Structure and fluctuations of single floating bilayers

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Outline

- Introduction: supported bilayers
- Fluctuations and interactions of bilayers
- X-ray scattering
- Results
  - Bilayers, protusion modes
  - Fluctuations and interaction between 2 bilayers
  - Destabilization under an electric field
**Vesicles, lamellar phases**

**Unilamellar vesicles**

- Normal and giant vesicles
- Fluctuations through contour analysis

**MLV’s, lamellar phases**

- Multilamellar vesicles (MLVs)
- Oriented samples
- Caillé theory $\rightarrow \sqrt{\kappa B}$
- Controlled osmotic pressure

Multilamellar stacks

Preparation: from solution + drying, spin-coating, freely suspended films,

Well oriented samples: mosaic spreading better than 0.01° by spin-coating

Bending rigidity and compressibility from full analysis of x-ray scattering

Unbinding

Consistency of specular/off-specular ?

Role of defects ?

Supported bilayers I

Adsorbed bilayers

AFM:

Polymeric cushions

DOPC + sphingomyelin bilayer
Supported bilayers II (x-rays)

Reflectivity

Model lipid raft Cholesterol/DOPC/Sphingomyelin/DPPE 20:40:39.5:0.5 + monosialoganglioside $G_{M1}$

Vesicle fusion via osmotic rupture

Diffraction

DPPE bilayer

Reich et al. *Biophys. J* 95 657 (2008), ID01

Tethered functional membranes

- Silane lipids (phytanyl chains) + lipid bilayer (DPhyPC 1,2-diphytanoyl-sn-glycero-3-phosphocholine) + proteins (valinomycin, gramicidin)
- Valinomycin is a small ion carrier peptide which selectively transports K+ ions from one side to the other
- Functional tethered lipid membranes on metals or semi-conductors


- Captors
- Resources of microelectronics, captors
Membranes spanning holes

iRSTV/biopuces, LETI, CEA Grenoble
300nm pores

Beerlink et al. *Langmuir* **24** 4952 (2008), BM05
Phosphatidylcholines with $C_{16}$, $C_{17}$, or $C_{18}$ chains
Sample preparation II

Langmuir-Blodgett + Langmuir Schaeffer deposition techniques

“Double bilayers”

“Grafted” or “OTS bilayers”, 1rst layer is a grafted octadecyltrichlorosilane self-assembled monolayer

Super-polished silicon wafers < 1Å rms, SESO, Aix en Provence

Structure and fluctuations of single floating bilayers
Membrane elasticity

- **Tension** $\gamma < 1 \text{mN/m}$?
- **bending modulus** $\kappa \approx 20 - 30k_B T$ in fluid phase
- **interaction potential** $U(z)$

\[
U(z) = P_h l_h \exp(-z/l_h) - \frac{H}{12\pi} \left( \frac{1}{z^2} - \frac{2}{(z + D)^2} + \frac{1}{(z + 2D)^2} \right) + c_{fl} \frac{(k_B T)^2}{\kappa z^2}
\]

- $4.10^7 \text{Pa} < P_h < 4.10^9 \text{Pa}$
- $l_h = 0.1 - 0.3 \text{nm}$
- $H \approx k_B T$
interaction potential in lamellar systems

- Measure distance for applied osmotic pressure (Rand, Parsegian)

\[ \frac{\pi k_B T}{16} \left( \frac{P_h}{\kappa \lambda} \right)^{1/2} \exp\left(-\frac{z}{2\lambda}\right) \]


No agreement to experimental data with Helfrich interaction → softer potential (Podgornik, Parsegian *Langmuir* **8** 557 (1992)):
Model Hamiltonian

X-ray scattering $\rightarrow$ correlation functions ($\langle \rho \rho \rangle$ or $\langle zz \rangle$)

\[ H = \sum_{i=1}^{2} \left[ \int d^{2}r \frac{1}{2} \left( \gamma_i \nabla^2 + \kappa_i \Delta^2 \right) (u_i(r)) \right] + \int d^{2}r \left[ U_{sm} (u_1 - u_0) + U_{sm} (u_2 - u_0) + U_{mm} (u_2 - u_1) \right] \]

\[ H = \sum_{q} \left[ \frac{1}{2} \sum_{i=1}^{2} (\tilde{a}(q) + B) |\tilde{u}_i(q)|^2 - A_1 \tilde{u}_1(q)\tilde{u}_s(-q) - A_2 \tilde{u}_2(q)\tilde{u}_s(-q) - B\tilde{u}_1(q)\tilde{u}_2(-q) \right] \]

with $\tilde{a}(i, q) = (A_i + \gamma_i q^2 + \kappa_i q^4)$
Correlation to the substrate (static)

Minimization of the Hamiltonian against $\tilde{u}_1(q)$ and $\tilde{u}_2(q)$:

$$\tilde{u}_1(q) = \frac{A_1 \tilde{a}_2(q) + B (A_1 + A_2)}{\tilde{a}_1(q) \tilde{a}_2(q) + B (\tilde{a}_1(q) + \tilde{a}_2(q))} \tilde{u}_s(q)$$

$$\tilde{u}_2(q) = \frac{A_2 \tilde{a}_1(q) + B (A_1 + A_2)}{\tilde{a}_1(q) \tilde{a}_2(q) + B (\tilde{a}_1(q) + \tilde{a}_2(q))} \tilde{u}_s(q)$$


General solution:

$$\langle u_i(r) u_i(0) \rangle = \frac{1}{2\pi} \frac{A_i^2}{\kappa_i^2} \sum_{j=1}^4 \left[ \lambda_{i,j} K_0(q_j r) + \frac{1}{2} \eta^2_{i,j} r K_1(q_j r) \right] \otimes \langle u_s(r) u_s(0) \rangle$$

$$\langle u_i(r) u_s(0) \rangle = \frac{1}{2\pi} \frac{A_i}{\kappa_i} \sum_{j=1}^4 \left[ \eta_{i,j} K_0(q_j r) \right] \otimes \langle u_s(r) u_s(0) \rangle$$

$$\langle u_i(r) u_j(0) \rangle = \frac{1}{2\pi} \frac{A_1 A_2}{\kappa_1 \kappa_2} \sum_{j=1}^4 \left[ \tau_{i,j} K_0(q_j r) + \frac{1}{2} \nu_{i,j} r K_1(q_j r) \right] \otimes \langle u_s(r) u_s(0) \rangle$$

Structure and fluctuations of single floating bilayers
Normal modes + equipartition of energy:

\[ \mathcal{H}_q = \frac{1}{2} \sum_{i=1}^{2} (\tilde{a}_i(q) + B) |\tilde{u}_i(q)|^2 - B\tilde{u}_1(q)\tilde{u}_2(-q) \]

\[ = \begin{pmatrix} \tilde{u}_1(-q) & \tilde{u}_2(-q) \end{pmatrix} \begin{pmatrix} a_1(q) & -B/2 \\ -B/2 & a_2(q) \end{pmatrix} \begin{pmatrix} \tilde{u}_1(q) \\ \tilde{u}_2(q) \end{pmatrix} \]

\[ \langle u_i(r)u_i(0) \rangle = k_B T \frac{1}{\kappa_i} \frac{1}{2\pi} \sum_{j=1}^{4} \alpha_{i,j} K_0(q_j r) \]

\[ \langle u_1(r)u_2(0) \rangle = k_B T \frac{B}{\kappa_1\kappa_2} \frac{1}{2\pi} \sum_{j=1}^{4} \iota_j K_0(q_j r) \]
Correlation functions

\[ \gamma = 1 \text{mN/m}, \kappa = 30k_B T, U = 10^{13} \text{J.m}^{-4} \]

Effect of \( \gamma \)

Effect of \( \kappa \)

Effect of \( U \)

Structure and fluctuations of single floating bilayers
Perturbation theory (Distorted Wave Born Approximation)
X-ray scattering - simplified DWBA

\[
\begin{align*}
\left( \frac{d\sigma}{d\Omega} \right) &= r_e^2 |t^{in}|^2 |t^{sc}|^2 (e_{in} \cdot e_{sc})^2 \left\langle \int dr_\parallel e^{iq_\parallel r_\parallel} \right. \\
& \quad \left\{ \tilde{\rho}_{sub} e^{iqz z_s (r_\parallel)} + \tilde{\rho}_{M1} e^{iqz (z^{th}_1 (r_\parallel) + z^{st}_1 (r_\parallel))} \\
& \quad + \tilde{\rho}_{M2} e^{iqz (z^{th}_2 (r_\parallel) + z^{st}_2 (r_\parallel))} \right\}^2 \\
R(q_z) &= R_F(q_z) \left| 1 + iqz \int \frac{\delta \rho(z)}{\rho_{sub} - \rho_w} e^{iqz z} dz \right|^2 \\
I &= R(q_z) + \text{diffuse in the specular direction}
\end{align*}
\]

Combined treatment
specular + off-specular

\[
I = \frac{l_0}{h_i w_i} \int \left[ \left( \frac{d\sigma}{d\Omega} \right)_{\text{ref}} + \left( \frac{d\sigma}{d\Omega} \right)_{\text{pert}} \right] R_{\text{res}}(\Omega) d\Omega
\]

\[
= \frac{l_0}{h_i w_i} \frac{4\pi \Delta \theta A r_e^2 |t^{in}|^2 |t^{sc}|^2 (e_{in} \cdot e_{sc})^2}{k_0} \\
\times \int_0^{+\infty} dx \cos(q_{x0} x) e^{-\frac{\Delta q_x^2 x^2}{2}} f(x, q_z)
\]

Structure and fluctuations of single floating bilayers
E = 27keV, \( \lambda = 0.46\,\text{Å} \), \( 0.015\,\text{mm} \times 0.5\,\text{mm} \) beam
Specular reflectivity: average structure normal to the interface

Off-specular reflectivity: lateral inhomogeneities, elastic properties ($\gamma, \kappa$) and interaction potentials
Background subtraction

Structure and fluctuations of single floating bilayers
\[ \langle z(0)z(r_{||}) \rangle = \sigma^2 \exp \left( \frac{r_{||}^2}{\xi^2} \right)^H \]

\[ \sigma = 4 \text{Å}, \ \xi = 1 \mu, \ H = 0.4 \]
Scattered intensity

Structure and fluctuations of single floating bilayers
Sensitivity to parameters

Effect of water bending rigidity

Effect of potential

Effect of water layer thickness

Structure and fluctuations of single floating bilayers
Reflectivity

Structure and fluctuations of single floating bilayers
Structural parameters

Two models:

- Gaussian model: 1 Gaussian for the headgroups and 1 Gaussian trough for the methyl groups.
- Fourier development of for symmetric bilayers:

\[ \rho(z) - \rho_w = \frac{F_0}{D} + \frac{2}{D} \sum_{h=1}^{h_{\text{max}}} F_h \cos \left( \frac{2\pi h z}{D} \right) \]

J. Daillant et al., PNAS 102, 11639, 2005

Structure and fluctuations of single floating bilayers
$C_{18}$ bilayer in the gel phase: protusion modes

- $U'' = 2.10^{12} \text{J.m}^{-4}$
- $\kappa = 300k_B T$
- $\gamma = 70 \text{mN/m}$


$\gamma_P = 50 \text{mN/m}$
Diffuse scattering OTS bilayers

Structure and fluctuations of single floating bilayers
Gel to fluid transition / structure

Structure and fluctuations of single floating bilayers
Gel to fluid transition / tension and rigidity

Double bilayer

OTS bilayer

In the bulk:  

\begin{align*}
\text{gel} & : C_{17} & 43 \\
\text{ripple} & : C_{17} & 50/52 \\
\text{fluid} & : C_{17} & 48/49.8 \\
\end{align*}
Structure and fluctuations of single floating bilayers

Less defects $\rightarrow$ smaller $U''$?
Collaboration with L. Leiserowitz, Weizmann Institute

Bovine cardiac phosphatidylcholine (BCPC), distribution for n:m acyl chains (n number of C atoms, m number of C=C bonds) of 23% 16:0; 1% 16:1; 6% 18:0; 13% 18:1; 43% 18:2; 1% 18:3; 6% 20:4 and 7% others

Segregated cholesterol bilayers with $10 \times 7.5 \text{Å}^2$ motif?

after Rapaport et al. *Biophys J* **81** 2729 (2001)
Summary

- Possible to study single fluctuating bilayers
- General method for x-ray scattering
- Bilayers: protusion modes
- Very soft potential
- More complicated systems can now be investigated

Thank you!