

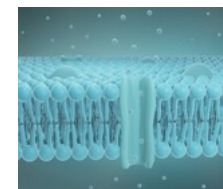
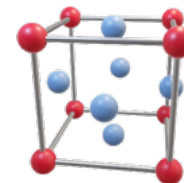
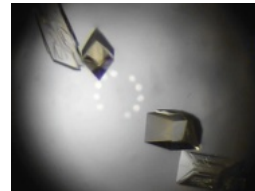


The Deuteration & Macromolecular Crystallization platform at ESS

Dr. Zoë Fisher

Team lead for the DEuteration & MAcromolecular Xtallography Platform ([DEMAX](#)) at ESS

Snr. Adjunct lecturer at Biology Department, Lund University



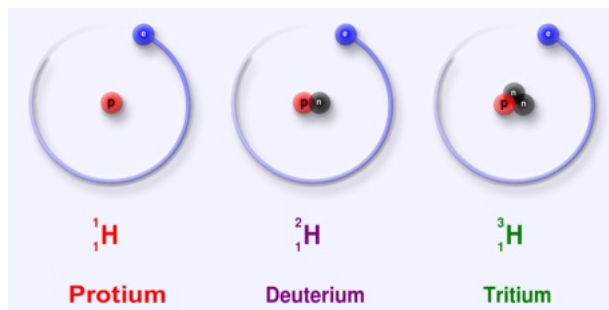
Deuteration is important & necessary for neutron experiments

- Molecules from living organisms are abundant in hydrogen, spec. ^1H isotope
- Deuteration: replacing endogenous ^1H with ^2H to greater or lesser extent through a variety of methods (H/D exchange, partial deuteration, perdeuteration)

Neutron scattering lengths and cross sections							
Isotope	conc	Coh b	Inc b	Coh xs	Inc xs	Scatt xs	Abs xs
H	---	-3.7390	---	1.7568	80.26	82.02	0.3326
^1H	99.985	-3.7406	25.274	1.7583	80.27	82.03	0.3326
^2H	0.015	6.671	4.04	5.592	2.05	7.64	0.000519
^3H	(12.32 a)	4.792	-1.04	2.89	0.14	3.03	0

<https://www.ncnr.nist.gov/resources/n-lengths/>

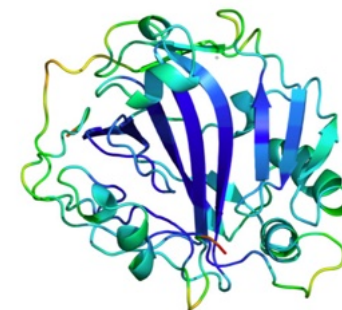
Natural abundance:
1 in 6420 H atoms are ^2H



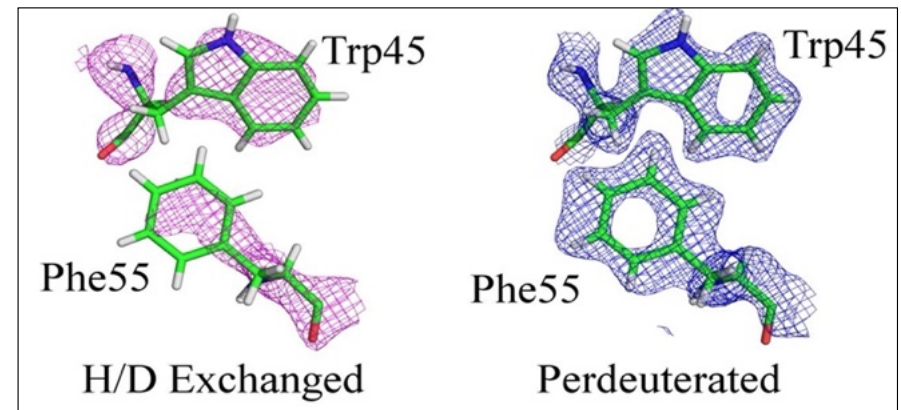
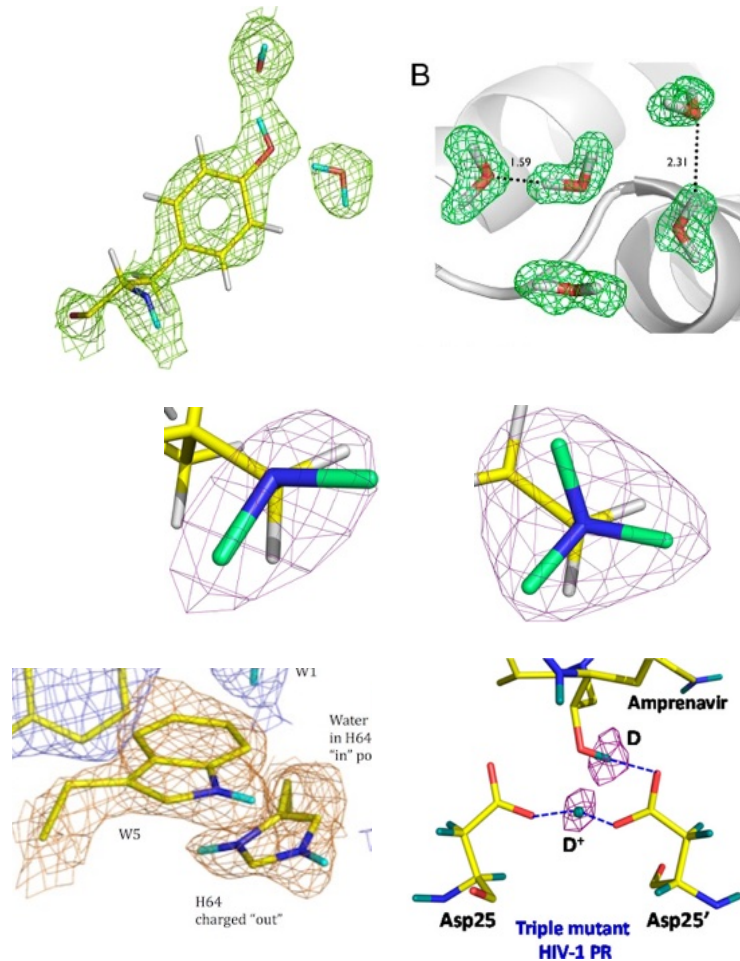
Carbon	C	1647	~ 350 amino acids
Hydrogen H	2565		
Nitrogen	N	465	
Oxygen	O	517	
Sulfur	S	21	

Formula: $\text{C}_{1647}\text{H}_{2565}\text{N}_{465}\text{O}_{517}\text{S}_{21}$

Total number of atoms: 5215



Determine position of H atoms in macromolecular structures



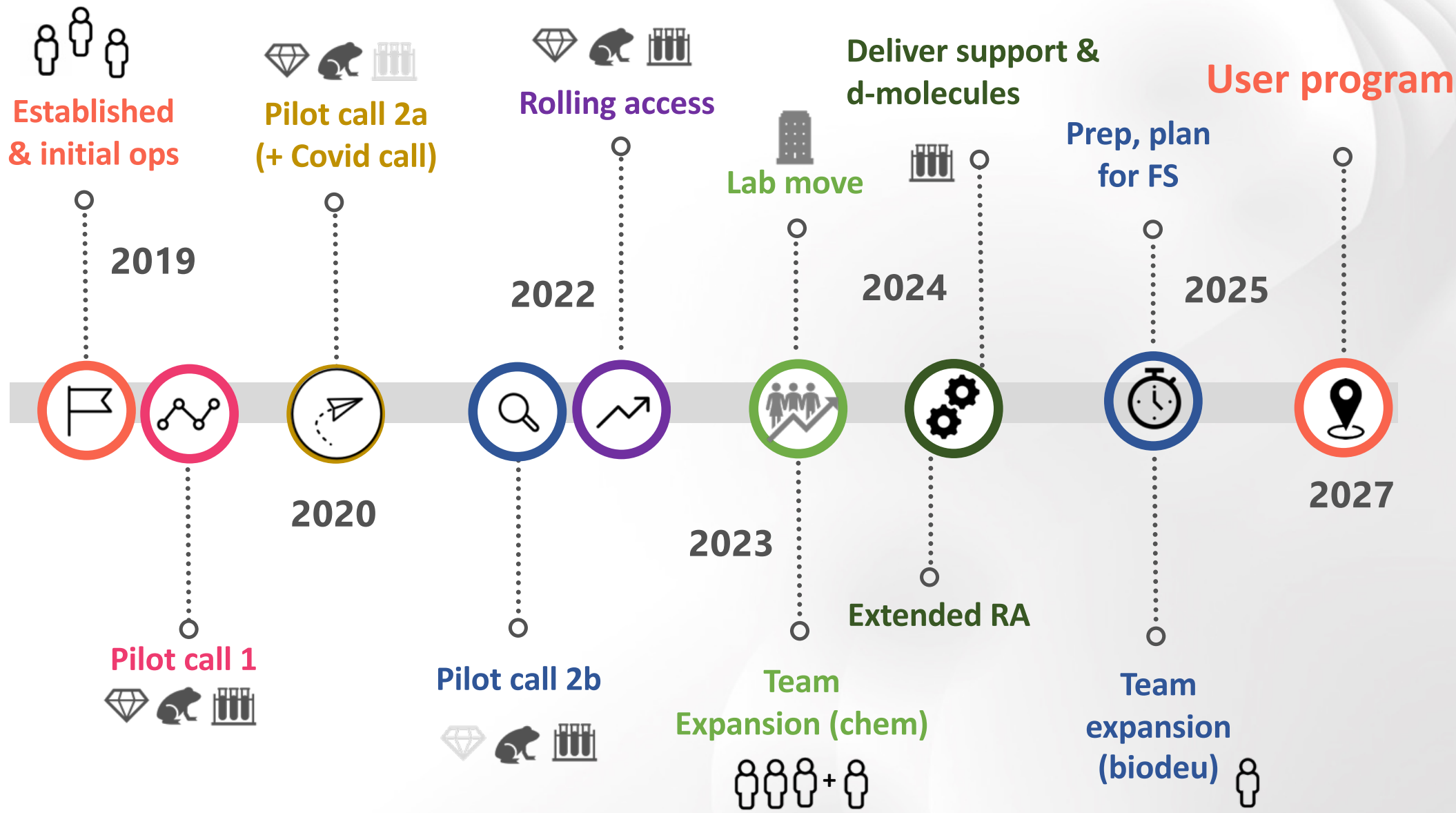
<https://journals.jps.jp/doi/pdf/10.7566/JPSCP.25.011003>

Limitations of biodeuteration

- Limited number of species tolerate D₂O – highly toxic in higher organisms (insects, mammals, plants) >30%
- Cells are not happy in D₂O: slow growth, low yields
- Requires a lot of very expensive D₂O and carbon source (e.g. glycerol-d8)
- And biophysical properties of d-proteins are altered (solubility for e.g.)

DEMAX overview

- DEMAX is the ESS user support lab that offers deuteration and crystallization service & support
- We are part of the CLS group in the Science Directorate
- We broadly support the chemistry, life science, and soft matter community with access to deuterated materials (small & large molecules) as well as large protein crystal growth



DEMAX Platform



Chemical Deuteration

- Small organic molecules, monomers
- Lipids (e.g. POPC, SOPC, POPE)
- Surfactants (e.g. sugar-based)
- Novel organic molecules for various applications



Biological Deuteration

- Deuterated biomass from *E. coli*, *B. braunii*, *P. pastoris*
- Recombinant soluble proteins, plasmid DNA, "other"
- Yeast-derived lipids (total, phospholipid)

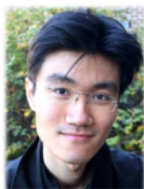


Protein Crystallization

- High- and low-throughput screening
- Fine screening in large volumes
- Support for room temperature crystal mounting & data collection
- X-ray testing (LU BAG at MAX lab)



Anna



Jia-Fei



LP3
0.7 FTE



D-lab (lipids)
0.2 FTE



Zoë

Labs are spread out LU, MV & ESS

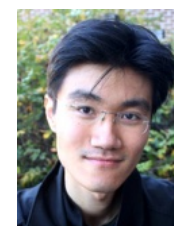


Lund, Sweden

Chemical Deuteration



Anna



Jia-Fei

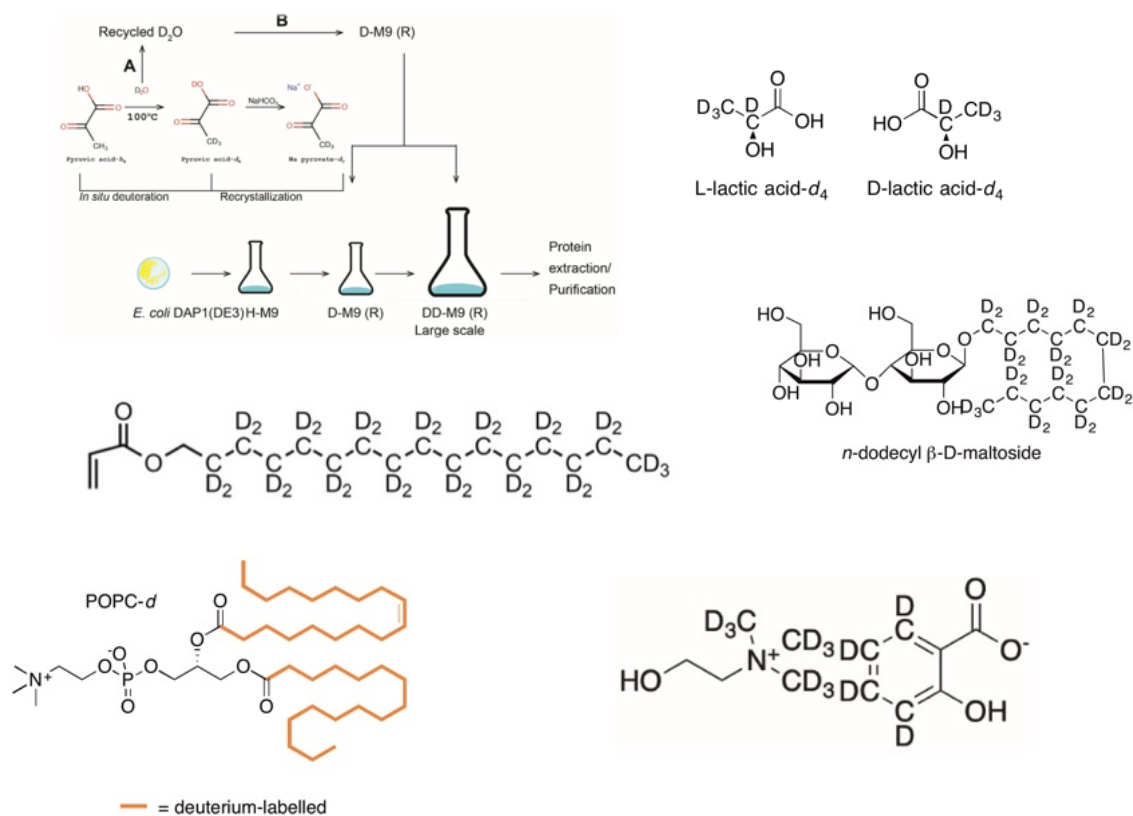


- Moved to ESS in June 2023
- Lab is up and running with essential equipment is in place for synthesis, separation, characterization.
- For some characterization needs (e.g. NMR) we have service arrangements with Red Glead & LU Chemistry.
- *In progress: Advion ESI-MS*

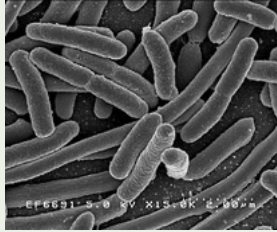
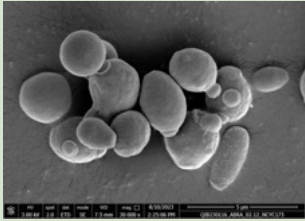
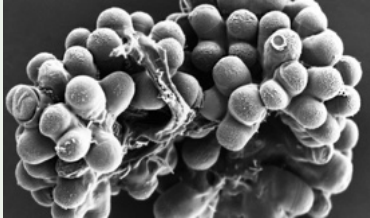


Deuterated organic molecules

H/D exchange, chemical & enzymatic synthesis of a range of small molecules (surfactants, monomers, alcohols, aldehydes, lipids, fatty acids etc.)



DEMAX offers biodeuteration from following:

Bacteria Escherichia coli (E. coli)	prokaryote 	Recombinant proteins Plasmid DNA
Yeast Pichia pastoris (P. pastoris)	eukaryote 	Lipids (total, phospholipid) (membranes, ergosterol, cholesterol)
Algae Botryococcus braunii (B. braunii)	eukaryote 	Total cell extract (lipids, oil, exopolysaccharides)

*All of these can tolerate up to ~99% D

Deuterated biolipids

Large scale production of *Pichia pastoris* (supported by LP3)

Total lipid extraction, separation phospholipid classes

Analysis: TLC, GC, MS

* temporarily housed at Kemicentrum, LU



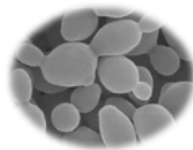
Hanna



Sophie



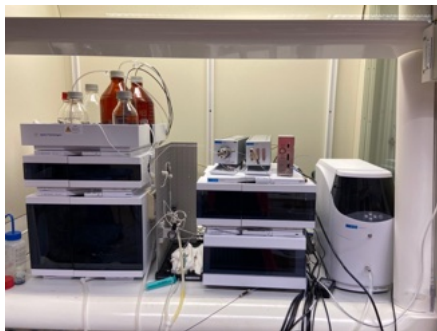
Pichia pastoris



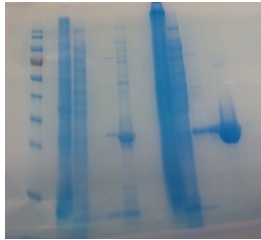
LUND
UNIVERSITY



NEUTRONS
FOR SOCIETY



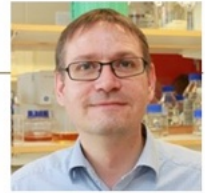
Deuterated biomolecules



Zoë



LP3 0.7 FTE



Wolfgang

- Essential ESS equipment in place, access agreement to be able to use LP3 labs & equipment
- LP3 research engineer supports some tasks related to biodeu (Swedish in-kind)
- Produce full or partially d-labeled biomass
- Cell Paste or purified recombinant proteins, plasmid DNA
- Check protein purity, yield (SDS-PAGE, UV/Vis), biophysical characterization tools for proteins (SEC-MALS, NanoDSF)

<https://www.lp3.lu.se>

Protein Crystallization

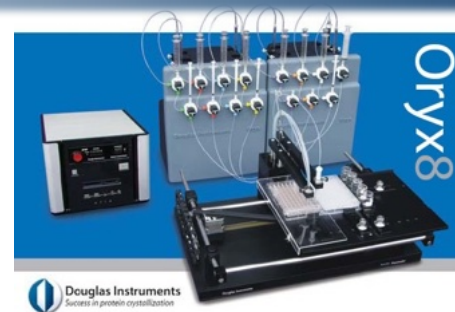
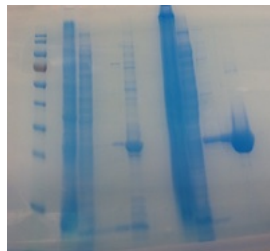
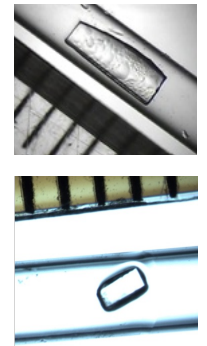


Zoë



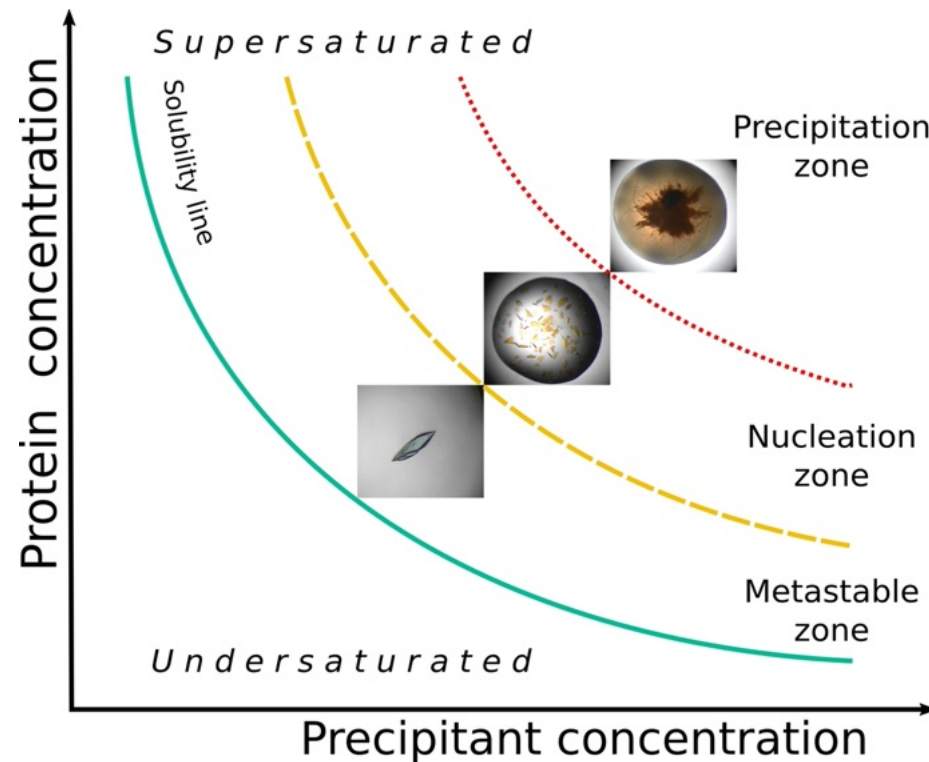
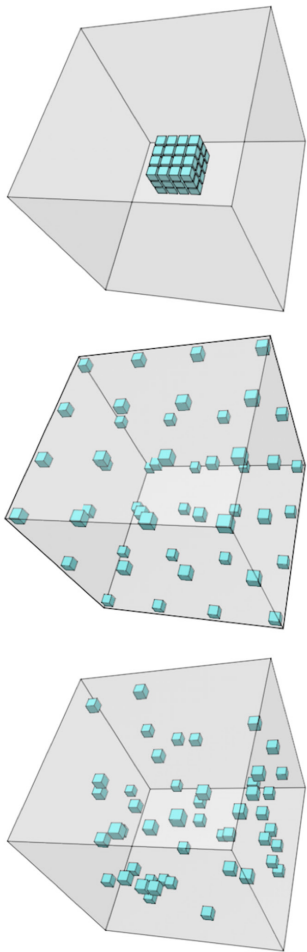
BAG access
to BioMAX

- Currently: co-located with the LP3 crystallization lab
- We offer low throughput optimization, often by hand or with custom screen & optimization using the Oryx8 – depends on maturity of project
- Part of LU BAG for BioMAX: test and/or collect RT (or cryo) X-ray diffraction data
- We support large single crystal growth, crystal prep for data collection (RT or cryo)
- Most users come with known conditions and “only” need help to increase volume
- ❖ Recommend to re-screen, especially if protein is partially or fully deuterated
- ❖ Recommend to check solubility, stability (pH, salt, buffer etc – NanoDSF)



Check concentration, purity, stability – then HT or fine screen = hopefully some good crystals that can be optimized!

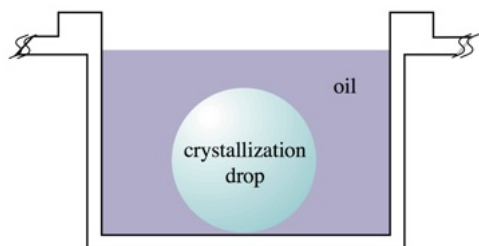
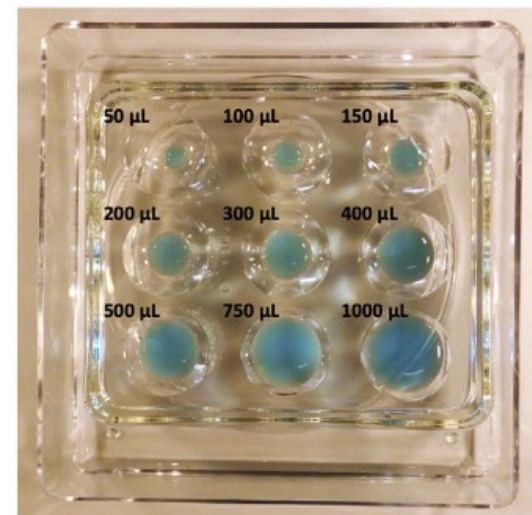
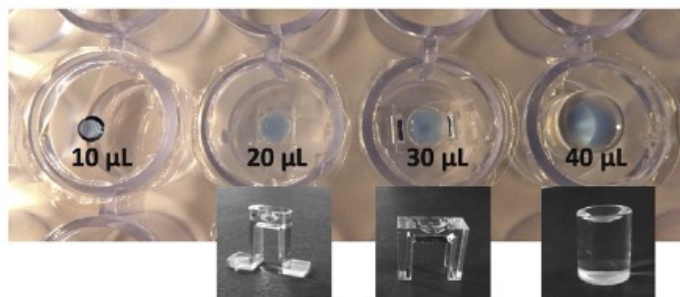
Crystal screening vs. optimization



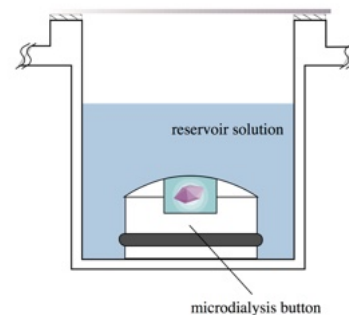
- ❖ pH
- ❖ Temperature
- ❖ Deuteration level
- ❖ Additives

Crystallization hardware

- Vapour diffusion



- Batch (under oil)



- Dialysis

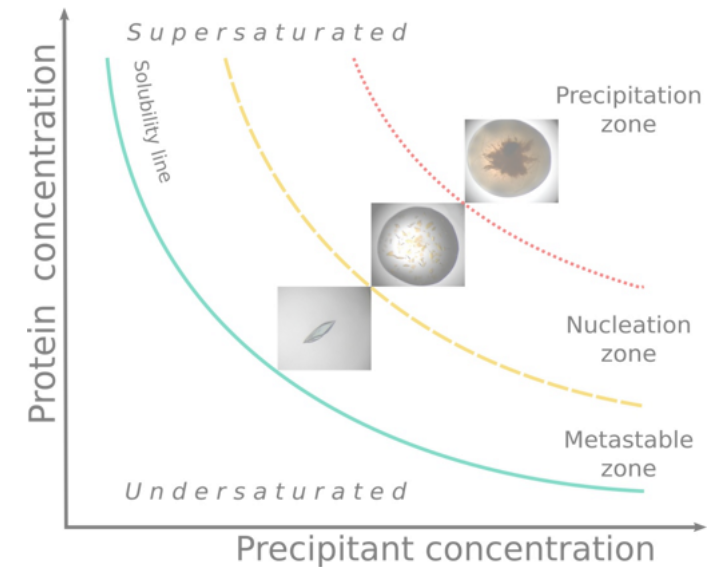
Modifications to basic methods

Can modify or adjust these methods by doing things that promote nucleation (formation of new crystals):

- Crystal seeding (micro or macro)

or simply growth:

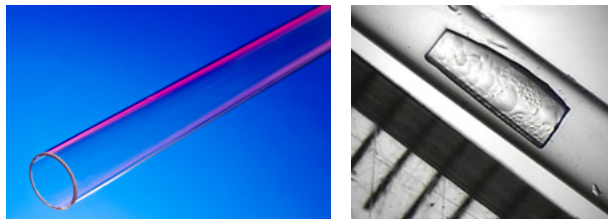
- Crystal feeding



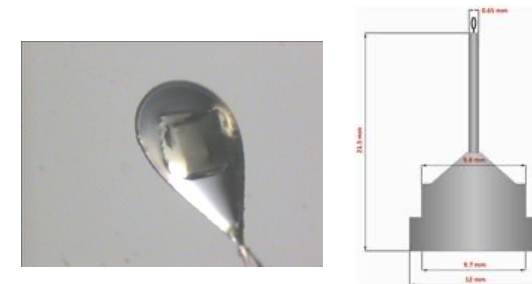
Preparing crystals for data collection

- We support both RT & cryo crystal mounting for testing and/or data collection
- Capillary mounting (RT) or Magnetic bases with capillary “cover” (RT) or the standard cryo loops, bases (cryo)

RT



CRYO



No damage from freezing

Tricky to do, need to practice and do it frequently

No cryoprotectants, SEE or LN2

Sensitive proteins degrade, radiation damage

Observe structure closer to physiological conditions

Can't make complexes or trap reaction intermediates

Easy to do, standardized mounts

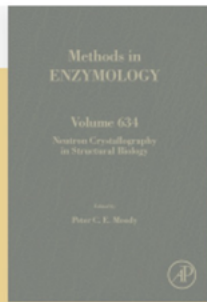
Need cryo conditions, freezing itself can damage xtal

Easy to store, preserve sensitive samples

Cryo-induced artefacts (glycerol, freeze-in conformations)

Protect from radiation damage

Need for special SEE, LN2 consumables



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Neutron Crystallography in Structural Biology

Edited by Peter C.E. Moody - Department of Molecular and Cell Biology and Leicester Institute of Structural Biology, University of Leicester, Leicester, United Kingdom

Volume 634,
Pages 2-389 (2020)

[Download full volume](#)



CHAPTER TWO

Large crystal growth for neutron protein crystallography

Monika Budayova-Spano^a, Katarina Koruza^{b,†}, Zoë Fisher^{b,c,*}

^aUniversité Grenoble Alpes, CEA, CNRS, IBS, Grenoble, France

^bDepartment of Biology, Lund University, Lund, Sweden

^cScientific Activities Division, European Spallation Source ERIC, Lund, Sweden

*Corresponding author e-mail address: zoe.fisher@ess.se

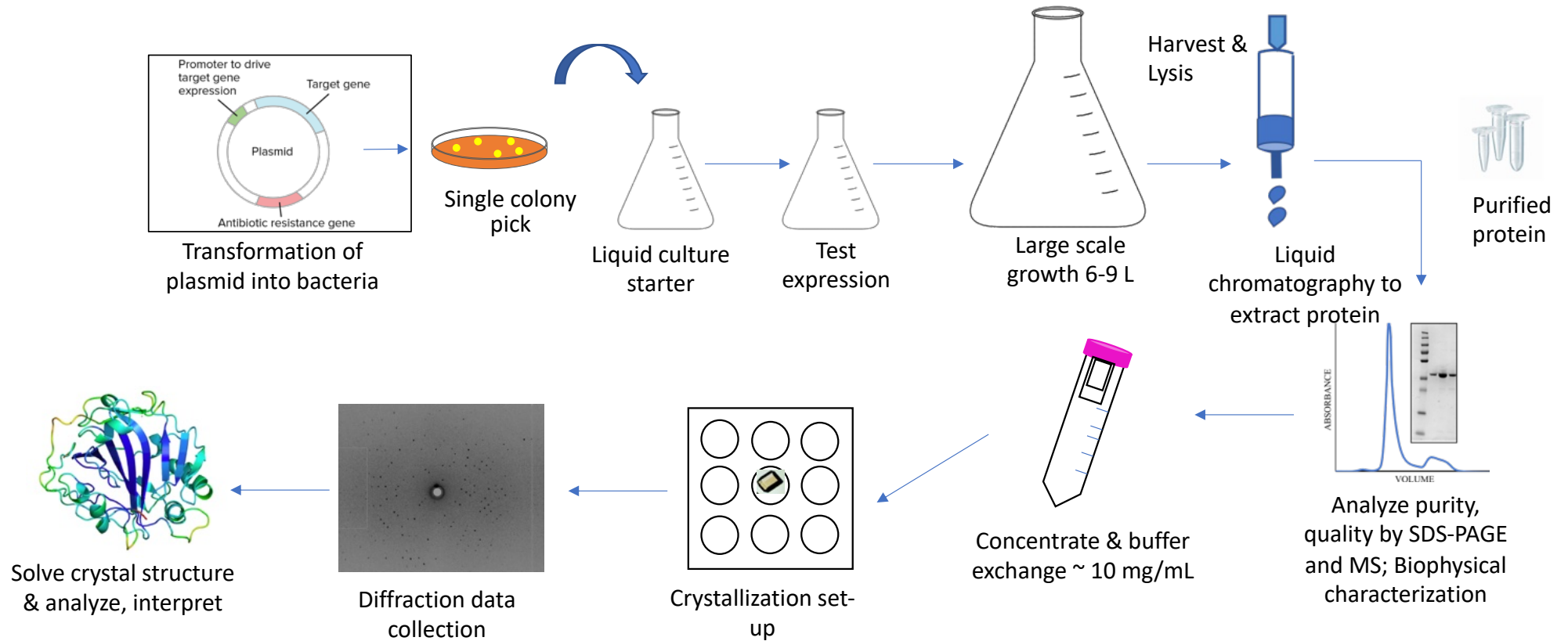
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<https://www.sciencedirect.com/bookseries/methods-in-enzymology/vol/634/suppl/C>

Borrowed & adapted from Dr. Swati Aggarwal
doi: 10.1016/j.pep.2021.105954

DEMAX supports the full NMX user journey



Talk to us about your project! demax@ess.eu or zoe.fisher@ess.eu

DEMAX product catalogue

demax@ess.eu

- Updated product catalogue is available on the DeuNet website

<https://deuteration.org/demax/>

- Also includes instructions for the dry shipper we use for sending perishables



Deuteration and Macromolecular Crystallisation Platform

Product List & Sample Shipping

August 2023

Biological: proteins, biomass, nucleic acids.....	2
Biological: purified lipid mixtures	2
Chemical: carboxylic acids, aldehydes, alcohols, alkyl halides.....	3
Chemical: surfactants	4
Chemical: phospholipids.....	6
Chemical: aromatic & heterocyclic aromatic molecules	7
Chemical: miscellaneous	9
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DEMAX Access

- Users have to submit a proposal.
- Proposal are subject to internal feasibility review and scientific (peer) review by a DEMAX panel.
- Access is free (for now) and granted upon acceptance of the proposal.
- In addition to user service, we also participate in collaborative projects & support other groups at ESS if needed.

User proposals

- Rolling access is currently open until end of September 2024
- User should register and submit proposals online
- Access is merit based and free of charge, not restricted to member nation status.
- Co-authorship vs acknowledgement

User Office / Dashboard Logge

Welcome to the ESS User Office Software

Now Open: Rolling access.

DEMAX is now extending Rolling Access for proposals requesting support for chemical & biological deuteration as well as support for protein crystallisation

For a list of molecules/support, please see our product catalogue [here](#).

We strongly encourage users to reach out at demax@ess.eu to discuss their project needs prior to submitting a proposal.

If you are interested in something that you don't see in the catalogue, please reach out to us at demax@ess.eu. We can do a feasibility review and see if it possible or we may be able to help through the Deuteration Network.

1st November 2022, 17:00 (CET): Rolling access opens
30th September 2024, 17:00 (CET): Rolling access closes
20th December 2024, 17:00 (CET): Final delivery of molecules

Pilot call for chemical and biodeuteration support from the DEMAX platform

JANUARY 10, 2022



The Deuteration and Macromolecular Crystallisation (DEMAX) platform at ESS supports neutron users from the soft matter, biology, life sciences and chemistry research areas. The neutron techniques that these communities typically use include small angle scattering, reflectometry, single crystal diffraction, and spectroscopy. For steady state ESS operations, DEMAX is currently developing three areas of support: Biological deuteration (e.g. cell paste, soluble proteins, lipids, membranes), Chemical deuteration (e.g. small organic molecules, surfactants, phospholipids), and Crystallisation (large protein crystal growth).

The following conditions apply to all DEMAX proposals:

Proposals must be submitted and fully completed online at ESS User Office portal: <https://useroffice.ess.eu>

All proposals will undergo a technical and internal scientific review.

There is no charge for the use of the platform. There is no charge for the use of the platform.

Materials at another neutron scattering facility (e.g. attach approved beamtime proposal).

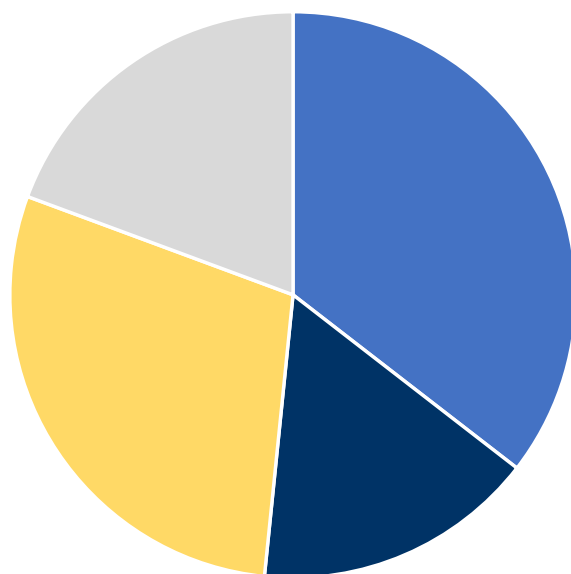
There is no charge, but we may ask that users pay for shipping & handling for dangerous good (e.g. dry ice).

Users must acknowledge the support of the DEMAX platform in any publications. Please read the publication guidelines [here](#).

The pilot call for chemical and biodeuteration support from the DEMAX platform starts on 1st November 2022 and has no specific deadline for proposal submission. We will aim to deliver all support to inform users if we can support their work as soon as possible after review.

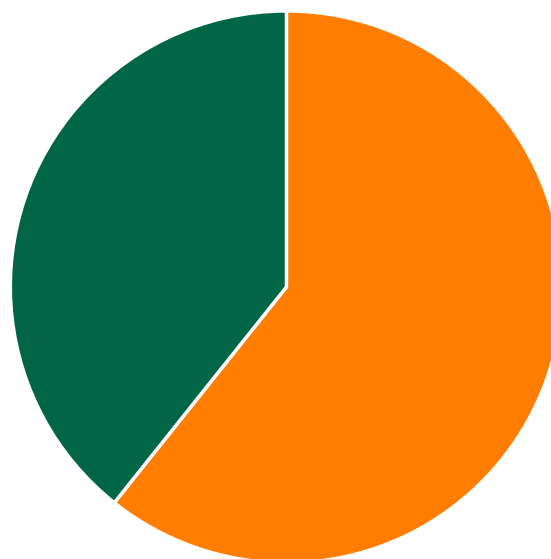
- Since starting (2019) we have now over **100 unique users**
- DEMAX has published or has under review **40 papers in peer-reviewed journals**
- In call 2b + Rolling Access we have received **31 proposals requesting 54 molecules/services** (accepted 28 proposals to deliver 48 molecules)

Intended neutron scattering technique



■ SANS ■ NPX ■ NR ■ Other

Type of deuteration required

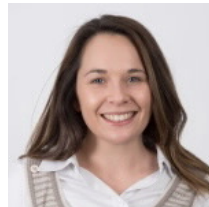


■ Chemical deuteration ■ Biological deuteration





Thanks to DEMAX, & LP3 & ESS



Anna Leung

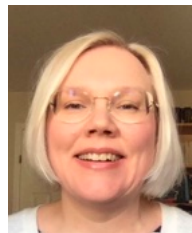


Zoë Fisher

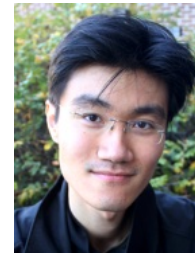


LUND UNIVERSITY

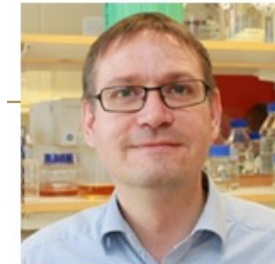
Wolfgang Knecht



Hanna Wacklin-Knecht



Jia-Fei Poon



Vetenskapsrådet

LP3 research engineers

