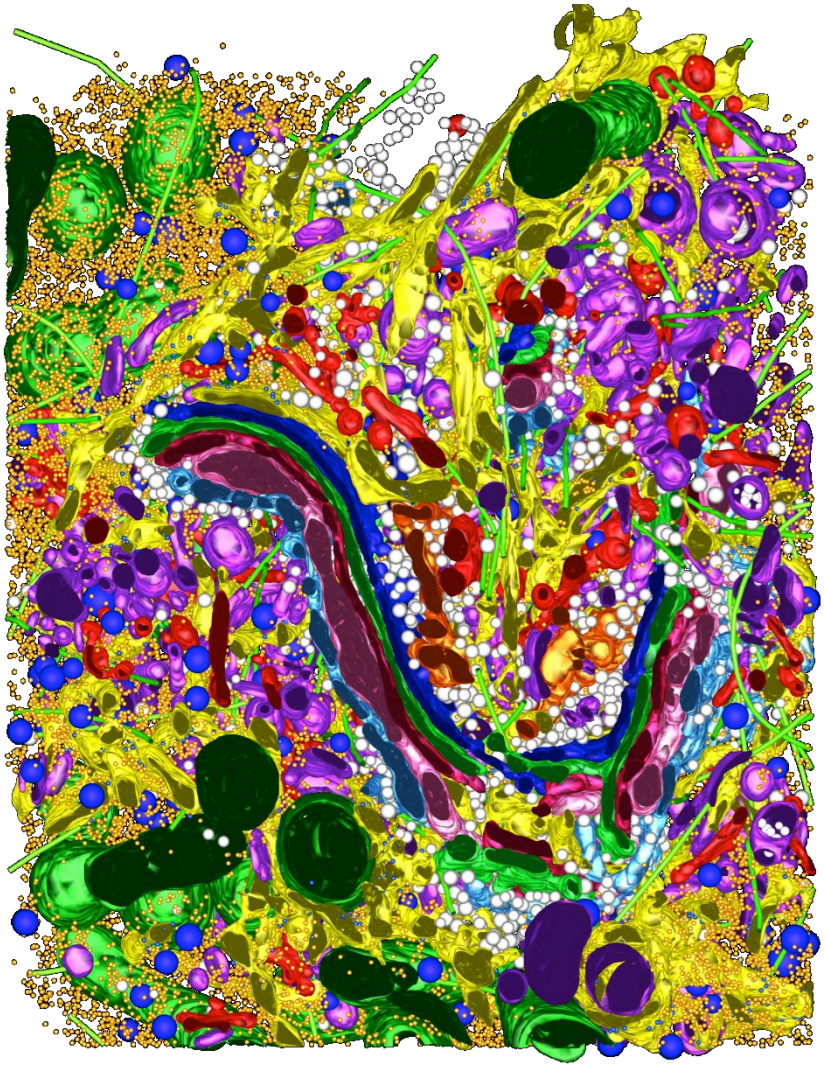
Unravelling the Complexity of Biological Samples using Isotopic Labelling

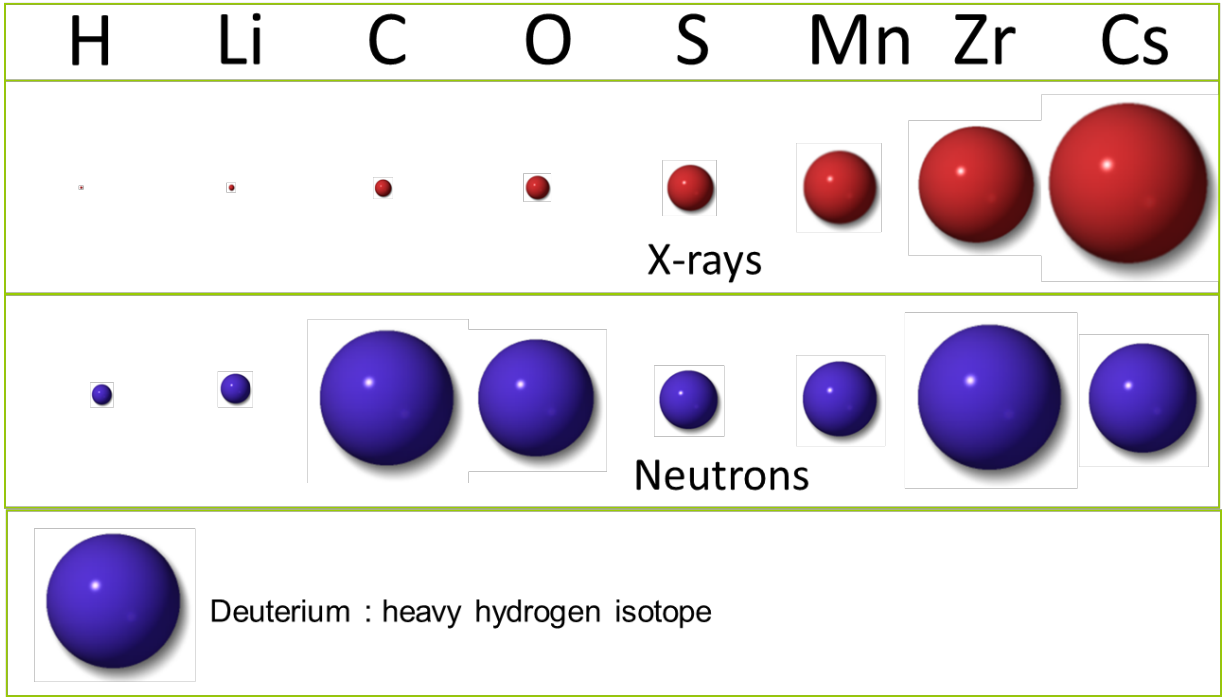
Luke Clifton
Luke.clifton@stfc.ac.uk

Biology is Complex



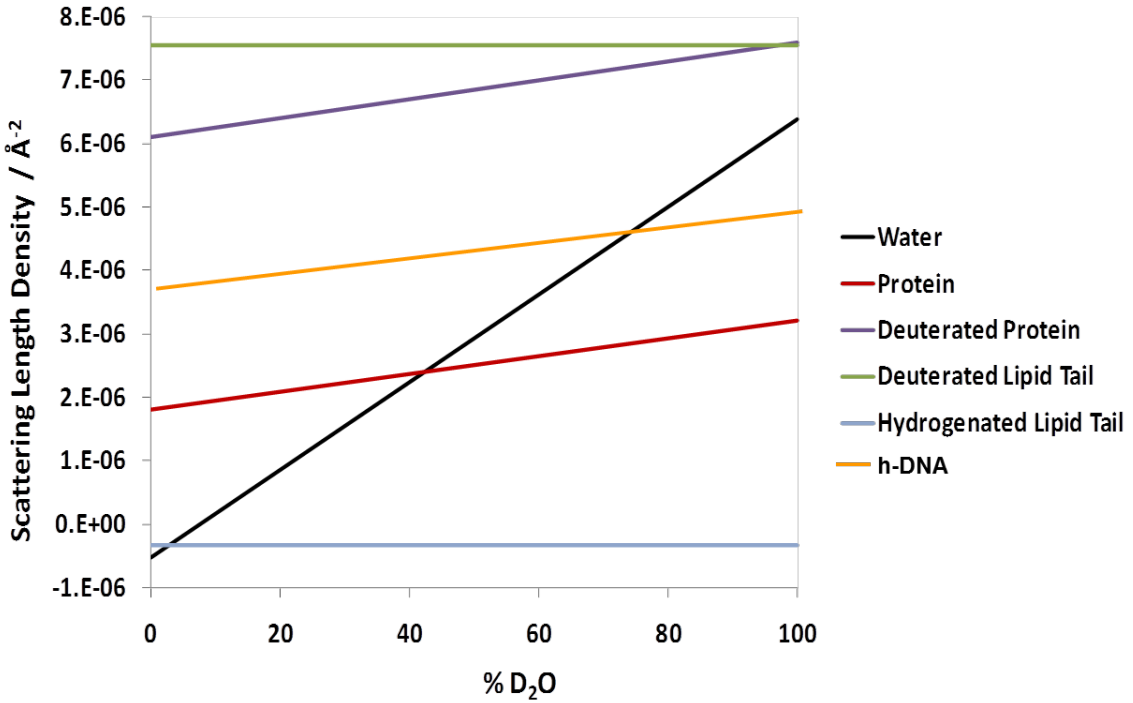
PNAS, 2001, 98, 5, 2399-2406

NS can easily differentiate different hydrogen isotopes



Element	Coherent Scattering Length (b)/ 10^{-5} Å
Hydrogen	-3.74
Deuterium	6.671
Carbon	6.646
Nitrogen	9.36
Oxygen	5.803
Sulphur	2.847
Phosphorous	5.13

Natural contrast between biomolecules is present due to ^{14}N



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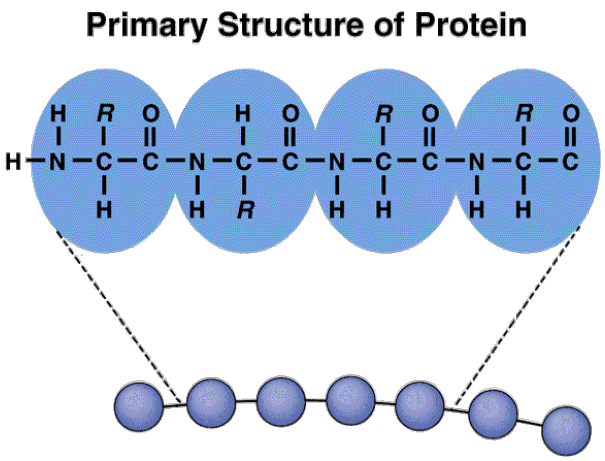
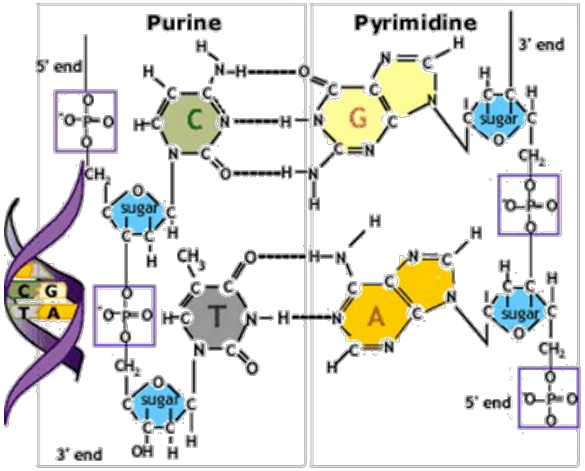
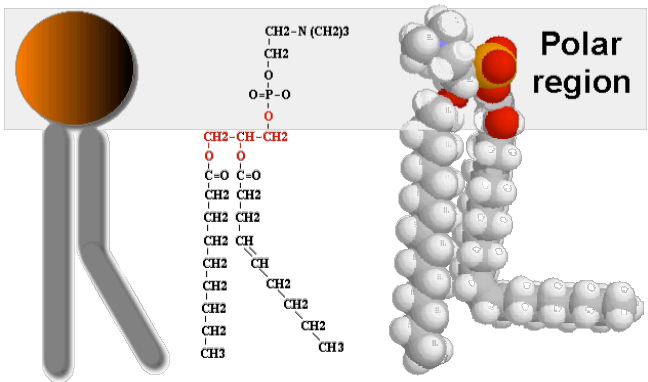
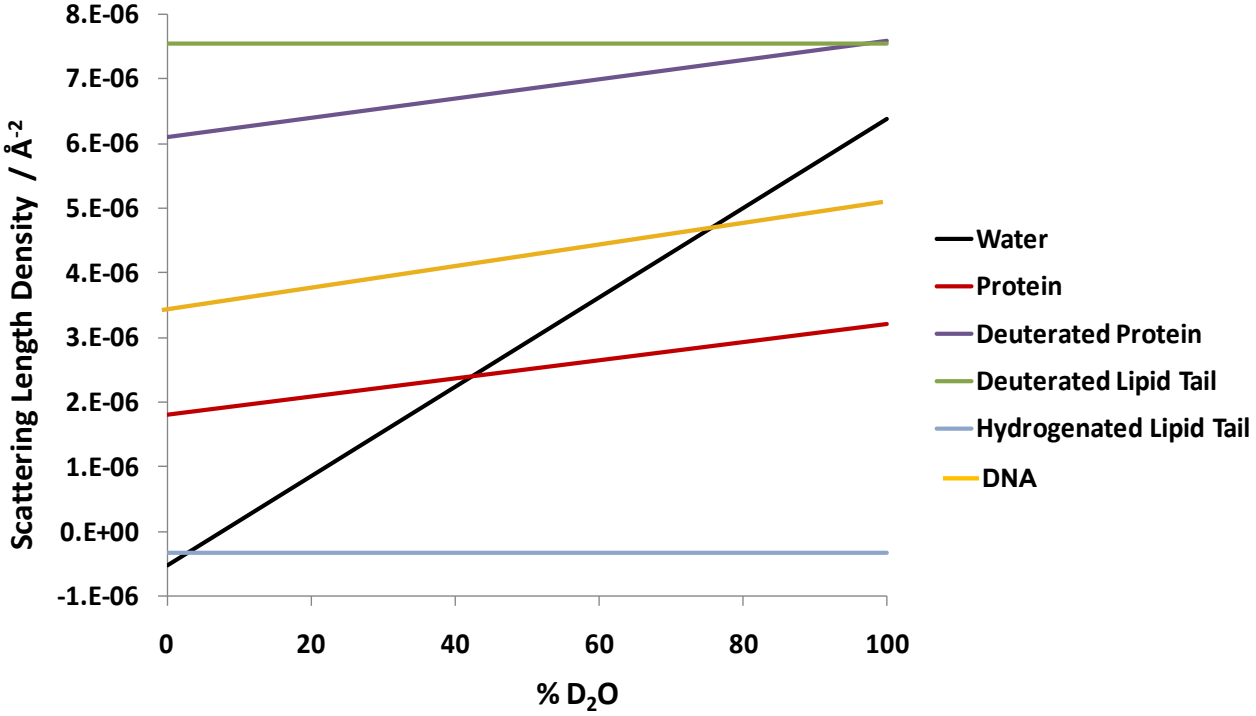
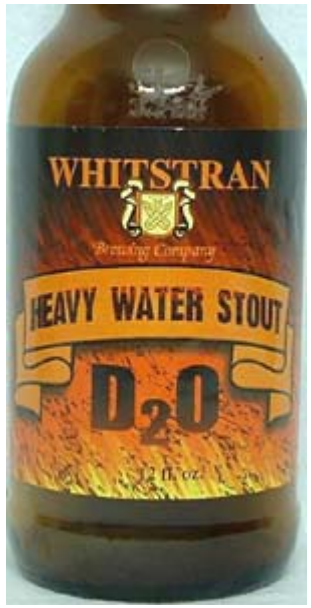


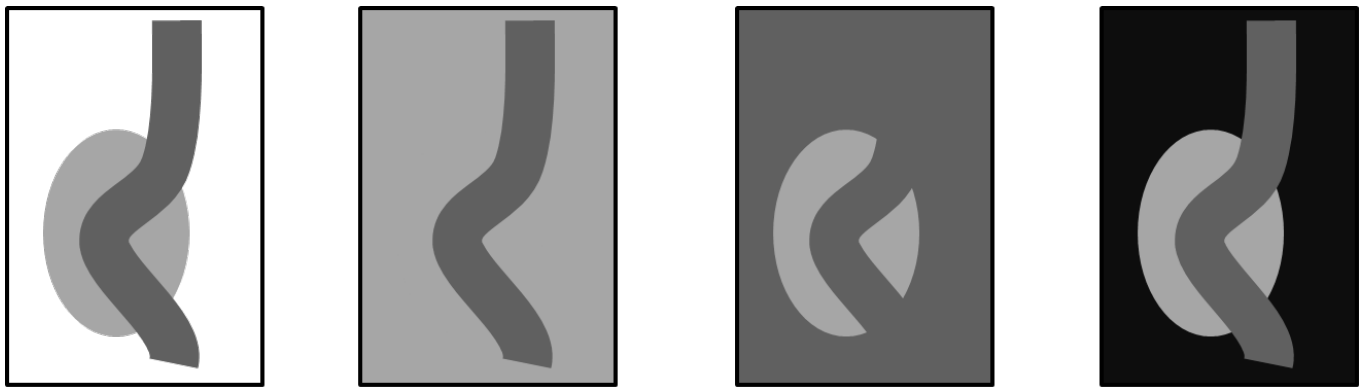




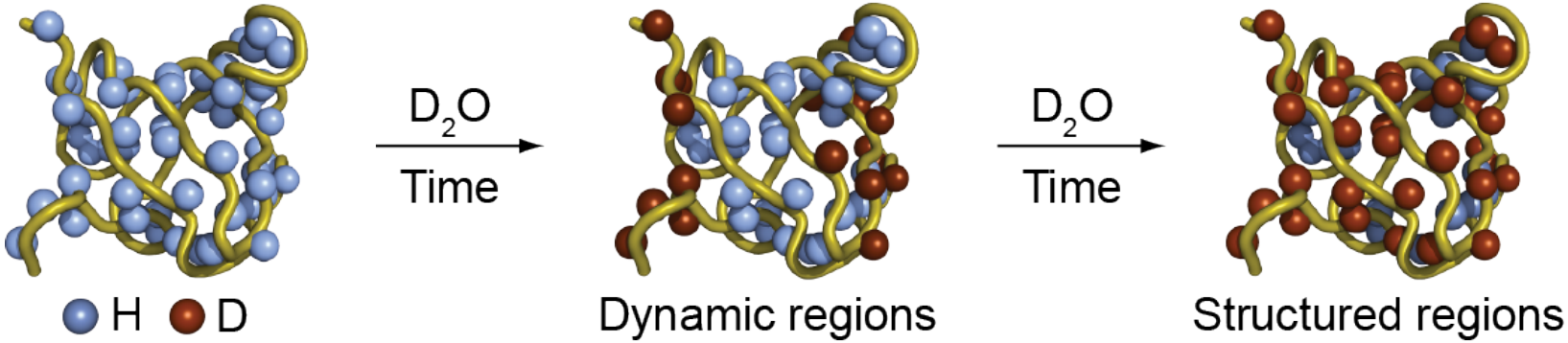
The easiest Labelling technique is to change the Labelling of the solution



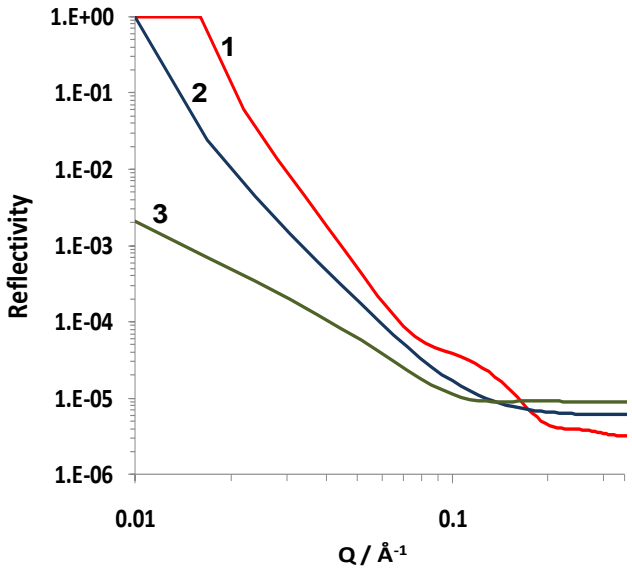


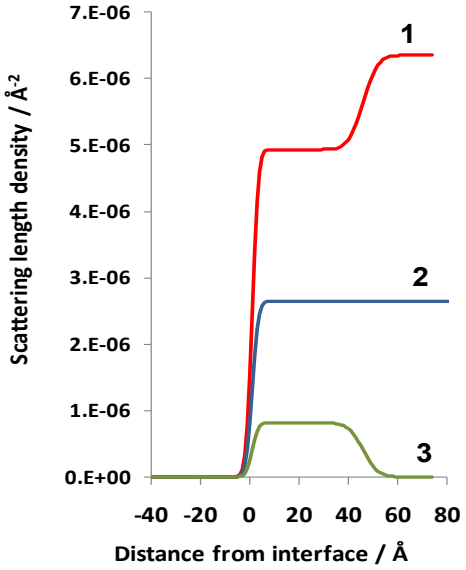


H/D Exchange

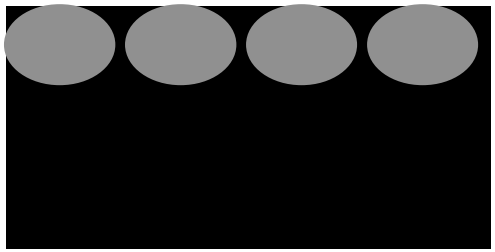

D_2O
Time
D_2O
Time
H
D
Dynamic regions
exchange rapidly
Structured regions
exchange slowly

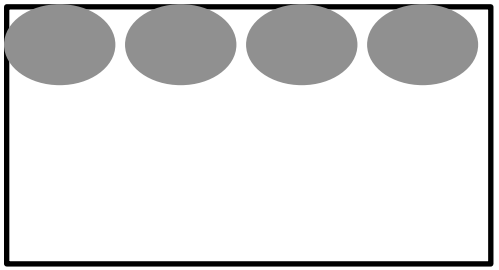
Matching sample and solution SLD





1. D_2O
2. 42% D_2O
3. H_2O





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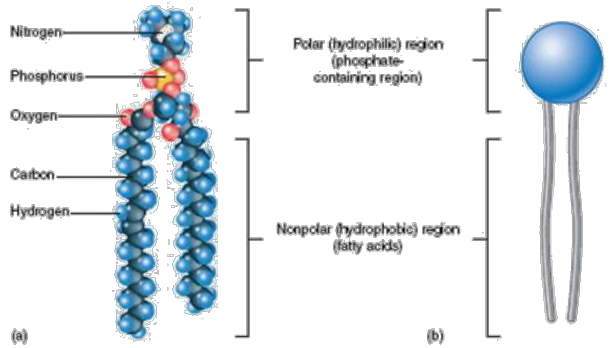
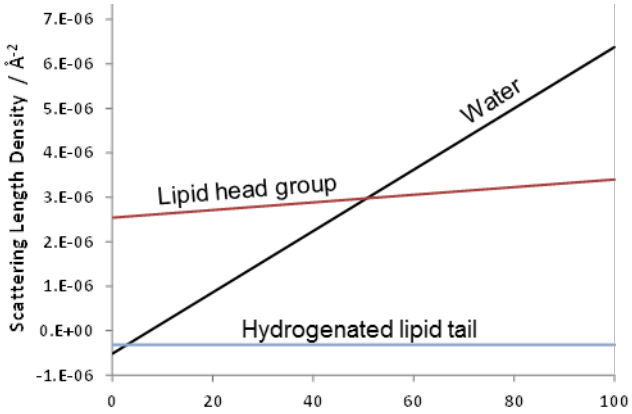
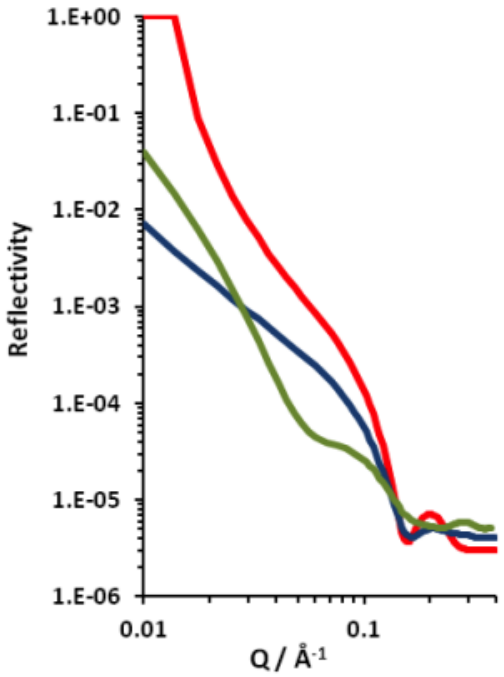
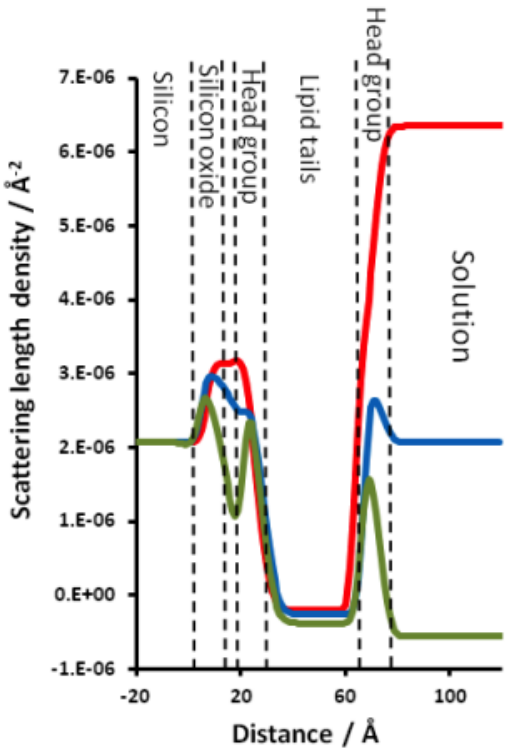
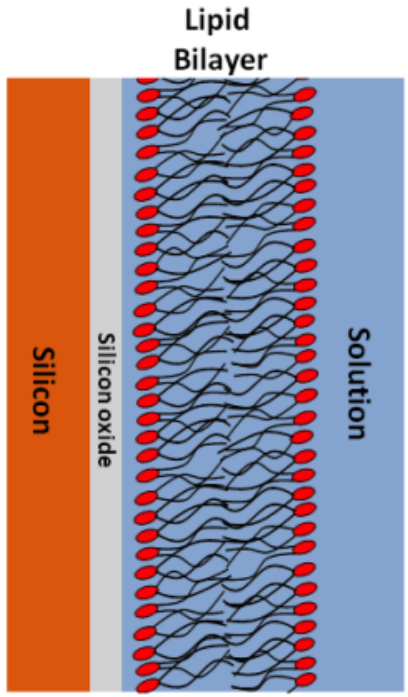


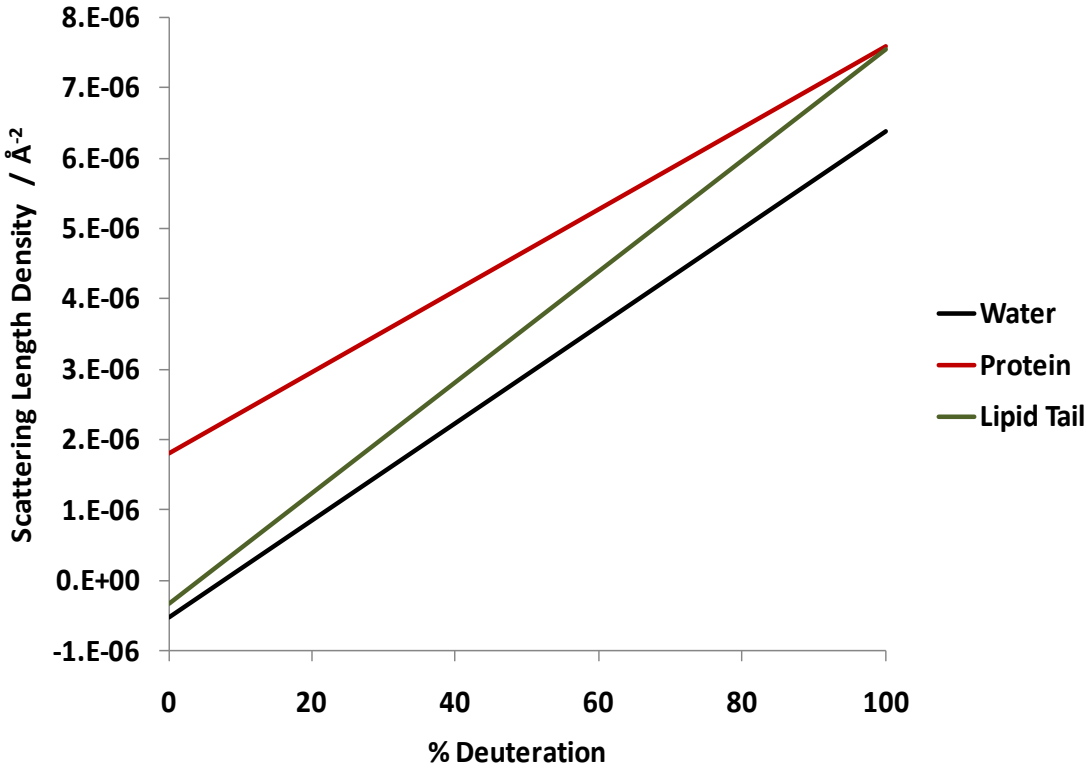


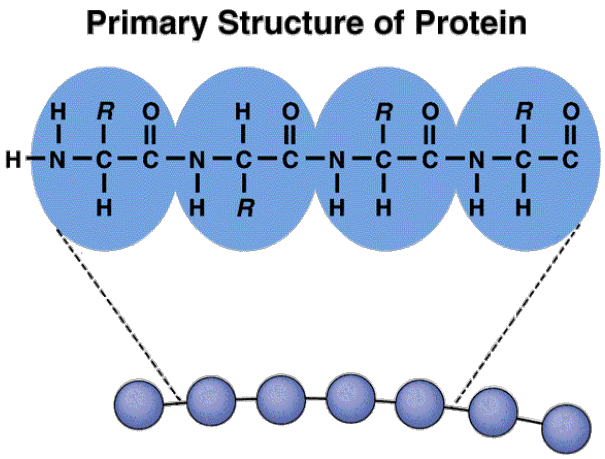


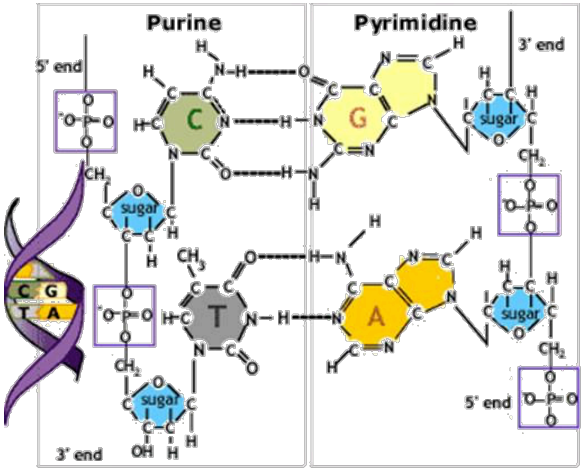


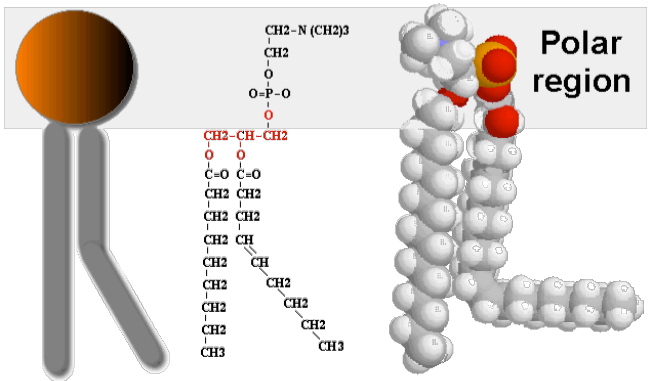


Isotopic Labelling Changes the SLD of Biomolecules









Where do you get your labelled samples?

Oxford Isotope Facility

The ISIS Isotope Facility (formerly the Oxford Isotope facility) is able to produce deuterated small molecules for ISIS and ILL experiments.

Details of what is possible are below. If you think you will need to use the services of the isotope facility for your experiment please tick the box on the ISIS proposal form when you submit a proposal, and also contact John Webster (john.webster@stfc.ac.uk) to discuss your requirements.

Materials categories:

Category A

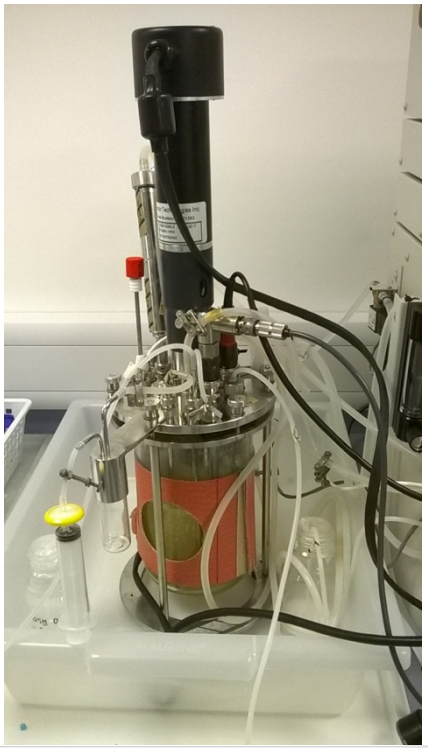
Perdeuterated fatty acids of most chain lengths from C6 to C20, including odd numbered chains, and the alcohol and bromoalkane versions of these. It is envisaged that up to 10 g of any compound could be made available instantly (a case will have to be made for quantities larger than 1g).

Sodium dodecyl sulphate. Some chain deuterated alkyl trimethylammonium bromides (C12, C14, C16, enquire about others). Some chain deuterated oligoethyleneglycol monoether non-ionic surfactants (enquire).

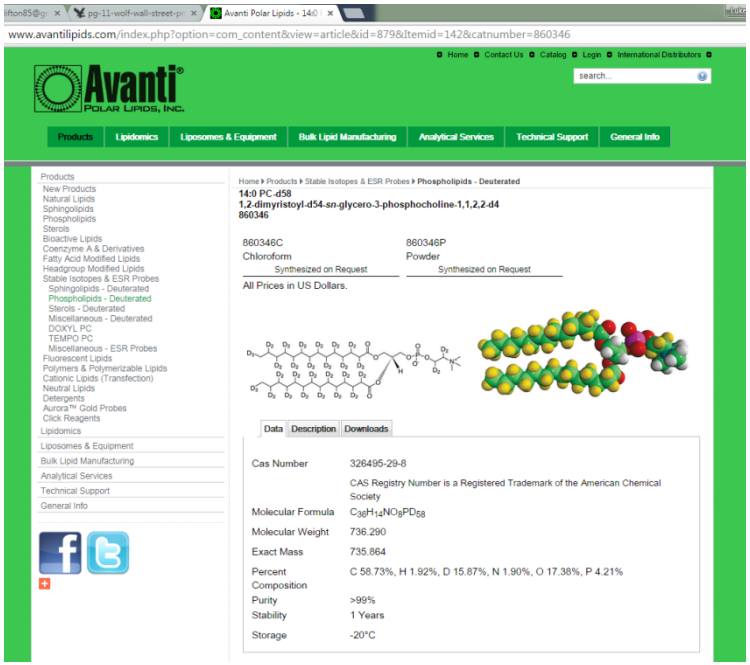
Category B

The compounds in this category include most compounds with a straight chain alkyl group, e.g. alkyl trichlorosilanes, alkane thiols, alkane sulphonates, dialkyl dimethyl ammonium halides.

Some dicarboxylic acids (C12, enquire about others) and their corresponding dialcohols and dibromides. Ethylhexanoic acid and corresponding alcohol and bromide.



Silantes
Stable Isotope Labeled Biomolecules



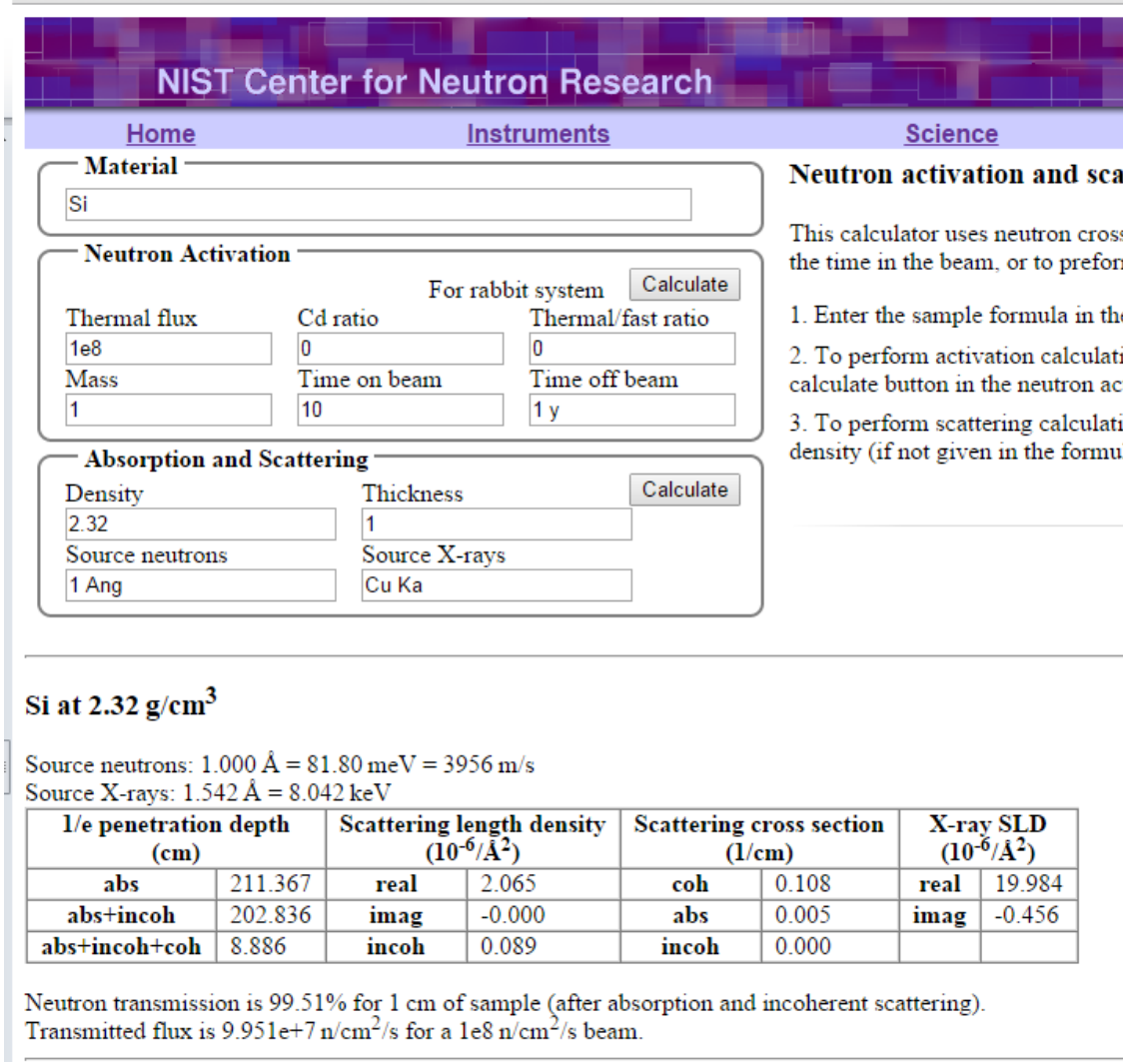
Starting an experiment : Calculating ρ

$$\rho = \frac{\sum b}{V}$$

Lipid / Solvent	Neutron scattering length density (ρ) (10^{-6} Å^{-2})
D_2O	6.35
H_2O	-0.56
Silicon	2.07
Silicon oxide (SiO_2)	3.41
Deuterated-tails (gel phase)	7.45
Hydrogenous-tails (gel phase)	-0.37
h-Protein in D_2O	~3.4
h-Protein in H_2O	~2.0
h-DNA in D_2O	~3.2
h-DNA in H_2O	~3.8

Web tools can help : NIST

https://www.ncnr.nist.gov/resources/activation/



NIST Center for Neutron Research

Home | Instruments | Science

Material

Si

Neutron Activation

For rabbit system — Calculate

Thermal flux	Cd ratio	Thermal/fast ratio
1e8	0	0
Mass	**Time on beam**	**Time off beam**
1	10	1 y

Absorption and Scattering

Calculate

Density	Thickness
2.32	1
Source neutrons	**Source X-rays**
1 Ang	Cu Ka

Neutron activation and sca

This calculator uses neutron cross
the time in the beam, or to prefor

1. Enter the sample formula in th
2. To perform activation calculati
calculate button in the neutron ac
3. To perform scattering calculati
density (if not given in the formu

Si at 2.32 g/cm^3

Source neutrons: 1.000 Å = 81.80 meV = 3956 m/s
Source X-rays: 1.542 Å = 8.042 keV

1/e penetration depth (cm)		Scattering length density (10^{-6}/Å^2)		Scattering cross section (1/cm)		X-ray SLD (10^{-6}/Å^2)	
abs	211.367	**real**	2.065	**coh**	0.108	**real**	19.984
abs+incoh	202.836	**imag**	-0.000	**abs**	0.005	**imag**	-0.456
abs+incoh+coh	8.886	**incoh**	0.089	**incoh**	0.000		

Neutron transmission is 99.51% for 1 cm of sample (after absorption and incoherent scattering).
Transmitted flux is 9.951e+7 n/cm^2/s for a 1e8 n/cm^2/s beam.

Biological Scattering Tool Website : Scattering Length Density Calculator

http://psldc.isis.rl.ac.uk/

Biomolecular Scattering Length Density Calculator

TITLE:

Pin-a

Type of biomolecule :

- (•) Protein / Peptide
- ( ) RNA (A,U,G,C)
- ( ) DNA(A,T,G,C)

Primary structure:

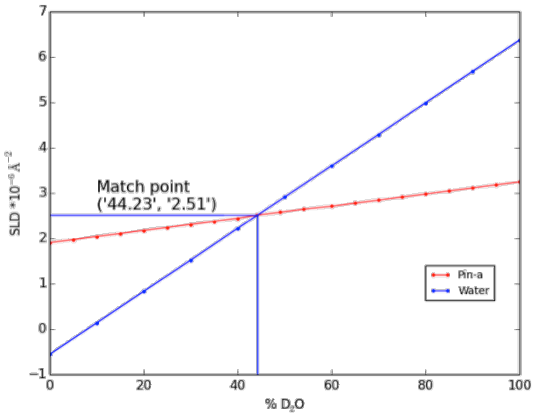
```
DV AGGGGAQQCP VETKLNSCRN YLLDRCSTMK DFPVTWRWWK WWKGGCQELL
GECCSRLGQM PPQCRCNIIQ GSIQGDLGGI FGFQRDRASK VIQEAKNLPP
RCNQGPPCNI PGTI
```

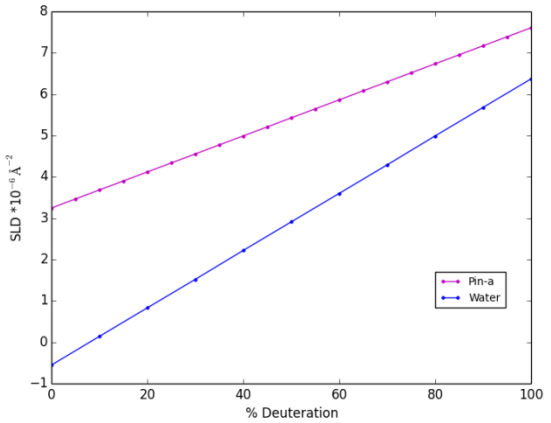
Submit

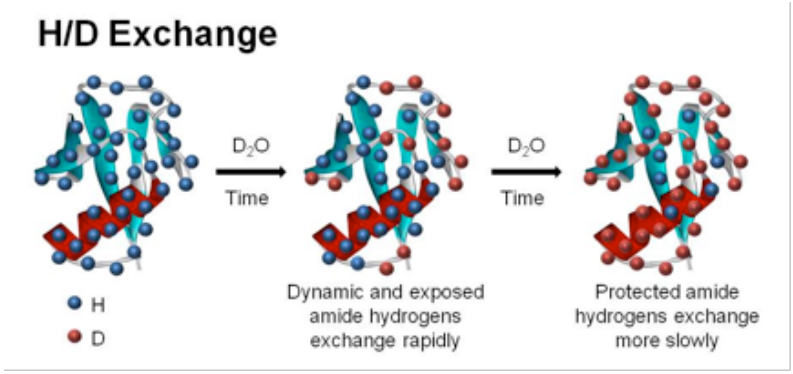
Reset

OPTIONS:

1. Solution D_2O% * 1 100
2. Deuteration % * 2 0
3. Exchange % * 3 90
4. Concentration of sample (mg/mL) 0

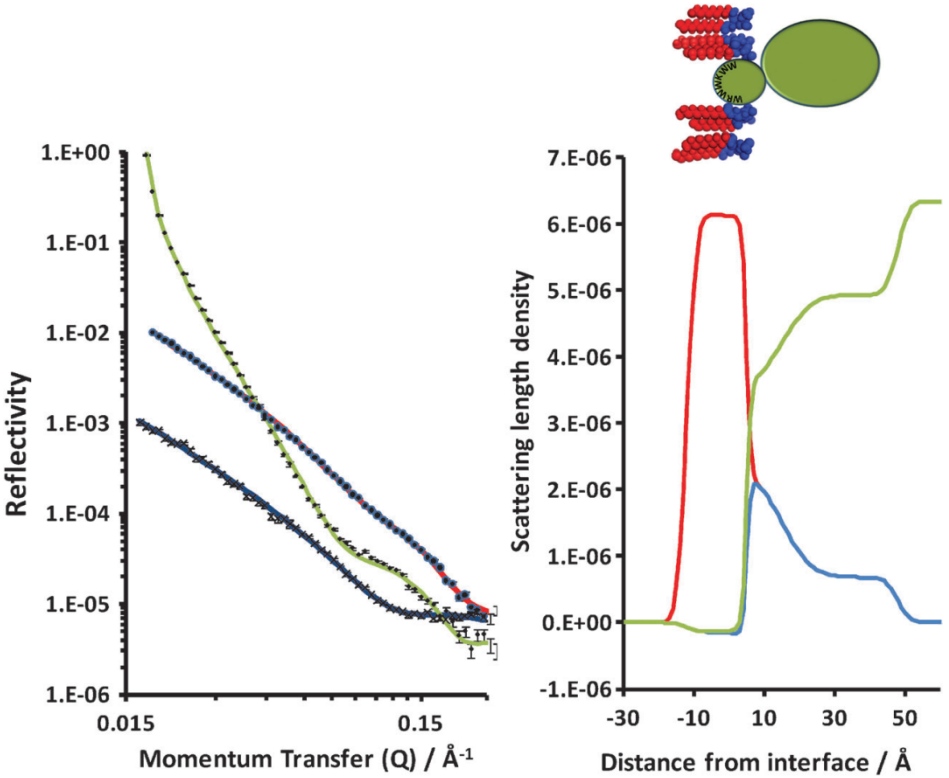






Choose your Contrasts

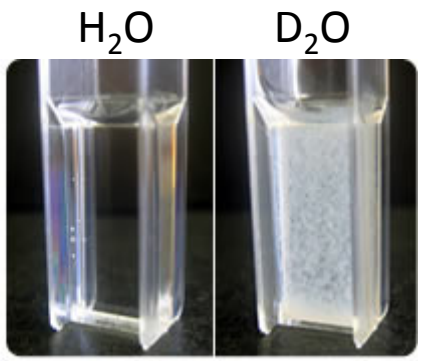
#	*Isotopic Contrast*	*Main Information Content of Reflectivity Data*	*Contrast Cartoon*
1	Deuterated-lipid : Hydrogenated-Protein on Null Reflective Water	Lipid component	
2	Hydrogenated-lipid : Hydrogenated-Protein on Null Reflective Water	Protein component	
3	Hydrogenated -lipid : Hydrogenated-Protein on D_2O	Solution and Hydrogenous material –both lipid and protein components	
4	Deuterated-lipid : Hydrogenated-Protein on D_2O	Solution and Protein component	
5	Hydrogenated -lipid : Deuterated-Protein on Null Reflective Water	Protein component	
6	Deuterated-lipid : Deuterated-Protein on Null Reflective Water	Deuterated material –both lipid and protein components	

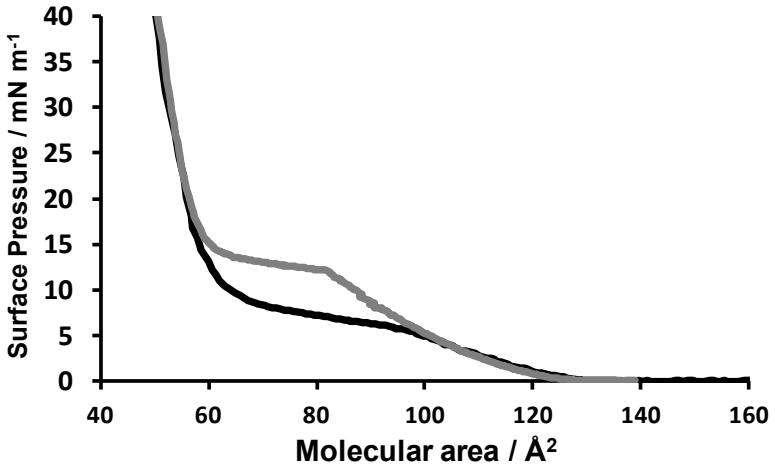


$$\rho_{fitted} = (\varphi_{lipid} \cdot \rho_{lipid}) + (\varphi_{protein} \cdot \rho_{protein}) + (\varphi_{water} \cdot \rho_{water})$$

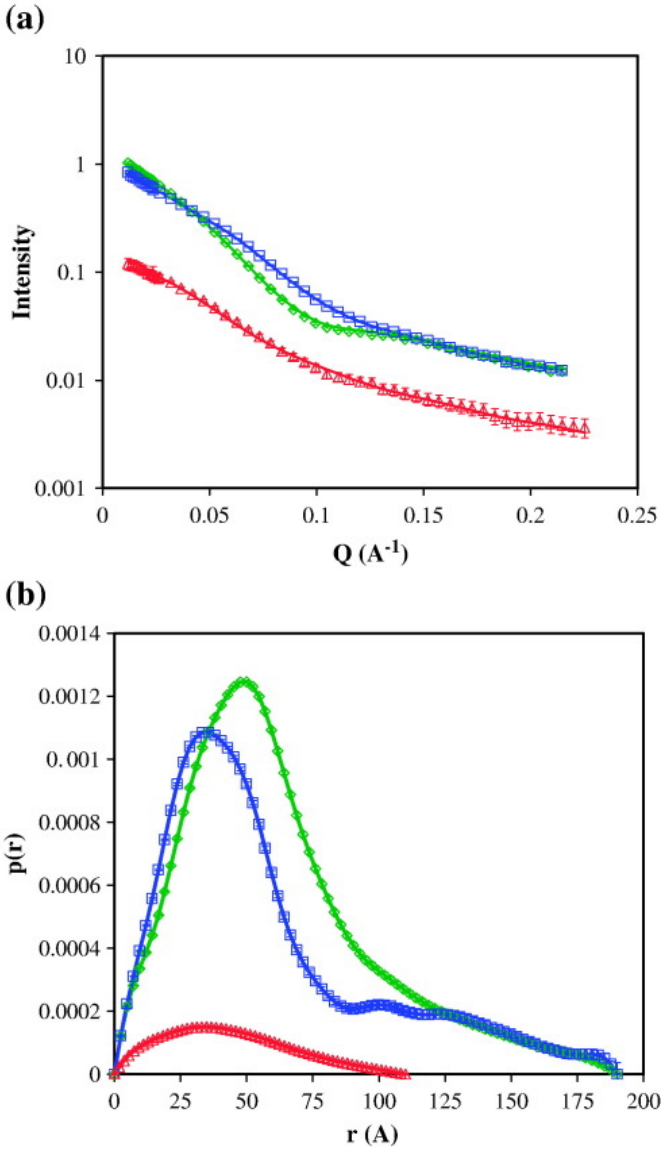
BEWARE

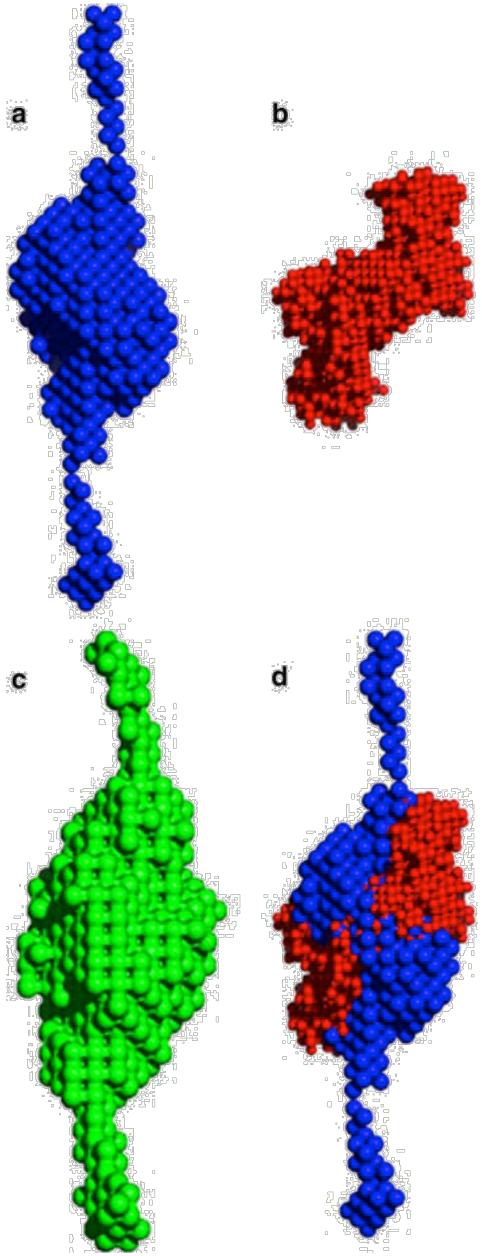
- D_2O and H_2O although chemically very similar are not the same!
    - Slight Differences in Nature of Hydrogen and Deuterium Bonding!
    - Due to more restricted O-D bond vibration vs. O-H, D_2O forms stronger dipole-dipole bind.
    - D_2O Melts at 3.7°C vs. 0°C.





Sample deuterium labelling is often required : SANS





Callow *et al*, J Molec Biol 2007, 369:177–185

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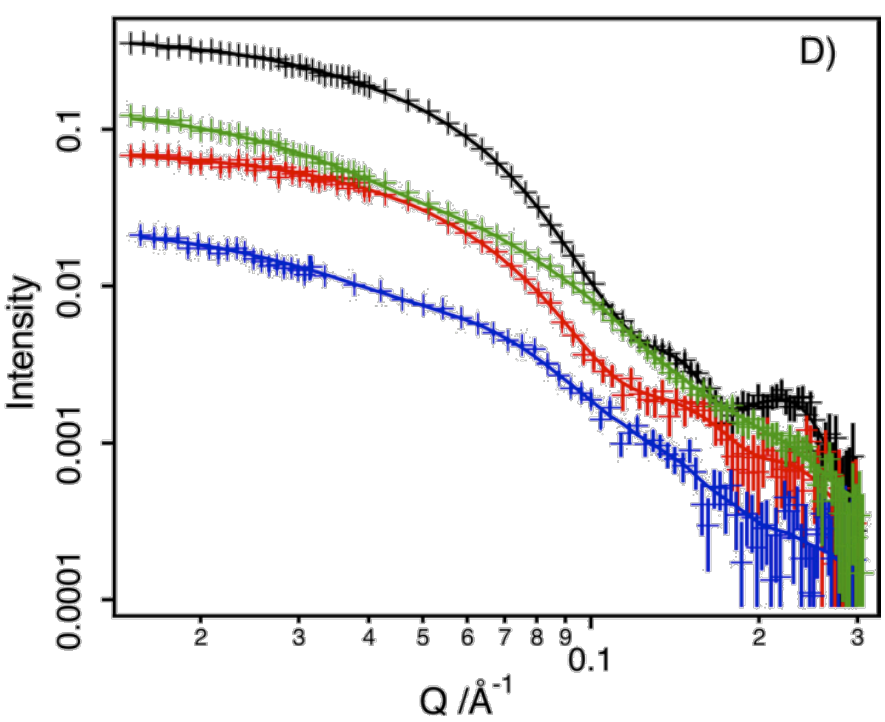
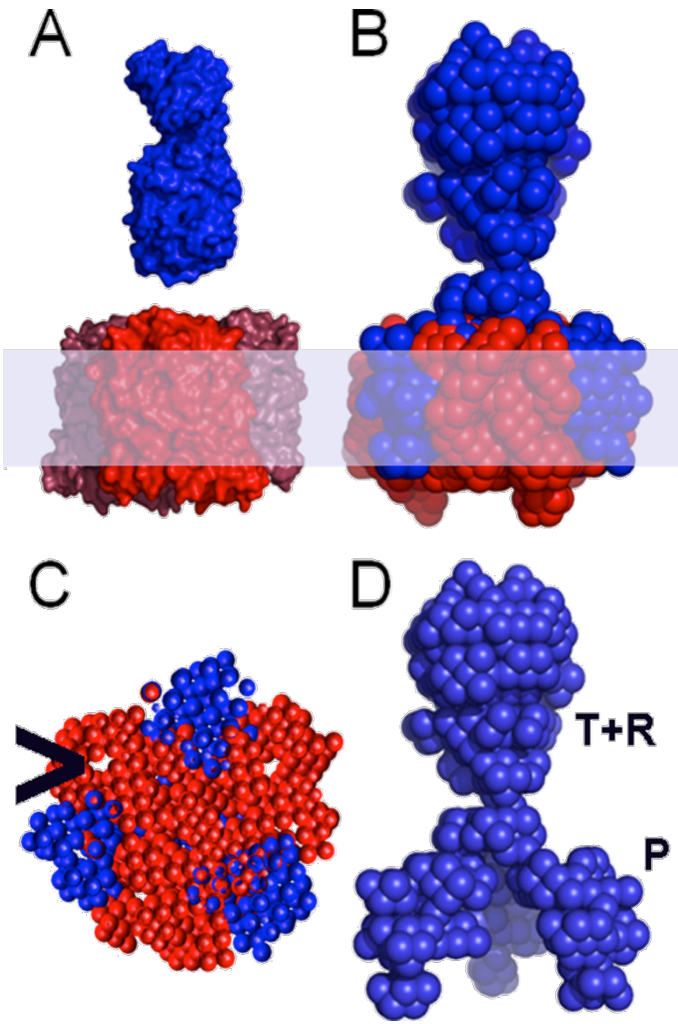
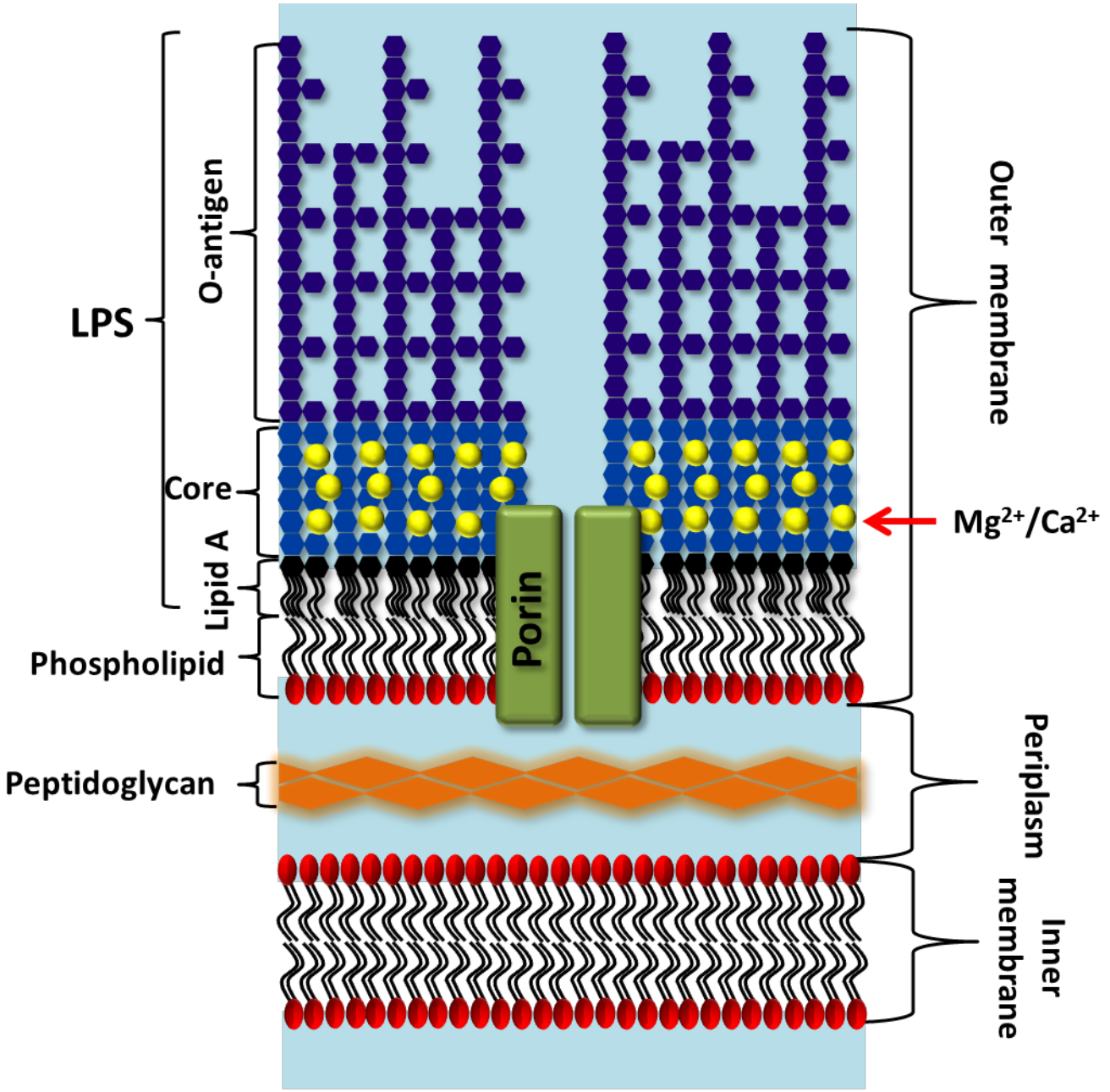


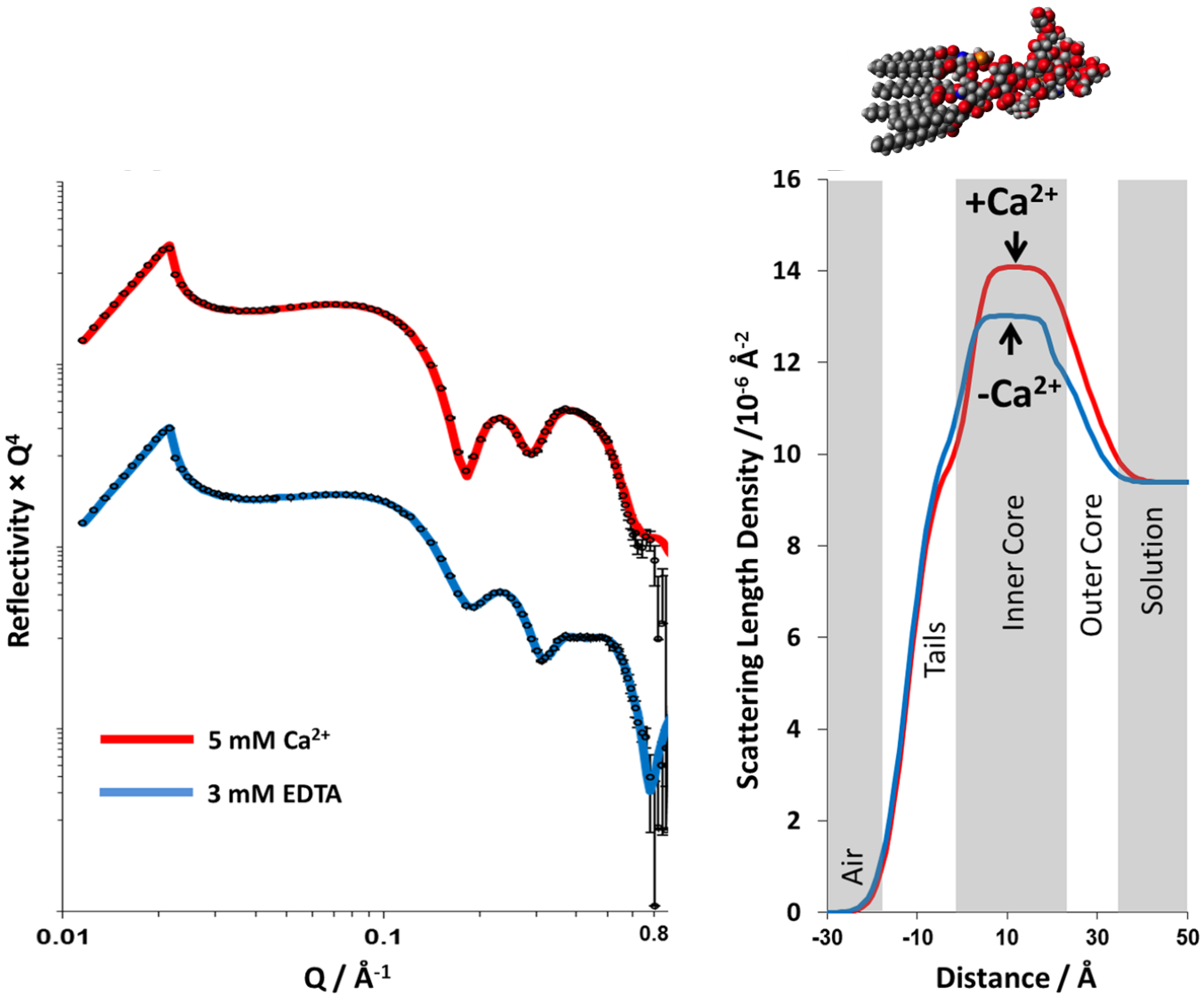


Clifton et al et al, J. Biol. Chem 2012

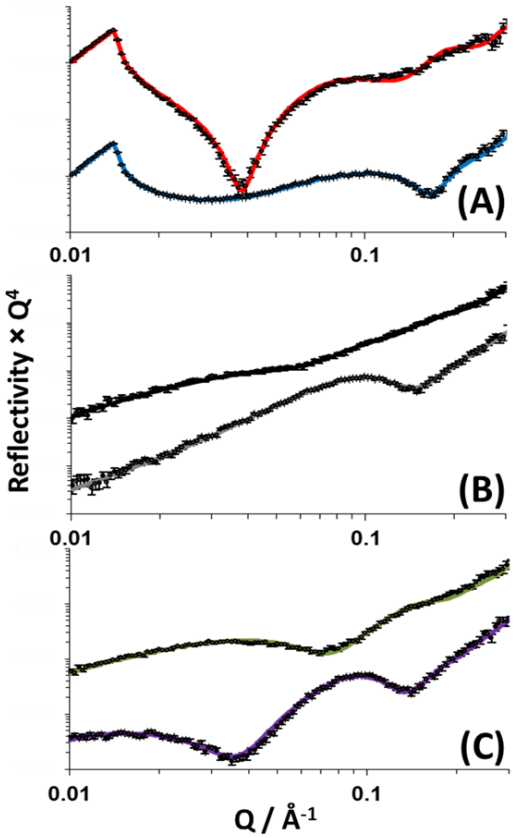
Sample labelling is often required : NR

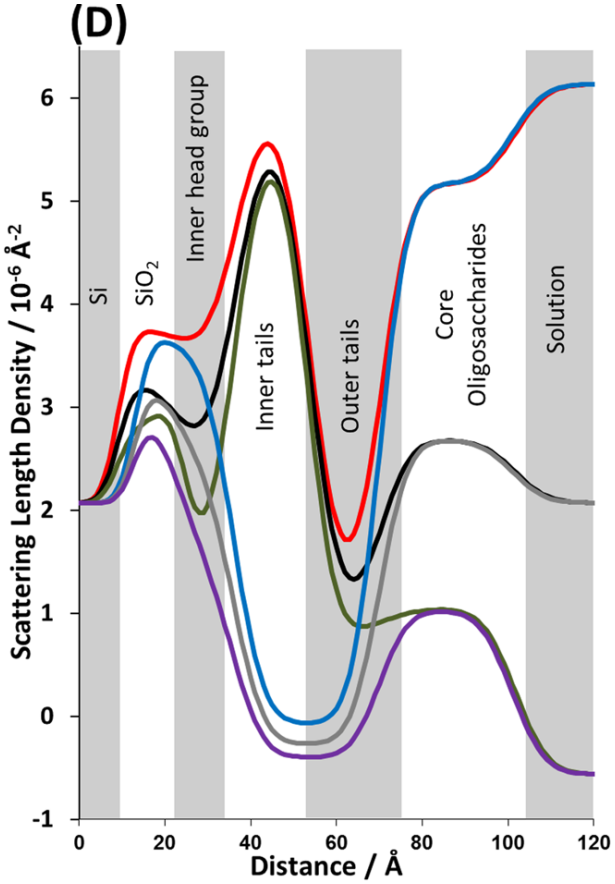


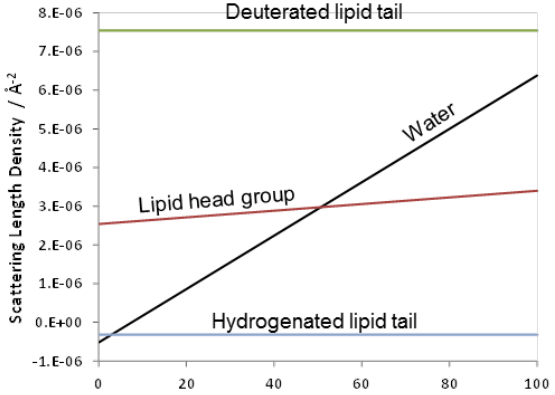
Sample labelling : X-rays find electron rich elements!

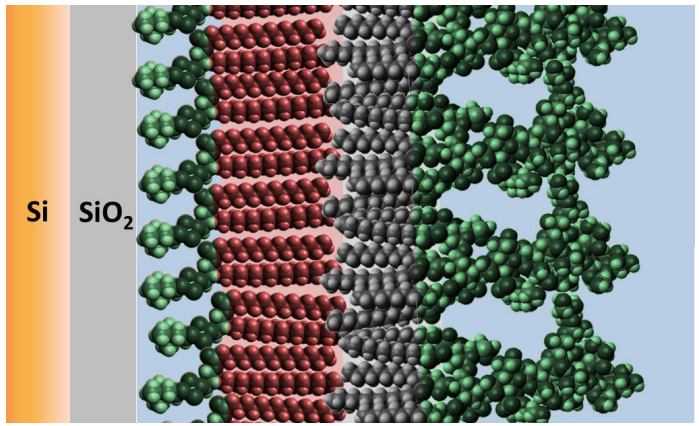


Sample deuterium labelling is often required : NR









Clifton *et al*, Langmuir, 2015, 31, 404-412.

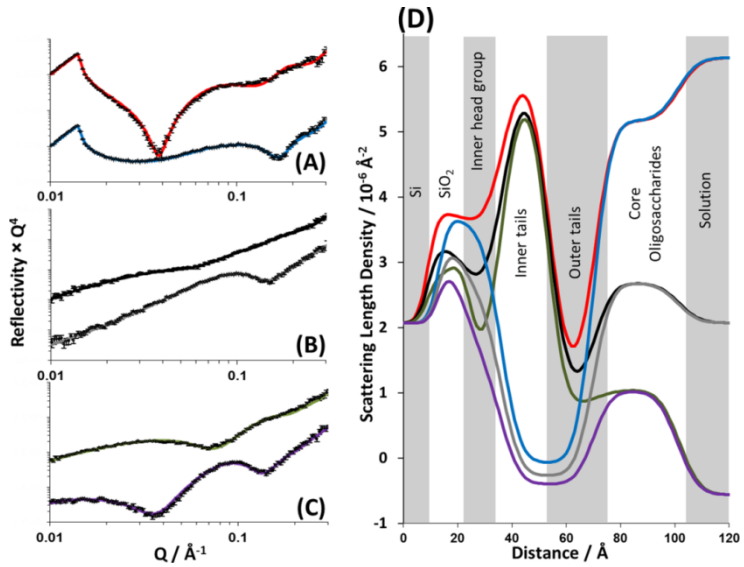
Determining Coverage and Asymmetry

$$\rho_{fitted\ D2O} = (\varphi_{h-lipid} \cdot \rho_{h-lipid}) + (\varphi_{d-lipid} \cdot \rho_{d-lipid}) + (\varphi_{D2O} \cdot \rho_{D2O})$$

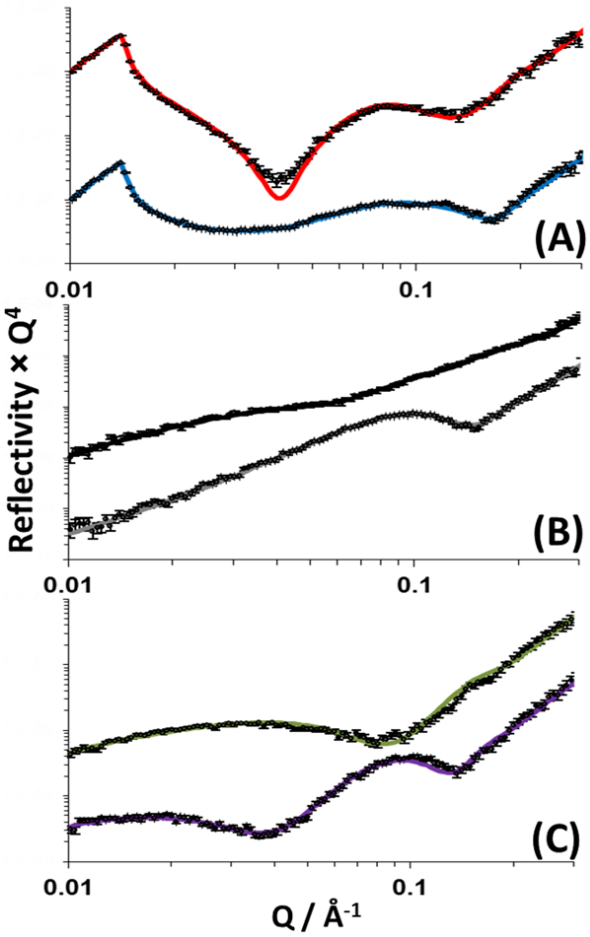
$$\rho_{fitted\ H2O} = (\varphi_{h-lipid} \cdot \rho_{h-lipid}) + (\varphi_{d-lipid} \cdot \rho_{d-lipid}) + (\varphi_{H2O} \cdot \rho_{H2O})$$

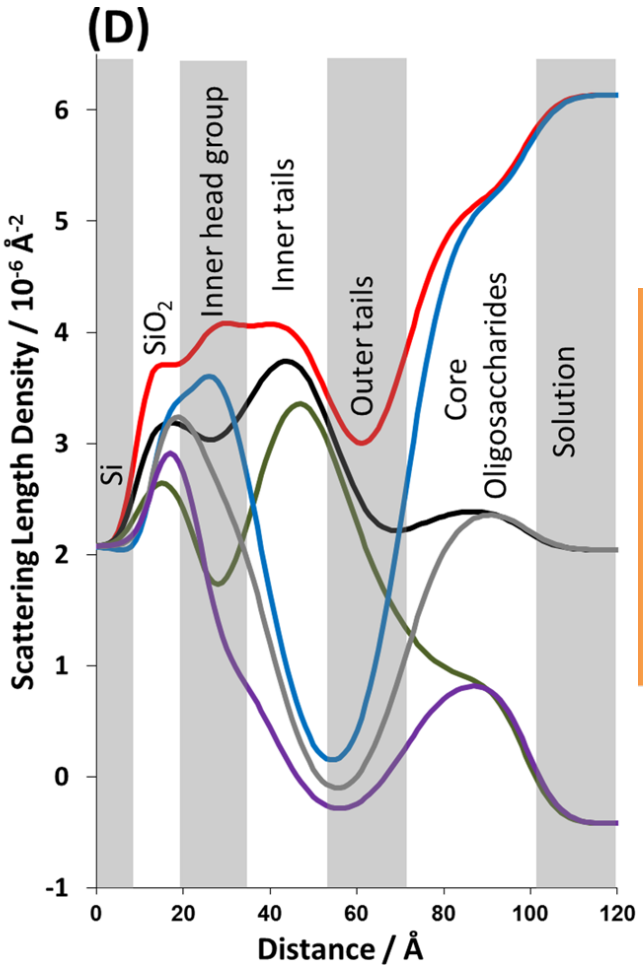
$$\varphi_{water} = \frac{(\rho_{fitted-D2O} - \rho_{fitted-H2O})}{(\rho_{D2O} - \rho_{H2O})}$$

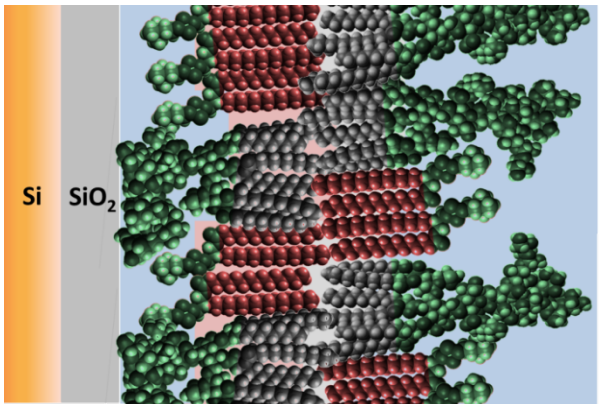
$$\varphi_{d-lipid} = \varphi_{lipid} \times \left(\frac{(((\rho_{fitted} - (\rho_{D2O}\varphi_{D2O})/\varphi_{lipid}) - \rho_{h-lipid\ tails})}{(\rho_{d-lipid\ tails} - \rho_{h-lipid\ tails})}\right)$$

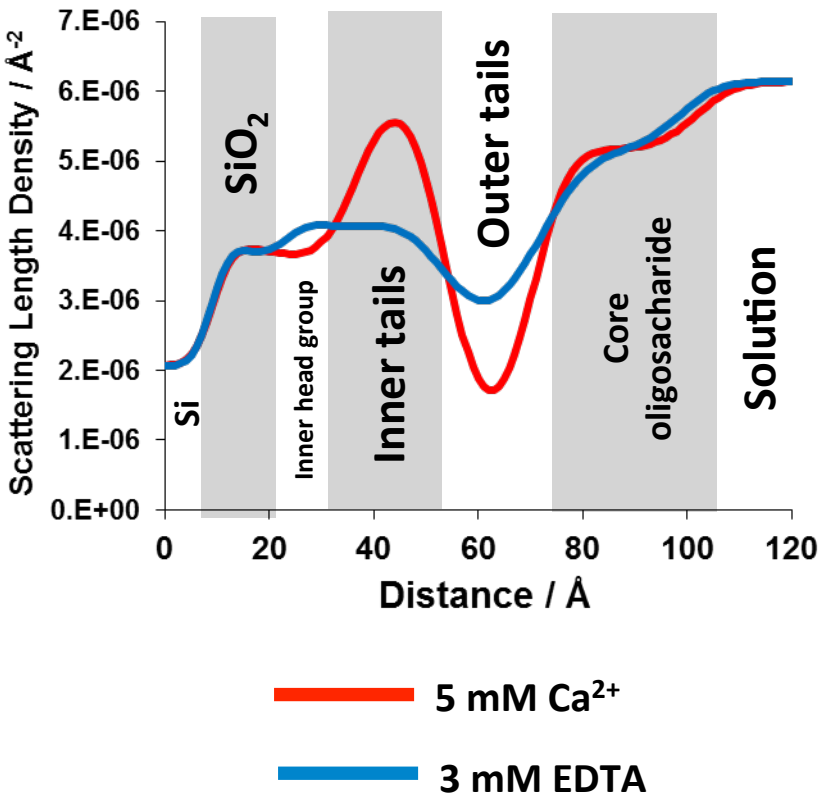


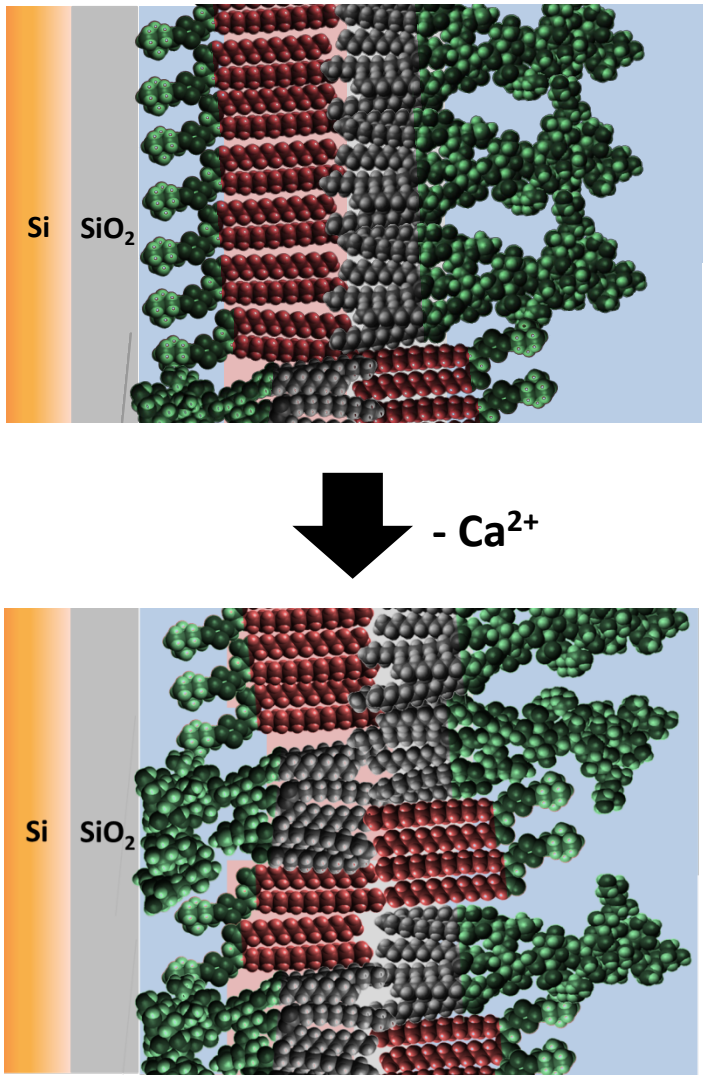
Asymmetric DPPC (inner leaflet) : Ra-LPS (outer leaflet) bilayer deposited on Silicon in EDTA containing buffer



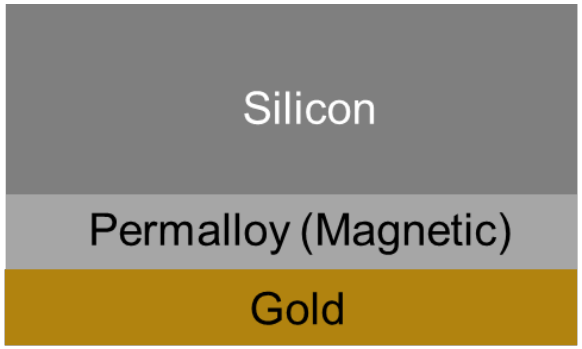


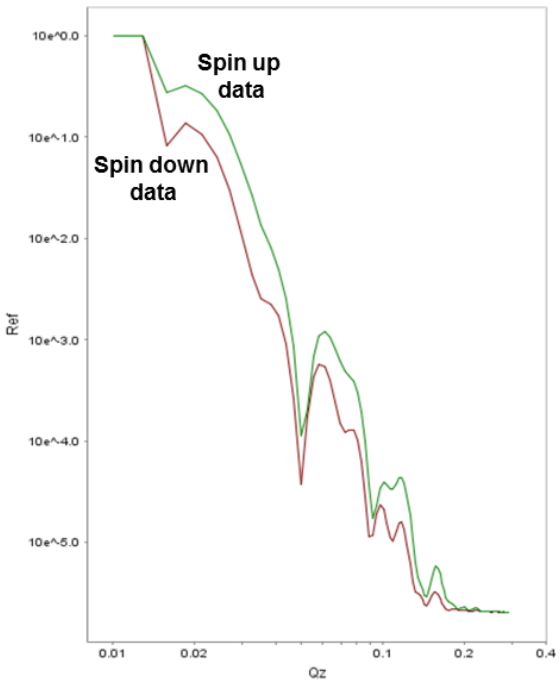


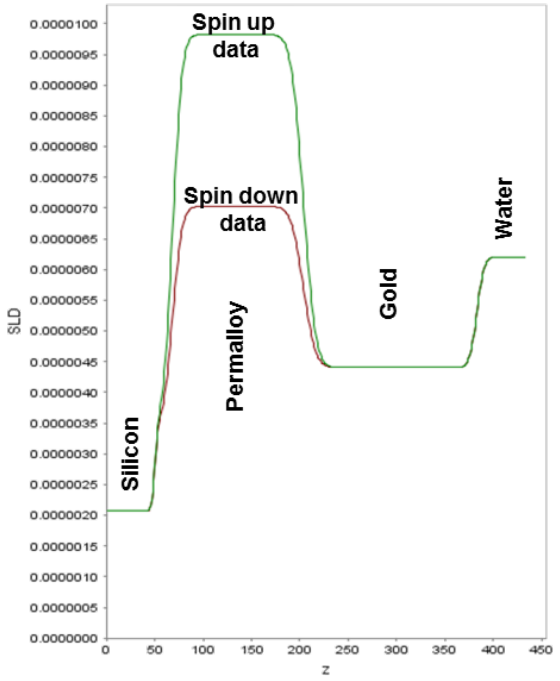

Scattering Length Density / Å-2
7.E-06
6.E-06
5.E-06
4.E-06
3.E-06
2.E-06
1.E-06
0.E+00
0
20
40
60
80
100
120
Distance / Å
Si
SiO2
Inner head group
Inner tails
Outer tails
Core oligosaccharide
Solution
5 mM Ca2+
3 mM EDTA


Si
SiO2
- Ca2+
Si
SiO2

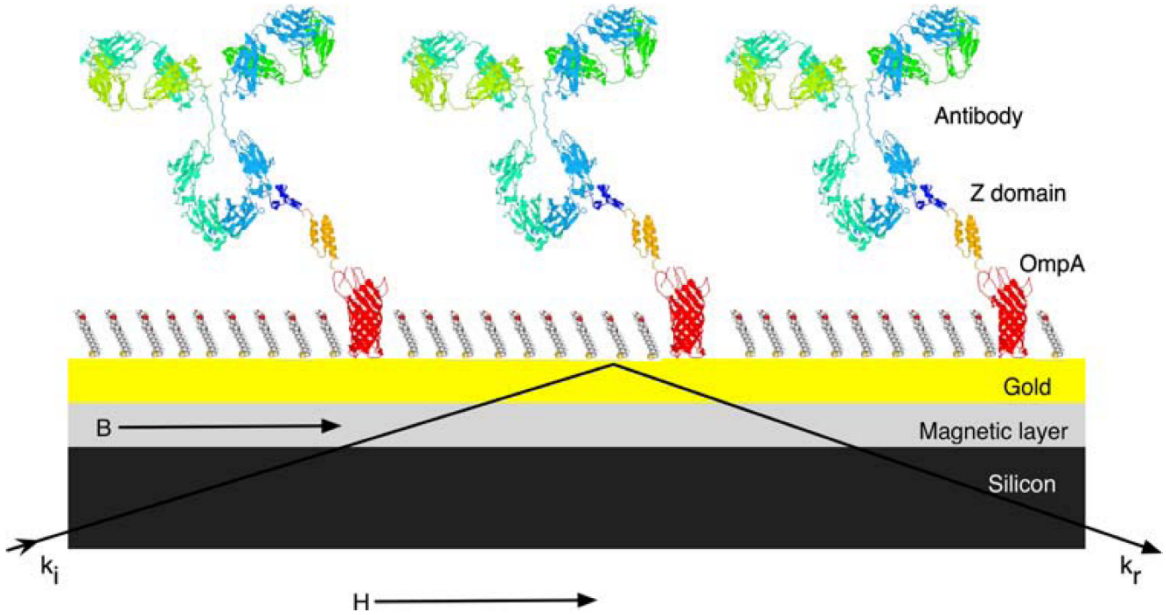
Magnetic Contrast

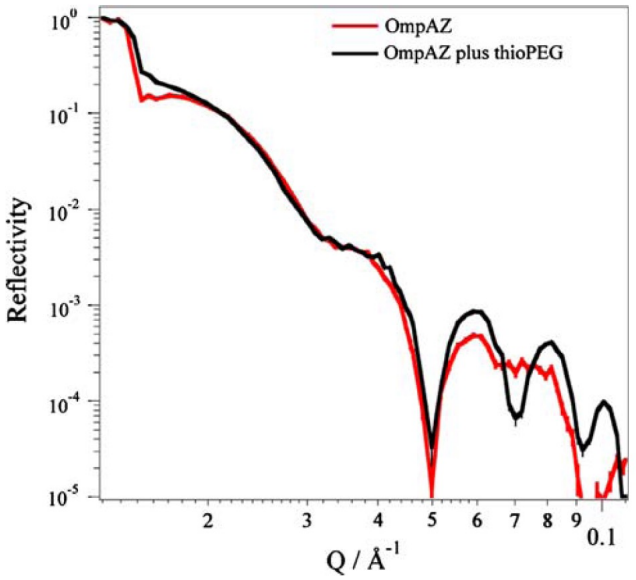


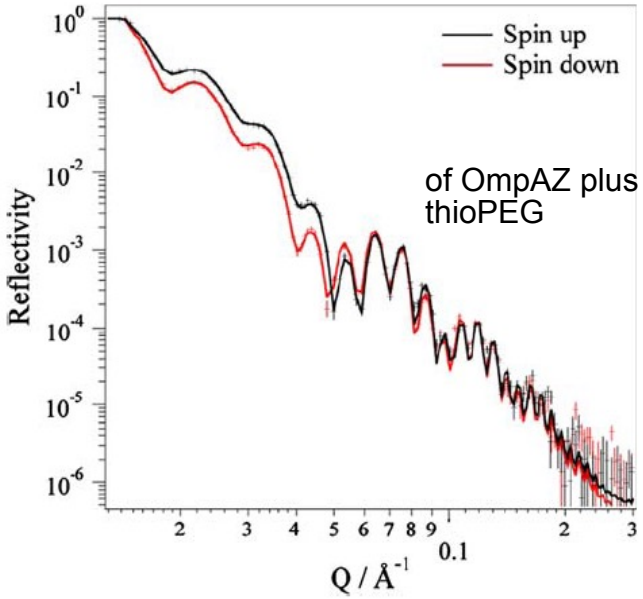




Magnetic Contrast

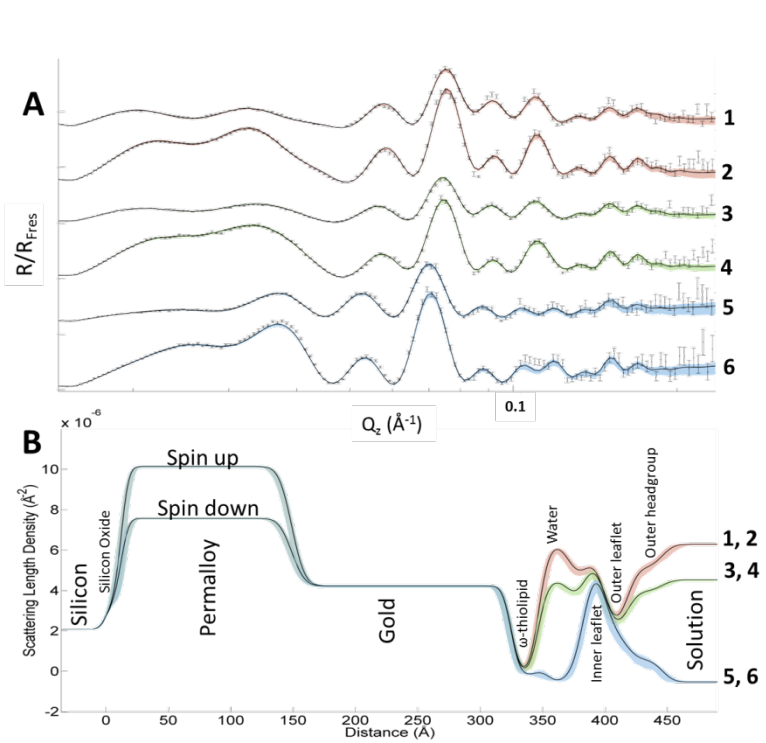


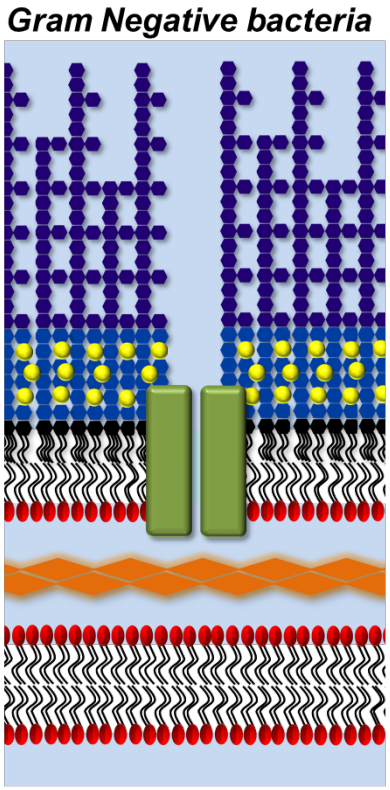


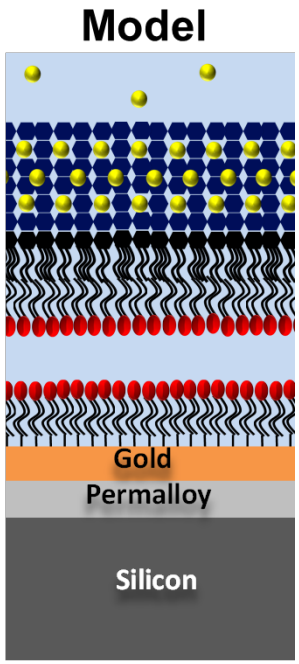


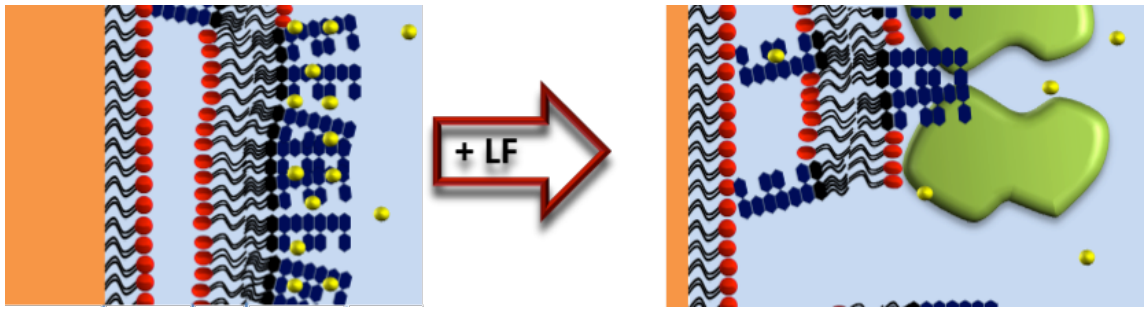
- Polarised neutron reflection used to probe the structure of an antibody on gold (separated by a thioPEG monolayer).
- Polarised neutrons are used as this provides a means of achieving extra contrast in samples having a magnetic metal layer (Fe or Ni) under the gold surface.
- This contrast is attained without resorting to hydrogen/deuterium exchange in the biological layer.

A.P. Le Brun et al Eur. Biophys. J. (2008) 37, 639



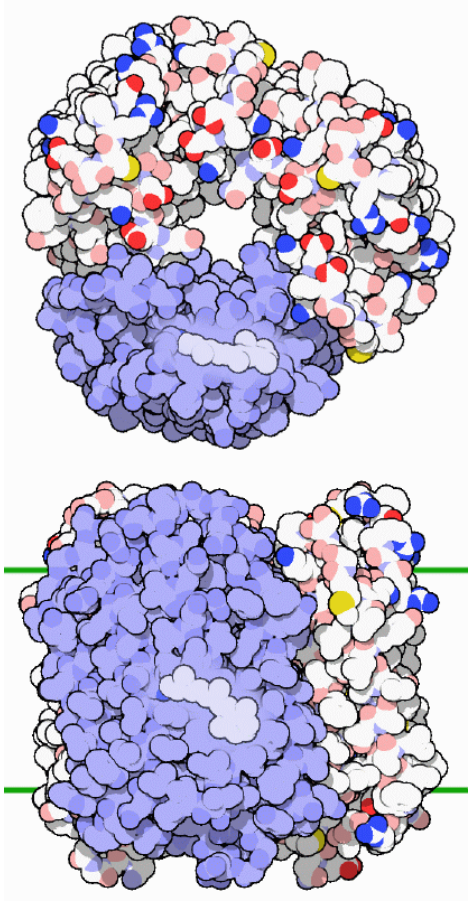


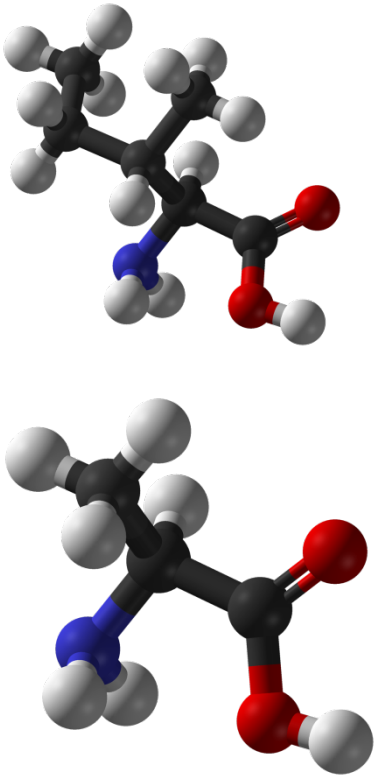


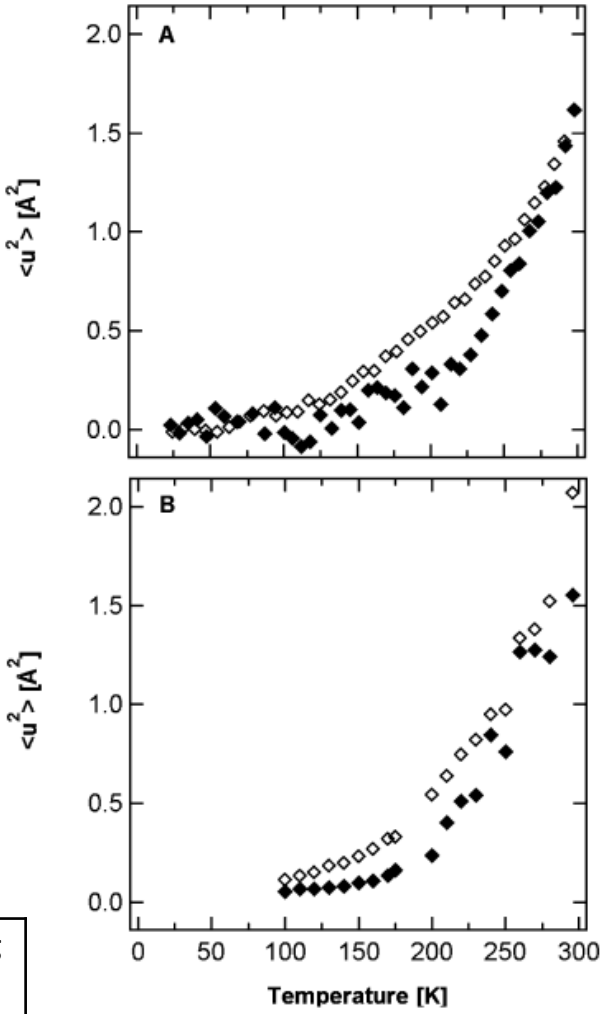


Clifton et al Angew. Chem. Int. Ed. (2015)

Sample deuterium labelling is often required : QENS







Element	Coherent Scattering Length (b_{coh})/ 10^{-5} Å	Incoherent Scattering Length (b_{inc})/ 10^{-5} Å
Hydrogen	-3.74	25.274
Deuterium	6.671	4.04

Wood et al, JACS, 2010, 4990-4991

Conclusions

- Isotopic Labelling is a powerful tool to examine complex biological structures with NS

- Solution labelling is by far the easiest way to match out components of a complex.

- Deuterium labelling of the samples is often require – if so label the cheapest part!

- Checks should be made to ensure labelling does not change the physiochemical properties of the samples.

Further Reading

- **Small angle neutron and X-ray scattering in structural biology, recent examples from the literature, 2008, Cameron Neylon, Eur Biophys J, DOI 10.1007/s00249-008-0259-2.**

- **Neutrons for biologists: a beginner's guide, or why you should consider using neutrons., 2009, J. Lakey, J. R. Soc. Interface, 6, Supp 5, S567-73.**

- **Examining protein-lipid complexes using neutron scattering. L Clifton, C. Neylon and J. H. Lakey, Lipid-Protein Interactions, Methods in Molecular Biology, 2013.**

- **Small Angle X-ray and Neutron Scattering from Solution of Biological Macromolecules, D. I. Svergun, M. H. Koch, P. A. Timmins, R. P. May.**

- **Small-angle scattering for structural biology-Expanding the frontier while avoiding the pitfalls. David Jacques and Jill Trewhella. Protein Science, 19, 642-657**