Composite membranes of polymeric and fluid lipid bilayers

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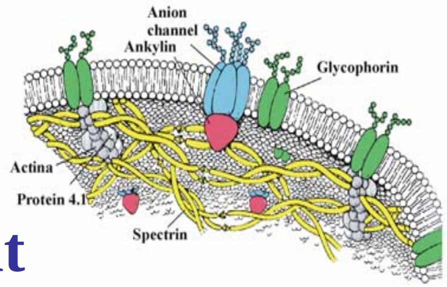
Membrane functions & biomedical applications

Biomedical applications

- Drug development
- Diagnostics
- Biosensors



Membrane functions

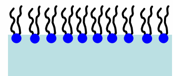
- Signal transduction
- Energy conversion
- Immune system
- Cell-cell recognition

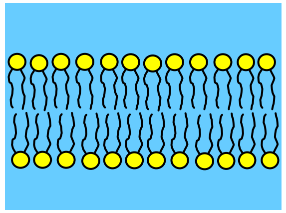
Measuring and manipulating the functions of cellular membranes at the molecular level

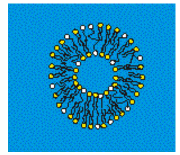
Synthetic model membranes

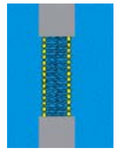
Roles of the model membrane systems

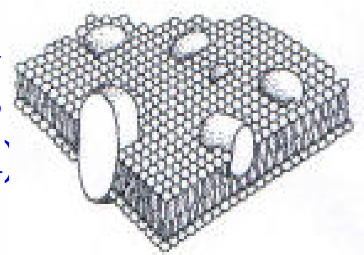
	(biological membrane)	(model membrane)
ca. 1900	lipidic	Langmuir monolayer
1925	bilayer structure	
1960s	lateral mobility asymmetry permeability	lipid vesicles (liposomes) planar bilayer (BLM)
1972	integral/ peripheral membrane proteins (fluid mosaic model)	planar bilayer (substrate supported)
From 1980s	more complex architecture (microdomains, caveolae, membrane skeleton, etc.)	giant vesicles

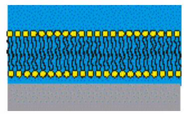


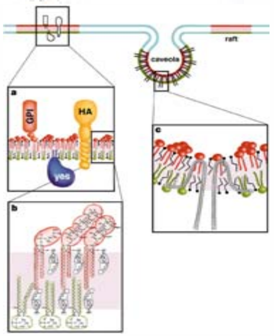








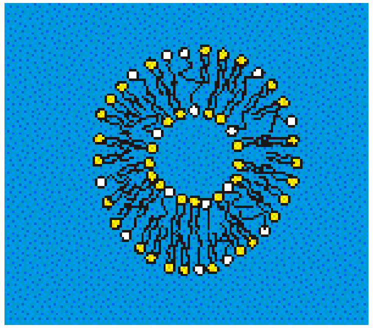




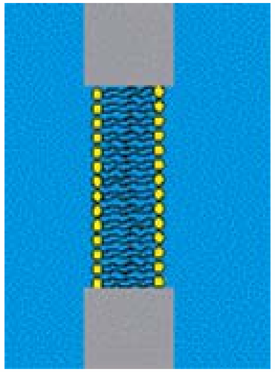
More complex structures and functions ⇄ **New model membranes**

Model systems of the biological membrane

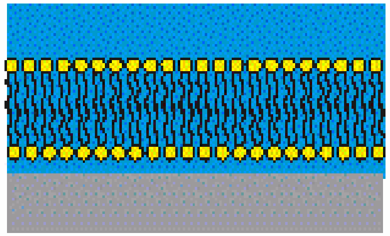
lipid vesicles (liposomes)



planar lipid bilayer (black lipid membrane: BLM)

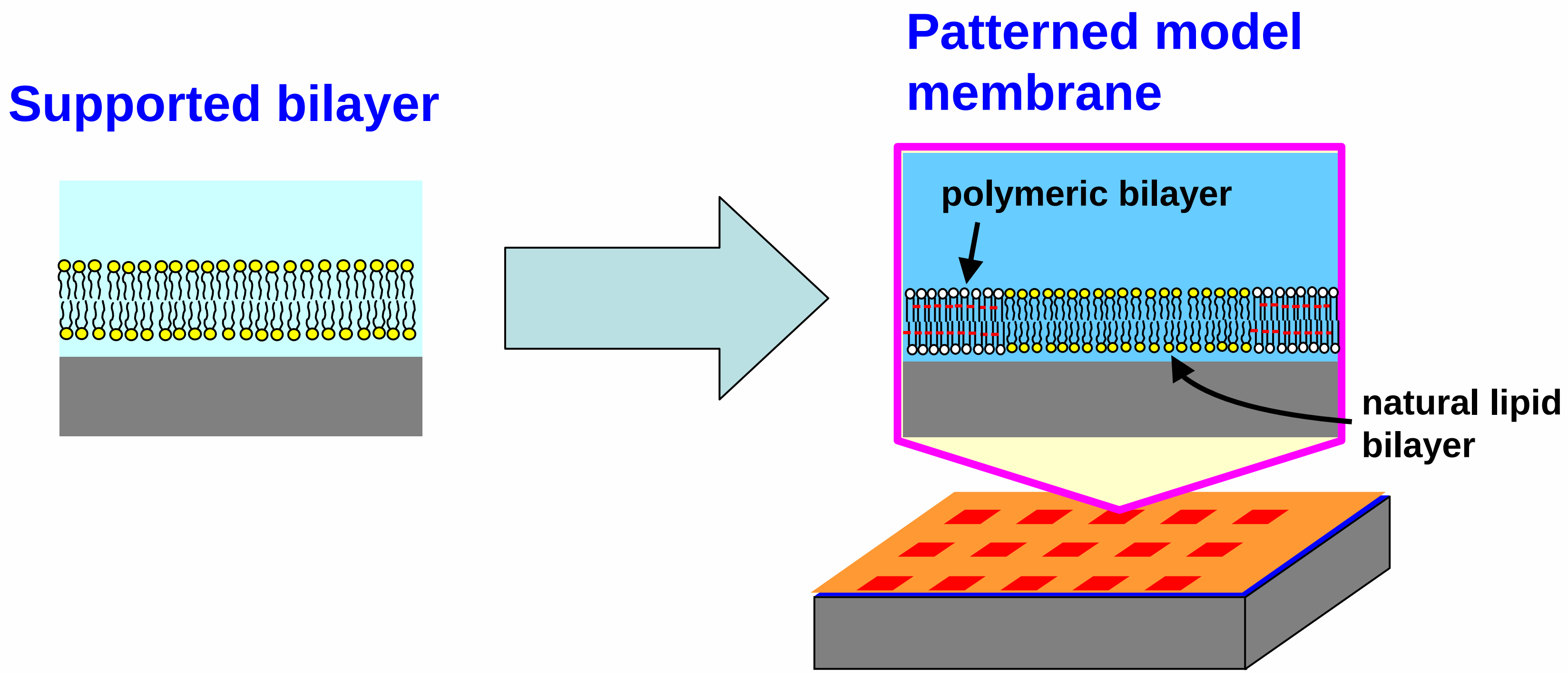


substrate supported lipid bilayer



1. Mechanical stabilization
2. Surface specific analytical techniques
3. Integration by micro-fabrication techniques

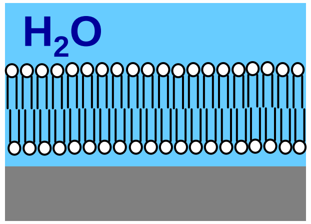
Generation of complex model membranes



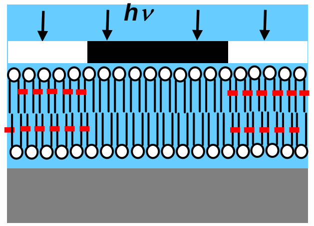
Micro-fabrication & Self-assembly

Fabrication of patterned lipid bilayers

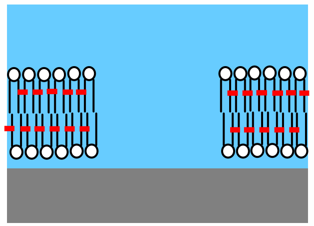
(1) Monomer lipid bilayer (LB/ LS method)



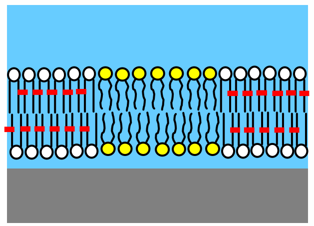
(2) Photopolymerization with a mask



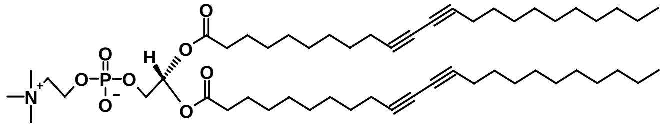
(3) Removal of monomer lipids



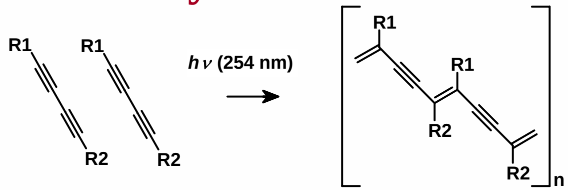
(4) Incorporation of new lipid bilayers



Polymerizable lipid

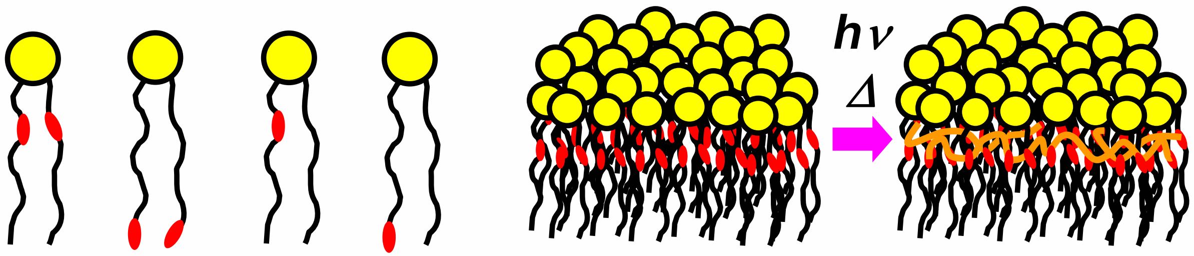


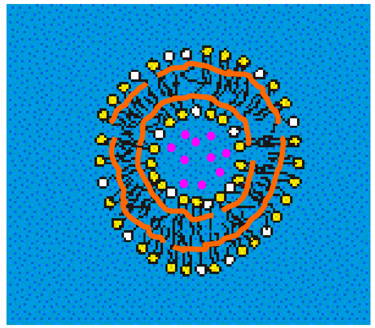
Polymerization

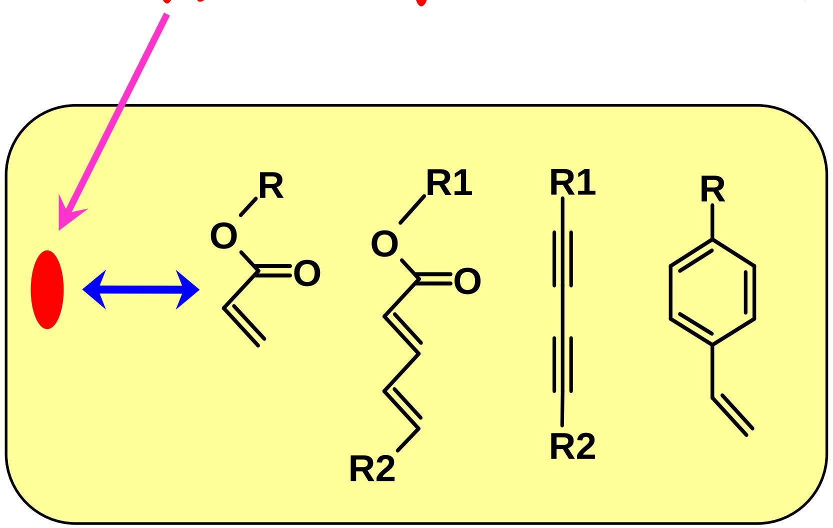


Polymerization of lipid bilayers

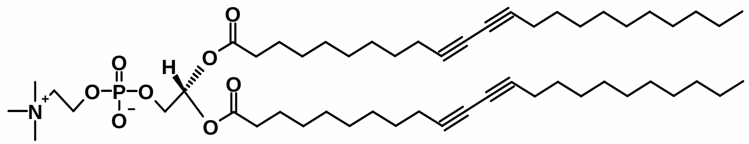
Synthetic polymerizable lipids: Originally developed for the stabilization of liposomes for drug delivery applications (Ringsdorf, O'Brien, Chapman, Regen, Tsuchida..)

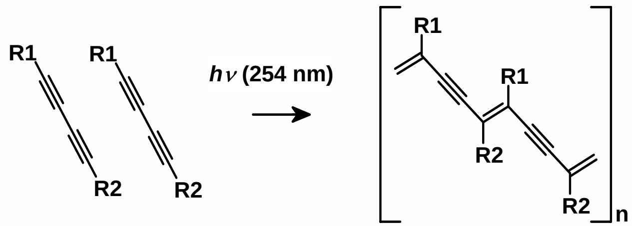






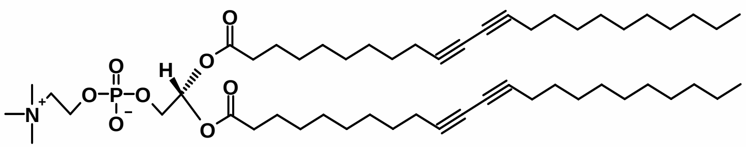
Diacetylene lipid (DiynePC)

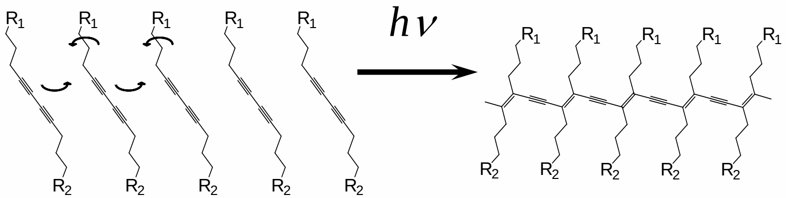




Polymerization in lipid bilayers

Diacetylene lipid (DiynePC)

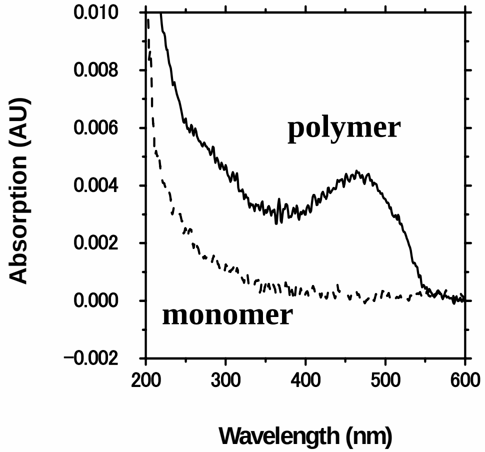




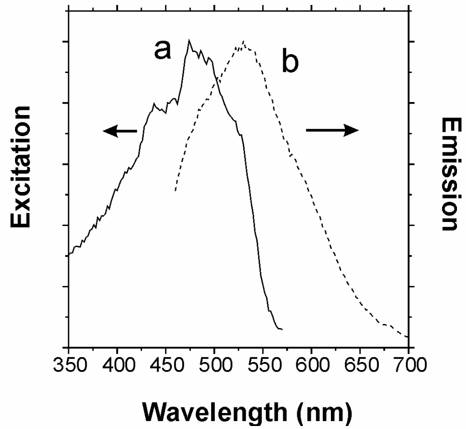
Unique features:

1. **Topochemical polymerization**
2. **Conjugated polymer backbone**

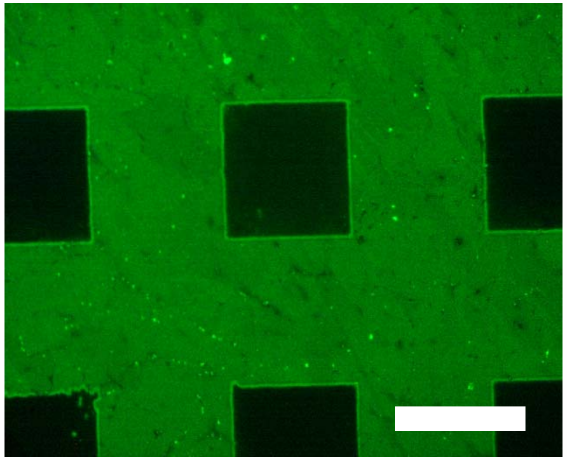
UV/visible absorption spectra

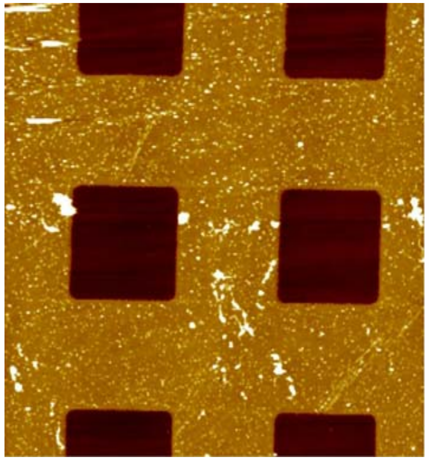


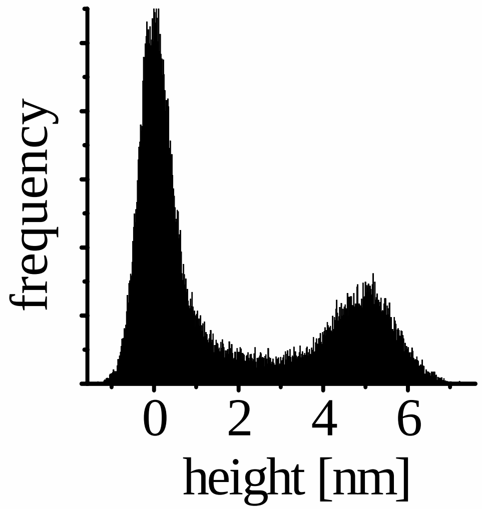
Fluorescence spectra



Patterned polymerization of DiynePC bilayers





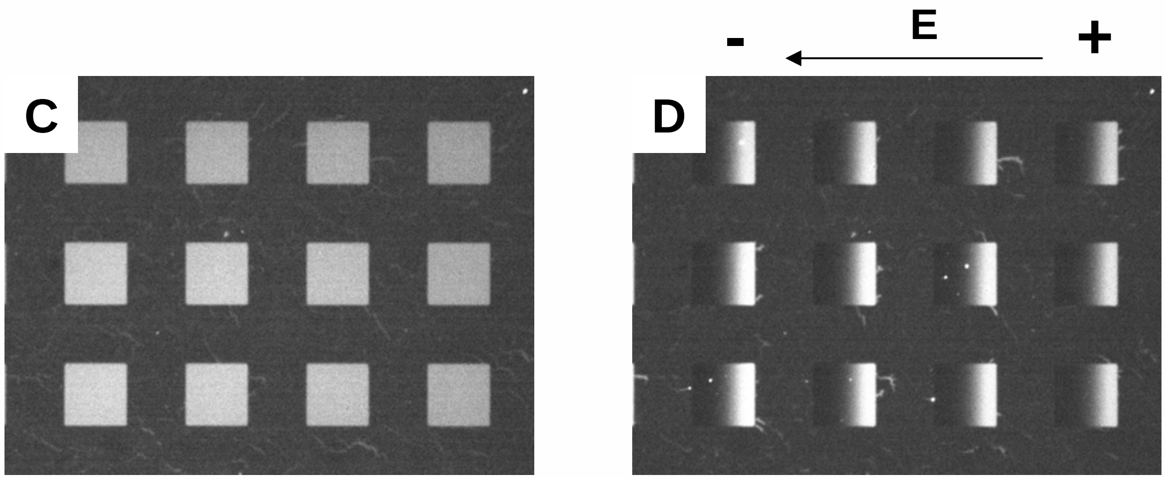


A polymerized Diyne-PC bilayer after lithographic UV light exposure and removal of monomers. The scale bar corresponds to 50 μm.

AFM observation of a lithgraphically polymerized DiynePC bilayer. The polymeric bilayer consist small domains. The size of corrals is 20 μm.

Okazaki *et al.* *Langmuir* **25**, 345 (2009)

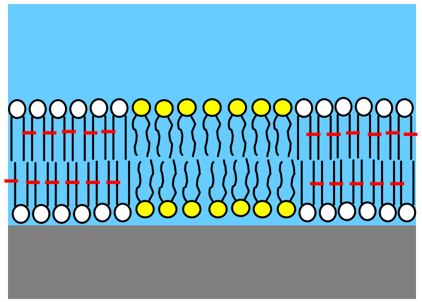
Fluid bilayers in polymeric bilayer corrals



Fluid lipid bilayers (egg-PC/ TR-PE) can be incorporated into the corrals, and application of an electric field induces concentration gradients. The size of corrals is 50 μm.

Roles of polymeric bilayer matrices

Unique feature

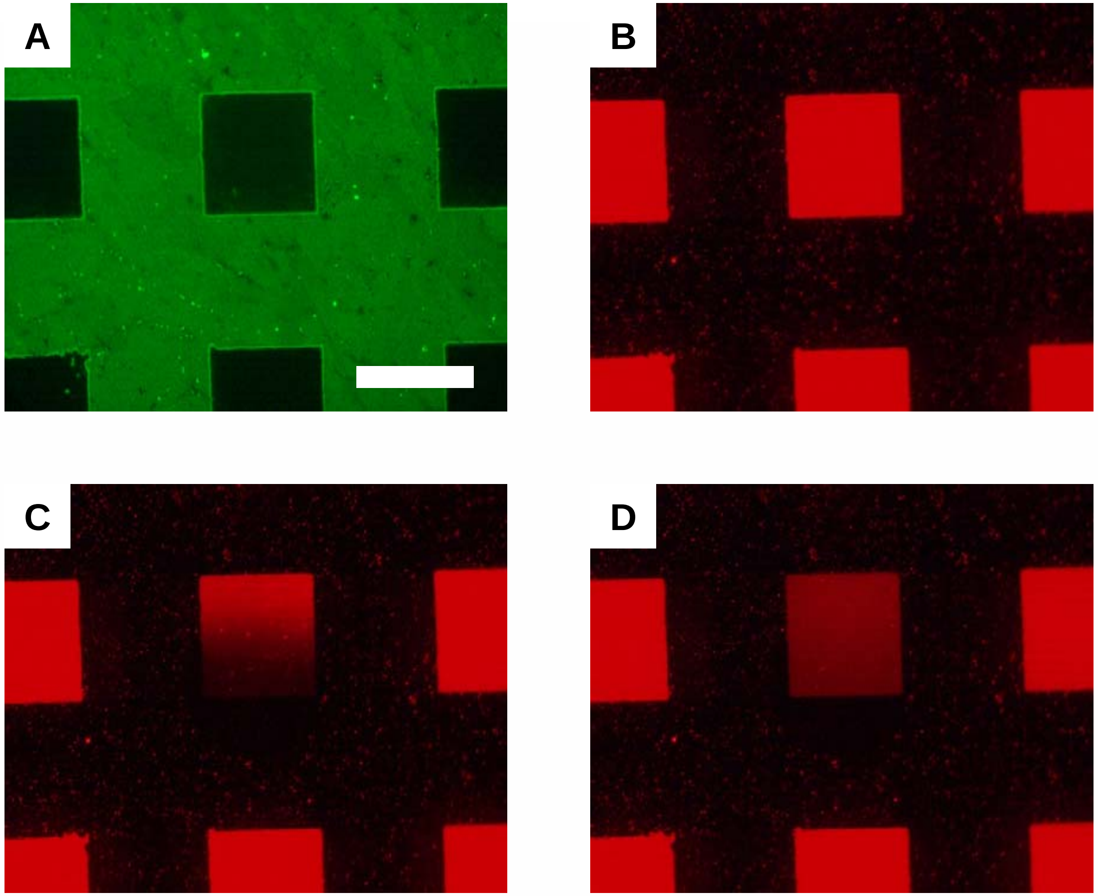


Polymeric and fluid bilayers are forming a continuous hybrid membrane

Roles of polymeric bilayers

- **Controlling the lateral distribution of molecules**
- **Stabilization of the model membrane**

Confinement of fluid bilayers in the corrals

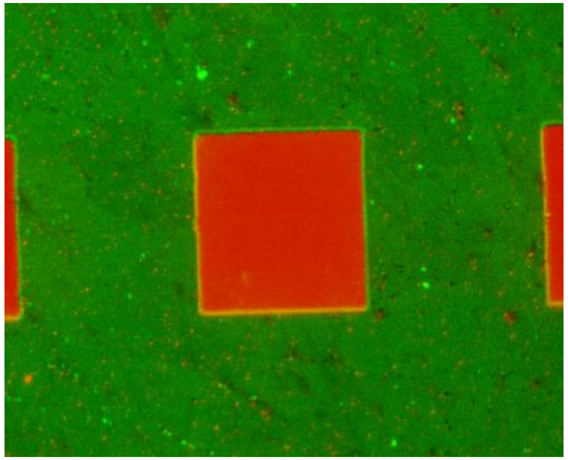


4 min after the photobleach

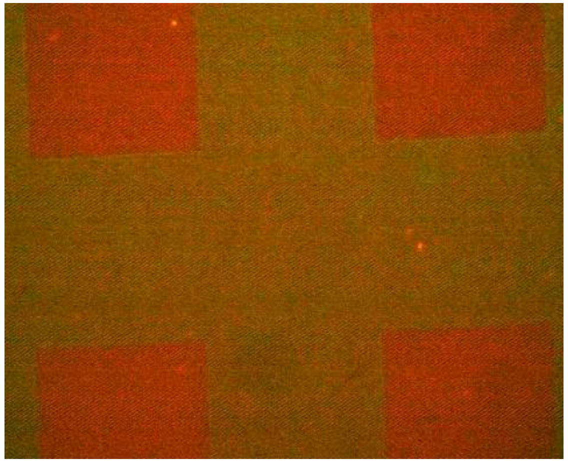
The bleached corral became homogeneously dark, indicating that lipid molecules are diffusing laterally within the corral. The scale bar corresponds to 50 μm.

Controlling the composition of bilayers

Large UV dose



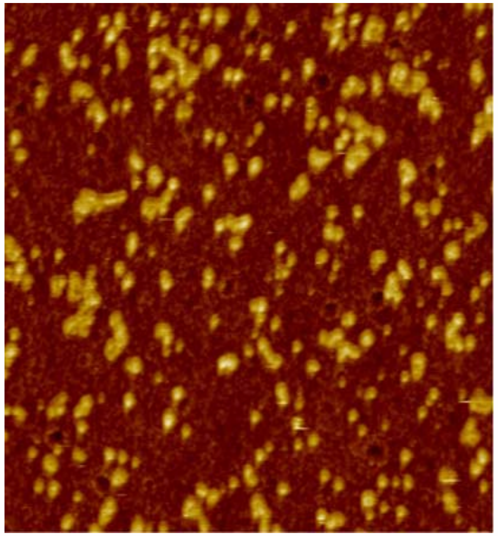
Small UV dose



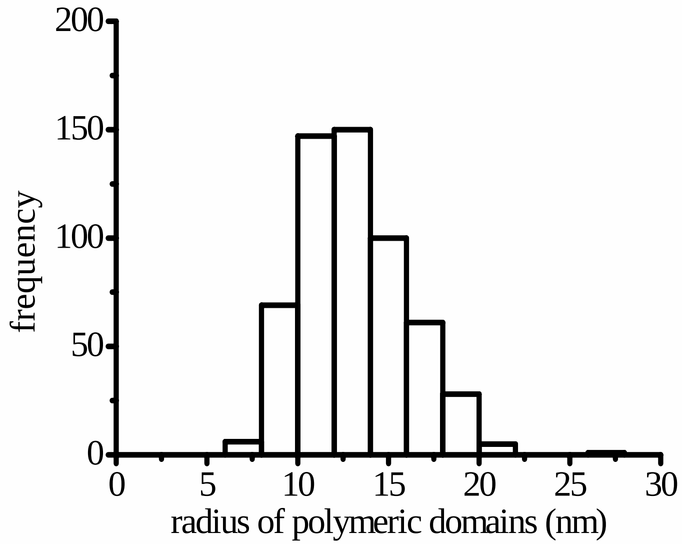
Polymeric and fluid bilayers can be integrated as sub-micrometer domains by modulating the degree of polymerization.

JP-Patent 4,150,793 (2008)
Morigaki *et al. Langmuir* **20**, 7729 (2004)

Size distribution of polymeric bilayer domains



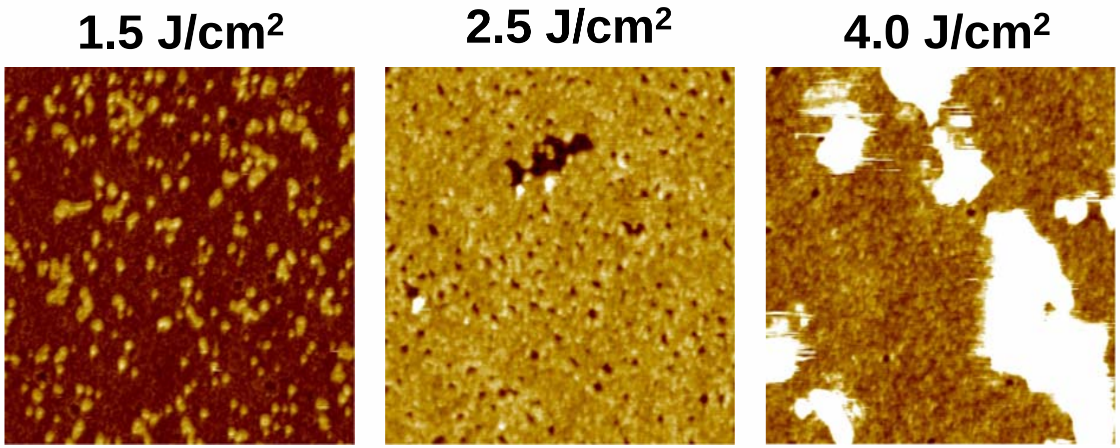
1 x 1 μm



The size distribution of the polymeric bilayer domains observed after the SDS treatment. The radius of domains was plotted in a histogram, assuming a circular shape. The influence of finite AFM probe curvatures is not corrected.

Okazaki *et al. Langmuir* **25**, 345 (2009)

Polymeric bilayers with different UV doses



AFM images (1 x 1μm^2) of polymeric bilayers prepared with different UV doses.
The samples were observed after the removal of monomers by SDS treatment.

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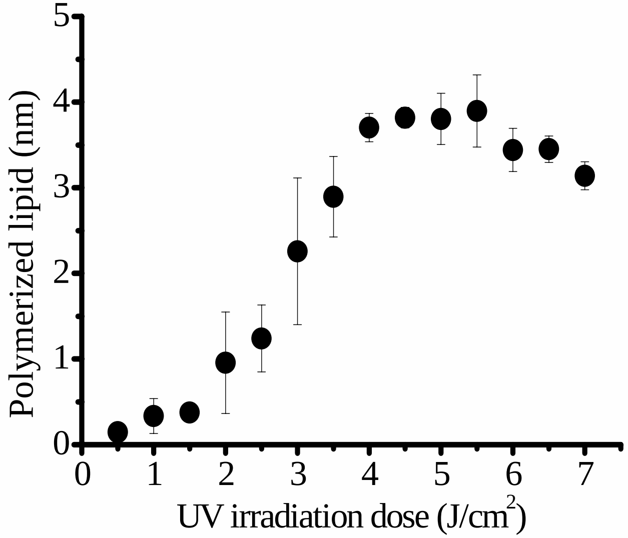
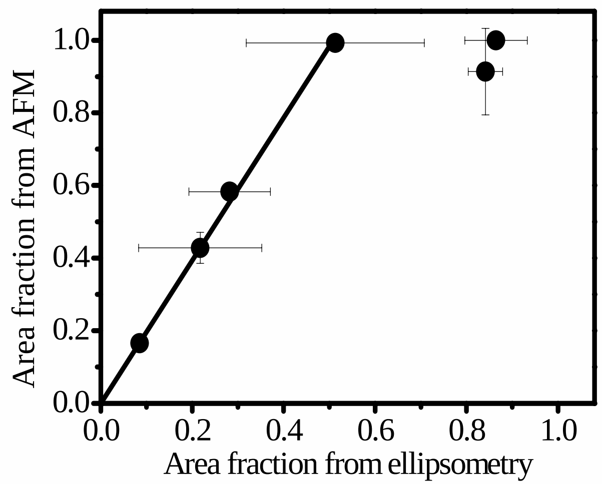
The amount of polymerized bilayers



Film thickness after the 0.1M SDS treatment (ellipsometry)



The area fractions of polymeric bilayers from AFM images were plotted versus those from the ellipsometry and fluorescence microscopy.

The amount of polymerized bilayers can be controlled by changing the applied UV dose for polymerization

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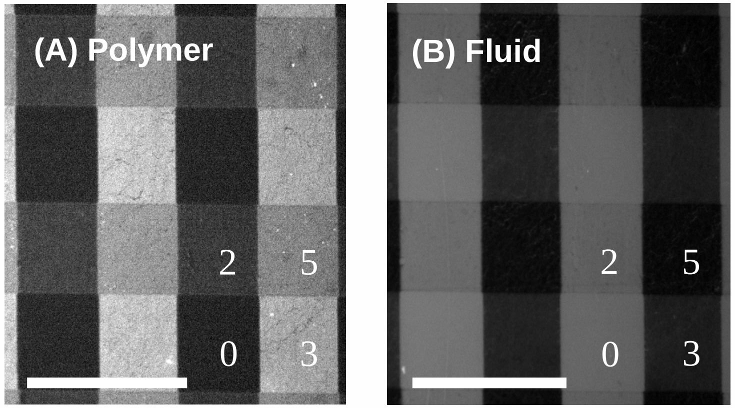
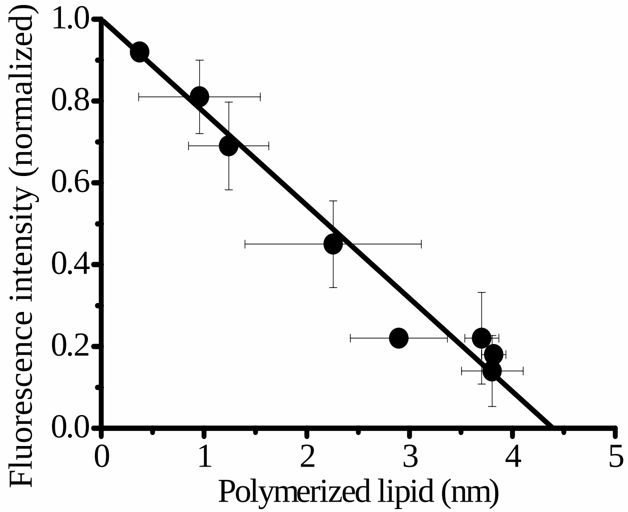
Composition of polymerized and fluid bilayers



Fluorescence microscopy images of a patterned hybrid SPB with spatially varied degree of polymerization (UV doses shown in J/cm^2).
(A) Fluorescence from polymeric DiynePC. (B) Fluorescence from fluid bilayers. The scale bars correspond to 100 μm.



The amount of fluid bilayers (normalized to full coverage) and polymeric bilayers (Determined by fluorescence microscopy)

The amount of fluid bilayers incorporated changed linearly with the amount of polymeric bilayers, suggesting the formation of a composite membrane.

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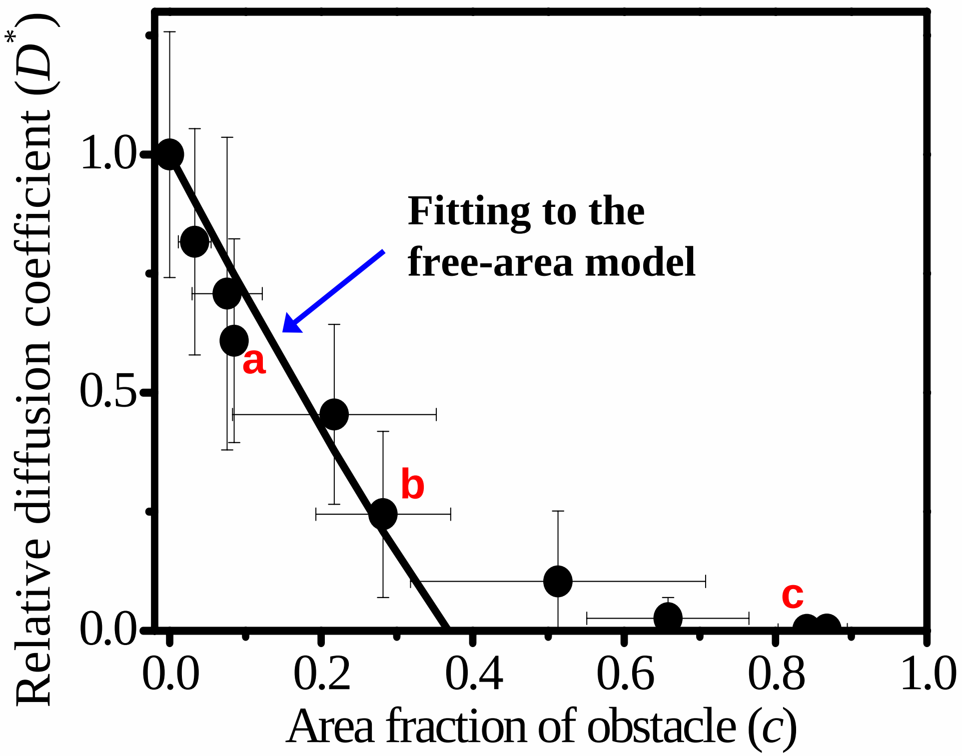
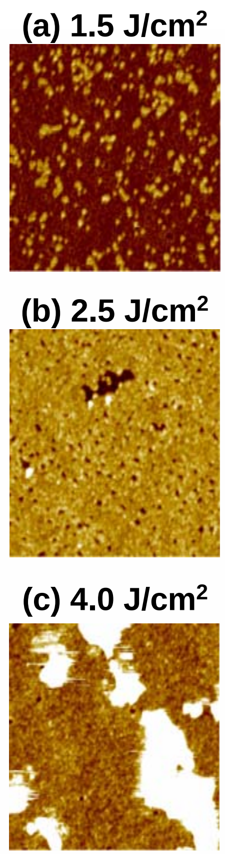
Obstructed diffusion by polymeric membranes





Diffusion coefficient of TR-PE was plotted as a function of the amount of polymeric bilayer in the composite membrane. Diffusion coefficients were determined by the fluorescence recovery after photobleaching (FRAP) method.

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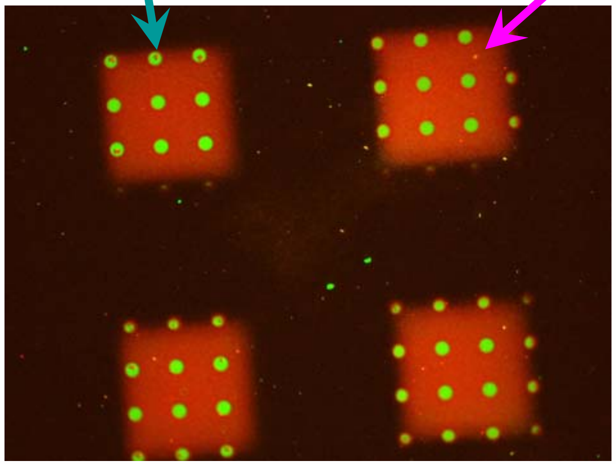
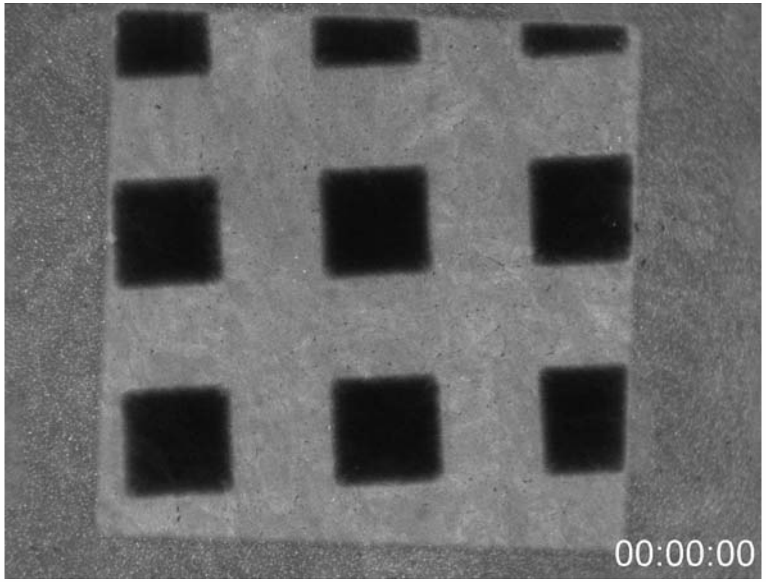
Phase separation of lipid membranes

Sphingomyelin/ cholesterol/DOPC

Sphingomyelin/ cholesterol (l_o) + DOPC (l_d)



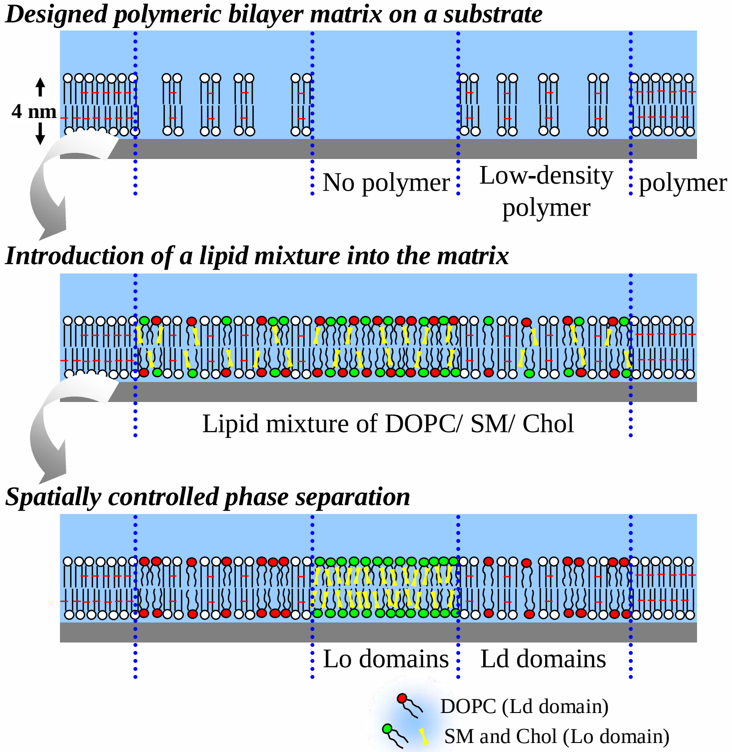
TIRFM image (TR-PE in the l_d phase)



From a mixture of DOPC/ sphingomyelin/ cholesterol, domains containing sphingomyelin/ cholesterol (l_o phase) and TR-PE (l_d phase) were enriched in polymer-free regions and partially polymeric bilayer regions, respectively.

Okazaki *et al. Langmuir* **26**, 4126 (2010)

Directed phase separation of lipid membranes



Possible mechanism

1) Polymer/ fluid junctions:

- membrane thickness
- bending elasticity

2) Domain sizes:

- restriction by polymeric bilayers
- Ostwald ripening

Roles of polymeric bilayer matrices

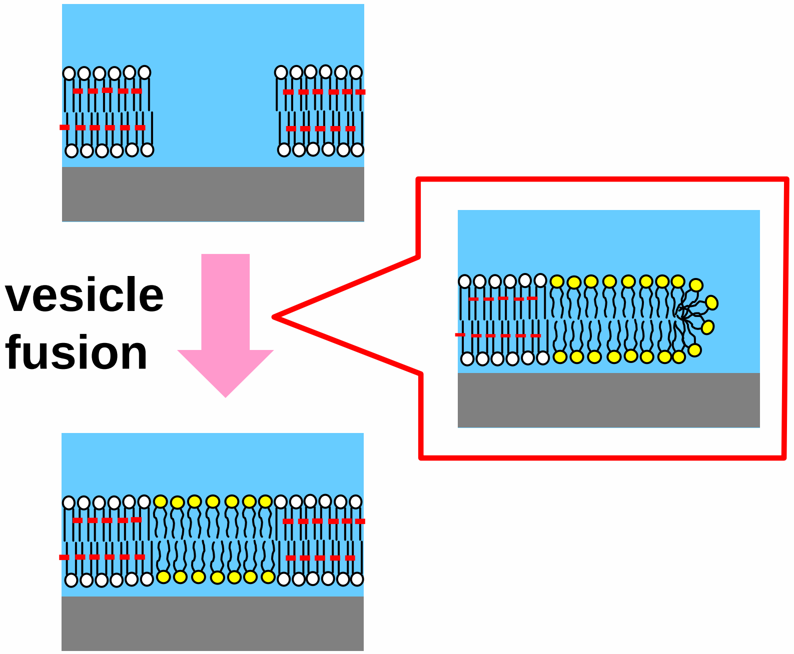
Unique feature

- Polymerized and fluid bilayers can be integrated at a desired composition by controlling the amount of polymerized bilayers.
- Polymeric bilayers can modulate the lateral diffusion of bound molecules and phase separation of l_o and l_d phases.

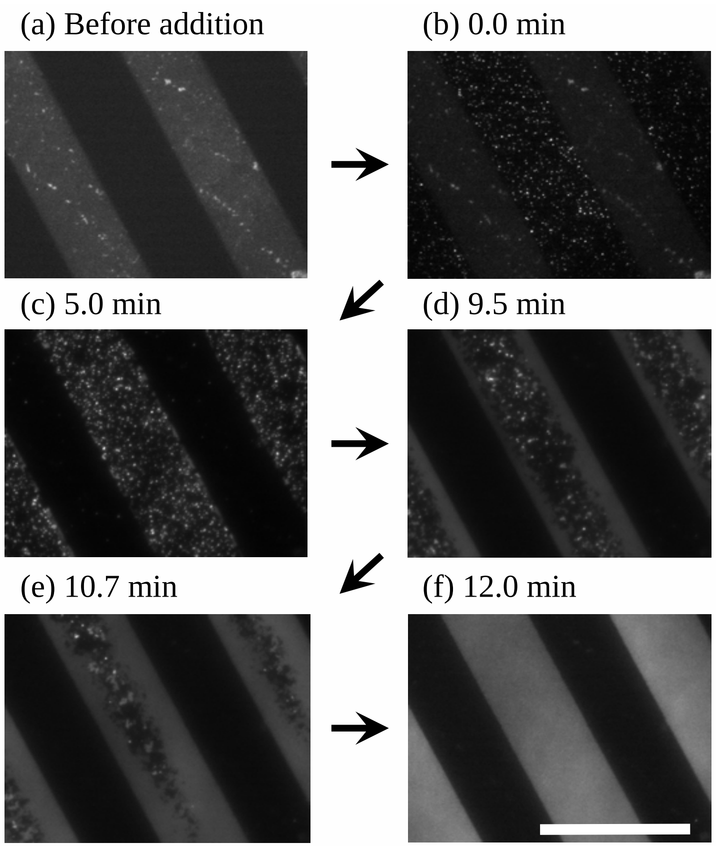
Roles of polymeric bilayers

- Controlling the lateral distribution of molecules
- Stabilization of the model membrane

Polymeric bilayer edge-induced vesicle fusion



Pre-formed polymeric bilayers induced the formation of planar bilayers by catalyzing the vesicle fusion process.

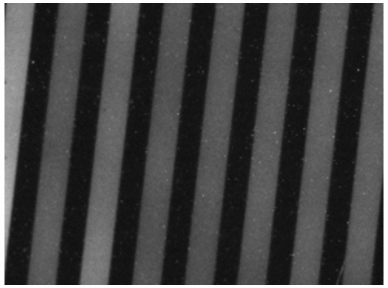


Total internal reflection fluorescence microscopy (TIR-FM) observation

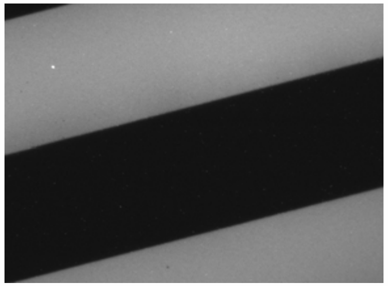
Okazaki *et al. Biophys. J.* **91**, 1757 (2006)

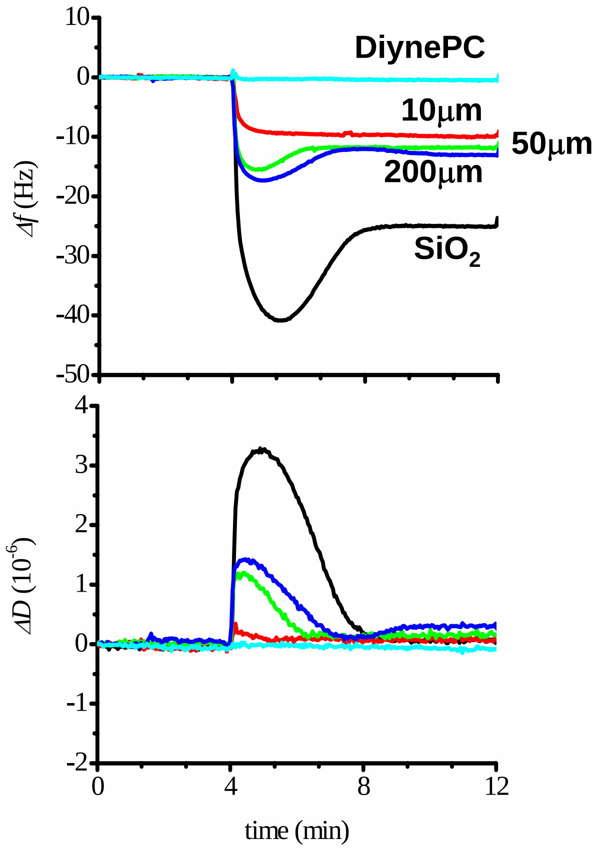
QCM-D results of catalyzed vesicle fusion

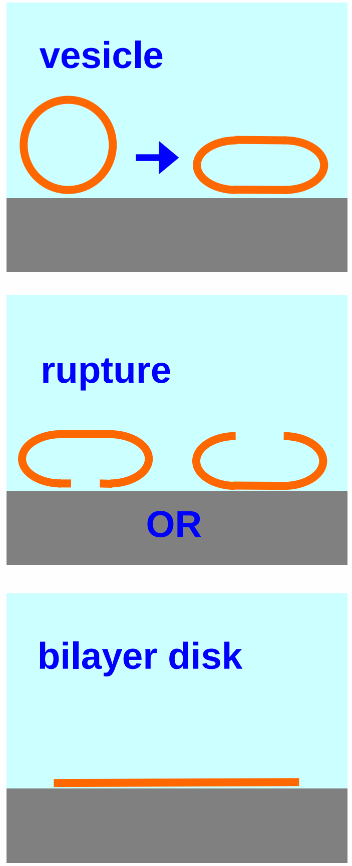
Stripe width: 10μm



Stripe width: 50μm







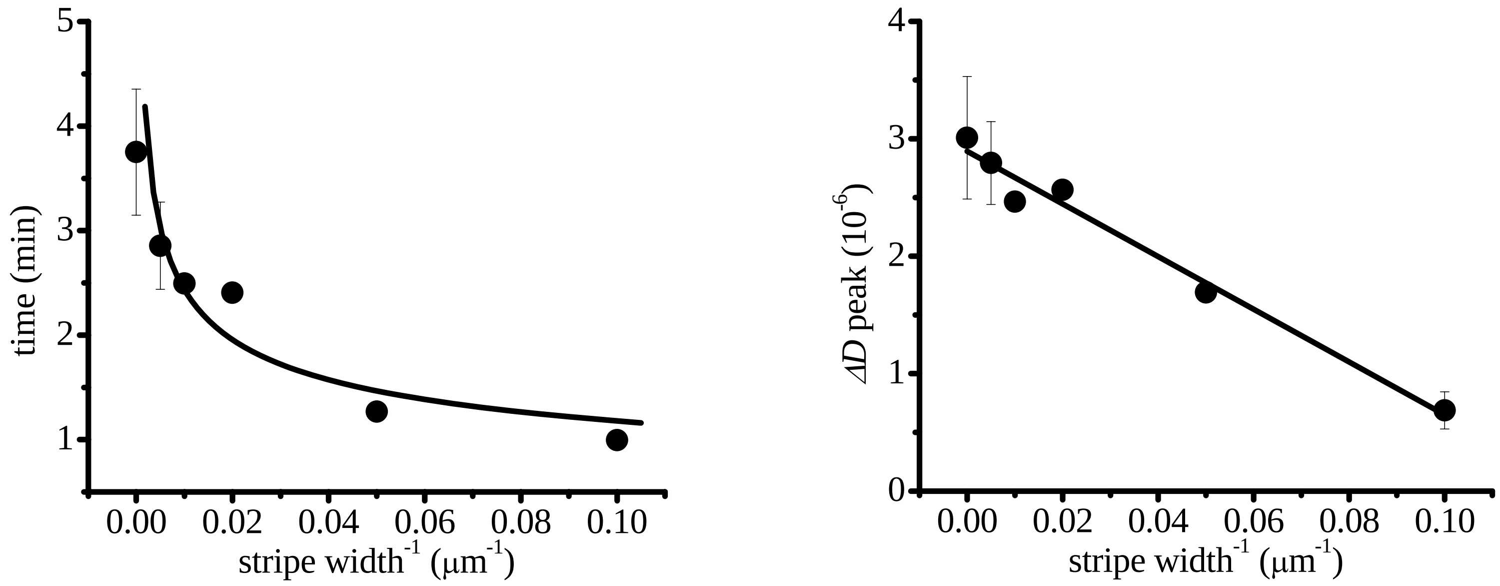
Patterned DiynePC bilayers were prepared on QCM-D sensors (SiO_2 coating) with different stripe width for comparing the effect of the density of bilayer edges on the vesicle fusion kinetics.

Okazaki *et al. Biophys. J.* 91, 1757 (2006)

QCM-D results of catalyzed vesicle fusion

(A) Time for membrane formation

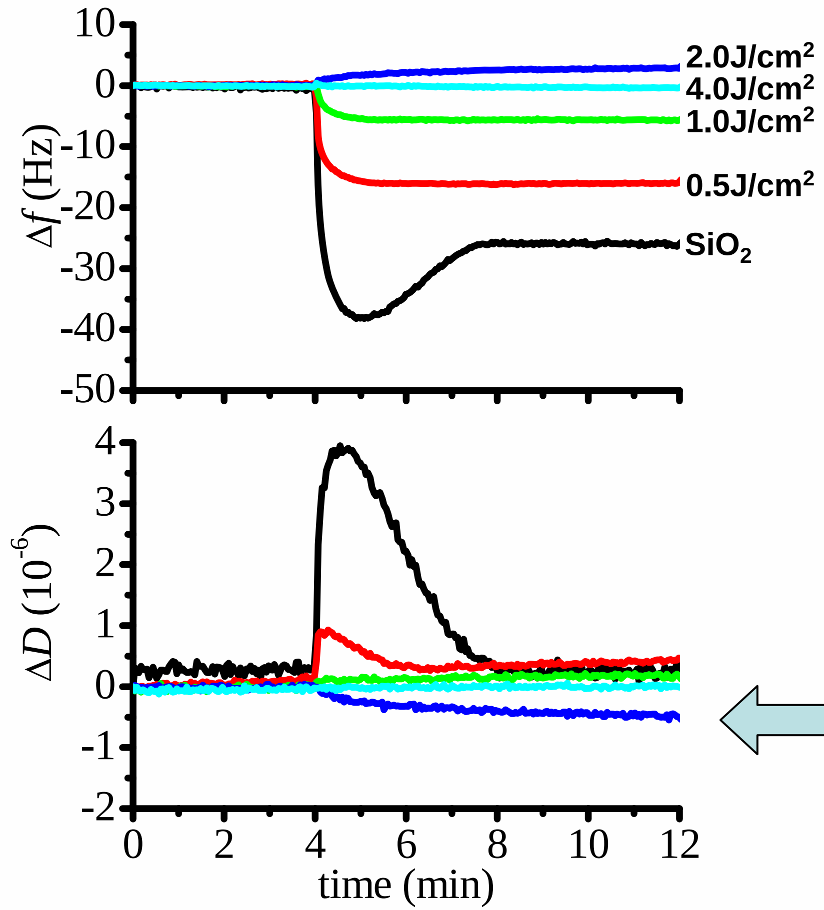
(B) Maximum ΔD in the vesicle fusion



Time for the vesicle fusion and intermediate ΔD maximum values were compared with DiynePC bilayer stripes having different width.

Okazaki *et al. Biophys. J.* 91, 1757 (2006)

QCM-D results of partially polymeric bilayers



Changes of the resonant frequency (Δf) and dissipation (ΔD) measured by the QCM-D during the vesicle fusion on SiO_2 (black) and polymerized DiynePC surfaces. The UV irradiation dose for the photopolymerization was 4.0 J/cm^2 (light-blue), 2.0 J/cm^2 (blue), 1.0 J/cm^2 (green) and 0.5 J/cm^2 (red), respectively.

Positive Δf and negative ΔD: Effects of bound water molecules at bilayer edges?

Okazaki *et al. Langmuir* **25**, 345 (2009)

Roles of polymeric bilayer matrices

Unique feature

- **Formation of planar bilayers was enhanced at the boundaries of polymeric bilayers.**
- **Defects formed in planar bilayers in polymer-free regions, whereas partially polymeric regions accumulated more lipid.**

Roles of polymeric bilayers

- **Controlling the lateral distribution of molecules**
- **Stabilization of the model membrane**

Summary of the results

- **Polymeric and fluid bilayers can be integrated as a continuous composite membrane.**
- **Polymeric bilayers act as obstacles for lateral migration of membrane-bound molecules.**
- **Polymeric bilayers can affect the spatial distribution of molecules by inducing guided phase separation of lipids.**
- **Junctions of polymeric and fluid bilayers play important roles in the formation and stability of membranes.**

In short:

Fluid bilayers are a confined 2D soft matter with a strong coupling with the boundaries

Acknowledgement

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AIST

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Noboru Yumoto

Kobe University

Hiromasa Imaishi

Institute for Molecular Science

Ryugo Tero, Tsuneo Urisu

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