STRUCTURAL PARAMETERS OF MACROSCOPICALLY FLAT LIPID MULTILAYERS ON A SILICA SOL SUBSTRATES

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Cell membrane model — phospholipid bilayer
Model lipids

1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC)

1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC)

Tabular properties:

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Formula</th>
<th>Mol. weight</th>
<th>Γ</th>
<th>$T_c$, °C</th>
<th>CAS Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSPC</td>
<td>$C_{44}H_{88}NO_8P$</td>
<td>790.145</td>
<td>438</td>
<td>55</td>
<td>816-94-4</td>
</tr>
<tr>
<td>SOPC</td>
<td>$C_{44}H_{86}NO_8P$</td>
<td>788.129</td>
<td>436</td>
<td>6</td>
<td>56421-10-4</td>
</tr>
</tbody>
</table>

How can one prepare a macroscopic lipid multilayer?

Langmuir-Blodgett technique

- **PRO**: widely used and relatively easy to implement;
- **PRO**: highly homogeneous lipid layers;
- **CON**: solid substrate does not correspond to actual cell body!
- **CON**: compression keeps lipid film homogeneity up to the monolayer only.

“Free” film on liquid substrate

- Relatively low homogeneity of the layers on clean water surface.
- Possibilities to use different water solutions as a substrates?
X19C beamline at NSLS, Brookhaven

\( \Phi = 10^{14} \) photons/s, \( E = 15000 \) eV, \( \Delta E/E \sim 10^{-3} \)

1 – thoroidal X-ray mirror; 2 – single-crystal monochromator; 3 – vibro-damping platform and sample chamber; 4 – \( 2\theta \)-rotation platform; 5 – two-slit collimator; 6 – \( \beta \)-rotation platform with detector slit.
Model-independent reconstruction of electron density profile
Extrapolation of the reflectivity asymptotic

Basic assumptions:

- Let $\delta(z) = \text{Re} \ 1 - \varepsilon(z)$ distribution contains $m$ special points of $n$-th order, where function $\delta(z)$ and its $n-1$ derivatives $\delta'(z) \ldots \delta^{(n-1)}(z)$ are continuous but $n$-th derivative suffers a step-like variation.
- Dielectric permittivity of external medium equals to vacuum ($\delta(z < z_{\text{min}}) \equiv 0$) while permittivity of substrate is constant ($\delta(z > z_{\text{max}}) \equiv \delta_+ \neq 0$).
- All discontinuity points $z_1 < z_2 < \cdots < z_m$ are of the same order.
- Absorption of the X-rays in a matter is neglected: $\text{Im} \ \varepsilon(z) \equiv 0$.

Reflectivity asymptotic in the frames of first-order Born approximation:

$$ r(q) \approx -k^2 \left( \frac{i}{2q} \right)^{2+n} \sum_{j=1}^{m} \Delta^{(n)}(z_j) \exp(2iqz_j), $$

$$ \Delta^{(n)}(z_j) \equiv \frac{d^n \delta}{dz^n}(z_j + 0) - \frac{d^n \delta}{dz^n}(z_j - 0), $$
Model-independent reconstruction of electron density profile

Searching for discontinuity points

Regularized Fourier-transform of the reflectivity curve

\[ F(x) = \frac{2^{2n+4}}{k^4 (q_{\text{max}} - q_{\text{min}})} \int_{q_{\text{min}}}^{q_{\text{max}}} \left[ q^{2n+4} R(q) - C \right] \cos(2qx) \, dq \]

\[ C = \frac{1}{q_{\text{max}} - q_{\text{min}}} \int_{q_{\text{min}}}^{q_{\text{max}}} q^{2n+4} R(q) \, dq, \quad q = k \sin \theta \]
**Model-independent reconstruction of electron density profile**

**Regularized merit function for numerical optimization:**

\[
MF = \frac{1}{N} \sum_j \left[ \frac{R_{\text{exp}}(\theta_j) - R_{\text{calc}}(\theta_j)}{R_{\text{exp}}(\theta_j)} \right]^2 + Q_1 \sum_{\substack{i=2,\ldots,N \atop i \neq i_1,\ldots,i_m}} (\delta_{i+1} - 2\delta_i + \delta_{i-1})^2 + Q_2 \left[ \sum_{i=i_2,\ldots,i_m} (\delta_{i+1} - \delta_i)^2 + \delta_1^2 \right]
\]

**Extraction of structural parameters**

\[
\rho_e(z) = \frac{\pi \delta(z)}{r_0 \lambda^2}, \quad A = \frac{\Gamma}{\int_{z_1}^{z_2} \rho(z)dz}
\]

where \( r_0 = 2.814 \times 10^{-5} \) Å, \( \Gamma \) – quantity of electrons per structure unit.
Lipid monolayer on a water substrate
Density profiles normalized by $\rho_{H2O} \approx 0.333 \text{ Å}^{-3}$
### Colloidal silica nanoparticles solutions

<table>
<thead>
<tr>
<th></th>
<th>D, nm</th>
<th>$\rho$, g/cm$^3$</th>
<th>$C_{SiO_2}$, %</th>
<th>$C_{NaOH}$, %</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>FM</td>
<td>5</td>
<td>1.1</td>
<td>16</td>
<td>0.3</td>
<td>9</td>
</tr>
<tr>
<td>SM-30</td>
<td>10</td>
<td>1.22</td>
<td>30</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>HS-40</td>
<td>12</td>
<td>1.27</td>
<td>40</td>
<td>0.3</td>
<td>9.5</td>
</tr>
<tr>
<td>TM-40</td>
<td>22</td>
<td>1.30</td>
<td>40</td>
<td>0.03</td>
<td>9</td>
</tr>
</tbody>
</table>


Volkov, Tikhonov et al. (IC RAS)
Surface effects on a clean silica sol

Example: sol SM-30, $\rho_{H_2O} \approx 0.333 \text{Å}^{-3}$

- Image-charge effect influences the particles near air/sol interface
- Debye screening length $\Lambda_D = \sqrt{\frac{\varepsilon_0 \varepsilon \frac{k_B T}{e}}{N_A e^2}} \sim 300 \ldots 500\text{Å}$.
- Air/“levitating ions”/depleted layer/SiO$_2$ particles/sol volume
- Approximate Na$^+$ surface density $(4.8 \pm 0.3) \times 10^{18} \text{m}^{-2}$

Phospholipid layer on a sol substrate

DSPC on SM-30, measurements over different time, $\rho_{H_2O} \approx 0.333 \text{ Å}^{-3}$

1-3 h

- Reflectivity
  - $10^{-8}$
  - $10^{-6}$
  - $10^{-4}$
  - $10^{-2}$
  - $10^{0}$
  - $10^{2}$
  - $10^{4}$

$q_z, \text{ Å}^{-1}$

1-3 h

- $\rho/\rho_{H_2O}$
  - $0.0$
  - $0.2$
  - $0.4$
  - $0.6$
  - $0.8$
  - $1.0$
  - $1.2$
  - $1.4$

$z, \text{ Å}$

$A = 55.7 \pm 0.7 \text{ Å}^2$

1–3 hrs: single layer, $d \approx 132 \text{ Å};$ SiO$_2$ particles are disappeared!
Phospholipid layer on a sol substrate

DSPC on SM-30, measurements over different time, $\rho_{H_2O} \approx 0.333 \, \text{Å}^{-3}$

- 1–3 hrs: single layer, $d \approx 132 \, \text{Å}$; SiO$_2$ particles are disappeared!
- $\approx 24$ hrs: periodic structure, $L = 68.0 \pm 2.1 \, \text{Å}$; $\Phi \approx 8 \, \text{Na}^+ \text{ ions/lipid molecule.}$
Phospholipid layer on a sol substrate
DSPC on SM-30, measurements over different time, $\rho_{H_2O} \approx 0.333 \, \text{Å}^{-3}$

$\rho / \rho_{H_2O}$

1–3 hrs: single layer, $d \approx 132 \, \text{Å}$; SiO$_2$ particles are disappeared!

$\approx 24$ hrs: periodic structure, $L = 68.0 \pm 2.1 \, \text{Å}$; $\Phi \approx 8 \, \text{Na}^+ \, \text{ions/lipid molecule}$.

$\approx 96$ hrs: multilayer, $L = 68.1 \pm 0.9 \, \text{Å}$; $\Phi \approx 10 \, \text{Na}^+ \, \text{ions/lipid molecule}$.

$A = 34.4 \pm 1.9 \, \text{Å}^2$
Influence of the substrate on multilayer properties

Reflectivity

$\rho / \rho_{H2O}$

$z, \text{Å}$

$\Lambda_D \approx 450 \text{ Å}$

$L = 72.2 \pm 1.8 \text{ Å}$

$A = 39.0 \pm 1.3 \text{ Å}^2$

$\Lambda_D \approx 400 \text{ Å}$

$L = 66.7 \pm 2.0 \text{ Å}$

$A = 33.7 \pm 1.3 \text{ Å}^2$

$\Lambda_D \approx 300 \text{ Å}$

$L = 68.3 \pm 1.8 \text{ Å}$

$A = 35.4 \pm 1.6 \text{ Å}^2$
Substrate doping with alkali ions
Sol FM-16 doped with Na⁺, pH≈11.5

- ΔpH = 1 decreases ΛD by $\sqrt{10}$ times;
- $L_1 \approx 35\text{Å}$, $A_{DSPC} = 44.9 \pm 1.7\text{Å}^2$ — crystalline monolayer;
- $A_{SOPC} = 65.3 \pm 2.5\text{Å}^2$ — liquid film;
- $d_2 \approx 20\text{Å} < d_{SiO_2}$ — probably lipid vesiculae with Na⁺ ions.

![Graphs showing reflectivity versus $q_z$, Å⁻¹ and density versus $z$, Å for DSPC and SOPC with Na⁺ doping.](image)
Ion screening effect: heavy alkali ions
DSPC on sol SM-30 doped with Cs\(^+\), pH\(\approx\) 11

- SiO\(_2\) particles layer is present!
- \(L_1 = 35 \pm 0.7\text{Å}, A = 40.2 \pm 2.0\text{Å}^2\) — almost ideal lipid monolayer.
- Approximate Cs\(^+\) surface density \((4.4 \pm 0.2) \times 10^{17}\text{m}^{-2}\)
Ion screening effect: saturated lipid salt
DSPC-Na salt on TM-40

- SiO$_2$ particles layer is present;
- $d_1 \approx 64\text{Å} \sim$ lipid bilayer.
- $A = 33.1 \pm 1.6\text{Å} \sim$ 2-dimensional lipid crystal.
### Cumulative table of lipid layer parameters

<table>
<thead>
<tr>
<th>Approx. structure</th>
<th>substrate</th>
<th>pH</th>
<th>phospholipid</th>
<th>$L$ (Å)</th>
<th>$A$ (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>monolayer</td>
<td>water</td>
<td>7</td>
<td>DSPC</td>
<td>37 ± 2</td>
<td>55.7 ± 0.7</td>
</tr>
<tr>
<td>monolayer</td>
<td>water</td>
<td>7</td>
<td>SOPC</td>
<td>36 ± 2</td>
<td>63.8 ± 1.3</td>
</tr>
<tr>
<td>bilayer in stack</td>
<td>FM</td>
<td>9</td>
<td>DSPC</td>
<td>72 ± 2</td>
<td>39.0 ± 1.3</td>
</tr>
<tr>
<td>bilayer in stack</td>
<td>SM-30</td>
<td>10</td>
<td>DSPC</td>
<td>68 ± 1</td>
<td>34.4 ± 0.9</td>
</tr>
<tr>
<td>bilayer in stack</td>
<td>HS-40</td>
<td>9.5</td>
<td>DSPC</td>
<td>67 ± 2</td>
<td>33.7 ± 1.3</td>
</tr>
<tr>
<td>bilayer in stack</td>
<td>TM-40</td>
<td>9</td>
<td>DSPC</td>
<td>68 ± 1</td>
<td>35.4 ± 1.6</td>
</tr>
<tr>
<td>monolayer</td>
<td>FM-16</td>
<td>11.5</td>
<td>DSPC</td>
<td>≈ 35</td>
<td>44.9 ± 1.7</td>
</tr>
<tr>
<td>monolayer</td>
<td>FM-16</td>
<td>11.5</td>
<td>SOPC</td>
<td>≈ 35</td>
<td>65.3 ± 2.5</td>
</tr>
<tr>
<td>monolayer</td>
<td>TM-40/Cs⁺</td>
<td>11</td>
<td>DSPC</td>
<td>35 ± 0.7</td>
<td>40.2 ± 2.0</td>
</tr>
<tr>
<td>bilayer</td>
<td>TM-40</td>
<td>9</td>
<td>DSPC-Na</td>
<td>64 ± 5</td>
<td>33.1 ± 1.6</td>
</tr>
</tbody>
</table>
Off-specular grazing incidence diffraction from DSPC multilayer
Preliminary data taken with laboratory source ($E = 8048$ eV), 25–30 June 2016
Phospholipid layer on the surface of silica sol compensates mirror charge effect, presumably due to the absorption of Na\textsuperscript{+} ions by lipid molecules from sol volume.

Lipid molecules within thick multilayer undergo spontaneous ordering effect under the influence of a surface electric field, leading to the formation of regular bilayer stack after a few days.

Quantity of bilayers depends on \( \Lambda_D \) at the surface and, consequently, substrate pH. Thus one can reduce bilayer number by increasing of sol pH.

Bilayer uniformity depends on surface electric field gradient and, consequently, size of SiO\textsubscript{2} nanoparticles in solution.

Layer of “levitating ions” on the air/sol interface prohibits the ion absorption from sol volume, effectively screening lipid layer from the substrate. This leads to the formation of thin monolayer instead of bilayer stack.
Further goals and perspectives

- Off-specular and off-plane GIXRD, X-ray fluorescence experiments (fine determination of lateral crystalline structure);
- Phospholipid mixtures and in-situ variation of substrate composition;
- Theoretical interpretation of spontaneous lipid stratification process (biophysicist needed);
- In situ membrane-protein conformation processes;
- Phospholipid layers at liquid/liquid interface.
THANK YOU FOR YOUR ATTENTION!