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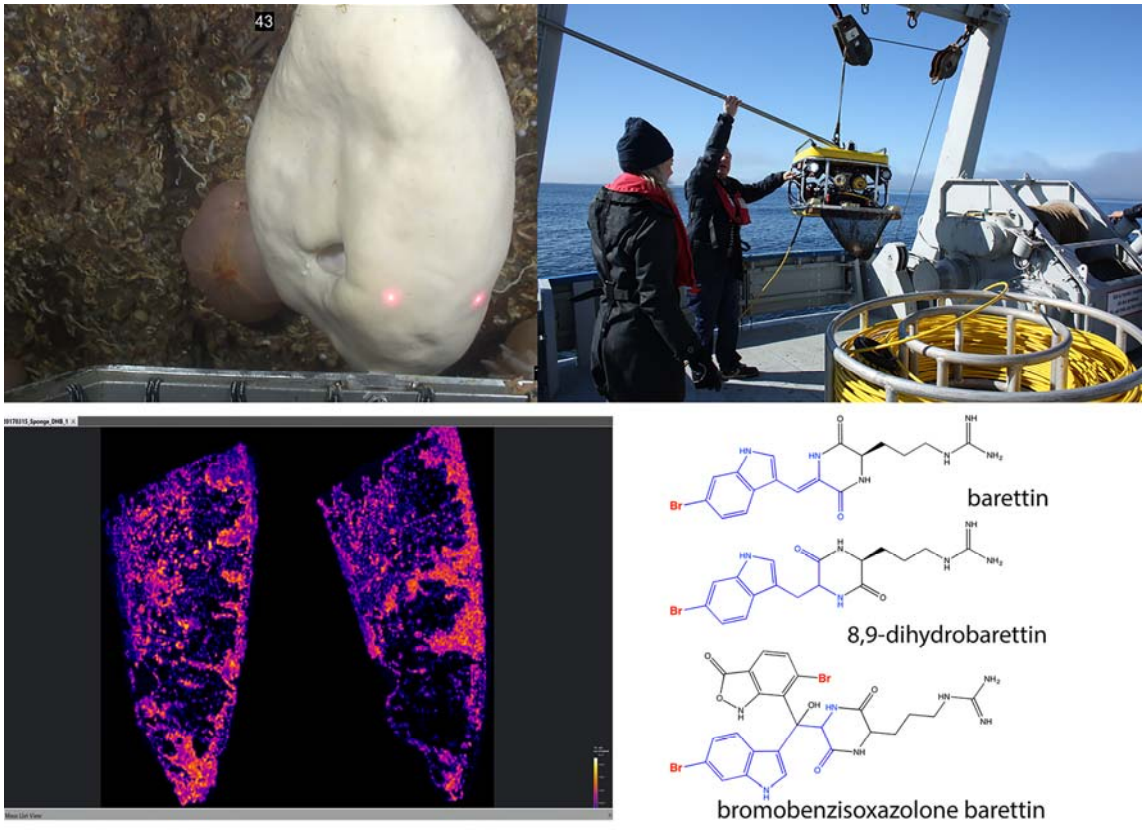
Annual Report 2016

Department of Medicinal Chemistry



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Front page illustration: Paco Cardenas

Introduction

The Department of Medicinal Chemistry (DMC) is organized into four different divisions: Analytical Pharmaceutical Chemistry, Molecular Imaging, Organic Pharmaceutical Chemistry and Pharmacognosy. At DMC there are also three platforms: PPP (Preclinical PET-MRI), SciLifeLab DDD Medicinal Chemistry – Lead Identification and ENABLE an EU IMI project working on the development of potential antibiotics against Gram-negative bacteria. During 2016, the department had 6 professors, 3 adjunct professors, 3 associate professor, 4 senior lectures, 2 research associates, 3 assistant professor, 28 researchers, 5 postdoctoral fellows and 35 PhD students. Three PhD students successfully defended their thesis and it was possible to admit 5 new PhD students at the department. The administrative staff Birgitta, Karin, Sandra and Linda as well as our systems administrator Sorin have continued their excellent works. Their effective support makes it possible for all of us at the department to maintain high output in research and teaching. A more detailed description and major findings of the separate research projects during 2016 are presented on the followings pages.
A good economy is vital to be able to maintain high quality in research and teaching activities. The economy at the department is in balance and during 2016 we received external grants at the same level as previous years. However, since more than half of our expenses are related to labor costs it is very alarming that we not get fully compensated for their salary increase. This means that all research groups must make every effort to keep and even further improve their performance in order to maintain or hopefully attract more external grants. Prof. Göransson has initiated plans to collaborate with Guangdong Provincial Hospital of Chinese Medicine and Chinese Academy of Chinese Medical Sciences China on research of traditional Chinese medicines.
In order to reduce costs, the department has decreased and to some extent rebuilt the premises to be functional. Furthermore, in order to improve the efficiency of administration, research and teaching at the department the board initiated a revision of the organization at the DMC. One proposal is that the DMC should be organized in research groups instead of divisions and also to form a unit for administration and development of the education at undergraduate and master levels. The working group for the revision of the organization will present a proposal and the board will decide in June 2017.
The Department of Medicinal Chemistry has extensive assignments in: the Master of Science in Pharmacy program, 300 hp, the Bachelor of Science in Pharmacy program), 180 hp Biomedical program, 240 hp and Master of Science in Chemical Engineering, 300 hp. Furthermore, we are also involved in two new programs, the Master in Drug Discovery, the Master in Pharmaceutical Modelling and the Master in Forensic Science. The teachers have worked on a new educational program for pharmacists, and we are looking forward to implementing the modernized and improved program. This task must be completed efficiently, as it diverts valuable time and resources from both teaching and research. In order to streamline the administration of written examinations and improve examination quality, we are trying to introduce computer-based examinations (CBE). Unfortunately, this has been seriously hampered by the shortage of invigilators provided by the central administration at Uppsala University. A more detailed description of undergraduate education at the Department of Medicinal Chemistry is given later in this annual report
We are pleased to note that the Pharmaceutical Student Council prize in 2016 for "Studentbemötande" was awarded to Anja Sandström and the prize for "Studentinflytande" to Jacob Haglöf. The researcher Charlotta Wallinder received the Limbiska prize for excellent pedagogic performance in the biomedical program.
One of our PhD students, Bogdan Mitran was in 2016 awarded the annual European Association of Nuclear Medicine (EANM) Eckert & Ziegler Abstract Award. Marc Stevens, a

previous PhD student in the PET research group has received a Postdoc scholarship from "Knut och Alice Wallenbergs stiftelse" for studies at the Stanford University (US).
During 2016, it has, as all previous years been a privilege to be the "prefekt" of the Department of Medicinal Chemistry. I want to thank everybody in the department for all their efforts and excellent work during the year.
I would also like to thank those who left the Department of Medicinal Chemistry during last years for their contributions to the achievements of the Department. It has been a great pleasure to collaborate with associate professor Jens Carlsson and his group members and we wish them all success in the future.

Curt Pettersson

Chairman
Department of Medicinal Chemistry

List of Contents

Organization

Department of Medicinal Chemistry

Head of Department
Curt Pettersson

Deputy Head of Department
Anders Karlén

Department board
Curt Pettersson, chairman
Sandra Bratt, technical/administrative (to August-2016)
Olof Jonsson, technical/administrative and secretary, deputy (to February-2016)
Birgitta Hellsing, technical/administrative and secretary, deputy (from March-2016)
Mats Larhed, teacher representative
Ulf Göransson, teacher representative
Olof Eriksson, teacher representative
Anja Sandström, teacher representative, deputy
Jakob Haglöf, teacher representative, deputy
Jonas Rydfjord, graduate student representative
Rebecka Isaksson, graduate student representative, deputy
Anna Joo, undergraduate student representative
Karin Karlsson, undergraduate student representative, deputy (to June-2016)
Tobias Haugmo, undergraduate student representative, deputy (from August-2016)

Professores emeriti
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Anders Hallberg
Gunnar Samuelsson
Lars-Olof Sundelöf
Douglas Westerlund

Senior lecturer emeriti
Uno Svensson

Director of graduate studies
Anders Backlund

Secretariat
Yosef Gebretsadkan
Birgitta Hellsing
Olof Jonsson
Karin Stenvall

Course Administration
Sandra Bratt
Agata Kostrzewa
Ulrika Rydberg
Linda Strandenhed

Computers/IT
Anders Backlund
Ola Åberg
Jakob Haglöf
Christian Sköld
Sorin Srbu

Analytical Pharmaceutical Chemistry
Head of Division
Curt Pettersson

Director of undergraduate studies
Curt Pettersson

Organic Pharmaceutical Chemistry
Head of Division
Mats Larhed

Directors of undergraduate studies
Ulrika Rosenström(75%)
Anja Sandström (25%)

Pharmacognosy
Head of Division
Anders Backlund

Director of undergraduate studies
Christina Wedén

Preclinical PET-MRI Platform
Head of Platform
Mats Larhed

SciLifeLab Drug Discovery and Development Platform
Vice Platform Director
Kristian Sandberg
SciLifeLab DDDp Lead Identification Facility
Facility Director
Mats Larhed
Head of Facility
Ulrika Yngve

ENABLE/European Gram Negative Antibacterial Engine
Head of Platform
Anders Karlén

Assignments of staff members

Cecilia Alsmark
- Member of the Committee for equality, Department of Medicinal Chemistry, Uppsala University

Gunnar Antoni
- Adjunct Professor in Pharmaceutical Radiochemistry
- Head of PET Centre Uppsala University hospital
- Sweden's representative in Expert group 14 in the European Pharmacopoeia
- Member of the Society of radiopharmaceutical sciences

Torbjörn Arvidsson
- Member of The Swedish Academy of Pharmaceutical Sciences
- Member of the Swedish Chemical Society
- Adjunct Professor in Applied Analytical Pharmaceutical Chemistry
- Scientific Expert, External relations and innovation support, Medical Products Agency
- Chairman of Expert group 10A in th European Pharmacopeia

Anders Backlund
- Head of division of Pharmacognosy, until February
- Honorary visiting professor at Kaohsiung Medical University
- Member of the ULLA ExCo
- Member of evaluation committé at Rannís - the Icelandic Research Council
- Member of the board of Uppsala University Center for Sustainable Development
- Member of the Editorial Board for "Acta Universitatis Upsaliensis" and the Ekman Foundation
- Member of the Editorial Board of "Phytochemical Analysis"
- Fellow of the Linnaean Society of London
- Fellow of the Willi Hennig Society
- Member of the International Association of Plant Taxonomists (IAPT)
- Member of the Society for Medicinal Plant Research (GA)

Jens Carlsson
- SciLifeLab Fellow

Olof Eriksson
- Secretary and board member, DIAB-IMAGE, European Association for the Study of Diabetes study group for biomedical imaging in diabetes
- Member of the European Association for the Study of Diabetes
- Member of the European Association for Nuclear Medicine
- Member of Uppsala Medical Society

Mikael Engskog
- Member of the Section for Pharmaceutical and Biomedical Analysis, at the Swedish Academy of Pharmaceutical Science
- Member of the Section for Analytical Chemistry, at the Swedish Chemical Society
- Member of the Metabolomics Society

Daniel Globisch
- Member of the German Chemical Society (GDCh)
- Member of the German Researcher Exchange Program (DAAD)
- SciLifeLab Fellow

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- Member of the Editorial Advisory Board "Peptidomics"
- Member of the International Council, Uppsala University
- Director of studies of postgraduate courses and member of the Postgraduate programmes committee, Scientific Domain of Medicine and Pharmacy, Uppsala University
- Member of the Swedish Academy of Pharmaceutical Sciences
- Member of the Swedish Chemical Society

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- Member of the Section for Analytical Chemistry, The Swedish Chemical Society

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- Member of the Royal Academy of Art and Sciences in Uppsala
- Member of the Royal Physiographic Society in Lund
- Member of the Royal Academy of Sciences
- Member of the Royal Academy of Engineering Sciences
- Member of the Royal Patriotic Society
- Member of the board of Åbo Akademi University
- Chairman of the Göran Gustavsson Foundation
- Member of the Scientific Advisory Board of the Government
- Member of the board of the Baltic Sea Foundation

Mikael Hedeland
- Member of the board of the Section for Pharmaceutical and Biomedical Analysis, at the Swedish Academy of Pharmaceutical Science
- Member of the Section for Analytical Chemistry, The Swedish Chemical Society
- Chairman of the European section of the Association of Official Racing Chemists (AORC)
- Member of the international board of AORC

Ylva Hedeland
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- Member of the Section for Analytical Chemistry, The Swedish Chemical Society

Anders Karlén
- Chairman of the Docentur committee within the Disciplinary Domain of Medicine and Pharmacy
- Deputy head of Department of Medicinal Chemistry
- Leader of the Managing Entity and co-coordinator of the IMI Project ENABLE
- Chairman of the board of the Medicinal Chemistry Section of the Swedish Academy of Pharmaceutical Sciences
- Member of the Pharmaceutical Faculty Committee
- Member of the American Chemical Society

Mats Larhed
- Deputy Vice President, Medicine and Pharmacy, Uppsala University
- Head of the Preclinical PET platform
- Chair of SciLifeLab Uppsala Steering Group
- Facility Director, SciLifeLab DDD, Medicinal Chemistry - Lead Identification
- National Director of EATRIS (European infrastructure for translational medicine)
- Member of the Board of Swedish Academy of Pharmaceutical Sciences
- Secretary of the Board of EIT Health Scandinavia

- Member of the American Chemical Society
- Member of the Editorial Board for ChemistryOPEN
- Member of the Royal Society of Sciences at Uppsala
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Luke Odell
- Member of The Swedish Academy of Pharmaceutical Sciences
- Member of the Swedish Chemical Society
- Member of the Editorial Board for Current Microwave Chemistry
- Member of the Editorial Board for Molbank

Anna Orlova
- Member of European Association of Nuclear Medicine
- Member of International Research group in Immuno-Scintigraphy and Therapy
- Member of International Society for Radiopharmaceutical Sciences
- Member of Board of Directors for WIPPET (Wallenberg Infrastructure Preclinical PET/MRI)
- Member of the editorial board of Scientific Reports
- Member of the Technical Advisory Board of Affibody AB, Solna
- Responsible for animal experiments at PPP, (Djurföreståndare)
- Member of laboratory animal protection group at Uppsala University

Curt Pettersson
- Head of Department of Medicinal Chemistry
- Head of division of Analytical Pharmaceutical Chemistry
- Director of undergraduate studies in analytical pharmaceutical chemistry
- Member of the Pharmaceutical Faculty Committee
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- Member of the board of Division of analytical chemistry of the Swedish Chemical Society

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- Member of the Swedish Academy of Pharmaceutical Sciences
- Member of the American Chemical Society
- Member of the Editorial Board of Frontiers in Chemical Biology

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- Member of The Swedish Academy of Pharmaceutical Sciences
- Member of the Swedish Chemical Society

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- Member of the student recruitment group (STURE), Faculty of Pharmacy, Uppsala University
- Member of the Program committee for the Master programme in Biomedicine, Faculty of Medicine, Uppsala University

Christina Wedén
- Director of undergraduate studies in pharmacognosy
- Member of the Academy of Culinary Arts and Meal Sciences

Scientific Reports

Analytical Pharmaceutical Chemistry

The research at the Division of Analytical Pharmaceutical Chemistry at the Department of Medicinal Chemistry is focused on separation science and mass spectrometry. The analytes of interest are drugs and their degradation products and metabolites as well as carbohydrates, peptides, proteins, amino acids and other small molecules.
The research is divided into two areas of importance: pharmaceutical analysis and bioanalysis. Over the last years the major emphasis has shifted from pharmaceutical analysis to bioanalysis.
Bioanalysis is the subdiscipline of analytical chemistry that covers the determination of drugs and their metabolites in biological systems. The research at the Division of Analytical Pharmaceutical Chemistry within this area covers investigation of the metabolic pattern of drugs in *in vivo* systems (i.e. human, horse), chiral and achiral analysis of drugs in the aquatic environment, the use of in vitro systems for production of metabolites as well as metabolomics studies in relation to diseases and nutrition.
Liquid chromatography hyphenated to high resolution mass spectrometry (LC-MS) and nuclear magnetic resonance (NMR) spectroscopy are the main techniques that are used within the projects in the bioanalysis field.

Development of Analytical Methods for Pharmaceutical Analysis

Research Group Leader: Curt Pettersson

Access to efficient analytical methods is a prerequisite in several steps in the drug discovery and development processes. Techniques for control of purity and identity of substances in chemical libraries, high speed analysis enabling fast screening of drug-receptor interactions as well as the physico-chemical characterization of drug candidates is of great importance in the early stages of drug development. Analytical methods are also necessary to secure that tablets and other pharmaceutical formulations contain the correct amount of active compounds and excipients. A very important area in drug development is the analysis of the enantiomeric drugs, *i.e.* drug molecules that can exist in two mirror image forms. The enantiomers of a molecule might have different pharmacokinetic, pharmacodynamic and toxicological properties which mean that one enantiomer may be responsible for the therapeutic effect, whereas the other may be inactive or even toxic.
Techniques such as liquid chromatography (LC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE) as well as MS and NMR are used in the projects within the pharmaceutical analysis area.
Our current work is focused on the following specific areas of importance:

- Analytical method development for metabolomics using NMR and MS
- Analysis of drugs in the environment
- Capillary electrophoresis for biomedical applications

Members of the group during 2016

Curt Pettersson, Professor
Ahmad Amini, Associate Professor
Torbjörn Arvidsson, Adjunct Professor
Albert Elmsjö, PhD student
Mikael Engskog, PhD, Researcher
Ida Erngren, PhD student
Lars Geurink, PhD student
Olle Gyllenhaal, Associate Professor
Jakob Haglöf, PhD, Junior Lecturer
Mikael Hedeland, Adjunct Professor
Ylva Hedeland, PhD, Senior Lecturer

Monika Johansson, Associate Professor
Lars B Nilsson, PhD, Researcher
Kristian Pirttilä, PhD student
Åke Stenholm, PhD student
Alfred Svan, PhD student
Cari Sänger-van de Griend, Associate Professor
Niklas Tyrefors, PhD, Researcher

Analytical method development for metabolomics using high resolution nuclear magnetic resonance spectroscopy and mass spectrometry

Curt Pettersson, Torbjörn Arvidsson, Mikael Engskog, Jakob Haglöf, Albert Elmsjö, Ida Erngren and Kristian Pirttilä

This multidisciplinary project aims to develop, establish and validate analytical methodologies for untargeted and targeted metabolomics investigations as well as to apply this platform in a diverse set of relevant international and national collaboration projects. We aim to find scientifically reliable workflows for detection, identification and quantification of metabolites (=small endogenous molecules) in biological samples derived from cells or biofluids. As the field of metabolomics is steadily becoming more and more advanced, and also being employed in a diverse set of research fields, we strongly believe in the need for robust, validated and accurate analytical methodology from design of experiments to evaluation of data. Metabolomic investigations of high scientific standards require the highest possible quality of data, thus making the analytical methodology a key point for success.

As a comparison to the other “omics” techniques, one could say that genetics and genomics capture events that might happen; proteomics capture events that are happening, while metabolomics captures events which have happened. Metabolomics thus provide real endpoint with biological meaning and thus holds a great promise for the future. From a technical viewpoint, metabolomics is a combination of analytical chemistry, statistics and bioinformatics tools that are used together or alone to perform (i) sample preparation, (ii) acquisition of data by mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, (iv) statistical analysis and, ultimately, (v) identification of significantly altered metabolites or pathways.

The analytical methodology is being developed and evaluated in several interesting ongoing studies through established national collaborators found at Uppsala University (UU) and Karolinska Institute (KI) as well as international collaborators at Queen Mary University of London. Of particular focus are metabolomic investigations in cancer research where the division is currently engaged in several different projects.The effect on the metabolome by radiation of cancer cells are being examined in collaboration with the Department of Radiation, Oncology and Radiation Sciences (UU, Marika Nestor), while the metabolic consequences of hearing loss caused by cisplatin treatment in patients are investigated with the Department of Surgical Sciences (UU, Göran Laurell and Pernilla Videhult Pierre). In collaboration with Maria Shoshan (KI, Cancer Center Karolinska) the division is looking at differences in the polar metabolome between parental and chemotherapy resistant ovarian cancer cell lines.

Furthermore, the division is engaged in projects related to neurotoxicology in collaboration with Eva Brittebo, molecular pharmacology with Robert Fredriksson and drug dependence with Mathias hallberg (all at the Department of Pharmaceutical Biosciences, UU), nutrition-based metabolomics (Ulf Risérus, UU, Department of Public Health and Caring Sciences) and pharmacometabolomics (Helena Jernberg-Wiklund, UU, Department of Immunology, Genetics and Pathaology)..

Publications 2014-2016

1. Aftab O, Engskog MK, Haglöf J, Elmsjö A, Arvidsson T, Pettersson C, Hammerling U, Gustafsson MG. NMR spectroscopy-based metabolic profling of drug-induced changes in vitro can discriminate between pharmacological classes. Journal of Chemical Information Modeling. 2014 Nov 24;54 (11): 3251-3258.

2. Elmsjö, A., Rosqvist, F., Engskog, M., Haglöf, J., Kullberg, J. et al. NMR-based metabolic profiling in healthy individuals overfed different types of fat: links to changes in liver fat accumulation and lean tissue mass. 2015 Nutrition & Diabetes, 5(19): e182-.

3. Engskog, M., Björklund, M., Haglöf, J., Arvidsson, T., Shoshan, M. et al. Metabolic profiling of epithelial ovarian cancer cell lines: evaluation of harvesting protocols for profiling using NMR spectroscopy. 2015 Bioanalysis, 7(2): 157-166.

4. Engskog, M., Haglöf, J., Arvidsson, T., Pettersson, C. LC-MS based global metabolite profiling: the necessity of high data quality. 2016 Metabolomics, 12(7).

Analysis of drugs in the environment

Curt Pettersson, Torbjörn Arvidsson, Mikael Hedeland and Alfred Svan

In the literature it has been reported that during the last few decades different analytical methods have been developed for about 150 pharmaceutical ingredients and related compounds in environmental matrices. Pharmaceuticals have been detected and quantified in different bodies of water, e.g. rivers and lakes, surface water, sewage treatment plant influent and effluent water, ground water, and even in drinking water. However, few of these methods focus on the metabolites, which can be just as equally or even more potent than the parent compounds. The occurrence, fate and effects of pharmaceutical compounds in the aquatic environment are poorly understood and the behaviour of chiral drugs in the environment is even more poorly understood. One reason for this is the difficulty to perform chiral analyses in environmental matrices at trace level concentrations.

An overall goal in this research field is to achieve an adequate elimination of drugs in wastewater plants or by other treatment, in a way that does not create harmful metabolites.

Our specific aim is to develop validated analytical methods for the detection, identification and quantification of drug substances and their metabolites as well as their stereoisomers that are of particular interest from an environmental point of view. Our second aim is to elucidate metabolic pathways for drugs in the environment. For this purpose, we cooperate with a research group in Berkeley California, which provides us with samples from their wetlands, designed and controlled to investigate the degradation of drug substances.

We are also interested in the challenges assosieted with environmental analyses, especially matrix effects, a major problem using complex matrices and mass spectrometry. Therefore, the ongoing project is focused on studying matrix effects, using matrices common within the environmental field and generic LC and SFC screening methods.

Publications 2014-2016

1.Svan, A., Hedeland, M., Arvidsson, T., Jasper, J., Sedlak, D. et al. (2015).
Rapid chiral separation of atenolol, metoprolol, propranolol and the zwitterionic metoprolol acid using supercritical fluid chromatography-tandem mass spectrometry - Application to wetland microcosms. Journal of Chromatography A, 1409: 251-258

2.Svan, A., Hedeland, M., Arvidsson, T., Jasper, J., Sedlak, D. et al. (2016).
Identification of transformation products from -blocking agents formed in wetland microcosms using LC-Q-ToF. Journal of Mass Spectrometry, 51(3): 207-218

IInvestigation of degradation products of toxic substances in fungal cultures by mass spectrometric techniques

Curt Pettersson, Torbjörn Arvidsson, Mikael Hedeland, Åke Stenholm

The presence of recalcitrant compounds in industrial and domestic wastewater streams leads to a continuous pollution of our environment. Among these substances, endocrine disrupting compounds (EDCs) and pharmaceutical active compounds (PhACs), are of special concern. EDCs mimic the action of natural hormones in water living organisms and PhACs may spread bacterial resistance to antibiotics and impair the health status of fish. Wastewater treatment plants (WWTPs), generally contain a biological step in which bacteria are used for biodegradation purposes. However, fungi have certain advantages over bacteria. Among these, a greater resistance to inhibitory components and a broad spectrum of non-specific enzymes are worth mentioning. The fungal enzymatic degradation of EDCs and PhACs is favorably studied using MS-techniques like UHPLC-Q-TOF and GC-MS by which both a quantification of the target compound and a qualitative analysis of the degradation products can be facilitated.

The goal in the project which is a collaboration between Uppsala University and GE Healthcare is to investigate the biodegradation of some selected EDCs and PhACs in a continuous bioreactor, using MS-techniques, at conditions that are beneficial for the growth of fungi mycelia and decline in target compound concentrations.

At present, the biodegradation of an EDC is studied. The compound of choice is nonylphenol polyethoxylate (NPEO). Continuous biodegradation experiments are conducted using the white rot fungus species Trametes versicolor (T.v) which is immobilized on polyurethane foam (PUF). The aim of this particular project is to determine the removal rate of NPEO and to tentatively identify degradation products.

Previously, similar biodegradation experiments were conducted using the same fungus species and the PhAc diclofenac. In those experiments, factors that influence fungal growth, choice of immobilization supports and suitable reactor residence times were determined. From this work, it was concluded that PUF is an excellent solid support for T.v. and that its strong adsorptive properties can be used for diclofenac removal purposes. 5 novel diclofenac biodegradation products were tentatively identified using UHPLC-Q-TOF MS and MS/MS.

Capillary electrophoresis for biomedical applications

Ylva Hedeland

The overall aim with this project is to develop general methods for analysis of protein isoforms in biological samples based on a top-down proteomics approach using capillary electrophoresis (CE) and high resolution mass spectrometry.

One focus has been to develop analytical methods that can support the diagnosis of renal function and enable differentiation between acute and chronic renal failure. A simple and reliable CE method for determination of iohexol, a glomerular filtration rate (GFR) marker, in plasma has earlier been developed and validated. A method for analysis of hemoglobin subtypes in order to enable differentiation between acute and chronic renal failure are under development.

The project has been performed in cooperation with Prof. Jonas Bergquist, Department of Chemistry, Uppsala University, Prof. Christian Neusüß at Aalen University, Germany and Dr Reidun Heiene at University of Utrecht, The Netherlands.

Oral presentations

“CE-MS of Hemoglobins” Christian Neusüß, Ylva Hedeland, Jonas Bergquist, IITP 2015 in Helsinki

Publications 2014-2016

1. Alexander Hellqvist, Reidun Heiene, Siegrid De Baere, Siska Croubels and Ylva Hedeland, "Development of a capillary electrophoretic method for determination of plasma clearance of iohexol in dogs and cats". Biomedical Chromatography Published online 8 aug 2014 DOI: 10.1002/bmc.3304

Virus and Vaccine Characterization with Capillary Electrophoresis

Cari Sänger -van de Griend (Uppsala University, Kantisto), Curt Pettersson (Uppsala University), Lars Geurink (Uppsala University and Janssen Vaccines & Prevention), Ewoud van Tricht, Martijn Schenning, Marta Germano (Janssen Vaccines & Prevention), Govert Somsen (Vrije Universiteit Amsterdam)

In the field of biopharmaceutical therapeutics faster and better characterization of therapeutic proteins is required. Important tools in this field are the Capillary Electrophoresis (CE) techniques. A few applications are well established in the field of therapeutic monoclonal antibodies, but there is an urgent need for further improvements. Also the methods for analysing viruses and vaccines need more accurate and precise analytical tools. The work performed currently in collaboration with Janssen Vaccines & Prevention in Leiden, the Netherlands, on the development of CE as a platform for viruses and vaccines is unique and pushes the frontiers for vaccine characterization. During 2016, several lectures and posters were presented at national and international symposia. The published work comprised the projects listed below.

Development of a capillary gel electrophoresis method for identification and quantification of hemagglutinin and other viral proteins in influenza *vaccines*

Influenza causes annual epidemics that are deadly to people in risk groups such as the elderly. Vaccines are available to protect against influenza virus infection and to reduce mortality rates. However, the antigenic properties of the influenza virus change rapidly as a result of antigenic drift. Consequently, antibodies will no longer be able to recognize the antigenic sites of hemagglutinin (HA) or neuraminidase (NA) -the membrane proteins exposed on the virus surface- and revaccination is required. The World Health Organization (WHO) makes yearly recommendations regarding the composition of the vaccines. For vaccine production, this means yearly changes in the product and/or manufacturing process, have to be made under significant time pressure.

A novel Capillary Gel Electrophoresis (CGE) method for the quantitative analysis of viral proteins in influenza virus and virosome samples was developed. The CGE method was validated for the quantification of influenza proteins HA1, HA2, NP, and M. CGE showed several advantages compared to the currently used methods for routine viral protein analysis, SRID, RP-HPLC, and SDS-PAGE. In contrast to SDS-PAGE, the CGE method allowed identification of virus and virosome influenza strains based on their specific protein profile. SDS-PAGE provided insufficient resolution to detect the differences in molecular weight between influenza virus strains and subtypes. The CGE results obtained for HA1 on virus and virosome samples corresponded quantitatively with results obtained with SRID, which is the established method for HA quantification. However, with CGE the total analysis time was much shorter than for SRID. The CGE method can handle complex samples containing cell debris and cell lysate, such as samples from upstream process. Such samples cannot be analysed directly with the current RP-HPLC method.

This work was finished in 2015. The developed CGE method is robust and provides quantitative analysis of HA in both downstream and upstream processing samples in an accurate and precise manner. In addition, the CGE method allows identification of influenza strains based on their specific electrophoretic profile. The CGE method is faster than SRID, and more precise and accurate than RP-HPLC. This work was published in Talanta [1]. During 2014 – 2016, Lectures [3, 4, 7 and 8] and posters [11, 13 – 15, 18, 21] were presented at KNCV SAC CE User Meeting, ATEurope, at The Analytical Challenge, HTC, ISC and at CEPharm.

Novel method for quantification of adenovirus particles in complex matrices using capillary electrophoresis

There is a distinct need for a fast, accurate and precise analytical method for the analysis of adenoviruses throughout the production process of vaccines. Current methods for the quantification of adenoviruses in vaccine products suffer from recovery issues due to the complexity of the matrix and/or long analysis times to reach sufficient analytical precision. A CE method was developed and is being implemented as alternative to quantitative polymerase chain reaction (qPCR) and anion exchange chromatography (AEX-HPLC). Adsorption issues, typically seen for LC-based method, were solved for CE in an extensive study. The method was successfully validated and applied to 5 representative samples from the process containing cell debris, cell lysate, residual DNA and proteins, and/or high salt concentrations. The results obtained with CE were in line with the result obtained with qPCR, however, the precision of the CE method was much better (2 and 6% RSD), compared to qPCR (between 6 and 25% RSD). Moreover, the fast analysis time of CE made it possible to run 35 samples within 1 day, while with qPCR the analysis time is up to 4 days.
During 2016, extensive work was performed on method transfer to different departments and sites and training of the scientists and technicians involved. Also, the applicability of the method was extended to Upstream Processing samples by adding a simple sample preparation step. The method was further extended for lower concentration samples by large volume injections followed by transient isotachophoretic in-capillary concentration. The method development is accepted for publication [2], a patent application is filed and several posters [17 - 24] and lectures [5 - 10] were presented during 2014 – 2016.

Capillary Gel Electrophoresis for Analysis of Polio Virus - Development and Qualification in four Days

Based on the previous work on Capillary Gel Electrophoresis (CGE), a strategy was developed for fast and effective method development. This strategy was applied to develop a method for identification and quantification of the viral proteins of three different polio strains. The strategy made it possible to systematically and efficiently develop a CGE method with only a few experiments required (≤ 5). During 2014 – 2016, posters were presented at ATEurope, CEPharm and at The Analytical Challenge [12, 16, 18 and 21] and the work was part of presentations [4], [6] and [7].

Publications 2014-2016

1. *New capillary gel electrophoresis method for fast and accurate identification and quantification of multiple viral proteins in influenza vaccines*, Ewoud van Tricht, Lars Geurink, Bojana Pajic, Johan Nijenhuis, Harold Backus, Marta Germano, Govert W. Somsen, Cari E. Sänger-van de Griend, **Talanta** 144 (2015) 1030–1035

2. *One single, fast and robust capillary electrophoresis method for the direct quantification of intact adenovirus particles in upstream and downstream processing samples*, Ewoud van Tricht, Lars Geurink, Harold Backus, Marta Germano, Govert W. Somsen, Cari E. Sänger-van de Griend, accepted in **Talanta** (2016)
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Bioanalysis of drugs and their metabolites, drug metabolite production and identification with mass spectrometry

Research Group Leader: Mikael Hedeland

Liquid chromatography - tandem mass spectrometry (LC-MS/MS) has become the most powerful technique for low-level determinations of drugs and their metabolites in biological fluids. As drug metabolites may be more active than the parent compound, or even toxic, it is of utmost importance to elucidate the metabolic pattern of a drug candidate in an early stage of drug development.

In qualitative and quantitative bioanalysis, it is necessary to use reference standards. However, the commercial availability of standards of drug metabolites is low. Production of reference compounds through classic organic synthesis is tedious and expensive and the use of *in vitro* systems based on microsomes does not usually provide sufficient amounts for a full characterization.

One specific application where access to reference standards of drug metabolites is of vital importance is horse racing doping control, which is carried out at the National Veterinary Institute (SVA). Many drugs are extensively metabolised in the horse prior to renal excretion. Thus, the only way of assessing the use of such a substance may be to identify a urinary metabolite in the cases where the concentration of the parent substance is too low. The internationally adopted criteria for mass spectrometric identification of a compound state that the chromatographic retention as well as the fragmentation pattern of the suspected substance must be compared with those of a characterised reference compound.

Fungi of the *Cunninghamella* species have earlier been shown to give metabolic patterns similar to those of mammals. Furthermore, these fungi are cheap and they can produce relatively large quantities of metabolites in a short period of time. One of the purposes of this project is to evaluate if *Cunninghamella* can be used to produce biologically relevant metabolites of different drugs.

The described research is conducted in collaboration between the Division of Analytical Pharmaceutical Chemistry at the Faculty of Pharmacy, Uppsala University, and the Department of Chemistry, Environment and Feed Hygiene at the National Veterinary Institute (SVA), Uppsala, Sweden. The mass spectrometric analyses are carried out at SVA, where a state-of-the-art collection of instruments is available. Furthermore, the staff at SVA has a long experience in mass spectrometric bioanalysis of drugs, from a scientific as well as a technical point of view.

Members of the group during 2016

Ulf Bondesson, Professor
Mikael Hedeland, Adjunct Professor
Annelie Hansson, PhD student

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Biomarker Discovery and the Link to Gut Microbiota Metabolism

Research Group Leader: Daniel Globisch

The Globisch laboratory focuses on the development of new methodologies at the interface of Chemistry and Biology to overcome limitations in the mass spectrometric analysis of small molecule metabolites in biological samples. These new methodologies are aimed at enhancing the scope of metabolomics-based research and simplify small molecule biomarker discovery through advanced quantitative and qualitative metabolite analysis. The group was established in September 2015 after Daniel was recruited by the SciLifeLab to start his independent research in the Division of Analytical Pharmaceutical Chemistry at the Department of Medicinal Chemistry.

The discovery of specific early-stage biomarkers, new drug targets and the development of new therapeutic interventions are crucial for disease prevention and management towards personalized medicine. Analysis of metabolites in human specimen comprises a high potential for identification of unknown biomarkers, which can readily be utilized for the development of new diagnostics. These diagnostics are urgently required for various cancer types as well as other metabolic diseases, for which no sensitive and reliable diagnostic tools are available. In some cases highly invasive biopsies are the only option for identifying the disease, which results in disease detection at an advanced stage and thus only allows for insufficient and late treatment. Therefore, new non-invasive, sensitive and selective diagnostics based on specific biomarkers are urgently required to improve health care and patient survival rates.

One of the most exciting scientific developments in the past decade has been the identification of the profound impact of the gut microbiota on mammalian physiology. The complex consortium of trillions of microbes provides a diverse range of biochemical and metabolic activities and plays a crucial role in mammalian processes such as co-metabolism, energy production, immune development, and epithelial homeostasis. However, analytical tools for the specific investigation of this co-metabolism are still missing. We will selectively investigate gut microbiota derived or modified metabolites to gain new insights in the link between gut microbiota and cancer development. Our highly interdisciplinary research projects will utilize techniques and strategies from various research fields including Chemical Biology, Organic Chemistry, Metabolomics, and Bioanalytical Chemistry for the selective mass spectrometric analysis of gut microbiota modified metabolites to accomplish the discovery of unknown biomarkers.

Members of the group during 2016

Dr. Daniel Globisch, Associate Professor / *Science For Life Laboratory* Fellow
Dr. Neeraj Garg, Researcher
Dr. Caroline Ballet, Postdoctoral Fellow

Our SciLifeLab Fellow Daniel Globisch has received a prestigious starting grant from Vetenskapsrådet with the title “Gut microbiota metabolism - Biomarker discovery for pancreatic and esophageal cancer”. This grant of 3.06 MSEK will finance his independent research for four years. His ongoing research projects to develop new methods at the interface of Chemistry and Biology to improve the investigation of metabolites in human samples besides are already supported by his SciLifeLab starting grant and a grant from Carl-Trygger-Stiftelsen for one postdoctoral position, which he has also received in 2016.

Organic Pharmaceutical Chemistry

At the Division of Organic Pharmaceutical Chemistry of the Department of Medicinal Chemistry, we perform basic research in both experimental and computational medicinal chemistry. Our research interests encompass a range of targets of pharmaceutical interest, including enzymes and membrane bound G-protein coupled receptors (GPCRs).

One of our primary themes is to identify novel and selective low molecular weight ligands for these targets. New strategies are developed for both the design and the synthesis of small, drug-like molecules. Lead compounds are optimized using computer-aided techniques and ADMET profiling, and are preferentially synthesized using high-speed chemistry. Major indications addressed are infections caused by HCV (Hepatitis C Virus), tuberculosis and a number of gram-negative bacteria. Method development in organic synthesis, including microwave flow applications and mechanistic studies of new palladium-catalyzed coupling reactions, is also performed. Furthermore, basic research on the transformation of biologically active peptides into more drug-like peptidomimetics are carried out, with special focus on the Renin/Angiotensin system and neuropeptides, such as Substance P 1-7.

Peptides to Peptidomimetics

Research Group Leader: Mats Larhed

Strategies for conversion of peptides into peptidomimetics. Peptides and proteins control all biological processes at some level, but the understanding of the relationships between structure and function is still to a large extent rudimentary. In recent years, a growing number of endogenous peptides have been identified and characterized. These peptides constitute valuable research tools and serve to gain insights on fundamental biological phenomena for the understanding of underlying mechanisms in various disease processes. Unfortunately, peptides, although often essential in the first phase of a drug discovery process, are not, with very few exceptions, useful as orally administrated therapeutics. They are not absorbed from the intestine, are metabolically unstable, and often lack specificity due to presentation of multiple pharmacophoric ensembles. To fully benefit from the massive new information provided from genomics and proteomics, it seems important to develop reliable strategies which allow for a systematic transformation of biologically significant peptides to small organic drug-like peptide mimetics. Until 1995, morphine and related opioids remained the only potent low molecular weight agonists known to activate receptors for peptides. More recently, after the pioneering work by Hirschman, Freidinger, Olson, Smith, Rich, and others, combinatorial chemistry and application of the dipeptidyl privilege structure concept have furnished e.g. orally bioavailable subtype-selective somatostatin receptor as well as melanocortin receptor agonists. These drug-like peptide receptor agonists, which are structurally very diverse from the endogenous peptides, almost exclusively emerged from stepwise modifications of antagonists, targeted screening (fragment-based, probabilistic design, chemogenomic approach, thematic analysis), or massive HTS campaigns.

Our approach to peptide mimetics is guided by the simple elegance which nature has employed in the molecular framework of proteinaceous species. Three basic building blocks, α-helices, β-sheets and reverse turns are utilized for the construction of all proteins. Peptides very frequently encompass reverse turn motifs (various β-turns and γ-turns), when interacting with their receptors. We and others realized, after analyzing a large collection of available 3D-structures of inhibitor/protease complexes, that small peptides and pseudopeptides, when acting as inhibitors of various protease families, often tend to adopt β-sheet structures. The design and synthesis of enzymatically stable peptide mimetic prosthetic units to replace these architectural motifs (reverse turns and β-sheets), and also less-well defined motifs, provides an opportunity to dissect and investigate complex structure-function relationships through the use of small synthetic conformationally restricted components. Thus, contrary to what is obtained from industrial screening programs, the strategy outlined herein should provide fundamental information on; a) the bioactive conformation of a target peptide when activating its receptor, b) the role of various motifs in the target peptide, and c) possible common binding features of importance for peptide receptor recognition and

receptor activation in general. Since metabolically stable peptidomimetics will be prepared and utilized instead of endogenous peptides, enzymatic processing and degradation will not be a major concern.

Secondary Structure Mimetics

Anders Hallberg, Anders Karlén, Mats Larhed, Gunnar Lindeberg, Christian Sköld, Ulrika Rosenström, Charlotta Wallinder, Rebecka Isaksson, Luke Odell, Jonas Sävmarker

Introduction: Drug design would benefit greatly from knowledge of the biologically active conformation of peptides. Since small linear peptides possess considerable conformational flexibility, and biophysical investigation of peptides in their natural environment is still in its infancy, the biologically active conformation has to be approached in a different way. The study of conformationally restricted analogues seems to be a worthwhile alternative.

Aim: To transform peptides into non-peptidic analogues by the iterative incorporation of well-defined secondary structure mimetics in target peptides which recognize receptors of unknown 3D structure.

Method: Our strategy comprises, in an iterative process: a) rigidification of the peptide and pharmacological evaluation, b) generation of a hypothesis of the bioactive conformation of the rigidified peptide by use of conformational analyses, c) incorporation of secondary structure mimetics and evaluation, d) elimination of non-essential molecular fragments followed by optimization, including, if relevant, structure optimization based on combinatorial chemistry to provide low molecular weight compounds. We aim to explore the potential of this strategy for the development of drugs acting on peptide receptors. This strategy, or modifications thereof, we believe should have a high generality and be applicable to numerous peptides, particularly in cases where the bioactive conformation comprises a well defined secondary structure motif. The octapeptide angiotensin II is a primary target suitable as a model peptide in the development and fine-tuning of the design strategy.

Angiotensin II Receptor Type 4 (IRAP) Inhibitors

Anders Hallberg, Mats Larhed, Anders Karlén, Gunnar Lindeberg, Karin Engen, Ulrika Rosenström, Christian Sköld, Jonas Sävmarker

The octapeptide angiotensin II is known as a potent effector of the renin-angiotensin system and the development of highly selective receptor ligands for this peptide has allowed the identification of several angiotensin II receptor subtypes: AT1, AT2, AT3 and AT4. Most of the known effects of angiotensin II can be attributed to the AT1 receptor (e.g. vasoconstriction). The relevance of the AT4 receptor, also known as the insulin-regulated amino peptidase (IRAP), is poorly understood and data regarding its properties mainly emerge from binding studies. The observed distribution of AT4 sites for angiotensin IV (the 3-8 fragment of ang II) indicated that this receptor/enzyme is present throughout several neuronal systems, and most striking is its location in motor nuclei and motor associated neurons. Most of the physiology of the AT4 system known so far, relates principally to cerebral vascular function and growth control of vascular tissues.

Aim: To design and synthesize selective AT4 receptor ligands / IRAP inhibitors and to characterize their mediation of CNS effects.

Method: Systematic cyclization and bicyclization of angiotensin IV followed by iterative incorporation of secondary structure mimetics as described in the project "Secondary structure mimetics." Small biased libraries of cyclised pseudopeptides are constructed in order to obtain information on the bioactive conformation of angiotensin IV and for the guidance of further design. As an alternative approach new lead compounds have been identified from a HTS screen of a small molecule library. Computational methods will guide the design process and the lead compounds will be systematically investigated to obtain more potent compounds. Side chains will be optimized by high-speed chemistry techniques.

Angiotensin II Receptor Type 2 Agonists

Anders Hallberg, Mats Larhed, Gunnar Lindeberg, Christian Sköld, Luke Odell, Charlotta Wallinder, Tamal Roy, Jonas Rydfjord, Jonas Sävmarker, Rebecka Isaksson, Marc Stevens, Jayendra Patel

Introduction: The role of the AT2 receptor is not yet fully understood. It has been suggested that the AT2 receptor is involved in renal function, growth, restinosis, wound healing cerebral blood flow control and control of bicarbonate secretion. While both selective and non-selective nonpeptidic AT1 receptor agonists have been developed recently, no examples of selective nonpeptidic AT2 agonists have been disclosed. Access to a selective AT2 agonist should constitute an important research tool in the effort to clarify the role of the AT2 receptor.

Aim: To design and synthesize selective nonpeptidic AT2 receptor agonists.

Method: We have established relevant AT1 and AT2 receptor assays that allow fast and efficient screening. A nonselective AT1/AT2 receptor agonist is used as starting point. Our strategy involves systematic modifications of nonselective agonists and in addition the application of the concept presented in the "secondary structure mimetics" project.

Members of the group during 2016

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Mats Larhed, Professor
Luke Odell, Assoc. Professor
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Marc Stevens, PhD student
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Charlotta Wallinder, Researcher
Ulrika Rosenström, Researcher
Tamal Roy, Post Doc
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Rebecka Isaksson, PhD student

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SciLifeLab DDD

Development and Mechanistic Understanding of Rapid Metal-Catalyzed Organic Reactions – Applications Involving Enzyme Inhibitors and ADMET privileged Compounds

Research Group Leader: Mats Larhed

Microwave-assisted organic synthesis: Developing lead structures with the goal to identify a drug candidate is seldom trivial and there is a constant demand for new, fast, efficient and reliable synthetic methods. In this context, tools that allow selective high-speed synthesis and convenient purification are highly desirable. Thus, the expectations placed on the preparative medicinal chemist today are not only to synthesize and purify every type of desired target structure, but also to do it quickly. To meet these high expectations, a set of emerging technologies have been developed, among them the use of controlled microwave irradiation as a convenient high-density energy source. The advantages of using sequential high-density microwave processing over traditional heating, or parallel methods, include shortest possible reaction times, high reaction control, faster hypothesis iterations and the possibilities to both change all parameters in the matrix and directly import achieved results into the design after each individual synthetic experiment. Reaction parameters such as heating time and temperature, different substrate concentrations and ratios, or solvents, catalysts or additives, can be rapidly evaluated. The rapid feedback encourages explorative work, providing quick results and increased productivity. Our previous work in the area of microwave-accelerated organic chemistry has resulted in a very large acceptance of this technology worldwide. In fact, you can today find dedicated microwave synthesizers in practically every single industrial or academic combinatorial / medicinal chemistry laboratory, making microwave heating the most utilized of all “combinatorial chemistry” technologies.
Metal-catalyzed transformations: Reactions catalyzed by soluble transition-metal complexes comprise a group of highly chemoselective transformations, which allow the formation of many kinds of carbon-carbon and carbon-heteroatom attachments that were previously very difficult to accomplish. However, the sometimes tedious pinpointing of the appropriate reaction components, together with the long reaction times (ranging from hours to days) frequently required for full conversions, have limited the exploitation of these protocols in many medicinal synthesis applications.

Aspartic protease inhibitors: There are four major classes of proteolytic enzymes: aspartic, serine, cysteine and metallo proteases. Enzymes from all these classes have been validated as targets for drug intervention in a wide array of diseases and syndromes, and a number of protease inhibitors have reached the market in the last decade. Protease inhibitors block an undesired cleavage of a peptide or protein substrate by binding, reversibly or irreversibly, to the active site of the protease. Hence, the inhibitors compete with the substrates. Aspartic proteases are characterized by their ability to hydrolyze peptide bonds with the aid of two catalytic aspartic acids in the active site. The cleavage mechanism most likely involves a nucleophilic attack by an activated water molecule at the scissile (hydrolyzable) peptide bond carbonyl carbon. One of the aspartic acids activates the water molecule while the other donates a proton to the amide nitrogen, creating a hydrogen-bond stabilized tetrahedral intermediate, which subsequently collapses into the carboxylic acid and amine cleavage products. The first aspartic protease used as a target protein in drug discovery was renin. Efforts were made in the 1970s and 1980s to develop renin inhibitors as a new class of anti-hypertensive drugs. During the search for renin inhibitors, substrate sequences where non-hydrolysable surrogates replaced the scissile bonds of the natural substrate were found to be effective blockers of enzyme function, especially when using replacements that can be considered to be analogues or mimics of the tetrahedral intermediate in the peptide cleavage. This strategy of using a central ‘transition-state’ isostere (e.g. –CH(OH)CH_2NH–) at the position where cleavage normally occurs was proven so effective that it has become the basis for the design of virtually all aspartic protease inhibitors. The aspartic proteases that have attracted most attention so far are renin, the HIV protease, the plasmepsins (malaria), the SAPs (candida infections) and β-secretase (Alzheimer’s disease).

HIV-1 Protease Inhibitors

Mats Larhed, Anders Hallberg

Introduction: Human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), is spreading at an alarming rate. Despite recent progress, a majority of HIV infected patients in low- and middle-income countries do not have access to proper treatment. The HIV-1 protease is a virally encoded homodimeric aspartyl protease responsible for the processing of the gag and gag/pol gene products, which enables the proper organization of the core structural proteins and the release of viral enzymes. Inhibition of HIV-1 protease leads to the production of immature, non-infectious viral particles. Today, several HIV-1 protease inhibitors have been approved for the treatment of AIDS. There is, however, a need for development of a new generation of inhibitors with high potency, with improved oral bioavailability and with reduced selection for resistance. The high cost of HIV therapy has also added to the importance of chemical readily accessible inhibitors.
Aim: To design and synthesize inhibitors to the aspartyl HIV-1 protease. To generate leads with high potency, selectivity and fair bioavailability for further development. To develop a strategy that allows production at a low cost.

Method: Structure-based design. The compounds synthesized are cocrystallized with the protease, and the structural information gives further design guidance in an iterative fashion. A large number of very potent transition-state analogues that have been extensively studied in vitro and in vivo have been developed. The relation between the chemical structures of these and the oral bioavailability is studied within the group at BMC. Inexpensive carbohydrate derivatives are used as chiral pools. We use stereoselective methods for the creation of libraries of masked *tert*-OH based inhibitors. Development of new microwave-enhanced high-speed synthesis methods are in progress.

ADMET-Tools for Medicinal Chemistry

Mats Larhed, Charlotta Wallinder, Jonas Sävmarker, Ahmed Adeyem

Introduction: Drug development is an extremely risky enterprise and a large fraction of all projects fail in the costly clinical phase. The major reasons behind termination of drug development programs in the pharmaceutical industry are non-optimal efficacy and safety profiles, which in many cases can be related to a failure to accurately predict, and poorly understood, pharmacokinetic (ADMET) properties (Absorption, Distribution, Metabolism, Elimination, Toxicity). An increased awareness of this problem has resulted in research organizations with large resources, such as big pharma, introducing ADMET profiling of drug-like compounds at an earlier stage in the drug discovery process. In contrast, academic groups as well as small spin off companies resulting from academic research generally lack ADMET competence and are therefore restricted to using costly and generic CROs offering standardized generic methodologies rather than those suitable for a specific project. This shortcoming limits the number of profiled compounds prior to clinical studies, reduces the value of innovative projects directed towards new targets, and decreases the likelihood for success.

Aim: To address the ADMET-problem by initiating collaborations where the ADMET profiles for new compound series are investigated before and immediately after their synthesis, using in silico and in vitro tools. Through this approach, the chemistry can be rapidly directed towards structures with the most promising ADMET properties without compromising their efficacy. To develop new innovative synthetic methods for ADMET privileged libraries. To implement the new innovative ADMET tools in novel, peer-reviewed academic collaborations with the goal of adding high quality scientific value to chemistry and biological discovery in the area of drug research, PET-imaging and chemical biology.

Method: New effective synthesis methods will be devised for the introduction of bioisosters and masking/blocking of problematic functionalities, accelerating the lead optimization process. In collaboration with Prof. Artursson and Prof. Ingelman-Sundberg, structure- (ADMET) property relationships will be established in order to identify optimal bioisosters for each ADMET property (membrane permeability, metabolic stability, uptake and efflux transporters, accessible drug

concentrations/binding and solubility) and selection of drug candidates, PET-tracers etc. of the highest quality.

High-Speed Medicinal Chemistry

Mats Larhed, Luke Odell, Ashkan Fardost, Linda Åkerbladh, Jonas Rydfjord

Introduction: Today there is an ever growing demand for new lead-like organic molecules for biological evaluation in the pursuit of new drugs. The combinatorial or high-throughput chemist is therefore under constant pressure to increase the compound production. In this reality, not only purification speed, but also reaction rate is of essence. Convenient methods to promote rapid reactions become important. New automatic microwave synthesizers constitute robust high-speed tools with the potential to help meet these demands, and to become efficient "superheating" devices in the combinatorial laboratory.

Aim: To explore microwaves as an efficient energy source for rapid solution phase combinatorial chemistry. To utilize high-density microwave irradiation for controlled release of gases from solids and liquids, and to use the liberated gases as central building blocks in high-speed metal-catalyzed synthesis. To apply the microwave "flash-heating" methodology in the synthesis of discrete and well characterized, high quality libraries of biologically interesting lead molecules. To employ a new concept for rapid lead optimization based on metal-catalysis target-assisted selection and preformed building blocks.

Method: The presented research project brings together investigations of new robust and very rapid microwave heated metal-catalyzed organic reactions for use in combinatorial chemistry, including reactions with carbon monoxide, the general rationale being optimization of lead structures. Microwave flash-heating, with a computer-controlled, dedicated single-mode microwave cavity designed for high-speed sequential synthesis, is exploited as a combinatorial niche technology.

Microwave-Assisted Metal Catalysis

Mats Larhed, Anders Hallberg, Jonas Sävmarker, Jonas Rydfjord, Peter Brandt, Bobo Skillinghaug

Introduction: Transition metal-catalyzed coupling reactions of aryl halides or pseudohalides have emerged as one of the most versatile types of carbon-carbon and carbon-heteroatom bond forming processes. Numerous elegant transformations in natural and non-natural product synthesis have been reported. Cross-couplings and Heck reactions constitute important tools in medicinal chemistry since they allow preparation of compounds substituted with a variety of functional groups, with diverse physicochemical properties, from a common precursor. Despite the extensive use of the Heck coupling, the reaction still suffers from severe limitations. These include unsatisfactory control of chemoselectivity, regioselectivity, stereoselectivity, double bond migration and selectivity in multifunctionalizations. Provided these factors could be controlled, the Heck reaction would have a considerably greater potential in selective organic synthesis and particularly in combinatorial organic chemistry. In addition, the possibility to perform metal-catalyzed chemistry in neat water or in flow-mode employing energy-efficient microwave heating appears attractive from a green perspective.

Aim: To develop new highly selective metal-catalysed coupling reactions. To investigate high-temperature water as an environmentally friendly reaction solvent. To develop continuous flow methodologies.

Method: In the Heck chemistry arena, we are focusing our research efforts on the oxidative addition, insertion and double bond migration processes, with the ultimate goal of developing robust and general synthetic methods. We investigate and expand the scope of chelation-controlled and ligand controlled Heck reactions. Furthermore, we are examining the unique properties of neat water at high temperature as the reaction medium. A profound mechanistic insight into metal-ligand interactions is a prerequisite for a successful programme. The use of microwave "flash-heating" for accelerating palladium-catalyzed coupling reactions is also examined, as well as continuous flow methodologies.

Green Palladium(II) Catalysis

Mats Larhed, Jonas Sävmarker, Ahmed Adeyem, Christian Sköld, Peter Brandt

Introduction: Research by R. F. Heck and T. Mizoroki in the early 1970s led to the discovery of the palladium(0)-catalyzed vinylic substitution reaction, nowadays commonly called the Heck reaction (Nobel Prize in Chemistry 2010). This highly versatile and useful carbon-carbon bond forming methodology using organo halides (or pseudohalides) as substrates has gained much interest over the years and is now a frequently employed synthetic tool. The palladium(II)-mediated version using organoboronic acids as arylmetal precursors did not cause much attention until the first catalytic protocols were reported by Uemura, Du and Jung. In 2004, we introduced the first ligand-modulated oxidative Heck reaction employing 2,9-dimethyl-1,10-phenanthroline (dmphen) to facilitate palladium reoxidation, to increase catalytic stability and to control the regioselectivity with electron-rich olefins. With bidentate nitrogen ligands, palladium loadings could be reduced and atmospheric air could be used as the sole reoxidant.

Aim: To develop new, green oxidative Heck reaction protocols, employing air for the essential Pd(II) recycling. To explore the scoop of the reaction methodology in medicinal chemistry projects. To develop continuous flow methodologies. To use the Pd(II)-bidentate nitrogen ligand catalytic system also for other classes of coupling reactions.

Method: We are directing our research work towards novel oxidative Heck couplings, enabling selective generation of secondary, tertiary and quaternary carbon centers from arylboronic acids. Moreover, we are examining the unique capacity of the Pd(II)-dmphen catalyst to produce arylpalladium(II) intermediates from arylboronic acids at room temperature. Furthermore, arylcarboxylic acids may now be employed as direct arylpalladium precursors. The reaction mechanism is investigated using direct ESI-MS and ESI-MS/MS analysis for detection and structural analysis of catalytic reaction intermediates.

Members of the group during 2016

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Ahmed Adeyem, PhD student
Peter Brandt, Researcher
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Publications 2014-2016

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Heterocyclic Chemistry

Luke Odell, Marc Stevens, Shiao Chow, Rajiv Sawant, Linda Åkerbladh

The vital importance of nitrogen heterocycles in organic chemistry and, in particular, in the pharmaceutical industry is without question. A recent analysis of the structural composition of FDA approved drugs revealed that 59% of all small-molecule drugs contain a nitrogen heterocycle. Moreover, the growing realization that compound collections do not provide an efficient sampling of chemical space has created a significant need for new and expedient methods for the preparation of heterocyclic scaffolds, particularly those that contain a sp^3 center. Our research is focused on various heterocyclic ring systems including indoles, indazoles, quinolinones, quinazolines as well as a number of saturated heterocycles. Our approach involves a mixture of different synthetic strategies including acid/base and transition-metal catalysis as well as multicomponent reactions. We are especially interested in the development of divergent and atom-efficient methodologies and exploiting new reactive intermediates.

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Theoretical investigations of transition metal-catalyzed reactions

Christian Sköld

Background: Information and models of the reaction steps in enzymatic reactions have proven to enable a rational approach in drug design by designing inhibitors that mimic key transitions states and high energy intermediates in the reaction. Using computational chemistry high quality model structures in the reaction steps can be identified and used for virtual screening aimed to identify new chemical starting points for enzyme inhibitors. When such a suitable chemical starting point is found that compound will serve as reference for the synthesis of structurally similar analogues to improve the properties of the compound. In this stage of the drug discovery process efficient carbon–carbon bond forming reactions are invaluable for both building the core structure and decorating the scaffold with efficient protein-interacting structural moieties. Palladium-catalyzed reactions are often employed and insights of the reaction mechanism of these reactions are important for development of efficient and useful reaction protocols. Key elements to that decide the efficiency and outcome of the reactions are the palladium ligand and solvent used, both of which effects are suitable to investigate by density functional theory calculations. The increased mechanistic understanding provides a foundation for the development of improved reaction protocols.

Aim: To investigate transition metal-catalyzed reaction mechanisms by means of density functional theory calculations for use in drug design and development and improvement of reaction protocols.

Method: We are currently focusing our efforts on both enzyme- and Pd(II)-catalyzed reactions to investigate their potential energy surface. In enzyme-catalyzed reaction investigations we identify relevant transition states and high energy intermediates and develop methods that can be used for screening and design of new enzyme inhibitors. In investigations of Pd(II)-catalyzed reactions we are currently implementing the Q2MM methodology to enable transfer from DFT to fast molecular mechanics calculations for the design of novel Pd ligand that aims to facilitate challenging chemical reactions.

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Antibacterial drug discovery

Research group leader: Anders Karlén

Mycobacterium tuberculosis (*Mtb*), the pathogen that causes tuberculosis, is estimated to affect one third of the world's population and the World Health Organization has declared the disease a global emergency. Serious challenges associated with the rising epidemic are multidrug-resistance and the growing number of people co-infected with *Mtb* and human immunodeficiency virus (HIV). Today's treatment consists of extensive chemotherapy, where complementary drugs are combined and administration periods stretch over several months. Side effects, in addition to the problems associated with patients discontinuing the treatment prematurely, add to the seriousness of the disease and there is therefore a need for new antitubercular drugs.

We have created RAPID (Rational Approaches to Pathogen Inhibitor Discovery), an integrated centre for structural biology and medicinal chemistry. This center was set up in 2003 and brings together medicinal chemistry, computational chemistry and structural biology groups at Uppsala University in a multi-disciplinarian effort with the aim to develop a new drug candidate against tuberculosis. Importantly, RAPID is also involved in the TB-related EU project, *More Medicine for Tuberculosis* (MM4TB, 2011-2015). This will give us the opportunity to maintain our network of collaborators and provides us with new targets and a future platform for TB drug discovery. Professor Alwyn Jones heads the center. The other principal investigators are Sherry Mowbray, Mats Larhed and Anders Karlén. Since its start in 2003 we have published more than 50 papers within the tuberculosis area and in methodology development.

RAPID scientists are active in the early phase of the drug discovery process. This includes target selection, protein expression, crystallographic studies, hit identification, assay development and evaluation of the inhibitory properties of compounds as well as design and synthesis of lead-like structures. Within the medicinal chemistry node we are responsible for the design and synthesis of small lead-like compounds that are required for inhibition studies, and for establishing structure-activity relationships (SAR). We are also involved in the hit identification process using computer-based virtual screening. In this approach

protein targets are screened against databases of small-molecule compounds to identify molecules that may interact with the target.

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Luke Odell, Research Associate
Christian Sköld, Research Associate
Johan Gising, Research Associate
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Design and synthesis of *Mtb* Ribonucleotide Reductase inhibitors

Anders Karlén, Mats Larhed, Johan Gising, Hiba Alogheli, Gunnar Lindeberg

Ribonucleotide reductase (RNR) catalyses the reduction of ribonucleotides to the corresponding deoxyribonucleotides and is an essential enzyme for DNA synthesis. The active enzyme is a tetramer composed of two large subunits (R1) and two small subunits (R2). R1 possesses the substrate and effector binding sites while R2 harbors a tyrosine radical essential for catalytic activity. The catalytic mechanism involves electron transfer between the radical in R2 and the active site in R1. The association of the subunits is therefore crucial for enzymatic activity. RNR is a well-known target for cancer therapy and antiviral agents and studies have also shown that RNR may be a promising target for development of new antitubercular drugs. In the RNR project, we have followed three strategies to identify RNR inhibitors.

The starting point for two of the approaches is the heptapeptide (Glu-Asp-Asp-Asp-Trp-Asp-Phe) corresponding to the C-terminal end of the R2 subunit. In the first approach a series of peptides based on an N-terminal truncation, an alanine scan and a novel statistical molecular design approach have been synthesized. A QSAR model has been built and an understanding of the requirements for molecular recognition has been developed. In the second approach which was based on modeling studies of the crystal structure of the R1/R2 complex from *S. typhimurium* we identified a benzodiazepine-based turn mimetic, and a set of novel compounds incorporating the benzodiazepine scaffold was synthesized. In the third approach a set of novel inhibitors have been discovered using a combined shape and structure based virtual screening approach. A series of compounds have been prepared based on one of the hits and these have also been evaluated for antibacterial activity.

Design and synthesis of *Mtb* 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) inhibitors

Anders Karlén, Mats Larhed, Peter Brandt, Martin Lindh, Bobo Skillinghaug, Fredrik Svensson, Shyamraj Dharavath, Christian Sköld, Luke Odell

The methylerythritol phosphate pathway to isoprenoids has attracted much attention lately as it has been shown to be a potential target for antimalarial and antibacterial drug discovery. The second enzyme in this pathway, 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), has been the focus of many of these investigations. The essentiality of DXR for *Mtb* has also recently been demonstrated. As a starting point for drug discovery in the DXR area two approaches have been applied. Both of these utilize the co-crystal structure between *Mtb* DXR and the known inhibitor fosmidomycin as determined within RAPID. Firstly, we have performed two independent structure-based virtual screens to identify hits that can be used as a starting point for X-ray crystallographic work and for synthesis. Secondly, we have used different structure-based design approaches for the design and synthesis of novel inhibitors. These studies have started from the crystal structure of fosmidomycin bound to *Mtb* DXR.

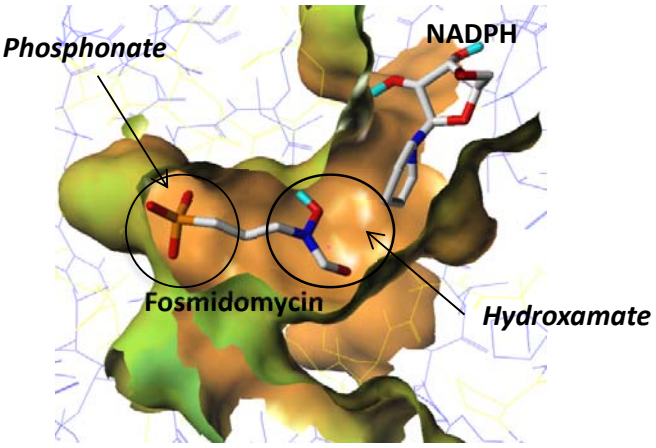

Figure 1. Crystal structure of fosmidomycin bound to *Mtb* DXR. Only the active site is shown for clarity and only part of the NADPH molecule is shown.

Fosmidomycin is presently in phase III studies for the treatment of malaria. Thus, fosmidomycin would seem to be the ideal candidate for development as an *Mtb* DXR inhibitor and as a potential lead compound in *Mtb* drug development. However, it lacks antibacterial activity and our aim is therefore to develop fosmidomycin analogues that can cross the *Mtb* cell wall while retaining high potency. In Figure 1, the binding of fosmidomycin and NADPH to *Mtb* DXR is seen. We have prepared fosmidomycin analogues using several different bioisosteres of the phosphonate and hydroxamate groups. However, the most promising modifications up to now have been to introduce aryl substituents in the α-position of fosmidomycin. This has produced analogues with submicromolar activity.

Design and synthesis of bacterial protease inhibitors

Anders Karlén, Mats Larhed, Peter Brandt, Johan Gising, Hiba Alogheli, Gunnar Lindeberg

Today, there are more than 20 protease inhibitor drugs approved by the FDA for the treatment of HIV/AIDS, hepatitis C infection, high blood pressure and type 2 diabetes. Approximately 25-30% of bacterial proteins are exported or expressed at the surface of the cell. Most secreted, and many membrane, proteins contain an N-terminal extension, the signal or leader peptide, which needs to be removed to form the mature protein. In bacteria, signal or leader peptidase 1 (LepB) is responsible for the majority of this

cleavage and is, therefore, an attractive target for the development of new antibacterial agents. The structure of a soluble truncated *E. coli* LepB has been determined in complex with a number of different inhibitors. We are presently running a medicinal chemistry programme based on the substrate for LepB using a classical peptide–to-peptidomimetic approach.

Computational medicinal chemistry

Research group leader: Anders Karlén

Computational medicinal chemistry has evolved into an important field within medicinal chemistry, and computational methods are used in almost all areas of drug design. Within the Department, the computational chemistry group works in close collaboration with the chemists in the different projects. We have a special focus on antibacterial and antiviral enzyme targets as well as GPCR targets. However, we also work on other targets with external collaborators. We predominantly use the techniques of conformational analysis, 3D-QSAR, molecular docking, virtual screening, and multivariate analysis. We have access to most of the important molecular modeling and computational chemistry tools. Much of our effort is spent on creating models that can be used to improve, for example, the activity of the compounds, or to identify compounds that can be used as starting points for drug discovery (hit identification). We are also developing methodology in the area of virtual screening in order to improve the performance of these approaches and to apply them to our projects. An increase in activity is not the only characteristic of a successful compound. Besides being non-toxic, it must also have other favorable features, such as good intestinal absorption and reasonably slow degradation (metabolism). We also try to model these properties with the help of computer-aided techniques.

Members of the group during 2016

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Christian Sköld, Associate Professor
Martin Lindh, PhD Student
Hiba Alogheli, PhD Student
Fredrik Svensson, PhD Student

Publications 2014-2016

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Peptides as starting points in drug discovery – Development of drug like peptides, peptidomimetics, bioisosteres, and synthetic methods for their development

Research Group Leader: Anja Sandström

Given the renewed interest of peptides within the pharmaceutical industry – as a result of the high success rate of peptide related pharmaceuticals, and massive new information provided from genomics, proteomics, and peptidomics – it is highly desirable that experience how to rationally transform biologically important peptides into drug-like molecules, i.e. modified peptides or peptidomimetics, is gained and that tools and strategies for such transformations are gathered. Peptides are often considered unsuitable as pharmaceuticals intended for oral administration due to the inherited drawbacks of the peptide structure as rapid degradation by proteolytic enzymes and low bioavailability. Thus, modified peptides or low-molecular weight drug-like molecules mimicking the action of bioactive peptides are highly needed. The term peptidomimetics is often used for such compounds. Rational design of peptidomimetics starting from peptides as lead compounds via a detailed probing of interactions between the peptides and the macromolecular drug-target is an alternative and a complementary approach to the high throughput screening procedures (HTS) that have coming to dominate industrial drug discovery strategies for hit and lead identification. In this research program two model systems have been heavily explored to study the overall transformation of bioactive peptides into drug-like molecules/peptidomimetics: HCV protease inhibitors derived from the important part of the natural peptide substrate and the neuropeptide Substance P 1-7 (SP_{1-7}). The starting point in both cases was peptides with a length of less than seven amino acids.

The overall aims of the project are a) to study the interaction between bioactive short peptides and their macromolecular targets, b) to develop orally bioavailable and drug-like peptides/peptidomimetics, and related chemical tools c) to use these molecules for the study of biological events related to the therapeutic area, and d) to develop general and efficient protocols for organic synthesis of modified peptides and peptidomimetic scaffolds.

Specific aims are to:

A. design and develop novel peptidomimetic hepatitis C virus (HCV) protease inhibitors with high potency, high selectivity and unique resistance profile.
B. design and develop drug-like peptides and/or peptidomimetics based on the neuropeptide Substance P 1-7 (SP_{1-7}) to be used as research tools for mechanistic investigations and target identification, and with potential use as analgestics for chronic neuropathic pain.
C. design and develop a novel carboxylic acid and amide bioisosteres with unique possibilities to fine tune physicochemical and pharmacokinetic properties as well as drug target interactions; that will serve as a new chemical tool in the development of orally bioavailable, efficient and safe drugs.
D. develop new synthetic methods for modification of peptides that should be compatible with solid-phase peptide synthesis (SPPS), and with special focus on methods useful for radioisotope-labeling of peptides for molecular imaging studies.

Members of the group during 2016

Anja Sandström, Associate Professor
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Anna Karin Belfrage, PhD-student
Johan Gising, Researcher
Hiba Alogheli, PhD student
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Publications 2014-2016

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The Hepatitis C project and carboxylic acid bioisosteres

Anja Sandström, Eva Åkerblom, Anders Karlén, Anna Karin Belfrage, Johan Gising, Hiba Alogheli, Per I Arvidsson, Sanjay Borhade, Prasad Wakchaure.

The interest in antiviral therapies has increased dramatically the last decades as shown by several successful market approvals in recent times, not least for hepatitis C. One important reason for this is that vaccines remain unavailable for many severe infectious diseases, including malaria, human immune-deficiency virus (HIV) and HCV. Unfortunately, the development of antiviral resistance runs side by side with the increased use of antiviral drugs. Today, is well known that a combination of antiviral drugs of different classes are needed to decrease the risk of escape mutants. For the treatment of chronic hepatitis C, which is caused by a virus recognized as the major cause of end-stage liver disease in the world and with a prevalence of 3% of the world population, the standard therapy for genotype 1 was recently augmented with several new direct acting antiviral drugs, including NS3 protease inhibitors, resulting in a high cure-rate for those that get the treatment. However, the long term success of this drug class might be challenged by the emergence of resistance. Single site mutations at protease residues R155, A156 and D168, confer resistance to almost all advanced inhibitors, and have frequently appeared in both in vitro and in vivo settings. Thus, efforts to design and develop the next generation of HCV protease inhibitors that retain activity against resistant variants must be taken into consideration. Moreover, efficient drugs for less common genotypes of the virus still remain to be developed.

We have developed several potent protease inhibitors of HCV NS3 over the years. The major achievements of our previous work were firstly the identification and exploration of C-terminal acylsulfonamides as bioisosteric replacements of the commonly used C-terminal carboxylic acid, and which now can be found in a marked drug and in several clinical candidates. Secondly, we discovered an influence of the helicase domain in the binding of protease inhibitors to the native full-length NS3 protein. Currently, we are concentrating our efforts to the development of unique HCV NS3 inhibitors that are different to those in late stages of clinical trials and on the market. More specifically, we are aiming at inhibitors targeting not only the wild type protease (gentotype 1), but also genotype 3, as well as drug resistant strains, and those within the “volume of the substrate” and thus potentially less susceptible to future drug resistance. Promising peptidomimetic lead compound classes have been developed, e.g. based on a heterocyclic beta sheet inducing scaffold, and will be further optimized with regard to potency as well as pharmacokinetic properties. In parallel with this project we are designing and synthesizing novel carboxylic acid bioisosteres, based on acylated sulfonimidamides, as well as characterizing their physicochemical properties.

The Neuropeptide Project

Anja Sandström, Anna Skogh, Gunnar Lindeberg, Rebecca Fransson.

Substance P 1-7 (SP_{1-7} = H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-OH) is the major bioactive metabolite of the well-known neuropeptide Substance P. The interest in this heptapeptide originates from the observation that it modulates, and in certain cases opposes the effects of the parent peptide, e.g. pain stimulation, inflammation, and the potentiating effect on opioid withdrawal symptoms. The physiological underlying mechanisms of SP_{1-7} at a molecular level, including receptor recognition, are still unclear. However, specific binding sites for SP_{1-7} in the rat and mice spinal cord and in certain brain regions have been identified. Even though the intriguing effects of SP_{1-7} have been known for quite some time SP_{1-7} has not previously been addressed in a medicinal chemistry program. Our early aims of this project was to develop stable and bioavailable peptidomimetics of SP_{1-7} be used as research tools in functional animal studies for a more thorough understanding of the physiological function of SP_{1-7}, including identification of its macromolecular target. Our initial efforts in this area included a thorough SAR study of the binding of SP_{1-7} and endomorphin-2 (EM-2) to the SP1-7-binding site by means of Ala-scans, truncation studies and C- and N-terminal modifications of the two target peptides, which resulted in the remarkable discovery of H-Phe-Phe-NH_2 as a high affinity ligand. Further studies and lead optimization in this project have resulted in both modified peptides and small-molecule dipeptidomimetics with strong analgesic effect in various animal models of neuropathic pain. Undeniably, there is a great need for new therapies that specifically targets neuropathic pain, since chronic neuropathic pain is an undertreated diagnosis with poor treatment options and which constitutes a major public health problem and a vast economic burden to society.

Several new types of less basic and constrained amino acid/dipeptide mimetics, including multidecorated heteroaryls, as well as synthetic protocols, are developed in this project. In vivo effects and pharmacokinetics properties are being evaluated both for small peptidomimetic analouges and modified peptides. In parallel with this project we are developing a novel method for N-capping and cyclization of peptides on solid phase which we believe will be useful also for isotope labelling of bioactive peptides.

Publications from Division members in 2014-2016, unrelated to the projects above

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Med. Chem. Commun. 2013, 4, 422-431.

Structure-based discovery of GPCR ligands

Research Group Leader: Jens Carlsson

Jens Carlsson studies complex biological systems at the atomic level using computer models and focuses in particular on G protein-coupled receptors (GPCRs). Several high-resolution structures of GPCRs have recently been determined, which has created intense interest in the research field regarding the possibility to discover ligands using structure-based methods. The main goal of the Carlsson group's research is to improve understanding of GPCR-ligand interactions at the atomic-level, with a vision to increase knowledge of receptor function and develop novel strategies for drug discovery. The group's projects are driven by computational chemistry and carried out in close collaboration with experimental groups.

Members of the group during 2016
Jens Carlsson, Associate Professor
Mariama Jaiteh, PhD student
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Publications 2016

Rodríguez D, Chakraborty S, Warnick E, Crane S, Gao ZG, O'Connor R, Jacobson KA and Carlsson J (2016) Structure-Based Screening of Uncharted Chemical Space for Atypical Adenosine Receptor Agonists. ACS Chem. Biol., 11:2763-2772

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Preclinical PET Platform (PPP)

Research at Preclinical PET Platform

At the Preclinical PET Platform (PPP) of the Department of Medicinal Chemistry, we bridge the gap between basic research in medicinal chemistry and clinical application of molecular imaging using Positron Emission Tomography (PET) and Single Photon Emission Tomography (SPECT) with simultaneously performed X-ray Computed Tomography (CT). We develop PET tracers for preclinical validation using state-of-the-art *in vivo* and *in vitro* methodologies. Our scanners include an integrated animal PET/SPECT/CT for small animal imaging, a high resolution Hamamatsu PET brain scanner for larger animals, as well as access to a clinical PET/CT scanner in collaboration with Uppsala University Hospital. During 2015, Prof. Mats Larhed received a grant from Kurt and Alice Wallenberg Foundation to build a national platform for preclinical in vivo imaging – WIPPET. A main part of the grant consists of the procurement of a state-of-the-art small animal integrated PET/MR instrument, which will be installed during 2016.

The main focus of PPP is on molecular imaging related to oncology, diabetes and neurodegenerative disorders, such as Alzheimer's disease (AD). Molecular imaging studies of other important diseases as well as radiolabelling technology studies have been performed during 2015.

Main research projects

- Diabetes
  - Beta cell imaging
- Molecular imaging and tracer development
  - Development of PET tracers for the study of angiotensin-2 receptor
  - Development of PET tracers for the study of fibrosis
  - Pre-clinical and clinical PET-CT *in vivo* and histomorphometrical investigations of bone response, and bone formation in connection with titanium implants and bone replacement.
  - Autoradiography study of angiogenesis in abdominal aortic aneurysm with [^{18}F]fluciclatide – an $\alpha_V\beta_3$ integrin ligand
  - Synthesis and preclinical evaluation of a ^{11}C-labelled libiguin - searching for a new brain receptor potentially involved in the regulation of sexual behaviors
- Neurodegeneration and other brain disorders
  - *In vitro* studies of central and systemic and Aβ-amyloidosis
  - Design and synthesis of a PET tracer for the study of the Vesicular Acetylcholine Transporter (VAChT)
  - Synthesis and radiolabelling of PET tracers for the study of Alzheimer's disease and trauma targeting the □-secretase enzyme (BACE-1)
  - Development of an antibody-based PET radioligand for Alzheimer's disease
  - Synthesis and preclinical evaluation of ^{11}C and ^{18}F- labelled tiophene derivatives as tracers for the study of Alzheimer's disease and systemic amyloidosis
- Oncology
  - Novel radionuclide imaging methods for molecular profiling of prostate cancer – a way for personalized therapy
  - Development of *in vitro* predictive assay for renal and hepatic uptake of conjugates for radionuclide molecular targeting.
- Radiolabelling technology
  - Development of methods for labelling synthesis with ^{11}CO

Members of PPP during 2016

Gunnar Antoni, Associate Professor
Veronika Asplund, Research Engineer
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Sara Bergman, PhD student
Jonas Eriksson, Scientist
Olof Eriksson, Associate Professor
Sergio Estrada, Scientist
Ewa Hellström-Lindahl, Associate Professor
Mats Larhed, Professor
Bogdan Mitran, MS student
Patrik Nordeman, PhD student
Anna Orlova, Associate Professor
Ulrika Rosenström, Scientist
Maria Rosestedt, PhD student
Ramkumar Selvaraju, PhD student
Marc Stevens, PhD student
Alf Thibblin, Assoc. Prof.
Irina Velikyan, Associate Professor
Ola Åberg, Scientist

Diabetes

Beta cell imaging

Research Group Leader: Olof Eriksson

Currently there exists no direct method for measuring the amount of insulin-producing cells (islet mass) in vivo. Today, islets mass in pancreas or at the site of islet transplantation is assessed by circulating biomarkers as for example c-peptide or glycated hemoglobin. However, these methodologies yields measurements which are delayed compared to changes in actual islet mass. When we measure a decrease in insulin producing capability, the corresponding islets may already be lost. The more direct approach of pancreatic biopsies for evaluation of BCM in patients is not practical due to invasiveness and risk of this procedure. Novel non-invasive methodologies for *in vivo* quantification of islet mass would therefore provide several advantages compared to current techniques.

Radiological modalities such as PET and SPECT offer the potential for direct non-invasive quantification of biological processes and tissues. The last decade has seen considerable investment in development of tracers aimed at quantification of islet mass in pancreas and transplanted islet grafts. Obviously such a methodology, when realized, would be of significance not only in relation to type 1 diabetes (T1D), but also to type 2 diabetes (T2D). The change in islet mass during the progress of T2D is not as drastic as in T1D, but the basic problem formulation of detecting successful prevention of decline or increase in islets due to intervention non-invasively is the same.

The major obstacle in imaging endogenous islet mass is related to the low proportion of islet tissue in pancreas (1-2%), combined with its heterogeneous distribution. Subsequently, this enterprise requires a PET tracer with very high specificity for islets. Much effort has been made to investigate several new and established tracers for the potential of *in vivo* islet imaging.

We study the *in vitro* and *in vivo* beta cell specificity of novel and established tracers, in preclinical animal models and in clinical studies. In addition, we work towards identifying novel beta cell specific targets and associated high affinity ligands by collaboration with the Department of Immunology, Genetics and Pathology, the Human Protein Atlas and AstraZeneca. The preclinical screening is performed using *in vitro* techniques such as cellular internalization and frozen tissue autoradiography on human donor material, acquired from the Nordic Network for Clinical Islet Isolation. *In vivo* scanning is performed in animal models of diabetes by means of a small animal PET/SPECT/CT scanner, and clinical PET/CT and PET/MR scanners Collaboration with the PET center at Uppsala University Hospital ensures rapid translation from preclinical to clinical studies.

Members of the group during 2016

Olof Eriksson, Associate Professor
Mohamed Altai, Postdoc
Ramkumar Selvaraju, Postdoc
Irina Velikyan, Associate Professor

Publications 2014-2016

1. Eriksson O, Selvaraju R, Willny M, Eich T, Carlbom L, Ahlström H, Tufvesson G, Lundgren T, Korsgren O. Positron Emission Tomography to assess the outcome of intraportal islet transplantation. Diabetes. 2016;65:2482-9.
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17. Eriksson O, Velikyan I, Selvaraju RK, Kandeel F, Johansson L, Antoni G, Eriksson B, Sörensen J, Korsgren O. Detection of Metastatic Insulinoma by Positron Emission Tomography with [68Ga]Exendin-4 - a case report. J Clin Endocrinol Metab. 2014;99:1519-24.
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Molecular imaging tracer development

Development of PET tracers for the study of angiotensin-2 receptor

Research Group Leader: Mats Larhed

The role and the biodistribution of the Angiotensin II AT2 receptor is not yet fully understood. The AT2 receptor is mainly expressed in foetal tissues and expression drops rapidly after birth. In the healthy adult, expression is concentrated to adrenal glands, uterus, ovary, vascular endothelium, heart and distinct areas of the brain. During pathological conditions such as myocardial infarction, brain ischemia, renal failure, and Alzheimer's disease up- regulation of the AT2 receptor has been reported. While selective Angiotensin II AT1 receptor tracers have been developed, the search for selective and efficient nonpeptidic AT2 ^{11}C-PET tracers continues. Access to metabolically stable AT2 receptor tracers should constitute an important research tool in the effort to clarify the role of the AT2 receptor in disease models.

The aim of this project is to design, synthesize and evaluate new selective nonpeptidic AT2 receptor PET tracers. We have established relevant AT1 and AT2 receptor assays that allow fast and efficient screening. A selective AT2 receptor agonist is used as the starting point for the development and our strategy involves systematic modifications of tracer candidates and radiolabelling with ^{11}CO. Series of unlabelled PET tracer candidates will be constructed using high speed organic chemistry based on innovative synthetic principles. Once important pharmaceutical properties such as drug solubility dissolution, absorption, distribution, metabolism, elimination and toxicity (ADMET) profiling have been established using Per Artursson's research platform, compound optimization will be performed and selected ADMET privileged PET candidates will undergo ^{11}C-radiolabelling and *in vitro* and *in vivo* testing. Despite the fact that candidate radiotracers often fail as a consequence of lack of metabolic stability and poor pharmacokinetics, recent breakthroughs in ADMET methods have not been fully utilized. Efficient synthesis of ADMET privileged PET AT2 tracer series will require high throughput analytical tools that allow rapid on line compound analysis. Magnetic spectroscopy imaging will be evaluated as an alternative to PET imaging.

Members of the group during 2016

Mats Larhed, Professor
Gunnar Antoni, Adjunct Professor
Sergio Estrada, Scientist
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Publications 2014-2016

1. M. Y. Stevens, S. Y. Chow, S. Estrada, J. Eriksson, V. Asplund, A. Orlova, B. Mitran, G. Antoni, M. Larhed, O. Åberg, L. R. Odell*: Synthesis of 11C-labelled Sulfonyl Carbamates via a Multicomponent Reaction Employing Sulfonyl Azides, Alcohols and [11C]CO. ChemistryOPEN, 5, (2016), 566–573.

Development of PET tracers for the study of fibrosis

Research Group leader: Gunnar Antoni

Gunnar Antoni, Olof Eriksson, Irina Velikyan, Ulrika Rosenström

Fibrosis is characterised by an increase and pathologic accumulation of collagen, a major constituent of the extracellular matrix. The main constituents in fibrosis are collagen type I and type II, forming fibrils composed of three α chains. The increase in collagen content found in fibrotic tissue is also combined with a remodelling process where the fibers are more cross-linked and aligned in one direction compared to normal extracellular matrix having a typical random direction of the collagen fibrils. This mechanically changes the properties of the tissue that becomes stiff. An increase in the ratio of collagen I to collagen II is also seen. In many chronic diseases fibrosis gives an important contribution to the symptoms and it is estimated that in USA up to 45% of all deaths can be related to disease involving fibrosis. All major organs can be affected by fibrosis with lungs, kidney and liver as particularly sensitive. In idiopathic pulmonary fibrosis (IPF) the etiology and pathogenesis is poorly understood. An excess of collagen is found early in the disease when clinical signs are minimal, with accumulation of collagen in alveols and interstitial space. The median survival time after diagnosis is only 36 months.

We intend to develop a non-invasive method for the study of fibrosis to be used in disease management to localize and quantify the fibrotic tissue. A peptide library will be designed and created based on binding affinity to the triple helical structure of collagen fibrils mimicking the collagen binding epitope of the immunoadherin glycoprotein VI. As a starting molecule is the peptide coined collagelin used which is modified to be labelled with ^{68}Ga as a NOTA chelate. This also gives the opportunity to label with ^{18}F in the form of FAl^{2+}. The labelled peptides will be evaluated in *in vitro* assays and *in vivo* using microPET. Biopsis of fibrotic tissue from patients will also be used to characterise the tracer candidates. The lipophilicity of the tracers can be modified with pegylation giving the option of directing the excretion to either a renal (hydrophilic) or hepatic (lipohilic) pathways to reduce the background radioactivity in the organ to be studied. It is thus likely that different tracers are needed for liver and kidney respectively.
In a collaboration with Rostock University, Germany, an animal model of pancreatic fibrosis will be studied using gallium-68 collagelin.

Irina Velikyan, Ulrika Rosenstrom, Thomas Bulenga, Olof Eriksson, Gunnar Antoni
Feasibility of multiple examinations using 68Ga-labelled collagelin analogues: organ distribution in rat for the extrapolation to the human organ and whole-body radiation dosimetry, *Pharmaceuticals* 9, 31 2016

Neurodegeneration and other brain disorders

Development of PET tracers for the study of neurodegeneration

Research Group Leaders: Gunnar Antoni and Mats Larhed

This program consists of four subprojects targeting different molecular aspects of neurodegeneration and the related potential causative processes inflammation and brain trauma.

Design and synthesis of PET tracers for the study of the Vesicular Acetylcholine Transporter (VAChT)

Gunnar Antoni, Sara Bergman, Sergio Estrada, Luke Odell, Mats Larhed, Alf Thibblin

Cognitive dysfunctions is either a hallmark and early manifestation or a late stage symptom in many neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, schizophrenia and progressive supranuclear palsy, frontotemporal dementia and Pick´s disease just to mention a few. Alzheimer's disease in particular is characterised by cognitive impairment and is today the most common cause for dementia. Due to the aging population Alzheimer's disease is an increasing healthcare problem with economical as well as social consequences, and not only affecting the patient but also influencing the quality of life among the family members.

The cholinergic systems together with the glutaminergic are the two main candidates involved in cognitive functions and the former is currently a target in symptomatic treatment of Alzheimer patients. It has also been shown that loss of cholinergic terminals better correlate to severity of cognitive impairments in Alzheimer patients than extracellular amyloid deposits measured as plaque load which further strengthens the hypothesis of cholinergic dysfunction as a cause for cognitive impairment.

A non-invasive diagnostic imaging approach using radiolabelled compounds for molecular imaging using PET is today the main modality for gaining insight into neurotransmission in the living brain by providing the tools for the study of complex chemical signalling systems that are responsible for normal brain functions. It is apparent that several neurotransmitter systems are involved in neurological disorders and in cognitive impairment, and access to PET tracers targeting different receptors, transporters and enzymes in the brain is of great importance for the understanding of normal brain functions as well as pathophysiological states.

VAChT is exclusively found in presynaptic neurons of the cholinergic system and is responsible for transport of newly synthesized acetylcholine into synaptic secretory vesicles and is one important marker for the integrity and function of the cholinergic system. Although the main interest is on brain VAChT expression, the peripheral cholinergic system is also a clinically important target such as in atrial fibrillation. A number of structural analogues based on the vesamicol or trozamicol templates have been labelled and investigated in in vitro and in vivo in animals as PET or SPECT tracers for VAChT. So far, no tracer sufficiently good for the intended purpose has been found and improvements in affinity, stability and pharmacokinetic properties are required.

The project aims at developing a selective and specific PET tracer with suitable characteristics that allow the in vivo study of VAChT in animals and humans using PET. The lead structures for ligands binding to VAChT are based on the benzovesamicol scaffold in which several positions have been identified with bulk tolerance. We will by structural modifications change lipophilicity and steric bulk at different positions generating a library of compounds for labelling with the short-lived positron emitting radionuclide carbon-11 (T½= 20.4 min) and potentially also fluorine-18 (T½= 109 min). Transition metal mediated ^{11}C-carbonylations will be the main chemical route for labelling which gives the option of introducing modifications both in the electrophilic and nucleophilic reagents used to build the labelled compounds..

Investigation of the tracer characteristics and biological functions of the labelled compounds are part of the project and standard in vitro binding assays are used for screening to select suitable candidates for more elaborated evaluations including in vivo animal studies using animal PET/CT.

Publications 2016

Roslin S, De Rosa M, Deuther-Conrad W, Eriksson J, Odell L.R, Antoni G, Brust P, Larhed M
Synthesis and in vitro evaluation of 5-substituted benzovesamicol analogs containing N-substituted amides as potential positron emission tomography tracers for the vesicular acetylcholine transporter
Bioorganic and Medicinal Chemistry in press 2017

Synthesis and radiolabelling of PET tracers for the study of Alzheimer's disease and trauma targeting the β-secretase enzyme (BACE-1)

Gunnar Antoni, Patrik Nordeman, Mats Larhed, Sergio Estrada.

Introduction: Alzheimer's disease (AD) is a neurodegenerative disease of the brain that is characterized by the progressive formation of insoluble amyloid plaques and fibrillary tangles. Plaques are extracellular constructs consisting primarily of aggregated Aβ42, a peptide fragment formed by the sequential proteolytic processing of β-amyloid precursor protein (APP) by two enzymes, β- and γ-secretase. β-Secretase (β-site APP cleaving enzyme or BACE-1), a novel type I transmembrane aspartyl protease whose identity remained elusive until 1999, is believed to be the key enzyme that commits APP catabolism to the amyloidogenic pathway. The amyloid hypothesis for treatment of Alzheimer's disease holds that upregulation of BACE-1 should promote deposition of long Aβ peptides and induce subsequent plaque formation in the brain. Methods for monitoring the progress of Alzheimers disease needs to be developed and one new promising concept concerns imaging of the BACE-1 concentration and location in the brain. The principal challenge is the construction of PET tracers that exhibit both high metabolic stability and ability to cross the blood-brain barrier (BBB) with high affinity to BACE-1.

Aim: To design and synthesize selective and stable non-peptidic β-secretase tracers. To investigate different strategies for ^{11}C labeling of BACE-1 PET tracers.

Method: Molecular modeling, enzyme-inhibitor docking and other computational methods, including molecular dynamic simulations, will guide the design process. Stereoselective synthetic strategies that allow for a systematic investigation and replacement of peptidomimetic prosthetic units carrying different bioisosteres will be employed. Radiolabeling will be conducted using ^{11}C monoxide.

Members of the group during 2016

Mats Larhed, Professor
Gunnar Antoni, Adjunct Professor
Sergio Estrada, Scientist
Patrik Nordeman, PhD student

Premixed calcium phosphate as a carrier for bone inducing factors - kinetics of bone regeneration studied with PET and SPECT

Research group leaders: Gunnar Antoni, Gry Hulsart Billström

Gry Hulsart Billstrom, Sune Larsson, Department of Surgical Sciences, Division of Orthopedics, Uppsala University, Jonas Åberg, Håkan Engqvist, Department of Technical Sciences and Division of Applied Materials Science, Uppsala University, Lars Gedda, Department of Oncology, Radiology and Clinical Immunology, Uppsala University, Sergio Estrada Platform for preclinical PET, Gunnar Antoni

Our group at the division of Orthopedics, is working in the field of tissue engineering and regenerative medicine with special emphasis on bone regeneration using cell-free injectable scaffolds. Our goal is to

develop and evaluate synthetic bone substitutes that induce bone, are highly biocompatible and that over time are resorbed and replaced by natural bone. Our aim is to use these biomaterials to heal large posttraumatic bone defects or provide healing when the normal bone formation is impaired. The materials we work with are hydrogels and injectable bone fillers containing calcium phosphates.
We have a close collaboration with two divisions at Ångström laboratory, i.e. the Division of Polymer Chemistry and the Division of Applied Materials Science. At present we are working on in vivo evaluation of injectable calcium phosphate cement where in the future bone-inducing factors will be added. In addition work is also being done on hydrogels made of modified hyaluronic acid as it is a potentially ideal biomaterial. It is abundant in the extracellular matrix and it is identical in all species. By modifying the material, we can derive a cross-linked stable hydrogel carrier for bone-inducing additives.

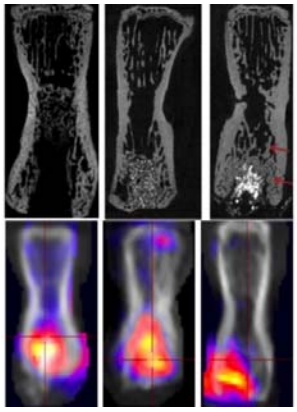

Figure 1: a rat-tail vertebral defect filled with bone substitutes. The lower picture show the osteoblast activity in the defect studied in SPECT with a radioactive tracer.

A study has been performed using PET using PET and SPECT to follow the kinetics of the bone regeneration process in rats using sodium [^{125}I]iodide, sodium [^{18}F]fluoride.
The kinetics of bone formation is measured both as release of [^{125}I]iodide from the synthetic bone substitutes with SPECT and as osteoblast activity quantified by PET and sodium [^{18}F]fluoride.
A manuscript has been submitted and a revised version to be sresubmitted 2017.

Synthesis and preclinical evaluation of 11C and 18F- labelled tiophene derivatives as tracers for the study of Alzheimer's disease and systemic amyloidosis.

Gunnar Antoni, Patrik Nordeman, Peter Nilsson, Per Hammarström, Gunilla Westermark

Pentameric thiophene scaffold, abbreviated LCOs (luminescent conjugated oligothiophenes)
show a striking specificity for protein aggregates associated with prion diseases and AD. These fluorescence probes bind to Aβ-deposits as well as prefibrillar Aβ assemblies and neurofibrillary tangles and exhibit distinct different emission spectra depending on which protein the molecule is bound to. In this project the prime objective is to label a library of tiophene derivative with ^{11}C and ^{18}F and investigate the specificity of binding and the potential of this class of compounds as PET tracers for the study of the different protein deposits found in AD patients. A potential novelty would be to distinguish by diagnostic imaging with PET between amyloid deposits and neurofibrillary tangles. Another interesting opportunity is to study systemic amyloidosis and be able to visualize and quantify amyloid deposits in organs such as, heart, liver, lung and kidney.

Publications 2016

Nordeman P, Johansson L, Bäck M, Estrada S, Hall H, Sjölander D, Westermark G, Westermark P, Nilsson L, Hammarstrom P, Nilsson P, Antoni G. ^{11}C and ^{18}F Radiolabeling of Tetra- and Pentathiophenes as PET-ligands for Amyloid Protein Aggregates, *ACS Medicinal Chemistry Letters Feb 2016*

Radiolabelling technology

Development of methods for labelling with synthesis with 11CO

Research Group Leader: Gunnar Antoni

Patrik Nordeman, Gunnar Antoni in collaboration with Prof. T. Skrydstrup, Aarhus University Denmark

Carbon monoxide in combination with transition metal catalysis has become a versatile reagent in organic synthesis. The carbonyl group is one of the most common functionalities in bioactive compounds and from a labelling perspective with ^{11}C an attractive position due to the expected high specific radioactivity and the option of a relatively simple process for creating a library of potential PET tracers for a certain in vivo binding site, such as a receptor protein. A new technique for the *ex situ* generation of carbon monoxide (CO) and its efficient incorporation in palladium catalyzed carbonylation reactions has been developed by Skrydstrup and co-workers at Aarhus university using a simple sealed two-chamber system. In this collaboration project we intend to translate this technology to synthesis with ^{11}CO and evaluate its usefulness. The importance is based on the technical simplicity compared with the existing methods for labelling synthesis with ^{11}CO.

Publications 2016

Andersen TL, Nordeman P, Christoffersen HF, Audrain H, Antoni G, Skrydstrup T
Application of methyl bisphospine-ligated palladium complexes for low pressure N-^{11}Cacetylations of peptides, *Angew Chem Int Ed* 56:x-y 2017 *in press*

DIVERSE CLINICAL AND PRECLINICAL PUBLICATIONS

1. Bodén R, Persson J, Wall A, Ekselius L, E-M Larsson, Antoni G.
   Striatal phosphodiesterase 10A and medical prefrontal cortical thickness in patients with schizophrenia: a PET and MRI study, *Trans. Psych* **7,** 2017
2. Somer EJ, Owenius R, Wall A, Antoni G, Thibblin A, Sörensen J
   The clinical safety, biodistribution and internal radiation dosimetry of [^{18}F]AH113804 in healthy adult volunteers, EJNMMI Research 6:87 2016
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5. Stevens M.Y, Chow S.Y, Estrada S, Eriksson J, Asplund V, Orlova A, Mitran B, Antoni G, Larhed M, Åberg O, Odel L.R
   Synthesis of 11C-labelled sulfonyl carbamates via a multicomponent reaction employing sulfonyl azides, alcohols and ^{11}CO, C*hemistryOpen* 5:566-573, 2016
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Positron Emission Tomography (PET) utilizing Pittsburgh compound B (PIB) for detection of amyloid heart deposits in hereditary transthyretin amyloidosis (ATTR)
J Nuc Cardiol 2016

7. Furmark T, Marteinsdottir I, Frick A, Heurling K, Tillfors M, Appel L, Antoni G, Hartvig P, Fischer H, Långström B, Eriksson E, Fredrikson M
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[^{11}C]UCB-A, a novel PET tracer for synaptic vesicle protein 2A, *Nuc Med Biol* 43:325-332 2016
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Imaging in vivo tau pathology in Alzheimer´s disease with THK5317 in a multimodal paradigm, *Eur J Med Mol Imaging,* 43:1686-1699 2016
10. Sehlin D, Fang X.T, Cato L, Antoni G, Lannfelt L, Syvänen S
Antibody-based PET imaging of amyloid beta in mouse models of Alzheimer's disease, *Nature Communication* 19;7:10759. 2016 doi: 10.1038/ncomms10759
11. Jaime Retamal, Jens Sörensen, Mark Lubberink, Fernando Suarez-Sipmann, João Batista Borges,Ricardo Feinstein, Sirpa Jalkanen, Gunnar Antoni, Göran Hedenstierna, Anne Roivainen, Anders Larsson, Irina Velikyan
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Am J Nucl Med Mol Imaging 2016;6(1):18-31
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Tracer kinetic analysis of (s)-^{18}F-THK5117 as a PET tracer for assessing tau pathology, *J Nuc Med* 57:574-581 2016

Oncology

Novel radionuclide imaging methods for detection and molecular profiling of prostate cancer – a way for personalized therapy

Research Group Leader: Anna Orlova

Molecular imaging techniques might improve treatment of prostate cancer by better staging, personalising patient management and/or evaluation of early response to therapy.

Correct staging of prostate cancer is crucial for patient management. Conventional anatomical imaging modalities (CT and MRI) tend to understage prostate cancer due to poor sensitivity to soft tissue metastases. The false-negative results contribute to a significant number of patients with extraprostatic disease undergoing non-curative surgery. The use of [^{18}F]FDG for imaging of malignant tumours by positron emission tomography (PET or PET/CT) provides excellent sensitivity in many cancers. However, the utility of this method for prostate cancer is limited because glucose utilisation is low and FDG uptake is insufficient in up to 81% of primary prostate cancers. Other metabolic PET tracers have shown some promising results in the clinic but have low selectivity.

An alternative approach to visualisation of prostate cancer is radionuclide targeting of the prostate tumour markers, e.g. PSMA or GPRP. Expression of prostate tumour markers is low in normal prostate tissue, but is increased in prostate cancer and correlates with prostate cancer progression. Targeting of PSMA is utilised for imaging of prostate cancer using ^{111}In-labelled ProstaScint (capromab pendetide), which is approved for clinical use by FDA. Still, imaging of PSMA can be improved by both optimizing radionuclide for labelling and by optimizing a tracer format (e.g. the use of small targeting proteins instead of bulky IgG).

It has been reported that GRPRs are expressed at high density on the cell membranes of prostatic intraepithelial neoplasias, primary PC and invasive prostatic carcinomas, whereas normal prostate tissue and, in most cases, benign prostate hyperplasia were predominantly GRPR-negative. Decapeptide analogues of the bombesin were predominantly evaluated for imaging of GRPRs and antagonistic analogues demonstrated advantages in molecular imaging over agonistic ones.

Our group works on development and pre-clinical evaluation of PSMA- and GRPR-targeting imaging agents. We have established collaboration with peptide chemists at Medicinal Chemistry Department for production of new tracers and with radiochemists at Institute of Immunology, Genetics and Pathology, Preclinical PET-MRI Platform and PET Center at Uppsala Hospital for development of the appropriate labeling methods.

Alternative treatments for of androgen-independent prostate cancer could be targeting against tyrosine kinase receptors family that are often overexpressed in advanced prostate cancers. This approach requires confirmation of the presence of receptors in cancer lesions and therapy monitoring for early response. This could be done by radionuclide diagnostic imaging.

The use of antibodies for diagnostics and therapy has a serious limitation. Antibodies are relatively bulky (150 kDa), which complicates their extravasation and penetration into malignant tissue. Blood clearance is also slow, which causes high background during imaging and high unspecific whole-body irradiation during therapy. Smaller antibody fragments provide better tumour-to-normal tissues radioactivity ratio than intact antibodies and size reduction is a proved approach to improvement of targeting properties of radionuclide probes for tumour imaging and treatment. The size of the immunoglobulin based tracers can only be reduced to 25 kDa for scFv or 15 kDa for domain antibodies. Affibody molecules are only half the size of the domain antibodies. Affibody molecules are three helical domain proteins of approximately 58 amino acids having a structure deriving from one domain of staphylococcal protein A. Our group participated in selection, evaluation and pre-clinical characterisation of Affibody molecules binding to different molecular targets relevant to prostate cancer, e.g. HER2, EGFR, IGF1R, HER3, VEGFR2, PDGFRβ. Preclinical data suggest that the affibody ligand provides at least one order of magnitude better imaging contrast (tumour-to-organ ratios) in murine xenograft model, than the best antibody fragments.

The comparison of imaging properties of anti-HER2 ligands as full length antibody trastuzumab and Affibody molecule ABY-025 demonstrated that high contrast image with Affibody molecule can be obtained in much shorter time after injection of radiolabeled ligand probe. Furthermore, clinical data show that ^{111}In- and ^{68}Ga-labelled anti-HER2 Affibody molecule may be used for imaging of HER2-expressing metastases cancer patients.

Members of the group during 2016

Anna Orlova, Professor
Zohreh Varasteh, PostDoc
Maria Rosestedt, PhD student
Bogdan Mitran, PhD student
Elin Linström, MSc student

Development of imaging agents for visualization of GRPRs

We have developed new method for affinity determination for binders to gastrin releasing peptide receptors (GRPR). The common methods are based on end-point experiments using cells that use to underestimate affinity for binders with picomolar affinities. Due to problems with purification and stabilisation of GRPR, it is impossible to determine affinity using the surface plasmon resonance. We proposed to use the measurements of real-time protein-cell interaction using the new device for in vitro characterisation, LigandTracer. We have demonstrated that affinity values measured using this method correlate with other measured parameters in vitro and in vivo.

We have studied influence of chelators, hydrophilizing linkers and radionuclides on the imaging properties of antagonistic bombesin analogue RM26. The receptor recognition part should be highly lipophilic for creating high affinity. This creates problems in biodistribution profile of binders, i.e. high degree of hepatic uptake and hepatobiliary excretion of radiolabeled peptides. Hepatic uptake obscures detection of liver metastases and hepatobiliary excretion - detection of lesions in abdomen. We hypothesised that by modification of the parts of the GRPR targeting peptide that do not participate in receptor recognition we can influence biodistribution profile. We have identified several factors that could influence hydrophilicity of these parts: chelators, linkers between chelator and peptide and radiometals. We have found that introduction of hydrophilic moieties do not influence peptides affinity to GRPR but significantly improve biodistribution properties by decreasing hepatobiliary excretion. We also have found that overall charge and geometry of metal-chelator complex has strong influence on biodistribution, and we concluded that in vivo properties should be evaluated properly when modifications in structure of peptide would be introduced or radioisotope should be exchanged. We have developed GRPR-targeting agents for PET labelled with ^{68}Ga, ^{18}F and for SPECT labelled with ^{111}In.

Members of the project group during 2016

Anna Orlova, Professor
Zohreh Varasteh, PostDoc
Bogdan Mitran, PhD student
Ulrika Rosenström, Researcher
Gunnar Lindeberg, Researcher
Mats Larhed, Professor
Jens Sörensen, Adjunct professor
Vladimir Tolmachev, Professor

Results were published in following full peer reviewed papers 1-6 (from publication list).

Development of PET tracer for imaging of PSMA

For the imaging of prostate specific membrane antigen (PSMA) we proposed to use capromab. This mAb recognises the intracellular domain of PSMA and binds just to cells with disrupted cellular membrane i.e.

dead cells. We hypothesised that when internalisation does not play any role in tumour uptake and retention of imaging agent the radiolabel could be non-residualizing (radiocatabolites leak from the cell after intracellular degradation). Normal excretory organs (liver and spleen for mAb) should have rapider clearance, but tumour uptake should not be influenced. We proposed to use PET for further improvement of imaging. We have demonstrated that capromab labelled with non-residualizing PET isotope ^{124}I visualised tumours better than capromab labelled with residualizing SPECT isotope ^{111}In.

Members of the project group during 2016

Anna Orlova, Professor
Bogdan Mitran, PhD student
Maria Rosestedt, PhD student
Vladimir Tolmachev, Professor

Results were published in following full peer reviewed papers 7 (from publication list).

Imaging of RTK using affibody molecules

1. Affibody-based imaging of RTK. We have developed a novel affibody for imaging of HER3 expression. A feasibility of in vivo imaging in tumours using ^{99m}Tc-labelled affibody was demonstrated. We have performed a comparative evaluation of two ^{111}In labelled affibodies with different lipophilicity of binding site. The best variant was labelled with ^{68}Ga for the imaging using PET. We also have developed affibody for PET and SPECT imaging of PDGFβR.
2. Refinement of labelling chemistry for anti-IGF-1R affibody was foreseen to reduce undesirable uptake and retention of radioactivity in liver and kidneys. We evaluated dose of injected protein, optimal molecular format, labelling methods, time of imaging after administration. We have demonstrated that introduction of hydrophilic moiety significantly decreased unspecific radioactivity uptake in liver. However, further reduction of hepatic and renal uptake by decreasing residualizing properties of radiolabel also decreased tumour uptake. We are planning to combine hydrophilic moiety with strongly residualizing radiometal label.
3. By analyses of our previous data on EGFR imaging using affibodies we had identified two mechanisms of hepatic uptake, i.e. receptor mediated that could be decreased by varying injected protein dose, and unspecific uptake that influenced by protein structure. We hypothesised that introduction of negatively charged hydrophilic modifications could supress unspecific uptake. We have identified labelling method that dramatically decrease hepatobiliary uptake. The optimal protocols were established and study on imaging of EGFR in murine model is ongoing.

Members of the project group during 2016

Anna Orlova, Professor
Maria Rosestedt, PhD student
Zohreh Varasteh, PostDoc
Bogdan Mitran, PhD student
Vladimir Tolmachev, Professor
Stefan Ståhl, Professor
John Löfblom, Lecturer
Torbjörn Gräslund, Lecturer
Mats Larhed, Professor

Results were published in following full peer reviewed papers 8-23 (from publication list).

Therapy monitoring of prostate cancer

Therapy monitoring could improve outcome in PCa by identification of non-responders. Targeting of RTK is often accompanied by changes in receptor expression. The anti-HER2 and anti-EGFR conjugates were

evaluated for detection of changes in molecular target expression in response to a targeted therapy. The response of several PC cell lines to targeted anti-RTK treatment was evaluated in vitro. Several kinds of targeting agents (mAbs, tyrosine kinase and HSP90 inhibitors) were evaluated. Receptor expression was measured using radiolabelled affibodies after treatment, and its correlation with the therapy response (degree of growth inhibition) was evaluated. We have demonstrated that only cells strongly affected by anti-RTK treatment responded with downregulation or stable HER2 expression. On the opposite, cells that showed a moderate response to treatment demonstrated an increased HER2 expression. We also observed significant up-regulation of EGFR expression in cells responding to anti-RTK therapy. In another study we modelled external irradiation treatment in vitro by acutely exposure of PC cells for external irradiation. We analysed the cell survival as well as their HER2 expression. The HER2-expression in cells responding to external therapy remained stable over 48 h, whereas the receptor expression in cells resistant to irradiation significantly increased. We also demonstrated that combination of external radiation and anti-HER2 therapy significantly improved therapy outcome. We conclude that HER2 and EGFR imaging could be a powerful tool in monitoring of therapy response in PC.

Members of the project group during 2016

Anna Orlova, Professor
Maria Rosestedt, PhD student

Results were published in following full peer reviewed papers 24-25 (from publication list).

Development of approaches for targeting therapy of cancer

The development of therapeutic agents for radionuclide targeting therapy is a challenge: the accumulation of radionuclide in tumour lesions should be sufficient to kill the tumour cells but healthy tissues should be protected from damage induced by radioactivity. The most sensitive organs are bone marrow and kidneys. Our group investigate the mechanisms of renal uptake of targeting therapeutic peptides and proteins with the aim to reduce dose to kidneys with radionuclide targeting therapy. We also investigate factors influencing retention of radioactivity in kidneys after renal clearance of targeting molecules.
One of the approaches to reduce renal uptake is fusion of targeting proteins to Albumin Binding Domain (ABD). Such fused proteins after binding to albumin have extended residence time in blood circulation remaining full length antibody, but are twice smaller than them and have better tissue penetration.
Another approach is development of non-residualizing labelling methods when after internalisation and intracellular degradation of targeting proteins radiocatabolites leak from the cells due to lipophilicity. This approach could be used if targeting molecules have very slow internalisation rate into tumour cells. We have developed several such labelling methods for therapeutic radionuclides, iodine-131 and rhenium.

Members of the project group during 2016

Anna Orlova, Professor
Maria Rosestedt, PhD student
Zohreh Varasteh, PostDoc

Results were published in following full peer reviewed papers 26-29 (from publication list).

Thesis defended

Zohreh Varasteh, 2014-10-31, Bombesin Antagonists for Targeting GRPR-Positive Tumors

Publications 2014-2016

1. Varasteh Z, Velikyan I, Lindeberg G, Sörensen J, Larhed M, Sandström M, Selvaraju RK, Malmberg J, Tolmachev V, Orlova A. Synthesis and characterization of a high affinity NOTA-conjugated bombesin antagonist for GRPR-targeted tumor imaging. Bioconjug Chem, 2013 Jul 17;24(7):1144-53.

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Reviews 2014-2016

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Agencies that support the work/Funding

Vetenskapsrådet 900 kSEK/y, 2013-2015
Cancerfonden 500 kSEK/y, 2015-2017
Knut and Alice Wallenberg, co-applicant, infrastructure grant
27 000 kSEK, 2015-2019

Radiolabelling technology

Development of methods for labelling with synthesis with 11CO

Research Group Leader: Gunnar Antoni

Patrik Nordeman, Mats Larhed, Gunnar Antoni

Carbon monoxide in combination with transition metal catalysis has become a versatile reagent in organic synthesis. The carbonyl group is one of the most common functionalities in bioactive compounds and from

a labelling perspective with ^{11}C an attractive position due to the expected high specific radioactivity and the option of a relatively simple process for creating a library of potential PET tracers for a certain in vivo binding site, such as a receptor protein. A new technique for the *ex situ* generation of carbon monoxide (CO) and its efficient incorporation in palladium catalyzed carbonylation reactions has been developed by Skrydstrup and co-workers at Aarhus university using a simple sealed two-chamber system. In this collaboration project we intend to translate this technology to synthesis with ^{11}CO and evaluate its usefulness. The importance is based on the technical simplicity compared with the existing methods for labelling synthesis with ^{11}CO.

Publications 2014-2016

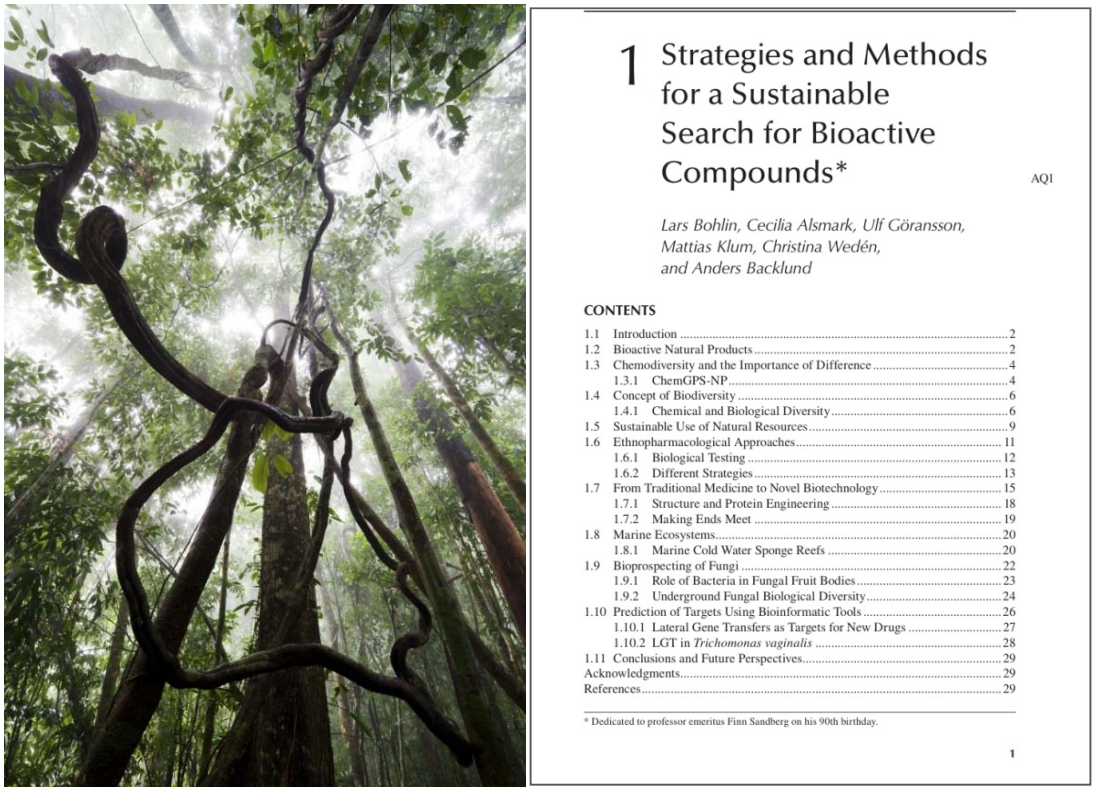
1. Thomas L. Andersen, Stig D. Friis, Hélène Audrain, Patrik Nordeman, Gunnar Antoni*, Troels Skrydstrup*
   Efficient ^{11}C-Carbonylation of Isolated Aryl Palladium Complexes for PET: Application to Challenging Radiopharmaceutical Synthesis
   J. Am. Chem. Soc. 2015, 137:1548–1555

2. Nordeman P, Friis SD, Andersen TL, Audrain H, Larhed M, Skrydstrup T, Antoni G
   Rapid and Efficient Conversion of $^{11}CO_2$ to ^{11}CO via Silacarboxylic Acids: Applications in Pd-Mediated Carbonylations
   Chemistry - A European Journal 21:17601-17604 2015

Pharmacognosy

Research at the Division of Pharmacognosy of the Department of Medicinal Chemistry is focused on bioactive substances of natural origin. We develop strategies for selection, isolation and characterisation with the objective to discover unique bioactive chemical structures with drug potential, and to reveal unknown targets, by studying the evolutionary structure-activity optimization in Nature. In addition to the possibility to discover new drug candidates for drug development, bioactive natural projects have potential as pharmacological tools, intermediates, or templates for synthesis of drugs. As a multidisciplinary division we conduct extensive national and international research collaborations in e.g. clinical pharmacology, marine chemical ecology, peptide chemistry, molecular biology, systematic botany, computational chemistry and structural biology.

Our research represents a modernization and renewal of a venerable discipline, pharmacognosy. With today's increased interest for environmental aspects, green chemistry, and a sustainable use of natural products, this renewal could have a strategic position in bridging chemistry and biology.



1 Strategies and Methods for a Sustainable Search for Bioactive Compounds* AQ1

Lars Bohlin, Cecilia Alsmark, Ulf Göransson, Mattias Klum, Christina Wedén, and Anders Backlund

CONTENTS

* Dedicated to professor emeritus Finn Sandberg on his 90th birthday.

1

Figure 1. Cover (photography by co-author M. Klum), and opening page for portal chapter by Bohlin L., Alsmark C., Göransson U., Klum M., Wedén C., & Backlund A. (2011) on “Strategies and methods for a sustainable search for bioactive compounds”. This chapter was written by the senior researchers at the division of pharmacogosy, and published in Bioactive Compounds from Natural Sources: Natural Products as Lead Compounds in Drug Discovery, edited by C. Tringali.

During 2016 there were two professors in pharmacognosy appointed; Anders Backlund and Ulf Göransson. The division is since March 2015 under management by professors Göransson and Backlund, of which the latter is initially taking up responsibility as head of division.

The ongoing projects are organised in two major groups, one focusing on the *chemistry and biology of ultra stable proteins* – led by professor Göransson – and the other on *methods of selection and target-finding of natural products based on phylogenetic and chemography* – led by professor Backlund.

During 2016 the group led by Dr. Paco Cárdenas as PI for the EU-funded project "SponGES" has development as a third group at the division with research in the field of natural products from marine sponges and their symbionts.

The Div. of Pharmacognosy at the Dept. of Medicinal Chemistry, Uppsala University
December 18th 2015.

From left to right, back row: Lars Bohlin, Ulf Göransson, Amit Jaisi, Erik Jacobsson, Cecilia Alsmark, Adam Strömstedt, Sandra Bratt*, Camilla Eriksson, Anna Koptina, Christina Wedén, Linda Strandenhed*, Birgitta Hellsing*, Paco Cárdenas, Nils-Otto Ahnfelt, Hesham el-Seedi, Anders Backlund.
Front row: Sohaib Mailk, Muhammad Shafiullah, Supun Mohotti, Taj Muhammad Khan, Lu Yang, Astrid Henz, Fardowsa Ibrahim. *Missing at the occasion:* Elisabet Vikeved,, Sungkyu Park

* administrative staff, employed at the department level

Peptide Chemical Biology

Research Group Leader: Ulf Göransson

Our research interest lies at the interface between chemistry and biology, and reflects our fascination of natural products and the possibilities these molecules represent. In particular, the research is focused on

peptides of natural origin, their discovery, biological effects, biochemistry, structure, and towards peptide chemical design and synthesis. The overall aim of our research is to develop naturally occurring peptide structures for applications in medicine or biotechnology, and to develop general methods to do so.

We focus on backbone cyclic peptides, such as cyclotides. These cyclic plant peptides represent an ideal scaffold for protein engineering because of their stability and ability to harness a wide variety of sequences and biological activities. Cyclotides consist of about 30 amino acid residues, of which six are cysteines that form three disulfide bonds arranged in a cystine knot (Figure 1). One aim of our research is to understand how we can exploit that scaffold and the way it is produced in plants, but also how the chemistry and biology of cyclotides can be applied to other families of peptides and proteins. After all, joining the N- and C- termini by an ordinary peptide bond seems perfectly logical and the seamless and knotted protein backbone confers an extraordinary stability. The exceptional chemical and biological stability that is inferred by cyclization favors their applications in drug design and discovery, where they may be used as carriers of less stable peptide sequences. Currently, the development in the field is geared towards the exploration of the biosynthetic pathways of cyclotides. The first examples of peptide ligases have been reported, and we have successfully been able to use purified enzyme fractions in preparative scale to produce the first cyclic peptides.

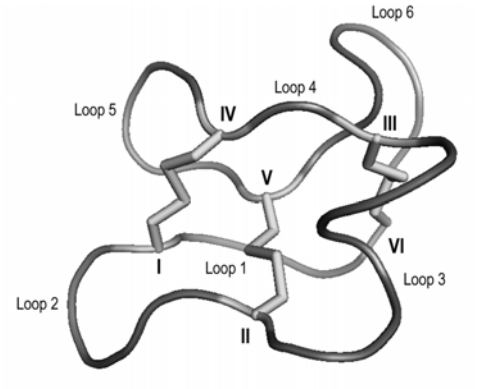


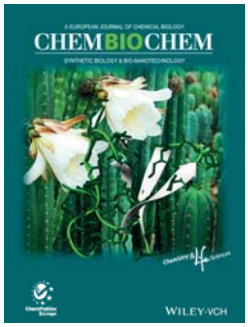
Figure 1. The cyclotide backbone*. Note the circular backbone and the cystine knot that define the cyclic cystine knot (CCK) motif. The variable loop regions (marked 1-6) between the cysteines (marked I-VI) are targets for protein engineering. The CCK motif is able to harness a number of biological activities: native cyclotides have been reported to have e.g. insecticidal, on-growth inhibitory, utero-contracting, HIV-inhibitory, trypsin inhibitory, and antibacterial activity.*

Currently, we aim at characterizing their antimicrobial properties in particular. Building on that knowledge and the methodology that we have developed, we have moved into the direction of design of cyclic peptides as specific binders to block protein-protein interactions and as antimicrobial agents. However, as our research group is expanding so are the research interests: during the last years we have entered into peptide toxin discovery exploiting peptidomics, combining next generation sequencing and mass spectrometry.

We collaborate with Prof Per-Johan Jakobsson at Karolinska Institutet, about peptides relevant in rheumatoid arthritis. Key collaborators include Prof Björn Hellman at the Dept of Pharmaceutical Biosciences, UU, on the antimutagenic effects of medicinal plants; Prof Dan I Andersson at the Dept of Medical Biochemistry and Microbiology, UU, on the antimicrobial effects of cyclotides.

The long standing interest of the chemistry in the marine environment has continued, and the strong research tradition on sponges is continued within the Bluegenics FP7 project, lead at UU by Prof Lars Bohlin. Dr Paco Cardenas worked in the Bluegenics project during the spring, to then formally moving to the Linnéuniversity to work on nemerteans. Nemerteans appear as a novel source of peptide toxins, and the project, has developed substantially. We have now identified the molecular targets of a new family of toxins. Key collaborators are Dr Håkan Andersson at Linnéuniversitetet, Dr Malin Strand at Swedish University of Agricultural sciences, and Prof Jan Tytgat, KU Leuven.

Our international collaborations include Dr Błażej Ślązak at the Polish academy of Sciences and Prof Elżbieta Kuta at the Jagiellonian University, Krakow, Poland. We have had a continued good collaboration with Drs Johan Rosengren and Richard Clark, and Prof David Craik at the University of Queensland, Australia. Dr Christian Gruber at the Medical University of Vienna, Prof Lars Skjeldal at The Norwegian University of Life Sciences should be mentioned among other international collaborators.



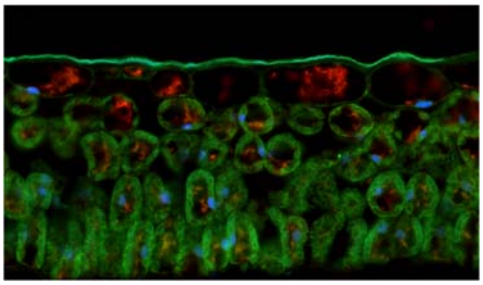


Figure 2. Some research highlights 2015. *At the top, the cover of the first book dedicated to cyclotides is shown. We contributed with Chapter 2, "Cyclotides in Violaceae". Our paper on a cactus antimicrobial peptide was selected for the cover of ChemBioChem. Another highlight in the cyclotide field was the use of antibodies for their staining: the panel below shows cyclotides (in red) in the epidermis of a leaf.* The VR Swedish Research Link project (750 kSEK, 2014-2016) to establish collaboration with University of Colombo, Sri Lanka is ongoing. Main applicant and the driving force behind this project is Sunithi Gunasekera.

We presented at several conferences during the year; highlights were the Nordic Natural Products meeting in Visby and the 3rd International Conference on Circular Peptides, which was held on Moreton Island. Taj Muhammed won the poster prize at the ICCP. We also presented at the American Peptide Symposium; the Australian Peptide Symposium; and the Modern Peptide Solid Phase meeting.

During 2015, four students the Uppsala Graduate School for Biomedical Research, and the Summer Research School (SOFOSKO) have been involved in our research. In addition, Martin Svahn did his Master research project at UQ in the Clark group.

Members of the group during 2016

Ulf Göransson, PhD, Professor
Sunithi Gunasekera, PhD
Adam Strömstedt, PhD
Erik Jacobsson, MSc, PhD student
Sohaib Malik, MSc, PhD student
Taj Muhammad Khan, MSc, PhD student
Sungkyu Park, MSc, PhD student
Camilla Eriksson, MSc, PhD student
Md Shafiullah, MSc, Research Assistant
Sanjeevan Rajendran, MSc, Guest PhD student University of Colombo
Supun Mohotti, MSc, Guest PhD student University of Colombo
Błażej Ślązak, MSc, Guest researcher (Jagiellonian University, Poland)
Aida Abd El-Wahed, Guest PhD student, El-Menoufia University
Javid Hussain, MSc, Research Assistant

Publications 2014-2016

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2. Strömstedt AA, Kristiansen, P; Sunithi Gunasekera S, Grob N, Skjeldal L, Göransson U. (2016) Selective membrane disruption by the cyclotide kalata B7: Complex ions and essential functional groups in the phosphatidylethanolamine binding pocket. Biochim Biophys Acta. 2016 Feb 12. pii: S0005-2736(16)30043-8. doi: 10.1016/j.bbamem.2016.02.013.

3. Boldbaatar D, Gunasekera S, El-Seedi H, Göransson U. (2015) Synthesis, Structural Characterization and Bioactivity of the Stable Peptide RCB-1 from *Ricinus communis* L. Journal of Natural Products, 78(11):2545-51.

4. Burman R, Yeshak MY, Larsson S, Craik DJ, Rosengren KJ, Göransson U. (2015) Distribution of circular peptides in plants: Large-scale mapping of cyclotides in the Violaceae. Frontiers in Plant Sciences 6:855. doi: 10.3389/fpls.2015.00855

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6. Carstens B, Rosengren, KJ, Gunasekera S, Schempp S, Bohlin L, Dahlström M, Clark R, Göransson U. (2015) Isolation, characterization and synthesis of the barrettides: Disulfide-containing peptides from the marine sponge *Geodia barretti*. Journal of Natural Products, 78(8):1886-93

7. Fernandes-Cerqueira C, Ossipova E, Gunasekera S, Hansson M, Mathsson L, Catrina A, Sommarin Y, Klareskog L, Lundberg K, Rönnelid J, Göransson U, Jakobsson PJ (2015) Targeting of anti-citrullinated protein/peptide antibodies in rheumatoid arthritis using peptides mimicking endogenously citrullinated fibrinogen antigens. Arthritis Research & Therapy, 10(17):155

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9. Dahlström M, Sjögren M, Jonsson PR, Göransson U, Lindh L, Arnebrant T, Pinori E, Elwing H, Berglin M (2015) Affinity states of biocides determine bioavailability and release rates in marine paints. Biofouling 31(2):201-210.

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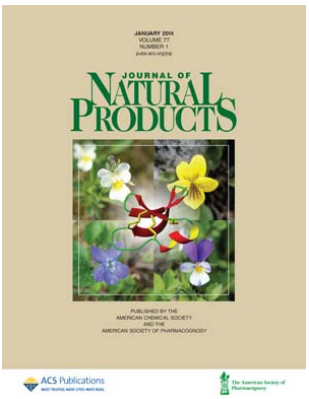
Reviews and Book chapters 2014-2016



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2. Burman R, Gunasekera S, Strömstedt AA, Göransson U. (2014) Chemistry and Biology of Cyclotides: Circular Plant Peptides Outside the Box. J Nat Prod. 77(3):724–736 Cover Feature

Agencies that support the work/Funding

Swedish Research Council, NT, 600 kSEK
Scientific Domain of Medicine and Pharmacy UU, 750 kSEK.

Molecular Pharmacognosy – Lateral gene transfers as targets for drugs against parasites

Research Group Leader: Cecilia Alsmark

The modern approach to drug discovery involves identification of possible drug targets by exploring the unique metabolism of individual pathogenic organisms. We have used bioinformatics to compare and contrast the role of lateral gene transfer (LGT) in shaping the genomes of important parasitic protozoa of man such as *Entamoeba histolytica, Leishmania spp Trypanosoma bruceii* and *Trichomonas vaginalis*. The goal was to identify the amount and types of genes affected and to investigate the degree to which LGT has influenced the evolution of these diverse parasites. The data has also shed light to one of the key questions in understanding evolution – the origin of the eukaryotic proteome.

The organisms chosen are major and increasingly-difficult-to-treat parasites affecting many millions of people yearly. Recent reports about failed treatment due to emerging resistant strains, highlights the urgent need for new drug targets. LGT provide attractive candidates as therapeutic leads – as genes acquired from bacteria by the parasite can be expected to be absent or structurally different from the genome of the human host. In collaboration with TIGR and Sanger Institutes we have made genome wide tree based screens for LGT in the genomes of *E. histolytica*, the trypanosomatides and *T. vaginalis*. In order to achieve an effective but reliable screen of these large datasets we combined rapid screening methods (such as homology searches and distance phylogeny) for LGT followed by a more detailed Baysian phylogenetic analysis of genes that pass the primary screen. All Bayesian trees were manually inspected and all cases where the tree topology show one of our chosen parasites clustered with prokaryote sequences separated from any other eukaryote by at least one well supported node was considered as a LGT in that specie for the gene analysed. The conservative selection thresholds singled out recent LGTs that probably only represent a subset of the complete transferome in our selected pathogens. The analyses showed that many of the metabolic differences between these parasites and man are due to LGT into the parasite genomes.

The LGTs are integrated into diverse metabolic pathways, including carbohydrate, nucleotide and amino acid metabolism. Thus, in the broadest sense LGT must be affecting the fitness of the recipient organism. The bacterial like-hemolysin acquired through LGT in *Entamoeba*

may be directly involved in virulence; they are commonly transferred among bacterial pathogens. Many of the LGTs detected lack a homologue in mammalian genomes, e.g. tagatose-6-phosphate kinase, that's active in galactose metabolism in *E. histolytica,* but not in human. Other LGTs, inferred by phylogeny as bacterial like, are likely to be structurally different to the ancestral eukarytotic homologue, for example isovaleryl-CoA dehydrogenase in the trypanosomadies.

The results also indicate strongly that recent gene transfers are but the tip of a potentially very large iceberg of gene transfers which over time have fundamentally shaped the content of eukaryotic genomes. Present work focus on developing and using analytical approaches to detect deeper transfers, to map this information onto protozoa metabolism, and to use this to begin to better understand the process of gene transfer over time *in silico* and *in vitro*. Better understanding of the metabolic impact of LGT in eukaryotes will guide us in the screen for potential drug targets.

Members of the group during 2016

Cecilia Alsmark, Researcher
Anna Koptina, PhD., Post doc

Publications 2014-2016

1. Sikora P, Andersson S, Winiecka-Krusnell J, Hallström B, **Alsmark C**, Troell K, Beser J, Arrighi RB. Genomic Variation in IbA10G2 and Other Patient-Derived *Cryptosporidium hominis* Subtypes. J Clin Microbiol. 2017 Mar;55(3):844-858. doi: 10.1128/JCM.01798-16. Epub 2016 Dec 21.

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3. Vikeved, E., Backlund, A. and **Alsmark, C**. (2016) The Dynamics of Lateral Gene Transfer in Genus *Leishmania* - A Route for Adaptation and Species Diversification. *PLoS Negl Trop Dis*. Jan 5;10(1)

4. Strese, Å., Backlund, A. and Alsmark, C. A recently transferred cluster of bacterial genes in Trichomonas vaginalis – lateral gene transfer and the fate of acquired genes. BMC Evolutionary Biology 14:119-122. 2014

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6. Hirt RP, Alsmark C, Embley TM. Lateral gene transfers and the origins of the eukaryote proteome: a view from microbial parasites. Curr Opin Microbiol. 2015 Feb;23:155-62.

7. Alsmark C, Foster PG, Sicheritz-Ponten T, Nakjang S, Martin Embley T, Hirt RP.Patterns of prokaryotic lateral gene transfers affecting parasitic microbial eukaryotes. Genome Biol. 2013 Feb 25;14(2):R19

Molecular Pharmacognosy - Methods and strategies of selection

Research Group Leader: Anders Backlund

In the process of developing new drugs more focus has lately been given to the process of selection and design of experiments, as opposed to the attempts in previous decades to use brute force to unravel drugability. These trends correspond with publications indicating that a significant proportion of new chemical entities registered by the FDA during the last few years are still derived directly from natural sources. With this project we attempt to develop methods of selection and tools for prediction, by combining insights from chemographic and phylogenetic analyses.

Life on Earth has one common history during which evolutionary forces have acted on living organisms and eventually producing the biological diversity displayed today. In parallel, these evolutionary forces have produced an immense chemical diversity of pre-validated, biologically active, chemical compounds present in nature. Hence, we have a chemical space occupied by compounds of natural origin, and an evolutionary space occupied by extant and extinct organisms. In the last year several major achievements have been made in this direction, within the project.

The ChemGPS-NPweb. During 2007 Josefin Rosén *née* Larsson (see publication list) completed the work on a global chemographic model describing the chemical space of natural products. With this model, a 'stable' map for exploring chemical space is established, and is available for studies of natural product. Using this, comparisons between properties of different groups of compounds can be made, volumes of chemical space with biologically active compounds can be identified, and evolutionary questions can be posed. With the purpose to make this tool available to scientists worldwide, a website with an interface allowing researchers to enter structure data as SMILES and retrieve prediction scores (corresponding to positions in 8D chemical space) was launched in 2008. The implementation of an industry-grade PCA tool, SIMCA-QP, in this implementation resulted in an application note published in 2010. In figure 3 below, the web interface is displayed.

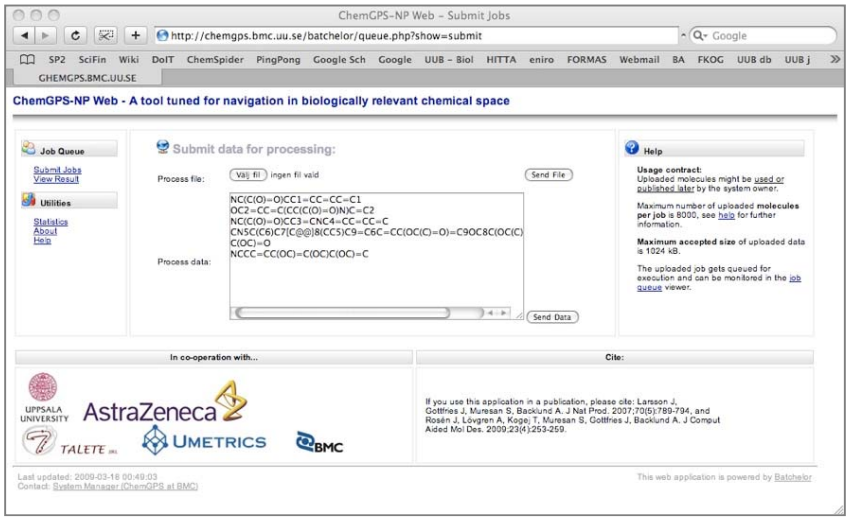

Figure 3. Web interface for ChemGPS-NPweb.

Since the launch in May 2008 more than 9 million computational tasks originating from more than 8700 users world-wide have been predicted via ChemGPS-NPweb.

In 2016 a strategic support from the scientific domain was obtained to develop and modernize the ChemGPS-NP website, which will be implemented during 2017.

Chemographic predictions and Euclidean distances. In 2013, the EU decided to support the ITN grant application "MedPlant". The ITN MedPlant is coordinated from University of Copenhagen, and includes one 'early stage researcher' (PhD-student) MSc. Astrid Henz to be stationed at Uppsala University under the supervision of Prof. Backlund, and a closely collaborating 'experienced researcher' (post-doc.) Dr. Rosa Buonfiglio, to be stationed at AstraZeneca R&D in Mölndal under the auspice of Dr. Thierry Kogej.

The recruitment process of both positions was finalized during 2014, and both beneficiaries have been in place during 2015 contributing greatly to work in the intersection between evolution and natural products chemistry.

During 2015 a very important study in the area of methodology and computational chemistry was published by Buonfiglio *et al.*, involving a large part of the research group. Here chemographic mapping and Euclidean distances were compared to structural fingerprint similarity indices, with regard to their predictive power on estimating biological activities from chemical structures. The main conclusion was that although both methods exhibited a strong ability to predict biological activity, the results were only partly overlapping, and hence complementary. The surprising precision found in ECFP_4 was balanced by the swift visualization in the ChemGPS-NP chemical propert space.
The 514 257 bioactive compounds retrieved from ChEMBL provided 826 281 data points, one for each biological activity experimentally determined for each compound, which were used as training- and validation sets .
From this study, it can also be argued that within one distance unit in ChemGPS-NP Euclidean distances, a high probability of retrieveing similar biological activity is found, hence giving a tentative "cut off" value for prospective studies attempting to propose biological activities for novel and previously untested compounds.

Shortly after, in the beginning of 2016, Dr. Buonfiglio obtained a position at an Italian chemoinformatics company stationed in Rome.

Connecting phylogenies and chemography. During 2013 two studies utilizing a phylogenetic or ecological approach were published from the group. In the first of these, a broad comparison between natural products from terrestrial and marine organisms was attempted. To obtain a relevant data-set partition, only organisms whose entire phylogenetic lineage was marine, were coded as such. The rational for this decision was that even if e.g. whales do live in a marine environment, their biosynthetic machinery has for millions of years been honed to provide functions for a terrestrial life mode. In this process it can be assumed that many of the functions crucial for a marine life mode has been lost.

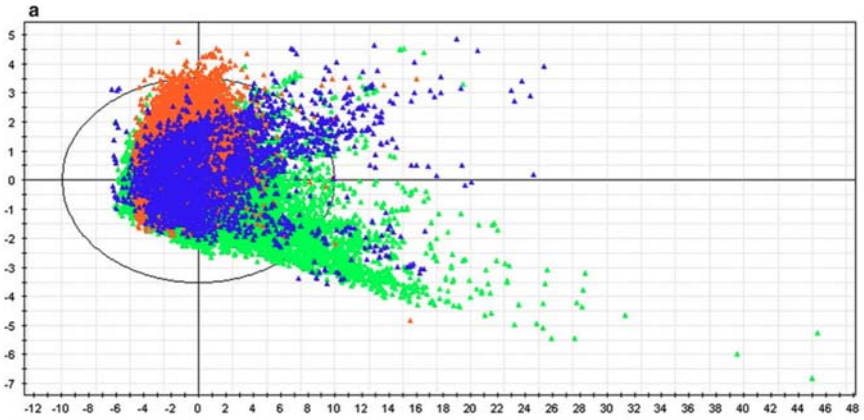

Figure 4. Results from chemographic mapping of marine (blue), terrestrial (green) and druglike (orange) compounds, compiled from literature. Plots clearly demonstrates differential coverage of multi-dimensional chemical property space. From Muigg *et al.*, 2013 in *Phytochemistry Reviews*.

Data compiled clearly demonstrates the differences between terrestrial and marine natural products chemistry (Figure 4), as well as both of these to a set of circa 50 000 'druglike compounds' from the Maybridge compound libraries.

In a second publication, a central question in natural products research – the integrity of a biological sample – was addressed using phylogenetic analysis. During the last few years an increasing interest in natural products from microorganisms such as bacteria and endophytic fungi has become evident in literature. The extensions of these observations, is naturally that when collecting a larger sample such as a macro-organism, e.g. a plant or an animal, we can also assume that within that sample a multitude of microorganisms is also housed.

In this study, samples of the soft coral *Alcyonium digitatum* were obtained from collaborators at the marine biology laboratories on Tjärnö at the Swedish west coast. The samples were sterilized with alcohol, after which a small sample under sterile conditions was extracted from the center of the coral colony. This sample was homogenized and dispersed on agar-plates prepared for bacteria cultivation. Bacterial colonies were retrieved, re-plated and cultivated to obtain adequate sample size, and subsequently DNA extracted and the two molecular markers 23S and 18S (segments encoding the large and small ribosomal subunits rRNA) sequenced. The hence obtained data from +50 bacterial strains, were co-analyzed with a reference data-set using phylogenetic analysis and BLAST sequence homology similarity searching. These analyses provide a completely congruent, and well corroborated, view of significant systematic diversity *inside* a small and supposedly homogenous sample. The results from the analyses are shown in Figure 5 below.

During 2016 the first PhD-student in the dual-degree program between Uppsala University and Kaohsiung Medical University, MSc. Kuei-Hung Lai, continued working with Prof. Backlund and Dr. Wedén as his supervisors at Uppsala University. MSc. Lais' project is focus on the study of triterpenoid specialized metabolites from fungi and sponges, several of which are used in traditional Chinese medicine. MSc. Lai returned from Uppsala to Kaohsiung Medical University to finalize the last part of his project with an aim for a dissertation during 2017.

During 2015 we welcomed the groups first PhD-student on a scholarship from China Scholarship Council; MSc. Lu Yang, from the Department of Complex Prescription of TCM, China Pharmaceutical University, Nanjing was working with Prof. Backlund as her supervisor at Uppsala University. MSc. Yangs' project adds a more pharmacological perspective to the chemographic exploration of natural products chemical property space. Here a traditional Chinese medicin known as ge-gen decoction and used for treatment of primary dymenorrhea is studied in detail with the aim to understand the pharmacological contributions from the many different ingredients. The work performed in Uppsala

includes interpretations from ChemGPS-NP and molecular docking studies in comparision with previously obtained pharmacological studies performed in China.

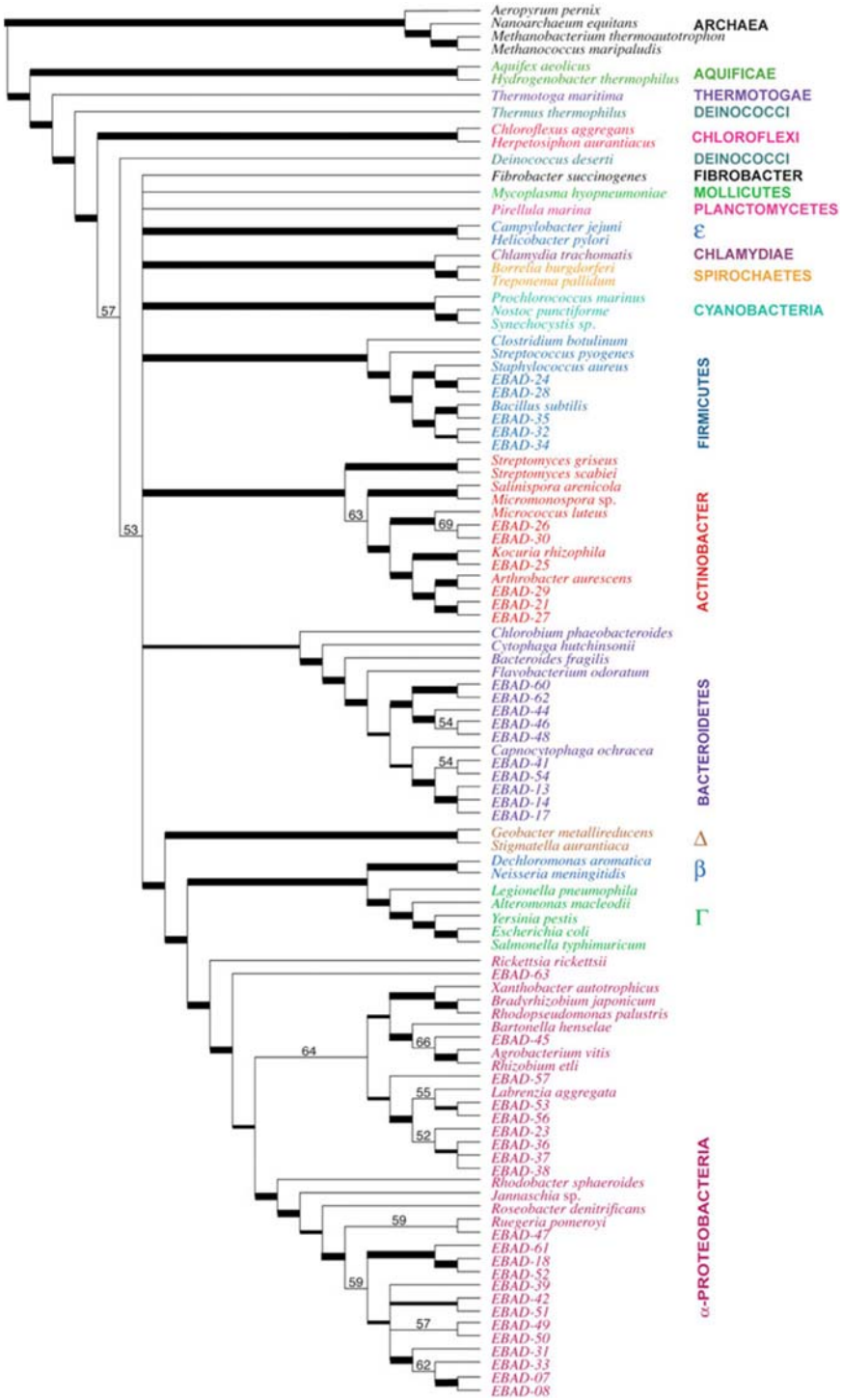

Figure 5. Phylogenetic tree a selected set of reference bacteria, and phylogenetic position of environmental samples from the interior of an *Acyonium* soft-coral (labels EBAD-#). This indicates not only that there is a wide diversity of bacteria living inside the coral, but also that these can be firmly assigned to evolutionary groupings by means of phylogenetic analysis. From Alsmark *et al.*, 2013 in *Phytochemistry Reviews*.

Members of the group during 2016

Anders Backlund, Professor
Christina Wedén, Lecturer and Director of Studies, PhD
Cecilia Alsmark, Assistant Professor
Thierry Kogej, researcher at AstraZeneca, PhD
Anna Koptina, postgraduate researcher, PhD
Rosa Buonfiglio, postgraduate researcher at AstraZeneca, PhD – left for position in industry, spring 2016
Elisabet Vikeved, MSc, PhD student
Astrid Henz, MSc, PhD student
Kuei-Hung Lai, MSc, dual degree PhD student
Lu Yang, MSc, visiting PhD student
Åke Strese, MSc, PhD student

Publications 2014-2016

1. Strese, Å., Backlund, A. and Alsmark, C.: A recently transferred cluster of bacterial genes in *Trichomonas vaginalis* – lateral gene transfer and the fate of acquired genes. *BMC Evolutionary* Biology 14:119-122 – 2014.

2. Koptina, A., Strese, Å., Backlund, A., and Alsmark, C.: Challenges to get axenic cultures of *Trichomonas* spp. – A new approach in eradication of contaminants and maintenance of laboratory microbiological cultures. *Journal of Microbiological Methods* 118:25-30 – 2015

3. Buonfiglio, R., Engkvist, O., Várkonyi, P., Henz, A., Vikeved, E., Backlund, A., and Kogej, T.: Investigating pharmacological similarity by charting chemical space. *Journal of Chemical Information and Modelling* DOI: 10.1021/acs.jcim.5b00375 – 2015

4. Vikeved, E., Backlund, A., and Alsmark C.: The dynamics of lateral gene transfer in genus *Leishmania* - a route for adaptation and species diversification. *PLoS Neglected Tropical Diseases 10(1): e0004326.* DOI:10.1371/journal.pntd.0004326 – 2016

5. Lee, J.-C., Chang, F.-R., Chen, S.-R., Wu, Y.-H., Hu, H.-C., Wu, Y.-C., Backlund, A., and Cheng, Y.-B.: Anti-Dengue virus constituents from Formosan Zoanthid *Palythoa mutuki*. *Marine Drugs* 14, 151; doi:10.3390/md14080151 – 2016

6. Lai, K.-H., Lu, M.-C., Du, Y.-C., el-Shazly, M., Backlund, A., Wu, T.-Y., Hsu, Y.-M., Henz, A., Yang, J.-C., Backlund, A., Chang, F.-R., and Wu, Y.-C.: Cytotoxic lanostanoids from *Poria cocos*. *Journal of Natural Products* – published on-line – 2016

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Umetrics, Talete Srl and Kode Chemoinformatics

Undergraduate Teaching

The Department of Medicinal Chemistry is involved in teaching at eight educational programmes: the Bachelor of Science in Pharmacy programme (180 hp), the Master of Science in Pharmacy programme (300 hp), the Biomedicine programme (180 hp), Master programme in Biomedicine (120 hp), Master Programme in Forensic Science, 120 hp, and Master of Science in Chemical Engineering (300 hp). In addition, the Department is actively participating in two of the dedicated masters-programmes at Faculty of Pharmacy: Drug management (120 hp) and Drug Discovery and development (120 hp), both requiring the degree of bachelor for admission, and thus forming the final two years of a masters degree. Furthermore, the students can specialise in Analytical chemistry, Organic chemistry or Pharmacognosy by taking electives courses and undergraduate projects (15 or 30 hp) in these disciplines. These programmes prepare the students for work in academia and pharmaceutical and biotechnical industries. The degree of Bachelor of Science in Pharmacy is the minimum requirement for a dispensing pharmacist position at a pharmacy.

All professors and lecturers at the Department are involved in lectures and seminars and are responsible for examination, whereas the PhD students are mainly involved in seminars and laboratory sessions. Our course administration plays an important role in the administration of courses and student contacts.

The Bachelor of Science in Pharmacy programme, 180 hp (Receptarieprogrammet)
The Department contributes with several courses in chemistry and pharmacognosy. The number of students attending this programme is approximately 35 each semester. The five courses given by the Department every semester are basic courses in pharmacognosy as well as analytical, general, medicinal and organic chemistry. Furthermore, the Department offers the student some elective courses in Bioanalytical Chemistry 7.5 hp; Drug Discovery based on Natural products 7.5 hp; Herbal remedies 7.5 hp; and the field course Global Pharmacy 7.5 hp. During the latter course the students travel to a country in which western school medicine can be compared with a living traditional medicine. During the last years the field part has taken place in Taiwan, but also Sri Lanka and Egypt have been receiving the course.

The Master of Science in Pharmacy programme, 300 hp (Apotekarprogrammet)
Each semester the Department presents nine mandatory courses for the circa 90 students at this programme: Drug-oriented general chemistry, Analytical pharmaceutical chemistry, Drug-oriented organic chemistry, Medicinal chemistry, Bioanalytical chemistry, Pharmacognosy, Drug synthesis, Pharmaceutical biotechnology and Product and process analytical chemistry. The aim is to provide a basic understanding of analytical, general and organic chemistry as well as pharmacognosy – the latter including natural products chemistry. Furthermore, the Department offers the student some elective courses in Bioanalytical chemistry 7.5 hp; Advanced organic chemistry and drug synthesis 15 hp, Drug discovery and development 7.5 hp, Computer aided drug design 7.5 hp, Drug Discovery based on Natural products 7.5 hp; Herbal remedies 7.5 hp; and the field course Global Pharmacy 7.5 hp. The undergraduate projects are integrated in the current research projects at the Department and prepare the student for work with drug development in the pharmaceutical chemistry as well as for subsequent PhD studies.

Biomedicine programme, 180 hp (Biomedicinprogrammet)
The Department's contribution to this programme (after revision of the programme in 2013) aims at providing fundamental knowledge of general, organic, and drug oriented chemistry and the courses given are Chemistry for biomedicine (15 hp) and Medicinal Chemistry (6 hp) integrated in the 12 hp course Pharmacology with medicinal chemistry. In this programme approximately 48 students are enrolled every year.

Master of Science in Chemical Engineering, 300 hp (Civilingenjörsprogrammet, kemiteknik)
Medicinal chemistry (7.5 hp) in the 6th semester is mandatory for about 10-20 students each year. For students in this programme the Department offers several elective courses (Analytical Pharmaceutical Chemistry; Drug analysis, Process monitoring, Drug Discovery based on Natural products and Computational medicinal chemistry). Senior staff members from the Department are frequently involved as experts and examiners in undergraduate projects performed by students at industrial or academic institutions during their last semester in the programme.

Master programme in Drug Management, 120 hp
(Masterprogrammet i läkemedelsanvändning)
In this programme the Division of pharmacognosy contributes with aspects on different medicinal systems, ethnopharmacology, and sustainable use of natural resources. The approach of the entire programme is to broaden the students' knowledge about all aspects of drug usage, from genetic variation in patients to social and cultural perspectives. Students at this programme will be prepared for positions ranging from education and academic research to taking office in governmental organisations.

Master programme in Drug Discovery and Development, 120 hp
(Masterprogrammet i läkemedelsutveckling)
In this programme the Division of organic pharmaceutical chemistry contributes with aspects on medicinal chemistry and drug discovery. The programme aims to deepen the knowledge of the students in areas of drug discovery and development. Students at this programme will be prepared for positions ranging from education and academic research to positions in pharmaceutical industry and biotech.

Master Programme in Forensic Science, 120 hp (Masterprogrammet i forensisk vetenskap)
The Division of analytical pharmaceutical chemistry provides, in cooperation with the division of toxicology at the Department of Pharmaceutical Biosciences, a mandatory course in Analytical Toxicology comprising 30 hp on the third semester of the master programme in Forensic Science. This program will provide deep knowledge and understanding of application of biomedical analysis techniques within the forensic field. The students at this program will be prepared for employments with a forensic focus ranging from education and academic research to positions within authority and industry.

Master Programme in Biomedicine, 120 hp (Masterprogram i Biomedicin)
The Division of organic chemistry presents two courses in Computational Medicinal Chemistry and Drug Discovery and Development, 7,5 hp each. The second course is in cooperation with the two other Departments at the Faculty of Pharmacy. The focus of the programme is biomedical sciences.

Master Programme in Pharmaceutical Modelling, 120 hp
The Master Programme in Pharmaceutical Modelling provides the student with theoretical and practical knowledge of the use of advanced modelling techniques in the various disciplines of drug discovery, development and usage. For students in this programme the Department is responsible for the following courses, Drug Discovery and Development, Computational Medicinal Chemistry and Advanced Molecular Modelling applied to Drug Discovery. The master programme attracts 20-30 students per year.

Centres and Facilities

RAPID

RAPID (Rational Approaches to Pathogen Inhibitor Discovery) is an integrated centre that brings together medicinal chemistry, computational chemistry and structural biology groups at Uppsala University with the overall aim to develop a new drug candidate against tuberculosis. RAPID is supported by the Swedish Foundation for Strategic Research (SSF), and by grants from VR (Swedish Science Research Council) VINNOVA and the EU (NM4TB project). Professor Alwyn Jones heads the center. The other principal investigators are Sherry Mowbray, Mats Larhed and Anders Karlén.

WIPPET

The aim of the WIPPET (Wallenberg Infrastructure Preclinical PET/MRI) infrastructure is to give Swedish scientists access to a preclinical small animal PET/MRI CT/SPECT scanners in an environment that provides expert technical and scientific competence. The WIPPET infrastructure will enhance multidisciplinary research at the interfaces between chemistry, biology and medicine, as well as within the field of PET. The WIPPET infrastructure is located at the Platform for Preclinical PET-MRI, which is equipped with a fully functional preclinical laboratory and radiochemistry facilities. Both scanners will be nationally available to Swedish scientists via Swedish Bioimaging. Professor Mats Larhed heads the center.

Facility for Medicinal Chemistry - Lead Identification, Drug Discovery and Development Platform, Science for Life Laboratories.

The objective for the Medicinal Chemistry Lead Identification-facility is to contribute to drug discovery projects with expertise in medicinal chemistry and to deliver an advanced lead compound with potency on the selected target, an ADME-profile that allows for adequate exposure in vivo and an acceptable toxicology profile. The projects are run in collaboration with Swedish academic groups. The advanced lead compound is thereafter planned to be used for proof-of-concept studies in animal models. The facility has during 2015 been active in several projects with design and synthesis of new drug-like compounds as well as project management and support regarding intellectual property.

Staff at the facilities during 2016

Ulrika Yngve, PhD
Johan Wannberg, PhD
Anna-Karin Belfrage, PhD
Neeraj Garg, PhD
Johanna Larsson, PhD

Awards and Appointments 2016

Lecturer Anja Sandström awarded The Pharmacy Student Representation Award for Student Attendance 2016.

Lecturer Jakob Haglöf awarded The Pharmacy Student Representation Award for Student Influence 2016.

Researcher Charlotta Wallinder awarded Limbic Prize for best pedagogy from the Biomedicine Program.

PhD student Bogdan Mitran awarded the yearly European Association of Nuclear Medicine (EANM) Eckert & Ziegler Abstract Award.

Marc Stevens, former PhD student in PET science at the Department of Medicinal Chemistry, has be Knut and Alice Wallenberg's Foundation awared a two-year post doctoral scholarship for assignment at the Stanford University (US).

List of Staff

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Division of Pharmacognosy, www.ilk.uu.se/forskning/fkog/
Division of Molecular Imaging, www.ilk.uu.se/research/ama_en/
Preclinical PET-MRI Platform, www.ilk.uu.se/platforms/ppp_en/
SciLifeLab Drug Discovery and Development Platform, www.ilk.uu.se/platforms/scilifelab-dddp/
ENABLE/European Gram Negative Antibacterial Engine, www.ilk.uu.se/platforms/enable/

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Mikael Hedeland
Ylva Hedeland
Monika Johansson
Anders Karlsson
Curt Pettersson
Kristian Pirttilä
Alfred Svan
Niklas Tyrefors
Douglas Westerlund

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Ahmed Adeyemi
Hiba Alogheli
Anna-Karin Belfrage
Peter Brandt
Jens Carlsson
Shiao Chow
Karin Engen
Neeraj Garg
Johan Gising
Anders Hallberg
Charles Hedgecock
Anneli Hällgren
Rebecka Isaksson
Mariama Jaiteh

Jon Kapla
Anders Karlén
Rebecka Klintenberg
Vivek Konda
Mats Larhed
Johanna Larsson
Gunnar Lindeberg
Martin Lindh
Pierre Matricon
Luke Odell
Gustav Olanders
Ulrika Rosenström
Sara Roslin
Jonas Rydfjord
Kristian Sandberg
Anja Sandström
Bobo Skillinghaug
Anna Skogh
Christian Sköld
Sorin Srbu
Marc Stevens
Lars-Olof Sundelöf
Uno Svensson
Jonas Sävmarker
Duc Duy Vo
Charlotta Wallinder
Johan Wannberg
Ulrika Yngve
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Aleksei Zeifman
Linda Åkerbladh

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Sergio Estrada
Mats Larhed
Ram Kumar Selvajaru
Ola Åberg

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Olof Eriksson
Örjan Lindhe
Bogdan Mitran
Anna Orlova
Maria Rosestedt

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Camilla Eriksson
Sunithi Gunasekera
Ulf Göransson
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Erik Jacobsson
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Gunnar Samuelsson
Md Shafiullah
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Åke Strese
Adam Strömstedt
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