Australian Government

ANSTO

Biomolecules

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Science. Ingenuity. Sustainability.

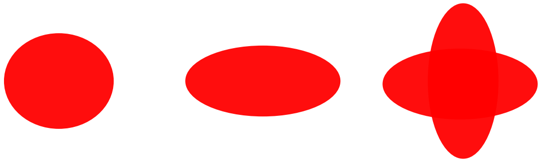
SMALL ANGLE SCATTEIRNG

Small Angle Scattering at ANSTO

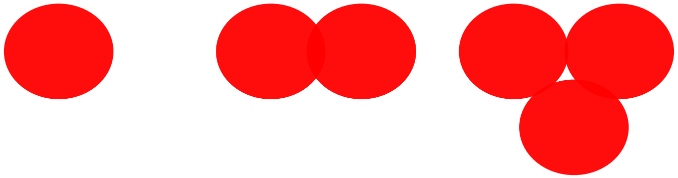
SAXS here!

Questions we answer:

Shape?

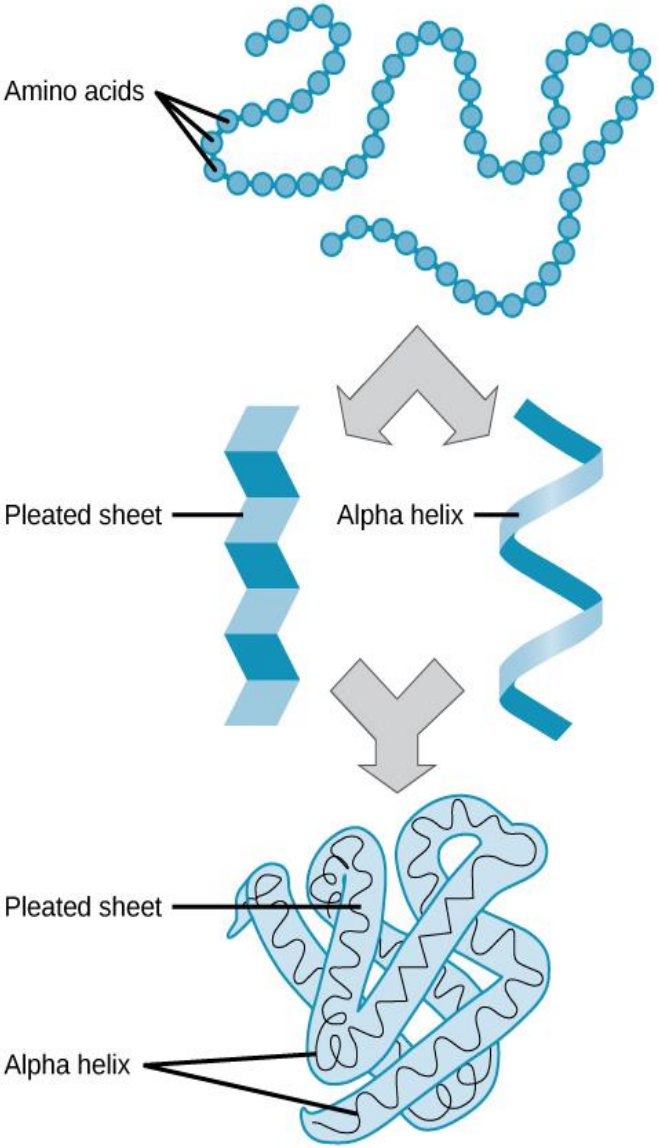


Association/Interactions?



SANS data over four decades in Q (KOOKABURRA & QUOKKA) = characterising lengths from 1nm to 10 micron

Proteins



Primary Protein structure
sequence of a chain of animo acids

Secondary Protein structure
hydrogen bonding of the peptide backbone causes the amino acids to fold into a repeating pattern

Tertiary protein structure
three-dimensional folding pattern of a protein due to side chain interactions

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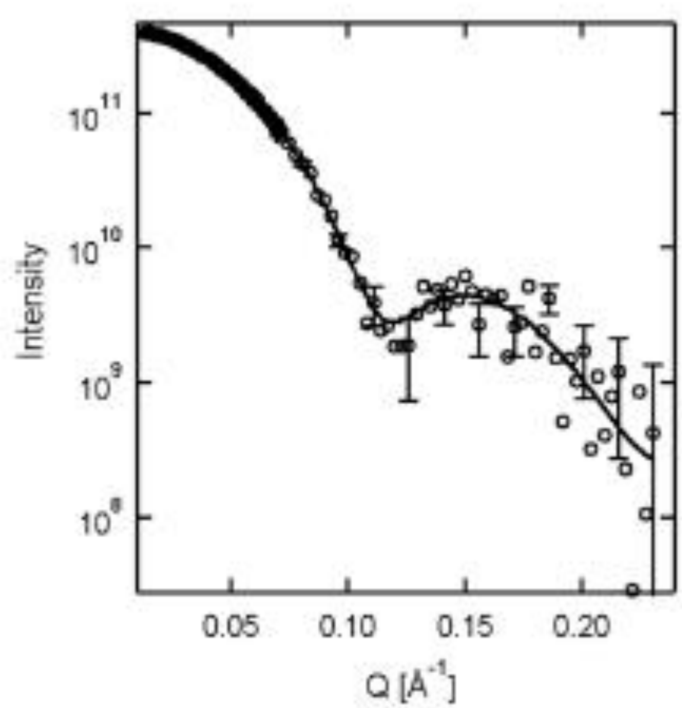
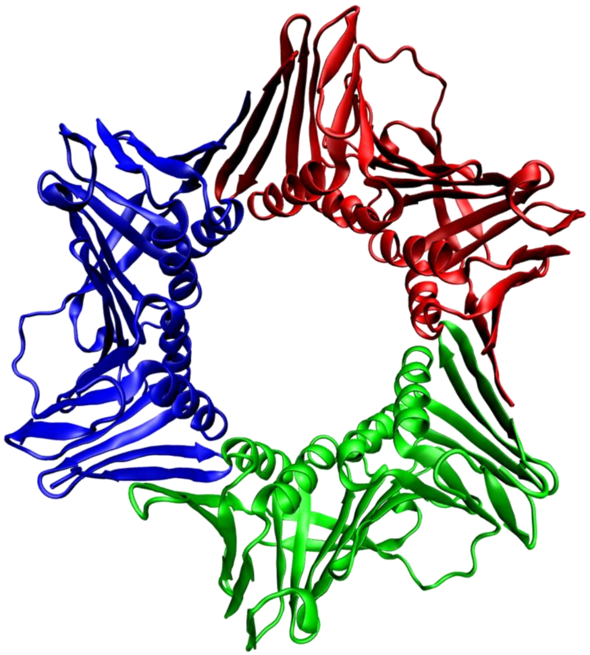
Proteins seen by small angle scattering



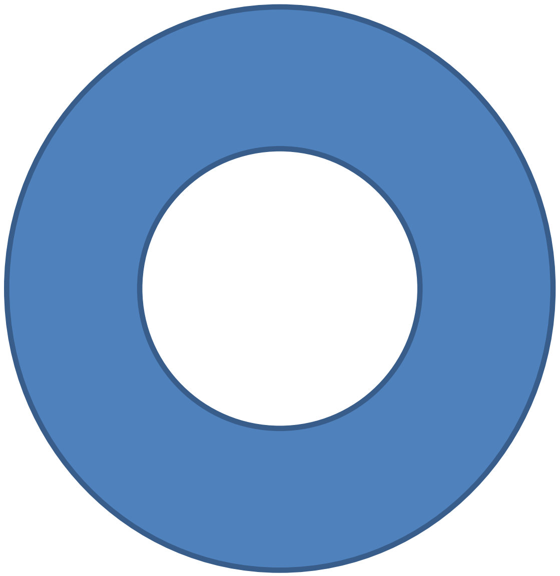
Typical small angle scattering data

Structure reconstruction from small angle data – DAMMIF from ATSAS suite

Proteins seen by small angle scattering



Protein 'seen by' X-ray crystallography



Protein 'seen by' Small Angle Scattering

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Why do protein small angle scattering?

- Is the crystal structure representative?
- What is the oligomerisation state?
- Do proteins interact in solution?
- What happens when we change conditions (eg salt, ligand, temperature, pH…)

X-rays & Neutrons

- Flux orders of magnitude X-rays>> neutrons
- Neutrons: no radiation damage, contrast variation

Before you start: Jeffries et al. (Nat Protoc (2016) 11(11): 2122-2153)
Trewhella et al. (Acta Cryst D. (2017) 73 (9):710-728)

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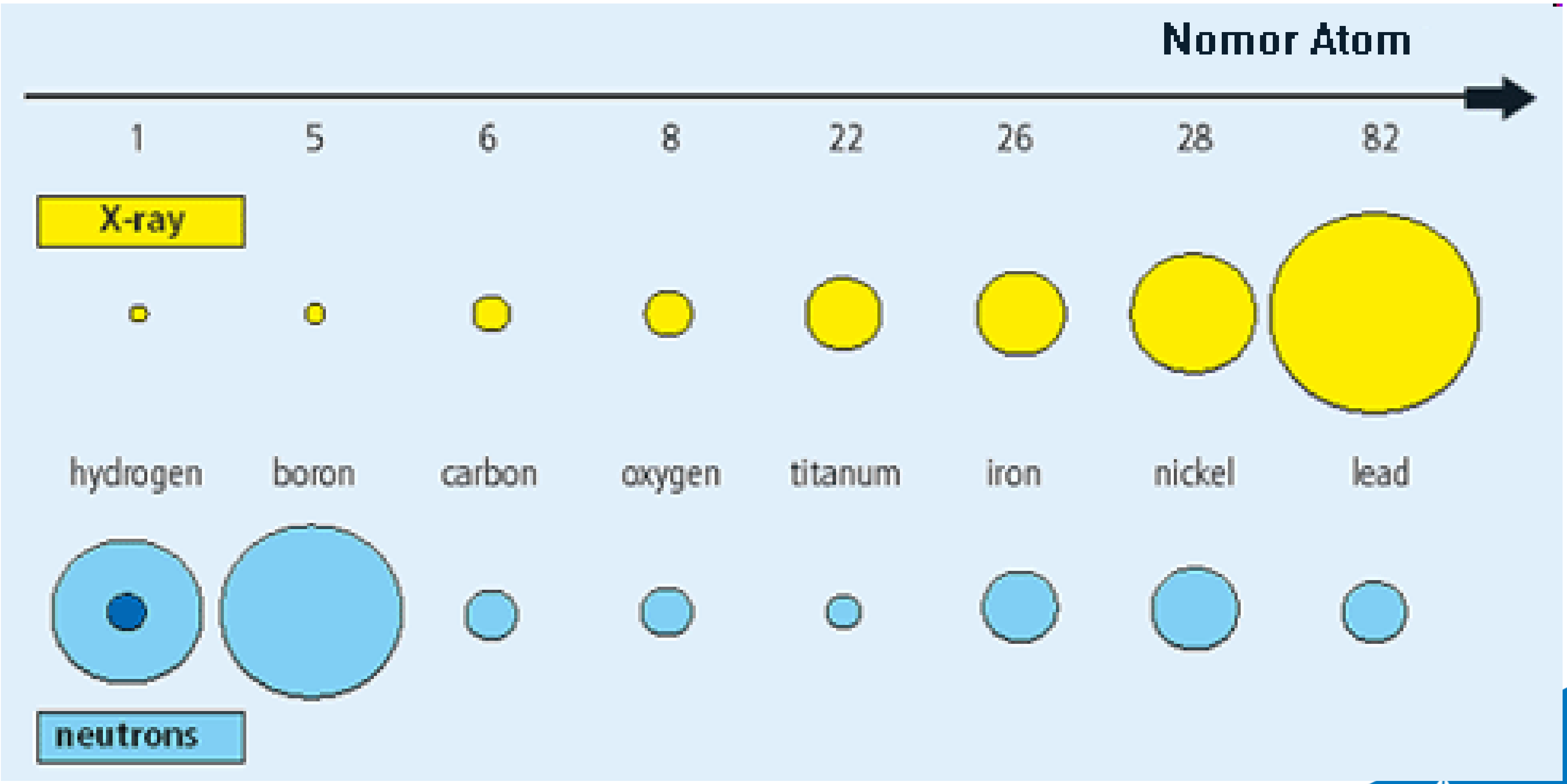
Neutrons / X-rays Scattering Lengths

Interact with nucleus not atomic cloud



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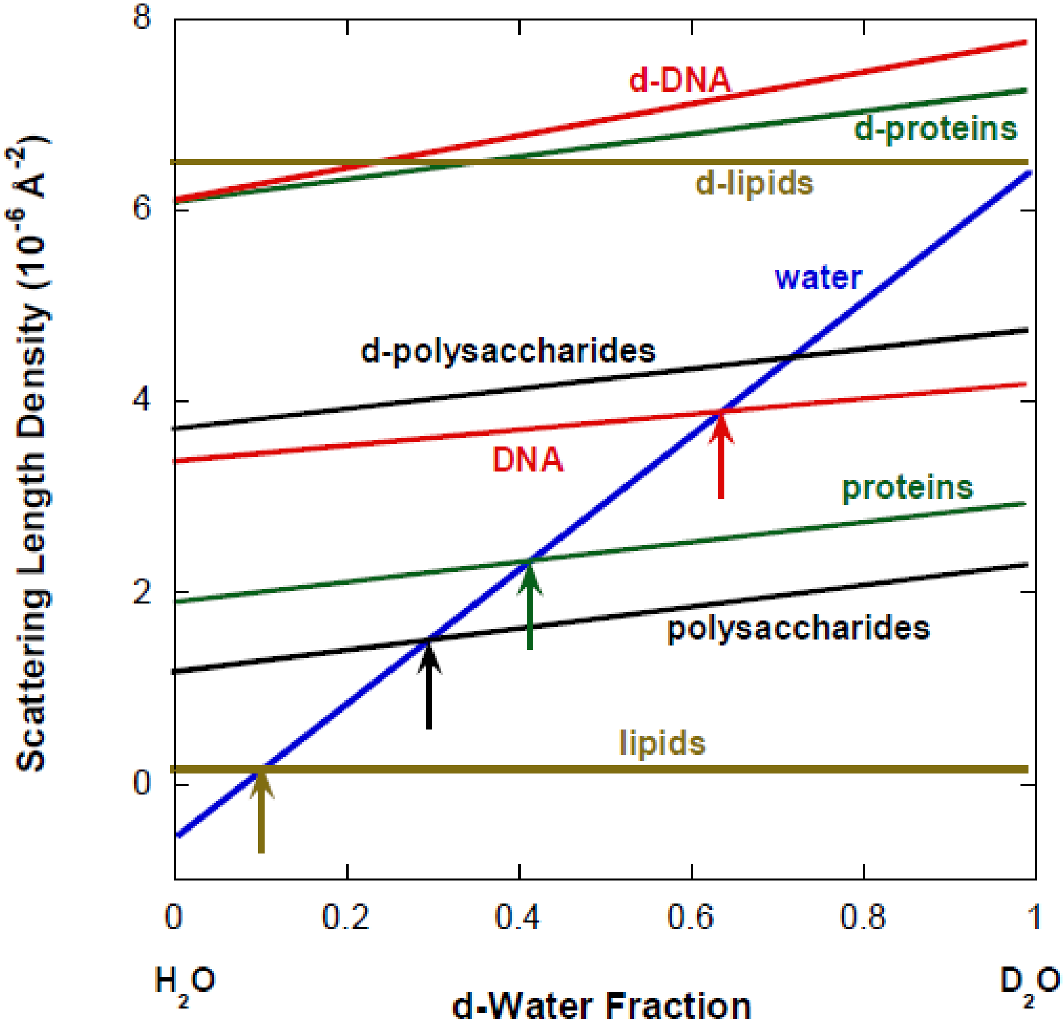
Optical contrast

Neutrons Scattering Length Densities & Contrast



Scattering Length Density Solvent

Contrast factor

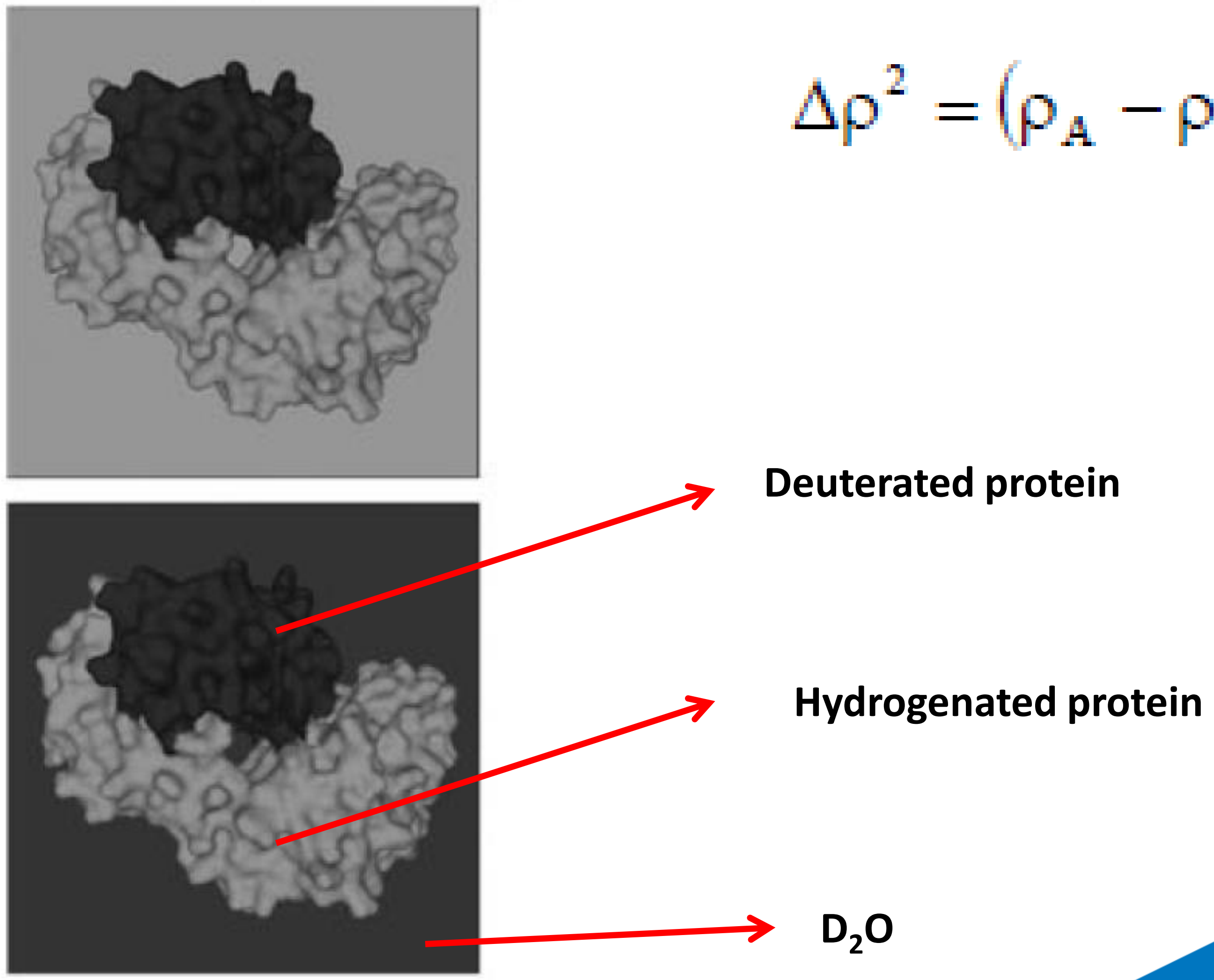
$$\Delta\rho^2 = (\rho_A - \rho_B)^2$$

Scattering Length Density Particle

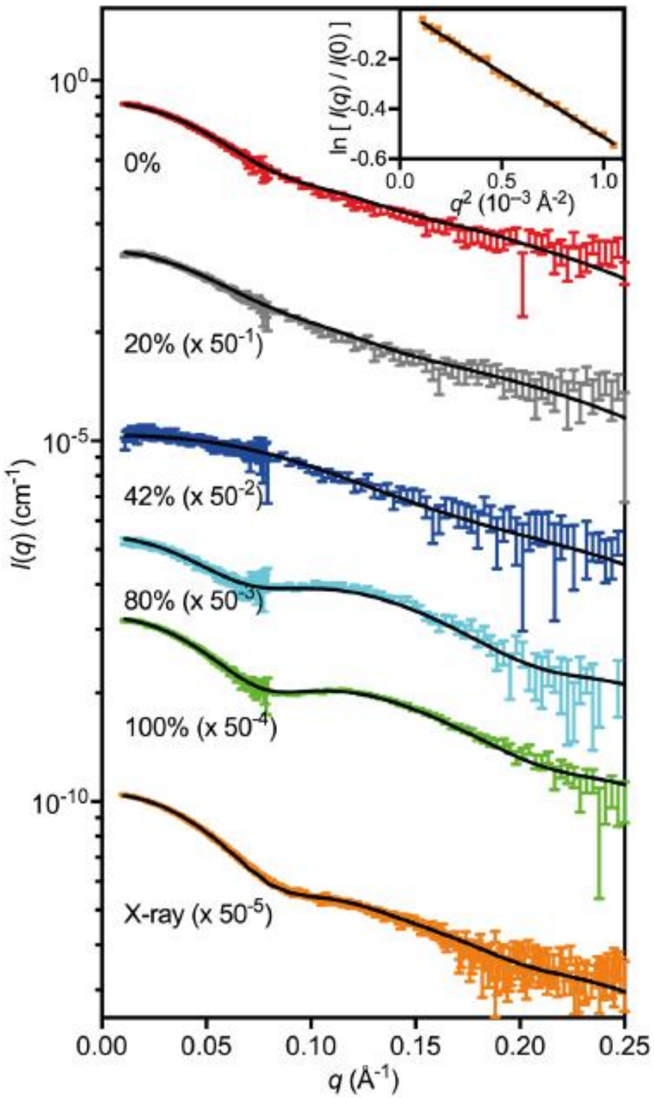
Mulch! Whitten et al. (J Appl Crystallogr 2008 41, 222-226)

Neutrons & ‘contrast matching’

$$\Delta\rho^2 = (\rho_A - \rho_B)^2$$



Studying protein interactions – SANS & Contrast Matching

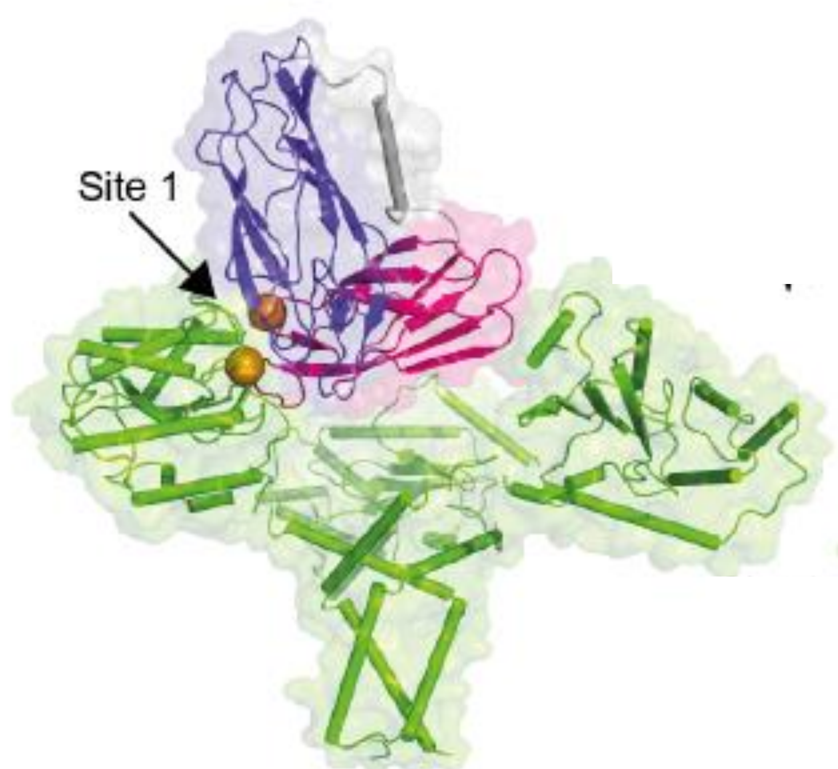


H_2O

↓

D_2O

ATSAS package – EMBL Hamburg



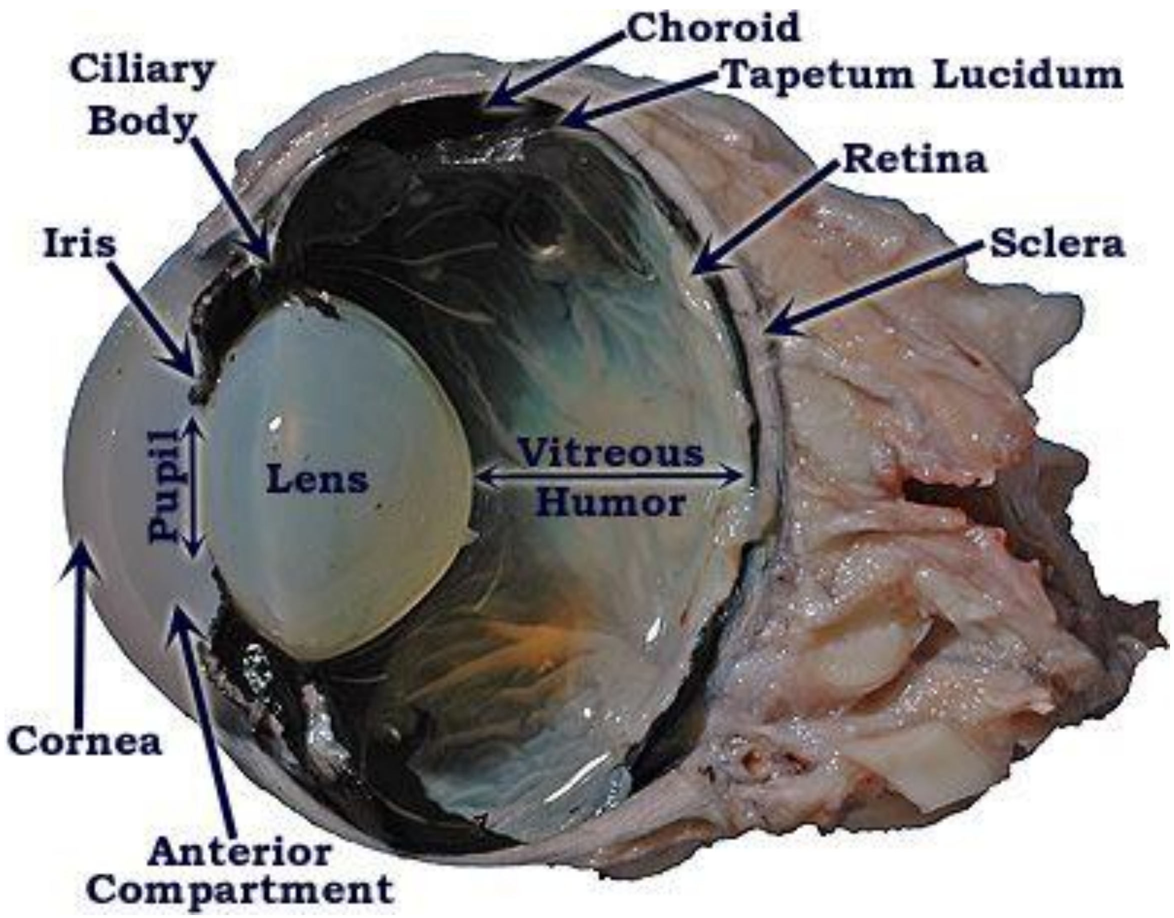
Furlong *et al.* J Biol Chem (2018)

Example of kinetic protein SANS

Masaaki Sugiyama

Rintaro Inoue

α-Crystallin – structural protein of mammalian eyes



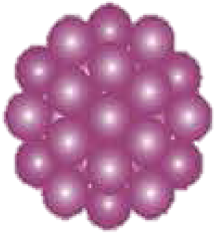
Schematic view of α-B-crystallin 26-mer:

Subunit exchange by contrast matching

Hydrogenated α-B-crystallin

Invisible in 40% D_2O

Deuterated α-B-crystallin



Invisible in D_2O

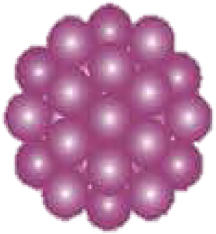
Inoue *et al.* (2016) Nat Sci Reports **6**: 29208

Subunit exchange by contrast matching

Hydrogenated α-B-crystallin

+

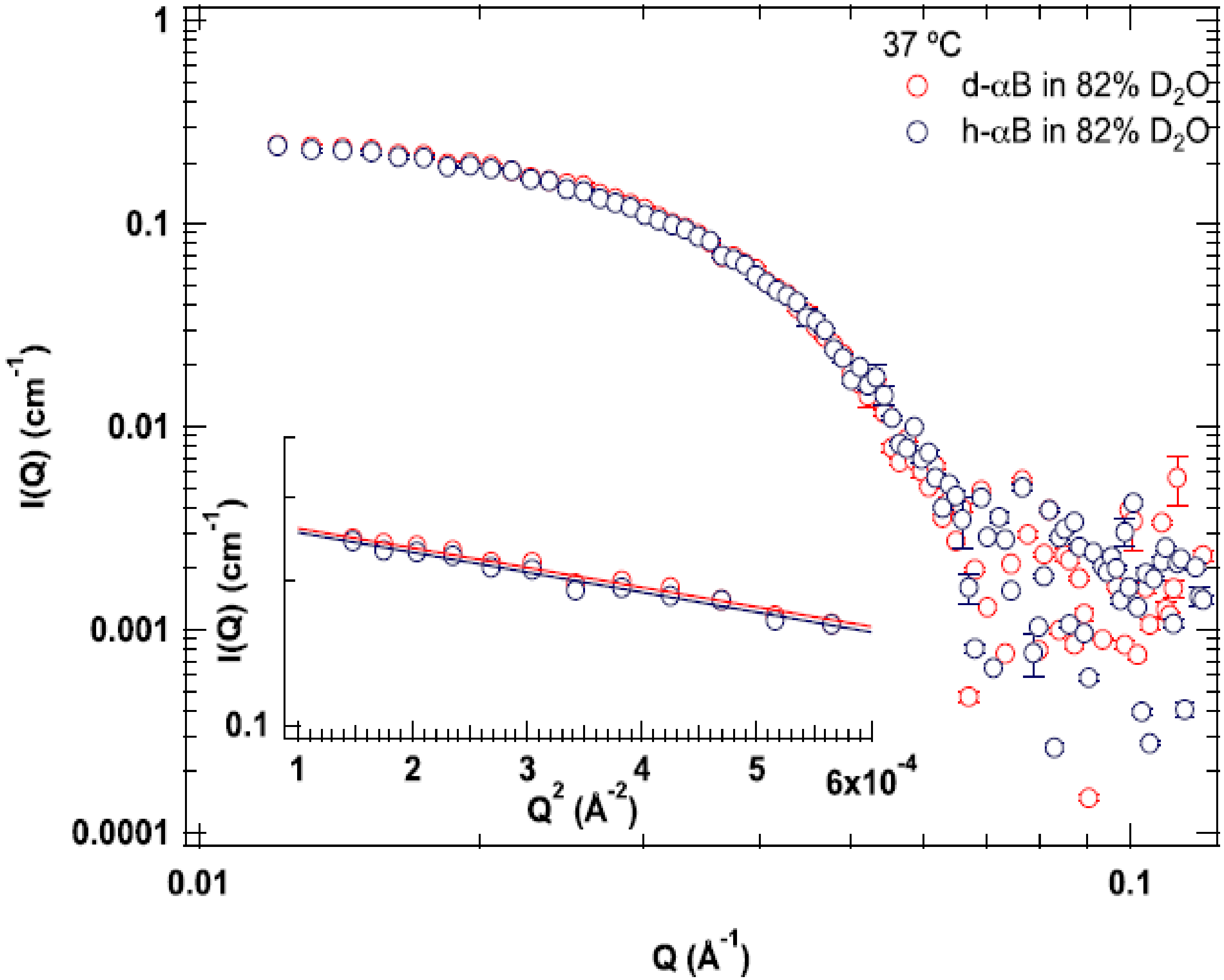
Deuterated α-B-crystallin

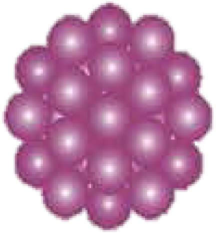


50/50 mixture matched out at 82% D_2O

Inoue *et al.* (2016) Nat Sci Reports **6**: 29208

Hydrogenated & deuterated a-B crystallin





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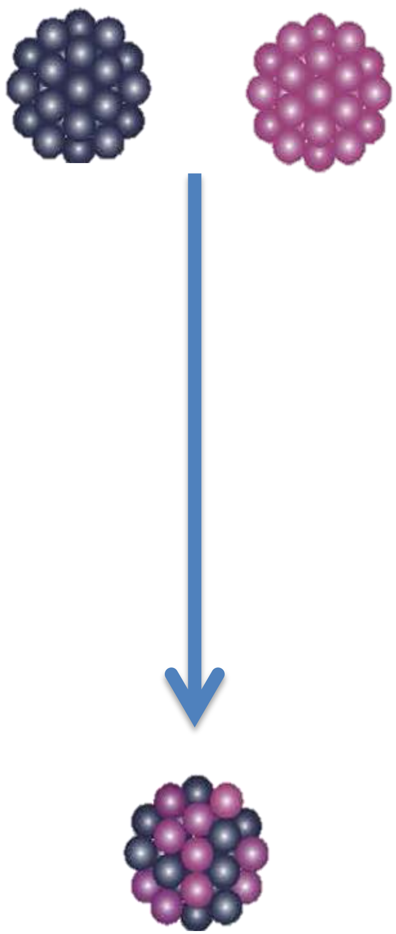
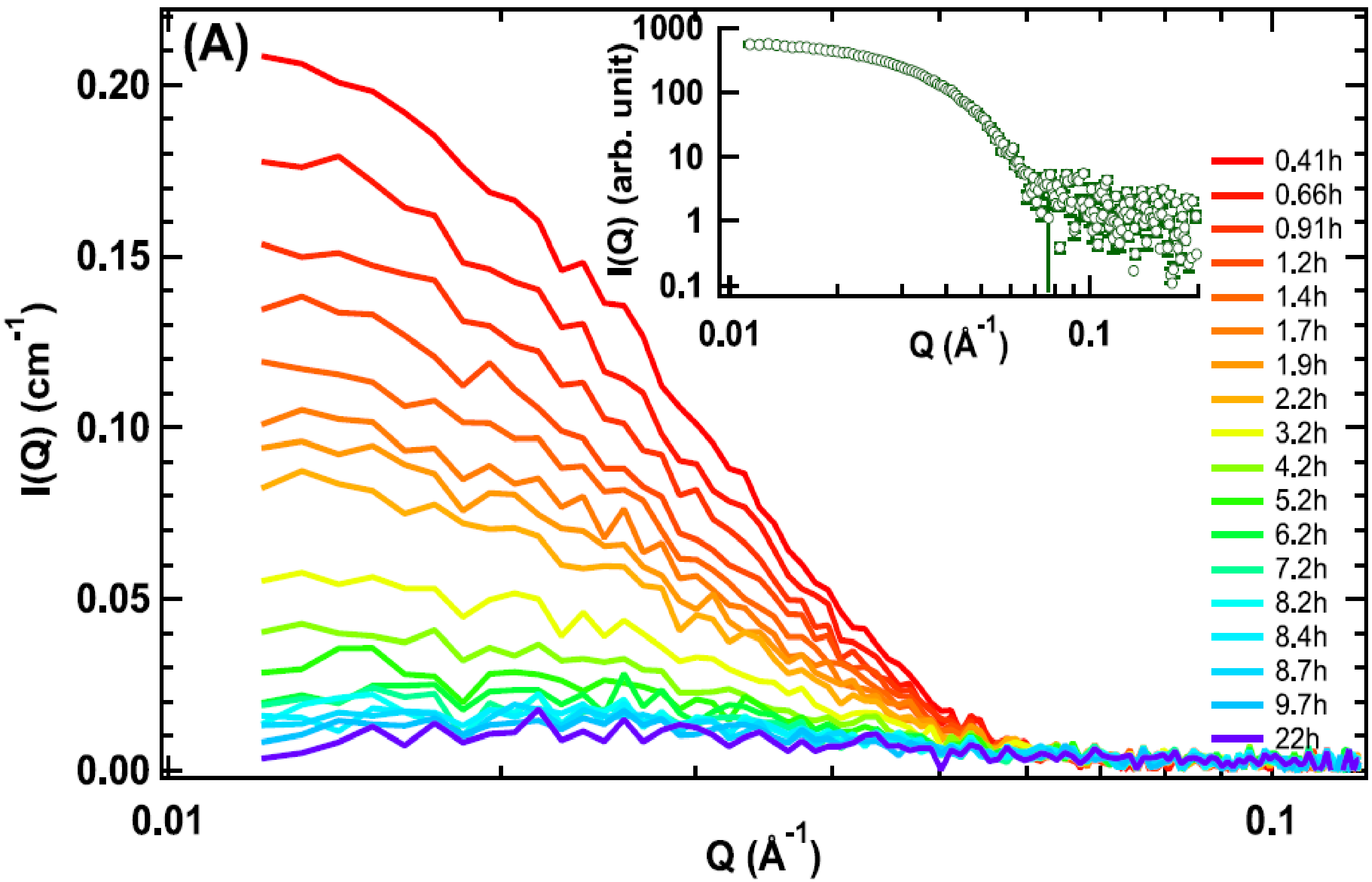
Time resolved Small Angle Neutron Scattering (QUOKKA)





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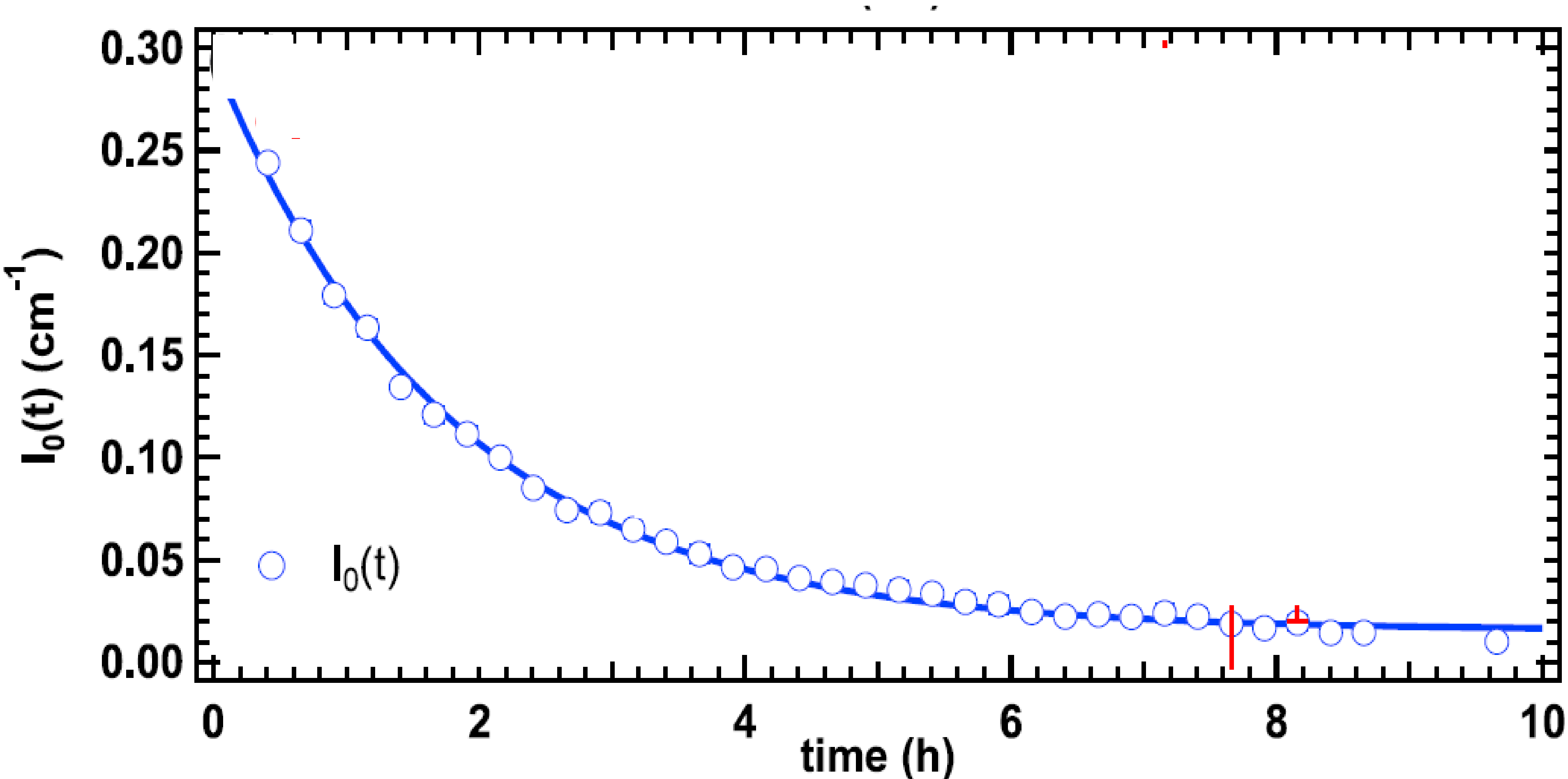
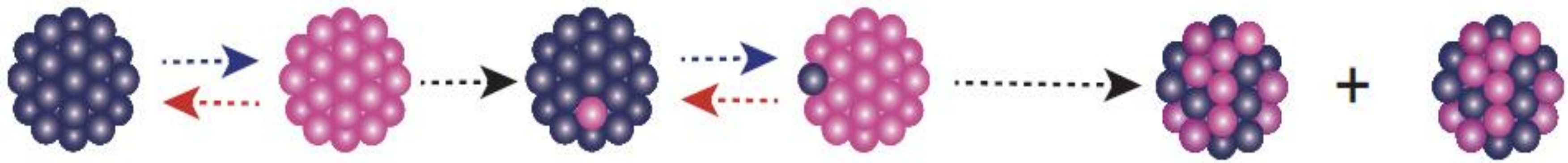
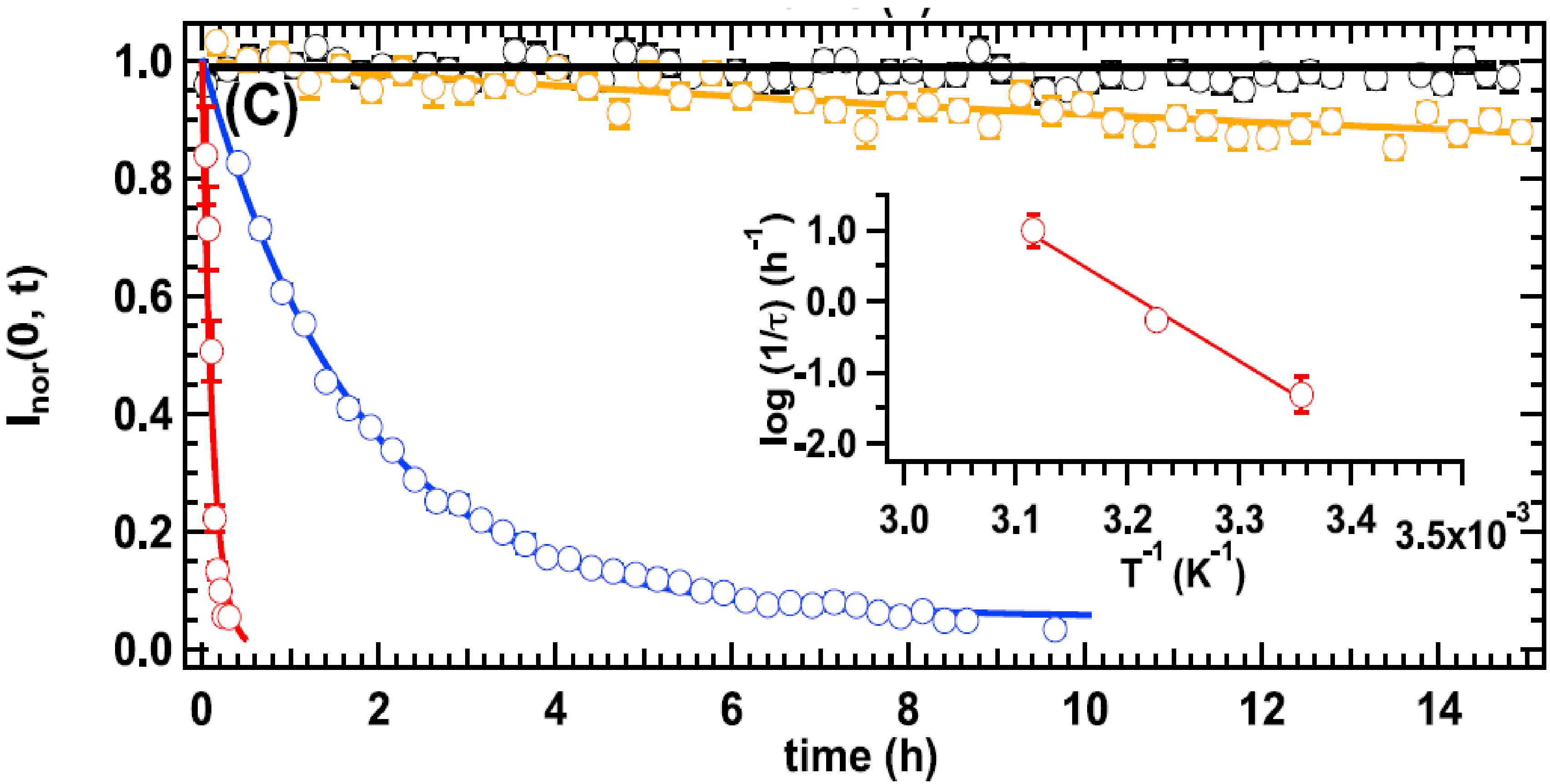
Time dependence of exchange

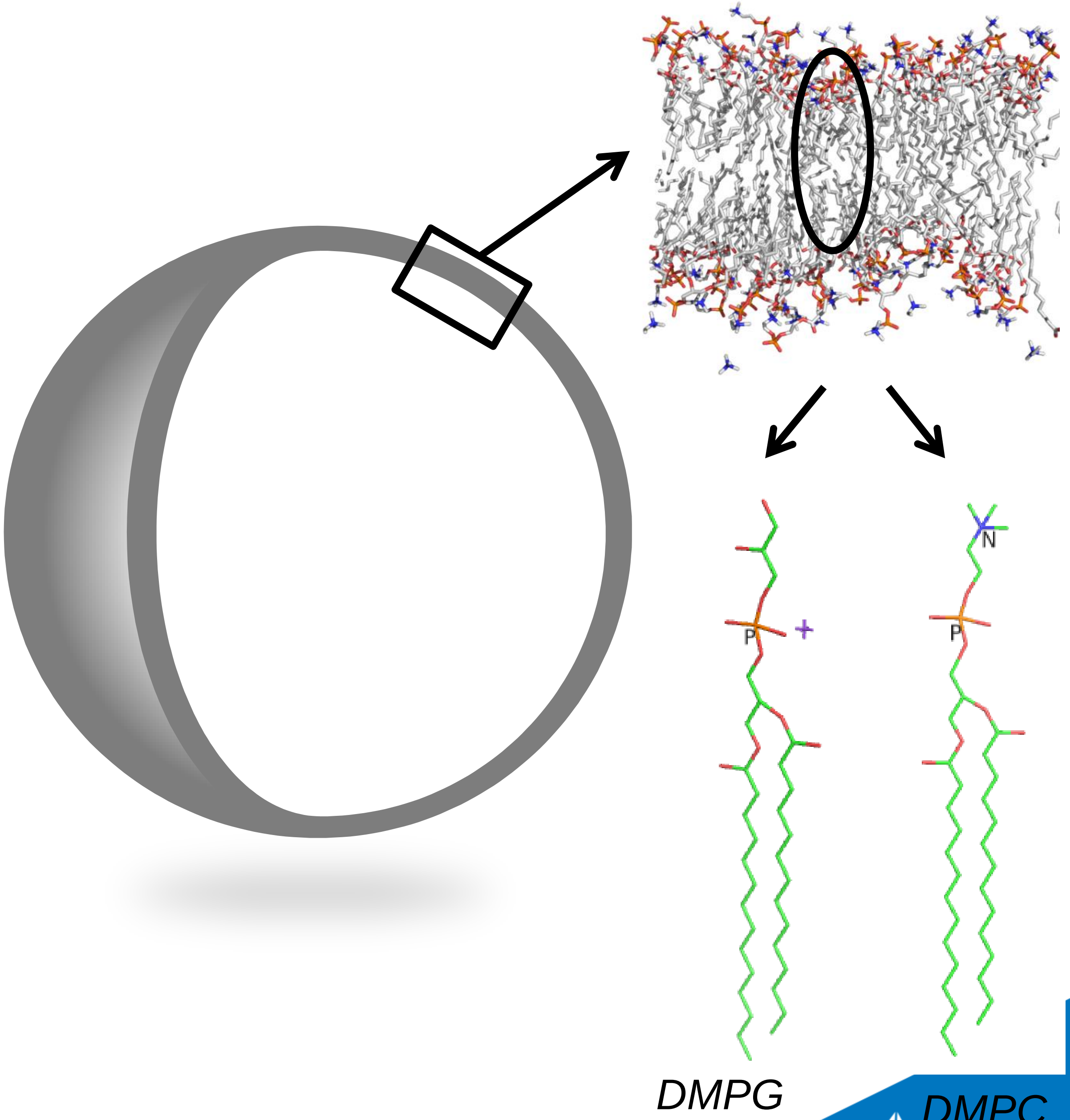


Mechanism of subunit exchange?

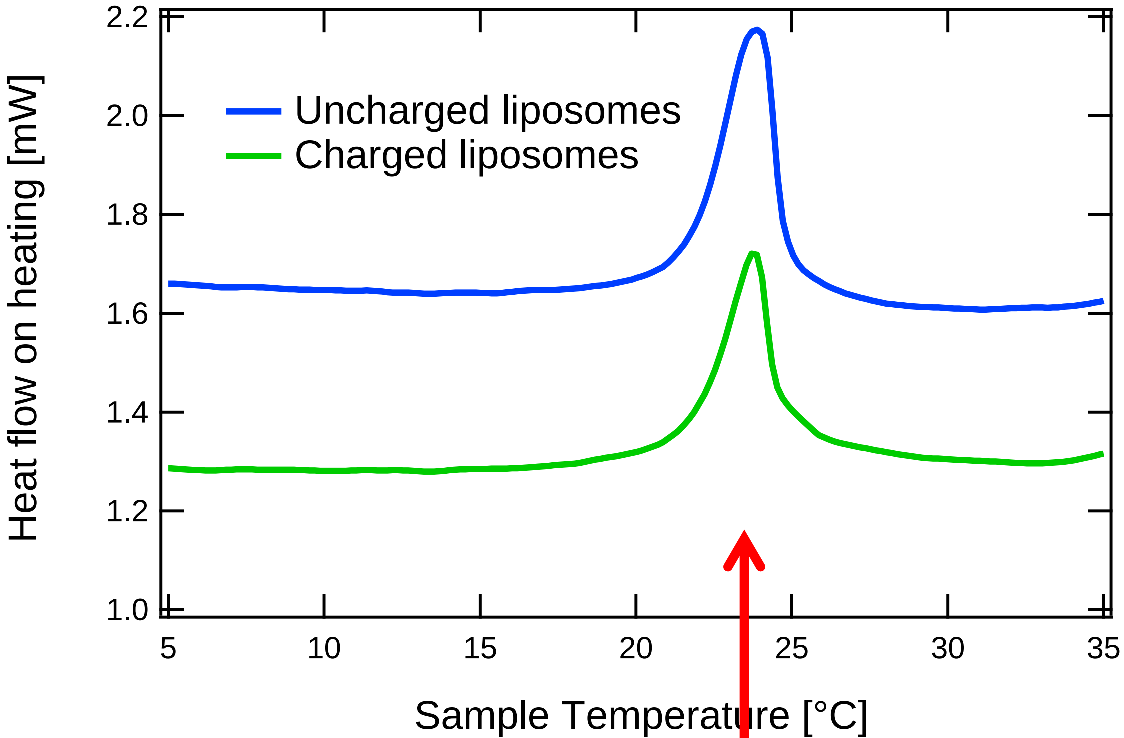




Liposomes

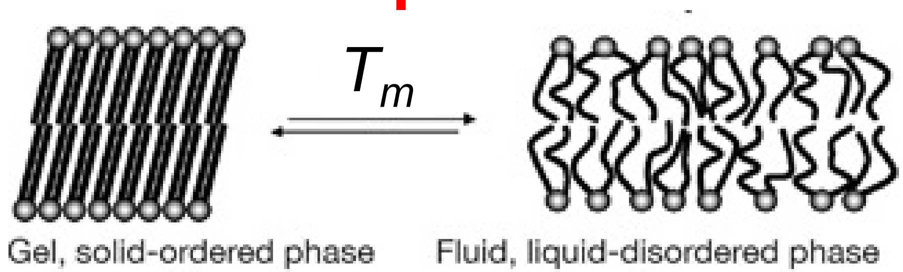


Determining T_m: Differential Scanning Calorimetry

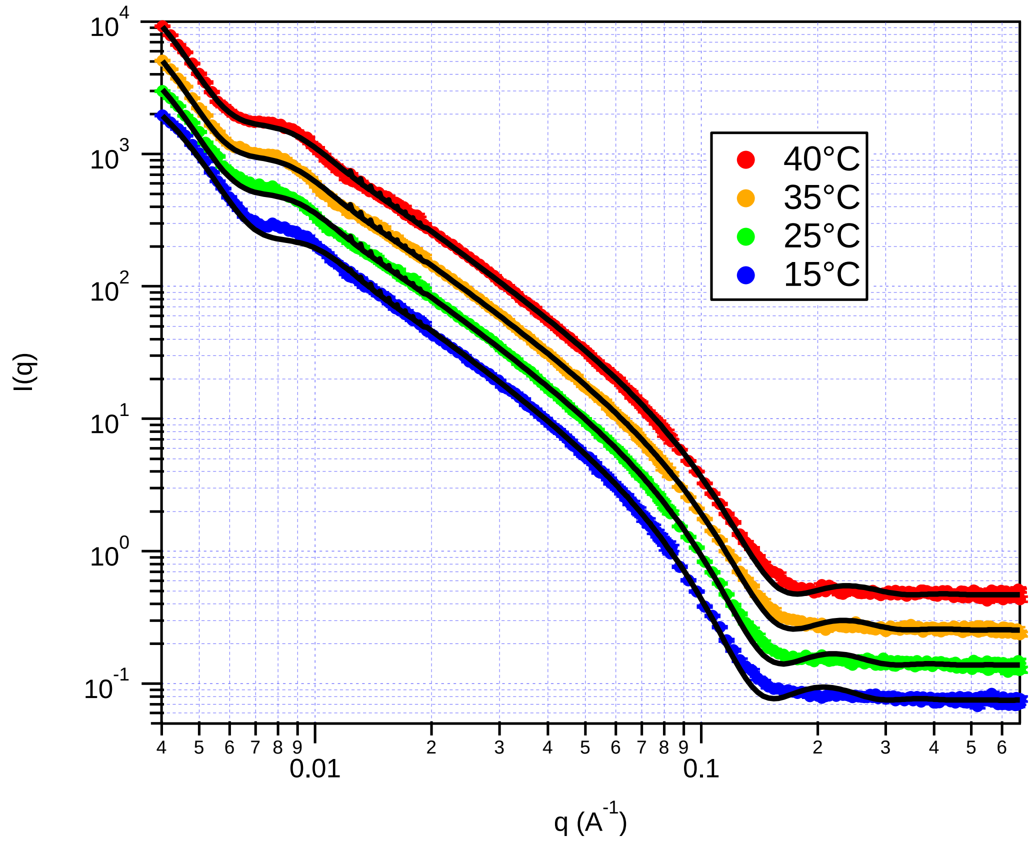


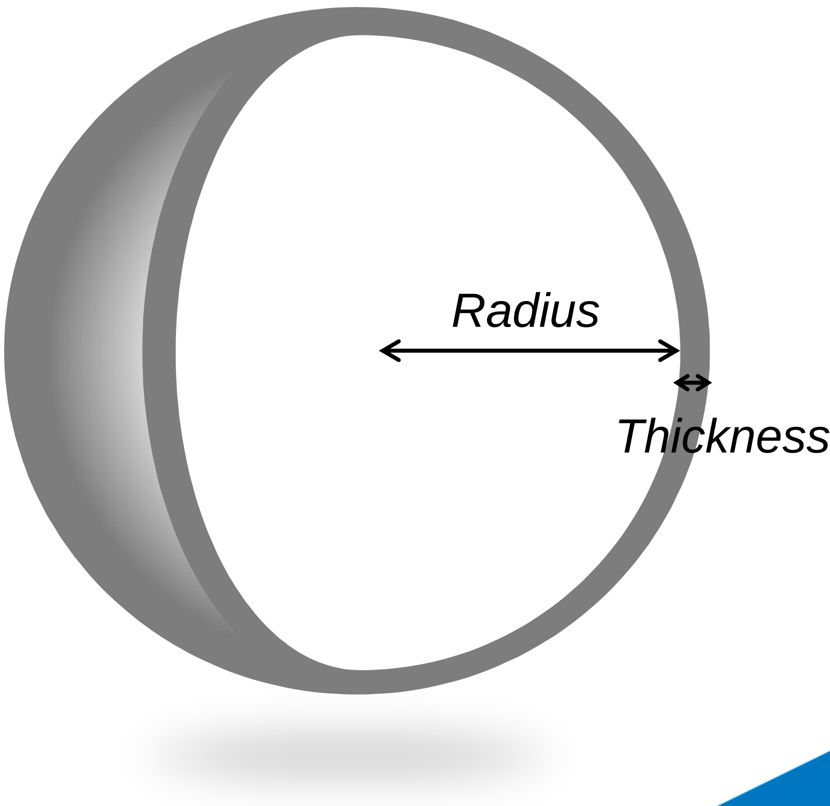
2 types of liposomes prepared:

- Uncharged (90% DMPC, 10% DMPG)
- Charged (50% DMPC, 50% DMPG)



SANS results – ‘Uncharged’ liposomes





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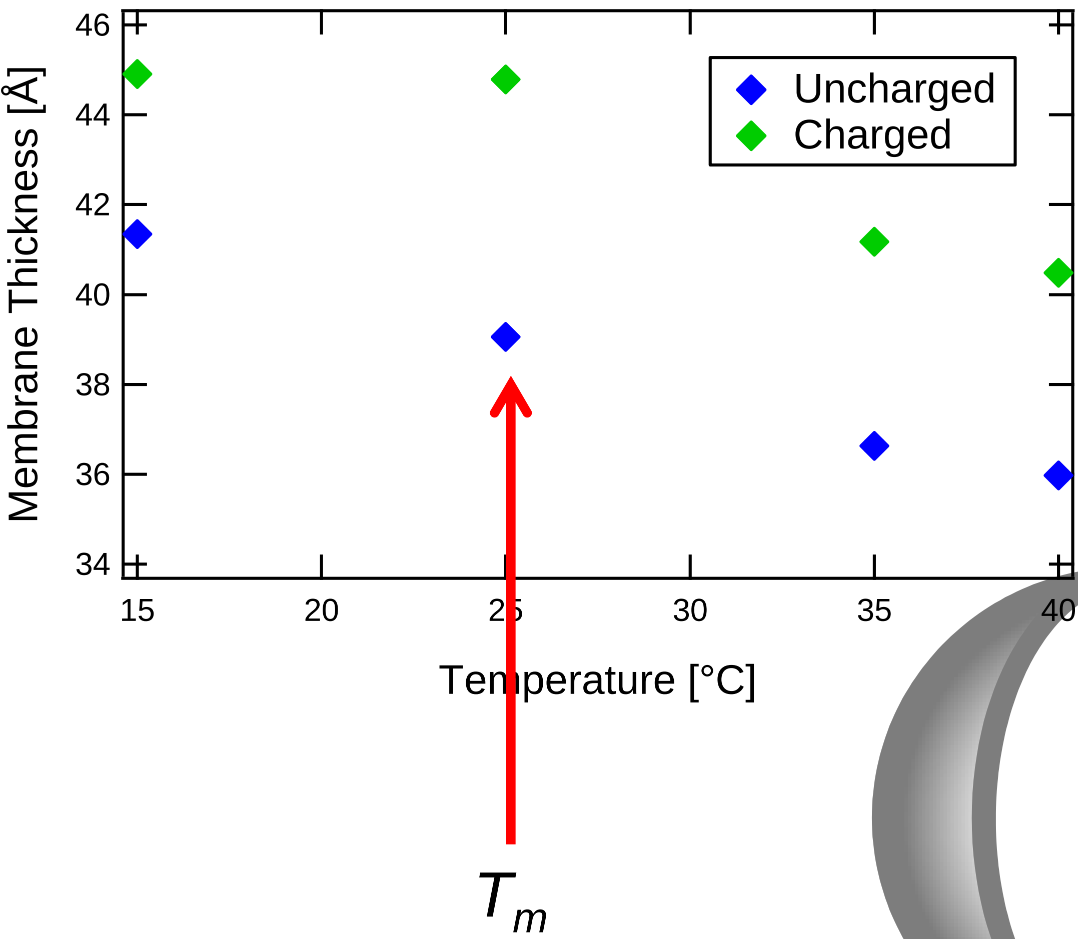
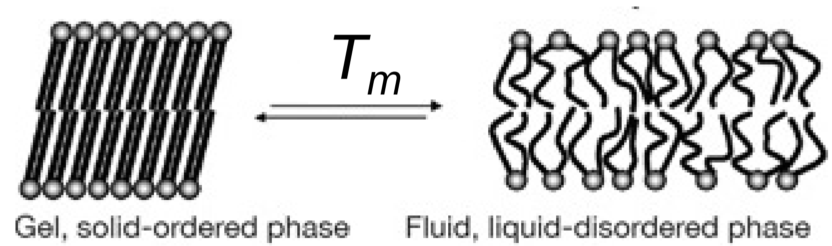
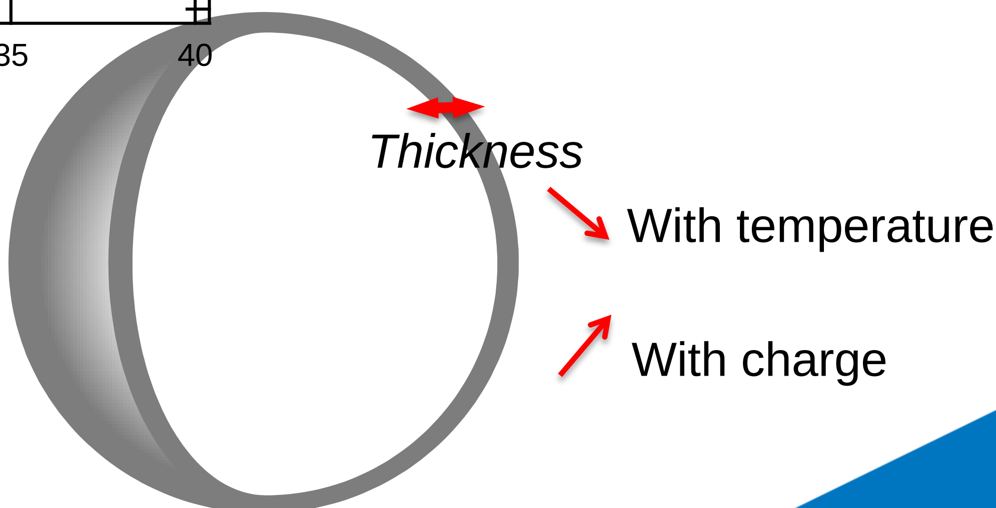
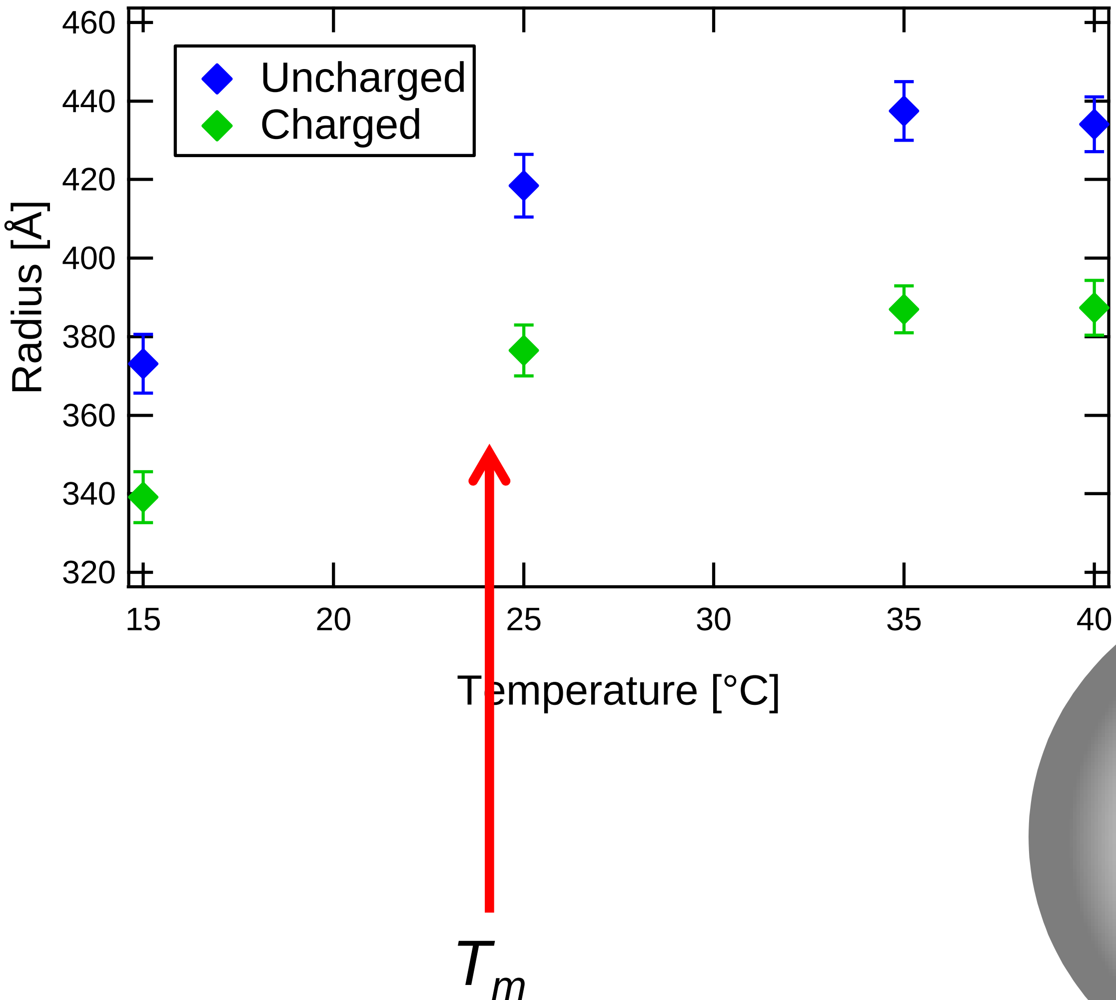
Membrane thickness from SANS data

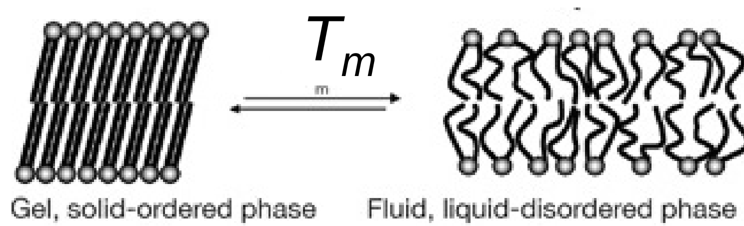


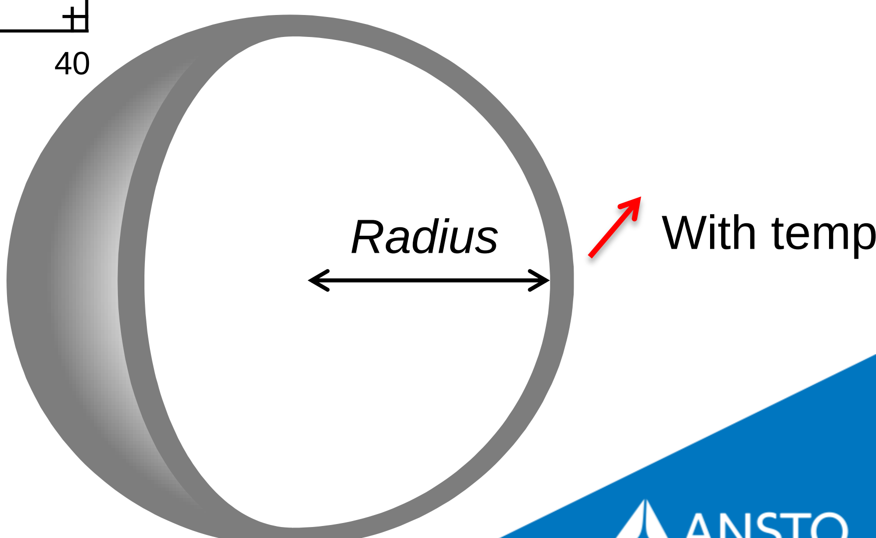




Liposome radius from SANS data







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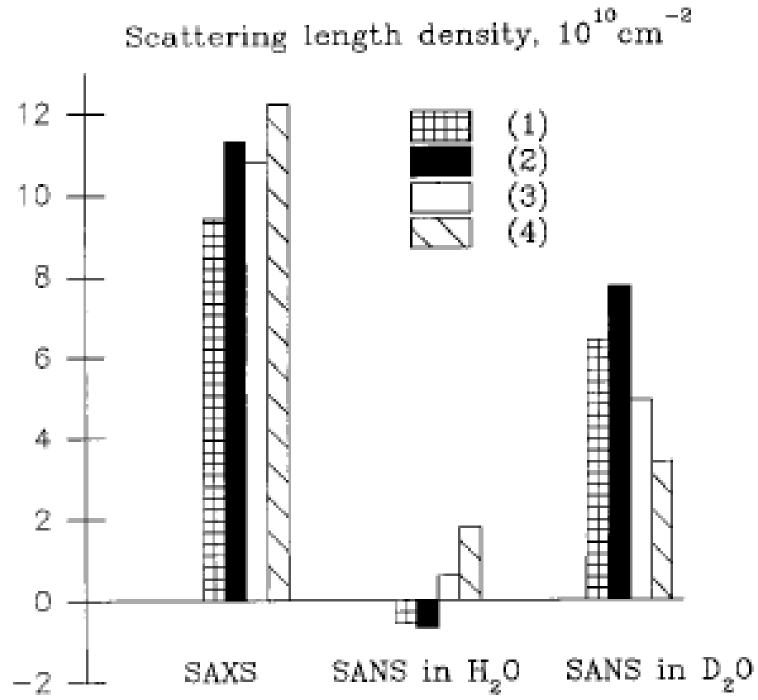
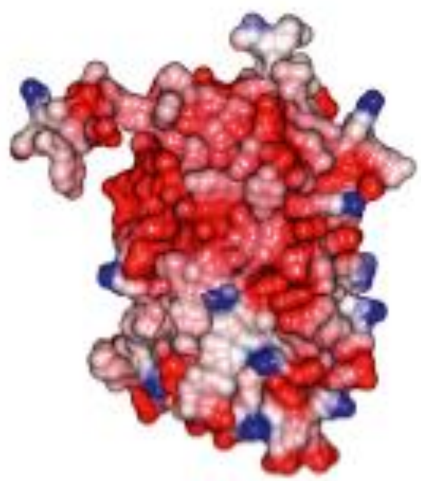
Hydration water



Svergun et al. PNAS 95 1998 2268-2272

SAXS, SANS in H_2O, SANS in D_2O
-> proteins have a hydration shell 5-20 % more dense than bulk



Kim et al. Biophys J 110 (2016) 2185-2194

REFLECTIVITY

Acknowledgments

Monash University:

Mei-Ling Han (AINSE PGRA)
Hsin-Hui Shen
Seong Hoong Chow
Jian Li
Tony Velkov

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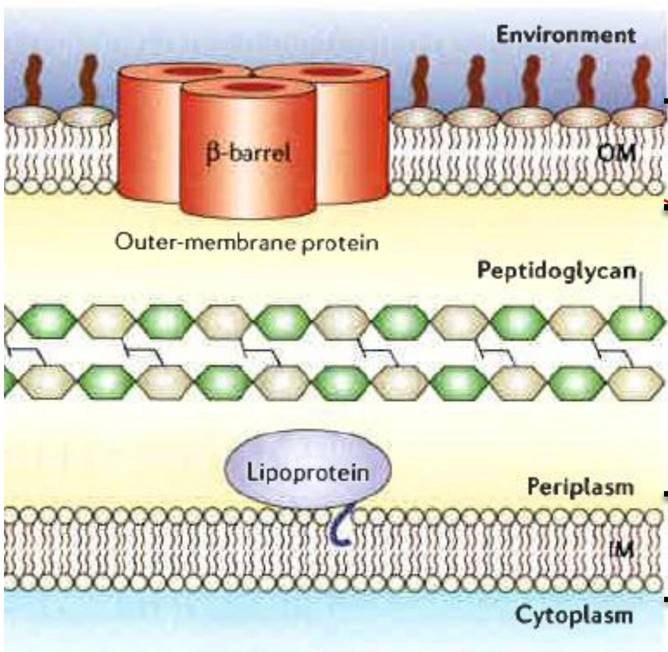
Anton le Brun

Publications:
M.-L. Han et al., *ACS Chemical Biology* (2018) **13:** 121

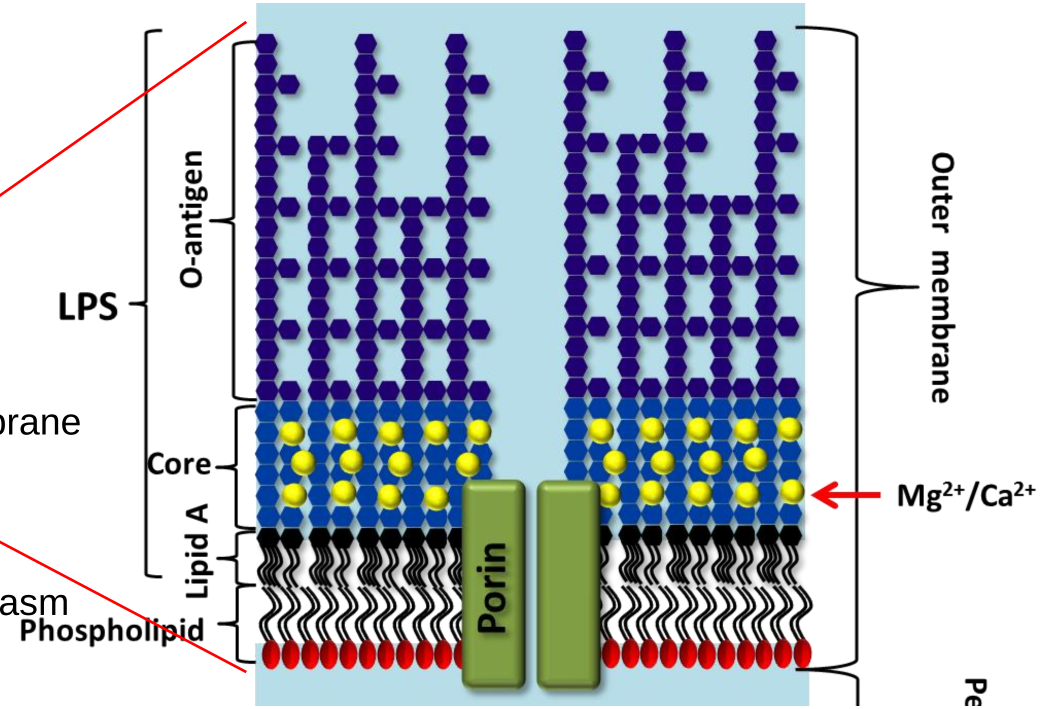
The Gram-negative cell envelope and the outer membrane



Outer membrane

Periplasm

Inner membrane



From: LA Clifton et al, *Angewandte Chemie International Edition* 2015, **54**: 11952-11955

From: N Ruiz, D Kahne & TJ Silhavy *Nature Reviews Microbiology* 2006, **4**:57-66

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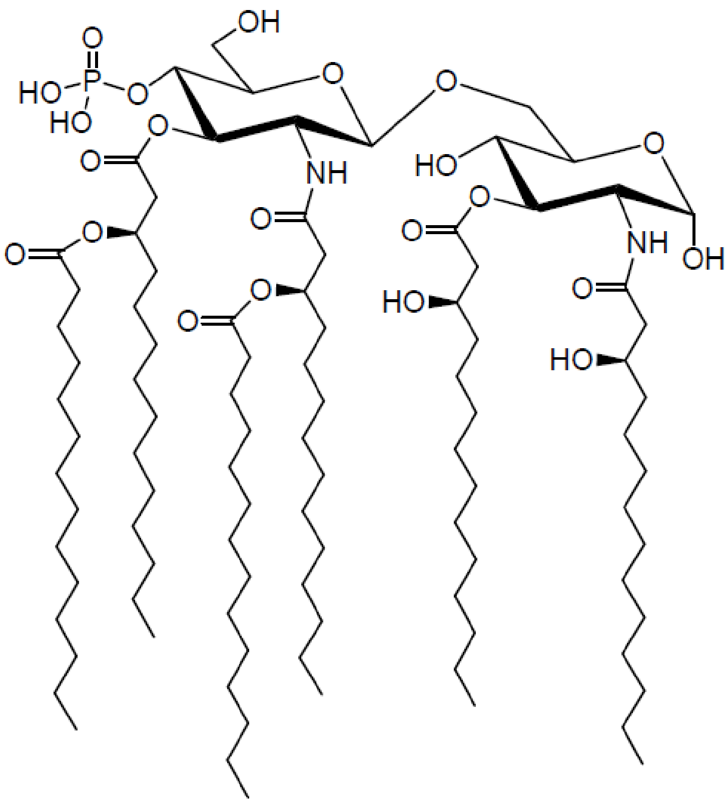
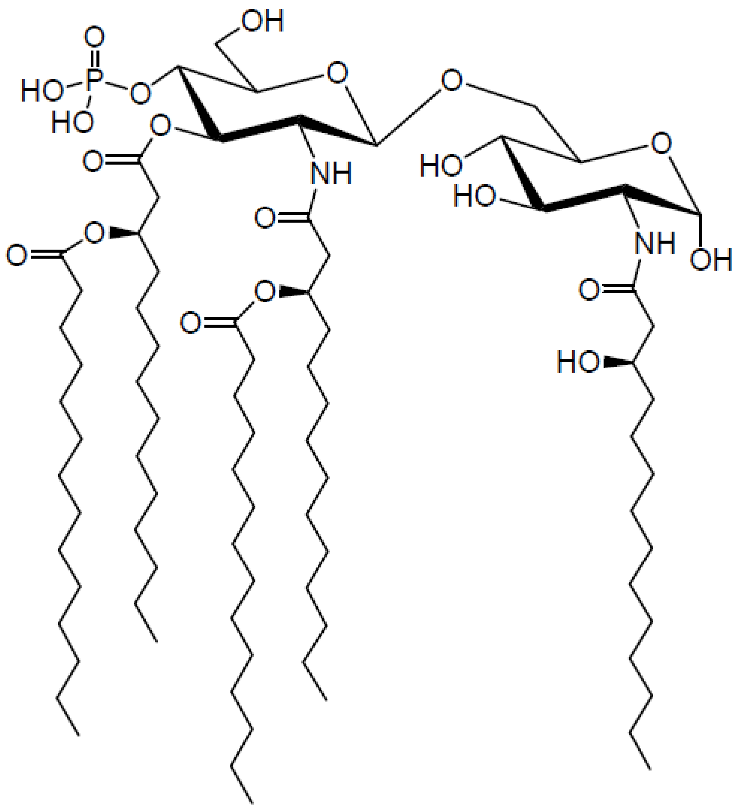
Lipid A



Hexa-acylated lipid A



Penta-acylated lipid A
Deacylation mediated by *pagL* in the Gram-negative bacterium *Pseudomonas aeruginosa* confers Polymyxin B resistance

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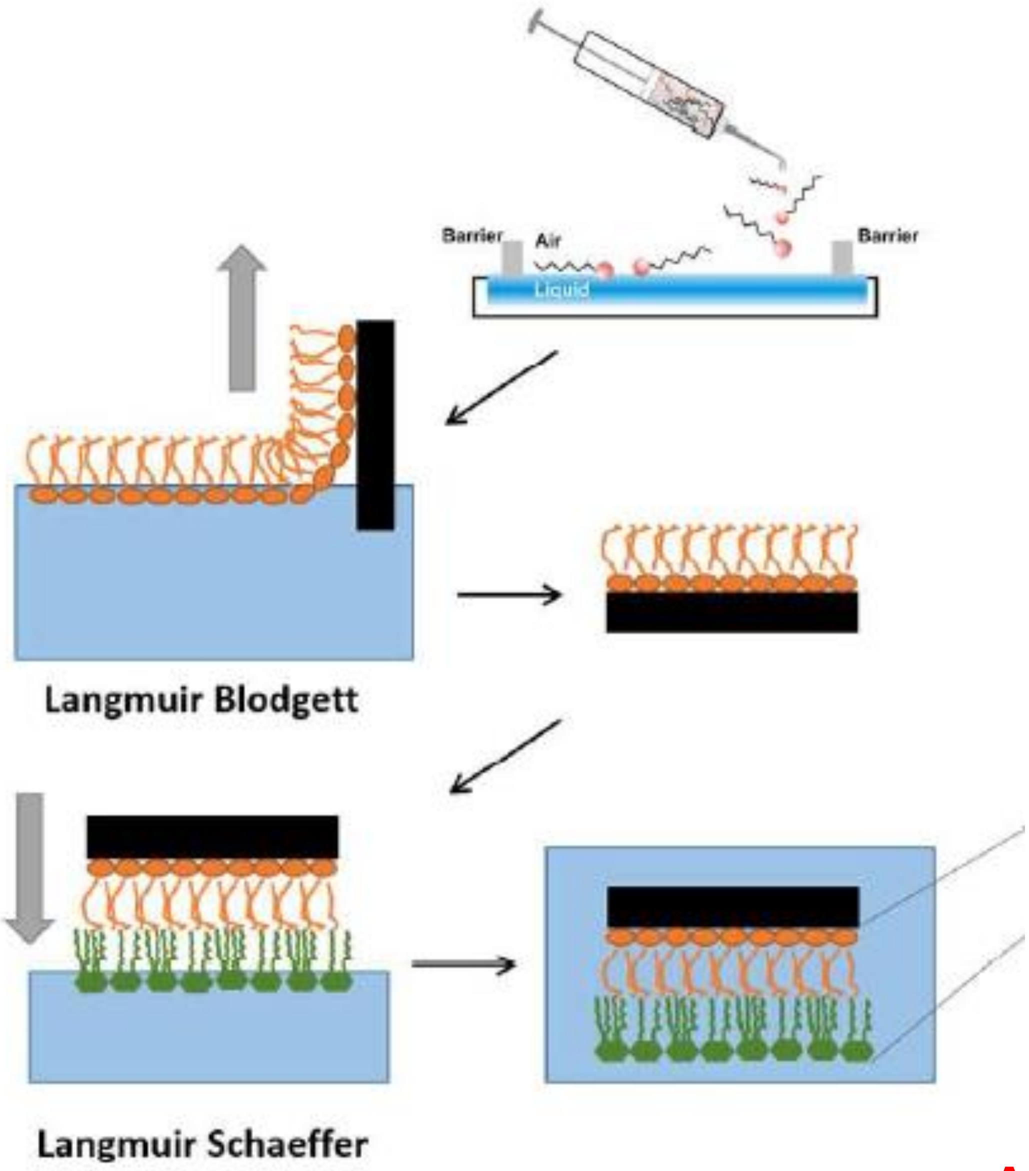
Preparing the membrane models



Langmuir-Blodgett and Langmuir-Schaefer dipping

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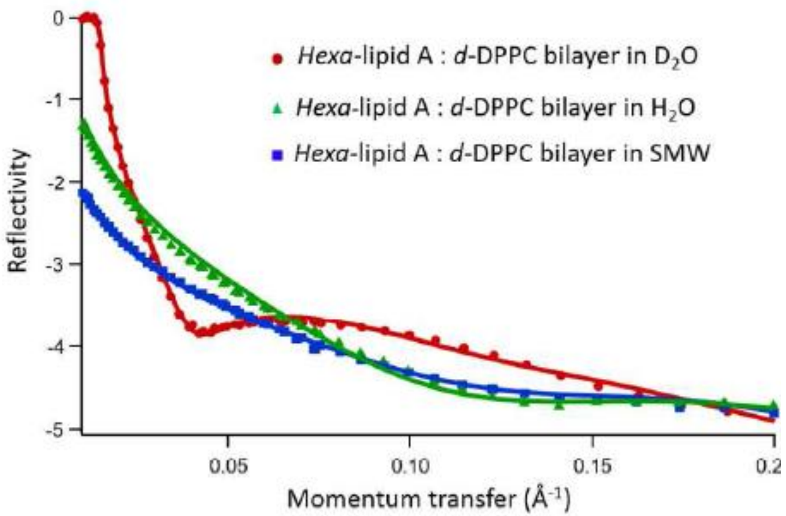
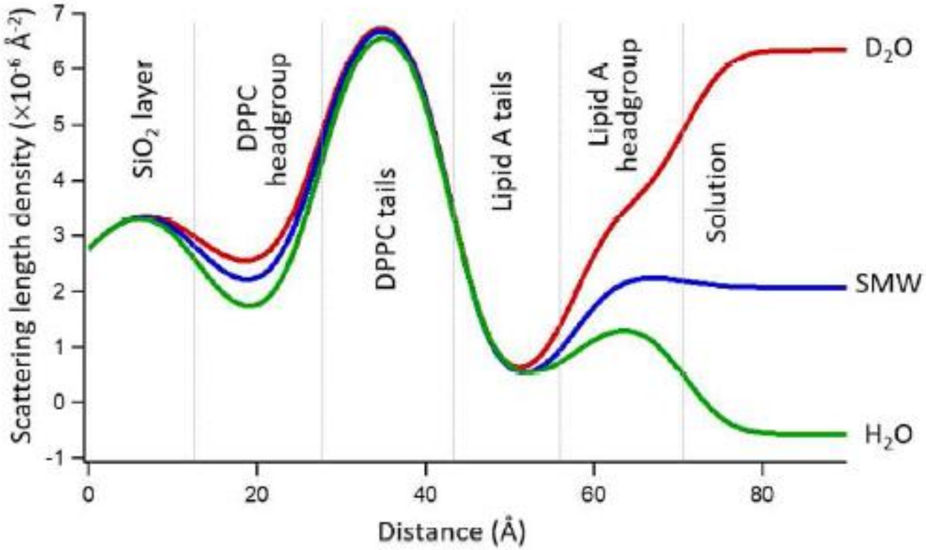
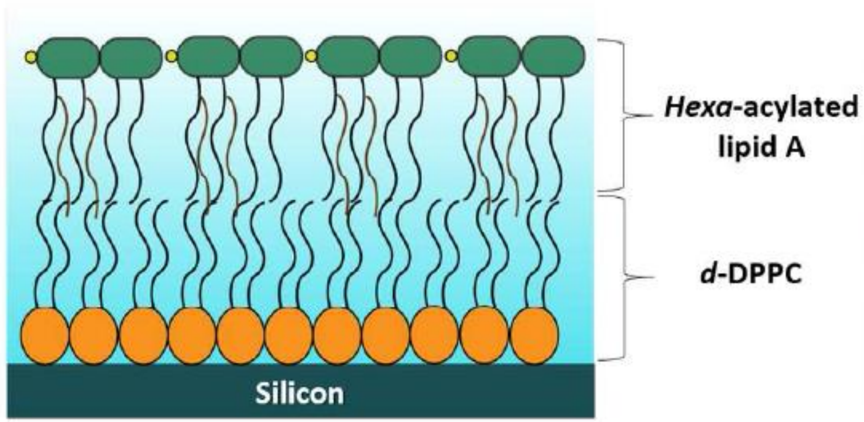
Model membranes of the OM







-> Fit with motofit (Andrew Nelson)

97 % surface coverage, total thickness of 59 Å

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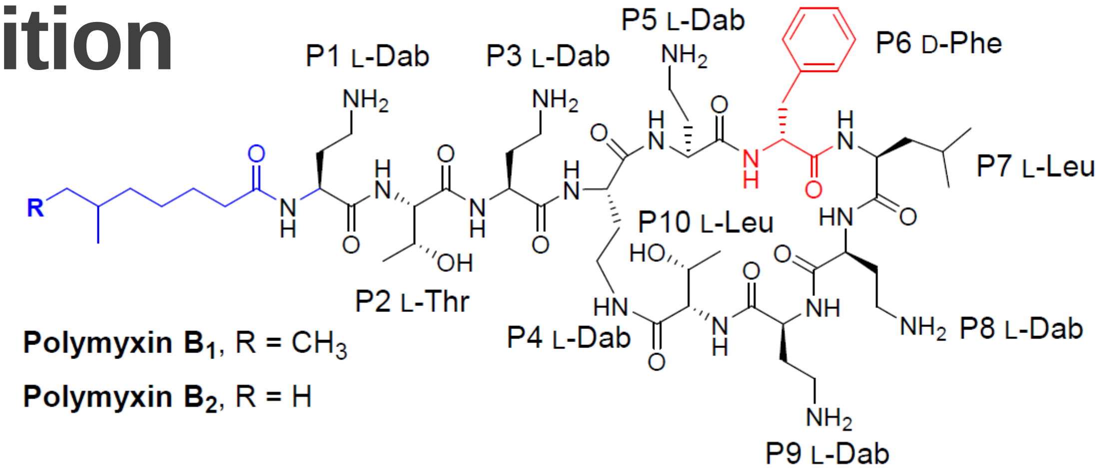
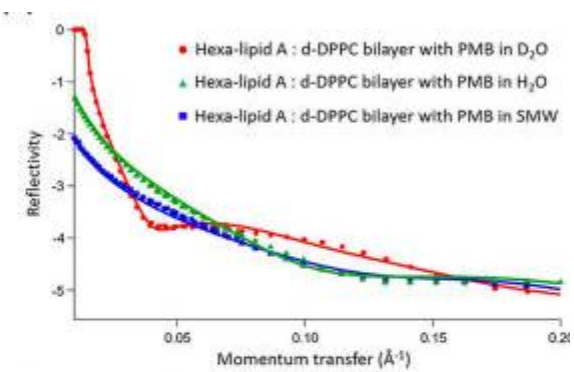
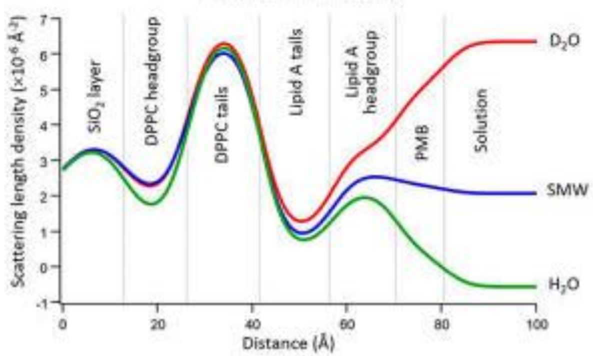
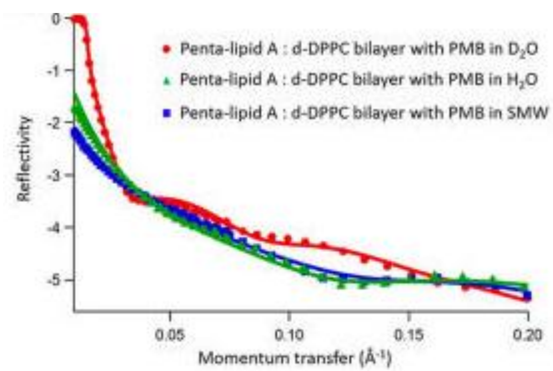
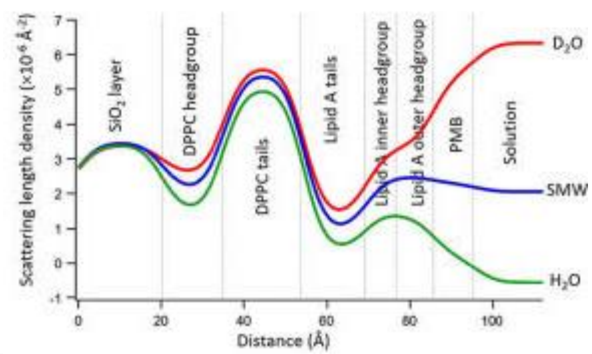
Polymyxin B addition

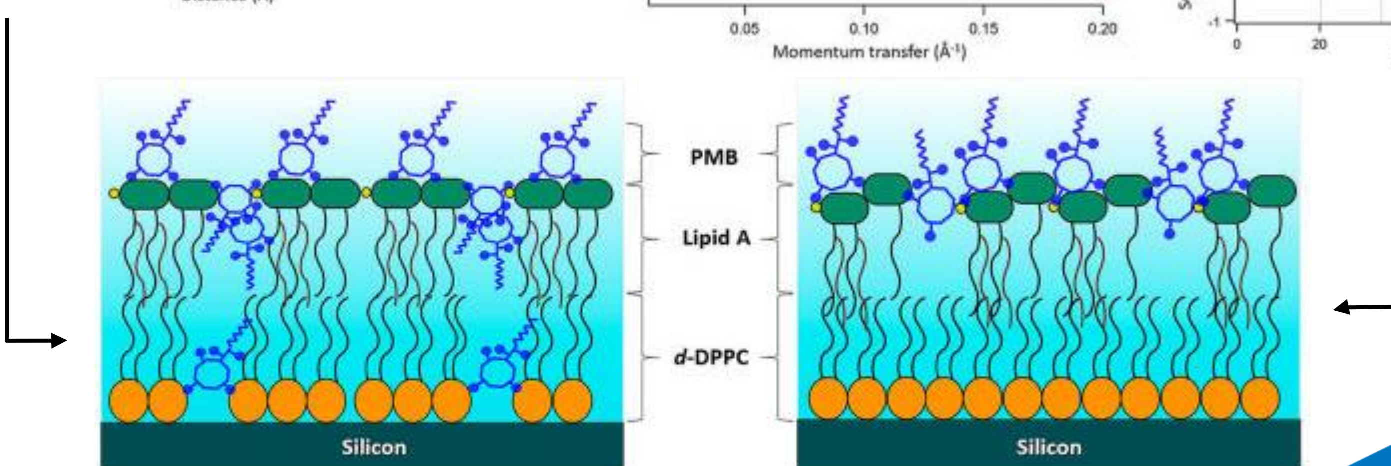












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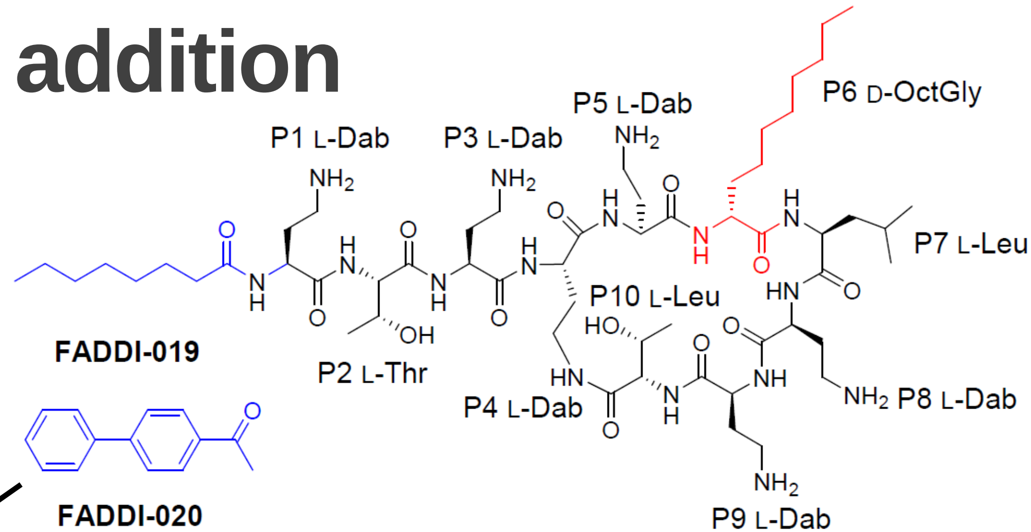
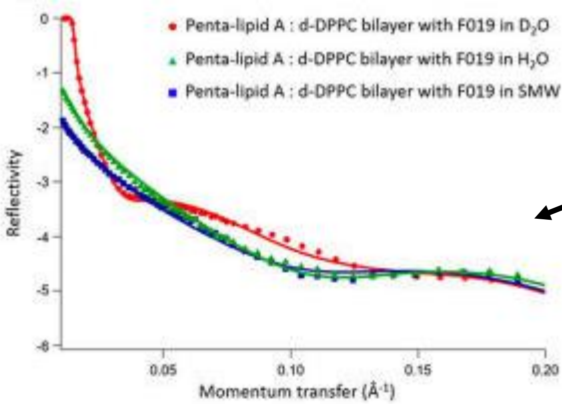
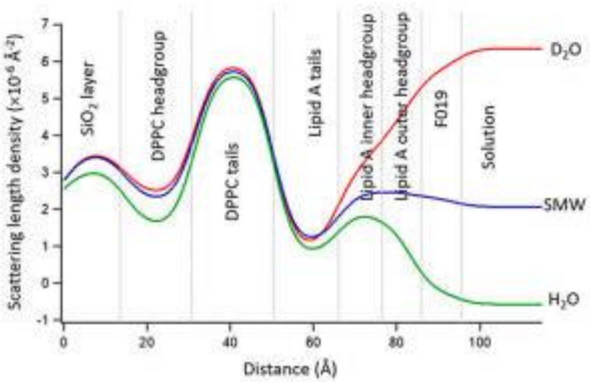
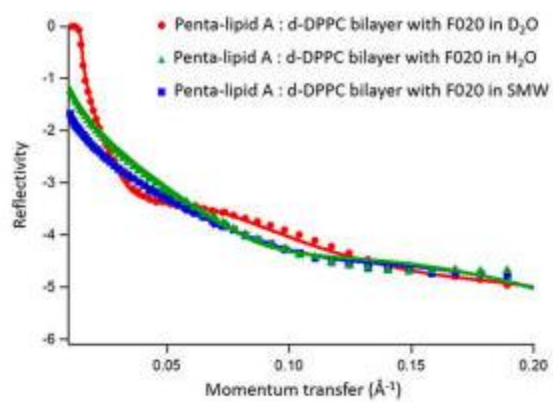
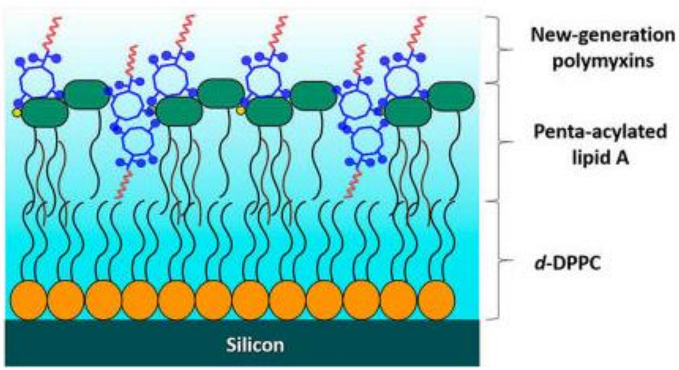
Modified Polymyxin addition

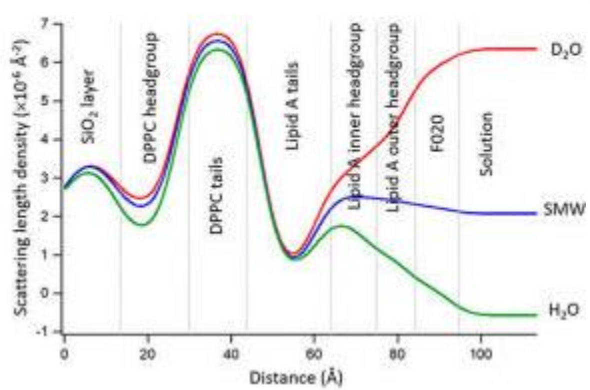












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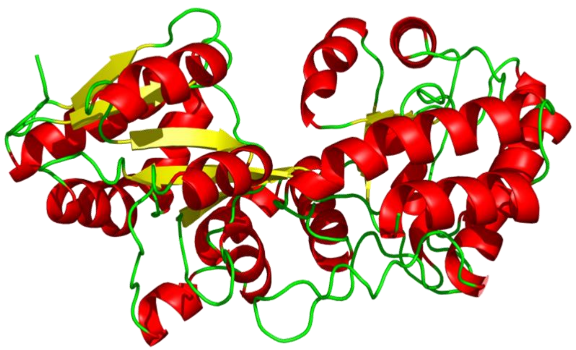
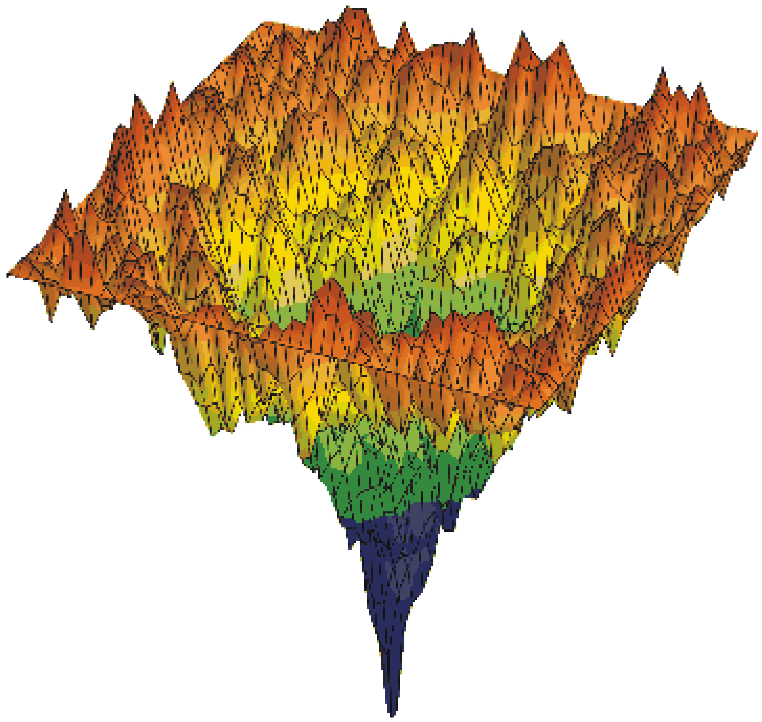
INELASTIC

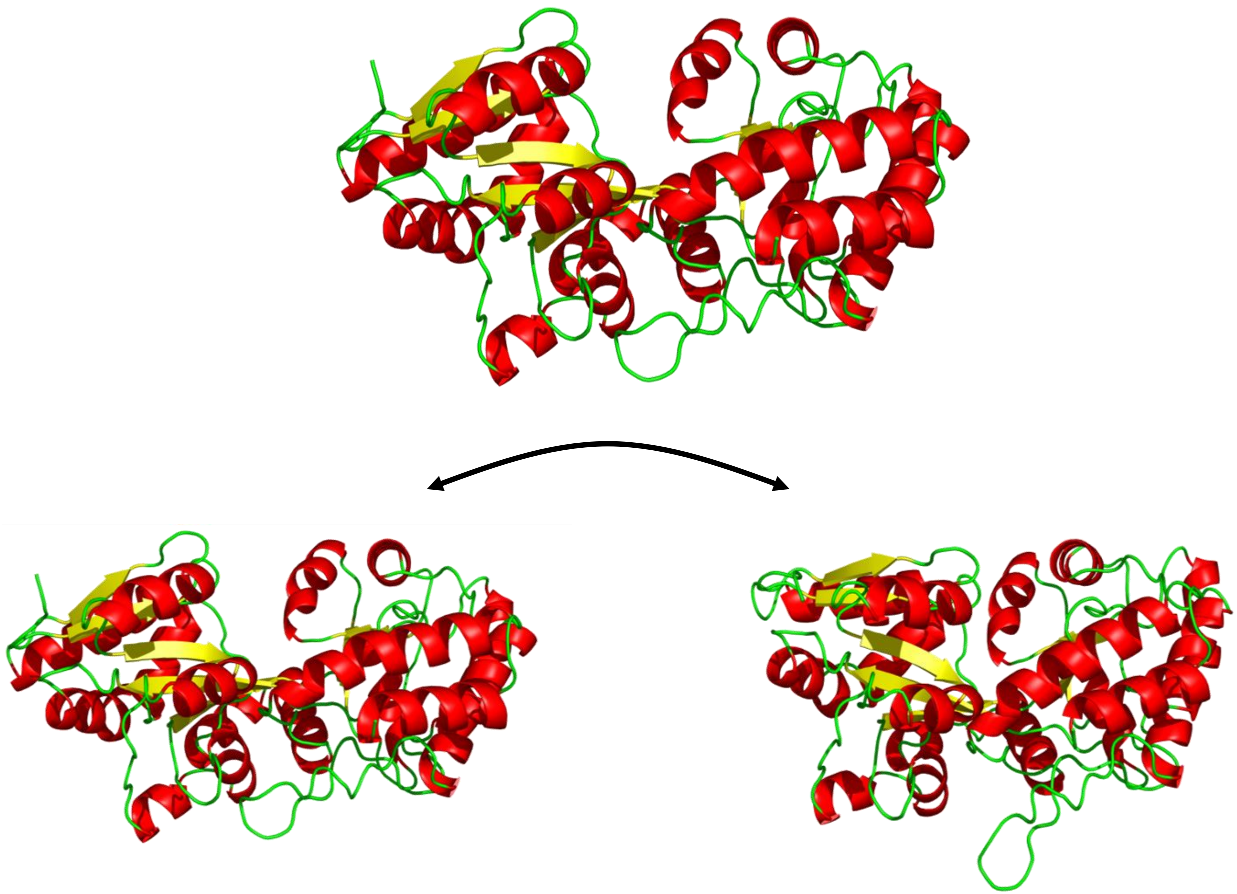
Protein dynamics & energy landscape

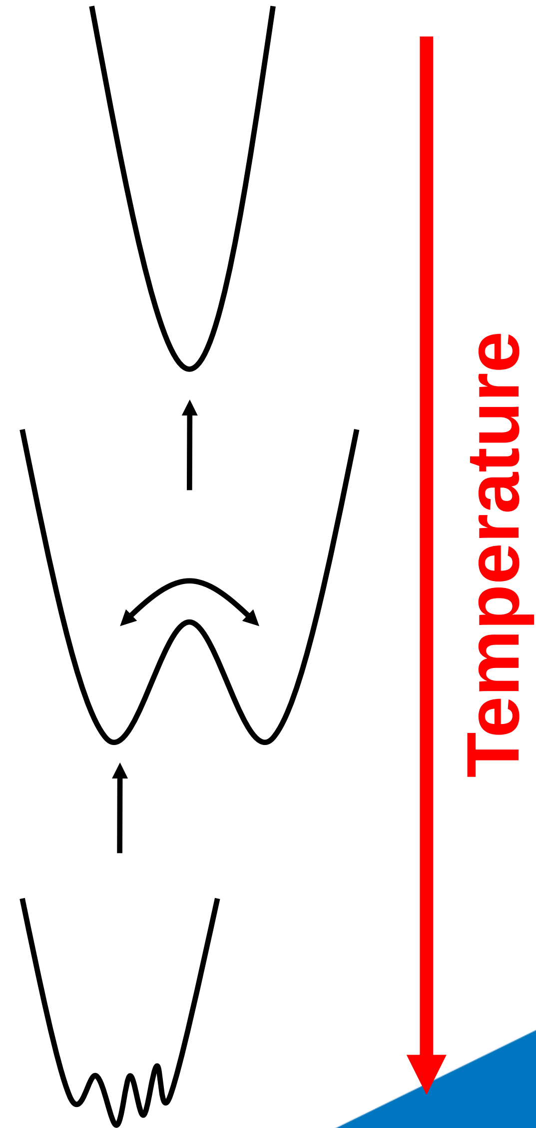




Temperature

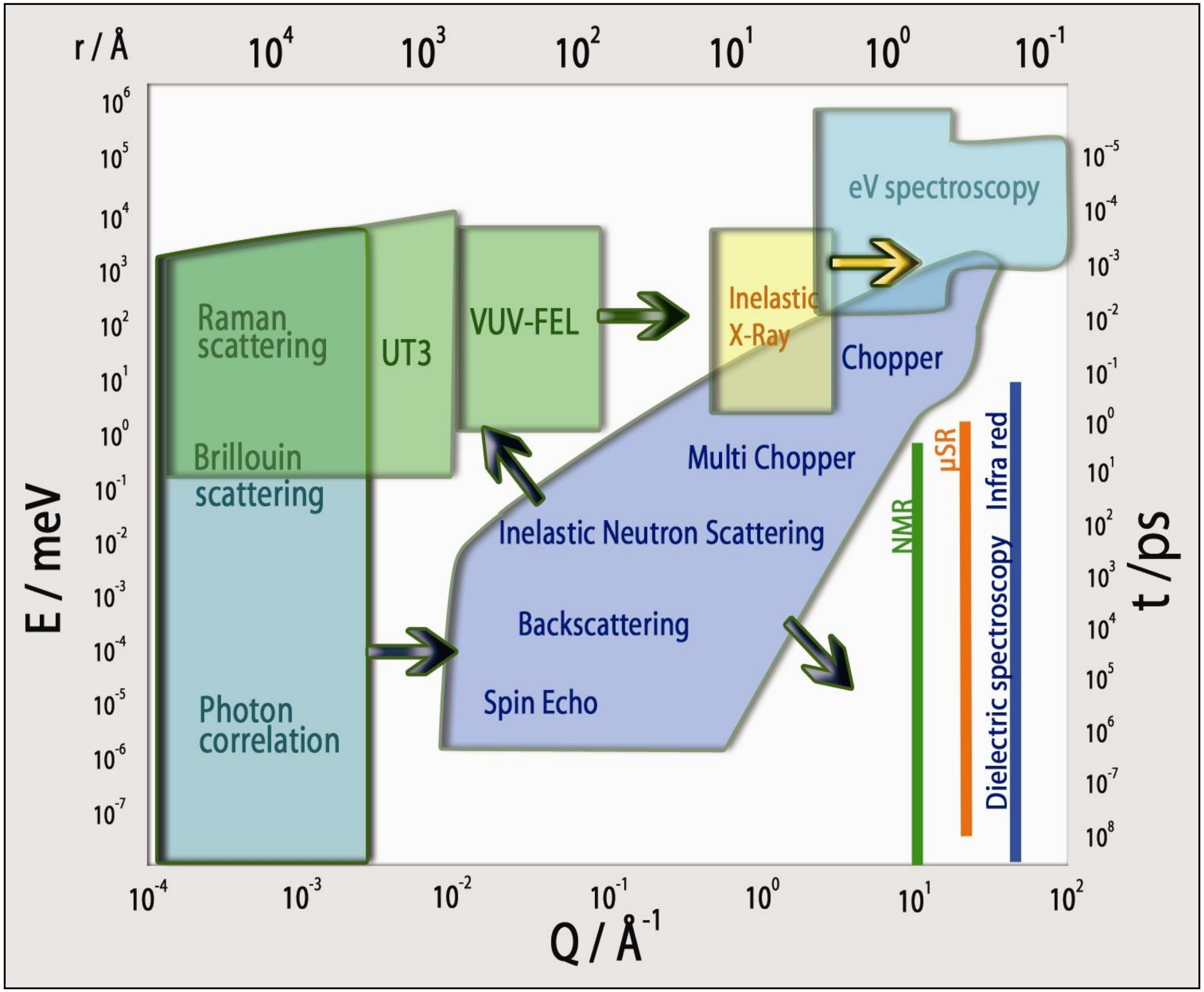
‘Tiers’ in the energy landscape





Frauenfelder *et al*. (1991) *Science* **254**, 1598

Timescales accessible by neutron scattering



From: www.neutron.neutron-eu.net

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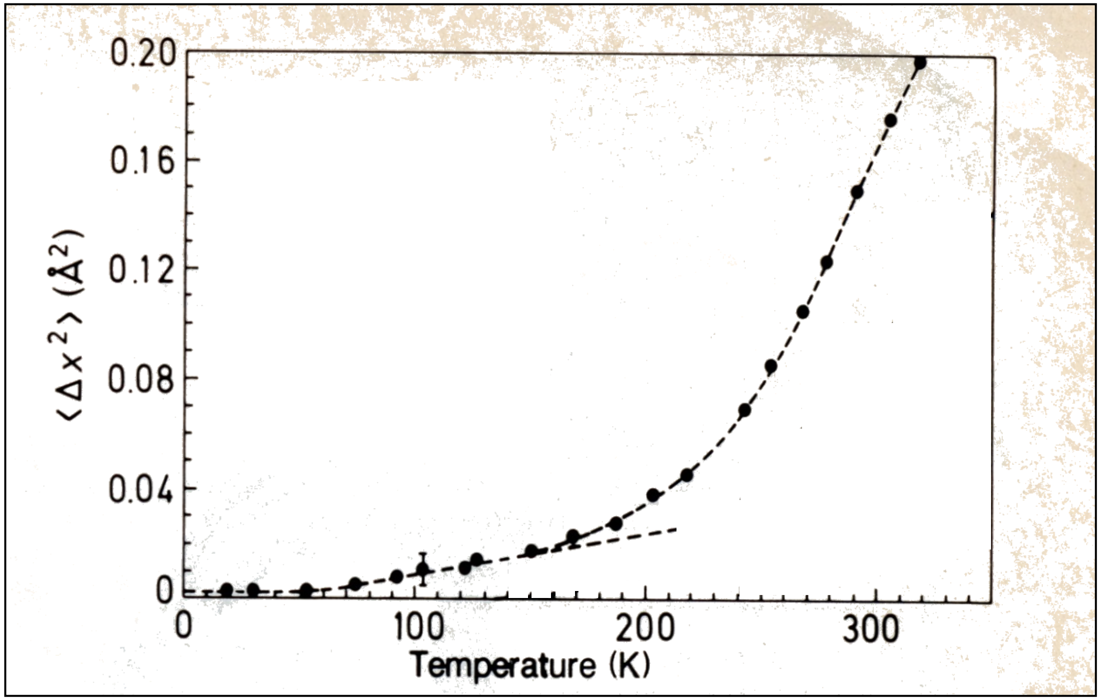
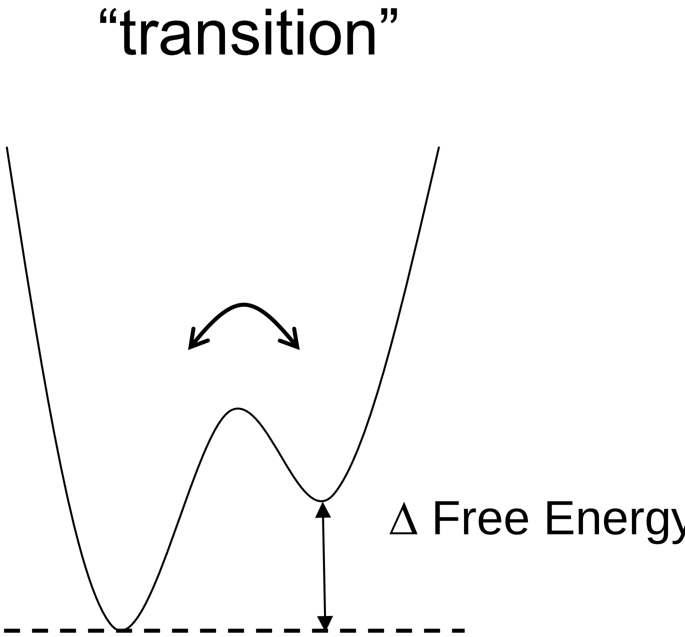
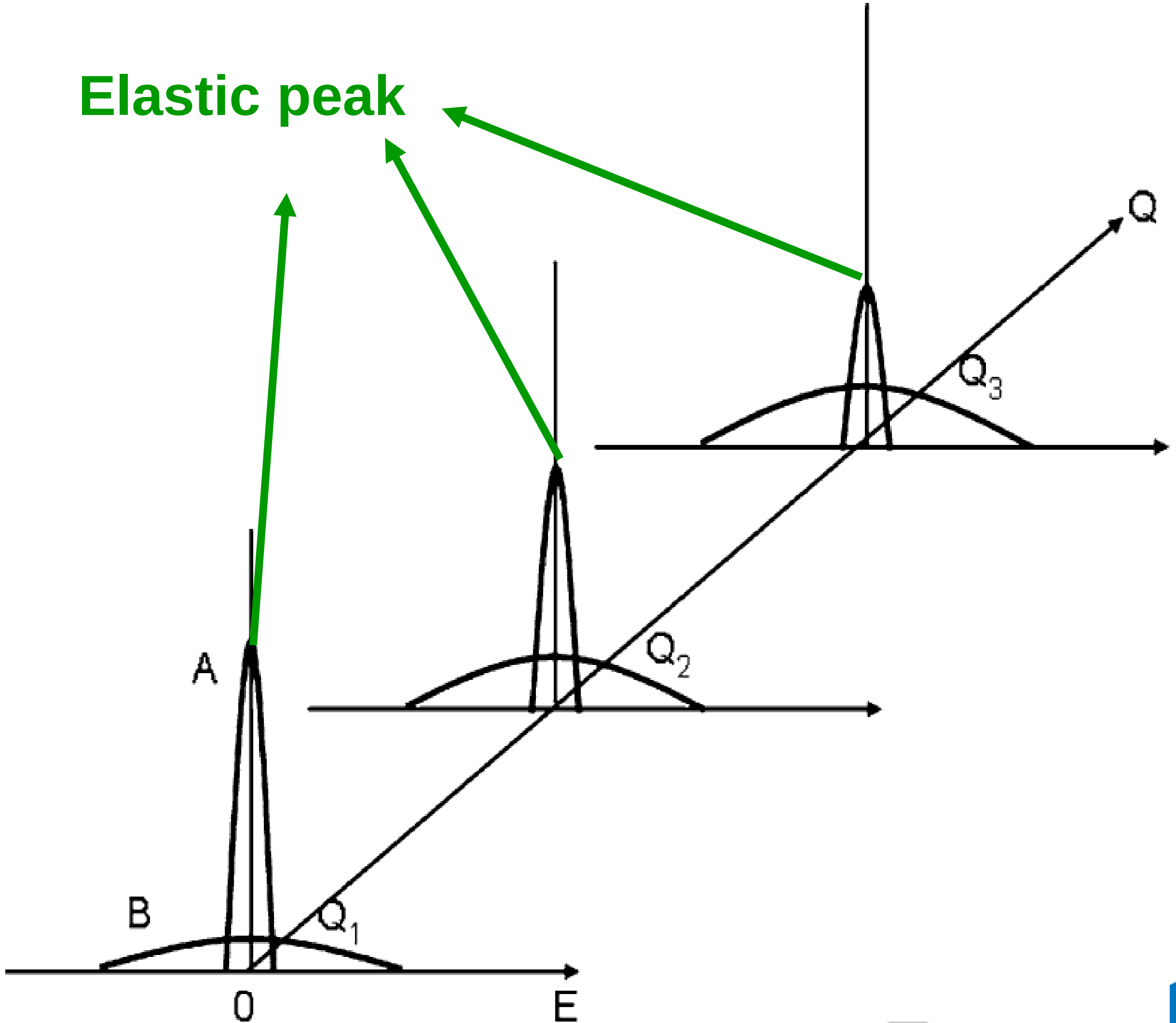
Protein dynamical transition



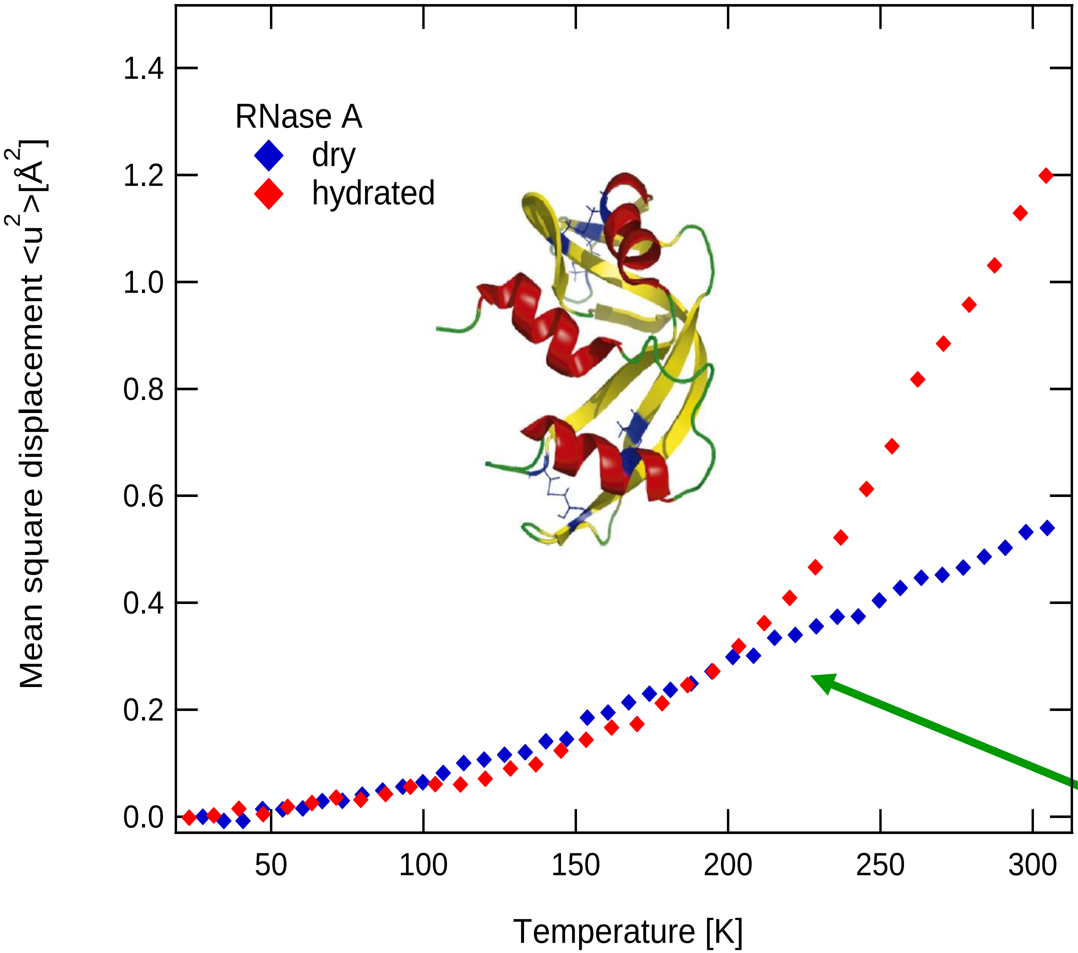


Doster *et al.* (1989) *Nature* **337**, 754-756

Neutron spectroscopy & ‘elastic scans’



Dry/Hydrated protein neutron scattering



backscattering neutron spectrometer ILL

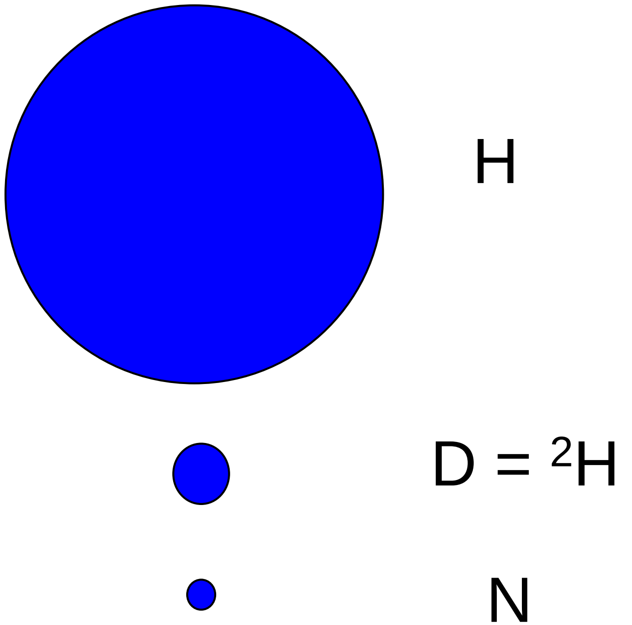
Timescale: ps-ns

dynamical transition

Wood *et al.* (2008) Chem Phys **345**, 305 - 314

Contribution to the scattering & samples

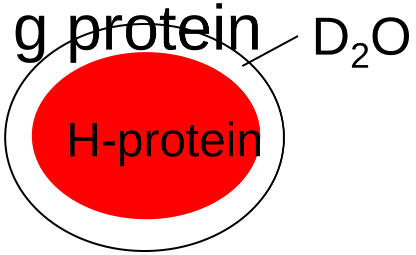
Neutron Incoherent
Scattering Cross
Sections drawn to scale



C,O,P,S invisible

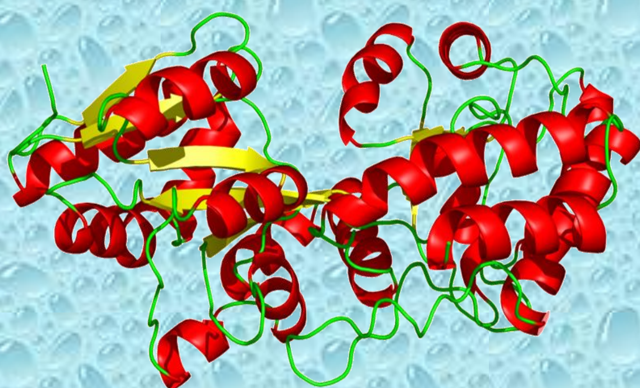
Hydrated powder
samples

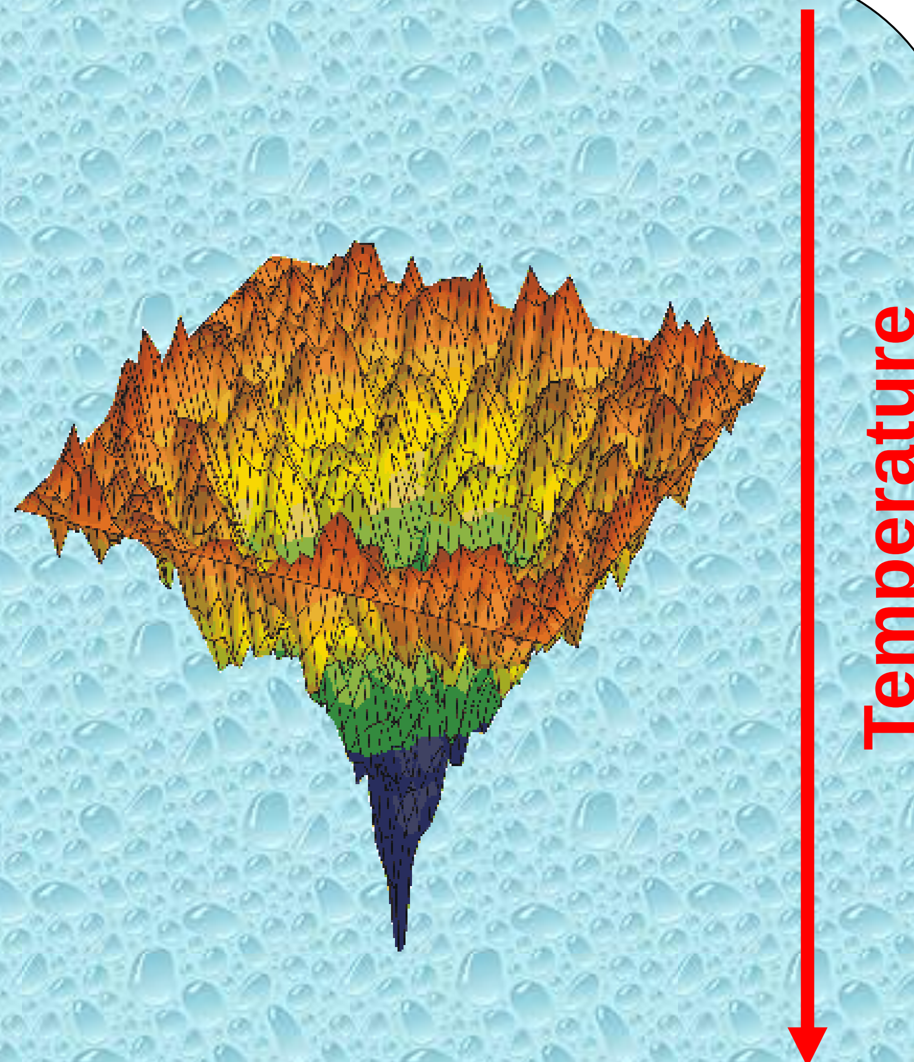
~ 0.4 g D_2O / g protein



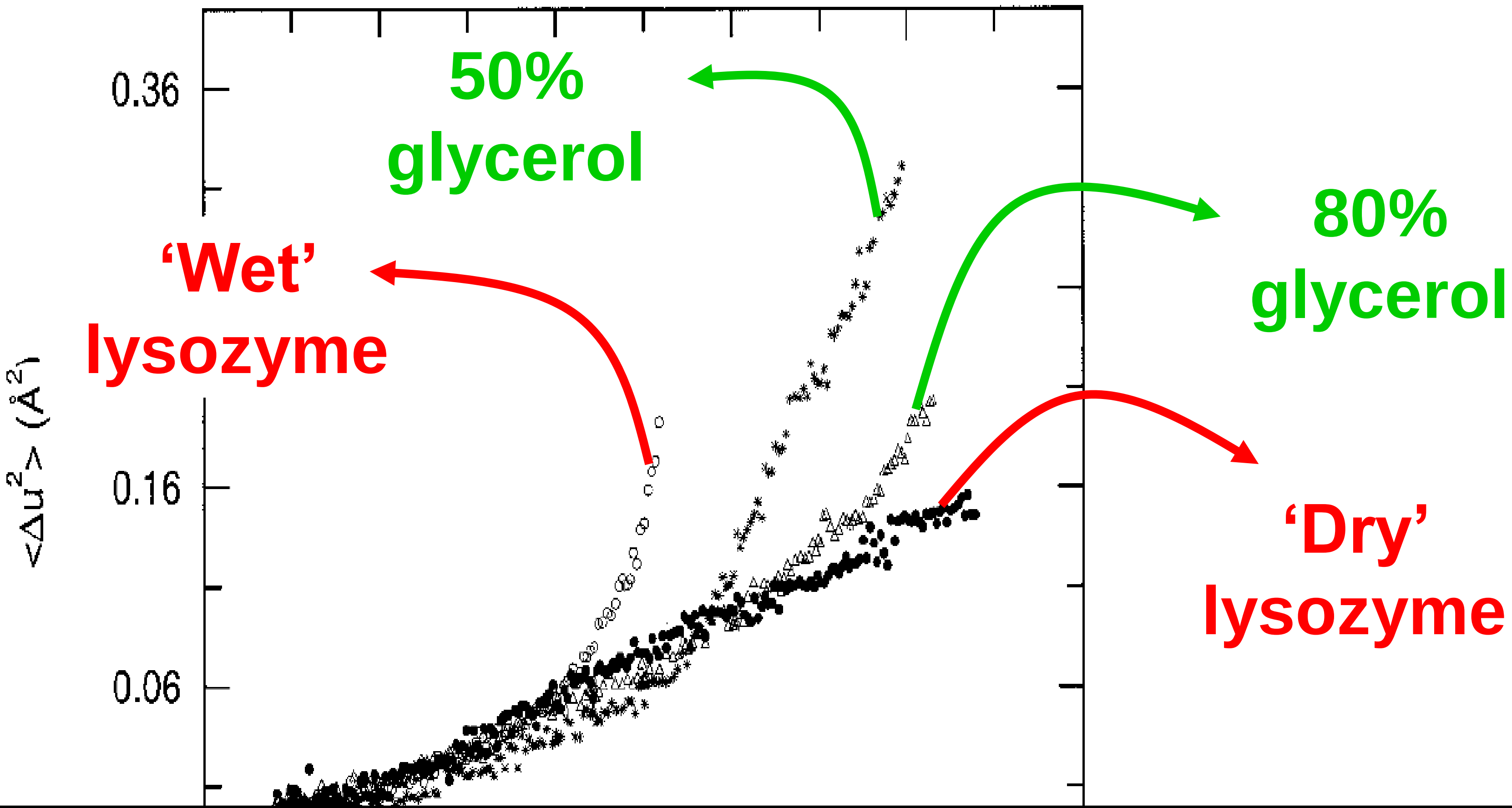
Protein dynamics

Water example 1 – protein hydration water





Proteins ‘Slaves’ to the water?



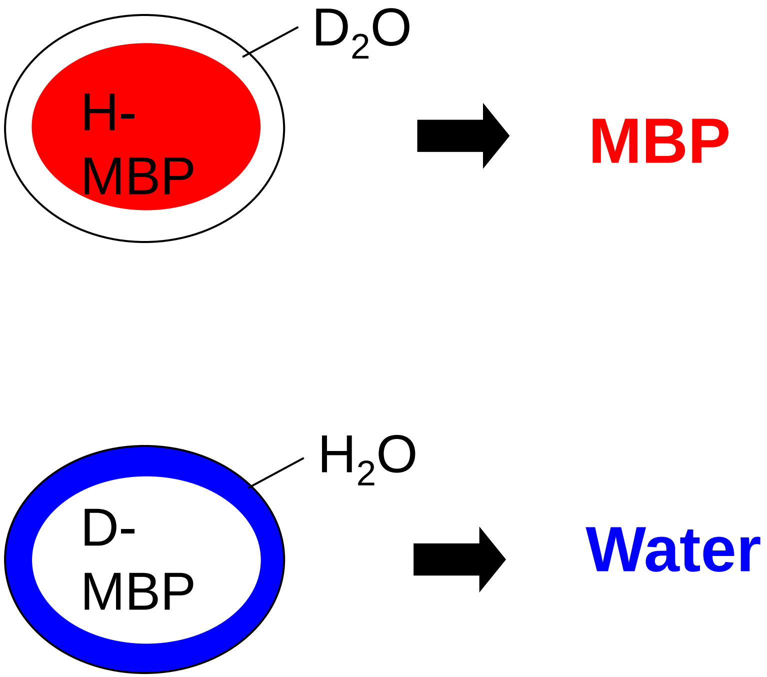
“...dynamics and functions are coupled to motions in the bulk solvent and the hydration shell”

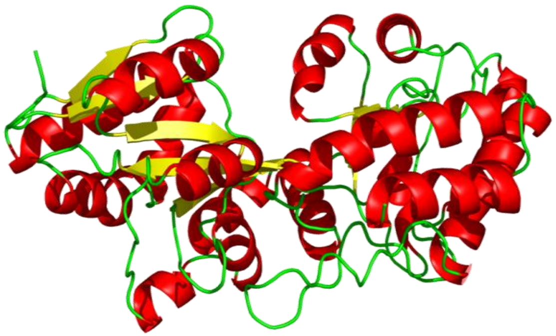
Fenimore *et al.* (2004), *Proc Natl Acad Sci USA,* **101**,14408

Tsai *et al.* (2000) Biophys J **130**, 2728 - 2732

Maltose Binding Protein (MBP): samples

Labelling Strategy





40 kDa

ILL G Zaccai, M Moulin, M Haertlein

IBS: A Frolich, M Weik

U of Perugia: A Paciaroni

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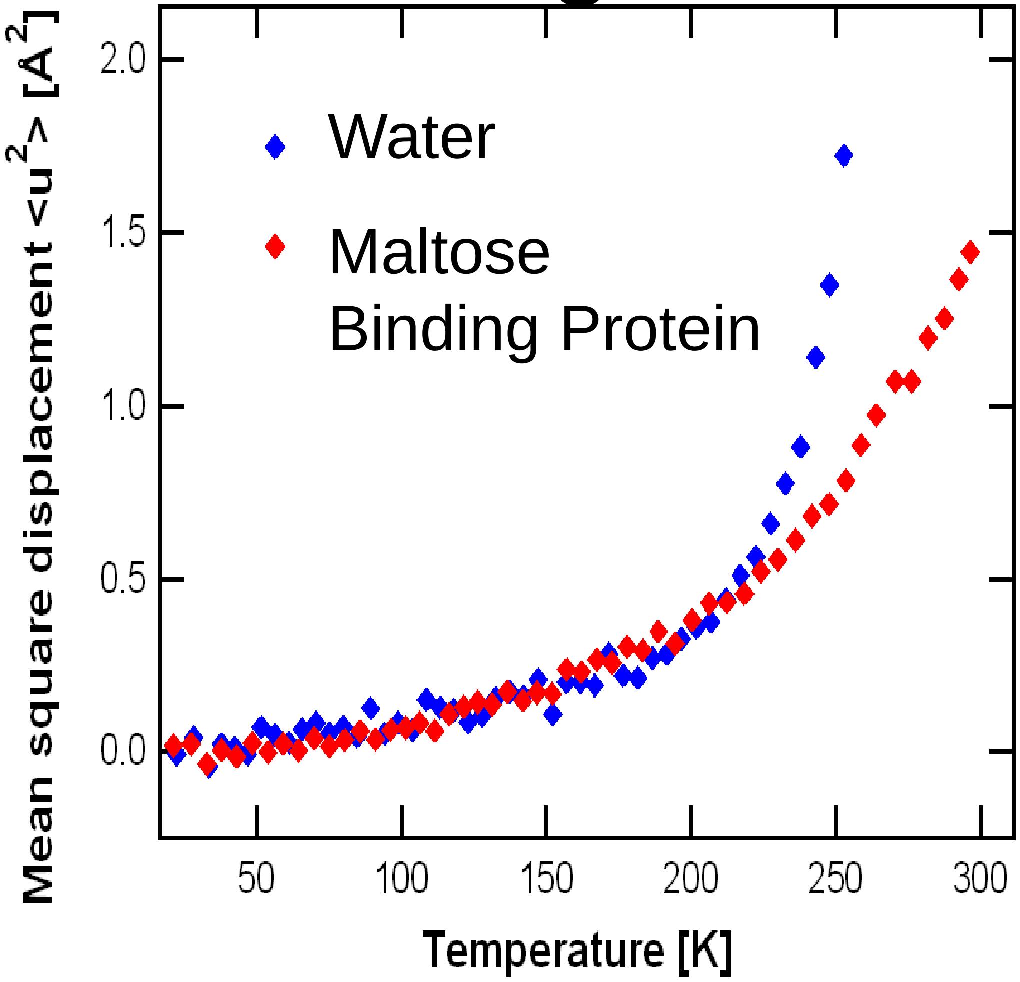
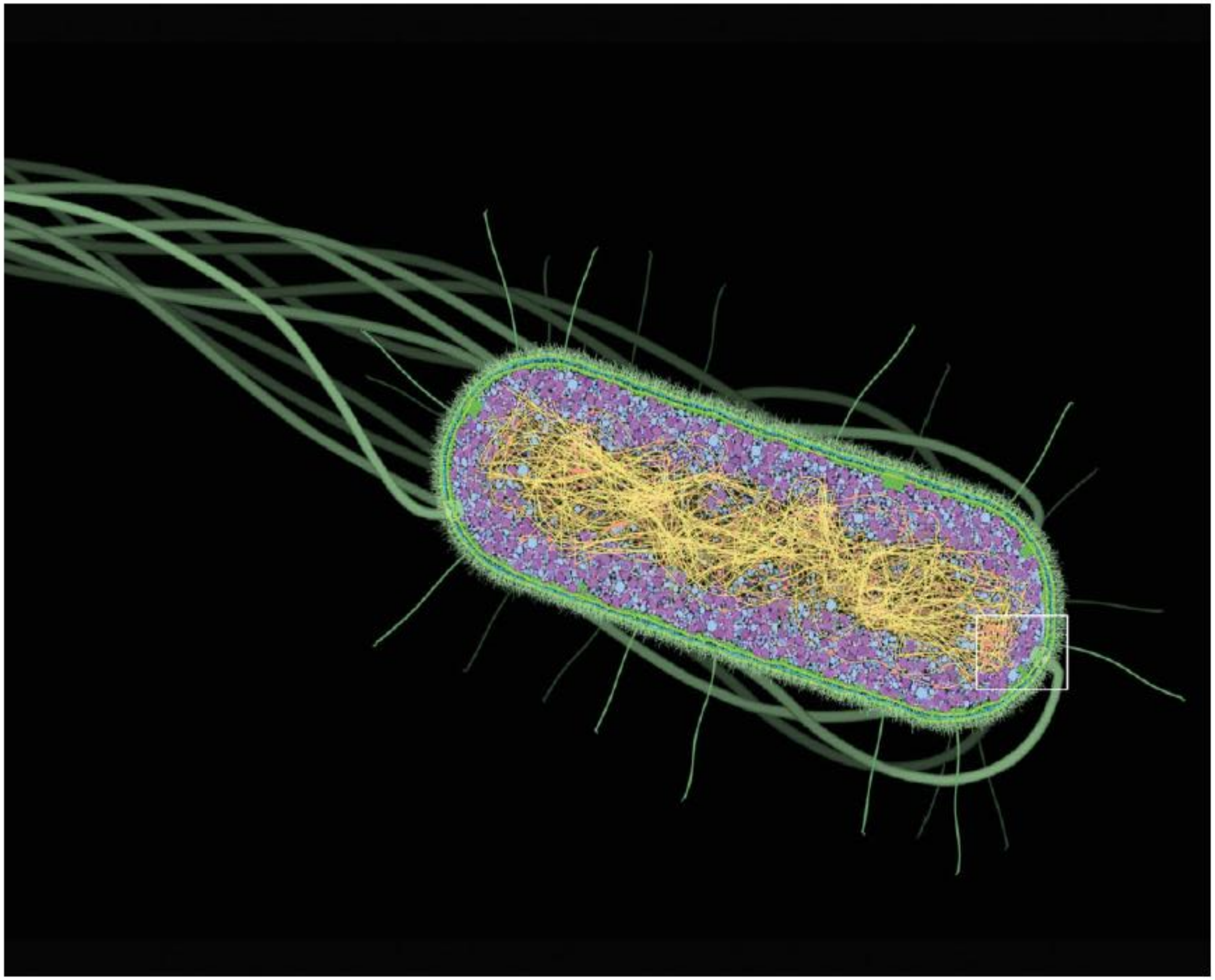
Maltose Binding Protein (MBP): neutron scattering



Wood *et al.* (2008) J Am Chem Soc **130**, 4586-4587

Water example 2 – water dynamics in cells



Deuterated cells, H_2O
- Deuterated cells, D_2O

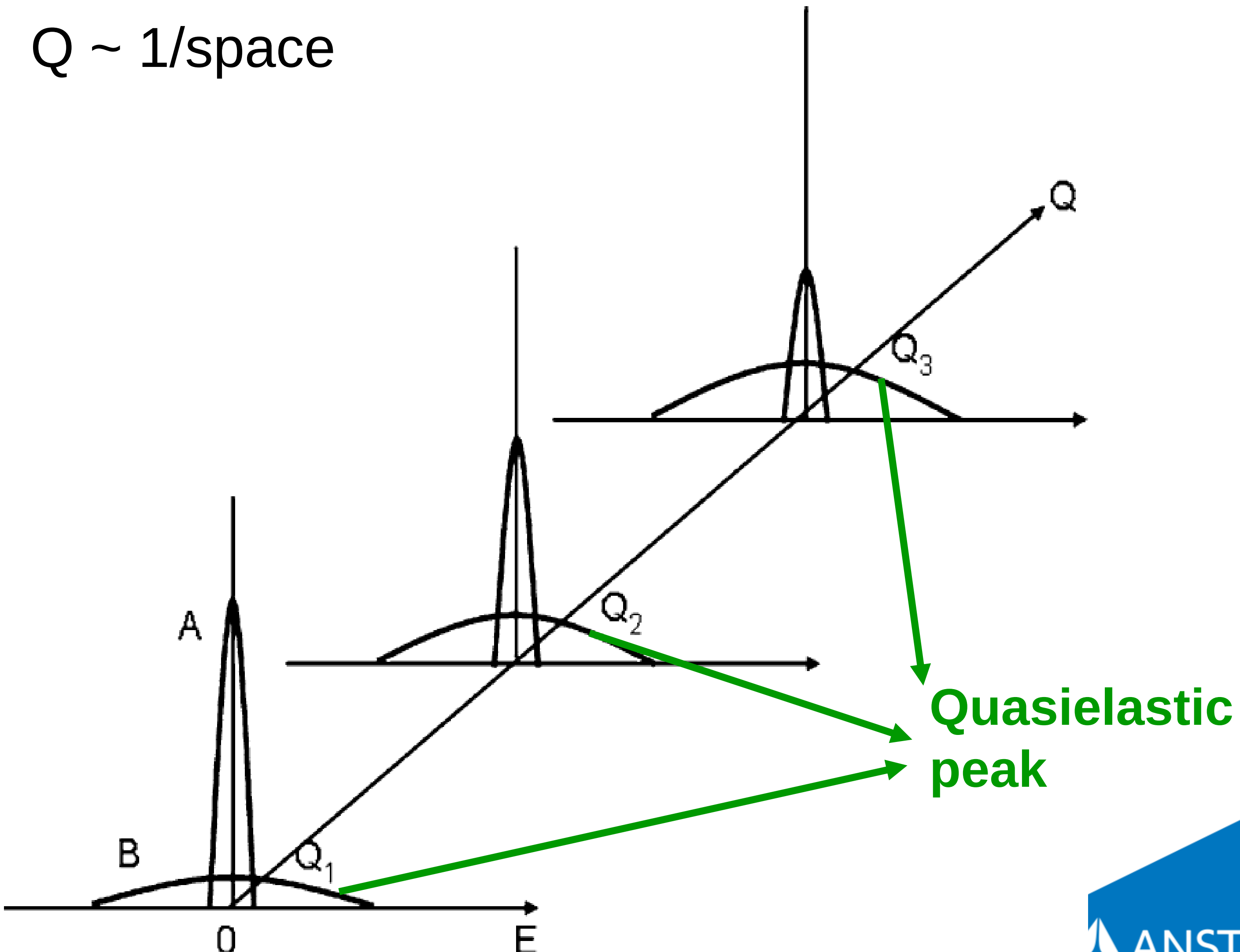
ILL/IBS Grenoble:
M Jasnin, M Moulin, M Haertlein, G Zaccai, M Tehei.

E. Coli cell, from Goodsell (2009) *Biochem and Mol Bio Edu*, 37, 325-332

Neutron spectroscopy

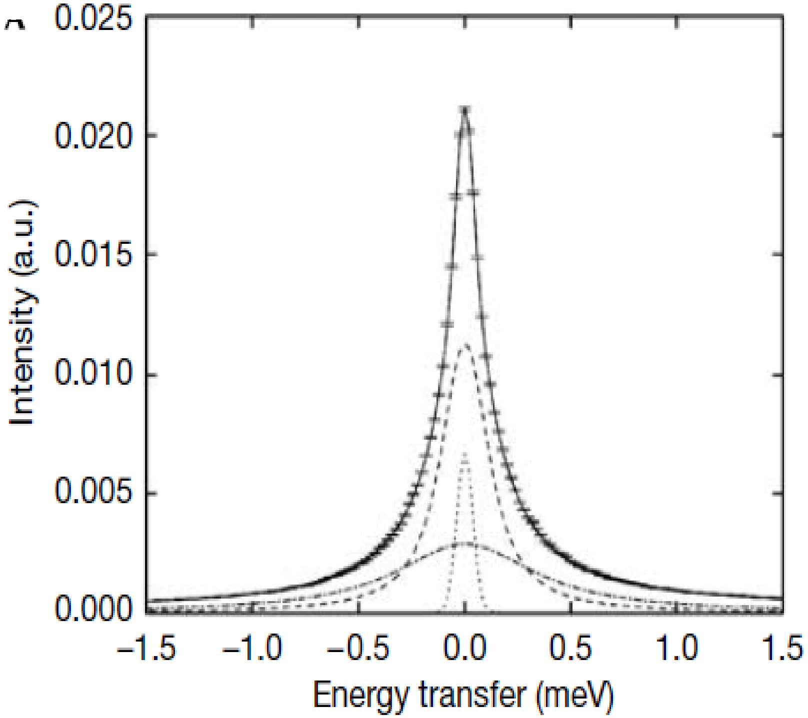
Q ~ 1/space

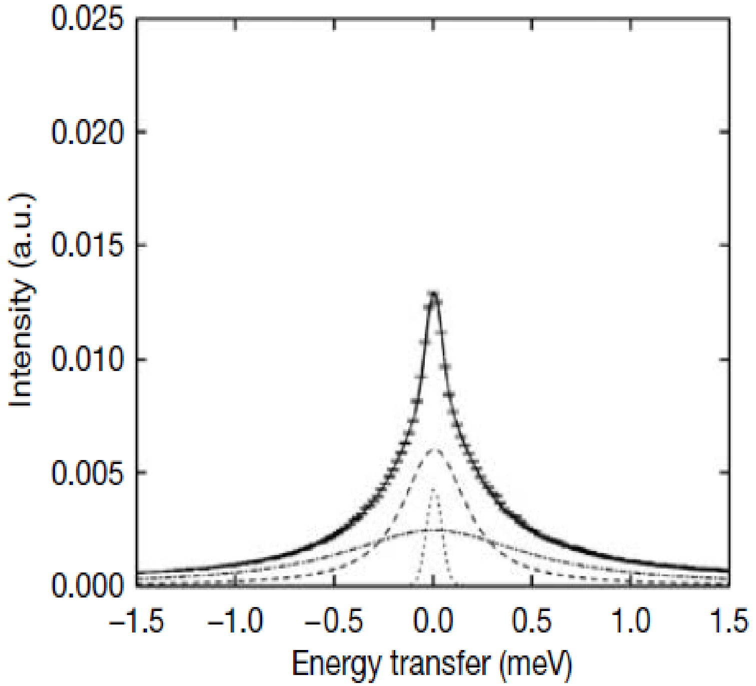
E ~ 1/time



Quasi-elastic neutron measurements

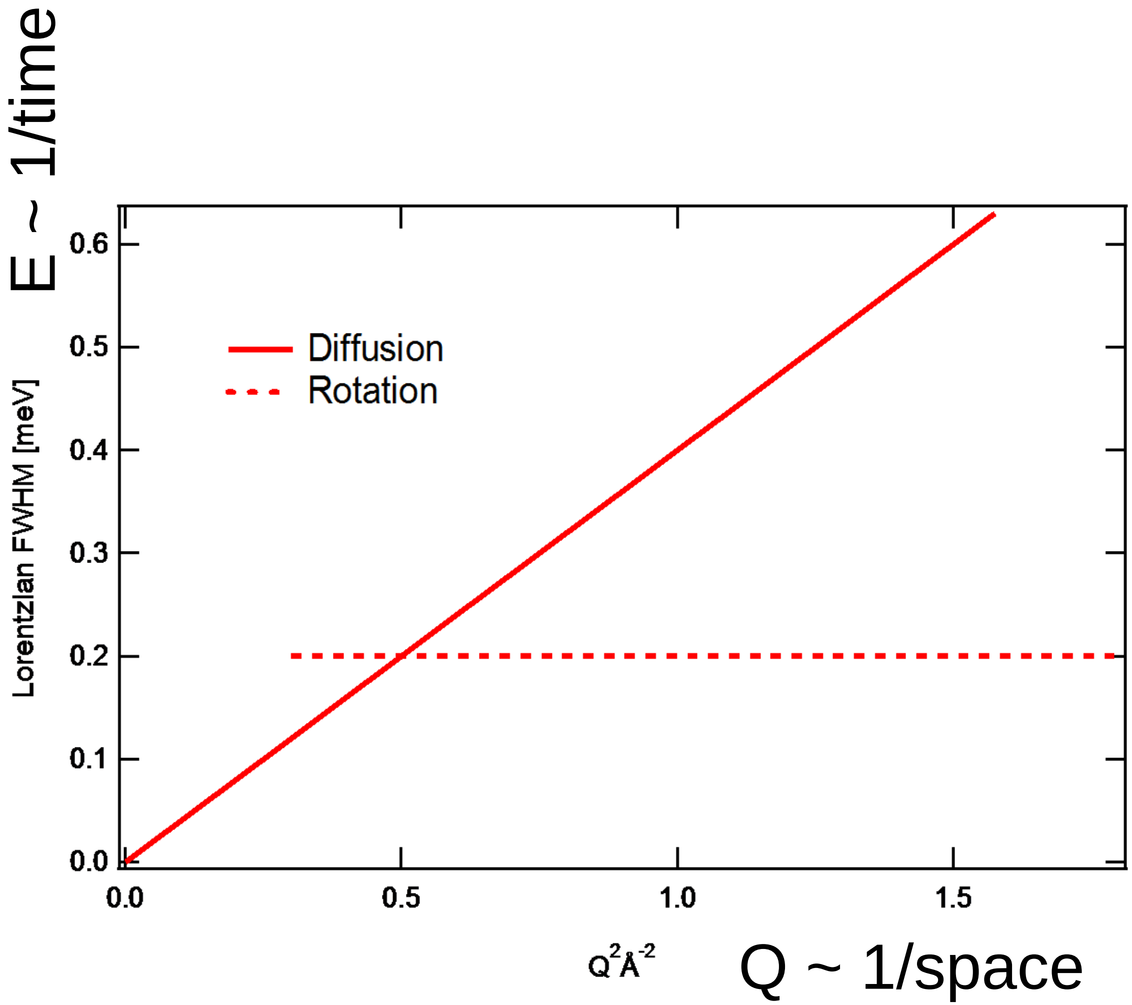
IRIS - ISIS, UK & IN6 - ILL, France

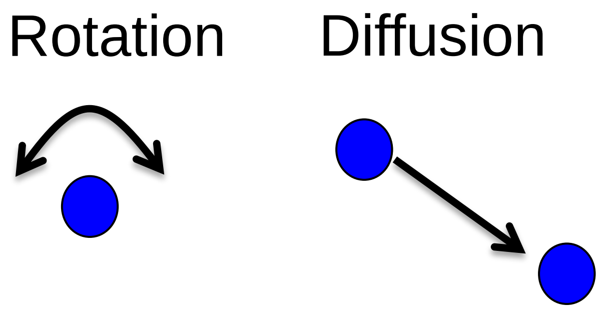




Jasnin *et al.* (2008) *EMBO J*, 9, 546

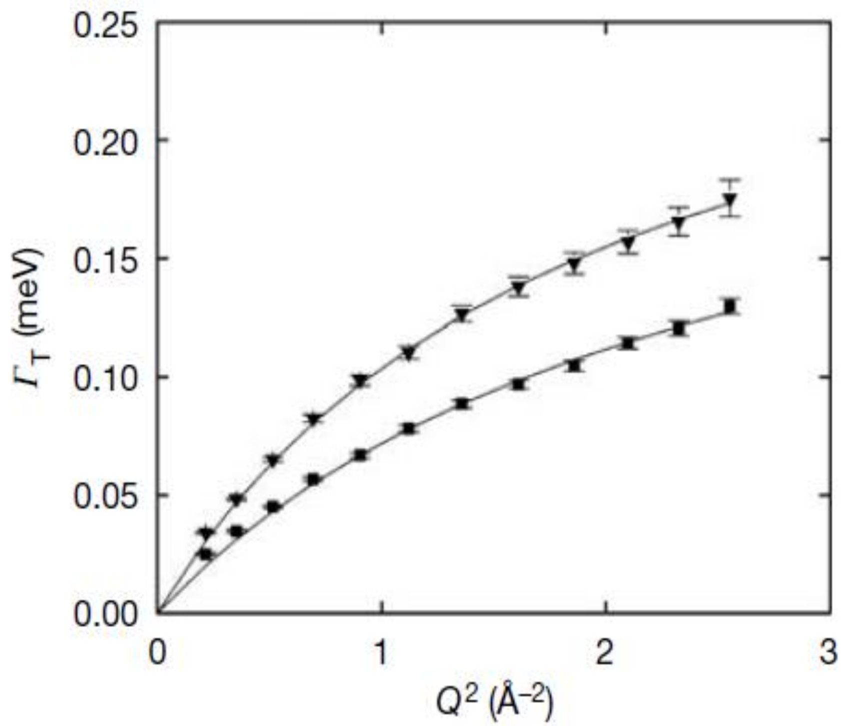
Interpreting QENS data





Quasi-elastic neutron measurements

IRIS - ISIS, UK & IN6 - ILL, France



"Jump diffusion model"

$$\Gamma_T = \frac{D_T Q^2}{1 + D_T Q^2 \tau_0}$$

Water in *E. Coli* cells

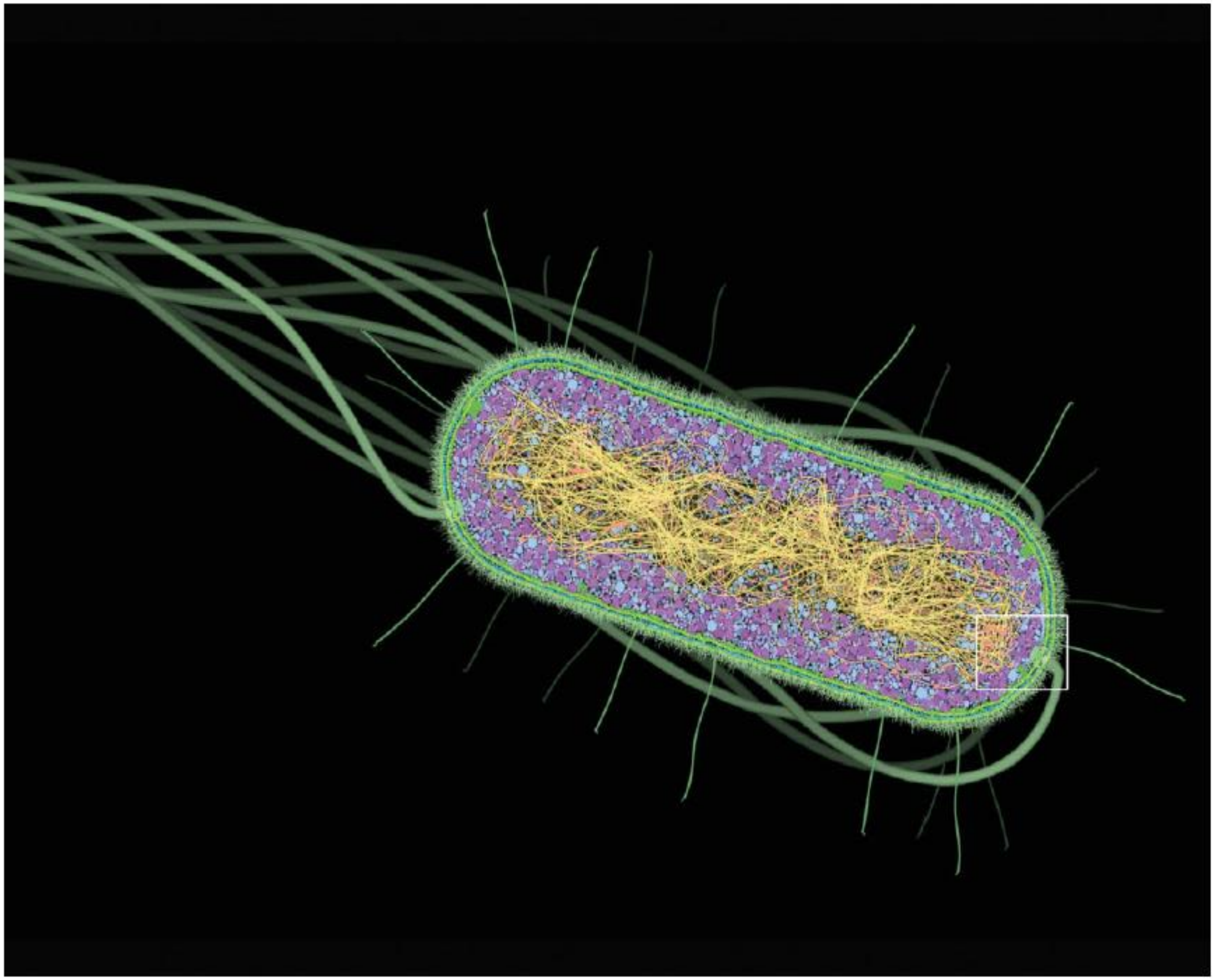
$$D_T = 1.5 \times 10^{-5} cm^2 s^{-1}$$

Buffer

$$D_T = 1.7 \times 10^{-5} cm^2 s^{-1}$$

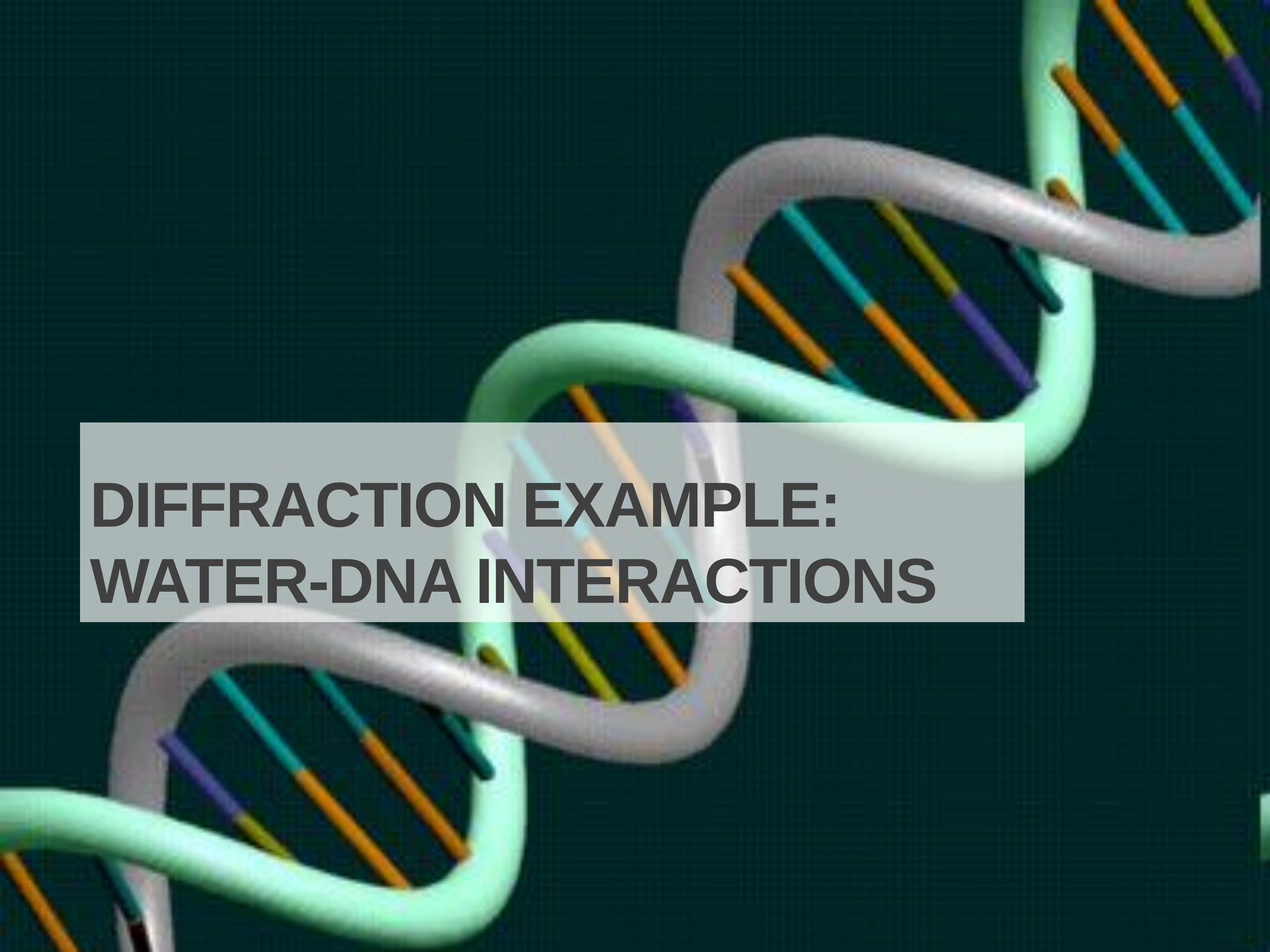
Jasnin *et al.* (2008) *EMBO J*, 9, 546

Water example 2 - conclusion



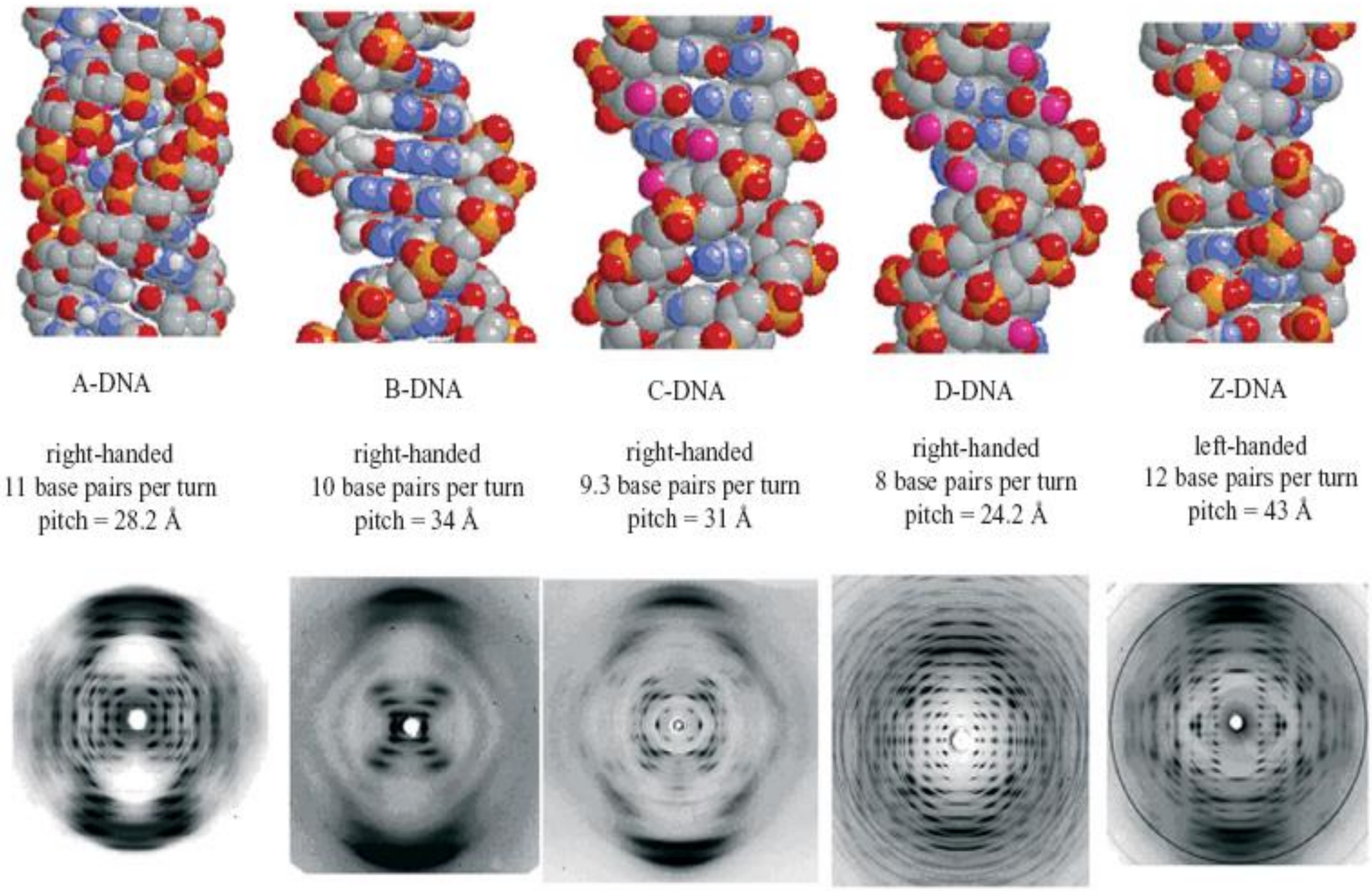
Significant amount of water has similar properties to bulk

E. Coli cell, from Goodsell (2009) *Biochem and Mol Bio Edu*, 37, 325-332



DIFFRACTION EXAMPLE: WATER-DNA INTERACTIONS

Water-DNA interactions



Fuller W, et al. (2004) *Philos Trans R Soc Lond B Biol Sci* **359**(1448)**:** 1237-1247; discussion 1247-1238

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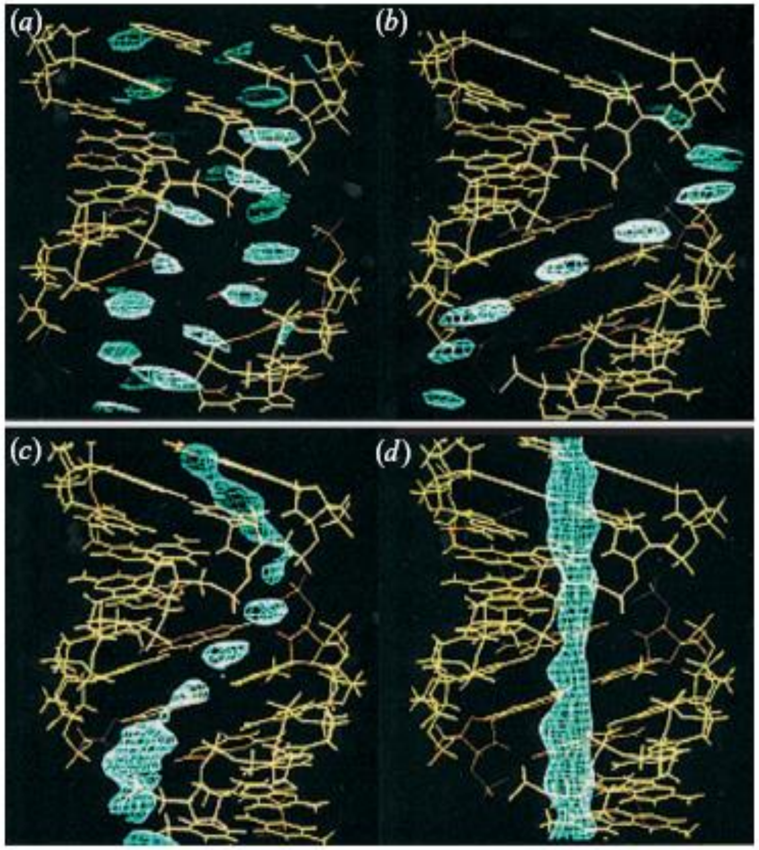
Water-DNA interactions



Fuller W, et al. (2004) *Philos Trans R Soc Lond B Biol Sci* **359**(1448)**:** 1237-1247; discussion 1247-1238

National Deuteration Facility, ANSTO