Determining the Structure of Bio-membranes by Neutron Diffraction and Reflectometry

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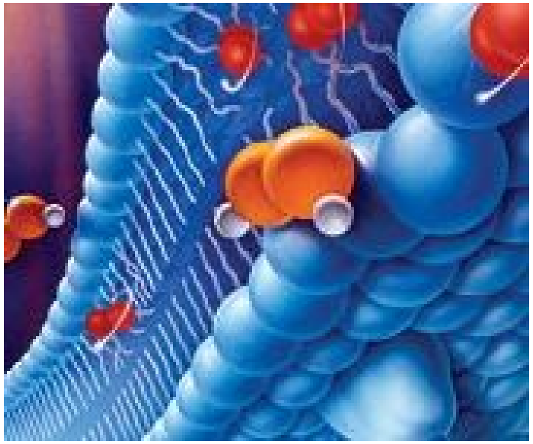
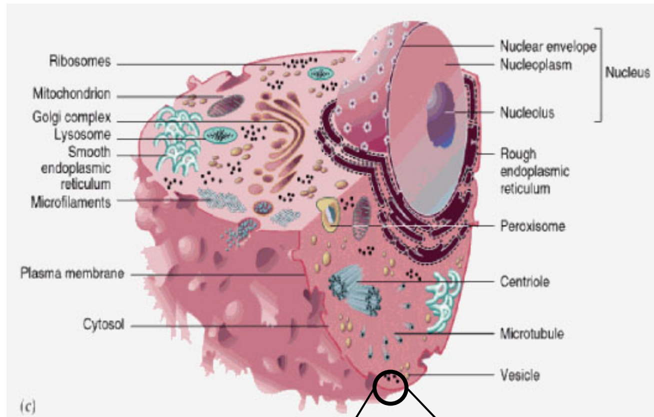
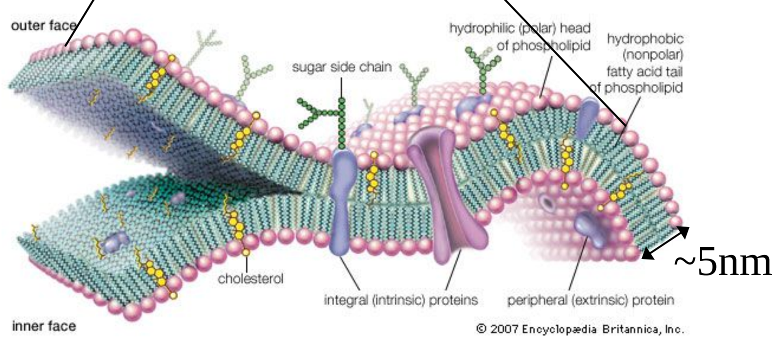


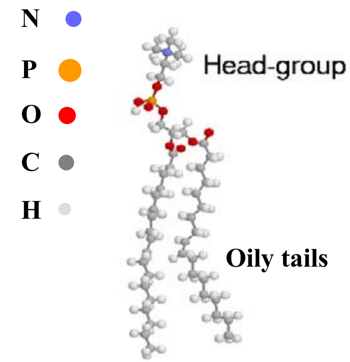
Cell membranes



Lipid bilayers

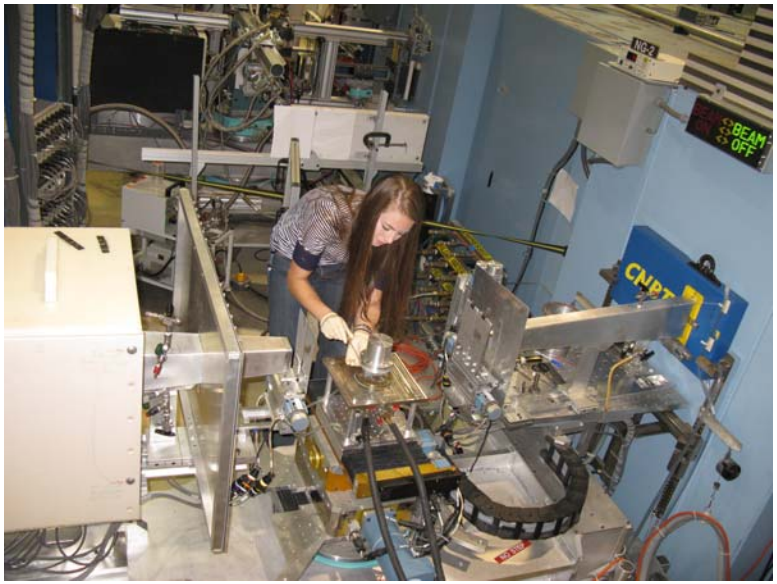


Lipids



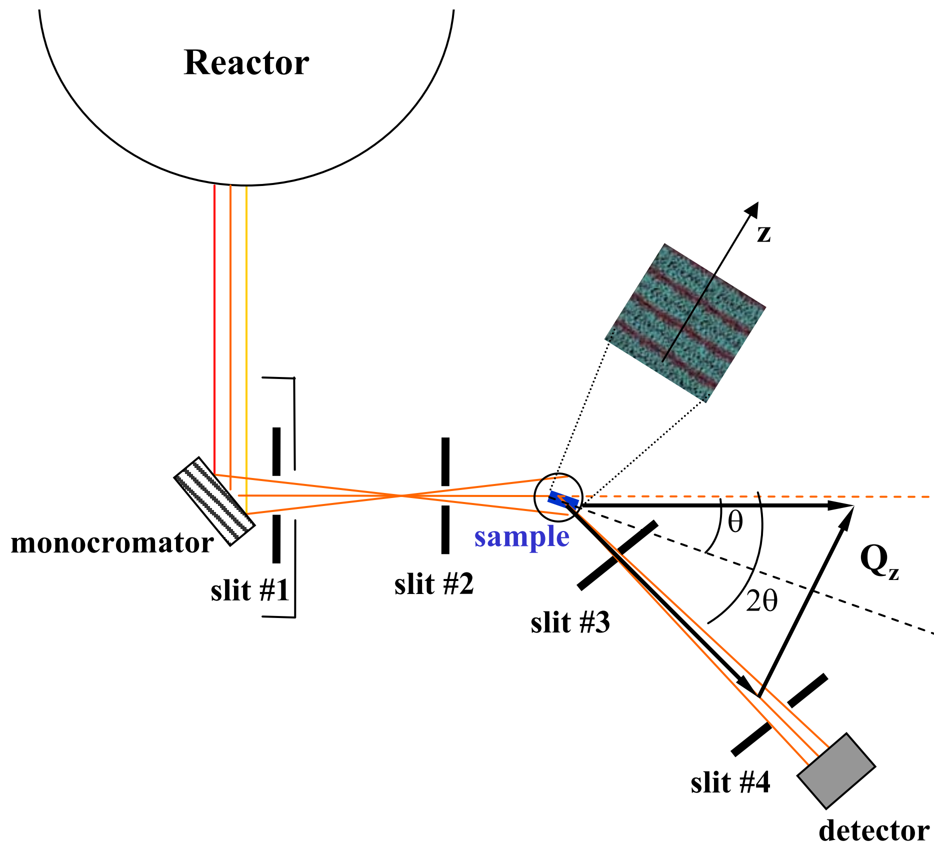
A neutron diffractometer/reflectometer

The Advanced Neutron Diffractometer/Refletometer
At the NIST Center for Neutron Research (NCNR)



$\lambda_n = 5$ Å
$\Delta\lambda/\lambda = 1\%$

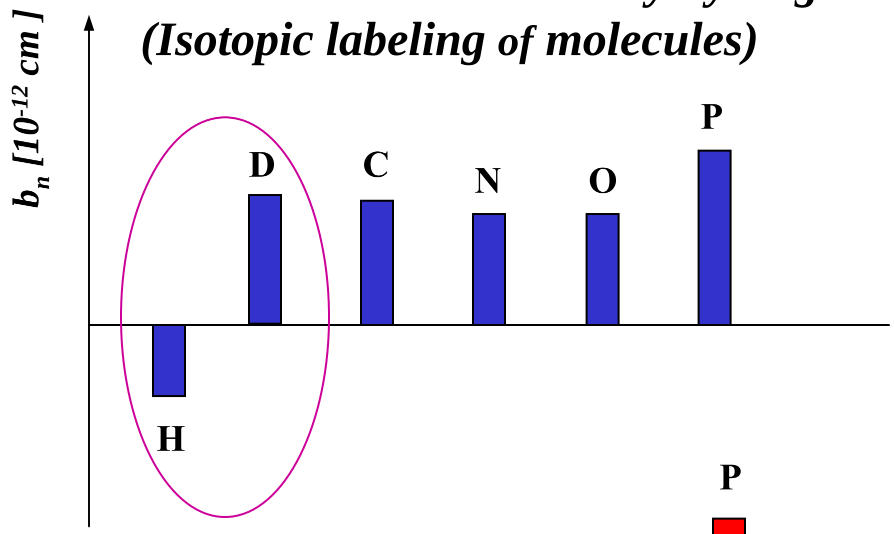
Schematic of the AND/R



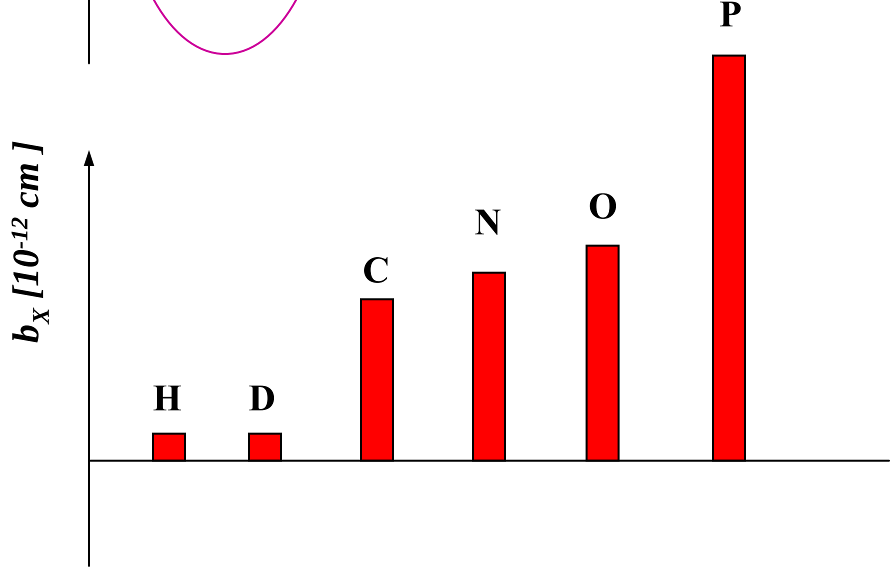
Scattering Lengths
Why neutrons when we have X-rays?

One can create contrast by hydrogen → deuterium exchange (Isotopic labeling of molecules)

Neutrons

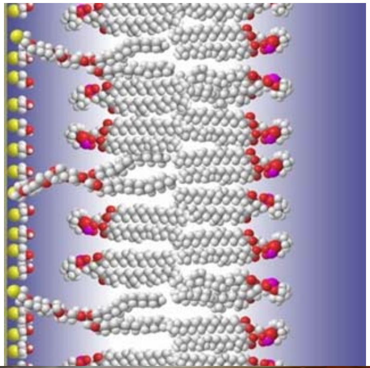
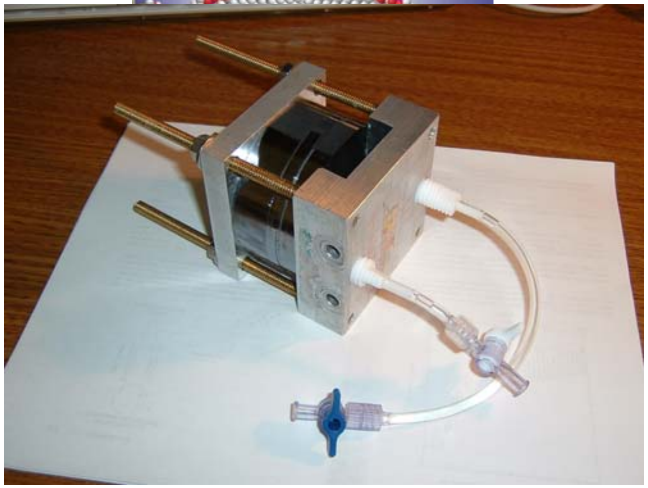


X-rays
$b_X \sim Z$

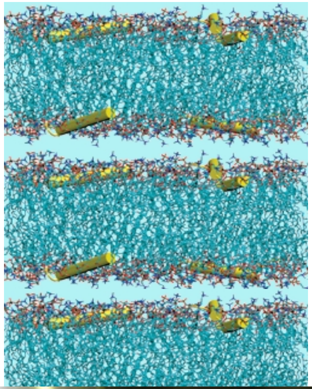
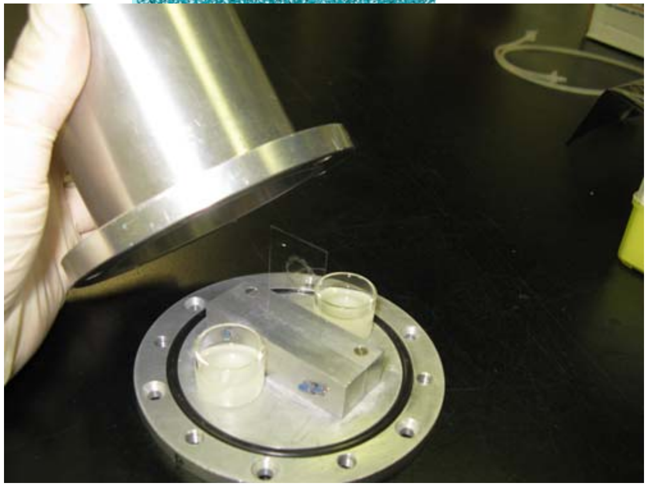


Single bilayer versus multilayers for the study of membrane structure

Single thethered membrane
-membrane fully hydrated in a ‘wet cell’

Oriented lipid stacks
-dry membranes hydrated from the vapor phase of a salt solution

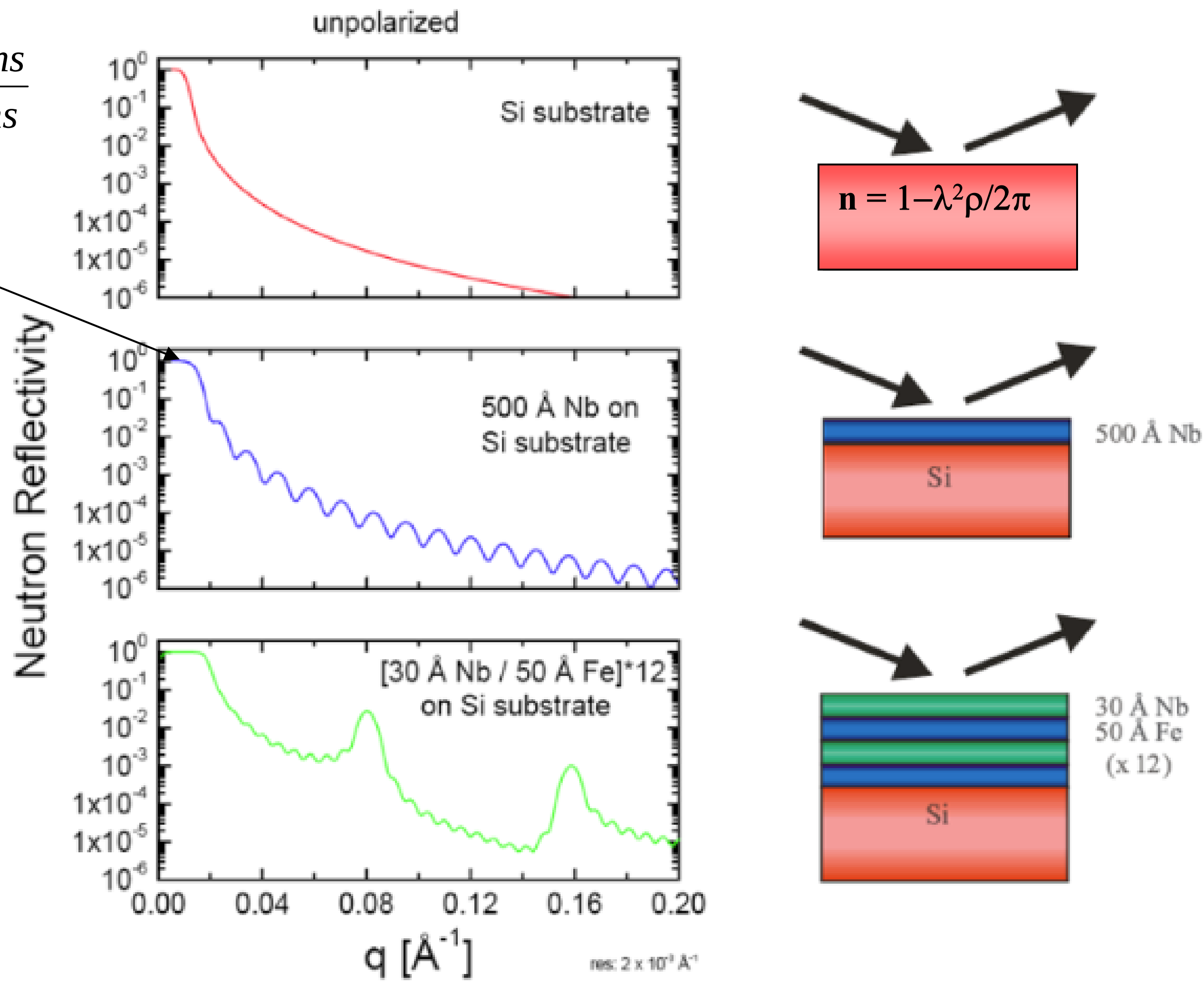



Reflectometry versus Diffraction.
What's the difference?…

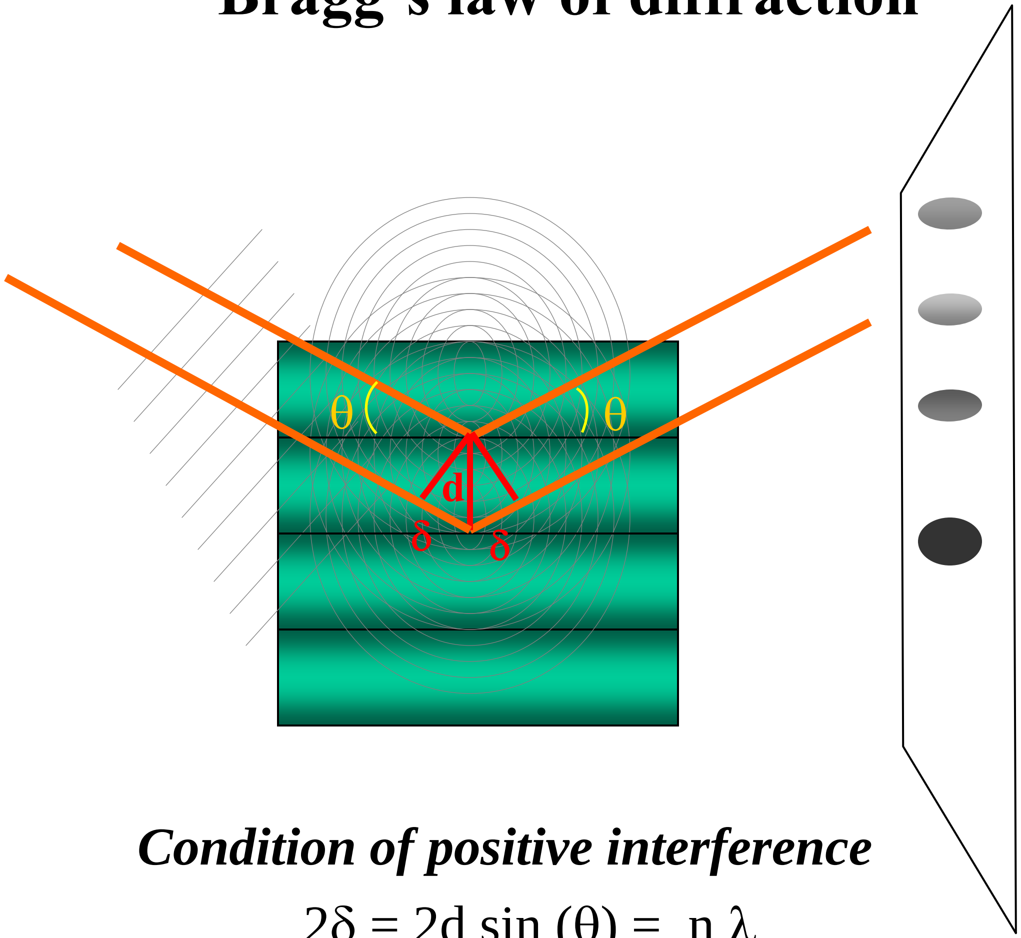
$$I = \frac{\#\,reflected\ neutrons}{\#\,incident\ neutrons}$$

Total reflection

(critical edge)



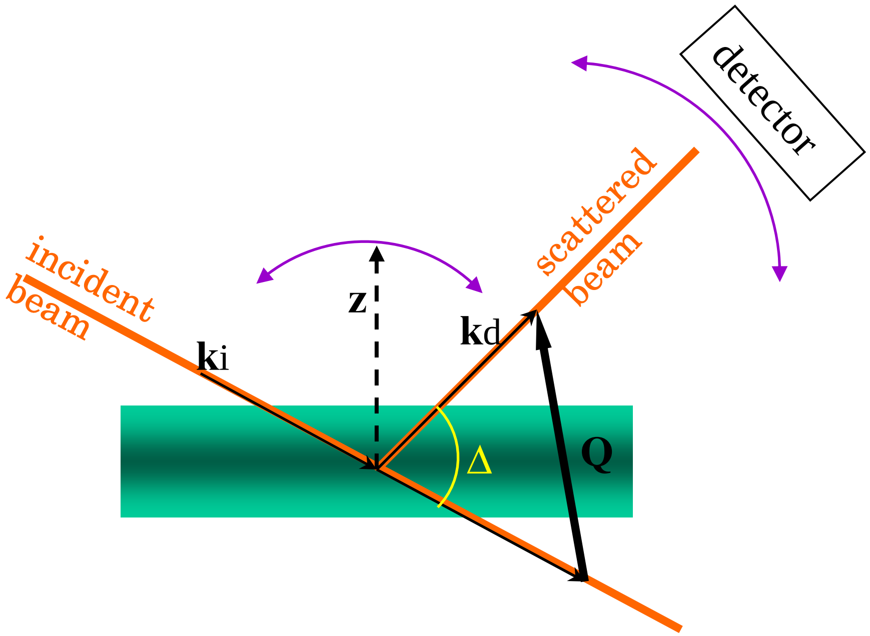
Bragg's law of diffraction



Condition of positive interference

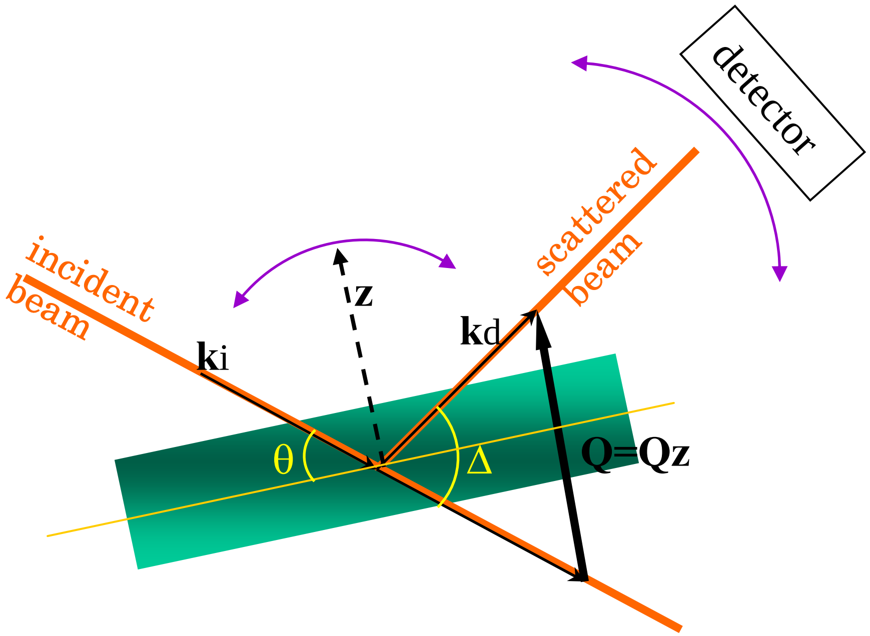
$$2\delta = 2d \sin(\theta) = n\lambda$$

Momentum transfer and planar geometry



$$Q = \frac{4\pi}{\lambda}\sin(\frac{\Delta}{2})$$

Momentum transfer and planar geometry

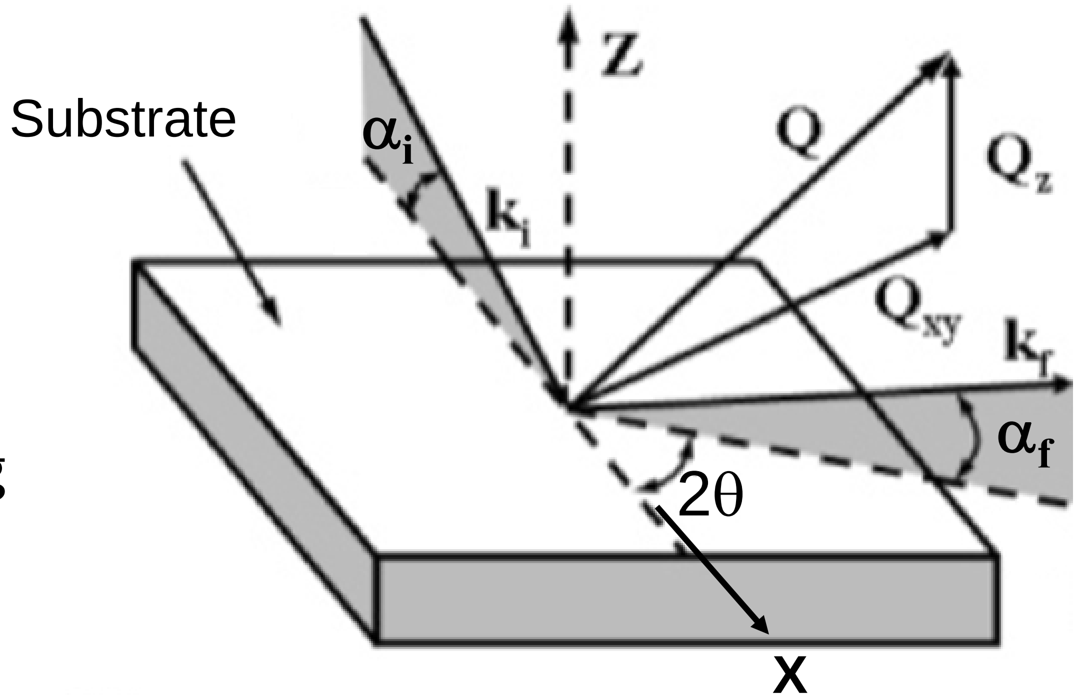


$$\Delta = 2\theta$$

$$Q = Q_z = \frac{4\pi}{\lambda}\sin(\theta)$$

Momentum transfer components

Basic parameters for general case, including $\alpha_i = 90°$:



2θ here not to be confused with scattering angle

$$q_x = \frac{2\pi}{\lambda}(\cos\alpha_f \cos(2\theta) - \cos\alpha_i)$$

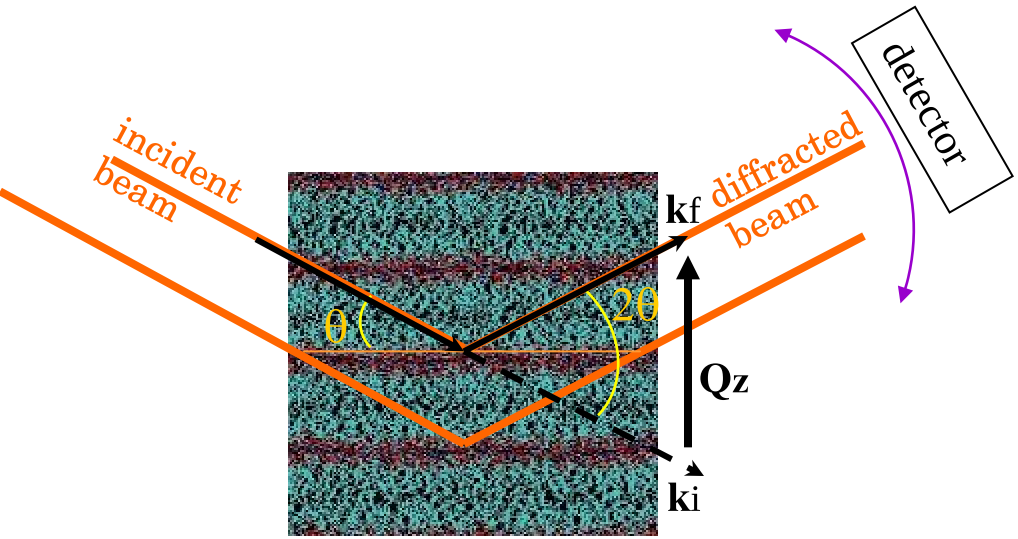
$$q_y = \frac{2\pi}{\lambda}\cos\alpha_f \sin(2\theta)$$

$$q_z = \frac{2\pi}{\lambda}(\sin\alpha_i + \sin\alpha_f)$$

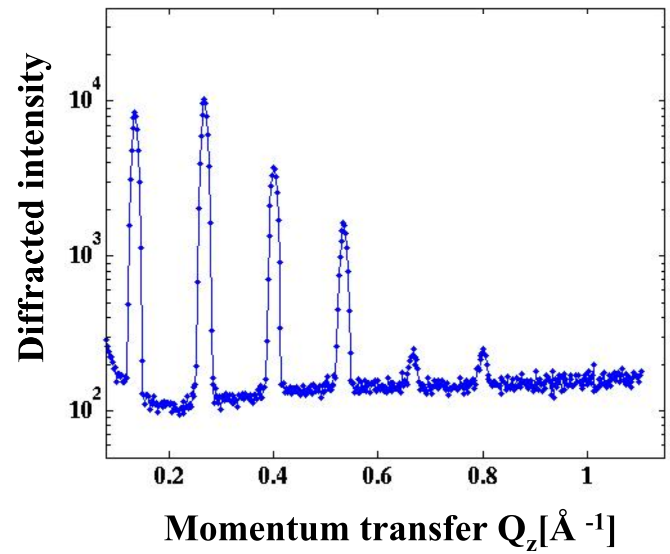
$q_r = (q_x^2 + q_y^2)^{1/2}$

For cylindrical geometry, sample invariant on rotation about Z.

Diffraction from lipid multilayers to determine the membrane structure



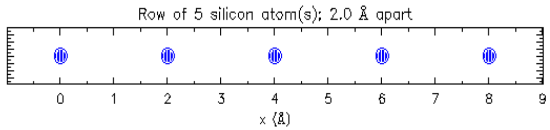
DOTAP at 93% relative humidity
~3000 layers

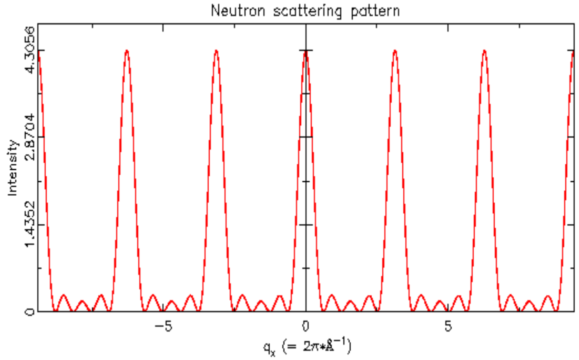


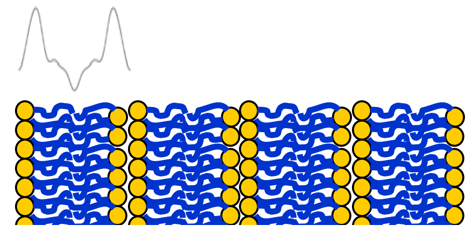
Bragg peaks @

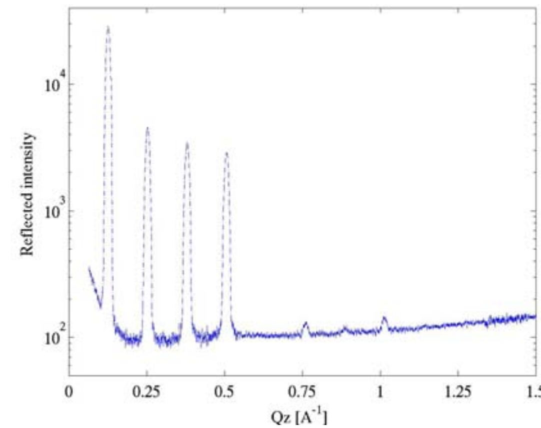
$$Q_z^n = \frac{4\pi}{\lambda}\sin(\theta_n) = n\frac{2\pi}{d}$$

The application of the convolution theorem to lipid multilayer diffraction









Row of atoms:
No atomic internal structure can be "seen" by the neutron

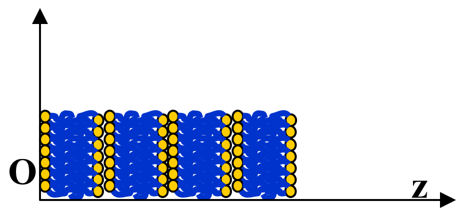
Stack of lipid bilayers:
The internal bilayer structure can be resolved by the neutron

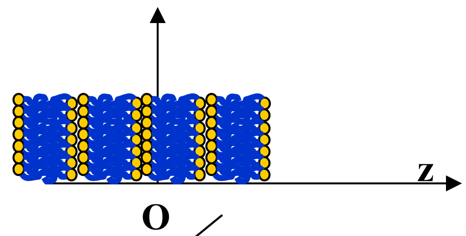
$$D(z)=\Sigma\delta(z-nd) © \rho_{\text{unit cell}}(z) \xrightarrow{\textit{Fourier transform}} s(q) = \mathcal{F}(\Sigma\,\delta(z-nd)) \,.\, \mathcal{F}(\rho_{\text{unit cell}})$$

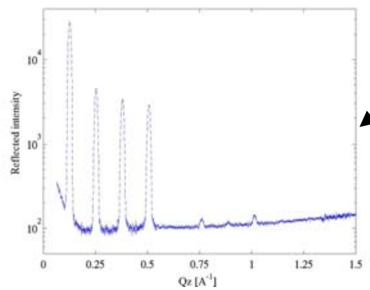
- **The diffraction peak intensities are modulated by the density distribution within the unit cell**

The phase problem

➢ ***What is measured:*** **Intensity** $= |s(Q_z)|^2 \sim |F(\rho_{\text{unit cell}})|^2 = \left| \int_{-d/2}^{d/2} \rho_{\text{unit cell}}(z)\, e^{izQ_z}\, dz \right|^2$







▪ **The intensity in reciprocal space in invariant under a shift of origin in real space**

For a centro-symmetric system **($\rho(z)=\rho(-z)$):**

$$s(Q_z) = \int_{-d/2}^{d/2} \cos(izQz)\, \rho(z)\, dz + \cancel{i \int_{-d/2}^{d/2} \sin(izQz)\, \rho(z) dz} \longrightarrow$$

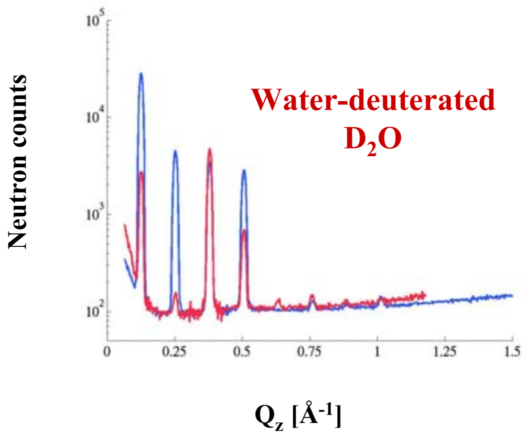
We determine phase of s(Qz) by H -> D contrast variations at known positions

▪ **There are only two possibilities: -1 and +1, corresponding to cos(0) and cos (180).**

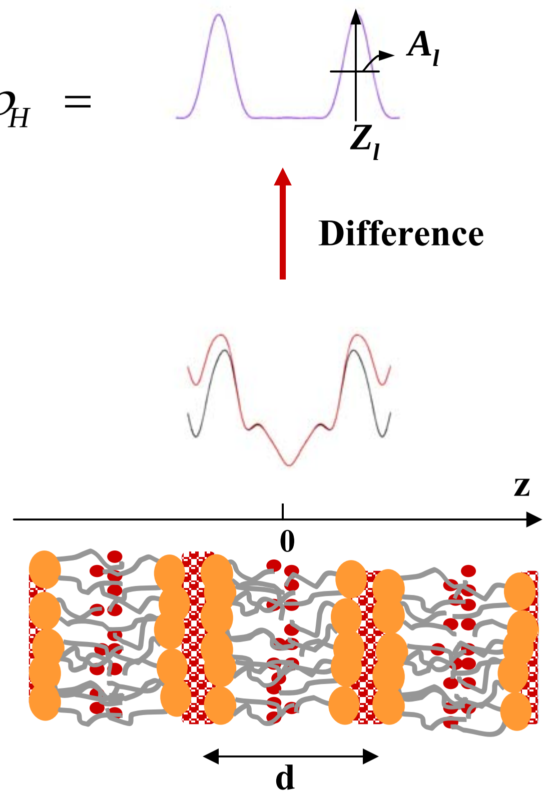
➢ ***Diffraction: signal sampled at*** $Q_z^n \longrightarrow$ **$\rho_{\text{unit cell}}(z)$= Fourier synthesis (+/-$\sqrt{I(n)}$)**

Using contrast to determine structural details from diffraction data

$$\rho_D(z) - \rho_H \quad =$$

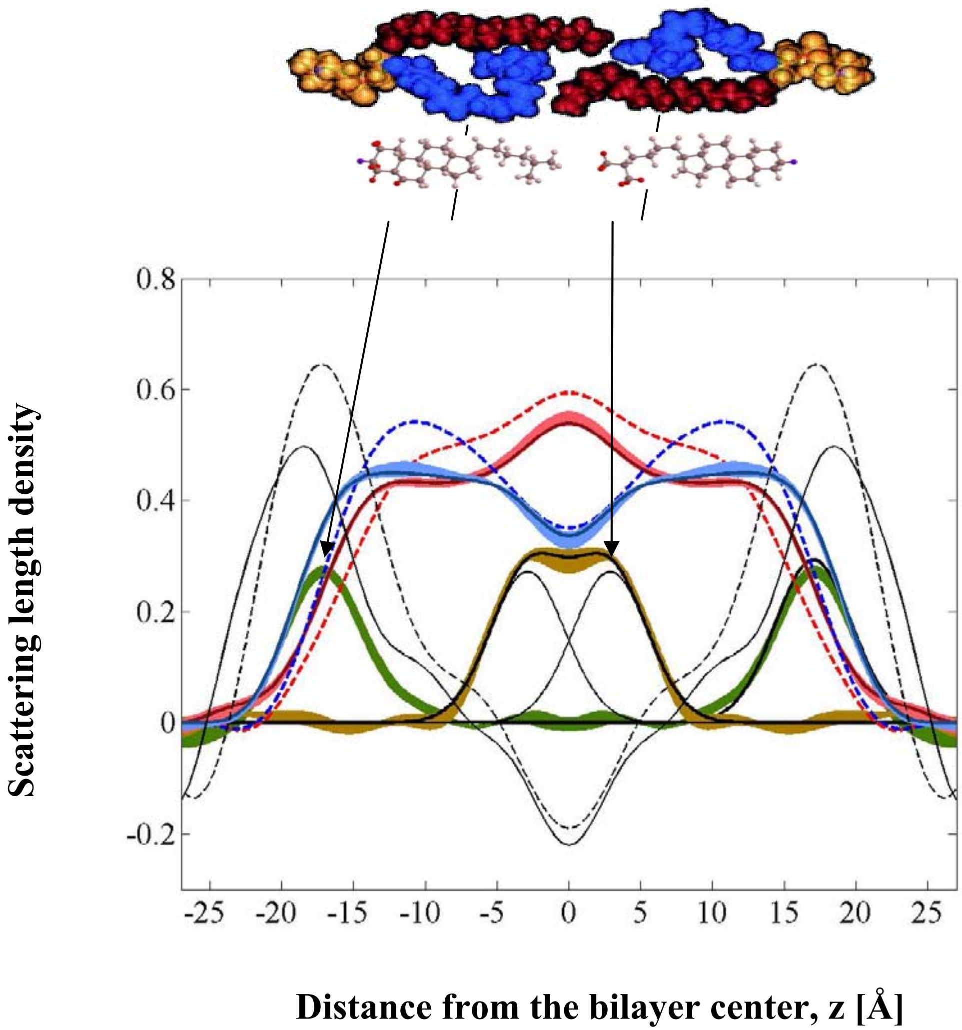


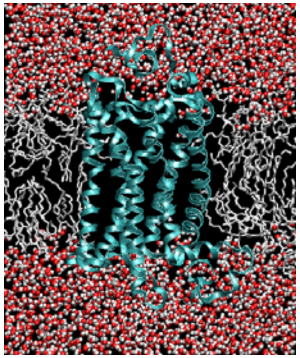
$$\rho_{H/D}(z) = \bar{\rho}_{H/D} + \frac{2}{d}\sum_n \overbrace{\pm\sqrt{I(n)}}^{F_{H/D}(n)}\cos\left(\frac{2\pi n z}{d}\right)$$



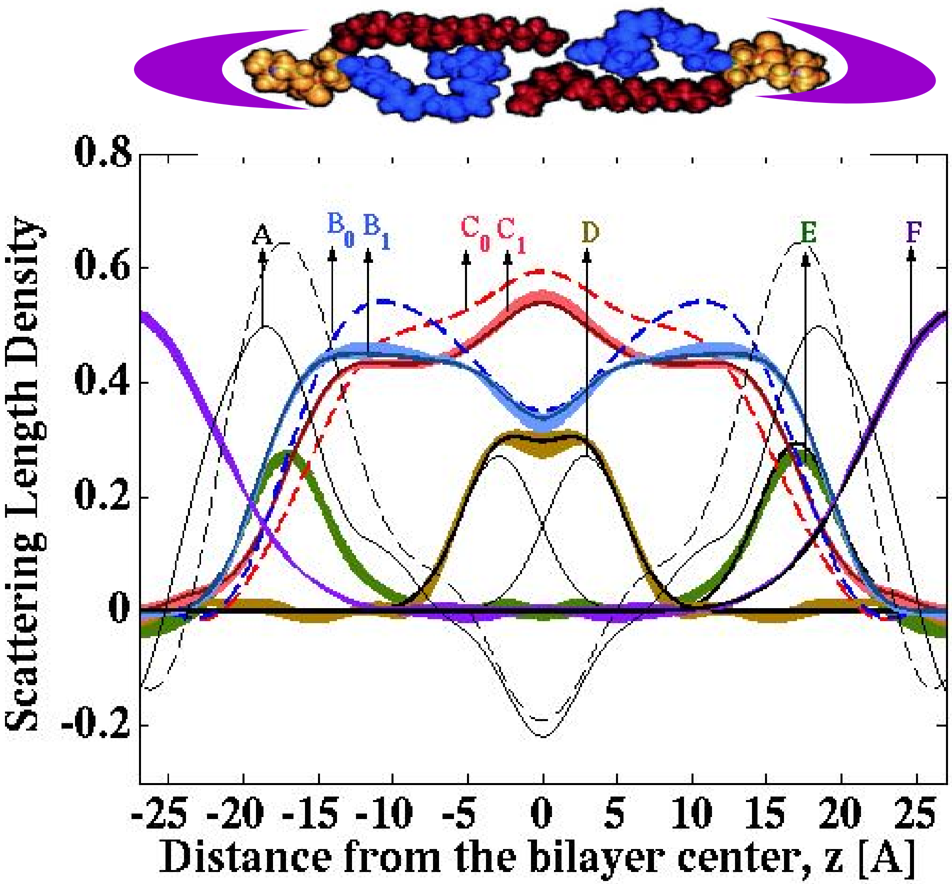
Examples of neutron diffraction experiments

Polyunsaturated lipids important for the function of brain receptors.





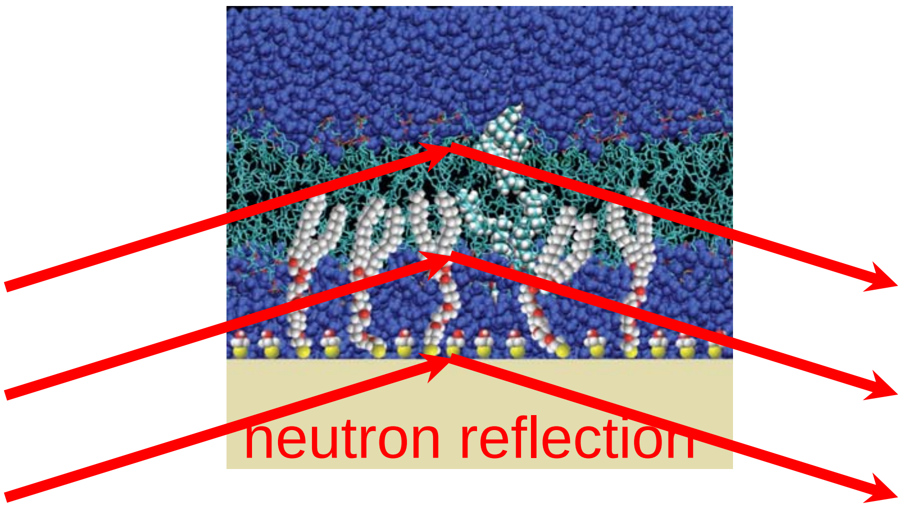
Rhodopsin/DHA

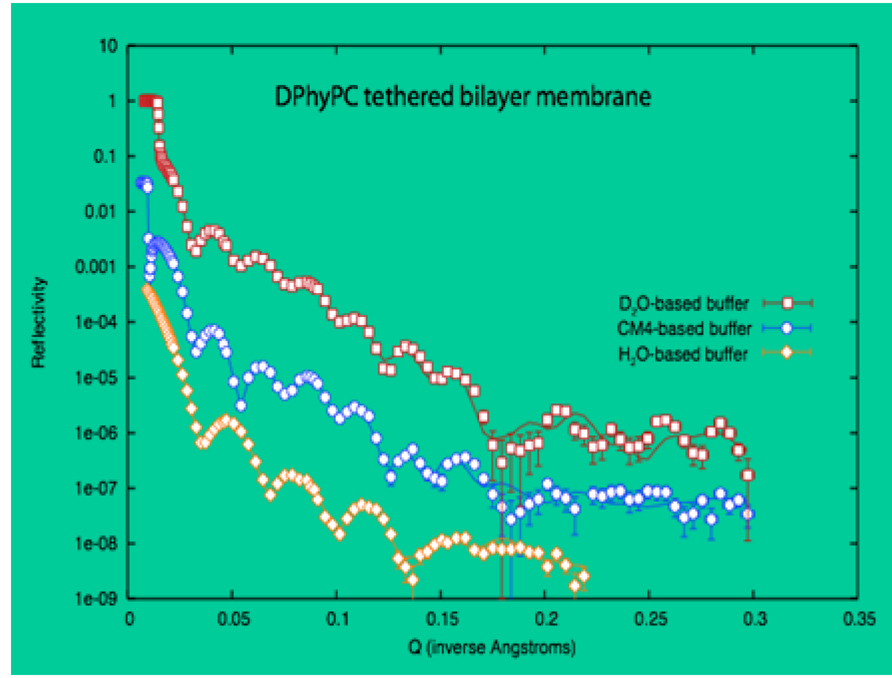


A – bilayer w. cholesterol
B0 - DHA chain, no cholesterol
B1 – DHA chain w. cholesterol
C0 – SA chain, no cholesterol
C1 – SA chain w. cholesterol
D – Cholesterol A-ring
E - Cholesterol CH3-tail
F- water

Designing reflectometry experiments

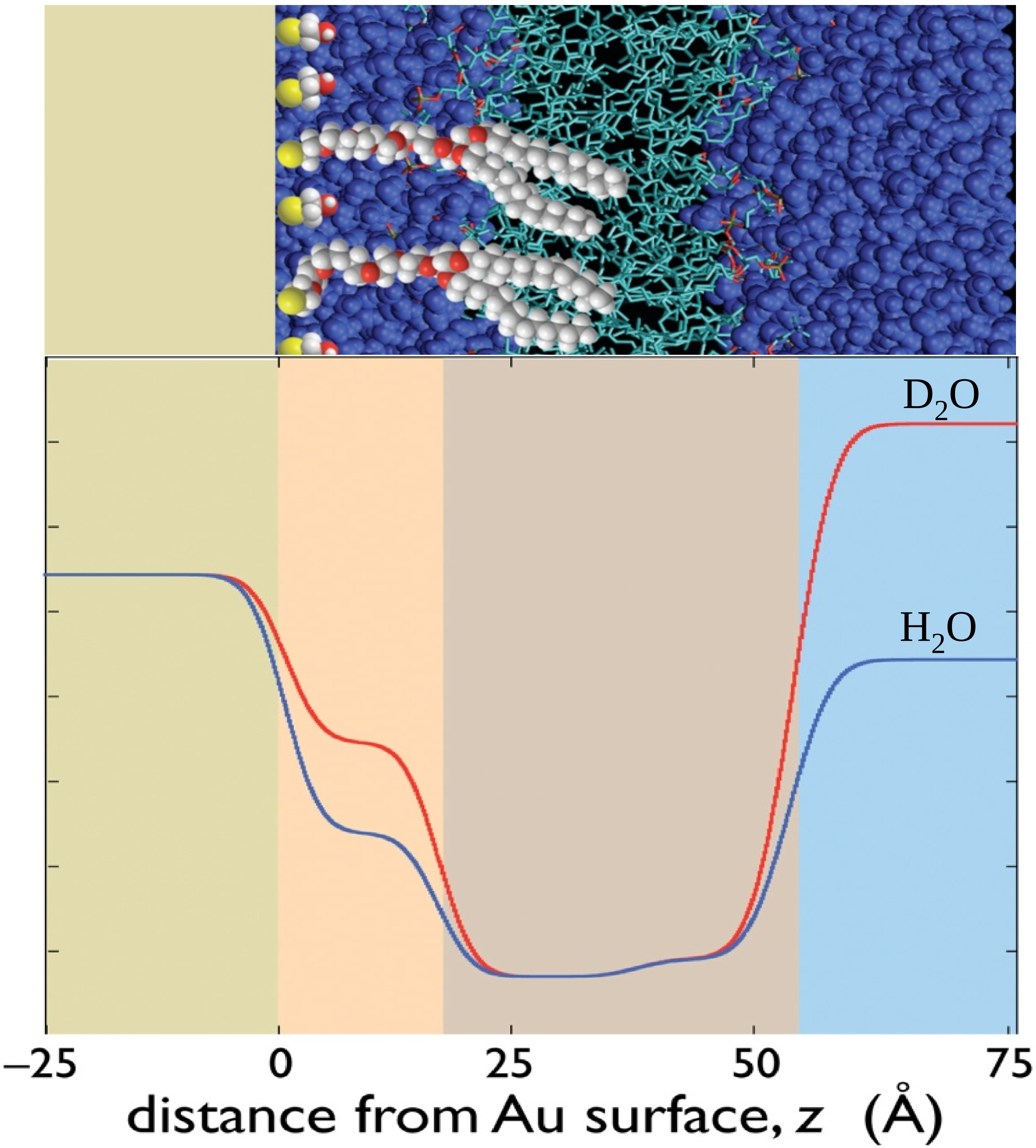
"tethered" lipid bilayer membrane (tBLM)



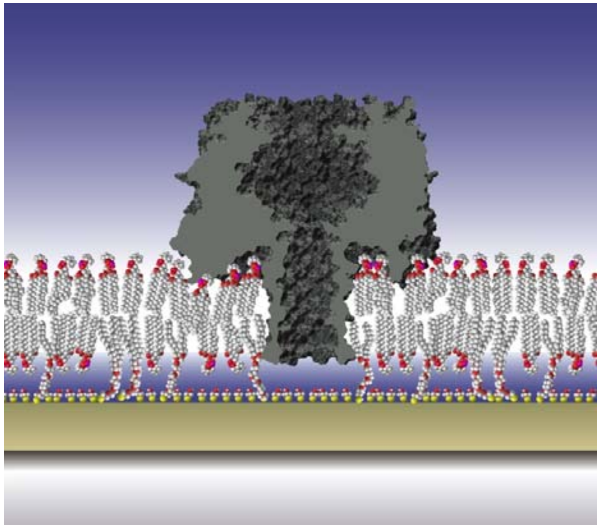


- Find an appropriate model to describe the layered molecular structure in terms of neutron Scattering Length Density distribution, in real space
- Fourier transform the model
- Fit the model to the data (Reflectivity vs. Qz) to find the SLD

Determining the SLD profile of the membrane



Summary



• To determine the 'in-depth' molecular architecture in model membranes one can use:

- reflectometry experiments on single supported membranes

- *membrane fully hydrated*
- *possibility of studying incorporated proteins with large extra-cellular domains*
- *requires an appropriate model for the molecular modeling*

- diffraction experiments on multilayers hydrated from vapor phase

- *higher structural resolution*
- *direct determination of the structure from the Bragg intensities*
- *membrane only partially hydrated (small inter-membrane space)*

Acknowledgements

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