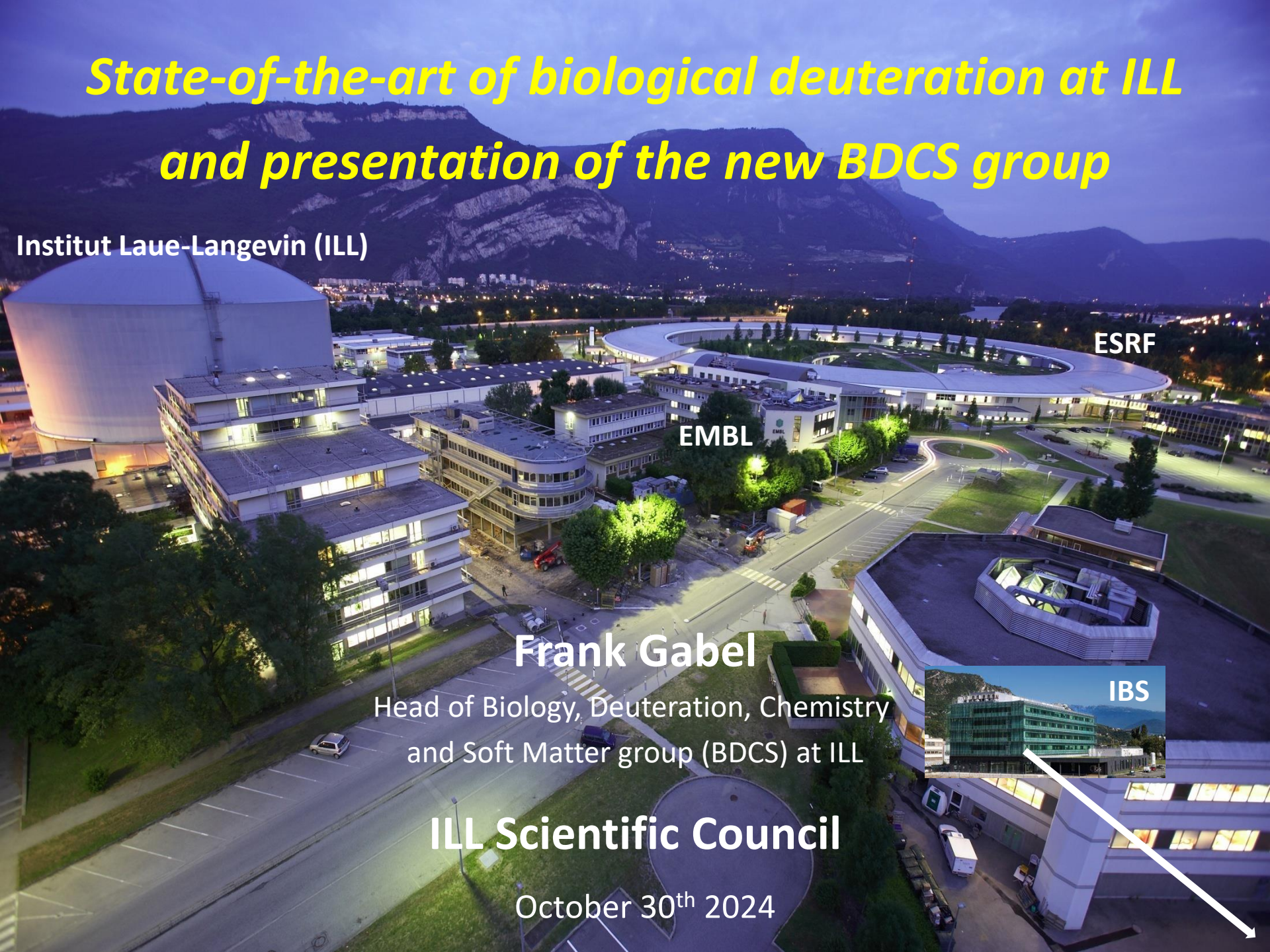
State-of-the-art of biological deuteration at ILL and presentation of the new BDCS group



Frank Gabel

Head of Biology, Deuteration, Chemistry and Soft Matter group (BDCS) at ILL

ILL Scientific Council

October 30th 2024

The D-and L-labs
and the new BDCS group
at ILL

The BDCS group (Biology, Deuteration, Chemistry and Soft Matter) at ILL: getting the best out of your neutron experiment!

Soft Matter Support Facilities (PSCM)

Infrastructure for ambitious large-scale soft matter research projects

The soft matter facilities at the ILL aim to offer a wide range of complementary techniques to the user community to allow improved sample preparation and beam-time optimisation.

Most studied topics

- Biomembranes and lipid assemblies
- Colloidal self-assembly (surfactants, polymers, microgels, etc…)
- Smart Coatings
- Structure, dynamics, and function of proteins

Our major equipment

Light scattering

- LS 3D static and dynamic light scattering
- ALV multiangle static and dynamic light scattering
- Cordouan portable light scattering
- Malvern particle size analyser

Solid and Liquid interfaces

- Beaglehole Picometer Light Ellipsometer
- Brewster Angle Microscope Nanofilm EP3
- 5+ Langmuir troughs
- Krüss K11 Tensiometer and DS114 Drop shape analyser
- Q-Sense Quartz Crystal Microbalance E4

Calorimetry and volumetry

- 2 Differential scanning calorimeters (solids and liquids)
- 2 Densitometers and sound velocity meters

Spectrophotometry

- UV-Vis spectrophotometer
- FT-IR spectrophotometer

Others

- Teclis Foamscan Foam analyser
- Anton-Paar MCR 501 Rheometer
- Brucker Time-Domain NMR
- Extruders for vesicle preparation

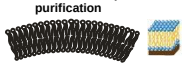
People: Leonardo Chiappisi, Martina Sandroni, Sandrine Verdon

ILL Lipid Lab (L-Lab)

Developing Advanced Models of Biological Membranes

- Neutron scattering techniques are ideally suited for the study of lipid bilayers that are major components of cellular membranes.
- There's a dearth for biologically relevant deuterated lipids which is both expensive and difficult to synthetise them through chemical synthesis.
- Ideally, reconstituted microbial lipids extracted and purified from cells grown under deuterated conditions should work as model cellular membranes.
- The L-Lab has been successful in optimising methods to extract, purify and characterise deuterated lipids produced in yeast and bacteria.

HPLC coupled to an ELS –detector for lipid purification



Recreating biomimicking model membranes

People: Krishna Batchu

Chemistry Lab Support Facilities

Enabling on-site sample handling and preparation

The laboratories are typically used for sample preparation, transfer to the sample holders, buffers preparation, substrates cleaning, sample dilution and deposition, sample hydration, dialysis, or thermal treatments before or during experiments. They are used by both soft and hard matter users.

Laboratory equipment

Besides standard equipment (fume hoods, scales, stirrers, pH-meters, ultrapure water, …) the laboratories are equipped with:

- Glovebox and glovebags under inert atmosphere
- Freeze dryer
- Cold room and -80 °C freezer
- Centrifuges (standard, large volumes)
- Ovens and high-temperature furnaces (up to 1600 °C)
- SafeTech fume hood for dry nanoparticles manipulation
- Schlenk line

Chemicals

A stock of standard chemicals is available. The needs for D_2O or specific chemicals are assessed before each experiment. The users can request chemicals to be ordered by ILL to be available onsite during the experiments. Costs will be invoiced after the experiment.

People: Martina Sandroni, Sandrine Verdon

ILL Deuteration Lab (D-lab)

Biomacromolecular Deuteration for Neutron Studies in Biology

The ILL D-Lab has been operating for more than 20 years as a dedicated platform to support the deuteration of biological molecules for neutron scattering experiments.

Activities of the D-Lab:

- Production of deuterated biomolecules with different labelling regimes for structural biology and dynamics via a proposal system.
- Method development activity for advanced labelling strategies.

Biological organisms used in the D-lab

E. coli

Yeast

Algae

Capability selections

- High cell-density culture for *E. coli*, yeast and algae.
- Purification of recombinant proteins and lipids by FPLC, HPLC and flash prep chromatography.
- Quality control of produced biomolecules.
- Characterisation of small molecules using an HPTLC system.
- Peptide synthesis, purification and characterisation.
- Protein crystallisation.

People: Valérie Laux, Juliette M. Devos, Martine Moulin

NEUTRONS FOR SOCIETY

PSB THE PARTNERSHIP FOR STRUCTURAL BIOLOGY

PSCM PARTNERSHIP FOR SOFT CONDENSED MATTER

Locations at ILL

Chemistry lab (Science Building)

Martina Sandroni

Sandrine Verdon

PSCM/ILL (Science Building)

Leonardo Chiappisi

Joint staff with PSB partners (CIBB building)

Florent Bernaudat

Jennyfer Gauthier

Lipid lab (Science Building)

Krishna Batchu

Technical support (Science Building)

Wayne Clancy

Assistant (Science Building)

Emily Ryan

Deuteration lab (CIBB Building)

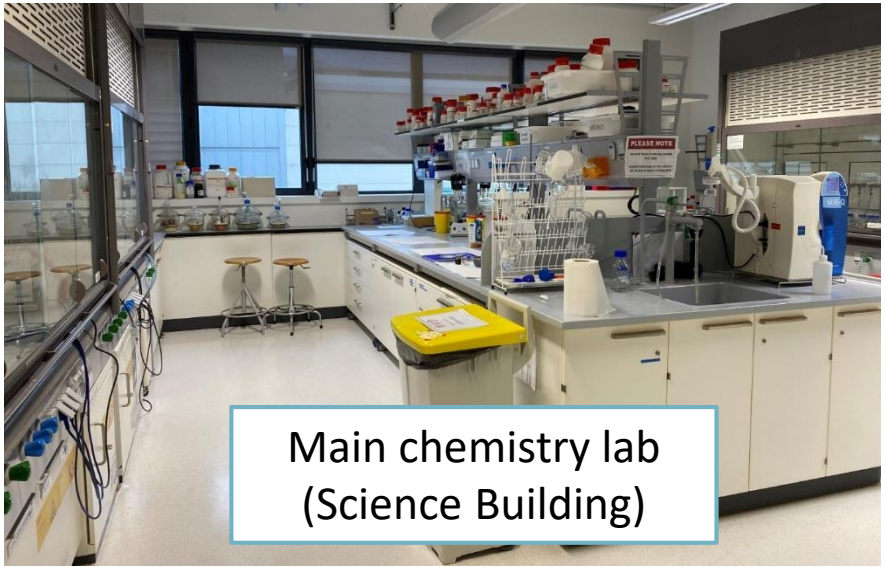
Martine Moulin

Juliette Devos

Valérie Laux

The ILL laboratories: chemistry…

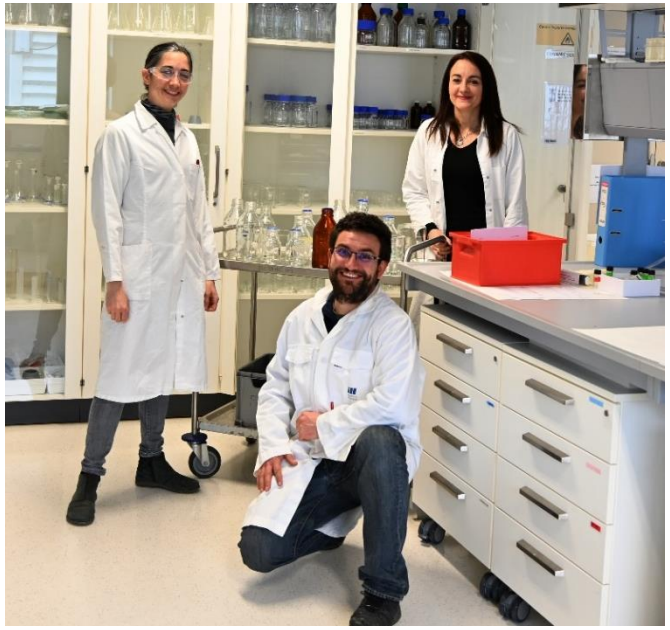
Science Building and Guide Halls



Main chemistry lab (Science Building)

FIG/IN5

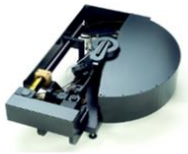
ILL22



https://www.ill.eu/de/users/support-labs-infrastructure/chemistry-laboratories

…and PSCM

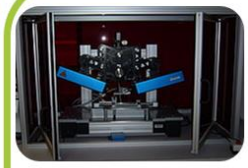
Partnership for Soft Condensed Matter
(with ESRF)


ALV multi-angle static and dynamic light scattering

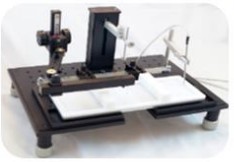

Malvern particle size and zeta-potential


Cordouan DLS

3D LS static and dynamic light scattering


Accurion Nanofilm EP3 BAM


Beaglehole picometer ellipsometer


5 different Langmuir troughs


Multi-cell DSC for liquid samples


DSC for solid samples


Density meter


Density and sound velocity meter


Krüss K11 Tensiometer


Q-sense E4 QCM-D


Lipid extraction facility


FT-IR spectrophotometer


UV-Vis spectrophotometer


Drop shape analysis / Contact angle


Teclis Foam analyser


Rheometer Physica MCR 501


Self-Diffusion NMR

THE EUROPEAN NEUTRON SOURCE

https://pscm-grenoble.eu/

Evolution of the D-lab

12/2021

Departure T. Forsyth as head of the Life Sciences Group

Retirement of M. Härtlein as head of Deuteration Lab

12/2021 to 12/2023

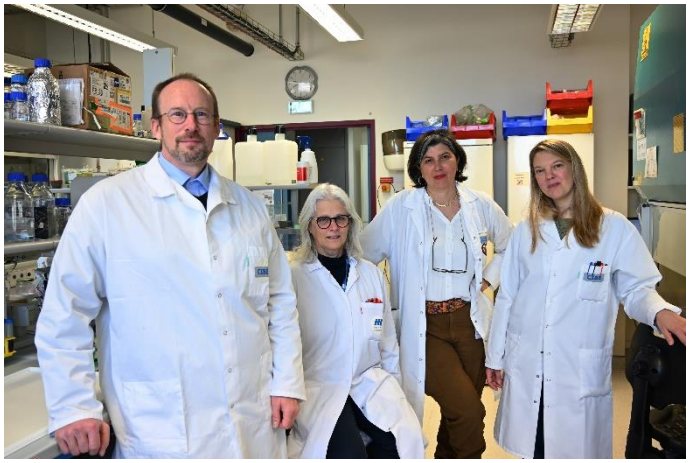
Jacques Jestin

M. Moulin V. Laux J. Devos

Co-responsibles of the deuteration platform

Since 12/2023

(5-year detachment from CEA)



Staff associated with L-lab staff over the years

Giovanna Fragneto

Science Director, ESS

Hanna Wacklin

Scientist, ESS

Anne Martel

Instrument responsible, D22, ILL

Rachel Morrison

Post-doc

Robin Delhom

PhD student

Alexis de Ghellinck

PhD student

Giacomo Corucci

PhD student

Ralf Schweins

LSS group leader

Krishna Batchu (2018)

EUROPEAN SPALLATION SOURCE (20%)

PARTNERSHIP FOR SOFT CONDENSED MATTER

Outreach and interaction with the user community and our local partners

Stimulating new collaborations within ILL and external user community:

- Co-supervision of ILL PhD students
- Welcoming visitors/scientists from collaborators
- AMBER research fellow project

© Communication office ILL

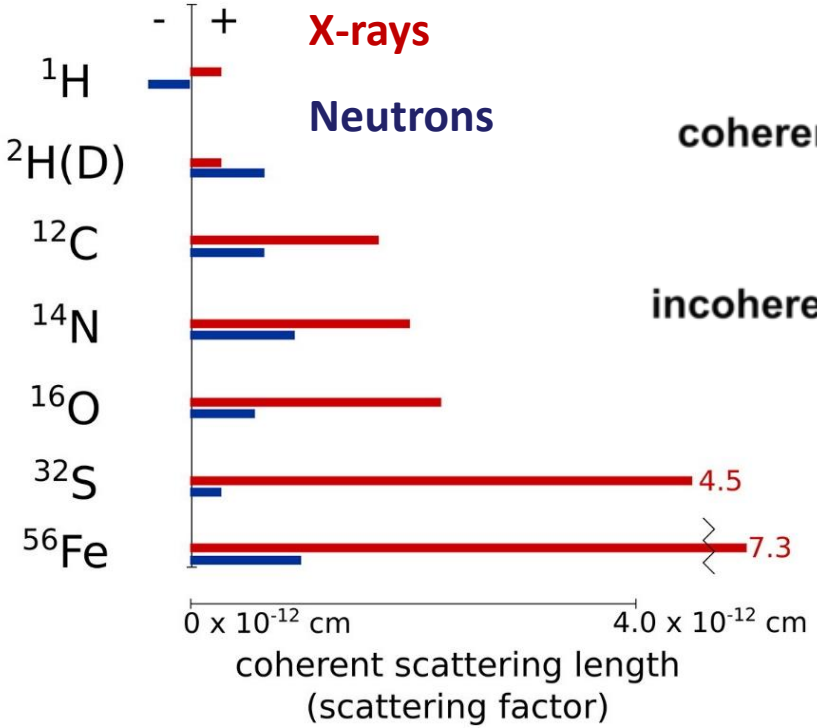
Promoting neutrons to the (local and global) user community:

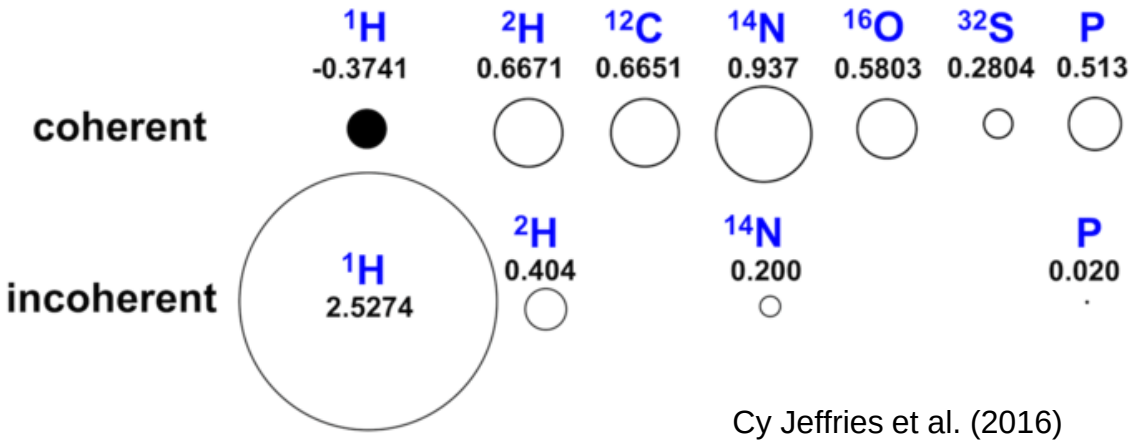
- PSCM User Meeting (February 2024)
- PSB Spotlight on “Neutrons in Biology” (June 2024)
- EMBO SAXS/SANS practical course (September 2024)
- Support-lab/DEUNET meetings

Why deuteration

for neutron scattering?

Neutron scattering in biology: why deuteration?





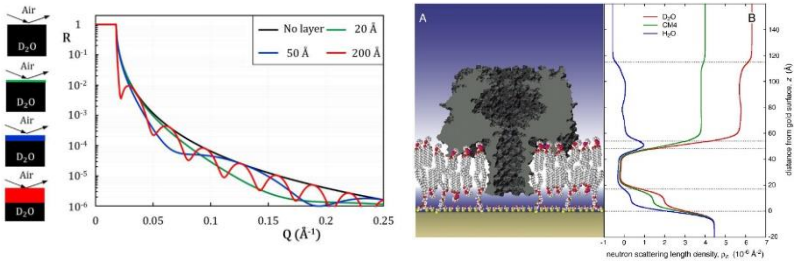
Cy Jeffries et al. (2016)
Nat. Protocols 11(11), 2122

Castellanos et al. (2017)
Compt. Struct. Biol. J. 15, 117-130

- Neutrons induce (almost) **no radiation damage**
- Low energy of neutrons allows to monitor **thermal motions in samples**
- Neutrons have spin and are **isotope-sensitive** (**H^{1}** vs **H^{2}=D**)
- **Structure** and **dynamics** can be probed (**coherent** vs **incoherent** scattering)

Different neutron scattering techniques in biology: what kind of deuteration makes sense?

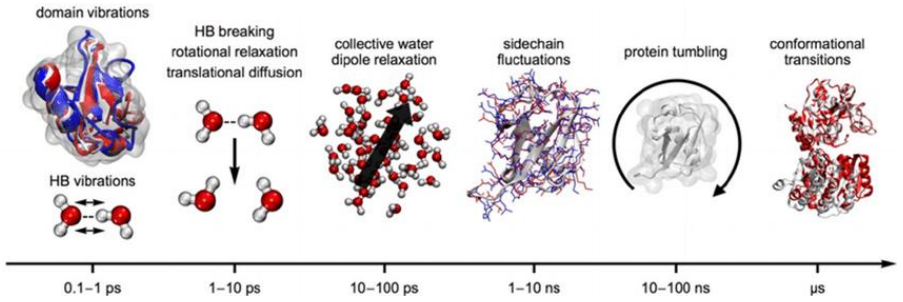
Reflectometry



Cousin & Fadda (2020) ***EPJ Web of Conferences*** 236, 04001

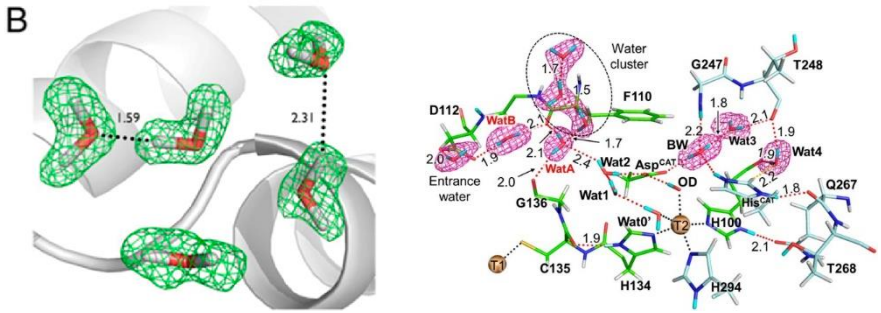
Heinrich & Lösche (2014) ***BBA*** 1838, 2341-2349

Spectroscopy



Xu & Havenith (2015) ***JCP*** 143, 170901

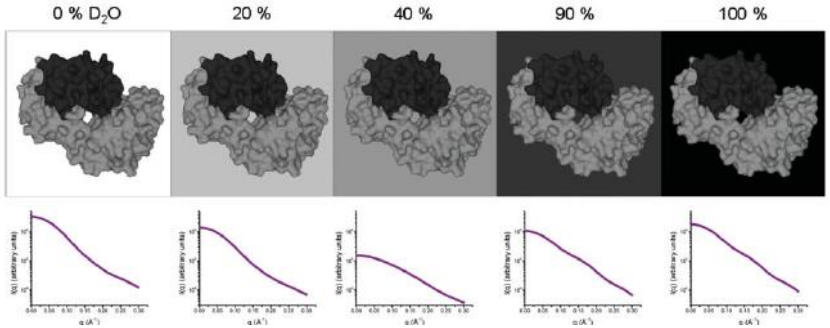
Crystallography



Oksanen et al. (2017) ***Molecules*** 22, 596

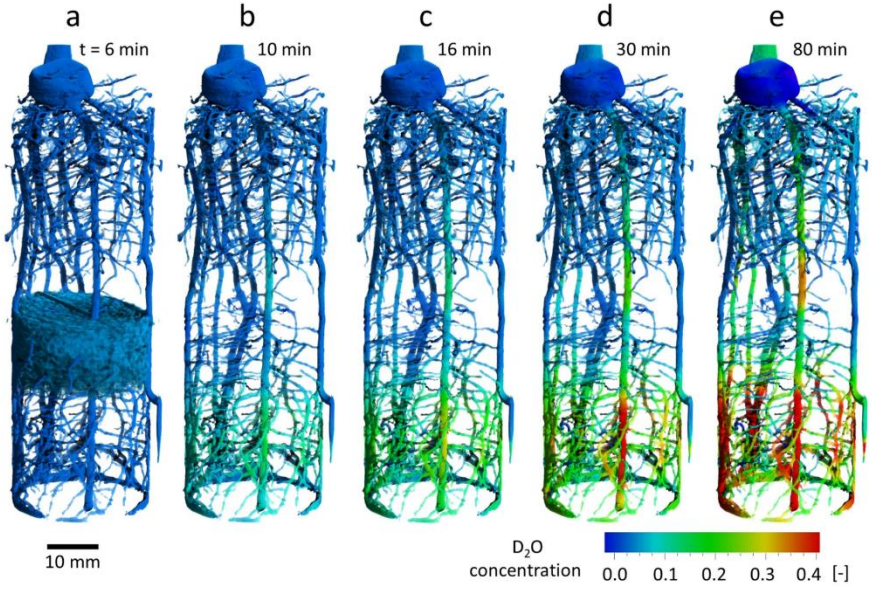
Fukuda et al. (2020) ***PNAS*** 117(8), 4071-4077

SANS



Jacques and Trewhella (2010) *Prot. Sci.* **19**, 642-657

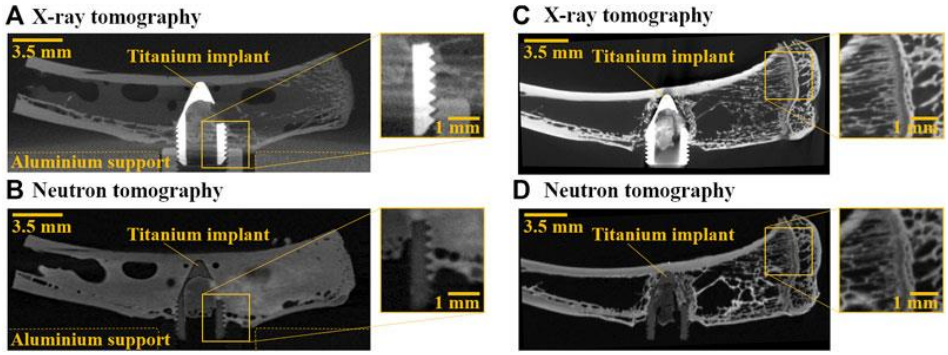
Neutron imaging:
water uptake in maize roots / bone structures



CONRAD (MLZ, Berlin)

Tötzke et al. (2021) ***Scientific Reports*** 11, 10578

(~100-200 µm)



NEXT (ILL, Grenoble)

Törnquist et al. (2021)

Physics in Medicine and Biology 66(13)

Tengattini et al. (2021)

Geomechanics for Energy and Environment 27, 100206

Getting to a 1 µm resolution!?

(ILL science strategy)

General expertise at D- and L-lab

> 20 years of biological deuteration and expertise at the ILL D-lab (and L-lab since 2017)

- **Deuteration of biomacromolecules** (proteins and nucleic acids):

  per-, matchout and specific deuteration regimes

- **Small molecules:** cholesterol, phospholipids, fucose…

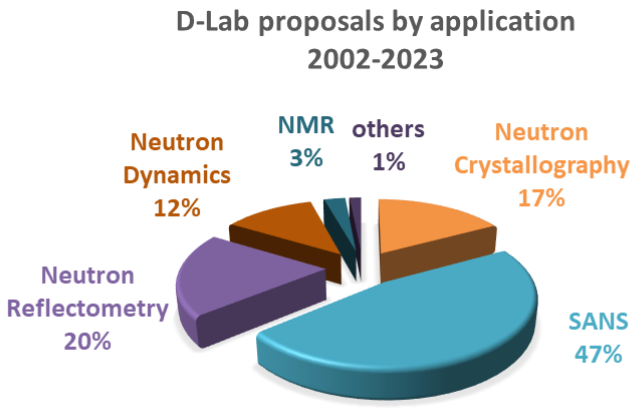
- **Biomass** for general use, plasmidic DNA

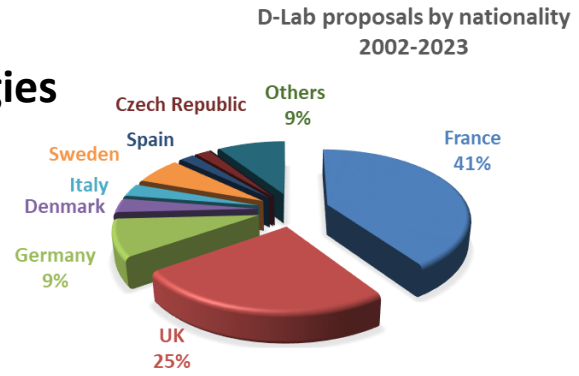
- **Advice** and **support** for sample preparation and crystal growth

- **Method development** activity for **advanced labelling strategies**

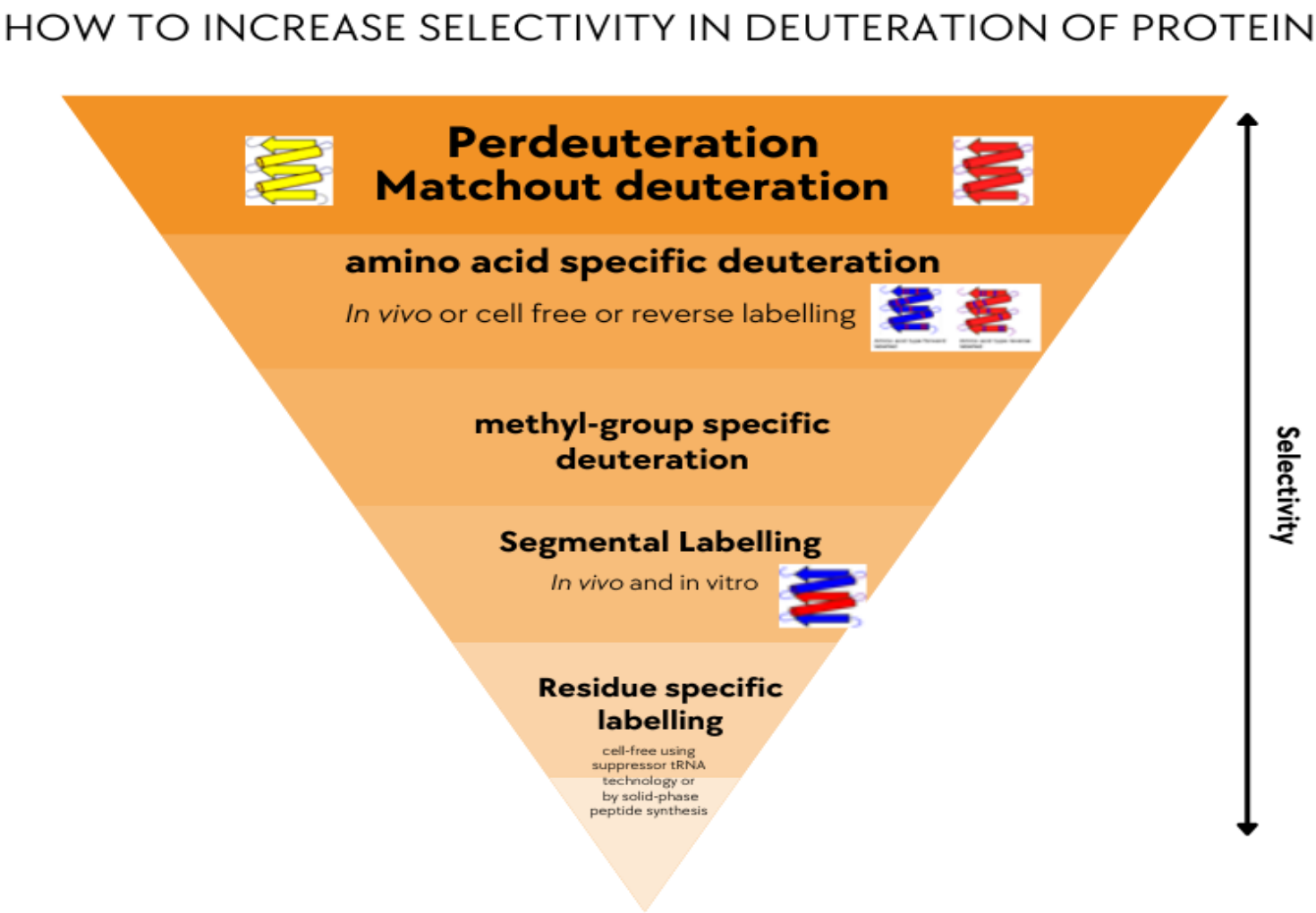
  (neutral lipids, sterols, hopanoids, peptides, cell free)

- **Teaching activities** (HERCULES, PSB, AILM…)

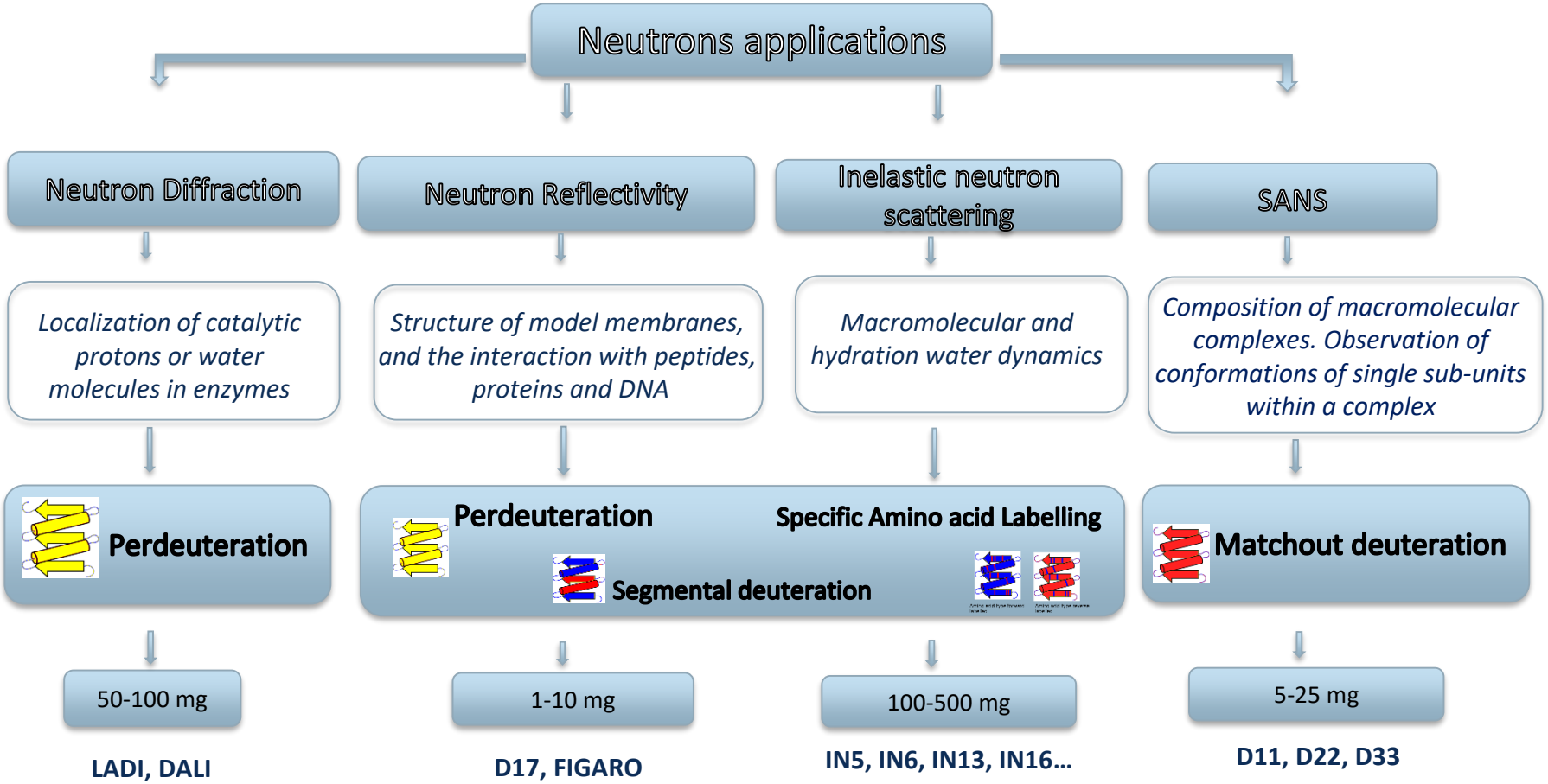




Overview of (biological) deuteration regimes



Choice of deuteration schemes and practical considerations



Principles and challenges of bio-deuteration

Effect of deuteration on higher organisms

	WATER	DEUTERIUM OXIDE
ATOMIC WEIGHT	18.01528 g/mol	20.0276 g/mol
BOILING POINT (25 °C)	100 °C	101.4 °C
DENSITY	997 kg/m³	1 105.9 kg/m³
FREEZING POINT (25 °C)	0 °C	3.8 °C

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

The growth of belladonna is inhibited by D_2O concentrations **above 50 per cent**.

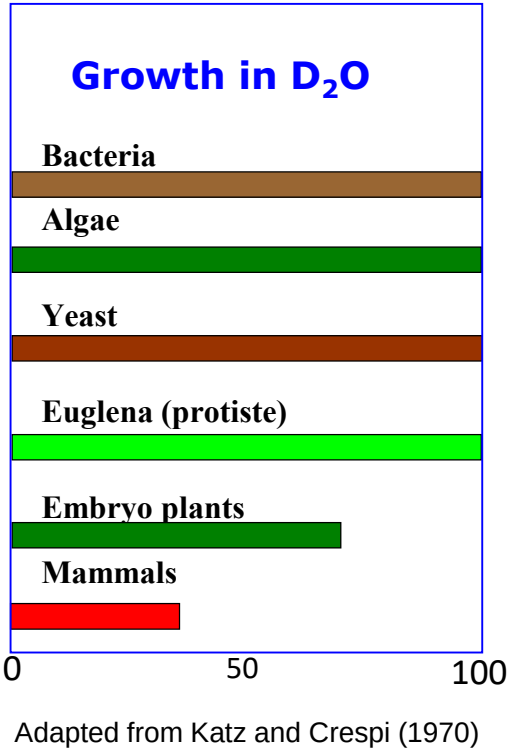
Systematic study of D_2O on mice
Barbour *et al.* (1934-1939)

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

In vivo toxicity of D_2O in humans was tested over a 4-month period when **0.5 %** of body water was replaced with D_2O without any adverse effect on the recipient
Steinberg et al. (1967)

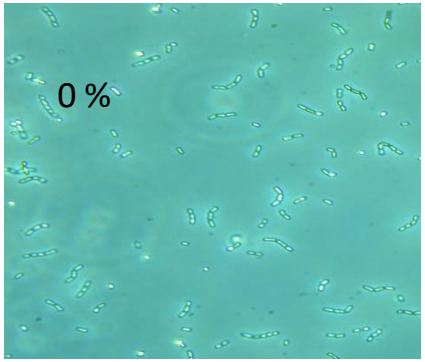
D_2O affects every process in the animal body (central nervous system, cardiac abnormalities and hormonal imbalances, disturbances in glucose metabolism…)

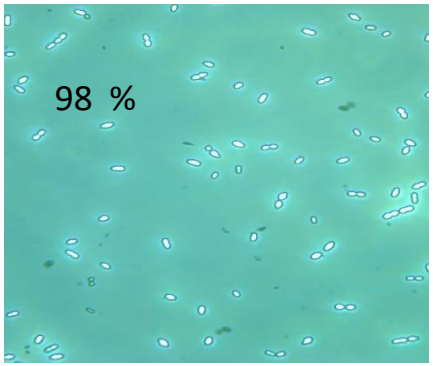
Adaptation of microorganisms to growth in D_2O: why is *in vivo* deuteration not straightforward?



Adapted from Katz and Crespi (1970)

Effects of deuterium on Cyanobacteria (*Synechococcus*)





Various morphological abnormalities such as discoloration, enlargement of cells or formation of clumps are visible

⇒ **adaptation may take weeks or several months !**

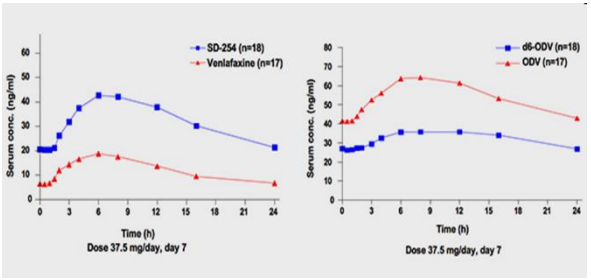
"Solvent Isotope Effect" (SIE) based on the properties of D_2O molecule as a whole, in particular its effects on the structure of water and the biological macromolecules.

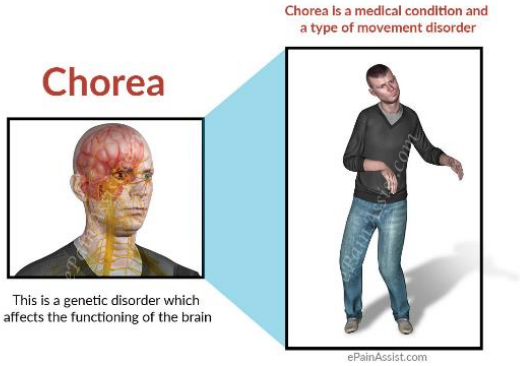
"Deuterium Isotope Effect« (DIE), resulting from the ability of D_2O to replace H with D in biological molecules. The C-D bond is several times stronger than the C-H bond and thus more resistant to enzymatic and even to chemical cleavage.

Deuteration does not always have undesired properties: “heavy hydrogen” drugs!

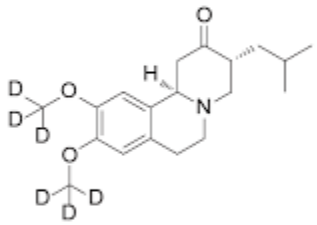
Selective replacements of hydrogen by deuterium result in new medicines that generally retain the full biochemical potency and selectivity of the original chemical entity that contains only hydrogen.

Pharmacokinetic properties are improved !





Deutetrabenazine



Deutetrabenazine (Austedo) is used for the treatment of chorea an abnormal involuntary movement disorder, associated with **Huntington’s disease**.

Deutetrabenazine is an isotopic isomer of **tetrabenazine** in which six hydrogen atoms have been replaced by deuterium atoms. The incorporation of deuterium slows the rate of drug metabolism, allowing **less frequent dosing**.

Teva Pharmaceuticals received approvals from the Food and Drug Administration to market deutetrabenazine in early 2017.

Drinking science: Deuterated ethanol and biochemical booze mimics

by **Craig Bettenhausen**

September 18, 2021 | A version of this story appeared in **Volume 99, Issue 34**

Putting the D in drinking

Ethanol gets the blame for hangovers and alcohol-related organ damage, but the main chemical culprit is a different molecule. As a first step in metabolizing ethanol, the body uses a liver enzyme called alcohol dehydrogenase to convert it to acetaldehyde, which is toxic."That acetaldehyde is a very bad actor," medicinal chemist Tony Czarnik tells Newscripts. "It cross-links your proteins, and that results in immune responses" that can cause inflammation, cirrhosis of the liver, esophageal cancer, and other ill effects.

Credit: William Ludwig/C&EN/Shutterstock

Alcohol alternatives: Chemists aim to keep the fun parts of drinking but leave the hangovers and organ damage behind.

Another enzyme, aldehyde dehydrogenase, comes along next and converts acetaldehyde to acetate, a relatively harmless molecule that the body can burn as fuel.

The problem is that acetaldehyde can accumulate in between those two enzyme-catalyzed steps, especially when heavy drinking swamps the whole alcohol-metabolizing system. Some people also have genetic factors that decrease the reaction rate or prevalence of aldehyde dehydrogenase. "That kind of got me thinking that if I can slow the rate of that first step, I can decrease the concentration of acetaldehyde present in your body at any given time," Czarnik says. He immediately thought that deuterated ethanol might do the trick.

MOST POPULAR IN FOOD

Welcome to the age of fermentation

What's in marshmallows, and how do the ingredients work together to make ooey-gooey treats?

Periodic Graphics: The chemistry of candy corn

Cadmium mapping would aid Colombian cocoa producers

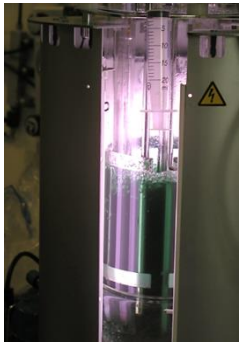
How is coffee decaffeinated, and is it safe to drink?

Specific expertise for tailor-made neutron samples

Specific expertise at the D- and L-labs

- **High cell density culture (HCDC) of bacteria (*E. coli*), yeast and algae**
- **Purification of recombinant proteins and lipids by FPLC, HPTLC and Flash Prep chromatography**
- **Characterization of small biomolecules *via* a HPTLC system**
- **Protein crystallization**
- **Peptide synthesis, purification and characterization**

HPTLC



Photobioreactor

Fermentor

Büchi chromatography

HPTLC

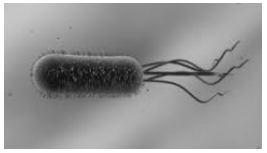
GAS CHROMATOGRAPH

HPLC

MASS SPECTROMETER

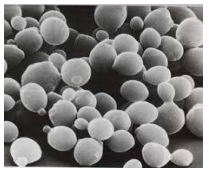
High Cell Density Cultures (HCDC)

- **Optimize volumetric yield**
- **Maximize biomass productivity**
- **Grow cells under controlled conditions**
- **Highest biomass/substrate yield** (≈ 1 g of cell paste / g of carbon source used)
- **€€€, in particular for perdeuteration!**

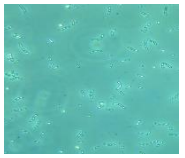


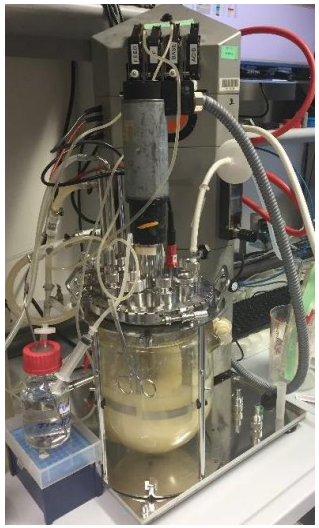
Bacterial expression systems

Yeast expression systems



Algae





D_2O = 1000 €/L

D_2O recycling process

Level of deuteration obtained ≈ **98%**
Savings: 1000 € /L around 20L/year are recycled: savings ≈ **20000 €**

Activated Charcoal

Distillation of D_2O

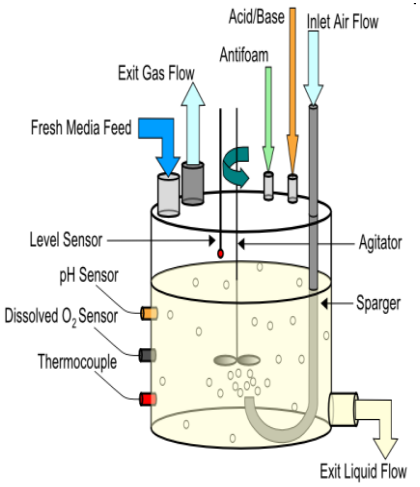
Recycled D_2O

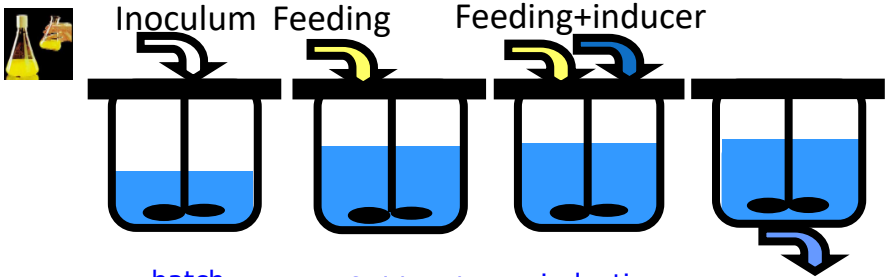
Bioreactor: recombinant fed-batch culture

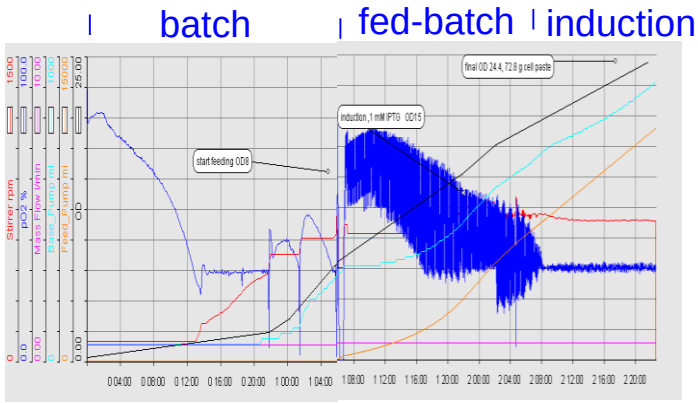
- Nutrients
  - D-glycerol = 25 €/g (50 g per fermentation)
  - D-methanol = 45 €/g (yeast culture)
  - D-amino acid = 1400 €/g (cell free)

  Fermentation run *(E.coli)* around **3500 €**

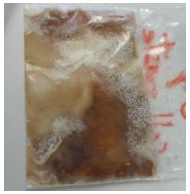
Perdeuterated (and matchout) labelling





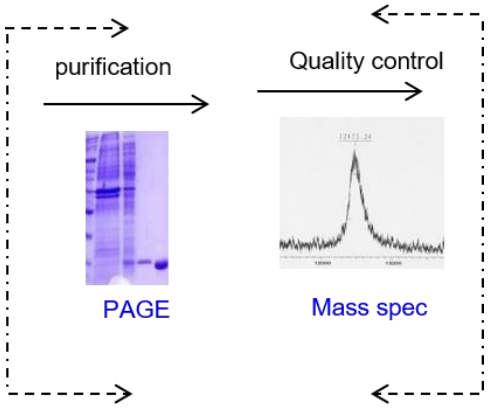


deuterated cell paste



Matchout Cost is more **reasonable** in comparison with perdeuterated culture:

- Use of recycled D_2O
- H-Glycerol as carbon source

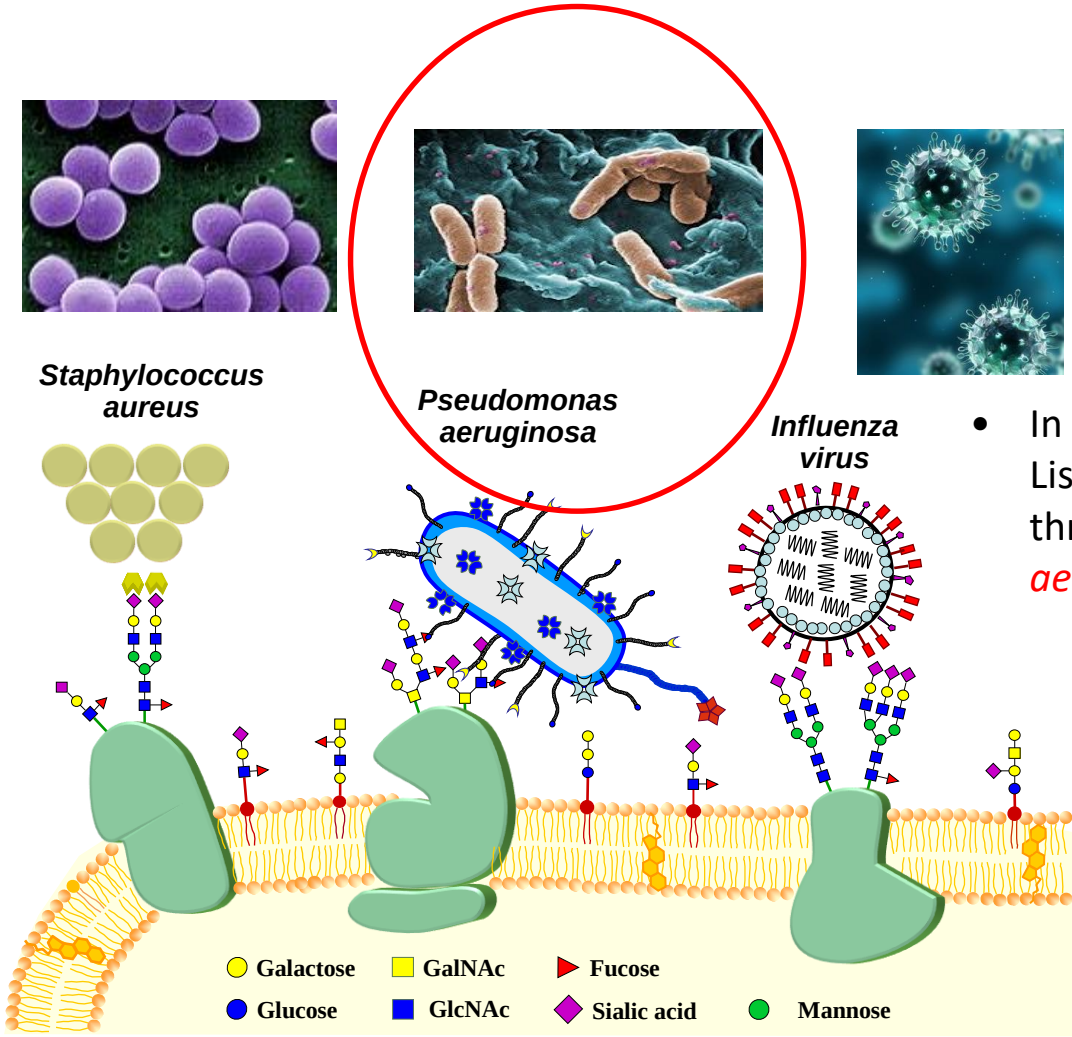


- **Only suited for organisms compatible with deuterated minimal media**
- **Optimization of protocols takes time**

Perdeuteration of biomolecules:

maximum contrast, minimum background!

Study of protein-carbohydrate interactions in bacterial infection



Between :
– glycans from the host cell (human)

– proteins from the pathogen : **LECTINS**

- In the WHO "2024 Bacterial Priority Pathogens List" (list of drug-resistant bacteria most threatening to human health): *Pseudomonas aeruginosa* – high priority pathogen

- Lectins : proteins that bind specifically and reversibly to glycans

- Many pathogens have lectins that can recognize specific sugars at the surface of human cells. First step of infection

Imberty & Varrot, *Curr. Opin. Struct. Biol.* 2008

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

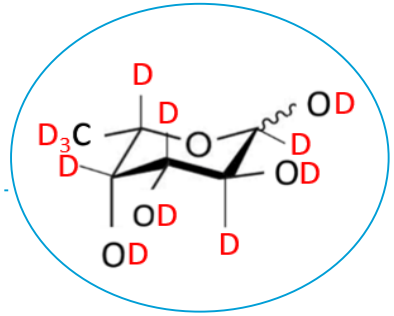
Synthetic glycobiology:

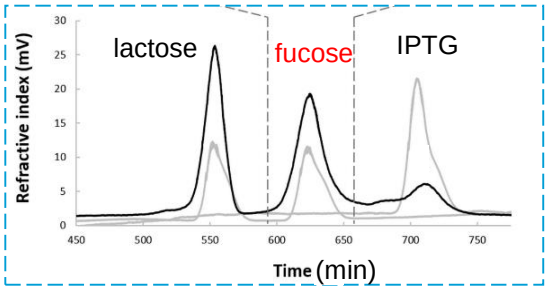
- A fucose-producing *E. coli* strain designed and enginereed at CERMAV
- Overexpressed and knocked-out genes

Collaboration CERMAV and ILL D-Lab.

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

- Adaptation to fully-deuterated growth medium
- High cell-density batch production
- Purification and characterization of deuterated fucose

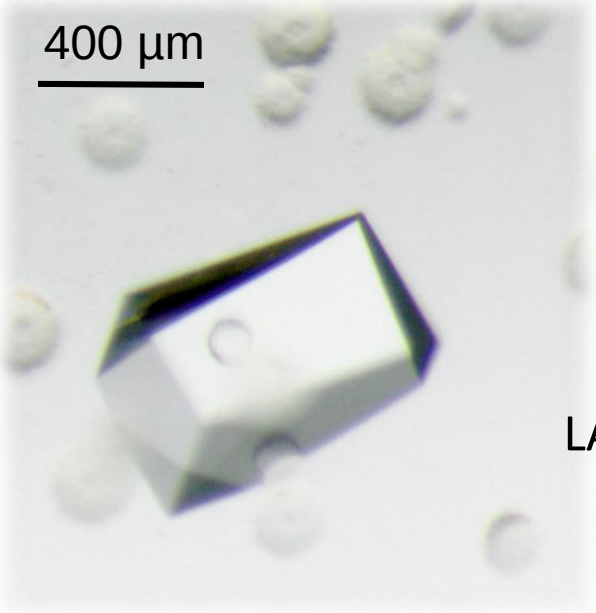




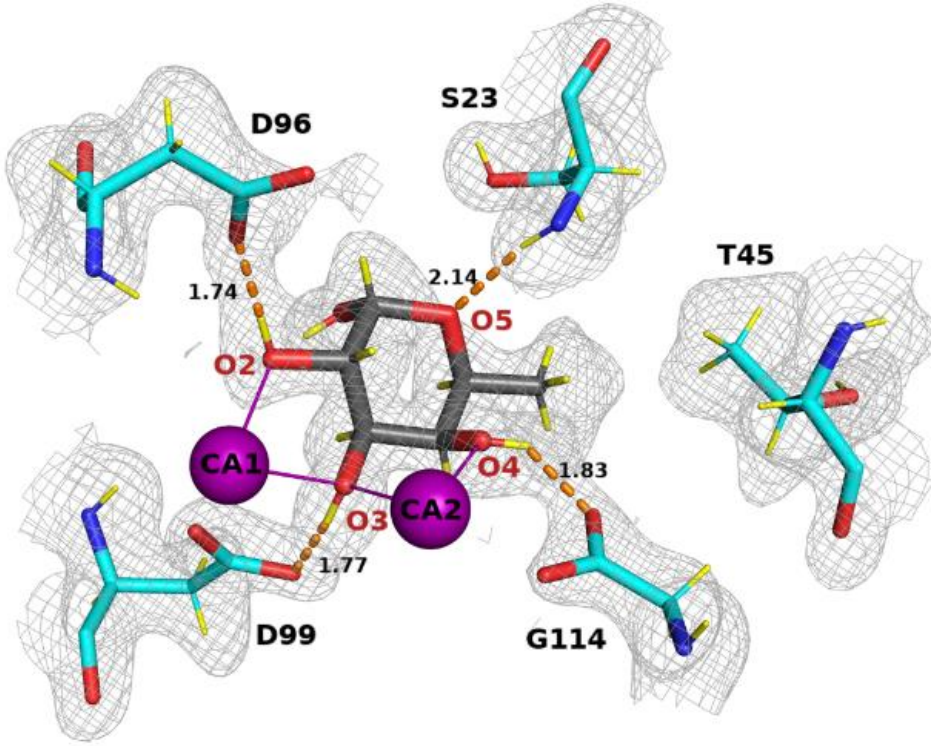
Gajdos et al. (2021) ***Glycobiology*** 31(2), 151-158.

Single crystal neutron diffraction

- Use of perdeurated LecB lectin and perdeuterated fucose (ILL D-Lab)



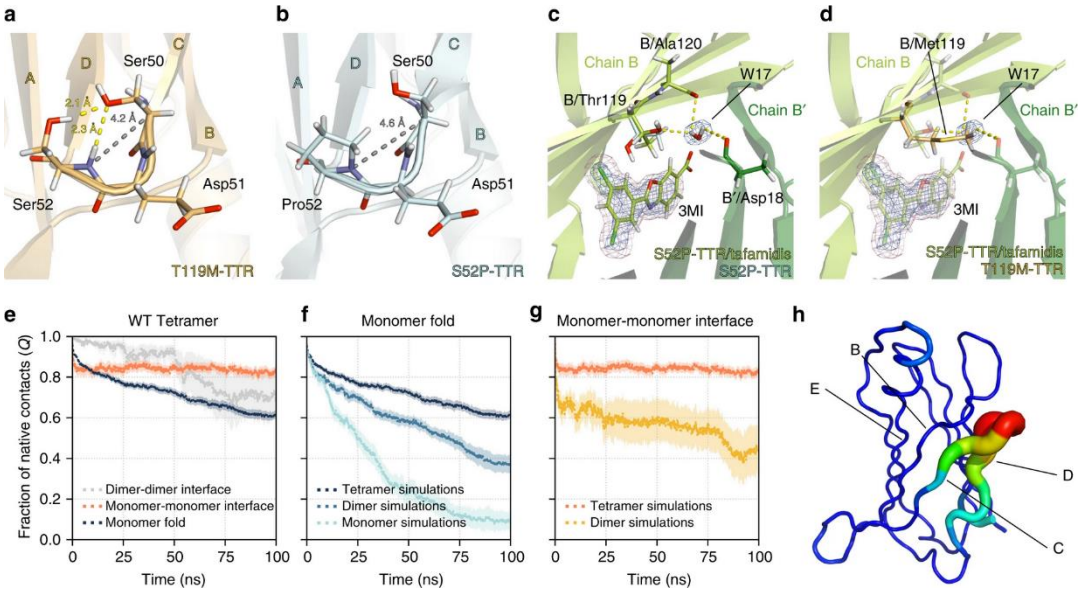
LADI-III



- Continuity of the neutron density map (H-bonding)
- Acidic residues in the binding site are all deprotonated
- Design of glycomimetics

Gajdos L. *et al.* (2022) ***Nat. Commun.*** 13(1), 194.

A molecular mechanism for transthyretin amyloidogenesis

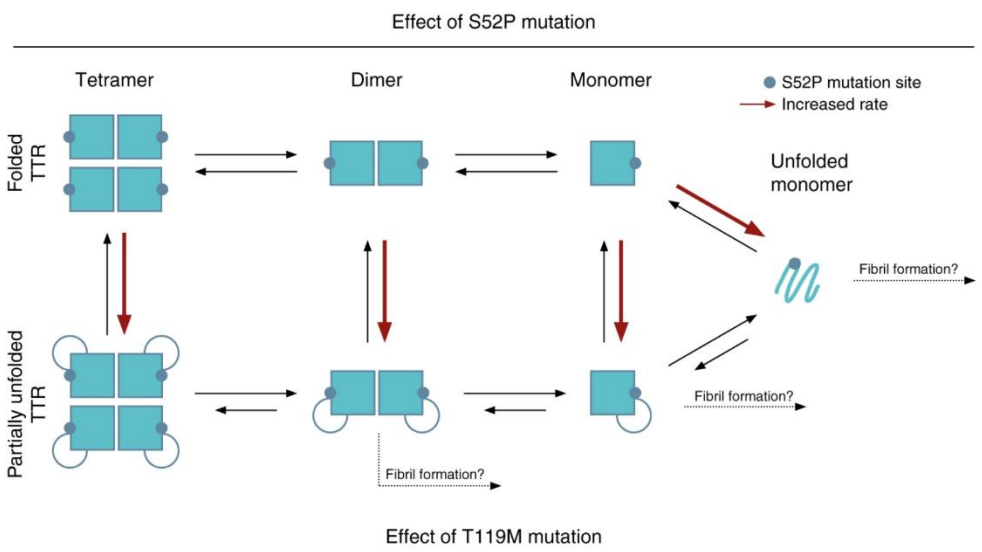


LADI III @ ILL (< 2 Å)

TTR mutations have beem identified in several severe familial amyloidoses: (e.g. polyneuropathy/myocardiopathy)

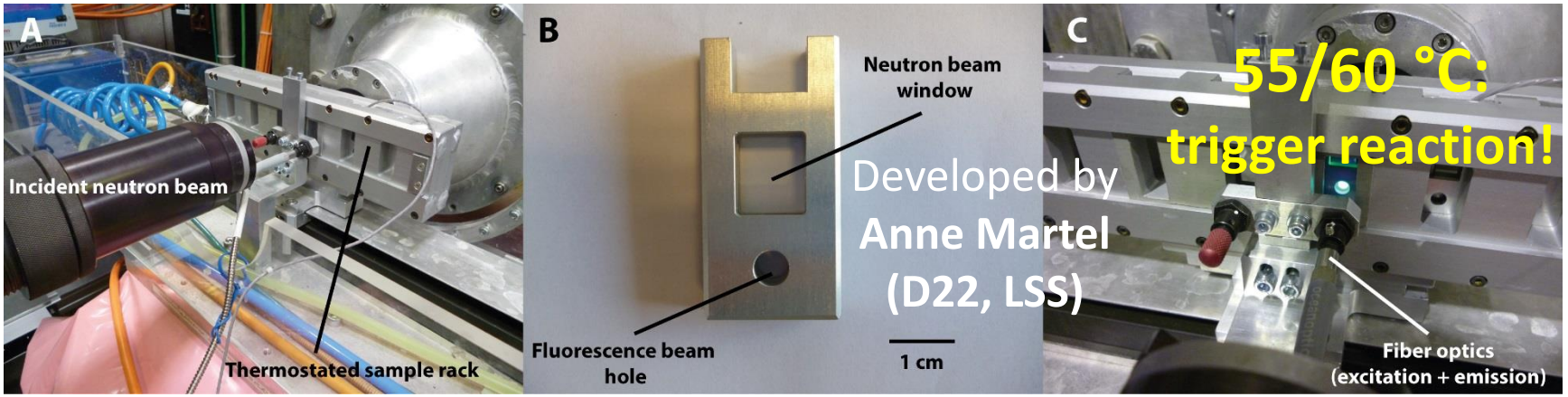
Perdeuterated protein

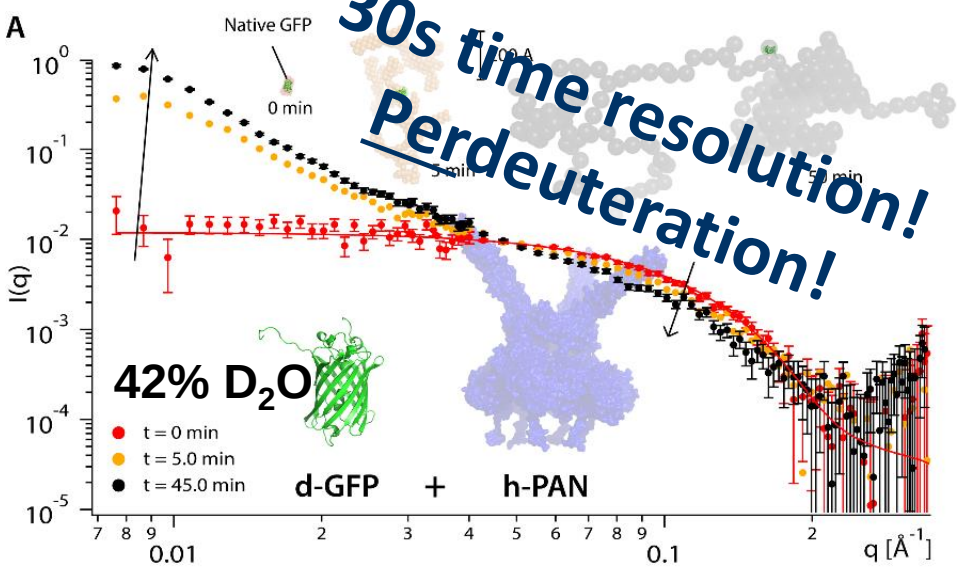
Atomic details of specific mutations induce local conformational flexibility: correlation with aggregation propensity?

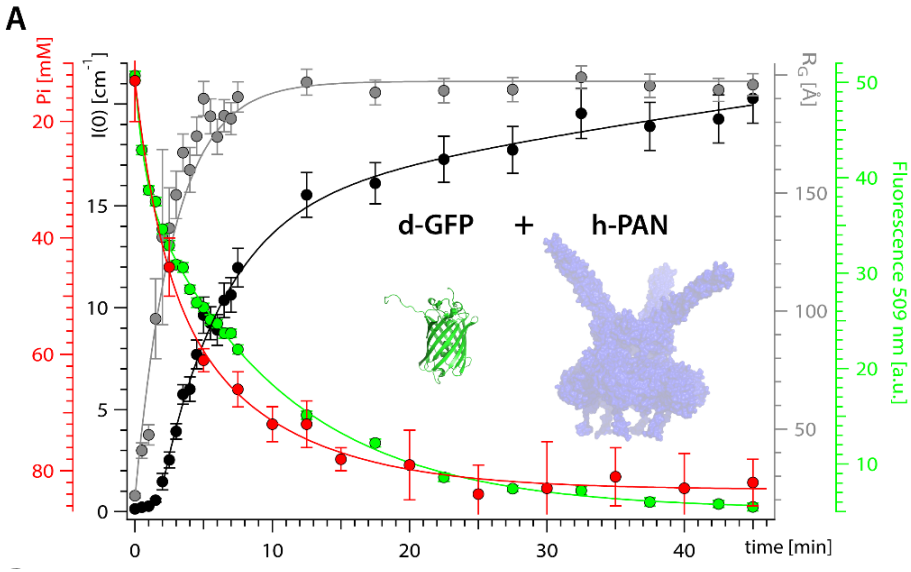


Yee et al. (2019) ***NatComm*** 10, 925

Perdeuteration for time-resolved (TR) SANS: watching protein degradation in real time

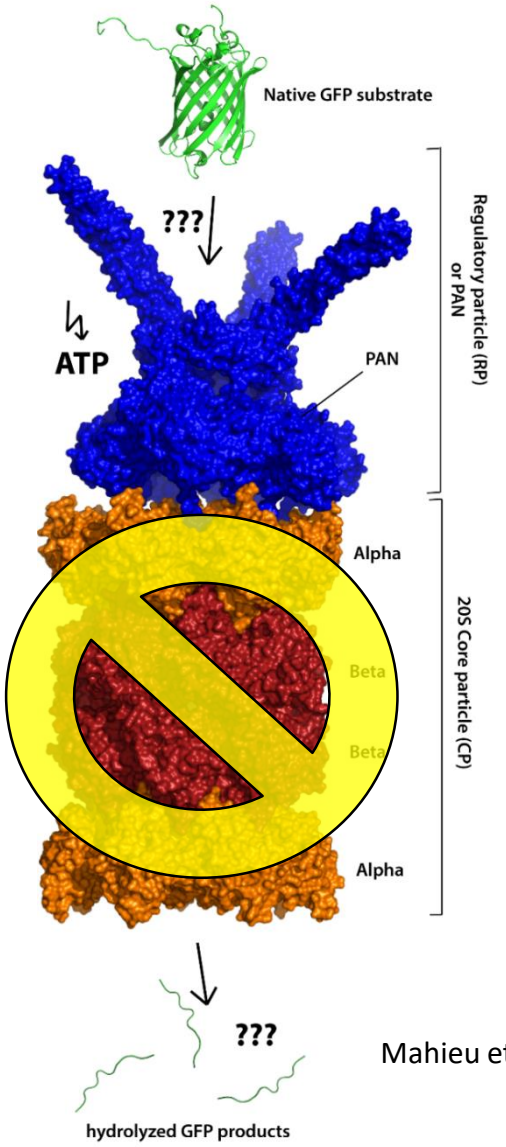


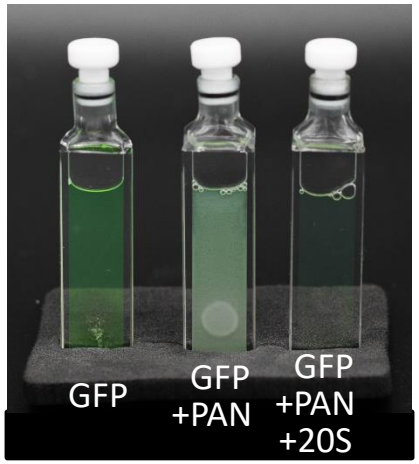


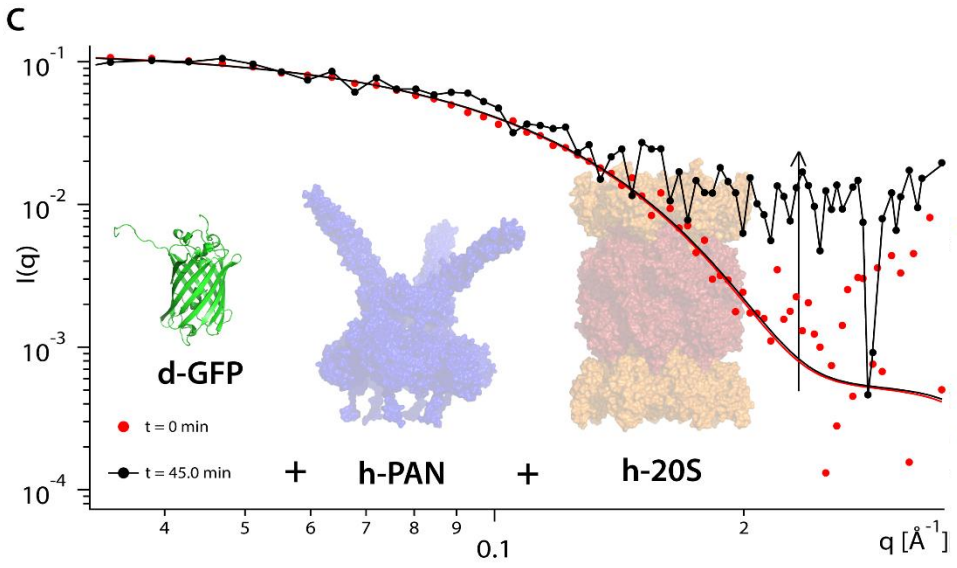


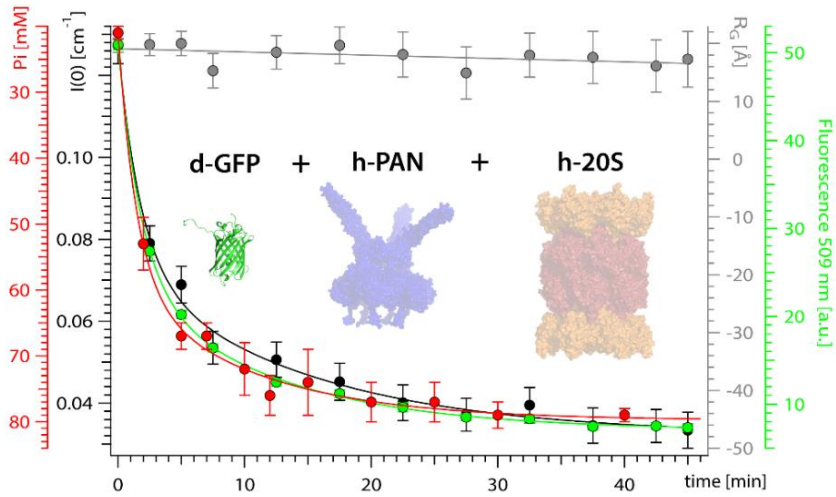
Ibrahim et al. (2017) ***Scientific Rep.*** 7, 40948

Watching protein degradation in solution by TR-SANS (II)





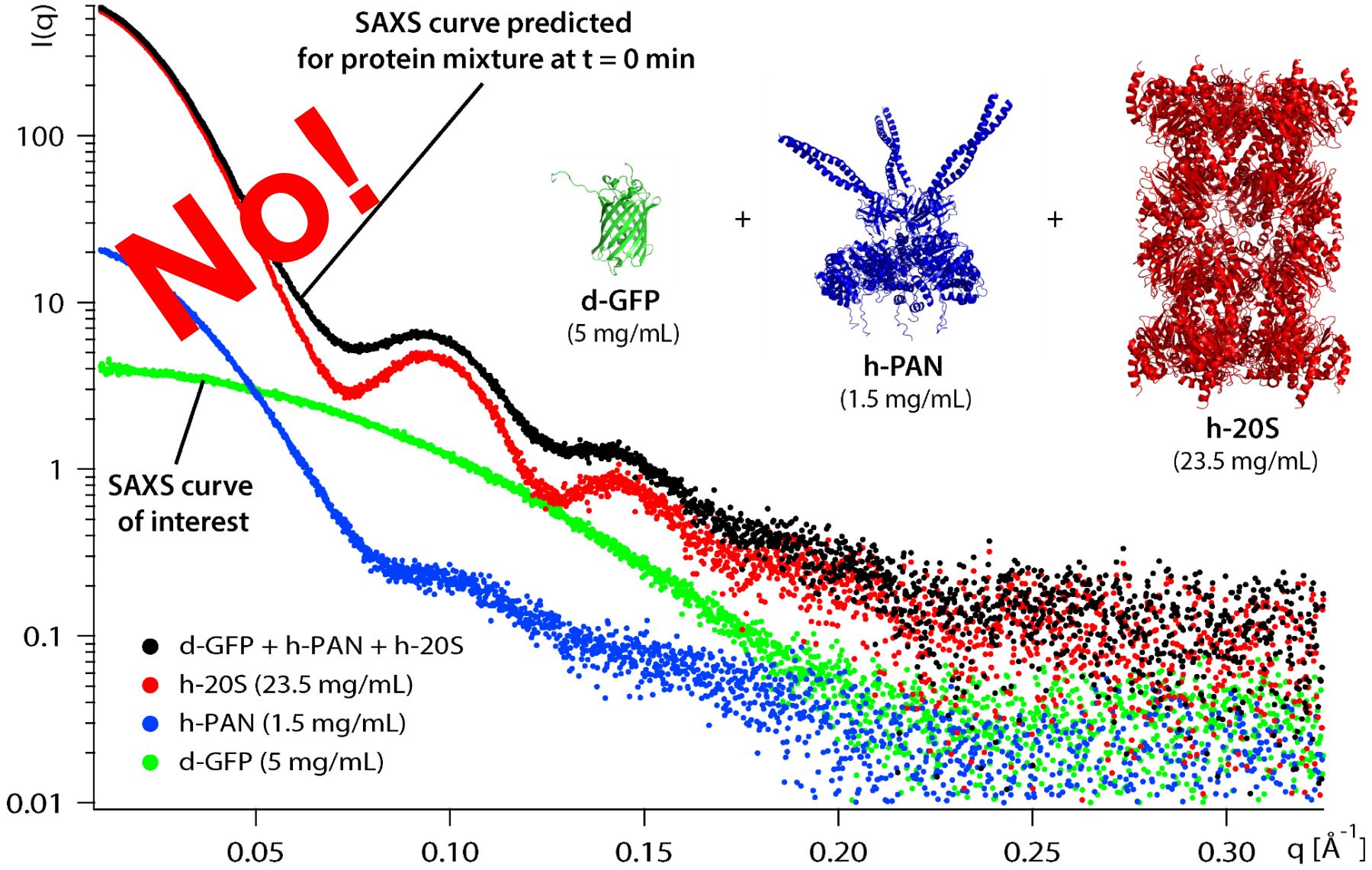




Mahieu et al. (2022) ***Biophys. J.*** 119(2), 375-388

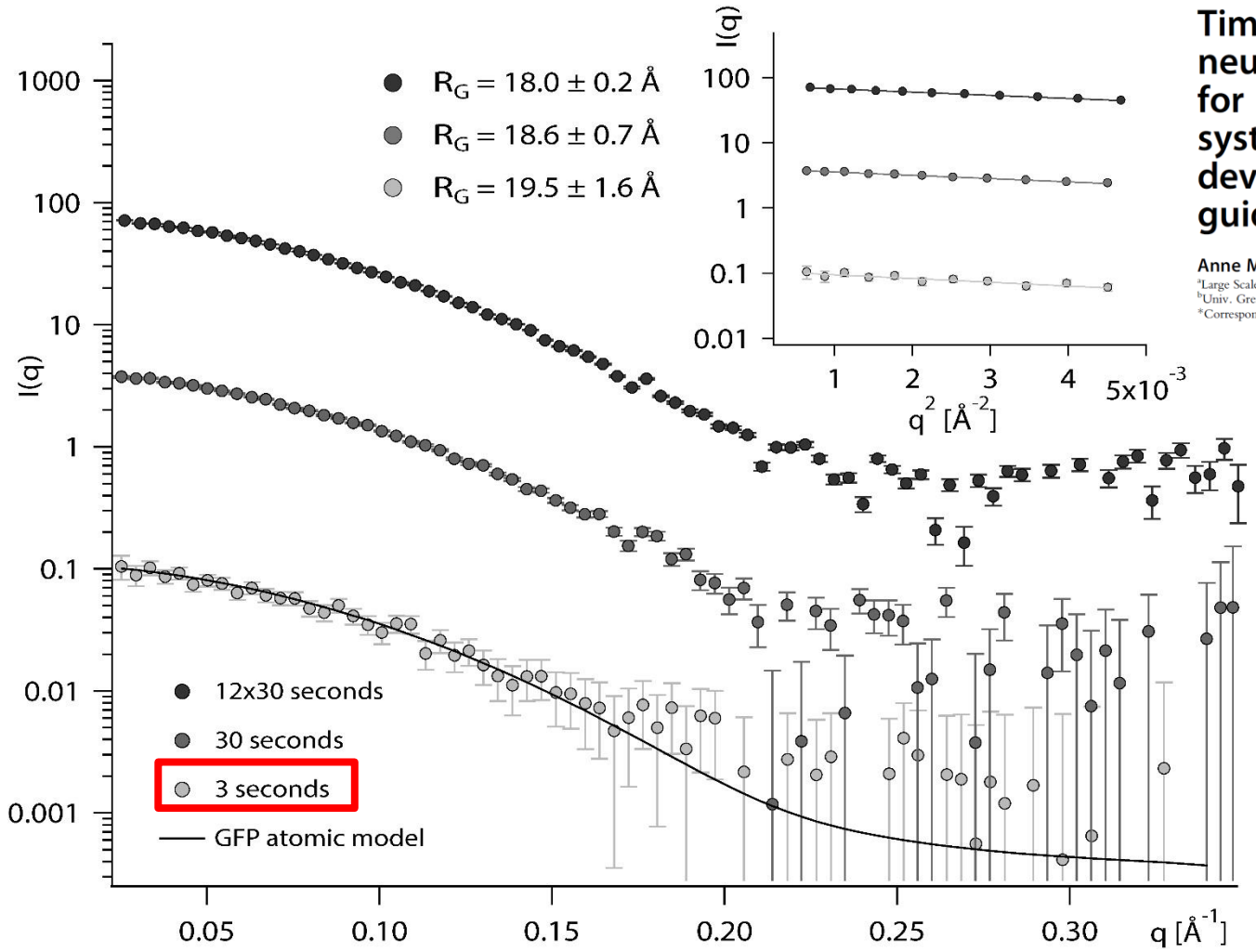
"ProtStretch"

Could this have been done by SAXS?



Mahieu et al. (2020) ***EPJ Web Conf.*** 236, 03002

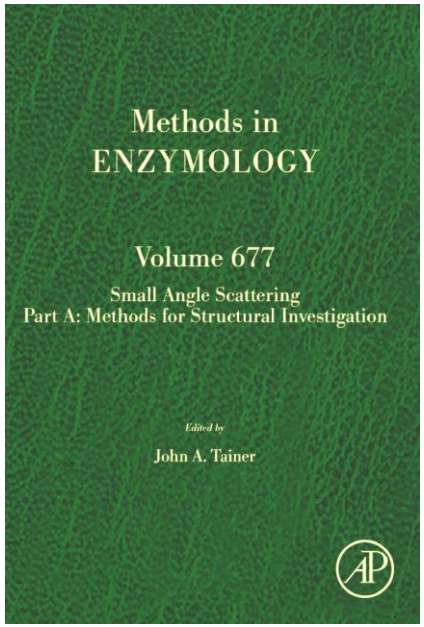
Best time-resolution achievable at a high-flux reactor (ILL) and on a top SANS instrument (D22)?



Time-resolved small-angle neutron scattering (TR-SANS) for structural biology of dynamic systems: Principles, recent developments, and practical guidelines

Anne Martel[a] and Frank Gabel[b,*]

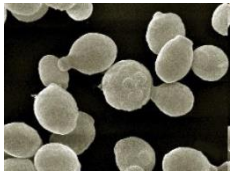
[a]Large Scale Structures Group, Institut Laue-Langevin, Grenoble, France
[b]Univ. Grenoble Alpes, CEA, CNRS, IBS, Grenoble, France
*Corresponding author: e-mail address: frank.gabel@ibs.fr



perdeuterated GFP (28 kDa), 42% D_2O, 2 mg/mL, D22

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

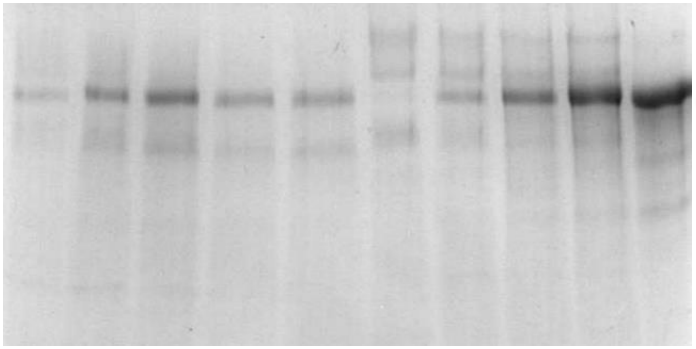
- High expression levels (intracellularly or secreted)
- Disulphide bonds formed during secretion
- Growth on “cheap” deuterated carbon sources (glycerol, methanol)



Expression test

Hydrogenated HSA Deuterated HSA

D1 D2 D3 D4 D5 D1 D2 D3 D4 D5

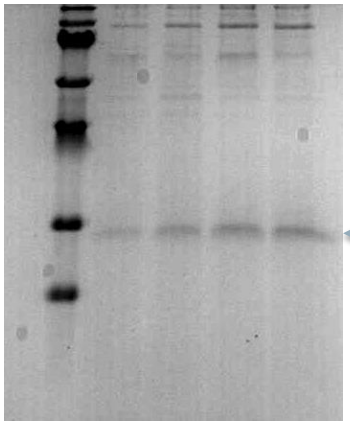


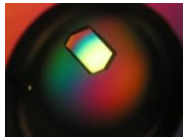
D= Day; HSA: Human Serum Albumin

(V. Laux)

Expression test

Perdeuterated lysozyme





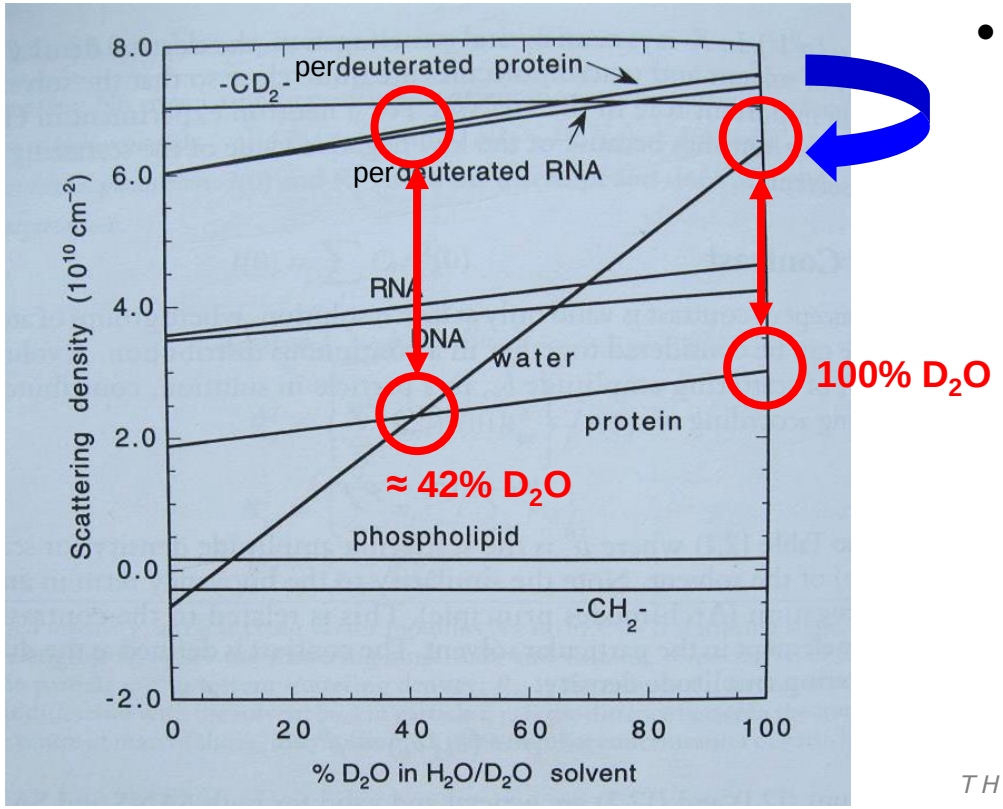
Matchout deuteration:

a handy tool for SANS!

The matchout labelling medium for proteins

Composition of growth medium

- ≈ 85% D_2O
- a hydrogenated ($$$!) carbon source



Advantages

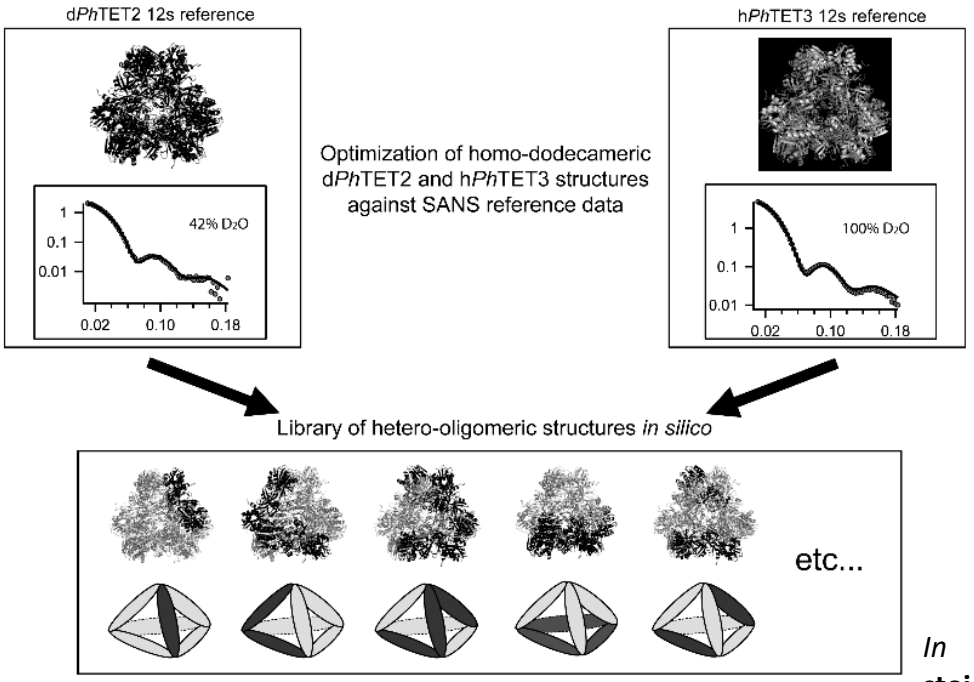
- The production of matchout protein is much more efficient than that of a perdeuterated protein in fully deuterated conditions.
- Significant cost advantages are due to the absence of deuterated carbon source needed in the culture medium.

Dunne et al. (2017) ***Eur. Biophys. J.*** 46(5), 425-432

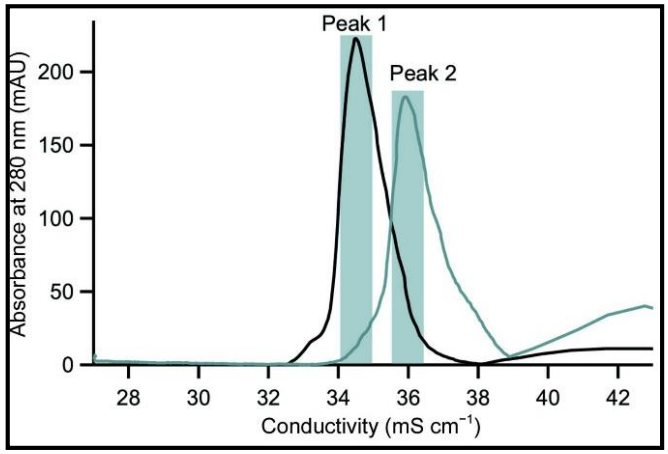
Protein deuteration not complete,

but only ≈75%!

Matching different subunits in a hetero-dodecameric aminopeptidase complex

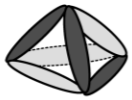
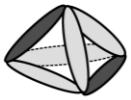
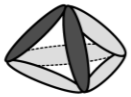
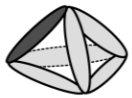
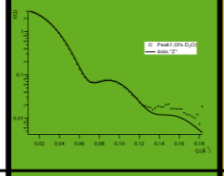
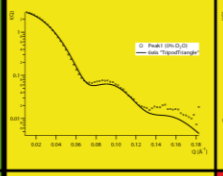
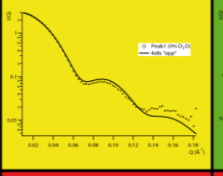
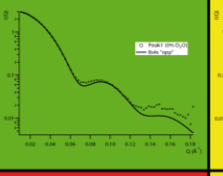
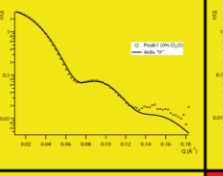
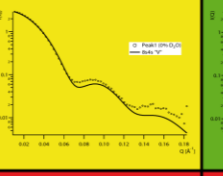
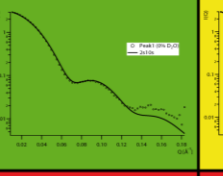
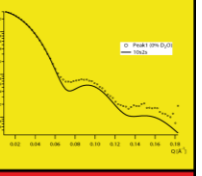
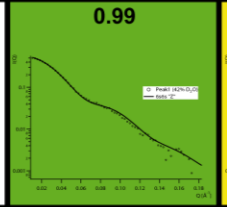
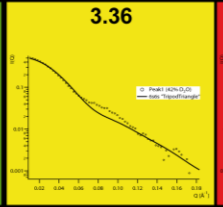
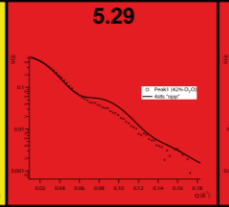
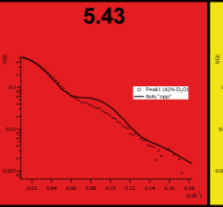
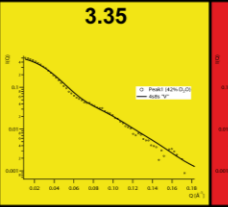
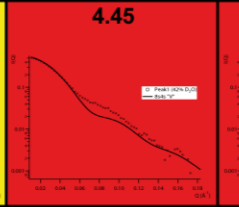
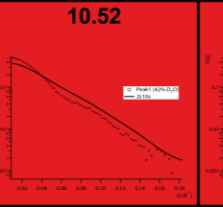
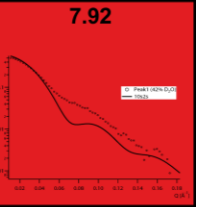
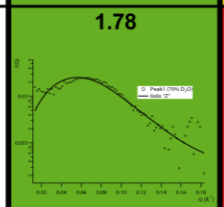
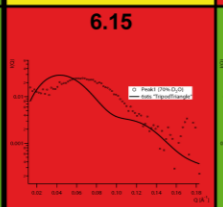
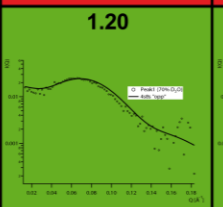
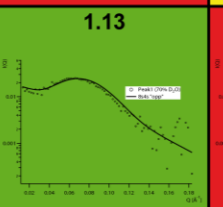
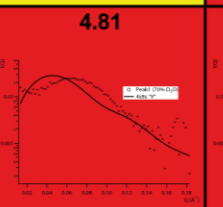
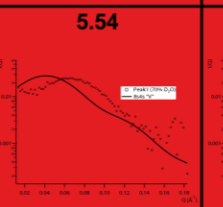
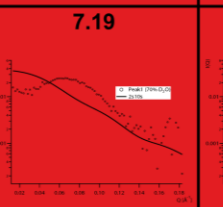
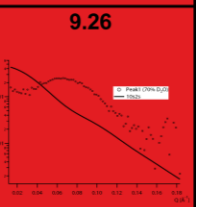
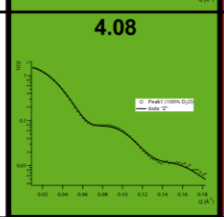
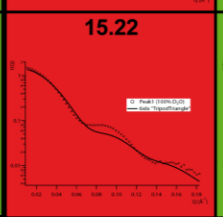
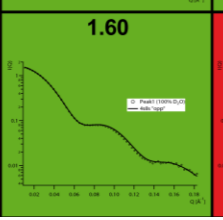
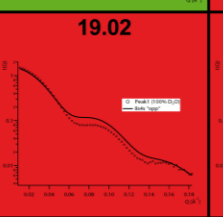
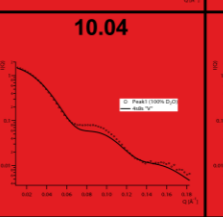
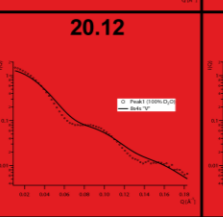
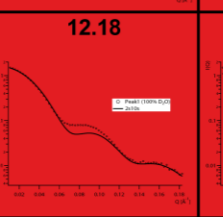
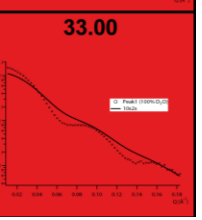


Preparation of **dTET2** (**deuterated**) and **hTET3** (**hydrogenated**). Reconstitution *in vitro* and separation by ion-exchange chromatography, based on charge.



In silico reconstitution of severals models with different **stoichiometry** and **topology** based on crystal structures of the two homo-dodecamers

Determination of stoichiometry and internal topology by contrast-variation SANS (D22, ILL)

Sample	6s6s "Z"	6s6s_Tripod_triangle	4s8s_opp	8s4s_opp	4s8s_V	8s4s_V	2s10s	10s2s
								
0%D_2O	2.93 	5.38 	3.31 	1.84 	5.02 	3.63 	3.46 	3.58 
42%D_2O	0.99 	3.36 	5.29 	5.43 	3.35 	4.45 	10.52 	7.92 
70%D_2O	1.78 	6.15 	1.20 	1.13 	4.81 	5.54 	7.19 	9.26 
100%D_2O	4.08 	15.22 	1.60 	19.02 	10.04 	20.12 	12.18 	33.00 

Appolaire et al. (2014) ***Acta Cryst. D*** D70(Pt 11), 2983-2993.

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%

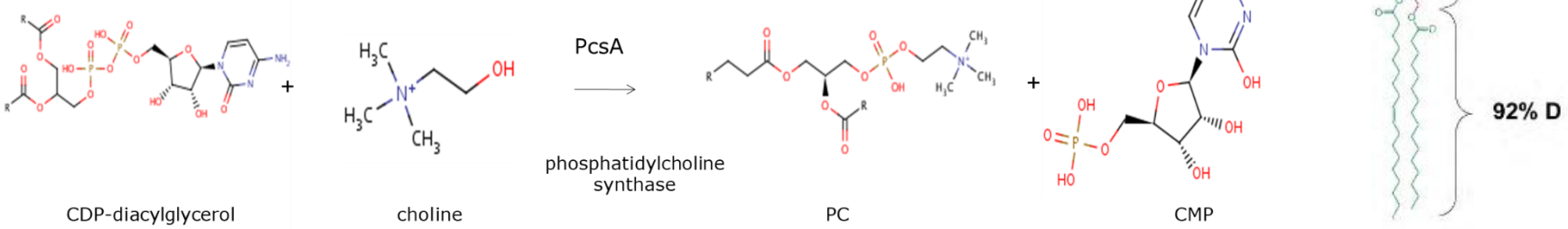
phosphatidylcholine (PC)

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.

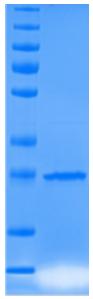
PC is synthesized from endogenous CDP-diacylglycerol and choline from the growth medium

Adaptation protocol was challenging!

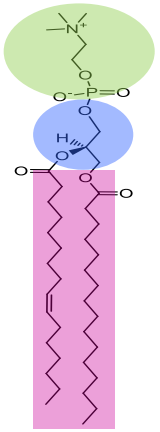


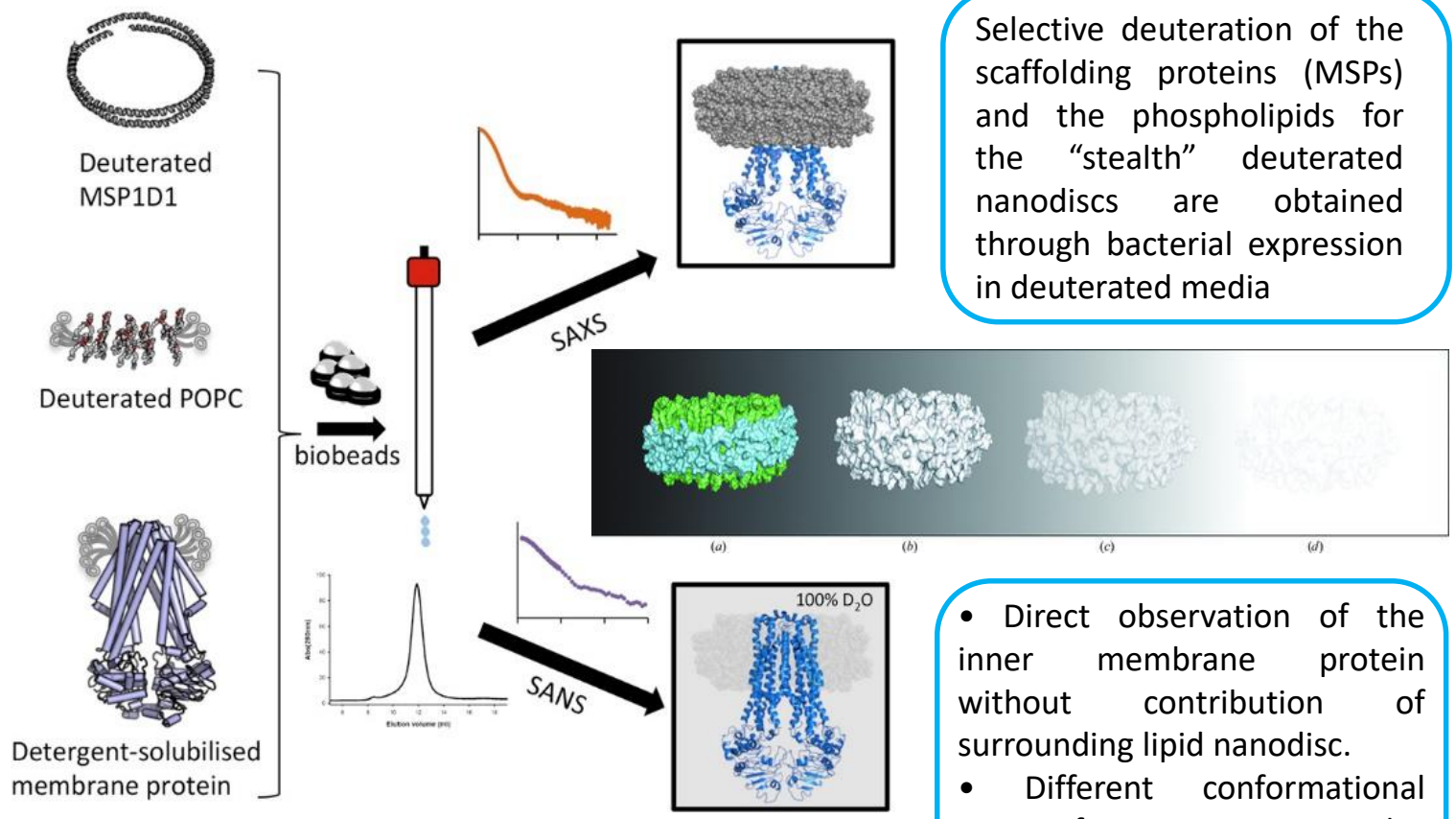
Maric et al. (2015) ***Appl. Microbiol. Biotechnol.*** 99(1), 241-254

Study of membrane proteins by SANS in “stealth” nanodiscs



Pure deuterated MSP1D1 protein





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

- Direct observation of the inner membrane protein without contribution of surrounding lipid nanodisc.
- Different conformational states of ABC transporter MsbA can be detected and modelled.

Maric et al. (2014) ***Acta Crystallogr D*** 70(Pt 2), 317-328

Maric et al. (2014) ***Appl. Microbiol. Biotechnol.*** 99(1), 241-254

Josts et al. (2018) ***Structure*** 26, 1072-1079

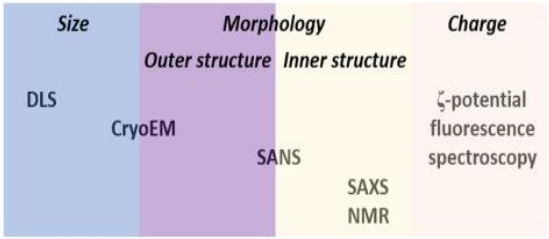
Nitsche et al. (2018) ***Communications Biology*** (1), 206

Developments at D/L-lab and by Anne Martel (LSS)

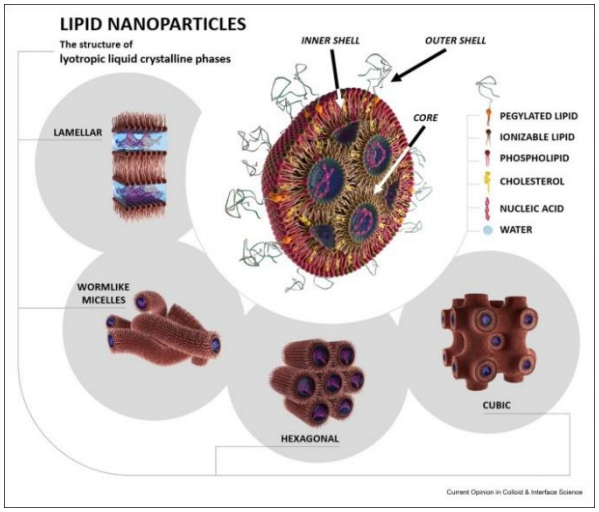
Lipid nanoparticles (LNP)

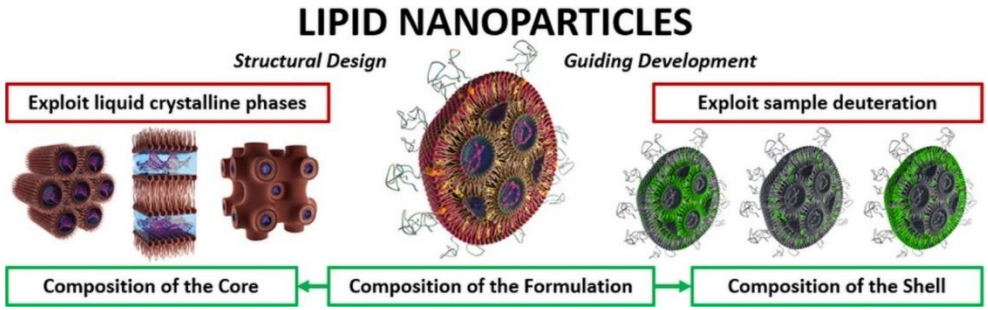
SAXS/**SANS** are used to show the respective location of **(deuterated) cholesterol** and **lipids**

The structural characterization of mRNA-containing LNPs (mRNA-LNPs) is crucial for a full understanding of the way in which they function: **mRNA vaccines!**



Several analytical and physical characterization techniques used to study LNP





LNPs are multicomponent systems consisting of a shell (PEG, DSPC and **Cholesterol**) and a core (the mRNA and ionizable lipid, such as MC3)

Particle size, shape and different spherical populations are determined and analyzed by SAXS and SANS.

The usage of deuterated lipids will enable to locate them.

Cárdenas et al. (2023) *Current Opinion in Colloid and Interface Science*, *66*, Article 101705

Specific deuteration:

focus on structure/dynamics of subsystems!

Amino acid-specific deuteration

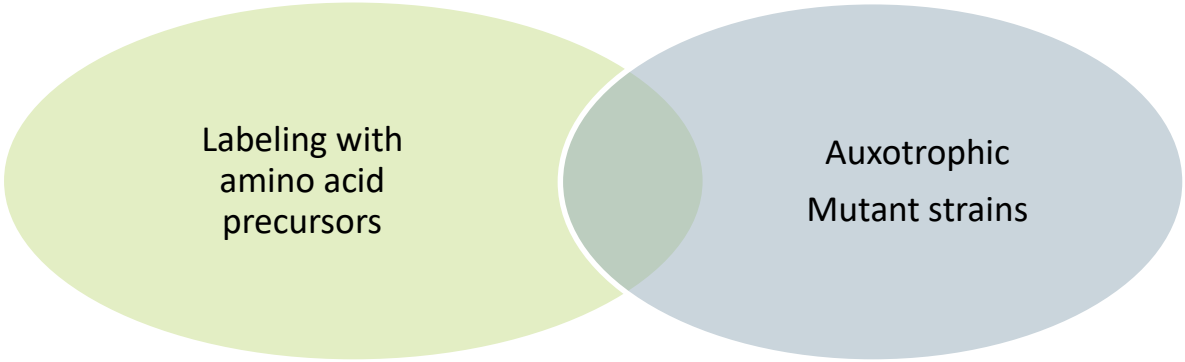
Incorporation of (a) selected **deuterated amino acid(s)** into a **hydrogenated protein background**

Incorporation of (a) selected **hydrogenated amino acid(s)** into a **deuterated protein background**

Occupancy/ Isotope scrambling

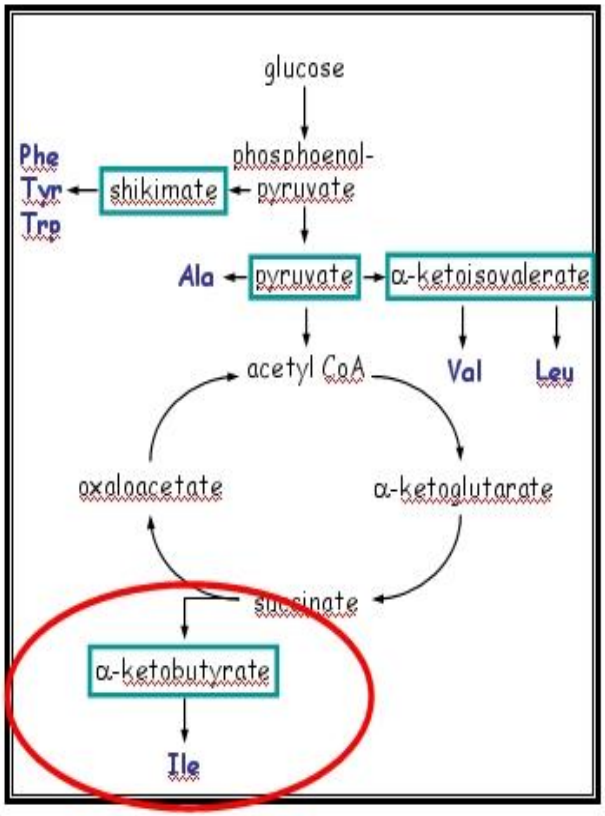
To obtain high occupancy

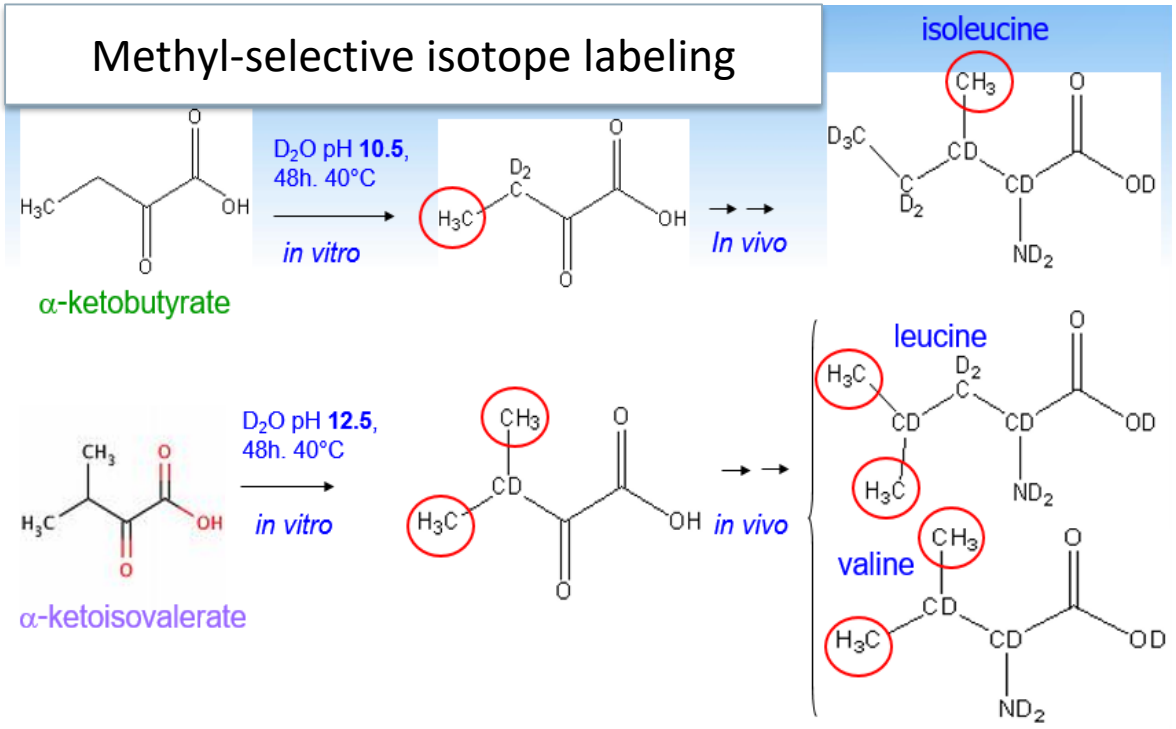
To avoid isotope scrambling



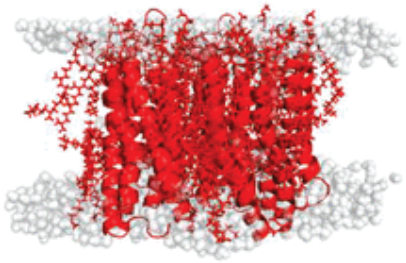
Selective labelling strategies using amino acid precursors (mainly flask)

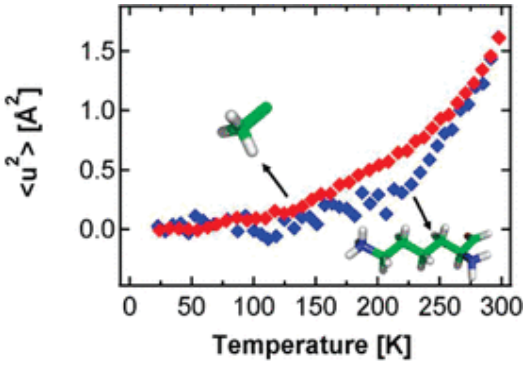
Methyl selective isotope labelling





“Inverse labelling” (H): dynamics of specific amino acids in proteins!





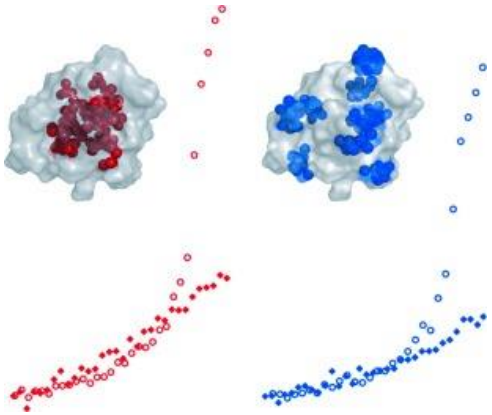
Specific deuteration of **methyl groups**

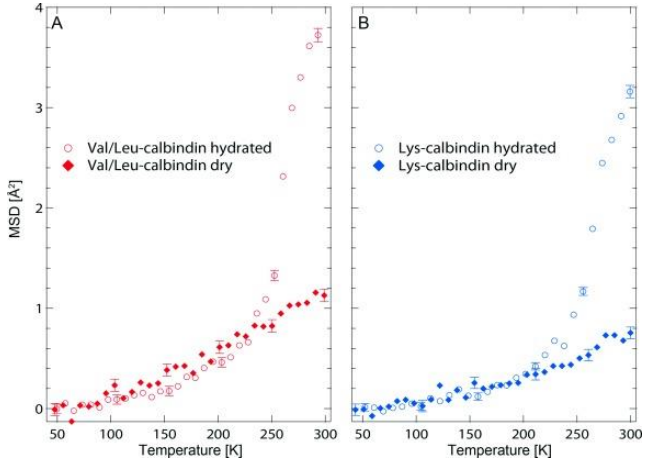
Wood et al. (2010) ***JACS*** 132(14), 4990-4991

IN16 (ILL)

Methyl and **lysine:**
“Inside” vs **“outside”** of a protein

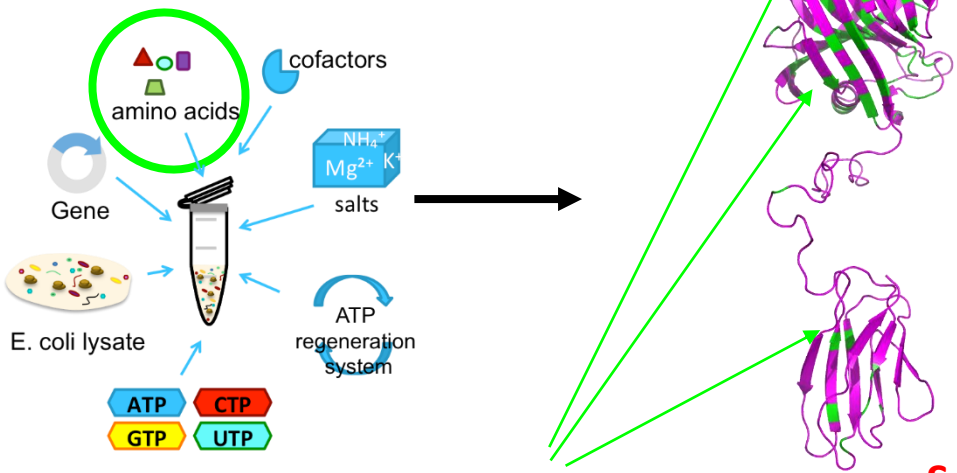
Wood et al. (2013)
Angew. Chem. Intl. 52(2), 665-668



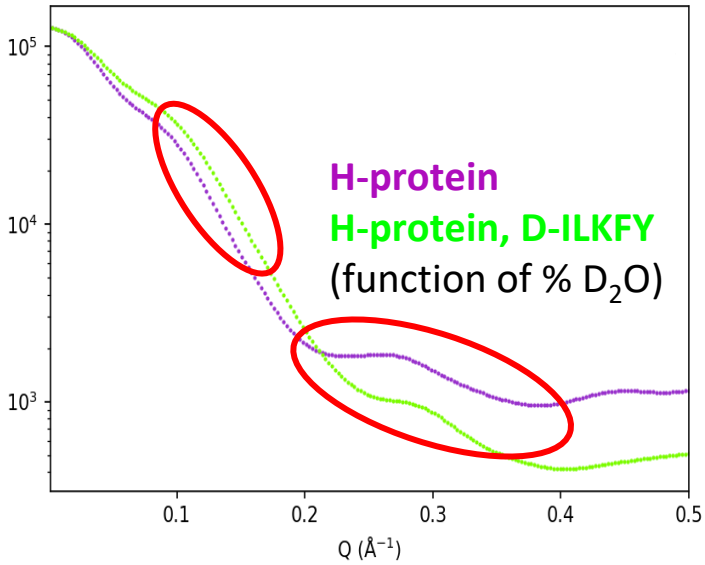


Cell-free protein deuteration:
adding amino-acid specific "tags" for SANS

Supplying deuterated amino acids to reaction mixture!



Specifically labelled protein!



Subtle, but significant changes of SANS curves!

High flux required!

ILL PhD projects Dr. Pau Bernadó (CNRS Montpellier)

Deuteration of whole organisms:

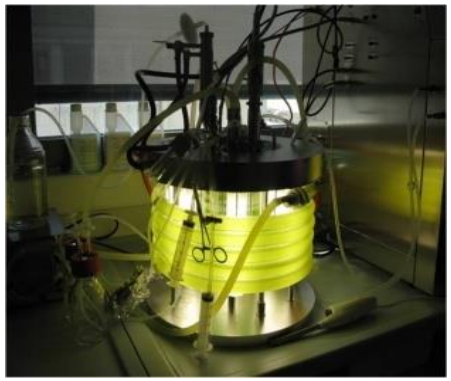
mimicking cellular environments realistically

as well as a precious source of biomolecules

Deuteration of whole organisms

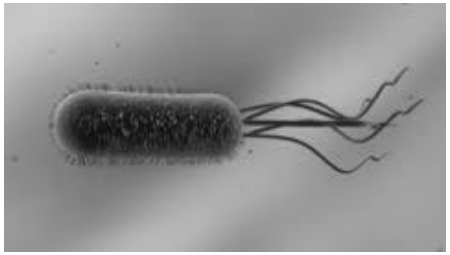
- Deuteration of algae (*Anabaena Chlorella Sorokiniana, Thalassiosira Pseudonana*) for the production of **deuterated biomass (D-lab)**

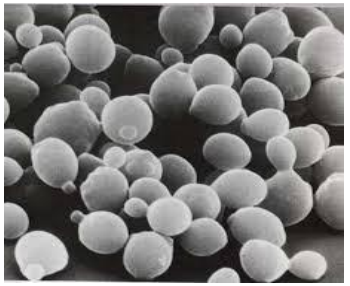
  No carbon source is needed



Photobioreactors

- Perdeuteration of yeast / *E. coli* for the production of of deuterated biomass and **lipids (L-LAB)**

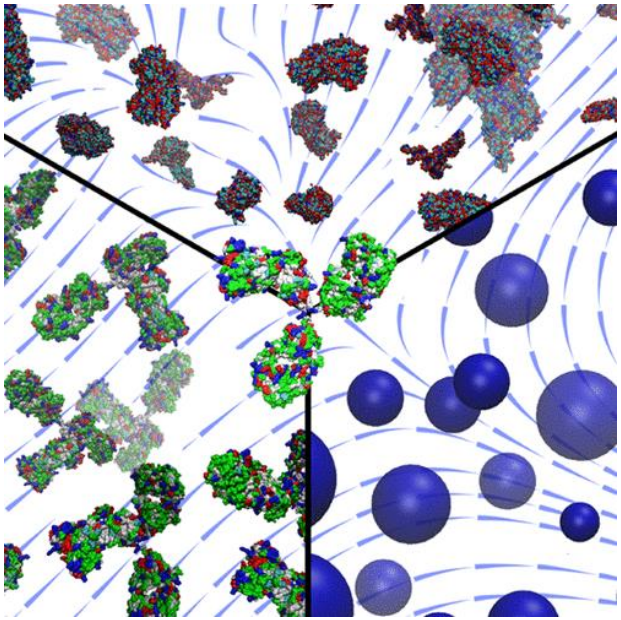


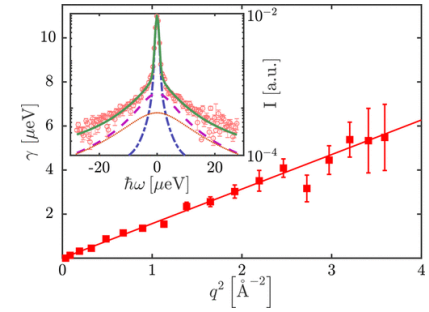


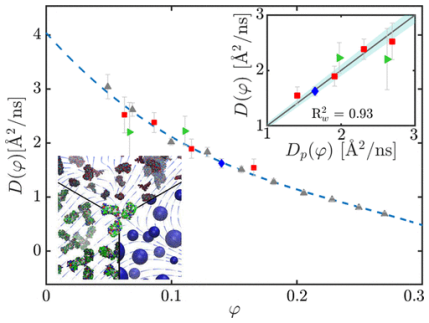
Mimicking a cellular environment by using a deuterated cell lysate

Hydrogenated immunoglobulin diffusion in deuterated *E. coli* cell lysate

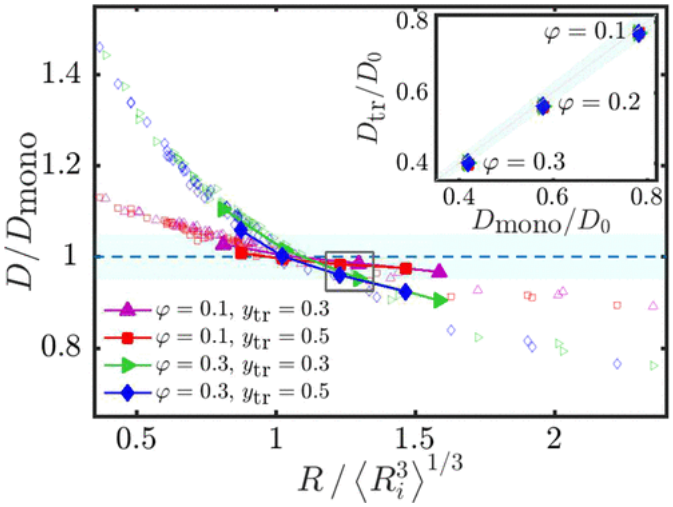
Obtained concentration ≈ 100-150 mg/mL (≈ 300-400 mg/mL *in vivo*)







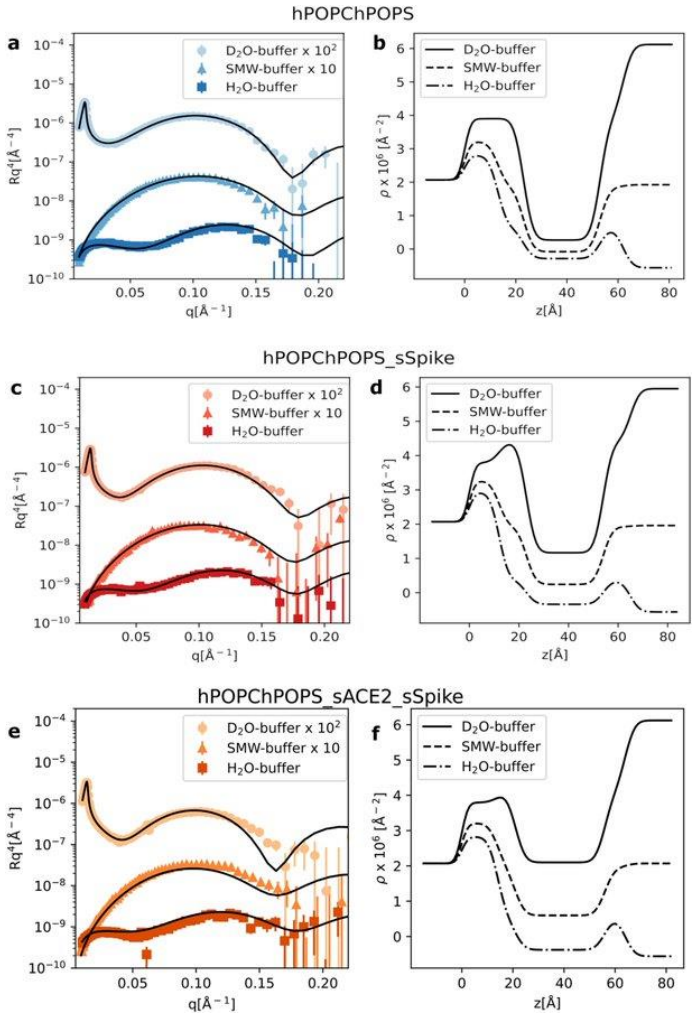
IN16B (ILL)



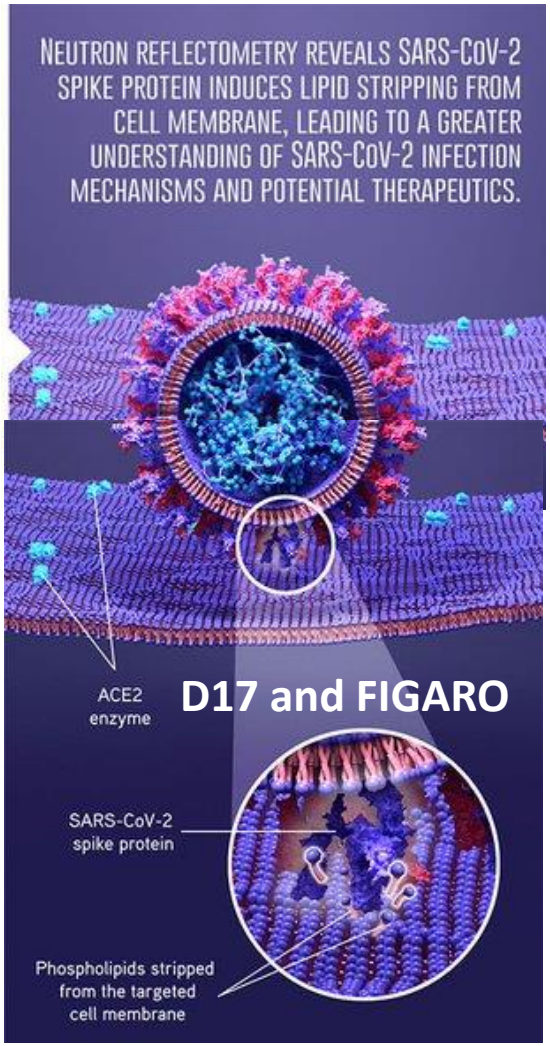
Modification of diffusion in crowded environments depends on the size of the proteins!

Grimaldo et al. (2019) ***J. Phys. Chem. Lett.*** 10, 1709

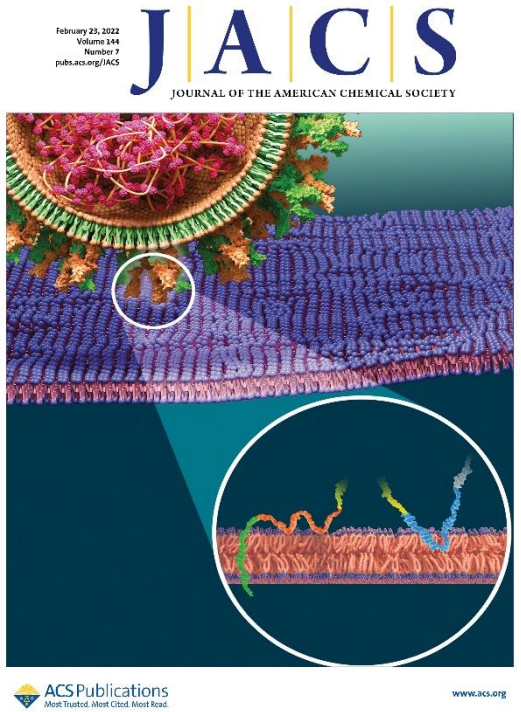
Lipid bilayer interaction of SARS-CoV2 spike protein



Luchini et al. (2021) ***Scientific Reports*** 11, 14867



Deuterated lipids from *Pichia pastoris* (ILL D/L-labs) (cholesterol from ANSTO)



FIGARO and D22

Santamaria et al. (2022) ***JACS*** 144(7)

Practical information,

access and perspectives

D-Lab and L-Lab catalogue

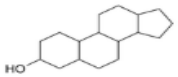
D-LAB CATALOGUE

Perdeuterated
Partially
deuterated
Selectively labelled
protein

P Deuterated Proteins

MBP
Myoglobin
Lysosyme
HSA
Synuclein

MP Model Proteins



Cholesterol
perdeuterated and
matchout
Campesterol
Hopanoid

S Sterols

Partially deuterated
peptide
Length 8 to 30 A.A

P Peptides

L-LAB CATALOGUE

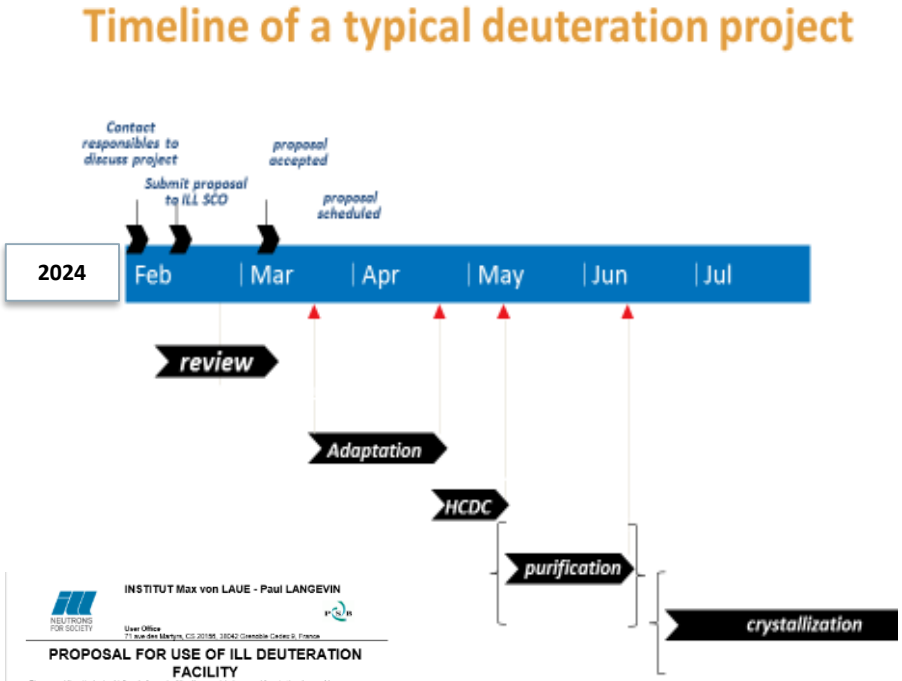
CsE3 circularized
MsP1D1
PC lipids

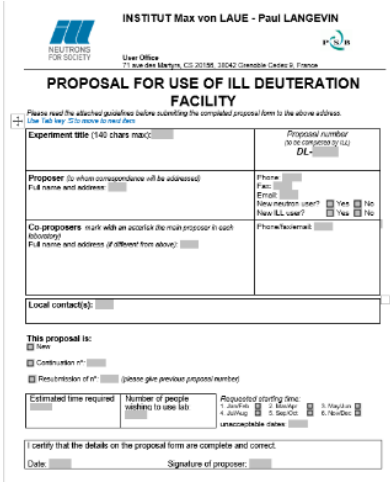
N Nanodiscs

Natural lipids mixture
(**H and D**) from
E.coli and yeast:
Phospholipids
(PE,PG, PC,PS, PI)
Neutral and polar lipids

L Lipids

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




INSTITUT Max von LAUE - Paul LANGEVIN

PROPOSAL FOR USE OF ILL DEUTERATION FACILITY

- access *via* peer-review system
- D-lab 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

https://www.ill.eu/users/support-labs-infrastructure/deuteration-laboratory

Contact: dlab-proposals@ill.fr

Access to L-lab platform

- Presently the platform is handling several internal and external requests
- Total phospholipid mixtures and class-specific phospholipid mixtures can be provided
- A total of 52 requests have been received between 2018 – 2024
- The platform has been heavily involved in handling COVID19-related science requests (total: 16)

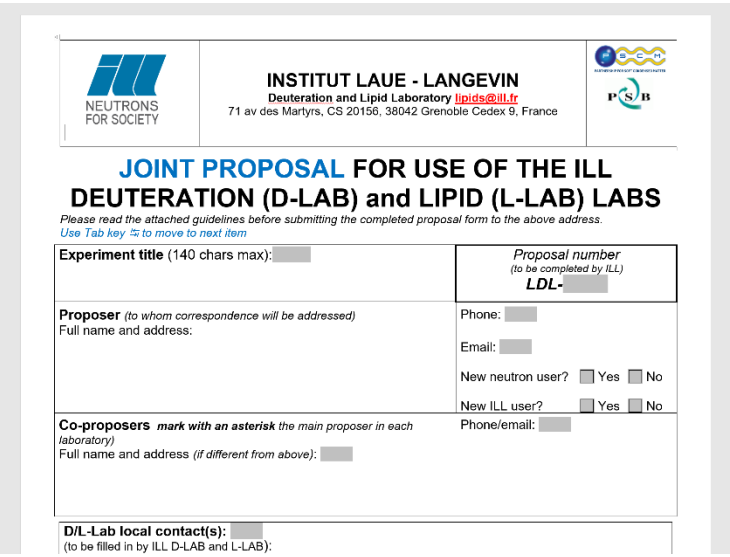
- The above request form could be accessed to

- https://www.ill.eu/users/scientific-groups/soft-matter-science-and-support/deuterated-lipids-at-the-ill

Contact: lipids@ill.fr

Improving interactions with the users

- Update of webpages (e.g. catalogue of molecules by L- and D-labs)
- Joint D/L-lab proposals
- Online safety training for chemistry lab
- Online proposal system for D- and L-labs



NEUTRONS FOR SOCIETY

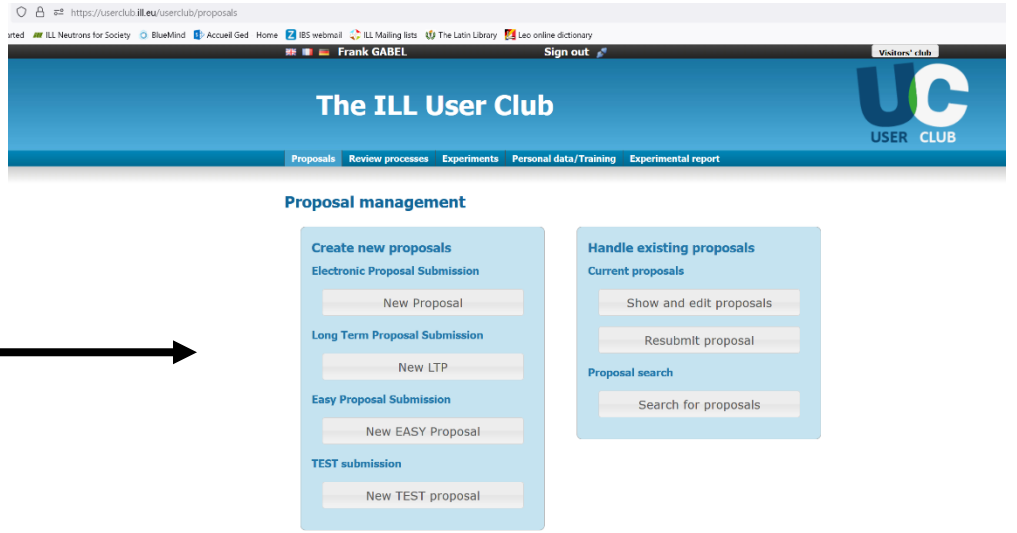
INSTITUT LAUE - LANGEVIN
Deuteration and Lipid Laboratory lipids@ill.fr
71 av des Martyrs, CS 20156, 38042 Grenoble Cedex 9, France

JOINT PROPOSAL FOR USE OF THE ILL DEUTERATION (D-LAB) and LIPID (L-LAB) LABS

Please read the attached guidelines before submitting the completed proposal form to the above address.
Use Tab key ⇆ to move to next item

Experiment title (140 chars max):	*Proposal number* (to be completed by ILL) **LDL-**
Proposer *(to whom correspondence will be addressed)* Full name and address:	Phone: Email: New neutron user? ☐ Yes ☐ No New ILL user? ☐ Yes ☐ No
Co-proposers ***mark with an asterisk*** *the main proposer in each laboratory)* Full name and address *(if different from above)*:	Phone/email:

D/L-Lab local contact(s):
(to be filled in by ILL D-LAB and L-LAB):



Thanks to BDCS staff for their great work and motivation and D/L-lab staff for contributions to my talk!

Questions? Access to labs? Requests for deuterated molecules?

Don’t hesitate to contact us!

Thank you very much

for your attention!