Lipid & membrane dynamics

Schematic representation of lipid motions in membranes

[Charitat et al., 2008 adapted from Sackmann 1995].

- rotation around chemical bonds, trans/gauche isomerizations (ps)
- rotation about axis (ns)
- wobbling (ns)
- lateral diffusion (μs)
- flip-flop (ms-s)
- undulatory motions of bilayer (ms-s)
Defining the fluid phase of membranes

Phase diagram of lipid membranes for DMPC vesicles as function of the stress history adapted from (Dix and Zasadny, 1995; Barroso and Harrigan, 1997; Harrigam and Dix, 1993).

(1) and (2): variation of temperature under zero or very low tension (0.16 mN/m).
(3) and (4): variation of temperature under moderate or high tension (0.2 or 2 mN/m).
(5): at 4°C for several days.
(6): at fixed temperature, under tension, ripple structure is eliminated.
Defining the fluid phase of membranes

Phase behavior of phospholipids. In the presence of aqueous buffer, a majority of membrane phospholipids form spontaneously solvated lipid bilayers that can exist in two distinct phases: gel and fluid. The gel phase has a diffusion coefficient \( D \approx 10^{-11} \text{ cm}^2/\text{s} \), while the fluid phase has a diffusion coefficient \( D \approx 5 \times 10^{-8} \text{ cm}^2/\text{s} \). The transition between these phases is denoted by \( T_m \).
Measuring membrane phase transitions

Differential Scanning Calorimetry (DSC) measures the difference in the amount of heat required to increase the temperature of a sample and reference cell as a function of T.

Heat flow = heat/time = \( q/t \)

Heating rate = temperature increase/time = \( \Delta T/t \)

\[
\frac{(q/t)/(\Delta T/t)}{= q/\Delta T = C_P = \text{heat capacity}}
\]

\[
\Delta H = \int_{T_1}^{T_2} \left( \frac{\partial H}{\partial T} \right)_P dT = \int_{T_1}^{T_2} C_P dT
\]

\[
\Delta S = \frac{\Delta H_{cal}}{T_m}
\]

- **Tm dependency on headgroup, chain length, saturation**

  - VDWs - tail length & saturation
  - Head-group interactions with each other & water
  - e.g. PE interacts with fewer waters which results in the ~30 degree increase in Tm.

### Phase Transition Temperatures for Glycerophospholipids

<table>
<thead>
<tr>
<th>Phosphatidylcholine</th>
<th>Phosphatidylglycerol (Sodium Salt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product</strong></td>
<td><strong>Tm (°C)</strong></td>
</tr>
<tr>
<td>12:0 PC (D LPC)</td>
<td>-2</td>
</tr>
<tr>
<td>13:0 PC</td>
<td>14</td>
</tr>
<tr>
<td>14:0 PC (DMPC)</td>
<td>24</td>
</tr>
<tr>
<td>15:0 PC</td>
<td>35</td>
</tr>
<tr>
<td>16:0 PC (DPPC)</td>
<td>41</td>
</tr>
<tr>
<td>17:0 PC</td>
<td>50</td>
</tr>
<tr>
<td>18:0 PC (DSPC)</td>
<td>55</td>
</tr>
<tr>
<td>19:0 PC</td>
<td>62</td>
</tr>
<tr>
<td>20:0 PC</td>
<td>66</td>
</tr>
<tr>
<td>21:0 PC</td>
<td>71</td>
</tr>
<tr>
<td>22:0 PC</td>
<td>75</td>
</tr>
<tr>
<td>23:0 PC</td>
<td>79.5</td>
</tr>
<tr>
<td>24:0 PC</td>
<td>80.3</td>
</tr>
<tr>
<td>16:1 PC</td>
<td>-36</td>
</tr>
<tr>
<td>18:1&amp;9 PC (DOPC)</td>
<td>-17</td>
</tr>
<tr>
<td>18:1&amp;8 PC</td>
<td>12</td>
</tr>
<tr>
<td>18:1&amp;6 PC</td>
<td>1</td>
</tr>
<tr>
<td>22:1&amp;3 PC</td>
<td>13</td>
</tr>
<tr>
<td>18:2 PC</td>
<td>-57</td>
</tr>
<tr>
<td>18:3 PC</td>
<td>-60</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table continued*
Phase transitions in mixed bilayers

**Figure 5.** High-sensitivity DSC heating scans of Acholeplasma laidlawii B elaidic acid-homogeneous intact cells, isolated membranes and extracted total membrane lipids dispersed as multilamellar vesicles in water.

**Figure 6.8** (a) DSC curves of mixtures of DMPE with DMPG in 0.1 M NaCl at two different pH values and various molar ratios. Solid line: experimental $C_p$ curves; dotted line: simulated $C_p$ curves. At pH = 2, the PG headgroup is almost completely protonated. (b) Pseudo-binary phase diagrams for the DMPE:DMPG system at pH 2 and 7. Triangles are $T(−)$ and $T(+) +$ values obtained from the simulation of the $C_p$ curve; circles are $T_{exp}(−)$ and $T_{exp}(+) +$ values obtained by the usual empirical procedure. The solid lines are the coexistence lines obtained by a nonlinear least square fit of the $T(−)$ and $T(+) +$ values using the four-parameter nonideal, nonsymmetric mixing model, yielding the nonideality parameters as indicated. (Adapted from Garidel, P., and Blume A., *Eur. Biophys. J.* 28, 629–638, 2000.)
Defining the fluid phase of membranes

Phase behavior of phospholipids. In the presence of aqueous buffer, a majority of membrane phospholipids form spontaneously solvated lipid bilayers that can exist in two distinct phases: gel and fluid. The gel phase is characterized by a lower diffusion coefficient ($D \sim 10^{-11} \text{ cm}^2/\text{s}$) compared to the fluid phase ($D \sim 5 \times 10^{-8} \text{ cm}^2/\text{s}$) at the transition temperature ($T_m$).
Measuring membrane diffusion fluorescence recovery after photobleaching (FRAP)

MOBILITY MEASUREMENT BY ANALYSIS OF FLUORESCENCE PHOTobleaching RECOVERY KINETICS

D. AXELROD, D. E. KOPPEL, J. SCHLESSINGER, E. ELSON, and W. W. WEBB
From the School of Applied and Engineering Physics, and Department of Chemistry, Cornell University, Ithaca, New York 14853

BIOPHYSICAL JOURNAL. Volume 16. 1976

doi: [10.1016/S0006-3495(76)8755-4]

\[ D = \frac{w^2}{4t_D} \]

where \( w \) is the radius of the beam and \( t_D \) is the "Characteristic" diffusion time.
Measuring membrane diffusion fluorescence correlation spectroscopy (FCS)

Thermodynamic Fluctuations in a Reacting System—Measurement by Fluorescence Correlation Spectroscopy

Douglas Magde,* Elliot Elson,† and W. W. Webb‡
Cornell University, Ithaca, New York 14850
(Received 10 July 1972)

The temporal correlations of thermodynamic concentration fluctuations have been measured in a chemically reactive system at equilibrium by observing fluctuations of the fluorescence of a reaction product. The experiment yields the chemical rate constants and diffusion coefficients and shows the coupling among them. Data are reported for binding of ethidium bromide to DNA.

\[
G_D(\tau) = \frac{1}{N} \left(1 + \frac{\tau}{\tau_D}\right)^{-1}
\]

(2)

\[
\tau_D = \frac{\omega^2}{4D}
\]

(3)
Lipid diffusion - Single particle tracking

Single-molecule total internal reflection fluorescence imaging

1. Specimen
2. Evanescent wave range
3. Cover slip
4. Immersion oil
5. Objective
6. Emission beam (signal)
Measuring membrane diffusion
Single Particle Tracking

POPE- AF488
POPC bilayer

peptide- Cy3

CLC- Cy5

\[
\langle r^2 \rangle = 4Dt
\]

\[
\text{MSD} \equiv \langle |x(t) - x_0|^2 \rangle = \frac{1}{N} \sum_{i=1}^{N} |x^{(i)}(t) - x^{(i)}(0)|^2
\]
## Biological Diffusion Constants

<table>
<thead>
<tr>
<th>System</th>
<th>$D \text{ (cm}^2 \text{ s}^{-1})$</th>
<th>$D \text{ (}\mu\text{m}^2 \text{ s}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small molecule in water</td>
<td>$1-1.5 \times 10^{-5}$</td>
<td>1000-1500</td>
</tr>
<tr>
<td>Small protein in water</td>
<td>$10^{-6}$</td>
<td>100</td>
</tr>
<tr>
<td>Phospholipid in membrane</td>
<td>$10^{-8}$ to $10^{-7}$</td>
<td>1-10</td>
</tr>
<tr>
<td>Protein in membrane</td>
<td>$10^{-10}$ to $10^{-7}$</td>
<td>0.01-10</td>
</tr>
</tbody>
</table>
TABLE I. Lateral Diffusion in Lipid Bilayers

<table>
<thead>
<tr>
<th>Diffusant</th>
<th>Molecular weight</th>
<th>D(cm²/s) in fluid DMPC</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid analog</td>
<td>~1,000</td>
<td>(5-10) × 10⁻⁸</td>
<td>Derzko and Jacobson [1980]</td>
</tr>
<tr>
<td>NBD-gramicidin S (cyclic)</td>
<td>1,150</td>
<td>2 × 10⁻⁸</td>
<td>Wu et al [1978]</td>
</tr>
<tr>
<td>Glycophorin</td>
<td>31,000 (2,500)a</td>
<td>1-2 × 10⁻⁸</td>
<td>Vaz et al [1982]</td>
</tr>
<tr>
<td>Bovine rhodopsin</td>
<td>37,000</td>
<td>2 × 10⁻⁸</td>
<td>Vaz et al [1982]</td>
</tr>
<tr>
<td>Acetylcholine receptor</td>
<td>~250,000</td>
<td>~2 × 10⁻⁸</td>
<td>Vaz et al [1982]</td>
</tr>
</tbody>
</table>

*Molecular weight of membrane spanning portion.

Figure 4. Lateral diffusion of protein and lipids as a function of protein density in the membrane. The labeled proteins were reconstituted in liposomes composed of DOPC/DOPG (3:1) at various protein-to-lipid ratios. The data were grouped in 9 bins logarithmically spread over the measured protein-to-lipid range. Each bin consists of at least 10 liposomes. The diffusion coefficients are shown for GlT ( ), and lipids ( ); LacS (•) and lipids (○); LacY (▲) and lipids (▲). The solid lines are linear fits for GlT ( ).

Diffusion in 2D vs. 3D

In 3D viscous liquid:
Stokes paradox - no creeping flow around a disk in 2D

In 2D viscous liquid:

\[ D = \frac{k_B T}{6\pi \mu R_0} \]

- \( D \) – diffusion coefficient
- \( \mu \) - solvent viscosity
- \( R_0 \) – solute radius
- \( k_B \) – Boltzmann’s constant
- \( T \) – temperature (K)

Saffman and Delbrück continuum hydrodynamic model for 2D

\[ D = \frac{k_B T}{6\pi \mu' (\ln(\frac{\mu h}{\mu'})) - \gamma) } \]

- \( h \) - membrane thickness
- \( \mu \) - viscosity
- \( r \) - radius
- \( \gamma \) - Euler-Mascharoni constant \( \approx 0.577 \)
- \( \mu' \) - bulk fluid viscosity
Membrane protein diffusion follows Saffran-Delbruck model

i.e. diffusion of membrane proteins does not depend strongly on size
Diffusion is slower in cellular membranes

Fig. 12 Range of diffusion coefficients. (a) Diffusion coefficients for different membrane proteins measured using fluorescence correlation spectroscopy in giant unilamellar vesicles showing dependence on protein size. The red line is a fit using the Saffman-Delbrück model which characterizes membrane diffusion as a function of the size of the diffusing molecule [52, 53]. (b) Diffusion coefficients for different membrane proteins measured using fluorescence recovery after photobleaching (FRAP) in the E. coli cell membrane. The red line is an empirical fit as a function of the number of transmembrane helices in the protein. The names refer to particular membrane proteins used in the experiments. (a) adapted from [54] and (b) adapted from [55]
Diffusion in cellular membranes demonstrates distinct behaviors

1. Stationary mode - $D < 4.6 \times 10^{-12} \text{ cm}^2/\text{s}$ (6%)
2. Simple Brownian diffusion mode (28%)
3. Directed diffusion with unidirectional motion (2%)
4. Confined diffusion within domains with Brownian diffusion between $D = 4.6 \times 10^{-12} \text{ cm}^2/\text{s}$ and $10^{-9} \text{ cm}^2/\text{s}$ (64%).
Cholesterol introduces a liquid ordered phase

Phase behavior of phospholipids. In the presence of aqueous buffer, a majority of membrane phospholipids form spontaneously solvated lipid bilayers that can exist in two distinct phases:

- **Gel Phase**
  - Diffusion coefficient: $D \approx 10^{-11} \text{ cm}^2/\text{s}$

- **Fluid Phase**
  - Diffusion coefficient: $D \approx 5 \times 10^{-8} \text{ cm}^2/\text{s}$

- **Liquid Ordered Phase (lo)**
  - Diffusion coefficient: $D \approx 2.5 \times 10^{-8} \text{ cm}^2/\text{s}$
Membrane heterogeneity - From lipid domains to curvature effects
Diffusion in liquid ordered phase is slower

<table>
<thead>
<tr>
<th>System</th>
<th>( \chi_{\text{cho}} )</th>
<th>( T (\text{°C}) )</th>
<th>( \text{Phase} )</th>
<th>( D (\text{cm}^2\text{s}^{-1}) )</th>
<th>Ratio</th>
<th>Method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPC</td>
<td>0</td>
<td>35</td>
<td>( \ell_\text{d} )</td>
<td>( 7.5 \times 10^{-8} )</td>
<td>–</td>
<td>FRAP</td>
<td>Rubenstein et al. (1979)</td>
</tr>
<tr>
<td>DMPC/Chol ( \geq 0.30 )</td>
<td>35</td>
<td>( \ell_\text{o} )</td>
<td>( 3.0 \times 10^{-8} )</td>
<td>2.5</td>
<td>–</td>
<td>FRAP</td>
<td>Aloeio et al. (1982)</td>
</tr>
<tr>
<td>DMPC</td>
<td>0</td>
<td>26</td>
<td>( \ell_\text{d} )</td>
<td>( 6.0 \times 10^{-8} )</td>
<td>–</td>
<td>FRAP</td>
<td>Vaz et al. (1985)</td>
</tr>
<tr>
<td>DMPC/Chol ( \geq 0.30 )</td>
<td>26</td>
<td>( \ell_\text{o} )</td>
<td>( 1.8 \times 10^{-8} )</td>
<td>3.3</td>
<td>–</td>
<td>–</td>
<td>Almeida et al. (1992)</td>
</tr>
<tr>
<td>DMPC</td>
<td>0</td>
<td>35</td>
<td>( \ell_\text{d} )</td>
<td>( 7.6 \times 10^{-8} )</td>
<td>–</td>
<td>FRAP</td>
<td>–</td>
</tr>
<tr>
<td>DMPC/Chol ( \geq 0.30 )</td>
<td>34</td>
<td>( \ell_\text{o} )</td>
<td>( 3.5 \times 10^{-8} )</td>
<td>2.2</td>
<td>–</td>
<td>–</td>
<td>Almeida et al. (1992)</td>
</tr>
<tr>
<td>DLPC</td>
<td>0</td>
<td>25</td>
<td>( \ell_\text{d} )</td>
<td>( 3 \times 10^{-8} )</td>
<td>–</td>
<td>FCS</td>
<td>Kollach et al. (1999)</td>
</tr>
<tr>
<td>DLPC/Chol 0.30</td>
<td>25</td>
<td>( \ell_\text{o} )</td>
<td>( 1 \times 10^{-8} )</td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DMPC</td>
<td>0</td>
<td>35</td>
<td>( \ell_\text{d} )</td>
<td>( 11 \times 10^{-8} )</td>
<td>–</td>
<td>pfg-NMR</td>
<td>Filipov et al. (2003)</td>
</tr>
<tr>
<td>DMPC/Chol 0.33</td>
<td>35</td>
<td>( \ell_\text{o} )</td>
<td>( 3 \times 10^{-8} )</td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SM</td>
<td>0</td>
<td>55</td>
<td>( \ell_\text{d} )</td>
<td>( 8 \times 10^{-8} )</td>
<td>–</td>
<td>pfg-NMR</td>
<td>Filipov et al. (2003)</td>
</tr>
<tr>
<td>SM/Chol 0.30–0.425</td>
<td>55</td>
<td>( \ell_\text{o} )</td>
<td>( 3.5 \times 10^{-8} )</td>
<td>2.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DOPC</td>
<td>0</td>
<td>30</td>
<td>( \ell_\text{d} )</td>
<td>( 10 \times 10^{-8} )</td>
<td>–</td>
<td>pfg-NMR</td>
<td>Filipov et al. (2003)</td>
</tr>
<tr>
<td>DOPC/Chol 0.33</td>
<td>30</td>
<td>( \ell_\text{o}(?) )</td>
<td>( 5 \times 10^{-8} )</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Measuring lipid dynamics and order by 2H NMR

Protons in lipids can be replaced by deuterium atoms
NMR provides information on the order and mobility of the molecule
$S_{CD}$ order parameter can be calculated from quadrupolar splitting
$R$ - relaxation parameters can inform on intramolecular dynamics
Fig. 1. (a) Immiscible liquid phases are directly visualized on the surface of giant unilamellar vesicles by fluorescence microscopy. (b) Distinct superposition of $^2$H NMR spectra demonstrates that liquid-crystalline ($L_{\alpha}$) and liquid-ordered ($L_o$) phases coexist in vesicle membranes. Distinguishing features of $L_{\alpha}$ ($L_o$) spectra are denoted by white (black) arrows as described in [54]. Membrane compositions are (a) 1:1 DPhyPC/DPPC + 50% cholesterol at 26°C and (b) 1:1 DOPC/DPPC-d62 + 20% cholesterol at 20°C. 

$^2$H NMR spectra of DPPC-d62 in membranes of various DOPC/DPPC-d62/Chol compositions acquired at 20°C with phase assignments as described in the text. Membrane compositions are in the form DOPC:DPPC-d62 plus % Chol: a, 4:1 plus 15%; b, 4:1 plus 40%; c, 2:1 plus 25%; d, 1:1 plus 15%; e, 1:2 plus 25%; f, 1:4 plus 10%. (g–l) Spectra for membranes of 1:2 DOPC/DPPC-d62 plus 20% Chol at the temperatures indicated.
Lipid phase diagrams of mixed systems

Fig. 6. Phase diagrams proposed in the literature for bilayer membranes containing binary mixtures of phospholipids and cholesterol.

Diagrams in (a and b) differ in their description of phases above $T_m$. In (a), regions of $L_\alpha$ and $L_o$ phases are separated by a coexistence region. Diagram (b) depicts a gradual transition between $L_o$ and $L_\alpha$ phases (denoted by the dotted line).

There is support for both types of diagrams in the literature.

https://doi.org/10.1016/j.bpj.2017.02.033
Key inventions developed over the years include: an optical detection system and fluid cell enabling contact mode AFM to operate in aqueous solution (Bio-AFM); dynamic mode AFM (DM-AFM), which oscillates the AFM tip to reduce friction while contouring the biological sample; force–distance curve-based AFM (FD-AFM), which contours the surface of a biological system while recording pixel-by-pixel a full force–distance curve; multiparametric AFM (MP-AFM), which contours the sample while mapping multiple physical or chemical properties; molecular recognition AFM (MR-AFM), which images and maps specific interactions of biological samples; multifrequency AFM (MF-AFM), which contours the sample while oscillating the cantilever tip at multiple frequencies, thus mapping various physical parameters; correlating advanced optical imaging and AFM (Opto-AFM) for the imaging of complex biological systems; high-speed AFM (HS-AFM), which speeds up the image acquisition time by a factor of ~1,000, providing access to dynamic processes in biology.

Most modes cross-fertilized each other, ultimately leading to combinatorial AFM. Images adapted from: Bio-AFM, ref. 28, Macmillan Publishers Ltd; DM-AFM, ref. 45, American Chemical Society; FD-AFM, ref. 76, Wiley; MP-AFM, ref. 78, Elsevier; MR-AFM, ref. 9, Cell Press; MF-AFM, ref. 46, Macmillan Publishers Ltd; Opto-AFM, ref. 145, The Company of Biologists; HS-AFM, ref. 122, Macmillan Publishers Ltd.
Fig. 2. AFM images of a DOPC/bSM/chol 1:1:1 supported lipid bilayer at 25 °C. a) The image shows homogeneous lipid bilayer patches (*) and smaller lipid bilayers in a phase coexistence state (#). b) Height difference between the homogeneous bilayer and the domains in a patch with phase coexistence. The height of the homogeneous bilayer is intermediate between the two domains in the small patch. The line section refers to the white line on the image.
Figure 1 | General overview of lateral heterogeneity in the plasma membrane.

a | Lipid raft domains are usually defined as small, highly dynamic and transient plasma membrane entities that are enriched in saturated phospholipids, sphingolipids, glycolipids, cholesterol, lipiddated proteins and glycosylphosphatidylinositol (GPI)-anchored proteins. Enrichment of these hydrophobic components endows these lipid domains with distinct physical properties; these include increased lipid packing and order, and decreased fluidity. In addition to membrane components, cortical actin plays an active part in domain maintenance and remodelling. Furthermore, membrane lipids are asymmetrically distributed in the inner and outer leaflets, which may further affect membrane organization. b | It is likely that membrane organization is not binary (that is, highly distinct raft and non-raft regions), but instead membranes consist of various raft-like and non-raft domains with distinct compositions and properties.
Nanodomains ("rafts") in cell membranes

Cells
Lateral heterogeneity
- Initiated by proteins and stabilized by lipids
- Driven by lipid immiscibility and phase separation?

Actin cytoskeleton

Lipid–lipid interactions
Sphingolipid–cholesterol interaction

C18-saturated PtdSer–cholesterol interaction

Transbilayer interdigation
Lipid phases in biological membranes via microscopy

https://doi.org/10.1016/j.chembiol.2013.11.009
a Lipid-associated membrane domains

Pure lipid clusters (glycolipid clusters, ceramide domains, etc.)

Lipid-mediated protein clusters (GPI domains, RAS domains, etc.)

Liquid–liquid phase separation of lipids

Protein-mediated lipid cluster

Unsaturated lipids Saturated lipids

Ordered domain Disordered domain

GM1 Cholera toxin
Lipid flip flop (transverse movement)

Kornberg & McConnell 1971

DOI: 10.1021/bi00783a003

Probability of flip flop is 0.00002/sec

Translation step for lateral diffusion is 3000/sec
Methods for lipid bilayer reconstitution

- Chloroform solubilized lipids are dried down under N\textsubscript{2} gas (no O\textsubscript{2} as lipids containing double bonds can become oxidized)

- The dried lipids are resuspended in buffer by sonication

- For reconstitution of detergent solubilized membrane proteins, the lipids are solubilized in a detergent buffer with a high CMC (e.g. CHAPS), then mixed with protein. The detergent is removed by dialysis or bio-beads.
Purifying membrane proteins

1. Native membrane
2. Solubilization
3. Purification
4. Reconstitution
   - Dialysis
   - Gel filtration
   - Dilution
   - Polystyrene beads
Fig. 1 Sketch representing the different preparation protocols for obtaining different model membranes. Abbreviations are: MLVs, multilamellar vesicles; SUVs, small unilamellar vesicles; LUVs, large unilamellar vesicles; LOVs, large oligolamellar vesicles; GUVs, giant unilamellar vesicles; PSM, planar supported membranes; LF, Langmuir films. The different colors in the PSM indicates lipid asymmetry.
Electroformation of GUVS

SUVs (~100nm) → SUV fusion via partial dehydration → Rehydration under AC electric field → GUVs (~10μm)
Measuring liposome sizes - cryo EM

A
Cu TEM grid
Li deposition
Plunge freeze under Ar
Liquid N$_2$

B
Cryo TEM holder
Sample in liquid N$_2$
Load sample
Close shutter
Transfer at -170 °C

E
400 nm EPL
(Walden et al.)

P radii

radius (nm)

400 nm 2:1 POPE/POPG

P radii

radius (nm)
Measuring liposome sizes - dynamic light scattering

\[ G_1(\tau) = 1 + \alpha(G_1(\tau))^2 \]

\[ G_2(\tau) = \exp(-Dq^2\tau) \]

\[ d = kT / 3\eta_s D \quad \text{Einstein-Stokes relation} \]

\[ G_2(\tau), G_1(\tau) : \text{Secondary and primary} \]

\[ \text{auto-correlation function(ACF)} \]

- \( D \): Diffusion coefficient
- \( \tau \): Correlation time
- \( q \): Scattering vector
- \( d \): Hydrodynamic diameter
- \( k \): Boltzmann's constant
- \( \eta \): Viscosity of solvent
- \( T \): Absolute temperature

Brownian motion of small particle

Brownian motion of large particle

Fluctuation of scattering light

Auto-correlation function (ACF)

Particle sizing

http://www.otsukael.com/product/detail/productid/23/category1id/2/category2id/2/category3id/32
Sizes of biological membranes

- SUV: <100 nm
- LUV: 100 ... 1000 nm
- MLV: up to several μm
- MVV: up to several μm

100 pm 1 nm 10 nm 100 nm 1 μm 10 μm 100 μm 1 mm

- Atom
- Small Molecules
- Lipids
- Proteins
- Virus
- Bacteria
- Organelles
- Eukaryotic Cells

Electron Microscope

Light Microscope

Eye
Supported membranes

- Supported bilayer on glass/silica has a hydration layer of ~10-20 Å.

https://doi.org/10.3389/fphys.2017.00063

The BLM preparation for electrical measurements of permeability, adapted from Tien (1968).
DOI: 10.1085/jgp.201812153
Scaffolded membranes

Nanodiscs - apolipoprotein (MSP)

MSP - Membrane scaffold protein

Protein in detergent micelles → Lipid-detergent micelles → MSP → Bio-Beads → Detergent removed with Bio-Beads → Assembled Nanodiscs

SMALPs - styrene maleic acid copolymers (SMA)


Saponin (Salipro)