Annual Report

Lund university Protein Production Platform (LP3) | 2017

Annual Report 2017

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.
During 2017, LP3 could recruit two additional staff scientists for protein production and bridging protein production and crystallography. In March 2017, LP3 received a new DSF instrument that added to LPs capabilities for protein characterization. LP3 received further funding in 2017, including funding from Lund University to acquire a new plate storage and imaging unit and a grant from BioCARE – A Strategic Research Area at Lund University, that will allow LP3 to open an additional research engineer position. LP3 intensified its collaboration with the BioMAX beamline at MAX IV and successfully applied together for beamtime to allow LP3 produced crystals to be screened at BioMAX. LP3 also consolidated its co-localization with the DEuteration and MAcromolecular Xtallization (DEMAX) unit of the European Spallation Source (ESS) by aligning working processes in the commonly used laboratories.
LP3 staff was in 2017 involved in both undergraduate and graduate teaching, as well as national and international conferences and networks of interest to the field.
As should be clear from the pages of this annual report 2017, LP3 continues to deliver value-adding services to Lund University researchers.

Wolfgang Knecht,
Manager LP3
Feb. 2018

Brief Facts 2017

45 users | 91 unique deliveries in 53 protein production projects | 158 protein crystallization plates | 10 visitors at LP3

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Content

Introduction

LP3 is a focal point for expertise and equipment for the entire process chain of production, purification, characterization and crystallization of proteins, or each individual step in the chain. LP3 is a service center that offers customer-adapted protein production, including stable isotope-labeled proteins, and crystallization, primarily for Lund University (LU), but also for the surrounding community. 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 five research engineers, of whom four are involved in protein production and one in protein crystallization. In 2017, 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) and an intern for 3 months. 10 visitors were associated with LP3 in 2017.
One additional staff position was filled in 2017 (start January 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. A Tecan liquid handling system for the preparation of crystallization screens is also available. Since 2016 LP3 also offers access to a Bioscreen C reader for recording microbial growth curves and in 2017 has added a new instrument for DSF.

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. Almut Kelber) and 6 members (Dr. Susanna Horsefield, Dr. Kajsa Paulsson, Prof. Mikael Akke, Dr. Tomas Lundqvist, Dr. Sindra Petersson-Årsköld, Katarina Koruza), one each from FoS, Faculty of Medicine (FoM), LTH, MAX IV 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 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

Services

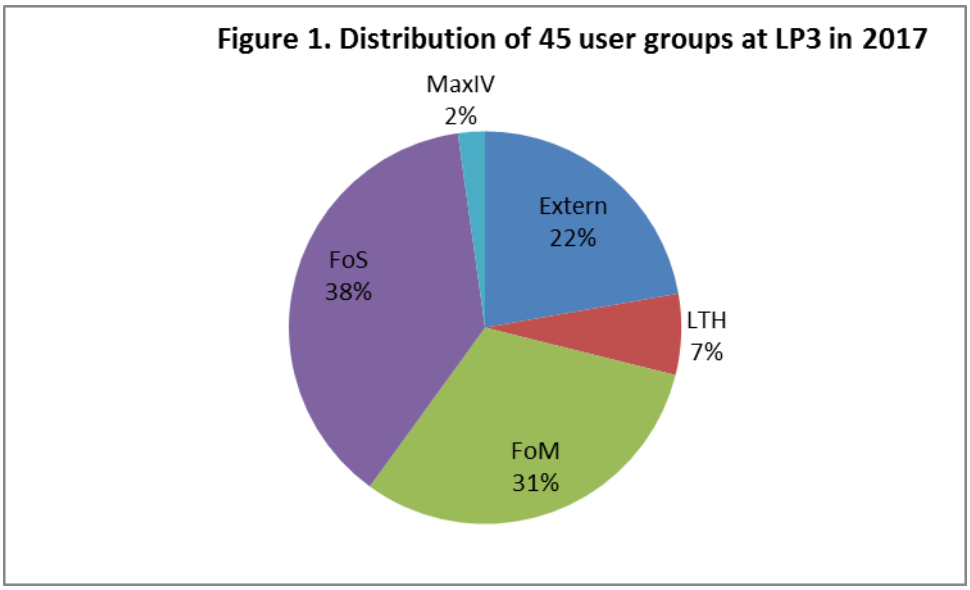
LP3 offers services for the entire process chain of production, purification, characterization and crystallization of proteins, or each individual step in the chain. LP3 can help with:

- 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
- Protein characterization (SEC, DSF, DSL)
- High-throughput & nanovolume protein crystallization
- Automated plate storage and imaging
- Microbiological growth monitoring (Bioscreen C)

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: 45 groups used LP3. Of these, 35 principal investigators came from LU and 10 were external. The distribution into different faculties is presented in Figure 1.
91 unique deliveries were made in 53 protein production projects and 158 protein crystallization plates (for 11 user groups) were processed. 10 visitors worked at LP3 for periods between a few days to up to one year, or are still associated with LP3. 4 user groups are using both protein production and crystallization at LP3.

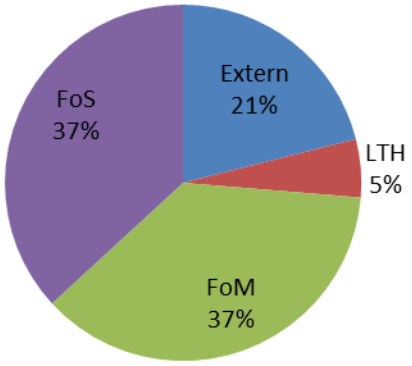
Protein Production: The table below shows the number and distribution of users in 2017. In brackets are the corresponding number given for 2016.

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

For 15 of the 38 principal investigators, it was the first time that they used LP3 to run a project.

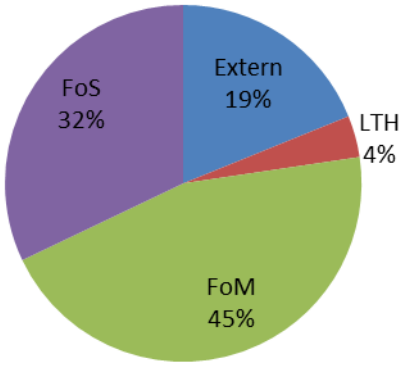
The distribution of users is illustrated in Figure 2.

Figure 2. Distribution of 38 user groups at LP3 (protein production)



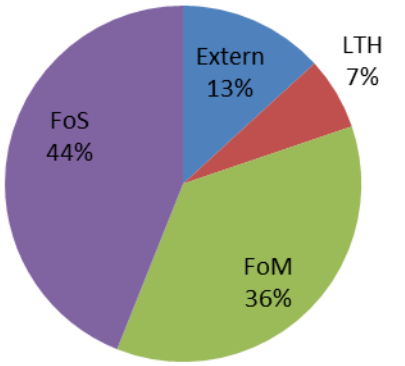
The distribution of projects is illustrated in Figure 3.

Figure 3. Distribution of 53 protein production projects at LP3



The distribution of deliveries is illustrated in Figure 4.

Figure 4. Distribution of 91 deliveries in protein production projects at LP3



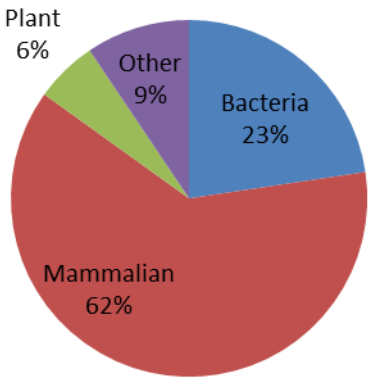
Figures 2. – 4. show that within protein production about ¾ of LP3s user groups, projects and deliveries are from and go to more or less equal parts to the FoS and FoM.

An increase in users with 38 vs 33 in 2016, as well as the total number of projects, 53 vs. 48 in 2016 can be seen.

All external users had projects in LP3 in one of its two areas of specialization, either the BEVS or stable isotope labeling (see long term strategy in the annual report 2016). On this note, about 40 % of all LP3 run projects use the BEVS and about 15 % of all LP3 run projects include stable isotope labeling. Compared to 2016 this means an increase in the percentage of BEVS projects by 8 % and the same level for stable isotope labeling. 5 projects, the same number as 2016, uses more than one expression system, meaning here to test expression in *E. coli* and BEVS in parallel.

The origin of the recombinant proteins in the projects run by LP3 is shown in Figure 5 below.

Figure 5. Distribution of the origin of proteins in the 53 protein production projects at LP3



Nearly 2/3 of all proteins are of mammalian (mostly human) origin. This emphasizes the need for more than just expression in *E. coli.* Recombinant protein expression in bacteria, is normally the cheapest and fastest way to produce recombinant proteins. However, many proteins, in particular complex mammalian proteins of biomedical interest (e.g. those with posttranslational modifications, membrane proteins, large protein complexes etc.) cannot be functionally produced in bacterial cells. It is therefore essential to have the ability to produce those proteins in eukaryotic expression hosts. The most effective eukaryotic expression systems are currently the BEVS that use insect cells as expression host, and mammalian cells (e.g., CHO and 293F). Insect cells are easy to handle, grow rapidly and can be easily scaled up, they are often the system of choice. This expression system has the best track record for subsequent crystallization of all eukaryotic expression systems. Insect cell based systems are capable of producing high-levels of proteins at lower running costs than mammalian cells. More than 50 % of the proteins of mammalian origin are expressed

in insect cells at LP3. The other proteins are comprised of proteins from virus, insect and bird origin.

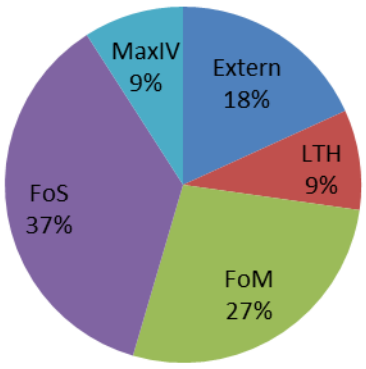
Protein Crystallization: The table below shows the number and distribution of users in 2017 and in brackets 2016.

Extern	2 (3)
LTH	1 (1)
FoM	3 (6)
FoS	4 (7)
MAX IV	1 (1)
Total	11 (18)

A total of 158 plates were processed in 2017. The comparable numbers are 262 plates in 2016 and 201 in 2015. Also the number of user groups has declined compared to the year before.

The distribution of users is illustrated in Figure 6.

Figure 6. Distribution of 11 user groups at LP3 (crystallization)



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 September and October 2017). It participates in relevant national and international networks and societies (e.g., PPNS (http://www.ppns.ki.se), ARBRE-MOBIEU (https://arbre-mobieu.eu), P4EU (https://p4eu.org) and 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 given at the following occasions in 2017:

- (Protein Production Network Sweden) PPNS meeting (Stockholm, February 2017)
- 3D imaging research school (Lund, March 2017)
- "SciLifeLab and LU resources" organized by organized by Molecular Recognition in Life (http://www.med.lu.se/morelife) (Lund, March 2017)
- MAX IV Laboratory User Meeting (UM17) (Lund, March 2017)
- Australian Centre for Neutron Scattering seminars (ANSTO - Lucas Heights, Sydney, April 2017)
- Deuteration for Neutron Scattering – DEUNET Workshop (Oxford, May 2017)
- Protein Science Day (Lund, May 2017)
- Dynamic structures: from atomic to cellular length scales (Lund University – University of Maryland Research Workshop) (Lund, May 2017)
- Infrastructure Fairs (FoM, September and October 2017, Lund and Malmö)
- LINX kickoff (September, Lund)

Conference attendance:

- Workshop "Advanced Isotopic labeling Methods for Integrated Structural Biology" (Grenoble, France March 2017)
- 21st Swedish Conference on Macromolecular Structure and Function (Tällberg, June 2017)

Results and /or proteins produced at the facility were used in the following 2017 publications:

1. Anderson, L.C., et al., Intact Protein Analysis at 21 Tesla and X-Ray Crystallography Define Structural Differences in Single Amino Acid Variants of

Human Mitochondrial Branched-Chain Amino Acid Aminotransferase 2 (BCAT2). J Am Soc Mass Spectrom, 2017. 28(9): p. 1796-1804.

2. Bagenholm, V., et al., Galactomannan Catabolism Conferred by a Polysaccharide Utilization Locus of Bacteroides ovatus: ENZYME SYNERGY AND CRYSTAL STRUCTURE OF A beta-MANNANASE. J Biol Chem, 2017. 292(1): p. 229-243.

3. Bukowska-Faniband, E. and L. Hederstedt, Transpeptidase activity of penicillin-binding protein SpoVD in peptidoglycan synthesis conditionally depends on the disulfide reductase StoA. Mol Microbiol, 2017. 105(1): p. 98-114.

4. Cassimjee, K.E., et al., Streamlined Preparation of Immobilized Candida antarctica Lipase B. ACS Omega, 2017. 2(12): p. 8674-8677.

5. Kulkarni, T.S., et al., Crystal structure of beta-glucosidase 1A from Thermotoga neapolitana and comparison of active site mutants for hydrolysis of flavonoid glucosides. Proteins, 2017. 85(5): p. 872-884.

6. Linares-Pasten, J.A., et al., Three-dimensional structures and functional studies of two GH43 arabinofuranosidases from Weissella sp. strain 142 and Lactobacillus brevis. Febs j, 2017. 284(13): p. 2019-2036.

7. Runnberg, R., S. Narayanan, and M. Cohn, Rap1 and Cdc13 have complementary roles in preventing exonucleolytic degradation of telomere 5' ends. Sci Rep, 2017. 7(1): p. 8729.

8. Weininger, U., Site-selective (13)C labeling of histidine and tryptophan using ribose. J Biomol NMR, 2017. 69(1): p. 23-30.

9. Weininger, U., Site-selective (13)C labeling of proteins using erythrose. J Biomol NMR, 2017. 67(3): p. 191-200.

10. Yang, J., et al., Improved molecular recognition of Carbonic Anhydrase IX by polypeptide conjugation to acetazolamide. Bioorg Med Chem, 2017. 25(20): p. 5838-5848.