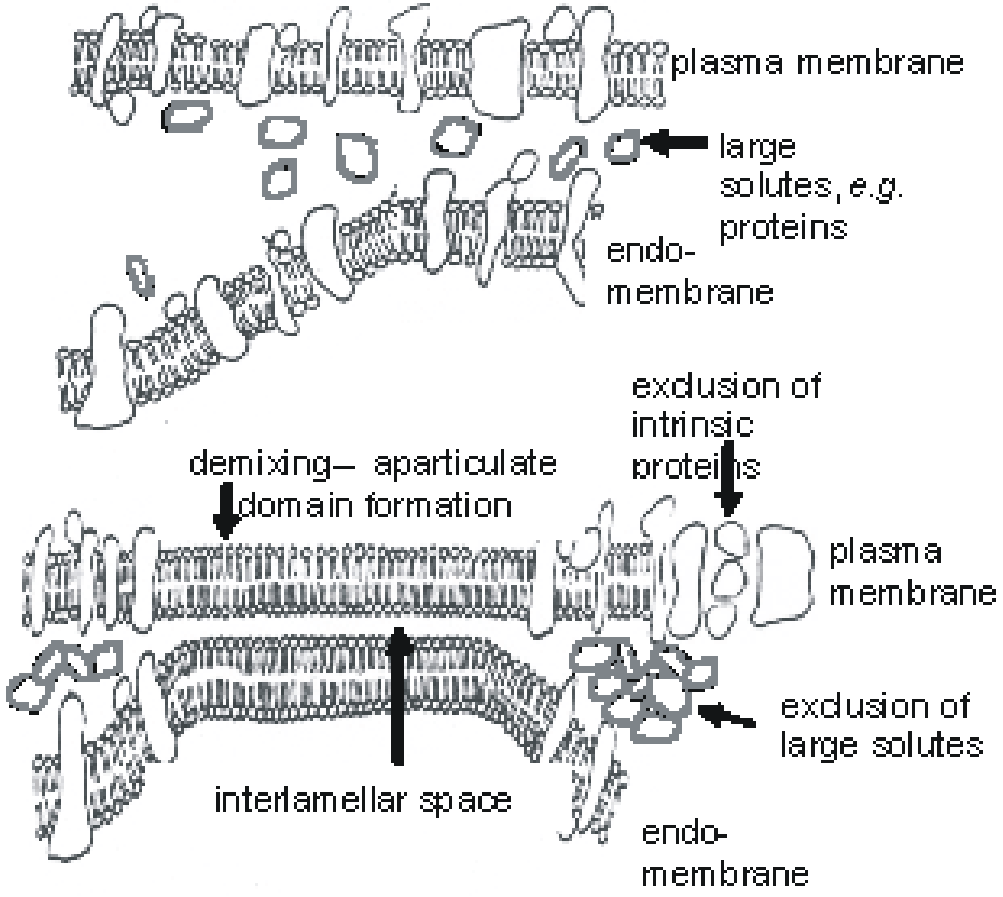
Background

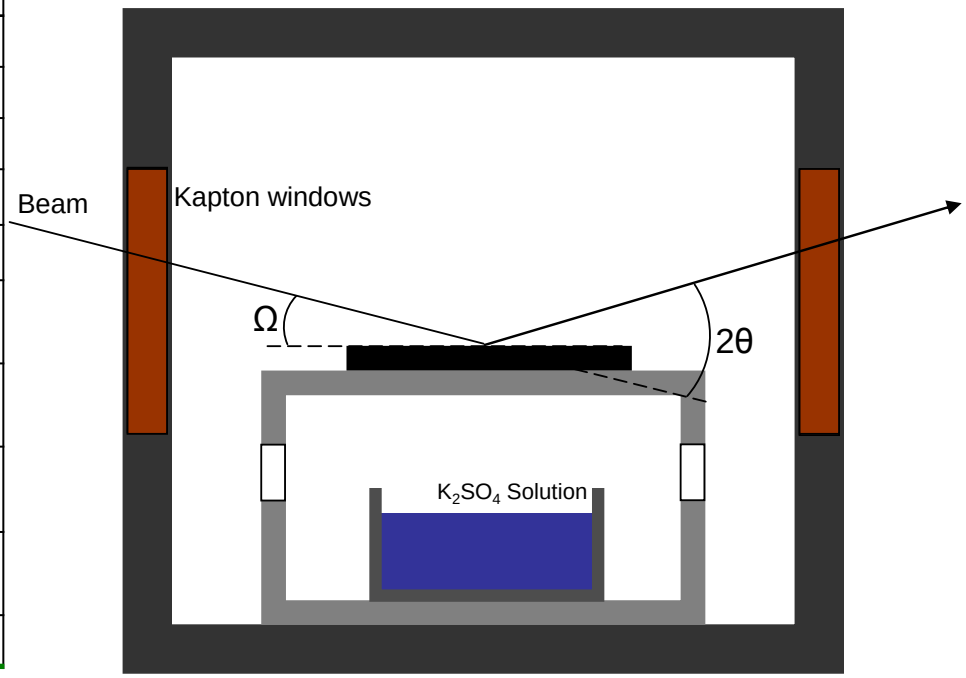
- Water replacement hypothesis
  - Specific interactions between sugar and lipid headgroups
  - Sugars replace water at low hydrations – hydrogen bonding
  - Sugars incorporated into plane of membrane provide lateral separation of lipids
- Hydration forces explanation
  - Non-specific osmotic, volumetric properties of the sugars preventing close approach



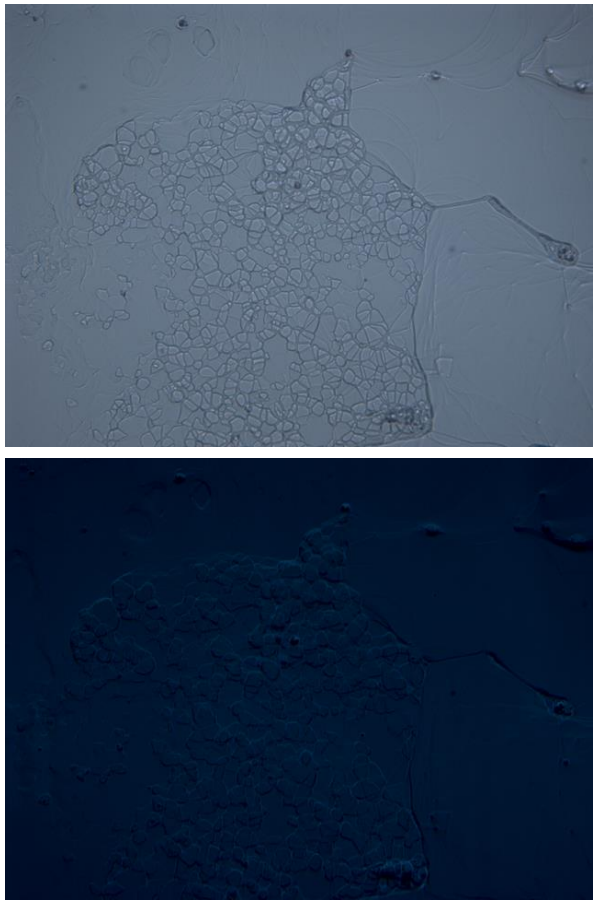
Setup

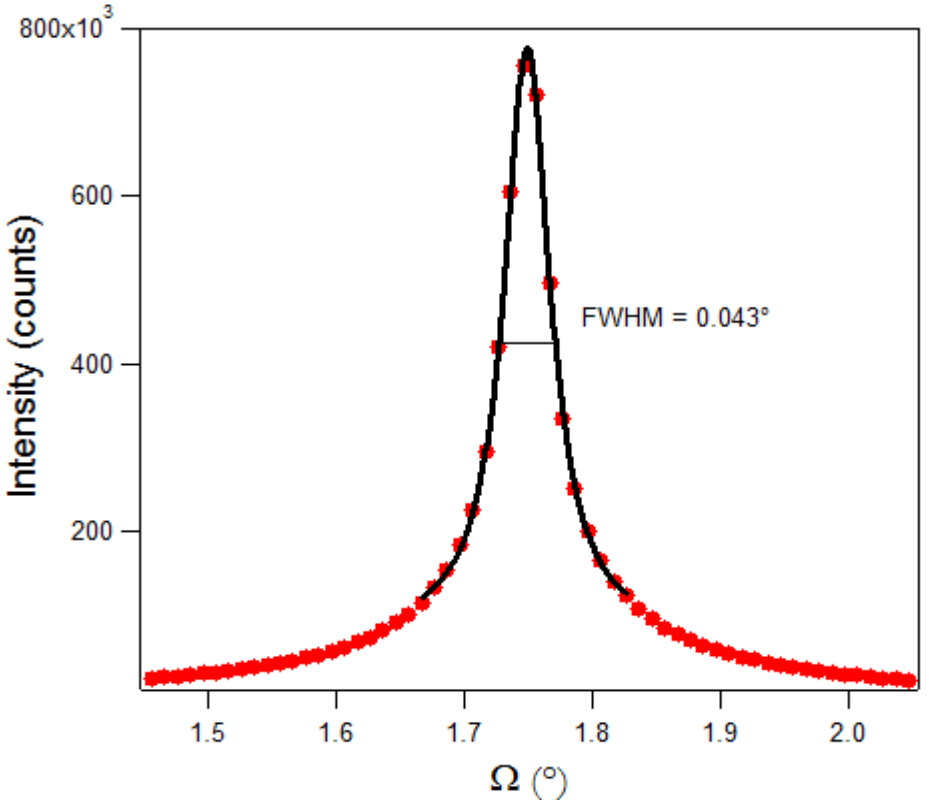
	Sample 1	Sample 2	Sample 3	Unit
Sample weight	0.8	1.0	0.3	mg
Area per lipid	72	72	72	$Å^2$
Sample area	2	2	2	cm^2
DOPC MW	786.15	786.15	786.15	$g\ mol^{-1}$
Mol lipid	1.02×10^{-6}	1.27×10^{-6}	3.82×10^{-7}	mol
Mol per bilayer	9.23×10^{-10}	9.23×10^{-10}	9.23×10^{-10}	mol
Weight per bilayer	7.25×10^{-7}	7.25×10^{-7}	7.25×10^{-7}	g
Number of bilayers	1103	1379	414	
Bilayer Thickness	50	50	50	Å
Film Thickness	5.5	6.9	2.1	µm

Reflectometer in crystallography mode



Sample quality

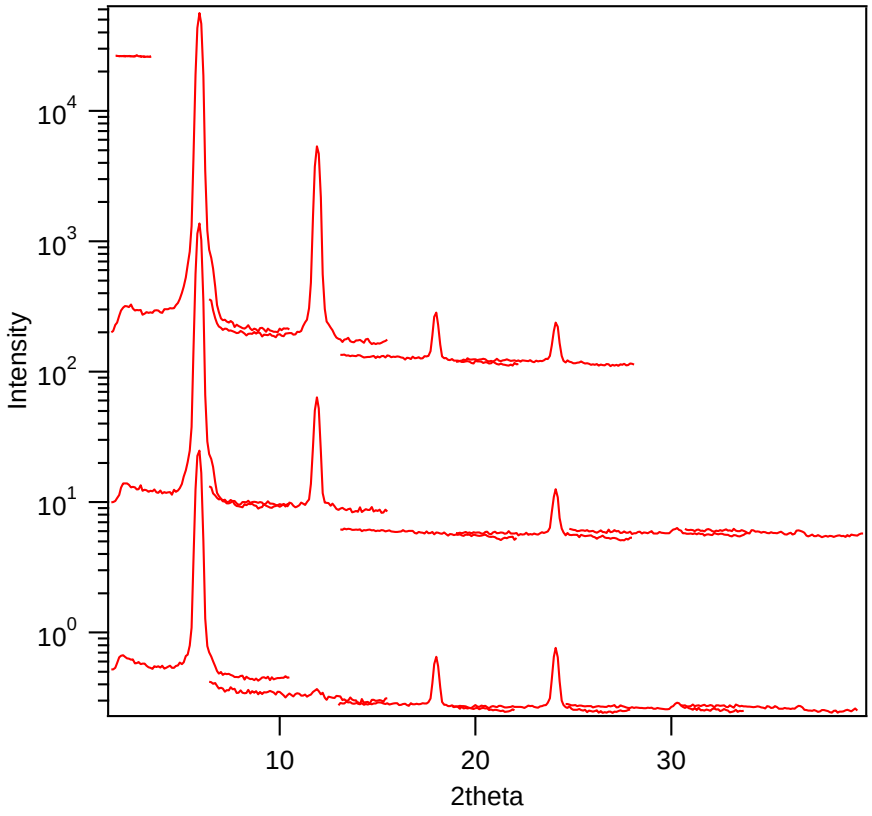


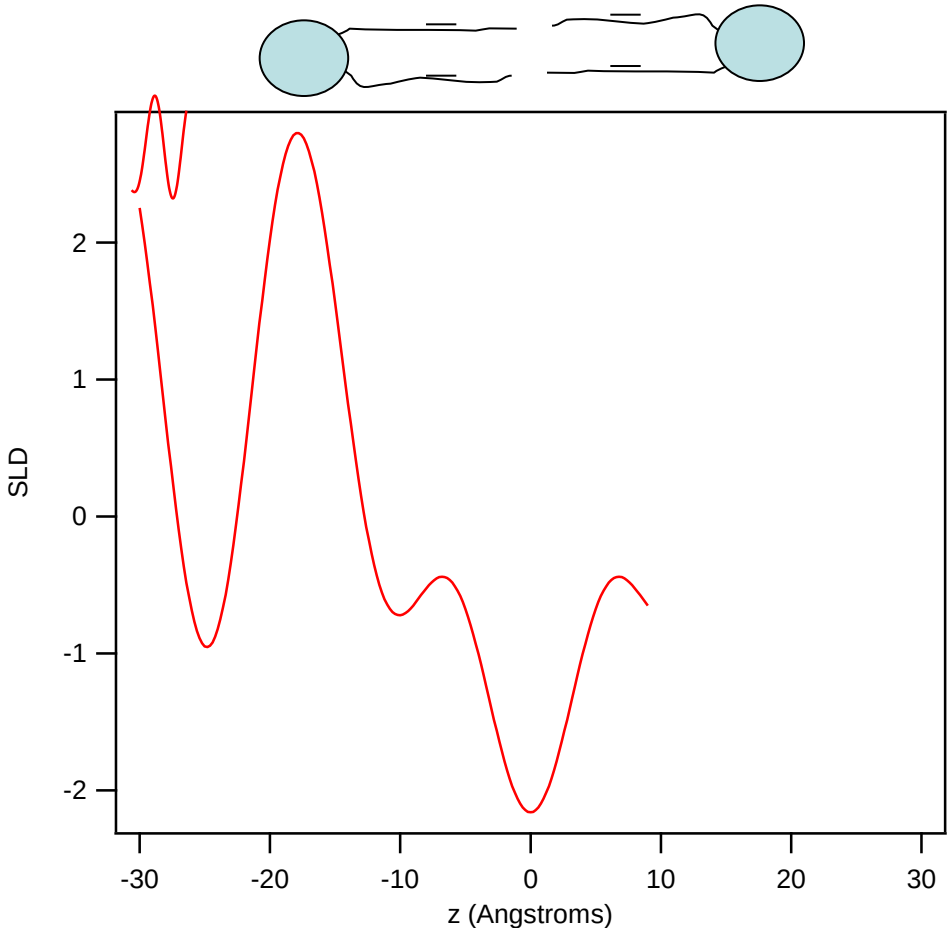


well-oriented layers = mosaic spreads of 0.5 degrees or less

Fourier synthesis

$$\rho^*(z) = \rho_0^* + \left(\frac{2}{dk}\right)\sum_{h=1}^{h_{max}} f(h)\cos\left(\frac{2\pi hz}{d}\right)$$

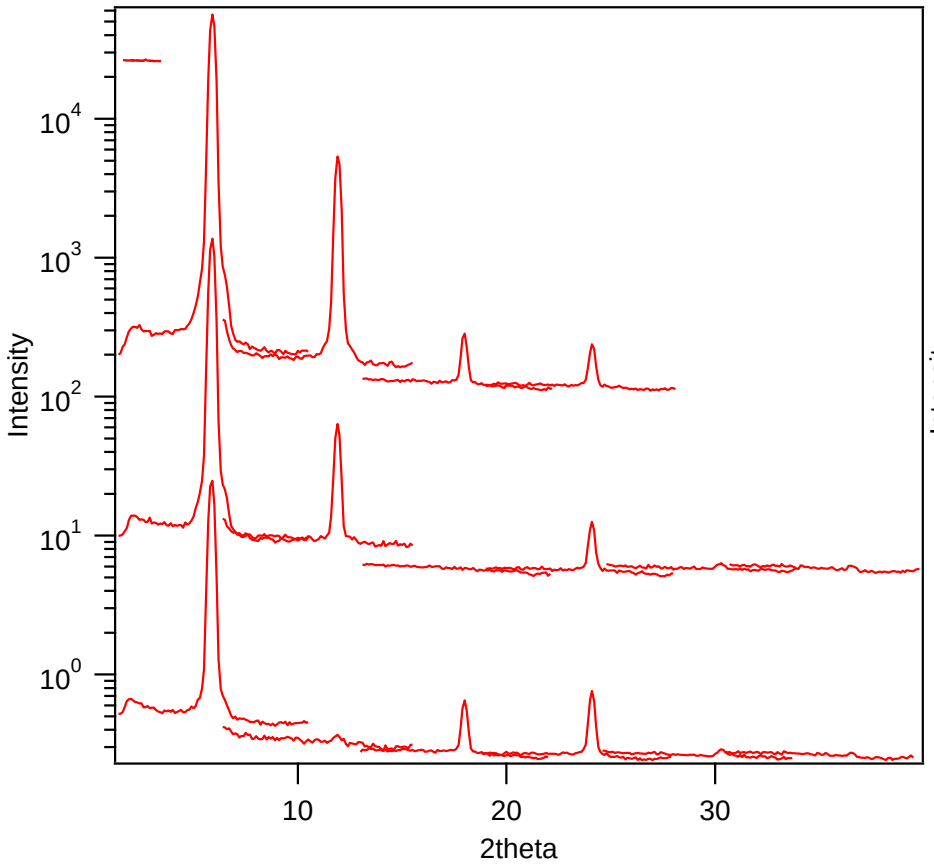


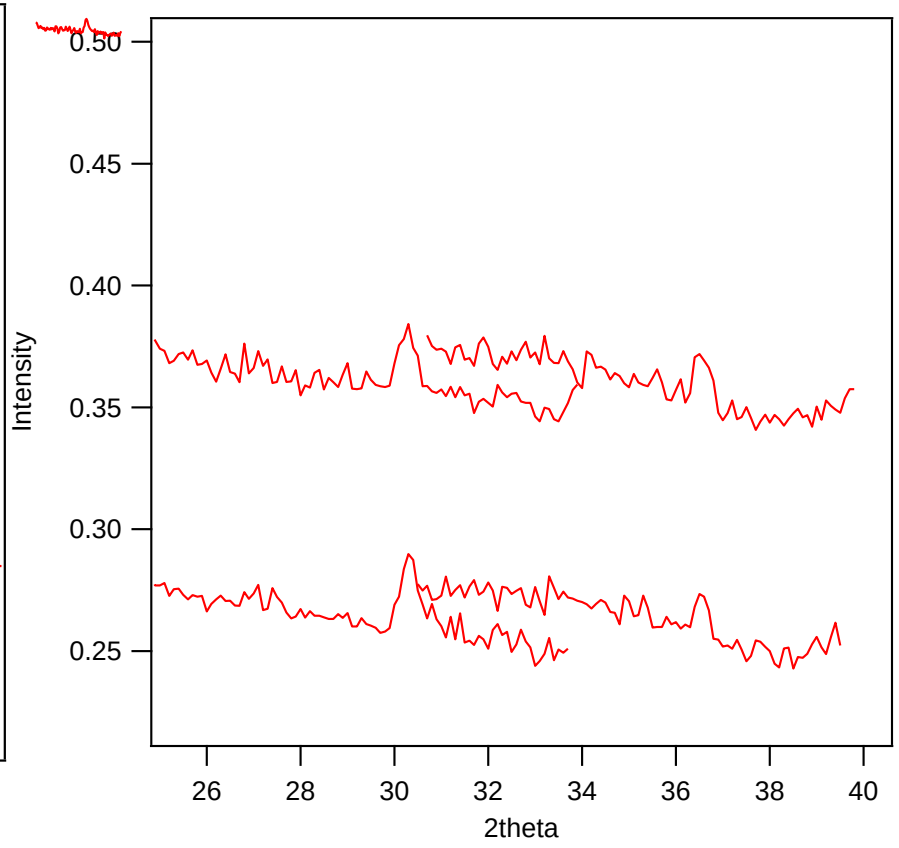


Data

$$\rho^*(z) = \rho_0^* + \left(\frac{2}{dk}\right)\sum_{h=1}^{h_{max}} f(h)\cos\left(\frac{2\pi hz}{d}\right)$$

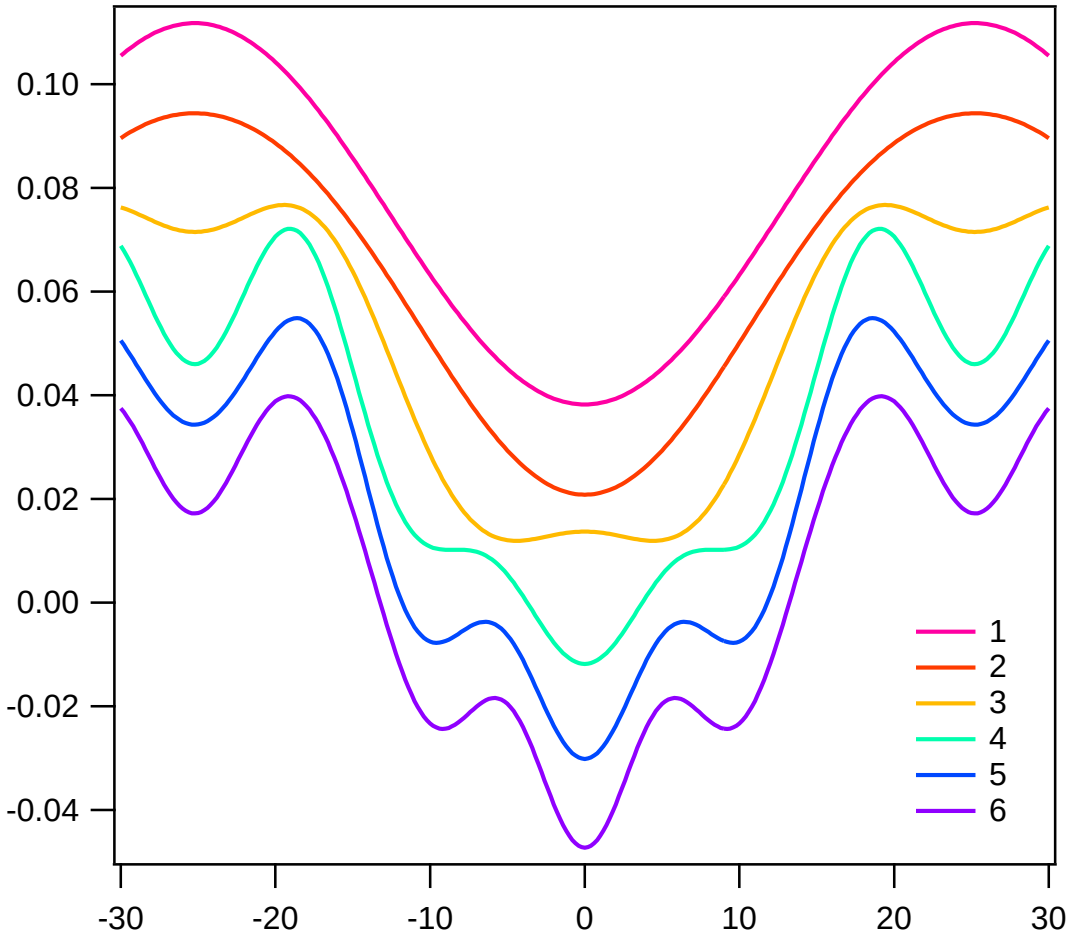
DOPC, RH=57%, 0, 50 & 100% D2O





Truncation errors

$$\rho^*(z) = \rho_0^* + \left(\frac{2}{dk}\right)\sum_{h=1}^{h_{\max}} f(h)\cos\left(\frac{2\pi hz}{d}\right)$$



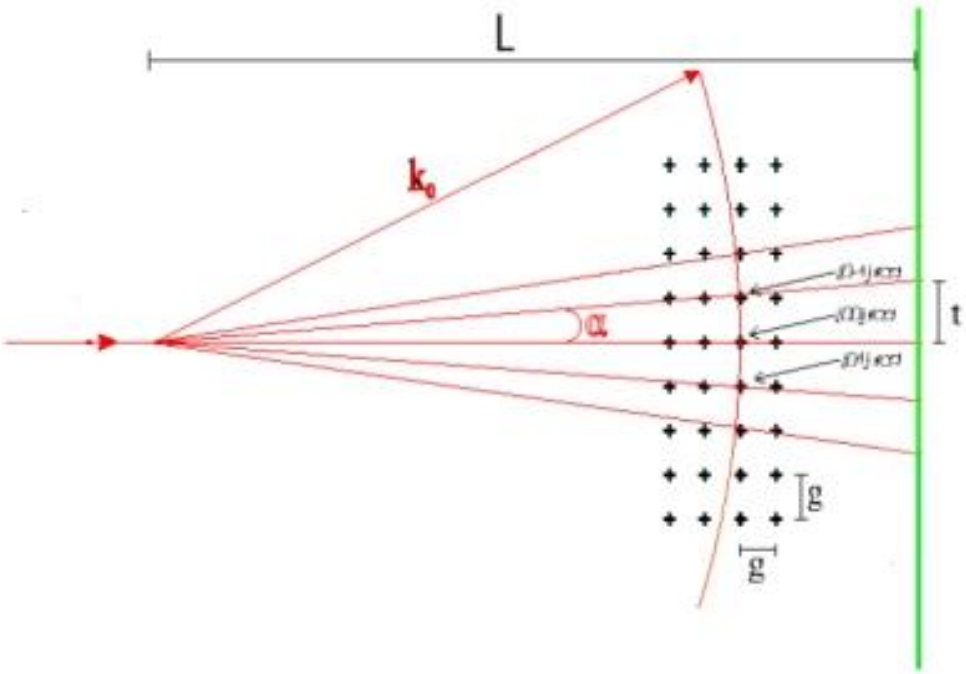
Lorentz Correction

$$\rho^*(z) = \rho_0^* + \left(\frac{2}{dk}\right)\sum_{h=1}^{h_{max}} f(h)\cos\left(\frac{2\pi hz}{d}\right)$$

$$f(h) = \sqrt{I(h)A(h)\sin\theta}$$

= sin(2θ) if integrating in θ
= sin(θ) if integrating in Q

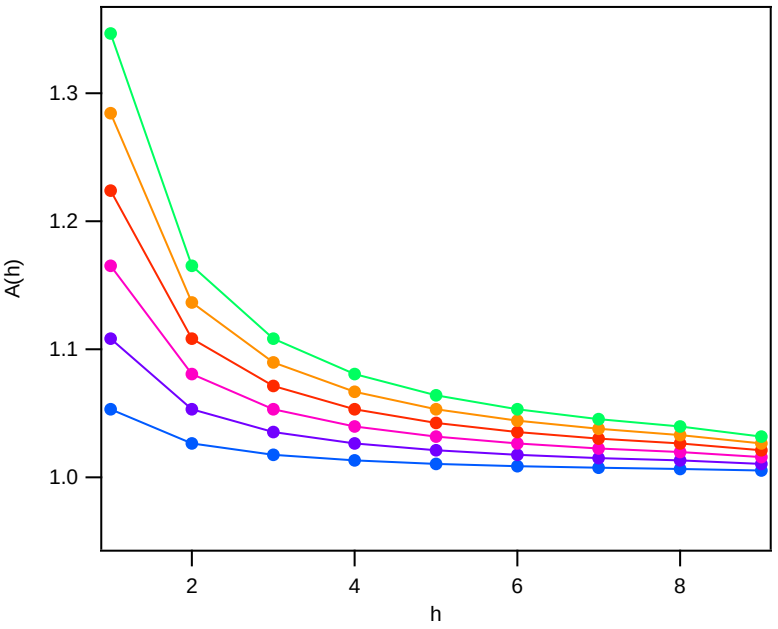
Nearly proportional to h^2 for unoriented MLV samples and to h for oriented samples

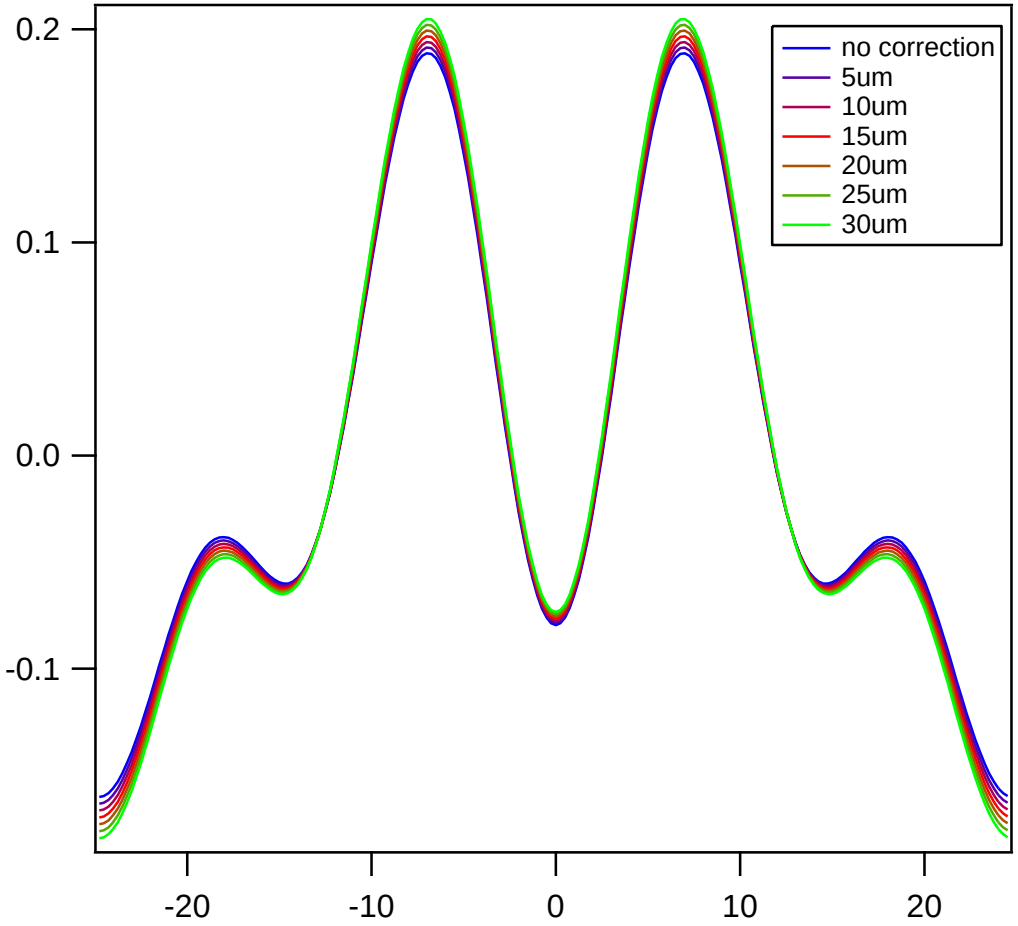


Absorption Correction

$$\rho^*(z) = \rho_0^* + \left(\frac{2}{dk}\right)\sum_{h=1}^{h_{max}} f(h)\cos\left(\frac{2\pi hz}{d}\right)$$

$$f(h) = \sqrt{I(h)A(h)\sin\theta}$$





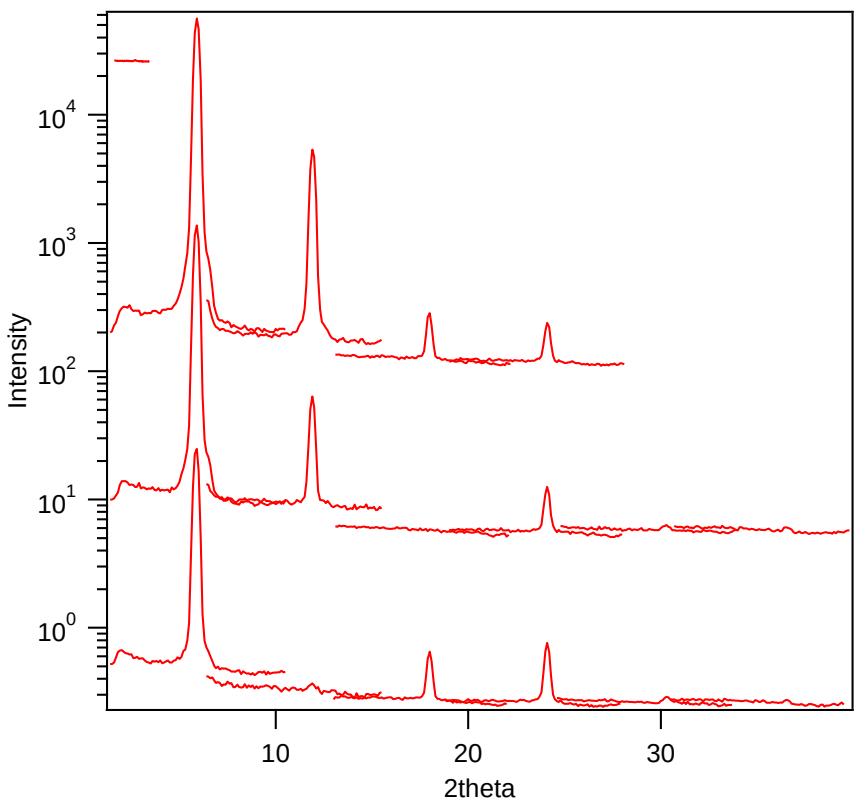
Phasing

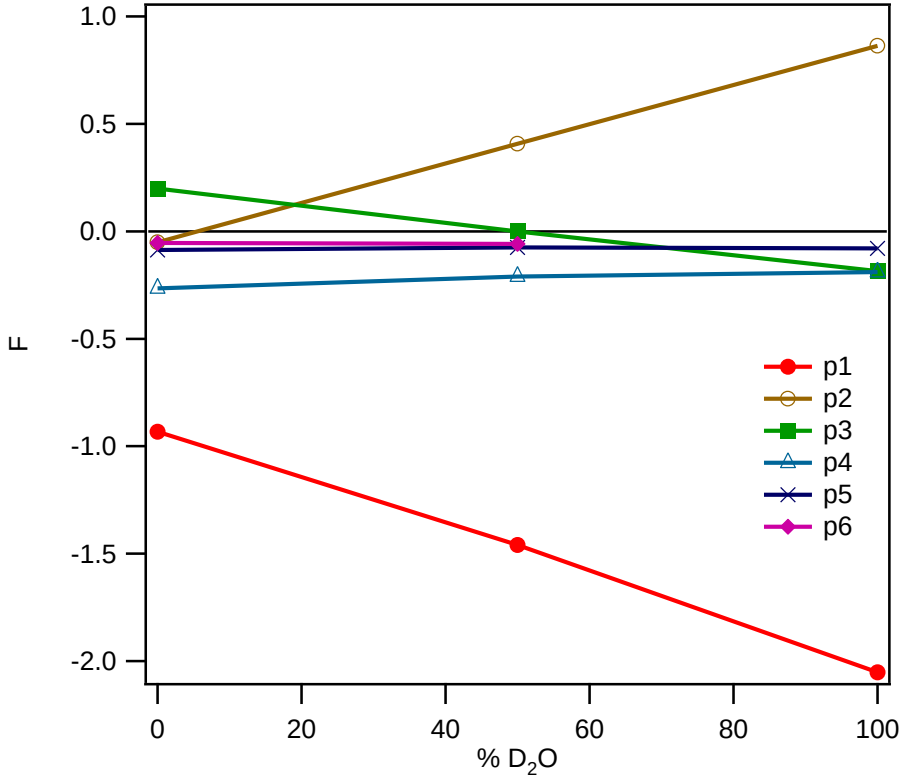
$$\rho^*(z) = \rho_0^* + \left(\frac{2}{dk}\right)\sum_{h=1}^{h_{max}} f(h)\cos\left(\frac{2\pi hz}{d}\right)$$

Phasing by water layer

- Adding D2O adds a positive value to the structure factor
- If structure factors goes through zero, need three ratios for unambiguous assignment
- Assumes water distribution is localised

- Best when near centre of symmetry
- More localised means easier to phase higher orders
- Covalently bound molecules give better localisation



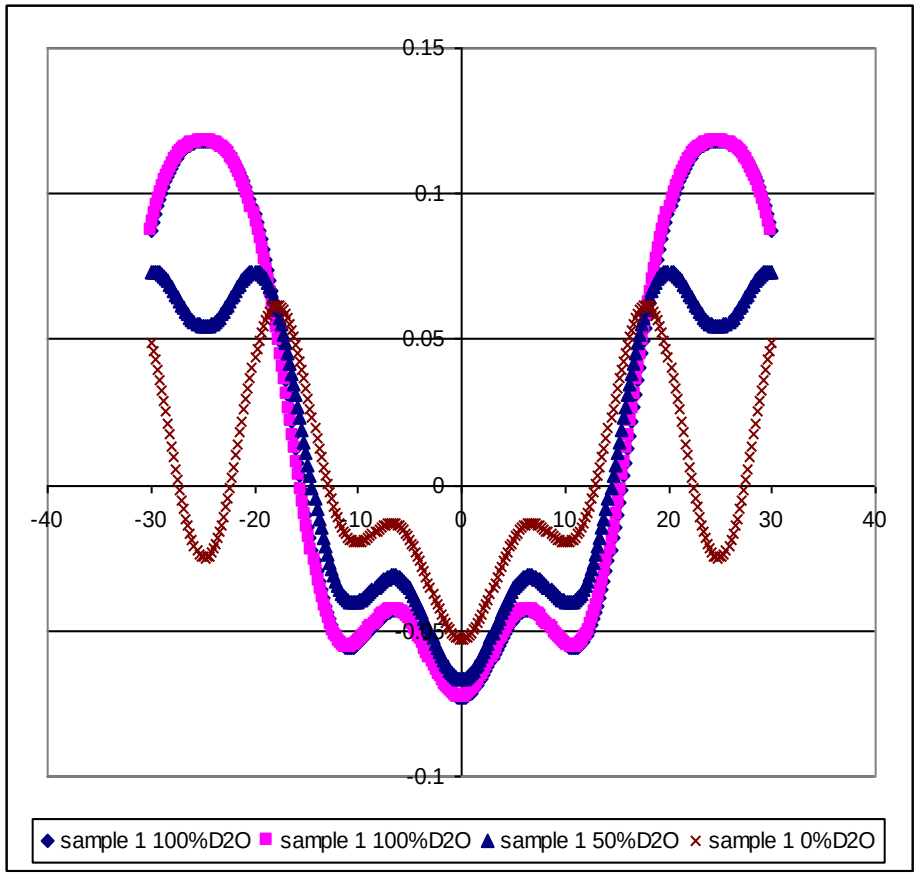


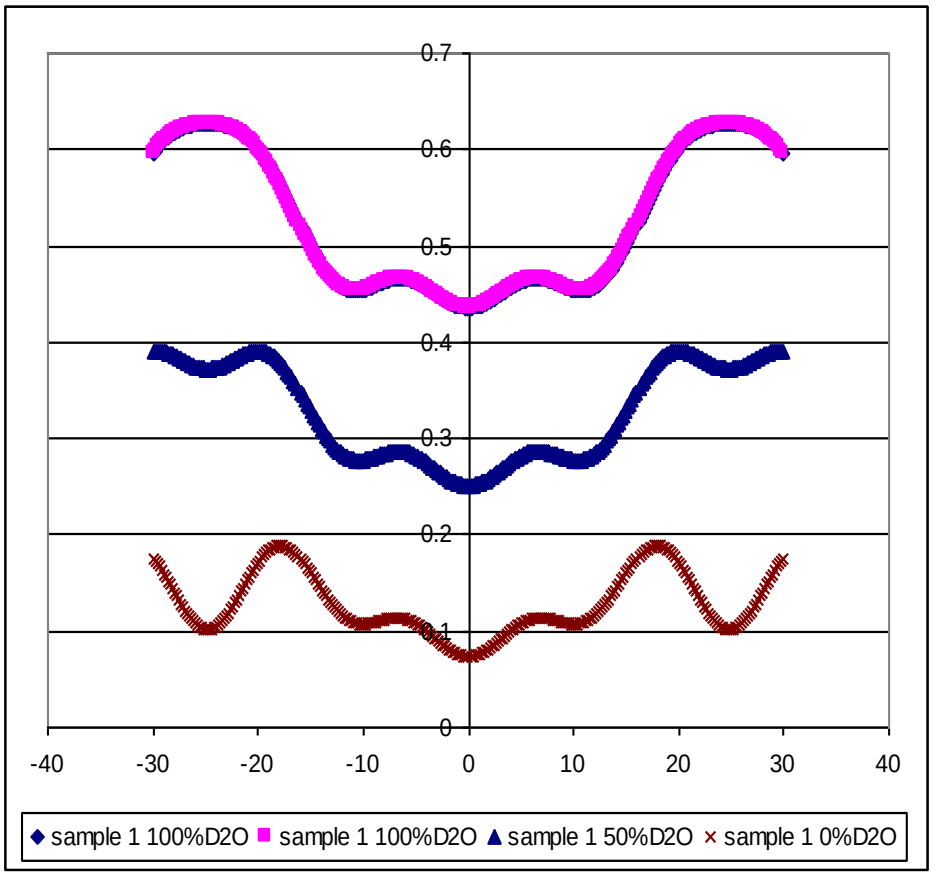
Scaling

$$\rho^*(z) = \rho_0^* + \left(\frac{2}{dk}\right)\sum_{h=1}^{h_{max}} f(h)\cos\left(\frac{2\pi hz}{d}\right)$$

Average SLD

$$\rho_{0i} = \frac{2}{Sd}[n_w b_w(1-f) + n_w f b_d + n_p b_p + b_{lip}] \qquad (3)$$



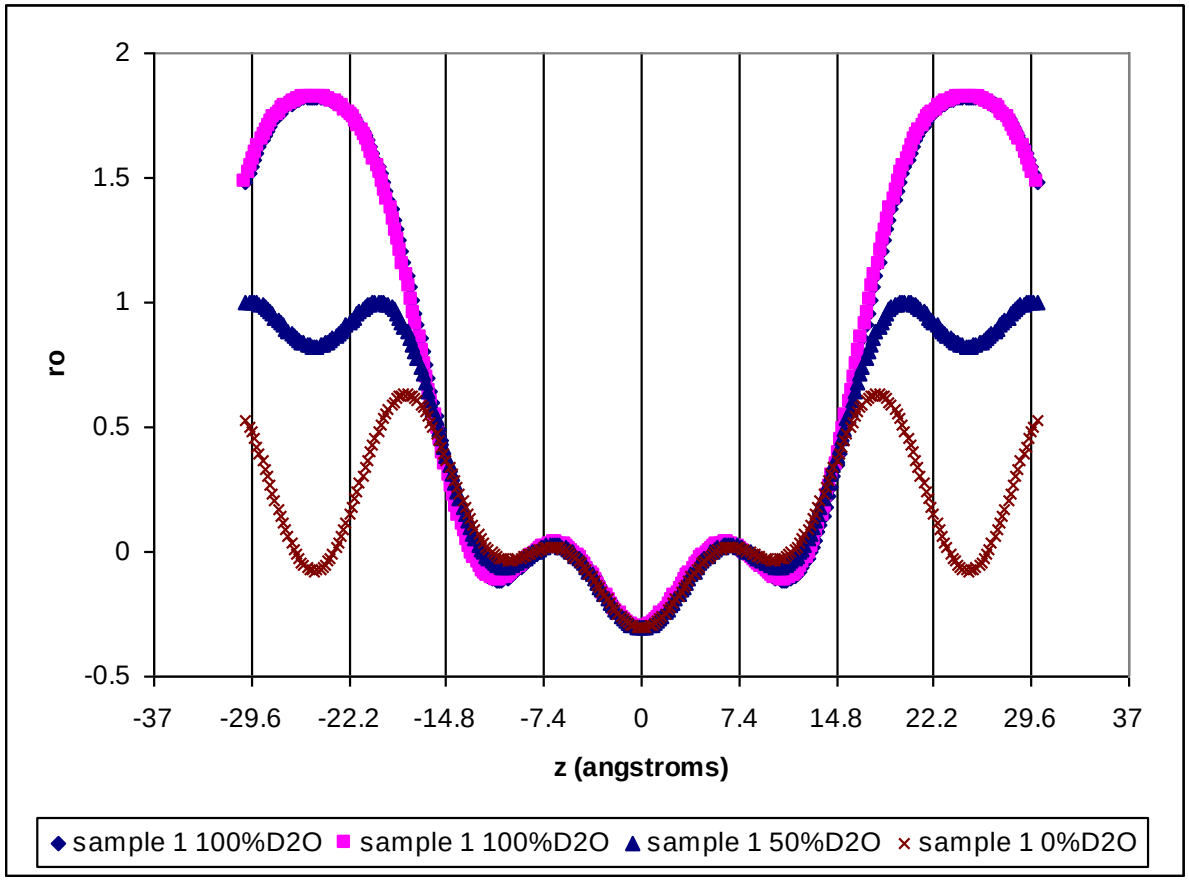


Scaling factors

$$\rho^*(z) = \rho_0^* + \left(\frac{2}{dk}\right)\sum_{h=1}^{h_{\max}} f(h)\cos\left(\frac{2\pi hz}{d}\right)$$

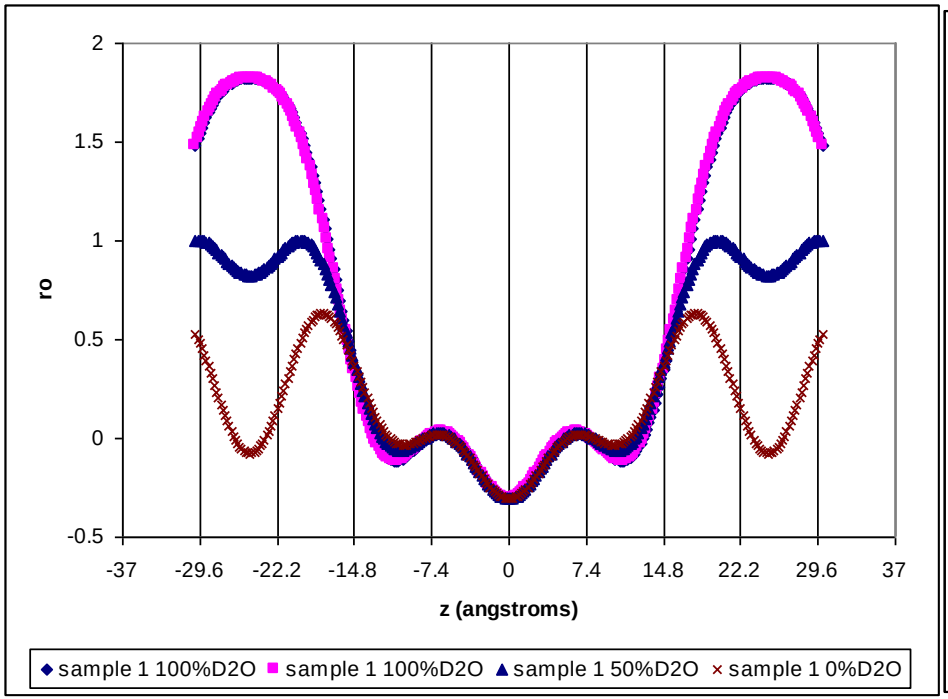
$$\left(\frac{2}{d}k_A\right)\sum_{h=1}^{h_{obs}} f_A(h)\cos(2\pi h z_1/d) - \left(\frac{2}{d}k_B\right)\sum_{h=1}^{h_{obs}} f_B(h)\cos(2\pi h z_1/d) = \Delta\rho^*$$

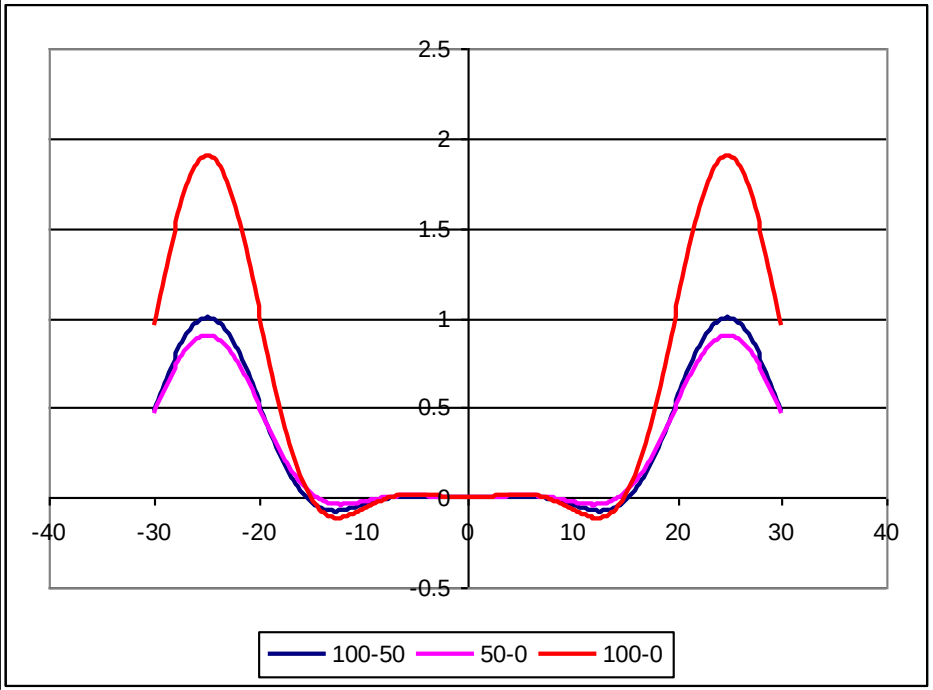
$$\left(\frac{2}{d}k_A\right)\sum_{h=1}^{h_{obs}} f_A(h)\cos(2\pi h z_2/d) - \left(\frac{2}{d}k_B\right)\sum_{h=1}^{h_{obs}} f_B(h)\cos(2\pi h z_2/d) = \Delta\rho^*$$



Results

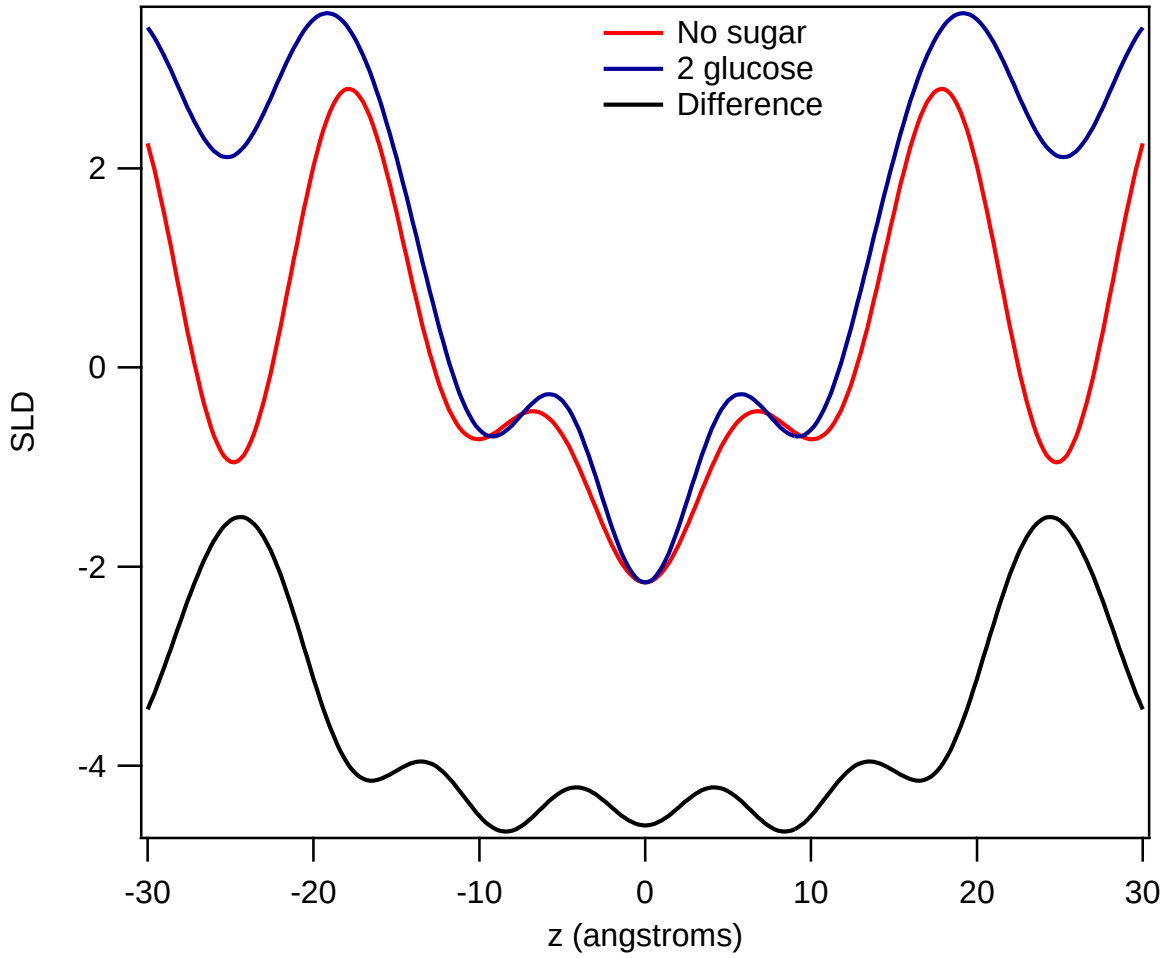
DOPC, RH=57%



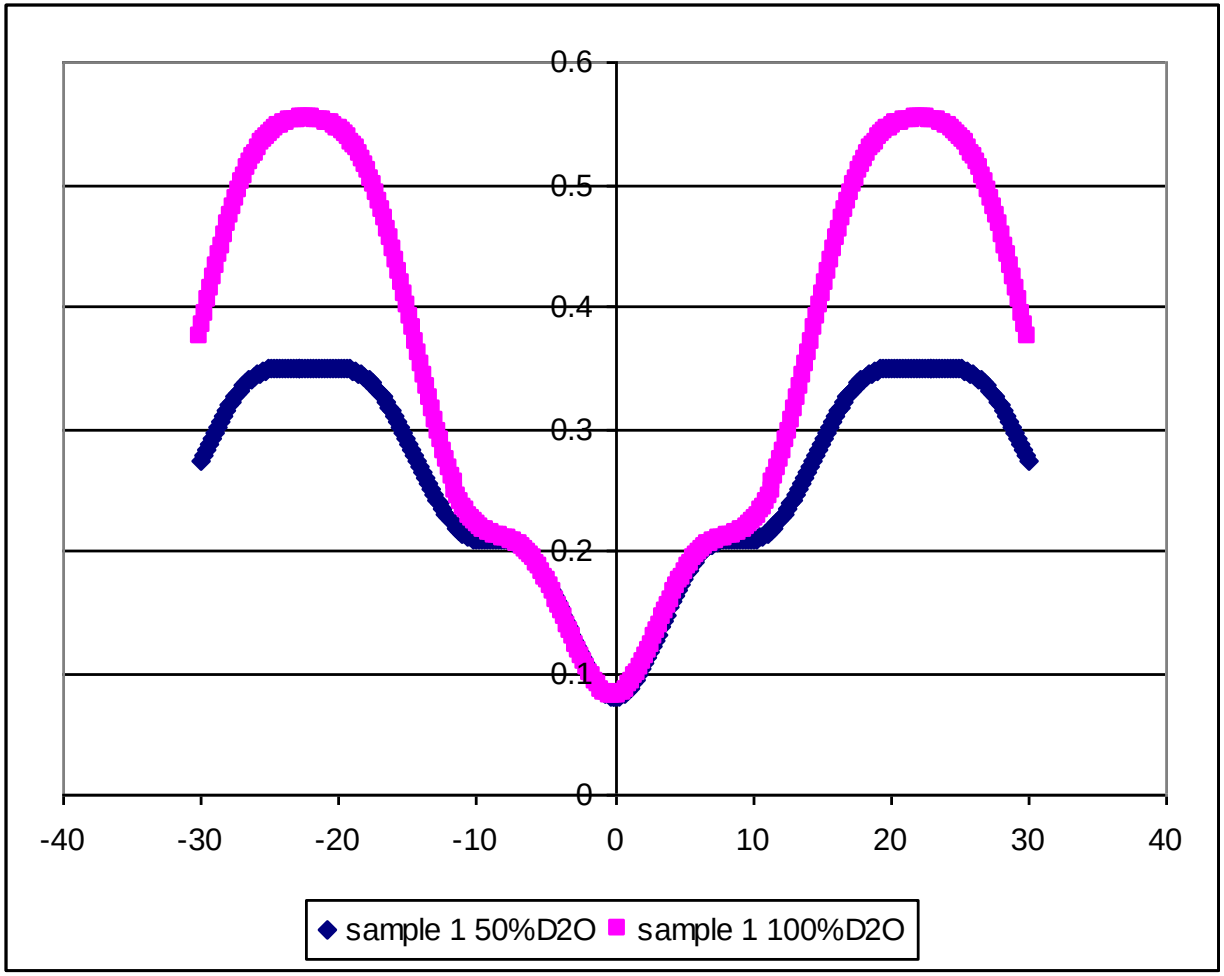


Results

DOPC, 100% H2O RH=57%, with and without glucose



DOPC, RH=11%, no sugar



Modelling

- Strip models
- Fluid minus models
  - Assumes bilayer doesn't change with hydration
  - Remove dependence on zeroth order structure factor
- Gaussian models
- Hybrid Gaussian model (Nagle group)
  - Methylene region is modelled as a constant plateau