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From Discovery to Innovation...

Looking at the biomembrane structure through the optics of neutron diffraction

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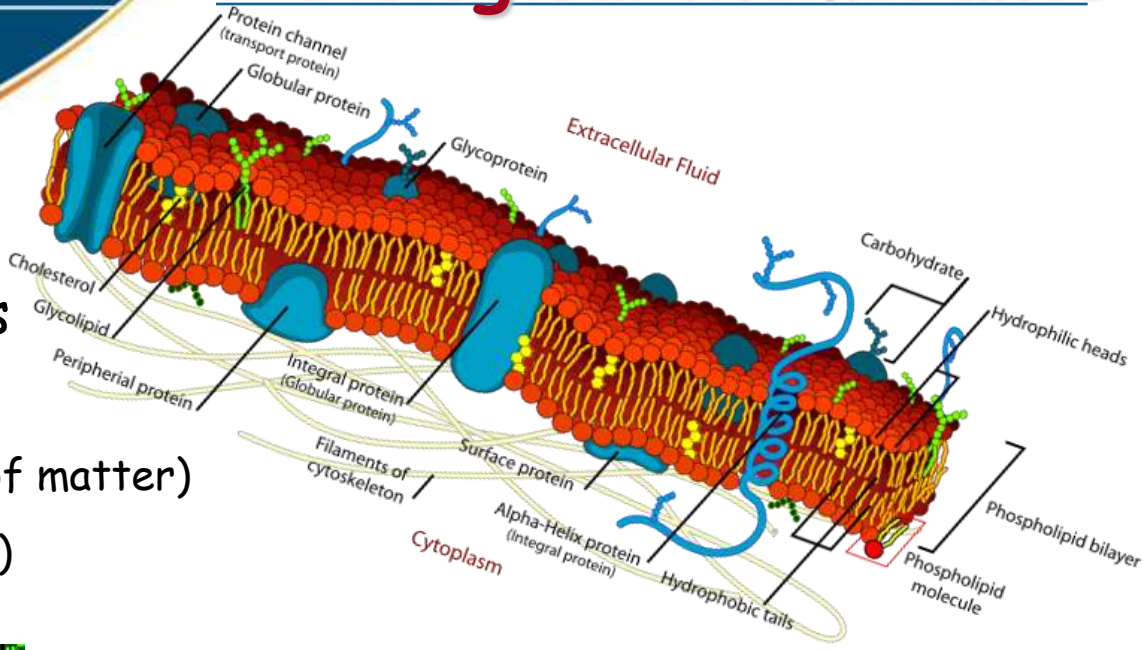
NRC – Canadian Neutron Beam Centre at Chalk River

Outline

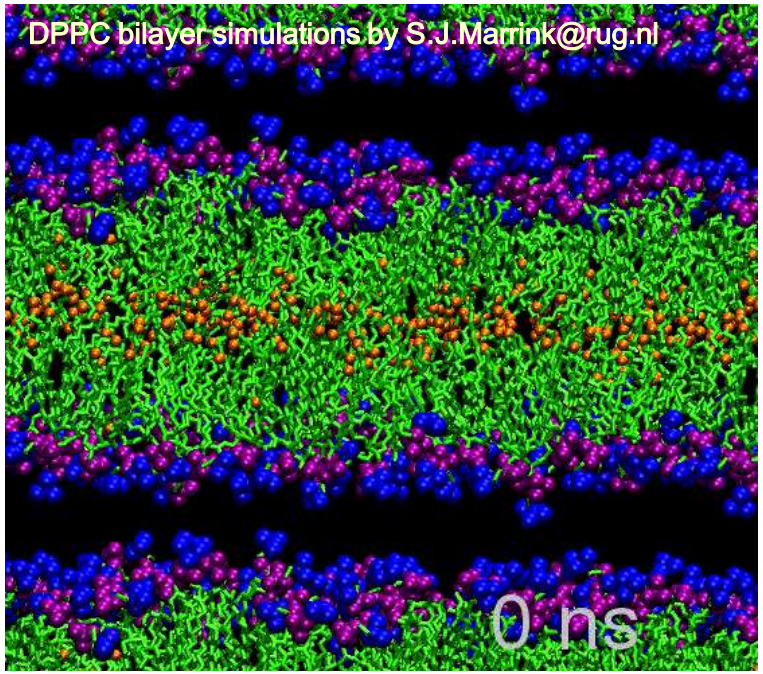
- Object: Biological Membranes
- Method: Small-Angle Neutron Diffraction
- Theory: Bragg's Law
  Rocking Curve
  Phase Determination
- Application: Lipid Bilayers Structure
  Water Distribution in LPS Bilayers
  Cholesterol Distribution in PUFA Bilayers

Biological Membranes



Biological membrane delivers

- Protection (separates cell)
- Selective permeability (transport of matter)
- Signaling (transport of information)

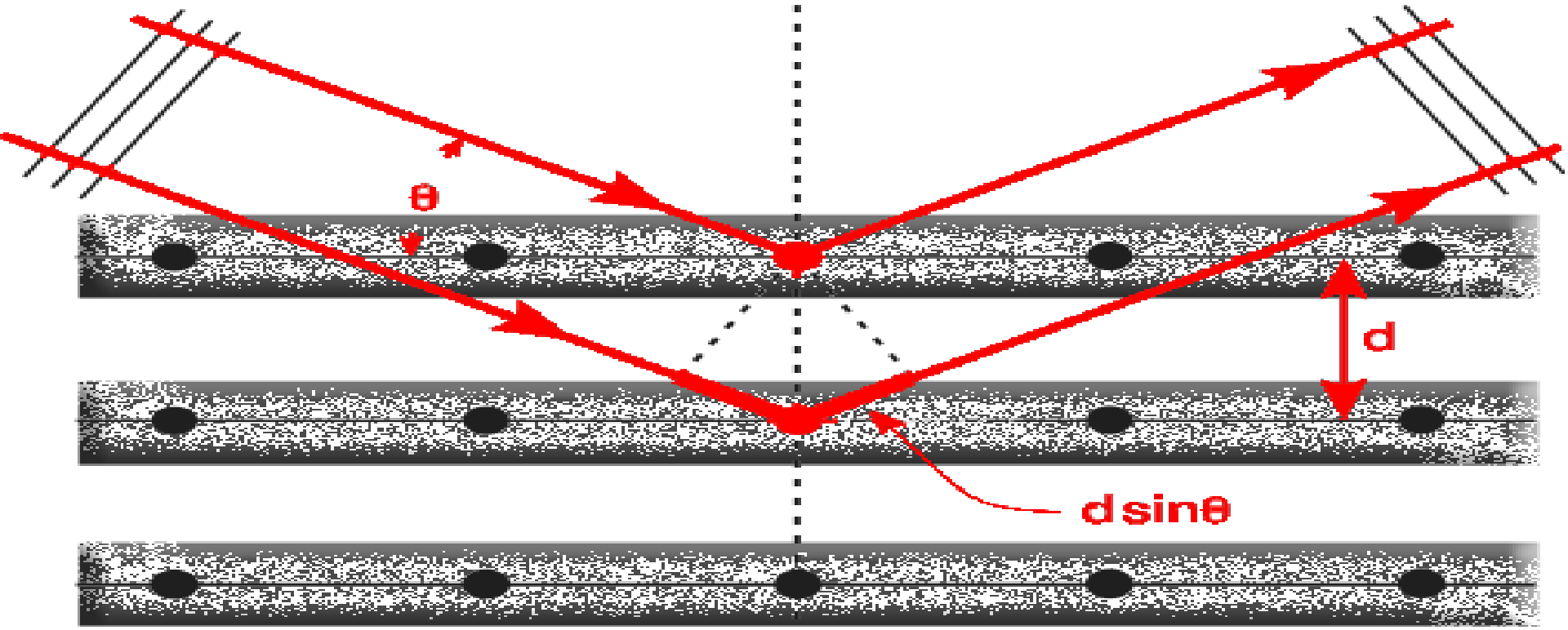


- Active functions are mainly provided by proteins
- Passive functions are determined by the structure of a lipid matrix
- Lipid matrix is a 2D liquid, where lipids and proteins diffuse almost freely

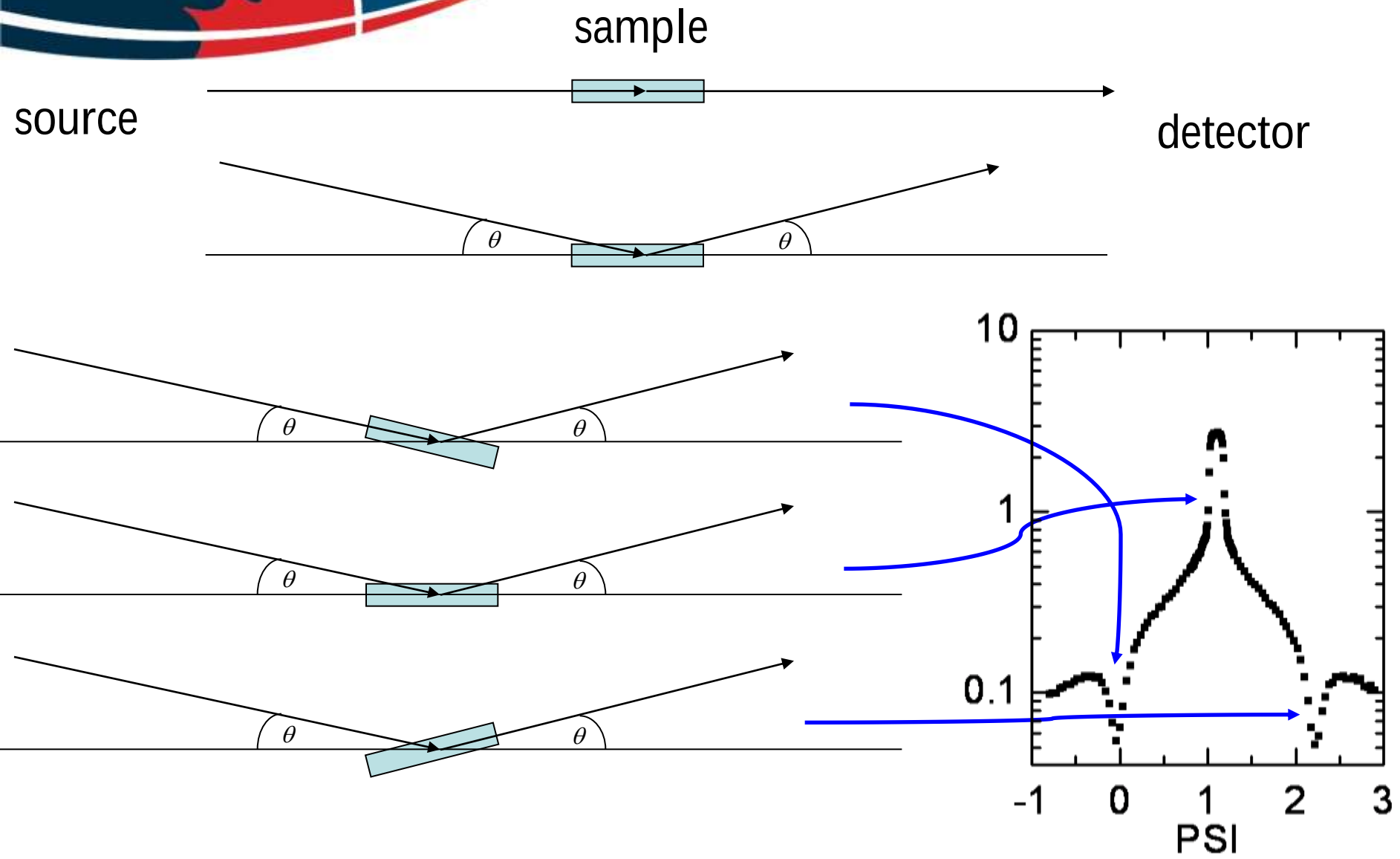
Bragg's Law

The two planes will scatter in phase if the path difference '$2d\sin(\theta)$' is a whole number of wavelengths '$n\lambda$':

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



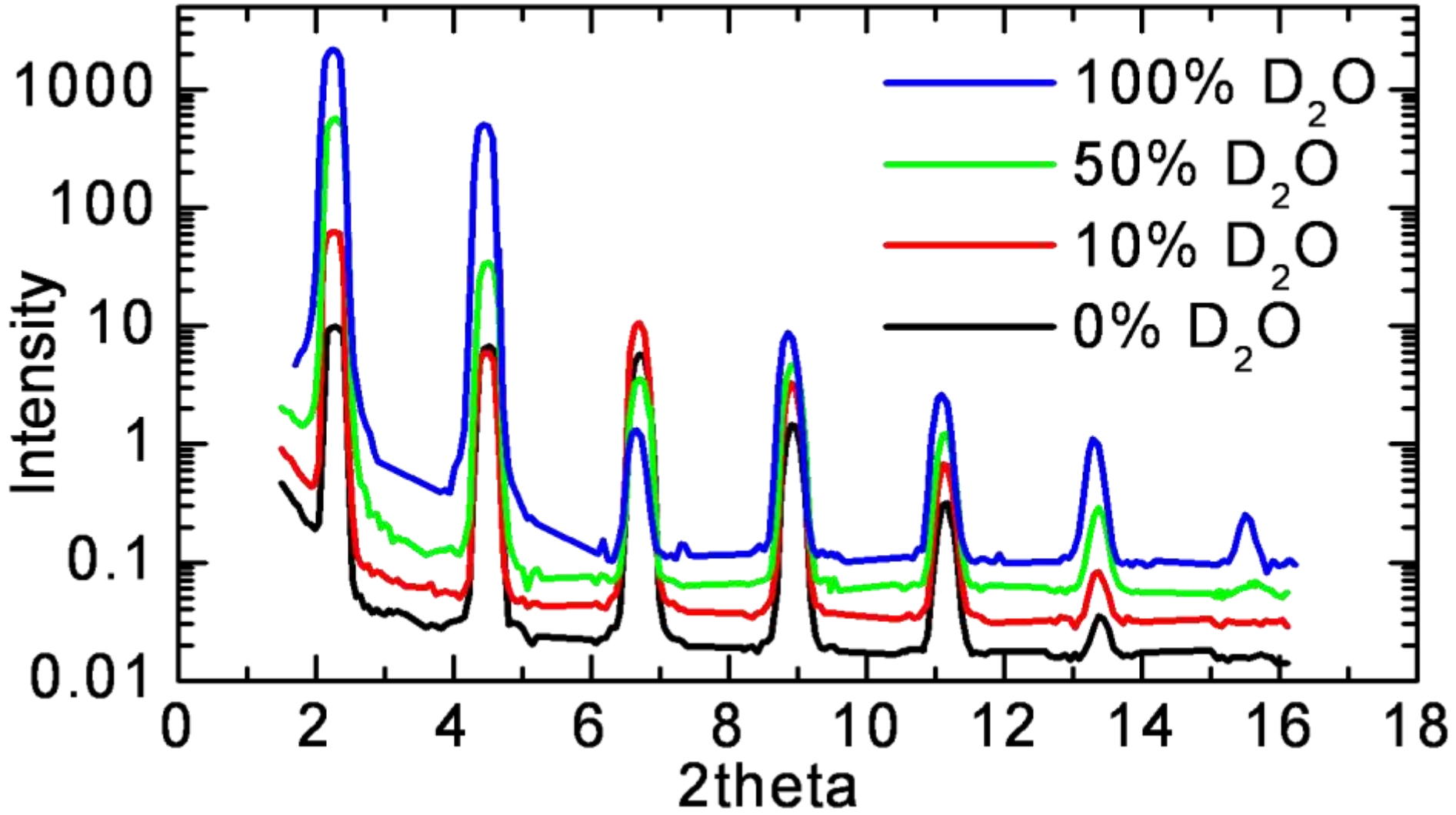
Rocking Curve



Diffraction Curve

$$I(q) \sim F^2(q)$$

$$F(q) \sim FT(\rho(z))$$



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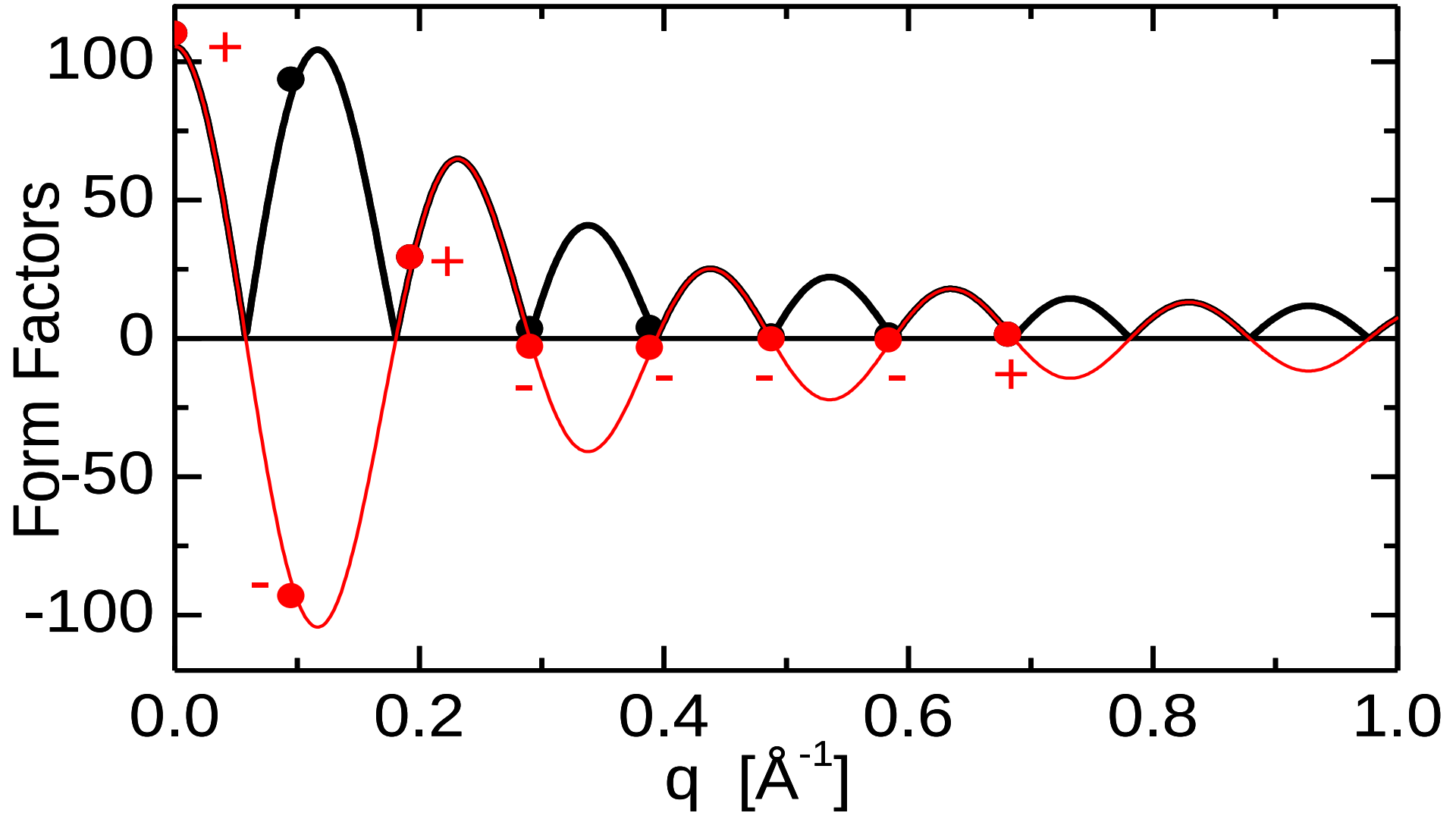
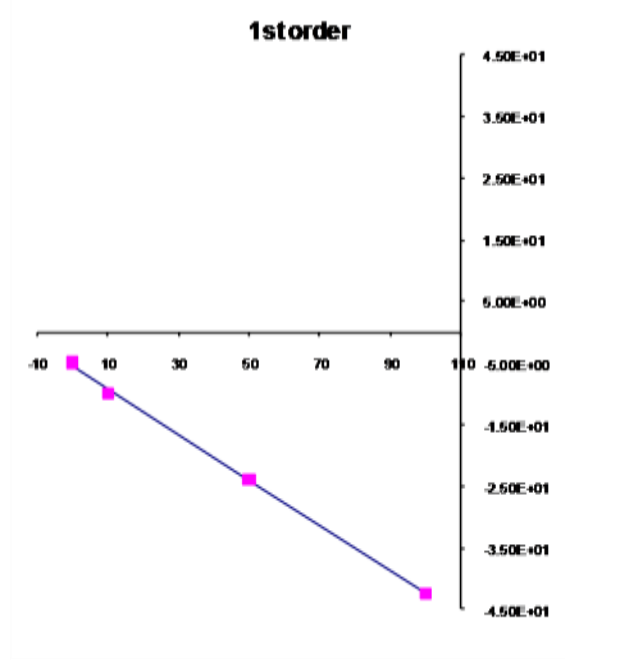
Phase Problem

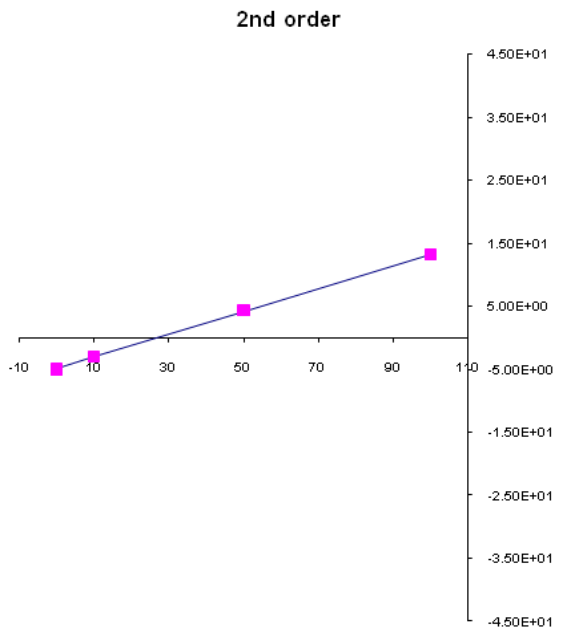
$$\rho(z) \sim FT\big(F(q)\big)$$

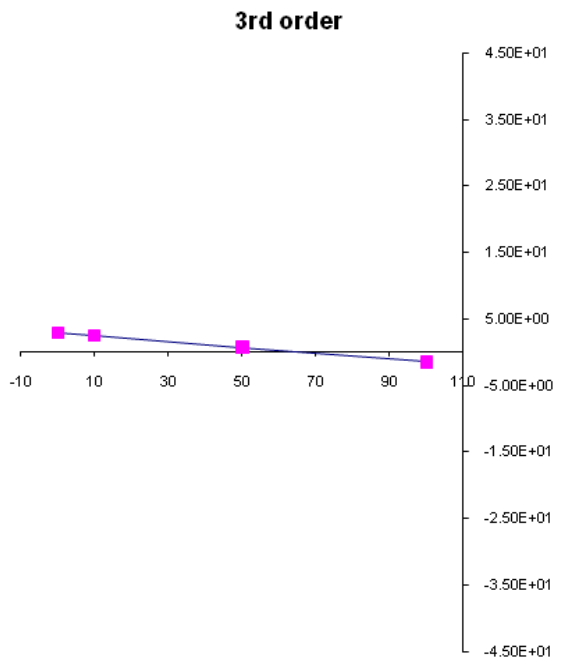
$$|F(q)| \sim \sqrt{I(q)}$$

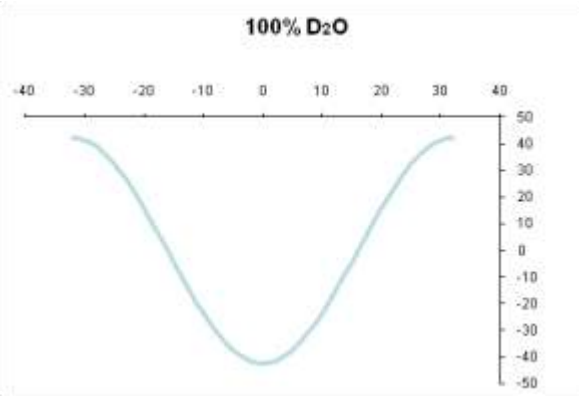


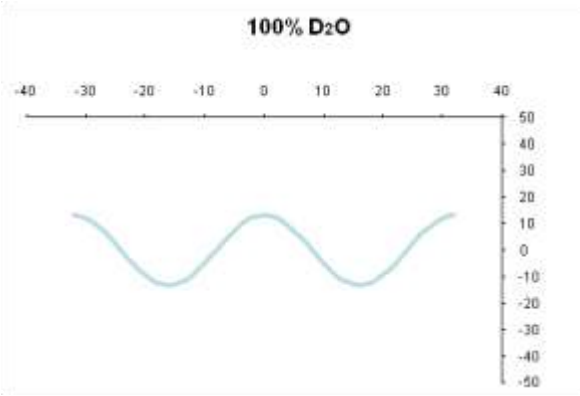
Phase Determination

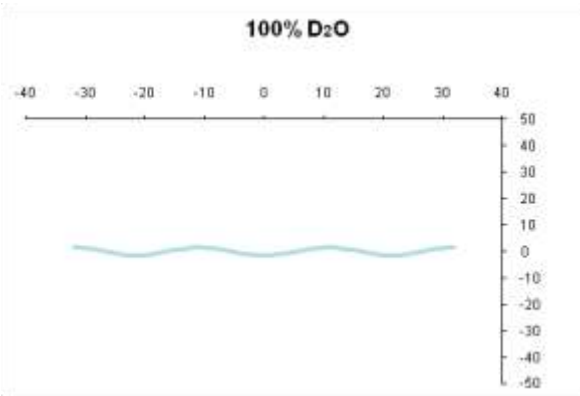












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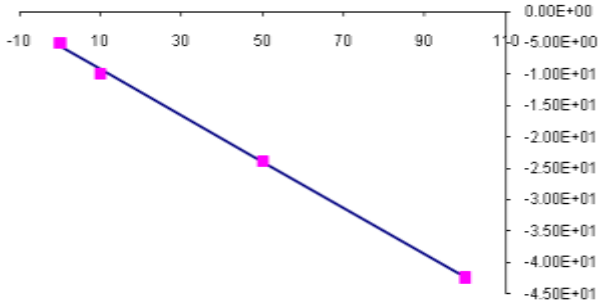
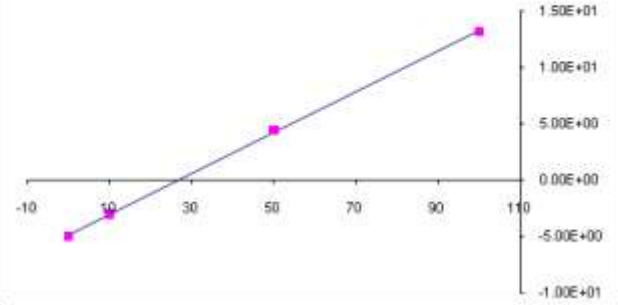
Phase Determination

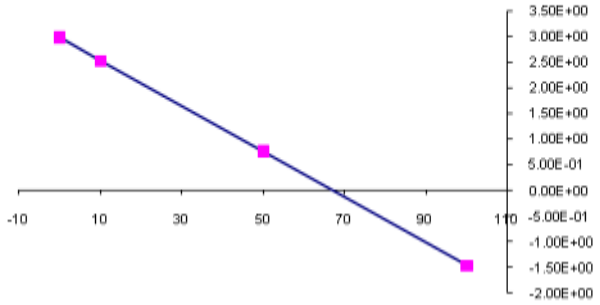
1st order



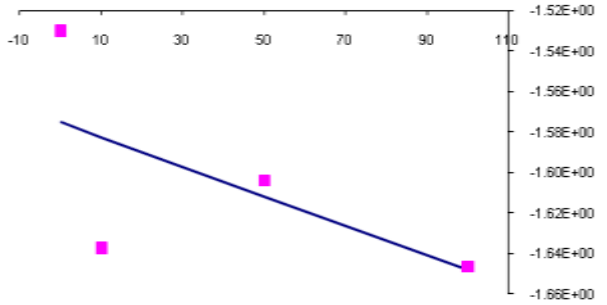
2nd order



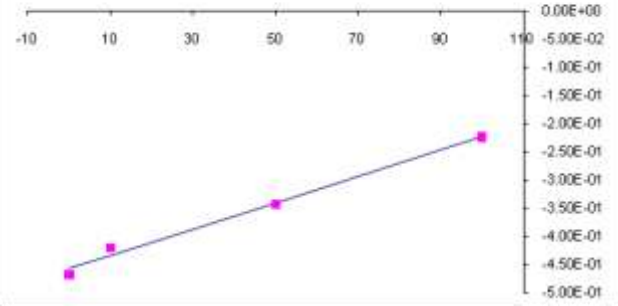
3rd order



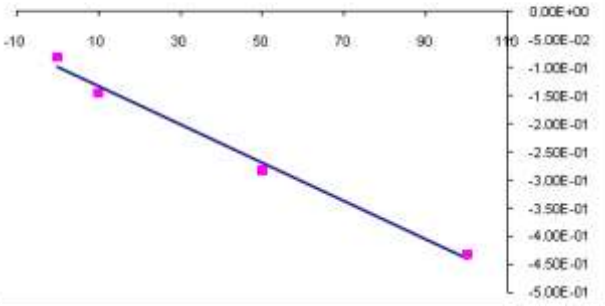
4th order



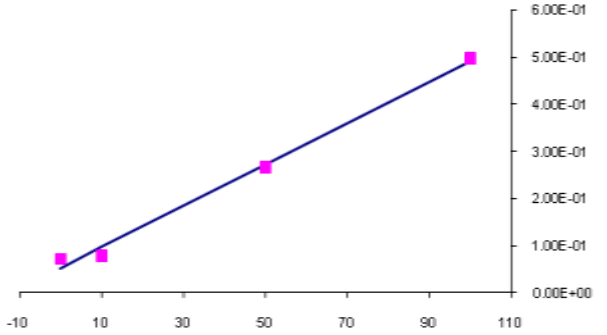
5th order



6th order

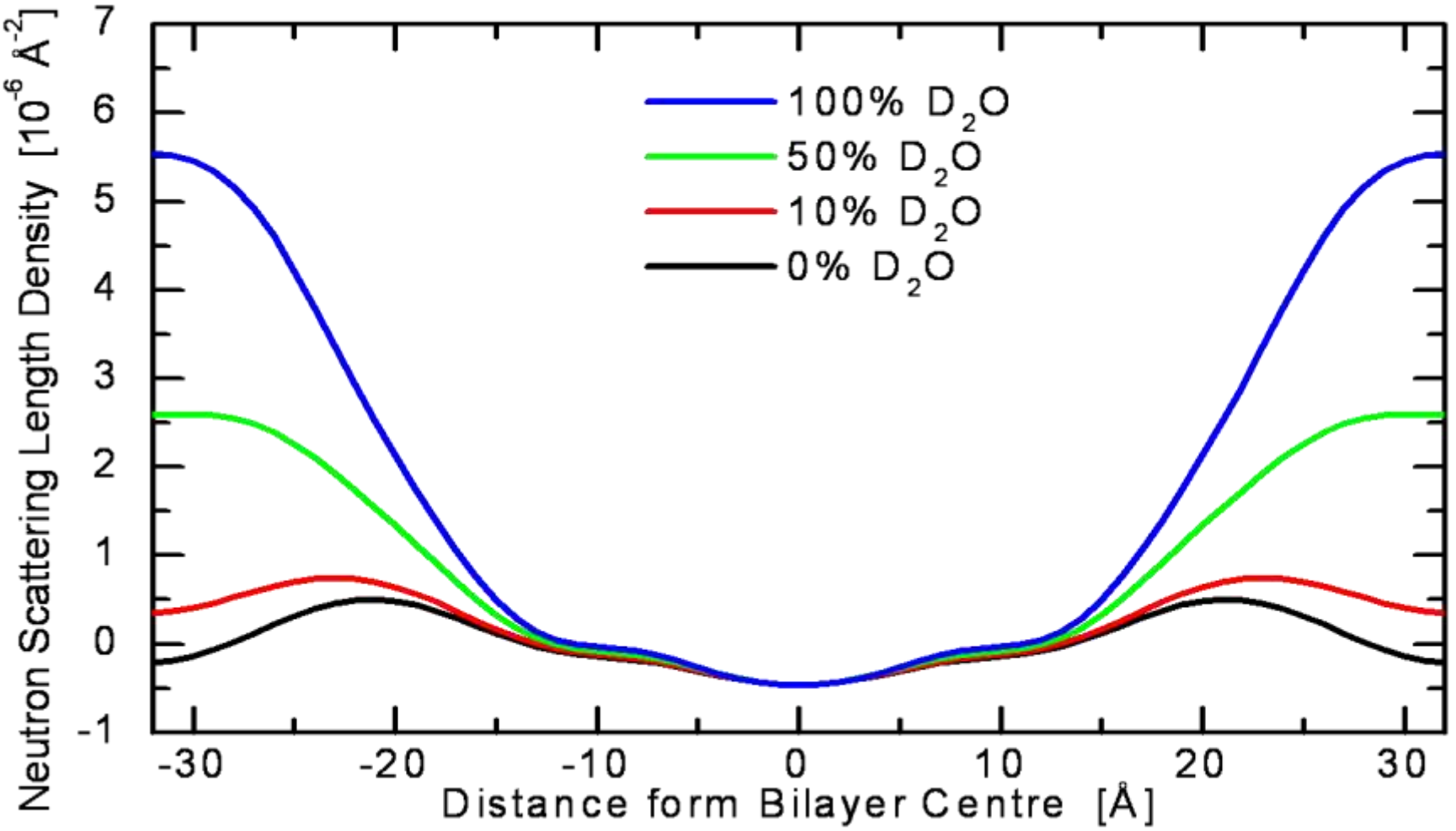


7th order



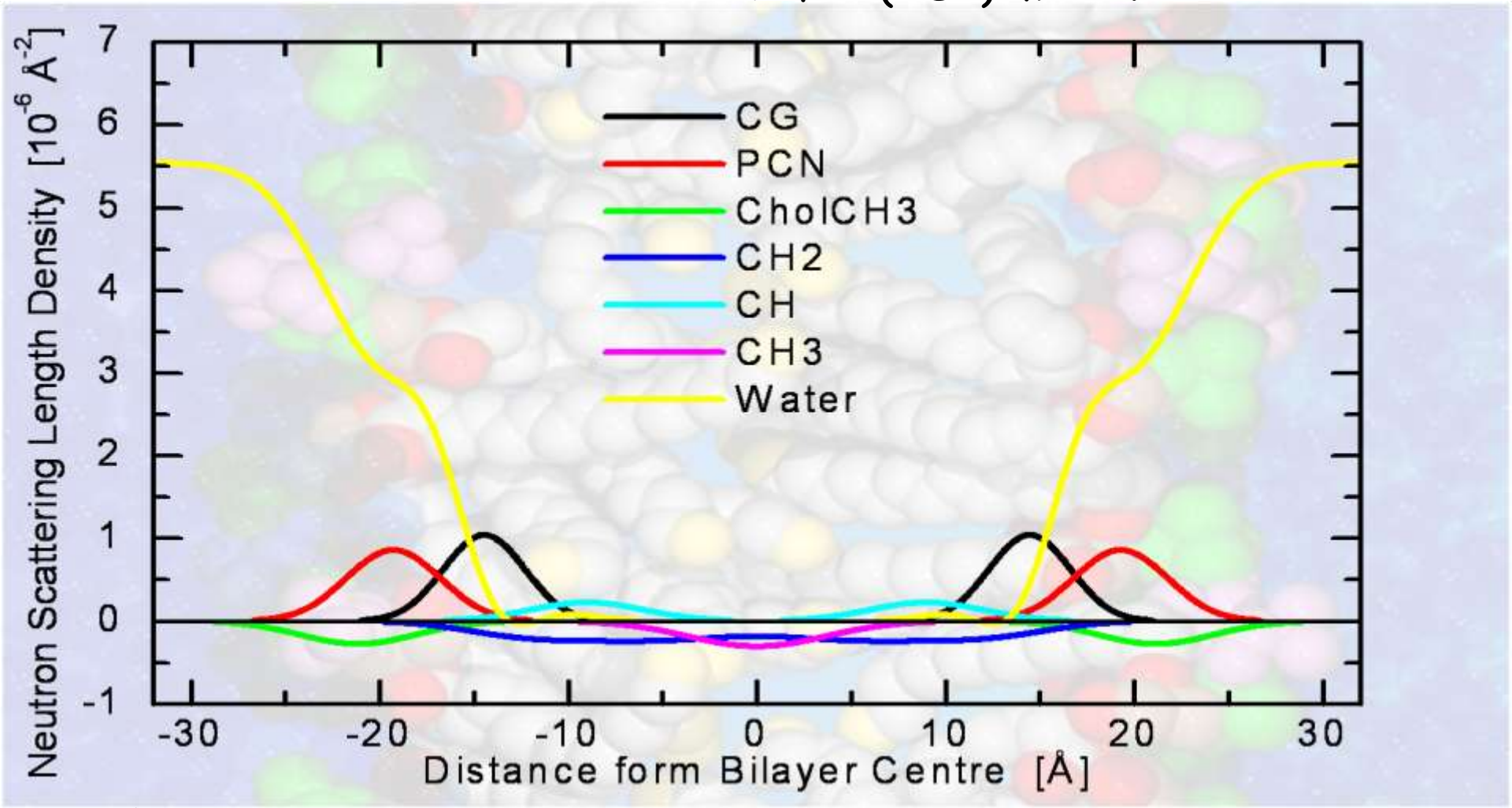
NSLD Reconstruction

$$\Delta\rho\ (z) = \frac{1}{D} F_0 + \frac{2}{D} \sum_{h=1} F_h \cos\left(\frac{2\pi hz}{D}\right)$$



Bilayer Structure

NSLD profiles could be decomposed into structural groups using an appropriate Scattering Density Profile (SDP) model.



N.Kučerka, J.F.Nagle, J.N.Sachs, S.E.Feller, J.Pencer A.J.Jackson, and J.Katsaras, Biophys. J (2008)

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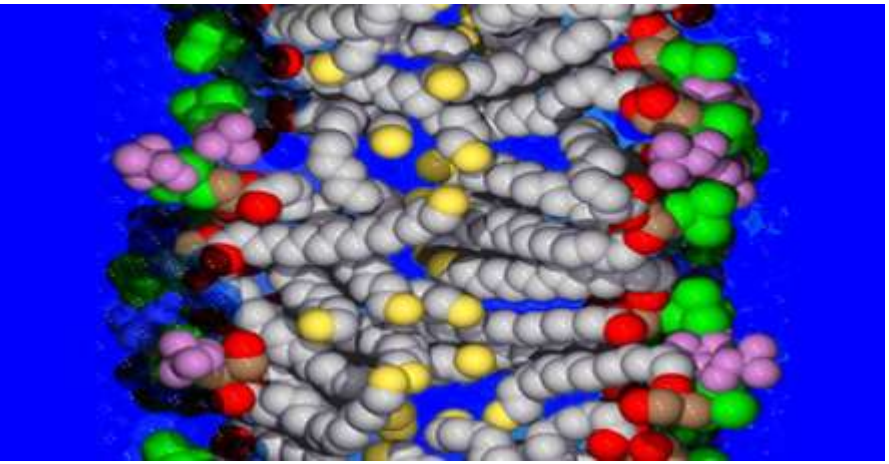
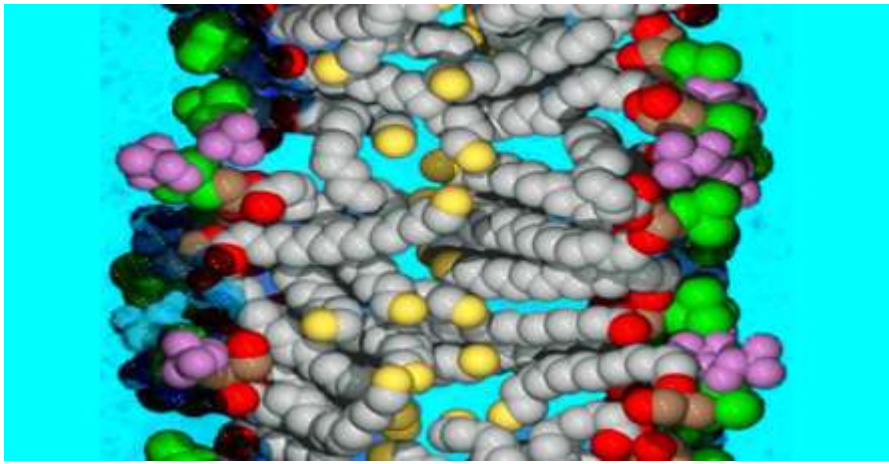
Water Distribution

The difference profiles of contrast varied SDPs provide distributions of bilayer/water probability.

$$\rho_1(z) = \rho_{W1} P_W(z) + \rho_B P_B(z)$$

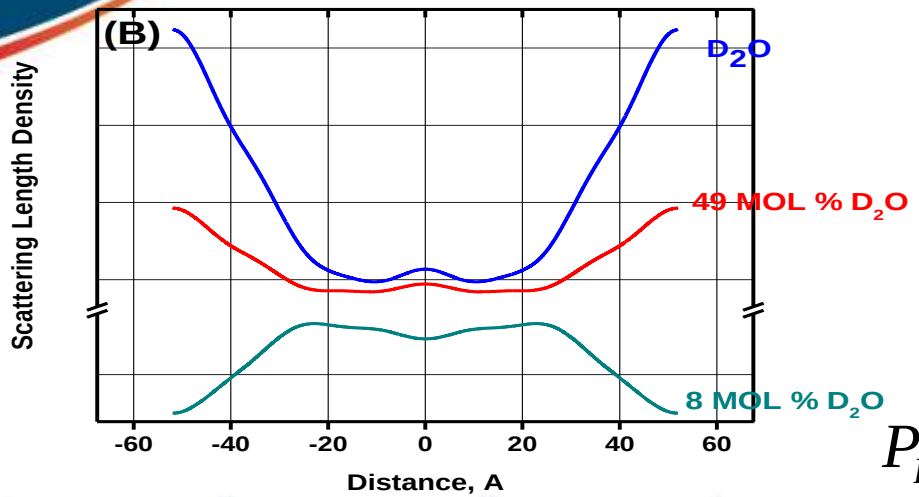


$$\rho_2(z) = \rho_{W2} P_W(z) + \rho_B P_B(z)$$



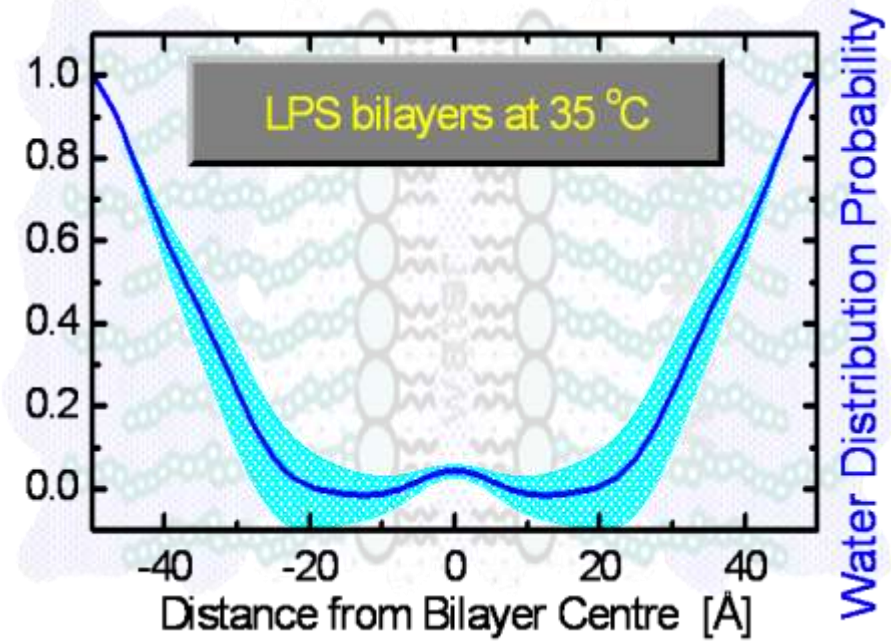
$$\rho_1(z) - \rho_2(z) = (\rho_{W1} - \rho_{W2}) P_W(z)$$

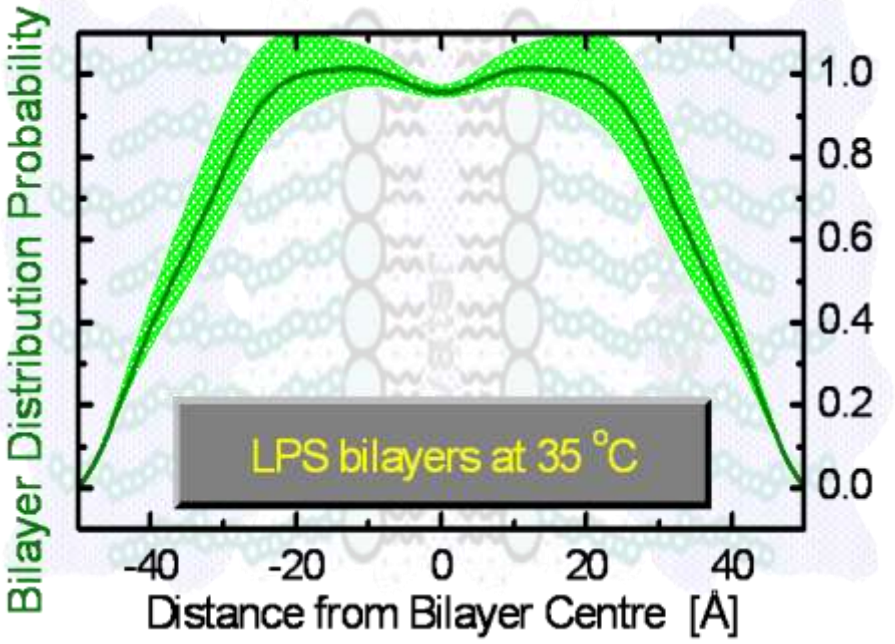
Water Distribution in Bacterial Membrane



$$P_W(z) = \frac{\rho_1(z) - \rho_2(z)}{\rho_{W1} - \rho_{W2}}$$

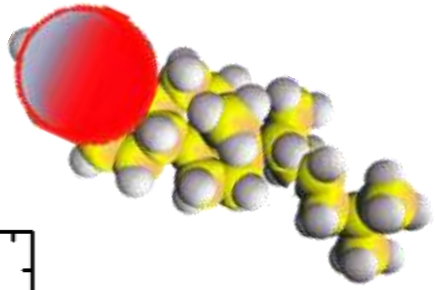
$$P_B(z) = 1 - P_W(z)$$

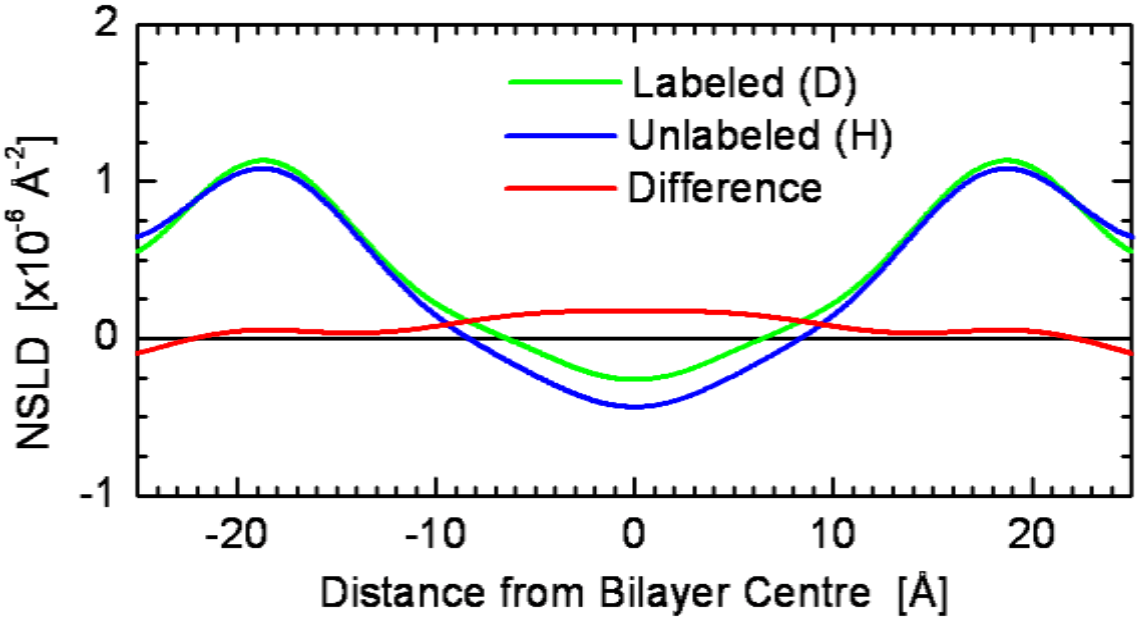




T. Abraham, S. Schooling, M.-P. Nieh, N. Kučerka, T.J. Beveridge and J. Katsaras, J PhysChemB 111 (2007)

Label Distribution





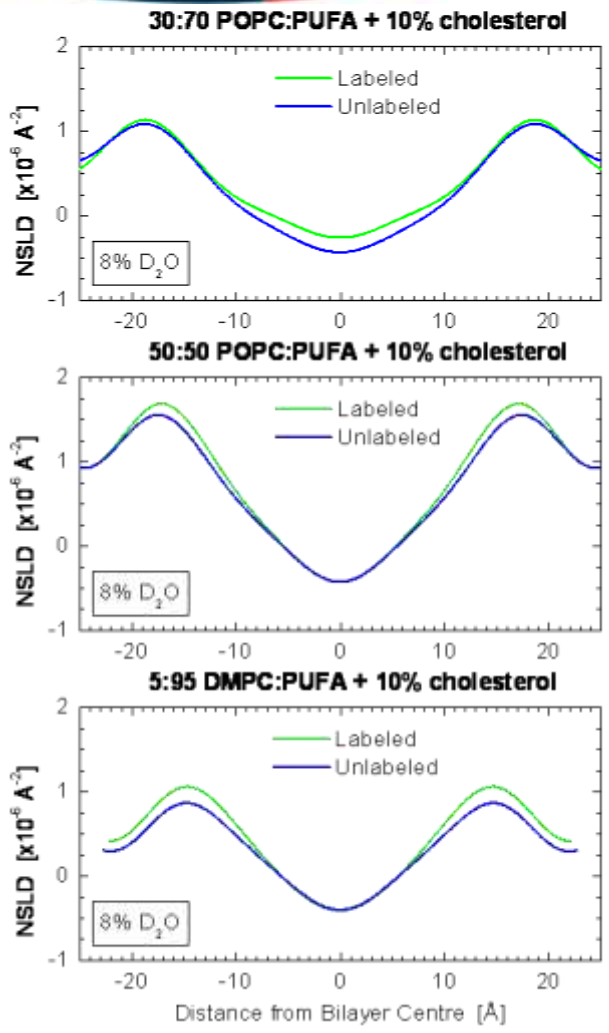
$$\rho_D(z) = \rho_B P_B(z) + \rho_W P_W(z) + \rho_{LD} P_L(z)$$

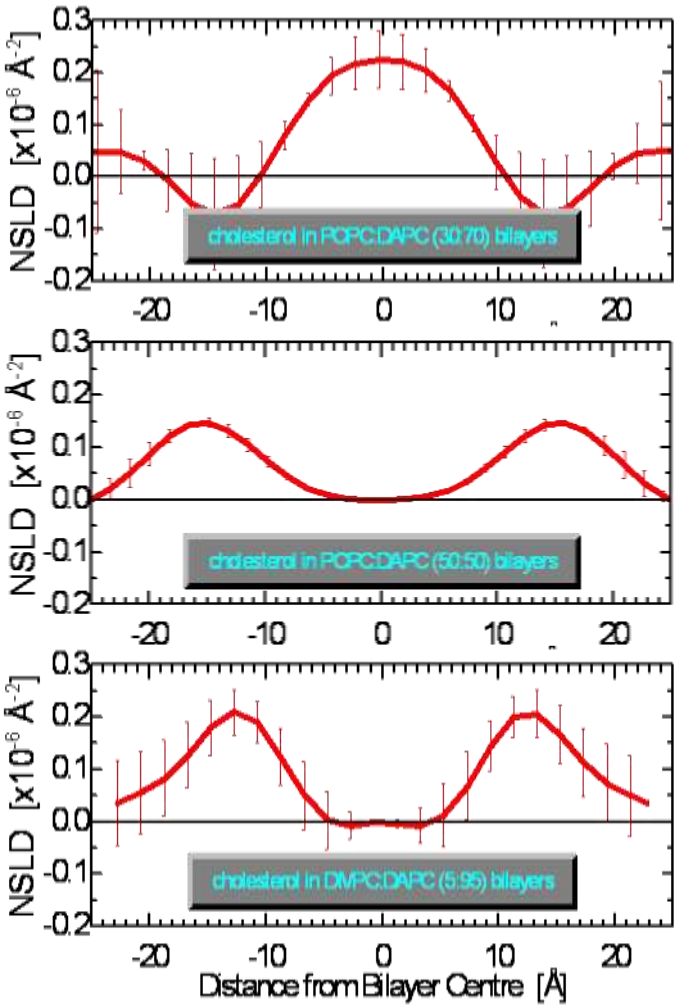
$$\rho_H(z) = \rho_B P_B(z) + \rho_W P_W(z) + \rho_{LH} P_L(z)$$

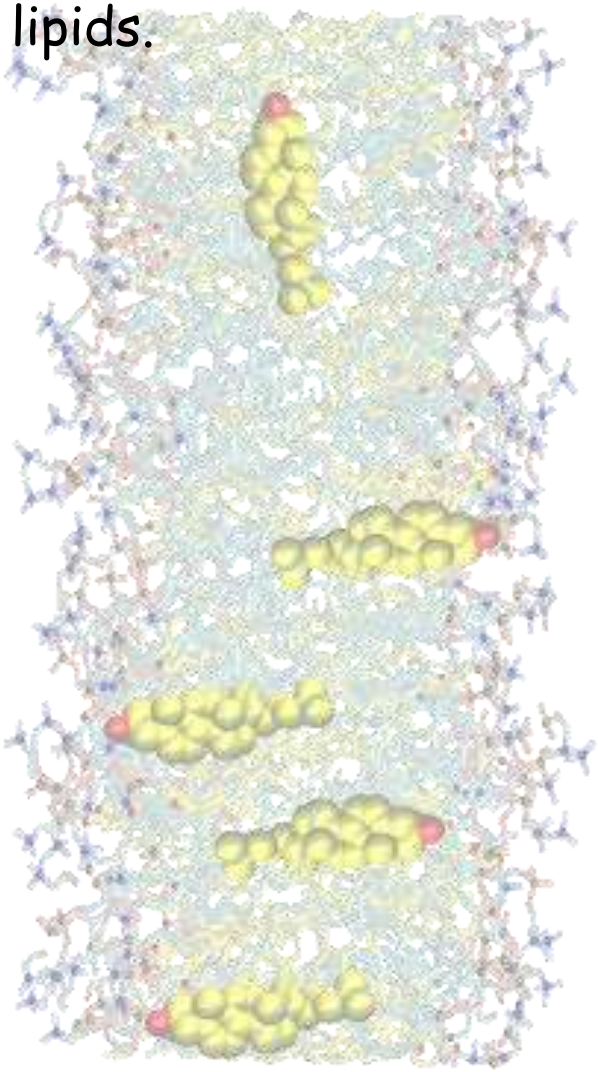
$$\rho_D(z) - \rho_H(z) = (\rho_{LD} - \rho_{LH}) P_L(z)$$

Cholesterol in PUFAs

Cholesterol can be made to change its orientation in model membranes by changing the ratio of PUFA to saturated chain lipids.







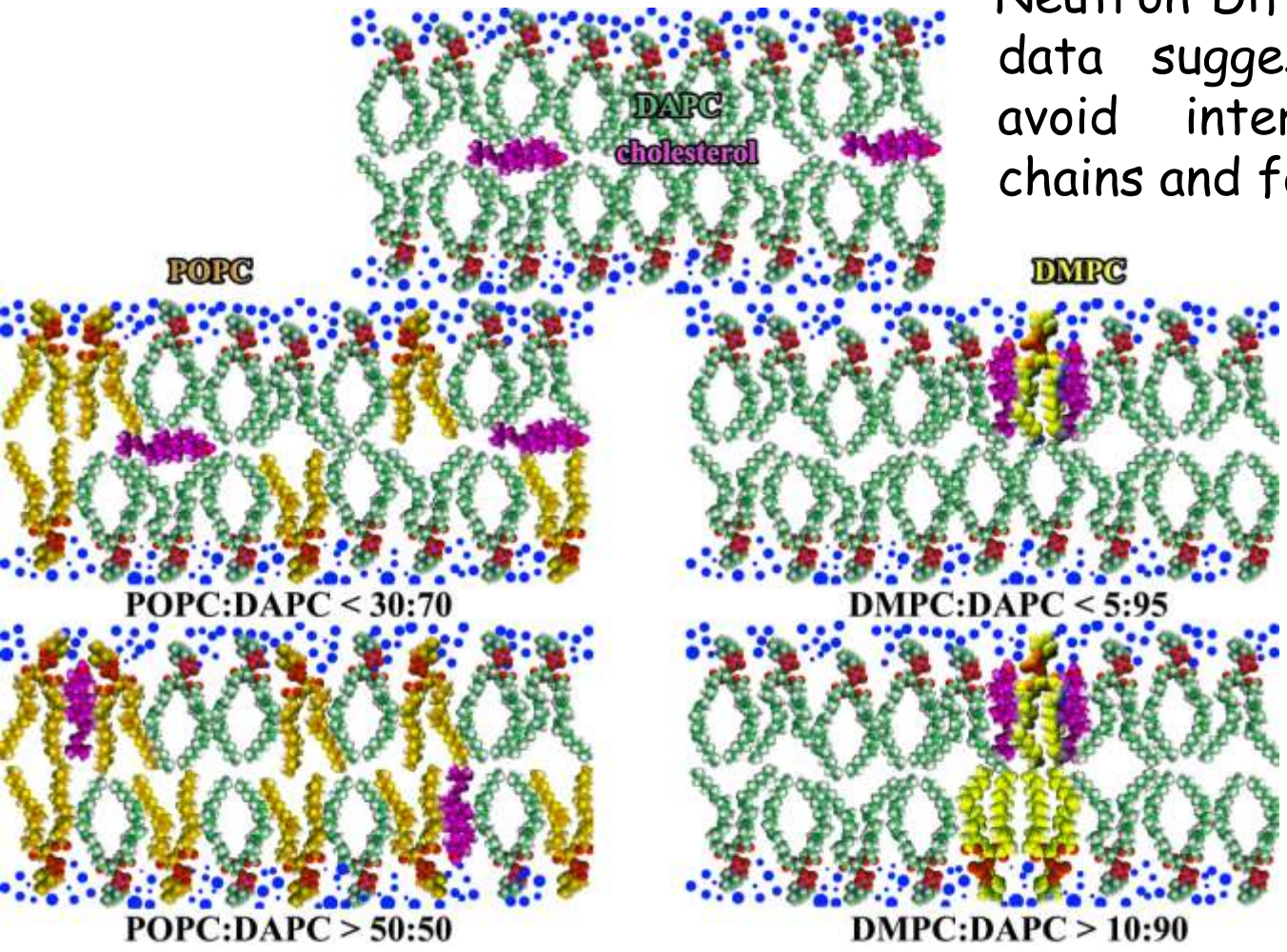
N. Kučerka, D. Marquardt, T.A. Harroun, M.-P. Nieh, S.R. Wassall and J. Katsaras, JACS 131 (2009)

Domains driven by lipids

In addition to convincingly demonstrating cholesterol's aversion to the disordered PUFA chains,

Neutron Diffraction experimental data suggest that DMPC also avoid interacting with PUFA chains and form domains,

i.e.: domain formation is driven by the aversion that certain lipids have for each other.



Kučerka,Marquardt,Harroun,Nieh,Wassall,de Jong,Schäfer,Marrink and Katsaras, Biochemistry (submitted)

Summary

- Small-Angle Neutron Diffraction is a valuable tool for structural biophysics
- Contrast variation provides water distribution profiles
- Specific labeling enhances resolution of determined structures

Collaborators

- Neutron Diffraction at CNBC, Chalk River, ON
  - John Katsaras
  - Mu-Ping Nieh
  - Thad Harroun
  - Thomas Abraham
- LPS extraction (University of Guelph, ON)
  - Erzsebet Papp-Szabo
  - Sarah Schooling
- MD simulations
  - Jonathan Sachs (U of Minnesota, Minneapolis, MN)
  - Siewert-Jan Marrink (U of Groningen, Netherlands)