放射光で調べる低pH誘起の脂質膜相転移

ーLα相からキュービック相へー

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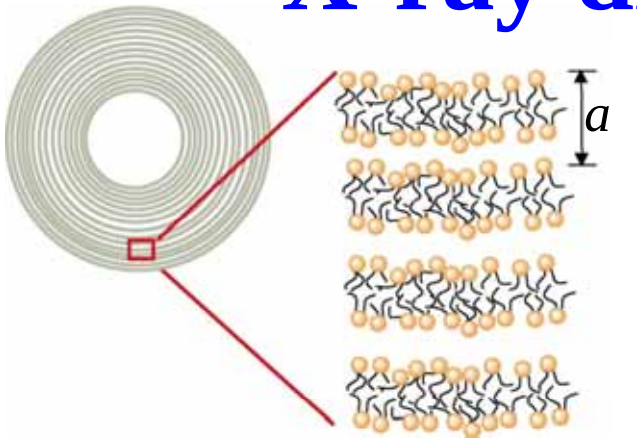
山崎昌一

JASRI　**太田昇**

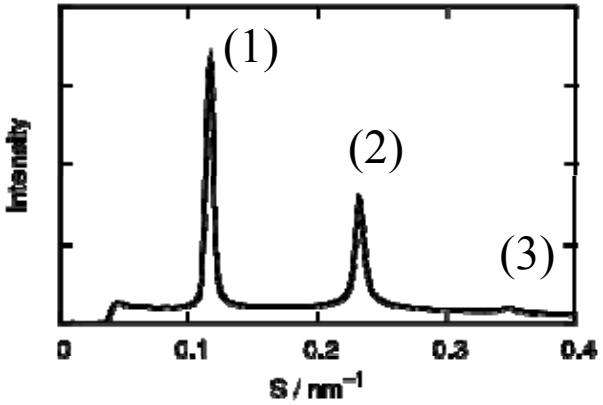
X-ray diffraction of lipids

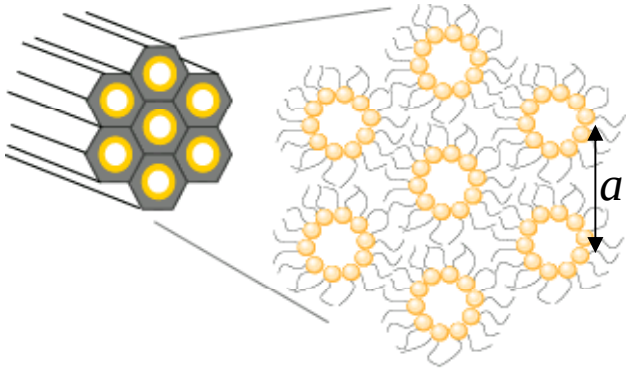


MLV (L_α phase)

1D periodic structure

$$S_h = \frac{h}{a}$$



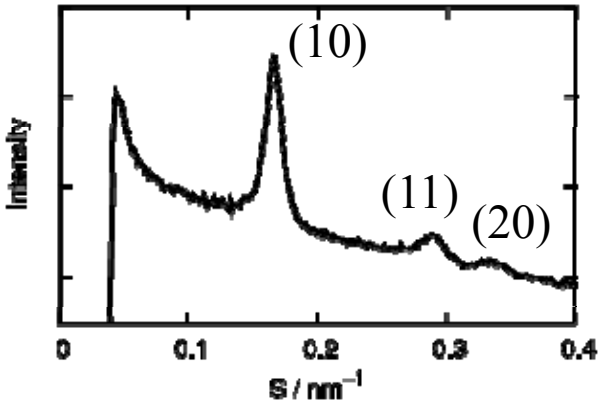


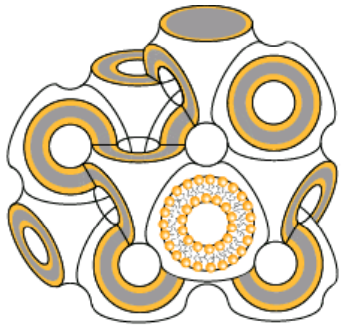
H_{II} phase

2D periodic structure

plane group: *p6mm*

$$S_{hk} = \frac{2}{\sqrt{3}} \frac{\sqrt{h^2 + hk + k^2}}{a}$$



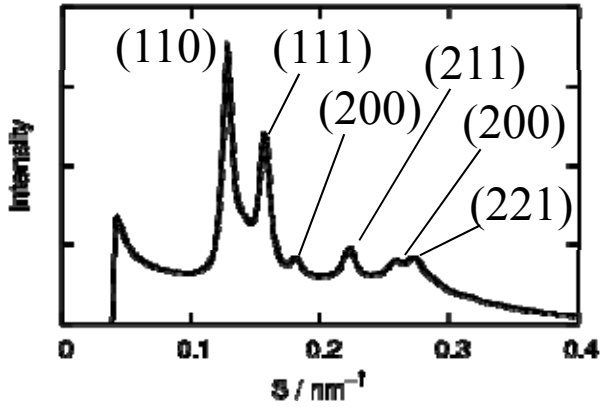


Q_{II}^D phase

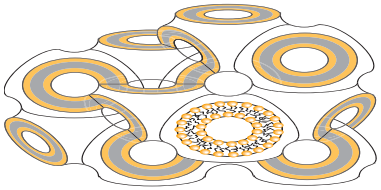
3D periodic structure

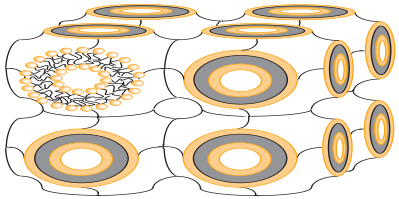
space group: $Pn\bar{3}m$

$$S_{hkl} = \frac{\sqrt{h^2 + k^2 + l^2}}{a}$$



Cubic Phase
Q_{II}^{D} phase





Cubic Phase
Q_{II}^{P} phase

Phase Transition between Q Phases and L_α phase of Biomembranes

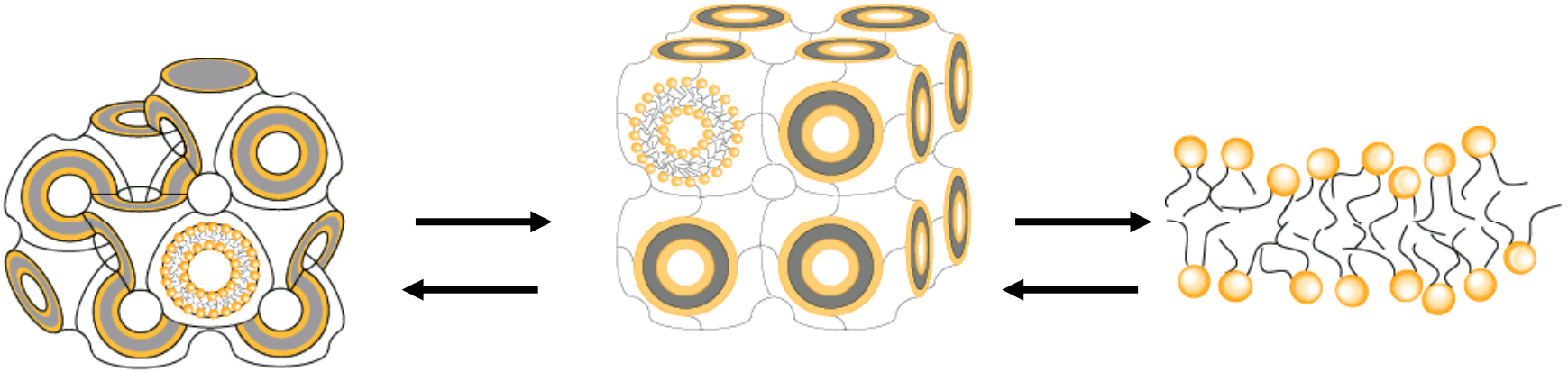
L_α Phase
Lamellar Bilayer Membrane

Elucidation of Mechanism of Phase Stability of Cubic Phase

$\Rightarrow$

1. Mechanism of phase transitions between cubic phases and Lα phase in cells (e.g., prolamellar body → thylakoids)
2. Mechanism of biomembrane dynamics of membrane fusion and fission
3. Crystallization of biomembranes using cubic phase of biomembranes

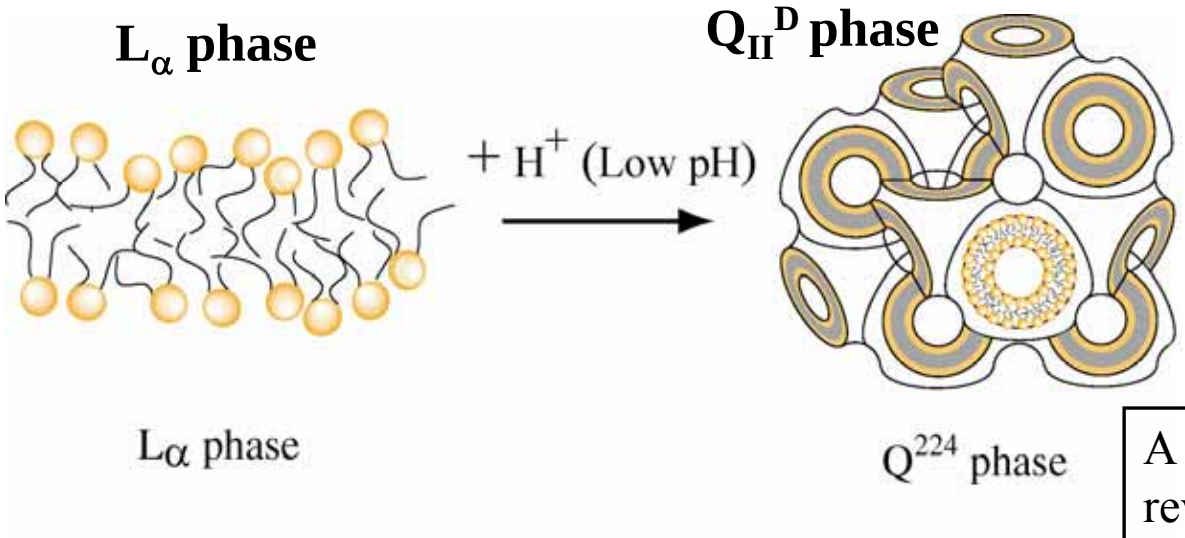
Effect of Electrostatic interactions on Stability of Cubic Phases of Biomembranes



Small ← **Electrostatic interactions due to Surface Charges of the membrane Interface** (Lipids or Peptides) → **Large**

BBA (1999) **1461**, 96
Biophys. J. (2001) **81**, 983
Langmuir (2003) **19**, 4745
Langmuir (2008) **24**, 3400

Low pH-induced Lα to Q phase transition in 20%DOPS/80%MO membrane



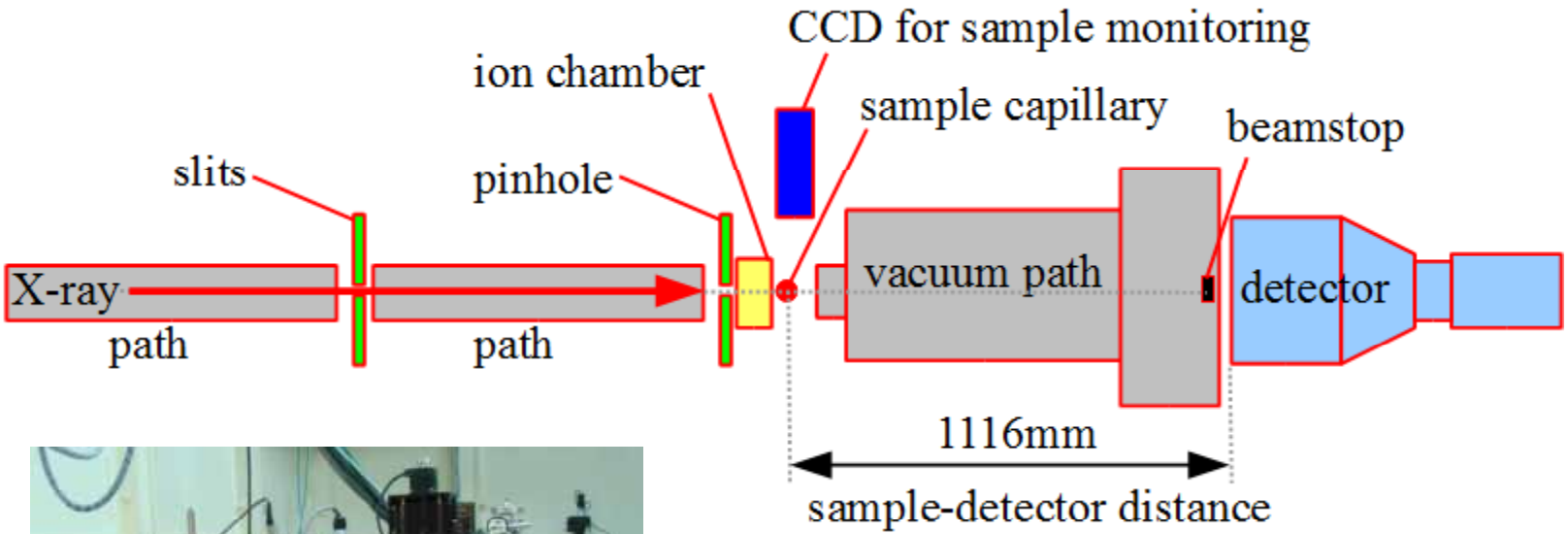
A change in pH can induce a reversible phase transition between the L_α and cubic phases of lipid membranes within 1 h.

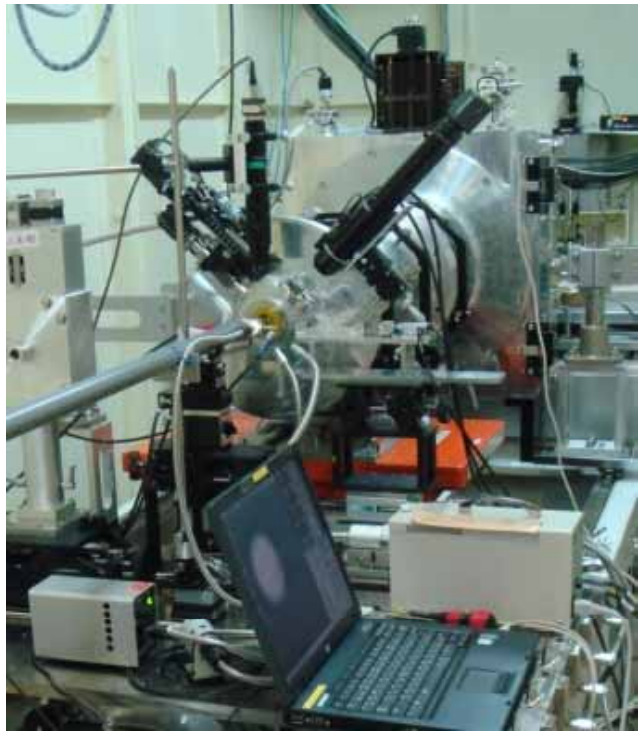
Langmuir 2008, **24**, 3400

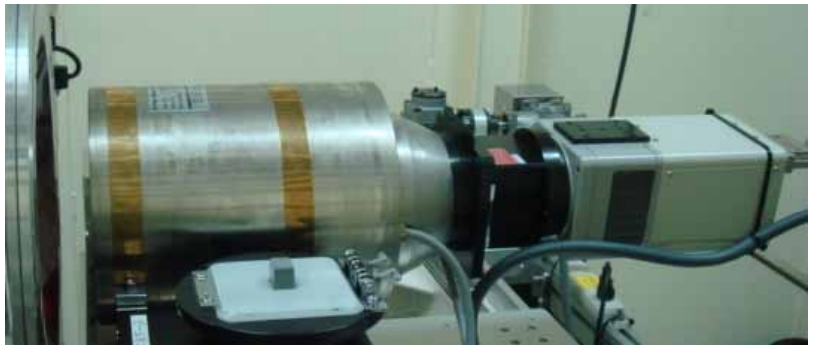
Kinetics measurements

J. Chem. Phys. (2011) 134: 145102

Experimental Setting

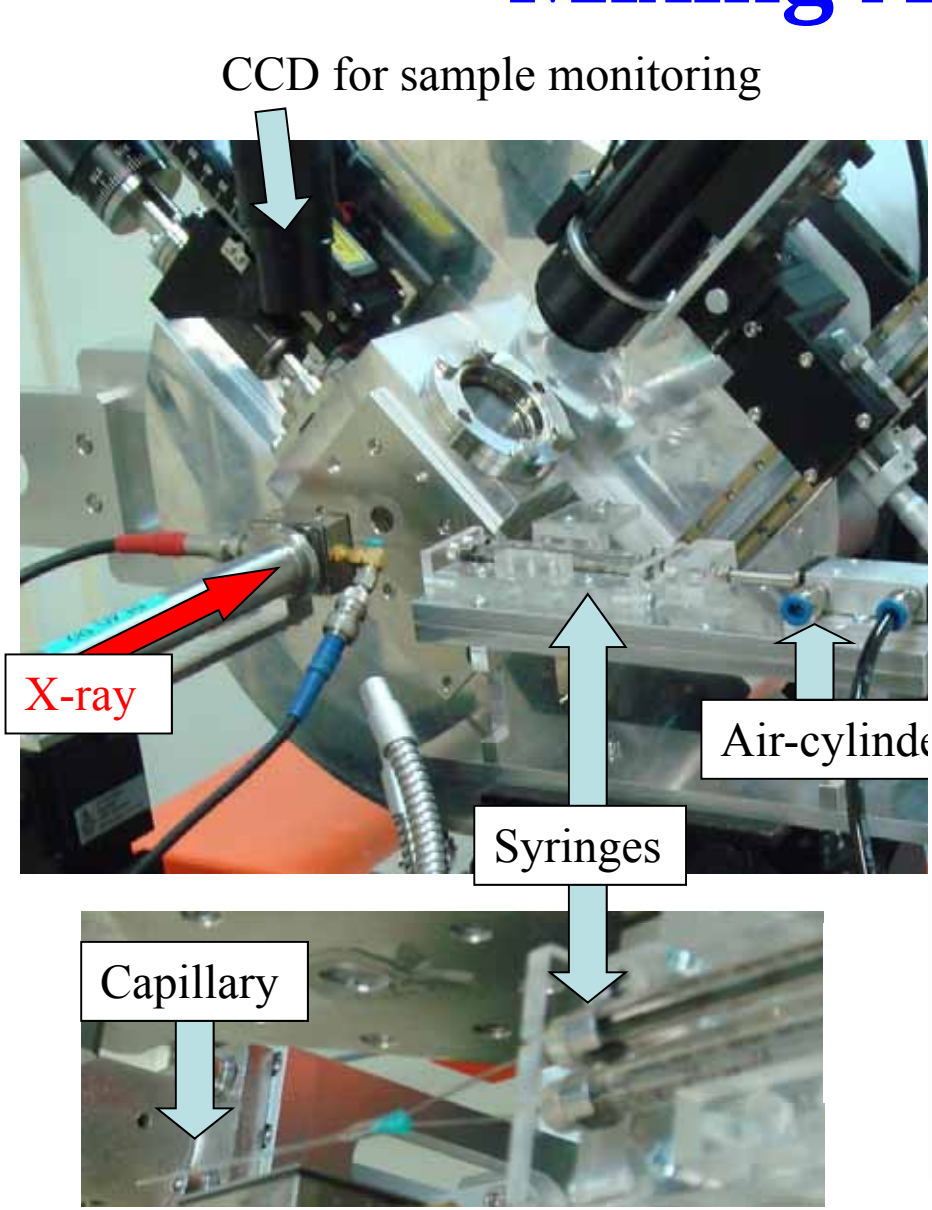


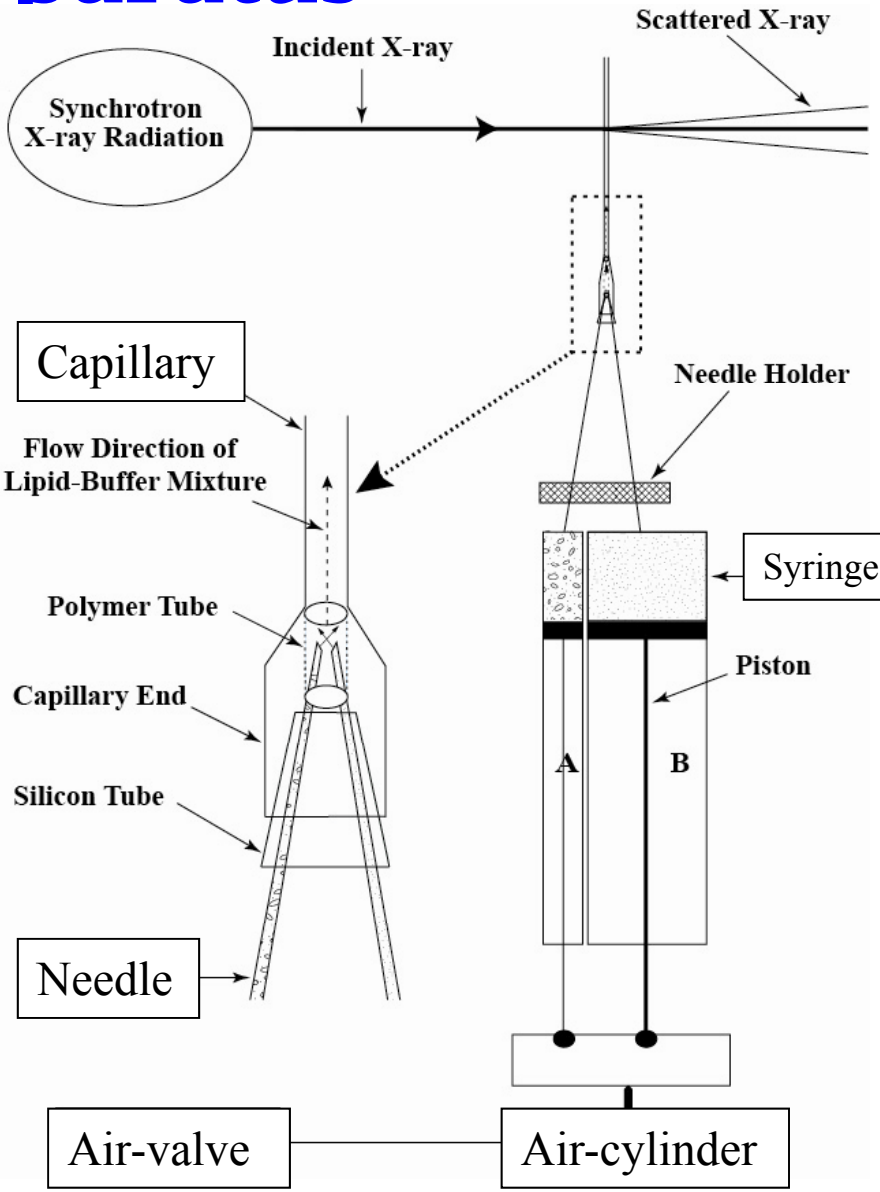




6-inch X-ray image intensifier (V5445P)
CCD camera (C4742-98-24A, Hamamatsu Photonics)

Mixing Apparatus





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Mixing MLV with Buffer

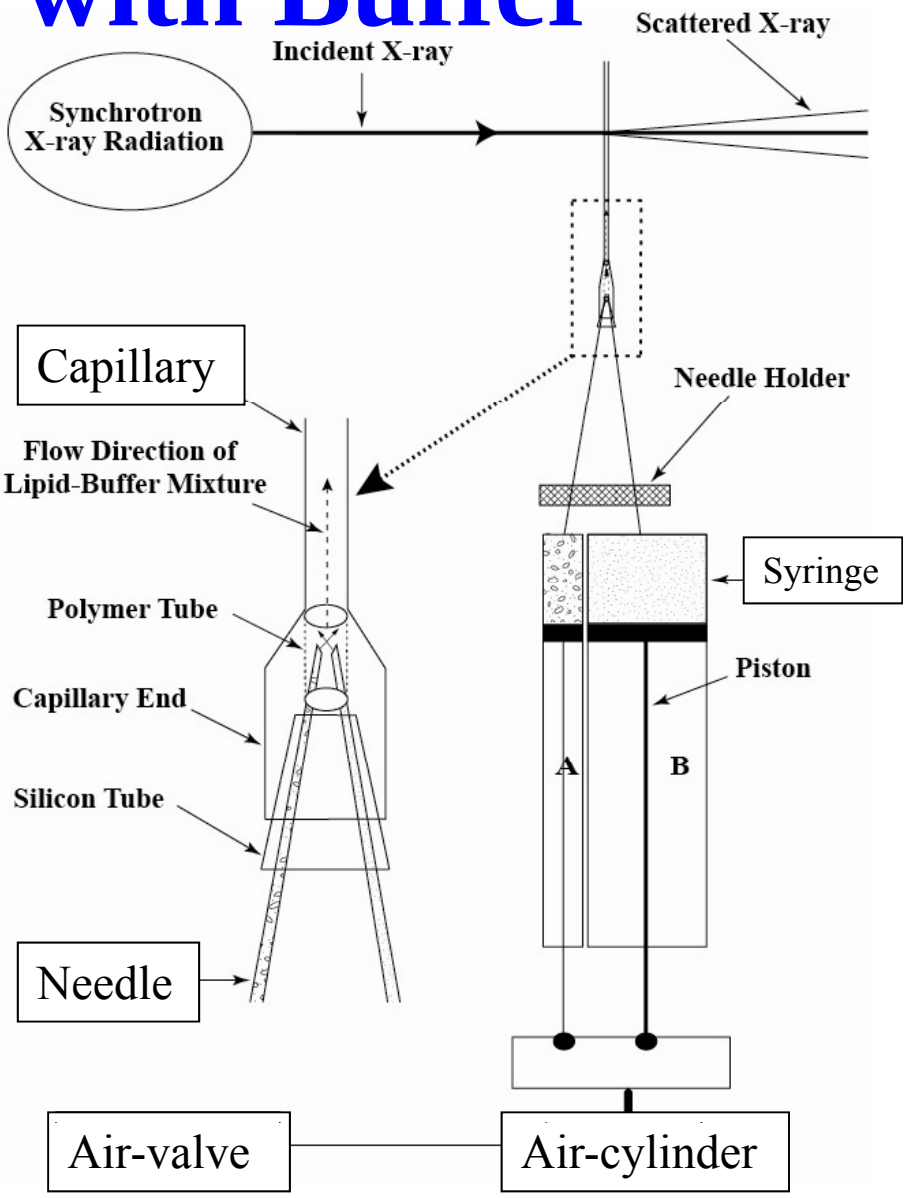
A 4μl

100mM **20%DOPS/80%MO MLV**
10mM ammonium acetate (pH 6.7)
100mM NaCl
5%w/v PEG6000

B 36μl

20mM citrate buffer (various pH)
100mM NaCl
5%w/v PEG6000

Final
lipid conc. 10mM
pH2.6-2.9



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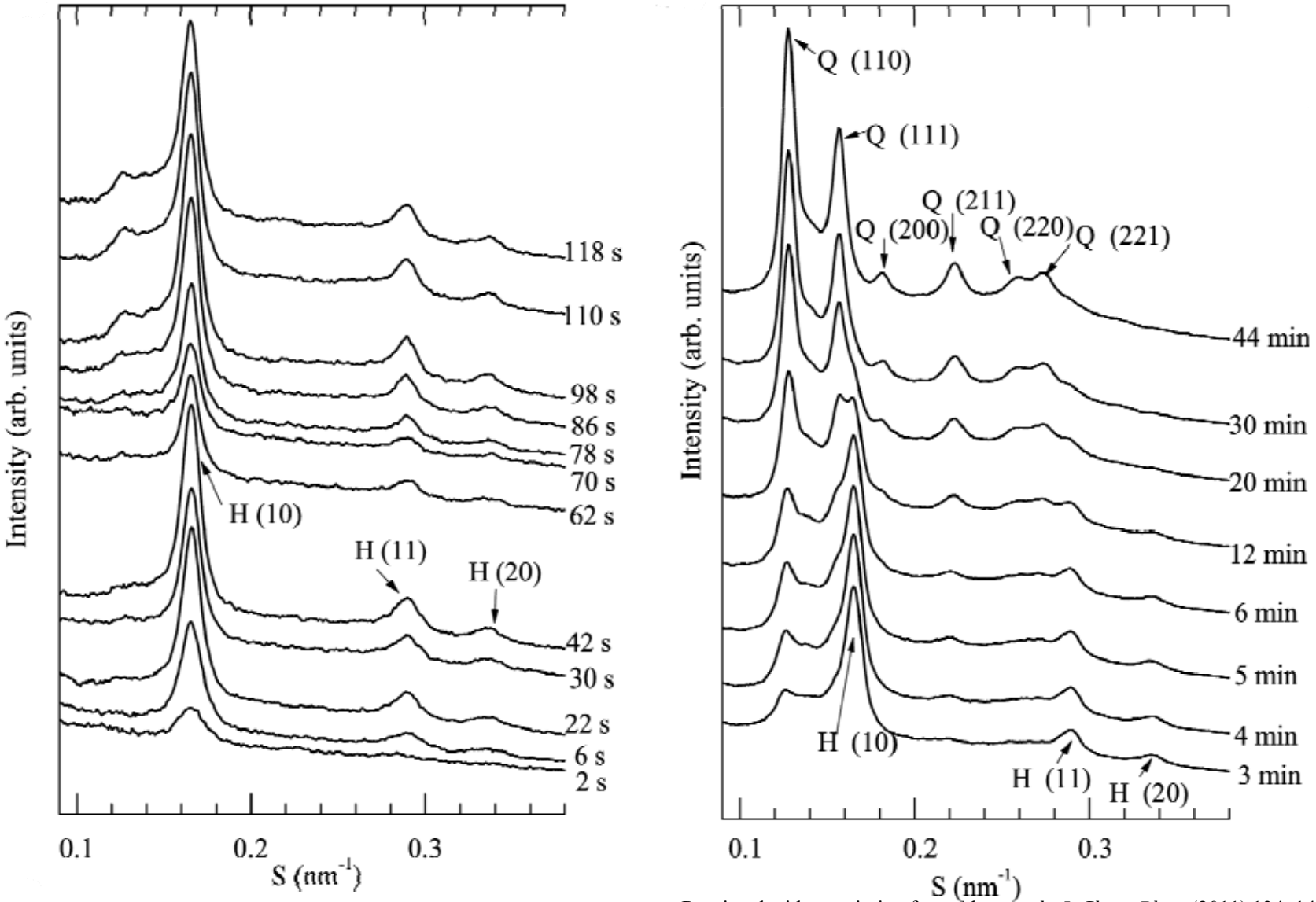
Kinetics of Low pH-induced L_α to Q phase transition in 20%DOPS/80%MO membrane pH 6.7 → pH 2.6



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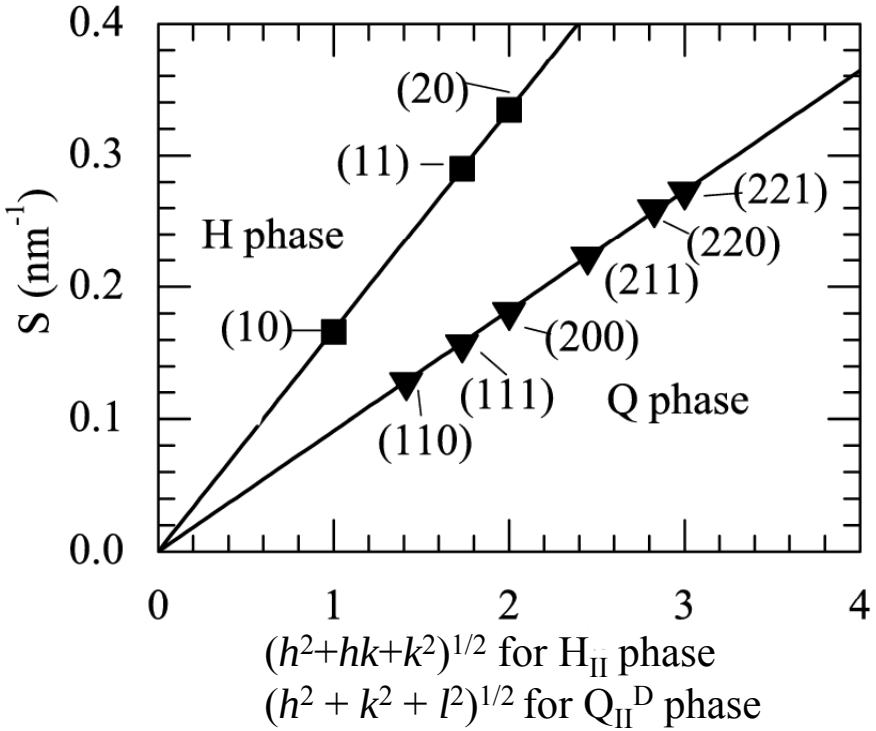
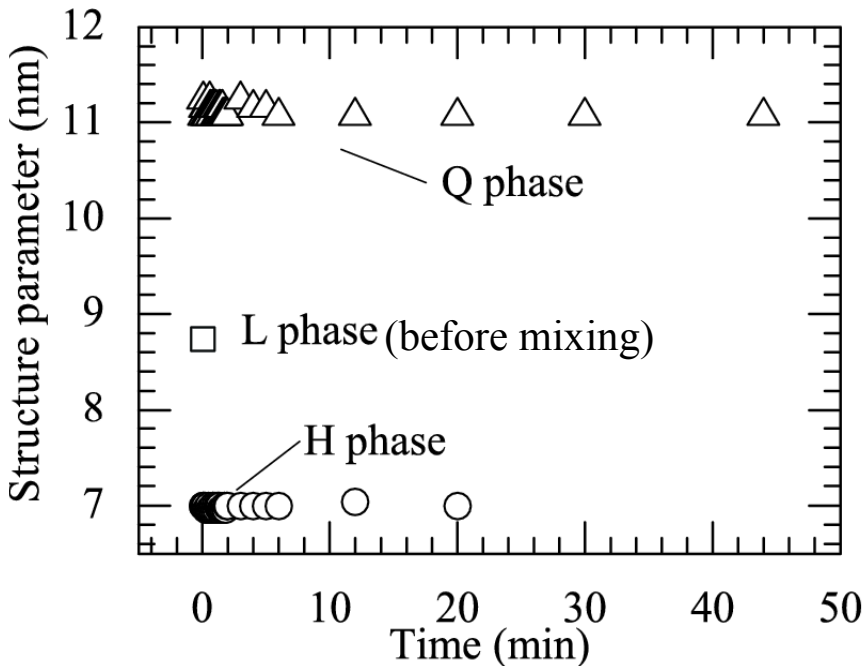
Indexing & Structure Parameters





H_{II} phase $\quad S_{hk} = \frac{2}{\sqrt{3}} \frac{\sqrt{h^2 + hk + k^2}}{a}$

Q_{II}^D phase $\quad S_{hkl} = \frac{\sqrt{h^2 + k^2 + l^2}}{a}$

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Singular Value Decomposition (SVD)

特異値分解

$$\mathbf{A} = \mathbf{U}\mathbf{S}\mathbf{V}^{\mathbf{T}}$$

A : m × n matrix.
U : m × n matrix. $\mathbf{U}^{\mathbf{T}}\mathbf{U}=\mathbf{I}_n$ **(n × n identity matrix)**
V : n × n matrix. $\mathbf{V}^{\mathbf{T}}\mathbf{V}=\mathbf{I}_n$ **(n × n identity matrix)**
S : n × n non-negative diagonal matrix. $(\mathbf{s}_1 \geq \mathbf{s}_2 \geq \cdots \geq \mathbf{s}_n \geq 0)$

データ行列Aがp個の成分からなるとき

$$\mathbf{A}_{ij} = \mathbf{A}(\mathbf{x}_i,\mathbf{t}_j) = \sum_{k=1}^{p} f_k(x_i)c_k(t_j)$$

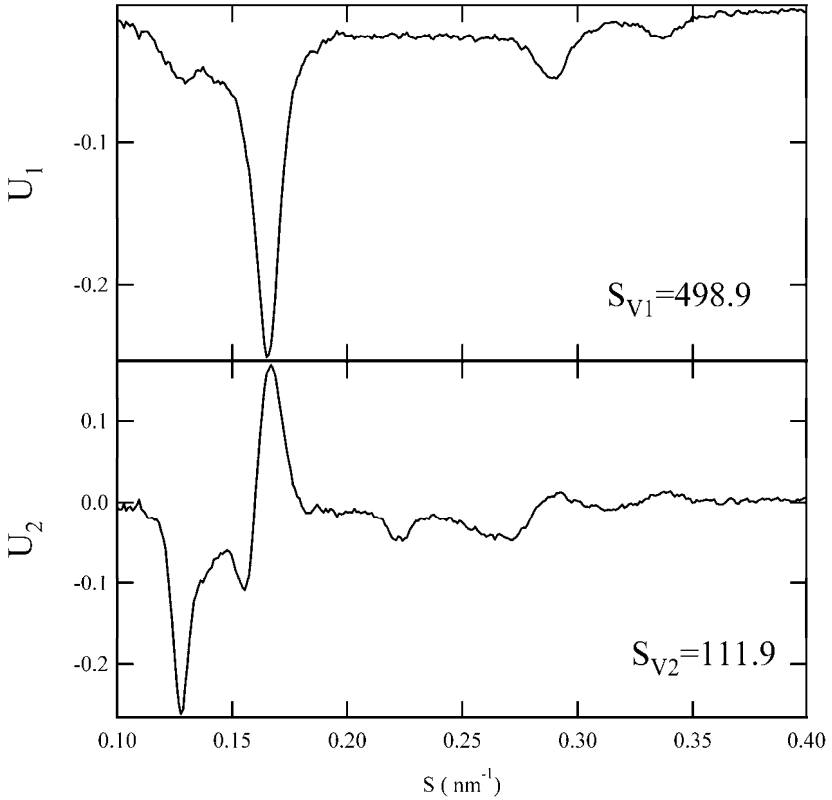
つまり

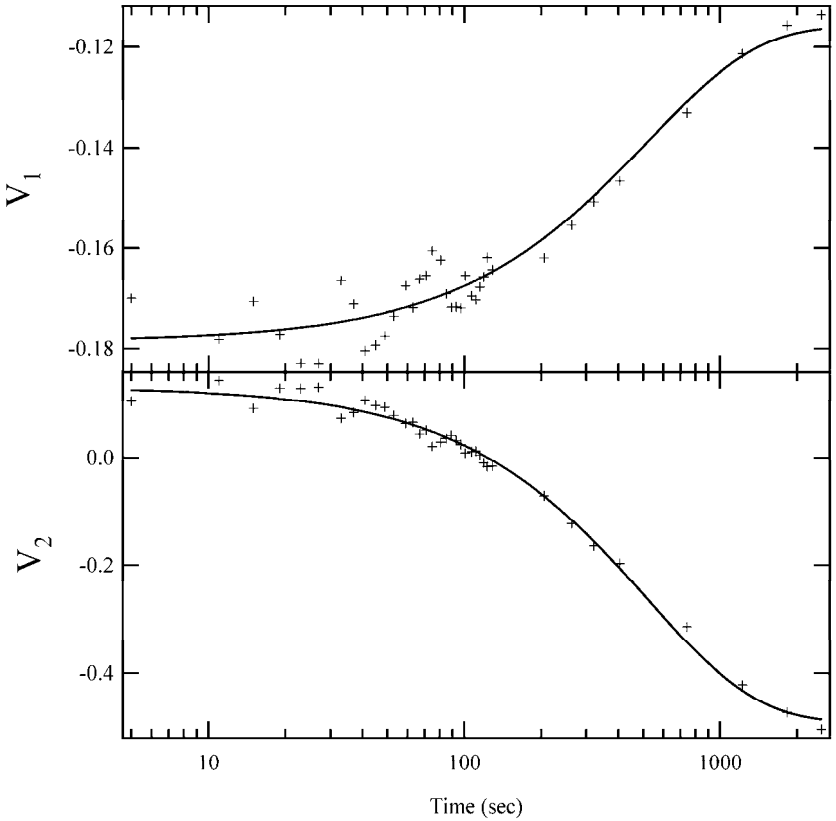
$$\mathbf{A} = \mathbf{F}\mathbf{C}^{\mathbf{T}}$$ **A : m × n matrix , F : m × p matrix, C : n × p matrix**

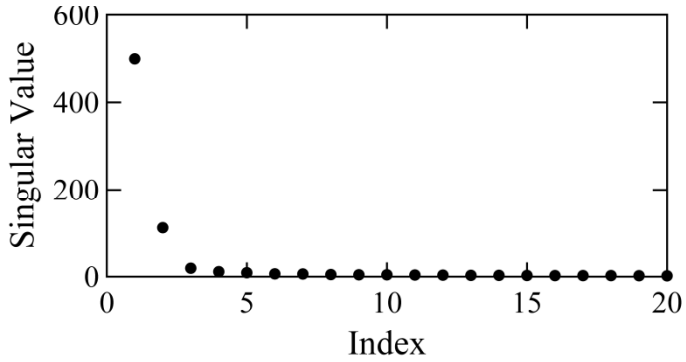
U、S、Vにモデルをあてはめることにより
FおよびCを再構成することができる

Result of SVD

$$I(S,t) \sim S_1 U_1(S) V_1(t) + S_2 U_2(S) V_2(t)$$







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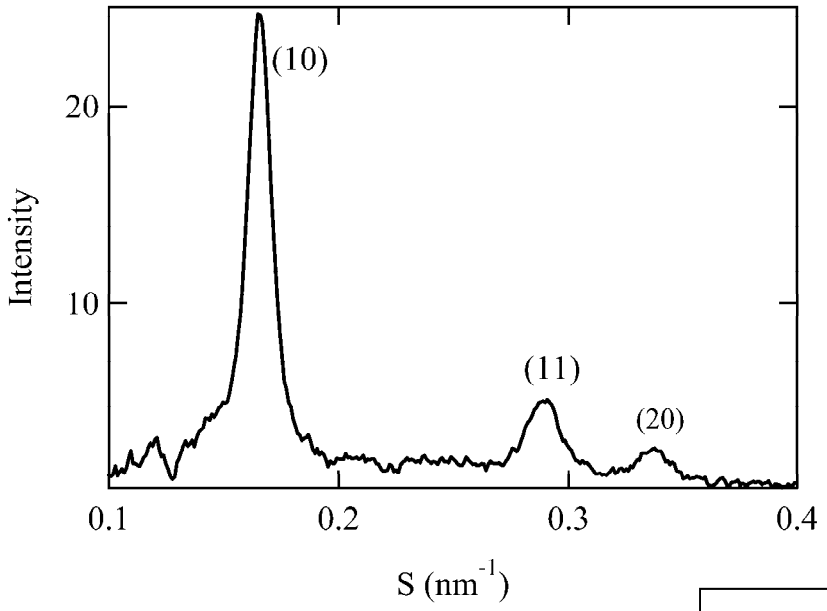
$$V_1 = C_{11} + C_{12} \exp(-kt)$$

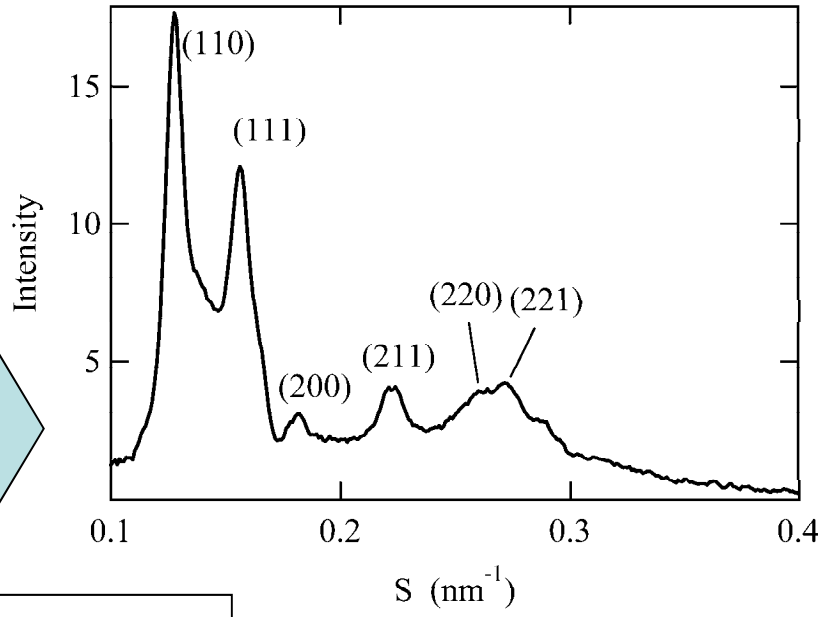
$$V_2 = C_{21} + C_{22} \exp(-kt)$$

Result of SVD

H_{II} phase



Q_{II}^{D} phase

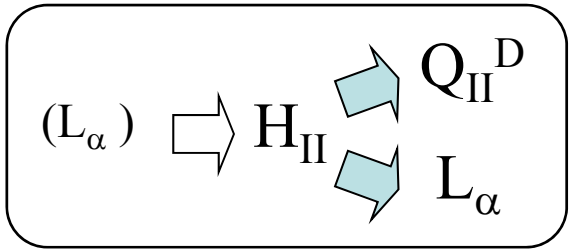


$k = 0.110\ min^{-1}$
$(0.00192\ sec^{-1})$

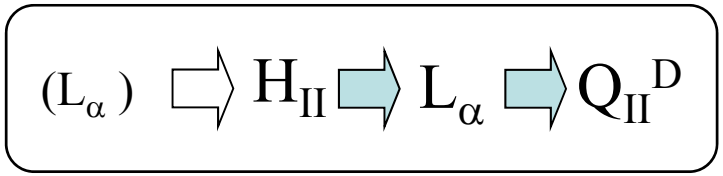
$(L_\alpha) \Rightarrow H_{II} \Rightarrow Q_{II}^{D}$

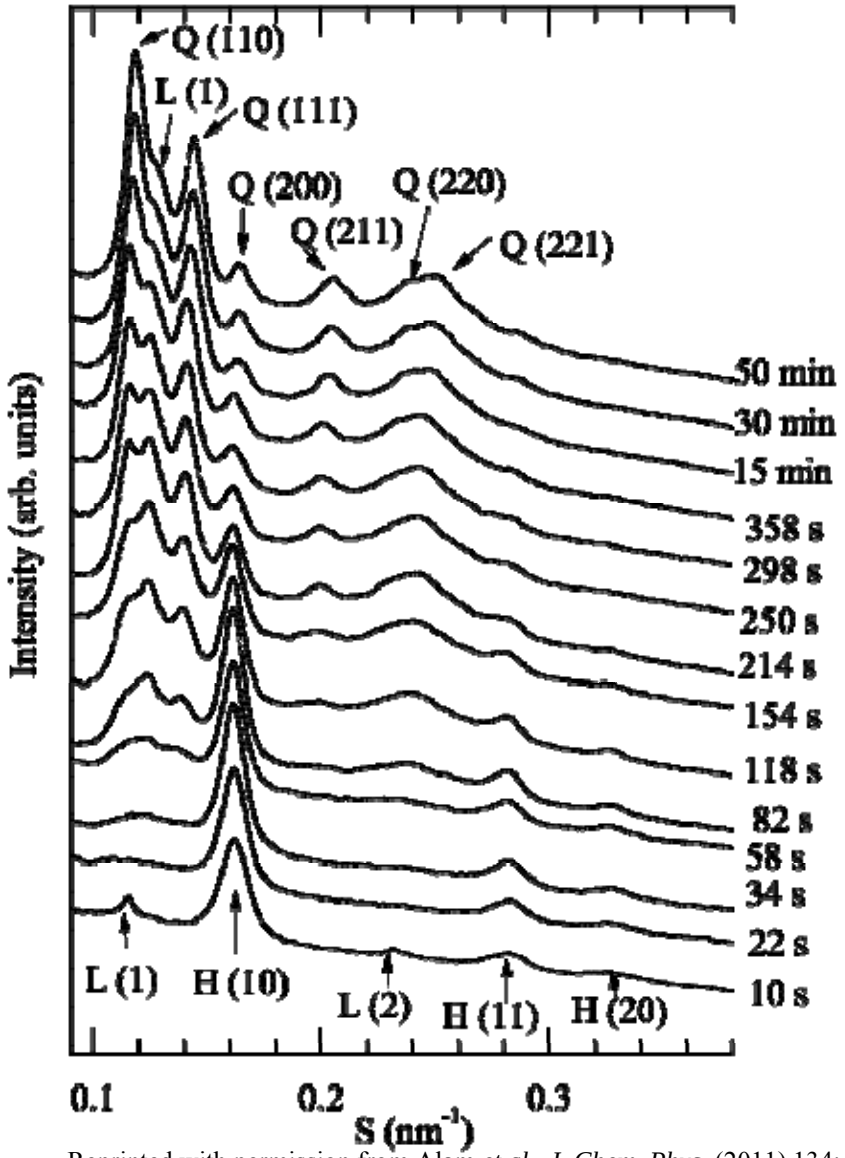
Kinetics of Low pH-induced Lα to Q phase transition in 20%DOPS/80%MO membrane

20%DOPS/80%MO-MLV
in **pH 6.7**
↓
pH 2.9



?





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pH dependence of structure parameter *a* and Rate constant *k* of H_{II} -> Q_{II}^{D} transition

Final pH	Equilibrium state Q_{II}^{D} and L_{α} phases *a* (nm)	Initial state H_{II} phase *a* (nm)	Rate constant of H_{II} -> Q_{II}^{D} *k* (min^{-1})
2.9	**12.0 (Q_{II}^{D}) , 8.0 (L_{α})**	**7.10**	**-**
2.8	**11.7 (Q_{II}^{D}) , 7.7 (L_{α})**	**7.05**	**-**
2.7	**11.4 (Q_{II}^{D})**	**6.99**	**0.138**
2.6	**11.0 (Q_{II}^{D})**	**6.92**	**0.110**

Mechanism of the L_α to the Q Phase Transitions

If we assume that the electrostatic interactions do not change and κ_m greatly, we can consider a following scenario.

Pure MO membrane; $|H_o|$ of the monolayer membrane is large

$\rightarrow \quad \bar{\kappa}_{bil} = 2(\bar{\kappa}_m - 4\kappa_m H_0 \xi) > 0$

$\rightarrow \quad \Delta F = F_{cubic} - F_{L\alpha} = 2(\bar{\kappa}_m - 4\kappa_m H_0 \xi) < K > A < 0$

$\rightarrow$ **Q Phase**

Electrostatic interactions between surface charges in the membrane interface increase

$\rightarrow |H_o|$ of the monolayer membrane $\downarrow$

$\rightarrow$ a decrease in $\bar{\kappa}_{bil}$

$\rightarrow$ a **Q to Lα phase transition** occurs at $\bar{\kappa}_{bil} = 0$.

Adv. Planar Lipid Bilayers and Liposomes, 9, 163-209, 2009, Yamazaki

Mechanism of the L_α to the H_{II} Phase Transitions

free energy difference

Marsh, *Biophys.J.* (1996) **70**, 2248

$$\Delta F_{H-L} = F_H - F_{L_\alpha} = \left(\mu_{ch} - 4\kappa_m H_0^{\,2}\right)A$$

μ_{ch}: packing energy of hydrocarbon chain (>0)

$|H_0| < \sqrt{\mu_{ch}/4\kappa_m}$: H_{II} phase is more stable

$|H_0| = \sqrt{\mu_{ch}/4\kappa_m}$: H_{II}-L_α transition

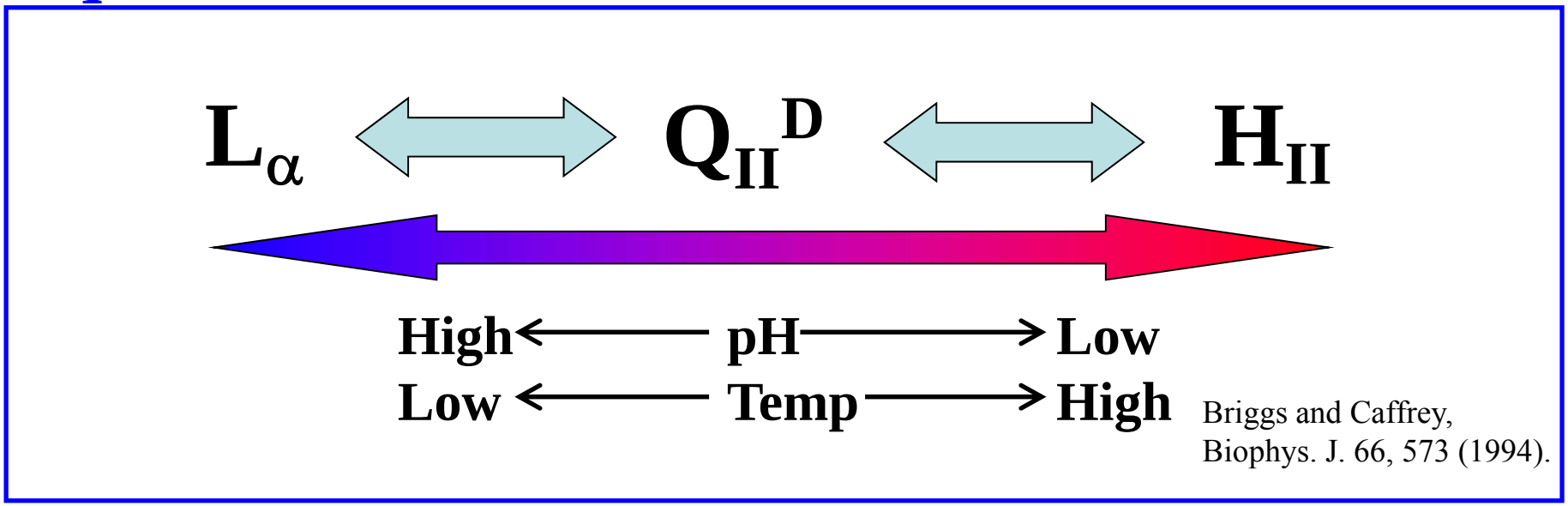
$|H_0| > \sqrt{\mu_{ch}/4\kappa_m}$: L_α phase is more stable

Electrostatic interactions between surface charges in the membrane interface increase

→ $|H_o|$ of the monolayer membrane ↓

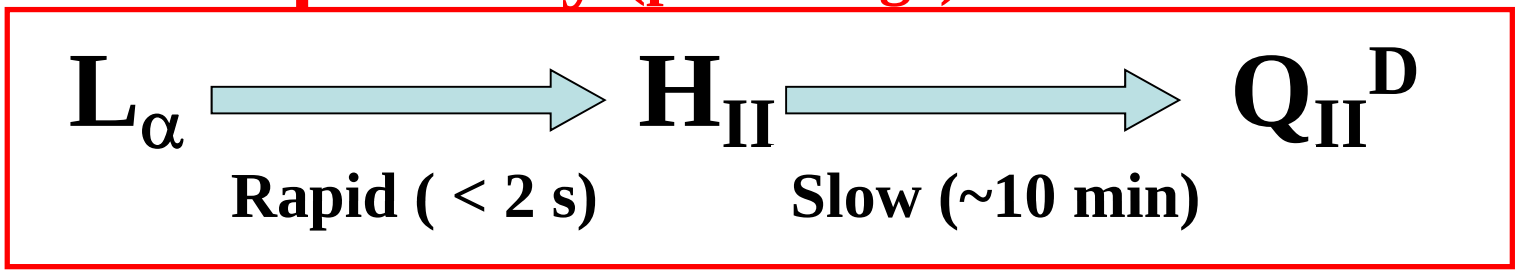
→ a **H_{II} to Lα phase transition** occurs.

Equilibrium state



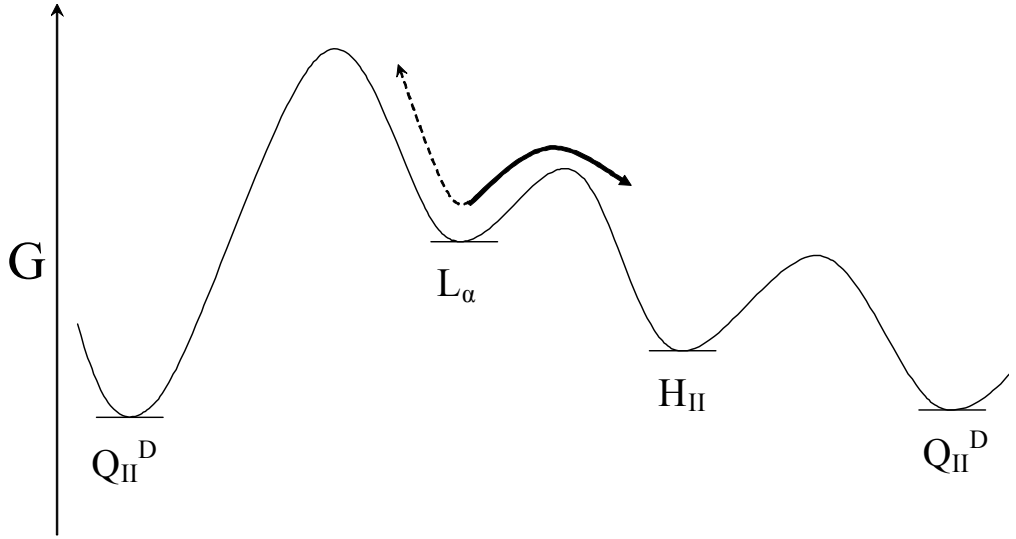
Briggs and Caffrey,
Biophys. J. 66, 573 (1994).

Kinetic pathway (pH change)



A kinetic pathway of the low-pH induced L_α to Q_{II}^D phase transition.

$$L_\alpha \xrightarrow[\text{Rapid (< 2 s)}]{} H_{II} \xrightarrow[\text{Slow (15~30 min)}]{} Q_{II}^D$$



If the **activation energy** of the **rate-determining step** from the L_α to Q_{II}^D phase is much larger than that from the L_α to H_{II} phase, the L_α phase first transforms into the H_{II} phase and then to the Q_{II}^D phase.

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<Summary>

For **final pH 2.6-2.9**, the **L_α phase** changed into the **hexagonal II (H_{II}) phase** completely less than 2 s after the mixing of low pH buffer with **multilamellar vesicle of 20%-DOPS/80%-MO** suspension (i.e., the initial step), and then the H_{II} phase slowly converted into the **Q_{II}^{D} phase** from 20-50 s and completely disappeared less than 15-30 min (i.e., the second step), which depended on the final pH.

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