Labelling strategies for small angle neutron scattering

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Life sciences group (ILL)

Isotope labelling of biomolecules

- It is important for many analytical and structural techniques, including NMR, Mass spectrometry and neutron applications.

- The particular sensitivity of neutrons to the isotopes of Hydrogen makes deuteration of biological system an essential tool for neutron scattering experiment.

- Neutron studies are greatly enhanced by production of labelled biological macromolecules.

Different deuterium labelling regimes

How to increase selectivity in deuteration of proteins ?

Perdeuteration

matchout deuteration
« random fractional deuteration »

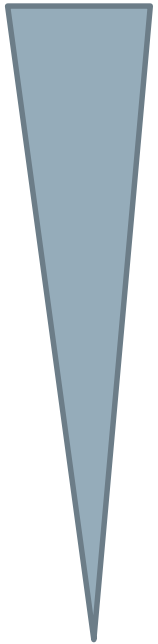
in vivo possible

amino acid specific deuteration
in vivo or cell-free

methyl-group specific deuteration
or reverse-labelling

segmental deuteration
in vitro and *in vivo*

Selectivity



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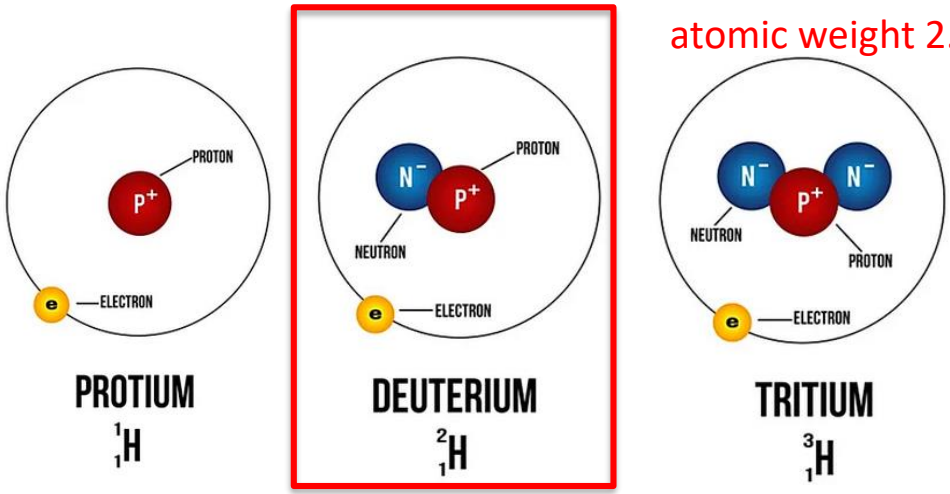
Hydrogen species

3 isotopes

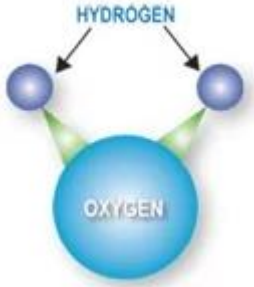
nucleus contains a proton and a neutron
atomic weight 2.01363



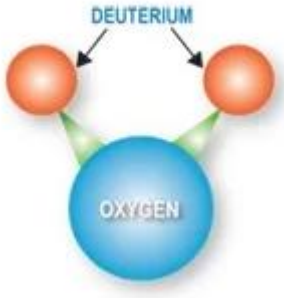
Natural abundance	99.985%	0.015%	trace
Half life	stable	stable	12.32 years

Properties of light and heavy water

H_2O



D_2O

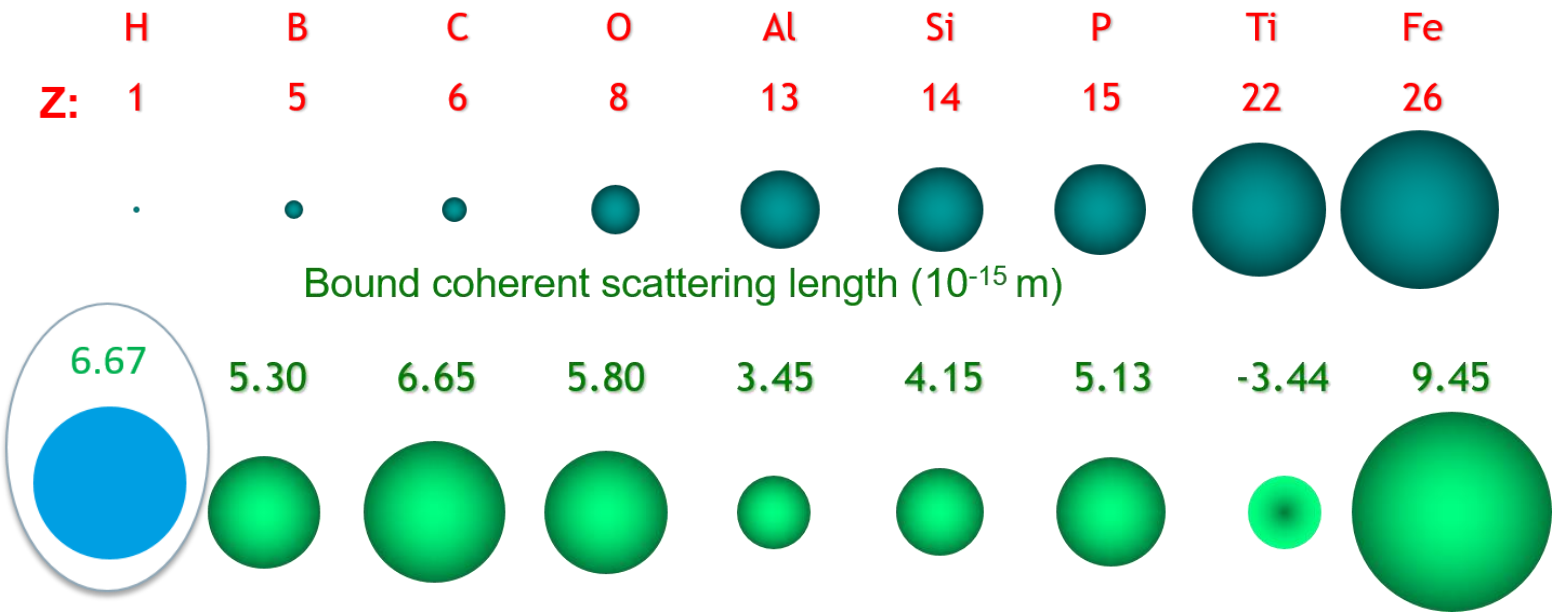


Melting point	0.00°C	3.81°C
Boiling point	100° C	101.42°C
Density (g ml^{-1})	1.00	1.10
Viscosity (20°C)	1.00	1.25
T °C max. density	3.98 °C	11.23°C

X-Ray and neutron scattering

X-ray : Scattering proportional to Z (Z = number of protons)

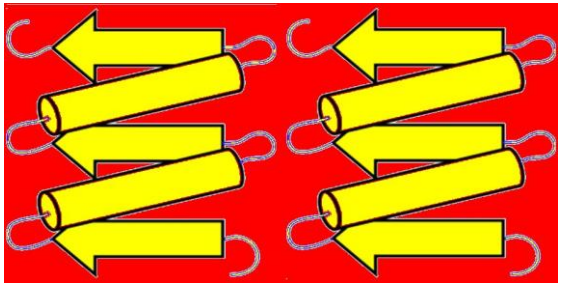
Neutron: Scattering not proportional to Z



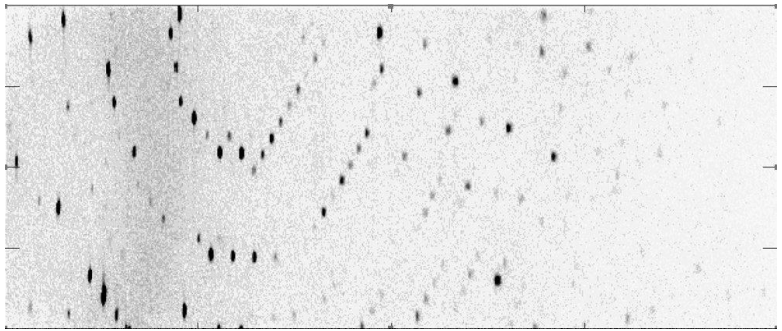
Mass number= number of protons + neutrons Ex: C: 14, deuterium: 2, N: 15

Role of deuterium in neutron scattering

- The replacement of hydrogen to deuterium introduce a strong and positive coherent scattering factor.
- It eliminates the hydrogen incoherent scattering contribution to the background and improves signal-to-noise, highlighting more informations.



Perdeuterated protein, D_2O solvent



D19: monochromatic larger crystals needed, smaller unit cells, but ultra-high resolution available

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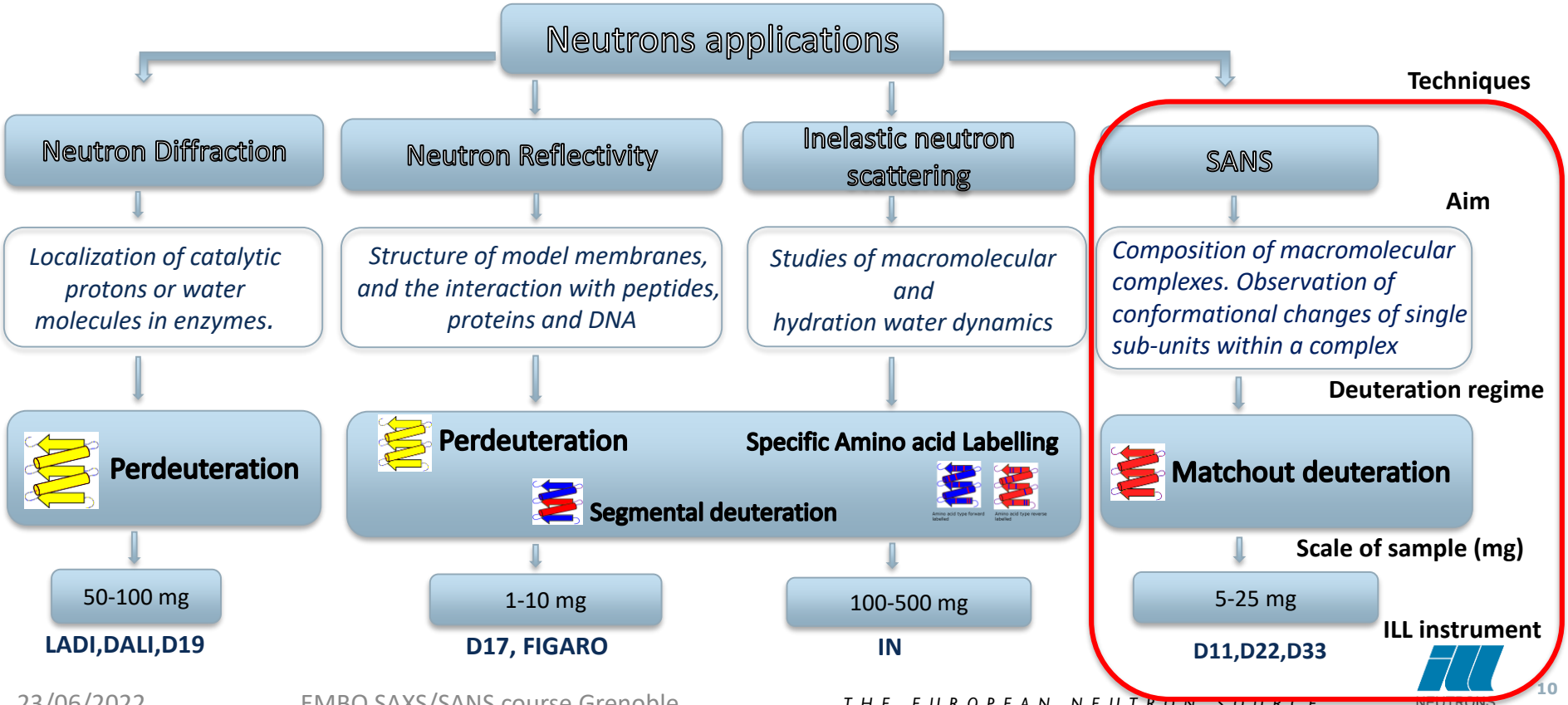
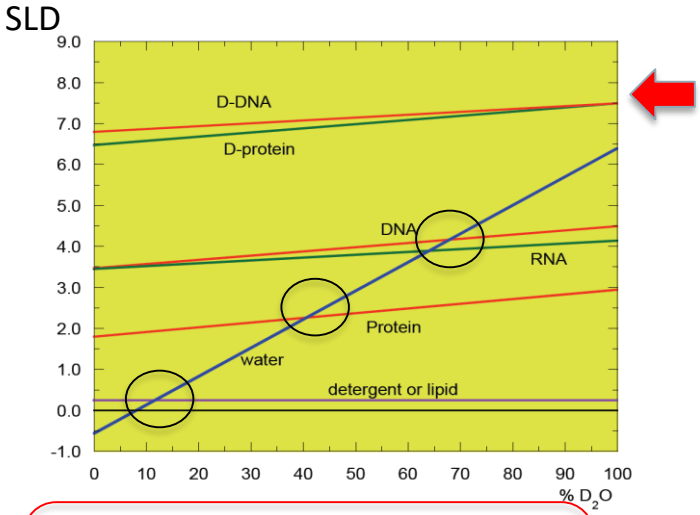
Deuteration of biomolecules for neutron applications



Deuteration to study protein-protein complexes by SANS

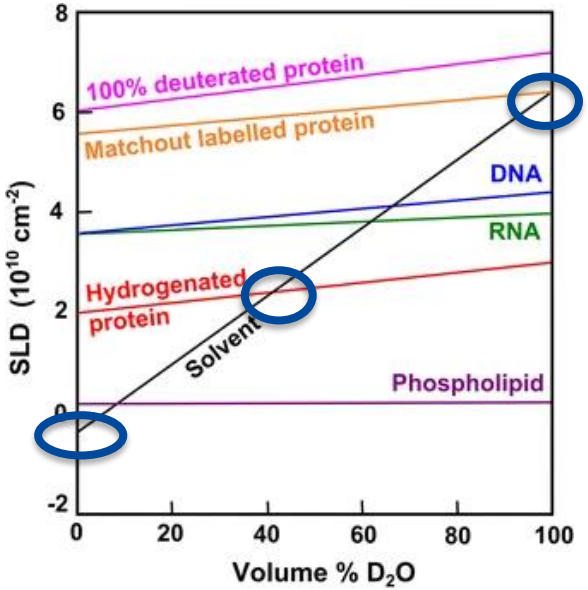
Ability to use D_2O /H_2O solvent contrast variation to selectively matchout specific parts of multi-component system

- Contrast variation can be performed by varying D_2O /H_2O solvent composition to matchout :
  - -hydrogenated protein (~40% D_2O),
  - -nucleic acid (~70% D_2O)
  - - lipid (~5-20% D_2O)
- This exploits the natural constrast that exists in H-complexes
- Perdeuterated protein has an SLD higher than that of D_2O and cannot be fully solvent matchout in SANS
- To distinguish between molecules having the same SLD in protein-protein complexes, **deuteration labelling is necessary** !



Goal: produce a protein having a SLD that is very close that of 100% D_2O

Random fractional deuteration (matchout labelling) for SANS



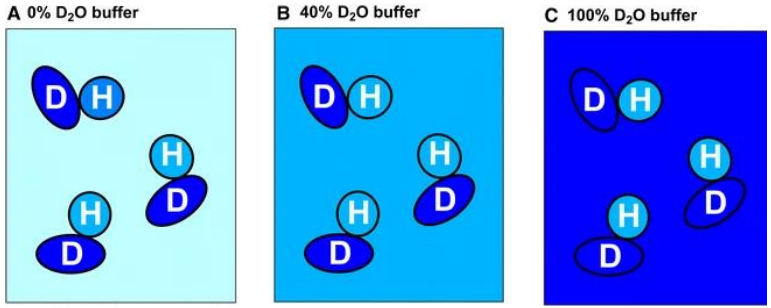
The scattering length densities (SLDs) of the four major biomolecules are depiected as a function of the volume percentage of D_2O

Matchout deuterium labelling of proteins for small-angle neutron scattering studies using prokaryotic and eukaryotic expression systems and high cell-density cultures

O. Dunne, M. Weidenhaupt, P. Callow, A. Martel, M. Moulin, S. J. Perkins, M. Haertlein & V. T. Forsyth

European Biophysics Journal **46**, 425–432 (2017) | Cite this article

Matchout regime for a protein-protein complex :



Growth medium required for matchout labelling:

- **about 85% D_2O**
- **an hydrogenated carbon source**

To obtain 75% D-protein matchout in pure D_2O

Advantages of the matchout labelling

For a complete SANS structural analysis, ONLY one component of the protein-protein complex needs to be matchout labelled

- SANS measurements at 3 different solvent contrasts will yield structural information :

  - at 100 % D_2O ⟹ on the unlabelled component
  - at 40 % D_2O ⟹ on the labelled component
  - at 0% D_2O ⟹ on the full complex

  Conformational changes and relative orientation of the 2 proteins in the complex

- The production of matchout protein is much more efficient than that of a perdeuterated protein in fully deuterated conditions.

- Significant cost advantages are due to the absence of deuterated carbon source needed in the culture medium.

Tables of content

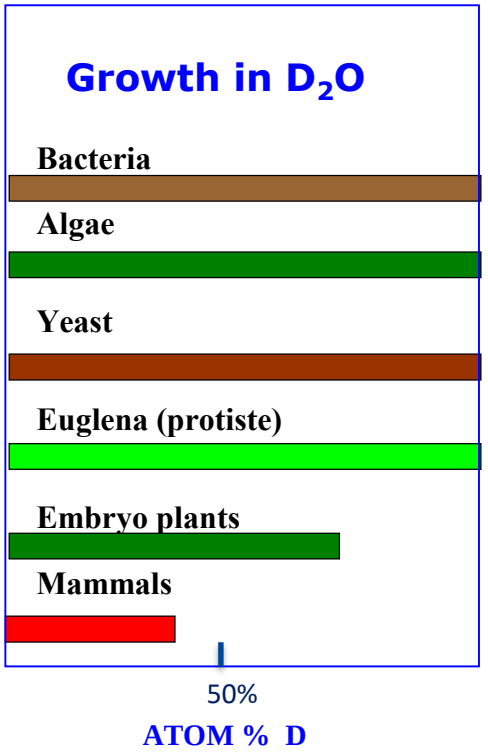
- Deuterium and D_2O properties
- Deuteration of biomolecules for neutron applications
- Effects of deuteration on living organisms
- Deuteration strategies
  - *In vivo* deuteration with recombinant organisms
  - *In vitro* deuteration
  - Deuterated peptide by solid phase peptide synthesis
- Deuterated samples used in recently published work
- Deuteration Lab platform– Access arrangements

Adaptation of microorganisms to growth in D_2O

- Bacteria, algae and yeasts can be gradually adapted to growth in D_2O by sequential subculturing into media containing increasing concentrations of D_2O.

$\Rightarrow$ may take several weeks or months !

- Mammals can not be adapted to a level above 40% D_2O



Adapted from Katz and Crespi (1970)

Early studies on deuterium effects

Phenomena associated with exposure to D_2O

D_2O effect on higher plants

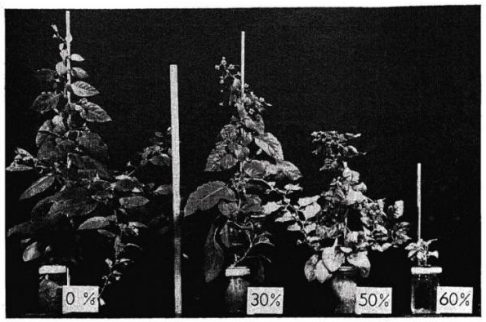
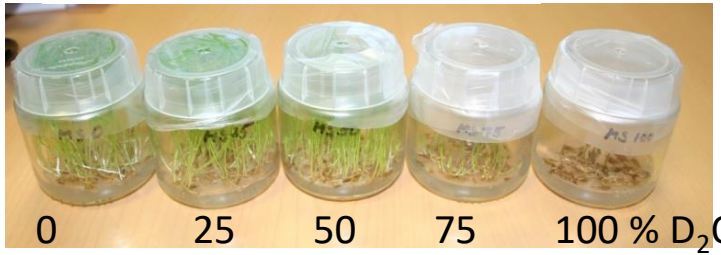


Fig. 3. Plants of *Atropa belladonna* grown hydroponically in nutrient solutions containing increasing concentrations of D_2O. [Uphaus *et al.* (29)]

Uphaus *et al.* 1965



D_2O effect on mice

Systematic study of D_2O on mice, Barbour *et al.* (1934-1939)

0-15% little change
15-20% hyperexcitability
20-25% aggressive, convulsions, increased body temperature
25-35% death

Why is *in vivo* deuteration not straightforward?

Effects of D_2O on living systems

" Solvent Isotope Effect " (SIE) based on the properties of D_2O molecule as a whole, in particular its effects on the structure of water and the biological macromolecules.

"Deuterium Isotope Effect" (DIE), resulting from the ability of D_2O to replace H with D in biological molecules. The C-D bond is several times stronger than the C-H bond and thus more resistant to enzymatic and even to chemical cleavage.

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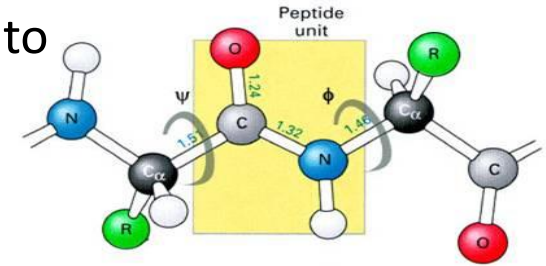
in vivo deuteration, the (only) way to obtain highly deuterated biological macromolecules

in proteins

~ 25% of labile H bound to nitrogen(N), Oxygen (O) and sulfur (S)



~ 75% of non-exchangeable H bound to carbon **(C)**

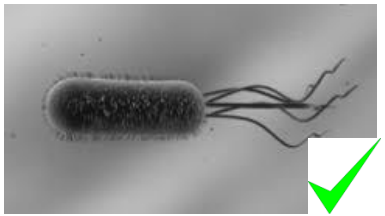
exchange by dialyzing against D_2O buffer or soaking crystals of hydrogenated protein in mother liquid containing D_2O

incorporation into proteins via protein biosynthesis:
In vivo Deuteration

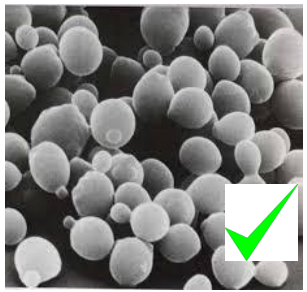
General deuteration strategy used in the ILL D-Lab for recombinant proteins

Perdeuteration and matchout labelling

- Choice of the growth medium
- Adaptation of recombinant microorganisms to deuterated media
- High cell density cultures (HCDC) of recombinant micro-organisms in deuterated minimal medium



Bacterial expression systems



Yeast expression systems

Growth media used for the deuteration

Complex media for flask culture

ex of seller for isotope labelling :

Silantes
Stable Isotope Labeled Biomolecules

CIL

(Cambridge isotope labelling)

Minimal medium for high cell density culture or alternatively to flask culture

Component	Initial concentration
$(NH_4)_2SO_4$	6.86 g/L
KH_2PO_4	1.56 g/L
$Na_2HPO_4 \cdot 2H_2O$	6.48 g/L
$(NH_4)_2$-H-citrate	0.49 g/L
$MgSO_4 \cdot 7H_2O$	0.25 g/L
Trace metal solution	1.0 mL/L
Glycerol	5.0 g/L

Trace metal solution
0.5 g/L $CaCl_2 \cdot 2H_2O$, 16.7 g/L $FeCl_3 \cdot 6H_2O$, 0.18 g/L $ZnSO_4 \cdot 7H_2O$, 0.16 g/L $CuSO_4 \cdot 5H_2O$, 0.15 g/L $MnSO_4 \cdot 4H_2O$, 0.18 g/L $CoCl_2 \cdot 6H_2O$, 20.1 g/L EDTA

Cost of a deuterated culture

Perdeuterated conditions

- D_2O = 450 €/L
- Nutrients:
  - D-glycerol = 25 €/g (50 g per fermentation)
  - D-methanol = 45 €/g (yeast culture)
  - D-amino acid = 1400 €/g (cell free)

Fermentation run around **2500 €**

Matchout conditions

The cost is more **reasonable** in comparison with perdeuterated culture: - Use of recycled D_2O
- H-Glycerol as carbon source

D_2O recycling process (setup by V.Laux)

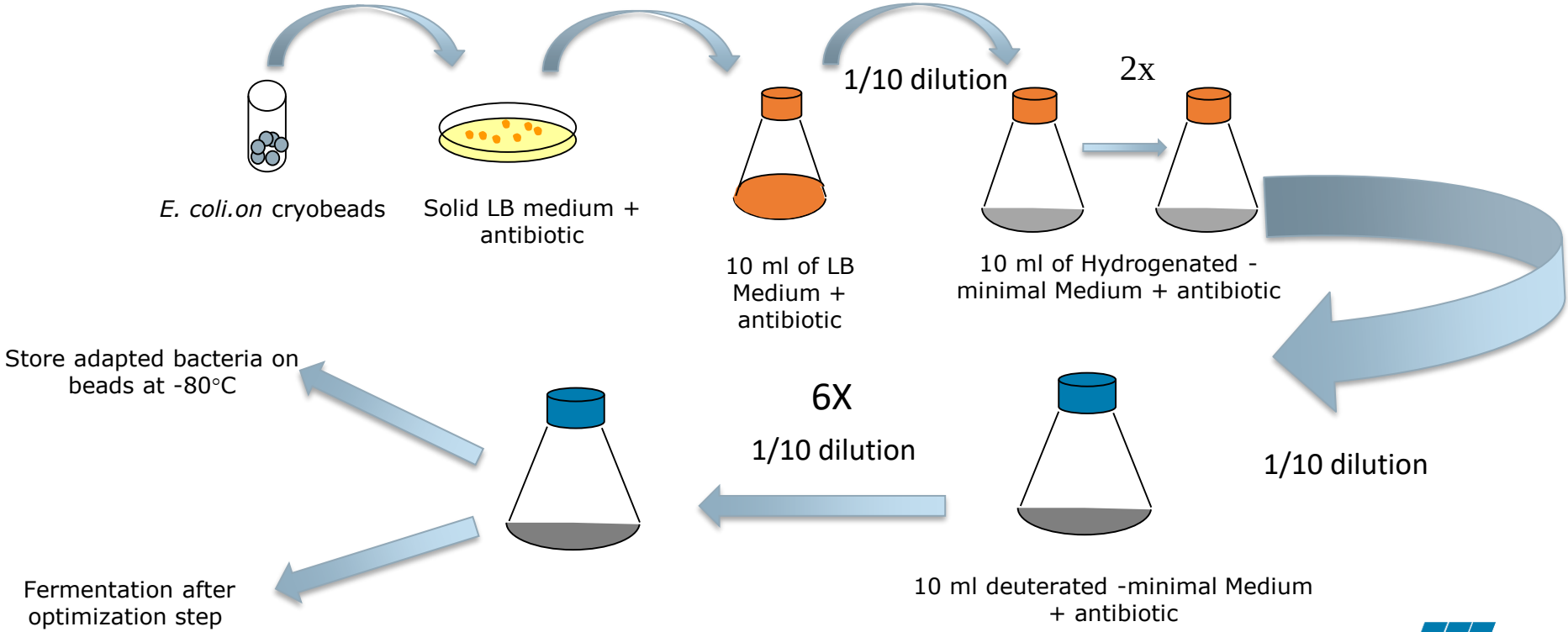
Level of deuteration obtained around **98%**
Savings: 450 € /L around 20L/year are recycled which gives a saving of **9000** €


Activated Charcoal


Distillation of D_20


Recycled D_2O

Adaptation of *E. coli* cells to deuterium



Optimisation of deuterated protein production

Perdeuteration or matchout labeling

Cell growth is typically slower (> 5 times slower) and yield is correspondly lower

The protein expression and its solubility are often affected in deuterated conditions

Optimisation of deuterated protein production at small scale
(medium composition, temperature and induction time)

Fermentation in High Cell Density Culture (HCDC)

High Cell Density Cultures (HCDC)

Objectives

- Optimize volumetric yield
- Maximize biomass productivity
  - Grow cells under controlled conditions
  - With the highest biomass/substrate yield (Expected yield is 1 g of cell paste per g of carbon source used)

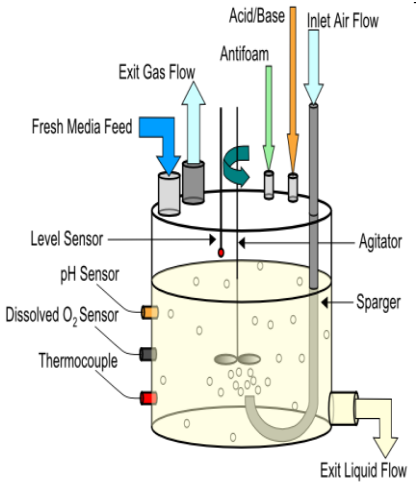
High cell density for *E.coli* culture

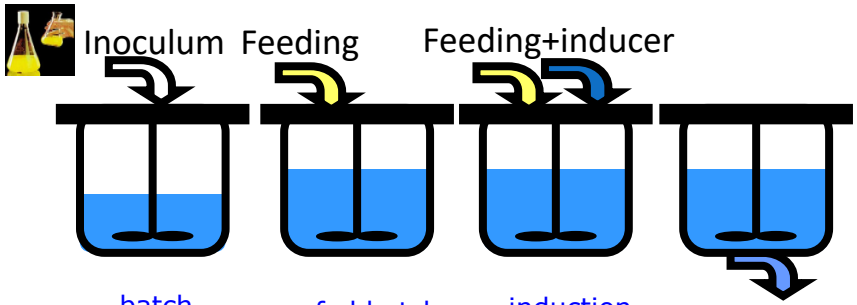
- 1.5L ≈50 to 85 g of cell paste ≈ 12L flask culture

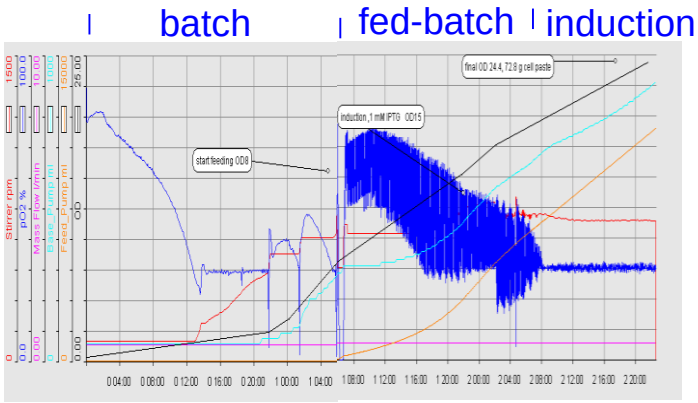
Recycling of D_2O

Bioreactor: recombinant fed-batch culture

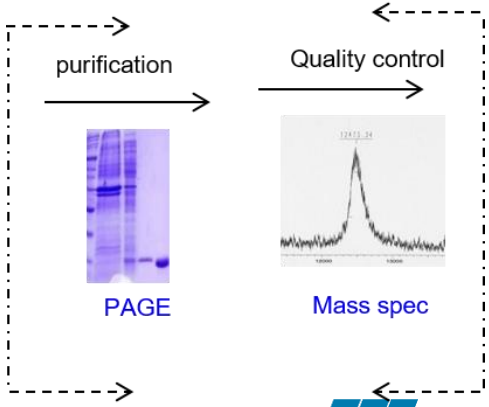
Perdeuterated and matchout labelling





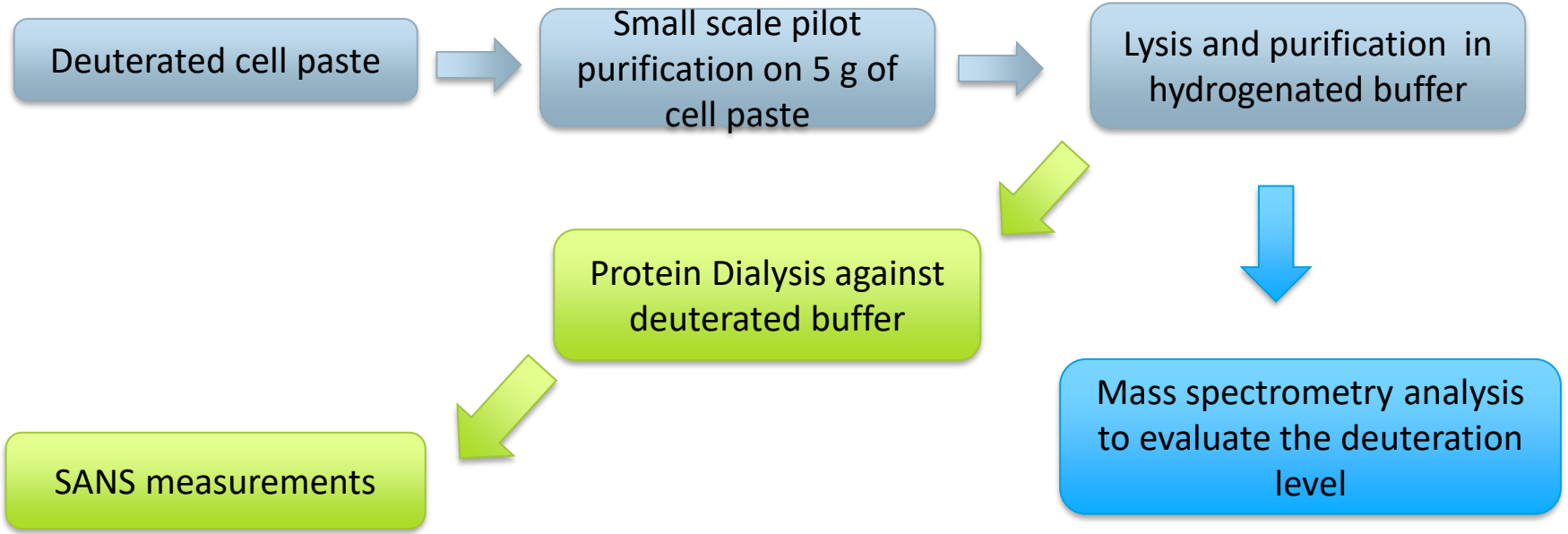


deuterated cell paste



Purification of deuterated protein

Perdeuteration or matchout labelling



Determination of protein deuteration level by mass-spectrometry

- Use ProtParam to get atomic composition, amino acid frequency and molecular weight of your H-protein from primary sequence
- i.e. Transthyretin (TTR):
- GAMGPTGTGESKCPLMVKVLDAVRGSPAINVAVHVFRKAADDTWEPFASGKTSESESGELHGLTTEEEFVEGIYKVEIDTKSYWKALGISPFHEHAEVVFTANDSGPRRYTIAALLSPYSYSTTAVVTNPKE
- Carbon C 635 **Hydrogen H 979** Nitrogen N 165 Oxygen O 201 Sulfur S 3

Ala (A)	13	4*	Leu (L)	7	10
Arg (R)	4	7	Lys (K)	8	9
Asn (N)	3	3	Met (M)	2	8
Asp (D)	5	3	Phe (F)	5	8
Cys (C)	1	3	Pro (P)	8	7
Gln (Q)	0	5	Ser (S)	12	3
Glu (E)	13	5	Thr (T)	12	5
Gly (G)	11	2	Trp (W)	2	8
His (H)	4	5	Tyr (Y)	5	7
Ile (I)	5	10	Val (V)	12	8

Total carbon-bound H: 761

* Number of C-bound hydrogens

Out of the 979 H in the TTR protein 761 are carbon bound (non-exchangeable).

Experimentally determined mass difference between the D-protein in H-buffer and the H-protein was 757 Da

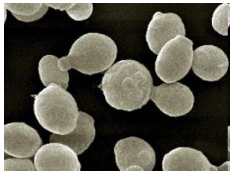
D protein in H-buffer	14994 Da
H protein	- 14237 Da
	757 Da

This implies that 757/761 x 100 = 99.47% of the carbon-bound H are deuterium.

Deuteration level is 99.47%

P. pastoris is a useful alternative to *E. coli* for the production of deuterated proteins

(V.Laux)

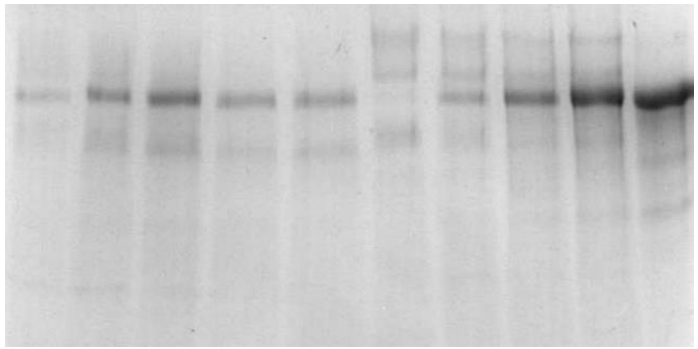


- High expression levels (intracellularly or secreted)
- Disulphide bonds formed during secretion
- Growth on “cheap” deuterated carbon sources (glycerol, methanol)

Expression test

Hydrogenated HSA Deuterated HSA

D1 D2 D3 D4 D5 D1 D2 D3 D4 D5

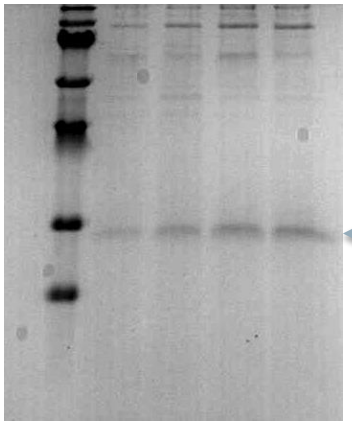


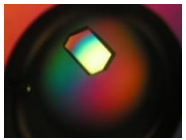
D= Day; HSA: Human Serum Albumin

Expression test

Perdeuterated lysozyme

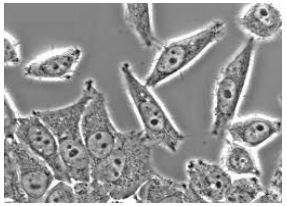
D1 D2 D3 D4





Deuteration in mammalian cells

Development project with Dr. Mizar Oliva



Mammalian cell expression systems

Advantages

- They are able to express membrane proteins and secreted proteins correctly folded
- They perform post translational modifications
- They produce a well defined glycosylation pattern

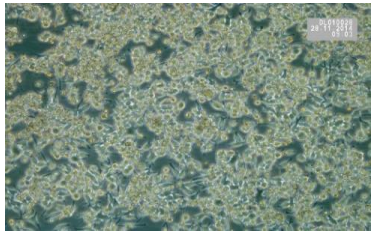
Inconvenients :

- They require a medium supplemented with sugars and amino acids
- Their expression yield is lower than bacteria and yeasts
- They have a low tolerance to D_2O

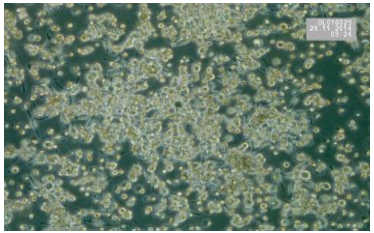
Toxicity of deuterium on mammalian living cells (HEK293)

Increasing amount of deuterium level in the medium

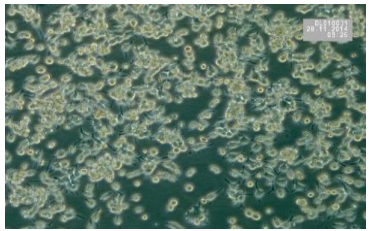
10 %



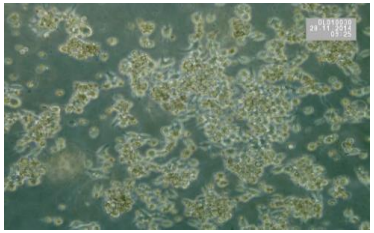
20 %



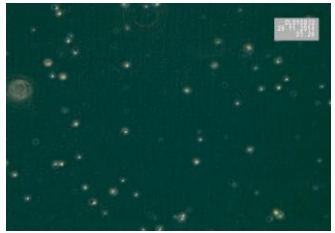
30 %



40 %



50%



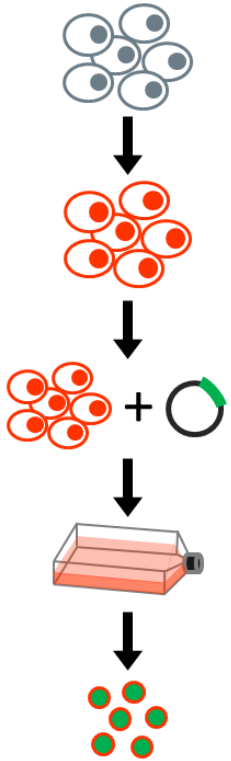
Protein deuteration in mammalian cells

In house development project for the D-Lab - with Dr. Mizar Oliva

Why?
- correct folding
- post translational modifications

Applications
- SANS
- reflectometry

Challenges
- complex medium
- isotope incorporation level



Cells growth in commercial medium

Cells growth in 'labelled' medium

Formulation of 'labelled' medium

1) Growth of yeasts & algae in fully labelled medium

2) Extraction & purification of labelled nutrients

Cells adapted in labelled medium & transfected with gene of interest

Protein production

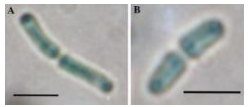
Protein purification & evaluation of labelling level

Deuteration of whole organisms

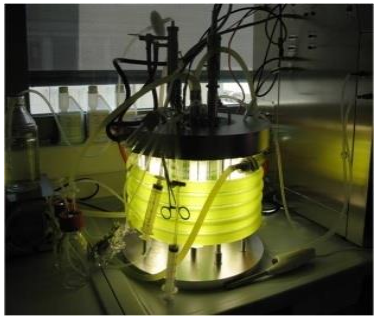
- Deuteration of algae (Anabaena Chlorella Sorokiniana, Thalassiosira Pseudonana) for the production of deuterated biomass
  No carbon source is needed

- Perdeuteration of Yeast/*E.coli* for the production of of deuterated biomass and lipids

- Perdeuteration of Thermosynechococcus elongatus for the production of phycocyanin



Photobioreactors



In vivo deuteration of biomolecules for neutron applications

Some challenges for sample preparation

- Toxicity of deuterium - reduced growth rates and expression yield of protein.
  ⟹ Adaptation of recombinant expression system needed
- Scale of samples required: 10mg – 100mg –1g ⟹ HCDC guarantee high volumetric yields for biomass and/or deuterated molecules
- High costs and availability of deuterated media components (except for “matchout” labelling)

Tables of content

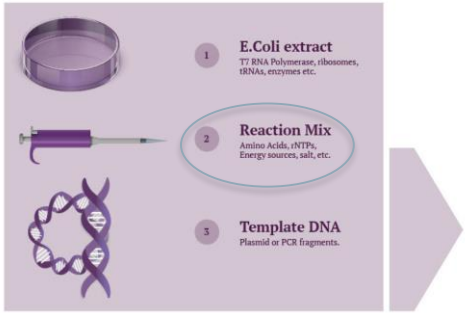
- Deuterium and D_2O properties
- Deuteration of biomolecules for neutron applications
- Effects of deuteration on living organisms
- Deuteration strategies
  - *In vivo* deuteration with recombinant organisms
  - *In vitro* deuteration (cell-free technique)
  - Deuterated peptide by solid phase peptide synthesis
- Deuterated samples used in recently published work
- Deuteration Lab platform– Access arrangements

Cell-free protein synthesis (CFPS)

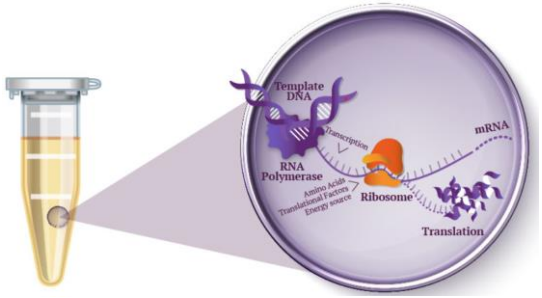
Production of difficult proteins: membrane proteins, cytotoxic proteins etc..

Synthelis
Your Cell-Free Partner

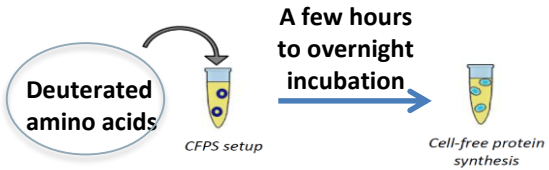
Preparation of the reaction mixture



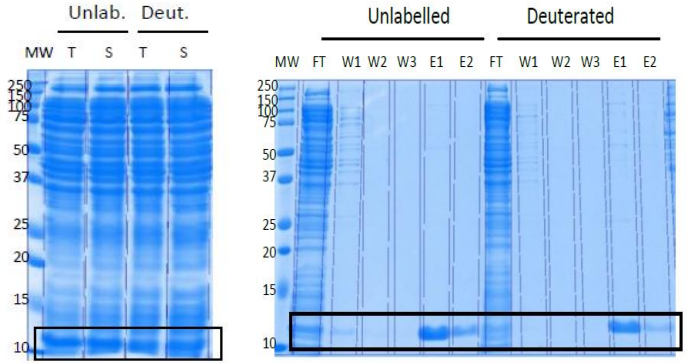
Protein expression



Replacement of hydrogenated A.A by deuterated A.A



CFPS: A simplified method for production of deuterated proteins

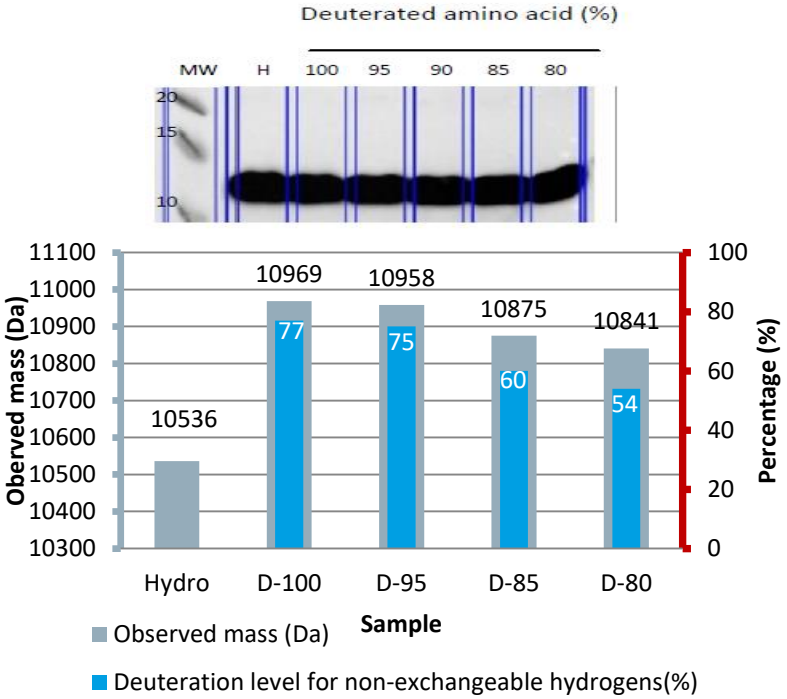


T total
S soluble

Coomassie stained SDS-PAGE gel for soluble expression and purification of hydrogenated and deuterated SDF1-α

(Dr V.Jugnarain)

Analysis of cell-free protein production (CFPS)



Advantages

- It is an alternative system for SANS experiment
- Possibility of doing a specific amino acid labelling

Inconvenients

- Limitation of quantity (250 - 300 µg/mL of CFPS reaction)
- Difficulty to reach 100% of deuteration

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  - Deuterated peptide by solid phase peptide synthesis
- Deuterated samples used in recently published work
- Deuteration Lab platform– Access arrangements

Selectively deuterated peptides production through Solid-Phase peptide Synthesis (SPPS)

In house development project for the D-Lab - with Valerie Laux

New capability for biological neutron scattering especially for SANS and NR studies

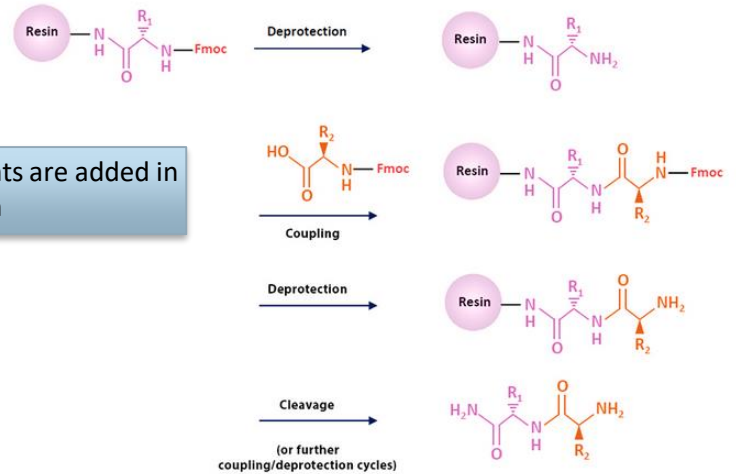
Example: studies of membrane interactions with anti-bacterial and anti-fungal peptides. They also play a role as anti-viral activity and anti-cancer activity (Hoskin&Rammaoorthy,2008)

Peptides of 8 to 37 amino acids in length were succesfully produced by SPPS

Selectively deuterated peptides production through Solid-Phase peptide Synthesis (SPPS)

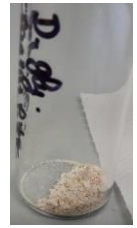
The starting material is bound to an inert solid support (resin)

Reactants are added in solution



General Scheme for Solid Phase Peptide Synthesis

Synthesis → Clivage → Precipitation

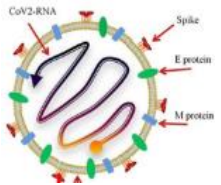


Purification on reverse phase chromatography

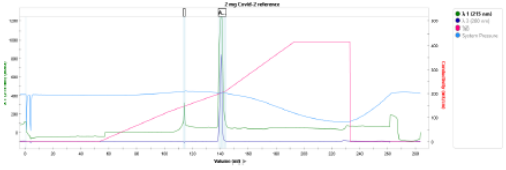
Mass spectrometry analysis

Production of SARS-CoV and SARS-CoV-2 peptides for interactions with model membranes

Deuterated SARS-CoV and SARS-CoV-2 nonapeptides from the E-protein C-terminus in all valine and the phenylalanine positions to study the interactions with bacterial and mammalian membrane mimics

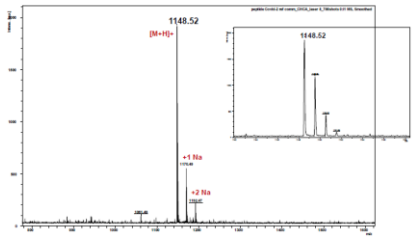


Collaboration A.Maestro
(Reflectometry)

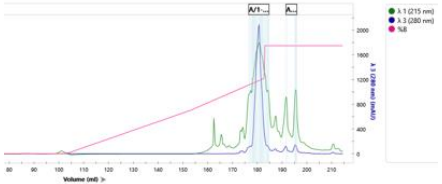


Expected monoisotopic mass (M) :
1147.60 Da

Observed monoisotopic mass (M+H)⁺ :
1148.52 Da
In agreement with intact peptide, seq. 1-9 (mass error : -0.09 Da)

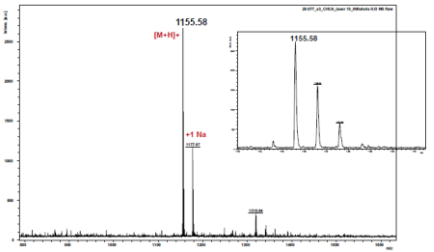


Peptide names	Peptide sequences	Observed Molecular weight (Da)
TK9-E/CD	T V Y V Y S R V K	1114,62
SK9-E/CD	S F Y V Y S R V K	1148,61
d_2TK9-E/CD	T **V** Y V Y S R V K	1121,67
d_2SK9-E/CD	S **F** Y V Y S R V K	1155,58
$d_{2,4,8}$TK9-E/CD	T **V** Y **V** Y S R **V** K	1137,71
$d_{2,4,8}$ SK9-E/CD	S **F** Y **V** Y S R **V** K	1171,68



Expected monoisotopic mass (M) :
1155.65 Da

Observed monoisotopic mass (M+H)⁺ :
1155.58 Da
In agreement with intact peptide, seq. 1-9 + 1D-Phe



Availability of deuterated amino acid with F-moc protected group

SIGMA-ALDRICH®

Commercially available 500 -1500 euro/g

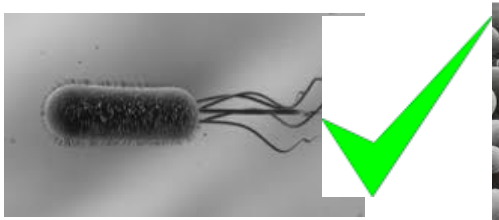
Arg, Leu, Ala, Val, Gly

Eurisotop
A CAMBRIDGE ISOTOPE LABORATORIES COMPANY

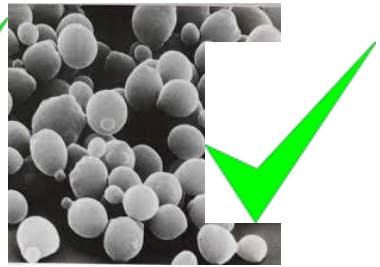
Custom synthesis Up to 16,000 euro/g

0.2 g of protected amino acid per labelled position is required to obtain 20 mg of pure peptide

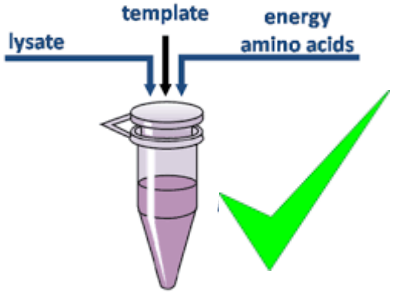
Deuteration approaches for SANS experiment



Bacterial expression systems

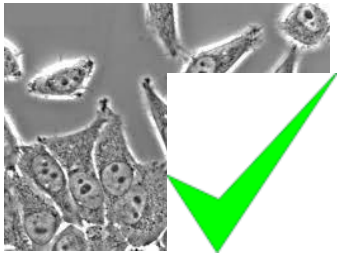


Yeast expression systems

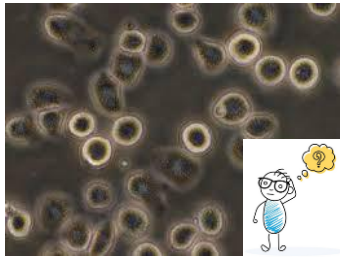


Cell-free (in vitro) synthesis

Peptide synthesis



Mammalian cell expression systems



Insect cell expression systems

Key points for deuteration

- Limited deuteration of biomolecules can be achieved by dialysis against deuterated buffer
- Full or high-level deuteration of macromolecules requires the growth of (recombinant) microorganisms in deuterated media (D_2O based; deuterated carbon source needed for perdeuteration of heterotrophs)
- Deuteration level can be tuned by composition of growth media (ie D_2O concentration in medium or type of carbon source)
- High cell density cultures guarantee high volumetric yields for biomass and/or deuterated molecules
- The purification of deuterated protein is carried out at the same way as hydrogenated protein.

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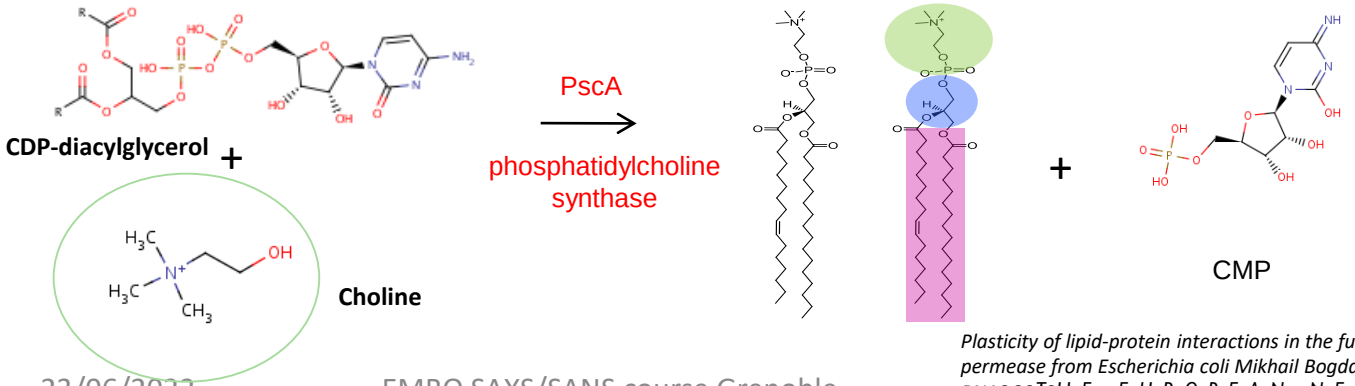
Matchout deuteration of phosphatidyl-choline (PC) using an engineered *E.coli* strain

major phospholipids in wt *E.coli*	
~~phosphatidylethanolamine (PE)~~	70%
phosphatidylglycerol (PG)	20-25%
cardiolipin (CL)	5-10%
phosphatidylcholine (PC)	

Strain AL95 (pss93::kanRlacY::Tn9, −PE cells) is devoid of PE and contains only the negatively charged major lipids, PG and CL .

Introduction of plasmid pAC-PCSlp-Sp-Gm (−PE +PC cells) allows expression of the *Legionella pneumophila* pcsA gene under induction control by arabinose of the promoter ParaB.

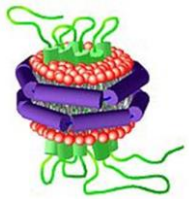
PC is synthesized from endogenous CDP-diacylglycerol and choline from the growth medium



*Plasticity of lipid-protein interactions in the function and topogenesis of the membrane protein lactose permease from Escherichia coli Mikhail Bogdanov, Philip Heacock, Ziqiang Guan, and **William Dowhan** PNAS 2010*

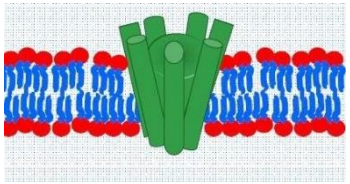
Study of membrane proteins by SANS

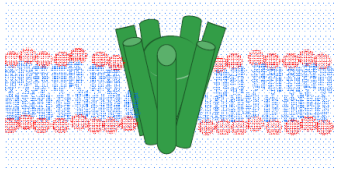
Use of *Stealth nanodiscs* as carrier system



A nanodisc is a synthetic model membrane system composed of phospholipids and belt proteins.

The goal is to use these “Stealth Nanodiscs” as a platform for low resolution structural studies of membrane proteins and their complexes in solution

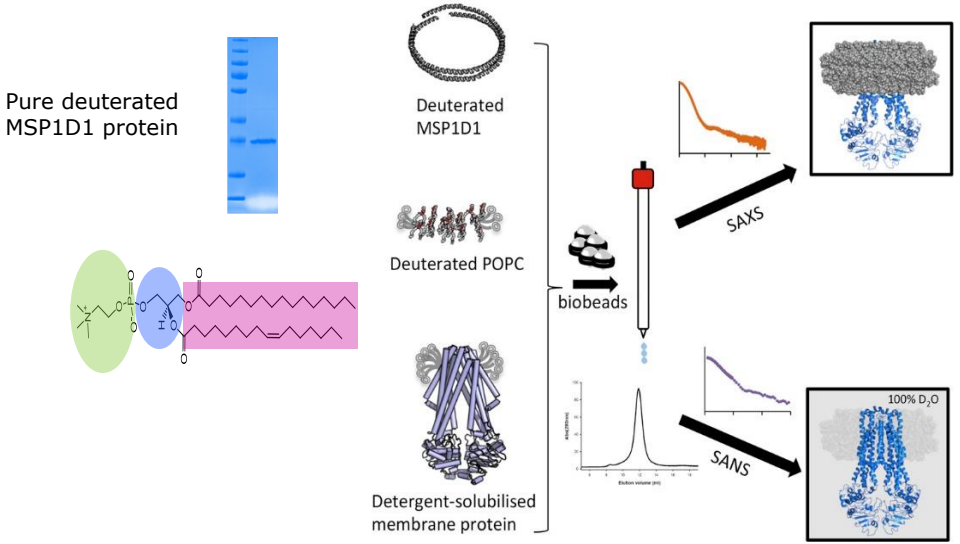


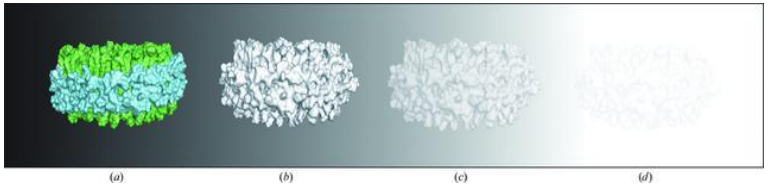


SANS
Collaboration with L.Arleth (Copenhagen)

Selective deuteration of the scaffolding proteins (MSPs) and the phospholipids for the “stealth” deuterated nanodiscs are obtained through bacterial expression in deuterated media

Study of membrane proteins by SANS





Maric S, Skar-Gislinge N, Midtgaard S, Thygesen MB, Schiller J, Frielinghaus H, Moulin M, Haertlein M, Forsyth VT, Pomorski TG, Arleth L., (2014), Stealth carriers for low-resolution structure determination of membrane proteins in solution. Acta Crystallogr D 70(Pt 2):317-28.

Maric S, Thygesen MB, Schiller J, Marek M, Moulin M, Haertlein M, Forsyth VT, Bogdanov M, Dowhan W, Arleth L, Pomorski TG. (2014), Biosynthetic preparation of selectively deuterated phosphatidylcholine in genetically modified Escherichia coli., Appl Microbiol Biotechnol. 2014 Oct 11.

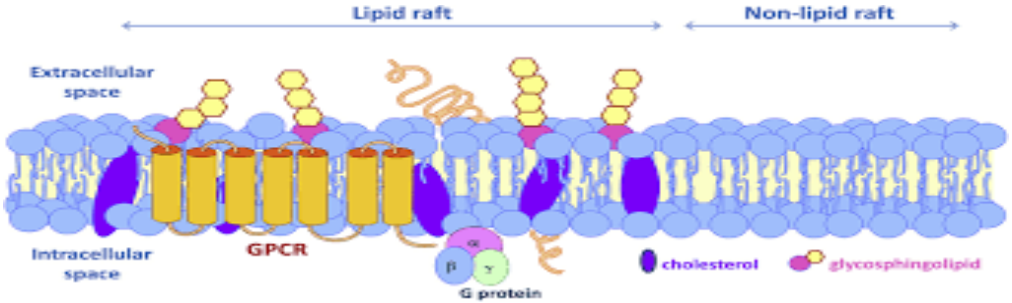
Josts I., Nitsche J., Maric S., Mertens H.D., Moulin M., Haertlein M., Svergun D.I., Busch S., Forsyth V.T., Tidow H. Conformational states of the ATP-binding cassette (ABC) transporter protein MsbA in lipid environment solution investigated by small-angle scattering using stealth carrier nanodiscs, *Structure* **26**, 1072-1079 (2018

Nitsche J., Josts I., Heidemann J., Mertens H.D., Maric S., Moulin M., M Haertlein M., Busch S., Forsyth V.T., Svergun D.I., Uetrecht C., Tidow H. Structural basis for activation of plasma-membrane Ca 2+-ATPase by calmodulin, *Communications Biology* 1 (**1**), 206 (2018).

Expression of deuterated cholesterol in genetically engineered *Pichia pastoris* cells

Harald Pichler

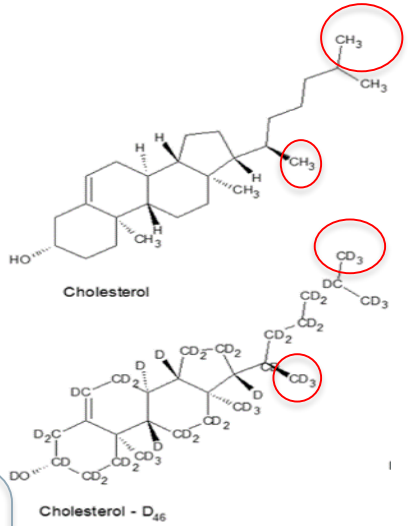
- **Cholesterol is an essential lipid component of mammalian membranes**
- It plays a pivotal role in determining the fluidity as well as the local architecture of the membrane
- It influences the structure, function and activity of a wide range of higher-eukaryotic integral membrane proteins
- Lipid rafts generally contain 3 to 5-fold the amount of cholesterol found in the surrounding bilayer- Lipid rafts play important role in signal transduction and act as a platform for virus entry



Hirz, M., Richter, G., Leitner, E. *et al.* A novel cholesterol-producing *Pichia pastoris* strain is an ideal host for functional expression of human Na,K-ATPase α3β1 isoform. *Appl Microbiol Biotechnol* **97,** 9465–9478 (2013).

Expression of deuterated cholesterol in genetically engineered *Pichia pastoris* cells

- *E.coli* and yeast cells do not synthesize cholesterol
- It is very difficult to produce perdeuterated cholesterol chemically
- Metabolically engineered strains of *Pichia pastoris* have been designed to produce cholesterol instead of its natural ergosterol
- This organism has been adapted to growth in deuterated minimal medium.
- d-cholesterol was purified, and characterised by mass spectrometry and gas chromatography.



The level of deuterium incorporation was greater than 98%.

The production of matchout cholesterol was also developped for SANS applications

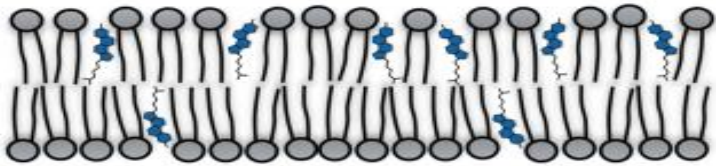
M.Moulin, G.A. Strohmeier, MHirz, K. C. Thompson, A. R. Rennie, R.A. Campbell, H. Pichler, S. Maric, V. T. Forsyth, M. Haertlein,Perdeuteration of cholesterol for neutron scattering applications using recombinant Pichia pastoris,Chemistry and Physics of Lipids,Volume 212,2018,Pages 80-87,.

Cholesterol – example of a metabolically engineered strains for the production of small deuterated biomolecules

- Hydrogenated cholesterol has about the same SLD as lipids and is **not distinguishable** from hydrogenated lipids
- **Perdeuterated cholesterol** provides **strong contrast** against all lipids.

Structural informations from neutron reflectometry studies: the location of deuterated cholesterol in a membrane bilayer

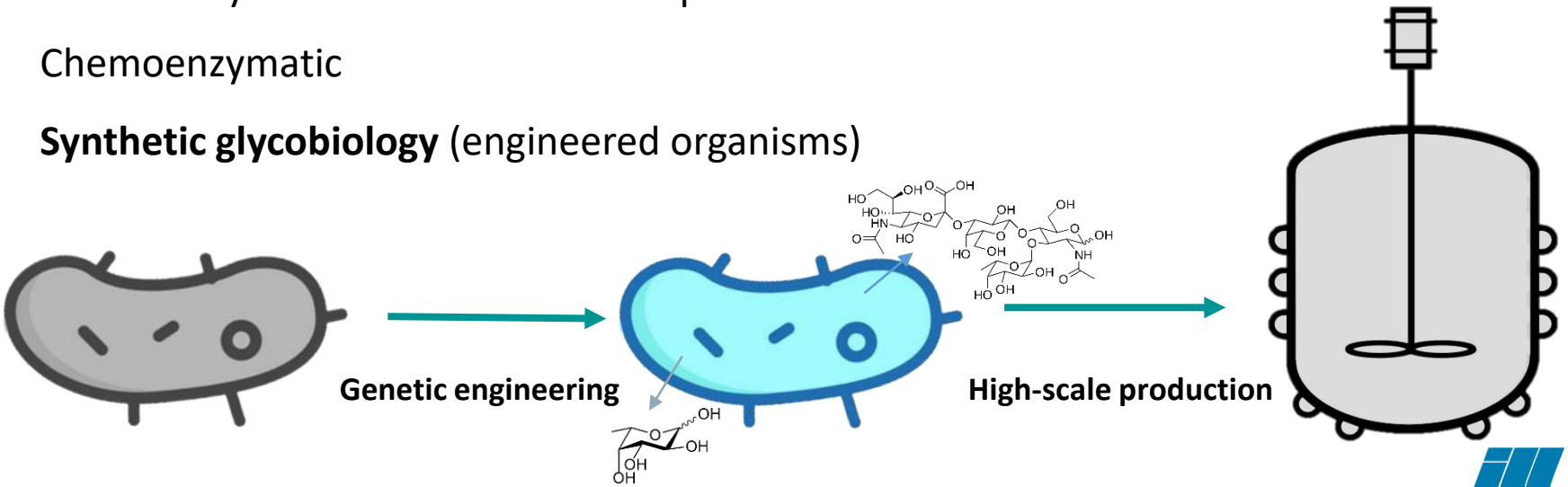
The analysis of the location of cholesterol within lipid bilayers composed of natural mixture of phosphatidylcholine (PC) demonstrates that cholesterol is **located closer to the lipid head group-tail interface** (Waldie *et al.,*2018).



Waldie, S., Moulin, M., Porcar, L. *et al.* The Production of Matchout-Deuterated Cholesterol and the Study of Bilayer-Cholesterol Interactions. *Sci Rep* **9,** 5118 (2019).
Y. Correa, S.Waldie, M.Thépaut, S. Micciulla, M. Moulin, et al.. SARS-CoV-2 spike protein removes lipids from model membranes and interferes with the capacity of high density lipoprotein to exchange lipids. *Journal of Colloid and Interface Science*, Elsevier, 2021, 602, pp.732-739

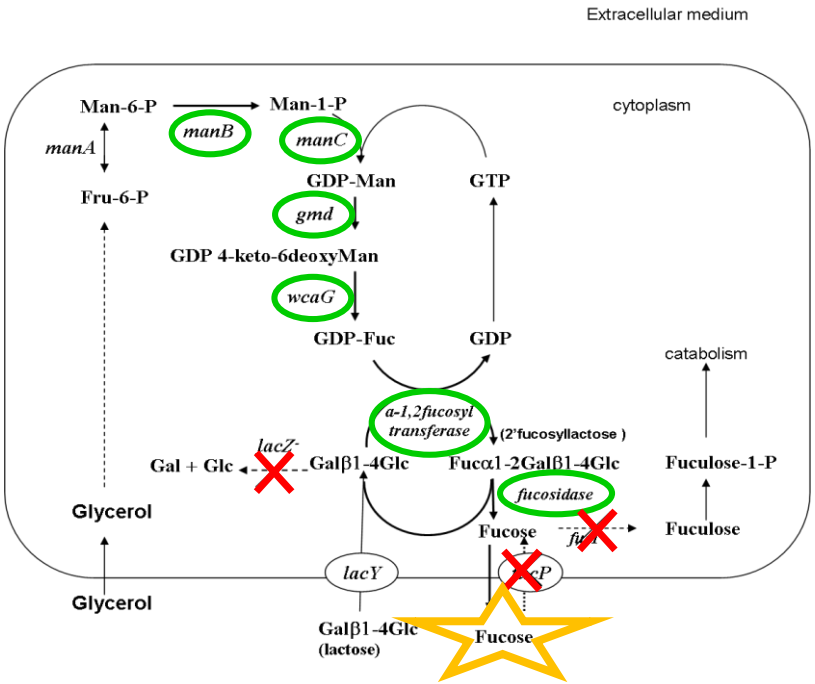
Production of perdeuterated carbohydrates

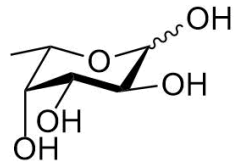
- Glucose-d_{12} from algea grown in D_2O, hydrolysis of cellulose
- Direct deuteration on Raney nickel catalyst
- Chemical synthesis from deuterated precursors
- Chemoenzymatic
- **Synthetic glycobiology** (engineered organisms)



In vivo production of L-fucose-d_{12} in *E. coli*

Fucose-producing strain of *E. coli* designed and engineered by Dr. Eric Samain at CERMAV





Overexpressed genes

manB: phosphomannomutase
manC: Man-1-P-guanyltransferase
gmd: GDP-Man 4,6-dehydratase
wcaG: GDP-L-fucose synthase
α-1,2-fucosidase
α-1,2-fucosyltransferase

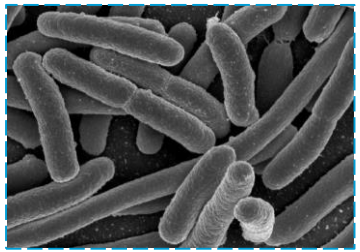
Knocked-out genes

lacZ: β-galactosidase
fucI: fucose isomerase
fucP: fucose permease

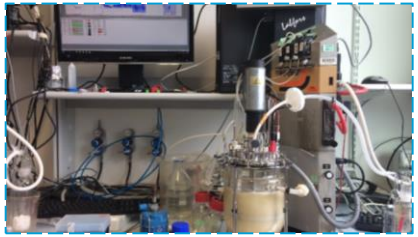
Production, purification and characterization of L-fucose-d_{12}

(J.Devos)

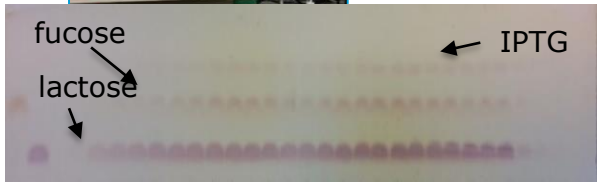
Adaptation to D_2O and glycerol-d_8



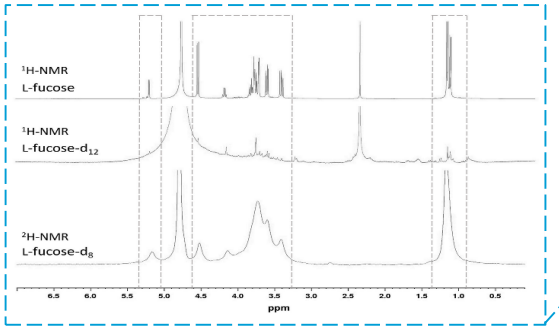
Batch production

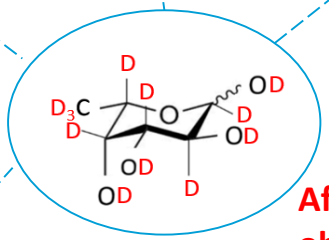


Purification



Characterization (Mass spec, NMR)



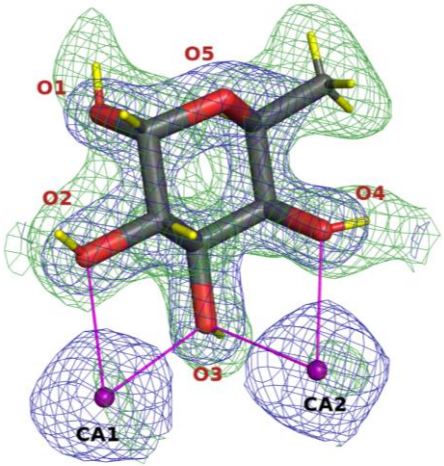


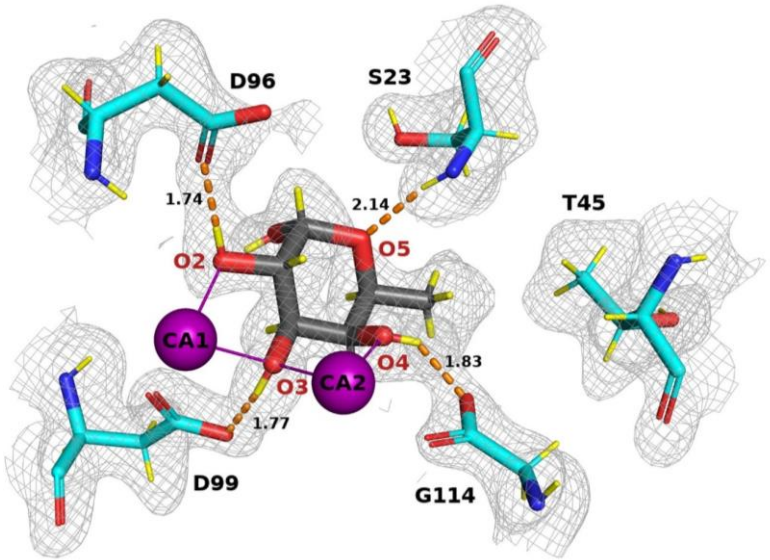
After purification, 220 mg of lyophilized Fuc-d12 was obtained from a culture using 1.5 L of D_2O and 45 g of deuterated glycerol

Perdeuterated fucose in the LecB binding site

LecB lectin
4 direct H-bonds (orange dashed lines) with the protein + hydrophobic interaction

- Electron density (1.4σ)
- Neutron density (2.2σ)





Gajdos L. et al., 2022, Nat Commun
Gajdos L. et al., 2021, Glycobiology

Tables of content

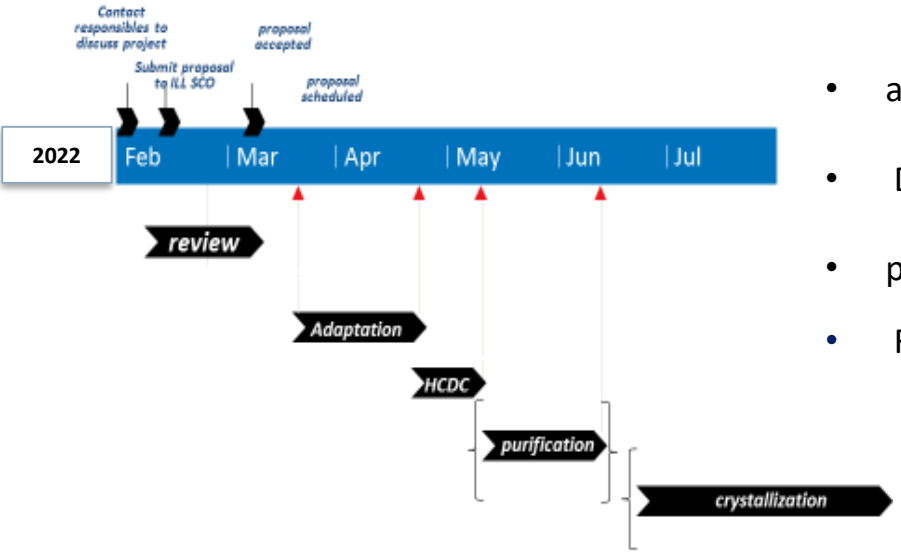
- Deuterium and D_2O properties
- Deuteration of biomolecules for neutron applications
- Effects of deuteration on living organisms
- Deuteration strategies
  - *In vivo* deuteration with recombinant organisms
  - *In vitro* deuteration
  - Deuterated peptide by solid phase peptide synthesis
- Deuterated samples used in recently published work
- Deuteration Lab platform– Access arrangements

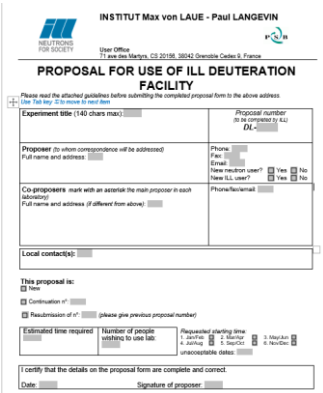
Deuteration Laboratory (D-Lab) platform for biological deuteration (ILL Life sciences group)

- **Deuterated proteins & other macromolecules** – different deuteration regimes (perdeuterated, matchout deuterated etc)
- **Small biomolecules** (eg perdeuterated/matchout cholesterol, triglycerides, fatty acids, fucose)
- **Crystallisation** / sample preparation

Deuteration Laboratory (D-Lab) platform for biological deuteration– Access arrangements

Timeline of a typical deuteration project





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PROPOSAL FOR USE OF ILL DEUTERATION FACILITY

- access via peer-review system
- Dlab proposals can be submitted at any time through ILL user office
- peer-reviewed by a panel of international experts within 2 weeks
- For accepted proposals, the ILL covers the costs of D_2O and deuterated carbon source

Deuteration Laboratory (D-Lab) platform for biological deuteration (ILL Life Sciences Group)

M.Moulin V.Laux J.Devos

Further information: http://www.ill.eu/deuteration

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Thank you for your attention !

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NEUTRONS FOR SOCIETY