Thermal Undulations of Bio-Membranes Studied by Neutron Spin-Echo

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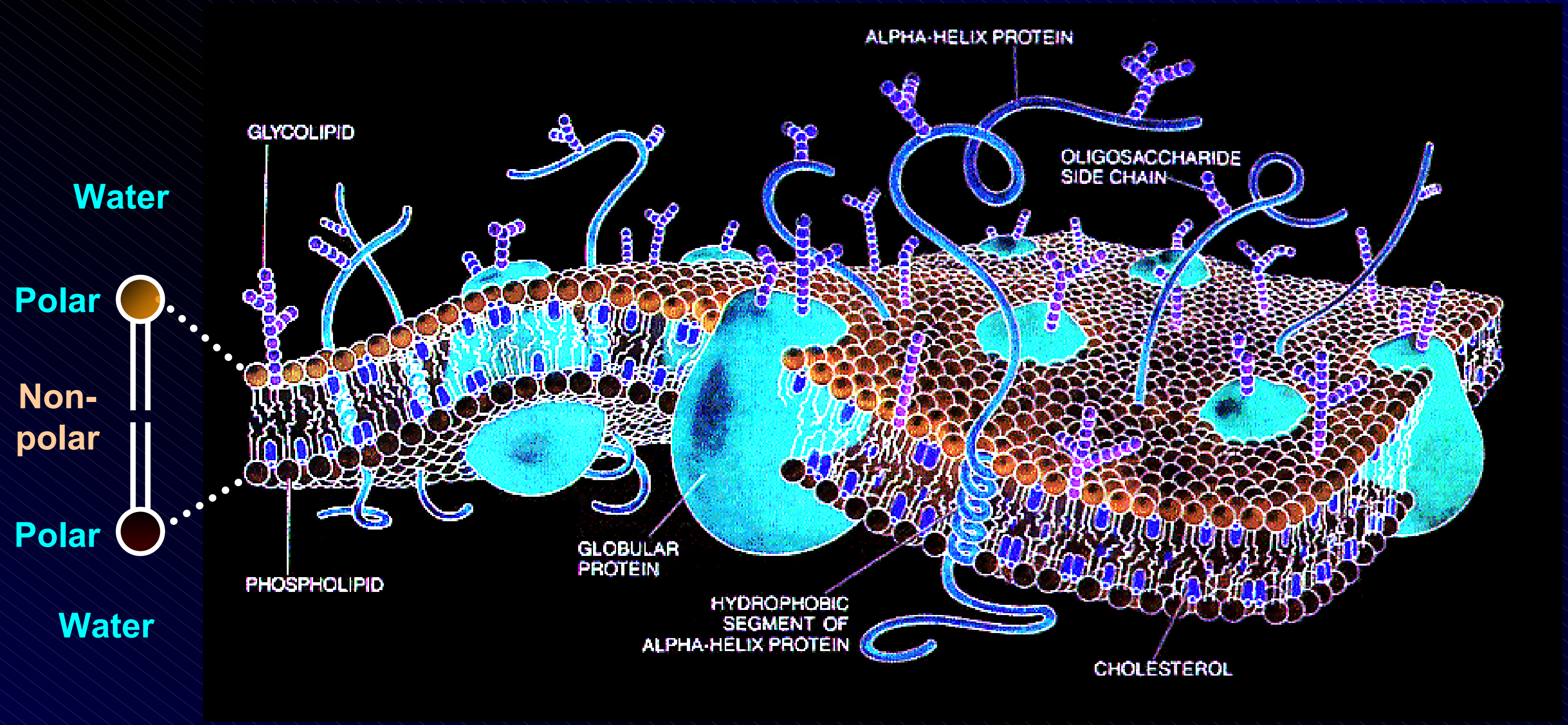
The cell membrane: thermal undulations



Essential Biological Functions

- Immune response
- Cell metabolism
- Neurotransmission
- Photosynthesis
- Cell adherence
- Cell growth and differentiation

Thermal undulations

- Immune response – contact time
- Cell adherence – undulation forces
- Cell mobility

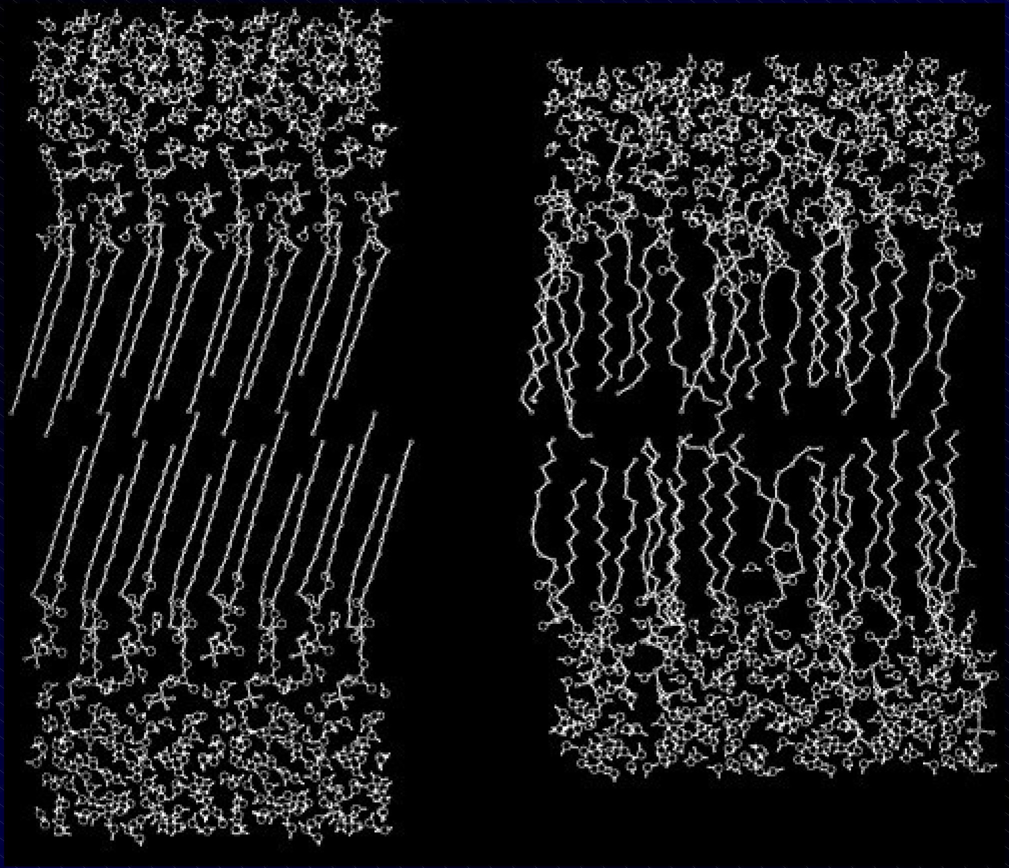
Thermal undulations

↓

Bending elasticity

Factors affecting the bending elasticity

Temperature: Liquid to Crystalline transition



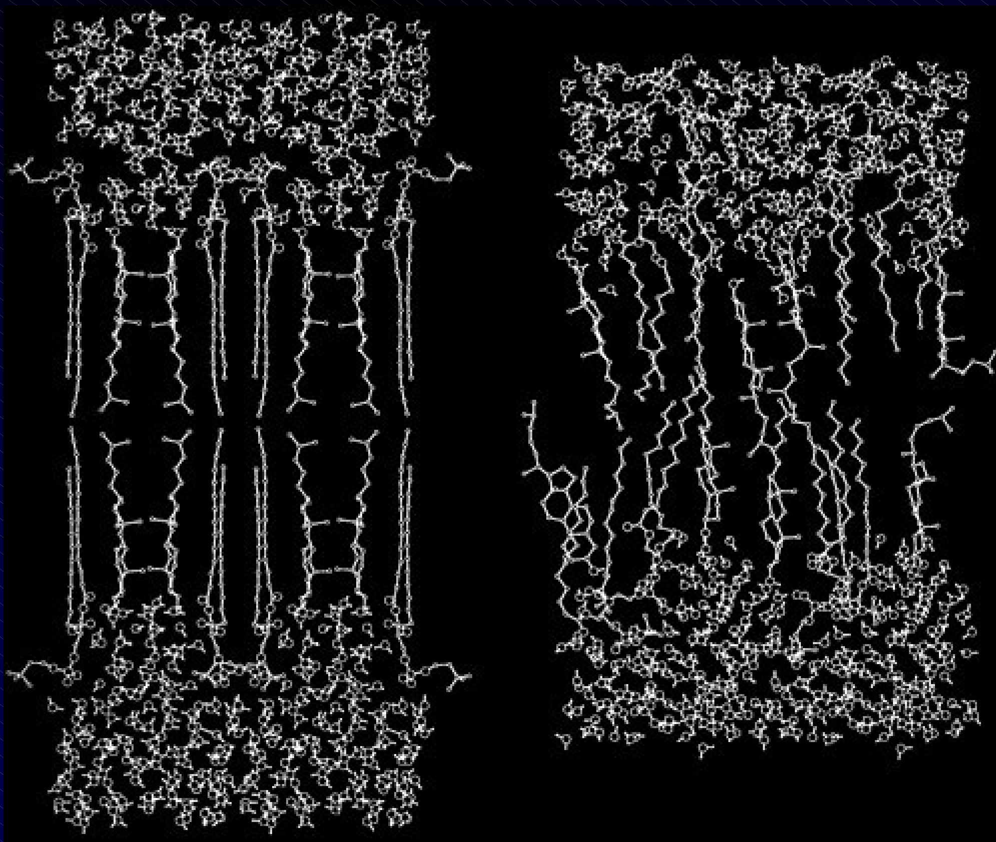
T < T_c *T* > T_c

(T_c = 24 ºC for DMPC)

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

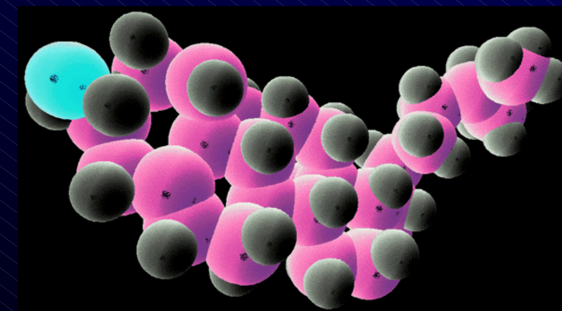
Factors affecting the bending elasticity

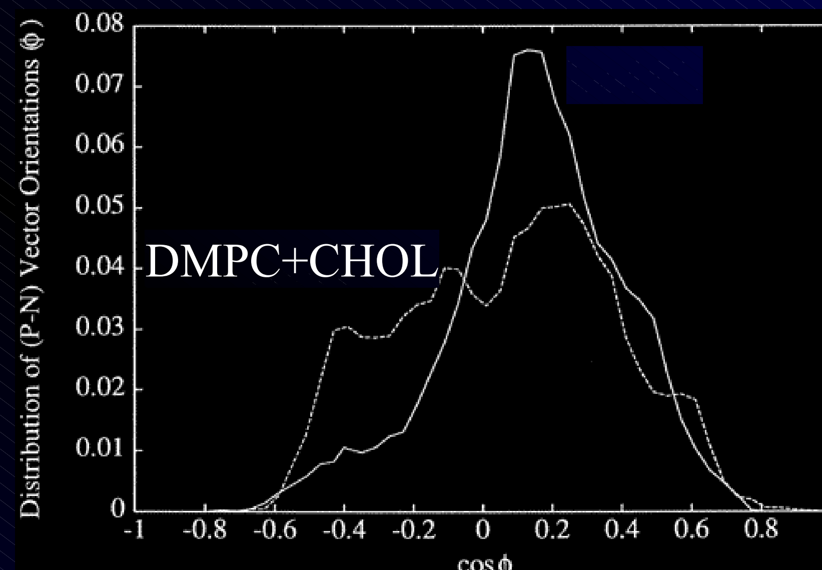
Presence of cholesterol



$T < T_c$ $T > T_c$

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

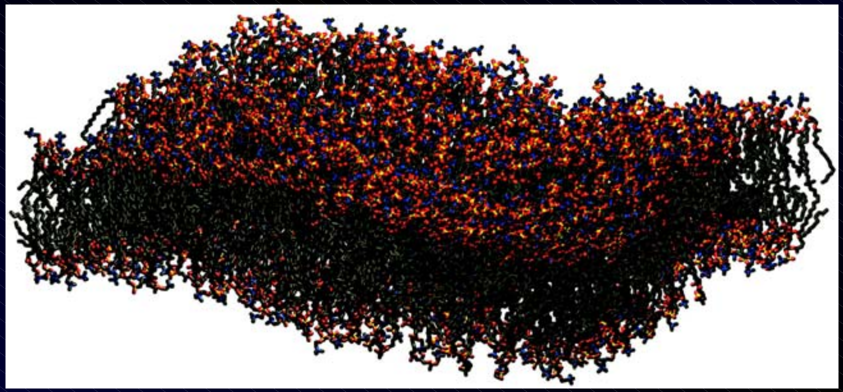




Distribution of the polar group orientations (P - N axis)

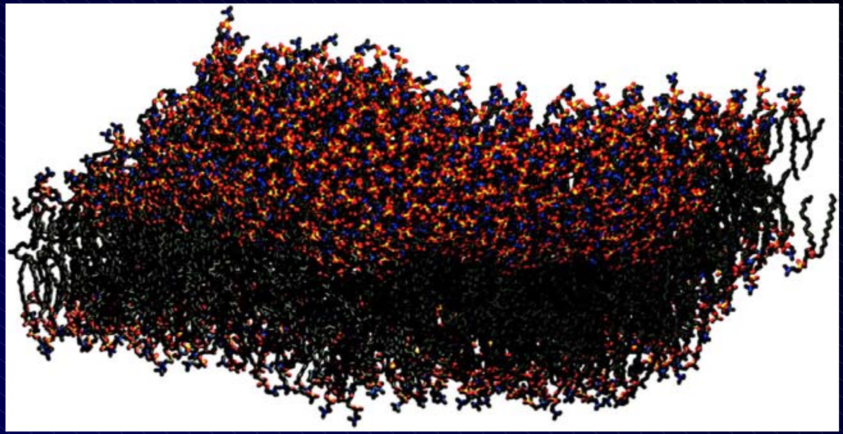
Computer simulations & bending elasticity

DPPC

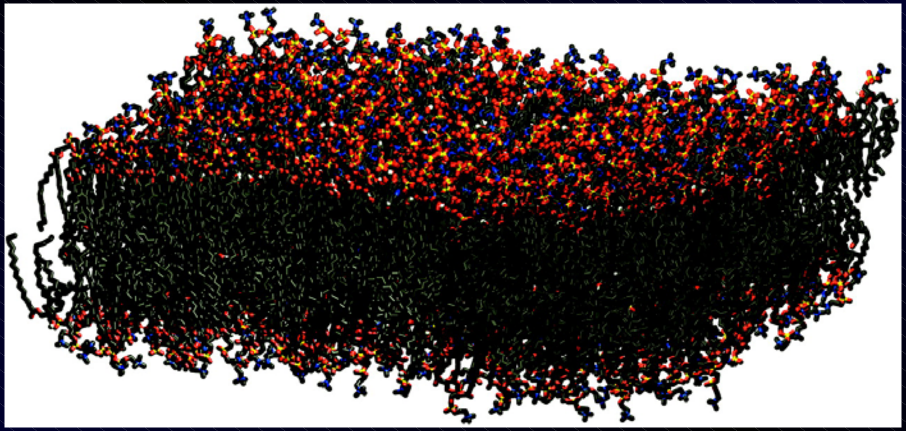


Bending elasticity in the presence of cholesterol

DPPC + 10% cholesterol



DPPC + 40% cholesterol

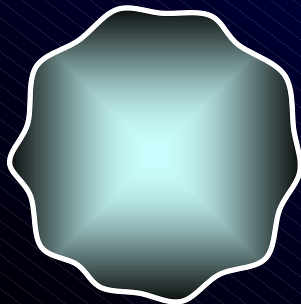


Hofsäß, E. Lindahl, O. Edholm, *Biophysical Journal,* 84: 2192, **2003**

Why is NSE ideal for this purpose?

Goal: Thermal undulations ⇔ Bending elasticity

Thermal undulations:
(highly localized)

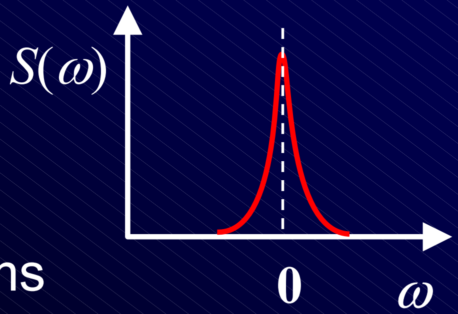


- Videomicroscopy
(large *T* & *L* scales)
- NMR transverse relaxation times
(wide *T* scale, relaxation model?)

NSE
T scale ~ 0.01 - 100 ns
L scale ~ 1 - 10 nm

NSE basics

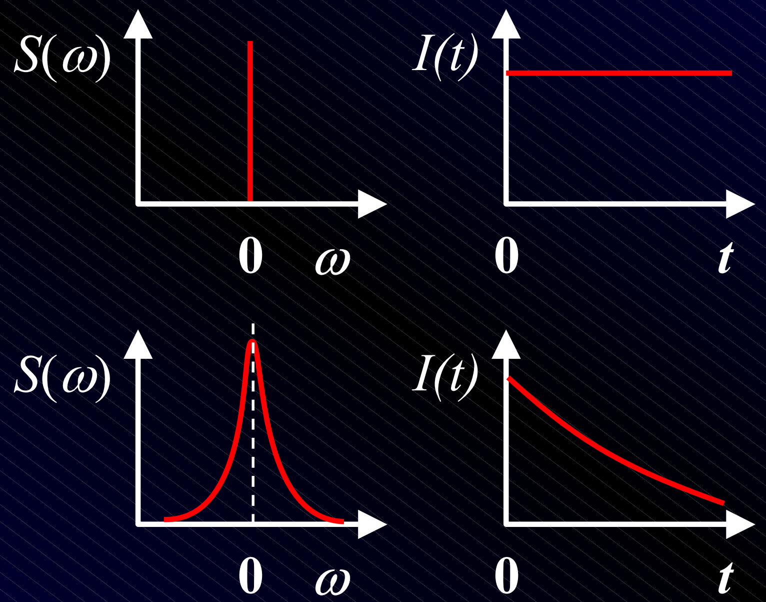
- **NSE is a quasielastic method:** small deviation from the elastic scattering



- **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:** Neutron precession in magnetic field. First proposed by Mezei in 1972. Yields the intermediate scattering function in the time domain *I*(*Q*,*t*):

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



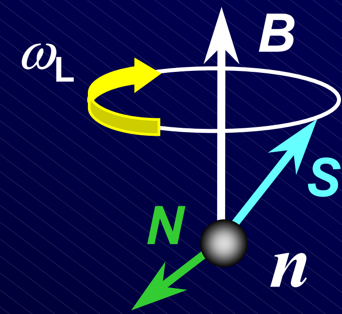
- **Fourier time range:** 0.01 to 200 ns

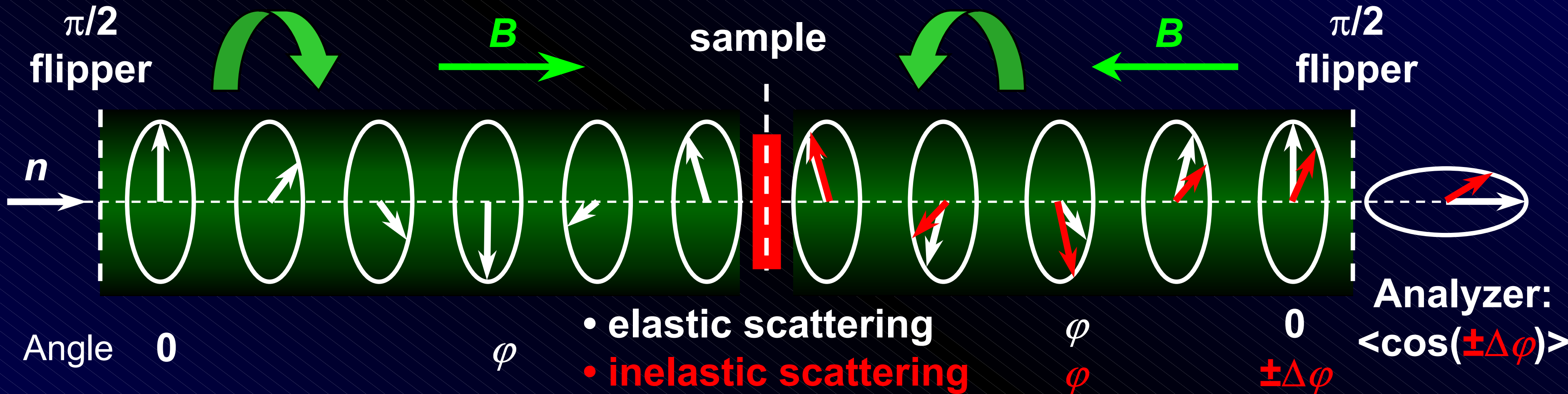
NSE walkthrough

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})

$$N = S \times B$$

$$\omega_L = gB$$





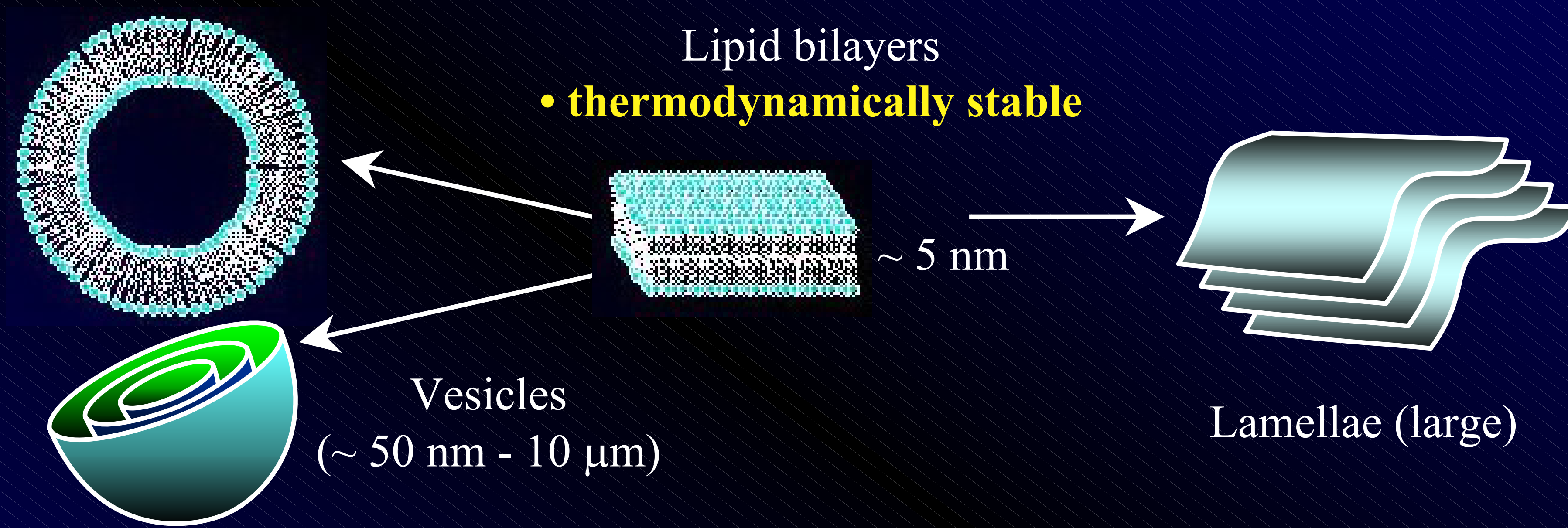
$$\varphi = gB\frac{L}{v}$$

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

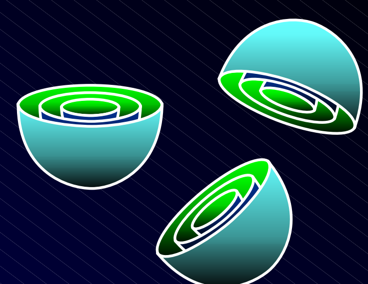
$$B = 0.5\ T,\ \ L = 2\ \mathrm{m}$$
$$\varphi \sim 1\times10^{6}\ \mathrm{rad}$$

$$\frac{\Delta v}{v} \approx \frac{2\pi}{\varphi} \approx 10^{-5}\ \ !$$

Lipid bilayers in aqueous environment



Model system:



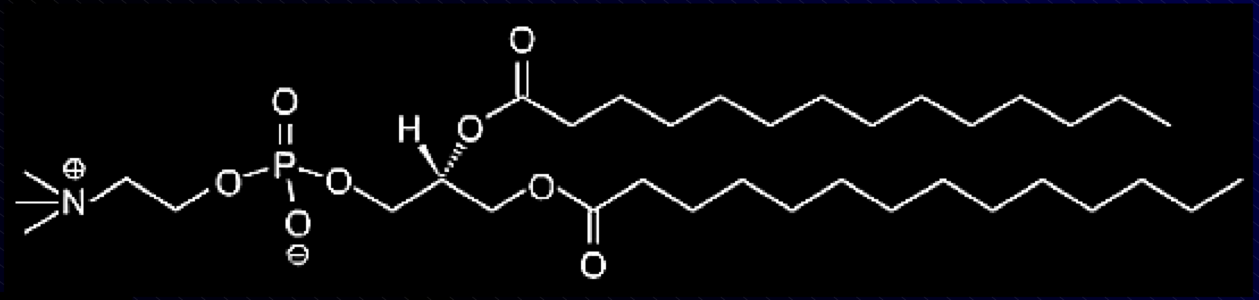
Vesicles

- Good scatterers
- Low concentration
- Mechanically stable

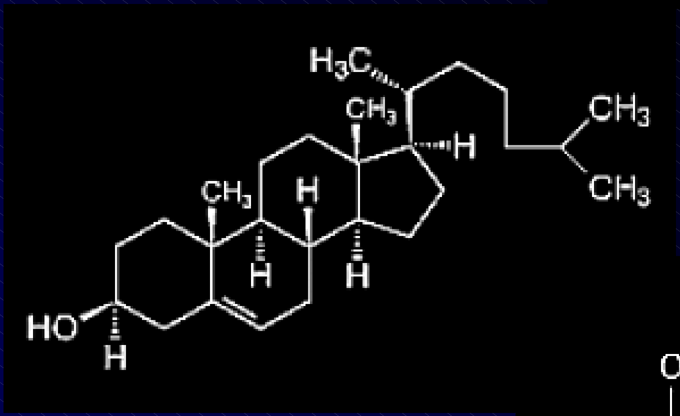
Ingredients

- 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, $CaCl_2$

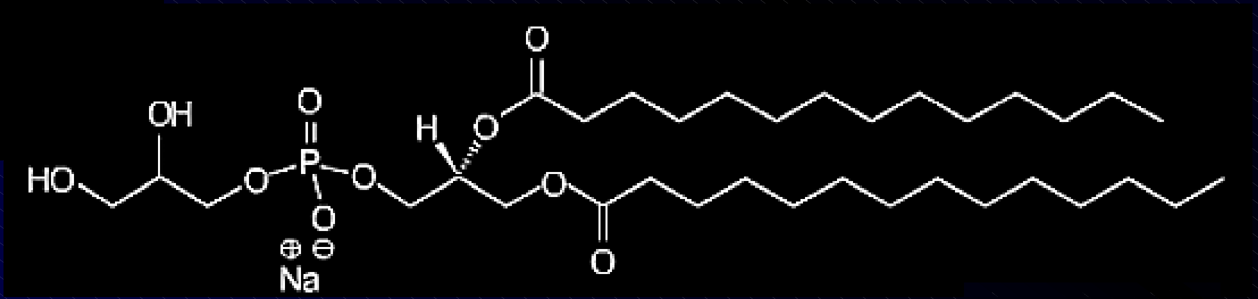
DMPC



Cholesterol



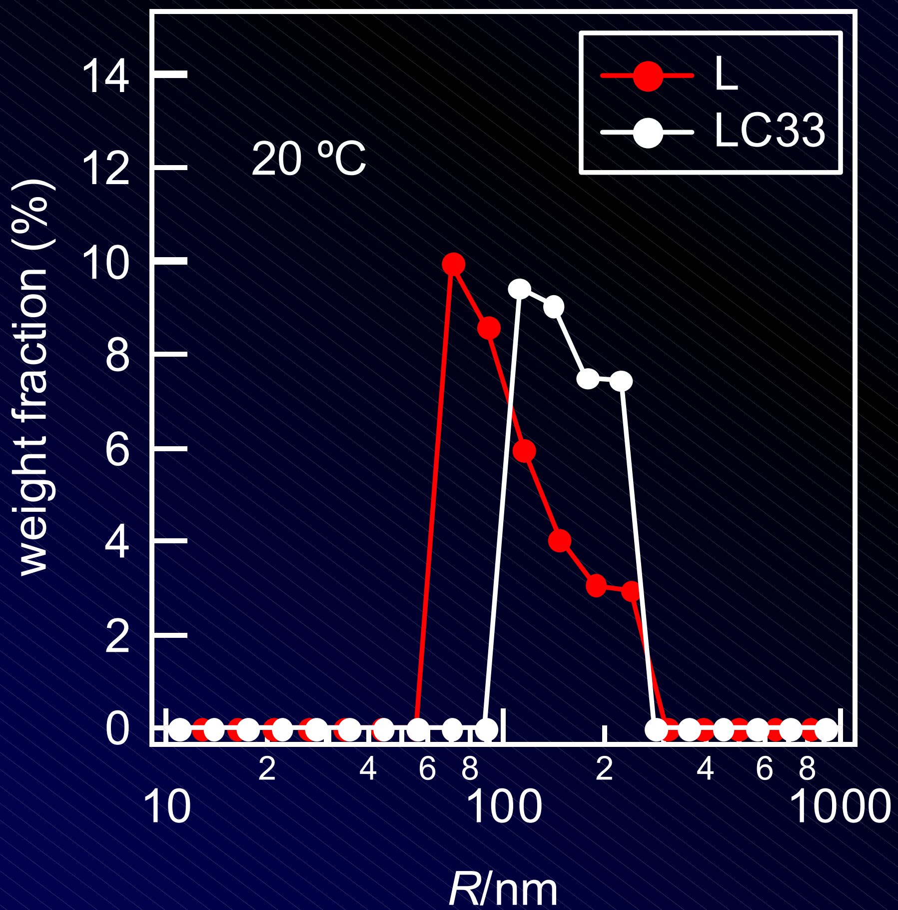
DMPG

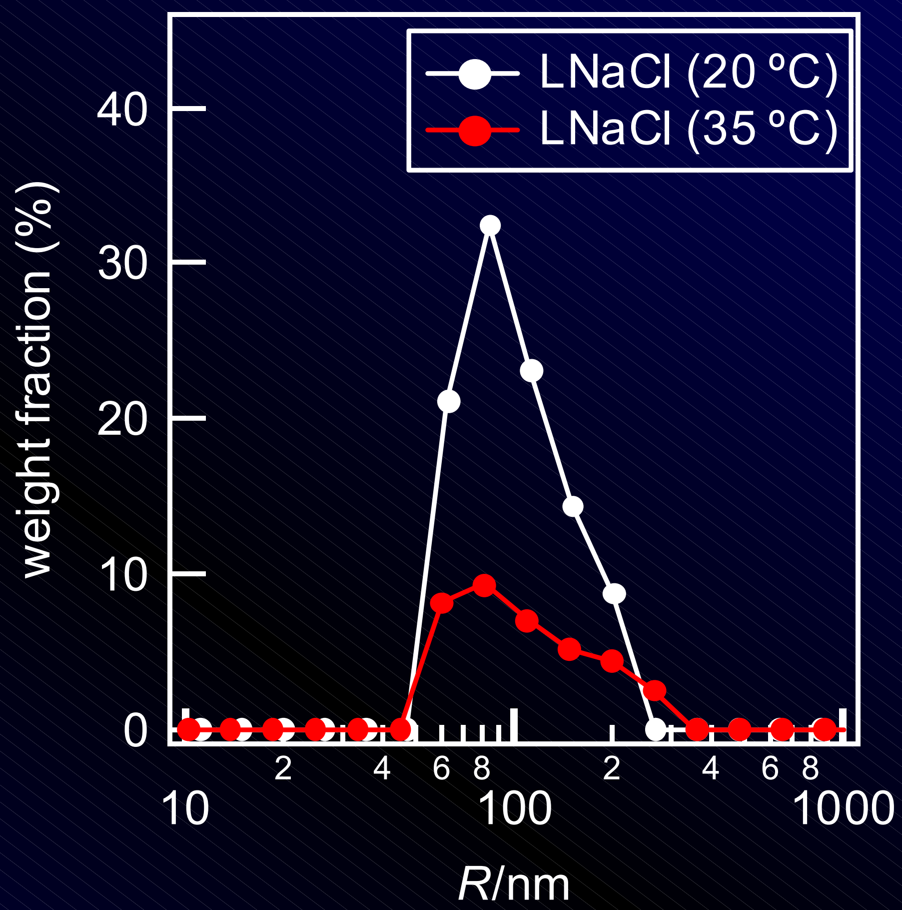


Vesicles compositions & preparation

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)

Dynamic Light Scattering (DLS)

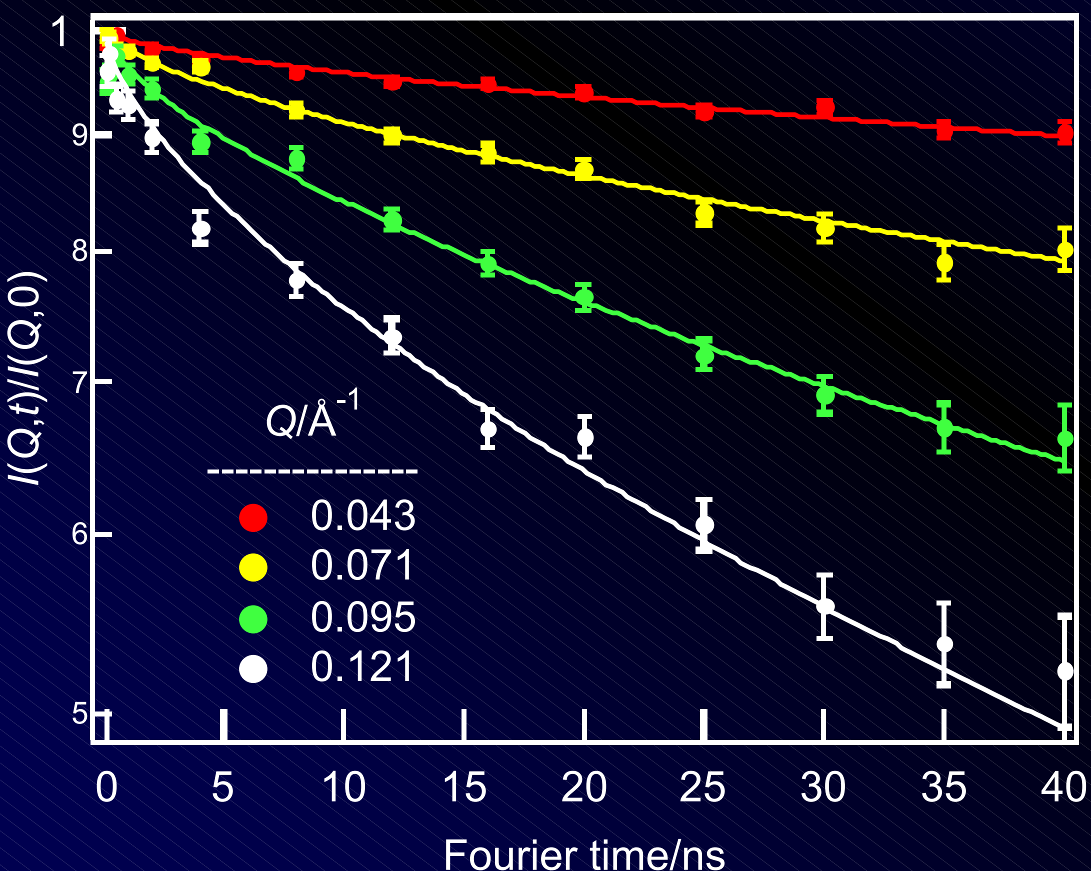




$R \approx 100$ nm

The decay of *I*(*Q*,*t*) measured by NSE

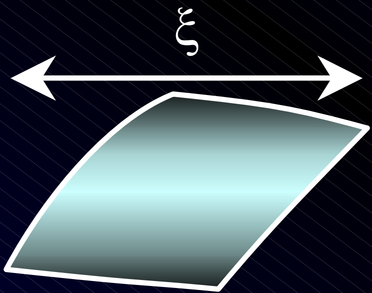
$$I(Q,t) = \int_{-\infty}^{\infty} S(Q,\omega)\cos(\omega t)\,d\omega$$



$$\frac{I(Q,t)}{I(Q,0)} = \exp\left[-(\Gamma t)^{\frac{2}{3}}\right]$$

L at 35 ºC

Zilman-Granek theory for thermal undulations



Membrane plaquette

Dynamic structure factor

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

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

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

static dynamic

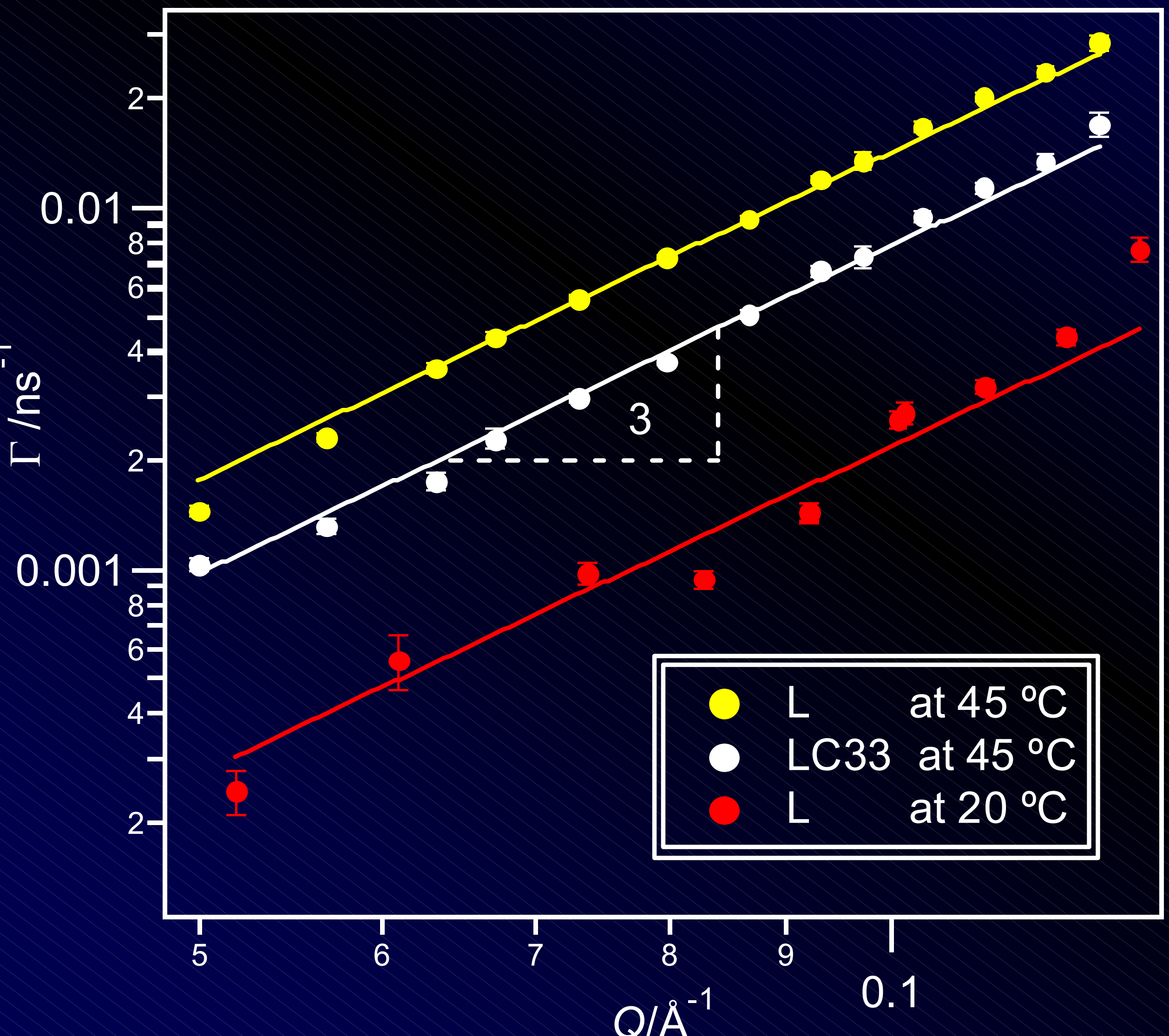
$$\left\langle\left[h(\vec{r},t)-h(\vec{r}',0)\right]^2\right\rangle=\Phi_0(\vec{r}-\vec{r}')+\Phi_0(\vec{r}-\vec{r}',t)$$

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

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*

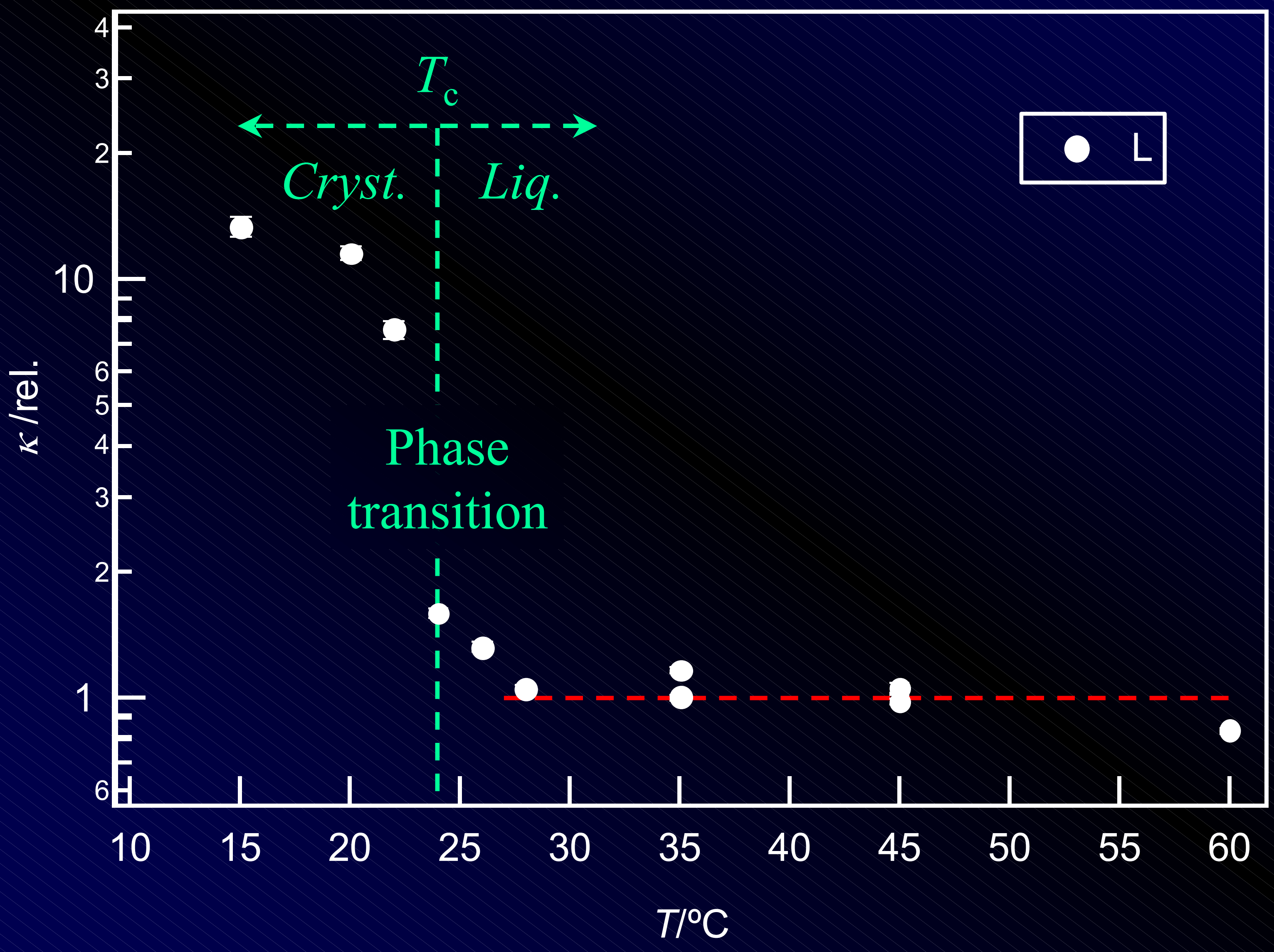


$$\Gamma \propto \sqrt{\frac{k_B T}{\kappa}} \frac{k_B T}{\eta} Q^3$$

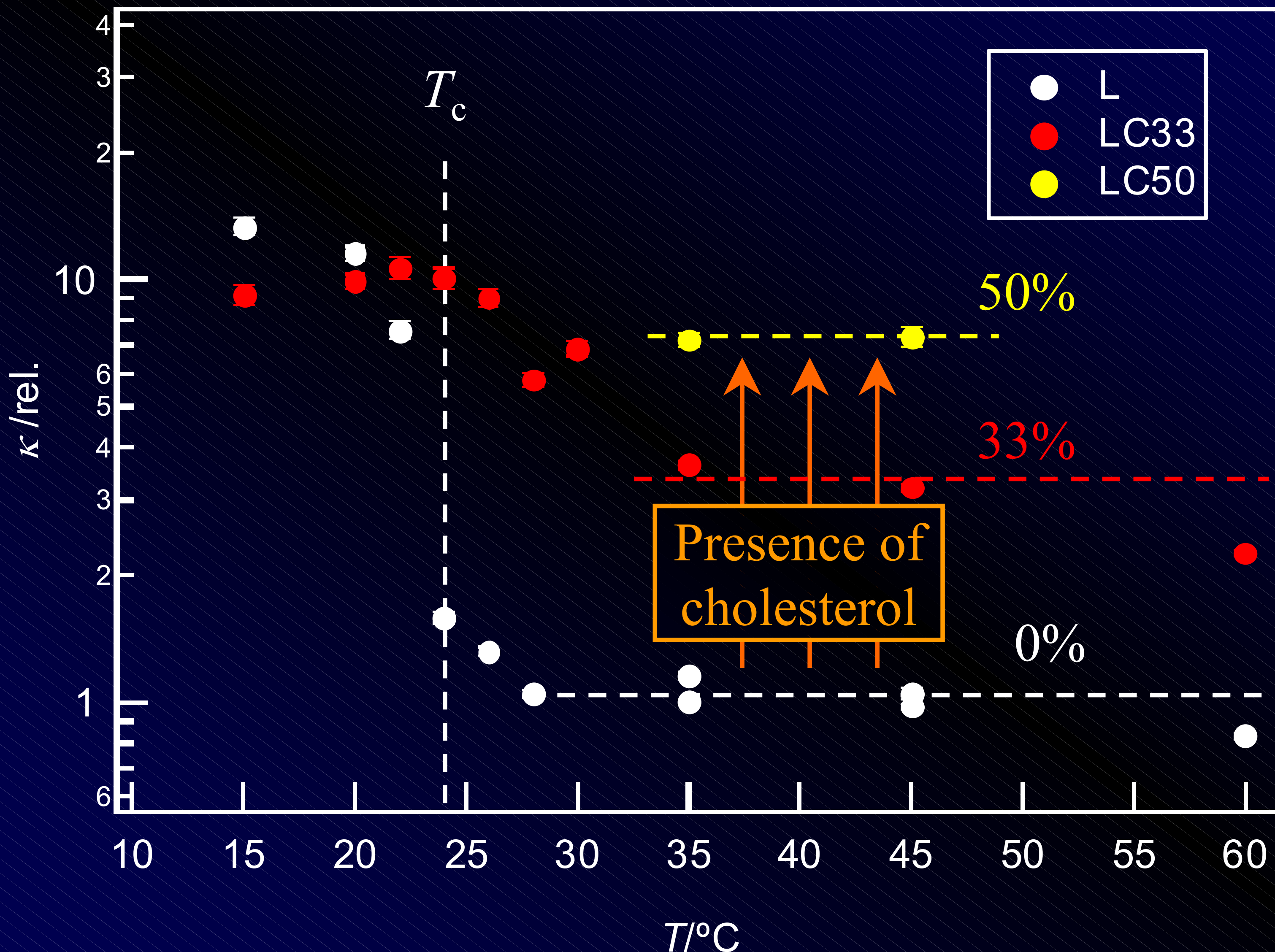
Bending elasticity

Sample	t/°C	$\eta_{D2O}\times10^3$ /N s m^{-2}	$\kappa/k_B T$ this work	$\kappa/k_B T$ 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)	

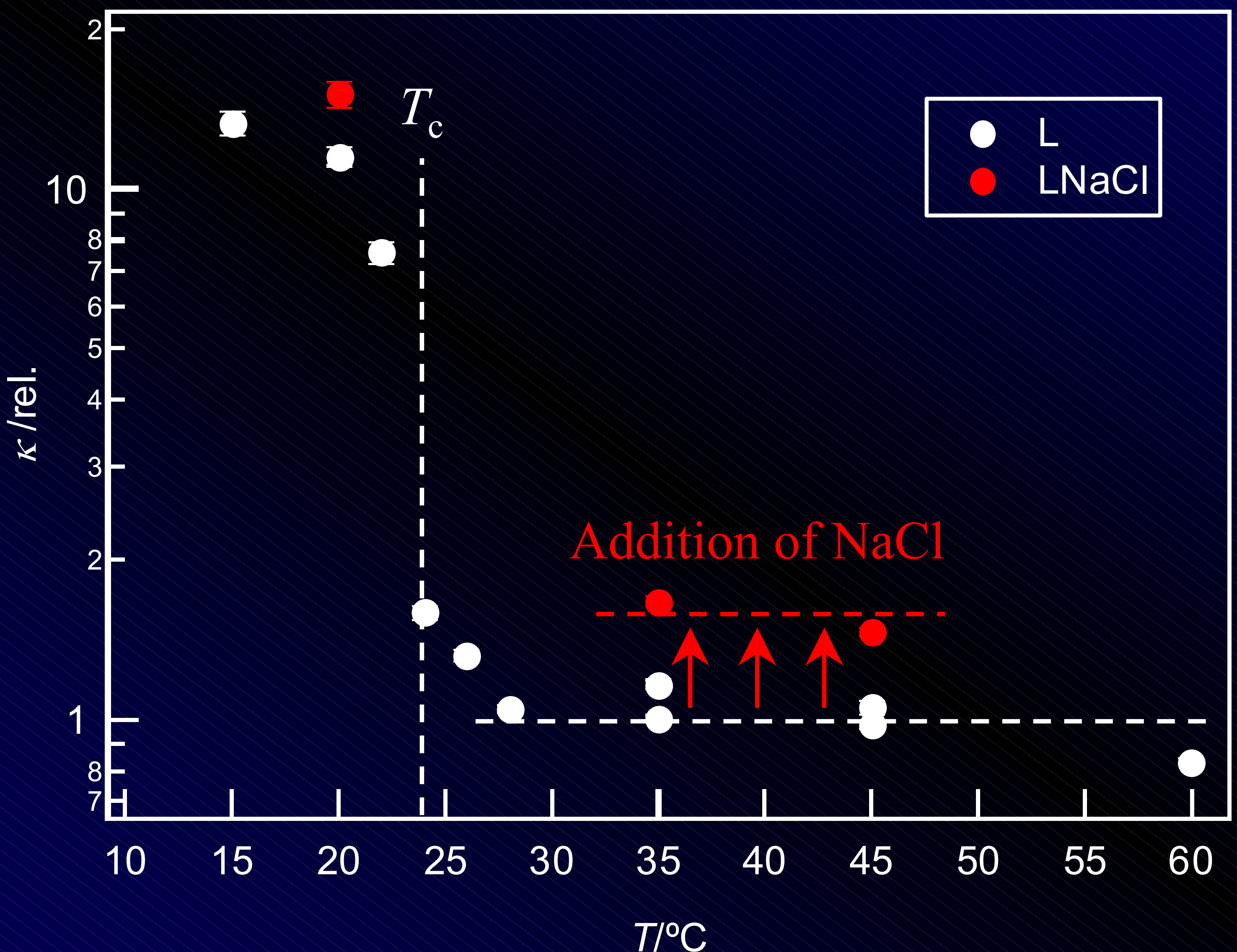
Temperature



Cholesterol

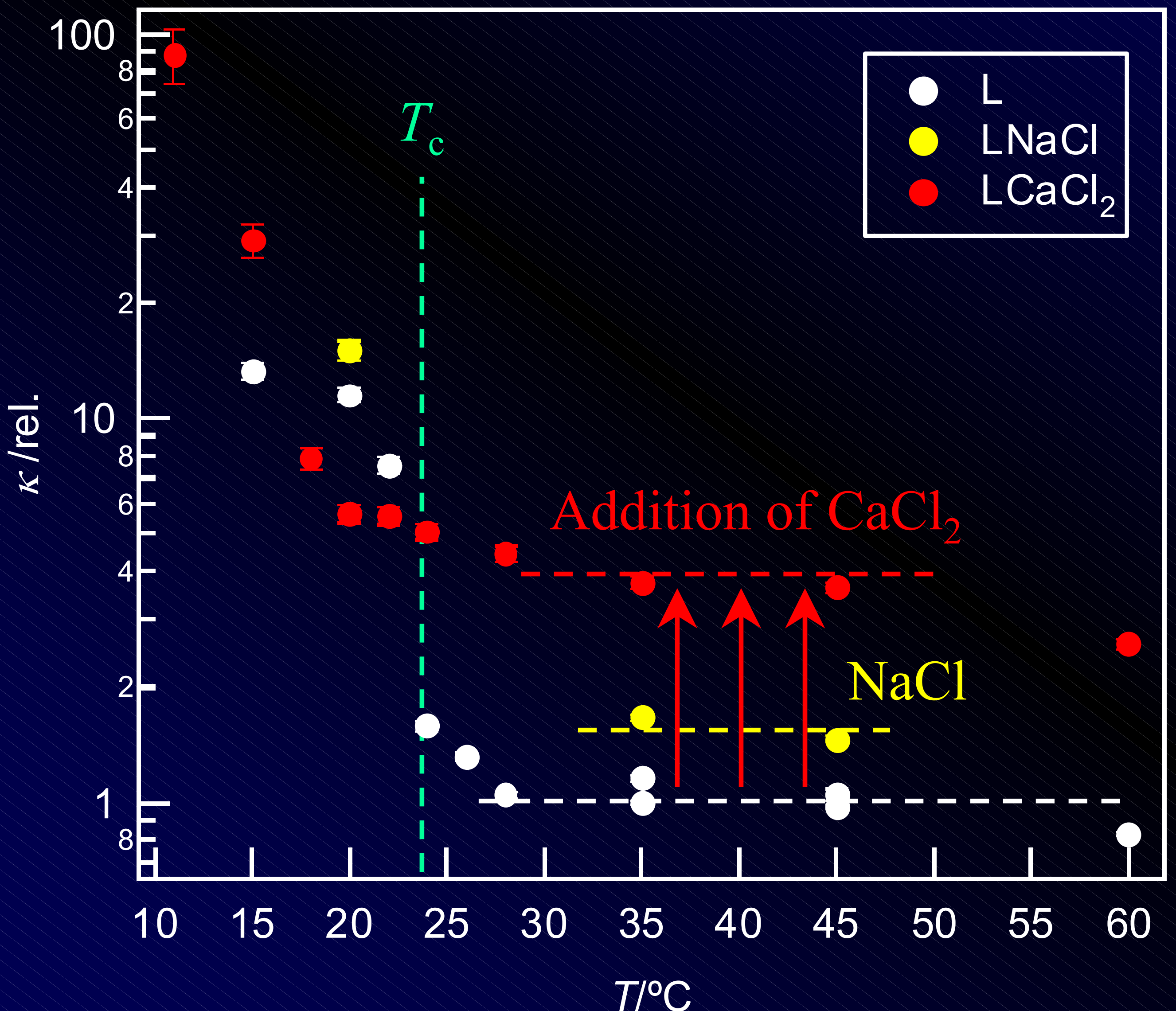


NaCl



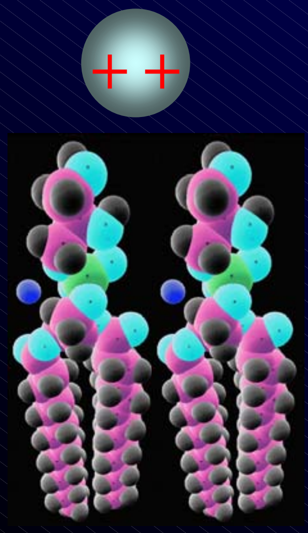
T_c
L
LNaCl
Addition of NaCl
κ /rel.
T/ºC
2
10
8
7
6
5
4
3
2
1
8
7
10
15
20
25
30
35
40
45
50
55
60

$CaCl_2$



100
8
6
4
2
10
8
6
4
2
1
8
κ /rel.
10
15
20
25
30
35
40
45
50
55
60
T/ºC
T_c
L
LNaCl
$LCaCl_2$
Addition of $CaCl_2$
NaCl

Ca^{2+}



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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

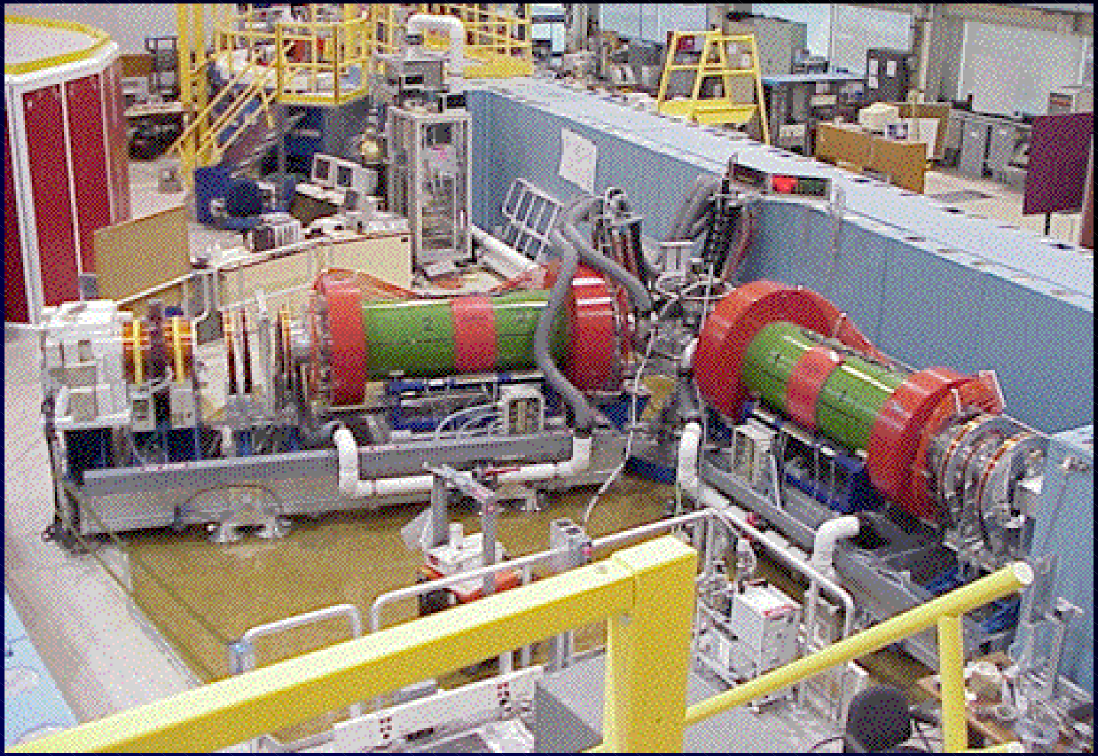
- **Cholesterol:**
- At $T < T_c$ cholesterol has negligible effect on κ
- At $T > T_c$ κ increases proportionally to the cholesterol concentration
- Cholesterol smears the sharp liquid-to-crystalline phase transition

- **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

NSE at NCNR, Gaithersburg, MD



NSE at NCNR, is currently the only operating NSE in North America. NCNR is a user facility

http://www.ncnr.nist.gov