SwedNess NNSP Intro Course in Neutron Scattering

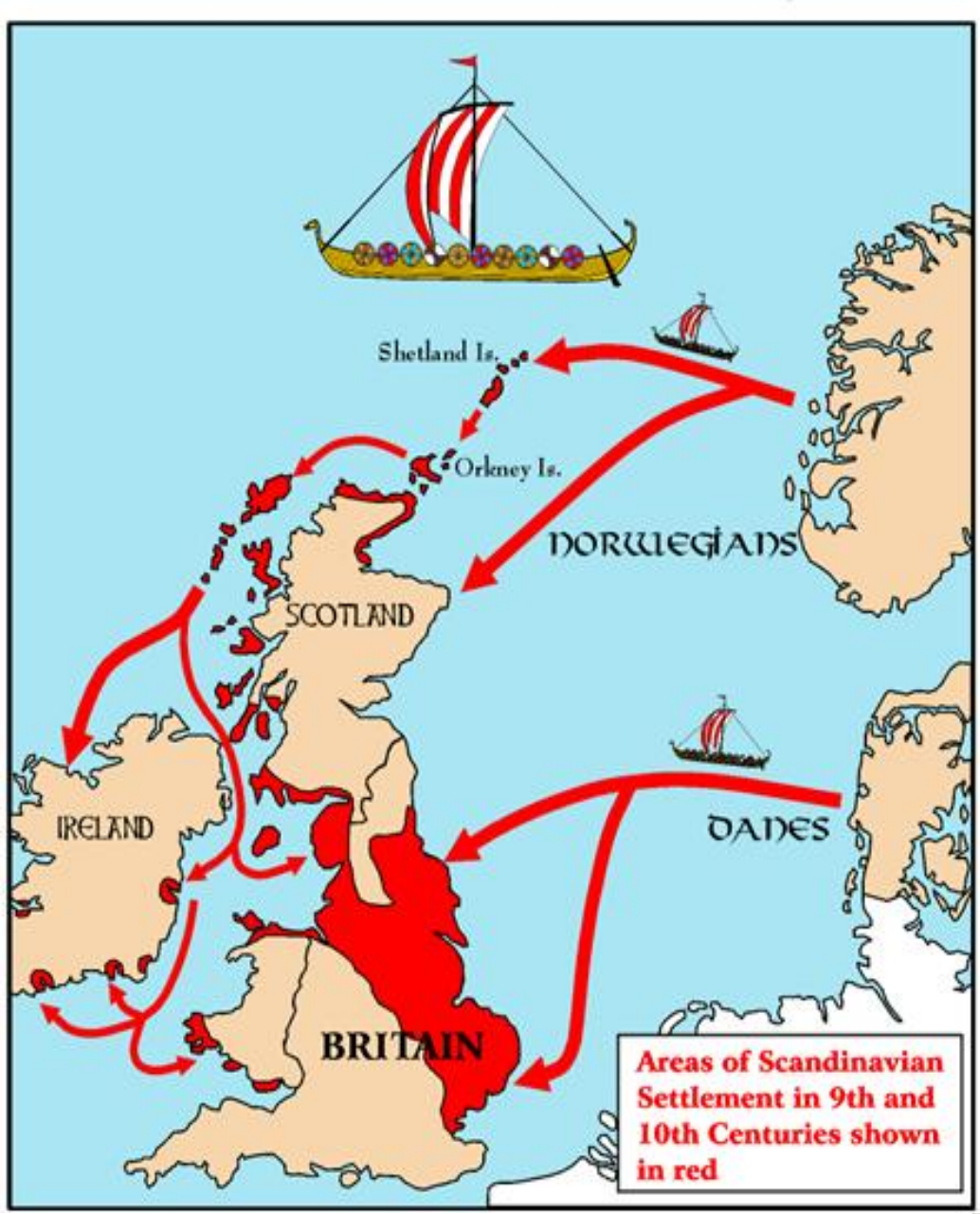
Tartu, Estonia 9-21 September 2017

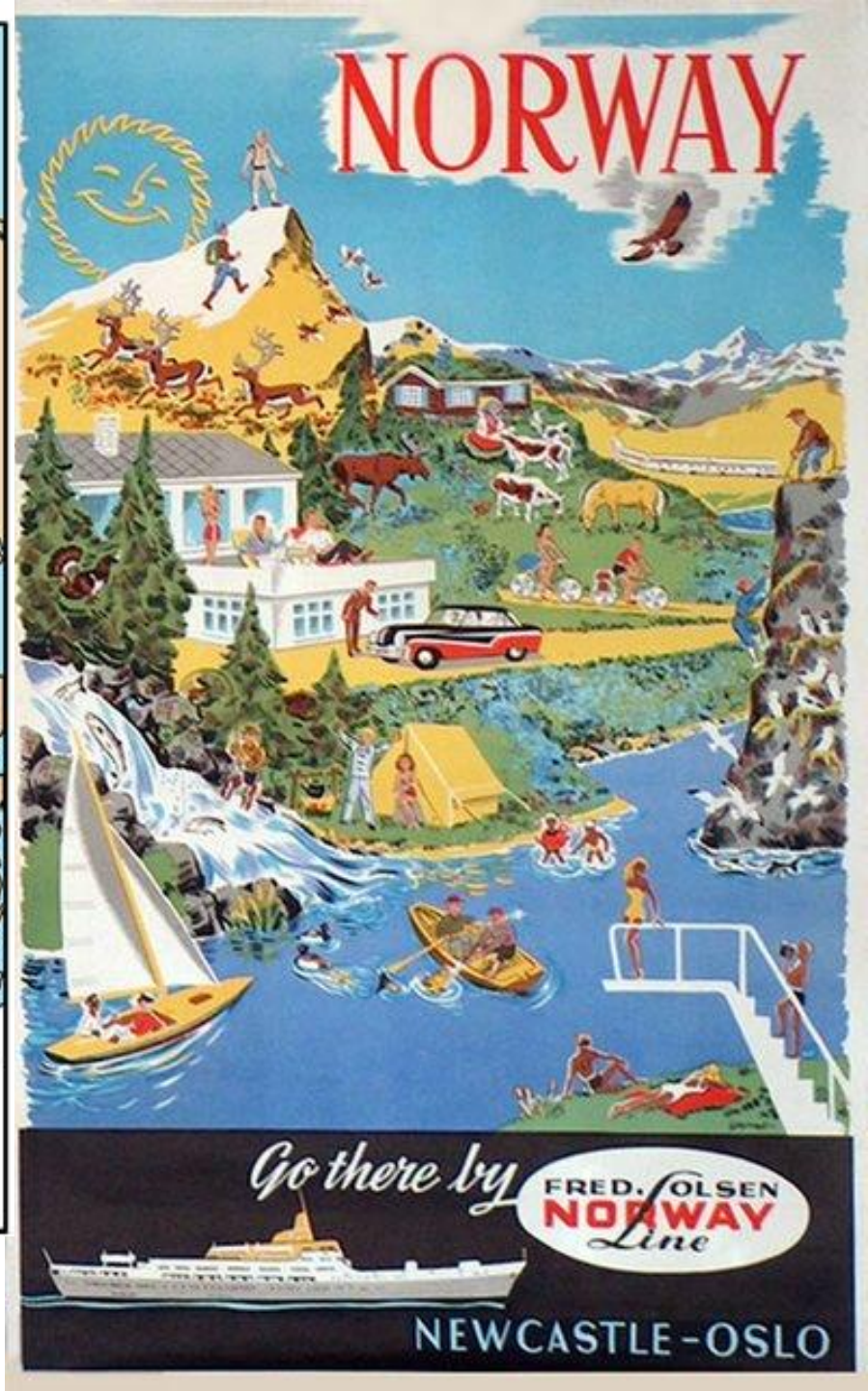
Neutrons for Life
Part 1

Jeremy Lakey

Medical School, Newcastle University, UK

Newcastle University


Shetland Is.
Orkney Is.
NORWEGIANS
SCOTLAND
IRELAND
DANES
BRITAIN
Areas of Scandinavian Settlement in 9th and 10th Centuries shown in red


NORWAY
Go there by
FRED. OLSEN
NORWAY
Line
NEWCASTLE-OSLO

A sense of scale

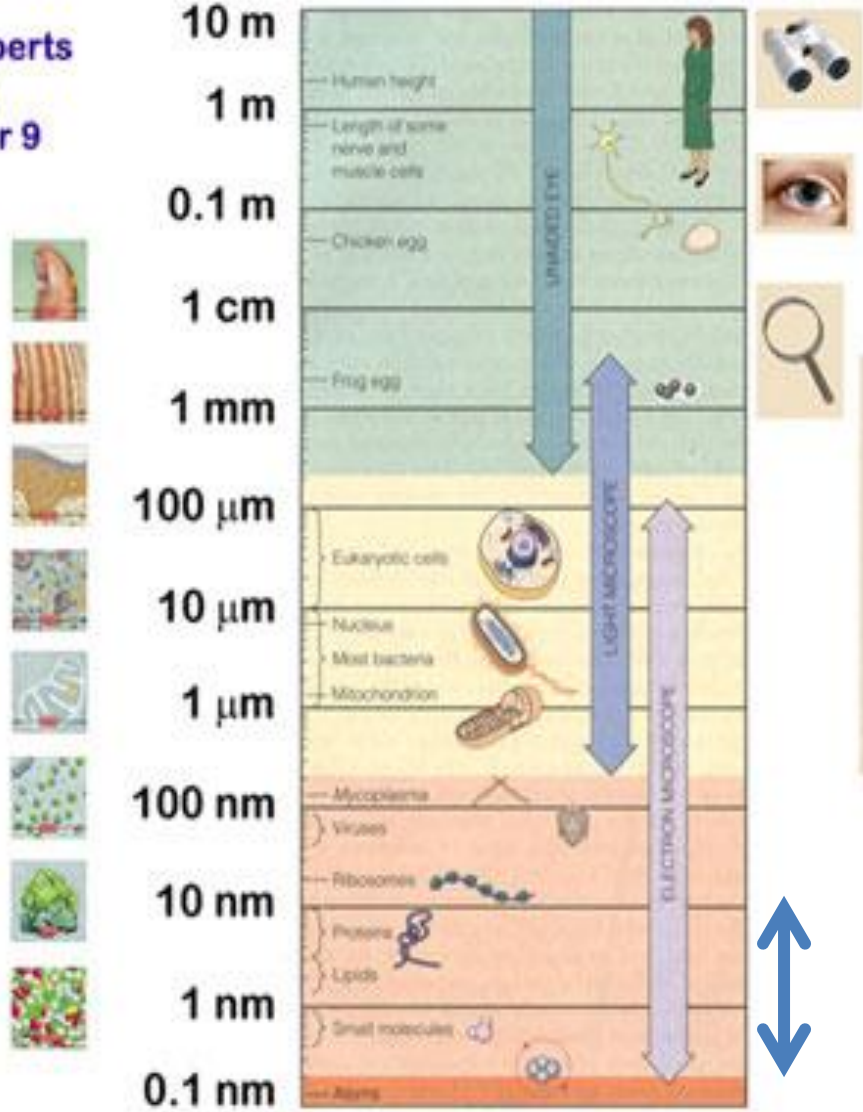
(see also http://www.jic.ac.uk/microscopy/scale.html)

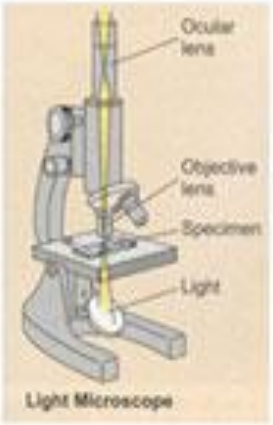
see Alberts (2007) chapter 9

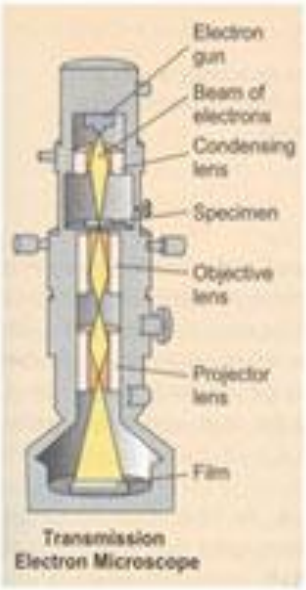
see Becker (2006) Chapter 1 & Appendix

Extend LM resolving power to see proteins

= future trend in LM





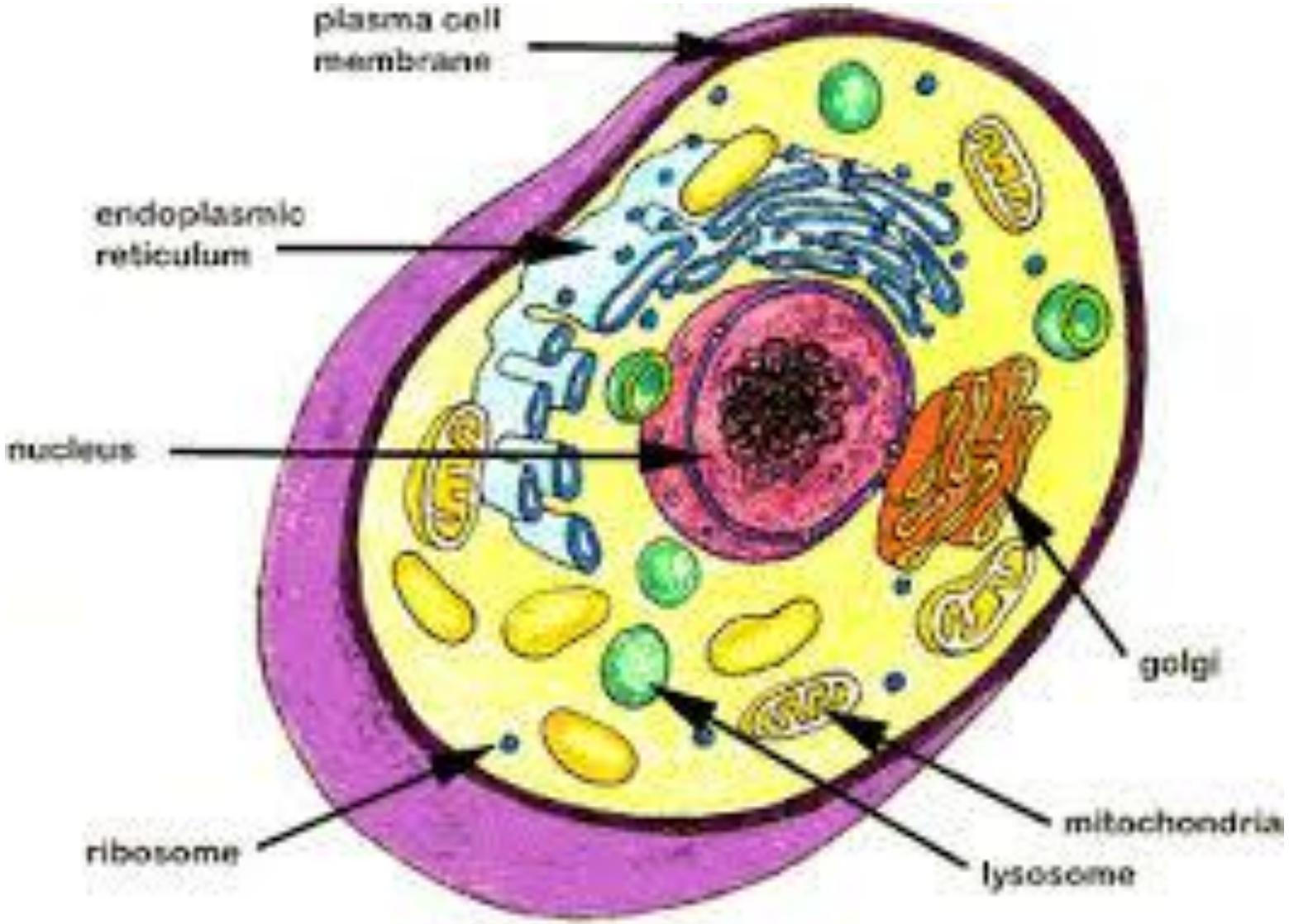


X-rays

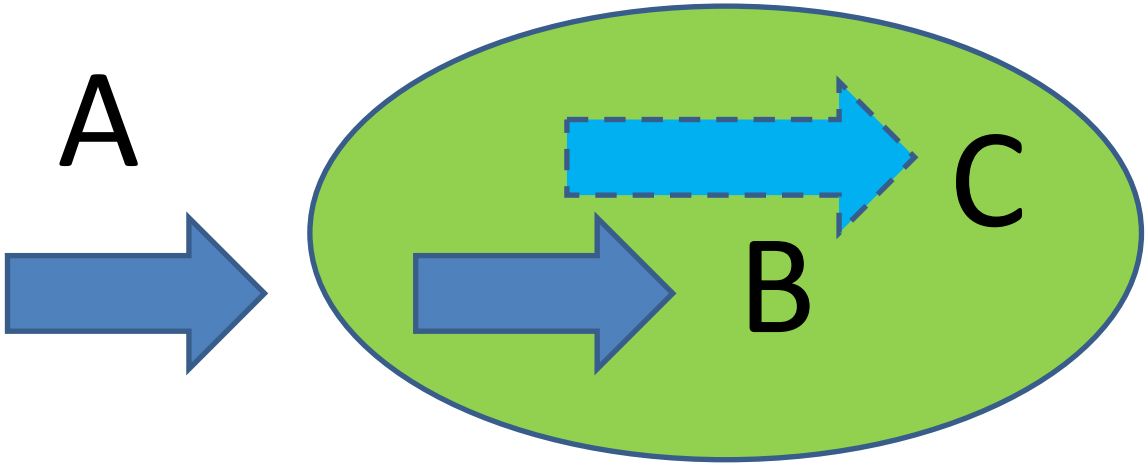
References: Alberts, B et al (2007) Molecular Biology of the Cell, chapter 9 Visualizing Cells. ISBN = 978-0815341055
Becker (2006) Thw World of the Cell, chapter 1 & Appendix. updated copy ISBN = 978-0321554185

The molecular scale in biology is the same as anywhere else.

- Bond lengths e.g. C-C ≈ 1Å
- Molecules (proteins, Nucleic acids) ≈ 1-10 nm
- Sub-cellular structures ≈ 10 -100 nm
- Cells ≈ 1-100 µm


plasma cell membrane
endoplasmic reticulum
nucleus
golgi
ribosome
lysosome
mitochondria

What do we want to know about molecular biology?



Example

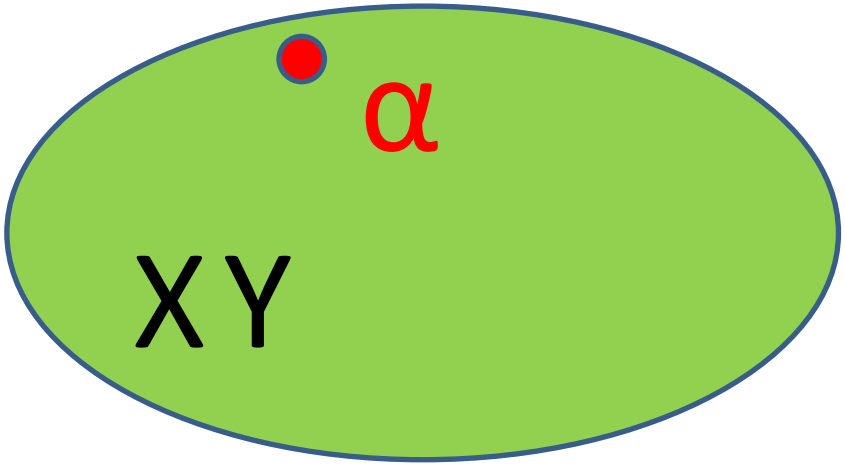
What is process B? (99% of effort) Cell division

Why does input A affect B? Cancer

Can we stop or increase B? Stop!

Can we make A cause C? Apoptosis

What data do we use?



X has a known function

X is in one part of the cell

X changes in a particular disease state.

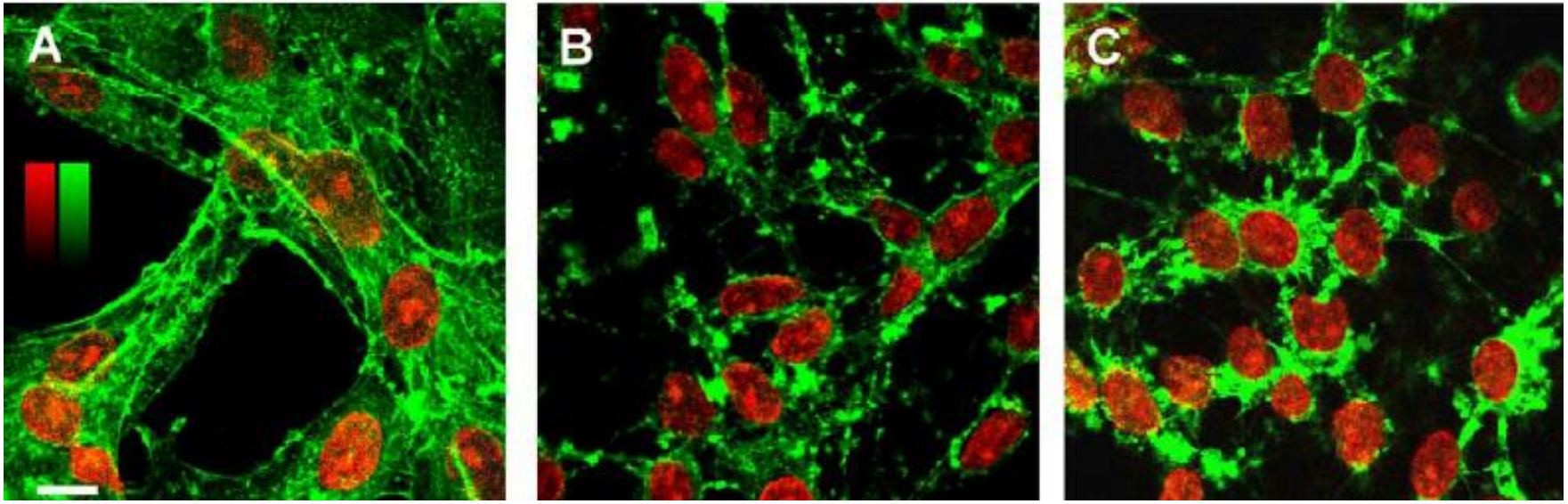
X interacts with Y

X changes the function of Y

Molecule α stops one of the above

We need methods to measure these changes.

- Effect of two molecules on the cell skeleton
- latrunculin A (0.6 µM, 15 min, Panel B) or with cytochalasin D (5 µM, 30 min, Panel C)



Scale bar = 10 µm

J Cell Sci. 2001 114(Pt 5):1025-36. Effects of cytochalasin D and latrunculin B on mechanical properties of cells. Wakatsuki, et al

We need to colour the cells

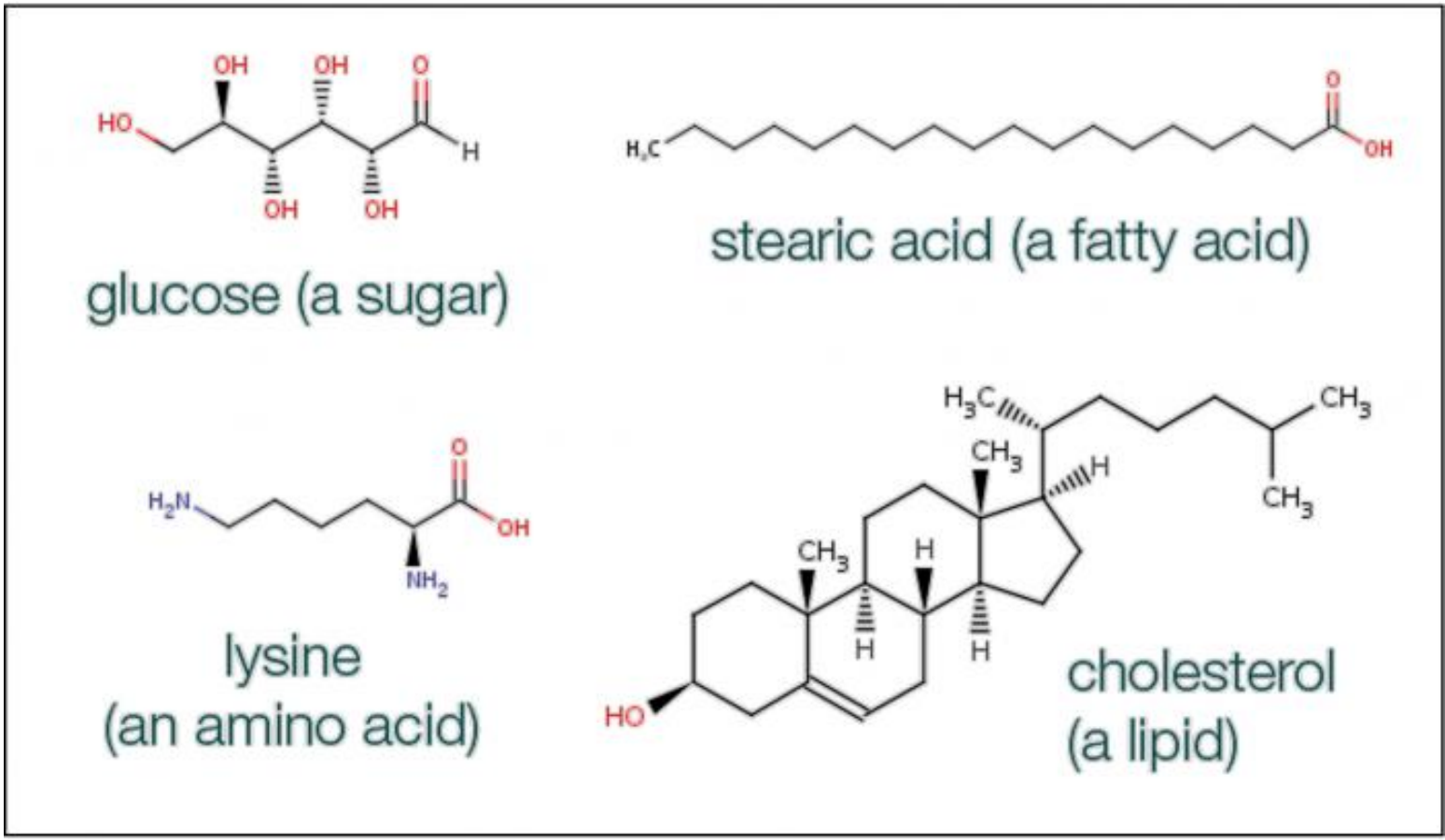
- Why?

- Biomolecules are made of similar elements and all look very similar.
- The molecular make up of cells is not obvious.

Biological building blocks

- Amino acids
- Lipids
- Sugars
- Salt
- Water

Hydrogens are not shown!



They make complex structures

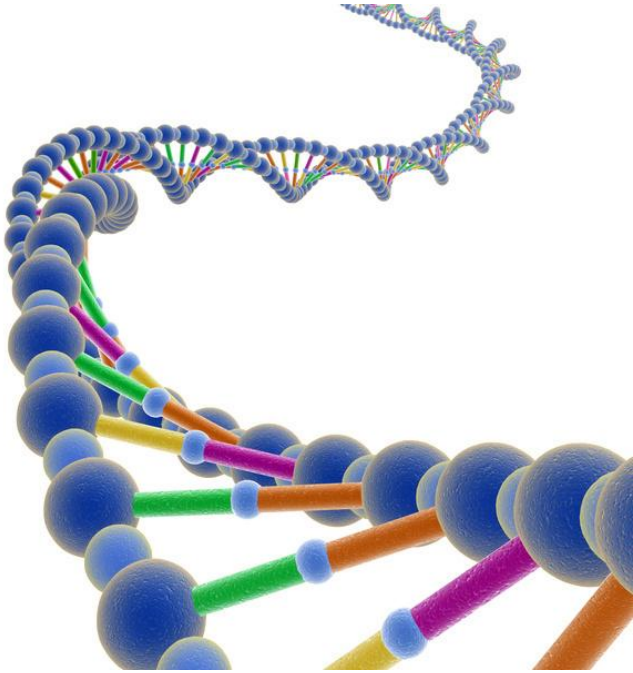
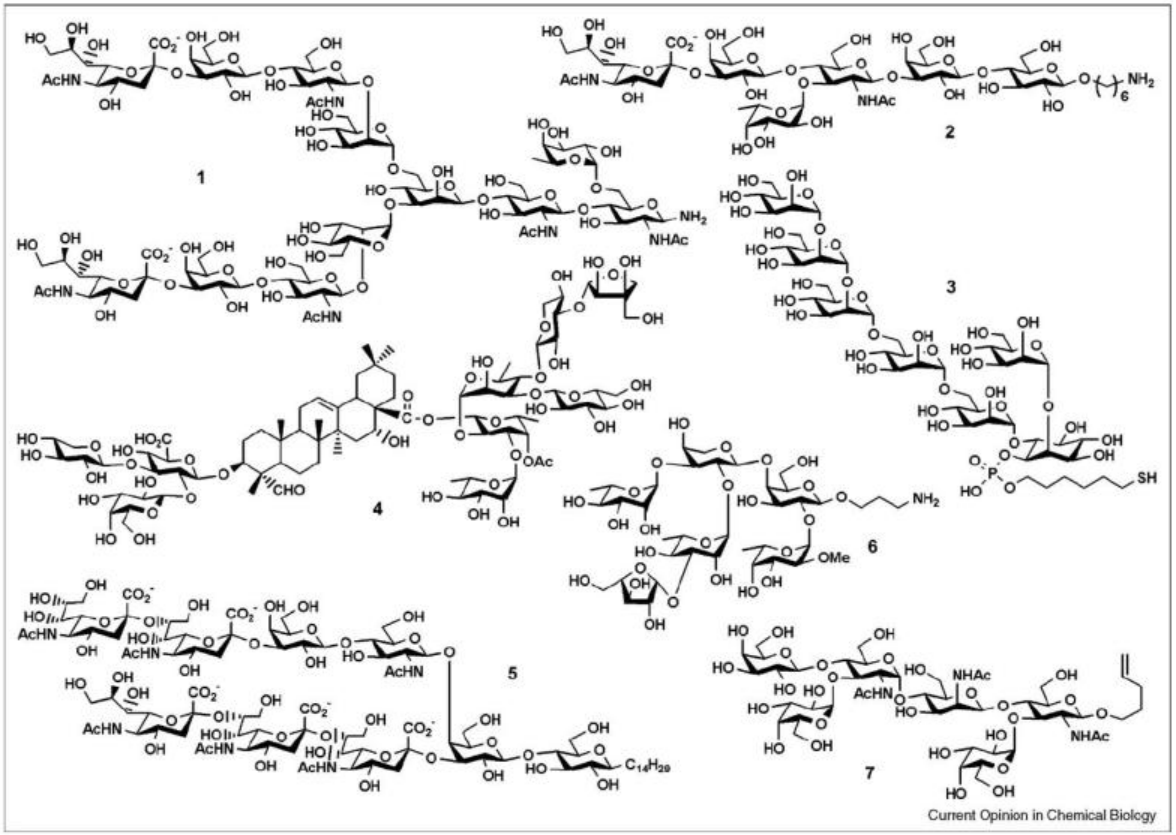
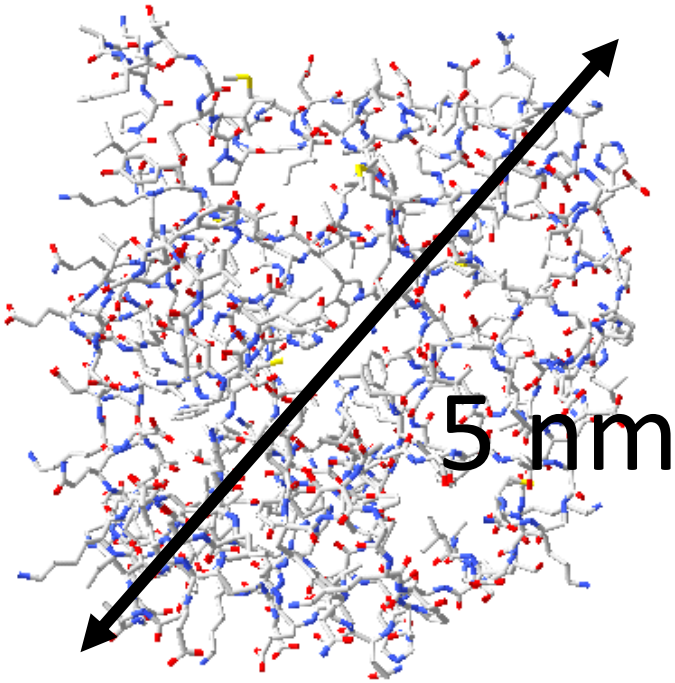


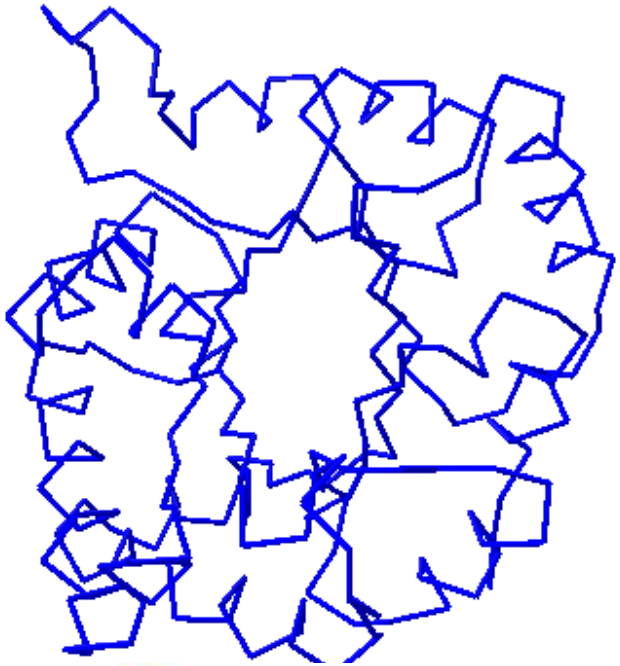
Figure 1

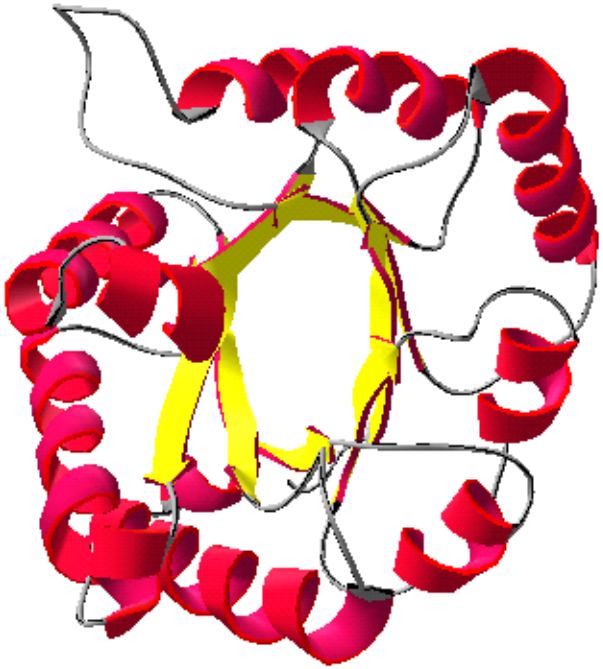


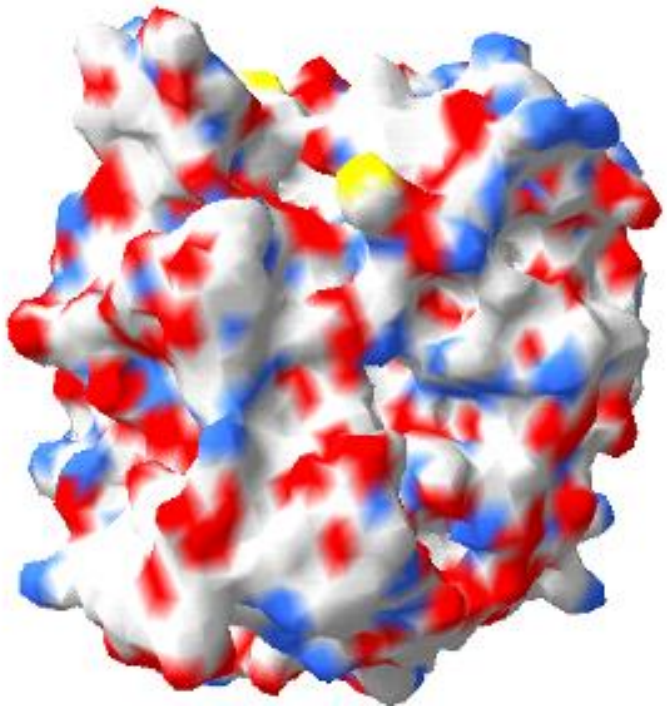
Structures of complex oligosaccharides obtained by chemical synthesis.



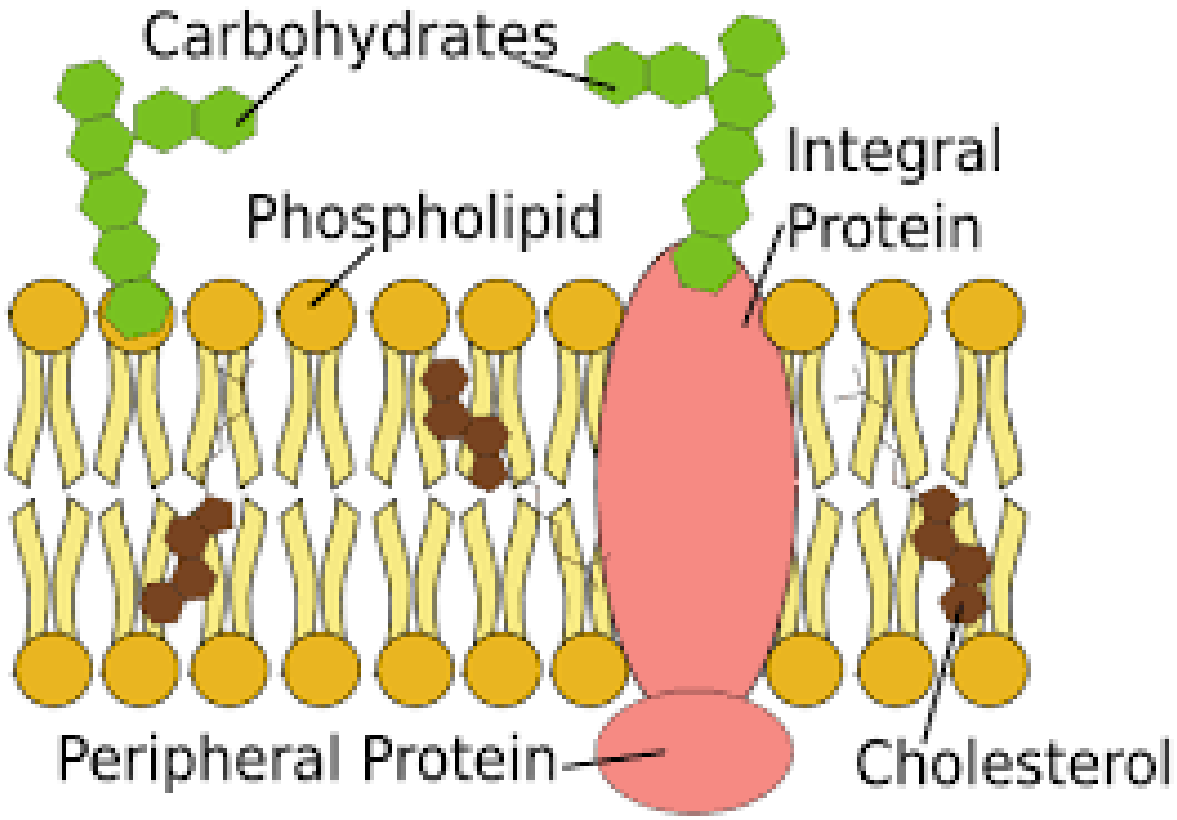
The same protein shown in different ways







Cell membrane

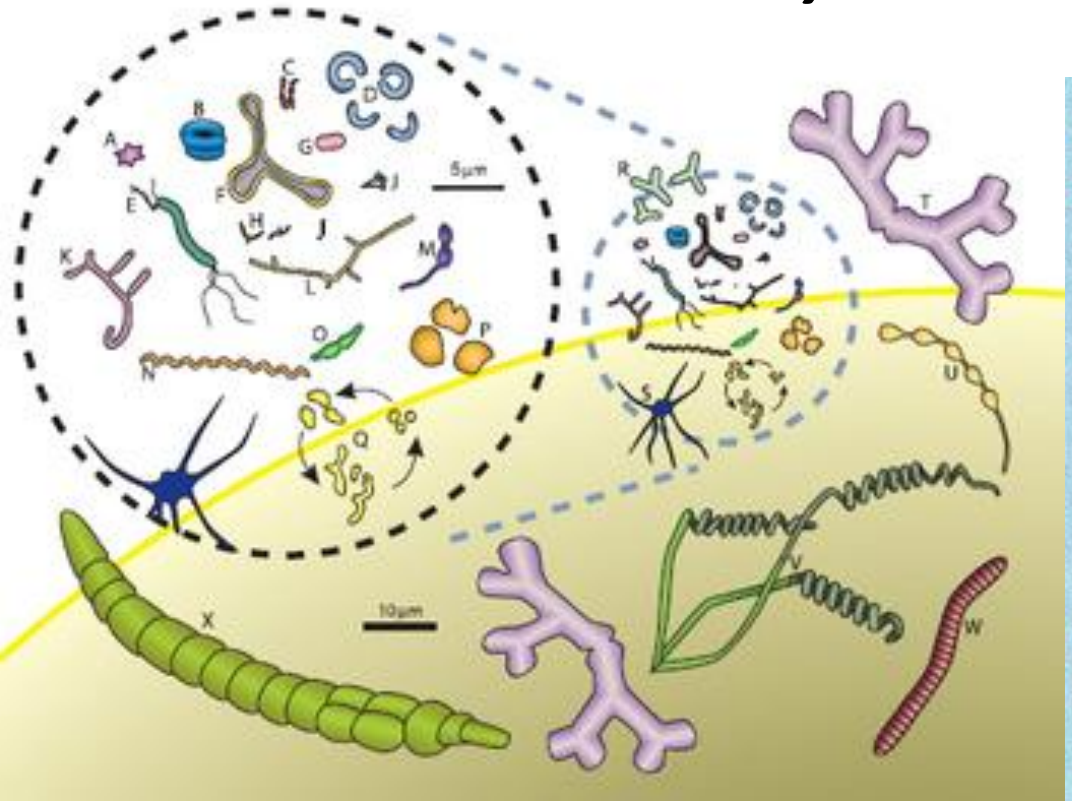


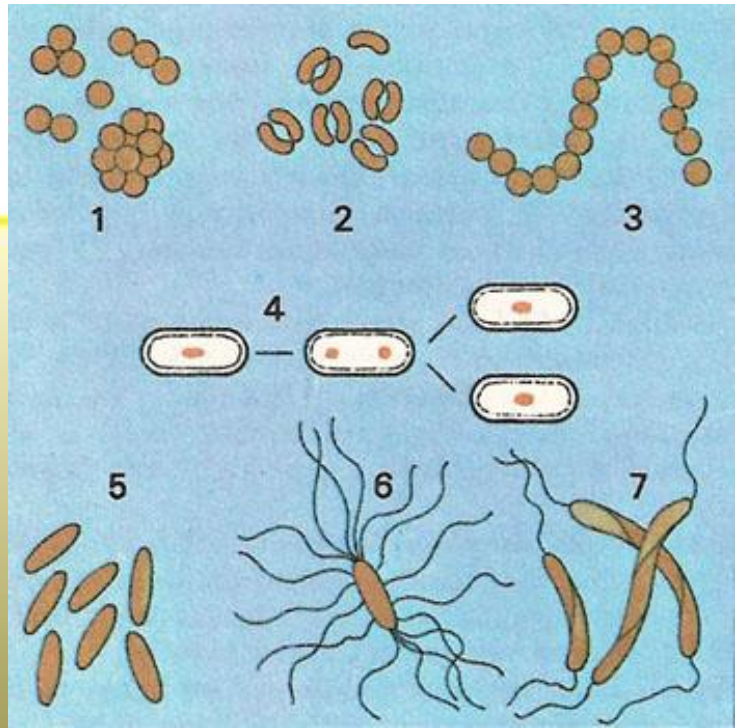
The same basic membrane design is found across biology so if we can add colour to this it will be very useful.

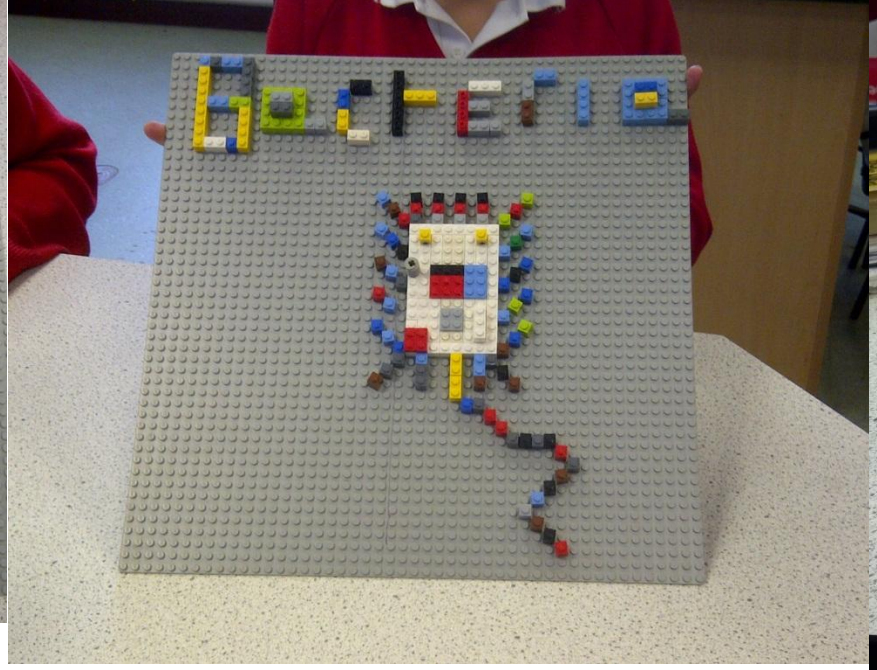
Newcastle University

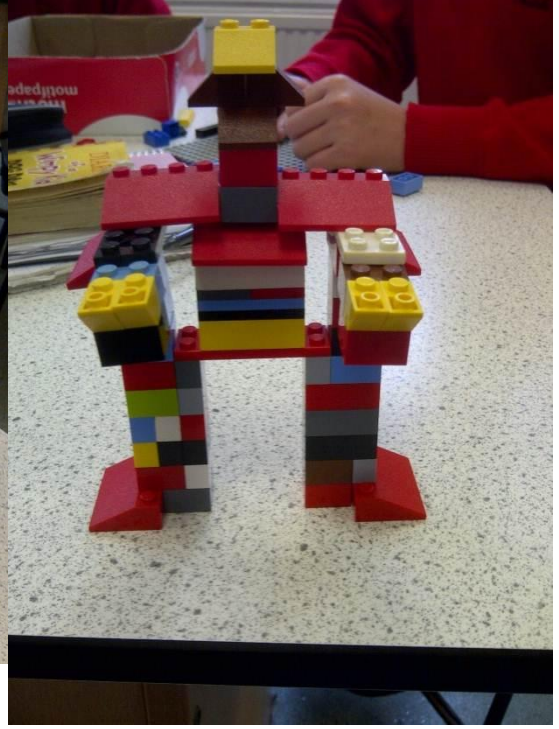
"To be brutally honest, few people care that bacteria have different shapes. Which is a shame, because the bacteria seem to care very much".

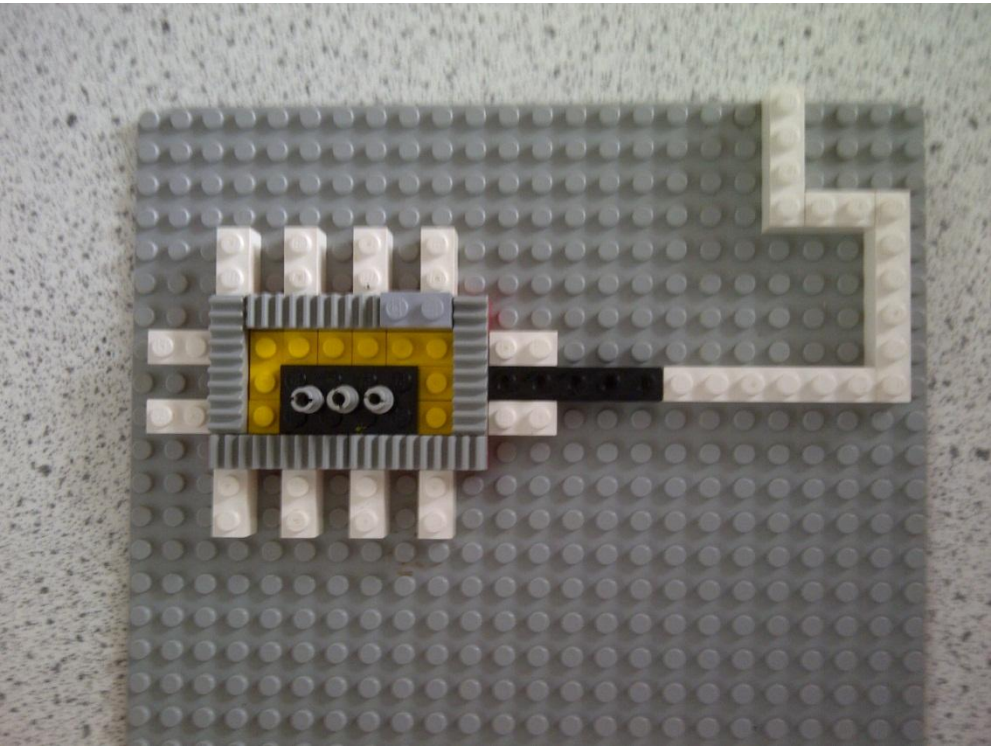
Kevin Young

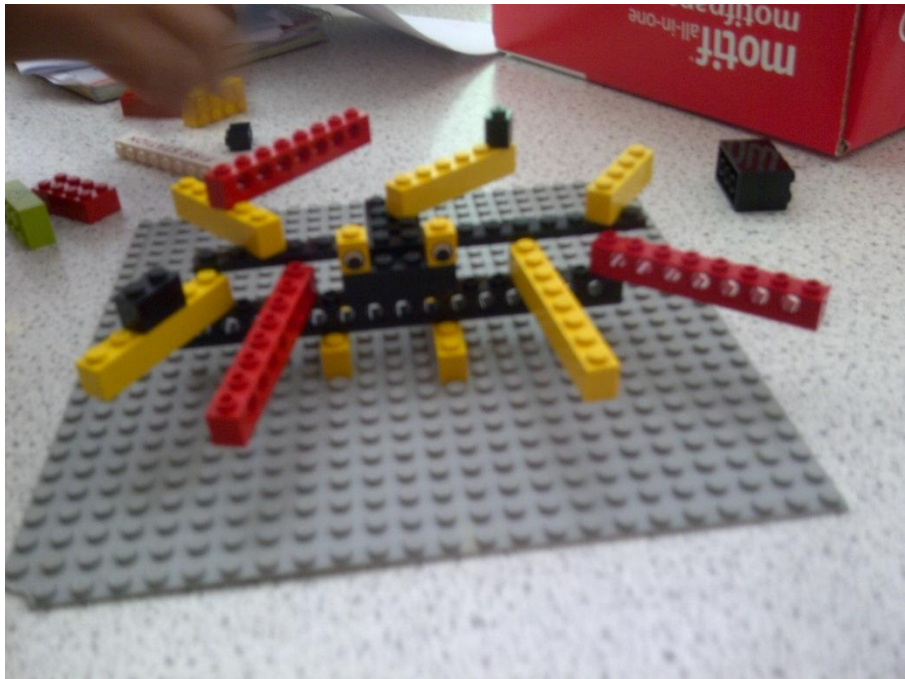





bacteria

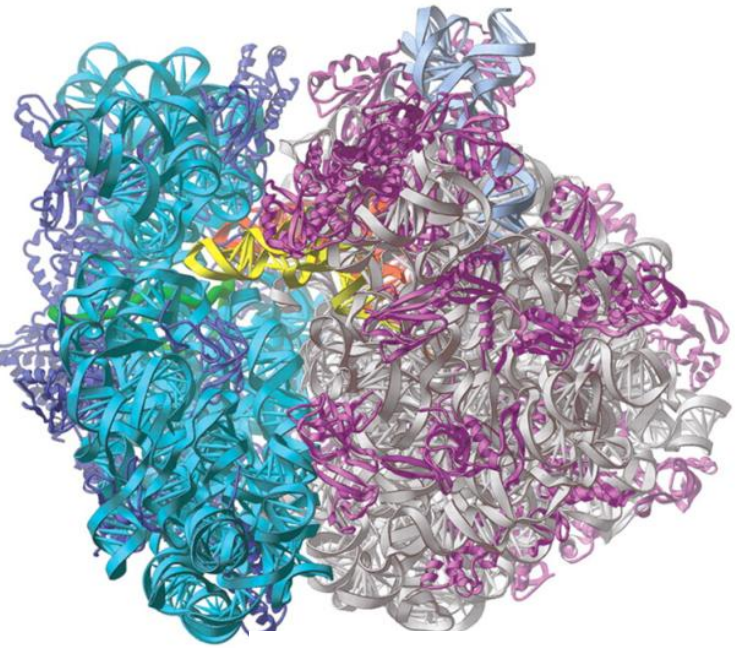





motif
all-in-one

X-ray crystallography can define large biological structures

20 nm

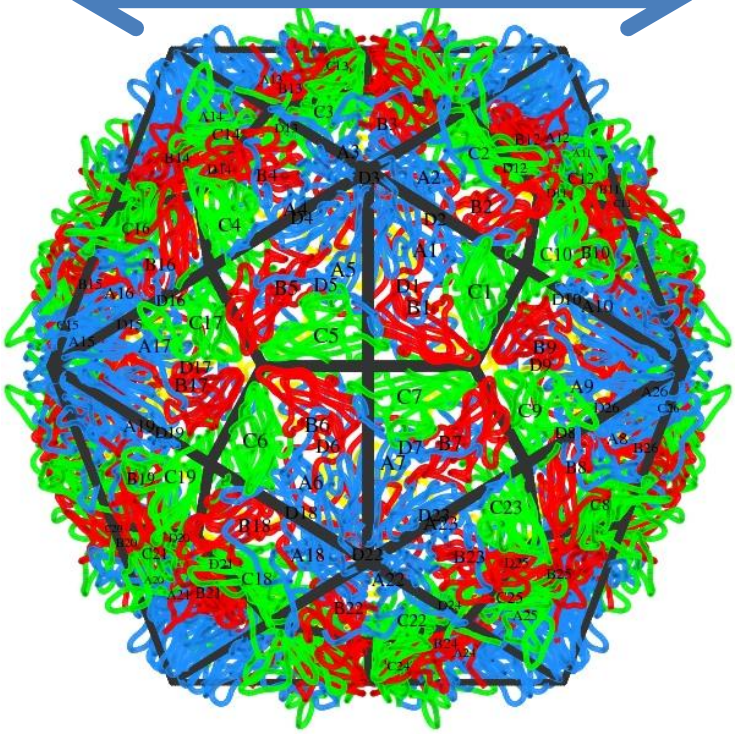


Ribosome

Selmer M,
Science. 2006 313; 1935-42.

Poliovirus ViperDB

36 nm

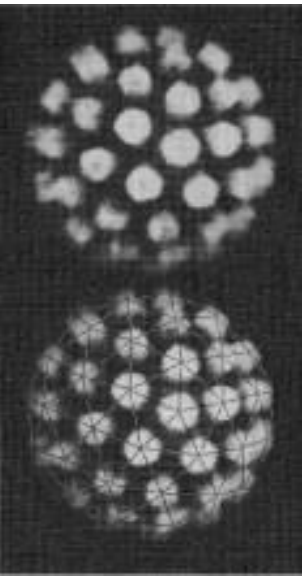


Filman DJ,
EMBO J. 1989 8:1567-79.

Electron microscopy

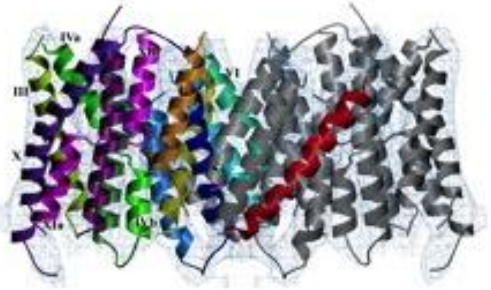
Electron crystallography

Virus

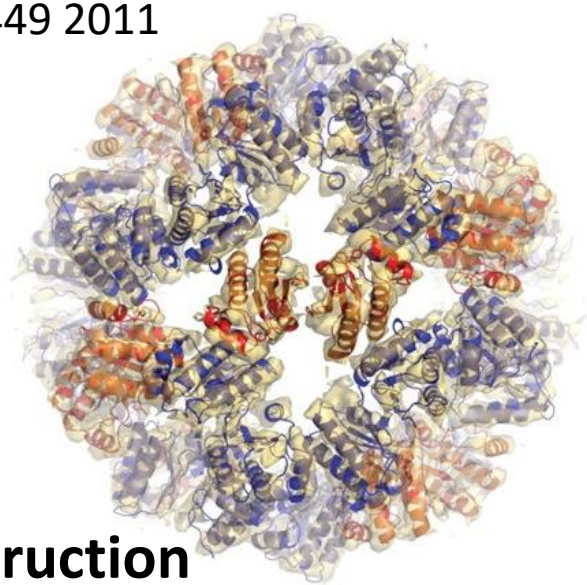


Aaron Klug ,
Nobel prize

Membrane protein



Goswami, EMBO JOURNAL 30
Pages: 439-449 2011

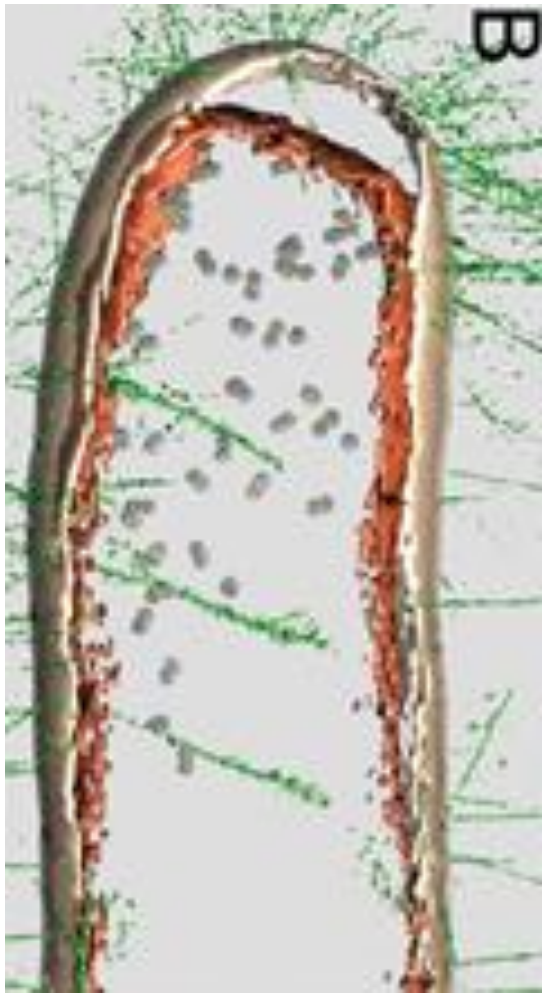


Single particle reconstruction

Marles-Wright J Science 322 (2008) 92-96

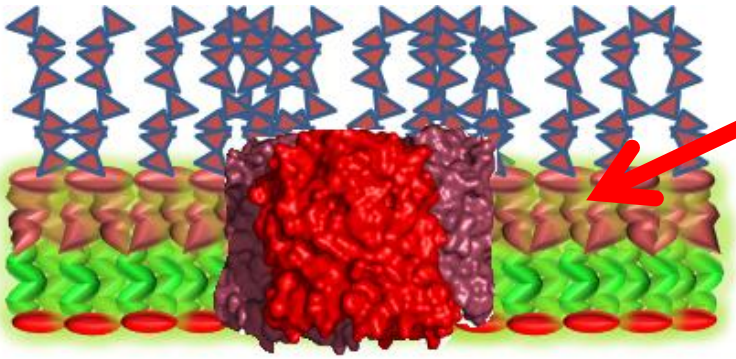
Electron Tomography

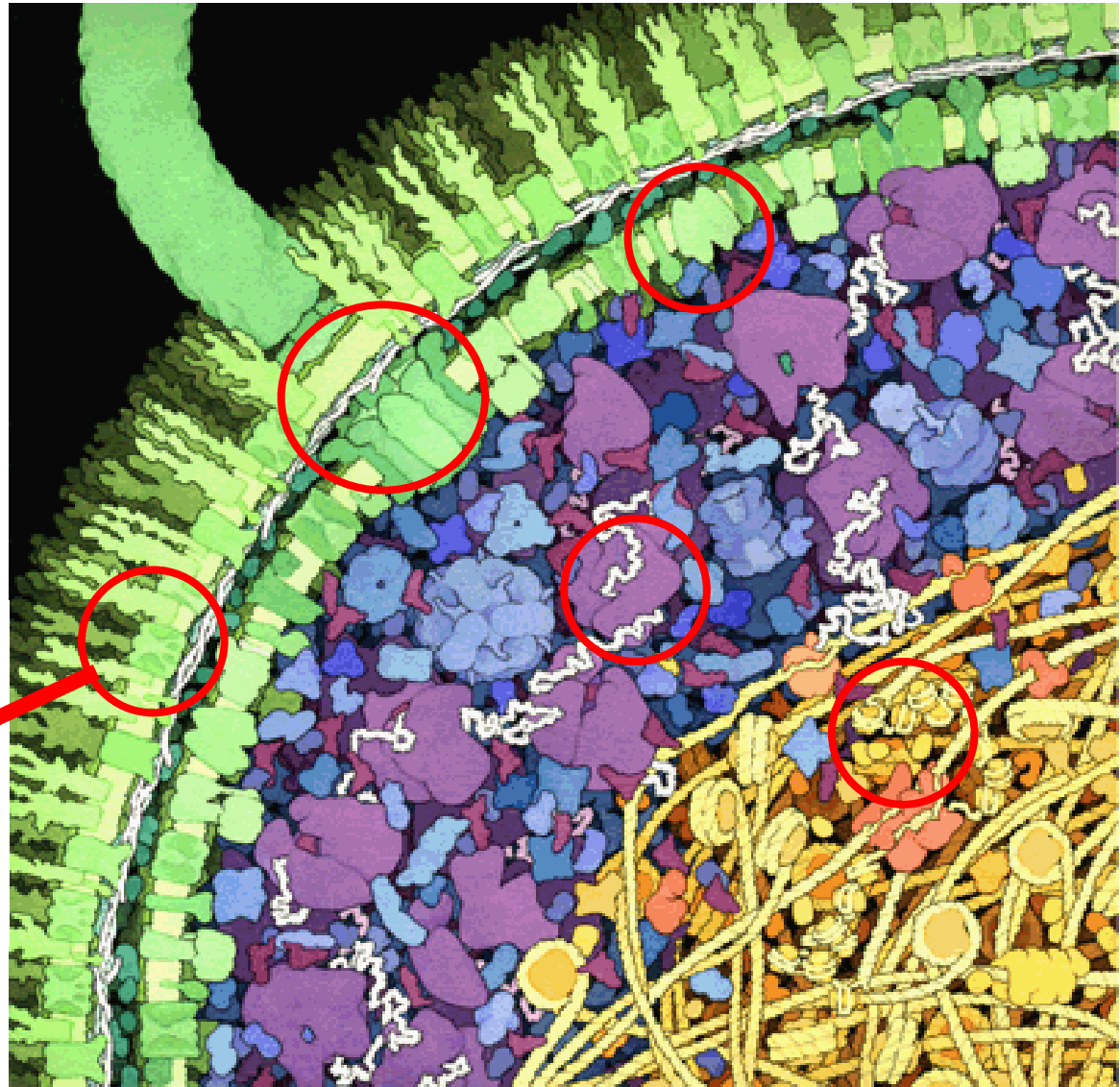
Ortiz et al. JCB 190 (4): 613



Why not just use X-rays and electrons?.

What we often lose in these methods are dynamics or molecular contexts.



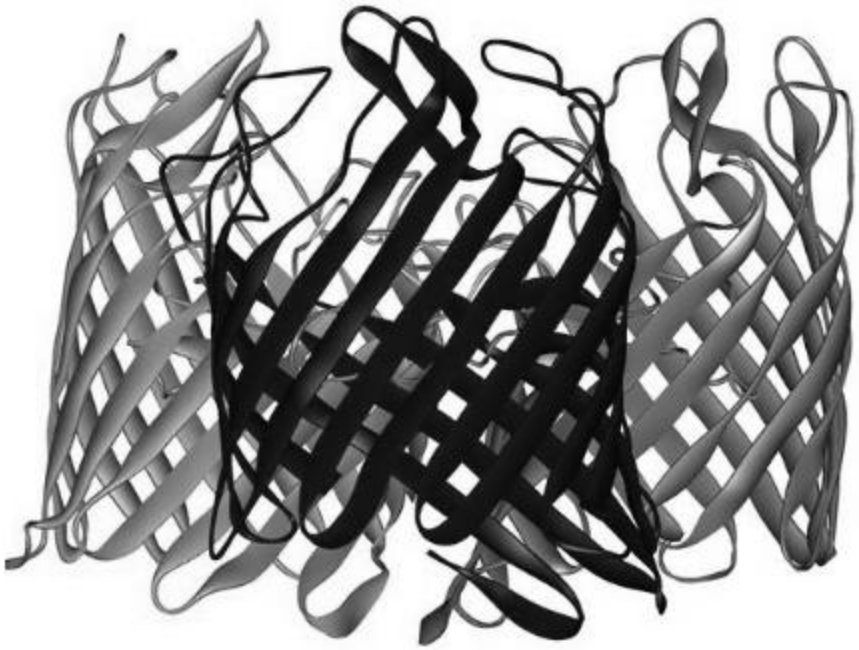



Can I help?
MR NEUTRO

Why can neutrons help?

- We can work in water.
- We can resolve dynamics.
- We can see Hydrogen
- We can change contrast
- We don’t damage the molecules.

OmpF Protein



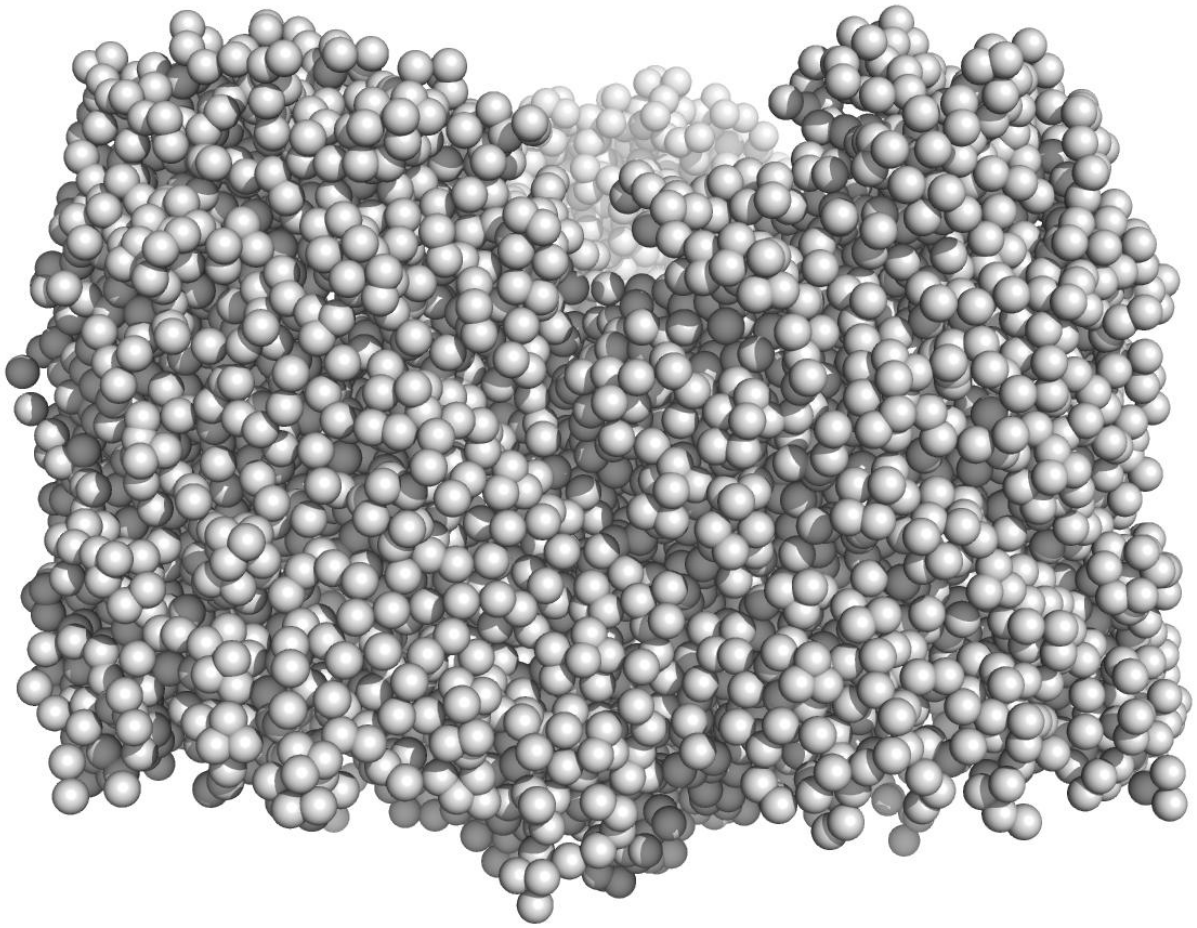
Side view

Top view

10 nm

OmpF Protein

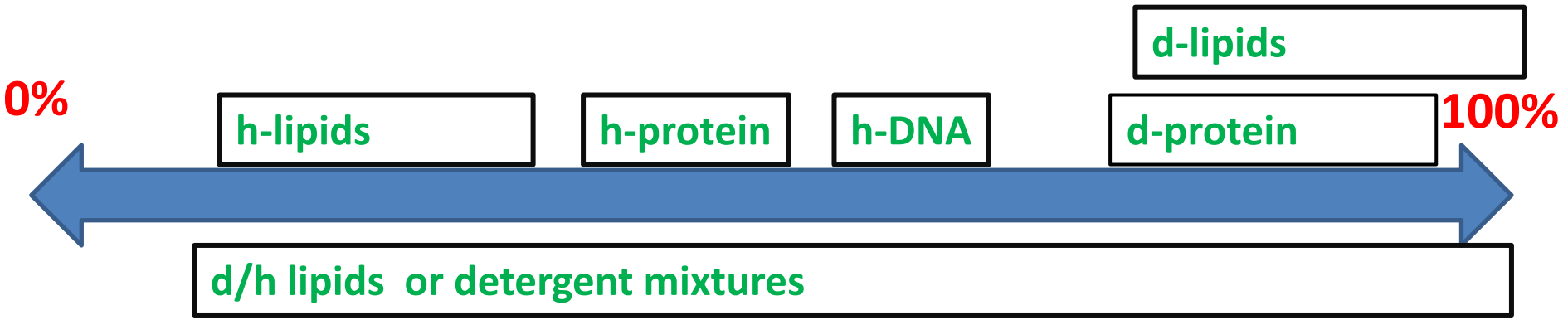
- OmpF Protein showing only the hydrogens but it’s monochrome grey.



The best things in life are free
But you can keep 'em for the birds and bees
Now give me contrast (that's what I want)
That's what I want (that's what I want)
That's what I want (that's what I want) yeah
That's what I want

The Beatles

The D_2O scale of bio-contrast



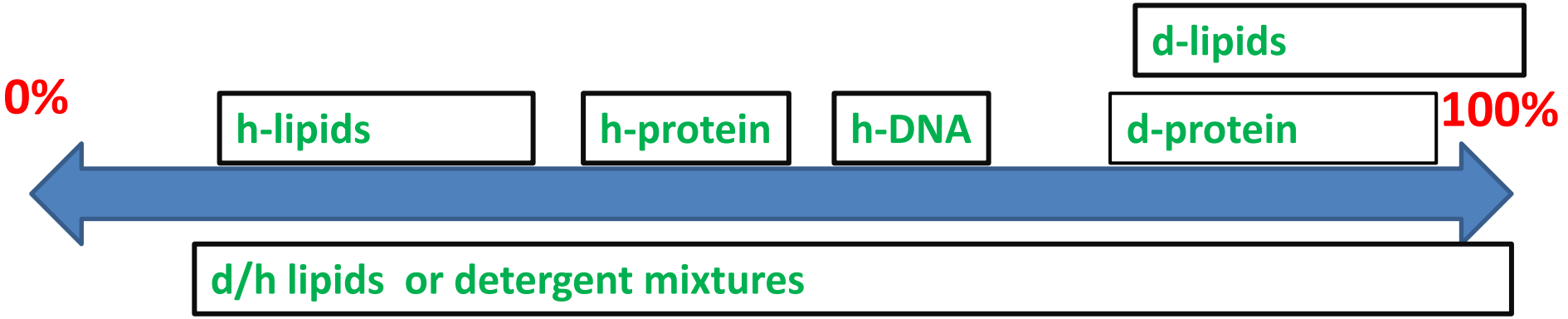
Scattering length density

Contrast matching- using the neutron “refractive index”

High refractive index glass in water is visible

High refractive index glass in high refractive index salt solution

The D_2O scale of bio-contrast



We can match any value on this axis using D_2O

Simple examples

- Seeing important water molecules.
- Seeing important membrane lipids.
- Seeing biology within complex apparatus
- Seeing Biology in complex chemical mixtures.

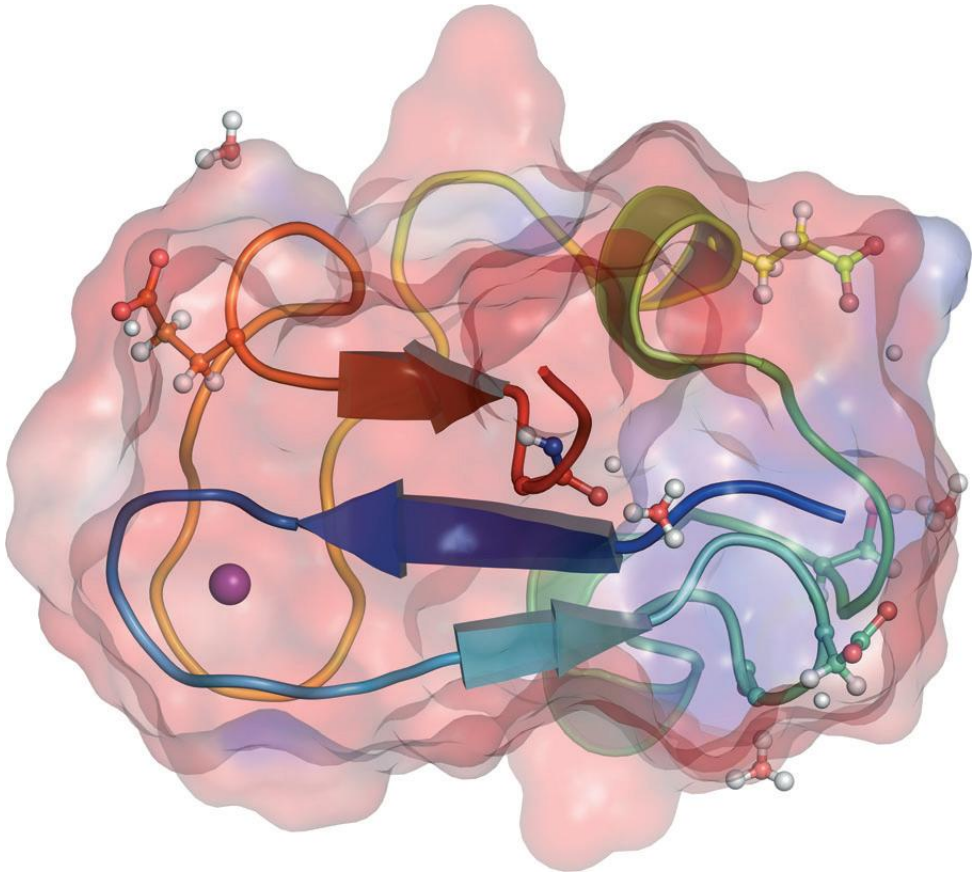
Angewandte
Communications

Protein Perdeuteration

DOI: 10.1002/anie.201207071

Near-Atomic Resolution Neutron Crystallography on Perdeuterated *Pyrococcus furiosus* Rubredoxin: Implication of Hydronium Ions and Protonation State Equilibria in Redox Changes**

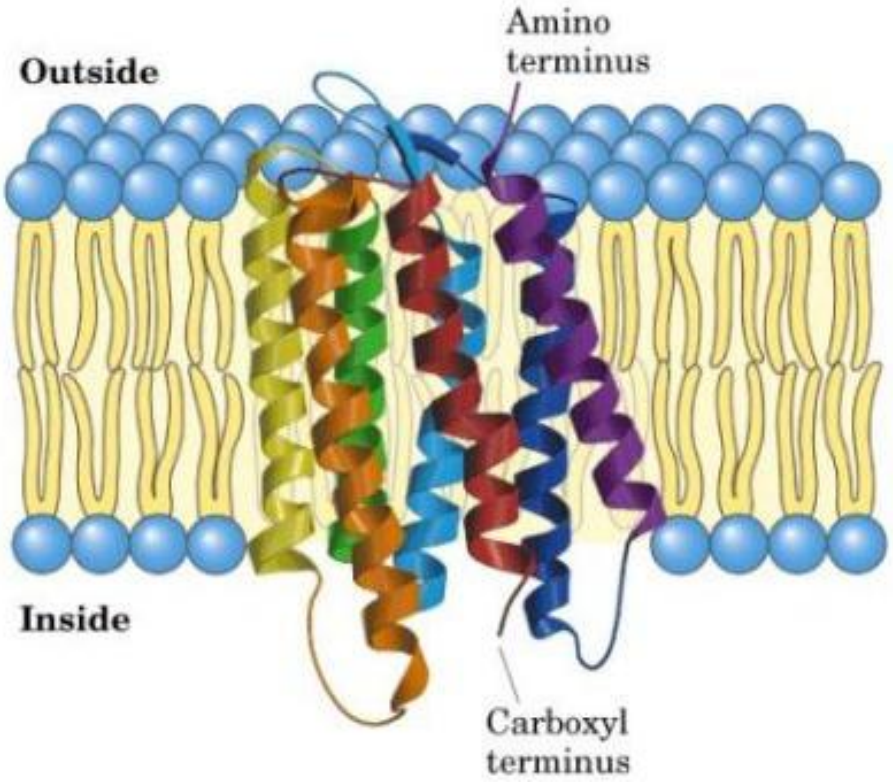
*M. G. Cuypers, S. A. Mason, M. P. Blakeley, E. P. Mitchell, M. Haertlein, and V. Trevor Forsyth**

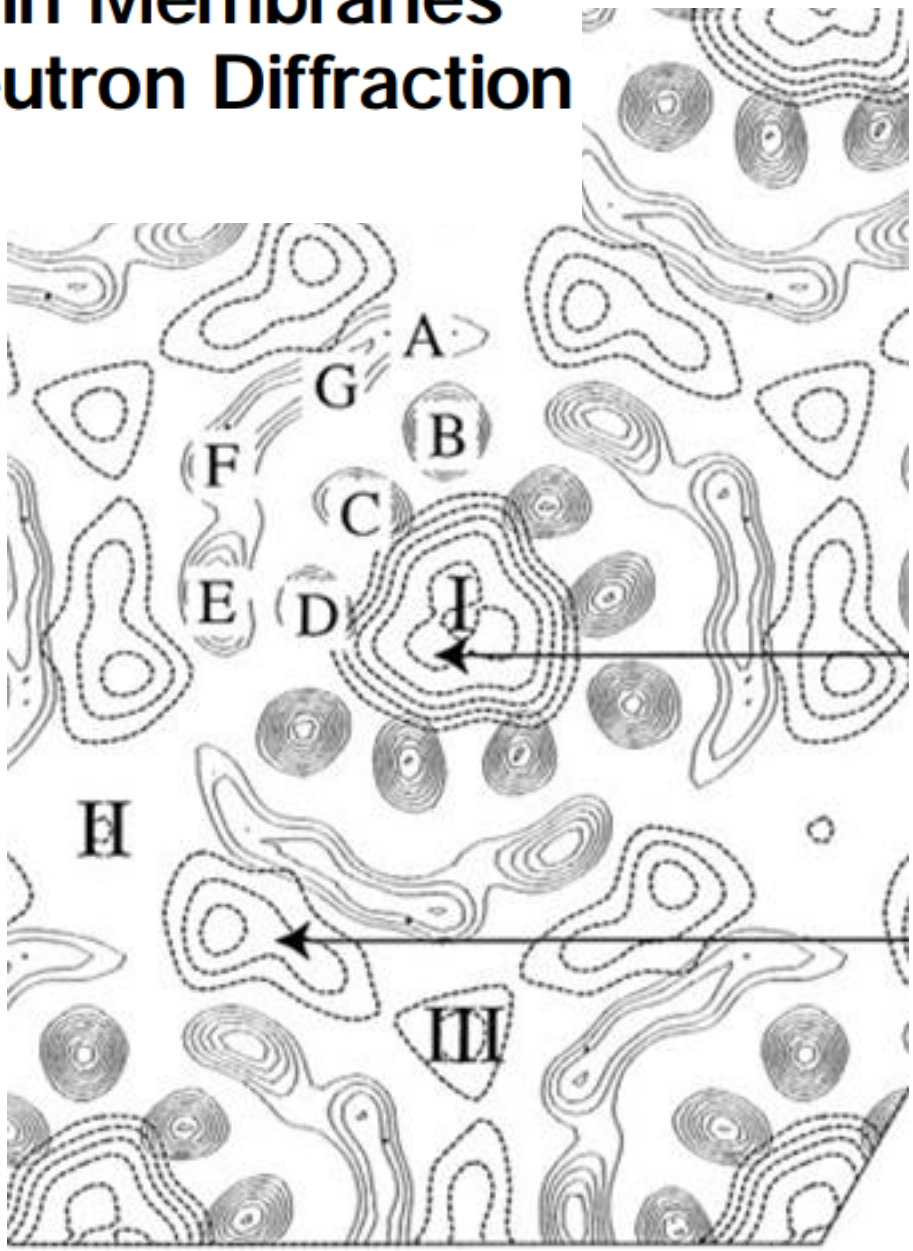


Molecular Cell, Vol. 1, 411–419, February, 1998, Copyright ©1998 by Cell Press

Localization of Glycolipids in Membranes by In Vivo Labeling and Neutron Diffraction

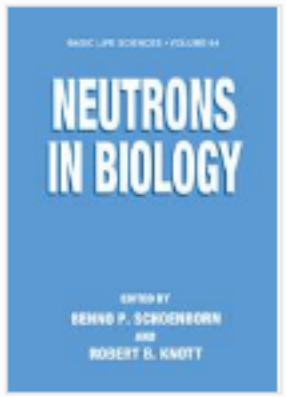
Martin Weik,*‖ Heiko Patzelt,‡‖
Giuseppe Zaccai,*†§ and Dieter Oesterhelt‡

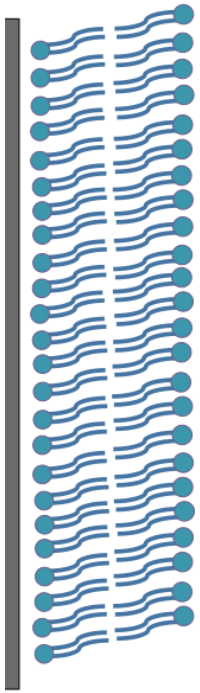


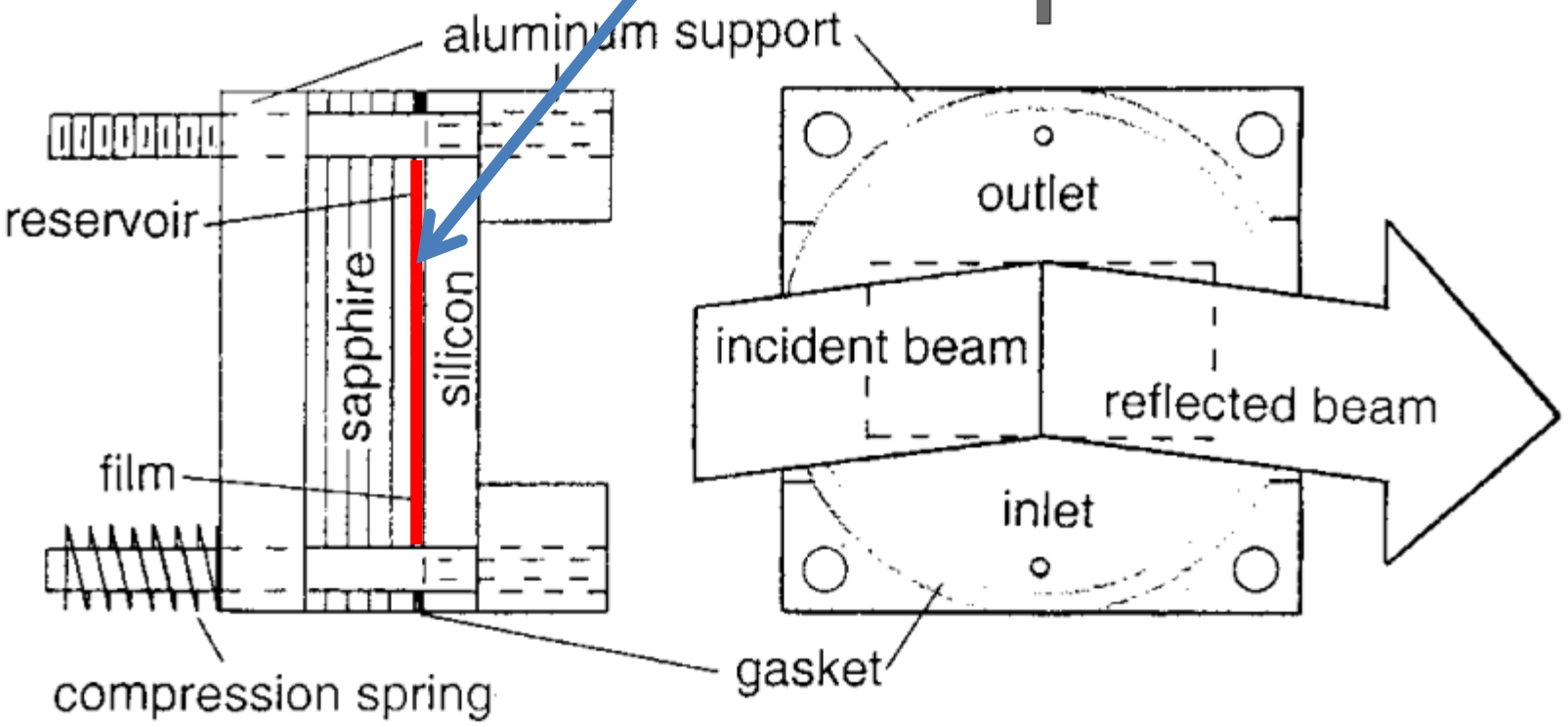


Neutron Reflectivity Studies of Single Lipid Bilayers Supported on Planar Substrates

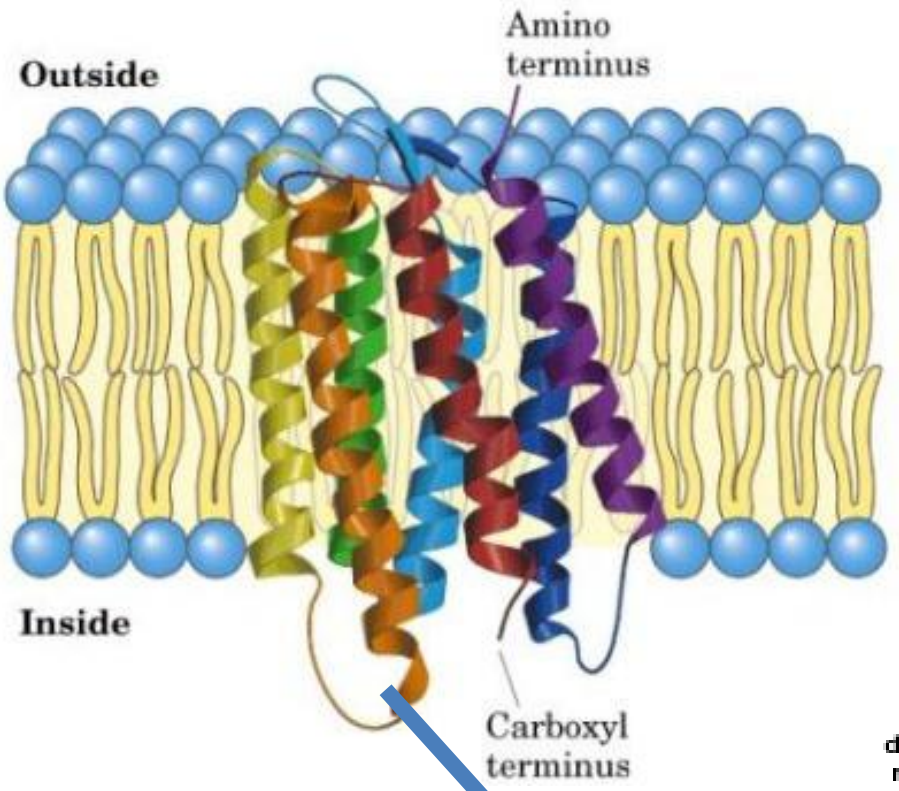
S. Krueger
B. W. Koenig
W. J. Orts
N. F. Berk
C. F. Majkrzak
K. Gawrisch

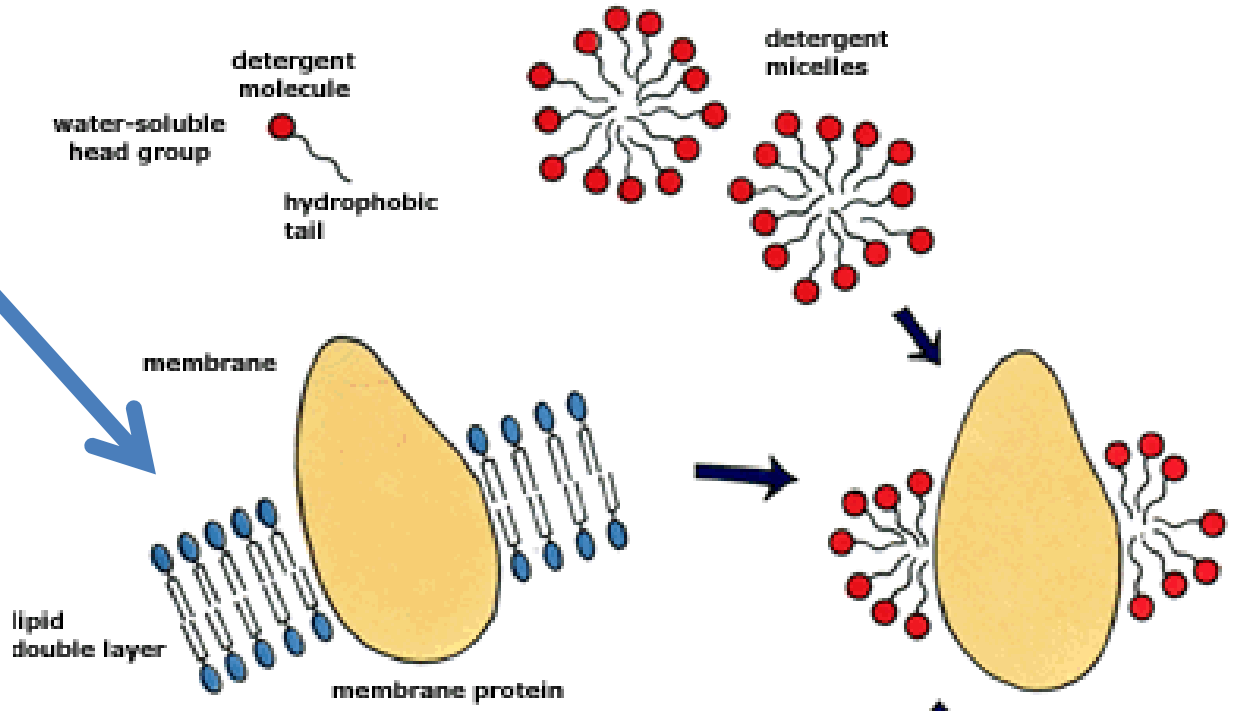






Purifying membrane proteins in detergent micelles.





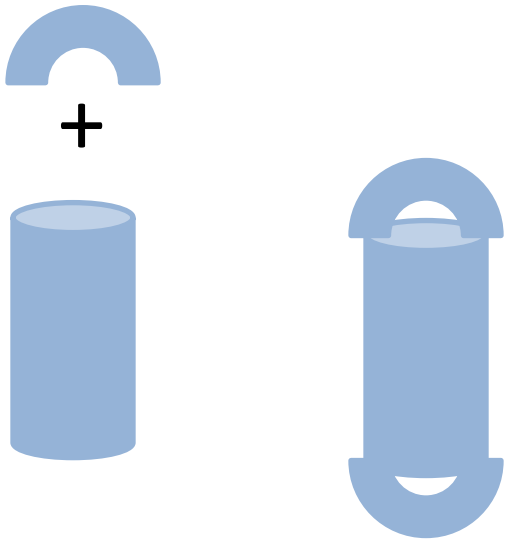
Contrast matching- using the neutron “refractive index”

High refractive index glass in water is visible

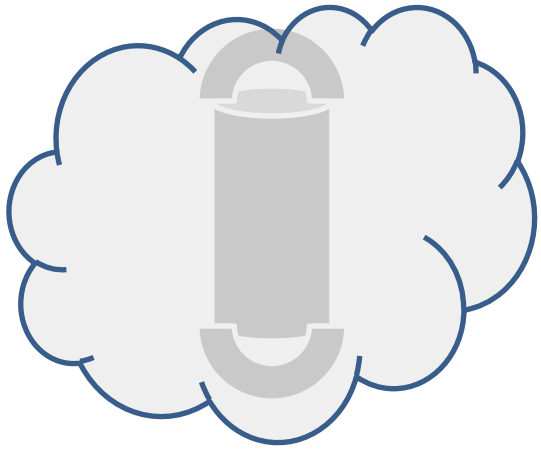
High refractive index glass in high refractive index salt solution

We want to solve a membrane protein complex made of two proteins

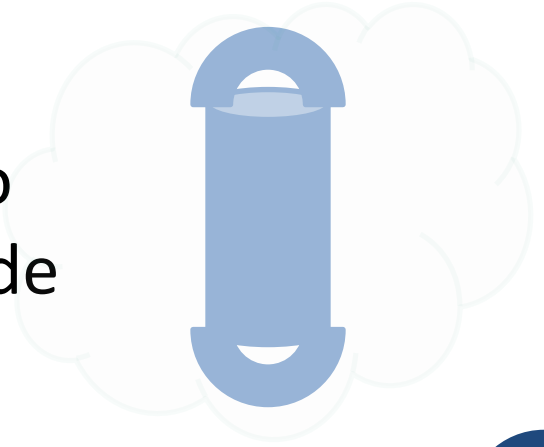
Membrane proteins have to be kept in solution by the use of detergent micelles which surround the protein.



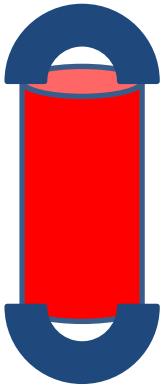
So X ray scattering would be dominated by detergent scattering.

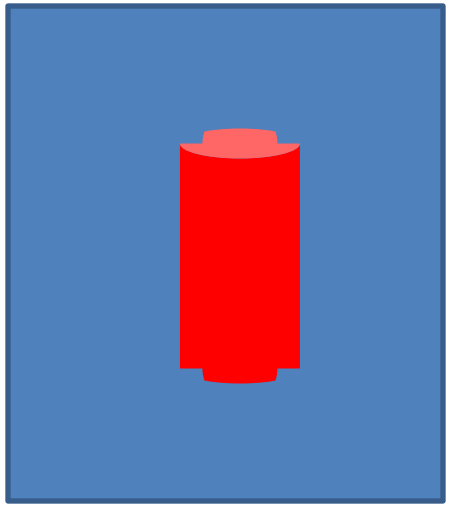


In a neutron experiment we can use deuterated detergents to match them to the water SLD, thus the detergent is made invisible.

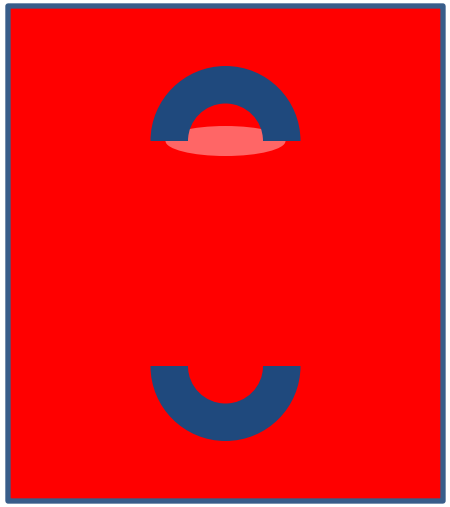


Then by making one protein deuterated we can make it visible when mixed with the natural protein





In H_2O

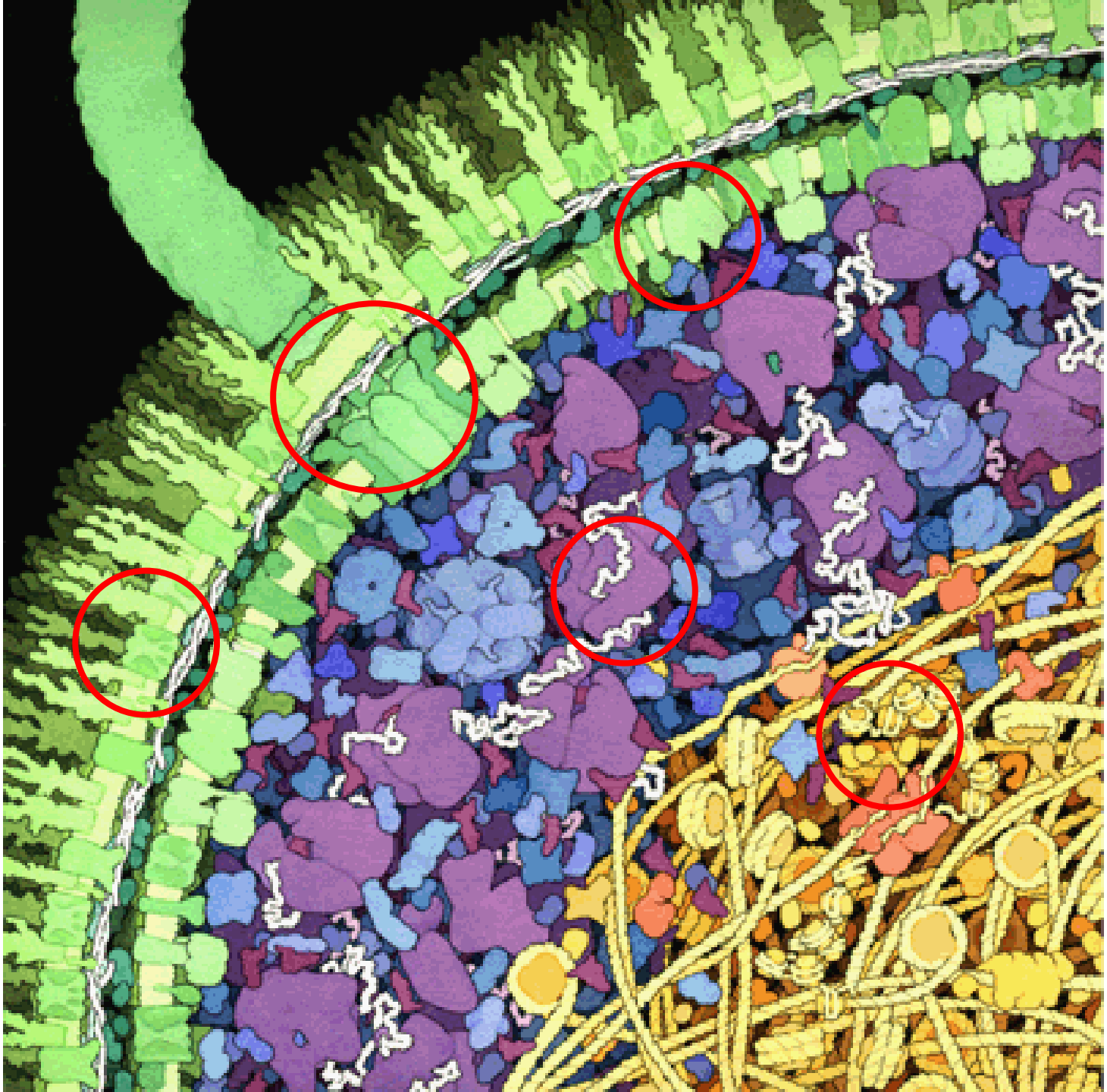


In D_2O

Thus we can resolve the different components

Contrast Matching- water background is adjusted by adding D_20

- We can make proteins in bacteria that are grown in H_20 or D_20 or mixtures.
- This can give proteins that match between 40-100% D_20
- Lipids/detergents can be deuterated so are useable in a range 12%-100% D_20
- ^1H Nucleic acids = 65% D_20



The Perils of Reductionism (1972)
Albert Szent-Gyorgi

Nobel Prize in Physiology or Medicine in 1937. He is credited with discovering vitamin C and the components and reactions of the citric acid cycle.

"My own scientific career was a descent from higher to lower dimension, led by a desire to understand life. I went from animals to cells to bacteria, from bacteria to molecules, from molecules to electrons.

The story had its irony, for molecules and electrons have no life at all.

On my way, the life I was trying to study ran out between my fingers."

PLOS BIOLOGY

The in vivo structure of biological membranes and evidence for lipid domains

Jonathan D. Nickels, Sneha Chatterjee, Christopher B. Stanley, Shuo Qian, Xiaolin Cheng, Dean A. A. Myles, Robert F. Standaert, James G. Elkins, John Katsaras

Published: May 23, 2017 • https://doi.org/10.1371/journal.pbio.2002214

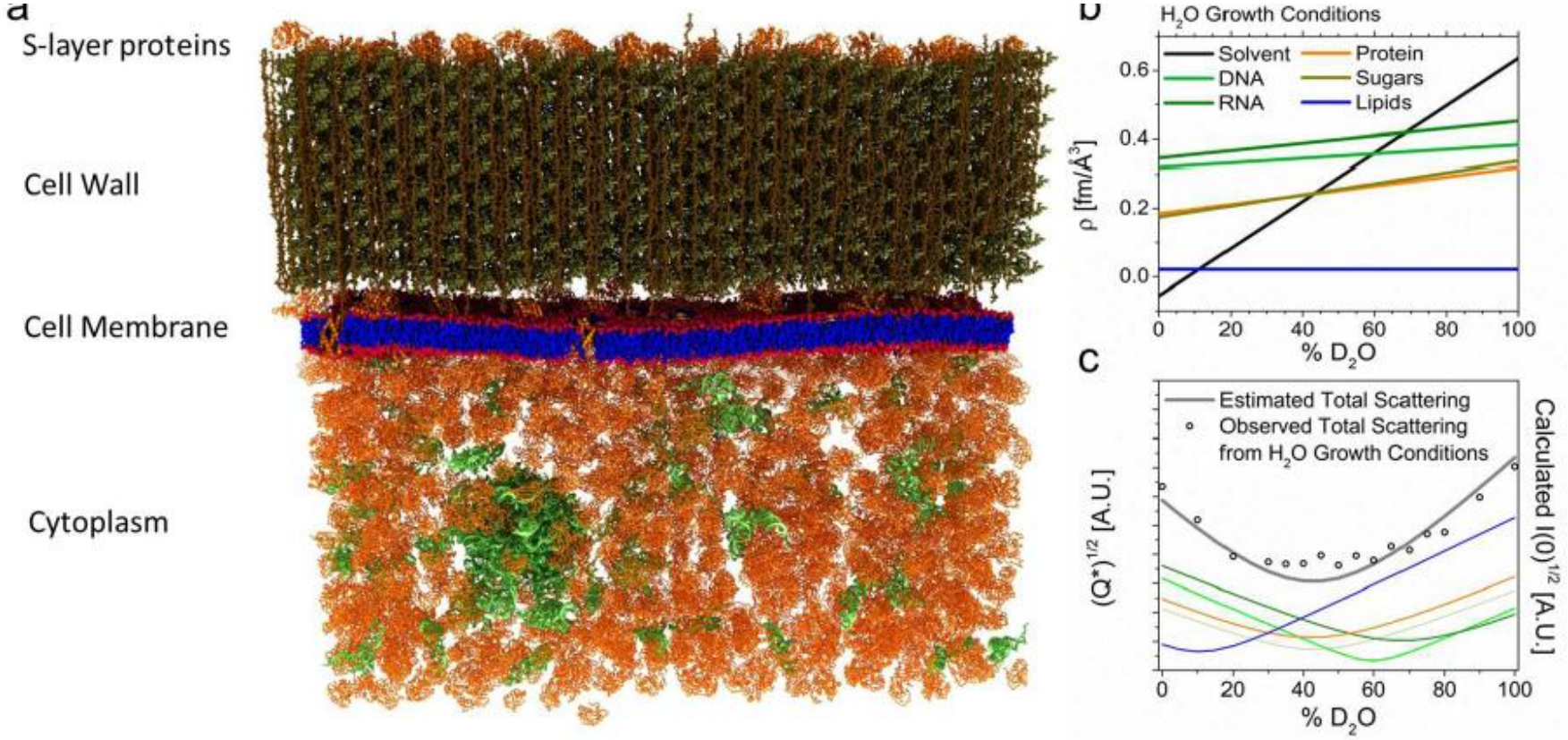


Fig 1. Envelope structure and scattering properties of *B. subtilis*. (a) Representation of the cell wall, the membrane and

Concluding thoughts

- Biophysics has many tools which are always cheaper than neutrons – use them first.
- Biological samples are often the most complex samples and often prepared on site.
- Very careful sample preparation is the key to using beam time effectively.
- You need to know the capabilities / limits / needs of each technique.
- Leave the neutron science to the specialists

Thank You

SwedNess NNSP Intro Course in Neutron Scattering

Tartu, Estonia 9-21 September 2017

Studying Bacterial Membrane Protein Complexes by the use of Contrasting Components

Jeremy Lakey

Institute for Cell and Molecular Biosciences

Newcastle University, UK

Why should we care?

The outer membrane is,

- a critical barrier to small antibiotics.
- site of action of alternative antibiotics (polymyxins).
- source of endotoxin which causes toxic shock syndrome
- the surface which interacts with the host organism

A simple, clear, but accurate model

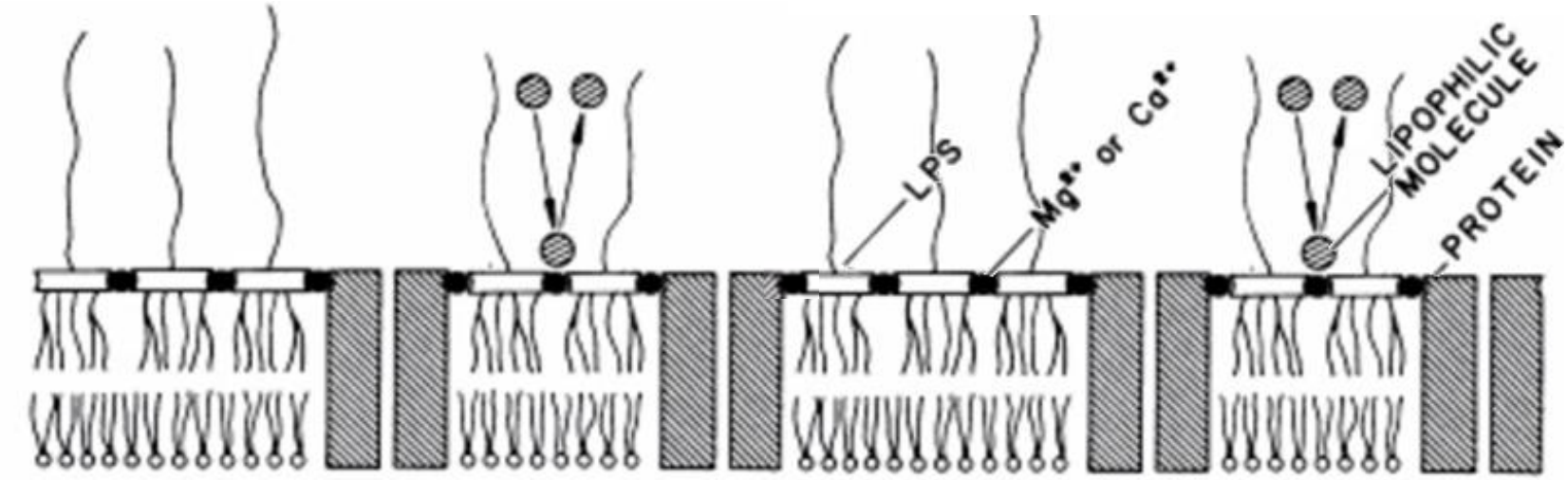
MICROBIOLOGICAL REVIEWS, Mar. 1985, p. 1–32
0146-0749/85/010001-32$02.00/0

Vol. 49, No. 1

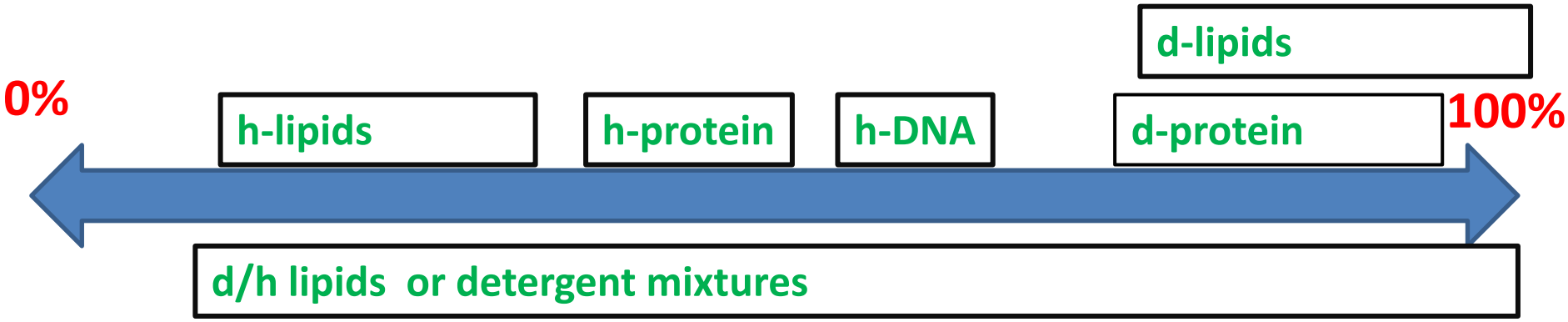
Molecular Basis of Bacterial Outer Membrane Permeability

HIROSHI NIKAIDO[1]* AND MARTI VAARA[2]

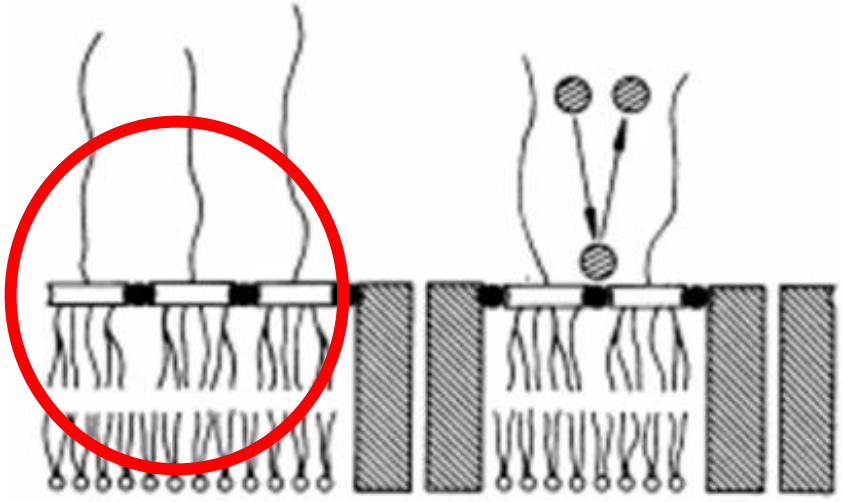
Department of Microbiology and Immunology, University of California, Berkeley, California 94720,[1] and National Public Health Institute, SF-00280 Helsinki 28, Finland[2]



The D_2O scale of bio-contrast



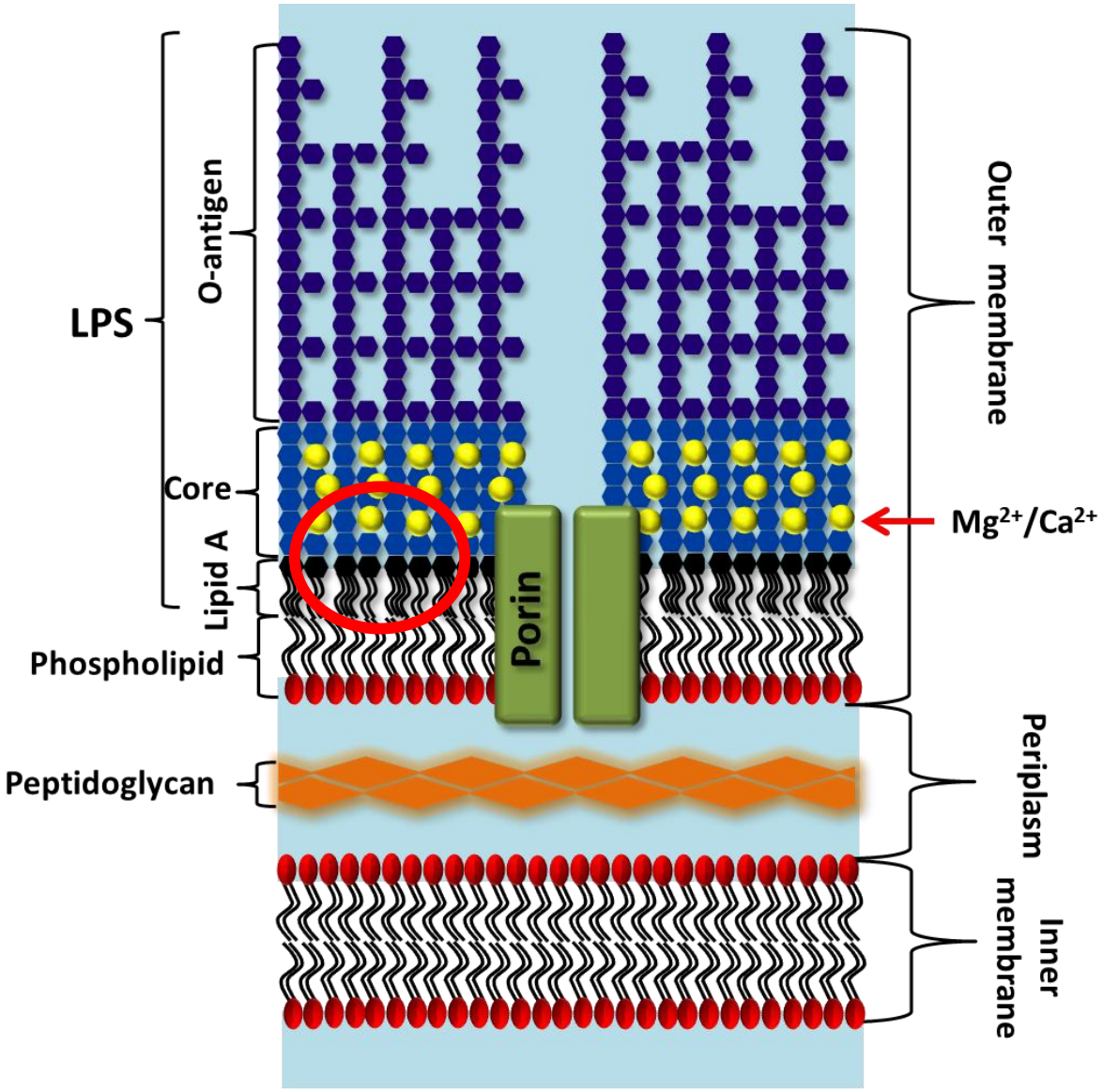
Part I
LPS – LPS interactions



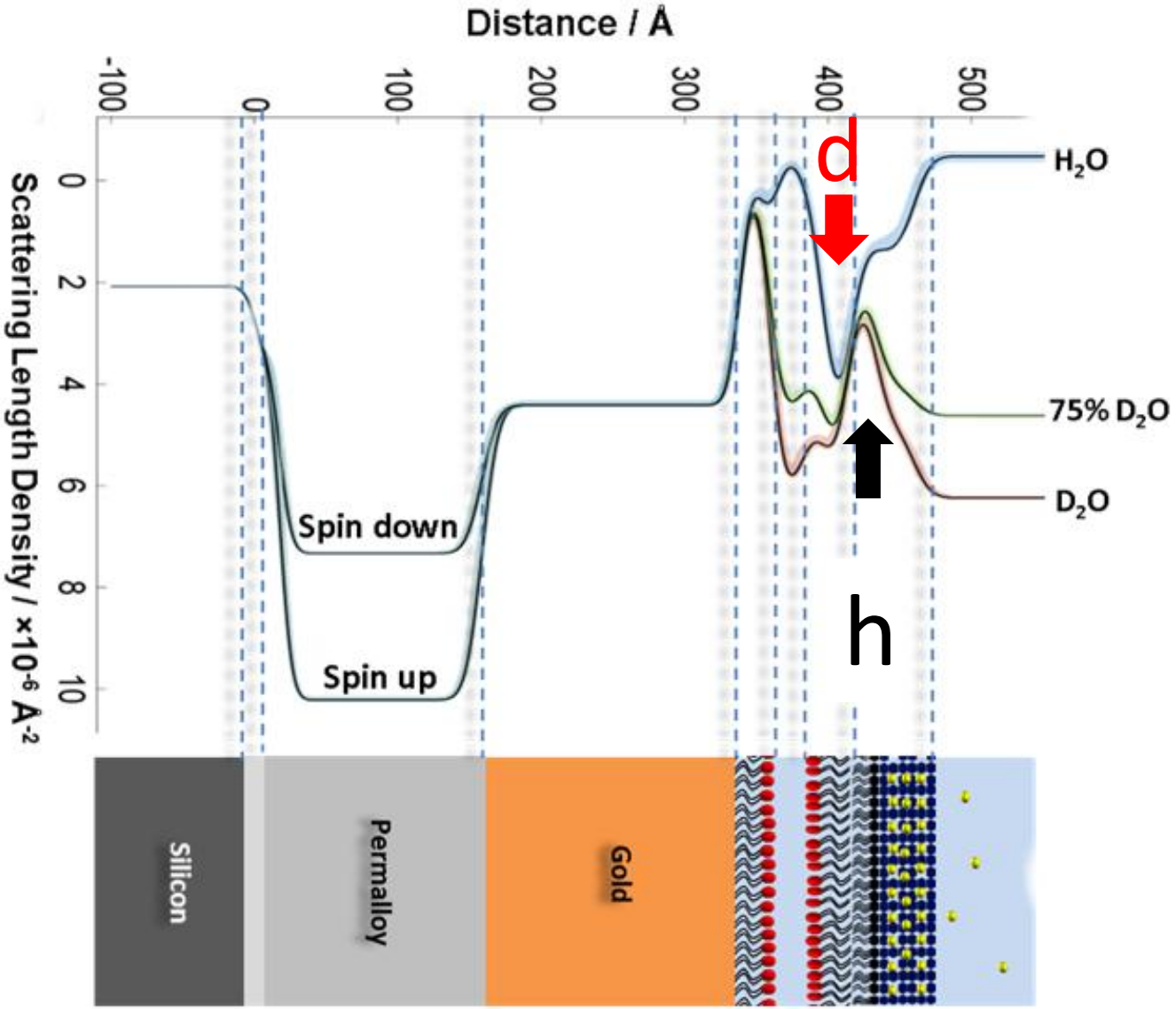
Bacteria are very small and complicated :

so we use *in vitro* models

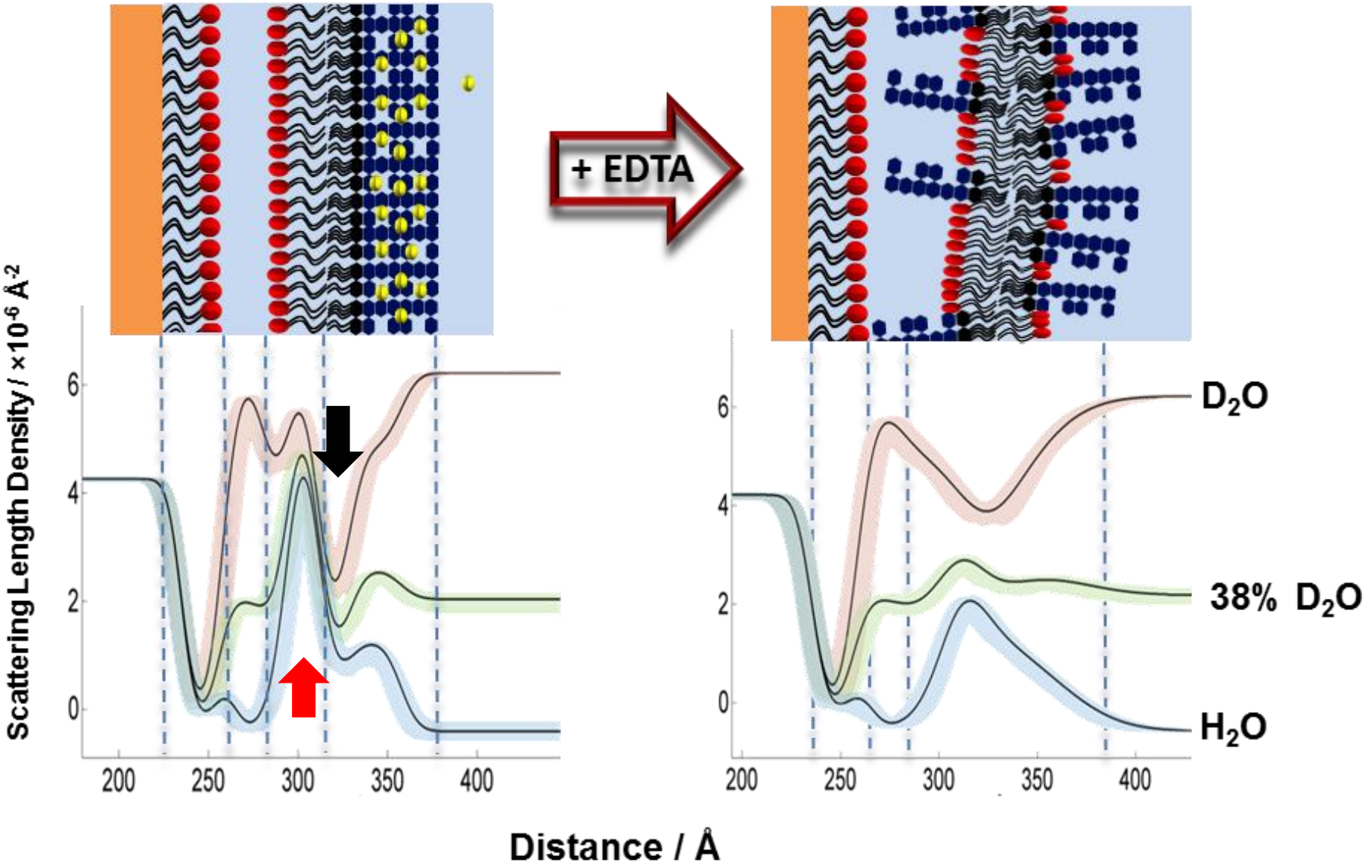
Outer membrane of Gram negative bacterium



Neutron scattering density profile using deuterated lipids, shows the model membrane to be highly asymmetric.



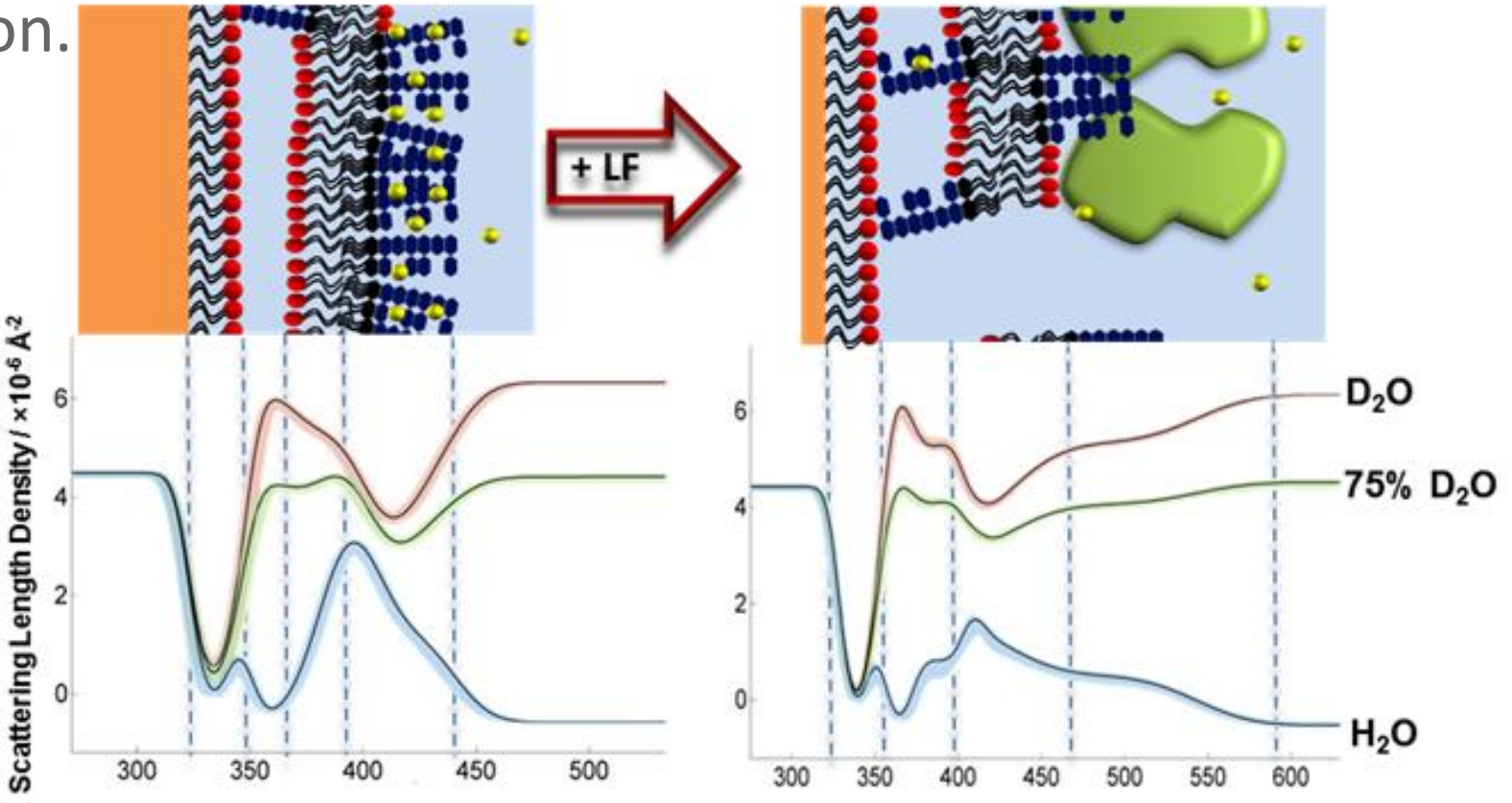
Removal of calcium ions – destroys asymmetry



Antimicrobial Proteins

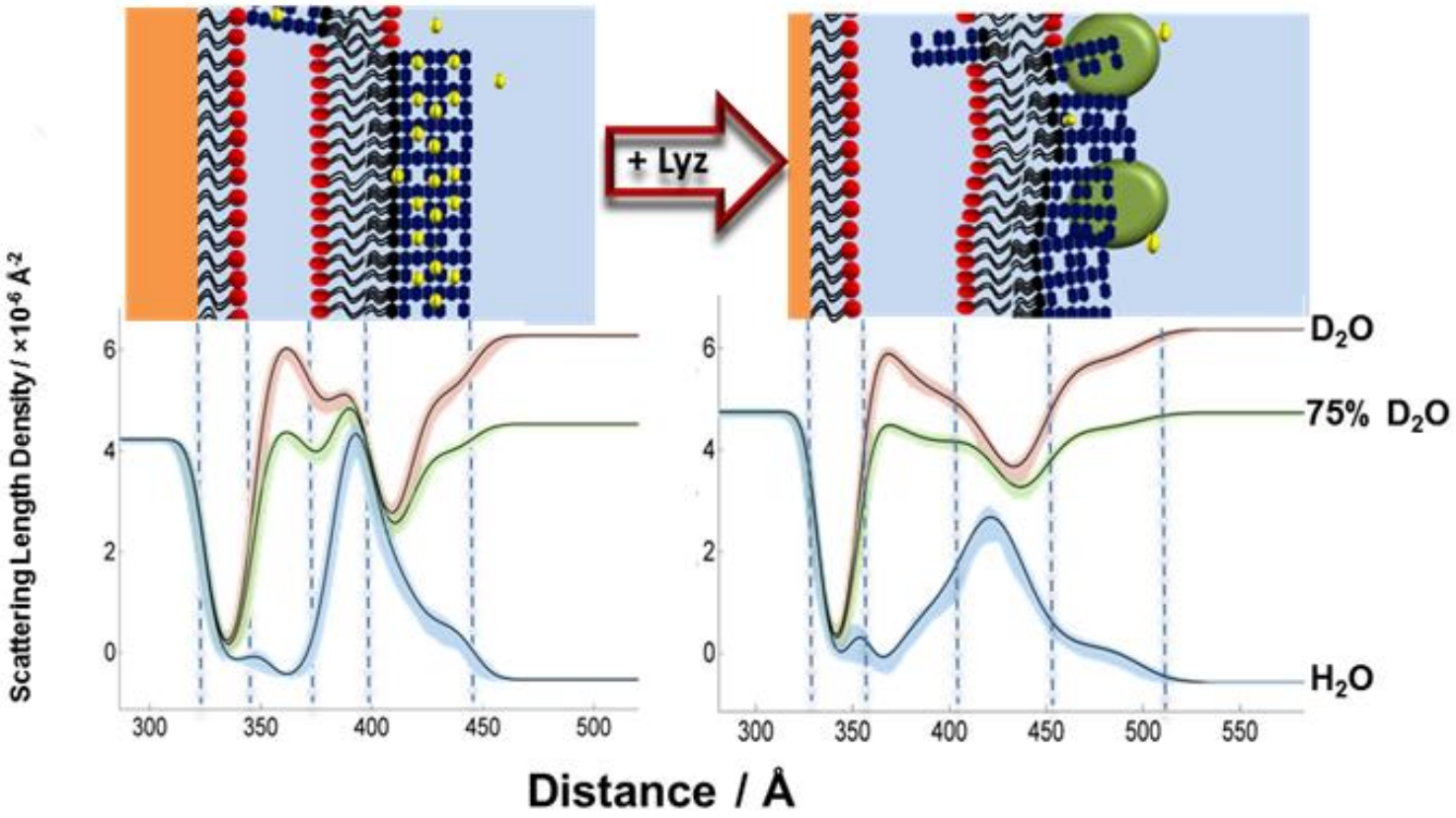
- Lactoferrin
- disrupts the divalent cation bridges between LPS molecules
- causing a release of LPS into the bulk solution.

Using h-DPPC

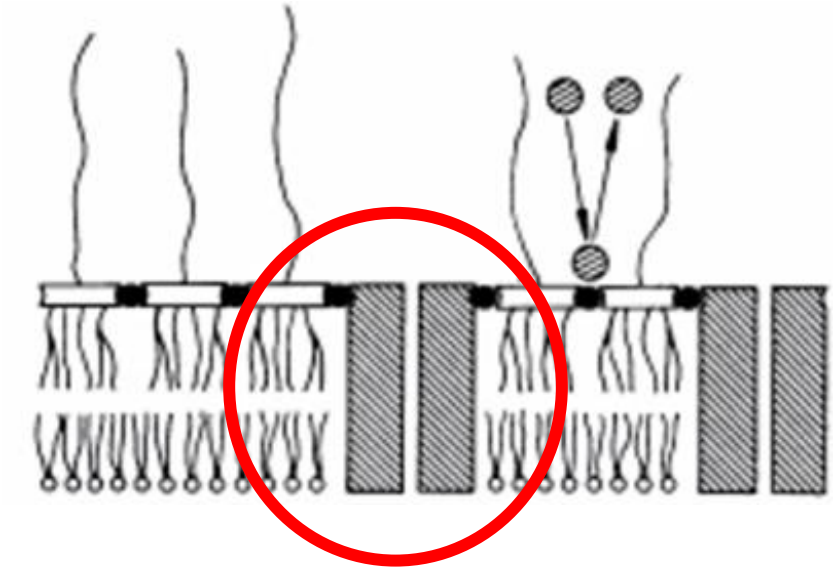


Antimicrobial Proteins

- Lysozyme
- When used without EDTA
- Binds to surface and does not disrupt LPS



Part II
Outer membrane protein – LPS interaction interaction



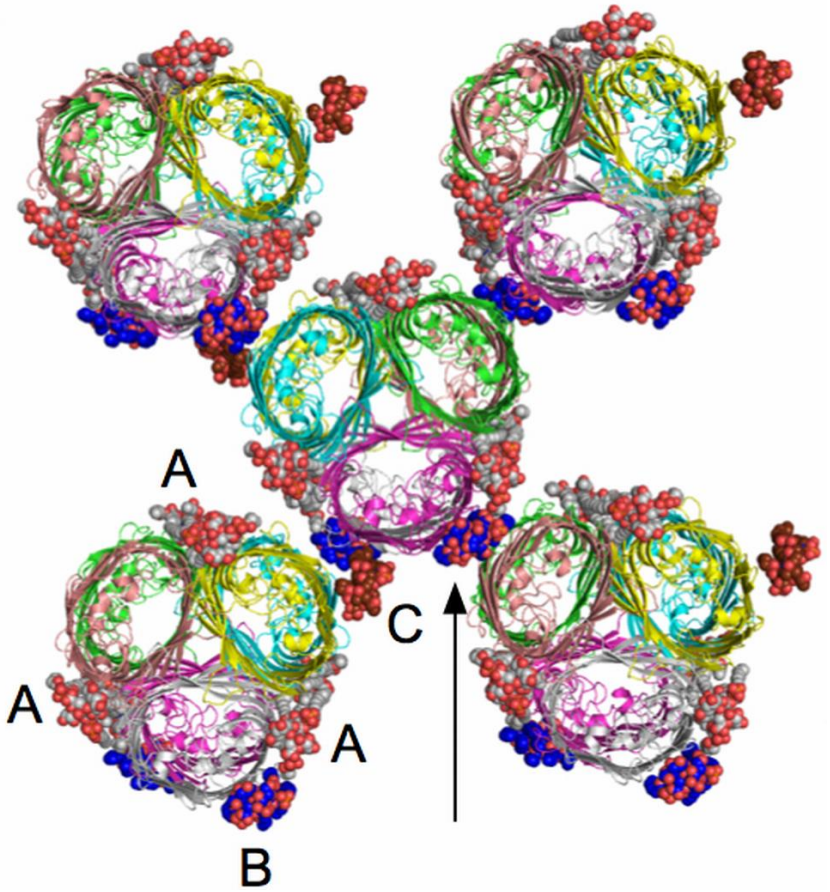
PNAS

Gram-negative trimeric porins have specific LPS binding sites that are essential for porin biogenesis

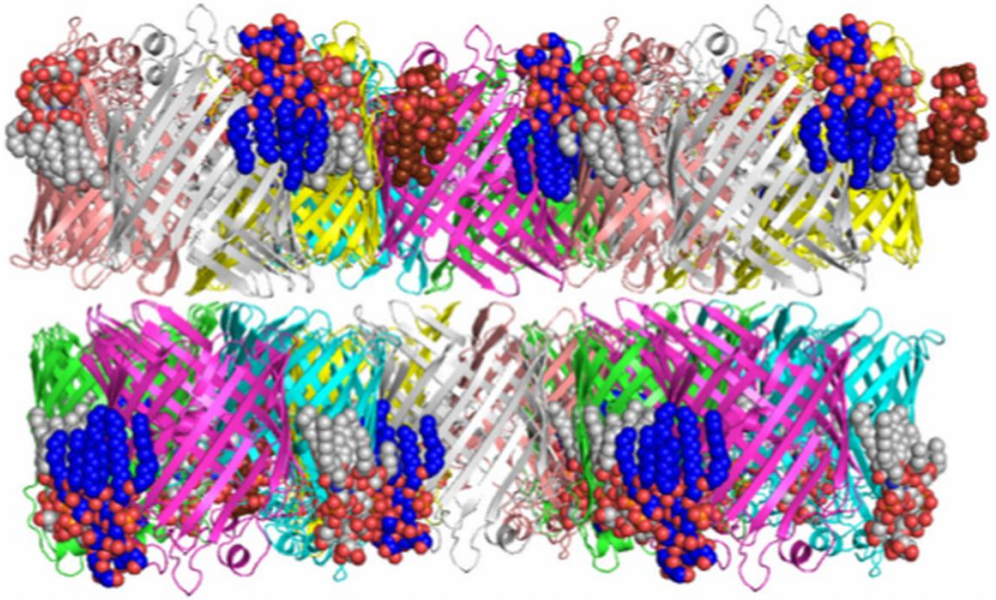
Wanatchaporn Arunmanee[a,1], Monisha Pathania[a,1], Alexandra S. Solovyova[a,b], Anton P. Le Brun[c], Helen Ridley[a], Arnaud Baslé[a], Bert van den Berg[a,2], and Jeremy H. Lakey[a,2]

[a]Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom; [b]Newcastle University Protein and Proteome Analysis, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom; and [c]National Deuteration Facility, Bragg Institute, Australian Nuclear Science and Technology Organisation, Kirrawee DC, NSW 2232, Australia

Edited by Hiroshi Nikaido, University of California, Berkeley, CA, and approved June 29, 2016 (received for review February 11, 2016)

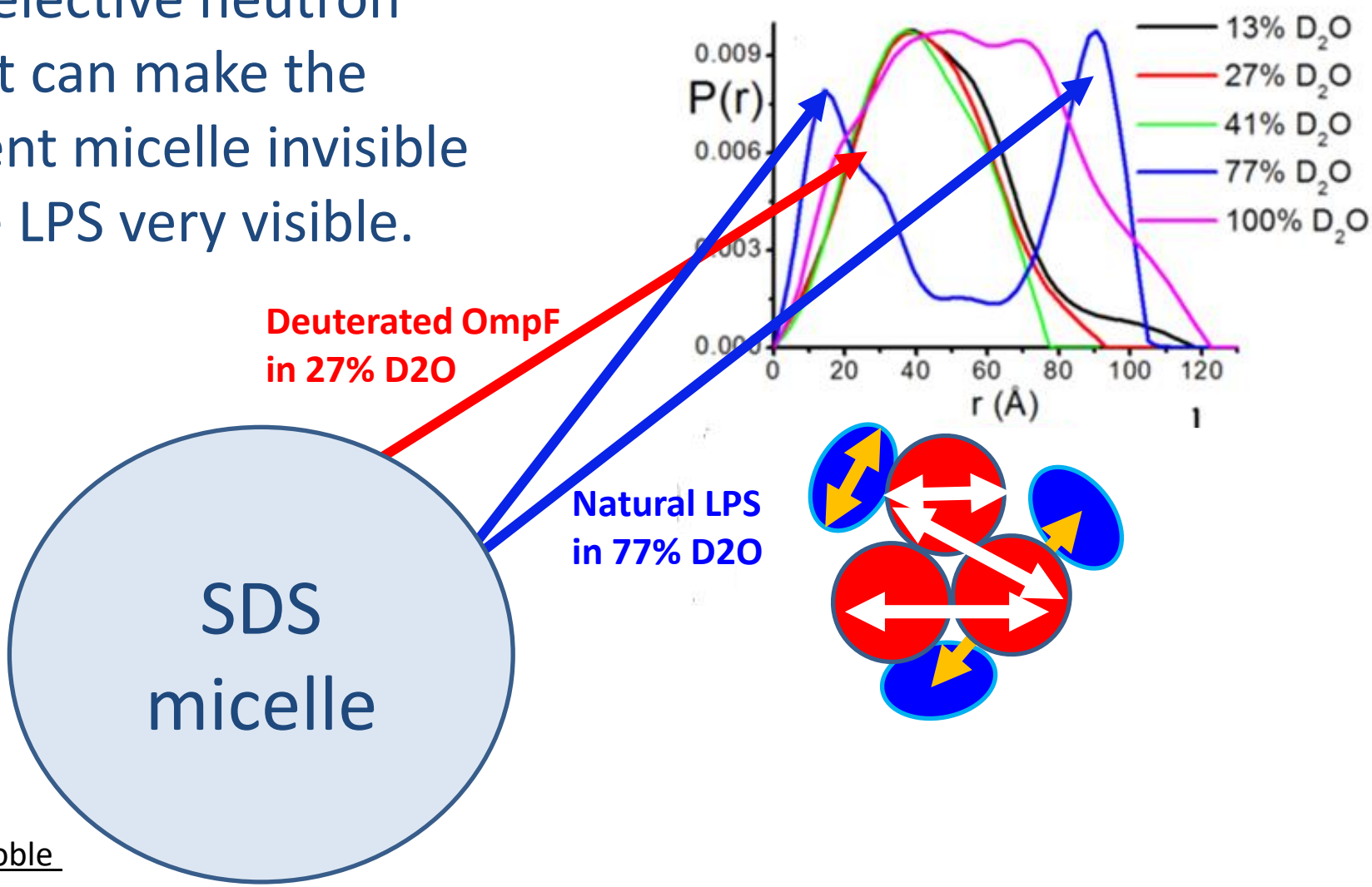


Structure of OmpE36 (*Enterobacter cloacae*) (1.45 Å) shows three LPS molecules.



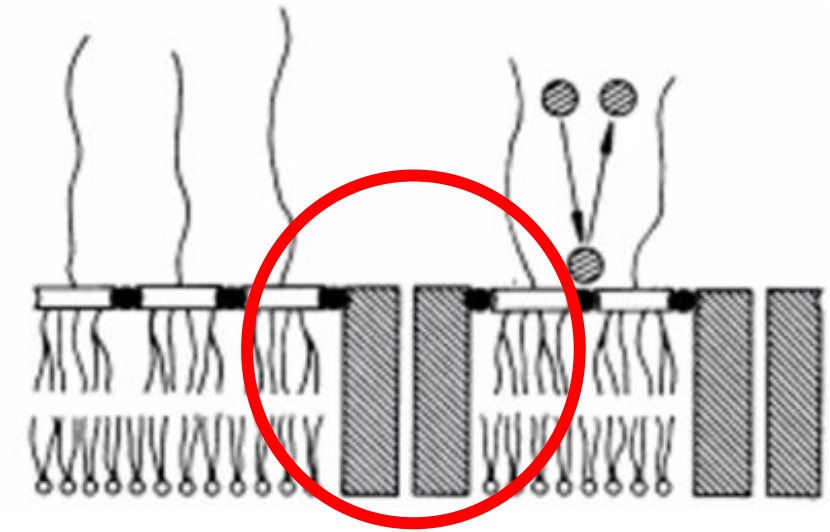
Small Angle Neutron Scattering confirms that, in solution, LPS binds at the periphery of OmpF

Using selective neutron contrast can make the detergent micelle invisible and the LPS very visible.



D22, ILL, Grenoble
Anne Martel

Part III
Outer membrane protein – Amphipol interaction



Trimeric porins

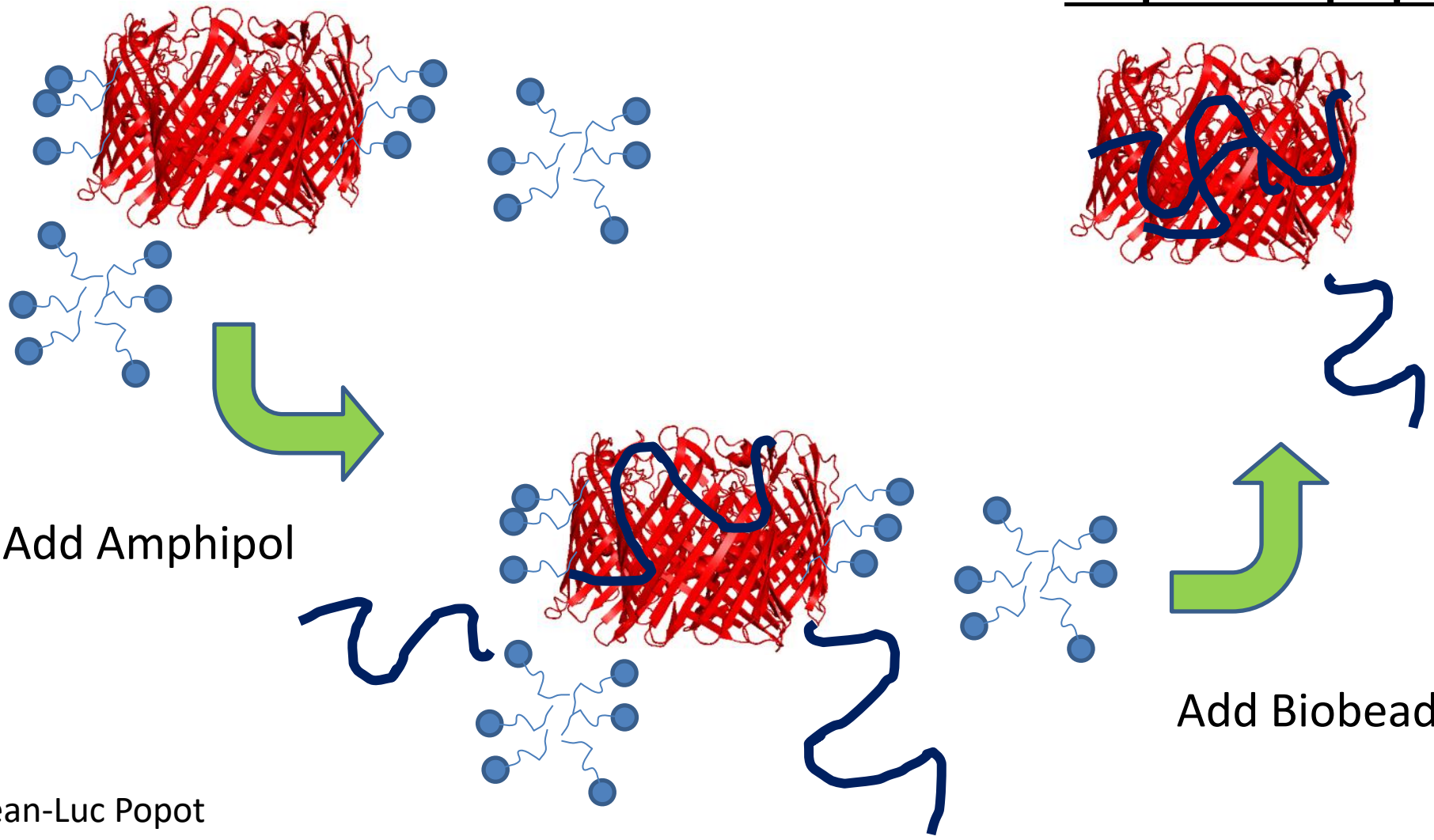
Arunmanee *et al* in preparation

Preparing OmpF in Amphipol

Newcastle University

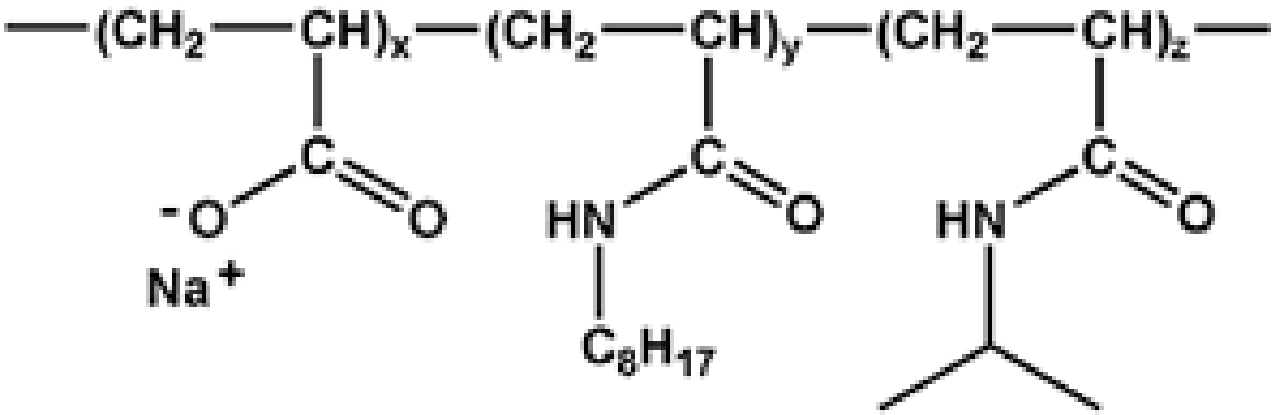
OmpF in detergent micelles

OmpF in Amphipol



Jean-Luc Popot

Amphipol A8-35

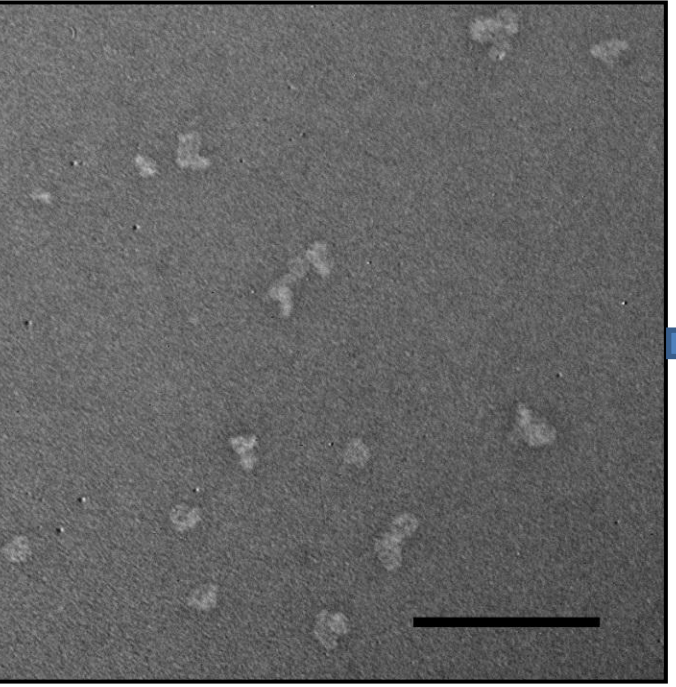


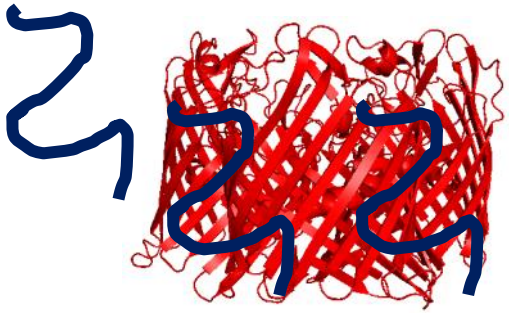
Amphipol A8-35 is a polymer with approx MW of 8kDa with a general chemical formula as below; x ≈ 0.35, y ≈ 0.25, and z ≈ 0.4.

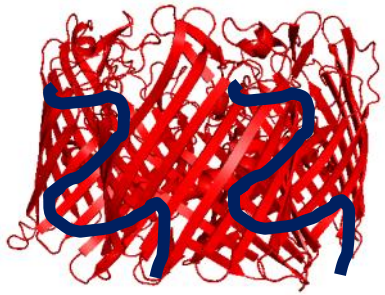
Gohon *et al* Biophys J. 2008 94: 3523–3537

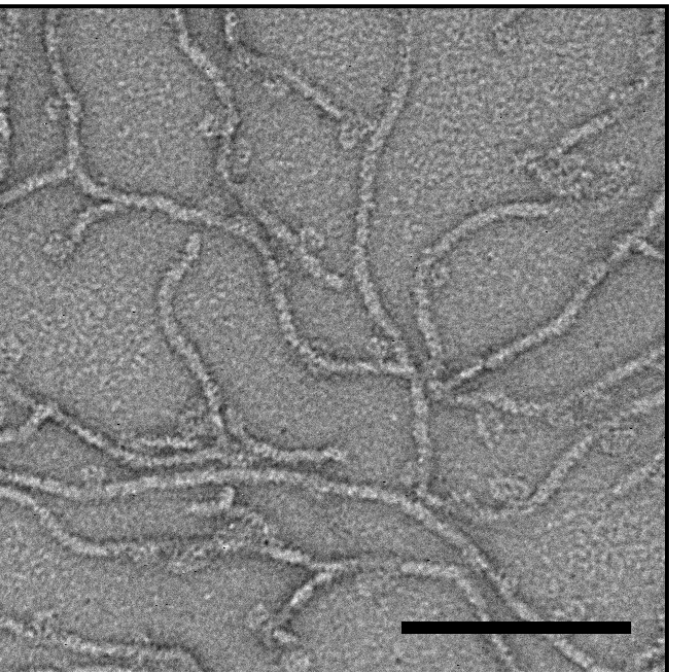
SLD of h-Amphipol = 1.06 x 10^{-6} $Å^{-2}$ =23.5% D2O

OmpF in Amphipol





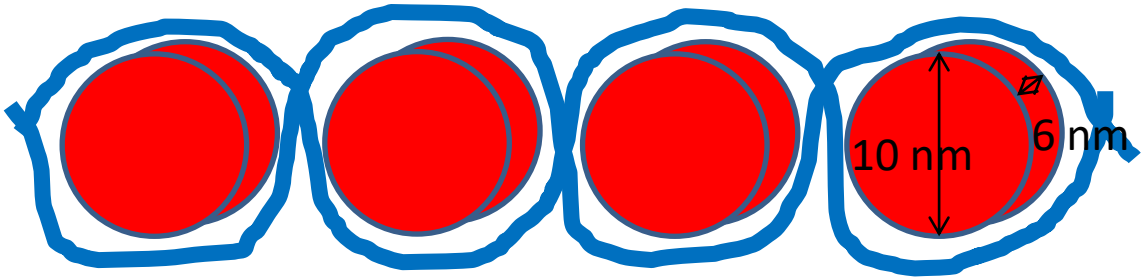




Hours -Days

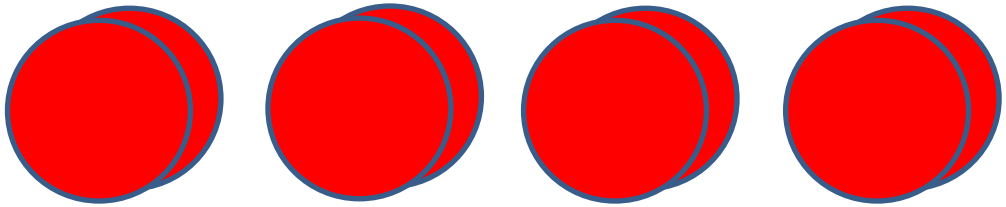
Where is the amphipol? Design of the SANS experiment.

0%, 50% and 100% D_2O



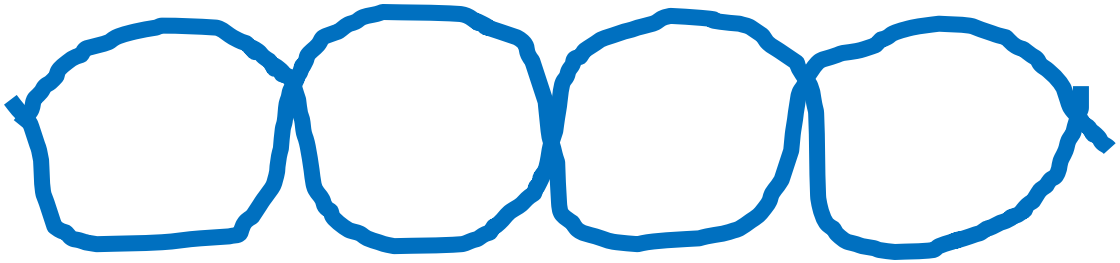
Side view

23.5% D_2O



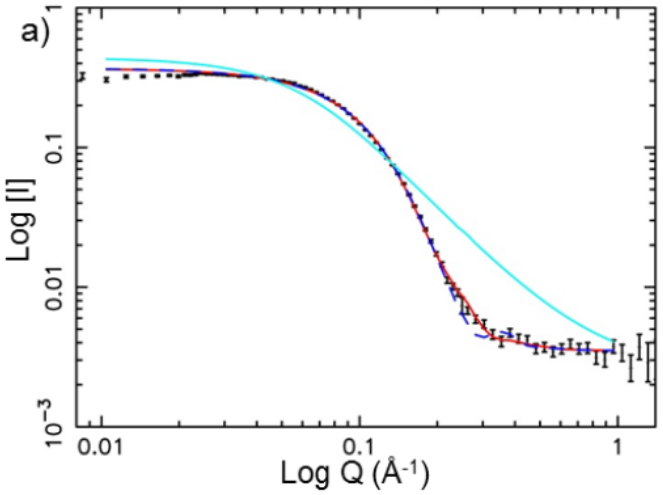
Side view

77% D_2O



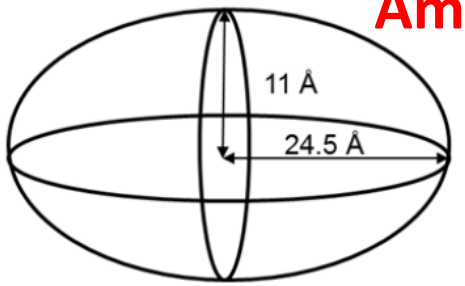
Side view

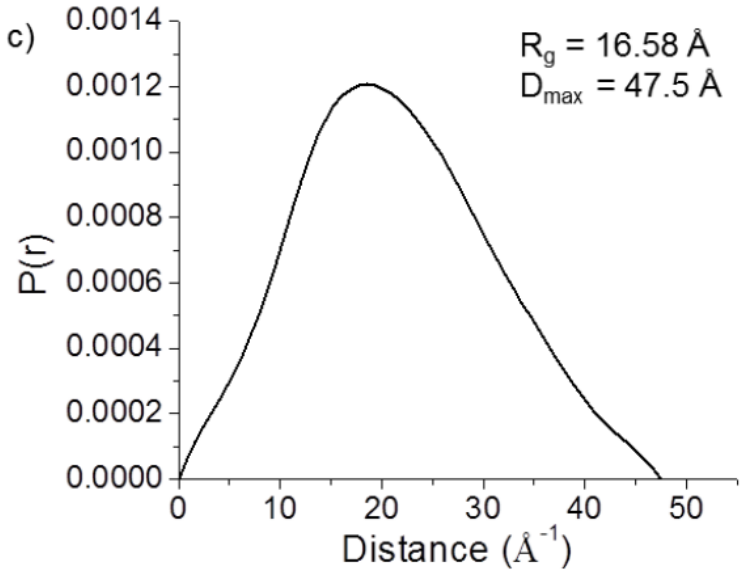
Where is the Amphipol?



b) **Experiment 1**

Amphipol alone in 100% D_2O



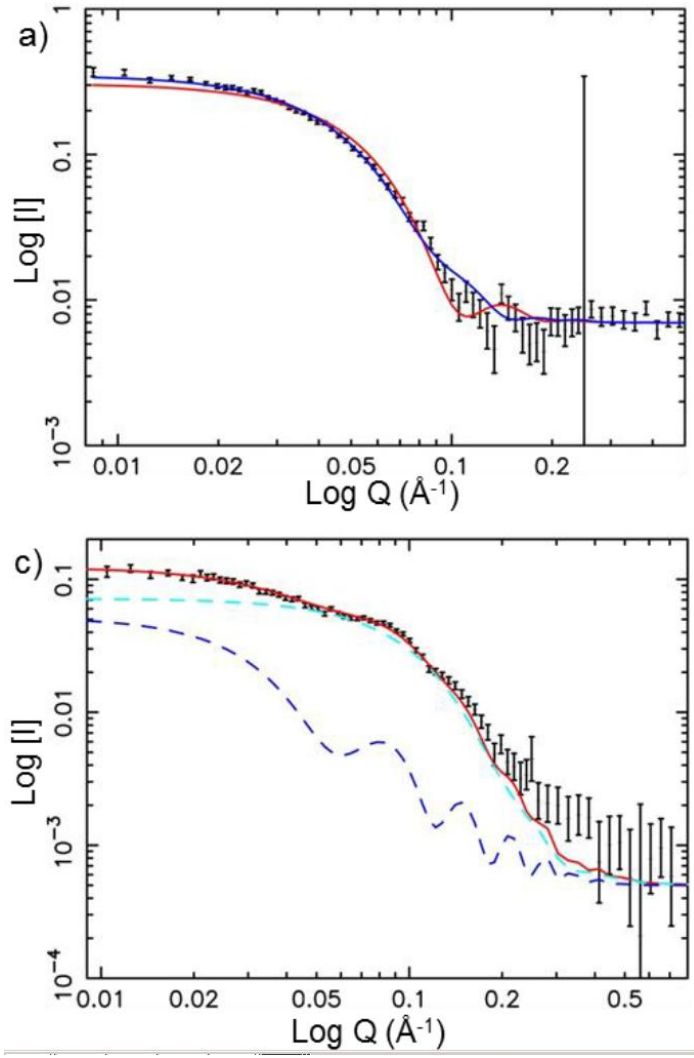


Amphipol forms oblate ellipsoid micelles with approx 1 Amphipol per micelle

SANS 2D at ISIS

Richard Heenan

Where is the Amphipol?

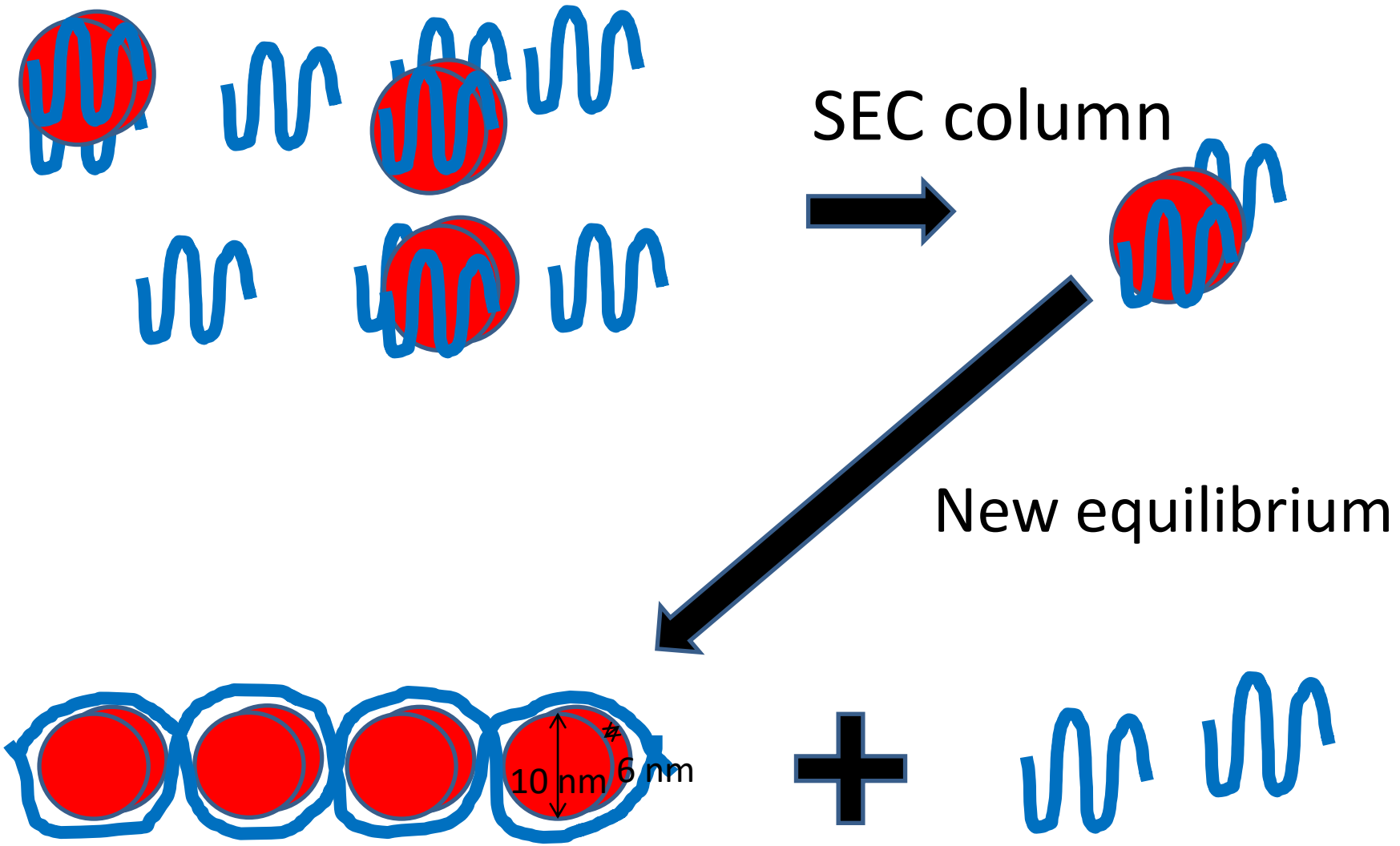


23.5% D_2O dOMPF only visible. Can be modelled as a disc

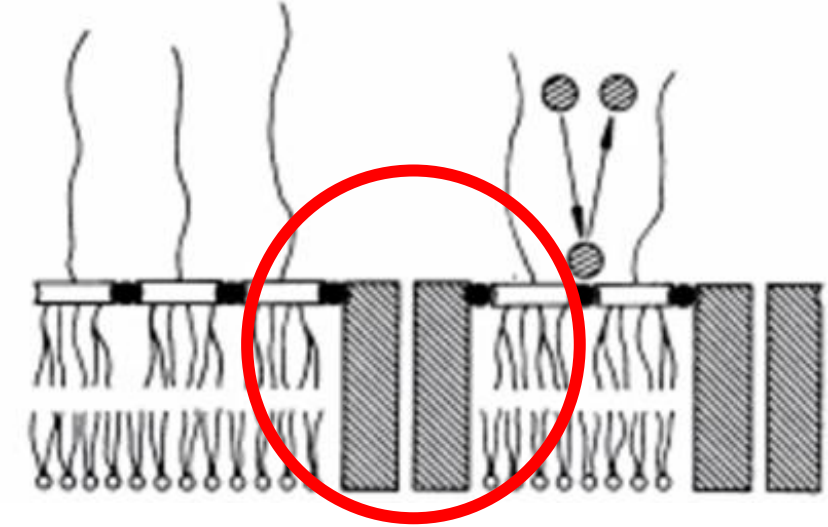
77% D_2O Amphipol only visible.

Can be modelled as a hollow tube plus micelles

Where is the Amphipol?

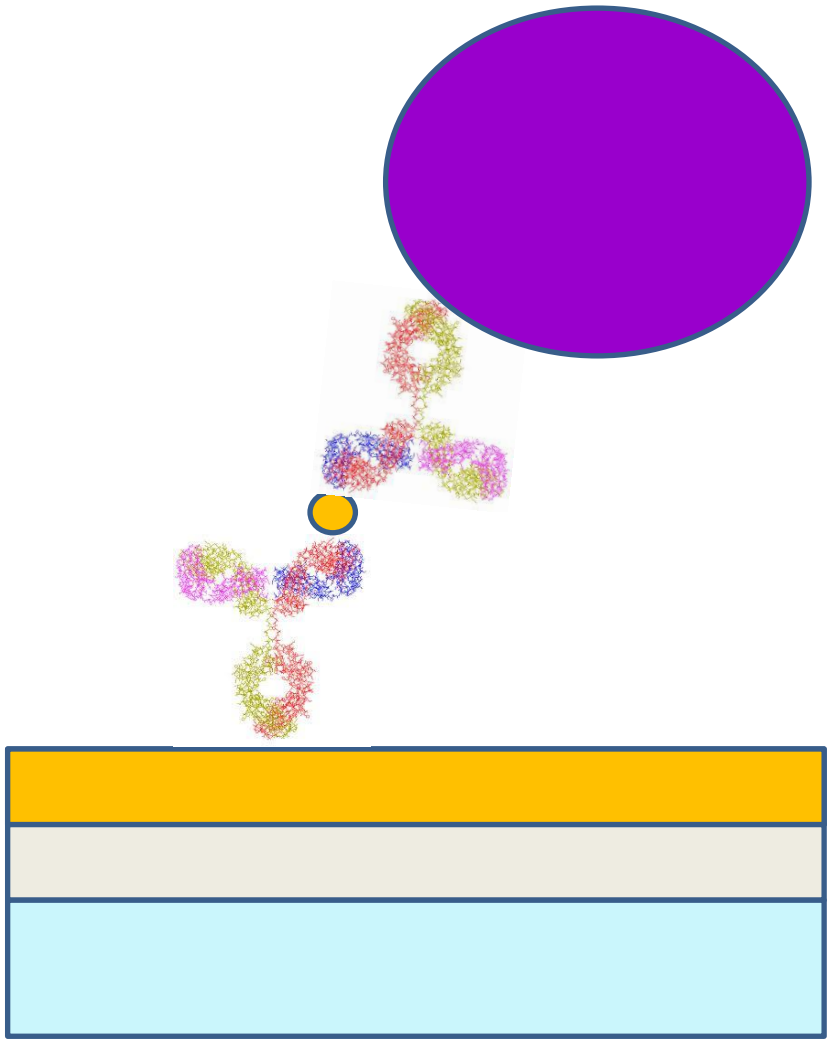


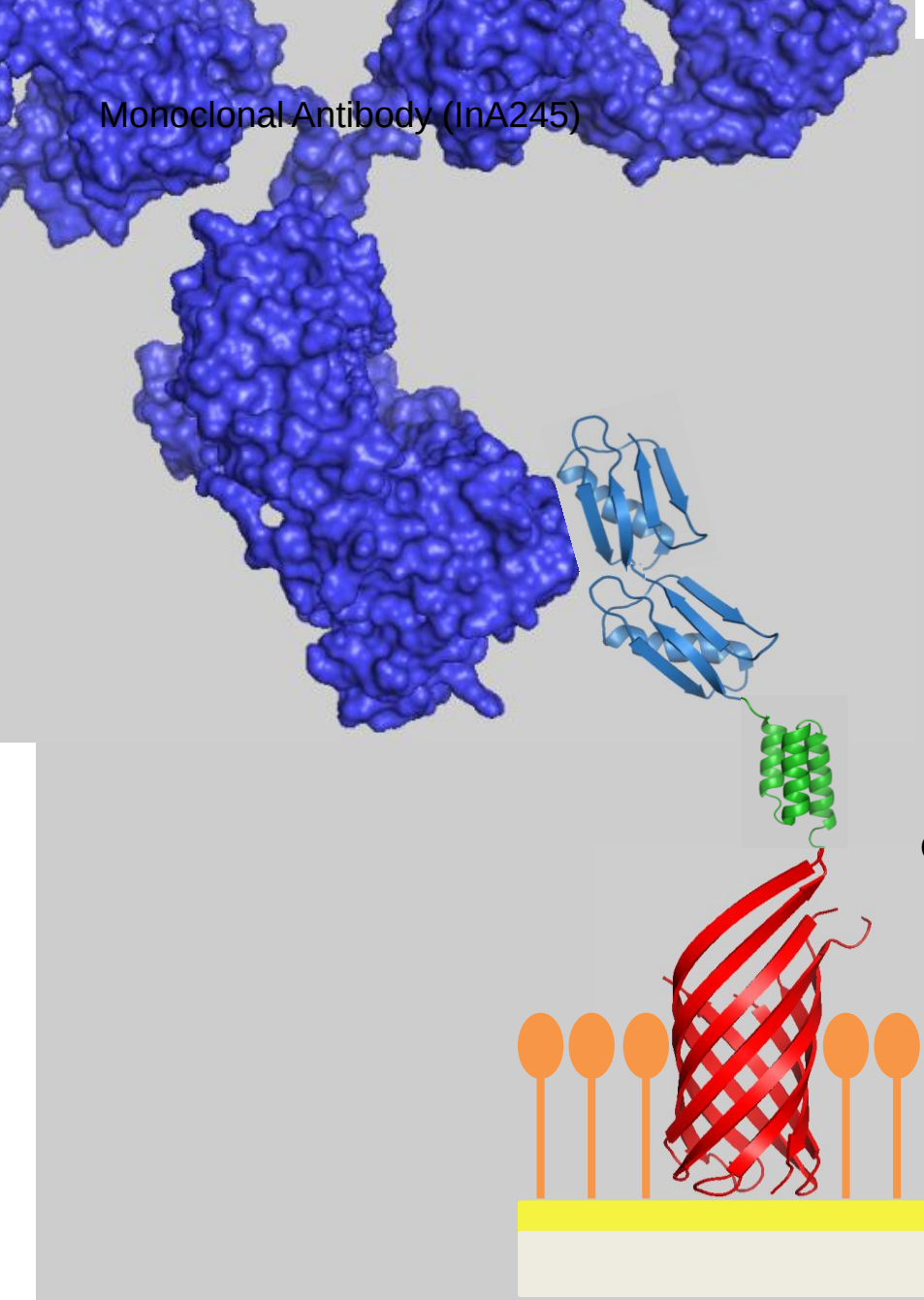
Part III
Outer membrane proteins in Biosensors



Why we sometimes have to measure complex layers by NR

A typical “sandwich” assay used in diagnostics.

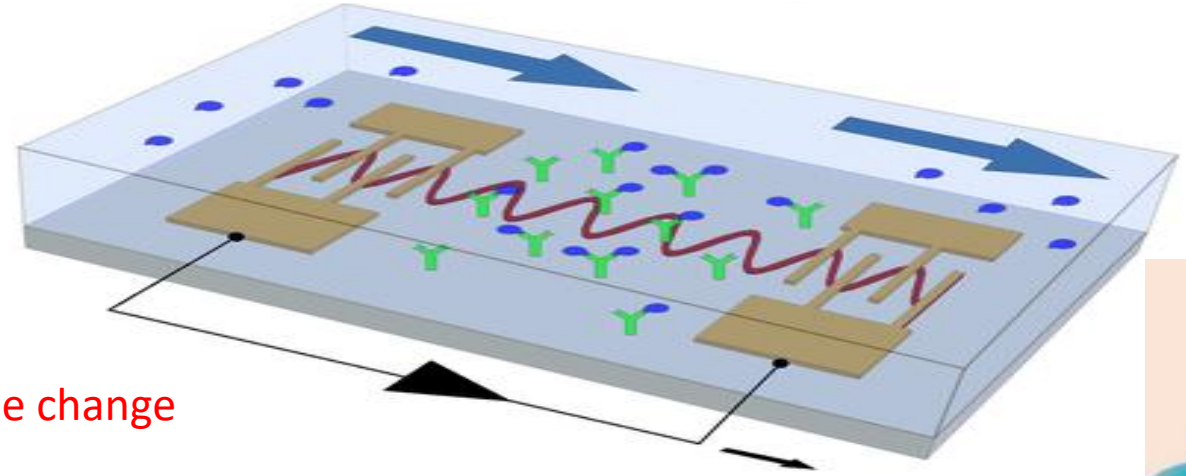




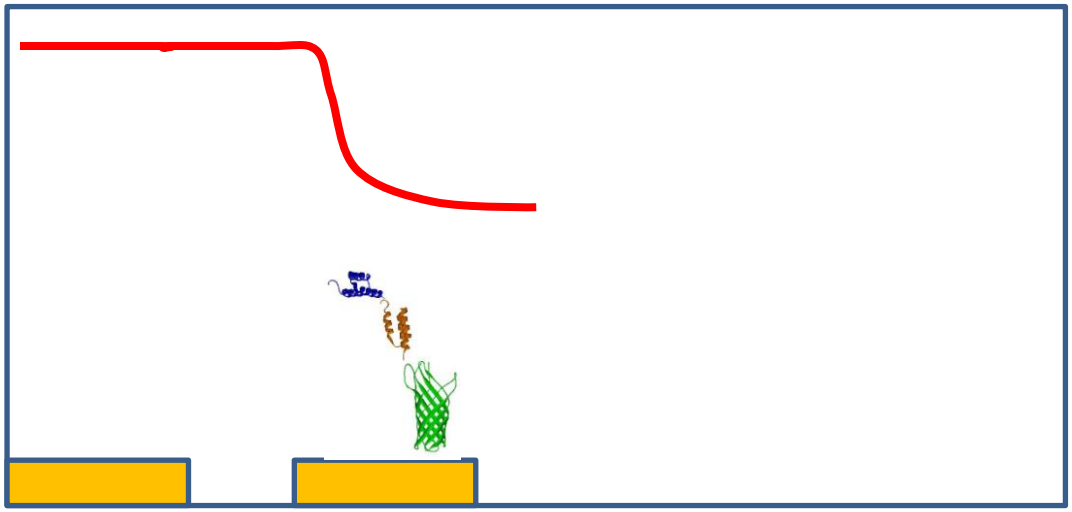
Self assembling layer based upon bacterial outer membrane proteins fused to antibody binding domains.
Achieves very high antibody density and activity plus low non specific binding

Why we sometimes have to measure complex layers by NR

•Biosensor based upon shear horizontal surface acoustic wave SH-SAW



Phase change

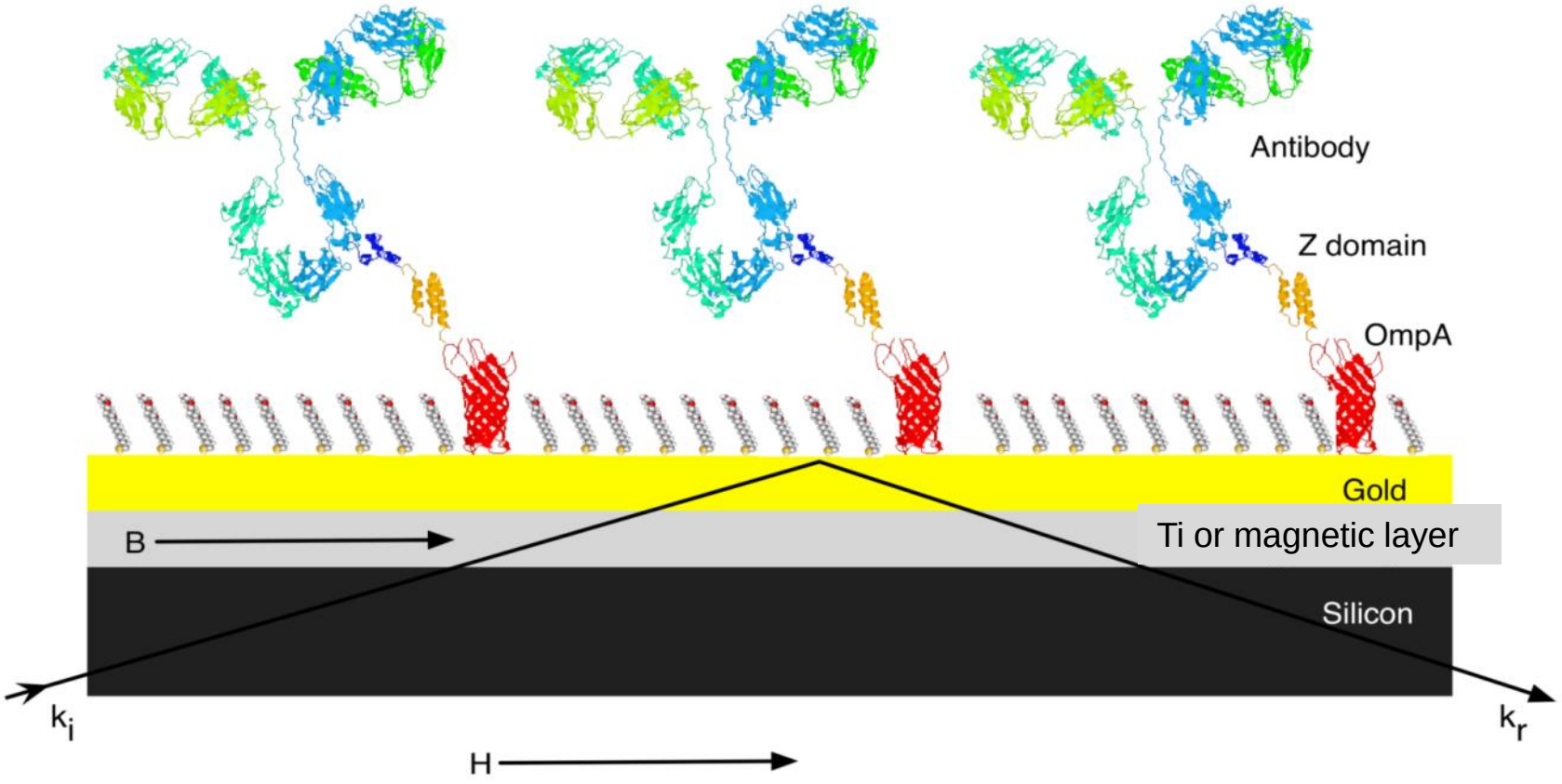


•Sensitive to Mass, viscosity, elasticity.

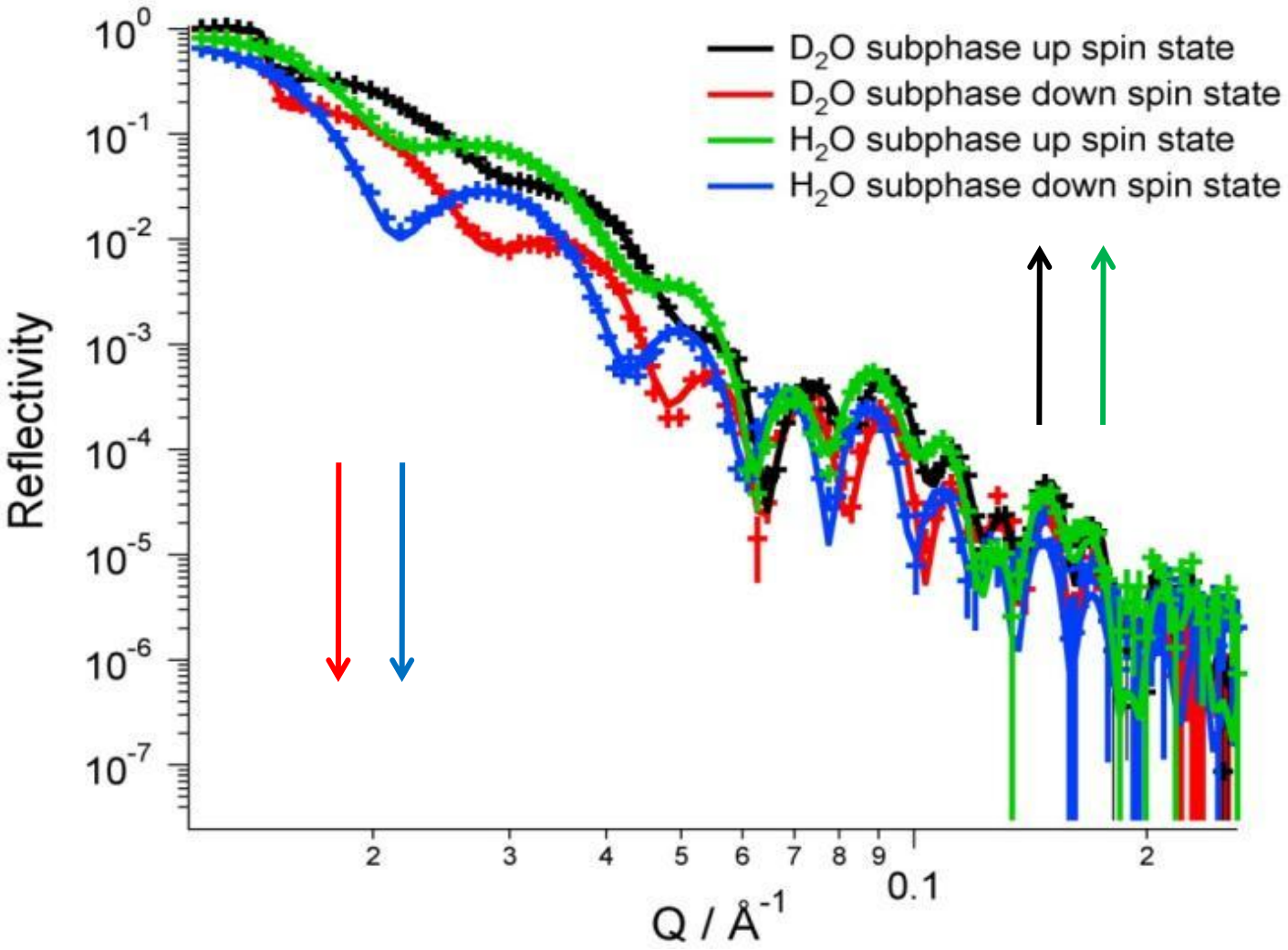
JRC Japan Radio Co., Ltd.

Orla PROTEIN TECHNOLOGIES

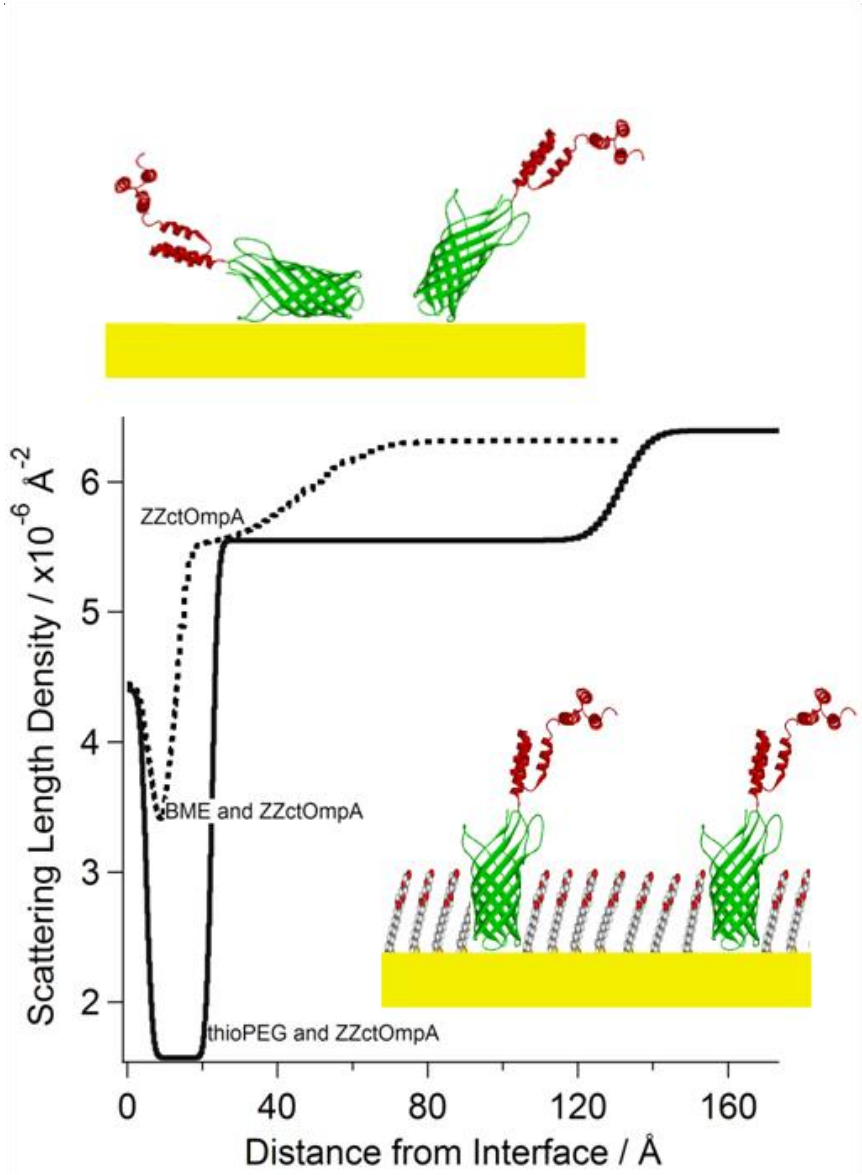
The array investigated by neutron reflection



Magnetic and solvent contrast

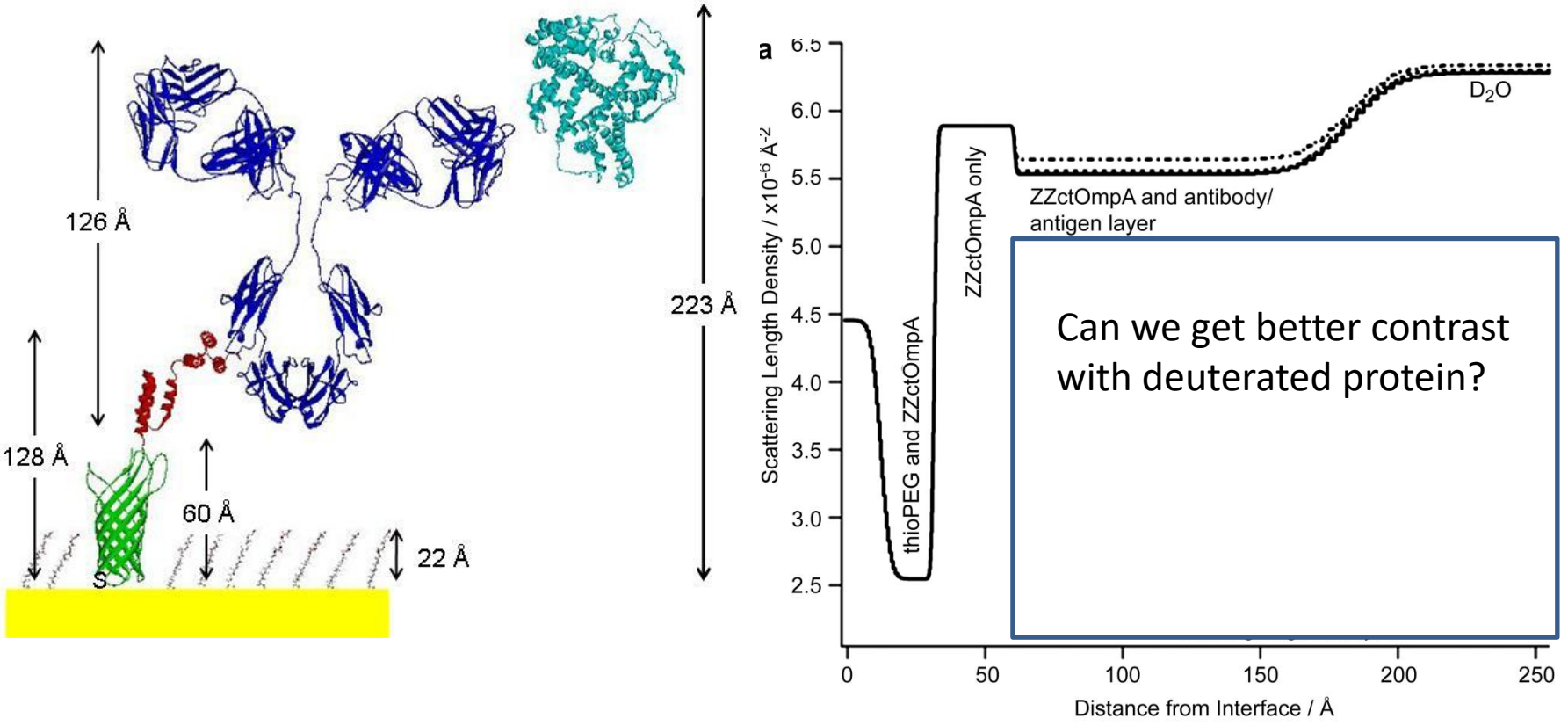


The filling molecule



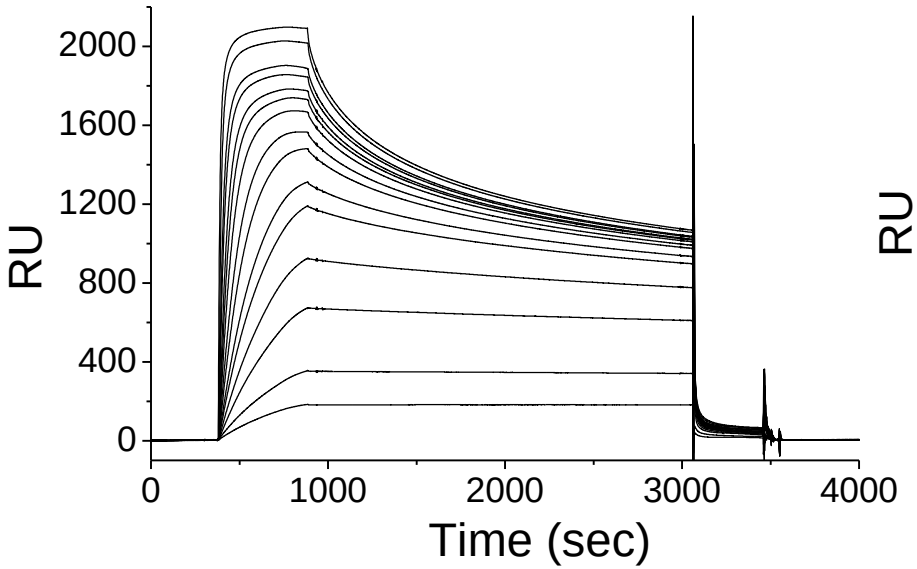
Neutron reflection showed the importance of having the filling molecule

Published data Le Brun, A.P., et al., *The structural orientation of antibody layers bound to engineered biosensor surfaces. Biomaterials., 2011 32(12): p. 3303-11.*

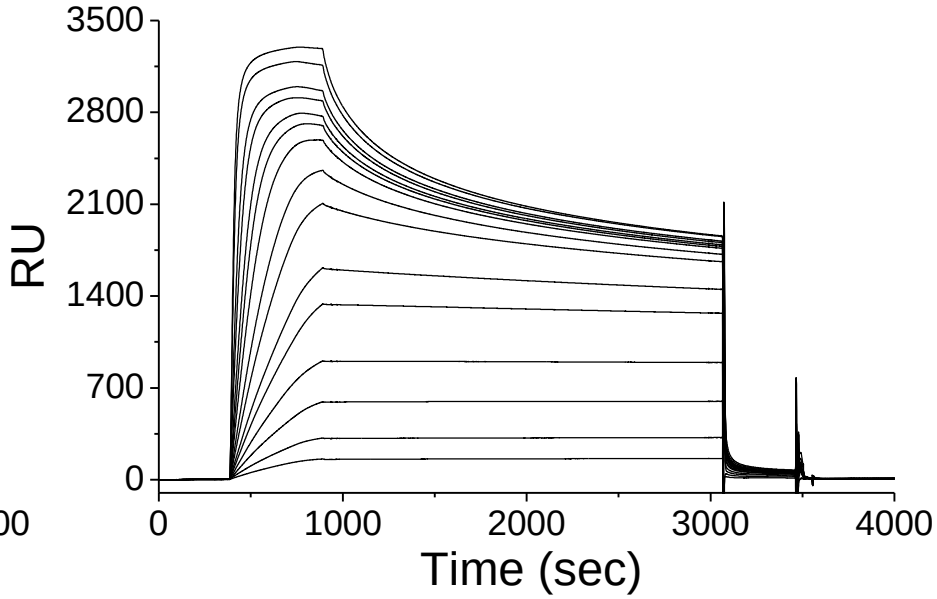


Antibody binding data from SPR (Biacore)

Hydrogenated Protein



Deuterated Protein



Antibody concentrations (from top) 300, 200, 100, 75, 50, 40, 30, 20, 15, 10, 8, 6, 4, 2, and 1 nM. Sensorgram is blank corrected (antibody injection minus buffer injection)

Hydrogenated Orla 85

k_{on} M^{-1} s^{-1}	k_{off} s^{-1}	K_d (nM)
4.29×10^5	7.45×10^{-4}	1.76

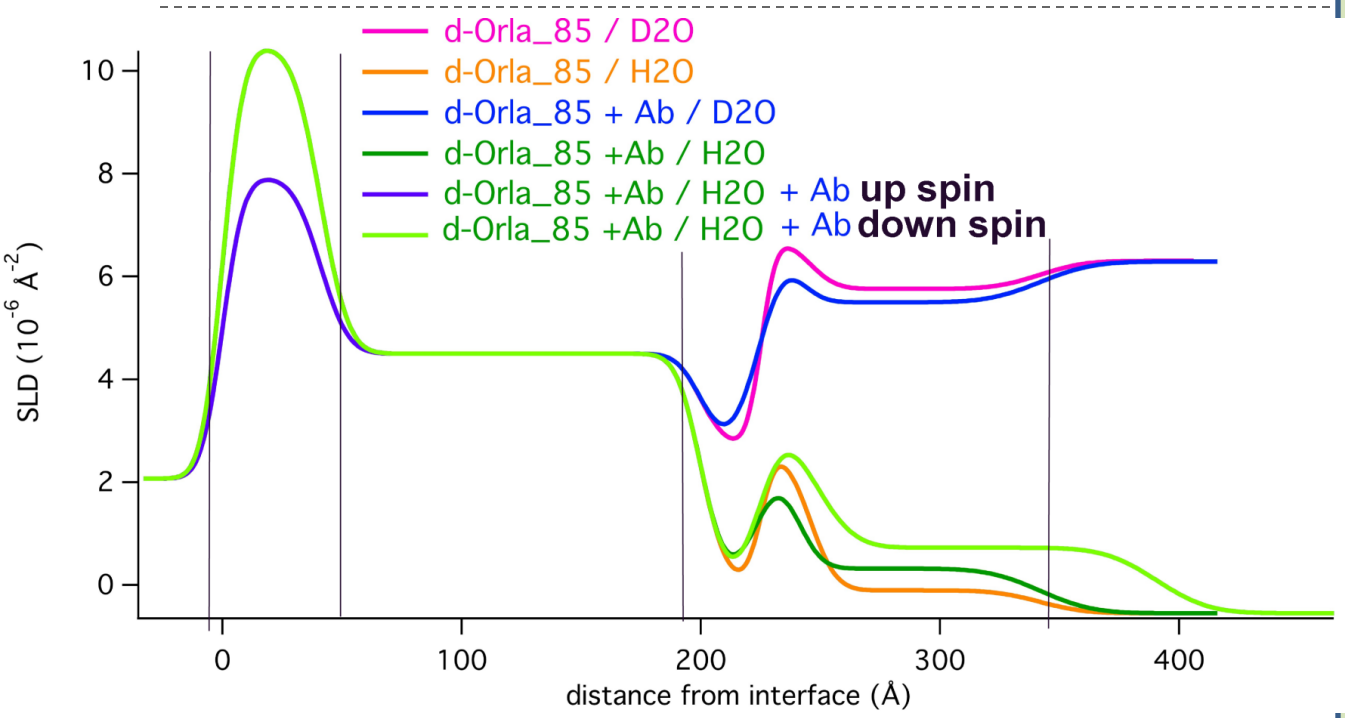
k_{on} M^{-1} s^{-1}	k_{off} s^{-1}	K_d (nM)
5.88×10^5	6.81×10^{-4}	1.15

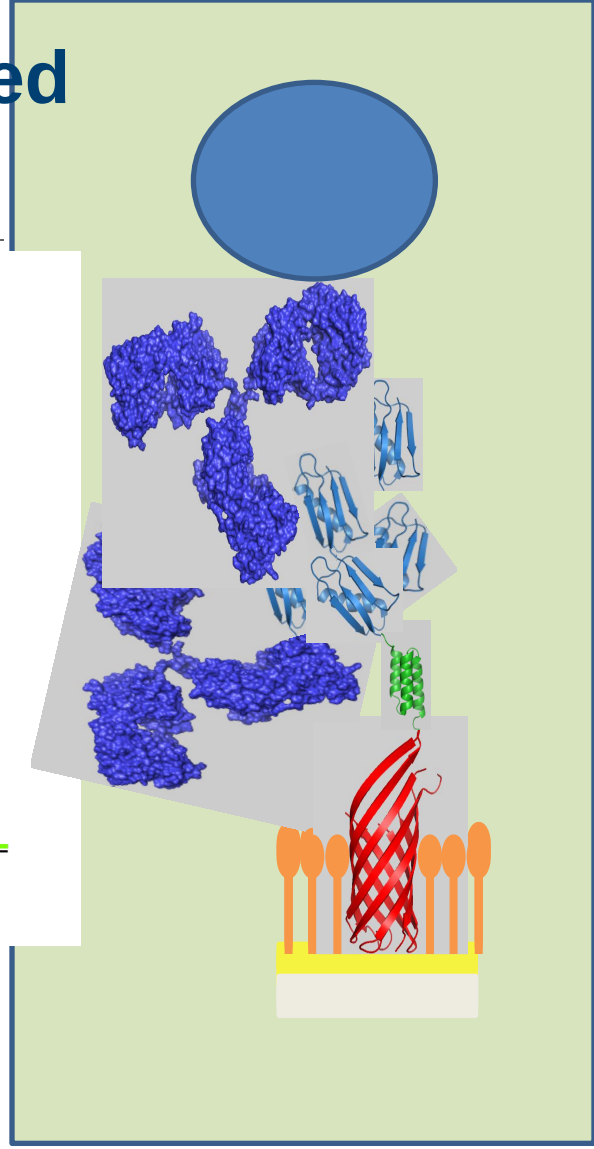
Deuterated Orla 85

k_{on} M^{-1} s^{-1}	k_{off} s^{-1}	K_d (nM)
2.91×10^5	7.52×10^{-4}	2.58

k_{on} M^{-1} s^{-1}	k_{off} s^{-1}	K_d (nM)
7.66×10^5	8.66×10^{-4}	1.13

Data from POLREF with the deuterated system





d-Orla85+filler = 160.4 ± 14.0 Å

d-Orla85+Ab = 142.3 ± 14.8 Å

Acknowledgements

Newcastle

Helen Waller
Alex Solovyova
Wanatchaporn Arunmanee
Chris Johnson
Tom Baboolal
Nicolo Paracini
Bert van den Berg
Monisha Pathania
Arnaud Basle

ISIS pulsed neutron source

Luke Clifton
Arwel Hughes
Christy Kinane
Tim Charlton
Richard Heenan
Sarah Rogers

ANSTO

Stephen Holt
Anton Le Brun

NIST

Frank Heinrich
Chuck Majkrzak

ILL

Phil Callow
Anne Martel
D-Lab

Institut de Biologie Physico-Chimique

Jean-Luc Popot

Thank you