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Heavy water effect on hydrated lipid systems in bicontinuous cubic phase

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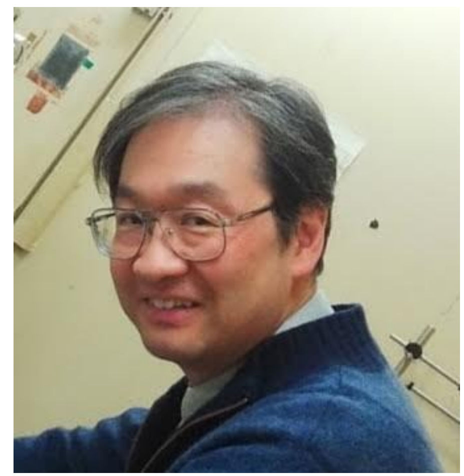
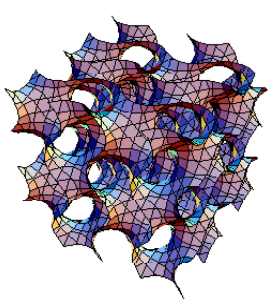
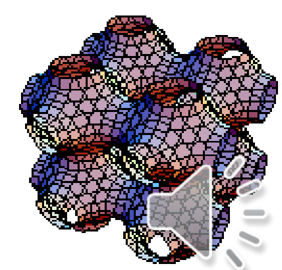




ESS Symposium on Crystallography for Soft Matter： 7-8 September 2015
Institute for Macromolecular Chemistry, Prague



Contrast variation method of neutron scattering

Example: structural study on short-chain phospholipid micelle in sugar alcohol solution

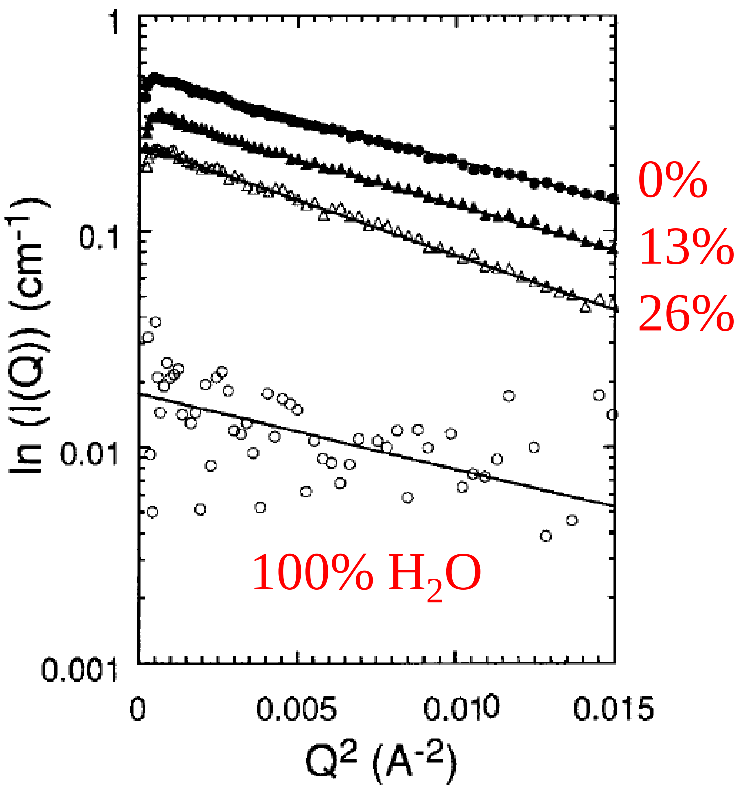


Fig. 4 Guinier plots for dC(6)PC micelles (50 mM) in the presence of 2 M sorbitol. The scattering intensities change with the H_2O/D_2O ratio. (●) 0, (▲) 13, (△) 26, and (○) 100 vol% H_2O

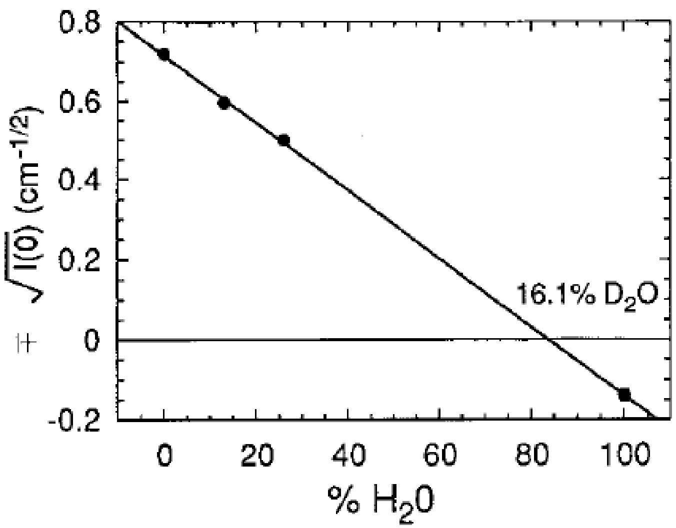
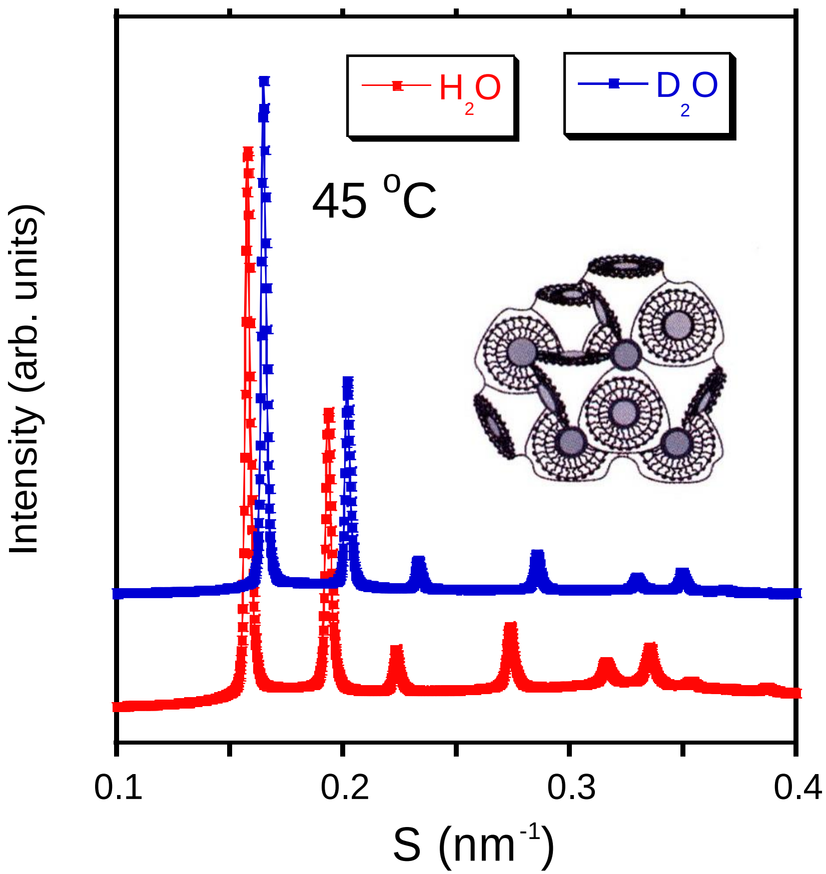
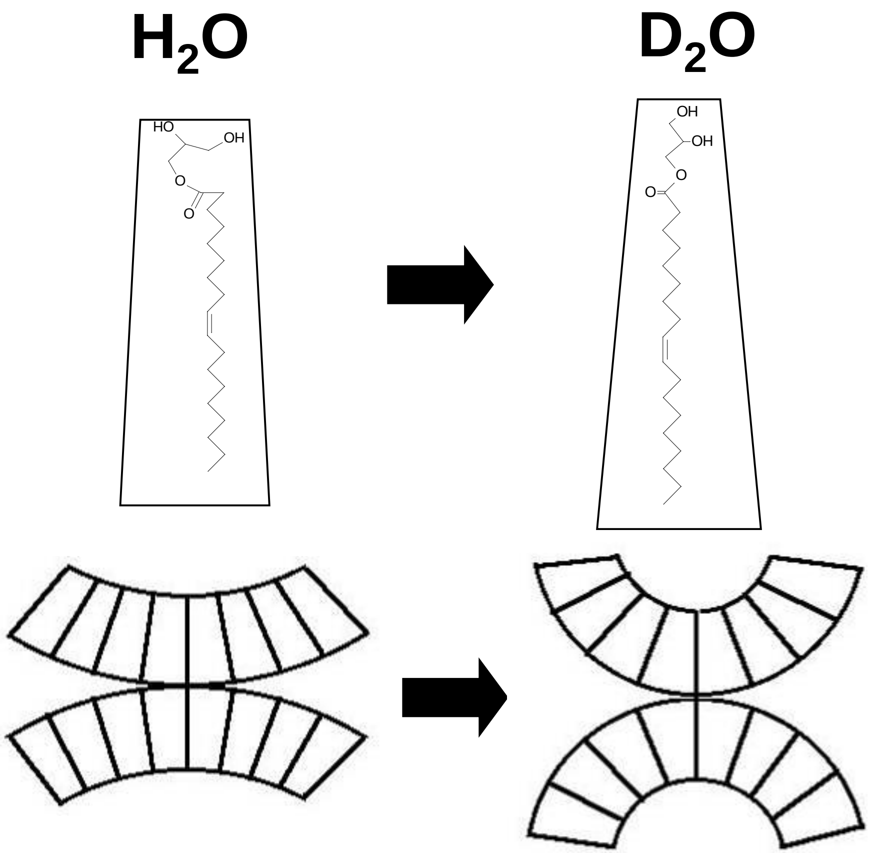


Fig. 5 Result of contrast variation: The zero-intensity intercept occurs at 16.1 vol% D_2O in the solvent

H. Takahashi, M. Imai, Y. Matsushita and I. Hatta
Small-angle neutron scattering study on short-chain phosphatidylcholine micelle in the presence of sorbitol
Prog. Colloid Polym. Sci. **106**, 223-227 (1997)

Main Points



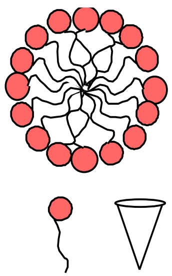


D_2O reduces the lattice constant of the cubic phase.

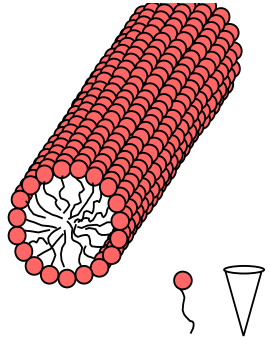
Molecular shape is changed !

Structures of Lipid Assemblies

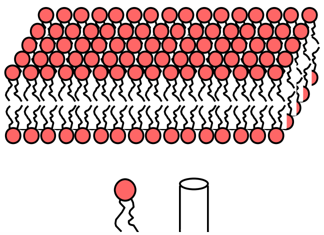
Spherical Micelle



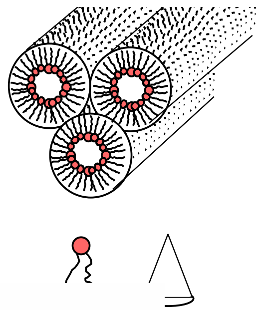
Rod Micelle



Lamellar (Bilayer)



Hexagonal II (H_{II})

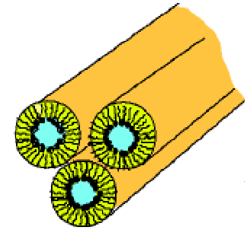


Bicontinuous Cubic

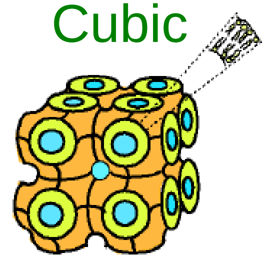
(Lipidic Cubic Phase)

Typical X-ray diffraction patterns

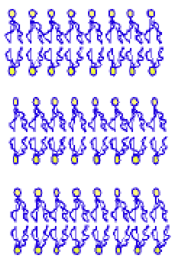
Hexagonal II (H_{II})

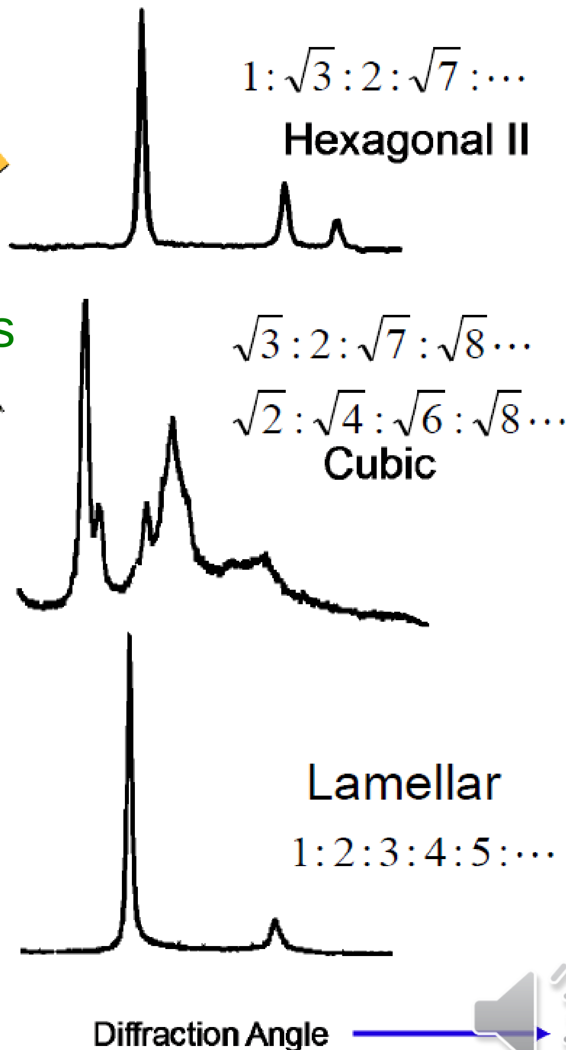


Bicontinuous Cubic



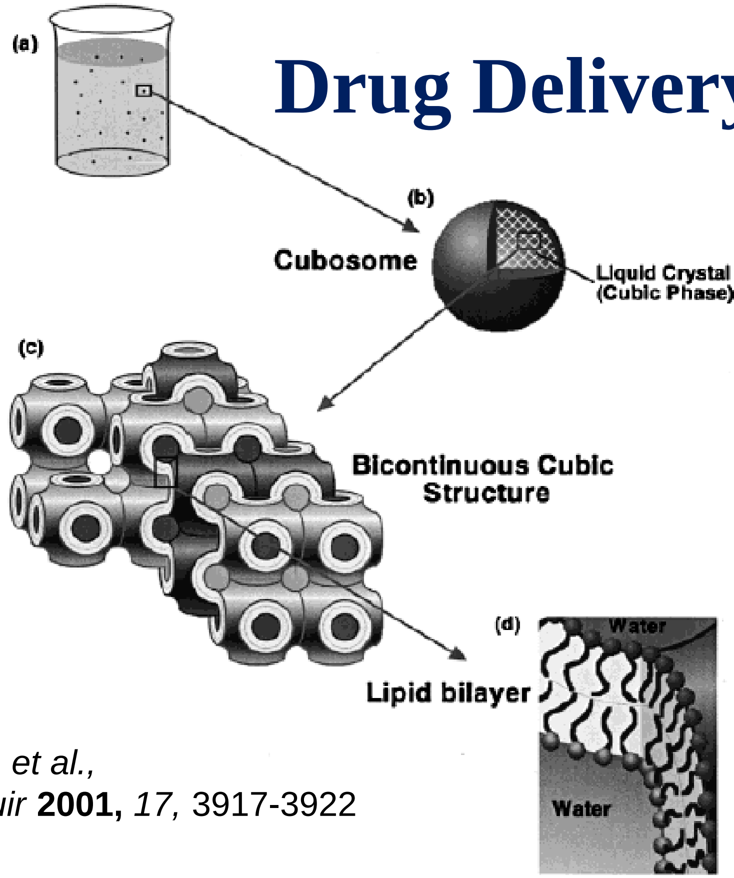
Lamellar



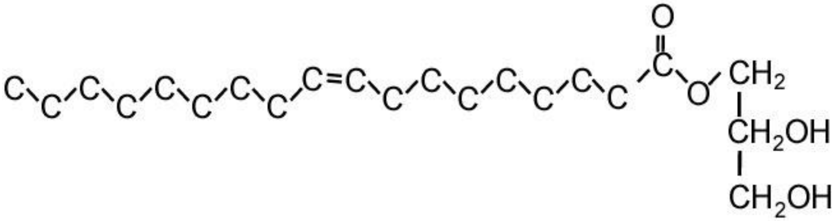


Application Area: Lipidic Cubic (Q) Phase

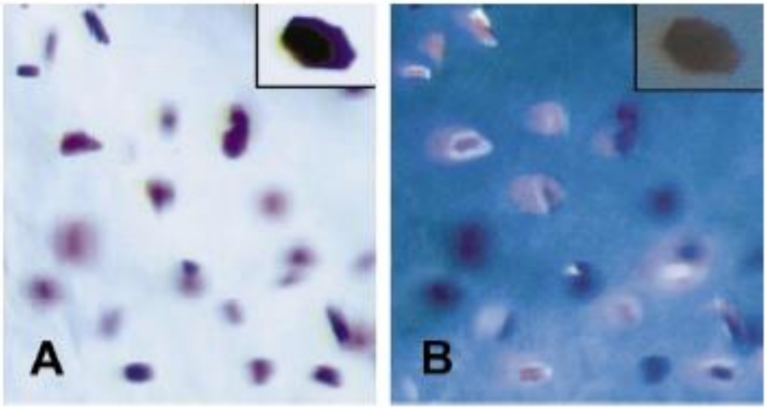
Drug Delivery

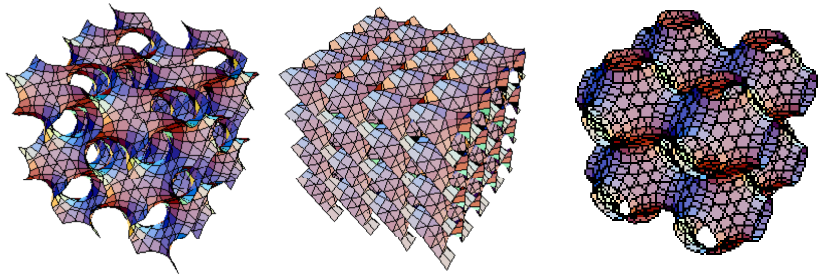


Nakano *et al.*,
Langmuir **2001,** *17,* 3917-3922



Crystallization of Membrane Protein





Ia3d Pn3m Im3m

Monoolein (MO)

1-monooleoyl-*rac*-glycerol

Heavy water effects

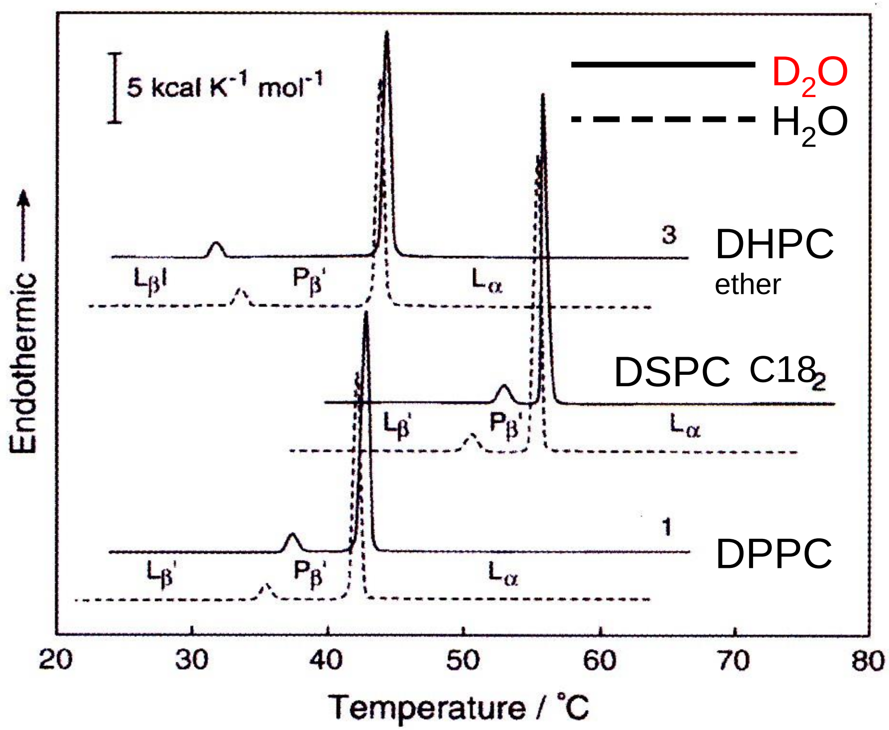
- Biological effects
  - High concentration D_2O is toxic for most mammals.
  - D_2O affects cell growth rate, circadian rhythm, *etc.*

- Effects of D_2O on lipid systems
  - D_2O induces about 0.5 ^{o}C increase of the main transition temperature of phospholipid bilayers.[a]
  - D_2O reduces the lamellar spacing of DPPC bilayers by about 0.01 nm. [b]

→ *Small change !*

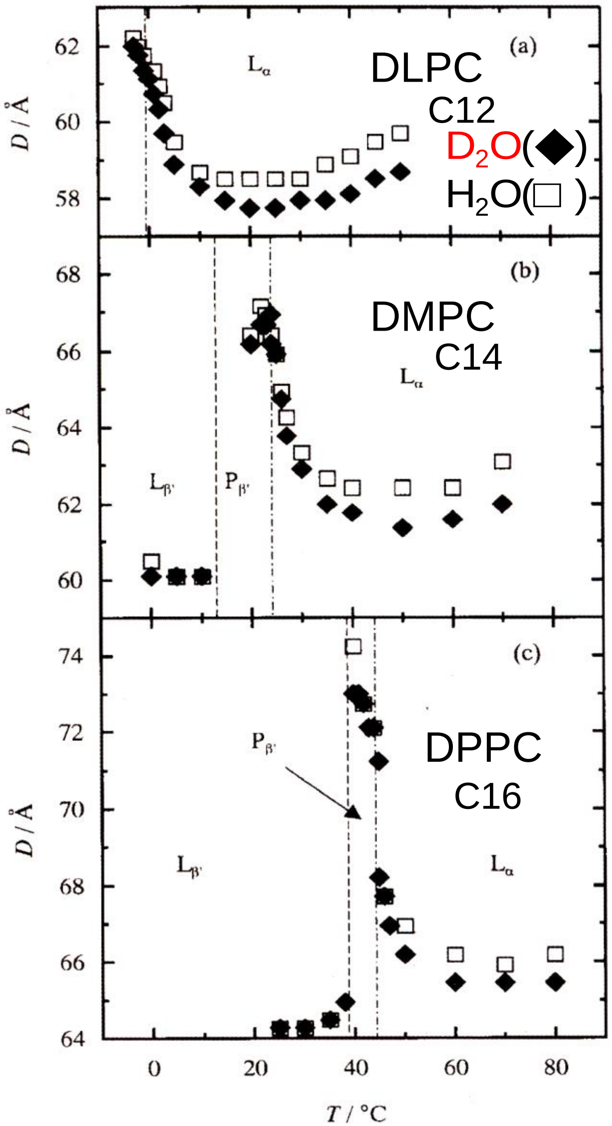
[a] Matsuki *et al*., *Biochem. Biophys. Acta* **1712** (2005) 92-100
[b] Kobayashi and K. Fukada, *Chem. Lett.* **27** (1998) 1105-1106

Previous studies for lipids



DSC heating thermograms for PC bilayers membranes.

H. Matsuki et al, *Biochim. Biophys. Acta* **1712** (2005) 92-100



Effect on the Lamellar spacing.

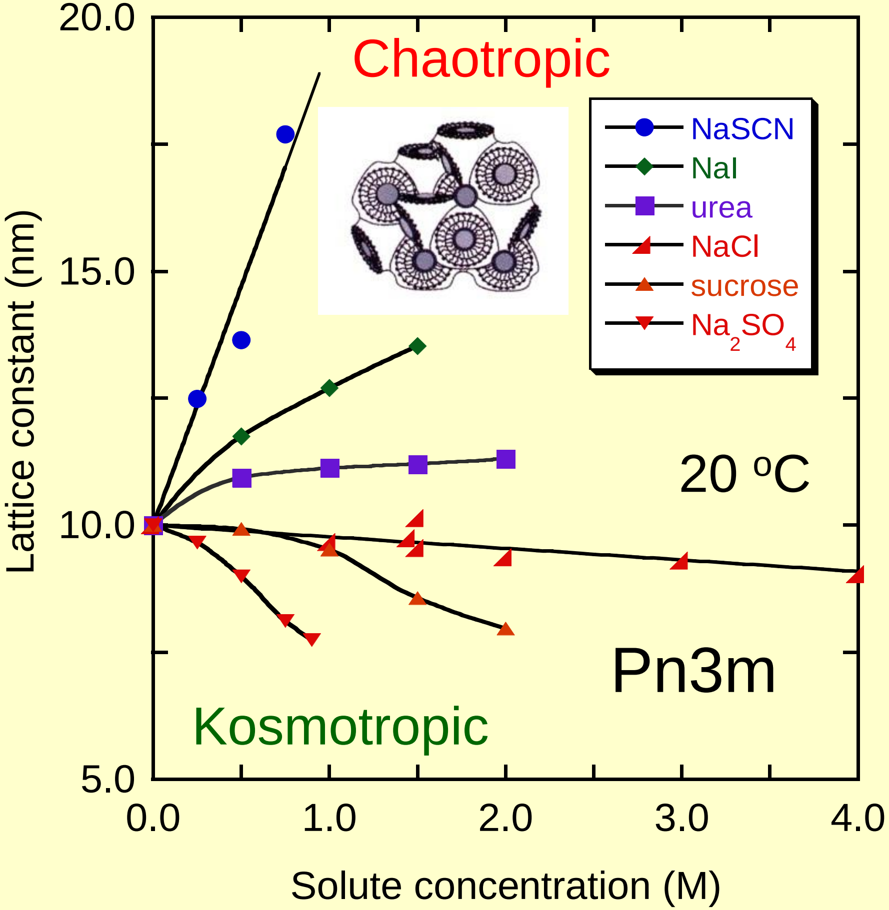
Y. Kobayashi and K. Fukada, *Chem. Lett.* **27** (1998) 1105-1106

Object of this study

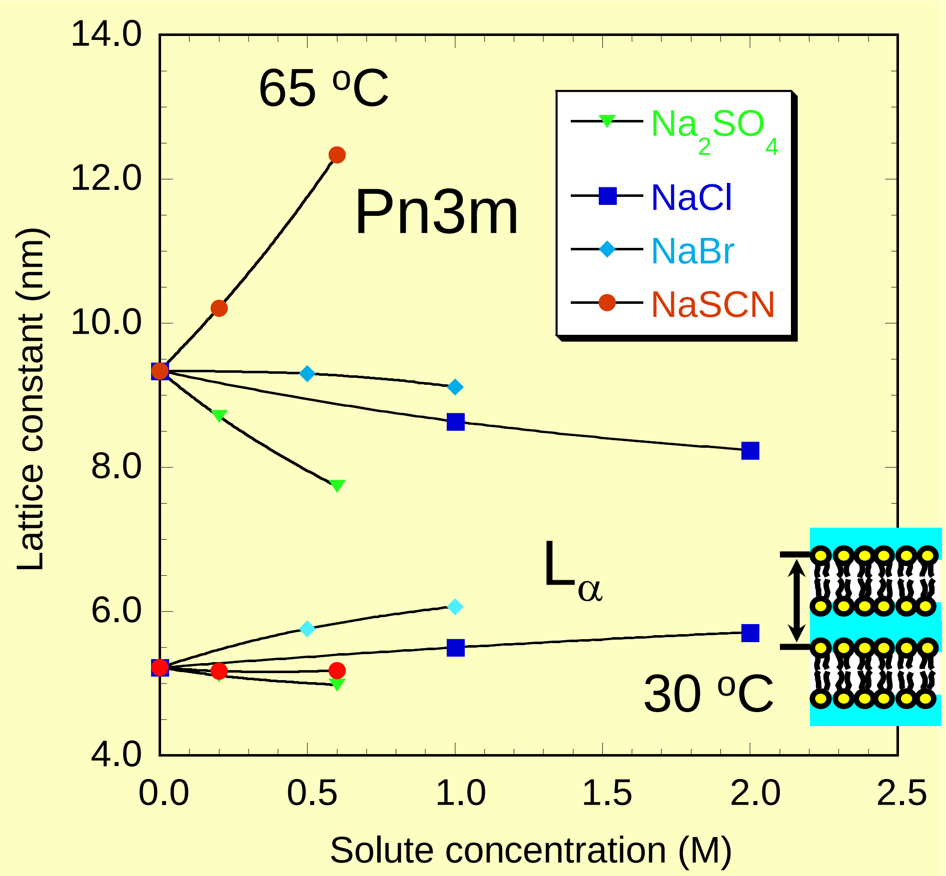
- To reveal how D_2O affects biomembranes, here we studied
  - The effect of D_2O on structural properties of lipid-water systems
  - For various phases
  - Using various lipid molecules
  - Paying attention to bicontinuous cubic phases

Cubic phase : Big Change
In line with Hofmeister series

[1] Monoolein (MO) Pn3m Cubic



[2] Monoelaidin (ME) Pn3m Cubic & L_α

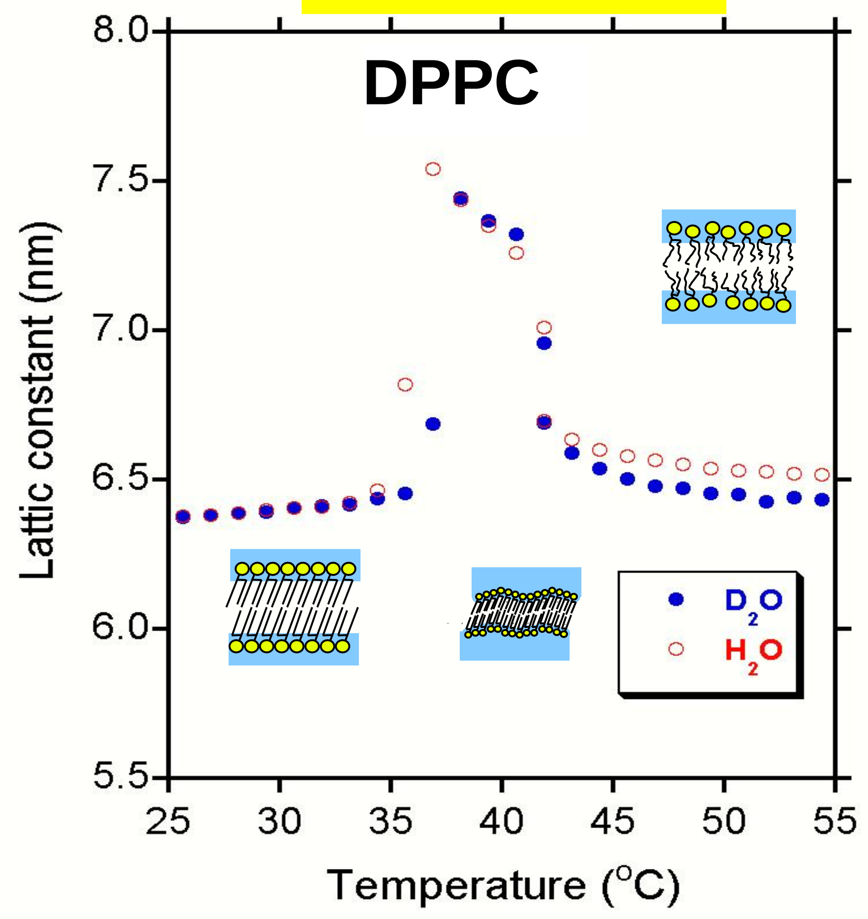


[1] Takahashi,H., *et al*., *Mol.Cryst.Liq.Cryst*. 374 (2000) 231-238.

[2] Takahashi,H., *et al*., I. *PCCP* 4 (2002) 2365-2370.

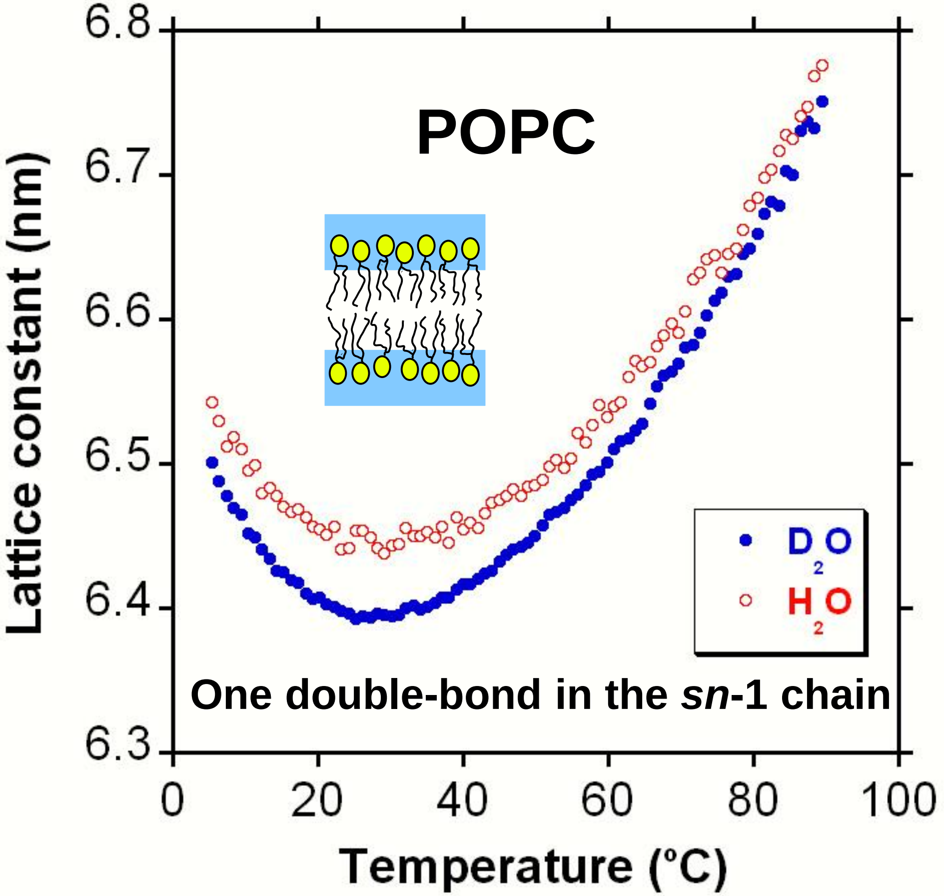
Effect of D_2O on the lamellar phase

Saturated Lipid



Liquid-crystalline phase
0.12 ± 0.01 nm

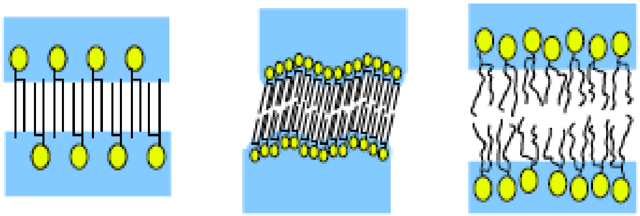
Unsaturated Lipid



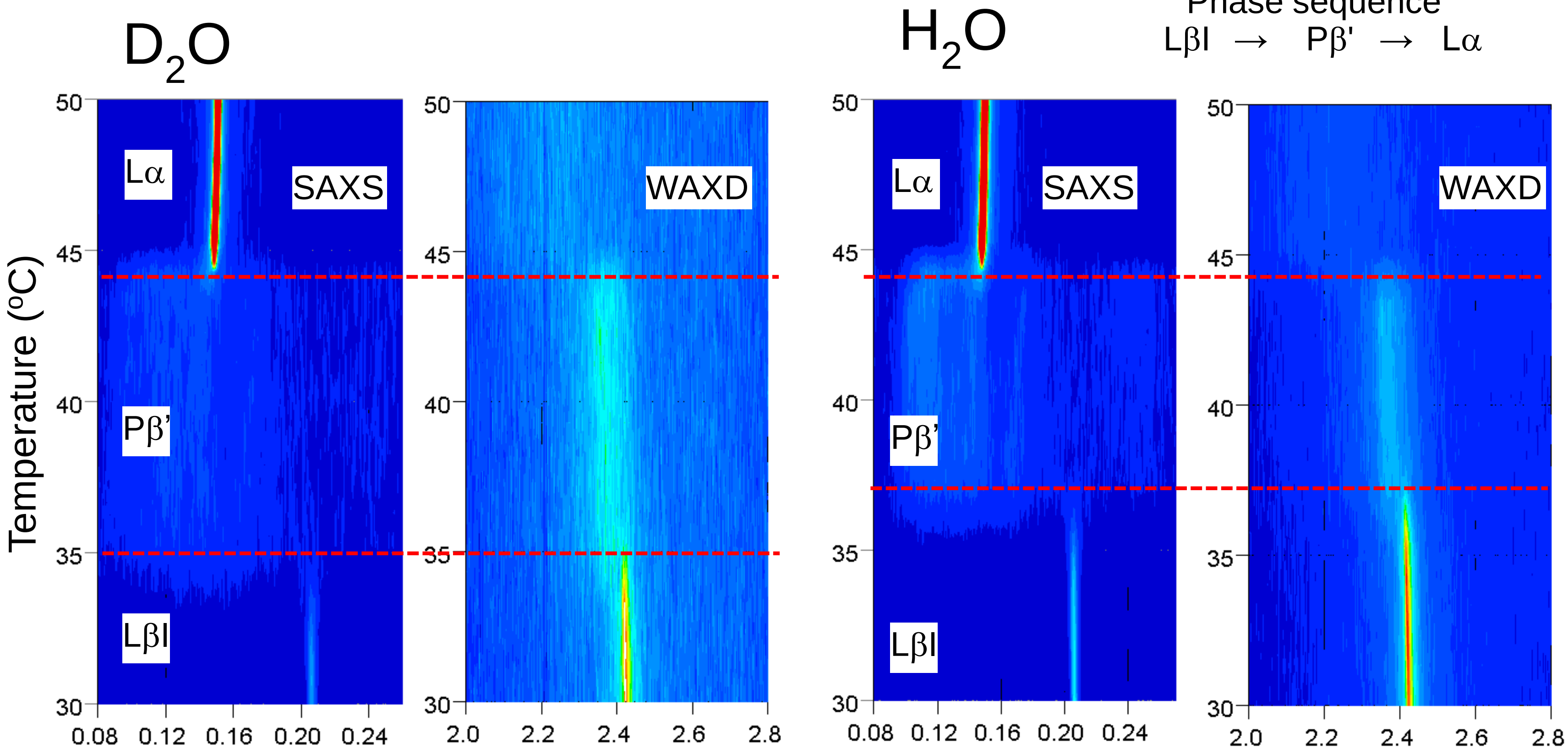
0.04 ± 0.01 nm (5-82 °C)
0.049 ± 0.006 nm (20-40 °C)

SAXS/WAXD of Photon Factory (Japan)

DHPC
(Dihexadecyl-phoshatidylcholine)



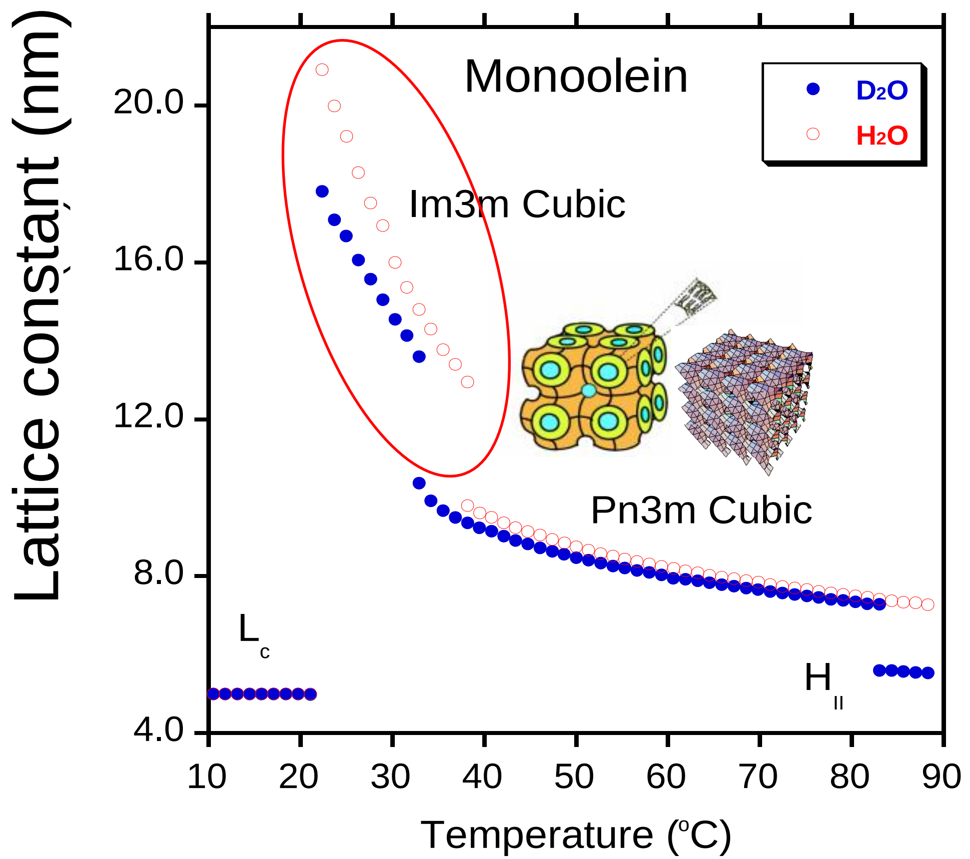
Phase sequence
Lβl → Pβ' → Lα

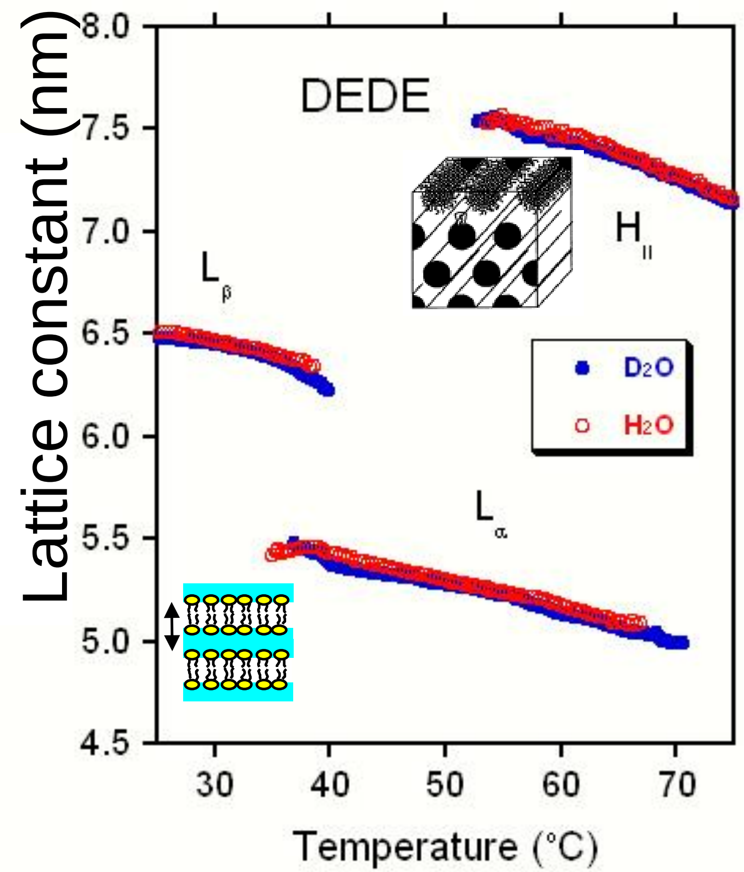


$$S = \frac{1}{d} = \frac{2\sin\theta}{\lambda}$$

Scan rate: 1.0 °C/min *Good agreement with the previous DSC results !*

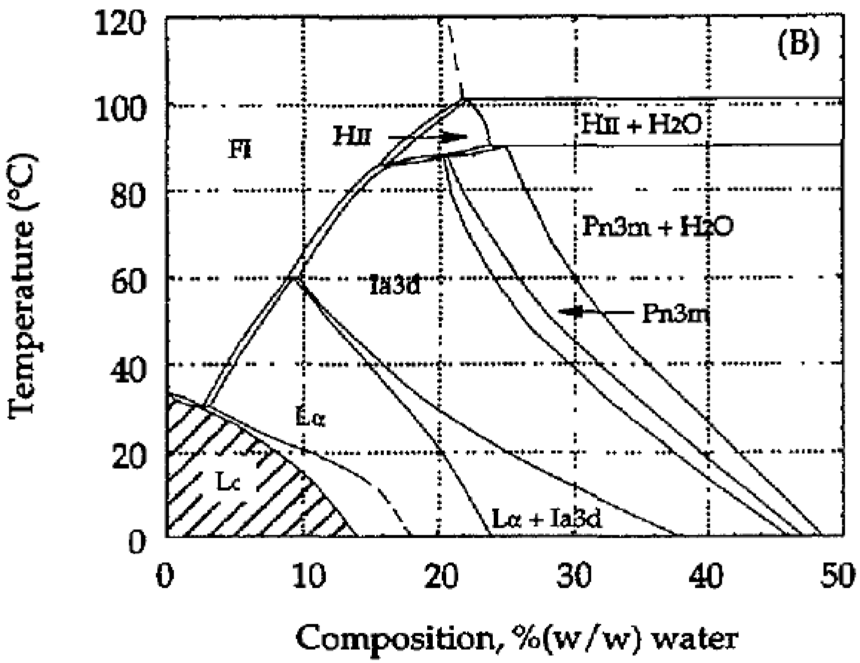
Effect of D_2O substitution for H_2O on the lattice constants of various phases

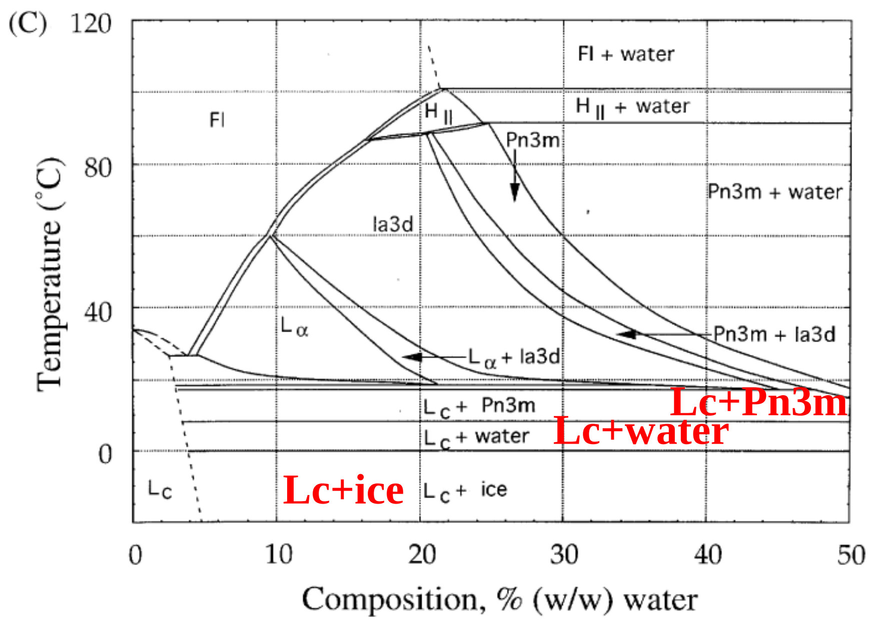




Big differences were observed in cubic phases!

Phase behavior of Monoolein in excess water



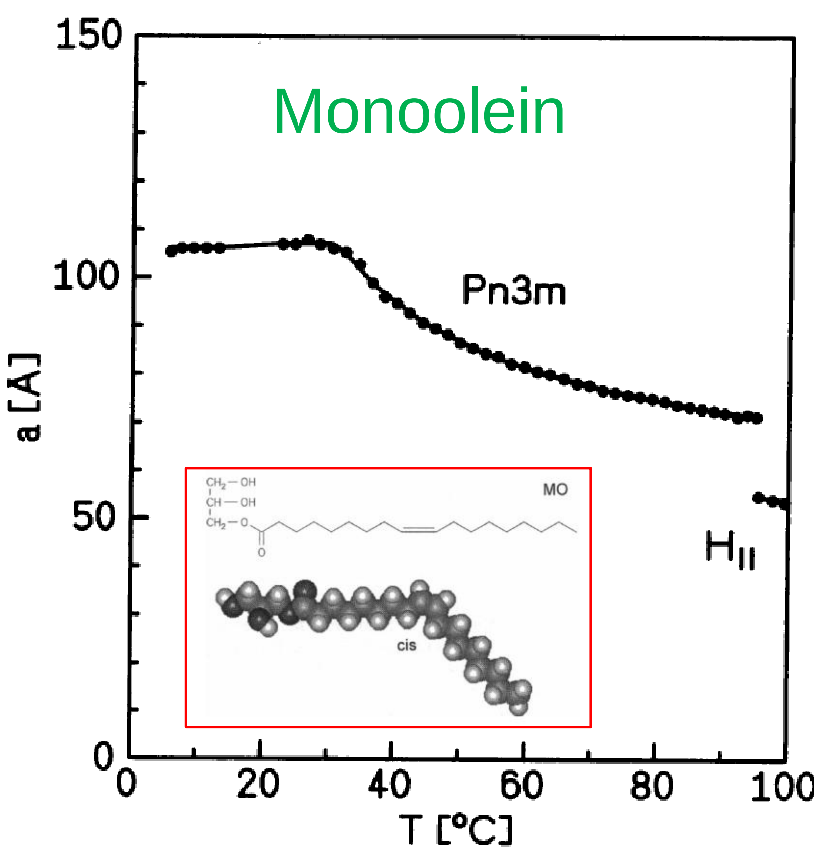


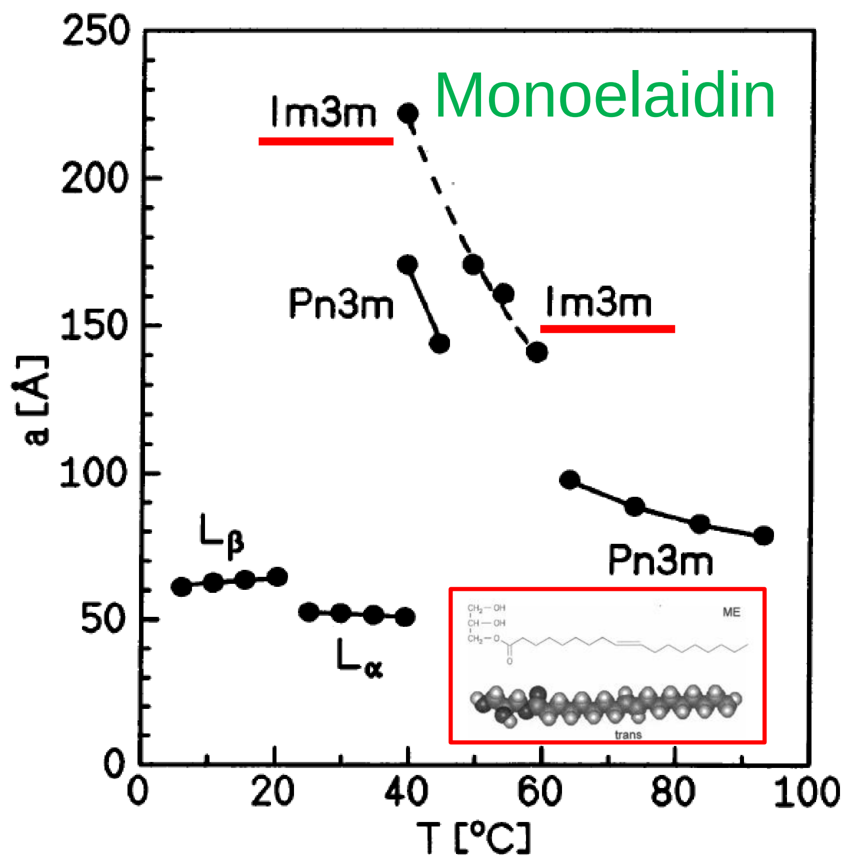
J. Briggs, H. Chung, M. Caffrey.
J. de Physique II, **6** (1996) 723-751.

H. Qiu, M. Caffrey,
Biomaterials **21** (2000) 223-234.

No Im3m Phase !

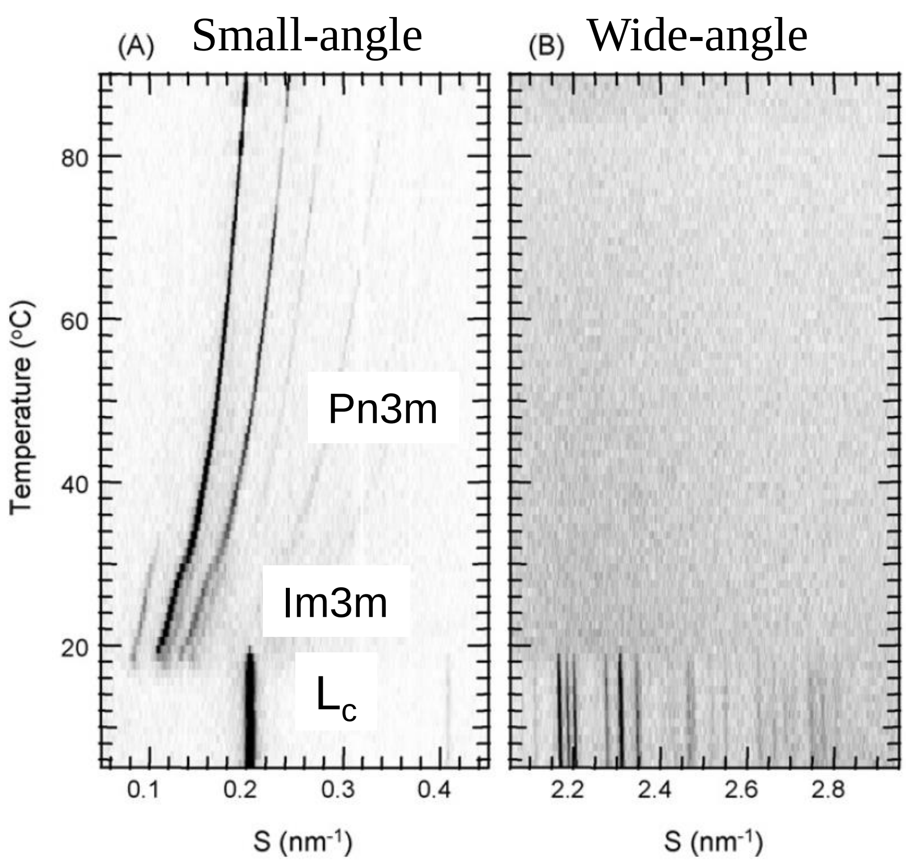
Phase sequences of Monoolein and Monoelaidin (Temp-scan)



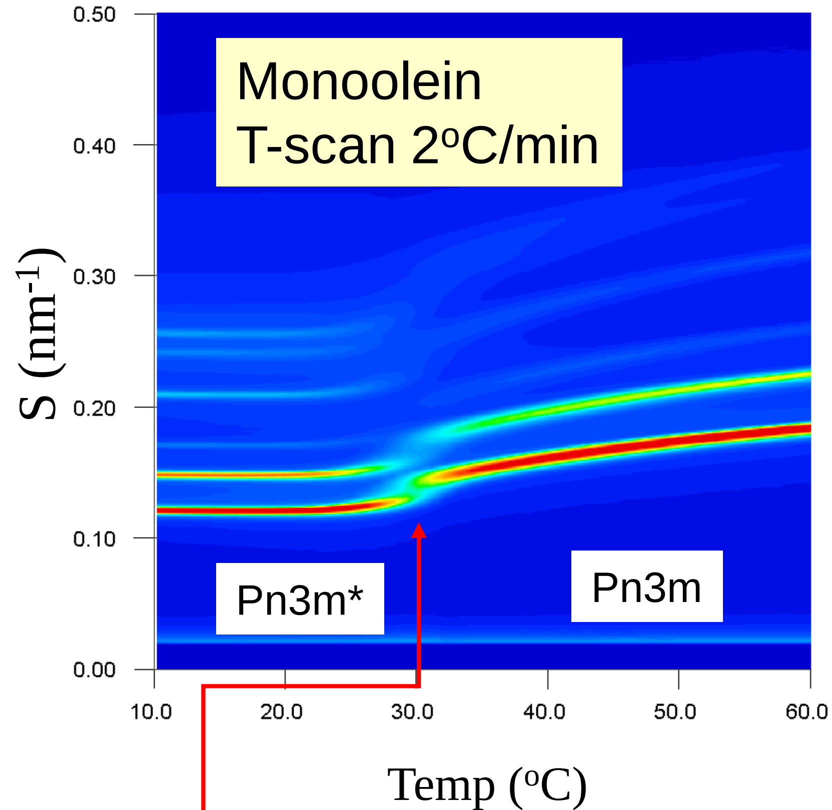


C. Czeslik, R. Winter, G. Rapp, K. Bartels, *Biophys J.* **68** (1995) 1423-1429.

Im3m & Two different Pn3m Phases ?



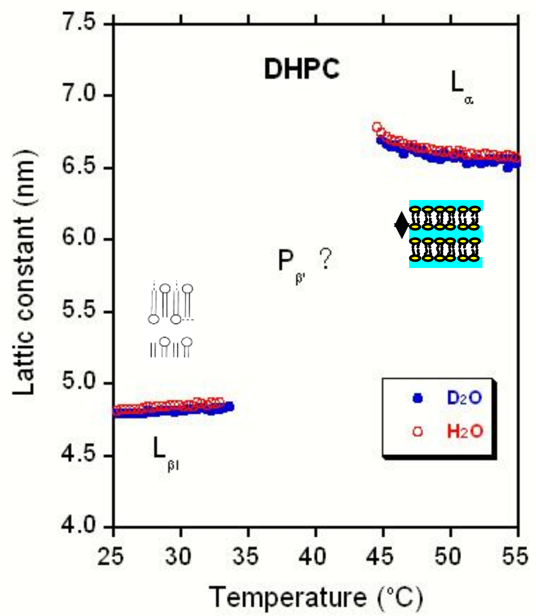
Monoolein dispersed in 12wt% DMSO solution T-scan: 2 ^{o}C/min

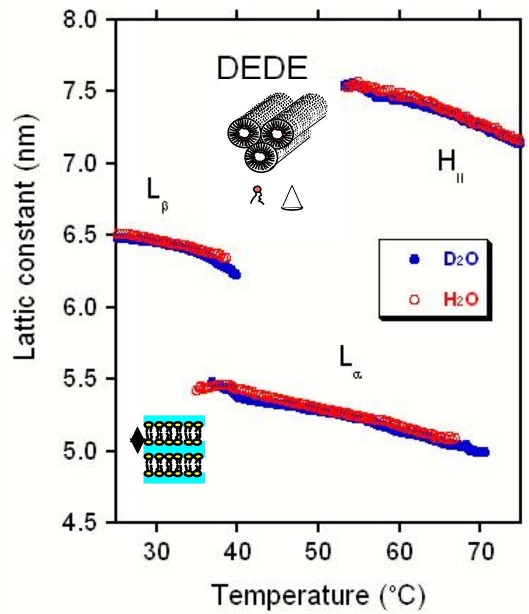


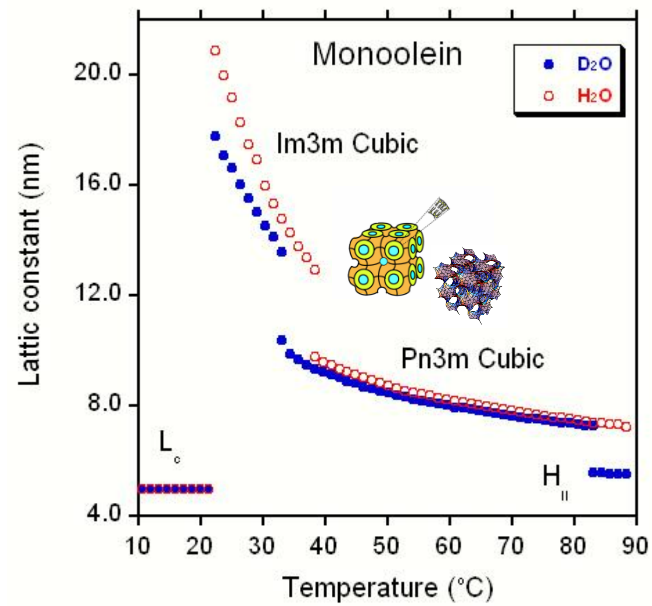
Phase Transition ?

S. Abe, H. Takahashi, *Chem. Phys. Lipids* **147** (2007) 59-68.

Effect of D2O on the various phases







Mean Values of Lattice Constants

	DHPC $L_{\beta'I}$	DHPC L_α	DEPE L_β	DEPE L_α	DEPE H_{II}	MO L_c	MO Im3m	MO Pn3m
H_2O	4.85	6.63	6.45	5.29	7.37	5.00	16.42	9.08
D_2O	4.82	6.59	6.40	5.21	7.37	5.00	14.12	8.28
D_2O/H_2O	0.994	0.994	0.992	0.985	1.00	1.00	0.860	0.912

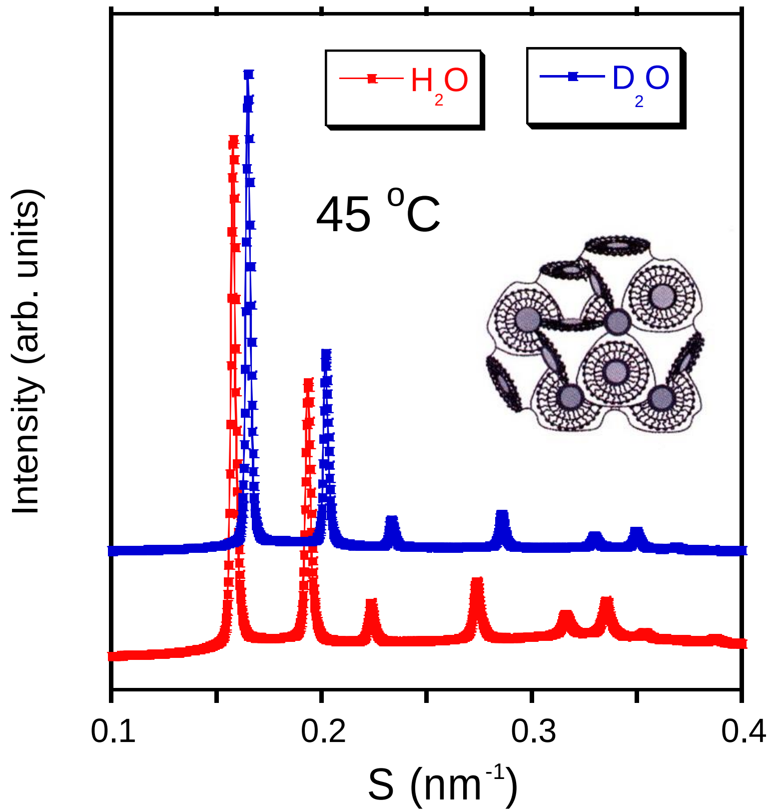
H. Takahahsi & K. Jojiki, *Chem. Lett.* **41** (2012) 1101-1103

Discussion

- D_2O reduces the lattice constants of cubic phases, being similar to Kosmotrope.
- Kosmotrope is believed to stabilize water-water interactions.
- Deuterium bond has been reported to be stranger than hydrogen bond.
- D_2O prefers D_2O than the headgroups of lipids as a partner to interact.
- If so, D_2O is expected to reduce the surface area at the interface.
- Next task is to examine above inference

Structural analysis using Garstecki – Hołyst Model

(*Langmuir*, 2002,Vol.18,pp.2519-2528)



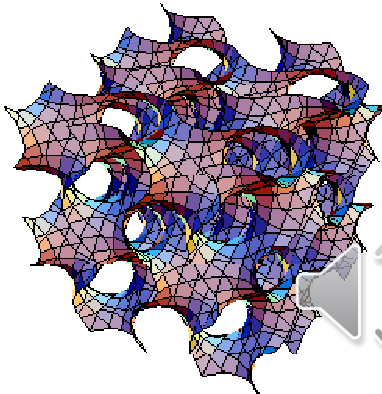
The schematic figure of Pn3m taken from H. Qiu and M. Caffrey, *Biomaterials*. 21 (2000) 223-234.

Assumptions

- Cubic Phase = Nodal Surface + Constant thickness of lipid bilayers
- Homogenous electron density of lipid bilayers

In this model, the bilayer thickness is only one fitting parameter

Nodal surface



$$\cos(X) + \cos(Y) + \cos(Z) = 0$$

$$\cos(X)\cos(Y)\cos(Z) + \sin(X)\sin(Y)\sin(Z) = 0$$

The parameters of the Garstecki – Hołyst Model

$$I_{hkl}^{(mod)} = M_{hkl}\left[F_{hkl}^{S*} \cdot A\right]^2$$

$$A = \frac{\sin\left(\alpha_{hkl}\pi\sqrt{h^2+k^2+l^2}\,(a/L)\right)}{\left(\alpha_{hkl}2\pi\sqrt{h^2+k^2+l^2}\right)}$$

L:Lattice constant
a:Bilayer thickness

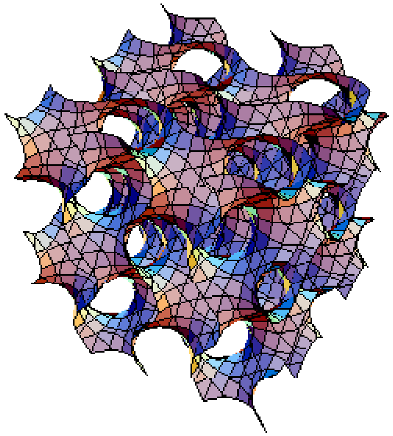


Table 2. The Scattering Data for the P Structure[a]

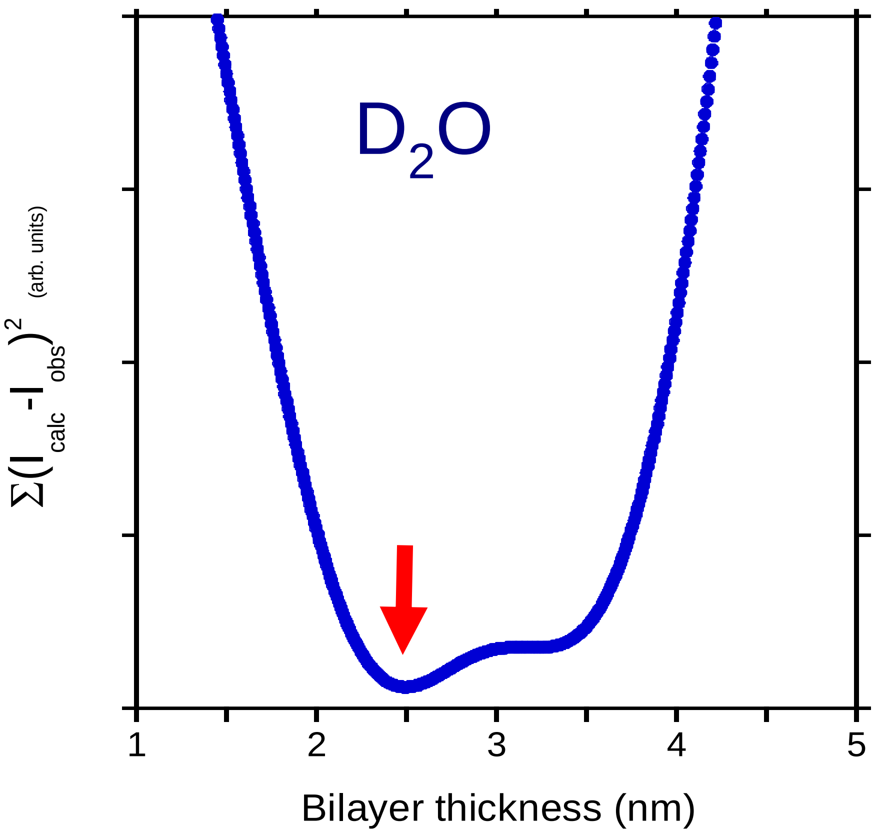
hkl	$\mathcal{M}_{hkl}$	F_{hkl}^{S*}	α_{hkl}
0 0 0	1	2.3458	
1 1 0	12	−0.4496	1.14
2 0 0	6	−0.5444	1
2 1 1	24	0.4565	1.03
3 1 0	24	0.0985	1
2 2 2	8	−0.4056	1
3 2 1	48	−0.2177	1
4 0 0	6	0.2454	1
4 1 1	24	−0.2425	1
3 3 0	12	0.2155	1
4 2 0	24	0.1580	1
3 3 2	24	0.2795	1
4 2 2	24	0.2536	1
5 2 1	48	0.0861	1
4 3 3	24	−0.2680	1
5 3 0	24	−0.1693	1
5 3 2	48	−0.1356	1
6 1 1	24	0.1577	1
5 4 1	48	0.1449	1
5 4 3	48	0.1795	1
5 5 4	24	−0.2070	1

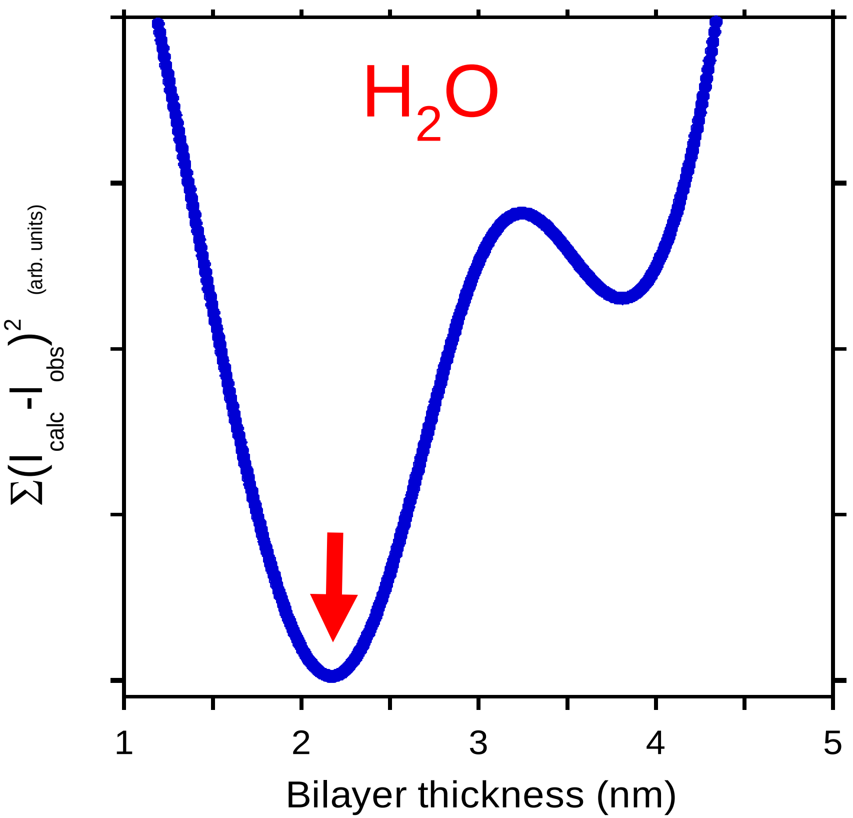
[a] The first column contains the hkl indices, the second the appropriate multiplicity factors for a powder spectrum, the third the dimensionless structure factors $F^{S*} = F^S/a^2$ for the zero-width base mathematical surface, and the fourth the α_{hkl} correction parameters for the isotropic MF.

(*Langmuir*, 2002,Vol.18,pp.2519-2528)

Results

The thickness in D2O is longer than that in H2O !

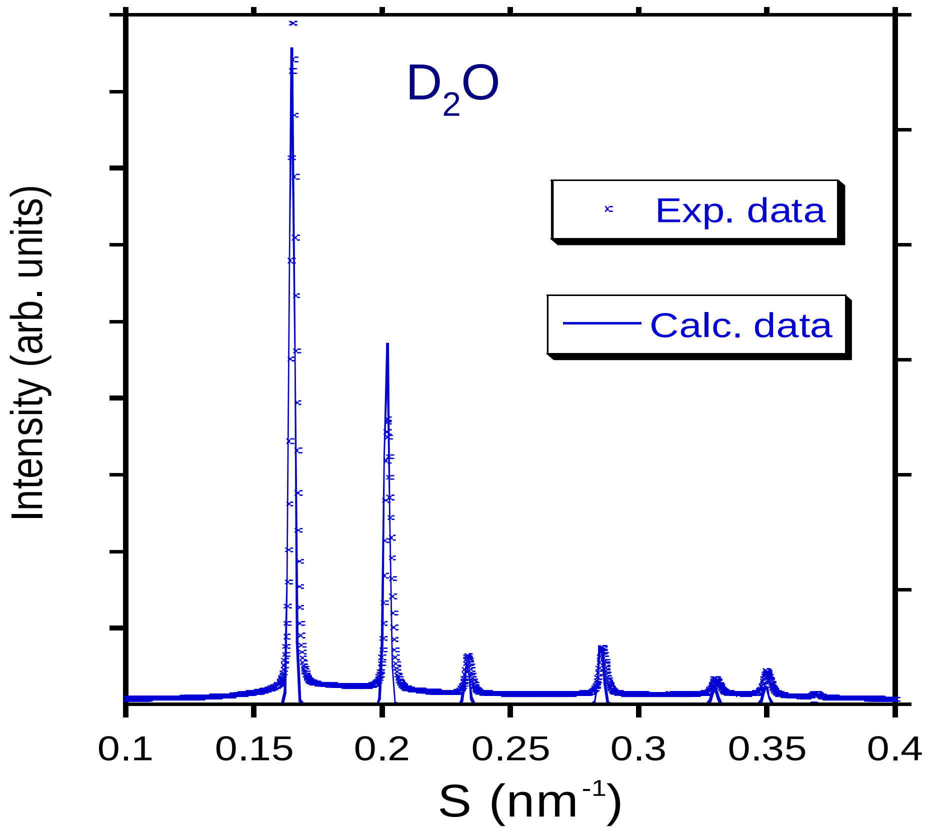


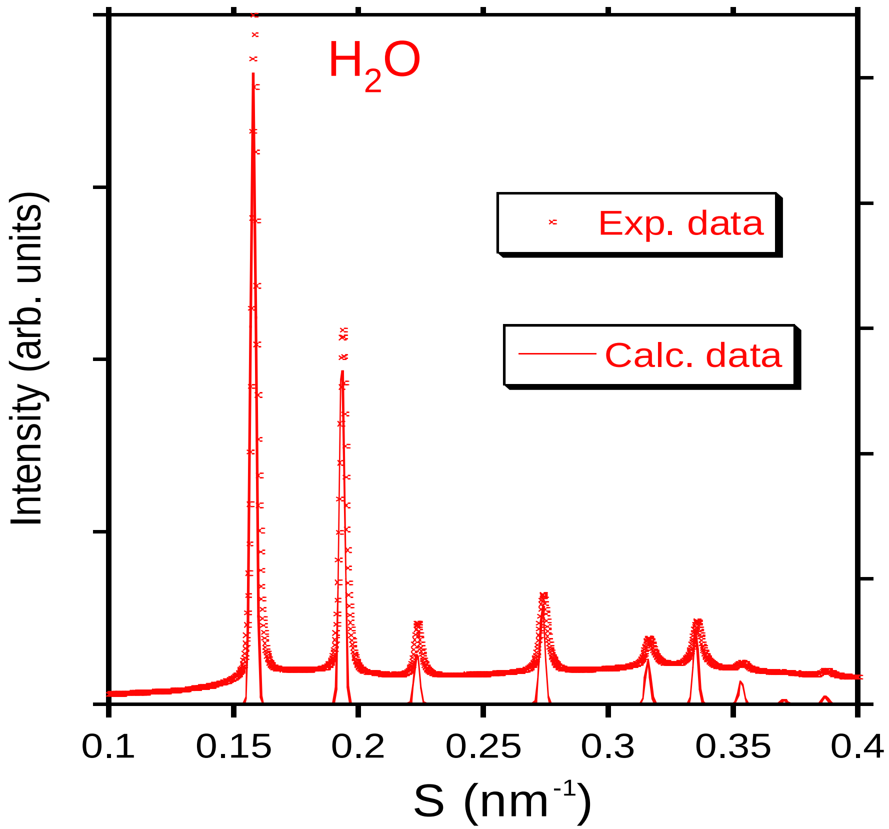


Thickness : 2.50 nm

Thickness : 2.17 nm

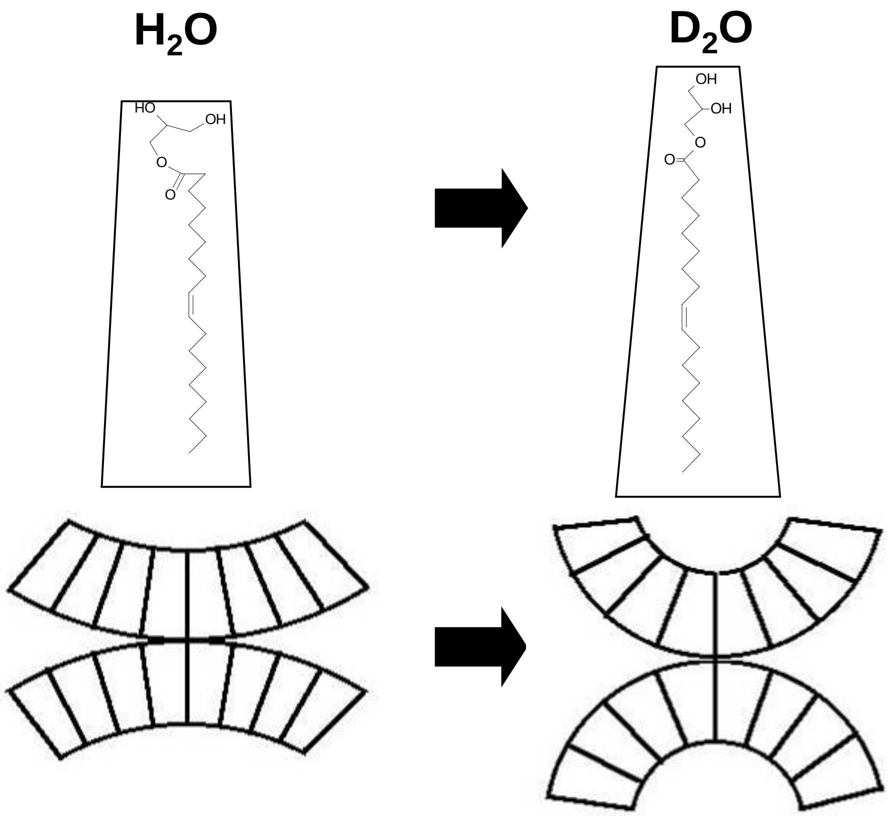
Calculated and observed diffraction patterns





Garstecki – Hołyst Model gives only intensities of each diffraction peak.
In the calculated patterns, the width of the peaks was assumed to be the same as that of the observed first diffraction peak.

Structural analysis using a model indicates Change of molecular shape in the cubic phase



Effective molecular shape is change

↓

Membrane curvature is change

↓

Lattice constant decreases

D_2O → Reduction of interfacial area

Summary

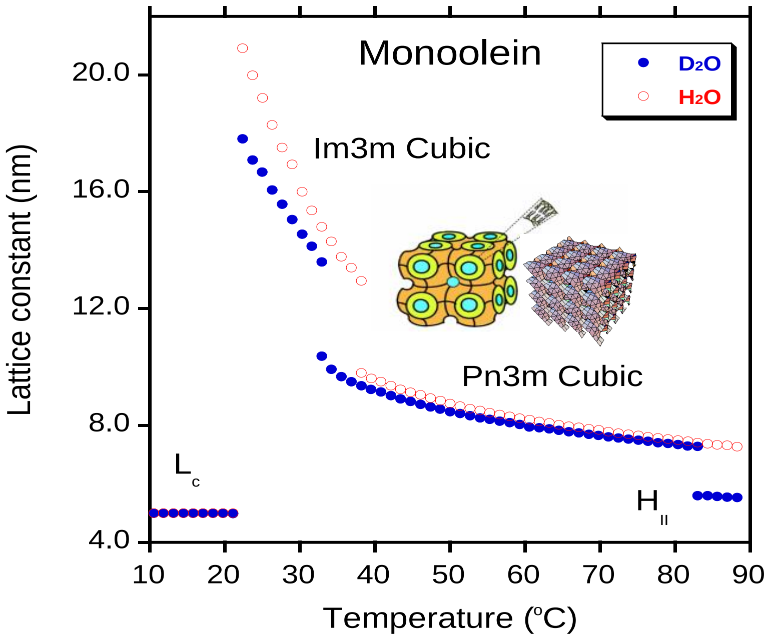
1. Large changes of the lattice constants are observed for lipidic cubic phases as a response to the presence of ions, co-solute or replacing solvent.

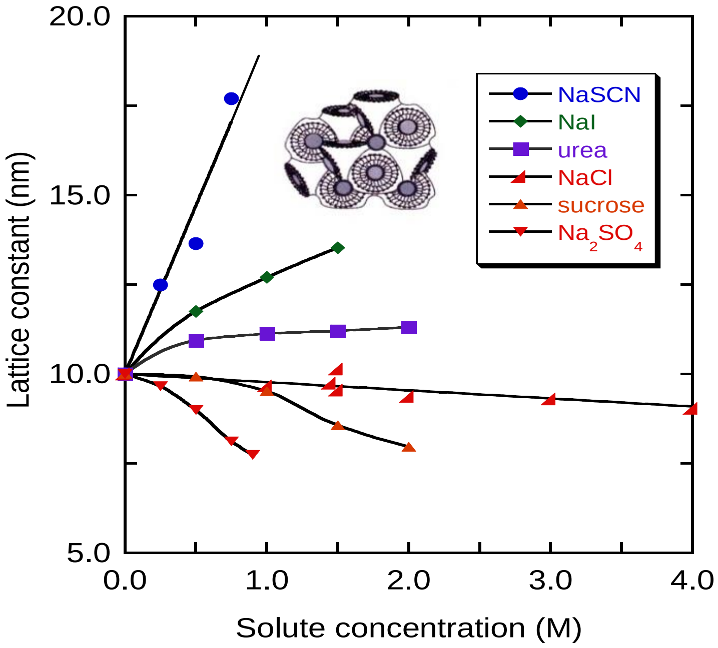
2. The replacement of H_2O by D_2O tends to reduce the molecular area of lipid (MO) at membrane interface.

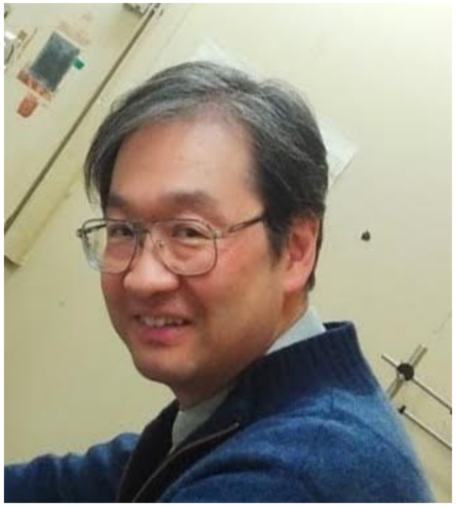
3. The lattice constant of cubic phase is extremely sensitive to the change the interaction at membrane interface.

Take-home message

When contrast variation by D_2O is used, especially for bicontinuous cubic phases, careful caution should be needed.







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