Annual Report

Lund university Protein Production Platform (LP3) | 2018

Annual Report 2018

The “new” LP3 was created as a center in June 2016 by the Faculty of Science, the Faculty of Medicine and LTH by combining the “old” LP3, specialized in protein production, with LU’s protein crystallization facility.
Two new research engineers started at LP3 during 2018, one with the main focus to bridge protein production and crystallization. In late autumn, LP3 received a new “plate hotel” with the capacity to store and automatically image crystallization plates, which will secure LP3 capabilities in this area for the next years. LP3 received further funding in 2018 from the Faculties of Science and Medicine to acquire new equipment for cell disruption. LP3 intensified its collaboration with the BioMAX beamline at MAX IV and we successfully re-applied together for beamtime to allow LP3 produced crystals to be screened at BioMAX. LP3 was co-applicant in a VR grant within the call “Grant for accessibility to infrastructure”. The granted application “FragMAX - a facility for high throughput fragment screening in drug development by X-ray crystallography” aims at setting up fragment screening at the BioMAX beamline with LP3 as the partner for crystallization and sample preparation.
LP3 staff was in 2018 involved in both undergraduate and graduate teaching, as well as national and international conferences and networks of interest to the field.
As this is the 3rd Annual report for LP3, the development in number of users, projects and deliveries and their distribution within Lund University and outside is also captured in this report.
As should be clear from the pages of this annual report 2018, LP3 continues to deliver value-adding services to Lund University researchers.

Wolfgang Knecht,
Manager LP3
Feb. 2019

Brief Facts 2018

57 users | 138 unique deliveries in 57 protein production projects | 164 protein crystallization plates | 13 visitors at LP3

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Content

Introduction

Lund Protein Production Platform (LP3) is a focal point for expertise and equipment for the entire process chain of production, purification, characterization, crystallization of proteins and their structure determination and refinement, or each individual step in the chain. LP3 is a service center that offers customer-adapted protein production, including stable isotope-labeled proteins, crystallization of proteins and structure determination of proteins primarily for Lund University (LU), but also for the surrounding community.
Since 2018, LP3 is part of Block Allocation Group (BAG) proposal of Lund researchers and has beamtime at the BioMAX beamline at MAX IV Laboratory. LP3 can therefore now also handle regular screening of user crystals at BioMAX and subsequent structure determination. LP3 is also a knowledge center for dissemination and exchange of new technologies and ideas within protein production and protein crystallization.

LP3's mission is to:

- offer open service and support, primarily to researchers at LU, with protein production, characterization and crystallization for their research projects.
- be responsible for a common and open infrastructure for protein production and crystallization, as well as to contribute actively to the interaction of LU with MAX IV, ESS and other relevant major research facilities, networks and initiatives.
- if needed, to act as LU's node in a national infrastructure in the protein science area.
- develop competence and methods in the area of protein sciences.
- serve the surrounding community (e.g. closely located large infrastructures, small biotech etc.).
- finance part of its operations (material and machine maintenance costs) by charging user fees and to increase this part of the funding over time.

The infrastructure is currently run by a manager (50 % FTE, senior lecturer) and six research engineers (of whom two are temporary positions), of whom four are

involved in protein production and two primarily in protein crystallization and protein structure determination. In 2018, the staffing also included 2 experts (10 %) in microbial protein production and crystallization as well as Dr. Z. Fisher (Head of DEMAX (ESS), assoc. lecturer at LU). 13 visitors were associated with LP3 in 2017.
One temporary staff position started January 2018 and another one in September 2018 with the main focus to bridge protein production and crystallization.

The center is fully equipped for protein production in *E. coli* and insect cells (Baculovirus Expression Vector System, BEVS). This includes flow hoods for sterile handling of cells, temperature-controlled shakers for culturing of cells (including access to temperature controlled rooms), centrifuges, cell homogenization equipment (e.g., French Press and sonicators). For purification there are several state of the art chromatography systems, including one Äkta Avant and two Äkta Purifier Systems. Equipment for SDS PAGE, Western blotting and other standard equipment for protein characterization and enzymatic activity assays is available at the center or within close proximity. All documentation is captured using electronic lab notebooks. For crystallization the facility is equipped with state-of-the-art nanolitre pipetting equipment with the recently-added capability to handle lipidic cubic phases for membrane proteins, as well as a "plate hotel" with the capacity to store and automatically image up to 600 plates, each with up to 288 crystallization trials. Tecan and a TTP (Dragonfly) liquid handling systems for the preparation of crystallization screens are also available. In 2018, LP3 acquired a new plate hotel. Together with the old plate hotel, this will allow LP3 in the future automated crystallization plate storage and imaging at 4 and 20 °C.
For more specifics on the capabilities and services of LP3, please see "Services below".

Placement of the infrastructure: LP3 is placed at the Biology Department (Biology Building A, Sölvegatan 35, 22362 Lund), within the Faculty of Science (FoS) at LU. LP3 is a separate entity within the existing administrative structure of the Department of Biology and follows the working and delegation principles of the FoS.

Leadership of the infrastructure: LP3 is governed by a board of one chairman (Prof. Anders Tunlid) and 6 members (Dr. Susanna Horsefield, Dr. Kajsa Paulsson, Prof. Mikael Akke, Dr. Uwe Mueller, Dr. Sindra Petersson-Årsköld, Katarina Koruza), one each from FoS, Faculty of Medicine (FoM), LTH, MAX IV laboratory and ESS (external member) and one student. The chairman is the dean or vice dean of the FoS. The daily business of the center is led by a manager (50 % FTE) (Dr. Wolfgang Knecht). The manager is supported in his function by additional experts (10 % FTE) (Currently Dr. Claes von Wachenfeldt (microbiological protein production and deputy manager LP3) and Dr. Derek Logan (crystallization).

For a description of the history of LP3 and a detailed outline of the long term strategy for LP3, please see the Annual report 2016 which is also available at www.lu.se/lp3.

Services

LP3 offers services for the entire process chain of production, purification, characterization, protein crystallization and protein structure determination and structure refinement or each individual step in the chain. LP3 can help with:

- Plasmids for protein production
- Recombinant protein production in bacterial (*E. coli*) or eukaryotic (insect) cells.
- Protein labeling (seleno-methionine incorporation, labeling with stable isotopes (2H, 13C, 15N), biotinylation, phosphorylation)
- Protein purification
- High-throughput & nanovolume protein crystallization
- Microbiological growth monitoring (Bioscreen C)
- Biophysical protein characterization by Size Exclusion Chromatography (SEC), Dynamic Light Scattering (DLS) and Differential Scanning Fluorimetry (DSF)
- Automated crystallization plate storage and imaging at 4 and 20 °C (in early 2019)
- Protein structure determination and refinement:
    - Application for beamtime, however LP3 is part of a BAG for BioMAX.
    - MX data collection at synchrotron beamline (BioMAX MAX IV)
    - Process x-ray data and determine and refine the structures
    - Cryo-EM screening, LP3 is part of a BAG for Cryo-EM in Stockholm

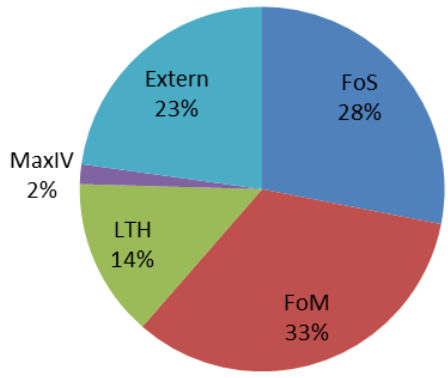
For details of current services, please see LP3 homepage: www.lu.se/lp3

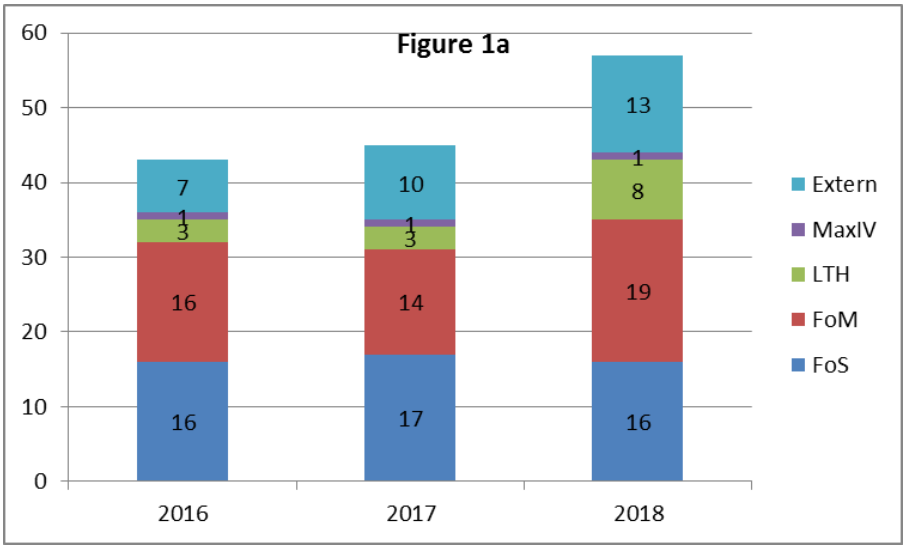
Users and projects

An overall user statistics will be reported here and a more detailed breakdown into protein production and the crystallization part will also be presented.

Overall: 57 groups used LP3 in 2018. Of these, 44 principal investigators came from LU and 13 were external. The distribution into different faculties and external users is presented in Figure 1. The development of user groups at LP3 over the last three years is shown in Figure 1a. The principal investigators in the external user group come from the ESS, other Swedish universities (e.g. in 2018: Karolinska Institute, Royal Institute of Technology, Stockholm University, Uppsala University, Swedish Agricultural University) and industry/biotech (e.g. in 2018: three companies).

Figure 1. Distribution of 57 user groups at LP3 in 2018





138 unique deliveries were made in 57 protein production projects and 164 protein crystallization plates (for 30 user groups) were processed. 13 visitors worked at LP3 for periods between a few days to up to one year, or are still associated with LP3. 13 user groups are using both protein production and crystallization at LP3. The last is a big increase towards 2017 with only 4 in this category. A new development in 2018 was that LP3 has now 8 projects in which LP3 does protein production and crystallization with the aim of structure determination at the BioMAX beamline for

mainly non-experts in protein crystallography. This has become possible due to regular beamtime at the BioMAX beamline and increased LP3 staffing for protein structure determination.

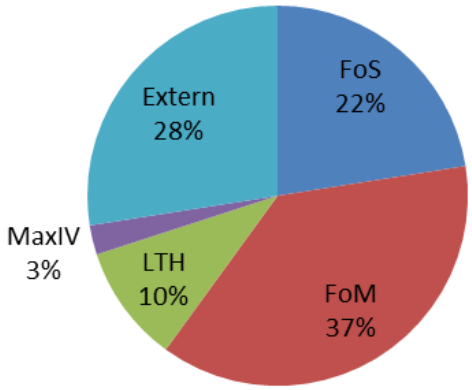
Protein Production: The table below shows the number and distribution of users in 2018. In brackets are the corresponding number given for previous years (2017, 2016).

		first project in LP3
Extern	**11** (8, 5)	**6** (5, 5)
LTH	**4** (2, 2)	**2** (1, 0)
FoM	**15** (14, 14)	**6** (5, 6)
FoS	**9** (14, 12)	**2** (4, 5)
MAX IV	**1** (0, 0)	**1** (0, 0)
Total	**40** (38, 33)	**17** (15,16)

For 17 of the 40 principal investigators, it was the first time that they used LP3 to run a project.

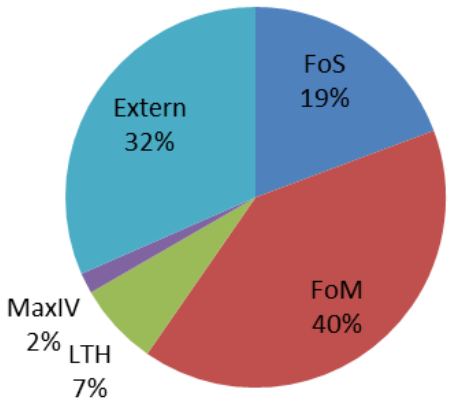
The distribution of users is illustrated in Figure 2.

Figure 2. Distribution of 40 user groups at LP3 (protein production)



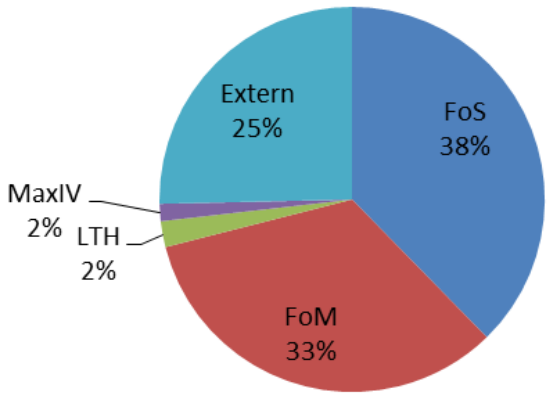
The distribution of projects is illustrated in Figure 3.

Figure 3. Distribution of 57 protein production projects at LP3



The distribution of deliveries is illustrated in Figure 4.

Figure 4. Distribution of 138 deliveries in protein production projects at LP3



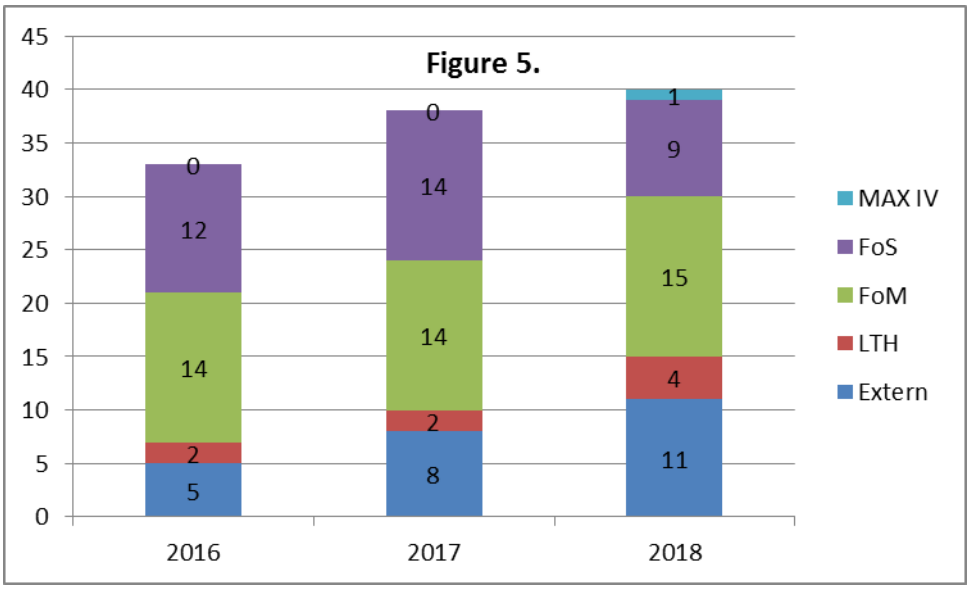
Figures 2. – 4. show that within protein production about 60 – 70 % of LP3s user groups, projects and deliveries are from and go to the FoS and FoM.

32 % or 14 % of all projects are connected to two areas of specialization of LP3, either the BEVS or stable isotope labeling, respectively (see long term strategy in the

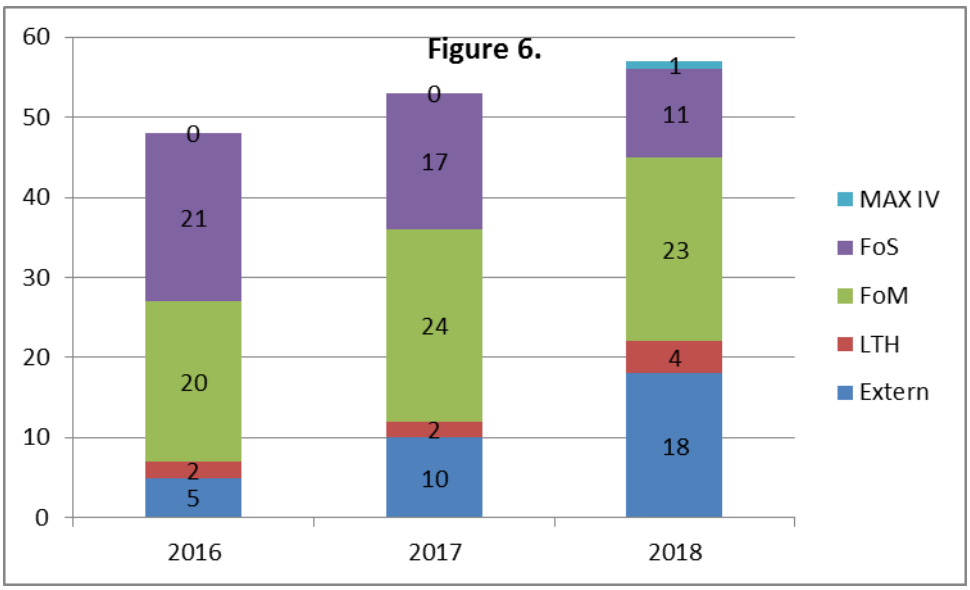
annual report 2016). 4 projects tested more than one expression system, meaning here to test expression in *E. coli* and BEVS in parallel.

As this is the 3rd Annual report for LP3, the 3 year development in protein production is summarized in this report:

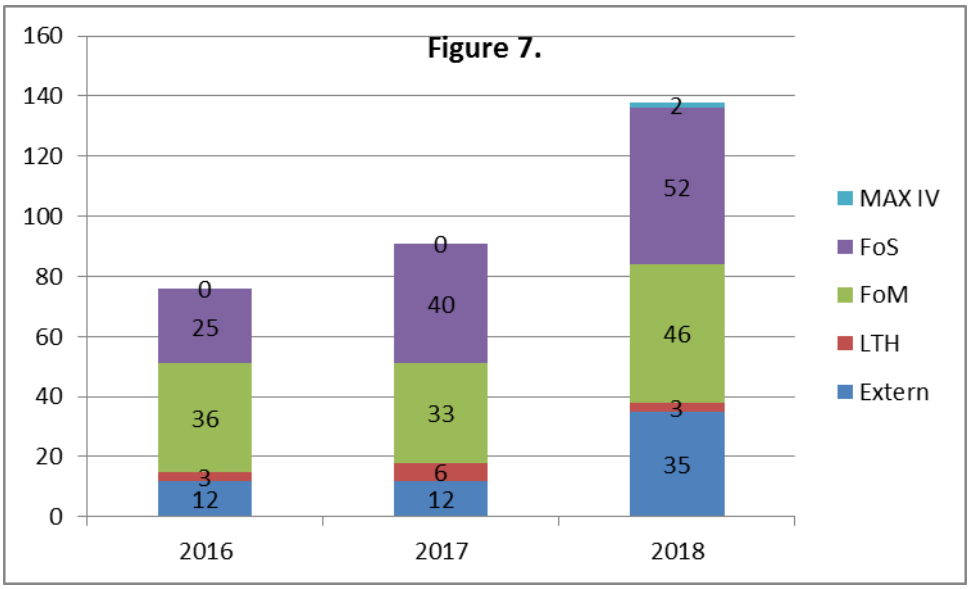
The distribution and number of users in protein production per year during 2016 - 2018 is illustrated in Figure 5.



The distribution and number of projects in protein production per year during 2016 - 2018 is illustrated in Figure 6.



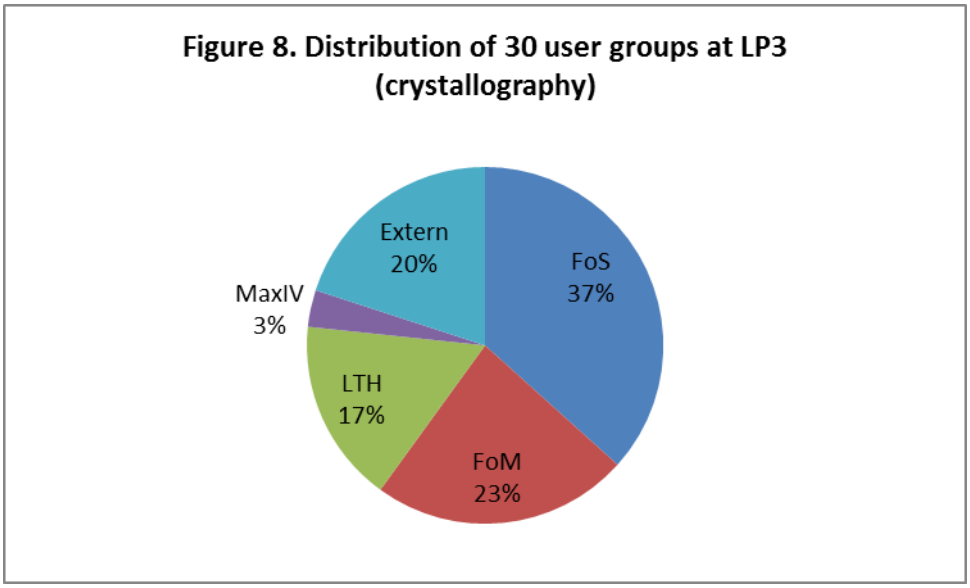
The distribution and number of deliveries in protein production projects per year during 2016 – 2018 is illustrated in Figure 7.



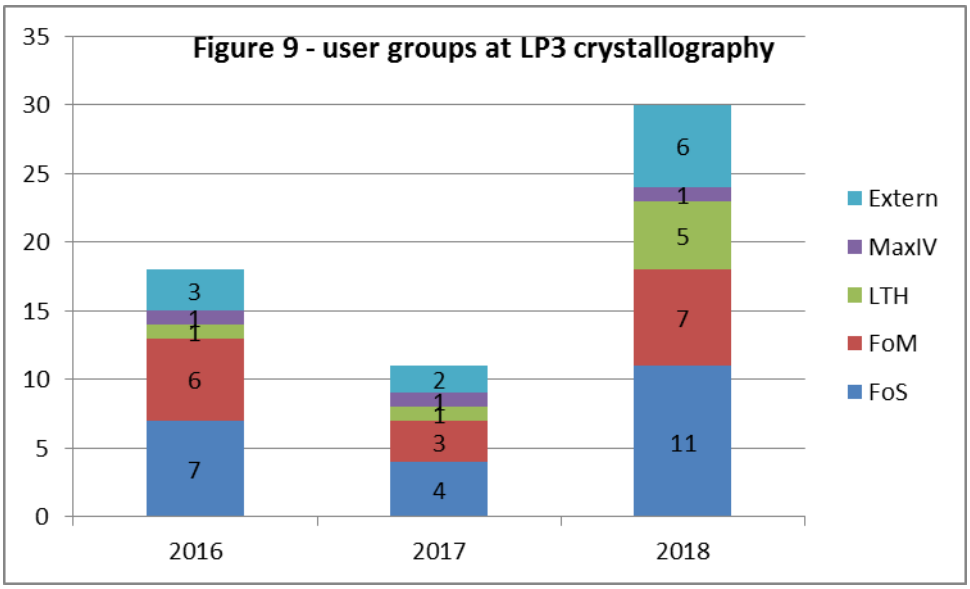
Protein Crystallization:

A total of 164 screening plates were processed in 2018 for 30 user groups. The comparable numbers of plates were 158 and 262 plates in 2017 and 2016, respectively. The distribution of user groups in crystallization and the development of the number and distribution of user groups during the last 3 years are shown in Figure 8 and 9.

The distribution of users of crystallography in LP3 is illustrated in Figure 8.



The distribution and number of users in protein crystallization per year during 2016 - 2018 is illustrated in Figure 9.



The sharp increase in user groups at LP3 crystallography between 2017 and 2018 can mainly be explained by that LP3 has now 8 projects in which LP3 does protein production and crystallization with the aim of structure determination at the BioMAX beamline for mainly non-experts in protein crystallography. This has become possible due to regular beamtime at the BioMAX beamline and increased LP3 staffing for protein structure determination.
Also more groups just use the biophysics instrumentation (DSF and DSL) at LP3 crystallography.

Visibility, access, outreach

LP3 presents its services, capabilities and new developments through Lund University-based homepages (www.lu.se/lp3) as well as by directed campaigns (e.g., LP3 was a part of the infrastructure fairs organized by the FoM in November 2018). LP3 participates in relevant national and international networks and societies, (e.g., Protein Production Network Sweden (PPNS) (http://www.ppns.ki.se), Association of Resources for Biophysical Research in Europe – Molecular Biophysics in Europe (ARBRE-MOBIEU) (https://arbre-mobieu.eu), Protein Production and Purification Partnership in Europe (P4EU) (https://p4eu.org) and Core Technologies for Life Sciences (CTLS) (http://www.ctls-org.eu/)). This is both for dissemination of LP3's work as well as for the exchange and adoption of new ideas and methods into LP3. LP3 staff participates in seminar series, research schools and conferences.

Presentations of LP3 were provided at the following occasions in 2018:

- MAX4ESSFUN Annual meeting (March 2018, Denmark)
- MAX4ESSFUN Summer school in Mölle (Mölle, June 2018)
- Swedish Chemical Society Meeting (June 2018, Lund)
- CTLS meeting (July 2018, Belgium)
- MAX IV Laboratory User Meeting (UM18) (Lund, September 2018)
- PPNS meeting (LP3 organizer) – satellite to Protein Science Day (Lund, October 2018)
- Research school QDETAILSS (Lund, October 2018)
- "Infrastructure fair" organized by the faculty of medicine (Lund, November 2018)
- P4EU meeting (December 2018, UK)
- Protein Production PhD course (Lund, December 2018)

Conference attendance:

- P4EU meetings (July 2018, Belgium and December 2018, UK)

Results and /or proteins produced at the facility were used in the following 2018 publications:

1 Akerstrom, B. *et al.* rA1M-035, a Physicochemically Improved Human Recombinant alpha1-Microglobulin, Has Therapeutic Effects in Rhabdomyolysis-Induced Acute Kidney Injury. *Antioxidants & redox signaling*, doi:10.1089/ars.2017.7181 (2018).

2 Del Giudice, R. & Lagerstedt, J. O. High-efficient bacterial production of human ApoA-I amyloidogenic variants. *Protein science : a publication of the Protein Society* **27**, 2101-2109, doi:10.1002/pro.3522 (2018).

3 Domingo-Espin, J., Nilsson, O., Bernfur, K., Del Giudice, R. & Lagerstedt, J. O. Site-specific glycations of apolipoprotein A-I lead to differentiated functional effects on lipid-binding and on glucose metabolism. *Biochimica et biophysica acta. Molecular basis of disease* **1864**, 2822-2834, doi:10.1016/j.bbadis.2018.05.014 (2018).

4 Kanje, S. *et al.* Protein Engineering Allows for Mild Affinity-based Elution of Therapeutic Antibodies. *Journal of molecular biology* **430**, 3427-3438, doi:10.1016/j.jmb.2018.06.004 (2018).

5 Koruza, K., Lafumat, B., Nyblom, M., Knecht, W. & Fisher, Z. From Initial Hit to Crystal Optimization with Microseeding of Human Carbonic Anhydrase IX—A Case Study for Neutron Protein Crystallography. *Crystals* **8**, 434 (2018).

6 Koruza, K., Lafumat, B., Vegvari, A., Knecht, W. & Fisher, S. Z. Deuteration of human carbonic anhydrase for neutron crystallography: Cell culture media, protein thermostability, and crystallization behavior. *Archives of biochemistry and biophysics* **645**, 26-33, doi:10.1016/j.abb.2018.03.008 (2018).

7 Mehaffey, M. R., Sanders, J. D., Holden, D. D., Nilsson, C. L. & Brodbelt, J. S. Multistage Ultraviolet Photodissociation Mass Spectrometry To Characterize Single Amino Acid Variants of Human Mitochondrial BCAT2. *Analytical chemistry* **90**, 9904-9911, doi:10.1021/acs.analchem.8b02099 (2018).

8 Rozman Grinberg, I. *et al.* Novel ATP-cone-driven allosteric regulation of ribonucleotide reductase via the radical-generating subunit. *eLife* **7**, doi:10.7554/eLife.31529 (2018).

9 Stolt-Bergner, P. *et al.* Baculovirus-driven protein expression in insect cells: A benchmarking study. *Journal of structural biology* **203**, 71-80, doi:10.1016/j.jsb.2018.03.004 (2018).

10 Wallerstein, J. & Akke, M. Minute Additions of DMSO Affect Protein Dynamics Measurements by NMR Relaxation Experiments through Significant Changes in Solvent Viscosity. *ChemPhysChem* **0**, doi:doi:10.1002/cphc.201800626 (2018).