Lipid Bilayers and Membrane Dynamics: Insight into Thickness Fluctuation

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Motivation

Lipid membranes are self-assembled highly flexible structures that have the ability to undergo an array of conformational and dynamic transitions which are essential for many biological functions.

Microscopic length scale:
Membrane stiffness and fluidity have been shown to have a large impact on cellular uptake and release.\(^{(2)}\)

Intermediate length scale:
Membrane thickness fluctuations have been proposed as a mechanism for pore formation.\(^{(3)}\)

Spectroscopic length scale:
Cell signal transduction is affected by molecular lateral diffusion within the lipid membrane.\(^{(1)}\)

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Membrane Dynamics

observation length scale

molecular motion
incoherent-quasi-elastic scattering
nuclear magnetic resonance etc.

collective motion
coherent-quasi-elastic scattering
photon correlation spectroscopy
The Dynamics in Lipid Bilayers

Molecular movements (incoherent movements of molecules)
- Lateral diffusion
- Rotation
- Vertical vibration

Coherent movements of molecular assemblies
- Stretching
- Compression
- Bending
- Protrusion
- Thickness fluctuations
Techniques to Measure Dynamics

- Molecular motion
- Protrusion
- Thickness fluctuations
- Stretching/Compression
- Capillary wave
Neutron Spin Echo

Schematic of the NG5-NSE Spectrometer

Motion of the neutron beam spins in the spectrometer

Elastic Scattering
Surfactant Membranes


- NSE measured $Q$ dependence of $D_{\text{eff}}$
- showing an increase at the membrane thickness length scale

Thickness fluctuations in lipid bilayers (theoretical studies)

Breathing model of a lipid bilayer by Miller

Amplitude of the fluctuations reaches $\approx 15$ Å or more from the geometrical constraints (volume conservation)

Thickness fluctuations by Hladky and Gruen

Thickness fluctuations occur, but the amplitude is small.

- Long wavelength fluctuation amplitude is negligible
- Short wavelength fluctuations ($< 30$ Å) are severely limited
- Intermediate wavelength fluctuation amplitude $< 10$ Å

Deformation free energy of bilayer membranes by Huang

$$\sqrt{\langle\text{Amplitude}^2\rangle} = \frac{k_B T}{\pi \gamma} \left\{ \tan^{-1} \left[ \frac{16 \pi^2 K_1 + h \gamma}{2 h \sqrt{K_1 B}} \right] - \tan^{-1} \left[ \frac{\gamma}{2 \sqrt{K_1 B}} \right] \right\} \approx 4.5$Å

Theoretically, thickness fluctuations exist, their amplitude is very small
Bilayer Preparation

(D54) DMPC = C14
(D62) DPPC = C16
(D70) DSPC = C18
Phospholipid Melting Temperature

Fluid phase above $T_m$

Gel phase, below $T_m$

Above $T_m$ lipid tails highly fluid, disordered and in constant motion

Below $T_m$ transition to a gel state, tails are fully extended with highly ordered packing
Small Angle Neutron Scattering

Peak shifts to higher Q with decreasing tail length

$q \sim 2\pi / \text{length scale}$

SANS and NSE are complimentary techniques

**SANS**
static “snapshot”
elastic scattering

$I(q) = \int S(q, \omega) d\omega$

**NSE**
dynamic “snapshot”
quasielastic scattering

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

*Scattering intensity is offset to highlight peak shift*
NSE : $I(q,t)$

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

Graphs showing $I(q,t)$ versus $t$ for different temperatures and phases:

- **DPPC 50ºC**
- **DMPC 25ºC**
- **DSPC 55ºC**
Surfactant Membrane Dynamics (bending)

Helfrich bending energy

Assuming the membrane is thin enough sheet, which is undulating

Zilman-Granek theory

Dynamics of a planar non-interacting Helfrich sheet

\[ I(q, t) = I(q, 0) \exp \left( -\frac{Gt}{2/3} \right) \]

\[ G = 0.025 \frac{k_B T}{k_BT \hbar q^3} \]

\( k \): bending modulus

\( \eta \): solvent viscosity

Watson-Brown theory

Extension of ZG theory including slipping of each monolayer

Inter-monolayer friction plays a role, where lateral compressibility \( k_m \) of membrane appears in dynamical equation

\[ \kappa \rightarrow \tilde{k} = \kappa + 2d^2 k_m \]
Bending motion is explained as a single membrane dynamics model

\[
\frac{I(q, t)}{I(q, 0)} = \exp \left[- \left(\Gamma t\right)^{\beta}\right]
\]

\(\Gamma\): decay rate, \(\beta=2/3\)

\[
\frac{\Gamma_{\text{Bend}}}{q^3} = 0.025\alpha\sqrt{\frac{k_B T}{\tilde{\kappa}}} \frac{k_B T}{\eta_{D_2O}}
\]

\(\tilde{\kappa}\): effective bending modulus,
\(\eta\): solvent viscosity, \(\alpha \approx 1\)

Considering slipping friction

\[
\tilde{\kappa} = \kappa + 2d^2k_m
\]

\[
k_m = \frac{24\kappa}{d_t}
\]

\[
\frac{\Gamma_{\text{Bend}}}{q^3} = 0.0058\sqrt{\frac{k_B T}{\kappa}} \frac{k_B T}{\eta_{D_2O}}
\]
\[ \Gamma_{\text{BEND}} = 0.025 \left( \frac{k_B T}{\kappa} \right)^{1/2} \frac{k_B T}{3 \eta_{D_2O}} q^3 \]

\[ \frac{\Gamma}{q^3} = \frac{\Gamma_{\text{BEND}}}{q^3} + \frac{\Gamma_{\text{TF}}}{q^3} \frac{1}{1 + (q - q_0)^2 \xi^2} \]

\( \Gamma \) deviates from the pure \( q^3 \) dependence expected from bending motions.

This peak occurs at the same \( q \) as the SANS dip position.

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Zilman-Granek
**NSE: Bending Modulus**

**Literature**


**Experiment**

![Graph showing experiment results for different lipids (DMPC, DPPC, DSPC) with temperature difference (T-Tm) in °C on the x-axis and bending modulus (k/k_BT) on the y-axis.](image)

**Table**

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Tm (°C)</th>
<th>T (°C)</th>
<th>(T - Tm) (°C)</th>
<th>(\kappa_c/k_BT)</th>
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<tbody>
<tr>
<td>14:0 PC</td>
<td>24</td>
<td>22</td>
<td>-2</td>
<td>100.0 ± 4.99</td>
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<tr>
<td></td>
<td>24</td>
<td>24</td>
<td>0</td>
<td>20.9 ± 0.61</td>
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<td>+4</td>
<td>13.9 ± 0.24</td>
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<td></td>
<td>35</td>
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<td>+11</td>
<td>15.3 ± 0.31</td>
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<tr>
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<td>45</td>
<td>45</td>
<td>+21</td>
<td>13.9 ± 0.44</td>
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<td>60</td>
<td>60</td>
<td>+36</td>
<td>8.2 ± 0.12</td>
</tr>
<tr>
<td>16:0 PC</td>
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<td>30</td>
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<td>49.6 ± 2.78</td>
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<tr>
<td></td>
<td>41</td>
<td>41</td>
<td>0</td>
<td>36.1 ± 1.49</td>
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<tr>
<td></td>
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<td>9.5 ± 0.18</td>
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<tr>
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<td>79.1 ± 3.23</td>
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<tr>
<td></td>
<td>60</td>
<td>60</td>
<td>+6</td>
<td>13.6 ± 0.24</td>
</tr>
</tbody>
</table>
\( q \) dependence of the decay rate: \( \text{Below vs. Above } T_m \)

Below \( T_m \)
DMPC
\( T = 16 \, ^\circ \text{C} \)

no enhancement =
suppressed thickness fluctuations

Enhancement =
thickness fluctuations

Above \( T_m \)
DMPC
\( T = 35 \, ^\circ \text{C} \)
$q$ dependence of the decay rate: 

**Lipid Tail Length**
NSE: Thickness fluctuations ($\Gamma / q^3$)

\[
\frac{\Gamma}{q^3} = \frac{\Gamma_{BEND}}{q^3} + \frac{\Gamma_{TF}}{q^3} \frac{1}{1 + (q - q_0)^2 \xi^2}
\]

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$\Gamma_{BEND} / q^3$: accounts for bending motions

$\Gamma_{TF}$: damping frequency of thickness fluctuations

$\xi$: Proportional to the amplitude of thickness fluctuations

$q_0$: SANS dip position (Lorentzian peak position)
Membrane Thickness Fluctuations

Time scale

Above $T_m$: independent of either temperature or tail length

$$\Gamma \frac{q^3}{q^3} = \Gamma_{BEND} \frac{q^3}{q^3} + \Gamma_{TF} \frac{1}{1+(q-q_0)^2 \xi^2}$$

An order of magnitude slower (than surfactant membranes)

$$\approx 100 \text{ ns}$$
Width of Lorentzian peak relates to the fluctuation amplitude

Mean amplitude = 3.7 Å ± 0.7 Å

Huang’s mean amplitude ≈ 4.5 Å

Lindahl & Edholm’s amplitude ≈ 5 Å

≈ 8 % of the membrane thickness; close to the value seen in surfactant membranes (≈ 12 %)

Suggests amplitude is defined by physical constraints, like volume conservation

Thickness Fluctuation Theory

Although membrane thickness fluctuations have not been previously measured Huang(7) has proposed a theory for thickness fluctuations in a lipid bilayer under the consideration of deformation free energy:

\[
\langle D^2 \rangle = \frac{k_B T}{2 \pi a K_1 C_2} \tan^{-1} \left[ \left( \frac{2\pi}{\lambda_0} \right)^2 + C_1 \right] \frac{1}{C_2} \tan^{-1} \left( \frac{C_1}{C_2} \right)
\]

\[C_1 = \frac{\gamma}{2aK_1}, \quad C_2 = \left( \frac{B}{a^2 K_1} \right)^{1/2}\]

\[D \approx 4.5\text{Å}\]

\[D = \text{thickness fluctuation amplitude}\]
\[B^# = \text{membrane compressibility}\]
\[\lambda_0 = \text{wavelength cut off}\]
\[d_m^* = \text{membrane thickness}\]
\[\kappa^+ = \text{bending modulus}\]
\[\gamma^# = \text{surface tension}\]

\[d_m = 2a, \quad \lambda_0 \approx a\]

+ from NSE measurements   * from SANS measurements   # from literature
Conclusions

- NSE was used to successfully measure lipid membrane thickness fluctuations.
- From SANS it is clear that these fluctuations appear at the length scale of the membrane thickness.
- The relaxation time $\approx 100$ ns and is independent of temperature and tail length.
  - An order slower than that observed in surfactant membranes.
- Amplitude is $\approx 8\%$ of the thickness, consistent with surfactant membranes ($12\%$).
  - Volume conservation may define the fluctuation amplitude.
- Below $T_m$, thickness fluctuations are not observed, suggesting total suppression of the mode or much slower relaxation times which are not accessible by the current setup.
- The experimental amplitude agrees well with both theory and simulation.
- FUTURE DIRECTION: What kind of effects do membrane associated molecules have on membrane dynamics such as thickness fluctuations?
Preliminary Data w/ Protein

T = 35°C
DMPC(NIST) DMPC(ILL) DMPC(ILL)
0.25mol% Ala 0.75mol% Ala 1.26mol% Ala 2.5mol% Ala

Alamethicin

Gramicidin

Compression
Bending

\[ \frac{I}{q} (\AA^3 \text{ns}^{-1}) \]

\[ q (\AA^{-1}) \]

\[ T = 35°C \]
DMPC(NIST) DMPC(ILL)
0.25mol% Gram 0.75mol% Gram 1.25mol% Gram 1.25mol% Gram
Preliminary Data w/ Protein

- DMPC(ILL)
- Ala/DMPC
- Gram/DPPC
- Gram/DMPC

Graphs showing:
- \( \kappa \) vs. [Protein] mol%
- \( \Gamma_F \times 10^{-3} \text{ ns}^{-1} \) vs. [Protein] mol%
- \( \xi^{-1} \times 10^{-3} \text{ Å}^{-1} \) vs. [Protein] mol%