Annual Report

Lund university Protein Production Platform (LP3) | 2016

Annual Report 2016

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. In July 2016, the DEuteration and MAcromolecular Xtallization (DEMAX) unit of the European Spallation Source (ESS) moved completely into Biology House A and co-localized with LP3. This move was accompanied by the appointment of Dr. Zoe Fisher, the head of DEMAX, as associated Senior Lecturer in structural biology at the Department of Biology and the signing of a 5 year contract between ESS and the Department of Biology, governing the usage of LP3 by DEMAX.

In November, Dr. Maria Gourdon started as research engineer at LP3, primarily supporting protein crystallization. Maria has a long track record in membrane protein work and crystallization and will expand LP3’s expertise in that area.

During the spring, a Bioscreen C plate reader for automated recording of microbial growth curves was added to the services offered by LP3 and an infrastructure grant to Prof. Lars Hederstedt in November will allow the placement of a Differential Scanning Fluorimetry reader at LP3 in 2017.

LP3 staff was in 2016 involved in both undergraduate and graduate teaching, as well as national and international conferences and networks of interest to the field.

LP3 services are fully used by Lund University researchers and a further increase in users, deliveries and projects can be foreseen. Within the protein production services, nearly 50 % were new users, running their first project within LP3.

As should be clear from the pages of this annual report 2016, LP3 continues to deliver value-adding services to Lund University researchers.

Wolfgang Knecht,
Manager LP3
Feb. 2017

Brief Facts 2016

43 users | 76 unique deliveries in 48 protein production projects | 262 protein crystallization plates | 11 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 even 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.

Long term strategy: LP3 operates on a fee-for-service basis where the LU user fee is comprised of consumables, materials, instrument costs, university overhead and a certain percentage of the staff time on the project in question. All documentation is captured using electronic lab books at LP3. End users will retain intellectual property rights and all results. LP3 however asks to be mentioned in the acknowledgements if results/proteins produced at the facility are published.

The expected long long-term developments within LP3 can be captured in 4 points, whereof points 2 and 3 are of special importance and interest in the context of the collaboration between LP3 and DEMAX (ESS):

1. LP3 is the only university platform in Sweden offering protein expression in insect cells (BEVS) to its users. This expression system has the best track record for subsequent crystallization of all eukaryotic expression systems [1], in particular certain membrane protein classes.

2. LP3 has a special focus on the development of more efficient and cost-effective methods for stable isotope labeling of proteins, in particular deuteration. For deuteration, LP3 partners with the co-localized DEMAX platform of the ESS to drive the development of production of both partially and perdeuterated proteins and other valuable bioreagents that can be isolated from the cell cultures grown at LP3 (e.g., membrane lipids). Access to perdeuterated natural lipids will open completely new avenues of research in the structural biology of membrane proteins that are complementary to X-ray crystallography to study, for example, the function of membrane proteins using neutron reflectometry and small angle scattering under physiological conditions. Additionally, the existing equipment at LP3 can be used for the biochemical synthesis (e.g., enzyme catalysis) of isotopically labeled small molecule ligands for structural biology and biomedical studies. LP3 plans to provide key tools and reagents for the commissioning of ESS instruments in biology from 2019 onwards. In parallel, LP3 will become a focal point for LU researchers to produce reagents and reproducible results in good time before user operations at the ESS in 2023, which will make them well-placed to win the highly competitive applications for beamtime access at the ESS and other neutron sources worldwide.

3. LP3 aims to increase its capacity and competence in the area of membrane protein crystallization. Despite the great biological importance of membrane proteins (e.g., for development of new drugs), their structural biology lags well behind that of soluble proteins because of difficulties in protein preparation and crystallization. However, publications in this area generally have very high impact. LP3 will support and assist researchers at LU who focus on membrane proteins. The expertise developed in expressing and purifying biological lipid samples as in point 2 above gives LP3 the

future potential to develop and optimize new methods for membrane protein production and crystallization. Also, equipment recently installed at LP3, or for which we plan to apply for future funding, will increase our capacity for membrane protein crystallization.

4. Quality characterization of recombinant produced proteins. LP3 aims to offer a characterization package of recombinant proteins from 2018 according to the recommendations of the Protein Production and Purification Partnership in Europe. (P4EU) and the European association for Core Technologies for Life Science (CTLS) [2,3].

1. Assenberg, R., Wan, P. T., Geisse, S. & Mayr, L. M. Advances in recombinant protein expression for use in pharmaceutical research. Current opinion in structural biology 23, 393-402, doi:10.1016/j.sbi.2013.03.008 (2013).
2. Lebendiker, M., Danieli, T. & de Marco, A. The Trip Adviser guide to the protein science world: a proposal to improve the awareness concerning the quality of recombinant proteins. BMC research notes 7, 585, doi:10.1186/1756-0500-7-585 (2014).
3. Raynal, B., Lenormand, P., Baron, B., Hoos, S. & England, P. Quality assessment and optimization of purified protein samples: why and how? Microbial cell factories 13, 180, doi:10.1186/s12934-014-0180-6 (2014).

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:

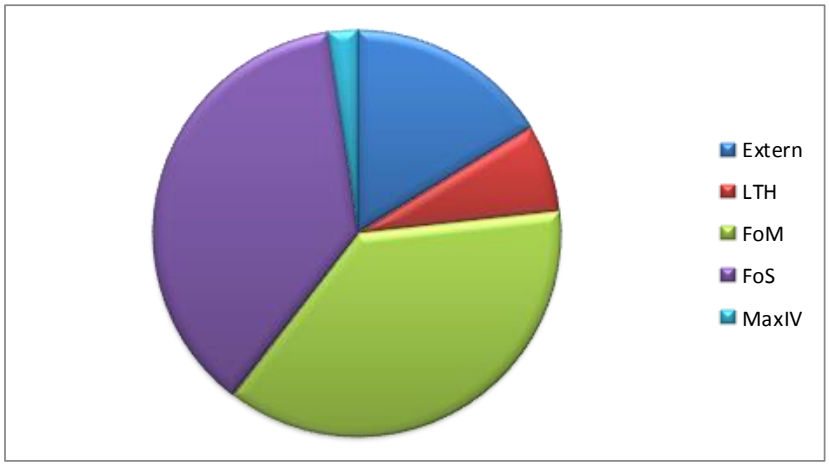
• Plasmids for protein production
• Recombinant protein production in bacterial (*E. coli*) or eukaryotic (insect) cells.
• Protein labeling (seleno-methionine incorporation, labeling with stable isotopes (2H, 13C, 15N), biotinylation, phosphorylation).
• Protein purification
• High-throughput & nanovolume protein crystallization
• Microbiological growth monitoring (Bioscreen C)

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

Users and projects

Due to LP3 being formed as a center first in June 2016, only very general overall user statistics will be reported here and a more detailed breakdown into the protein production part and the crystallization part will follow below.

Overall: 43 groups used LP3. Of these, 36 principal investigators came from LU and 7 were external. The distribution into different faculties is presented in the figure below.



76 unique deliveries were made in 48 protein production projects and 262 protein crystallization plates were processed. 11 visitors worked at LP3 for periods between a few days to up to one year, or are still associated with LP3.

Protein Production: The table below shows the number and distribution of users in 2016. In brackets are the corresponding number given for 2015.

		first project in LP3
Extern	5 (3)	5
LTH	2 (2)	0
FoM	14 (9)	6
FoS	12 (11)	5
MAX IV	(1)	
Total	33 (26)	16

For 16 of the 33 principal investigators, it was the first time that they used LP3 to run a project.

All external users had projects in LP3 in one of its two areas of specialization, either the BEVS or stable isotope labeling (see also points 1. and 2. under Long term

strategy in the introduction). On this note, about 1/3 of all LP3 run projects use the BEVS and about 1/6 of all LP3 run projects include stable isotope labeling.

A clear increase in users with 33 vs 26 in 2015, as well as the total number of projects, 48 vs. 30 in 2015 can be seen. It also reflects the trend that an individual user trusts the platform with more than one project. There is also an increasing trend to test protein expression in more than one expression system, meaning here to test expression in *E. coli* and BEVS in parallel.

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

Extern	3
LTH	1
FoM	6
FoS	7
MAX IV	1
Total	18

A total of 262 plates were processed in 2016. In 2015, 351 plates were processed; however this reported number included around 150 plates from SARomics Biostructures AB that administered and used the facility until 2015, and such plates are not included in the statistics from 2016. Therefore a relative increase of usage by LU researchers (and their external collaborators, e.g., ESS and within MAX4ESSFUN) can be seen in an increase of the numbers of plates by about 60 in 2016.

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 Infrastructure of the Month in May 2016 at the FoM). It participates in relevant national and international networks and societies, (e.g., PPNS, SwedStruct, P4EU and CTLS). 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 Ph.D. courses (e.g., ESS scientific seminars, 3D imaging research school, PhD course protein factories).

Presentations of LP3 were given at the following occasions in 2016:

- 3D imaging research school (Lund, January 2016)
- Protein Science Day (Lund, May 2016)
- ESS seminar (Lund, May 2016)
- MAX4ESSFUN Annual Meeting 2016 (Lund, October 2016)
- Synchrotron and Neutron Scattering in Biomaterials and Soft (Malmö, October 2016)
- CALS seminar (Lund, November 2016)
- Tandem Talk (Lund, November 2016)
- ESS STAP meeting (Lund, December 2016)

Conference attendance: CTLS & P4EU meeting (Heidelberg, June 2016)

Results and /or proteins produced at the facility were used in the following 2016 publications:

1 Badarau, A. et al. Context matters: The importance of dimerization-induced conformation of the LukGH leukocidin of Staphylococcus aureus for the generation of neutralizing antibodies. mAbs 8, 1347-1360, doi:10.1080/19420862.2016.1215791 (2016).

2 Domingo-Espin, J. et al. Dual Actions of Apolipoprotein A-I on Glucose-Stimulated Insulin Secretion and Insulin-Independent Peripheral Tissue Glucose Uptake Lead to Increased Heart and Skeletal Muscle Glucose Disposal. Diabetes 65, 1838-1848, doi:10.2337/db15-1493 (2016).

3 Johansson, R. et al. Structural Mechanism of Allosteric Activity Regulation in a Ribonucleotide Reductase with Double ATP Cones. Structure (London, England : 1993) 24, 906-917, doi:10.1016/j.str.2016.03.025 (2016).

4 Kakkar, V. et al. The S/T-Rich Motif in the DNAJB6 Chaperone Delays Polyglutamine Aggregation and the Onset of Disease in a Mouse Model. Molecular cell, doi:10.1016/j.molcel.2016.03.017 (2016).

5 Kirscht, A. et al. Crystal Structure of an Ammonia-Permeable Aquaporin. PLoS biology 14, e1002411, doi:10.1371/journal.pbio.1002411 (2016).

6 Lizatovic, R. et al. A De Novo Designed Coiled-Coil Peptide with a Reversible pH-Induced Oligomerization Switch. Structure (London, England : 1993) 24, 946-955, doi:10.1016/j.str.2016.03.027 (2016).

7 Manzoni, F. et al. Perdeuteration, crystallization, data collection and comparison of five neutron diffraction data sets of complexes of human galectin-3C. Acta crystallographica. Section D, Structural biology 72, 1194-1202, doi:10.1107/s2059798316015540 (2016).

8 Mutahir, Z. et al. Gene duplications and losses among vertebrate deoxyribonucleoside kinases of the non-TK1 Family. Nucleosides, nucleotides & nucleic acids 35, 677-690, doi:10.1080/15257770.2016.1143557 (2016).

9 Orozco Rodriguez, J. M., Nesrini, M., Christiansen, L. S. & Knecht, W. Expression of tomato thymidine kinase 1 by means of the baculovirus expression vector system. Nucleosides, nucleotides & nucleic acids 35, 691-698, doi:10.1080/15257770.2016.1139126 (2016).

10 Rutardottir, S. et al. Structural and biochemical characterization of two heme binding sites on alpha1-microglobulin using site directed mutagenesis and molecular simulation. Biochimica et biophysica acta 1864, 29-41, doi:10.1016/j.bbapap.2015.10.002 (2016).