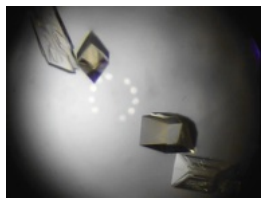
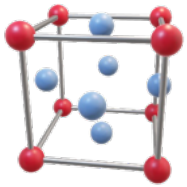
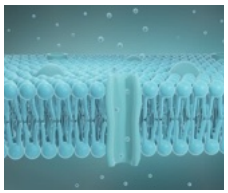
The Deuteration & Macromolecular Crystallization platform at ESS

Dr. Zoë Fisher

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

Snr. Adjunct lecturer at Biology Department, Lund University



ECM24 – NMX First Science workshop

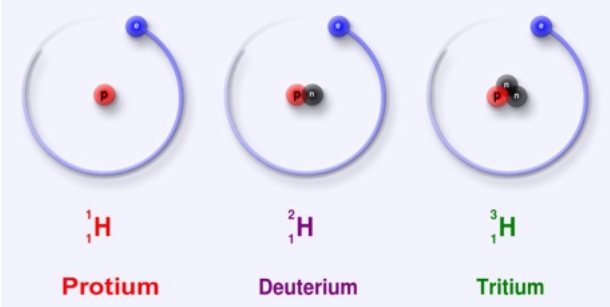
Deuteration is important & necessary for neutron experiments

- Molecules from living organisms are abundant in hydrogen, spec. ^{1}H isotope
- Deuteration: replacing endogenous ^{1}H with ^{2}H 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 ^{2}H

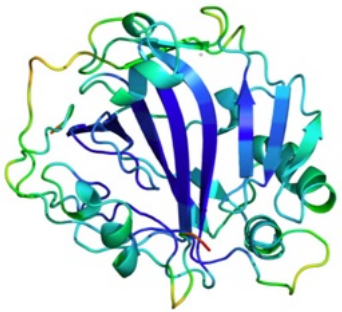


Carbon	C	1647
Hydrogen H	2565	
Nitrogen	N	465
Oxygen	O	517
Sulfur	S	21

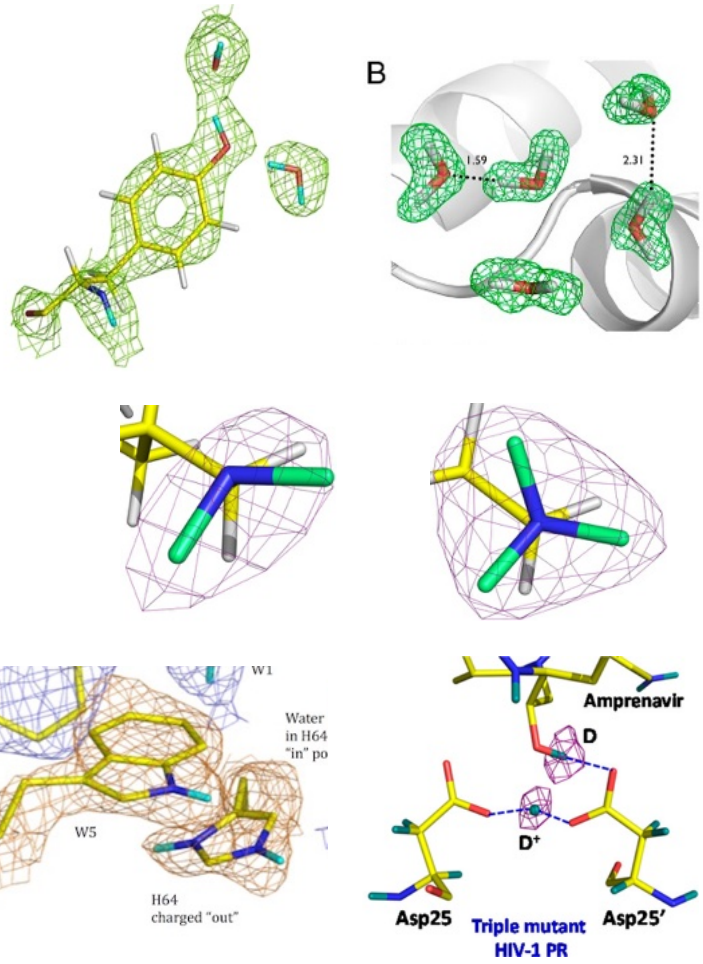
Formula: $C_{1647}H_{2565}N_{465}O_{517}S_{21}$

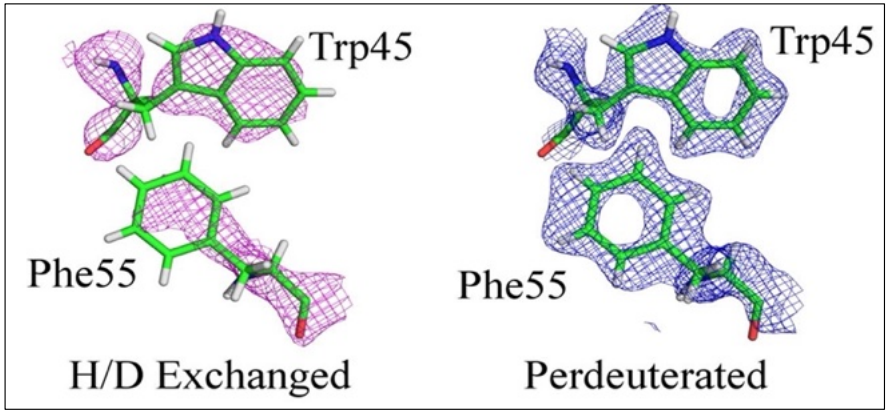
Total number of atoms: 5215

~ 350 amino acids



Determine position of H atoms in macromolecular structures





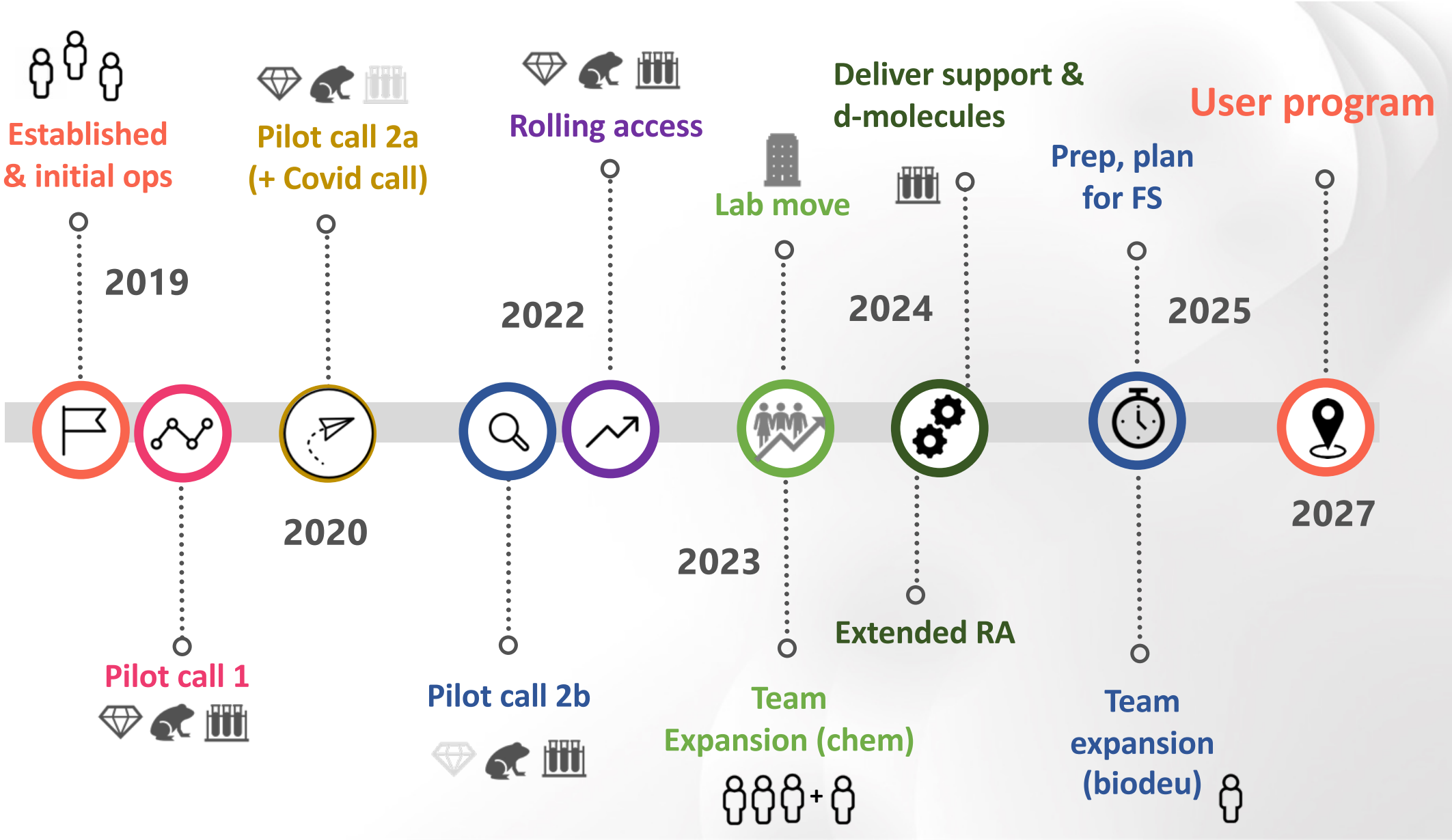
https://journals.jps.jp/doi/pdf/10.7566/JPSCP.25.011003

Limitations of biodeuteration

- Limited number of species tolerate D_2O – highly toxic in higher organisms (insects, mammals, plants) >30%
- Cells are not happy in D_2O: slow growth, low yields
- Requires a lot of very expensive D_2O 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


Established & initial ops
2019
Pilot call 1
Pilot call 2a (+ Covid call)
2020
Rolling access
2022
Pilot call 2b
Lab move
2023
Team Expansion (chem)
Deliver support & d-molecules
2024
Extended RA
Prep, plan for FS
2025
Team expansion (biodeu)
User program
2027

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

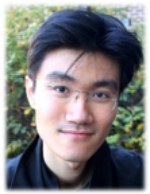
Biological Deuteration

- 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



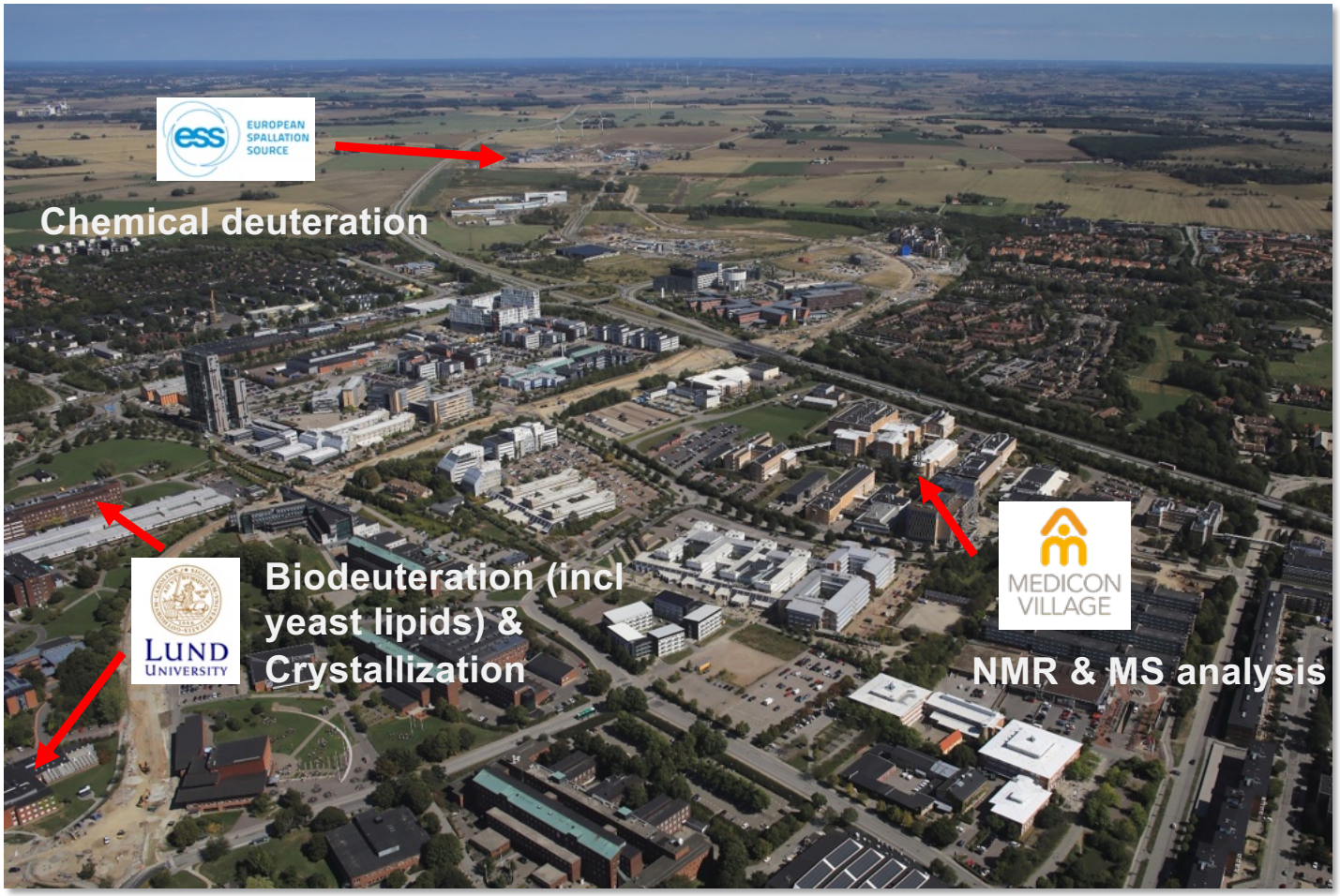
Jia-Fei

LP3
0.7 FTE

D-lab (lipids)
0.2 FTE

Zoë

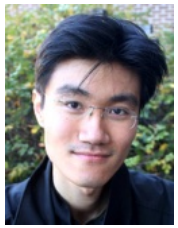
Labs are spread out LU, MV & ESS



Lund, Sweden

Chemical Deuteration

Anna

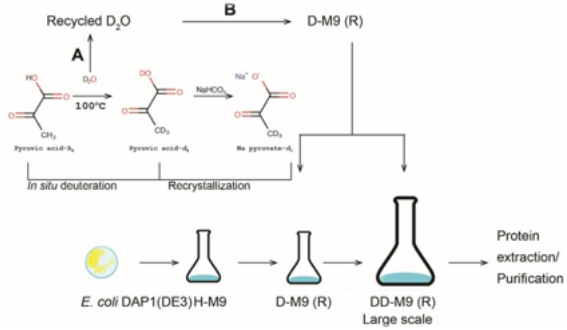


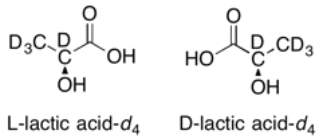
Jia-Fei

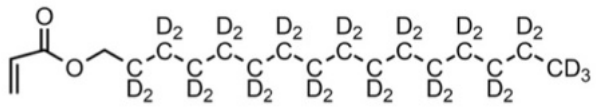
- Moved to ESS in June 2023
- Lab is up and running with essential equipment is in place for synthesis, separation, characterization.
- For some characterization needs (e.g. NMR) we have service arrangements with Red Glead & LU Chemistry.
- *In progress: Advion ESI-MS*

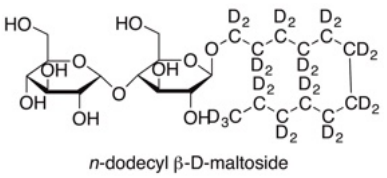
Deuterated organic molecules

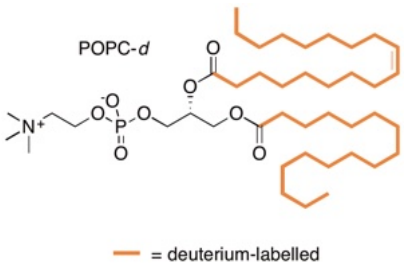
H/D exchange, chemical & enzymatic synthesis of a range of small molecules (surfactants, monomers, alcohols, aldehydes, lipids, fatty acids etc.)

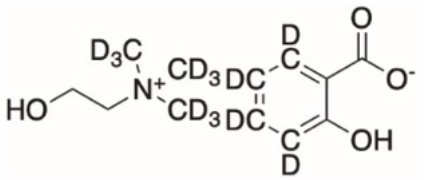




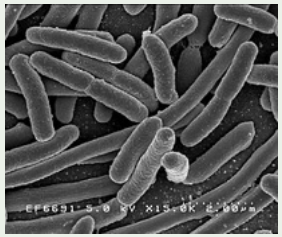
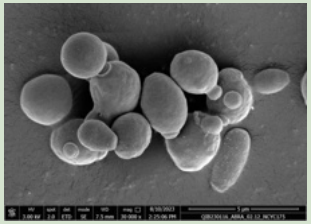
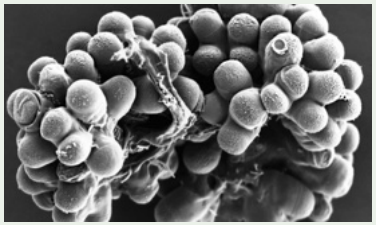






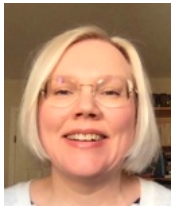


DEMAX offers biodeuteration from following:

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

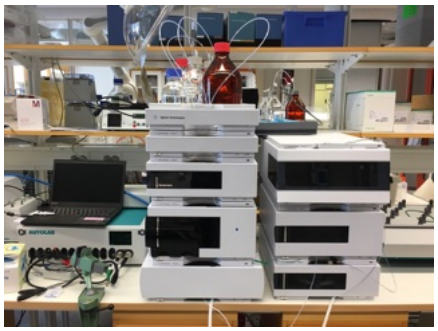


Hanna

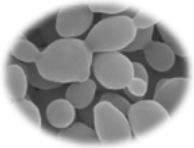
Sophie

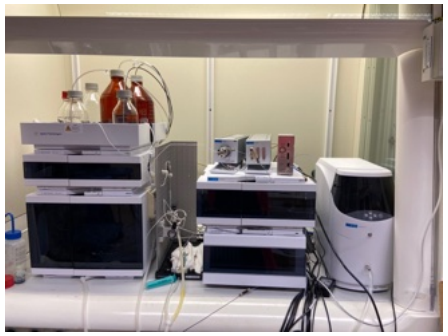
Large scale production of *Pichia pastoris* (supported by LP3)
Total lipid extraction, separation phospholipid classes
Analysis: TLC, GC, MS

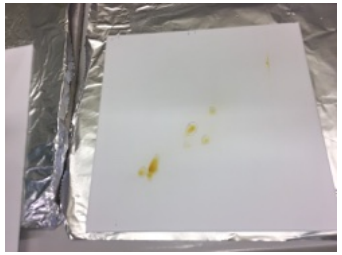
** temporarily housed at Kemicentrum, LU*



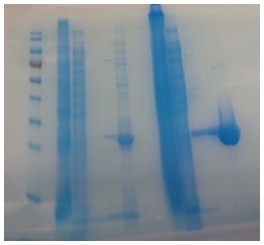
Pichia pastoris

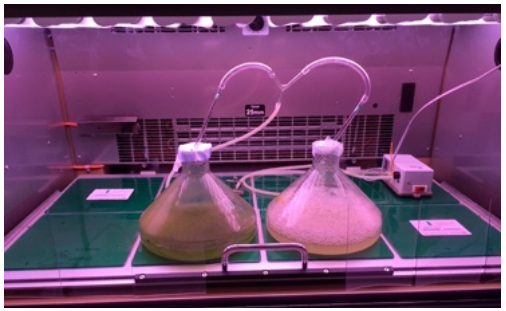


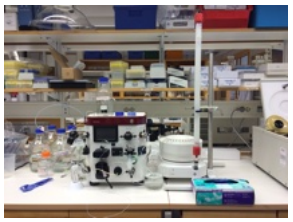


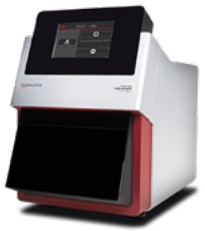


Deuterated biomolecules







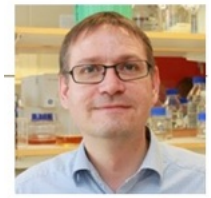


LP3
Lund Protein-Production Platform

Zoë

LUND UNIVERSITY

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

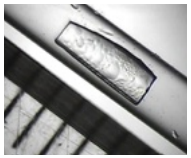
LUND UNIVERSITY

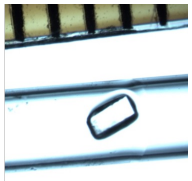
BAG access to BioMAX

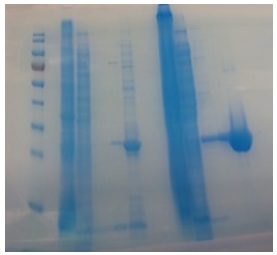
- Currently: co-located with the LP3 crystallization lab
- We offer low throughput optimization, often by hand or with custom screen & optimization using the Oryx8 – depends on maturity of project
- Part of LU BAG for BioMAX: test and/or collect RT (or cryo) X-ray diffraction data
- We support large 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)

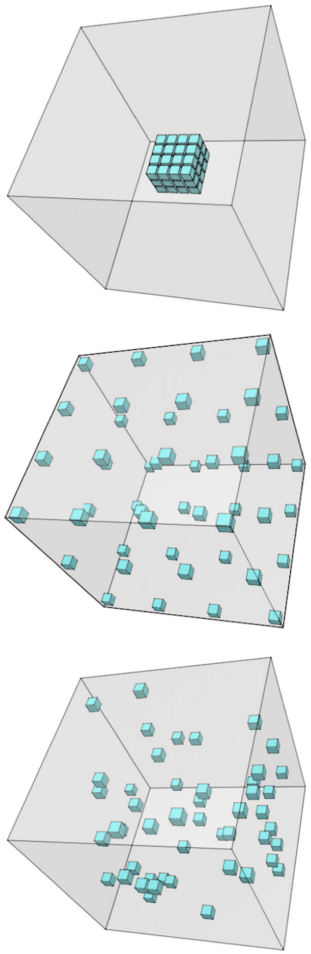


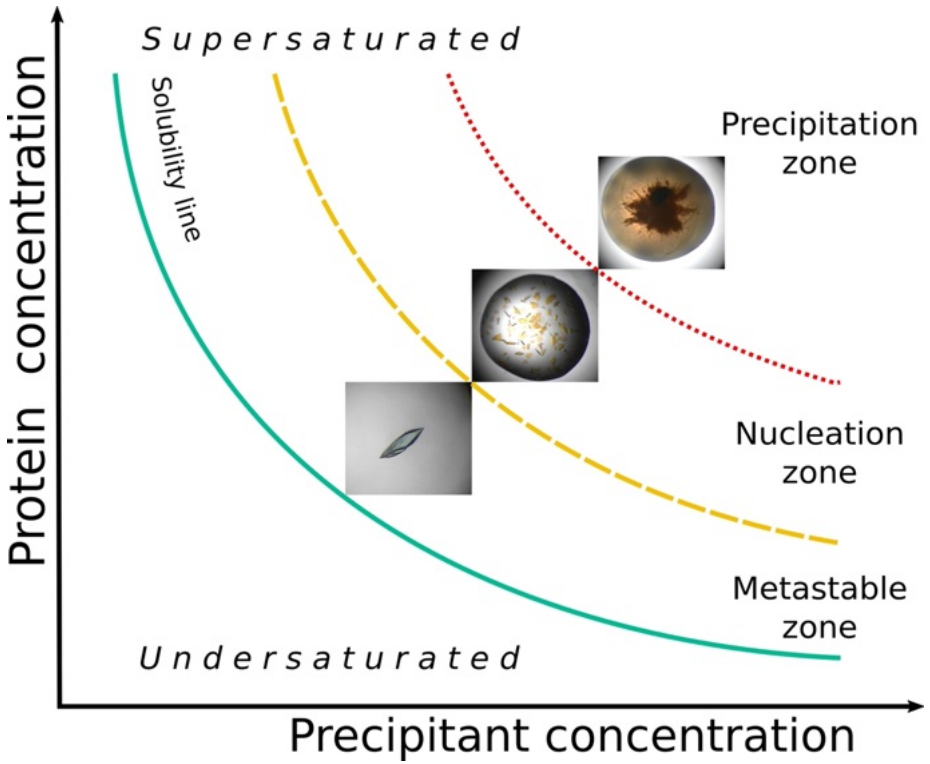




Check concentration, purity, stability – then HT or fine screen = hopefully some good crystals that can be optimized!

Crystal screening vs. optimization



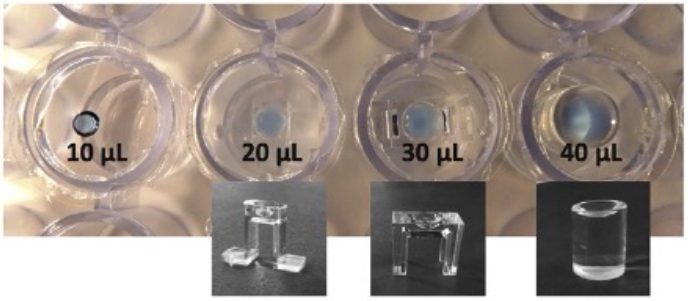


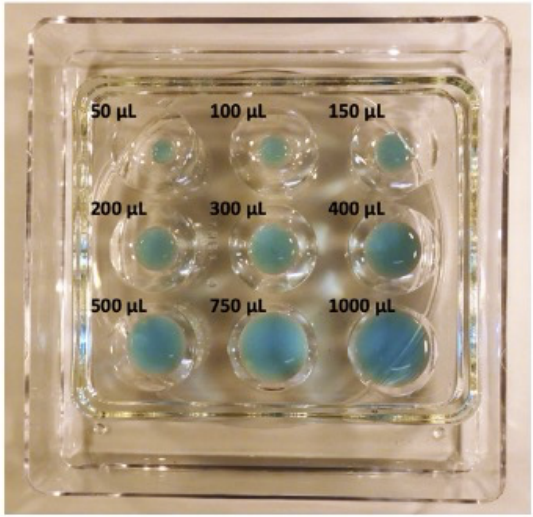
- pH
- Temperature
- Deuteration level
- Additives

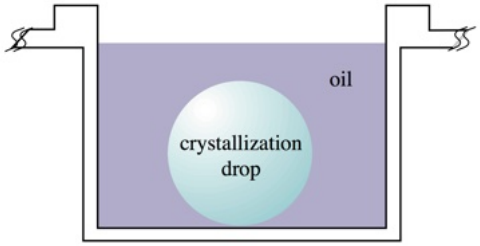
https://doi.org/10.1016/bs.mie.2019.11.015

Crystallization hardware

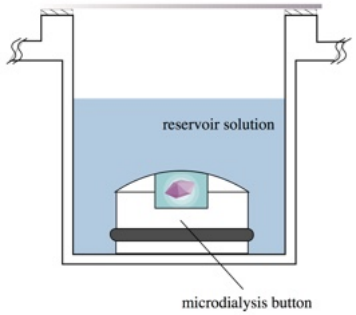
- Vapour diffusion







- Batch (under oil)



- Dialysis

https://hamptonresearch.com/growth_101_lit.aspx

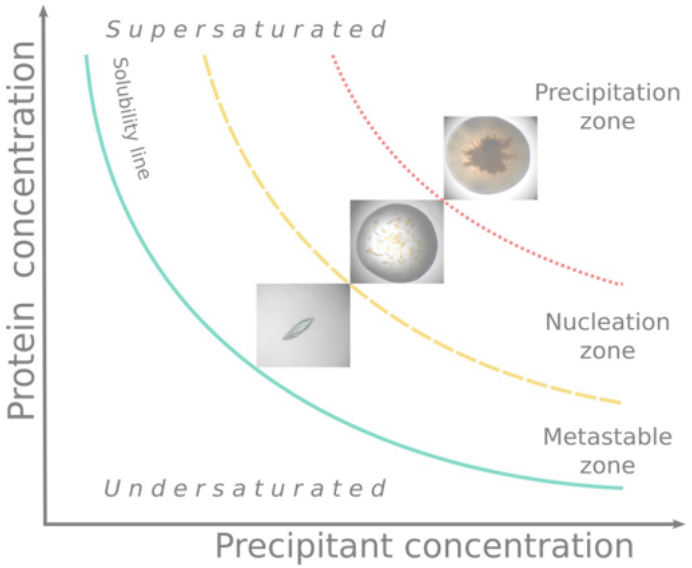
Modifications to basic methods

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

- Crystal seeding (micro or macro)

or simply growth:

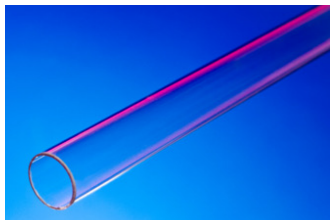
- Crystal feeding



Preparing crystals for data collection

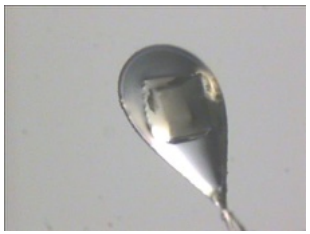
- We support both RT & cryo crystal mounting for testing and/or data collection
- Capillary mounting (RT) or Magnetic bases with capillary “cover” (RT) or the standard cryo loops, bases (cryo)

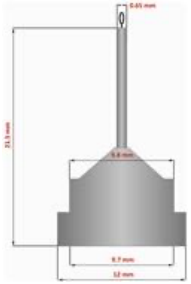
RT



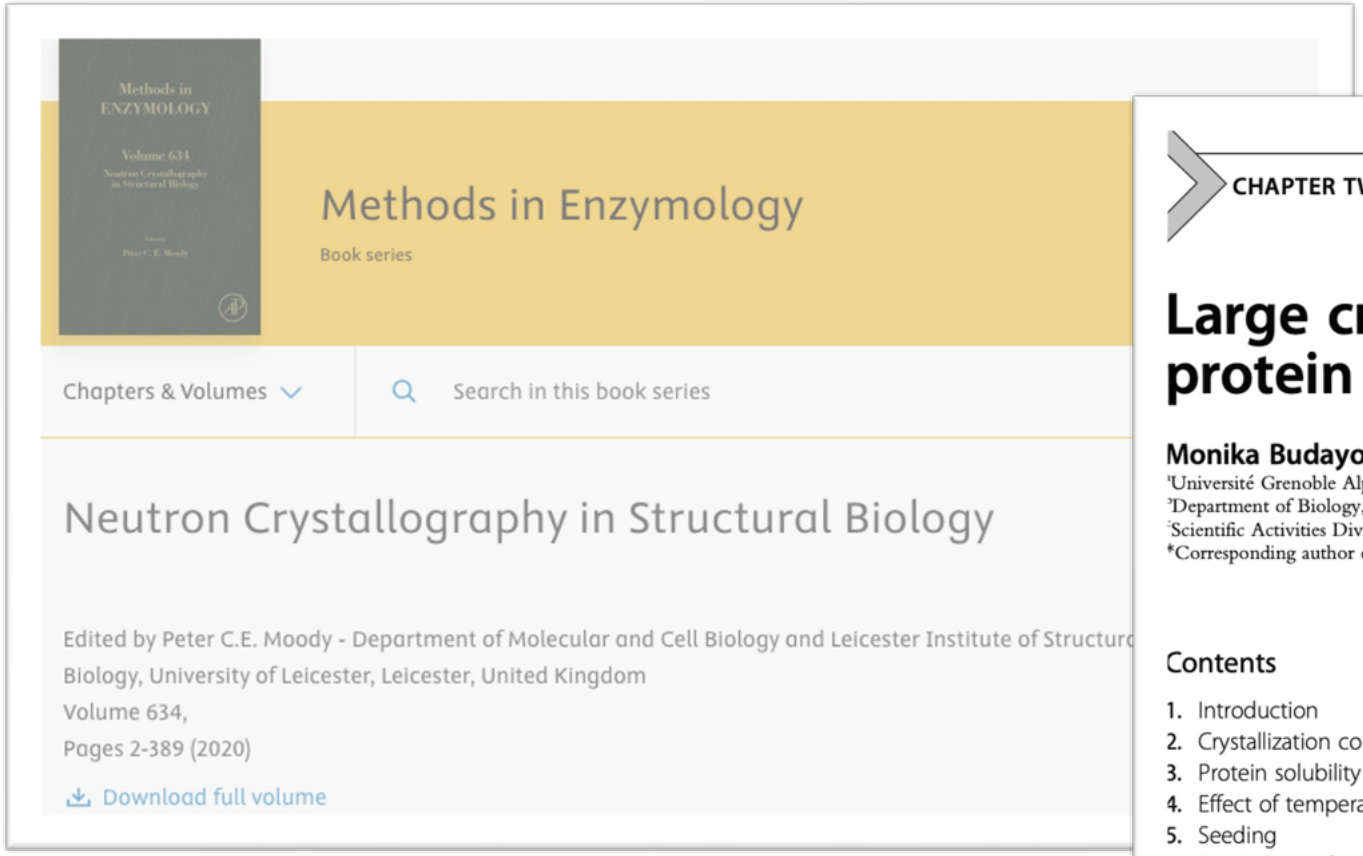
No damage from freezing	**Tricky to do, need to practice and do it frequently**
No cryoprotectants, SEE or LN2	**Sensitive proteins degrade, radiation damage**
Observe structure closer to physiological conditions	**Can’t make complexes or trap reaction intermediates**

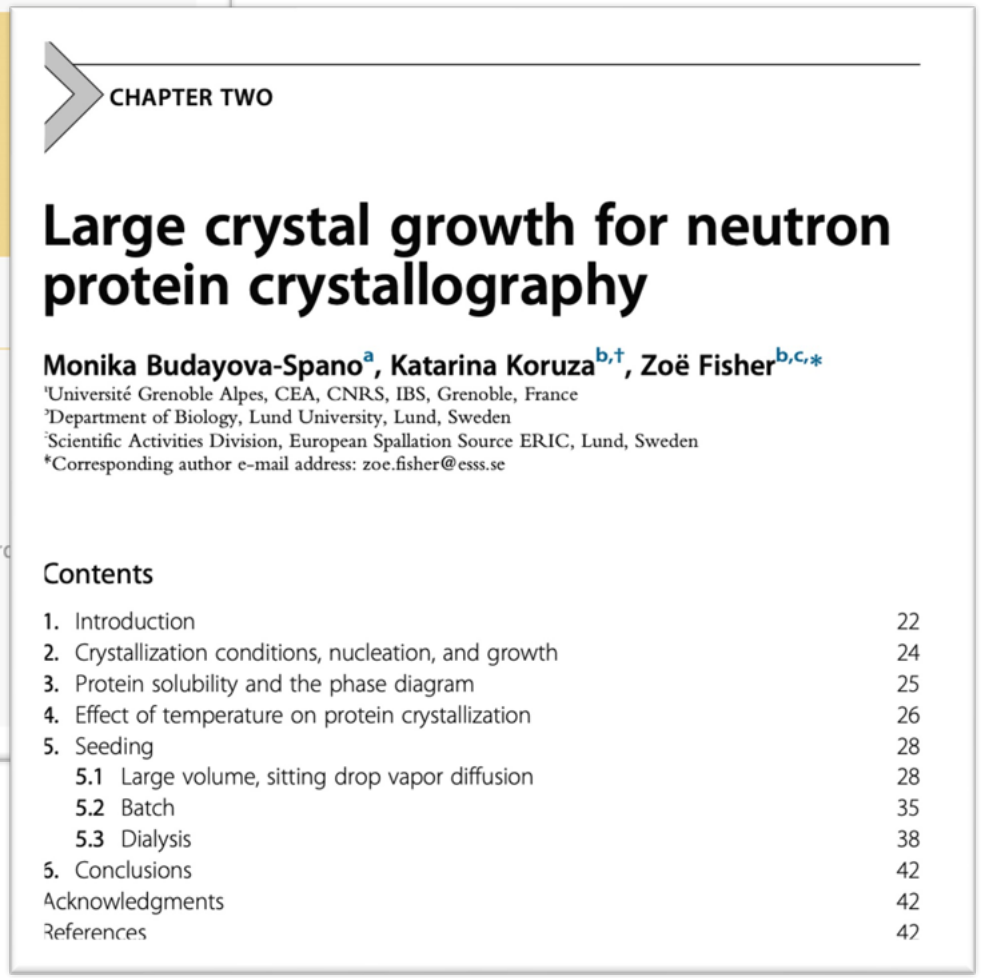
CRYO





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**





CHAPTER TWO

Large crystal growth for neutron protein crystallography

Monika Budayova-Spano[a], **Katarina Koruza**[b,†], **Zoë Fisher**[b,c,*]
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[2]Department of Biology, Lund University, Lund, Sweden
[3]Scientific Activities Division, European Spallation Source ERIC, Lund, Sweden
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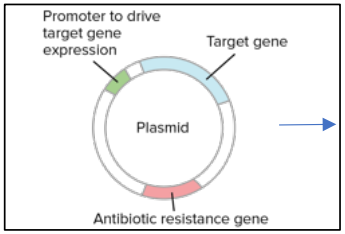
Contents

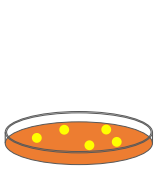
https://www.sciencedirect.com/bookseries/methods-in-enzymology/vol/634/suppl/C

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

DEMAX supports the full NMX user journey



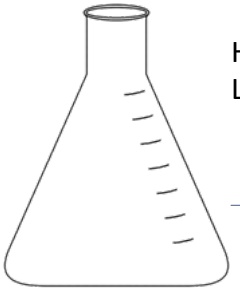
Transformation of plasmid into bacteria



Single colony pick

Liquid culture starter

Test expression

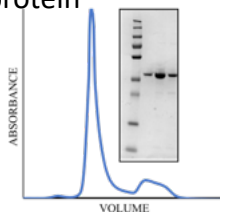


Large scale growth 6-9 L

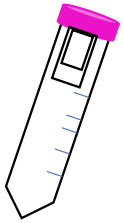
Harvest & Lysis

Liquid chromatography to extract protein

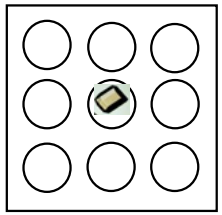
Purified protein



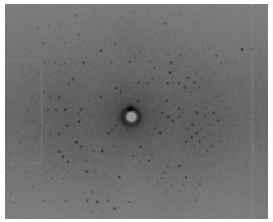
Analyze purity, quality by SDS-PAGE and MS; Biophysical characterization



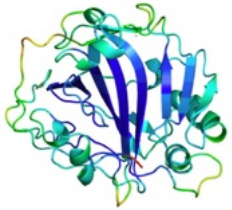
Concentrate & buffer exchange ~ 10 mg/mL



Crystallization set-up



Diffraction data collection



Solve crystal structure & analyze, interpret

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

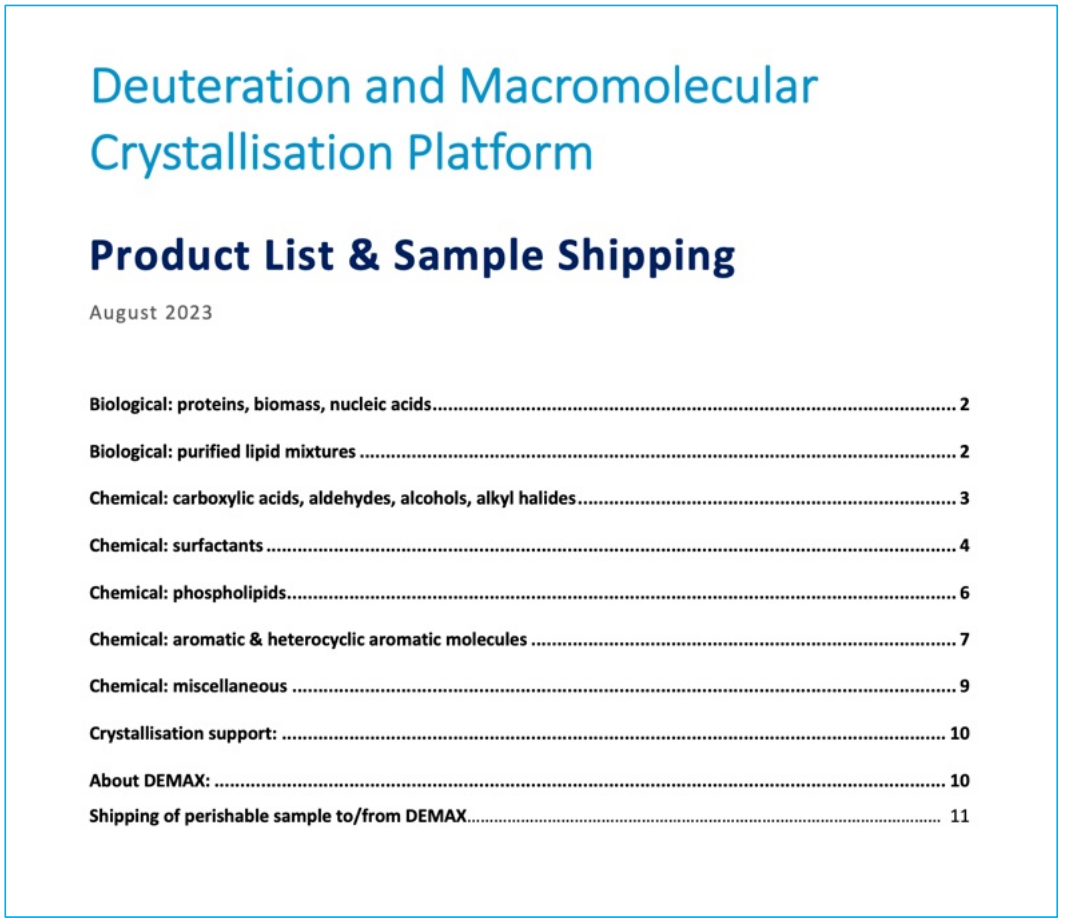
DEMAX product catalogue

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

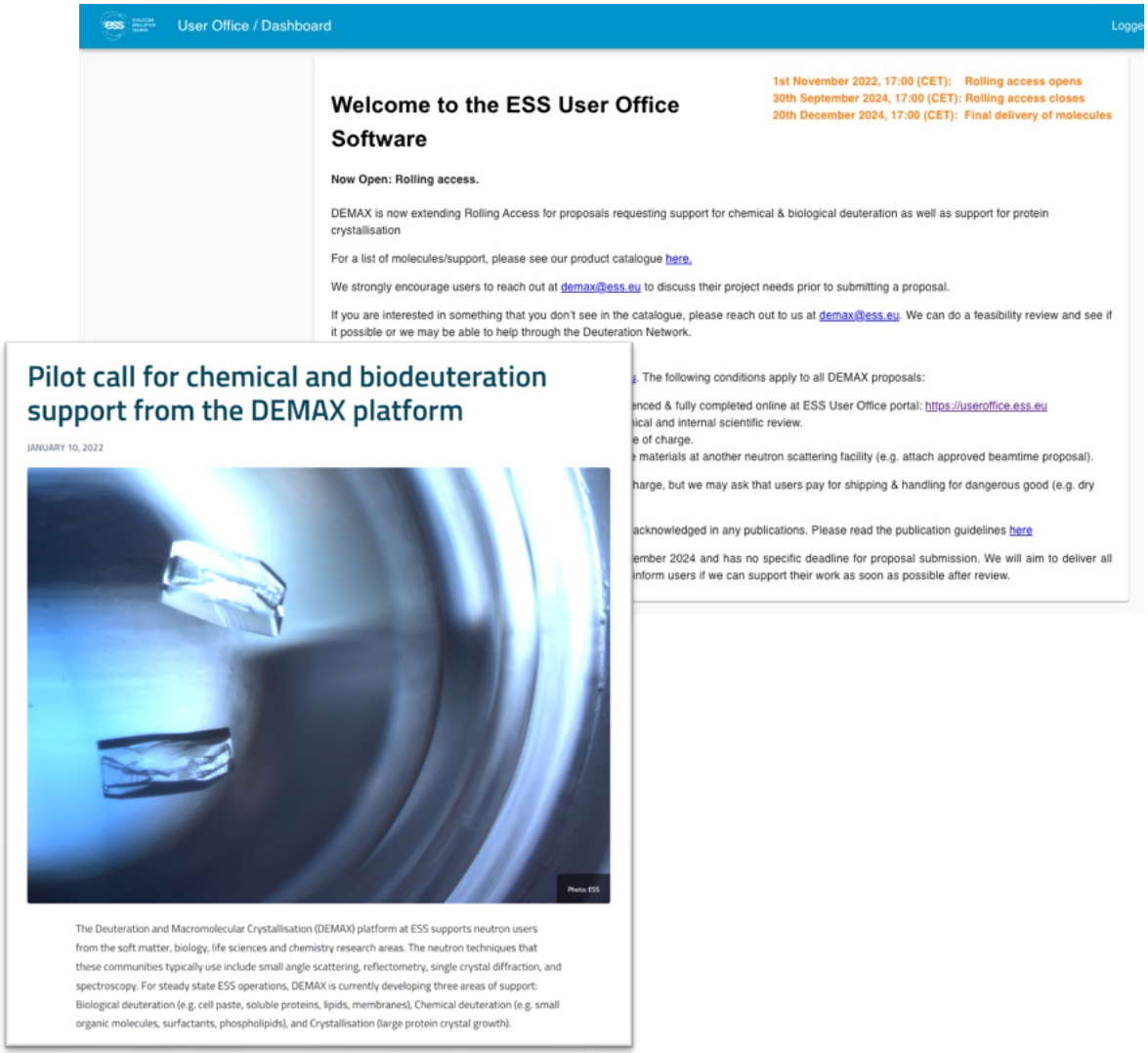
DEMAX Access

- 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

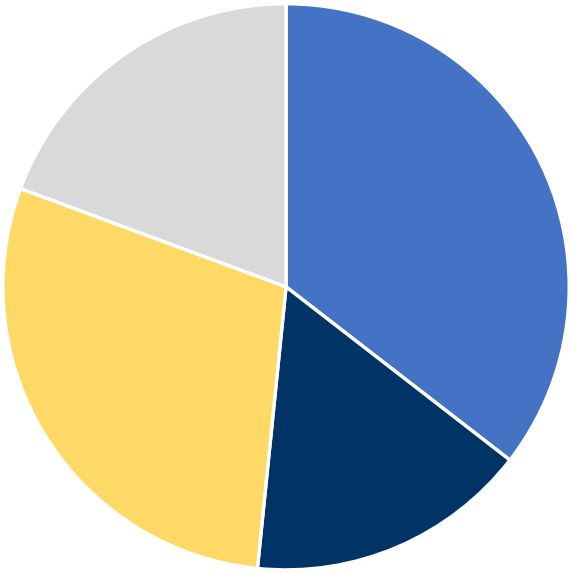
useroffice.ess.eu



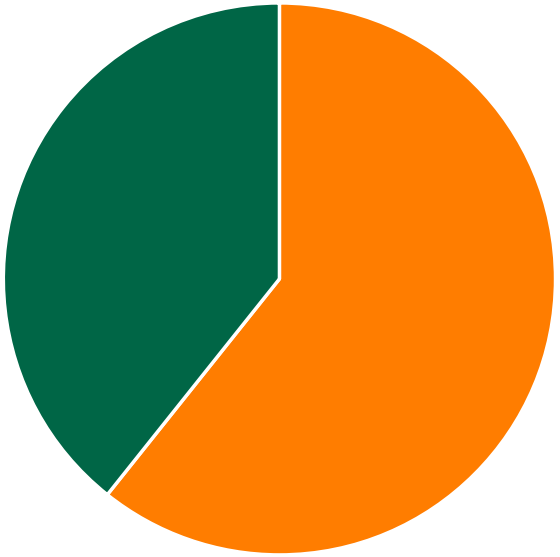
https://europeanspallationsource.se/node/247917

- 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



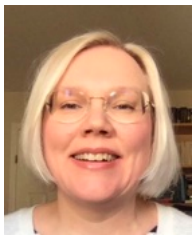
Type of deuteration required



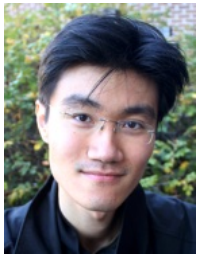
Thanks to DEMAX, & LP3 & ESS

Anna Leung

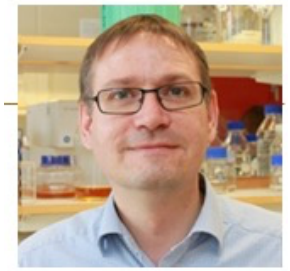
Zoë Fisher



Hanna Wacklin-Knecht



Jia-Fei Poon



Wolfgang Knecht

LP3 research engineers