UPPSALA
UNIVERSITET

Annual Report 2016

Department of Pharmaceutical Biosciences

Fastställd 2017-09-07

Address:

Department of Pharmaceutical Biosciences
Uppsala University
PO Box 591
SE-751 24 UPPSALA
SWEDEN
Phone: +46 18 – 471 4010

www.farmbio.uu.se

E-mail to a member of the staff:
firstname.lastname@farmbio.uu.se

Editor:
Björn Hellman

Authors:
The scientific reports were written by the different research groups at the department.

Introduction

This annual report highlights the research activities in the Department of Pharmaceutical Biosciences during 2016. The research areas cover many different areas, including research on basic mechanisms of drug action, drug dependency and adverse health effects, as well as studies on drug metabolism, pharmacokinetics and pharmacodynamics. In addition, there is also a significant focus on pharmaceutical bioinformatics and proteomics, as well as pharmacometric modeling and simulations used in drug development. Some research groups are developing tools directly applicable to the pharmaceutical industry, while others are studying issues such as drug dependency and environmental contaminants with important socioeconomically implications. Also 2016, special commitments were made on our image platform based on a HCA/HCR equipment for high content analysis/screening. This platform is used by several research groups at the Department in different types of projects studying different types of end-points in cellular systems (including tissues). This report confirms that the research groups continue to have national and international collaborations within their research areas. Another important activity is the teaching we promote, and most of our junior lecturers, senior lecturers, and professors, and also some of our researchers, are heavily engaged in our education on various programs (focusing on pharmacy students). Most of our education is research-based and it is our aim to ensure that there is a synergy between teaching and research. Besides providing professional pharmacy education, the Department also offers graduate pharmacy students specialization in clinical pharmacy.

Funding

Apart from the block grants, all research activities in most cases also require external funding from national and international research councils and pharmaceutical companies. For example, during the last 2 years, major (sometimes smaller) research grants were obtained from various national and international research councils and foundations such as the Swedish Foundation for Strategic Research (SSF), the Swedish Research Council (SRC/VR and SRC/Formas), EU Innovative Medicines Initiative, Research and Innovation for Sustainable Growth (VINNOVA), the Swedish Association of the Pharmaceutical Industry (LIF), Drugs for Neglected Diseases (DNDi), Kjell and Märta Beijers Foundation, Carl Tryggers Foundation, Science for Life Laboratory (SciLife Lab), Swedish Council for Working Life and Social Research, Swedish Institute, Uppsala University, the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly, Svenska Spel Research Council, Berzelii Centre for Biotechnological Research, the Research Council of Swedish Criminal Care, the Swedish Governmental Agency for Innovation Systems, Agricultural Sciences and Spatial Planning, Genentech, EU FP7 Health, Innovative Medicines Initiative (IMI), Swedish Cancer Society, the National Board of Health and Welfare, National Institute of Health (NIH), AFA Insurance, the Swedish Brain Foundation, eSSENCE, SeRC, Japanese Society for Promotion of Science, Fredrik and Ingrid Thuring foundation, and

the Facias Foundation. Many projects also have financial support from pharmaceutical companies such as AstraZeneca, Janssen Pharmaceuticals, Novartis, Pfizer and Roche.

Organisation and financial review

The Department is organised on the basis of our main core activities, i.e., research, teaching and the collected support activities (i.e., management, finance/staff administration, education administration and infrastructure). All support activities are funded by a percentile overhead on salaries and operating costs. The government provides the basic funding for teaching and research. The peer-reviewed scientific publications, PhD examinations and the external grants provide the foundation for any decisions concerning the allocation of university funding of research. The revenues from research councils, EU, foundations and pharmaceutical companies are very important to increase the research activities at the Department. Below follows a short summary of incomes and expenditures 2016.

Major incomes 2016	*(kSEK)*
Research and graduate education- government	37 960
Research grants – research councils	34 596
Research – commissioned	22 892
Education – basic and advanced level –government	44 386

Major expenditures 2016	*(kSEK)*
Staff costs	69 720
Operating expenses etc.	13 929
Premises	14 590
University/faculty support activities	14 755
Library	2 796
Depreciation	4 815
Travels	2 871

Core facilities and other engagements

Apart from being involved in regular research and teaching activities, our Department also host two major core facilities, NRMSI and UUBF. We are also involved in the platform UPPNEX hosted by the SciLifeLab. Short descriptions of these facilities and platforms are presented below under “Collaborations with other universities and/or other parts of Uppsala University”. These two were put together under one heading because NRMSI, UUBF and UPPNEX have collaborations not only with our Department and other Departments at the Faculty of Pharmacy, Faculty of Medicine, Disciplinary Domain of Science and Technology (Uppsala University), but also with Karolinska Institutet, Stockholm University, University of Lund, etc.

The Department is also engaged in “the third mission”, mainly by hosting and leading the activities of U-FOLD. In addition, the Department of Pharmaceutical Biosciences

has also been one of the participants in the EU-supported education project SafeSciMet, involving both the Academy and Pharmaceutical Industry in several European countries. We are also one of the participants in SweTox, a national resource centre for academic interdisciplinary collaboration within toxicology sciences. Since our engagement in SafeSciMet was discontinued 2016, this activity will not be further mentioned in this report, but short descriptions of U-FOLD and SweTox are also given below.

Collaborations with other universities and/or other parts of Uppsala University: Apart from the fact that most research groups have regular collaborations with other research groups within our own department, as well as with other departments at the Disciplinary Domain of Medicine and Pharmacy; the University Hospital; Swedish University of Agricultural Sciences etc., we also host some core facilities/platforms that have rather extensive collaborations with other departments at Uppsala University. Research collaboration with other universities is also taking place through EU and national funding (e.g., STINT - The Swedish Foundation for International Cooperation in Research and Higher Education). There are also other collaborative possibilities through, e.g., the ULLA Consortium. Many collaborations are also driven by investigator networks, for example in areas such as tropical infectious diseases.

The National Resource for Mass Spectrometry Imaging (NRMSI) was founded at Uppsala University in 2010 with funds provided by the Swedish Research Council-Research Infrastructure in 2010 and Uppsala University-Infrastructure in 2013. The mission of the resource is to accelerate the adoption of MALDI and DESI technologies in the biological and medical research community. NRMSI provides access to advanced technologies and promotes interactions between scientists who are experts in these technologies and researchers conducting focused biological investigations. Such collaborations provide new insights into the targeted biological problems and a better understanding of health and disease at the molecular level. NRMSI uses state-of-the art mass spectrometry technologies and cutting-edge research to help projects in e.g., drug discovery and development, neuroscience, oncology and pathology applications and will during 2017 upgrade its MSI instrument for 11.6 MSEK. The NRMSI was 2015 awarded an Infrastructure Research Fellow grant from the Swedish Foundation for Strategic Research for 2016-2020 (15 MSEK), and 2016 a SciLife Lab Pilot Instrument grant for 2016-2018 (6 MSEK).

Uppsala University Behavioural Facility (UUBF) was established in 2011 through the strategic funding from the Disciplinary Domaine of Medicine and Pharmacy. UUBF functions as a non-profit core facility that administers and organizes experimental behavioral testing of mice, rats and fish with the aim to optimize access to well validated tests for research groups at Uppsala University as well as for external researchers, and promote the further development of behavioural tests. Services offered include experimental design, statistical processing and interpretation of results, rental of equipment including advanced recording equipment, and full performance of experiments.

The Bioinformatics Compute and Storage facility (UPPNEX) at Science for Life Laboratory (SciLifeLab) is headed from the Department of Pharmaceutical Biosciences. The facility provides high-performance computing and storage resources, maintain relevant bioinformatics software and data (e.g. reference genomes), and offer educational courses and user support. The facility had over 800 active projects during 2015 with computational resources hosted at UPPMAX - Uppsala University's resource

for high-performance computing and a node in the Swedish National Infrastructure for Computing (SNIC).

The Swedish Toxicology Sciences Research Center (Swetox) is based on a consortium of eleven Swedish universities (including UU through our Department). Swetox was established as a national resource for academic interdisciplinary collaboration within toxicology sciences in 2013 and it promotes research, education and societal interactions related to chemicals, health and environment. A Swetox hub at Södertälje was established as a research centre in modern laboratory facilities for safety assessment of pharmaceuticals that were closed-down by AstraZeneca in 2012.

External collaboration and outreach: Our rather extensive collaboration with the Pharmaceutical Industry in various projects is an important example of our external collaborations. Most of the projects are related to pharmacometrics/pharmacokinetic issues, but also other research groups at the Department have such collaborations. We also have some collaborations with regulatory agencies (most notably the Medical Products Agency, also located here in Uppsala), and other types of agencies like the WHO-associated Uppsala Monitoring Center (who are dealing with issues related to Pharmacovigilance).

Many of the researchers at the Department are also involved in national and international societies helping the development of the respective field and providing opportunities for advancing science. Much of this work is also to provide meeting and dissemination opportunities, both as conferences and symposia, as well as internet presence.

Another type of activity that has been going on for a couple of years now, are the activities arranged by U-FOLD. This is a forum/network for research on addiction to medical products and illegal drugs. U-FOLD is the link between research, prevention and treatment activities in Uppsala regarding different types of addiction. The network brings broad experience and different perspectives and the major aim of the activities is to contribute to greater understanding and a common approach regarding the underlying chain of events leading to addiction. U-FOLD, which has been led by senior professor Fred Nyberg since the network was initiated about five years ago, will most likely move from our Department to a more central chancellery at UU within a couple of years.

Future development

The years ahead promise many changes in terms of research and education and one can only hope that we will be successful in our attempts to get our different types of activities financially supported by both governmental funding of undergraduate teaching and research, as well as external fundings, so that we can keep the high level of research and teaching also in the future. What we do know is that our Department is under a state of transition. Some research areas will most likely decrease their research activities in the near future and replaced by new ones when new profesors have been recruited. Other research groups/research areas have slightly changed focus after the retirement of the professors that had led these groups for decades, but most of our research groups are continuing their research following the strategy and research profiles characterizing the groups for many years, with new group leaders after retired professors. Some research groups are very strong (both when it comes to funding's, and number of staff members and PhD students), other groups are clearly smaller. Two new professors forming their own research groups were recruited 2016, and these groups (Molecular

Neuropharmacology led by professor Robert Fredriksson, and Medical Mass Spectrometry led by professor Per Andrén) will probably increase their activities and staff during the next few years.

Two senior lecturer/associate professors, one in Pharmacokinetics (Ulrika Simonsson) and one in Biological Research on Drug Dependence (Mathias Hallberg) were promoted to professors during 2016, and eleven PhD students at the Department received their PhD this year (Igor Bazov, Brendan Bender, Salim Bouchene, Steve Choy, Oskar Clewe, David Khan, Anders Kristoffersson, Annika Linqvist (Borgs), Elin Svensson, Shima Momeni and Rikke Meldgaard).

Uppsala August 30, 2017

Björn Hellman
Head of Department

List of Contents

Organisation 2016

Chairman
Björn Hellman

Deputy chairman
Mats Karlsson

Department board
Björn Hellman, *chairman*
Mats Karlsson*, teacher representative*
Margareta Hammarlund-Udenaes*, teacher representative, deputy*
Maria Kjellsson*, teacher representative, deputy*
Siv Jönsson, *teacher representative*
Maria Swartling, *teacher representative*
Ola Spjuth, *teacher representative*
Eva Brittebo, *teacher representative*
Karin Tjäder*, technical/administrative representative, deputy*
Magnus Efverström, *technical/administrative representative*
Erik Nylander, *graduate student representative*
Ida Netterberg*, graduate student representative*
Emelie De Geer, *student representative*
Nikolina Bolander, *student representative, deputy*
Marina Rönngren*, technical/administrative representative*
Agneta Hortlund, *economical coordinator*, *adjunct (until 2016-06-30)*
Martin Grentzelius, *economical coordinator*, *adjunct (from 2016-01-01)*
Mikaela Andersson, *secretary, adjunct*

Professors
Per Andrén (from 2016-03-01)
Eva Brittebo
Robert Fredriksson
Lena Friberg
Margareta Hammarlund-Udenaes
Mathias Hallberg (from 2016-03-01)
Björn Hellman
Mats Karlsson
Ingrid Nylander
Ulrika Simonsson (from 2016-03-01)
Jarl Wikberg (until 2016-09-30)
Kjell Wikvall (until 2016-03-31)

Professor emeriti
Lennart Dencker
Lennart Palzow
Kjell Wikvall (from 20160401)

Senior Professors
Georgy Bakalkin
Fred Nyberg
Ernst Oliw (until 2016-12-31)
Jarl Wikberg (from 2016-10-01)

Adjunct Professors
Jan Kehr (until 2016-06-30)

Senior lecturers
Per Andrén (until 2016-02-29), *Associate professor*
Jörgen Bengtsson
Lena Bergström, *Associate professor*
Agneta Freijs
Mathias Hallberg (until 2016-02-29), *Associate professor*
Andrew Hooker
Oskar Karlsson*, *Associate professor*
Maria Kjellsson, *Associate professor*
Elisabet Nielsen
Maria Norlin
Ulrika Simonsson (until 2016-02-29), *Associate professor*
Ola Spjuth (from 2016-07-01), *Associate professor*
Anne-Lie Svensson
Erika Roman, *Associate professor*

Lecturer
Ola Spjuth (until 2016-06-30), *Associate professor*

Adjunct Lecturer
Markus Friden

Junior lecturers
Ann-Marie Falk
Lena Klarén
Emelie Lefvert*(until 2016-06-10)
Viktoria Lind
Emma Lundkvist
Maria Swartling
Tomas Friman*
Lisa Puigvert Fredriksson
Emelie Karlsson*
Frida Moqvist*

Directors of undergraduate studies
Lena Bergström
Jörgen Bengtsson
Eva Brittebo
Ann-Marie Falk
Mathias Hallberg
Björn Hellman
Elisabet Nielsen
Anne-Lie Svensson
Maria Swartling
Jarl Wikberg (until 2016-11-30)
Ola Spjuth (from 2016-12-01)
Maria Norlin

Postdocs, Researchers and PhD students
Listed in the scientific reports

Laboratory staff
Jessica Dunhall
Lena Norgren

Administration and service
Mikaela Andersson
Johanna Axnér (until 2016-08-31)
Ulrica Bergström
Magnus Efverström
Sigrid Engström* (from 2016-03-01)
Martin Grenzelius
Emma Holmberg* (until 2016-08-31)
Anna Hillbom* (2016-08-22 to 2016-12-31
Agneta Hortlund (until 2016-06-30)
Elisabeth Jonsson
Hedvig Nilsén* (from 2016-08-08)
Marina Rönngren
Karin Tjäder

Safety officers
Magnus Efverström
Lena Norgren
Marina Rönngren
Henrik Wadensten
Sviatlana Yahorava

The work environment group
Björn Hellman, *chairman*
Sigrid Engström, *secretary*
Eva Brittebo
Jessica Dunhall
Magnus Efverström
Alfhild Grönblad
Patrik Källback
Emilia Lekholm
Erik Melander
Lena Norgren
Marina Rönngren
Lova Segerström, *gender equality representative*
Karin Tjäder

Working group on post-graduate studies
Margareta Hammarlund-Udenaes, *chairman*
Ari Brekkan
Maria Norlin
Marina Rönngren, *secretary*

*Temporary positions

Scientific Reports 2016

Research area: Drug Safety and Toxicology

Eva Brittebo

http://farmbio.uu.se/research/researchgroups/dst/

The research group Drug Safety and Toxicology is focusing on mechanisms of adverse effects of environmental contaminants as well as pharmaceuticals and traditionally used medicinal plants, by the use of various *in vitro* and *in vivo* models. The major objective of our studies is to increase the knowledge about mechanisms of actions of toxicants in order to improve predictions of hazards and risks for humans. The studies are presently focusing on the following research areas: Neurotoxicology, Genetic toxicology, and Developmental toxicology.

The neurotoxicology studies aim to elucidate mechanisms of long-term cognitive impairments and neurodegeneration in the adult brain following exposure to toxicants. An important issue is the concept of early life exposure and neurotoxicity manifested late in life. Another project focuses on a troublesome adverse effect (dyskinesia) of L-DOPA pharmacotherapy for patients with Parkinson disease. This project is based on various imaging techniques including MALDI –TOF imaging mass spectrometry (MALDI IMS) for the topographical elucidation of proteins, neuropeptides and neurotransmitters in the brain. In addition, the direct delivery of therapeutic agents and toxicants to the brain via intranasal administration is being examined.

The studies on genetic toxicology are based on the Comet Assay for evaluating DNA-strand breaks in individual cells. An important issue in the safety evaluation of drugs is if there are threshold doses or not for agents found to be genotoxic *in vitro* or *in vivo*. In addition, gene-environment-epigenome interactions are studied using various epigenetic tools.

The studies on developmental toxicology are focusing on the molecular basis of toxicant-induced disruptions that lead to irreversible changes in adults. An important issue is how specific gene regulation changes during developmental sensitivity periods *in vivo* can be replicated in cell lines *in vitro*.

Members of the group during 2016

Eva Brittebo, Professor
Björn Hellman, Professor
Lennart Dencker, Professor emeritus
Malin Andersson, PhD, Associate Professor, Researcher
Oskar Karlsson, PhD, Associate Professor, Senior lecturer (25 %)
Paula Pierozan, PhD, research scholarship holder
Michael Stigson, PhD, Associate Professor, Researcher
Lisa Ersson, PhD student
Daiane Cattani, PhD student (exchange program from Brazil)
Elena Piras, PhD student (parental leave)
Jasem A Shemaili, PhD student
Emelie Karlsson, MSci, Research assistant
Johanna Eriksson, MSci, Research assistant (part time)
Lena Norgren, Technician
Hans Lindén, Senior project coordinator (part time)
Ulla Mårs PhD (part time)

Publications 2014 – 2016

1. Lundqvist J, Hellman B, Oskarsson A. (2016) Fungicide prochloraz induces oxidative stress and DNA damage in vitro. Food Chem Toxicol. 91:36-41.
2. Al Shemaili J, Parekh K A, Newman R A, Hellman B, Woodward C, Adem A, et al. (2016) Pharmacokinetics in Mouse and Comparative Effects of Frondosides in Pancreatic Cancer. Marine Drugs. 14:115.
3. Karlsson, O., Michno, W., Ransome, Y., and Hanrieder, J. (2016) MALDI imaging delineates hippocampal glycosphingolipid changes associated with neurotoxin induced proteopathy following neonatal BMAA. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics. [Epub ahead of print]
4. Karlsson, O., Rodosthenoues, R., Jara, C., Brennan, K., Wright, RO., Baccarelli, AA. and Wright., R. (2016) Detection of long noncoding RNAs in human breast milk extracellular vesicles: Implications for early child development. Epigenetics.11:721-729.
5. Andersson, M., Karlsson, O., Banack, S., and Brandt, I. (2016) Transfer of the developmental neurotoxin β-N-methylamino-L-alanine (BMAA) via milk to nursed offspring: Studies by mass spectrometry and image analysis. Tox Lett. 258:108-14.
6. Dencker L, Hellmann K, Mochel J, Senel S, Tyden E, Vendrig J C, et al. (2016) Position Paper : EUFEPS Network on Veterinary Medicines Initiative: An interdisciplinary forum to support Veterinary Pharmacology and promote the development of new pharmaceuticals for Animal Health. Eur J Pharm Sci. 91:I-VII.
7. Schweizer, N, T Viereckel, C J.A. Smith-Anttila, K Nordenankar, E Arvidsson, S Mahmoudi, A Zampera, H Wärner Jonsson, J Bergquist, D Lévesque, Å Konradsson-Geuken, M Andersson, S Dumas and Å Wallén-Mackenzie (2016) Reduced Vglut2/Slc17a6 gene expression levels throughout the mouse subthalamic nucleus causes cell loss and structural disorganization followed by increased motor activity and decreased sugar consumption. eNeuro. 3(5).
8. Bergman HM, Lundin E, Andersson M, Lanekoff I. (2016) Quantitative mass spectrometry imaging of small-molecule neurotransmitters in rat brain tissue sections using nanospray desorption electrospray ionization. Analyst 141:3686-95
9. Karlsson, O., Segerström, L., Sjöback, R., Nylander, I., and Boren, M. (2016) qPCR based mRNA quality score show intact mRNA after heat stabilization. Biomolecular Detection and Quantification. 7:21-26.
10. Karlsson, O., and Roman, E. (2016) Dose-dependent effects of alcohol administration on behavioral profiles in the multivariate concentric square field™ (MCSF) test. Alcohol. 50:51-56
11. Scholz B, Doidge AN, Barnes P, Hall J, Wilkinson LS, Thomas KL. (2016) The regulation of cytokine networks in hippocampal CA1 differentiates extinction from those required for the maintenance of contextual fear memory after recall. PLoS One. 11:e0153102
12. Klint H, Lejonklou MH, Karimullina E, Rönn M, Lind L, Lind PM, Brittebo E. (2016) Low-dose exposure to bisphenol A in combination with fructose increases expression of genes regulating angiogenesis and vascular tone in juvenile Fischer 344 rat cardiac tissue. Ups J Med Sci.13:1-8.
13. Karlsson O, Jiang L, Ersson L, Malmström T, Ilag LL, Brittebo EB. (2015) Environmental neurotoxin interaction with proteins: Dose-dependent increase of free and protein-associated BMAA (β-N-methylamino-L-alanine) in neonatal rat brain. Sci Rep. 5:15570.
14. Karlsson O, Berg AL, Hanrieder J, Arnerup G, Lindström AK, Brittebo EB (2015) Intracellular fibril formation, calcification, and enrichment of chaperones, cytoskeletal, and intermediate filament proteins in the adult hippocampus CA1 following neonatal exposure to the nonprotein amino acid BMAA. Arch Toxicol. 89:423-36.
15. Shariatgorji M, Nilsson A, Källback P, Karlsson O, Zhang X, Svenningsson P, Andren PE. (2015) Pyrylium Salts as Reactive Matrices for MALDI-MS Imaging of Biologically Active Primary Amines. J Am Soc Mass Spectrom. 26:934-9.
16. Jernerén F, Söderquist M, Karlsson O. (2015) Post-sampling release of free fatty acids - effects of heat stabilization and methods of euthanasia. J Pharmacol Toxicol Methods 71:13-20.
17. Karlsson O, Colombo G, Roman E. (2015) Low copulatory activity in selectively bred Sardinian alcohol-nonpreferring (sNP) relative to alcohol-preferring (sP) rats. Ups J Med Sci. 120:181-9.
18. Sun, Y., Jergil, M., Stockling, K., Hellmold, H., Dencker, L. et al. (2014). Short-Term Embryonic Stem Cell Exposure Assay As a Tool to Predict Developmental Toxicity. Birth defects research. Clinical and molecular teratology, 100:402-402.

19. Stingl, C., Söderquist, M., Karlsson, O., Boren, M., Luider, T. (2014) Uncovering ex-vivo degradation of peptides and neuropeptides during sample extraction in rat brain tissue by oxygen-18 labelling. J Proteome Res. 13:2807-2817
20. Hanrieder, J., Karlsson, O., Brittebo, EB. Malmberg, P., and Ewing, AG. (2014) Probing the Lipid Chemistry of Neurotoxin Induced Hippocampal Lesions Using ToF-SIMS Imaging. Surf Interf Anal. 46:375–378
21. Karlsson O, Bergquist J, Andersson M. (2014) Quality measures of imaging mass spectrometry aids in revealing long-term striatal protein changes induced by neonatal exposure to the cyanobacterial toxin ctermethylamino-L-alanine (BMAA). Mol Cell Proteomics. 13:93-104.
22. Ritchie H, Svensson CH, Nilsson MF, Webster WS. (2014) A comparison of drug-induced cardiotoxicity in rat embryos cultured in human serum or protein free media. J Pharmacol Toxicol Methods. 70:276-82
23. Nilsson MF, Webster WS. (2014) Effects of macrolide antibiotics on rat embryonic heart function in vitro. Birth Defects Res B Dev Reprod Toxicol.101:189-98.
24. Hanrieder J, Gerber L, Persson Sandelius Å, Brittebo EB, Ewing AG, Karlsson O. (2014) High resolution metabolite imaging in the hippocampus following neonatal exposure to the environmental toxin BMAA using ToF-SIMS. ACS Chem Neurosci. 5:568-75.
25. Fransson M, Piras E, Wang H, Burman J, Duprez I, Harris RA, LeBlanc K, Magnusson PU, Brittebo E, Loskog AS. (2014) Intranasal delivery of central nervous system-retargeted human mesenchymal stromal cells prolongs treatment efficacy of experimental autoimmune encephalomyelitis. Immunology. 142:431-41.
26. Karlsson O, Jiang L, Andersson M, Ilag LL, Brittebo EB. (2014) Protein association of the neurotoxin and non-protein amino acid BMAA (β-N-methylamino-L-alanine) in the liver and brain following neonatal administration in rats. Toxicol Lett. 226:1-5.
27. Al Shemaili J, Mensah-Brown E, Parekh K, Thomas SA, Attoub S, Hellman B, Nyberg F, Adem A, Collin P, Adrian TE (2014) Frondoside A enhances the antiproliferative effects of gemcitabine in pancreatic cancer. Eur. J. Cancer, 50, 1391-1398.
28. Boldbaatar D, El-Seedi HR, Findakly M, Jabri S, Javzan B, Chodash U, Göransson U, Hellman B (2014). Antigenotoxic and antioxidant effects of the Mongolian medicinal plant Leptopyrum fumarioides (L): an in vitro study. J. Ethnopharmacol., 155:599-606.

Reviews 2014 – 2016

1. Karlsson, O. and Lindquist NG. (2016) Melanin and neuromelanin binding of drugs and chemicals: toxicological implications. Arch Toxicol. 90(8):1883-91
2. Karlsson, O., and Baccarelli, AA. (2016) Environmental Health and Long Non-coding RNAs. Curr Envir Health Rpt. 3:178-87
3. Karlsson, O. and Hanrieder, J. (2016) Imaging mass spectrometry in drug development and toxicology. Arch Toxicol. Dec 8 [Epub ahead of print]
4. Webster WS, Nilsson M, Ritchie H. (2014) Therapeutic drugs that slow the heart rate or early rat embryos. Is there a risk for the human? Curr Pharm Des. 20:5364-76.

Agencies that supported the work/Funding 2016

The Swedish Research Council (VR-Medicine), The Swedish Research Council FORMAS, EU Innovative Medicines Initiative (IMI), Carl Tryggers Stiftelse, Fredrik och Ingrid Thurings Stiftelse, The Nilsson-Ehle Endowments and Magnus Bergvalls Stiftelse

Other commitments/assignments of group members 2016

Eva Brittebo: Member of the Quality Advisory Board on issues of Uppsala University quality management. Member of the Faculty of Pharmacy Recruitment Group. Member of the Executive Committee for the SafeSciMET education and training program in safety sciences for medicines. Member of the Swetox Consortium Board. Member of the Editorial Board of Environmental Toxicology and Pharmacology, Director of studies, advanced level in toxicology.

Björn Hellman: Head of Department; Member of the local committee for scholarships at the Faculty of Pharmacy; Director of studies, basic and advanced level in toxicology.

Oskar Karlsson: Member of the Editorial Boards of Scientific Reports, Epigenomics, and Journal of Environmental Toxicology

Projects

Neurodegeneration following neonatal exposure to neurotoxins (Eva Brittebo, Oskar Karlsson, Lisa Ersson, Daiane Cattani, Malin Andersson, Birger Scholtz in collaboration with Marie Andersson and Ingvar Brandt, Dept. of Environmental Toxicology, Uppsala University; Mikael Enskog and Curt Petterson, Dept. of Medicinal Chemistry, Uppsala University; Anna-Lena Berg, AstraZenca, Södertälje; Jörg Hanrieder, University of Gothenburg, and Leopold Ilag, Liying Jiang, Stockholm University, and Ariane Zamoner, Universidade Federal de Santa Catarina, Brazil)

Neurodegenerative disease is a major health issue with increasing incidences in an ageing population. Such disease has a multi-factorial etiology involving interplay between genetic and environmental risk factors e.g. neurotoxic contaminants. BMAA (beta-N-methylamino-L-alanine) is a neurotoxic amino acid suggested to contribute to a particular neurodegenerative disease in humans. Our studies demonstrated a poor transfer of radiolabelled BMAA across the blood-brain barrier (BBB) with no specific localisation in discrete brain regions in adult mice. In neonatal mice, however, there was an efficient transport across the BBB and a selective uptake of radioactivity in the hippocampus and striatum. Neonatal exposure to BMAA also induced cognitive impairments such as reduced spatial learning and memory abilities in adulthood. In addition, neonatal rat pups treated with a high dose BMAA showed acute neuronal cell death in the hippocampus. This brain area is important for cognitive function. Histopathological and ultrastructural analysis identified intracellular fibril formation, neuronal degeneration, cell loss, calcium deposits and astrogliosis in the hippocampus of adult animals following exposure during the brain development. Moreover, lower doses of BMAA caused distinct impairments in learning and memory function in adult animals following neonatal exposure without acute morphological changes in the brain.

Proteomic analysis of the adult hippocampus demonstrated an enrichment of chaperones, cytoskeletal and intermediate filament proteins, and proteins involved in the antioxidant defense system in the damaged area. Moreover, developmental exposure to BMAA resulted in increased protein ubiquitination in the adult hippocampus indicating that BMAA may induce protein aggregation. MALDI-TOF imaging mass spectrometry demonstrated that developmental exposure to BMAA induced changes in the expression of many proteins in the damaged hippocampus. In addition, time-of-flight secondary ion mass spectrometry based imaging revealed BMAA-induced localization of phosphatidylcholine lipids in the damaged hippocampus. In another study, we characterized changes of major intermediary metabolites in serum following neonatal exposure to BMAA using a non-targeted metabolomic approach. NMR data indicated that intermediary metabolites associated with energy metabolism and amino acid metabolism were changed in serum of BMAA-treated neonatal rats.

Autoradiographic imaging of ^{14}C-labelled BMAA demonstrated a distinct uptake of radioactivity in tissues with a high rate of cell turnover and/or protein synthesis. Ultra-high performance liquid chromatography-tandem mass spectrometry analysis demonstrated a dose-dependent increase of protein-associated BMAA in neonatal rat tissues. BMAA was also associated to proteins in the brain, especially

in the hippocampus. The level in the brain was, however, considerably lower compared to the liver that is not a target organ for BMAA. In contrast to the liver there was a significantly increased level of protein-association of BMAA in the hippocampus and other brain areas following repeated administration suggesting that the degradation of BMAA-associated proteins may be lower in neonatal brain than in the liver. Another study showed that secretion of radiolabelled BMAA into milk is an elimination pathway in lactating mothers. Following secretion of radiolabelled L-BMAA into milk, the levels of radioactivity in the brains of the suckling neonatal mice significantly exceeded the levels in the maternal brains. *In vitro* studies using the mouse mammary epithelial cell line confirmed a more efficient influx and efflux of L-BMAA than of D-BMAA in cells, suggesting enantiomer-selective transport. Competition experiments with other amino acids suggest that the amino acid transporters LAT1 and LAT2 may contribute to the transport of L-BMAA into milk.

Glyphosate, a synthetic derivative of glycine, is the most widely used broad-spectrum herbicide in the world. A research group at the Universidade Federal de Santa Catarina, Brazil has recently demonstrated that exposure to a glyphosate formulation during the development induces an acute excitotoxicity in the neonatal rodent hippocampus. In collaboration with the Brazilian research group we are examining the long-term effects in the adult brain following neonatal exposure to a glyphosate formulation using MALDI-TOF imaging mass spectrometry. The effects of glyphosate on differentiating neuronal cells are also studied using a High Content Analysis/High Content Screening system.

Nasal transfer of therapeutic agents (Eva Brittebo and Elena Piras, in collaboration with Moa Fransson and Angelica Loskog, Dept. of Immunology, Genetics and Pathology, Uppsala University)

The nasal olfactory pathway is a potential route of delivery of therapeutic agents that do not easily pass the blood-brain barrier. The olfactory neurons have direct contact with the external environment via dendrites in the nasal mucus and with the brain via axons that reach the olfactory bulb without synaptic connections. The olfactory transfer of therapeutic agents into the brain is a novel principle for drug delivery. Intranasal administration of CNS-targeting modified CD4+ T cells significantly reduced disease symptoms as well as decreased IL-12 mRNA in a mouse model of brain inflammation - experimental autoimmune encephalomyelitis (EAE). Immunohistochemical markers for myelination and reactive astrogliosis confirmed recovery in mice treated with engineered Tregs. Furthermore, Treg-treated symptom-free mice recovering from EAE were rechallenged with a second EAE-inducing inoculum but remained healthy, demonstrating a sustained effect of engineered Tregs. In another study, intranasal administration of engineered mesenchymal stromal cells (MSCs) expressing a CNS-targeting receptor significantly reduced disease symptoms of EAE. Mice treated with CNS-targeting MSCs were resistant to further EAE induction whereas non-targeted MSCs did not give such persistent effects. This demonstrates that intranasal delivery of central nervous system-retargeted human mesenchymal stromal cells can prolong treatment efficacy in experimental autoimmune encephalomyelitis.

Evaluation of genotoxic/antigenotoxic effects of dietary anti-oxidants, traditionally used medicinal plants and other compounds of naturally origin (Björn Hellman Jasem A Shemaili, Lena Norgren, in collaboration with Ulf Göransson, Dept. of Medicinal Chemistry, Uppsala University, Abdu Adem, and Thomas Edward Adrian, UAE University Al Ain)

In this project, we have mainly been evaluating the genotoxic and anti-genotoxic effects of some plants used in traditional medicine in Ethiopia and other countries, and in these studies, we also include fractions of extracts and/or pure compounds from extracts. Following up clinical studies showing that intake of β-carotene and other antioxidants from the diet was found to be associated with a lower level of oxidative DNA damage in mononuclear leukocytes, and we have also recently published a study on the effect of β-carotene on catechol-induced DNA damage in mouse lymphoma cells. Our most recent study on compounds of natural origin is a study on Frondoside A (a triterpenoid glycoside isolated from the Atlantic sea cucumber *Cucumaria frondosa*) which was found to enhance the anti-cancer effects of gemcitabine, a finding, which in the future may turn out to be of clinical benefit for patients with pancreatic cancer.

Evaluation of the potential genotoxicity of drinking water (Björn Hellman, Lena Norgren in collaboration with Agneta Oskarsson and Karin Winberg, Swedish Agricultural University)

In this project evaluating the potential genotoxicity of water samples intended to be used as drinking water, we have introduced HepG2-cells as an additional cell line to be used in our tests. The latter cells are liver cells of human origin, commonly used when screening for toxicological profiles of xenobiotics *in vitro*. The main objective of the drinking water project (which we only are a small part of) is to develop methods for assessment of hazardous chemicals in drinking water by integrating chemical analysis and in vitro toxicity testing.

Environmental exposures and epigenetic mechanisms (Oskar Karlsson, Paula Pierozan, in collaboration with Andrea Baccarelli, Dept. of Environmental Health Sciences, Columbia University, USA, Russ Hauser, Dept. of Environmental Health, Harvard University, USA, Anders Glynn and Per-Ola Danerud, Swedish National Food Agency, and Cecilia Berg Dept. of Organism Biology, Uppsala University).

There has been a rapidly increasing interest in whether environmental factors modulate the establishment and maintenance of epigenetic modifications, and thereby affect gene expression and phenotype. Chemical pollutants, dietary components, temperature changes and other external stressors can have long-lasting effects on development, metabolism and health, and maybe even in subsequent generations. Although the underlying mechanisms remain largely unknown, particularly in humans, mechanistic insights are emerging from experimental model systems, which may have implications for understanding disease and development. We aim to combine experimental and epidemiological research to study gene-environment-epigenome interactions. In particular, our research focuses on developmental origins of health and disease with an emphasis on environmental exposures and epigenetic mechanisms (DNA methylation and noncoding RNA). The projects concern the effects of environmental exposures such as endocrine disrupting chemicals, flame retardants, pesticides, metals, particulate air pollution, as well as drugs, psycho-social stressors and ethnical disparities. Ongoing efforts include investigation of multigenerational epigenetic inheritance.

Imaging Mass Spectrometry study of basal ganglia levels of neuropeptides in L-DOPA-induced dyskinesia in experimental Parkinson's disease (Malin Andersson in collaboration with Anna Ljungdahl, Madelene Svedin, Kristen Burnum (PNLL) USA, Jonas Bergquist, Uppsala University)

In this project, we study Parkinson's disease (PD), which is characterized by degeneration of dopaminergic neurons and accompanied by a dramatic loss of dopamine in the striatum, particularly in the putamen. About 1% of the population over 65 years suffers from PD and the cardinal symptoms that include akinesia, bradykinesia, muscle rigidity, and tremor. Dopamine replacement therapy with L-DOPA is currently the most effective pharmacotherapy for patients with PD, however with PD disease progression and long-term L-DOPA treatment complications occur in many patients. The main complications are troublesome motor complications such as "wearing off" fluctuations and L-DOPA-induced dyskinesia. Despite large efforts in the field of dyskinesia research, the difficulties we face today remain as how to dissociate the molecular changes that arise by the motor execution of dyskinesia from causative changes that induce or predispose to dyskinesias. Our group studies the brain structures of the basal ganglia for molecular correlates of the incidence and severity of dyskinesia in an experimental model of Parkinson's disease. We focus on neuropeptides and proteins involved in the development of L-DOPA-induced dyskinesias in experimental Parkinson's disease. Here we use MALDI-TOF imaging mass spectrometry (IMS) for unbiased assessment and topographical elucidation of neuropeptides and proteins in the basal ganglia of high and low dyskinetic animals. MALDI-IMS has the unique advantage of high sensitivity, high molecular specificity, and the detection of hundreds of molecular species in a single tissue section. Indeed, several dynorphins and enkephalins have been detected in these studies, including dynorphin B, dynorphin A(1-8), alpha-neoendorphin, MetEnkRF, MetEnkRGL, PEnk (198-209, 219-229).

Imaging MALDI mass spectrometry characterization of opioid peptides after a single dose cocaine or morphine (Malin Andersson in collaboration with Emma Gustafsson, Jonas Bergquist, areUppsala University, Jan Rodriguez Parkitna and Ryszard Przewłocki, Polish Academy of Science)

Drugs of abuse causes rapid changes in neurotransmission, for example release followed by synthesis of opioid peptides in different nuclei of the basal ganglia. In this study, we examine the localization and time course of opioid peptides after an acute dose of either cocaine or morphine.

MALDI mass spectrometry based molecular phenotyping of CNS and PNS glial cells for prediction in mammalian brain tissue (Malin Andersson in collaboration with Jörg Hanrieder, Grzegorz Wicher, Karin Forsberg Nilsson, Ping Sui, Jonas Bergquist Uppsala University, and Åsa Fex-Svenningsen SDU, Denmark)

These are several similar studies that examine the use of differential protein expression profiling of mammalian neural cells by means of MALDI TOF MS. MALDI MS profiling analysis is rapid, sensitive, robust and specific for large biomolecules in complex matrices. A new straightforward methodology was developed for direct characterization of rodent CNS glial cells using MALDI MS based intact cell mass spectrometry. Molecular phenotyping enables the characterization of cell growth stages, (stem) cell differentiation as well as probing cellular responses towards different stimulations. On-going work includes profiling of glioblastoma multiforme-derived cell lines for the prediction of cancer severity to facilitate diagnosis.

Development of new in situ enzyme histochemistry-MALDI imaging method (Malin Andersson in collaboration with Georgy Bakalkin, Jonas Bergquist, Maria Norlin, Uppsala University; Åsa Fex-Svenningsen, SDU, Denmark; Kristin Burnum-Johnson, PNLL, USA, and Jörg Hanrieder, Gothenborg University.

Currently we focus on the development of a new MALDI imaging method for the identification of effective enzyme inhibitor and mapping the effect on neuropeptide bioconversion in different brain areas. In addition, functional studies are carried out using live cell imaging and High Content Analyses (HCA) of an in vitro model of Parkinson disease.

Developmental Neurotoxicology in vitro (Birger Scholz and Henrik Alm)

The issue of in-vitro extrapolation to in-vivo conditions is of great importance in predictive toxicology considering how resource-intensive animal studies are. Unfortunately, the majority of todays *in silico* and *in vitro* assays suffer from weak predictive power for more complex toxicological endpoints. The mechanistic information from the postnatal sensitivity project is to be applied to the development of a range of more representative and specific *in vitro* tests for developmental neurotoxicology. This project investigates if and under what in-vitro conditions the observed developmental sensitivity period specific gene regulation changes can be replicated in cell lines (immortalized somatic neural cell lines and teratocarcinoma derived cell lines). In order to make the *in vitro* tests as relevant as possible for possible future hazard identification, they are conducted with and optimized for High Content Analysis/High Content Screening

Research area: Medical Mass Spectrometry

Per Andrén

http://farmbio.uu.se/research/researchgroups/mms

The research group focus on new approaches in mass spectrometry imaging (MSI), i.e. matrix-assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI)–MSI of biological tissue sections, and peptidomics, the comprehensive study of endogenous peptides.

MSI is a novel technique used to determine the spatial distribution of molecular species in biological tissue sections *in situ*. The technology allows analysis and visualization of endogenous proteins, peptides and neurotransmitters, as well as drugs and their metabolites, in their native biochemical states within the same tissue section with high molecular specificity. Molecular images are created by rasterizing over the sample while collecting MS or tandem MS (MS/MS) spectra from every position at a chosen spatial resolution. The localization pattern from individual molecular species present on the tissue surface can then be extracted and positioned on the original histological image with the abundances represented by a concentration dependent color scale.

Peptidomics involves the comprehensive analysis of the endogenous peptide content of a certain cell, organ, body fluid, or organism. It complements molecular biology approaches in its ability to characterize the processing of translation products, including changes in expression or posttranslational modifications (PTMs) of peptides and small proteins. By comparing the proteins and peptides in samples of diseased tissue with those in normal tissue, differential expression patterns can be detected that may lead to the identification of novel biomarkers.

The objective of our research is to utilize MSI and peptidomics approaches to study neurochemical processes in Parkinson's disease (PD) and specifically L-Dopa-induced dyskinesias (LID). The aim is to define neuropeptides, proteins and neurotransmitters that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, and to determine which of these molecules are regulated by loss of dopamine signaling, as well as to investigate their expression patterns in subjects with and without LID symptoms.

Understanding the relationship between pharmacokinetics and pharmacodynamics is crucial in the development of effective drug therapies. Current technologies only provide information on the total amount of drug in the whole tissue with no possibility to address micro-environmental localization of the compound or metabolic derivatives. The applications of MSI in drug discovery studies provide a new tool to accurately measure local tissue concentrations of drug/drug metabolite in context with efficacy achieved with the targeted biology or in the context of safety.

Our laboratory is equipped with the latest separation and MS technologies and we purchased new instruments (12.6 MSEK) during 2016. The laboratory has four MALDI-MSI instruments; one Ultraflextreme and one Rapiflex TOF/TOF (Bruker Daltonics), one MALDI/DESI/ESI Q-TOF Synapt G2si (Water Corp.), one MALDI/ESI FTICR (Bruker Daltonics) and one electrospray ionization mass spectrometer, LTQ (Thermo Scientific).

Members of the group during 2016

Per Andrén, Professor
Anna Nilsson, Researcher
Henrik Wadensten, Researcher
Mohammadreza Shariatgorji, Researcher
Patrik Källback, PhD student
Theodosia Vallianatou, PhD student
Elva Fridjonsdottir, PhD student

Publications 2014 – 2016

1. Bourdenx M, Nilsson A, Wadensten H, Fälth M, Li Q, Crossman AR, Andrén PE, Bezard E (2014) Abnormal structure-specific peptide transmission and processing in a primate model of Parkinson's disease and l-DOPA-induced dyskinesia. Neurobiol Dis. 62:307-312.
2. Akhtar MN, Southey BR, Andrén PE, Sweedler JV, Rodriguez-Zas SL (2014) Accurate assignment of significance to neuropeptide identifications using Monte Carlo k-permuted decoy databases. PLoS One.;9(10):e111112.
3. Swales JG, Tucker JW, Strittmatter N, Nilsson A, Cobice D, Clench MR, Mackay CL, Andren PE, Takáts Z, Webborn PJ, Goodwin RJ. Mass spectrometry imaging of cassette-dosed drugs for higher throughput pharmacokinetic and biodistribution analysis (2014) Anal Chem. 86:8473-8480.
4. Akhtar MN, Southey BR, Andrén PE, Sweedler JV, Rodriguez-Zas SL (2014) Identification of best indicators of peptide-spectrum match using a permutation resampling approach. J Bioinform Comput Biol. 12(5):1440001.
5. Shariatgorji M, Nilsson A, Goodwin RJA, Källback P, Schintu N, Zhang X, Crossman AR, Bezard E, Svenningsson P, Andren PE (2014) Direct Targeted Quantitative Molecular Imaging of Neurotransmitters in Brain Tissue Sections. Neuron. 84: 697–707.
6. Watanabe H, Fitting S, Hussain MZ, Kononenko O, Iatsyshyna A, Yoshitake T, Kehr J, Alkass K, Druid H, Wadensten H, Andren PE, Nylander I, Wedell DH, Krishtal O, Hauser KF, Nyberg F, Karpyak VM, Yakovleva T, Bakalkin G (2015) Asymmetry of the Endogenous Opioid System in the Human Anterior Cingulate: a Putative Molecular Basis for Lateralization of Emotions and Pain. Cereb Cortex. 25: 97-108.
7. Nilsson CL, Mostovenko E, Lichti CF, Ruggles K, Fenyo D, Rosenbloom KR, Hancock WS, Paik YK, Omenn GS, LaBaer J, Kroes RA, Uhlén M, Hober S, Vegvari A, Andrén PE, Sulman EP, Lang FF, Fuentes M, Carlsohn E, Emmett MR, Moskal JR, Berven FS, Fehniger TE, Marko-Varga G (2015) Use of ENCODE Resources to Characterize Novel Proteoforms and Missing Proteins in the Human Proteome. J Proteome Res. 14:603-8.
8. McDonnell LA, Römpp A, Balluff B, Heeren RM, Albar JP, Andrén PE, Corthals GL, Walch A, Stoeckli M (2015) Discussion point: reporting guidelines for mass spectrometry imaging. Anal Bioanal Chem. 407:2035-2045.
9. Cobice DF, Goodwin RJ, Andren PE, Nilsson A, Mackay CL, Andrew R (2015) Future technology insight: mass spectrometry imaging as a tool in drug research and development. Br J Pharmacol. 172:3266-83
10. Shariatgorji M, Nilsson A, Källback P, Karlsson O, Zhang X, Svenningsson P, Andren PE (2015) Pyrylium Salts as Reactive Matrices for MALDI-MS Imaging of Biologically Active Primary Amines. J Am Soc Mass Spectrom. 2015 26:934-9.
11. Nilsson A, Goodwin RJ, Swales JG, Gallagher R, Shankaran H, Sathe A, Pradeepan S, Xue A, Keirstead N, Sasaki JC, Andren PE, Gupta A (2015) Investigating nephrotoxicity of polymyxin derivatives by mapping renal distribution using mass spectrometry imaging. Chem Res Toxicol. 28:1823-30.
12. Wang X, Zhang Y, Nilsson CL, Berven FS, Andrén PE, Carlsohn E, Horvatovich P, Malm J, Fuentes M, Végvári Á, Welinder C, Fehniger TE, Rezeli M, Edula G, Hober S, Nishimura T, Marko-Varga G (2015) Association of chromosome 19 to lung cancer genotypes and phenotypes. Cancer Metastasis Rev. 34:217-226.
13. Lodén H, Shariatgorji M, Nilsson A, Andrén PE (2015) An introduction to MS imaging in

drug discovery and development. Bioanalysis. 7:2621-2627.
14. Goodwin RJ, Nilsson A, Mackay CL, Swales JG, Johansson MK, Billger M, Andrén PE, Iverson SL (2016) Exemplifying the Screening Power of Mass Spectrometry Imaging over Label-Based Technologies for Simultaneous Monitoring of Drug and Metabolite Distributions in Tissue Sections. J Biomol Screen. 21:187-193.
15. Ashton S, Song YH, Nolan J, Cadogan E, Murray J, Odedra R, Foster J, Hall PA, Low S, Taylor P, Ellston R, Polanska UM, Wilson J, Howes C, Smith A, Goodwin RJ, Swales JG, Strittmatter N, Takáts Z, Nilsson A, Andren P, Trueman D, Walker M, Reimer CL, Troiano G, Parsons D, De Witt D, Ashford M, Hrkach J, Zale S, Jewsbury PJ, Barry ST (2016) Aurora kinase inhibitor nanoparticles target tumors with favorable therapeutic index in vivo. Sci Transl Med. 8:325ra17.
16. Källback P, Nilsson A, Shariatgorji M, Andrén PE (2016) msIQuant - Quantitation Software for Mass Spectrometry Imaging Enabling Fast Access, Visualization, and Analysis of Large Data Sets. Anal Chem. 88:4346-4353.
17. Shariatgorji M, Strittmatter N, Nilsson A, Källback P, Alvarsson A, Zhang X, Vallianatou T, Svenningsson P, Goodwin RJ, Andren PE (2016) Simultaneous imaging of multiple neurotransmitters and neuroactive substances in the brain by desorption electrospray ionization mass spectrometry. Neuroimage. 136:129-138.
18. Shariatgorji M, Nilsson A, Bonta M, Gan J, Marklund N, Clausen F, Källback P, Loden H, Limbeck A, Andrén PE (2016) Direct imaging of elemental distributions in tissue sections by laser ablation mass spectrometry. Methods. 104:86-92

Reviews 2014 – 2016

1. Shariatgorji M, Svenningsson P, Andrén PE (2014) Mass Spectrometry Imaging, an Emerging Technology in Neuropsychopharmacology. Neuropsychopharmacology. 39:34-49.
2. El Magraoui F, Eisenacher M, Schrötter A, Kuhlmann K, Heinsen H, Andrén PE, Nilsson P, Häggmark A, Schmitz G, Verhaert P, Borchers C, Yoo JS, Lee BH, Meyer, HE, Grinberg LT (2014) Developing new methods to answer old and new questions in neurodegenerative diseases. Proteomics. 14:1308-1310.
3. Goodwin RJA, Shariatgorji M, Andren PE (2015) MALDI Mass Spectrometry Imaging of Dopamine and PET D1 and D2 Receptor Ligands in Rodent Brain Tissues. In: Dopamine Receptor Technologies, Neuromethods. Ed. Tiberi, M. Vol. 96, pp 177-196, Springer New York, pp 177-196
4. Nilsson A, Goodwin RJ, Shariatgorji M, Vallianatou T, Webborn PJ, Andrén PE (2015) Mass Spectrometry Imaging in Drug Development. Anal Chem. 87:1437-1455.

Agencies that supported the work/Funding 2016

The Swedish Research Council (VR); NT (2014–6215), MH (2013–3105) and RFI (2009-6050); AstraZeneca Global DMPK; FP7-PEOPLE-2013- Marie Curie Initial Training Networks (ITN); Uppsala University – Infrastructure, the Swedish Foundation for Strategic Research and Science for Life Laboratory.

Other commitments/assignments of group members 2016

Per Andrén: Swedish Academy of Pharmaceutical Sciences, Section for Drug Analysis (member of the board); Swedish Proteomics Society (member of the board); Journal of Proteomics (editorial board); European Proteomics Association (EuPA) Open Proteomics (editorial board), Cooperation in Science and Technology (COST) Action; Mass Spectrometry Imaging (member of the management committee).

Projects

(I) Functional neuroproteomic changes associated with L–Dopa–induced dyskinesia in Parkinson´s disease (Collaboration with Per Svenningsson, Karolinska Institutet, Erwan Bezard, Univ. of Bordeaux 2, France)

The main objective of the present research is to study neurochemical processes in Parkinson disease and specifically L-Dopa-induced dyskinesias (LID). No treatment exists yet for the management of LID, a debilitating complication of L-dopa therapy for PD. The aim is to define neuropeptides, proteins, neurotransmitters and endogenous metabolites that are differentially expressed in the basal ganglia complex of animals with striatal dopamine depletions, with and without LID. Project supported by VR-MH grant 2013–3105.

(II) Mass Spectrometry Imaging in Drug Discovery – Targeting drugs, metabolites, peptides and neurotransmitters

General objectives include development of MALDI-MSI in the following health research and drug discovery areas, a) ADME, high-resolution imaging the spatial distribution and absolute concentration of unlabeled drugs within tissue micro-compartments, b) Safety Toxicology, monitoring of molecular toxicology in organs (kidney), c) Blood-Brain Barrier (BBB), determine BBB profile for drug candidates in the early stages of the drug development, d) Neuroscience, absolute quantitation of neurotransmitters directly in tissue sections, e) msIQuant, MSI software development for quantitation of drugs, metabolites and endogenous compounds directly in tissue sections. The application of these methods will provide new tools aiding pharmaceutical development, as well as for clinical and experimental model systems. Project supported by VR-NT grant 2014–6215.

(III) Identification and characterization of protein-protein interactions in cerebrospinal fluid and brain tissue from Parkinson's disease (PD) patients and experimental PD models (Collaboration with Per Svenningsson, Karolinska Institutet)

Using surface plasmon resonance technique (Biacore 3000) coupled to mass spectrometry technology, new protein partners of α-synuclein and parkin have been captured and identified in cerebrospinal fluid or post-mortem human tissue from PD patients. In addition to using native α-synuclein and parkin, mutated forms of these proteins seen in familiar forms of PD will be immobilized on the sensor chip and used as baits. Project supported by VR-MH grant 2013–3105 and 2011-4722.

(IV) Fine mapping the spatial distribution and concentration of unlabeled drugs within tissue micro-compartments using imaging mass spectrometry (Collaboration with AstraZeneca)

In respiratory inhalation drug discovery projects, one key objective is to optimize retention of compound in the lung and consequently achieve duration of effect. Current technologies only provide information on the total amount of compound in the whole lung with no possibility to address micro-environmental localization of the compound or metabolic derivatives. The application of MALDI-MSI in such studies would provide a new tool to accurately measure local tissue concentrations of drug/drug metabolite in context with efficacy achieved with the targeted biology or in the context of safety. Such technology could provide new and important information. Project supported by collaboration agreement with AstraZeneca.

(V) Characterization of drug-induced kidney toxicity using MALDI-MSI (Collaboration with AstraZeneca)

The project is aimed at developing a routine methodology for the use of MALDI imaging in problem solving of toxicity questions. It will include preparation of tissue material, interpretation of the data generated and structure elucidation of new analytes. Project supported by collaboration agreement with AstraZeneca.

Research area: Molecular Neuropharmacology

Robert Fredriksson

The research area Molecular Neuropharmacology is a new at our Department and led by professor Robert Fredriksson. The old research area/group led by senior professor Ernst Oliw is now organized within Roberts group. The research area Molecular neuropharmacology is therefore divided into the following two research groups:

1. Molecular neuropharmacology
2. Biochemical pharmacology

Molecular Neuropharmacology

Robert Fredriksson

http://www.farmbio.uu.se/forskning/researchgroups/molekylar-neurofarmakologi/ (under construction)

Our research group is investigating which role transporters from the Solute Carrier (SLC) family plays in sustaining various aspects of cell homeostasis. We put most of our efforts into SLCs with high expression in the central nervous system and specifically those that are potential amino acid transporters. There is a large gap in our understanding regarding how SLCs regulate levels of neurotransmitters and amino acids in neurons, and how this regulation impacts the physiology and the nervous system. SLCs are also important in mediating uptake of drugs and for distribution of drugs within the body, most prominently by mediating passage of certain compounds over the blood brain barrier.

The SLC family has currently 430 known members in human and approximately one third of these, over 120 proteins, are still orphan meaning they have no known substrate that they are transporting. Also, for even more of the SLC transporters the physiological functions are not known. Most of the SLCs are expressed on the plasma membrane and are hence accessible for pharmaceutical compounds. Therefore, they are considered to be part of the drugable genome and our ultimate goal is to evaluate the SLCs as potential drug targets or as helper proteins to aid in designing drugs that are specifically taken up by certain cell types or tissues. Increased understanding of these mechanisms would enable SLCs to be used for controlling distribution and uptake of drugs, something that is poorly utilized at present.

We use transgenic mice and fruit flies (*Drosophila melanogaster*) as our primary *in vivo* modells to study SLCs. Mainly, we use tissue specific knockout and knock down models and investigate the effect of removal of a certain SLC through behavioral and biochemical methods. Because the genes we are studying are of unknown function “omics” tools are our key technologies, were we investigate effects of gene removal on the metabolome, transcriptome and proteome. This helps us to place the SLC into functional pathways which, together with bioinformatics analysis can guide us towards identification of the endogenous substrate and subsequent deorphanization. We express the SLCs in *in-vitro* systems, both eukaryotic cell-lines and *Xenopus laevis* oocytes to measure uptake of possible substrates. We use two electrode voltage clamp electrophysiology as well as labeled substrates to measure uptake. We also use histology, molecular biology, proximity ligation assays and imaging techniques to identify which cells express a certain SLC as well as to identify their subcellular site of expression.

Members of the group during 2016

Robert Fredriksson, professor
Sonchita Bagchi, Postdoc
Karin Nordenankar, Postdoc
Tanya Aggarwal, Postdoc
Sofie Hellsten, Postdoc
Nadine Schweizer, Postdoc
Emelie Perland, PhD student
Emilia Lekholm, PhD student
Rekha Tripathi, PhD student
Mikaela Eriksson, PhD student

Publications 2014-2016

1. Alsiö J, Rask-Andersen M, Chavan RA, Olszewski PK, Levine AS; Fredriksson R, Schiöth HB. Exposure to a high-fat high-sugar diet causes strong up-regulation of proopiomelanocortin and differentially affects dopamine D1 and D2 receptor gene expression in the brainstem of rats. *Neurosci Lett. 2014 Jan 24;559:18-23*
2. Roshanbin S, Hellsten SV, Tafreshiha A, Zhu Y, Raine A, Fredriksson R. PAT4 is abundantly expressed in excitatory and inhibitory neurons as well as epithelial cells. *Brain Res. 2014 Apr 4;1557*
3. Brooks SJ, Nilsson EK, Jacobsson JA, Stein DJ, Fredriksson R, Lind L, Schiöth HB.BDNF polymorphisms are linked to poorer working memory performance, reduced cerebellar and hippocampal volumes and differences in prefrontal cortex in a Swedish elderly population. *PLoS One. 2014 Jan 23;9(1):e82707*.
4. Krishnan A, Almén MS, Fredriksson R, Schiöth HB. Insights into the origin of nematode chemosensory GPCRs: putative orthologs of the Srw family are found across several phyla of protostomes. *PLoS One. 2014 Mar 24;9(3):e93048*
5. Bagchi S, Ali-Baomar H, Al-Walai S, Fredriksson R. Histological analysis of SLC38A6 (SNAT6) expression in mouse brain shows selective expression in excitatory neurons with high expression in the synapses. *PLoS One 2014 Apr 21;9(4):e95438*.
6. Rask-Andersen M, Philippot G, Moschonis G, Dedoussis G, Manios Y, Marcus C, Fredriksson R, Schiöth HB. CDKAL1-Related Single Nucleotide Polymorphisms Are Associated with Insulin Resistance in a Cross-Sectional Cohort of Greek Children. *PLoS One. 2014 Apr 2;9(4):e93193*
7. Williams JM, Goergen P, Phad G, Fredriksson R, Schiöth HB. The Drosophila Kctd-family homologue Kctd12-like modulates male aggression and mating behavior. *Eur J Neurosci. 2014 Aug;40(3):2513-26*.
8. Almén MS, Nilsson EK, Jacobsson JA, Kalnina I, Klovins J, Fredriksson R, Schiöth HB. Genome-wide analysis reveals DNA methylation markers that vary with both age and obesity. *Gene. 2014 Sep 10;548(1):61-7*.
9. Williams MJ, Goergen P, Rajendran J, Zheleznyakova G, Hägglund MG, Perland E, Bagchi S, Kalogeropoulou A, Khan Z, Fredriksson R, Schiöth HB. Obesity-linked homologues TfAP-2 and Twz establish meal frequency in Drosophila melanogaster. *PLoS Genet. 2014 Sep 4;10(9)*
10. Krishnan A, Dnyansagar R, Almén M, Williams MJ, Fredriksson R, Manoj N, Schiöth HB. The GPCR repertoire in the demosponge Amphimedon queenslandica : insights into the GPCR system at the early divergence of animals. *BMC Evol Biol. 2014 Dec 21;14(1):270*
11. Carosu V, Hagglund MG, Badiali L, Bagchi S, Roshanbin S, Tauseef Ahmad, Schioth HB, Fredriksson R. The G protein-coupled receptors GPR162 is widely distributed in the CNS and highly expressed in hypothalamus and in hedonic feeding areas. *Gene. 2014 Dec 10;553(1):1-6.*
12. Corell M, Wicher G, Radomska KJ, Dağlıkoca ED, Godskesen RE, Fredriksson R, Benedikz E, Magnaghi V, Fex Svenningsen A. GABA and its B-receptor are present at the node of Ranvier

in a small population of sensory fibers, implicating a role in myelination. *J Neurosci Res. 2015 Feb;93(2):285-95.*

13. Hägglund MG, Hellsten SV, Bagchi S, Philippot G, Löfqvist E, Nilsson VC, Almkvist I, Karlsson E, Sreedharan S, Tafreshiha A, Fredriksson R. Transport of L-glutamine, L-alanine, L-arginine and L-histidine by the neuron-specific Slc38a8 (SNAT8) in CNS (*J Mol Biol. 2015 Mar 27;427(6 Pt B):1495-512*).
14. Nilsson EK, Schultes B, Ernst B, Voisin S, Sällman Almén M, Fredriksson R, Schiöth HB, Benedict C Roux-En Y. Gastric Bypass Surgery Induces Genome-Wide Promoter-Specific Changes in DNA Methylation in Whole Blood of Obese Patients (*PLoS One. 2015 Feb 24;10(2):e0115186*)
15. Carosu V, Le Grevés M, Shirazi Farad S, Haitina T. Olszewski PK, Alsiö, J, Shiöth HB, Fredriksson R. The orphan G protein-coupled receptor gene Gpr178 is evolutionary conserved and altered in response to acute changes in food intake (*PLoS One. 2015 Jun 5;10(6):e0122061*).
16. Zheleznyakova GY, Nilsson EK, Kiselev AV, Maretina MA, Tishchenko LI, Fredriksson R, Baranov VS, Schiöth HB. Methylation levels of SLC23A2 and NCOR2 genes correlate with spinal muscular atrophy severity. (*PLoS One. 2015 Mar 30;10(3):e0121964*)
17. Krishnan A, Mustafa A, Almén MS, Fredriksson R, Williams MJ, Schiöth HB. Evolutionary hierarchy of vertebrate-like heterotrimeric G protein families. (*Mol Phylogenet Evol. 2015 Oct;91:27-40*)
18. Williams MJ, Eriksson A, Shaik M, Voisin S, Yamskova O, Paulsson J, Thombare K, Fredriksson R, Schiöth HB. The Obesity-Linked Gene Nudt3 Drosophila Homolog Aps Is Associated With Insulin Signaling. (*Mol Endocrinol. 2015 Sep;29(9):1303-19*)
19. Caruso V, Sreedharan S, Carlini VP, Jacobsson JA, Haitina T, Hammer J, Stephansson O, Crona F, Sommer WH, Risérus U, Lannfelt L, Marcus C, Heilig M, de Barioglio SR, Fredriksson R, Schiöth HB. mRNA GPR162 changes are associated with decreased food intake in rat, and its human genetic variants with impairments in glucose homeostasis in two Swedish cohorts. (*Gene* 2016 *Jan 29. pii: S0378-1119(16)00106-2*).
20. Rask-Andersen M, Sällman Almén M, Jacobsson JA, Ameur A, Moschonis G, Dedoussis G, Marcus C, Gyllensten U, Fredriksson R, Schiöth HB. Determination of obesity associated gene variants related to TMEM18 through ultra-deep targeted re-sequencing in a case-control cohort for pediatric obesity (*Genet Res (Camb). 2015 Sep 14;97:e16)*.
21. Williams JM, Klockars K, Eriksson A, Voisin S, Wiemerslage L, Dnyansagar R, Kasagiannis A, Ambrosi A, Hallqvist E, Fredriksson R, Schiöth HB. The *Drosophila ETV5* homologue *Ets96B*: Molecular link between obesity and bipolar disorder. (*PLoS Genet. 2016 Jun 9;12(6):e1006104*)
22. Wiemerslage L, Gohel PA, Hilmarsson TG, Mickael M, Fredriksson R, Williams JM, Schiöth HB. The Drosophila ortholog of TMEM18 Regulates Insulin and Glucagon-like Signaling. (*J Endocrinol. 2016 Jun;229(3):233-43*).
23. Herisson FM, Waas JR, Fredriksson R, Schiöth HB, Levine AS, Olszewski PK.Oxytocin Acting in the Nucleus Accumbens Core Decreases Food Intake. (*J Neuroendocrinol. 2016 Apr;28(4)*)
24. Perland E, Lekholm E, Eriksson MM, Bagchi S, Arapi V, and Fredriksson R. The putative SLC transporters Mfsd5 and Mfsd11 are abundantly expressed in the mouse brain and havepotential role in energy homeostasis. (*PLoS One. 2016 Jun 7;11(6):e0156912*)
25. Roshanbin S, Lindberg FA, Lekholm E, Eriksson MM, Perland E, Åhlund J, Raine A, Fredriksson R. Histological characterization of orphan transporter MCT14 (SLC16A14) shows abundant expression in mouse CNS and kidney. (*BMC Neurosci. 2016 Jul 1;17(1):43*)
26. Lekholm E, Perland E, Eriksson MM, Hellsten SV, Lindberg FA, Rostami J, Fredriksson R. Putative membrane-bound transporters MFSD14A and MFSD14B are neuronal and affected by nutrient availability. (*Front Mol Neurosci. 2017 Jan 25;10:11*)
27. Perland E, Hellsten SV, Lekholm E, Eriksson MM, Arapi V, Fredriksson R. The novel membrane-bound proteins MFSD1 and MFSD3 are putative SLC transporters affected by altered nutrient intake. (*J Mol Neurosci. 2017 Feb;61(2):199-214*)
28. Syk M, Ramklint M, Fredriksson R, Ekselius L, Cunningham JL. Elevated Total Plasma-Adiponectin is Stable Over Time in Young Women with Bulimia Nervosa. (*Eur Psychiatry. 2016 Dec 29;41:30-36*)

Reviews and book chapters 2014-2016

1. Caruso V, Lagerström MC, Olszewski PK, Fredriksson R, Schiöth HB. Synaptic changes induced by melanocortin signaling: role in memory, nociception and mood disorders. (*Nat Rev Neurosci. 2014 Jan 20;15(2):98-110*).
2. *In-situ* proximity ligation assay. Bagchi S, Fredriksson R#, Wallén-Mackenzie Å#. (*Methods Mol Biol. 2015;1318:149-59*). #Equal contribution
3. International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G Protein–Coupled Receptors. Hamann J, Aust G, Araç D, Engel FB, Formstone C, Fredriksson R, Hall RA, Harty BL, Kirchhoff C, Knapp B, Krishnan A, Liebscher I, Lin H-H, Martinelli DC, Monk KR, Peeters MC, Piao X, Prömel S, Schöneberg T, Schwartz TW, Singer K, Stacey M, Ushkaryov YA, Vallon M, Wolfrum U, Wright MW, Xu L, Langenhan T, Schiöth HB (*Pharmacol Rev. 2015 Apr;67(2):338-67*)

Agencies that supported the work/Funding 2016

The Swedish Research Council (VR-Medicine), The Swedish Research Council (VR-Natural and engineering sciences), The Swedish Brain Foundation, The Novo Nordisk Foundation, The Swedish Society for Medical Research, Olle Engkvist Byggmästare Foundation, Åhlens Foundation, Gunvor and Josef Anérs Foundation, Magnus Bergvalls Foundation and Fredrik och Ingrid Thurings Foundation

Projects

(I) The role of Sodium Coupled Neutral Amino Acid Transporters in regulating the Glutamine/Glutamate/GABA cycle.

Glutamate and GABA are the two most widespread neurotransmitters in the nervous system, and there are several intricate systems regulating their concentration levels. Many diseases, such as anxiety, epilepsy and substance dependency are currently treated with drugs targeting components of these systems. The glutamate/glutamine/GABA cycle is fundamental for regulating levels of glutamate and GABA in the brain. This process has probably evolved as means to fine tune and strictly control the strength of synaptic signalling at glutamatergic synapses, to avoid the neurotoxic effects, known as excitotoxicity, of glutamate. This cycle is known to be controlled by various transporters from the solute carrier superfamily number 38 (SLC38), called SNATs, although the exact SNATs responsible for the various parts of this cycle is unknown. Possibly, this differs between different types of neurons and other cells of the brain. We are using transgenic mice and gene knockdown in fruit fly (*Drosophila melanogaster*) to elucidate which SNATs are important in which cells of the brain. There are currently 11 known SNATs and all are to certain extents expressed in the brain. By knocking down individual SNATs and analys intracellular concentrations of amino acids and neurotransmitters using metabolomics, we can identify which intracellular levels are controlled by which transporter in a certain type of cells. The metabolomics are done in collaboration with the metabolomics platform at SciLife labs and with the group of Professor Curt Petterson at department of pharmaceutical chemistry at Uppsala University. We are also performing behavioural analysis in both mice and fly to elucidate the behavioural consequences of removal of a certain transporter.

(II) Understanding the role of a novel transporter as a sensor of amino acid availability

In our investigation of SNATs in the nervous system (See project I) we identified one member of this family with exclusively intracellular expression. We have found that it regulates protein synthesis and metabolic rate in neurons (and possibly other cells) through the mTORC pathway. We have transgenic mice as well as transgenic flies with this SNAT removed and we are currently investigating the physiological consequences of this gene loss. We are also growing primary neuronal cultures from the transgenic mice and investigating how they respond to amino acid starvation and other stress conditions and compare this to cells from wild type mice. We are also using proteomics and transcriptomics analysis, in collaboration with SciLife labs in Uppsala, to investigate in which functional networks the SNAT plays role.

(III) Understanding the role of a novel nutrient sensor in neuro degeneration

Impaired energy metabolism in neurons is a fundamental factor in many neurodegenerative diseases. This impaired metabolism causes, among other things, oxidative stress and excitotoxicity in neurons, accelerating the neuronal cell death and disease progression. We are investigating a novel sensor for energy status (See project (II)) in several cellular models for neurodegeneration, to test the hypothesis that this SNAT is important for the response to nutrient stress. We do this in primary neuronal cultures from mice lacking this SNAT as well in neuronal cell-lines where the SNAT of interest have been knocked down using siRNA or the CRISPR/CAS9 method.

(IV) Physiological characterization of a novel putative sugar transporter in the kidney: a possible regulator of water balance

In a phenotypic screen in fruit fly (*Drosophila melanogaster*) in lines with orphan SLCs knocked out, we identified a putative transporter that resulted in a sever oedema phenotype. We have subsequently found out that this putative transporter is expressed in the kidney in flies as well as in mice and have a role in osmoregulation. We are currently expressing this transporter in oocytes from the frog *Xenopus laevis* to identify the substrate(s) this transporter is able to transport. Our current hypothesis is that it transports carbohydrate molecules such as some or several forms of sugar. This would be novel system regulating sugar and water balance in the body and could potentially be important as drug target, to control water as well as sugar levels in the body.

(V) A global approach to understand the functional interplay between transporters in the brain

Our preliminary analysis has shown that out of the 430 transporters from the SLC family, over 75% are expressed in the adult brain. Many of these lacks known substrate of transport and there is a considerably overlap between the substrate profiles between those with known substrate, meaning that each biomolecule can be transported by many different transporters. This redundancy makes functional studies difficult. Although there are over 320 SLCs known to be expressed in the brain, it is likely so that each individual cell only expresses a subset of these transporters and our hypothesis is that there exist unique fingerprints of transporters expressed on the different cell types in the brain. If this is true it makes it impossible to fully understand the transporter capabilities of cell, without knowing this fingerprint. We will use single cell RNA sequencing in collaborating with the next generation sequencing platform at SciLife labs in Uppsala. We will sequence the RNA from several thousand of random cells from the mouse brain, and by using advanced clustering approaches and machine learning (in collaboration with Ola Spjuth at the department) identify groups of cells with similar pattern of expression regarding SLCs. We will subsequently identify expression of markers for neuronal cell types as well as from non-neuronal cells to identify the phenotype(s) of the cells within each cluster. This would provide the first map of the combination of SLCs expressed in a given type of cell in the brain.

Biochemical Pharmacology

Ernst Oliw

Arachidonic acid and other polyunsaturated fatty acids are bioactivated in humans, plants and fungi to local hormones and other signal molecules. Prostaglandins contribute to fever, pain, inflammation and cancer development, and regulate physiological processes. Common drugs such as aspirin, acetaminophen (paracetamol, APAP) and ibuprofen reduce symptoms of disease by inhibition of the biosynthesis of prostaglandins. Jasmonates are chemical analogues to prostaglandins and regulate important functions in plants and fungi.

The goal of our research is to investigate the mechanism of oxygenation and bioactivation of fatty acids in eukaryotic cells and to determine their biological function of these metabolites. We investigate mainly two groups of oxygenating enzymes: (i) lipoxygenases and (ii) DOX-CYP fusion proteins with dioxygenase (DOX) and cytochrome P450 (CYP) domains.

(i)) Lipoxygenases: Lipoxygenases contain iron as the catalytic metal in human, plant and some fungal enzymes. We focus our basic research on the first described manganese-lipoxygenases. They are secreted by the Take-all fungus (*Gäumannomyces graminis*) of wheat and the Rice blast fungus (Magnaporthe oryzae). We have crystallized the enzymes and determined their 3D structures. We now investigate the mechanism of biosynthesis of jasmonates in F. oxysporum f sp tulipae.

(ii) DOX-CYP fusion proteins: The DOX-CYP family consists of at least six subfamilies. LDS oxidize oleic and linoleic acids to a series of vicinal diols (e.g., 5,8-dihydroxy-, 7,8-dihydroxyoctadecadienoic acids) and hydroperoxides (e.g., 8-hydroperoxy- and 10-hydroperoxylinoleic acidd), which likely function as sporulation hormones. Recent discoveries are the 8*R*-, 8*S*-,9R- and 9S-dioygenase and allene oxide synthase of human and plants pathogens causing Valley fever and Septoria tritici blotch.

Members of the group during 2016

Ernst H. Oliw, MD, PhD, Senior professor
Yang Chen, scientist
Fredrik Jerneren, scientist

Publications 2014 – 2016

1. Wennman, A., Oliw, E. H., and Karkehabadi, S. (2014) Crystallization and a preliminary crystallographic analysis of manganese-lipoxygenase. Acta Crystallogr. F70*,*522-525.
2. Wennman, A., Karkehabadi, S., and Oliw, E. H. (2014) Kinetic investigation of the rate-limiting step of manganese- and iron-lipoxygenases. Arch. Biochem. Biophys. 555-556, 9-15
3. Oliw, E. H., and Wennman, A. (2014) Chiral phase-HPLC separation of hydroperoxyoctadecenoic acids and their biosynthesis by fatty acid dioxygenases. Methods Mol. Biol. 1208, 85-95
4. Oliw, E. H., and Wennman, A. (2015) Chiral phase-HPLC separation of hydroperoxyoctadecenoic acids and their biosynthesis by fatty acid dioxygenases. *Methods Mol. Biol.* 1208, 85-95
5. Sooman, L., and Oliw, E. H. (2015) Discovery of a novel linoleate dioxygenase of Fusarium oxysporum and linoleate diol dynthase of Colletotrichum graminicola. *Lipids* 50, 1243-125
6. Wennman, A., Jernerén, F., Magnuson, A., and Oliw, E. H. (2015) Expression and characterization of manganese lipoxygenase of the rice blast fungus reveals prominent sequential lipoxygenation of alpha-linolenic acid. *Arch. Biochem. Biophys.* 583, 87-95
7. Wennman, A., Magnuson, A., Hamberg, M., and Oliw, E. H. (2015) Manganese lipoxygenase of Fusarium oxysporum and the structural basis for biosynthesis of distinct 11-hydroperoxy stereoisomers. *J. Lipid Res.* ***56****, 1606-1615*
8. Sooman, L., Wennman, A., Hamberg, M., Hoffmann, I., and Oliw, E. H. (2016) Replacement of two amino acids of 9R-dioxygenase-allene oxide synthase of Aspergillus niger inverts the chirality of the hydroperoxide and the allene oxide. *Biochim. Biophys. Acta* **1861**, 108-118
9. Alberti, J. C., Mariani, M., de Caraffa, V. B. B., Gambotti, C., Oliw, E. H., Berti, L., and Maury, J. (2016) A functional role identified for conserved charged residues at the active site entrance of lipoxygenase with double specificity. *J Mol Catal B-Enzym* **123**, 167-173
10. Wennman, A., Oliw, E. H., Karkehabadi, S., and Chen, Y. (2016) Crystal structure of manganese lipoxygenase of the rice blast fungus Magnaporthe oryzae. *J. Biol. Chem. e-pub 2016 Jan 18* **291**, 8130-8139
11. Oliw, E. H., Arago, M., Chen, Y., and Jerneren, F. (2016) A new class of fatty acid allene oxide formed by the DOX-P450 fusion proteins of human and plant pathogenic fungi, C. immitis and Z. tritici. *J. Lipid Res.* **57**, 1518-1528
12. Chen, Y., Wennman, A., Karkehabadi, S., Engstrom, A., and Oliw, E. H. (2016) Crystal structure of linoleate 13R-manganese lipoxygenase in complex with an adhesion protein. *J Lipid Res* **57**, 1574-1588

Agencies that supported the work/Funding 2016

The Swedish Medical Research Council (03X-06523) and The Knut and Alice Wallenberg Foundation (KAW 2004.123).

Projects

(I) *Characterization of heme-containing fatty acid dioxygenases and P450 fusion enzymes of human and plant pathogens (Yang Chen, Fredrik Jernerén, Ernst Oliw)*

Fungi are severe pathogens of man and can be devastating for important crops. Aspergillus causes farmer´s lung disease and invasive aspergillosis of immunocompromized patients. Rice blast disease is caused by *Magnaporthe oryzae*, and destroys ~25% of rice crops worldwide. Aspergillus and *M. oryzae* contain cyclooxygenase-related enzymes, diol synthases and dioxygenases, which transform linoleic acid into hydroperoxides and dihydroxy fatty acids. Our aim is to characterize the enzymes by enzyme expression, gene targeting and by studies on their biological importance.

(i) Characterization of 8R- and 8S-dioxygenases linked to allene oxide synthases of the human pathogen *Coccidiodes immitis* and the plant pathogen *Zymospetoria tritici*. We are now investigating the reaction mechanisms and the mechanisms of biosynthesis of allene oxides.

(ii) Charaterization of all unique DOX-CYP fusions enzymes of the 10 top pathogens in molecular biology. Three have already been characterized and we now focus on Z. tritici and the mechanism of biosynthesis of jasmonic acid.

(iii) EPR analysis of the protein radicals of CYP-DOX fusion enzymes. The Tyr residues of recombinant dioxygenases with a catalytic Tyr radical will be labeled with deuterium to conclusively identify that a Tyr radical is formed during catalysis. Purification of 9S-dioxygernase-allene oxide synthase for crystallization.

(II) *Characterization of the 3D structures of manganese-lipoxygenases with substrate (Yang Chen and Ernst Oliw)*

Our aim is to study the reaction mechanisms of lipoxygenases by determination of the the 3D structure with linoleic acid in the active site. We will focus on the novel lipoxygenases of *M. oryazae.*
The latter forms crystals in a reproducible way.

(III) Mechanism of biosynthesis of jasmonates by Fusarium oxysporum f sp tulipae in collaboration with prof Mats Hamberg, Karolinska instutitet.

Our aim is to identify the key steps in oxidation and transformatio of linolenic acid to jasmonates.

Research area: Neuropharmacology and Biological Research on Addiction

Ingrid Nylander

The research area Neuropharmacology and Biological Research on Addiction have three different research groups focusing on the role of basic neurology, and physiological and pathophysiologial mechanisms leading to addiction and neurodegenerative diseases. The research area is divided into the three following subgroups:

1. Neuropharmacology, Addiction and Behavior
2. Biological Research on Drug Dependence
3. Molecular Neuropsychopharmacology

Neuropharmacology, Addiction and Behaviour

Ingrid Nylander, Lena Bergström, Erika Roman, Anne-Lie Svensson

http://farmbio.uu.se/research/researchgroups/nab

In general, the research projects are devoted to studies on basic neurobiology, experimental behavioural neuroscience, physiological and pathophysiological mechanisms in the brain, and neuropharmacology of relevance for substance use disorders, especially alcohol use disorder, gambling disorder and neurodegenerative diseases. Of special interest in the research group is to combine neurobiological and behavioural evaluation to investigate how the association between neurobiology, behavioural characteristics, such as risk-taking behaviour, and drug consumption patterns can determine individual high or low risk to develop addiction. A number of behavioural models within the field of neuroscience and neuropharmacology are employed including tests for assessment of neonatal development, exploratory behaviour, locomotor activity, risk taking behaviour, self-administration, learning and memory and the multivariate concentric square field™ (MCSF) test in combination with multivariate data analysis approaches. Neurobiological assessment includes methods to analyse effects on receptors, transmitters and mRNA in tissue samples and brain slices but also analysis of transmitter release and re-uptake patterns using *in vivo* chronoamperometric analysis using Fast Analytical Sensing Technology (FAST). *In vitro* work is used to examine detailed cellular mechanisms. Specific research activities within the group are described shortly under projects.

The experimental behavioural neuroscience is led by Dr Erika Roman. The current line of research is threefold; 1) validation and use of the multivariate concentric square field™ (MCSF) test; a technique with an ethoexperimental approach that in one and the same test situation includes a variety of physical situations meaning risk, safety, exploratory incentives etc. that permit measurements of general activity, exploration, approach/avoidance in open versus sheltered areas and the motivation for seeking reinforcers. The purpose of the multivariate design is to illustrate a personality profile including how this profile is altered under various forms of challenges. The MCSF test is used for behavioural profiling of rats, mice and zebrafish. Studies on zebrafish are performed in collaboration with Dr S Winberg (Department of Neuroscience), 2) studies of the neurobiological basis for individual differences in risk-related behaviours and the association between voluntary alcohol intake, addiction processes and response to treatment, and 3) studies of individual differences in emotional and risk-related behaviors, and associations with impulsivity and gambling behavior, and underlying neurobiological correlates, including studies of the endogenous opioid system and brain activity using resting state fMRI.

The neurobiology and neuropharmacology projects are led by Dr Ingrid Nylander and Dr Anne-Lie Svensson, respectively. Current projects include studies of individual differences in

vulnerability for risk consumption of alcohol or alcohol use disorder and in response to pharmacotherapy. Alcohol addiction is a complex trait and the phenotype related to vulnerability for addiction is based on the interaction of multiple genes and environmental factors. Of particular interest for us is the impact of early environmental factors, such as rearing environment and the consequences of adolescent drug intake, and the association between behavioural characteristics, such as risk-taking behaviour, and later drug consumption. It is hypothesized that disruption of early developmental processes in transmitter networks either by rearing factors or drug intake early in life, causes long-term changes in brain function and behaviour that, in turn, affects alcohol consumption later in life. In the projects, experimental models are used in combination with extensive evaluation of neurobiological and behavioural consequences. We collaborate with Drs E Comasco, L Oreland (Department of Neuroscience) and K Nilsson (Centre for Clinical Research, Västerås) in projects that include investigation of how epigenetic processes are involved in long-term consequences of exposure to various early-life environmental factors.

Another line of research investigates the role of cannabinoids and neurosteroids and endocannabinoids for neurogenesis and for interactive processes that are ongoing in neurodegenerative disorders, like Alzheimer's disease (AD). Since AD is associated with excitotoxicity, oxidative stress and neuroinflammation, the research line emphasis on neuroprotective properties of neurosteroids and endocannabinoids against different toxic insults in *in vitro* cell models.

Members of the group during 2016

Ingrid Nylander, Professor
Lena Bergström, Associate Professor, Senior Lecturer
Erika Roman, Associate Professor, Senior Lecturer
Anne-Lie Svensson, Senior Lecturer
Viktoria Lind, Junior Lecturer
Lova Segerström, Researcher
Sarah Holst, Researcher
Linnea Granholm, PhD student
Stina Lundberg, PhD student
Shima Momeni, Researcher
Marita Berg, Technician
Matilda Persson, Research assistant
Nikita Tjernström, Research assistant

Publications 2014 - 2016

1. Momeni S, Sharif M, Ågren G, Roman E. Individual differences in risk-related behaviors and voluntary alcohol intake in outbred Wistar rats. Behavioural Pharmacology (2014) 25 206-215
2. Palm S, Momeni S, Lundberg S, Nylander I, Roman E. Risk-assessment and risk-taking behavior predict potassium- and amphetamine-induced dopamine response in the dorsal striatum of rats. Frontiers in Behavioral Neuroscience (2014) 8 236
3. Momeni S, Roman E. Subgroup-dependent effects of voluntary alcohol intake on behavioral profiles in outbred Wistar rats. Behavioural Brain Research (2014) 275 288-296
4. Palm S and Nylander I. Dopamine release dynamics change during adolescence and after voluntary alcohol intake. Plos One (2014) 9 (5): e96337
5. Granholm L, Roman E, Nylander I. Single housing during early adolescence causes time-, area- and peptide-specific alterations in endogenous opioids of rat brain. Br J Pharmacol (2015) 172 606-614
6. Karlsson O, Colombo G, Roman E. Low copulatory activity in selectively bred Sardinian alcohol-nonpreferring (sNP) relative to alcohol-preferring (sP) rats. Ups J Med Sci (2015) 120 181-189
7. Magara S, Holst S, Lundberg S, Roman E, Lindskog M. Altered explorative strategies and reactive coping style in the FSL rat model of depression. Front Behav Neurosci (2015) 9 89

8. Momeni S, Segerström L, Roman E. Supplier-dependent differences in intermittent voluntary alcohol intake and response to naltrexone in Wistar rats. Front Neurosci (2015) 9 424
9. Palm S and Nylander I. Alcohol-induced changes in opioid peptide levels in adolescent rats are dependent on housing conditions. Alcohol Clin Exp Res (2015) 38 (12) 2978-2987
10. Granholm L, Rowley S, Ellgren M, Segerström L, Nylander I. Impact of adolescent ethanol exposure and adult amphetamine self-administration on evoked striatal dopamine release in male rats. Psychopharmacology (2015) 232 4421-4431
11. Comasco E, Todkar A, Granholm L, Nilsson KW, Nylander I. Alpha 2a-Adrenoceptor Gene Expression and Early Life Stress-Mediated Propensity to Alcohol Drinking in Outbred Rats. Int. J. Environ. Res. Public Health (2015) 12 7154-7171
12. Engel JA, Nylander I, Jerlhag E. A ghrelin receptor (GHS-R1A) antagonist attenuates the rewarding properties of morphine and increases opioid peptide levels in reward areas in mice. European Neuropsychopharmacology (2015) 25:2364-2371
13. Vrettou M, Granholm L, Todkar A, Nilsson KW, Wallén-Mackenzie Å, Nylander I, Comasco E. Ethanol affects limbic and striatal presynaptic glutamatergic and DNA methylation gene expression in outbred rats exposed to early-life stress (2015) Addiction Biol doi:10.1111/adb.12331
14. Bendre M, Comasco E, Nylander I, Nilsson KW, Effect of voluntary alcohol consumption on *Maoa* expression in the mesocorticolimbic brain of adult male rats previously exposed to prolonged maternal separation. Transl Psychiatry (2015) 5, e690; doi:10.1038/tp.2015.186
15. Segerström L, Gustavsson J, Nylander I. Minimizing Postsampling Degradation of Peptides by a Thermal Benchtop Tissue Stabilization Method. Biopreservation and Biobanking (2016) 14(2):172-179
16. Karlsson O, Segerström L, Sjöback R, Nylander I, Borén M. qPCR based mRNA quality score show intact mRNA after heat stabilization. Biomolecular Detection Quantification (2016) 7:21-26
17. Todkar A, Granholm L, Aljumah M, Nilsson KW, Comasco E, Nylander I. HPA axis gene expression and DNA methylation profiles in rats exposed to early life stress, adult volntary ethanol drinking and single housing. Frontiers Mol Neurosci (2016) doi: 10.3389/fnmol.2015.00090
18. Karlsson O, Roman E. Dose-dependent effects of alcohol administration on behavioural profiles in the MCSF test. Alcohol (2016), 50, 51-56
19. Segerström L, Roman E. Response: Commentary: Supplier-dependent differences in intermittent voluntary alcohol intake and response to naltrexone in Wistar rats. Frontiers in Neuroscience (2016), 10, 442
20. Lundberg S, Martinsson M, Nylander I, Roman E. Altered corticosterone levels and social play behavior after prolonged maternal separation in adolescent male but not female Wistar rats. Hormones and Behavior (2017), 87, 137-144

Reviews 2014 – 2016

1. Lundberg S, Martinsson M, Nylander I, Roman E. Altered corticosterone levels and social play behavior after prolonged maternal separation in adolescent male but not female Wistar rats. Hormones and Behavior (2017), 87, 137-144
2. Wood CM, Nicolas CS, Choi SL, Roman E, Nylander I, Fernandez-Teruel A, Colombo G, Chstagnier D, Wafford KA, Collingridge GL, Wildt SJ, Conway-Campbell BL, Robinson ESJ, Loge D. Prevalence and influence of cys407* Grm2 mutation in Hannover-derived Wistar rats: mGu2 receptor loss links to alcohol intake, risk taking and emotional behaviour. Neropharmacology 115 (2017) 128-138.

National publications (Text books) 2014 – 2016

1. Franck J & Nylander I (Eds.) Beroendemedicin (2nd ed.), Studentlitteratur, 2015
2. Nylander I. Beroendemekanismer. In Beroendemedicin, 2nd ed. (Franck & Nylander, Eds.), Studentlitteratur, 2015
3. Roman E. Djurexperimentell metodik. In Beroendemedicin, 2nd ed. (Franck & Nylander, Eds.), Studentlitteratur, 2015

4. Svensson A, Nikotin. In Beroendemedicin, 2nd ed. (Franck & Nylander, Eds.), Studentlitteratur, 2015

Dissertations 2014 – 2016

1. Sara Palm *Early environment, adolescent alcohol drinking and neurobiological responses to drugs*, 2014. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 190. ISSN 1651-6192.
2. Shima Momeni *Profiling of risk-related behaviors in relation to voluntary alcohol intake and neurobiology,* 2014. Faculty of Pharmacy, Uppsala University Licentiate Theses, 47.
3. Shima Momeni *Individual differences in behavior, neurochemistry and pharmacology associated with voluntary alcohol intake*, 2015. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 205. ISSN 1651-6192.
4. Linnea Granholm *Neurobiological Consequences of Social Conditions and Alcohol Exposure in Adolescent rats*, 2015. Faculty of Pharmacy, Uppsala University Licentiate Theses, 48.

Agencies that supported the work/Funding 2016

The Swedish Research Council (Nylander); The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly (two separate projects; Nylander and Roman); The Facias Foundation (Roman); Svenska Spels Research Council (Roman), Swedish Brain Foundation (Roman), Gahlins Foundation (Roman).

Other commitments/assignments of group members 2016

Lena Bergström: Member of the Academic Senate, Uppsala University

Ingrid Nylander: Grant committee member Alcohol Research Council of the Swedish Alcohol Retailing Monopoly; Member of the Faculty of Pharmacy committee; Vice chairman, the recruitment committee Faculty of Pharmacy; Chairman, the committee for undergraduate education Faculty of Pharmacy; Vice Dean and board member, Disciplinary Domain of Medicine and Pharmacy

Erika Roman: International Adjunct Associate, Department of Psychology, Indiana University Purdue University at Indianapolis, USA; Review Editor: Frontiers in Addictive Disorders and Behavioural Dyscontrol; Board member, The Society for Swedish Alcohol and Drug Research; Board member, Uppsala University Laboratory Animal Resources; Member of the Animal Welfare Body, Uppsala University; Member of the Research Training Committee (KUF), Uppsala University; Approved Supervisor by the Swedish Board of Agriculture; Member of the expert panel, Swedish Centre for Animal Welfare; Member of the assembly of electors, Uppsala University; One of three coordinators of Uppsala University Behavioural Facility (UUBF), Uppsala University

Lova Segerström: Animal facility warden; Member of the gender equality committee

Anne-Lie Svensson: Member of the committee for undergraduate education (GRUFF) at the Faculty of Pharmacy, Uppsala University; Chairman, the gender equality committee at the Disciplinary Domain of Medicine and Pharmacy, Uppsala University; Member of the assembly of electors, Uppsala University; Member of the Academic Senate, Uppsala University.

Projects

(I) The impact of early life environment on the brain and the stress axis (Linnea Granholm, Lova Segerström, ***Ingrid Nylander****)*

Early life adversity: We have previously shown that adverse experiences early in life cause long-term neurobiological and behavioural alterations. Currently, we study the mechanisms underlying long-term consequences of early life stress. A rodent maternal separation (MS) model is used to simulate rearing

conditions that are associated with resilience (after short MS) or vulnerability (after longer MS) in terms of adult risk consumption. We investigate transmitter networks relevant for reward and reinforcement, for example, endogenous opioid, glutamate and monoamine circuits. In collaboration with Dr E Comasco (Nylander co-supervisor to PhD students Megha Bendre and Maria Vrettou in these studies), we assess the effects of early-life stress on expression and methylation of genes involved in regulation of stress and reward.
Adolescent drug intake: We study the long-term effects of adolescent drug exposure on opioid networks and on drug consumption in adulthood. The impact of social deprivation in single housing drinking paradigms has been evaluated. Alcohol-induced effects are evaluated both after voluntary drinking during adolescence and with administration of a given dose alcohol. Currently we examine the effects of adolescent alcohol and also the combined alcohol and nicotine exposure on opioid networks.

(II) In vivo and in vitro studies of drug-induced effects in the brain (Anne-Lie Svensson, ***Ingrid Nylander****)*

Effects of drug exposure or voluntary alcohol drinking on for example dopamine are examined *in vivo* by chronoamperometry with Fast Analytical Sensing Technology (FAST). FAST offers unique advantages as compared to *in vivo* microdialysis: high temporal resolution and close spatial orientation enables a second-by-second analysis instead of several minutes, high sensitivity allows measurement of resting levels, high specificity and the microelectrodes cause minimal brain damage. Of special interest is the dorsal striatum, a brain area involved in transition from controlled to compulsive drug taking behaviour. It is also used to correlate *in vivo* dopamine dynamics with behaviour, e.g. risk taking or risk assessment. In vitro studies focus on cellular mechanisms and effects of nicotine and alcohol exposure.

*(III) Method development (Lova Segerström****, Ingrid Nylander****)*

Post-mortem metabolism is a major concern in the analysis of opioid peptides in biological samples and we have established a a reliable, reproducible and easy to use procedure for rapid and efficient enzyme inactivation in tissue samples using the bench top Stabilizor T1 instrument (Denator AB, Gothenburg, Sweden) for heat stabilization of brain tissue. We have optimized the measurements of opioid peptides in the brain to achieve detection of peptide levels more similar to the *in vivo* concentration.

*IV) Neurosteroids and Alzheimer's disease: Mechanistic studies of neuroprotection and amyloid-*β*-modulation (****Anne-Lie Svensson****)*

Neurosteroids are endogenous modulators of neuronal functions responsible for many biological and pathophysiological effects. Plausible links between neurosteroids and neurodegenerative disorders, like Alzheimer´s disease (AD), are suggested. Accumulation of amyloid-β induced by toxic events in cells might be able to reduce the synthesis of neuroprotective neurosteroids, thus favour/support neurodegenerative processes. In ongoing studies the neuroprotective properties of neurosteroids and their metabolites, against amyloid-β-induced toxicity are investigated. Since neurosteroids most likely affects neuronal and glial cells differently, their effects on amyloid-β-induced toxicity are also investigated in numerous cell types.

(V) Behavioural profiling of animals exposed to early environmental stress and adolescent alcohol consumption (Linnea Granholm, Stina Lundberg, ***Erika Roman, Ingrid Nylander****)*

Current experiments analyse the short- and long-term behavioural consequences of rearing in different environmental settings and of long-term alcohol consumption. The project comprises development of animal experimental models to assess maternal behaviour, interactions between the dam and offspring and play behaviour. In addition, the MCSF test is employed to examine behavioural profiles after different rearing conditions and before and after adolescent alcohol consumption. Individual behavioural profiling in animals subjected to various rearing conditions and alcohol early in life in controlled experimental groups enables distinction between rearing-induced and drug-induced consequences for behaviour later in life. It is also examined whether and how altered behaviour relate to vulnerability for drug addiction.

*(VI) Development and validation of the MCSF test (Stina Lundberg, Matilda Persson, Nikita Tjernström, Svante Winberg****, Erika Roman)***

The multivariate concentric square field™ (MCSF) test is a technique with an ethoexperimental approach that in one and the same test situation includes a variety of physical situations meaning risk, safety, exploratory incentives etc. that permit measurements of general activity, exploration, approach/avoidance in open versus sheltered areas and the motivation for seeking reinforcers. The purpose of the multivariate

design is to gather information that taken together should illustrate a personality profile including how this profile is altered under various forms of challenges. The MCSF test is under continuous development. On-going work aims to i) validate the test arena set up for zebrafish, ii) study the impact of pharmacological substances on behavioural profiles, and iii) develop plug-in units for studies of motivated behaviours in rodents and expanding the use of the MCSF for studies of learning and memory.

*(VII) Behavioural profiling of selectively bred alcohol-preferring and alcohol-avoiding rodent lines (Stina Lundberg, Richard Bell, Nicholas Grahame, Tiebing Liang, Giancarlo Colombo, Petri Hyytiä, **Erika Roman**)*

Genetic aspects of alcohol use disorders have been modelled using rats and mice selectively bred for extremes of alcohol preference and voluntary alcohol intake. Several different lines of rodents have been selectively bred for high and low oral alcohol preference and intake. These lines show similar alcohol drinking phenotypes but have different genetic and environmental backgrounds and may therefore display diverse behavioural traits as seen in human alcohol dependent individuals. This project involves behavioural characterization of selectively bred alcohol preferring and non-preferring rat and mouse lines as well as congenic rat lines using the MCSF test. Moreover, the response to natural reinforcing stimuli is characterized in selectively bred alcohol preferring and non-preferring rat lines. This extensive behavioural characterization enables a deeper understanding of behavioural traits of importance for understanding of alcohol use disorders and related addiction processes.

*(VIII) Ethoexperimental studies of appetitive and consummatory mechanisms related to natural rewarding stimuli and drugs of abuse (Matilda Persson, Bengt J Meyerson (deceased 2015), **Erika Roman**)*

The project aims at exploring basic mechanisms of reinforcing stimuli with special focus on differentiating appetite for seeking reinforcers such as food, sexual activity and drugs of abuse from consummatory behaviours. On-going studies assess the animal's motivation for passing the risk area and reach reinforcement by increasing the resistance of passing. The association between natural rewards, such as sexual activity and food intake, and drugs of abuse, i.e. alcohol, is subject for examination. The hypothesis is that reward motivated behaviours are different in animals with different voluntary intake of drugs of abuse.

*(IX) The role of individual differences in drug-seeking and drug-intake behaviour and associated neurobiological effects of relevance to vulnerability for addiction (Shima Momeni, **Lena Bergström**, **Erika Roman**)*

We use experimental methods to examine the neurobiological basis for individual differences in risk-related behaviours and the association between voluntary alcohol intake, addiction processes and response to treatment. With regard to neurobiology, focus is on the cannabinoid, opioid and dopamine systems. A multivariate behavioural approach with an ethological foundation that incorporates several aspects of the behavioural repertoire and evolutionary conserved behaviours is used. Risk-related behaviours are of importance for liability for excessive alcohol intake and also affect the response to drug treatment. The impact of individual differences in risk-related behaviours on voluntary alcohol intake and CB1 and opioid receptor density are investigated. We also study the effects of alcohol on FAAH and MAGL enzyme activity, i.e. enzymes metabolizing the endogenous cannabinoids anandamide and 2-AG.

*(X) Experimental studies of gambling disorder (Sarah Holst, Kent W Nilsson, **Erika Roman**)*

There is an urgent need for valid animal models for studying the neurobiological and behavioral associations between behavioral traits and decision-making of relevance for gambling disorder. Herein ethoexperimental approaches (the MCSF and novel cage tests) are used for studies of individual differences in emotional and risk-related behaviors, and impulsivity and gambling behavior in combination with studies of the underlying neurobiological correlates and brain activity using resting state fMRI.

Biological Research on Drug Dependence

Mathias Hallberg and Fred Nyberg

http://farmbio.uu.se/research/researchgroups/brdd/biolbero

The first objective of our research is to improve the understanding of brain mechanisms of relevance for the etiology of drug addiction and to develop strategies for relevant treatment. Studies of drug effects on brain circuits involved in reward and dependence are combined with attempts to develop peptidomimetics reducing the expression of opioid withdrawal, an important aspect in the treatment of opioid addiction. Studies are also aimed to find strategies to repair drug-induced brain damages, i.e. using peptidomimetics or growth factors counteracting apoptosis and stimulating neurogenesis. As animal models do not reflect all complexity of addiction current research also involves epigenetic adaptations and regulation of plastic neuropeptide genes in human brain. The second objective of our research is a) to discover new classes of analgesics with potential use in the treatment of neuropathic pain and b) to discover unique cognitive enhancers. These two subprojects are derived from findings in the tachykinin and renin-angiotensin systems.

Members of the group during 2016

Mathias Hallberg, Professor
Fred Nyberg, Senior Professor
Alfhild Grönbladh, PhD
Sofia Zelleroth, PhD student
Erik Nylander, PhD student
Erika Brolin, PhD student
Shanti Diwakarla, Postdoc
Anna Lesniak, Postdoc
Stina Silander, Project administrator
Myron Zaluha, Project manager

Publications 2014 – 2016

1. Righard L, Carlsson-Jonsson A, Nyberg F. (2014) Enhanced levels of immunoreactive β-casomorphin-8 in milk of breastfeeding women with mastitis. Peptides. 51:54-8.
2. Borhade S, Rosenström U, Sävmarker J, Lundbäck T, Jenmalm-Jensen A, Sigmundsson K, Axelsson H, Svensson F, Konda V, Sköld C, Larhed M, Hallberg M. (2014) Inhibition of Insulin-regulated aminopeptidase (IRAP) by arylsulfonamides. Chemistry Open. 3(6):256-63.
3. Guimond M-O, Hallberg M, Gallo-Payet N, Wallinder C. (2014) Saralasin and sarile are AT_2 receptor agonists. Med Chem Lett. 5:1129-1132.
4. Heddini U, Bohm-Starke N, Grönbladh A, Nyberg F, Nilsson KW, Johannesson U. (2014) Serotonin receptor gene (5HT-2A) polymorphism is associated with provoked vestibulodynia and comorbid symptoms of pain. J Sex Med. 11(12):3064-71.
5. Carlsson-Jonsson A, Gao T, Hao JX, Fransson R, Sandström A, Nyberg F, Wiesenfeld-Hallin Z, Xu XJ. (2014) N-terminal truncations of substance P 1-7 amide affect its action on spinal cord injury-induced mechanical allodynia in rats. Eur J Pharmacol. 738:319-25.
6. Rhodin A, von Ehren M, Skottheim B, Grönbladh A, Ortiz-Nieto F, Raininko R, Gordh T, Nyberg F. (2014) Recombinant human growth hormone improves cognitive capacity in a pain patient exposed to chronic opioids. Acta Anaesthesiol Scand. 58(6):759-65.
7. Fransson R, Nordvall G, Bylund J, Jonsson A, Kratz J M, Svensson R, Artursson P, Hallberg M, Sandström A (2014) Exploration and pharmacokinetic profiling of phenylalanine based carbamates as novel substance P 1-7 analogues. ACS Med Chem Lett. 5(12):1272-7.

8. Wallinder C, Sköld C, Botros M, Guimond MO, Hallberg M, Gallo-Payet N, Karlén A, Alterman M. (2014) Interconversion of Functional Activity by Minor Structural Alterations in Nonpeptide AT2 Receptor Ligands. ACS Med Chem Lett. 2014 6(2):178-82.
9. Pettersson FD, Hellgren C, Nyberg F, Åkerud H, Sundström-Poromaa I. (2015) Depressed mood, anxiety, and the use of labor analgesia. Arch Womens Ment Health. 19:11-6.
10. Muresanu DF, Sharma A, Lafuente JV, Patnaik R, Tian ZR, Nyberg F, Sharma HS. (2015) Nanowired Delivery of Growth Hormone Attenuates Pathophysiology of Spinal Cord Injury and Enhances Insulin-Like Growth Factor-1 Concentration in the Plasma and the Spinal Cord. Mol Neurobiol. 52(2):837-45
11. Nehlin C, Nyberg F, Öster C. (2015) The patient's perspective on the link between ADHD and substance use: a qualitative interview study. J Atten Disord. 19(4):343-50.
12. Heddini U, Bohm-Starke N, Grönbladh A, Nyberg F, Nilsson KW, Johannesson U. (2014) Serotonin receptor gene (5HT-2A) polymorphism is associated with provoked vestibulodynia and comorbid symptoms of pain. J Sex Med. 11(12):3064-71. doi: 10.1111/jsm.12685. Epub 2014 Sep 1.
13. Grönbladh A, Hallberg M. (2015) GTPγS-autoradiography for studies of opioid receptor functionality. Methods Mol Biol. 1230:169-76.
14. Jonsson A, Fransson R, Haramaki Y, Skogh A, Brolin E, Watanabe H, Nordvall G, Hallberg M, Sandström A, Nyberg F. (2015) Small constrained SP1-7 analogs bind to a unique site and promote anti-allodynic effects following systemic injection in mice. Neuroscience. 298:112-9.
15. Watanabe H, Fitting S, Hussain MZ, Kononenko O, Iatsyshyna A, Yoshitake T, Kehr J, Alkass K, Druid H, Wadensten H, Andren PE, Nylander I, Wedell DH, Krishtal O, Hauser KF, Nyberg F, Karpyak VM, Yakovleva T, Bakalkin G (2015) Asymmetry of the endogenous opioid system in the human anterior cingulate: a putative molecular basis for lateralization of emotions and pain. Cereb Cortex. 25:97-108.
16. Alho H, Jansen, J., Krajci, P., Littlewood, R., Runarsdottir, V. & Nyberg, F. (2015) Misuse and diversion of agonist opioid treatment medicines: assessment of the scale of the problem and review of the changing environment for care in the Nordic countries. Heroin Addiction and Related Clinical Problems. 17, 5, 43-49.
17. Kalliomaki M-L, Sandblom G, Hallberg M, Grönbladh A, Gunnarsson U, Gordh T, Ginya H, Nyberg F. (2016) Genetic susceptibility to postherniotomy pain. The influence of polymorphisms in the Mu opioid receptor, TNF-alfa, GRIK3, GCH1, BDNF and CACNA2D3 genes. Scandinavian Journal of Pain. 12: 1-6.
18. Diwakarla S, Nylander E, Grönbladh A, Vanga SR, Khan YS, Gutiérrez-de-Terán H, Ng L, Sävmarker J, Lundbäck T, Jenmalm-Jensen A, Andersson H, Engen K, Rosenström U, Larhed M, Åqvist J, Chai SY, Hallberg M. (2016) Binding to and inhibition of insulin-regulated aminopeptidase (IRAP) by macrocyclic disulfides enhance spine density. Molecular Pharmacol. 89: 413-424.
19. Engen K, Rosenström U, Axelsson H, Konda V, Dahllund L, Otrocka M, Sigmundsson K, Nikolaou A, Vauquelin G, Hallberg M, Jenmalm Jensen A, Lundbäck T, Larhed M. (2016) Novel insulin-regulated aminopeptidase inhibitors identified through small molecule screening. Assay Drug Dev Technol. 14: 180-93.
20. Lesniak A, Aarnio M, Jonsson A, Norberg T, Nyberg F, Gordh T. High-throughput screening and radioligand binding studies reveal monoamine oxidase-B as the primary binding target for d-deprenyl. Life Sci. 2016 May 1;152:231-7.
21. Nehlin C, Nyberg F, Jess K. Brief Intervention Within Primary Care for At-Risk Gambling: A Pilot Study. J Gambl Stud. 2016 Dec;32(4):1327-1335.
22. Philippot G, Nyberg F, Gordh T, Fredriksson A, Viberg H. Short-term exposure and long-term consequences of neonatal exposure to Δ(9)-tetrahydrocannabinol (THC) and ibuprofen in mice. Behav Brain Res. 2016 Jul 1;307:137-44.
23. Pettersson FD, Hellgren C, Nyberg F, Åkerud H, Sundström-Poromaa I. Depressed mood, anxiety, and the use of labor analgesia. Arch Womens Ment Health. 2016 Feb;19(1):11-6.
24. Grönbladh A, Nylander E, Hallberg M. (2016) The neurobiology and addiction potential of anabolic androgenic steroids and effects of growth hormone. Brain Res Bull. 126:127-137.

25. Diwakarla S, Nylander E, Grönbladh A, Vanga S, Khan Y, Gutiérrez-de-Terán H, Savmarker J, Ng L, Pham V, Lundbäck T, Jenmalm-Jensen A, Svensson R, Artursson P, Zelleroth S, Engen K, Rosenström U, Larhed M, Aqvist J, Chai S Y, Hallberg M. (2016) Aryl sulfonamide inhibitors of insulin-regulated aminopeptidase enhance spine density in primary hippocampal neuron cultures. ACS Chem Neurosci. 7: 1383-1392.
26. Nylander E, Grönbladh A, Zelleroth S, Diwakarla S, Nyberg F, Hallberg M. (2016) Growth hormone is protective against acute methadone-induced toxicity by modulating the NMDA receptor complex. Neuroscience. 339: 538-547.

Reviews 2014 – 2016

1. Nyberg F. (2014) Structural plasticity of the brain to psychostimulant use. Neuropharmacology. 87:115-24.
2. Pope HG Jr, Wood RI, Rogol A, Nyberg F, Bowers L, Bhasin S. (2014) Adverse health consequences of performance-enhancing drugs: An Endocrine Society scientific statement. Endocr Rev. 35(3):341-75.
3. Hallberg M. (2015) Neuropeptides: Metabolism to Bioactive Fragments and the Pharmacology of Their Receptors. Med Res Rev. 35: 464-519.
4. Nyberg F, Grönbladh A, Enhamre E, Jonsson A, Johansson J, Hallberg M. (2016) The effect of growth hormone on the brain. In: Neuroendocrine Disorders in Children. Eds: Dattani MT, Hindmarsh P, Robinson I, Carr L, Wiley. Chapter 3, 30-41.
5. Larhed M, Hallberg M, Hallberg A. (2016) Nonpeptide AT2R agonists. Med Chem Reviews. 51: 69-82.

Agencies that supported the work/Funding 2016

Swedish Research Council - Medicine and Health; Hjärnfonden; Kjell and Märta Beijers Foundation; Carl Tryggers Foundation; Science for Life Laboratory (SciLifeLab); Swedish Council for Working; Life and Social Research; Precision Science System; Swedish Foundation for Strategic Research; Berzelii Centre for Biotechnological Research; Swedish Institute, Visby Program; Disciplinary Domain of Medicine and Pharmacy; The Alcohol Research Council of the Swedish Alcohol Retailing Monopoly; The Research Council of Swedish Criminal Care.

Other commitments/assignments of group members 2016

Mathias Hallberg: Deputy Chairman of the Board for Education (GRUFF), Faculty of Pharmacy, Member of the Steering Committee/Reference group for the development of the Language Workshop, Uppsala University, Deputy Chairman of the Student Recruitment Committee, Faculty of Pharmacy. Referee: Curr Protein Pept Sci.

Fred Nyberg: Member of the Governmental Advisory Board for Addictive drugs (ANDT-S Advisory Board). Member of the Uppsala University Center for Studies of the Religion in the Society since 2006 and the National Center for Mens Violence against women since 2006. Member of the Board for the Medical Committee of the Swedish Criminal Care. Member of the Board of the Alcohol Research Council of the Swedish Alcohol Retailing Monopoly
Member of the Board of The Research Council of the Swedish Criminal Care.
President of the International Narcotics Research Conference (INRC) from 2014-2017.
Member of Editorial Board of Scientific journals: Peptides, Open J Endocrinology (Editor in Chief), Pharmacology-on-line, J Musc Skel. Pain.
PI at the Uppsala Berzelii Technology Center for Neurodiagnostics; PI at the Linne project Impact of Religion: Challenges for Society, Law and Democracy;

Projects

(I) Studies on neuropeptides, neurohormones and steroids in relation to opioid sensitivity and chronic pain (including animal experimental models, in vitro cell cultures and clinical studies). In clinical studies the project directs to assessment of genetic polymorphism in various transmitter systems combined with

quantification of neuropeptide levels in various body fluids. Peptides chosen for analysis includes the tachykinins and opioid peptides. In animal studies models for nociceptive, neuropathic pain are chosen.

(II) Studies on neuropeptides, neurohormones and steroids (in particular anabolic androgenic steroids = AAS) in relation to drug dependence (including experimental animal models, in vitro cell cultures and clinical studies). In clinical studies the project directs to assessment of genetic polymorphism in various transmitter systems combined with quantification of neuropeptide levels in various body fluids. Peptides chosen for analysis includes the tachykinins and opioid peptides. In animal studies models to investigate opiate tolerance and withdrawal and drug self-administration (in collaboration with other laboratories) are used. Endogenous peptides with high potency to attenuate withdrawal reactions have been identified and serve as basis for design and synthesis of peptides and non-peptides that may be further developed to act as drugs in the treatment of opiate addiction. In studies of effects of AAS on the brain neurochemical technologies (radioimmunoassays, autoradiograhy, Western blot, etc.) are combined with various behavioural assays.

(III) Studies on the functions of growth hormone (GH) and prolactin (PRL) and their receptors in the central nervous system (including experimental animal models, in vitro cell cultures and clinical studies). Receptors for GH have been identified in the brain in areas of relevance for many of the known effects of GH on the central nervous system (CNS). Beneficial effects of GH on cognitive functions are recorded by the assessment of memory and cognition using the Water maze in conjunction with various neurobiological techniques.

(IV) Studies on synthetic compounds acting on the angiotensin II AT2 receptor and the angiotensin IV receptor, insulin-regulated aminopeptidase. Receptor assays animal models are used to guide synthesis and design of peptide and non-peptide analogues. The objective is a) to discover new classes of analgesics with potential use in the treatment of neuropathic pain and b) to discover unique cognitive enhancers. These two subprojects are derived from findings in the tackykinin and renin-angiotensin systems (RAS) that we have studied for a long period. The substance P metabolite substance P (1-7) alleviates pain and drug-like substances mimicking substance P (1-7) have been discovered that powerfully alleviate pain in animal models. In parallel selective drug-like angiotensin II AT2 receptor antagonists are made as potential analgesics.

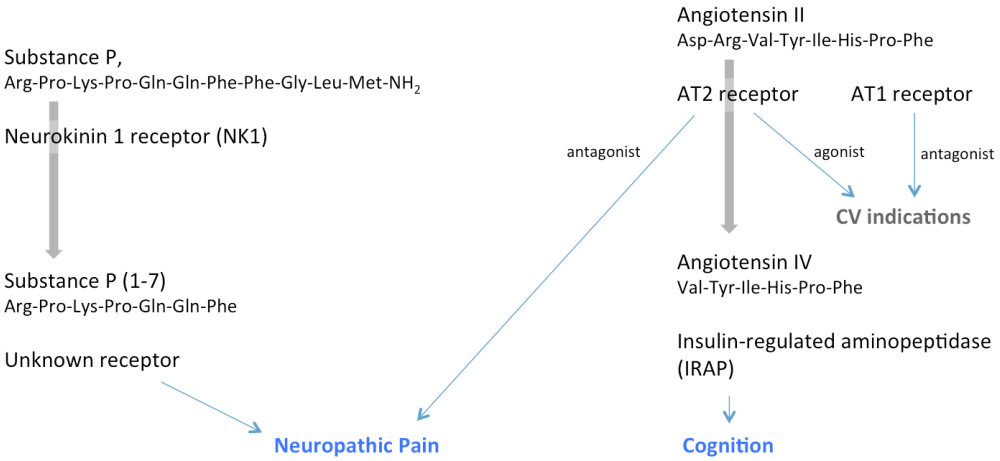

Figure: Degradation of bioactive neuropeptides may lead to fragments with different biological activities as compared to the parent peptide (Hallberg, 2014 Med Res Rev).

Angiotensin IV (angiotensin 3-8), a fragment derived from the hypertensive angiotensin II, improves memory after i.c.v. injection in rats. Potent drug-like compounds that mimic the effects of angiotensin IV and e.g. increase dendritic spine density in primary hippocampal neuronal cultures and that display neuroprotective effects have been identified. The density and morphological architecture of dendritic spines correlate with parameters associated with cognition. These compounds, aimed at serving as potential cognitive enhancers inhibit the proposed molecular target for angiotensin IV, insulin-regulated aminopeptidase (IRAP). At present considerable research efforts are devoted to these drug discovery programs that now involve both national and international collaborations.

Molecular Neuropsychopharmacology

Georgy Bakalkin

http://farmbio.uu.se/research/researchgroups/brdd/molneupsyfarm/

The main goal for the research group on Molecular Neuropsychopharmacology is to increase the understanding of molecular and epigenetic mechanisms underlying the development of alcoholism and substance addiction, and to develop treatment strategies. Identification of biomarkers and new therapeutic targets for the treatment of developmental psychiatric disorders is also in focus. Specific research activities within the group are described under projects.

Members of the group during 2016

Georgy Bakalkin, PhD, Senior professor
Tatiana Yakovleva, PhD, Senior scientist
Hiroyuki Watanabe, PhD, Research scientist
Daniil Sarkisyan, PhD, Research scientist
Lada Stålhandske, PhD, Research scientist
Wei Sun, Ph.D., Postdoctoral scientist
Xingwu Zhou, PhD, Postdoctoral scientist
Igor Bazov, PhD, Postdoctoral scientist
Olga Kononenko, PhD student

Publications 2014 – 2016

1. Smeets CJ, Zmorzyńska J, Melo MN, Stargardt A, Dooley C, Bakalkin G, McLaughlin J, Sinke RJ, Marrink SJ, Reits E, Verbeek DS. Altered secondary structure of Dynorphin A associates with loss of opioid signalling and NMDA-mediated excitotoxicity in SCA23. Hum Mol Genet. 2016 Jul 1;25(13):2728-2737. PubMed PMID: 27260403.
2. Schumann G, Liu C, O'Reilly P, Gao H, Song P, Xu B, Ruggeri B, Amin N, Jia T, Preis S, Segura Lepe M, Akira S, Barbieri C, Baumeister S, Cauchi S, Clarke TK, Enroth S, Fischer K, Hällfors J, Harris SE, Hieber S, Hofer E, Hottenga JJ, Johansson Å, Joshi PK, Kaartinen N, Laitinen J, Lemaitre R, Loukola A, Luan J, Lyytikäinen LP, Mangino M, Manichaikul A, Mbarek H, Milaneschi Y, Moayyeri A, Mukamal K, Nelson C, Nettleton J, Partinen E, Rawal R, Robino A, Rose L, Sala C, Satoh T, Schmidt R, Schraut K, Scott R, Smith AV, Starr JM, Teumer A, Trompet S, Uitterlinden AG, Venturini C, Vergnaud AC, Verweij N, Vitart V, Vuckovic D, Wedenoja J, Yengo L, Yu B, Zhang W, Zhao JH, Boomsma DI, Chambers J, Chasman DI, Daniela T, de Geus E, Deary I, Eriksson JG, Esko T, Eulenburg V, Franco OH, Froguel P, Gieger C, Grabe HJ, Gudnason V, Gyllensten U, Harris TB, Hartikainen AL, Heath AC, Hocking L, Hofman A, Huth C, Jarvelin MR, Jukema JW, Kaprio J, Kooner JS, Kutalik Z, Lahti J, Langenberg C, Lehtimäki T, Liu Y, Madden PA, Martin N, Morrison A, Penninx B, Pirastu N, Psaty B, Raitakari O, Ridker P, Rose R, Rotter JI, Samani NJ, Schmidt H, Spector TD, Stott D, Strachan D, Tzoulaki I, van der Harst P, van Duijn CM, Marques-Vidal P, Vollenweider P, Wareham NJ, Whitfield JB, Wilson J, Wolffenbuttel B, Bakalkin G, Evangelou E, Liu Y, Rice KM, Desrivières S, Kliewer SA, Mangelsdorf DJ, Müller CP, Levy D, Elliott P. KLB is associated with alcohol drinking, and its gene product β-Klotho is necessary for FGF21 regulation of alcohol preference. Proc Natl Acad Sci U S A. 2016 Dec 13;113(50):14372-14377. PubMed PMID: 27911795; PubMed Central PMCID: PMC5167198.
3. Winham SJ, Preuss UW, Geske JR, Zill P, Heit JA, Bakalkin G, Biernacka JM, Karpyak VM. Associations of prodynorphin sequence variation with alcohol dependence and related traits are phenotype-specific and sex-dependent. Sci Rep. 2015 Oct 27;5:15670. doi: 10.1038/srep15670. PubMed PMID: 26502829; PubMed Central PMCID: PMC4621530.
4. Smeets CJ, Jezierska J, Watanabe H, Duarri A, Fokkens MR, Meijer M, Zhou Q, Yakovleva T, Boddeke E, den Dunnen W, van Deursen J, Bakalkin G, Kampinga HH, van de Sluis B, Verbeek DS. Elevated mutant dynorphin A causes Purkinje cell loss and motor dysfunction in

spinocerebellar ataxia type 23. Brain. 2015 Sep;138(Pt 9):2537-52. doi: 10.1093/brain/awv195. PubMed PMID: 26169942.
5. Sarkisyan D, Hussain MZ, Watanabe H, Kononenko O, Bazov I, Zhou X, Yamskova O, Krishtal O, Karpyak VM, Yakovleva T, Bakalkin G. Downregulation of the endogenous opioid peptides in the dorsal striatum of human alcoholics. Front Cell Neurosci. 2015 May 12;9:187. doi: 10.3389/fncel.2015.00187. PubMed PMID: 26029055; PubMed Central PMCID: PMC4428131.
6. Maximyuk O., Khmyz V., Lindskog C.-J., Vukojević V., Ivanova T., Bazov I., Hauser K.F., Bakalkin G. and Krishtal O. Plasma membrane poration by opioid neuropeptides: a possible mechanism of pathological signal transduction. Cell Death and Disease. 2015 Mar 12;6:e1683.
7. Ruggeri B., Nymberg C., Vuoksimaa E., Lourdusamy A., Wong C.P., Carvalho F.M., Jia T., Cattrell A., Macare C., Banaschewski T., Barker G.J., Bokde A.L., Bromberg U., Büchel C., Conrod P.J., Fauth-Bühler M., Flor H., Frouin V., Gallinat J., Garavan H., Gowland P., Heinz A., Ittermann B., Martinot J.L., Nees F., Pausova Z., Paus T., Rietschel M., Robbins T., Smolka M.N., Spanagel R., Bakalkin G., Mill J., Sommer W.H., Rose R.J., Yan J., Aliev F., Dick D., Kaprio J., Desrivières S., Schumann G.; IMAGEN Consortium. Association of protein phosphatase PPM1G with alcohol use disorder and brain activity during behavioral control in a genome-wide methylation analysis. Am. J Psych. 2015 Jun;172(6):543-52.
8. Chizhmakov I, Kulyk V, Khasabova I, Khasabov S, Simone D, Bakalkin G, Gordienko D, Verkhratsky A, Krishtal O. Molecular mechanism for opioid dichotomy: bidirectional effect of μ-opioid receptors on P2X3 receptor currents in rat sensory neurones. Purinergic Signal. 2015 Jan 16.
9. Henriksson R, Bäckman CM, Harvey BK, Kadyrova H, Bazov I, Shippenberg TS, Bakalkin G. PDYN, a gene implicated in brain/mental disorders, is targeted by REST in the adult human brain. Biochim Biophys Acta. 2014 Nov;1839(11):1226-32. doi: 10.1016/j.bbagrm.2014.09.001.
10. Bhandage AK, Jin Z, Bazov I, Kononenko O, Bakalkin G, Korpi ER, Birnir B.GABA-A and NMDA receptor subunit mRNA expression is altered in the caudate but not the putamen of the postmortem brains of alcoholics. Front Cell Neurosci. 2014 Dec 5;8:415. doi: 10.3389/fncel.2014.00415.
11. Sui P, Watanabe H, Ossipov MH, Bakalkin G, Artemenko K, Bergquist J. Proteomics of neuropathic pain: proteins and signaling pathways affected in a rat model. J Proteome Res. 2014 Sep 5;13(9):3957-65. doi: 10.1021/pr500241q.
12. Jin Z, Bhandage AK, Bazov I, Kononenko O, Bakalkin G, Korpi ER, Birnir B. Expression of specific ionotropic glutamate and GABA-A receptor subunits is decreased in central amygdala of alcoholics. *Front Cell Neurosci*. 2014 Sep 16;8:288. doi: 10.3389/fncel.2014.00288.
13. Ahmed AS, Ahmed M, Li J, Gu HF, Bakalkin G, Stark A, Harris HE. Proteasome inhibitor MG132 modulates inflammatory pain by central mechanisms in adjuvant arthritis. Int J Rheum Dis. 2014 Apr 5. doi: 10.1111/1756-185X.12353. Epub ahead of print. PMID: 24702728.
14. Jin Z, Bhandage AK, Bazov I, Kononenko O, Bakalkin G, Korpi ER, Birnir B. Selective increases of AMPA, NMDA, and kainate receptor subunit mRNAs in the hippocampus and orbitofrontal cortex but not in prefrontal cortex of human alcoholics. Front Cell Neurosci. 2014 Jan 29;8:11. doi: 10.3389/fncel.2014.00011. eCollection 2014.

Reviews 2014 – 2016

1. Jin Z, Bhandage AK, Bazov I, Kononenko O, Bakalkin G, Korpi ER, Birnir B. Selective increases of AMPA, NMDA, and kainate receptor subunit mRNAs in the hippocampus and orbitofrontal cortex but not in prefrontal cortex of human alcoholics. Front Cell Neurosci. 2014 Jan 29;8:11. doi: 10.3389/fncel.2014.00011. eCollection 2014.
2. Bazov, I, Bakalkin, G. Prodynorphin Epialleles. *Epigenetics and Neuroendocrinology*. Springer International Publishing, 2016. 43-76.

Dissertations 2016

1. Igor Bazov. *Epigenetic Dysregulations in the Brain of Human Alcoholics: Analysis of Opioid Genes,* 2016. Faculty of Pharmacy, Uppsala University Doctoral Theses, ISSN 1651-6192; 209

Agencies that supported the work/Funding 2016

The Swedish Council for Working Life and Social Research; The Swedish Governmental Agency for Innovation Systems; The Swedish Research Council; The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning.

Other commitments/assignments of group members 2016

Georgy Bakalkin: Editor in Addiction Biology journal

Projects

(I) Integrated genetic and epigenetic approach to developmental psychiatric disorders: analysis of human blood and brain

Environmental stimuli influence the developmental trajectories of neural circuits from birth through adolescence. Exposure to harmful environmental stimuli during these developmental stages may result in increased vulnerability to psychiatric disorders. These effects are suggested to be partly dependent on genotype and mediated by epigenetic mechanisms. We aim to identify biomarkers and new therapeutic targets for the treatment of developmental psychiatric disorders, primarily alcohol dependence. We will perform genome-wide analysis of DNA methylation in blood from 2000 adolescents part of the IMAGEN study on factors that influence mental health in adolescents (http://www.imagen-europe.com/en/the-imagen-study.php), and of genotype, DNA methylation and gene expression in brain from circa 400 controls at different developmental stages and adult alcohol dependents. Loci associated with phenotypic traits relevant to alcohol dependence, DNA methylation and gene expression in the IMAGEN sample and diagnosis, DNA methylation and gene expression in the brain sample will be considered candidate biomarkers for alcohol dependence. Mechanisms underlying these associations will be considered candidate therapeutic targets for the treatment of alcohol dependence.

(II) Alcohol binge drinking – induced cognitive impairment in health and working life: genetic vulnerability and novel pharmacotherapy

Alcohol binge drinking patterns and excessive alcohol consumption have severe effects on public health and working life in Sweden and its Nordic neighbors. Delayed impairment of cognitive capabilities and executive functions are the most deteriorating consequence of heavy drinking. We examine the novel pathophysiological mechanism of this impairment, and develop clinically manageable strategies to identify subpopulations at risk and effective pharmacotherapy to treat alcohol-produced cognitive impairment. According to the mechanism, cycles of alcohol intoxication and withdrawal impair cognition through dysregulations in neurotransmission that is controlled by the endogenous opioid systems (EOS) including kappa-opioid receptor (KOPr) and its ligands dynorphins, prodynorphin (PDYN) products. 1) We examine whether genetic variants of the KOPr/*OPRK1* and *PDYN* genes contribute to alcohol use, abuse and dependence, and to alcohol-induced cognitive impairments. Population-based cohorts of young adult monozygotic and dizygotic twin pairs and office workers/social drinkers are analysed. 2) By conducting human post-mortem studies, we are identifing and characterizing dysregulations in the opioid and glutamate neurotransmitter systems, potential targets for pharmacological interference, in human heavy alcohol drinkers; and analyze underlying epigenetic mechanism. We focus on epigenetic modifications that may cause long-lasting behavioral alterations. 3) We plan to use animals models to mimic binge alcohol - induced impairment of cognitive functions and evaluate if these detrimental effects are reversed by available and novel pharmacological means. Characterization of the novel mechanism of alcohol binge drinking - induced cognitive impairment will open new possibilities for identification of subpopulations at risk, and for therapeutic intervention with opioid or glutamate antagonists.

(III) Pathogenic mechanisms of human neuropeptide mutations: implications for regulation of the anti-reward system

Much of our knowledge about functions of genes and proteins in human brain has come from analysis of rare human and mouse mutations. Such mutations represent entry points into novel signaling pathways and molecular mechanisms. In collaboration with Dr. Dineke Verbeek, Dept. Genetics, Univ. Med. Center Groningen, The Netherlands, we have identified missense mutations in the human prodynorphin gene (*PDYN*) coding for a precursor to opioid peptides dynorphins (Bakalkin et al., 2010). These mutations cause profound neurodegeneration in the cerebrum and cerebellum underlying the development of spinocerebellar ataxia type 23, a dominantly inherited neurodegenerative disorder. Remarkably, seven out of eight mutations are located in dynorphins, which also have non-opioid neurodegenerative activities.

This is the first finding on neuropeptide mutations underlying human neuropathology. The best-established function of the dynorphins in the brain is regulation of the anti-reward system by inducing dysphoria that counterbalances positive hedonic effects of endorphins and enkephalins, and addictive substances. Analysis of PDYN mutations could uncover novel molecular and cellular mechanisms of anti-reward - reward regulation that are generally unknown. We focus on two mechanisms. First, the mutations may impair correct folding of PDYN molecules in the endoplasmic reticulum, resulting in PDYN aggregation, cellular stress and the unfolded protein response. Long-lasting activation of the unfolded protein response by mutant PDYNs, or by wild-type - PDYN excessively produced under pathological conditions such as substance addiction, may lead to neuronal dysfunction, atrophy and neurodegeneration. Second, *PDYN* mutations may enhance non-opioid pathogenic activities of dynorphins. Our previous analysis suggests that dynorphins may mediate communications between neurons through non-receptor excitatory mechanism. This mechanism may be engaged in pathogenic effects of upregulated wild-type - dynorphins and mutant peptides. Our general goal is to understand pathogenic mechanisms of PDYN mutations and, in the following studies to evaluate whether wild-type - PDYN and dynorphins engage these mechanisms to regulate reward circuits under normal conditions and in substance addiction when these neuropeptides are excessively produced. We explore pathogenic mechanisms underlying actions of wild-type- and mutant-PDYN in cellular and in vitro biochemical/ biophysical studies, and are planning to use in vivo transgenic mice expressing human wild-type- or pathogenic mutant-PDYN that have been produced by Dr. Verbeek. Generalized pathological changes including cerebral cortical and subcortical atrophy, and agenesis of corpus callosum in patients caring PDYN mutations emphasize the fundamental role of neuropeptides in brain functions, and propose that the essential molecular mechanisms are affected; the phenomenon that requires detailed investigation. Dissection of these mechanisms is critically important for understanding of reward – anti-reward, substance addiction, depression and chronic pain, all neuropathological conditions in which dynorphins play a critical role, as well as for the neuropeptide and neurodegeneration fields in general because of the novelty of mechanisms identified.

Research area: Pharmaceutical Bioinformatics

Ola Spjuth and Jarl Wikberg

http://farmbio.uu.se/research/researchgroups/pb

During the year we carried out several projects involving large-scale predictive modeling, including a study that investigated the effect of dataset sizes on predictive performance and modelling time for LogD and solubility. We also developed the SciLuigi workflow framework, inspired by elements of the flow-based programming paradigm, which enables reproducible and agile data analysis on high-performance and cloud computing e-infrastructures. We also carried out large-scale structure-based virtual screening on the Big Data framework Apache Spark, showing good parallel efficiency (87%) when running in a public cloud environment. The work on confidence measures in predictive modeling was continued in collaboration with primarily AstraZeneca and SweTox. The XMetDB open access database for xenobiotic metabolism was published. Our joint project with Uppsala Academic Hospital on long-read single molecule sequencing for analysis of drug resistance in BCR-ABL1 for chronic myelogenous leukemia (CML) was further developed, assessed, and implemented in the clinic.

Our work within the PhenoMeNal H2020 project (2015-2018) included contributions for on-demand cloud-agnostic virtual research environments in metabolomics with a microservice architecture based on Docker and Kubernetes. Work to demonstrate the scalability and interoperability of workflows on top of this architecture is ongoing. A new EU H2020 project 'OpenRiskNet' was funded and initiated. The project will create and establish an openly accessible e-infrastructure for prediction of safety of existing and new molecular entities for health and environmental sustainability.

Studies on the metabolism and pharmacokinetics of the libiguins were conducted and supply chains for raw-materials for synthesis of libiguins were established.

Members of the group during 2016

Jarl Wikberg, Professor
Ola Spjuth, Associate Professor
Maris Lapins, PhD, Researcher
Wesley Schaal, PhD, Researcher
Jonathan Alvarsson, PhD, Researcher
Marco Capuccini, PhD student
Samuel Lampa, PhD student
Martin Dahlö, PhD student
Arvid Berg, Software engineer
Staffan Arvidsson, Software engineer
Anders Larsson, PhD, Software engineer
Valentin Georgiev, PhD, Software engineer
Polina Georgieva, PhD, Web technician
Aleh Yahorau, Technician

Publications 2014-2016

1. S. Lampa, J. Alvarsson, and O. Spjuth. (2016) Towards Agile Large-Scale Predictive Modelling in Drug Discovery with Flow-Based Programming Design Principles. Journal of Cheminformatics, 8:67.
2. O. Spjuth, P. Rydberg, E. L. Willighagen, C. T. Evelo and N. Jeliazkova. (2016) XMetDB: an open access database for xenobiotic metabolism. Journal of Cheminformatics, 8:47.
3. J. Alvarsson, S. Lampa, W. Schaal, C. Andersson, J.E.S Wikberg, and O. Spjuth. (2016) Large-scale ligand-based predictive modelling using support vector machines. Journal of Cheminformatics, 8:39.
4. S. Simeon, O. Spjuth, M. Lapins, S. Nabu, N. Anuwongcharoen, V. Prachayasittikul, J.E.S. Wikberg, C. Nantasenamat. (2016) Origin of aromatase inhibitory activity via proteochemometric modeling. PeerJ, 4:e1979
5. O. Spjuth, J. Hastings, J. Dietrich, J. Heikkinän, N. Pedersen, J. Hottenga, S. Ripatti, P. Burton, I. Fortier, C. van Duijn, E. Wichmann, J. Rung, M. McCarthy, M. Allen, E. Raulo, I. Prokopenko, J. Karvanen, M. Perola, M. Kolz, E. J.C. de Geus, G. Willemsen, P. Magnusson, J-E. Litton, J. Palmgren, M. Krestyaninova, and J. Harris. (2016) Harmonising and linking biomedical and clinical data across disparate data archives to enable integrative cross-biobank research. European Journal of Human Genetics, 24, 521528
6. Fossen T, Yahorau A, Yahorava S, Raharinjato F, Razafimahefa S, Rasoanaivo P, Wikberg JE. (2016) New Polyfunctional Phragmalin Limonoids from Neobeguea mahafalensis. Planta Med. 82(11-12):1087-95.
7. Nagaev, I., Andersen, M., Olesen, M., Nagaeva, O., Wikberg, J., Mincheva-Nilsson, L., Andersen, GN. (2016) Resistin Gene Expression is Downregulated in CD4(+) T Helper Lymphocytes and CD14(+) Monocytes in Rheumatoid Arthritis Responding to TNF-alpha Inhibition. Scandinavian Journal of Immunology, vol. 84, ss. 229-236
8. Shoombuatong, W., Nabu, S., Simeon, S., Prachayasittikul, V., Lapins, M., Wikberg J.E.S., Nantasenamat, C. (2016) Extending proteochemometric modeling for unraveling the sorption behavior of compound-soil interaction. Chemometrics and Intelligent Laboratory Systems, vol. 151, ss. 219-227
9. Simeon, S., Anuwongcharoen, N., Shoombuatong, W., Malik, A., Prachayasittikul, V., Wikberg J.E.S., Nantasenamat, C. (2016) Probing the origins of human acetylcholinesterase inhibition via QSAR modeling and molecular docking. PeerJ, vol. 4
10. Simeon, S., Shoombuatong, W., Anuwongcharoen, N., Preeyanon, L., Prachayasittikul, V., Wikberg J.E.S., Nantasenamat, C. (2016) osFP: a web server for predicting the oligomeric states of fluorescent proteins. Journal of Cheminformatics, vol. 8
11. M. Dahlö, F. Haziza, A. Kallio, E. Korpelainen, E. Bongcam-Rudloff, and O. Spjuth. (2015) BioImg.org: A catalogue of virtual machine images for the life sciences. Bioinformatics and Biology Insights 2015:9 125-128
12. Eklund, M., Norinder, U., Boyer, S., Carlsson, L. (2015) The application of conformal prediction to the drug discovery process. Annals of Mathematics and Artificial Intelligence, vol. 74, ss. 117-132
13. R. C. Grafström, P. Nymark,V. Hongisto, O. Spjuth, R. Ceder, E. Willighagen, B. Hardy, S. Kaski and P. Kohonen. (2015) Toward the Replacement of Animal Experiments through the Bioinformatics-driven Analysis of Omics Data from Human Cell Cultures Alternatives to Laboratory Animals ATLA, 43(5):325–332
14. Nabu, S., Nantasenamat, C., Owasirikul, W., Lawung, R., Isarankura-Na-Ayudhya, C. et al. (2015) Proteochemometric model for predicting the inhibition of penicillin-binding proteins. Journal of Computer-Aided Molecular Design, vol. 29, ss. 127-141
15. E. Ahlberg, O. Spjuth, C. Hasselgren, and L. Carlsson. (2015) Interpretation of Conformal Prediction Classification Models. In Statistical Learning and Data Sciences, vol. 9047 of Lecture Notes in Computer Science. Springer International Publishing, pp. 323–334.
16. A. Siretskiy, L. Pireddu, T. Sundqvist, and O. Spjuth. (2015) A quantitative assessment of the Hadoop framework for analyzing massively parallel DNA sequencing data. Gigascience. 2015 Jun 4; 4:26.
17. M. Capuccini, L. Carlsson, U. Norinder and O. Spjuth. (2015) Conformal Prediction in Spark:

Large-Scale Machine Learning with Confidence. IEEE/ACM 2nd International Symposium on Big Data Computing (BDC), Limassol, pp. 61-67.
18. B. T. Moghadam, J. Alvarsson, M. Holm, M. Eklund, L. Carlsson, and O. Spjuth. (2015) Scaling predictive modeling in drug development with cloud computing. J. Chem. Inf. Model., 2015, 55 (1), pp 19-25
19. A. Gholami, E. Laure, P. Somogyi, O. Spjuth, S. Niazi, J. Dowling. (2015) Privacy-Preservation for Publishing Sample Availability Data with Personal Identifiers. Journal of Medical and Bioengineering, Vol. 4, No. 2, pp. 117-126.
20. Alvarsson, J., Eklund, M., Andersson, C., Carlsson, L., Spjuth, O., & Wikberg, J. E. (2014). Benchmarking Study of Parameter Variation When Using Signature Fingerprints Together with Support Vector Machines. Journal of chemical information and modeling, 54(11), 3211-3217.
21. Alvarsson, J., Eklund, M., Engkvist, O., Spjuth, O., Carlsson, L., Wikberg, J. E., & Noeske, T. (2014). Ligand-based target prediction with signature fingerprints. Journal of chemical information and modeling, 54(10), 2647-2653.
22. A. Siretskiy and O. Spjuth. (2014) HTSeq-Hadoop: Extending HTSeq for Massively Parallel Sequencing Data Analysis using Hadoop. In e-Science, 2014 IEEE 10th International Conference on, vol. 1, pp. 317–323.
23. Andersen, M., Olesen, M., Nagaev, I., Nagaeva, O., Wikberg, J. et al. (2014). Adalimumab (Humira (R)) normalizes melanocortin receptor subtype 2, 3, and 4 expression in CD8+, CD14+, and CD19+leucocyte subsets in rheumatoid arthritis. Scandinavian Journal of Rheumatology, vol. 43, ss. 25-26
24. P. Kohonen, R. Ceder, I. Smit, V. Hongisto, B. Fadeel, O. Spjuth, and R. Grafström. (2014) The fields of Cancer Biology, Toxicology and Alternative Methods Development Go Hand-in-Hand. Basic Clin Pharmacol Toxicol. 115(1):50-8.
25. Eklund, M., Norinder, U., Boyer, S., & Carlsson, L. (2014). Choosing Feature Selection and Learning Algorithms in QSAR. Journal of chemical information and modeling, 54(3), 837-843.
26. Grigorjeva, L., Liepinsh, E., Razafimahefa, S., Yahorau, A., Yahorava, S., Rasoanaivo, P., & Wikberg, J. E. (2014). Semisynthesis of libiguin A and its analogues by translactonization of phragmalin. The Journal of Organic Chemistry, 79(9), 4148-4153.
27. O. Spjuth, J. Heikkinen, J-E Litton, J. Palmgren, and M. Krestyaninova. (2014) Data integration between Swedish national clinical health registries and biobanks using an availability system. In Data Integration in the Life Sciences, Lecture Notes in Computer Science. Springer International Publishing, pp. 32-40.
28. Nantasenamat, C., Simeon, S., Owasirikul, W., Songtawee, N., Lapins, M., Prachayasittikul, V., & Wikberg, J. E. (2014). Illuminating the origins of spectral properties of green fluorescent proteins via proteochemometric and molecular modeling. Journal of computational chemistry, 35(27), 1951-1966.
29. Razafimahefa, S., Mutulis, F., Mutule, I., Liepinsh, E., Dambrova, M., Cirule, H., & Wikberg, J. E. (2014). Libiguins A and B: novel phragmalin limonoids isolated from Neobeguea mahafalensis causing profound enhancement of sexual activity. Planta medica, 80(4), 306-314.

Reviews 2014 – 2016

1. O. Spjuth, E. Bongcam-Rudloff, J. Dahlberg, M. Dahl, A. Kallio, L. Pireddu, F. Vezzi, E. Korpelainen. Recommendations on e-Infrastructures for next-generation sequencing. (2016) GigaScience, vol. 5, no. 1, pp. 1-9
2. O. Spjuth, E. Bongcam-Rudloff, G. C. Hernandez, L. Forer, M. Giovacchini, R. V. Guimera, A. Kallio, E. Korpelainen, M. Kandula, M. Krachunov, D. P. Kreil, O. Kulev, P. P. Labaj, S. Lampa, L. Pireddu, S. Schönherr, A. Siretskiy, and D. Vassilev. (2015) Experiences with workflows for automating data-intensive bioinformatics. Biology Direct. 10(1):43.

Dissertations 2014 – 2016

1. Jonathan Alvarsson, *Ligand-based Methods for Data Management and Modelling*, 2015. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, ISSN 1651-6192; 200

Agencies that supported the work/Funding 2016

The Swedish Research Council; Swedish Institute; eSSENCE, SeRC, EU Horizon 2020

Other commitments/assignments of group members 2016

Ola Spjuth: Co-Director, UPPMAX High Performance Computing center. Head of Bioinformatics Compute and Storage Facility at Science for Life Laboratory.

Projects

(I) Pharmacology of the libiguins (Jarl Wikberg et al.)
Studies on the mechanisms of action for the effects of libiguins on sexual behaviour; in part a collaboration with Philippe Rasoanaivo, IMRA, Antananarivo, Madagascar, Gunnar Antoni, PET-centre, Uppsala University and Aigars Jirgensons, IOS, Riga, Latvia.

(II) Isolation, structural determination and pharmacology of novel natural and semi-synthetic compounds (Jarl Wikberg et al.)
Studies devoted to the isolation, structural determination of novel natural compounds and semi-synthetic derivatives therefrom; collaborations with Torgils Fossen, Centre for Pharmacy, Department of Chemistry University of Bergen, Bergen, Norway, Philippe Rasoanaivo, IMRA, Antananarivo, Madagascar, and Aigars Jirgensons, IOS, Riga, Latvia.

(III) Proteochemometrics (Jarl Wikberg et al.)
Studies directed to further development of proteochemometics: Large scale proteochemometrics and integration of 3D-molecular modelling with proteochemometric modelling. Wet laboratory experimentations in relation to development of antivirals directed towards dengue and Japanese encephalitis viruses; in part a collaboration Mahidol University, Salaya, Thailand.

(IV) Large-scale Predictive Modelling in Drug Discovery (Ola Spjuth et al.)
This project aims at developing computational methods, tools and predictive models to aid the drug discovery process on large data sets. Methods include ligand-based and structure-based methods such as QSAR (machine learning) and docking, with applications including prediction of drug safety, toxicology, interactions, target profiles and secondary pharmacology. Collaboration with Andreas Hellander at the Department of Information Technology, UU as well as Lars Carlsson, Ernst Ahlberg at AstraZeneca R&D and Ulf Norinder at SweTox.

(V) Predictive toxicology and site-of-metabolism (Ola Spjuth et al.)
Studies on informatics and predictive modeling in toxicology and site-of-metabolism. Collaboration with Lars Carlsson at AstraZeneca R&D; Egon Willighagen at Maastricht University NL; Nina Jeliazkova at Ideaconsult BG; Catrin Hasselgren, UCB and Leadscope, US; Roland Grafström at Karolinska Institutet the OpenTox and OpenRiskNet consortia.

(VI) Large-scale computing for medical metabolomics (Ola Spjuth et al.)
The PhenoMeNal Horizon 2020 project develops a standardised e-infrastructure for analysing medical metabolic phenotype data. This comprises development of standards for data exchange, pipelines, computational frameworks and resources for the processing, analysis and information-mining. Collaboration with the PhenoMeNal consortium.

(VII) Sequence-based diagnostics of drug resistance (Ola Spjuth et al.)
Studies devoted to translating bioinformatics-based analysis of long-read amplicon sequencing data to clinical diagnostics with applications in chronic myelogenous leukemia (CML) and multi-drug resistant bacteria. Collaboration with Åsa Melhus at Department of Medical Sciences and Uppsala Academic Hospital, Adam Ameur at National Genomics Infrastructure, Lucia Cavelier at Department of Genetics and Pathology.

Research area: Pharmacometrics

Mats Karlsson

http://farmbio.uu.se/research/researchgroups/pharmacometrics

Pharmacometric research focuses on nonlinear mixed effects (”population”) models. Such models describe data, generally the response-time profiles observed in a clinical trial, by a basic model, accounting for the general structure of the underlying system, and a set of hierarchical variability components, accounting for variability between subjects, within subjects over time and remaining between observation variability. Research at the pharmacometrics group can be divided into four main areas. First, development and evaluation of methods for efficient and robust model building. This involves development of estimation algorithms, methods for model diagnosis and sequential procedures for model building. The result of the research, when applicable, is made available as free software. Second, so-called platform models are being developed for the use in specific therapeutic areas or for particular therapeutic/pharmacological principles. Such a model may involve the time-course of a biomarker or a system of biomarkers during normal, diseased and/or provoked situations. The third research area concerns utilization of the developed models for the purpose of designing studies, deciding upon dosing strategies and other developmental decisions. Fourth, we also do analyses of dose-concentration-response data from trials to understand therapies with existing drugs with the aim of allowing improved therapy.

Members of the group during 2016

Mats Karlsson, Professor
Lena Friberg, Professor
Ulrika Simonsson, Professor
Andrew Hooker, Senior Lecturer
Elisabet Nielsen, Senior Lecturer
Maria Kjellsson, Senior Lecturer
Martin Bergstrand, Researcher
Oskar Clewe, Researcher
Eva Germovsek, Researcher
Nick Holford, Researcher
Jonas Häggström, Researcher
Siv Jönsson, Researcher
Kristin Karlsson, Researcher
Anders Kristoffersson, Researcher
Joakim Nyberg, Researcher
Elodie Plan, Researcher
Sebastian Ueckert, Researcher
Kajsa Harling, System Developer
Rikard Nordgren, System Developer
Svetlana Freiberga, Research Assistant
Gunnar Yngman, Research Assistant
Britt Jansson, Lab Engineer
Agneta Ekholm, Administrative Assistant
Chayan Acharya, Post-doctoral fellow
Yasunori Aoki, Post-doctoral fellow
Margreke Brill, Post-doctoral fellow
Chenhui Deng, Post-doctoral fellow
Thomas Dorlo, Post-doctoral fellow

Gopichand Gottipati, Post-doctoral fellow
Elham Haem, Post-doctoral fellow
Anna Largajolli, Post-doctoral fellow
Philippe Pierrillas, Post-doctoral fellow
Wanchana Ungphakorn, Post-doctoral fellow
Sven von Dijkman, Post-doctoral fellow
Sebastian Wicha, Post-doctoral fellow
Gülbeyaz Yildiz, Post-doctoral fellow
João Abrantes, PhD Student
Oskar Alskär, PhD Student
Brendan Bender, PhD Student
Henrik Bjugård Nyberg, PhD Student
Salim Bouchene, PhD Student
Ari Brekkan, PhD Student
Chunli Chen, PhD student
Steve Choy, PhD student
Anne-Gaëlle Dosne, PhD student
Benjamin Guiastrennec, PhD Student
Ana Kalezic, PhD student
David Khan, PhD Student
Siti Maisharah Sheikh Ghadzi, PhD Student
Moustafa Mahmoud Abdellatif Ibrahim, PhD student
Rikke Meldgaard, PhD Student
Ida Netterberg, PhD Student
Jesmin Permal, PhD Student
Yevgen Ryeznik, PhD Student
Emilie Schindler, PhD Student
Marina Senek, PhD student
Eric Strömberg, PhD Student
Elin Svensson, PhD Student
Robin Svensson, PhD Student
Anders Thorsted, PhD Student
Shijun Wang, PhD Student
Gustaf Wellhagen, PhD Student
Sreenath Madathil Krishnan, PhD Student
Lénaïg Tanneau, PhD Student
Chenyan Zhao, PhD Student
Simon Buatois, visiting PhD student
Anke Kip, visiting PhD student
Stein Schalkwijk, visiting PhD student
Bruna Torres, visiting PhD student

Publications 2014 – 2016

1. Aoki Y, De Sterck H. Numerical study of unbounded capillary surfaces. Pacific J Math. 2014;267(1):1-34.
2. Aoki Y, Hayami K, De Sterck H, Konagaya A. Cluster Newton Method for Sampling Multiple Solutions of Underdetermined Inverse Problems: Application to a Parameter Identification Problem in Pharmacokinetics. Siam J Sci Comput. 2014;36(1):B14-B44.
3. Bender B, Leipold DD, Xu K, Shen BQ, Tibbitts J, Friberg LE. A mechanistic pharmacokinetic model elucidating the disposition of trastuzumab emtansine (T-DM1), an antibody-drug conjugate (ADC) for treatment of metastatic breast cancer. Aaps J. 2014;16(5):994-1008.
4. Bergstrand M, Nosten F, Lwin KM, Karlsson MO, White NJ, Tarning J. Characterization of an in vivo concentration-effect relationship for piperaquine in malaria chemoprevention. Science translational medicine. 2014;6(260):260ra147.

5. Davies Forsman L, Schon T, Simonsson US, Bruchfeld J, Larsson M, Jureen P, Sturegard E, Giske CG, Angeby K. Intra- and extracellular activities of trimethoprim-sulfamethoxazole against susceptible and multidrug-resistant Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2014;58(12):7557-9.
6. De Cock RF, Allegaert K, Sherwin CM, Nielsen EI, de Hoog M, van den Anker JN, Danhof M, Knibbe CA. A neonatal amikacin covariate model can be used to predict ontogeny of other drugs eliminated through glomerular filtration in neonates. Pharm Res. 2014;31(3):754-67.
7. Hamberg AK, Wadelius M, Friberg LE, Biss TT, Kamali F, Jonsson EN. Characterizing variability in warfarin dose requirements in children using modelling and simulation. Br J Clin Pharmacol. 2014;78(1):158-69.
8. Hennig S, Karlsson MO. Concordance between criteria for covariate model building. J Pharmacokinet Pharmacodyn. 2014;41(2):109-25.
9. Johansson AM, Ueckert S, Plan EL, Hooker AC, Karlsson MO. Evaluation of bias, precision, robustness and runtime for estimation methods in NONMEM 7. J Pharmacokinet Pharmacodyn. 2014;41(3):223-38.
10. Lacroix BD, Karlsson MO, Friberg LE. Simultaneous Exposure-Response Modeling of ACR20, ACR50, and ACR70 Improvement Scores in Rheumatoid Arthritis Patients Treated With Certolizumab Pegol. CPT Pharmacometrics Syst Pharmacol. 2014;3:e143.
11. Marklund M, Stromberg EA, Laerke HN, Knudsen KE, Kamal-Eldin A, Hooker AC, Landberg R. Simultaneous pharmacokinetic modeling of alkylresorcinols and their main metabolites indicates dual absorption mechanisms and enterohepatic elimination in humans. J Nutr. 2014;144(11):1674-80.
12. Mohamed AF, Cars O, Friberg LE. A pharmacokinetic/pharmacodynamic model developed for the effect of colistin on Pseudomonas aeruginosa in vitro with evaluation of population pharmacokinetic variability on simulated bacterial killing. J Antimicrob Chemother. 2014;69(5):1350-61.
13. Moller JB, Kristensen NR, Klim S, Karlsson MO, Ingwersen SH, Kjellsson MC. Methods for Predicting Diabetes Phase III Efficacy Outcome From Early Data: Superior Performance Obtained Using Longitudinal Approaches. CPT Pharmacometrics Syst Pharmacol. 2014;3:e122.
14. Plan EL. Modeling and simulation of count data. CPT Pharmacometrics Syst Pharmacol. 2014;3:e129.
15. Quartino AL, Karlsson MO, Lindman H, Friberg LE. Characterization of endogenous G-CSF and the inverse correlation to chemotherapy-induced neutropenia in patients with breast cancer using population modeling. Pharm Res. 2014;31(12):3390-403.
16. Ravva P, Karlsson MO, French JL. A linearization approach for the model-based analysis of combined aggregate and individual patient data. Stat Med. 2014;33(9):1460-76.
17. Roge RM, Klim S, Kristensen NR, Ingwersen SH, Kjellsson MC. Modeling of 24-hour glucose and insulin profiles in patients with type 2 diabetes mellitus treated with biphasic insulin aspart. J Clin Pharmacol. 2014;54(7):809-17.
18. Steven Ernest C, 2nd, Nyberg J, Karlsson MO, Hooker AC. Optimal clinical trial design based on a dichotomous Markov-chain mixed-effect sleep model. J Pharmacokinet Pharmacodyn. 2014;41(6):639-54.
19. Svensson EM, Dooley KE, Karlsson MO. Impact of lopinavir-ritonavir or nevirapine on bedaquiline exposures and potential implications for patients with tuberculosis-HIV coinfection. Antimicrob Agents Chemother. 2014;58(11):6406-12.
20. Svensson EM, Karlsson MO. Use of a linearization approximation facilitating stochastic model building. J Pharmacokinet Pharmacodyn. 2014;41(2):153-8.
21. Ueckert S, Plan EL, Ito K, Karlsson MO, Corrigan B, Hooker AC, Alzheimer's Disease Neuroimaging I. Improved utilization of ADAS-cog assessment data through item response theory based pharmacometric modeling. Pharm Res. 2014;31(8):2152-65.
22. van der Graaf PH, Friberg LE. CPT: Pharmacometrics & Systems Pharmacology Publishes Its 100th Article. CPT Pharmacometrics Syst Pharmacol. 2014;3:e104.
23. Zvada SP, Denti P, Donald PR, Schaaf HS, Thee S, Seddon JA, Seifart HI, Smith PJ, McIlleron

HM, Simonsson US. Population pharmacokinetics of rifampicin, pyrazinamide and isoniazid in children with tuberculosis: in silico evaluation of currently recommended doses. J Antimicrob Chemother. 2014;69(5):1339-49.
24. Zvada SP, Denti P, Sirgel FA, Chigutsa E, Hatherill M, Charalambous S, Mungofa S, Wiesner L, Simonsson US, Jindani A, Harrison T, McIlleron HM. Moxifloxacin population pharmacokinetics and model-based comparison of efficacy between moxifloxacin and ofloxacin in African patients. Antimicrob Agents Chemother. 2014;58(1):503-10.
25. Alskar O, Bagger JI, Roge RM, Knop FK, Karlsson MO, Vilsboll T, Kjellsson MC. Semimechanistic model describing gastric emptying and glucose absorption in healthy subjects and patients with type 2 diabetes. J Clin Pharmacol. 2015.
26. Bjornsson MA, Friberg LE, Simonsson US. Performance of nonlinear mixed effects models in the presence of informative dropout. Aaps J. 2015;17(1):245-55.
27. Bjornsson MA, Norberg A, Kalman S, Simonsson US. A Recirculatory Model for Pharmacokinetics and the Effects on Bispectral Index after Intravenous Infusion of the Sedative and Anesthetic AZD3043 in Healthy Volunteers. Anesth Analg. 2015;121(4):904-13.
28. Bogo R, Farah A, Johnson AC, Karlsson KK, Pedersen NL, Svartengren M, Skjonsberg A. The role of genetic factors for hearing deterioration across 20 years: a twin study. J Gerontol A Biol Sci Med Sci. 2015;70(5):647-53.
29. Brokjaer A, Kreilgaard M, Olesen AE, Simonsson US, Christrup LL, Dahan A, Drewes AM. Population pharmacokinetics of morphine and morphine-6-glucuronide following rectal administration - A dose escalation study. Eur J Pharm Sci. 2015;68:78-86.
30. Clewe O, Aulin L, Hu Y, Coates AR, Simonsson US. A multistate tuberculosis pharmacometric model: a framework for studying anti-tubercular drug effects in vitro. J Antimicrob Chemother. 2016;71(4):964-74.
31. Clewe O, Goutelle S, Conte JE, Jr., Simonsson US. A pharmacometric pulmonary model predicting the extent and rate of distribution from plasma to epithelial lining fluid and alveolar cells-using rifampicin as an example. Eur J Clin Pharmacol. 2015;71(3):313-9.
32. Clewe O, Karlsson MO, Simonsson US. Evaluation of optimized bronchoalveolar lavage sampling designs for characterization of pulmonary drug distribution. J Pharmacokinet Pharmacodyn. 2015;42(6):699-708.
33. Dosne AG, Bergstrand M, Karlsson MO. A strategy for residual error modeling incorporating scedasticity of variance and distribution shape. J Pharmacokinet Pharmacodyn. 2016;43(2):137-51.
34. Gaudreau P, Hayami K, Aoki Y, Safouhi H, Konagaya A. Improvements to the cluster Newton method for underdetermined inverse problems. Journal of Computational and Applied Mathematics. 2015;283(0):122-41.
35. Jonsson S, Simonsson US, Miller R, Karlsson MO. Population pharmacokinetics of edoxaban and its main metabolite in a dedicated renal impairment study. J Clin Pharmacol. 2015;55(11):1268-79.
36. Juul RV, Rasmussen S, Kreilgaard M, Christrup LL, Simonsson US, Lund TM. Repeated Time-to-event Analysis of Consecutive Analgesic Events in Postoperative Pain. Anesthesiology. 2015;123(6):1411-9.
37. Kagedal M, Karlsson MO, Hooker AC. Improved precision of exposure-response relationships by optimal dose-selection. Examples from studies of receptor occupancy using PET and dose finding for neuropathic pain treatment. J Pharmacokinet Pharmacodyn. 2015;42(3):211-24.
38. Kagedal M, Varnas K, Hooker AC, Karlsson MO. Estimation of drug receptor occupancy when non-displaceable binding differs between brain regions - extending the simplified reference tissue model. Br J Clin Pharmacol. 2015;80(1):116-27.
39. Karaiskos I, Friberg LE, Pontikis K, Ioannidis K, Tsagkari V, Galani L, Kostakou E, Baziaka F, Paskalis C, Koutsoukou A, Giamarellou H. Colistin Population Pharmacokinetics after Application of a Loading Dose of 9 MU Colistin Methanesulfonate in Critically Ill Patients. Antimicrob Agents Chemother. 2015;59(12):7240-8.
40. Karlsson M, Janzen DL, Durrieu L, Colman-Lerner A, Kjellsson MC, Cedersund G. Nonlinear mixed-effects modelling for single cell estimation: when, why, and how to use it. BMC Syst Biol. 2015;9:52.
41. Khan DD, Lagerback P, Cao S, Lustig U, Nielsen EI, Cars O, Hughes D, Andersson DI, Friberg LE. A mechanism-based pharmacokinetic/pharmacodynamic model allows prediction of

antibiotic killing from MIC values for WT and mutants. J Antimicrob Chemother. 2015;70(11):3051-60.
42. Korell J, Martin SW, Karlsson MO, Ribbing J. A model-based longitudinal meta-analysis of FEV1 in randomized COPD trials. Clin Pharmacol Ther. 2016;99(3):315-24.
43. Kreeftmeijer-Vegter AR, Dorlo TP, Gruppen MP, de Boer A, de Vries PJ. Population pharmacokinetics of levamisole in children with steroid-sensitive nephrotic syndrome. Br J Clin Pharmacol. 2015;80(2):242-52.
44. Kristoffersson AN, Friberg LE, Nyberg J. Inter occasion variability in individual optimal design. J Pharmacokinet Pharmacodyn. 2015;42(6):735-50.
45. Martial LC, Bruggemann RJ, Schouten JA, van Leeuwen HJ, van Zanten AR, de Lange DW, Muilwijk EW, Verweij PE, Burger DM, Aarnoutse RE, Pickkers P, Dorlo TP. Dose Reduction of Caspofungin in Intensive Care Unit Patients with Child Pugh B Will Result in Suboptimal Exposure. Clin Pharmacokinet. 2015.
46. Niebecker R, Jonsson S, Karlsson MO, Miller R, Nyberg J, Krekels EH, Simonsson US. Population pharmacokinetics of edoxaban in patients with symptomatic deep-vein thrombosis and/or pulmonary embolism-the Hokusai-VTE phase 3 study. Br J Clin Pharmacol. 2015;80(6):1374-87.
47. Nyberg J, Bazzoli C, Ogungbenro K, Aliev A, Leonov S, Duffull S, Hooker AC, Mentre F. Methods and software tools for design evaluation in population pharmacokinetics-pharmacodynamics studies. Br J Clin Pharmacol. 2015;79(1):6-17.
48. Panoilia E, Schindler E, Samantas E, Aravantinos G, Kalofonos HP, Christodoulou C, Patrinos GP, Friberg LE, Sivolapenko G. A pharmacokinetic binding model for bevacizumab and VEGF165 in colorectal cancer patients. Cancer Chemother Pharmacol. 2015;75(4):791-803.
49. Roge RM, Klim S, Ingwersen SH, Kjellsson MC, Kristensen NR. The Effects of a GLP-1 Analog on Glucose Homeostasis in Type 2 Diabetes Mellitus Quantified by an Integrated Glucose Insulin Model. CPT Pharmacometrics Syst Pharmacol. 2015;4(1):e00011.
50. Swat MJ, Moodie S, Wimalaratne SM, Kristensen NR, Lavielle M, Mari A, Magni P, Smith MK, Bizzotto R, Pasotti L, Mezzalana E, Comets E, Sarr C, Terranova N, Blaudez E, Chan P, Chard J, Chatel K, Chenel M, Edwards D, Franklin C, Giorgino T, Glont M, Girard P, Grenon P, Harling K, Hooker AC, Kaye R, Keizer R, Kloft C, Kok JN, Kokash N, Laibe C, Laveille C, Lestini G, Mentre F, Munafo A, Nordgren R, Nyberg HB, Parra-Guillen ZP, Plan E, Ribba B, Smith G, Troconiz IF, Yvon F, Milligan PA, Harnisch L, Karlsson M, Hermjakob H, Le Novere N. Pharmacometrics Markup Language (PharmML): Opening New Perspectives for Model Exchange in Drug Development. CPT Pharmacometrics Syst Pharmacol. 2015;4(6):316-9.
51. Svensson EM, Acharya C, Clauson B, Dooley KE, Karlsson MO. Pharmacokinetic Interactions for Drugs with a Long Half-Life-Evidence for the Need of Model-Based Analysis. Aaps J. 2015.
52. Svensson EM, Murray S, Karlsson MO, Dooley KE. Rifampicin and rifapentine significantly reduce concentrations of bedaquiline, a new anti-TB drug. J Antimicrob Chemother. 2015;70(4):1106-14.
53. Venkatakrishnan K, Friberg LE, Ouellet D, Mettetal JT, Stein A, Troconiz IF, Bruno R, Mehrotra N, Gobburu J, Mould DR. Optimizing oncology therapeutics through quantitative translational and clinical pharmacology: challenges and opportunities. Clin Pharmacol Ther. 2015;97(1):37-54.
54. Acharya C., Hooker A.C., Turkyilmaz G.Y., Jonsson S., Karlsson M.O. A diagnostic tool for population models using non-compartmental analysis: The ncappc package for R. Computer methods and programs in biomedicine. 2016;127:83-93.
55. Alskar O., Bagger J.I., Roge R.M., Knop F.K., Karlsson M.O., Vilsboll T., Kjellsson M.C. Semimechanistic model describing gastric emptying and glucose absorption in healthy subjects and patients with type 2 diabetes. J Clin Pharmacol. 2016;56(3):340-8.
56. Aoki Y., Nordgren R., Hooker A.C.. Preconditioning of Nonlinear Mixed Effects Models for Stabilisation of Variance-Covariance Matrix Computations. AAPS J. 2016;18(2):505-18.
57. Aoki Y., Sundqvist M., Hooker A.C., Gennemark P. PopED lite: An optimal design software for preclinical pharmacokinetic and pharmacodynamic studies. Computer methods and programs in biomedicine. 2016;127:126-43.

58. Bonate P.L., Ahamadi M., Budha N., de la Pena A., Earp J.C., Hong Y., Karlsson M.O., Ravva P., Ruiz-Garcia A., Struemper H., Wade J.R. Methods and strategies for assessing uncontrolled drug-drug interactions in population pharmacokinetic analyses: results from the International Society of Pharmacometrics (ISOP) Working Group. J Pharmacokinet Pharmacodyn. 2016;43(2):123-35.
59. Brekkan A., Berntorp E., Jensen K., Nielsen E.I., Jonsson S. Population pharmacokinetics of plasma-derived factor IX: procedures for dose individualization. J Thromb Haemost. 2016;14(4):724-32.
60. Brill M.J., Svensson E.M., Pandie M., Maartens G., Karlsson M.O. Confirming model-predicted pharmacokinetic interactions between bedaquiline and lopinavir/ritonavir or nevirapine in patients with HIV and drug-resistant tuberculosis. Int J Antimicrob Agents. 2017;49(2):212-7.
61. Chae J.W., Teo Y.L., Ho H.K., Lee J., Back H.M., Yun H.Y., Karlsson M.O., Kwon K.I., Chan A. BSA and ABCB1 polymorphism affect the pharmacokinetics of sunitinib and its active metabolite in Asian mRCC patients receiving an attenuated sunitinib dosing regimen. Cancer Chemother Pharmacol. 2016;78(3):623-32.
62. Chen C., Ortega F., Alameda L., Ferrer S., Simonsson U.S. Population pharmacokinetics, optimised design and sample size determination for rifampicin, isoniazid, ethambutol and pyrazinamide in the mouse. Eur J Pharm Sci. 2016;93:319-33.
63. Choy S., de Winter W., Karlsson M.O., Kjellsson M.C. Modeling the Disease Progression from Healthy to Overt Diabetes in ZDSD Rats. AAPS J. 2016;18(5):1203-12.
64. Choy S., Kjellsson M.C., Karlsson M.O., de Winter W. Weight-HbA1c-insulin-glucose model for describing disease progression of type 2 diabetes. CPT Pharmacometrics Syst Pharmacol. 2016;5(1):11-9.
65. Clewe O., Aulin L., Hu Y., Coates A.R., Simonsson U.S. A multistate tuberculosis pharmacometric model: a framework for studying anti-tubercular drug effects in vitro. J Antimicrob Chemother. 2016;71(4):964-74.
66. Deng C., Plan E.L., Karlsson M.O. Approaches for modeling within subject variability in pharmacometric count data analysis: dynamic inter-occasion variability and stochastic differential equations. J Pharmacokinet Pharmacodyn. 2016;43(3):305-14.
67. Dickstein Y., Leibovici L., Yahav D., Eliakim-Raz N., Daikos G.L., Skiada A., Antoniadou A., Carmeli Y., Nutman A., Levi I., Adler A., Durante-Mangoni E., Andini R., Cavezza G., Mouton J.W., Wijma R.A., Theuretzbacher U., Friberg L.E., Kristoffersson A.N., Zusman O., Koppel F., Dishon Benattar Y., Altunin S., Paul M. consortium A. Multicentre open-label randomised controlled trial to compare colistin alone with colistin plus meropenem for the treatment of severe infections caused by carbapenem-resistant Gram-negative infections (AIDA): a study protocol. BMJ Open. 2016;6(4):e009956.
68. Dosne A.G., Bergstrand M., Harling K., Karlsson M.O. Improving the estimation of parameter uncertainty distributions in nonlinear mixed effects models using sampling importance resampling. J Pharmacokinet Pharmacodyn. 2016;43(6):583-96.
69. Dosne A.G., Bergstrand M., Karlsson M.O. A strategy for residual error modeling incorporating scedasticity of variance and distribution shape. J Pharmacokinet Pharmacodyn. 2016;43(2):137-51.
70. Dosne AG, Niebecker R, Karlsson MO. dOFV distributions: a new diagnostic for the adequacy of parameter uncertainty in nonlinear mixed-effects models applied to the bootstrap. J Pharmacokinet Pharmacodyn. 2016;43(6):597-608.
71. Germovsek E., Kent A., Metsvaht T., Lutsar I., Klein N., Turner M.A., Sharland M., Nielsen E.I., Heath P.T., Standing J.F. Development and Evaluation of a Gentamicin Pharmacokinetic Model That Facilitates Opportunistic Gentamicin Therapeutic Drug Monitoring in Neonates and Infants. Antimicrobial agents and chemotherapy. 2016;60(8):4869-77.
72. Guiastrennec B., Sonne D.P., Hansen M., Bagger J.I., Lund A., Rehfeld J.F., Alskar O., Karlsson M.O., Vilsboll T., Knop F.K., Bergstrand M. Mechanism-Based Modeling of Gastric Emptying Rate and Gallbladder Emptying in Response to Caloric Intake. CPT Pharmacometrics Syst Pharmacol. 2016;5(12):692-700.
73. Henin E., Bergstrand M., Weitschies W., Karlsson M.O. Meta-analysis of Magnetic Marker Monitoring Data to Characterize the Movement of Single Unit Dosage Forms Though the Gas-

trointestinal Tract Under Fed and Fasting Conditions. Pharmaceutical research. 2016;33(3):751-62.
74. Hennig S., Svensson E.M., Niebecker R., Fourie P.B., Weiner M.H., Bonora S., Peloquin C.A., Gallicano K., Flexner C., Pym A., Vis P., Olliaro P.L., McIlleron H., Karlsson M.O. Population pharmacokinetic drug-drug interaction pooled analysis of existing data for rifabutin and HIV PIs. J Antimicrob Chemother. 2016;71(5):1330-40.
75. Juul R.V., Nyberg J., Lund T.M., Rasmussen S., Kreilgaard M., Christrup L.L., Simonsson U.S. A Pharmacokinetic-Pharmacodynamic Model of Morphine Exposure and Subsequent Morphine Consumption in Postoperative Pain. Pharmaceutical research. 2016;33(5):1093-103.
76. Karaiskos I., Friberg L.E., Galani L., Ioannidis K., Katsouda E., Athanassa Z., Paskalis H., Giamarellou H. Challenge for higher colistin dosage in critically ill patients receiving continuous venovenous haemodiafiltration. Int J Antimicrob Agents. 2016;48(3):337-41.
77. Khan D.D., Friberg L.E., Nielsen E.I. A pharmacokinetic-pharmacodynamic (PKPD) model based on in vitro time-kill data predicts the in vivo PK/PD index of colistin. J Antimicrob Chemother. 2016;71(7):1881-4.
78. Korell J., Martin S.W., Karlsson M.O., Ribbing J. A model-based longitudinal meta-analysis of FEV1 in randomized COPD trials. Clin Pharmacol Ther. 2016;99(3):315-24.
79. Krekels E.H., Niebecker R., Karlsson M.O., Miller R., Shimizu T., Karlsson K.E., Ruff C.T., Simonsson U.S., Jonsson S. Population Pharmacokinetics of Edoxaban in Patients with Non-Valvular Atrial Fibrillation in the ENGAGE AF-TIMI 48 Study, a Phase III Clinical Trial. Clinical pharmacokinetics. 2016;55(9):1079-90.
80. Kristoffersson A.N., David-Pierson P., Parrott N.J., Kuhlmann O., Lave T., Friberg L.E., Nielsen E.I. Simulation-Based Evaluation of PK/PD Indices for Meropenem Across Patient Groups and Experimental Designs. Pharmaceutical research. 2016;33(5):1115-25.
81. Lindqvist A., Jonsson S., Hammarlund-Udenaes M. Exploring Factors Causing Low Brain Penetration of the Opioid Peptide DAMGO through Experimental Methods and Modeling. Mol Pharm. 2016;13(4):1258-66.
82. Mohamed A.F., Kristoffersson A.N., Karvanen M., Nielsen E.I., Cars O., Friberg L.E. Dynamic interaction of colistin and meropenem on a WT and a resistant strain of Pseudomonas aeruginosa as quantified in a PK/PD model. J Antimicrob Chemother. 2016;71(5):1279-90.
83. Moller J.B., Kristensen N.R., Klim S., Karlsson M.O., Ingwersen S.H., Kjellsson M.C. Methods for Predicting Diabetes Phase III Efficacy Outcome From Early Data: Superior Performance Obtained Using Longitudinal Approaches. CPT Pharmacometrics Syst Pharmacol. 2016;5(7):388-9.
Muliaditan M., Davies G.R., Simonsson U.S., Gillespie S.H., Della Pasqua O. The implications of model-informed drug discovery and development for tuberculosis. Drug Discov Today. 2016.
84. Nguyen T., Mouksassi M.S., Holford N., Al-Huniti N., Freedman I., Hooker A.C, John J., Karlsson M.O., Mould D.R., Perez Ruixo J.J., Plan E.L., Savic R., van Hasselt J., Weber B., Zhou C., Comets E., Mentre F. Model Evaluation Group of the International Society of Pharmacometrics Best Practice C. Model Evaluation of Continuous Data Pharmacometric Models: Metrics and Graphics. CPT Pharmacometrics Syst Pharmacol. 2016.
85. Novakovic A.M., Krekels E.H., Munafo A., Ueckert S., Karlsson M.O. Application of Item Response Theory to Modeling of Expanded Disability Status Scale in Multiple Sclerosis. AAPS J. 2017;19(1):172-9.
86. Nyberg J., Karlsson K.E., Jonsson S., Yin O., Miller R., Karlsson M.O., Simonsson U. Edoxaban Exposure-Response Analysis and Clinical Utility Index Assessment in Patients With Symptomatic Deep-Vein Thrombosis or Pulmonary Embolism. CPT Pharmacometrics Syst Pharmacol. 2016;5(4):222-32.
87. Nyman E., Rozendaal Y.J., Helmlinger G., Hamren B., Kjellsson M.C., Stralfors P., van Riel N.A., Gennemark P., Cedersund G. Requirements for multi-level systems pharmacology models to reach end-usage: the case of type 2 diabetes. Interface Focus. 2016;6(2):20150075.
88. Oosten AW, Abrantes JA, Jonsson S, de Bruijn P, Kuip EJ, Falcao A, van der Rijt CC, Mathijssen RH. Treatment with subcutaneous and transdermal fentanyl: results from a population pharmacokinetic study in cancer patients. European journal of clinical pharmacology. 2016;72(4):459-67.

89. Oosten A.W., Abrantes J.A., Jonsson S., Matic M., van Schaik R.H., de Bruijn P., van der Rijt C.C., Mathijssen R.H. A Prospective Population Pharmacokinetic Study on Morphine Metabolism in Cancer Patients. Clinical pharmacokinetics. 2016.
90. Parkinson J., Hamren B., Kjellsson M.C., Skrtic S. Application of the integrated glucose-insulin model for cross-study characterization of T2DM patients on metformin background treatment. Br J Clin Pharmacol. 2016;82(6):1613-24.
91. Sadiq M.W., Nielsen E.I., Khachman D., Conil J.M., Georges B., Houin G., Laffont C.M., Karlsson M.O., Friberg L.E. A whole-body physiologically based pharmacokinetic (WB-PBPK) model of ciprofloxacin: a step towards predicting bacterial killing at sites of infection. J Pharmacokinet Pharmacodyn. 2016.
92. Schindler E., Amantea M.A., Karlsson M.O., Friberg L.E. PK-PD modeling of individual lesion FDG-PET response to predict overall survival in patients with sunitinib-treated gastrointestinal stromal tumor. CPT Pharmacometrics Syst Pharmacol. 2016;5(4):173-81.
93. Senek M., Nielsen E.I., Nyholm D. Levodopa-entacapone-carbidopa intestinal gel in Parkinson's disease: A randomized crossover study. Mov Disord. 2016.
94. Strömberg, E., Nyberg, J., Hooker, A. (2016). The effect of Fisher information matrix approximation methods in population optimal design calculations. Journal of Pharmacokinetics and Pharmacodynamics, 43(6): 609-619 DiVA
95. Svensson E.M., Acharya C., Clauson B., Dooley K.E., Karlsson M.O. Pharmacokinetic Interactions for Drugs with a Long Half-Life-Evidence for the Need of Model-Based Analysis. AAPS J. 2016;18(1):171-9.
96. Svensson E.M., Dosne A.G., Karlsson M.O. Population Pharmacokinetics of Bedaquiline and Metabolite M2 in Patients With Drug-Resistant Tuberculosis: The Effect of Time-Varying Weight and Albumin. CPT Pharmacometrics Syst Pharmacol. 2016;5(12):682-91.
97. Ueckert S., Karlsson M.O., Hooker A.C. Accelerating Monte Carlo power studies through parametric power estimation. J Pharmacokinet Pharmacodyn. 2016;43(2):223-34.
98. Ungphakorn W., Malmberg C., Lagerback P., Cars O., Nielsen E.I., Tangden T. Evaluation of automated time-lapse microscopy for assessment of in vitro activity of antibiotics. J Microbiol Methods. 2017;132:69-75.
99. Ungphakorn W., Tangden T., Sandegren L., Nielsen E.I. A pharmacokinetic-pharmacodynamic model characterizing the emergence of resistant Escherichia coli subpopulations during ertapenem exposure. J Antimicrob Chemother. 2016;71(9):2521-33.
100. Vildhede A., Mateus A., Khan E.K., Lai Y., Karlgren M., Artursson P., Kjellsson M.C. Mechanistic Modeling of Pitavastatin Disposition in Sandwich-Cultured Human Hepatocytes: A Proteomics-Informed Bottom-Up Approach. Drug metabolism and disposition: the biological fate of chemicals. 2016;44(4):505-16.
101. Zecchin C., Gueorguieva I., Enas N.H., Friberg L.E. Models for change in tumour size, appearance of new lesions and survival probability in patients with advanced epithelial ovarian cancer. Br J Clin Pharmacol. 2016;82(3):717-27.

Reviews 2014 – 2016

19. Bender BC, Schindler E, Friberg LE. Population pharmacokinetic-pharmacodynamic modelling in oncology: a tool for predicting clinical response. Br J Clin Pharmacol. 2015;79(1):56-71.
20. Kostewicz ES, Aarons L, Bergstrand M, Bolger MB, Galetin A, Hatley O, Jamei M, Lloyd R, Pepin X, Rostami-Hodjegan A, Sjogren E, Tannergren C, Turner DB, Wagner C, Weitschies W, Dressman J. PBPK models for the prediction of in vivo performance of oral dosage forms. Eur J Pharm Sci. 2014;57:300-21.
21. Ribba B, Holford NH, Magni P, Troconiz I, Gueorguieva I, Girard P, Sarr C, Elishmereni M, Kloft C, Friberg LE. A review of mixed-effects models of tumor growth and effects of anticancer drug treatment used in population analysis. CPT Pharmacometrics Syst Pharmacol. 2014;3:e113.
22. Valitalo P, Ranta VP, Hooker AC, Kokki M, Kokki H. Population pharmacometrics in support of analgesics studies. Acta Anaesthesiol Scand. 2014;58(2):143-56.

Dissertations 2016

1. Steve Choy. *Semi-mechanistic models of glucose homeostasis and disease progression in type 2 diabetes*, 2016. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 210, ISSN 1651-6192.
2. Rikke Meldgaard Røge. *Pharmacometric Models of Glucose Homeostasis in Healthy Subjects and Diabetes*, 2016. Patients Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 212, ISSN 1651-6192.
3. Salim Bouchene. *Physiologically Based Pharmacometric Models for Colistin and the Immune Response to Bacterial Infection*, 2016. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 213, ISSN 1651-6192.
4. Elin M Svensson. *Pharmacometric Models to Improve Treatment of Tuberculosis*, 2016. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 214, ISSN 1651-6192.
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6. Brendan Bender. *Pharmacometric Models for Antibody Drug Conjugates and Taxanes in HER2+ and HER2- Breast Cancer*, 2016. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 217, ISSN 1651-6192.
7. Oskar Clewe. *Novel Pharmacometric Methods for Informed Tuberculosis Drug Development*, 2016. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 222, ISSN 1651-6192.
8. Anne-Gaëlle Dosne. *Improved Methods for Pharmacometric Model-Based Decision-Making in Clinical Drug Development*, 2016. Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Pharmacy, 223, ISSN 1651-6192.

Agencies that supported the work/Funding 2016

AstraZeneca; Genentech; EU FP7 Health; Innovative Medicines Initiative (IMI); Horizon 2020; Janssen Pharmaceuticals; Joint Programming Initiative on Antimicrobial Resistance (JPIAMR); Novartis; Pfizer; Roche; Servier; Swedish Cancer Society; Swedish Foundation for Strategic Research; The National Board of Health and Welfare; The Swedish Research Council; National Institute of Health; AFA Försäkring. The Center for Interdisciplinary Mathematics (CIM), Uppsala University.

Other commitments/assignments of group members 2016

Lena Friberg**:** Deputy Editor-in-chief, CPT: Pharmacometrics & Systems Pharmacology; Organizing committee, PAGE conference, Lisbon 2016 and Budapest 2017; Executive committee, WCoP 2016 and 2020 conferences; Scientific committee, 2nd international workshop on clinical pharmacology of anticancer drugs, Madrid 2017; Secretary, International Society of anti-infective pharmacology (ISAP); Board member, The Joint ASA and ISoP Interest Group on Statistics and Pharmacometrics (SxP); Board member, Faculty of Pharmacy:; Board member, EIT Health Uppsala University

Andrew Hooker**:** Organizing committee, Population Optimum Design of Experiments (PODE) conference. Primary organizer, PODE conference, 2016. Board member, Center for Interdisciplinary Mathematics (CIM), Uppsala University.

Siv Jönsson**:** Department Board Member; Organizing committee, chair scientific program, PAGE conference, Lisbon 2016 and Budapest 2017;; Board Member: Swedish Academy of Pharmaceutical Sciences, Section for Pharmacokinetics and Drug Metabolism

Mats Karlsson**:** Deputy Head of Department; Department Board Member; Editor Journal of Pharmacokinetics and Pharmacodynamics; Editorial Board on Clin Pharmacol Ther, Eur J Pharm Sci, Basic Clin Pharmacol Toxicol, CPT: Pharmacometrics & Systems Pharmacology. Board Member DDMoRe Foundation.

Maria Kjellsson**:** Department Board Member, Board Member: Swedish Academy of Pharmaceutical Sciences, Section for Pharmacokinetics and Drug Metabolism

Elisabet Nielsen**:** Executive Committee member, EPASG, ESCMID PK/PD Study Group, Programme Committee ECCMID, Vienna 2017

Ulrika Simonsson**:** Board Member: CPTR Regulatory Science Consortium, Critical Path to TB Drug Regimens. Clinical Disease Progression Modeling Workgroup. http://cptrinitiative.org/; Board member of IF´s stiftelse, Swedish Academy of Pharmaceutical Sciences.

Projects

Methodological research

(I) Optimal design/Clinical trial design (Martin Bergstrand, Andrew Hooker, Mats Karlsson, Joakim Nyberg)

There are two principle ways in which models can be used to evaluate and optimize clinical and pre-clinical experiments. The first is by simulation of a set of proposed designs from a model (or set of models), followed by evaluation of those resulting data sets using metrics of interest. The simulations, repeated many times with different random seeds, provide information about the expected results of various different designs (for example, measures of the precision and bias of parameter estimates, or the power to detect a drug effect of a specific size). With this methodology we have investigated, for example, differences in randomization schemes for dose-finding trials where it was found that dose-randomized trials are more powerful in characterizing the underlying relation compared to concentration-randomized trials. In most instances, this increase in power can be achieved with a similar or lower number of observed side effects.

The second way of evaluating and optimizing trial designs is through the use of optimal experimental design methodologies. These methods often rely on calculations of an Information Matrix (e.g. the Fisher Information Matrix), which characterizes the information content of any possible design. Each design evaluation is much quicker than clinical trial simulation, thus one can investigate the landscape of possible designs (within constraints) potentially available for an experiment, and even optimize a design based on this information. We have developed methods and software (PopED) that utilize these methods with both local and global design criteria (e.g. E-family optimal designs, which take into account the underlying uncertainty in a pharmacometric model description of a biological system. Additionally, while optimal design is often focused on optimization of sampling times in an experiment, the methodology can be applied to other aspects of trial designs, such as the dose administered or the length of run-in, treatment and wash-out phases of an experiment, these aspects are investigated in our research. Further, we have investigated the extension of optimal design methodology to optimize a study for other interesting quantities such as power, as opposed to the traditional optimization based on model parameter estimation uncertainty.

The two methods of evaluating and optimizing trial designs can be combined to evaluate and explore adaptive optimal designs. In these types of trial designs, interim analyses are used to update models used to describe the system being investigated and then to use this information to re-optimize the next cohort of patients coming into a study. With combined simulation/optimization one can explore the adaptation and optimization rules one will use in an adaptive trial. We are currently developing such a tool (MBAOD), and are investigating the use of such designs in, for example, pedatric bridging studies and time-to-event type studies.

(II) Model building and parameter estimation (Andrew Hooker, Mats Karlsson, Sebastian Ueckert)

Pharmacometric models are based on (patho-) physiological and pharmacological knowledge. The complexity and heterogeneity of biological data makes the knowledge about, and development of, statistical data analysis methods a central part of this scientific field. There are many benefits to using

pharmacometric models in the analysis of data from clinical trials, for example the ability to handle sparse data and to integrate different types of observations into one model; however, these models are complex and intrinsically non-linear which presents technical challenges in model building and estimation.

One main challenge is to reduce the time it takes to develop these models. With complex, non-linear models and data from a clinical trial that can have thousands of data points from hundreds of patients with multiple response variables, computer runtimes become non-ignorable. Generally, run-times can be divided into short (minutes), intermediate (hours to days) and long (days to months). The number of runs in a complete analysis tends to range between 30 and many hundred. We are investigating the implementation and automation of important modelling tasks through the use of new algorithms developed in our research group. Additionally, we are developing new methods of model building and new algorithm development that can shorten run times and the number of steps needed in the model building process.

Other areas of active research include the influence on parameter estimates of single observations and rational and statistically correct algorithms for adding explanatory variables, .i.e. covariates, to the models.

(III) Diagnostic tools (Andrew Hooker, Mats Karlsson, Siv Jönsson)

A main problem for complex pharmacometric models and data is to evaluate how well the models fit the data. For the assessment of an adequate description of data, methods of evaluations can be based on model predictions, residuals, simulations from the model, simulations followed by evaluation and simulations followed by full re-estimation. We are developing diagnostic tools based on all of these principles and for both continuous and categorical type data. Knowing about model and parameter uncertainty is often crucial in model-informed decision-making. We are developing methods for diagnosing existing methods of uncertainty estimates as well as developing new methods for both model and parameter uncertainty and how these can be applied in decision making.

(IV) Software development (Andrew Hooker, Mats Karlsson, Kajsa Harling, Rikard Nordgren)

One integral part of all of our research activities is the implementation of the methods developed in freely available software to facilitate a wider and consistent use of the new algorithms.

The main software developed by the group is PsN (http://psn.sf.net), Xpose (http://xpose.sf.net), PopED (http://poped.sf.net), PopED lite (http://www.bluetree.me/PopED_lite.html), NCAPPC (https://cran.r-project.org/web/ packages/ncappc/) and MBAOD (https://github.com/andrewhooker/MBAOD) .

(V) Pharmacodynamic modelling of discrete outcomes (Andrew Hooker, Siv Jonsson, Mats Karlsson, Joakim Nyberg, Elodie Plan, Sebastian Ueckert)

For many diseases, the main outcome is of discrete nature: stage category, symptom severity, number of events, or occurrence of events. In pharmacometrics, we distinguish (non-)ordered categorical data, count data, and (repeated) time-to-event data. Models handling this type of data are based on probabilities and, even if they have been around for ~20 years in pharmacometrics, are still not widely used and subject to important innovations. In this project we aim to study and develop new methodologies for discrete data, in order to better describe disease progression, characterize exposure-response with a higher power, as well as simulate clinical trials in a more realistic manner.

In terms of applications, sleep stages have been analysed using Markov models in patients with insomnia. Pain scores rated on a Likert scale by neuropathic patients have also been modelled by including features for under-dispersion and serial correlation to count models. Daily numbers of seizures have been used in the investigation of over-dispersion and Markov patterns in count data. Simultaneous characterization of drug effect on severity and time to acid reflux events has been made possible by a repeated time-to-categorical event model.

Methodology-wise, parametric time-to-event models have been compared to semi-parametric Cox proportional hazard models and an approach to simulate large scale unbiased repeated time-to-event data

has been developed. Methods to handle within-subject variability in count models were studied since these data are often collected on a regular basis in clinical trials, resulting in the time and length of potential occasions to not be predefined and a dynamic implementation of inter-occasion variability as well as stochastic differential equations were proposed.

The performance of estimation methods available for discrete models was also explored. We have studied how Laplace behaves in situations with non-even distributions of ordered categories as well as for different Poisson-type models. In another study, the accuracy of parameter estimation with SAEM and importance sampling was compared to the one of Laplace in repeated time-to-event models where the frequency of individuals with events was low. We have also conducted a study investigating all methods available in NONMEM 7 for all types of discrete models.

We also introduced to the field the item response theory (IRT) approach, allowing to connect subscores of a composite scale to a continuous latent disease variable. Such models have now been applied in the group to Alzheimer's (with ADAS-cog), Schizophrenia (with PANSS), multiple sclerosis (with EDSS), and we are presently working with Parkinson's (with UPDRS) and COPD (with EXACT).

Currently, we are interested in the implementation of hidden Markov models (HMM) in NONMEM, enabling to characterize non-observed discrete stages. Through simulations, we demonstrate how time and drug effects can be investigated on the transition probability from a disease state to another and how correlation can be explored in bivariate models. An ongoing application for HMMs concerns the exploration of antidrug antibodies (see *Pharmacometric modelling of biologic medicinal products*).

(V) Mechanism-based pharmacokinetic models (Martin Bergstrand, Mats Karlsson)

Clinical pharmacokinetic experiments typically measure drug concentrations in plasma only. As a consequence, pharmacokinetic models, used in drug development, aim to describe observations of drug concentration in plasma with minimum model complexity. Such models have limited capacity for extrapolations and to predict concentration-time profiles in tissues and organs. Also, mechanistic insight about drug disposition dependence of factors related to individual organs and tissues may not be possible to incorporate in a fully satisfactory manner. Physiologically-based pharmacokinetic (PBPK) models, which have a structure based on anatomy, can provide predictions in tissues and organs. However, because of their complexity, such models are traditionally not used for analysing clinical data. We have demonstrated that PBPK model parameters can be estimated based on clinical observations and are currently investigating the possibilities for improving such a combined "bottom-up and top down approach". We have showed that for a relatively simple PBPK model such analyses can become feasible by using informative prior information about physiology and drug-related parameters. To further improve such an approach we are combining information about (co-)variability in organ and tissue properties from a data base representing physiological values for about 30000 subjects, tissue composition models and models for relating drug molecular properties and in vitro data to expected behaviour in tissues and organs.

A mechanism-based approach has also been applied to better understand the processes involved in oral absorption. Several factors influencing oral absorption vary along the GI tract, e.g. pH, active influx and efflux transporters, gut wall metabolism. Models characterizing gastro intestinal transit and absorption properties along the GI region can be especially useful in the case of modified release formulations and/or substances with slow dissolution rate. Mechanism based models have been used for prospective population predictions of plasma concentrations based on in vitro dissolution data.

(VI) Pharmacometric modelling of biologic medicinal products (Siv Jönsson, Andrew Hooker, Mats Karlsson, Elodie Plan)

Biological medicinal products are becoming an important contributor in the treatment of many diseases, e.g. multiple sclerosis, rheumatoid arthritis, cancer, psoriasis. Characterization of biologics benefit from pharmacometric modelling, since they exhibit complex disposition characteristics, quite different to the processes and pathways utilized for small molecules, e.g. monoclonal antibodies exhibit target mediated drug disposition (TMDD).

Available TMDD models describe the formation of one complex (a dimer), but in reality further complexes may be formed (trimers, hexamers, etc.), as described for IgE and omalizumab. We aim to explore and develop alternative TMDD models for the interaction between a target and drug, taking into account formation of different complexes. Furthermore, to explore study design options for studies in different stages of drug development, optimal design methodology is applied to TMDD models.

A complicating factor for biologics is the occurrence of antidrug antibodies (ADA), which may affect the pharmacokinetic and efficacy features. The identification of ADAs is usually confounded by the presence of the drug itself and therefore the result from an analysis is that ADA is present, absent or unknown (missing information). Thus, there is a need to develop adequate methods to incorporate the ADA information in pharmacometric models. Currently, we are using mixed hidden Markov models (MHMM) to model the underlying unobservable ADA states (see Pharmacodynamic modelling of discrete outcomes).

Applied research/Disease areas

(I) Antibiotics (Lena Friberg, Mats Karlsson, Elisabet Nielsen)

Antibiotics are considered one of the greatest discoveries of modern therapeutic medicine and have turned previously fatal diseases into treatable minor illnesses. Today, treatment failures due to multidrug-resistant bacteria are becoming more frequently observed. The evolution of resistance is a natural phenomenon; however, the use and misuse of antibiotics will accelerate this phenomenon. We aim to advance the understanding of pharmacokinetic-pharmacodynamic relationships for antibiotics of value for a more streamlined drug development process and an improved therapeutic use of clinically available antibiotics that maximize the efficacy and minimize resistance development.

Today, dosing regimens are typically selected based on PK/PD indices that discard information on dynamic changes in the drug-bacteria interaction. Mechanism-based models describing time-kill curves from in vitro experiments form the basis for the modelling. The models have shown to be applicable across drugs and bacteria strains (including clinical isolates), for both static and dynamic concentration experiments, for different sizes of start inocula, for mixtures of wild-type and resistant bacteria, for drug combinations, and for predicting competition experiments of wild-type and mutants. Based on developed models, optimal experimental design techniques are applied to find experimental protocols that increase the efficiency of both pre-clinical and clinical studies. Typically, the PKPD characterisation of antibiotics is done based on pre-clinical data and high performing translational methods are thus central in the assessment of appropriate drug use. While the methodology based on PK/PD indices has been shown to be sensitive to experimental design, misspecification of the MIC, and differences in PK characteristics, the use of a mechanism-based PKPD modelling approach in dose selection has been suggested for increased robustness and extrapolation potential, especially for special patient populations. To further increase the translatability of pre-clinical results, our current research aim to incorporate the effect of the innate immune response in the model predictions. Pharmacometric models are developed describing the activation and effect of the innate immune system following endotoxin and/or bacterial exposures. Such models could be combined with the PKPD models and help to improve the understanding of the development of manifest inflammations/infections and how dosing regimens should be designed to optimize the efficacy and at the same time minimize the emergence of resistance.

Polymyxins have regained interest in recent years to overcome antibiotic drug resistance. We have assayed clinical data on colistin and CMS using an in-house developed LC-MS-MS method to understand the PK in different patient populations and the need for a loading dose. Whole-body Physiology-based Pharmacokinetic (WBPBK) models for CMS, colistin and ciprofloxacin have been developed based on data from various sources, including patients, healthy volunteers and several animal species. Such models can be used to understand the time-courses of the antibiotics, and thereby the bacterial killing, in different tissues.

One strategy to overcome and prevent emergence of resistance is to use antibiotic combination therapy. However, the search for effective combinations is challenging given the number of permutations of doses

that could be tested. The use of alternative methods, such as digital time-lapse microscopy, to economically screen for potentially effective combinations are currently investigated. Further, for combinations, the drug-drug concentration ratio will vary over time depending on the PK profiles of the drugs. In this situation the use of mechanism-based modelling, that describes the combined effect on the bacterial killing, taking the time-aspect of PK as well as PD into account, is highly advantageous. The developed models can hence facilitate the translation of in vitro information to in vivo.

(II) Infectious diseases (Martin Bergstrand, Mats Karlsson, Ulrika Simonsson)

Plasmodium falciparum, the human immunodeficiency virus (HIV), and Mycobacterium tuberculosis (TB) are three devastating pathogens in tropical areas. Due to the geographical overlap of malaria, HIV and TB prevalence, the diseases are likely to co-exist in a great number of individuals. For these individuals, there is an obvious need for concomitant use of antimalarial, antiretroviral and antitubercular drugs. Drug-drug interactions may result from concurrent administration of drugs leading to diminished therapeutic efficacy of or increased toxicity from one or more of the administered drugs. Drug-drug interactions are an important concern in the management of patients with HIV because of the large number of antiretroviral drugs and other drugs that are required by these patients for the management of co-morbidities and opportunistic infections. Combination therapy has also been introduced in the management of malaria and TB, to overcome drug resistance. The exposure response relationships are not fully understood for the management of malaria and TB whereas there is more knowledge in the field of HIV. Better understanding of the exposure response relationships in malaria and TB is urgently needed. Our research focus also in suggesting dosing recommendations for children based on scaling from adult data or evaluation of pediatric data. Limited information is available in the literature on drug-drug interactions between the artemisinin antimalarial drugs and other drugs such as antiretrovirals or antitubercular drugs; consequently, the extent of such interactions is not fully known.

Activities of CYP enzymes and consequently drug-drug interactions occurring due to their inhibition or induction can be studied by using probe drugs. The pharmacokinetics of probes and drugs under investigation can be described by mathematical models in order to characterise and quantify the interaction.

We have developed enzyme turnover models to describe the time course of induction of different CYP450 enzymes by different artemisinin derivatives. One focus of the work has been to compare the potential for drug-drug interactions among the artemisinin drugs to choose a derivative that is suitable for combination therapy from a drug-drug interaction perspective.

One commonly used antitubercular drug is rifampicin which is known to induce CYP450 enzymes. Our work has involved quantitative analysis by modelling the pharmacokinetics of other drugs metabolised by these enzymes and which are used in HIV treatment. The need for potential dose adjustment has been evaluated for example nevirapine and lopinavir. For example, the population pharmacokinetics of nevirapine in HIV-infected patients taking nevirapine-based antiretroviral therapy in the presence and absence of the antitubercular drug rifampicin has been evaluated.

Pharmacokinetic drug-drug interactions can possibly be compensated for by dose adjustment of the target drug. The developed nevirapine model was used for simulations of different doses of nevirapine which revealed that increasing the dose of nevirapine to 300 mg twice daily elevated nevirapine concentrations above sub-therapeutic levels in most patients, with minimum exposure above the recommended maximum concentration. We have also investigated the population pharmacokinetics of lopinavir in TB/HIV co-infected children taking lopinavir/ritonavir in a ratio of 1:1 in the presence of the antitubercular drug rifampicin, with that of lopinavir in HIV-infected children taking lopinavir/ritonavir in a ratio of 4:1. Increasing the ritonavir dose in the TB/HIV co-infected children resulted in model predicted lopinavir trough concentrations above the recommended minimum therapeutic concentration.

Malaria was estimated to cause 800,000 deaths and 225 million cases worldwide in 2010. The mortality has recently been decreasing and is expected to decrease further due to more widespread use of effective treatment with drugs from the artemisinin class. However, a possible emerging resistance to these drugs might counteract this positive development. Drug resistance has appeared repeatedly within the area of

malaria chemotherapy and drastically hampered our ability to fight the disease. It has been hypothesised that such development could have been avoided and or delayed with a better treatment regimen. We are conducting research with the aim of optimising anti-malarial treatment regimens with regards to both short and long-term outcome. Pharmacometric models have been used for translational simulations of expected treatment outcome in vulnerable populations such as children and pregnant and to optimize the treatment regimen.

(III) Type 2 Diabetes (Mats Karlsson, Maria Kjellsson)

Diabetes is a chronic disease, affecting more than 220 million people worldwide and the "diabetic epidemic" is projected to affect 366 million people by 2030, of which more than 90% will suffer from type 2 diabetes (T2D). T2D occurs when the body does not effectively respond to insulin and is unable to produce enough insulin to account for the inefficient use of insulin. The result is elevated blood glucose which is toxic and eventually lead to complications; e.g. cardio-vascular diseases (CVD) and chronic kidney disease (CKD). The aim with most anti-diabetic treatment is symptomatic, bringing glucose down to healthy concentrations. Diagnosis of diabetes is mainly based on fasting plasma glucose (FPG) but also glycosylated haemoglobin (HbA1c). The success of treatments is assessed on both FPG and HbA1c but also on dynamic glucose after provocation studies. Our research stands on three legs: 1) models of dynamic glucose after glucose provocations, 2) models of dynamic HbA1c and 3) models of long-term complications.

Provocation studies are used to characterize the functionality of the glucose-insulin system and could vary greatly in design from clamping of glucose or insulin by variable rate infusions, graded glucose infusions, intravenous bolus or oral administration of glucose or meals challenges. We have developed several integrated models with simultaneous analysis of dynamic glucose and insulin after such provocations. These models, which include production, disposition and control (homeostatic) mechanisms of the system, have shown to realistically simulate outcomes of short dynamic glucose provocations at the raw data level. These models have been developed to describe both healthy and pre-diabetic subjects as well as patients with T2D and have been coupled with models of incretin hormone secretion, sub-cutaneous insulin absorption, glucose gut absorption, pre-hepatic insulin and hepatic extraction ratio of insulin. We are focusing our current research on including mechanism of glucagon release and glucagon effects on glucose and insulin as well as a fully mechanistic whole-body integrated glucose homeostasis model. This model should include elements of disease progression from healthy to overtly diabetic through the pre-diabetic state.

Long-term clinical trials in T2D patients mainly focus on HbA1c. HbA1c is the fraction of the haemoglobin, in red blood cells (RBC) that has been glycosylated. This is a naturally occurring reaction depending on the amount of glucose in plasma; the higher the glucose concentration, the higher the HbA1c. As the life-span of RBCs ranges from 2 to 4 months, the HbA1c supplies a measurement of the sustained glycaemic control. Several models have been developed in-house to describe the relationship between HbA1c and FPG or mean plasma glucose (MPG) either using empirical descriptions or mechanistic approaches using knowledge about RBC life-span. Also models acknowledging the mechanism of insulin sensitivity, glucose production and disposition and changes in beta-cell mass or function in relation to weight loss has been developed. Currently we are investigating how study design can be improved using model-based analysis.

The overall endpoint of most anti-diabetic treatments is to lower the risk of long-term complications, such as CVD, retinopathy and CKD. Long term studies commonly involve assessments of the risk of CVD or CKD progression in relation to elevated levels of HbA1c or FPG. We are developing parametric risk models, using data from the National Diabetes Registry (NDR, Sweden) to characterise the relationship between CVD/CKD and time-varying covariates such as HbA1c and other biomarkers, i.e. blood pressure and weight, with the aim to predict CVD/CKD after changes in key biomarkers.

All models have been developed for the purpose of being used to quantify changes in the system following interventions (drug administration, diet changes, etc.) and associate these changes with known or hypothesized mechanisms of impact of the system. Further the models are intended as tools for

hypothesis generation regarding single or combined interventions as well as clinical trial design optimization.

(IV) Oncology (Lena Friberg, Mats Karlsson)

Within the oncology area, we are working on PK and PKPD models describing the time-courses of biomarkers, drug-induced toxicity, tumor size measurements, tumor activity measurements (SUV, standard uptake value) and overall survival. The models are aimed to be mechanism-based and be applicable across different drugs, and thereby valuable in development of new and existing drugs, including individualization of the therapy. By integrating information of different variables into a modelling framework the variables' relations and predictive value can be tested, and a better overview of both desired and adverse effects from a changed dosing regimen can be obtained. The models can also be used to explore different concepts of study design in oncology. This type of modelling framework, including biomarkers, side-effects, tumor response and survival, has been developed for sunitinib in gastrointestinal stromal tumors and for axinitib in renal cell carcinoma. Different metrics of tumor size, both constant and time-varying, as well as one dimension (diameter) vs. three-dimensional (volume) are evaluated for predicting overall survival. We also have ongoing projects around immuno-therapies, e.g. in quantifying potential biomarkers and their relationship to tumor growth and shrinkage.

Projects are on-going around extensions and applications of a semi-physiological model that can describe neutropenia for numerous anticancer drugs. We have for example quantified the relationship between IL-6, CRP and febrile neutropenia. For TDM-1, an integrated model that includes platelets and the liver enzymes ASAT and ALAT has been developed, which has been applied to explore alternative dosing regimens. For TDM-1, we are also developing models for Patient Reported Outcomes (PRO), which is an increasingly used component for comparison of drug treatments during oncology drug development, by application of Item Response Theory.

Different design aspects are also being evaluated, e.g. how the frequency of neutrophil counts affect the possibility to predict nadir and myelosuppression recovery and how the tumor measurement frequency influence the predictive capacity of OS, as well as how model-based adaptive designs could be valuable for efficient characterization of drug combinations.

(V) Progressive disorders (Lena Friberg, Andrew Hooker, Mats Karlsson, Elodie Plan, Ulrika Simonsson)

Progressive neurological disorders such as Alzheimer's disease and Parkinson's disease represent challenges in many respects. The slow progression may make it difficult to assess in which aspect -a disease-modifying or symptomatic manner- the treatment impacts the disease. How to best design and analyse trials in these disorders, but also for diseases with relapsing progression patterns such as Multiple Sclerosis and Schizophrenia, are the topics of our investigations.

Further, the severity of most of these diseases are followed over time using clinical scoring scales that are formed from tests or questions probing individual aspects of the disease. Rather than using the overall clinical scale, we are developing methodologies and models based on item response theory (IRT), where each item (i.e. test or question) is modelled individually as a function of a latent variable of the disease-related disability.

(VI) Immunological disorders (Lena Friberg, Mats Karlsson)

Multiple Sclerosis is both a complex and chronic neurological disease of the CNS. The natural course of MS is slow and difficult to monitor clinically. The overall aim of this project is to establish the first population, data driven, MS disease progression model in order to construct a mathematical modelling platform where the interplay between the majority of relevant aspects of the disease, such as time course of disability progression, relapse rate dynamics, time course of the imaging data, time course of lymphocytes and population characteristics are incorporated. Model building involves sequential development of (i) separate models for all components of interest (disease progression, relapse rate dynamics, MRI dynamics and lymphocytes including CD4+, CD8+) and (ii) the covariate model, which

explore the underlying patient factors influencing within- and between-subject variability in treatment response. The final anticipated model shall enable simultaneous characterization of the interplay between relapse rate dynamics, total CD4+ and CD8+ lymphocytes and their ratio, and MRI readout dynamics in the evolution of disease and, most importantly, link the time-course of MRI and clinical outcome (relapse rate and disability) and lymphocyte data.

In rheumatoid arthritis a range of variables of the disease are summarized into a clinical endpoint for evaluation of drug response - the dichotomous ACR20 score. Integrated longitudinal transition models with dropout are useful for understanding the outcome of different dosing schedules and by expanding such models to also include the more stringent ACR70 criteria more information can be preserved. To increase the information on the concentration-effect relationship in the available data, a longitudinal transition model describing the probability of ACR20, ACR50 and ACR70 responses have been developed.

(VII) Model-based individualized drug therapy (Siv Jönsson, Mats Karlsson, Elisabet Nielsen)

Variability in pharmacokinetics and pharmacodynamics are common and might result in suboptimal treatment in the individual patient. In our research we aim to facilitate model-based treatment individualization and advance the methodologies used for dose individualization based on therapeutic drug monitoring (TDM). Ongoing projects relate to dose individualization of antibiotics in critically ill patients, levodopa treatment in patients with Parkinson's disease and Factor VIII replacement therapy in Haemophilia A patients.

Hemophilia is a group of hereditary genetic disorders impairing blood coagulation. Hemophilia A and B refer to the coagulation factor VIII and IX deficiency, respectively, whereof hemophilia A is the more common although rare: in Sweden 14 of 100 000 boys and men have hemophilia, whereof 80 % has hemophilia A. By substitution therapy with the coagulation factors, on-demand or prophylactically, the disease status can be controlled. In prophylactic treatment of factor VIII and IX, pharmacokinetic (PK) tailored dose individualization, i.e. the individual dose is based on Bayesian estimation using pharmacokinetic information and a population PK model, is promoted and appears to be a good approach for reducing the total doses administered. We are involved in a clinical study where the benefit of PK tailored dosing for prophylactic FVIII treatment is evaluated in clinical practice. Our contribution in the project refers to the Bayesian estimation of the dose. We will also explore alternative approaches in the dose individualization involving exposure-response models. Furthermore, for factor IX we have re-developed a population PK model and, based on the model, explored alternative clinical practically sampling schedules to be used in PK tailoring.

Research area: Steroid Biochemistry

Maria Norlin & Kjell Wikvall

http://farmbio.uu.se/research/researchgroups/farmaceutisk-cellbiologi/steroidbiokemi/

Our research is focused on the properties and regulation of enzymatic processes involving steroids. Steroids and steroid-related genes and enzymes, producing biologically active metabolites, are vital for human physiology. Many steroids or steroid-related compounds are used as drugs. We apply our expertise in biochemistry and molecular biology to obtain knowledge of relevance for drug discovery and development and drug management. In particular we focus on steroids. We study metabolic conversions, gene regulation and cellular effects of steroids. Primary missions and goals are to find new drug targets, understand the mechanisms for effects of steroids and steroid-related enzymes, compare effects of endogenous molecules and drugs, examine effects of drugs and drug candidates on steroid-related processes and study molecular mechanisms behind adverse drug effects. Our research activities, focusing on basic research, provide important information that increases the understanding of mechanisms for steroid action. This should improve drug management (e.g. counteract/avoid adverse side-effects) and can be used for development of novel, better drugs.

Members of the group during 2016

Maria Norlin, PhD, Associate Professor
Kjell Wikvall, MD, PhD, Professor
Ida Emanuelsson, PhD student
Mokhtar Almokhtar, PhD student
Ahmad Zayny, PhD student
Kerstin Rönnqvist, research assistant
Lisa Fredriksson Puigvert, lecturer
Tomas Friman, lecturer
Emelie Karlsson, lecturer
Abdulkader Aljabar, trainee

Publications 2014 – 2016

1. Wicher G, Norlin M. Estrogen-mediated regulation of steroid metabolism in rat glial cells; effects on neurosteroid levels via regulation of CYP7B1-mediated catalysis. *J Steroid Biochem Mol Biol. 145, 21-27 (2015)*
2. Almokhtar M, Wikvall K, Ubhayasekera SJ, Bergquist J, Norlin M. Motor neuron-like NSC-34 cells as a new model for the study of vitamin D metabolism in the brain. *J Steroid Biochem Mol Biol. 158:178-188 (2016)*
3. Olsson PO, Gustafsson R, in 't Zandt R, Friman T, Maccarana M, Tykesson E, Oldberg Å, Rubin K, Kalamajski S. The Tyrosine Kinase Inhibitor Imatinib Augments Extracellular Fluid Exchange and Reduces Average Collagen Fibril Diameter in Experimental Carcinoma. *Mol Cancer Ther. 15: 2455-2464 (2016)*
4. Wegler C, Wikvall K, Norlin M. Effects of osteoporosis-inducing drugs on vitamin D-related gene transcription and mineralization in MG-63 and Saos-2 cells. *Basic Clin Pharmacol Toxicol. 119: 426-442 (2016)*
5. Norlin M, Lundqvist J, Ellfolk M, Hellström Pigg M, Gustafsson J, Wikvall K. Vitamin D_3-metabolism in primary human dermal fibroblasts: drug-mediated gene regulation. *Basic Clin Pharmacol Toxicol.* 120: 59-63 (2017) *doi: 10.1111/bcpt.12612. Epub 2016 May 20*

Other commitments/assignments of staff members 2016

Kjell Wikvall: Chair of the Scholarships committee for the Faculty of Pharmacy;

Member of the Scholarships committee for Uppsala University.

Maria Norlin: Senior member of the Departmental Committee for PhD education (FUG); Study director in Pharmaceutical Biochemistry.

Tomas Friman: Member of the Electronic literature (e-literature) Committee.

Projects

(I) Functions of steroids and steroid-metabolizing enzymes for hormonal signalling and cellular viability

This research concerns steroids involved in hormonal signalling, sex hormone biosynthesis and brain function. The studies are focused on physiological and pharmacological control of steroid levels, effects of metabolic events and regulation of gene expression. The project concerns endogenous steroids, including vitamin D, steroid drugs and drugs affecting steroid hormone receptors. The studies include mechanisms of importance for estrogenic and androgenic signalling. Enzymes that regulate the concentration of neuroactive steroids in the brain may be future targets for therapy of importance for abnormal cell growth, immune function or in neurodegenerative conditions. Some of our current studies involve enzymes and genes of importance for the levels of neurosteroids in neurons and glial cells. Regulation of hormone metabolism in the nervous system by endogenous steroids and pharmaceutical compounds is also studied.

Steroids may affect growth and differentiation in several tissues. Thus properties of steroids may be of interest in a wide range of normal and disease conditions, e g in neuroprotection or cancer therapy. We study effects of steroids such as enzymatically formed oxysterols (cholesterol derivatives), hormones including vitamin D and vitamin D-like compounds on cellular survival and growth. These studies particularly focus on cells of the central nervous system.

(II) Bioactivation and metabolism of vitamin D including vitamin D-mediated effects on cellular function

This research is focused on enzymes and genes of importance for vitamin D bioactivation, metabolism and function. Effects on these processes by endogenous and pharmacological compounds are studied. Many drugs result in adverse side-effects, including increased risk of bone disease. We study the mechanisms by which anti-inflammatory, anti-viral and anti-epileptic drugs affects cells, with particular focus on the bone and the central nervous system. Vitamin D is needed for regulation of calcium levels in the body and vitamin D deficiency leads to skeletal diseases such as rickets in children and osteomalacia/osteoporosis in adults. The biologically active form, 1α,25-dihydroxyvitamin D3, is formed through metabolic activation. The activated form of vitamin D blocks cell division and increases cell differentiation. Vitamin D analogues (synthetic compounds with vitamin D-like effects) are used in the treatment of psoriasis and are of interest in development of new cancer therapy. In addition, epidemiological data during recent years have indicated that vitamin D may have many more targets than previously known. Altered vitamin D levels in plasma have been linked to a number of different conditions including neuropsychiatric and neurodegenerative disease. For these reasons, it is important to obtain more knowledge about the enzymes and genes in activation and metabolism of vitamin D and the roles of the formed metabolites. Part of this project is focused on cellular effects of vitamin D and vitamin D analogues in order to explore previously unknown functions of these compounds.

Research area: Translational Pharmacokinetics/ Pharmacodynamics

Margareta Hammarlund-Udenaes

http://farmbio.uu.se/research/researchgroups/tPKPD

Our research focuses on understanding the fundamental and functional aspects of pharmacokinetics-pharmacodynamics (PKPD) in health and disease. Within the area of translational PKPD we address conversion of pharmacokinetic data from the preclinical to the clinical setting. This is strongly needed for optimizing drug discovery processes and promoting efficient treatment of CNS diseases. We are interested in brain drug delivery in relation to transport processes of both small and large molecules across the blood brain barrier (BBB), where a quantitative role of nanocarriers is specifically studied. Using the concepts and methodologies developed for the brain we are also exploring drug distribution in the lung. This is important for the design and evaluation of lung targeted drugs which may be administered by inhalation. For this purpose, methods are being developed to help analyzing the pharmacologically relevant unbound concentrations in brain and other tissues. Our work also emphasizes the importance of bridging the expertise within academia and pharmaceutical industry in order to seek excellence in method development for better therapeutics treating CNS and lung disorders.

Members of the group during 2016

Jessica Dunhall, Laboratory Assistant
Markus Fridén, Adjunct Lecturer
Pia Frisk, MSc in Pharmacy, PhD student
Sofia Gustafsson, MSc in Biomedicine, PhD student
Margareta Hammarlund-Udenaes, PhD, Professor
Yang Hu, MSc in Pharmacy, PhD student
Irena Loryan, MD, PhD, Researcher
Erik Melander, MSc in Pharmacy, PhD student
Nebojsa Mihajlica, MSc in Pharmacy, PhD student
Johanna Sulku, MSc in Pharmacy, PhD student

Publications 2014 – 2016

1. Loryan I, Sinha V, Mackie C, Van Peer A, Drinkenburg W, Vermeulen A, Morrison D, Monshouwer M, Heald D, Hammarlund-Udenaes M. Mechanistic understanding of brain drug disposition to optimize the selection of potential neurotherapeutics in drug discovery. Pharm Res. 31(8):2203-19 (2014).
2. Alassaad A, Bertilsson M, Gillespie U, Sundström J, Hammarlund-Udenaes M, Melhus H. The effects of pharmacist intervention on emergency department visits in patients 80 years and older: subgroup analyses by number of prescribed drugs and appropriate prescribing. PLoS One. **9**(11) (2014).
3. Chen X, Loryan I, Payan M, Keep RF, Smith DE, Hammarlund-Udenaes M. Effect of transporter inhibition on the distribution of cefadroxil in rat brain. Fluids Barriers CNS. 11(1):25 (2014).
4. Friden M, Wennerberg M, Antonsson M, Sandberg-Stall M, Farde L and Schou M. Identification of positron emission tomography (PET) tracer candidates by prediction of the target-bound fraction in the brain. EJNMMI research 4:50 (2014).
5. Varadharajan S, Winiwarter S, Carlsson L, Engkvist O, Anantha A, Kogej T, Friden M, Stalring J and Chen H. Exploring In Silico Prediction of the Unbound Brain-to-Plasma Drug Concentration Ratio: Model Validation, Renewal, and Interpretation. Journal of pharmaceutical sciences (2014).

6. Alassaad A, Melhus H, Hammarlund-Udenaes M, Bertilsson M, Gillespie U and Sundstrom J. A tool for prediction of risk of rehospitalisation and mortality in the hospitalised elderly: secondary analysis of clinical trial data. BMJ open 5:e007259 (2015).
7. Backstrom E, Lundqvist A, Boger E, Svanberg P, Ewing P, Hammarlund-Udenaes M and Friden M. Development of a novel lung slice methodology for profiling of inhaled compounds. J Pharm Sci (2015) [e-pub ahead of print].
8. Loryan I, Sinha V, Mackie C, Van Peer A, Drinkenburg WH, Vermeulen A, Heald D, Hammarlund-Udenaes M and Wassvik CM. Molecular properties determining unbound intracellular and extracellular brain exposure of CNS drug candidates. Mol Pharm 12:520-532 (2015).
9. Sadiq MW, Uchida Y, Hoshi Y, Tachikawa M, Terasaki T and Hammarlund-Udenaes M. Validation of a P-Glycoprotein (P-gp) Humanized Mouse Model by Integrating Selective Absolute Quantification of Human MDR1, Mouse Mdr1a and Mdr1b Protein Expressions with In Vivo Functional Analysis for Blood-Brain Barrier Transport. PloS One 10:e0118638 (2015).
10. Boger E, Ewing P, Eriksson UG, Fihn BM, Chappell M, Evans N and Friden M. A novel in vivo receptor occupancy methodology for the glucocorticoid receptor: toward an improved understanding of lung pharmacokinetic/pharmacodynamic relationships. J Pharmacol Exp Ther353:279-287 (2015).
11. Loryan I, Melander E, Svensson M, Payan M, Konig F, Jansson B, and Hammarlund-Udenaes M, In-depth neuropharmacokinetic analysis of antipsychotics based on a novel approach to estimate unbound target-site concentration in CNS regions: link to spatial receptor occupancy. Mol Psychiatry 21, 1527-1536 (2016).
12. Lindqvist A, Jonsson S, and Hammarlund-Udenaes M, Exploring Factors Causing Low Brain Penetration of the Opioid Peptide DAMGO through Experimental Methods and Modeling. Mol Pharm 13, 1258-1266 (2016).
13. Lindqvist A, Friden M, and Hammarlund-Udenaes M, Pharmacokinetic considerations of nanodelivery to the brain: Using modeling and simulations to predict the outcome of liposomal formulations. Eur J Pharm Sci 92, 173-182 (2016).
14. Backstrom E, Lundqvist A, Boger E, Svanberg P, Ewing P, Hammarlund-Udenaes M, Friden M, Development of a Novel Lung Slice Methodology for Profiling of Inhaled Compounds. Journal of pharmaceutical sciences 105, 838-845 (2016).
15. Melander E, Eriksson C, Jansson B, Goransson U, and Hammarlund-Udenaes M, Improved method for quantitative analysis of the cyclotide kalata B1 in plasma and brain homogenate. Biopolymers 106, 910-916 (2016).
16. Backstrom E, Boger E, Lundqvist A, Hammarlund-Udenaes M, and Friden M, Lung Retention by Lysosomal Trapping of Inhaled Drugs Can Be Predicted In Vitro With Lung Slices. Journal of pharmaceutical sciences 105, 3432-3439 (2016).
17. Lindqvist A, Rip J, van Kregten J, Gaillard PJ, and Hammarlund-Udenaes M, In vivo Functional Evaluation of Increased Brain Delivery of the Opioid Peptide DAMGO by Glutathione-PEGylated Liposomes. Pharm Res 33, 177-185 (2016).

Reviews, books and book chapters 2014 – 2016

1. Hammarlund-Udenaes M., De Lange E.C., and Thorne R.G., Eds. Drug delivery to the brain. Physiological concepts, methodologies and approaches, Springer, New York Heidelberg Dordrecht London, 2014.
2. Hammarlund-Udenaes M. Pharmacokinetic Concepts in Brain Drug Delivery. In M. Hammarlund-Udenaes, E.C.M. de Lange, and R.G. Thorne (Eds.), Drug Delivery to the Brain Physiological concepts, methodologies and approaches, Springer, New York, Heidelberg, Dordrecht, London, 2014, pp. 127-161.
3. Hammarlund-Udenaes M. In Vivo Approaches to Assessing the Blood–Brain Barrier. In G. Fricker, M. Ott, and A. Mahringer (eds.), The blood-brain barrier (BBB), Springer Berlin Heidelberg, Heidelberg, New York, Dordrecht, London, 2014, pp. 21-48.
4. Loryan I. and Hammarlund-Udenaes M. Drug Discovery Methods for Studying Brain Drug Delivery and Distribution. In M. Hammarlund-Udenaes, E.C.M. de Lange, and R.G. Thorne (eds.), Drug Delivery to the Brain Physiological concepts, methodologies and approaches, Springer New York, Heidelberg, Dordrecht, London, 2014, pp. 271-316.

5. de Lange EC and Hammarlund-Udenaes M. Translational aspects of blood-brain barrier transport and central nervous system effects of drugs: from discovery to patients. Clin Pharmacol Ther 97:380-394 (2015).
6. Hammarlund-Udenaes M, Intracerebral microdialysis in blood-brain barrier drug research with focus on nanodelivery. Drug Discov Today Technol 20, 13-18 (2016).

Agencies that supported the work/Funding 2016

Swedish Research Council; AstraZeneca; Grünenthal

Other commitments/assignments of staff members 2016 (Update)

Margareta Hammarlund-Udenaes: Associate Editor of Pharmaceutical Research, Editorial Advisory Board member of the Journal of Pharmaceutical Sciences and of Fluids and Barriers of the CNS, Member of the Executive Committee of the European Federation of Pharmaceutical Sciences with responsibility regarding Education and Training 2015 - 2016. Vice Chair of the Research Education Committee of the area of Medicine and Pharmacy - 2015, Member of the Committee of the Faculty of Pharmacy.

Projects

(I) Concepts and method development regarding drug delivery to the brain (Irena Loryan and Margareta Hammarlund-Udenaes)

Drug transport across the BBB and cellular barriers with subcellular distribution in the brain parenchyma are key processes of interest. Moreover, brain pharmacokinetic processes are also investigated at the level of its regions. In order to enhance the mechanistic understanding of brain target-site pharmacokinetics, several advanced methods are being developed by the group. Neuropharmacokinetic parameters such as unbound brain-to-plasma concentration ratio (Kp, uu, brain), unbound volume of distribution in brain (Vu,brain), and permeability clearance into the brain (CLin), are descriptors of BBB function and intracerebral distribution established by the group. Kp, uu, cell is another unique parameter characterizing the extent of cellular barrier transport. These parameters are identified by means of systematic PKPD analyses.For quantitative evaluation of BBB transport we are using cerebral microdialysis, recognized as the “gold standard” for measurement of unbound drug concentration in the brain. An alternative strategy is based on combination of high-throughput in vitro techniques, such as brain homogenate equilibrium dialysis and brain slices.

(II) Translational aspects of brain drug distribution in health and disease (Sofia Gustafsson, Irena Loryan and Margareta Hammarlund-Udenaes in collaboration with Stina Syvänen and Martin Ingelsson, Dept Public Health and Caring Sci., Uppsala Univ)

The interpretation and translation of neuropharmacokinetic data might be even more challenging during disease conditions, where a disrupted integrity and function of the BBB is apparent. Recent findings even point towards dysfunctional BBB as being the cause of neurodegenerative disease etiology and progression. As a result, dysfunction in BBB processes might lead to altered brain pharmacokinetics of CNS drugs as well as peripherally acting drugs, which normally have a very low brain penetrance, resulting in unpredicted CNS effect or side effect profiles. Moreover, CNS disorder pathology usually affects certain areas of the brain, which might result in regional differences of brain drug distribution and binding.

Hence, our research aims to investigate differences in drug distribution to and within the brain as well as drug binding in separate brain regions both in health and disease, preclinically and clinically. By combining and comparing in vitro and in vivo experiments with clinical studies the current project strives to increase the understanding of pharmacokinetics and disease implication on brain drug distribution. Results from in vitro techniques as well as microdialysis and non-invasive imaging techniques, such as positron emission tomography (PET), is integrated and used to address the current issues.

(III) The role of pericytes for brain drug distribution (Nebojsa Mihajlica, Margareta Hammarlund-Udenaes in collaboration with Christer Betsholtz, Dept Immunol, Genet and Pathol, Uppsala Univ).

Endothelial cells of the BBB represent a key component of the neurovascular unit (NVU), which also includes other types of cells such as pericytes, astrocytes, vascular smooth muscle cells, microglia and neurons.

Although the anatomical relationship between pericytes and endothelial cells suggests their close interaction, the relative contribution of the pericytes is still not sufficiently elucidated. Interaction between cells within the NVU is essential for the normal functioning of the central nervous system, whilst impairments in their communication can result in the development of many pathological conditions.

The principal aim of this project is to improve our understanding of the mechanisms of the NVU regarding the drug distribution into brain parenchyma, with special emphasis on the contribution of pericytes. Hence, better understanding of pericyte role in BBB regulation and drug distribution into the CNS has a great potential for the improvement of current pharmacotherapy of neurodegenerative diseases and other pathological conditions associated with BBB impairment.

(IV) *Biomolecular drugs and nanocarriers (Annika Lindkvist, Erik Melander, Yang Hu and Margareta Hammarlund-Udenaes in collaboration with Drs Elizabeth de Lange, Leiden Univ, Pieter Gaillard and Jaap Rip, to-BBB, Leiden, the Netherlands, and Ulf Göransson, Div Pharmacognosy, Uppsala Univ)*.

Peptides and proteins play a crucial role in the regulation of brain activity in health and disease conditions. They are therefore promising candidates in the development of new neurotherapeutics. Understanding the use of large molecules and their interaction with the barriers of the CNS is crucial in order to succeed in the clinic.

One promising group of peptides is the cyclic peptides originating from different plant families. These peptides exhibit exceptional stability due to a cyclic cysteine knot forming disulfide bonds. Their cyclic nature makes them less susceptible to degradation by proteases, causing a great interest from a drug development perspective.

Our research focuses on the pharmacokinetics of brain delivery of peptides and the possible quantitative benefits of using nanocarrier systems, including their effect on the pharmacodynamic outcome. In vivo preclinical studies are performed to estimate the modulation of penetration across the BBB using targeted liposomes. For authentication of the principles, physiologically based pharmacokinetics population modeling is applied.

(V) Optimizing brain penetration of drug candidates (Irena Loryan and Margareta Hammarlund-Udenaes, in collaboration with Dr Eddie Hoppe, Grünenthal, Aachen, Germany)

The combinatory mapping approach developed by our group can be used as a BBB screening toolbox for selection of candidate drugs in early drug discovery by pharmaceutical companies.

In addition, a present project is focused on identification of desirable physicochemical properties for CNS penetration using computational models, aiming to facilitate discovery and development of novel neurotherapeutics. Integration of overall findings is directed towards the development of physiologically based mathematical models of brain drug disposition.

(VI) Drug distribution in the lung (Erica Bäckström, Elin Boger (Marie Curie program IMPACT, University of Warwick), Markus Fridén (AstraZeneca), and Margareta Hammarlund-Udenaes)

This is a project started in 2013 in collaboration with AstraZeneca. The treatment of asthma and chronic obstructive pulmonary diseases (COPD) was revolutionized by the introduction of inhaled corticosteroids (ICS) and bronchodilators such as beta adrenergics and anti-muscarinics. For these drug classes topical delivery by inhalation has provided an efficient means of overcoming systemically mediated side-effects, that previously limited the dosing and therapeutic response.

Despite the historical success of inhalation medicines and the significant share (10 %) of the global drug market, there is very little known about the fundamental prerequisites for a drug molecule to be retained in and exert a localized effect in the lung when administered by inhalation.

Our research aims to study basic mechanisms of drug distribution in the lung which include non-specific tissue binding, lysosomal trapping and carrier-mediated membrane transport as well as the profound influence of blood perfusion. We are employing a lung slice methodology, equilibrium dialysis of lung tissue and in vivo methodologies to study the extent of drug distribution in the lung and the absorption half-life of inhaled drugs.

As an indicator of the unbound and pharmacologically active drug concentration we are collaborating with industrial and academic partners on measurement of target occupancy in the lung after inhalation using mass-spectrometry or positron emission tomography (PET). Along the same lines we are also developing physiologically based pharmacokinetic (PBPK) models to better explain and predict the possible advantage of the inhaled route of drug delivery.

(VII) Clinical Pharmacy Research (Johanna Sulku, Elisabet Nielsen and Margareta Hammarlund-Udenaes in collaboration with Håkan Melhus, Dept Med Sci, Ulrika Gillespie, Uppsala Univ Hospital, and Hirsh Koyi, Gävle)

We are interested in evaluating the impact of clinical pharmacist interventions in medical care.

The purpose of this research is to see whether, and in what way the contributions of clinical pharmacy services in the hospital ward changes patient treatment and status. This research area is new in Sweden and important for the development of clinical pharmacy in this country. A seminal paper was published in 2009 in Arch Intern Med, which received much attention. Here we showed that clinical pharmacist intervention reduced costs and decreased the number of readmissions to hospital. The research is further oriented towards appropriate use of medicines in chronic obstructive lung disease.

Undergraduate Teaching 2016

The work with revision of the Master of Science in Pharmacy program, including renewal as well as improved training in oral and written communication, lab skills and professional training continued. Many of teachers within the department of Pharmaceutical Biosciences took an active part in the faculty work during 2016. Lena Bergström and Ann-Marie Falk contributed to the development of training in communication skills in the revised program. Jörgen Bengtsson, one of the three key teachers within "PRåM" (pedagogiska rådet vid Medicine & Pharmacy), contributed in the organization of work shops for the staff with the main goals to stimulate pedagogical development and dissemination of good ideas within Medicine & Pharmacy. Jörgen Bengtsson, Ann-Marie Falk and Emma Lundkvist led the project "RefSa" (reflekterande sammanhållande seminarier), a student oriented project aiming at giving the students an improved overall picture of their program, to prepare for future professional life and to increase student-teacher interactions. Jörgen Bengtsson and Emma Lundkvist continued with their faculty assignments as coordinators of apotekare, receptarie and master programs, respectively. Erika Roman contributed to the work in a project that aimed to improve the guidelines for bachelor and master projects within the faculty. Ann-Marie Falk and Ingrid Nylander was part of a project group, lead by Shima Momeni, that in October initiated work with a new complementary program and a test, respectively, for pharmacists that aim to apply for a Swedish licence as a pharmacist. Maria Swartling was the faculty representative in a project group working with implementation of interprofessional training in programs within the disciplinary domain Medicine & Pharmacy. Mathias Hallberg was vice chairman in the "STURE" (student recruiting at the Faculty of Pharmacy). Ingrid Nylander was vice chairman in the newly initiated Alumni association "Alumnföreningen Farmis".

The major part of the undergraduate teaching is within the two Pharmacy programs. During 2016 the extent of undergraduate teaching was 505 hst (full-time equivalents) representing 46% of the total number of hst within the Faculty of Pharmacy. In addition, the department is involved in teaching at the Master of Science in Chemical Engineering with specialization in drugs and the Biomedical program. Students attending internet-based courses given by the department comprised 80 hst during 2016.

Pharmaceutical Biosciences comprises a number of courses, e.g. Drug development and Drug usage, Drug metabolism and safety, Gene technology, Infection Biology, Microbiology and immunology, Pharmaceutical Molecular Biology with Bioinformatics, Pharmaceutical Biochemistry, Pharmaceutical bioinformatics, Pharmacology, Pharmacokinetics, Pharmacotherapy, Physiology, Toxicology, Drug metabolism and Safety assessment. A mix of traditional teaching and problem-based learning with lectures, laboratory sessions, seminars, workshops and computer sessions characterizes the teaching. In addition, the teachers are involved in interdisciplinary training in laboratory practice, communication skills and professional development.

Teaching at the basic level

The main teaching at the basic level is within the Bachelor of Science in Pharmacy program that comprises three years studies (180 hp) and the first years of the Master of Science in Pharmacy program, see below. Completed studies at the Bachelor program provide the necessary theoretical and practical competence that is required to apply for a pharmacist license as *receptarie*. The teachers also instruct in undergraduate projects

(15 hp) at the basic level. These projects are individual and they are examined by an oral presentation and a written report. During 2016, the teachers within the department supervised 71% (45 students) of the total number of undergraduate projects within the Bachelor of Science in Pharmacy program. Some basic level courses are open for other students than pharmacy students and attract both students at other programs at Uppsala University or other universities and also professionals.

Teaching at the advanced level

The department gives courses at the Master of Science in Pharmacy program that comprises five years studies (300 hp). Completed studies at the programs provide the necessary theoretical and practical competence that is required to apply for a pharmacist license as *apotekare*. The teachers instruct in undergraduate projects (30 hp) at the advanced level. These projects are individual and are examined by an oral presentation and a written report. The majority of the projects are laboratory-based and involve the student in ongoing research projects. During 2016, the teachers supervised 65% (62 students) of the total number of undergraduate projects within the Master of Science in Pharmacy program.

The teachers lead and teach a number of elective courses and single subject courses at the advanced level. These courses mirror research profiles within the department, such as Bioinformatics, Clinical pharmacy, Drug metabolism and safety, Drug addiction and Pharmacokinetics. The courses attract a large number of students, not only pharmacy students but also other students and professionals showing the proper prerequisites. During 2016, 74% (296 students) of the students within the Master of Science in Pharmacy program and 76% (76 students) of the students within the Bachelor of Science in Pharmacy program participated in elective courses given by the department.

The teachers also contribute to the teaching within several master programs within the Faculty of Pharmacy, Drug development, Drug management and safety and Clinical Pharmacy, and at master programs at the Faculty of Medicine, Infection biology and Forensic Science. The one-year post-graduate program in Clinical Pharmacy is the only one in the Nordic countries attracting students from all over Sweden and also from Norway.

Uppsala 2017-06-01

Ingrid Nylander

Course List 2016

List of courses on basic and advanced (second cycle) levels

Abuse and Addiction, 7,5 c
Acute Intoxications and Clinical Toxicology Second cycle, 7.5 c
Advanced Pharmacotherapy Second cycle, 7.5 c
Adverse Drug Reactions and Pharmacovigilance Second cycle, 7.5 c
Analytical Toxicology Second cycle, 30 c
Applied Pharmaceutical Bioinformatics Second cycle, 5 c
Applied Pharmaceutical Structural Bioinformatics Second cycle, 5 c
Applied Pharmacotherapy, Pharmacokinetics and Therapeutics Second cycle, 15 c
Biochemistry of Gene Regulation Second cycle, 7.5 c
Clinical Attachment and Service Development Second cycle, 18 c
Clinical Drug Trials with Applied Biostatistics Second cycle, 7.5 c
Clinical Pharmacokinetics and Pharmacodynamics Second cycle, 7.5 c
Clinical Pharmacy, 7.5 c
Degree Project in Drug Discovery and Development Second cycle, 30 c
Degree Project in Drug Management, 15 c
Degree Project in Drug Management Second cycle, 30 c
Degree Project in Pharmaceutical Biochemistry, 15 c
Degree Project in Pharmaceutical Biochemistry Second cycle, 30 c
Degree Project in Pharmaceutical Bioinformatics Second cycle, 30 c
Degree Project in Pharmaceutical Bioscience Second cycle, 20 c
Degree project in Pharmaceutical Pharmacology Second cycle, 30 c
Degree project in Pharmacokinetics, 15 c
Degree Project in Pharmacokinetics Secnd cycle, 30 c
Degree project in Pharmacokinetics D Second cycle, 30 c
Degree Project in Pharmacology, 15 c
Degree Project in Pharmacotherapy, 15 c
Degree Project in Pharmacotherapy Second cycle, 30 c
Degree Project in Pharmacotherapy D Second cycle, 30 c
Degree Project in Toxicology, 15 c
Degree Project, Toxicology D Second cycle, 30 c
Drug Dependence Mechanisms, Prevention of Cannabis Abuse (Contract education) 7,5 c
Drug Development and Drug Usage, 7.5 c
Drug Management Second cycle, 7.5 c
Drugs and Dependence, Advanced Course Second cycle, 7.5 c
Drugs and the Elderly Second cycle, 7.5 c
Drugs during Pregnancy and Lacation, Second cycle, 7.5 c
Drug Safety and Pharmacovigilance, Second cycle, 7.5 c
Evidence Based Clinical Pharmaceutical Methods Second cycle, 12 c
Models for Biological Systems Second cycle, 7.5 c
Molecular Biology with Focus on Drug Therapy, 7.5c
Molecular Mechanisms for Enzymatic Activation Second cycle, 7.5 c
Molecular Pharmacology, 7.5 c
Neuropharmacology Second cycle, 7.5 c
Pharmaceutical Biochemistry, 9 c
Pharmaceutical Biochemistry and Cell Biology, 7.5 c
Pharmaceutical Bioinformatics Second cycle, 7.5 c
Pharmaceutical Bioinformatics (Contract Education), 7.5c
Pharmaceutical Molecular Biology with Bioinformatics, 7.5c
Pharmacokinetics, 3 c
Pharmacokinetics, 7.5 c
Pharmacokinetics and Statistics, 9 c
Pharmacology, 15 c
Pharmacology, 16.5 c
Pharmacology for engineering students, 7.5 c

Pharmacotherapy, 7.5 c
Pharmacotherapy in Self-Treatment, 9 c
Preclinical Safety Assessment and Pharmacovigilance, 7.5c
Research Project in Clinical Pharmacy Second cycle, 15 c
Toxicology B, 7.5 c
Toxicology for Engineering Students, 7.5 c
Toxicology, Advanced Course D Second cycle, 30 c
Toxicology, Drug Metabolism and Safety Assessment, 4.5 c
Toxicology, Drug Metabolism and Safety Assessment, 7.5 c
Veterinary Pharmacology Second cycle, 7.5 c

Research Education 2016

The Department has a high priority for research training. The aim is to prepare the PhD students to become independent researchers able to make significant contributions to academia and/or pharmaceutical industry, and to take on highly qualified professional tasks. The Department has two weekly seminar series for PhD students and young scientists – one series on pharmacokinetics/pharmacometrics and one series on basic biomedical research. Here, the PhD students can improve their oral presentation skills and their ability to discuss research, which is an important goal within the scientific training.

The 4-year PhD program consists of research work and a number of courses, with a total course requirement of 30 points (one semester full-time). Most PhD students take more courses than that. The PhD thesis is a doctoral thesis consisting of separately published articles with a comprehensive introductory summary. A PhD thesis typically contains 4 – 5 scientific papers, of which at least half are published in scientific journals at the time of the public thesis defence. Besides research activities, the PhD students participate at international conferences, of which one presentation at an international conference is obligatory during the program.

Many PhD students also perform important tasks in undergraduate teaching, comprising around 20 % of their time, making the time for a PhD exam be around 5 years in total. The teaching assignments give the students a broader knowledge base than their own research area. It also gives them leadership and communication skills that are of importance in further professional life. PhD students may also tutor master students in experimental research work. At the end of 2016, there were 54 PhD students registered at the Department, and 11 PhD students who defended their theses during the year.

The Research Education Group at the Department actively structures the application procedure for PhD student positions, especially by contributing to the process of establishing new positions and selecting new PhD students. The group consists of two representatives who are teachers and one PhD student. The group, through the Chair, also follows the training by requesting yearly follow up document from a meeting between each PhD student and supervisor, where issues regarding coursework, progression in the research project, possible change of the scope of research and thereby updates of the research plan, as well as how the communication between student and supervisor is functioning, are evaluated. Dr Erika Roman represents the Department in the Research Education Committee of the area of Medicine and Pharmacy, hereby connecting the Department to central decision making.

Uppsala 2017-08-29

Margareta Hammarlund-Udenaes

Awards 2016

Per Andrén received an Infrastructure Grant (2016-2020) from the Swedish Foundation for Strategic Research (SSF) "Research, Infrastructure Fellow" in Mass Spectrometry Imaging, 15 MSEK. Top-ranked.

Per Andrén also received a Pilot Mass Spectrometry Imaging Infrastructure grant (2016-2019) from the Science for Life Laboratory (SciLifeLab), 6 MSEK. Top-ranked of all applicants.

Linnea Granholm et al. received the International Narcotic Research Conference Poster Prize.

Lena Friberg received the International Society of Pharmacometrics (ISoP) Innovation award.

Margareta Hammarlund-Udenaes became an Honorary Member of the Swedish Academy of Pharmaceutical Sciences in 2016.

Sarah Holst, Erika Roman et al. received the Svenska Spel Research Council Poster Prize.

Mats Karlsson received the Lily and Sven Thuréus Award. The prize is awarded by The Royal Society of Sciences at Uppsala.

Mats Karlsson also became an Elected Honorary Fellow of the International Society of Pharmacometrics (ISoP).

Patrik Källback, Vallianatou T, Barré F, Nilsson A, Shariatgorji M, Andrén PE were given the price for the best poster at Mass Spectrometry Imaging Society, OurCon IV conference in Ustron, Poland, October 17-21, 2016.

Elin Svensson received the 2017 Rosenön Award. The prize is awarded by the Section of Pharmacokinetics and Drug Metabolism, The Swedish Pharmaceutical Society.