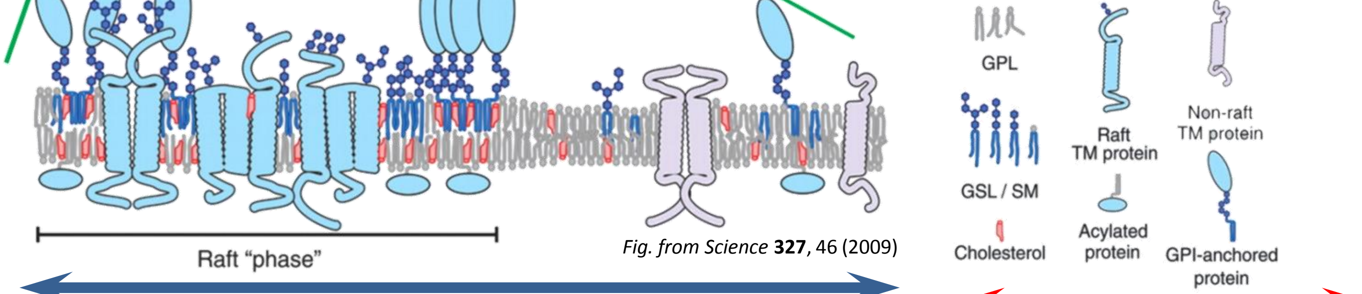


Fig. from Science **327**, 46 (2009)

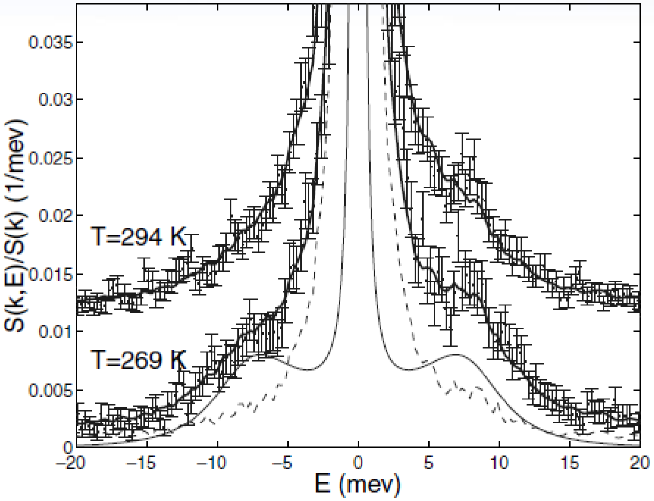
Slow dynamics:
Lipid domain (rafts), formation, diffusion, evolution and structural changes

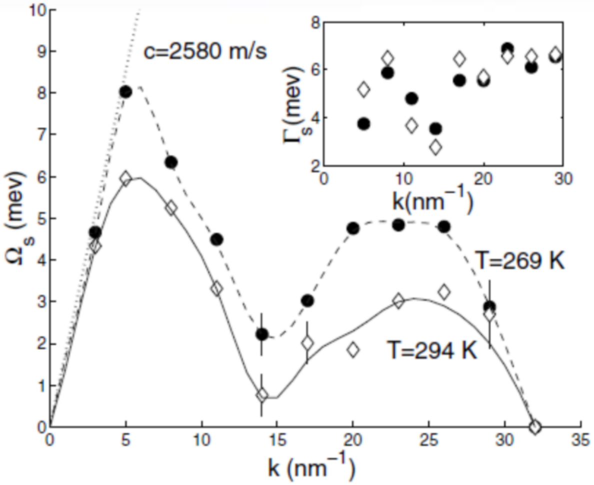
Ultrafast dynamics:
Protein and lipid Movements/vibrations, lateral and transmembrane transport

IXS
Fast dynamics on molecular scale:
picoseconds

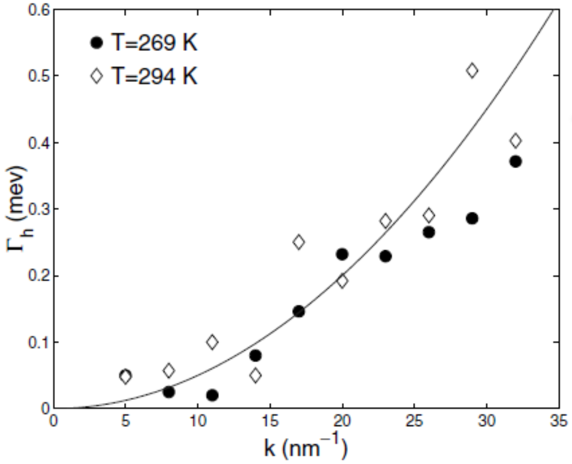
History of IXS/INS studies of biomembranes

IXS study (ESRF) of DLPC at -4°C and 21°C





S.H. Chen et al. Phys. Rev. Lett. **86** (2001) 740

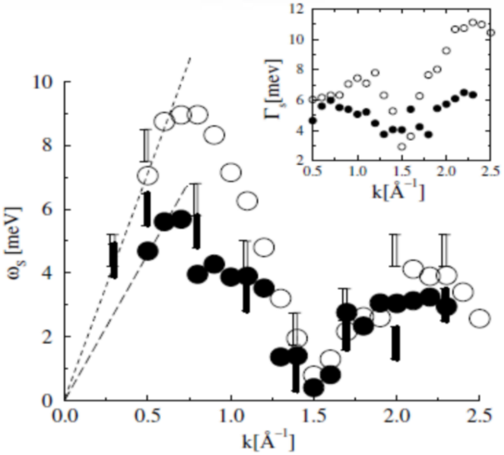


The model for the IXS fit:

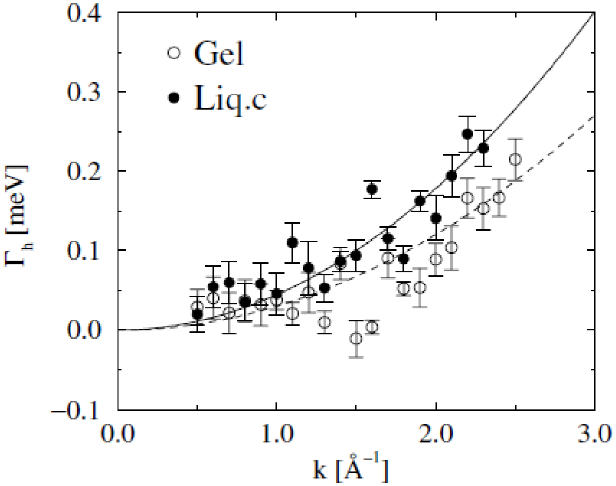
$$\frac{S(q,\omega)}{S(q)} = \frac{1}{\pi}\left\{A_o \frac{\Gamma_h}{\omega^2+\Gamma_h^2} + A_s\left[\frac{\gamma_s + b(\omega+\omega_s)}{(\omega+\omega_s)^2+\gamma_s^2} + \frac{\gamma_s - b(\omega-\omega_s)}{(\omega-\omega_s)^2+\gamma_s^2}\right]\right\}$$

History of IXS/INS studies of biomembranes

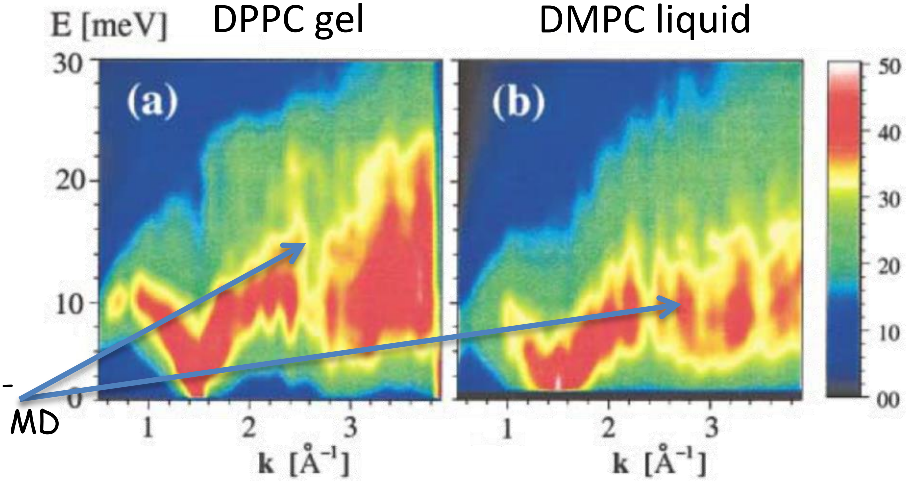
MD of DPPC gel(ο) and DMPC liquid (•)



M. Tarek et al. Phys. Rev. Lett. **87** (2001) 238101



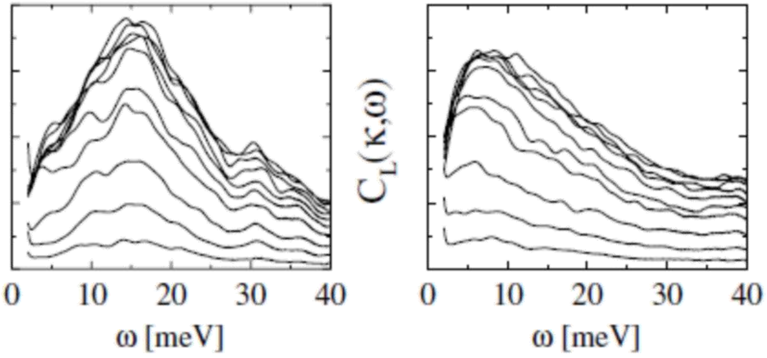
Extra mode not detected in the IXS



DPPC gel

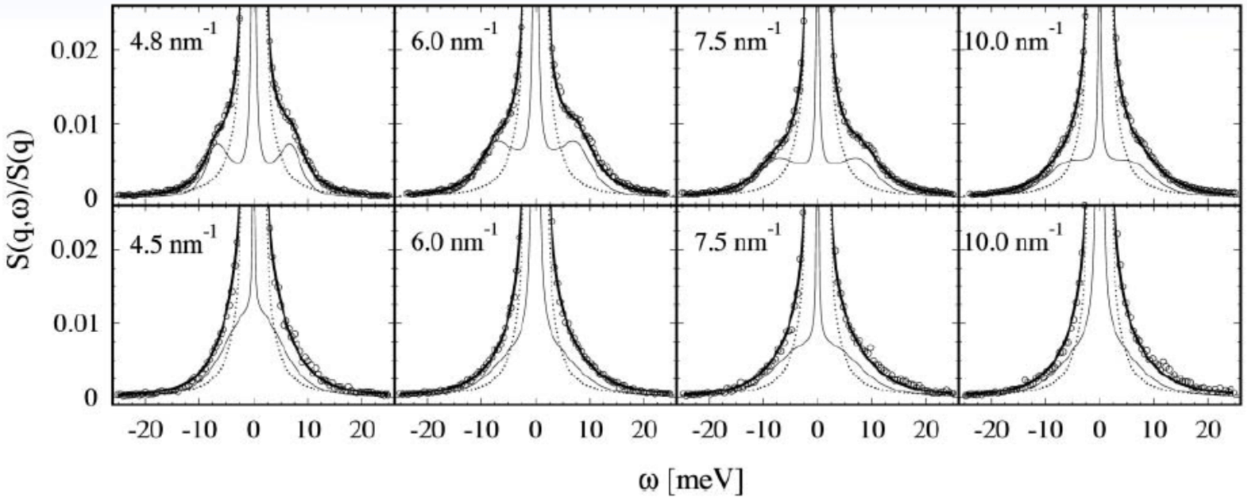
DMPC liquid

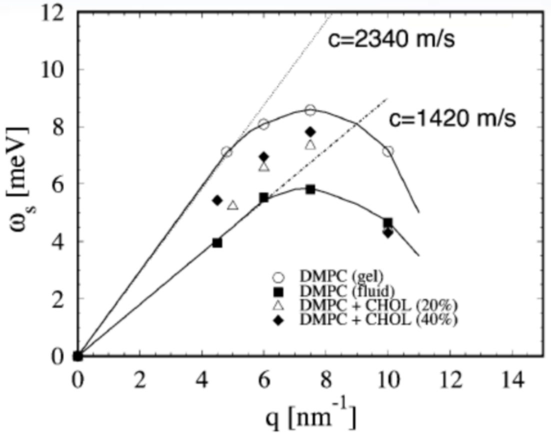
Evidence for a non-dispersive mode in MD simulations



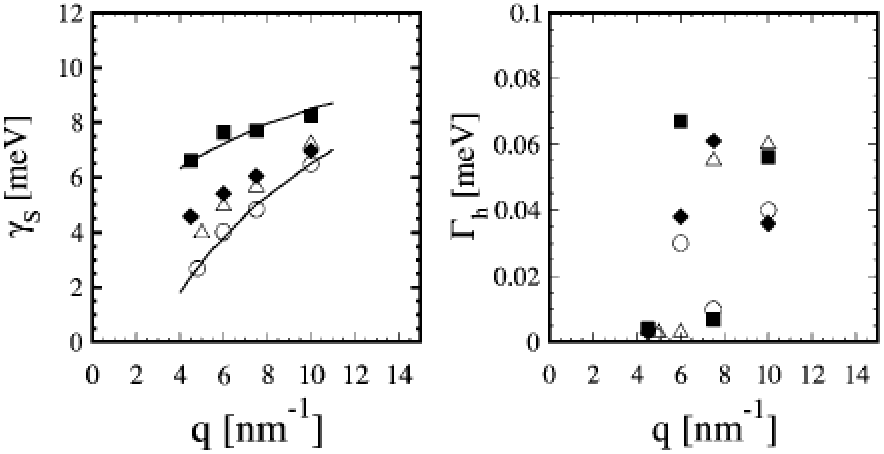
History of IXS/INS studies of biomembranes

IXS study of DMPC at 17°C and 35°C





T. Weiss et al. Biophys. J. **84** (2003) 3767



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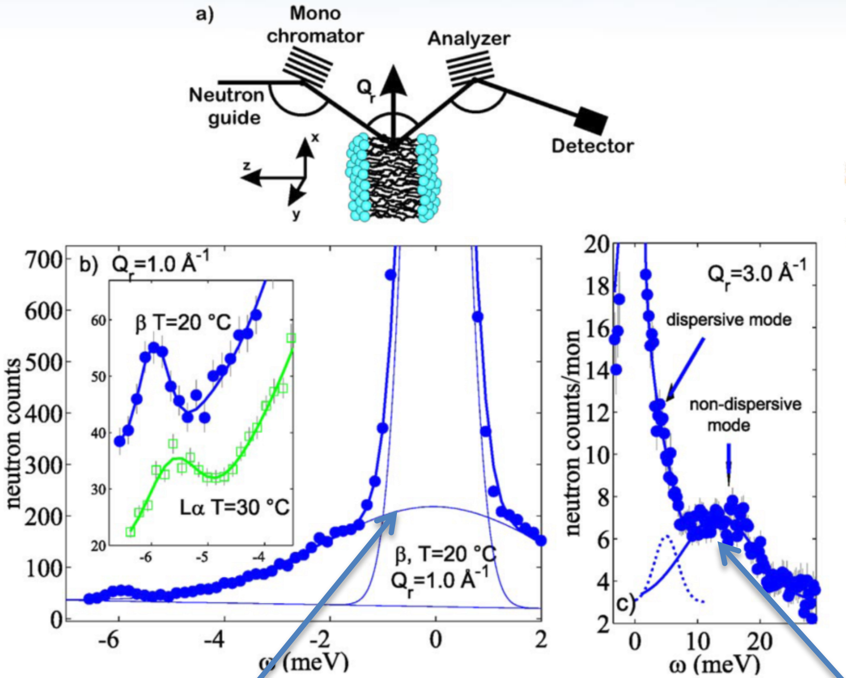
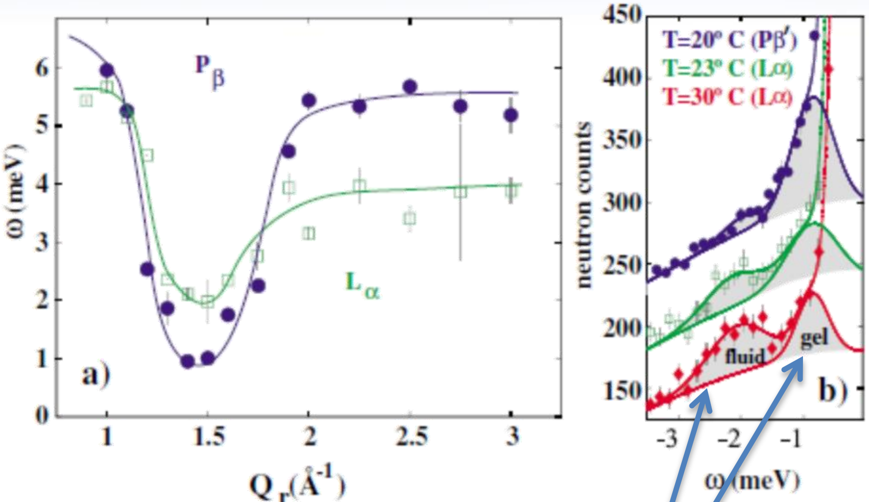
History of IXS/INS studies of biomembranes

INS study of DMPC at 17°C and 35°C





Quasielastic peak

Extra excitation: unclear whether dispersive or not

Need two excitations to fit data: erroneously attributed to the coexistence of phases

The model to fit INS data

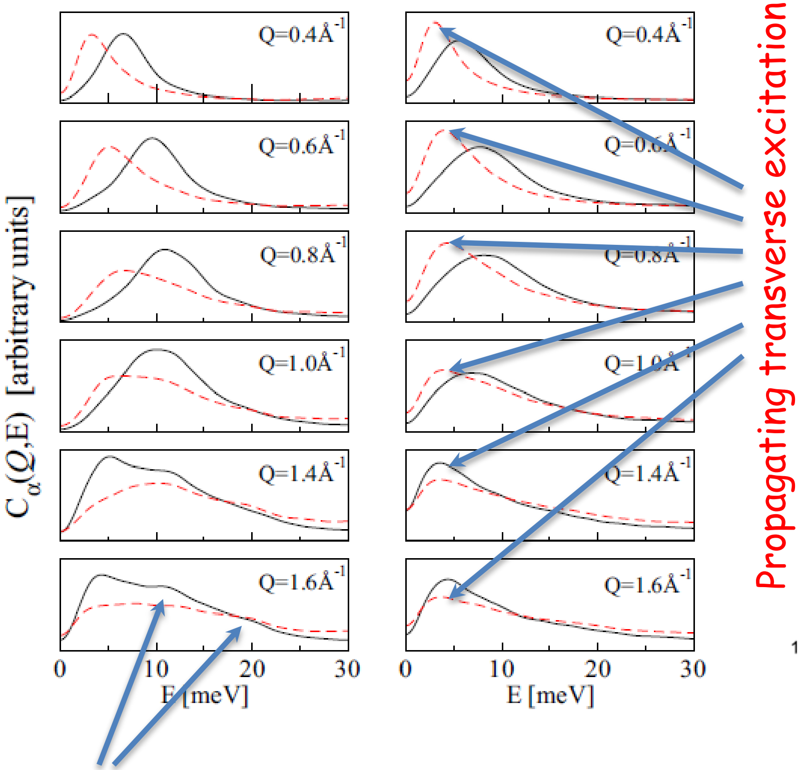
$$\frac{S(Q,\omega)}{S(Q)} = \frac{1}{\pi}\left[A_0 \frac{\Gamma_h}{\omega^2+\Gamma_h^2} + A_s\left(\frac{\gamma_s + b(\omega+\omega_s)}{(\omega+\omega_s)^2+\gamma_s^2} + \frac{\gamma_s - b(\omega-\omega_s)}{(\omega-\omega_s)^2+\gamma_s^2}\right)\right].$$

M. C. Rheinstadter et al. Phys. Rev. Lett. 93 (2004) 108107

Recent MD studies of biomembranes

Longitudinal (-) and transverse (-) currents:

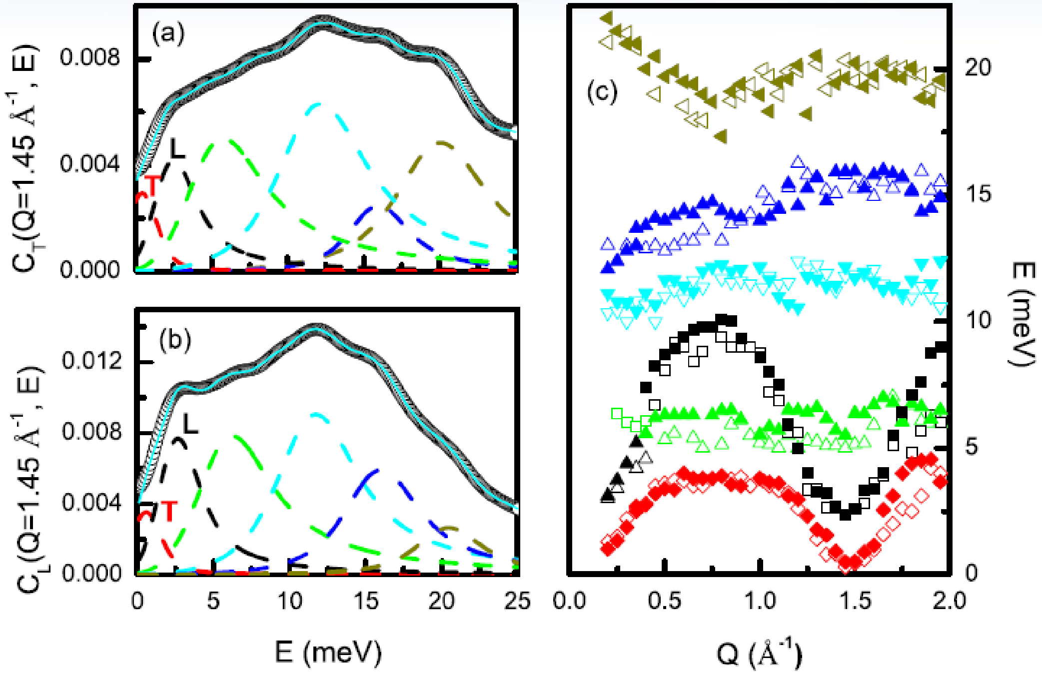
DMPC gel DMPC liquid

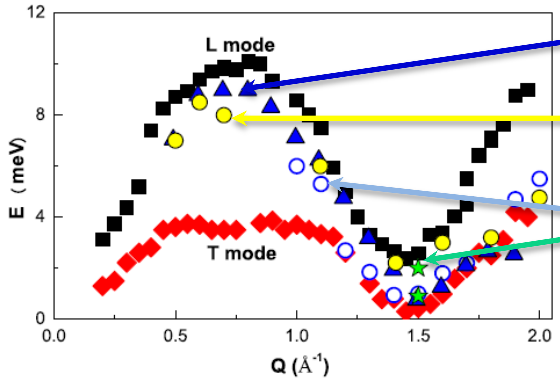


Propagating transverse excitation

Extra excitations

Six excitations in total below 25 meV





M. Tarek et al. Phys. Rev. Lett. **87** (2001) 238101

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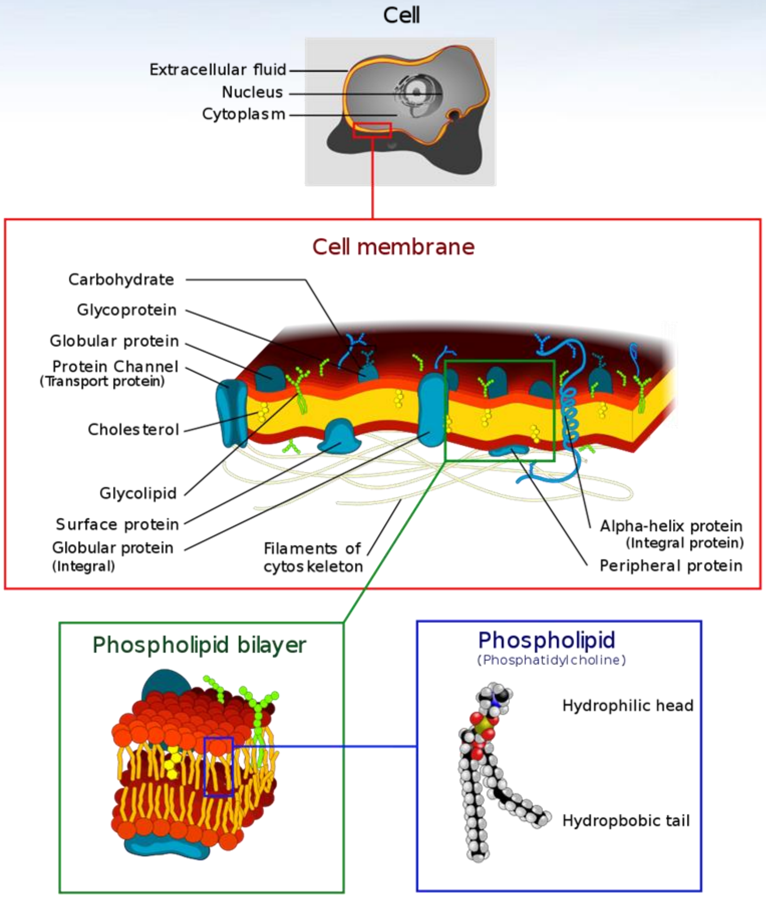
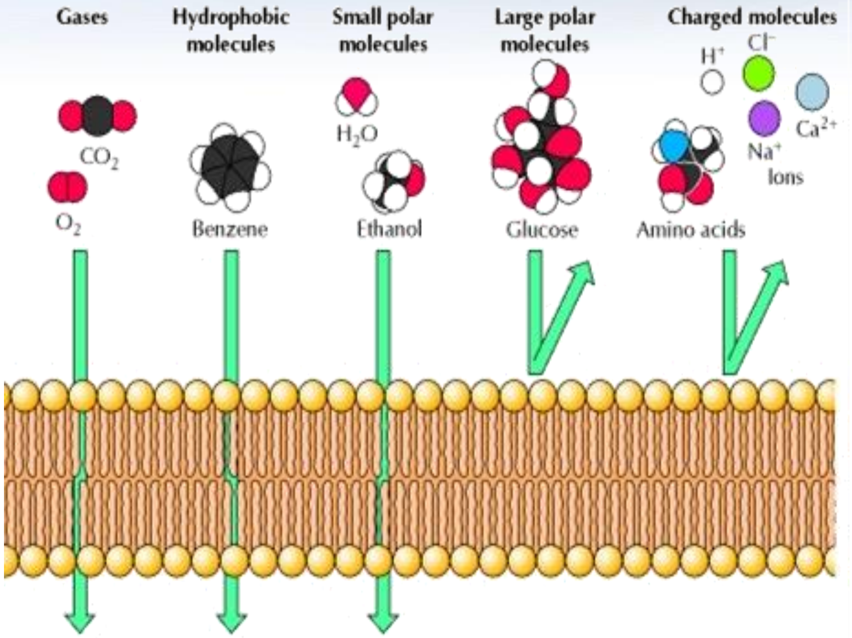
V. Conti Nibali et al. Phys.Rev. E 89 050301(R) (2014)

Lessons learned from previous studies

- Different parts of lipid molecules contribute to different excitations, which all can, in principle, be probed by inelastic scattering
- Lipid membranes can support long-wave transverse acoustic-like excitations in both liquid and gel phases
- Generalized three effective eigenmode (GTEE) theory is not an appropriate model to fit the inelastic scattering data
- The oversimplified interpretations of collective motions in membranes in terms of simple liquids were incorrect and had lead to misunderstanding of lipid dynamics, which resulted in a field stagnation
- Lack of connection between the observed dynamics and biological functions

Passive transport in lipid membranes





Permeability of phospholipid bilayers:
Gases, hydrophobic molecules, and small polar uncharged molecules → can diffuse through

Larger polar molecules and charged molecules cannot

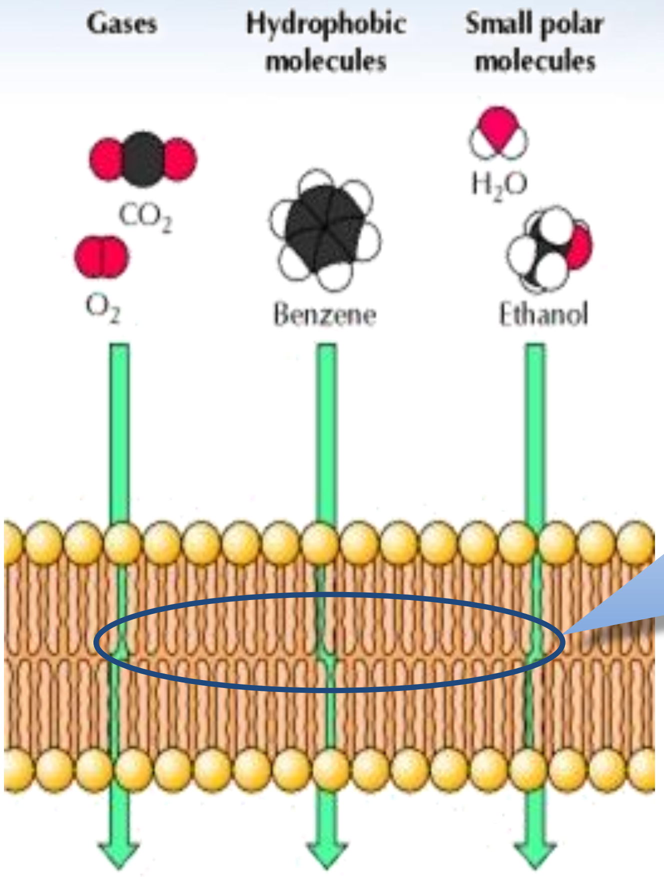
The Cell: A Molecular Approach. 2nd ed. Cooper GM. 2000.

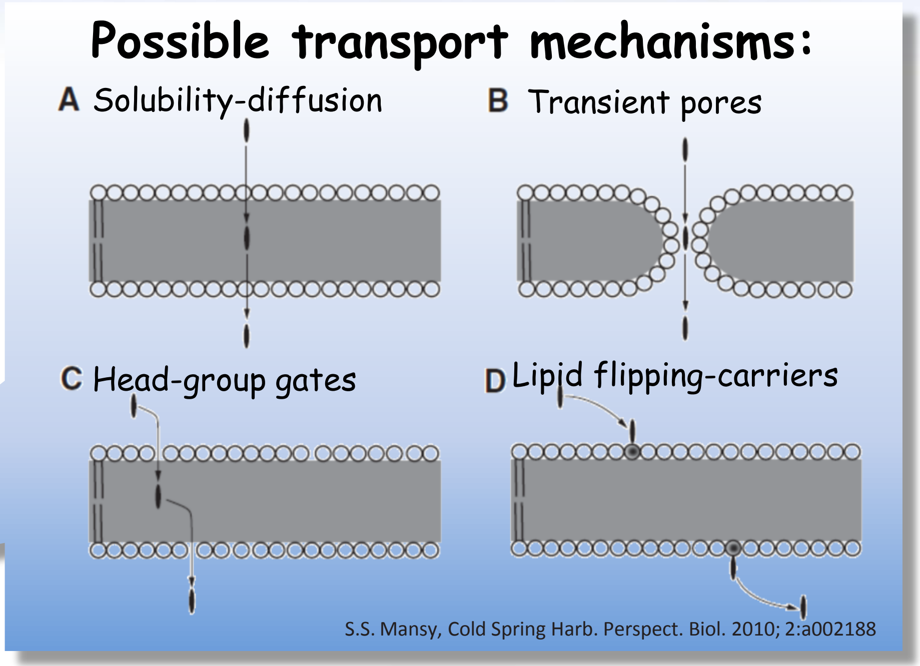
Many factors control the permeation

- solute nature
- molecule type and size
- membrane thickness

Exact mechanism is unknown

Passive transport mechanisms




Possible transport mechanisms:

S.S. Mansy, Cold Spring Harb. Perspect. Biol. 2010; 2:a002188

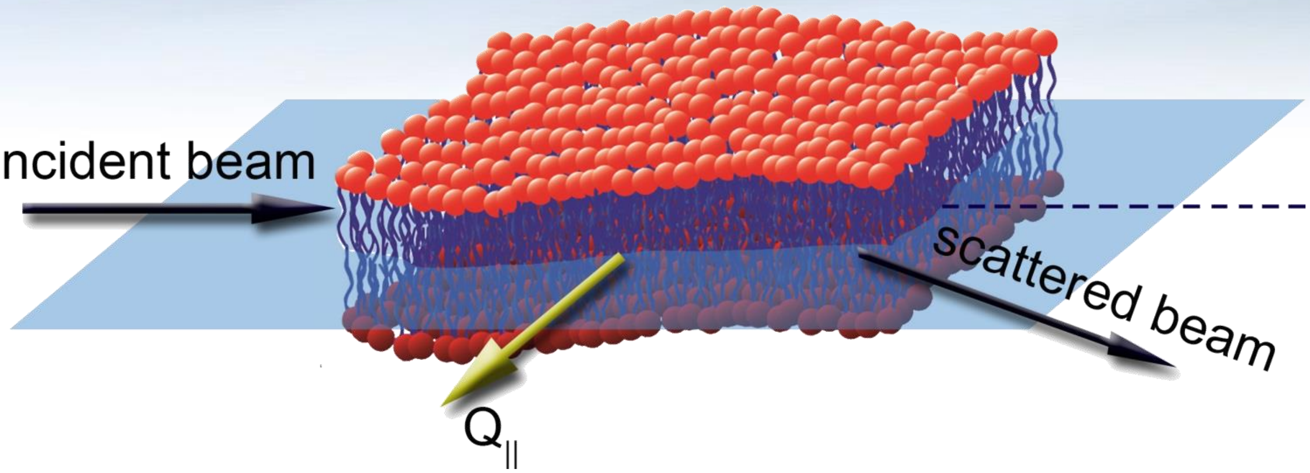
IXS is perfect to study ultrafast **thermally-triggered** phonon-mediated transport mechanisms

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

Transient Pore Model
for unsaturated lipids

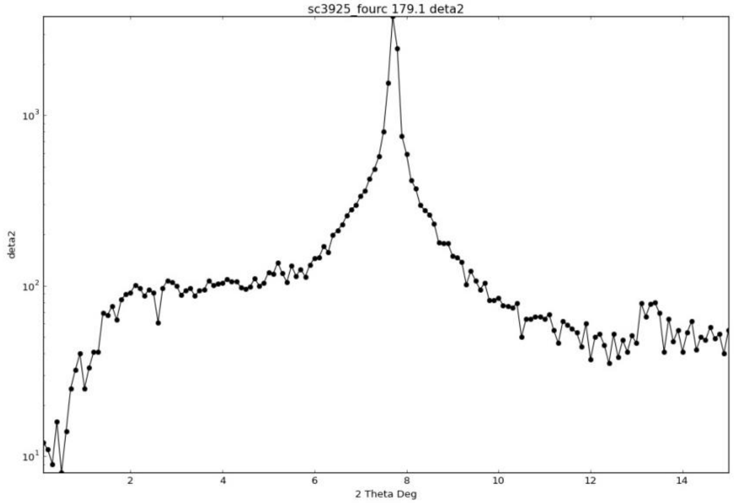
Packing Defect Model
for saturated lipids

IXS measurements: DPPC lipid



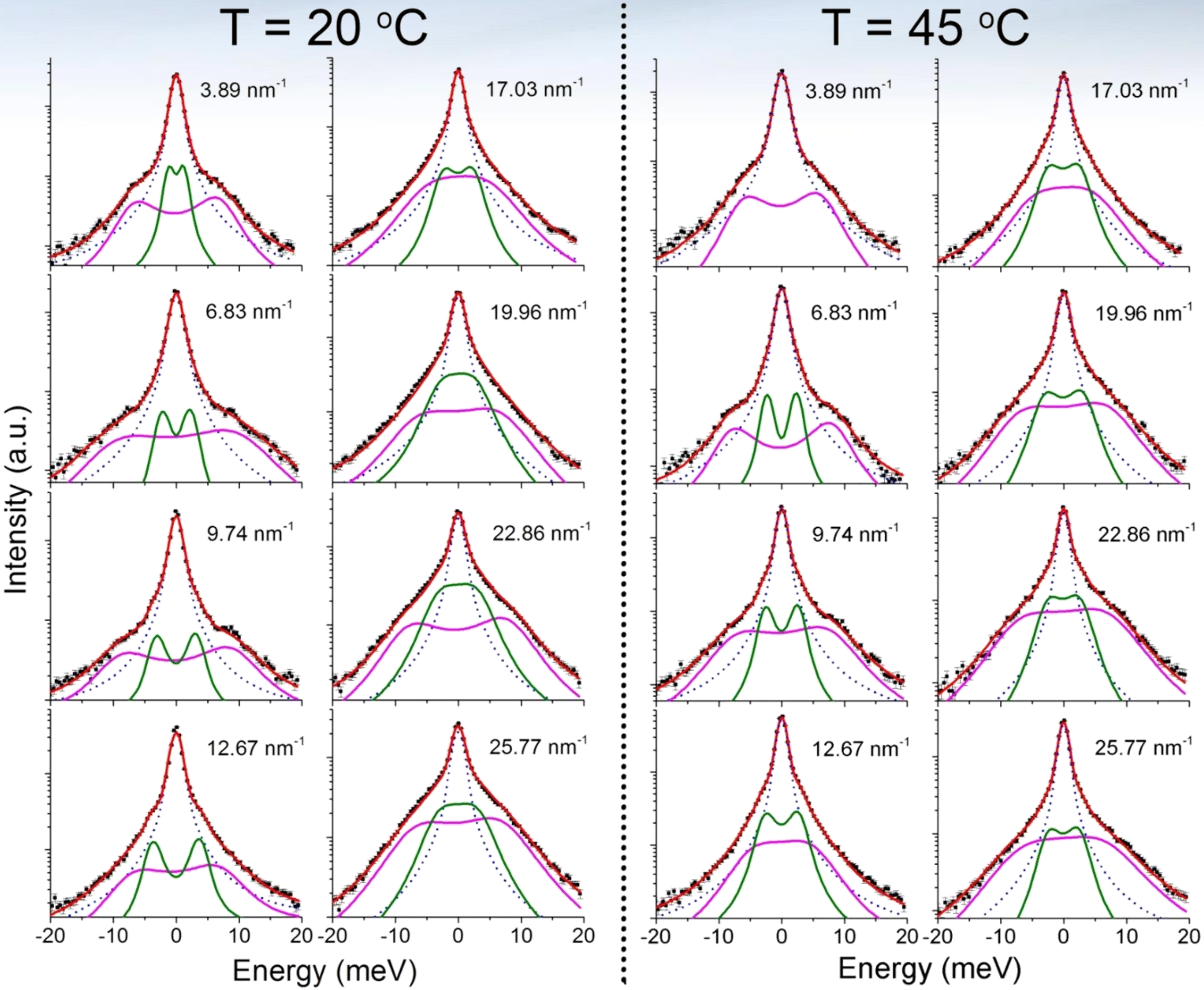
- DPPC main transition temperature: 41 °C
- DPPC measured at 20 °C and 45 °C; E = 21.78 KeV, Relative humidity ~ 97%

Example of S(Q,0)



Q_{peak} ~ 14.5 nm^{-1} for 45 °C corresponds to $A_L = 65.4 \pm 1\ A^2$
→ fully hydrated DPPC

IXS data: DHO modeling

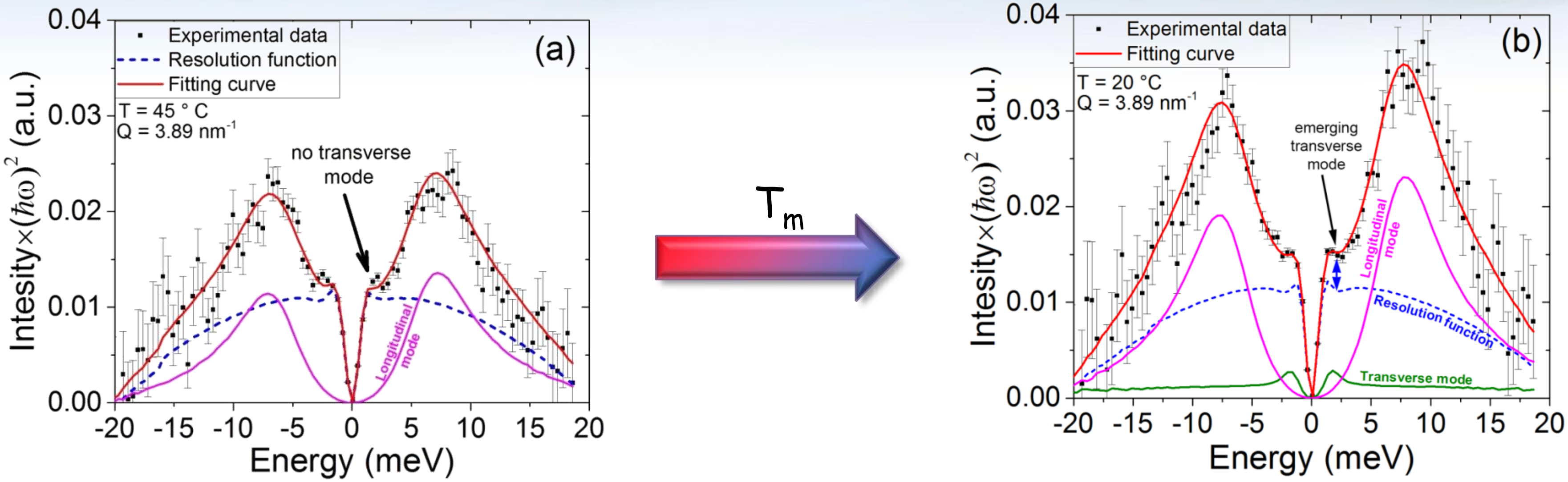


M. Zhernenkov et al. *Nat. Commun.* **7**, 11575 (2016)

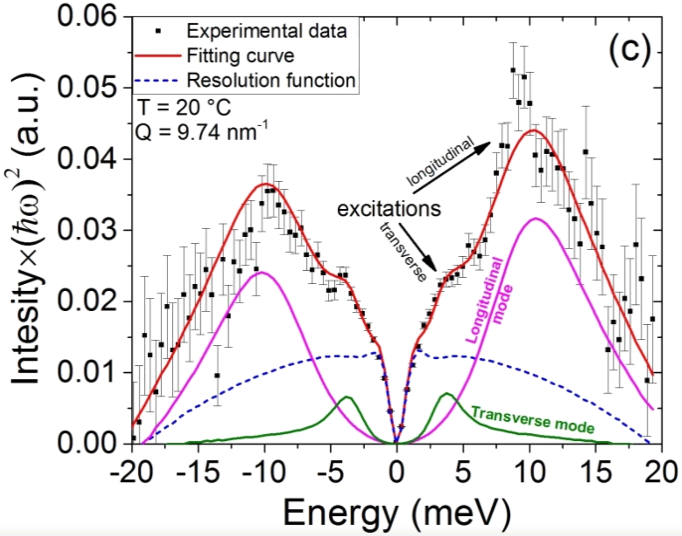
Evidence for transverse excitations

Fluid phase

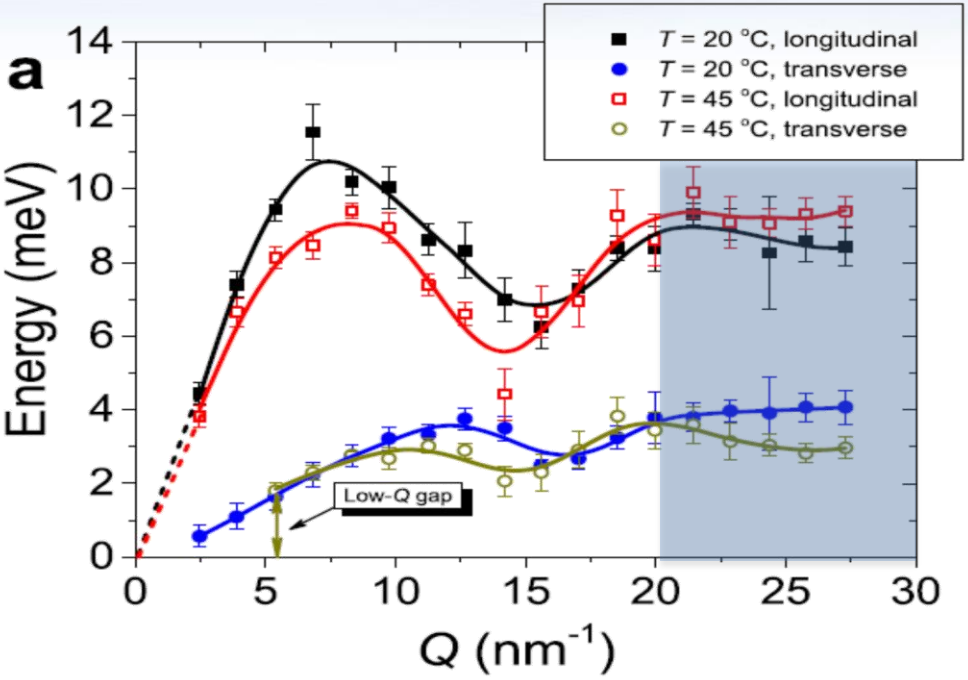
Gel phase



Gel phase, at high Q → evident transverse mode

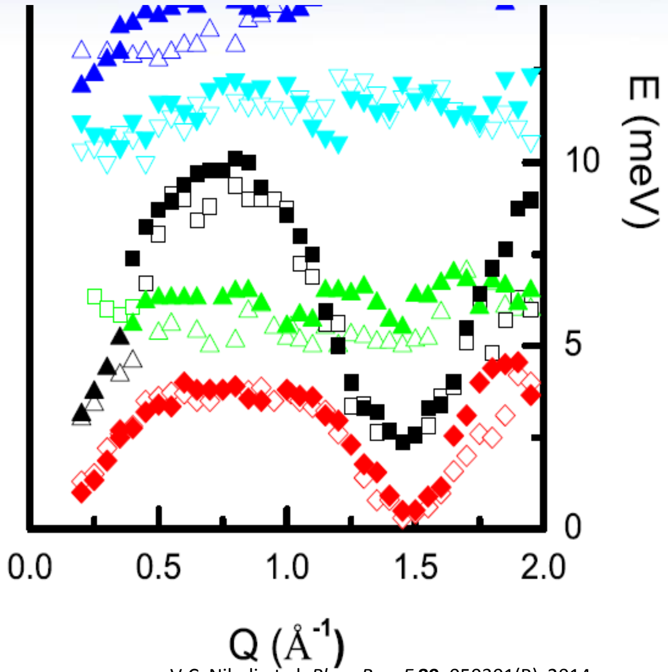


IXS data: dispersion curves



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

Gel phase: MD simulation



V.C. Nibali et al. *Phys. Rev. E* **89**, 050301(R), 2014

Fast sound from longitudinal mode:
2532 ± 190 m/s for 20 C
2241 ± 437 m/s for 45 C
→ Speed of sound agreed with MD

The minimum in the longitudinal dispersion
at Q_{min} ~ 15.4 nm^{-1} for 20 C; A_L = 55.6±1 $Å^2$
Q_{min} ~ 14.2 nm^{-1} for 45 C; A_L = 65.4±1 $Å^2$
→ agrees well with literature…

The discovery of the low-Q phononic gap!

Low-Q phononic gap

Low-Q phononic gap

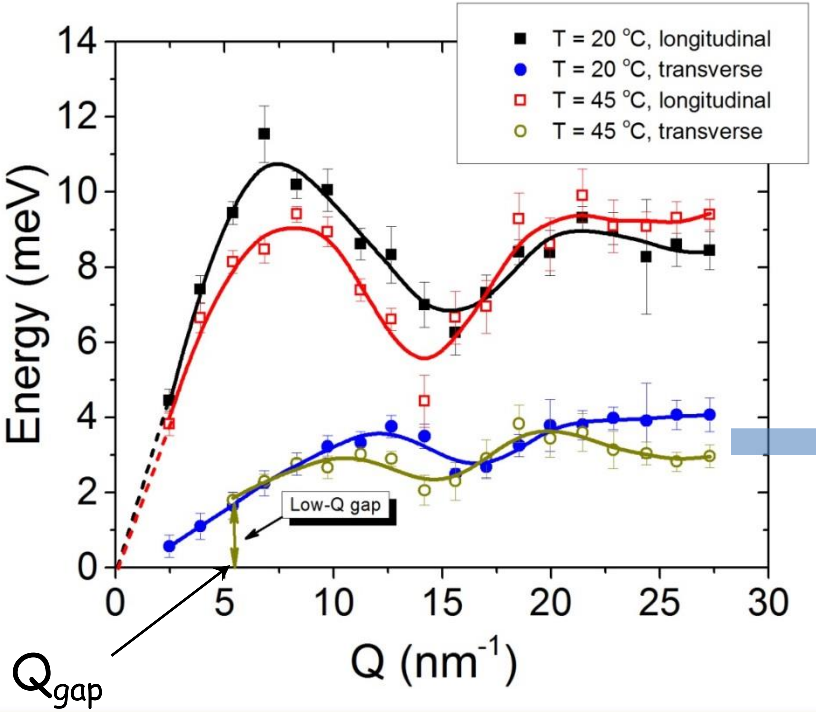
The cut-off value Q_{gap}
(at which gap occurs)

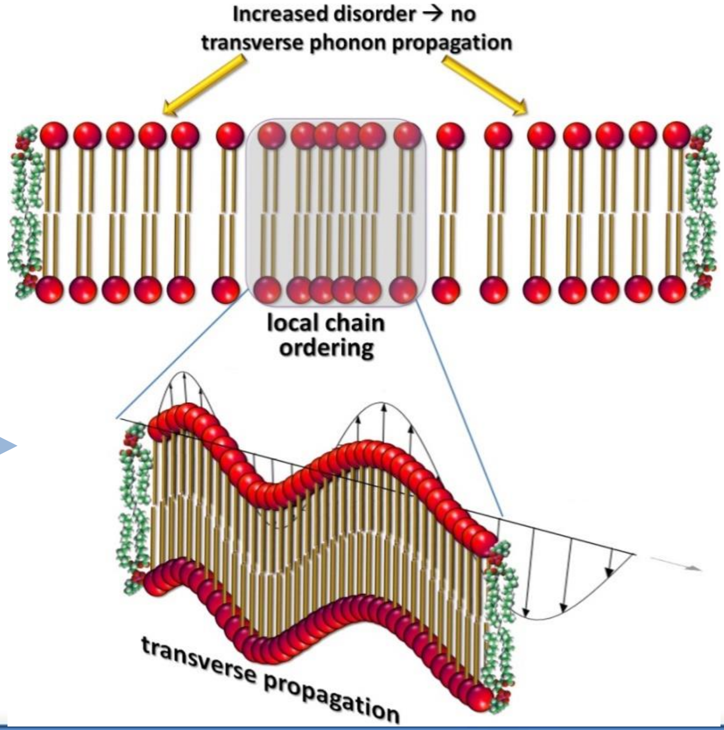
No phonon propagation
at $Q < Q_{gap}$

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)*

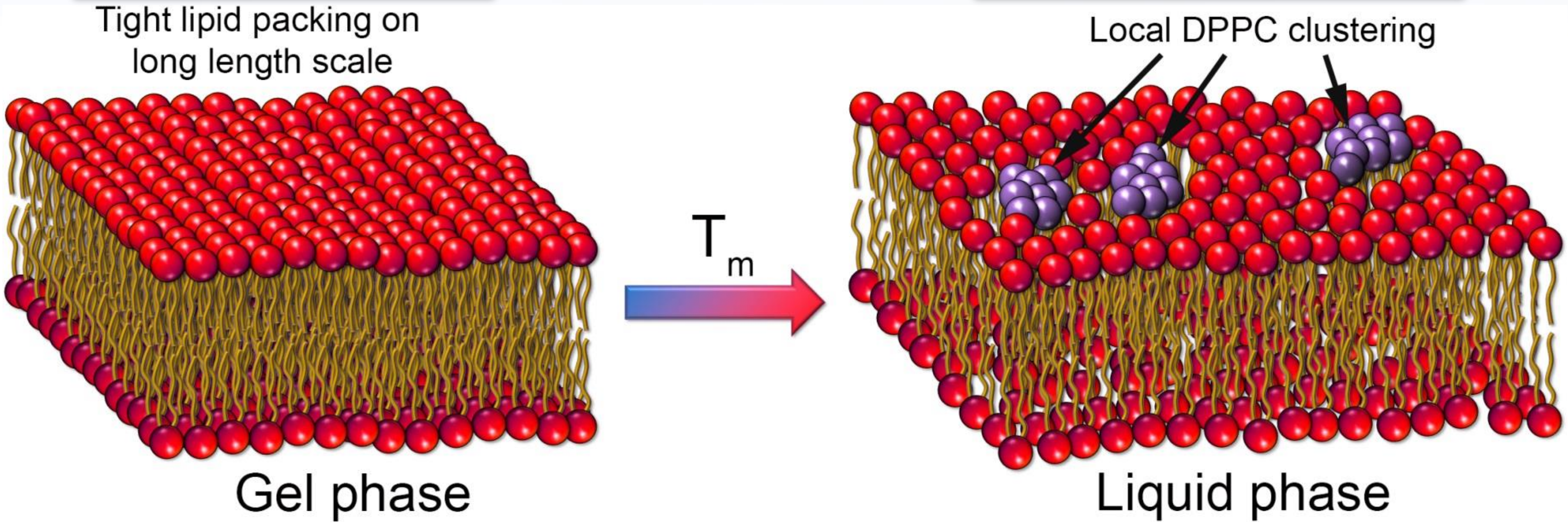


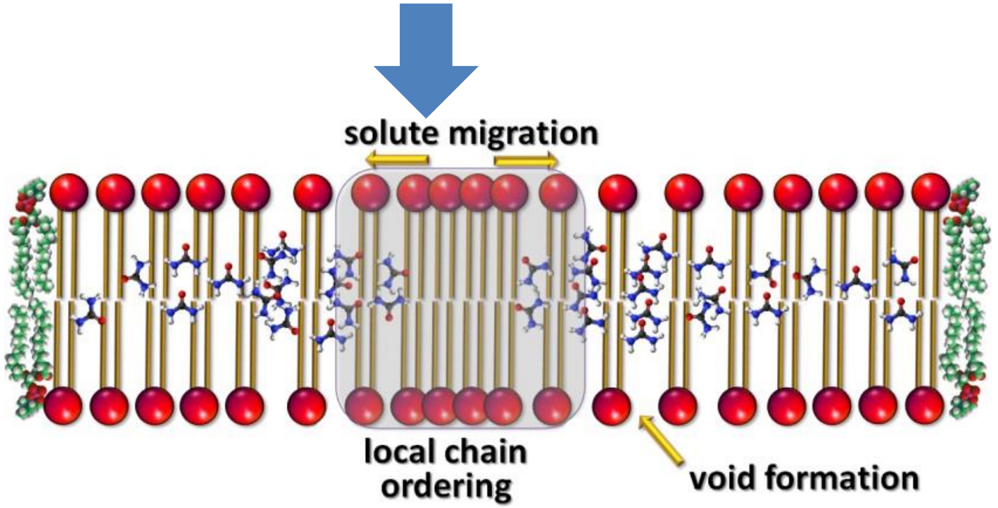


Phonon-mediated nm-scale clustering

NO phononic gap

Low-Q phononic gap





- 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

Outlook

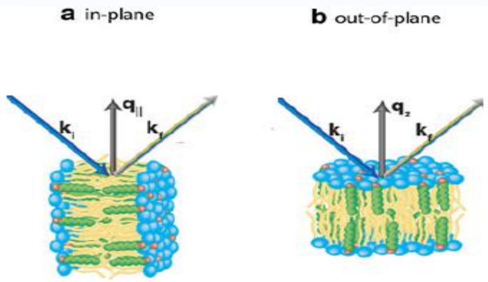
IXS machines readily available for users virtually exhausted their capabilities to advance study of biomembranes.

So we need better
RESOLUTION!

Outlook: Interesting physics lies at low energies and low Q!

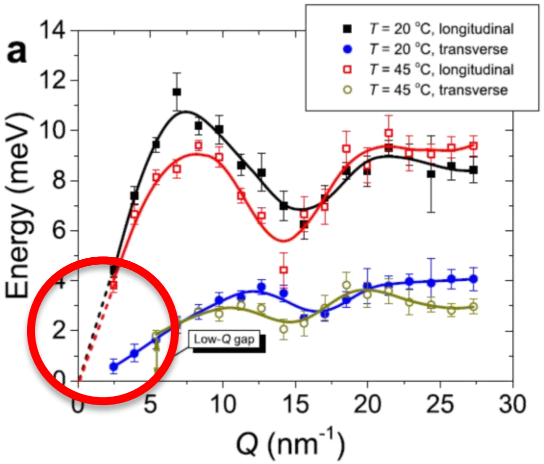
Better
RESOLUTION

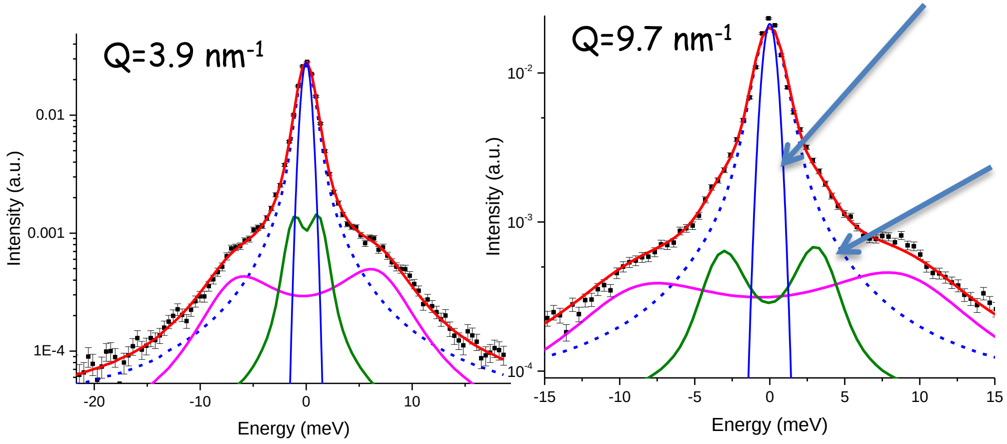
Energy
Q
Line shape



✓ Out-of-plane dynamics in lipids – never studied before:
Require Q resolution of <0.1 nm^{-1}

✓ Multicomponent systems (binary, ternary, with proteins), gap physics
Require both Q and E resolution ~0.1 (nm^{-1}, meV)





Gaussian function @FWHM 1 meV

Resolution function at ID28@ESRF

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THANK YOU!