Biomimetic Lipid Membranes for the Study of Membrane Protein Structure

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Goal: Prepare model oriented lipid bilayers with incorporated membrane proteins to be studied with x-ray and neutron diffraction.

Part I: Study lipid membranes and the effect of charge and pH on sample alignment and phase behavior.

Part II: Study how the antimicrobial peptide, Gramicidin A, resides in the lipid membranes.
Membranes

Lipids

- polar headgroup
- oily chains

Cells

- Nuclear envelope
- Nucleoplasm
- Nucleolus
- Rough endoplasmic reticulum
- Peroxisome
- Centriole
- Cytosol
- Vesicle

- Cholesterol
- Integral (membrane) proteins
- Peripheral (membrane) protein
Comparison of Lipid Structures

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Structure</th>
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<tbody>
<tr>
<td>POPC</td>
<td><img src="image" alt="POPC Structure" /></td>
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<tr>
<td>POPG</td>
<td><img src="image" alt="POPG Structure" /></td>
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<tr>
<td>DOPC</td>
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<tr>
<td>EDOPC</td>
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<tr>
<td>DOPE</td>
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<tr>
<td>DOTAP</td>
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Introduction: Lipid Structure
Microscope: PCPG (80:20)

Before Hydration

After Hydration
Measuring diffraction from oriented lipid multilayers

- θ–2θ scans for determining lipid membrane structure (in projection on the z-axis)

\[ Q_z = \frac{4\pi}{\lambda} \sin(\theta) = n \frac{2\pi}{d} \]

- θ scans for determining the degree of orientation
pH Effect on PG

Basic Environment: pH 8.2

Acidic Environment: pH 5.2

\[ Q_z = \frac{4\pi}{\lambda} \sin(\theta) = n \frac{2\pi}{d} \]

Additional Diffraction Peaks

Phase separation of POPG from POPC in an acidic environment.
X-Ray Diffraction Results

PCPG(1:1) vs. PCPG(8:2) vs. POPG alone

Repeat Distances
- PCPG (8:2): 52.6 +/- 0.07 Å
- PCPG (1:1): 52.1 +/- 0.05 Å
- POPG alone: 50.7 +/- 0.05 Å

FWHM
- PCPG (8:2): 0.066 +/- 0.003
- PCPG (1:1): 0.044 +/- 0.004
- POPG: 0.041 +/- 0.001
X-Ray Diffraction Results

PCPG/GrA(50/1) vs. PCPG/GrA(25/1) vs. PCPG(1:1)

Repeat Distances

PCPG (1:1): 52.1 +/- 0.05 Å
PCPG+GrA (50:1): 49.2 +/- 0.05 Å
PCPG+GrA (25:1): 49.0 +/- 0.05 Å

FWHM

PCPG: 0.044 +/- 0.004
PCPG+GrA (50:1): 0.072 +/- 0.003
PCPG+GrA (25:1): 0.076 +/- 0.003
Circular Dichroism

CD Signal for an Alpha Helix Configuration

Gramicidin A in POPC, PCPG (1:1), and DOTAP

36 residues

α-Helix

β₆₋₃-Helix

Neutron Diffraction

Advanced Neutron Diffractometer/Reflectometer
NIST Center for Neutron Research
What we measure

Intensity(n) = \( \frac{1}{n^2} N^2 \left| F(n) \right|^2 \)

for large number of bilayers, \( N \)

Fourier Sum

\[
\rho(z) = \frac{1}{d} \sum_n |F_n| \cos \left( \frac{2\pi n z}{d} \right)
\]

What we want to determine

SLD Profiles for POPC+GrA in 20% D2O and H2O

Neutron Diffraction Data

POPCh+Gramicidin A (25:1) (H2O vs. 20% D2O)

Water Distribution for POPC+GrA

\[ \rho(z)_{D} - \rho(z)_{H} \]
Neutron Diffraction Data

SLD Profiles and Water Distributions for POPC+GrA and POPC alone

\[ d = 53.5 \pm 0.08 \text{ Å (POPC alone)} \]
\[ d = 52.8 \pm 0.05 \text{ Å (POPC+GrA (25:1))} \]
Conclusions

- Improve on sample quality by hydration and the amount of charged lipid.
- Solvent pH plays an important role in the structure and properties of a lipid membrane.
- Neutron Diffraction and CD data indicate that Gramicidin A is incorporated into the membrane.
  - Suggests transmembrane, channel forming conformation.
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References:


