Reconstruction of a transmembrane protein tetraspanin (CD9) into lipid bilayer by interaction of ganglioside GM3 and tetraspanin

Glycobiology World Congress

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Tokyo University of Science, Japan

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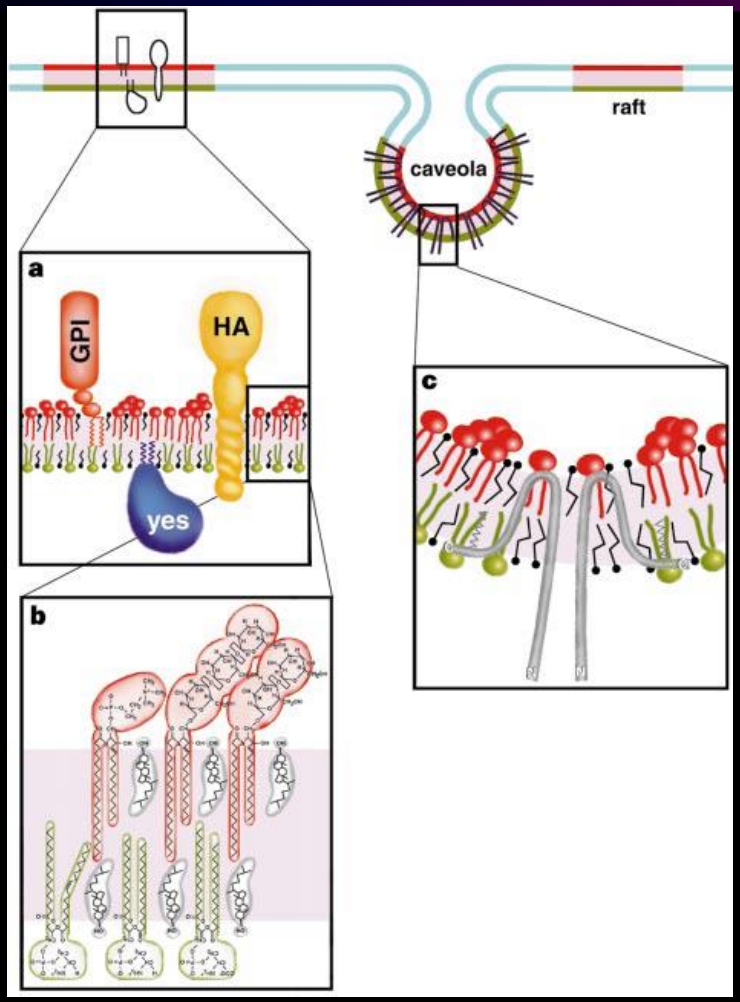
Model for the organization of rafts

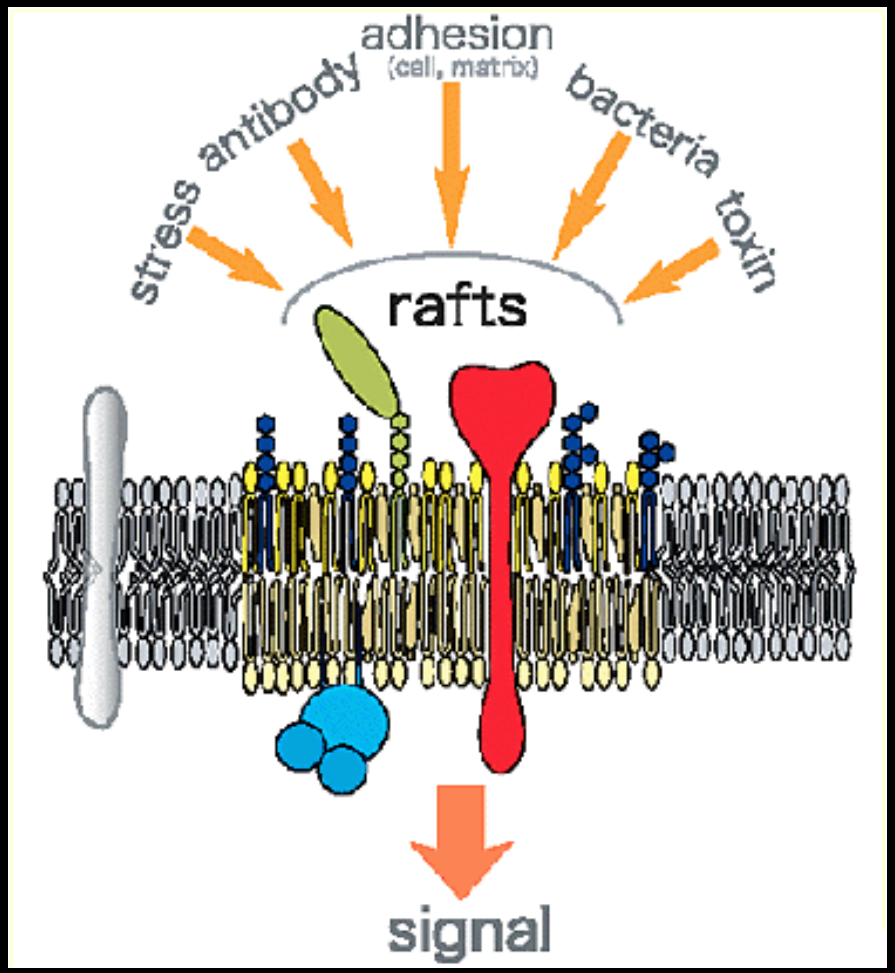
Kai Simons & Elina Ikonen, NATURE |VOL 387 | 5 JUNE 1997



The rafts (red) segregate from the other regions (blue) of the bilayer.

Rafts contain proteins attached to the exoplasmic leaflet of the bilayer by their GPI anchors, proteins binding to the cytoplasmic leaflet by acyl tails (the Src-family kinase Yes is shown), or proteins associating through their transmembrane domains, like the influenza virus proteins neuraminidase and haemagglutinin (HA).

Function of ganglioside rafts



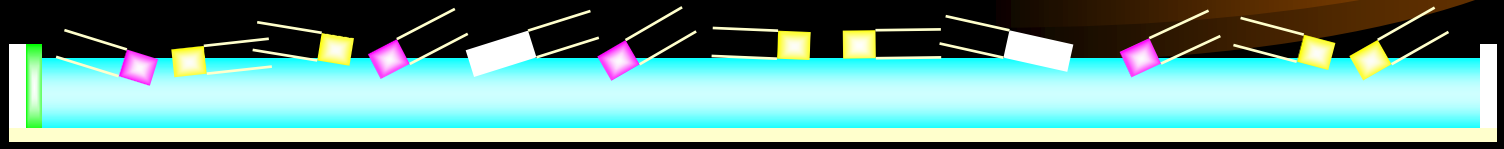
On outer membrane of raft, it is very clear that many ligands link to the corresponding receptors.

How is the signal transduced on inner membrane of raft?

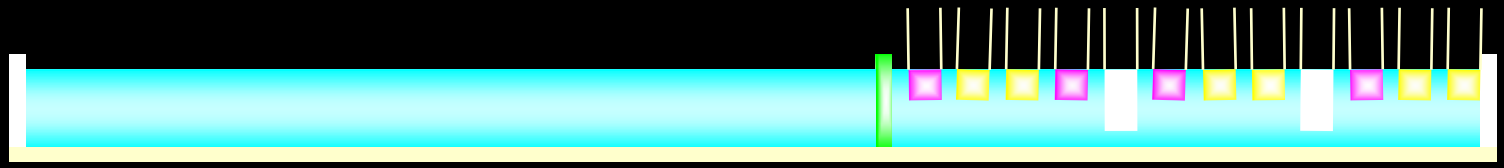
For example, autophosphorylation enzyme or cutting enzyme of signal peptides.

<u>How is the enzyme recluited?</u>

Preparation monolayers by Langmuir-Blodgett technique



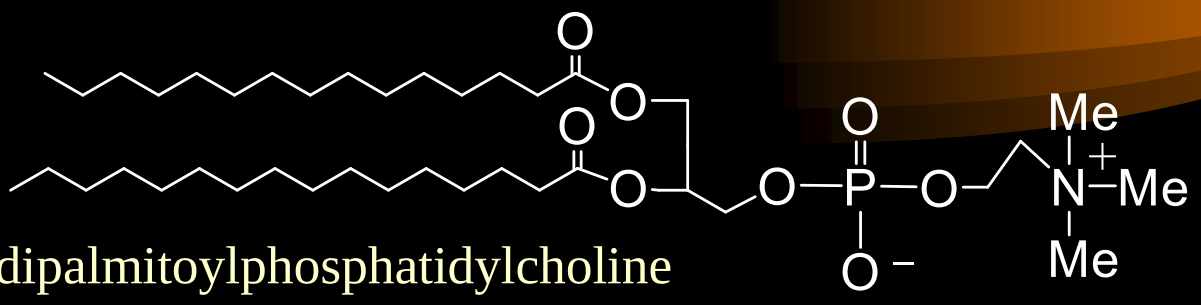
Lipids solution were spread on the subphase using microsyringe.



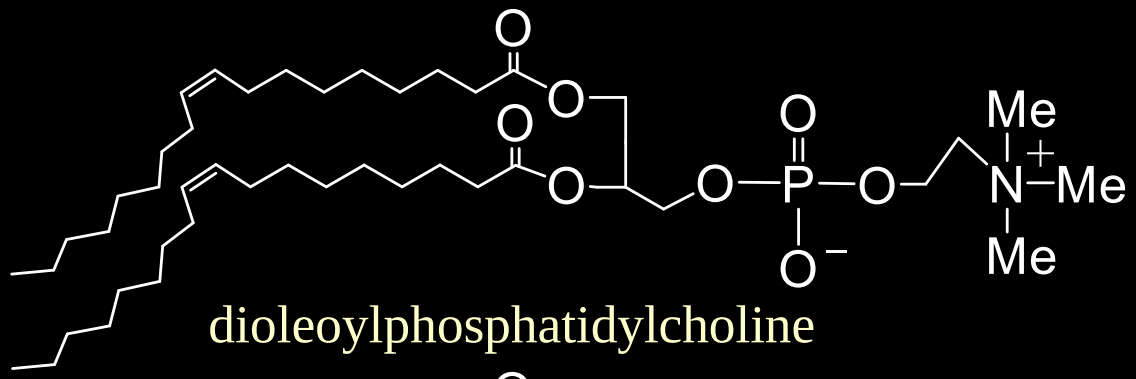
After evaporation of the solvent, the monolayer was compressed at 50 cm^2 / min.

→ **The half side of cell membrane can be obtained.**

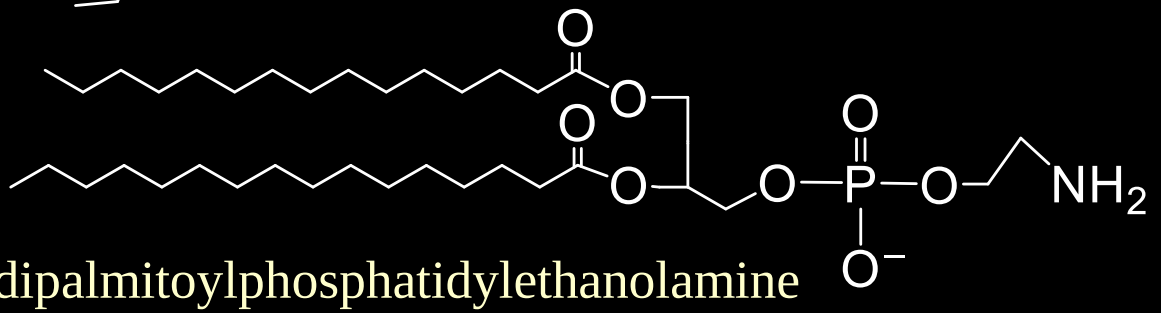
Molecular structures of lipids for L-B film preparation



DPPC dipalmitoylphosphatidylcholine



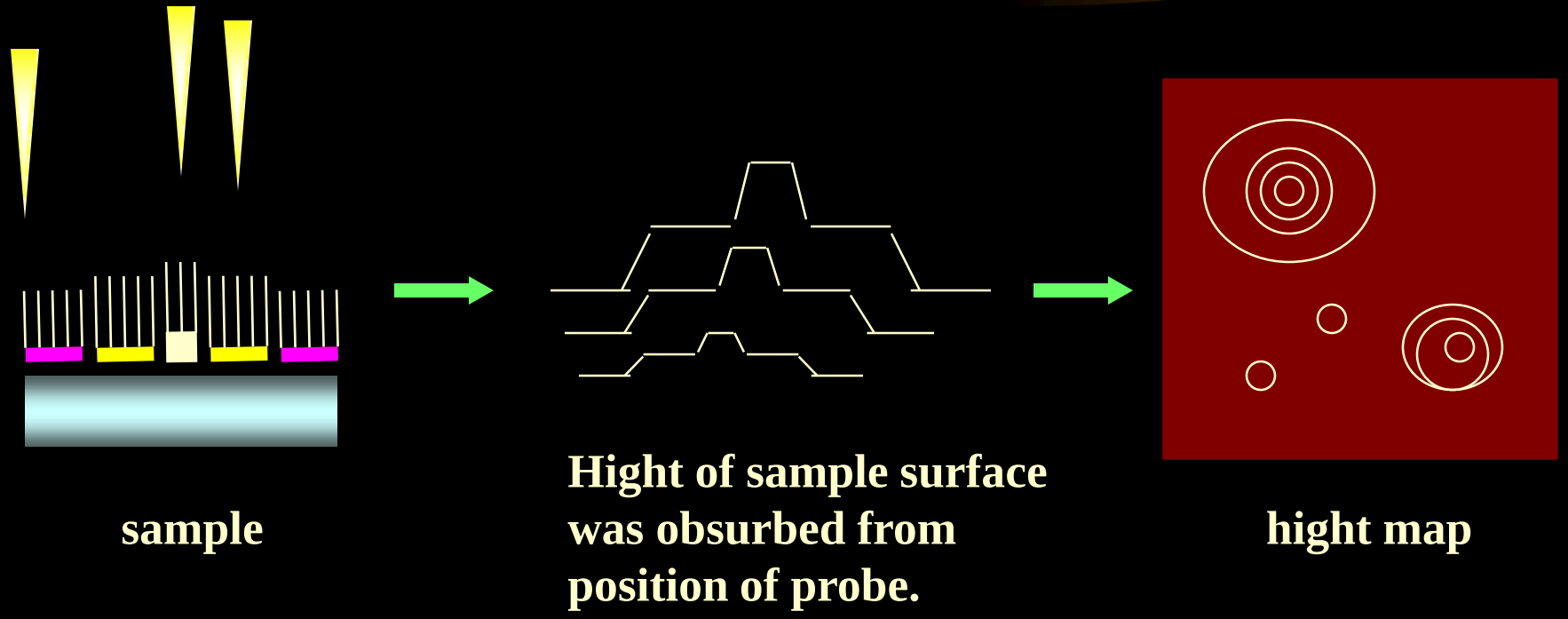
DOPC dioleoylphosphatidylcholine



DPPE dipalmitoylphosphatidylethanolamine

About atomic force microscopy (AFM)

Atomic Force between probe and sample is able to measure.
Same value of atomic force means same distance.



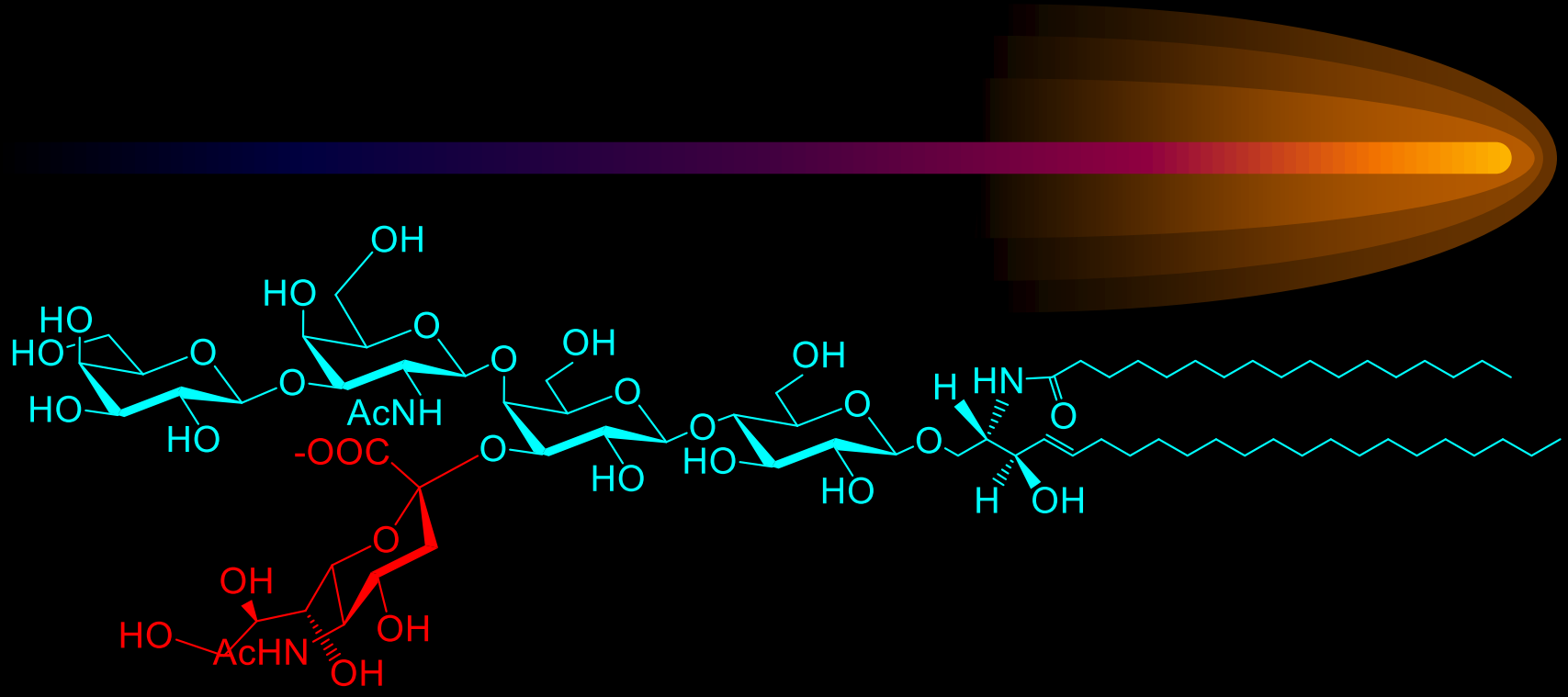
sample

Hight of sample surface was obsurbed from position of probe.

hight map

Resolusion: about 0.2 nm

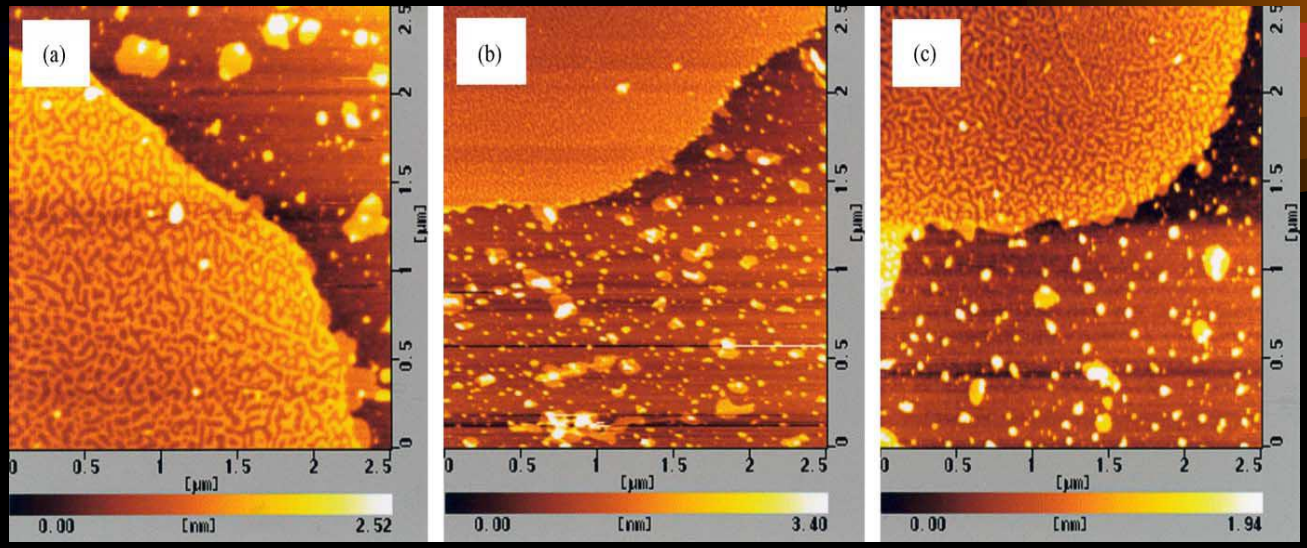
Chemical structure of ganglioside GM1



Ganglioside was composed of carbohydrate part and lipid part (Ceramide).

Ganglioside GM1 has a sialyl α2→3 galactose residue.

Membrane properties of binary and ternary systems of ganglioside GM1/DPPC/DOPC



Effect of surface pressure on the AFM images for the GM1/DPPC/DOPC (2:9:9) monolayer. Surface pressure: (a) 30 mN/m; (b) 35 mN/m; (c) 40 mN/m.

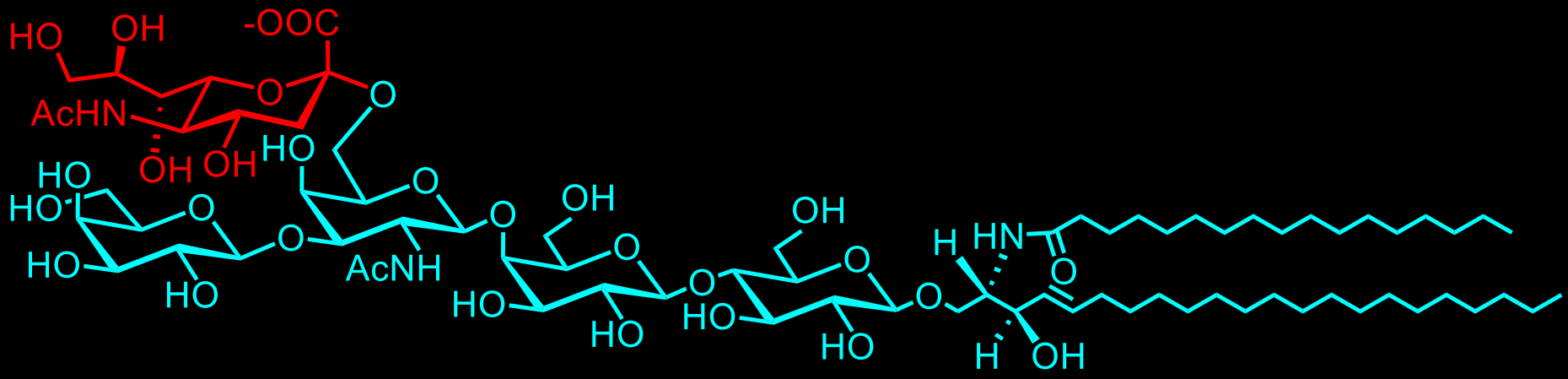
Conclusion: The percolation pattern in the GM1/DPPC/DOPC monolayer changed as the surface pressure was varied.

Yumiko Ohta, Shoko Yokoyama, Hideki Sakai, Masahiko Abe
Colloids and Surfaces B: Biointerfaces 34 (2004) 147–153.

1. Research on ganglioside GM1a raft

- GM1α is not commercially available, We must synthesize it.
- GM1α in DOPC/DPPC monolayers and hybrid bilayers are prepared.
- Distribution of GM1α is investigated by AFM.

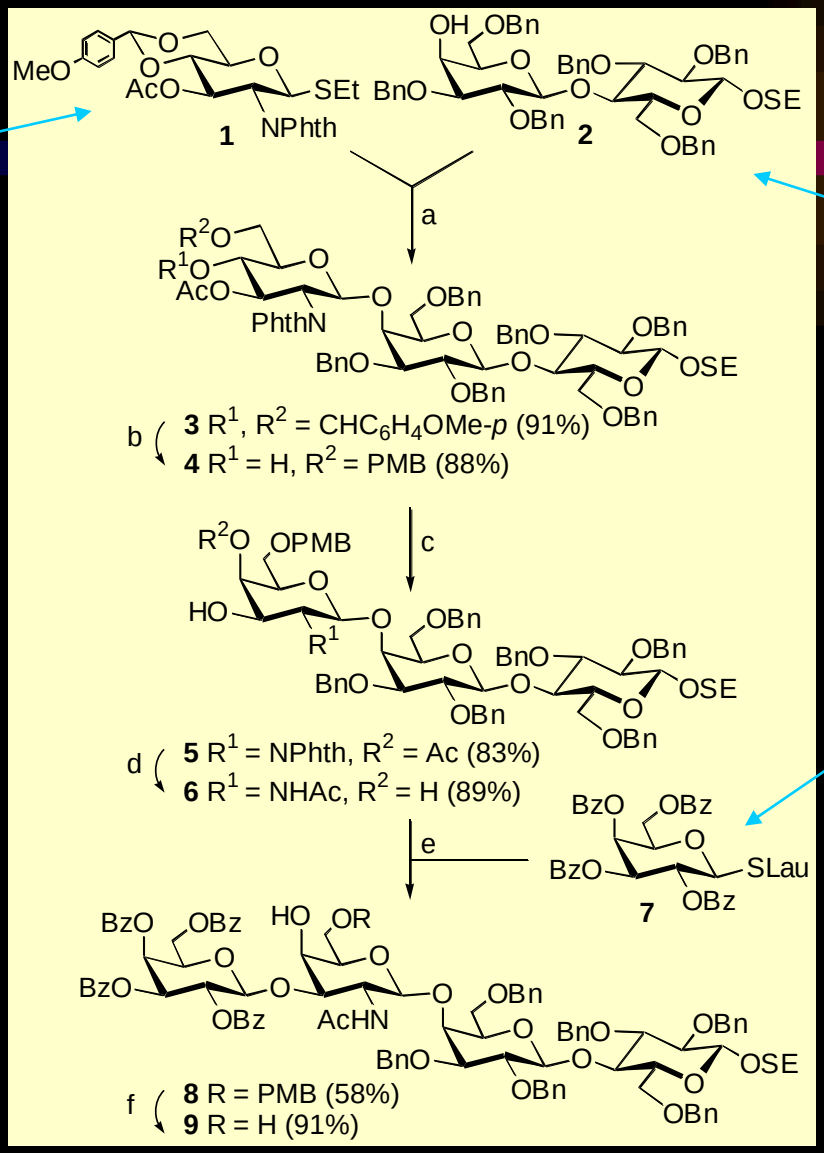
Chemical structure of GM1α



α-Series ganglioside containing a sialyl α-2→6-*N*-acetylgaractosamine residue are exclusively localized on cholinergic neurons.

Chemical synthesis of GM1α (1)

6 steps from glucosamine

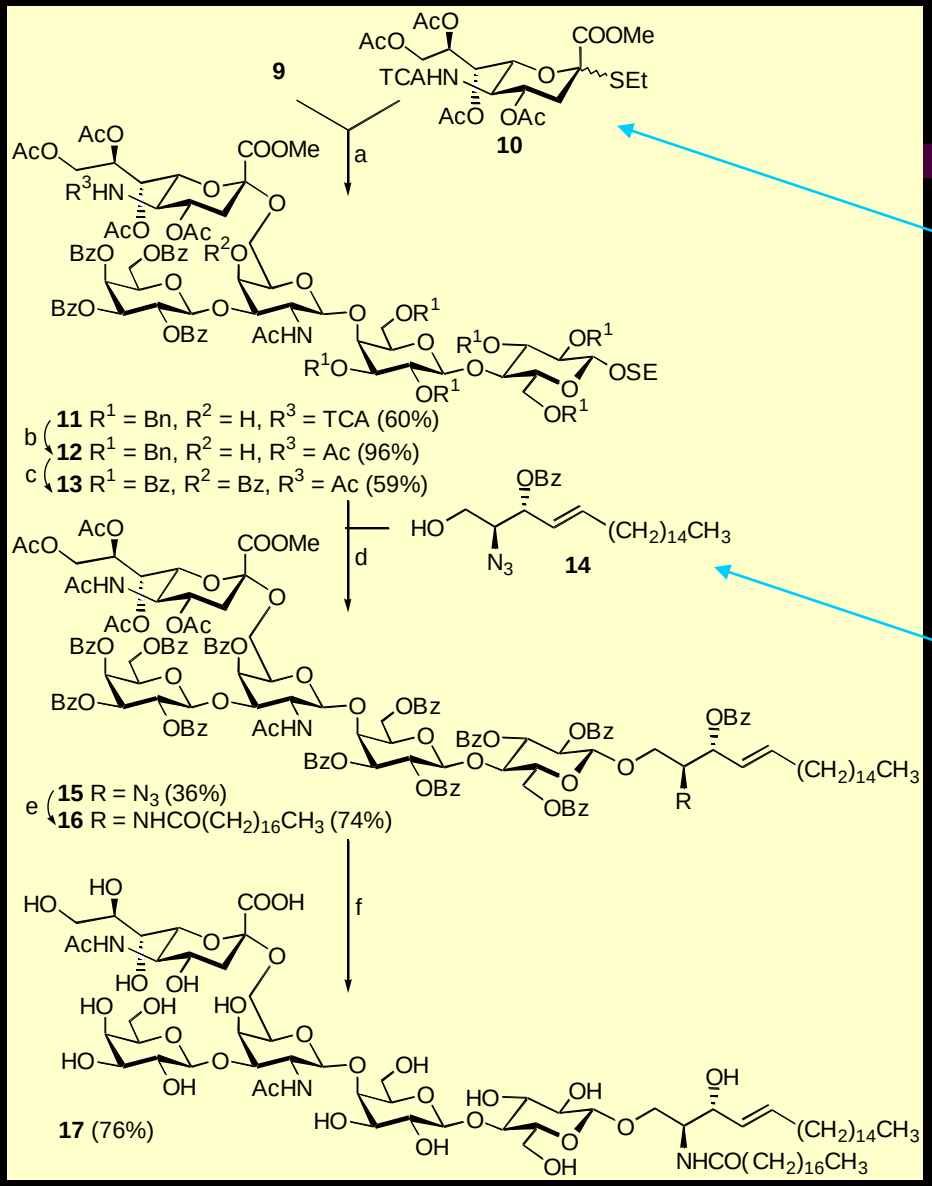


7 steps from lactose

4 steps from galactose

23 steps

Chemical synthesis of GM1α (2)

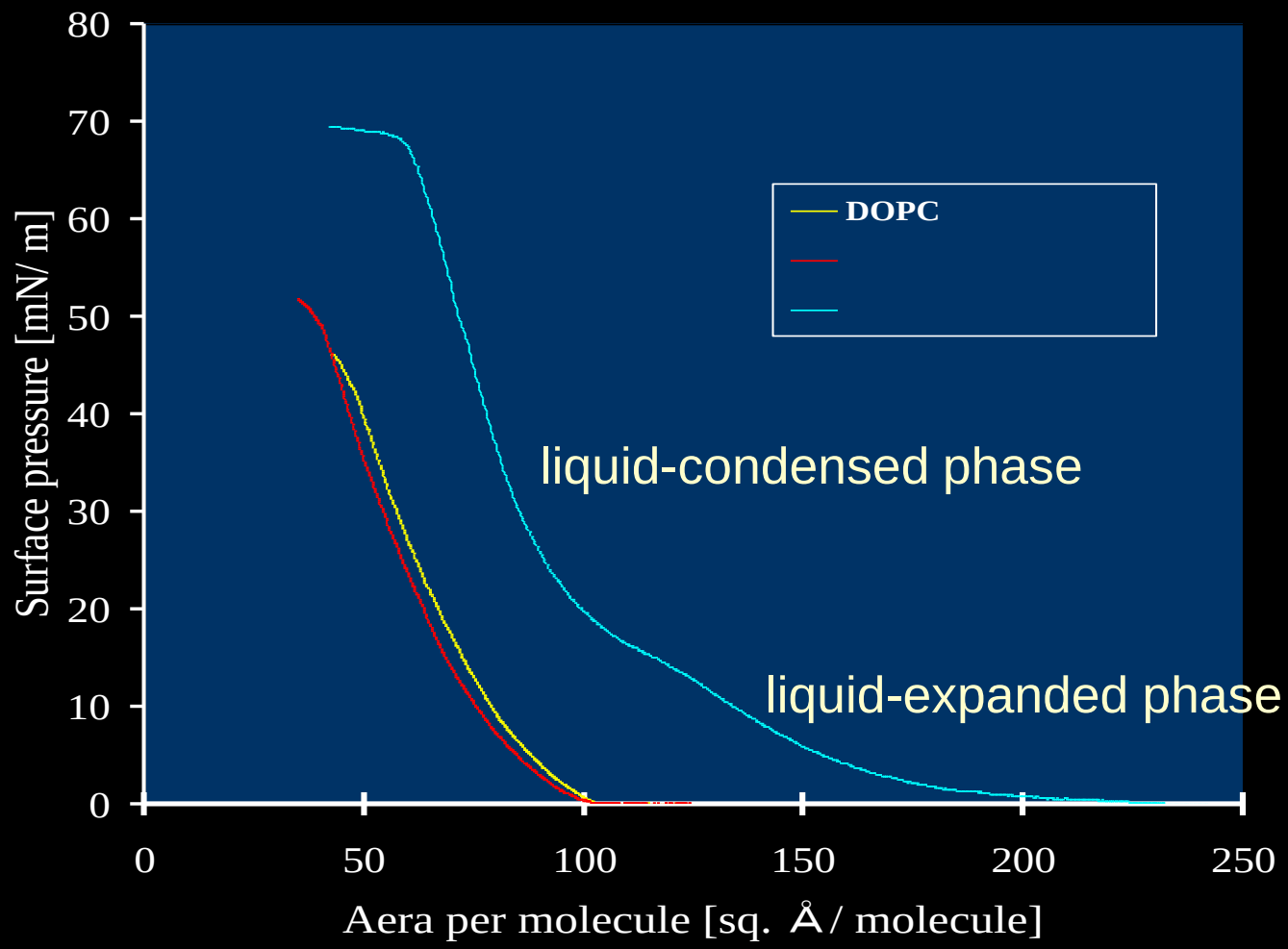


9 steps from glucosamine

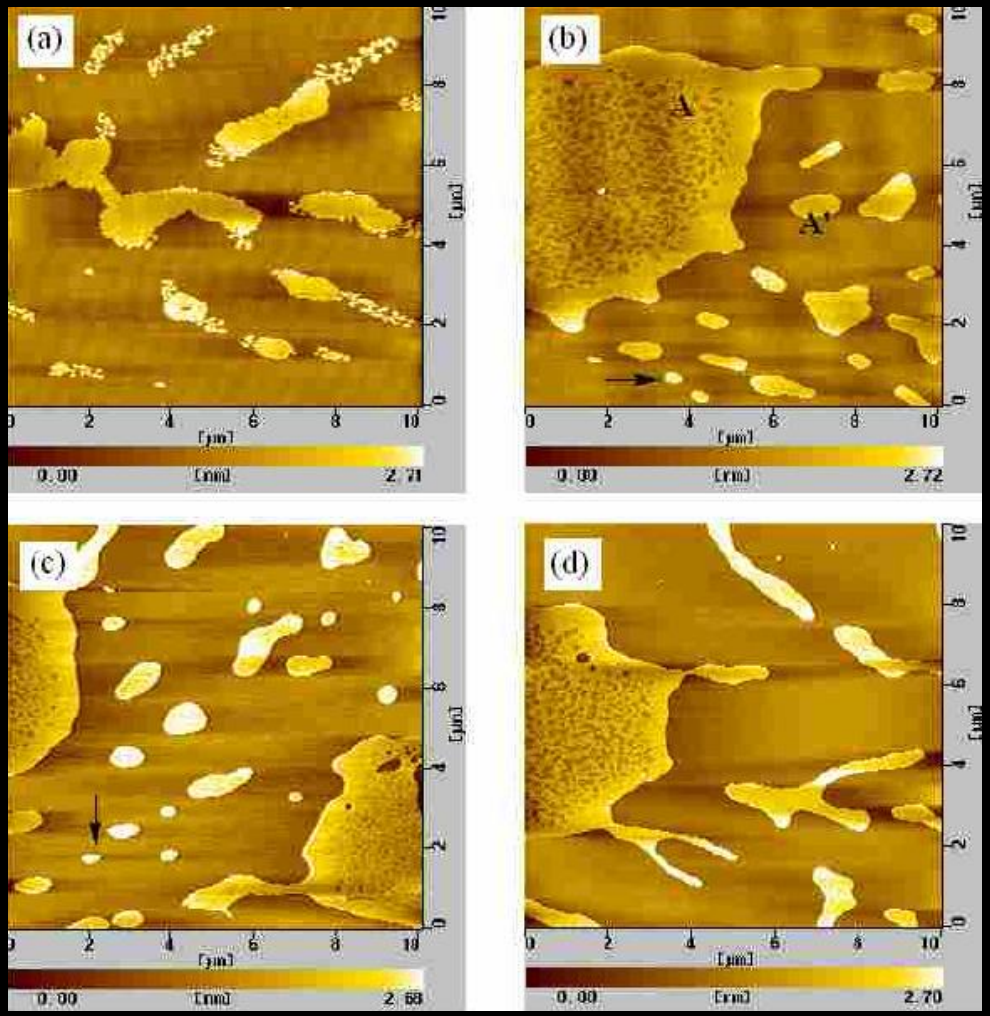
7 steps from galactose

Total steps：45
（33 steps were known in publication.）

Surface pressure vs. area per molecule isotherms for GM1a/DOPC/DPPC(1:9:9)



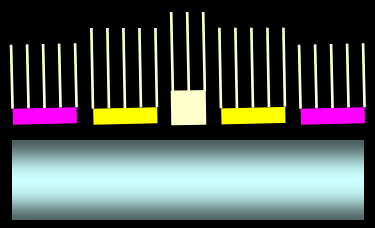
AFM images for GM1α/DOPC/DPPC (1:9:9) monolayers



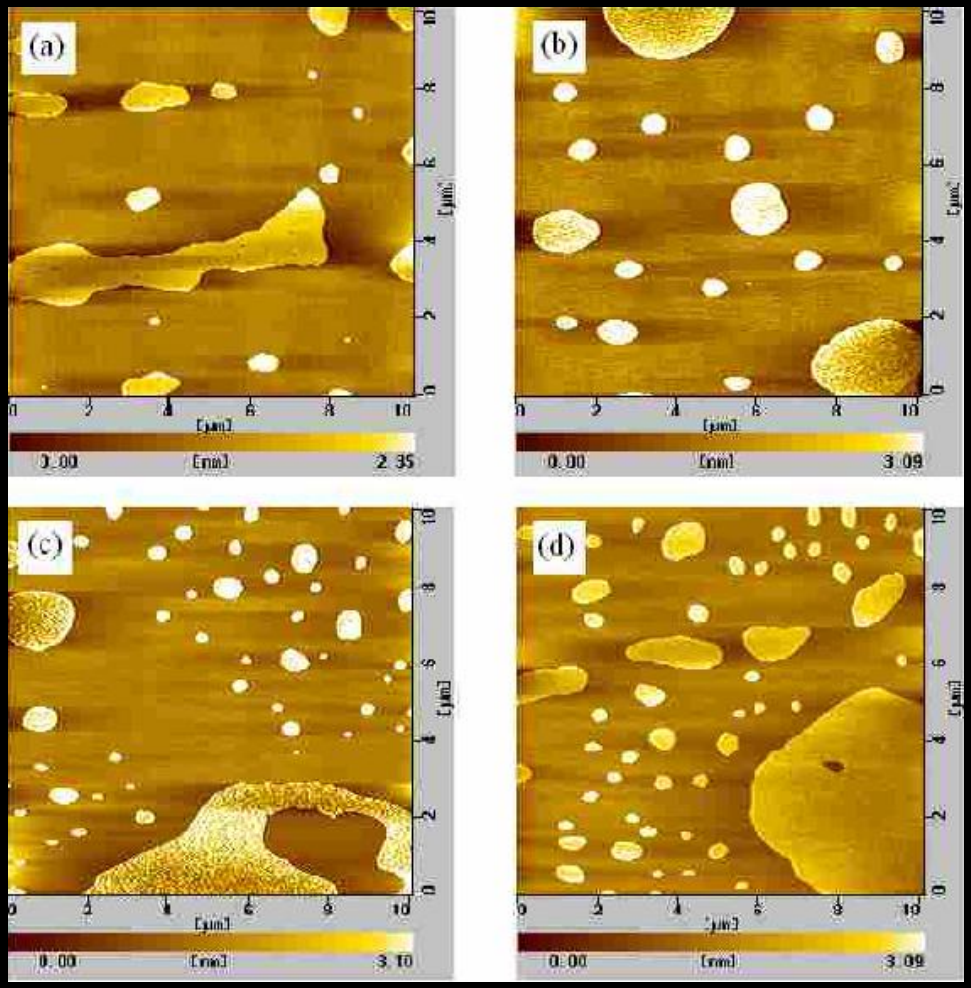
a) 15 mN/m
b) 25 mN/m
c) 30 mN/m
d) 40 mN/m

A, A': DPPC-rich domains
→: GM1α-raft

Subphase: 2.0mM NaCl

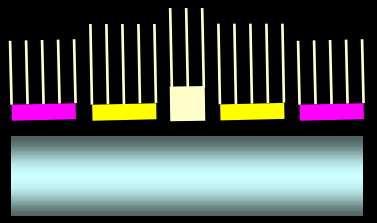


AFM images for GM1α/DOPC/DPPC (1:9:9) monolayers on physiologycal saline

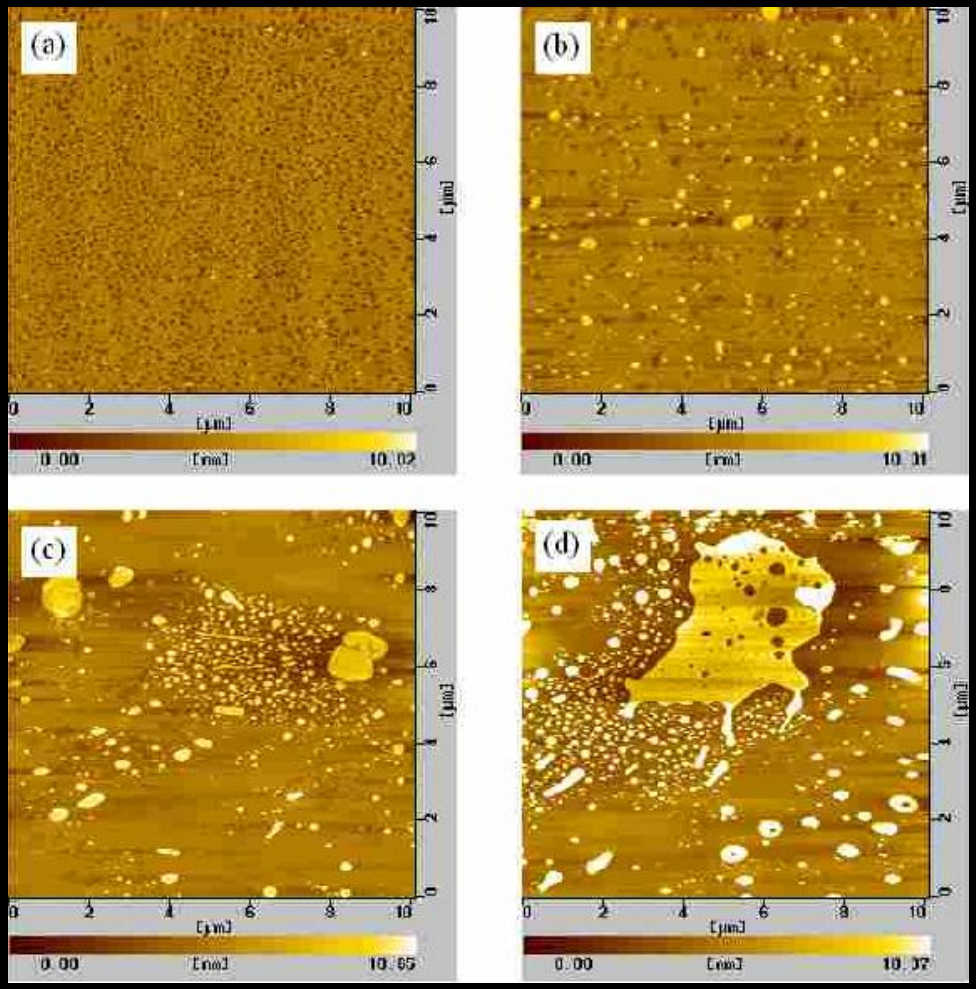


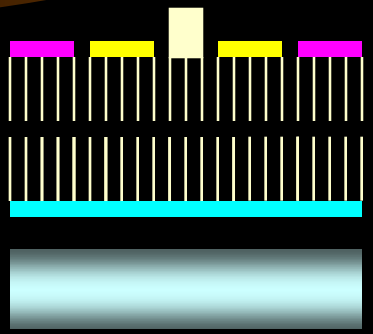
a) 15 mN/m
b) 25 mN/m
c) 30 mN/m
d) 40 mN/m

Subphase: 157 mM NaCl



AFM images for hybrid bilayers of GM1α/DOPC/DPPC (1:9:9)





a) 15 mN/m
b) 20 mN/m
c) 25 mN/m
d) 30 mN/m

First layer: DPPE
Subphase: 157 mM NaCl

Second layer:
GM1α:DOPC:DPPC=1:9:9
Subphase: 2.0 mM NaCl

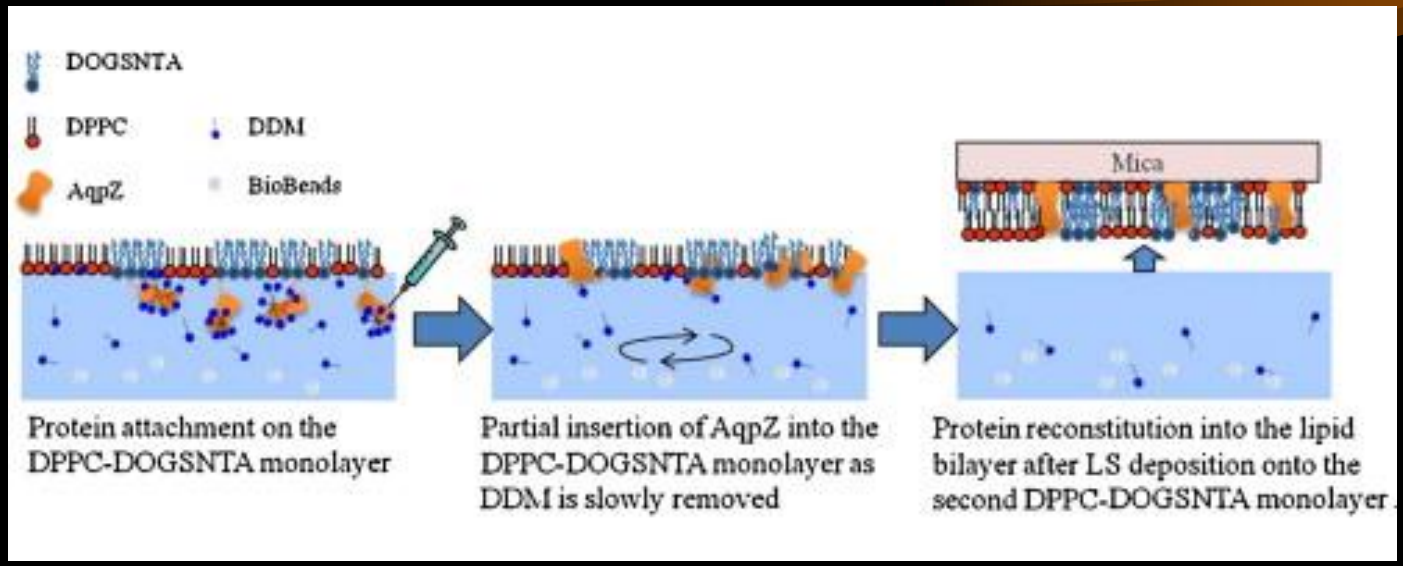
Self-assembly by surface pressure

- GM1α/DOPC/DPPC ternary monolayer and hybrid bilayer obviously shows GM1α-rafts.
- The raft was associated, separated and associated again, according to increase of surface pressure.
- The association of rafts causes enzyme-localization on signal transduction.

2. Reconstruction of transmembrane protein

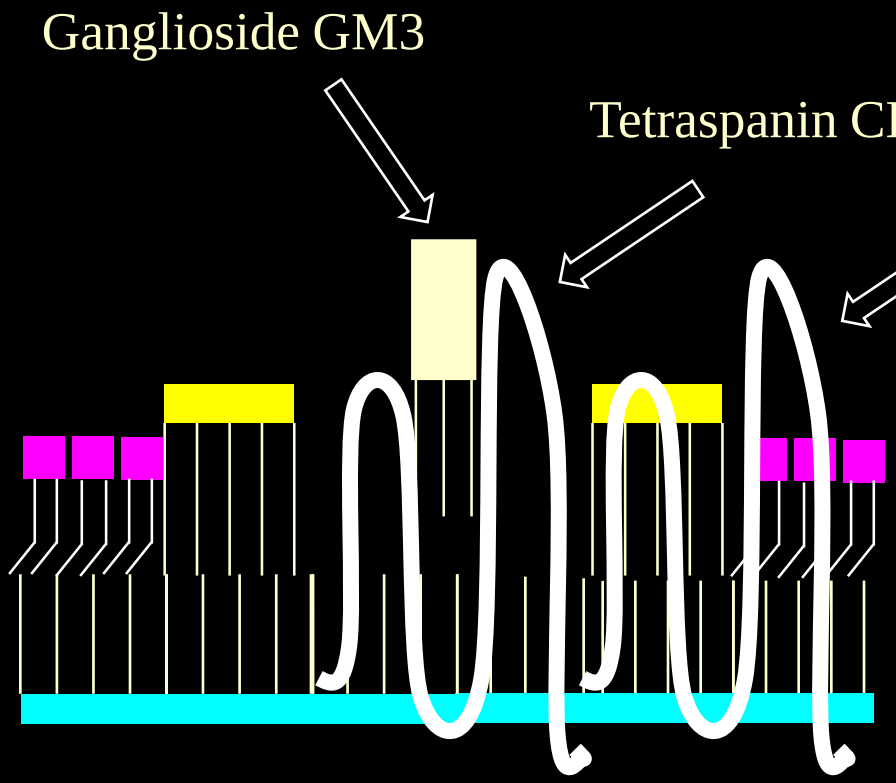
A novel method of AquaporinZ incorporation via binary-lipid Langmuir monolayers

Guofei Suna, Hu Zhoub, Yi Li a, Kandiah Jeyaseelanc, Arunmozhiarasi Armugamc, Tai-Shung Chung, Colloids and Surfaces B: Biointerfaces **89** (2012) 283– 288.



The incorporation of Histidine-tagged AquaporinZ by Nickel chelating lipids is investigated with Langmuir–Blodgett technology for the first time. Detergent removal by BioBeads in the Langmuir–Blodgett system is studied. AquaporinZ and detergent interaction during protein insertion is elaborated and a protein incorporation mechanism is proposed.

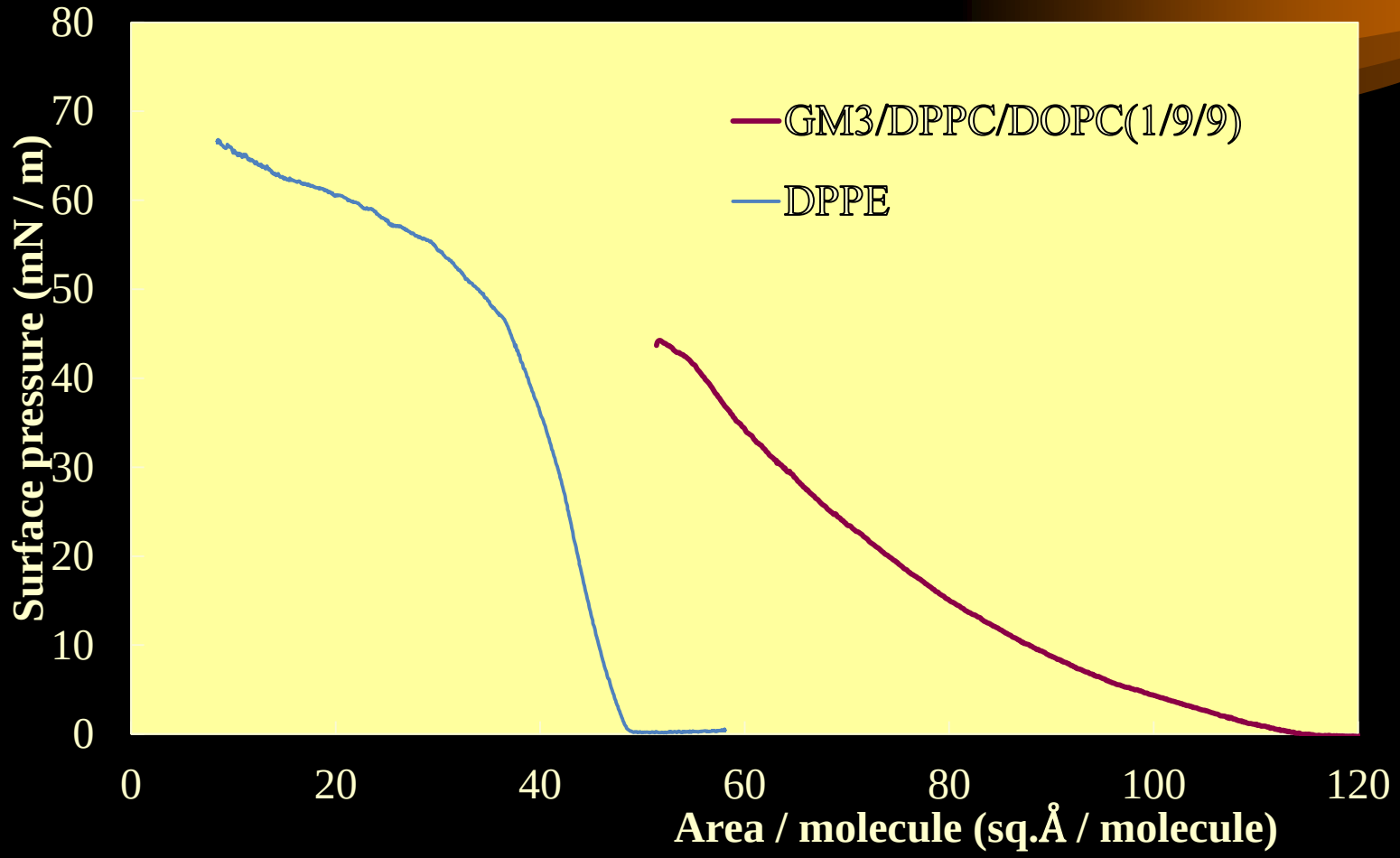
GM3/CD9/CD81 complex



The complex regulates cell growth, proliferation.

The complex regulates also cancer cell proliferation.

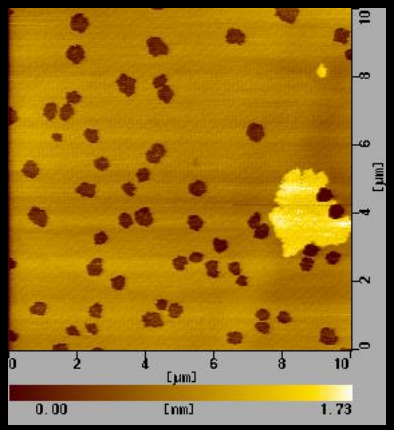
Surface pressure vs. area per molecule isotherms for GM3/DPPC/DOPC(1:9:9) ternary monolayer

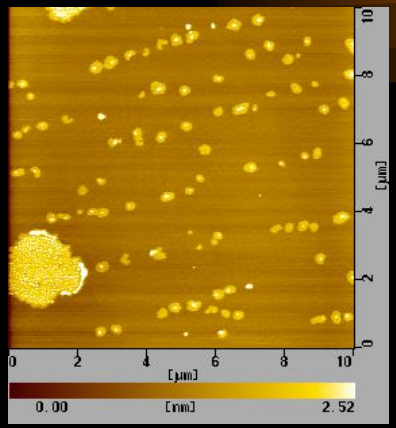


AFM images for GM3/DOPC/DPPC (1:9:9) monolayers

Surface pressure:
10 mN/m

9 images / 9 points



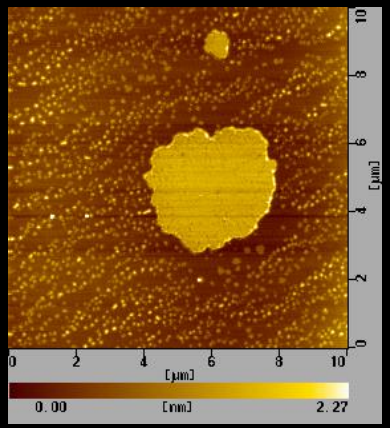


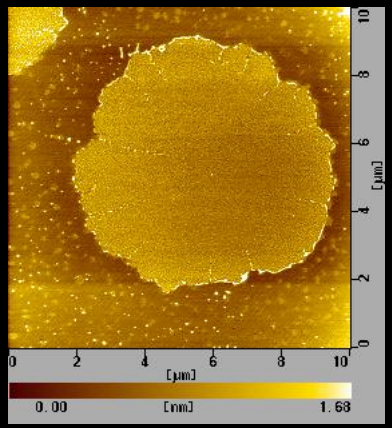
Surface pressure:
20 mN/m

9 images / 9 points

Surface pressure:
30 mN/m

8 images / 9 points

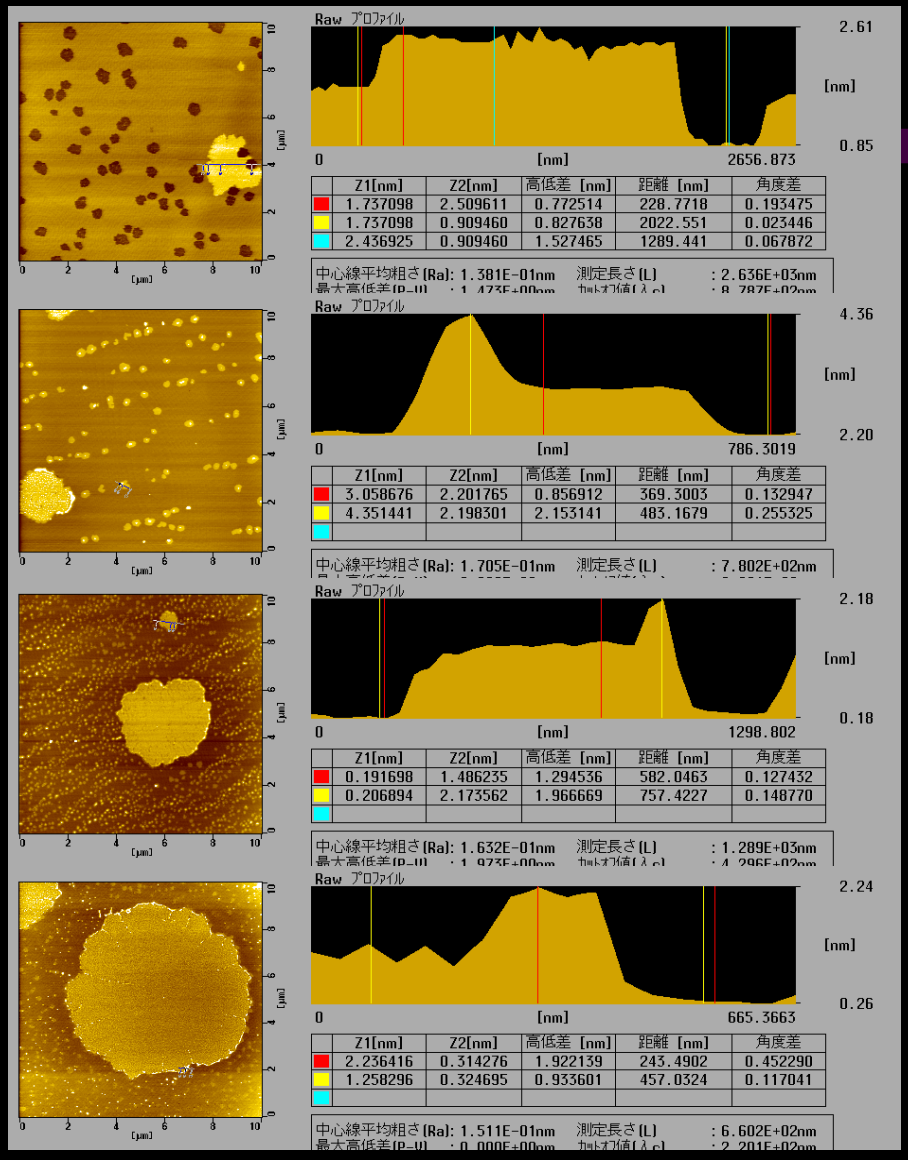




Surface pressure:
40 mN/m

9 images / 9 points

Cross-sectional study of rafts



Surface pressure: 10 mN/m

Hight: 0.9±0.1 nm

Surface pressure: 20 mN/m

Hight: 0.8±0.1 nm, 2.1±0.2 nm

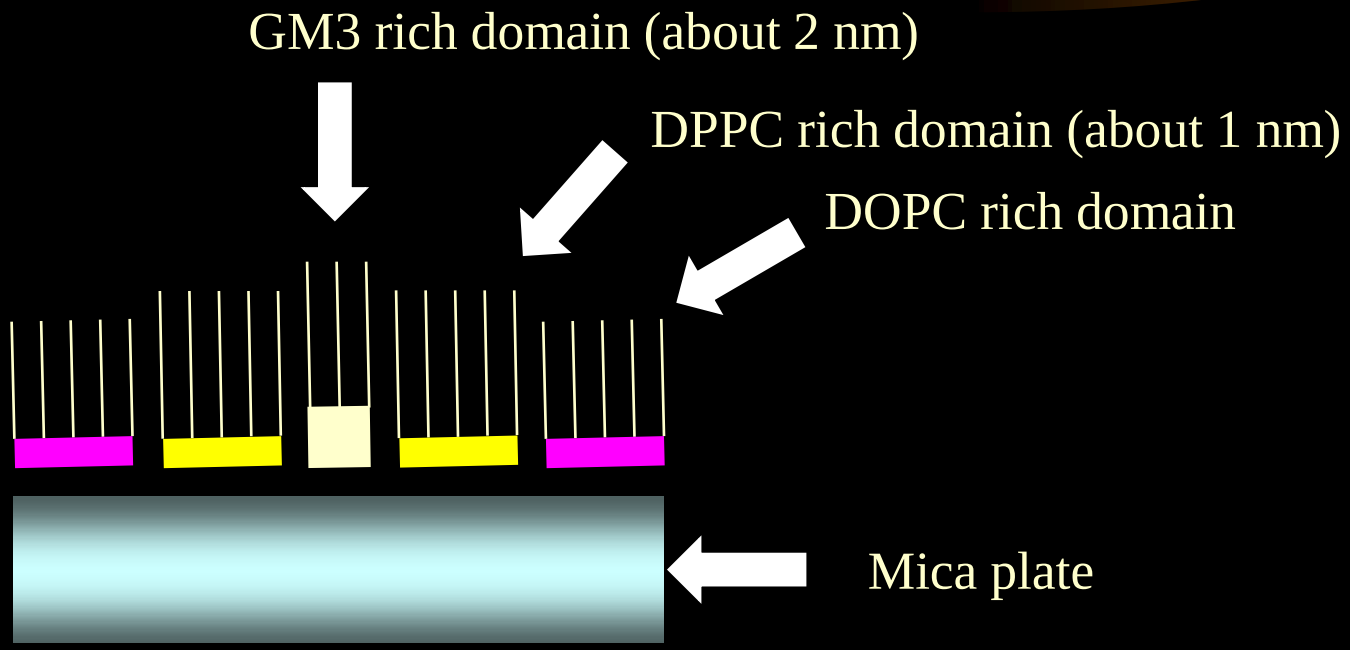
Surface pressure: 30 mN/m

Hight: 0.5±0.2 nm, 1.7±0.5 nm

Surface pressure: 40 mN/m

Hight: 0.5±0.2 nm, 1.6±0.3 nm

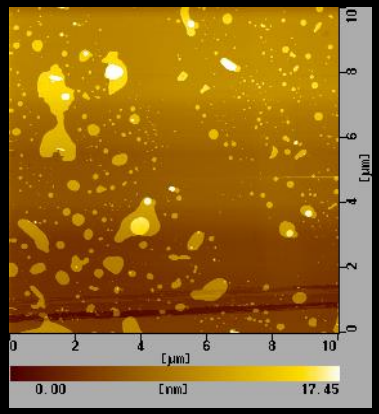
Proposed structure of ganglioside raft

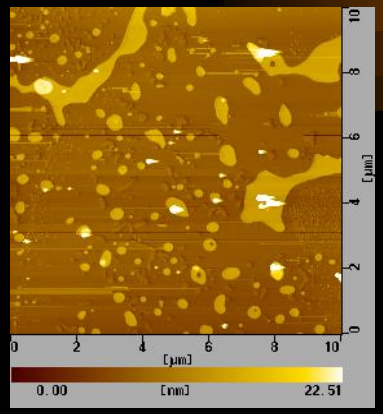


AFM images for hybrid bilayers of GM3/DOPC/DPPC (1:9:9)

Surface pressure:
10 mN/m

6 images / 9 points



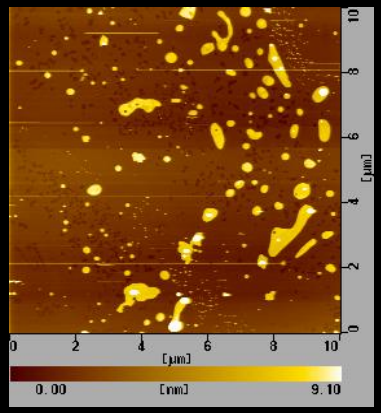


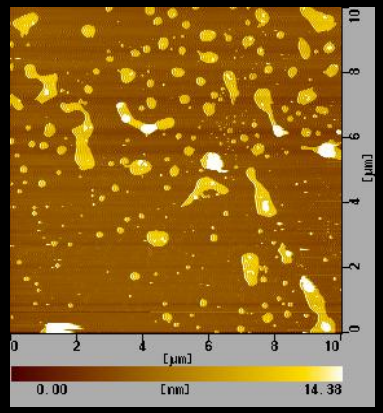
Surface pressure:
20 mN/m

7 images / 9 points

Surface pressure:
30 mN/m

6 images / 9 points

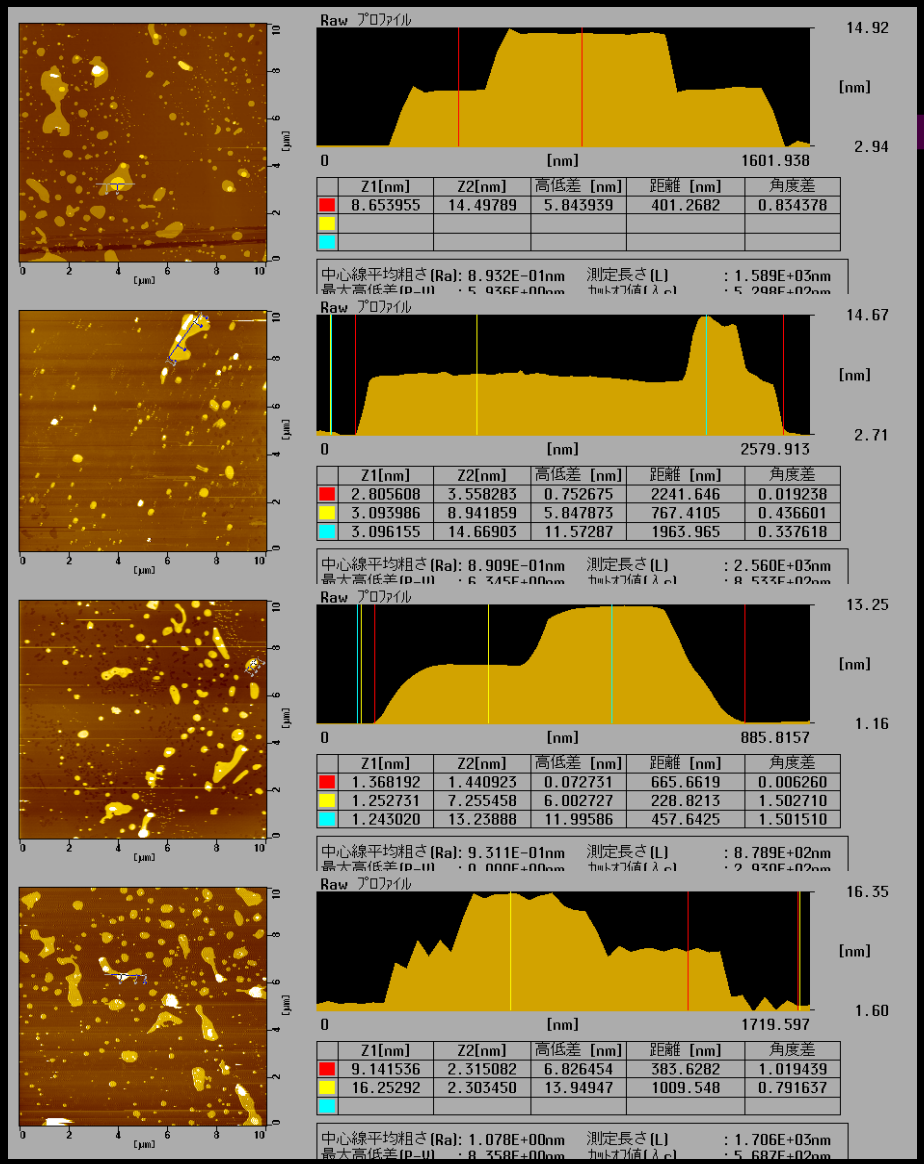




Surface pressure:
40 mN/m

5 images / 9 points

Cross-sectional study of rafts



Surface pressure: 10 mN/m

Hight: 6.3±0.7 nm, 11.5±0.4 nm

Surface pressure: 20 mN/m

Hight: 6.7±0.6 nm, 12.5±0.2 nm

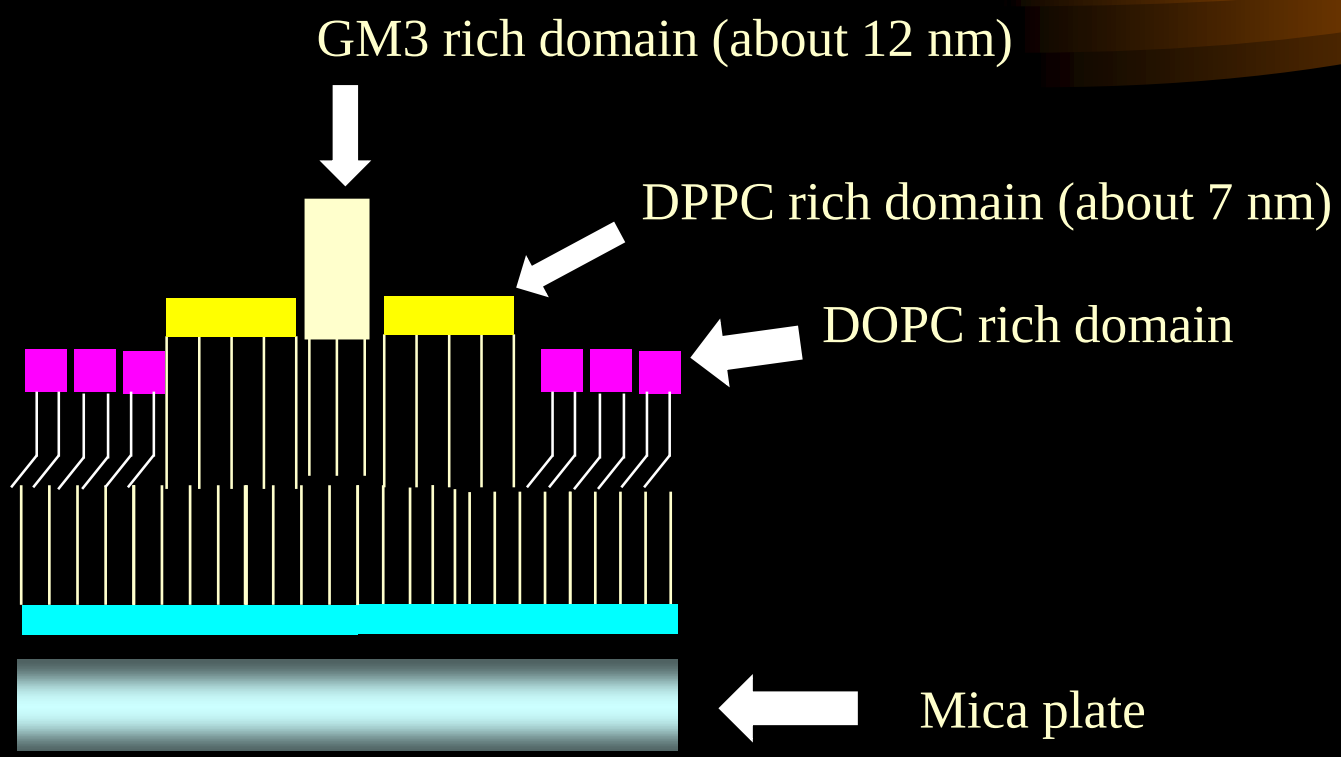
Surface pressure: 30 mN/m

Hight: 6.2±0.4 nm, 11.7±0.5 nm

Surface pressure: 40 mN/m

Hight: 6.5±0.5 nm, 11.2±0.3 nm

Proposed structure of ganglioside raft

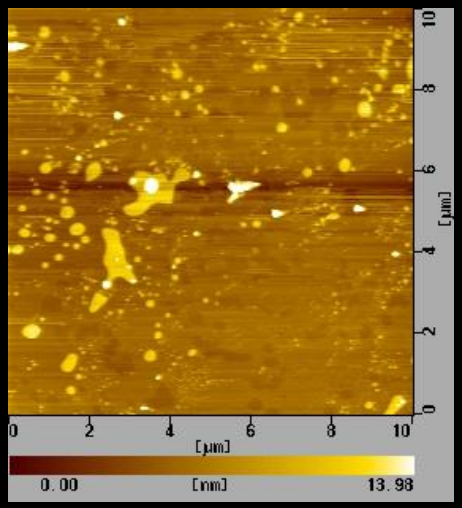


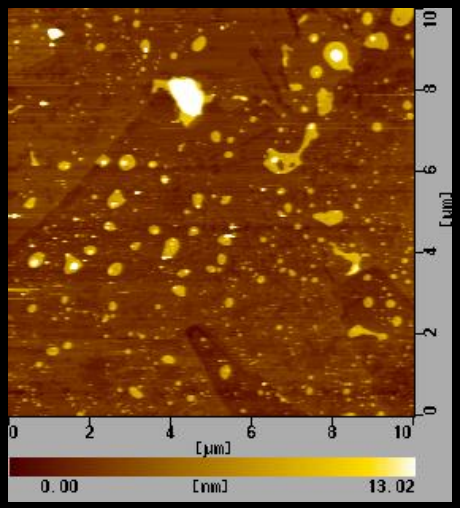
Reconstruction of tetraspanin (CD9) into lipid bilayer

The hybrid bilayer was kept in CD9 solution (2 μg/ml) for 5 min.

Surface pressure:
30 mN/m

5 images / 9 points were changed.



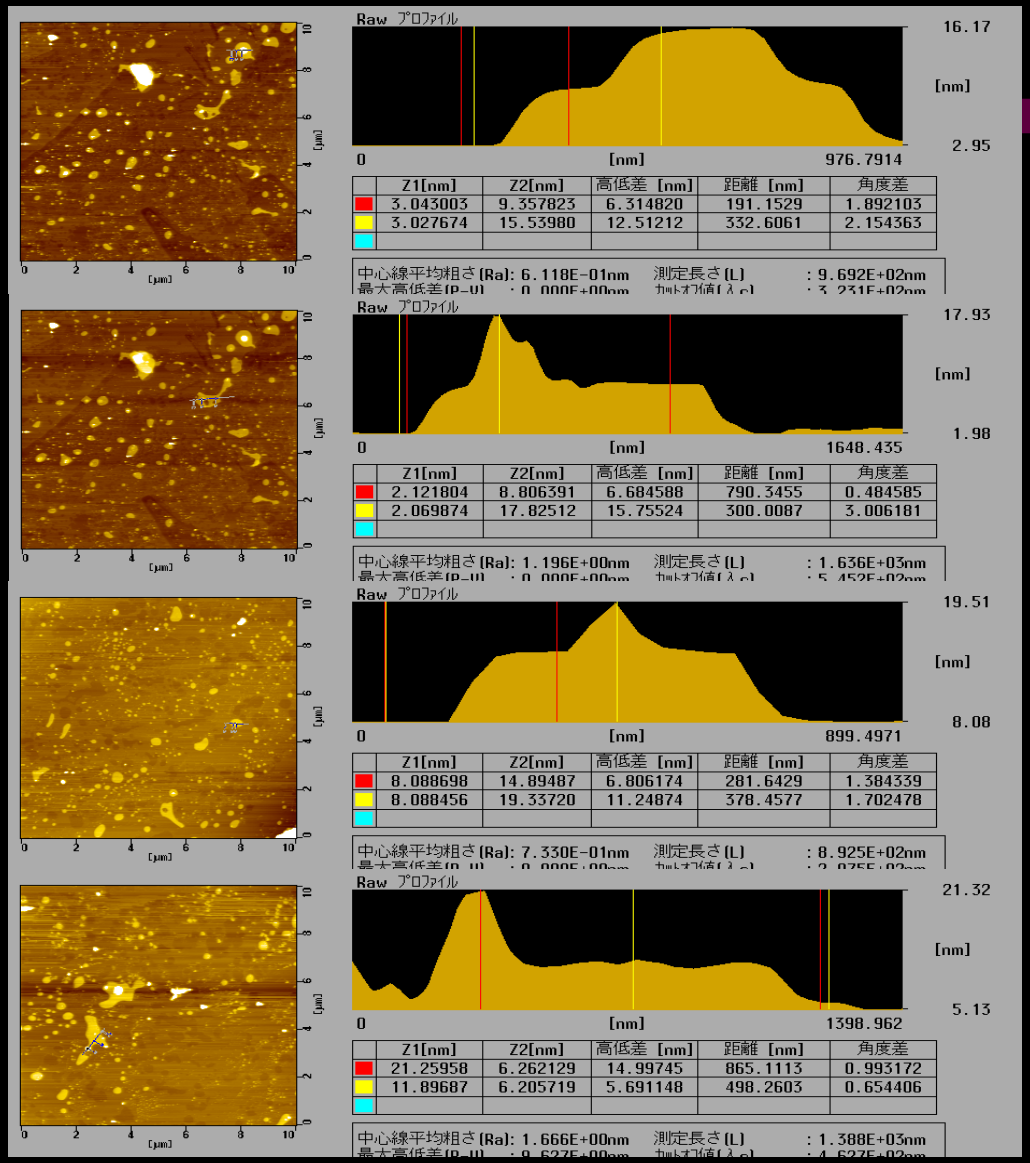


Surface pressure:
40 mN/m

2 images / 9 points were no change.

7 images / 9 points were changed.

Cross-sectional study of rafts reconstructed by tetraspanin



Surface pressure: 30 mN/m

Hight: 6.1±0.5 nm, 11.7±1.1 nm

Surface pressure: 30 mN/m

Hight: 6.1±0.5 nm, 16.4±0.9 nm

Surface pressure: 40 mN/m, 2 / 9

Hight: 6.4±0.5 nm, 11.3±0.8 nm

Surface pressure: 40 mN/m, 7 / 9

Hight: 6.4±0.5 nm, 15.6±1.7 nm

Proposed structure of ganglioside raft with tetraspanin