Nanoscale Imaging of Artificial Membranes using Atomic Force Microscopy
Center of Structural Biology
CBS
http://www.cbs.cnrs.fr

Single Molecule Biophysics Group
Structure and dynamics of membrane assemblies

Atomic Force Microscopy  Single Molecule Tracking
OUTLINES

- How to mimic biological membranes
  *Supported lipid bilayers (SLB) and Atomic Force Microscopy (AFM)*

- Development of a membrane biosensor based on porous silicon

- Direct incorporation of solubilized proteins into supported lipid bilayers
Requirements for Membrane in Biosensor

Mimicking the membrane composition
Continuous membrane
Separation of 2 compartments
Stability
Membrane decoupling from the substrate

Adapted from Reimhult and Kumar (2007) TiBS
### Methods used to form lipid membrane on substrates

**Large Unilamellar Vesicle (LUV) Fusion**

![Diagram of LUV Fusion](image.png)
Characterization of Membrane-inspired biosensor

Quartz Crystal Microbalance with Dissipation (QCMD)

Surface Plasmon Resonance Spectroscopy

Fluorescence

Small angle X-rays scattering

Atomic Force Microscopy
AFM Imaging in aqueous buffer

Electrostatic force
Repulsive
(0.1 to 1 µm)

Van der Walls
Attractive Force (~Å)

Force applied during scanning < 100 pN
Silicium Nitride k<100 mN/m
From the intact cell to ... the molecule

Vertical (0.1 nm) and lateral (0.5 nm) resolution
Outstanding method for membrane characterization

Development of a membrane-inspired biosensor on porous silicon

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Characterization of the ability of drugs to cross membranes

Porous silicon

Optical properties (fluorescence, reflectivity)

Tunable structural properties (surface, volume, size of the pores)

Silicon chemistry (surface modification)

Reservoir of buffer
Preparation of the porous silicon

Anodization conditions

- P type silicon
- Solution of HF/Ethanol (3/1 in volume)
- Current density 22.5 mA/cm$^2$ for 5 min
- Thermal oxidation 450°C for 2 hours then treatment with NaOH (1M)

Si + 6F$^- + 2h^+ + 2H^+ \rightarrow SiF_6^{2-} + H_2
Preparation of the porous silicon

**Anodization conditions**

- Pores diameter 5-9 nm
- Thickness 3.7 µm

Si + 6F\(^-\) + 2h\(^+\) + 2H\(^+\) → SiF\(_6\)\(^{2-}\) + H\(_2\)

22.5 mA/cm\(^2\)
Optical properties of porous silicon
Sensing using Optical Reflectivity from Fabry-Pérot Layers

Fabric Perot interference pattern
\[ m\lambda = 2nL \]

\( n \) is the refractive index, \( L \) the thickness, \( \lambda \) the wavelength
Optical properties of porous silicon
Sensing using Optical Reflectivity from Fabry-Pérot Layers

Fabry Perot interference pattern
\[ m\lambda = 2nL \]

- \( n \) is the refractive index,
- \( L \) the thickness,
- \( \lambda \) the wavelength

Light source to spectrometer

Interference fringes

Fourier transform

- peak amplitude = index contrast \( 2\rho_a\rho_b \)
- peak position = effective optical thickness \( 2nL \)
Supported lipid bilayer by vesicle fusion

- Extrusion
- Multilamellar vesicle (MLV) → Liposome → SLB
- EggPC + 10% DOTAP/DPTAP + 0.1% Rhodamine-DHPE
- Dioleoyl-TrimethylAmmonium-Propane
- EggPC L-α-phosphatidylcholine

Aqueous medium
EggPC/DOTAP
Supported Lipid Bilayer

99.9% coverage
EggPC/DOTAP
Supported Lipid Bilayer

FRAP

99.9% coverage

D = 1.58 µm²/s
Mobile fraction = 78%
25% of the human genome encode transmembrane proteins. Target of 70% of the commercially available drugs.

**In a Structural point of view**

~ 200 structures available in the PDB (~ 20 eukaryotic).

Beta barrels (porin)  
Alpha helix (GPCR)

**In a nano-biotechnological point of view**
How to incorporate transmembrane proteins within artificial bilayers?

- Proteoliposome fusion
- Tethered proteins reconstitution
- Incorporation
Direct Incorporation of transmembrane proteins within artificial bilayers

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Ensemble, prenons le cancer de vitesse.
Direct incorporation of protein into SLB

Direct incorporation of protein into liposomes destabilized with glycosylated detergent

Reconstitution from fully solubilized samples

Direct incorporation of protein into SLB

1. SLB destabilization
   [detergent] \sim \text{cmc}

2. Incorporation

3. AFM Imaging
   of non crystalline proteins
Control SLB treated with detergent

15 min incubation with detergent (1.5 x cmc at 20°C)

- SLB are stable above the cmc with low cmc detergent and more resistant than liposomes.
- Both gel and fluid phases are preserved.

n-Dodecyl-β-D-Thiomaltopyranoside (DOTM)
cmc = 0.05 mM

n-Dodecyl-β-D-Maltopyranoside (DDM)
cmc = 0.2 mM

Octyl-β-D-glucopyranoside (OG)
cmc = 17 mM

DOPC/DPPC (1:1)

Dioleoyl-phosphatidylcholine

Dipalmitoyl-phosphatidylcholine
Incorporation of proteins from the photosynthetic apparatus of bacteria

LC-LH1
*Rhodobacter spheroides*

MW 300 kDa

LH2
*Rhodopseudomonas acidophila*

MW 110 kDa

LH, Light-Harvesting
Incorporation of RC-LH1 from *Rhodobacter spheroides*

**Experimental procedure**
500 ng (1.5 picomole) in 0.075 mM DOTM, 150 mM KCl, 10 mM Tris pH 7.4
15 min incubation with RC-LH1

Contact mode

1 μm x 1 μm
Incorporation of RC-LH1 from *Rhodobacter spheroides*
Incorporation of RC-LH1 from *Rhodobacter spheroides*
Incorporation of RC-LH1 from *Rhodobacter spheroides*

Proteins diffuse in the fluid phase and segregate in the lipid bilayer

Formation of quasi-crystalline areas
Incorporation of LH2 from *Rhodopseudomonas acidophila*

Experimental procedure
100 ng (1 picomole) in 0.075 mM DOTM, 150 mM KCl, 10 mM Tris pH 7.4
15 min incubation with LH2

∅ = ~ 6 nm

80 nm scan

Biophysical J (2006) 91, 3268-3275
Calcium effect on protein incorporation

5 mM CaCl₂

FosCholine-16
Calcium effect on protein incorporation

<table>
<thead>
<tr>
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<th>- Ca²⁺</th>
<th>+ Ca²⁺</th>
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<tbody>
<tr>
<td>δh DOPC-mica</td>
<td>5.67 ± 0.56</td>
<td>4.36 ± 0.25</td>
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<tr>
<td>δh DPPC -mica</td>
<td>6.69 ± 0.35</td>
<td>5.37 ± 0.14</td>
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Incorporation of the lactose permease Lac Y

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M. Teresa Montero
Jordi Hernández-Borrell
Direct incorporation of the permease Lac Y

Abramson et al. Science, 2003

1-Palmitoyl-2-oleoyl-phosphatidylglycerol (POPG)
1-Palmitoyl-2-oleoyl-phosphatidylethanolamine (POPE)

POPE:POPG (3:1) mixture

Calcium buffer
TM-AFM
Detergent effect on POPE/POPG bilayer

n-Dodecyl-β-D-Maltopyranoside (DDM)(2 cmc)
Direct incorporation of the permease Lac Y

Images acquired in TM-AFM in calcium buffer.

Summary

Low amount of protein (picomole range)
Unique orientation
Incorporation in the fluid phase and diffusion (weak interaction with the substrate)

Lateral resolution below the nanometer range (subunit of oligomers can be delineated).

Suitable for functional and nano-biotechnological applications

→ Fill-in (continuous bilayer)
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