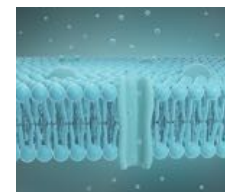
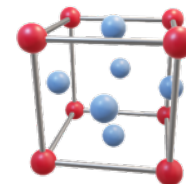
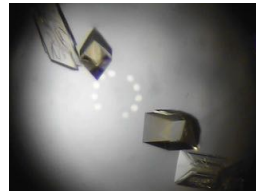


# DEMAX

## The Deuteration and Macromolecular Crystallization Platform



# Outline

- DEMAX overview & background
- DEMAX today: pillars, people, premises
- Overview of capabilities by pillar
- Summary of pilot calls & KPI
- Next steps
- DEUNET

# DEMAX overview

- DEMAX is the ESS user support lab that offers deuteration and crystallization service & support
- Entered operations in 2019
- We drive a mix of user support & (grant-funded) research activities
- We maintain & operate our biology and chemistry labs in support of ESS & user community
- *Our efforts are aligned to enable high impact science on ESS instruments in life science, soft matter, chemistry*



# ESS (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,
- 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)



Zoë



Anna



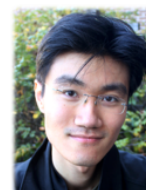
Hanna



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Jenny



Jia-Fei

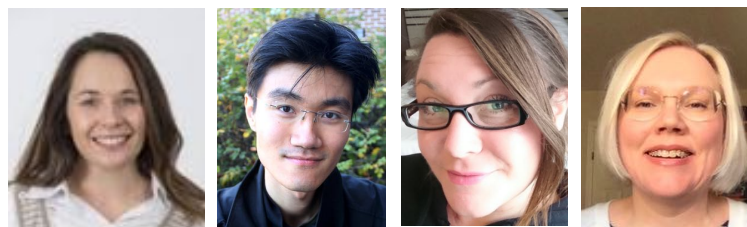


New PD

Labs are spread out LU (LP3), MV, ESS



# Chemical Deuteration



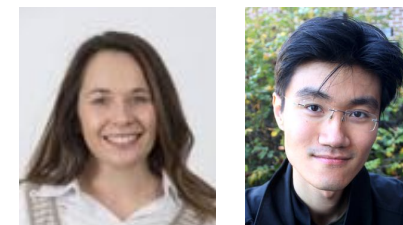
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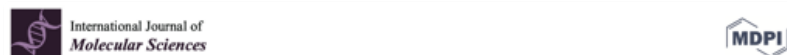
- Functioning chemistry lab with essential equipment is in place for synthesis, separation, characterization.
- For some characterization needs (e.g. NMR, MS) we have service arrangements with Red Glead & LU Chemistry.



# Organic deuteration chemistry

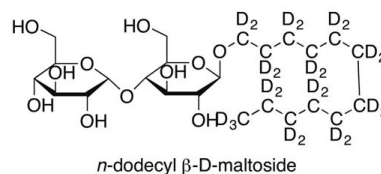
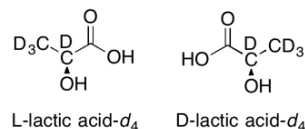
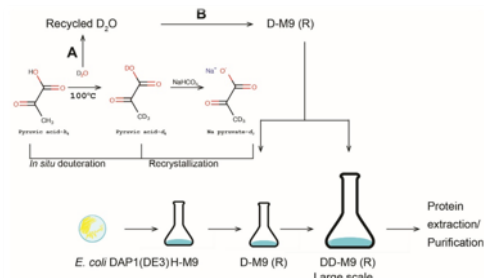


H/D exchange, chemical & enzymatic synthesis of small molecules (surfactants, monomers), lipids, fatty acids etc.



## 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>



<https://pubs.acs.org/journal/acsodf>

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>

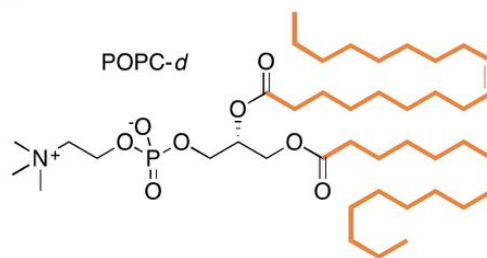
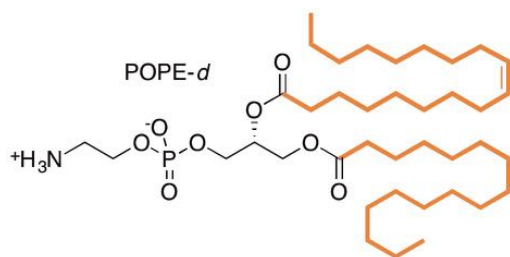
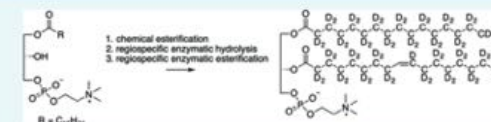
Read Online

ACCESS |

Metrics & More

Article Recommendations

Supporting Information



— = deuterium-labelled

# Biological lipid production

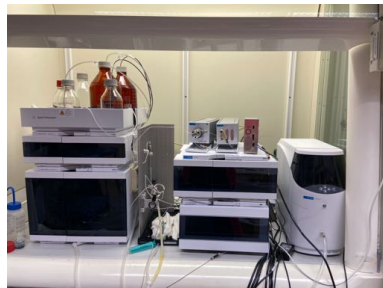


Large scale production (LP3), extraction/purification  
Total lipid extracts, non-polar lipid separation, total phospholipid extracts & sterols; Analysis (TLC, GC, MS)  
In development: Separation of phospholipid classes, optimization of reverse-phase HPLC

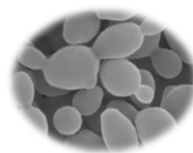
Analytical (LP3 loan)



Preparative RP



*Pichia pastoris*



GC-FID



Prep TLC

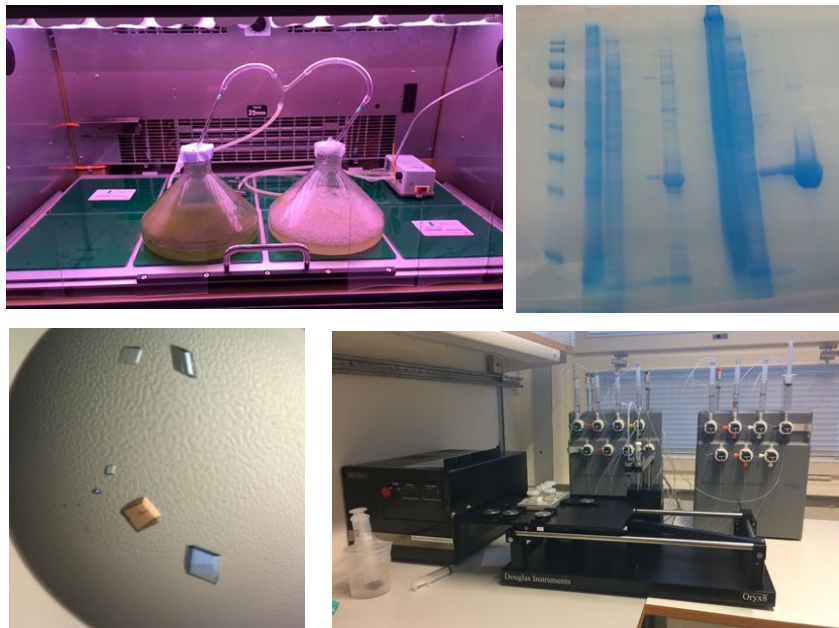




# Biological Deuteration & Crystallization



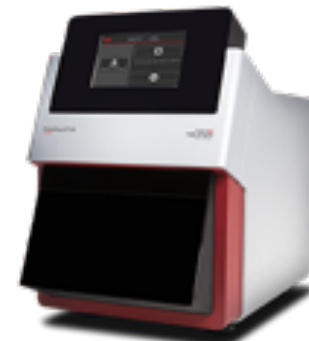
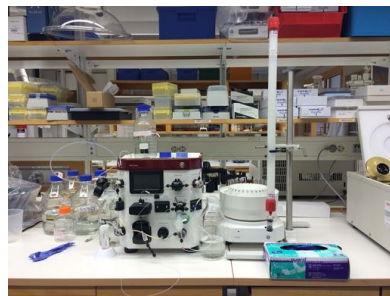
0.5 RT



- Co-located with LP3 in Biology Department, LU
- Essential ESS equipment in place, access agreement to be able to use LP3 labs & equipment
- Research technician (0.5 FTE)
- Part LU BAG for BioMAX

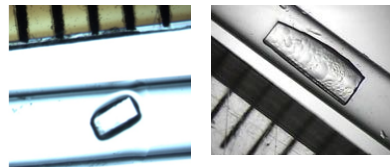
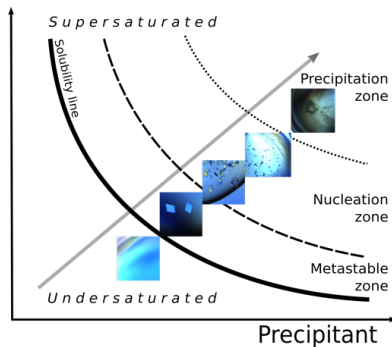
# Biological deuteration

- We can produce full or partially deuterated biomass from bacteria (*E. coli*), algae (*Botryococcus braunii*), and yeast (*P. pastoris*)
- Extract & purify recombinant proteins, plasmid DNA from *E. coli*; extract & separate lipids from yeast (chem lab), cell extract from algae, D-altonone
- Protein purification, check yield & purity (SDS-PAGE, UV/Vis)
- Biophysical characterization tools for proteins to measure solubility & stability (DLS, Thermofluor, NanoDSF)



# Protein Crystallization

- Large single crystals (>0.5 mm<sup>3</sup> today)
- Low and high-throughput screening with Oryx8
- Large crystal growth (optimization) by hand (vapour diffusion, large sitting drops) or machine (dialysis with Opticryst)
- Methods commonly used: seeding, feeding, dialysis, counter-diffusion (capillary) crystallization, VD, batch, temperature control
- X-ray testing – BAG with LP3, regular access BioMAX

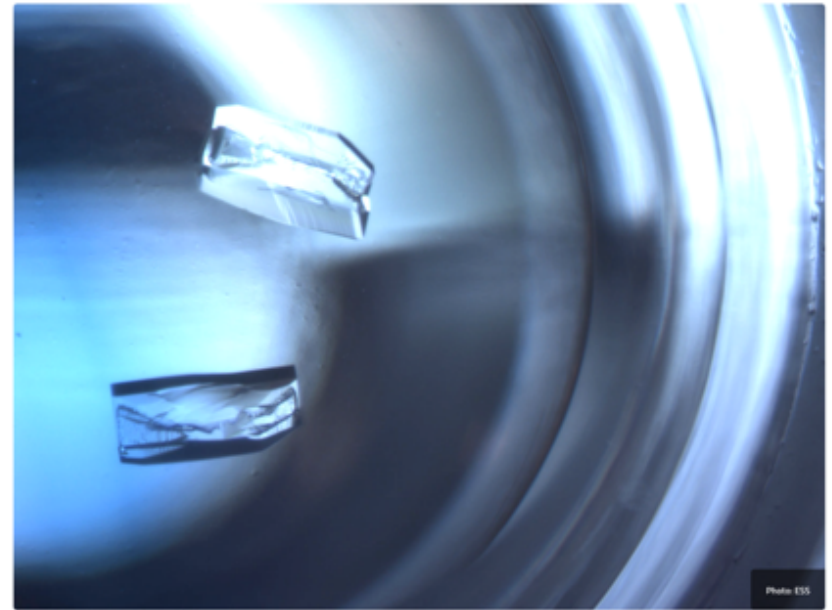


# User support

- 3 pilot calls for user proposals (2019, 2020, 2022)
- Access is proposal based pending feasibility & scientific merit review
- User support is a mix of service & collaborations and free of charge (for now)
- The final pilot call will be linked to First Science on ESS instruments

## Pilot call for chemical and biodeuteration support from the DEMAX platform

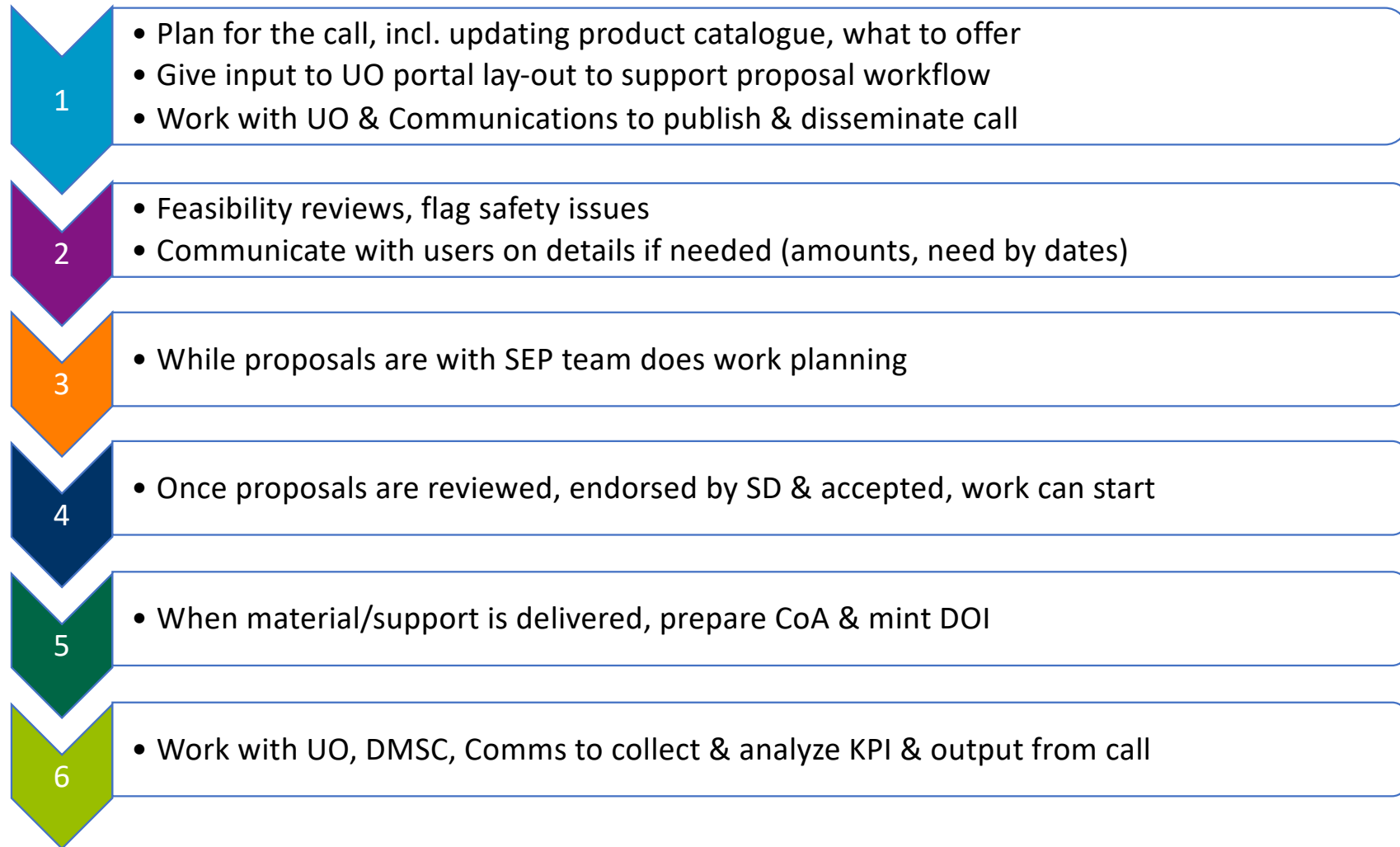
JANUARY 10, 2022



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).

[useroffice.ess.eu](https://useroffice.ess.eu)

# Proposal workflow within DEMAX team



# Materials & services offered in Pilot call 2'b':



- **Biodeuteration:**

*E. coli*, *Botryococcus braunii*, *Pichia pastoris* cell paste  
Recombinant protein expression & plasmid DNA



- **Chemical deuteration:**

Small organic molecules (e.g. sodium pyruvate, D- or L-lactic acid)  
Lipids (e.g. chain-deuterated POPC, SOPC, POPE)  
Surfactants (sugar-based or other) & Other small organic molecules



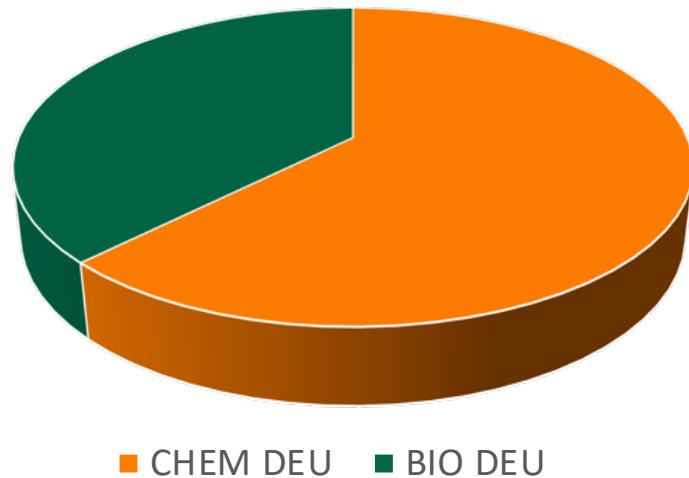
- **Expression of interest invited for:**

Crystallisation & Biological lipid mixtures (yeast)

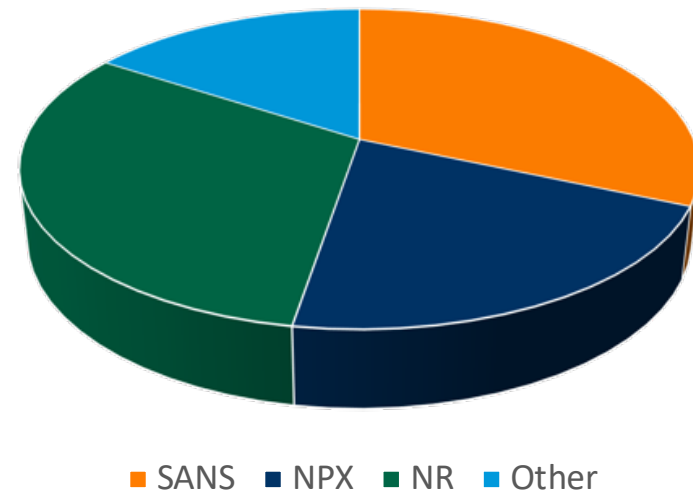
# 2022 3<sup>rd</sup> Proposal Round

- 16 proposals received and assessed, asking for 32 materials/support
- All passed feasibility reviews, with a few caveats
- A few are internal proposals for making e.g. oleic acid & POPC
- 10 Chem, 4 bio (+ 1 chem), 2 EoI (yeast-derived lipids)

Type of molecule/support requested

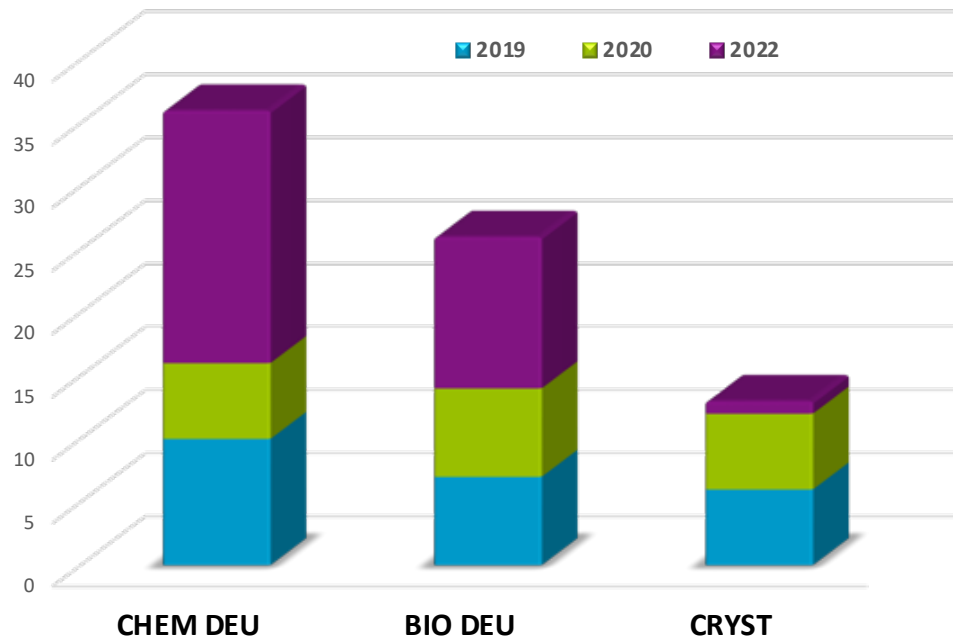


Intended neutron method

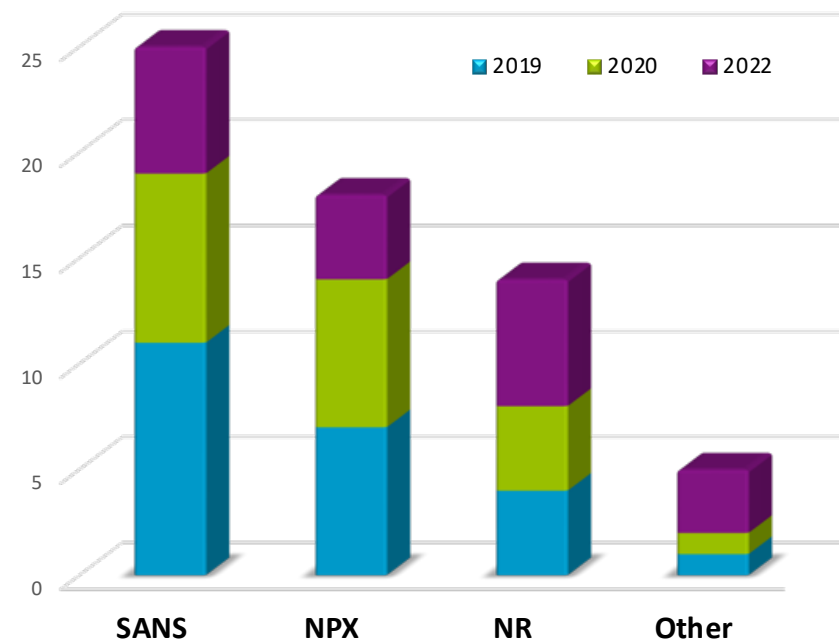


# Request of # of molecules by pillar & intended neutron method stable over 3 calls

# of molecules requested by pillar (2019, 2020, 2022)



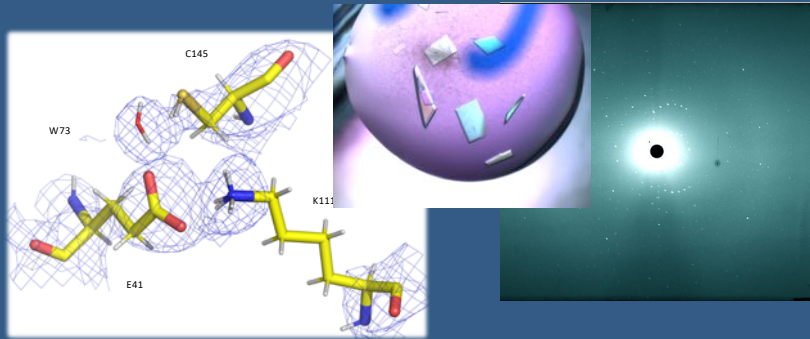
Intended neutron method (2019, 2020, 2022)



52 proposals, 54 unique users, 88 molecules requested  
26 papers published since entering ops in 2019



# Some highlights from 2<sup>nd</sup> pilot call



Large crystals of NitN amidase from Antarctic polyextremophile to study enzymatic mechanism. NPX data collected at LADI (ILL), X-ray data from BioMAX (MAX IV)

Journal of Colloid and Interface Science 581 (2021) 292–298

Contents lists available at ScienceDirect

**Journal of Colloid and Interface Science**

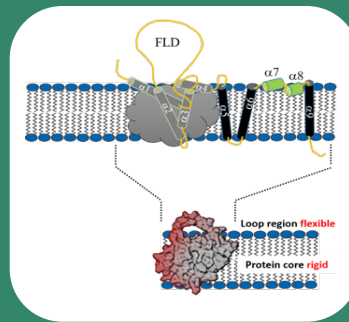
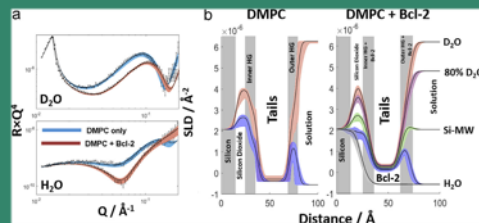
journal homepage: [www.elsevier.com/locate/jcis](http://www.elsevier.com/locate/jcis)

**Complex by design: Hydrotrope-induced micellar growth in deep eutectic solvents**

Adrian Sanchez-Fernandez<sup>a,\*</sup>, Anna E. Leung<sup>b</sup>, Elizabeth G. Kelley<sup>c</sup>, Andrew J. Jackson<sup>b,d</sup>

<sup>a</sup> Food Technology, Engineering and Nutrition, Lund University, Box 124, 221 00 Lund, Sweden  
<sup>b</sup> European Spallation Source, Box 176, 221 00 Lund, Sweden  
<sup>c</sup> The NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, MD 20899-8562, USA  
<sup>d</sup> Division of Physical Chemistry, Lund University, Box 124, 221 00 Lund, Sweden

GRAPHICAL ABSTRACT



## communications biology

ARTICLE

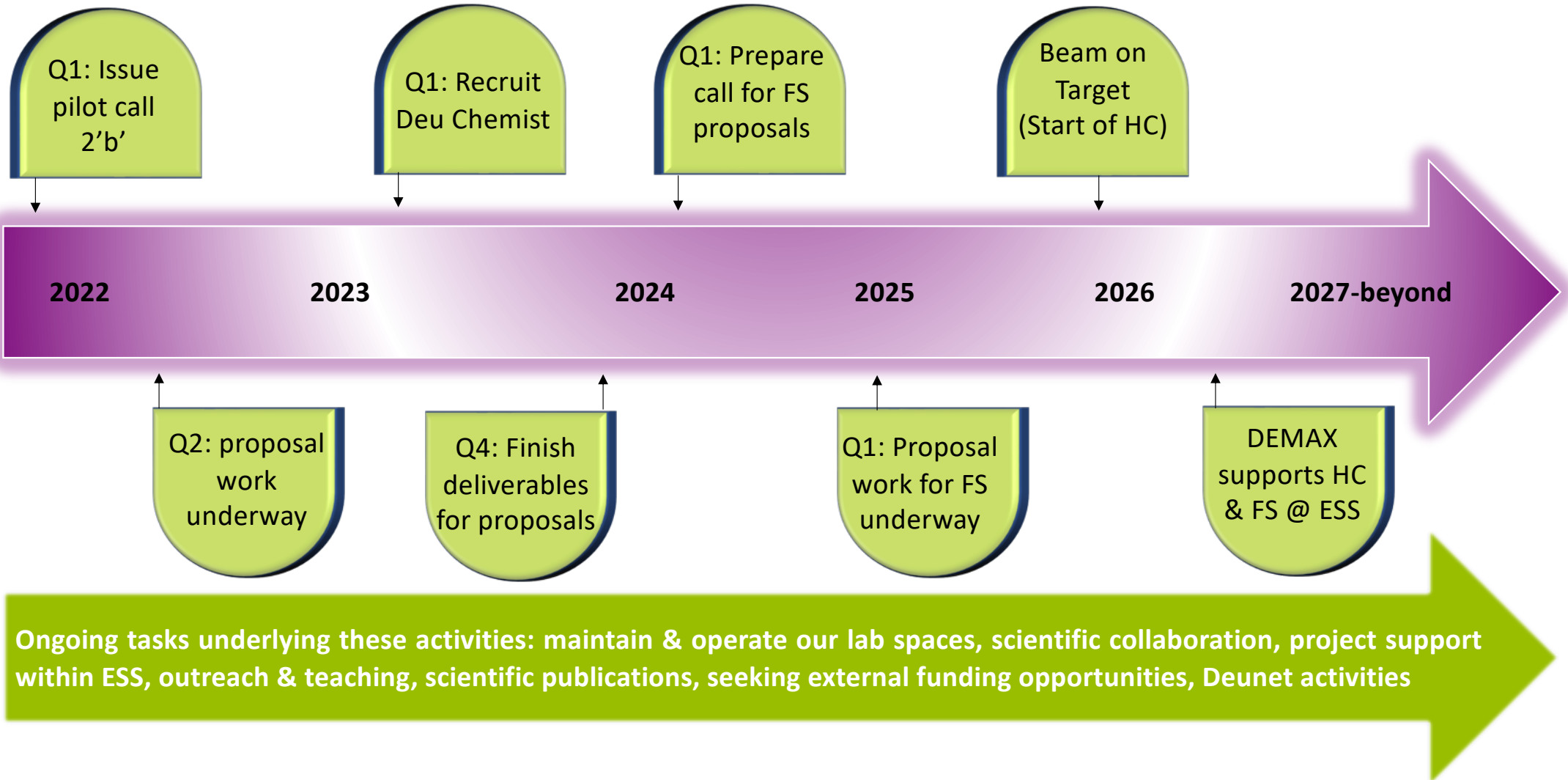
Check for updates

<https://doi.org/10.1038/s42003-021-02032-1> OPEN

## Neutron reflectometry and NMR spectroscopy of full-length Bcl-2 protein reveal its membrane localization and conformation

Ameeq Ul Mushtaq<sup>1,6</sup>, Jörgen Ådén<sup>1,6</sup>, Luke A. Clifton<sup>2</sup>, Hanna Wacklin-Knecht<sup>3,4</sup>, Mario Campana<sup>2</sup>, Artur P. G. Dingeldein<sup>1</sup>, Cecilia Persson<sup>5</sup>, Tobias Sparrman<sup>1</sup> & Gerhard Gröbner<sup>1,5\*</sup>

# Looking forward





*Deunet is now a truly international network*

brightness



Polymer synthesis

Chemical synthesis



Chemical & biological deuteration, crystal growth



Chemical & biological deuteration

Lipid biodeuteration (PSCM), biodeuteration (D-lab)



UNIVERSITY OF MARYLAND | NIST  
INSTITUTE FOR BIOSCIENCE  
& BIOTECHNOLOGY RESEARCH

insight | innovation | application



Department of Organic Chemistry  
Graduate School of Pharmaceutical Sciences,  
Kyoto University

LUND  
UNIVERSITY



中國散裂中子源  
China Spallation Neutron Source

SONGSHAN LAKE  
MATERIALS LABORATORY

# Deunet activities & initiatives



- Created a board with representation from all facilities, regular meetings
- Volunteer members formed executive board (chair, 2 x co-chairs, secretary) & Communications team appointed
- Comms: Updated deuteration survey prepared & updates to website are coming (support from LENS for this)
- Annual meeting planned for September of all facilities in the network
- Discussing possibilities of having an in person “user meeting” – idea: have it as a satellite meeting to a larger meeting (e.g. MLZ user meeting in 2023)



[www.deuteration.net](http://www.deuteration.net)  
deuteration\_net



# Thanks to DEMAX, & LP3 & ESS



Hanna Wacklin-Knecht



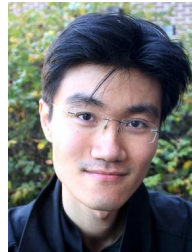
Anna Leung



Zoë Fisher



Jenny Andersson



Jia-Fei Poon



Wolfgang Knecht



Vetenskapsrådet



# Q&A – extract from charge:

- How do we demonstrate that our services are relevant and useful to the user communities, and how do we show “value added” to the scientific profile of ESS - not only to our user communities but also to ESS upper management?
- Reflecting on the first three pilot calls published: what worked well/not so well?
- Looking forward to the period 2024-2027: should we continue with pilot calls or move to a rolling access scheme?
- What scientific areas should DEMAX prioritise/specialise in now to best benefit future ESS neutron users? What time percentage should be dedicated to established, robust procedures vs. method development? One idea is to split between ‘services catalogue’ (offered every call) and ‘new’ (rolling access).
- How do we incorporate workload from regular proposal rounds with longer-term projects (e.g. hosting postdocs, PhD students)? Should we incorporate long-term deuteration proposals for projects that run year after year, as opposed to submitting the same proposal year after year?
- With the exception of the planned recruitment of a deuteration chemist in 2023, staffing will continue to be flat in the foreseeable future. How do we set expectations from ESS and the users for when and how much support we can provide?