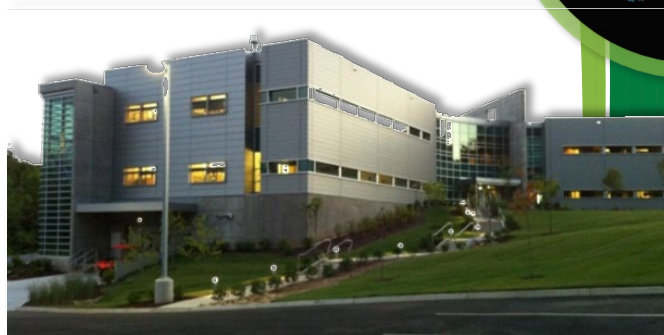
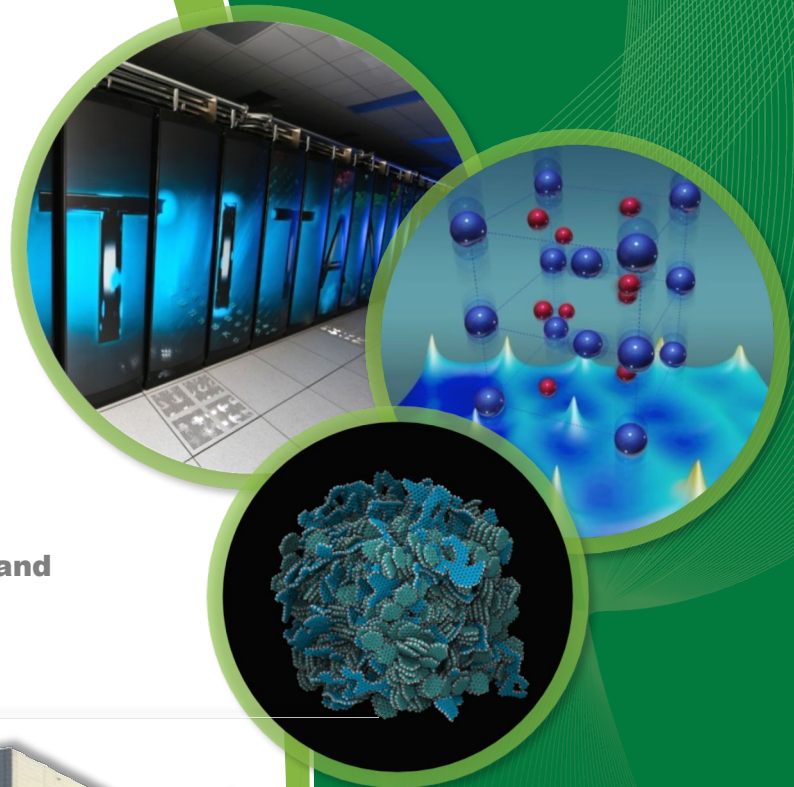


# Membrane Lateral Heterogeneity

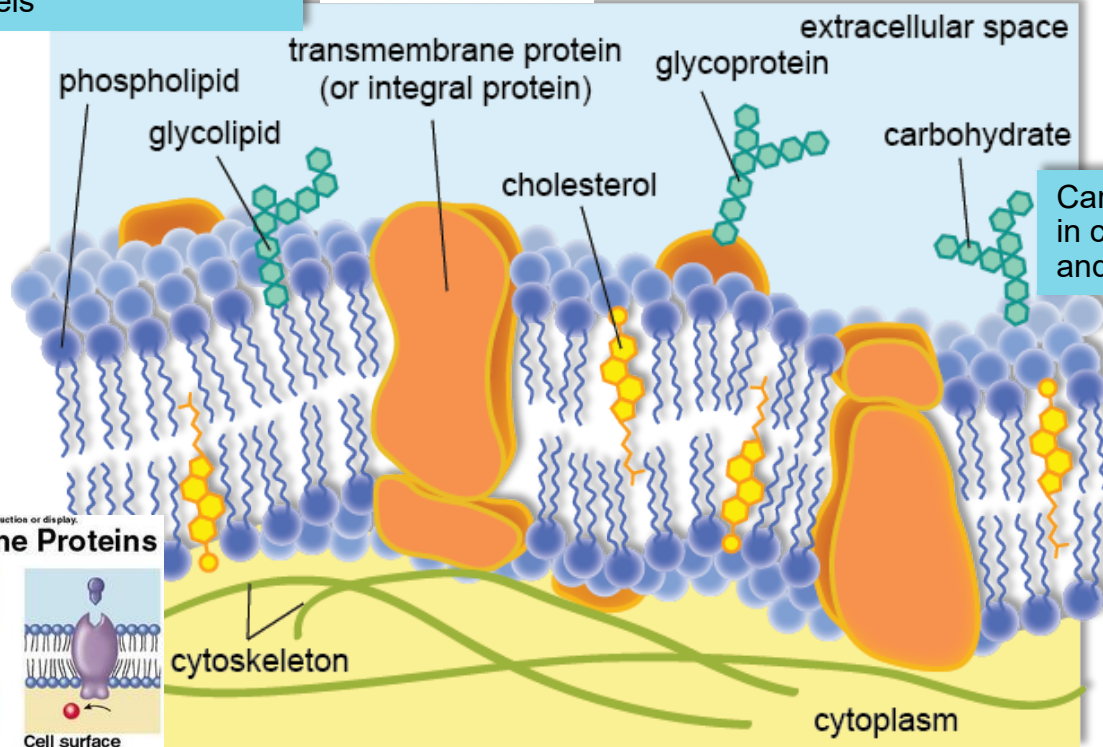
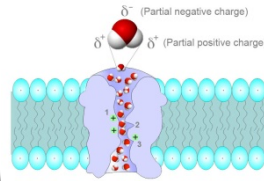
**John Katsaras**

**Shull Wollan Center – a Joint Institute for Neutron Sciences and Neutron Scattering Division, Oak Ridge National Laboratory**



# What Good are Membranes? – see examples

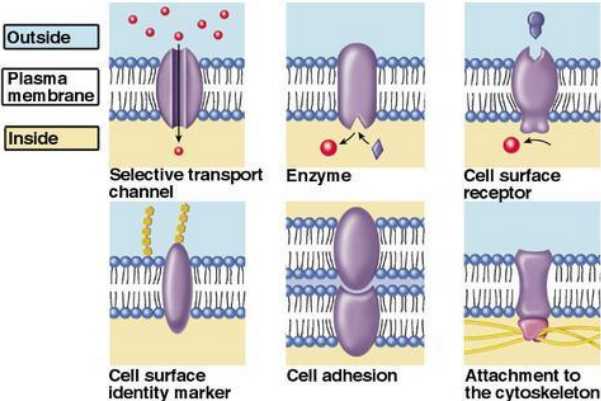
Plasma membrane separates cell's Interior from its aqueous environment, and acts as a selective barrier allowing the traffic of molecules in and out of the cell via protein channels



Carbohydrates used in cell-cell recognition, and immune system

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## Functions of Plasma Membrane Proteins

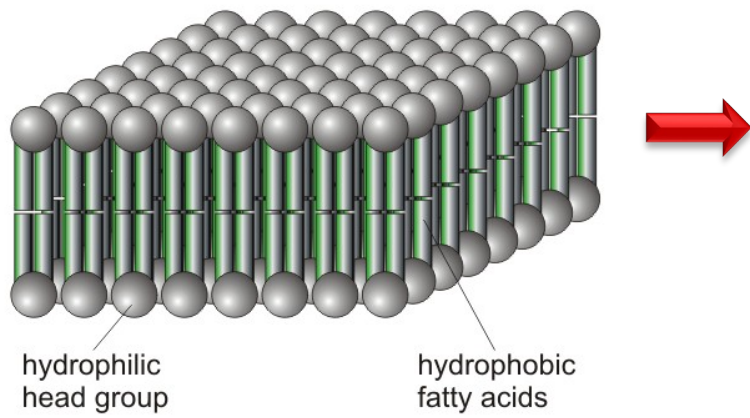


# Membrane Structure Evolution – 1925 - present

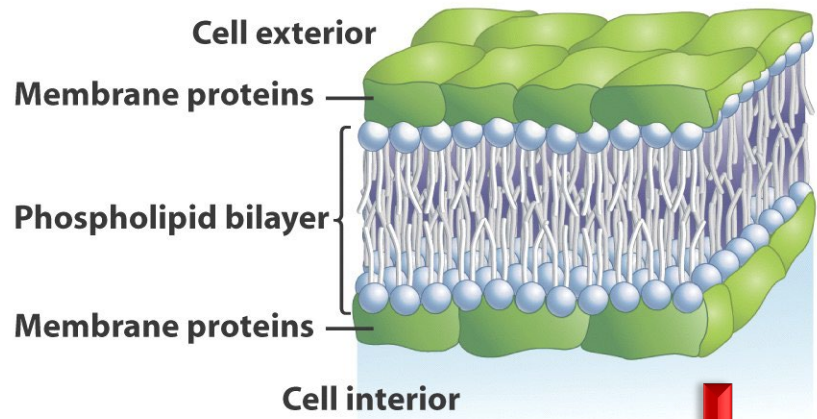
Gorter, E. and Grendel, F. 1925. On bimolecular layers of lipoids on the chromocytes of the blood. *J. Exp. Med.* **41**:439-443.

1935 Sandwich Model by Danielli and Davson. In 1957, Robertson stated that the bilayer is found in all membranes, including those of organelles.

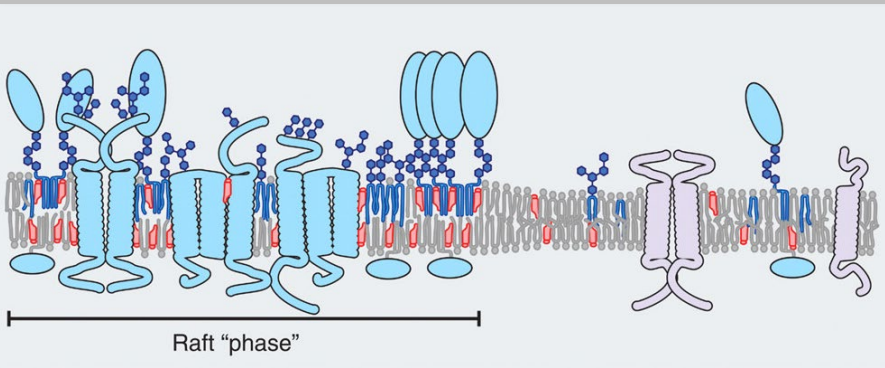
Phospholipid bilayer



(a) Sandwich model



Lingwood and Simons, *Science* 327, 46 (2010).



(b) Fluid-mosaic model

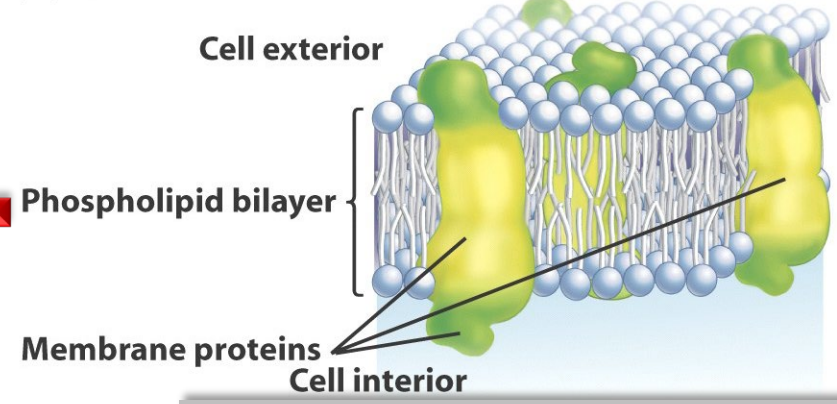
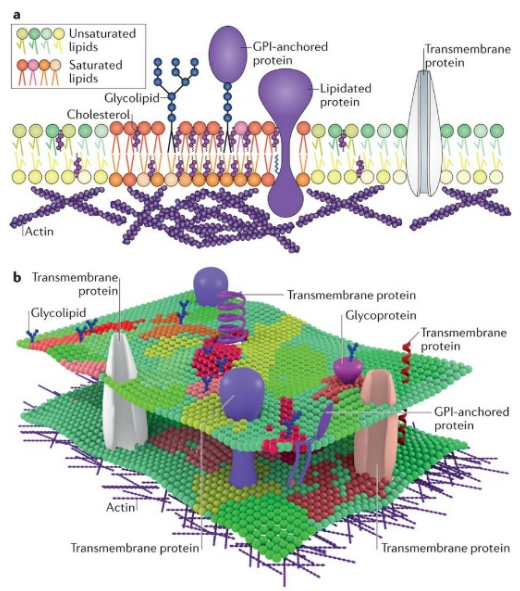


Figure 6-19 Biolo Singer, S. J. and Nicolson, G. L. 1972. The fluid mosaic model of the structure of cell membranes. *Science* **175**:720-731

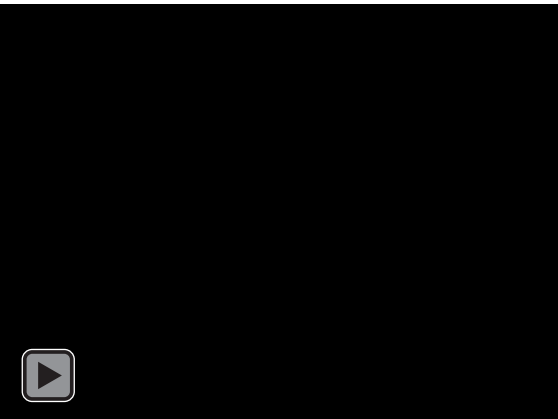
# Biological Membranes – dynamic 2D sheets rich in hydrogen

## Model of Plasma Membrane

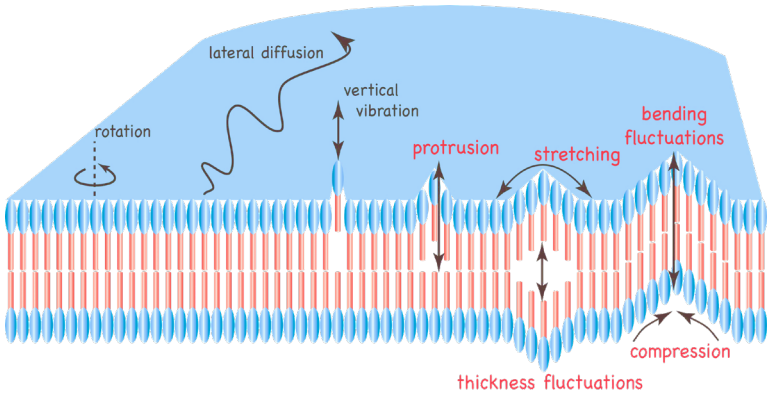


Nature Reviews | Molecular Cell Biology

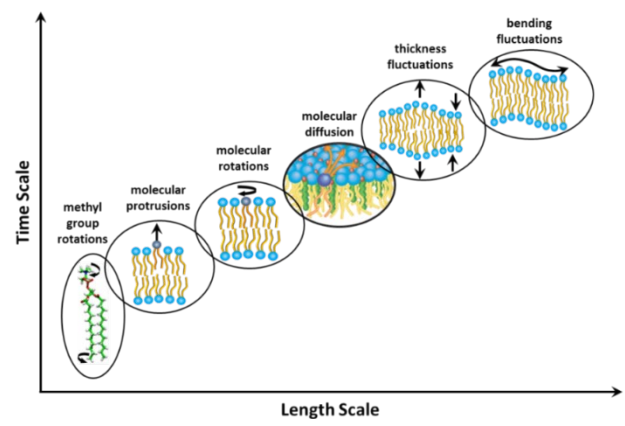
## Domain Formation in a Model Membrane



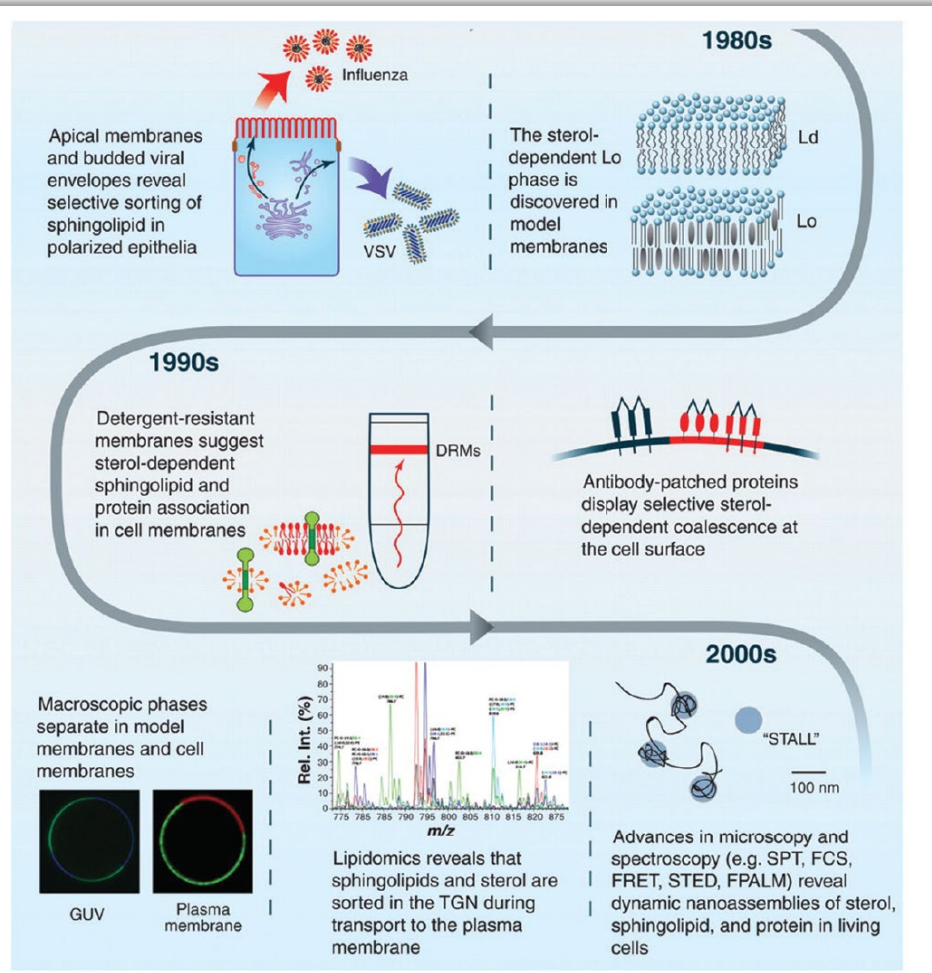
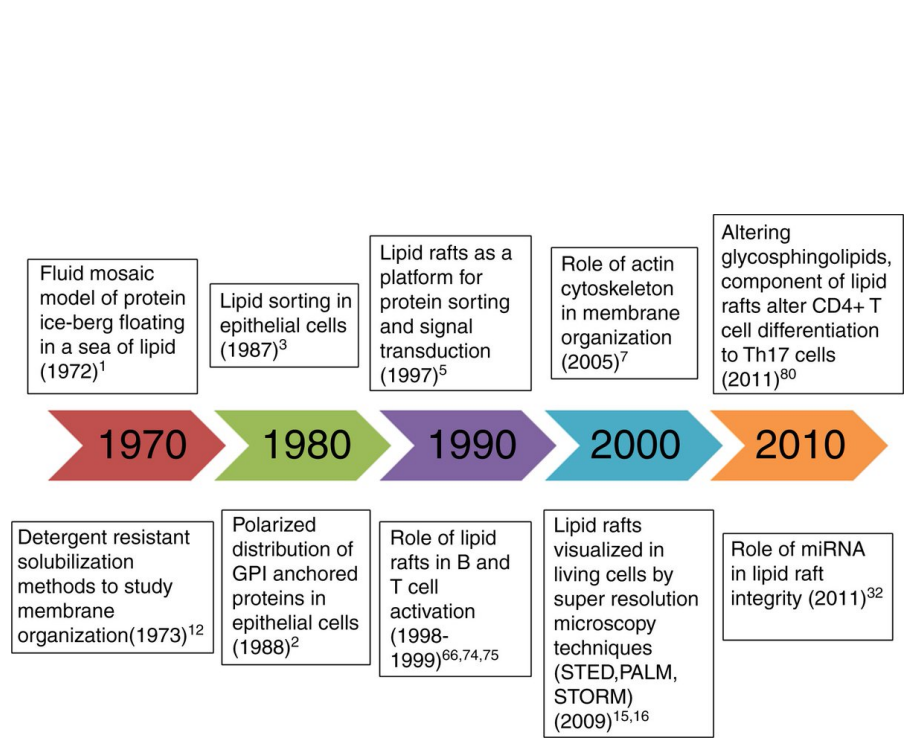
## Membrane Dynamics



← molecular motion → collective motion →  
 incoherent-quasi-elastic scattering  
 nuclear magnetic resonance  
 etc.      coherent-quasi-elastic scattering  
 photon correlation spectroscopy  
 etc.



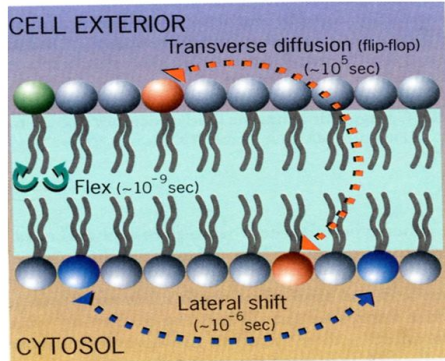
# Lipid Raft Timeline – lipid rafts inferred through different studies over 4 decades



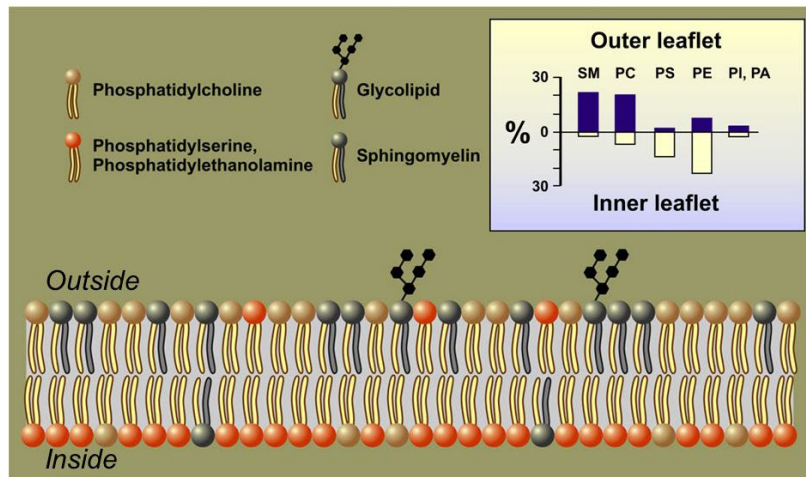
# What We Know Of Biological Membranes – asymmetric, heterogeneous, dynamic, etc.

## Fluidity of the Lipid Membrane

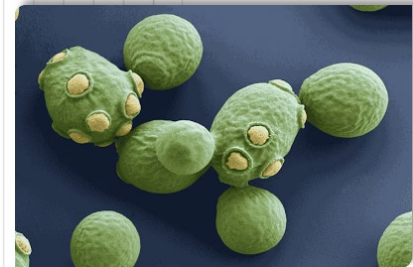
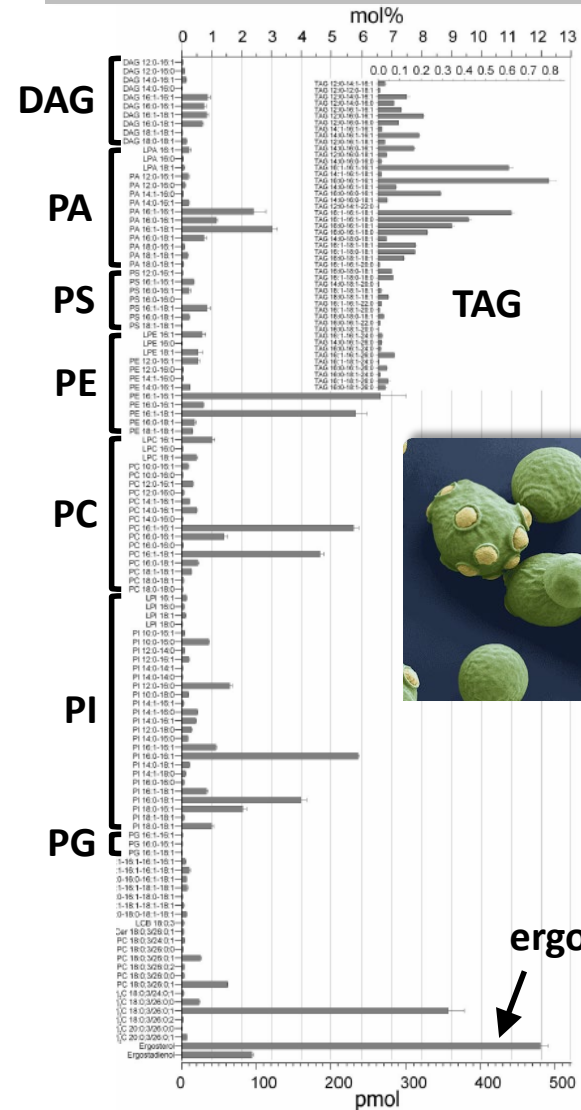
At physiological temperatures the lipids in the membrane bilayer are very dynamic exhibiting **vibrational**, **rotational** ( $10^{-9}$  sec), **lateral movement** ( $10^{-6}$  sec) & **"flip-flop"** ( $10^5$  sec or every 28 hr).



Phospholipids are distributed asymmetrically in the plasma membrane

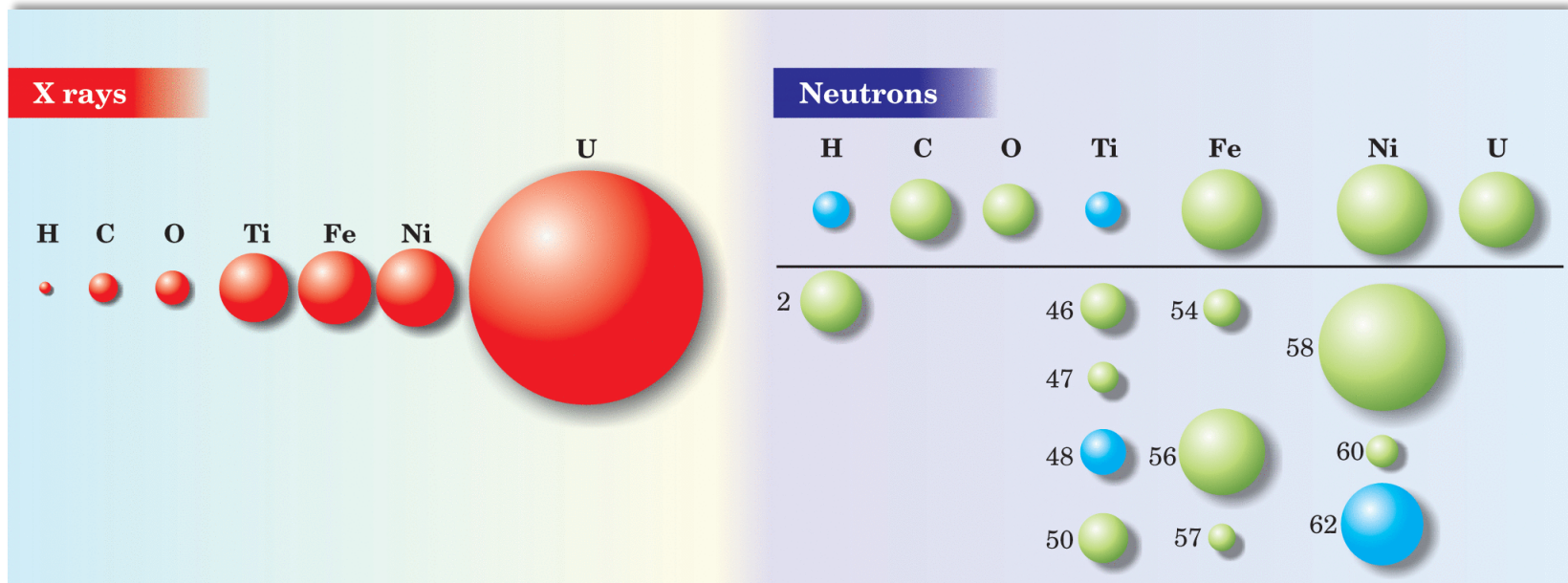


## Yeast *S. Cerevisiae* Lipidome



Ejsing et al., 2009 PNAS 106:2136

# Neutron and X-Ray Cross Sections – neutrons scatter from nuclei and x-rays from electrons



Bound Atom Scattering Lengths and Cross Sections for Typical Elements in Biomaterials

Atom	Nucleus	$b_c$ ( $10^{-12}$ cm)	$\sigma_c$ ( $10^{-24}$ cm <sup>2</sup> )	$\sigma_i$ ( $10^{-24}$ cm <sup>2</sup> )	$\sigma_{abs}^*$ ( $10^{-24}$ cm <sup>2</sup> )
Hydrogen	<sup>1</sup> H	-0.374	1.76	79.7	0.33
Deuterium	<sup>2</sup> H	0.667	5.59	2.01	0
Carbon	<sup>12</sup> C	0.665	5.56	0	0
Nitrogen	<sup>14</sup> N	0.930	11.1	0	1.88
Oxygen	<sup>16</sup> O	0.580	4.23	0	0
Fluorine	<sup>19</sup> F	0.556	4.03	0	0
Phosphorous	P <sup>†</sup>	0.513	3.31	0	0.17
Chlorine	Cl <sup>†</sup>	0.958	11.53	5.9	33.6

$$\sigma_{coh} = 4\pi b_c^2$$



Cross  
Section

Scattering  
Length

$$\sigma_{tot} = \sigma_{coh} + \sigma_{inc}$$

# Neutron Contrast – changing the neutron contrast of a system through the substitution of H for D

$$I(\lambda, \theta) = I_0(\lambda) \Delta\Omega \eta(\lambda) T V \frac{d\sigma}{d\Omega}(\mathbf{Q})$$

$I(\lambda, \theta)$ : # of neutrons scattered  
 $I_0(\lambda)$ : # of incident neutrons  
 $\Delta\Omega$ : # of neutrons scattered into solid angle element  
 $\eta(\lambda)$ : detector efficiency  
 $T$ : sample transmission and volume  
 $V$ : differential cross-section  
 $\frac{d\sigma}{d\Omega}(\mathbf{Q})$ : differential cross-section

$$\frac{d\sigma}{d\Omega}(\mathbf{Q}) = N_p V_p^2 (\Delta\delta)^2 P(\mathbf{Q}) S(\mathbf{Q}) + B_{inc}$$

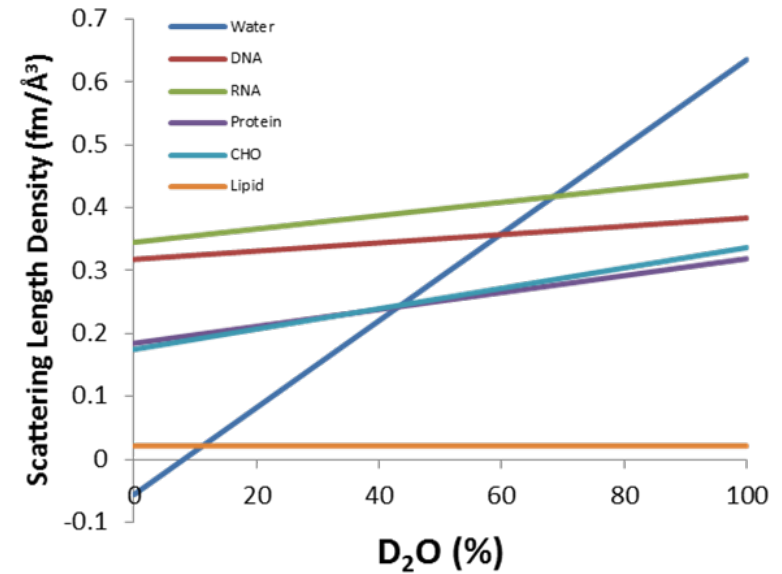
$N_p$ : # of scattering particles  
 $V_p$ : volume of one scattering particle  
 $\Delta\delta$ : neutron contrast  
 $P(\mathbf{Q})$ : form factor  
 $S(\mathbf{Q})$ : structure factor  
 $B_{inc}$ : incoherent background, no Q-dependence

$$(\Delta\delta)^2 = (\delta_{lipid} - \delta_{water})^2 \neq 0 \text{ scattering}$$

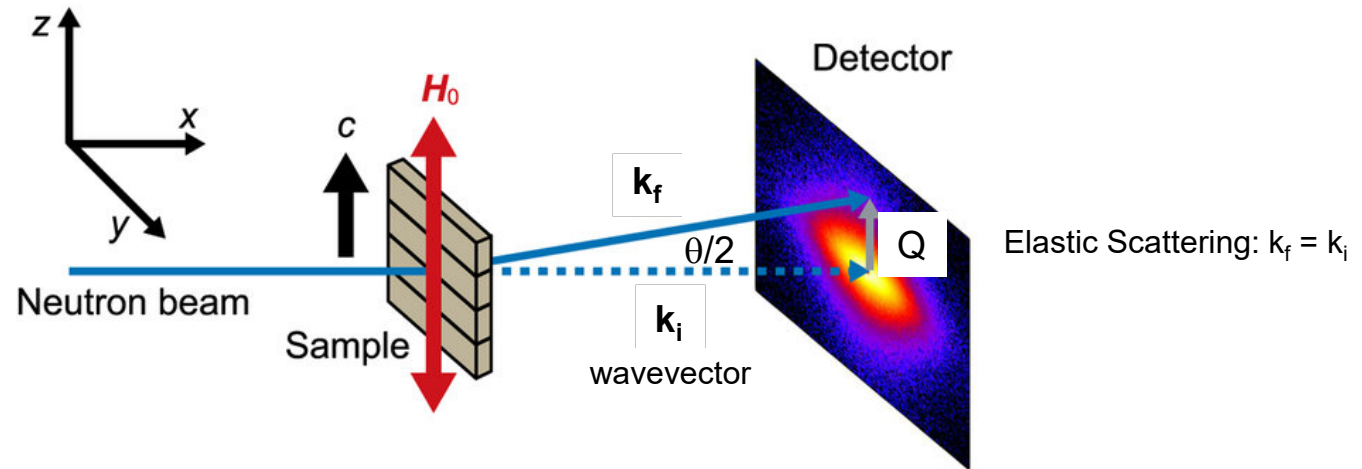
$$(\Delta\delta)^2 = (\delta_{lipid} - \delta_{water})^2 = 0 \text{ no scattering}$$

$\frac{d\sigma}{d\Omega} = \# \text{ of neutrons scattered into a solid angle element } d\Omega \text{ per unit time}$

- sample dependent terms (thousands \$)
- instrument specific terms (millions \$\$\$)



# Small Angle Neutron Scattering (SANS) – $Q = 2\pi/d$ ( $\text{nm}^{-1}$ )



$$q \text{ (nm}^{-1}\text{)} = |\mathbf{q}| = \mathbf{k}_f - \mathbf{k}_i = 4\pi n/\lambda [\sin(\theta/2)]$$

↑  
Refractive index ( $n$ ) for neutrons slightly  $< 1$

Recall Bragg's Law

$$\lambda = 2d \sin(\theta/2)$$

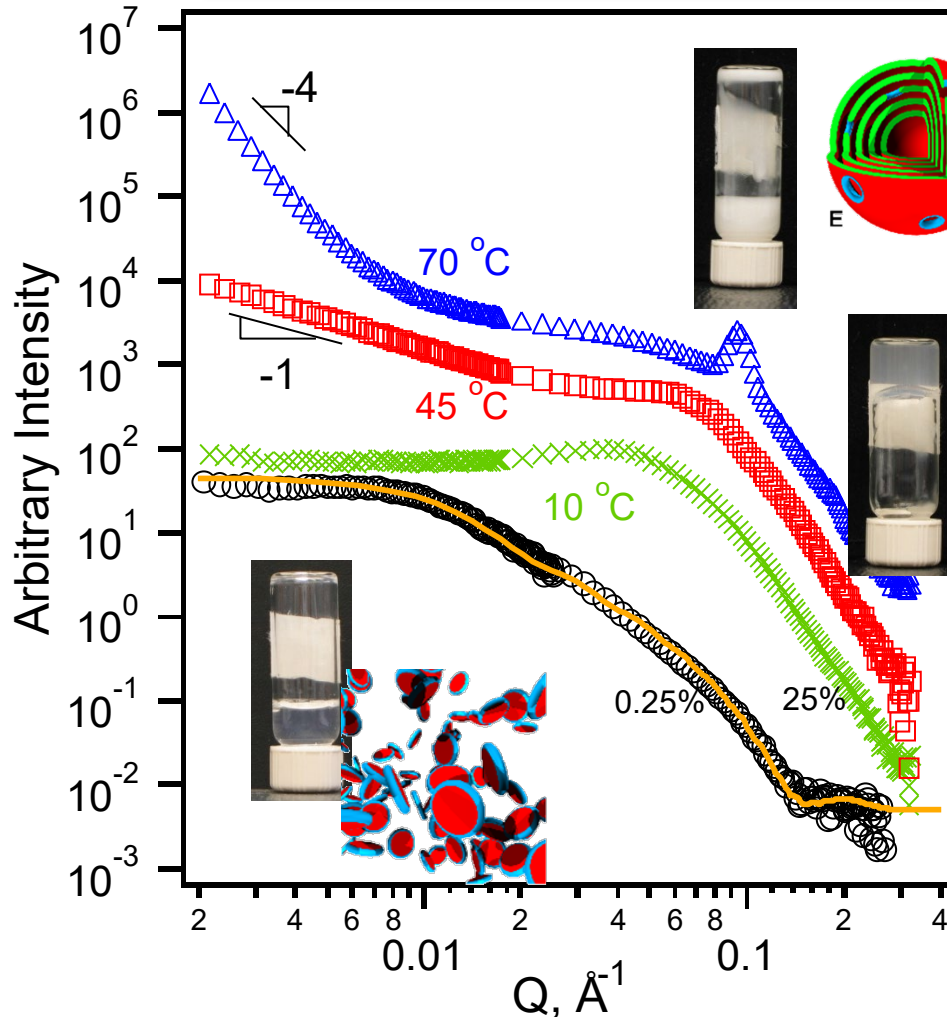
Substituting  $q$  into BL we get:

$$q = 2\pi/d \text{ (nm}^{-1}\text{)} \text{ (a useful expression)}$$

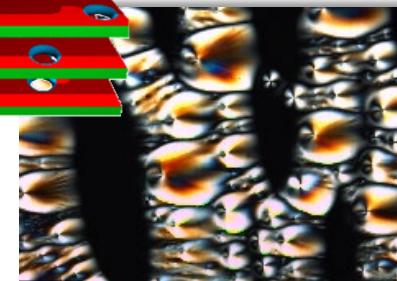
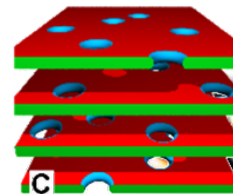
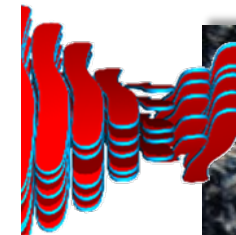
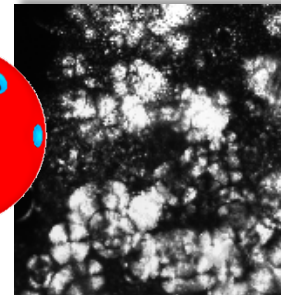
# SANS, Optical Microscopy, and Visual Inspection - developing a consistent story

$q = \text{DMPC (14:0 PC)/DHPC (6:0 PC)} = 3.2$

$I(Q) \sim Q^{-a}$

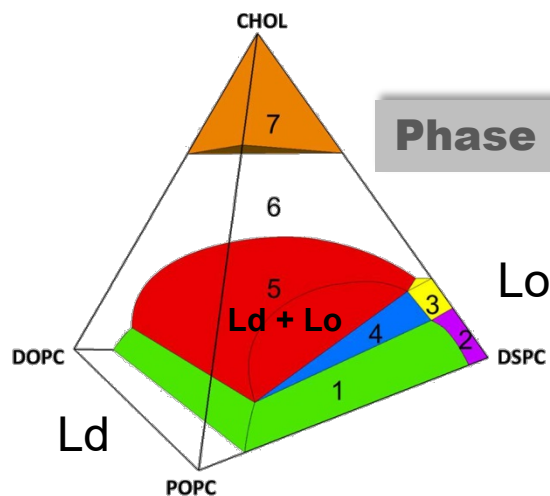


$Q^{-1}$  Long 1D Objects i.e., rods  
 $Q^{-2}$  Smooth 2D Objects (e.g., lamellar sheets), Gaussian Chains etc.  
 $Q^{-3}$  3D Fractal Objects etc. (e.g., proteins)  
 $Q^{-4}$  3D Objects with Smooth Surfaces



# A Canonical Phase Separating Lipid Mixture – high and low temperature melting lipids with cholesterol

34.6% D<sub>2</sub>O and 65.4% chain-deuterated DSPC-d<sub>70</sub>. When randomly mixed there is no SANS signal. DSPC, (DOPC+POPC), and Chol, in a 39/39/22 ratio.

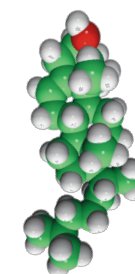
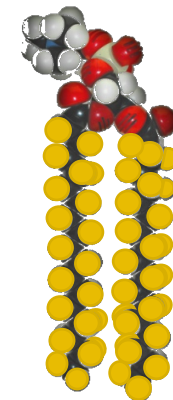
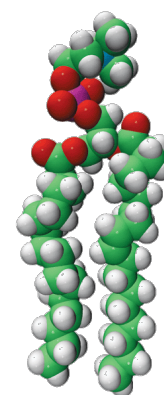
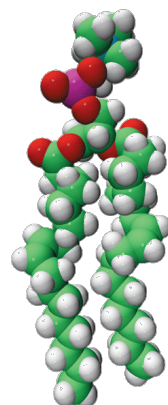


DOPC

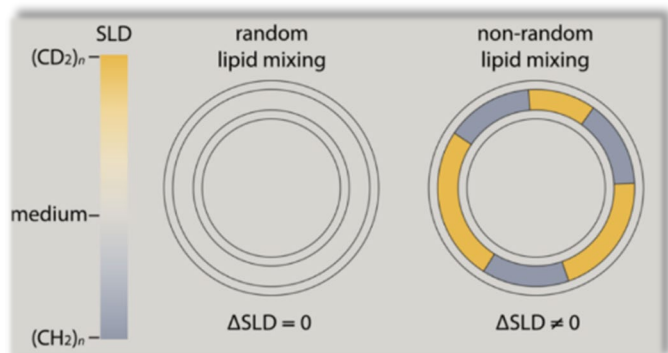
POPC

DSPC

Cholesterol



**Low-Melting Lipids**      **High-Melting Lipid**

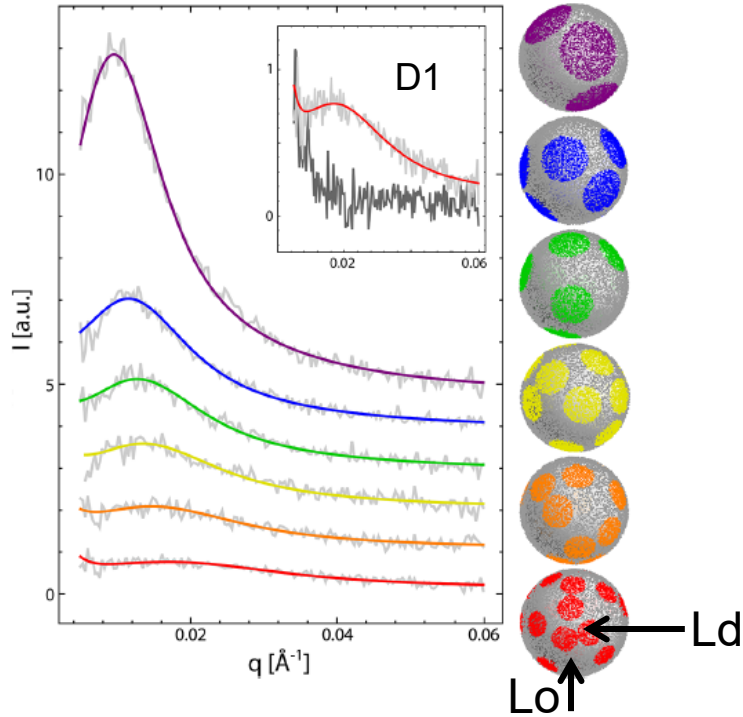


$$(\Delta\delta)^2 = (\delta_{\text{lipid}} - \delta_{\text{water}})^2 \neq 0 \text{ scattering}$$

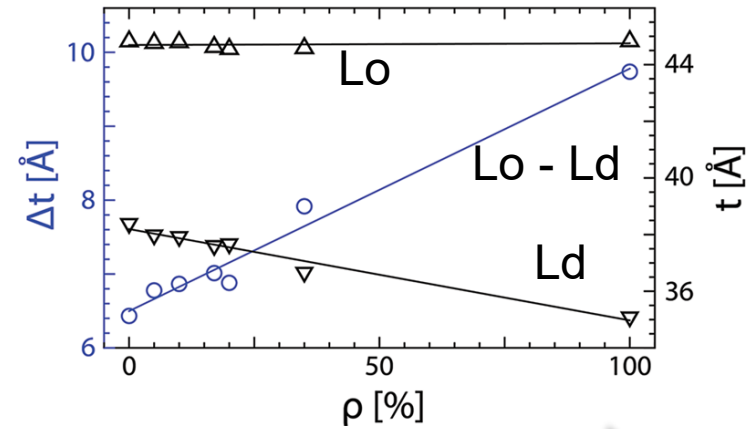
$$(\Delta\delta)^2 = (\delta_{\text{lipid}} - \delta_{\text{water}})^2 = 0 \text{ no scattering}$$

# Detecting Nanoscopic Domains – SANS and deuterated lipids

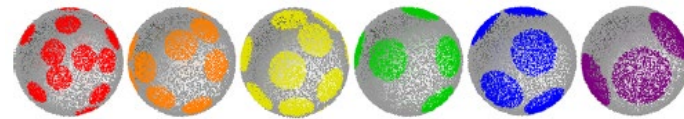
## Fits to 20 °C SANS Data



## Lo and Ld Bilayer Thicknesses



**DOPC**

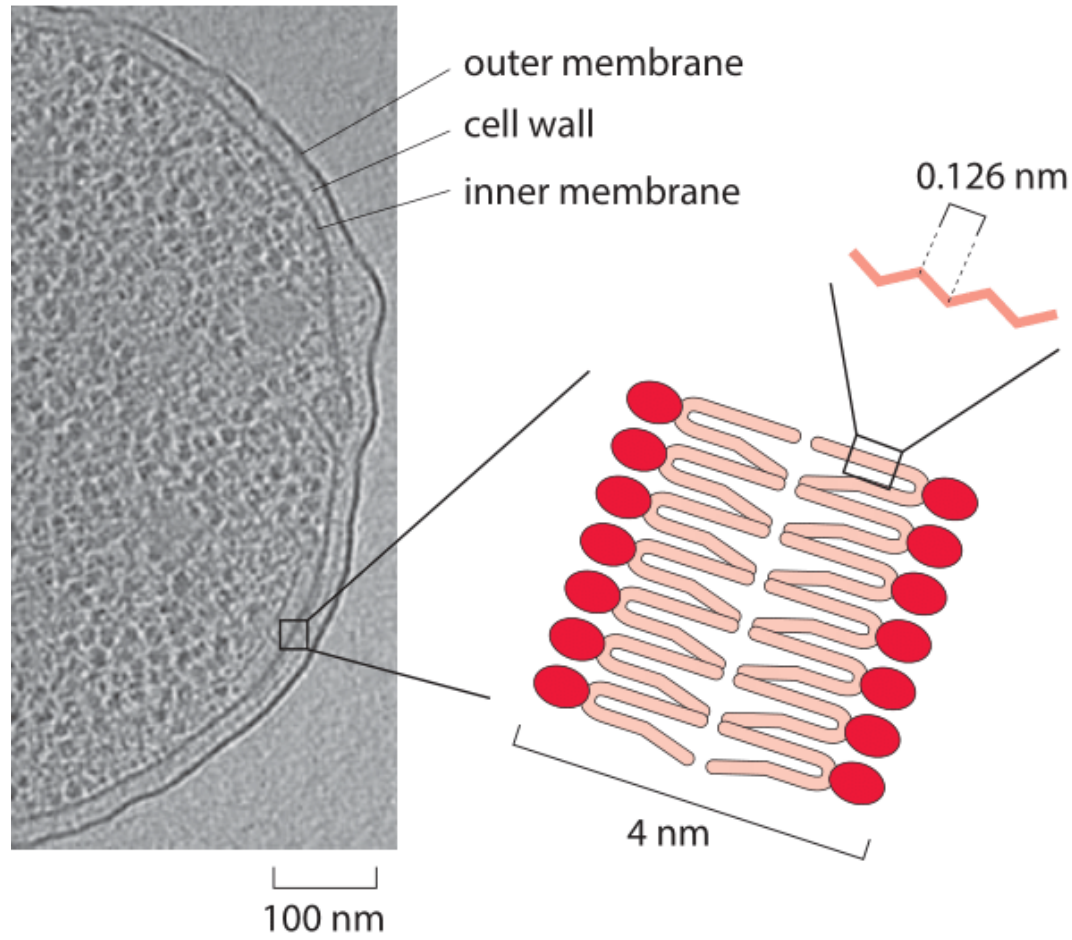


**Line Tension**

F. A. Heberle et al., *J. Am. Chem. Soc.* **135**, 6853 (2013)

# Experimental Evidence of Membrane Thickness of a Biological Organism – frozen membrane by EM

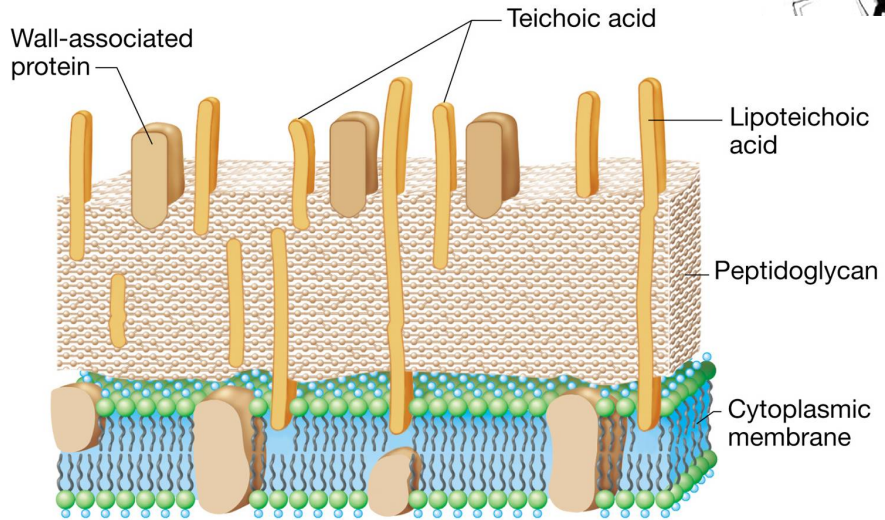
## Electron Micrograph of *E. coli* Membrane



# Gram Positive vs Gram Negative Bacteria – 1 vs 2 membranes

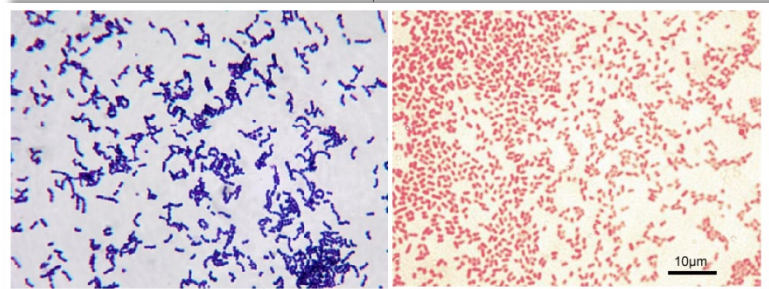
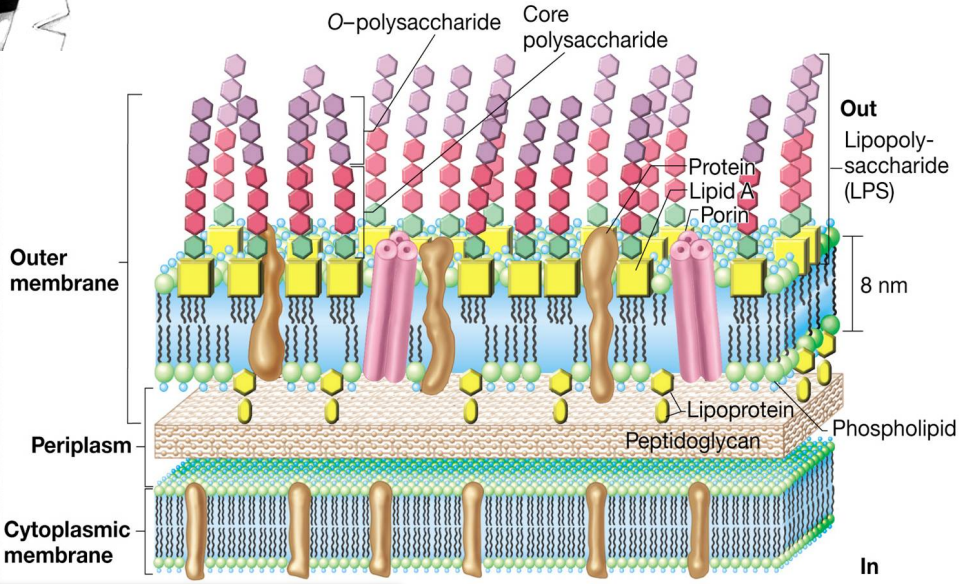


## Gram Positive



(b)

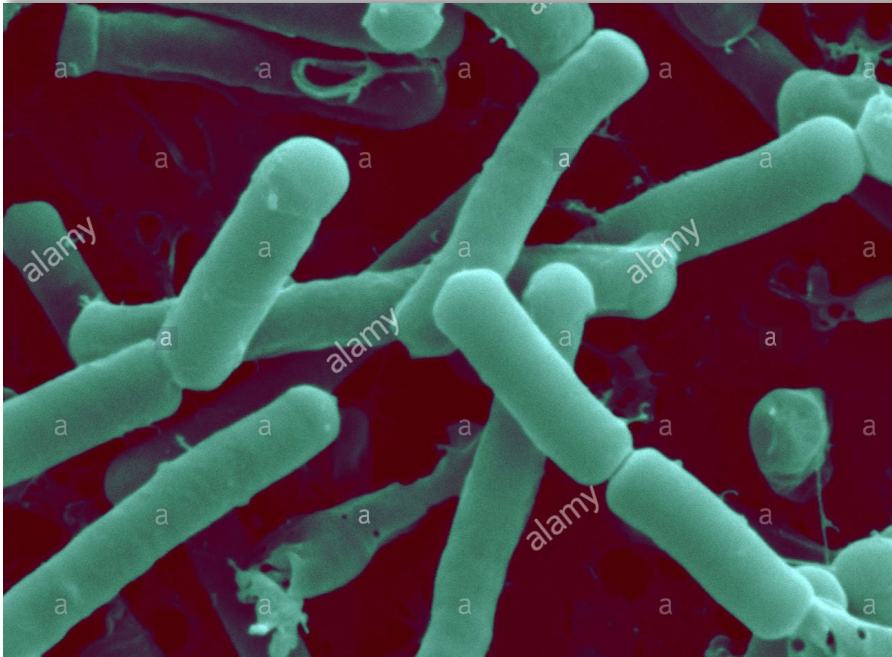
## Gram Negative



Gram Positive Bacteria

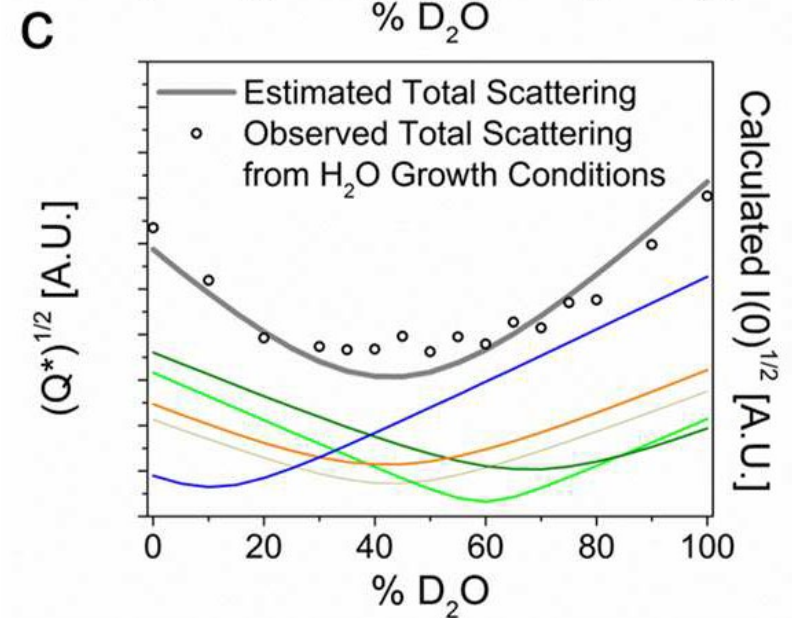
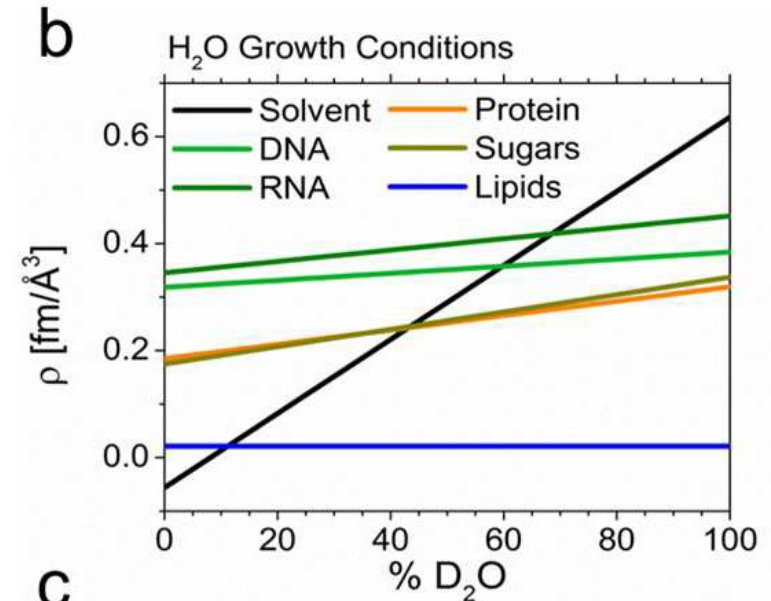
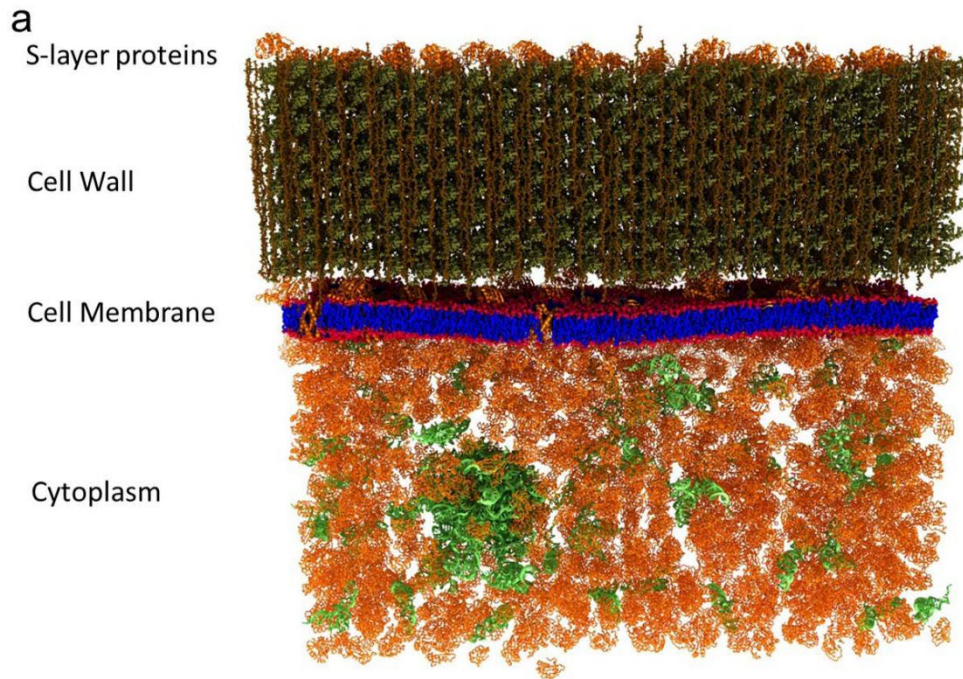
Gram Negative Bacteria

# Bacillus subtilis – a gram positive bacteria

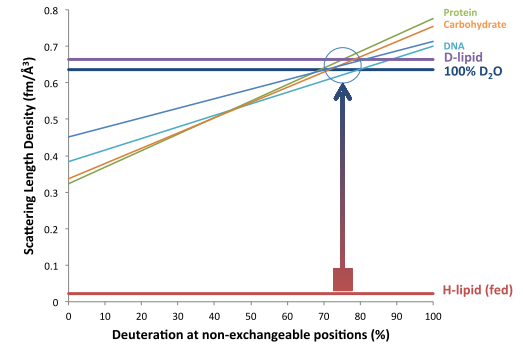
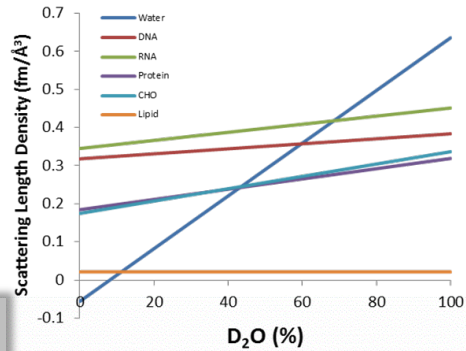


- **B. subtilis** is found in the human gut, a variety of foods and soil.
- Considered a Gram-positive equivalent of *E. coli*.
- Highly amenable to genetic manipulation.
- Bacillus Genetic Stock Center provides a mutant library, expediting some of the genetic manipulations.

# Envelope Structure of Total Scattering From *B. subtilis* – estimated and measured scattering

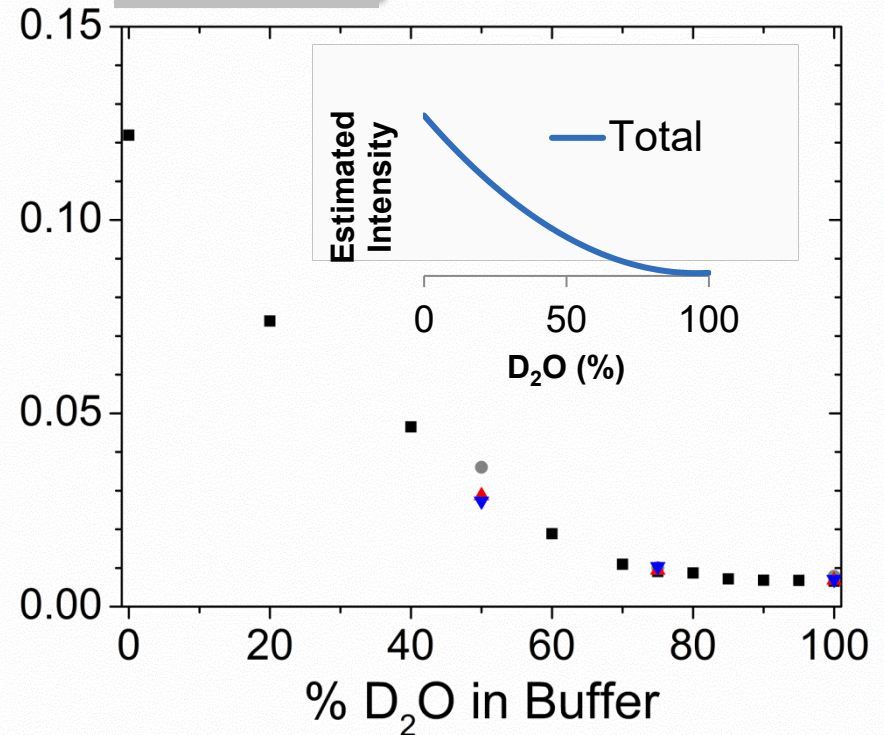
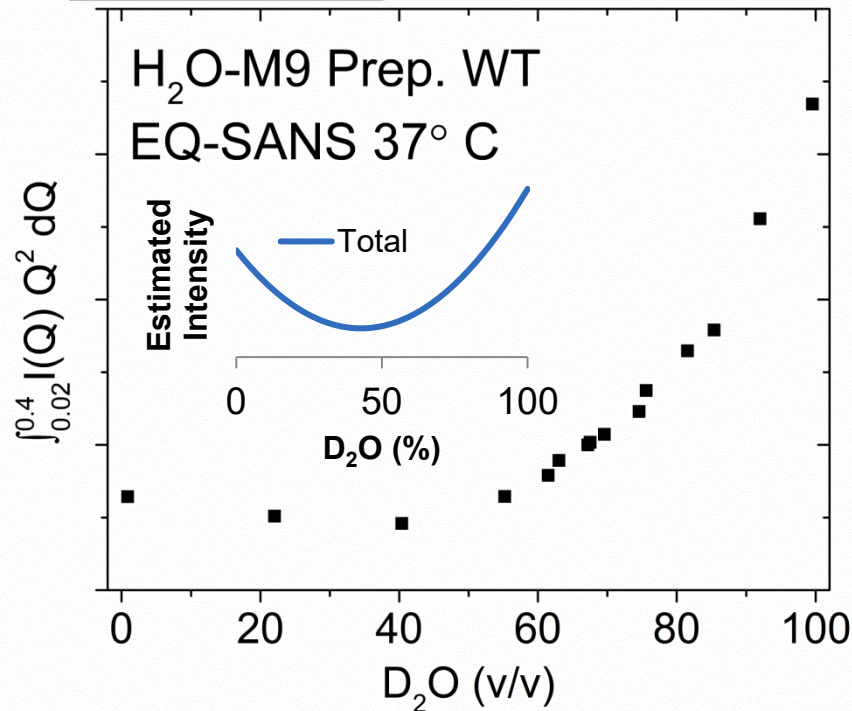


# SANS Data from *B. subtilis* Grown in 90% D<sub>2</sub>O and 100% H<sub>2</sub>O – integrated neutron intensities vs % D<sub>2</sub>O

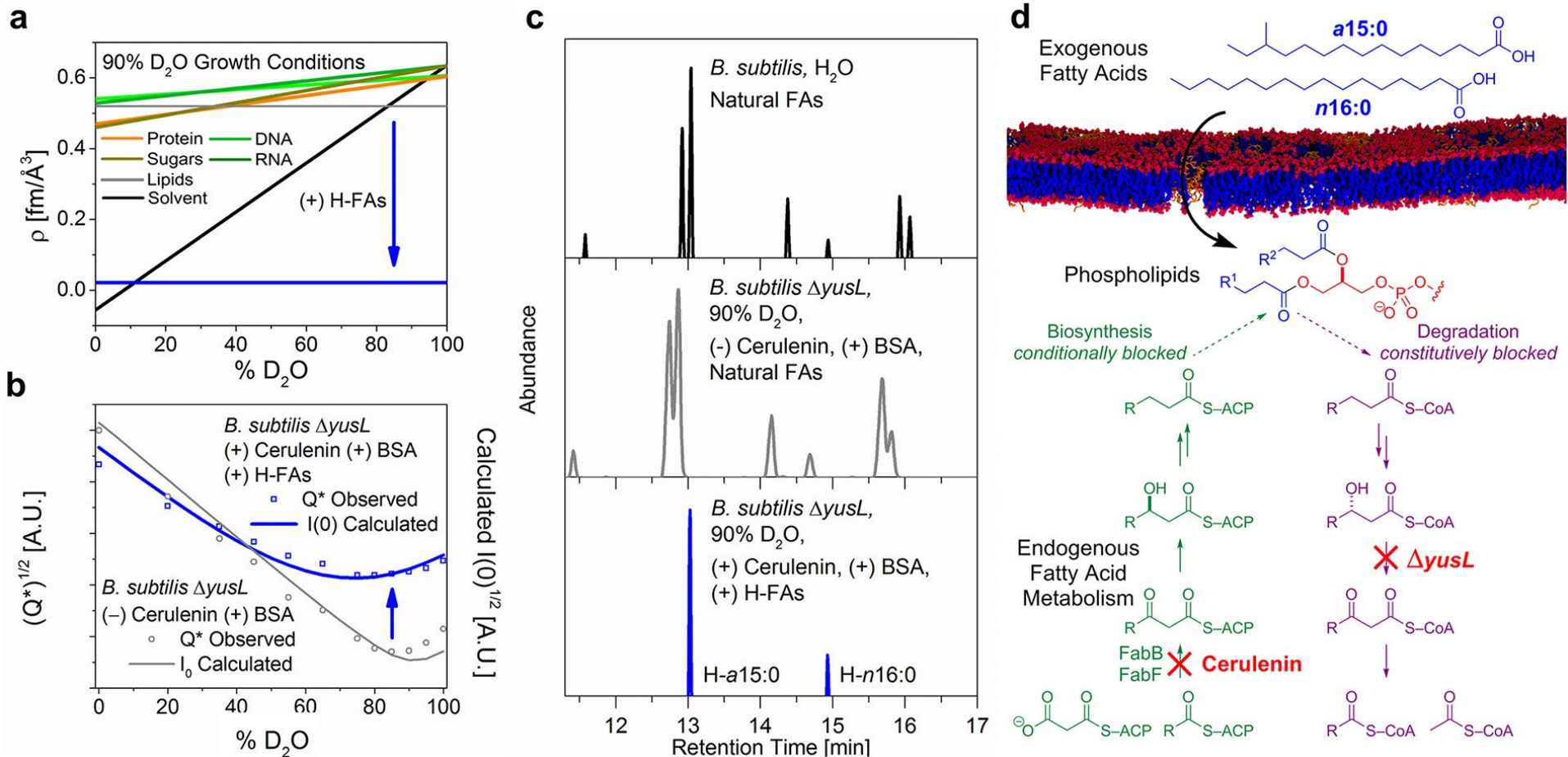


**100% H<sub>2</sub>O**

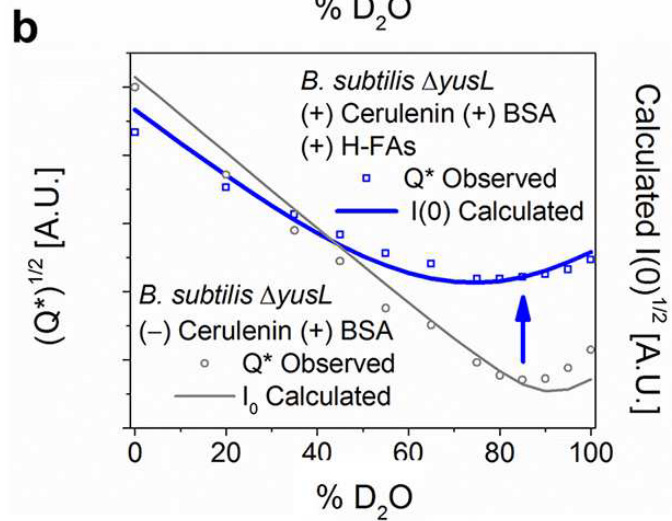
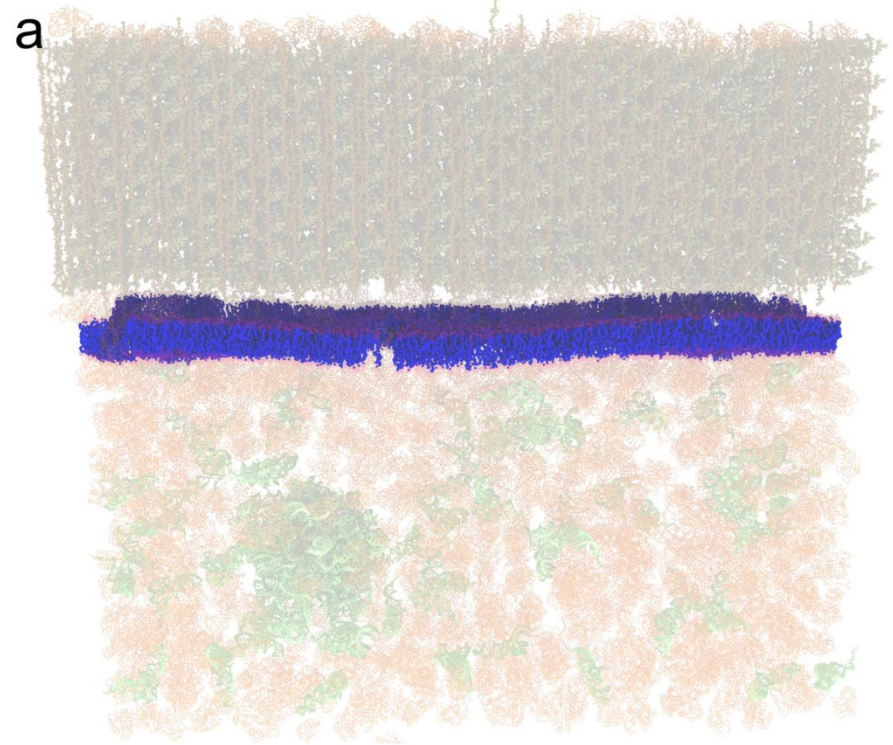
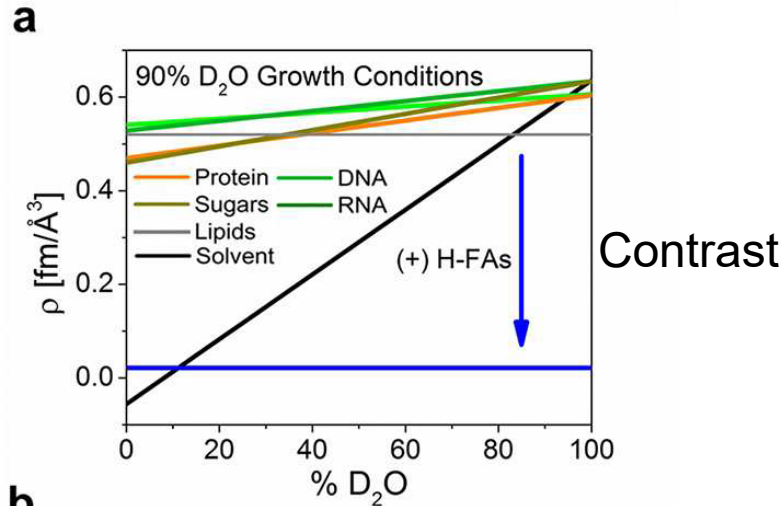
**90% D<sub>2</sub>O**



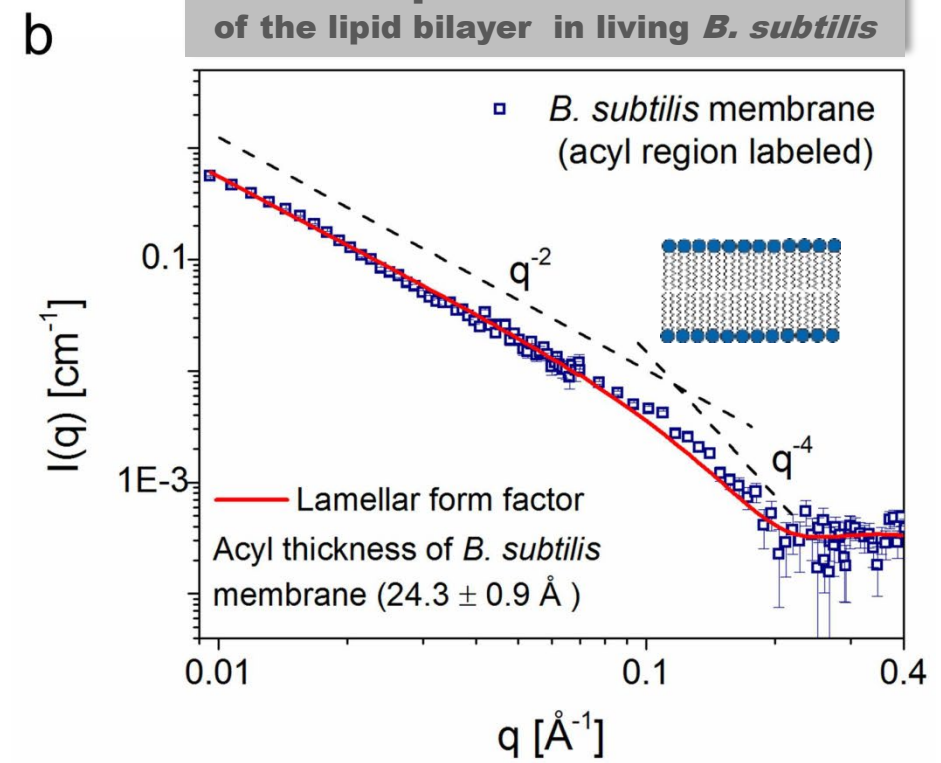
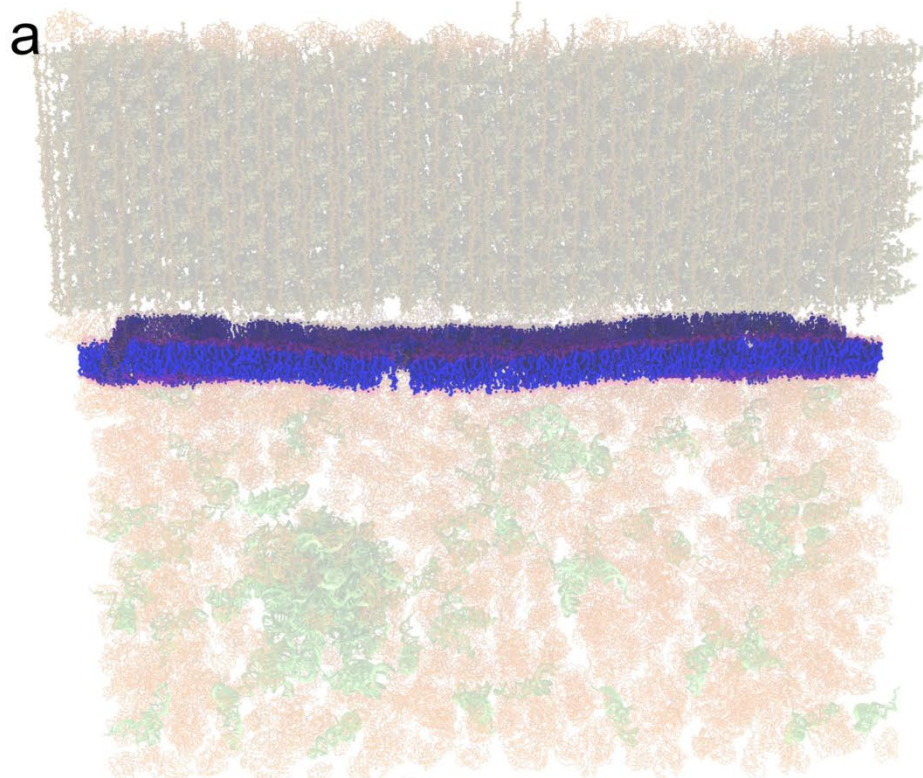
# Controlling Membrane Contrast – creating neutron contrast by feeding bacteria protiated FAs



# Scattering Length Densities and Total Scattering from *B. subtilis* – neutrons only “see” the membrane!

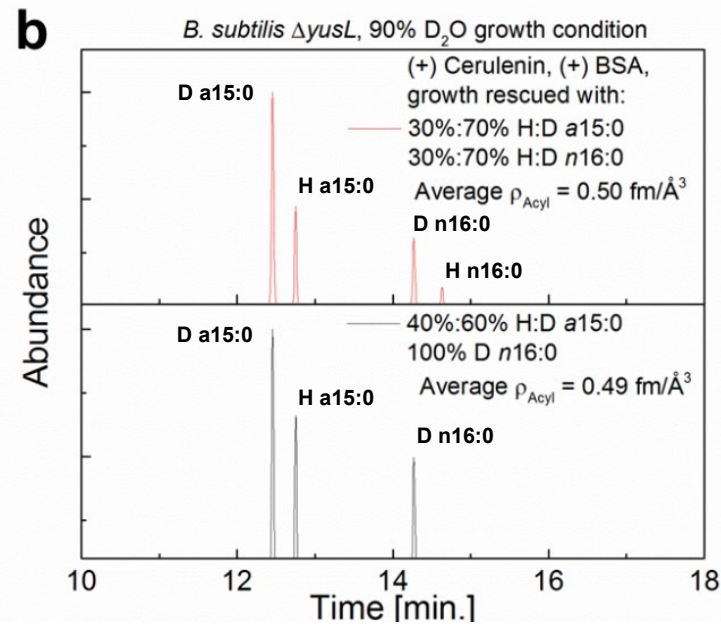
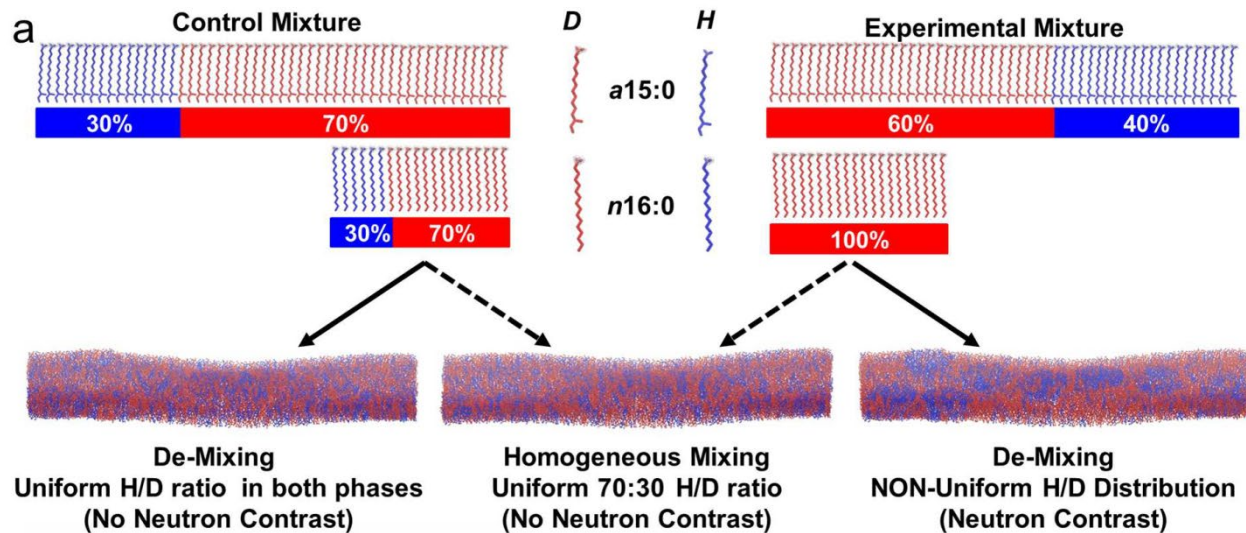


# First Ever Measurement of a Living Membrane – 24.3 +/- 0.9 Å thick

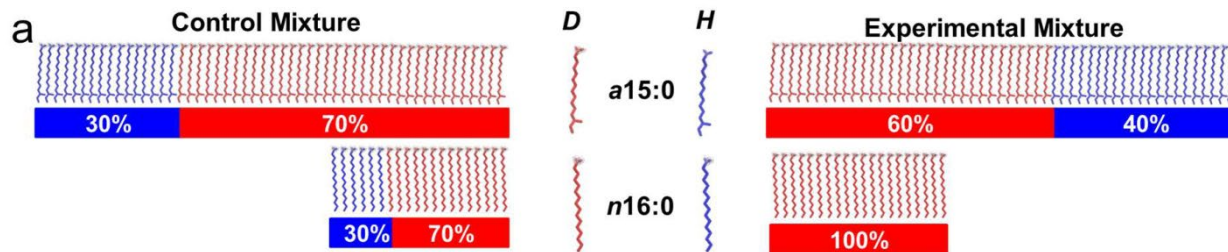
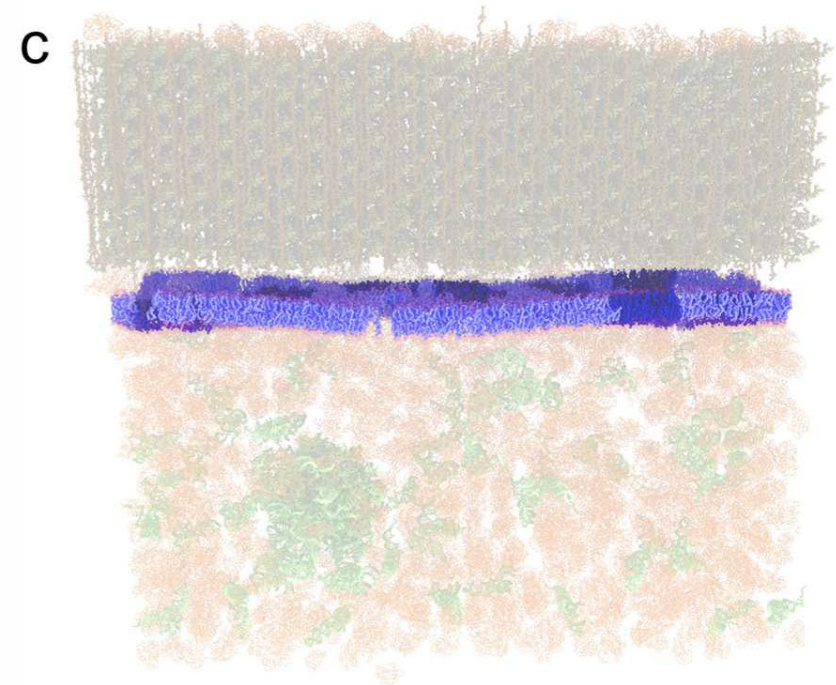
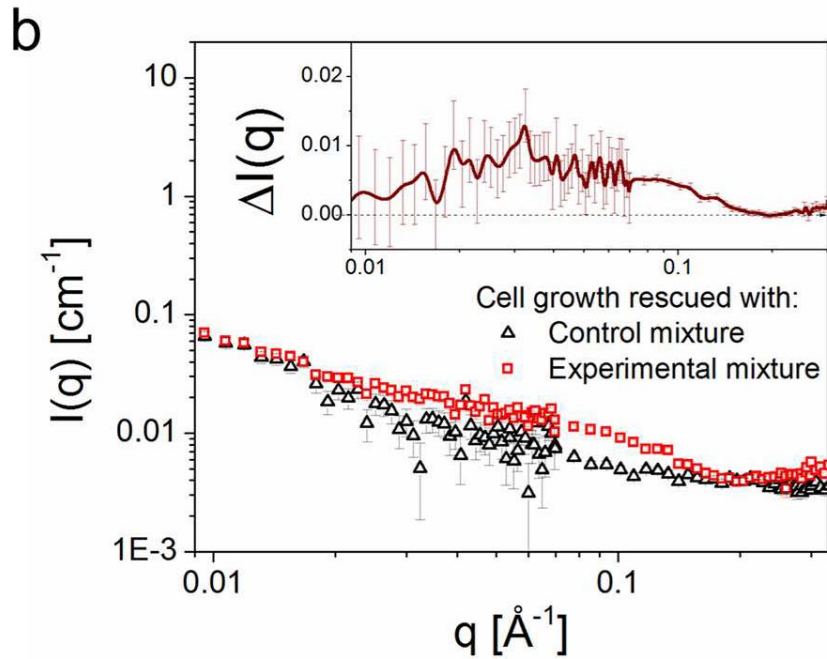


Nickels et al., *PLoS Biology* 15, e2002214 (2017)

# Contrast Variation Strategy to Detect Nanodomains – creating in-plane contrast



# Direct Observation of < 40 nm Lipid Domains in *B. subtilis* – lipid domains also exist in non-eukaryotic systems

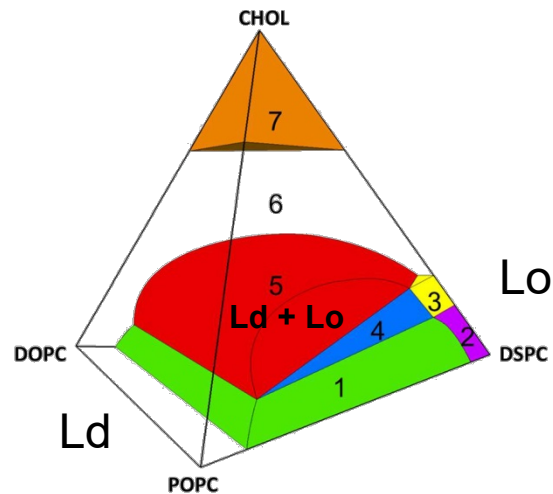


Nickels et al., *PLoS Biology* **15**, e2002214 (2017)

# Questions

Is it useful to know the phase diagram of the A Canonical Phase Separating Lipid Mixture? Why?

## Phase Diagram at 20 °C



Region 1 (green), 2-phase Ld + L $\beta$  coexistence

Region 2 (purple) 1-phase L $\beta$ (L $\beta$ ')

Region 3 (yellow) 2-phase Lo + L $\beta$

Region 4 (blue) 3-phase Lo + Ld + L $\beta$

Region 5 (red) 2-phase Ld + Lo

Region 6 (white) 1-phase varying continuously between Ld and Lo

Region 7 (orange) 2-phase crystalline cholesterol monohydrate + cholesterol-saturated Lo phase

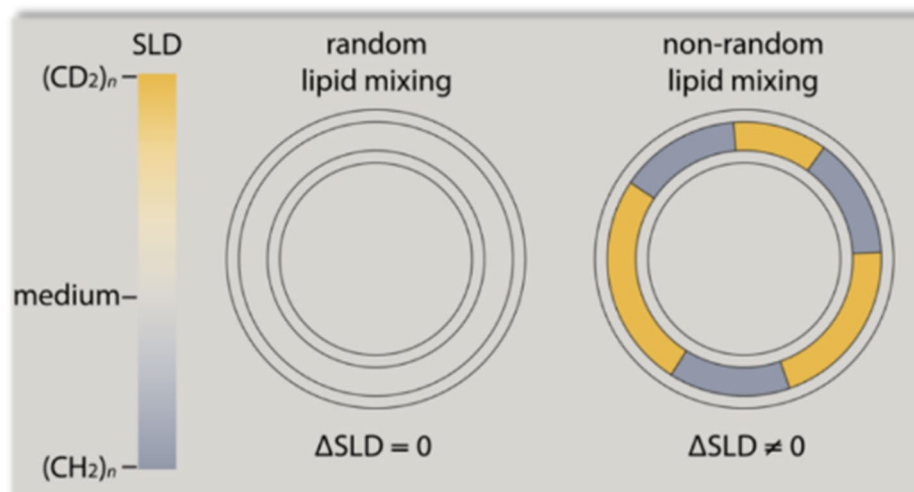
# Questions

For ULVs, SANS arises from three components:

- (i) a mean component from SLD contrast between the average bilayer composition and the solvent.
- (ii) a radial component from SLD variation in the direction normal to the bilayer plane (approximated by two slabs corresponding to the headgroup and acyl chain regions).
- (iii) a lateral component from SLD variation within the plane of the bilayer.

SLDs of the solvent and acyl chains can be controlled experimentally by adjusting the ratio of D<sub>2</sub>O/H<sub>2</sub>O in the aqueous medium, and the ratio of deuterated/protiated chains in the bilayer.

We matched the average SLD of the solvent, headgroup, and acyl chain region (Tables S1 and S2, SI) using a 34.6% D<sub>2</sub>O medium, and 65.4% of chain-perdeuterated DSPC-d70 (expressed as a percentage of total DSPC).



**F. A. Heberle et al., *J. Am. Chem. Soc.* 135, 6853 (2013)**

$$(\Delta\delta)^2 = (\delta_{\text{lipid}} - \delta_{\text{water}})^2 \neq 0 \text{ scattering}$$
$$(\Delta\delta)^2 = (\delta_{\text{lipid}} - \delta_{\text{water}})^2 = 0 \text{ no scattering}$$

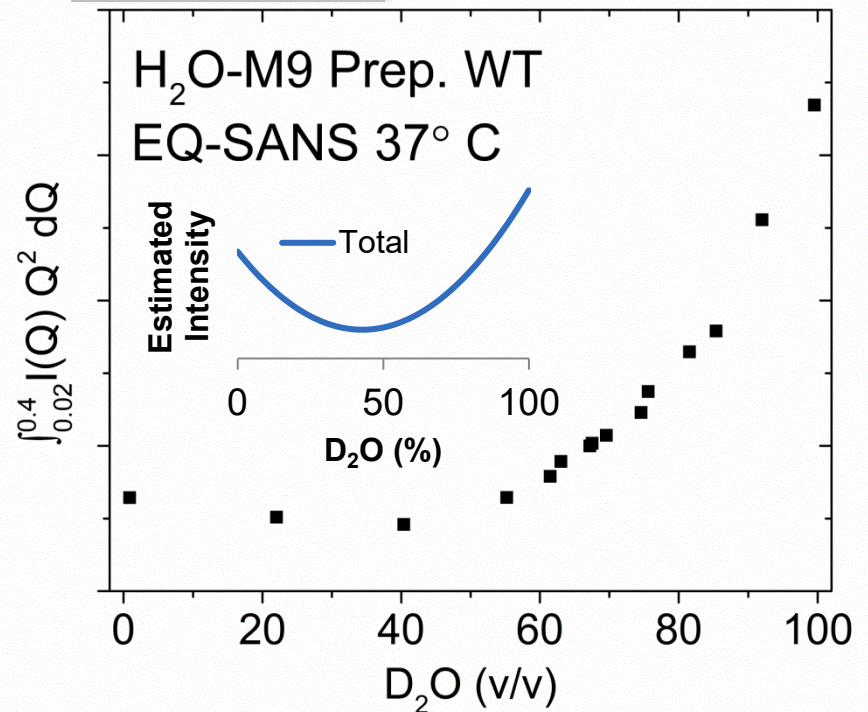
# Questions

You can calculate the SLD of the membrane and water to determine the contrast match conditions. How would you determine the contrast match condition experimentally?

Table S2. Neutron scattering lengths, molecular volumes at 60 °C, and corresponding scattering length densities of species relevant to this study

Molecule	Chem. Formula	$b$ [fm]	$V$ [Å <sup>3</sup> ]	SLD [fm/Å <sup>3</sup> ]
PC headgroup	C <sub>10</sub> H <sub>18</sub> NO <sub>8</sub> P	60.1	331 <sup>a</sup>	0.181
DSPC chains	C <sub>34</sub> H <sub>70</sub>	-35.8	1017 <sup>b</sup>	-0.035
DSPCd <sub>70</sub> chains	C <sub>34</sub> D <sub>70</sub>	692.9	1017 <sup>b</sup>	0.681
DOPC chains	C <sub>34</sub> H <sub>66</sub>	-20.8	1003 <sup>c</sup>	-0.021
POPC chains	C <sub>32</sub> H <sub>64</sub>	-26.6	953 <sup>b</sup>	-0.028
cholesterol	C <sub>27</sub> H <sub>46</sub> O	13.3	630 <sup>d</sup>	0.021
water	H <sub>2</sub> O	-1.68	30.4	-0.055
heavy water	D <sub>2</sub> O	19.15	30.5	0.628
34.6% heavy water	H <sub>1.31</sub> D <sub>0.69</sub> O	5.53	30.4	0.181
11.7% heavy water	H <sub>1.77</sub> D <sub>0.23</sub> O	0.761	30.4	0.025

100% H<sub>2</sub>O



# Questions

How can you improve on the experiment by Heberle et al.?

The average SLD of the solvent, headgroup, and acyl chain region using a **34.6% D<sub>2</sub>O medium**, and 65.4% of chain-perdeuterated DSPC-d70 (expressed as a percentage of total DSPC).

**Table S1.** Compositions of lipid samples. Further details can be found in <sup>7b</sup>.

Mole Fraction:	DSPC	POPC	Chol.	d83-DSPC	d70-DSPC	d44-POPC	d31-POPC	d41-Chol.	Solvent
L <sub>D</sub>	0.09	0.79	0.12	-	-	-	-	-	D <sub>2</sub> O
L <sub>O</sub>	0.49	0.26	0.25	-	-	-	-	-	D <sub>2</sub> O
Domains	0.39	0.39	0.22	-	-	-	-	-	D <sub>2</sub> O
Domains-L <sub>D</sub>	-	-	-	0.39	-	0.38	0.01	0.22	93.5% D <sub>2</sub> O
Domains-L <sub>O</sub>	-	-	-	0.18	0.21	0.18	0.21	0.22	64.5% D <sub>2</sub> O

**Table S2.** Calculated neutron scattering length densities of headgroup and acyl regions at 20°C for the phases present in each sample. These values are derived from volume data compiled in <sup>7b</sup> from <sup>43</sup>. Atomic scattering lengths were taken from the NIST database <sup>44</sup>.

	L <sub>D</sub> Head Group [fm/Å <sup>3</sup> ]	L <sub>D</sub> Acyl [fm/Å <sup>3</sup> ]	L <sub>O</sub> Head Group [fm/Å <sup>3</sup> ]	L <sub>O</sub> Acyl [fm/Å <sup>3</sup> ]	Solvent [fm/Å <sup>3</sup> ]
L <sub>D</sub>	0.18	0.00	-	-	0.63
L <sub>O</sub>	-	-	0.18	0.00	0.63
Domains	0.18	0.00	0.18	0.00	0.63
Domains-L <sub>D</sub>	0.58	0.38	0.58	0.58	0.58
Domains-L <sub>O</sub>	0.38	0.38	0.38	0.58	0.38

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## Bilayer Thickness Mismatch Controls Domain Size in Model Membranes

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J. Am. Chem. Soc. 2013, 135, 6853–6859



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## Mechanical Properties of Nanoscopic Lipid Domains

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J. Am. Chem. Soc. 2015, **137**, 15772–15780



RESEARCH ARTICLE

## The in vivo structure of biological membranes and evidence for lipid domains

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