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

**Bending elasticity**

**Topologies of the lipid bilayers**

- **Micelles** (~ 2 nm)
- **Bicelles** (~ 20 nm)
- **Lipid bilayers** (~ 5 nm)
- **Unilamellar vesicles** (~ 50 nm - 10 μm)
- **Multilamellar vesicles** (~ 50 nm - 10 μm)

**Lamellae (large)**

**DMPC:**
Factors that may affect the bending elasticity

Temperature: liquid to crystalline transition \((T_c = 24 \, ^\circ C \text{ for DMPC})\)

Presence of cholesterol

\(T < T_c\)  \(T > T_c\)


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

Small Angle Neutron Scattering (SANS)

Elastic scattering $\Rightarrow$ 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 $I(Q,t)$:

  $$ I(Q,t) = \int S(Q,\omega) \cos(\omega t) d\omega $$

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

\[2\sin^4 \frac{q}{l} = Q\]
**NSE walkthrough**

Neutrons possess spin and magnetic moment. Larmor frequency of precession in magnetic fields depends on $\mathbf{B}$ only ($g = 1.83 \times 10^8 \text{ s}^{-1} \text{ T}^{-1}$)

$$N = S \times B$$

$$\omega_L = gB$$

Angle $0$ $\varphi$ $\pm \Delta \varphi$

- elastic scattering
- inelastic scattering

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

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

$B = 0.5 \text{ T}, \ L = 2 \text{ m}$

$\varphi \sim 1 \times 10^6 \text{ rad}$

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

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

**Ingredients**

- 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC), $t_{\text{trans}} = 24 \degree \text{C}$
- 1,2-Dimyristoyl-sn-Glycero-3-[Phospho-rac-(1-glycerol)] (Sodium Salt) (DMPG)
- Cholesterol
- NaCl, CaCl$_2$

**DMPC**

**Cholesterol**

**DMPG**
Vesicles compositions & preparation

<table>
<thead>
<tr>
<th>For all samples</th>
<th>- total lipids = 2 wt.%, DMPG/DMPC = 5 mol.%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>L</strong></td>
<td>- DMPC and DMPG in D₂O</td>
</tr>
<tr>
<td><strong>LC33</strong></td>
<td>- cholesterol/total lipids = 33 mol.%</td>
</tr>
<tr>
<td><strong>LC50</strong></td>
<td>- cholesterol/total lipids = 50 mol.%</td>
</tr>
<tr>
<td><strong>LNaCl</strong></td>
<td>- NaCl added to L at 50 mM</td>
</tr>
<tr>
<td><strong>LCaCl₂</strong></td>
<td>- CaCl₂ added to L at 30 mM</td>
</tr>
</tbody>
</table>

**Method**
- Extrusion through a filter (200 - 400 nm pores)

**Background**
- D₂O

**Resolution**
- carbopack (elastic scatterer)

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Small Angle Neutron Scattering (SANS)

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SANS from DMPC vesicles in D₂O

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**R/Å** 1002

polydisp (0.1) 0.32

shell/Å 42

SLD core/Å² 6.4×10⁻⁶

SLD shell/Å² 1.0×10⁻⁶

SLD solvent/Å² 6.4×10⁻⁶

Bkg/cm⁻¹ 0.07
Dynamic Light Scattering (DLS)

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

\[ I(Q,t) = \int S(Q,\omega) \cos(\omega t) d\omega \]

\[ \frac{I(Q,t)}{I(Q,0)} = \exp\left[-(\Gamma t)^{\frac{3}{2}}\right] \]
Zilman-Granek theory for thermal undulations

Dynamic structure factor
\[ S(\vec{Q}, t) = \left\langle \sum_{i,j} \sum_{k} e^{i\vec{Q}(\vec{r}_i(t) - \vec{r}_j(0))} \right\rangle \]

lateral undulation
\[ \vec{R}_l(t) = \vec{r}_l(t) + z_l(t) \]

perpendicular undulation

Membrane plaquette

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

(weak deformations, \( \nabla h \ll 1 \))

Dynamic structure factor
\[ S(\vec{Q}, t) = \frac{1}{a^4} \int d^2 r \int d^2 \vec{r} e^{i\vec{Q}(\vec{r} - \vec{r}')} e^{-\frac{Q^2}{2} [h(\vec{r}, t) - h(\vec{r}', 0)]^2} \]

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

Dynamic
\[ I(\vec{Q}, t) = I(Q, 0) \exp \left[ - (\Gamma t)^{\frac{3}{2}} \right], \quad \Gamma = 0.025 \gamma \frac{k_B T}{k} \frac{k_B T}{\eta} Q^3 \]


![Relaxation rate of I(Q, t) as a function of Q](image)
### Bending elasticity

<table>
<thead>
<tr>
<th>Sample</th>
<th>$t/°C$</th>
<th>$\eta_{020} \times 10^3$ /N s m$^2$</th>
<th>$\kappa/k_B T$ this work</th>
<th>$\kappa/k_B T$ ref.</th>
<th>method</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>35</td>
<td>0.871</td>
<td>15.3 ± 0.31</td>
<td>13 – 31 (30 °C)</td>
<td>NMR, VM</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.871</td>
<td>13.2 ± 0.20</td>
<td>13 – 31 (30 °C)</td>
<td>NMR, VM</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.714</td>
<td>12.9 ± 0.18</td>
<td>13 – 31 (40 °C)</td>
<td>VM</td>
</tr>
<tr>
<td>LC33</td>
<td>20</td>
<td>1.25</td>
<td>129.7 ± 5.3</td>
<td>150 (20 °C)</td>
<td>VM</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>0.871</td>
<td>48.1 ± 1.3</td>
<td>96 - 98 (30 °C)</td>
<td>VM</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.714</td>
<td>42.4 ± 0.91</td>
<td>73 (40 °C)</td>
<td>VM</td>
</tr>
<tr>
<td>LC50</td>
<td>35</td>
<td>0.871</td>
<td>94.9 ± 3.2</td>
<td>146 (30 °C)</td>
<td>VM</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.714</td>
<td>96.7 ± 5.3</td>
<td>88 (40 °C)</td>
<td>VM</td>
</tr>
</tbody>
</table>

### Temperature

![Temperature graph](image_url)
Cholesterol

Addition of NaCl
Summary

• **NSE probes short time and length scales:**
  - Convenient for studies on thermal fluctuations of bio-membranes

• **Temperature:**
  - Liquid-to-crystalline transition increases $\kappa$ by an order of magnitude
  - At $T > T_c$ temperature effect is weak

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

• **Electrolytes:**
  - Presence of 50 mM NaCl increases $\kappa$ by a factor of 1.5
  - Presence of 30 mM CaCl$_2$ increases $\kappa$ 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)

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