Bending Elasticity of Bio-Membranes Studied by Neutron Spin-Echo

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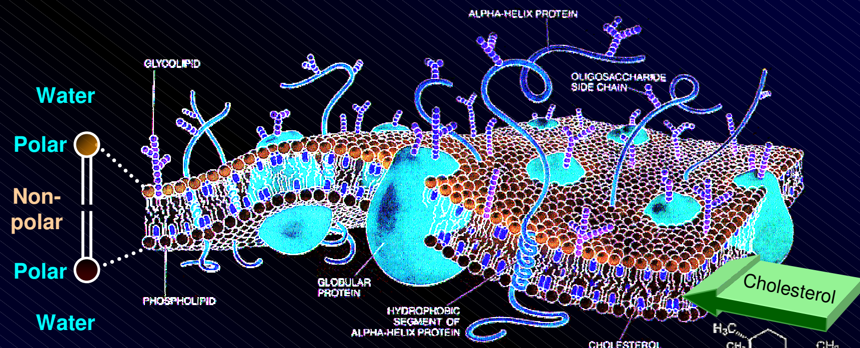
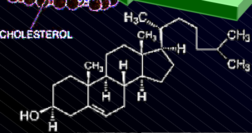
In this presentation …

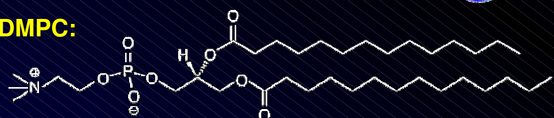
- **Thermal undulations of cell membrane**
- **Neutron Spin Echo (NSE)**
  - Why NSE is ideal for this study. Energy resolution
  - How NSE works. Principles
- **Experimental system. Results & Discussion**
- **Summary**

The Cell Membrane





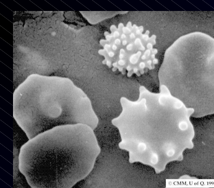
DMPC:



(1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine)

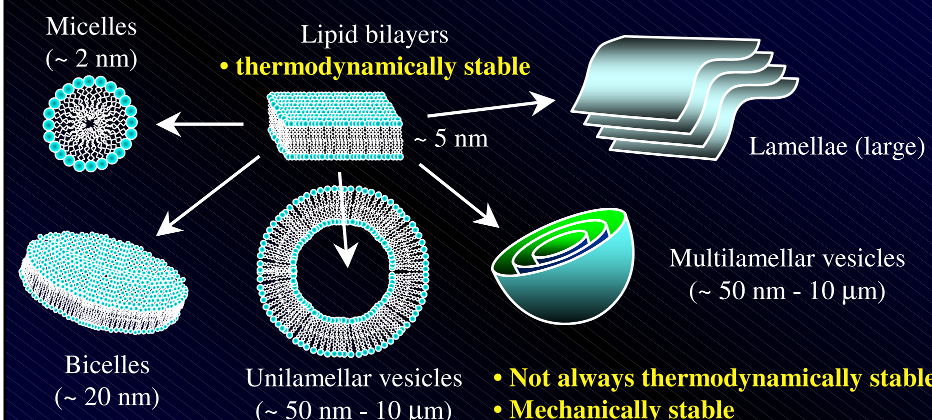
Thermal undulations:
↓
Bending elasticity

- Biosensing – contact time
- Cell adherence – undulation
- Cell mobility

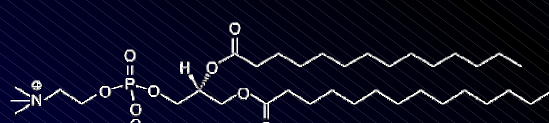


Blood Cells

Topologies of the lipid bilayers



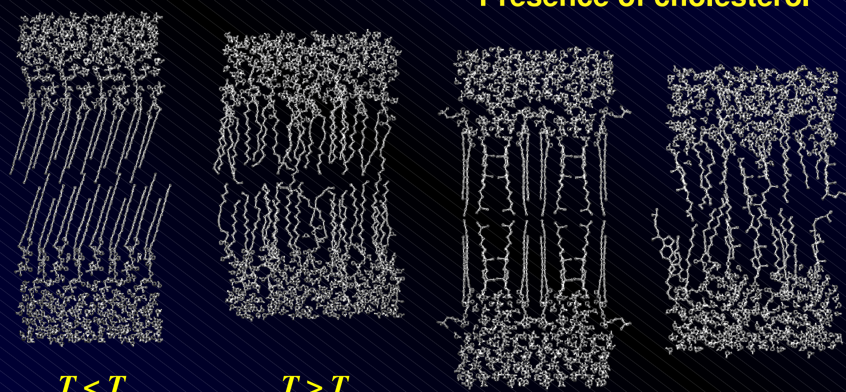
DMPC:



Factors that may affect the bending elasticity

Temperature: liquid to crystalline transition (T_c = 24 ºC for DMPC)

Presence of cholesterol



$T < T_c$ $T > T_c$ $T < T_c$ $T > T_c$

R. R. Gabdoulline, *J. Phys. Chem.*, 100:15942, **1996**

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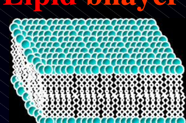
How to measure bending elasticity

Lipid bilayer



Properties:

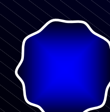
- Interfacial tension ⟷
- Lateral elasticity ⟷
- Bending elasticity

Bending elasticity

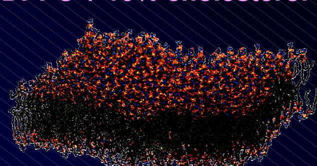
⇓

Thermal undulations
(highly localized)

- Videomicroscopy: large *T* & *L* scales
- NMR transverse relaxation times: wide *T* scale, relaxation model?
- Dynamic light scattering: *T* scale > 100 ns
- Computer simulations: not fast enough



DPPC + 10% cholesterol



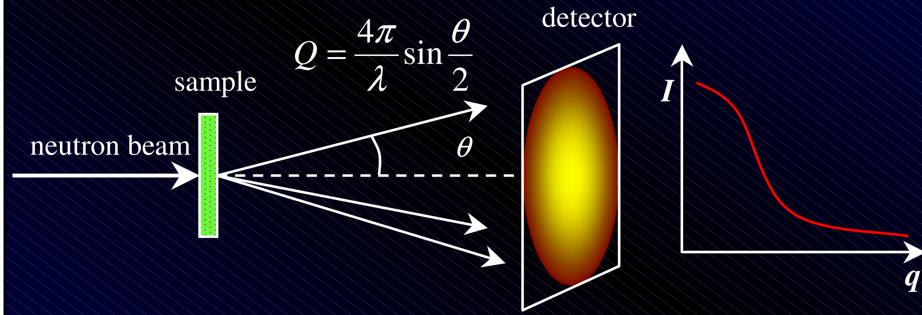
NSE:

T scale ~ 0.01 - 100 ns

L scale ~ 1 - 10 nm

Hofsäß, E. Lindahl, O. Edholm, *Biophysical Journal,* 84: 2192, **2003**

Small Angle Neutron Scattering (SANS)

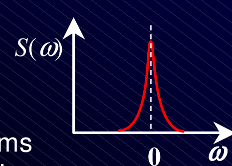


Elastic scattering ⇒ static structure

Dynamics?

NSE basics

- **NSE is a quasielastic method:** small deviation from the elastic scattering
- **Energy transfer:** $\omega = 10^{-5} - 10^{-2}$ **meV**

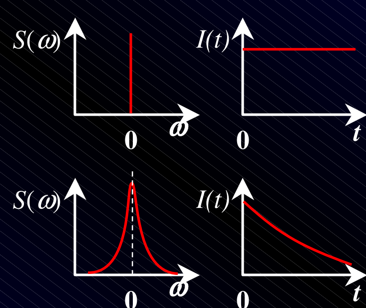


- **Goals:**
  - Micellar systems in solution
  - Undulations of lipid membranes and thin films
  - Intra-molecular diffusion of proteins and polymers
  - Dynamics of polymer melts and glasses
  - Other thermal fluctuations of the soft matter

- **Principle:** Precession in magnetic field. Each neutron has a personal stopwatch. Yields the intermediate scattering function in the time domain *I*(*Q*,*t*):

$$I(Q,t) = \int_{-\infty}^{\infty} S(Q,\omega)\cos(\omega t)\,d\omega$$

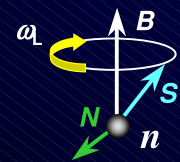
- **Fourier time range:** 0.01 to 200 ns

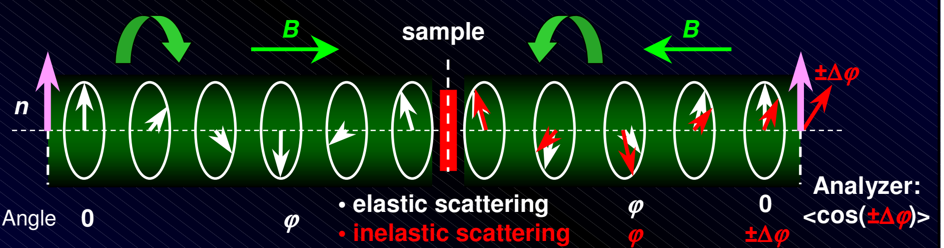


NSE walkthrough

Neutrons posses spin and magnetic moment. Larmor frequency of precession in magnetic fields depends on ***B*** only (g =1.83×10^{8} s^{-1}T^{-1})

$$N = S \times B$$
$$\omega_L = gB$$





$$\varphi = gB\frac{L}{v} \qquad \Delta\varphi = gBL\left(\frac{1}{v} - \frac{1}{v'}\right) = \frac{gBL\Delta v}{v^2} = \varphi\frac{\Delta v}{v}$$

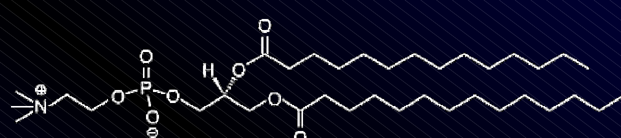
$B = 0.5\ T,\ \ L = 2$ m φ ~ 1×10^{6} rad

$$\frac{\Delta v}{v} \approx \frac{2\pi}{\varphi} \approx 10^{-5} \qquad I(Q,t) = \int_{-\infty}^{\infty} S(Q,\omega)\cos(\omega t)\,d\omega$$

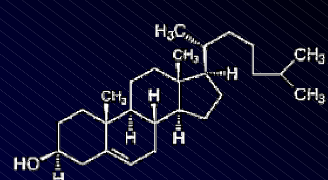
Ingredients

- 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC), t_{trans}= 24 ºC
- 1,2-Dimyristoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt) (DMPG)
- Cholesterol
- NaCl, $CaCl_2$

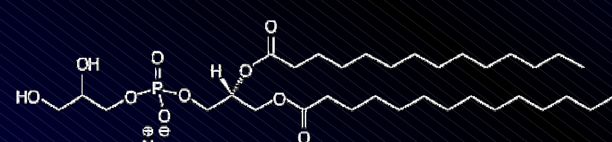
DMPC



Cholesterol



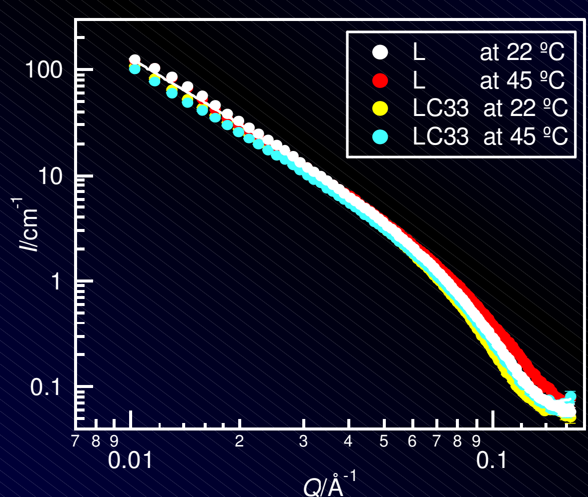
DMPG



Vesicles compositions & preparation

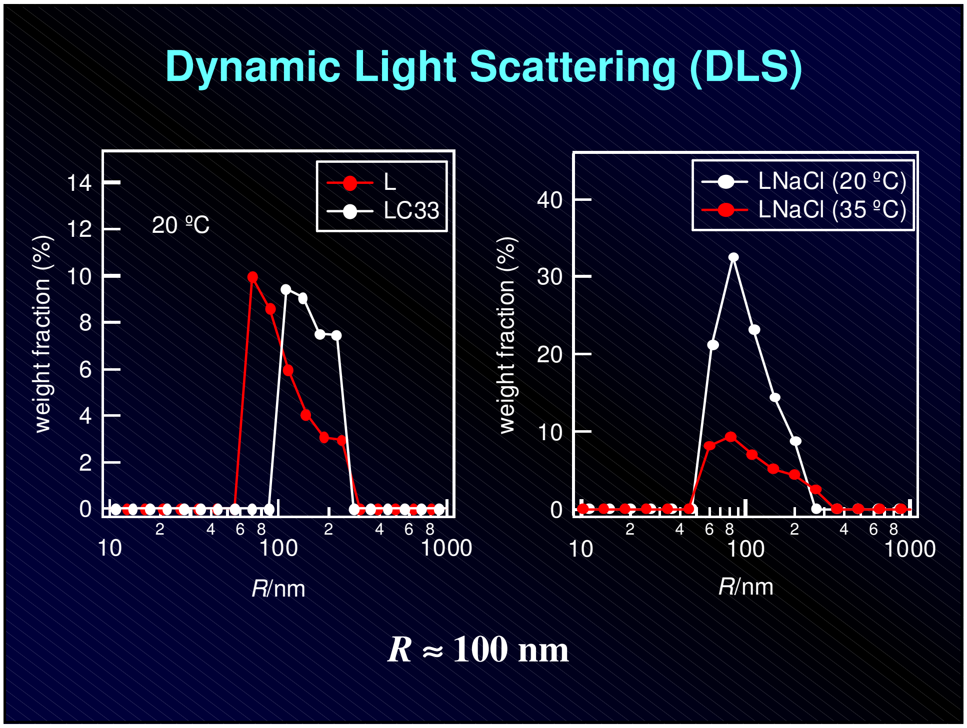
For all samples	- total lipids = 2 wt.%, DMPG/DMPC = 5 mol.%
L	- DMPC and DMPG in D_2O
LC33	- cholesterol/total lipids = 33 mol.%
LC50	- cholesterol/total lipids = 50 mol.%
LNaCl	- NaCl added to L at 50 mM
$LCaCl_2$	- $CaCl_2$ added to L at 30 mM
Method	- Extrusion through a filter (200 - 400 nm pores)
Background	- D_2O
Resolution	- carbopack (elastic scatterer)

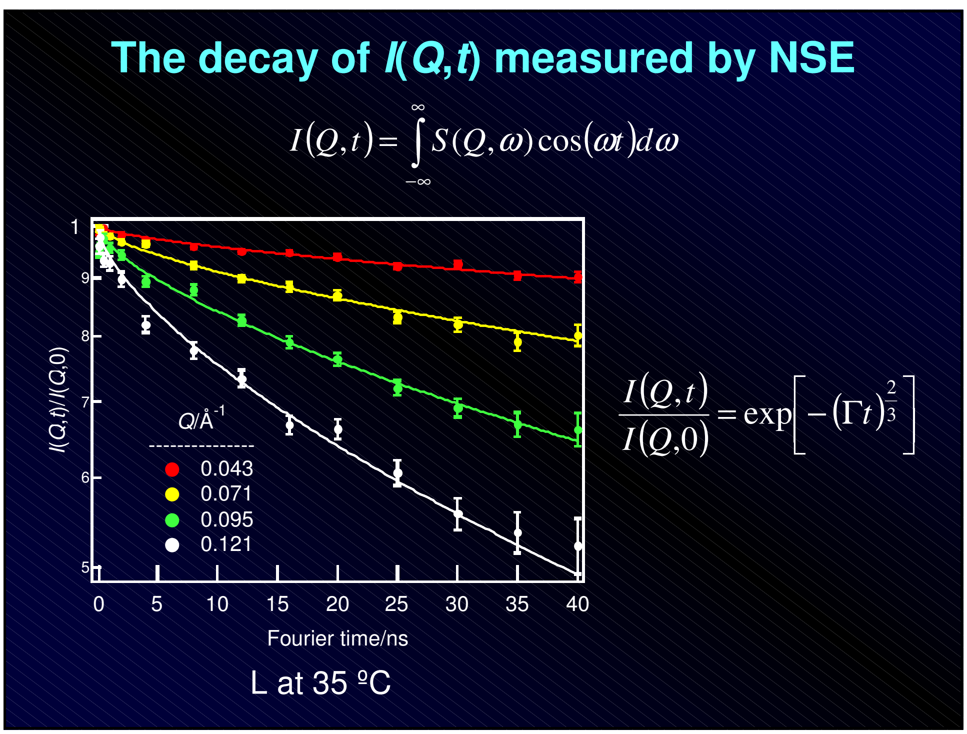
Small Angle Neutron Scattering (SANS)



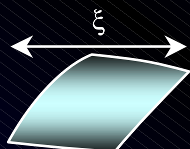
R/Å	1002
polydisp (0,1)	0.32
shell/Å	42
SLD core/$Å^{-2}$	6.4×10^{-6}
SLD shell/$Å^{-2}$	1.0×10^{-6}
SLD solvent/$Å^{-2}$	6.4×10^{-6}
Bkg/cm^{-1}	0.07

SANS from DMPC vesicles in D_2O


Dynamic Light Scattering (DLS)
20 ºC
L
LC33
weight fraction (%)
R/nm
LNaCl (20 ºC)
LNaCl (35 ºC)
weight fraction (%)
R/nm
R ≈ 100 nm


The decay of I(Q,t) measured by NSE
$I(Q,t)=\int_{-\infty}^{\infty} S(Q,\omega)\cos(\omega t)\,d\omega$
$\frac{I(Q,t)}{I(Q,0)}=\exp\left[-(\Gamma t)^{\frac{2}{3}}\right]$
I(Q,t)/I(Q,0)
Q/Å-1
0.043
0.071
0.095
0.121
Fourier time/ns
L at 35 ºC

Zilman-Granek theory for thermal undulations



Membrane plaquette

Dynamic structure factor

$$S(\vec{Q},t)=\left\langle \sum_{i,j} e^{i\vec{Q}[\vec{R}_i(t)-\vec{R}_j(0)]} \right\rangle$$

lateral

$$\vec{R}_i(t)=\vec{r}_i(t)+z_i(t)$$

perpendicular

Helfrich bending Hamiltonian (small deformations, $\nabla h << 1$)

$$H=\frac{1}{2}\kappa\int d^2r\left[\nabla^2 h(\vec{r})\right]^2$$

$$z_i(t)=h(\vec{r}_i(t),t)$$

amplitude

$$S(\vec{Q},t)=\frac{1}{a^4}\int d^2r\int d^2r' e^{i\vec{Q}_{\parallel}(\vec{r}-\vec{r}\,')} e^{-\frac{Q_z^2}{2}\left\langle [h(\vec{r},t)-h(\vec{r}\,',0)]^2\right\rangle}$$

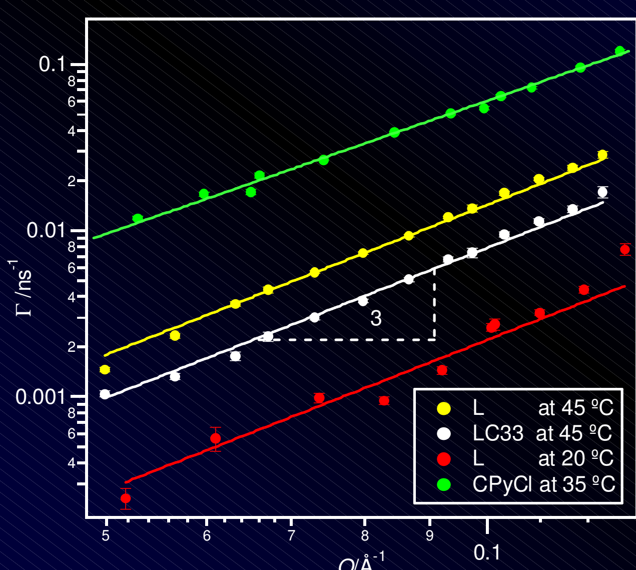
static dynamic

$$\left\langle [h(\vec{r},t)-h(\vec{r}\,',0)]^2\right\rangle = \Phi_0(\vec{r}-\vec{r}\,')+\Phi_0(\vec{r}-\vec{r}\,',t)$$

$$I(Q,t)=I(Q,0)\exp\left[-(\Gamma t)^{\frac{2}{3}}\right],\quad \Gamma=0.025\gamma_k\sqrt{\frac{k_BT}{\kappa}}\frac{k_BT}{\eta}Q^3$$

A. G. Zilman, R. Granek, *Phys. Rev. Lett.*, 77:4788, **1996**
A. G. Zilman, R. Granek, *Chemical Physics*, 284:195, **2002**

Relaxation rate of *I*(*Q*,*t*) as a function of *Q*



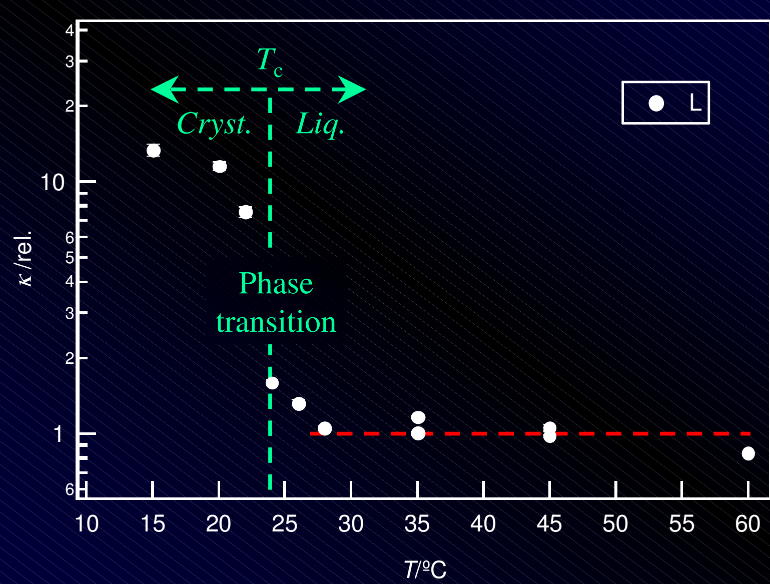
$$\Gamma \propto \sqrt{\frac{k_BT}{\kappa}}\frac{k_BT}{\eta}Q^3$$

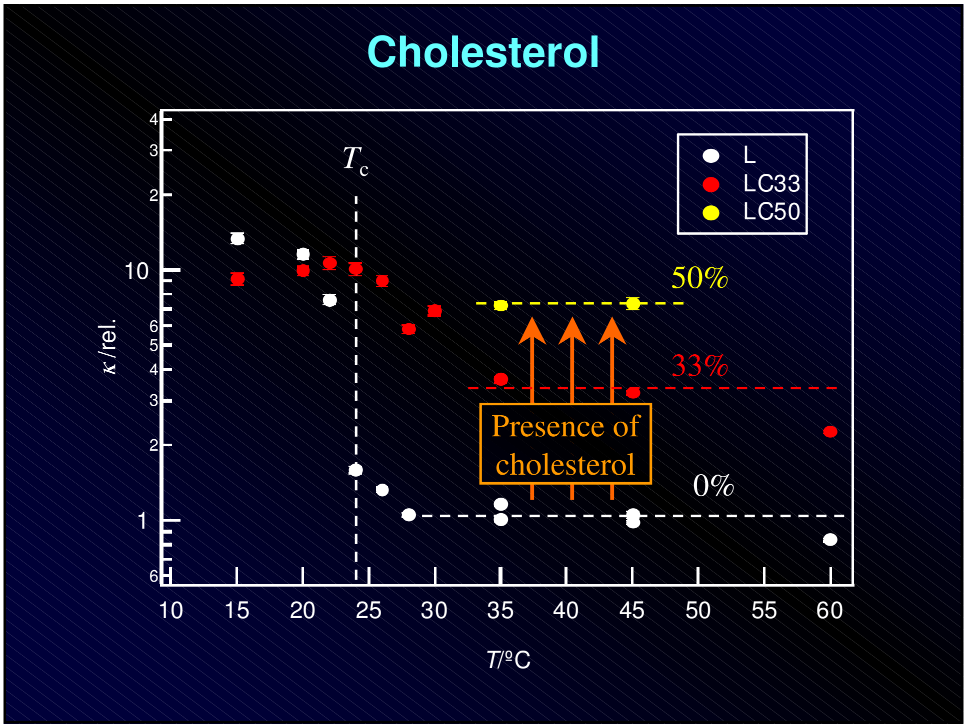
$$\Gamma \propto \sqrt{\frac{k_BT}{\kappa}}\frac{k_BT}{\eta}Q^{\frac{8}{3}}$$

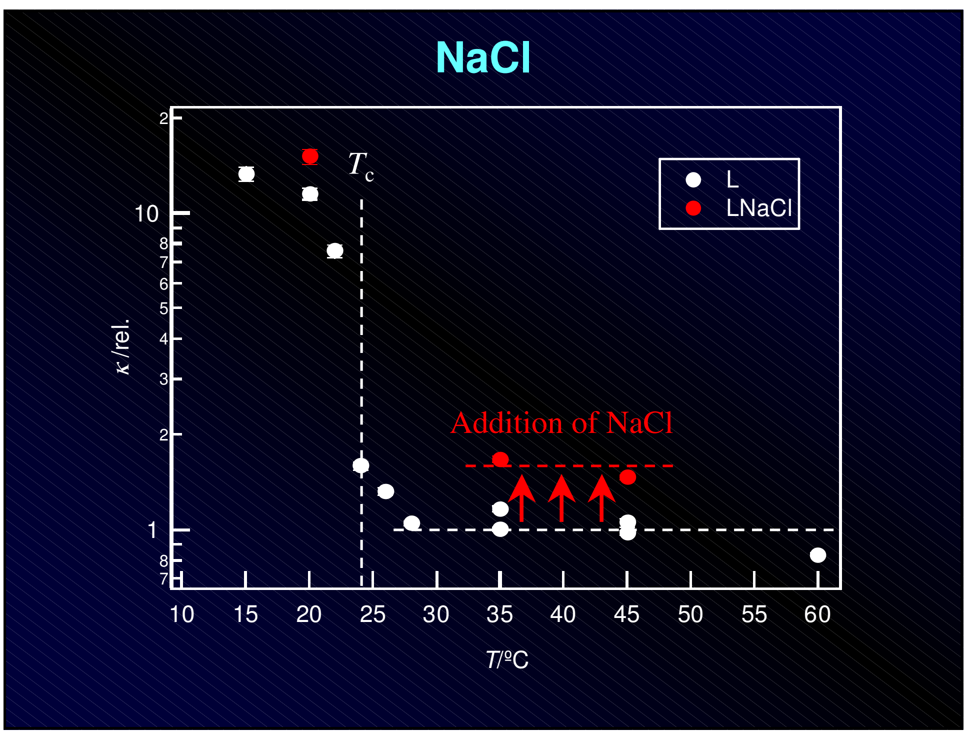
Bending elasticity

Sample	t/°C	$\eta_{D2O}\times10^3$ /N s m^{-2}	κ/k_BT this work	κ/k_BT ref.	method
L	35	0.871	15.3 ± 0.31	13 – 31 (30 °C)	NMR, VM
	35	0.871	13.2 ± 0.20	13 – 31 (30 °C)	
	45	0.714	12.9 ± 0.18	13 – 31 (40 °C)	
LC33	20	1.25	129.7 ± 5.3	150 (20 °C)	VM
	35	0.871	48.1 ± 1.3	96 - 98 (30 °C)	
	45	0.714	42.4 ± 0.91	73 (40 °C)	
LC50	35	0.871	94.9 ± 3.2	146 (30 °C)	VM
	45	0.714	96.7 ± 5.3	88 (40 °C)	

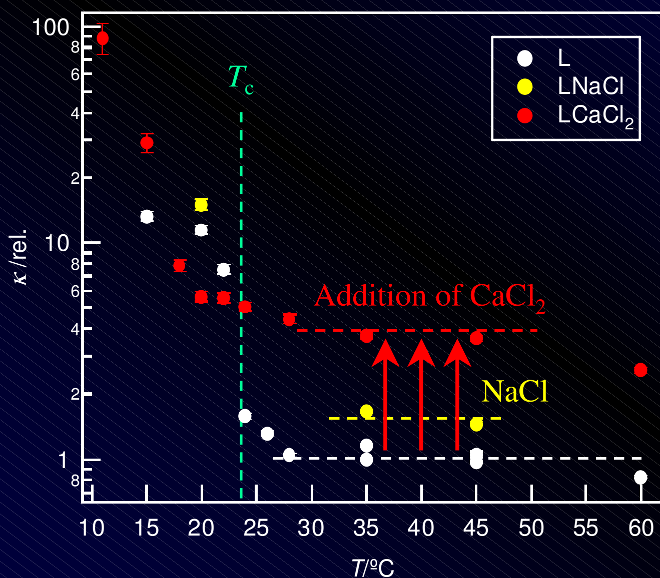
Temperature

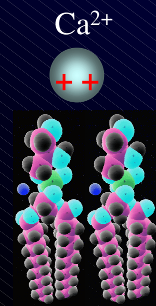



Cholesterol
T_c
L
LC33
LC50
50%
33%
0%
Presence of cholesterol
κ /rel.
T/ºC
10
15
20
25
30
35
40
45
50
55
60


NaCl
T_c
L
LNaCl
Addition of NaCl
κ /rel.
T/ºC
10
15
20
25
30
35
40
45
50
55
60

$CaCl_2$





Summary

- **NSE probes short time and length scales:**
  - Convenient for studies on thermal fluctuations of bio-membranes
- **Temperature:**
  - Liquid-to-crystalline transition increases κ by an order of magnitude
  - At $T > T_c$ temperature effect is weak
- **Cholesterol:**
  - At $T < T_c$ cholesterol has negligible effect on κ
  - At $T > T_c$ κ increases proportionally to the cholesterol concentration
  - Cholesterol smears the sharp liquid-to-crystalline phase transition
- **Electrolytes:**
  - Presence of 50 mM NaCl increases κ by a factor of 1.5
  - Presence of 30 mM $CaCl_2$ increases κ by a factor of 4 and shifts T_c to lower values
- **Suggestions for future studies:**
  - Other electrolytes, pH etc.
  - Effect of other constituents in the lipid bilayers (e.g proteins, other lipids)

Acknowledgements: Dr. Dan Neumann @ NCNR, NIST