Neutron Scattering and Lateral Membrane Organization

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ORNL is managed by UT-Battelle for the US Department of Energy

APS – Scientific Challenges to Elimination of HEU in Civilian Reactors Workshop
Properties of Neutrons

Neutrons are **Neutral** particles. They:
- Are highly penetrating
- Can be used as non-destructive probes
- Are used to study samples in complex environments

Neutrons have a **Magnetic** moment and **Spin**. Used:
- To study magnetic structure and fluctuations
- Form polarized beams
- Used for coherent and incoherent scattering

Neutrons “see” **Nuclei**. They:
- Are sensitive to light atoms
- Differentially sensitive to isotopes of the same element
- Exploit contrast variation to differentiate complex structures

**X-ray (L) and Neutron (R) Radiographs**

\[
\text{n} + {^3}\text{He} \rightarrow {^3}\text{H} + \text{p} \\
\text{n} + {^{10}}\text{B(F}_3\text{)} \rightarrow {^7}\text{Li} + {^4}\text{He}
\]
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### Biomaterials

<table>
<thead>
<tr>
<th>Atom</th>
<th>Nucleus</th>
<th>$b_c$ ($10^{-12}$ cm)</th>
<th>$\sigma_c$ ($10^{-24}$ cm$^2$)</th>
<th>$\sigma_l$ ($10^{-24}$ cm$^2$)</th>
<th>$\sigma_{abs}$ ($10^{-24}$ cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>$^1$H</td>
<td>-0.374</td>
<td>1.76</td>
<td>79.7</td>
<td>0.33</td>
</tr>
<tr>
<td>Deuterium</td>
<td>$^2$H</td>
<td>0.667</td>
<td>5.59</td>
<td>2.01</td>
<td>0</td>
</tr>
<tr>
<td>Carbon</td>
<td>$^{12}$C</td>
<td>0.665</td>
<td>5.56</td>
<td>0</td>
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<tr>
<td>Nitrogen</td>
<td>$^{14}$N</td>
<td>0.930</td>
<td>11.1</td>
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<tr>
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Membrane Chronology


Robertson, J. D. 1957. New observations on the ultrastructure of the membranes of frog peripheral nerve fibers. J. Biophys. Biochem. Cytol. 3:1043-1047. Put forth the notion that the bilayer is found in all membranes, including organelles.

(a) Sandwich model
- Cell exterior
- Membrane proteins
- Phospholipid bilayer
- Cell interior

(b) Fluid-mosaic model
- Cell exterior
- Phospholipid bilayer
- Membrane proteins
- Cell interior


Functional Lipid Domains – much indirect, but no direct experimental evidence

- The existence of rafts remains controversial.
- Lipid domains are now thought to be nanoscopic in size and transient.
- X-rays do not offer sufficient contrast for detecting lipid domains.
- Neutrons offer the real possibility to detect nanoscopic lipid domains (wavelength ~1-5 Å).
Detecting Nanoscopic Domains using SANS – how small can SANS detect?

**PROBLEM:** How to determine the lateral structure of a bilayer in a scattering experiment?

**INSIGHT:** Deuterated and protiated lipid chains have a large NSLD contrast. Signal from acyl chain segregation can be isolated through contrast matching.

**ANALYSIS:** Monte Carlo simulation of pair-distance distribution function, for spherical shell with domains

**IMPACT:** SANS is a probe free technique with spatial sensitivity to raft-sized domains (5-100 nm)

\[
(\Delta \delta)^2 = (\delta_{\text{lipid}} - \delta_{\text{water}})^2 \neq 0 \text{ scattering} \\
(\Delta \delta)^2 = (\delta_{\text{lipid}} - \delta_{\text{water}})^2 = 0 \text{ no scattering}
\]

![Diagram of lipid mixing and SLD contrast](image)

**Table: Bound Atom Scattering Lengths and Cross Sections for Typical Elements in Biomaterials**

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DSPC, (DOPC+POPC), and Chol, in a 39/39/22 ratio
34.6% D$_2$O and 65.4% chain-deuterated DSPC-d$_{70}$. When randomly mixed there is no SANS signal. DSPC, (DOPC+POPC), and Chol, in a 39/39/22 ratio.
Bilayer Thickness Mismatch between Ld and Lo Phases

Fits to 20 °C SANS Data


Lo and Ld Bilayer Thicknesses

DOPC

Line Tension

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Selective deuteration has been employed to isolate the scattering of individual phases, revealing key mechanical parameters, such as the rigidity of each phase and the interfacial tension between them.

**Impact:** NSE in conjunction with high performance computing is the only technique capable of determining the mechanical properties of nanoscopic domains, structures thought to be involved in imparting biological function.

The Domain Interface – molecular insights from MD simulations

Impact: NSE in conjunction with high performance computing is the only technique capable of determining the mechanical properties of nanoscopic domains, which are thought to be involved in imparting biological function.

**DOMAINS ARE IN-REGISTER ACROSS BILAYER LEAFLETS**

- **Nickels et al., J. Am. Chem. Soc. 137, 15772 (2015)**
B. subtilis – a gram positive bacteria

- B. subtilis is found in the human gut, a variety of foods and soil.
- Considered a Gram positive equivalent of E. coli.
- Highly amenable to genetic manipulation.
- Bacilllus Genetic Stock Center provides a mutant library, expediting some of the genetic manipulations.
Gram Positive vs Gram Negative Bacteria – one membrane vs two

Gram Positive
- Wall-associated protein
- Teichoic acid
- Lipoteichoic acid
- Peptidoglycan

Gram Negative
- O-polysaccharide
- Core polysaccharide
- Protein A Porin
- Lipoprotein
- Lipopolysaccharide (LPS)

8 nm
Contrast Matching of Different Classes of Biomolecules – making them invisible or visible to neutrons

SLDs from H Biomolecules

SLDs from Biomolecules as a Function of %D

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SANS Data from *B. subtilis* Grown in Minimal Glucose Media at 90% D$_2$O and 100% H$_2$O – integrated neutron intensities vs % D$_2$O.
Selective Isotopic Labeling of the Membrane – creating neutron contrast

All major classes of biomolecules can be contrast-matched to D$_2$O and disappear when appropriately D-labeled.

Bacterial Cells Disappear in D$_2$O

We knocked out the cells’ ability to make and destroy FAs.

The cells grow on 2 fed FAs.

We now have complete control over composition and labeling of the membrane FAs, hence contrast with medium, cell and each other.

Making Cells “Invisible” to Neutrons

Engineering Cells

Nickels et al., PLoS Biology (under review)
Figure S2. Cell stability: optical density (OD) and manual cell count. A) OD at 600 nm was used to quantify cell stability versus time for B. subtilis cells before and after SANS measurements were taken, showing a drop of 5% over the course of the 4 hour measurement. A continuous OD measurement was made offline on equivalently treated cells showing a similar 7% drop over 4 hours. B) Cell counts were made using a hemocytometer were consistent with the OD measurements.
SANS “sees” the Membrane of \textit{B. subtilis} – first scattering experiment of its kind

\textbf{SANS Experiment} – observation of the lipid bilayer of live \textit{B. subtilis} bacteria

\textbf{SANS Instruments Used}

- EQ-SANS @ SNS
- Bio-SANS @ HFIR

\textbf{Nickels et al., PloS Biology (under review)}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{sans_experiment}
\end{figure}
Introducing Contrast in the Plane of the Membrane – model free way of determining the presence/absence of nanodomains

Contrast Matched – no scattering

Non-Contrast Matched – scattering
SANS “sees” Lipid Organization as Large as 30 nm in the Membrane of Living *B. subtilis* – first direct evidence of lipid partitioning in the membrane of a living system.

Nickels et al., *PLoS Biology* (under review)