Nanotechnology in Agrochemicals
Challenges and Opportunities

13th June, 2019

Agform

- UK based R&D laboratories and formulation plant
- Research chemists and regulatory experts
- Development of improved agrochemical delivery systems
- Application of novel formulation technologies to generic-proprietary products

Nanotechnology?

- EU Regulatory Definition of Nanotechnology

  <100 nm

- Pharmaceutical Definition of Nanotechnology

  <1 micron

TransCel® Technology

TRANSCEL® ADVANTAGES

Advantages of TransCel® technology over conventional SC's.

- High adhesiveness.
- Increased bioavailability due to enhanced solubility[1] and dissolution rate[2].
- High physical stability due to absence of aggregation and crystal growth.
- Improved biological performance.

Eqn. 1:

$$\log \frac{C_S}{C_\alpha} = \frac{2\sigma V}{2.303 RT \rho r}$$

Ostwald–Freundlich equation

Eqn. 2:

$$\frac{dc}{dt} = \frac{D.A}{h}(C_s - C_b)$$

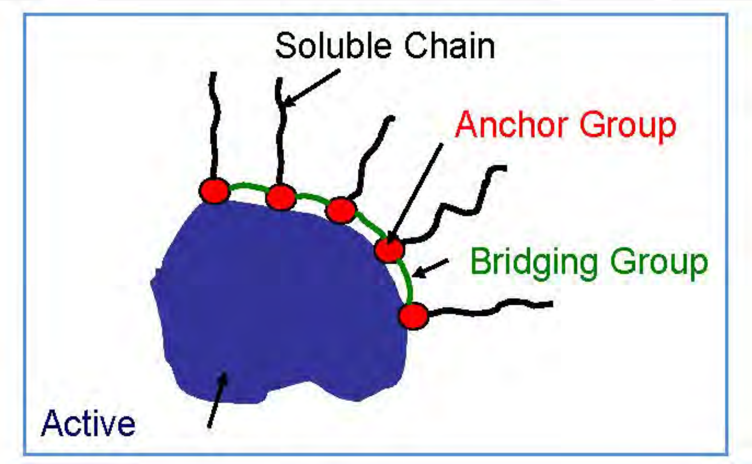
Noyes-Whitney equation

Particle Size Stability and Zeta Potential

Active Ingredient	Dispersant	Zeta Potential (mV)	Particle Size (nm)	Particle Size Stability (2 Weeks RT, nm)
Isoproturon	polyetheralkanol amine comb polymer	-0.0378	342	382
Isoproturon	polymethyl methacrylate – polyethylene glycol graft copolymer	-33.0	364	1420
Isoproturon	polymerised form of methacrylic acid	-34.1	237	1150
Isoproturon	graft copolymer of polymethylmethacrylate backbone and PEO side chains	-5.12	452	2930
Isoproturon	sodium salt of naphthalene sulfonate condensate	-52.3	224	527

TRANSCEL® OVERVIEW

Stabilisation of nano-particles during processing, storage and application critical.



- Nano-particles coated in polymeric layer
- Stable on processing, storage and dilution
- Production uses conventional equipment
- Scaled up to 1,000 litre batches
- Applicable to actives <100 ppm aqueous solubility

Efficacy Overview

- Agform's TransCel® technology produces formulations of insoluble agrochemical actives, as nano-sized suspensions.
- Unlike ordinary suspension concentrates, with particle size ranges of 2-5 microns, TransCel® formulations have particle size ranges of 0.2 – 0.5 microns.
- The increase in biological activity provided by TransCel® technology has been demonstrated over years of greenhouse, field trial and commercial use. Products utilizing this technology include Blutron®, a herbicide mixture of isoproturon and diflufenican, Ascent®, a herbicide mixture of flufenacet and diflufenican, Cachet®, a herbicide containing diflufenican and Oxe®, a new fungicide product containing azoxystrobin, formulated as a nano-sized suspension.

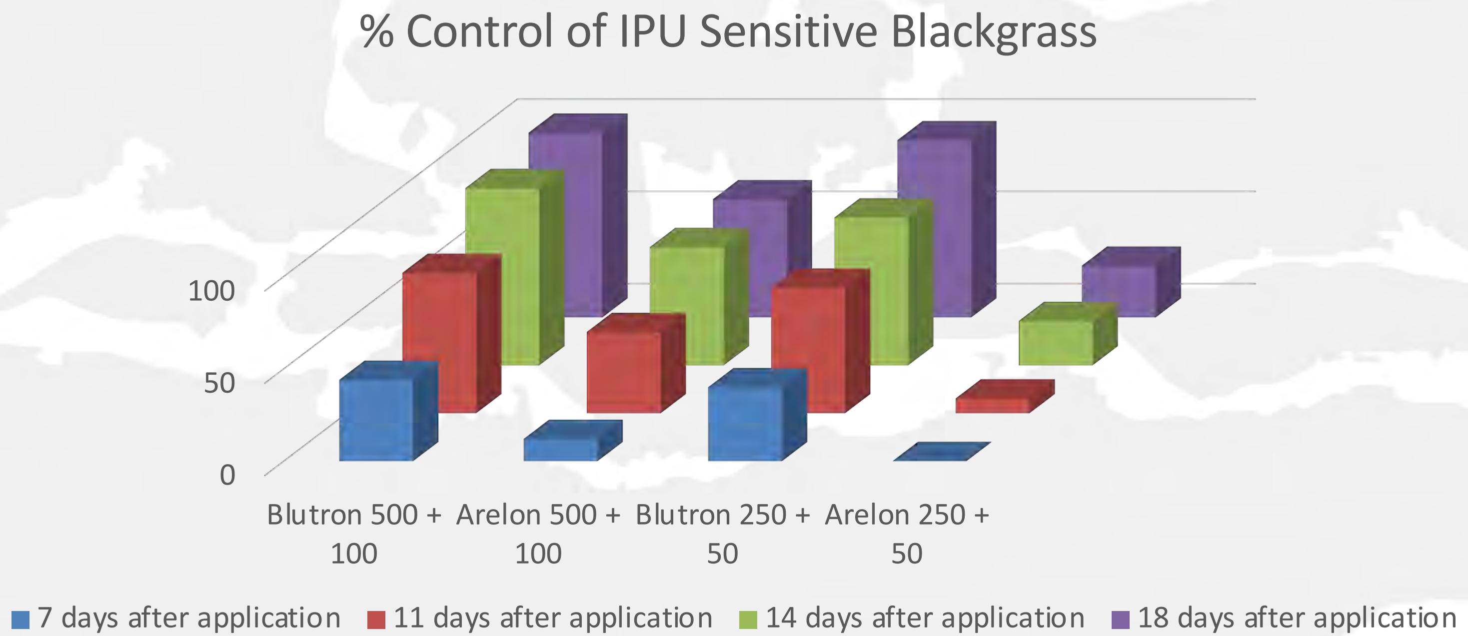
Future Development

- Exploring the benefits to other actives
- Application to (some) herbicides, fungicides and insecticides
- Excellent results with azoxystrobin
- 25 EU field trials over two years indicate comparable levels of fungal control at less than half rate of Amistar® using TransCel® technology

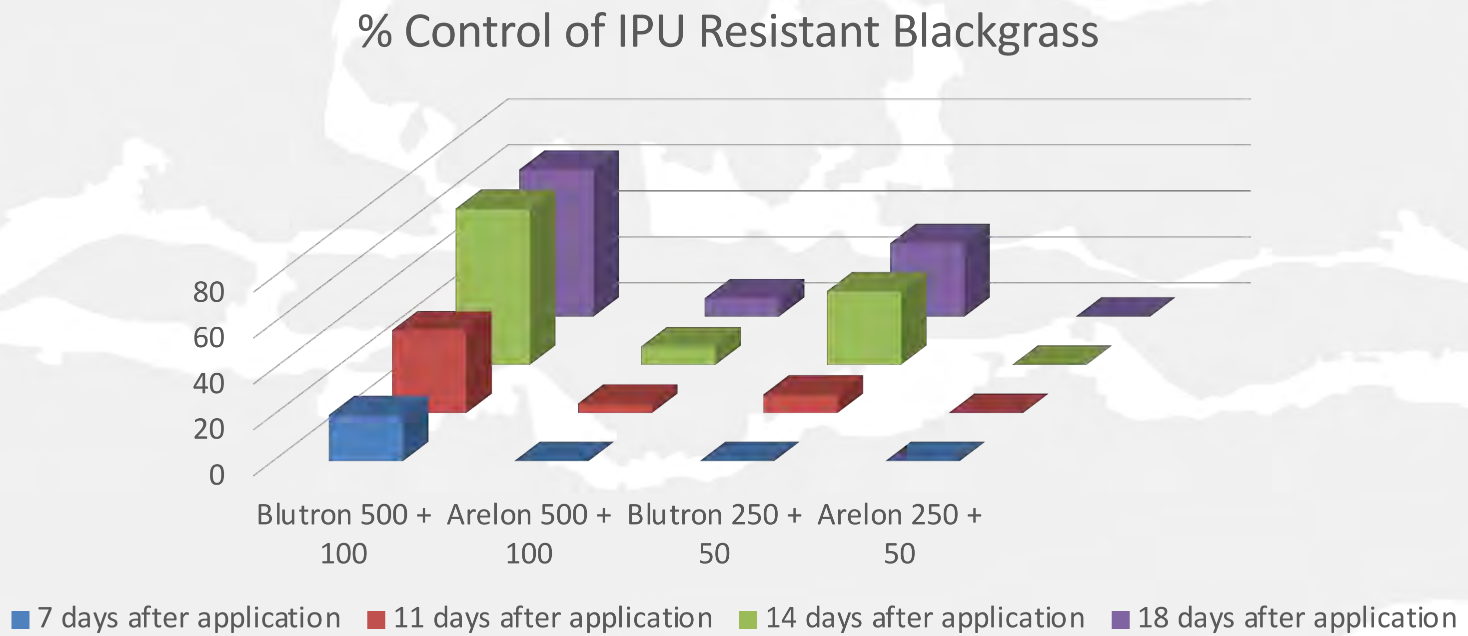
Herbicide Trials Results

Blutron®
with TransCel® Technology

Blutron™ Greenhouse Trials

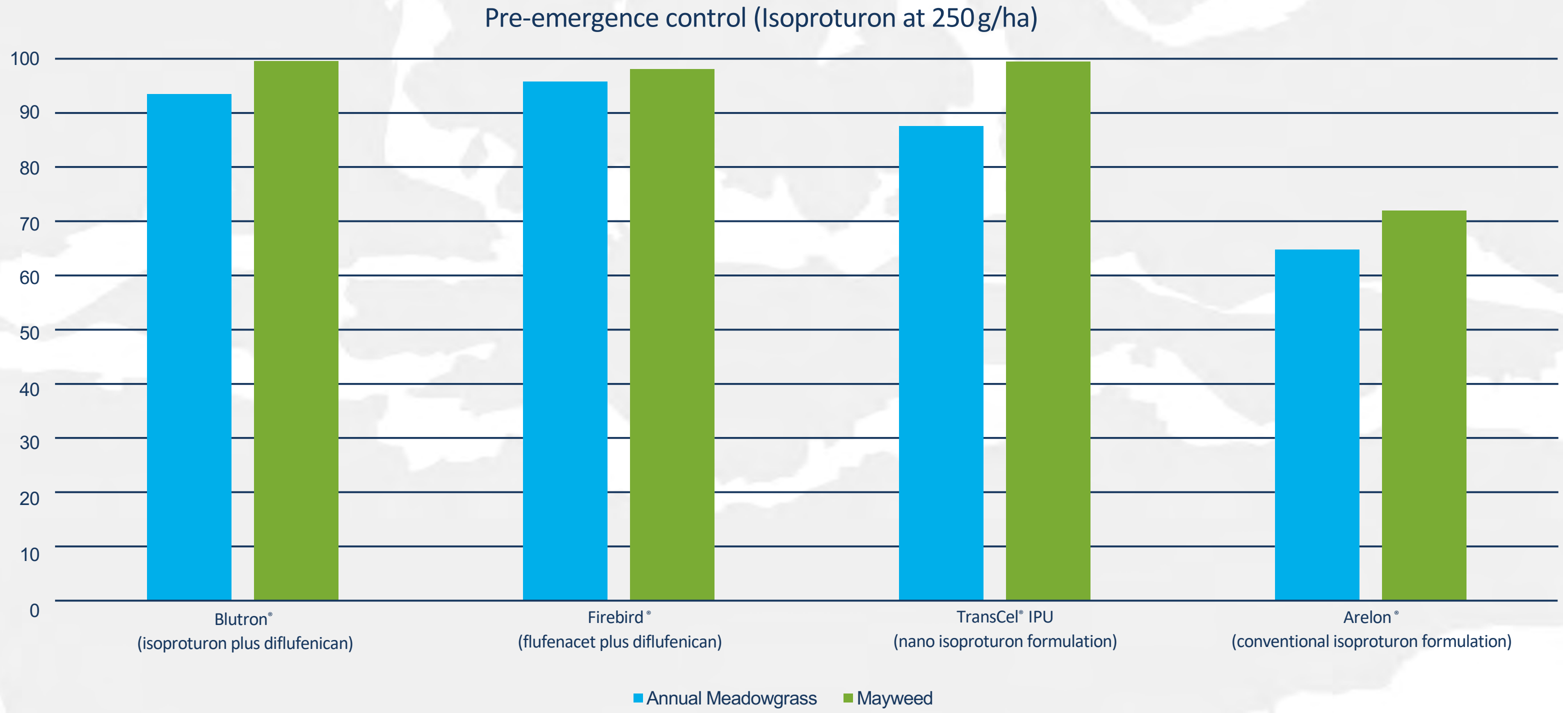


Blutron™ Greenhouse Trials

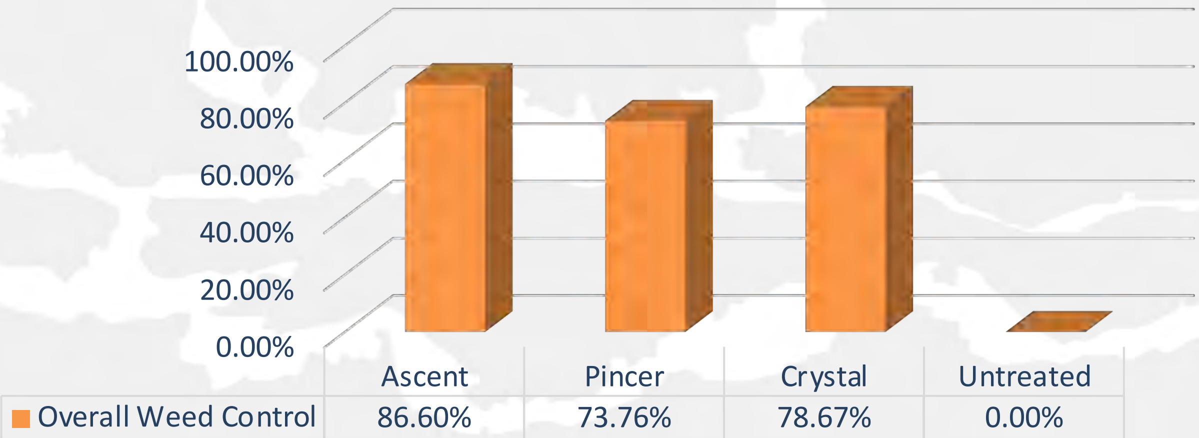


Blutron®
with TransCel® Technology

2008 TransCel® Field Trials

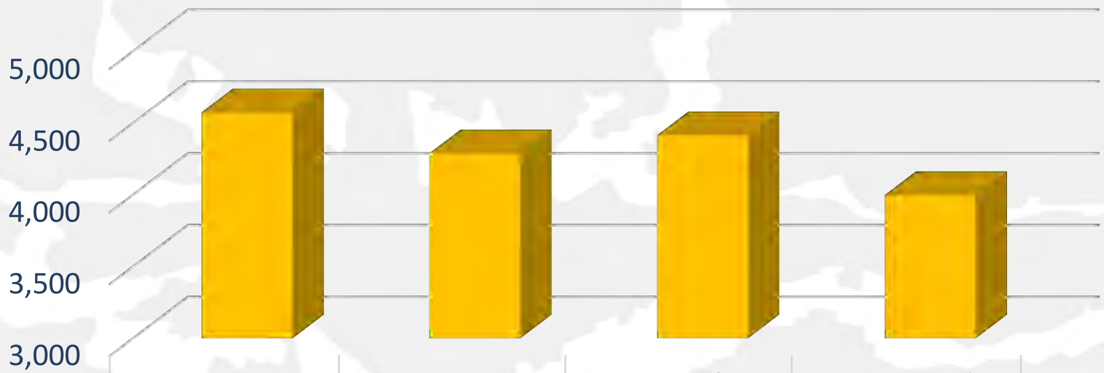


Efficacy Results 2017-2018 – 10 Sites



Ascent®
with TransCel® Technology

Small Plot Crop Yield - 10 sites



	Ascent	Pincer	Crystal	Untreated
Yield - kg/ha	4,575	4,289	4,415	3,998

Ascent®
with TransCel® Technology

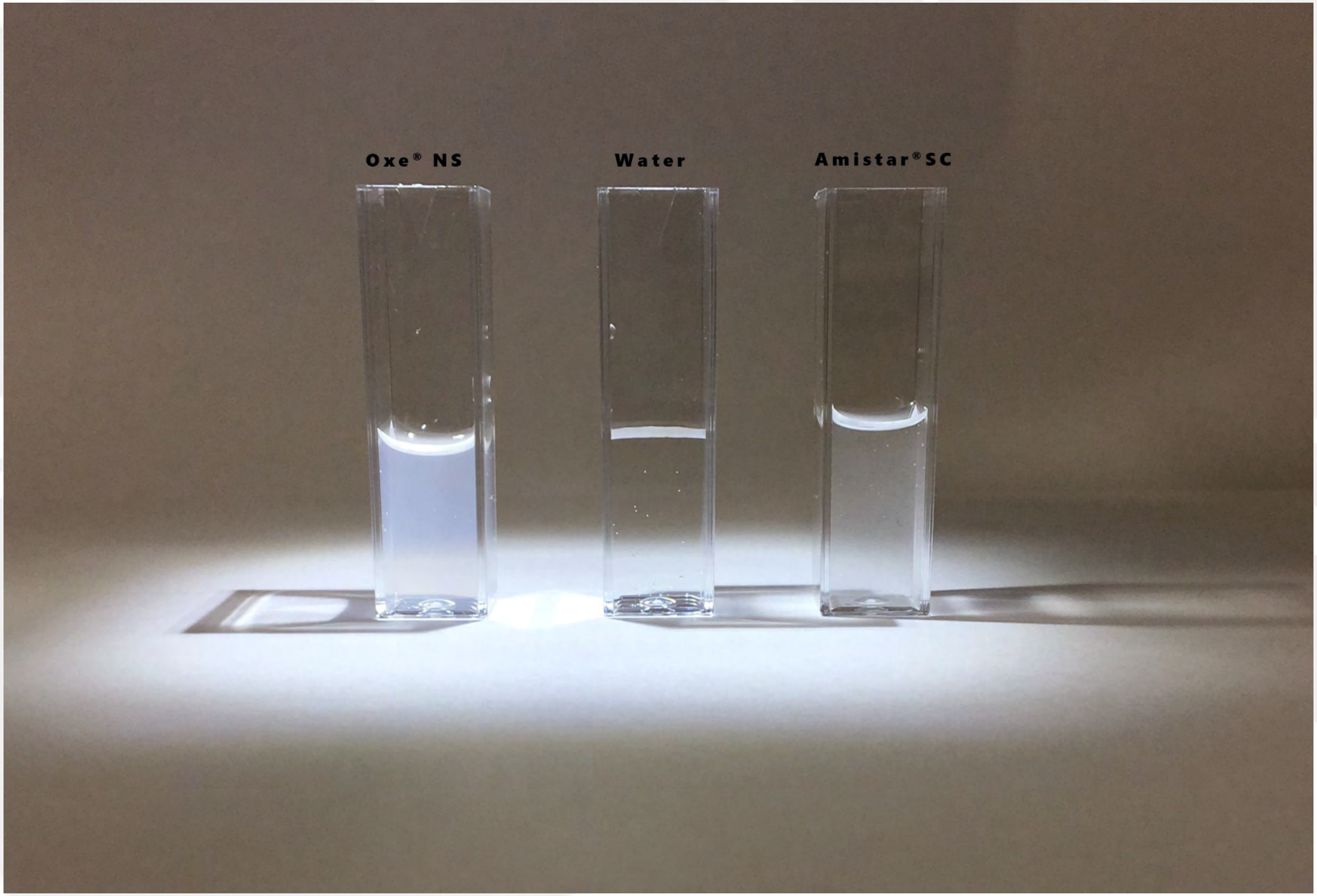
Ascent Yield Benefits

Product	Cost of Treatment/ha*	Yield Improvement Value/ha**
Ascent®	£32	£80
Crystal®	£50	£57
Pincer®	£30	£40

*   Distributor price
**Assuming wheat price of £137/tonne

Fungicide Trials Results

Oxe®
With TransCel® technology



Oxe® With TransCel® technology

Executive Summary

Oxe® at all rates was safe to winter wheat & winter barley in all trials over both seasons. No adverse effects on yield recorded

2015:

- Target Wheat: SEPTTR, (PUCCSS), Barley: RHYNSE, (PUCCSS)
- Low disease levels in the North, limited data produced suggested use rate for Oxe® of 94-125 gai/ha
- Poor disease year for RHYNSE
- High disease levels and good data from the southern trials
- Oxe® at 94 gai/ha provided similar control to Amistar ®/Ortiva® against SEPTTR & PYRNTE

2016:

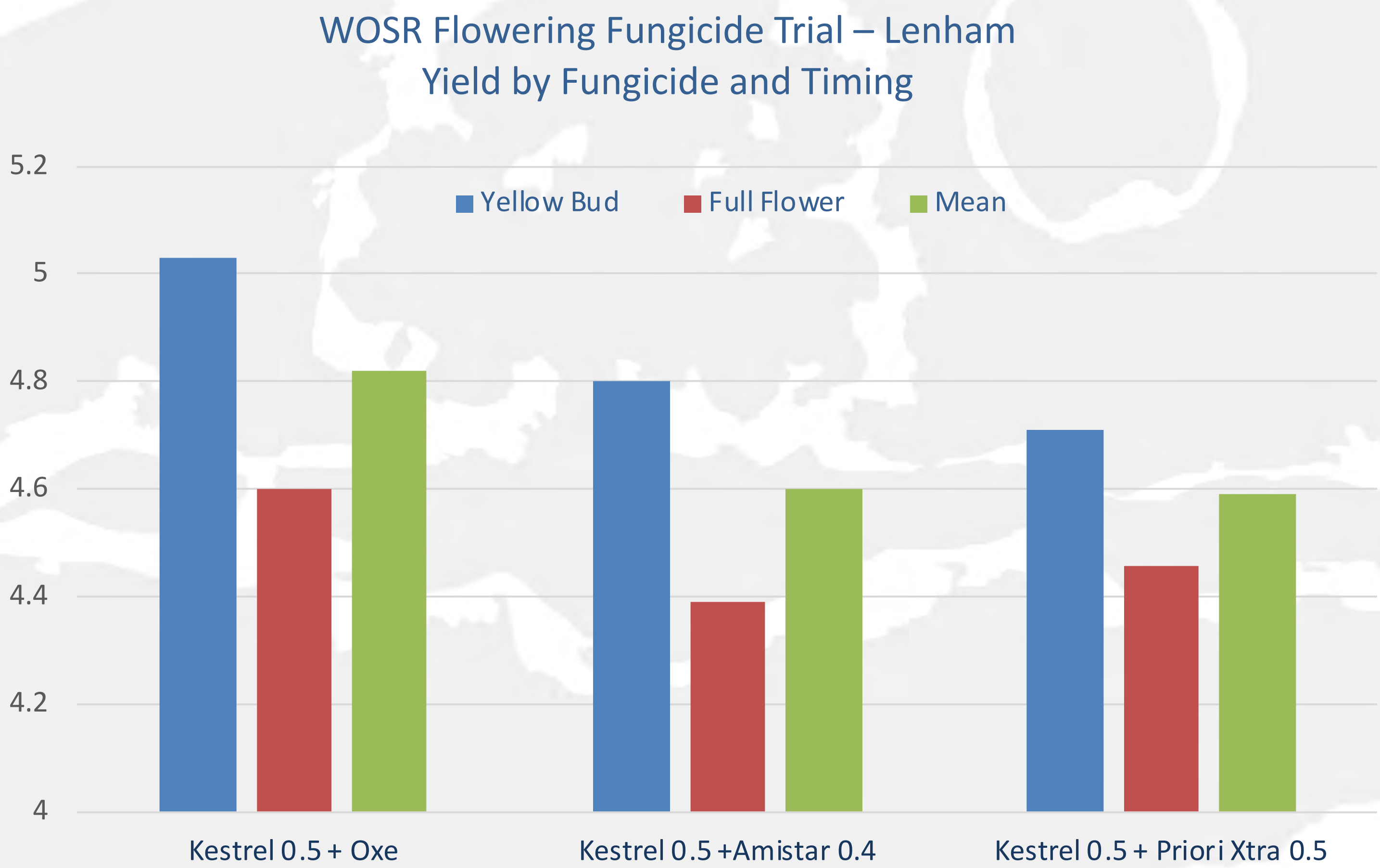
- Target Wheat: SEPTTR (PUCCSS), Barley: PYRNTE (PUCCSS)
- Good disease levels in many trials in both North & South
- In general across both zones, 125 gai/ha of Oxe® was required to consistently match the disease control seen from Amistar ® / Ortiva ® against SEPTTR & PYRNTE

Oxe®
With TransCel® technology

2018 Grower Trials Results

TransCel® Azoxystrobin – Spring 2018

Winter Oil Seed Rape Yield



Yield (t/ha) @ 9% m.c.

Untreated = 4.62t/ha

Oxe® With TransCel® technology

Oxe® Yield Benefits

Winter Oil Seed Rape

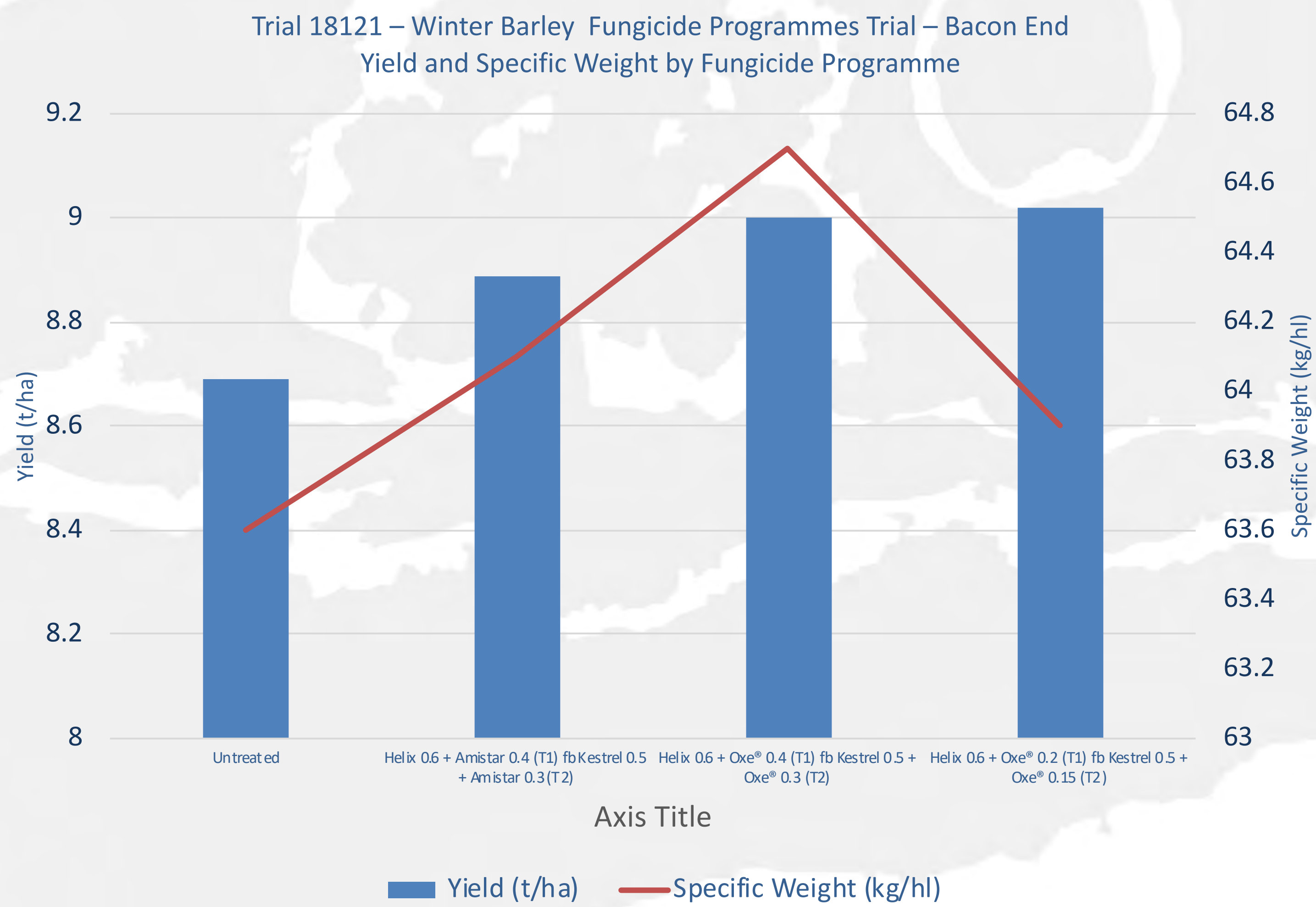
Fungicide Programme	Timing		Mean
	Yellow Bud	Full Flower	
Kestrel 0.5 + Oxe® 0.4	5.03	4.60	**4.82**
Kestrel 0.5 + Amistar 0.4	4.80	4.39	**4.60**
Kestrel 0.5 + Priori Xtra 0.5	4.71	4.46	**4.59**
Mean	**4.85**	**4.48**	

Product	Yield Improvement Value/ha*
Oxe®	£60

*Assuming oil seed rape price of £300/tonne

TransCel® Azoxystrobin – Spring 2018

Winter Barley Yield



Oxe®
With TransCel® technology

Oxe® Yield Benefits

Winter Barley

Product	Specific Weight (kg/hl)	Yield Improvement Value/ha*
Oxe®	64.7	£51
Oxe® - half rate	63.9	£55
Amistar®	64.1	£33

*Assuming barley price of £165/tonne

Solid state NMR Application to Vesicles & Soils

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Acknowledgments

- Ollie Levers
- Prof Jon Lloyd, Dept of Life Sciences

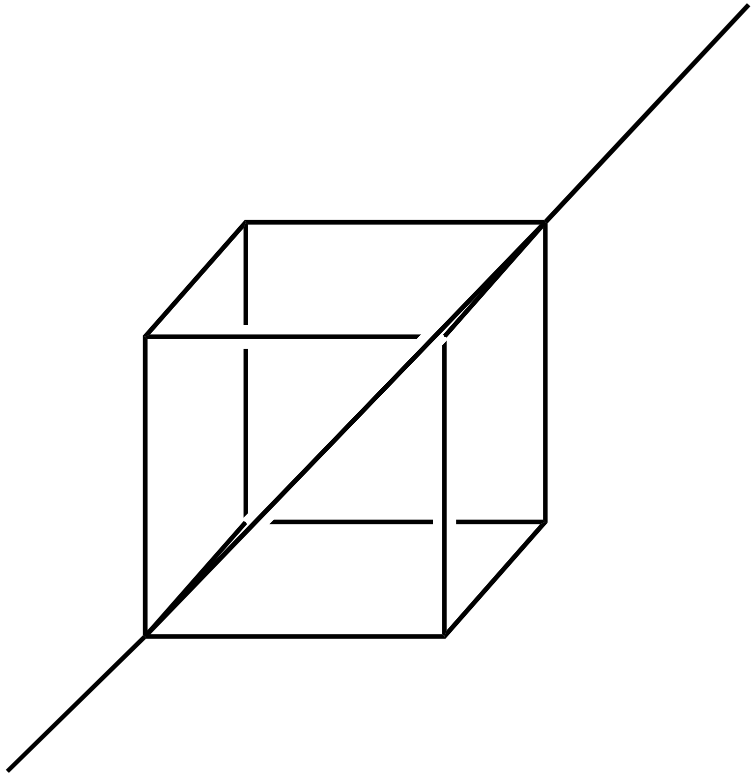
- Prof O. Ces, Dept of Chemistry
- Dr N.J. Brooks, Dept. of Chemistry

- Why solid state NMR spectroscopy?
- Solid state NMR of phospholipids – liposome (vesicles)
- Solid state NMR of model soils and the role of lignin

Why solid state NMR spectroscopy?

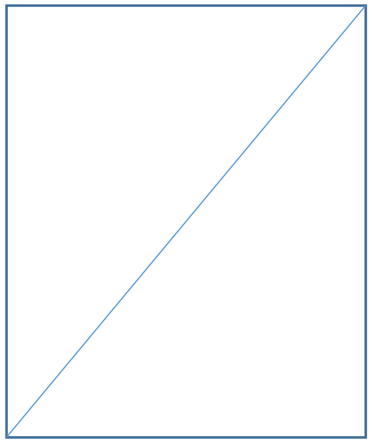
- Dissolution changes nature of materials e.g. crystal
- Solid state intrinsically useful to examine
- Some solids do not dissolve. Or act of dissolution destroys them e.g. wood, xlinked polymer, rock
- Very useful for anisotropic and amorphous materials e.g. liquid xtal, membrane proteins, silicon

Magic Angle (MAS)



Same angle as the diagonal in a cube between the origin (0,0,0) and the (1,1,1) point.

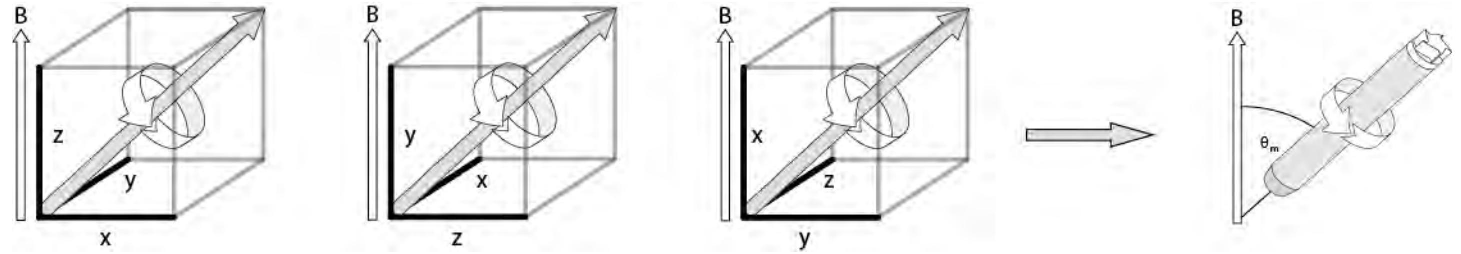
Equilateral square based pyramid



A4 paper

Solid state differs from solution state NMR by

- Dipolar Coupling
- Chemical Shift Anisotropy (CSA)
- Quadrupolar Interactions



Dipolar coupling, CSA and quadrupolar have $3\cos^2\theta - 1$ dependences

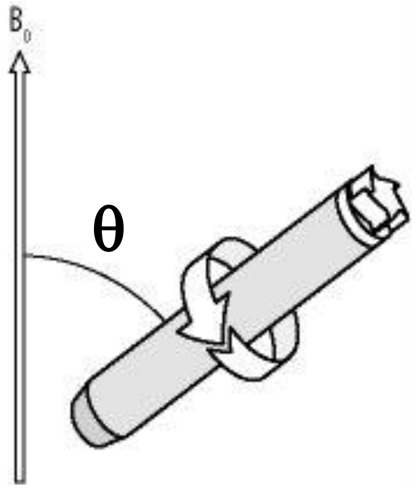
Removed by Magic Angle Spinning, MAS ($\theta \approx 54.74^o$)

Broadening mechanisms are also averaged by molecular motions

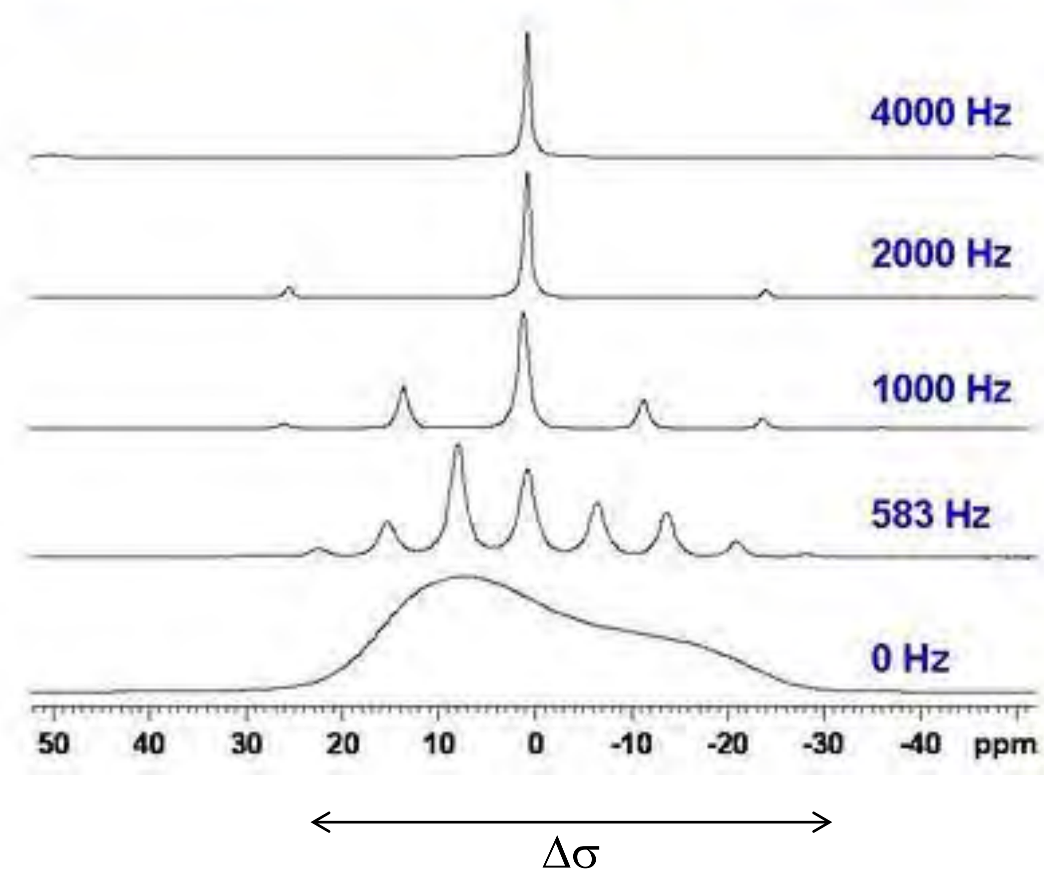
MAS spinning

- Spinning side bands are therefore a manifestation of partial removal of the chemical shift anisotropy
- Spun on air turbine, at magic angle
- Spun on a air turbine made of ceramic

  (e.g. zirconia)
- Magic angle ≈ 54.74°
- speeds can now be up to 110kHz



MAS
^{31}P MAS $NH_4H_2PO_4$



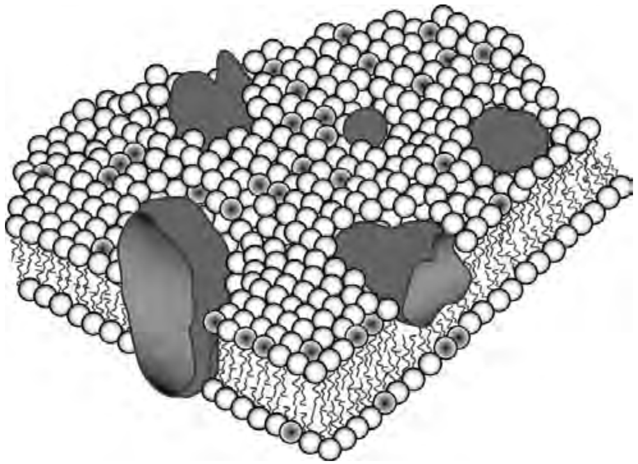
$\omega_r >> \Delta\sigma$

$\omega_r \approx \Delta\sigma$

$\omega_r << \Delta\sigma$

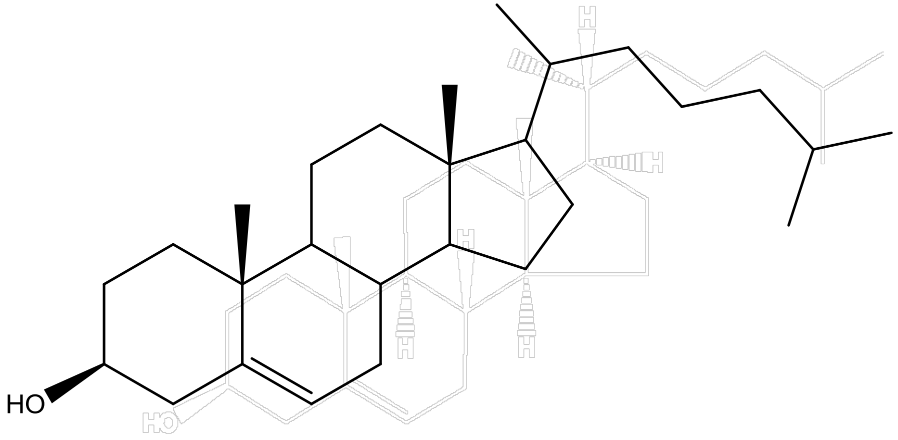
static

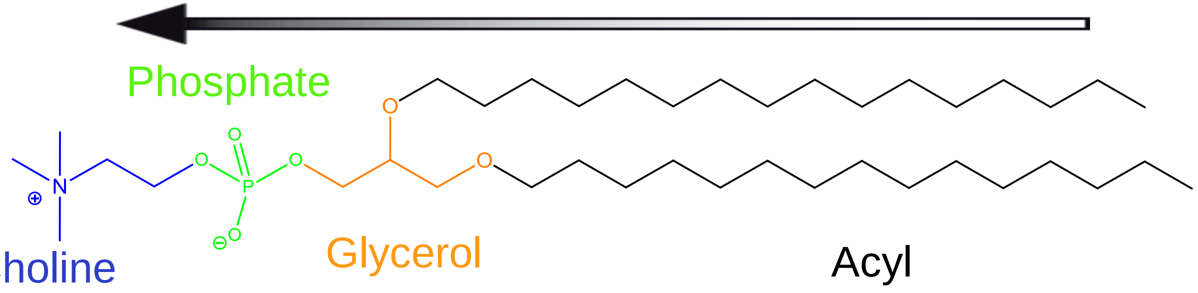
Phospholipid Bilayers



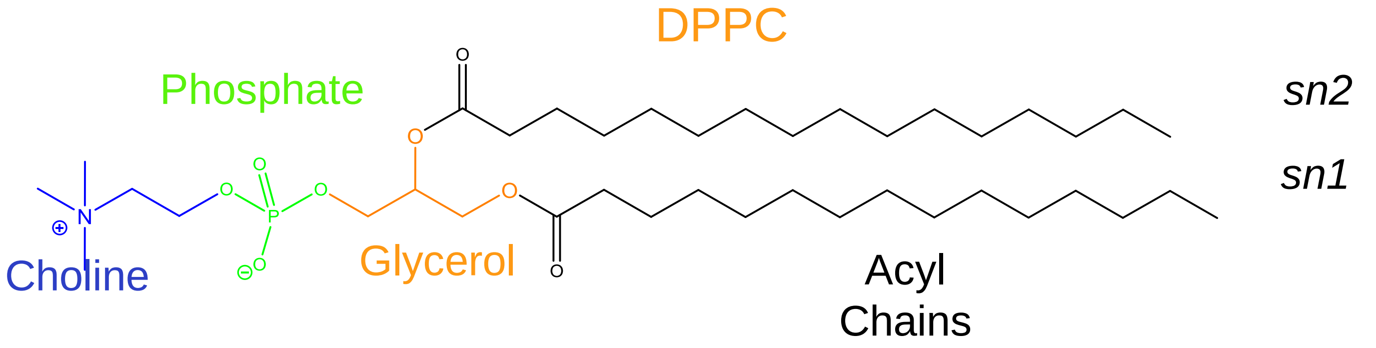
Singer, SJ; Nicolson, GL. “Fluid mosaic model of the structure of cell membranes”. *Science*. 175 (23): 720-731. 1972.

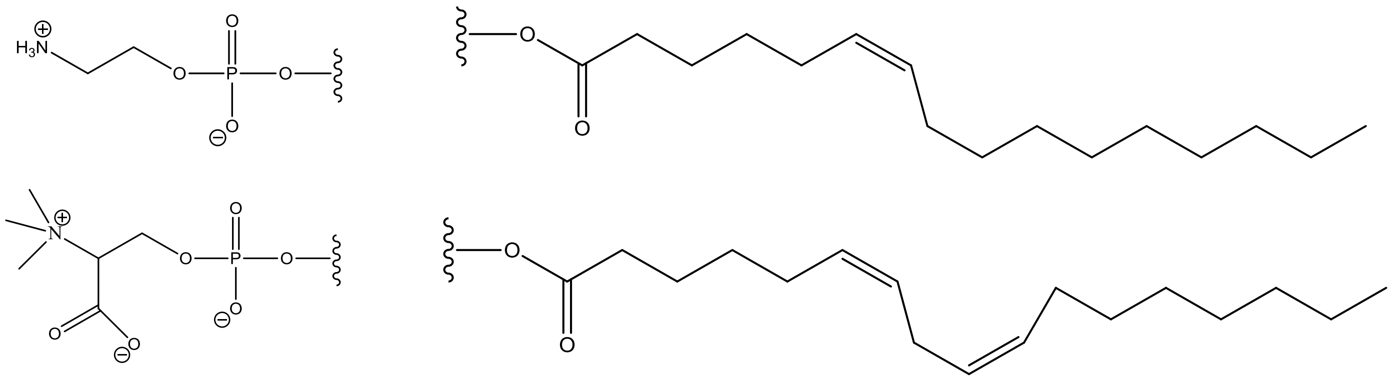
Cholesterol (Chol)





DPPC



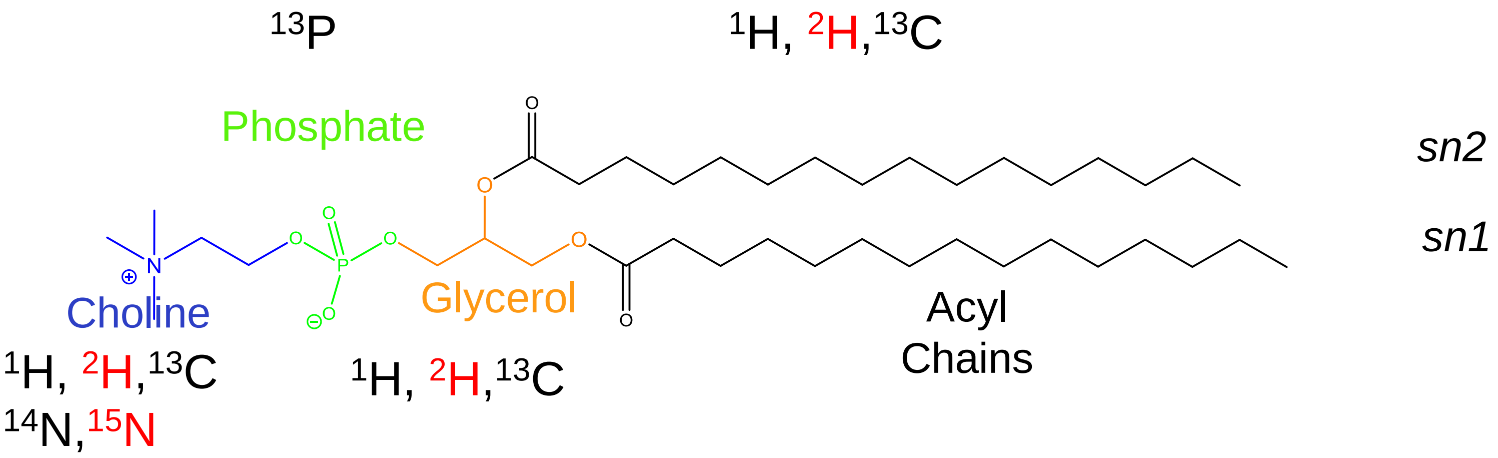


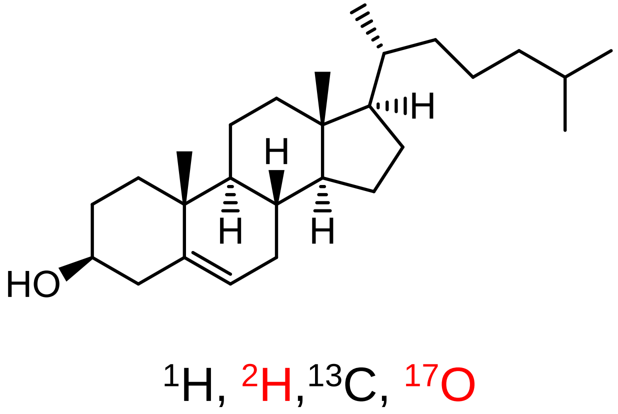
•Neutral e.g.
PE, sugar
•Charged e.g.
PS

- Saturated, unsaturated
- Different chain lengths
- Symmetrical, unsymmetrical
- Ester, ether

In pure state characterised by a gel (L_β) to fluid (L_α) liquid crystalline transition (thermodynamic) temp. T_m.

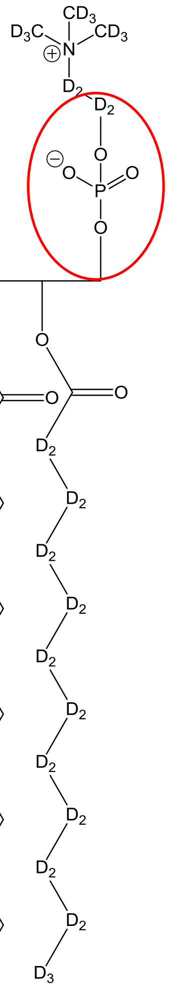
Multinuclear NMR

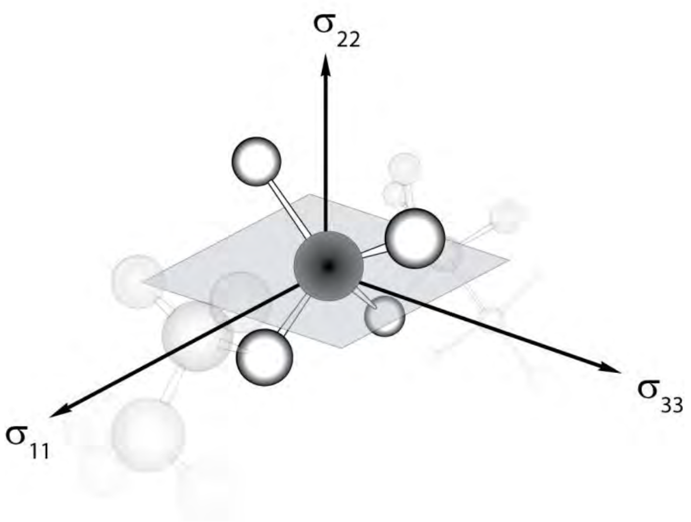


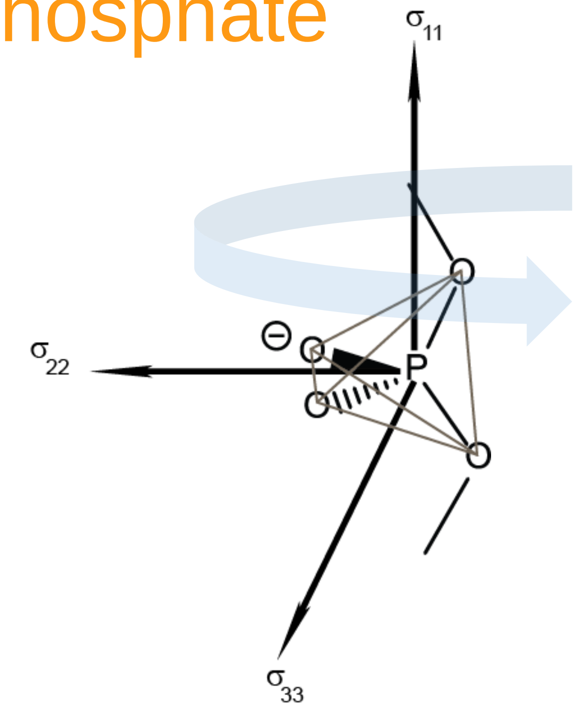


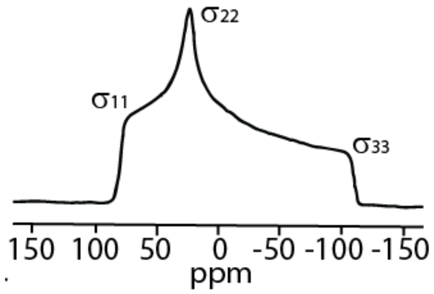
- Natural abundance
- Stable isotope

^{31}P Chemical Shielding Tensors of Phosphate



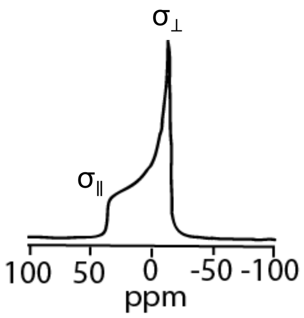






Crystal – powder

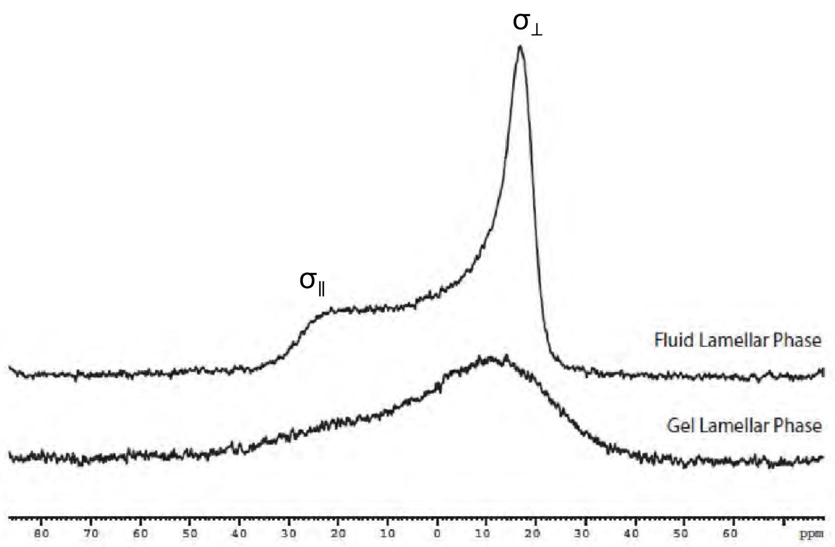
- No rotation

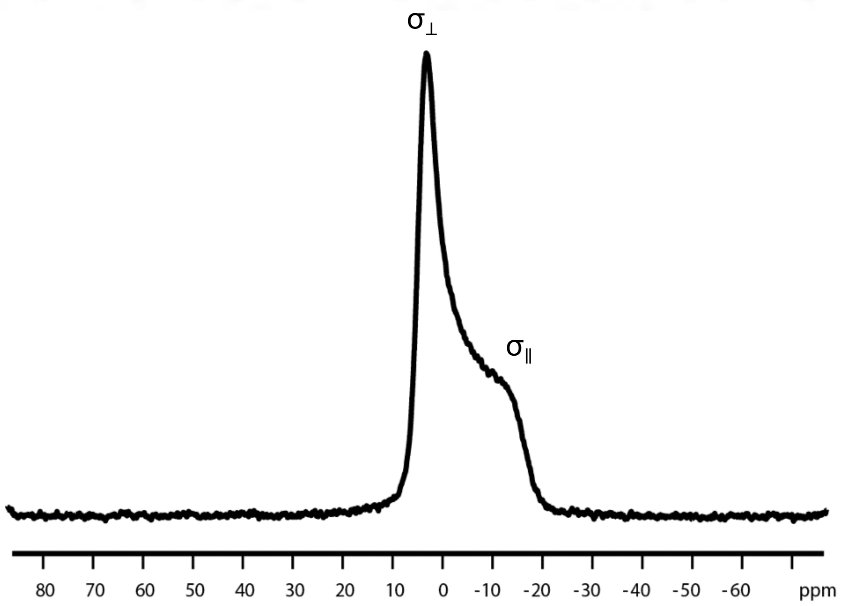


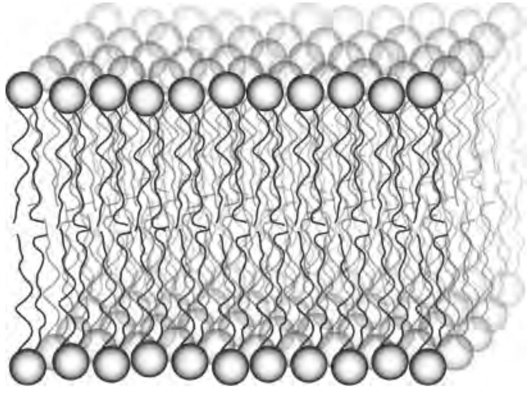
Liquid Crystal

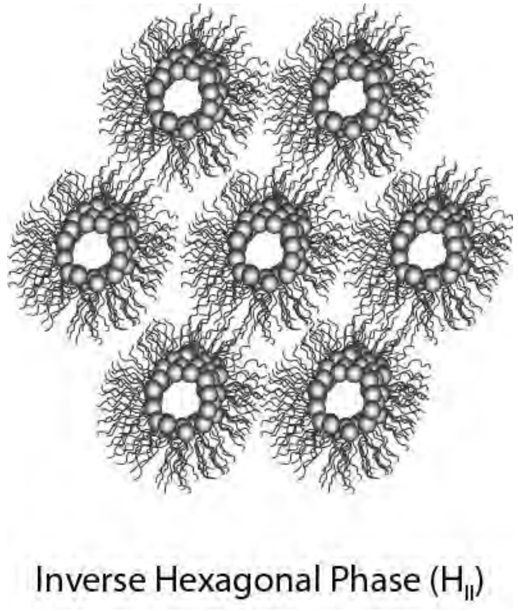
- Rapid axial rotation

^{31}P NMR

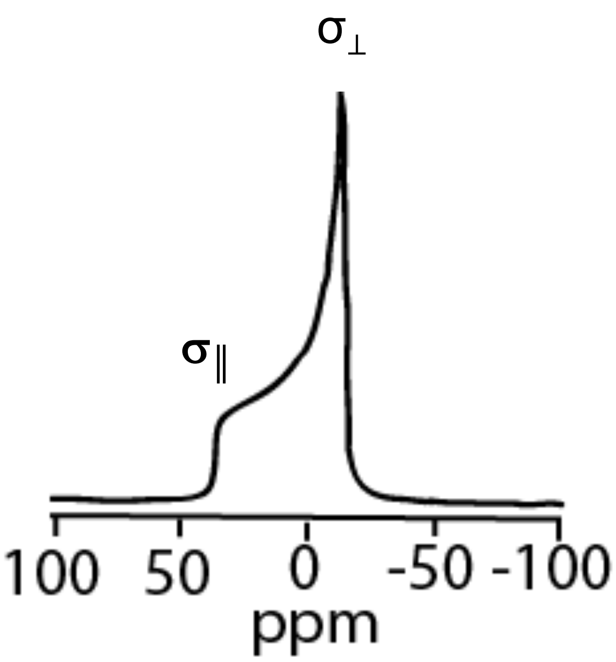

σ⊥
σ∥
Fluid Lamellar Phase
Gel Lamellar Phase
80 70 60 50 40 30 20 10 0 10 20 30 40 50 60 ppm


σ⊥
σ∥
80 70 60 50 40 30 20 10 0 -10 -20 -30 -40 -50 -60 ppm

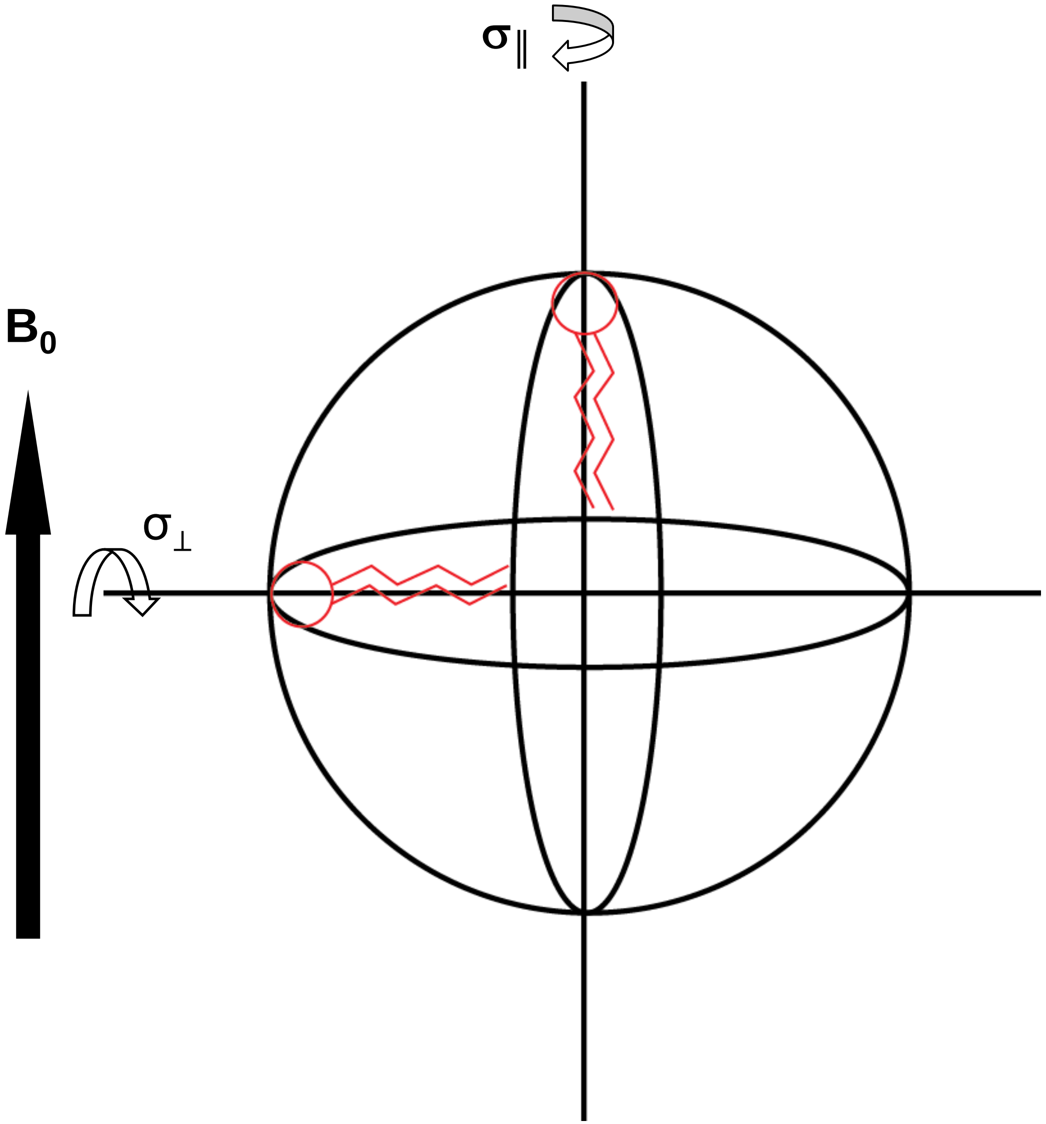



Inverse Hexagonal Phase (H_{II})

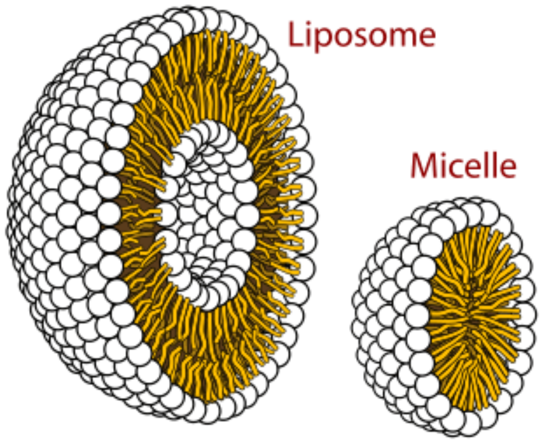
Static - ^{31}P CSA Powder Patterns

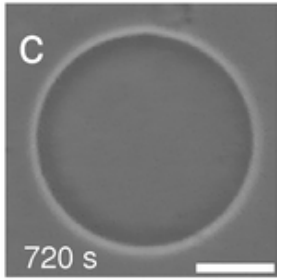


- Axial rotation of phospholipid and partial diffusion over a spherical vesicle
- Partial averaging of CSA tensor



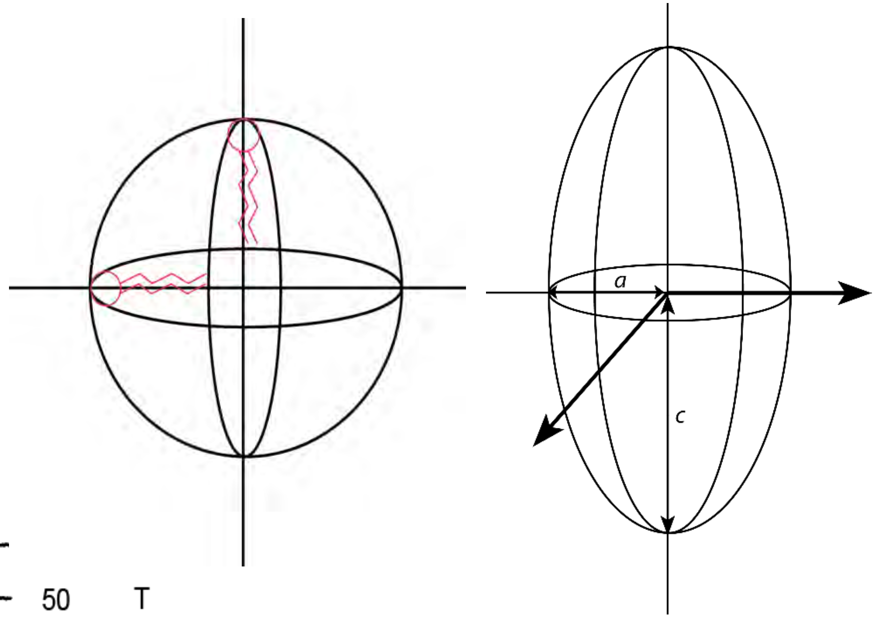
Diffusion over a spherical vesicle

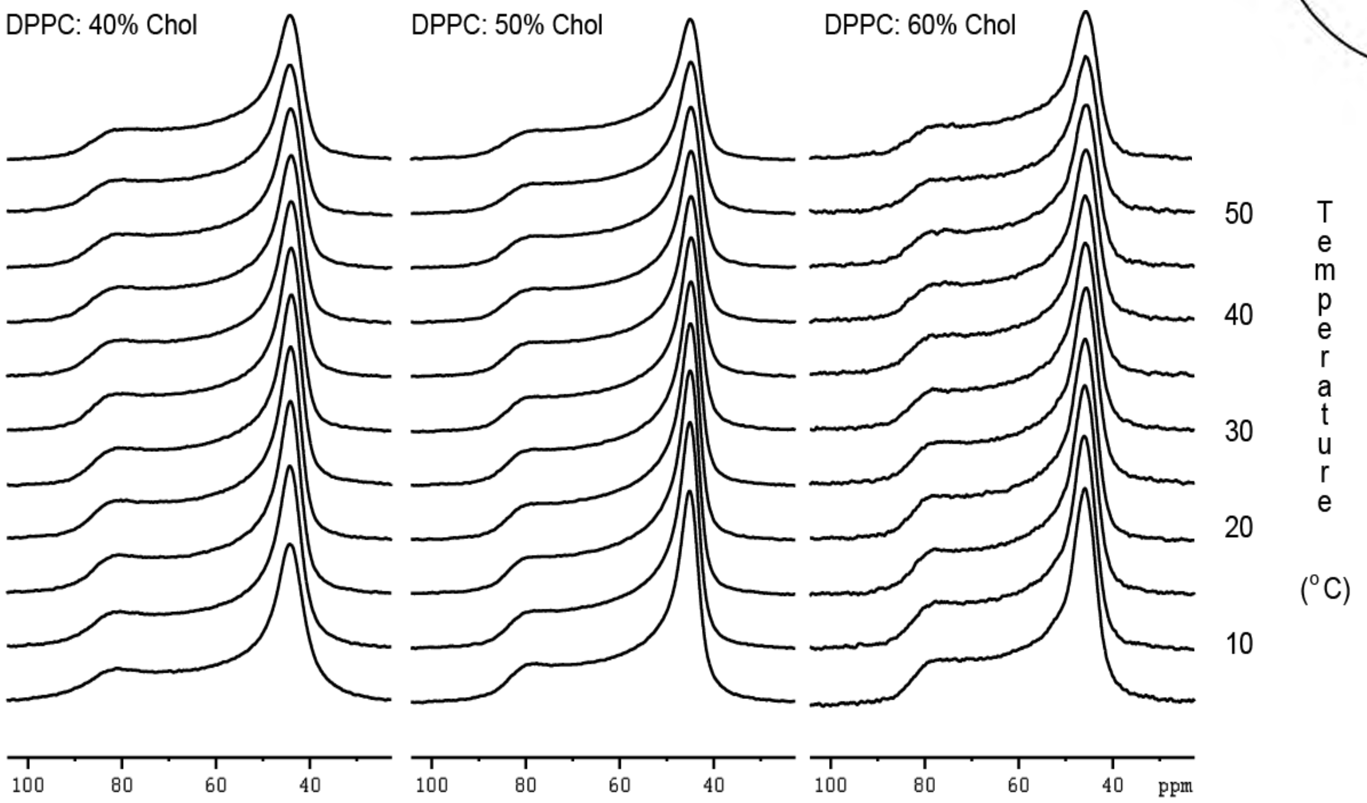




Static - ^{31}P NMR of Binary System

- Liquid crystalline phase behavior
- Distortion under magnetic field – mag. susceptibility

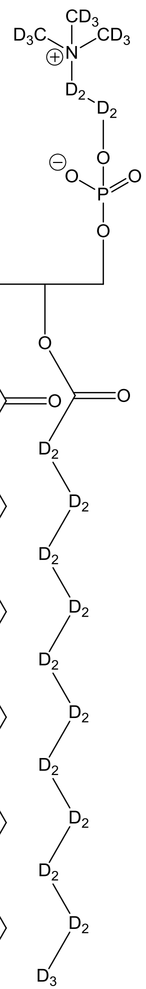




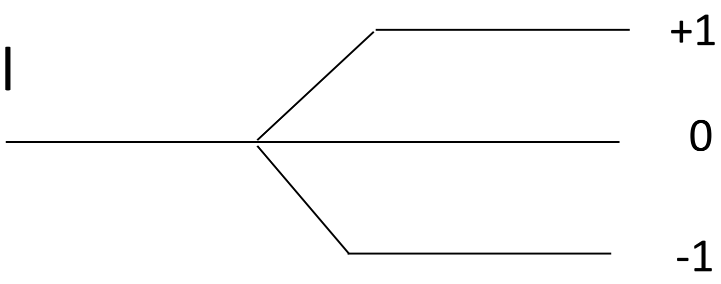
Helfrich, Wolfgang *Zeits. Naturfors*. (1973) C 28 (11): 693.

^2H NMR

- Anisotropy in the phospholipid bilayer makes conventional NMR difficult
- Use deuterated phospholipids – along acyl chains or headgroup

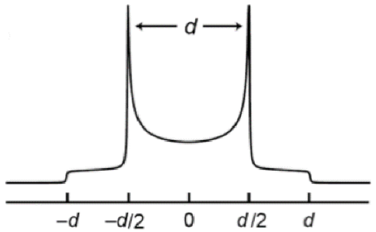


Energy level diagram



I = I

For every ^2H
Two transitions



Pake powder pattern

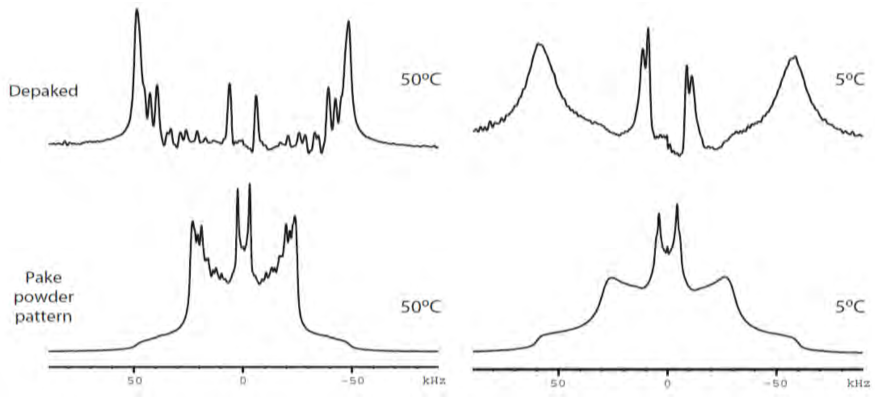
Static - Deuterium NMR – *Order Profiles*

Quadrupolar splittings partially averaged by motion in a magnetic field

Order profiles from deuterium quadrupolar splittings

$$\Delta v Q = \frac{3}{2}\left(\frac{e^2 qQ}{h}\right)\left(\frac{3\cos^2\theta - 1}{2}\right)$$

Powder patterns "de-Paked" to remove angular dependence

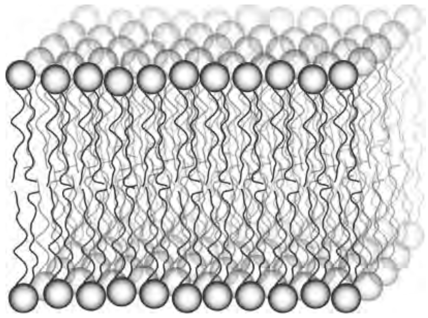


- Phase coexistence
- Area per lipid and bilayer thickness
- Rigidity of bilayers

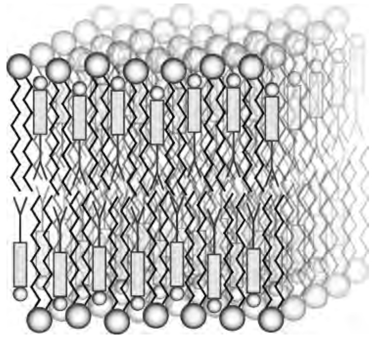
Helfrich, Wolfgang *Zeits. Naturfors*. (1973) C 28 (11): 693.
Seelig *Q. Review Biophys*. (1977), **10,** 353
Brown *Chem. Phys. Lipids.* (1994), **73,** 159

Static – ^{2}H NMR Types of Lamellar Phase

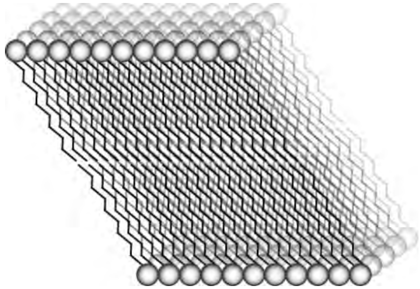
Most biologically significant - lamellar phase



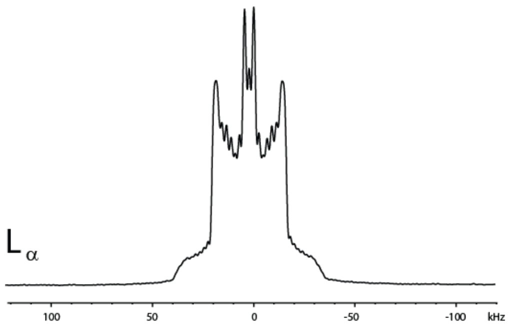
Liquid Crystal Phase, L_α

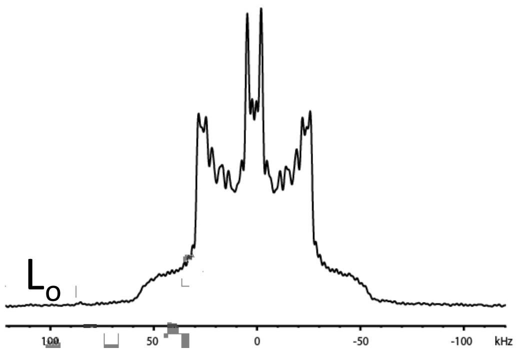


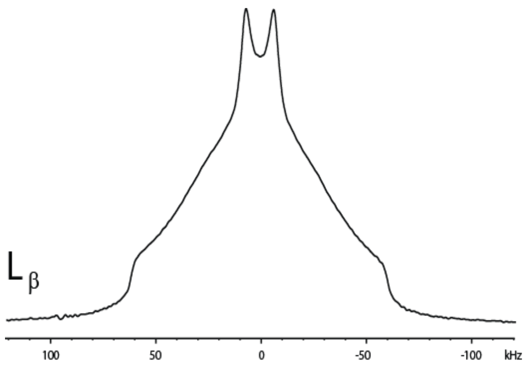
Liquid Crystal Phase, L_o



Gel Phase, L_β

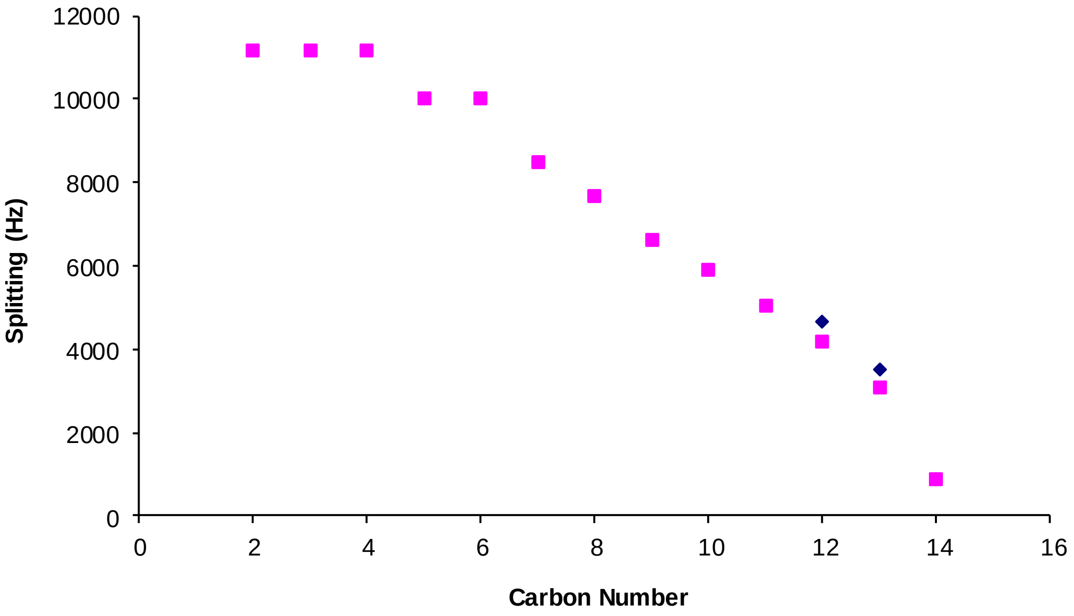


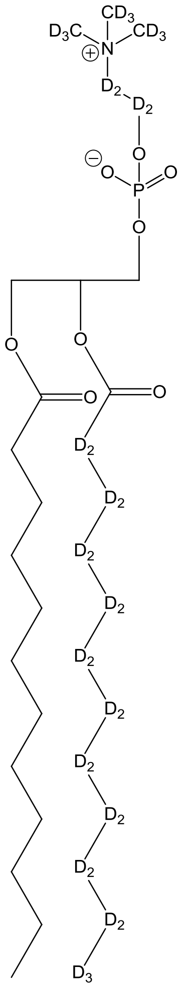


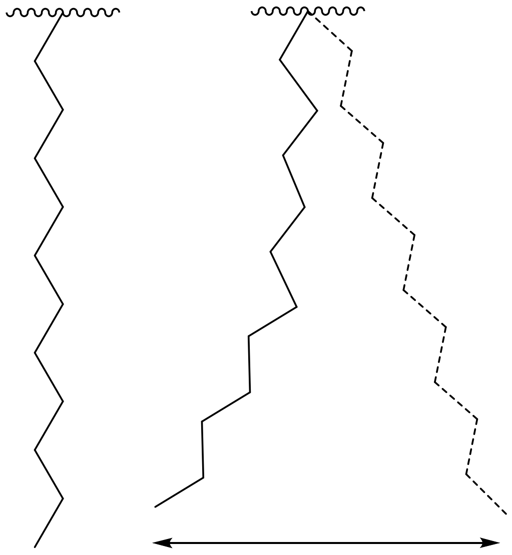


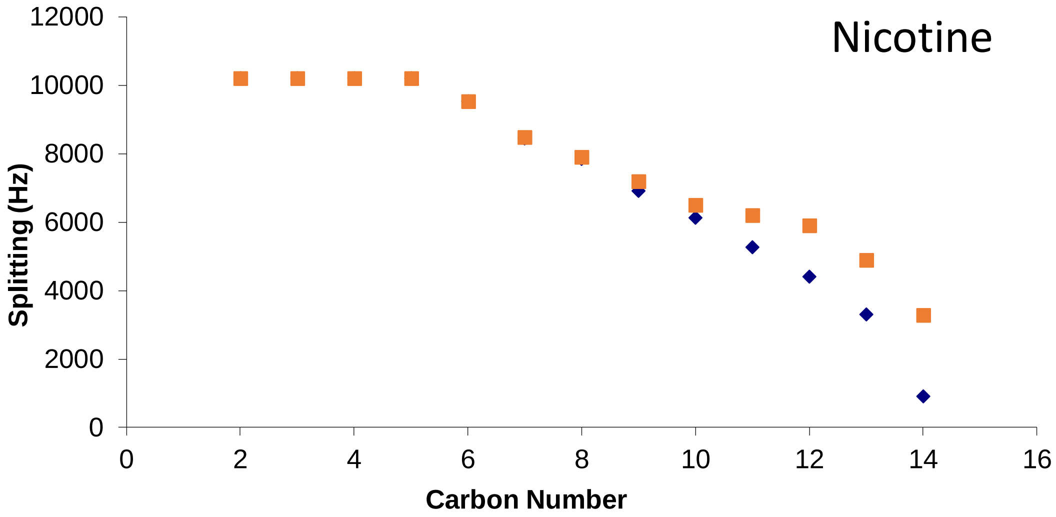
^{2}H NMR Order Parameter

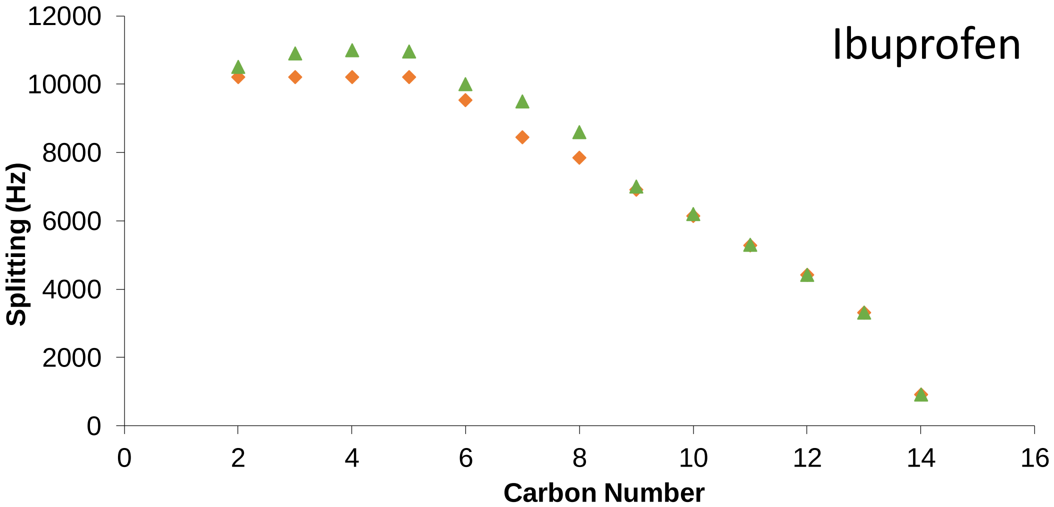
- Plot of splitting vs. carbon number down chain
- Order parameter profile
- Bilayer thickness, area per lipid

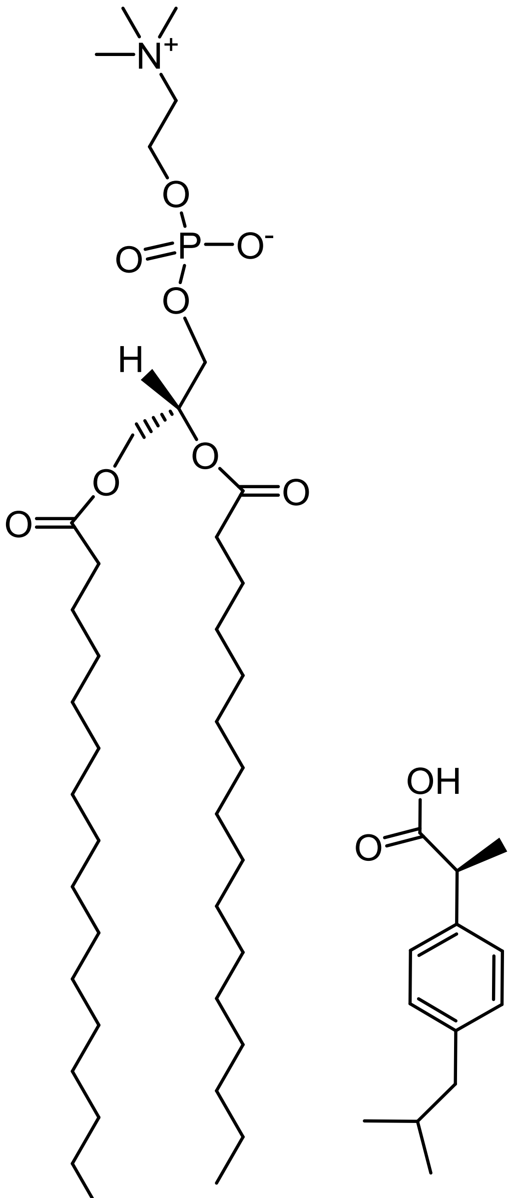




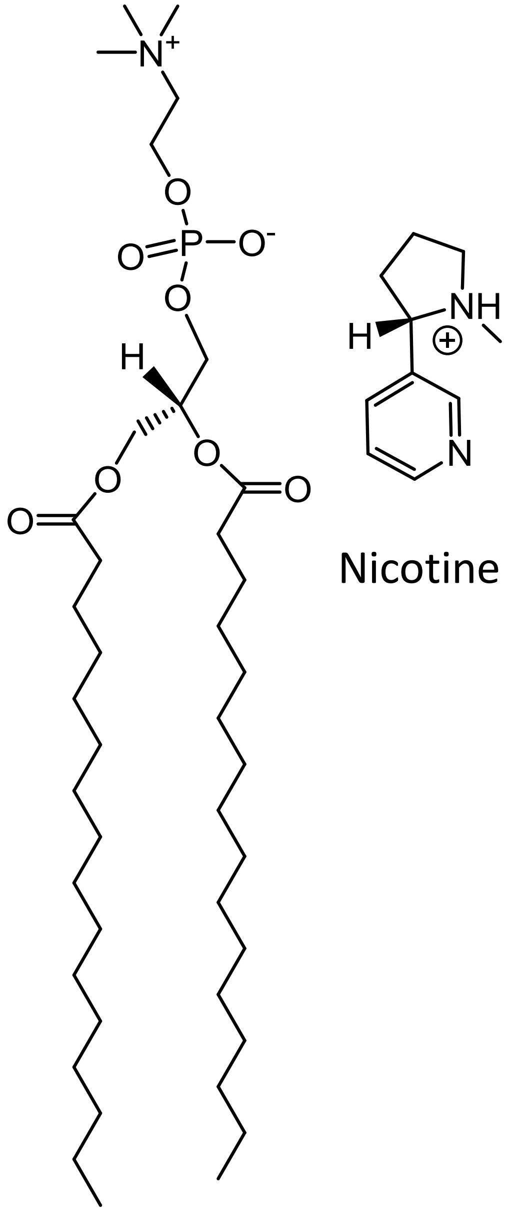



Nicotine
12000
10000
8000
6000
4000
2000
0
0
2
4
6
8
10
12
14
16
Splitting (Hz)
Carbon Number

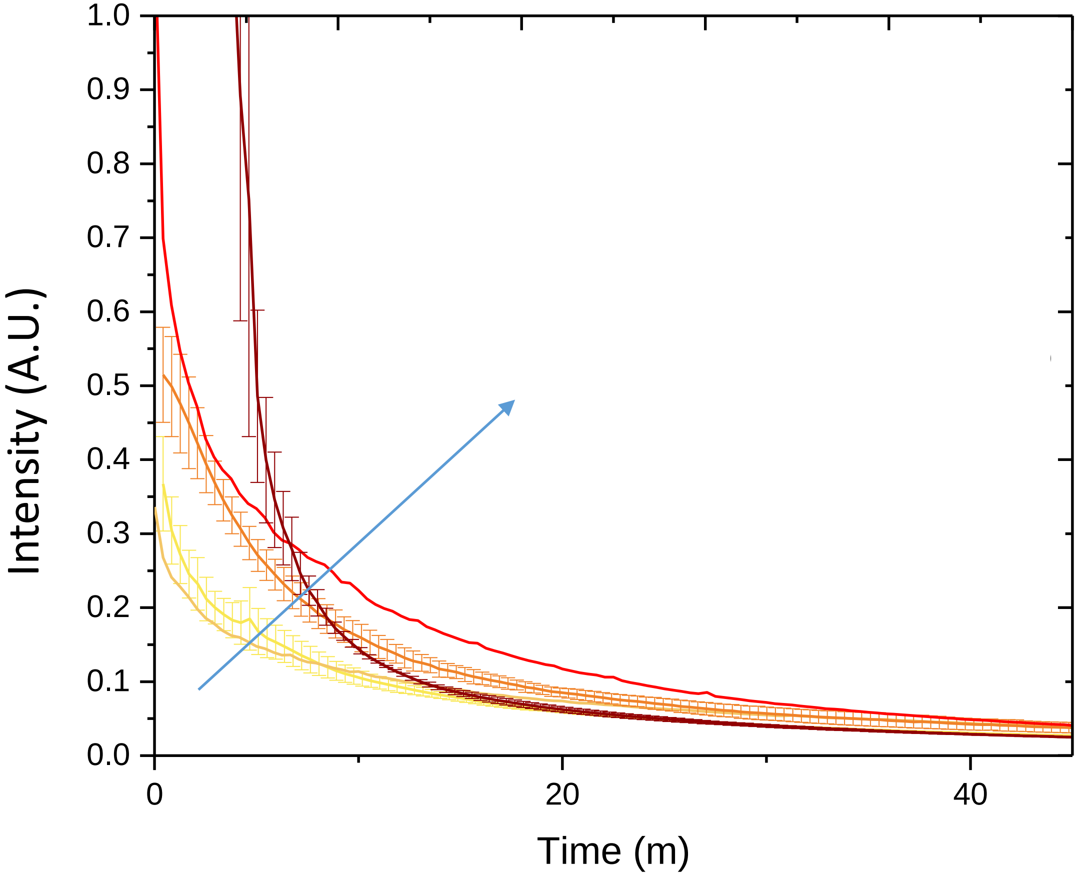

Ibuprofen
12000
10000
8000
6000
4000
2000
0
0
2
4
6
8
10
12
14
16
Splitting (Hz)
Carbon Number


N+
O
O
P
O-
O
H
O
O
O
O
OH
O

Ibuprofen

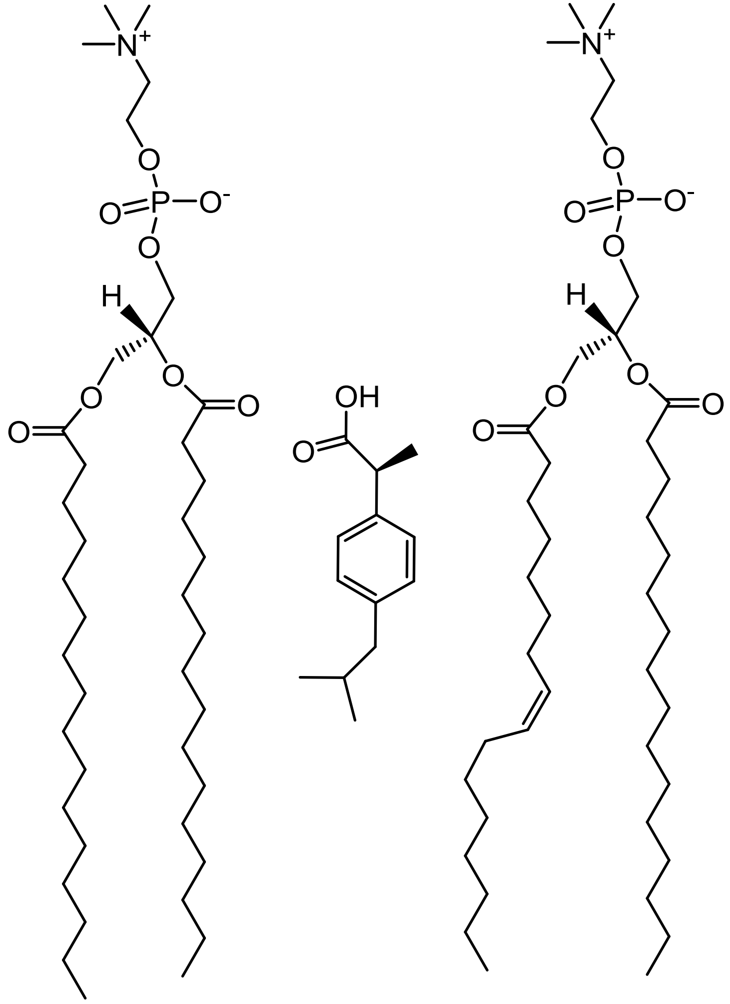

N+
O
O
P
O-
O
H
O
O
O
O
H
NH
N
Nicotine

Control Release Kinetics of Drugs



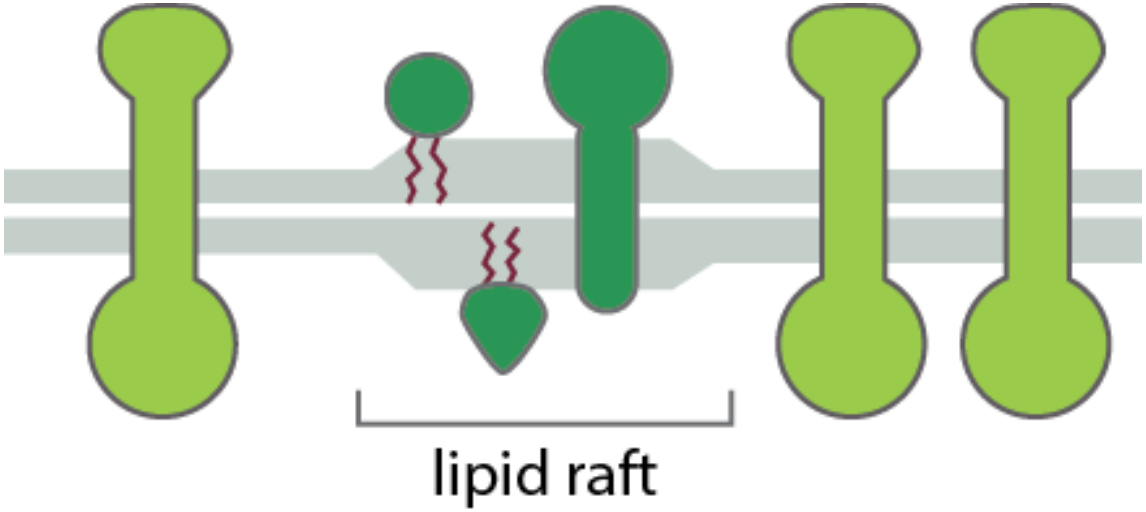
Increasing ratio of unsaturated:saturated lipid

- Location of drug in bilayer
- By changing lipid composition changes release kinetics

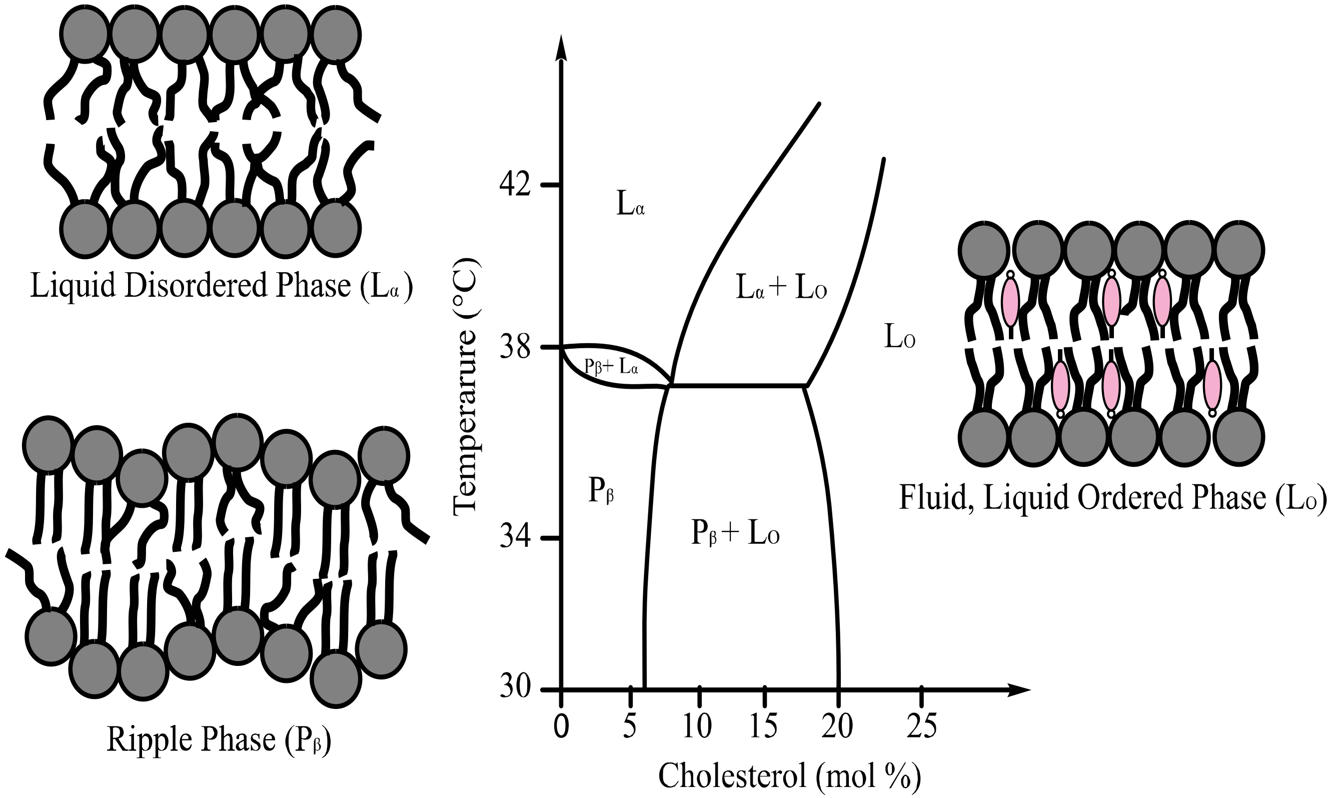


Saturated Unsaturated

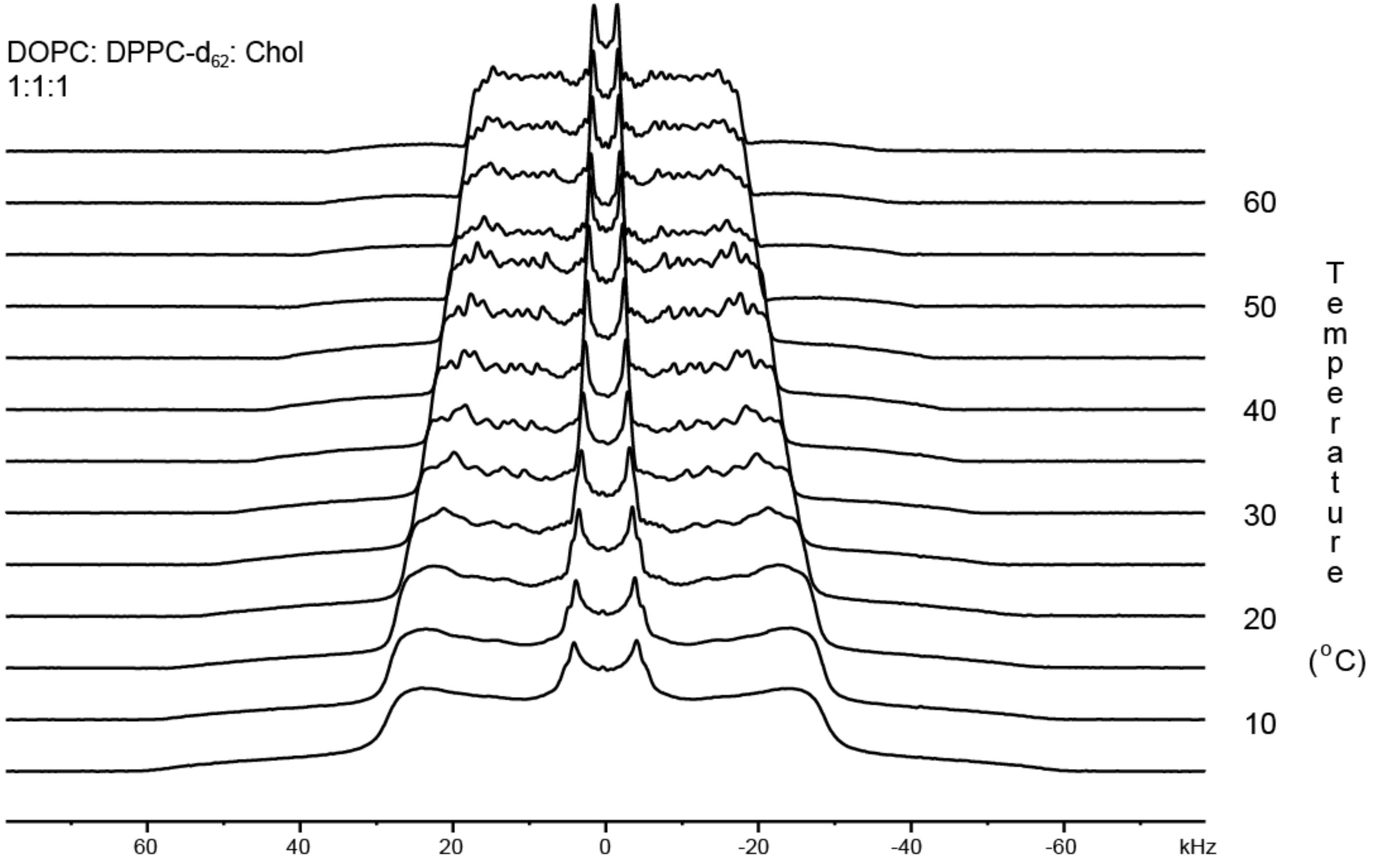
The Raft Concept now as Written in Stone



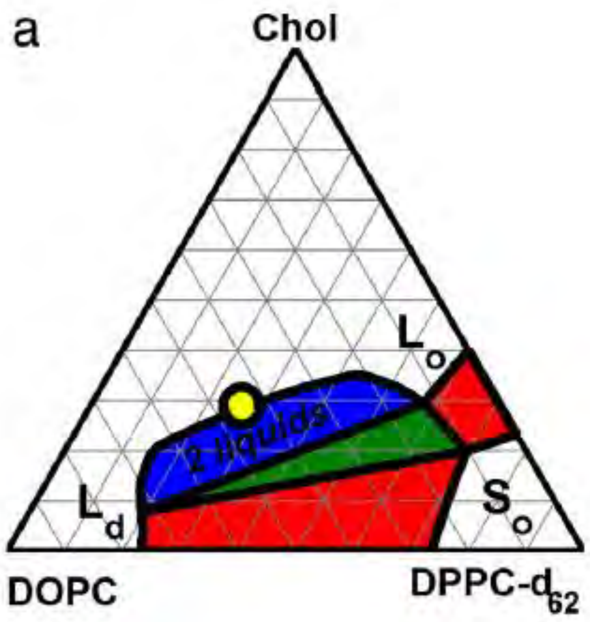
Molecular Biology of the Cell. 4th ed. Alberts B, Johnson A, Lewis J, et al. New York: Garland Science; 2002.

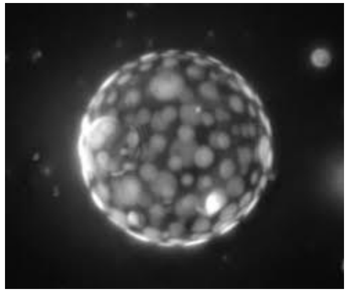

Liquid Disordered Phase (Lα)
Ripple Phase (Pβ)
Fluid, Liquid Ordered Phase (Lo)
Temperarure (°C)
42
38
34
30
0
5
10
15
20
25
Cholesterol (mol %)
Lα
Lα + Lo
Lo
Pβ+ Lα
Pβ
Pβ + Lo

^2H NMR Ternary Phase Diagram



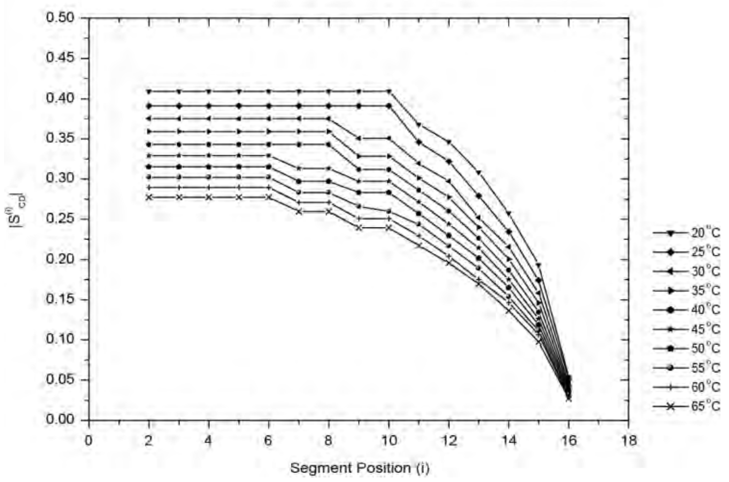
Ternary Phase Diagram

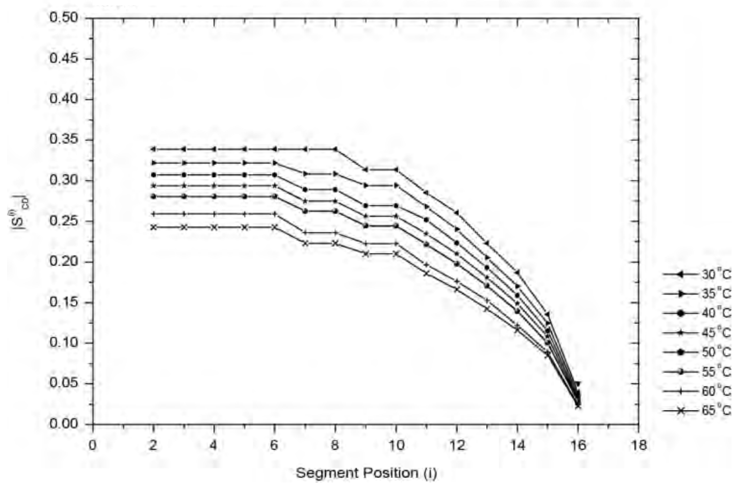


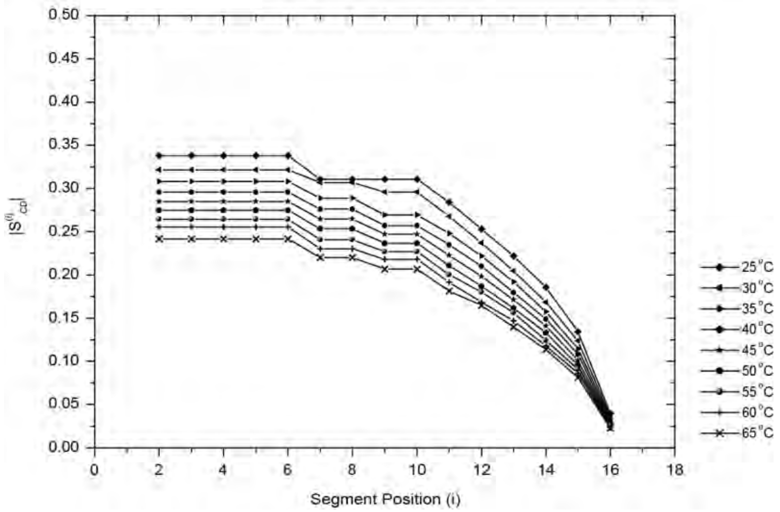


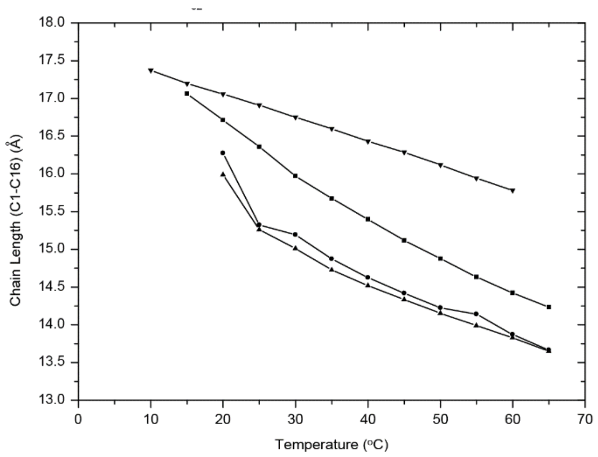
Clarke et al. *Biophysical J.* 2006 **90**, 2383
Clarke et al. *Soft Matter,* 2009, **5**, 369

Order Parameters

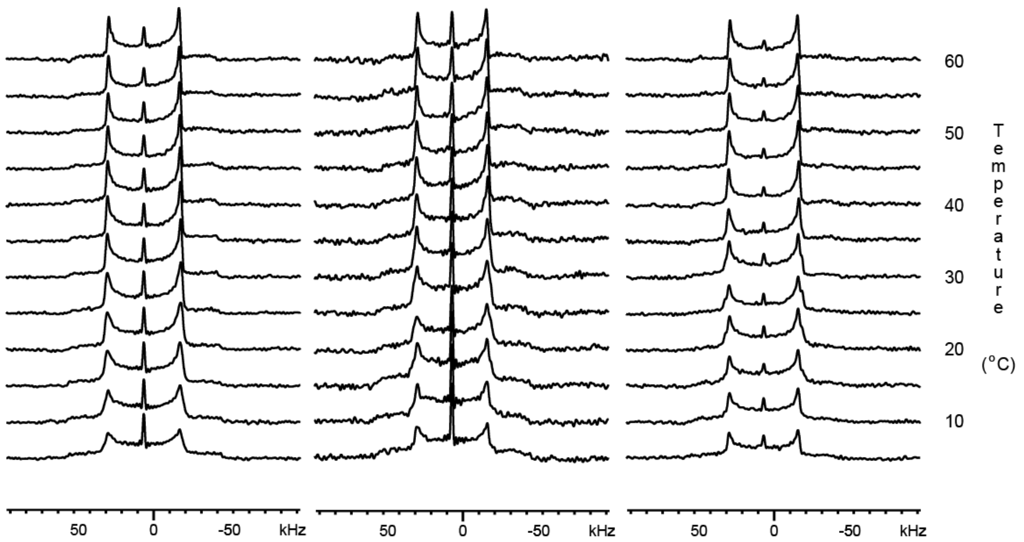


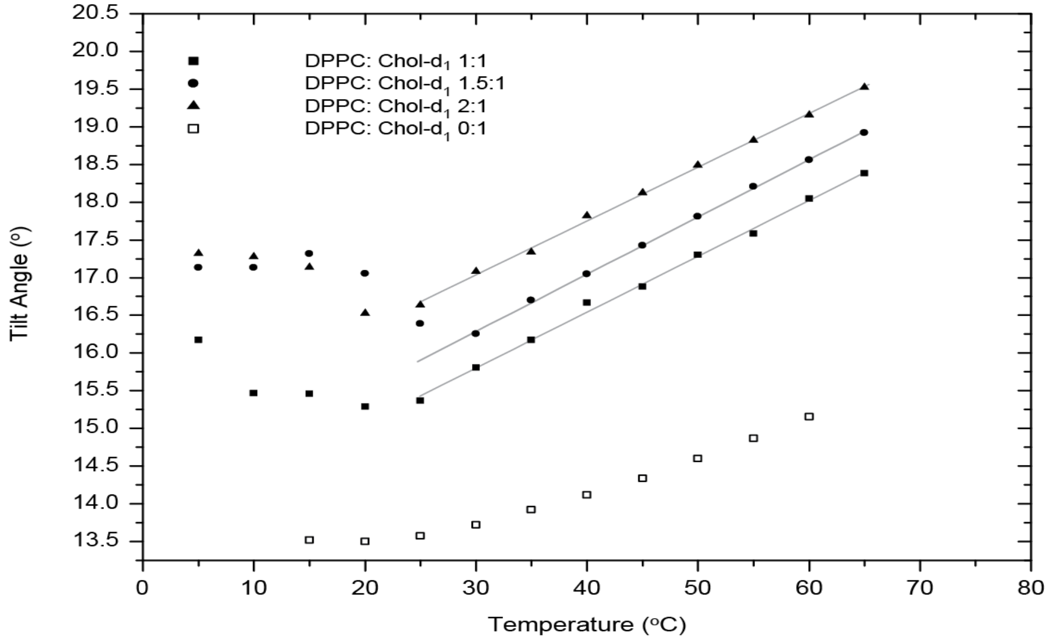


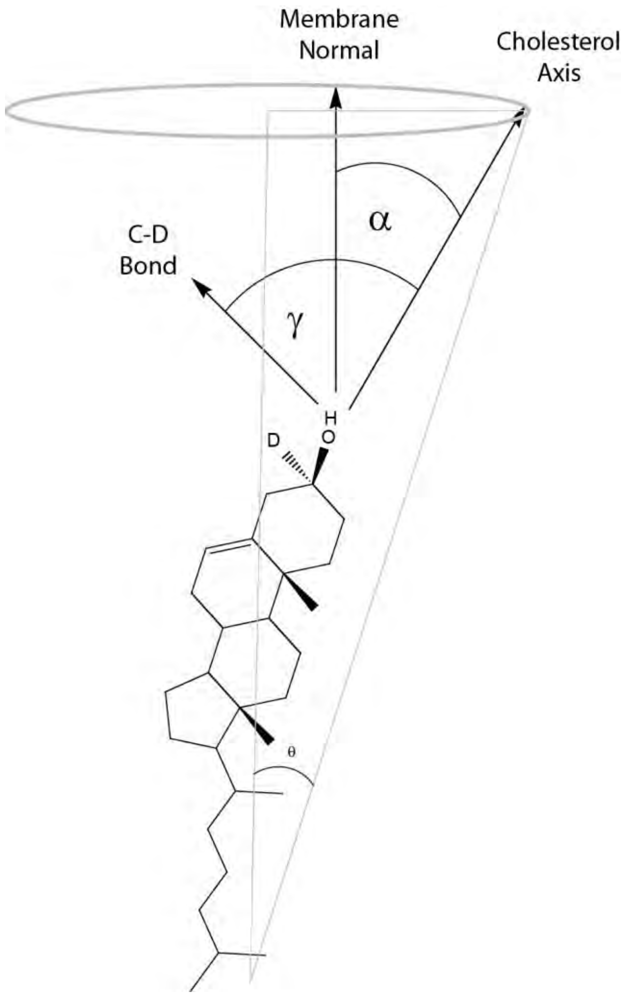




^{2}H Cholesterol Tilt Angle







$$S_\alpha = \frac{\frac{1}{2}\int_0^\pi \sin\alpha \exp(-\alpha^2/2\alpha_0^2)(3\cos^2\alpha - 1)d\alpha}{\int_0^\pi \sin\alpha \exp(-\alpha^2/2\alpha_0^2)d\alpha}$$

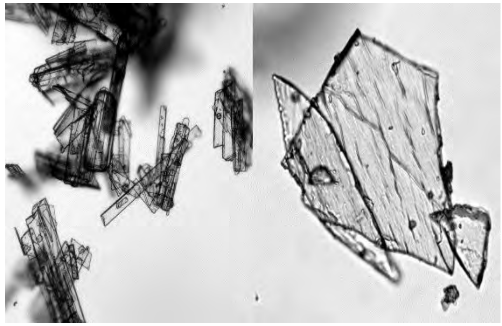
Oldfield et al.. *Biochemistry,* (1978) **17**, 2727
Clarke et al. *Soft Matter, (*2014), **5**, 369

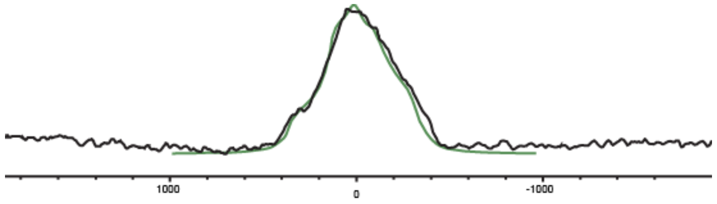
^{17}O-Cholesterol Crystal

Two known crystal forms:

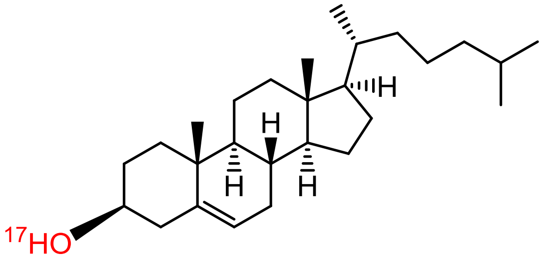
- Anhydrous (P_1, needle)
- Monohydrate (P_1, plate)

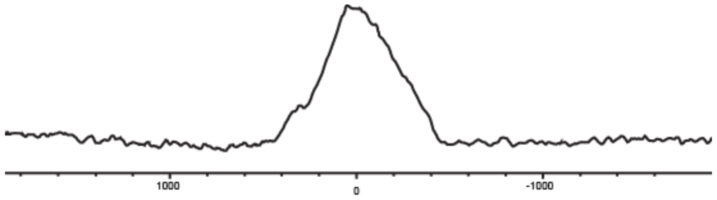
- occurs within the body
- e.g. gallbladder, eye etc.





δ_{iso}= -12.1ppm
C_Q = 3.9 MHz
η_Q = 0.41





Galactopyranoside
δ_{iso}= 6.4ppm
C_Q = 9.1 MHz
η_Q = 0.90

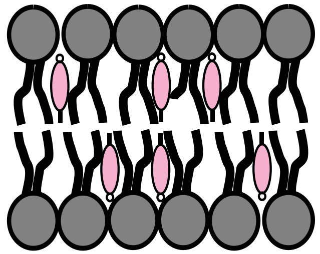
MAS

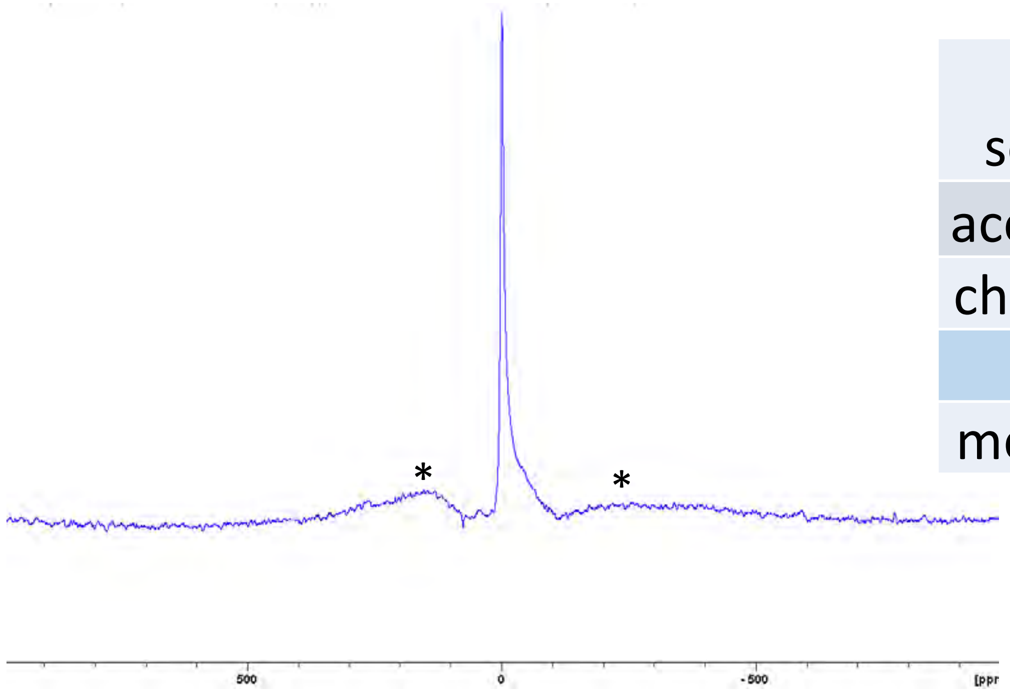
Sheih et al.. *Acta Cryst.*, (1981) **B37**, 1538
Lemaitre et al. *Solid State NMR* 26 (2004) 215
Sefzik et al. *Chem. Phys. Lett* 434 (2007) 312
Boykin *^{17}O NMR Spectroscopy in Organic Chemistry* CRC Press

Cholesterol in the bilayer

In plasma membrane chol form up to 50mol%

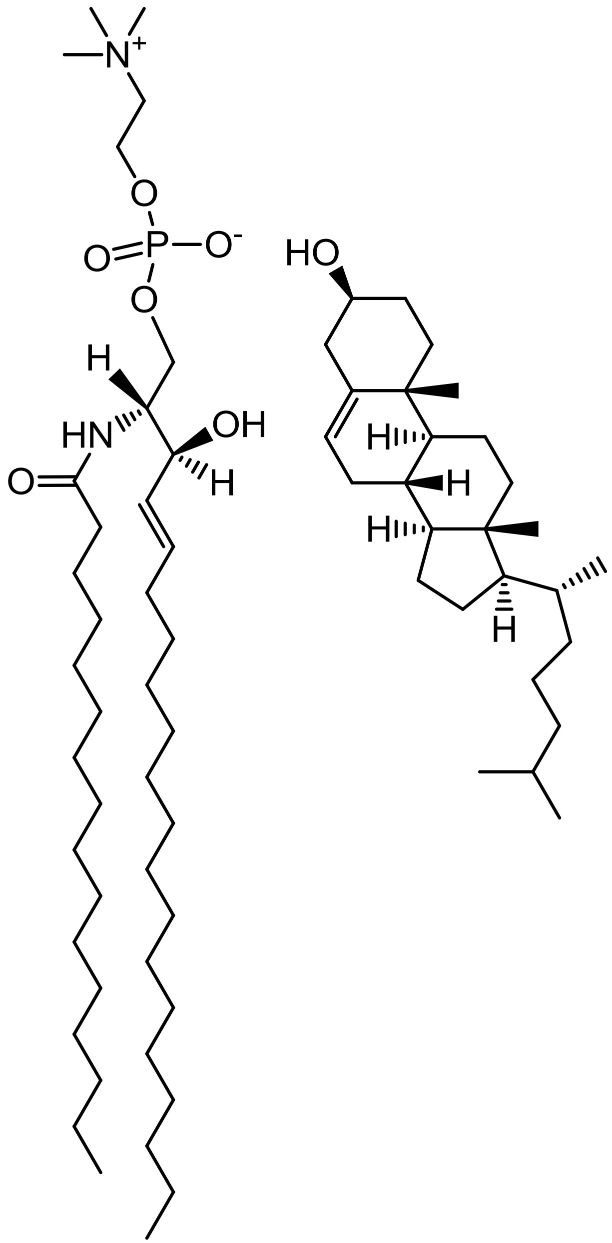
- rapid axial/lateral diffusion collapse to a sharp line

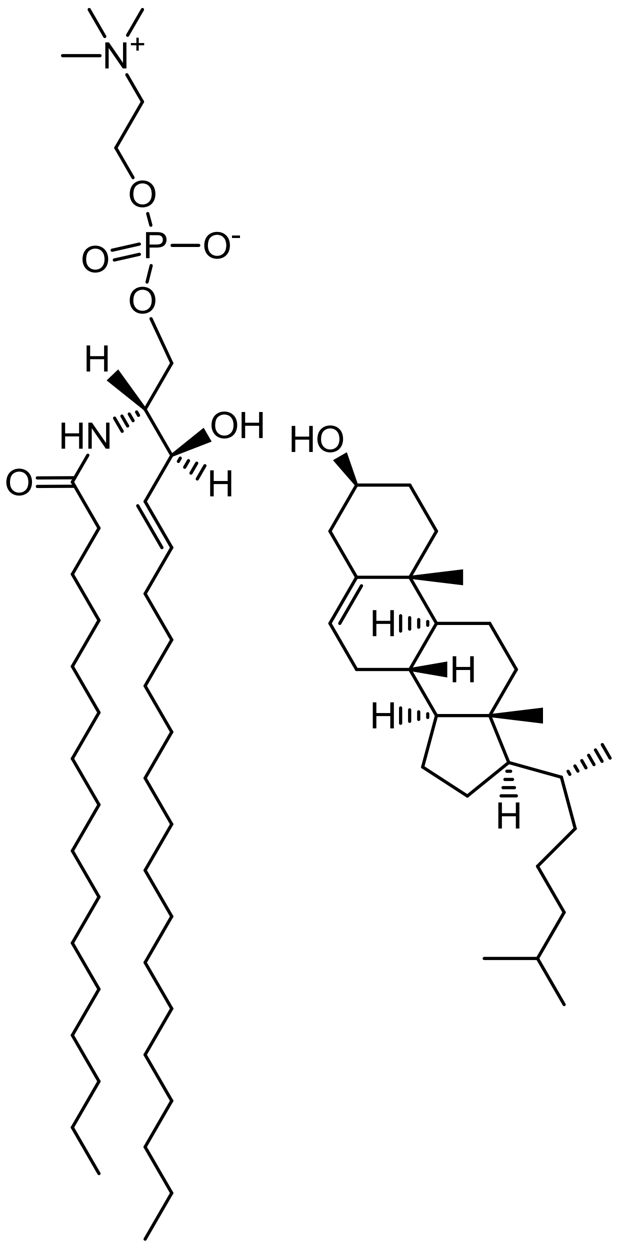




static

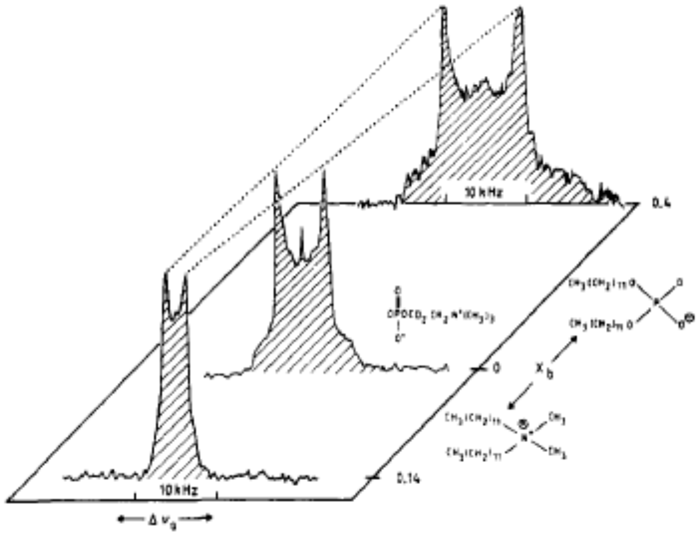
solution	$\delta(H_2O)$	linewidth Hz
acetonitrile	38.8	700
chloroform	38.7	1840
CCl_4	35.7	900
membrane	-0.17	780

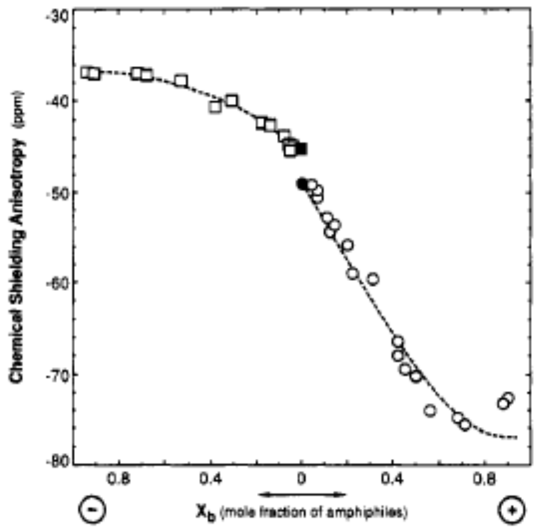


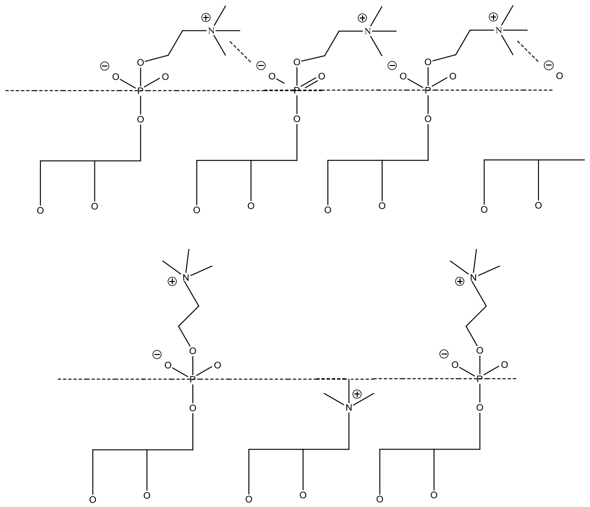


^{14}N NMR- molecular "voltmeter"

- Determination of the headgroup orientation
- I=1 for ^{14}N same as ^{2}H NMR
- Splitting of the Pake powder pattern indicative of the tilt of the headgroup
- By altering the local charge in the bilayer – P-N orientation can be altered

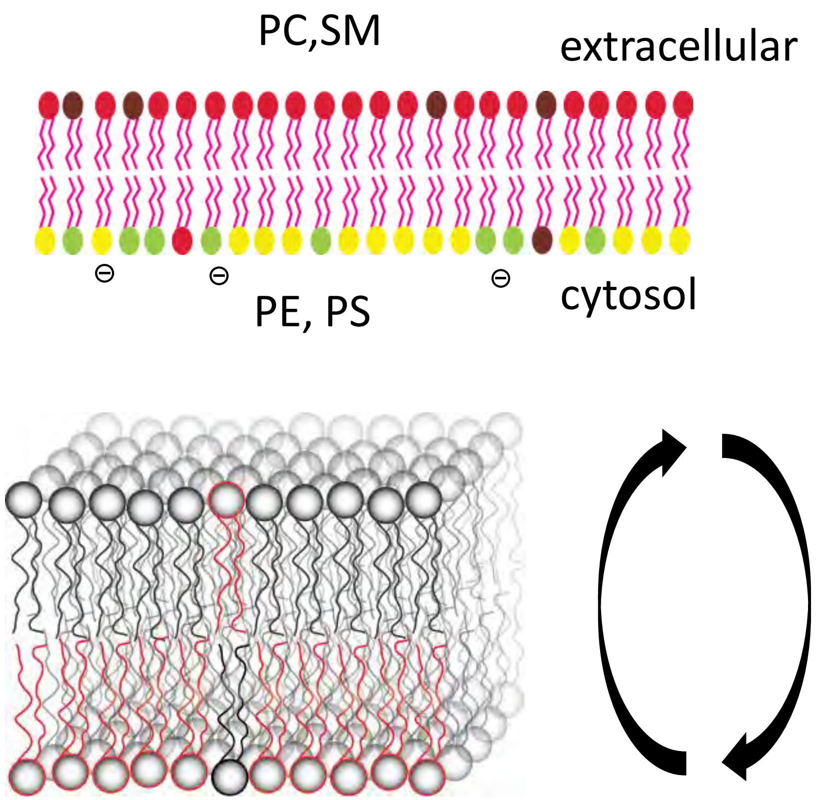






Phospholipid Asymmetry in Membranes

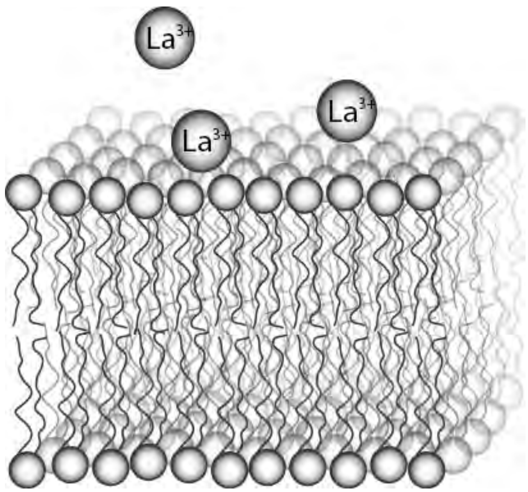
- Biological membranes are asymmetric in phospholipid composition
- Outside is phosphatidyl-choline (PC), sphingomyelin (SM)
- Inside is phosphatidyl-ethanolamine (PE) and negatively charged lipids e.g. phosphatidylserine (PS)
- What is the rate of flip flop?
- How is it maintained?
- Why?

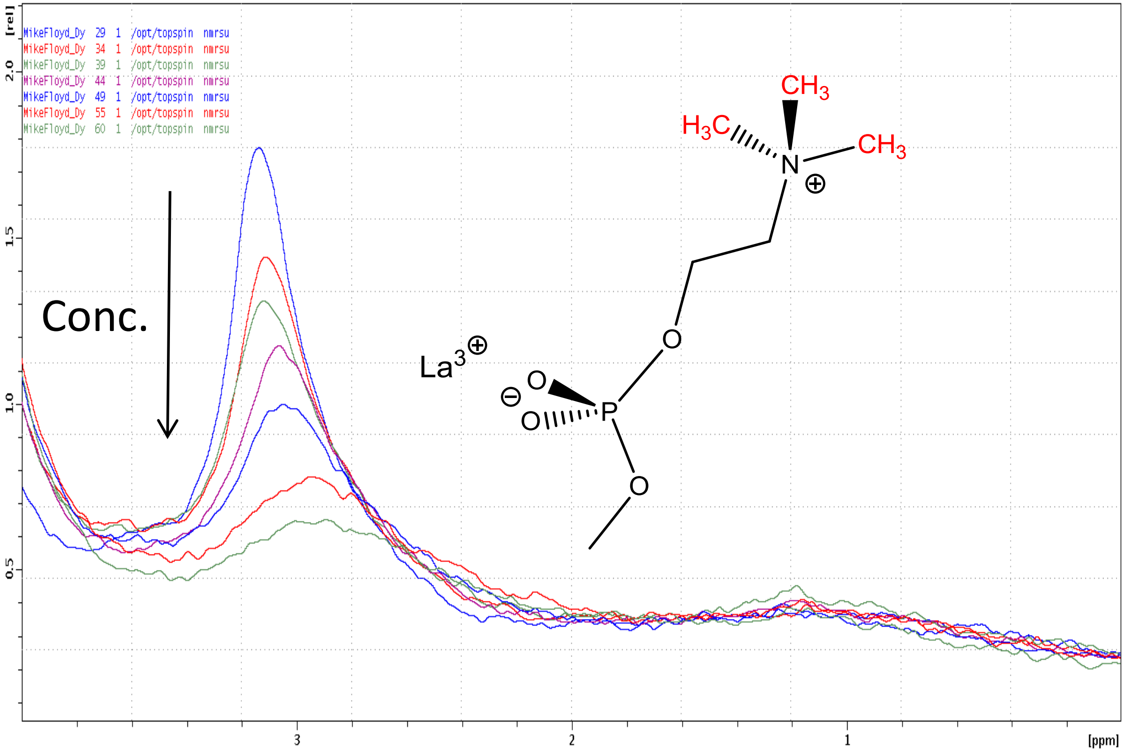


J. M. Sanderson *Mol. Memb. Bio.*, (2012) 29 118–143

J. Liu, and J.C. Conboy *J. Am. Chem. Soc.*, (**2004)**, 126, 8376

Lanthanide Shift Reagent





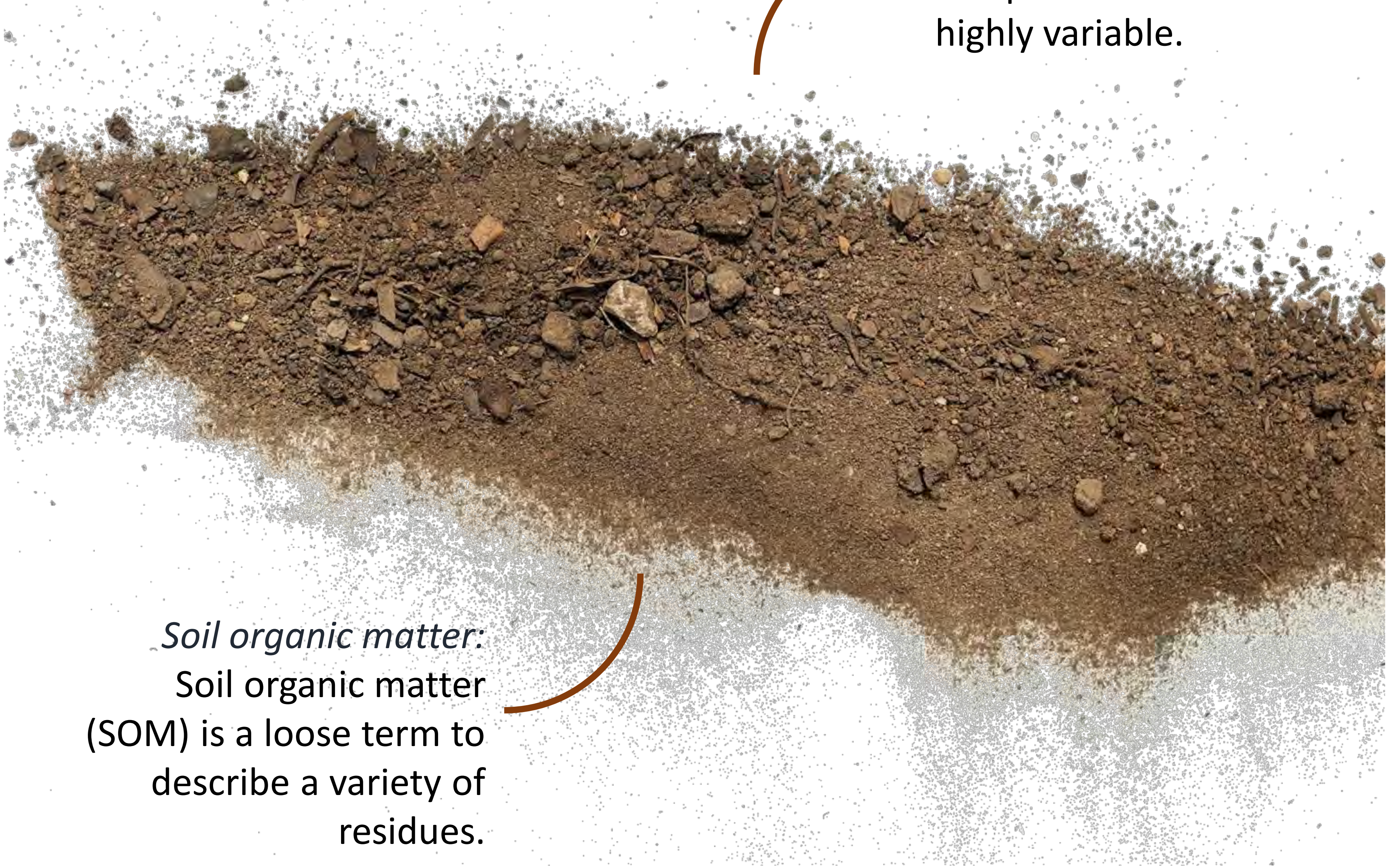
- La shift reagents
- Causes chemical shift change and line broadening

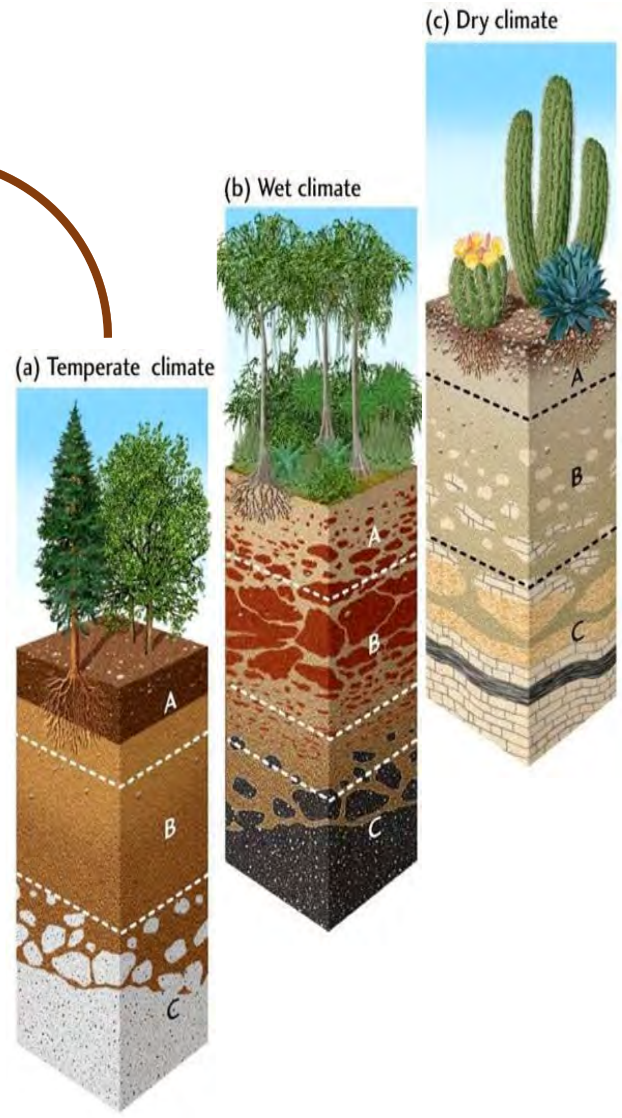
Conclusions

- Multinuclear NMR can be exploited to determine phase behaviour
- Can use it to determine bending rigidity of bilayer, chain length and area per chain
- ^{17}O cholesterol has been introduced for the first time in bilayers
- Sensitive to different hydrogen bonding environments – dependent upon lipids
- Can be used to determine “location” of molecules within bilayer
- Can be used to determine flip-flop rates

What is soil?

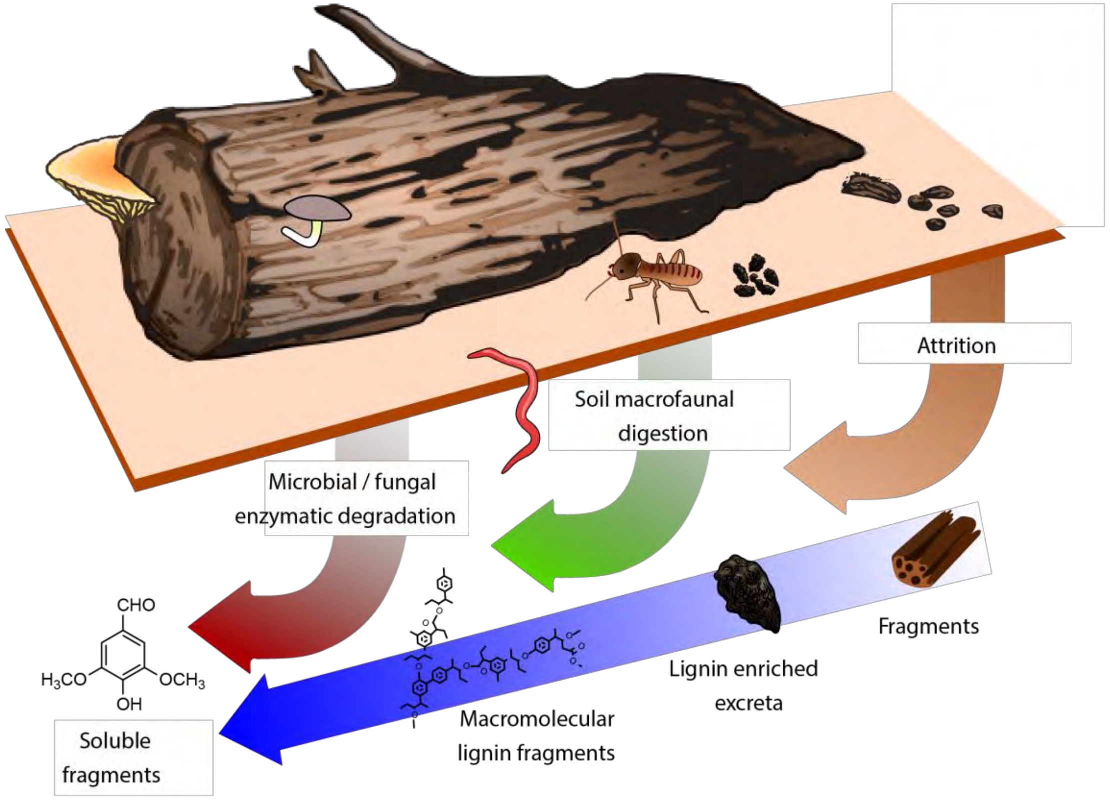
Sand, Silt & Clay:
The inorganic component of soil is highly variable.



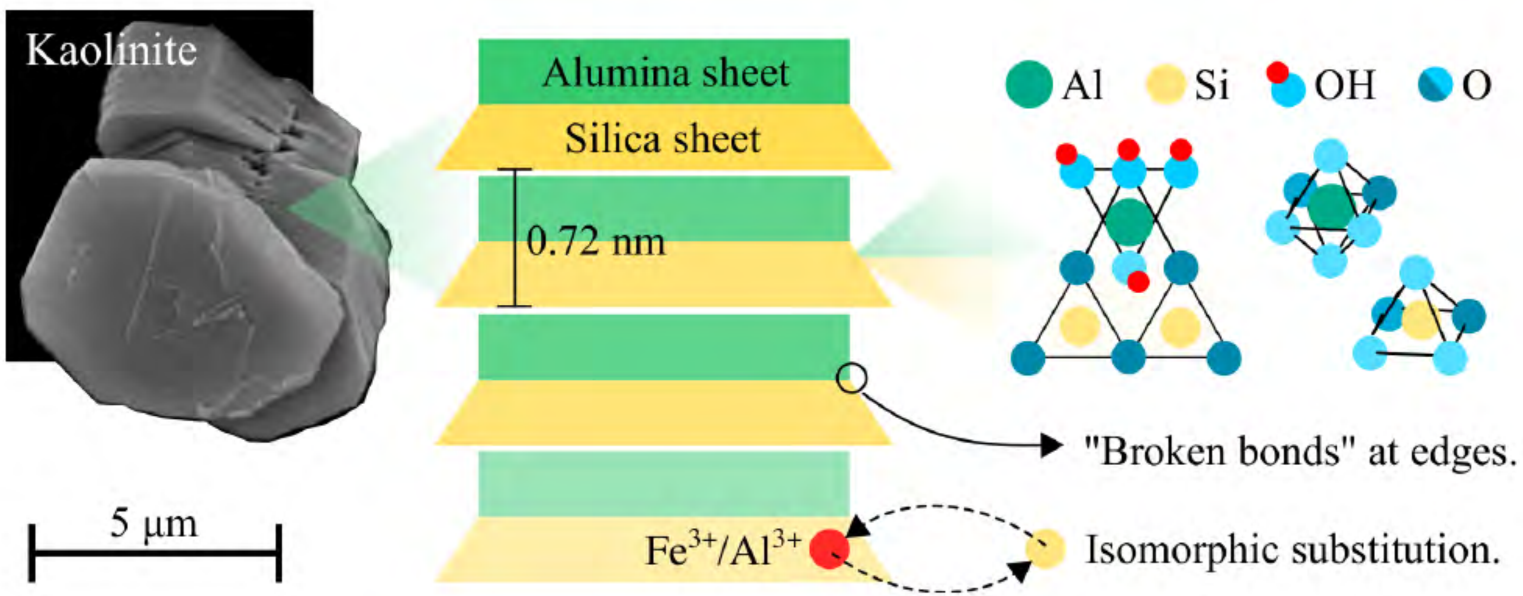


Soil organic matter:
Soil organic matter (SOM) is a loose term to describe a variety of residues.

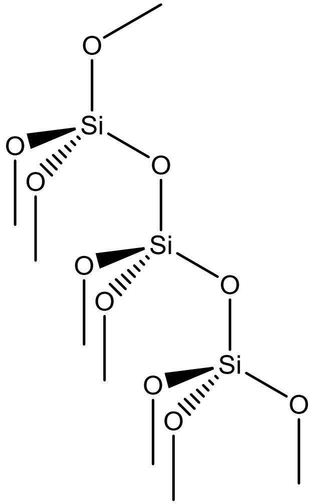
Usually porous, structured, layered. Very diverse, but are generally classified into 12 major classes.


Attrition
Soil macrofaunal digestion
Microbial / fungal enzymatic degradation
CHO
H_3CO
OCH_3
OH
Soluble fragments
Macromolecular lignin fragments
Lignin enriched excreta
Fragments


100
90
80
70
60
50
40
30
20
10
Percent Clay
Percent Silt
Percent Sand
CLAY
SILTY CLAY
SANDY CLAY
CLAY LOAM
SILTY CLAY LOAM
SANDY CLAY LOAM
LOAM
SANDY LOAM
SILT LOAM
LOAMY SAND
SAND
SILT

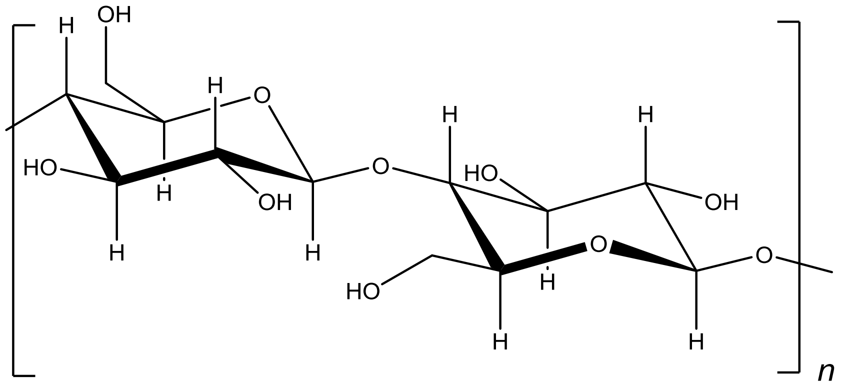


1:1 sheet aluminosilicate

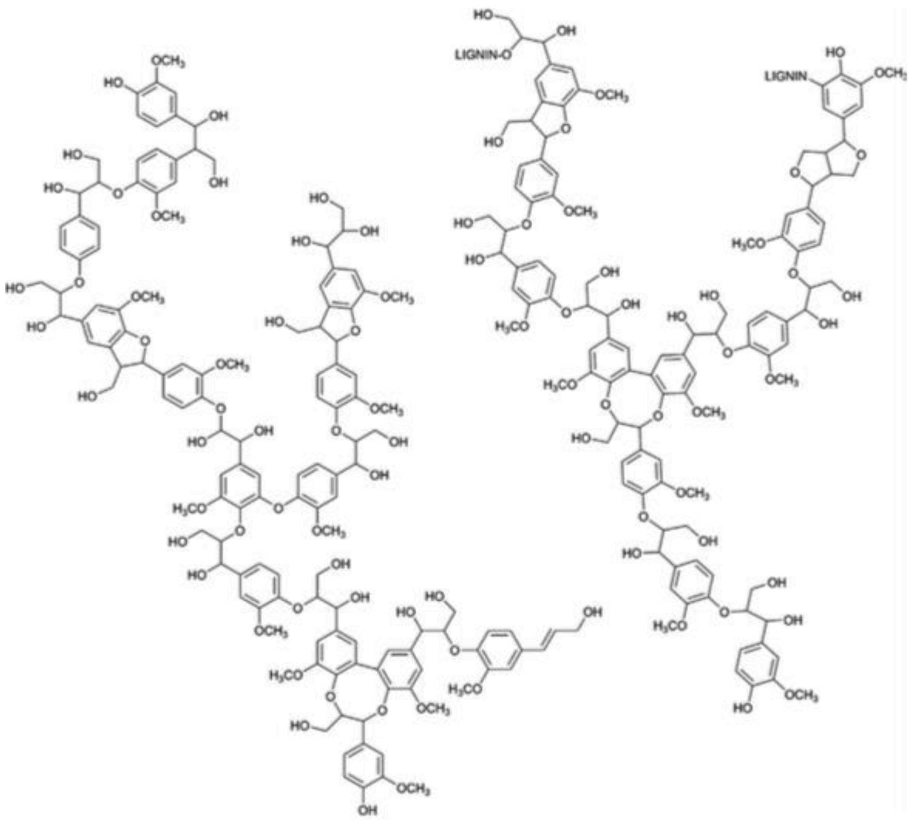


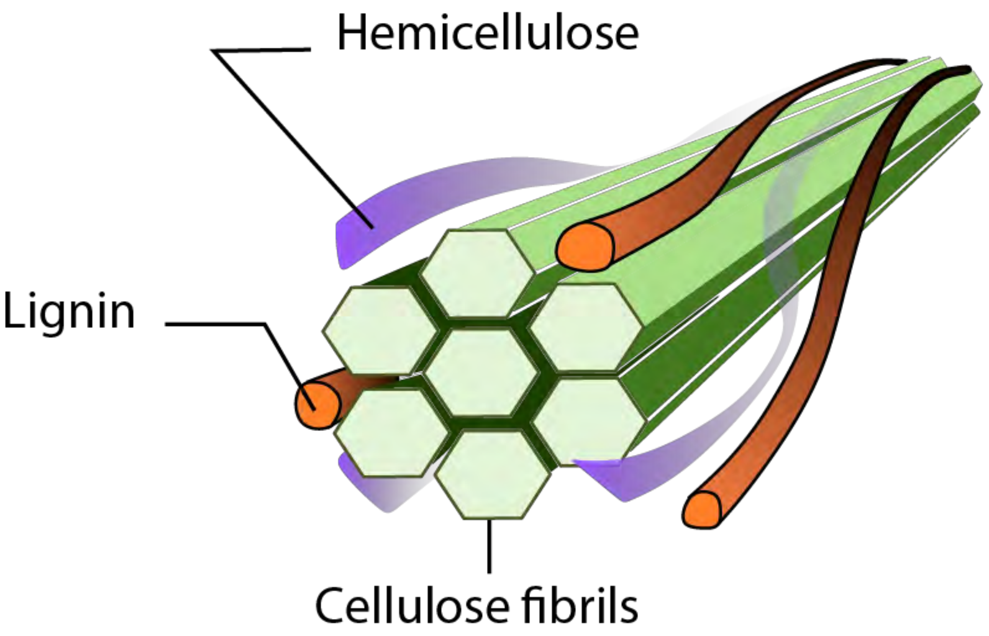
1:1 α-quartz

Cellulose



Lignin





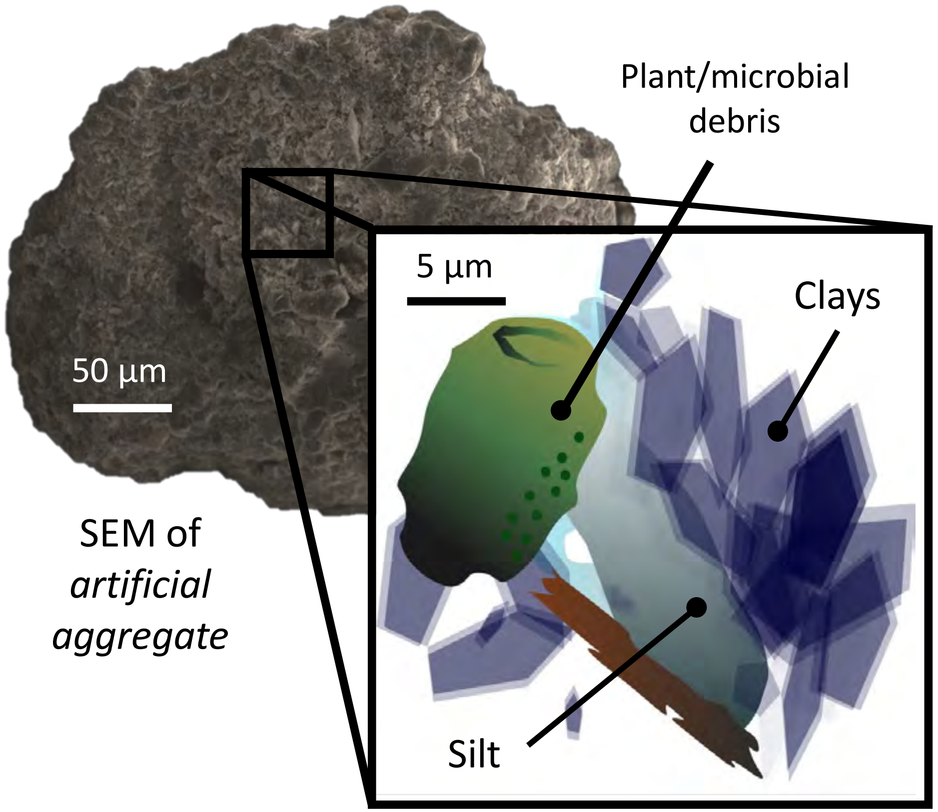
Soil organic matter (SOM) is key

What *does* Soil Organic Matter *Do*?

- Binds materials together into **aggregates.**
- Creates pores and microenvironments.
- Holds water.
- Is a source of nutrients and carbon for organisms.

What *is* SOM made of?
- Insoluble plant and animal residues
- Microbial and fungal products
- Chars
- Soluble brown goo ‘humic substances’

What is an aggregate?



SEM of *artificial aggregate*

Microaggregate
Primary particles of silt, clay and 'humus'.

- Aggregates contain **particulate organic matter.**
  - Bulk of carbon in the soil.
  - Most sensitive indicators of land use change.
- Data about aggregation is **empirical and qualitative**.
  - Difficult for modelling/engineering applications.
- Interactions **between primary particles** are not well understood…
  - Interactions are weak and occur over small surface area.

Soil additives for aggregation

Particulate organic matter

Lignin (bio refinery waste)

- Beech vs Spruce feedstock
- Biopolymer in wood

Biochar

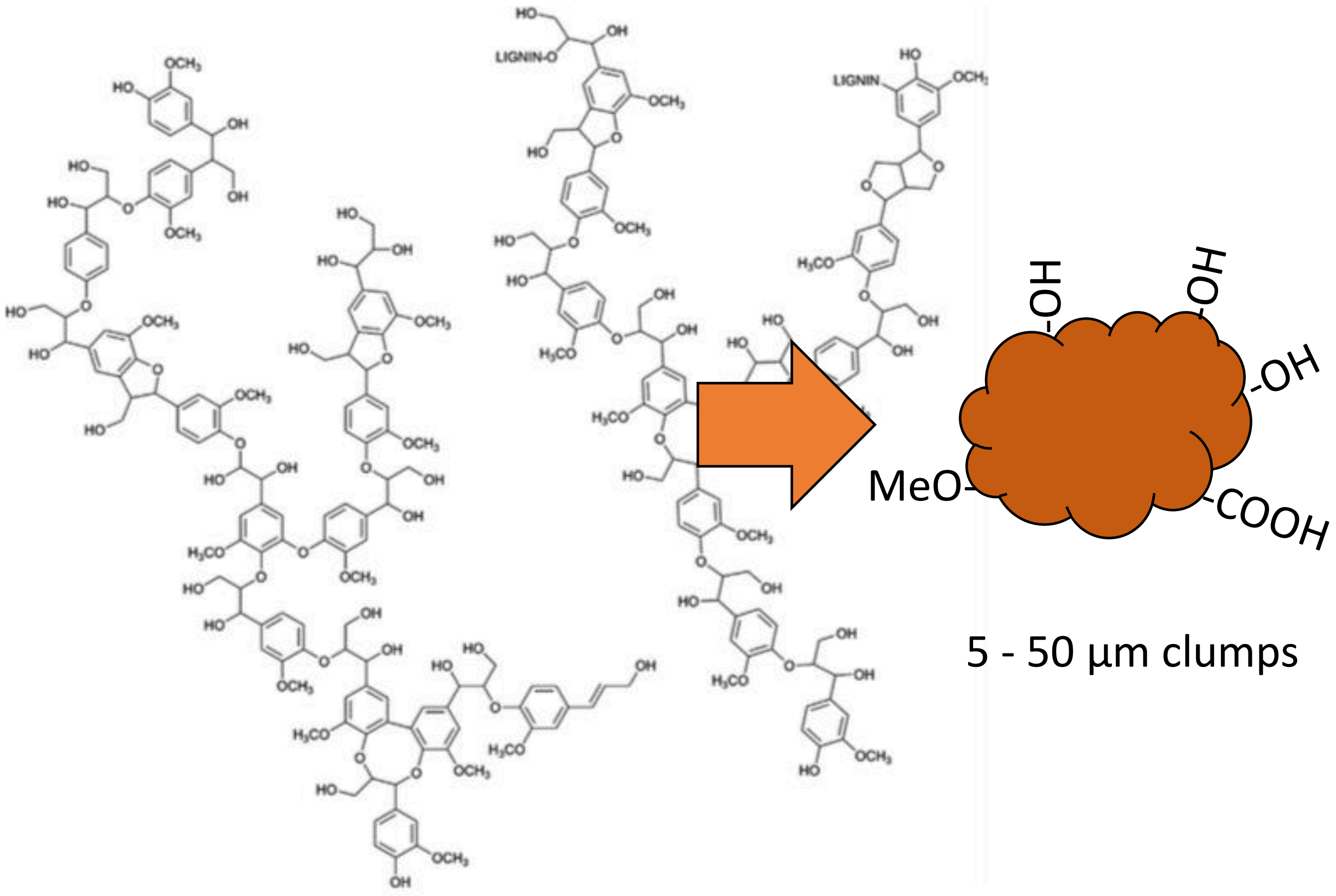
- Pyrolysis (charcoal)

Cellulose

- Bulk of root and shoot material

Wood fragments

Micro plastic



5 - 50 µm clumps

C. Crestini, M. Crucianelli, M. Orlandi, R. Saladino, Catal. Today 156 (2010) 8.

Soil additives for aggregation

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Cellulose

- Bulk of root and shoot material

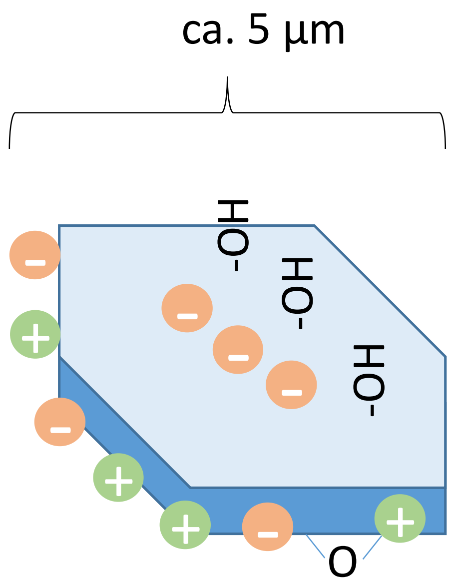
Wood fragments

Micro plastic

+

Kaolinite

- Clay present in many highly weathered soils.
- Any other clay clay or oxide.



Soil additives for aggregation

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Wood fragments

Micro plastic

+

Kaolinite

- Clay present in many highly weathered soils
- Any other clay clay or oxide

→

Aggregate

- Look for evidence of an interaction
- Test stability to water (slaking)

How to make an artificial soil

Increasing lignin added →

Component (g)	0 %	0.5 %	1.0 %	5.0 %	Compost 5.0 % + comp.	No Fe_2O_3 5.0 % NoOx
Quartz (sand)	80	80	80	80	80	80
Fine-quartz (silt)	13	13	13	13	13	14
Kaolinite (clay)	5	5	5	5	5	5
Mica (mineral)	1	1	1	1	1	1
Iron oxide (oxides)	1	1	1	1	1	0
Compost (POM)*	0	0	0	0	0.38	0
Spruce/Beech Lignin	0	0.5	1	5	5	5

*POM = particulate organic matter

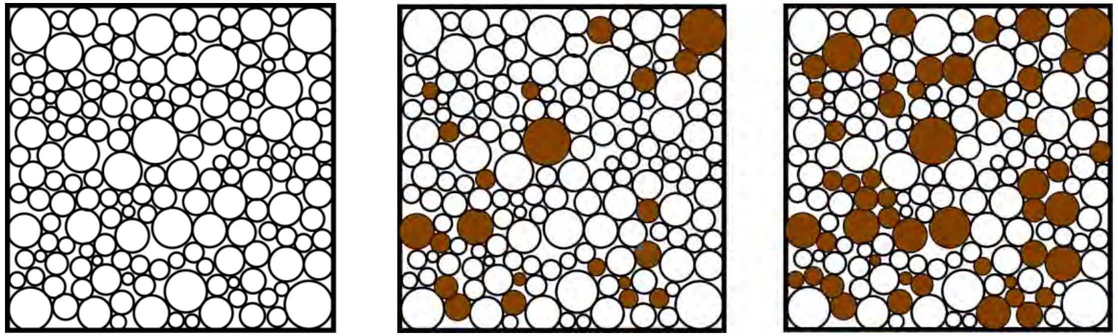
1. Soils are mixed for 4 hours in a rotary mixer
2. 100 mls of 0.01 $CaCl_2$ is added and mixing is continued
3. Soils are dried to constant weight at 40 °C
4. Soils are broken up
5. Soils are ‘aged’: wetted slowly, mixed, dried

Soil mechanics

Looking for evidence of clay - additive interactions.

Alec Skempton

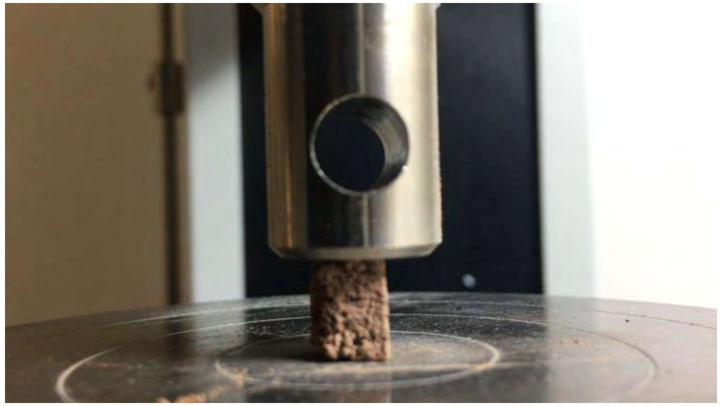
Imperial College, home of soil mechanics.



10mm

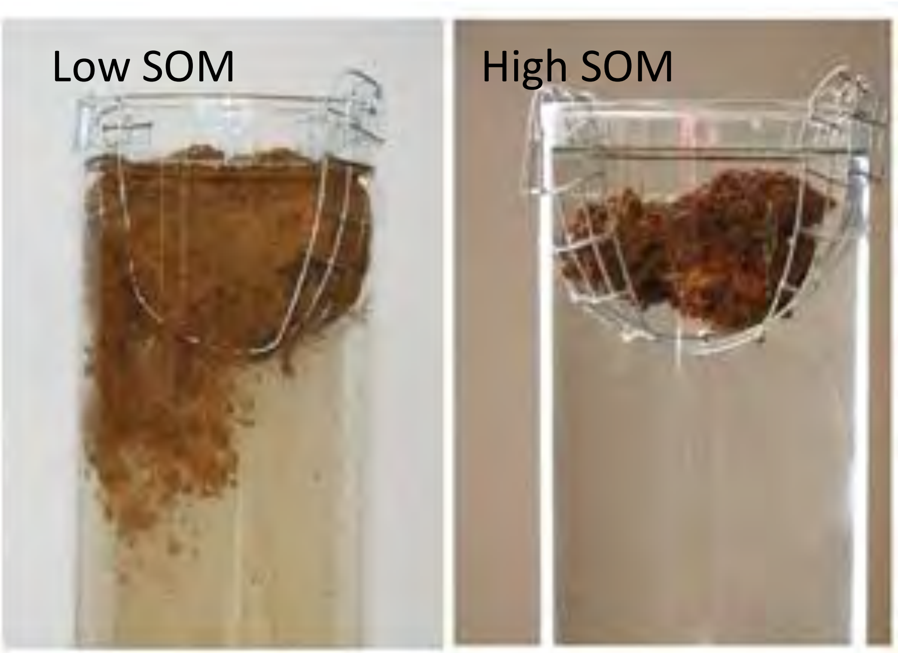
Artificial aggregates made from additives and kaolinite mixed at different ratios.

Standard Soil Tests

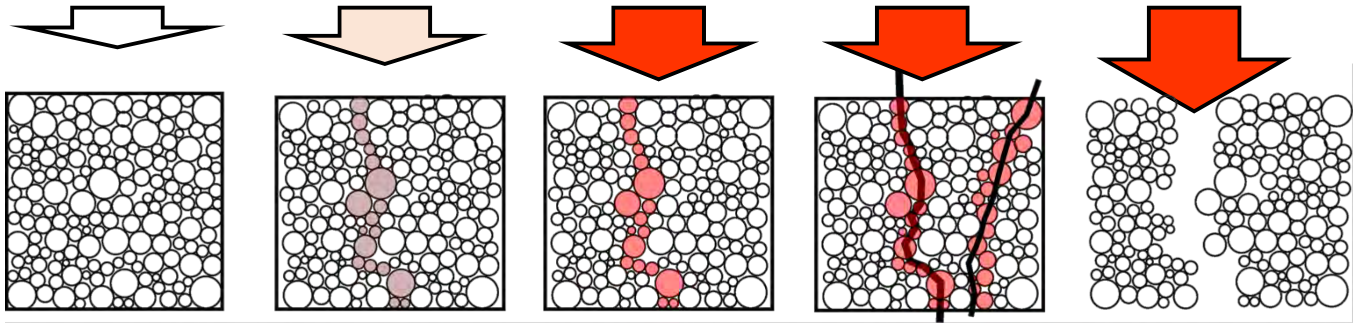


Unconfined uniaxial compression (crushing)

Mechanical Sieving



Slake Test: soil aggregation



Compressive strength

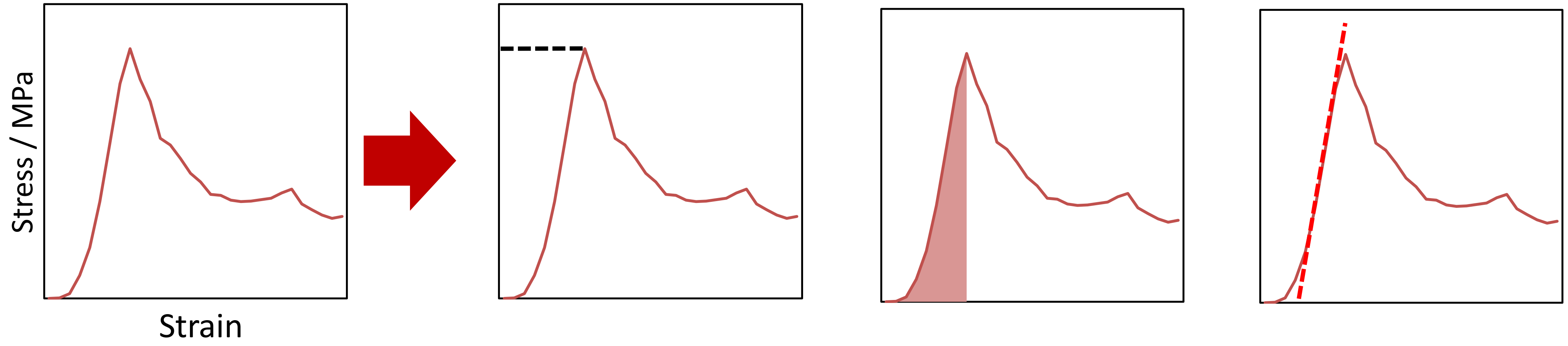
Force/unit area, required to rupture the cube.

Toughness

This is the energy/unit volume required to cleave the cube.

Young’s modulus

This is a measure of the stiffness/plasticity.



Finding evidence of an interaction…

Strengthened composites

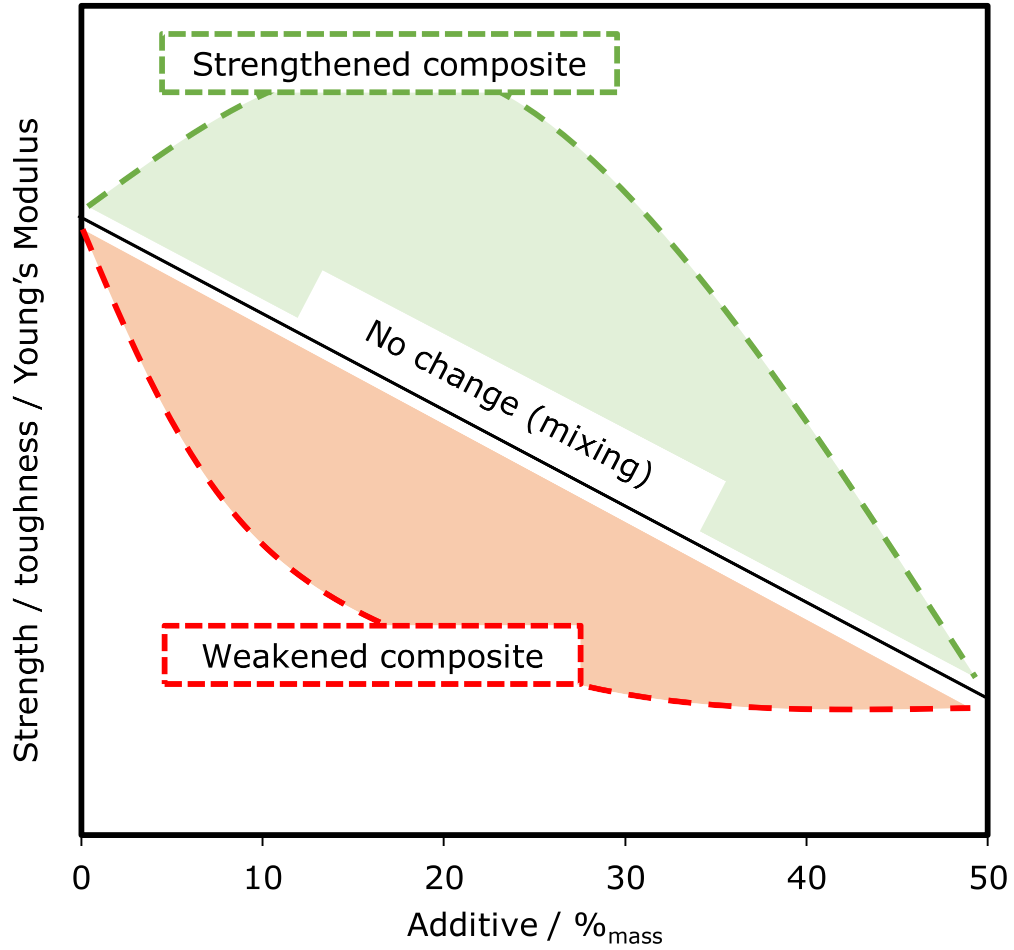
- Adhesive interactions, **the composite exceeds the strength of the individual components.**

Weakened composites

- Interactions are absent or repulsive.

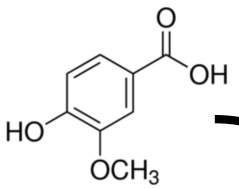
No interaction

- The strength is proportional to the amount of added additive.



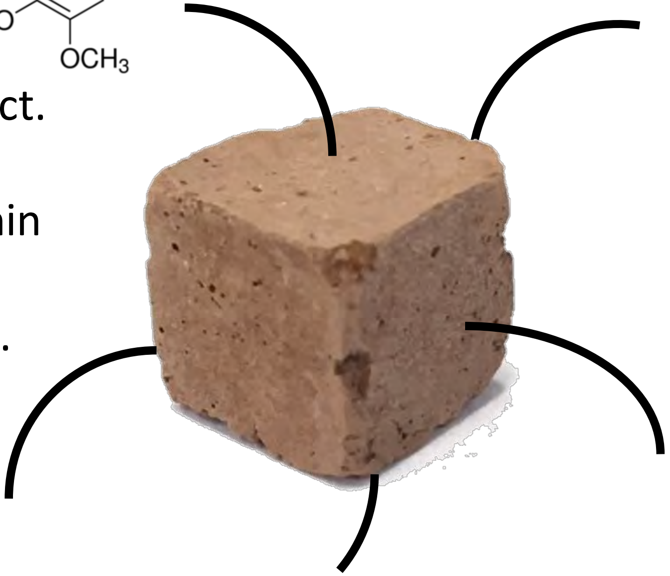
Conclusions from crushing cubes

A new method to investigate interactions between clays and particulate organic matter.



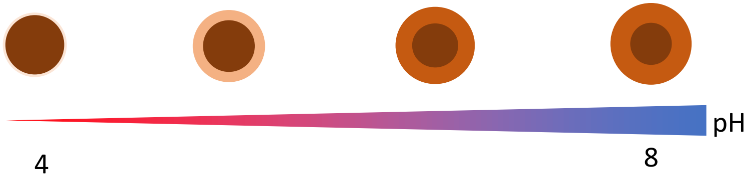
Soluble fragments and ions
(Dissolved organic matter)

- Dissolved glucose has no effect.
- Dissolved lignin fragments reduced the strength of a lignin – kaolinite composite.
- Ca^{2+} > Na^{+} to modify strength.



Lignin - Solution pH

- Raising the pH increases the strength lignin residues due to the formation of a soluble component.

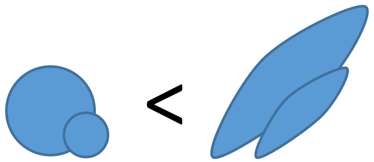


Surface functionality.

- Functional groups such as COOH appear to increase kaolinite – POM interactions.
- Likely to be a contribution from a variety of functional groups.

Porosity

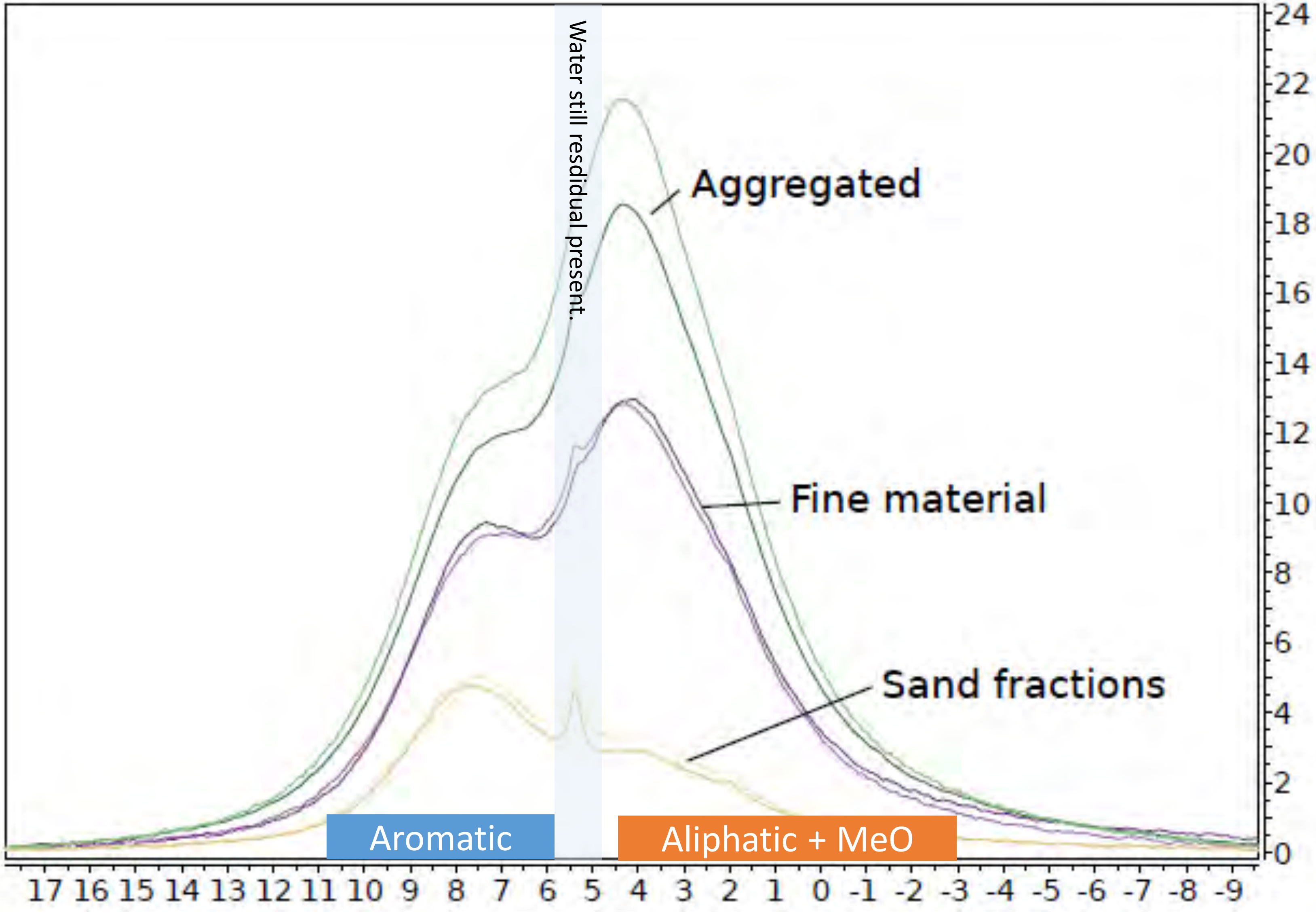
- Porosity weakens all composites.
- Reduction indicates adhesive interaction.



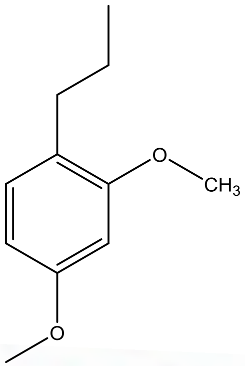
Particle size

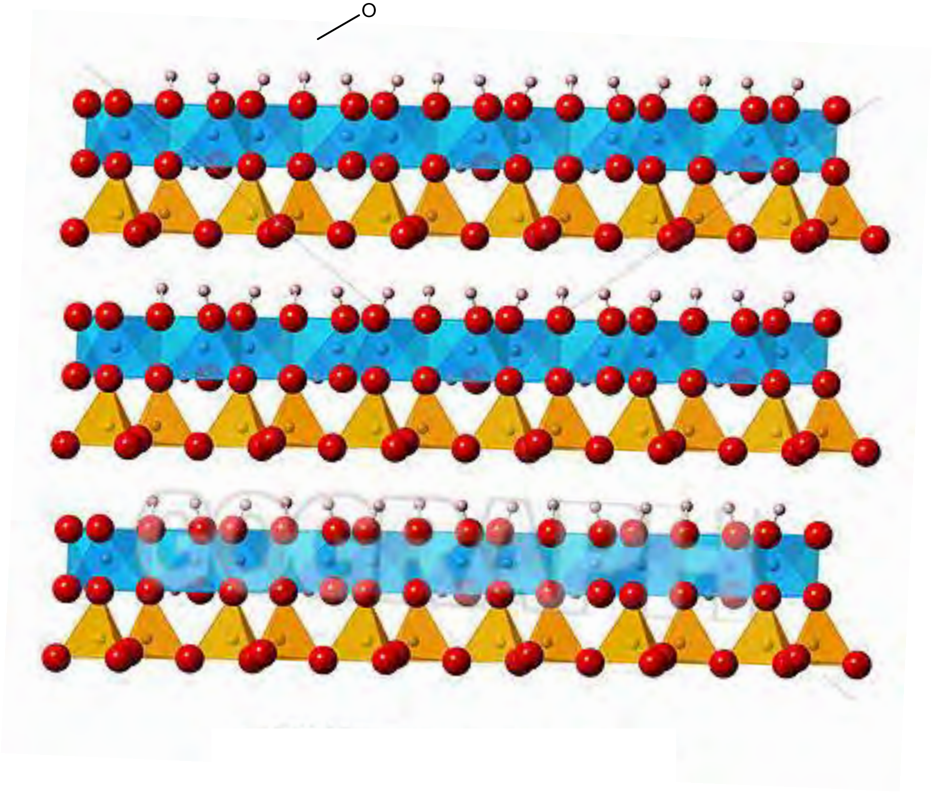
- Particle size has a minor effect within a range (≈ 5- 100 µm) **UNLESS,** things are fibre like (cellulose or wood fragments).

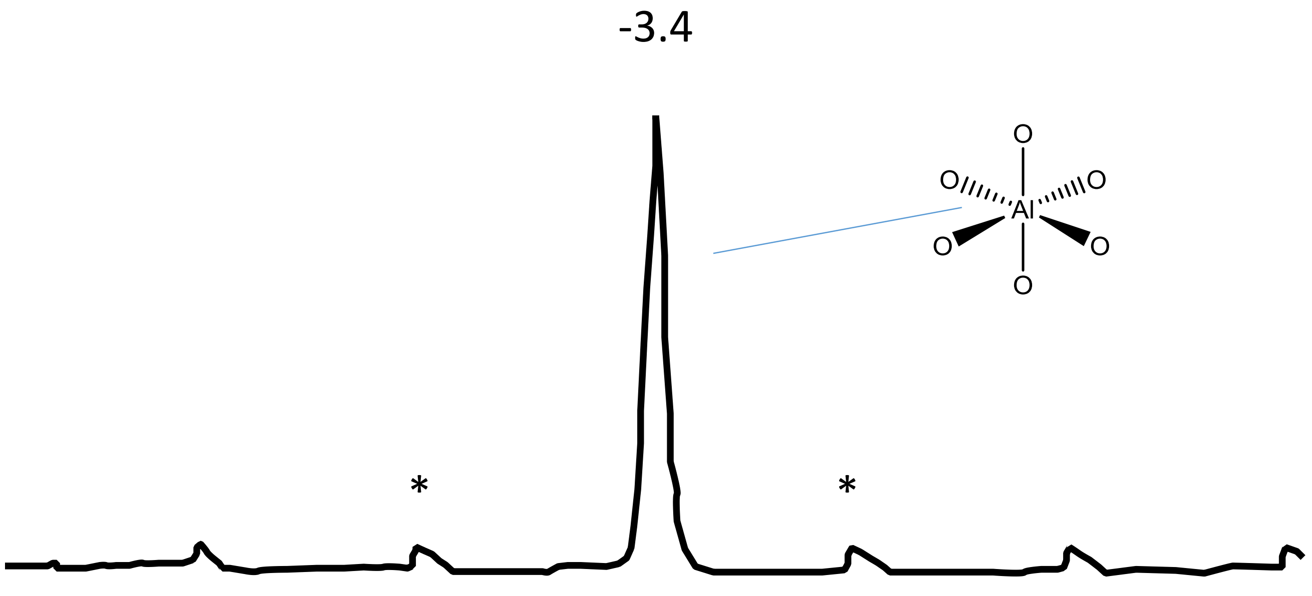
^1H MAS NMR of Freeze dried & D_2O exchanged soil

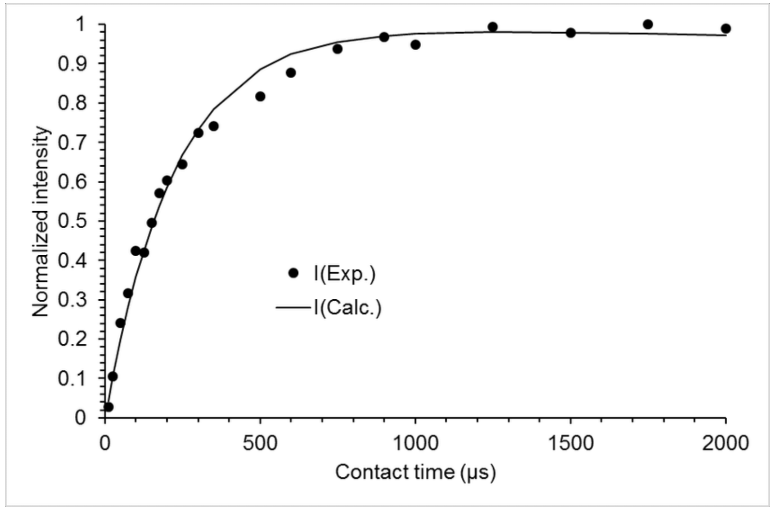


^{27}Al- ^{1}H CP MAS kaolinite









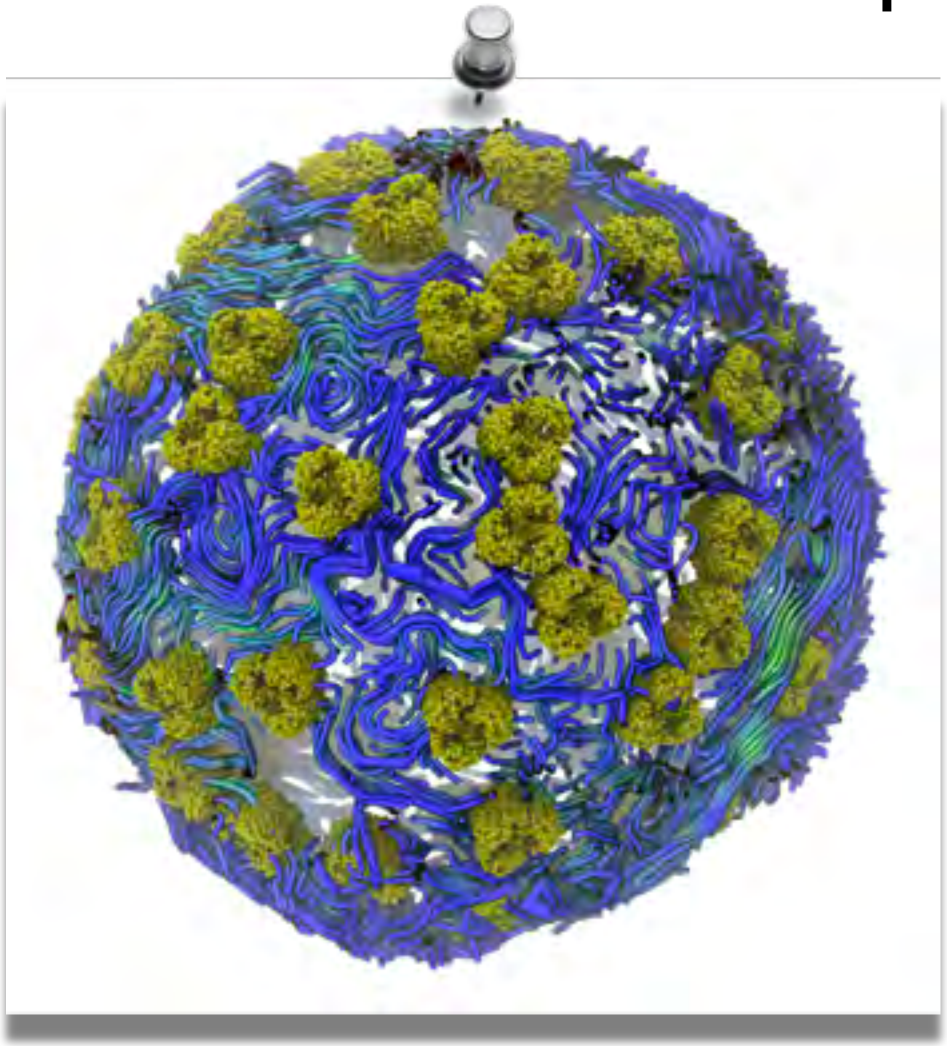
Thank you for listening.

Climate-KIC

Institute of Chemical Biology

Grantham Institute for Climate Change

Focussing the Computational Microscope on Bacterial Cell Envelopes



Syma Khalid
S.Khalid@soton.ac.uk

Syngenta 2019

Our aim is to develop a virtual Gram-negative bacterial cell envelope

- This is an on-going project involving many collaborators, both computational & experimental.

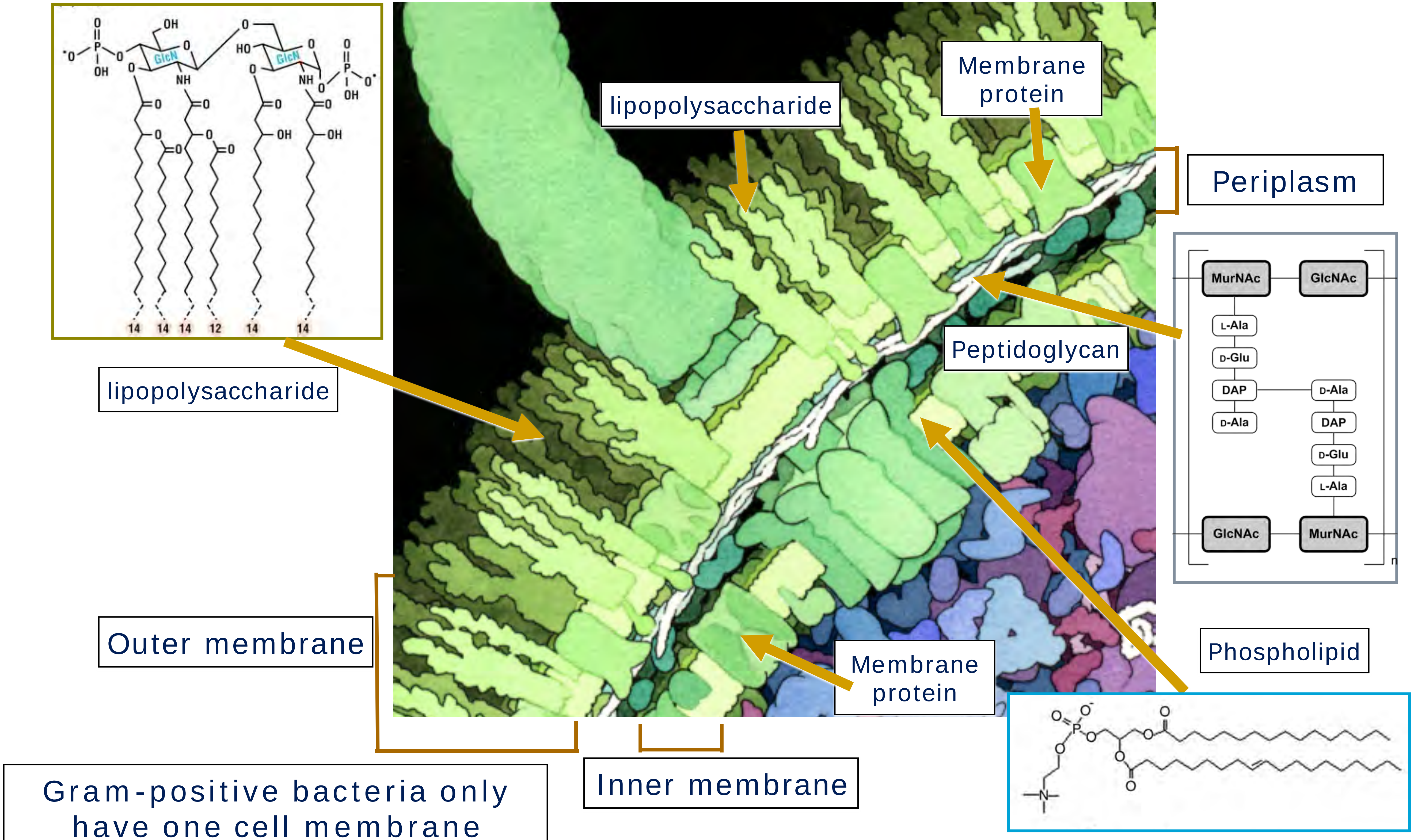
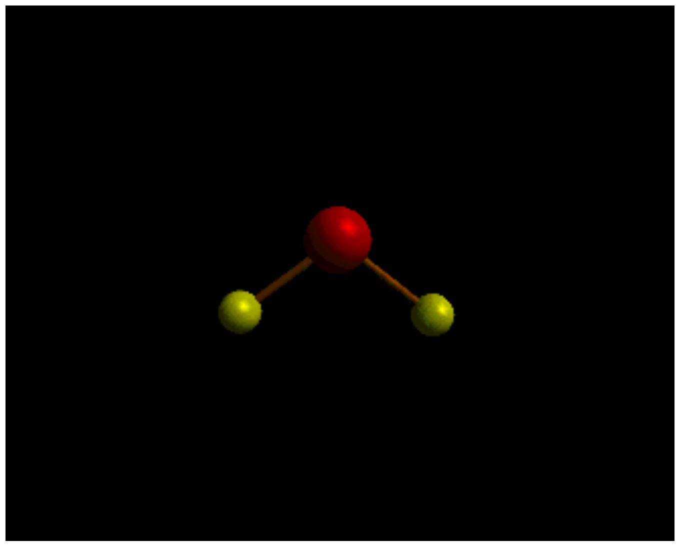


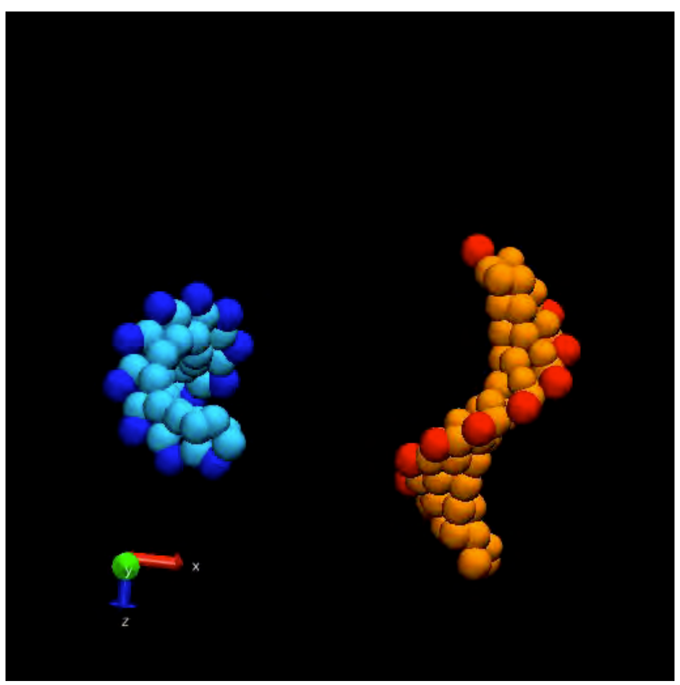
Illustration of *E.coli* membrane kindly provided by David Goodsell

Classical simulation methods

Molecular Dynamics is now an established technique for studying biological molecules

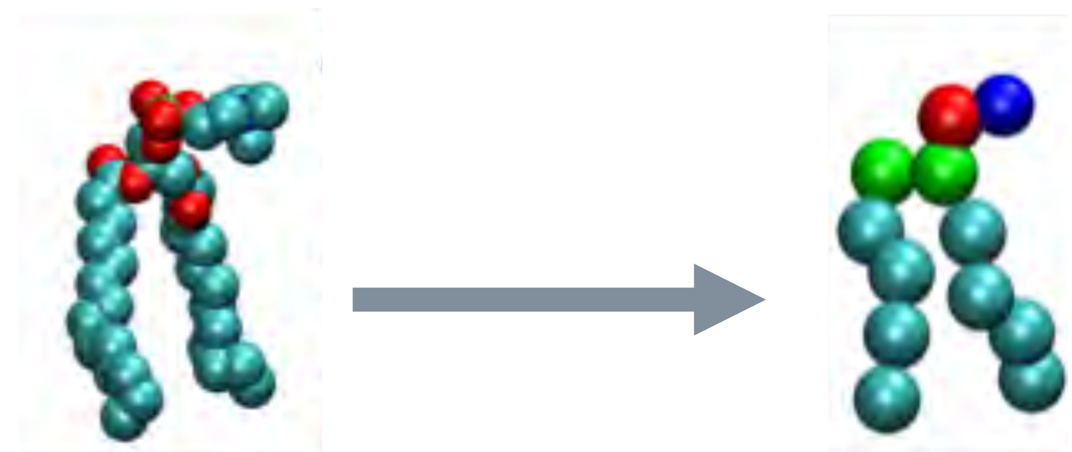


Thermodynamic properties are accessible



Balls connected by springs: classical, Newtonian mechanics ($F = ma$)

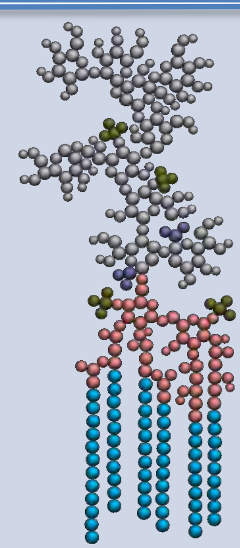
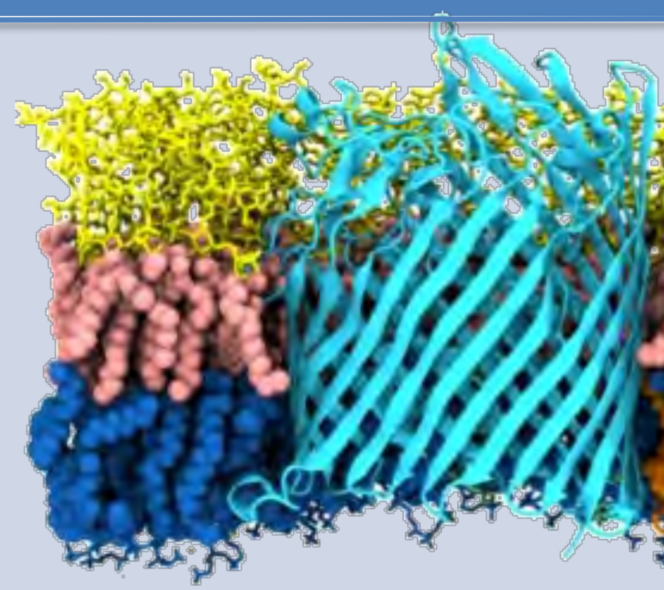
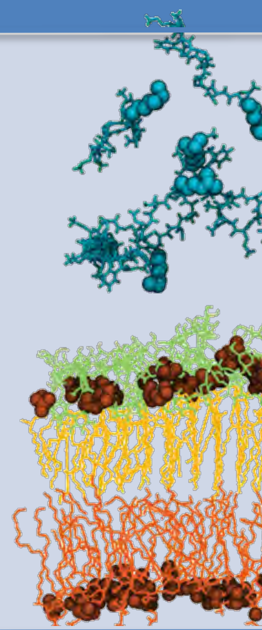
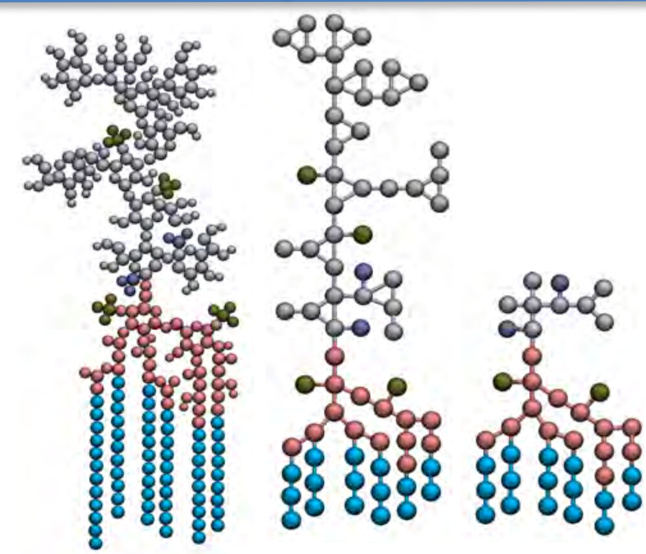
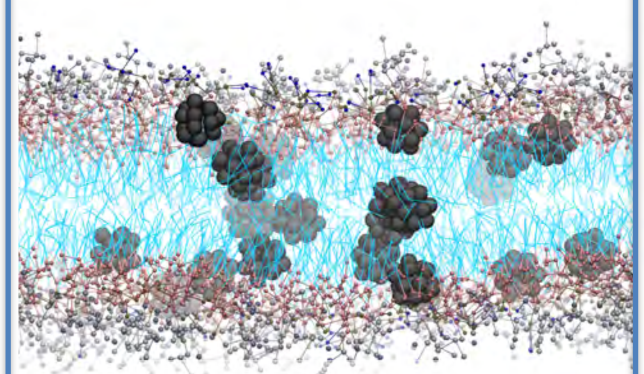
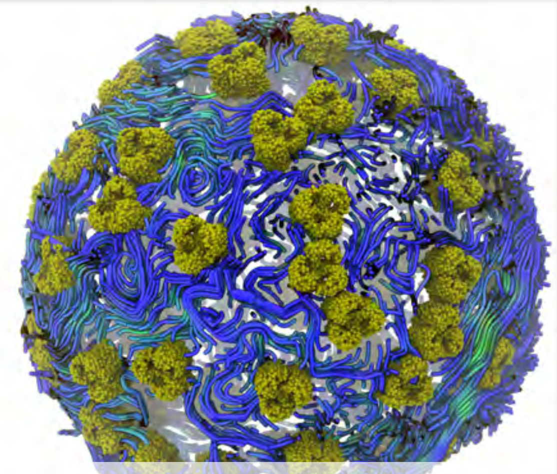
Resolution of MD simulations



Atomistic *Coarse-grained*

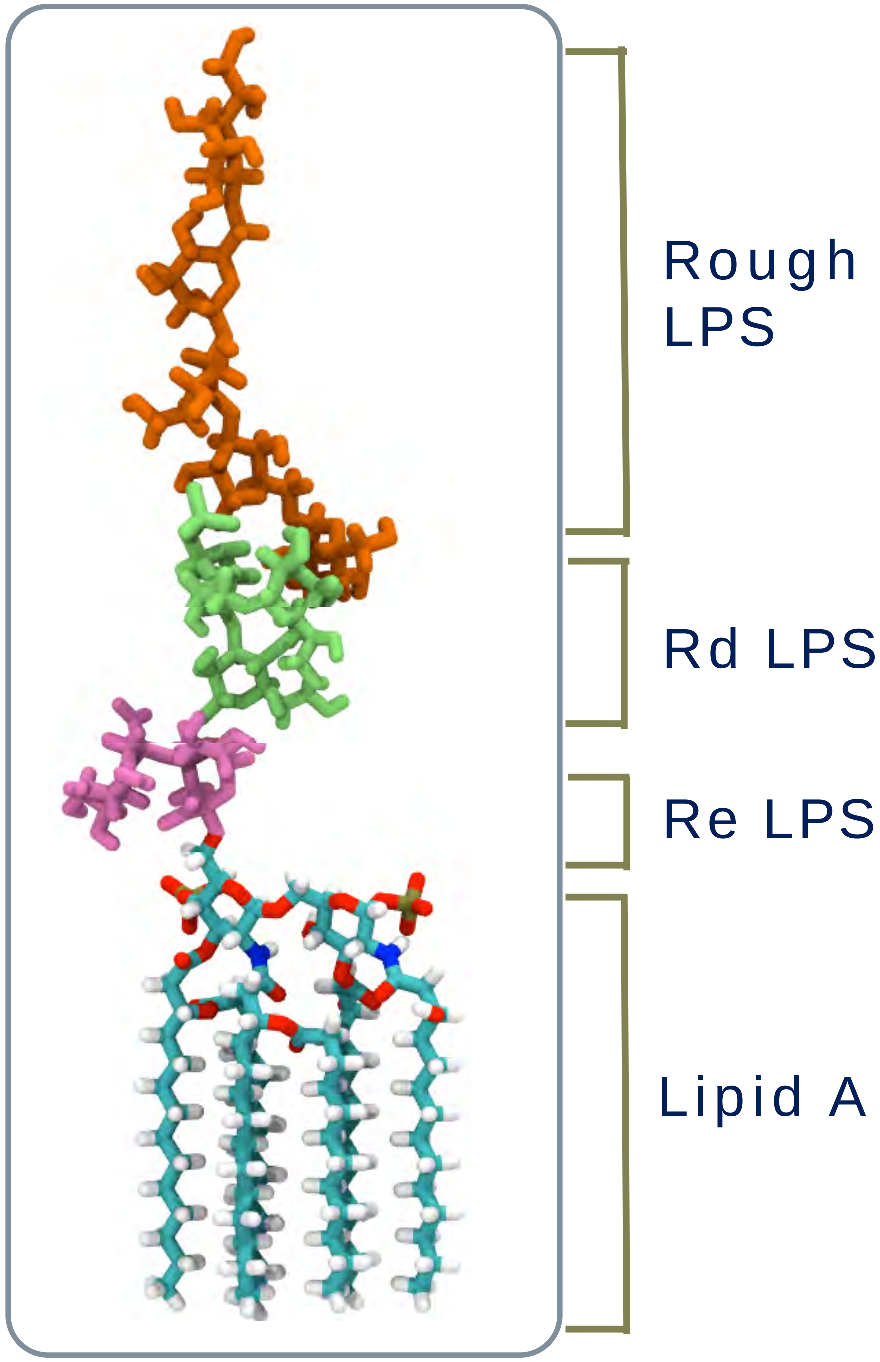
Particularly successful for studying membrane proteins in a range of environments

Our modelling of the outer membrane to date

	Outer membrane		
Atomistic Piggot *et al*, J Phys Chem B, 2011 & BBA, 2013, Berglund *et al*, PLoS Comp Biol, 2015, Samsudin et al, Structure 2015, 2019, Ortiz-Suarez et al, Biophys J, 2016, Carpenter et al, JPCL, 2016, Dror et al, Science 2019		 OMPs	 AMPs
Coarse-grain Khalid *et al* Biochem Soc Trans, 2015, Hsu *et al*, J Phys Chem B, Holdbrook *et al*, PLoS ONE, Hsu et al JPCL, 2018, Shearer et al JCTC, 2019, Jefferies at al, J Phys Chem B, 2019		 Nanoparticles	 Large systems

The outer membrane: atomistic models

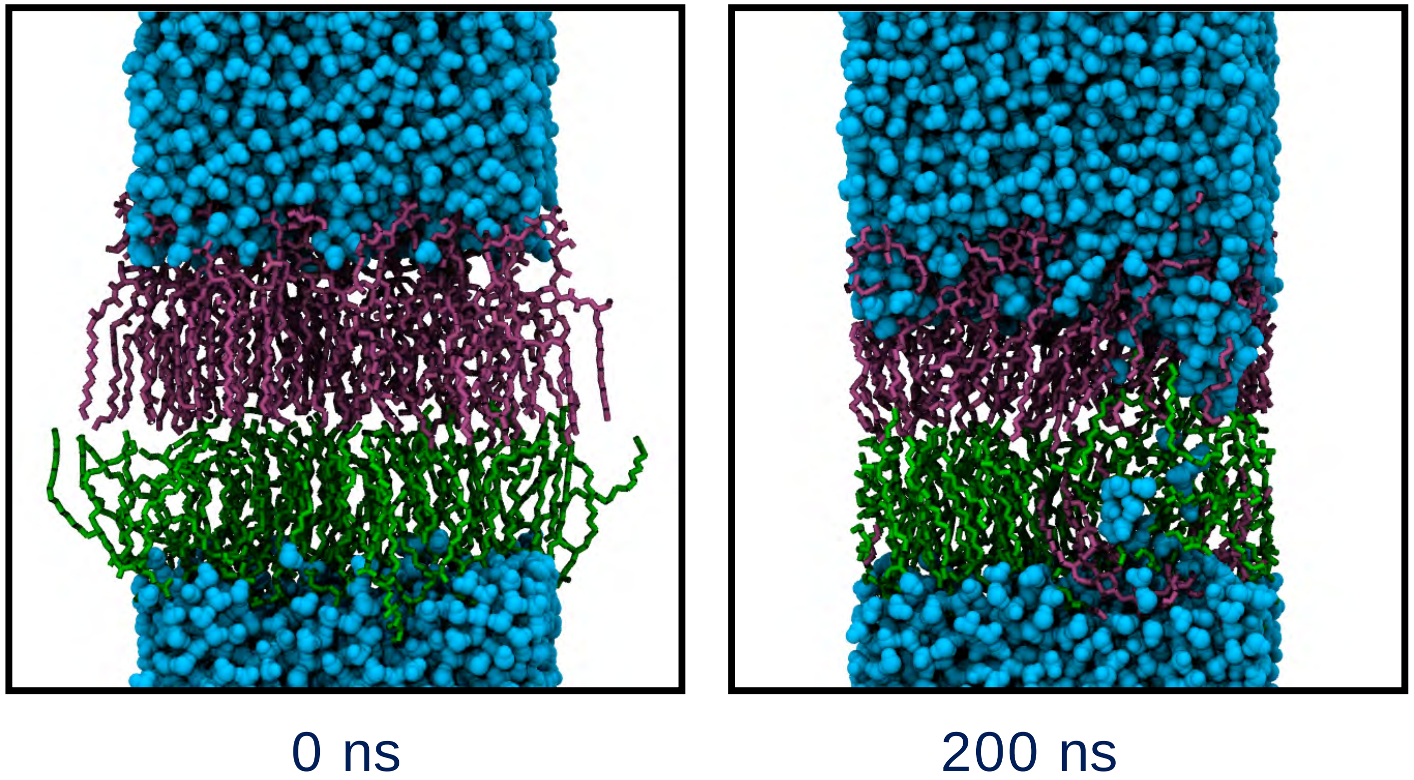
- We have developed an atomistic-level model of the *E.coli* outer membrane:
    - Inner leaflet: combination of phospholipids that vary in headgroup (size and charge) and tails (length and saturation)
    - Outer leaflet: lipopolysaccharide
    - GROMOS53A6 force field within GROMACS4
    - Validation against experimental and simulation data



Piggot *et al*. J Phys Chem B, 2011

Collaboration with Jeremy Lakey (Newcastle)

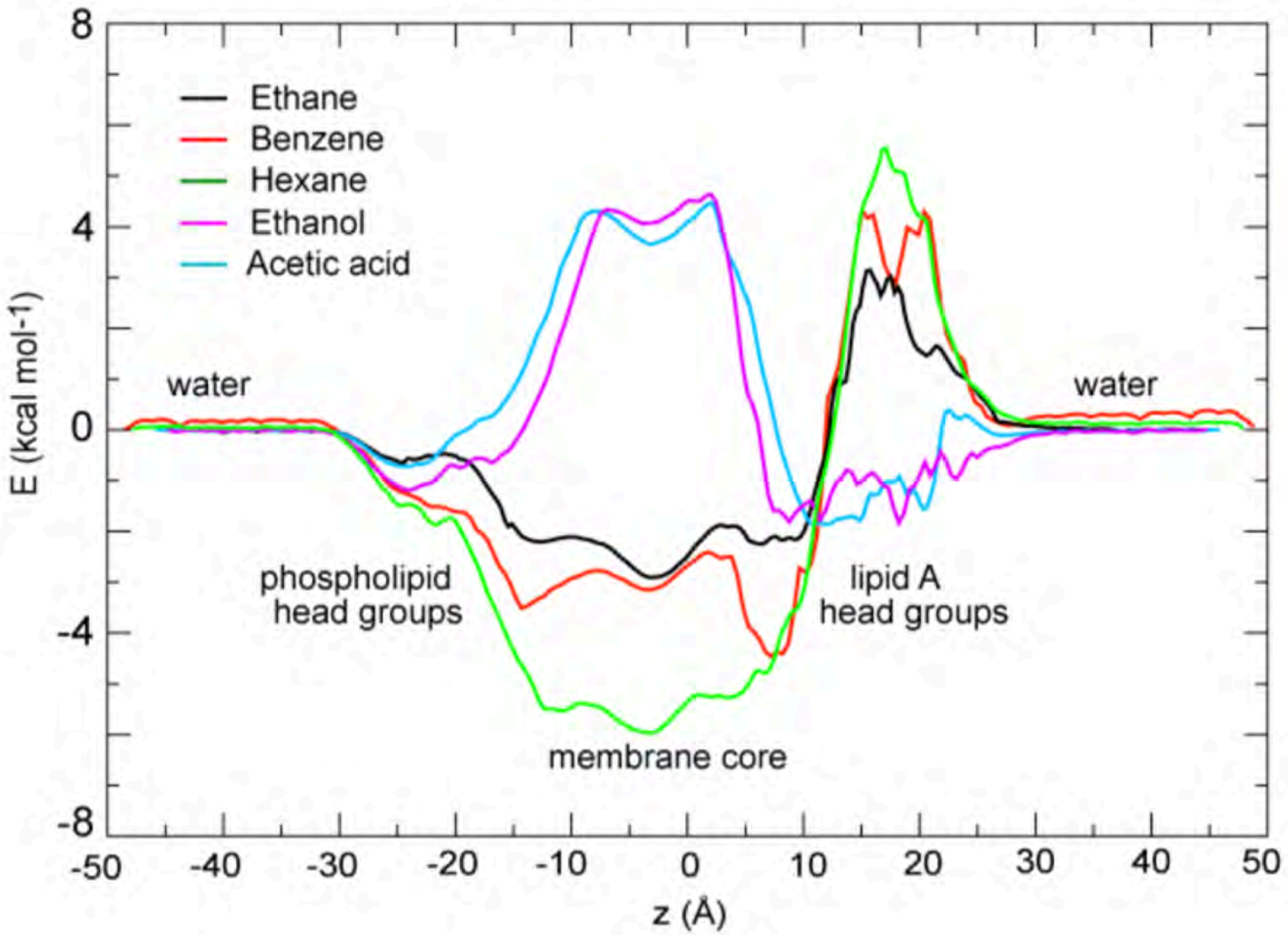
Replacement of divalent cations with 2 x monovalent cations

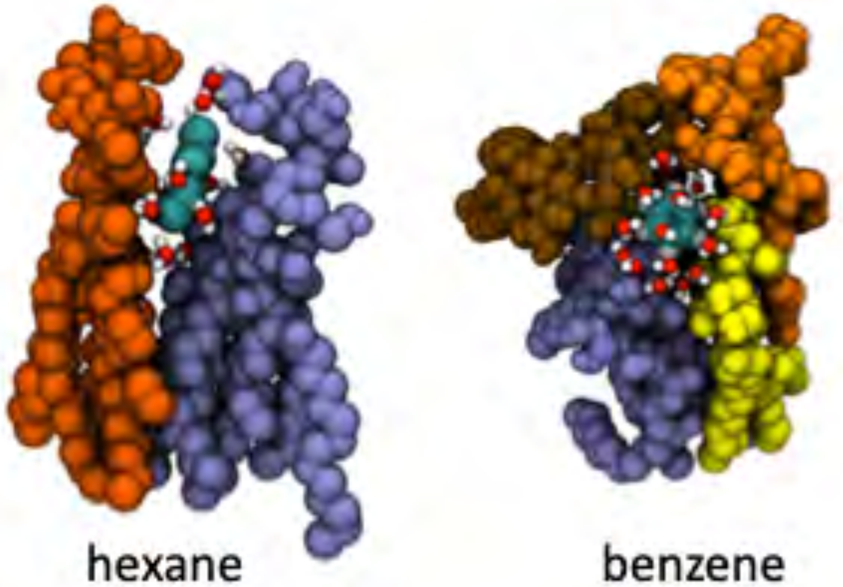


Clifton *et al.* Angewandte Chemie, 2015

Establishing an energetic baseline for permeation

- We have employed umbrella sampling to calculate the differences in free energy profiles for permeation of a range of chemical moieties across the outer membrane
- We see distinctly asymmetric profiles. The level of detail we can access enables us to build up a molecular picture of permeation.



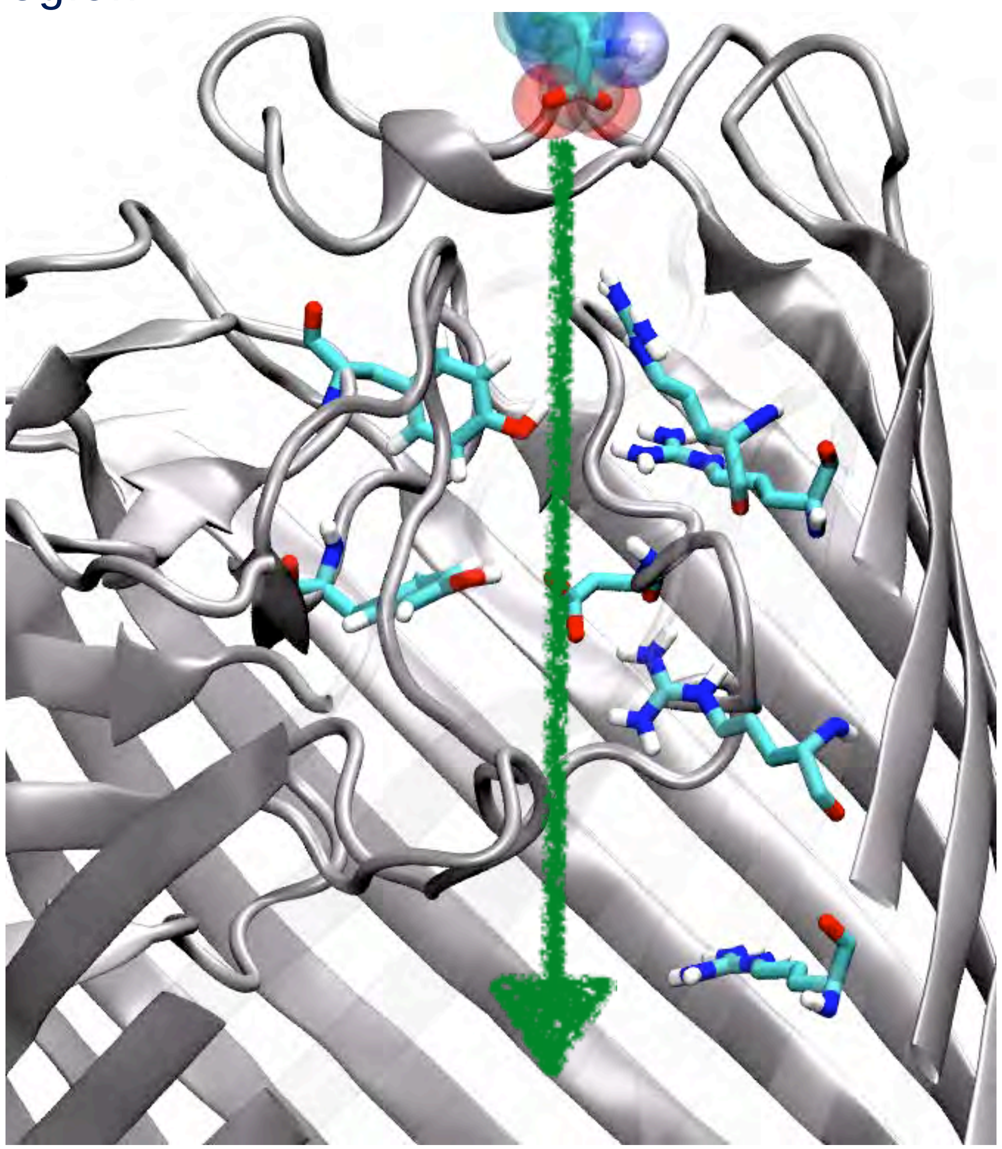


- But how many antibiotics really permeate directly across the outer membrane?

The outer membrane: proteins

- Some of the channel proteins in the outer membrane of have been shown to allow the passage of antibiotics.
- This is surely an energetically easier route to take, compared to directly across the OM?

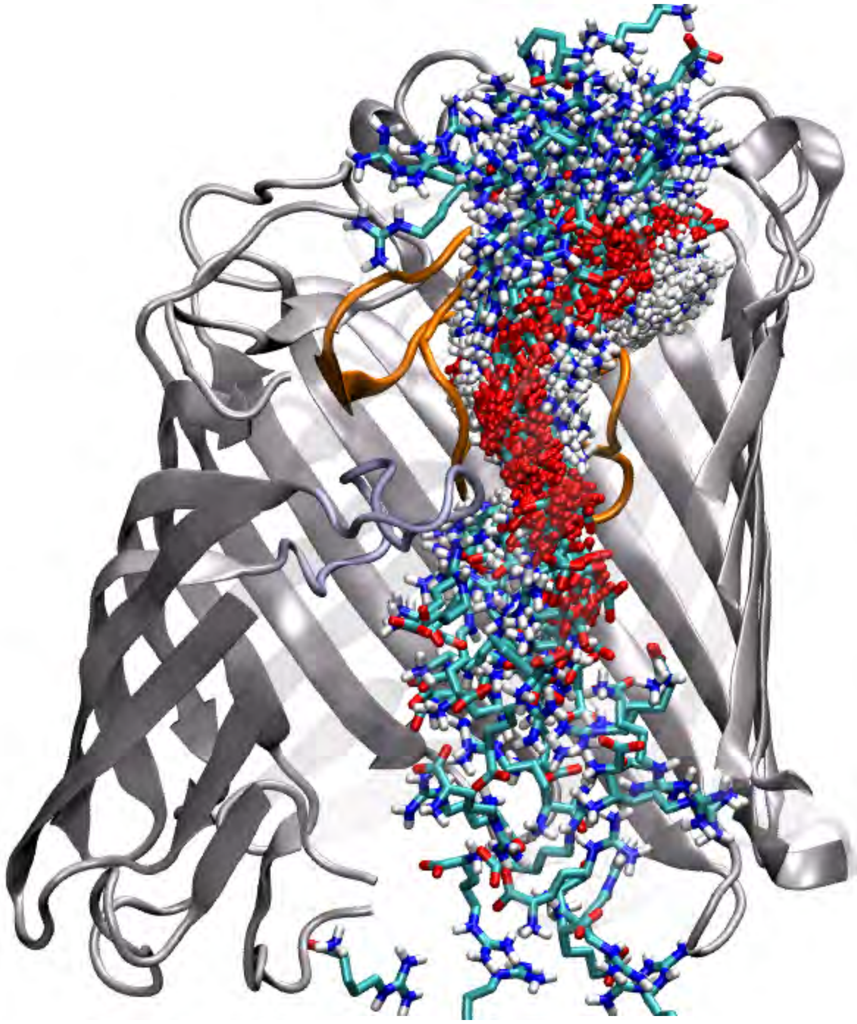
Steered MD simulations to provide potential pathways through the pore region



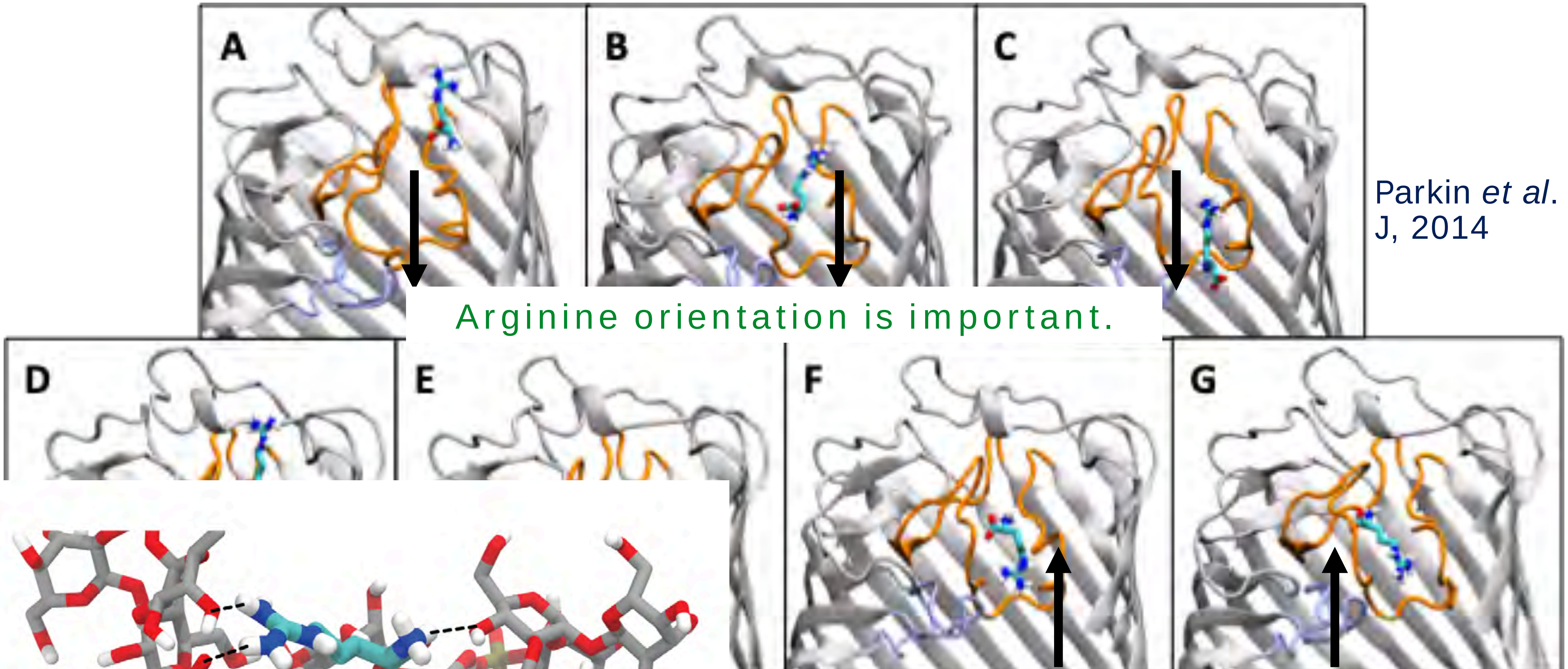
Parkin *et al*. Biophys J, 2014

Equilibrium MD simulations using snapshots from SMD as starting points.

25 x 200 ns simulations



The OprD channel protein from *P. aeruginosa*

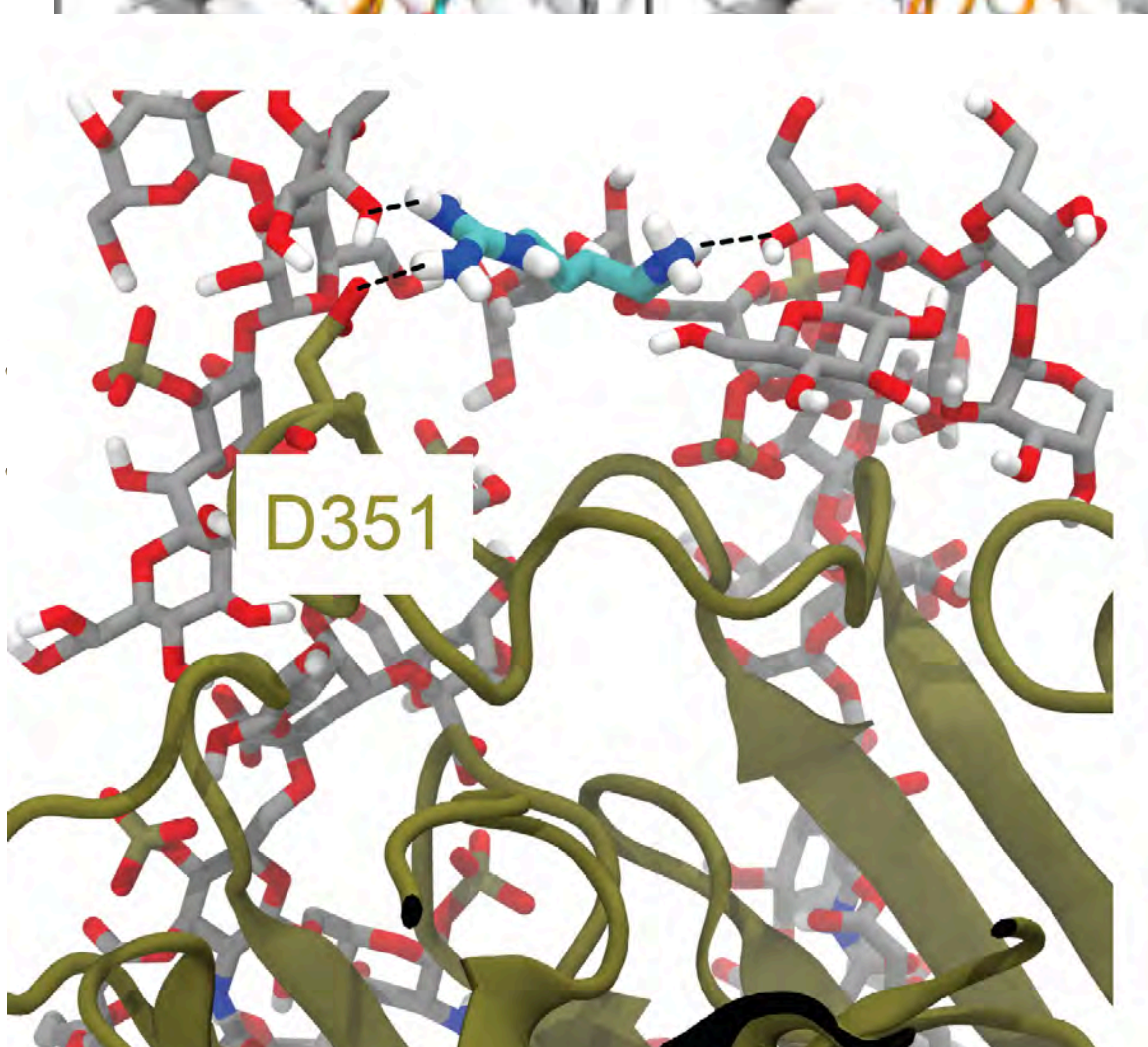


Parkin *et al*. Biophys J, 2014

Arginine orientation is important.

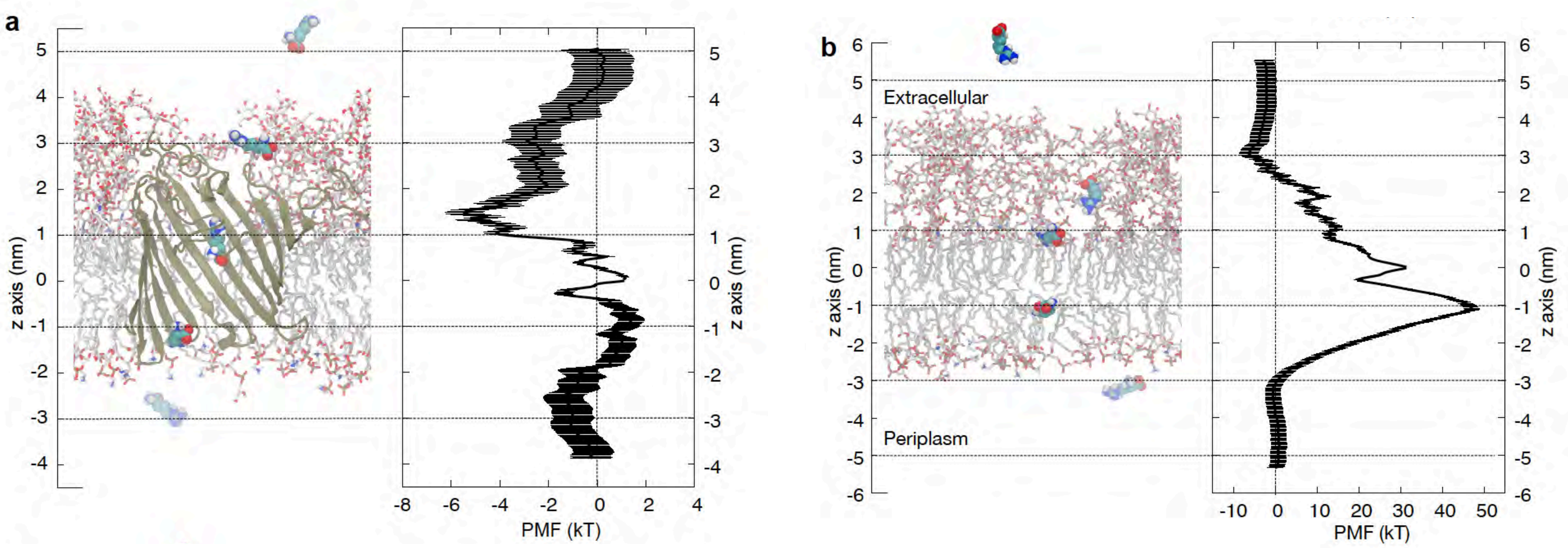
pass through the highlighted region within 10 ns

he same region for > 100 ns



Samsudin and Khalid J Phys Chem B, 2019

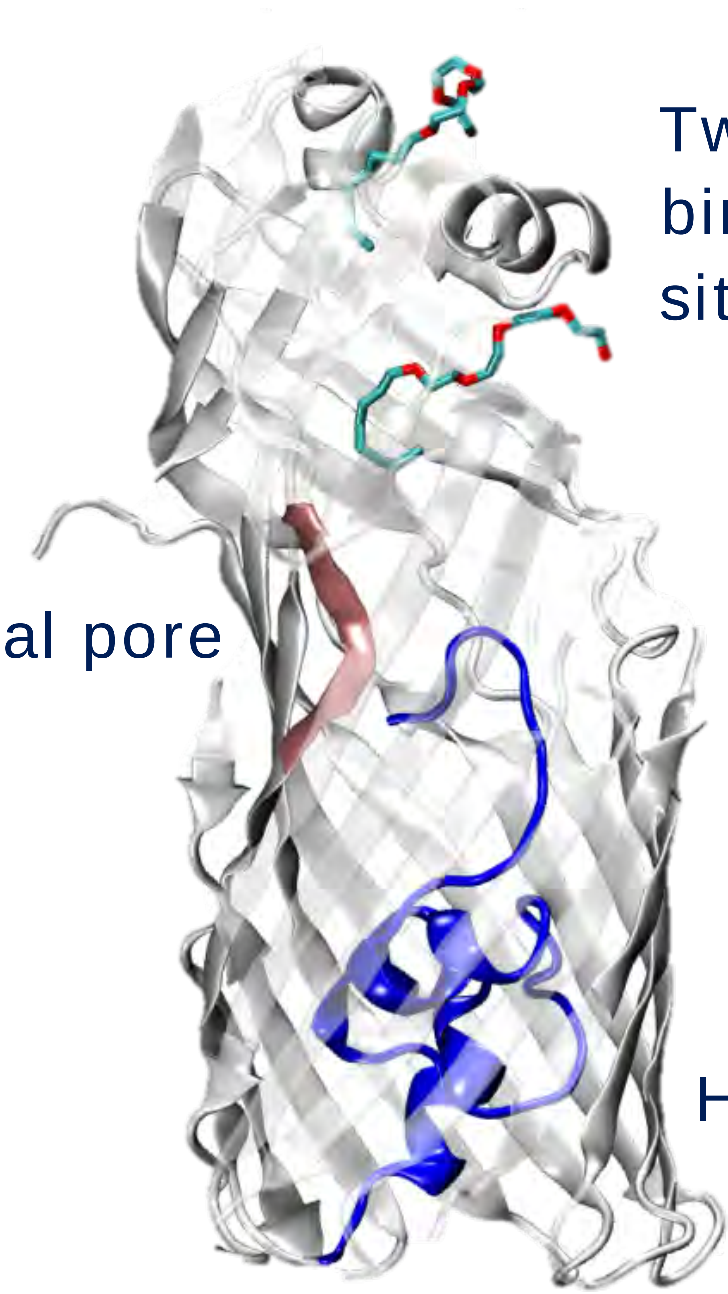
Energetics of arginine passage through OprD

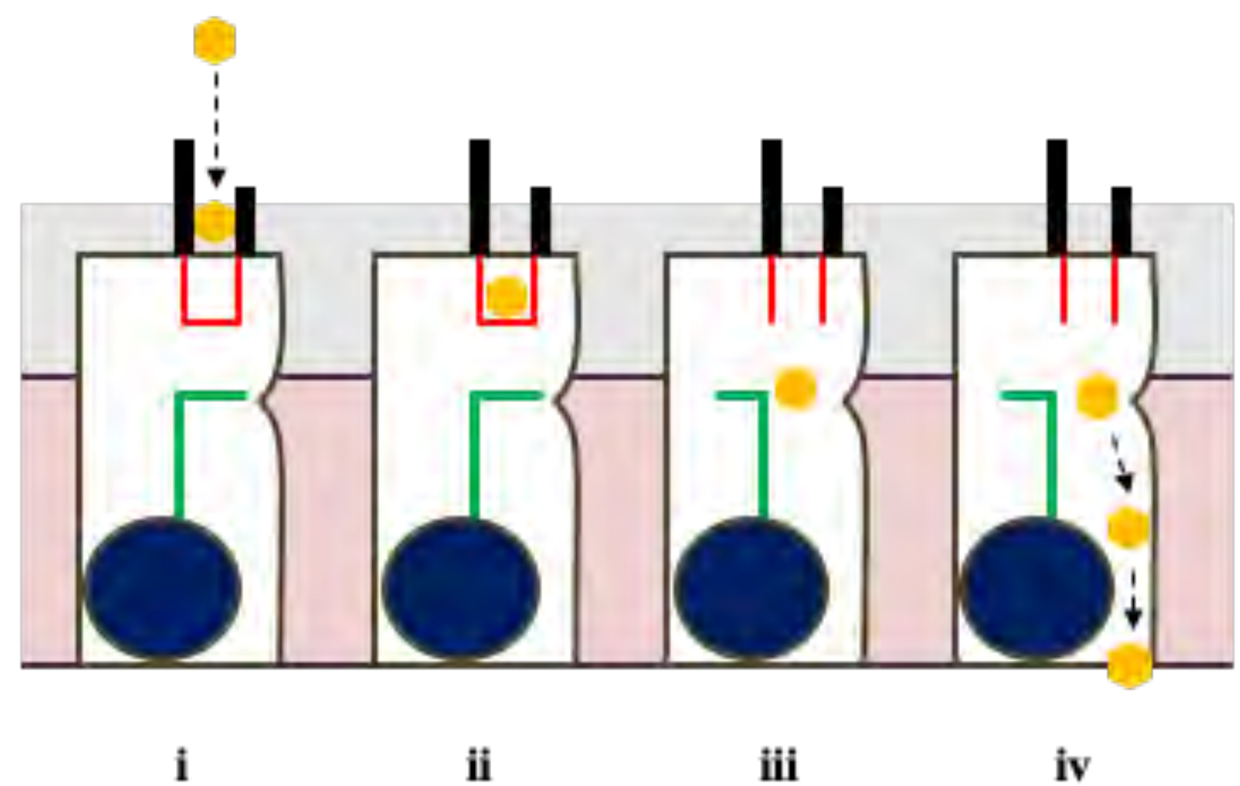


- Arginine is in an energetically favourable state within the OprD pore regions
- In contrast it experiences a huge barrier as it attempts to permeate directly across the OM
- It is highly likely that the same is true of antibiotics- we are working on calculating free energy profiles for antibiotics through a range of outer membrane proteins.

Benzene passage through TodX

- How does benzene get across the outer membrane?





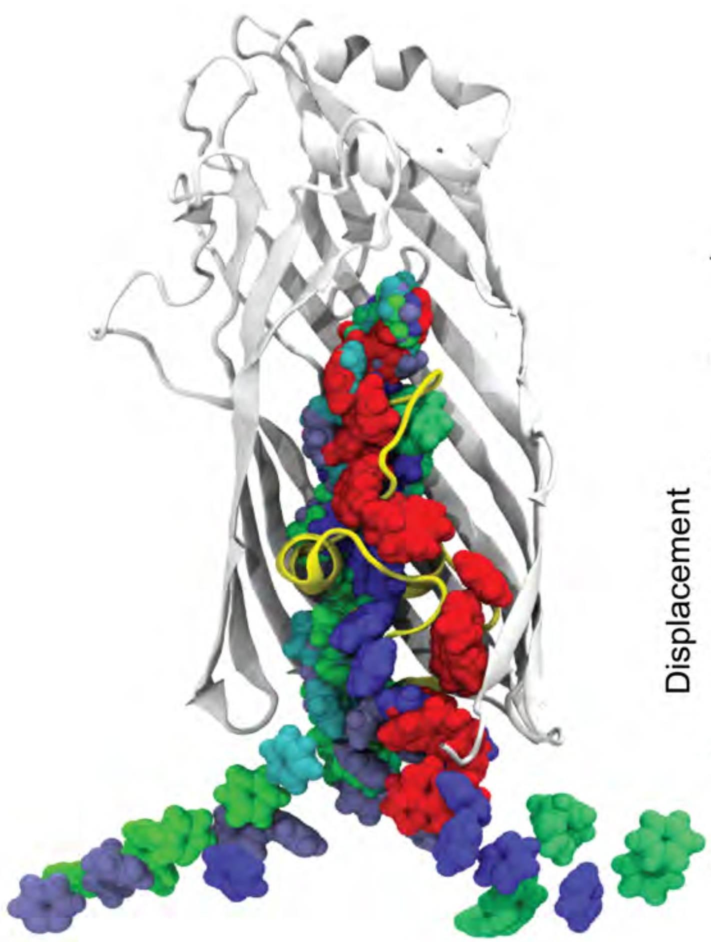
Proposed binding site based on structural and mutational data

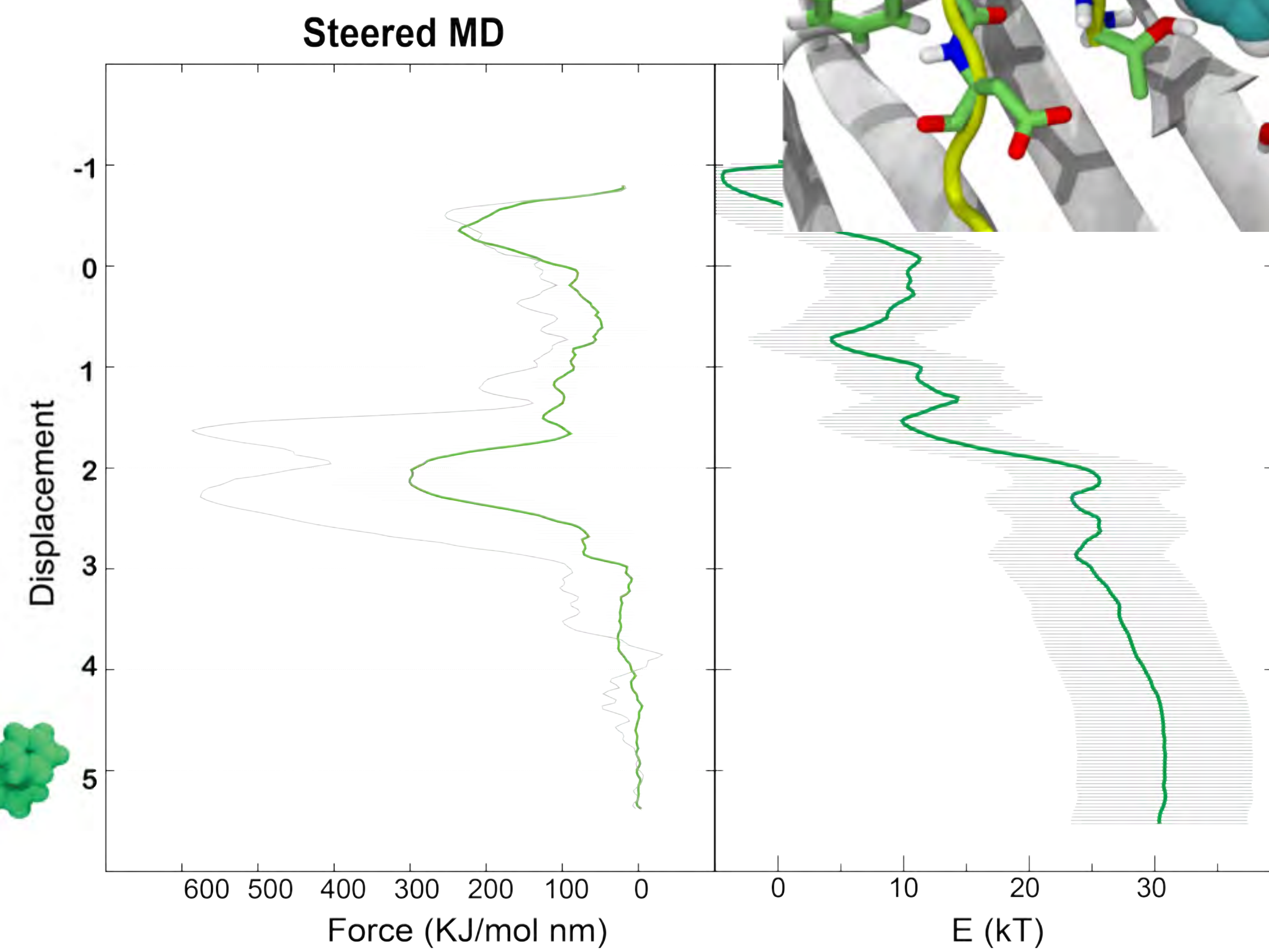
- Why does TodX have two pores?

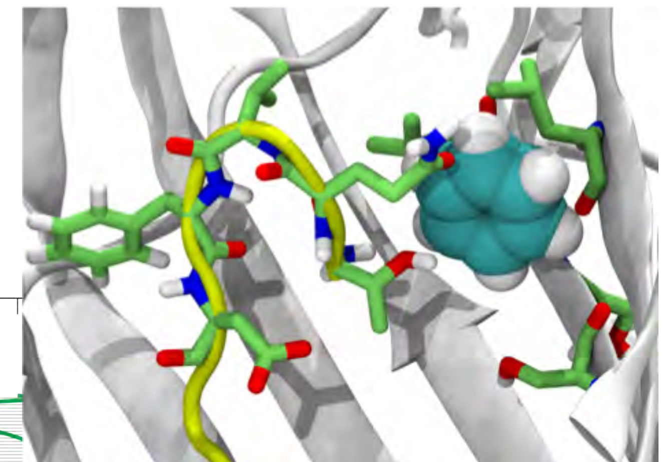
Benzene passage through TodX

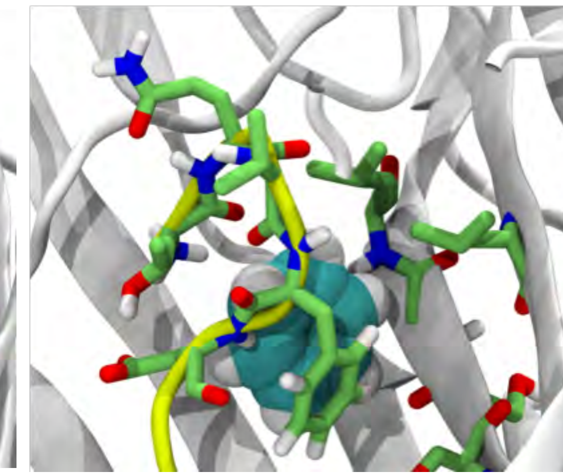
- We are using a combination of steered MD and Umbrella sampling to determine the barriers to benzene permeation.

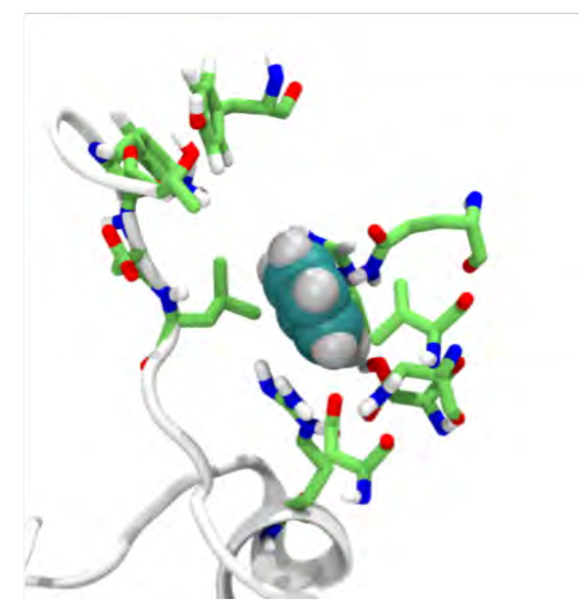
- The lateral pore remains a mystery...











- We are beginning to identify features of the protein that are important for benzene permeation

Polymyxin B1 and the *E. coli* inner membrane

- How does it achieve cell lysis at the inner membrane?
- How does Polymyxin get across the outer membrane?

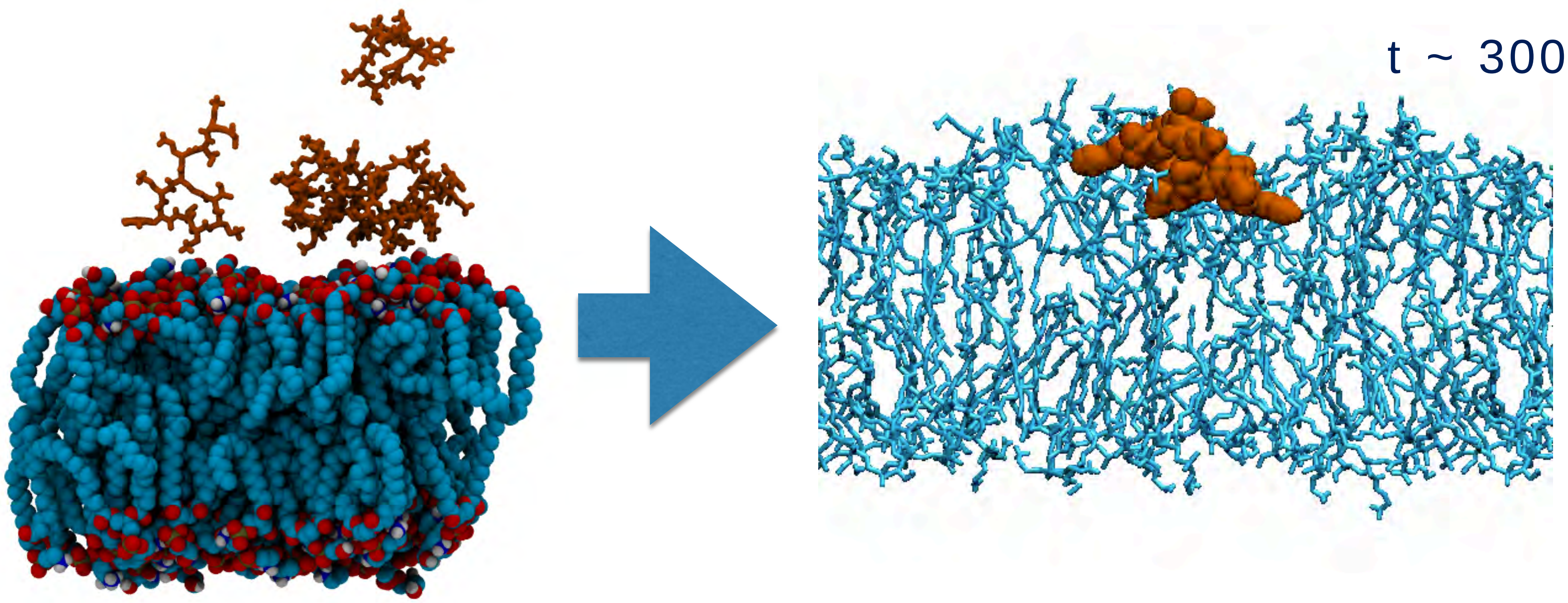
detailed membrane models needed

Polymyxin action on the inner membrane:

t= 0 ns

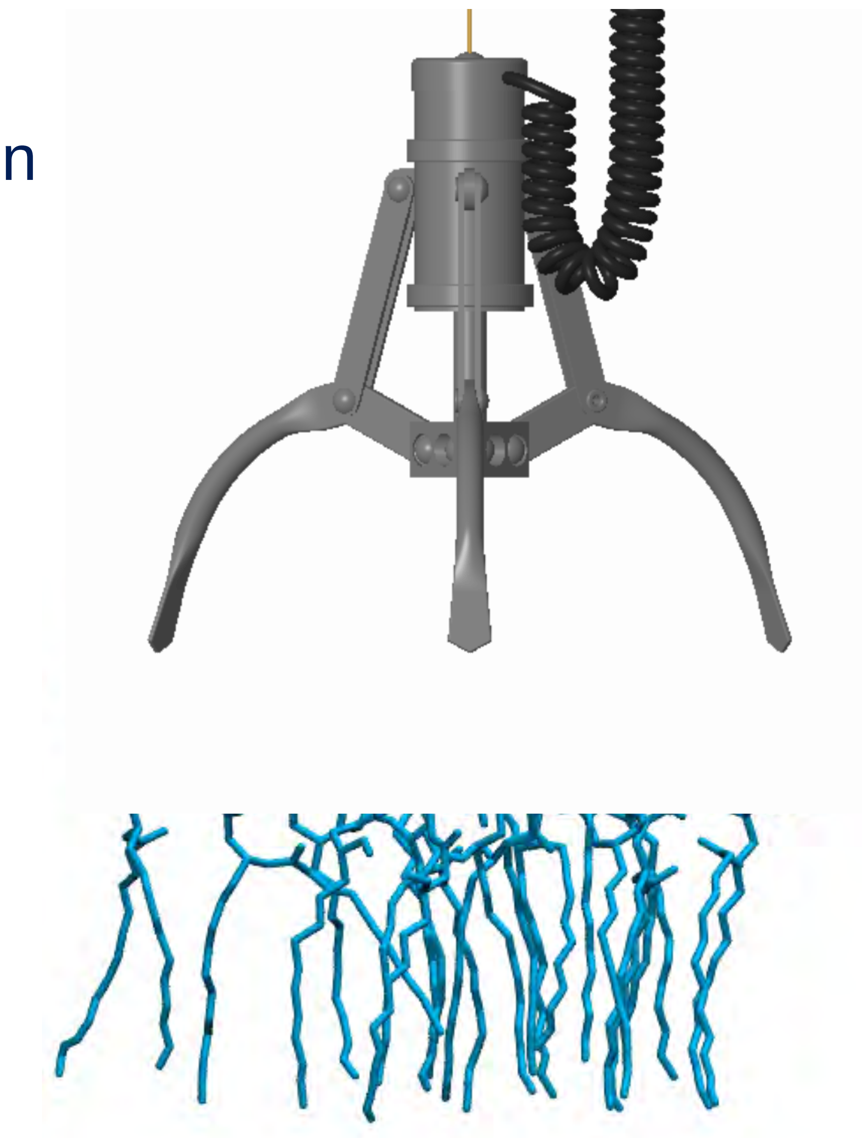
7 x PMB

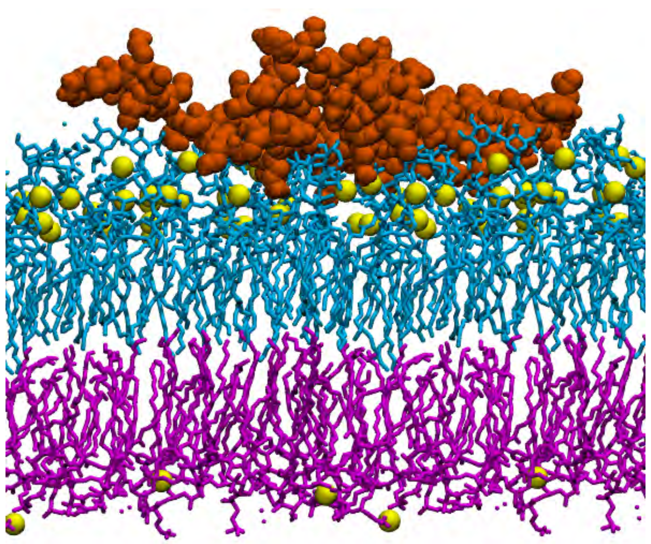
t ~ 300 ns



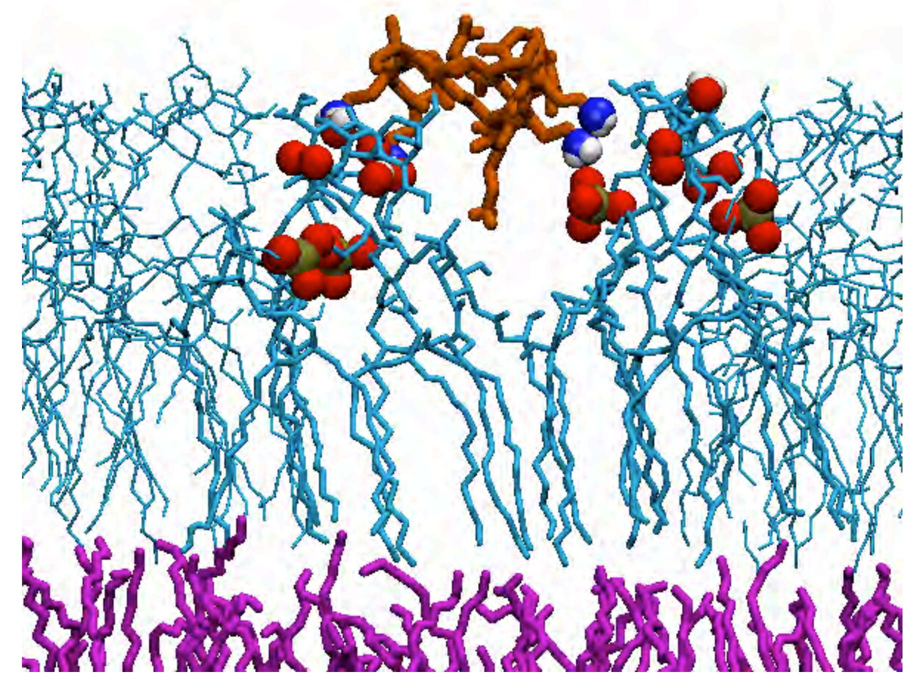
- Polymyxin aggregates and then easily inserts into the membrane, and is fairly mobile
- Insertion causes destabilisation of the inner membrane.

Direct passage of antibiotics through the OM

- How does Polymyxin  nbrane?



2 x 1 microsecond simulations

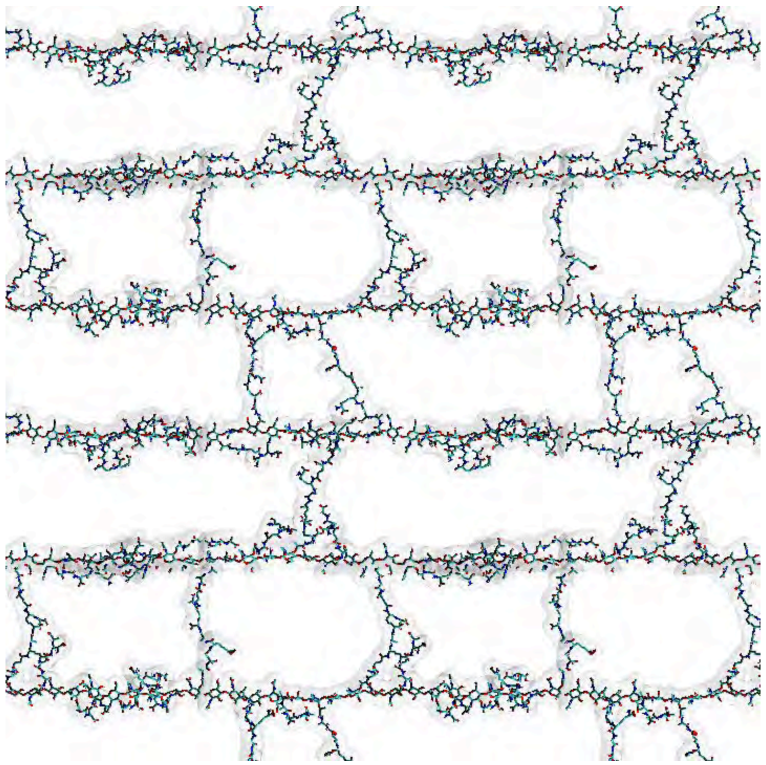


- LPS forms multiple electrostatic interactions with Polymyxin B1
- LPS diffuses very slowly

Berglund et al, *PLoS Comp. Biol*, 2015

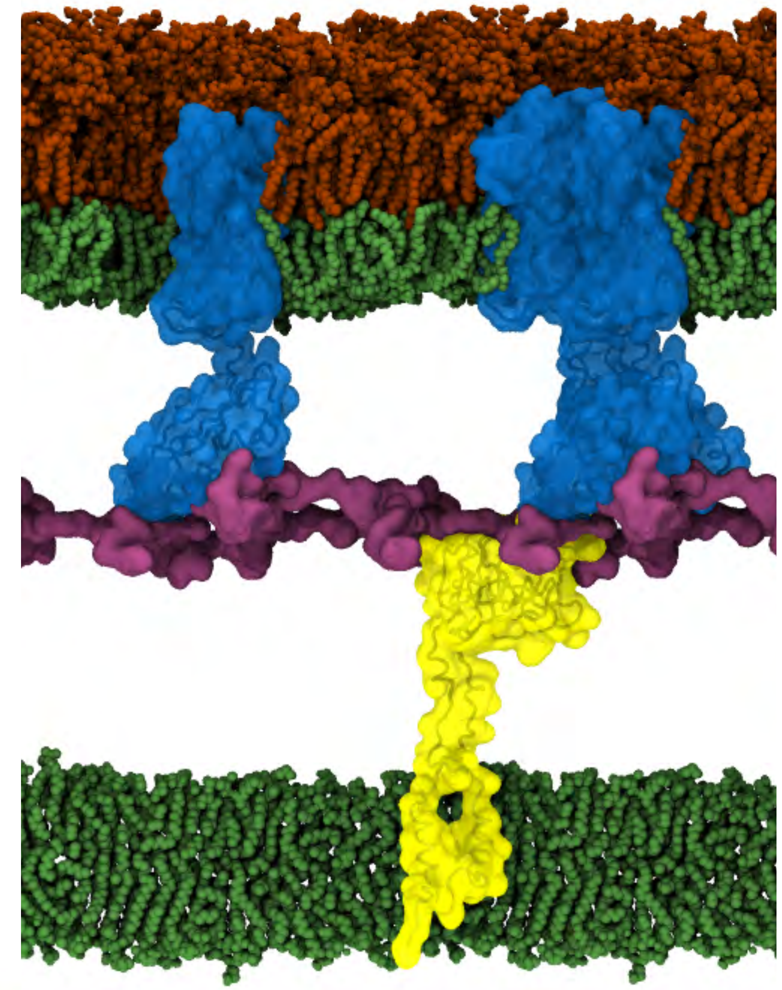
Considering the whole envelope

We are now simulating systems which include two or three compartments of the cell envelope



We can study how molecules move through all compartments.

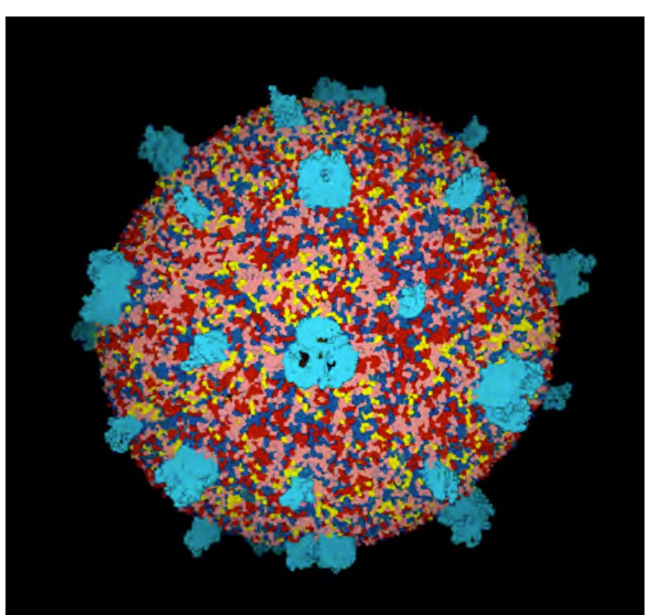
Our models and expertise can be extended to other species.

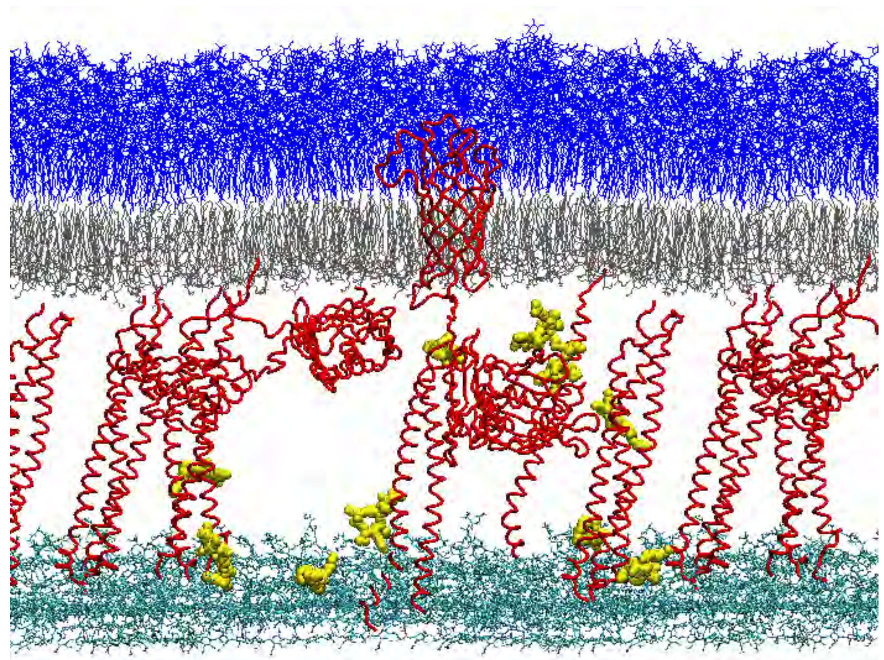


Our atomistic model of *E. coli* PGN

NAG-NAM peptide units

Boags et al, Structure, 2019

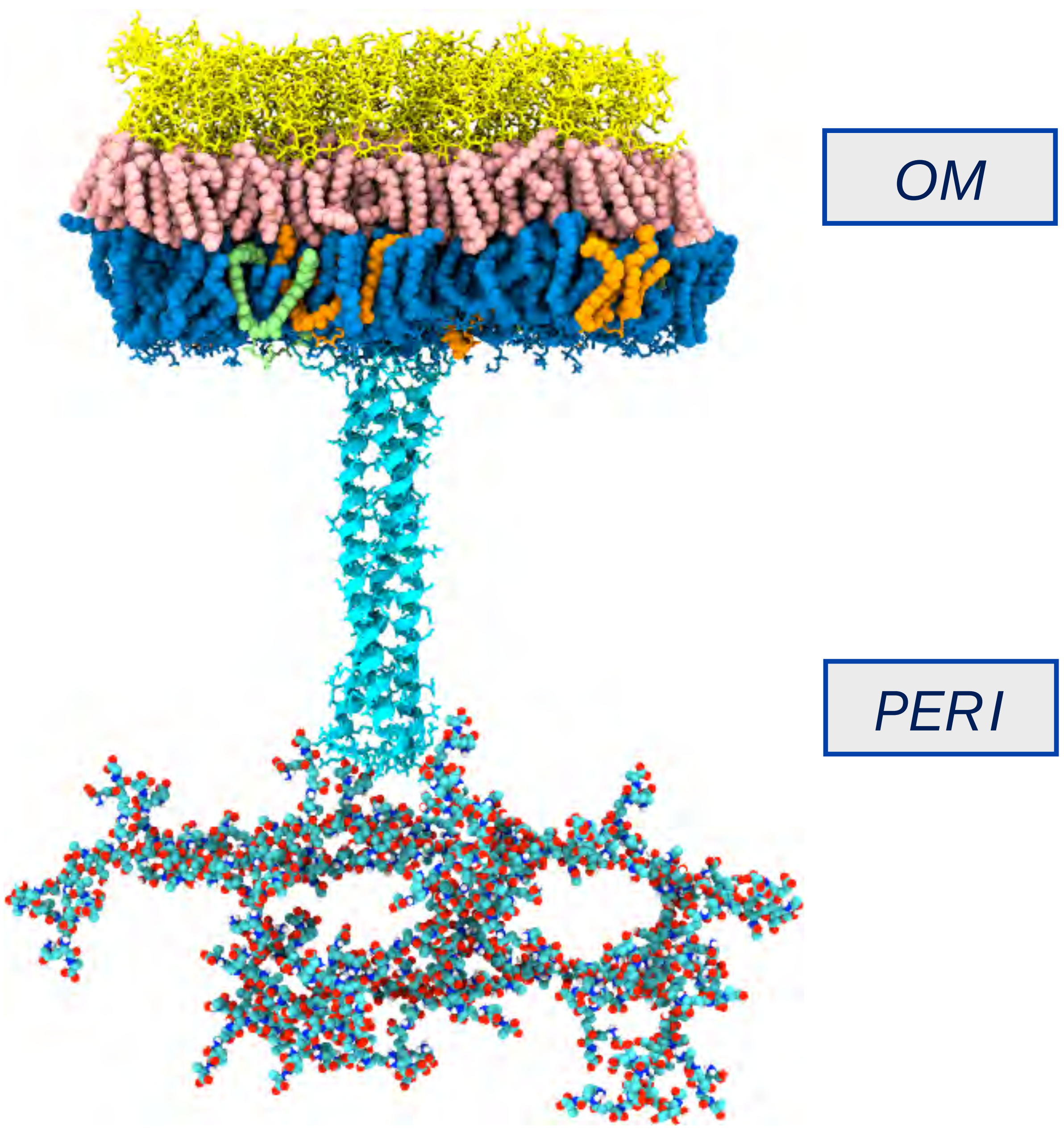




Pedebos & Khalid (in preparation)

Summary and future work

- We are working towards developing a ‘virtual bacterial cell envelope’
- Essential for the computational study of proteins native to bacterial membranes and also the action of antimicrobial agents



- Atomistic & CG models of LPS have been developed.
- Future work includes:
  - adding additional details of the peptidoglycan layer.
  - adding proteins
- Thus we are working towards a model of the complete bacterial cell envelope.

Acknowledgments

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- Conrado Pedebos
- Jonathan Shearer
- Damien Jefferies
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- Bob Hancock (British Columbia)
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- Jeremy Lakey (Newcastle)
- Wonpil Im (LeHigh)