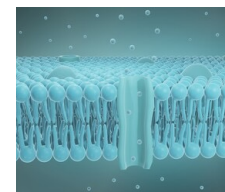
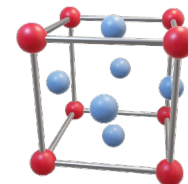
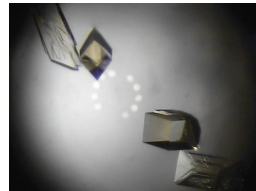


# DEMAX

## The Deuteration and Macromolecular Crystallization Platform at ESS



# What is DEMAX?

- DEMAX is the ESS user support lab that offers deuteration and crystallization service & support – aimed at chemistry, soft matter, life science neutron community
- We drive a mix of method development, support & research activities
- Access to (non-commercial) deuterated materials & large protein crystals is a bottleneck for doing neutron scattering experiments = support makes high impact science possible!

# Past, present, future...

- We entered Initial Operations in 2019
- Issued 3 pilot calls, offering different things
- Currently we have Rolling Access as it is some time before the next formal call (spec. biodeu, crystallization support – but EoI are welcome!)
- Next call: in conjunction with hot commissioning and/or friendly users on ESS instruments (late 2024?)

**Rolling Access now available for deuteration & crystallisation support from the DEMAX platform**



<https://useroffice.ess.eu/SignIn>


<https://europeanspallationsource.se/node/247917>




# DEMAX Platform




Users can collaborate with us, get advice + access to expertise (us and within DeuNet)

 **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)

Extended team  
(incl postdocs & tech support  
From LP3/LU)



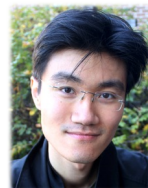
Zoë



Anna



Hanna



Jia-Fei



Jenny



Sophie

+



0.75 FTE

LU/LP3



# Chemical Deuteration



Anna

Jia-Fei

Hanna

Sophie

Jenny



- Main activities are organic chemistry & lipid production (yeast)
- Essential equipment & capabilities in place, some analysis off-site (e.g. NMR, MS) – RG & LU
- Chem labs are moving to site (4+ mo. downtime)

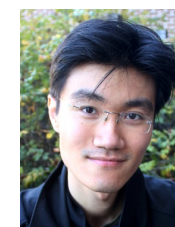


# Organic deuteration chemistry

Deuteration, H/D exchange, chemical & enzymatic synthesis of small molecules (surfactants, monomers, aromatic & heterocyclic molecules, lipids, fatty acids etc.



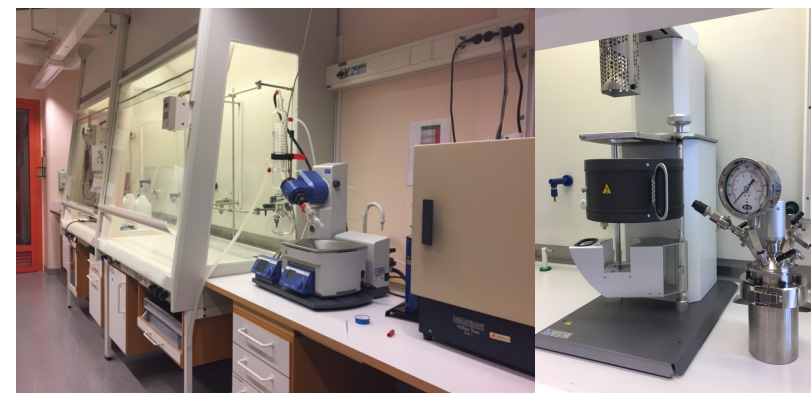
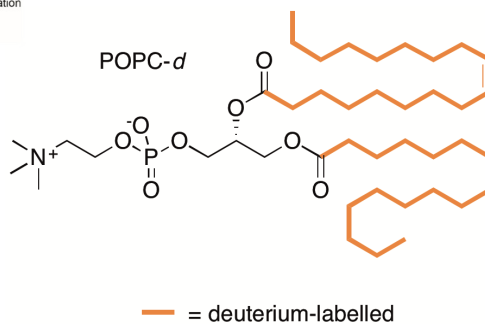
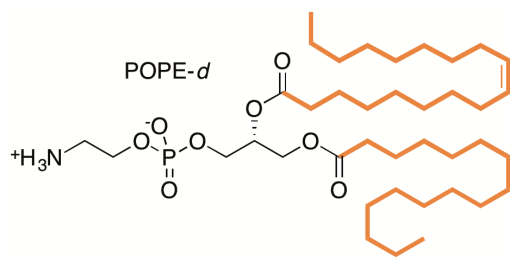
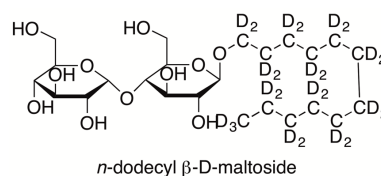
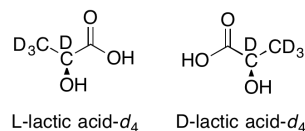
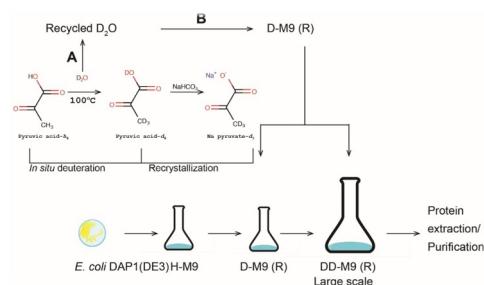
Anna



Jia-Fei

Article  
**Evolving *Escherichia coli* Host Strains for Efficient Deuterium Labeling of Recombinant Proteins Using Sodium Pyruvate- $d_3$**

Vinardas Kelpšas <sup>1</sup>, Anna Leung <sup>2</sup> and Claes von Wachenfeldt <sup>1,\*</sup>



<http://pubs.acs.org/journal/acscdf>

Article

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

Oliver Bogojevic and Anna E. Leung\*

Cite This: <https://dx.doi.org/10.1021/acsomega.0c02823>

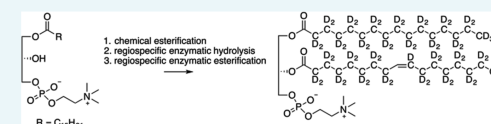
Read Online

ACCESS |

Metrics & More

Article Recommendations

Supporting Information



# Biological lipid production



Hanna



Jenny



Sophie

Large scale production of *P. pastoris* (LP3)

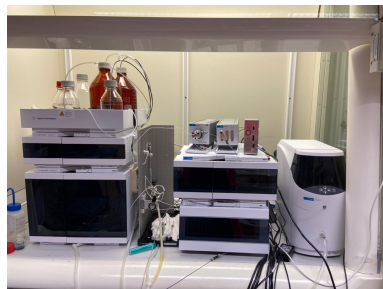
Total lipid extraction, non-polar lipid separation, total phospholipid extracts & sterols; Analysis: TLC, GC, MS

In development: Separation of phospholipid classes, optimization of reverse-phase HPLC

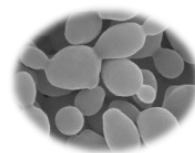
Analytical (LP3 loan)



Preparative RP



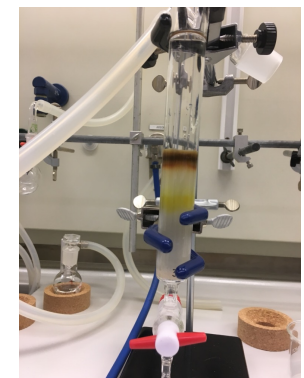
*Pichia pastoris*



GC-FID



Prep TLC



# Biological Deuteration & Crystallization



Zoë



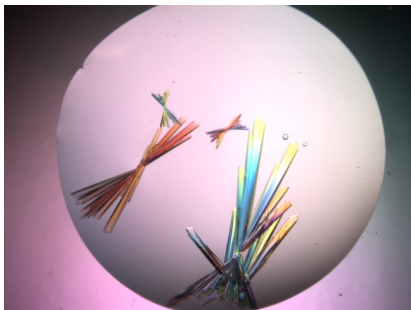
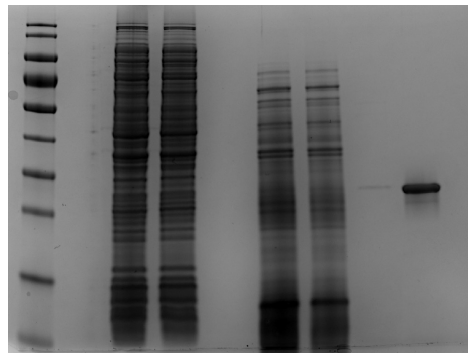
LUND  
UNIVERSITY



EUROPEAN  
SPALLATION  
SOURCE



0.75 RT

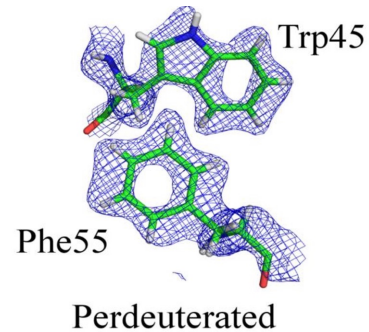
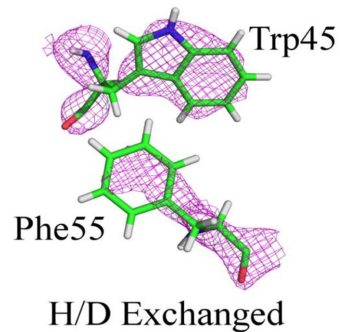
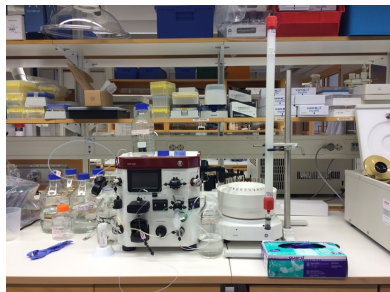
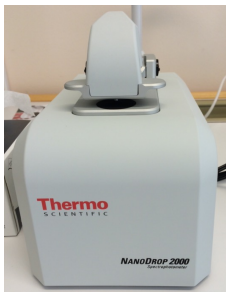


- Co-located with LP3 in Biology Department, LU
- ESS equipment in place, access agreement to be able to use departmental and LP3 labs & equipment
- LP3 provides Swedish in-kind DLS to DEMAX



# Biological deuteration

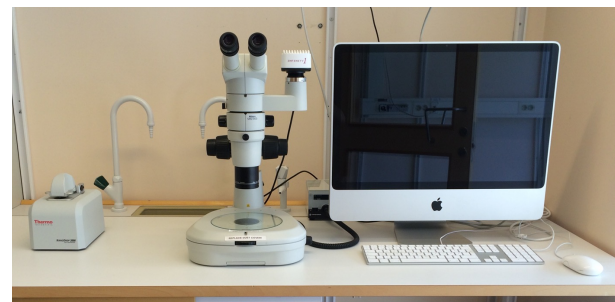
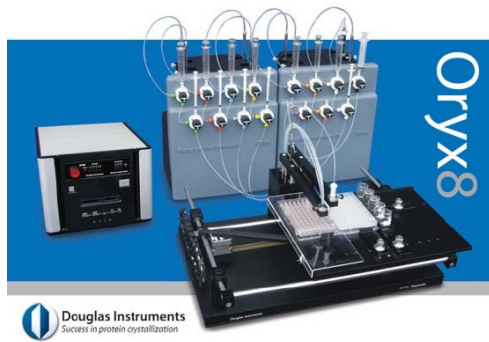
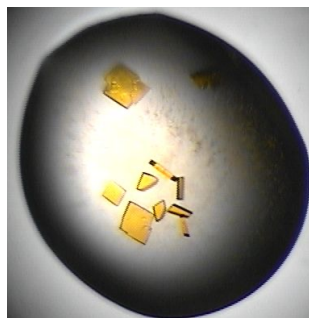
- We can produce full or partially deuterated biomass from bacteria (*E. coli*), algae (*Botryococcus braunii*), and yeast (*P. pastoris*) – deuteration level according to need (99.9%, match-out 65 – 85%, 20-30% H/D exchange)
- Recombinant protein production from user-supplied plasmid, plasmid DNA, yeast derived lipid extraction & separation, total cell extract from algae (D-altone)
- Protein purification, yield & purity & stability (LC, SDS-PAGE, UV/Vis, NanoDSF)



# Protein Crystallization Lab

(also located at Biology Dept in LP3 labs)


- ESS have equipment to support the full workflow (from receiving a protein, screening, optimization, to a mounted crystal for data collection)
- Support VD, dialysis, batch in a range of volumes – temp control
- Part of LU-BAG with LP3 for cryo or RT screening & data collection on user crystals at BioMAX beamline



# The DEMAX workflow

- Access is handled through proposal calls
- Proposals undergo internal safety & feasibility review, followed by external scientific peer review
- Upon review & acceptance & endorsement by SD, support is provided free to user
- We negotiate amounts, level of D, "need by date" and get to work!
- Once sample is made & fully analyzed, we ship it to users and provide a CoA (and associated DOI)

# Sample shipment

 **EUROPEAN SPALLATION SOURCE**

Dry shipper care & handling

General info:

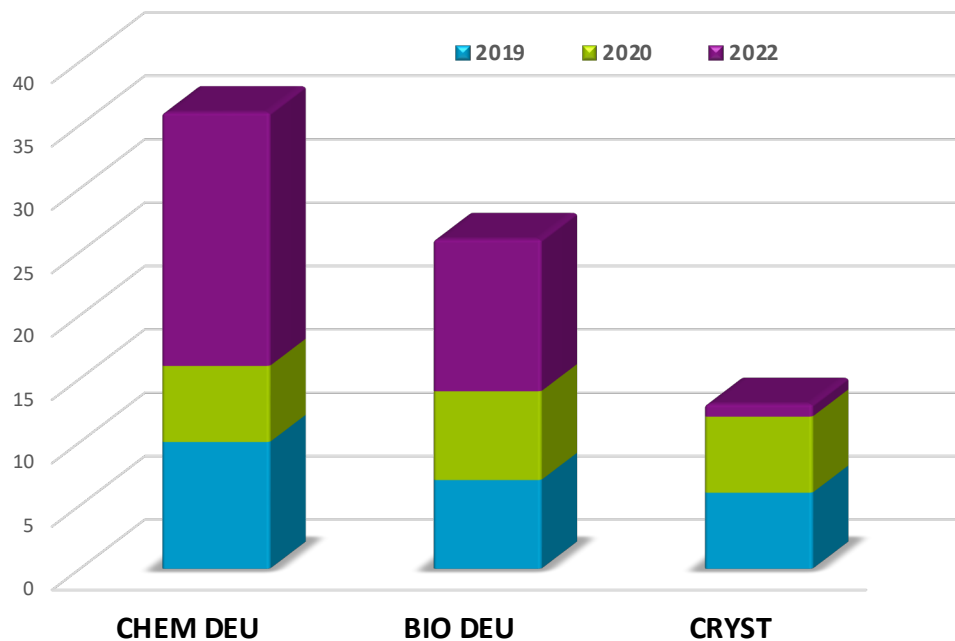
- DEMAX uses dry shippers to send perishable samples. Dry shippers are used for safe transport of samples and are designed to keep your samples at  $-190\text{ }^{\circ}\text{C}$  for an extended period of time (10 – 21 days if treated properly). These are preferred to dry ice for transport as a correctly prepared dry shipper does *not* fall under dangerous goods regulation.
- We typically will send your samples as overnight express but in case something goes wrong and shipment is delayed, using the dry shipper ensures that your samples arrive unspoiled.
- Please keep the dewar upright and in the shipping container with the foam lid on when not inserting/removing samples. The foam lid has a notch allowing it to slide over the canister rod, please place the lid properly and don't force it if you feel resistance.
- Upon arrival, remove your samples by lifting out the canister seated in the core of the vessel (use insulated gloves or tongs to retrieve your samples from the canister).
- Close up the dry shipper & shipping case and return to us with ordinary freight (any carrier of your choosing – DHL, TNT, Fedex etc). Please include a tracking number and check with your local contact which address to send it to.
- Make sure the "up" arrow and "fragile" sign is visible on the shipping case when attaching shipping labels.
- If you would like to borrow our dry shipper to send your sample on for an experiment, you are welcome to do so. Please ask for permission and also handle the shipper according to the instructions below.

- Free of charge the user but we ask that they return the container
- Users are welcome to borrow it to send their samples onward to their experiment/beamtime

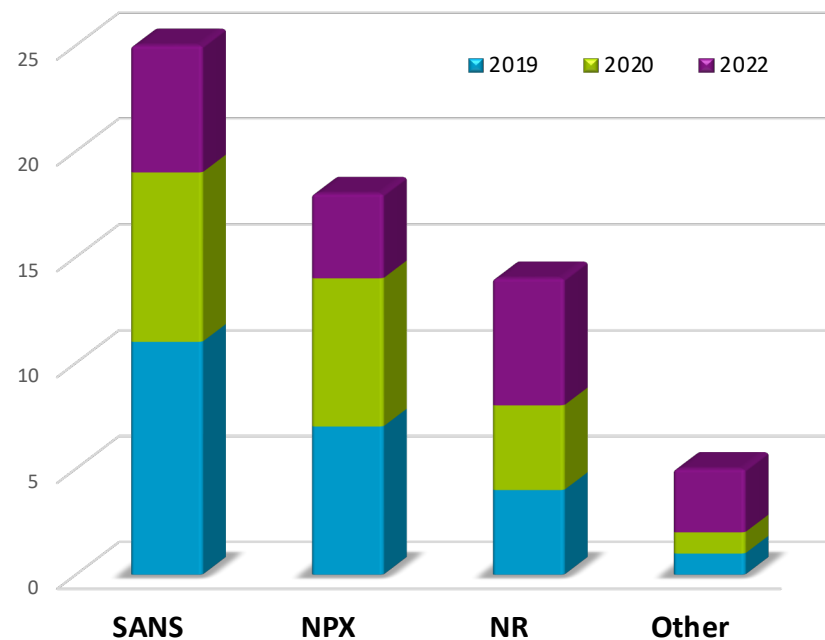
- Dry shipper have many benefits vs dry ice
- Long temperature stability (10-21 days), no dangerous goods for shipping, low cost as sent as ordinary freight (100 Euro within Europe)



Molecules requested by area



Intended neutron method



56 proposals, 57 unique users, 93 molecules requested  
28 papers published since entering ops in 2019

# DEMAX product catalogue

- Product list updated in 2023, will soon be available on the DeuNet website
- Ask me if you are interested in a copy (PDF)

- Older version :

<https://deuteration.net/2021/11/24/deuteration-and-macromolecular-crystallisation-demax-at-ess/>

**Talk to us!**  
**demax@ess.eu**

## Deuteration and Macromolecular Crystallisation Platform

### Product List

February 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  |
| Crystallisation support: .....                                      | 10 |

# Outlook

- Operations are stable and we are learning and growing with every pilot call
- Breadth of science we can support is good but we need some “defense in depth” (lean staffing = single point of failure)
- Lab move & getting up and running on site will be a major focus for the rest of 2023
- DEMAX support needs to be integrated with instrument & facility schedule and review procedures in the future
- Next recruitment bio(deuteration) person that can focus on cell culture & yeast lipid and/or protein extraction and purification



# Thanks to DEMAX, & LP3 & ESS



Hanna Wacklin-Knecht



Anna Leung



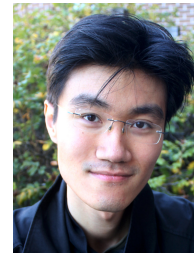
Zoë Fisher



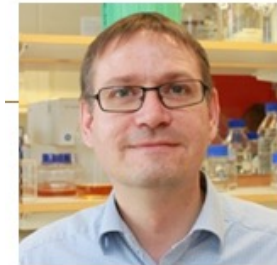
Jenny Andersson



Sophie Ayscough



Jia-Fei Poon



Wolfgang Knecht  
+ LP3 team



Vetenskapsrådet

