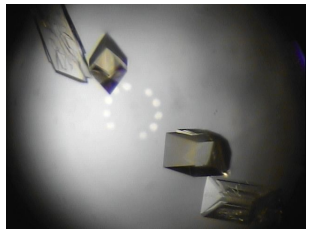
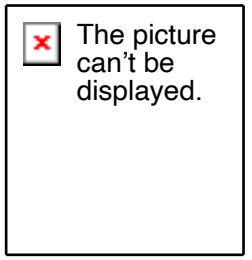
The Deuteration & Macromolecular Crystallization platform at ESS

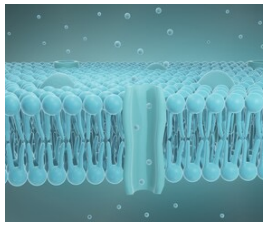
Dr. Zoë Fisher

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

Snr. Adjunct lecturer at Biology Department, Lund University



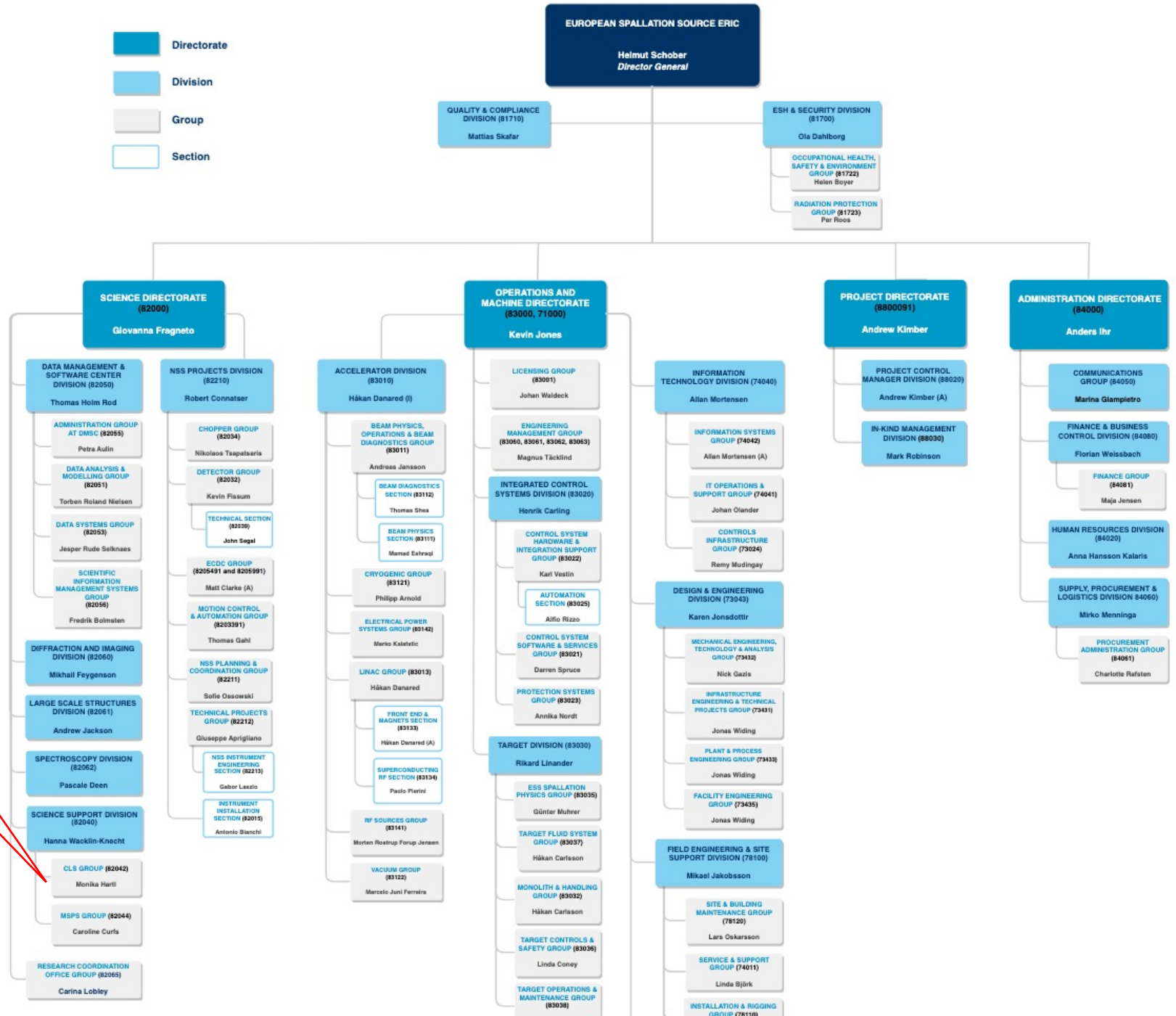


LOKI First Science workshop
6 September 2024

DEMAX overview

- Established in 2019, DEMAX is the ESS user support lab that offers deuteration and crystallization service & support
- We support the chemistry, life science, and soft matter community with access to deuterated materials and (large) protein crystals
- Goal is to help users get the appropriate samples to do meaningful & advanced neutron experimetns

DEMAX is part of the CLS group



DEMAX Platform

Chemical Deuteration

- Small organic molecules, monomers
- Lipids (e.g. POPC), surfactants (e.g. sugar-based), detergents (DDM)
- Aldehydes, alcohols, acrylates etc.
- Novel organic molecules for various applications

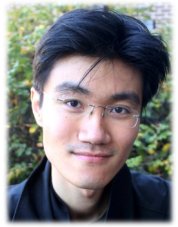
Biological Deuteration

- 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



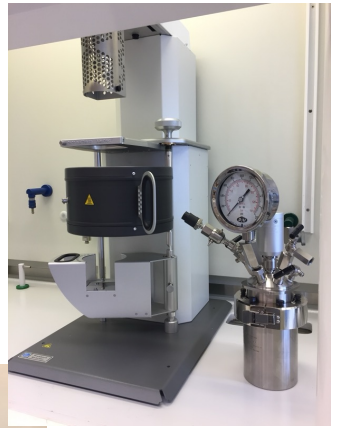
Jia-Fei

LP3
0.7 FTE

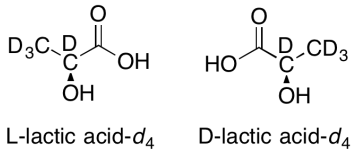
D-lab (lipids)
0.2 FTE

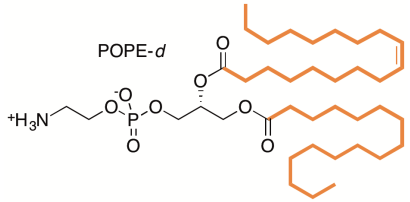
Zoë

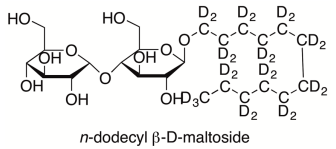
Different kinds of deuteration: chem vs. bio

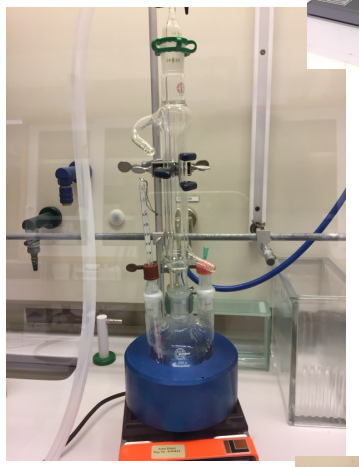


- **Chemical deuteration**: organic synthesis of small molecules using either commercial deuterated precursors and deuterated solvents, or make the precursors/monomers in the lab using Parr reactor (pressure, temp, catalyzed H/D exchange).

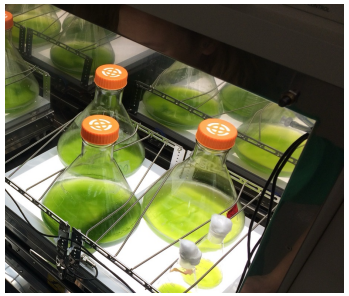


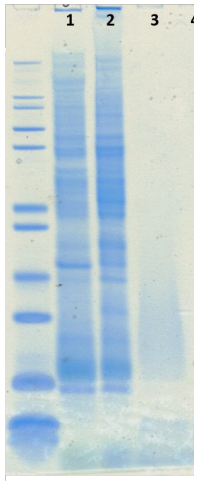


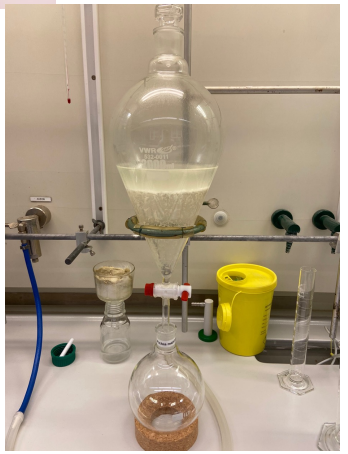




- **Biological deuteration**: production of molecules under deuterated conditions in living cells (e.g. yeast for lipids, bacteria for protein/DNA, algae for cell extract preparation). Prepare cell culture media in D_2O and feed cells d-labeled carbon source (e.g. glycerol)

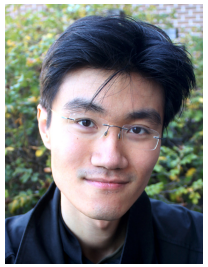






Chemical Deuteration

Anna



Jia-Fei

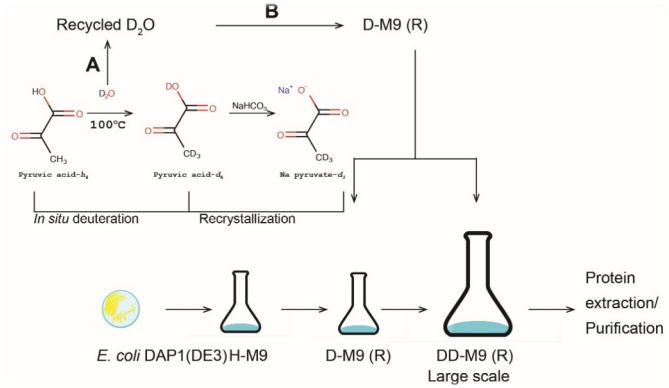
MEDICON VILLAGE

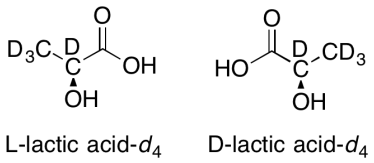
EUROPEAN SPALLATION SOURCE

- 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*

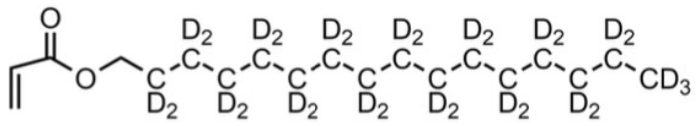
Deuterated organic molecules

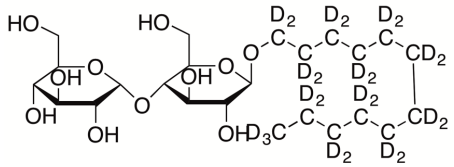
H/D exchange, chemical & enzymatic synthesis of a range of small molecules (surfactants, monomers, alcohols, aldehydes, lipids, fatty acids etc.)



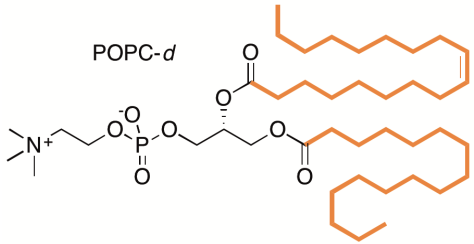


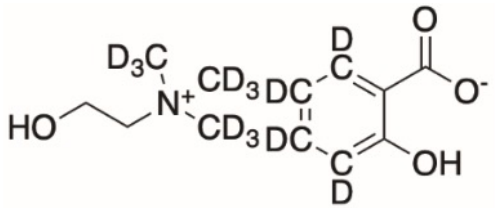
L-lactic acid-d_4 D-lactic acid-d_4



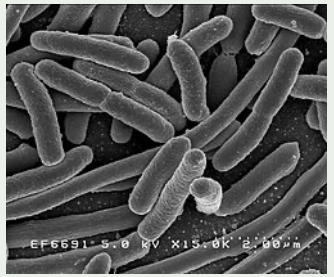
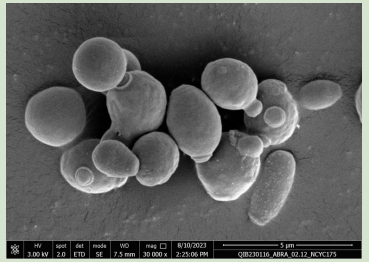
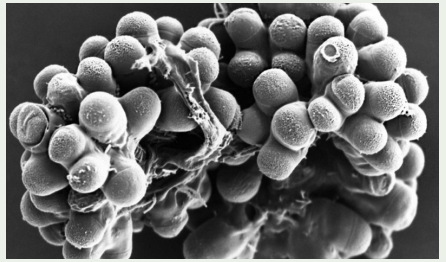


n-dodecyl β-D-maltoside



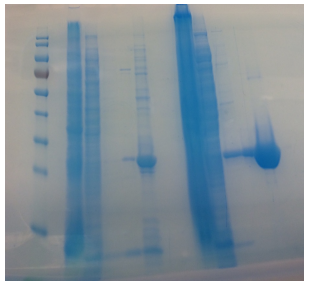


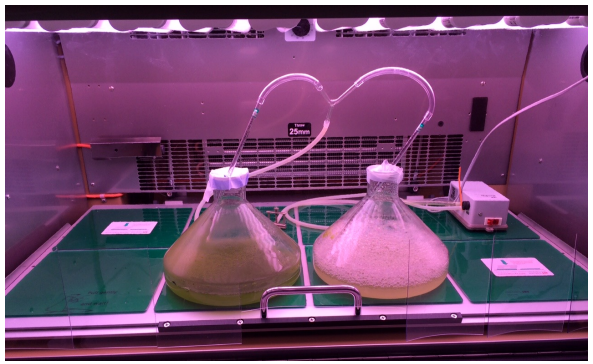
DEMAX offers biodeuteration from following:

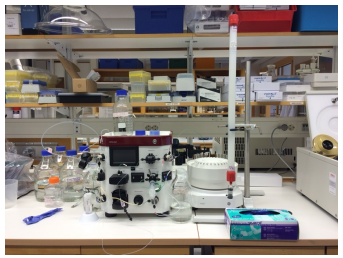
Bacteria **Escherichia coli (E. coli)**	**prokaryote**		**Recombinant proteins** **Plasmid DNA** *(cellulose)*
Yeast **Pichia pastoris (P. pastoris)**	**eukaryote**		**Lipids (total, phospholipid)** (*membranes, ergosterol, cholesterol*)
Algae **Botryococcus braunii (B. braunii)**	**eukaryote**		**Total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

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

Deuterated biomolecules

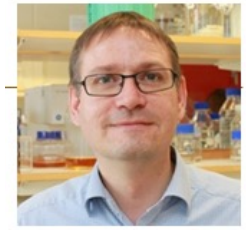






Zoë

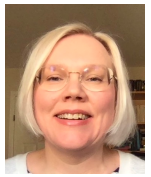
LP3 0.7 FTE



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, proteins & DNA
- Yeast-derived lipids

Hanna



Sophie

- Check protein purity, yield (SDS-PAGE, UV/Vis), biophysical characterization tools for proteins (SEC-MALS, NanoDSF)

LP3
Lund Protein-Production Platform

https://www.lp3.lu.se

PROTEIN PRODUCTION SWEDEN
National Research Infrastructure

DEMAX product catalogue

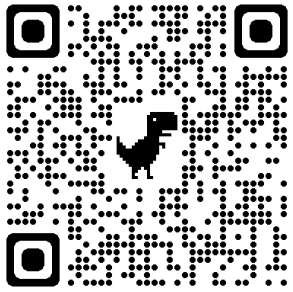
demax@ess.eu

- 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

DeuNet



Deuteration and Macromolecular Crystallisation Platform

Product List & Sample Shipping

August 2023

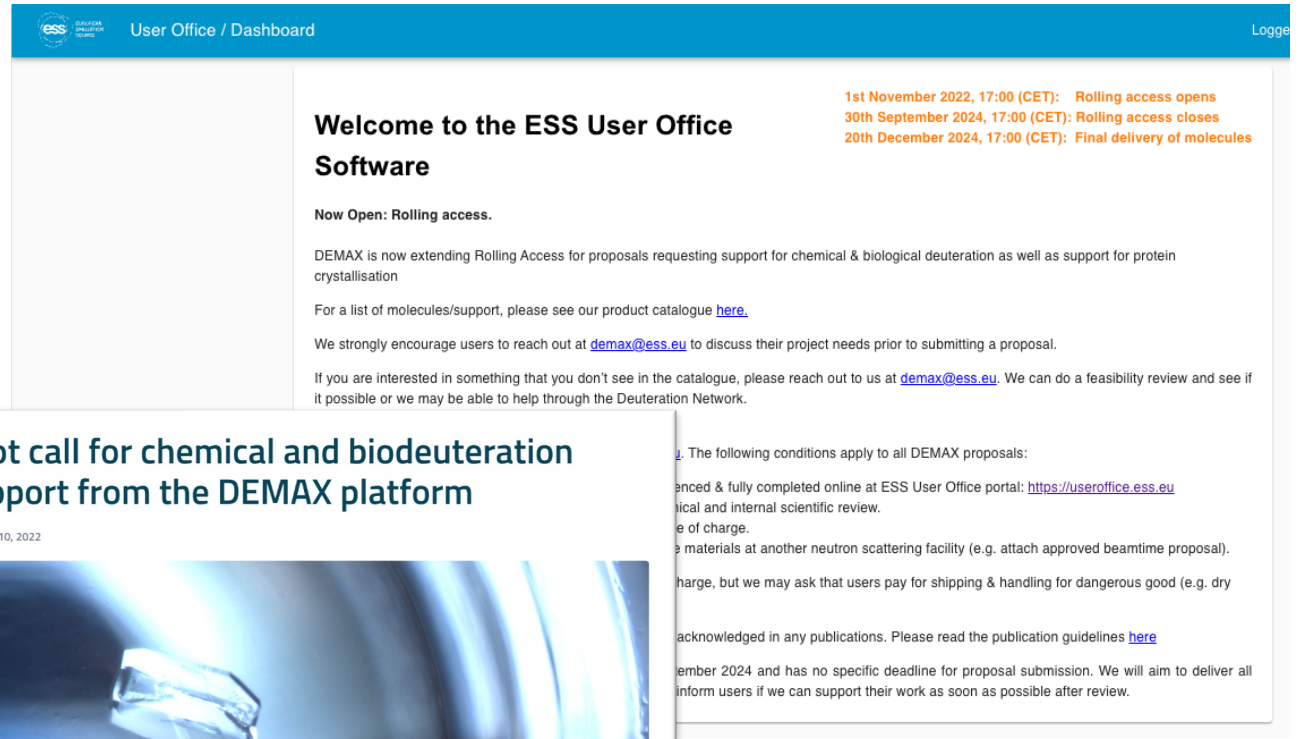
DEMAX Access

- 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

useroffice.ess.eu

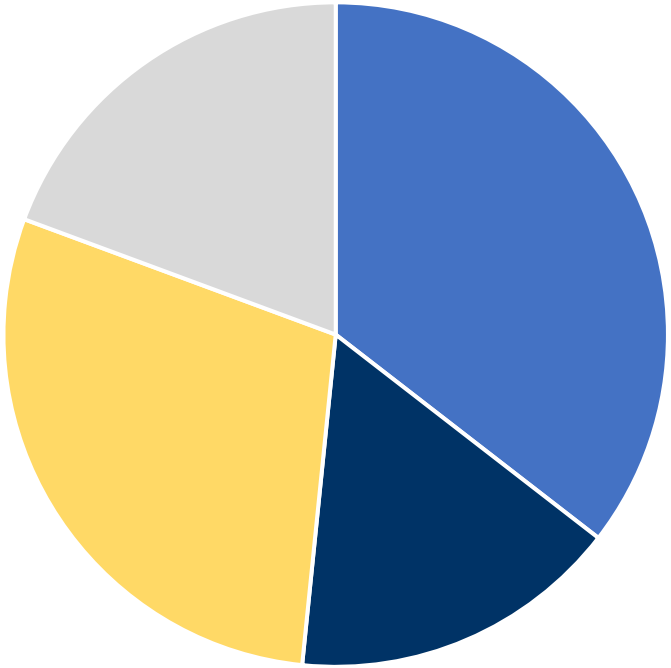
- Rolling access is currently open until end of September 2024
- Users should register and submit proposals online
- Access is merit based not restricted to member nation status (for now).
- Co-authorship vs acknowledgement



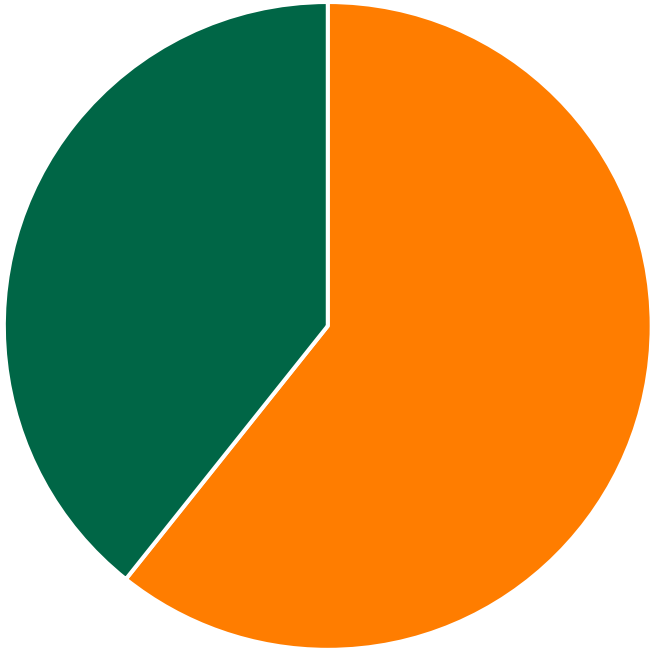
https://europeanspallationsource.se/node/247917

- 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

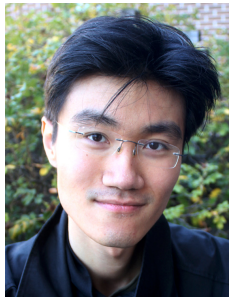


Thanks to DEMAX, & LP3 & ESS

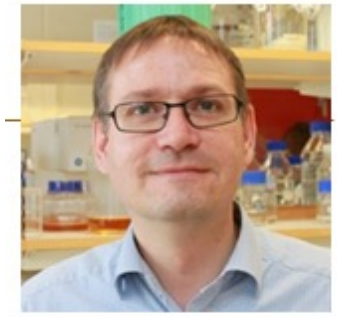
Anna Leung

Zoë Fisher

Hanna Wacklin-Knecht



Jia-Fei Poon



Wolfgang Knecht

LP3 research engineers