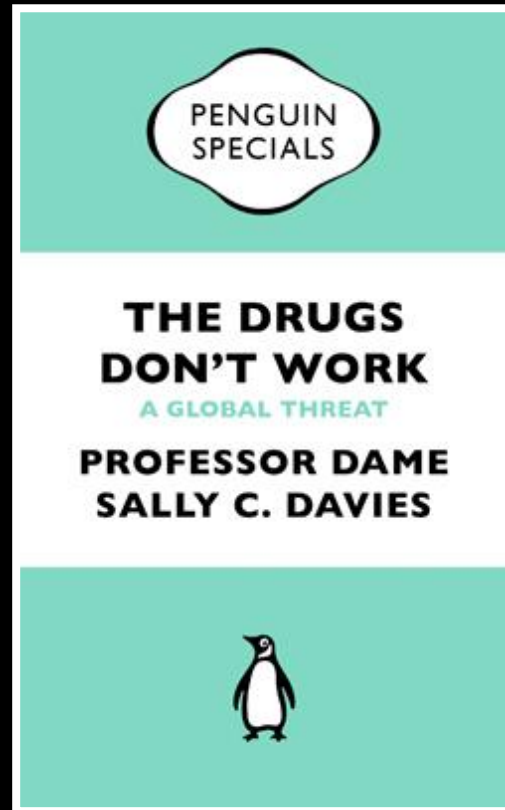


Drugs, Bugs & Neutrons – where are we and where do we go from here?

Dave Barlow, Pharmacy Department,
King's College London

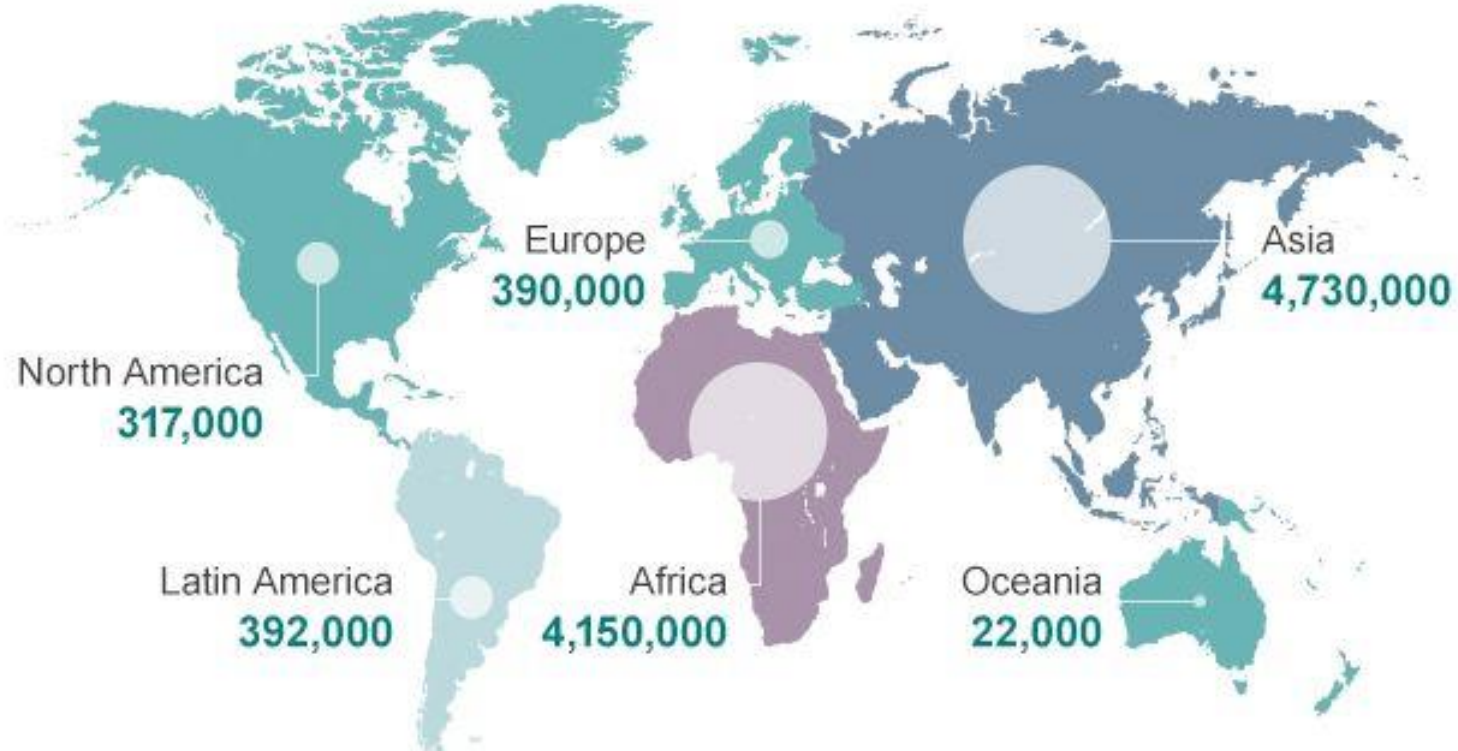
The Future and Next Generation Capabilities of Accelerator-driven Neutron and Muon Sources

ANTIBIOTIC RESISTANCE



THE ANTIBIOTIC APOCALYPSE

Yearly death toll by 2050



WHO review 2014

Deaths due to antimicrobial resistance:

UK: 10,000 / year

USA: 25,000 / year

Globally: ~1,000,000 / year

ANTIFUNGAL DRUGS



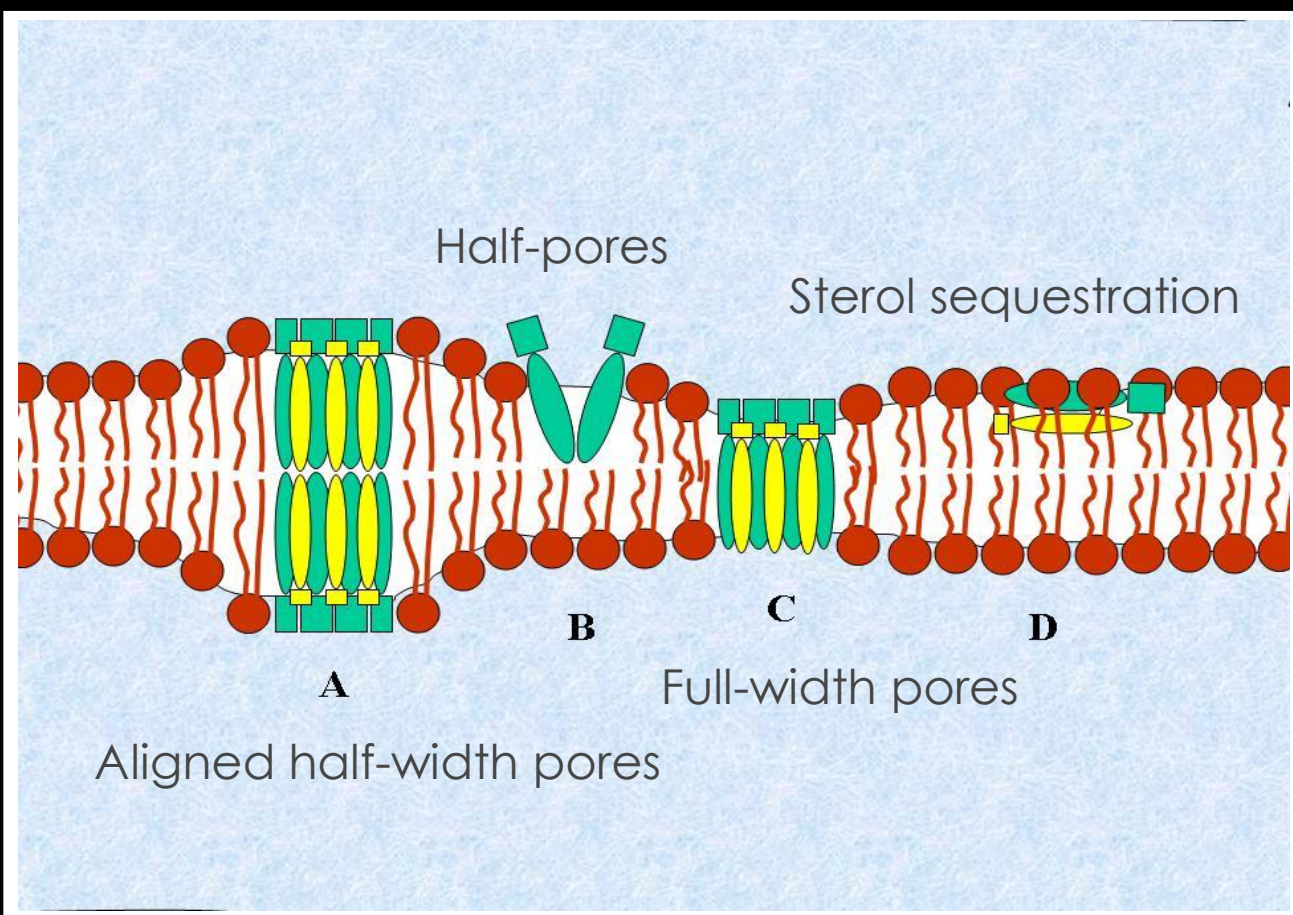
Polyene macrolides (1955) Amphotericin B

Azoles (1980) Fluconazole

Echinocandins (1980) Caspofungin



AMPHOTERICIN – MECHANISM?

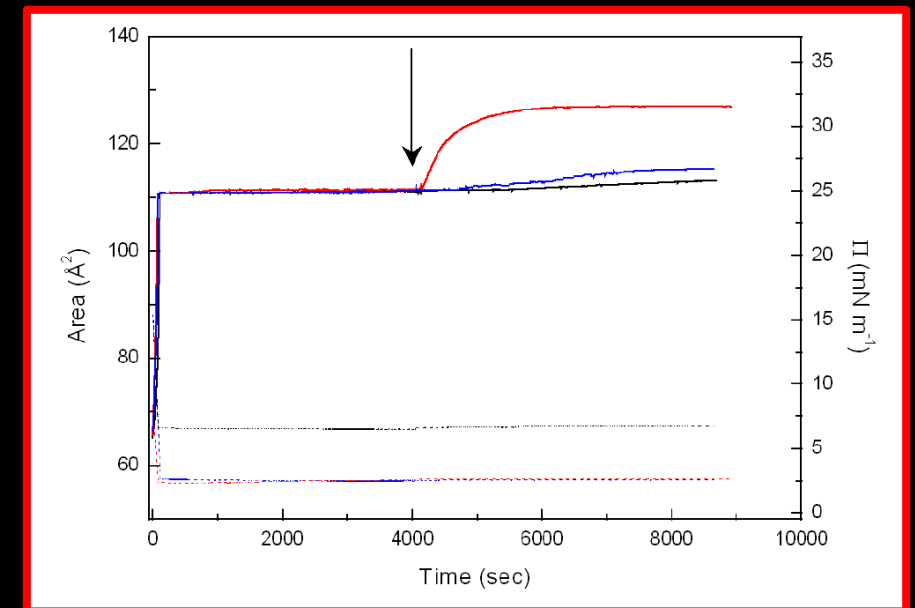


- Acts on fungal cell membranes ...
- ... also on host cell membranes (leading to kidney/damage failure)
- Increasing resistance (necessitating higher doses (with increased incidence of side-effects))
- Need to probe at **molecular level** using **non-invasive** technique
- Use **model** membrane with **sensible composition**
- Use **clinical doses** of drug

NEUTRON SCATTERING STUDIES

- Neutron reflectivity (lipid/sterol monolayers; Surf / Inter @ISIS, Figaro @ILL)
- Small angle neutron scattering (lipid/sterol vesicles LoQ @ISIS)
- Stopped-flow kinetic studies (free drug vs Amphotec[®] LoQ, Sans2d @ISIS)

Sample	Time post injection / sec	$L / \text{\AA}$	$d / \text{\AA}$
POPC/Chol	0	41	58
	5	42	58
	120	44	62
	1500	44	62
POPC/Erg	0	41	58
	5	42	58
	10	44	62
	1500	44	62



NEUTRON SCATTERING STUDIES

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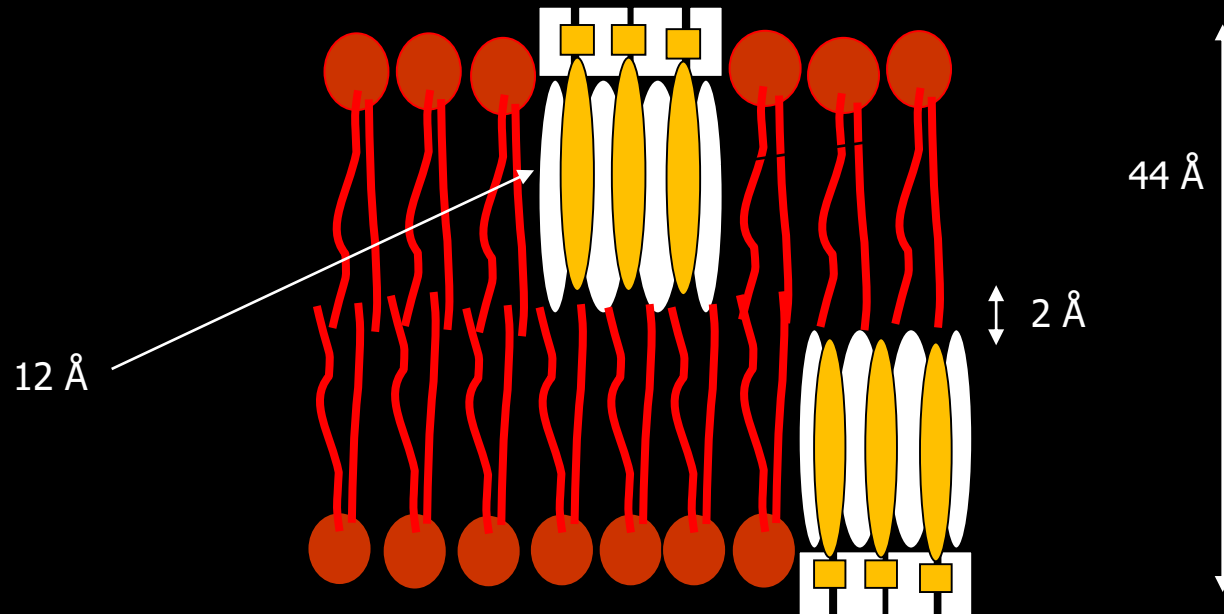
Neutron diffraction
(stacked lipid/sterol lamellae @ILL)

Foglia *et al* (2012) *Sci Rep* **2**:778

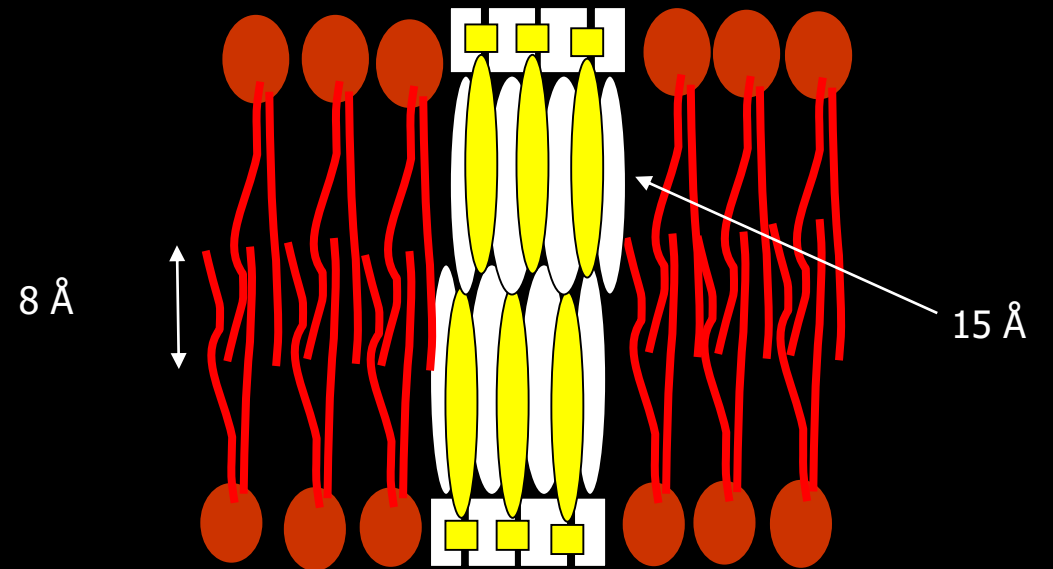


TRANSMEMBRANE ION CHANNELS

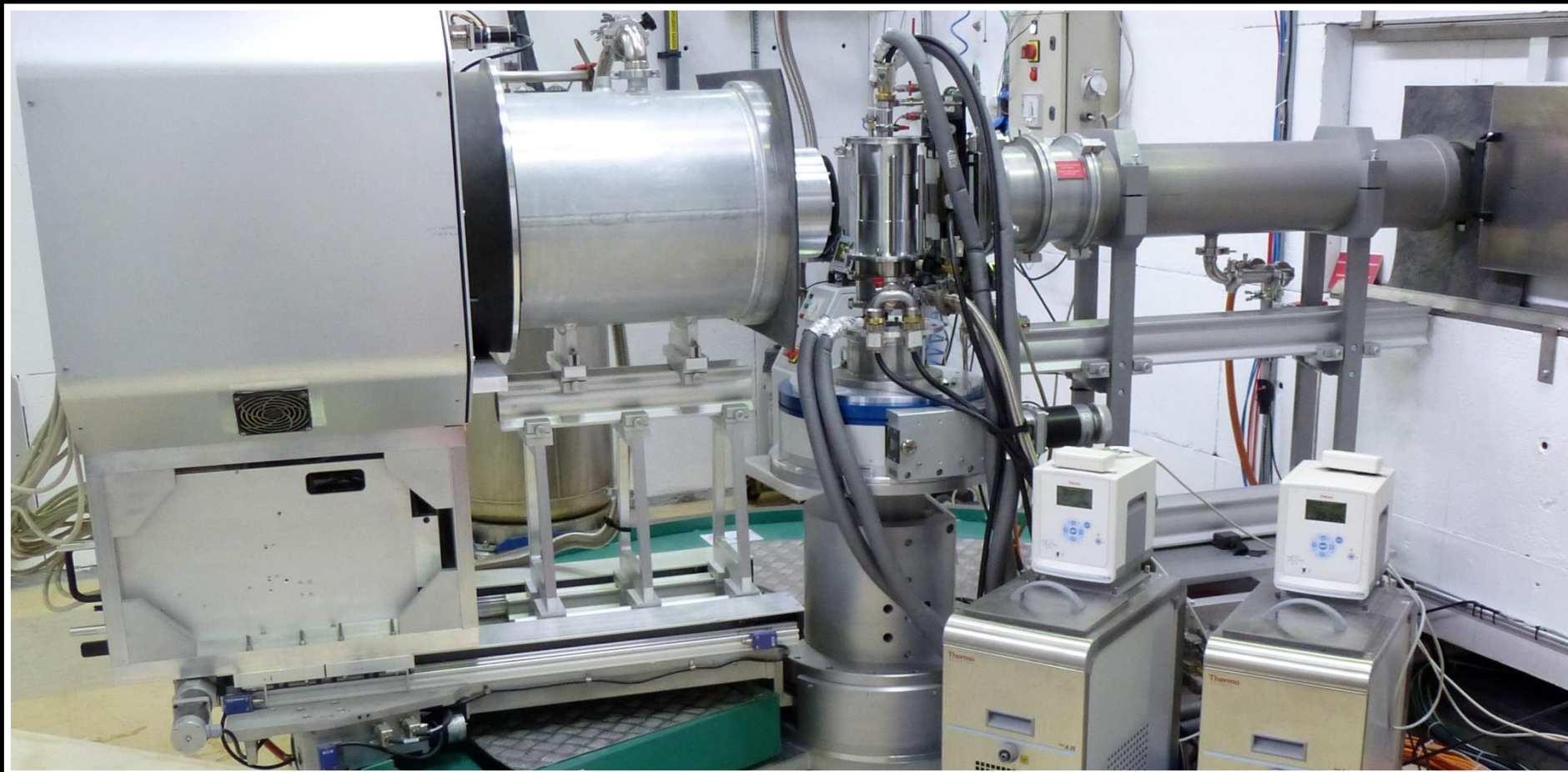
POPC-cholesterol-AmB



POPC-ergosterol-AmB



NEUTRON DIFFRACTOMETER



PHOENIX

BACTERIAL BIOFILMS

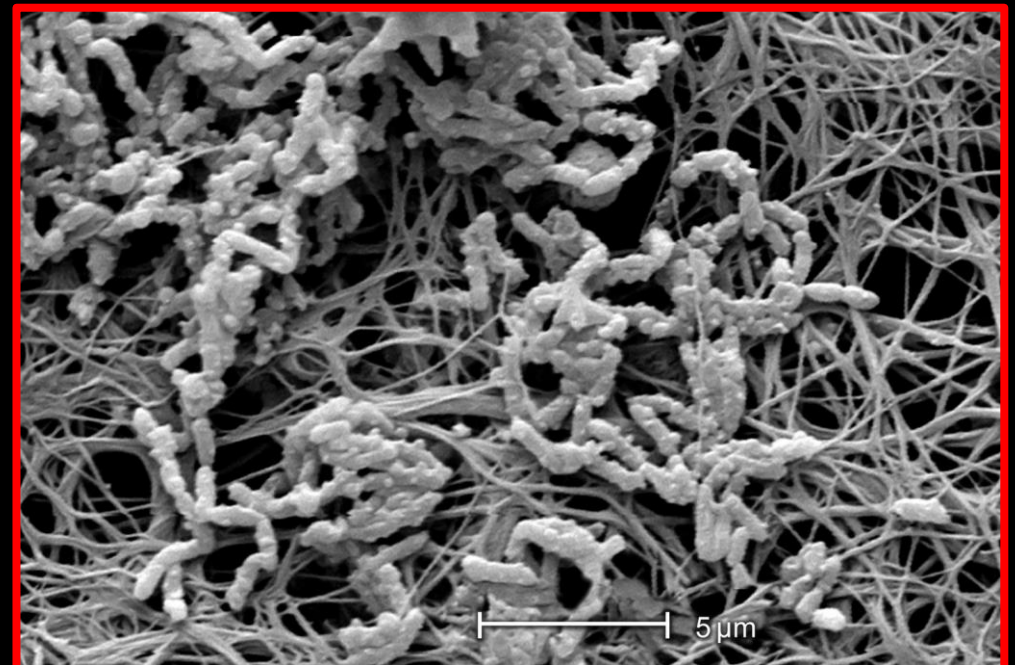
USA NIH report 65% of all microbial infections & 80% of chronic infections due to biofilm (spread)

1 in 25 chance of nosocomial infection; 1 in 7 chance of infection by antibiotic resistant microbe

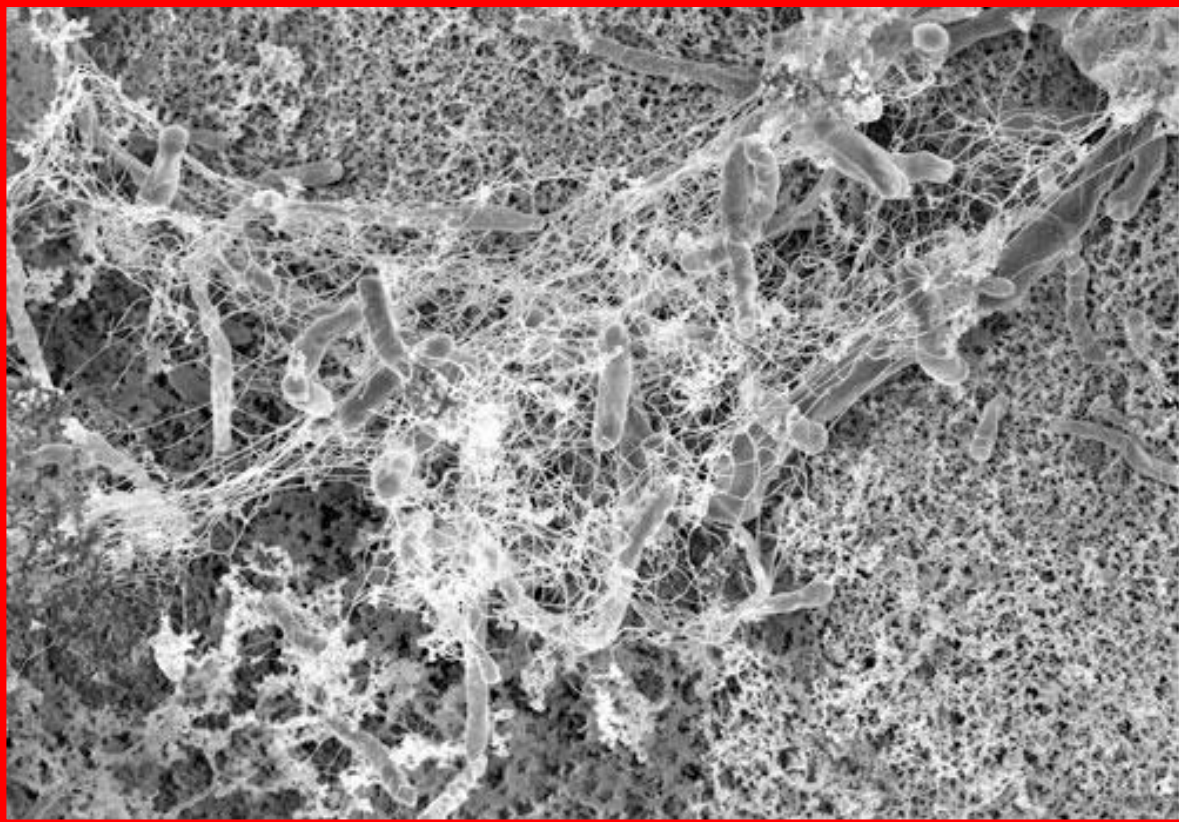
Cost to NHS = £2 billion per annum

Cost to UK foods industry = £70 billion per annum

Cost to consumer products sector = \$2.8 trillion



BACTERIAL BIOFILMS



Water (97%)

Microbes (2-5%)

DNA/RNA (1-2%)

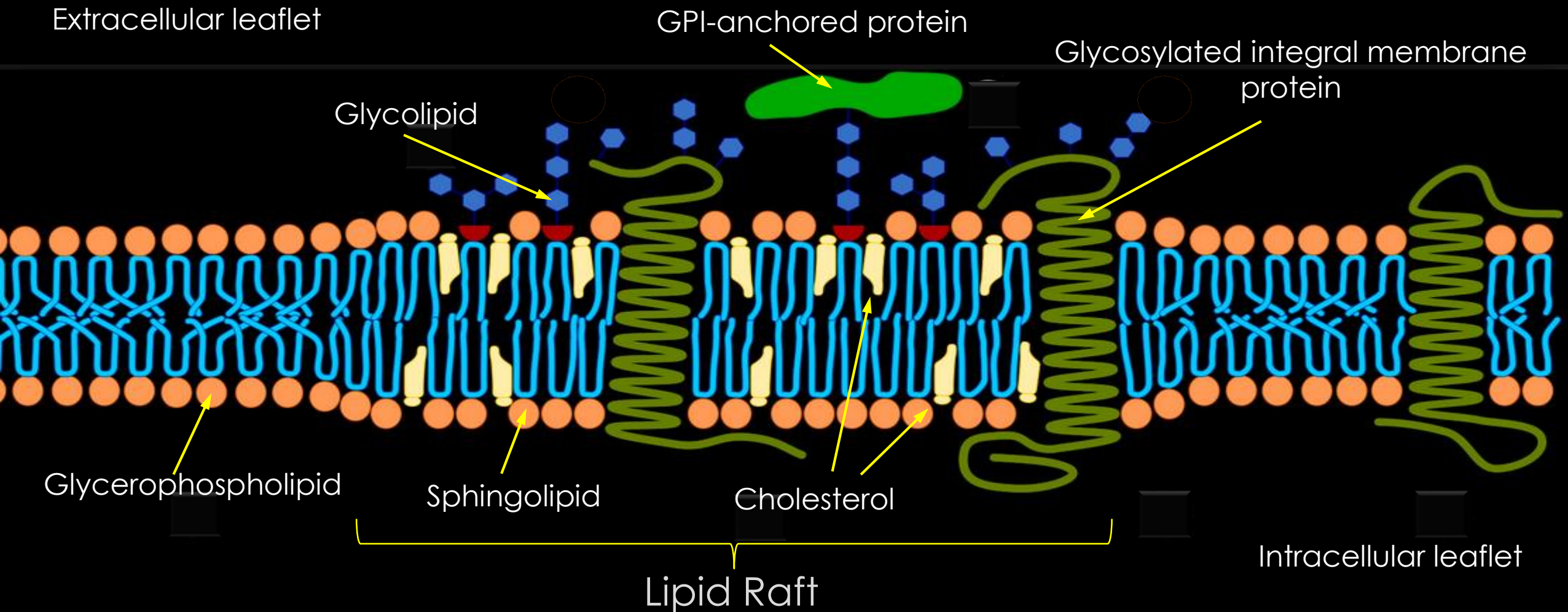
Proteins (1-2%)

Polysaccharides (1-2%)

EXPANDED CL 2 LAB FACILITIES



MEMBRANE RAFTS



MEMBRANE RAFTS

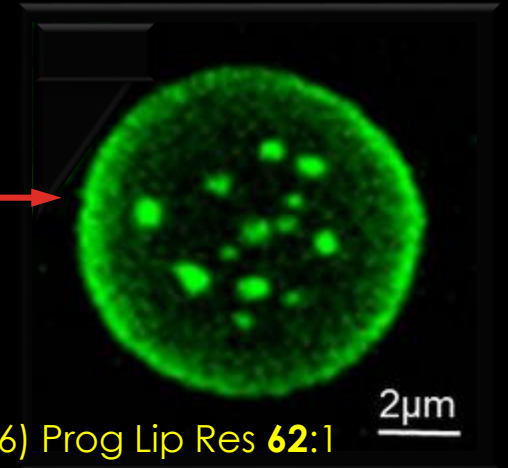
Lipid raft disorder implicated in numerous pathological conditions:

Spherocytosis – a genetic membrane fragility disorder of erythrocytes

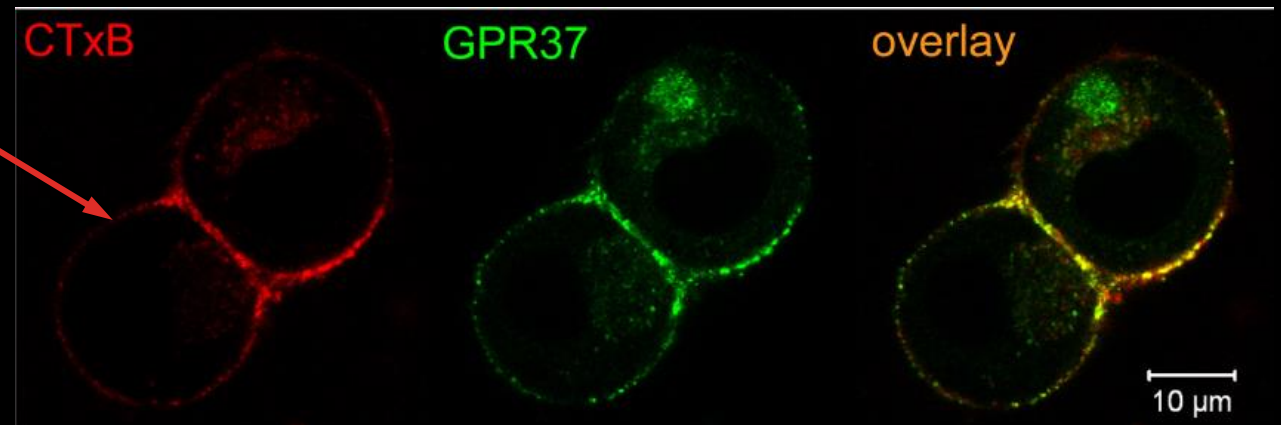
Cholesterol enrichment in rafts promotes NF- κ B receptor activated release of pro-inflammatory cytokines and chemokines (**Atherosclerosis**)

Rafts may provide a platform for formation of neurotoxic proteins like amyloid β (**Alzheimer's disease**) and prion protein (**Creutzfeldt-Jakob disease**), and GPR-37 (**juvenile Parkinson's disease**)

Changes in raft lipid content can prevent apoptosis induced by death receptors (**Breast Cancer**)



Carquin *et al* (2016) *Prog Lip Res* **62**:1



Gregorsson Lundius *et al* (2014) *J Biol Chem* **289**:4660

MEMBRANE RAFTS

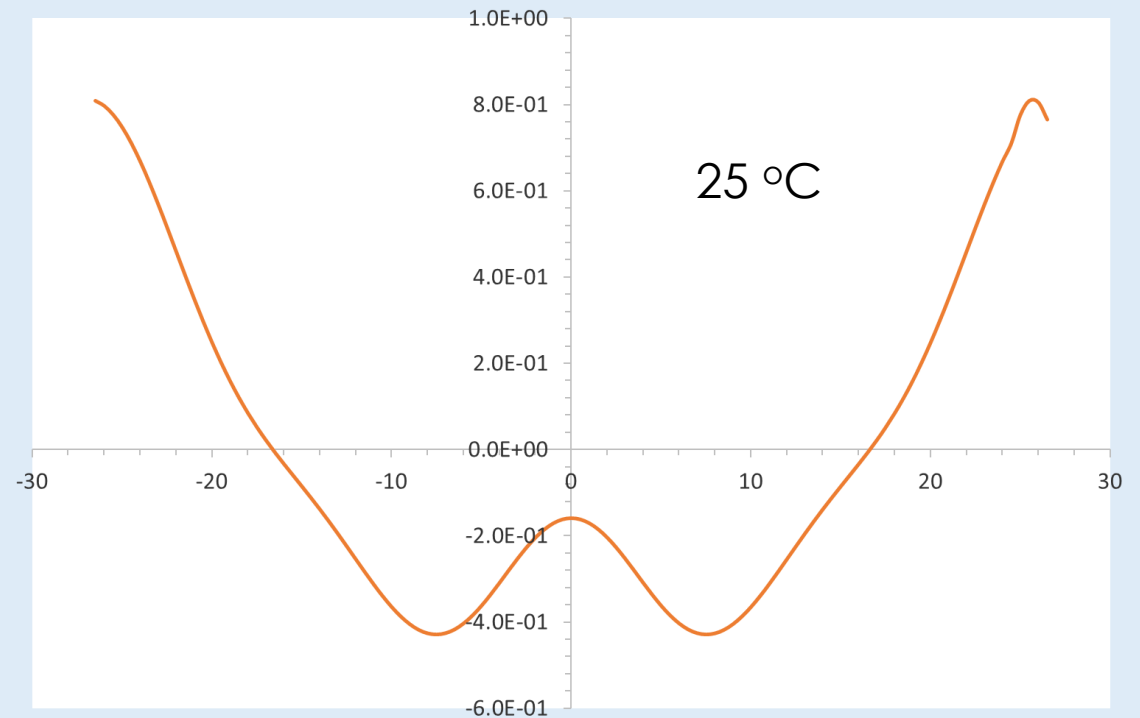
Lamellar Neutron Diffraction (D16)

2:2:1 DPPC-DOPC-Cholesterol multilayers
supported on silicon wafers

Studied at 100% and 80% RH in 100% D₂O,
50:50 D₂O:H₂O & 100% H₂O

Studied at temperatures of 15 °C, 20 °C, 25 °C

25 °C, *d*-spacing 53.8 Å



MEMBRANE RAFTS

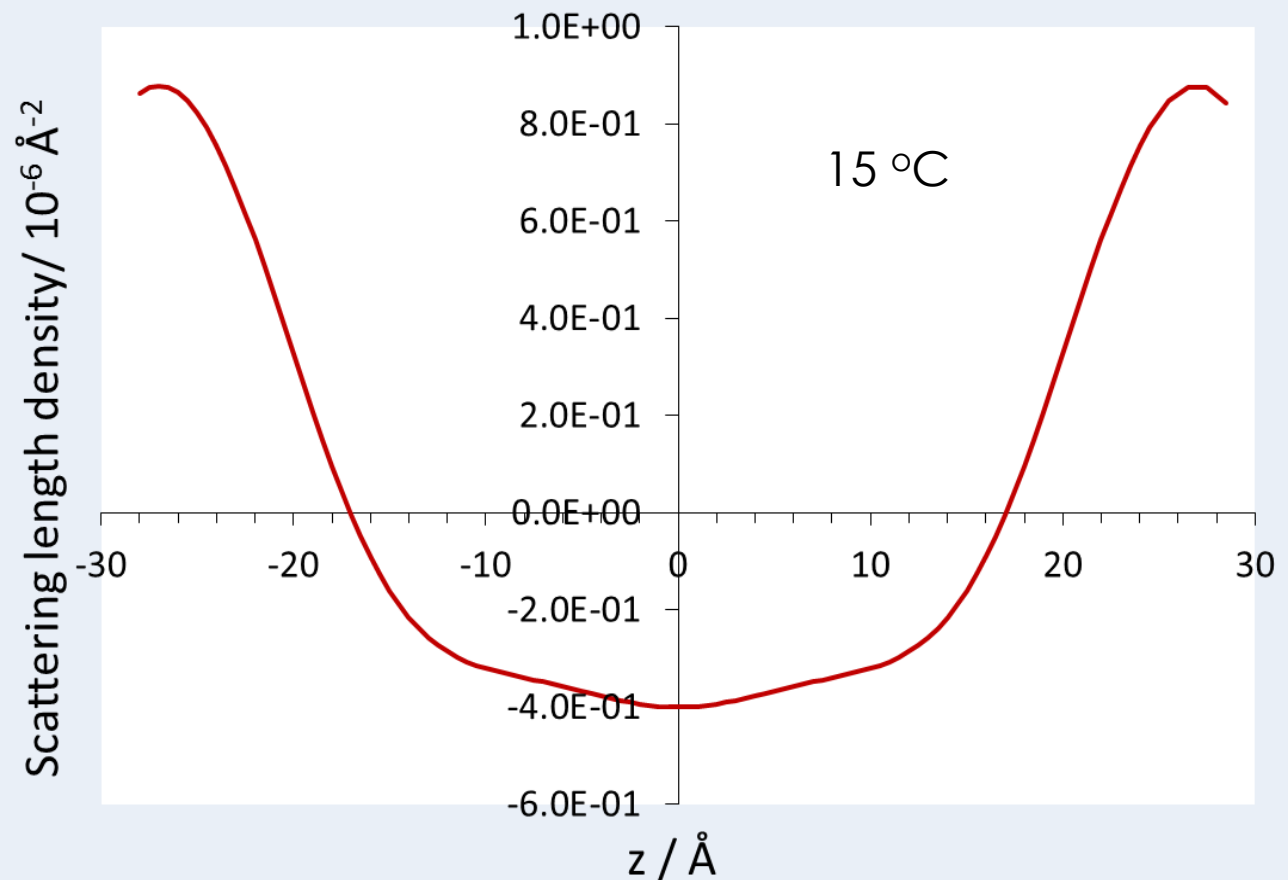
Lamellar Neutron Diffraction (D16)

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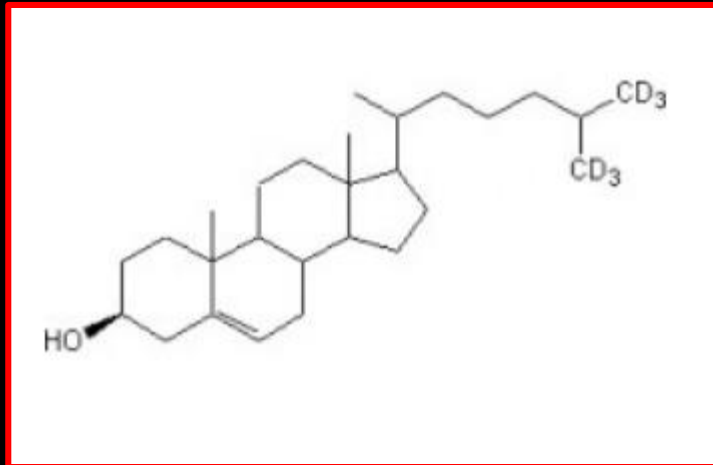
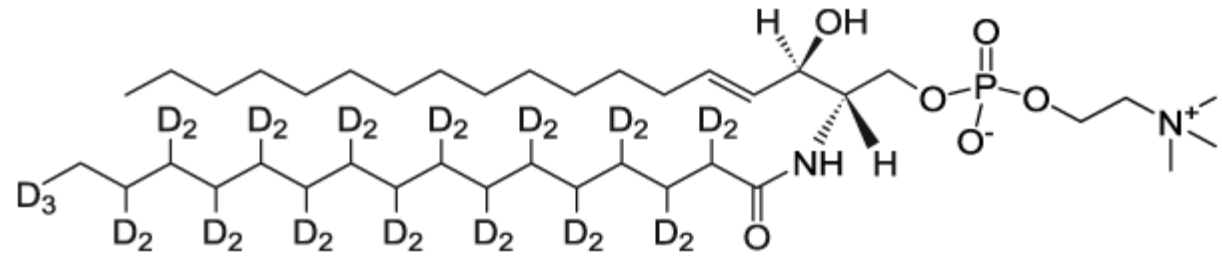
Studied at temperatures of 15 °C, 20 °C, 25 °C

15 °C, *d*-spacings 53.8 Å, 59.2 Å



EXOTIC DEUTERATED LIPIDS

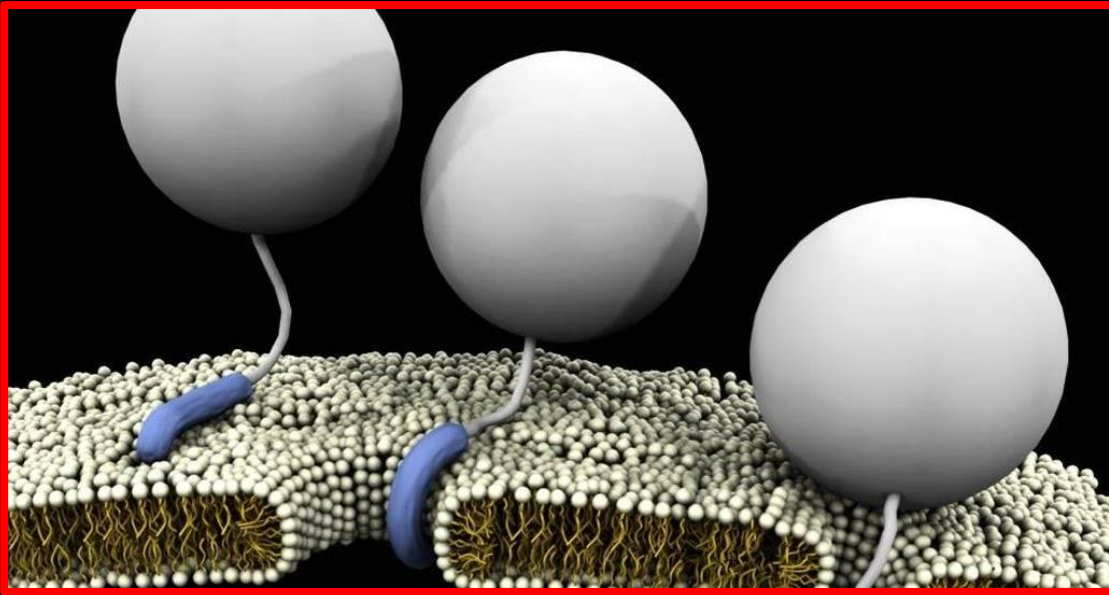
N-palmitoyl- d_{31} -*D*-erythro-sphingosylphosphorylcholine
(10 mg = £1,500)



Perdeuterated cholesterol
(d_6 -cholesterol, 10 mg for £140)

BIOPHARMACEUTICAL DRUG DELIVERY SYSTEMS

TAT peptide

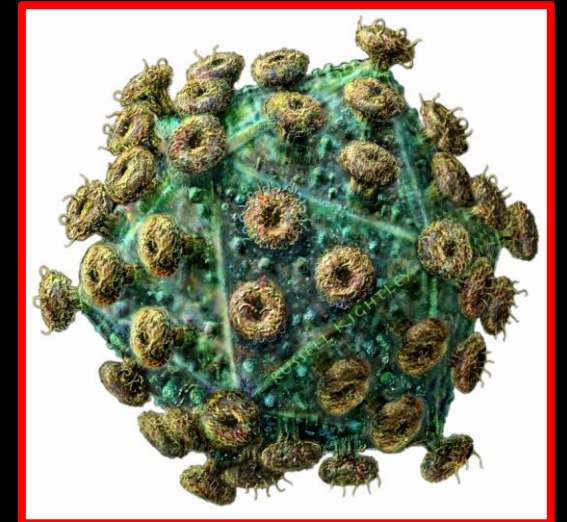


Cell penetrating peptides – mediate passive translocation across cell membranes

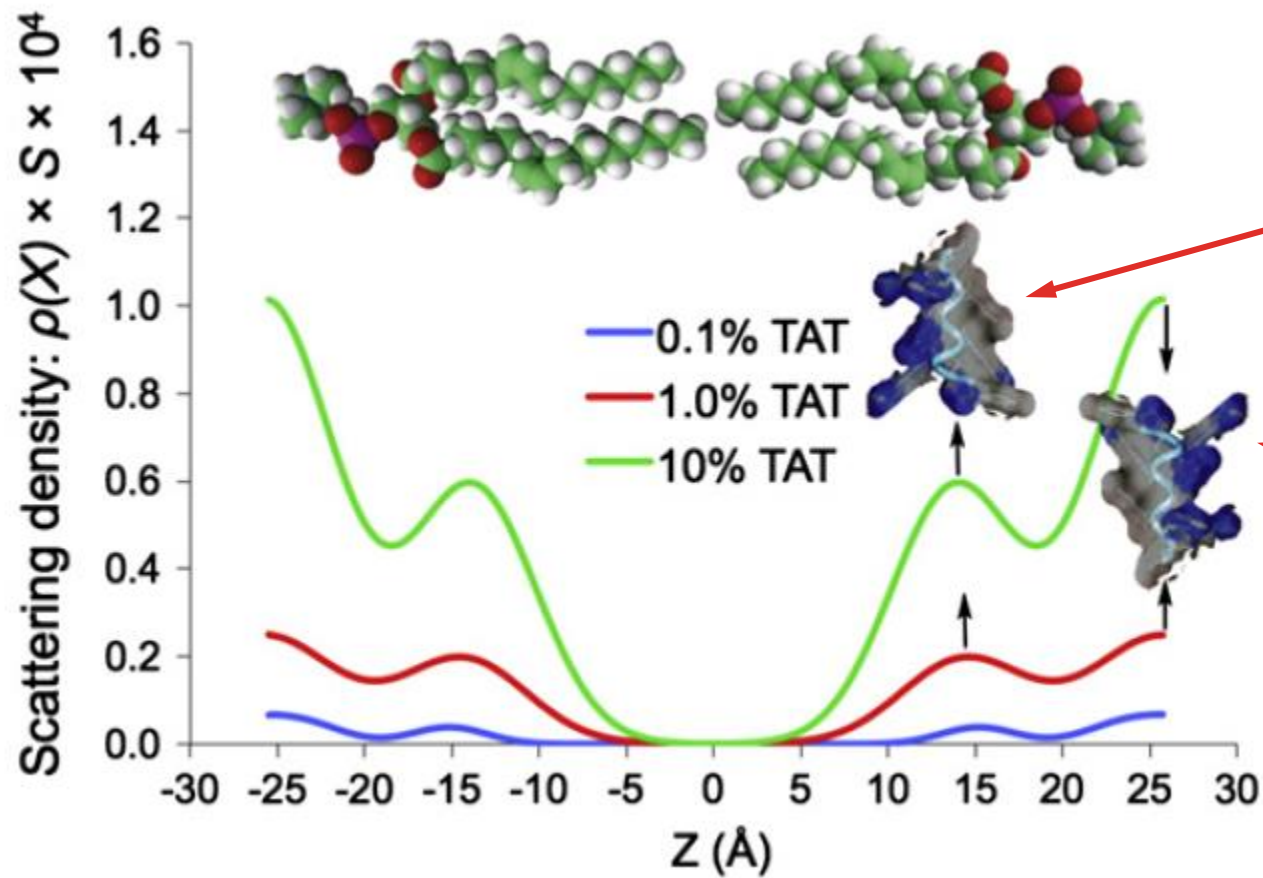
Potential for use in non-invasive delivery of macromolecules into cells

One of best characterised – a peptide derived from the transactivator of transcription protein (TAT protein) from HIV-1

Minimal sequence of TAT protein capable of mediating cellular uptake:
 $^{47}\text{YGRKKRRQRR}^{57}$ – the TAT peptide



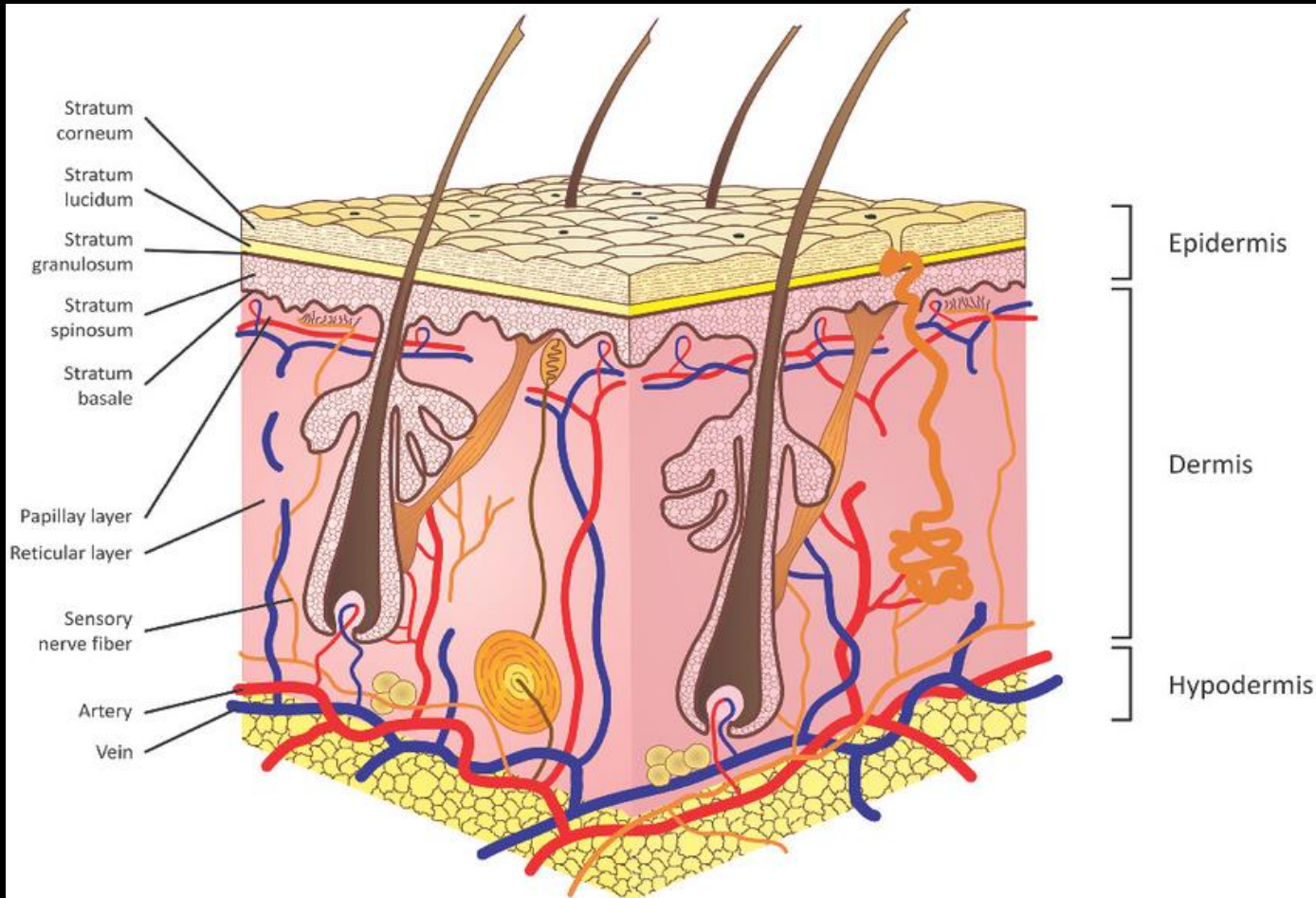
BIOPHARMACEUTICAL DRUG DELIVERY SYSTEMS



TAT peptide inserted into the lipid bilayer, just beneath the glycerol moiety

TAT peptide within the aqueous space between lipid bilayers

STRATUM CORNEUM

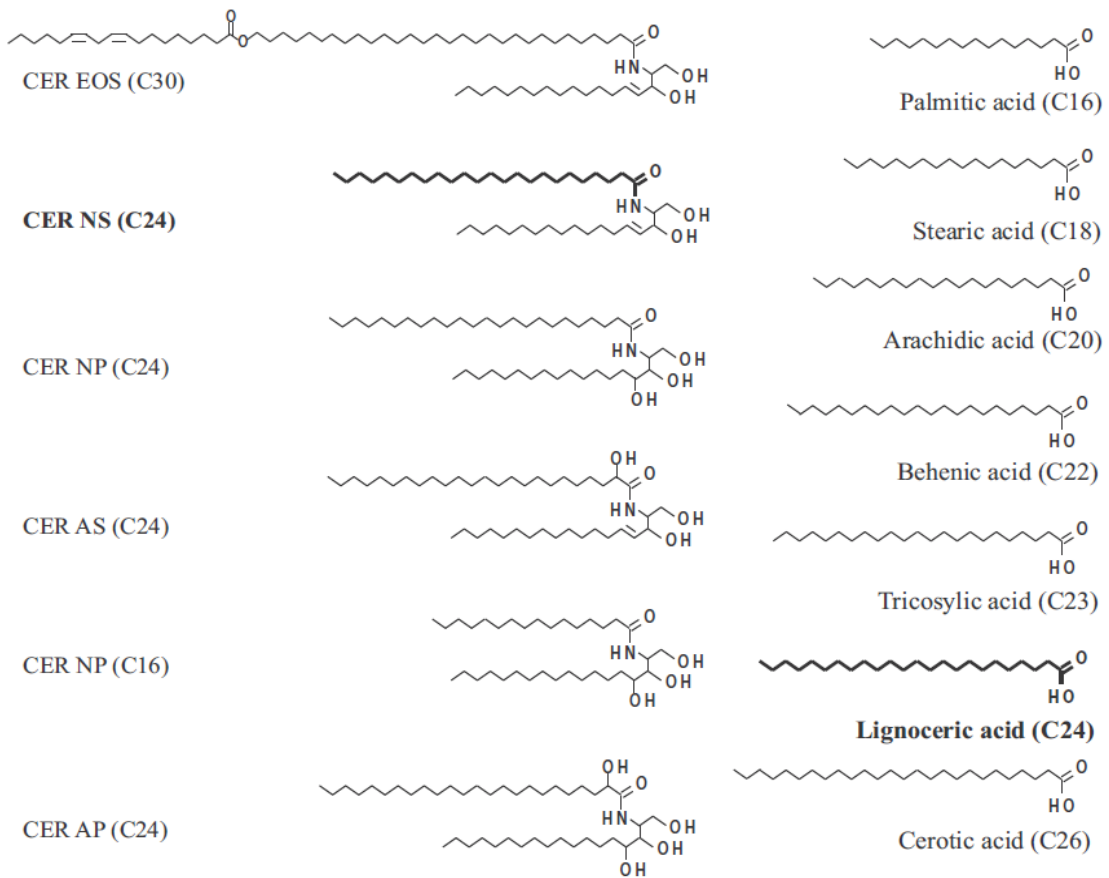


Primary skin barrier

Relevant to topical drug delivery

Compromised in psoriasis, eczema, atopic dermatitis, rosacea

STRATUM CORNEUM



Lipid lamellae comprising mixtures of ceramides, fatty acids and cholesterol

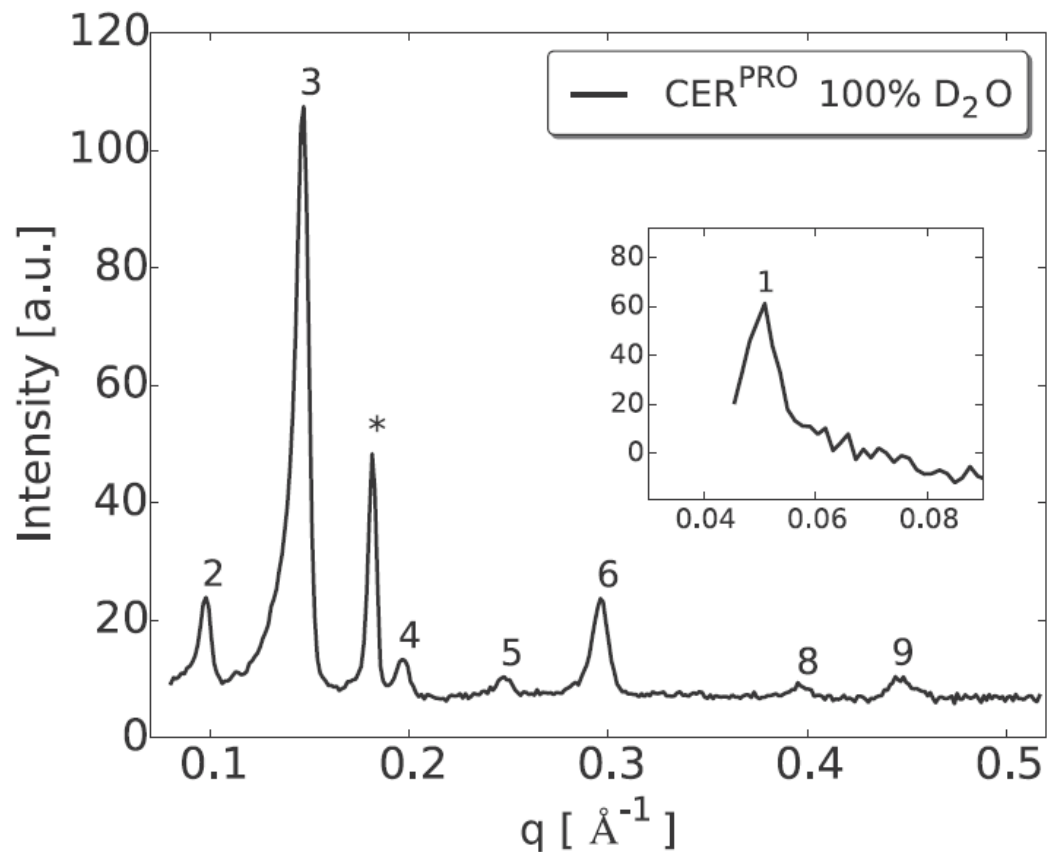
STRATUM CORNEUM

Description	Synthetic Lipids	Molar Ratio (%)
CER name and chain length	CER EOS (C30)	13.3
	CER NS (C24)	12
	CER NP (C24)	3.7
	CER AS (C24)	1
	CER NP (C16)	2
	CER AP (C24)	1.3
	CERmix	Total 33.3
CHOL	CHOL (C27)	33.3
	palmitic acid (C16)	0.6
	stearic acid (C18)	1.3
	arachidic acid (C20)	2.6
	behenic acid (C22)	14.2
FFA name and chain length	tricosylic acid (C23)	1.7
	lignoceric acid (C24)	11.5
	cerotic acid (C26)	1.4
	FFA	Total 33.3
	Total	CER:CHOL:FFA

Highly complex multicomponent system – not amenable to study by XRD

Nor by reflectivity studies of bilayers or monolayers

STRATUM CORNEUM

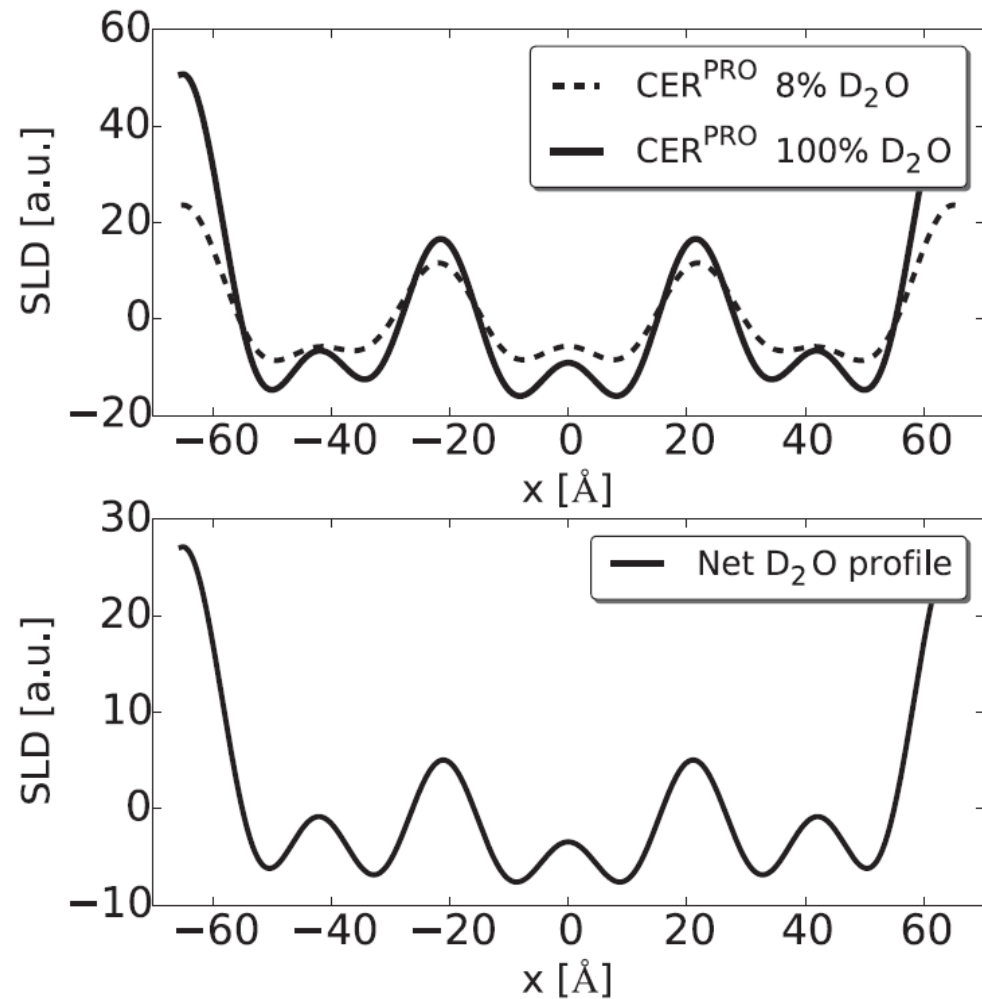


Lamellar Neutron Diffraction (D16)

Highly ordered system with nine orders of reflection (+ cholesterol peak^{*})

Mojumdar *et al* (2015) *Biophys J* **108**:2670.

STRATUM CORNEUM



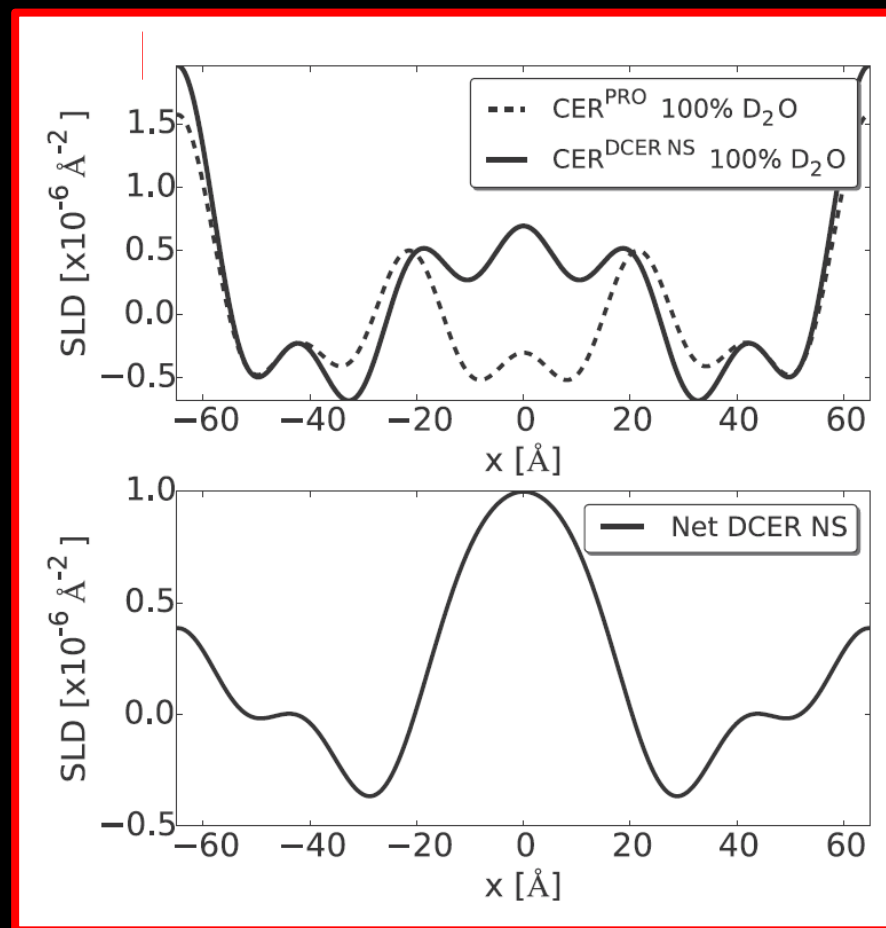
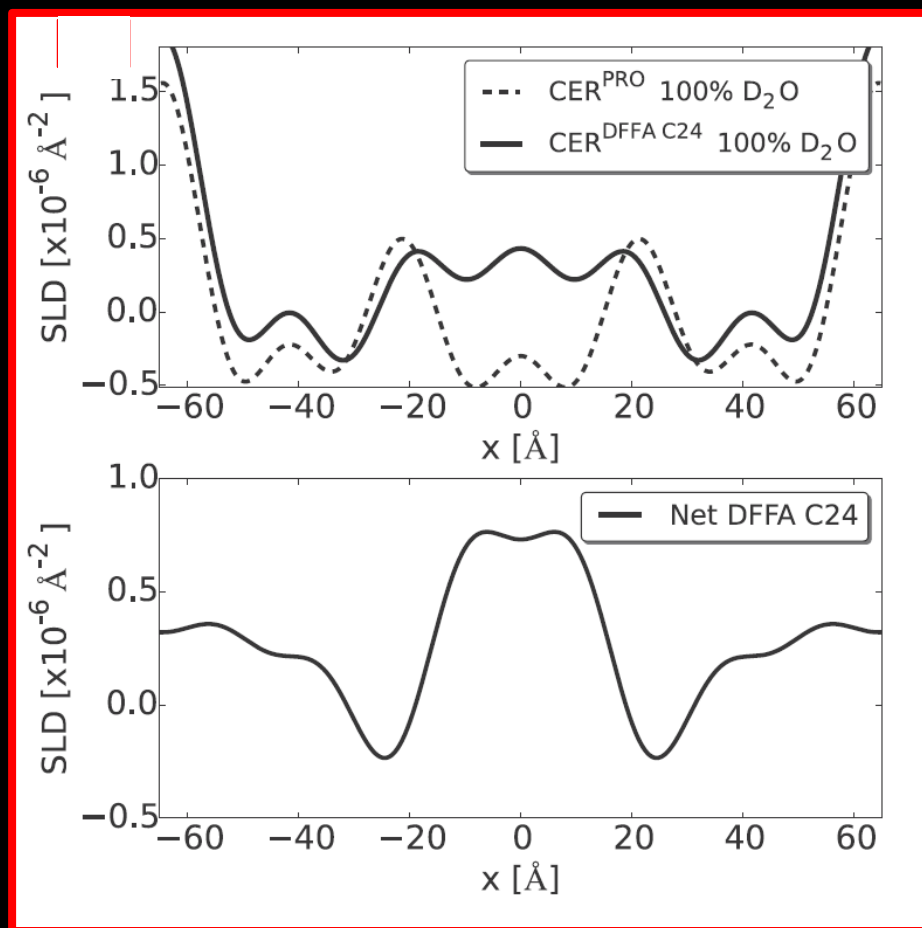
Relative SLD profiles of the CER^{PRO} mixture hydrated and measured at 8% and 100% D₂O (in 100% RH)

Difference profile showing the water SLD.

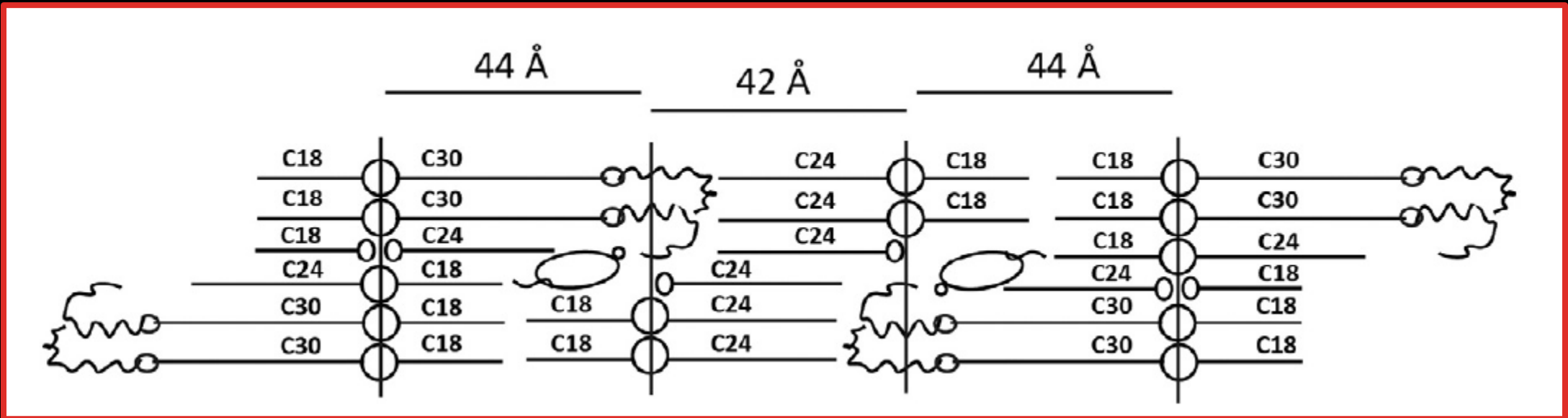
The 4 head group regions seen here clearly indicate a 3-layer arrangement in the LPP.

STRATUM CORNEUM

CER^{PRO} and C24 FFA at centre of the unit cell

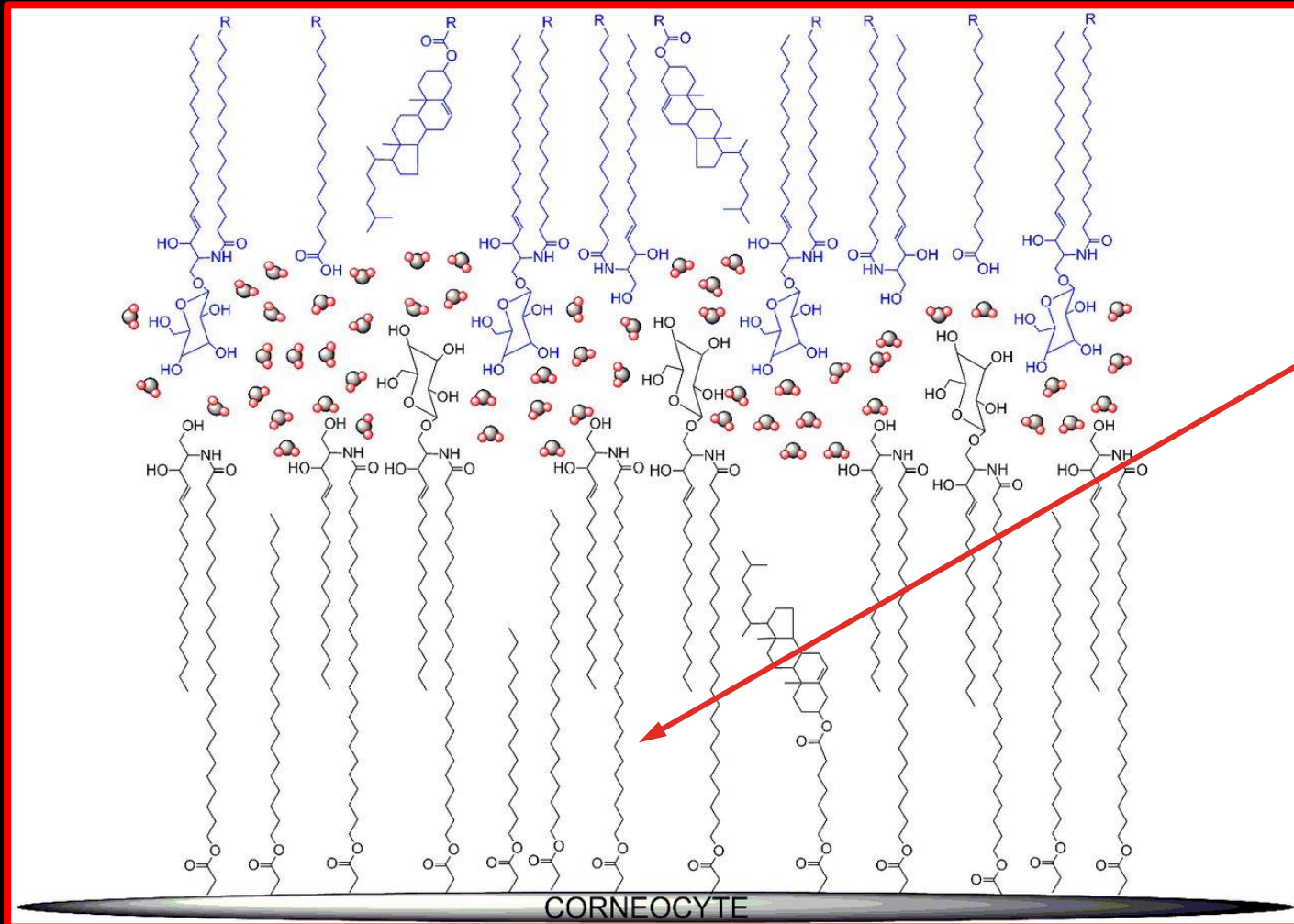


STRATUM CORNEUM



Proposed structure of the Long Periodicity Phase

STRATUM CORNEUM



Corneocyte lipid envelope

Ceramides & FFA anchored to cell membrane – serves to organise the lipid lamellae

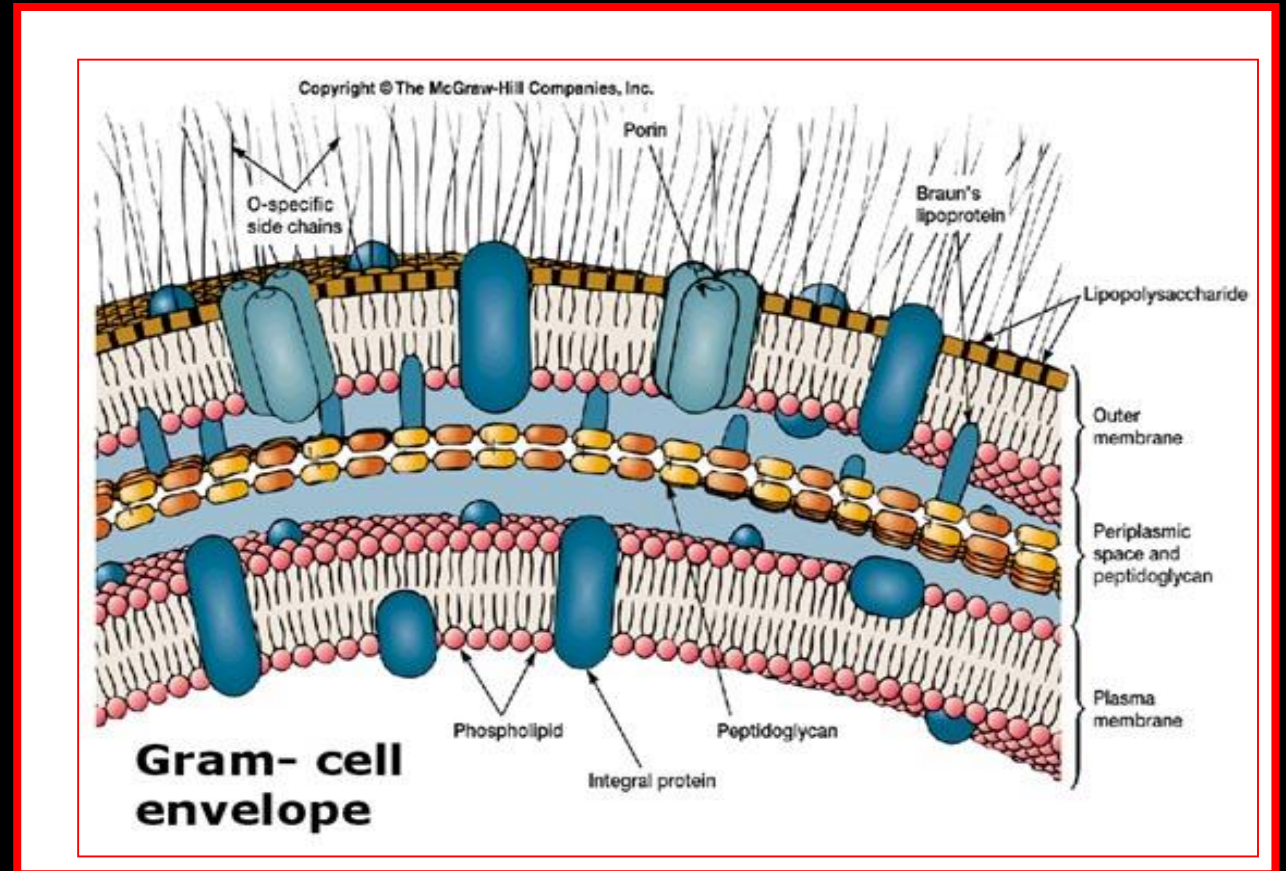
21ST CENTURY MEMBRANE RESEARCH

Current studies use a myriad of **indirect** physical methods ...

... but frequently yield **equivocal** and sometimes entirely **contradictory** results

Establish an **international centre of excellence** for membrane structural biology at ISIS ...

... expanding its current portfolio to include **lamellar diffraction**, and providing for production of **exotic deuterated lipids**, with **expanded bio-lab facilities** for work on pathogenic materials



ACKNOWLEDGEMENTS



Science & Technology Facilities Council

ISIS



GlaxoSmithKline



EPSRC

Engineering and Physical Sciences
Research Council



THANK YOU FOR LISTENING!