DEMAX (DEuteration and MAcromolecular Xtallization) is the ESS user support lab within the Scientific Activities Division that offers deuteration and crystallization service & support. Our support is aligned to enable high impact science in life science, soft matter, and chemistry on ESS instruments.

A. Outcome of first proposal call

In the first proposal round, 2019, we received 19 proposals collectively asking for 31 deliverables. These were a mix of chemicals, proteins, lipids, mixtures of molecules, and support for protein crystal growth. The proposals break down in CHEM, BIO and CRYST categories as follows: 10 CHEM, 7 BIO (2 yeast lipids, 5 protein expression in *E. coli*), 6 CRYST. We rejected 3 proposals based on safety/feasibility concerns, 9 were accepted "as is", and negotiated partial/pilot support for an additional 7. For BIO and CHEM the molecules included a range such as d-proteins, POPC, POPE, DDM, oleic acid, pyruvate, alpha-&beta-maltopyranosides, and a very large amount of yeast lipids (phospholipids). We have completed 7 proposals, 4 will conclude soon and the others will continuations in 2020-2021. Details on users and statistics will be presented in the talk.

Along with the delivered molecule, we also provide a certificate of analysis (CoA) to the user – similar to NDF, ANSTO. The CoA may contain details of the molecule synthesized, analysis, purity, and level of D incorporated. The CoAs are uploaded to zenodo.org and assigned a DOI. Access to the CoA is embargoed for three years from the date of upload; after that, it is publicly available.

** In the webcast presentation, slide 12 skipped. We would like to point out to the STAP that this slide highlighted some of the materials we made in the first call that already made it so (successful) beamtime/experiments. Besides successful experiments that will surely lead to publications, some materials were used in graduate studies: the pyruvate was a key part of a PhD student's research and the use of it in cell culture ended up as a chapter in his final thesis; the POPC was used in membrane protein studies that are part of a current PhD student's research.

The DEMAX team experienced the following challenges and list them here as "lessons learned": 1) Feasibility reviews are important and take time; 2) External reviews were done manually (in Excel) and was a large burden; 3) we were very optimistic and accepted too many proposals; 4) we did not adequately accound for downtime in operations due to back-ordered items, supplier issues with starting materials, and holiday coverage at LU during summer.
We have learned and are adjusting as follows: 1) Current call was modified to build in time to allow for thorough internal feasibility reviews; 2) We stretched the execution period to a year and set aside time for reviews, holidays, experiment planning & procurement; 3) We limit and vary what we offer to allow room for R&D and time to expand and develop our team and methods.

We would like the STAP to comment on this way of working and our mitigation steps we took to address our challenges in the first call.

B. Process and status of second (current) call

DEMAX is using the new ESS user office software and have been involved with defining and developing the platform for our needs in the current call, but also for the entire ESS going forward. Details of the portal and how it works will be shown by SCUO. This collaboration between DMSC, SCUO and DEMAX has been very productive.

For the second pilot call issued at the end of 2019, we offered biodeuteration (lipids, cell paste from E. coli/yeast/algae, labeled proteins) and crystallization. We received 17 proposals, including 6 Expressions of Interest (EoIs) for chemical deuteration. Reviews are ongoing and users will be notified of the outcome by the end of May 2020. Some requests will be easy to fulfill as we have some requested materials already prepared. Others will not be able to be done at ESS at this time and we are considering asking other deuteration labs within DEUNET if they could help us execute these requests.

We would like comments and/or advice on how to proceed with proposals that will be accepted: with regards to the current pandemic, many facilities closing, and user beamtimes being postponed indefinitely. How do we prioritize our tasks and choose what to work on the meantime? Do we work on some appropriate proposals where we can prepare and safely store the materials until later? What kinds of internal/ongoing type activities should we instead work on? For e.g. we can prioritize research, data analysis & publications, grant applications, documenting lab protocols, and coordinate the LENS priority actions. Can we balance methods development (R&D) while also delivering on selected projects that we know we can prepare now and freeze for later (e.g. bacterial/yeast/algal cell pellets, prepare deuterated DNA, lipids etc.).

C. Location and working in the lab(s)

Staff density and a shortage of fume hood and bench space was a problem in the second half of 2019 (~3 SULF members and 3 DEMAX members working full-time in the lab). Changing the division of the fumehoods to favour DEMAX worked well, as has SULF spending more time on-site for installation work. The current travel and visitor restrictions to the lab has also eased space limitations. Once on-site lab installation is further along, SULF will move staff and equipment, relieving the crowding of people and equipment. With Oliver leaving end of May and Anna going on leave in June/July, the DEMAX team in the chemistry lab will be reduced to Hanna and maternity hire.

There are advantages and disadvantages to the chemical deuteration lab being housed at MV. We can currently work with SULF and other colleagues like FLUCO and this provides community, safety, and mitigates working alone. We have good interactions with other companies like RedGlead in the building and we can use some expensive equipment such as NMR and mass spectrometers. Paying access fees to use NMR and MS frees up our own resources for smaller equipment and removes the burden of maintenance. We also have access to other chemists for assistance/advice). On the other hand, the distance from the rest of ESS leads to detachment and loss of knowledge of what is going on at ESS site; we are invisible to management as we are “out of sight”. Efforts were made to investigate *alternative premises* (e.g. Chemistry Dept at LU) but have so far not been successful. LU Chem Dept is short of space and there are entire floors soon cut off due to renovations; the length of leases to corporate tenants is too short for us (two-year limit) as we need a long term stable home. We are still stuggling to find reliable GC-MS service/access. We have been using the GC-MS at

LU Ecology as part of a grant collaboration. While they have excellent equipment and expertise, we found the access unsuitable for large numbers of DEMAX samples. For this we may use GC-FID in the future for standard lipid quantification and quality control. However, we need access to GC-MS for identification of new samples/components and determination of the deuteration level. As the sample preparation for GC takes significant amount of time, an automated sample preparation workbench (~20k€) is being considered to save time.

We would like the STAP to comment on our current premises and set-up for external services.

D. Current research and development, projects, collaboration

DEMAX staff are involved to varying extents in scientific collaborations and regularly publish papers related to research and method development. Below follow specific projects that related to DEMAX and externally funded activities that we are explicitly involved with:

Concluded projects:
SINE2020 concluded in October 2019. SINE2020 WP5 (Deuteration) successfully completed all deliverables and was used as one of the most successful highlights of the consortium. The DEUNET homepage and communications are currently done by ESS (http://www.deuteration.net). Partners within DEUNET have agreed to support participation in DEUNET meetings and related LENS activities (see below). WP6 (Crystallogenesis) also concluded successfully with several method development manuscripts published. **iNEXT** (http://www.inext-eu.org) concluded in 2019. ESS participated in JRA2 " Enabling technologies for integral membrane protein systems" and set up the lipid separation and analysis at ESS using iNEXT funding.

Ongoing projects:
Anna is leading the **BrightnESS^2** deuteration pilot project and she and Oliver have made excellent progress. They synthesized chain-deuterated POPC-d_{63} and provided it to the STFC partners for NR experiments in February. They also prepared additional POPC-d_{63} and provided it to a user from a 2019 DEMAX proposal and plan to deliver more to another user in May.

Within **LENS** (League of advanced European Neutron Sources), one of the four currently proposed priority actions "Role of cell membranes in health and disease" is led from ESS with confirmed participation from DEUNET partners ILL, STFC and JCNS, and possibly MLZ, HZG and PSI. The aim is to develop experimental and analytical strategies that allow disease processes related to global health challenges to be investigated at the molecular level. Specifically the goals are: 1) Develop a library of deuterated membrane and membrane-interacting compounds for reconstitution of native-like cell membrane environments to model both diseased and healthy cells; 2) Automating sample handling to provide the high repeatability and throughput, while reducing the amounts of sample required. DEMAX is working on the first common goal of deuterating relevant lipids of mitochondrial membranes (POPE, Oliver). ESS will investigate commercial sample handling robots for future applications in reflectometry (SE team). A third potential area is the use of polarized neutrons (QENS, NSE, GINSES) for the study of cell membranes. DEMAX will provide deuterated lipids for the feasibility studies (Hanna). The goal is to work towards a Horizon Europe proposal in 2021 by organizing workshops with relevant user communities later in 2020.

Hanna is involved with two **VR-funded projects**: 1) 2016-01164 "Role of membrane lipids and sterols in antifungal susceptibility, resistance and virulence mechanisms in pathogenic yeast", PI Wolfgang Knecht, LU Biology; and 2) 2016-06963 "Organization of mitochondrial membranes under oxidative stress: Implications for their active role in regulation of apoptosis", PI Gerhard Gröbner, Umeå University in collaboration with STFC (Luke Clifton).

Hanna is also involved with overseeing the **PhD** work of a student at LU (Manuel Orozco). His project involves reconstitution of membrane-bound enzymes for neutron scattering studies (Main supervisor W. Knecht, ESS co-supervisor Z. Fisher, ILL co-supervisor G. Fragneto).

E. Staffing, recruitment

Currently we are four in the team; three from June 2020 onwards when Oliver goes to graduate school. Zoë (GL) is alone in doing crystallization and biodeuteration; Hanna is in charge of lipid extraction and separation from yeast; Anna takes care of organic synthesis; Oliver has been working on enzyme synthesis work and supported Anna on some tasks until May 2020. Our contract with LP3 covers some support (0.5 FTE technician) to DEMAX for deuterated yeast production for lipids and help with deuterated algae growth maintenance.

We are recruiting a 1-year deuteration chemist as parental leave replacement for Anna, who will take leave from June/July 2020. The idea is that this person will do general chem lab activities and also support Hanna for the LENS PA in the lipid purification/analysis work. We are also working to recruit a second full time chemist that should start working with us at the beginning of Q2 of 2021. We are working on a job description and will trigger the necessary steps after the one-year deuteration chemist is in place. Following previous advice from the STAP, we have focused our efforts on expanding our capacity in synthesis of deuterated organic small molecules.

By Q2 of 2021, there will be 3 people in the chemistry lab of DEMAX working on organic synthesis and yeast lipid extraction/characterization. This puts biodeuteration and crystallization far behind in terms of capacity for growing cells, producing deuterated proteins, DNA, and even other cellular products as well as protein crystallization activities. Currently the effort dedicated to these "biology" activities come to ~0.4 FTE of Zoë and 0.5 FTE shared technician with LP3. Considering current proposals (2019-2021), and even looking ahead to First Science, we can expect that demand for biodeuteration will be on the same level as chemical deuteration and our staffing levels need to reflect that. We really need a dedicated resource to focus on biodeuteration – both taking care off cell culturing but also protein and DNA production and purification. Part of Zoë's time can continue to be spent on crystallization.

Our preference is to have the second chemist overlap in skills with Anna so that both of them can run projects independently and in parallel. Does the STAP think it could be useful to send a new chemist to another D-lab (e.g. NDF at ANSTO) for training purposes?
We would also like a comment from the STAP on our current and future needs for dedicated biologist to run biodeuteration for cell production, DNA/protein purification, and crystallization.