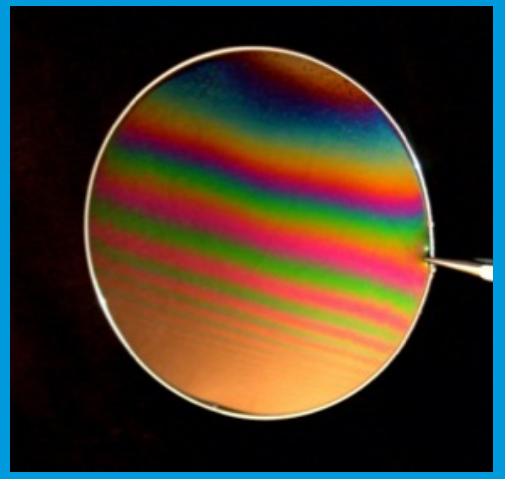
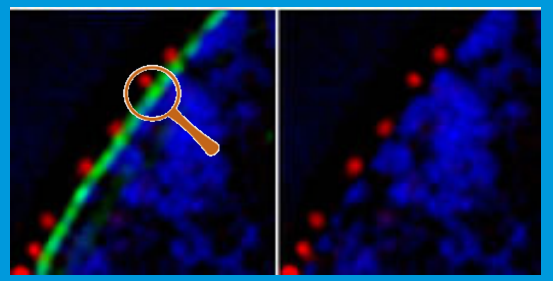
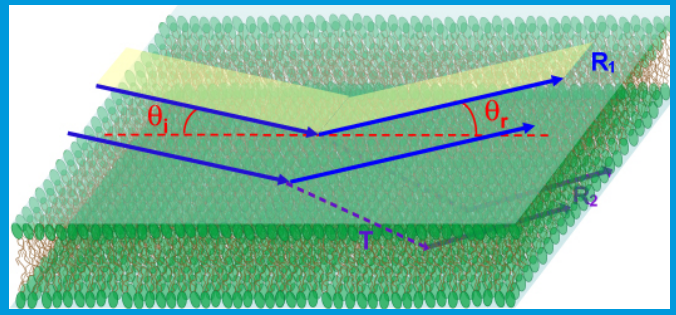
Membrane deuteration and internal contrast





Examples of applications in NR
(incl. lipolytic enzymes!)



HANNA WACKLIN-KNECHT, ESS DEMAX PLATFORM

2021-06-17

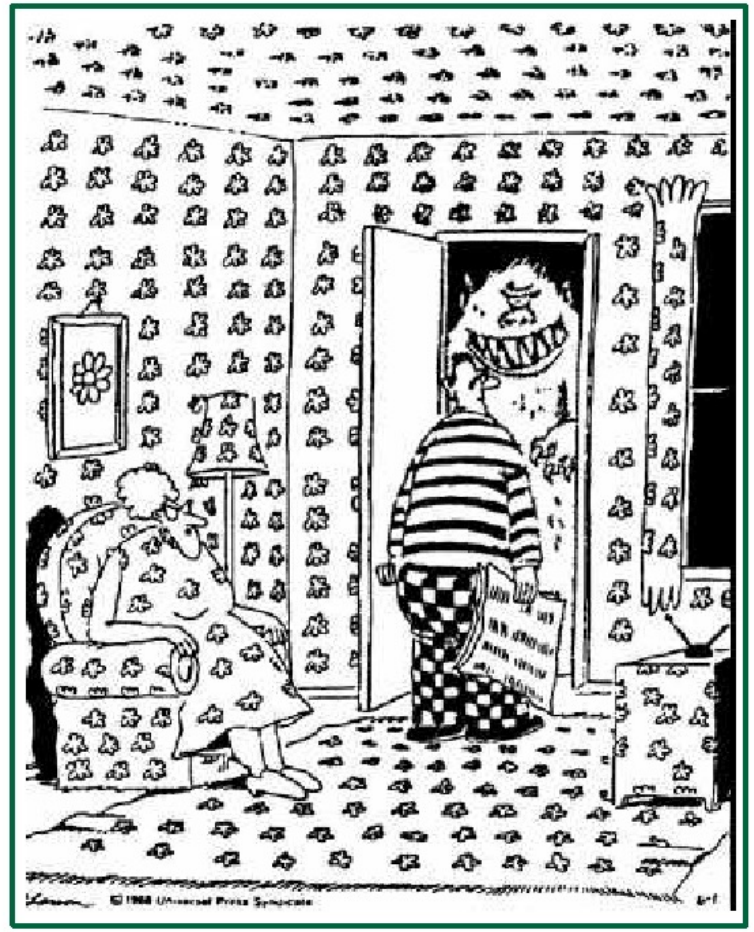
We will look today at:

1. Contrast variation in membrane samples – why and how?
2. Deuteration of membrane lipids
3. Data analysis considerations
4. Example 1: Phospholipase A_2 enzymes
5. Example 2: Lipid asymmetry
6. Example 3: Sterol extraction by Amphotericin B

2

Contrast variation – why and how?

$$\text{Contrast} = (\rho_{\text{object}} - \rho_{\text{environment}})^2$$



When the monster came,
Lola remained undetected.

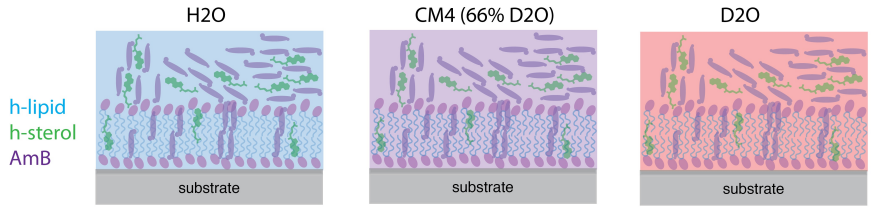
Harold, of course, was immediately devoured.

1 Contrast variation in membranes

Solvent contrast, membrane deuteration or magnetic contrast?

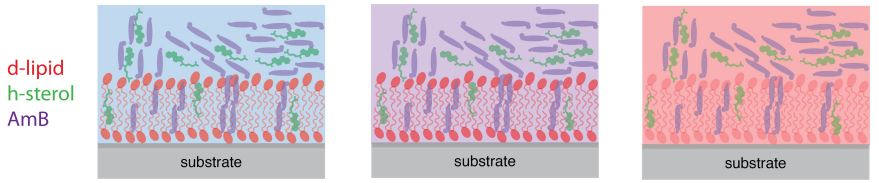
A) Solvent contrast variation:

Same sample measured in solvents with a different degree of deuteration (0-100% D_2O) -> simultaneous fit to structure in principle solves phase problem and allows SLD profile to be determined. *-> overall membrane structures, solvent content*



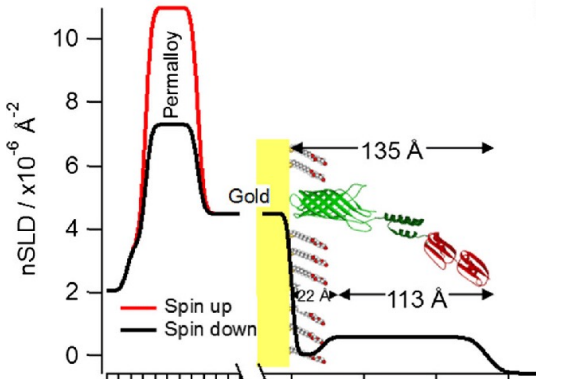
B) Membrane deuteration:

Multicomponent membranes -> selective deuteration allows composition and component distribution to be determined (e.g. two different lipids, or a protein and lipids) *-> combine with A)*



C) Magnetic contrast and polarised neutron reflection:

Permalloy layer has different SLD for up/down spins-> two different measurement contrasts – longer measurement times, but no contrast matching possible *–> combine with A) and or B).*



A.P. Le Brun, A. Soliakov, D.S.H. Shah, et al., Biomedical Microdevices 17(3) (2015) 49.

2 Lipid deuteration

Biological, enzyme catalysed and synthetic chemistry

Organic synthesis of lipids

Pure molecular species in mg-g quantities, partial or per-deuteration, contrast matching ->complex synthesis routes

PC, (PG), tri-, di-, monoglycerides
Fatty acids: saturated + C18:1c9

Enzyme-catalysed synthesis of lipids

Lipases and phospholipases shorten lipid synthesis routes due to regio- and enantiospecificity –> immobilisation increases yield

PC, PE

Biological deuteration of lipids in cell cultures

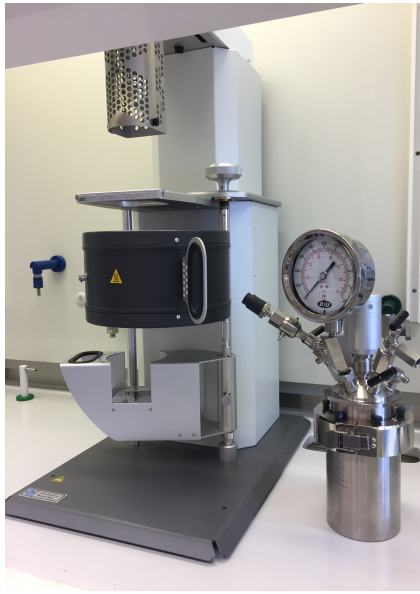
Microorganisms such as yeast, bacteria, algae can grow in deuterated media -> 10-500mg biological lipid mixtures with native fatty acyl chain distribution in per-deuterated, partially deuterated or contrast matched forms. Separation of components by chromatography.

Yeast, E.Coli

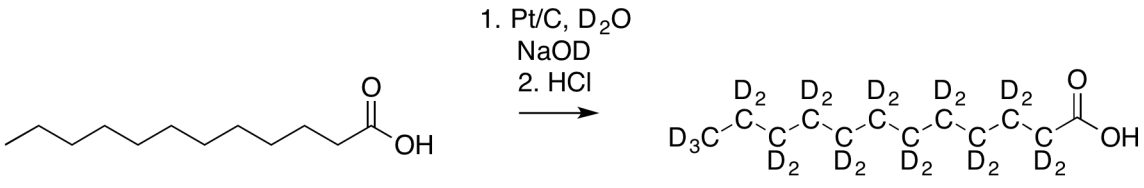
Phospholipids: PC, PE, PS, PG, PI, CL
Sterols: Ergosterol, cholesterol (squalene, lanosterol)
Glycerolipids: TG, DG, MG

2 Lipid deuteration

Organic synthesis of lipids



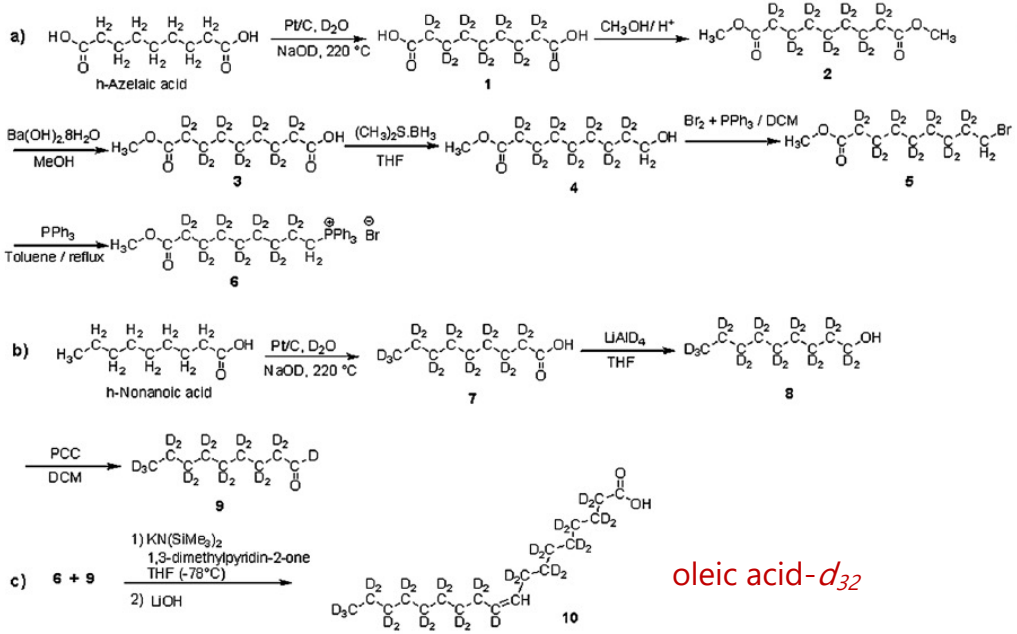
Perdeuteration of saturated fatty acids by hydrothermal H/D exchange:



lauric acid

lauric acid-d_{23}

Deuteration of un-saturated fatty acids from saturated precursors:



oleic acid-d_{32}

Special Issue Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 22 May 2013, Revised 12 June 2013, Accepted 12 June 2013 Published online in 16 July 2013 Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3088

Synthesis of deuterated [D_{32}]oleic acid and its phospholipid derivative [D_{64}]dioleoyl-*sn*-glycero-3-phosphocholine†‡

Tamim A. Darwish,[a]* Emily Luks,[a] Greta Moraes,[a] Nageshwar R. Yepuri,[a] Peter J. Holden,[a] and Michael James[a,b,c]

Biologically deuterated oleic acid-d_{33} commercially available from Merck since 2019 ~3k€ per gram.

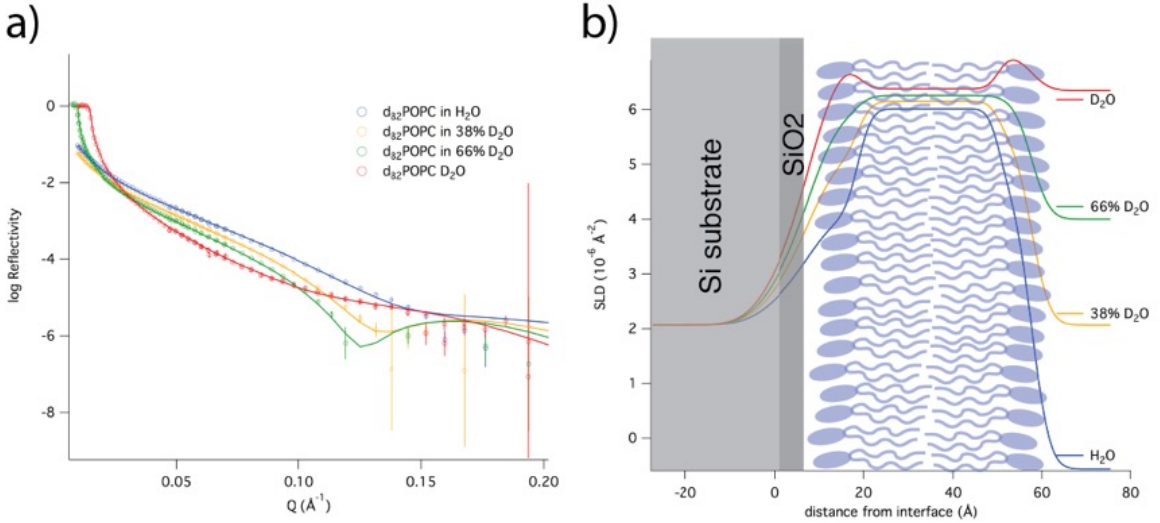
2 Lipid deuteration

Organic synthesis of PC lipids

ChemPubSoc Europe
DOI: 10.1002/cplu.201500452
CHEMPLUSCHEM Full Papers

Synthesis of Perdeuterated 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine ([D_{82}]POPC) and Characterisation of Its Lipid Bilayer Membrane Structure by Neutron Reflectometry

Nageshwar R. Yepuri,[a] Tamim A. Darwish,*[a] Anwen M. Krause-Heuer,[a] Anna E. Leung,[a] Robin Delhom,[b, c] Hanna P. Wacklin,*[b, d] and Peter J. Holden[a]



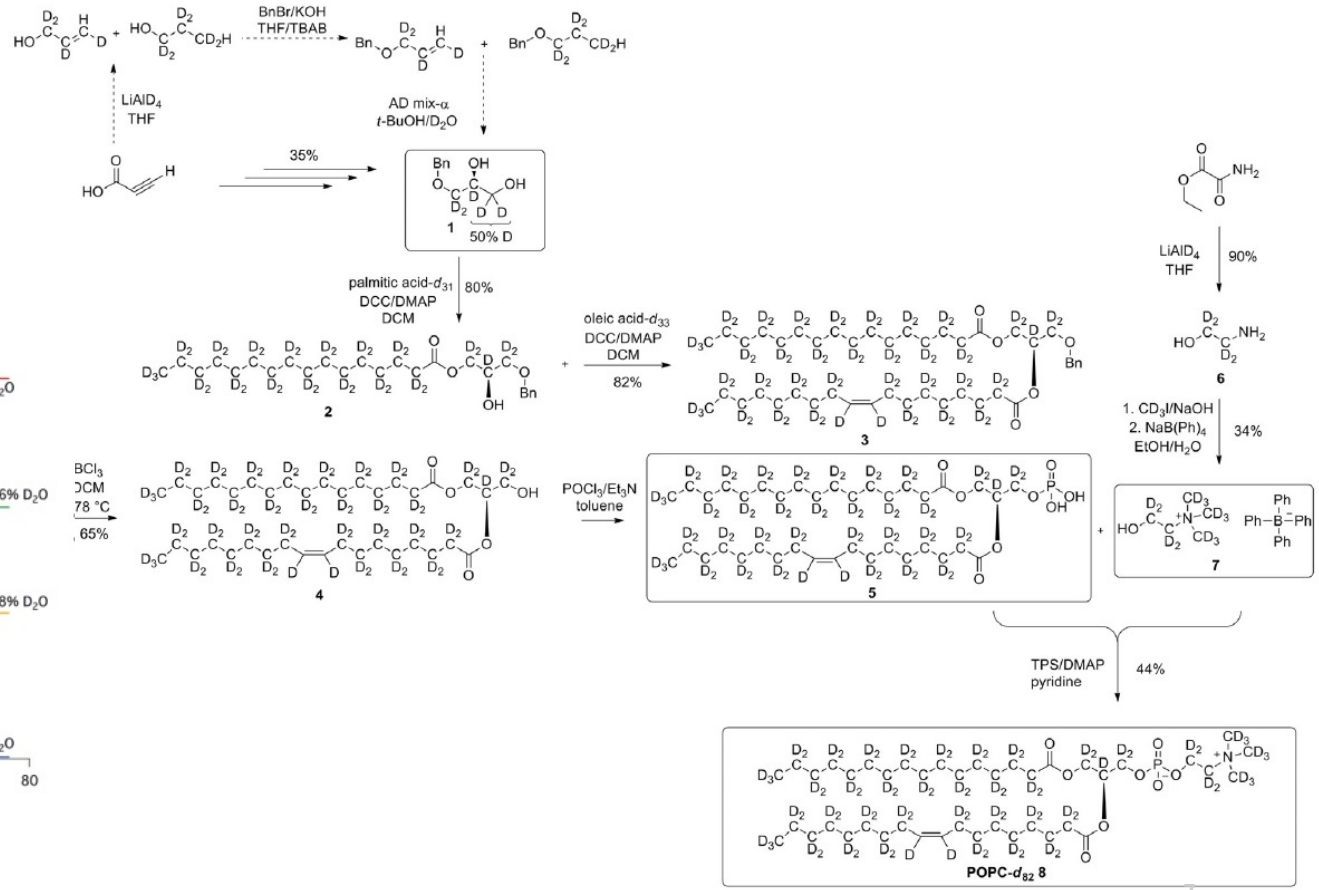
ANSTO – NDF/Platypus

nanomaterials
MDPI
Article

The Antifungal Mechanism of Amphotericin B Elucidated in Ergosterol and Cholesterol-Containing Membranes Using Neutron Reflectometry

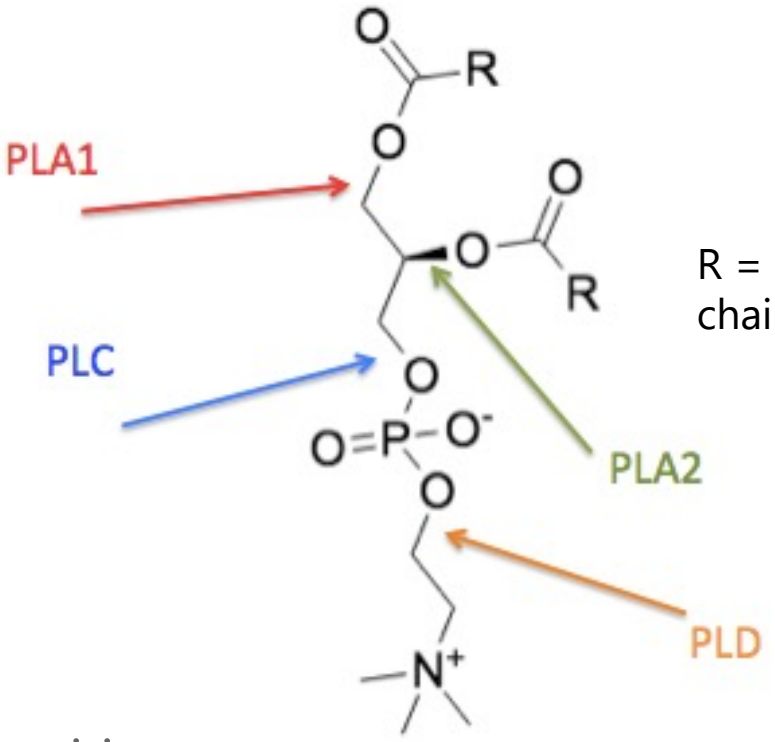
Robin Delhom [1,2,3], Andrew Nelson [4], Valerie Laux [1], Michael Haertlein [1], Wolfgang Knecht [3,5], Giovanna Fragneto [1] and Hanna P. Wacklin-Knecht [2,6,*]

Nanomaterials 2020, 10, 2439 doi:10.3390/nano10122439



2 Lipid deuteration

Enzymes for lipid synthesis



Enzymatic Synthesis

- CLEAN and GREEN – few by products/no toxic chemicals
- Highly specific – shortens reactions/purifications
- Immobilised enzymes can be reused

Application to lipid deuteration:

- different lipolytic enzymes attack selectively in different positions
- Can be used to swap d-fatty acids h-fatty acids

Commercial lipolytic enzymes available:

- Lipases (1,3 specific), PLA_2

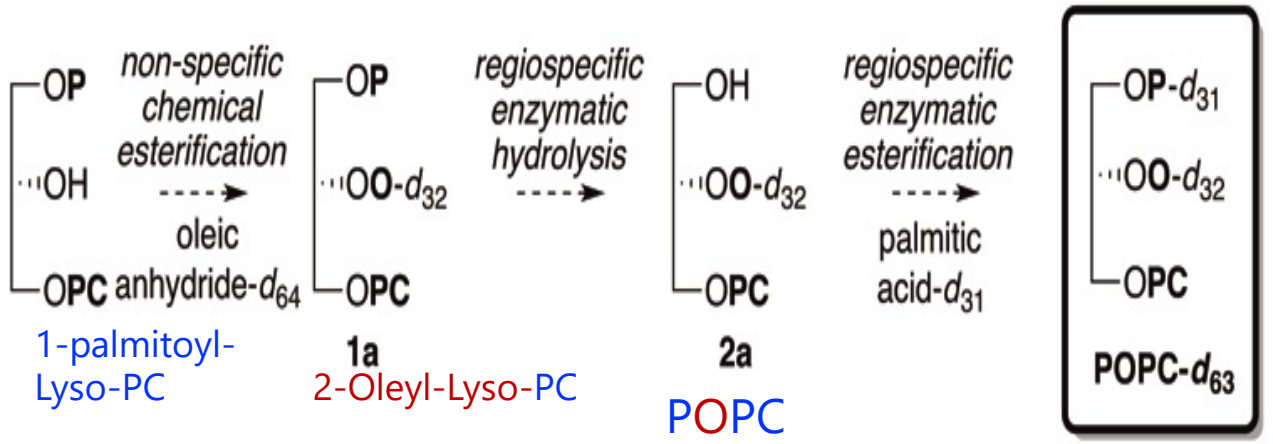
This project received funding from the European Union's Horizon 2020 programme under grant agreements No 654000 & 823867

2 Lipid deuteration

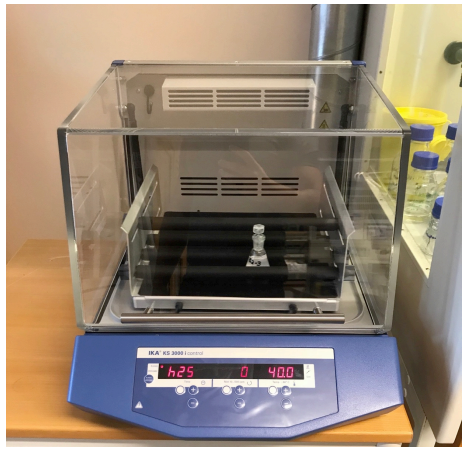
Enzymes for lipid synthesis

Practical considerations:

- Acyl-migration
- Enzyme & water activity
- Solubility & Mixing
- Reaction monitoring
- Enzyme immobilisation



P = palmitoyl; **O** = oleoyl; **PC** = phosphocholine. Chirality is not shown but the starting lipid is enantiopure and conservation of enantiopurity is expected throughout the synthesis.



http://pubs.acs.org/journal/acsodf Article

Enzyme-Assisted Synthesis of High-Purity, Chain-Deuterated 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine

Oliver Bogojevic and Anna E. Leung*

Cite This: https://dx.doi.org/10.1021/acsomega.0c02823 Read Online

This project received funding from the European Union's Horizon 2020 programme under grant agreements No 654000 & 823867

2 Lipid deuteration

Biological lipid deuteration – P. Pastoris yeast

- *Pichia Pastoris* yeast (used for protein production) grows well in minimal D_2O-media with glycerol-d_8 as carbon source
- Growth either in Shaker flasks or bioreactors yields 100-1000mg per-deuterated lipids
- *P. pastoris* produces a significant amount of C18:2 and C18:3 lipids (Δ12 and Δ15 desaturate enzymes)
- Deuterated *P. pastoris* produces the same phospholipid composition but fewer polyunsaturated lipids and less ergosterol
- Difference in PUFA chains reduced at lower growth T in D_2O
- Growth rate/Kinetic isotope effect behind difference.

OPEN ACCESS Freely available online PLOS ONE

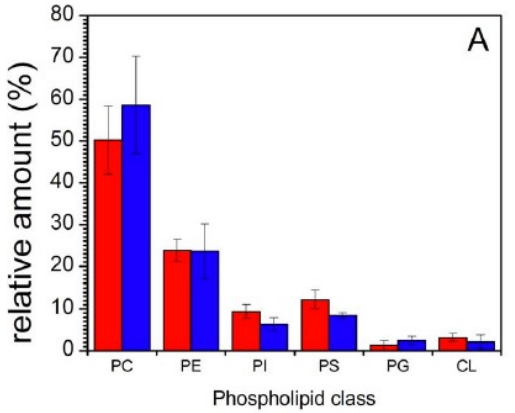
Production and Analysis of Perdeuterated Lipids from *Pichia pastoris* Cells

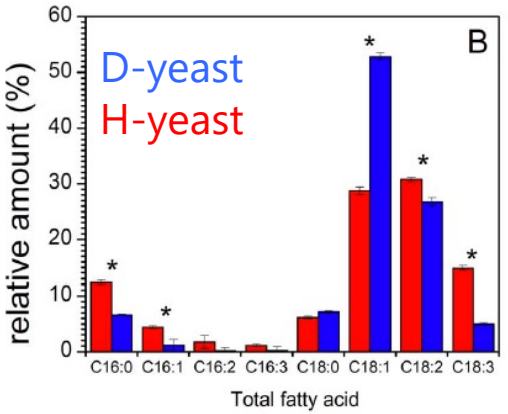
Alexis de Ghellinck[1,2], Hubert Schaller[3], Valérie Laux[1], Michael Haertlein[1], Michele Sferrazza[2], Eric Maréchal[4], Hanna Wacklin[5,6], Juliette Jouhet[4]*, Giovanna Fragneto[1]

1 Institut Laue-Langevin, Grenoble, France, 2 Service des polymères, Université Libre de Bruxelles, Brussels, Belgium, 3 Institut de Biologie Moléculaire des Plantes du CNRS, Strasbourg, France, 4 Laboratoire de physiologie cellulaire et végétale, CNRS/CEA/Univ. Grenoble Alpes/INRA, Grenoble, France, 5 European Spallation Source ESS AB, Lund, Sweden, 6 Chemistry Department, University of Copenhagen, Copenhagen, Denmark

Abstract

Probing molecules using perdeuteration (i.e deuteration in which all hydrogen atoms are replaced by deuterium) is extremely useful in a wide range of biophysical techniques. In the case of lipids, the synthesis of the biologically relevant unsaturated perdeuterated lipids is challenging and not usually pursued. In this work, perdeuterated phospholipids and sterols from the yeast *Pichia pastoris* grown in deuterated medium are extracted and analyzed as derivatives by gas chromatography and mass spectrometry respectively. When yeast cells are grown in a deuterated environment, the phospholipid homeostasis is maintained but the fatty acid unsaturation level is modified while the ergosterol synthesis is not affected by the deuterated culture medium. Our results confirm that the production of well defined natural unsaturated perdeuterated lipids is possible and gives also new insights about the process of desaturase enzymes.





2 Lipid deuteration

Lipid extraction and purification – yeast

- *Phospolipids are not overexpressed by cells*

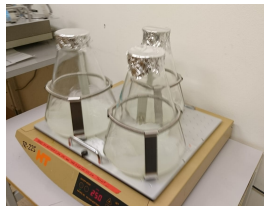
-> Large scale growth/extraction/purification

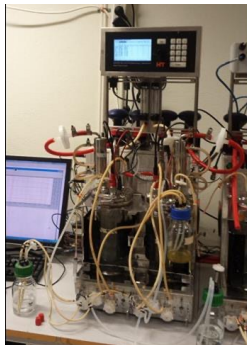
- Main cost glycerol-d_8
- Non-polar/phospholipid lipid separation
- *Growth conditions affect lipid composition*

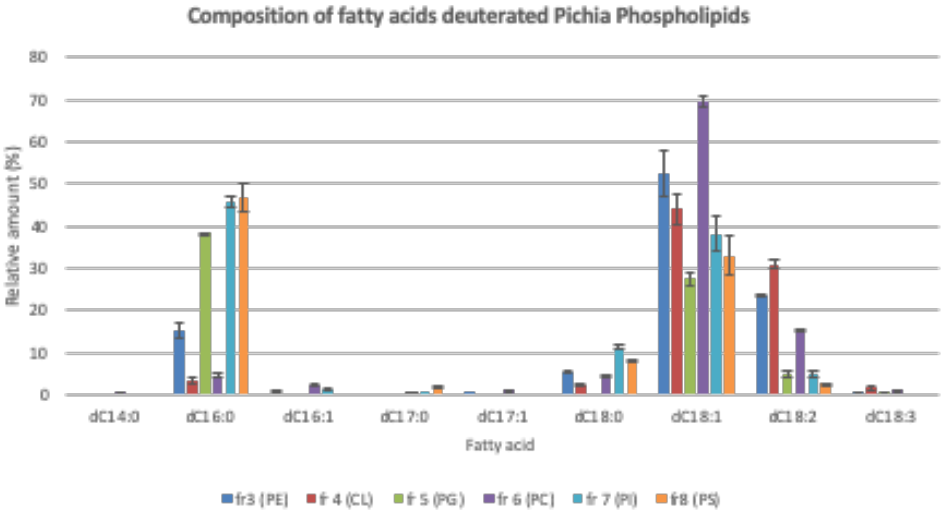
-> Analysis of composition (TLC, GC, MS)

- PC, CL and ergosterol/cholesterol* available purified from ESS and ILL.

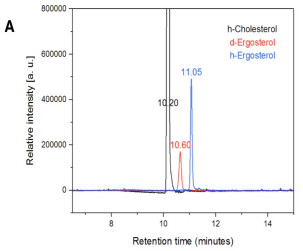
Shaker flasks
Fermentor/bioreactors
-> 100mg - 1 g of lipids



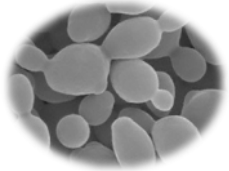


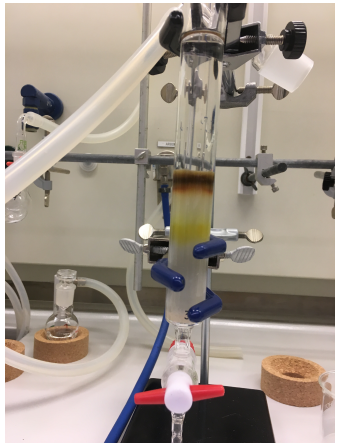


Extraction/separation

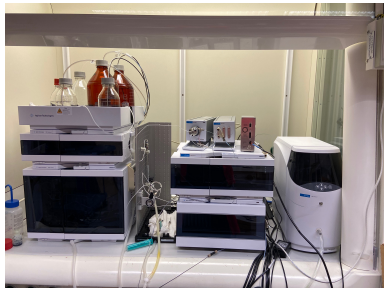


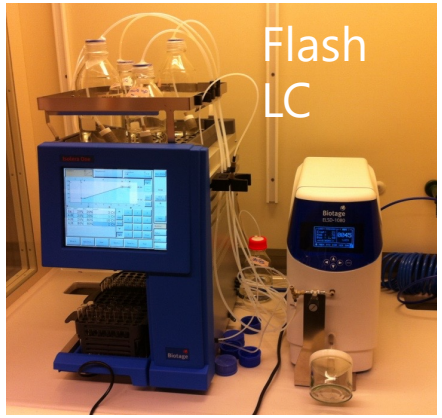
Pichia pastoris

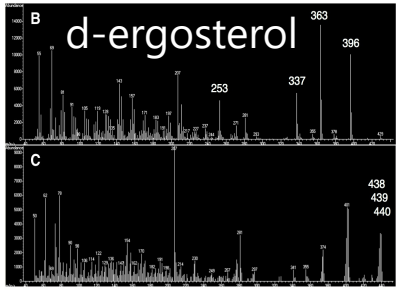


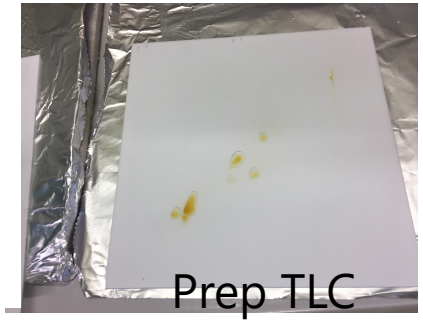


Prep HPLC









*M. Moulin, et al., Perdeuteration of cholesterol for neutron scattering applications using recombinant Pichia pastoris, Chemistry and Physics of Lipids (2018).

2 Lipid deuteration

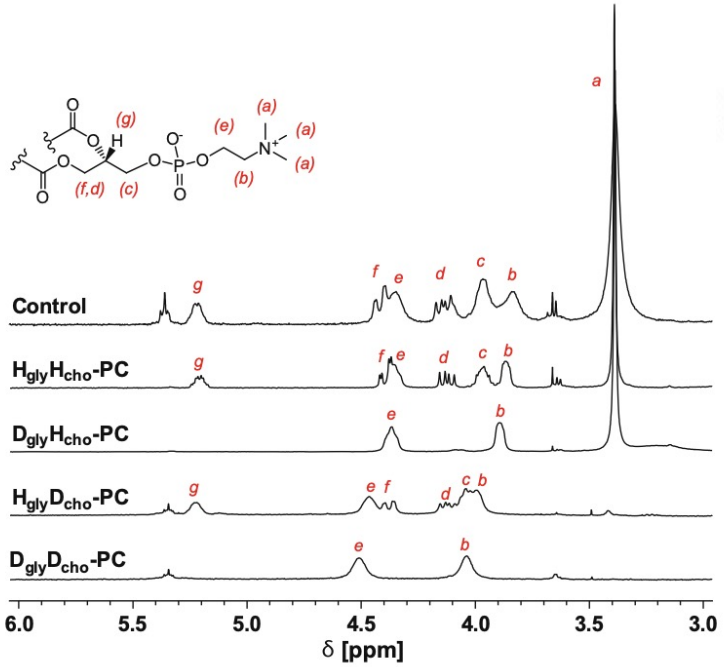
Biological lipid deuteration – E.Coli

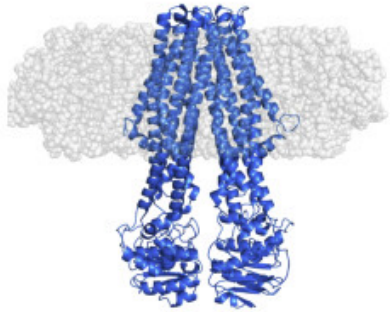
Appl Microbiol Biotechnol (2015) 99:241–254
DOI 10.1007/s00253-014-6082-z

BIOTECHNOLOGICAL PRODUCTS AND PROCESS ENGINEERING

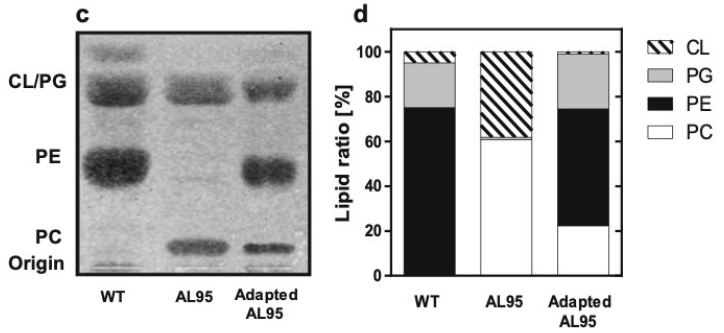
Biosynthetic preparation of selectively deuterated phosphatidylcholine in genetically modified *Escherichia coli*

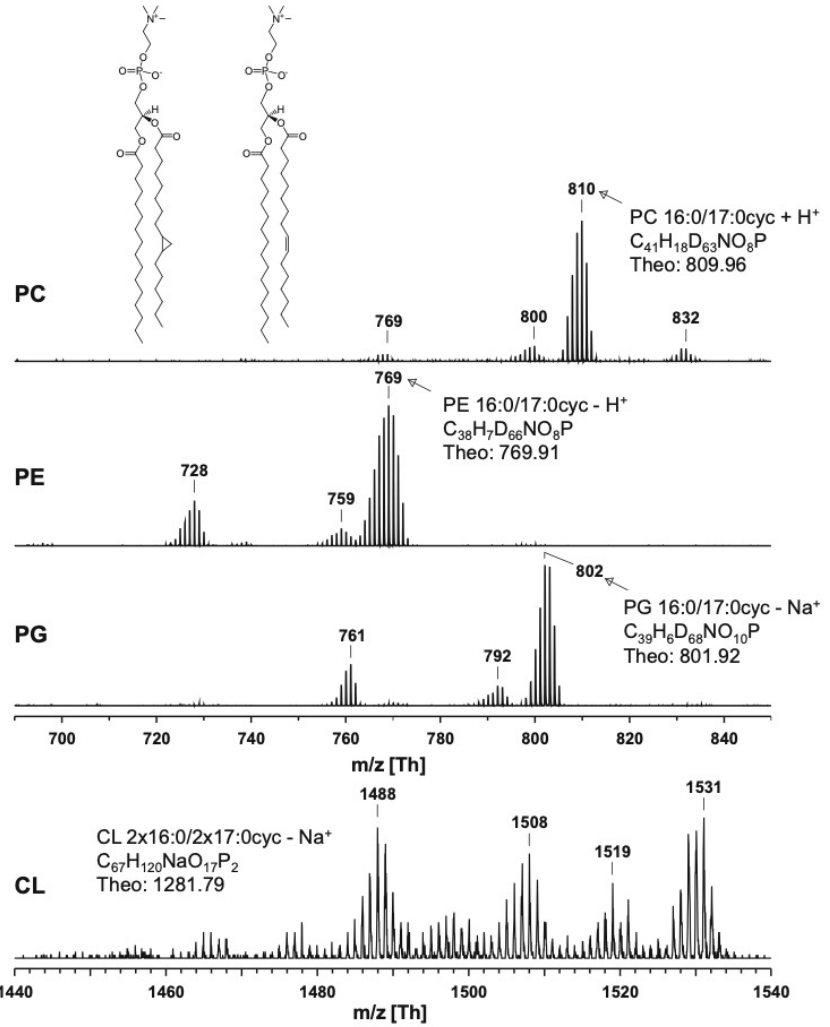
Selma Maric · Mikkel B. Thygesen · Jürgen Schiller · Magdalena Marek · Martine Moulin · Michael Haertlein · V. Trevor Forsyth · Mikhail Bogdanov · William Dowhan · Lise Arleth · Thomas Günther Pomorski





I. Josts, J. Nitsche, S. Maric, et al., Structure 26(8) (2018) 1072-1079.e4.

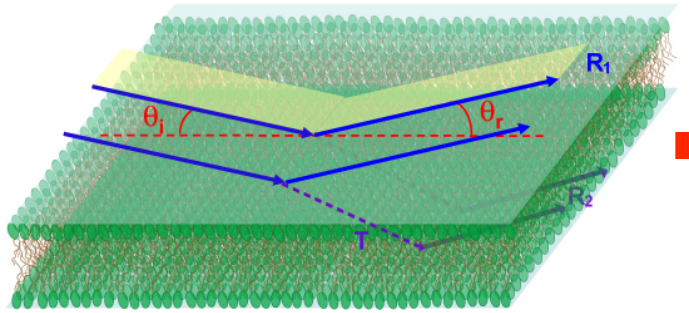


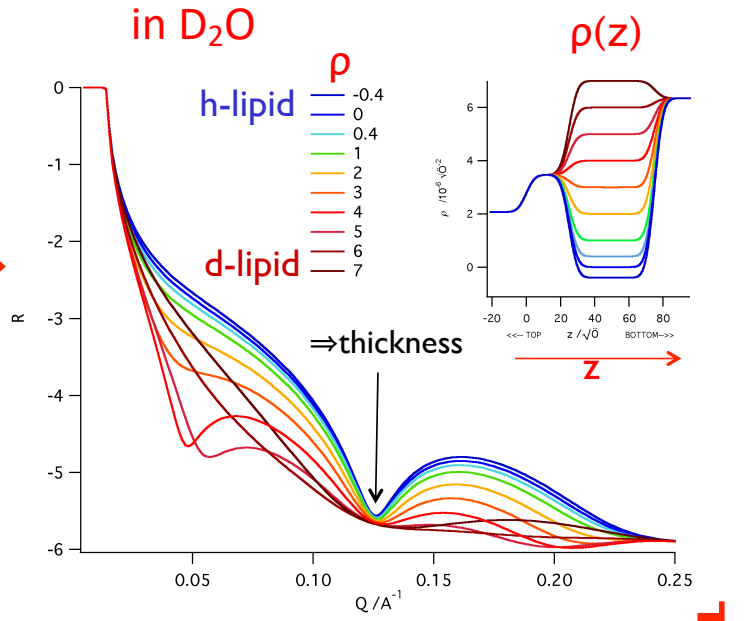


Deuteration in Neutron reflectometry

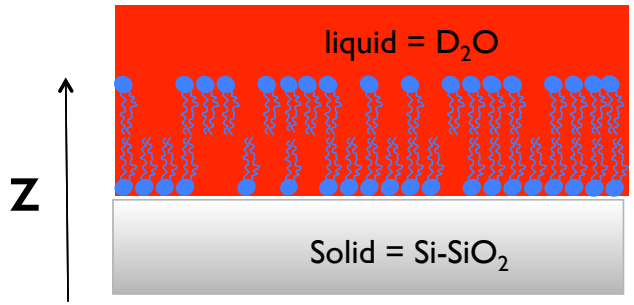
Selective deuteration of lipids to create contrast between components

Composition sensitivity ~ 3-5 Vol%

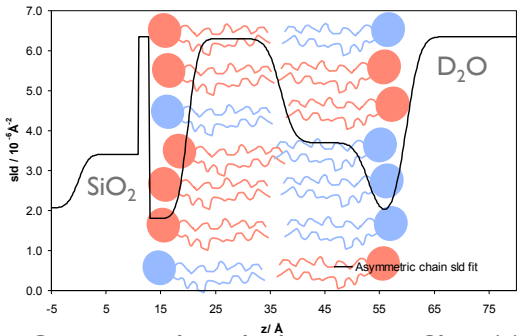




1-2Å resolution



Model structure of lipid membrane
= layered model of structure & composition

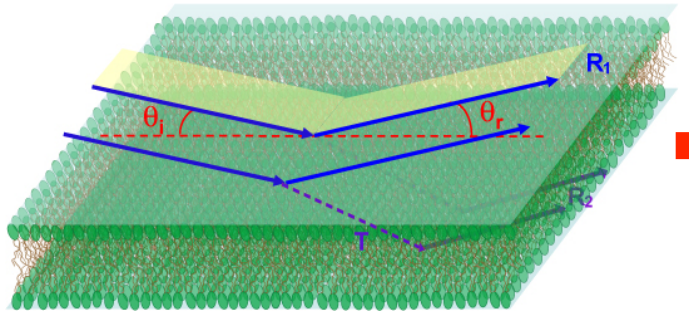


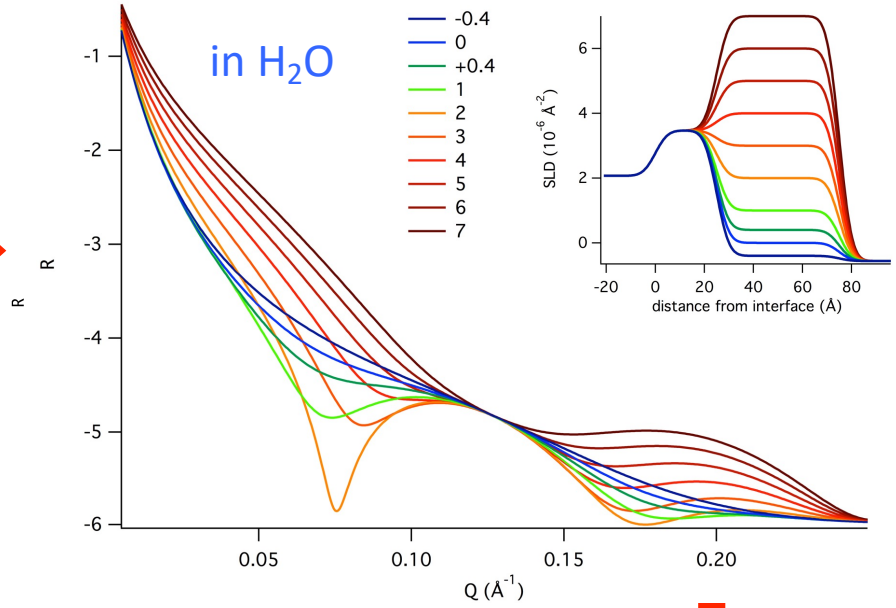
Scattering length density profile ρ(z)
= neutron refractive index profile

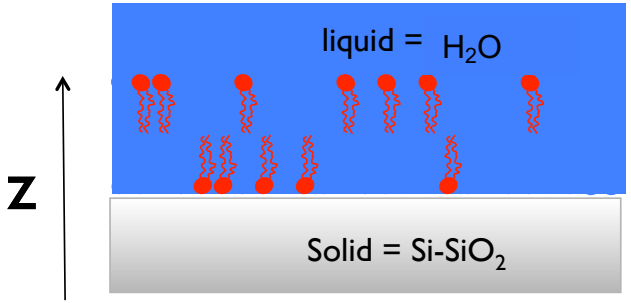
Deuteration in Neutron reflectometry

Solvent contrast variation to highlight/contrast match out components

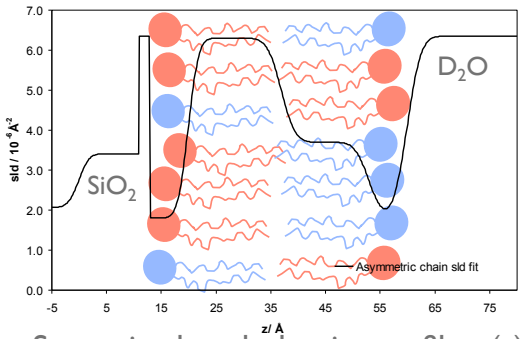
Contrast determines sensitivity







Model structure of lipid membrane
= layered model of structure & composition



Scattering length density profile ρ(z)
= neutron refractive index profile

3 Data analysis considerations

What do you need to know (A) and do (B) for NR analysis?

A) The expected SLD of your molecule(s)

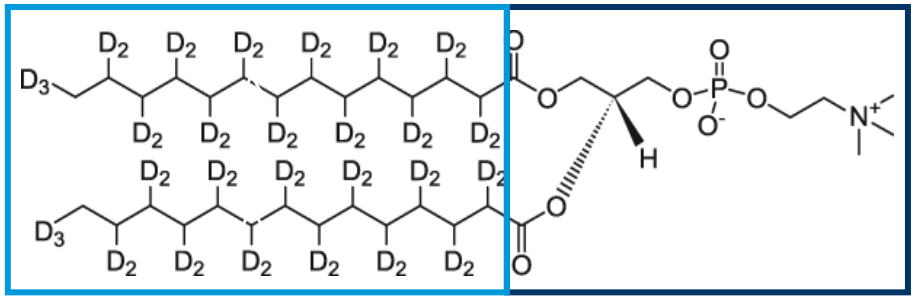
- Degree of deuteration
- Chemical composition
- Molecular structure
- Molecular volume or density
- Exchange of protons with solvent

$$SLD = \frac{sum\ of\ b_{coh}}{V_m}$$

B) Stoichiometry/geometry and interpretation

- Constant lipid area per molecule (heads/tails)
- Space-filling/geometry
- Analysis of experimental and fitting uncertainties

chains head



Armen, R.S., O.D. Uitto, and S.E. Feller, *Phospholipid component volumes: Determination and application to bilayer structure calculations.* Biophysical Journal, 1998. **75** 734-744.

Group	*V (DPPC)/ Å^3*	*V (DOPC)/ Å^3*	*V (POPC)/ Å^3*
CH_3	53.57±0.91	52.79±0.4	50.41±0.77
CH_2	27.93±0.1	28.13±0.09	28.24±0.17
C=C		45.91±0.69	42.1±2.31
COO Carbonyl	43.58±1.05	37.4±1.28	38.43±1.47
C_3H_5 Glycerol backbone	65.47±2.11	81.62±2.96	72.48±2.28
PO_4 Phosphate	66.36±2.95	51.05±6.31	52.12±2.23
$C_5H_{13}N$ Choline	107.62±2.49	129.68±5.25	120.68±2.98
H_2O	29.5±0.5	29.5±0.5	29.5±0.5
Total	1215.79±14.27	1322.19±20.52	1255.78±17.12
Experimental [7-10]	1232	1295	1267

[7] Costigan, S.C., P.J. Booth, and R.H. Templer, BBA-Biomembranes, 2000. **1468** 41-54.
[8] Wiener, M.C. and S.H. White, Biophysical Journal, 1992. **61** 434-447.
[9] Small, D.M., *The Physics and Chemistry of Lipids*. 1986, Plenum Press.
[10] Nagle, J.F. and M.C. Wiener, BBA, 1988. **942** 1-10

3 Data analysis considerations

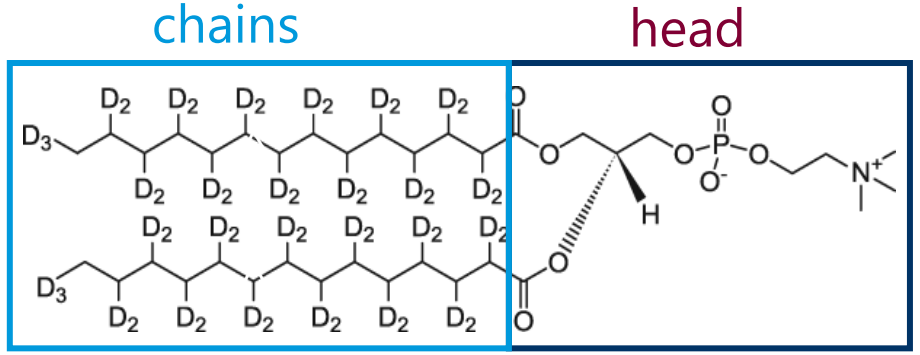
What do you need to know (A) and do (B) for NR analysis?

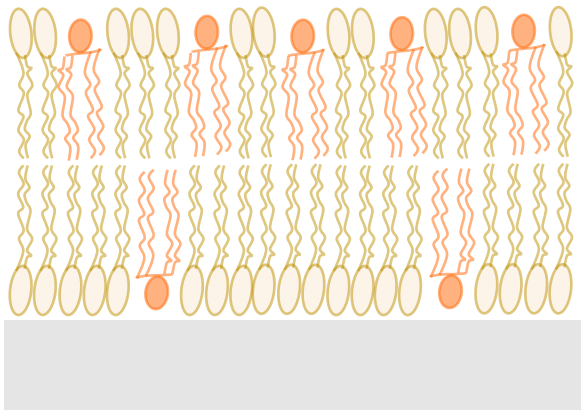
A) The expected SLD of your molecule(s)

- Degree of deuteration
- Chemical composition/purity
- Chemical formula
- Molecular volume or density
- Exchange of protons with solvent

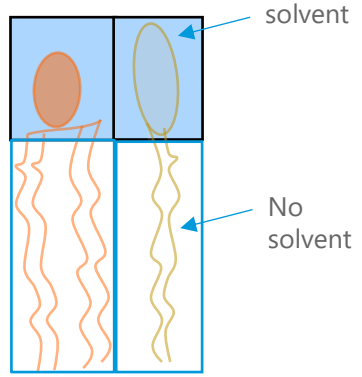
B) Stoichiometry/geometry and interpretation

- Constant lipid area per molecule (heads/tails)
- Space-filling/geometry
- Analysis of experimental and fitting uncertainties!!!





SLD rough thick vol%



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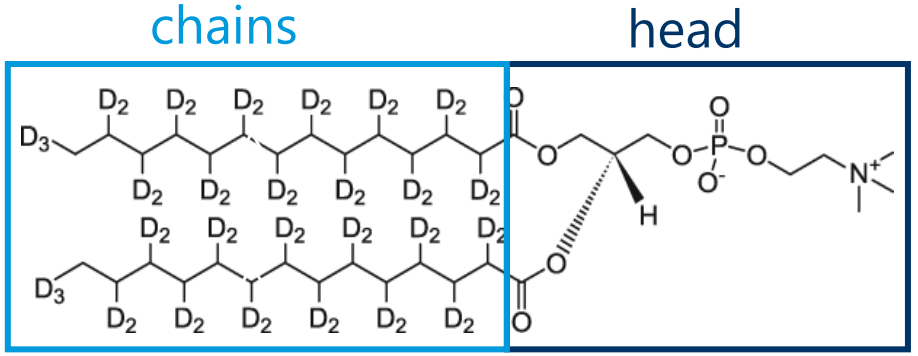
$$A = \frac{V_m}{\phi * t}$$



		SLD	rough	thick	vol%
	layer	ρ/Å^{-2}	σ/Å	t/ Å	ϕ
D2O	head	1.8±0.3	2±1	6±1	0.83±0.2
	chains	6.8±0.3	2±1	28±1	0.85±0.4
	head	1.8±0.3	2±1	6±1	0.83±0.2
	head	1.8±0.2	2±1	6±2	0.83±0.2
H2O	chains	6.8±0.05	2±1	28±2	0.85±0.05
	head	1.8±0.2	2±1	6±2	0.83±0.2

Global fit to both conrasts: All parameters are the same in both solvents (exept solvent SLD and background)

3 Data analysis considerations

What do you need to know (A) and do (B) for NR analysis?

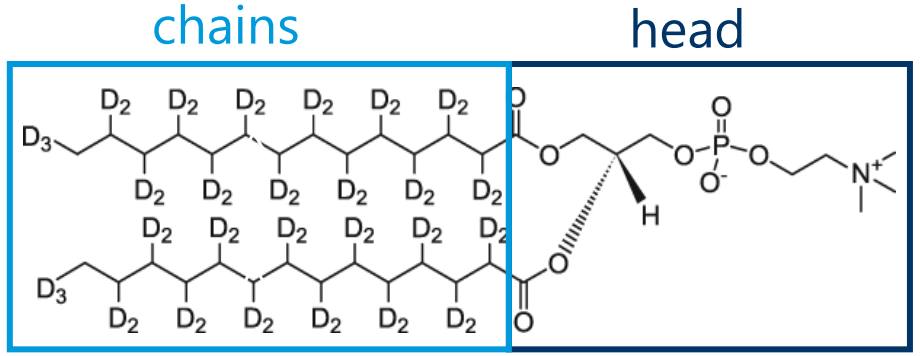
A) The expected SLD of your molecule(s)

- Degree of deuteration
- Chemical composition/purity
- Chemical formula
- Molecular volume or density
- Exchange of protons with solvent

B) Stoichiometry/geometry and interpretation

- Constant area per lipid molecule (heads/tails)
- Space-filling/geometry
- Analysis of experimental and fitting uncertainties
- Best contrast/smallest error for each parameter

$$A = \frac{V_m}{\phi * t}$$



	layer	V_m/ Å$_3$	t/ Å	ϕ	A/ Å^2
D2O	head	326	6±1	0.83±0.2	66±20
	chains	1555	28±1	0.85±0.4	65±15
	head	326	6±1	0.83±0.2	66±20
H2O	head	326	6±2	0.83±0.2	66±15
	chains	1555	28±2	0.85±0.05	65±5
	head	326	6±2	0.83±0.2	66±15

"Area per molecule": includes any solvent present and should be the same for heads and tails (not necessarily for both leaflets).

3 Data analysis considerations

SLD of yeast phospholipids from analysed lipid composition

Headgroups:

	Chemical formula in H_2O	Chemical formula in D_2O	Vm ($Å^3$)	relative amount (%)
h-PC	$C_{10}O_8H_{18}PN$	$C_{10}O_8H_{18}PN$	323	53.1
h-PE	$C_7O_8H_{12}PN$	$C_7O_8H_9D_3PN$	235	22.2
h-PS	$C_8O_{10}H_{11}P$	$C_7O_8H_8D_3P$	335	13.5
h-PI	$C_{11}O_{13}H_{16}P$	$C_{11}O_{13}H_{11}D_5P$	372	11.1
d-PC	$C_{10}O_8D_{18}PN$	$C_{10}O_8D_{18}PN$	323	48.2
d-PE	$C_7O_8D_9H_3PN$	$C_7O_8D_{12}PN$	235	20.1
d-PC	$C_7O_8D_8H_3P$	$C_8O_{10}D_{11}P$	335	17.6
d-PI	$C_{11}O_{13}D_{11}H_5P$	$C_{11}O_{13}D_{16}P$	372	14.0

Proton exchange of headgroups in different solvents:

CM4 = 66% D_2O (SLD 4.0), CmSi = 38% D_2O (SLD 2.07)

	D_2O	CM4	CMSi	H_2O
h-PC	1.86	1.86	1.86	1.86
h-PE	3.99	3.47	3.11	2.66
h-PI	3.25	3.02	2.85	2.64
h-PS	3.92	3.39	3.00	3.52
Averaged ρ				
	D2O	CM4	CMSi	H2O
	2.82	2.60	2.44	2.25

Chains:

Chains	***C16:0***	***C16:1***	***C16:2***	***C16:3***	***C18:0***	***C18:1***	***C18:2***	***C18:3***	
Volume	443,72	436,17	425,82	415,47	499,58	492,43	453,95	471,73	
% in h-lipids	12	5	2	1	7	29	31	16	
%in d-lipids	6	1	0,5	0,5	7	53	27	6	***averaged***
sld h ($x10^{-6}$)	-0,386	-0,201	-0,031	0,149	-0,359	-0,212	-0,66	0,096	-0.143
sld d ($x10^{-6}$)	7,36	6,72	6,57	6,41	6,94	6,77	7,045	6,496	6.61

Higher than POPC due to PUFAs

3 Data analysis considerations

Global fitting of NR data contrasts

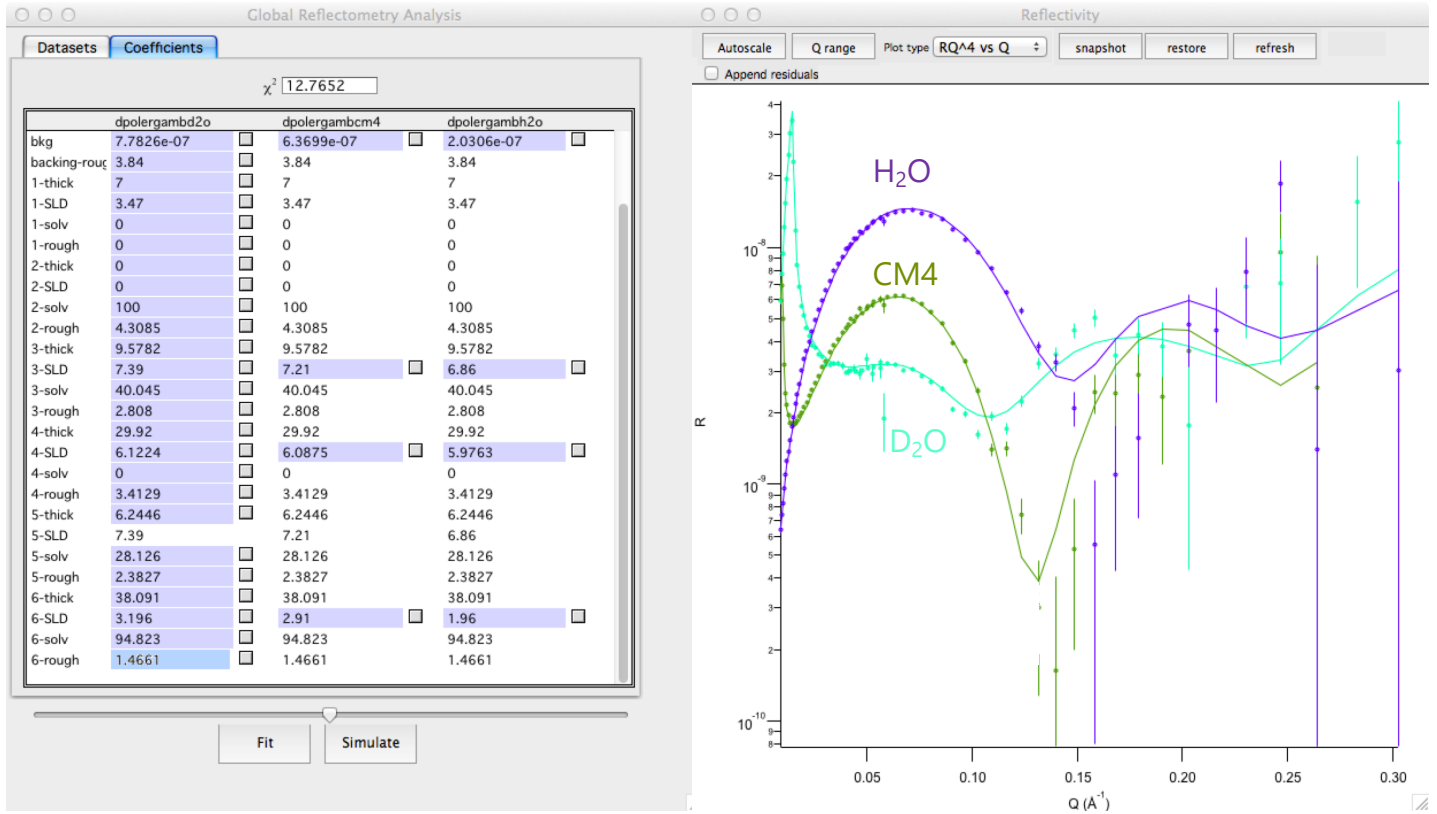
Fits are constrained so that only sld changes due to solvent contrast and other exchangeable protons are allowed.

Supported bilayers: for best resolution and confidence to bilayer structure, each SiO_2 substrate should be characterised clean and used as as reference in the fits (layer 1).

Deuterated yeast phospholipid bilayer model

Layers:

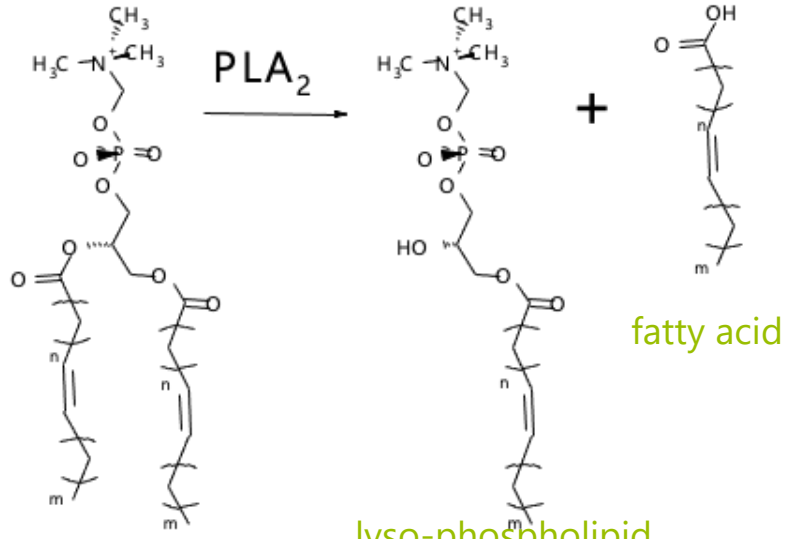
1: SiO_2
2: ~~solvent layer~~
3: lipid heads
4: lipid chains+AmB
5: lipid heads
6: AmB/dErg

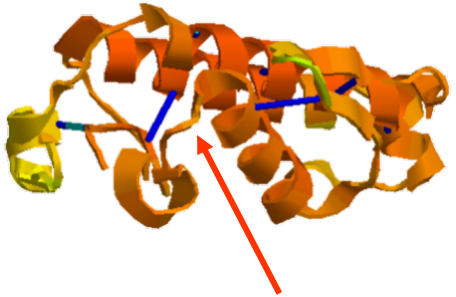


4 Examples 1: Phospholipase A_2

Understanding interfacial enzyme catalysis by selective phospholipid deuteration

PLA_2 breaks down phospholipids in digestion, inflammation, venoms, cell signalling etc.

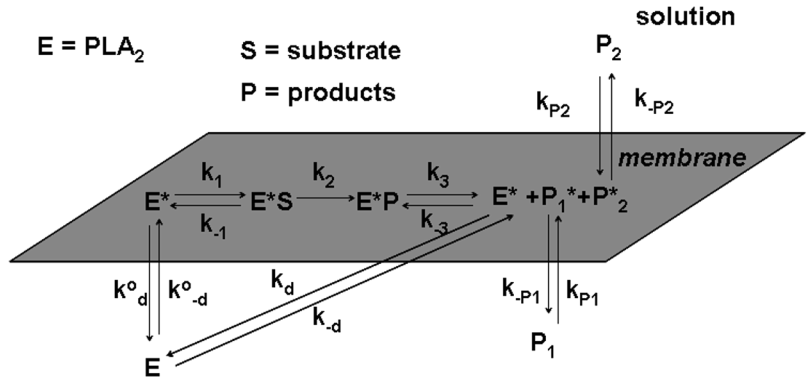




Naja Naja Naja (Cobra)

Mw	13219
Vmol/ Å^3	15451

Interfacial enzyme catalysis:



⇒ enzyme crystal structure is not enough to understand mechanism of PLA_2

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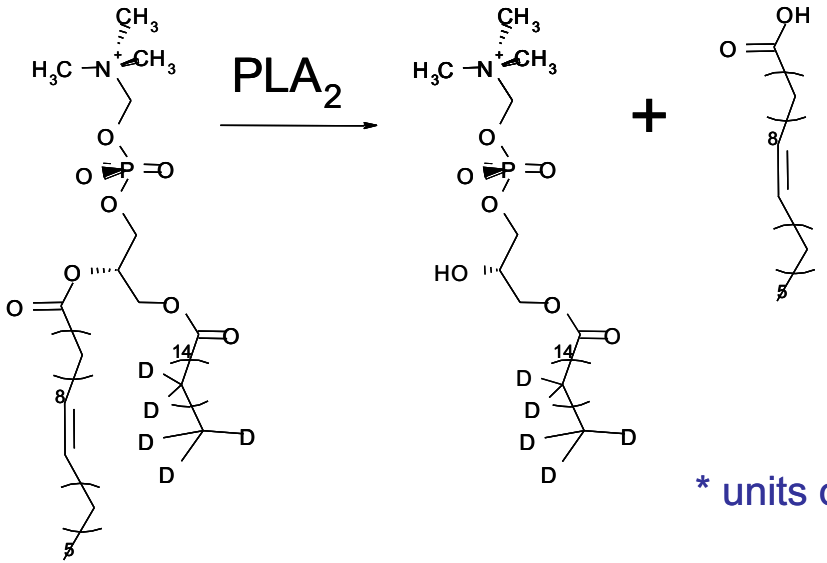
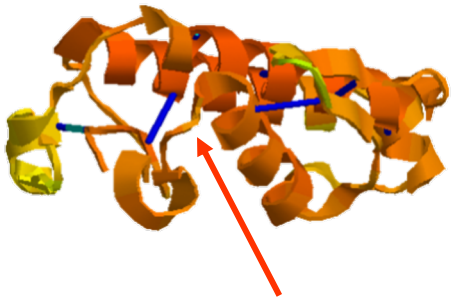
d_{31}-POPC ⇒ d_{31}-lyso-PC + oleic acid

ρ = 3.17 ρ = 6.44 ρ = - 0.2*



* units of 10^{-6} Å^{-2}

⇒ neutron reflection to reveal what happens to reaction products



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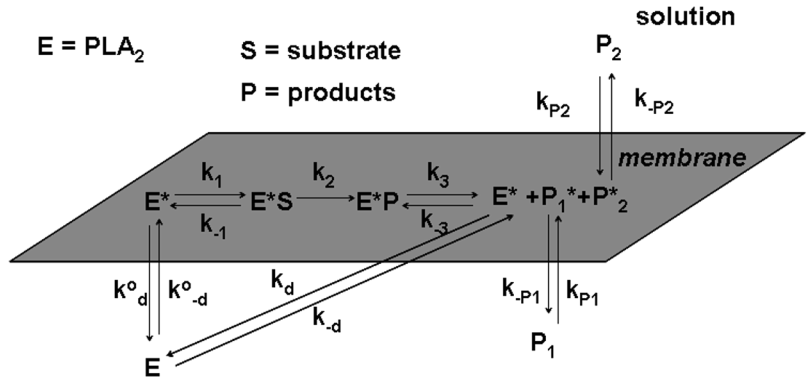

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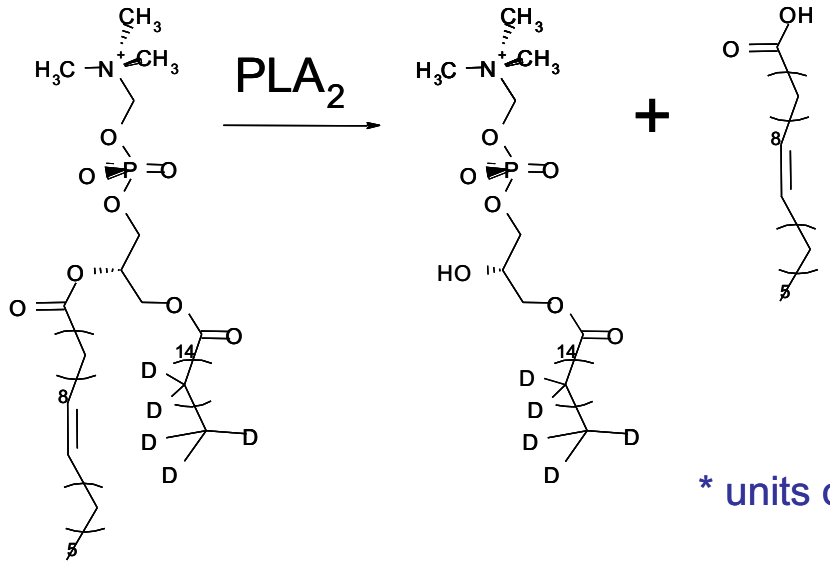

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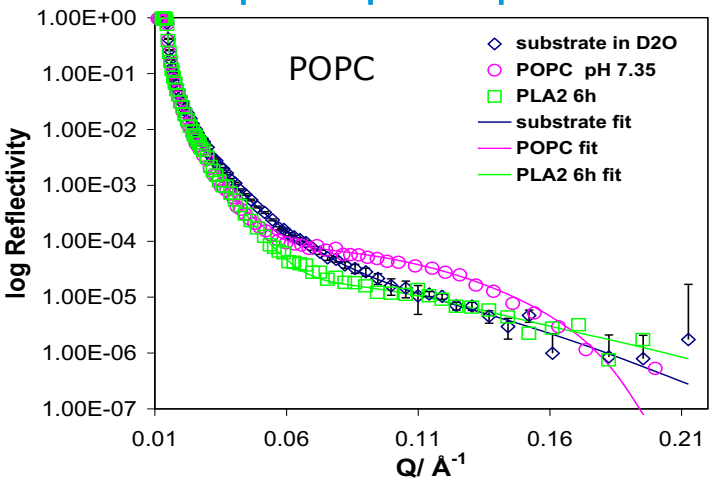
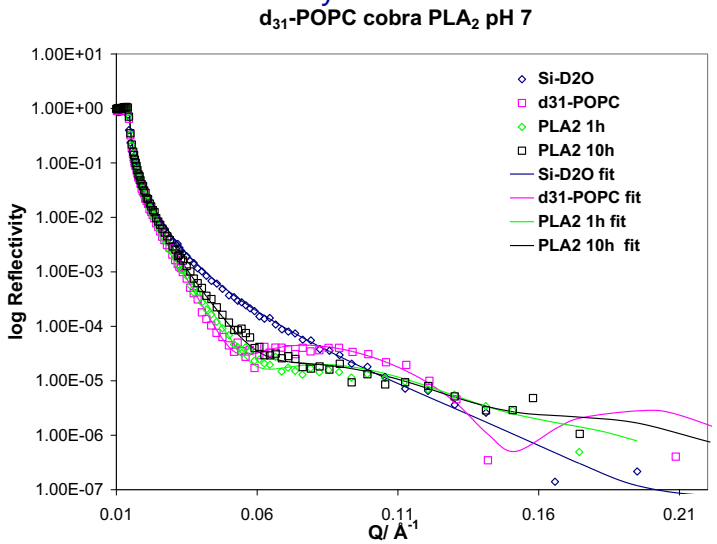


h-POPC in D_2O : reflectivity drops as ~50% of lipid membrane is destroyed



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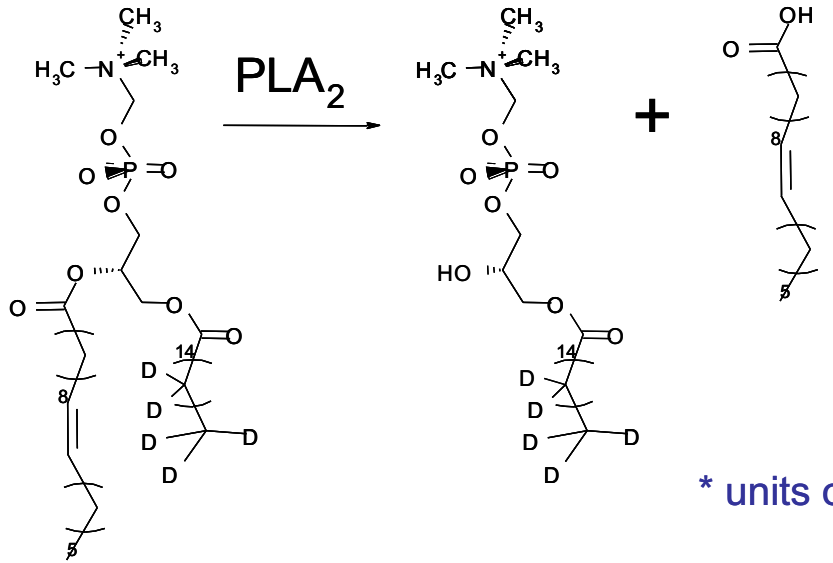

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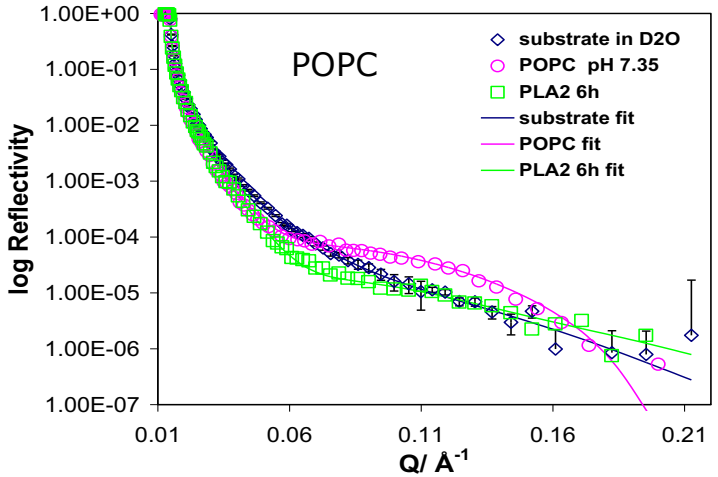

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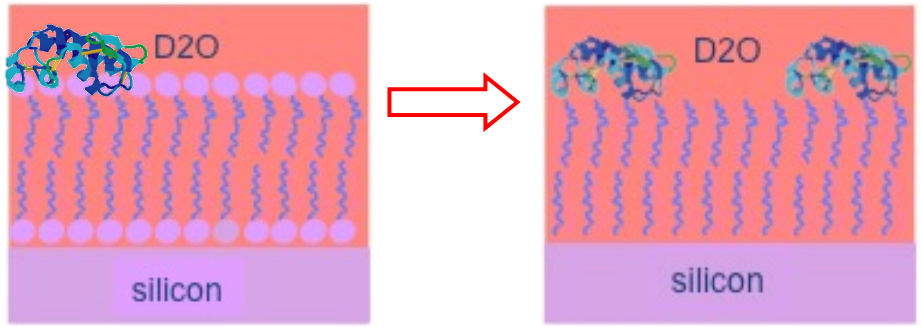

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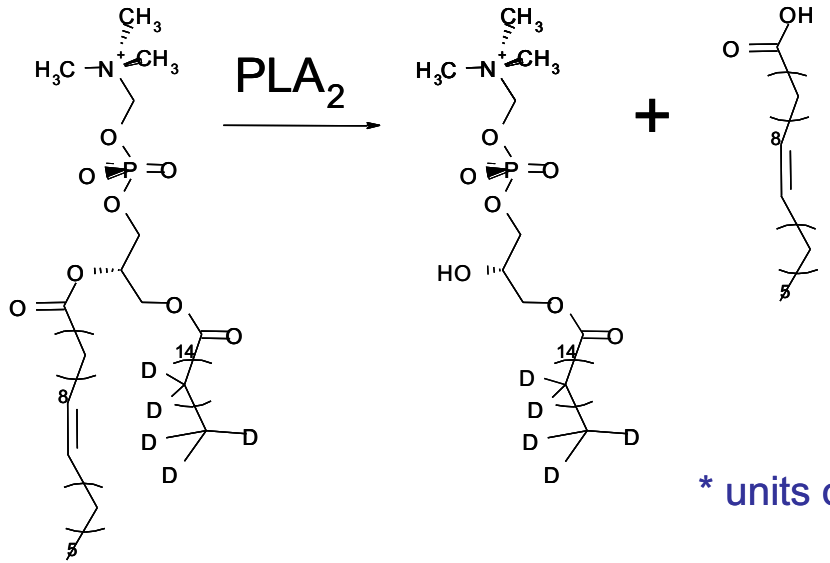

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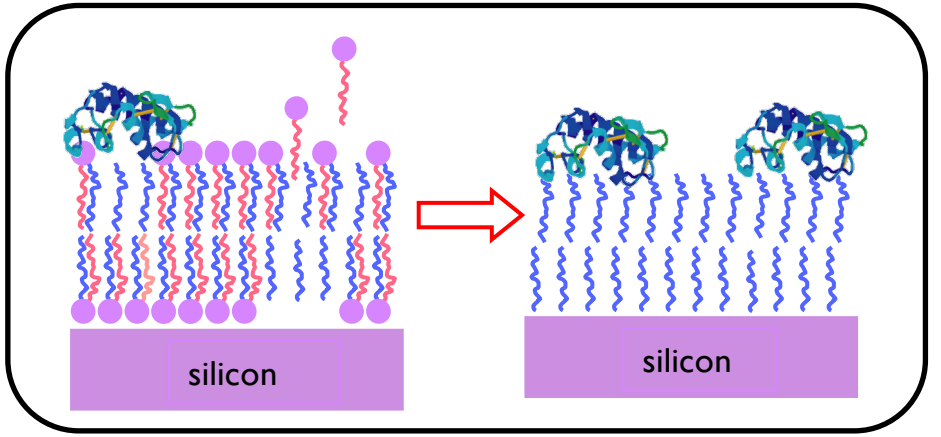

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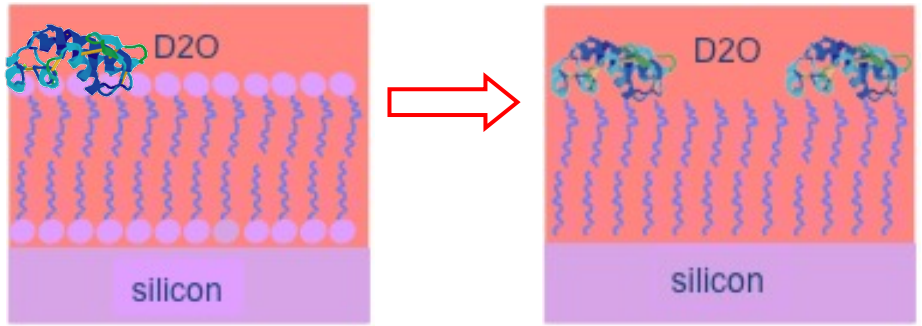

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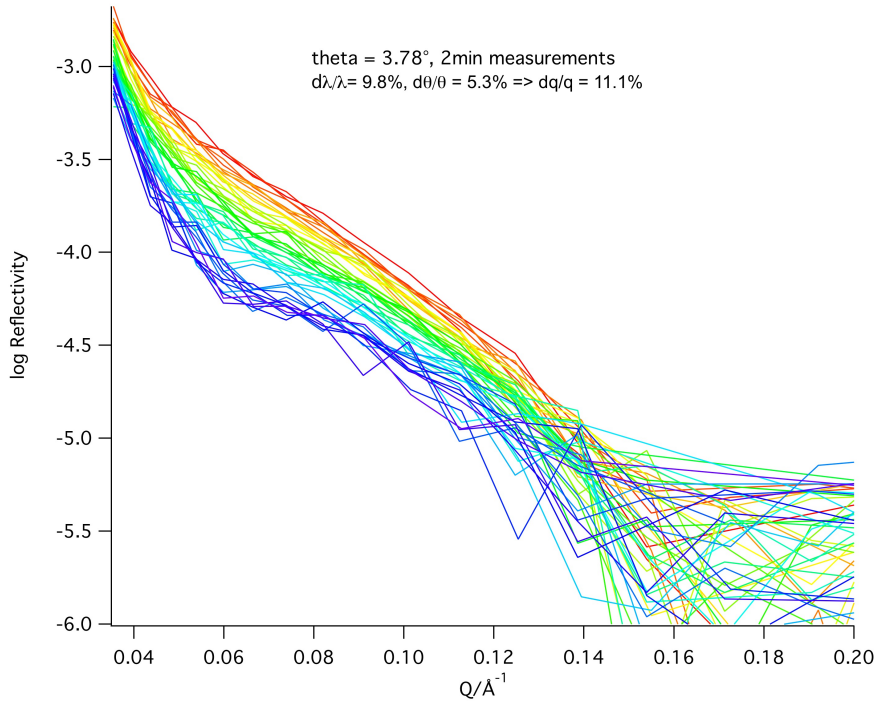
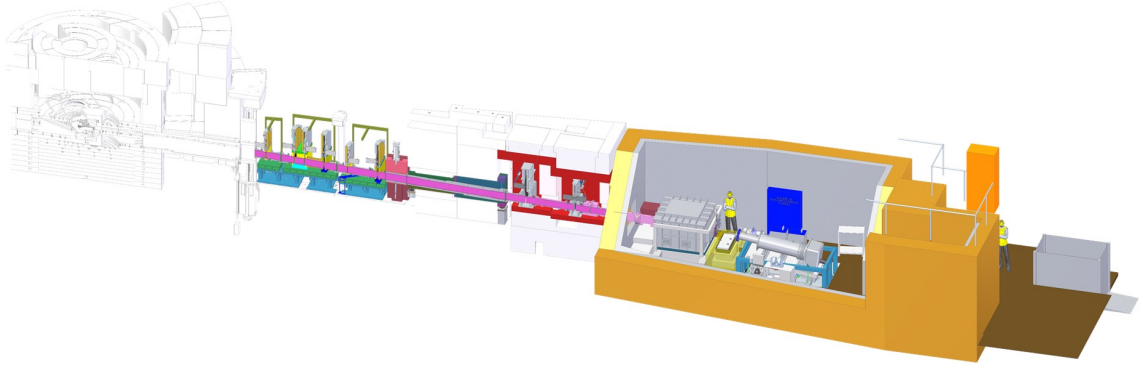
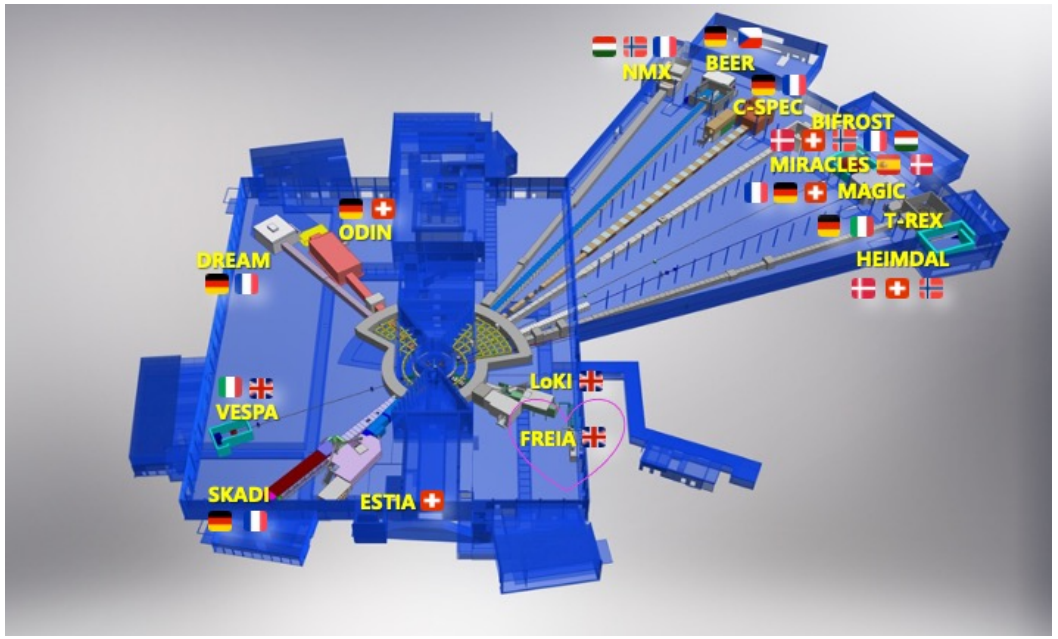
Wacklin et al. (2007) BBA – Biomembranes 1768, 1036-49

Kinetics

location of enzyme and membrane structure as function of time?







- Measuring changing membrane structure and composition every 2 min (FIGARO @ ILL).
- On FREIA @ ESS we will be able to measure on sub-second timescales!

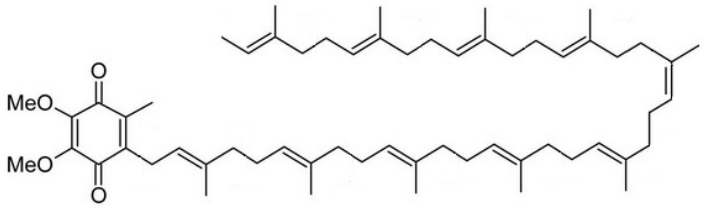
5 Examples 2: Membrane asymmetry

Resolving lipid membrane inner structure

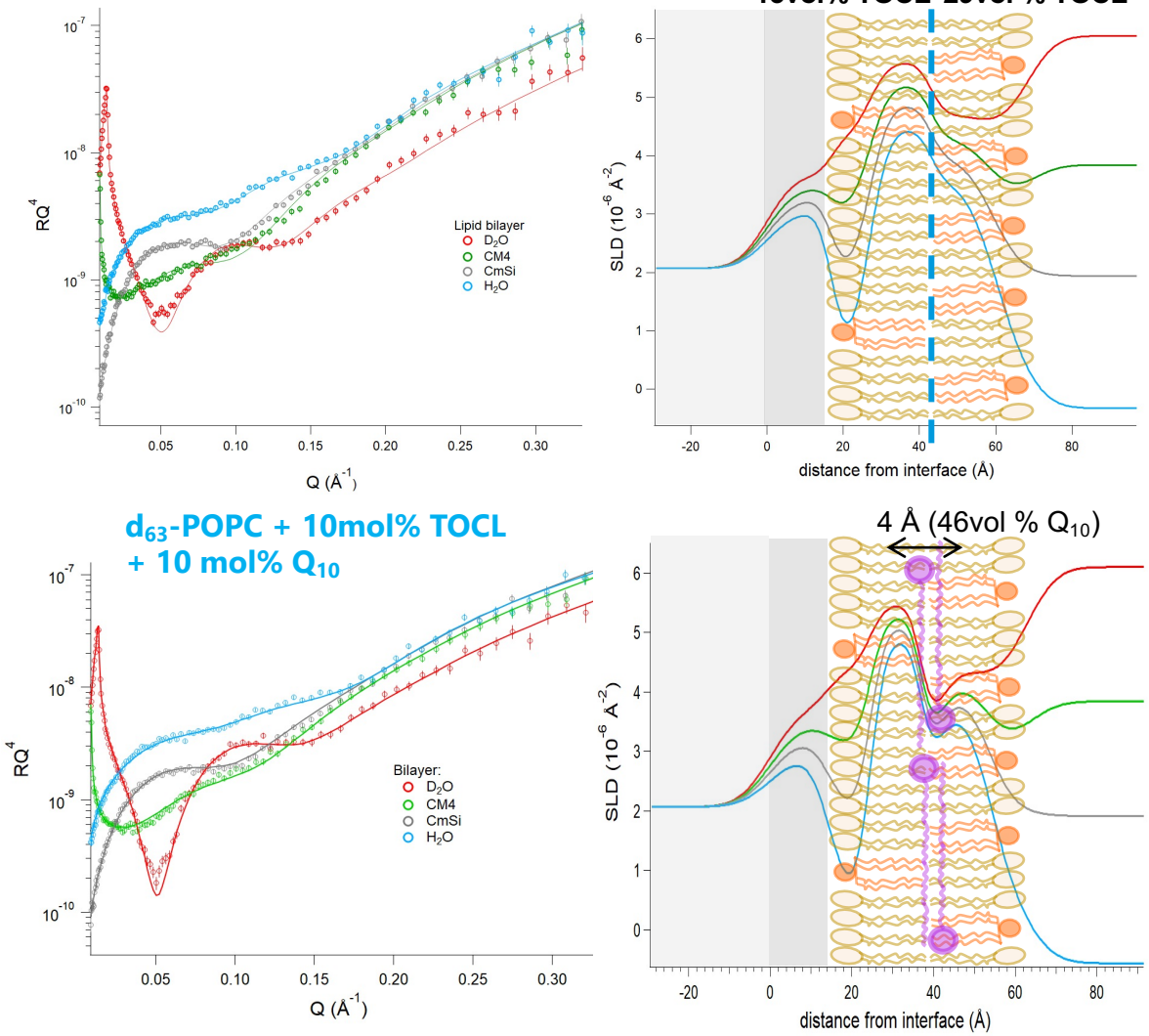
Lipid mixtures can self-assemble into asymmetric supported bilayers

- Cardiolipin is negatively charged and repelled by SiO_2 surfaces
- CL asymmetry detected using chain-deuterated POPC.

Ubiquinone Q_{10} is a long hydrophobic electron acceptor for many enzymes in mitochondrial membranes.



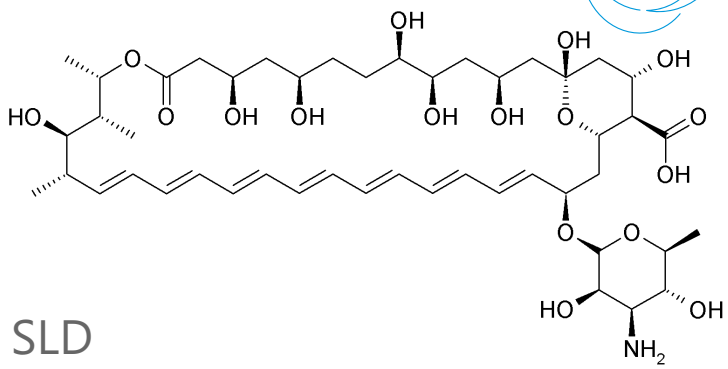
- Location in centre of membranes resolved by NR.



6 Example 3: Amphotericin B

Detecting antibiotic insertion and sterol extraction

- AmB is a membrane-binding antimykotic drug that inserts into lipid bilayers
- AmB exchanges 13 of 47 protons with aqueous solvents
- sld varies in different solvent contrasts
- AmB has contrast to both hydrogenous and deuterated lipids in all contrasts
- AmB is contrast matched to silicon substrates and CmSi!
- AmB solvent exchange can be analysed to resolve membrane insertion



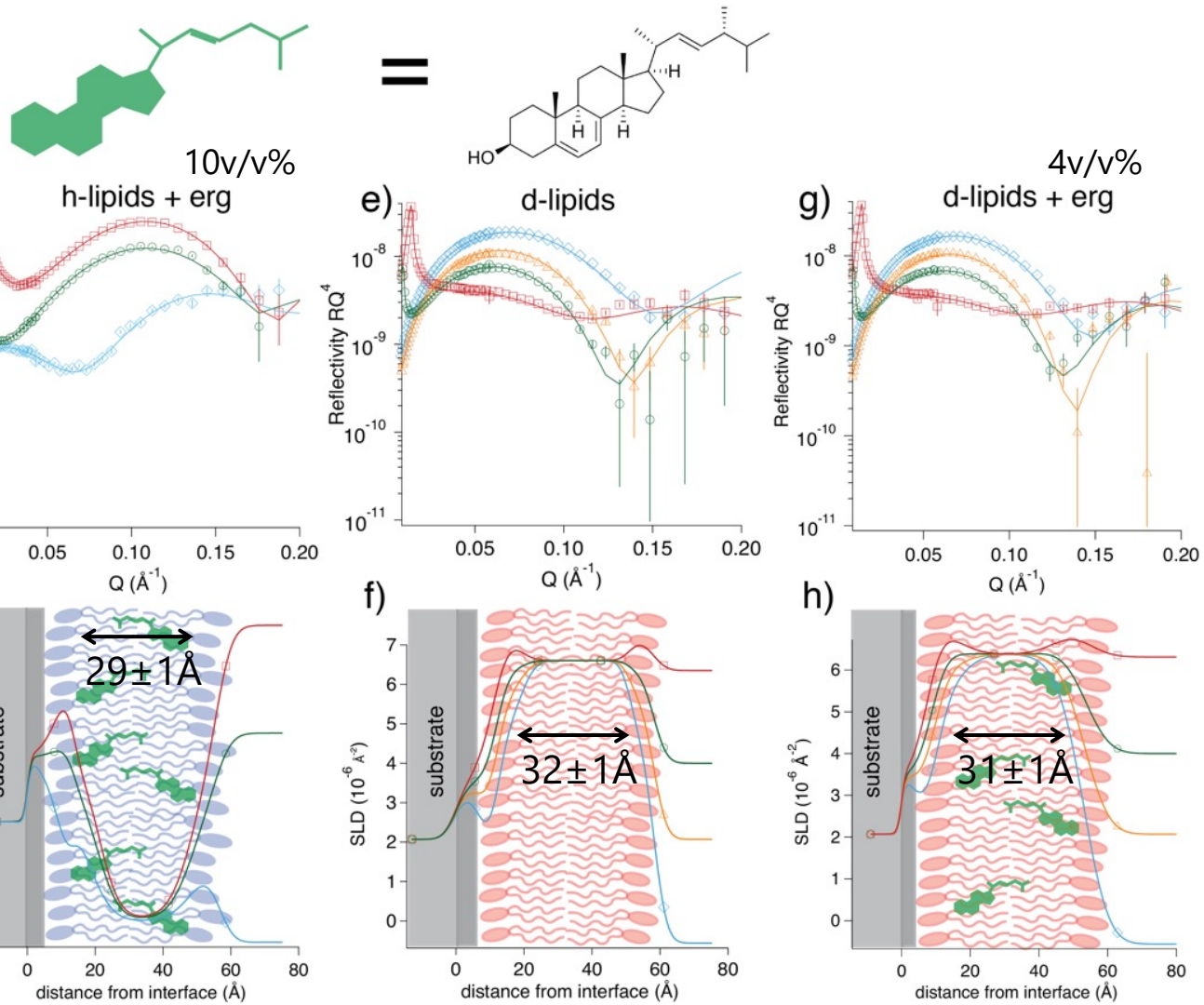
SLD

H_2O -0.56	CmSi (38% D_2O) 2.07	CM4 (66% D_2O) 4.0	D_2O 6.35
1.52	2.03	2.52	2.87

6 Example 3: Amphotericin B

Structure of *P.Pastoris* yeast phospholipid membranes ± native ergosterol

ILL - FIGARO

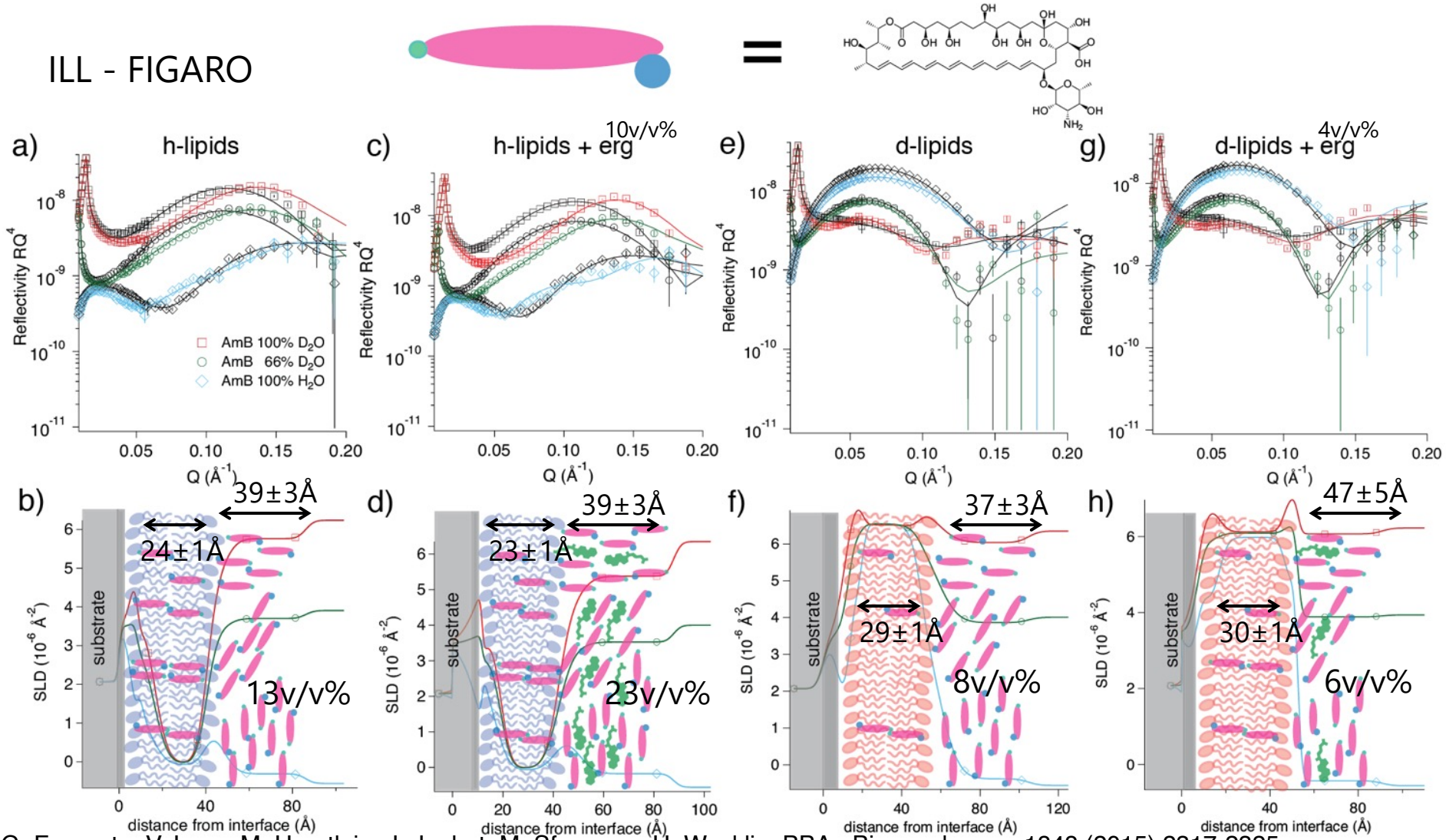


A. de Ghellinck, G. Fragneto, V. Laux, M. Haertlein, J. Jouhet, M. Sferrazza, H. Wacklin, BBA - Biomembranes 1848 (2015) 2317-2325.

6 Example 3: Amphotericin B

AmB inserts into yeast lipid bilayers and extracts ergosterol into "sponge-layer"

ILL - FIGARO



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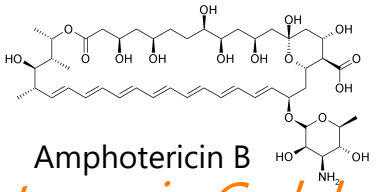
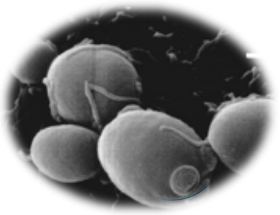
6 Example 3: Amphotericin B

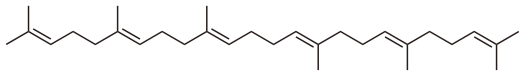
Linking genes and lipid composition to Amphotericin B resistance in C.glabrata



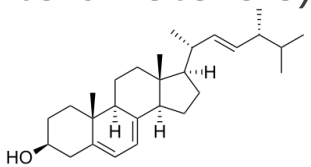


C. glabrata is a human pathogen attacking immunocompromised patients.

- *C.glabrata* used iRNA library of strains to up/down regulate genes
- Phospholipid/fatty acid composition shows minor changes
- AmB resistant C. glabrata accumulates squalene (1st precursor to all sterols)

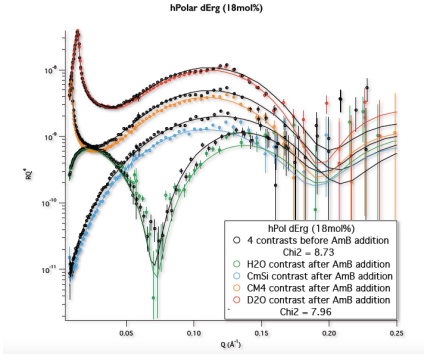


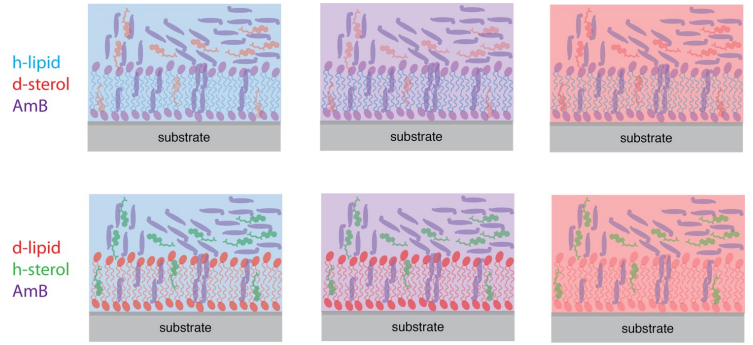
squalene

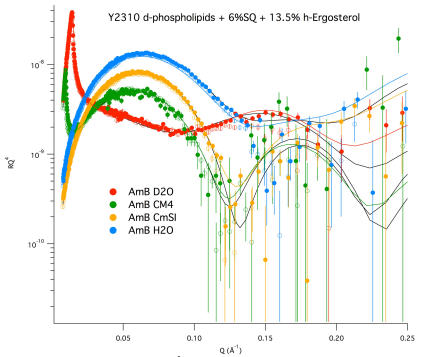


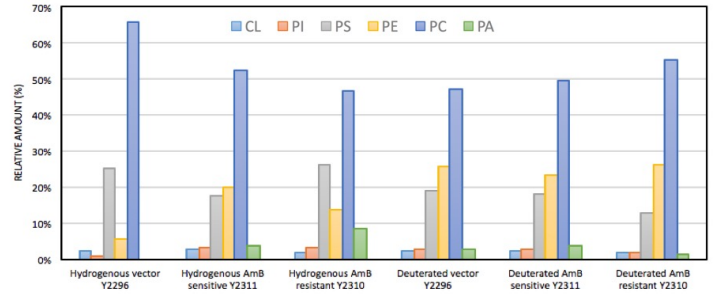
ergosterol

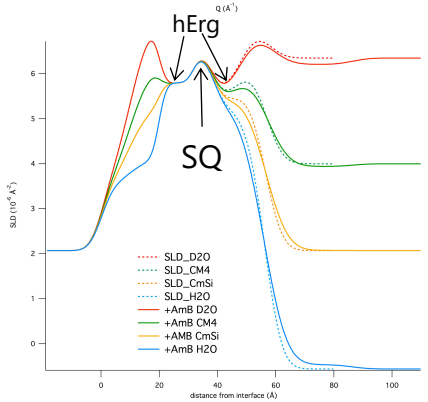
- NR: AmB extracts d_{44}ergosterol but this is partly blocked by squalene.
- *C.glabrata resistance correlates with lower ergosterol extraction*

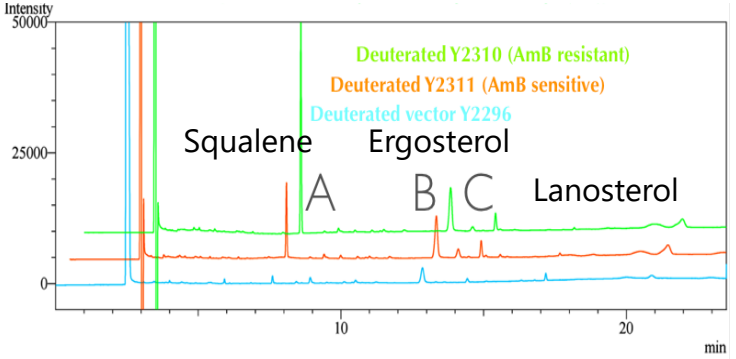












7 Conclusion

Lipid deuteration is a powerful tool to understand membrane structure and function

Deuteration of a range of membrane lipids is possible synthetically, enzymatically and biologically.

- Synthetic lipids can be made in large quantities, but only few available and methods are time-consuming €€€
- Biological lipid extracts containg many lipid classes and a range of fatty acid chains – more native like models of cell membranes but purification of components is challenging €€€
- Contrast matched/selectively labelled lipids can be produced both synthetically and biologically
- *Analysis of deuteration, purity and molecular composition is important!*

Deuteration allows the high resolution of NR and SANS to be fully exploited:

- Detection of lipid arrangement in multicomponent membranes, e.g. asymmetry
- Changes in membrane lipid composition/arrangement
- Penetration of proteins/drugs etc.
- *Solvent contrast variation important for reducing ambiguity in analysis*
- Magnetic contrast variation for fragile samples where solvent exchange not possible, but long PNR measurements.
- *The analysis is as good as the information/assumptions used!*

5 What types of support is available at ESS?

demax@ess.eu

Deuteration and crystallisation services are in operation since 2019:

ESS Deuteration and Macromolecular Crystallization (DEMAX) Platform

LP3 Lund Protein-Production Platform

Chemical Deuteration

- Catalystic H/D exchange
- Organic (chemical) synthesis
- Enzyme synthesis
- Cell lipid extraction, separation and analysis)
- NMR, GC-MS (Red Glead, LU)

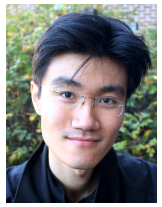
Biological Deuteration

- Deuterated biomass (algae, bacteria, yeast)
- Deuterated protein expression, purification
- Biophysical characterization (DLS, nanoDSF, Thermoflour)
- ^{2}H-incorporation ESI-MS (KI)

Macromolecular Crystallization

- High- and Low-throughput screening
- Optimization (fine-screening, scale-up, temperature)
- Testing & data collection with X-rays @ MAX IV lab (with LP3)

LP3 biotechnician



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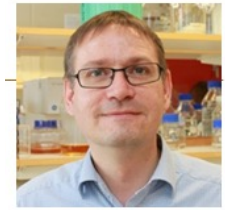


Wolfgang Knecht

DEUNET international deuteration network

UNIVERSITY OF MARYLAND | NIST
INSTITUTE FOR BIOSCIENCE & BIOTECHNOLOGY RESEARCH
insight | innovation | application

OAK RIDGE National Laboratory

Science & Technology Facilities Council
ISIS

Chemical synthesis

EUROPEAN SPALLATION SOURCE

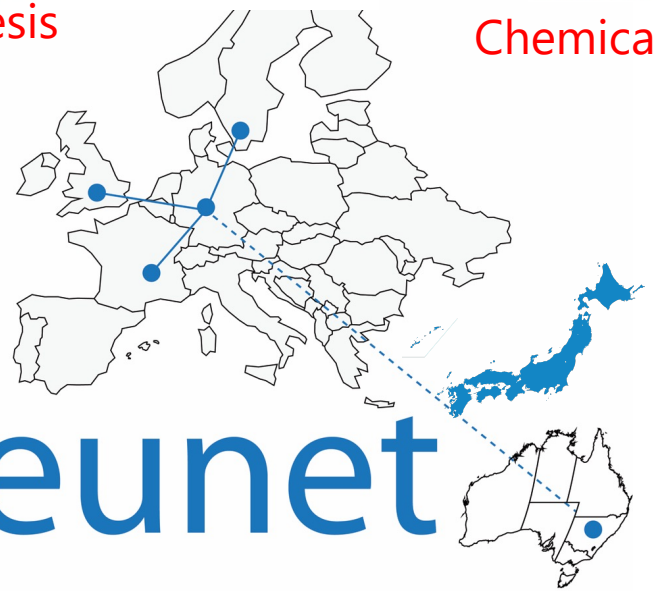
LP3
Lund Protein-Production Platform

LUND UNIVERSITY

Chemical & biological deuteration, crystal growth

JÜLICH FORSCHUNGSZENTRUM

Polymer & chem synthesis



Deunet

NEUTRONS FOR SCIENCE

Lipid biodeuteration (PSCM), biodeuteration (D-lab)

J-PARC MLF

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Further reading:

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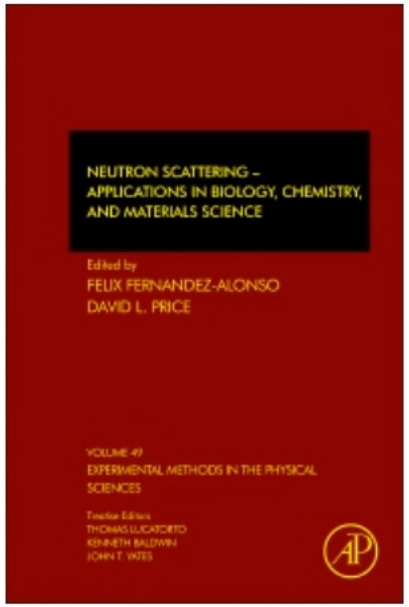

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Questions?