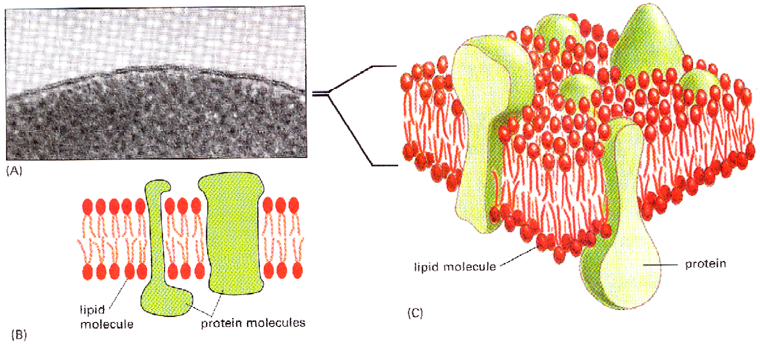
脂質膜上でのナノドメインの静的および動的構造

Y. Sakuma and M. Imai

Ochanomizu University

Cell Membranes



Components of cell membrane (red blood cell)

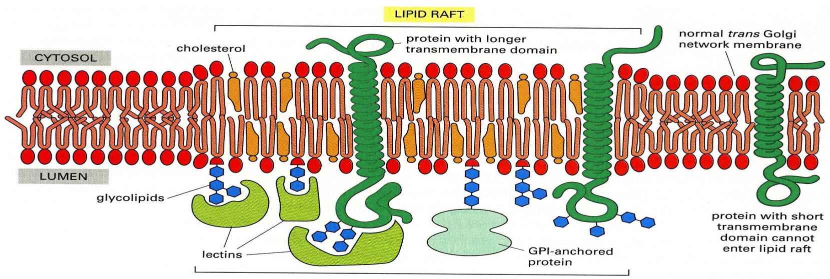
Protein 49.2%

Lipid 43.6%

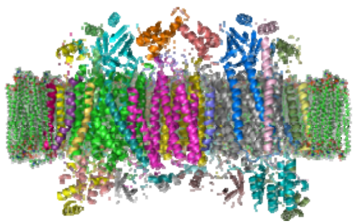
- Phospholipid 32.5%
- Cholesterol 11.1%

Carbohydrate 7.2%

- Glycoprotein 6.7%
- Glycolipid 0.5%



Lateral diffusion and electron transfer in the mitochondrial inner membrane



Cytochrome c oxidaze

M =140,000

$D = 8.3 \times 10^{-14}$ m^2/s

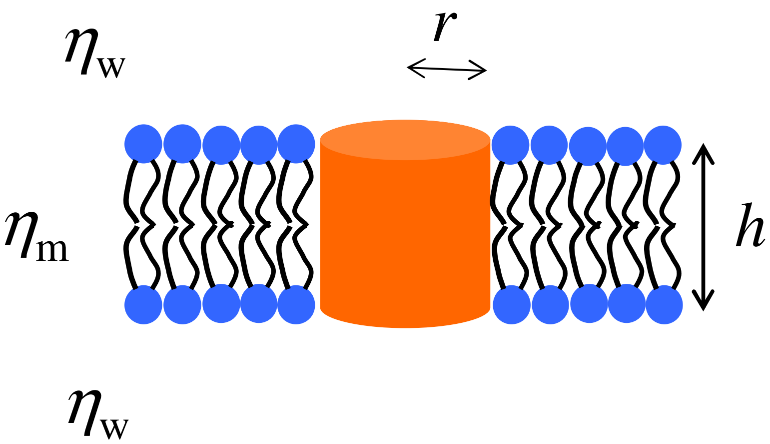
Cytochrome c

M = 12,000

$D = 1.0 \times 10^{-12}$ m^2/s

C. Hackenbrock, TIBS (1981)

Saffman-Delbrück Model

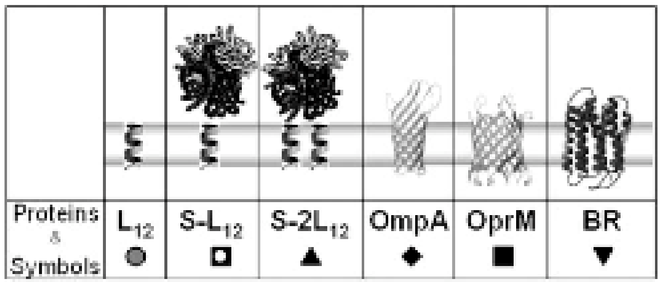


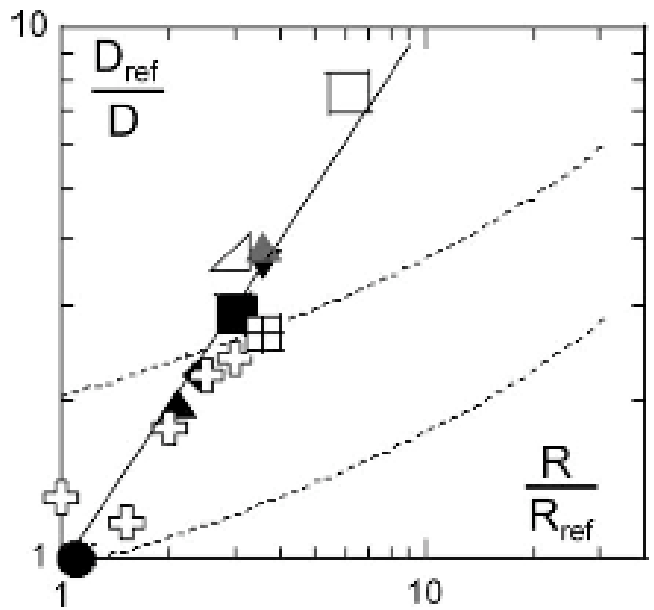
$$r << \frac{\eta_m h}{\eta_w} = r^*$$

$$D(r) = \frac{k_B T}{4\pi \eta_m h}\left[\ln\left(\frac{\eta_m}{\eta_w}\frac{h}{r}\right) - \gamma\right]$$

Lateral mobility of proteins in liquid membranes revisited

Y. Gambin*[†], R. Lopez-Esparza*, M. Reffay*, E. Sierecki[‡], N. S. Gov[§], M. Genest[¶], R. S. Hodges[¶], and W. Urbach*

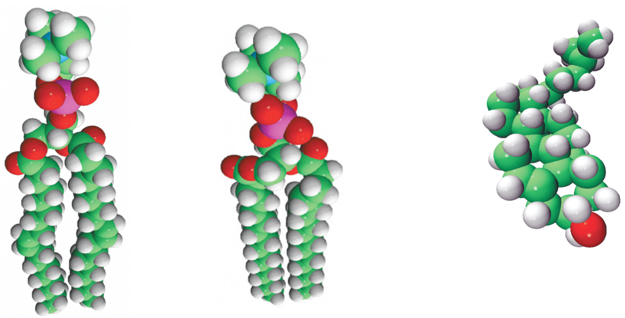


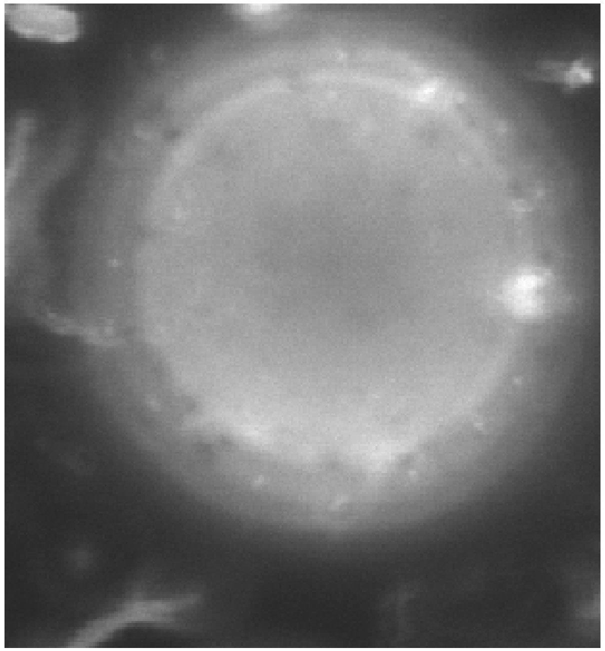


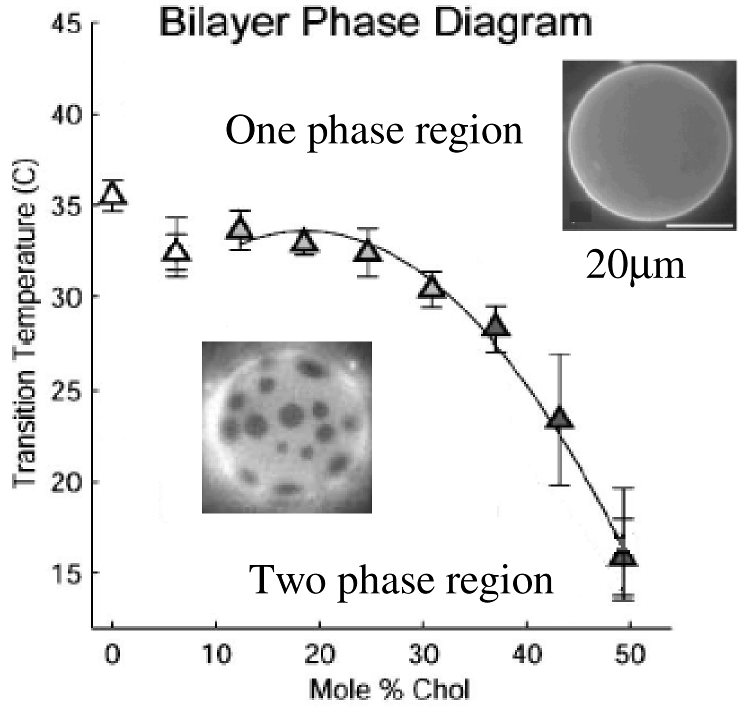
Lateral Phase Separation in Ternary Lipid Membrane

Unsaturated and Saturated Phospholipids and Cholesterol

DOPC DPPC Cholesterol







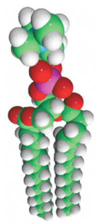
S. Veatch and S. Keller,
Phys. Rev. Lett. 89, 268101 (2002).

Preparation of Nanometer-Sized Domains

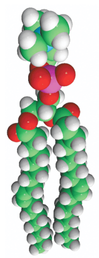
Preparation of Small Unilamellar Vesicle

[sample]

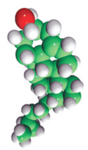
· **DPPC**
(saturated lipid)



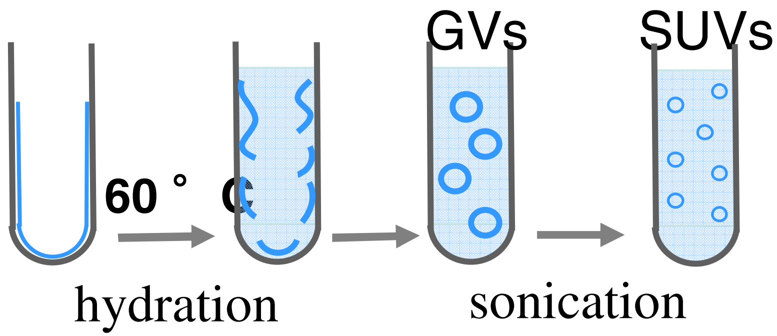
· **DOPC**
(unsaturated lipid)



· **cholesterol**

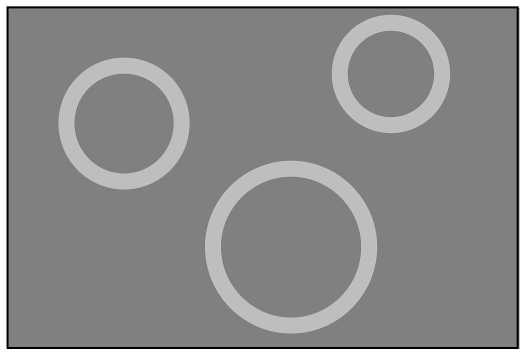


Gentle Hydration Method



Estimation of SUV size

[SANS under film contrast]



Nanometer Sized Vesicle

$$I(q) = N\int_0^\infty f(r)P(q,r)dr$$

$$P(q) = 16\pi^2 \Delta\rho^2 \left\{R^3 f_0(qR) - (R-\delta)^3 f_0(q(R-\delta))\right\}^2$$

$$f_0(x) = (\sin x - x\cos x)/x^3$$

$I(q)$: Intensity

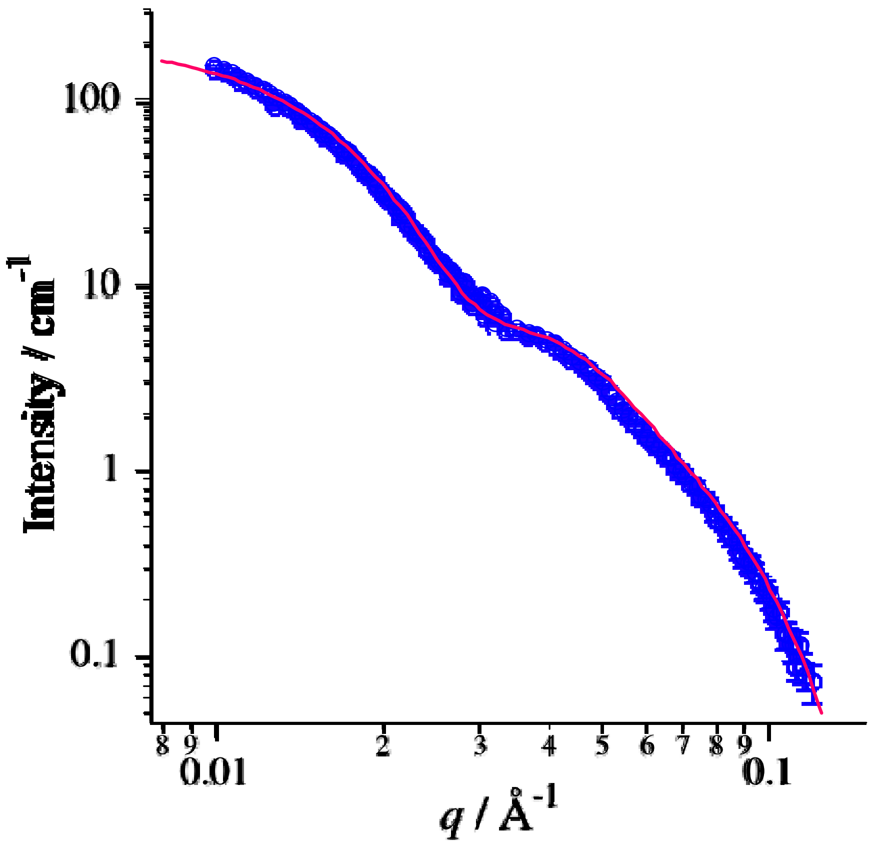
$P(q)$:form factor

q : scattering vector (= $4\pi \sin\theta/\lambda$)

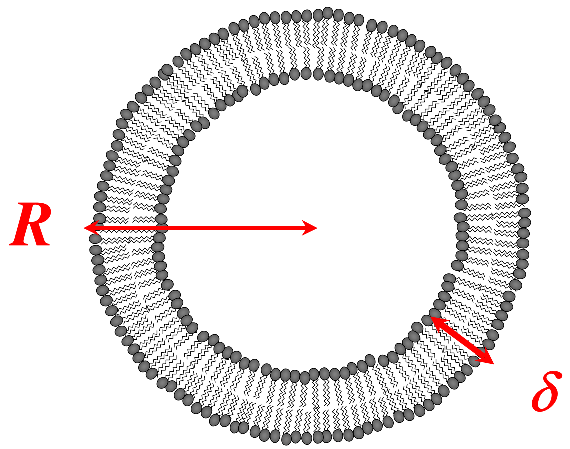
$\Delta\rho$: scattering length density

$f(r)$: Schulz distribution of vesicle radius

p : size polydispersity (= $\langle R^2\rangle/\langle R\rangle^2 - 1$)



Instrument : SANS-U (ISSP, JPN)



Fitting results

R = 11.2 nm

δ = 4.0 nm

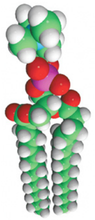
p = 0.3

Structure of nanometer sized domains

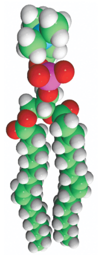
Matching contrast of neutron scattering

[sample]

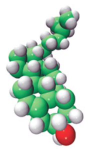
- **DPPC-*d*62**
  (deuterated saturated lipid)



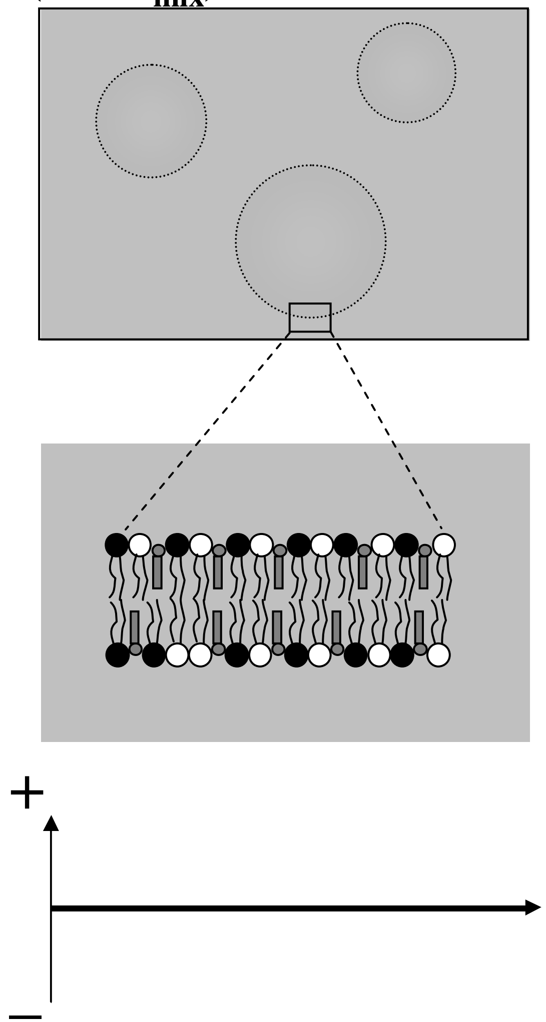
- **DOPC**
  (unsaturated lipid)



- **cholesterol**



One phase region
($T > T_{mix}$)



Structure of nanometer sized domains

Matching contrast of neutron scattering

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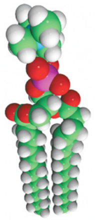

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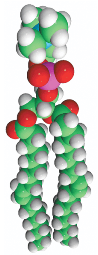
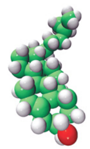
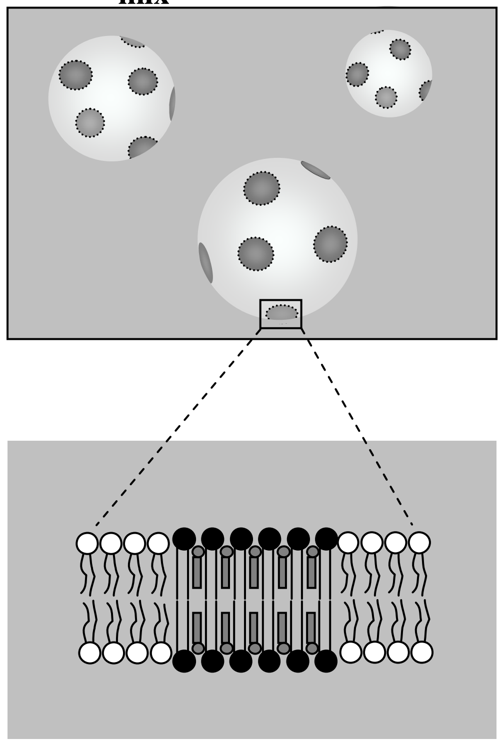
(unsaturated lipid)

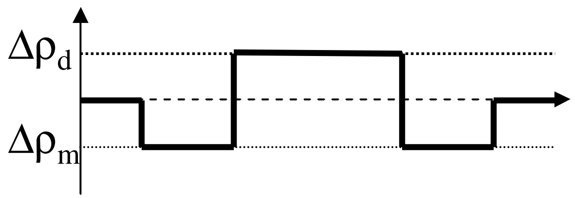


▪ **cholesterol**

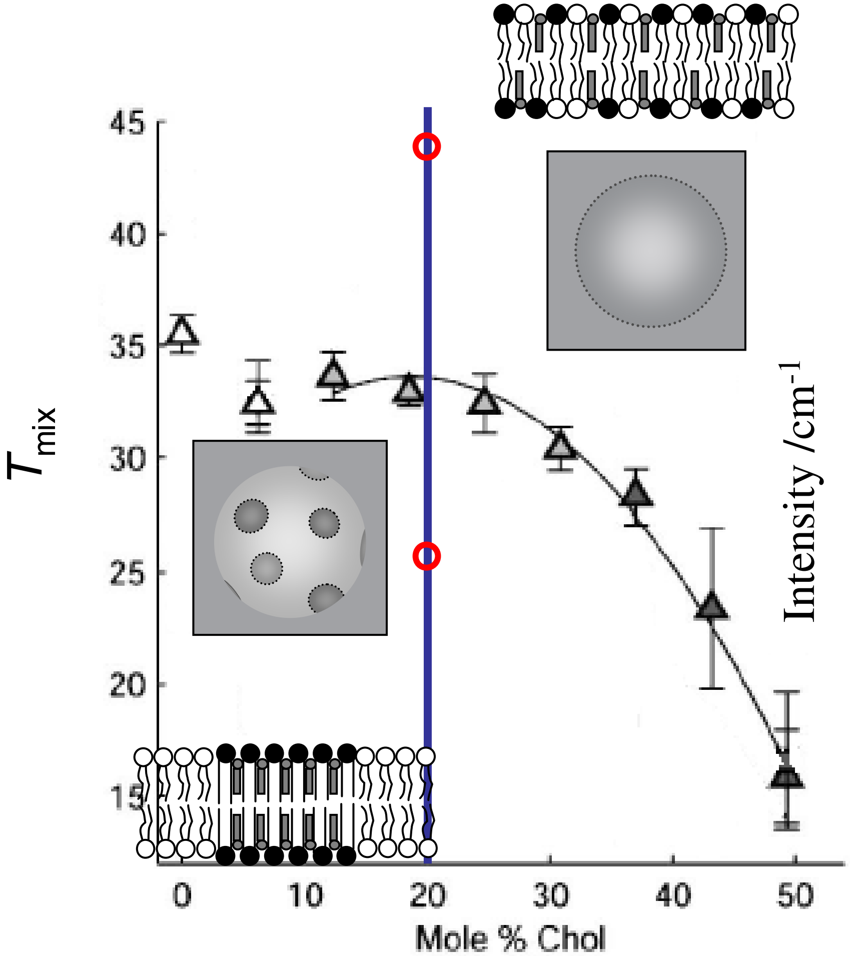


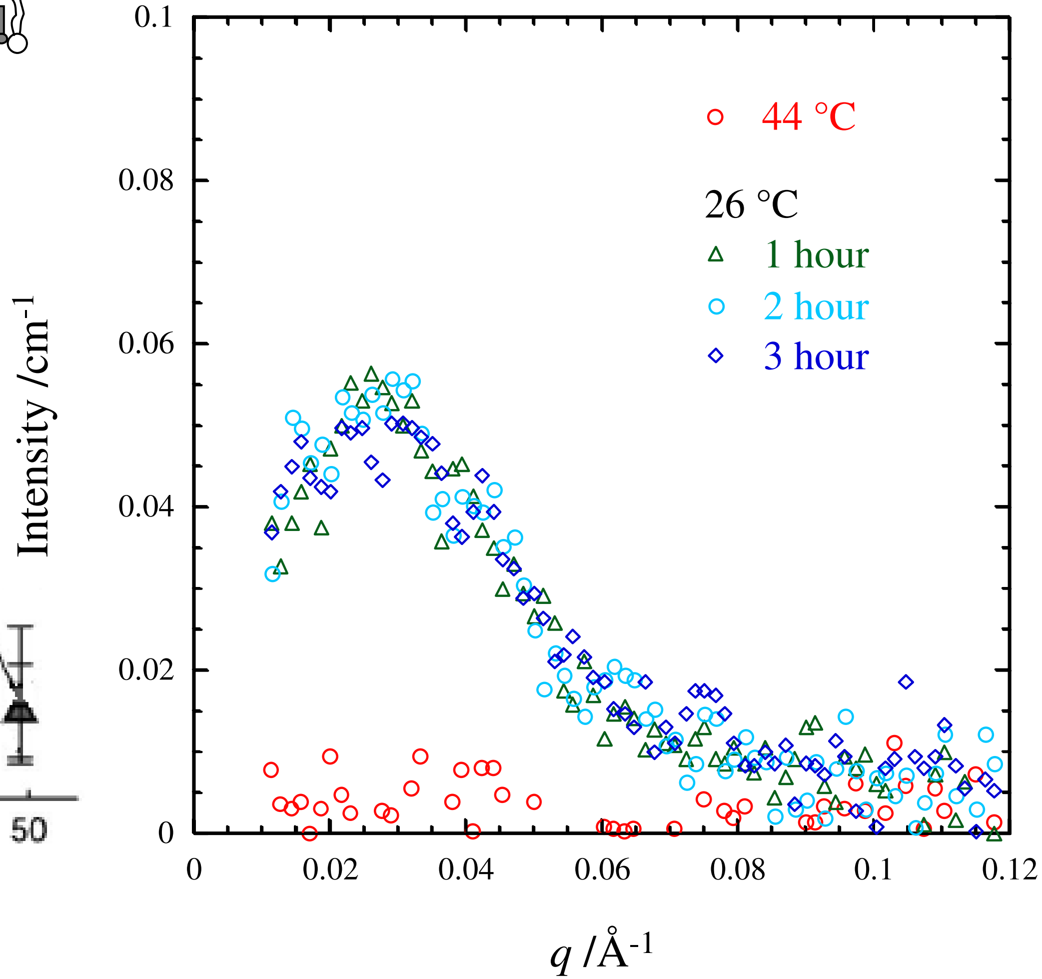
Two phase region
(***T*** **<** $\boldsymbol{T}_{\mathbf{mix}}$)





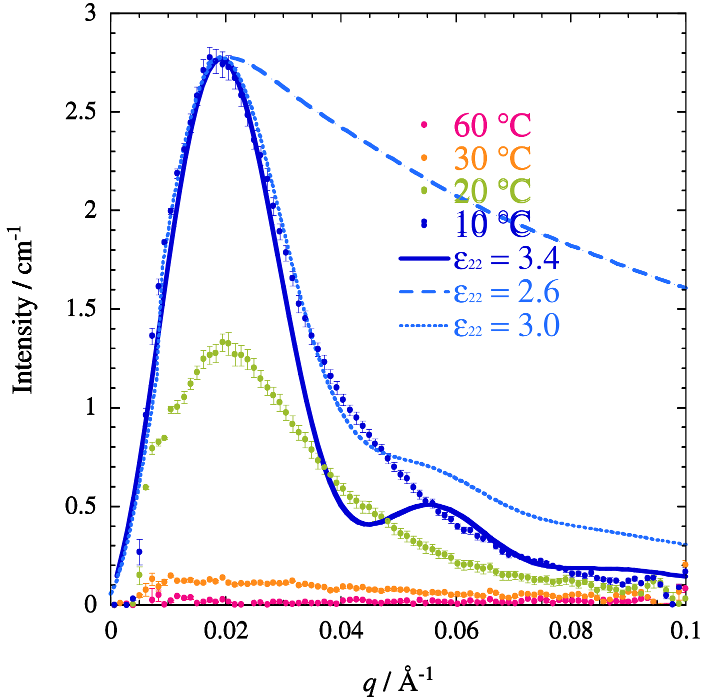
SANS Profiles of the SUVs at the Contrast Matching Point





Nanometer Sized Domains

Temperature dependence of SANS profiles



Instrument : SANS-U (ISSP, JPN)

Molecular Dynamics Simulation

Red particle(domain) : 750
Green particle(matrix) : 750

[potential]

- Lennard-Jones potential

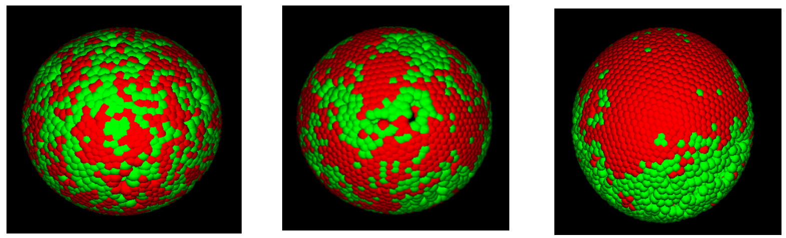
$$U_{LJ} = 4\varepsilon_{ij}\left\{\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right\}$$

$$\varepsilon_{11} = \varepsilon_0$$

$$\varepsilon_{22} = 2.6\varepsilon_0 \sim 3.4\varepsilon_0$$

$$\varepsilon_{12} = \sqrt{\varepsilon_1 \cdot \varepsilon_2}$$

$$\varepsilon_{22} = \frac{k_B T}{T^*}$$



$\varepsilon_{22} = 2.6$ 3.0 3.4

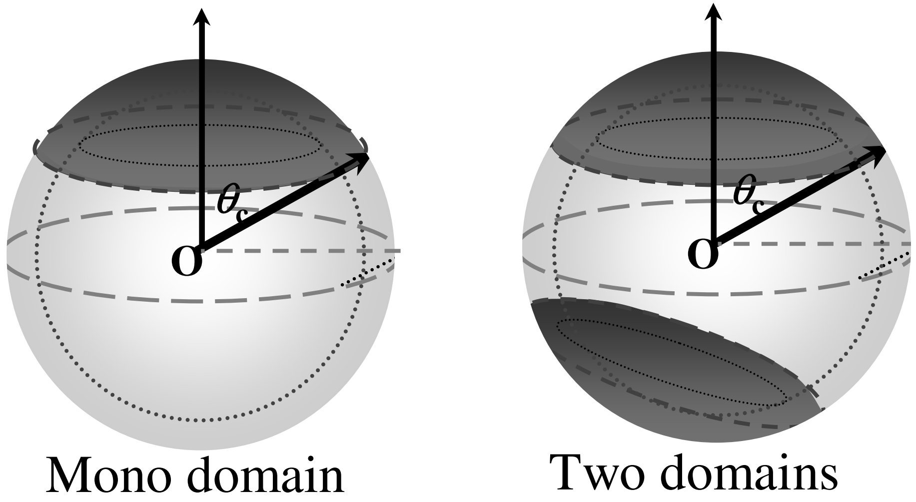
[Model scattering function]

$$\rho(\boldsymbol{r}) = \sum_{i=1}^{N} \delta(\boldsymbol{r} - \boldsymbol{r}_i)$$

$$\rho_q = \int \rho(\boldsymbol{r}) e^{-i\boldsymbol{q}\cdot\boldsymbol{r}} d\boldsymbol{r}$$

$$S(\boldsymbol{q}) = \frac{1}{N}\left\langle \rho_q \cdot \rho_{-q} \right\rangle$$

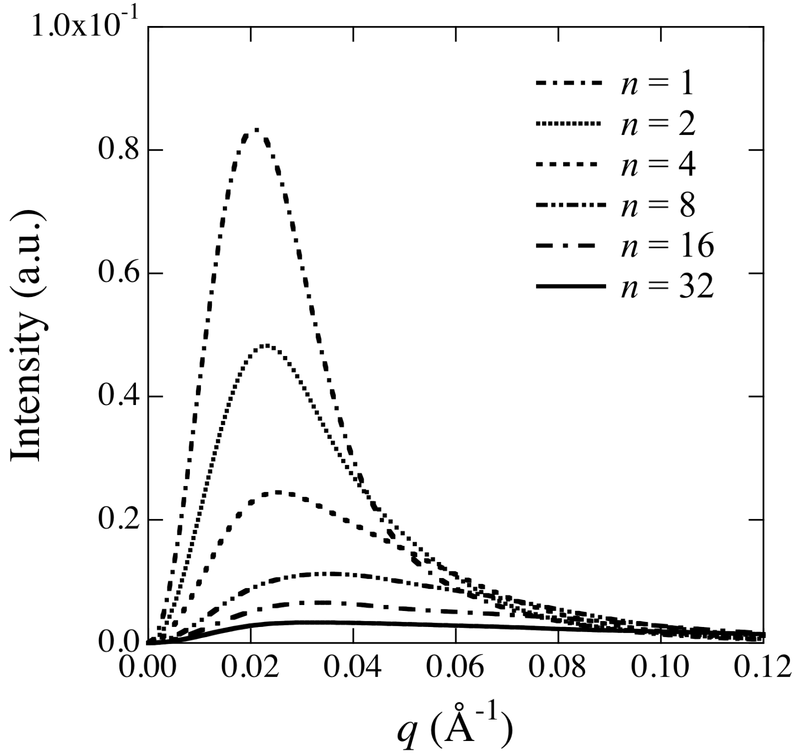
Monte Carlo Simulation

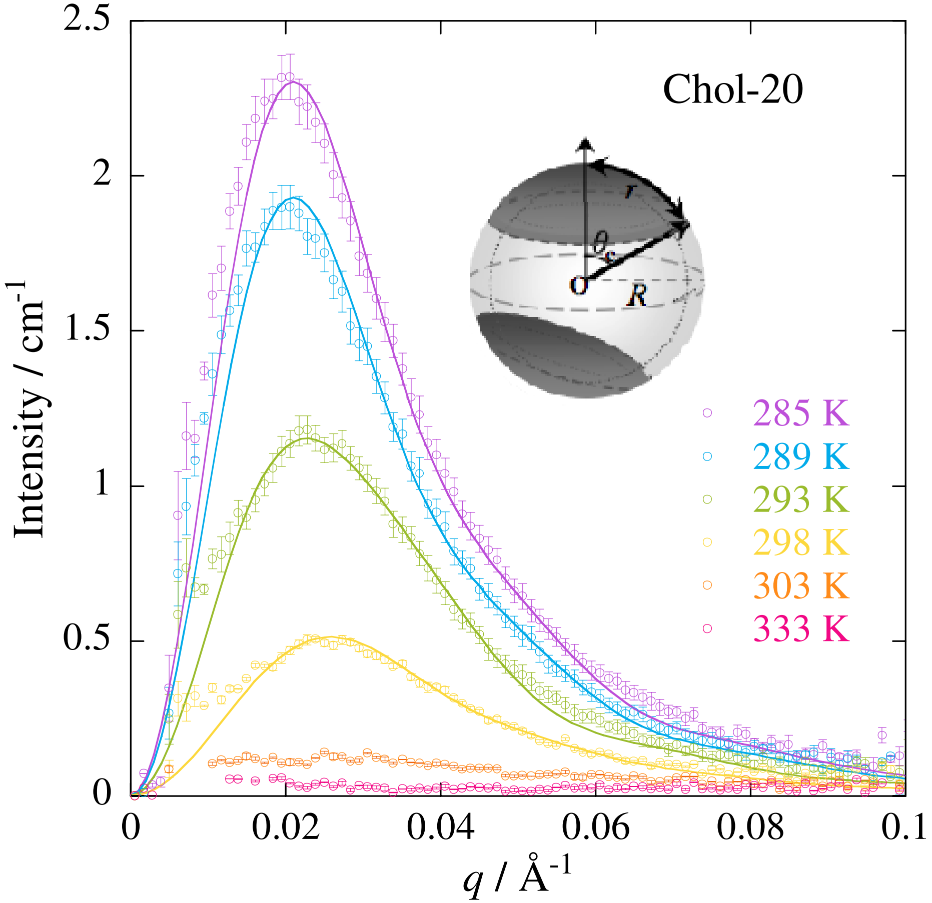


$$P(q) = \sum_{\alpha,\beta} \rho_\alpha \rho_\beta S_{\alpha\beta}(q)$$

$$S_{\alpha\beta}(q) = \frac{N_\alpha}{N}\delta_{\alpha\beta} + \frac{N_\alpha N_\beta}{N^2} 4\pi\rho \int_0^{r_{\max}} r^2 g_{\alpha\beta}(r) \frac{\sin(qr)}{qr} dr$$

α : domain particle
β : matrix particle
N_α : number of domain particle
N_β : number of matrix particle
N : total number of particle
$g_{\alpha\beta}(r)$: partial radial distribution function




Chol-20
r
θc
O
R
285 K
289 K
293 K
298 K
303 K
333 K
Intensity / cm-1
q / Å-1
0
0.5
1
1.5
2
2.5
0
0.02
0.04
0.06
0.08
0.1

$$\Delta\rho_d = \frac{\phi^d_{d\text{-DPPC}}\Delta\rho_{d\text{-DPPC}} + \phi^d_{\text{DOPC}}\Delta\rho_{\text{DOPC}} + \phi^d_{\text{Chol}}\Delta\rho_{\text{Chol}}}{\phi^d_{d\text{-DPPC}} + \phi^d_{\text{DOPC}} + \phi^d_{\text{Chol}}}$$

$\Delta\rho_{\text{d-DPPC}}=3.36 \times 10^{14}\ \text{m}^{-2}$

$\Delta\rho_{\text{DOPC}}= -2.22 \times 10^{14}\ \text{m}^{-2}$

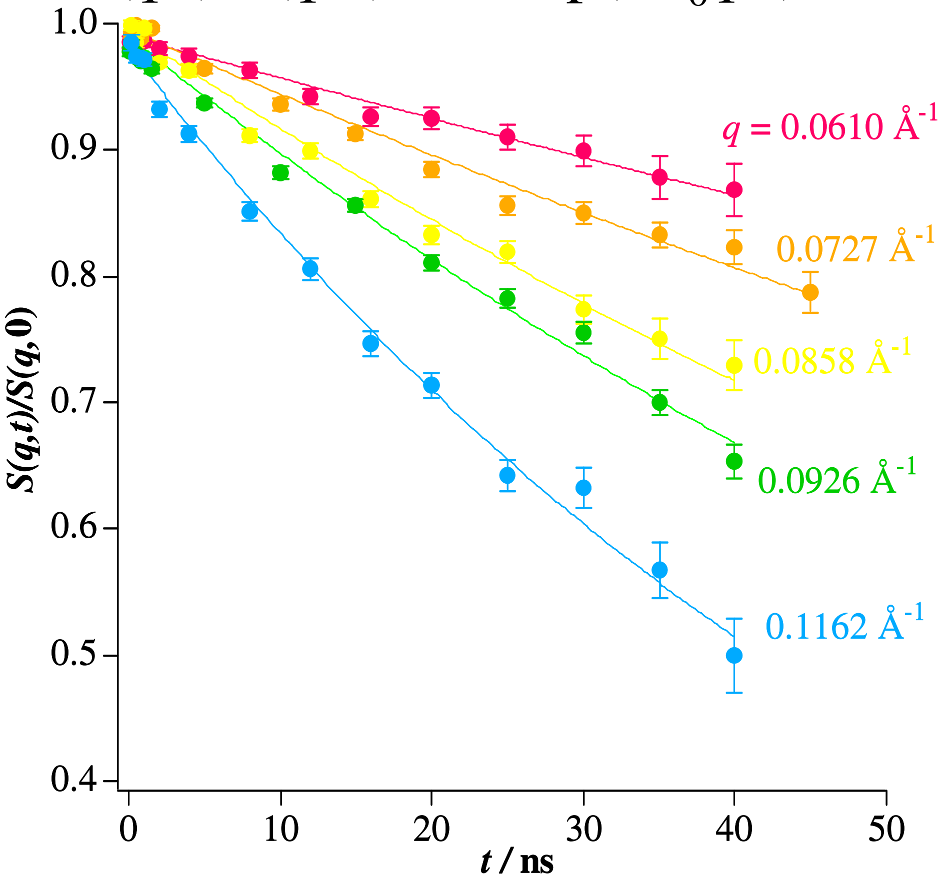
$\Delta\rho_{\text{Chol}}= -2.31 \times 10^{14}\ \text{m}^{-2}$

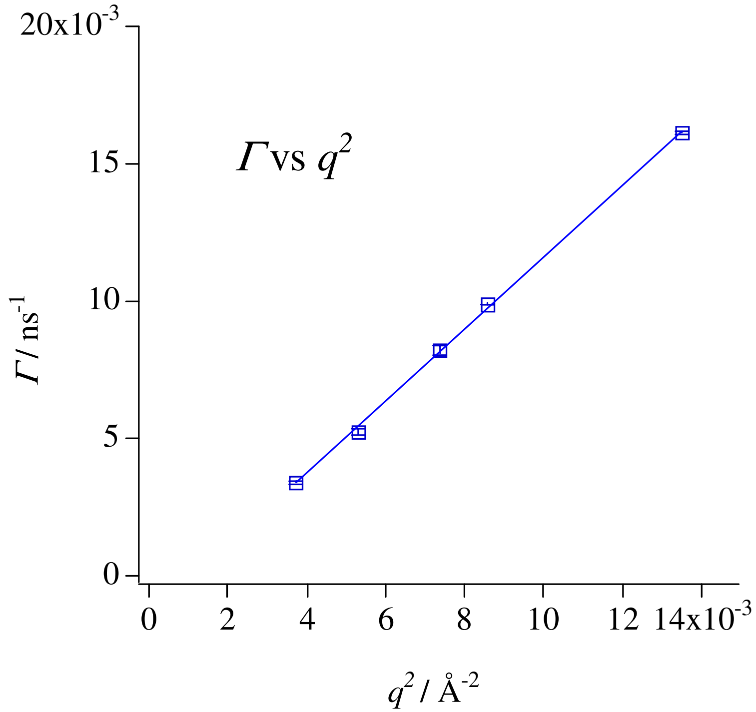
Dynamic Structure of Phase Separated Vesicles

$$S(q,t)/S(q,0) = \text{A}\exp(-D_0q^2t) + (1\text{-A})\exp(-(D_0+D_d)q^2t)$$

Diffusion of Vesicles

$$S(q,t)/S(q,0) = \text{A}\exp(-D_0q^2t)$$





$D_0 = (1.31 \pm 0.02)\text{x}10^{-11}\ \text{m}^2/\text{s}$

Instrument : NG5-NSE (NIST, USA)

conclusion

Using a contrast matching technique, a neutron spin echo spectroscopy and a phase separation on nanometer-sized vesicle we addressed static and dynamic structures of nanometer-sized domains on membrane.

Nanometer-sized domains are agitated by the thermal fluctuations and the composition depends on the temperature.

The diffusion coefficient of nanometer-sized domains in fluid membranes is almost constant versus the domain size, which well described by the hydrodynamic model.