New Insights on the Interaction of Flavonoids with Biomimetic Membranes

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Overview

- Playing with “Poor” Diffraction Data
- Global Analysis of Lipid Bilayer Structure
- Flavonoids’ Interaction with Biomimetic Membranes
- Flavonoids’ Protective Properties in Mitochondrial Stress
- Outlook: Combining Structural & Simulation Data
From Cells to Vesicles

Life bases on Biomembranes

Liposome (MLV)

Phospholipid Membrane Stack

Electron Density Profile

$\rho(z)$

$1 - 5 \text{ mm}$
The Information Given in Diffraction Peak

- **Position**: $d$-spacing
- **Width**: crystallite size
- **Height**: electron density contrast
- **Shape**: disorder type

$$FWHM = \frac{0.89 \cdot \lambda}{L} \approx \frac{\lambda}{N \cdot d}$$
The Information Given in Diffraction Peak

\[ \tilde{\rho}(z) = \sum_{h=1}^{h_{\text{max}}} \alpha_h F_h \cos(\frac{2\pi z}{d}) \]

What do I need?

1.) 4 diffraction peaks
2.) The correct phase combination: - - + -
Why are Four Diffraction Orders Enough?

A

B

-30 -20 -10 0 10 20 30

-30 -20 -10 0 10 20 30

electron density (a.u.)

z (Å)

18 19 20 21 22 23 24 25 26

26 25 24 23 22 21 20 19 18

1Å

A

B

1 2 3 4 5 6 7 8

included orders

1A
Electron Density Profile Determination

-30 -20 -10 0 10 20 30

-1.0 -0.5 0.0 0.5 1.0

z (Å)

rel. electron density (a.u.)

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5

z (Å)

rel. electron contrasts (a.u.)

lipid/water dispersion
(multilamellar liposomes)

background: water & capillary scattering

h = 1

h = 2

h = 3

h = 4

log(I) (a.u.)

s (Å⁻¹)

0.01 0.02 0.03 0.04 0.05 0.06 0.07

100 10 1

1000

100

h = 4

Lorentzian fit

I (a.u.)

s (Å⁻¹)

0.057 0.058 0.059 0.060 0.061 0.062

100 1000

h = 1

h = 2

h = 3

h = 4

background: water & capillary scattering

lipid/water dispersion
(multilamellar liposomes)
Bilayer Models: Vittorio Luzzati’s Idea

\[ d_{B\_Luzzati} = \Phi_L \, d \quad \sim d_{HH} \]
Modelling Bilayers: Strips and Gaussians

\[ |F(q)|^2 = 2\pi \left[ 2\sigma_H \exp(-\sigma_H^2 q^2/2) \cos(qz_H) - \sigma_C \rho_R \exp(-\sigma_C^2 q^2/2) \right]^2 \]
Interpolation of „Poor“ Data

\[ \rho(z) = \rho_s + \sum_{n=1}^{\text{max}} \alpha_n F_n \cos\left( \frac{2\pi h z}{d} \right) \]  \hspace{1cm} (1)

\[ F(q)^2 = 2\pi \left[ 2\sigma_{nn} \exp(-\sigma_{nn}^2 q^2 / 2) \cos(qz_{nn}) - \sigma_{C} \rho, \exp(-\sigma_{C}^2 q^2 / 2) \right]^2 \]  \hspace{1cm} (2)
Playing with Contrast

\[ d \]

\[ d/4 \]

\[ d \]

\[ d/2 \]
Taking a Look to F1 and F2

Decreasing of Hydration

\[ |F(q)|^2 = 2\pi \left[ 2\sigma_H \exp\left(-\sigma_H^2 q^2 / 2\right) \cos(q z_H) - \sigma_C \rho_R \exp\left(-\sigma_C^2 q^2 / 2\right) \right]^2 \]
2 Peak Estimation of Bilayer Thickness

\[ z_H = \pm \frac{d}{2\pi} \cdot \arccos\left( \frac{c_1 - \sqrt{8 (r_F c_3)^2 + 8 (c_2 - r_F c_4) \cdot (r_F c_3) + c_1^2}}{4 r_F c_3} \right) = \Phi_L \]

with \( r_F = \frac{F_1}{F_2} \)

\[ c_1 = 2\sigma_H \exp\left(-2\pi^2 \sigma_H^2/d^2\right) \]

\[ c_2 = -\sigma_C |\rho_r| \exp\left(-2\pi^2\sigma_C^2/d^2\right) \]

\[ c_3 = 2\sigma_H \exp\left(-8\pi^2\sigma_H^2/d^2\right) \]

\[ c_4 = -\sigma_C |\rho_r| \exp\left(-8\pi^2\sigma_C^2/d^2\right) \]
Some Examples: POPE, POPC and DMPC

Bilayer parameters at full hydration.

<table>
<thead>
<tr>
<th></th>
<th>POPE&lt;sup&gt;a&lt;/sup&gt; (30 °C)</th>
<th>2-peak method</th>
<th>3-peak method</th>
<th>POPC&lt;sup&gt;b&lt;/sup&gt; (2° C)</th>
<th>3-peak method</th>
<th>DMPC&lt;sup&gt;c&lt;/sup&gt; (30 °C)</th>
<th>2-peak method</th>
</tr>
</thead>
<tbody>
<tr>
<td>( z_H ) (Å)</td>
<td>20.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.3</td>
<td>20.0 (19.8)</td>
<td>20.2</td>
<td>20.8 (20.4)</td>
<td>17.3</td>
<td>19.1</td>
</tr>
<tr>
<td>( \sigma_H ) (Å)</td>
<td>3.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.3 (4.1)</td>
<td>3.6</td>
<td>4.8 (4.6)</td>
<td>3.0</td>
<td>3.1&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>( \sigma_C ) (Å)</td>
<td>4.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.3 (8.9)</td>
<td>4.8</td>
<td>6.9 (7.3)</td>
<td>4.5</td>
<td>4.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>(</td>
<td>\rho_r</td>
<td>)</td>
<td>1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.91&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.01 (1.01)</td>
<td>0.73</td>
<td>1.30 (1.24)</td>
</tr>
</tbody>
</table>

Real and Inverse Space

Lattice & its disorder

Motif = Crystal

\[ x = \text{Lattice & its disorder} \]

\[ \bigcirc \]

\[ \text{Motif} = \text{Crystal} \]
The Global Model for Fluid Lamellar Phases


What Do You Get?

\[ d_B = 2 \left( z_H + 2\sigma_H \right) \]
\[ d_W = d - d_B \]
\[ d_C = d_B/2 - d_H \]
\[ d_C = z_H - 4.1 \text{ Å} \]

\[ A = (V_L + V_H)/d_C \]

Geometrical method

\[ \eta \propto 1/\sqrt{(B K_c)} \]

B: bulk compression modulus
K_c: bilayer bending modulus
Experimental Insights into Flavonoid-Biomembrane Interactions (Didem Sanver & Andrew Nelson)

RCVs at 40 V s⁻¹ of a pure DOPC-coated Pt/Hg electrode (black line) in the presence of
(a) quercetin (b) naringenin (c) kaempferol (d) hesperetin (e) tiliroside (f) (+) – catechin (g) rutin (h) naringin at concentrations of 10 µmol dm⁻³ (red line) and 35 µmol dm⁻³ (green line) in PBS at pH 7.4.
Structural data on pure DOPC MLVs and DOPC with 6% of flavonoids
Structural data on pure DOPC MLVs and DOPC with 6% of flavonoids (II)

<table>
<thead>
<tr>
<th></th>
<th>DOPC</th>
<th>DOPC/Rutin</th>
<th>DOPC/Quercitin</th>
<th>DOPC/Tiliroside</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d$ (Å)</td>
<td>62.48</td>
<td>62.39</td>
<td>63.13</td>
<td>62.03</td>
</tr>
<tr>
<td>$d_{HH}$ (Å)</td>
<td>36.2</td>
<td>35.7</td>
<td>35.4</td>
<td>34.8</td>
</tr>
<tr>
<td>$d_{W}$ (Å)</td>
<td>26.3</td>
<td>26.6</td>
<td>27.8</td>
<td>27.7</td>
</tr>
<tr>
<td>$\sigma$ (Å)</td>
<td>5.3</td>
<td>6.1</td>
<td>6.5</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Temperature ramp 15 to 65 °C

6 mol% Tiliroside
Flavonoids’ Protective Properties in Mitochondrial Stress (Christine Bosch)

All flavonols markedly **attenuated** the hydrogen peroxide induced mtDNA damage in skin fibroblast cells after a pre-incubation period of 24 hours at concentrations of 10µM or higher.

At 25 and 50µM, **isorhamnetin** and **quercetin** further reduced mtDNA damage to 47-52% and 32-47%, respectively, whereas **kaempferol** showed a maximal reduction to 67% mtDNA damage.

Inhibition of H₂O₂ generation by **isorhamnetin** was much stronger compared to quercetin and **kaempferol**.
Global Fitting of the Mitochondrial Model Membranes

![Graph showing intensity vs. q (nm⁻¹)]

- PCPE-Isorhamnetin
- PCPE-Kaempferol
- PCPE-Quercetin
- PCPE
Structural Results on the Structural Influence of Flavonoids
Overview on Membrane Fluidization

PCPE

PCPE-Kaempferol

PCPE-Quercetin

PCPE-Isorhamnetin
Outlook: Combining Structural & Simulation Data

(A) DOPC/20 mol% Genistein
(B) DOPC/ 20 mol% Daidstein

Thank You for Your Attention!

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