Determining the Structure of Bio-membranes by Neutron Diffraction and Reflectometry

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Cell membranes

Lipid bilayers

Lipids

~5nm
The Advanced Neutron Diffractometer/Refletometer
At the NIST Center for Neutron Research (NCNR)

$\lambda_n = 5 \, \text{Å}$
$\Delta \lambda/\lambda = 1\%$

Schematic of the AND/R
Scattering Lengths
Why neutrons when we have X-rays?

One can create contrast by hydrogen → deuterium exchange (Isotopic labeling of molecules)

Neutrons

X-rays $b_X \sim Z$

$H$ $D$ $C$ $N$ $O$ $P$

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Single bilayer versus multilayers for the study of membrane structure

**Single thethered membrane**
- membrane fully hydrated in a ‘wet cell’

**Oriented lipid stacks**
- dry membranes hydrated from the vapor phase of a salt solution
Reflectometry versus Diffraction. What’s the difference?…

\[ I = \frac{\text{# reflected neutrons}}{\text{# incident neutrons}} \]

Total reflection (critical edge)

\[ n = 1 - \frac{\lambda^2 \rho}{2\pi} \]
Bragg’s law of diffraction

Condition of positive interference

\[ 2\delta = 2d \sin (\theta) = n \lambda \]
Momentum transfer and planar geometry

\[ Q = \frac{4\pi}{\lambda} \sin\left(\frac{\Delta}{2}\right) \]
Momentum transfer and planar geometry

\[ \Delta = 2\theta \]

\[ Q = Q_z = \frac{4\pi}{\lambda} \sin(\theta) \]
Basic parameters for general case, including $\alpha_i = 90^\circ$:

$$q_r = (q_x^2 + q_y^2)^{1/2}$$

For cylindrical geometry, sample invariant on rotation about Z.

$$q_x = \frac{2\pi}{\lambda}(\cos \alpha_f \cos(2\theta) - \cos \alpha_i)$$

$$q_y = \frac{2\pi}{\lambda}\cos \alpha_f \sin(2\theta)$$

$$q_z = \frac{2\pi}{\lambda}(\sin \alpha_i + \sin \alpha_f)$$
Diffraction from lipid multilayers to determine the membrane structure

DOTAP at 93% relative humidity
~3000 layers

Bragg peaks

\[ Q_n^z = \frac{4\pi}{\lambda} \sin(\theta_n) = n \frac{2\pi}{d} \]
The application of the convolution theorem to lipid multilayer diffraction

\[ D(z) = \sum \delta(z-nd) \odot \rho_{\text{unit cell}}(z) \xrightarrow{\text{Fourier transform}} s(q) = F(\sum \delta(z-nd)) \cdot F(\rho_{\text{unit cell}}) \]

- The diffraction peak intensities are modulated by the density distribution within the unit cell.

Row of atoms:
*No atomic internal structure can be “seen” by the neutron*

Stack of lipid bilayers:
*The internal bilayer structure can be resolved by the neutron*
The phase problem

- **What is measured:** Intensity = \(|s(Qz)|^2 \sim |F(\rho_{\text{unit cell}})|^2 \int_{-d/2}^{d/2} \rho_{\text{unit cell}}(z) e^{izQz} dz|^2\)

- The intensity in reciprocal space is invariant under a shift of origin in real space.

For a centro-symmetric system (\(\rho(z) = \rho(-z)\)):

\[s(Qz) = \int_{-d/2}^{d/2} \cos(izQz) \rho(z) dz + i \int_{-d/2}^{d/2} \sin(izQz) \rho(z) dz\]

- There are only two possibilities: -1 and +1, corresponding to \(\cos(0)\) and \(\cos(180)\).

- **Diffraction:** signal sampled at \(Q_z^n \rightarrow \rho_{\text{unit cell}}(z) = \text{Fourier synthesis} (\pm \sqrt{I(n)})\)
Using contrast to determine structural details from diffraction data

\[
\rho_D(z) - \rho_H = \frac{\sum_{n=1}^{\infty} \pm I(n) \cos \left( \frac{2 \pi n z}{d} \right)}{d}
\]

Water-deuterated \( D_2O \)

Neutron counts

\( Q_\lambda [\text{Å}^{-1}] \)

Difference
Examples of neutron diffraction experiments

Polyunsaturated lipids important for the function of brain receptors.

Scattering length density

Distance from the bilayer center, $z$ [Å]

Rhodopsin/DHA
A – bilayer w. cholesterol
B0 - DHA chain, no cholesterol
B1 – DHA chain w. cholesterol
C0 – SA chain, no cholesterol
C1 – SA chain w. cholesterol
D – Cholesterol A-ring
E - Cholesterol CH3-tail
F- water

Scattering Length Density

Distance from the bilayer center, $z$ [Å]
Designing reflectometry experiments

- Find an appropriate model to describe the layered molecular structure in terms of neutron Scattering Length Density distribution, in real space
- Fourier transform the model
- Fit the model to the data (Reflectivity vs. Qz) to find the SLD

“tethered” lipid bilayer membrane (tBLM)
Determining the SLD profile of the membrane
Summary

- To determine the ‘in-depth’ molecular architecture in model membranes one can use:

- reflectometry experiments on single supported membranes
  - membrane fully hydrated
  - possibility of studying incorporated proteins with large extra-cellular domains
  - requires an appropriate model for the molecular modeling

- diffraction experiments on multilayers hydrated from vapor phase
  - higher structural resolution
  - direct determination of the structure from the Bragg intensities
  - membrane only partially hydrated (small inter-membrane space)
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