Biomembranes

- Macromolecular assemblies of lipids
- Bilayer physical properties.
- Biomembrane composition
- Cholesterol is a membrane plasticizer
- Lipid miscibility
- Molecular dynamics
- Membrane bilayer asymmetry
- Lipid Rafts
- Physical basis of membrane permeability

http://glutxi.umassmed.edu/grad.html
### The Common Biological Fatty Acids

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Common Name</th>
<th>Systematic Name</th>
<th>Structure</th>
<th>mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>Lauric acid</td>
<td>Dodecanoic acid</td>
<td>CH₃(CH₂)₁₀COOH</td>
<td>44.2</td>
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<tr>
<td>14:0</td>
<td>Myristic acid</td>
<td>Tetradecanoic acid</td>
<td>CH₃(CH₂)₁₂COOH</td>
<td>52</td>
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<tr>
<td>16:0</td>
<td>Palmitic acid</td>
<td>Hexadecanoic acid</td>
<td>CH₃(CH₂)₁₄COOH</td>
<td>63.1</td>
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<tr>
<td>18:0</td>
<td>Stearic acid</td>
<td>Octadecanoic acid</td>
<td>CH₃(CH₂)₁₆COOH</td>
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<td>20:0</td>
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<td>Eicosanoic acid</td>
<td>CH₃(CH₂)₁₈COOH</td>
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<td>22:0</td>
<td>Behenic acid</td>
<td>Docosanoic acid</td>
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<td>24:0</td>
<td>Lignoceric acid</td>
<td>Tetracosanoic acid</td>
<td>CH₃(CH₂)₂₂COOH</td>
<td>84.2</td>
</tr>
</tbody>
</table>

#### Unsaturated fatty acids (all double bonds are cis)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Common Name</th>
<th>Systematic Name</th>
<th>Structure</th>
<th>mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:1</td>
<td>Palmitoleic acid</td>
<td>9-Hexadecenoic acid</td>
<td>CH₃(CH₂)₊₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋个百分</td>
<td>13.4</td>
</tr>
<tr>
<td>18:2</td>
<td>Linoleic acid</td>
<td>9,12-Octadecadienoic acid</td>
<td>CH₃(CH₂)₊₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋个百分</td>
<td>9</td>
</tr>
<tr>
<td>18:3</td>
<td>α-Linolenic acid</td>
<td>9,12,15-Octadecatrienoic acid</td>
<td>CH₃(CH₂)₊₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋个百分</td>
<td>17</td>
</tr>
<tr>
<td>18:3</td>
<td>γ-Linolenic acid</td>
<td>6,9,12-Octadecatrienoic acid</td>
<td>CH₃(CH₂)₊₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋个百分</td>
<td>49.5</td>
</tr>
<tr>
<td>20:4</td>
<td>Arachidonic acid</td>
<td>5,8,11,14-Eicosatetraenoic acid</td>
<td>CH₃(CH₂)₊₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋เชียงใหม่</td>
<td>54</td>
</tr>
<tr>
<td>20:5</td>
<td>EPA</td>
<td>5,8,11,14,17-Eicosa-14,15-dienoic acid</td>
<td>CH₃(CH₂)₊₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋₋╴</td>
<td>39</td>
</tr>
</tbody>
</table>

#### Phosphatidic acid

Water

Ethanolamine

Choline

Serine

myo-inositol

Glycerol

Phosphatidylethanolamine

Phosphatidylcholine (lecithin)

Phosphatidylserine

Phosphatidylcholine

Phosphatidylinositol

Phosphatidylglycerol

<table>
<thead>
<tr>
<th>NAME OF R3</th>
<th>FORMULA OF R3</th>
<th>NAME OF PHOSPHOLIPID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>–H</td>
<td>Phosphatidic acid</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>–CH₂CH₂NH₃⁺</td>
<td>Phosphatidylethanolamine</td>
</tr>
<tr>
<td>Choline</td>
<td>–CH₂CH₂(NH₃)⁺</td>
<td>Phosphatidylcholine (lecithin)</td>
</tr>
<tr>
<td>Serine</td>
<td>–CH₂CH(NH₃⁺)COO⁻</td>
<td>Phosphatidylserine</td>
</tr>
<tr>
<td>myo-inositol</td>
<td></td>
<td>Phosphatidylinositol</td>
</tr>
<tr>
<td>Glycerol</td>
<td>–CH₂CH(OH)CH₂OH</td>
<td>Phosphatidylglycerol</td>
</tr>
</tbody>
</table>
**Lipid Composition of Biological Membranes**

**TABLE 10-1** Approximate Lipid Compositions of Different Cell Membranes

<table>
<thead>
<tr>
<th>LIPID</th>
<th>LIVER CELL PLASMA MEMBRANE</th>
<th>RED BLOOD CELL PLASMA MEMBRANE</th>
<th>MYELIN</th>
<th>MITOCHONDRION (INNER AND OUTER MEMBRANES)</th>
<th>ENDOPLASMIC RETICULUM</th>
<th>E COLI BACTERIUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>17</td>
<td>23</td>
<td>22</td>
<td>3</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>7</td>
<td>18</td>
<td>15</td>
<td>25</td>
<td>17</td>
<td>70</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
<td>4</td>
<td>7</td>
<td>9</td>
<td>2</td>
<td>5</td>
<td>trace</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>24</td>
<td>17</td>
<td>10</td>
<td>39</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>19</td>
<td>18</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Glycolipids</td>
<td>7</td>
<td>3</td>
<td>28</td>
<td>trace</td>
<td>trace</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>22</td>
<td>13</td>
<td>8</td>
<td>21</td>
<td>27</td>
<td>30</td>
</tr>
</tbody>
</table>

*Note, molecular weight of cholesterol = 386.7 while that of a typical PC ≈ 760. Thus a phospholipid : cholesterol ratio of 1 : 1 by mass ≈ 1 : 2 as a molar ratio.*
Lipids are amphipathic molecules whose packing is governed by 2 factors:
1) the hydrophobic effect
2) lipid shape

\[ \text{PO}_4 \text{O}_3 \text{C} \text{H}_2 \text{C} \text{H}_2 \text{N} + \text{H}_3 \text{C} \text{C}_3 \text{H}_3 \text{C}_3 \text{H}_3 \text{C}_3 \text{H}_3 \text{N} \]

\( \mu^\circ \) of acetone in water is low because acetone can form H-bonds and ionic interactions with water.

\( \mu^\circ \) of 2-methyl propane in water is high because 2MP is nonpolar. To minimize unfavorable interactions with water, 2MP will form droplets minimizing molecular contact with water.

At the air-water interface, the hydrophobic tails of a lipid monolayer avoid association with the water by extending into the air.
Lipid shape affects packing

The concept of lipid shape and its effects on lipid packing. In a cylindrical lipid, the cross-sectional area of the lipid headgroup is about equal to that of the chains; a lipid of this type will pack as a bilayer. In a cone-shaped lipid, the cross-sectional area of the lipid headgroup is less than that of the chains; a lipid of this type will pack in the hexagonal H₃ phase.
Glycerophospholipids spontaneously form lipid bilayers when dispersed in water

A. Electron micrograph of a multilamellar phospholipid vesicle in which each layer is a lipid bilayer (After Bangham, Cambridge Univ)
B. An electron micrograph of a liposome. Its wall, as the diagram indicates, consists of a bilayer (After Stoekenius, UCSF)

Bilayer organization

DMPC is dimyristoyl-phosphatidylcholine (C14)
DLPC is dilauryl-phosphatidylcholine (C12)

FIGURE 10 The solid line shows the electron density profile for DMPC obtained by fitting the electron density model to the form factor data in Fig. 8. The individual contributions to the total electron density of the phosphatidylcholine PC group, the carbonyl-glycerol CG groups, the terminal methyl M groups, and the baseline function $\rho_b$ are shown by broken lines on the left side of the figure.

FIGURE 11 The solid line shows the electron density profile for DLPC obtained by fitting the electron density model to the form factor data in Fig. 9. The individual contributions to the total electron density of the phosphatidylcholine PC group, the carbonyl-glycerol CG groups, the terminal methyl M groups, and the baseline function $\rho_b(z)$ are shown by broken lines on the left side of the figure.

**Physical properties of membranes**

**Paraffin Waxes** consist mostly of straight chain hydrocarbons and are available in a wide variety of melting points ranging from 120 to 160 degrees fahrenheit. Paraffin waxes are mainly identified in the candle industry by melt point and oil content.

Wax melting is an endothermic reaction (heat is absorbed from surroundings).

---

**Monitoring the melting process by Differential Scanning Calorimetry**

DSC measures the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material, as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate.

The temperatures of the sample and reference are controlled independently using separate, identical heat sources. The temperatures of the sample and reference are made identical by varying the power input to the two heat sources; the energy required to do this is a measure of the enthalpy or heat capacity changes in the sample relative to the reference.
Temperature

Heat flow

Exothermic

Endothermic

melting a wax
(scan from low to high temp)

freezing a wax
(scan down in T)

inert sample material

In fact the baselines of the melting and freezing curves would be superimposable - they are separated here for illustration purposes only.
Cholesterol is a relatively flat molecule and serves as a membrane plasticizer.
Physical properties of membranes

A

Dimyristoyl PC bilayers

mol% cholesterol

mol% cholesterol

\(0\)

\(20\)

\(5\)

\(27\)

\(10\)

\(34\)

\(15\)

\(40\)

\(50\)

\(0\)

\(40\)

\(80\)

\(0\)

\(50\)

\(100\)

°C

B

\(5\)

\(10\)

\(15\)

\(20\)

\(27\)

\(34\)

\(40\)

\(50\)

MOL % CHOLESTEROL

Heat of transition, \(J/\text{mol}

FIGURE 1: Temperature dependence of the apparent partial specific volume (\(P_a\)) for pure DPPC and DOPC bilayers and a series of cholesterol-containing DPPC bilayers. The featureless curve with the highest \(P_a\) is that of DOPC. Pure DPPC is the curve with the lowest \(P_a\) at low temperatures. The remaining curves, from the bottom up at lower temperatures, are for DPPC bilayers containing 20, 23, 25, 29, 33, 40, 45, and 50 mol % cholesterol.

Biochemistry 1983, 22, 5797-5807
Lipid Miscibility

A  DOL/DSL

B  DOL/SM

C  DOL/SM/CHOL

D  SM

Studies of the Relationship between Bilayer Water Permeability and Bilayer Physical State²

A. Carruthers* and D. L. Melchior*
The less viscous (the fluid or $\alpha$ phase) is labeled by a fluorescent dye (Texas Red-DPPE).
DMPC and sphingomyelin are miscible in artificial lipid bilayers

Fig. 1. $\Delta h$-DSC thermograms for membranes composed of DMPC and pure-C16:0-SM at a ratio of (a) 10:0, (b) 8:2, (c) 6:4, (d) 4:6, (e) 2:8, or (f) 0:10.

J. Phys. Soc. Jpn., Vol. 73, No. 12, December, 2004

M. ShibaKami et al.

Cholesterol preferentially associates with sphingomyelin in DMPC/sphingomyelin bilayers

Fig. 2. Transition temperature ($T_m$) (top) and enthalpy (bottom) of pure-C16:0-SM/DMPC membrane as a function of mole fraction of pure-C16:0-SM.

Fig. 3. $\Delta h$-DSC thermograms for membranes composed of pure-C16:0-SM and DMPC with a molar ratio of 6:4 (A) or 4:6 (B) with (a) 0, (b) 9, (c) 16, (d) 23, or (e) 33 mol% of cholesterol.
Fluorescence recovery after photobleaching illustrates the lateral mobility of lipid molecules
lipids diffuse $\approx 1 \mu m / sec$

$1 \mu m = 1 \times 10^{-4} cm$

$$D_m = \frac{0.5\lambda^2}{t_{av}}$$

$$= \frac{0.5 \times 1 \times 10^{-8}}{1}$$

$$= 0.5 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1}$$

$k \approx 2 \frac{D_m}{\lambda^2}$

$t_{0.5} = 0.693/k$

for $\lambda = 3.5 \mu m$

$k = 0.164 \text{ s}^{-1}$ and

$t_{0.5} = 4.23 \text{ sec}$

$\tau = 6 \text{ sec}$
Phospholipid distribution between hemileaflets of the lipid bilayer

Lipid localization in biological membranes has been carried out primarily via chemical or enzymatic modification, via exchange techniques, and in some cases by immunochemical procedures.

The digestion of phospholipids in the outer monolayer of a membrane by exogenous phospholipases may reveal the distribution of phospholipids between the two membrane layers. e.g.

• Phospholipase A2 - releases fatty acids from the second carbon group of glycerol
• Phospholipase C - cleaves phospholipids just before the phosphate group
• Phospholipases D - produces phosphatidic acid from phosphatidylcholine.
• Sphingomyelinase breaks SM into phosphocholine and ceramide.

Lipid binding proteins (e.g. BSA) or lipid exchange vehicles (small unilamellar vesicles) can show which lipids, chemically modified lipids or enzymatically cleaved lipids are in the external hemileaflet.

What effects could complicate this analysis?
Spherical giant unilamellar vesicles (GUVs; 30-100 μm diameter) are made by electroformation of a ternary mixture of cholesterol with phospholipids of both high (DPPC; d(16:0)PC) and low (DOPC; d(18:1)PC) melting temperatures. The vesicle membranes are initially uniform at high temperature and phase-separate into two liquid phases when vesicle suspensions are placed on a precooled microscope stage. The less viscous Lα phase is labeled by fluorescent dye (Texas Red-DPPE). The composition and viscosity of the two phases depend on the composition and temperature of the entire vesicle.


Figure 2. Grayscale fluorescence images (left) are filtered and thresholded (middle). White regions are identified as domains. Those within a specified size range, ellipticity, and distance from the edge are retained. Circles (right image) identify those domains successfully identified through five successive frames. Mean square displacement data for domains with radii of 1-1.5 μm are shown on the right. Both vesicles have a composition of 1:2 DOPC/DPPC + 30% Chol; T 10 °C (top) and 20 °C (bottom). Note the factor of 10 difference in diffusion coefficients. The scale bar is 40 μm.

Lipid Rafts

The term raft denotes relatively large and long-lived domains composed of specific lipid species such as sphingomyelin (SM), cholesterol (Cho), and glycolipids in biological membranes. They are suggested to accumulate certain membrane proteins (often anchored by saturated alkyl chains) that could, for example, greatly enhance signaling cascades or crucially interfere with many other functions.

![Lipid Raft Diagram](image)


However, several studies using artificial membranes indicate:

1. SM & Cho do not have a specific affinity for one-another but rather, associate because they are geometrically excluded from other phases of the membrane.

   *Biophysical Journal Volume 86, 2004, pp1510–1520*

2. Addition of Triton may create ordered domains in a homogeneous fluid membrane, which are, in turn, Triton resistant upon subsequent membrane solubilization. Hence, detergent-resistant membranes should not be assumed to resemble biological rafts in size, structure, composition, or even existence. Functional rafts may not be steady phenomena; they might form, grow, cluster or breakup, shrink, and vanish according to functional requirements, regulated by rather subtle changes in the activity of membrane disordering or ordering compounds.

   *Biophysical Journal Volume 83, 2002, pp2693–2701*

3. Cholesterol depletion in fibroblasts reduces sphingolipid domain abundance but cholesterol remains evenly distributed throughout the plasma membrane and is not enriched in sphingolipid domains. This rules out cholesterol-sphingolipid interactions as the driving force for plasma membrane organization in cells. Sphingolipid domains are disrupted by drugs that depolymerize the actin cytoskeleton, suggesting that lipid organization involves the cytoskeleton.

   *Frisz et al., 2013 Sphingolipid Domains in the Plasma Membranes of Fibroblasts Are Not Enriched with Cholesterol J. Biol. Chem. 288, pp16855-16861*
The Cell Membrane as a Barrier to Solute Movement

1 Diffusion = Random Walk (Fig 1)

Figure 1 Simulation of the diffusion process. Three successive stages are shown of molecules moving by random walks from: A. The first position where all molecules are at one side of the barrier. B. An intermediate stage. C. An equilibrium distribution
2 Chemical Potential

The chemical potential of a molecule comprises those components of a molecule \((j)\) that enable it to perform work.

a. Concentration, \(C_j\) (osmotic work)

b. Charge, \(Z_j e \psi\) where

\[ Z = \text{valence (electrical work)} \]
\[ e = \text{electron charge} \]
\[ \psi = \text{electric potential} \]

c. Volume, \(V_j\) (work against applied pressure)

d. Mass, \(m_j\) (gravitational work)

e. Chemical structure (chemical work)

Nobel, 1974 shows that chemical potential of \(j\) \((\mu_j)\)

\[
\mu_j = \mu_j^\circ + R T \ln C_j + Z_j e F \psi + V_j P + m_j g h
\]

\(\mu_j^\circ\) = chemical potential of substance \(j\) in standard state when \(\psi = 0, h = 0, P\) and \(T\) are standard and \(C_j = 1\text{M}\) in a particular solvent. As gravity and \(\Delta P\) unimportant here,

\[
\mu_j = \mu_j^\circ + R T \ln C_j + Z_j F e \psi
\]
3  Equilibrium Distributions

3.1 The Partition coefficient, $K$

Imagine glycerol is added to a mixture of oil and water. The mixture is shaken until the concentrations of glycerol in oil and water no longer change (equilibrium is achieved). The mixture is allowed to stand (phase separation occurs) and the oil and water phases are assayed for glycerol content.

At equilibrium, glycerol$_{\text{oil}}$ is in equilibrium with glycerol$_{\text{water}}$

\[ \text{i.e. } \mu_{\text{j}}^{o}_{\text{oil}} = \mu_{\text{j}}^{o}_{\text{water}} \]

As glycerol is uncharged, the electrical term disappears.

\[
\begin{align*}
\mu_{\text{j}}^{o}_{\text{water}} + RT\ln C_{\text{j}}^{o}_{\text{water}} &= \mu_{\text{j}}^{o}_{\text{oil}} + RT\ln C_{\text{j}}^{o}_{\text{oil}} \\
\mu_{\text{j}}^{o}_{\text{water}} - \mu_{\text{j}}^{o}_{\text{oil}} &= RT\ln \left[ \frac{C_{\text{j}}^{o}_{\text{oil}}}{C_{\text{j}}^{o}_{\text{water}}} \right] \\
o r \quad K_{\text{oil/water}} &= e^{\frac{\mu_{\text{j}}^{o}_{\text{water}} - \mu_{\text{j}}^{o}_{\text{oil}}}{RT}}
\end{align*}
\]

i.e. $K$ is determined by differences in standard state chemical potential of $j$ in oil and water

\[
K_{\text{oil/water}} = \exp \left[ (\mu_{\text{j}}^{o}_{\text{water}} - \mu_{\text{j}}^{o}_{\text{oil}})/RT \right]
\]

Each $\mu_{\text{j}}^{o}$ determined by energetics of interaction between $j$ and solvent

glycerol has three - OH groups resulting in strong H-bonding to H$_2$O and is thus in a more energetically favorable state in H$_2$O

\[
\therefore \quad \mu_{\text{j}}^{o}_{\text{water}} < \mu_{\text{j}}^{o}_{\text{oil}} \\
\therefore \quad K_{\text{oil/water}} < 1.
\]
4 Basal Permeabilities

Permeability depends on:
1. Partitioning into the membrane - $K_j$ (a and c)
2. Mobility within the membrane - $\mu_j$ (b)
3. Membrane thickness $\lambda$
5 Transbilayer diffusion is a first order process

transbilayer solute flux = \( J_{12} = k(C_{1}^{aq} - C_{2}^{aq}) \) mol.L\(^{-1}\).s\(^{-1}\)

![Graph showing relative signal vs time in seconds and fractional equilibration vs time in seconds]

mols of substrate crossing the membrane per sec

* molar flux into a unit volume of cells: \( J_{12} = mL \cdot k(C_{1}^{aq} - C_{2}^{aq}) \) mol.s\(^{-1}\)
* flux across a unit surface area: \( J_{12} = P \cdot A(C_{1}^{aq} - C_{2}^{aq}) \) mol.s\(^{-1}\)

hence, \( mL \cdot k = PA \)

\{A = surface area in cm\(^2\); P = permeability coefficient in cm.s\(^{-1}\); C=mol.cm\(^{-3}\}\}

At steady state, flow across membrane = flow within membrane

\( J_{12} = \frac{D_{m}}{\lambda} A(C_{1}^{m} - C_{2}^{m}) \)

where \( D_{m} = \) diffusion coefficient for movement in membrane \( \sim \mu_{j} \)

At any temperature, a molecule has a higher chemical potential in a higher-concentration area, and a lower chemical potential in a low concentration area. Movement (diffusion) of molecules from high to lower chemical potential is accompanied by a release of free energy. This movement is therefore a spontaneous process.
\[ J_{12} = \frac{D_m}{\lambda} A \left( C_{1}^{m} - C_{2}^{m} \right) \]

Concentrations just within membrane are related to those just outside by the partition coefficient \( K \)

\[ K = \frac{C^m}{C^{aq}} \text{ thus } C^m = K C^{aq} \]

\[ J_{12} = \frac{KD_m}{\lambda} A \left( C_{1}^{aq} - C_{2}^{aq} \right) \]

The original permeability expression was:

\[ \text{flux}_{1 \rightarrow 2} = J_{12} = P A \left( C_{1}^{aq} - C_{2}^{aq} \right) \]

\[ \therefore P = \frac{KD_m}{\lambda} \]

Thus, permeability is positively related to \( K \) (and \( D_m \))

We will now use measurements of the permeability of human red blood cells to a variety of small compounds to determine whether we can understand more about the physical properties of membrane lipid bilayers.
Fig 2 shows a plot of log P vs. log K where P is the permeability of red cells to substances and K is Partition coefficient for species in hexadecane/water.

The data are listed and numbered in Table A.1
There is reasonable agreement!
However, low MW species lie above line e.g. H₂O
high MW species lie below line (see Table 1 for molecular species)

**Why? Is Dₘ greater for small species?**
Assuming K is identical to that for hexadecane and H₂O and assuming λ = 40 Å, Dₘ is calculated and shown in Fig 3

\[ D_m = \frac{P\lambda}{K} \]

\[ D_m < D_{\text{water}} \text{ and is inversely proportional to MW!} \]
If we then refine this and make plot (Figure 4) of log $D_m$ vs diffusant volume (van der Waal’s vol), the relationship is better

$$\log D_m = m_v \cdot V + \log D_m^{v=0}$$

(slope · diffusant volume + y_intercept)

or,

$$\log D_m = \log D_m^{v=0} - m_v \cdot V$$

where:

- $m_v =$ slope
- $\equiv$ size selectivity of membrane

for these data

$m_v = 0.0517 \text{ mol cm}^{-3}$

$D_m^{v=0} = 1.3 \times 10^{-5} \text{ cm}^2\text{s}^{-1}$

($D_m$ for infinitely small species)

**Note**  Another way of writing the expression

$$\log D_m = \log D_m^{v=0} - m_v \cdot V$$

is

$$D_m = D_m^{v=0} e^{-(2.303 m_v \cdot V)}$$

You will see why this expression is useful later!
Now let's size correct the permeability data:

The size corrected permeability coefficient \( P^{\text{c}} \) is obtained by simply multiplying the measured permeability coefficient, \( P \), by the factor

\[ 10^{m_v \times V} \]

(remember \( m_v \) and \( V \) from Fig 4)

\[ P^{\text{c}} = \frac{KD^{v=0}}{\lambda} = P \cdot 10^{m_v \cdot V} \]

Thus a plot of \( \log(P \cdot 10^{0.0517 \times V}) \) versus \( \log K_{\text{hexadecane}} \)

Much better fit! The slope of the linear regression fit is almost unity!

Size and solubility almost completely account for membrane permeability!!

5. Why is diffusion dependent upon size?

Fig 5 shows a plot of \( \log D \) vs \( \log \) vol for molecular species in hexadecane, a polymer and the red cell membrane.

Comparison of the size-dependence of diffusion in n-hexadecane, in a polymer and within the human red cell membrane. The upper points (1-7) and curve are for diffusion in hexadecane at 25°C. The lower points (8-13) are for diffusion in poly(methyl acrylate) at 45°C. The lower line is the regression line for diffusion within the red cell membrane reproduced from Fig. 4. Diffusants are numbered as follows: 1, water; 2, methane; 3, ethane; 4, n-propane; 5, n-hexane; 6, n-heptane; 7, n-octane; 8, methyl-acetate; 10, n-propyl-acetate; 11, n-butyl-acetate; 12, methanol; 13, benzene.

Diffusion in the membrane and polymer displays a very strong dependence on vol.
6.1 Stokesian diffusion:
for sphere of radius \( r \) moving through a fluid
\[
D = \frac{kT}{(6 \pi \eta r)}
\]
where \( k = \) boltzman constant
\( 6 \pi \eta r = \) factor to account for frictional drag through a viscous fluid
for a sphere of vol \( V \left( \frac{4}{3} \pi r^3 \right) \) in a given fluid at a fixed Temp
\[
D = \text{constant} \cdot \frac{1}{\text{vol}^3}
\]
explains why diffusion in H\(_2\)O and hexadecane shows only slight dependence on size!

6.2 Non-Stokesian Diffusion
e.g. as in polymers.
Polymer scientists have attempted to explain diffusion in terms of free volumes or "holes". e.g. van der Waal volumes for hydrocarbon solvents are frequently < 65% Total volume
i.e. > 35% volume is empty!!
Holes of free volume are distributed over many different transient holes of different sizes. For diffusion, it is important that a molecule of vol. \( V \) can find a hole \( \geq V \) adjacent to it!

Probability of this, \( f_V \)
\[
f_V = e^{-\left( \frac{V}{V_{avg}} \right)}
\]
where \( V_{avg} = \) mean volume of hole.
Overall diffusion coefficient is then proportional to

(i) total number holes \( N_t \)
(ii) rate of appearance and disappearance of holes \( j \)
(iii) \( fV \)

\[ \therefore D = \text{constant} \cdot N_t \cdot j \cdot fV \]

For a given medium \( N_t \) and \( j \) are constants

\[ D = \text{constant} \cdot e^{-\left(\frac{V}{V_{avg}}\right)} \]

Returning to slide 54 we can see that this is equivalent to

\[ D_m = D_m^{V=0} e^{-\left(2.303m_v \cdot V\right)} = D = \text{constant} \cdot e^{-\left(\frac{V}{V_{avg}}\right)} \]

\[ e^{-\left(2.303m_v \cdot V\right)} = e^{-\left(\frac{V}{V_{avg}}\right)} \therefore \frac{V}{V_{avg}} = 2.303m_v \cdot V \therefore \frac{1}{V_{avg}} = 2.303m_v \]

Hence in this simple model,

\[ V_{avg} = \frac{1}{2.303m_v} = \frac{1}{2.303 \times 0.0517} \text{cm}^3/\text{mol} = 8.4 \text{ cm}^3/\text{mol} \]

This volume \( \approx \) van der Waal’s volume of a methylene group of a hydrocarbon which is less than the van der Waal’s volume of water (10.6 cm\(^3\)/mol)!!

\[ \therefore \text{explains steep size dependence of Dm in red cells!} \]
Table A.1

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Number</th>
<th>vdWvol</th>
<th>Mr</th>
<th>P</th>
<th>Khex</th>
<th>Dmnm</th>
<th>SizecorrectedP</th>
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<tr>
<td>Ethanediol</td>
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<td>36.5</td>
<td>62</td>
<td>2.90E-05</td>
<td>1.70E-05</td>
<td>6.82E-07</td>
<td>2.24E-03</td>
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<td>31.9</td>
<td>46.07</td>
<td>2.10E-03</td>
<td>5.70E-03</td>
<td>1.47E-07</td>
<td>9.36E-02</td>
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<tr>
<td>Glycerol</td>
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<td>51.4</td>
<td>95.12</td>
<td>1.60E-07</td>
<td>2.00E-06</td>
<td>3.20E-08</td>
<td>7.27E-05</td>
</tr>
<tr>
<td>n-Hexanol</td>
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<td>72.9</td>
<td>102.18</td>
<td>8.70E-03</td>
<td>1.30E-02</td>
<td>2.68E-09</td>
<td>51.10334</td>
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<tr>
<td>Methanol</td>
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<td>21.7</td>
<td>33.05</td>
<td>3.70E-03</td>
<td>3.80E-03</td>
<td>3.86E-07</td>
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</tr>
<tr>
<td>n-Propanol</td>
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<td>42.2</td>
<td>60.1</td>
<td>6.50E-03</td>
<td>3.30E-02</td>
<td>7.88E-08</td>
<td>9.88E-01</td>
</tr>
<tr>
<td>Urea</td>
<td>9</td>
<td>32.6</td>
<td>60.6</td>
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<td>3.50E-06</td>
<td>8.80E-08</td>
<td>3.73E-05</td>
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<tr>
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<td>10</td>
<td>10.6</td>
<td>18.02</td>
<td>1.20E-03</td>
<td>4.20E-05</td>
<td>1.14E-05</td>
<td>4.24E-03</td>
</tr>
</tbody>
</table>

Data for Fig. 5
Water    | 1 | 10.60 | 4.22E-05 |
methane  | 2 | 15.77 | 2.37E-05 |
etane   | 3 | 27.04 | 1.78E-05 |
propane  | 4 | 38.31 | 1.54E-05 |
hexane  | 5 | 68.73 | 7.5E-06  |
heptane | 6 | 78.87 | 6.46E-06 |
octane  | 7 | 90.14 | 5.62E-06 |
methyl acetate | 8 | 42.82 | 1E-07  |
ethyl acetate | 9 | 52.96 | 5.62E-08 |
propyl acetate | 10 | 63.10 | 1.78E-08 |
butyl acetate | 11 | 74.37 | 1E-08  |
methanol | 12 | 22.54 | 5.62E-07 |
benzene | 13 | 47.32 | 2.09E-08 |

FIGURE 5: Physical state and water permeability of various LUV types vs. temperature. (A) DSC thermogram (top) and rate of volume change (bottom) in LUVs consisting of DOL and DSL (1:1 molar ratio). Conditions as in Figure 3B. DSC scan rate, 5 °C/min. (B) DSC thermogram (top) and rate of volume change (bottom) of LUVs consisting of DOL and egg yolk sphingomyelin (1:1 ratio by weight). DSC scan rate, 10 °C/min. Conditions of water flux determinations as in Figure 3B. (C) Physical state (DSC thermogram, top) and rate of volume change (bottom) in DOL/egg yolk sphingomyelin/cholesterol LUVs (1:1:0.5 molar ratio assuming sphingomyelin molecular weight = 700). DSC scan rate, 10 °C/min. (D) Physical state (DSC thermogram, top) and rate of volume change (bottom) in egg yolk sphingomyelin LUVs. DSC scan rate, 10 °C/min. With volume change determinations, each point represents the mean ± SE of at least four separate determinations. Where error bars are not seen, the error is less than the size of the point.